Furan- and Pyran-Based Heterocycles as Subtype-Selective Ligands of the Estrogen Receptor

Synthesis and Biological Characterisation

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Jochen Zimmermann

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Prüfungsausschuss:	Prof. Dr. J. Heilmann	(Vorsitzender)
	Prof. Dr. E. von Angerer	(Erstgutachter)
	Prof. Dr. B. König	(Zweitgutachter)
	Prof. Dr. R. Gschwind	(Drittprüfer)

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List of Abbreviations

AcOH	acetic acid
ACTH	adrenocorticotropic hormone
AF	activation function
AIB1	amplified in breast cancer 1
AMP	adenosine monophosphate
AP1	activator protein 1
app	approximately
aq	aqueous
ATCC	American Type Culture Collection
ATP	adenosine triphosphate
bps	base pairs
BSA	bovine serum albumine
BuLi	n-butyllithium
CBP	CREB binding protein
CC	column chromatography
$CDCl_3$	chloroform-d_1
CDI	carbonyl diimidazole
cf	confer
conc	concentrated
CREB	Ca^{2+}/camp response element-binding protein
CRH	corticotropin releasing hormone
ctFCS	charcoal treated FCS
DBD	DNA binding domain
DCC	dextran choated charcoal
DCE	dichloroethane
DCM	dichloromethane
dec	decomposition
DES	diethylstilbestrol
DHP	3,4-dihydro-2H-pyran
DIAD	diisopropyl azodicarboxylate

DMAP	4-(N,N-dimethylamino)pyridine
DMEM	Dulbecco's modified eagle medium
DMF	N,N-dimethylformamide
DMSO-d_6	dimethylsulfoxide, entirely deuterated
DNA	desoxyribonucleic acid
DPN	diarylpropionitrile; 2,3-bis(4-hydroxyphenyl)propionitrile
DTE	*erythro*-1,4-dimercapto-2,3-butandiol
DTT	*threo*-1,4-dimercapto-2,3-butandiol
E2	17ß-estradiol
EDTA	ethylendiamintetraacetic acid
e.g.	exempli gratia (lat. = for instance)
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
EMEM	Eagle's minimum essential medium
EORTC	European organization for research and treatment of breast cancer
EpRE	electrophile response element
ER	estrogen receptor
ERE	estrogen response element
EtOAc	ethyl acetate
EtOH	ethanol
FCS	fetal calf serum
Fig	figure
FSH	follicle-stimulating hormone
FT	fourier transformation
GnRH	gonadotropin releasing hormone
GRIP1	glucocorticoid receptor-interacting protein 1
h	hour
HAP	hydroxylapatite
HAS	human albumine serum
HAT	histone acetyltransferase
HDAC	histone deacetylase
HER	human epidermal growth factor receptor
Hsp	heat-shock protein
Hz	Hertz

IC_{50}	inhibitory concentration leading to a 50% decrease of mediated effect
ICI	ICI 182.780; fulvestrant
i.e.	id est; (lat. = that is to say)
IGF-1	insulin-like growth factor-1
IGFBP-4	insulin-like growth factor binding protein-4
IR	infrared
kDa	kiloDalton
KHMDS	potassium 1,1,1,3,3,3-hexamethyl-disilazane; potassium bis(trimethylsilyl)amide
LDA	lithium diisopropylamide
LBD	ligand binding domain
LH	luteinizing hormone
log P	logarithm of the octanol-water partition coefficient
m-CPBA	*meta*-chloroperbenzoic acid
MAPK	mitogen-activated protein kinase
MeOH	methanol
MeOD-d_4	methanol, entirely deuterated
min	minute
MS	mass spectrum
MW	molecular weight
N-CoR	nuclear recepetor corepressor
NF-κB	necrosis factor kappa B
NH	no hormone
NMR	nuclear magnetic resonance
NR	nuclear receptor
OD_{600}	optical density at 600nm wavelength
p	significance
p160	160kDa protein
p300	300kDa protein
$P450_{arom}$	cytochrome P450 aromatase
p.a.	pro analysis
PBS	phosphate buffered saline
PI3-K	phosphatidylinositol 3-kinase
PKA	protein kinase A

PKC	protein kinase C
PPA	polyphosphoric acid
PP_i	inorganic pyrophosphate
ppm	parts per million
ppv	parts per volume
PTSA	*para*-toluenesulfonic acid
RAC3	receptor-associated coactivator 3
RBA	relative binding affinity
rms	root means square
RTP	relative transcriptional potency
s	second
sat.	saturated
SERD	selective estrogen receptor downregulator
SERM	selective estrogen receptor modulator
SMRT	silencing mediator for retinoid and thyroid hormone receptor
S_N2	bimolecular nucleophilic substitution
SRC	steroid receptor coactivator
TBS	*tert*-butyldimethylsilyl
TBDMSCl	*tert*-butyldimethylsilyl chloride
T/C	treated vs. control
THC	(R,R)-5,11-*cis*-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol
THF	tetrahydrofuran
THP	4-(2-tetrahydro-2H-pyranyl)
TIF2	transcription intermediary factor-2
TLC	thin layer chromatography
TMS	tetramethylsilane
Tris	tris(hydroxymethyl)-aminoethane
TTN	thallium(III) nitrate
UV	ultraviolet
vs	versus
v/v	volume per volume

A Introduction

1 Cancer

1.1 Characteristics of benign and malignant tumours

Cancer can be considered as a disease of certain cells in humans, animals and also in plants. It comprises several distinct types of malignant tumours and subtypes thereof can be found within specific organs. The medical term for cancer or tumour is neoplasm, which means an autonomously and uncontrolled growing mass of abnormal endogenous cells [Pschyrembel, 1990].

Cancers are classified according to the tissue and cell type from which they arise. Tumours arising from epithelial cells are termed carcinomas, which make 90% of all human cancers, whereas those arising from mesenchymal cells (e.g. connective or muscle tissues) are termed sarcomas. The leukemias, a subdivision of the sarcomas, are derived from hemopoietic cells. In contrast to other tumours they do not form solid masses, but grow as individual cells in the blood. [Alberts et al., 1994].

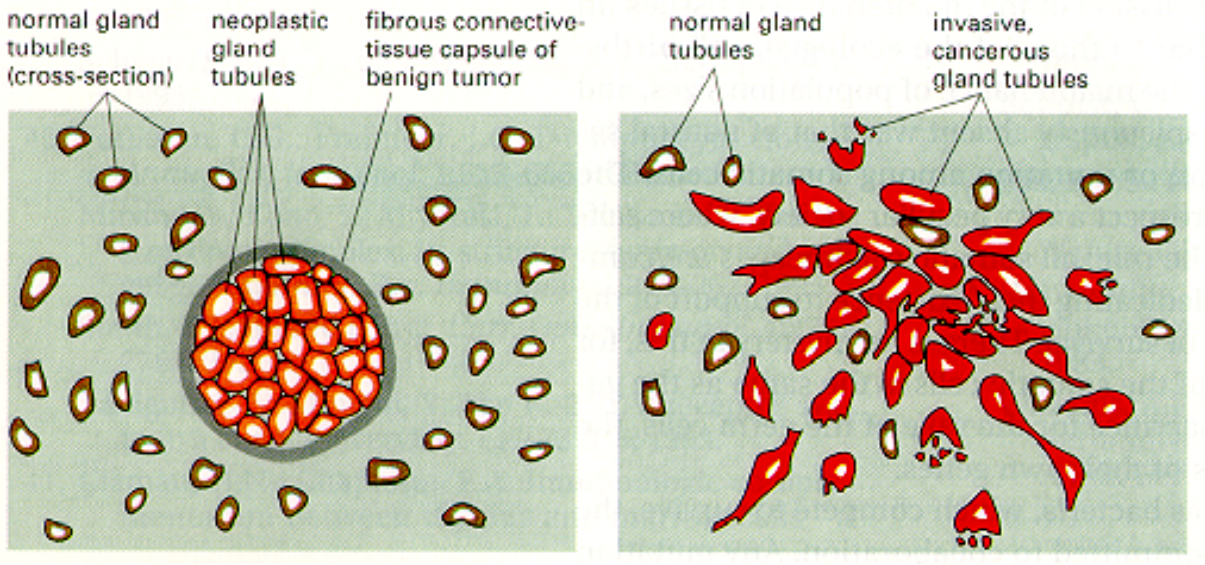


Figure A1: The contrast between an adenoma (benign) and adenocarcinoma (malignant)

Benign tumours remain localised in the original organs and are usually separated from normal tissue by fibrous capsules. They only cause serious medical problems, if their sheer bulk

presses on surrounding tissues or if they secret excess amounts of biologically active substances [Darnell et al., 1986].

Malignant tumours are characterised by several hallmarks, which are proposed to be common features in all forms of human tumours: genomic instability, unlimited proliferation, autonomy towards growth signals, resistance to apoptosis, sustained angiogenesis, tissue invasion and metastasis. Each of these physiological traits is acquired during tumour development. They are directly or indirectly interrelated with each other and may occur at different timepoints during tumour progression, depending on the number und type of genetic changes [Hanahan and Weinberg, 2000].

Changes in the DNA sequence of cells (mutagenesis) – if not hereditary – may be caused by environmental, chemical or biological agents:

- high energetic radiation, causing chromosome breaks and translocations
- chemical carcinogens, causing simple local changes in the nucleotide sequence
- oncogenic viruses, which are capable of introducing foreign DNA into cells [Alberts et al., 1994].

The transformation of a normal into a neoplastic cell requires more than one genetic alteration. It results from the stepwise accumulation of mutations in two broad groups of growth regulatory genes. Proto-oncogenes, either cytoplasmic or nuclear, mutate to oncogenes and their encoded proteins are known to excessively activate cell proliferating pathways. Tumour suppressor genes become inactivated as a result of mutation and the cell looses important control instances on its progression through the cell cycle [Boerner et al., 2002].

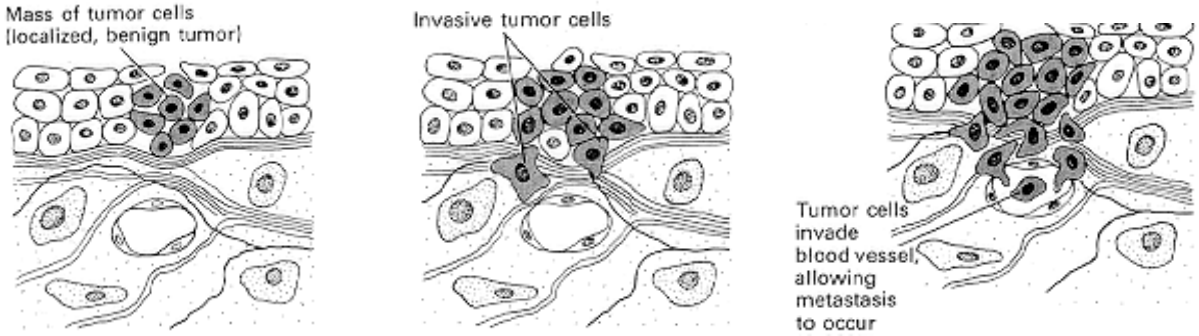


Figure A2: Stages in tumour growth and metastasis

One of the major characteristics of malignant tumours is the capability to spread beyond its original site, to disseminate and form secondary tumours or metastases elsewhere in the body. This implies the ability of the cancer cells to break through the basal membrane, to cross surrounding tissues, to enter the bloodstream or the lymphatic system and to survive and prolif-

erate in a new environment (cf. figure A2) [Darnell et al., 1986]. From a medicinal point of view this aspect complicates a successful treatment and the complete eradication of such malignancies, leaving the chance (and the fear in patients) to relapse due to unrecognised metastases.

Cancer was recorded the second frequent cause of death in Germany after cardiovascular diseases (cf. figure A3). In the year 2003, two hundred and nine thousand people (111.000 male and 98.000 female) died as a consequence of malignant tumours. This makes 25% of the total number of deaths. In the male population the most common cancer deaths were caused by carcinomas of the digestive organs followed by carcinomas of the respiratory system, whereas women predominantly died of carcinomas of the digestive organs and the mammary gland [Statistische Bundesamt, 2005].

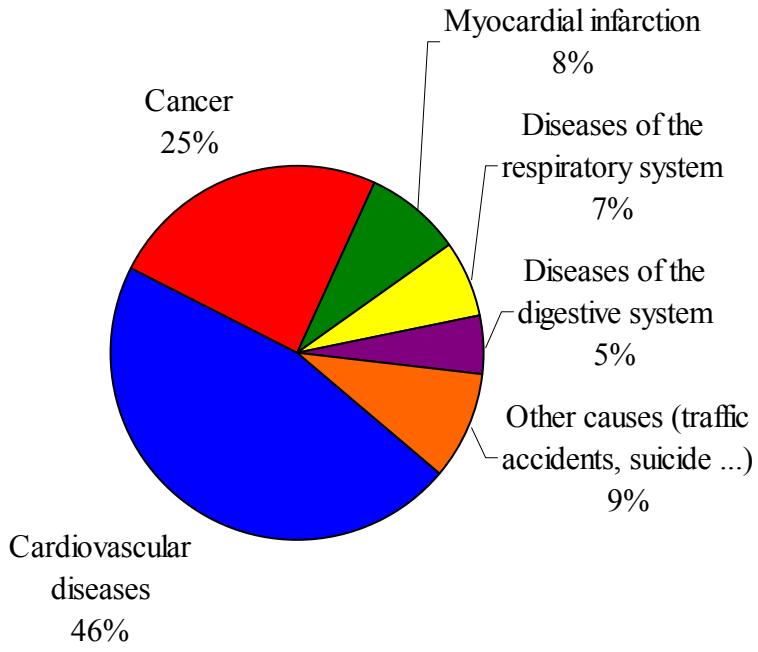


Figure A3: Death causes in Germany 2003

1.2 Breast Cancer

Breast cancer is the most common type of cancer among women all over the world (Ferlay et al., 2004). In Germany, 47.500 new cases of breast cancer are diagnosed each year. In other words, approximately 10% of all German women will develop breast cancer at some stage during their lifetime. The mammary carcinoma makes 25% of all cancer incidences among women, with more than one third (34%) being under the age of 60 years. More than 60% of

the new cases can be cured. When the tumours are diagnosed at a early stage the average relative 5-years survival rate is about 76%. However, about 18.000 women die annually from this disease [Bertz et al., 2004]. Breast cancer among men occurs relatively seldom. It makes less than 1% of all mammary carcinomas in the western civilization and 0.2 – 0.5% of all cancers in male [Jungmayr, 2004]

Nearly all forms of breast cancer arise from glandular tissue (adenocarcinoma). Most invasive breast cancers (> 80%) develop from ductal carcinomas *in situ*. It is characterised as malignant epithelial cells within the mammary ductal system without evidence of invasion. Lobular carcinomas *in situ* constitute approximately 10%. They are often only identified incidentally, because this form of cancer lacks both clinical and mammographic signs in its early stage. Medullary, mucinous or tubular carcinomas occur less often, but provide better prognoses [Van Poznak and Seidman, 2002]

Epidemiologic studies indicate that the incidence of breast cancer is influenced by environmental, endocrine and familial factors. Alcohol abuse, the intake of antioxidant vitamins or phytoestrogens, high fat consumption and/or overweight may contribute to the incidence of breast cancer, though the results reported in literature are contradictory. In contrast, childlessness, early menarche, late menopause, late first full-term pregnancy, long duration of hormone replacement therapy or high-dose oral contraceptives especially in BRCA mutation carriers are associated to increase the risk of breast cancer. [Cade et al., 1998; Clemons and Goss, 2001, Gabrick et al., 2000; Gapstur et al., 1999; Holmes et al., 1999; Smith-Warner et al., 1998].

Inherited predisposition to breast cancer is conferred to two recently identified genes, BRCA1 and BRCA2 [Hall et al., 1990; Miki et al., 1994; Wooster et al., 1994 and 1995]. Individuals, who are born with mutations in either of these genes, show a higher susceptibility to develop breast or ovarian cancer, but the magnitude of the estimated lifetime risk is controversial and can be modified by external factors such as hormonal cofactors or modifier genes [Narod, 2002]. BRCA1/2 mutations are thought to be associated with 5-10% of all breast cancers [Van Poznak and Seidman, 2002]. Attempts to identify a third breast cancer susceptibility gene (BRCA3) have so far been unsuccessful [Narod and Foulkes, 2004].

Up to now, mutations in BRCA1/2 can not be linked to the development of sporadic, non-hereditary forms of breast cancer. However, several studies provide evidence that both BRCA genes encode tumour suppressors, that are responsible for the maintenance of genomic stability and the regulation of cell growth and differentiation. Functional analyses of the encoded

nuclear phosphoproteins revealed their participation in DNA damage repair and transcriptional regulation. The role of BRCA in these processes is supported by the identification of autonomous transcription functions and by protein interactions with a variety of transcription activators and repressors [Zheng et al., 2000]. A false expression or an improperly regulated activity of the BRCA1/2 gene products might contribute not only to the formation of breast cancer but other cancers as well.

It was reported that BRCA1 mediates the repression of the transcriptional activity of the estrogen receptor, which may imply a potential role in the estrogen-signalling pathway [Fan et al., 1999]. Once completely understood this finding may explain why BRCA1/2 exert distinct tumour suppressive properties in the breast and ovarian tissue and consequently, why tumours arise preferably in these tissues. Finally, this knowledge could lead to the design of new strategies for the treatment and prevention of breast cancer.

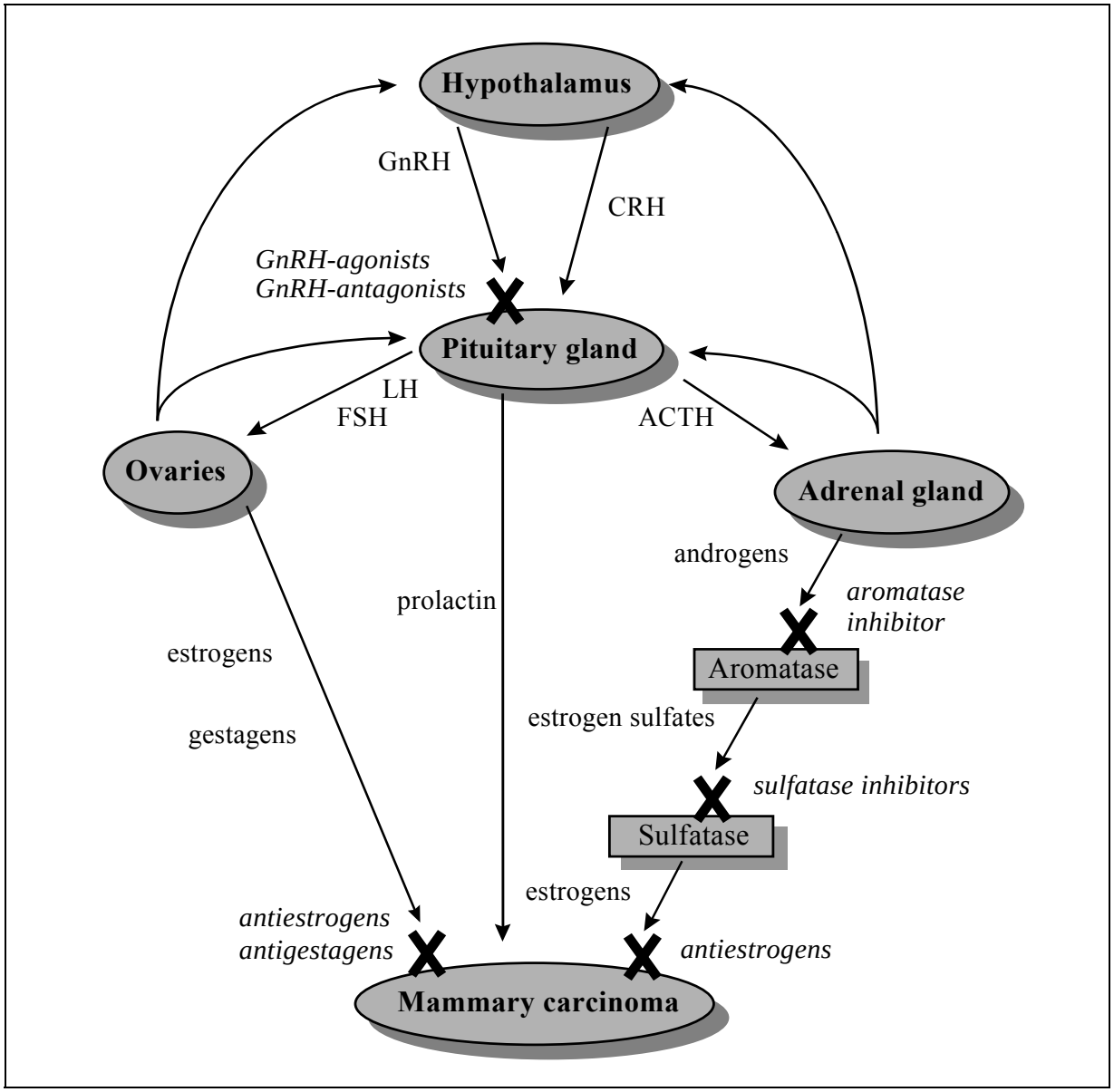
2 Endocrine Therapy of Breast Cancer

Due to the high biological complexity of breast cancer there is no general treatment for this disease. Surgery (i.e. mastectomy or lumpectomy), radiotherapy, hormone therapy, chemotherapy or a combination may be used, depending on the stage of tumour development, type and size of the tumour, and the general health state and age of the patient.

Hormones, especially estrogens, are understood to play an important role in the development and progression of the majority of breast cancers. Their effects are mediated by receptors for the female sexual hormones estradiol and progesterone. Approximately two third of all mammary carcinoma tumours express estrogen (ER) and progesterone receptors (PR), which are well established predictive factors for the likelihood of response to endocrine therapy [Hopp and Fuqua, 2003]. Therefore, the intervention in the endocrine system with the objective to block estrogen synthesis and function is an important option for the treatment of hormone receptor positive breast cancers (cf. figure A4).

Generally, the endocrine therapy of the mammary carcinoma can be divided into two categories: ablative and additive treatment modalities. The ablative therapy is directed towards the removal of the sources of steroids, which are primarily the ovaries in premenopausal women (ovariectomy) and the adrenal gland (adrenalectomy) in postmenopausal women. The principle of the additive therapy is the administration of drugs which interfere with the release of

estrogens, their biosynthesis or their interaction with the ER. In the latter regard, estrogen production persists, but the hormonal effects on the target cancer cell are blocked [Hayes, 2002]. The following sections shall provide an overview over the most promising approaches in endocrine breast cancer therapy and outline recent developments.



Scheme A4: Illustration of the hormone dependency of the mammary carcinoma and the interventions in the endocrine system [modified; von Angerer, 1996]

2.1 Gonadotropin Releasing Hormone (GnRH) Analogues

Initially, ablative hormone therapies were accomplished by surgical or radiation induced ablation of the hormone producing organs. This irreversible procedure has been largely replaced

by the use of pharmaceutical agents whose effects are reversed once the administration of the drugs has been stopped. [Miller, 2004].

In premenopausal women chemical estrogen deprivation can be obtained by administration of GnRH agonists. These peptides are protease-resistant analogues of the natural dekapeptide GnRH, that is also known as luteinising hormone-releasing hormone (LHRH) [Schally et al., 1971]. The latter is produced in the hypothalamus and secreted in a time and concentration regulated fashion into the portal blood system. GnRH interacts with membrane bound receptors (GnRH receptors) in the anterior pituitary gland and stimulates the biosynthesis and secretion of both gonadotropic hormones LH and FSH. These act on the ovaries and regulate the steroid production. Circulating gonadal steroids, in turn, exert both negative and positive feedback actions and thus modulate GnRH and gonadotropin release in the hypothalamus and pituitary gland [Kaiser et al., 1997]. Continuous administration of high doses of GnRH (agonists) leads after an initial overstimulation of release of gonadotropins to a desensitisation and downregulation of GnRH receptors. The consequence is the suppression of ovarian estrogen production to postmenopausal levels [Klijn et al., 2001].

Goserelin (Zoladex®), Buserelin (Profact®), and Leuprorelin (Carcinil®) represent examples of GnRH analogues. Goserelin have been shown to be therapeutically as effective as surgical ovarian ablation in premenopausal women with hormone-sensitive advanced breast cancers [Taylor et al., 1998]. The combination of a GnRH agonists with tamoxifen is superior to a monotherapy with GnRH agonists. This combined estrogen blockade prolongs the progression-free survival and increases both response rate and response duration [Klijn et al., 2001]. Subsequent substitution of tamoxifen for an aromatase inhibitor results in a further reduction of serum estrodiol levels and, clinically, in a prolonged therapeutic remission in a reasonable proportion of patients [Cheung et al., 2001].

The role of GnRH analogues has also been studied extensively in the adjuvant therapy of early breast cancers in premenopausal women. In comparison to cytotoxic chemotherapy ovarian suppression with or without concurrent tamoxifen has shown fewer distressing side effects and equivalent or superior results in terms of disease-free and overall survival at 5-6 years follow-up [Jakesz et al., 2002; Jonat et al., 2002]. The administration of GnRH agonists compared to tamoxifen treatment as well as their use in addition to chemotherapy are subjects of several ongoing clinical trials, whose first results look very promising [reviewed in Sharma et al., 2005].

Thus, GnRH analogues (alone or in combination with other endocrine agents) should be considered as a relevant treatment option in the standard endocrine therapy of premenopausal patients with both early and advanced mammary carcinomas.

2.2 Aromatase Inhibitors

In premenopausal women the ovaries are the main site of estrogen production. After the menopause estrogens are predominantly produced through conversion of adrenal androgens in different peripheral tissues, including skin, muscle, fat and bone [Sasano and Harada, 1998]. Estrogen synthesis also occurs in the normal mammary adipose tissue as well as in breast tumours. Breast cancers in postmenopausal women show the potential to produce sufficient amounts of estrogen to maintain tumour growth, which is explained by a high aromatase activity in these tissues [Miller, 1997].

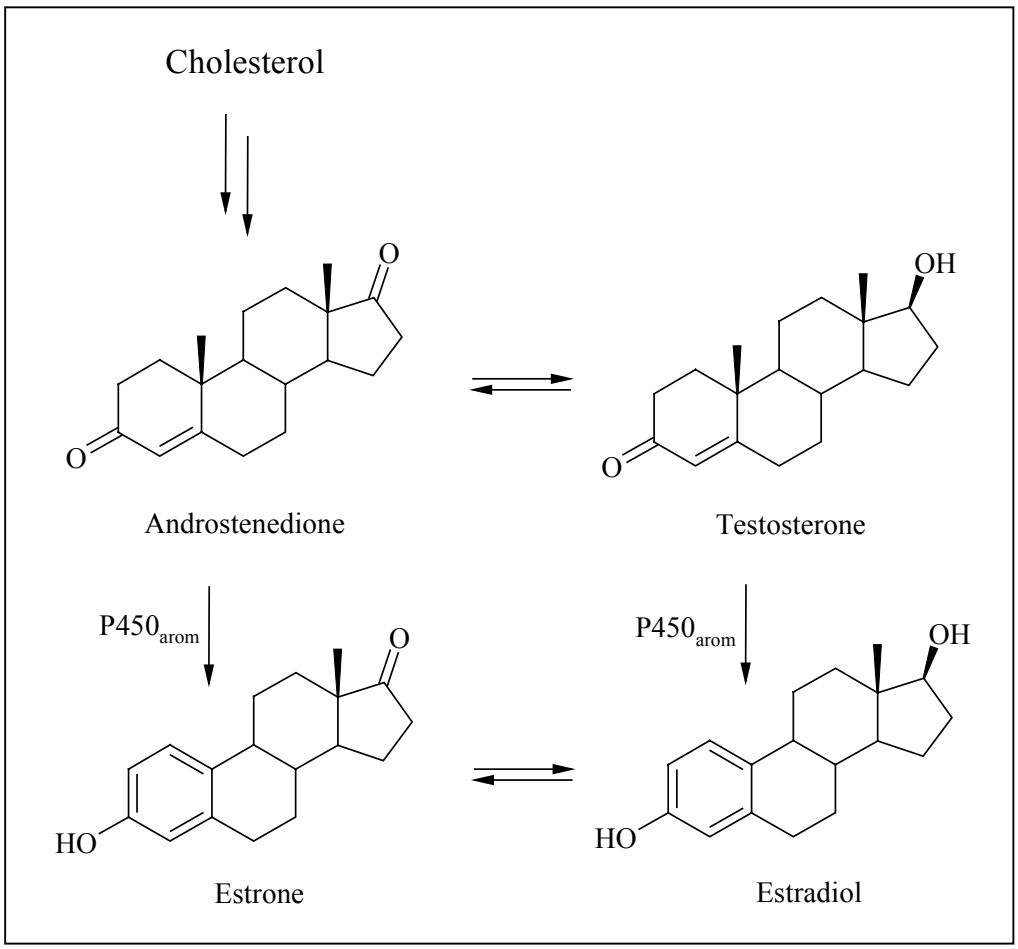
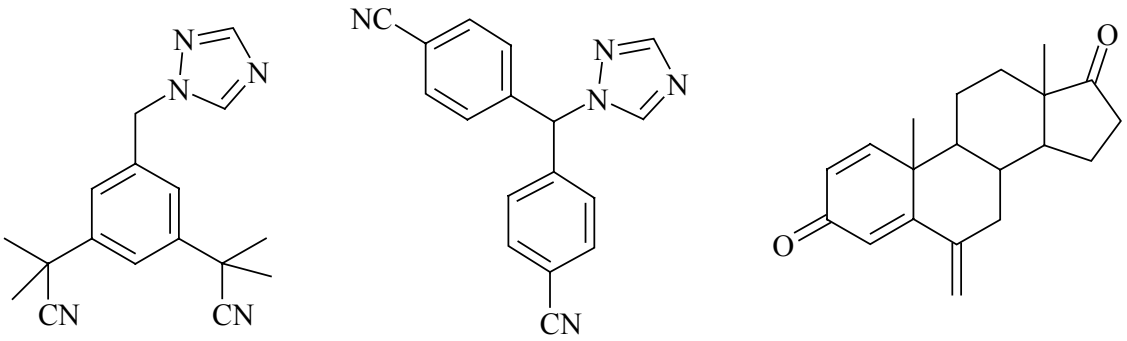


Figure A5: Estrogen biosynthesis

The aromatase is an enzyme complex ($P450_{arom}$) existing of the cytochrom P450 aromatase and the flavoprotein NADPH cytochrome P450 reductase. It catalyses the last step in a series of reactions in estrogen biosynthesis. Androstenedione and testosterone are aromatised by oxidative demethylation to estrone and estradiol, respectively (cf. figure A5), utilizing 3 mol of molecular oxygen and 3 mol of the reduced cofactor NADPH [Simpson et al., 1994]. Aromatase inhibitors, especially those of the new generations, are developed to specifically block this reaction without affecting the synthesis of other steroids, such as corticoids or gestagens.

Aromatase inhibitors can be categorised into steroidal and non-steroidal agents. They represent three generations of evolution, with each generation reflecting increased specificity and higher potency [Miller, 2004]. Steroidal inhibitors are substrate analogues based on the structure of androstenedione. They are converted by the normal catalytic mechanism of the aromatase into reactive intermediates that inactivate the enzyme by covalent and irreversible binding. Once the enzyme is inactivated the duration of inhibitory effect is dependent on the synthesis of new aromatase [Brodie, 2003]. Exemestane (Aromasin®) is the most prominent example and marketed as an aromatase inactivator.
Anastrozole (Arimidex®) and letrozole (Femara®) represent the current lead types of non-steroidal aromatase inhibitors. These agents suppress aromatase activity by a different kind of mechanism. They block the catalytic function of the enzyme in a reversible manner, for the N(4)-atom of their triazole heterocycle coordinates as sixth ligand with the iron in the substrate binding site. Molecular modelling studies show a particularly good fit of these drugs within the ligand binding pocket [Brodie, 2003; Recanatini et al., 2002]. These properties confer both anastrozole and letrozole high potency and great specificity.



Ananstrozole Letrozole Exemestane

Figure A6: Third-generation aromatase inhibitors

Both steroidal and nonsteroidal aromatase inhibitors have shown clinical efficacy over conventional therapies in the treatment of breast cancer and superiority over previous generations of inhibitors [Buzdar et al., 1998; Dombernowsky et al., 1998; Gershanovich et al., 1998; Kaufmann et al., 2000]. Anastrozole, letrozole, and exemestane were introduced as potent and selective third-generation aromatase inhibitors into the market for the endocrine therapy of patients with tumour progression after tamoxifen treatment. These agents have also shown to be superior or equivalent to tamoxifen in first-line settings [Bonneterre et al., 2001, Mouridsen et al., 2003, Paridaens et al., 2004] and have been approved recently as first-line therapy for the treatment of postmenopausal women with metastatic ER-positiv breast cancer [Brueggemeier, 2005].

In further clinical trials these three agents were evaluated as options for the adjuvant therapy of early breast cancer. The results of the ATAC trial (anastrozole alone or in combination with tamoxifen vs. tamoxifen in patients with newly diagnosed breast tumours) were recently completed after more than 5 years. Anastrozole proved to be significantly superior to tamoxifen in terms of disease-free survival, time to recurrence, distant metastases and contralateral breast cancers. Additionally, anastrozole was associated with fewer acceptable side effects [ATAC Trialist Group, 2002 and 2005]. Letrozole and exemestane were tested in patients who had previously completed a 2-5-year course of tamoxifen and were disease-free. Both agents also demonstrated a significant reduction of breast cancer in comparison to tamoxifen [Coombes et al., 2004, Goss et al., 2003]. Unfortunately, up to now there exist no sufficient data on long term efficacy, safety aspects with respect to toxicity, quality of life, and organ effects, and the optimal duration of treatment of aromatase inhibitors to estimate their full potential in the adjuvant setting [Mouridsen and Robert, 2005]. In the future, however, aromatase inhibitors may, if not yet, change a paradigm in endocrine therapy and replace tamoxifen completely as standard therapy for breast cancer in postmenopausal women.

2.3 Antiestrogens and SERMs

Since more or less 30 years tamoxifen has been the treatment of choice for all stages of hormone-sensitive breast cancer in pre- and postmenopausal woman. Tamoxifen provides effective palliation in patients with advanced disease and reduces the risk of recurrence and death when given as adjuvant therapy [Osborne, 1998]. It is characterised by its ability to bind to the estrogen receptor and to inhibit the growth of hormone-dependent breast tumours. How-

ever, tamoxifen exerts estrogen agonistic action on other tissues, such as bone, blood and endometrium. The preservation of bone mineral density [Powles et al., 1996] and the reduction of cholesterol and other lipids in the blood [Love et al., 1994] are advantageous virtues of the drug, whereas the stimulation of endometrial hyperplasia and, thus, an increased risk for the development of endometrium cancer is an undesirable side effect [Fisher et al., 1994]. For this reason, new compounds with an altered agonistic profile and the potential to enhance the efficacy and reduce the toxicity of tamoxifen were designed. These compounds are termed selective estrogen receptor modulator (SERM), which refers to their capacity to have alternative effects on different target tissues [Johnston and Howell, 2002].

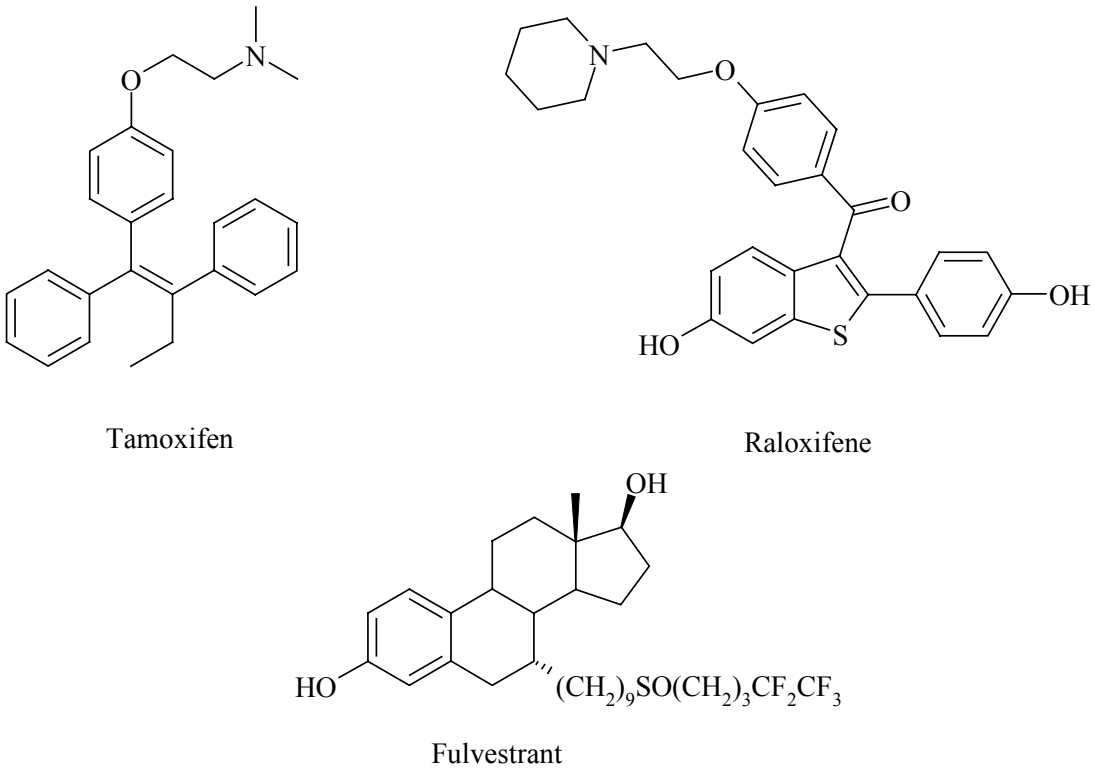


Figure A7: Structures of clinically relevant antiestrogenes

The newer SERMs can be divided into two groups: triphenylethylene-based tamoxifen-like compounds, such as toremifene, droloxifene and idoxifene, and fixed-ring tamoxifen analogues, such as raloxifene, arzoxifene, EM-800 and ERA-923 [Johnston and Howell, 2002]. Although each of these compounds offer pharmacological and pharmacodynamic benefits over tamoxifen in preclinical trials, none of these antiestrogenes have shown yet any significant advantage in clinical trials for advanced breast cancer in terms of efficacy and tolerability. Also the possibility of cross resistance to tamoxifen may limit their potential usefulness in

the treatment of advanced disease following adjuvant tamoxifen therapy [Howell et al., 2000]. A much greater potential may exist in the adjuvant or chemopreventive setting, where an improved SERM profile on bone, lipid metabolism and the endometrium would be of maximum benefit [Johnston and Howell, 2002]. Presently, raloxifene is the only clinically relevant SERM beside tamoxifen and is used for the prevention of osteoporosis.

Mammary carcinomas acquire resistance to tamoxifen treatment followed by tamoxifen stimulated tumour proliferation, which is closely linked to the partial estrogenicity of the drug. The pure antiestrogen fulvestrant (Faslodex®) is completely devoid of estrogenic activity and capable of antagonising tamoxifen-resistant advanced breast tumours in postmenopausal patients [Robertson et al., 2003]. Contrary to tamoxifen, the administration of fulvestrant significantly reduces ER and PR expression in a dose-dependent manner [Robertson et al., 2001], suggesting a mode of action different from conventional SERMs.

Besides the suppression of estrogen-mediated gene transcription (discussed in detail in chapter A3), a rapid degradation of the highly labile ER-fulvestrant complex and consequently a downregulation of cellular ER protein levels is observed [Morris and Wakeling, 2002]. Further, a reduced shuttling of the ER from the cytoplasm to the nucleus is observed [Dauvois et al., 1993], which completes the full spectrum of antagonistic fulvestrant action. Thus, fulvestrant is not only described as a pure antiestrogen but also as a selective estrogen receptor downregulator (SERD).

The efficacy and tolerability of fulvestrant (250mg monthly injection) was compared to the aromatase inhibitor anastrozole (1mg oral administration daily) in postmenopausal women with advanced breast carcinoma, who had progressed after prior endocrine therapy. The results from a combined analysis of two phase III studies show that fulvestrant is well tolerated with moderate adverse effects and at least as effective as anastrozole in terms of progression and response rates [Robertson et al., 2003]. Based on these data, fulvestrant was approved as second-line therapy for postmenopausal ER-positive advanced breast cancer in Germany in March 2004 [Bertsche and Schulz, 2005].

Surprisingly, the effectiveness of fulvestrant in comparison to tamoxifen in the first-line treatment of advanced breast cancer was almost similar [Howell et al., 2004], despite the high superiority of fulvestrant in preclinical trials and the complete different mechanism of action. However, patients who have derived clinical benefit from fulvestrant treatment and have started progressing again, retain sensitivity to subsequent endocrine therapy (e.g. aromatase inhibitors) [Vergote et al., 2003]. Thus, the concerns that the downregulating mechanism of

fulvestrant may lead to an end-point of hormonal therapy could be relieved. The effectiveness of the sequential use of fulvestrant after previous therapy with aromatase inhibitors or other endocrine agents remains to be answered.
In future it is of great interest to evaluate the role of fulvestrant in first-line setting and to find its potential position in the endocrine therapy cascade. The use of fulvestrant in the adjuvant treatment of early breast cancer and its effectiveness in premenopausal women are also important aspects to be addressed in current or planned studies. A further aspect is the combination of fulvestrant with aromatase inhibitors or signal transduction inhibitors, such as gefitinib or trastuzumab, that may enhance the therapeutic response to endocrine therapy [Piccart-Gebhart and Loi, 2005].

2.4 Growth Factor Receptor Directed Therapies

Hormone therapy is an effective and relatively non-toxic treatment of ER positive breast cancer, but ultimately most tumours develop resistance upon tamoxifen treatment. This acquired resistance almost certainly occurs not only through loss of ER, but, as a result of ER-mediated suppression of cell signalling, tumours adapt to alternative signalling pathways. Crosstalk between the ER and the epidermal growth factor (EGF) receptor family is evidently one of the molecular mechanisms of antiestrogen resistance [Osborne et al., 2005]. Other growth factors, such as the insulin growth factor 1 (IGF-1), have also been shown to activate the ER and stimulate the growth of breast cancer cells via the ER [Hafner et al., 1996].
The EGFR family comprises four members of transmembrane receptors (EGFR/ErbB1/HER1, ErbB2/HER2/c-neu, ErbB3/HER3 and ErbB4/HER4) with tyrosine kinase activity modulating a variety of cellular functions such as proliferation, migration and survival. Binding of specific ligands to the extracellular receptor domain results in homodimerisation or heterodimerisation with other members of the EGFR family. This dimerisation mediates tyrosine kinase activation and receptor autophosphorylation at six tyrosine residues in the intracellular domain, which initiates the kinase signalling cascades [Tikhomirov and Carpenter, 2003]. 25-30% of breast cancers are associated with the expression of excessive amounts of these receptors, particularly EGFR and HER2, which makes them attractive therapeutic targets for the treatment of breast cancer [Slamon et al., 1987].
Gefitinib (Iressa®) (cf. figure A8) is an orally active EGFR-selective tyrosine kinase inhibitor that blocks ATP binding at the ATP binding site leading to an inhibition of downstream sig-

nalling pathways. This blockade results not only in retardation of cell cycle progression but also in the induction of apoptosis in EGFR-expressing tumour cells [Okubo et al., 2004]. Gefitinib inhibits the proliferation of breast cancer cells *in vitro* and *in vivo* [Chan et al., 2001; Moulder et al., 2001] and was shown to be effective in breast cancer cells, that have developed resistance to fulvestrant [McClelland et al., 2001]. In addition, gefitinib additively increases the antiproliferative effect of fulvestrant in ER positive breast cancer cells [Okubo et al., 2004]. In future this combination can possibly enhance the response rates in breast cancer therapy.

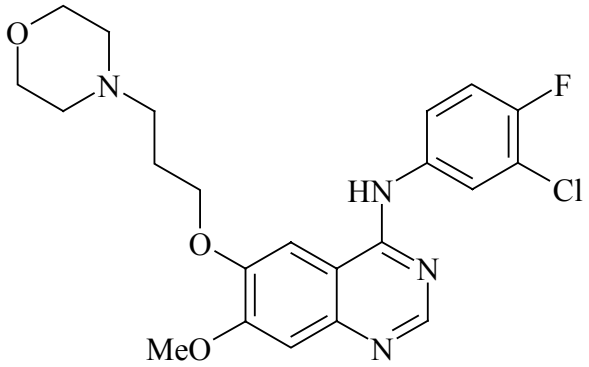


Figure A8: Chemical structure of gefitinib

Trastuzumab (Herceptin®) is a recombinant humanised monoclonal antibody that binds specifically with high affinity to the extracellular domain of the HER2 receptor and blocks its signalling function. Its clinical efficacy and favourable safety profile in HER2-overexpressing advanced breast cancer have been shown when administered as single agent in second- and first-line therapy [Cobleigh et al., 1999; Vogel et al., 2002]. Trastuzumab even potentiates the efficacy of standard cytotoxic chemotherapy, but it demonstrated a significantly increased incidence of symptomatic cardiac toxicity when used simultaneously with anthracycline-based chemotherapy [Slamon et al., 2001; Marty et al., 2005]. Up to now there are no current investigations that study the combination of trastuzumab with endocrine agents. However, with the introduction of gefitinib and trastuzumab the era of breast cancer therapy based on the disruption of non-ER signal transduction pathways has been opened.

3 The Estrogen Receptor

3.1 Structure of the Estrogen Receptor

The estrogen receptor, known as the two subtypes ERα and ERβ, belongs to the superfamily of nuclear hormone receptors (NR). It is a ligand-inducible transcription factor that can initiate gene transcription by interaction with specific estrogen response elements (ERE) of the DNA [Tsai and O'Malley, 1994; Weatherman et al., 1999].
Like other NR family members, the ER has a molecular organisation consisting of six distinct functional domains A through F (cf. figure A9). The N-terminal A/B-domains of ERα and ERβ differ markedly in length from each other and have the lowest degree of sequence similarity. This domain harbours the transcriptional activation function AF-1, which contributes to ligand-independent transcription activation [Enmark and Gustafsson, 1998].

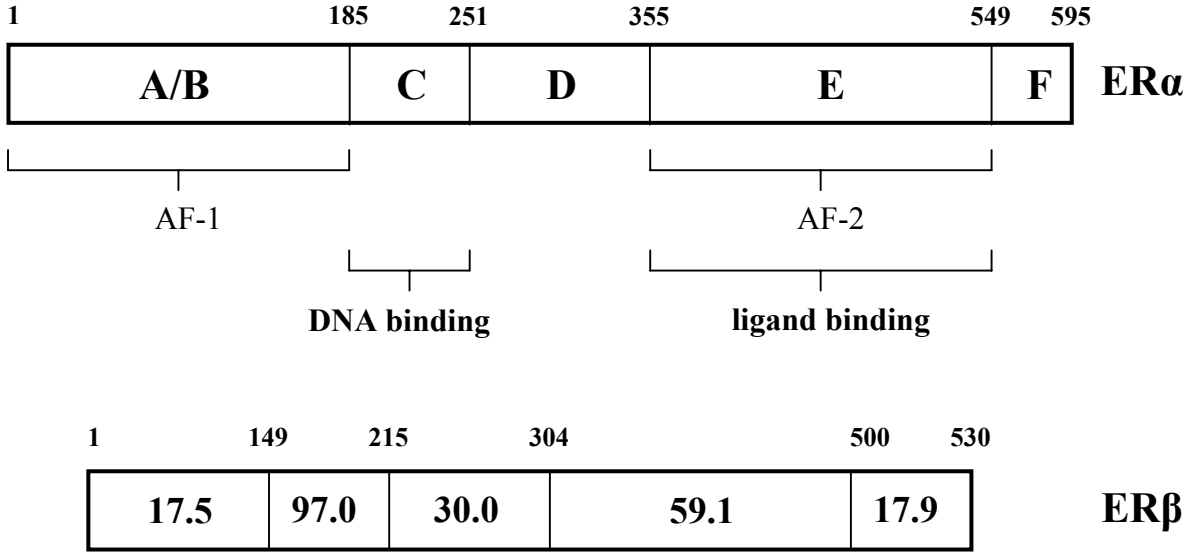


Figure A9: Schematic comparison of the human full-length ERα und ERβ

The numbers above each receptor represent the number of amino acid. The numbers inside the respective boxes represent the percentage of identity in the amino acid sequence.

The adjacent DNA binding domain (DBD) consists of 66 amino acids and is the most conserved region. Two zinc finger motifs, in which eight highly conserved cysteine residues coordinate two zinc atoms, are responsible for DNA binding and receptor dimerisation. The P-box refers to a sequence of amino acids within the N-terminal zinc finger, which is involved in the recognition of specific base pairs of the ERE. The D-box refers to a amino acid se-

quence in the C-terminal zinc finger, which is responsible for the interaction between the two receptor monomers and subsequently dimer formation. These protein-protein and protein-DNA interactions together contribute to the stabilization of the receptor-DNA-complex [Petterson and Gustafsson, 2001]. ERα binds with high affinity and specificity to an consensus ERE, which comprises two inverted palindromic half-sites separated by three intervening nucleotides (5'-AGGTCAnnnTGACCT-3') [Schwabe et al., 1993]. The three-dimensional structure of the ERβ-DBD-complex has not been determined yet, but the high amino acid identity of 97% to ERα suggests a similar structure.

The domain E or ligand binding domain (LBD) is relatively large and locates the second ligand-dependent activation function AF-2. It mediates ligand binding, receptor dimerisation, coregulator recruitment and transcriptional activation of target gene expression. The LBD is composed of twelve α-helices (H1-H12) and two antiparallel β-sheets (S1 and S2). Three of these α-helices (H5/6, H9 and H10) form an antiparallel central core layer that is sandwiched between two additional layers of helices (H1-4 and H7, H8, H11). This three-layered helical arrangement creates a wedge-shaped molecular scaffold that maintains a relatively large ligand binding cavity at the narrower end of this wedge. The ligand binding cavity is entirely excluded form the external environment of the LBD. The remaining secondary structural elements (H12 and S1, S2) are located near the ligand binding site and flank the main three-layered structure [Brzozowski et al., 1997].

The functions of the domains D and F are to a large extent unknown. The domain D appears to serve as a hinge between the DBD and the LBD, offering a high flexibility to these essential domains within the ER. A third activation function, AF-2a, was postulated in this region and has been shown to have constitutive activity in the absence of both AF-1 and AF-2 [Norris et al., 1997]. The C-terminal F domain is poorly conserved in ERα and ERβ. Deletion and mutation studies have suggested a role in influencing the transactivation capacity of the receptor [Montano et al., 1995].

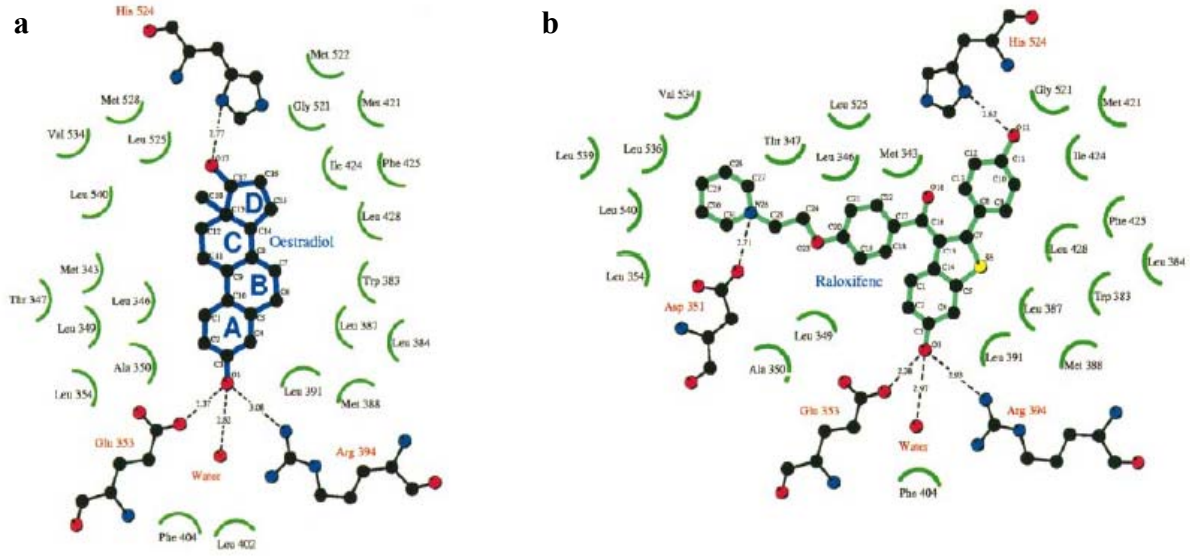
ERα and ERβ differ from each other with respect to their overall structure and their tissue distribution. The full length ERα protein consists of 595 amino acids with a molecular weight of 66kDa, whereas its isoform ERβ is somewhat smaller and comprises 530 amino acids with a molecular weight of 59kDa. Both human ERs show a homology in total amino acid sequence of 47%, which is considerably low for two receptor subtypes, particularly in the LBD [Enmark and Gustafsson, 1998]. Despite a sequence homology of 55% in the LBD, ERα and ERβ differ only from two amino acids in the ligand binding pocket: Leu 384 and Met 421 in

ERα correspond to Met336 and Ile 373 in ERβ [Pike et al., 1999]. This slight alteration explains, on the one hand, the very high affinity and specificity of estradiol to both receptor subtypes [Kuiper et al., 1997] and, on the other hand, the volume difference of the liganded binding pocket (490Å^3 for ERα and 390Å^3 for ERβ) [Pike et al., 1999].

Both ER isoforms have been shown to possess distinct tissue distribution profiles. ERα regulates the development and maintenance of both male and female reproductive organs and is predominantly expressed in malignant mammary carcinoma. In opposite, ERβ is the dominant isoform in the breast and in benign breast tumours, and is also found in a variety of other tissues including the cardiovascular system, the reproductive organs, the brain, and the skeleton [Enmark and Gustafsson, 1998; Gustafsson and Warner, 2000].

3.2 The Molecular Basis for Agonistic and Antagonistic ER Action

In the recent years several crystal structures of both ERα and ERβ with a variety of natural and synthetic ligands have been reported [Brzozowski et al., 1997; Pike et al., 1999 and 2001; Shiau et al., 1998 and 2002; Tanenbaum et al., 1998]. The LBD of the ER presents the centre of interest when studying the structural aspects of agonistic and antagonistic ER action. Agonists and antagonists bind at the same binding site within the hydrophobic core of the LBD. A strong ligand binding to the receptor is granted by a combination of specific hydrogen bonds and several hydrophobic interactions.



Scheme A10: Schematic representation of estradiol and raloxifene in the ligand binding cavity of ERα [Brzozowski et al., 1997]

The schematic representation of estradiol in the ERα LBD (cf. scheme A10a) outlines two distinctive hydrogen bonds of the two hydroxy groups at both ends of the molecule. A multiple hydrogen bond interaction is formed to the carboxylate of Glu353, the guanidinium group of Arg394 and an additional water molecule. Another single hydrogen bond is formed to an imidazole nitrogen of the highly flexible His524 residue. The remainder of the molecule participates in a number of non-polar contacts with surrounding residues. The SERM raloxifene (cf. scheme A10b) is accommodated within the ligand binding pocket in a similar manner, but with the difference, that the side chain makes additional hydrophobic interactions and is anchored to the receptor by a direct hydrogen bridge between the carboxylate of Asp351 and the piperidine ring nitrogen [Brzozowski et al., 1997].

The principal difference in the agonistic and antagonistic action via the ER lies in the capability to stimulate or inhibit the transcriptional activation functions AF-1 and AF-2. Herein, the orientation of helix H12 has a crucible role with regard to the recruitment of transcription co-regulating proteins.

In a receptor complex liganded with pure agonists, such as E2 or DES, H12 seals the ligand binding cavity like a lid and generates an interaction surface for the recruitment of essential coactivator. In contrast, the bulky side chain of SERMs, such as raloxifene or tamoxifen, is too long to be contained within the confines of the ligand binding cavity. It protrudes from the binding pocket and displaces H12 into a hydrophobic groove formed by parts of the helices H3 and H5/6. In this way it masks key residues essential for the interaction with the NR box of coactivator proteins and antagonizes their recruitment [Brzozowski et al., 1997; Shiau et al., 1998]. The crystal structure of raloxifene in the rERβ LBD demonstrates an identical picture [Pike et al., 1999]. The displacement of helix H12 is generally accepted as the molecular mechanism on both ER subtypes for antagonists with a bulky side chain (cf. figure A11).

Pure antagonists, such as fulvestrant and related compounds, are characterized by a distinctive longer side chain than SERMs, with additional functionalities at the outer extension. From an crystal structure of an pure antagonist (ICI 164,384) in the ERβ LBD it has been shown, that this extended side chain sterically prevents the alignment of helix H12 over the ligand binding cavity and, in addition, precludes H12 from adopting its alternative orientation along the coactivator binding groove as seen in the SERM complex [Pike et al., 2001]. This “double blocking” of both H12 positions on the surface of the LBD implicates two possible mechanisms for full antagonism. ICI binding does not result in the blockade of the coactivator binding site by the H12 displacement and thus, the recruitment of corepressors could be facilitated [Pike et al., 2001]. In fact, both NR coactivator and corepressor utilize only slightly different

binding motifs and share almost the same hydrophobic groove between H3 and H5/6 as their protein interaction site (cf. section A3.3). A second possible mechanism considers, that the displaced and "liberated" helix H12 is able to somehow neutralize the function of AF-1 by interfering directly with the correct spatial positioning of AF-1 [Pike et al., 2001].

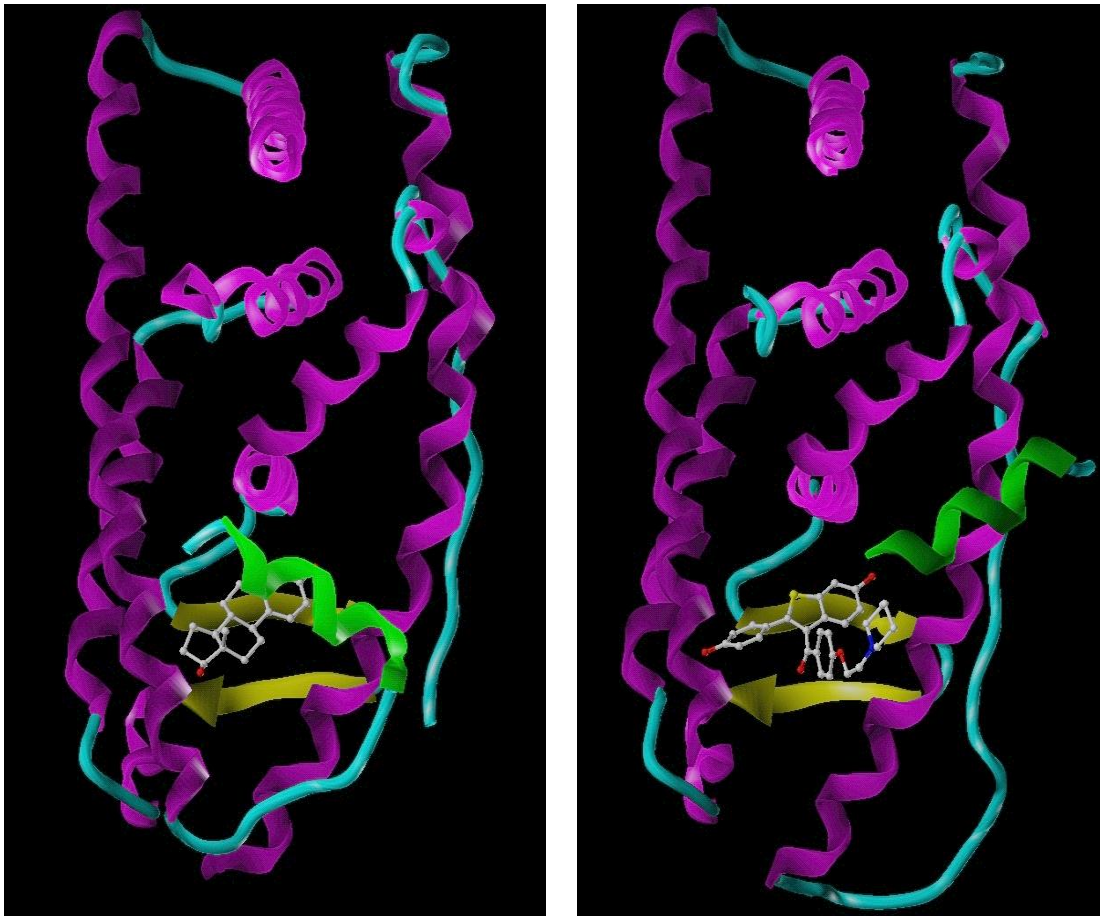


Figure A11: Positioning of helix H12 in the ERα-LBD complexed with estradiol (left) and raloxifene (right) [modified by Dr. A. Botzki, University of Regensburg]

The α-helix H12 is coloured green. The remainder of α-helices and random coils of the LBD are depicted in violet or cyan, respectively. β-sheets are coloured in yellow. The random coil between helices H11 and H12 was left for clarity reasons.

The impact of pure antiestrogens on AF-1 in the distant A/B domain and how they suppress AF-1 mediated effects is unknown to a large extent. The same applies to the corepressor recruitment to an antiestrogen complexed ER. Furthermore, one has to bear in mind, that an ERβ complex was the basis for the present discussion and similar conformational perturbations were assumed for pure antiestrogenes acting on ERα. However, among other possible explanations (inhibition of DNA binding or receptor degradation) both above mentioned considerations offer attractive hypotheses for full antagonism, but further studies are required to

either confirm these explanations and/or to determine the precise involvement of the AF-2 in the antagonism of ICI related compounds on the ER.

The crystal structure of the ERβ-LBD complexed with THC reveals that ligand binding can stabilize yet another conformation of the helix H12. THC (cf. figure A12) is an ERα agonist and a pure ERβ antagonist lacking a long, bulky side chain like the known ERα antagonists [Meyers et al., 1999]. In this complex, H12 adopts a kind of mid-position that partially seals the ligand binding cavity and also only partially occludes the coactivator recognition surface. The reason for this distinctive position of H12 can be found in the difference of the amino acid sequence between ERα and ERβ. A number of hydrophobic contacts with residues in the helices H3, H5, H6 and H11 force H12 into the described position and provide a certain stabilisation [Shiau et al., 2002].

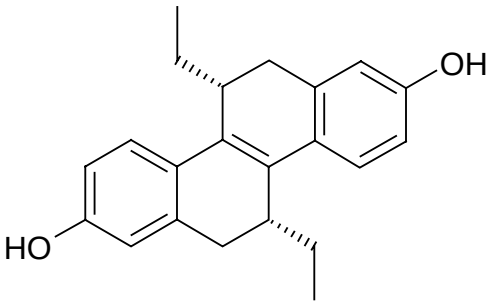


Figure A12: (R,R)-5,11-*cis*-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol (THC)

A model was postulated that provides an suitable explanation for the full antagonism of THC at ERβ. It is based on the hypothesis that in the unliganded receptor the helix H12 is in equilibrium between an active and inactive conformation. Ligands that bind to the ER affect the transcriptional activity by shifting this dynamic equilibrium rather than inducing a single static conformation. Thus, pure agonists shift the conformational equilibrium of H12 in favour of the active agonist bound conformation and stimulate AF-2 activity by increasing the affinity of the LBD for coactivators. THC achieves it antagonistic effect on ERβ through its failure to make essential interactions involving key residues in helix H11, that would normally stabilize the active agonist bound conformation of H12. This leads to an shift of the equilibrium towards the inactive conformation and a stabilisation of helix H12 in a position that is non-permissive for coactivators recruitment [Greene et al., 2004; Shiau et al., 2002].
This kind of antagonism is termed “passive antagonism”, because the conformation of the LBD is not directly changed by the interaction of structural features of the ligand with secon-

dary elements of the receptor [Greene et al., 2004]. Passive antagonism may not be unique to THC and ERβ, as there are examples of other NR ligands that act as antagonists even though they are smaller than the endogenous agonists of these NRs and incapable of inducing conformational changes by steric hindrance [Souque et al., 1995]. But it needs further detailed investigations with other passive acting antagonists to confirm this novel mode of antagonism at the ERβ and to study unresolved issues in the structural linkage between ligand and transcription activation.

3.3 Molecular Pathways to Transcription Activation

In the absence of hormones, the ER exists as an inactive complex with chaperone proteins, such as the heat-shock protein Hsp90 and Hsp70, which help to maintain the receptor in an appropriate conformation to respond rapidly to hormonal signals [Pratt and Toft, 1997]. Upon ligand binding this oligomeric complex dissociates, allowing ERα and ERβ to homo- or heterodimerize, interact directly with EREs in the promoter of estrogen responsive genes and activate transcription through the activation functions AF-1 and/or AF-2. The promoter-bound ER stimulates transcriptional initiation by facilitating the formation of a stable preinitiation complex containing RNA polymerase II and other basal transcription factor [Bagchi, 2003]. The precise mechanism by which the receptor achieves this effect is not determined yet, but a number of coregulators are associated to be involved in the activation of the transcription process.

Coregulators are coactivators or corepressors which enhance or suppress the transcriptional activity of NRs, respectively. Coactivators are multifunctional proteins that can act independently from each other or in large complexes and some of them have enzymatic activity (histone acetyltransferase activity). The p160 family of coactivators was first identified and consists of three closely related members: SRC-1, SRC-2 (TIF2, GRIP1) and SRC-3 (ACTR, AIB1, p/CIP, RAC3, TRAM-1). CBP and its homologue p300 belong to another group of potential coactivators which serve as secondary coactivators through their direct interaction with p160 proteins. These coactivators are characterised by three highly conserved LxxLL motifs termed the NR-box that mediates the interaction with the hydrophobic groove in the LBD of the ER. Coactivators are associated to function as signalling intermediates between the ligand-bound receptor and the basal transcription machinery [Edwards, 2000; Rowan and O'Malley, 2003].

Despite a large number of coactivators only a few corepressors are reported. Among these N-CoR and SMRT are the most intensively studied. Similar to coactivator, they contain an slightly extended hydrophobic motif, that was shown to bind to identical regions in the LBD of NRs [Xu et al., 2002]. Corepressors are thought to recruit proteins with HDAC activity, resulting in deacetylation of histones and subsequently in the repression of basal transcription. Up to now, only little is known about the nature of corepressor interaction with steroid receptors, especially when complexed with an antagonist [Edwards, 2000; Rowan and O'Malley, 2003].

Besides the ERE-mediated transcriptional activation, ER can also regulate ligand-dependent gene expression by binding to non-classical promoter elements that contain non-consensus ERE or ERE half-sites [Harrington et al., 2003]. ER is also able to alter transcription at other promoter sites (e.g. AP1, Sp1, EpRE, NF-κB) without directly binding to the DNA [Montano et al., 1998; Ray et al., 1997; Saville et al., 2000]. ERα and ERβ stimulate gene expression from AP1 enhancer elements in an indirect manner by interacting with the DNA-bound transcription factors fos and jun. The response at the AP1 promoter is dependent on the ER subtype and the nature of the ligand. ERα and ERβ were shown to display opposite effects when complexed with E2: ERα bound E2 activated gene transcription, whereas ERβ bound E2 inhibited transcription [Paech et al., 1997].

Additionally, the transcriptional activity of the ER can be stimulated by phosphorylation of specific serine residues in the N-terminal AF-1. A number of signal transduction pathways are known to mediate this process, including PKA, PKC or growth factor (EGF or IGF-1) signaling via MAPK or Akt/PI3-K. This pathway crosstalk, that can occur in the absence or presence of respective receptor ligand, is well studied for ERα [Smith, 2003]. Although ERβ lacks a functional AF-1, it was shown that phosphorylation events enhance the binding of the coactivator SRC-1 in the AF-1 domain suggesting an alternative mechanism for ligand-independent ERβ activation [Tremblay et al., 1999].

Membrane bound ERs have been identified, but at present the precise location, structure and function of these receptors are unclear. They are associated to activate kinase pathways similar to G-protein coupled receptor. Future studies of these membrane-mediated events in our workgroup will broaden our knowledge of the molecular mechanisms underlying the transcriptional activation of the ER.

4 Recent Advances in the Development of ERβ Selective Ligands

The discovery of the second ER subtype (ERβ) in 1996 [Mosselman et al., 1996] together with its distinct tissue distribution and transcriptional properties (cf. section A3) has led to an intense interest in developing cell- and tissue-selective agents, that might display a pharmacological profile different from non-selective compounds. Although a number of steroidal and non-steroidal SERMs with good potency and selectivity for ERα has been reported [Meegan and Lloyd, 2003], only a few ERβ-selective compounds are known. The latter shall be reviewed in the following section.

Phytoestrogens were the first ERβ-selective compounds characterised [Kuiper et al., 1998]. The isoflavone genistein (cf. figure A13) is the most prominent and most intensive studied representative of the class of phytoestrogens. Genistein behaves as a full agonist via ERα, being even more efficacious than E2, and as partial agonist via ERβ. Its slightly higher potency via ERβ is in good agreement with its modest binding selectivity (~20-fold) for this receptor subtype [Barkhem et al., 1998; Kuiper et al., 1998]. The co-crystallization with ERβ reveals a hydrogen bridge of the phenolic hydroxyl group with the Glu305-Arg346-water triad at the one end and an hydrogen-bonding interaction of the hydroxyl group at C7 with His475. The remaining OH-group does not interact with the protein but forms an intramolecular hydrogen bond with the adjacent carbonyl group. The helix H12 adopts a similar binding mode as observed in THC-ERβ-complex [Pike et al., 1999].

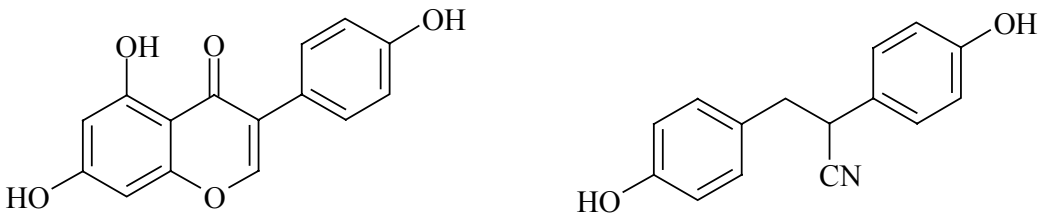


Figure A13: Genistein and 2,3-bis(4-hydroxyphenyl)propionitrile (DPN)

Genistein is the most potent phytoestrogen, as studies with daidzein and its natural estrogenic metabolite equol demonstrate [Muthyala et al., 2004]. Constraining the ring system of the naturally occuring isoflavone phytoestrogens through introduction of oxa- or thia-bridges results in tetracyclic compounds, that retain ERβ selectivity [Miller et al., 2003].

DPN shows a substantially higher level of ERβ affinity and selectivity than genistein. It has a 70-fold ERβ RBA selectivity and a 78-fold higher potency in activating ERβ compared to ERα in a cell-based transcription assay. In contrast to genistein, DPN is a full agonist on ERβ. Replacement of the CN group with acetylene or a polar function leads to a decrease in ERβ selectivity. This suggests that the nitrile functionality represents the optimal combination of linear geometry and polarity and it is essential for ERβ selectivity [Meyers et al., 2001].

Scientists at the Wyeth Research Institute have investigated a series of diphenolic benzofurans, benzoxazoles and benzisoxazoles as ERβ selective ligands. The most selective and potent compounds are depicted in figure A14, with ERβ binding affinities as high as estradiol and RBA selectivities exceeding 100-fold. The depicted 2-phenyl benzoxazoles ERB-041 shows a selectivity of 226-fold for ERβ. Vinyl or cyanomethyl substituents in 7-position proved to be most appropriate for this high selectivity. From the crystal structures of the ERβ complexed with the 7-substituted benzoxazole and benzofuran, respectively, it became clear that these substituents extend into the relatively narrow groove formed by Ile373, Ile376 and Phe377. A substitution of ERβ Ile373 by a methionine, that corresponds to ERα Met421, is hypothesized to lead to a combination of electrostatic and steric repulsion associated with the methionine side chain. This results in an enhanced ERβ selectivity. The crystallography studies have also confirmed that helix H12 of ERβ maintains an agonist-like conformation, which is consistent with the fact that these compounds behave as full agonist on ERβ [Collini et al., 2004; Malamas et al., 2004].

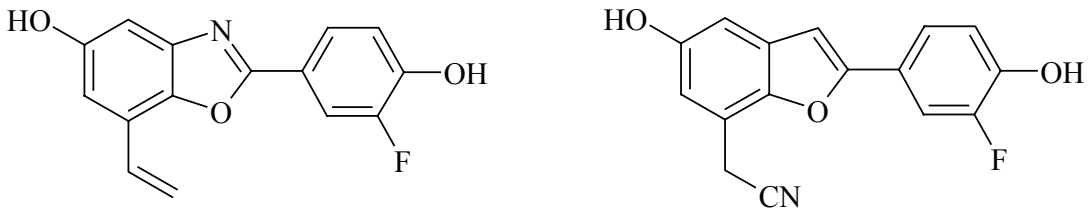


Figure A14: The most potent 7-substituted 2-phenyl benzoxazole ERB-041 and 2-phenyl benzofuran

The estrogenic or antiestrogenic character of the most selective derivatives in this class of compounds was evaluated in a cell-based transcription assay and *in vivo* models. In the transcription assay human osteosarcoma cells (SAOS-2) were used, which were manipulated to overexpress ERβ. When the increase in IGFBP-4 mRNA, a marker for ERβ activity, was

measured all compounds proved to be full agonists. The majority of the ERβ selective agonists displayed *in vivo* profiles consistent with these data. They were inactive in models of typical estrogen actions (rodent uterine weight, bone mineral density), but active in a model of inflammation. This suggests, that this class of compounds may have the utility in treating chronic inflammatory diseases while lacking the undesireable side effects of nonselective estrogen agonists [Collini et al., 2004; Malamas et al., 2004].
Furthermore, compounds with a similar scaffold such as 2-phenyl benzothiophenes [Schopfer et al., 2002] or 2-phenyl indazoles [De Angelis et al., 2005] were also found to be selective ligands for ERβ. The benzothiophenes showed only weak selectivity in binding and transcription assays, whereas the indazoles were highly selective agonists for ERβ. However, they were not able to compete with the above described benzoxazoles or benzofurans, probably because they lack the essential substituent in 7-position necessary for the interaction with the ERβ Ile373.

The unconventional scaffold of 4-hydroxy-biphenyl-carbaldehyde oxime was investigated and found to give ERβ selective agonists when equipped with appropriate substituents. The best oxime (cf. figure A15) had almost identical affinity and selectivity to genistein. Molecular modelling studies suggest, that the phenol was acting as the A-ring and the oxime group as the C-ring of genistein, forming an hydrogen bond to the histidine imidazole ring [Yang et al., 2004].

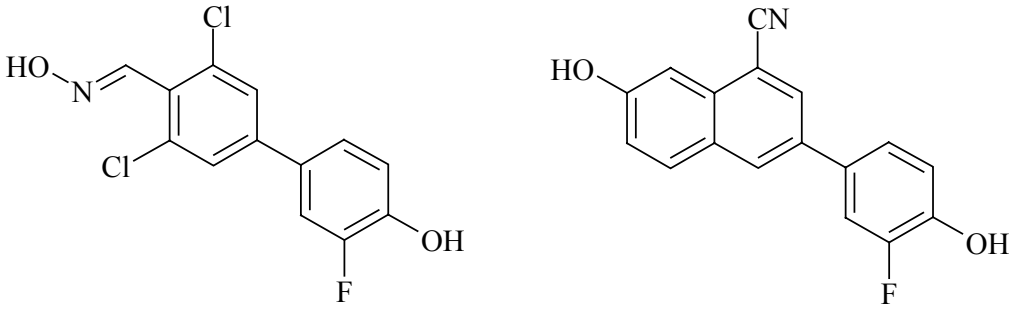


Figure A15: ERβ selective biphenyl-carbaldehyde oxime and 2-phenylnaphalene

Recently two crystal structures of the ERβ complexed with the isosteric 2-hydroxy-6-(4-hydroxphenyl)naphthalene were published. These structures provide a binding mode for phenylnapthalenes that is similar to the binding mode of the biphenyl-carbaldehyde oxime proposed from modelling studies. The substituents (CN, CH=O, vinyl, ethyl) are most suitable

when they are located in 1- or 8-position of the naphthalene ring and, thus, capable of interacting with residues in the narrow groove which incorporates ERβ Ile373. The phenylnaphthalenes were slightly superior to the isosteric oximes with respect to ERβ selectivity and agonistic potency. The most selective compounds of this class (cf. figure A15) showed no evidence of ERα activity *in vivo*, but were shown to be active in two inflammation models [Mewshaw et al., 2005].

The key to subtype selective ligands of the ER lies in the differences between two amino acids in the ligand binding pocket that can be exploited for a structure-based approach to highly ERβ selective steroidal ligands. A vinyl substituent in 8β-position between the B- and C-ring of estradiol extends into the direction of ERβ Met336, that provides additional space in the ERβ binding pocket compared to ERα. This steroid exhibits a 180-fold selectivity of both RBA and RTP (relative transcriptional potency) for ERβ and was also selectively effective in a number of *in vivo* experiments [Hillisch et al., 2004].

The first example of a pure ERβ antagonist with ERα activity was THC (cf. figure A12). Substituted triazines were identified as another class of antagonists, which displayed modest potency and selectivity for ERβ. In a cell-based transcription assay, these compounds profiled as full antagonist at ERβ and weak partial agonists at ERα, activating the reporter gene with only 10-20% of the efficiency of estradiol. When these agents were tested for their antiestrogenic potency at ERα, they demonstrated an inhibition of E2 mediated gene transcription of 60-80%. The structure and substitution pattern of these compounds differ from those of conventional ER ligands (cf. figure A16).

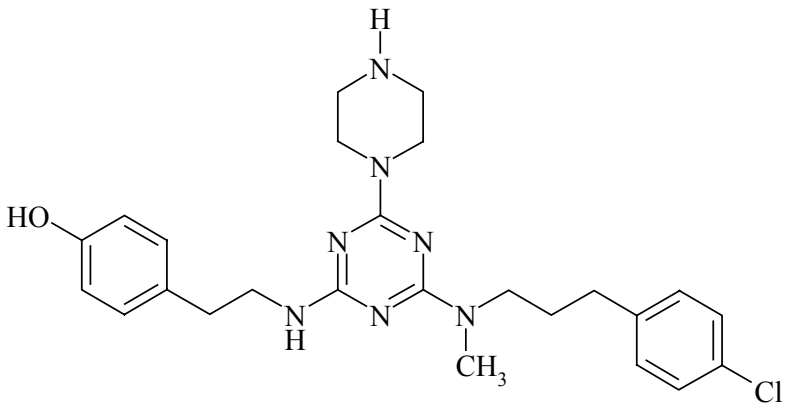


Figure A16: Structure of the triazine-based ERβ selective SERM

The crystal structure of one of these compounds complexed with the ERβ LBD demonstrates features similar to other ER antagonist structures with the basic side chain interacting with ERβ Asp303 (ERα Asp351). Favourable interactions with Met336 and Ile373 can be associated with the selectivity for ERβ [Henke et al., 2002]. However, THC remains up to now the only reported ERβ selective antagonist, that acts through silent antagonism and shows agonistic but no antagonistic effects on ERα.

5 Objectives

The discovery of an estrogen binding protein in target tissues about 50 years ago provided a rational basis for the endocrine therapy of breast cancer. After the approval of tamoxifen as drug for treatment of hormone-dependent mammary carcinomas antiestrogen therapy has become an established treatment modality for this malignancy. More recently an isoform of the estrogen receptor was discovered and characterised. It was termed ERβ to discriminate it from the original ERα. Both receptor forms share many similarities but show a different distribution in the body and, consequently, differ in their functions which are not yet completely assigned to the two subtypes ERα and ERβ. The objective of this study is the search for compounds with a preference for one of the two subtypes of the estrogen receptor and the conversion of these agents to pure antagonists. Pure antiestrogens with selectivity for ERα would be the drugs of choice for the treatment of breast cancer patients who had responded to tamoxifen but have become resistant to this drug. It also would be desirable to have potent ERβ-selective antagonists as a tool to study the functions of this receptor form.

Studies reported in the literature have shown that a variety of agents with different chemical structures bind to both forms of the estrogen receptor without significant preference for one of them. However, some classes of compounds display selectivity to a certain extent. Among these are five-membered heterocycles with preference for ERα and some phytoestrogens with preference for ERβ. The preference for one or the other receptor form depends mainly on the structure of the core, which usually carries two hydroxylated phenyl rings. In order to discover new structures that have not yet been investigated as ligands for the estrogen receptor α virtual screening should be performed to select promising structures from a large database. These compounds should either be commercially available or easily be synthesised for pri-

mary testing. Compounds with sufficient binding affinity for the estrogen receptor should then be used as lead.

In parallel, heterocycles with known selectivity for ERα such as triphenylfurans should be modified to convert them into pure antiestrogens. Basically these modifications comprise the removal of one of the phenyl rings leading to 2,5- and 2,4-bis(4-hydroxyphenyl)furans, respectively, and the introduction of long aliphatic side chains with diverse functional groups. A variety of benzanellated five-membered heterocycles had been studied previously in our group before ERβ was discovered. These compounds which derive from 2-phenyl-benzo[b]furan and -benzo[b]thiophene should be re-evaluated in respect to their selectivity for one or the other ER subtype. Ring enlargement of the benzofurans leads to the 1-benzopyran system which offers two different positions for the introduction of appropriate side chains. Representative examples of the 3-phenylbenzopyran system should be synthesised and compared with the analogous benzo[b]furans in respect to binding affinities for the two estrogen receptor subtypes and antiestrogenic potency.

The biological evaluation of the new ligands of the estrogen receptor should comprise the determination of binding affinities for a native estrogen receptor isolated from calf uteri and for the two recombinant human estrogen receptor isoforms α and β. Compounds with sufficient affinity should be tested for antiproliferative activity in estrogen-sensitive human MCF-7 breast cancer cells. In order to detect non-specific cytotoxic actions all compounds will be tested in hormone-independent MDA-MB-231 breast cancer cells in parallel. For the quantification of estrogenic and antiestrogenic activities a genetically modified MCF-7/2a subline should be used. These cells are stably transfected with a luciferase reporter gene under the control of an estrogen responsive element (ERE). Depending on the experimental setup this *in-vitro* system allows the determination of both agonist and antagonist activities. However, these activities cannot be clearly assigned to one of the two receptor subtypes because MCF-7 cells contain both receptors with the beta-form as the minor fraction. This uncertainty should not affect the outcome of this study which is primarily aimed to the discovery of new agents useful for the treatment of hormone-dependent breast cancer. Subtype-selective agents that emerge from this study can be used as tool for further investigations on the functional role of the estrogen receptor isoforms α and β.

B Biological and Pharmacological Test System

1 *In vitro* Assays

1.1 Radiometric Binding Assay

The radiometric binding assay is a standard procedure in many academic and industrial research groups to determine the relative binding affinity (RBA) of new substances with potential estrogenic or antiestrogenic activity. It is an indirect method based on the competitive displacement of the tritium labelled physiological ligand 17β-estradiol ([^{3}H]-estradiol) and circumvents the synthesis and application of radioactive test compounds.

Increasing concentrations of inhibitor compete with the tracer molecule [^{3}H]-estradiol, applied in constant concentration, for the single binding site at the ER. The degree of displacement of [^{3}H]-estradiol from the receptor is direct proportional to the relative binding affinity of the competitor.

The origin of receptor material used in this binding assay varies. A natural source of receptor is the cytosol prepared from uteri of immature animals such as calves [Walter et al., 2004], lambs [Stauffer et al., 2001] or rats [Katzenellenbogen et al., 1973; Williams et al., 1974], which possess low levels of endogenous estrogen. This cytosol contains both estrogen receptor subtypes ERα and ERβ, but the predominantly expressed receptor in the uterus is ERα [Enmark and Gustafsson, 1998].

Full-length, human receptors ERα and ERβ expressed as recombinant proteins in baculovirus infected insect cells are also used for this purpose. With these proteins it was possible to establish a new binding assay in our research group to assign the affinity and selectivity of certain compounds to one or the other ER isoform.

Depending on the receptor source different work-up procedures are required to separate the excess of radioactivity. In case of the receptor containing cytosol unbound [^{3}H]-estradiol is removed by dextran-coated charcoal (DCC), following the recommendation of EORTC [1973]. The pretreatment with dextran (60-90kDa) is necessary to close the large pores of the charcoal and reach effective absorption of excess [^{3}H]-estradiol and other small molecules that are found in the cytosol, but not of the receptor-ligand-complex. The DCC method fails when the recombinant receptors are used. These proteins are substantially smaller than those

obtained from natural sources and are removed together with unbound [^{3}H]-estradiol, so that no receptor-bound radioactivity can be detected. For this reason the receptor-ligand-complex formed during equilibration is absorbed with hydroxylapatite (HAP) and subsequently washed free of any unbound radioactivity. The HAP pellet is resuspended and counted for tritium activity in a liquid scintillation counter [Ke et al., 1998; Leake and Habib, 1987]

For each binding assay carried out with either natural or recombinant receptors control and background values are determined. In the control experiment the maximum number of binding sites is determined by using exclusively tritium labelled estradiol as ligand. The background experiment takes into account any low affinity binding sites, such as other lipids or proteins, that especially come along with the preparation of the cytosol and might be responsible for unspecific and irreversible binding of estradiol or test compounds. For this reason, an excess concentration of the unlabeled estradiol is incubated with [^{3}H]-estradiol and so the reversible equilibrium shifted quantitatively favouring the binding of "cold" ligand to the receptor. Finally, after treatment with DCC or HAP, only unspecific binding is recorded and this background activity is subtracted from all other measurements.

All new compounds and unlabeled estradiol are tested within a broad range of concentrations to identify the molar concentration required to decrease the specific radioligand binding by 50% (IC_{50}-value). The RBA of each competitor is calculated as the ratio of IC_{50}-value of estradiol to IC_{50}-value of competitor, multiplied by 100.

1.2 Proliferation Assay with Human Mammary Carcinoma Cell Lines

Since receptor binding affinities do not allow conclusions about the hormonal activity of potential antitumour agents, the antiproliferative effect of these substances on human mammary carcinoma cell lines is determined using a computerised microculture chemosensitivity assay based on the quantification of cellmass by staining cells with crystal violet. [Bernhardt et al., 1992]

Two different human breast cancer cell lines have been used. The MCF-7 cell line was established from a pleural effusion from a disseminated breast carcinoma of a 69 years old patient [Soule et al., 1973]. It is characterised by a high content of estrogen receptors (cf. table B1) and is therefore used to demonstrate an estrogen receptor mediated effect of test compounds.

The MDA-MB-231 cell line is hormone-independent and is derived from the pleural effusion of a 51 years old woman with recidivous adenocarcinoma of the breast [Cailleau et al., 1974].

Its low content of estrogen receptors (cf. table B1) excludes a receptor mediated drug action, but it allows the detection non-specific cytotoxic action of test compounds.

Cell line	Passage	ER[a]	PR[a]
MDA-MB-231	32[b]	3	1
	37[b]	2	7
MCF-7	154[b]	2	39
	166[b]	119	8
	177[b]	148	5
	194[c]	101	57
	198[c]	280	17
	202[c]	247	1
	213[c]	489	130

Table B1: Steroid receptor content of two human breast cancer cells

[a] concentration of receptor in fmol/mg soluble protein
[b] [Bernhard et al., 1992]
[c] [Leichtl, 1994]

The ER content of the hormone sensitive MCF-7 cells varies with the number of passages. Constant culture conditions indicate an increase in ER content with increasing numbers of passages [Leichtl, 1994]. Shortly after rethawing, the ER content is at a limit of detection [Bernhardt et al., 1992]. For this reason, cells are submitted to the chemosensitivity assay not before three or four passages.

In the proliferation assay the MCF-7 cells are stimulated with estradiol in a concentration of 1nM. This resembles the physiological concentration of the hormone in the malignant breast cancer tissue. With this method the chemosensitivity assay gives reproducible results independent from the passage number and thus independent from the ER content [Walter et al., 2002]. The assay is performed as single-point determination in ctFCS supplemented medium. The duration of incubation after substance addition is between 200 and 250 hours and the cells are fixed shortly before confluence.

The proliferation assay with the hormone-independent MDA-MB-231 cells is also performed as single-point determination. The concentrations of test compounds are 1µM, 5µM and

10µM and incubation lasts 90 to 100 hours. Since these cells lack the estrogen receptor, estradiol was omitted and untreated FCS was added to the medium (cf. section E3.2.5).
Each compound is tested in duplicate or triplicate in two or three independent experiments. Fulvestrant and 4-hydroxytamoxifen are used as references. In order to distinguish inhibitory effects from cytocidal drug action T/C-values are corrected for the initial cell density, which refer to the average cellmass at the time of drug addition.

1.3 Luciferase Assay

The luciferase assay is a convenient, rapid and very sensitive method for the determination of the hormonal activity of test compounds *in vitro*. The assay is based on the transfection of an suitable eukaryotic cell line with an ERE controlled luciferase reporter gene, that was isolated from the North American firefly *Photinus pyralis* [de Wet et al., 1987].

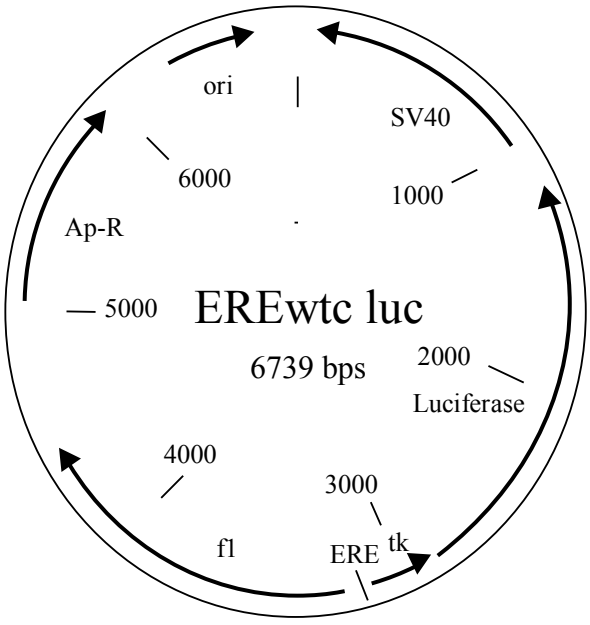


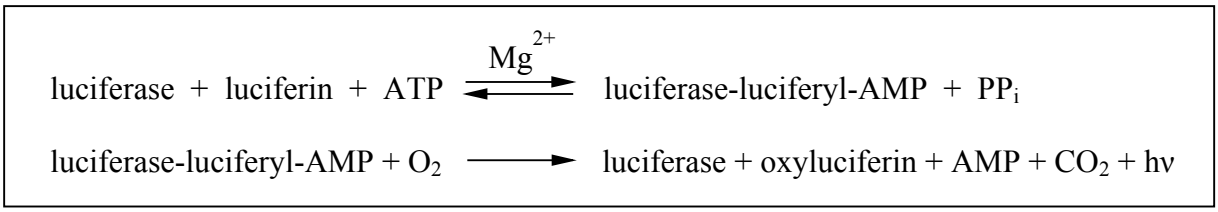
Figure B2: Plasmid chart of 'EREwtc luc' [Meyer et al., 1994]

ori: origin of replication
Ap-R: β-lactamase gene (encodes for ampicillin resistance, that is essential for the replication in E. coli)
SV40: Polyadenylation signal of the big T-antigene from simian virus 40 (SV40; important for m-RNA stability)
f1: Fragment from the F1 phage (enables the single-strand expression of the plasmid)
tk: Promotor of the thymidin-kinase-gene from Herpes simplex
ERE: Estrogen response element from the Vitellogenin A2-gene from Xenopus laevis
luc: Luciferase gene from Photinus pyralis (encodes for the enzyme luciferase)

The plasmid 'EREwtc luc' (cf. figure B2) consisting of 6739 base pairs harbours the luciferase reporter gene and sequences with promoter and enhancer activity. This reporter construct has been integrated into the genome of the estrogen receptor positive MCF-7 cell line together with the pWLneo vector, which is essential for the selection of stable transfectants and neomycin resistance. This new MCF-7 cell line has been termed MCF-7/2a [Hafner, 1996].

When estrogenic agents, like estradiol, are added to MCF-7/2a cells, they exert their effects exclusively by activation of gene transcription. They bind to the estrogen receptor and induce transcription of the luciferase gene by the interaction as ligand-receptor-dimers with the ER sensitive enhancer sequence of the ERE. Antiestrogens consequently inhibit the estrogen receptor mediated process.

The expressed luciferase enzyme catalyses a reaction sequence known as firefly luminescence (cf. scheme B3). In nature, bioluminescence is a widely observed phenomenon in animal kingdom, especially in various marine organisms [Hoffmann, 1981]. The enzyme requires luciferin as substrate and forms in the presence of ATP and magnesium enzyme-bound luciferyl-adenosine monophosphate. During a second reaction, this enzyme-substrate-complex undergoes oxidative decarboxylation, which results in the production of oxyluciferin, AMP and visible light [White et al., 1980; including a detailed mechanistic study].



Scheme B3: Chemistry of the firefly luminescence

The light emission of the firefly luminescence can be detected within the wavelength range of 490 and 630nm, depending on the nature of the luciferase [Hoffmann, 1981]. The reaction catalysed by the *Photinus pyralis* luciferase emits yellow-green light at pH 7.5 to 8.5 with the peak emission at 560nm [de Wet et al., 1987]. Changes in the pH-value have not only an influence on the emission spectrum of the luminescence, but also on its efficiency. The quantum yield in weak alkaline medium is almost quantitative [Seliger and McElroy, 1960].

However, the duration of the firefly luminescence is rather short. The initial intensity declines by 50% after 15s and by 90% after one minute. The addition of the coenzyme A (also part of the Promega's luciferase-assay-system) has proven to increase the half-life of the luciferase

activity from 15 seconds to 16 minutes and enhances the quantum yield of the light emission. [Hafner, 1996].

For the investigation of agonistic or antagonistic activity the compounds are incubated alone or in combination with estradiol in culture medium containing MCF-7/2a cells. According to the expression kinetics, the luciferase expression in stably transfected MCF-7 cells reaches its maximum after an incubation period of 50h. Due to this relatively short incubation period the growth conditions of this assay are of minor importance.

The luciferase activity of a certain compound can be estimated by comparison with the estradiol reference at a concentration of 1nM (=100%) and an expression experiment without hormones (NH). Potent antiestrogens decrease the luciferase activity stimulated by estradiol below the basal level. Dose-response curves allow the calculation of IC_{50}-values, which reflect the antagonistic activity of these compounds. This assay is performed in triplicate for each drug concentration in two independent experiments.

2 *In vivo* Assay: Uterotrophic and Antiuterotrophic Test

In recent years the numbers of *in vivo* experiments was cut down tremendously due to the development of reliable *in vitro* screening systems. The determination of estrogenic or antiestrogenic activity of antitumor agents in the luciferase assay gave results that were in good accordance with *in vivo* observations [Biberger, 1996; Walter, 2002]. Nevertheless, it is still of great importance to investigate the endocrine activity of very potent compounds in animals to get information on drug resorption and elimination. For example, non-steroidal pure antiestrogens often suffer from poor bioavailability. This fact prompted us to choose the mouse uterine weight test as the *in vivo* test system.

The uterotrophic and antiuterotrophic test is based on the stimulating effects of estrogens on uterus growth in immature, female NMRI mice. The test compounds are applied subcutaneously on three consecutive days, either alone or in combination with estradiol. For the estimation of the obtained effects two control groups are treated with either estradiol (=100%) or the vehicle (olive oil) alone. At the end of the experiment the uteri are excised, dried and weighed.

Estrogenic effects of the test compounds are calculated from the ratio of uterine dry weight to body weight of the respective animal. Simultaneous administration of estradiol allows the determination of antiestrogenic activity of compounds.

C Synthesis and Biological Characterisation of New Ligands for the Estrogen Receptor

1 Virtual Screening for New Lead Structures

Virtual screening of chemical databases is a rather new method for finding new lead compounds and has emerged as an alternative and complementary approach to experimental high-throughput screening. It can be considered as a powerful computational filter for reducing the size of a chemical library that will be further experimentally investigated [Walters et al., 1998].

The application of virtual screening approaches depends on the availability of a three-dimensional (3D) structure of the biological target and the detailed knowledge about the localisation and the geometry of the ligand binding site. In case of the ER this knowledge can be deduced from several X-ray structures with the co-crystallised natural substrate or synthetic organic ligands.

A further prerequisite for virtual screening approaches is a 3D virtual compound library containing drug-like molecules, which are either commercially available or readily synthetically accessible. Additionally, the hits from computational approaches should have physicochemical properties that allow favourable pharmacokinetics. Lipinski's empirical "rule of 5" can be regarded as a valuable filtering method to eliminate compounds with undesirable physicochemical properties (MW > 500g/mol, log P >5, more than 5 H-bond donors and 10 H-bond acceptors) [Lipinski et al., 2001]. Based on Lipinski's "rule of 5", Dr. A. Botzki [2004] compiled a 3D library suitable for virtual screening from the commercially available ChemACX database Version 5.5 (CambridgeSoft Corp., Cambridge, MA, USA). The resulting database comprises more than 196.000 different compounds that were used for the structure-based virtual screening for new ER lead structures.

1.1 Search New ER Ligands Using the Computer Programme LUDI

As a starting point for this lead discovery process the coordinates for the E2-ERα LBD (pdb-code: 1ERE), DES-ERα LBD (pdb-code: 3ERD) and THC-ERβ LBD (pdb-code: 1L2J) from

the brookhaven protein data bank were obtained. The receptor proteins are recorded as multi-domain structures. After deletion of all non-protein components, the corresponding domains of each structure were superimposed over all Cα-atoms using SYBYL 6.8 (Tripos Inc, St. Louis, MO, USA) on an Indigo workstation running IRIX 6.5: Based on this alingment one representative domain was chosen. Full models with complete amino acid sequences were constructed by inserting missing residues into joining loops and mutating incomplete amino acid residues. The resulting models with all hydrogen atoms added were energetically minimised using the MMFF94 force field with the Powell gradient method.

The subsequent database screening was performed with the structure-based design software LUDI (Accelrys Inc., San Diego, CA, USA). Prior to screening, a sphere with a selectable radius needs to be defined. It should represent the ligand binding region where all interactions between ligand and receptor are considered. For this LUDI approach a sphere with a radius of 5Å within the active site of the ER comprising 20-23 amino acids was set up. The centre of this sphere was determined as the centre of the co-crystallised ligand of the corresponding LBD. The ligand itself was subsequently removed for the LUDI run.

For all functional groups of the receptor exposed to the binding region, putative interaction sites in space were generated by LUDI according to rules which have been derived from composite crystal-field environments compiled with appropriate small molecule crystal data (Cambridge Structural Database) [Botzki, 2004]. The programme tries to fit each database molecule into these interaction sites in the pre-defined binding pocket. All 3D structures retrieved from the database are treated as rigid bodies independent of the presence of rotatable bonds. For each successfully docked ligand, LUDI estimates the expected binding affinity by an empirical scoring function, which approximates the interaction between the ligand and the amino acid residues of the binding pocket. The minimal scoring value of 300 equals a predicted K_i-value of 1mM [Botzki, 2004].

For the LUDI performance to design ligands for the ER the values of the most important LUDI parameters were as follows: The maximum rms distance of the fit between the fragment and the interaction sites was 0.5Å. The number of lipophilic and polar interaction sites per protein atom was set to 30 and the minimal contact surface between ligand and protein was set to 70%. All other parameters were set to default values.

Performing a LUDI run with the constructed ChemACX database and the ERα LBDs resulted in 785 hits for 1ERE and 699 hits for 3ERD. The LUDI run with the ERβ LBD resulted in only 40-48 hits even with different sets of run parameters, which reflects the smaller volume of the ligand binding pocket of ERβ. The candidate molecules were ranked according to their

LUDI scores. In each case 17β-estradiol and derivatives thereof reached the highest score values (ca. 700-600) indicating that LUDI generated reasonable and useful results. Apart from steroidal structures many of the retrieved molecules with good LUDI scores resembled structural features which are already known from high affinity ER ligands, including DES- or hexestrol-like structures or heterocycles with benzimidazole-, indole- or chinolin-like scaffolds. Therefore, we concentrated on bridged bicyclic core structures or compounds with a 1,1-diaryl motif. Finally, four compounds (cf. figure C1) of these hits were selected for determining ER binding affinities according to high scores values and commercial availability. One hit with a dibenzobicyclo[2.2.2]octadiene scaffold (cf. compound **4a** in scheme C2; LUDI score: 659) was chosen to be synthesised and subsequently modified with additional hydroxy groups, that are known to be essential for good ligand binding to the ER.

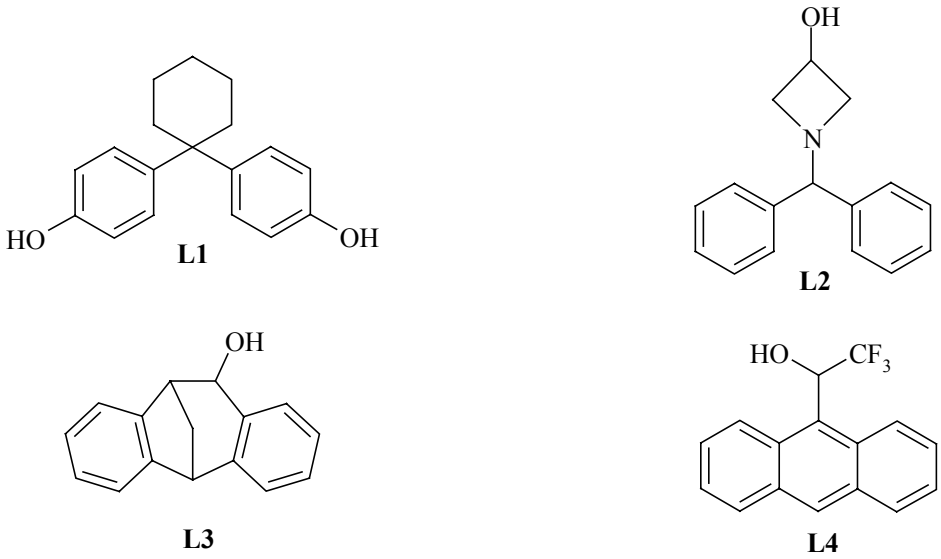


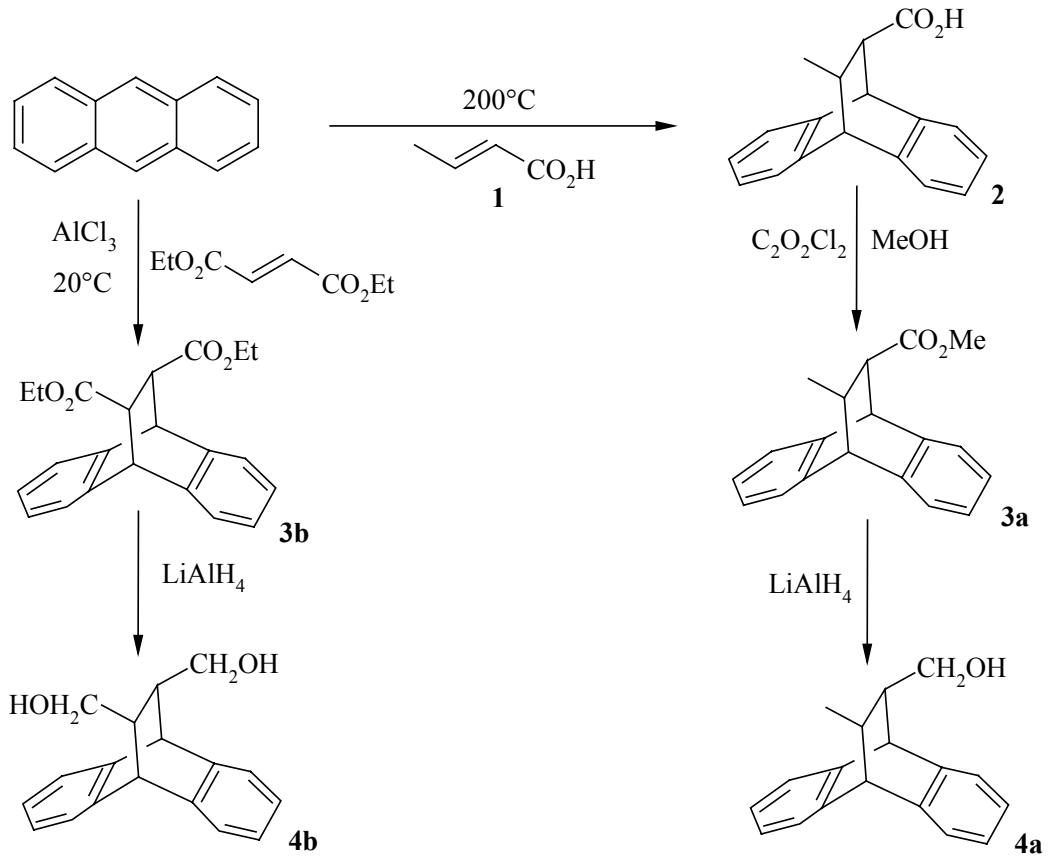
Figure C1: Chemical structures of the selected ligands from virtual screening

L1 = 1,1-Bis(4-hydroxyphenyl)cyclohexane (LUDI score: 610), L2 = 1-(Diphenylmethyl)-3-hydroxyazetidine (561), L3 = 5,11-Dihydro-10-hydroxy-dibenzo[a,d]bicyclo[3.2.1]octadiene (563), L4 = 9-(1-Hydroxy-2,2,2-trifluoro-ethyl)anthracene (535)

1.2 Synthesis and Biological Characterisation of Ligands Derived From Virtual Screening

The access to dibenzobicyclo[2.2.2]octadiene-based compounds, or alternatively termed ethanoanthracenes, can be achieved by appropriate Diels-Alder reactions with anthracene or derivatives thereof. Anthracene is considered as a relatively electron-rich diene and reaction with suitably substituted electron-poor dienophiles should provide the envisioned Diels-Alder compounds.

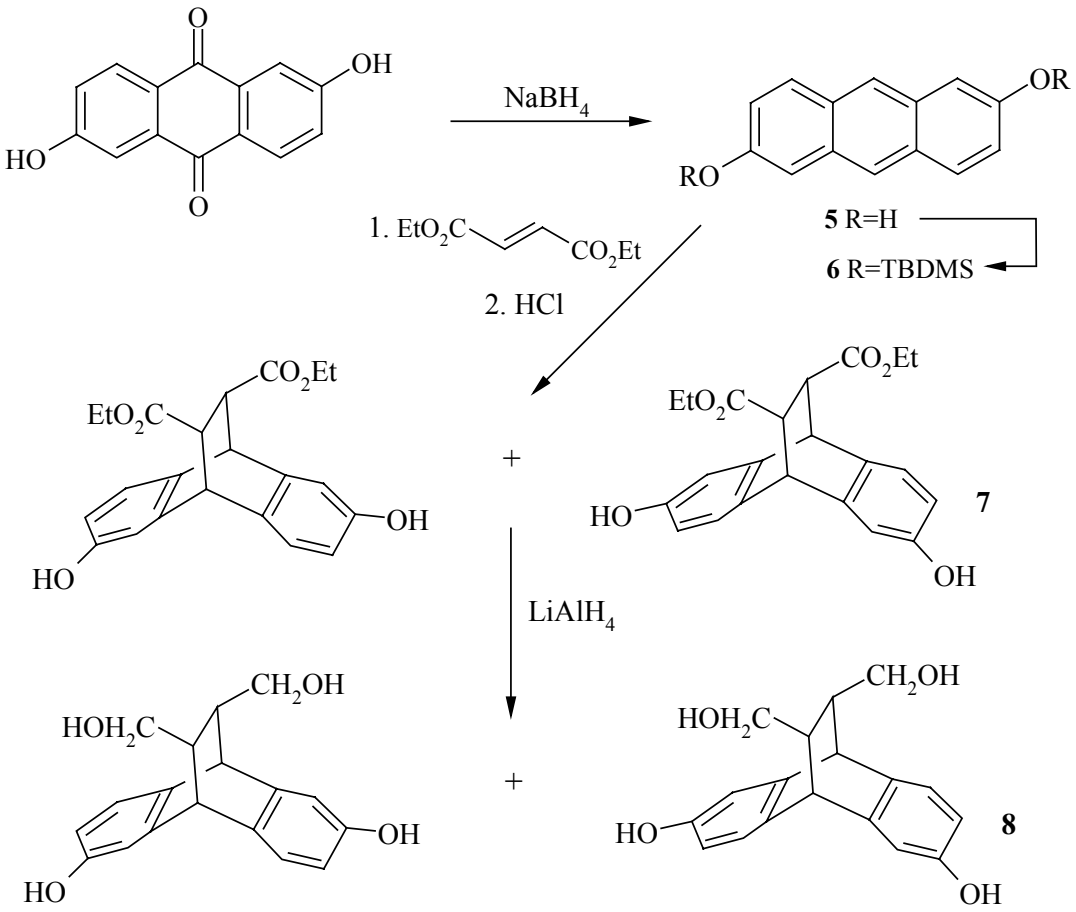
In his Ph.D. thesis Dr. J. Kochansky [1971] obtained the unsubstituted Diels-Alder compound **4a** by heating anthracene and ethyl crotonate in a sealed, thick walled glass tube followed by reduction of the intermediate ester. On a small scale and with ordinary heating this Diels-Alder reaction yielded only unreacted starting material, even with addition of $AlCl_3$. In contrary, in the presence of $AlCl_3$ diethyl fumarate reacted readily with anthracene to the Diels-Alder adduct **3b** at ambient temperature [Yates and Eaton, 1960; Furuta et al., 1986]. The electron withdrawing strength of one ester function was evidently too low to get this reaction going. Therefore, ethyl crotonate was hydrolysed to crotonic acid **1** and the subsequent high temperature reaction with anthracene under solvent free conditions gave the desired compound **2**, as originally described by O. Diels and K. Alder [1931]. Compound **2** was also obtained with the same moderate yield when the reaction was performed in refluxing xylene, but the reaction time (40h) was significantly longer. A direct reduction of the carboxy ethanoanthracene **2** to the alcohol **4a** using Pd/C and H_2 proved not to be successful [Falorni et al., 1999]. Thus, **2** was esterified under standard conditions to compound **3a** and finally, both esters **3a** and **3b** were reduced with $LiAlH_4$ to give the corresponding alcohols **4a** and **4b**, respectively (cf. scheme C2).



Scheme C2: Synthesis of unsubstituted ethanoanthracenes

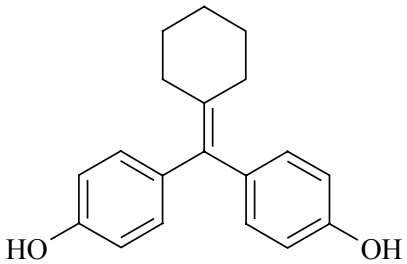
The synthesis of ethanoanthracenes bearing two additional phenolic hydroxy groups (cf. scheme C3) started from commercially available anthraflavic acid, which was reduced with $NaBH_4$ to 2,6-dihydroxyanthracene **5** [Boldt, 1967]. The phenols were protected as *tert*-butyldimethylsilyl ethers to improve the solubility of compound **5** [Petti et al., 1988], which then underwent Diels-Alder reaction with diethyl fumarate in refluxing xylene over 65h. The phenolic hydroxy groups were deprotected under acidic conditions to increase the polarity of the product , which makes it separable from the excess fumarate. The C_2-symmetric ethanoanthracene **7** was obtained in good yield as a mixture of two regioisomeric racemates, which were not considered for further separation.

Attempts to accelerate this reaction by addition of $AlCl_3$ resulted in partial deprotection of **6**, but not in the formation of product. Trials to submit crotonic acid or its corresponding ethyl ester to Diels-Alder reactions with **5** under various conditions let only to the isolation of unreacted starting material. The final reduction with $LiAlH_4$ afforded the ethanoanthracene **8** as a racemic mixture of two regioisomers.

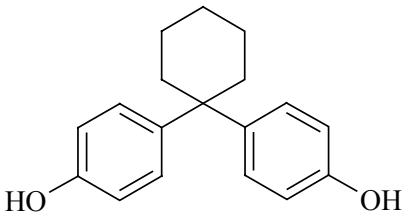


Scheme C3: Synthesis of hydroxylated ethanoanthracenes

The synthesised ethanoanthracenes **4a**, **4b** and **8** and the four purchased ligands **L1**-**L4** were tested for their binding affinity to both ER subtypes. None of the tested compounds, except **L1**, were able to displace E2 from the receptor binding site up to concentrations of 20µM Compound **L1** bound to the ER with RBA values of 0.95 to ERβ and 0.08 to ERα and showed a 12-fold selectivity for ERβ. The ability of **L1** to bind to the ER was not surprising, because a similar structural feature is well-known from cyclofenil (cf. figure C4), a non-steroidal estrogen with high binding affinity for the ER.



Cyclofenil



L1

Figure C4: Structural comparison of cyclofenil and compound **L1**

In contrast to **L1,** the cyclohexane ring in cyclofenil is linked with the bis(4-hydroxyphenyl)methylene group by a doublebond. It has recently been demonstrated, that for good ER binding a sp^2 carbon is preferential over an tetrahedral orientation of the phenol rings as in compound **L1**. Additionally, the complete elimination of the spacer between the hydrophobic core and the phenols resulted in a further decrease of binding affinity down to **L1** levels [Muthyala et al., 2003a]. Besides the tetrahedral orientation of the phenyl rings, the missing phenolic hydroxy groups almost certainly account for the immeasurably low binding affinity of **L2**. At least one hydroxy-substituted phenyl ring to mimic the phenolic A-ring of E2 is an essential part in almost every ER ligand. This structural feature can hardly be compensated by a different hydroxy pattern.

Although virtual screening with LUDI proposed a good fit of the ethanoanthracene **4a** in the ligand binding pocket, its binding affinity was too low to be measured in the radioligand binding assay. This also applies to the bridged compounds **L3** and **4b**, which all lack a phenolic hydroxy group in a position appropriate for interaction with polar amino acids such as glutamate, arginine or histidine. Even the modified ethanoanthracene **8** with two phenolic functions does not bind. The dibenzobicyclic system forms a very rigid structure, that provides only few possible orientations in the ligand binding site. The reduced flexibility of this system

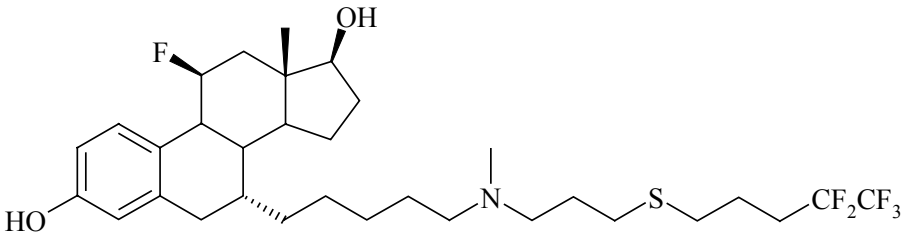
requires an optimal geometry and an optimal substitution pattern for a good fit in the ligand binding pocket. This fact has also become apparent from recent SAR studies with compounds based on a bicyclo[3.3.1]nonane scaffold [Muthyala et al., 2003b].

In conclusion, none of the investigated compounds provided satisfactory results with respect to ER binding for various discussed reasons. The high binding affinities proposed by LUDI resulted from an overestimation of hydrophobic interactions of the ligand with the receptor. Detailed investigations on compounds containing either a bridged bicyclic core structure or a 1,1-diaryl motif have been reported and confirm the low binding affinities of these types of compounds. Therefore, a further computer-aided or chemical refinement of the identified structures was not considered.

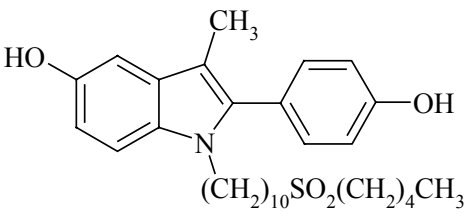
2 Antiestrogens Based on a 2,5-Diphenylfuran Scaffold

Steroidal fulvestrant is the only pure antiestrogen on the market for the treatment of breast cancer. Unfortunately fulvestrant possesses no selectivity for ERα, the predominant ER subtype in malignant mammary carcinomas. The basis for the development of selective antiestrogens is the structural core of the ligand, which positions the substituents for favourable interactions with key residues of the receptor. Virtual database screening provided only limited success for the identification of new lead structures, that can be further modified to selective ER ligand.

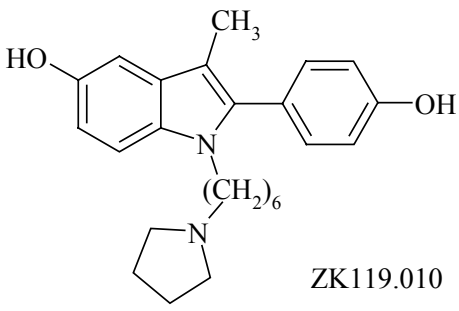
In the literature several non-steroidal structures have been identified as ER ligands sharing the common feature of a 5-membered heterocyclic core with two or three hydroxy-substituted aromatic rings attached to it. Besides diaryl- [Nishiguchi et al., 2002] or triarylpyrazoles [Stauffer et al., 2000 and 2001], triarylfurans demonstrated high binding affinity and selectivity for ERα [Mortensen et al., 2001]. Exchanging one of the aromatic rings in this triarylfuran system for long aliphatic side chains might lead to ERα selective compounds, that show complete antagonism in the absence of estrogenic side effects.



ZK191.703



ZK164.015



ZK119.010

Figure C5: Aliphatic side chains as part of potent steroidal and non-steroidal antiestrogens

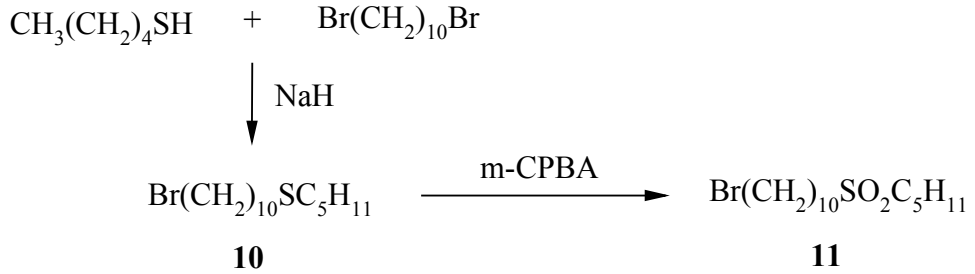
Three aliphatic side chains that should guarantee full antagonism were adopted from anti-estrogens that have demonstrated good results in previous studies of our research group. In general, these side chains have a length of 15 or 16 atoms depending on the carrier molecule, with one or two appropriate functional groups in defined positions.

The functional side chains possess either a sulfanyl- or sulfonyl group in a distance of 9 or 10 carbon atoms from the core. The bifunctional side chain has in addition to the sulfanyl group a basic methylamine function in place of the methylene group in position 6 or 7. This function was introduced in analogy to the side chain of the 2-phenylindol based antiestrogen ZK119.010 (cf. figure C5). Scientists in the research laboratories of Schering in Berlin have used this bifunctional side chains with an additional terminal fluorination to improve the structure of the antiestrogen fulvestrant. The resulting steroid ZK191.703 (cf. figure C4) has shown an increased bioavailability in comparison to fulvestrant [Hoffmann and Sommer, 2005]. A fourth side chain with a spacer of 6 methylene groups between the core and a terminal pyrrolidine ring, as it is known from ZK119.010, was also synthesised.

2.1 Synthesis of 3,4-Dialkyl-2,5-diphenylfurans

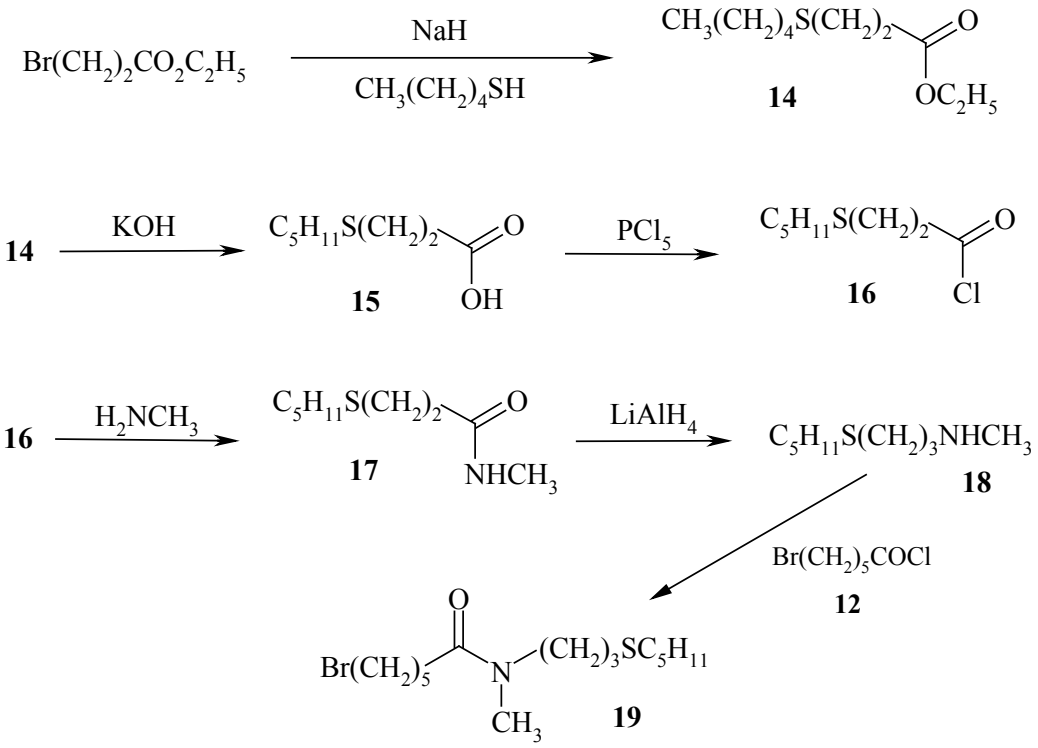
2.1.1 Synthesis of Side Chains

The monofunctional side chains were synthesised from pentanthiolate that was generated with sodium hydride and added to a 4-fold excess of 1,10-dibromodecane [Biberger, 1996]. Despite this large excess of dibromoalkane in a highly diluted solution the formation of **10** was accompanied by the disubstituted by-product. However, both compounds could be easily separated from each other and unconverted dibromoalkane by column chromatography. The sulfide **10** was oxidised quantitatively to the sulfone **11** with m-CPBA (cf. scheme C6).



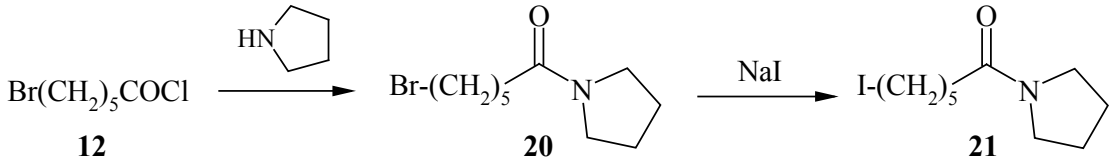
Scheme C6: Synthesis of the monofunctional side chains

The synthesis of the bifunctional side chain required a multistep reaction sequence (cf. scheme C7) starting from ethyl 3-bromopropionate, that was converted by nucleophilic substitution with pentanthiolate to the corresponding sulfide **14**. The deprotonated thioalcohol attacked exclusively the brominated carbon leaving the ester function untouched. Subsequent ester hydrolysis to **15**, chlorination with PCl_5 to **16**, and reaction with aqueous methylamine afforded the amide **17**, that was reduced to amine **18** using $LiAlH_4$ [Walter, 2002]. The overall yield of these five reactions was 33% due to the low yield of the final amide reduction. The preparation of the side chain (compound **19**) was completed by reaction of the secondary amine **18** with the activated ω-bromohexanoic acid **12**. Due to the high reactivity of the acid chloride function, substitution of the terminal bromine was not observed.



Scheme C7: Synthesis of the bifuncitional side chain

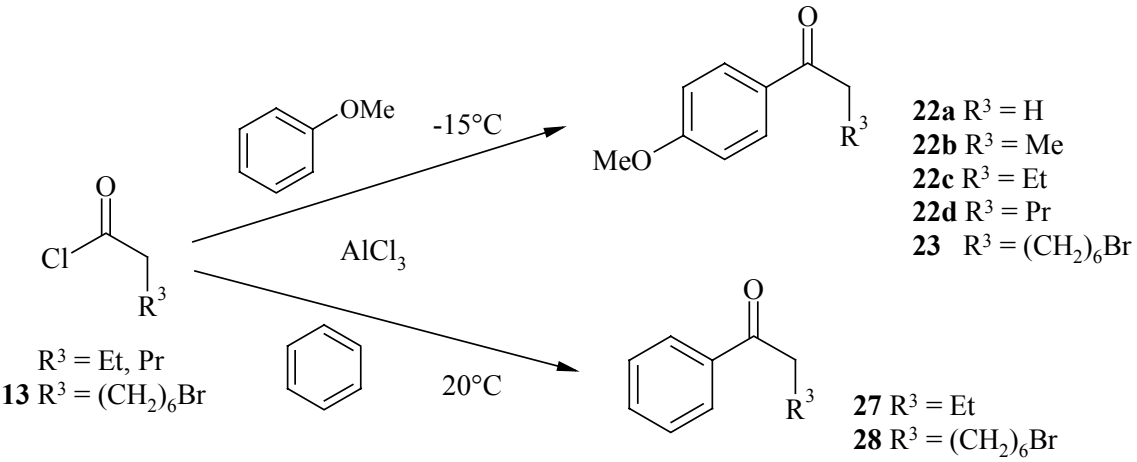
The fourth side chain **20** was obtained from the same substitution reaction of the acid chloride **12** with the cyclic secondary amine pyrrolidine. For further reactions, the terminal bromine in compound **20** was replaced by iodine as better leaving group using the Finkelstein reaction (side chain **21**) (cf. figure C8).



Scheme C8: Synthesis of pyrrolidine containing side chain

2.1.2 Synthesis of Ketone Precursors

The 2,5-diphenylfuran structure provides two equivalent positions in the aromatic furan ring to be linked with the aliphatic side chain. The C-3 position of the furan is known for its limited reactivity because of the rather low acidity of the β-protons. Reactions at this position require either harsh conditions or appropriate substituents have to be introduced before the furan ring is formed [Hou et al., 1998]. For this reason the side chains were introduced on an early stage and kept throughout the whole furan synthesis. The synthetic strategy to the desired 3,4-dialkyl-2,5-diphenylfurans was the formation of 1,4-dicarbonyl compounds from arylketones and α-bromoarylketones and subsequent acid catalysed cyclisation.

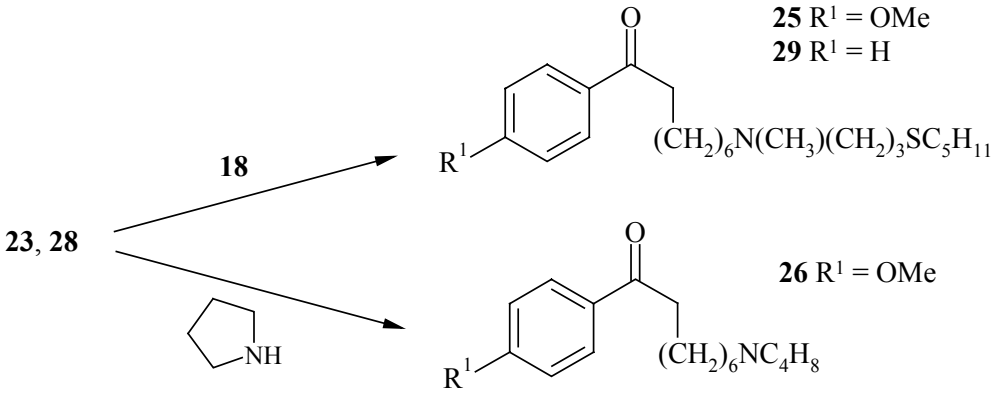


Scheme C9: Synthesis of alkyl aryl ketones by Friedel-Crafts acylation

Throughout this work the R groups in depicted molecules are assigned to the alphabetical letters a through e as follows: **a** ≡ R=H; **b** ≡ R=Me; **c** ≡ R=Et; **d** ≡ R=Pr; **e** ≡ R=Bu

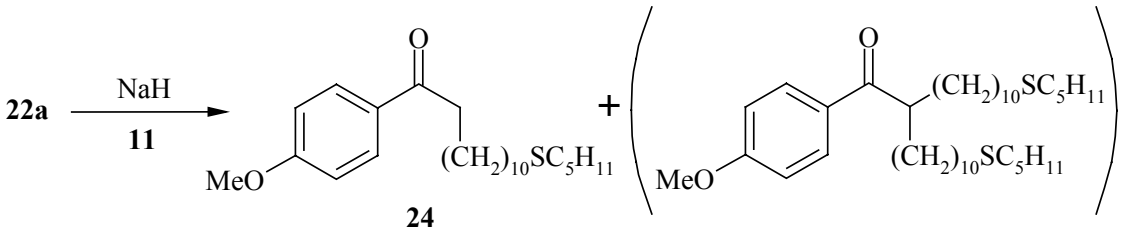
The Friedel-Crafts acylation is a very efficient method for the preparation of alkyl aryl ketones from an acid chloride and an aromatic compound in the presence of a Lewis acid. The acid chlorides described in this work were generally obtained from the reaction of oxalyl chloride with the respective carboxylic acids. Anhydrous $AlCl_3$ was the Lewis acid of choice

for the Friedel-Crafts reactions and a variety of ketones were prepared in high yield. The 4-methoxyphenylketones **22c**, **22d**, and **23** were synthesised from anisole at a temperature of -15°C. The methoxy substituent of the anisole exclusively directed the acylation in *para*-position on the aromatic ring. 4-Methoxyacetophenone **22a** and 4-methoxypropiophenone **22b** are commercially available starting materials. The unsubstituted phenylketones **27** and **28** were prepared from benzene at room temperature due to the high melting point of the aromatic substrate (cf. scheme C10). The synthesised ω-bromoketones **23** and **28** were further reacted with the secondary amines **18** and pyrrolidine, respectively, to give the ketones **25**, **26**, and **29** with the complete side chain attached.



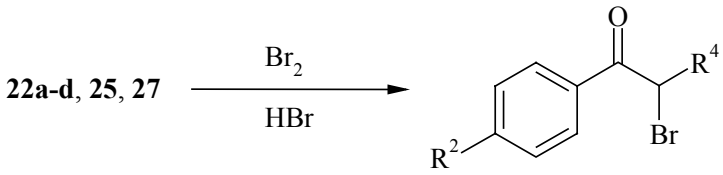
Scheme C10: Introduction of the amine function

The 4-methoxyphenylketone **24** carrying the monofunctional side chains with the sulfanyl group was prepared by nucleophilic substitution from 4-methoxyacetophenone **22a** and the side chain **10**. The reaction was carried out between –45°C and -10°C, because higher temperatures facilitated the formation of a disubstituted by-product. The factors that account for the formation of this side product are not well understood, as the positive inductive effect of the alkyl group in the monosubstituted product actually leads to a decrease of CH-acidity of the α-protons (cf. scheme C11).



Scheme C11: Synthesis of the ketone precursor **24**

The α-bromoketone **30a-d**, **31**, and **32** were prepared by direct bromination of the above described alkyl aryl ketones **22a-d**, **25**, and **27** with equimolar amounts of elemental bromine (cf. scheme C12). Acid catalysis of this reaction prevented multiple bromination, because the negative inductive effect of the introduced bromine atom slows the rate determining step of enol formation and, thus, prevents the attack of another bromine. The bromination reactions were performed either in glacial acidic acid or a mixture of dioxan and diethylether depending on the solubility of the starting material. An intra- or intermolecular substitution of the α-bromine in compound **31** by the basic nitrogen in the side chain was not observed.



30a-d R^2 = OMe, R^4 = H, Me, Et, Pr
31 R^2 = OMe, R^4 = $(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$
32 R^2 = H, R^4 = Et

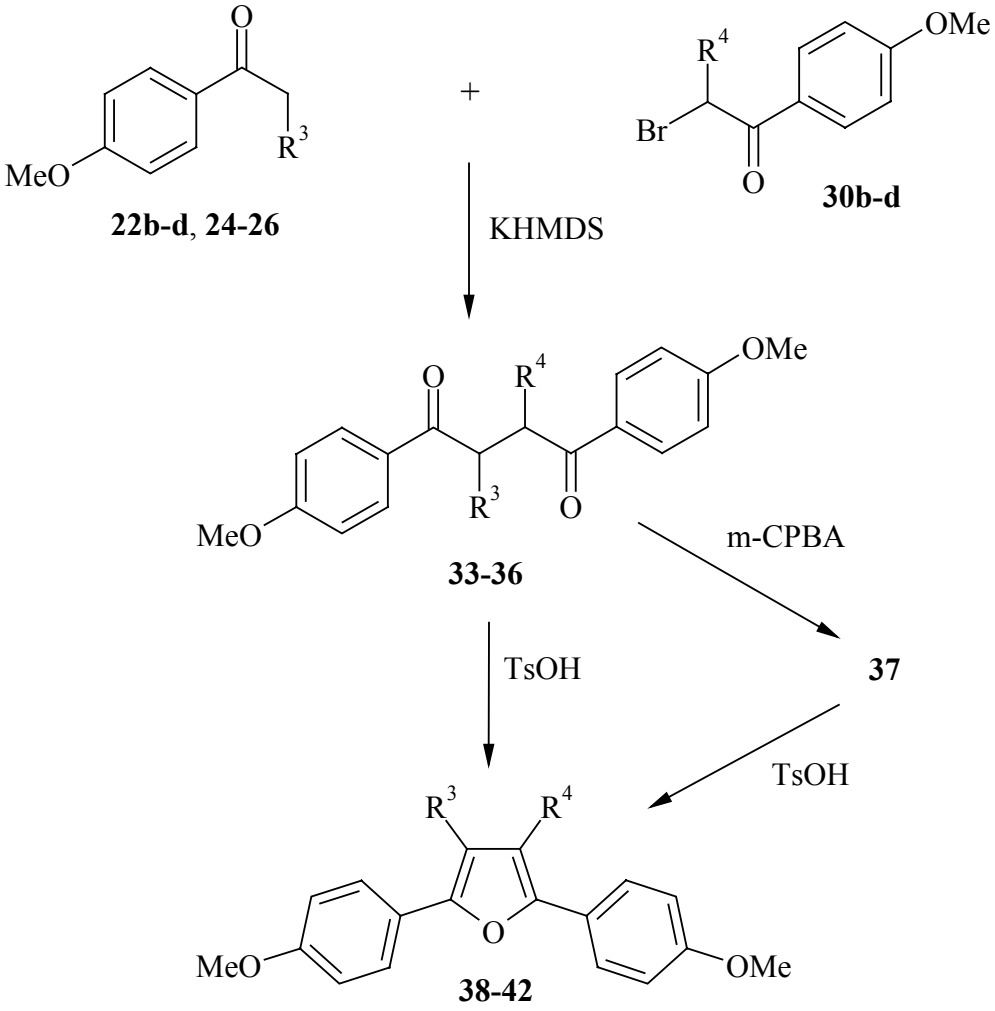
Scheme C12: Synthesis of α-bromoketones

2.1.3 Synthesis of 3,4-Dialkyl-2,5-diphenylfurans

The synthesised ketones **22b-d, 24-26** and α-bromoketones **30b-d** were fused to the 1,4-dicarbonyl compounds **33-36** by a S_N2 reaction with potassium bis(trimethylsilyl)amide (KHMDS) as base. Performing the reactions at temperatures between –50°C and –40°C guaranteed the exclusive formation of the desired products, which were usually obtained as diastereomeric mixtures. These diastereomers were not separated, because the subsequent cyclisation involves a re-trigonalisation of the generated stereocentres (cf. scheme C13). The NMR spectra of the 1,4-carbonyl compounds showed an unexpected strong low field shift of the methin protons to about 4ppm, which might result from an influence of the aromatic rings on the magnetic environment of these protons.

The sulfanyl groups in the monofunctional side chain were oxidised to the sulfones **37** using m-CPBA. The oxidation was performed at this stage of synthesis, because after the cyclisation oxidation would also affect the furan ring. An earlier introduction might also cause problems, as the sulfonyl group increases the CH-acidity of the adjacent α-protons.

The subsequent cyclisation of the 1,4-dicarbonyl compounds **33-37** to the furans **38-42** was achieved in good yields with catalytic amounts of 4-toluenesulfonic acid or somewhat more than one equivalent when a basic nitrogen was present in the molecule (cf. scheme C13).

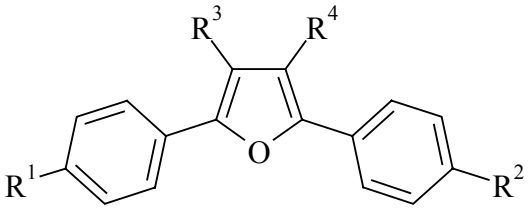


33b-d, **38b-d** $R^3 = R^4$ = Me, Et, Pr
34b-d, **39b-d** $R^3 = (CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$, R^4 = Me, Et, Pr
35b,c, 40b,c $R^3 = (CH_2)_6NC_4H_8$, R^4 = Me, Et
36b-d, 41b $R^3 = (CH_2)_{10}SC_5H_{11}$, R^4 = Me, Et, Pr
37b-d, 42b-d $R^3 = (CH_2)_{10}SO_2C_5H_{11}$, R^4 = Me, Et, Pr

Scheme C13: Synthesis of 3,4-dialkyl-2,5-diphenylfurans

Finally, the methoxy-protected furans **38-42** were demethylated using a 5-fold excess of boron tribromide in dry dichlormethane yielding the free phenols **43-50**. The product ratio of monohydroxy- to bis(hydroxyphenyl)furan was strongly dependent on the reaction time. Demethylation of one of the methoxy groups was complete within several minutes. Demethyla-

tion of the second methoxy took between 3 and 24 hours. An overview over the synthesised 3,4-dialkyl-2,5-diphenylfurans is presented in table C14.



43-50

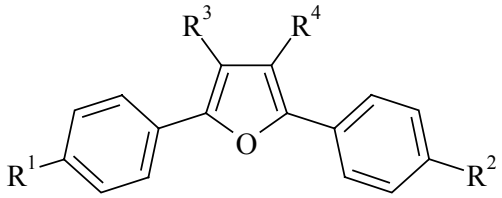
Compound	R^1	R^2	R^3	R^4
43b	OH	OH	Me	Me
43c	OH	OH	Et	Et
43d	OH	OH	Pr	Pr
44b	OH	OH	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	Me
44c	OH	OH	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	Et
44d	OH	OH	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	Pr
45b	OH	OH	$(CH_2)_6NC_4H_8$	Me
45c	OH	OH	$(CH_2)_6NC_4H_8$	Et
46b	OH	OH	$(CH_2)_{10}SC_5H_{11}$	Me
47b	OH	OH	$(CH_2)_{10}SO_2C_5H_{11}$	Me
47c	OH	OH	$(CH_2)_{10}SO_2C_5H_{11}$	Et
47d	OH	OH	$(CH_2)_{10}SO_2C_5H_{11}$	Pr
48b	OH	OMe	Me	Me
48c	OH	OMe	Et	Et
48d	OH	OMe	Pr	Pr
49b	OH	OMe	$(CH_2)_{10}SC_5H_{11}$	Me
50b	OH	OMe	$(CH_2)_{10}SO_2C_5H_{11}$	Me
50c	OH	OMe	$(CH_2)_{10}SO_2C_5H_{11}$	Et
50d	OH	OMe	$(CH_2)_{10}SO_2C_5H_{11}$	Pr

Table C14: Overview over the synthesised 3,4-dialkyl-2,5-diphenylfurans

2.2 Biological Characterisation of the 3,4-Dialkyl-2,5-diphenylfurans

2.2.1 Determination of Affinity and Selectivity for the ER

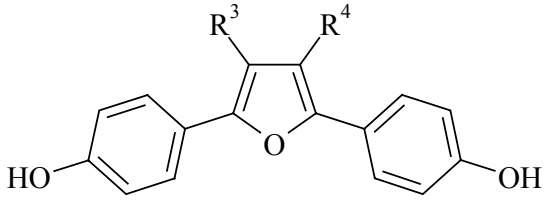
The synthesised series of mono- and diphenolic 3,4-dialkyl-2,5-diphenylfurans were tested for their binding affinities to the ER and their selectivity for one of the two ER subtypes. The binding curves of all test compounds were comparable in shape to the binding curve of E2, but shifted to higher concentrations, which indicates a competitive displacement of the physiological ligand from the receptor binding site. The RBA values were determined by two methods using either calf uterus cytosol as receptor source or the recombinant human ERα and ERβ. The RBA values shown are the means of two or three independent experiments.
First, the simple alkyl derivatives **43b-d** and **48b-d** were investigated in respect to their affinity to the ER in the cytosol assay. The diphenols **43** showed higher affinity than their corresponding monophenols **48**. For this reason only the diphenols were considered for selectivity studies. All three 2,5-diphenylfurans **43** demonstrated a preference for ERα. The highest RBA values for both subtypes were found for the ethyl derivative **43c**, which bound by a factor of 4 stronger to ERα. The results of both assays with cytosol and recombinant proteins are listed in table C15.



Compound	R^1, R^2	R^3, R^4	RBA (cytosol)	RBA (ERα)	RBA (ERβ)	Binding ratio α/β
43b	OH, OH	Me, Me	0.30	1.5	0.33	4.5
43c	OH, OH	Et, Et	1.5	23	5.6	4.1
43d	OH, OH	Pr, Pr	0.58	8.4	4.5	1.9
48b	OH, OMe	Me, Me	0.04	n. d.	n. d.	n. d.
48c	OH, OMe	Et, Et	0.51	n. d.	n. d.	n. d.
48d	OH, OMe	Pr, Pr	0.44	n. d.	n. d.	n. d.

Table C15: Relative binding affinities of simple alkyl derivatives of 2,5-diphenylfurans

Binding studies of the 2,5-diphenylfurans with aliphatic substituents in position C-3 and C-4 revealed that one alkyl group can be substituted by a long functionalised side chain without loss of selectivity for ERα. The preference for ERα is mainly due to the low affinities of these compounds for ERβ. The degree of selectivity was quite variable ranging from 2.3 to 240. By far the highest binding ratio of 240 was observed for compound **45b** with the pyrrolidinohexyl side chain. Generally, the highest selectivity for ERα was achieved with the compounds **44b**, **45b**, and **47b** bearing a methyl substituent. Going from the methyl to the ethyl group the binding selectivities dropped.



Comp.	R^3	R^4	RBA (cytosol)	RBA (ERα)	RBA (ERβ)	Binding ratio α/β
44b	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	Me	2.0	13	1.1	12
44c	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	Et	1.9	18	3.4	5.3
44d	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	Pr	0.54	18	2.7	6.7
45b	$(CH_2)_6NC_4H_8$	Me	1.2	24*	0.10*	240
45c	$(CH_2)_6NC_4H_8$	Et	2.1	42	2.8	15
46b	$(CH_2)_{10}SC_5H_{11}$	Me	0.01	0.07	0.03	2.3
47b	$(CH_2)_{10}SO_2C_5H_{11}$	Me	0.09	1.6	0.10	16
47c	$(CH_2)_{10}SO_2C_5H_{11}$	Et	0.11	0.90	0.25	3.6
47d	$(CH_2)_{10}SO_2C_5H_{11}$	Pr	0.11	0.71	0.28	2.5
OH-tam			23	13	7.7	1.7
ICI			5.2	5.3	3.1	1.7

Table C16: Relative binding affinities of 3,4-dialkyl-2,5-phenylfurans with functionalised side chains

* Mean value of four independent experiments

Compound **46b** with the sulfanyl function incorporated in the side chain was hardly capable of displacing E2 from the receptor binding site. Somewhat higher but still rather low binding affinities were observed for the sulfone series **47**. Much higher RBA values especially for ERα were found for the compounds of series **44** and **45** with a basic nitrogen function implemented in their side chain. This indicates an additional ionic interaction of this nitrogen atom with ERα Asp351 of the receptor. The highest RBA value of 42 was obtained for compound **45c**. Again, the monophenols showed lower binding affinities in the cytosol assay than the corresponding diphenols and were therefore not considered for detailed selectivity studies. The RBA values of the diphenolic 3,4-dialkyl-2,5-phenylfurans together with values for fulvestrant (ICI) and 4-hydroxytamoxifen (OH-Tam) from both assays are summarised in table C16.

Molecular modelling studies were performed with compound **44c** to investigate the binding mode of the 3,4-dialkyl-2,5-diphenylfurans in the ERα binding pocket. For this purpose, **44c** was slightly modified by cutting off the terminal section of the long aliphatic side chain after the C-8 atom. The starting conformation of the modified compound **44c** was generated from random conformational searches followed by full energy minimisation using the Tripos force field with the Powell algorithm. The lowest-energy conformer of **44c** was then manually adjusted in the modified (cf. section C1.1) raloxifene-ERα LBD (pdb entry: 1ERR) using selected atoms of the raloxifene structure. Once prepositioned, raloxifene was deleted and the ligand-receptor-complex energetically optimised utilizing the MMFF94 force field with the Powell gradient method implemented in the modeling program SYBYL 6.8.
The final model of **44c** in the ligand binding pocket of ERα is depicted in figure C17 showing important interacting residues within a radius of 2.5Å of the ligand. The general orientation of the compound within the binding site is determined by the long aliphatic side chain, that aligns itself along helix H11 and finds its way out of the binding cavity. Thus, the C-2 phenyl ring mimics the A-ring of E2 with the phenolic hydroxyl group forming hydrogen bonds to Arg394 and Glu353. The hydroxyl group of the phenyl ring at C-5 also is located within hydrogen bonding distance of the residue His524. The favourable orientation of **44c** with contacts to both hydrogen bonding sites of the receptor provides a rational explanation for the discussed binding affinities of the monophenolic 2,5-diphenylfuran derivatives. Furthermore, from the docking model the ionic interaction of the basic nitrogen in the side chain with Asp351 can be confirmed. Conclusions on the selectivity of the compounds based on the 2,5-diphenylfuran scaffold cannot be drawn from this model.

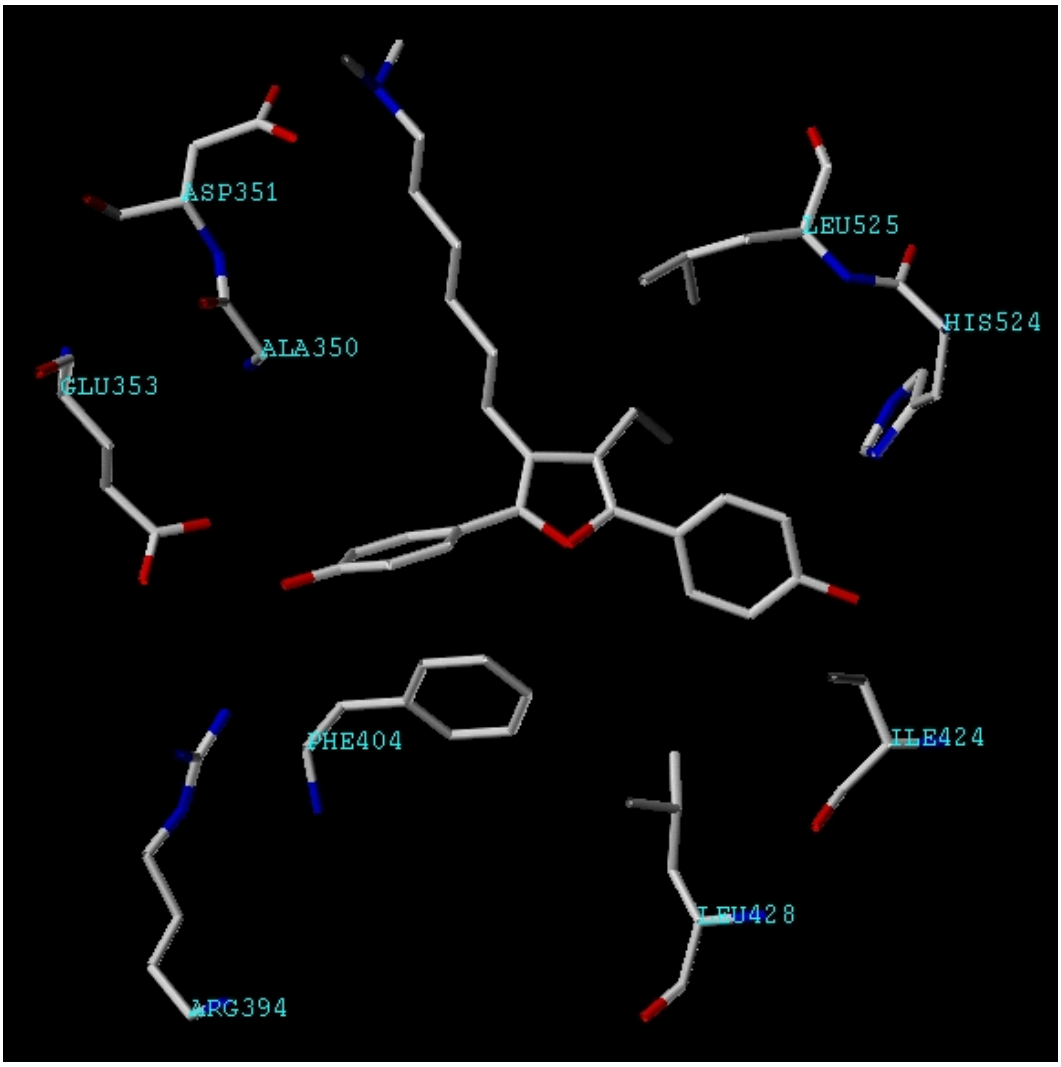


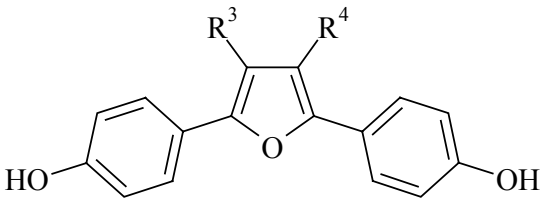
Figure C17: The modified 3,4-dialkyl-2,5-diphenylfuran **44c** docked in the ERα LBD

2.2.2 Determination of Antiproliferative Activity

The antiproliferative activity of the synthesised compounds were determined in the ER-positive MCF-7 mammary carcinoma cell line. This assay was performed using estradiol as growth stimulating hormone in a nearly physiological concentration of 1nM. The monophenolic derivatives **48-50** of 2,5-diphenylfurans were not considered for this evaluation due to the reduced binding affinities compared to their corresponding diphenols.

The compounds **43b-d** of the series with simple alkyl substituents showed no inhibitory effect on cell growth (data not shown). In contrast, the compounds carrying a long functionalised side chain demonstrated a dose-dependent growth inhibition. The IC_{50}-values from this assay are listed in table C18. The antiproliferative effects of the 2,5-diphenylfurans were mainly

influenced by the structure of the side chain. The strongest inhibitory effect with IC_{50}-values of 22nM and 53nM, respectively, was found for compounds **44c** and **44d** with the bifunctional side chain. For the sulfone and the pyrrolidinohexyl series the antiproliferative activity was reduced by one or two order of magnitude. Compound **46b** proved to be inactive (IC_{50}-value >10µM).



Comp.	R^3	R^4	MCF-7 IC_{50} [µM]	MDA-MB 231 IC_{50} [µM]
44b	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	Me	0.2	2.0
44c	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	Et	0.022	2.0
44d	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	Pr	0.053	2.1
45b	$(CH_2)_6NC_4H_8$	Me	2.1	2.5
45c	$(CH_2)_6NC_4H_8$	Et	0.7	2.7
46b	$(CH_2)_{10}SC_5H_{11}$	Me	>10	>10
47b	$(CH_2)_{10}SO_2C_5H_{11}$	Me	1.7	>10
47c	$(CH_2)_{10}SO_2C_5H_{11}$	Et	0.6	5.8
47d	$(CH_2)_{10}SO_2C_5H_{11}$	Pr	0.7	5.8
OH-tam			0.012	6.4
ICI			0.004	>10

Table C18: Antiproliferative effects of 3,4-dialkyl-2,5-phenylfurans with functionalised side chains in MCF-7 and MDA-MB 231 breast cancer cells

In order to exclude a non-specific cytotoxic action all 2,5-diphenylfurans were tested in hormone-independent MDA-MB 231 breast cancer cells. Their growth was not inhibited by these agents in concentrations up to 1µM. However, at higher concentrations all compounds exerted cytotoxic effects, but the IC_{50}-values in the hormone-independent cell line exceeded the one in the MCF-7 cells at least by the factor of 10. The only exceptions were the compounds **45b**

and **45c**. In fact, **45b** was equally active in both cell lines what makes an non-specific action of this derivative likely (cf. table C18).

2.2.3 Determination of Estrogenic and Antiestrogenic Activity *in vitro*

The diphenolic 3,4-dialkyl-2,5-diphenylfurans were tested for their estrogenic and antiestrogenic activity in the luciferase assay using hormone-dependent MCF-7/2a cells that have been stably transfected with a luciferase reporter gene under the control of an ERE. The antiestrogenic activity was determined by simultaneous treatment of the cells with 1nM E2 and the respective 2,5-diphenylfuran in various concentration.

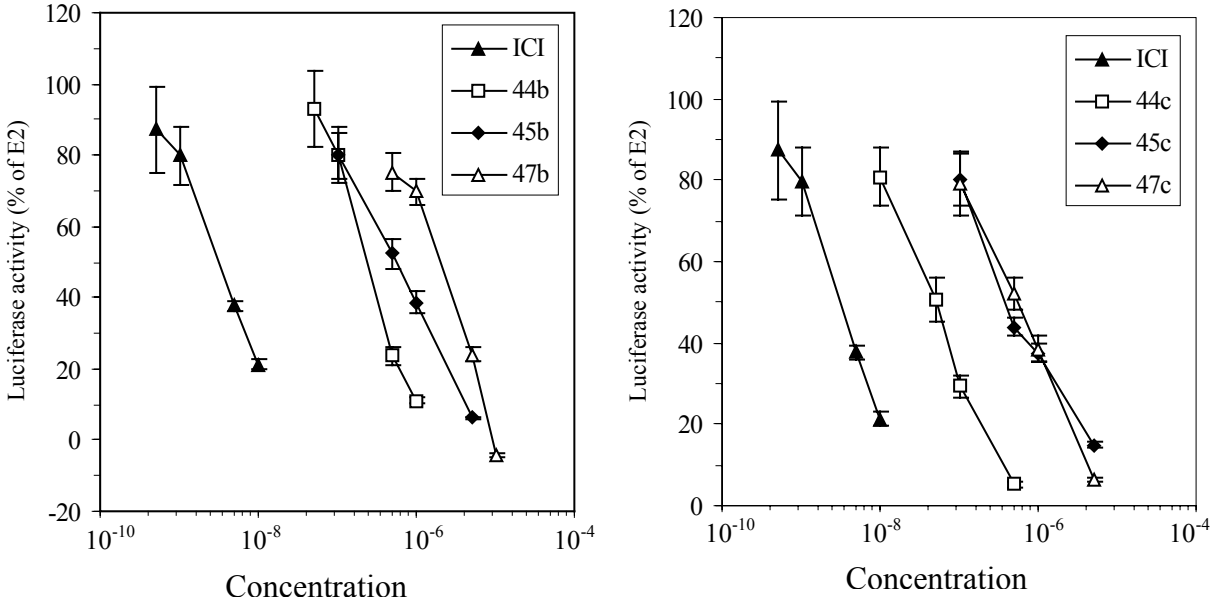
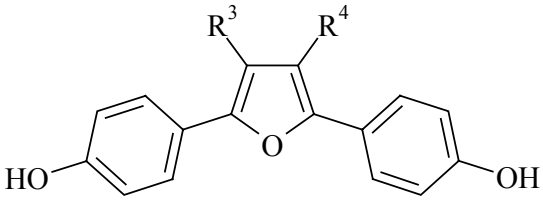


Figure C19: Antiestrogenic effects of 3,4-dialkyl-2,5-diphenylfurans with functionalised side chains in MCF-7/2a cells

The simple alkyl derivatives **43b-d** of 2,5-diphenylfurans showed no antiestrogenic effects at a concentration of 1µM (data not shown). All compounds with an aliphatic side chain, except **46b**, inhibited the E2 stimulated luciferase expression in a dose-dependent manner (cf. figure C19). The lowest IC_{50}-values of 50nM and 67nM, respectively, were obtained for the compounds **44c** and **44d** with the bifunctional side chain. Their antiestrogenic activity was by one order of magnitude lower than that of fulvestrant. All other compounds **45b-c** and **47b-d** inhibited gene transcription with IC_{50}-values that were by one or two orders of magnitude

higher. The ethyl derivatives were by a factor of 10 more potent than the corresponding methyl derivatives in each series of compounds. All IC_{50}-values (cf. table C20) were in good accordance with those from the chemosensitivity assay in MCF-7 cells, but there was no obvious correlation between the potencies in the luciferase assay and the RBA of these agents for ERα. A possible explanation is the difference in the experimental conditions of the two assays. The binding assay was performed in a cell-free system, whereas the transactivation assay required intact cells.

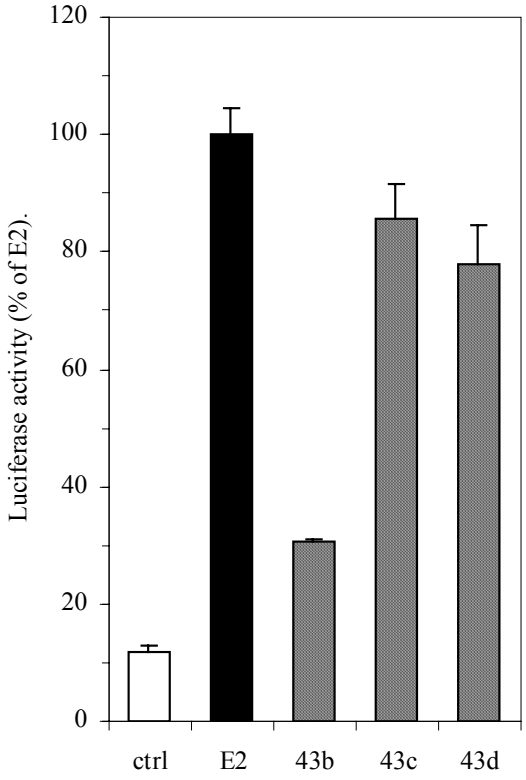


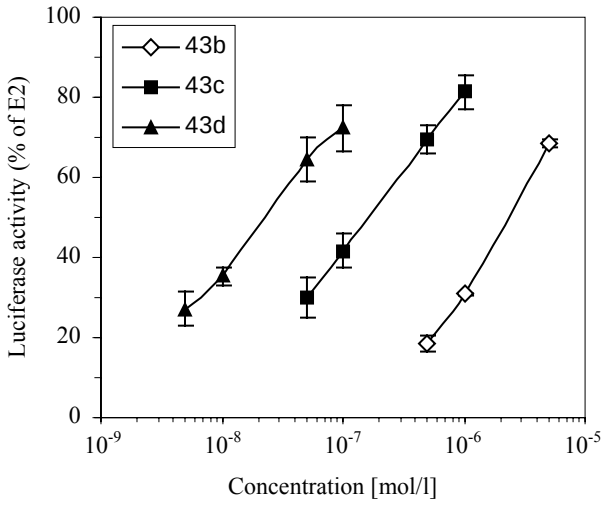
Comp.	R^3	R^4	IC_{50} [µM]
44b	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	Me	0.4
44c	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	Et	0.050
44d	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	Pr	0.067
45b	$(CH_2)_6NC_4H_8$	Me	1.5
45c	$(CH_2)_6NC_4H_8$	Et	0.4
46b	$(CH_2)_{10}SC_5H_{11}$	Me	>10
47b	$(CH_2)_{10}SO_2C_5H_{11}$	Me	3,2
47c	$(CH_2)_{10}SO_2C_5H_{11}$	Et	0.6
47d	$(CH_2)_{10}SO_2C_5H_{11}$	Pr	0.7
OH-tam			0.004
ICI			0.003

Table C20: Antiestrogenic activity of 3,4-dialkyl-2,5-phenylfurans with functionalised side chains in MCF-7/2a cells

The estrogenic potency of all test compounds was determined in a similar assay at a concentration of 1µM in the absence of estradiol. At this concentration the simple alkyl derivatives **43b-d** produced full estrogenic response with exception of compound **43b,** which bound only weakly to the ER (cf. table C21 left). For an exact determination of the estrogenic activity of

these compounds dose-response curves were determined. The EC_{50}-values showed an increase in agonistic potency by the factor of 10 with increasing chain length (cf. table C21 right). This behaviour did not correlate with the binding affinities of these agonist.





Comp.	R^3	R^4	EC_{50} [µM]
43b	Me	Me	2.3
43c	Et	Et	0.2
43d	Pr	Pr	0.02

Table C21: Estrogenic activity of the 3,4-dialkyl-2,5-phenylfurans **43b-d** in MCF-7/2a cells

ctrl = control; left: luciferase activity measured at a compound concentration of 1µM without E2 stimulation; right: Dose-response curves without E2 stimulation and the corresponding EC_{50}-values

The 2,5-diphenylfurans with functionalised side chains showed no agonistic activity at a concentration of 1µM. All values except that of compound **46b** were below that of the control cells. Luciferase activity below baseline levels are characteristic for potent antiestrogens and indicate the blockade of AF-1 mediated ligand-independent activation of the ER, that is responsible for the basal luciferase activity in the control cells. Thus, **46b** cannot be characterised as an antiestrogen, which is in accordance with the low binding affinity and proliferative activity.

For an estimation of the residual estrogenic activity of these agents the levels of luciferase expression were compared with those of the partial antiestrogen 4-hydroxytamoxifen and the pure antiestrogen fulvestrant (cf. figure C22). The compounds **44b-d** with the bifunctional side chain can be characterised as pure antiestrogens with a suppression of luciferase expres-

sion comparable to that of fulvestrant. The relative luciferase activities of compounds **45b** and **45c** with the pyrrolidinohexyl side chain exceed that of 4-hydroxytamoxifen and confirm the observation that the length of the side chain is an important factor for antagonism. Contrary to previous studies with sulfones based on different core structures the sulfones **47b-d** did not reach the level of fulvestrant. In this respect they resemble 4-hydroxytamoxifen.

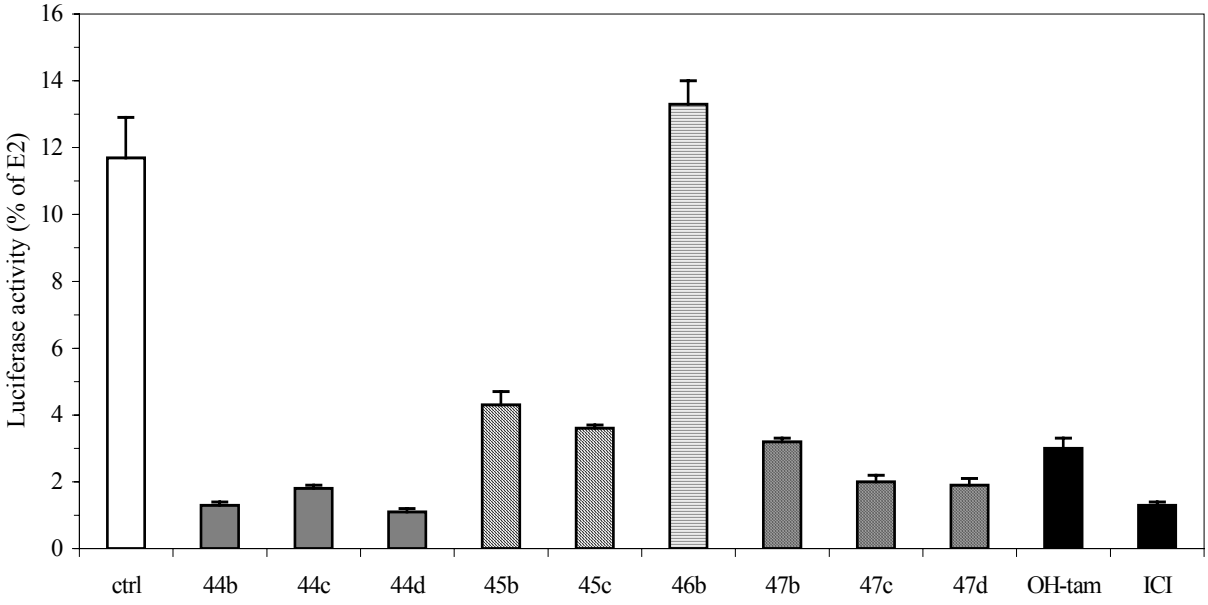


Figure C22: Suppression of basal luciferase activity by 3,4-dialkyl-2,5-diphenylfurans with functionalised side chains in MCF-7/2a cells

The value for estradiol at a concentration of 1nM was set to 100% (not shown).
ctrl = control

2.2.4 Determination of Estrogenic and Antiestrogenic Activity *in vivo*

The uterine growth in immature mice is a typical model for the determination of estrogenic (uterotrophic) and antiestrogenic (antiuterotrophic) activity of test compounds *in vivo*. *In vitro*, compound **44b** was shown to be a potent antiestrogen without any residual estrogenic activity. For this reason it was chosen to be tested as representative example of this series in the uterine weight test. This should provide first information about the activity of 2,5-diphenylfuran-based antiestrogens in a mammalian organism, since most of the non-steroidal pure antiestrogens suffer from low bioavailability.

The drug was administered in doses of 6.0 and 30.0mg/kg body weight, respectively, either alone or in combination with estradiol. At the higher concentration the antiestrogen **44b** suppressed the estradiol stimulated uterus growth by 74% and demonstrated no significant estro-

genic effects (cf. table C23). This result confirmed that the compound is also a pure antiestrogen *in vivo*. The reference drug fulvestrant is also devoid of estrogenic activity when given alone, but requires only 1.2mg/kg body weight in the antiuterotrophic test to completely suppress uterus growth in the animals. Considering that **44b** was not the most potent 2,5-diphenylfuran it can be assumed that the antiestrogenic effect *in vivo* can be enhanced by the ethyl or propyl derivative **44c** and **44d**. Furthermore it has been shown that a terminal fluorination of the side chains, which has not yet been performed in this series, increases bioavailability and, thus, the antiestrogenic activity [Golob, 1999].

Comp.	Dose (mg/kg)	Uterotrophic test		Antiuterotrophic test	
		Rel. uterus weight ± SD	Estrogenic effect [%]	Rel. uterus weight ± SD	Antiestrogenic effect [%]
Control	–	21.1 ± 3.2	–	21.1 ± 3.2	–
Estradiol	0.005	58.5 ± 10.9	100	58.5 ± 10.9	–
44b	6.0	22.2 ± 3.7	3	52.8 ± 9.2	15
44b	30	21.5 ± 5.1	1	30.9 ± 10.3	74*
Control	–	19.4 ± 4.9	–	19.4 ± 4.9	–
Estradiol	0.01	55.3 ± 8.3	100	55.3 ± 8.3	–
ICI	0.05	n. d.	–	51.5 ± 8.8	11
ICI	0.25	n. d.	–	36.7 ± 8.1	52*
ICI	1.2	n. d.	–	19.5 ± 3.5	100*
ICI	6.0	10.8 ± 1.8	-28	17.0 ± 3.8	107*

Table C23: Estrogenic and antiestrogenic activity of **44b** *in vivo*

* Significant ($p < 0.01$) in comparison to the estradiol group; ICI = fulvestrant

2.3 Conclusion

It could be shown that the 2,5-diphenylfuran scaffold is a suitable structural core for the development of non-steroidal ligands, that have high affinity and selectivity for the ERα. One hydroxy group in *para*-position on each aromatic ring proved to be necessary for high receptor affinities. The implementation of long functionalised side chain retained ERα selectivity

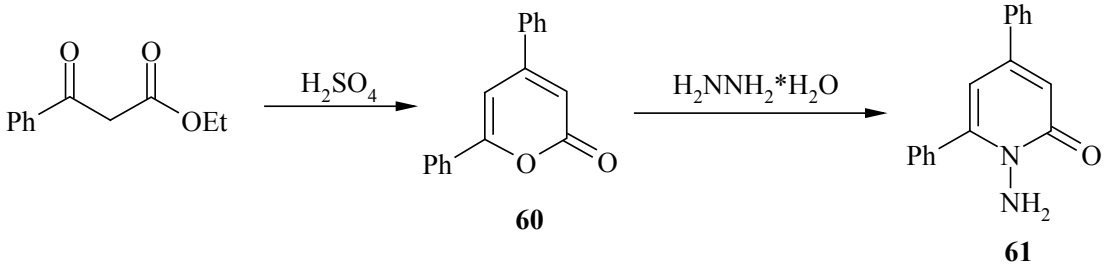
and lead to pure estrogen antagonists with good *in vitro* and *in vivo* activity. In a transcription assay all these furan-based compounds completely antagonised the effect of estradiol on gene activation. When they were given alone the luciferase activity was below that of the control cells, which indicates the total blockade of ER-mediated action. The lowest IC_{50}-values were obtained from compounds bearing a 6-[N-methyl-N-(3-pentylsulfanyl-propyl)amino]hexyl side chain and an ethyl **44c** or propyl substituent **44d**. The results from the luciferase assay were in good accordance with those obtained in the chemosensitivity assay with the human MCF-7 breast cancer cells.

3 Antiestrogens Based on a 2,4-Diphenylfuran Scaffold

Compounds based on a 2,5-diphenylfuran scaffold can be turned into pure antiestrogens with good *in vitro* and *in vivo* activity by attaching a functionalised side chain. The bifunctional 6-[N-methyl-N-(3-pentylsulfanyl-propyl)amino]hexyl side chain proved to be most suitable in this respect. For this reason this side chain was considered to be implemented in 3- or 5-position of the 2,4-diphenylfuran leading to two sets of isomeric furan-based antiestrogens, which might give rise to enhanced ER selectivity and potency. Up to now there exist no reports in the literature that investigated 2,4-diphenylfurans as ligands for the ER.

3.1 Synthesis of 3,5-Dialkyl-2,4-diphenylfurans

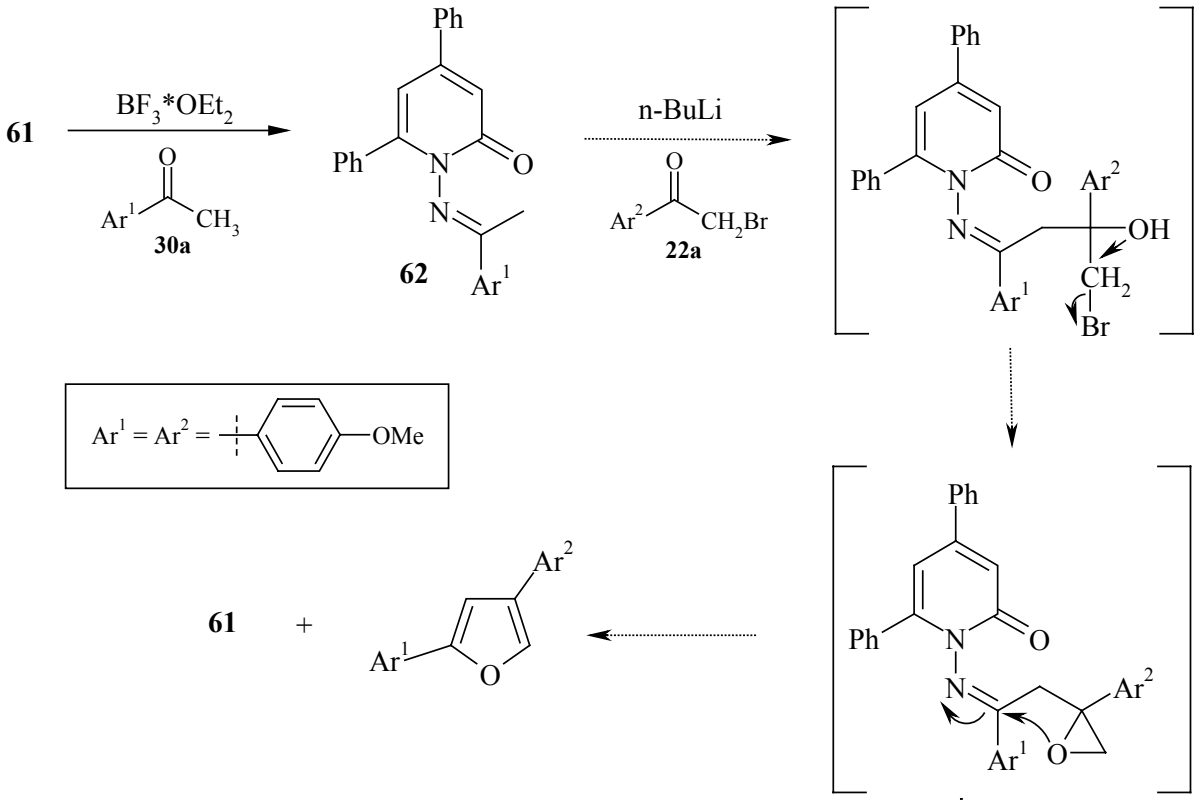
The introduction of the aliphatic side chain as one of the last step of the synthesis was not considered for 2,5-diphenylfuran, but it offers an attractive route to 2,4-diphenylfurans carrying the side chains in α-position of the furan ring. An efficient regioselective synthesis of 5-unsubstitued 2,4-diarylfurans was reported by Molina et al. [1983] using aryl methyl ketones and phenylacyl bromides. This two-step process required 1-amino-4,6-diphenyl-2-pyridone **61** as auxiliary, which could be recovered for reuse in high yield. Compound **61** was prepared by a literature procedure [Katritzky et al., 1979] from ethyl benzoylacetate, that was first condensed in concentrated sulfuric acid over 3 weeks to the pyrone **60** and subsequently converted to the pyridone **61** using hydrazine hydrate (cf. scheme C24).



Scheme C24: Synthesis of the auxiliary 1-amino-4,6-diphenyl-2-pyridone **61**

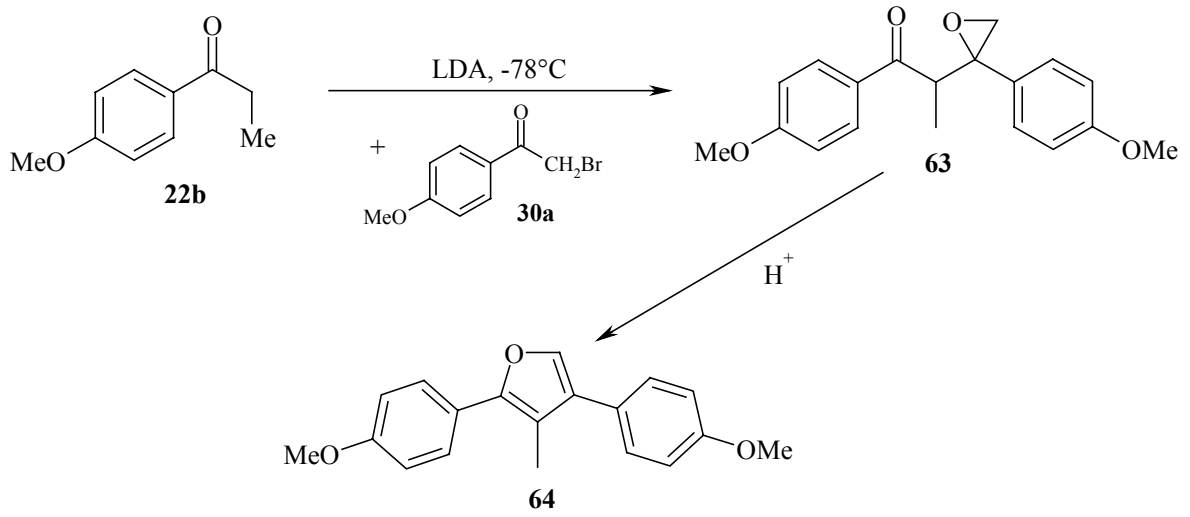
The following, acid catalysed, transformation of **61** with 4-methoxyacetophenone to the corresponding ketimine **62** let exclusively to the isolation of either the unconverted pyridone or

its hydrochloride salt. Modification of this procedure by using boron trifluoride afforded the desired product **62**, but it was obtained only in low yield and separation from the starting material proved to be difficult (cf. scheme C25).



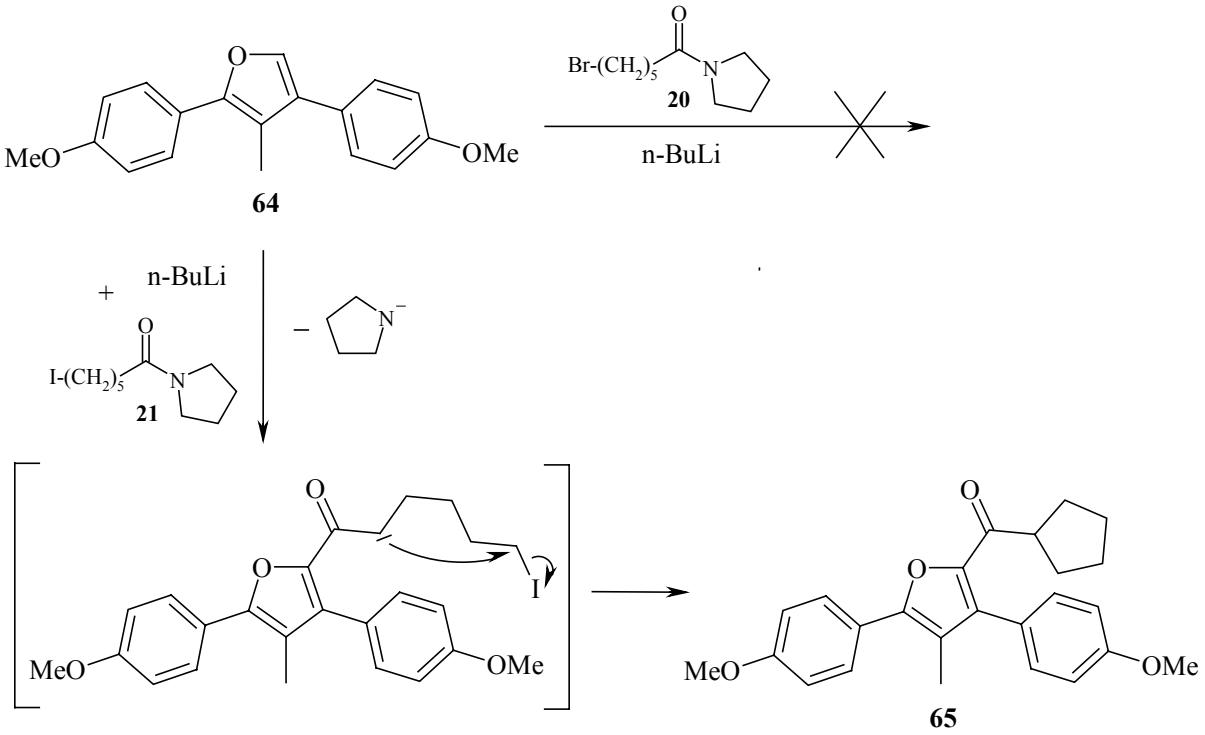
Scheme C25: Attempted auxiliary mediated synthesis of 3,5-unsubstituted 2,4-diphenylfurans

Molina's synthetic route involved the furan formation via an intermediary epoxide (cf. scheme C25). It was possible to isolate a similar epoxide (**63**) in good yield directly from the reaction of 4-methoxypropiophenone with 4-methoxyphenylacyl bromide using LDA as base at –78°C [Bartroli et al., 1995]. This epoxide was converted to the 5-unsubstituted furan **64** by adding the epoxide **63** to a slightly acidic solution of chloroform (cf. scheme C26). Due to the fast decomposition of furan **64** during the reaction, the compound was isolated in a low yield. NMR analysis revealed that **64** was highly unstable in solution and it was completely decomposed after 36h.



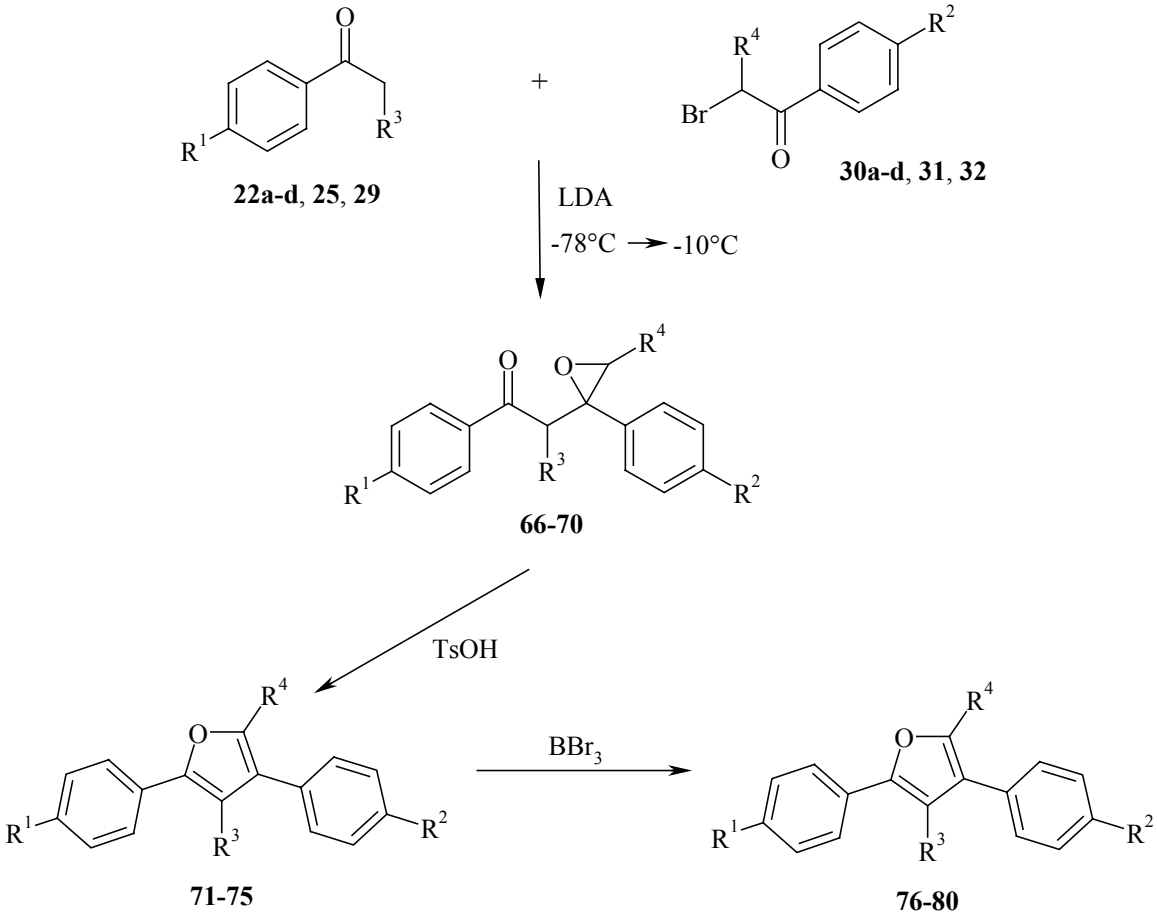
Scheme C26: Synthesis of the 5-unsubstituted 2,4-diphenylfuran **64**

Nevertheless, compound **64** was used to introduce an aliphatic side chain in α-position by lithiation with n-BuLi and subsequent treatment of the lithiated furan with the side chain **20** at temperatures slightly below 0°C. No product formation was observed even when the temperature was raised to room temperature, which facilitated the decomposition of the starting material **64**. For this reason the side chain **20** was equipped with a better leaving group (cf. section C2.1.1), which was thought to accelerate the product formation by enhanced reactivity.



Scheme C27: Formation of 5-cyclopentylcarbonyl substituted 2,4-diphenylfuran

Using the new side chain **21** as electrophile the result was almost the same with the exception that the by-product **65** with a cyclopentylcarbonyl substituent was formed (cf. scheme C27). A proposed mechanism for this synthesis involves the attack of the lithiated furan **64** at the amide carbonyl in **21** and the release of a pyrrolidine anion. The heterocycle now acts as a base and abstracts one α-proton adjacent to the carbonyl function. The resulting anion substitutes intramolecularly the iodine in a S_N2-type reaction leading to the five-membered ring found in compound **65**.



66a-d, **71a-d** $R^1 = R^2 = OMe$; $R^3 = R^4 = H$, Me, Et, Pr
67, **72** $R^1 = R^2 = OMe$; $R^3 = SC$; $R^4 = Et$
68, **73** $R^1 = H$; $R^2 = OMe$; $R^3 = SC$; $R^4 = Et$
69, **74** $R^1 = OMe$; $R^2 = H$; $R^3 = SC$; $R^4 = Et$
70, 75 $R^1 = R^2 = OMe$; $R^3 = Et$; $R^4 = SC$

76a-d $R^1 = R^2 = OH$; $R^3 = R^4 = H$, Me, Et, Pr
77 $R^1 = R^2 = OH$; $R^3 = SC$; $R^4 = Et$
78 $R^1 = H$; $R^2 = OH$; $R^3 = SC$; $R^4 = Et$
79 $R^1 = OH$; $R^2 = H$; $R^3 = SC$; $R^4 = Et$
80 $R^1 = R^2 = OH$; $R^3 = Et$; $R^4 = SC$

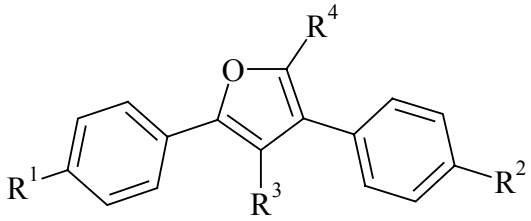
Scheme C28: Synthesis of 3,5-dialkyl-2,4-diphenylfurans

$SC = (CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$

In principle, 2,4-diphenylfurans were accessible via appropriate epoxides, but the direct introduction of the functional side chain into the aromatic ring failed. Therefore a similar strategy

as applied in the synthesis of 2,5-diphenylfurans was pursued and the side chain attached to appropriate ketones and α-bromoketones prior to the ring formation (cf. section C2.1.2). By this method both series of 2,4-diphenylfurans with the side chain in position C-3 and C-5 of the furan ring were accessible (cf. scheme C28).

The ketones **22a-d**, **25** and **29** were deprotonated with LDA at –78°C and the α-bromoketones **30-32** were subsequently added. At this temperature the addition of the produced enolates to the carbonyl function is faster than the bromide substitution (note in contrast the slightly higher temperature of –45°C for the preparation of the isomeric 1,4-dicarbonyl compounds). Raising the reaction temperature to a maximum of –10°C results in intramolecular substitution of the bromine substituent and formation of the epoxide precursors **66-70** in high yields. Only the epoxide prepared from 4-methoxyacetophenone **22a** and 4-methoxyphenacyl bromide **30a** could not be isolated, because it readily cyclised to the corresponding furan **71a**. All other epoxides were converted without further purification with 4-toluenesulfonic acid to the corresponding 2,4-diphenylfurans **71-75**. The action of boron tribromide finally afforded the free phenols **76-80**, which are presented in table C29.



76-80

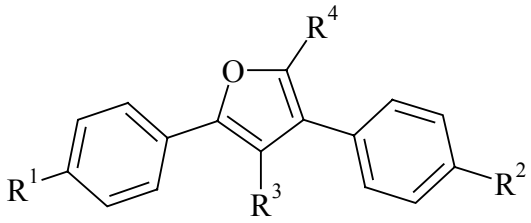
Comp.	R^1	R^2	R^3	R^4
76a	OH	OH	H	H
76b	OH	OH	Me	Me
76d	OH	OH	Et	Et
76d	OH	OH	Pr	Pr
77	OH	OH	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	Et
78	H	OH	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	Et
79	OH	H	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	Et
80	OH	OH	Et	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$

Table C29: Overview over the synthesised 3,5-dialkyl-2,4-diphenylfurans

3.2 Biological Characterisation of the 3,5-Dialkyl-2,4-diphenylfurans

3.2.1 Determination of Affinity and Selectivity for the ER

The synthesised 3,5-dialkyl-2,4-diphenylfurans **76-80** were tested in a radioligand binding assay using either calf uterus cytosol and the recombinant human ERα and ERβ. In analogy to the 2,5-diphenylfurans, an 3,5-unsubstituted 2,4-diphenylfuran **76a** and simple alkyl derivatives **76b-d** were investigated for the binding affinity and selectivity for the ER. The obtained RBA values of all tested compounds are listed in table C30.



Comp.	R^1	R^2	R^3	R^4	RBA (cytosol)	RBA (ERα)	RBA (ERβ)	Binding ratio β/α
76a	OH	OH	H	H	0.03	0.07	1.5	21
76b	OH	OH	Me	Me	6.6	21	76	3.6
76c	OH	OH	Et	Et	12	18	28	1.6
76d	OH	OH	Pr	Pr	3.1	3.7	8.9	2.4
77	OH	OH	SC	Et	3.6	8.0	8.5	1.1
78	H	OH	SC	Et	1.5	3.3	1.7	0.5
79	OH	H	SC	Et	2.9	6.9	7.1	1.0
80	OH	OH	Et	SC	9.3	11.3	9.3	0.8
Genistein					n. d.	0.82	18	22
ICI					5.2	5.3	3.1	0.6

Table C30: Relative binding affinities of the 3,5-dialkyl-2,4-phenylfurans

SC = $(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$

Contrary to the 2,5-diphenylfurans, all four 2,4-diphenylfurans **76a-d** showed a preference for ERβ. The highest selectivity for ERβ was obtained by the unsubstituted furan **76a** with a bind-

ing ratio of 21, which is comparable to the ERβ selective agonist genistein. However, the RBA values of **76a** for both receptor subtypes were quite low. Especially the very low affinity for ERα lead to this relatively high selectivity. The alkyl derivatives **76b-d** showed only moderate selectivities for ERβ up to 3.6-fold, but generally much higher binding affinities The highest RBA value for ERβ was found for the methyl derivative **76b** with an affinity of 76% of that of E2. The compounds **76b** and **76c** displayed the best figures for ERα, comparable to that of the corresponding diethyl derivative **43c** of the 2,5-diphenylfuran series. The drop of selectivity when going from methyl to ethyl can be explained by the strong decrease in affinity for ERβ. The propyl substituents in position C-3 and C-5 lead to a further reduction of affinity for both receptor subtypes.

The 2,4-diphenylfurans **77-80** carrying a long functionalised side chain were almost completely devoid of selectivity to either of the two ER subtypes. In comparison to the corresponding 2,5-diphenylfuran **44c**, the reduced affinity for ERα and the preferential binding of the 2,4-diphenylfuran scaffold to ERβ account for the loss of selectivity. These data and the data from the previous investigated 2,5-diphenylfurans show, that the main determinant of selectivity is the structural core of the ligand, whereas the functional side chain has only minor influence on selectivity. The comparison of **77** with **80** revealed that the position of the long side chain in the furan ring had no effect on affinity and selectivity of the ligands. The RBA values of both compounds were approximately 10 for both ERα and ERβ. Elimination of one of the phenolic hydroxy groups caused a decrease of binding affinity, which was more pronounced for compound **78**. This indicates that the phenyl ring in position C-2 of the compounds **77-79** might mimic the A-ring of E2.

Molecular modelling studies were performed with a modified structure of the compound **80** as described in the previous section (cf. section C2.2.1) to investigate the binding mode of the 2,4-diphenylfurans. The final model of **80** in the ligand binding pocket of ERα is depicted in figure C31 showing important interacting residues within a radius of 2.5Å of the ligand. This modelling study shows that the two phenolic hydroxy groups and the basic nitrogen in the side chain of **80** form hydrogen bonds to the conserved polar amino acids Glu353, Arg394, His524, and Asp351. Due to the single possible orientation of the long side chain outside of the binding site, the phenyl ring attached to carbon C-4 of the furan core takes over the role of the A-ring in E2. The amino acids ERα Leu384 and ERα Met421 are replaced by Met336 and Ile373 in the ligand binding pocket of ERβ. An obvious influence of these amino acids on

ligand binding, which might explain the differences in selectivity between the 2,4- and 2,5-diphenylfurans, could not be deduced from these docking results.

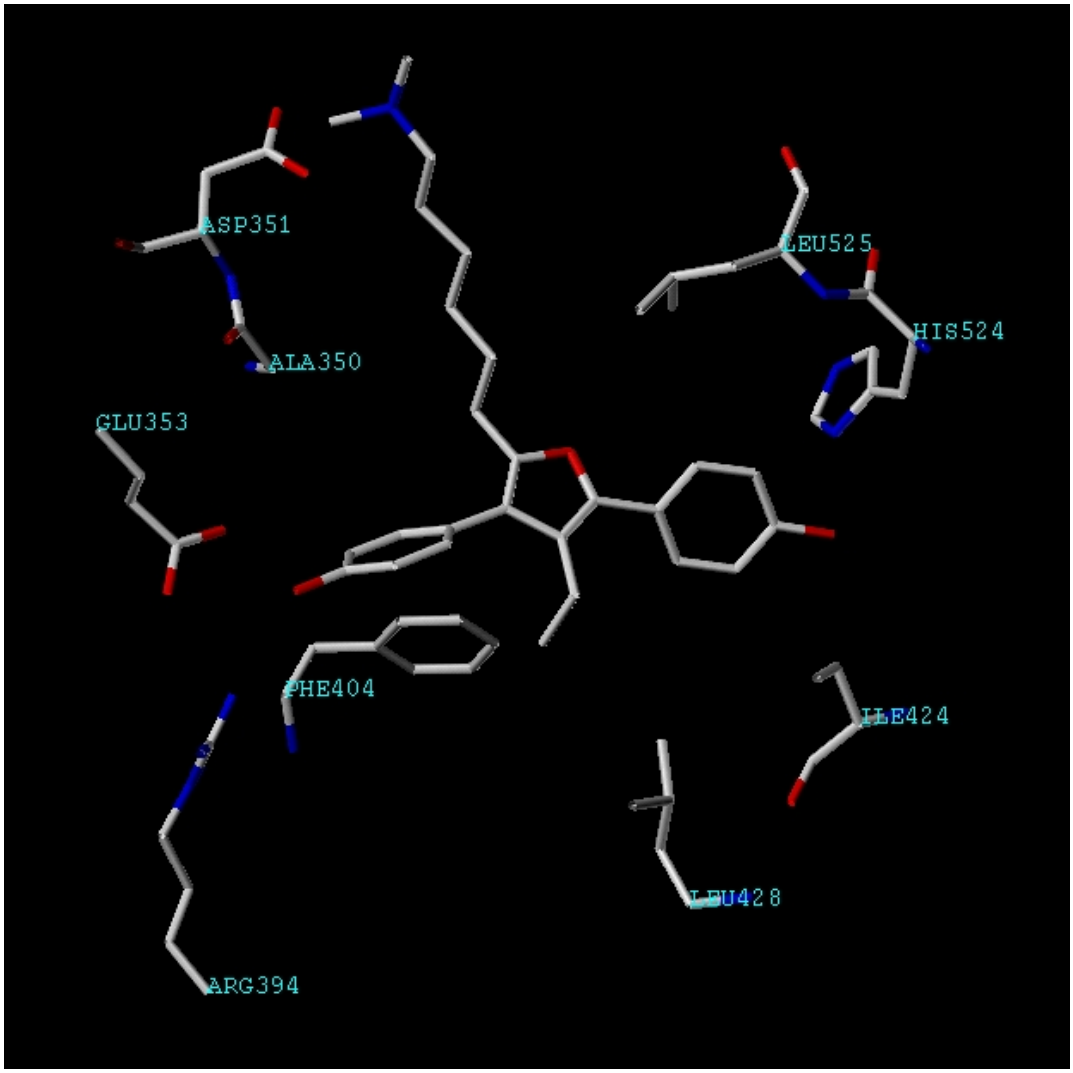


Figure C31: The modified 3,5-dialkyl-2,4-diphenylfuran **80** docked in the ERα LBD

The three diphenylfuran-based ligands **44c**, **77** and **80** used for docking are depicted in an overlay picture in figure C32, which clearly outlines the distinct orientations of the two phenyl rings and the ethyl substituent. The proposed orientation of the 2,4-diphenylfuran **77** with the side chain attached to carbon C-3 between the two phenolic rings could be confirmed through molecular modelling. The phenyl ring in position C-2 mimics the A-ring of E2 and its hydroxy group builds hydrogen bridges to Glu353 and Arg394. The distant phenyl ring is directed away from His524, what makes an hydrogen bond to the imidazole impossible. Also a hydrogen bond of the second phenolic hydroxy group to backbone atoms or to other polar residues in the ligand binding pocket like ERα Thr347, as suggested from modelling studies

with triphenylfurans by the Katzenellenbogen group [Mortensen et al., 2001], is not likely. This assumption is supported by the fact, that the hydroxy group at the 3-phenyl ring hardly contributes to the binding (cf. table C30). A ligand comparison in picture C32 suggests that there might be space for a third phenolic ring in place of the ethyl substituent, which can contact His524 via an hydrogen bond and, thus, contribute to the RBA to the ER. In this context it would be very interesting to investigate the ER binding characteristics of the tris(4-hydroxyphenyl) derivative of compound **77**.

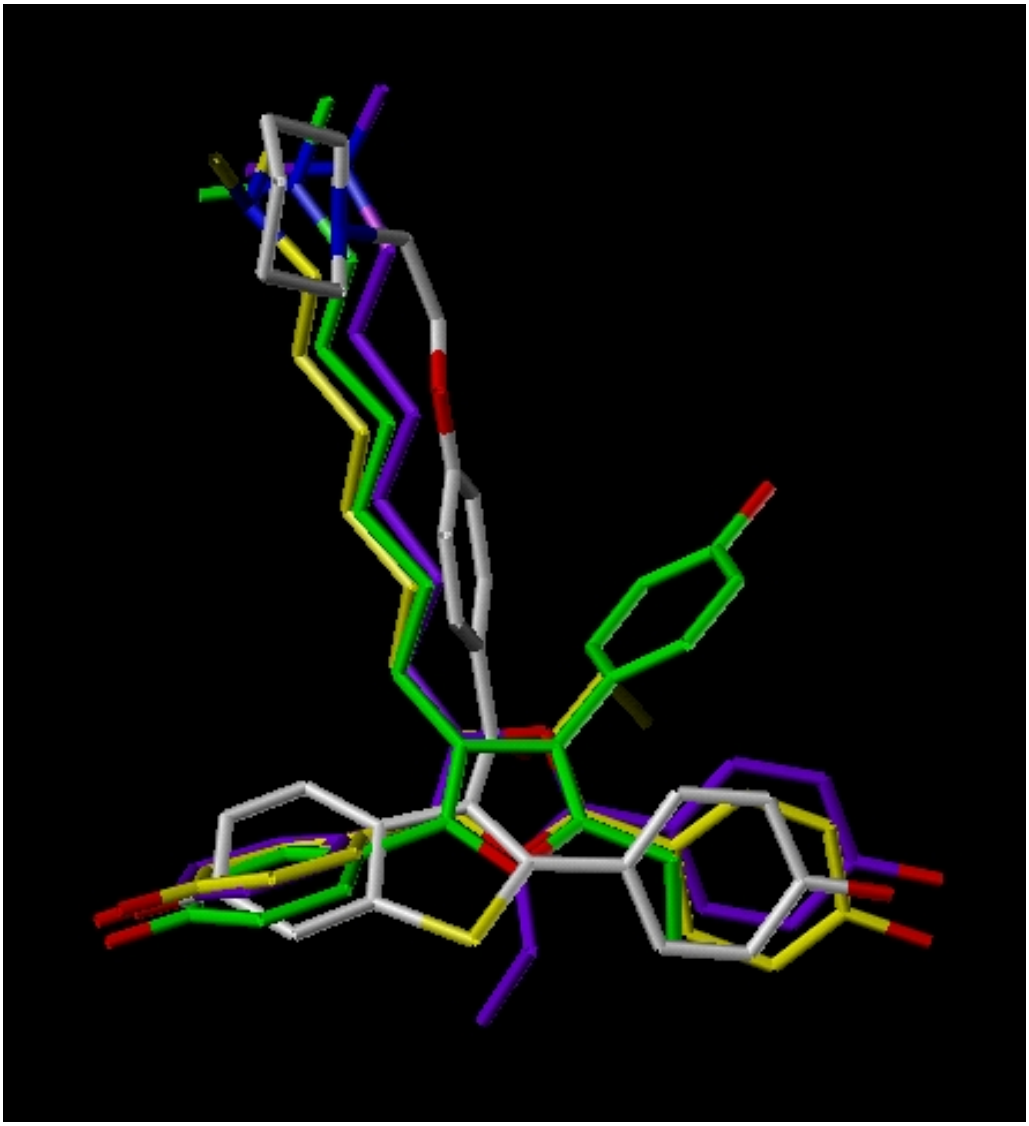


Figure C32: Overlay picture of the modified ligands based on a diphenylfuran scaffold

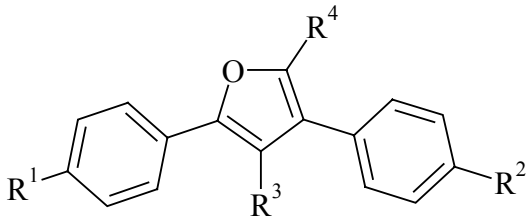
Yellow = 2,5-diphenylfuran **44c**; Green = 2,4-diphenylfuran **77**; Purple = 2,4-diphenylfuran **80**; White = raloxifene

In comparison to the diphenylfurans, raloxifene shows a slightly shifted orientation in the ligand binding pocket which results from a combination of the reduced flexibility of the ben-

zothiophene core, the different structure of the side chain and the significant smaller oxygen-oxygen-distance (11.7Å in raloxifene, 12.8-13.2Å in the diphenylfurans) of the distant phenolic hydroxy groups.

3.2.2 Determination of Antiproliferative Activity

The antiproliferative activities of the synthesised 2,4-diphenylfurans were determined in the ER-positive MCF-7 mammary carcinoma cell line. The assay was performed using estradiol as growth stimulating hormone in a nearly physiological concentration of 1nM. Except compound **76b**, all compounds lacking the functional side chain showed either no effect on cell growth or caused a further stimulation of the E2-mediated cell proliferation (data not shown). Compound **76b** demonstrated a weak inhibition of MCF-7 cell growth, which might be due to the non-specific cytoxicity of the drug, because a similar effect was observed in hormone-independent MDA-MB 231 cells.



Comp.	R^1	R^2	R^3	R^4	MCF-7 IC_{50} [µM]	MDA-MB 231 IC_{50} [µM]
76b	OH	OH	Me	Me	6.9	7.4
77	OH	OH	SC	Et	0.017	1.7
78	H	OH	SC	Et	1.8	1.9
79	OH	H	SC	Et	0.2	2.1
80	OH	OH	Et	SC	0.039	1.1
ICI					0.004	>10

Table C33: Antiproliferative effects of the 3,5-dialkyl-2,4-phenylfurans in MCF-7 and MDA-MB 231 breast cancer cells

SC = $(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$

In contrast, the compounds **77**-**80** with a functional side chain exclusively demonstrated an inhibition of the MCF-7 cell growth in dependence on the applied drug concentration. The IC_{50}-values from this assay are listed in table C33. The diphenolic compounds **77** and **80** achieved the strongest inhibitory effects with IC_{50}-values of 17nM and 39nM, respectively, which were in the same range as those of the corresponding 2,5-diphenylfuran **44c** (cf. table C18 in chapter C2.2.2). The antiproliferative activity of compound **79** lacking the second hydroxy function was reduced by one order of magnitude. Compound **78** even required a concentration in the micromolar range to reach an 50% inhibition of the E2-stimulated cell growth.

Cytotoxic effects of the 2,4-diphenylfurans were determined in the ER-negative MDA-MB 231 breast cancer cell line. The four compounds **77**-**80** inhibited the growth of these cells at concentrations higher than 1µM (cf. table C33). The IC_{50}-values for the compounds **77**, **79**, and **80** exceeded the values in the ER-positive MCF-7 cells by a factor of 10 or 100, which excludes cytotoxicity and indicates an ER-mediated drug action. Compound **78**, however, exerted equal activity in both cell lines, which makes an non-specific, cytotoxic effect on cellular growth for this derivative likely.

3.2.3 Determination of Estrogenic and Antiestrogenic Activity

All 3,5-dialkyl-2,4-diphenylfurans were tested for estrogenic and antiestrogenic activity in the luciferase assay using hormone-dependent MCF-7/2a cells that have been stably transfected with a luciferase reporter gene under the control of an ERE. The antiestrogenic activity was determined by simultaneous treatment of the cells with 1nM E2 and the respective 2,4-diphenylfuran in various concentration.

The 2,4-diphenylfurans **76a**-**d** with two short alkyl groups showed no antiestrogenic effects at a concentration of 1µM (data not shown). The compounds **77**-**80** equipped with a long side chain inhibited the E2-stimulated luciferase expression in a dose-dependent manner (cf. figure C34). The highest antiestrogenic activity was exerted by the diphenolic compounds **77** and **80**. Their almost identical IC_{50}-values of 18nM and 21nM, respectively, were comparable to that of fulvestrant or the corresponding 2,5-diphenylfuran **44c**. Both compounds **78** and **79** with only one hydroxy function antagonised the effect of estradiol on gene activation in MCF-7/2a cells, but their IC_{50}-values were higher by a factor of 10 and 50, respectively, than

that of the corresponding diphenol **77**. All IC_{50}-values in the luciferase assay (cf. table C35) were in good accordance with those from the chemosensitivity assay in MCF-7 cells.

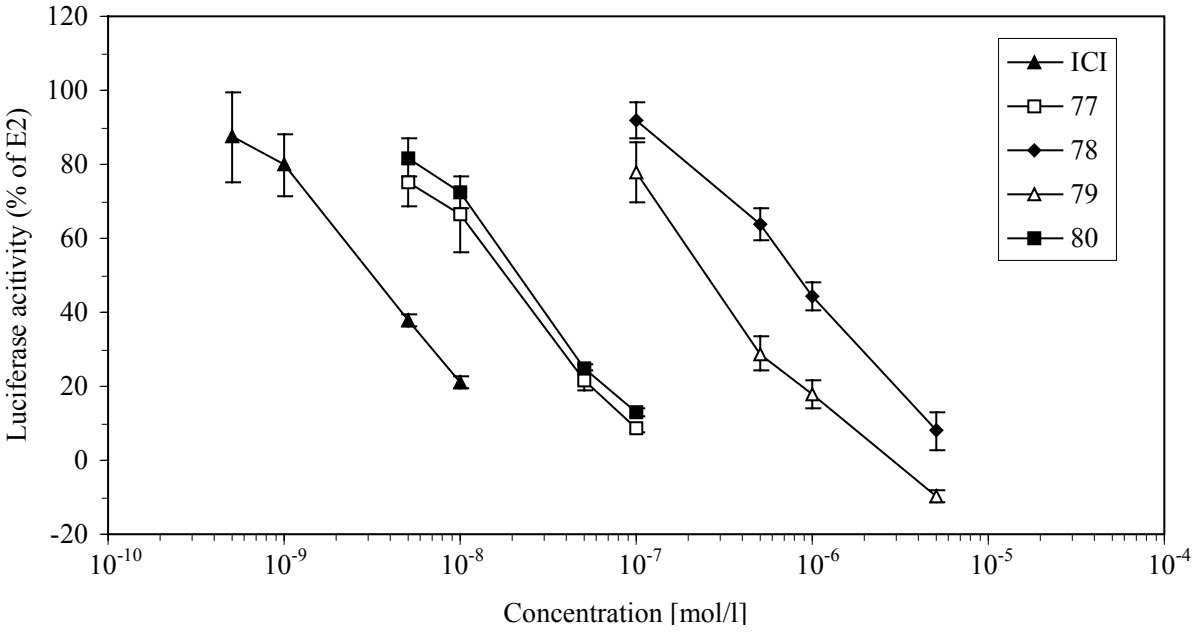
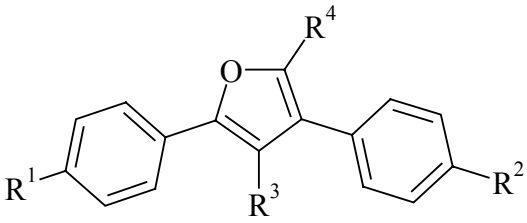


Figure C34: Antiestrogenic activity of 3,5-dialkyl-2,4-diphenylfurans with functionalised side chains in MCF-7/2a cells



Comp.	R^1	R^2	R^3	R^4	IC_{50} [µM]
77	OH	OH	SC	Et	0.018
78	H	OH	SC	Et	0.9
79	OH	H	SC	Et	0.2
80	OH	OH	Et	SC	0.021
ICI					0.004

Table C35: Antiestrogenic activity of 3,5-dialkyl-2,4-diphenylfurans with functionalised side chains in MCF-7/2a cells

The estrogenic potency of all 2,4-diphenylfurans was determined in a similar assay at a concentration of 1µM in the absence of estradiol. At this concentration the simple 3,5-dialkyl substituted 2,4-diphenylfurans **76b-d** produced full estrogenic response. The 3,5-unsubstituted compound **76a,** which bound only poorly to ERα and ERβ, stimulated luciferase expression only to a maximum of 37% at a concentration of 10µM.

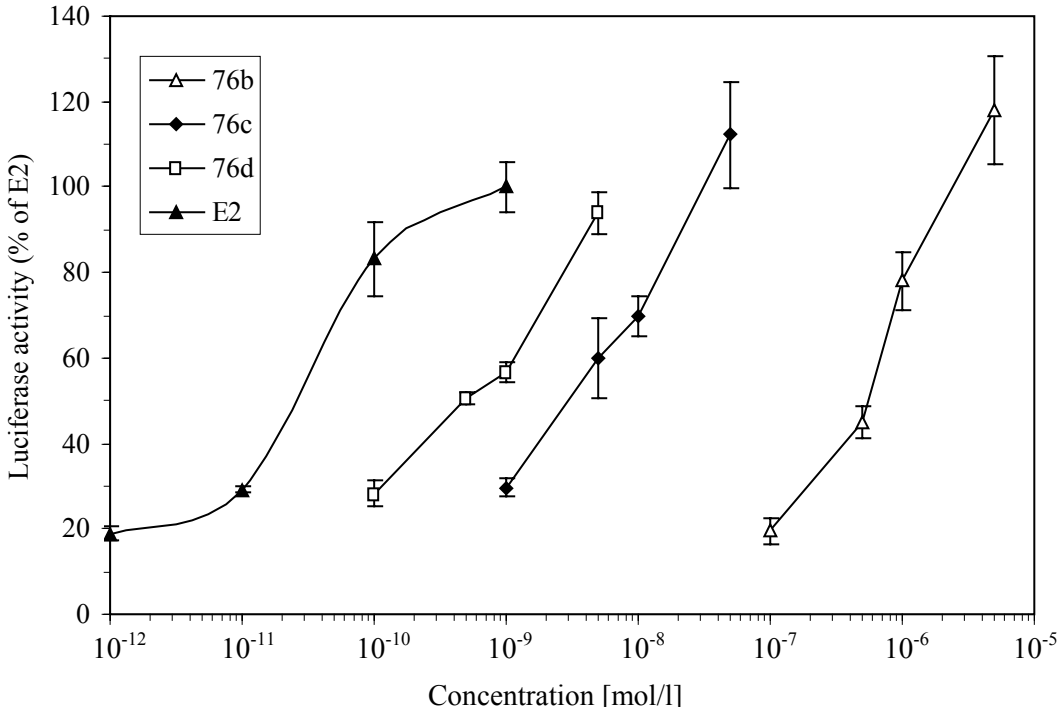
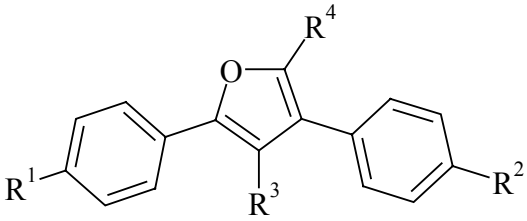


Figure C36: Estrogenic activity of the 3,5-dialkyl-2,4-diphenylfurans **76a-d**



Comp.	R^1	R^2	R^3	R^4	EC_{50} [nM]
76a	OH	OH	H	H	–
76b	OH	OH	Me	Me	560
76c	OH	OH	Et	Et	2.9
76d	OH	OH	Pr	Pr	0.5
Estradiol					0.02

Table C37: Estrogenic activity of the 3,5-dialkyl-2,4-diphenylfurans **76a-d** in MCF-7/2a cells

For an exact evaluation of the estrogenic activity of the compounds **76b-d** dose-response curves (cf. figure C36) were measured and the EC_{50}-values calculated (cf. table C37). Although the binding affinity to the ER was relatively weak compared to the derivatives **76b** and **76c**, compound **76d** produced an EC_{50}-value in the subnanomolar range, only one order of magnitude higher than that of estradiol. The EC_{50}-value of **76c** was by the factor of 6 higher than that of **76d**. All three derivatives reach a maximum stimulation of luciferase expression and can therefore be characterised as full agonist.

The 2,4-diphenylfurans **77-80** with functionalised long side chains showed no agonistic activity at a concentration of 1µM. All values were below that of the control cells (cf. figure C38). Luciferase activity below baseline levels is characteristic for potent antiestrogens and indicates the blockade of AF-1 mediated ligand-independent activation of the ER, that is responsible for the basal luciferase activity in the control cells.

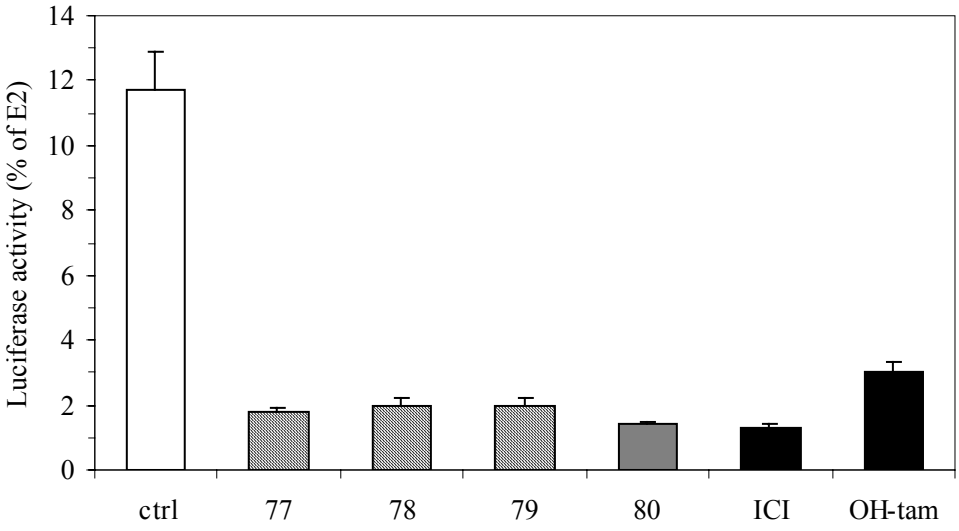


Figure C38: Suppression of basal luciferase activity by 3,5-dialkyl-2,4-diphenylfurans with functionalised side chains in MCF-7/2a cells

The value for estradiol at a concentration of 1nM was set to 100%.
ctrl = control

For a better estimation of the residual estrogenic activity of these agents the levels of luciferase expression were compared with those of the partial antiestrogen 4-hydroxytamoxifen and the pure antiestrogen fulvestrant. Compound **80** with the bifunctional side chain in position 5 of the furan ring can be characterised as pure antiestrogen, because it suppressed luciferase expression to the same extent as fulvestrant did. The compounds **77-79** with the side chain in position C-3 were also devoid of agonist activity though their suppres-

sion was somewhat weaker, but still better than that of the partial antiestrogen 4-hydroxy-tamoxifen.

3.3 Conclusion

It was possible to prepare 2,4-diphenylfuran-based antiestrogens bearing the bifunctional 6-[N-methyl-N-(3-pentylsulfanyl-propyl)amino]hexyl side chain at position C-3 and C-5, respectively, in the aromatic furan ring. The biological evaluation of these compounds demonstrated, that the positions of substituents in the furan ring strongly influence the affinity and selectivity for the ER. In comparison to the 2,5-diphenylfurans, the isomeric 2,4-diphenylfurans have completely lost their selectivity for ERα. However, the antiproliferative and antiestrogenic potency of the 2,5-diphenylfuran was retained. For receptor binding only the phenyl ring at C-2 proved to be important, because it mimics the 3-hydroxy group in E2. Investigations by molecular modelling have shown that 2,4-diphenylfurans with the long side chain in position 3 adopt an orientation in the binding pocket in which a hydrogen bridge between His524 and a phenolic hydroxy group is no longer possible and other polar interactions are excluded. This binding is supported by experimental data. All of the derivatives with functionalised side chains were characterised as pure antiestrogenes. The 3,5-dipropyl-2,4-diphenylfuran **76d** was found to be a potent non-steroidal estrogen with an activity one order of magnitude lower than that of estradiol in the luciferase assay.

4 Benzo[b]furans and Benzo[b]thiophenes

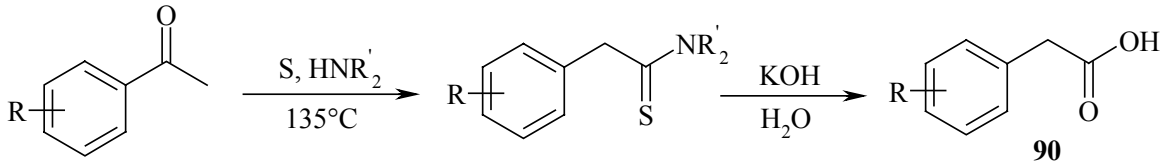
The furan ring as core is also found in the 2-phenylbenzo[b]furan structure, which can be derived from the 2,4-diphenyl- or 2,5-diphenylfurans by fusing one of the phenyl rings with the furan heterocycle. The 2-phenylbenzo[b]furan system as scaffold for ER ligands leaves only one position in the heterocycle free to be equipped with a side chains. 5- and 6-Hydroxy-2-(4-hydroxyphenyl)benzo[b]furans with different types of substituents in position C-3 have been investigated as ER ligands in our research group previously [Erber, 1991; Leichtl, 1994]. However, some functionalised side chains that have recently been developed (cf. chapter C2.1.1) have not yet been introduced into the 6-hydroxybenzo[b]furan system. The lack of knowledge of a second functional ER subtype prevented studies on the selectivity of 2-phenylbenzofuran-based ligands at that time. Therefore, 6-hydroxy-2-(4-hydroxyphenyl-benzo[b]furans substituted with aliphatic side chains containing functional groups were synthesised and characterised for their biological activity. Together with them, 5-hydroxy derivatives were re-evaluated in terms of affinity to ERβ and selectivity for one of the ER subtypes.
In this context, some recently synthesised 3-alkyl 2-phenylbenzo[b]thiophenes with hydroxy groups in position C-5 and C-6 were also investigated for their ERβ selectivity and their estrogenic activity in the luciferase assay. In order to complete the series of benzo[b]thiophenes a new synthesis for 5-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene was developed.

4.1 Synthesis

4.1.1 Synthesis of 6-Hydroxy-2-(4-hydroxyphenyl)benzo[b]furans

The synthesis of 6-hydroxy-2-(4-hydroxyphenyl)benzo[b]furans was based on the synthetic route developed by Dr. S. Erber [1989]. This synthesis involved the preparation of 2,4-dimethoxyphenylacetic acid from 2,4-dimethoxyacetophenone via the Willgerodt-Kindler reaction and subsequent Friedel-Crafts acylation with anisol to a trimethoxy-substituted diaryl ethanone.
The Willgerodt-Kindler reaction comprises the reduction of the carbonyl function in acetophenones to a methylene group and the oxidation of a methyl group to a carboxyl function. The reaction is not limited to acetophenones but is also applicable to alkyl aryl ketones with

longer alkyl chains. The major product has the same number of carbon atoms as the starting ketone and the carbonyl function has moved to the terminal methyl group. Thioamides are common intermediates in the Kindler modification of the Willgerodt reaction, which are usually not isolated and hydrolysed to the desired arylacetic acid under strongly alkaline conditions (cf. figure C39). Due to the large number of substrates a unique mechanism can not be assigned to this reaction [Brown, 1975].



Scheme C39: The Willgerodt-Kindler reaction

The Willegrodt-Kindler reaction for the preparation of 2,4-dimethoxyphenylacetic acid **90** using morpholine as secondary amine followed by hydrolysis of the thioamide with aqueous potassium hydroxide afforded the product only in low yield (< 20%). Furthermore, the purified product usually contained sulfur impurities up to 5% and the undesired by-product **91** (cf. figure C40) was isolated in substantial quantities. The structure of the 3,4-diarylthiophene derivatives **91** was concluded from combined ^{1}H and ^{13}C-NMR analysis.

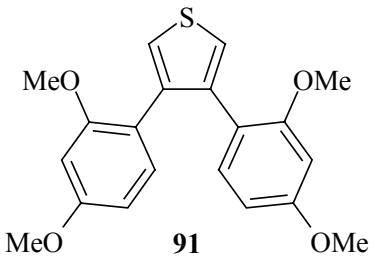
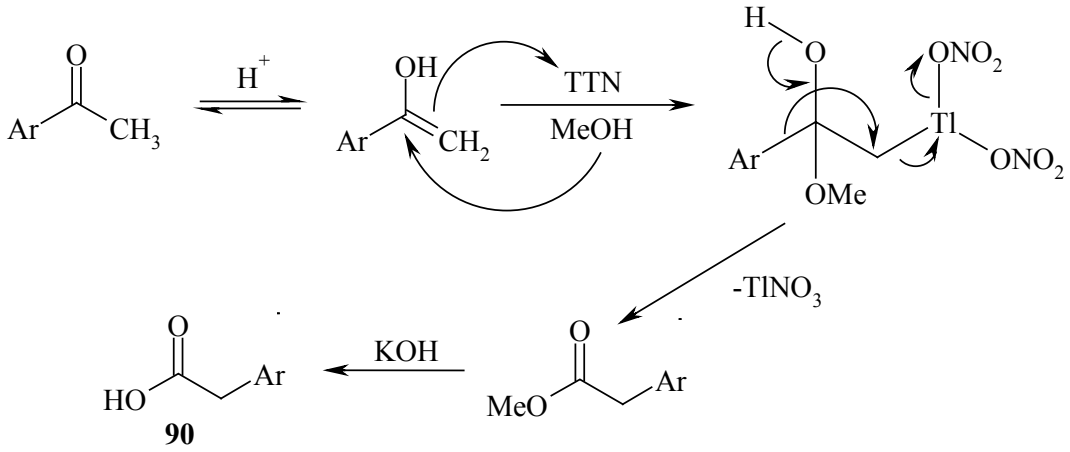


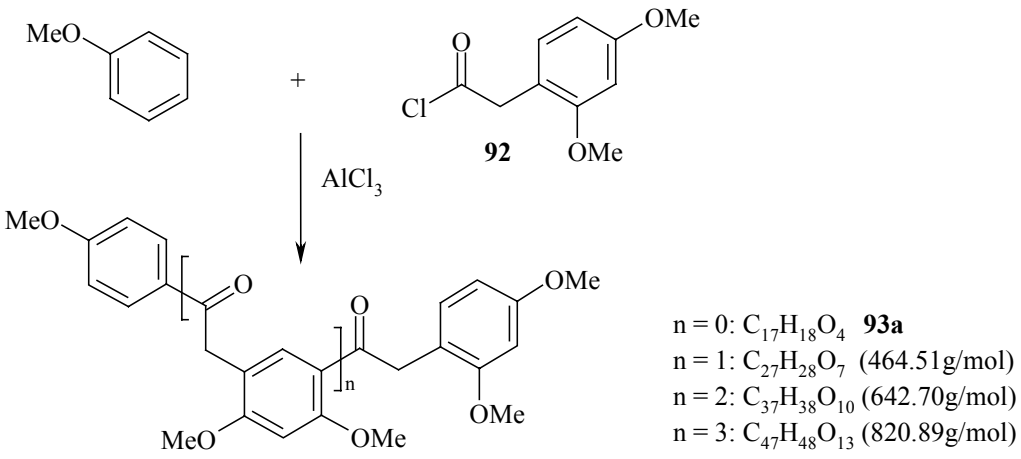
Figure C40: Structure of 3,4-bis(2,4-dimethoxyphenyl)thiophene

For these reasons the Willgerodt-Kindler reaction was not refined, but replaced by a direct oxidation of the 2,4-dimethoxyacetophenone with thallium(III) nitrate (TTN) using methanol and perchloric acid [Alesso et al., 1992]. A slightly modified variant of this procedure gave compound **90** in an overall yield of 75% after hydrolysis of the intermediate methyl 2,4-dimethoxyphenylacetate. The mechanism of this transformation was shown with ^{14}C-labelling experiments to proceed via an 1,2-aryl migration [McKillop et al., 1971]. Acid-catalysed eno-

lisation followed by oxythallation leads to an unstable alkylthallium dinitrate. Decomposition of this intermediate proceeds via migration of the aryl substituent, resulting in formation of the methyl arylacetate and simultaneous reduction of thallium(III) to thallium(I) (cf. scheme C41). Progress and end of the reaction are indicated by the precipitation of white thallium(I) nitrate.



Scheme C41: Mechanism of the thallium(III) nitrate oxidation

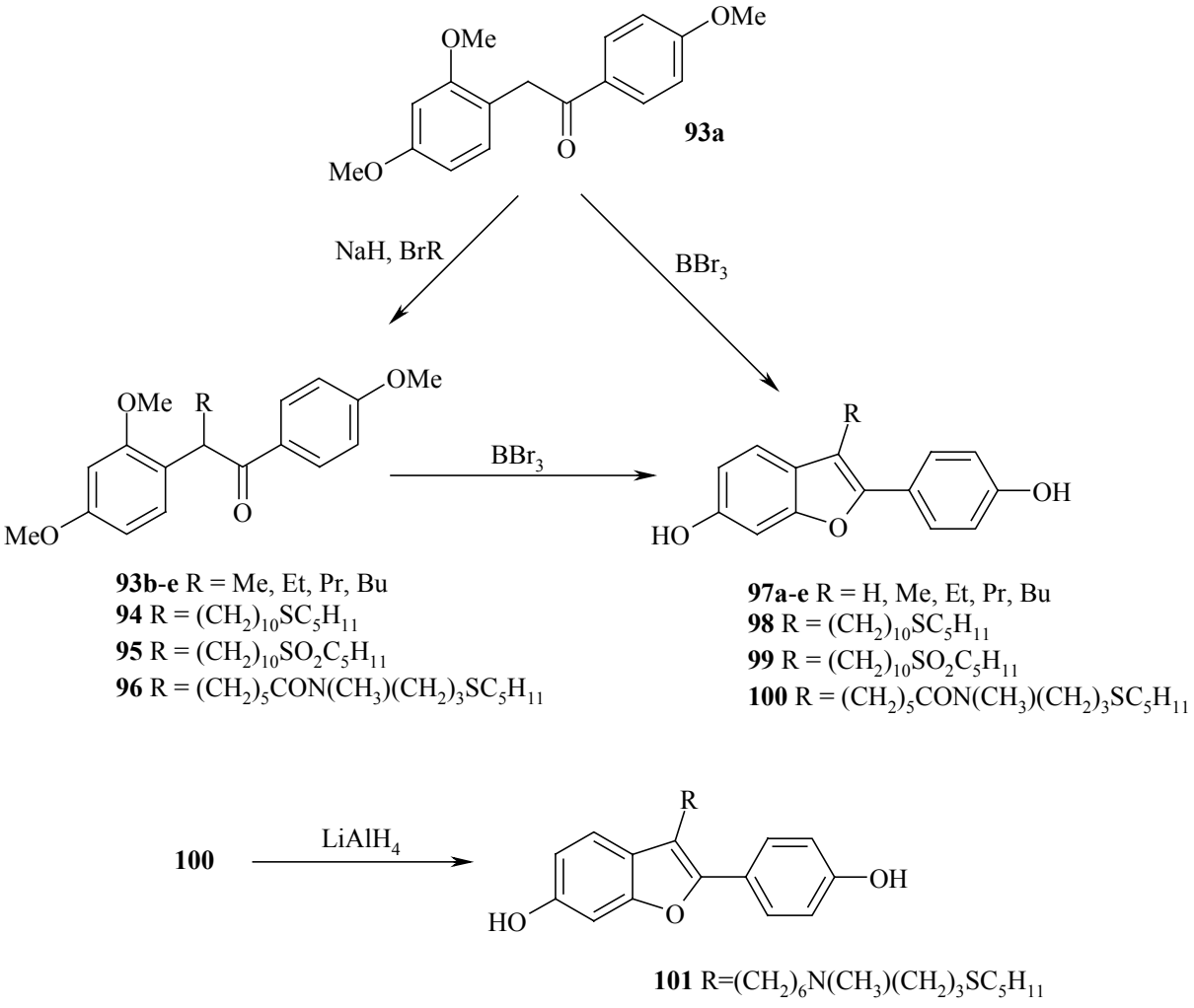


Scheme C42: Polymeric by-products of the Friedel-Crafts acylation

The resulting 2,4-dimethoxyphenylacetic acid **90** was converted into the acid chloride **92**, which was used in a Friedel-Crafts acylation reaction with anisole and $AlCl_3$ to prepare the 1,2-diarylethanone **93a**. Anisole was used as solvent to guarantee the reaction of **92** with the substrate instead of with itself or the reaction product. The latter are more activated than anisole in C-5 position of the aromatic ring because of the two methoxy groups in *ortho*- and *para*-position. In fact, the Friedel-Crafts reaction using only two equivalents of anisole in di-

chloroethane resulted in the formation of polymeric by-products containing two, three, and four monomers of **92** (cf. figure C42).

Compound **93a** was the key intermediate for the preparation of 3-substituted 2-phenyl-benzo[b]furans. Small alkyl groups or long aliphatic side chains with various functions were introduced by alkylation of the enolate of **93a** with the appropriate alkyl bromides, including the side chains **10**, **11**, and **19**. The resulting 1-alkyl-1,2-diarylethanones **93-96** were subjected to ether cleavage with an excess of boron tribromide, upon which the cyclisation to the benzo-furans **97-100** occurred. Obviously, BBr_3 reacted not only with the methoxy groups of the molecule but also catalysed, as a Lewis acid, the nucleophilic attack of the hydroxy group in the *ortho*-position at the carbonyl function. The subsequent elimination of water was driven by the formation of the heteroaromatic system. Benzofuran **101** was generated by reduction of the amide function in compound **100** with $LiAlH_4$ (cf. scheme C43). The free phenolic benzo[b]furan derivatives are sensitive towards light and/or air.



Scheme C43: Synthesis of 3-substituted 6-hydroxy-2-(4-hydroxyphenyl)benzo[b]furans

4.1.2 A New Synthesis of 5-Hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene

A previously used route to 5-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophenes was restricted to derivatives with short alkyl groups in position C-3 and was not very efficient [Erber, 1989]. Thus, a new approach to this system was investigated.

The synthesis of 5-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene started from commercially available 4-bromobenzenethiol, which was deprotonated with potassium carbonate and reacted in a S_N2 substitution with bromoacetaldehyde diethyl acetal to compound **102** (cf scheme C45) [Graham et al., 1989]. A by-product of this reaction was bis(4-bromophenyl)-disulfane **103** (cf. figure C44), that resulted from an oxidative coupling of two 4-bromobenzenethiol molecules. As oxidising agent could have served the oxygen in the air.

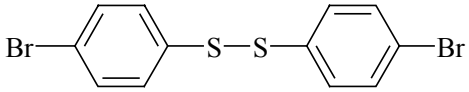
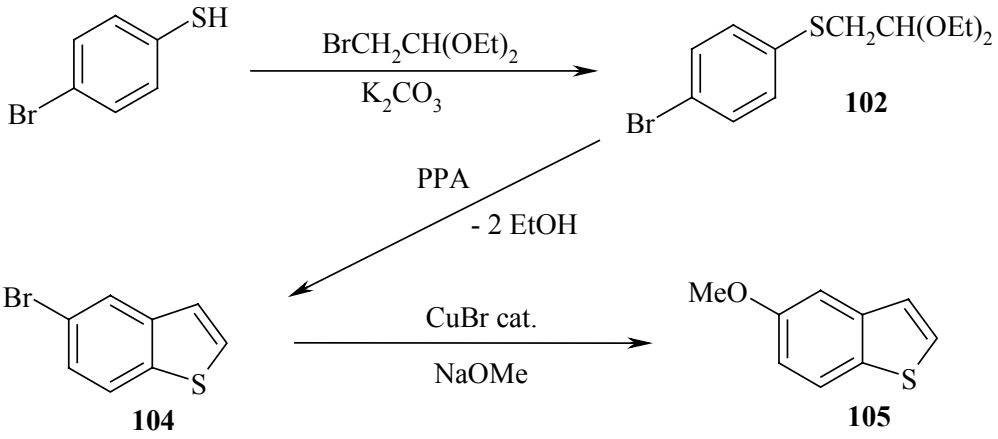


Figure C44: Bis(4-bromophenyl)disulfane **103**

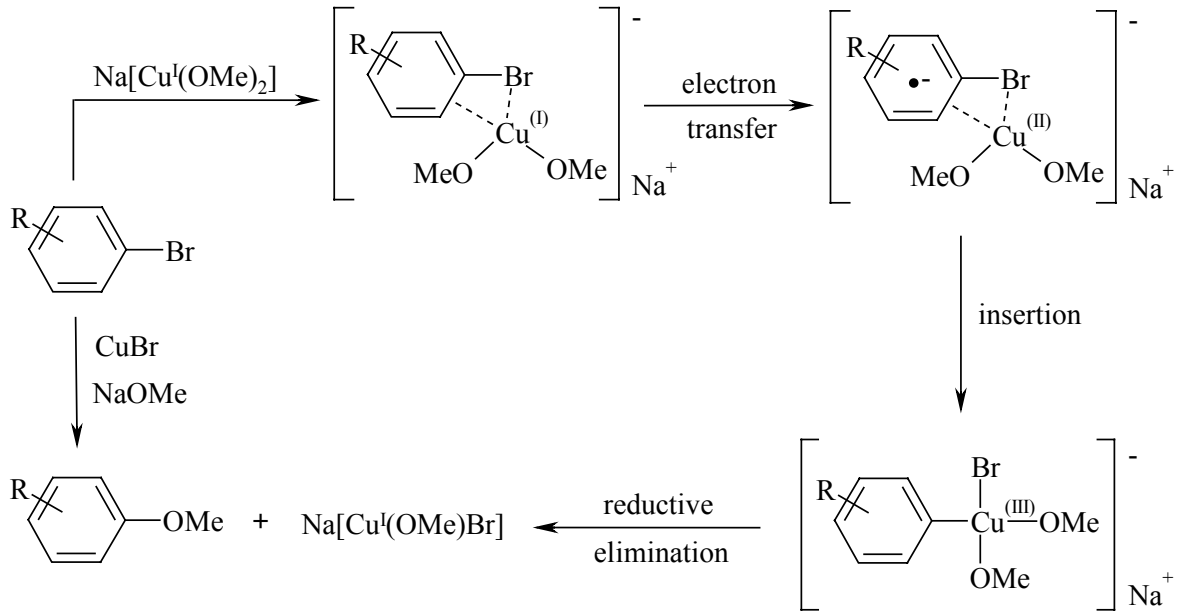
Compound **102** was cyclised to 5-bromobenzo[b]thiophene **104** with polyphosphoric acid in refluxing chlorobenzene [Plé et al., 1988]. This cyclisation involves the nucleophilic attack of the aromatic ring at the acetal carbon and the generation of the heterocyclic system through elimination of a two molecules of ethanol.



Scheme C45: Synthesis of 5-methoxybenzo[b]thiophene **105**

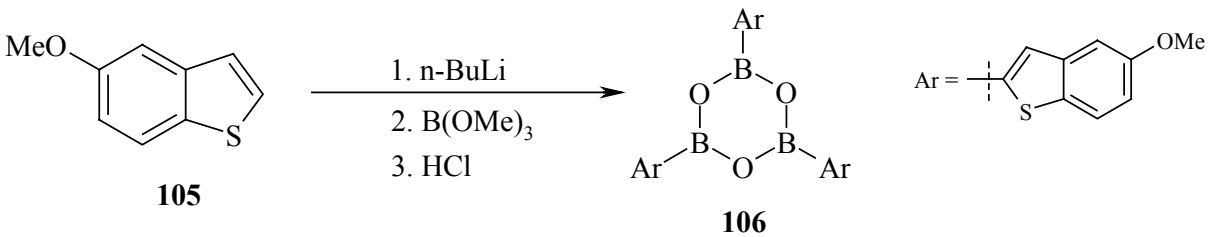
The following nucleophilic aromatic substitution of the bromide with sodium methoxide gave the aryl methyl ether **105** (cf. scheme C45). Aalten and co-workers [1989] thoroughly studied

a number of parameters that influence this copper-catalysed *ipso*-substitution of unactivated aromatic substrates (substrates without strong electron withdrawing substituents). They found, that sodium methoxide is required in high concentration for a fast reaction and cuprate-like intermediates such as $Na^+[Cu(OMe)_2]^-$ are the reactive catalysts. On the basis of their results they proposed that the reaction proceeds rather via an intimate electron transfer mechanism than a free radical (cf. scheme C46).



Scheme C46: Mechanism of the copper-catalysed S_NAr substitution

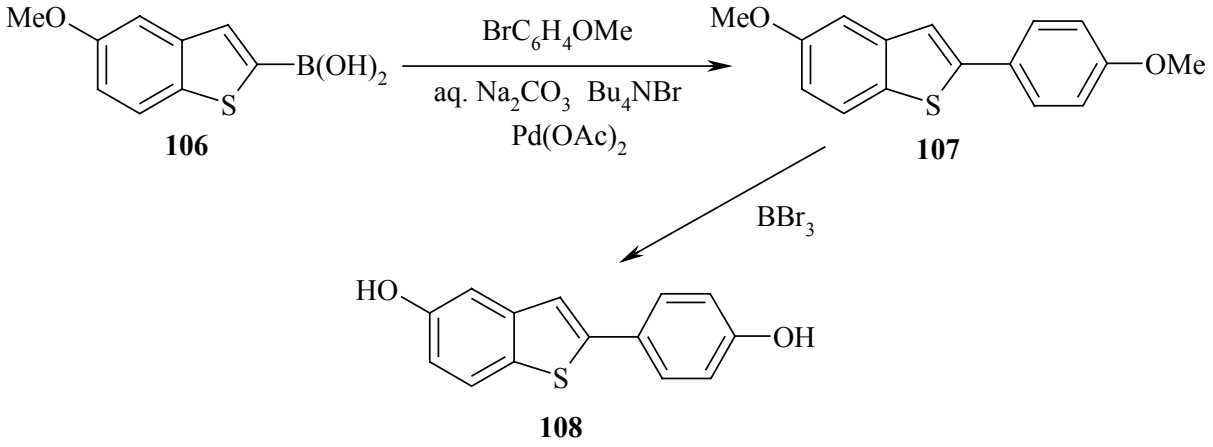
Compound **105** was converted into the corresponding 5-methoxybenzo[b]thiophene 2-boronic acid **106** as reagent for the following Suzuki coupling. The boronic acids was prepared in three steps: Lithiation of **105** with n-BuLi at –78°C, followed by reaction with trimethyl borate and hydrolysis of the resulting boronic ester under acidic condition. The boronic acid was isolated in good yield as the cyclic boronic acid anhydride (cf. scheme C47), which formed upon dehydration of the acid .



105 **106**

Scheme C47: Synthesis of the 5-methoxybenzo[b]thiophene 2-boronic anhydride **106**

The boronic acid anhydride **106** was used as such in the Suzuki coupling reaction to prepare 5-methoxy-2-(4-methoxyphenyl)benzo[b]thiophene **107**. The reaction was performed according to a recently described procedure [Leadbeater and Marco, 2003] within 5 minutes at 150°C using water as solvent and palladium acetate as catalyst. The formation of a black undissolvable residue indicates a reduction of Pd^{2+} (probably by water) to the active species Pd^0. Tetrabutylammonium bromide acts as a phase transfer catalyst and is thought to enhance the rate of the coupling reaction by activating the boronic acid as $ArB(OH)_3^-NR_4^+$ [Leadbeater and Marco, 2003]. The synthesis of 5-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene **108** was completed by deprotection of the phenolic hydroxy groups with boron tribromide (cf. scheme C48).



Scheme C48: Suzuki coupling and final deprotection

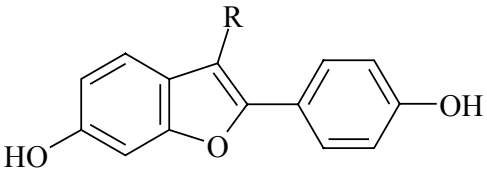
4.2 Biological Characterisation

4.2.1 Biological Characterisation of the Benzo[b]furans

4.2.1.1 Determination of Affinity and Selectivity for the ER

The 5- and 6-hydroxy-2-(4-hydroxyphenyl)benzo[b]furans were tested for their binding affinities to the ER and their selectivity for one of the two ER subtypes. The binding curves of all test compounds were comparable in shape to the binding curve of E2, but shifted to lower or higher concentrations, which indicates a competitive displacement of the physiological ligand from the receptor binding site. The RBA values were determined by two methods using either calf uterus cytosol as receptor source or the recombinant human ERα and ERβ. The

RBA values shown are the means of two or three independent experiments and are listed in table C49 and C50.



Comp.	R	RBA (cytosol)	RBA (ERα)	RBA (ERβ)	Binding ratio β/α
97a	H	0.13	2.6	46	18
97b	Me	0.19	1.0	5.7	5.7
97c	Et	1.0	2.7	8.3	3.1
97d	Pr	2.9	13	26	2.0
97e	Bu	1.5	3.6	11	3.1
98	$(CH_2)_{10}SC_5H_{11}$	0.09	0.26	0.62	2.4
99	$(CH_2)_{10}SO_2C_5H_{11}$	1.4	2.9	4.2	1.4
100	$(CH_2)_5CON(CH_3)(CH_2)_3SC_5H_{11}$	1.4	5.2	7.9	1.5
101	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	3.9	30	19	0.6
A1[*]	$(CH_2)_6NC_4H_8$	16	40	20	0.5

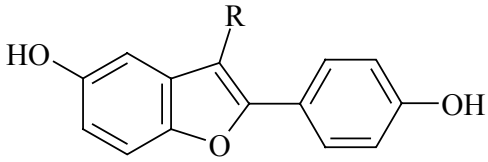
Table C49: Relative binding affinities of the 6-hydroxy-2-(4-hydroxyphenyl)benzo[b]furans

[*] Synthesised by Dr. S. Erber, 1989

All benzofuran derivatives with the hydroxy group in position C-6 displayed a selectivity for ERβ, except compound **101** and **A1**. The highest selectivity demonstrated the 3-H benzofuran **97a** being 18-fold for ERβ. In the series of benzofurans substituted with short alkyl groups **97a-e** the selectivity decreased with increasing chain length. In this respect the butyl substituted compound **97e** marked an exception, because the decrease of relative binding affinity from propyl to butyl was more pronounced for ERα. In this series the maximum binding to ERα (RBA-value: 13) was produced by the propyl derivatives **97d**, whereas the 3-H benzofuran **97a** bound strongest to ERβ (RBA-value: 46).

In the series of 6-hydroxybenzofurans with functionalised side chains the selectivities for ERβ were less pronounced. As mentioned above, compound **101** and **A1** showed a preference for ERα. The common feature of the latter is the basic nitrogen in position 7 in the aliphatic side

chain, which is known to a form hydrogen bridge to an aspartate in both receptor subtypes. This additional hydrogen bridge probably accounts for the relatively high RBA-values of these two compounds. The compound **99** with the sulfone side chain was superior over compound **98** with the sulfanyl group in the side chain and compound **100** having an amide bond in the side chain bound less strongly to both ERs as the corresponding amine **101**. These observations were made before with antiestrogens based on the stilbene- and 2-phenylindole structure [Golob et al., 1999; Walter et al., 2002].



Comp.	R	RBA (ERα)	RBA (ERβ)	Binding ratio β/α
B1[a]	$(CH_2)_6NC_4H_8$	32	53	1.7
B2[b]	$(CH_2)_{10}SO_2C_5H_{11}$	4.3	8.8	2.0
B3a[a]	H	5.2	109	21
B3b[a]	Me	15	93	6.2
B3c[a]	Et	51	85	1.7
B3d[a]	Pr	63	117	1.9
B3e[a]	Bu	5.2	61	12

Table C50: Relative binding affinities of the 5-hydroxy-2-(4-hydroxyphenyl)benzo[b]furans

[a] Synthesised by Dr. S. Erber, 1989
[b] Synthesised by Dr. S. Leichtl, 1994

The 5-hydroxybenzofuran derivatives were exclusively selective for ERβ, even compound **B1** with the basic nitrogen in the side chain. The order of selectivity was almost identical to the corresponding 6-hydroxybenzofurans, only the butyl derivative **B3e** showed a higher binding ratio. There was a marked difference between the compounds with the hydroxy group in position C-6 and the 5-hydroxy analogues, especially between the compounds with small alkyl groups. The RBA values of the 5-hydroxy derivatives were by one order of magnitude higher than those of their isomers. Thus, the ligand binding pocket of the ER provides a better fit for the 5-hydroxybenzofurans, in which the hydroxy group and the alkyl substitutent are located at the same side of the ligand. The unsubstituted 6-hydroxy derivative **97a** marks the only

exception in the series of 6-hydroxybenzofurans. The lack of an alkyl group in position C-3 makes it possible to flip around the longitudinal axis of the molecule and to adopt the same orientation in the binding site as the 5-hydroxybenzofurans without steric hindrance. This behaviour provides a rational explanation for the binding affinities of **97a**, that differ only by a factor of 2 from that of the corresponding 5-hydroxy analogue. The difference in electron density between the oxygen and the sp^2-carbon atom is reflected by the factor of 2.

Molecular modelling studies were performed with the 5- and 6-hydroxy-2-(4-hydroxy-phenyl)benzo[b]furans derivatives **A1** and **B1** with a pyrrolidinohexyl side chain and the corresponding indole ZK119.010. These three structures were docked into the hERα-LBD (1ERR) as described in the previous section (cf. section C2.2.1). The lowest-energy conformation of each ligand-receptor-complex and the raloxifene crystal structure were superpositioned using the Cα-atoms of the amino acid residues within a sphere of 4Å radius around the ligands. The resulting alignment is depicted in figure C51 showing the amino acid residues of only one structure within a radius of 2.5Å of the ligand. The residues of the three other protein structures were omitted for clarity reasons.

The general binding mode of the two benzofuran structures and the 2-phenylindole is identical to that of raloxifene. The phenyl ring of the bicycle mimics the A-ring of E2 and the phenolic hydroxy group at this bicycle forms hydrogen bonds with the polar amino acids Glu353 and Arg394. The second hydroxy function at the phenyl ring at C-2 can also form a hydrogen bridge to imidazole nitrogen of His524, which is turned into the ligand binding cavity and points towards the hydroxy group of the ligand. The basic nitrogen in each side chain is located within hydrogen bonding distance of the carboxy group of Asp351. A hydrogen bond Asp351 provides an rational explanation for the higher binding affinity of these four compounds to the ER compared to other ligands with aliphatic side chains, that lack the nitrogen function.

The bicyclic cores of the two benzofurans and the indole are rotated out of the plane that is represented by the benzothiophene structure. This rotation is probably caused by the different lengths of the side chains. In order to allow the formation of an hydrogen bridge between the N-atom and Asp351, the somewhat shorter pyrrolidinohexyl side chain needs to be directed further into the direction of this amino acid. This can be only achieved by rotation of the ligand around its longitudinal axis, when hydrogen bonds to Glu353, Arg394 and His524 keep the core of the ligand in a fixed position. The 5-hydroxy-substituted benzofuran showed

the strongest rotation out of this plane, because the hydrogen bonding contacts to glutamate and arginine forces the ligand deeper into the binding pocket.

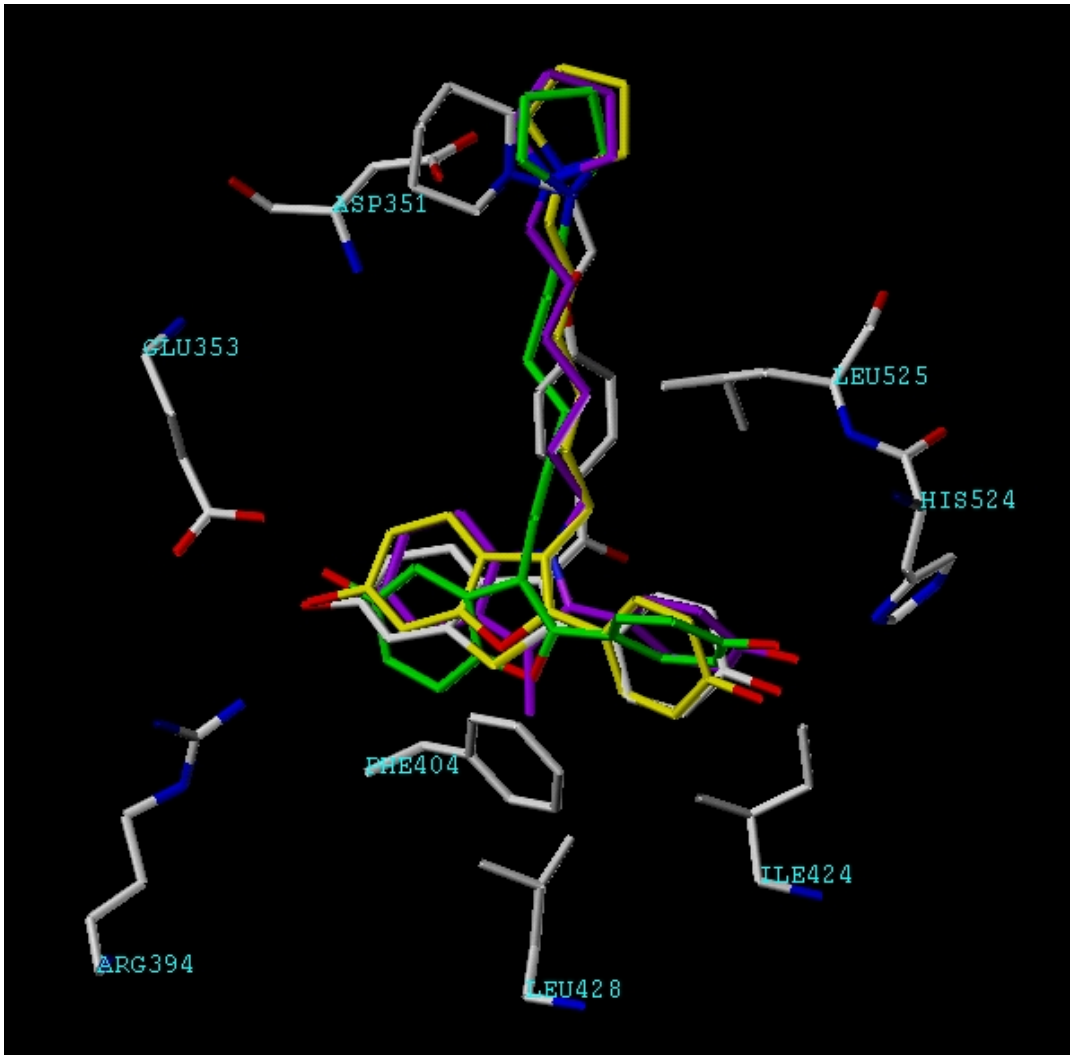


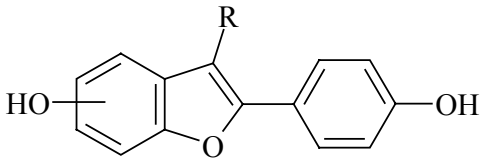
Figure C51: Comparison of four structurally similar antiestrogens in the ERα-LBD

Yellow = 6-hydroxy-2-(4-hydroxyphenyl)-3-pyrrolidinohexylbenzo[b]furan **A1**;
Green = 5-hydroxy-2-(4-hydroxyphenyl)-3-pyrrolidinohexylbenzo[b]furan **B1**;
Purple = ZK119.010; White = raloxifene

4.2.1.2 Determination of Antiproliferative Activity

The antiproliferative activity of the 6-hydroxybenzofuran derivatives was determined in the same assay as described above using the ER-positive MCF-7 mammary carcinoma cell line. In parallel, the compounds were tested in hormone-independent human MDA-MB 231 mammary tumour cells in order to find out whether the effects on cell growth are mediated by the ER or are due to a general cytostatic activity of the test compounds. The previously described 5-hydroxybenzofurans **B1** and **B2** bearing long side chains were re-evaluated for comparison.

The simple alkyl derivatives of the 6-hydroxybenzofurans **97a-e** showed no inhibitory effects on cellular growth in both cell lines up a concentration of 10µM (data not shown). The compounds substituted with functionalised side chains demonstrated antiproliferative effects in MCF-7 cells. The activities of the 6-hydroxylated compounds **A1** and **99** were almost similar to the 5-hydroxy analogues **B1** and **B2** with corresponding side chains. The compounds with a basic nitrogen in the side chain, that possess the highest binding affinities, inhibited the growth of the MCF-7 cells in submicromolar concentrations. The lowest IC_{50}-value of 0.1µM was obtained for compound **101** substituted with the bifunctional side chain. The IC_{50}-values of the compounds **98**-**100** and **B2** were higher by one order of magnitude (cf. table C52).



Comp.	R	OH	MCF-7 IC_{50} [µM]	MDA-MB 231 IC_{50} [µM]
98	$(CH_2)_{10}SC_5H_{11}$	6	3.5	7.6
99	$(CH_2)_{10}SO_2C_5H_{11}$	6	0.7	>10
100	$(CH_2)_5CON(CH_3)(CH_2)_3SC_5H_{11}$	6	3.3	8.1
101	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	6	0.1	1.9
A1[a]	$(CH_2)_6NC_4H_8$	6	0.3	5.9
B1[a]	$(CH_2)_6NC_4H_8$	5	0.2	2.4
B2[b]	$(CH_2)_{10}SO_2C_5H_{11}$	5	1.2	>10

Table C52: Antiproliferative effects of 2-phenylbenzo[b]furans with functionalised side chains in MCF-7 and MDA-MB 231 breast cancer cells

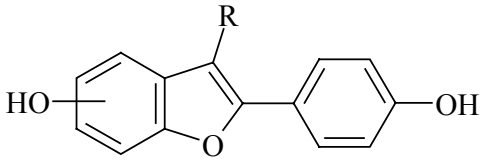
[a] Synthesised by Dr. S. Erber, 1989
[b] Synthesised by Dr. S. Leichtl, 1994

The inhibitory effect of the compounds with side chains containing either a basic nitrogen or a sulfonyl group was by a factor of 10 lower in the hormone-independent MDA-MB 231 cells than that in MCF-7 cells. The antiproliferative action of these drugs is mediated by the ER. In contrast, the IC_{50}-values of **98** and **100** were only by a factor of 2 higher in the ER-negative

cell line, so that non-specific effects of these derivative must be taken into consideration (cf. table C52).

4.2.1.3 Determination of Estrogenic and Antiestrogenic Activity

The 2-phenylbenzofuran derivatives were tested for their estrogenic and antiestrogenic activity in the luciferase assay using hormone-dependent MCF-7/2a cells as described above. The antiestrogenic activity was determined by simultaneous treatment of the cells with 1nM E2 and the respective benzofuran in various concentrations. The IC_{50}-values are summarised in table C53.



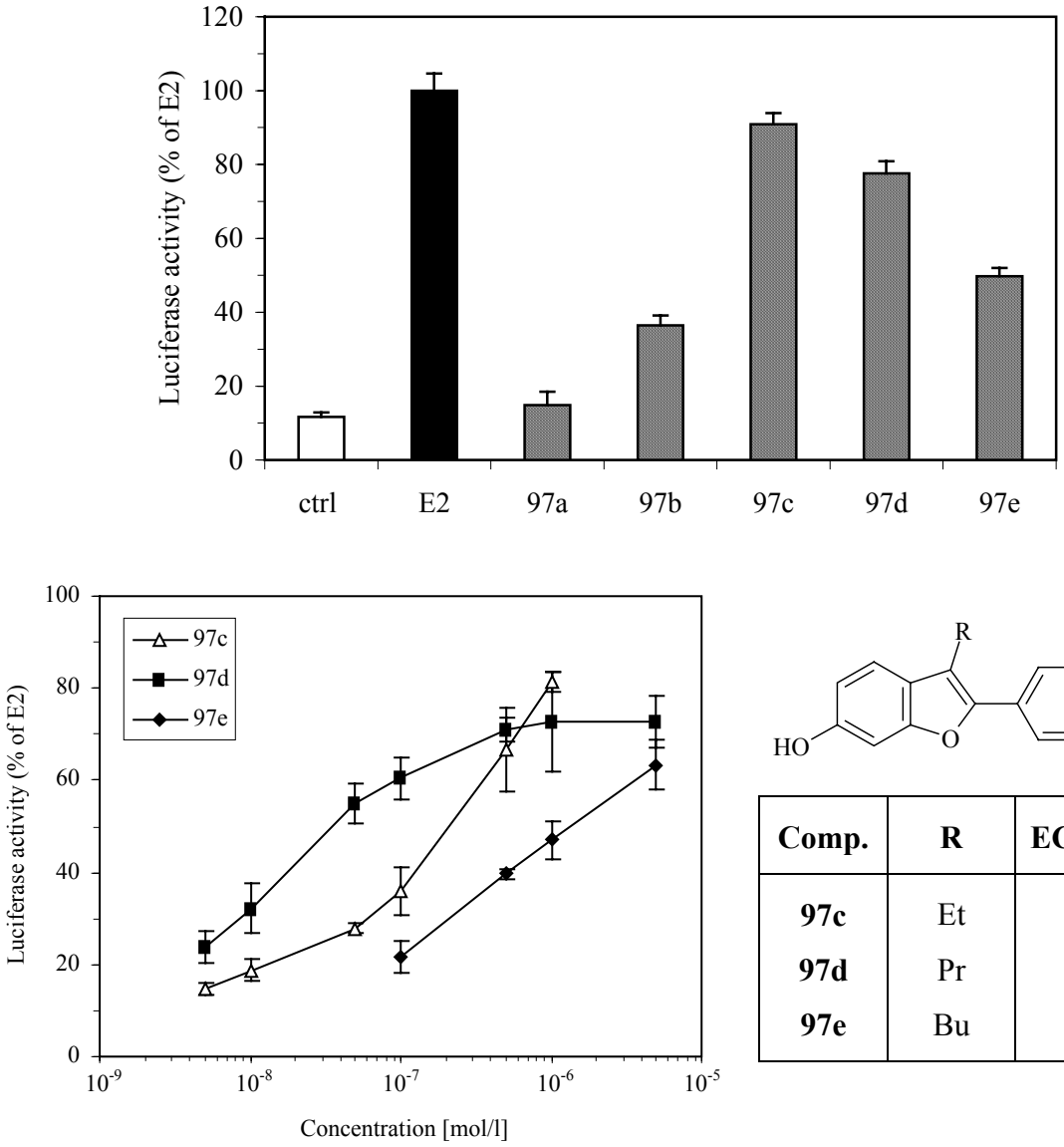
Compound	R	OH	IC_{50} [µM]
98	$(CH_2)_{10}SC_5H_{11}$	6	–
99	$(CH_2)_{10}SO_2C_5H_{11}$	6	0.5
100	$(CH_2)_5CON(CH_3)(CH_2)_3SC_5H_{11}$	6	1.7
101	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	6	0.2
A1[a]	$(CH_2)_6NC_4H_8$	6	0.3
B1[a]	$(CH_2)_6NC_4H_8$	5	0.1
B2[b]	$(CH_2)_{10}SO_2C_5H_{11}$	5	0.8

Table C53: Antiestrogenic activity of 2-phenylbenzo[b]furans with functionalised side chains in MCF-7/2a cells

[a] Synthesised by Dr. S. Erber, 1989
[b] Synthesised by Dr. S. Leichtl, 1994

The simple alkyl derivatives **97a-d** showed no antiestrogenic effects at a concentration of 1µM (data not shown), but all compounds with an long functional side chain, except **98**, inhibited the E2-stimulated luciferase expression in a dose-dependent manner. In analogy to the chemosensitivity assay with MCF-7 cells, the IC_{50}-values of both the 5-hydroxy and 6-hydroxybenzofuran derivatives differed not much within a small range of concentration. The highest activity was achieved by the compounds containing a basic nitrogen in position 7

in the side chain, followed by the sulfones **99** and **B1**, and compound **100** with the amide function incorporated in the aliphatic side chain. Compound **98**, which bound poorly to the ER, antagonised the effect of estradiol on gene activation by only 15% at a concentration of 1µM.



Comp.	R	EC_{50} [µM]
97c	Et	0.2
97d	Pr	0.04
97e	Bu	1.4

Figure C54: Estrogenic activity of 6-hydroxy-2-phenylbenzofurans with simple alkyl groups in MCF-7/2a cells

The estrogenic potency of all test compounds was determined in a similar assay at a concentration of 1µM in the absence of estradiol. At this concentration not all alkyl derivatives **97a-e** produced full estrogenic response (cf. figure C54 top). Only the compounds **97c-e** substituted with alkyl groups of more than two carbon atoms achieved a relative luciferase expression of

50% and were considered for dose-reponse correlations (cf. figure C54 bottom). The propyl derivative **97d** exerted the strongest estrogenic effect, but stimulated gene transcription only to a maximum of 70%. Its EC_{50}-value was by a factor of 5 lower than that of the ethyl derivative **97c**. The low activity of the butyl derivative **97c** corresponds well with its binding affinity to ERα.

The estrogenic activities of the 2-phenylbenzofurans with functional side chains were also determined in a concentration of 1μM and the levels of luciferase expression compared with those of fulvestrant and 4-hydroxytamoxifen. The 6-hydroxy derivatives **98**-**101** suppress basal gene transcription in MCF-7/2a cells to the level of fulvestrant and can therefore be characterised as pure estrogen antagonists. The derivatives **A1**, **B1**, and **B2** did not reach this level. The incomplete blockade of the luciferase expression by **B2** is analogous to the 2,5-diphenylfurans with the same sulfone side chain, but there was no obvious explanation for this behaviour. In both series of 2-phenylbenzofurans the partial antagonistic character of the pyrrolidinohexyl side chain was confirmed again (cf. figure C55).

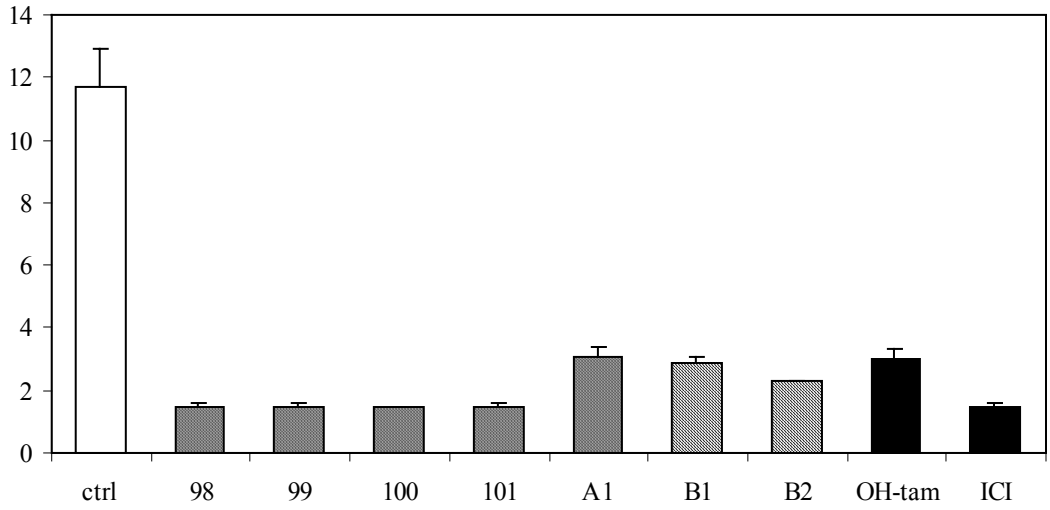


Figure C55: Suppression of basal luciferase activity by 2-phenylbenzo[b]furans with functionalised side chains in MCF-7/2a cells

The value for estradiol at a concentration of 1nM was set to 100%.
ctrl = control;

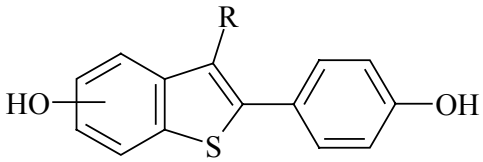
4.2.2 Biological Characterisation of the Benzo[b]thiophenes

4.2.2.1 Determination of Affinity and Selectivity for the ER

Two series of 2-phenylbenzo[b]thiophenes with an hydroxy group in position C-5 or C-6 were evaluated in terms of their affinity and selectivity for ERβ. Except compound **108** all

these benzothiophenes substituted with small alkyl groups in position C-3 were synthesised by Dr. S. Erber [1989].

The 6-hydroxy-2-(4-hydroxyphenyl)benzothiophene derivative **C2a** showed the highest selectivity for ERβ in the binding assay with recombinant human receptor proteins. Its selectivity of 16 was somewhat smaller than that of the ERβ agonist genistein. The unsubstituted 5-hydroxy isomer **108** was the compound with the second highest selectivity for ERβ. In both series the RBA to ERβ decreased with increasing length of the alkyl groups in position C3. This directly affected the ERβ selectivity of these compounds, because the affinity to ERα was influenced inversely by the chain length. The highest RBA to ERα demonstrated the ethyl derivatives **C1c** and **C2c**. These data reflect the smaller internal volume of ERβ and its preference for smaller molecules, whereas ERα can bind somewhat bigger molecules with increased lipophilicity. The highest RBA-values for ERβ were achieved by the 3-H and 3-methyl substituted benzothiophenes, which bound twice as strong as the physiological ligand estradiol (cf. table C56).



Compound	OH	R	RBA (cytosol)	RBA (ERα)	RBA (ERβ)	Binding ratio β/α
108	5	H	3.5	28	221	7.9
C1b*	5	Me	n. d.	56	198	3.5
C1c*	5	Et	n. d.	94	130	1.4
C1d*	5	Pr	n. d.	48	65	1.3
C2a*	6	H	n. d.	11	172	16
C2b*	6	Me	n. d.	69	186	2.7
C2c*	6	Et	n. d.	68	146	2.1
C2d*	6	Pr	n. d.	45	82	1.8
Genistein			n. d.	0.82	18	22

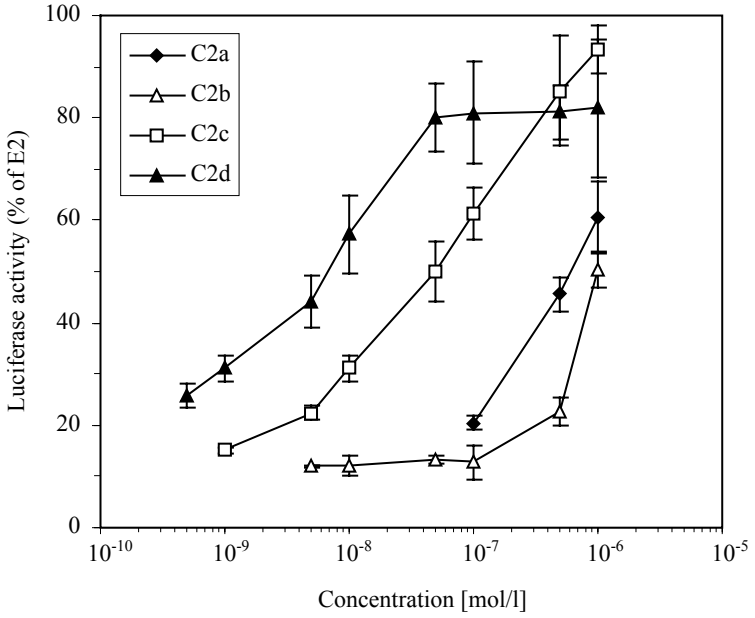
Table C56: Relative binding affinities of 2-phenylbenzo[b]thiophenes

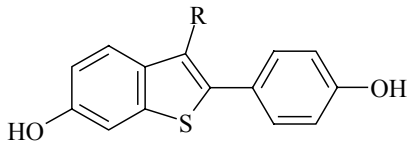
* Synthesised by Dr. S. Erber, 1989

4.2.2.2 Determination of Estrogenic and Antiestrogenic Activity

The 2-phenylbenzothiophenes were also tested for the estrogenic activity in the luciferase assay with stably transfected MCF-7/2a cells. Dose-response curves were determined up to a concentration of 1µM, because at higher concentrations the cytotoxic effect of the compounds killed the cells.

In analogy to the corresponding 6-hydroxy-2-phenylbenzofurans, the 6-hydroxy-2-phenyl-benzothiophenes demonstrated estrogenic effects in dependence on the length of the alkyl substitutent. The highest potency was found for the propyl derivative **C2d** with an EC_{50}-value of 7nM. The 3-H benzothiophene **C2a** and the methyl derivative **C2b** were by a factor of 100 less active (cf. figure C57).





Comp.	R	EC_{50} [µM]
C2a	H	0.6
C2b	Me	1.0
C2c	Et	0.05
C2d	Pr	0.007

Figure C57: Estrogenic activity of 6-hydroxy-2-phenylbenzothiophenes in MCF-7/2a cells

There was no obvious correlation with the binding affinities to ERα, which is the dominant ER isoform in MCF-7 cells. However, the estrogenic activity of these compounds correlated inversely with the binding affinities to ERβ: the higher the affinity to ERβ, the lower the agonistic potency. This implies an antagonistic effect of the 6-hydroxy-2-phenylbenzothiophenes mediated via ERβ, which has previously been shown to inhibit cell proliferation in MCF-7 cells [Paruthiyil et al., 2004]. However, one has to consider, that the luciferase assay is not based on cell proliferation and that the concentration of both ER subtypes in the MCF-7/2a cell line was not determined.

The estrogenic potency of the 5-hydroxylated 2-phenylbenzothiophenes was by far lower than that of their 6-hydroxy analogues. This is in accord with previous observations, which showed that **C1c** and **C1d** act as partial agonists both *in vitro* and *in vivo* [von Angerer and Erber, 1992].

4.3 Conclusion

A number of agonists and antagonists based on the 6-hydroxy-2-(4-hydroxyphenyl)-enzo[b]furan scaffold were prepared by an improved method. These benzofurans, that are substituted in position C-3 with simple alkyl groups or long functionalised side chain, were studied together with the previously synthesised 5-hydroxy analogues in terms of their affinity and selectivity for the ER. With exception of the compounds **101** and **A1**, both series of compounds demonstrated preference for ERβ with a 21-fold selectivity as the maximum, that is similar to that of the ERβ selective agonist genistein. The compounds with the hydroxy group in position C-5 generally bound with higher affinity to both receptor subtypes than the corresponding 6-hydroxy derivatives. The antiproliferative and antiestrogenic activities of the 2-phenylbenzofurans were lower than those of the diphenylfurans. This can be exemplified by derivative **101** bearing the bifunctional 6-[N-methyl-N-(3-pentylsulfanyl-propyl)amino]hexyl side chain with a decrease in activity by one order of magnitude. In the luciferase assay the 6-hydroxy-2-phenylbenzo[b]furans with short alkyl groups proved to be agonists, whereas the derivatives with long functionalised side chains behaved as pure antiestrogens.

Furthermore, a new synthesis for 5-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene comprising six reaction steps was developed. The 2-phenylbenzothiophenes substituted with short alkyl groups and hydroxy groups in either position C-5 or C-6 bound with significantly higher affinity to both ER subtypes than the corresponding 2-phenylbenzofurans and showed a comparable selectivity for ERβ. The 6-hydroxy derivatives were found to be partial ER agonists, whose estrogenic potency was influenced by the length of the alkyl substituent and the affinity to ERβ.

5 Antiestrogens Based on a Benzopyran(one) scaffold

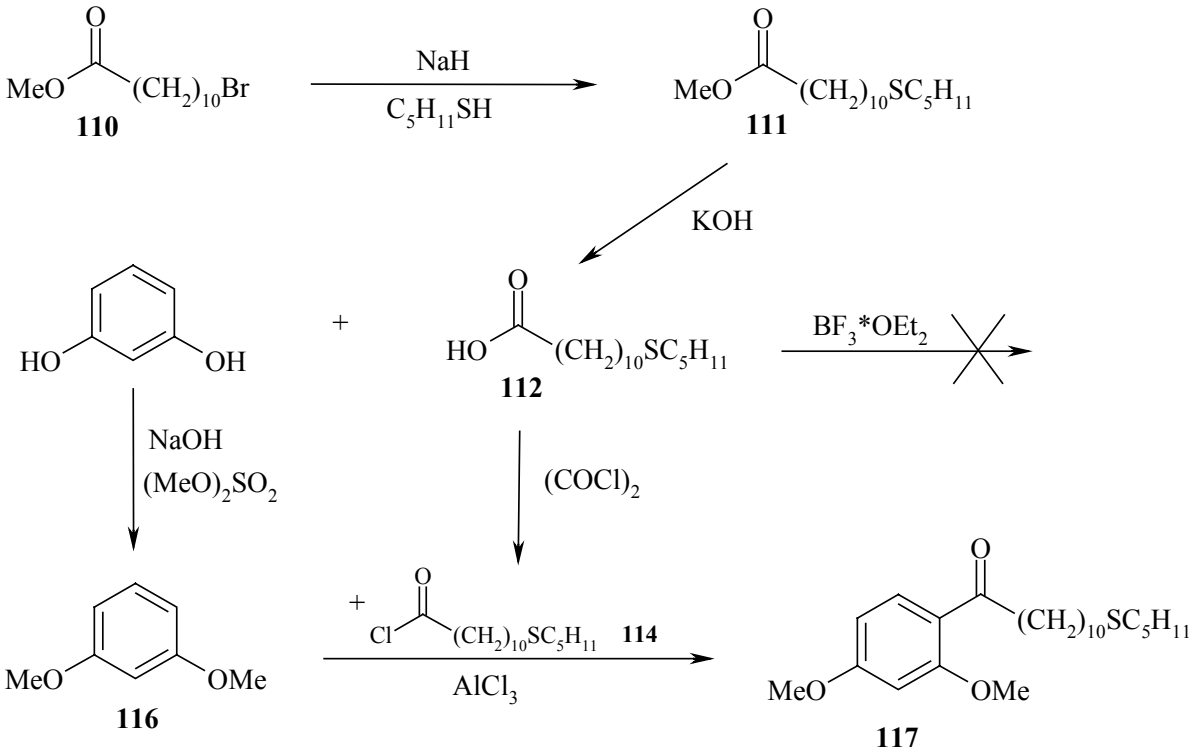
The investigations on the structure-activity relationship of antiestrogens based on the diphenylfuran and 2-phenylbenzo[b]furan structure lead to a ring enlargement of the benzofuran heterocycle by one carbon atom. The 1-benzopyran structure is known since the development of the selective estrogen receptor modulator EM-800 [Gauthier et al., 1997] and it has recently become a matter of intensive interest for many research groups working in the field of estrogen receptor modulation [Amari et al., 2004, Bury et al., 2002, McKie et al., 2004]. However, there are no studies reported in the literature that describe structure-activity relationships of the benzopyran scaffold with side chains typical for the pure antiestrogens fulvestrant or its newer analogues [Hoffmann and Sommer, 2005]. Therefore, such side chains should be introduced in position C-4 of the 3-phenylbenzopyrans and –benzopyranones. The biological activity of the resulting compounds should be evaluated in terms of their affinity and selectivity for the estrogen receptor and their antiproliferative and antiestrogenic potency in human mammary tumour cells.

5.1 Synthesis

5.1.1 Synthesis of 1-Benzopyran-2-ones

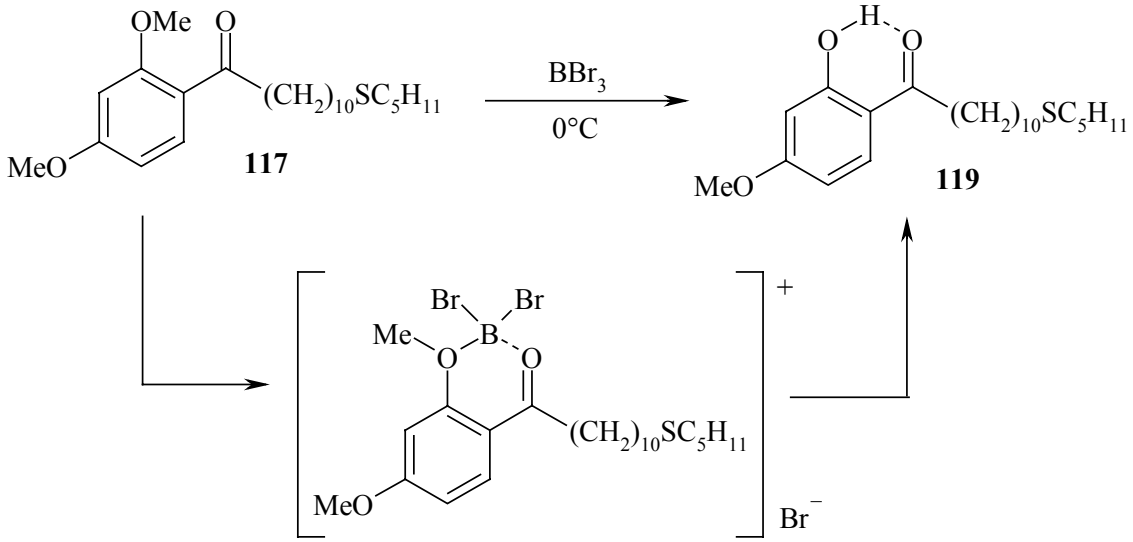
The strategy for the preparation of antiestrogens based on a benzopyranone core structure was similar to the synthesis of the diphenylfurans. Alkyl aryl ketones bearing the long aliphatic side chains were considered as appropriate precursors for the preparation of the desired compounds.

The synthesis of the respective ketone with the monofunctional alkyl side chain started from ω-bromoundecanoic acid. The carboxy function was converted into the corresponding methyl ester **110** and deprotected again after the introduction of the terminal pentyl sulfanyl group by a S_N2 reaction. The resulting acid **112** was reacted with resorcinol and boron trifluoride etherate, but the expected alkyl aryl ketone did not form. Therefore, the acid was activated as acid chloride **114** and the Friedel-Crafts reaction conducted with the dimethyl ether of resorcinol **116** and $AlCl_3$ to give the desired ketone **117** in good overall yield (cf. scheme C58).



Scheme C58: Synthesis of the alkyl aryl ketone **117** with monofunctional side chain

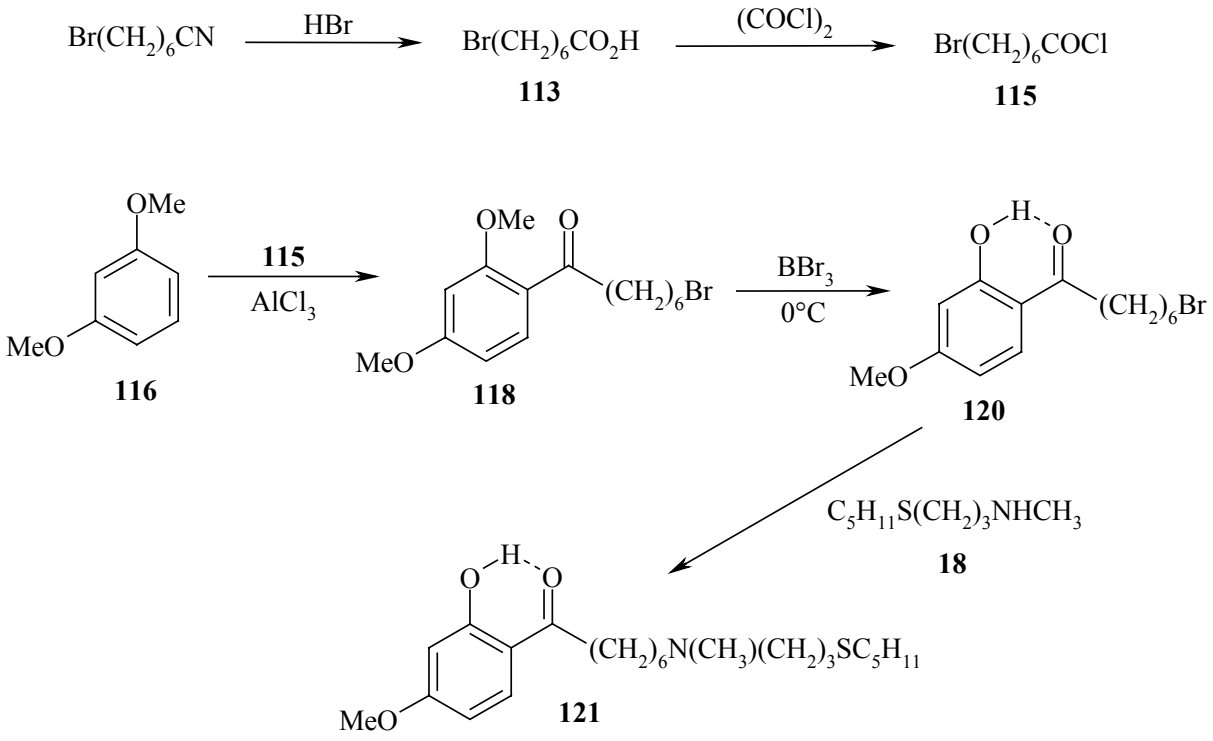
The key step in the reaction sequence leading to the chromenone system was the selective demethylation of the *ortho*-methoxy group in **117**. The action of boron tribromide on the dimethoxy compound **117** at 0°C led to quantitative formation of the monohydroxy compound **119** within 30 minutes (cf. scheme C59).



Scheme C59: Selective *ortho*-demethylation

In this reaction the stabilising effect of the adjacent carbonyl function was exploited. The carbonyl group facilitates the selective *ortho*-demethylation by stabilising the intermediated complex with boron tribromide (cf. scheme C59). In the final product **119** the carbonyl group forms an intramolecular hydrogen bridge to the *ortho*-hydroxy group, which can be observed through the shift of the carbonyl band in the IR spectrum to lower energy. A demethylation mediated by $AlCl_3$ as described by Horie et al. [1991] did not lead to the expected product.

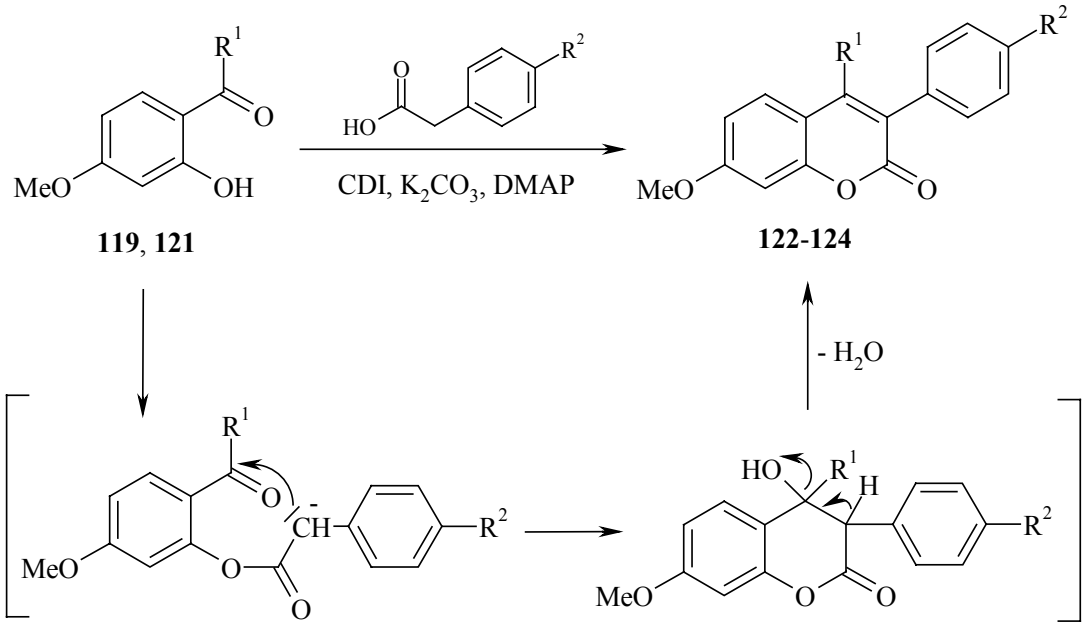
The synthesis of the 2-hydroxyphenyl ketone with a bifunctional aliphatic side chain started from ω-bromoheptanitrile, which was hydrolysed under strongly acidic conditions to the corresponding acid **113**. Conversion to the acid chloride and Friedel-Crafts acylation afforded the dimethoxy compound **118**, which was selectively demethylated with BBr_3 as described above. The side chain of the alkyl aryl ketone **121** was completed by substitution of the terminal bromine in **120** with the amine **18** (cf. scheme C60).



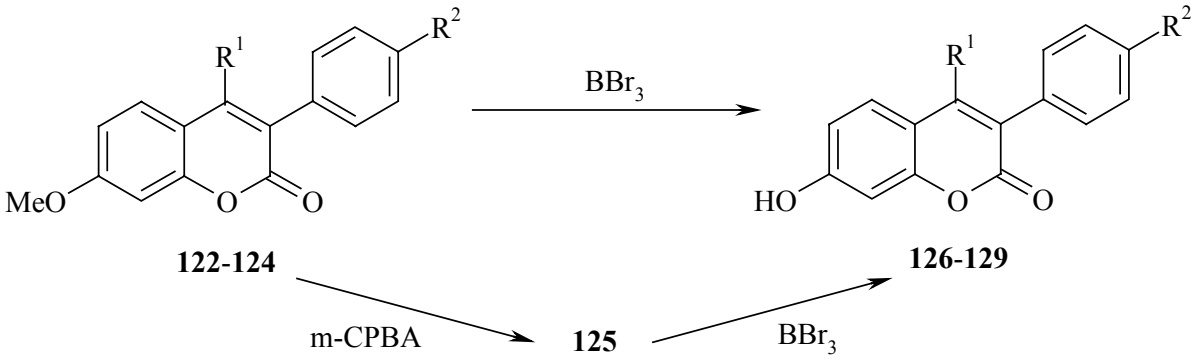
Scheme C60: Synthesis of the 2-hydroxyphenyl ketone **121** with bifunctional side chain

The formation of the 3-aryl benzopyrane core structure was achieved by condensation of the 2-hydroxyphenyl ketones **119** and **121** with phenylacetic acid or its 4-methoxy analogue in the presence of anhydrous K_2CO_3 and DMAP (cf. scheme C61) [McKie et al., 2004]. The

phenylacetic acids were activated with carbonyl diimidazole prior to the addition of the other reagents. The reaction did not lead to complete conversion, which is dependent on the phenylacetic acid used. Unconverted starting material can be recovered by chromatography. The reaction proceeds via O-acylation of the activated phenylacetic acid and abstraction of one of the benzylic protons by K_2CO_3. The resulting enolate adds to the second carbonyl group and the subsequent elimination of water is driven by the formation of the aromatic system.



Scheme C61: Formation of the 3-phenylbenzopyranones



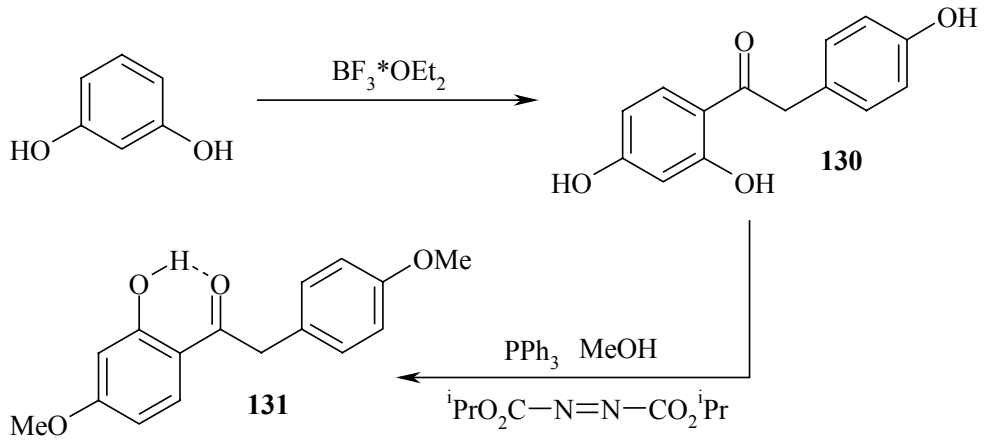
122 $R^1 = (CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$, R^2 = H
123 $R^1 = (CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$, R^2 = OMe
124 $R^1 = (CH_2)_{10}SC_5H_{11}$, R^2 = OMe
125 $R^1 = (CH_2)_{10}SO_2C_5H_{11}$, R^2 = OMe
126 $R^1 = (CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$, R^2 = H
127 $R^1 = (CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$, R^2 = OH
128 $R^1 = (CH_2)_{10}SC_5H_{11}$, R^2 = OH
129 $R^1 = (CH_2)_{10}SO_2C_5H_{11}$, R^2 = OH

Scheme C62: Demethylation of the 3-phenylbenzopyranones

After the cyclisation reaction the sulfide in the side chain of the benzopyranone **124** was oxidised with m-CPBA to the corresponding sulfone **125**. Finally, the deprotection of the methoxy groups with boron tribromide afforded the free phenols **126**-**129** (cf. scheme C62), which are presented in table C70 at the end of section C5.2.

5.1.2 Synthesis of 1-Benzopyrans

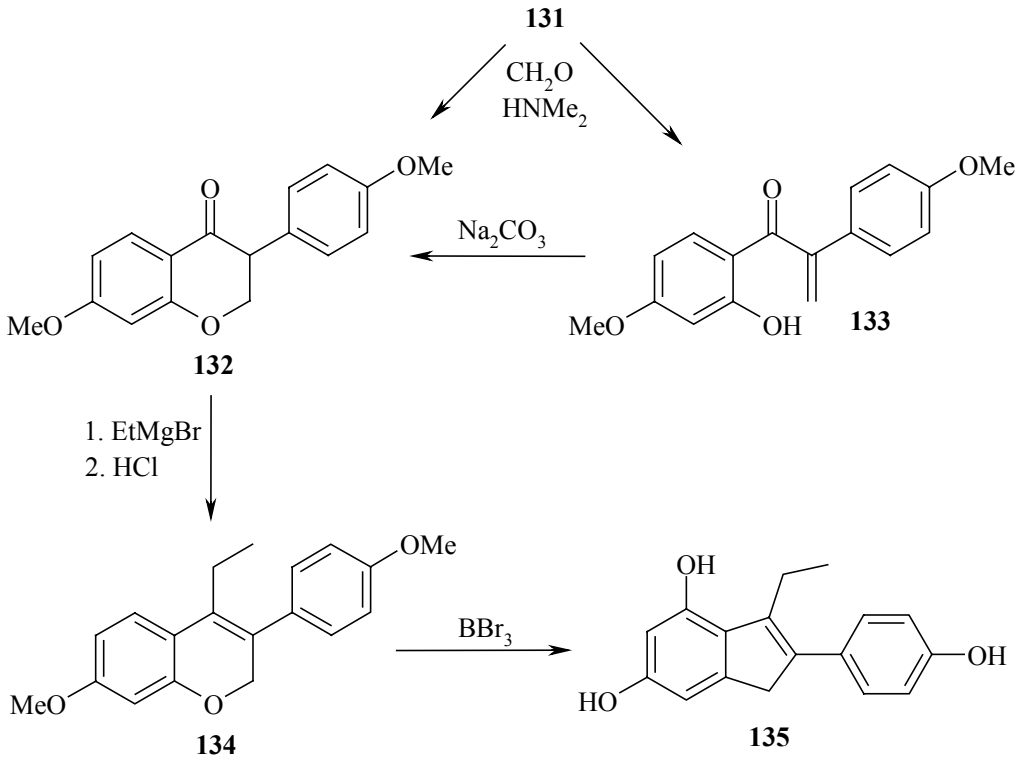
The synthesis of the benzopyrans started with a modified procedure of the Friedel-Crafts acylation of resorcinol and 4-hydroxyphenylacetic acid using freshly distilled boron trifluoride etherate as Lewis acid and solvent [Wähälä and Hase, 1991]. Two of the hydroxy groups of the resulting trihydroxydeoxybenzoin **130** were transferred into methyl ethers under Mitsunobu conditions [Dushin and Danishefsky, 1992]. The *ortho*-hydroxy group of **130** remained unaffected due to the stabilisation by an intramolecular hydrogen bond with the carbonyl function (cf. scheme C63).



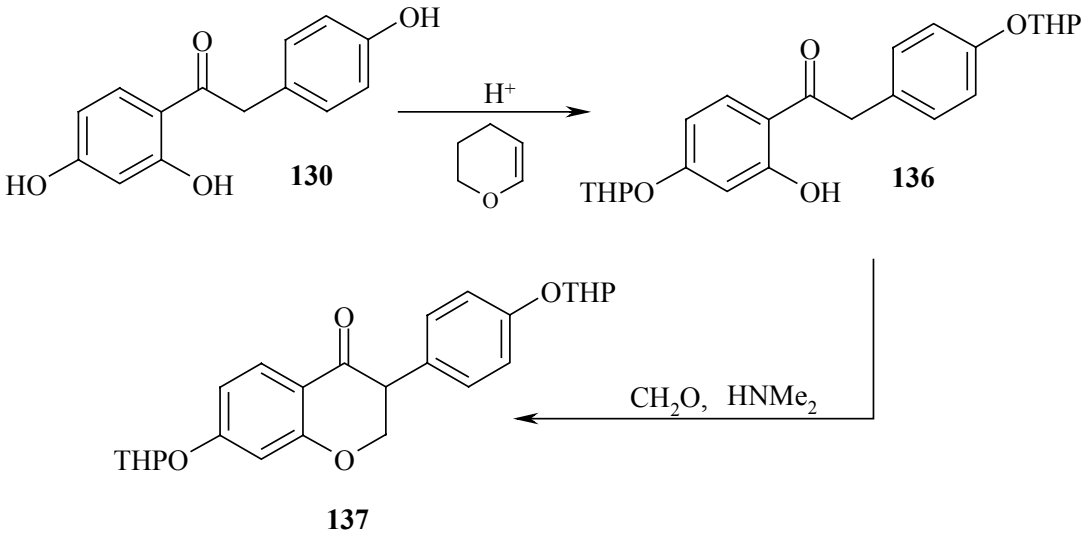
Scheme C63: Synthesis of the protected deoxybenzoin **131**

The respective isoflavanone **132** was obtained by the Mannich reaction of **131** in the presence of paraformaldehyde and three equivalents of aqueous dimethylamine. When only one equivalent of the secondary amine was used, as described by Gandhidasan et al. [1982], the propenone intermediate **133** was isolated as main product. Compound **133** could be quantitatively converted into the isoflavanone **132** by reaction with 4% ethanolic Na_2CO_3. The subsequent Grignard reaction and elimination of water under acidic condition gave the alkylated benzopyran **134**. The final deprotection of the methoxy groups with boron tribromide did not

afford the desired free phenolic product, but instead the indene **135** through a ring opening and recyclisation sequence (cf. scheme C64).



Scheme C64: Synthesis leading to the 2-phenylindene **135**

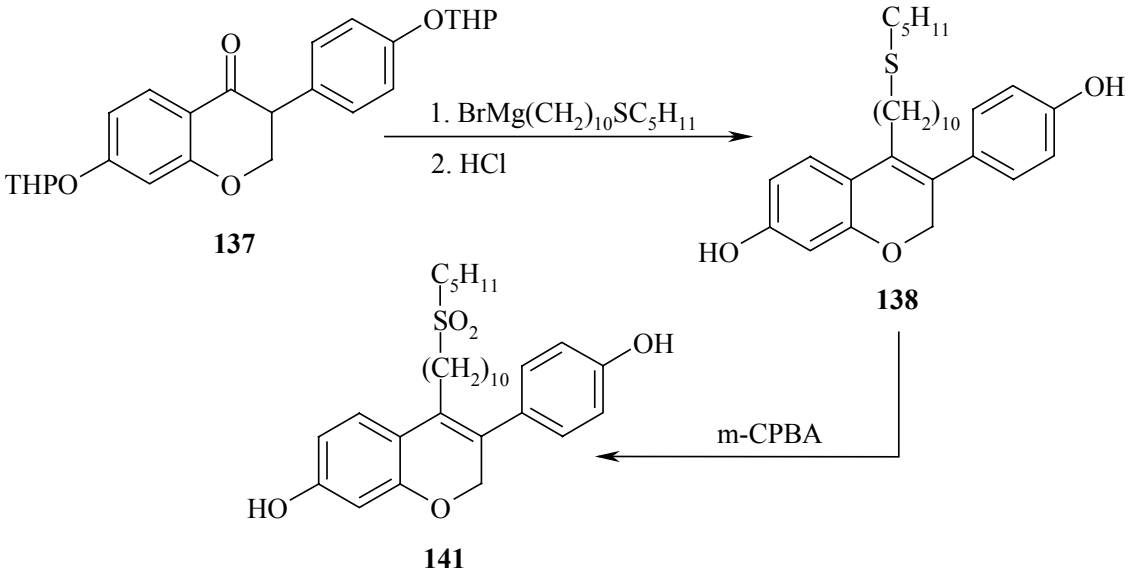


Scheme C65: Synthesis of the THP-protected isoflavanone **137**

Due to the problem with the final demethylation either a new demethylation proceducre or a new protecting group strategy was needed. The failure of the methoxy cleavage with pyridin-

ium hydrochloride led to THP-protection of the phenolic hydroxy groups of the trihydroxydeoxybenzoin **130**, as described in the synthesis of EM-800 [Gauthier et al., 1997]. This was achieved with catalytic amounts of TsOH and 3,4-dihydropyran (DHP) as reagent and solvent. The protected deoxybenzoin **136** was converted into the corresponding isoflavanone **137** using the Mannich reaction as described above (cf. scheme C65).

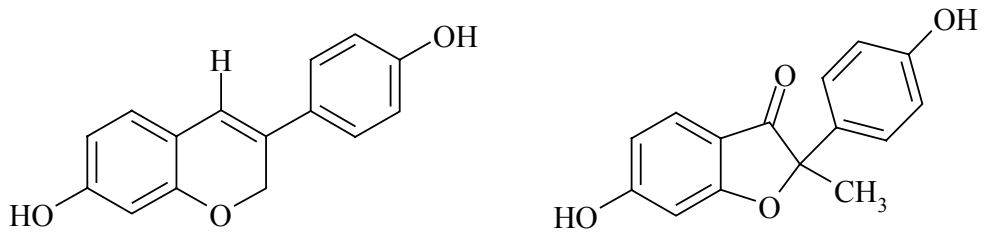
The aliphatic side chain **10** was introduced by a Grignard reaction into the isoflavanone **137**. Acidic work-up of the intermediary tertiary alcohol led to elimination of one molecule water and cleavage of the THP protecting group to give the desired benzopyran **138**. Subsequent oxidation of the sulfanyl group in the side chain of **138** afforded the corresponding sulfone **141** (cf. scheme C66).



137 **138** **141**

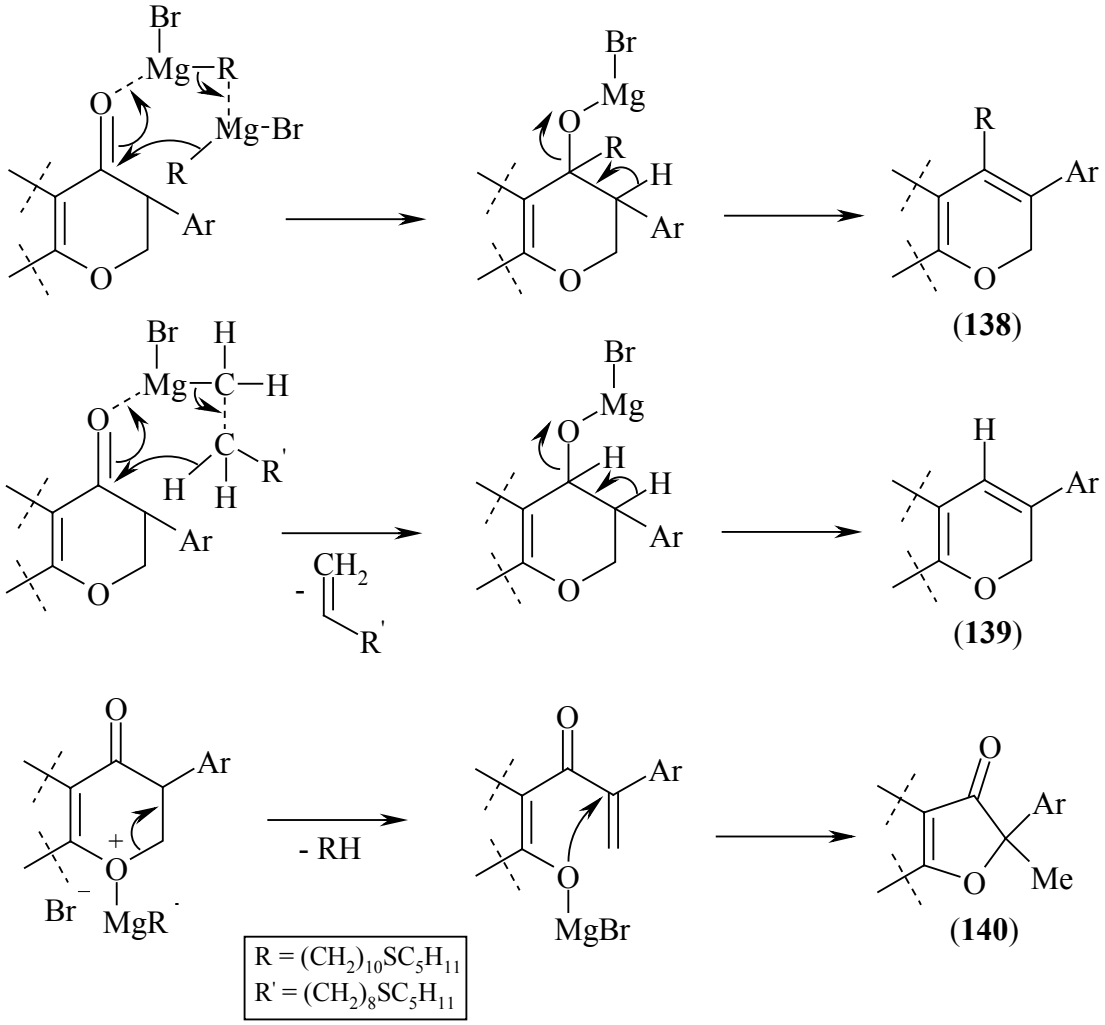
Scheme C66: Grignard reaction and oxidation of the side chain

The Grignard reaction yielded the two by-products **139** and **140**. The mechanism of the Grignard reaction and the formation of both by-products is outlined in scheme C68.



139 **140**

Figure C67: By-products of the Grignard reaction



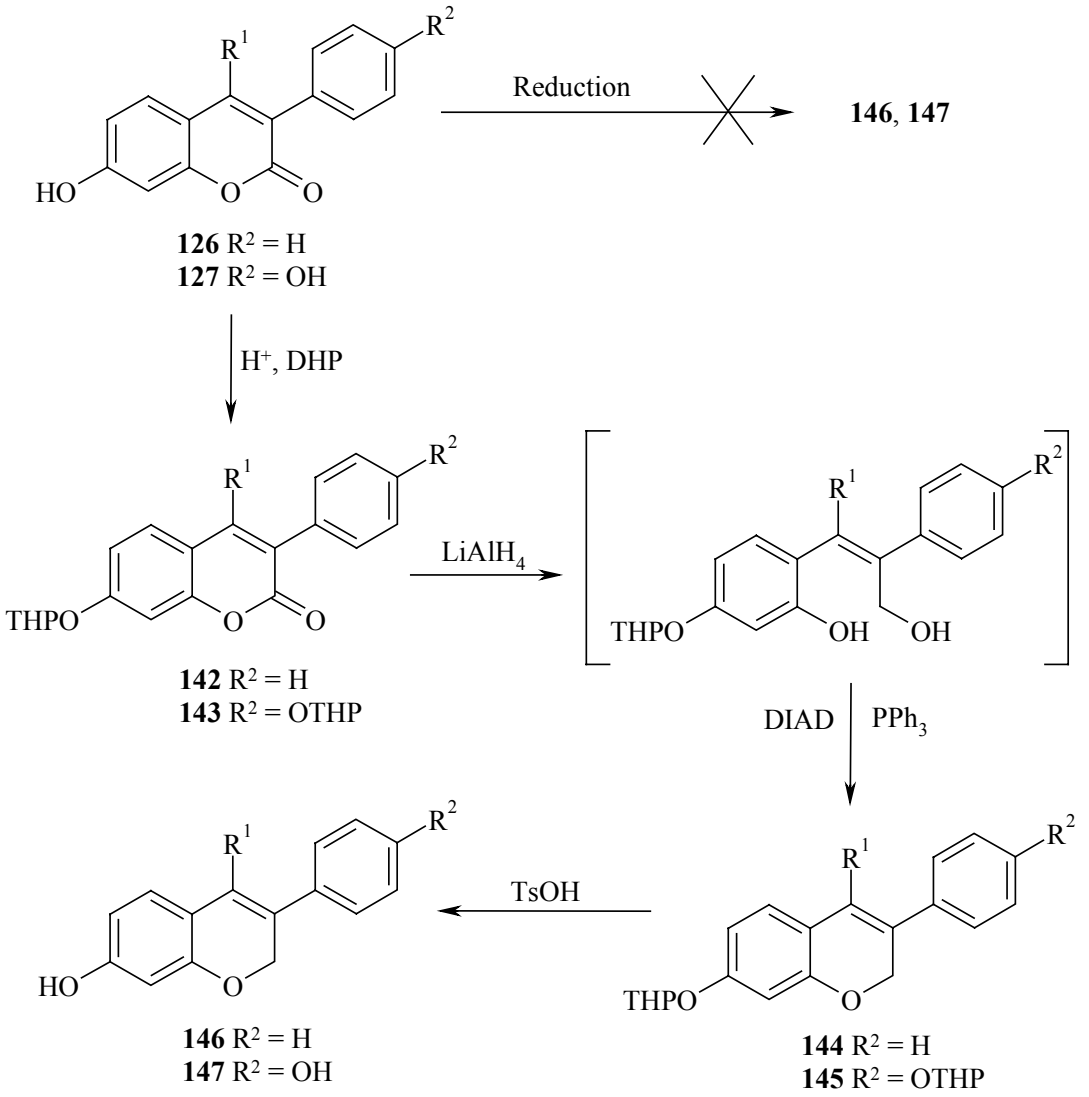
Scheme C68: Mechanisms of the formation of the products in the Grignard reaction

The reduction product **139** was possibly formed due to steric demands of both the carbonyl compound **137** and the Grignard reagent with the long alkyl group. In analogy to the Meerwein-Ponndorf-Verley reduction one of the β-hydrides of Grignard reagent was transferred to the carbonyl group via a six-membered transition state. The elimination of water during the aqueous acidic work-up was facilitated by the formation of the conjugated system in **139**.

The Mg-atom of the Grignard reagent can also coordinate with the ether-oxygen of the isoflavanone **137** and effect ring opening. The oxygen now has two possibilities to attack the double bond and regenerated the cyclic system: a 6-endo-trig cyclisation would have regenerated compound **137**, whereas a 5-exo-trig attack of the terminal double bond led to the isolated compound **140** (cf. scheme C68).

The introduction of the bifunctional side chain into the isoflavanone **137** was not successful through a Grignard reaction. Therefore, a reduction of the lactone functionality of the corre-

sponding 3-phenylbenzopyranones **126** and **127** and subsequent acid catalysed recyclisation was considered. Both the use of $LiAlH_4$ [Bury et al., 2002] and of DIBAL-H [Alberola et al., 1983] as reducing agents led to a complete decomposition of the starting material, probably due to the presence of unprotected hydroxy functions.



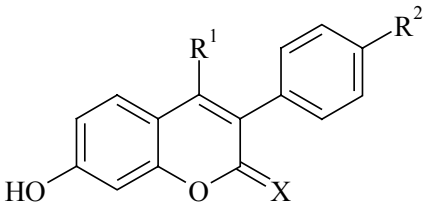
Scheme C69: Synthesis of the benzopyran with the bifunctional side chains

$R^1 = (CH_2)_6N(CH_3)(CH_2)SC_5H_{11}$

Consequently, the free phenolic hydroxy groups were protected as THP-ethers to give the compounds **142** and **143**. These protected benzopyranones were readily reduced with $LiAlH_4$ to the corresponding diols, which were recyclised under Mitsunobu conditions to give the THP-protected benzopyrans **144** and **145** in good overall yield [Carlock and Mack, 1978].

Finally, the free phenols **146** and **147** were obtained from treatment of the THP-ethers with 4-toluenesulfonic acid in methanol (cf. scheme C69).

The synthesised benzopyranones and benzopyrans that were submitted to the biological evaluation are presented in table C70.



Compound	X	R^1	R^2
126	O	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	H
127	O	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	OH
128	O	$(CH_2)_{10}SC_5H_{11}$	OH
129	O	$(CH_2)_{10}SO_2C_5H_{11}$	OH
138	H, H	$(CH_2)_{10}SC_5H_{11}$	OH
141	H, H	$(CH_2)_{10}SO_2C_5H_{11}$	OH
146	H, H	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	H
147	H, H	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	OH
139	H, H	H	OH

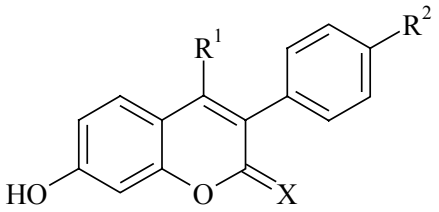
Table C70: Overview over the synthesised benzopyran(one)s

5.2 Biological Characterisation of the Benzopyran(one)s

5.2.1 Determination of Affinity and Selectivity for the ER

The synthesised benzopyrans and benzopyranones were tested for their binding affinities to the ER and their selectivity for one of the two ER subtypes. The binding curves of all test compounds were comparable in shape to the binding curve of E2, but shifted to higher concentrations, which indicates a competitive displacement of the physiological ligand from the receptor binding site. The RBA values were determined by two methods using either calf

uterus cytosol as receptor source or the recombinant human ERα and ERβ. The RBA values shown in table C71 are the means of two or three independent experiments.



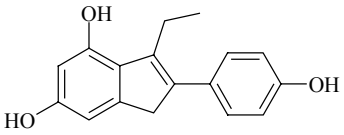
Comp.	X	R^1	R^2	RBA (cytosol)	RBA (ERα)	RBA (ERβ)	Binding ratio α/β
126	O	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	H	8.3	16	7.0	2.3
127	O	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	OH	8.3	24	5.7	4.3
128	O	$(CH_2)_{10}SC_5H_{11}$	OH	0.32	0.73	0.51	1.4
129	O	$(CH_2)_{10}SO_2C_5H_{11}$	OH	4.4	5.2	2.9	1.8
138	H, H	$(CH_2)_{10}SC_5H_{11}$	OH	0.31	1.1	1.0	1.1
141	H, H	$(CH_2)_{10}SO_2C_5H_{11}$	OH	3.8	5.5	3.7	1.5
146	H, H	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	H	5.1	16	14	1.1
147	H, H	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	OH	8.4	42	29	1.4
139	H, H	H	OH	0.34	0.53	4.35	0.12
135				0.60	1.0	1.5	0.67
ICI				5.2	5.3	3.1	1.7

Table C71: Relative binding affinities of the benzopyran(one)s

All the benzopyrans and benzopyranones substituted with a long functionalised side chain in position C-4 demonstrated a slight preference for the ERα. In contrast, the unsubstituted benzopyran **139** showed a 8-fold selectivity for ERβ due to a rather low binding affinity to ERα. The methylene and the carbonyl group in position 2, which is the only structural difference between the benzopyrans and benzopyranones, weakly affect the affinity and subtype selectivity of the compounds. The lipophilic methylene group favours the interaction with amino acid residues in both receptor subtypes more than the polar carbonyl function, as it can be concluded from the higher binding affinities of the benzopyrans. Contrary to the binding

concluded from the higher binding affinities of the benzopyrans. Contrary to the binding affinities, the selectivities of the benzopyrans were somewhat lower than those of the corresponding benzopyranones. This finding reflects, that the carbonyl group rather than the methylene group contributes to ERα selectivity, because ligand binding pocket of ERα tolerates the carbonyl function better than ERβ.

The benzopyranone **127** with the bifunctional side chain displayed the highest selectivity for ERα (4.3-fold). The highest binding affinities in both series were found for the compounds bearing the bifunctional side chain with a basic nitrogen in position 7, which probably forms a hydrogen bond to ERα Asp351 or ERβ Asp303. A comparison of the binding affinities of the mono- and diphenolic derivatives **146** and **147** in the benzopyran series indicates that both hydroxy functions are involved in hydrogen bonding to the glutamate, arginine and histidine, respectively, of ERα and ERβ. The binding data of the corresponding benzopyranones **126** and **127** allow no definite statement in this context. The sulfones **129** and **141** in each series of compounds bound stronger to the ER than the corresponding sulfides **128** and **138**.

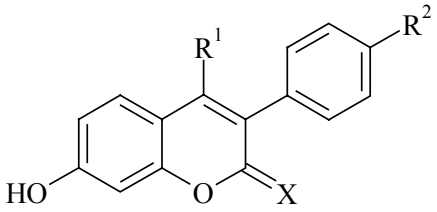
The indene **135** displayed a 1.5-fold selectivity for ERβ, but the binding affinities to both ER subtypes in comparison to the structurally related 6-hyroxybenzofurans and 6-hydroxybenzothiophenes (cf. table C49 and C56) were much lower.

5.2.2 Determination of Antiproliferative Activity

The antiproliferative activities of the synthesised 3-phenylbenzopyrans and -benzopyranones were determined in the ER-positive MCF-7 mammary carcinoma cell line. The assay was performed using estradiol as growth stimulating hormone in a nearly physiological concentration of 1nM.

All the compounds equipped with long functionalised side chains inhibited the cellular growth of these cells with IC_{50}-values within a range of 30 and 500nM (cf. table C72). The sulfones **129** and **141** displayed equal activities, which can be rationalised by the similar RBA values. The antiproliferative effect of the corresponding sulfides **128** and **138** was lower by a factor of ten. The benzopyran **147** that bound with the highest affinity to the ER exerted the strongest inhibitory effect in MCF-7 cells with an IC_{50}-value of 30nM. Its monophenolic derivatives **146** was slightly less active, which was in good accordance with the data from the binding assay. Surprisingly, the activities of the benzopyranones **126** and **127** with the bifunctional side chain were reduced by one order of magnitude in comparison to the corresponding ben-

zopyrans **146** and **147**, although the binding affinities were similar. This discrepancy might be due to the different experimental conditions under which these assays were performed: cell-free conditions for the binding assay and intact cells for the proliferation assay.



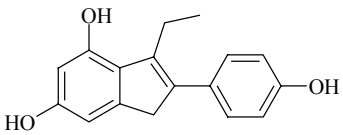
Comp.	X	R^1	R^2	MCF-7 IC_{50} [µM]	MDA-MB 231 IC_{50} [µM]
126	O	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	H	0.2	1.6
127	O	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	OH	0.2	1.6
128	O	$(CH_2)_{10}SC_5H_{11}$	OH	0.4	6.0
129	O	$(CH_2)_{10}SO_2C_5H_{11}$	OH	0.05	5.6
138	H, H	$(CH_2)_{10}SC_5H_{11}$	OH	0.5	6.2
141	H, H	$(CH_2)_{10}SO_2C_5H_{11}$	OH	0.06	1.9
146	H, H	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	H	0.08	2.1
147	H, H	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	OH	0.03	2.0
139	H, H	H	OH	1.5	1.4
135				> 10	> 10
ICI				0.004	>10

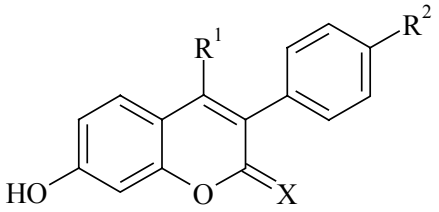
Table C72: Antiproliferative effects of the benzopyran(one)s in MCF-7 and MDA-MB 231 breast cancer cells

The benzopyran **139** without a substituent in position C-4 showed an inhibition of MCF-7 cell growth, which is very likely caused by non-specific cytotoxic effects of this compound, because similar activity was observed in the assay with ER-negative MDA-MB 231 cells. Generally, the inhibitory effect of the benzopyran(one)s with functionalised side chains on these hormone-independent MDA-MB 231 cells was at least by a factor of 10 smaller than that on the MCF-7 cells, which makes a non-specific drug action of these agents unlikely. The indene

135 showed no inhibition of cell proliferation in both cell lines up to a concentration of 10µM.

5.2.3 Determination of Estrogenic and Antiestrogenic Activity

The 3-phenylbenzopyrans and -benzopyranones with a long functionalised side chain were tested for their estrogenic and antiestrogenic activity in the luciferase assay using the ER-positive MCF-7/2a cells The antiestrogenic activity was determined by simultaneous treatment of these cells with 1nM E2 and the respective benzopyran(one) in various concentration. The IC_{50}-values calculated from the dose-response curves were similar to those obtained from the chemosensitivity assay with MCF-7 cells (cf. table C73), except for the monophenolic benzopyran **146**, whose inhibitory effect on E2-stimulated luciferase expression was by a factor of 2 higher and, thus, in the same range as the diphenolic derivative **147**. Compound **147** displayed the strongest antiestrogenic effect, which was by a factor of 10 lower than that of the steroidal antiestrogen fulvestrant.



Comp.	X	R^1	R^2	IC_{50} [µM]
126	O	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	H	0.2
127	O	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	OH	0.2
128	O	$(CH_2)_{10}SC_5H_{11}$	OH	0.4
129	O	$(CH_2)_{10}SO_2C_5H_{11}$	OH	0.05
138	H, H	$(CH_2)_{10}SC_5H_{11}$	OH	0.3
141	H, H	$(CH_2)_{10}SO_2C_5H_{11}$	OH	0.06
146	H, H	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	H	0.04
147	H, H	$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$	OH	0.03
ICI				0.003

Table C73: Antiestrogenic activity of the benzopyran(one)s in MCF-7/2a cells

The estrogenic potency of the benzopyran(one)s was determined in a similar assay at a concentration of 1µM in the absence of estradiol. At this concentration none of the compounds tested showed agonistic activity. The luciferase expression levels were below that of the control cells (cf. figure C74), what is characteristic for potent antiestrogens. It indicates the blockade of a ligand-independent activation of the ER, that is responsible for the basal luciferase activity in the control cells. For a better estimation of the residual estrogenic activity the levels of luciferase expression were compared with those of the partial antiestrogen 4-hydroxytamoxifen and the pure antiestrogen fulvestrant. All the tested benzopyrans and benzopyranones, except the compounds **126** and **146**, suppressed luciferase expression to the same extent as fulvestrant did. The two monophenols **126** and **146** were also devoid of agonist activity though their suppression was somewhat weaker, but still better than that of the partial antiestrogen 4-hydroxytamoxifen.

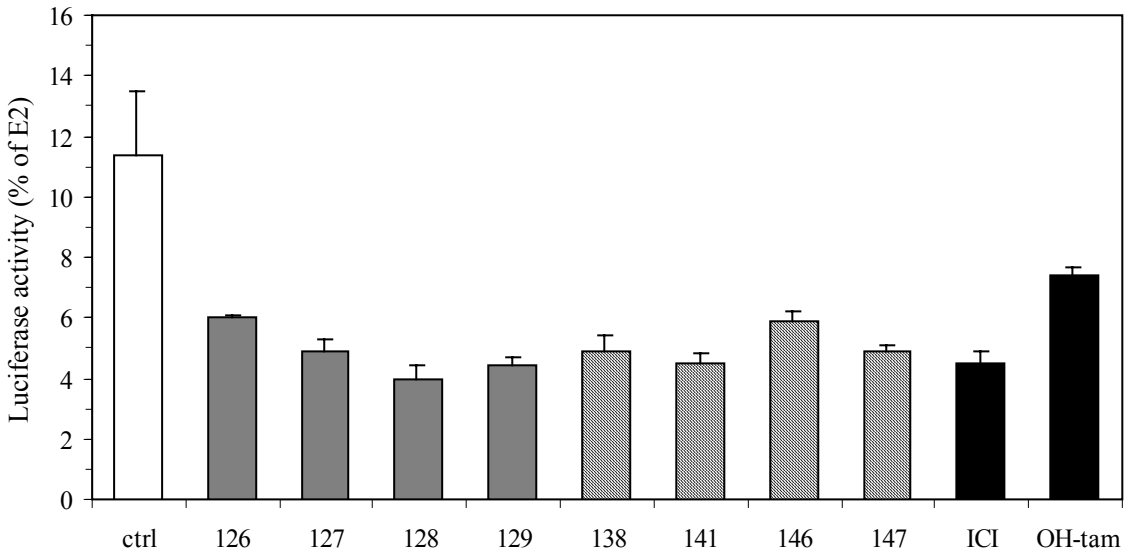


Figure C74: Suppression of basal luciferase activity by benzopyran(one)s with functionalised side chains in MCF-7/2a cells

The value for estradiol at a concentration of 1nM was set to 100%.
ctrl = control

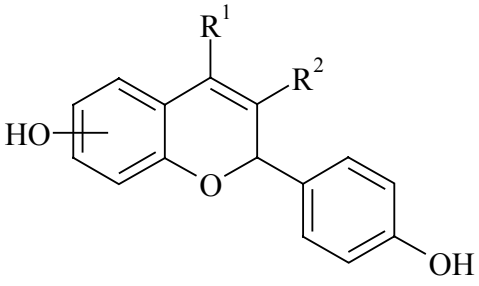
5.3 Conclusion

It was possible to introduce aliphatic side chains with various functional groups into position C-4 of 3-phenylbenzopyrans and –benzopyranones. All the synthesised compounds were characterised as potent estrogen antagonists without residual estrogenic activity. They showed

a preference for ERα contrary to antiestrogens based on the 2-phenylbenzo[b]furan, whose heterocyclic core is by one carbon atom smaller. The antiproliferative and antiestrogenic potency of the 3-phenylbenzopyran(one)s was by one order of magnitude higher than that of the corresponding 2-phenylbenzofurans. In comparison to the 2,5-diphenylfurans the selectivity of the 3-phenylbenzopyran(one)s for ERα was slightly reduced. The antagonistic potency of the benzopyran **146** with the bifunctional side chain was comparable to that of the corresponding 2,5-diphenylfurans, whereas that of the benzopyranones was by one order of magnitude smaller. However, in both series of benzopyrans and benzopyranones the sulfones and sulfides were by a factor of 10 more active than in the 2,5-diphenylfuran series, which was in good accordance with the higher binding affinities. Compound **129** with the sulfonyl group in the side chain was the most active in the class of antiestrogens based on the 3-phenylbenzopyranone scaffold.

5.4 Attempted synthesis of 2-Phenyl-Substituted 1-Benzopyrans

The introduction of a long functionalised side chain in position C-4 of the 3-phenylbenzopyrans scaffold has led to potent antiestrogens, but hardly to a preference for ERα. Thus, further investigations on structure-activity relationships of benzopyrans with a different arrangement of the substitutents at the heterocyclic core were considered. Therefore, the synthesis to 2-phenyl-benzopyrans (cf. figure C75) with a short alkyl group in position C-3 and a functionalised side chain in position C-4 was initiated, but could not be completed for synthetic reasons.

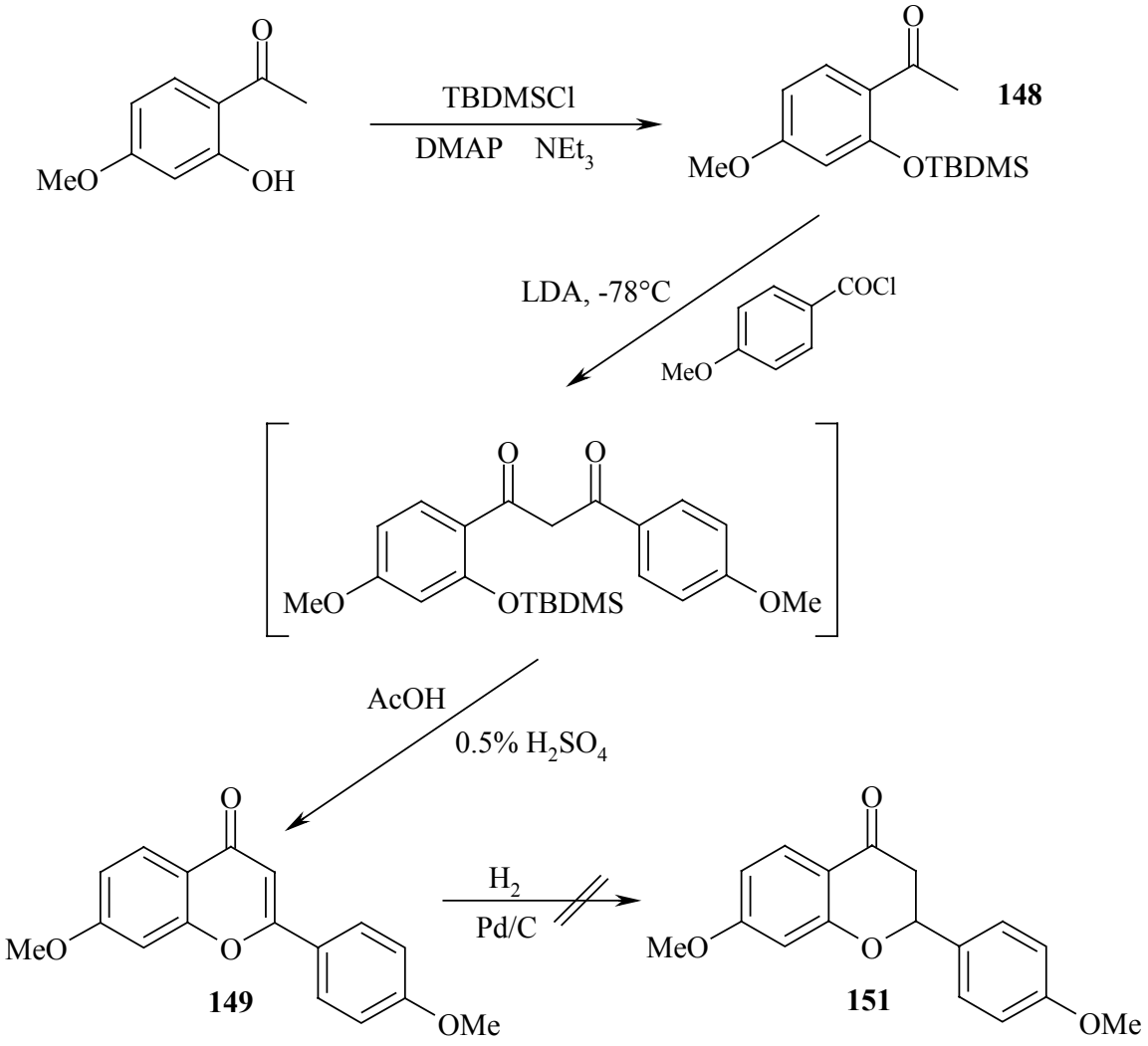


R^1 = functionalised side chain
R^2 = alkyl

Figure C75: Substituted 2-Phenylbenzopyrans

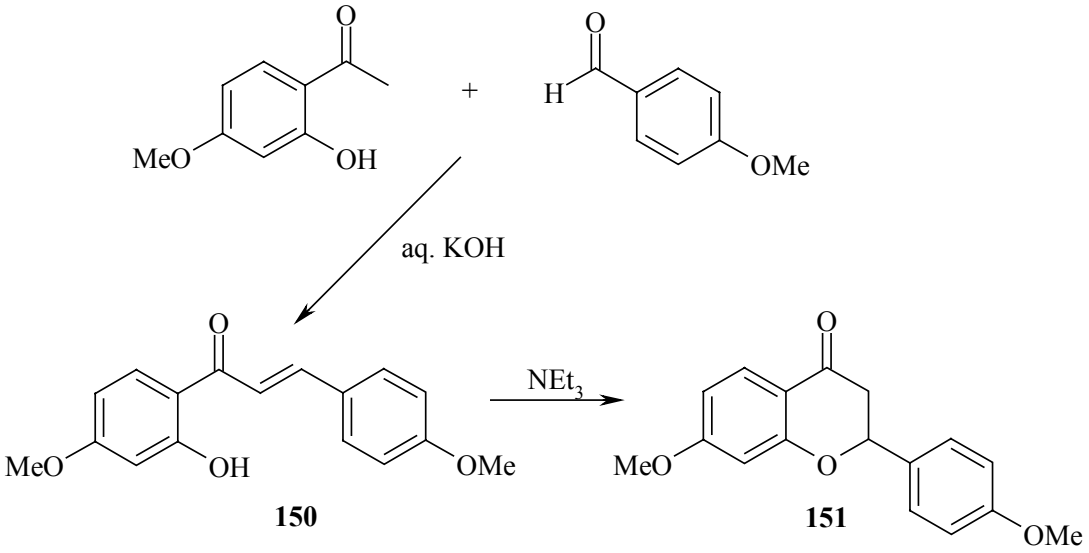
The first attempt to 2-phenylbenzopyrans started from commercially available 2-hydroxy-4-methoxyacetophenone, which was protected at the hydroxy group using *tert*-butyldimethyl-

silylchloride and DMAP. The resulting silylether **148** was acylated with 4-methoxyanisic acid chloride using LDA at –78°C. Treatment of the intermediary 1,3-propandione with glacial acetic acid containing 0.5% H_2SO_4 resulted in cleavage of the siloxy group followed by cyclisation and dehydration to the flavone **149** [Ismail and Aziem, 2001]. Unfortunately, the required flavanone **151** was not accessible through Pd/C catalysed hydrogenation (cf. scheme C76).

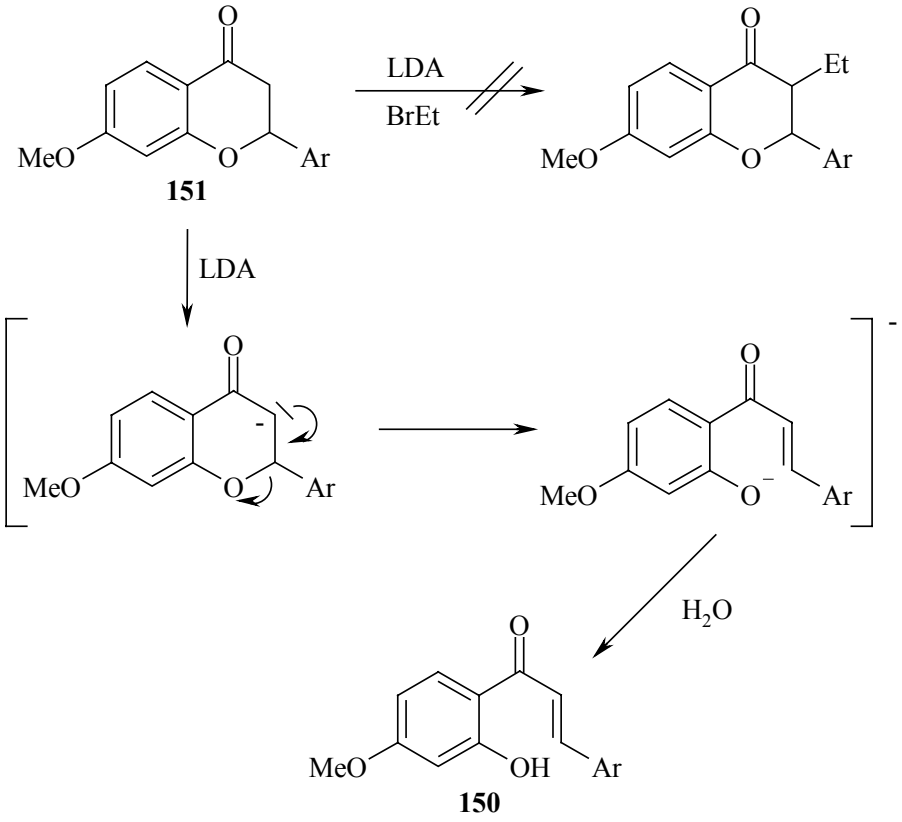


Scheme C76: Synthetic approach to 2-phenylbenzopyran via flavones

Flavanones can also be synthesised by cyclisation of the corresponding chalcones using acidic or alkaline conditions. The chalcone precursor **150** was prepared by a Claisen-Schmidt condensation of 2-hydroxy-4-methoxyacetophenone with 4-methoxybenzaldehyde [Salmen, 2003], which was isomerised into the flavanone **151** by refluxing with equimolar amounts of triethylamine in ethanol (cf. scheme C77) [Aitmambetov et al., 2001].



Scheme C77: Synthesis of the flavanone **151**



Scheme C78: Re-isomerisation to the chalcone **150**

The subsequent attempt to introduce an alkyl substituent in position C-3 of the flavanone **151** with LDA and an alkyl halide resulted in quantitative re-isomerisation to the chalcone **150**.

Upon deprotonation in α-position of the carbonyl group, the expected enolate did not form. Instead, the free electron pair was stabilised by formation of the conjugated chalcone system. The phenolate as good leaving group facilitated this reaction, which was irreversible under the conditions used (cf. scheme C78).

D Summary and Discussion

The objective of this study was the search for new agents, that can be applied to the treatment of hormone-dependent mammary carcinomas. These compounds should display a preference for one of the two estrogen receptor isoforms ERα and ERβ, which have been shown to possess distinct tissue distribution profiles and functions in the body. The ERα was found to be the predominant estrogen receptor protein in malignant mammary tumours. Steroidal fulvestrant is the only pure antiestrogen in the clinics for the treatment of this malignancy, but it lacks subtype selectivity. Thus, pure antiestrogens with selectivity for ERα would be the drugs of choice for the treatment of breast cancer patients, who have become resistant to prior endocrine therapy. Potent ERβ-selective antagonists would represent an useful tool to elucidate the functions of this receptor isoform, which are not yet completely understood.

In order to discover new structures that have not yet been investigated as ligands for the ER, virtual screening with the software programme LUDI was performed to select promising structures from a large database. Five compounds containing either a bridged bicyclic core structures or a 1,1-diaryl motif were chosen for primary testing. An ethanoanthracene-based compound was synthesised through the Diels-Alder reaction and synthetically modified with phenolic hydroxy groups. None of the investigated compounds produced sufficient binding affinity for the estrogen receptor for various discussed reasons including a disadvantageous geometry and reduced flexibility. The high binding affinities proposed by LUDI resulted from an overestimation of hydrophobic interactions of the ligands with the receptor. The results of this investigation have been confirmed by reported studies on compounds with similar structural features. Therefore, a further computer-aided or chemical refinement of the identified structures was not considered.

1 Synthesis

A number of non-steroidal compounds have been identified as ER ligands with a variable degree of subtype selectivity for ERα. The preference for one or the other receptor isoform depends mainly on the structure of the core. In this study furan- or pyran-based heterocycles

linked with two hydroxylated phenyl rings were chosen as carrier molecules. These core structures, including 2,5- and 2,4-diphenylfurans, 2-phenylbenzo[b]furans and 3-phenyl-1-benzopyrans, were chemically modified with long aliphatic side chains incorporating appropriate functional groups in order to convert them into pure antiestrogens.
The functionalised side chains that should guarantee full antagonism were adopted from 2-phenylindole- and stilbene-based antiestrogens that have demonstrated good results in previous studies of our research group. Three of them comprise a length of 16 atoms with one or two functional groups in defined positions. The two monofunctional side chains possess a sulfanyl or sulfonyl group in position 11 from the core. The bifunctional side chain has in addition to this sulfanyl group a basic methylamine function in place of the methylene group in position 7. A fourth side chain with a spacer of 6 methylene groups between the core and a terminal pyrrolidine ring was also synthesised.

In the synthesis of antiestrogens based on the 2,5-bis(4-hydroxphenyl)furan scaffold the side chains were completely introduced prior to the furan cyclisation, because the C-3 position in the aromatic furan ring is known for its limited reactivity due the rather low acidity of the β-protons. The synthetic strategy to the desired 3,4-dialkyl-2,5-diphenylfurans comprised the formation of 1,4-dicarbonyl compounds from arylketones and α-bromoarylketones and subsequent acid catalysed cyclisation. The free phenols were obtained by demethylation of the methoxy-protected furans with BBr_3.

The 2,4-diphenylfuran system provides the position C3 and C-5 in the furan ring to be linked with the long side chain. Both isomers were accessible via the synthesis of appropriate epoxide intermediates. The direct introduction of the complete functional side chain into position C-5 of the aromatic was attempted, but failed for various discussed reasons. Therefore a similar strategy as applied in the synthesis of 2,5-diphenylfurans was pursued and the side chain attached to appropriate ketones and α-bromoketones prior to the ring formation. The epoxides were prepared from the corresponding enolates of the aryl ketones, which exclusively added to the carbonyl function of the α-bromoketones at –78°C and then intramolecularly substituted the bromine atom when the temperature had been raised to –10°C. In analogy to the 2,5-diphenylfurans, the epoxides were converted into the 3,5-dialkyl-2,4-bis(4-hydroxyphenyl)furans by acid catalysed cyclisation and cleavage of the methoxy protecting groups.

A variety of benzanellated five-membered heterocycles have been synthesised in our group, but some functionalised side chains have not been introduced into 6-hydroxy-2-(4-hydroxy-

phenyl)benzo[b]furans, yet. The 2-phenylbenzo[b]furan system leaves only one position in the heterocycle free to be equipped with an aliphatic side chain. These 3-alkylated benzofurans were prepared by a modified procedure. For the preparation of 2,4-dimethoxyphenylacetic acid from 2,4-dimethoxyacetophenone the Willgerodt-Kindler reaction, which produced low yields of product and substantial amounts of a thiophene by-product, was replaced by an oxidation utilizing thallium(III) nitrate. The phenylacetic acid derivative was reacted in a Friedel-Crafts acylation with anisole to a 1,2-diarylethanone, which was subsequently α-alkylated with short alkyl groups or the respective functional side chains. The action of BBr_3 on these alkylated ketones resulted in cleavage of the methyl ethers and cyclisation to heteroaromatic system.
Furthermore, a synthesis of 5-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene comprising a sequence of six reactions was developed to complete a series of benzothiophenes, that were re-evaluated in respect to their selectivity for one or the other ER subtype.

Ring enlargement of the benzofuran system lead to 3-phenyl-substituted 1-benzopyran-2-ones and the reduced 1-benzopyran analogues, which were linked with the functional side chain in position C-4. The precursors for the synthesis of the benzopyranones were 2-hydroxphenylketones already carrying the long aliphatic side chains. These were prepared by Friedel-Crafts acylation with 1,3-dimethoxybenzene and the respective acid chlorides followed by a selective demethylation of the *ortho*-methoxy group with BBr_3. The benzopyranone core was constructed by condensation of the 2-hydroxyphenylketones with carbonyl diimidazole activated derivatives of phenylacetic acid in the presence of anhydrous K_2CO_3 and DMAP.
The 1-benzopyrans with the monofunctional side chains were synthesised by introduction of the side chain with the Grignard reaction into THP-protected benzopyran-4-ones, which were prepared from the corresponding deoxybenzoin by the Mannich reaction. The 1-benzopyrans with the bifunctional side chain were synthesised by reduction of the THP-protected 1-benzopyran-2-ones and subsequent re-cyclisation under Mitsunobu conditions.

2 Biological Characterisation

The biological characterisation of the new ligands of the estrogen receptor comprises the determination of binding affinities for a native estrogen receptor isolated from calf uteri and for the two recombinant ER subtypes α and β. Compounds with sufficient affinity were tested for

antiproliferative activity in estrogen-sensitive human MCF-7 breast cancer cells and in hormone-independent MDA-MB-231 breast cancer cells in order to detect non-specific cytotoxic drug actions. The estrogenic and antiestrogenic activity of the test compounds was quantified in the luciferase assay using hormone-dependent MCF-7/2a cells that have been stably transfected with a luciferase reporter gene under the control of an estrogen response element.
The radiometric binding assay with the full-length human receptors ERα and ERβ was established as a new assay in our research group. It allows the determination of the binding affinity and selectivity for one or the other ER isoform. The work-up to separate the excess of radioactivity differs from the assay applying the natural receptor source, because the recombinant receptors are substantially smaller and are removed by the DCC method. Therefore the receptor-ligand-complex formed during equilibration is absorbed with hydroxylapatite (HAP) and is subsequently washed free of any unbound radioactivity. After resuspension of the HAP pellet the bound radioactivity can be counted.

Prior to the synthesis of ligands with long functionalised side chains compounds substituted with simple alkyl groups were investigated for their binding affinities to the ER. This should provide information about the preference of the core structure for one or the other receptor isoform. The binding data of this initial study showed, that the 2,5-diphenylfurans displayed a preference for ERα, whereas the isomeric 2,4-diphenylfurans were selective for the other estrogen receptor subtype. Both the benzo[b]furans and the benzo[b]thiophenes demonstrated also a preference for ERβ with a similar degree of selectivity as the 2,4-diphenylfurans. The series of benzothiophenes displayed much higher binding affinities for both ER subtypes than the analogous benzofurans. Obviously, the sulfur in the heterocyclic ring provides a more favourable interaction with the receptor than the smaller oxygen atom. The highest selectivities of about 20-fold for ERβ was found for the unsubstituted derivatives in each series, which reflects the smaller internal volume of the ERβ ligand binding pocket. With increasing length of the alkyl substituents attached to the core the selectivity for both ER isoforms dropped.
In the luciferase assay these compounds were found to be agonists. In general, their estrogenic potency increased with increasing length of the alkyl substituent from methyl to propyl. The 3,5-dipropyl-2,4-bis(4-hydroxyphenyl)furan **76d** was the most potent estrogen. It produced full agonistic response in MCF-7/2a cells with an EC_{50}-value of 0.5nM, that was only one order of magnitude higher than that of the natural female sex hormone estradiol. Most of the tested agonists, especially the compounds of the series of benzofurans and benzothiophenes, did not stimulated luciferase expression to the maximum level. The estrogenic activity of the

6-hydroxy-2-phenylbenzothiophenes correlated inversely with the binding affinities to ERβ, which implies an inhibitory effect mediated via ERβ.

On the basis of this study appropriate long aliphatic side chains with diverse functional groups were introduced into the diphenylfuran and benzofuran system in order to develop pure antiestrogens. Beyond this, the benzofuran core heterocycle was extended by one carbon atom and also equipped with these functionalised side chains. The detailed *in vitro* characterisation of these novel ligands showed, that both the diphenylfuran system and the benzanellated furan and pyran scaffold are appropriate structural cores for the development of pure antiestrogens. The antagonistic potency of these compounds depends on the core structure and the type of the functional side chain.

The compounds carrying the monofunctional side chains with the sulfanyl group were usually less active than the corresponding sulfones. The presence of an additional basic nitrogen function in the side chain generally produced a further increase in the antagonistic potency of the compounds. The compounds with a pyrrolidinohexyl side chain suppressed the basal luciferase expression in MCF-7/2a cells only to the level of the partial antiestrogen 4-hydroxytamoxifen, which confirmed the partial antagonistic character of this side chain. Almost all compounds carrying side chains with a length of 16 carbon atoms, especially those with the two functional groups incorporated, achieved suppression levels comparable to that of pure antiestrogen fulvestrant.

The influence of the core on the antiproliferative and antiestrogenic activity of the compounds became evident by comparison of the 2,5-diphenylfurans and the 3-phenylbenzopyrans. The activities of the sulfones in the 2,5-diphenylfuran series were by one order of magnitude lower than those of the compounds with the bifunctional side chain. In contrast, the activity of the sulfone in the 3-phenylbenzopyran series was only reduced by a factor of 2 compared to its bifunctional analogue. Basically, the structure of the core determines the exact position of the ligand in the active site of the receptor, which in turn determines the alignment of the side chain and the optimal interactions of the incorporated functional groups with respective amino acids of the receptor. The comparison of the corresponding sulfides of the 2,5-diphenylfuran and the 3-phenylbenzopyran series adds further evidence to this finding.

What effects has the introduction of a long functional side chain on the affinity and subtype selectivity of the compounds? The structural core of the ligand was found to be the main determinant of selectivity for ERα or ERβ, whereas the functional side chain had only minor

influence. In the series of the 2,5-diphenylfurans the selectivity for ERα was retained when one alkyl group was replaced by the long side chain. Contrary, the β-selectivity of the 2,4-diphenylfurans and benzofurans was diminished or completely lost. This shows that the long functionalised side chain fits better into the binding pocket of ERα than ERβ. In the series of the 2,5-diphenylfurans an influence of short alkyl groups on subtype selectivity was observed. The selectivity for ERα dropped substantially when the methyl group was replaced by ethyl. The various functional groups of the long aliphatic side chains affected the affinity to the both ER subtypes to the same extent. The binding affinities of sulfides were generally lower than that of the corresponding sulfones and the compounds with side chains incorporating a basic nitrogen generally bound with the highest affinities to the estrogen receptor. This finding correlates with the antiestrogenic potencies discussed above and shows that strong receptor binding is prerequisite for a high activity mediated by the ER.

The antiestrogens with the bifunctional side chain were generally the most potent compounds in each series. For comparison their data are presented in table D1.

Ar-$(CH_2)_6N(CH_3)(CH_2)_3SC_5H_{11}$						
Comp.	Ar	**RBA (ERα)**	**RBA (ERβ)**	**Sel. α/β**	**Antiprolif. activity IC_{50} [µM]**	**Antiestrog. activity IC_{50} [µM]**
44c	2,5-Diphenylfuran-3-yl	18	3.4	5.3	0.02	0.05
77	2,4-Diphenylfuran-3-yl	8.0	8.5	0.9	0.02	0.02
80	2,4-Diphenylfuran-5-yl	11	9.3	1.2	0.04	0.02
101	Benzofuran-3-yl	30	19	1.6	0.1	0.2
147	1-Benzopyran-4-yl	42	29	1.4	0.03	0.03
	Fulvestrant	5.3	3.1	1.7	0.004	0.003

Table D1: Comparison of the antiestrogens with the bifunctional side chain in respect to binding affinity, selectivity, antiproliferative and antiestrogenic activity

The 2,5-bis(4-hydroxyphenyl)furan **44c** with an ethyl substituent was the only pure antiestrogen with a marked prefernce for ERα. Its antiproliferative and antiestrogenic activity were each only by one order of magnitude lower than that of fulvestrant, but the selectivity for ERα was by a factor of 3 higher. The isomeric 2,4-bis(4-hydroxyphenyl)furans **77** and **80** with the

side chain in position C-3 and C-5 were both as potent as compound **44c**, but devoid of selectivity for one or the other ER isoform. The 7-hydroxy-3-(4-hydroxyphenyl)-1-benzopyran **147** displayed a higher binding affinity than the diphenylfurans, but no significant selectivity for ERα. Its antiproliferative and antiestrogenic activity, however, were in the same range as those of the diphenylfurans. The corresponding antiestrogen **101** based on the 6-hydroxy-2-(4-hydroxyphenyl)benzo[b]furan scaffold was by one order of magnitude less active than the benzopyran derivative **147**, despite its comparable binding affinity. A possible explanation for these discrepancies between binding data and cellular activities might be the difference in the experimental conditions of the assays. The binding assay was performed in a cell-free system, whereas in the antiproliferation and transactivation assay intact cells were used.

3 Investigations on the Binding Mode

Molecular modelling studies were performed to investigate the binding mode of the synthesised antiestrogens in the active site of the receptor and to rationalise the results of the *in vitro* evaluations. The antiestrogen models show, that the overall orientation of ligands with a bulky side chain is determined by the alignment of this side chain, which is too long to be haboured within the confines of the ligand binding cavity. It adopts the only possible position by reorientating helix H12 and protruding it from the binding pocket. The exact position of the core of the ligand is then determined by the hydrophobic ligand-receptor interaction within the binding site and two hydrogen bonds to the conserved polar amino acids.

Both hydroxy groups in the two aromatic rings of the 2,5-diphenylfurans can form hydrogen bonds to Arg394 and Glu353 on one side of the ligand binding pocket and to the imidazole nitrogen of His524 on the other. This was confirmed by the lower binding affinities of some monophenolic derivatives in this series. The basic nitrogen in aliphatic side chain is capable of forming an hydrogen bridge to Asp351, which explains the higher affinity of the compounds with the bifunctional or pyrrolidinohexyl side chain.

Due to the different arrangement of the substituents in the isomeric 2,4-bis(4-hydroxyphenyl)furan **77**, which carries the side chain in position C-3 of the furan ring, only the hydrogen bond to Arg394 and Glu353 is formed. A hydrogen bond of the second phenyl ring to His524 or to other polar residues in the ligand binding pocket can be excluded. This finding is supported by a comparison of the binding data of the diphenolic compound and its monophe-

nolic analogues, which shows, that the hydroxy group at the 3-phenyl ring hardly contributes to the binding. The complete profile of compound **77** proves, that it is not prerequisite for antiestrogens to be bound by hydrogen bonds to both Arg/Glu and His in the ligand binding pocket to give rise to high activity.

The models of the benzanellated furan structures show, that the phenyl rings of the bicycle mimic the A-ring of estradiol and the phenolic hydroxy groups at these bicycles form hydrogen bonds to both Glu353 and Arg394. The second hydroxy functions at the C-2 phenyl rings are also in hydrogen bonding distance to the imidazole nitrogen of His524, although the oxygen-oxygen-distance of the phenolic hydroxy groups in the benzofuran is about 1Å shorter than that in the diphenylfuran structure. The hydrogen bridges of the basic nitrogen atoms to the carboxy group of Asp351 are confirmed by the higher RBA-values of the corresponding compounds containing a methylamine group in the side chain. The 5-hydroxy-substituted benzofuran adopts a slightly different position than the 6-hydroxy analogue, because the hydrogen bonding contacts to glutamate and arginine forces the ligand deeper into the binding pocket. In comparison to the diphenylfurans the reduced flexibility of the benzofuran scaffold is compensated by the relatively large space in binding pocket and the flexibility of the receptor protein in order to provide a good fit for both series of ligands.

The binding mode of antiestrogens is mainly determined by the alignment of bulky side chains, whereas agonists can avoid high energy conformations caused by steric hindrance or unfavourable ligand-receptor interactions by rotations around the longitudinal axis of the molecule. The observed binding data of the unsubstituted 6-hydroxybenzofuran **97a**, which fit into the series of the 5-hydroxy analogues, make this behaviour likely. A 180° flip around the longitudinal axis of the molecule leads to an orientation similar to the 5-hydroxybenzofurans and probably results in favoured interactions with the amino acids in the active site. Furthermore, small compounds such as the benzofuran **97a** can also benefit from the rotation around the transverse axis of the molecule as additional option to adopt the most favourable orientation in the binding pocket. The latter orientation has been proved for genistein by x-ray cristallography.

E Experimental Section

1 Materials and General Methods

1.1 Chemistry

Chemicals:

The majority of the starting compounds was obtained from ALDRICH, FLUKA and MERCK.

Column - and thin-layer chromatography:

Column chromatography (CC) and thin-layer chromatography (TLC) were performed using the following stationary phases:

CC: MERCK 10832 Geduran® SI 60 silica gel, particle size 0.063 – 0.200mm
FLUKA 6300 aluminiumoxide, neutral (Typ 507 C), particle size 0.05 – 0.15mm

TLC: MERCK 5554 TLC aluminium sheets silica gel 60 F_{254}
MERCK 5550 TLC aluminium sheets aluminium oxide 60 F_{254} neutral (type E)

Elemental analysis:

Elemental analyses of crystalline compounds were performed by the microanalytical laboratory of the University of Regensburg.

Infrared spectroscopy:

Infrared spectra were recorded on a BRUKER Tensor27 FT-IR-spectrometer with ATR-unit. The wave number ν is given in cm^{-1}. The following abbreviations are used to show the intensities of the bands: w = weak; m = moderate; s = strong; br = broad

Melting points:

Melting points were determined on a BÜCHI 510 melting point apparatus and are uncorrected.

Mass spectrometry:

Mass spectra were performed by the analytical laboratory of the University of Regensburg.

Nuclear magnetic resonance spectroscopy:

^{1}H-Nuclear resonance spectra (^{1}H-NMR) and ^{13}C-nuclear resonance spectra (^{13}C-NMR) were recorded on a BRUKER AVANCE300 spectrometer at 300.13MHz and 75.46MHz, respectively, and standardised using the significant signal of the solvents chloroform-d_1, dimethylsulfoxide-d_6 or methanol-d_4. Tetramethylsilane was added as internal standard control. The chemical shift δ is given in ppm. The following abbreviations are used for the characterisation of the peaks: s = singlet; d = duplet; dd = duplet of duplet; t = triplet; q = quartet; quin = quintet; sex = sextet; m = multiplet; br = broad; $^{(n)}$J = coupling constant over n bonds

1.2 Biochemistry

Biochemicals and reagents:

Bradford reagent, protein-assay (BIO-RAD LABORATORIES GmbH)
Charcoal Norit A (SERVA)
Dextran 60, MW: 60000 – 90000 (SERVA)
Dulbecco's Modified Eagles Medium, w/o phenol-red (GIBCO)
Fetal Calf Serum (BIOCHROM)
Geneticin ® (CALBIOCHEM)
Gentamycin (PAN SYSTEMS)
Glutardialdehyde for spectroscopy (MERCK)
Hydroxylapatite Fast Flow (CALBIOCHEM)
L-Glutamine solution, 29mg/ml, 100-fold concentrate (MERCK)
Luciferase Assay System E1500 (PROMEGA)
McCoy's 5A Medium (SIGMA)
Minimal Essential Medium Eagle, w/o sodium hydrogencarbonate (SIGMA)
Penicillin-G sodium-salt, 1647 U/mg (SIGMA)
Rotiszint ecoplus scintillation fluid (ROTH)
Streptomycin sulfate, 750 U/mg (SIGMA)
Trypsin 0.05% with 0.02% EDTA (BOEHRINGER)

Biological material and cell lines

Calf uteri:	For the preparation of cytosol used in the binding studies (butcher's shop LISTL, Regensburg)

ERα:	Human recombinant full length estrogen receptor α (INVITROGEN, formerly PANVERA)
ERβ:	Human recombinant full length estrogen receptor β (INVITROGEN, formerly PANVERA)
MCF-7:	Hormone-dependent human mammary carcinoma cell line (AMERICAN TYPE CULTURE COLLECTION)
MCF-7/2a:	Stably transfected MCF-7 cell clone (Frank Hafner, University of Regensburg)
MDA-MB-231:	Hormone-independent human mammary carcinoma cell line (ATCC)
NMRI mice:	Female, immature mice (CHARLES RIVER WIGA, Sulzfeld)

Buffers and solutions:

Bouin solution:	sat. aq. picric acid (15 ppv) 35% aq. formaldehyde solution (5 ppv) glacial acetic acid (1 ppv)
Bradford reagent:	250mg SERVA Blue G 250ml 95% EtOH 500ml 35% phosphoric acid 250ml water diluted with water (1:5, v/v) before usage
charcoal suspension:	for the binding assay: 0.8% charcoal Norit A and 0.008% dextran 60 in Tris-buffer (pH 7.5) for serum treatment: 5.0% charcoal Norit A and 0.05% dextran 60 in Tris-buffer (pH 7.4)
ER binding buffer:	10mM tris(hydroxymethyl)-aminomethane 10% glycerol 2mM DTT 1mg/ml BSA adjusted to pH 7.5 with HCl
ERα wash buffer:	40mM tris(hydroxymethyl)-aminomethane 100mM KCl adjusted to pH 7.5 with HCl
ERβ wash buffer:	40mM tris(hydroxymethyl)-aminomethane adjusted to pH 7.5 with HCl

HAP equilibration buffer:	50mM tris(hydroxymethyl)-aminomethane adjusted to pH 7.4 with HCl
HAP slurry:	HAP in HAP equilibration buffer (1:1, v/v)
PBS-buffer:	8.0g/l NaCl 2.0g/l KCl 1.0g/l $Na_2HPO_4 \cdot 2H_2O$ 0.15g/l $NaH_2PO_4 \cdot H_2O$ 0.2g/l KH_2PO_4
TED-Mo-buffer:	10.0mM tris(hydroxymethyl)-aminomethane 10.0mM sodium molybdate 1.0mM EDTA 0.5mM DTE adjusted to pH 7.4 with HCl
Tris-buffer (pH 7.4):	10.0mM tris(hydroxymethyl)-aminomethane adjusted to pH 7.4 with HCl
Tris-buffer (pH 7.5):	10.0mM tris(hydroxymethyl)-aminomethane 1.0mM EDTA 3.0mM sodium azide adjusted to pH 7.5 with HCl

Consumable items:

6-Well-macroplates, sterile (FALCON)

96-Well-microtiterplates, sterile (GREINER)

Plastic Pasteur pipettes (RENNER)

Polystyrene centrifuge tubes 55476 (SARSTEDT)

Polystyrene cuvettes 67742 (SARSTEDT)

Polypropylene tubes, sterile (GREINER)

Reaction vessels (EPPENDORF)

Cell culture flasks, 75 cm^2, sterile (NUNC)

Reference compounds:

17β-estradiol:	1,3,5(10)-estratrien-3,17β-diol (SIGMA)
[2,4,6,7-^{3}H]-17β-estradiol:	[2,4,6,7-^{3}H]-1,3,5(10)-estratrien-3,17β-diol (NEW ENGLAND NUCLEAR and AMERSHAM BIOSCIENCES LTD.)

4,4-hexestrol:	*meso*-3,4-bis(4-hydroxyphenyl)hexane
4-hydroxytamoxifen:	(Z)-1-[4-(2-dimethylaminoethoxy)phenyl]-1-(4-hydroxy-phenyl)-2-phenylbut-1-ene kindly provided by Prof. Dr. Peter W. Jungblut from Hannover
Fulvestrant (ICI 182.780):	7α-[9-(4,4,5,5,5-pentafluoropentylsufinyl)nonyl]-17β-estradiol kindly provided by Prof. Dr. Martin R. Schneider from Berlin (SCHERING AG)

Technical devices:

CO_2-Incubator Auto-Zero (HERAEUS)
CO_2-Incubator Biocenter 2001 (SALVIS)
Centrifuge Biofuge 13 (HERAEUS)
Centrifuge Minifuge T (HERAEUS)
Fluorescence spectrophotometer LS 50B (PERKIN ELMER)
Liquid scintillation counter LS6500 (BECKMANN)
Luminometer Lumat LB 9501 (BERTHOLD)
Microplate Autoreader EL 309 (BIO-TEK)
Microscope Diavert (LEITZ)
Milli Q Water System (MILLIPORE)
pH-meter 530 (WTW)
Ultracentrifuge TGA-45 (KONTRON)
Ultraturrax homogenisator (IKA)
UV spectrophotometer Uvikon 930 (KONTRON)

2 Chemical Methods and Analytical Data

2.1 Synthesis of Ligands Derived From Virtual Screening

2.1.1 Bridged Anthracene Derivatives

Crotonic acid (**1**)

Ethyl crotonate (100mmol) and potassium hydroxide (175mmol) in a mixture of EtOH/H_2O (3:1 v/v, 160ml) were refluxed for 4h. The solution was cooled to room temperature, concen-

trated and the white residue was dissolved in water. Upon acidification with conc. HCl and standing at room temperature slowly a white solid formed, that was collected by filtration and washed with small volumes of cold water.

Colourless solid; yield: 94%

Melting point: 70-71°C

$C_4H_6O_2$ (86.09)

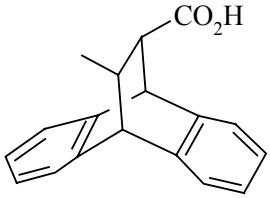
IR: ν (cm^{-1}) = 3400-2300 (m, br; -COOH), 1684 (s; C=O), 1649 (s; C=C)

Analysis: Calculated: C: 55.81 H: 7.03

Found: C: 55.72 H: 6.62

^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.83 (dd; 3H; ^{3}J = 6.9Hz, ^{4}J = 1.6Hz; -C<u>H</u>$_3$), 5.79 (qd; 1H; ^{3}J = 15.5Hz, ^{4}J = 1.6Hz; =C<u>H</u>-CO_2H), 6.83 (qd; 1H; ^{3}J = 6.9Hz, ^{3}J = 15.5Hz; =C<u>H</u>-CH_3), 12.10 (s; 1H; -COO<u>H</u>)

trans-*11-Carboxy-9,10-dihydro-12-methyl-9,10-ethanoanthracene* (**2**)



Anthracene (11mmol) and crotonic acid (11mmol) were mixed in a small, sealed flask and heated with stirring at 200°C for 6h. The resulting brown melt was cooled and boiled in 2N NaOH solution. The mixture was filtered and acidified with 2N H_2SO_4. The precipitated solid was collected by suction, washed with cold water and recrystallised from toluene.

Colourless solid; yield: 59%

Melting point: 188-189°C

$C_{18}H_{16}O_2$ (264.32)

IR: ν (cm^{-1}) = 3300-2500 (m, br; -COOH), 1700 (s; C=O)

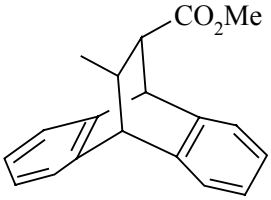
Analysis: Calculated: C: 81.79 H: 6.10

Found: C: 81.75 H: 6.59

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.81 (d; 3H; ^{3}J = 6.9Hz; -C<u>H</u>$_3$), 2.07 (dd; 1H; ^{3}J = 2.3Hz, ^{4}J = 5.4Hz; -C<u>H</u>-CO_2H), 2.20-2.29 (m; 1H; -C<u>H</u>-CH_3), 4.08 (d; 1H; ^{3}J =

2.2Hz; -CH-phenyl), 4.58 (d; 1H; ^{3}J = 2.2Hz; -CH-phenyl), 7.02-7.14 (m; 4H; phenyl-H), 7.21-7.38 (m; 4H; phenyl-H), 12.23 (s; 1H; -COOH)

trans-*9,10-Dihydro-11-methoxycarbonyl-12-methyl-9,10-ethanoanthracene* (**3a**)



Oxalyl chloride (7.6mmol) was added to *trans*-11-carboxy-9,10-dihydro-12-methyl-9,10-ethanoanthracene **2** (3.8mmol) in dry benzene (10ml) and heated at 50°C for 2h until the gas evolution ceased. The solvent and excess oxalyl chloride were distilled off and the resulting colourless oil [100%, IR: ν (cm^{-1}) = 1795 (s; C=O)] was redissolved in dry benzene. Dry MeOH (7.6mmol) was added and the reaction stirred at 55°C for 2h. The mixture was cooled to room temperature and diluted with diethyl ether (50ml). This etheral solution was washed with 10% $NaHCO_3$ and water, dried over Na_2SO_4 and concentrated *in vacuo*.
The crude product was purified by column chromatography (SiO_2; DCM/petroleum ether 40-60 1:1, v/v).

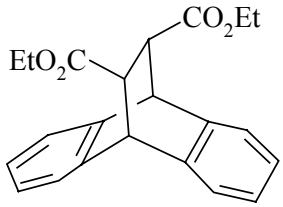
Colourless oil; yield: 81%

$C_{19}H_{18}O_2$ (278.35)

IR: ν (cm^{-1}) = 1734 (s; C=O)

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.82 (d; 3H; ^{3}J = 6.6Hz; -CH-CH$_3$), 2.19 (dd; 1H; ^{3}J = 2.2Hz, ^{4}J = 5.2Hz; -CH-CO_2CH_3), 2.20-2.29 (m; 1H; -CH-CH_3), 3.54 (s; 3H; -CO_2CH_3), 4.10 (d; 1H; ^{3}J = 2.2Hz; -CH-phenyl), 4.59 (d; 1H; ^{3}J = 2.2Hz; -CH-phenyl), 7.03-7.15 (m; 4H; phenyl-H), 7.20-7.40 (m; 4H; phenyl-H)

trans-*11,12-Diethoxycarbonyl-9,10-dihydro-9,10-ethanoanthracene* (**3b**)



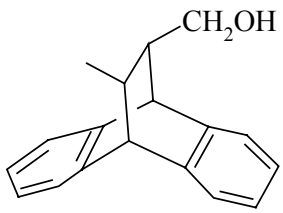
Anthracene (10.0mmol) and ethyl fumarate (10.0mmol) were dissolved in DCM (150ml) and then anhydrous $AlCl_3$ (20.0mmol) added in small portions. The reaction was stirred at room temperature for 18h before it was quenched with $Na_2CO_3x10H_2O$ (20mmol). As soon as the gas evolution had stopped, anhydrous Na_2CO_3 (5g) were added, the mixture filtered and washed with portions of DCM. Subsequently the solvent was removed *in vacuo*.
The resulting yellow solid was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:10, v/v) followed by recrystallisation from a small volume of ethyl acetate.

Colourless needles; yield: 85%
Melting point: 104°C
$C_{22}H_{22}O_4$ (350.41)

IR:	ν (cm^{-1}) = 1724 (s; C=O)		
Analysis:	Calculated:	C: 75.41	H: 6.33
	Found:	C: 75.38	H: 5.92

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.21 (d; 6H; ^{3}J = 7.1Hz; -CH_2-C<u>H</u>$_3$), 3.42 (s; 2H;-C<u>H</u>-CO-), 3.97-4.15 (m; 4H; -C<u>H</u>$_2$-CH_3), 4.73 (s; 2H; -C<u>H</u>-phenyl), 7.05-7.15 (m; 4H; phenyl-<u>H</u>), 7.18-7.26 (m; 2H; phenyl-<u>H</u>), 7.32-7.35 (m; 2H; phenyl-<u>H</u>)

trans-*9,10-Dihydro-11-hydroxymethyl-12-methyl-9,10-ethanoanthracene* (**4a**)



To a suspension of $LiAlH_4$ (3.8mmol) in dry THF (8ml) was added dropwise *trans*-9,10-dihydro-11-methoxycarbonyl-12-methyl-9,10-ethanoanthracene **3a** (2.5mmol) in dry THF (8ml). The reaction mixture was refluxed for 3h and subsequently hydrolysed with water (25ml). The white precipitate was dissolved with HCl and the aqueous phase was extracted with diethyl ether (2x 25ml). The combined organic phases were washed with water and dried over Na_2SO_4. Finally the solvent was removed *in vacuo*.
The crude product was purified by column chromatography (SiO_2; DCM) and recrystallised from toluene.

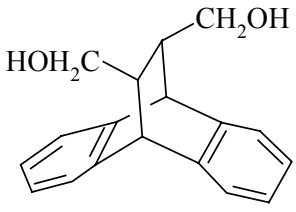
Colourless solid; yield: 93%
Melting point: 146-147°C
$C_{18}H_{18}O$ (250.34)

IR: ν (cm^{-1}) = 3305 (m, br; O-H)

Analysis: Calculated: C: 86.36 H: 7.25
Found: C: 86.17 H: 6.63

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.76 (d; 3H; ^{3}J = 6.7Hz; -CH-C<u>H</u>$_3$), 1.18-1.36 (m; 2H; -C<u>H</u>-CH$_3$, -C<u>H</u>-CH$_2$-), 2.71-2.80 and 3.06-3.13 (m; 2H; -C<u>H</u>$_2$-OH), 3.98 (d; 1H; ^{3}J = 2.0Hz; -C<u>H</u>-phenyl), 4.31 (d; 1H; ^{3}J = 2.0Hz; -C<u>H</u>-phenyl), 4.64 (t; 1H; ^{3}J = 5.2Hz; -O<u>H</u>), 7.05-7.13 (m; 4H; phenyl-<u>H</u>), 7.23-7.31 (m; 4H; phenyl-<u>H</u>)

trans-*9,10-Dihydro-11,12-dihydroxymethyl-9,10-ethanoanthracene* (**4b**)



Preparation from *trans*-11,12-diethoxycarbonyl-9,10-dihydro-9,10-ethanoanthracene **3b** (7.0mmol) as described for compound **4a**, using 3eq of $LiAlH_4$. The crude product was purified by column chromatography (SiO_2; DCM/petroleum ether 40-60 1:1, v/v) followed by recrystallisation from EtOH (99%).

Colourless crystals; yield: 84%

Melting point: 196-197°C

$C_{18}H_{18}O_2$ (266.34)

IR: ν (cm^{-1}) = 3272 (m, br; O-H)

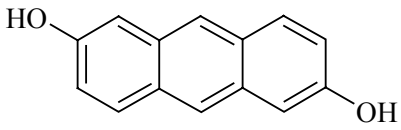
Analysis: Calculated: C: 81.17 H: 6.81

Found: C: 80.95 H: 6.40

^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.26 (dt; 2H; ^{3}J = 2.0Hz, ^{3}J = 5.6Hz; -C<u>H</u>-CH_2-), 2.71-2.80 (m; 2H; -C<u>H</u>$_2$-OH), 3.08-3.15 (m; 2H; -C<u>H</u>$_2$-OH), 4.35 (s; 2H; -C<u>H</u>-phenyl), 4.68 (t; 2H; ^{3}J = 5.1Hz; -O<u>H</u>), 7.05-7.11 (m; 4H; phenyl-<u>H</u>), 7.25-7.30 (m; 4H; phenyl-<u>H</u>)

2.1.2 Hydroxylated Bridged Anthracene Derivatives

2,6-Dihydroxyanthracene (**5**)



2,6-Dihydroxyanthraquinone (10mmol) was suspended in 2N Na_2CO_3 solution (150ml) and $NaBH_4$ (127mmol) was slowly added in small portions. After an period of 3h, until the gas evolution came to an end, the mixture was heated under reflux for about 10min. After cooling, the reaction mixture was acidified with conc. HCl. The precipitated solid was collected by suction and redissolved in acetone. The solvent was dried over Na_2SO_4 evaporated under reduced pressure.

The black crude product was purified by column chromatography (SiO_2; DCM/ethyl acetate 5:1, v/v) and recrystallised from EtOH (99%).

Yellow plates; yield: 74%

Melting point: >250°C

$C_{14}H_{10}O_2$ (210.23)

IR: ν (cm^{-1}) = 3257 (m, br; O-H)

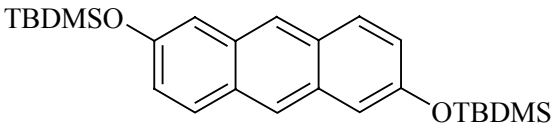
Analysis: Calculated: C: 79.99 H: 4.79

Found: C: 79.49 H: 4.87

^{1}H-NMR (DMSO-d_6): δ (ppm) = 7.09 (dd; 2H; ^{3}J = 9.0Hz, ^{4}J = 2.3Hz; phenyl-<u>H</u>^{3}, phenyl-<u>H</u>^{7}), 7.15 (d; 2H; ^{4}J = 2.3Hz; phenyl-<u>H</u>^{1}, phenyl-<u>H</u>^{5}), 7.84 (d; 2H; ^{3}J =

9.0Hz; phenyl-H[4], phenyl-H[8]), 8.15 (s; 2H; phenyl-H[9], phenyl-H[10]), 9.67 (s; 2H; -OH)

*2,6-Bis(*tert*-butyldimethylsiloxy)anthracene* (**6**)



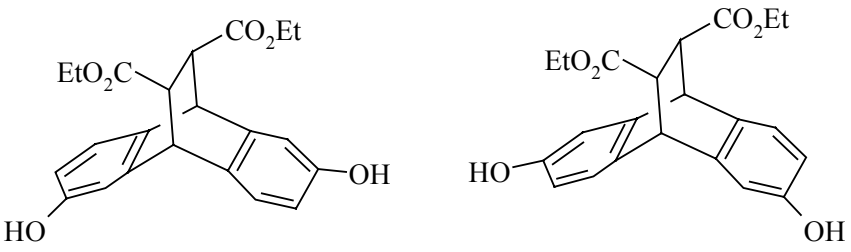
TBDMSCl (15.0mmol) in dry DMF (30ml) was added to a solution of 2,6-dihydroxyanthracene **5** (5.0mmol) and triethylamine (15.0mmol) in dry DMF (50ml). The mixture was stirred at 35°C overnight (15h) and finally poured onto ice-water. The aqueous phase was extracted with DCM (3x50ml). The combined organic phases were washed with water and brine (50ml each). After drying over Na_2SO_4 the solvent was evaporated.
Purification of the crude product was achieved by column chromatography (neutral Al_2O_3; DCM/petroleum ether 40-60 1:10, v/v).

Orange solid; yield: 65%
Melting point: 119-121°C
$C_{26}H_{38}O_2Si_2$ (438.76)

Analysis:	Calculated:	C: 71.17	H: 8.73
	Found:	C: 71.43	H: 7.67

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.27 (s; 12H; -Si-CH_3), 1.03 (s; 18H; -Si-C(-CH_3)), 7.08 (dd; 2H; ^{3}J = 9.0Hz, ^{4}J = 2.4Hz; phenyl-H[3], phenyl-H[7]), 7.26 (d; 2H; ^{4}J = 2.5Hz; phenyl-H[1], phenyl-H[5]), 7.83 (d; 2H; ^{3}J = 9.0Hz; phenyl-H[4], phenyl-H[8]), 8.17 (s; 2H; phenyl-H[9], phenyl-H[10])

(+/-)-trans-*11,12-Diethoxycarbonyl-9,10-dihydro-2,6-dihydroxy-9,10-ethanoanthracene* (**7**)



A mixture of 2,6-bis(*tert*-butyldimethylsiloxy)anthracene **6** (1.14mmol) and ethyl fumarate (11.4mmol) in xylene (30ml) were heated at reflux temperature for 65h. The solvent was removed under reduced pressure and the residue taken up in MeOH (20ml). Conc. HCl (1ml) was added and the reaction stirred at room temperature for 2h. After addition of water and DCM (20ml each) the layers were separated and the aqueous phase extracted with DCM once again. The combined organic layers were washed with water and dried over Na_2SO_4. The solvent was removed *in vacuo*.

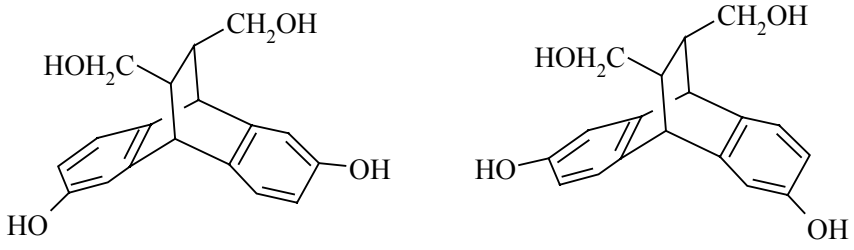
The remaining brown oil was purified by column chromatography (SiO_2; DCM/ethyl acetate 1:10, v/v) yielding the final product as a mixture of two regioisomeric racemates: anti-isomers (9R, 10R, 11R, 12R) and (9S, 10S, 11S, 12S); syn-isomers (9R, 10R, 11S, 12S) and (9S, 10S, 11R, 12R)

Slightly brown solid; yield: 85%

$C_{22}H_{22}O_6$ (382.41)

^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.15 (t; 6H; ^{3}J = 7.0Hz; -CH_2-C<u>H</u>$_3$), 1.16 (t; 6H; ^{3}J = 7.1Hz; -CH_2-C<u>H</u>$_3$), 3.16 (s; 4H; -C<u>H</u>-CO-), 3.91-4.10 (m; 8H; -C<u>H</u>$_2$-CH_3), 4.49 (s; 4H; -C<u>H</u>-phenyl), 6.41-6.46 (m; 4H; phenyl-<u>H</u>^{3}, phenyl-<u>H</u>^{7}) 6.63 (d; 2H; ^{4}J = 2.3Hz; phenyl-<u>H</u>^{1}, phenyl-<u>H</u>^{5}), 6.78 (d; 2H; ^{4}J = 2.3Hz; phenyl-<u>H</u>^{1}, phenyl-<u>H</u>^{5}), 6.98 (d; 2H; ^{3}J = 8.0Hz; phenyl-<u>H</u>^{4}, phenyl-<u>H</u>^{8}), 7.15 (d; 2H; ^{3}J = 8.0Hz; phenyl-<u>H</u>^{4}, phenyl-<u>H</u>^{8}), 9.20 (s; 2H; -OH), 9.24 (s; 2H; -OH)

(+/-)-*trans-9,10-Dihydro-2,6-dihydroxy-11,12-dihydroxymethyl-9,10-ethanoanthracene* (**8**)



Preparation from (+/-)-*trans*-11,12-Diethoxycarbonyl-9,10-dihydro-2,6-dihydroxy-11,12-dihydroxmethyl-9,10-ethanoanthracene **7** (0.78mmol) as described for compound **4a**, using 3eq of $LiAlH_4$. The crude product was purified by column chromatography (SiO_2; ethyl acetate) followed by recrystallisation from ethyl acetate/petroleum ether 40-60, yielding the final

product as a mixture of two regioisomeric racemates: anti-isomers (9R, 10R, 11R, 12R) and (9S, 10S, 11S, 12S); syn-isomers (9R, 10R, 11S, 12S) and (9S, 10S, 11R, 12R)

Colourless solid; yield: 59%

Melting point: 211-214 (dec.)

$C_{18}H_{18}O_4$ (298.34)

IR: ν (cm^{-1}) = 3358 (m, br; O-H), 3134 (m, br; O-H)

Analysis: Calculated: C: 72.47 H: 6.08

Found: C: 69.48 H: 5.86

^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.17 (t; 4H; ^{3}J = 7.0Hz; -C<u>H</u>-CH_2-), 2.69-2.77 und 3.05-3.12 (m; 8H; ^{3}J = 7.1Hz; -CH-C<u>H_2</u>-), 4.05 (s; 2H; -C<u>H</u>-phenyl), 4.07 (s; 2H; -C<u>H</u>-phenyl), 4.60 (t; 4H; ^{3}J = 4.9Hz; -O<u>H</u>), 6.42 (dd; 4H; ^{3}J = 7.9Hz, ^{3}J = 2.3Hz; phenyl-<u>H</u>^{3}, phenyl-<u>H</u>^{7}) 6.64 (d; 2H; ^{4}J = 2.3Hz; phenyl-<u>H</u>^{1}, phenyl-<u>H</u>^{5}), 6.67 (d; 2H; ^{4}J = 2.3Hz; phenyl-<u>H</u>^{1}, phenyl-<u>H</u>^{5}), 6.99 (d; 4H; ^{3}J = 7.9Hz; phenyl-<u>H</u>^{4}, phenyl-<u>H</u>^{8}), 9.04 (s; 2H; -OH), 9.07 (s; 2H; -OH)

2.2 Synthesis of 3,4-Dialkyl-2,5-diarylfurans

2.2.1 Synthesis of Aliphatic Side Chains

2.2.1.1 Synthesis of Monofunctional Side Chains

1-Bromo-10-(pentylsulfanyl)decane (**10**)

Under nitrogen atmosphere pentanethiol (100mmol) in dry DMF (100ml) was added dropwise to a suspension of sodium hydride (60% suspension in paraffin; 110mmol) in dry DMF (110ml) and stirred till the gas evolution ceased. The resulting mixture was filled into a dropping funnel, slowly added to a DMF solution (300ml; 50°C) of 1,10-dibromodecane (300mmol) and stirred at this temperature for another two hours. Excess sodium hydride was decomposed by the addition of water and the product extracted with three portions of ethyl acetate. The organic extract was washed with water and brine and dried over Na_2SO_4. The solvent was removed *in vacuo*.

Unreacted starting material, desired product and the by-product 1,10-bis(pentylsulfanyl)-decane were separated by column chromatography (SiO_2; DCM/petroleum ether 40-60 1:10, v/v). Neither the starting material nor the two products showed quenching of fluorescence on TLC, but staining the TLC plates with sublimating iodine in an iodine chamber reveals the starting material as pink spot and the two sulfur-containing products as yellow spots. The excess of starting material can be recovered quantitatively.

Colourless oil; yield: 27%

$C_{15}H_{31}BrS$ (323.38)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.90 (t; 3H; ^3J = 6.9Hz; -C<u>H</u>$_3$), 1.28-1.45 (m; 16H; -(C<u>H</u>$_2$)$_2$-CH$_3$, -(C<u>H</u>$_2$)$_6$-), 1.52-1.61 (m; 4H; -C<u>H</u>$_2$-CH$_2$-S-CH$_2$-C<u>H</u>$_2$-), 1.85 (quin; 2H; ^3J = 6.9Hz; Br-CH$_2$-C<u>H</u>$_2$-), 2.50 (t; 4H; ^3J = 7.3 Hz; -C<u>H</u>$_2$-S-C<u>H</u>$_2$-), 3.40 (t; 2H; ^3J = 6.9Hz; Br-C<u>H</u>$_2$-)

1-Bromo-10-(pentylsulfonyl)decane (**11**)

A solution of *meta*-chloroperbenzoic acid (27.8mmol) in chloroform (100ml) was added dropwise to 1-brom-10-(pentylsulfanyl)decane **10** (13.9mmol) in chloroform (100ml) and stirred at room temperature for 1h. The mixture was poured into sat. $NaHCO_3$ (200ml) solution and stirred vigorously for 15min. The layers were separated and the organic layer was washed with sat. $NaHCO_3$, water and brine. After drying over Na_2SO_4 the solvent was evaporated.

The obtained product was sufficiently pure without further purification. An analytical sample was recrystallised from ethanol (99%).

Colourless solid; yield: 99%

Melting point: 65-67°C

$C_{15}H_{31}BrO_2S$ (355.38)

Analysis: Calculated: C: 50.70 H: 8.79

Found: C: 50.77 H: 8.43

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.93 (t; 3H; ^3J = 7.1Hz; -C<u>H</u>$_3$), 1.31-1.51 (m; 16H; -(C<u>H</u>$_2$)$_2$-CH$_3$, -(C<u>H</u>$_2$)$_6$-), 1.79-1.91 (m; 6H; -C<u>H</u>$_2$-CH$_2$-SO$_2$-CH$_2$-C<u>H</u>$_2$-, Br-CH$_2$-C<u>H</u>$_2$-), 2.95 (t; 4H; ^3J = 8.1 Hz; -C<u>H</u>$_2$-SO$_2$-C<u>H</u>$_2$-), 3.42 (t; 2H; ^3J = 6.9Hz; Br-C<u>H</u>$_2$-)

2.2.1.2 Synthesis of Bifunctional Side Chains

2.2.1.2.1 General Method for the Preparation of Acid Chlorides

Oxalyl chloride (2eq) was added to the respective carboxylic acid (1eq) in dry benzene and heated at 50°C for about 2h until the gas evolution ceased. The solvent and excess oxalyl chloride were distilled off under reduced pressure.

6-Bromohexanoic acid chloride (**12**)

Colourless oil; yield: 98%

$C_6H_{10}BrClO$ (213.50)

IR: ν (cm^{-1}) = 1793 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.47-1.57 (m; 2H; -C$\underline{H}_2$-), 1.75 (quin; 2H; ^{3}J = 7.5Hz; -CO-CH$_2$-C$\underline{H}_2$-), 1.89 (quin; 2H; ^{3}J = 7.1Hz; -C$\underline{H}_2$-CH$_2$-Br), 2.92 (t; 2H; ^{3}J = 7.2Hz; -CO-C$\underline{H}_2$-), 3.41 (t; 2H; ^{3}J = 6.6Hz; -C$\underline{H}_2$-Br)

8-Bromooctanoic acid chloride (**13**)

Colourless oil; yield: 100%

$C_8H_{14}BrClO$ (213.51)

IR: ν (cm^{-1}) = 1795 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.32-1.49 (m; 6H; -(C$\underline{H}_2$)$_3$-), 1.72 (quin; 2H; ^{3}J = 7.3Hz; -CO-CH$_2$-C$\underline{H}_2$-), 1.85 (quin; 2H; ^{3}J = 7.0Hz; -C$\underline{H}_2$-CH$_2$-Br), 2.89 (t; 2H; ^{3}J = 7.3Hz; -CO-C$\underline{H}_2$-), 3.41 (t; 2H; ^{3}J = 6.8Hz; -C$\underline{H}_2$-Br)

2.2.1.1.2 Preparation of the Amine Function

Ethyl 3-(pentylsulfanyl)propionate (**14**)

Under nitrogen atmosphere and at room temperature, pentanethiol (138mmol) in dry DMF (140ml) was added dropwise to a suspension of sodium hydride (60% suspension in paraffin; 166mmol) in dry DMF (100ml) and stirred till the gas evolution ceased. Then, a DMF solution (140ml) of ethyl 3-bromopropionate (138mmol) was added dropwise and the resulting

solution was stirred at this temperature for another two hours. Excess sodium hydride was decomposed by the addition of water and the product extracted with ethyl acetate (3x200ml). The organic extract was washed with water and brine. After drying over Na_2SO_4 the solvent was removed *in vacuo*.
Purification was achieved by column chromatography (SiO_2; DCM/petroleum ether 40-60 1:1, v/v).

Colourless oil; yield: 82%
$C_{10}H_{20}O_2S$ (204.33)
IR: ν (cm^{-1}) = 1738 (s; C=O)
^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.90 (t; 3H; ^3J = 7.1Hz; -$(CH_2)_4$-C$\underline{H}_3$), 1.27 (t; 3H; ^3J = 7.1Hz; -CH_2-C$\underline{H}_3$), 1.31-1.39 (m; 4H; -(C$\underline{H}_2$)$_2$-CH_3), 1.54-1.64 (m; 2H; -C$\underline{H}_2$-CH_2-S-), 2.50 (t; 2H; ^3J = 7.3Hz; -$(CH_2)_3$-C$\underline{H}_2$-S-), 2.59 (t; 2H; ^3J = 7.4Hz; -C$\underline{H}_2$-CO-), 2.78 (t; 2H; ^3J = 7.4Hz; -S-C$\underline{H}_2$-); 4.15 (q; 2H; ^3J = 7.1Hz; -O-C$\underline{H}_2$-)

3-(Pentylsulfanyl)propionic acid (**15**)

Ethyl 3-(pentylsulfanyl)propionate **14** (112mmol) and potassium hydroxide (196mmol) in a mixture of EtOH/H_2O (3:1 v/v, 200ml) were refluxed for 4h. The solution was cooled to room temperature and concentrated. The remaining white residue was dissolved in water. This aqueous phase was washed with ether (100ml) before it was acidified with conc. HCl. Then, the product was extracted from the aqueous phase with 3 portions of ether (3x100ml). The combined organic extracts were washed with water and brine. After drying over Na_2SO_4 the solvent was evaporated.

Slighly yellow oil; yield: 98%
$C_8H_{16}O_2S$ (176.27)
IR: ν (cm^{-1}) = 3600 bis 2500 (s, br; COOH), 1711 (s; C=O)
^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.90 (t; 3H; ^3J = 7.1Hz; -$(CH_2)_4$-C$\underline{H}_3$), 1.25-1.43 (m; 4H; -(C$\underline{H}_2$)$_2$-CH_3), 1.59 (quin; 2H; ^3J = 7.3Hz; -C$\underline{H}_2$-CH_2-S-), 2.54 (t; 2H; ^3J = 7.3Hz; -$(CH_2)_3$-C$\underline{H}_2$-S-), 2.66 (t; 2H; ^3J = 7.1Hz; -C$\underline{H}_2$-COOH), 2.78 (t; 2H; ^3J = 7.1Hz; -S-C$\underline{H}_2$-)

3-(Pentylsulfanyl)propionic acid chloride (**16**)

Under nitrogen, phosphorus pentachloride (110mmol) was added in small portions to 3-(pentylsulfanyl)propionic acid **15** (110mmol) and stirred for 0.5h at room temperature and another hour at 50°C. The produced phosphorus oxychloride was distilled off under reduced pressure. The remaining oil was three to four times resuspended in dry benzene (40ml) and the benzene was evaporated again to remove any residual $POCl_3$ completely.

Yellow oil; yield: 100%

$C_8H_{15}ClOS$ (194.72)

IR: ν (cm^{-1}) = 1797 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.87 (t; 3H; ^3J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.22-1.38 (m; 4H; -(C<u>H</u>$_2$)$_2$-CH_3), 1.55 (quin; 2H; ^3J = 7.3Hz; -C<u>H</u>$_2$-CH_2-S-), 2.50 (t; 2H; ^3J = 7.3Hz; -$(CH_2)_3$-C<u>H</u>$_2$-S-), 2.78 (t; 2H; ^3J = 7.2Hz; -S-C<u>H</u>$_2$-), 3.14 (t; 2H; ^3J = 7.2Hz; -C<u>H</u>$_2$-COCl)

N-Methyl-3-(pentylsulfanyl)propionamide ***(17)***

Sodium hydroxide (231mmol) was dissolved into an 40% aqueous solution of methylamine (1.1mol, 93ml) and cooled to – 10°C. 3-(Pentylsulfanyl)propionic acid chloride **16** (110mmol) was added dropwise, so that the inner temperature could be kept below 20°C. After complete addition the reaction mixture was acidified with 15% HCl and extracted with DCM (3x100ml). The combined organic phases were washed with water and brine, dried over Na_2SO_4 and the solvent was evaporated.

The crude product was purified by column chromatography (SiO_2; DCM/ethyl acetate 1:1, v/v). TLC plates were stained with sublimating iodine to reveal the product as yellow spot.

Oranges oil; yield: 89%

$C_9H_{19}NOS$ (189.32)

IR: ν (cm^{-1}) = 3298 (s, br; N-H), 1647 (s; C=O; amide I), 1561 (s; N-H; amide II)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.90 (t; 3H; ^3J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.20-1.41 (m; 4H; -(C<u>H</u>$_2$)$_2$-CH_3), 1.59 (quin; 2H; ^3J = 7.1Hz; -C<u>H</u>$_2$-CH_2-S-), 2.45 (t; 2H;

^3J = 7.2Hz; -$(CH_2)_3$-C<u>H</u>$_2$-S-), 2.53 (t; 2H; ^3J = 7.2Hz; -C<u>H</u>$_2$-CO-), 2.79-2.83 (m; 5H; -S-C<u>H</u>$_2$-,-NH-C<u>H</u>$_3$), 5.86 (s, br; 1H; -N<u>H</u>-CH_3)

N-Methyl-3-(pentylsulfanyl)propylamine (**18**)

Under dry nitrogen atmosphere, N-Methyl-3-(pentylsulfanyl)propionamide **17** (50.0mmol) in dry THF (50ml) was added dropwise to a suspension of $LiAlH_4$ (75.0mmol) in dry THF (75ml). The reaction mixture was refluxed for 3h and then with cooling in an ice-water bath hydrolysed with water (100ml) and sat. $NaHCO_3$ (50ml). The organic components were extracted into ethyl acetate (3x75ml) and this organic phase was washed with 3 portions of 2N HCl (3x75ml). The combined acidic phases were rebasified with 2N NaOH and extracted again with ethyl acetate (3x75ml). The combined organic phases of the second extraction were washed with water and dried over Na_2SO_4. Finally the solvent removed under reduced pressure.

Orange oil; yield: 46%

$C_9H_{21}NS$ (175.33)

IR: ν (cm^{-1}) = 3304 (m, br; N-H);

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.90 (t; 3H; ^3J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$) 1.28-1.42 (m; 4H; -(C<u>H</u>$_2$)$_2$-CH_3), 1.59 (quin; 2H; ^3J = 7.3Hz; -C<u>H</u>$_2$-CH_2-S-), 1.78 (quin; 2H; ^3J = 7.3Hz; -C<u>H</u>$_2$-CH_2-NH-), 2.44 (s; 3H; -NH-C<u>H</u>$_3$); 2.51 (t; 2H; ^3J = 7.4Hz; -C<u>H</u>$_2$-S-), 2.57 (t; 2H; ^3J = 7.4Hz; -NH-$(CH_2)_2$-C<u>H</u>$_2$-S-), 2.68 (t; 2H; ^3J = 7.0Hz; -C<u>H</u>$_2$-NH-)

2.2.1.2.3 Introduction of the Amine Function

At room temperature the acid chloride (1eq) was added dropwise to dry DCM solution of the respective secondary amine (1-2eq) alone or in combination with a tertiary amine (1eq) as proton scavenger and stirred for another 6-7h. The reaction mixture was poured into water and extracted with two portion of diethyl ether. The combined organic phases were washed with water and brine, dried and the solvent evaporated.

6-Bromohexanoic acid methyl-[3-(pentylsulfanyl)propyl]amide (**19**)

Preparation from N-methyl-3-(pentylsulfanyl)propylamine **16** (22.0mmol), N,N-diisopropyl-ethylamine (22.0mmol) and 6-bromohexanoic acid chloride **17** (22.0mmol). The crude red oil was purified by column chromatography (SiO_2; DCM/ethyl acetate 10:1, v/v). TLC plates were stained with sublimating iodine to reveal the product as yellow spot.

Yellow oil; yield: 67%

$C_{15}H_{30}BrNOS$ (352.38)

IR: ν (cm^{-1}) = 1643 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^3J = 7.1Hz; -$(CH_2)_4$-C$\underline{H}_3$) 1.25-1.41 (m; 4H; -(C$\underline{H}_2$)$_2$-CH_3), 1.45-1.73 (m; 6H; -S-CH_2-C$\underline{H}_2$-, -CO-CH_2-(C$\underline{H}_2$)$_2$-), 1.75-1.94 (m; 4H; -C$\underline{H}_2$-CH_2-Br-, -C$\underline{H}_2$-CH_2-N-), 2.30-2.38 (m; 2H; -CO-C$\underline{H}_2$-), 2.47-2.53 (m; 4H; -C$\underline{H}_2$-S-C$\underline{H}_2$-), 2.92/2.99 (s; 3H; -N-C$\underline{H}_3$; E/Z), 3.37-3.47 (m; 4H; -C$\underline{H}_2$-Br, -C$\underline{H}_2$-N-)

N-(6-Bromohexanoyl)pyrrolidine (**20**)

Preparation from 6-bromohexanoic acid chloride **17** (23.4mmol) and pyrrolidine (46.8mmol). The crude product was sufficiently pure without further purification.

Yellowish oil; yield: 88%

$C_{10}H_{18}BrNO$ (248.16)

IR: ν (cm^{-1}) = 1637 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.43-1.54 (m; 2H; -C$\underline{H}_2$-) 1.68 (quin; 2H; ^3J = 7.7Hz; -C$\underline{H}_2$-CH_2-CO-), 1.80-2.00 (m; 6H; -C$\underline{H}_2$-CH_2-Br-, -(C$\underline{H}_2$-CH_2)$_2$-N-), 2.27 (t; 2H; ^3J = 7.3Hz; -CO-C$\underline{H}_2$-), 3.38-3.48 (m; 4H; -C$\underline{H}_2$-Br, -C$\underline{H}_2$-N-C$\underline{H}_2$)

2.2.1.2.4 Finkelstein Reaction

N-(6-Iodohexanoyl)pyrrolidine (**21**)

N-(6-Bromohexanoyl)pyrrolidine **21** (23.4mmol) and sodium iodine (93.6mmol) in acetone (100ml) were refluxed for 24h. The acetone was removed and the residue was taken up in diethyl ether (100ml). The organic solution washed with water, 10% sodium thiosulphate and brine. The solvent was dried over Na_2SO_4 and evaporated. The crude product required no additional purification.

Orange oil; yield: 85%

$C_{10}H_{18}INO$ (295.16)

IR: ν (cm^{-1}) = 1637 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.40-1.51 (m; 2H; -C<u>H</u>$_2$-) 1.68 (quin; 2H; ^{3}J = 7.7Hz; -C<u>H</u>$_2$-CH$_2$-CO-), 1.80-2.00 (m; 6H; -C<u>H</u>$_2$-CH$_2$-I-, -(C<u>H</u>$_2$-CH$_2$)$_2$-N-), 2.27 (t; 2H; ^{3}J = 7.5Hz; -CO-C<u>H</u>$_2$-), 3.20 (t; 2H; ^{3}J = 7.0Hz; -C<u>H</u>$_2$-I-), 3.41 (t; 2H; ^{3}J = 6.8Hz; -C<u>H</u>$_2$-N-), 3.46 (t; 2H; ^{3}J = 6.8Hz; -C<u>H</u>$_2$-N-),

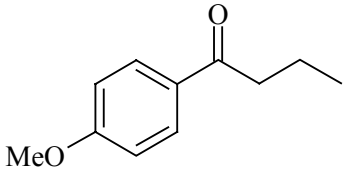
2.2.2 Synthesis of Alkylarylketone Precursors

1-(4-Methoxyphenyl)ethan-1-one (4-methoxyacetophenone; **22a**) and *1-(4-methoxyphenyl)-propan-1-one (4-methoxypropiophenone*; **22b**) are commercially available starting materials.

2.2.2.1 Preparation by Friedel-Crafts Acylation

Under nitrogen atmosphere at 0°C, the respective acid chloride (1.1-1.2eq) was added to a suspension of anhydrous aluminium(III)-chloride (1.1-1.2eq) in dry DCE and stirred at room temperature for 30min. The mixture was cooled to –15°C and the respective aromatic compound (1eq) in dry DCE added dropwise. The reaction was maintained at this temperature for 2h before it was hydrolysed with water/conc. HCl (3:1, v/v). The layers were separated and the aqueous phase extracted with DCM. The combined organic phases were washed with water, sat. $NaHCO_3$-solution and brine, dried over Na_2SO_4 and concentrated *in vacuo.*

1-(4-Methoxyphenyl)butan-1-one (**22c**)



Preparation from dry anisole (92.5mmol) and butyryl chloride (111mmol). The product was sufficiently pure without further purification.

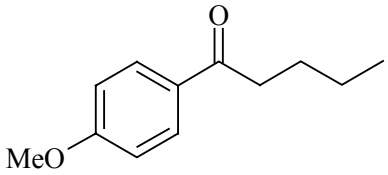
Colourless oil; yield: 95%

$C_{11}H_{14}O_2$ (178.23)

IR: ν (cm^{-1}) = 1674 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.00 (t; 3H; ^3J = 7.4Hz; -CH_2-C$\underline{H}_3$), 1.75 (sex; 2H; ^3J = 7.5Hz; -C$\underline{H}_2$-CH_3), 2.89 (t; 2H; ^3J = 7.4Hz; -CO-C$\underline{H}_2$-), 3.87 (s; 3H; -O-C$\underline{H}_3$), 6.93/7.95 (AA'BB'; 4H; ^3J = 8.9Hz; phenyl-$\underline{H}$)

1-(4-Methoxyphenyl)pentan-1-one (**22d**)



Preparation from dry anisole (92.5mmol) and pentanoyl chloride (111mmol). The product was sufficiently pure without any further purification.

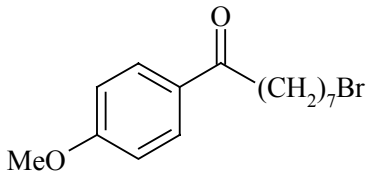
Colourless oil; yield: 99%

$C_{12}H_{16}O_2$ (192.26)

IR: ν (cm^{-1}) = 1675 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.95 (t; 3H; ^3J = 7.3Hz; -CH_2-C$\underline{H}_3$), 1.41 (sex; 2H; ^3J = 7.5Hz; -C$\underline{H}_2$-CH_3), 1.71 (quin; 2H; ^3J = 7.5Hz; -CO-CH_2-C$\underline{H}_2$-), 2.89 (t; 2H; ^3J = 7.4Hz; -CO-C$\underline{H}_2$-), 3.87 (s; 3H; -OC$\underline{H}_3$), 6.93/7.95 (AA'BB'; 4H; ^3J = 8.8Hz; phenyl-$\underline{H}$)

8-Bromo-1-(4-methoxyphenyl)octan-1-one (**23**)



Preparation from dry anisole (40.7mmol) and 8-bromo-octanoic acid chloride **13** (44.8mmol). The crude product was recrystallised from EtOH (99%).

Colourless crystals; yield: 94%

Melting point: 46-47°C

$C_{15}H_{21}BrO_2$ (313.24)

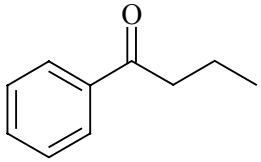
IR: ν (cm^{-1}) = 1669 (s, C=O)

Analysis: Calculated: C: 57.52 H 6.76

Found: C: 57.90 H 6.84

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.37-1.50 (m; 6H; -(C<u>H</u>$_2$)$_3$-), 1.73 (quin; 2H; ^{3}J = 7.3Hz; -CO-CH$_2$-C<u>H</u>$_2$-), 1.86 (quin; 2H; ^{3}J = 7.0Hz; -C<u>H</u>$_2$-CH$_2$-Br), 2.91 (t; 2H; ^{3}J = 7.3Hz; -CO-C<u>H</u>$_2$-), 3.40 (t; 2H; ^{3}J = 6.8Hz; -C<u>H</u>$_2$-Br), 3.87 (s; 3H; -O-C<u>H</u>$_3$), 6.93/7.94 (AA'BB'; 4H; ^{3}J = 8.9Hz; phenyl-<u>H</u>)

1-Phenylbutan-1-one (**27**)



Preparation from dry benzene (50.0mmol) and butyryl chloride (55.0mmol). Due to the melting point of benzene the entire reaction was carried out at room temperature. The product was purified by column chromatography (SiO_2; DCM/petroleum ether 40-60 1:5, v/v).

Colourless oil; yield: 92%

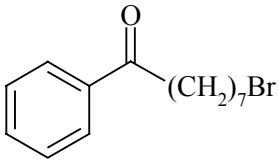
$C_{10}H_{12}O$ (148.21)

IR: ν (cm^{-1}) = 1683 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.01 (t; 3H; ^{3}J = 7.4Hz; -CH$_2$-C<u>H</u>$_3$), 1.77 (sex; 2H; ^{3}J = 7.4Hz; -C<u>H</u>$_2$-CH$_3$), 2.95 (t; 2H; ^{3}J = 7.3Hz; -CO-C<u>H</u>$_2$-), 7.43-7.48 (m;

2H; phenyl-H^3, phenyl-H^5), 7.52-7.58 (m; 1H; phenyl-H^4), 7.95-7.98 (m; 2H; phenyl-H^2, phenyl-H^6)

8-Bromo-1-phenyloctan-1-one (**28**)



Preparation form dry benzene (20.0mmol) and 8-bromooctanoic acid chloride **13** (22.0mmol). Due to the melting point of benzene the entire reaction was carried out at room temperature. The product was purified by column chromatography (SiO_2; DCM/petroleum ether 40-60 1:25, v/v).

Colourless oil; yield: 79%

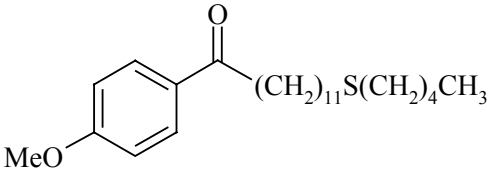
$C_{14}H_{19}BrO$ (283.21)

IR: ν (cm^{-1}) = 1677 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.38-1.50 (m; 6H; -(CH$_2$)$_3$-), 1.75 (quin; 2H; ^{3}J = 7.2Hz; -CO-CH$_2$-CH$_2$-), 1.86 (quin; 2H; ^{3}J = 7.1Hz; -CH$_2$-CH$_2$-Br), 2.97 (t; 2H; ^{3}J = 7.4Hz; -CO-CH$_2$-), 3.41 (t; 2H; ^{3}J = 6.7Hz; -CH$_2$-Br), 7.43-7.49 (m; 2H; phenyl-H^3, phenyl-H^5), 7.53-7.59 (m; 1H; phenyl-H^4), 7.95-7.98 (m; 2H; phenyl-H^2, phenyl-H^6)

2.2.2.2 Preparation by Nucleophilic Substitution

1-(4-Methoxyphenyl)-12-(pentylsulfanyl)dodecan-1-one (**24**)



Under nitrogen atmosphere and with ice cooling, 4-methoxyacetophenone **22a** (55.8mmol) in dry DMF (50ml) was added dropwise to a DMF suspension (50ml) of sodium hydride (60% suspension in paraffin, 55.8mmol) and stirred for 1h. After the reaction mixture had been

cooled to about –45°C, 1-bromo-10-(pentylsulfanyl)decane **10** (18.6mmol) in dry DMF (50ml) was slowly added and the stirring continued for 1h at this temperature and for another 5h at –10°C until the electrophile was completely consumed.

The resulting solution was partitioned between water (200ml) and ethyl acetate (200ml) and the layers separated. The aqueous layer was extracted two times with ethyl acetate (100ml). The combined organic layers were washed with water (100ml) and brine (100ml) and dried over Na_2SO_4.The solvent was removed under reduced pressure.

Purification of the crude product was achieved by column chromatography (SiO_2, ethyl acetate/petroleum ether 40-60 1:10, v/v).

Colourless plates; yield: 60%

Melting point: 48-49°C

$C_{24}H_{40}O_2S$ (392.64)

IR: ν (cm^{-1}) = 1673 (s; C=O)

Analysis: Calculated: C: 73.42 H 10.27

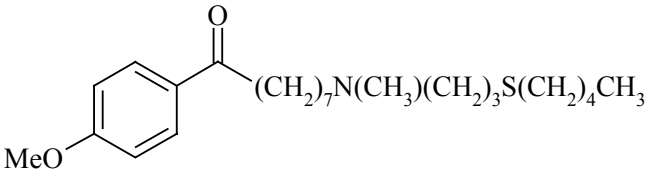
Found: C: 73.35 H 10.33

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.90 (t; 3H; ^{3}J = 6.9Hz; -C<u>H</u>$_3$), 1.27-1.77 (m; 24H; -(C<u>H</u>$_2$)$_3$-CH$_3$, -(C<u>H</u>$_2$)$_9$-CH$_2$-S-), 2.49 (t; 4H; ^{3}J=7.4Hz; -C<u>H</u>$_2$-S-C<u>H</u>$_2$-), 2.90 (t; 2H; ^{3}J = 7.4Hz; -C<u>H</u>$_2$-CO-), 3.87 (s; 3H; -O-C<u>H</u>$_3$), 6.93/7.94 (AA'BB'; 4H; ^{3}J = 8.9Hz; phenyl-<u>H</u>)

2.2.2.3 Introduction of the Amine Function

A solution of the respective ω-bromoketone (1eq) and the respective secondary amine (1-2eq) alone or in combination with triethylamine (1eq) in dry EtOH (20ml) was refluxed for 24h. The mixture was cooled, poured into 10% sodium bicarbonate solution (50ml) and extracted with EtOAc (3x50ml). The combined organic phases were washed with water and brine (50ml each) and dried over Na_2SO_4.

1-(4-Methoxyphenyl)-8-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}octan-1-one (**25**)



Preparation from 8-bromo-1-(4-methoxyphenyl)octan-1-one **23** (9.5mmol) and N-methyl-3-(pentylsulfanyl)propylamine **16** (9.5mmol). Purification of the crude product was achieved by column chromatography (SiO_2; ethyl acetate/MeOH 3:1, v/v).

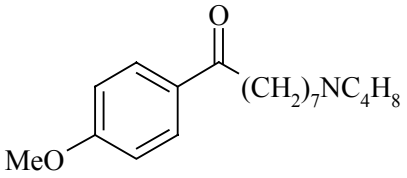
Yellow oil; yield: 66%

$C_{24}H_{41}NO_2S$ (407.66)

IR: ν (cm^{-1}) = 1678 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^3J = 7.1Hz; -$(CH_2)_4$-C$\underline{H}_3$), 1.22-1.41 (m; 10H; -(C$\underline{H}_2$)$_2$-CH_3, -(C$\underline{H}_2$)$_3$-), 1.50-1.63 (m; 4H; -C$\underline{H}_2$-CH_2-N-, -C$\underline{H}_2$-CH_2-S-), 1.72 (quin; 2H; ^3J = 7.3Hz; -N-CH_2-C$\underline{H}_2$-CH_2-S-), 1.84 (quin; 2H; ^3J = 7.3Hz; -CO-CH_2-C$\underline{H}_2$-), 2.33 (s; 3H; -N-C$\underline{H}_3$), 2.44-2.60 (m; 8H; -C$\underline{H}_2$-N-C$\underline{H}_2$-CH_2-C$\underline{H}_2$-S-C$\underline{H}_2$), 2.90 (t; 2H; ^3J = 7.4Hz; -CO-C$\underline{H}_2$-), 3.87 (s; 3H; -OC$\underline{H}_3$), 6.93/7.94 (AA'BB'; 4H; ^3J = 8.9Hz; phenyl-$\underline{H}$)

1-(4-Methoxyphenyl)-8-pyrrolidinyloctan-1-one (**26**)



Preparation from 8-bromo-1-(4-methoxyphenyl)octan-1-one **23** (9.5mmol) and pyrrolidine (19.0mmol). From NMR analysis the crude product was sufficiently pure for further transformations.

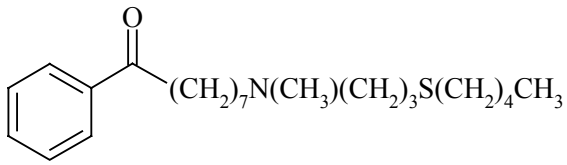
Brownish oil; yield: 96%

$C_{19}H_{29}NO_2$ (303.45)

IR: ν (cm^{-1}) = 1673 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.36-1.43 (m; 6H; -(C$\underline{H}_2$)$_3$-), 1.57 (quin; 2H; ^3J = 7.2Hz; -C$\underline{H}_2$-CH_2-N-), 1.71 (quin; 2H; ^3J = 7.3Hz; -CO-CH_2-C$\underline{H}_2$-), 1.78-1.87 (m; 4H; -(C$\underline{H}_2$-CH_2)$_2$-N-), 2.50 (t; 2H; ^3J = 7.8Hz;-C$\underline{H}_2$-N-), 2.60 (s, br; 4H; -C$\underline{H}_2$-N-C$\underline{H}_2$-), 2.91 (t; 2H; ^3J = 7.3Hz; -CO-C$\underline{H}_2$-), 3.87 (s; 3H; -OC$\underline{H}_3$), 6.93/7.94 (AA'BB'; 4H; ^3J = 8.9Hz; phenyl-$\underline{H}$)

8-{N-Methyl-N-[3-(pentylsulfanyl)-propyl]-amino}-1-phenyloctan-1-one (**29**)



Preparation from 8-bromo-1-phenyloctan-1-one **28** (10.2mmol) and N-methyl-3-(pentylsulfanyl)propylamine **16** (10.2mmol). Purification of the crude product was achieved by column chromatography (SiO_2; ethyl acetate/MeOH 6:1, v/v).

Yellow oil; yield: 58%

$C_{24}H_{41}NO_2S$ (407.66)

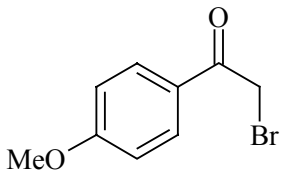
IR: ν (cm^{-1}) = 1677 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^3J = 7.0Hz; -$(CH_2)_4$-C$\underline{H}_3$), 1.31-1.43 (m; 10H; -(C$\underline{H}_2$)$_2$-CH_3, -(C$\underline{H}_2$)$_3$-), 1.50 (quin; 2H; ^3J = 7.2Hz;-C$\underline{H}_2$-CH_2-S-), 1.58 (quin; 2H; ^3J = 7.2Hz;-C$\underline{H}_2$-CH_2-N-), 1.74 (quin; 2H; ^3J = 7.5Hz; -N-CH_2-C$\underline{H}_2$-CH_2-S-), 1.79 (quin; 2H; ^3J = 7.5Hz; -C$\underline{H}_2$-CH_2-CO-), 2.26 (s; 3H; -N-C$\underline{H}_3$), 2.38 (t; 2H; ^3J = 7.5Hz; -NC$\underline{H}_2$-), 2.48 (merged t; 2H; -NC$\underline{H}_2$-), 2.51 (t; 2H; ^3J = 7.3Hz; -SC$\underline{H}_2$-), 2.54 (t; 2H; ^3J = 7.3Hz; -SC$\underline{H}_2$-), 2.97 (t; 2H; ^3J = 7.4Hz; -CO-C$\underline{H}_2$-), 7.43-7.49 (m; 2H; phenyl-$\underline{H}^3$, phenyl-$\underline{H}^5$), 7.53-7.59 (m; 1H; phenyl-$\underline{H}^4$), 7.94-7.98 (m; 2H; phenyl-$\underline{H}^2$, phenyl-$\underline{H}^6$)

2.2.3 Synthesis of α-Bromoketone Precursors

A solution of alkylarylketone (1eq) in conc. acetic acid or a mixture of diethyl ether/dioxan (1:2, v/v) was supplemented with 5-10 drops of HBr (48%) and cooled in an ice bath to 0°C. Then, bromine (1eq) was added in such a rate, that the reaction mixture could decolorise after each addition. After stirring at this temperature for another 30min, the reaction mixture was poured onto ice-water (100ml). If the product formed crystals, it was collected by suction and washed free of bromine with cold water. Otherwise, the aqueous phase was extracted with ether (3x100ml) and the combined organic phases were washed with water (2x100ml) and brine (100ml) After drying over Na_2SO_4 the solvent was evaporated.

2-Bromo-1-(4-methoxphenyl)ethan-1-one (**30a**)



Preparation from commercially available 4-methoxyacetophenone **22a** (100mmol), using diethyl ether/dioxan as solvent mixture. Purification by recrystallisation from EtOH (99%).

Colourless needles; yield: 74%

Melting point: 68-69°C

$C_9H_9BrO_2$ (229.07)

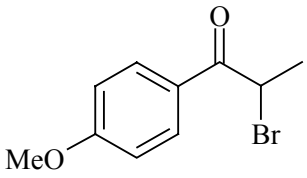
IR: ν (cm^{-1}) = 1686 (s; C=O)

Analysis: Calculated: C: 47.19 H 3.96

Found: C: 47.10 H 4.01

^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.89 (s; 3H; -O-C<u>H</u>$_3$), 4.40 (s; 2H; -C<u>H</u>$_2$-Br), 6.96/7.97 (AA'BB'; 4H; ^{3}J = 9.0Hz; phenyl-<u>H</u>)

2-Bromo-1-(4-methoxphenyl)propan-1-one (**30b**)



Preparation from commercially available 4-methoxypropiophenone **22b** (200mmol), using acetic acid as solvent. Purification by recrystallisation from EtOH (99%).

Colourless crystals; yield: 74%

Melting point: 66°C

$C_{10}H_{11}BrO_2$ (243.10)

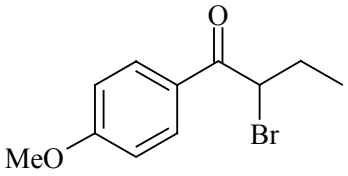
IR: ν (cm^{-1}) = 1665 (s; C=O)

Analysis: Calculated: C: 49.41 H 4.56

Found: C: 49.39 H 4.49

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.89 (d; 3H; ^{3}J = 6.6Hz; -C<u>H</u>$_3$), 3.88 (s; 3H; -O-C<u>H</u>$_3$), 5.26 (q; 1H; ^{3}J = 6.6Hz; -C<u>H</u>-Br), 6.96/8.01 (AA'BB'; 4H; ^{3}J = 9.0Hz; phenyl-<u>H</u>)

2-Bromo-1-(4-methoxphenyl)butan-1-one (**30c**)



Preparation from 1-(4-methoxyphenyl)butan-1-one **22c** (87.8mmol), using acetic acid as solvent. Purification by recrystallisation from EtOH (99%).

Colourless crystals; yield: 70%

Melting point: 48-49°C

$C_{11}H_{13}BrO_2$ (257.13)

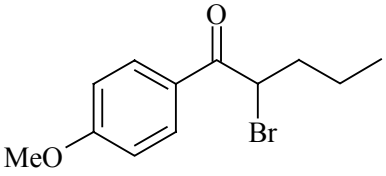
IR: ν (cm^{-1}) = 1662 (s; C=O)

Analysis: Calculated: C: 51.38 H 5.10

Found: C: 51.04 H 5.14

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.07 (t; 3H; ^{3}J = 7.4Hz; -C<u>H</u>$_3$), 2.05-2.31 (m; 2H; -C<u>H</u>$_2$-), 3.89 (s; 3H; -O-C<u>H</u>$_3$), 5.04 (dd; 1H; ^{3}J = 6.5Hz, ^{3}J = 7.8Hz; -C<u>H</u>-Br); 6.97/8.01 (AA'BB'; 4H; ^{3}J = 9.1Hz; phenyl-<u>H</u>)

2-Bromo-(4-methoxyphenyl)pentan-1-one (**30d**)



Preparation from 1-(4-methoxyphenyl)pentan-1-one **22d** (50.0mmol), using conc. acetic acid as solvent. Purification by column chromatography (SiO_2; DCM/petroleum ether 40-60 1:3, v/v) and recrystallisation from EtOH (99%).

Colourless solid; yield: 77%

Melting point: 49-50°C

$C_{12}H_{15}BrO_2$ (271.15)

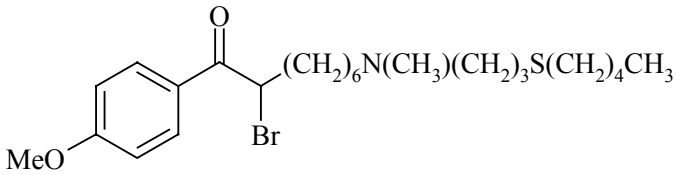
IR: ν (cm^{-1}) = 1664 (s; C=O)

Analysis: Calculated: C: 53.16 H 5.58

Found: C: 52.98 H 5.35

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.98 (t; 3H; ^{3}J = 6.9Hz; C<u>H</u>$_3$-), 1.34-1.65 (m; 2H; -C<u>H</u>$_2$-CH_3), 2.03-2.23 (m; 2H; -CHBr-C<u>H</u>$_2$-), 3.88 (s; 3H; -O-C<u>H</u>$_3$), 5.12 (dd; 1H; ^{3}J = 6.6Hz, ^{3}J = 7.6Hz; -C<u>H</u>-Br), 6.96/8.00 (AA'BB'; 4H; ^{3}J = 9.0Hz; phenyl-<u>H</u>)

2-Bromo-1-(4-methoxyphenyl)-8-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}octan-1-one (**31**)



Preparation from 1-(4-methoxyphenyl)-8-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}-octan-1-one **25** (3.7mmol), using diethyl ether/dioxane as solvent mixture and 5% $NaHCO_3$ solution for the work-up. The crude product was purified by column chromatography (SiO_2; ethyl acetate/MeOH 3:1, v/v).

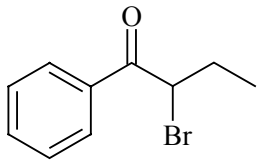
Yellow oil; yield: 80%

$C_{24}H_{40}BrNO_2S$ (486.55)

IR: ν (cm^{-1}) = 1678 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^{3}J = 7.0Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.25-1.53 (m; 12H; -(C<u>H</u>$_2$)$_2$-CH_3, -(C<u>H</u>$_2$)$_4$-), 1.58 (quin; 2H; ^{3}J = 7.3Hz; -S-CH_2-C<u>H</u>$_2$-), 1.74 (quin; 2H; ^{3}J = 7.3Hz; -S-CH_2-C<u>H</u>$_2$-CH_2-N-), 2.04-2.26 (m; 2H; -CHBr-C<u>H</u>$_2$-), 2.21 (s; 3H; -N-C<u>H</u>$_3$-), 2.31 (t; 2H; ^{3}J = 7.3Hz; -N-C<u>H</u>$_2$-), 2.41 (t; 2H; ^{3}J = 7.3Hz; -N-C<u>H</u>$_2$-), 2.50 (t; 2H; ^{3}J = 7.3Hz; -S-C<u>H</u>$_2$-), 2.53 (t; 2H; ^{3}J = 7.3Hz; -N-C<u>H</u>$_2$-), 3.89 (s; 3H; -O-C<u>H</u>$_3$), 5.10 (dd; 1H; ^{3}J = 6.9Hz, ^{3}J = 7.4Hz; -C<u>H</u>-Br), 6.96/8.00 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>)

2-Bromo-1-phenylbutan-1-one (**32**)



Preparation from 1-phenylbutan-1-one **27** (45.0mmol), using acetic acid as solvent. The product was sufficiently pure without any further purification.

Yellow oil; yield: 47%

$C_{10}H_{11}BrO$ (227.10)

IR: ν (cm^{-1}) = 1683 (s; C=O)

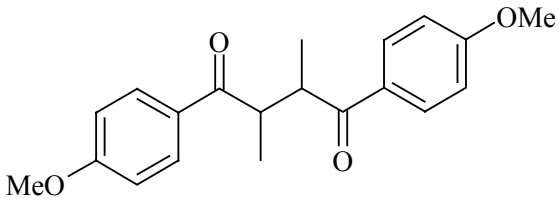
^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.09 (t; 3H; ^3J = 7.4Hz; C$\underline{H}_3$-), 2.07-2.33 (m; 2H; -C$\underline{H}_2$-), 5.08 (dd; 1H; ^3J = 6.4Hz, ^3J = 7.8Hz; -C$\underline{H}$-Br); 7.47-7.53 (m; 2H; phenyl-$\underline{H}^3$, phenyl-$\underline{H}^5$), 7.58-7.63 (m; 1H; phenyl-$\underline{H}^4$), 8.01-8.05 (m; 2H; phenyl-$\underline{H}^2$, phenyl-$\underline{H}^6$)

2.2.4 Synthesis of 1,4-Dicarbonyl Compounds

2.2.4.1 General Prodecure

Under nitrogen and with cooling (~ -45°C), KHMDS (1.1eq of a 0.5M solution in toluene) was added to a solution of an alkylaryketone (1.0eq) in dry DMF. After stirring for 1h, the respective α-bromoketone (1.1eq) in dry DMF was added dropwise. After the addition stirring was continued at –45°C for 2h. Finally the reaction mixture was hydrolysed with water and extracted three times with ethyl acetate. The combined organic layers were washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent and subsequent purification by column chromatography or recrystallisation often afforded the product as a mixture of stereoisomers – two enantiomers (2R,3R; 2S,3S) and the respective *meso*-compound in case of symmetrical compounds or two pairs of diasteromeric enantiomers (2R,3R; 2S,3S; 2R,3S; 2S,3R) –, which were not separated because chirality is lost in the following cyclisation step.

1,4-Bis(4-methoxyphenyl)-2,3-dimethylbutan-1,4-dione (**33b**)



Preparation from 4-methoxypropiophenone **22b** (6.5mmol) and 2-bromo-(4-methoxyphenyl)-propan-1-one **30b** (7.2mmol). The yellow crude product was recrystallised from ethyl acetate and petroleum ether 40-60.

Colourless solid; yield: 63%

Melting point: 117°C

$C_{20}H_{22}O_4$ (326.39)

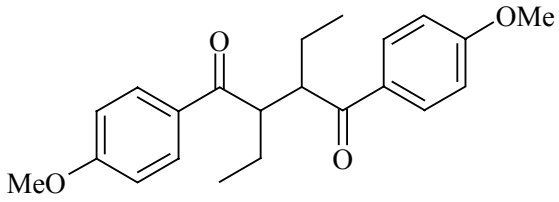
IR: ν (cm^{-1}) = 1659 (s; C=O)

Analysis: Calculated: C: 73.60 H 6.79

Found: C: 73.79 H 7.13

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.27 (d; 6H; ^3J = 6.8Hz; C<u>H</u>$_3$-), 3.86 (s; 6H; -O-C<u>H</u>$_3$), 3.87-3.94 (m; 2H; -C<u>H</u>-), 6.93/7.97 (AA'BB'; 8H; ^3J = 9.1Hz; phenyl-<u>H</u>)

2,3-Diethyl-1,4-bis(4-methoxyphenyl)butan-1,4-dione (**33c**)



Preparation from 1-(4-methoxyphenyl)butan-1-one **22c** (8.4mmol) and 2-bromo-(4-methoxyphenyl)butan-1-one **30c** (9.2mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60, 1:5, v/v).

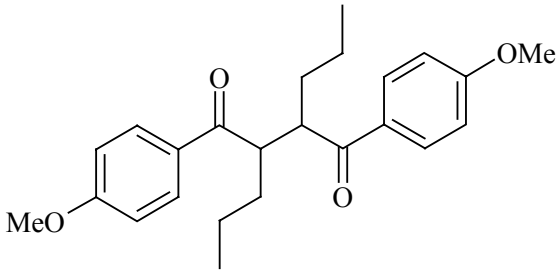
Colourless oil; yield: 94%

$C_{22}H_{26}O_4$ (354.45)

IR: ν (cm^{-1}) = 1664 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.82 (t; 6H; ^{3}J = 7.4Hz; C<u>H</u>$_3$-CH$_2$-), 1.75-1.85 (m; 4H; -C<u>H</u>$_2$-CH-), 3.86 (s; 6H; -O-C<u>H</u>$_3$), 3.94-4.02 (m; 2H; -C<u>H</u>-CH$_2$-), 6.91/7.95 (AA'BB'; 8H; ^{3}J = 8.8Hz; phenyl-<u>H</u>)

1,4-Bis(4-methoxyphenyl)-2,3-dipropylbutan-1,4-dione (**33d**)



Preparation from 1-(4-methoxyphenyl)pentan-1-one **22d** (4.4mmol) and 2-bromo-(4-methoxyphenyl)pentan-1-one **30d** (4.8mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60, 1:8, v/v).

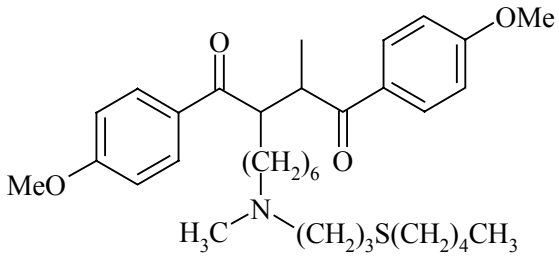
Colourless oil; yield: 89%

$C_{24}H_{30}O_4$ (382.50)

IR: ν (cm^{-1}) = 1660 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.84 (t; 6H; ^{3}J = 7.2Hz; C<u>H</u>$_3$-CH$_2$-), 1.05-1.33 (m, 4H, CH$_3$-C<u>H</u>$_2$-), 1.65-1.79 (m; 4H; -C<u>H</u>$_2$-CH-), 3.85 (s; 6H; -O-C<u>H</u>$_3$), 3.91-3.99 (m; 2H; -C<u>H</u>-CH$_2$-), 6.90/7.92 (AA'BB'; 8H; ^{3}J = 9.0Hz; phenyl-<u>H</u>)

1,4-Bis(4-methoxyphenyl)-3-methyl-2-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}-hexyl}butan-1,4-dione (**34b**)



Preparation from 1-(4-methoxyphenyl)-8-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}-octan-1-one **25** (3.7mmol) and 2-bromo-(4-methoxyphenyl)propan-1-one **30b** (4.1mmol). Sat. sodium bicarbonate solution was used for the aqueous work-up. The crude product was purified by column chromatography (SiO_2; ethyl acetate/methanol 5:1, v/v).

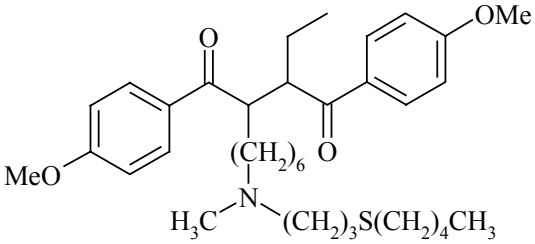
Yellow oil; yield: 72%

$C_{34}H_{51}NO_4S$ (569.85)

IR: ν (cm^{-1}) = 1669 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^3J = 7.0Hz; -$(CH_2)_4$-C$\underline{H}_3$), 1.15-1.48 (m; 15H; -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_4$-(CH_2)-N-, -CH-C$\underline{H}_3$), 1.58 (quin; 2H; ^3J = 7.3Hz; -C$\underline{H}_2$-CH_2-S-), 1.67-1.85 (m; 4H; -N-CH_2-C$\underline{H}_2$-CH_2-S-, -CH-C$\underline{H}_2$-), 2.23 (s; 3H; -N-C$\underline{H}_3$), 2.31-2.56 (m; 8H; -C$\underline{H}_2$-N-C$\underline{H}_2$-CH_2-C$\underline{H}_2$-S-C$\underline{H}_2$-), 3.85 (s; 3H; -O-C$\underline{H}_3$), 3.86 (s; 3H; -O-C$\underline{H}_3$), 3.87-4.01 (m; 2H; -C$\underline{H}$-CH_3, -C$\underline{H}$-$(CH_2)_6$-N-), 6.91/7.92 (AA'BB'; 4H; ^3J = 8.9Hz; phenyl-$\underline{H}$), 6.92/7.96 (AA'BB'; 4H; ^3J = 8.9Hz; phenyl-$\underline{H}$)

3-Ethyl-1,4-bis(4-methoxyphenyl)-2-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-butan-1,4-dione (**34c**)



Preparation from 1-(4-methoxyphenyl)-8-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}-octan-1-one **25** (1.7mmol) and 2-bromo-(4-methoxyphenyl)butan-1-one **30c** (1.9mmol). Sat. sodium bicarbonate solution was used for the aqueous work-up. The crude product was purified by column chromatography (SiO_2; ethyl acetate/methanol 9:1, v/v).

Yellow oil; yield: 51%

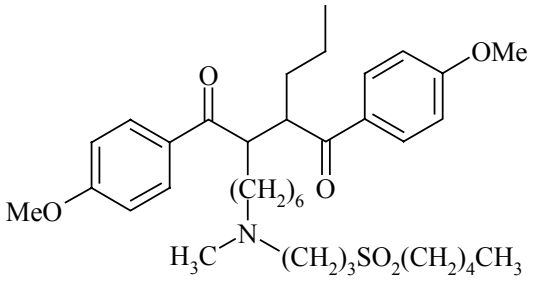
$C_{35}H_{53}NO_4S$ (583.87)

IR: ν (cm^{-1}) = 1666 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.81 (t; 3H; ^3J = 7.1Hz; -CH_2-C$\underline{H}_3$), 0.89 (t; 3H; ^3J = 7.0Hz; -$(CH_2)_4$-C$\underline{H}_3$), 1.21-1.47 (m; 12H; -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_4$-(CH_2)-N-),

1.58 (quin; 2H; ^{3}J = 7.2Hz; -C<u>H</u>$_2$-CH$_2$-S-), 1.69-1.85 (m; 6H; -N-CH$_2$-C<u>H</u>$_2$-CH$_2$-S-, -CH-C<u>H</u>$_2$-CH$_3$, -CH-C<u>H</u>$_2$-), 2.19 (s; 3H; -N-C<u>H</u>$_3$), 2.28 (t; 2H; ^{3}J = 7.4Hz; -N-C<u>H</u>$_2$-), 2.41 (t; 2H; ^{3}J = 7.4Hz; -N-C<u>H</u>$_2$-), 2.50 (t; 2H; ^{3}J = 7.3Hz; -S-C<u>H</u>$_2$-), 2.50 (t; 2H; ^{3}J = 7.3Hz; -S-C<u>H</u>$_2$-), 3.85 (s; 3H; -O-C<u>H</u>$_3$), 3.86 (s; 3H; -O-C<u>H</u>$_3$), 3.87-4.02 (m; 2H; -C<u>H</u>-CH$_2$-CH$_3$, -C<u>H</u>-(CH$_2$)$_6$-N-), 6.90/7.93 (AA'BB'; 4H; ^{3}J = 8.9Hz; phenyl-<u>H</u>), 6.91/7.94 (AA'BB'; 4H; ^{3}J = 8.9Hz; phenyl-<u>H</u>)

1,4-Bis(4-methoxyphenyl)-2-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-3-propylbutan-1,4-dione (**34d**)



Preparation from 1-(4-methoxyphenyl)-8-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}-octan-1-one **25** (2.5mmol) and 2-bromo-(4-methoxyphenyl)pentan-1-one **30d** (2.8mmol). Sat. sodium bicarbonate solution was used for the aqueous work-up. The crude product was purified by column chromatography (SiO_2; ethyl acetate/methanol 9:1, v/v).

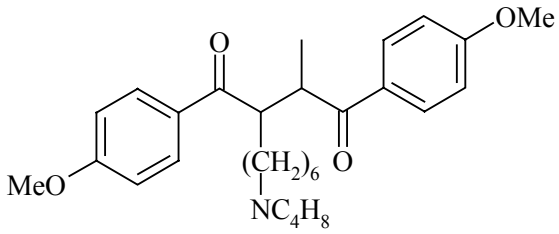
Yellow oil; yield: 48%

$C_{36}H_{55}NO_4S$ (597.90)

IR: ν (cm^{-1}) = 1666 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.83 (t; 3H; ^{3}J = 7.1Hz; -(CH$_2$)$_2$-C<u>H</u>$_3$), 0.89 (t; 3H; ^{3}J = 7.0Hz; -(CH$_2$)$_4$-C<u>H</u>$_3$), 1.07-1.47 (m; 14H; -C<u>H</u>$_2$-CH$_3$, -(C<u>H</u>$_2$)$_2$-CH$_3$, -(C<u>H</u>$_2$)$_4$-(CH$_2$)-N-), 1.57 (quin; 2H; ^{3}J = 7.3Hz; -C<u>H</u>$_2$-CH$_2$-S-), 1.70-1.79 (m; 6H; -N-CH$_2$-C<u>H</u>$_2$-CH$_2$-S-, -CH-C<u>H</u>$_2$-, -CH-C<u>H</u>$_2$-), 2.20 (s; 3H; -N-C<u>H</u>$_3$), 2.28 (t; 2H; ^{3}J = 7.3Hz; -N-C<u>H</u>$_2$-), 2.39 (t; 2H; ^{3}J = 7.3Hz; -N-C<u>H</u>$_2$-), 2.50 (t; 2H; ^{3}J = 7.3Hz; -S-C<u>H</u>$_2$-), 2.50 (t; 2H; ^{3}J = 7.3Hz; -S-C<u>H</u>$_2$-), 3.85 (s; 6H; -O-C<u>H</u>$_3$), 3.88-3.98 (m; 2H; -C<u>H</u>-CH$_2$-CH$_3$, -C<u>H</u>-(CH$_2$)$_6$-N-), 6.90/7.91 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 6.90/7.92 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>)

1,4-Bis(4-methoxyphenyl)-3-methyl-2-(6-pyrrolidinylhexyl)butan-1,4-dione (**35b**)



Preparation from 1-(4-methoxyphenyl)-8-pyrrolidinyloctan-1-one **26** (3.8mmol) and 2-bromo-(4-methoxyphenyl)propan-1-one **30b** (4.2mmol). Sat. sodium bicarbonate solution was used for the aqueous work-up. The crude product was purified by column chromatography (SiO_2; DCM/methanol 1:1, v/v).

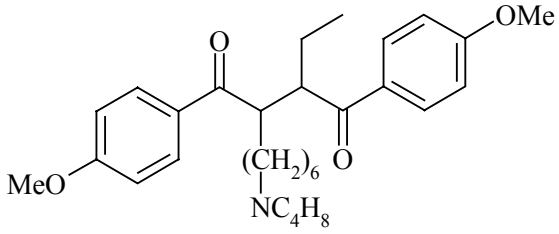
Yellow oil; yield: 67%

$C_{29}H_{39}NO_4$ (465.63)

IR: ν (cm^{-1}) = 1667 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.20-1.26 (m; 11H; -CH-C<u>H</u>$_3$, -(C<u>H</u>$_2$)$_4$-), 1.40-1.49 (m; 2H; -C<u>H</u>$_2$-CH$_2$-N-), 1.71-1.82 (m; 4H; -N-(CH$_2$-C<u>H</u>$_2$)-), 2.38 (t; 2H; ^{3}J = 7.8Hz; -N-C<u>H</u>$_2$-), 2.49 (s, br; 4H; -N-(C<u>H</u>$_2$)-), 3.85 (s; 3H; -O-C<u>H</u>$_3$), 3.86 (s; 3H; -O-C<u>H</u>$_3$), 3.89-4.03 (m; 2H; -C<u>H</u>-CH$_3$, -C<u>H</u>-(CH$_2$)$_6$-N-), 6.91/7.93 (AA'BB'; 4H; ^{3}J = 8.9Hz; phenyl-<u>H</u>), 6.92/7.97 (AA'BB'; 4H; ^{3}J = 8.9Hz; phenyl-<u>H</u>)

3-Ethyl-1,4-bis(4-methoxyphenyl)-2-(6-pyrrolidinylhexyl)butan-1,4-dione (**35c**)



Preparation from 1-(4-methoxyphenyl)-8-pyrrolidinyloctan-1-one **26** (4.8mmol) and 2-bromo-(4-methoxyphenyl)butan-1-one **30c** (5.3mmol). Sat. sodium bicarbonate solution was used for the aqueous work-up. The crude product was purified by column chromatography (SiO_2; DCM/methanol 1:1, v/v).

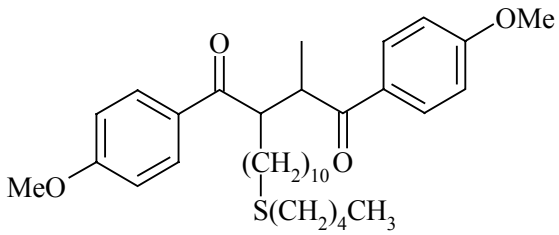
Yellow oil; yield: 49%

$C_{30}H_{41}NO_4$ (479.66)

IR: ν (cm^{-1}) = 1667 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.81 (t; 3H; ^{3}J = 7.5Hz; -CH_2-C$\underline{H}_3$), 1.18-1.25 (m; 6H; -(C$\underline{H}_2$)$_3$-), 1.41-1.51 (m; 2H; -C$\underline{H}_2$-CH_2-N-), 1.71-1.85 (m; 8H; -C$\underline{H}_2$-CH_3, -CH-C$\underline{H}_2$, -N-(CH_2-C$\underline{H}_2$)-), 2.39 (t; 2H; ^{3}J = 7.8Hz; -N-C$\underline{H}_2$-), 2.51 (s, br; 4H; -N-(C$\underline{H}_2$)-), 3.85 (s; 3H; -O-C$\underline{H}_3$), 3.86 (s; 3H; -O-C$\underline{H}_3$), 3.92-4.01 (m; 2H; -C$\underline{H}$-CH_2-, -C$\underline{H}$-(CH_2)$_6$-N-), 6.90/7.93 (AA'BB'; 4H; ^{3}J = 8.7Hz; phenyl-$\underline{H}$), 6.91/7.94 (AA'BB'; 4H; ^{3}J = 8.7Hz; phenyl-$\underline{H}$)

1,4-Bis(4-methoxyphenyl)-3-methyl-2-[10-(pentylsulfanyl)decyl]butan-1,4-dione (**36b**)



Preparation from 1-(4-methoxyphenyl)-12-(pentylsulfanyl)dodecan-1-on **24** (1.3mmol) and 2-bromo-(4-methoxyphenyl)propan-1-one **30b** (1.4mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:10, v/v).

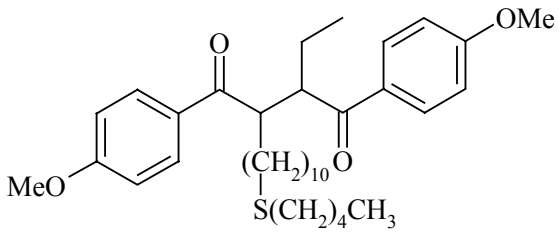
Light yellow oil; yield: 71%

$C_{34}H_{50}O_4S$ (554.83)

IR: ν (cm^{-1}) = 1668 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^{3}J = 7.0Hz; -(CH_2)$_4$-C$\underline{H}_3$), 1.18-1.75 (m; 27H; -(C$\underline{H}_2$)$_3$-CH_3, -(C$\underline{H}_2$)$_9$-, -CH-C$\underline{H}_3$), 2.48 (t; 2H; ^{3}J = 7.4Hz; -C$\underline{H}_2$-S-), 2.49 (t; 2H; ^{3}J = 7.4Hz; -S-C$\underline{H}_2$-), 3.85 (s; 3H; -O-C$\underline{H}_3$), 3.86 (s; 3H; -O-C$\underline{H}_3$), 3.87-4.03 (m; 2H; -C$\underline{H}$-(CH_2)$_{10}$-, -C$\underline{H}$-CH_3), 6.91/7.93 (AA'BB'; 4H; ^{3}J = 9.1Hz; phenyl-H); 6.92/7.96 (AA'BB'; 4H; ^{3}J = 9.1Hz; phenyl-H)

3-Ethyl-1,4-bis(4-methoxyphenyl)-2-[10-(pentylsulfanyl)decyl]butan-1,4-dione (**36c**)



Preparation from 1-(4-methoxyphenyl)-12-(pentylsulfanyl)dodecan-1-on **24** (2.6mmol) and 2-bromo-(4-methoxyphenyl)butan-1-one **30c** (2.9mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:10, v/v).

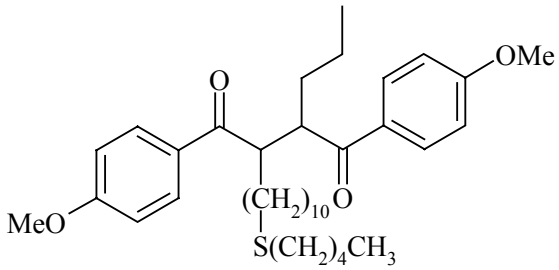
Light yellow oil; yield: 50%

$C_{35}H_{52}O_4S$ (568.86)

IR: ν (cm^{-1}) = 1667 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.85 (t; 3H; ^3J = 7.4Hz; -CH_2-C$\underline{H}_3$), 0.93 (t; 3H; ^3J = 7.0Hz; -$(CH_2)_4$-C$\underline{H}_3$), 1.14-1.45 (m; 18H; -(C$\underline{H}_2$)$_2$-CH_3, -(C$\underline{H}_2$)$_7$-), 1.54-1.66 (m; 4H; -C$\underline{H}_2$-CH_2-S-CH_2-C$\underline{H}_2$-), 1.75-1.89 (m; 4H; -CH-C$\underline{H}_2$-, -CH-C$\underline{H}_2$-$(CH_2)_9$-), 2.52 (t; 2H; ^3J = 7.4Hz; -C$\underline{H}_2$-S-), 2.53 (t; 2H; ^3J = 7.4Hz; -S-C$\underline{H}_2$-), 3.89 (s; 6H; -O-C$\underline{H}_3$), 3.95-4.05 (m; 2H; -C$\underline{H}$-$(CH_2)_{10}$-, -C$\underline{H}$-CH_2-), 6.94/7.96 (AA'BB'; 4H; ^3J = 8.9Hz; phenyl-H); 6.95/7.97 (AA'BB'; 4H; ^3J = 8.9Hz; phenyl-H)

1,4-Bis(4-methoxyphenyl)-2-[10-(pentylsulfanyl)decyl]-3-propylbutan-1,4-dione (**36d**)



Preparation from 1-(4-methoxyphenyl)-12-pentylsulfanyl-dodecan-1-on **24** (3.8mmol) and 2-bromo-(4-methoxyphenyl)-pentan-1-one **30d** (4.2mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:10, v/v).

Light yellow oil; yield: 55%

$C_{36}H_{54}O_4S$ (582.88)

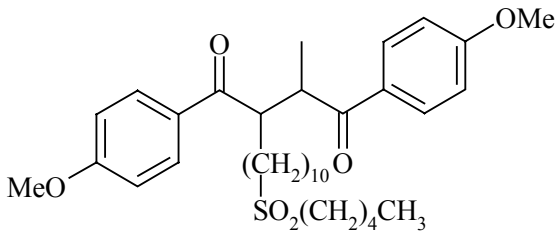
IR: ν (cm^{-1}) = 1665 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.84 (t; 3H; ^3J = 7.3Hz; -CH_2-C<u>H</u>$_3$), 0.89 (t; 3H; ^3J = 7.0Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.18-1.41 (m; 20H; -C<u>H</u>$_2$-CH_3, -$(C$<u>H</u>$_2)_2$-CH_3, -$(C$<u>H</u>$_2)_7$-), 1.50-1.62 (m; 4H; -C<u>H</u>$_2$-CH_2-S-CH_2-C<u>H</u>$_2$-), 1.84-1.91 (m; 4H; -CH-C<u>H</u>$_2$-, -CH-C<u>H</u>$_2$-$(CH_2)_9$-), 2.48 (t; 2H; ^3J = 7.5Hz; -C<u>H</u>$_2$-S-), 2.49 (t; 2H; ^3J = 7.5Hz; -S-C<u>H</u>$_2$-), 3.85 (s; 6H; -O-C<u>H</u>$_3$), 3.93-3.99 (m; 2H; -C<u>H</u>-$(CH_2)_{10}$-, -C<u>H</u>-CH_2-), 6.90/7.91 (AA'BB'; 4H; ^3J = 8.9Hz; phenyl-H); 6.90/7.92 (AA'BB'; 4H; ^3J = 8.9Hz; phenyl-H)

2.2.4.2 Oxidation of the Side Chain Sulfur

At room temperature a solution of *meta*-chloroperbenzoic acid (2.0eq) in chloroform was added dropwise to a solution of the respective thioether (1.0eq) in chloroform and stirred at room temperature for 4h. The mixture was poured onto sat. $NaHCO_3$ solution and stirred vigorously for 15min. The layers were separated and the organic layer was washed with sat. $NaHCO_3$, water and brine. After drying over Na_2SO_4 the solvent was evaporated.

1,4-Bis(4-methoxyphenyl)-3-methyl-2-[10-(pentylsulfonyl)decyl]butan-1,4-dione (**37b**)



Preparation from 1,4-bis(4-methoxyphenyl)-3-methyl-2-[10-(pentylsulfanyl)decyl]butan-1,4-dione **36b** (0.59mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:2, v/v).

Yellow oil; yield: 92%

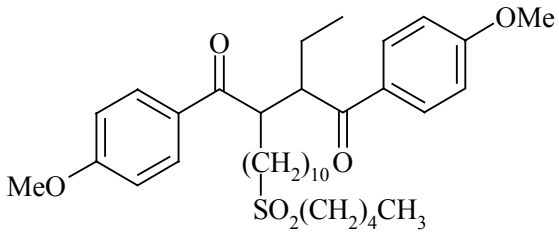
$C_{34}H_{50}O_6S$ (586.83)

IR: ν (cm^{-1}) = 1668 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.94 (t; 3H; ^3J = 7.0Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.20-1.49 (m; 23H; -$(C$<u>H</u>$_2)_2$-CH_3, -$(C$<u>H</u>$_2)_8$-, -CH-C<u>H</u>$_3$), 1.52-1.75 (m; 4H; -C<u>H</u>$_2$-CH_2-SO_2-

CH_2-C<u>H</u>$_2$-), 2.94 (t; 2H; ^3J = 8.1Hz; -C<u>H</u>$_2$-SO_2-), 2.95 (t; 2H; ^3J = 8.1Hz; -SO_2-C<u>H</u>$_2$-), 3.87 (s; 3H; -O-C<u>H</u>$_3$), 3.88 (s; 3H; -O-CH_3), 3.90-4.02 (m; 2H; -C<u>H</u>-$(CH_2)_{10}$-, -C<u>H</u>-CH_3), 6.93/7.95 (AA'BB'; 4H; ^3J = 9.1Hz; phenyl-H), 6.94/7.99 (AA'BB'; 4H; ^3J = 9.1Hz; phenyl-H)

3-Ethyl-1,4-bis(4-methoxyphenyl)-2-[10-(pentylsulfonyl)decyl]butan-1,4-dione (**37c**)



Preparation from 3-ethyl-1,4-bis(4-methoxyphenyl)-2-[10-(pentylsulfanyl)decyl]butan-1,4-dione **36c** (0.88mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:3, v/v).

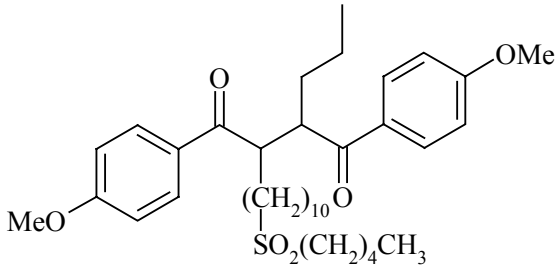
Yellow oil; yield: 72%

$C_{35}H_{52}O_6S$ (600.86)

IR: ν (cm^{-1}) = 1666 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.86 (t; 3H; ^3J = 7.5Hz; -CH_2-C<u>H</u>$_3$), 0.96 (t; 3H; ^3J = 7.0Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.15-1.52 (m; 18H; -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_7$-), 1.75-1.89 (m; 8H; -CH-C<u>H</u>$_2$-, -CH-C<u>H</u>$_2$-$(CH_2)_9$-, -C<u>H</u>$_2$-CH_2-SO_2-CH_2-C<u>H</u>$_2$-), 2.96 (t; 2H; ^3J = 8.1Hz; -C<u>H</u>$_2$-SO_2-), 2.97 (t; 2H; ^3J = 8.1Hz; -SO_2-C<u>H</u>$_2$-), 3.89 (s; 3H; -O-C<u>H</u>$_3$), 3.90 (s; 3H; -O-C<u>H</u>$_3$), 3.95-4.05 (m; 2H; -C<u>H</u>-$(CH_2)_{10}$-, -C<u>H</u>-CH_2-), 6.94/7.96 (AA'BB'; 4H; ^3J = 8.9Hz; phenyl-H); 6.95/7.98 (AA'BB'; 4H; ^3J = 8.9Hz; phenyl-H)

1,4-Bis(4-methoxyphenyl)-2-[10-(pentylsulfonyl)decyl]-3-propylbutan-1,4-dione (**37d**)



Preparation from 1,4-bis(4-methoxyphenyl)-2-[10-(pentylsulfanyl)decyl]-3-propylbutan-1,4-dione **36d** (1.34mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:3, v/v).

Yellow oil; yield: 40%

$C_{36}H_{54}O_6S$ (614.88)

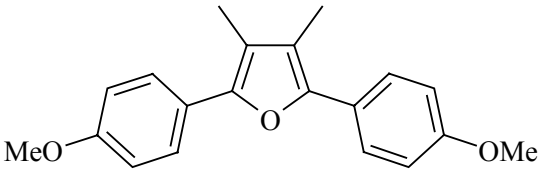
IR: ν (cm^{-1}) = 1665 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.84 (t; 3H; ^3J = 7.3Hz; -CH_2-C$\underline{H}_3$), 0.92 (t; 3H; ^3J = 7.0Hz; -$(CH_2)_4$-C$\underline{H}_3$), 1.12-1.47 (m; 20H; -C$\underline{H}_2$-CH_3, -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_7$-), 1.68-1.88 (m; 8H; -CH-C$\underline{H}_2$-, -CH-C$\underline{H}_2$-$(CH_2)_9$-, -C$\underline{H}_2$-CH_2-SO_2-CH_2-C$\underline{H}_2$-), 2.92 (t; 2H; ^3J = 8.1Hz; -C$\underline{H}_2$-SO_2-), 2.93 (t; 2H; ^3J = 8.1Hz; -SO_2-C$\underline{H}_2$-), 3.85 (s; 6H; -O-C$\underline{H}_3$), 3.87-3.98 (m; 2H; -C$\underline{H}$-$(CH_2)_{10}$-, -C$\underline{H}$-CH_2-), 6.90/7.91 (AA'BB'; 4H; ^3J = 8.9Hz; phenyl-H); 6.90/7.92 (AA'BB'; 4H; ^3J = 8.9Hz; phenyl-H)

2.2.5 Cyclisation to 3,4-Dialkyl-2,5-bis(4-methoxyphenyl)furans

A solution of the respective 1,4-dicarbonyl compound (1.0eq) and 4-toluenesulfonic acid monohydrate (0.3eq or 1.3eq for compounds with amine functionality) in toluene was stirred at 95°C for 3h. After cooling the dark-brown reaction mixture was filtered and the solvent was removed under reduced pressure.

2,5-Bis(4-methoxyphenyl)-3,4-dimethylfuran (**38b**)



Preparation from 1,4-bis(4-methoxyphenyl)-2,3-dimethylbutan-1,4-dione **33b** (4.1mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:5, v/v).

Colourless solid; yield: 97%

Melting point: 109°C

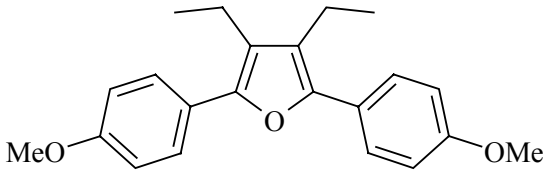
$C_{20}H_{20}O_3$ (308.38)

Analysis: Calculated: C: 77.90 H 6.54

Found: C: 77.99 H 6.74

^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.20 (s; 6H; furan-C<u>H</u>$_3$), 3.84 (s; 6H; -O-C<u>H</u>$_3$), 6.96/7.61 (AA'BB'; 8H; ^{3}J = 8.9Hz; phenyl-<u>H</u>)

3,4-Diethyl-2,5-bis(4-methoxyphenyl)furan (**38c**)



Preparation from 2,3-diethyl-1,4-bis(4-methoxyphenyl)butan-1,4-dione **33c** (7.9mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:4, v/v).

Colourless solid; yield: 89%

Melting point: 71-73°C

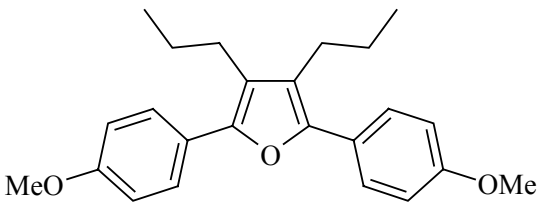
$C_{22}H_{24}O_3$ (336.43)

Analysis: Calculated: C: 78.54 H 7.19

Found: C: 78.25 H 6.90

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.26 (t; 6H; ^{3}J = 7.5Hz; -CH_2-C<u>H</u>$_3$), 2.65 (q; 4H; ^{3}J = 7.5Hz; -C<u>H</u>$_2$-CH_3), 3.85 (s; 6H; -O-C<u>H</u>$_3$), 6.96/7.61 (AA'BB'; 8H; ^{3}J = 8.9Hz; phenyl-<u>H</u>)

2,5-Bis(4-methoxyphenyl)-3,4-dipropylfuran (**38d**)



Preparation from 1,4-bis(4-methoxyphenyl)-2,3-dipropylbutan-1,4-dione **33d** (3.9mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:9, v/v).

Colourless solid; yield: 79%

Melting point: 70-72°C

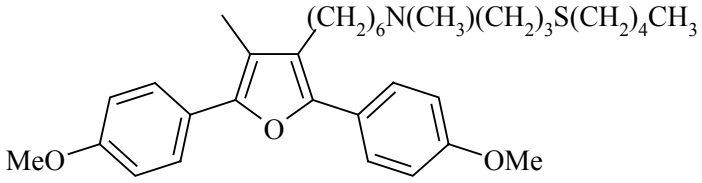
$C_{24}H_{28}O_3$ (364.49)

Analysis: Calculated: C: 79.09 H 7.74

Found: C: 78.59 H 7.79

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.03 (t; 6H; ^{3}J = 7.3Hz; -CH_2-C$\underline{H}_3$), 1.63 (sex; 4H; ^{3}J = 7.5Hz; -CH_2-C$\underline{H}_3$), 2.57 (t; 4H; ^{3}J = 8.1Hz; -C$\underline{H}_2$-CH_2-), 3.85 (s; 6H; -O-C$\underline{H}_3$), 6.96/7.60 (AA'BB'; 8H; ^{3}J = 8.9Hz; phenyl-$\underline{H}$)

2,5-Bis(4-methoxyphenyl)-4-methyl-3-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}-hexyl}furan (**39b**)



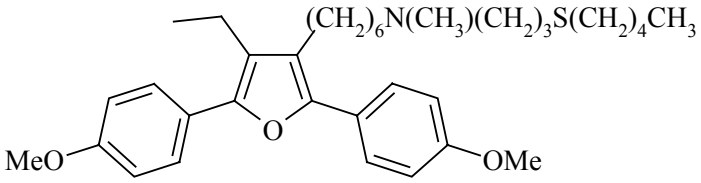
Preparation from 1,4-bis(4-methoxyphenyl)-3-methyl-2-{6-{N-methyl-N-[3-(pentylsulfanyl)-propyl]amino}hexyl}butan-1,4-dione **34b** (2.6mmol). The crude product was purified by column chromatography (SiO_2; toluene/methanol 20:1, v/v).

Colourless oil; yield: 53%

$C_{34}H_{49}NO_3S$ (551.83)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^{3}J = 7.1Hz; -$(CH_2)_4$-C$\underline{H}_3$), 1.22-1.65 (m; 14H; -$(C\underline{H}_2)_3$-CH_3, -N-CH_2-$(C\underline{H}_2)_4$-), 1.76 (quin; 2H; ^{3}J = 7.3Hz; -N-CH_2-C$\underline{H}_2$-CH_2-S-), 2.20 (s; 3H; furan-C$\underline{H}_3$), 2.22 (s; 3H; -N-C$\underline{H}_3$), 2.34 (t; 2H; ^{3}J = 7.3Hz; -N-C$\underline{H}_2$-), 2.44 (t; 2H; ^{3}J = 7.4Hz; -C$\underline{H}_2$-N-), 2.50 (t; 2H; ^{3}J = 7.3Hz; -C$\underline{H}_2$-S-), 2.53 (t; 2H; ^{3}J = 7.4Hz; -S-C$\underline{H}_2$-), 2.59 (t; 2H; ^{3}J = 7.8Hz; furan-C$\underline{H}_2$-), 3.84 (s; 3H; -O-C$\underline{H}_3$), 3.84 (s; 3H; -O-C$\underline{H}_3$), 6.95/7.58 (AA'BB'; 4H; ^{3}J = 9.0Hz; phenyl-$\underline{H}$), 6.95/7.61 (AA'BB'; 4H; ^{3}J = 9.0Hz; phenyl-$\underline{H}$)

4-Ethyl-2,5-bis(4-methoxyphenyl)-3-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}-hexyl}furan (**39c**)



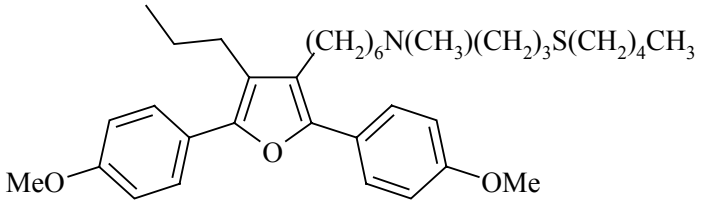
Preparation from 3-ethyl-1,4-bis(4-methoxyphenyl)-2-{6-{N-methyl-N-[3-(pentylsulfanyl)-propyl]amino}hexyl}butan-1,4-dione **34c** (1.1mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/methanol 5:1, v/v).

Yellow oil; yield: 88%

$C_{35}H_{51}NO_3S$ (565.86)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^{3}J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.25 (t; 3H; ^{3}J = 7.5Hz; -CH_2-C<u>H</u>$_3$), 1.28-1.66 (m; 14H; -(C<u>H</u>$_2$)$_3$-CH_3, -N-CH_2-(C<u>H</u>$_2$)$_4$-, 1.75 (quin; 2H; ^{3}J = 7.3Hz; -N-CH_2-C<u>H</u>$_2$-CH_2-S-), 2.21 (s; 3H; -N-C<u>H</u>$_3$), 2.33 (t; 4H; ^{3}J = 7.5Hz; -C<u>H</u>$_2$-N-C<u>H</u>$_2$-), 2.42 (t; 4H; ^{3}J = 7.3Hz; -C<u>H</u>$_2$-S-C<u>H</u>$_2$-), 2.51 (q; 2H; ^{3}J = 7.7Hz; furan-C<u>H</u>$_2$-), 2.62 (t; 2H; ^{3}J = 7.5Hz; furan-C<u>H</u>$_2$-), 3.85 (s; 6H; -O-C<u>H</u>$_3$), 6.96/7.59 (AA'BB'; 4H; ^{3}J = 8.9Hz; phenyl-<u>H</u>), 6.96/7.61 (AA'BB'; 4H; ^{3}J = 8.9Hz; phenyl-<u>H</u>)

2,5-Bis(4-methoxyphenyl)-3-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-4-propylfuran (**39d**)



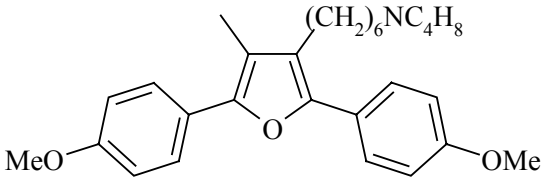
Preparation from 1,4-bis(4-methoxyphenyl)-2-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]-amino}hexyl}-3-propylbutan-1,4-dione **34d** (0.82mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/methanol 5:1, v/v).

Yellow oil; yield: 76%

$C_{36}H_{53}NO_3S$ (579.88)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.85 (t; 3H; ^{3}J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 0.99 (t; 3H; ^{3}J = 7.4Hz; -CH_2-C<u>H</u>$_3$), 1.18-1.65 (m; 16H; -(C<u>H</u>$_2$)$_3$-CH_3, -N-CH_2-(C<u>H</u>$_2$)$_4$-, -C<u>H</u>$_2$-CH_3), 1.72 (quin; 2H; ^{3}J = 7.3Hz; -N-CH_2-C<u>H</u>$_2$-CH_2-S-), 2.18 (s; 3H; -N-C<u>H</u>$_3$), 2.31 (t; 2H; ^{3}J = 7.3Hz; -C<u>H</u>$_2$-N-), 2.40 (t; 2H; ^{3}J = 7.3Hz; -C<u>H</u>$_2$-N-), 2.44-2.57 (m; 8H; -C<u>H</u>$_2$-S-C<u>H</u>$_2$-, -C<u>H</u>$_2$-furan-C<u>H</u>$_2$-), 3.81 (s; 6H; -O-C<u>H</u>$_3$), 6.91/7.55 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 6.91/7.56 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>)

2,5-Bis(4-methoxyphenyl)-4-methyl-3-(6-pyrrolidinylhexyl)furan (**40b**)



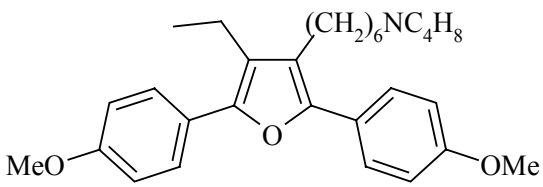
Preparation from 1,4-bis(4-methoxyphenyl)-3-methyl-2-(6-pyrrolidinylhexyl)butan-1,4-dione **35b** (1.7mmol). The crude product was purified by column chromatography (SiO_2; DCM/methanol 1:1, v/v).

Colourless oil; yield: 69%

$C_{29}H_{37}NO_3$ (447.62)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.34-1.47 (m; 4H; -(C<u>H</u>$_2$)$_2$-), 1.49-1.65 (m; 4H; -N-CH_2-C<u>H</u>$_2$-, furan-CH_2-C<u>H</u>$_2$-), 1.75-1.84 (m; 4H; -N-(CH_2-C<u>H</u>$_2$)$_2$-), 2.20 (s; 3H; furan-C<u>H</u>$_3$), 2.42-2.62 (m; 8H; furan-C<u>H</u>$_2$-, 3x -C<u>H</u>$_2$-N-), 3.85 (s; 6H; -O-C<u>H</u>$_3$), 6.96/7.59 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 6.96/7.61 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>)

4-Ethyl-2,5-bis(4-methoxyphenyl)-3-(6-pyrrolidinylhexyl)furan (**40c**)



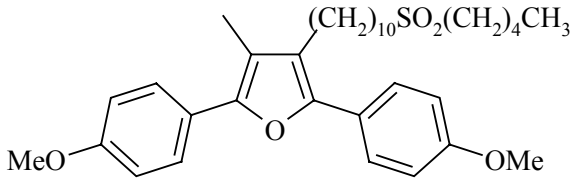
Preparation from 3-ethyl-1,4-bis(4-methoxyphenyl)-2-(6-pyrrolidinylhexyl)butan-1,4-dione **35c** (2.4mmol). The crude product was purified by column chromatography (SiO_2; DCM/methanol 1:1, v/v).

Yellow oil; yield: 78%

$C_{30}H_{39}NO_3$ (461.65)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.24 (t; 3H; ^{3}J = 7.5Hz; -CH_2-C$\underline{H}_3$), 1.33-1.66 (m; 8H; -(C$\underline{H}_2$)$_4$-), 1.76-1.84 (m; 4H; -N-(CH_2-C$\underline{H}_2$)$_2$-), 2.43-2.67 (m; 10H; -C$\underline{H}_2$-furan-C$\underline{H}_2$-, 3x -C$\underline{H}_2$-N-), 3.85 (s; 6H; -O-C$\underline{H}_3$), 6.96/7.59 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-$\underline{H}$), 6.96/7.61 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-$\underline{H}$)

2,5-Bis(4-methoxyphenyl)-4-methyl-3-[10-(pentylsulfanyl)decyl]furan (**41b**)



Preparation from 1,4-bis(4-methoxyphenyl)-3-methyl-2-[10-pentylsulfanyl)decyl]butan-1,4-dione **36b** (0.55mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:10, v/v).

Slightly yellow solid; yield: 54%

Melting point: 45-47°C

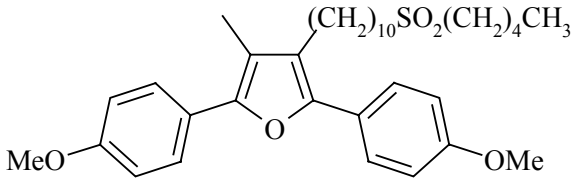
$C_{34}H_{48}O_3S$ (536.81)

Analysis: Calculated: C: 76.07 H 9.01

Found: C: 75.71 H 8.87

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.94 (t; 3H; ^{3}J = 7.1Hz; -(CH_2)$_4$-C$\underline{H}_3$), 1.29-1.64 (m; 22H; -(C$\underline{H}_2$)$_3$-CH_3, -(C$\underline{H}_2$)$_8$-), 2.24 (s; 3H, furan-C$\underline{H}_3$), 2.54 (t; 4H; ^{3}J=7.4Hz; -C$\underline{H}_2$-S-C$\underline{H}_2$-), 2.63 (t; 2H; ^{3}J = 7.8Hz; furan-C$\underline{H}_2$-), 3.89 (s; 6H; -O-C$\underline{H}_3$), 7.00/7.63 (AA'BB'; 4H; ^{3}J = 8.5Hz; phenyl-$\underline{H}$), 7.00/7.65 (AA'BB'; 4H; ^{3}J = 8.5Hz; phenyl-$\underline{H}$)

2,5-Bis(4-methoxyphenyl)-4-methyl-3-[10-(pentylsulfonyl)decyl]furan (**42b**)



Preparation from 1,4-bis(4-methoxyphenyl)-3-methyl-2-[10-(pentylsulfonyl)decyl]butan-1,4-dione **37b** (0.55mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:2, v/v).

Slightly yellow solid; yield: 67%

Melting point: 87-88°C

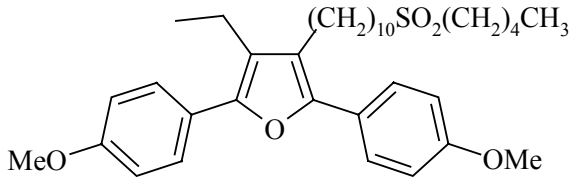
$C_{34}H_{48}O_5S$ (568.82)

Analysis: Calculated: C: 71.79 H 8.51

Found: C: 71.39 H 8.29

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.96 (t; 3H; ^3J = 7.0Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.26-1.64 (m; 18H; -(C<u>H</u>$_2$)$_2$-CH_3, -(C<u>H</u>$_2$)$_7$-), 1.81-1.93 (m; 4H;-C<u>H</u>$_2$-CH_2-SO_2-CH_2-C<u>H</u>$_2$-), 2.24 (s; 3H, furan-C<u>H</u>$_3$), 2.63 (t; 2H; ^3J = 7.9Hz; furan-C<u>H</u>$_2$-), 2.97 (t; 4H; ^3J = 8.1Hz; -C<u>H</u>$_2$-SO_2-C<u>H</u>$_2$-), 3.89 (s; 6H; -O-C<u>H</u>$_3$), 6.99/7.63 (AA'BB'; 4H; ^3J = 8.8Hz; phenyl-<u>H</u>), 7.00/7.65 (AA'BB'; 4H; ^3J = 8.8Hz; phenyl-<u>H</u>)

4-Ethyl-2,5-bis(4-methoxyphenyl)-3-[10-(pentylsulfonyl)decyl]furan (**42c**)



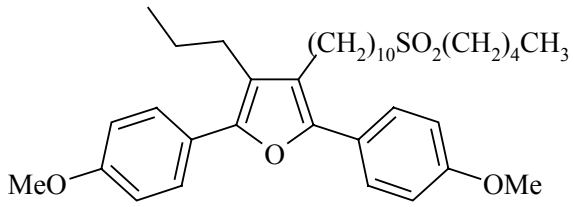
Preparation from 3-ethyl-1,4-bis(4-methoxyphenyl)-2-[10-(pentylsulfonyl)decyl]butan-1,4-dione **37c** (0.60mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:4, v/v).

Yellow oil; yield: 94%

$C_{35}H_{50}O_5S$ (582.84)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.92 (t; 3H; ^{3}J = 7.0Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.25 (t; 3H; ^{3}J = 7.5Hz; -CH_2-C<u>H</u>$_3$), 1.28-1.48 (m; 16H; -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_6$-), 1.60 (quin; 2H; ^{3}J = 7.8Hz; furan-CH_2-C<u>H</u>$_2$-), 1.78-1.89 (m; 4H; -C<u>H</u>$_2$-CH_2-SO_2-CH_2-C<u>H</u>$_2$-), 2.56-2.67 (m; 4H; -C<u>H</u>$_2$-furan-C<u>H</u>$_2$-), 2.93 (t; 4H; ^{3}J = 8.1Hz; -C<u>H</u>$_2$-SO_2-C<u>H</u>$_2$-), 3.85 (s; 6H; -O-C<u>H</u>$_3$), 6.95/7.60 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 6.96/7.61 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>)

2,5-Bis(4-methoxyphenyl)-3-[10-(pentylsulfonyl)decyl]-4-propylfuran (**42d**)



Preparation from 1,4-bis(4-methoxyphenyl)-2-[10-(pentylsulfonyl)decyl]-3-propylbutan-1,4-dione **37c** (0.55mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:4, v/v).

Yellow oil; yield: 79%

$C_{36}H_{52}O_5S$ (596.87)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.92 (t; 3H; ^{3}J = 7.0Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.03 (t; 3H; ^{3}J = 7.4Hz; -CH_2-C<u>H</u>$_3$), 1.26-1.48 (m; 16H; -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_6$-), 1.54-1.69 (m; 4H; -C<u>H</u>$_2$-CH_2-furan-CH_2-C<u>H</u>$_2$-), 1.78-1.89 (m; 4H; -C<u>H</u>$_2$-CH_2-SO_2-CH_2-C<u>H</u>$_2$-), 2.54-2.61 (m; 4H; -C<u>H</u>$_2$-furan-C<u>H</u>$_2$-), 2.93 (t; 4H; ^{3}J = 8.1Hz; -C<u>H</u>$_2$-SO_2-C<u>H</u>$_2$-), 3.85 (s; 6H; -O-C<u>H</u>$_3$), 6.95/7.60 (AA'BB'; 8H; ^{3}J = 8.8Hz; phenyl-<u>H</u>)

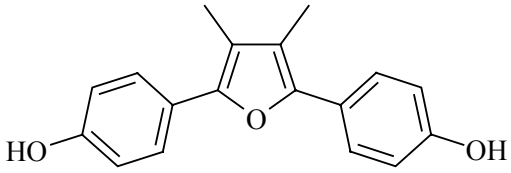
2.2.6 Demethylation of the Protected Furans

Under nitrogen and with stirring, a solution of the protected furan (1.0eq) in dry DCM was added dropwise to a solution of boron tribromide (5.0eq) in dry DCM, that had been cooled to –5°C. After the addition the cooling bath was removed and stirring continued for 0.5-24h.

With cooling and vigorous stirring, sat. sodium bicarbonate solution was added slowly until the gas evolution ceased, followed by addition of the same volume of ethyl acetate. The aqueous phase was separated and extracted with three portions of ethyl acetate. The combined organic layers were washed with water and brine. After drying over Na_2SO_4 the solvent was removed *in vacuo*.

2.2.6.1 Demethylation to 3,4-Dialkyl-2,5-bis(4-hydroxyphenyl)furans

2,5-Bis(4-hydroxyphenyl)-3,4-dimethylfuran (**43b**)



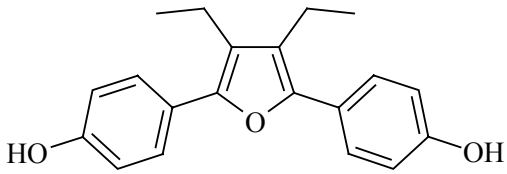
Preparation from 2,5-bis(4-methoxyphenyl)-3,4-dimethylfuran **38b** (3.9mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 3h.
Purification by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:5, v/v) and recrystallisation from DCM.

Slightly blue solid; yield: 66%
Melting point: 219°C (dec.)
$C_{18}H_{16}O_3$ (280.32)

IR:	ν (cm^{-1}) = 3302 (w, br; O-H)		
Analysis:	Calculated:	C: 77.12	H 5.75
	Found:	C: 76.74	H 5.95
MS:	m/z (%) = 280 (100; $M^{+\bullet}$), 159 (14; $[M\text{-}HOC_6H_4CO]^{+\bullet}$), 140 (9; $M^{2+\bullet}$), 121 (3; $HOC_6H_4CO^{+\bullet}$)		
HRMS:	Calculated for $C_{18}H_{16}O_3$:		280.1099
	Found:		280.1096 ± 0.0002

^{1}H-NMR (DMSO-d_6): δ (ppm) = 2.11 (s; 6H; -C<u>H</u>$_3$), 6.83/7.45 (AA'BB'; 8H; ^3J = 8.8Hz; phenyl-<u>H</u>), 9.58 (s; 2H; -O<u>H</u>)

3,4-Diethyl-2,5-bis(4-hydroxyphenyl)furan (**43c**)

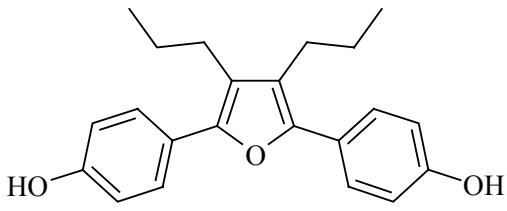


Preparation from 3,4-diethyl-2,5-bis(4-methoxyphenyl)furan **38c** (3.5mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 3h.
Purification by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:4, v/v) and recrystallisation from a mixture of ethyl acetate and petroleum ether 40-60.

Slightly green solid; yield: 26%
Melting point: 199-200°C
$C_{20}H_{20}O_3$ (308.38)

IR:	ν (cm^{-1}) = 3243 (w, br; O-H)		
Analysis:	Calculated:	C: 77.90	H 6.54
	Found:	C: 77.62	H 6.35
MS:	m/z (%) = 308 (100; $M^{+\bullet}$), 293 (5; $[M\text{-}CH_3]^{+\bullet}$), 187 (5; $[M\text{-}HOC_6H_4CO]^{+\bullet}$), 154 (5; $M^{2+\bullet}$), 121 (10; $HOC_6H_4CO^{+\bullet}$)		
HRMS:	Calculated for $C_{20}H_{20}O_3$:	308.1412	
	Found:	308.1407 ± 0.0003	
^{1}H-NMR (DMSO-d_6):	δ (ppm) = 1.13 (t; 6H; ^3J = 7.4Hz; -C<u>H</u>$_3$), 2.52 (q; 4H; ^3J = 7.4Hz; -C<u>H</u>$_2$-), 6.81/7.41 (AA'BB'; 8H; ^3J = 8.5Hz; phenyl-<u>H</u>), 9.56 (s; 2H; -O<u>H</u>)		

2,5-Bis(4-hydroxyphenyl)-3,4-dipropylfuran (**43d**)



Preparation from 2,5-bis(4-methoxyphenyl)-3,4-dipropylfuran **38d** (3.0mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 3h.

Purification by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:4, v/v) and recrystallisation from a mixture of ethyl acetate and petroleum ether 40-60.

Slightly brown solid; yield: 66%

Melting point: 153-155°C

$C_{22}H_{24}O_3$ (336.43)

IR: ν (cm^{-1}) = 3165 (w, br; O-H)

Analysis: Calculated: C: 78.54 H 7.19

Found: C: 78.38 H 7.05

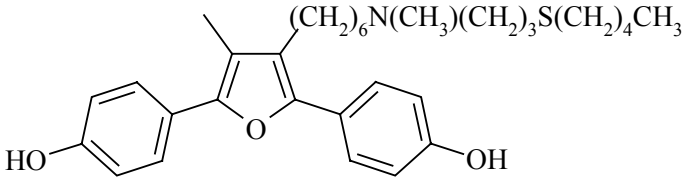
MS: m/z (%) = 336 (100; $M^{+\bullet}$), 307 (5; $[M\text{-}CH_2CH_3]^{+\bullet}$), 168 (7; $M^{2+\bullet}$), 121 (14; $HOC_6H_4CO^{+\bullet}$)

HRMS: Calculated for $C_{22}H_{24}O_3$: 336.1725

Found: 336.1721 ± 0.0003

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.96 (t; 6H; ^3J = 7.3Hz; -CH_2-C<u>H</u>$_3$), 1.52 (sex; 4H; ^3J = 7.4Hz; -CH_2-C<u>H</u>$_2$-CH_3), 2.53 (furan-C<u>H</u>$_2$-, merged in DMSO-signal), 6.84/7.44 (AA'BB'; 8H; ^3J = 8.6Hz; phenyl-<u>H</u>), 9.59 (s; 2H; -O<u>H</u>)

2,5-Bis(4-hydroxyphenyl)-4-methyl-3-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}-hexyl}furan (**44b**)



Preparation from 2,5-bis(4-methoxyphenyl)-4-methyl-3-{6-{N-methyl-N-[3-(pentylsulfanyl)-propyl]amino}hexyl}furan **39b** (0.96mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 3h.

The crude product was purified twice by column chromatography (SiO_2; methanol/ethyl acetate 1:5, v/v and methanol/ethyl acetate 1:10, v/v).

Yellow oil; yield: 40%

$C_{32}H_{45}NO_3S$ (523.78)

IR: ν (cm^{-1}) = 3400-2600 (w, br; -OH)

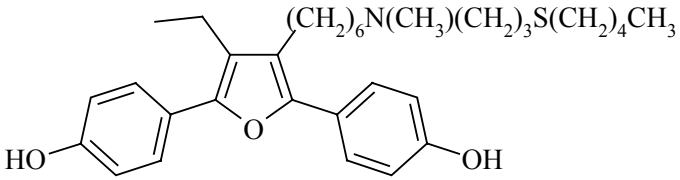
MS: m/z (%) = 524 (100; $[MH]^+$), 454 (2; $[MH\text{-}CH_3(CH_2)_2CHCH_2]^+$), 422 (8; $[MH\text{-}S(CH_2)_5]^+$), 380 (3; $[MH\text{-}CH_2{=}CHCH_2SC_5H_{11}]^+$), 176 (18; $[CH_3(CH_2)_4S(CH_2)_3NH_2(CH_3)]^+$)

HRMS: Calculated for $C_{32}H_{45}NO_3S$: 523.3122

Found: 523.3122 ± 0.0005

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.86 (t; 3H; ^3J = 7.1 Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.22-1.64 (m; 14H; -$(C\underline{H}_2)_3$-CH_3, -N-CH_2-$(C\underline{H}_2)_4$-), 1.83 (quin; 2H; ^3J = 7.4Hz; -N-CH_2-$C\underline{H}_2$-CH_2-S-), 2.12 (s; 3H; furan-C<u>H</u>$_3$), 2.31 (s; 3H; -N-C<u>H</u>$_3$), 2.40-2.59 (m; 10H; -C<u>H</u>$_2$-N-C<u>H</u>$_2$-CH_2-C<u>H</u>$_2$-S-C<u>H</u>$_2$, furan-C<u>H</u>$_2$-), 6.85/7.48 (AA'BB'; 4H; ^3J = 8.5Hz; phenyl-<u>H</u>), 6.85/7.51 (AA'BB'; 4H; ^3J = 8.5Hz; phenyl-<u>H</u>)

4-Ethyl-2,5-bis(4-hydroxyphenyl)-3-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-furan (**44c**)



Preparation from 4-ethyl-2,5-bis(4-methoxyphenyl)-3-{6-{N-methyl-N-[3-(pentylsulfanyl)-propyl]amino}hexyl}furan **39c** (0.85mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 3h.

The crude product was purified by column chromatography (SiO_2; methanol/DCM 1:10, v/v).

Yellow solid; yield: 34%

Melting point: 50-52°C

$C_{33}H_{47}NO_3S$ (537.80)

IR: ν (cm^{-1}) = 3400-2600 (w, br; -OH)

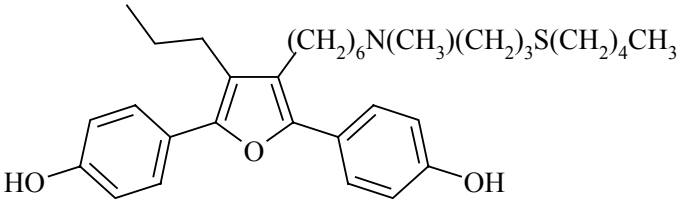
MS: m/z (%) = 538 (100; $[MH]^+$), 468 (2; $[MH\text{-}CH_3(CH_2)_2CHCH_2]^+$), 436 (10; $[MH\text{-}S(CH_2)_5]^+$), 394 (4; $[MH\text{-}CH_2{=}CHCH_2SC_5H_{11}]^+$), 176 (14; $[CH_3(CH_2)_4S(CH_2)_3NH_2(CH_3)]^+$)

HRMS: Calculated for $C_{33}H_{47}NO_3S$: 537.3277

Found: 537.3272 ± 0.0005

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.85 (t; 3H; ^{3}J = 7.1 Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.16 (t; 3H; ^{3}J = 7.4Hz; -CH_2-C<u>H</u>$_3$), 1.23-1.52 (m; 14H; -(C<u>H</u>$_2$)$_3$-CH_3, -N-CH_2-(C<u>H</u>$_2$)$_4$-), 1.75 (quin; 2H; ^{3}J = 6.7Hz; -N-CH_2-C<u>H</u>$_2$-CH_2-S-), 2.31 (s; 3H; -N-C<u>H</u>$_3$), 2.45-2.73 (m; 15H; -C<u>H</u>$_2$-N-C<u>H</u>$_2$-CH_2-C<u>H</u>$_2$-S-C<u>H</u>$_2$, -N-C<u>H</u>$_3$, -C<u>H</u>$_2$-furan-C<u>H</u>$_2$-), 6.85/7.45 (2x AA'BB'; 4H; ^{3}J = 8.6Hz; phenyl-<u>H</u>), 9.62 (s; 2H; -O<u>H</u>)

2,5-Bis(4-hydroxyphenyl)-3-{6-{N-Methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-4-propylfuran (**44d**)



Preparation from 2,5-bis(4-methoxyphenyl)-3-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]-amino}hexyl}-4-propylfuran **39d** (0.85mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 3h.

The crude product was purified by column chromatography (SiO_2; methanol/DCM 1:10, v/v).

Yellow solid; yield: 31%

Melting point: 47-49°C

$C_{34}H_{49}NO_3S$ (551.83)

IR: ν (cm^{-1}) = 3400-2600 (w, br; -OH)

MS: m/z (%) = 551 (100; $M^{+\bullet}$), 420 (75; $[M-(CH_2)_2S(CH_2)_4CH_3]^{+\bullet}$), 210 (40; $[M-(CH_2)_2S(CH_2)_4CH_3]^{2+\bullet}$), 188 (31; $[M-CH_3(CH_2)_4S(CH_2)_3N-(CH_3)=CH_2]^{+\bullet}$), 121 (17; $HOC_6H_4CO^{+\bullet}$)

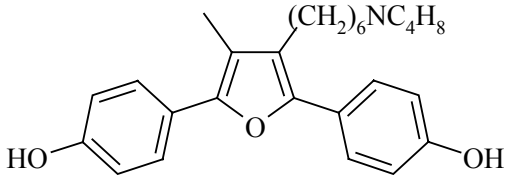
HRMS: Calculated for $C_{34}H_{49}NO_3S$: 551.3433

Found: 551.3427 ± 0.0006

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.85 (t; 3H; ^{3}J = 7.0 Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 0.98 (t; 3H; ^{3}J = 7.3Hz; -CH_2-C<u>H</u>$_3$), 1.23-1.60 (m; 16H; -(C<u>H</u>$_2$)$_3$-CH_3, -N-CH_2-(C<u>H</u>$_2$)$_4$-, furan-CH_2-C<u>H</u>$_2$), 1.72 (quin; 2H; ^{3}J = 7.0Hz; -N-CH_2-C<u>H</u>$_2$-CH_2-S-), 2.34 (s; 3H; -N-C<u>H</u>$_3$), 2.44-2.63 (m; 12H; -C<u>H</u>$_2$-N-C<u>H</u>$_2$-CH_2-C<u>H</u>$_2$-S-

C<u>H</u>$_2$, -C<u>H</u>$_2$-furan-C<u>H</u>$_2$-), 6.86/7.45 (AA'BB'; 8H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 9.61 (s; 2H; -O<u>H</u>)

2,5-Bis(4-hydroxyphenyl)-4-methyl-3-(6-pyrrolidinylhexyl)furan (**45b**)



Preparation from 2,5-bis(4-methoxyphenyl)-4-methyl-3-(6-pyrrolidinylhexyl)furan **40b** (1.2mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 7h.

The crude product was purified twice by column chromatography (SiO_2; methanol/DCM 1:5→1:2, v/v) followed by recrystallisation from ethyl acetate/petroleum ether 40-60.

Colourless solid; yield: 46%

$C_{27}H_{33}NO_3$ (419.57)

Melting point: 112-114°C

IR: ν (cm^{-1}) = 3400-2600 (w, br; -OH)

Analysis: Calculated: C: 77.29 H 7.93 N 3.34

Found: C: 75.69 H 8.01 N 2.96

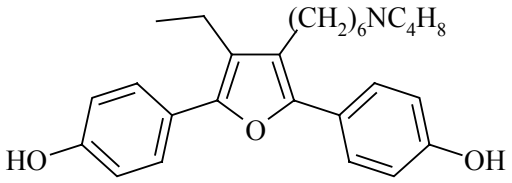
MS: m/z (%) = 419 (80; $M^{+\bullet}$), 404 (2; $[M\text{-}CH_3]^{+\bullet}$), 348 (4; $[M\text{-}NH(CH_2)_4]^{+\bullet}$), 298 (3; $[M\text{-}HOC_6H_4CO]^{+\bullet}$), 279 (4; $[M\text{-}(CH_2)_5N\text{-}(CH_2)_4]^{+\bullet}$), 140 (15; $(CH_2)_5N(CH_2)_4^{+\bullet}$), 121 (12; $HOC_6H_4CO^{+\bullet}$), 84 (100; $CH_2{=}N(CH_2)_4^{+}$), 70 (3; $N(CH_2)_4^{+\bullet}$)

HRMS: Calculated for $C_{27}H_{33}NO_3$: 419.2460

Found: 419.2455 ± 0.0004

^{1}H-NMR (DMSO-d$_6$): δ (ppm) = 1.13-1.57 (m; 8H; -(C<u>H</u>$_2$)$_4$-), 1.60-1.69 (m; 4H; -N-(CH$_2$)$_2$-(C<u>H</u>$_2$)$_2$-), 2.12 (s; 3H; -C<u>H</u>$_3$), 2.30-2.39 (m; 6H; -C<u>H</u>$_2$-N-(C<u>H</u>$_2$)$_2$-), 2.52 (furan-C<u>H</u>$_2$-, merged in DMSO-signal), 6.84/7.43 (AA'BB'; 4H; ^{3}J = 8.3Hz; phenyl-<u>H</u>), 6.84/7.46 (AA'BB'; 4H; ^{3}J = 8.3Hz; phenyl-<u>H</u>), 9.60 (s, 2H, -O<u>H</u>)

4-Ethyl-2,5-bis(4-hydroxyphenyl)-3-(6-pyrrolidinylhexyl)furan (**45c**)



Preparation from 4-ethyl-2,5-bis(4-methoxyphenyl)-3-(6-pyrrolidinylhexyl)furan **40c** (1.84mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 7h.

The crude product was purified twice by column chromatography (SiO_2; methanol/DCM 1:5→1:2, v/v) followed by recrystallisation from ethyl acetate/petroleum ether 40-60.

Colourless solid; yield: 30%

$C_{28}H_{35}NO_3$ (433.59)

Melting point: 99-102°C

IR: ν (cm^{-1}) = 3400-2600 (w, br; -OH)

Analysis: Calculated: C: 77.56 H 8.14 N 3.23

Found: C: 75.75 H 8.46 N 2.94

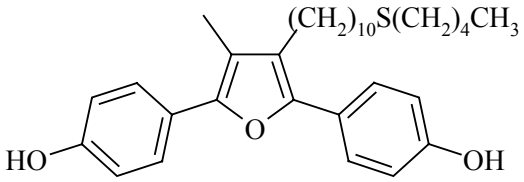
MS: m/z (%) = 433 (49; $M^{+\bullet}$), 404 (7; $[M\text{-}CH_2CH_3]^{+\bullet}$), 362 (4; $[M\text{-}NH(CH_2)_4]^{+\bullet}$), 312 (3; $[M\text{-}HOC_6H_4CO]^{+\bullet}$), 293 (3; $[M\text{-}(CH_2)_5N\text{-}(CH_2)_4]^{+\bullet}$), 140 (15; $(CH_2)_5N(CH_2)_4^{+\bullet}$), 121 (15; $HOC_6H_4CO^{+\bullet}$), 84 (100; $CH_2{=}N(CH_2)_4^{+}$), 70 (3; $N(CH_2)_4^{+\bullet}$)

HRMS: Calculated for $C_{28}H_{35}NO_3$: 433.2617

Found: 433.2607 ± 0.0002

^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.16 (t; 3H; ^{3}J = 7.5Hz; -CH_2-C$\underline{H}_3$), 1.35-1.56 (m; 8H; -(C$\underline{H}_2$)$_4$-), 1.60-1.70 (m; 4H; -N-(CH_2)$_2$-(C$\underline{H}_2$)$_2$-), 2.31-2.41 (m; 6H; -C$\underline{H}_2$-N-(C$\underline{H}_2$)$_2$-), 2.52-2.59 (m, 4H, -C$\underline{H}_2$-furan-C$\underline{H}_2$-), 6.84/7.44 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-$\underline{H}$), 6.85/7.45 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-$\underline{H}$), 9.61 (s, 2H, -O$\underline{H}$)

2,5-Bis(4-hydroxyphenyl)-4-methyl-3-[10-(pentylsulfanyl)decyl]furan (**46b**)



Preparation from 2,5-bis(4-methoxyphenyl)-4-methyl-3-[10-(pentylsulfanyl)decyl]furan **41b** (0.28mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 3h.

The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:5, v/v).

Colourless solid; yield: 65%

Melting point: 70-71°C

$C_{32}H_{44}O_3S$ (508.76)

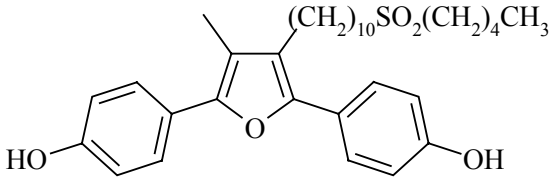
IR(KBr): ν (cm^{-1}) = 3381 (m, br; O-H)

MS: m/z (%) = 509 (100; $[MH]^+$), 439 (11; $[MH\text{-}CH_3(CH_2)_2CHCH_2]^+$), 407 (20; $[MH\text{-}S(CH_2)_5]^+$)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.90 (t; 3H; ^3J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.22-1.62 (m; 22H; -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_8$-CH_2-S-), 2.19 (s; 3H, furan-C<u>H</u>$_3$), 2.51 (t; 4H; ^3J = 7.4Hz; -C<u>H</u>$_2$-S-C<u>H</u>$_2$-), 2.58 (t; 2H; ^3J = 7.2Hz; furan-C<u>H</u>$_2$-), 6.88 (d; 2H; ^3J = 8.8Hz; phenyl-<u>H</u> ortho to -OH), 6.94 (d; 2H; ^3J = 8.5Hz; phenyl-<u>H</u> ortho to -OH), 7.53 (br; 4H; phenyl-<u>H</u> meta to -OH)

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.85 (t; 3H; ^3J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.24-1.51 (m; 22H; -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_8$-CH_2-S-), 2.12 (s; 3H, furan-C<u>H</u>$_3$), 2.45 (t; 4H; ^3J = 7.3Hz; -C<u>H</u>$_2$-S-C<u>H</u>$_2$-), 2.55 (furan-C<u>H</u>$_2$-; merged in DMSO-signal), 6.84/7.43 (AA'BB'; 4H; ^3J = 8.6Hz; phenyl-<u>H</u>), 6.85/7.46 (AA'BB'; 4H; ^3J = 8.5Hz; phenyl-<u>H</u>), 9.58 (s; 1H; -OH), 9.59 (s; 1H; -O<u>H</u>)

2,5-Bis(4-hydroxyphenyl)-4-methyl-3-[10-(pentylsulfonyl)decyl]furan (**47b**)



Preparation from 2,5-bis(4-methoxyphenyl)-4-methyl-3-[10-(pentylsulfonyl)decyl]furan **42b** (0.29mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 3h.

The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 2:3, v/v).

Colourless solid; yield: 51%

$C_{32}H_{44}O_5S$ (540.76)

IR(KBr): ν (cm^{-1}) = 3387 (m, br; O-H)

MS: m/z (%) = 558 (21; $[M+NH_4]^+$), 541 (100; $[MH]^+$), 407 (20; $[MH-SO_2(CH_2)_5]^+$), 267 (3; $[MH-CH_3(CH_2)_9SO_2(CH_2)_3CHCH_2]^+$)

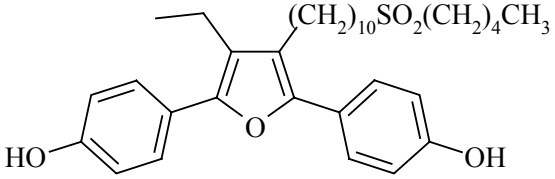
HRMS: Calculated for $C_{32}H_{44}O_5S$: 540.2906

Found: 540.2906 ± 0.0007

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.96 (t; 3H; ^3J = 7.0Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.26-1.65 (m; 18H; -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_7$-CH_2-CH_2-SO_2-), 1.84-1.94 (m; 4H; -C<u>H</u>$_2$-CH_2-SO_2-CH_2-C<u>H</u>$_2$-), 2.24 (s; 3H, furan-C<u>H</u>$_3$), 2.62 (t; 2H; ^3J = 7.9Hz; furan-C<u>H</u>$_2$-), 3.00 (t; 4H; ^3J = 8.1Hz; -C<u>H</u>$_2$-SO_2-C<u>H</u>$_2$-), 6.92 (d; 2H; ^3J = 8.8Hz; phenyl-<u>H</u> ortho to -OH), 6.94 (d; 2H; ^3J = 8.8Hz; phenyl-<u>H</u> ortho to -OH), 7.58 (br; 4H; phenyl-<u>H</u> meta to -OH)

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.87 (t; 3H; ^3J = 7.0Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.25-1.51 (m; 18H; -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_7$-CH_2-CH_2-SO_2-), 1.61-1.68 (m; 4H; -C<u>H</u>$_2$-CH_2-SO_2-CH_2-C<u>H</u>$_2$-), 2.12 (s; 3H, furan-C<u>H</u>$_3$), 2.55 (furan-C<u>H</u>$_2$-; merged in DMSO-signal), 3.03 (t; 4H; ^3J = 8.0Hz; -C<u>H</u>$_2$-SO_2-C<u>H</u>$_2$-), 6.84/7.43 (AA'BB'; 4H; ^3J = 8.5Hz; phenyl-<u>H</u>), 6.84/7.46 (AA'BB'; 4H; ^3J = 8.5Hz; phenyl-<u>H</u>), 9.59 (s; 1H; -O<u>H</u>), 9.60 (s; 1H; -O<u>H</u>)

4-Ethyl-2,5-bis(4-hydroxyphenyl)-3-[10-(pentylsulfonyl)decyl]furan (**47c**)



Preparation from 4-ethyl-2,5-bis(4-methoxyphenyl)-3-[10-(pentylsulfonyl)decyl]furan **42c** (0.57mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 3h.

The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:2, v/v).

Yellow oil; yield: 63%

$C_{33}H_{46}O_5S$ (554.79)

IR: ν (cm^{-1}) = 3401 (m, br; O-H)

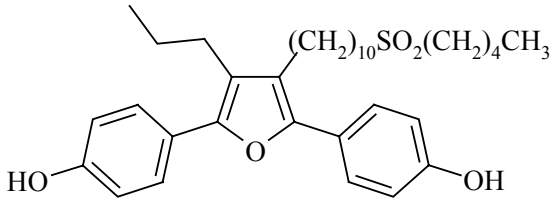
MS: m/z (%) = 554 (100; $M^{+\bullet}$), 420 (7; $[M\text{-}SO_2(CH_2)_5]^+$), 293 (6; $[M\text{-}(CH_2)_9SO_2(CH_2)_4CH_3]^{+\bullet}$), 121 (11; $HOC_6H_4CO^{+\bullet}$)

HRMS: Calculated for $C_{33}H_{46}O_5S$: 554.3066

Found: 554.3065 ± 0.0001

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.87 (t; 3H; ^3J = 7.0Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.16 (t; 3H; ^3J = 7.4Hz; -CH_2-C<u>H</u>$_3$), 1.26-1.38 (m; 16H; -(C<u>H</u>$_2$)$_2$-CH_3, -(C<u>H</u>$_2$)$_6$-CH_2-CH_2-SO_2-), 1.51-1.60 (m; 2H; -C<u>H</u>$_2$-CH_2-furan), 1.61-1.70 (m; 4H; -C<u>H</u>$_2$-CH_2-SO_2-CH_2-C<u>H</u>$_2$-), 2.53-2.60 (m, 4H, -C<u>H</u>$_2$-furan-C<u>H</u>$_2$-), 3.03 (t; 4H; ^3J = 8.0Hz; -C<u>H</u>$_2$-SO_2-C<u>H</u>$_2$-), 6.84/7.44 (AA'BB'; 4H; ^3J = 8.8Hz; phenyl-<u>H</u>), 6.85/7.45 (AA'BB'; 4H; ^3J = 8.8Hz; phenyl-<u>H</u>), 9.59 (s; 2H; -O<u>H</u>)

2,5-Bis(4-hydroxyphenyl)-3-[10-(pentylsulfonyl)decyl]-4-propylfuran (**47d**)



Preparation from 2,5-bis(4-methoxyphenyl)-3-[10-(pentylsulfonyl)decyl]-4-propylfuran **42d** (0.46mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 3h.

The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:3, v/v).

Yellow oil; yield: 54%

$C_{34}H_{48}O_5S$ (568.81)

IR: ν (cm^{-1}) = 3333 (m, br; O-H)

MS: m/z (%) = 568 (100; $M^{+\bullet}$), 434 (12; $[M\text{-}SO_2(CH_2)_5]^+$), 307 (5; $[M\text{-}(CH_2)_9SO_2(CH_2)_4CH_3]^{+\bullet}$), 121 (11; $HOC_6H_4CO^{+\bullet}$)

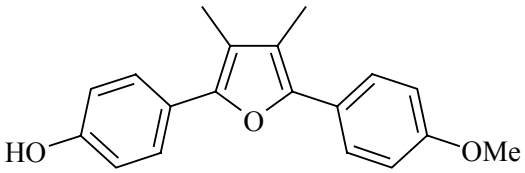
HRMS: Calculated for $C_{34}H_{48}O_5S$: 568.3222

Found: 568.3218 ± 0.0003

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.82 (t; 3H; ^3J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 0.93 (t; 3H; ^3J = 7.3Hz; -$(CH_2)_2$-C<u>H</u>$_3$), 1.21-1.36 (m; 16H; -$(CH_2)_2$-(C<u>H</u>$_2$)$_2$-CH_3, -(C<u>H</u>$_2$)$_6$-CH_2-CH_2-SO_2-), 1.41-1.53 (m; 4H; -C<u>H</u>$_2$-CH_2-furan-CH_2-C<u>H</u>$_2$-), 1.56-1.66 (m; 4H; -C<u>H</u>$_2$-CH_2-SO_2-CH_2-C<u>H</u>$_2$-), 2.50 (-C<u>H</u>$_2$-furan-C<u>H</u>$_2$-, merged in DMSO-signal), 2.99 (t; 4H; ^3J = 8.1Hz; -C<u>H</u>$_2$-SO_2-C<u>H</u>$_2$-), 6.79/7.39 (AA'BB'; 4H; ^3J = 8.6Hz; phenyl-<u>H</u>), 6.80/7.40 (AA'BB'; 4H; ^3J = 8.6Hz; phenyl-<u>H</u>), 9.54 (s; 2H; -O<u>H</u>)

2.2.6.2 Demethylation to 3,4-Dialkyl-2-(4-hydroxyphenyl)-5-(4-methoxyphenyl)furans

2-(4-Hydroxyphenyl)-5-(4-methoxyphenyl)-3,4-dimethylfuran (**48b**)



By-product from the preparation of 2,5-bis(4-hydroxyphenyl)-3,4-dimethylfuran **43b**. Separation from the main product and purification was achieved by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:5). Subsequently the product was recrystallised from ethyl acetate/petroleum ether 40-60.

Colourless solid; yield: 17%

Melting point: 126-127°C

$C_{19}H_{18}O_3$ (294.35)

IR: ν (cm^{-1}) = 3316 (w, br; O-H)

Analysis: Calculated: C: 77.53 H 6.16

Found: C: 77.48 H 5.86

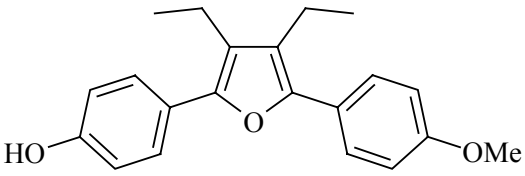
MS: m/z (%) = 294 (100; $M^{+\bullet}$), 279 (56; $[M\text{-}CH_3]^{+\bullet}$), 147 (8; $M^{2+\bullet}$)

HRMS: Calculated for $C_{19}H_{18}O_3$: 294.1256

Found: 294.1255 ± 0.0003

^{1}H-NMR (DMSO-d_6): δ (ppm) = 2.13 (s; 3H; -C<u>H</u>$_3$), 2.14 (s; 3H; -C<u>H</u>$_3$), 3.79 (s; 3H; -O-C<u>H</u>$_3$), 6.86/7.48 (AA'BB'; 4H; ^{3}J = 8.7Hz; HO-phenyl-<u>H</u>), 7.02/7.57 (AA'BB'; 4H; ^{3}J = 8.9Hz; MeO-phenyl-<u>H</u>), 9.62 (s; 1H; -O<u>H</u>)

3,4-Diethyl-2-(4-hydroxyphenyl)-5-(4-methoxyphenyl)furan (**48c**)



By-product from the preparation of 3,4-diethyl-2,5-bis(4-hydroxyphenyl)furan **43c**. Separation from the main product and purification was achieved by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:4). Subsequently the product was recrystallised from ethyl acetate/petroleum ether 40-60.

Colourless needles; yield: 62%

Melting point: 106-107°C

$C_{21}H_{22}O_3$ (322.40)

IR: ν (cm^{-1}) = 3385 (w, br; O-H)

Analysis: Calculated: C: 78.24 H 6.88

Found: C: 78.14 H 7.20

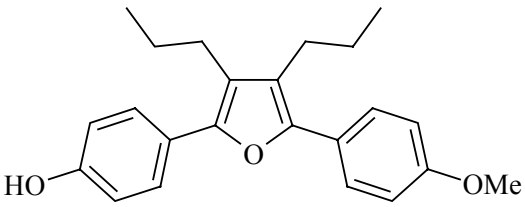
MS: m/z (%) = 322 (100; $M^{+\bullet}$), 307 (14; $[M\text{-}CH_3]^{+\bullet}$), 161 (8; $M^{2+\bullet}$), 135 (5; $CH_3OC_6H_4CO]^{+\bullet}$), 121 (5; $HOC_6H_4CO]^{+\bullet}$)

HRMS: Calculated for $C_{21}H_{22}O_3$: 322.1569

Found: 322.1571 ± 0.0002

^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.18 (t; 6H; ^{3}J = 7.4Hz; -C<u>H</u>$_3$), 2.58 (q; 2H; ^{3}J = 7.5Hz; -C<u>H</u>$_2$-), 2.59 (q; 2H; ^{3}J = 7.3Hz; -C<u>H</u>$_2$-), 3.80 (s; 3H; -O-C<u>H</u>$_3$), 6.86/7.47 (AA'BB'; 4H; ^{3}J = 8.8Hz; HO-phenyl-<u>H</u>), 7.03/7.56 (AA'BB'; 4H; ^{3}J = 8.8Hz; MeO-phenyl-<u>H</u>), 9.56 (s; 1H; -O<u>H</u>)

2-(4-Hydroxyphenyl)-5-(4-methoxyphenyl)-3,4-dipropylfuran (**48d**)



By-product from the preparation of 2,5-bis(4-hydroxyphenyl)-3,4-dipropylfuran **43d**. Separation from the main product and purification was achieved by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:4). Subsequently the product was recrystallised from ethyl acetate/petroleum ether 40-60.

Colourless solid; yield: 26%

Melting point: 107-109°C

$C_{23}H_{26}O_3$ (350.46)

IR: ν (cm^{-1}) = 3181 (w, br; O-H)

Analysis: Calculated: C: 78.83 H 7.48

Found: C: 78.37 H 7.36

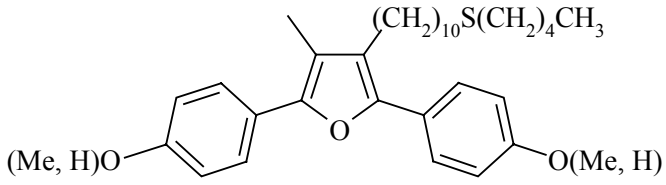
MS: m/z (%) = 350 (100; $M^{+\bullet}$), 335 (2; $[M\text{-}CH_3]^{+\bullet}$), 321 (3; $[M\text{-}CH_3CH_2\text{-}]^{+\bullet}$), 175 (9; $M^{2+\bullet}$), 135 (4; $CH_3OC_6H_4CO]^{+\bullet}$), 121 (5; $HOC_6H_4CO]^{+\bullet}$)

HRMS: Calculated for $C_{23}H_{26}O_3$: 350.1882

Found: 350.1878 ± 0.0003

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.97 (t; 6H; ^3J = 7.3Hz; -CH_2-C<u>H</u>$_3$), 1.53 (sex; 4H; ^3J = 7.6Hz; -CH_2-C<u>H</u>$_2$-CH_3), 2.53 (furan-C<u>H</u>$_2$-, merged in DMSO-signal), 3.79 (s; 3H; -O-C<u>H</u>$_3$), 6.85/7.46 (AA'BB'; 4H; ^3J = 8.8Hz; HO-phenyl-<u>H</u>), 7.03/7.56 (AA'BB'; 4H; ^3J = 9.0Hz; MeO-phenyl-<u>H</u>), 9.59 (s; 1H; -O<u>H</u>)

2-(4-Hydroxyphenyl)-5-(4-methoxyphenyl)-4-methyl-3-[10-(pentylsulfanyl)decyl]furan (**49b**)



By-product from the preparation of 2,5-bis(4-hydroxyphenyl)-4-methyl-3-[10-(pentylsulfanyl)decyl]furan **46b**. Separation from the main product and purification was achieved by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:5). The product was obtained as a mixture of two isomers.

Colourless solid; yield: 27%

Melting point: 47-49°C

$C_{33}H_{46}O_3S$ (522.79)

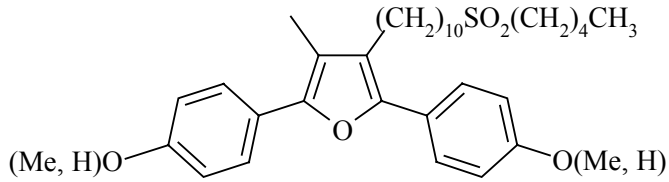
IR(KBr): ν (cm^{-1}) = 3399 (m, br; OH)

Analysis: Calculated: C: 75.82 H 8.87

Found: C: 75.60 H 8.74

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.90 (t; 3H; ^3J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.22-1.62 (m; 22H; -$(C\underline{H}_2)_3$-CH_3, -$(C\underline{H}_2)_8$-CH_2-S-), 2.20 (s; 3H, furan-C<u>H</u>$_3$), 2.48-2.57 (m; 4H; ^3J = 7.4Hz; -C<u>H</u>$_2$-S-C<u>H</u>$_2$-), 2.58 (t; 2H; ^3J = 7.7Hz; furan-C<u>H</u>$_2$-), 3.84 (s; 3H; -O-C<u>H</u>$_3$), 6.88/7.54 (AA'BB'; 4H; ^3J = 8.7Hz; HO-phenyl-<u>H</u>), 6.95/7.60 (AA'BB'; 4H; ^3J = 8.8Hz; MeO-phenyl-<u>H</u>)

2-(4-Hydroxyphenyl)-5-(4-methoxyphenyl)-4-methyl-3-[10-(pentylsulfonyl)decyl]furan (**50b**)



By-product from the preparation of 2,5-bis(4-hydroxyphenyl)-4-methyl-3-[10-(pentylsulfonyl)decyl]furan **47b**. Separation from the main product and purification was achieved by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 2:3). The product was obtained as a mixture of two isomers.

Colourless solid; yield: 29%

Melting point: 100-102°C

$C_{33}H_{46}O_5S$ (540.76)

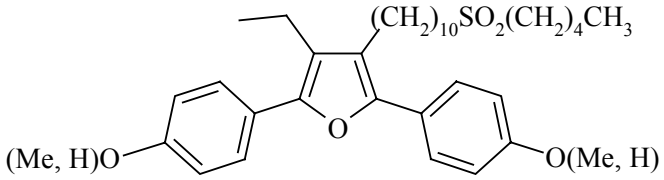
IR(KBr): ν (cm^{-1}) = 3397 (s, br; OH)

Analysis: Calculated: C: 71.44 H 8.36

Found: C: 70.94 H 8.27

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.96 (t; 3H; ^{3}J = 7.0Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.26-1.64 (m; 18H; -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_7$-CH_2-CH_2-SO_2-), 1.81-1.94 (m; 4H; -C<u>H</u>$_2$-CH_2-SO_2-CH_2-C<u>H</u>$_2$-), 2.24 (s; 3H, furan-C<u>H</u>$_3$), 2.63 (t; 2H; ^{3}J = 7.8Hz; furan-C<u>H</u>$_2$-), 3.00 (t; 4H; ^{3}J = 8.1Hz; -C<u>H</u>$_2$-SO_2-C<u>H</u>$_2$-), 3.89 (s; 3H; -OMe); 6.94/7.57 (AA'BB'; 4H; ^{3}J = 8.8Hz; HO-phenyl-<u>H</u>), 7.00/7.64 (AA'BB'; 4H; ^{3}J = 8.8Hz; MeO-phenyl-<u>H</u>)

4-Ethyl-2-(4-hydroxyphenyl)-5-(4-methoxyphenyl)-3-[10-(pentylsulfonyl)decyl]furan (**50c**)



By-product from the preparation of 4-ethyl-2,5-bis(4-hydroxyphenyl)-3-[10-(pentylsulfonyl)-decyl]furan **47c**. Separation from the main product and purification was achieved by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:2). The product was obtained as a mixture of two isomers.

Colourless solid; yield: 25%

$C_{34}H_{48}O_5S$ (568.81)

IR: ν (cm^{-1}) = 3383 (m, br; O-H)

MS: m/z (%) = 568 (100; $M^{+\bullet}$), 434 (5; $[M\text{-}SO_2(CH_2)_5]^+$), 307 (4; $[M\text{-}(CH_2)_9SO_2(CH_2)_4CH_3]^{+\bullet}$), 135 (4; $MeOC_6H_4CO^{+\bullet}$), 121 (5; $HOC_6H_4\text{-}CO^{+\bullet}$)

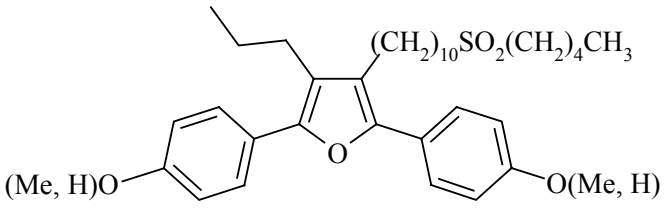
HRMS: Calculated for $C_{34}H_{48}O_5S$: 568.3222

Found: 568.3220 ± 0.0004

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.87 (t; 3H; ^{3}J = 7.0Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.17 (t; 3H; ^{3}J = 7.4Hz; -CH_2-C<u>H</u>$_3$), 1.26-1.38 (m; 16H; -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_6$-CH_2-CH_2-SO_2-), 1.52-1.60 (m; 2H; -C<u>H</u>$_2$-CH_2-furan), 1.61-1.70 (m; 4H; -C<u>H</u>$_2$-CH_2-SO_2-CH_2-C<u>H</u>$_2$-), 2.53-2.60 (m, 4H, -C<u>H</u>$_2$-furan-C<u>H</u>$_2$-), 3.03 (t; 4H; ^{3}J = 8.0Hz; -C<u>H</u>$_2$-SO_2-C<u>H</u>$_2$-), 3.32 (s; 3H; -O-C<u>H</u>$_3$), 6.85/7.45

(AA'BB'; 4H; ^3J = 8.7Hz; HO-phenyl-H), 7.03/7.56 (AA'BB'; 4H; ^3J = 8.9Hz; MeO-phenyl-H), 9.60 (s; 1H; -OH)

2-(4-Hydroxyphenyl)-5-(4-methoxyphenyl)-3-[10-(pentylsulfonyl)decyl]-4-propylfuran (**50d**)



By-product from the preparation of 2,5-bis(4-hydroxyphenyl)-3-[10-(pentylsulfonyl)decyl]-4-propylfuran **47d**. Separation from the main product and purification was achieved by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:3). The product was obtained as a mixture of two isomers.

Yellow oil; yield: 22%

$C_{35}H_{50}O_5S$ (582.84)

IR: ν (cm^{-1}) = 3391 (m, br; O-H)

MS: m/z (%) = 582 (100; $M^{+\bullet}$), 448 (8; $[M\text{-}SO_2(CH_2)_5]^+$), 321 (5; $[M\text{-}(CH_2)_9SO_2(CH_2)_4CH_3]^{+\bullet}$), 135 (7; $MeOC_6H_4CO^{+\bullet}$), 121 (9; $HOC_6H_4\text{-}CO^{+\bullet}$)

HRMS: Calculated for $C_{35}H_{50}O_5S$: 582.3379

Found: 582.3382 ± 0.0003

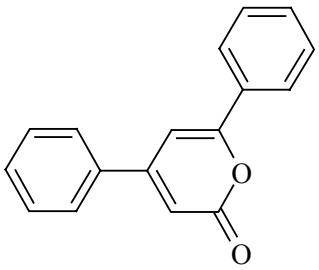
^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.85 (t; 3H; ^3J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 0.96 (t; 3H; ^3J = 7.3Hz; -$(CH_2)_2$-C<u>H</u>$_3$), 1.24-1.39 (m; 16H; -$(CH_2)_2$-(C<u>H</u>$_2$)$_2$-CH_3, -(C<u>H</u>$_2$)$_6$-CH_2-CH_2-SO_2-), 1.43-1.56 (m; 4H; -C<u>H</u>$_2$-CH_2-furan-CH_2-C<u>H</u>$_2$-), 1.59-1.69 (m; 4H; -C<u>H</u>$_2$-CH_2-SO_2-CH_2-C<u>H</u>$_2$-), 2.50 (-C<u>H</u>$_2$-furan-C<u>H</u>$_2$-, merged in DMSO-signal), 3.02 (t; 4H; ^3J = 8.0Hz; -C<u>H</u>$_2$-SO_2-C<u>H</u>$_2$-), 3.30 (s; 3H; -O-C<u>H</u>$_3$), 6.83/7.44 (AA'BB'; 4H; ^3J = 8.7Hz; HO-phenyl-H), 7.01/7.54 (AA'BB'; 4H; ^3J = 8.9Hz; MeO-phenyl-H), 9.59 (s; 1H; -OH)

2.3 3,5-Dialkyl-2,4-bis(4-hydroxyphenyl)furans

2.3.1 Procedures and Compounds of Unsuccessful Pathways

2.3.1.1 Attempted Auxilliary Mediated Furan Synthesis

4,6-Diphenyl-1-pyran-2-one (**60**)



A mixture of ethyl benzoylacetate (300mmol) and conc. sulfuric acid (30ml) was stirred at room temperature for 20 days. The dark-green viscous mass was poured onto ice and 25% sulfuric acid was used to transfer the whole lot. The acidic water was decanted from the organic precipitate. The latter was taken up in water (500ml) and extracted with three portions of ethyl acetate (3x250ml). The combined organic phases were washed with water and dried over Na_2SO_4. Evaporation of the solvent and recrystallisation of the brown crude product from EtOH (99%) afforded the desired compound.

Slightly yellow crystals; yield 38%

$C_{17}H_{12}O_2$ (248.28)

Melting point: 136°C (lit: 138°C; [Katritzky et al., 1979])

IR: ν (cm^{-1}) = 1698 (s; C=O)

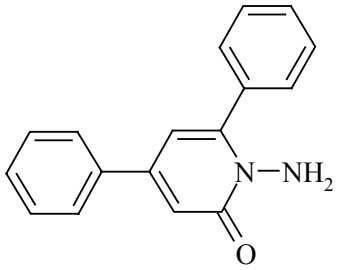
Analysis: Calculated: C: 82.24 H 4.87

Found: C: 82.11 H 4.61

^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.47 (d; 1H; ^{4}J = 1.5Hz; -C<u>H</u>-), 6.96 (d; 1H; ^{4}J = 1.5Hz; -C<u>H</u>-), 7.45-7.53 (m; 6H; phenyl-<u>H</u>), 7.62-7.67 (m; 2H; phenyl-<u>H</u>), 7.87-7.93 (m; 2H; phenyl-<u>H</u>)

^{13}H-NMR ($CDCl_3$): δ (ppm) = 101.38 (CH), 109.26 (CH), 125.76 (2x CH), 126.73 (2x CH), 128.97 (2x CH), 129.25 (2x CH), 130.70 (CH), 130.93 (CH), 131.53 (C), 136.04 (C), 155.61 (C), 160.37 (C), 162.69 (C=O)

1-Amino-4,6-diphenylpyridin-2-one (**61**)



A mixture of 4,6-diphenyl-1-pyran-2-one **60** (50mmol) and hydrazine hydrate (50ml) in EtOH (100ml) were refluxed for 4h. The reaction mixture was poured onto ice-water (300ml) and the precipitate collected by suction and washed with water. The crude product was recrystallised from benzene.

Colourless needles; yield: 50%

$C_{17}H_{14}N_2O$ (262.31)

Melting point: 163-164°C (lit: 166°C; [Katritzky et al., 1979])

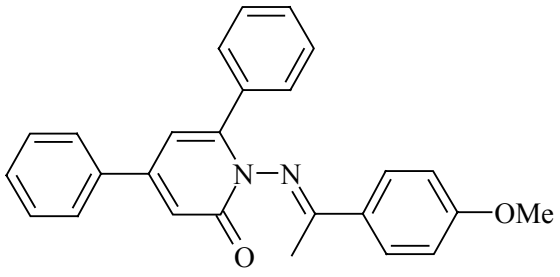
IR:	ν (cm^{-1}) = 3271 (w; N-H), 1636 (s; C=O)			
Analysis:	Calculated:	C: 77.84	H 5.38	N 10.68
	Found:	C: 77.79	H 5.40	N 10.71

MS: m/z (%) = 262 (85; $M^{+\bullet}$), 261 (100; $[MH]^{+\bullet}$), 247 (15; $[M\text{-}NH_2]^{+\bullet}$)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 5.04 (s, br; 2H; -NH_2), 6.46 (d; 1H; ^4J = 2.2Hz; -C<u>H</u>-), 6.87 (d; 1H; ^4J = 2.2Hz; -C<u>H</u>-), 7.42-7.62 (m; 10H; phenyl-<u>H</u>)

^{13}H-NMR ($CDCl_3$): δ (ppm) = 107.04 (CH), 113.54 (CH), 126.81 (2x CH), 128.44 (2x CH), 128.98 (2x CH), 129.01 (2x CH), 129.41 (CH), 129.55 (CH), 133.90 (C), 137.57 (C), 147.69 (C), 150.09 (C), 161.06 (C=O)

1-[1-(4-Methoxyphenyl)ethylideneamino]-4,6-diphenylpyridin-2-one (**62**)



A mixture of 4-methoxyacetophenone (3.8mmol) and boron triflouride etherate (3.8mmol) in dry THF (10ml) was refluxed for 2h. Then, 1-amino-4,6-diphenylpyridin-2-one **61** (3.8mmol) in dry THF (5ml) was added dropwise and refluxing continued for 2h. The reaction mixture was poured into water (25ml) and extracted with ethyl acetate (3x25ml). The combined organic layers were washed with water and dried over Na_2SO_4. The brown crude solid was purified by column chromatography (SiO_2; DCM/ethyl acetate 1:3, v/v).

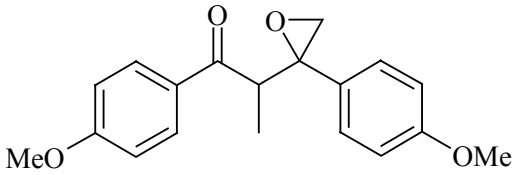
Yellow solid; yield: 16%

$C_{25}H_{22}N_2O_2$ (382.46)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.23 (s; 3H; -C<u>H</u>$_3$), 3.82 (s; 3H; -O-C<u>H</u>$_3$), 6.55 (d; 1H; ^{4}J = 2.2Hz; -C<u>H</u>-), 6.94 (d; 1H; ^{4}J = 2.2Hz; -C<u>H</u>-), 7.34-7.54 (m; 10H; phenyl-<u>H</u>), 6.85/7.69 (AA'BB'; 4H; ^{3}J = 9.0Hz; phenyl-<u>H</u>)

2.3.1.2 Synthesis of a 5-Unsubstituted Furan

1,2-Epoxy-3-(4-methoxybenzoyl)-2-(4-methoxyphenyl)butane (**63**)



Preparation from 1-(4-methoxyphenyl)propan-1-one **22b** (15.2mmol) and 2-bromo-1-(4-methoxyphenyl)ethan-1-one **30a** (15.2mmol) following the procedure described in section E2.3.2. Purification of the crude product by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:5, v/v) afforded the desired compound as diastereomeric mixture.

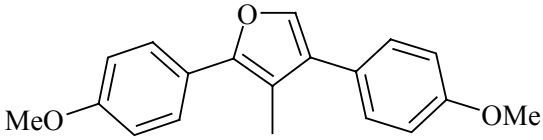
Colourless oil; yield 75%

$C_{19}H_{20}O_4$ (312.37)

IR: ν (cm^{-1}) = 1672 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.25 (d; 3H; ^{3}J = 7.1Hz; -CH-C<u>H</u>$_3$), 2.91/3.17 (AB; 2H; ^{3}J = 4.9Hz; -O-C<u>H</u>$_2$-), 3.75 (s; 3H; -O-C<u>H</u>$_3$), 3.87 (s; 3H; -O-C<u>H</u>$_3$), 4.00 (q; 1H; ^{3}J = 7.1Hz; -C<u>H</u>-CH$_3$), 6.77/7.20 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 6.93/7.96 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>)

2,4-Bis(4-methoxyphenyl)-3-methylfuran (**64**)



Chloroform, that was slightly acidified with aqueous H_2SO_4 (0.5M, 5ml), was heated to 55°C and 1,2-epoxy-3-(4-methoxybenzoyl)-2-(4-methoxyphenyl)butan **63** (12.8mmol) in $CHCl_3$ (250ml) added dropwise. After stirring at this temperature for 1h, the reaction mixture was cooled to ambient temperature, washed with sodium bicarbonate solution and dried over Na_2SO_4. Purification was achieved by recrystallisation from EtOH (99%) and column chromatography of the mother liquor (SiO_2; DCM/petroleum ether 40-60 1:1, v/v).

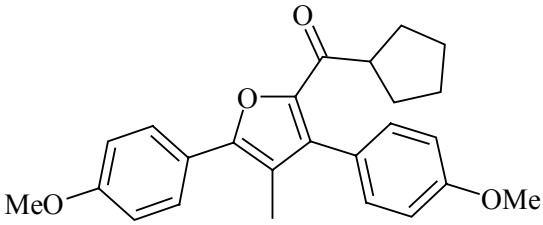
Colourless crystals; yield: 38%

$C_{19}H_{18}O_3$ (294.35)

Analysis:	Calculated:	C: 77.53	H 6.16
	Found:	C: 76.93	H 6.01

^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.26 (s; 3H; -C<u>H</u>$_3$), 3.84 (s; 3H; -O-C<u>H</u>$_3$), 3.85 (s; 3H; -O-C<u>H</u>$_3$), 6.96/7.34 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 7.44 (s; 1H; furan-<u>H</u>), 6.98/7.59 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>)

5-Cyclopentylcarbonyl-2,4-bis(4-methoxyphenyl)-3-methylfuran (**65**)



n-BuLi (1.6M in hexane, 1.1eq) in dry THF (10ml) was cooled to –15°C and 2,4-bis(4-methoxyphenyl)-3-methylfuran **64** (3.4mmol) in dry THF (40ml) added. The solution was stirred at this temperature for 2h until N-(6-iodohexanoyl)pyrrolidine **21** (3.7mmol) in dry THF (10ml) was added. The reaction mixture was stirred at –15°C for 2h, warmed to room temperature within 2h and stirred overnight. Then, water (50ml) was added and the aqueous phase extracted with ethyl acetate (3x 50ml). The combined organic layers were washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent and purification by col-

umn chromatography yielded the unsubstituted furan educt as colourless solid (58%) and this unexpected product.

Yellow oil; yield: 26%

$C_{25}H_{26}O_4$ (390.48)

IR: ν (cm^{-1}) = 1661 (s; C=O)

MS: m/z (%) = 390 (79; $M^{+\bullet}$), 349 (69; $[M\text{-}CH_2CH{=}CH_2]^{+\bullet}$), 321 (100; $[furan\text{-}CO]^{+\bullet}$), 294 (60; $[furan\text{-}H]^{+\bullet}$), 265 (72; $[294\text{-}CHO]^{+\bullet}$), 135 (32; $[MeOC_6H_4CO]^{+\bullet}$), 69 (21; $[CH(CH_4)_2]^{+\bullet}$), 41 (29; $CH_2CH{=}CH_2]^{+\bullet}$)

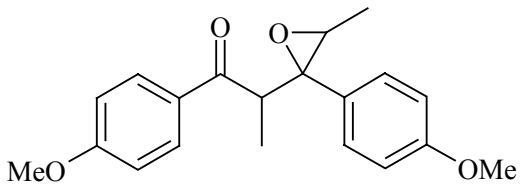
^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.52-1.57(m; 4H; -($C\underline{H}_2$)$_2$-), 1.82-1.92 (m; 4H; -$C\underline{H}_2$-CH-$C\underline{H}_2$), 2.13 (s; 3H; -$C\underline{H}_3$), 3.58 (quin; 1H; ^3J = 8.0Hz; -CO-C$\underline{H}$-), 3.85 (s; 3H; -O-$C\underline{H}_3$), 3.88 (s; 3H; -O-$C\underline{H}_3$), 6.98/7.33 (AA'BB'; 4H; ^3J = 8.8Hz; phenyl-$\underline{H}$), 7.02/7.72 (AA'BB'; 4H; ^3J = 8.8Hz; phenyl-$\underline{H}$)

^{13}H-NMR ($CDCl_3$): δ (ppm) = 10.39 (CH_3), 26.36 (2x CH_2), 29.58 (2x CH_2), 47.13 (CH), 55.25 (CH_3), 55.37 (CH_3), 113.54 (2x CH), 114.22 (2x CH), 118.11 (C), 123.48 (C), 124.16 (C), 127.93 (2x CH), 131.14 (2x CH), 136.08 (C), 145.40 (C-O), 151.32 (C-O), 159.32 (C-O), 159.73 (C-O), 192.71 (C=O)

2.3.2 Synthesis of the Epoxide Precursors

Under nitrogen atmosphere at –78°C, a solution of alkylarylketone (1eq) in dry THF was added dropwise to LDA (2M in THF, 1eq) and the mixture was stirred for 0.5h. Then, the respective α-bromoketone (1eq) in dry THF was added and stirring continued at this temperature for 1.5h. Subsequently, the reaction mixture was warmed to –10°C and kept stirring for another 1.5h. The mixture was hydrolysed by the addition of water (75ml) and the aqueous phase extracted with ethyl acetate (3x 75ml). The combined organic layers were washed with water and brine After drying over Na_2SO_4 the solvent was evaporated *in vacuo*.

2,3-Epoxy-4-(4-methoxybenzoyl)-3-(4-methoxyphenyl)pentane (**66b**)



Preparation from 1-(4-methoxyphenyl)propan-1-one **22b** (12.2mmol) and 2-bromo-1-(4-methoxyphenyl)propan-1-one **30b** (12.2mmol). The crude mixture of diastereomers was used in the cyclisation step.

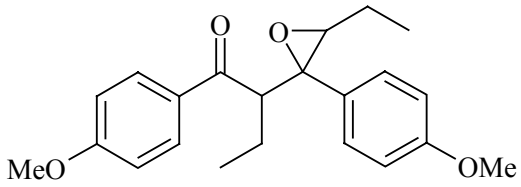
Yellow oil; yield: 98%

$C_{20}H_{22}O_4$ (326.39)

IR: ν (cm^{-1}) = 1672 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.03 (d; 3H; ^{3}J = 5.4Hz; -O-CH-C<u>H</u>$_3$), 1.26 (d; 3H; ^{3}J = 7.1Hz; -CO-CH-C<u>H</u>$_3$), 3.34 (q; 1H; ^{3}J = 5.4Hz; -O-C<u>H</u>-), 3.75 (s; 3H; -O-C<u>H</u>$_3$), 3.79 (q; 1H; ^{3}J = 7.2Hz; -C<u>H</u>-CH$_3$), 3.87 (s; 3H; -O-C<u>H</u>$_3$), 6.77/7.08 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 6.93/7.95 (AA'BB'; 4H; ^{3}J = 9.0Hz; phenyl-<u>H</u>)

3,4-Epoxy-5-(4-methoxybenzoyl)-4-(4-methoxyphenyl)heptane (**66c**)



Preparation from 1-(4-methoxyphenyl)butan-1-one **22c** (11.2mmol) and 2-bromo-1-(4-methoxyphenyl)butan-1-one **30c** (11.2mmol). The crude mixture of diastereomers was used in the cyclisation step.

Yellow oil; yield: 99%

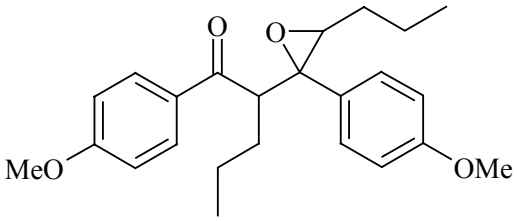
$C_{22}H_{26}O_4$ (354.45)

IR: ν (cm^{-1}) = 1671 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.88 (t; 3H; ^{3}J = 7.3Hz; -CH$_2$-C<u>H</u>$_3$), 0.92 (t; 3H; ^{3}J = 7.3Hz; -CH$_2$-C<u>H</u>$_3$), 1.14-1.28 (m; 2H; -C<u>H</u>$_2$-CH$_3$), 1.64-1.80 (m; 1H;

-CO-CH-C$\underline{H}_a$H$_b$-), 1.92-2.07 (m; 1H; -CO-CH-CH$_a$$\underline{H}_b$-), 3.14 (t; 1H; ^{3}J = 6.2Hz;-O-C$\underline{H}$-), 3.53 (dd; 1H; ^{3}J = 4.7Hz, ^{3}J = 9.6Hz; -CO-C$\underline{H}$-), 3.76 (s; 3H; -O-C$\underline{H}_3$), 3.87 (s; 3H; -O-C$\underline{H}_3$), 6.77/7.07 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-$\underline{H}$), 6.93/7.96 (AA'BB'; 4H; ^{3}J = 8.9Hz; phenyl-$\underline{H}$)

4,5-Epoxy-6-(4-methoxybenzoyl)-5-(4-methoxyphenyl)nonane (**66d**)



Preparation from 1-(4-methoxyphenyl)pentan-1-one **22d** (5.2mmol) and 2-bromo-1-(4-methoxyphenyl)pentan-1-one **30d** (5.2mmol). The crude mixture of diastereomers was used in cyclisation step.

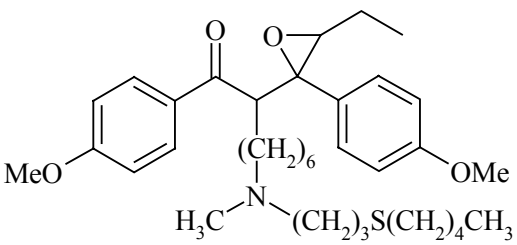
Yellow oil; yield: 99%

$C_{24}H_{30}O_4$ (382.50)

IR: ν (cm^{-1}) = 1671 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.84 (t; 3H; ^{3}J = 7.5Hz; -CH$_2$-C$\underline{H}_3$), 0.86 (t; 3H; ^{3}J = 7.5Hz; -CH$_2$-C$\underline{H}_3$), 1.17-1.31 (m; 4H; -C$\underline{H}_2$-CH$_3$), 1.34-1.47 (m; 2H; -O-CH-C$\underline{H}_2$-), 1.53-1.66 (m; 1H; -CO-CH-C$\underline{H}_a$H$_b$-), 1.92-2.03 (m; 1H; -CO-CH-CH$_a$$\underline{H}_b$-), 3.18 (dd; 1H; ^{3}J = 5.2Hz, ^{3}J = 6.8Hz;-O-C$\underline{H}$-), 3.62 (dd; 1H; ^{3}J = 4.2Hz, ^{3}J = 9.9Hz; -CO-C$\underline{H}$-), 3.76 (s; 3H; -O-C$\underline{H}_3$), 3.87 (s; 3H; -O-C$\underline{H}_3$), 6.77/7.07 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-$\underline{H}$), 6.92/7.94 (AA'BB'; 4H; ^{3}J = 8.9Hz; phenyl-$\underline{H}$)

3,4-Epoxy-5-(4-methoxybenzoyl)-4-(4-methoxyphenyl)-11-{N-methyl-N-[3-(pentylsulfanyl)-propyl]amino}undecane (**67**)



Preparation from 1-(4-methoxyphenyl)-8-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}-octan-1-one **25** (3.0mmol) and 2-bromo-1-(4-methoxyphenyl)-butan-1-one **30c** (3.0mmol). Sat. sodium bicarbonate solution was used for the aqueous work-up. The crude mixture of diastereomers was used in the following cyclisation step.

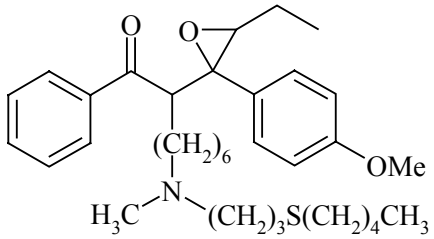
Yellow oil; yield:97%

$C_{35}H_{53}NO_4S$ (583.87)

IR: ν (cm^{-1}) = 1674 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^3J = 7.4Hz; -CH_2-C$\underline{H}_3$), 0.92 (t; 3H; ^3J = 7.5Hz;-CH_2-C$\underline{H}_3$), 1.13-1.48 (m; 14H; -(C$\underline{H}_2$)$_2$-CH_3, -N-CH_2-(C$\underline{H}_2$)$_4$-, -O-CH-C$\underline{H}_2$-), 1.53-1.66 (m; 3H; ^3J = 7.3Hz; -S-CH_2-C$\underline{H}_2$-, -CO-CH-C$\underline{H}_a$H$_b$-), 1.72 (quin; 2H; ^3J = 7.4Hz; -N-CH_2-C$\underline{H}_2$-CH_2-S-), 1.91-2.01 (m; 1H; -CO-CH-CH_aH$_b$-), 2.18 (s; 3H; -N-C$\underline{H}_3$-), 2.26 (t; 2H; ^3J = 7.6Hz; -N-C$\underline{H}_2$-), 2.38 (t; 2H; ^3J = 7.4Hz; -N-C$\underline{H}_2$-), 2.50 (t; 2H; ^3J = 7.4Hz; -S-C$\underline{H}_2$-), 2.51 (t; 2H; ^3J = 7.3Hz; -S-C$\underline{H}_2$-), 3.12 (t; 1H; ^3J = 6.2Hz; -O-C$\underline{H}$-), 3.60 (dd; 1H; ^3J = 4.2Hz, ^3J = 9.9Hz; -CO-C$\underline{H}$-), 3.76 (s; 3H; -O-C$\underline{H}_3$), 3.87 (s; 3H; -O-C$\underline{H}_3$), 6.77/7.06 (AA'BB'; 4H; ^3J = 8.8Hz; phenyl-$\underline{H}$), 6.93/7.94 (AA'BB'; 4H; ^3J = 8.9Hz; phenyl-$\underline{H}$)

5-Benzoyl-3,4-epoxy-4-(4-methoxyphenyl)-11-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}-undecane (**68**)



Preparation from 8-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}-1-phenyloctan-1-one **29** (6.0mmol) and 2-bromo-1-(4-methoxyphenyl)butan-1-one **30c** (6.0mmol). Sat. sodium bicarbonate solution was used for the aqueous work-up. The crude mixture of diastereomers was used in the following cyclisation step.

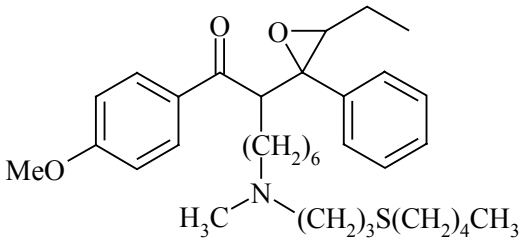
Yellow oil; yield: 90%

$C_{34}H_{51}NO_3S$ (553.85)

IR: ν (cm^{-1}) = 1673 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^{3}J = 7.4Hz; -CH_2-C<u>H</u>$_3$), 0.92 (t; 3H; ^{3}J = 7.5Hz; -CH_2-C<u>H</u>$_3$), 1.13-1.48 (m; 14H; -(C<u>H</u>$_2$)$_2$-CH_3, -N-CH_2-(C<u>H</u>$_2$)$_4$-, -O-CH-C<u>H</u>$_2$-), 1.53-1.66 (m; 3H; ^{3}J = 7.3Hz; -S-CH_2-C<u>H</u>$_2$-, -CO-CH-C<u>H</u>$_a$H$_b$-), 1.72 (quin; 2H; ^{3}J = 7.4Hz; -N-CH_2-C<u>H</u>$_2$-CH_2-S-), 1.91-2.01 (m; 1H; -CO-CH-CH_a<u>H</u>$_b$-), 2.18 (s; 3H; -N-C<u>H</u>$_3$-), 2.26 (t; 2H; ^{3}J = 7.6Hz; -N-C<u>H</u>$_2$-), 2.38 (t; 2H; ^{3}J = 7.4Hz; -N-C<u>H</u>$_2$-), 2.50 (t; 2H; ^{3}J = 7.4Hz; -S-C<u>H</u>$_2$-), 2.51 (t; 2H; ^{3}J = 7.3Hz; -S-C<u>H</u>$_2$-), 3.12 (t; 1H; ^{3}J = 6.2Hz; -O-C<u>H</u>-), 3.65 (dd; 1H; ^{3}J = 4.2Hz, ^{3}J = 9.9Hz; -CO-C<u>H</u>-), 3.76 (s; 3H; -O-C<u>H</u>$_3$), 6.78/7.08 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 7.43-7.48 (m; 2H; phenyl-<u>H</u>^{3}, phenyl-<u>H</u>^{5}), 7.53-7.58 (m; 1H; phenyl-<u>H</u>^{4}), 7.93-7.98 (m; 2H; phenyl-<u>H</u>^{2}, phenyl-<u>H</u>^{6})

3,4-Epoxy-5-(4-methoxybenzoyl)-11-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}-4-phenylundecane (**69**)



Preparation from 1-(4-methoxyphenyl)-8-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}-octan-1-one **25** (4.0mmol) and 2-bromo-1-phenylbutan-1-one **32** (4.0mmol). Sat. sodium bicarbonate solution was used for the aqueous work-up. The crude mixture of diastereomers was used in the following cyclisation step.

Yellow oil; yield: 91%

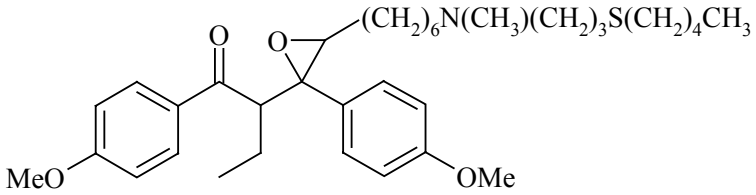
$C_{34}H_{51}NO_3S$ (553.85)

IR: ν (cm^{-1}) = 1673 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^{3}J = 7.3Hz; -CH_2-C<u>H</u>$_3$), 0.92 (t; 3H; ^{3}J = 7.4Hz;-CH_2-C<u>H</u>$_3$), 1.15-1.41 (m; 14H; -(C<u>H</u>$_2$)$_2$-CH_3, -N-CH_2-(C<u>H</u>$_2$)$_4$-, -O-CH-C<u>H</u>$_2$-), 1.53-1.65 (m; 3H; -S-CH_2-C<u>H</u>$_2$-, -CO-CH-C<u>H</u>$_a$H$_b$-),

1.73 (quin; 2H; ^3J = 7.3Hz; -N-CH_2-C$\underline{H}_2$-CH_2-S-), 1.92-2.02 (m; 1H; -CO-CH-$CH_a\underline{H}_b$-), 2.17 (s; 3H; -N-C$\underline{H}_3$-), 2.26 (t; 2H; ^3J = 7.5Hz; -N-C$\underline{H}_2$-), 2.39 (t; 2H; ^3J = 7.3Hz; -N-C$\underline{H}_2$-), 2.50 (t; 2H; ^3J = 7.5Hz; -S-C$\underline{H}_2$-), 2.51 (t; 2H; ^3J = 7.3Hz; -S-C$\underline{H}_2$-), 3.16 (dd; 1H; ^3J = 6.2Hz; ^3J = 6.2Hz; -O-C$\underline{H}$-), 3.67 (dd; 1H; ^3J = 4.0Hz, ^3J = 9.7Hz; -CO-C$\underline{H}$-), 3.87 (s; 3H; -O-C$\underline{H}_3$), 6.92/7.93 (AA'BB'; 4H; ^3J = 9.1Hz; phenyl-$\underline{H}$), 7.14-7.29 (m; 5H; phenyl-$\underline{H}$)

4,5-Epoxy-3-(4-methoxybenzoyl)-4-(4-methoxyphenyl)-11-{N-methyl-N-[3-(pentylsulfanyl)-propyl]amino}undecane (**70**)



Preparation from 1-(4-methoxyphenyl)butan-1-one **22c** (3.5mmol) and 2-bromo-1-(4-methoxyphenyl)-8-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}octan-1-one **31** (3.5mmol). Sat. sodium bicarbonate solution was used for the aqueous work-up. The crude mixture of diastereomers was used in the following cyclisation step.

Yellow oil; yield:98%

$C_{35}H_{53}NO_4S$ (583.87)

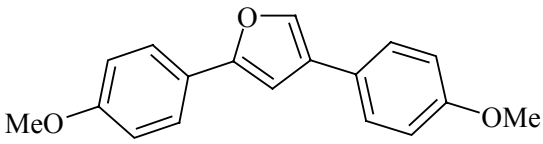
IR: ν (cm^{-1}) = 1673 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.87 (t; 3H; ^3J = 7.1Hz; -CH_2-C$\underline{H}_3$), 0.89 (t; 3H; ^3J = 6.9Hz; -CH_2-C$\underline{H}_3$), 1.19-1.43 (m; 14H; -(C$\underline{H}_2$)$_2$-CH_3, -N-CH_2-(C$\underline{H}_2$)$_5$-), 1.58 (quin; 2H; ^3J = 7.3Hz; -S-CH_2-C$\underline{H}_2$-), 1.66-1.80 (m; 3H; -CH-C$\underline{H}_a$H$_b$-CH_3, -N-CH_2-C$\underline{H}_2$-CH_2-S-), 1.91-2.01 (m; 1H; -CH-$CH_a\underline{H}_b$-CH_3), 2.19 (s; 3H; -N-C$\underline{H}_3$-), 2.27 (t; 2H; ^3J = 7.3Hz; -N-C$\underline{H}_2$-), 2.39 (t; 2H; ^3J = 7.3Hz; -N-C$\underline{H}_2$-), 2.50 (t; 2H; ^3J = 7.3Hz; - S-C$\underline{H}_2$-), 2.52 (t; 2H; ^3J = 7.3Hz; -S-C$\underline{H}_2$-), 3.17 (dd; 1H; ^3J = 5.2Hz, ^3J = 6.6Hz; -O-C$\underline{H}$-), 3.53 (dd; 1H; ^3J = 4.4Hz, ^3J = 9.6Hz; -CO-C$\underline{H}$-), 3.77 (s; 3H; -O-C$\underline{H}_3$), 3.87 (s; 3H; -O-C$\underline{H}_3$), 6.77/7.06 (AA'BB'; 4H; ^3J = 8.8Hz; phenyl-$\underline{H}$), 6.93/7.95 (AA'BB'; 4H; ^3J = 8.9Hz; phenyl-$\underline{H}$)

2.3.3 Cyclisation to 3,5-Dialkyl-2,4-bis(4-methoxyphenyl)furans

A solution of the respective epoxide (1.0eq) and 4-toluenesulfonic acid monohydrate (0.3eq or 1.3eq for compounds with amine functionality) in chloroform was stirred at reflux for 2h. After cooling to ambient temperature the reaction mixture was washed with sodium bicarbonate solution, water and brine. The solvent was dried over Na_2SO_4 and removed under reduced pressure.

2,4-Bis(4-methoxyphenyl)furan (**71a**)



Preparation from 1-(4-methoxyphenyl)ethan-1-one **22a** (10.0mmol) and 2-bromo-1-(4-methoxyphenyl)ethan-1-one **30a** (10.0mmol) as described in section E2.3.2. The desired epoxide intermediate could not be isolated, because it readily cyclised to the furan, that was purified by recrystallisation from a MeOH/DCM mixture.

Yellow crystals; yield 59%

$C_{18}H_{16}O_3$ (280.32)

Melting point: 190-193°C

Analysis: Calculated: C: 77.12 H 5.75

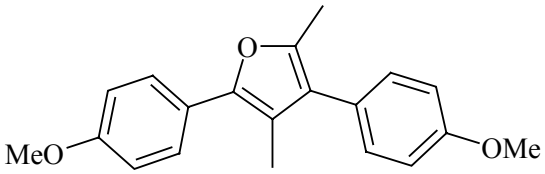
Found: C: 76.95 H 5.85

MS: m/z (%) = 280 (100; $M^{+\bullet}$), 251 (12; $[M\text{-}CHO]^{+\bullet}$), 237 (23; $[M\text{-}CH_3\text{-}CO]^{+\bullet}$), 135 (9; $[MeOC_6H_4CO]^{+\bullet}$)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.84 (s; 3H; -O-C$\underline{H}_3$), 3.85 (s; 3H; -O-C$\underline{H}_3$), 6.78 (d; 1H; ^{4}J = 0.8Hz; furan-$\underline{H}^3$), 6.93/7.64 (AA'BB'; 4H; ^{3}J = 8.9Hz; phenyl-$\underline{H}$), 6.94/7.45 (AA'BB'; 4H; ^{3}J = 8.9Hz; phenyl-$\underline{H}$), 7.63 (d; 1H; ^{4}J = 0.8Hz; furan-$\underline{H}^5$),

^{13}H-NMR ($CDCl_3$): δ (ppm) = 55.4 (2x CH_3), 102.5 (CH), 114.2 (2x CH), 114.3 (2x CH), 123.9 (C), 125.3 (2x CH), 127.0 (2x CH), 127.9 (2x C), 136.5 (CH), 154.8 (C-O), 158.8 (C-O), 159.2 (C-O)

2,4-Bis(4-methoxyphenyl)-3,5-dimethylfuran (**71b**)



Preparation from 2,3-epoxy-4-(4-methoxybenzoyl)-3-(4-methoxyphenyl)pentane **66b** (12.0mmol). Purification was achieved by column chromatography (SiO_2; DCM/petroleum ether 40-60 1:2, v/v).

Colourless solid; yield 79%

$C_{20}H_{20}O_3$ (308.38)

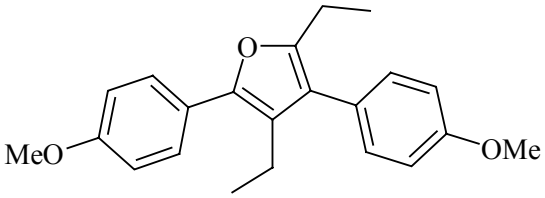
Melting point: 127-128°C

Analysis: Calculated: C: 77.90 H 6.54

Found: C: 77.72 H 6.46

^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.14 (s; 3H; -C<u>H</u>$_3$), 2.33 (s; 3H; -C<u>H</u>$_3$), 3.84 (s; 3H; -O-C<u>H</u>$_3$), 3.85 (s; 3H; -O-C<u>H</u>$_3$), 6.97/7.22 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 6.96/7.57 (AA'BB'; 4H; ^{3}J = 8.9Hz; phenyl-<u>H</u>)

3,5-Diethyl-2,4-bis(4-methoxyphenyl)furan (**71c**)



Preparation from 3,4-epoxy-5-(4-methoxybenzoyl)-4-(4-methoxyphenyl)heptane **66c** (11.1mmol). Purification was achieved by column chromatography (SiO_2; DCM/petroleum ether 40-60 1:2, v/v).

Colourless solid; yield 80%

$C_{22}H_{24}O_3$ (336.43)

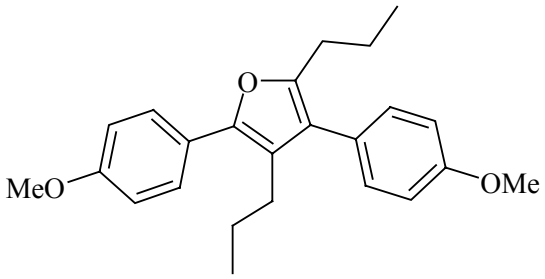
Melting point: 85-86°C

Analysis: Calculated: C: 78.54 H 7.19

Found: C: 78.26 H 7.14

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.02 (t; 3H; ^{3}J = 7.5Hz; -C<u>H</u>$_3$), 1.22 (t; 3H; ^{3}J = 7.5Hz; -C<u>H</u>$_3$), 2.57 (q; 2H; ^{3}J = 7.5Hz; -C<u>H</u>$_2$-CH$_3$), 2.61 (t; 2H; ^{3}J = 7.5Hz; -C<u>H</u>$_2$-CH$_3$), 3.84 (s; 3H; -O-C<u>H</u>$_3$), 3.85 (s; 3H; -O-C<u>H</u>$_3$), 6.94/7.21 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 6.95/7.57 (AA'BB'; 4H; ^{3}J = 8.9Hz; phenyl-<u>H</u>)

2,4-Bis(4-methoxyphenyl)-3,5-dipropylfuran (**71d**)



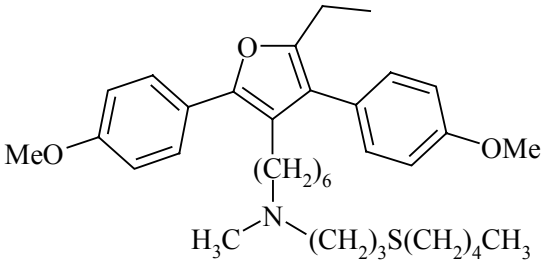
Preparation from 4,5-epoxy-6-(4-methoxybenzoyl)-5-(4-methoxyphenyl)nonane **66d** (5.1mmol). Purification was achieved by column chromatography (SiO_2; DCM/petroleum ether 40-60 1:2, v/v).

Colourless oil; yield 78%

$C_{24}H_{28}O_3$ (364.49)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.80 (t; 3H; ^{3}J = 7.3Hz; -C<u>H</u>$_3$), 0.91 (t; 3H; ^{3}J = 7.4Hz; -C<u>H</u>$_3$), 1.40 (sex; 2H; ^{3}J = 7.4Hz; -C<u>H</u>$_2$-CH$_3$), 1.66 (sex; 2H; ^{3}J = 7.4Hz; -C<u>H</u>$_2$-CH$_3$), 2.50 (t; 2H; ^{3}J = 7.8Hz; -C<u>H</u>$_2$-CH$_2$-CH$_3$), 2.55 (t; 2H; ^{3}J = 7.5Hz; -C<u>H</u>$_2$-CH$_2$-CH$_3$), 3.84 (s; 3H; -O-C<u>H</u>$_3$), 3.85 (s; 3H; -O-C<u>H</u>$_3$), 6.94/7.19 (AA'BB'; 4H; ^{3}J = 8.7Hz; phenyl-<u>H</u>), 6.95/7.56 (AA'BB'; 4H; ^{3}J = 8.9Hz; phenyl-<u>H</u>)

5-Ethyl-2,4-bis(4-methoxyphenyl)-3-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-furan (**72**)



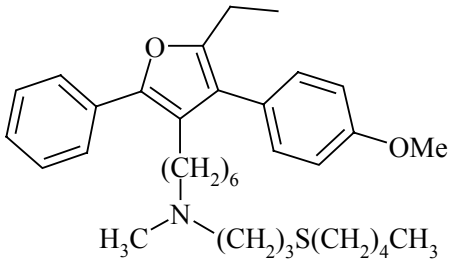
Preparation from 3,4-epoxy-5-(4-methoxybenzoyl)-4-(4-methoxyphenyl)-11-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}undecane **67** (2.9mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/methanol 9:1, v/v).

Yellow oil; yield: 55%

$C_{35}H_{51}NO_3S$ (565.86)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^{3}J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.22 (t; 3H; ^{3}J = 7.5Hz; -CH_2-C<u>H</u>$_3$), 1.12-1.43 (m; 12H; -(C<u>H</u>$_2$)$_2$-CH_3, -N-CH_2-(C<u>H</u>$_2$)$_4$-), 1.57 (quin; 2H; ^{3}J = 7.3Hz; -C<u>H</u>$_2$-CH_2-S-), 1.75 (quin; 2H; ^{3}J = 7.3Hz; -N-CH_2-C<u>H</u>$_2$-CH_2-S-), 2.17 (s; 3H; -N-C<u>H</u>$_3$), 2.23 (t; 2H; ^{3}J = 7.6Hz; -N-C<u>H</u>$_2$-), 2.38 (t; 2H; ^{3}J = 7.3Hz; -N-C<u>H</u>$_2$-), 2.47-2.55 (m; 6H; -C<u>H</u>$_2$-S-C<u>H</u>$_2$-, furan-C<u>H</u>$_2$-), 2.61 (q; 2H; ^{3}J = 7.5Hz; -C<u>H</u>$_2$-CH_3), 3.84 (s; 3H; -O-C<u>H</u>$_3$), 3.85 (s; 3H; -O-C<u>H</u>$_3$), 6.95/7.19 (AA'BB'; 4H; ^{3}J = 8.7Hz; phenyl-<u>H</u>), 6.95/7.56 (AA'BB'; 4H; ^{3}J = 8.9Hz; phenyl-<u>H</u>)

5-Ethyl-4-(4-methoxyphenyl)-3-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-2-phenylfuran (**73**)



Preparation from 5-benzoyl-3,4-epoxy-4-(4-methoxyphenyl)-11-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}undecane **68** (3.5mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/methanol 19:1, v/v).

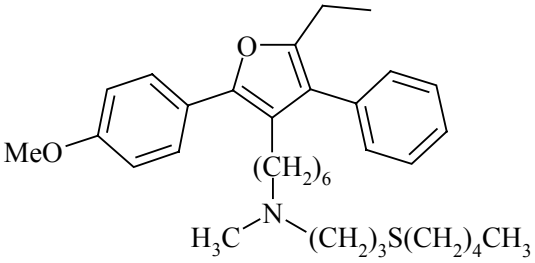
Yellow oil; yield: 52%

$C_{33}H_{49}NO_2S$ (535.83)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^{3}J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.23 (t; 3H; ^{3}J = 7.5Hz; -CH_2-C<u>H</u>$_3$), 1.11-1.45 (m; 12H; -(C<u>H</u>$_2$)$_2$-CH_3,-N-CH_2-(C<u>H</u>$_2$)$_4$-), 1.57 (quin; 2H; ^{3}J = 7.2Hz; -C<u>H</u>$_2$-CH_2-S-), 1.72 (quin; 2H; ^{3}J = 7.3Hz; -N-CH_2-C<u>H</u>$_2$-CH_2-S-), 2.17 (s; 3H; -N-C<u>H</u>$_3$), 2.23 (t; 2H; ^{3}J = 7.5Hz;

-N-C<u>H</u>$_2$-), 2.24 (t; 2H; ^{3}J = 7.3Hz; -N-C<u>H</u>$_2$-), 2.49 (t; 2H; ^{3}J = 7.4Hz; -S-C<u>H</u>$_2$-), 2.51 (t; 2H; ^{3}J = 7.3Hz; -S-C<u>H</u>$_2$-), 2.57 (t; 2H; ^{3}J = 7.8Hz; furan-C<u>H</u>$_2$), 2.61 (q; 2H; ^{3}J = 7.5Hz; -C<u>H</u>$_2$-CH$_3$), 3.86 (s; 3H; -O-C<u>H</u>$_3$), 6.95/7.20 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 7.23-7.27 (m; 1H; phenyl-<u>H</u>^{4}), 7.38-7.43 (m; 2H; phenyl-<u>H</u>^{3}, phenyl-<u>H</u>^{5}), 7.61-7.65 (m; 2H; phenyl-<u>H</u>^{2}, phenyl-<u>H</u>^{6})

5-Ethyl-2-(4-methoxyphenyl)-3-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-4-phenylfuran (**74**)



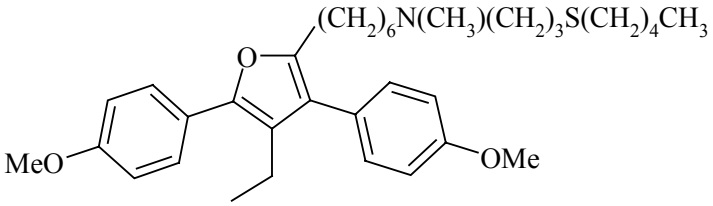
Preparation from 3,4-epoxy-5-(4-methoxybenzoyl)-11-{N-methyl-N-[3-(pentylsulfanyl)-propyl]amino}-4-phenylundecane **69** (3.6mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/methanol 19:1, v/v).

Yellow oil; yield: 65%

$C_{33}H_{49}NO_2S$ (535.83)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^{3}J = 7.1Hz; -(CH$_2$)$_4$-C<u>H</u>$_3$), 1.23 (t; 3H; ^{3}J = 7.5Hz; -CH$_2$-C<u>H</u>$_3$), 1.11-1.42 (m; 12H; -(C<u>H</u>$_2$)$_2$-CH$_3$, -N-CH$_2$-(C<u>H</u>$_2$)$_4$-), 1.57 (quin; 2H; ^{3}J = 7.3Hz; -C<u>H</u>$_2$-CH$_2$-S-), 1.72 (quin; 2H; ^{3}J = 7.3Hz; -N-CH$_2$-C<u>H</u>$_2$-CH$_2$-S-), 2.16 (s; 3H; -N-C<u>H</u>$_3$), 2.21 (t; 2H; ^{3}J = 7.5Hz; -N-C<u>H</u>$_2$-), 2.37 (t; 2H; ^{3}J = 7.3Hz; -N-C<u>H</u>$_2$-), 2.49 (t; 2H; ^{3}J = 7.3Hz; -S-C<u>H</u>$_2$-), 2.51 (t; 2H; ^{3}J = 7.3Hz; -S-C<u>H</u>$_2$-), 2.55 (t; 2H; ^{3}J = 7.8Hz; furan-C<u>H</u>$_2$), 2.63 (q; 2H; ^{3}J = 7.5Hz; -C<u>H</u>$_2$-CH$_3$), 3.85 (s; 3H; -O-C<u>H</u>$_3$), 6.96/7.56 (AA'BB'; 4H; ^{3}J = 9.1Hz; phenyl-<u>H</u>), 7.26-7.43 (m; 5H; phenyl-<u>H</u>)

3-Ethyl-2,4-bis(4-methoxyphenyl)-5-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-furan (**75**)



Preparation from 4,5-epoxy-3-(4-methoxybenzoyl)-4-(4-methoxyphenyl)-11-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}undecane **70** (3.4mmol). The crude product was purified by column chromatography (SiO_2; ethyl acetate/methanol 9:1, v/v).

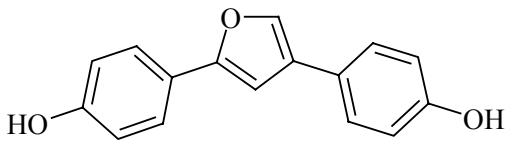
Yellow oil; yield: 64%

$C_{35}H_{51}NO_3S$ (565.86)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^{3}J = 7.0Hz; -$(CH_2)_4$-C$\underline{H}_3$), 1.01 (t; 3H; ^{3}J = 7.5Hz; -CH_2-C$\underline{H}_3$), 1.19-1.48 (m; 10H; -(C$\underline{H}_2$)$_2$-CH_3, -N-CH_2-(C$\underline{H}_2$)$_3$-), 1.53-1.66 (m; 4H; -C$\underline{H}_2$-CH_2-S-, furan-CH_2-C$\underline{H}_2$-), 1.74 (quin; 2H; ^{3}J = 7.3Hz; -N-CH_2-C$\underline{H}_2$-CH_2-S-), 2.19 (s; 3H; -N-C$\underline{H}_3$), 2.28 (t; 2H; ^{3}J = 7.3Hz; -N-C$\underline{H}_2$-), 2.40 (t; 2H; ^{3}J = 7.3Hz; -N-C$\underline{H}_2$-), 2.47-2.59 (m; 8H; -C$\underline{H}_2$-S-C$\underline{H}_2$-, -C$\underline{H}_2$-furan-C$\underline{H}_2$-), 3.84 (s; 3H; -O-C$\underline{H}_3$), 3.85 (s; 3H; -O-C$\underline{H}_3$), 6.95/7.20 (AA'BB'; 4H; ^{3}J = 8.7Hz; phenyl-$\underline{H}$), 6.95/7.56 (AA'BB'; 4H; ^{3}J = 8.9Hz; phenyl-$\underline{H}$)

2.3.4 Demethylation of the Protected Furans

2,4-Bis(4-hydroxyphenyl)furan (**76a**)



Preparation from 2,4-bis(4-methoxyphenyl)-furan **71a** (1.4mmol) as described in section E2.2.6. To dissolve the protected furan a large volume of hot dichloromethane was required, and the final solution was quickly added to boron tribromide at room temperature. The reaction mixture was stirred at room temperature for 24h.

First, the crude product was purified by column chromatography (SiO_2; ethyl acetate/DCM 40-60 1:15, v/v). To remove any soluble impurities the obtained solid was further treated with boiling chloroform and, after cooling, recovered by filtration.

Light orange solid; yield 62%

$C_{16}H_{12}O_3$ (252.27)

Melting point: > 230°C (dec.)

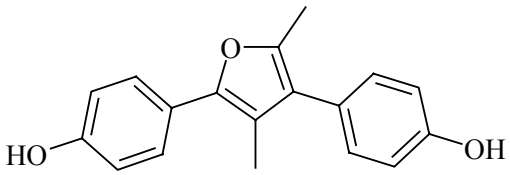
IR: ν (cm^{-1}) = 3355 (w, br; O-H)

Analysis: Calculated: C: 76.18 H 4.79

Found: C: 75.90 H 4.57

^{1}H-NMR (DMSO-d_6): δ (ppm) = 6.79/7.44 (AA'BB'; 4H; ^3J = 8.5Hz; phenyl-H), 6.83/7.55 (AA'BB'; 4H; ^3J = 8.6Hz; phenyl-H), 7.09 (s; 1H; furan-H^3), 7.97 (s; 1H; furan-H^5), 9.46 (s; 1H; -OH), 9.66 (s; 1H; -OH)

2,4-Bis(4-hydroxyphenyl)-3,5-dimethylfuran (**76b**)



Preparation from 2,4-bis(4-methoxyphenyl)-3,5-dimethylfuran **71b** (4.9mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 8h.

Purification was achieved by column chromatography (SiO_2; ethyl acetate/DCM 1:15, v/v) and recrystallisation from a EtOAc/DCM mixture.

Light orange solid; yield 80%

$C_{18}H_{16}O_3$ (280.32)

Melting point: 211-213°C (dec.)

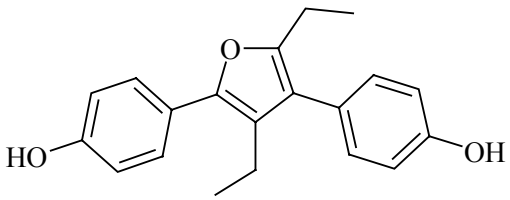
IR: ν (cm^{-1}) = 3299 (w, br; O-H)

Analysis: Calculated: C: 77.13 H 5.75

Found: C: 77.33 H 5.80

^{1}H-NMR (DMSO-d_6): δ (ppm) = 2.05 (s; 3H; -CH_3), 2.26 (s; 3H; -CH_3), 6.84/7.11 (AA'BB'; 4H; ^3J = 8.4Hz; phenyl-H), 6.84/7.40 (AA'BB'; 4H; ^3J = 8.5Hz; phenyl-H), 9.46 (s; 1H; -OH), 9.56 (s; 1H; -OH)

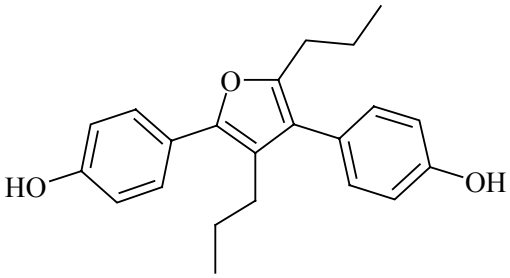
3,5-Diethyl-2,4-bis(4-hydroxyphenyl)furan (**76c**)



Preparation from 3,5-diethyl-2,4-bis(4-methoxyphenyl)furan **71c** (4.5mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 8h.
Purification was achieved by column chromatography (SiO_2; ethyl acetate/DCM 1:15, v/v) and recrystallisation from DCM and few drops of ethyl acetate.

Light orange solid; yield 72%
$C_{20}H_{20}O_3$ (308.38)
Melting point: 108-110°C
IR: ν (cm^{-1}) = 3255 (w, br; O-H)
Analysis: Calculated: C: 77.90 H 6.54
Found: C: 77.78 H 6.20
^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.92 (t; 3H; ^{3}J = 7.4Hz; -C<u>H</u>$_3$), 1.15 (t; 3H; ^{3}J = 7.5Hz; -C<u>H</u>$_3$), 2.44-2.57 (-C<u>H</u>$_2$-furan-C<u>H</u>$_2$-, merged in DMSO-signal), 6.82/7.08 (AA'BB'; 4H; ^{3}J = 8.5Hz; phenyl-<u>H</u>), 6.84/7.39 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 9.46 (s; 1H; -O<u>H</u>), 9.58 (s; 1H; -O<u>H</u>)

2,4-Bis(4-hydroxyphenyl)-3,5-dipropylfuran (**76d**)



Preparation from 2,4-bis(4-methoxyphenyl)-3,5-dipropylfuran **71d** (3.0mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 8h.
Purification was achieved by column chromatography (SiO_2; ethyl acetate/DCM 1:15, v/v) and recrystallisation from DCM and few drops of ethyl acetate.

Orange solid; yield 68%

$C_{22}H_{24}O_3$ (336.43)

Melting point: 116-118°C

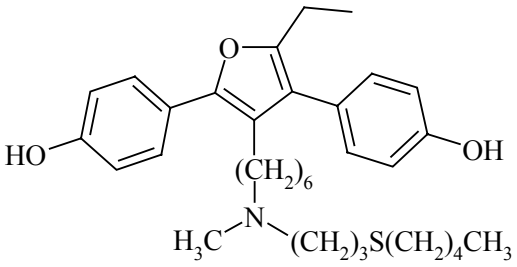
IR: ν (cm^{-1}) = 3316 (w, br; O-H)

Analysis: Calculated: C: 78.54 H 7.19

Found: C: 78.16 H 6.74

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.73 (t; 3H; ^{3}J = 7.3Hz; -C<u>H</u>$_3$), 0.85 (t; 3H; ^{3}J = 7.5Hz; -C<u>H</u>$_3$), 1.29 (sex; 2H; ^{3}J = 7.6Hz; -C<u>H</u>$_2$-CH$_3$), 1.57 (sex; 2H; ^{3}J = 7.4Hz; -C<u>H</u>$_2$-CH$_3$), 2.42-2.51 (-C<u>H</u>$_2$-furan-C<u>H</u>$_2$-, merged in DMSO-signal), 6.82/7.06 (AA'BB'; 4H; ^{3}J = 8.5Hz; phenyl-<u>H</u>), 6.84/7.38 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 9.45 (s; 1H; -O<u>H</u>), 9.57 (s; 1H; -O<u>H</u>)

5-Ethyl-2,4-bis(4-hydroxyphenyl)-3-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-furan (**77**)



Preparation from 5-ethyl-2,4-bis(4-methoxyphenyl)-3-{6-{N-methyl-N-[3-(pentylsulfanyl)-propyl]amino}hexyl}furan **72** (1.6mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 8h.

The crude product was purified by column chromatography (SiO_2; methanol/DCM 1:15, v/v).

Orange solid; yield: 68%

Melting point: 41-44°C

$C_{33}H_{47}NO_3S$ (537.80)

IR: ν (cm^{-1}) = 3400-2600 (w, br; -OH)

MS: m/z (%) = 537 (100; $M^{+\bullet}$), 466 (12; $[M\text{-}C_5H_{11}]^{+\bullet}$), 435 (10; $[M\text{-}S(CH_2)_5]^{+\bullet}$), 406 (97, $[furan\text{-}(CH_2)_6N(CH_3)=CH_2]^{+\bullet}$), 393 (5, $[furan\text{-}(CH_2)_6NH(CH_3)]^{+\bullet}$), 362 (9, $[furan\text{-}(CH_2)_5=CH_2]^{+\bullet}$), 293 (6, [fu-

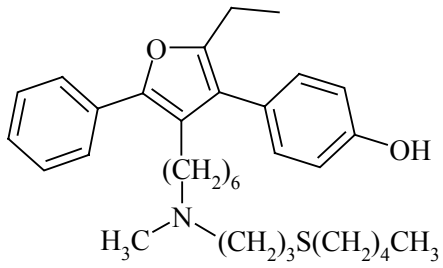
ran=CH_2]$^{+\bullet}$), 244 (7; [$C_5H_{11}S(CH_2)_3N(CH_3)(CH_2)_4$=$CH_2$]$^{+\bullet}$), 203 (33; [244-$C_3H_5$]$^{+\bullet}$)

HRMS: Calculated for $C_{33}H_{47}NO_3S$: 537.3277

Found: 537.3273 ± 0.0002

^{1}H-NMR (MeOD-d_4): δ (ppm) = 0.90 (t; 3H; ^{3}J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.19 (t; 3H; ^{3}J = 7.5Hz; -CH_2-C<u>H</u>$_3$), 1.11-1.42 (m; 12H; -(C<u>H</u>$_2$)$_2$-CH_3, -N-CH_2-(C<u>H</u>$_2$)$_4$-), 1.57 (quin; 2H; ^{3}J = 7.2Hz; -C<u>H</u>$_2$-CH_2-S-), 1.78 (quin; 2H; ^{3}J = 7.4Hz; -N-CH_2-C<u>H</u>$_2$-CH_2-S-), 2.35 (s; 3H; -N-C<u>H</u>$_3$), 2.44 (t; 2H; ^{3}J = 8.0 Hz; -N-C<u>H</u>$_2$-), 2.50-2.58 (m; 8H; -C<u>H</u>$_2$-S-C<u>H</u>$_2$, -C<u>H</u>$_2$-furan-C<u>H</u>$_2$-), 2.64 (t; 2H; ^{3}J = 7.8 Hz; -N-CH_2-), 6.82/7.42 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 6.83/7.08 (AA'BB'; 4H; ^{3}J = 8.6Hz; phenyl-<u>H</u>)

5-Ethyl-4-(4-hydroxyphenyl)-3-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-2-phenylfuran (**78**)



Preparation from 5-ethyl-4-(4-methoxyphenyl)-3-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]-amino}hexyl}-2-phenylfuran **73** (1.8mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 8h.

The crude product was purified by column chromatography (SiO_2; methanol/DCM 1:25, v/v).

Yellow oil; yield: 26%

$C_{33}H_{47}NO_2S$ (521.80)

IR: ν (cm^{-1}) = 3400-2600 (w, br; -OH)

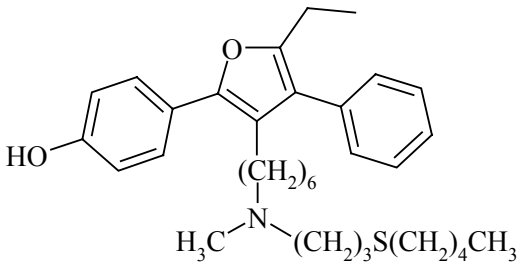
MS: m/z (%) = 521 (64; $M^{+\bullet}$), 450 (12; [M-C_5H_{11}]$^{+\bullet}$), 419 (7; [M-$S(CH_2)_5$]$^{+\bullet}$), 390 (100, [furan-$(CH_2)_6N(CH_3)$=CH_2]$^{+\bullet}$), 346 (9, [furan-$(CH_2)_5$=CH_2]$^{+\bullet}$), 277 (6, [furan=CH_2]$^{+\bullet}$), 244 (8; [$C_5H_{11}S(CH_2)_3$N-

$(CH_3)(CH_2)_4=CH_2]^{+\bullet}$), 195 (19, [furan-$(CH_2)_6N(CH_3)=CH_2]^{2+\bullet}$), 188 (55; $[C_5H_{11}S(CH_2)_3N(CH_3)=CH_2]^{+\bullet}$), 105 (23; $[C_6H_5CO]^{+\bullet}$)

HRMS: Calculated for $C_{33}H_{47}NO_2S$: 521.3328
Found: 521.3329 ± 0.0002

^{1}H-NMR (MeOD-d_4): δ (ppm) = 0.91 (t; 3H; ^{3}J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.21 (t; 3H; ^{3}J = 7.5Hz; -CH_2-C<u>H</u>$_3$), 1.15-1.42 (m; 10H; -(C<u>H</u>$_2$)$_2$-CH_3, furan-CH_2-(C<u>H</u>$_2$)$_3$-), 1.50 (quin; 2H; ^{3}J = 7.8Hz; -C<u>H</u>$_2$-CH_2-N-), 1.58 (quin; 2H; ^{3}J = 7.3Hz; -C<u>H</u>$_2$-CH_2-S-), 1.91 (quin; 2H; ^{3}J = 7.4Hz; -N-CH_2-C<u>H</u>$_2$-CH_2-S-), 2.51-2.65 (m; 8H; -C<u>H</u>$_2$-S-C<u>H</u>$_2$, -C<u>H</u>$_2$-furan-C<u>H</u>$_2$-), 2.68 (s; 3H; -N-C<u>H</u>$_3$), 2.84 (t; 2H; ^{3}J = 8.2 Hz; -N-CH_2-), 3.04 (t; 2H; ^{3}J = 8.0 Hz; -N-C<u>H</u>$_2$-), 6.85/7.22 (AA'BB'; 4H; ^{3}J = 8.7Hz; phenyl-<u>H</u>), 7.22-7.28 (m; 1H; phenyl-<u>H</u>^{4}), 7.37-7.42 (m; 2H; phenyl-<u>H</u>^{3}, phenyl-<u>H</u>^{5}), 7.59-7.62 (m; 2H; phenyl-<u>H</u>^{2}, phenyl-<u>H</u>^{6})

5-Ethyl-2-(4-hydroxyphenyl)-3-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-4-phenylfuran (**79**)



Preparation from 5-ethyl-2-(4-methoxyphenyl)-3-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]-amino}hexyl}-4-phenylfuran **74** (2.2mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 8h.
The crude product was purified by column chromatography (SiO_2; methanol/DCM 1:19, v/v).

Yellow oil; yield: 62%
$C_{33}H_{47}NO_2S$ (521.80)

IR: ν (cm^{-1}) = 3400-2600 (w, br; -OH)

MS: m/z (%) = 521 (69; $M^{+\bullet}$), 450 (12; [M-$C_5H_{11}]^{+\bullet}$), 419 (8; [M-$S(CH_2)_5]^{+\bullet}$), 390 (100, [furan-$(CH_2)_6N(CH_3)=CH_2]^{+\bullet}$), 346 (11, [furan-$(CH_2)_5=CH_2]^{+\bullet}$), 277 (11, [furan=$CH_2]^{+\bullet}$), 244 (11; $[C_5H_{11}S(CH_2)_3N$-

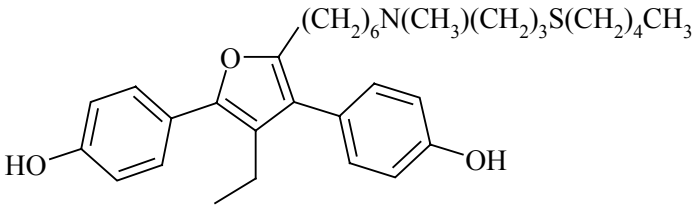
$(CH_3)(CH_2)_4=CH_2]^{+\bullet}$), 188 (63; $[C_5H_{11}S(CH_2)_3N(CH_3)=CH_2]^{+\bullet}$), 121 (23; $[HOC_6H_4CO]^{+\bullet}$)

HRMS: Calculated for $C_{33}H_{47}NO_2S$: 521.3329

Found: 521.3329 ± 0.0002

^{1}H-NMR (MeOD-d_4): δ (ppm) = 0.90 (t; 3H; ^{3}J = 7.0Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.20 (t; 3H; ^{3}J = 7.5Hz; -CH_2-C<u>H</u>$_3$), 1.11-1.47 (m; 12H; -(C<u>H</u>$_2$)$_2$-CH_3, furan-CH_2-(C<u>H</u>$_2$)$_4$-), 1.58 (quin; 2H; ^{3}J = 7.2Hz; -C<u>H</u>$_2$-CH_2-S-), 1.84 (quin; 2H; ^{3}J = 7.5Hz; -N-CH_2-C<u>H</u>$_2$-CH_2-S-), 2.49-2.65 (m; 13H; -C<u>H</u>$_2$-S-C<u>H</u>$_2$, -C<u>H</u>$_2$-furan-C<u>H</u>$_2$-, -N-C<u>H</u>$_3$, -N-CH_2-), 2.83 (t; 2H; ^{3}J = 8.0 Hz; -N-C<u>H</u>$_2$-), 6.84/7.44 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 7.25-7.35 (m; 3H; phenyl-<u>H</u>$^{3-5}$), 7.39-7.46 (m; 2H; phenyl-<u>H</u>^{2}, phenyl-<u>H</u>^{6})

3-Ethyl-2,4-bis(4-hydroxyphenyl)-5-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-furan (**80**)



Preparation from 3-ethyl-2,4-bis(4-methoxyphenyl)-5-{6-{N-methyl-N-[3-(pentylsulfanyl)-propyl]amino}hexyl}furan **75** (2.2mmol) as described in section E2.2.6. The reaction mixture was stirred at room temperature for 8h.

The crude product was purified by column chromatography (SiO_2; methanol/DCM 1:19, v/v).

Orange solid; yield: 63%

Melting point: 39-42°C

$C_{33}H_{47}NO_3S$ (537.80)

IR: ν (cm^{-1}) = 3400-2600 (w, br; -OH)

MS: m/z (%) = 537 (91; $M^{+\bullet}$), 466 (12; $[M-C_5H_{11}]^{+\bullet}$), 435 (4; $[M-S(CH_2)_5]^{+\bullet}$), 406 (100, $[furan-(CH_2)_6N(CH_3)=CH_2]^{+\bullet}$), 362 (18, $[furan-(CH_2)_5=CH_2]^{+\bullet}$), 293 (28, $[furan=CH_2]^{+\bullet}$), 244 (10; $[C_5H_{11}S(CH_2)_3N-(CH_3)(CH_2)_4=CH_2]^{+\bullet}$), 203 (22; $[244-C_3H_5]^{+\bullet}$), 188 (45; $[C_5H_{11}S-(CH_2)_3N(CH_3)=CH_2]^{+\bullet}$), 121 (15; $[HOC_6H_4CO]^{+\bullet}$)

HRMS: Calculated for $C_{33}H_{47}NO_3S$: 537.3277
Found: 537.3272 ± 0.0005

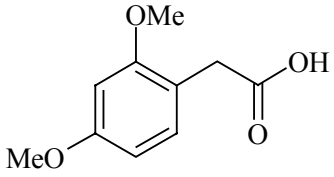
^{1}H-NMR (MeOD-d_4): δ (ppm) = 0.90 (t; 3H; ^{3}J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 0.97 (t; 3H; ^{3}J = 7.5Hz; -CH_2-C<u>H</u>$_3$), 1.28-1.42 (m; 10H; -$(C\underline{H}_2)_2$-CH_3, -N-CH_2-$(C\underline{H}_2)_3$-), 1.53-1.88 (m; 6H; -N-CH_2-C<u>H</u>$_2$-CH_2-S-CH_2-C<u>H</u>$_2$-, furan-CH_2-C<u>H</u>$_2$-), 2.50-2.59 (m; 8H; -C<u>H</u>$_2$-S-C<u>H</u>$_2$, -C<u>H</u>$_2$-furan-C<u>H</u>$_2$-), 2.62 (s; 3H; -N-C<u>H</u>$_3$), 2.80 (t; 2H; ^{3}J = 8.1Hz; -N-C<u>H</u>$_2$-), 2.95 (t; 2H; ^{3}J = 8.0Hz; -N-CH_2-), 6.83/7.42 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 6.84/7.08 (AA'BB'; 4H; ^{3}J = 8.5Hz; phenyl-<u>H</u>)

2.4 Benzo[b]furans and Benzo[b]thiophenes

2.4.1 3-Alkyl-2-(4-hydroxyphenyl)benzo[b]furans

2.4.1.1 Synthesis of Precursors

2,4-Dimethoxyphenylacetic acid (**90**)



At room temperature and with stirring 2,4-dimethoxyacetophenone (29.5mmol) was dissolved in dry MeOH (60ml), supplemented with $HClO_4$ (70% w/w, 10ml) and solid thallium trinitrate trihydrate (29.5mmol) added in portions. Immediately, the formation of white $TlNO_3$ was observed and continuous stirring for further 10min turned the reaction mixture into slightly orange. The solid was removed by filtration and the filtrate poured into water (150ml). This aqueous phase was extracted with three portions of DCM (75ml). The combined organic phases were washed with water (75ml) and brine (75ml) and dried over Na_2SO_4. Evaporation of the solvent gave methyl 2,4-dimethoxyphenylacetate as a slightly orange oil.

The crude ester was taken up in EtOH (75ml) and treated with aqueous NaOH (2M, 25ml) for 6h. The solution was concentrated and the residue dissolved in water (75ml). The aqueous phase was washed once with chloroform and acidified with conc. HCl. Upon cooling to +4°C

the desired product precipitated. It was collected by suction and washed twice with small volumes of cold water. It did not required any further purification.

Beige crystals; yield: 75%

Melting point: 109-110°C

$C_{10}H_{12}O_4$ (196.20)

IR: ν (cm^{-1}) = 3300-2400 (w, br; -COOH), 1711 (s; C=O)

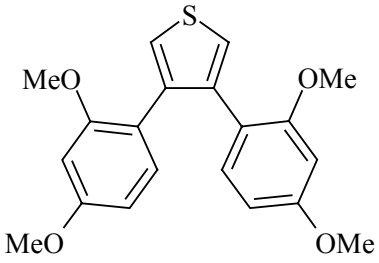
Analysis: Calculated: C: 61.21 H 6.17

Found: C: 60.84 H 5.94

MS: m/z (%) = 196 (37; $M^{+\bullet}$), 151 (100; $[M\text{-}CO_2H]^{+\bullet}$), 121 (30, $[M\text{-}CO_2H\text{-}CH_2O]^{+\bullet}$), 91 (9, $[M\text{-}CO_2H\text{-}2x\ CH_2O]^{+\bullet}$), 77 (7, $[C_6H_5]^{+\bullet}$)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.59 (s; 2H; -C<u>H</u>$_2$-), 3.80 (s; 6H; -O-C<u>H</u>$_3$), 6.45 (m; 2H; phenyl-<u>H</u>^{3}, phenyl-<u>H</u>^{5}), 7.09 (d; 1H; ^{3}J = 8.6Hz; phenyl-<u>H</u>^{6})

3,4-Bis(2,4-dimethoxyphenyl)thiophene (**91**)



By-product from the preparation of compound **90** via the Willgerodt-Kindler reaction. It crystallised from acetone upon standing at room temperature.

Colourless needles; yield: 29%

Melting point: 101°C

$C_{20}H_{20}O_4S$ (356.44)

Analysis: Calculated: C: 67.39 H 5.66 S 8.77

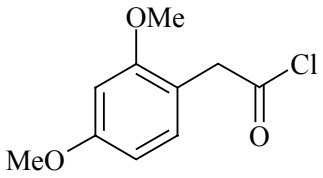
Found: C: 67.37 H 5.52 S 8.44

MS: m/z (%) = 356 (100; $M^{+\bullet}$), 341 (36; $[M\text{-}CH_3]^{+\bullet}$), 178 (33; $M^{2+\bullet}$)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.59 (s; 6H; -O-C<u>H</u>$_3$), 3.91 (s; 6H; -O-C<u>H</u>$_3$), 6.52-6.56 (m; 4H; phenyl-<u>H</u>^{3}, phenyl-<u>H</u>^{5}), 7.34 (s; 2H; thiophen-<u>H</u>^{2}, thiophen-<u>H</u>^{5}), 7.56 (d; 2H; ^{3}J = 9.3Hz; phenyl-<u>H</u>^{6})

^{13}H-NMR ($CDCl_3$): δ (ppm) = 55.44 (2x CH_3), 55.59 (2x CH_3), 99.06 (2x CH), 105.22 (2x CH), 116.97 (2x C), 124.64 (2x CH), 129.17 (2x CH), 138.36 (2x C), 156.86 (2x C-O), 159.96 (2x C-O)

2,4-Dimethoxyphenylacetic acid chloride (**92**)



Preparation from 2,4-dimethoxyphenylacetic acid **90** (39.1mmol) as described in the general procedure (cf. section E2.2.1.2.1).

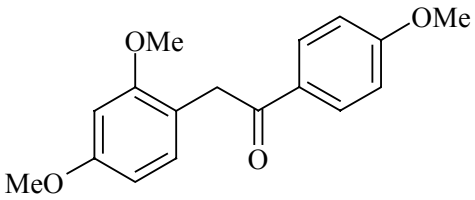
Yellow oil; yield: 98%

$C_{10}H_{11}ClO_3$ (214.65)

IR: ν (cm^{-1}) = 1799 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.77 (s; 6H; -O-C$\underline{H}_3$), 4.01 (s; 2H; -C$\underline{H}_2$-), 6.42 (m; 2H; phenyl-$\underline{H}^3$, phenyl-$\underline{H}^5$), 7.02 (d; 1H; ^{3}J = 8.7Hz; phenyl-$\underline{H}^6$)

2-(2,4-Dimethoxyphenyl)-1-(4-methoxyphenyl)ethan-1-one (**93a**)



At 0°C, solid anhydrous aluminium chloride (46.0mmol) was added in small portions to 2,4-dimethoxyphenylacetic acid chloride **92** (38.3mmol) dissolved in dry anisole (150ml) and stirred at room temperature for 3.5h. The reaction mixture was poured into an ice-cold mixture of water and conc. HCl (3:1, v/v; 300ml). The aqueous phase was extracted with DCM (3x100ml), washed with water. The combined organic phases were dried over Na_2SO_4. The solvent and excess anisole were removed under reduced pressure. The resulting red solid was purified by column chromatography (SiO_2; DCM) and recrystallisation from EtOH (99%).

Colourless crystals; yield: 76%

Melting point: 103-104°C

$C_{17}H_{18}O_4$ (286.33)

IR: ν (cm^{-1}) = 1674 (s; C=O)

Analysis: Calculated: C: 71.31 H 6.34

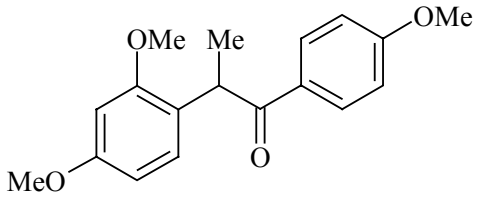
Found: C: 71.22 H 5.92

^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.77 (s; 3H; -O-C$\underline{H}_3$), 3.79 (s; 3H; -O-C$\underline{H}_3$), 3.86 (s; 3H; -O-C$\underline{H}_3$), 4.16 (s; 2H; -C$\underline{H}_2$-), 6.45 (m; 2H; phenyl-$\underline{H}^3$, phenyl-$\underline{H}^5$), 7.07 (d; 1H; ^{3}J = 8.2Hz; phenyl-$\underline{H}^6$), 6.92/8.01 (AA'BB'; 4H; ^{3}J = 8.9Hz; phenyl-$\underline{H}$)

2.4.1.2 Synthesis of α-Alkylated 1,2-Diarylethanones

At 0°C, a solution of 2-(2,4-dimethoxyphenyl)-1-(4-methoxyphenyl)ethan-1-one **93a** (1eq) in dry DMF was added dropwise to a suspension of sodium hydride (60% in paraffin, 1.2eq) in dry DMF and the mixture was stirred at room temperature for 1h. The reaction mixture was cooled in ice again and the respective haloalkane (1.2eq) in dry DMF added. After stirring at room temperature for another hour excess NaH was hydrolysed with water and the aqueous phase extracted with three portions of diethyl ether. The combined organic extracts were washed with water and brine After drying over Na_2SO_4 the solvent was evaporated.

2-(2,4-Dimethoxyphenyl)-1-(4-methoxyphenyl)propan-1-one (**93b**)



Preparation from 2-(2,4-dimethoxyphenyl)-1-(4-methoxyphenyl)ethan-1-one **93a** (1.6mmol) and iodomethane (2.4mmol). The crude product was purified by column chromatography (SiO_2; DCM) and recrystallised from EtOH (99%).

Colourless crystals; yield 89%

Melting point: 59-60°C

$C_{18}H_{20}O_4$ (300.35)

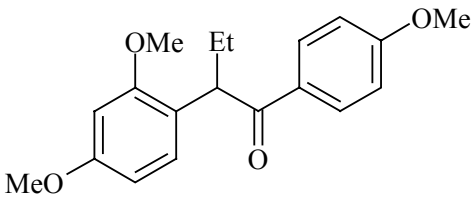
IR: ν (cm^{-1}) = 1672 (s; C=O)

Analysis: Calculated: C: 71.98 H 6.71

Found: C: 71.67 H 6.41

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.41 (d; 3H; ^3J = 6.9Hz; -C$\underline{H}_3$), 3.75 (s; 3H; -O-C$\underline{H}_3$), 3.81 (s; 3H; -O-C$\underline{H}_3$), 3.87 (s; 3H; -O-C$\underline{H}_3$), 4.96 (q; 1H; ^3J = 6.9Hz; -C$\underline{H}$-CO-), 6.39 (dd; 1H; ^3J = 8.5Hz; ^4J = 2.5Hz; phenyl-$\underline{H}^5$), 6.46 (d; 1H; ^4J = 2.5Hz; phenyl-$\underline{H}^3$), 7.01 (d; 1H; ^3J = 8.4Hz; phenyl-$\underline{H}^6$), 6.84/7.97 (AA'BB'; 4H; ^3J = 8.9Hz; phenyl-$\underline{H}$)

2-(2,4-Dimethoxyphenyl)-1-(4-methoxyphenyl)butan-1-one (**93c**)



Preparation from 2-(2,4-dimethoxyphenyl)-1-(4-methoxyphenyl)ethan-1-one **93a** (7.0mmol) and iodoethane (10.5mmol). The crude product was purified by column chromatography (SiO_2; DCM).

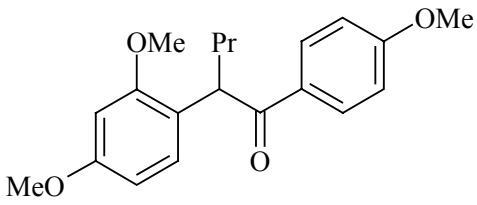
Colourless oil; yield 85%

$C_{19}H_{22}O_4$ (314.38)

IR: ν (cm^{-1}) = 1671 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.87 (t; 3H; ^3J = 7.3Hz; -C$\underline{H}_3$), 1.66-1.80 (m; 1H; -C$\underline{H}_a$H$_b$-CH$_3$), 2.03-2.17 (m; 1H; -CH$_a\underline{H}_b$-CH$_3$), 3.74 (s; 3H; -O-C$\underline{H}_3$), 3.81 (s; 3H; -O-C$\underline{H}_3$), 3.89 (s; 3H; -O-C$\underline{H}_3$), 4.84 (t; 1H; ^3J = 7.2Hz; -C$\underline{H}$-CO-), 6.39 (dd; 1H; ^3J = 8.5Hz; ^4J = 2.4Hz; phenyl-$\underline{H}^5$), 6.45 (d; 1H; ^4J = 2.4Hz; phenyl-$\underline{H}^3$), 7.04 (d; 1H; ^3J = 8.5Hz; phenyl-$\underline{H}^6$), 6.84/7.97 (AA'BB'; 4H; ^3J = 9.0Hz; phenyl-$\underline{H}$)

2-(2,4-Dimethoxyphenyl)-1-(4-methoxyphenyl)pentan-1-one (**93d**)



Preparation from 2-(2,4-dimethoxyphenyl)-1-(4-methoxyphenyl)ethan-1-one **93a** (2.4mmol) and 1-bromopropane (3.6mmol). The crude product was purified by column chromatography (SiO_2; DCM).

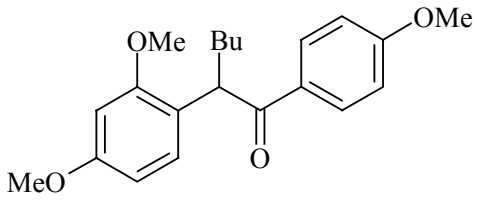
Colourless oil; yield 83%

$C_{20}H_{24}O_4$ (328.41)

IR: ν (cm^{-1}) = 1672 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.90 (t; 3H; ^3J = 7.4Hz; -C$\underline{H}_3$), 1.14-1.41 (m; 2H; -C$\underline{H}_2$-CH$_3$), 1.66-1.78 (m; 1H; -CH-C$\underline{H}_a$H$_b$-), 2.02-2.14 (m; 1H; -CH-CH$_a\underline{H}_b$-), 3.74 (s; 3H; -O-C$\underline{H}_3$), 3.81 (s; 3H; -O-C$\underline{H}_3$), 3.89 (s; 3H; -O-C$\underline{H}_3$), 4.87 (t; 1H; ^3J = 7.2Hz; -C$\underline{H}$-CO-), 6.39 (dd; 1H; ^3J = 8.5Hz; ^4J = 2.4Hz; phenyl-$\underline{H}^5$), 6.44 (d; 1H; ^4J = 2.4Hz; phenyl-$\underline{H}^3$), 7.03 (d; 1H; ^3J = 8.5Hz; phenyl-$\underline{H}^6$), 6.84/7.96 (AA'BB'; 4H; ^3J = 9.0Hz; phenyl-$\underline{H}$)

2-(2,4-Dimethoxyphenyl)-1-(4-methoxyphenyl)hexan-1-one (**93e**)



Preparation from 2-(2,4-dimethoxyphenyl)-1-(4-methoxyphenyl)ethan-1-one **93a** (2.4mmol) and 1-bromobutane (3.6mmol). The crude product was purified by column chromatography (SiO_2; DCM).

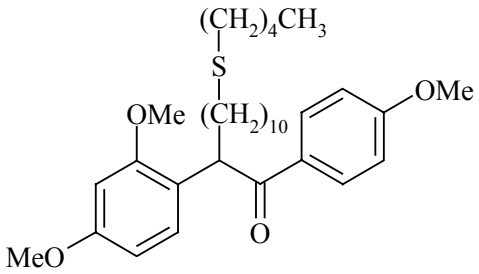
Colourless oil; yield 85%

$C_{21}H_{26}O_4$ (342.44)

IR: ν (cm^{-1}) = 1672 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.86 (t; 3H; ^{3}J = 7.4Hz; -C<u>H</u>$_3$), 1.10-1.42 (m; 4H; -(C<u>H</u>$_2$)$_2$-CH$_3$), 1.66-1.78 (m; 1H; -CH-C<u>H</u>$_a$H$_b$-), 2.05-2.17 (m; 1H; -CH-CH$_a$<u>H</u>$_b$-), 3.74 (s; 3H; -O-C<u>H</u>$_3$), 3.81 (s; 3H; -O-C<u>H</u>$_3$), 3.88 (s; 3H; -O-C<u>H</u>$_3$), 4.88 (t; 1H; ^{3}J = 7.2Hz; -C<u>H</u>-CO-), 6.39 (dd; 1H; ^{3}J = 8.5Hz; ^{4}J = 2.4Hz; phenyl-<u>H</u>^{5}), 6.45 (d; 1H; ^{4}J = 2.4Hz; phenyl-<u>H</u>^{3}), 7.04 (d; 1H; ^{3}J = 8.5Hz; phenyl-<u>H</u>^{6}), 6.84/7.97 (AA'BB'; 4H; ^{3}J = 9.0Hz; phenyl-<u>H</u>)

2-(2,4-Dimethoxyphenyl)-1-(4-methoxyphenyl)-12-(pentylsulfanyl)dodecan-1-one (**94**)



Preparation from 2-(2,4-dimethoxyphenyl)-1-(4-methoxyphenyl)ethan-1-one **93a** (5.2mmol) and 1-bromo-10-(pentylsulfanyl)decane **10** (6.3mmol). The crude product was purified by column chromatography (SiO_2; DCM/petroleum ether 40-60 1:1, v/v).

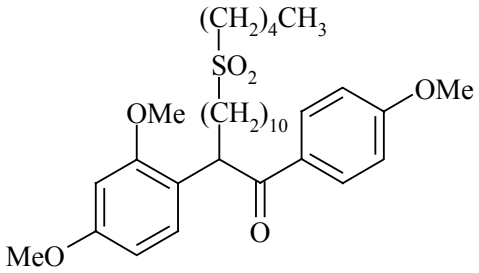
Colourless oil; yield 76%

$C_{32}H_{48}O_4S$ (528.79)

IR: ν (cm^{-1}) = 1672 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.90 (t; 3H; ^{3}J = 7.1Hz; -C<u>H</u>$_3$), 1.23-1.41 (m; 18H; -(C<u>H</u>$_2$)$_2$-CH$_3$, -CH-CH$_2$-(C<u>H</u>$_2$)$_7$-), 1.51-1.63 (m; 4H; -C<u>H</u>$_2$-CH$_2$-S-CH$_2$-C<u>H</u>$_2$-), 1.65-1.74 (m; 1H; -CH-C<u>H</u>$_a$H$_b$-), 2.01-2.12 (m; 1H; -CH-CH$_a$<u>H</u>$_b$-), 2.48 (t; 2H; ^{3}J = 7.4Hz; -C<u>H</u>$_2$-S-), 2.49 (t; 2H; ^{3}J = 7.4Hz; -C<u>H</u>$_2$-S-), 3.74 (s; 3H; -O-C<u>H</u>$_3$), 3.81 (s; 3H; -O-C<u>H</u>$_3$), 3.89 (s; 3H; -O-C<u>H</u>$_3$), 4.92 (t; 1H; ^{3}J = 7.3Hz; -C<u>H</u>-CO-), 6.39 (dd; 1H; ^{3}J = 8.5Hz; ^{4}J = 2.4Hz; phenyl-<u>H</u>^{5}), 6.45 (d; 1H; ^{4}J = 2.4Hz; phenyl-<u>H</u>^{3}), 7.06 (d; 1H; ^{3}J = 8.3Hz; phenyl-<u>H</u>^{6}), 6.84/7.98 (AA'BB'; 4H; ^{3}J = 9.0Hz; phenyl-<u>H</u>)

2-(2,4-Dimethoxyphenyl)-1-(4-methoxyphenyl)-12-(pentylsulfonyl)dodecan-1-one (**95**)



Preparation from 2-(2,4-dimethoxyphenyl)-1-(4-methoxyphenyl)ethan-1-one **93a** (5.2mmol) and 1-bromo-10-(pentylsulfonyl)decane **11** (6.3mmol). The crude product was purified by column chromatography (SiO_2; DCM).

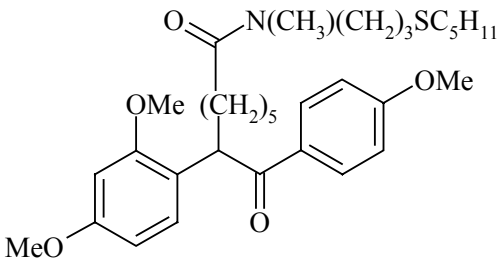
Slighly yellow oil; yield 96%

$C_{32}H_{48}O_6S$ (560.79)

IR: ν (cm^{-1}) = 1671 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.92 (t; 3H; ^3J = 7.1Hz; -C$\underline{H}_3$), 1.23-1.48 (m; 18H; -(C$\underline{H}_2$)$_2$-CH$_3$, -CH-CH$_2$-(C$\underline{H}_2$)$_7$-), 1.62-1.73 (m; 1H; -CH-C$\underline{H}_a$H$_b$-), 1.76-1.89 (m; 4H; -C$\underline{H}_2$-CH$_2$-SO$_2$-CH$_2$-C$\underline{H}_2$-), 2.01-2.13 (m; 1H; -CH-CH$_a\underline{H}_b$-), 2.93 (t; 4H; ^3J = 7.4Hz; -C$\underline{H}_2$-SO$_2$-), 3.74 (s; 3H; -O-C$\underline{H}_3$), 3.81 (s; 3H; -O-C$\underline{H}_3$), 3.89 (s; 3H;-O-C$\underline{H}_3$), 4.91 (t; 1H; ^3J = 7.3Hz;-C$\underline{H}$-CO-), 6.39 (dd; 1H; ^3J = 8.3Hz; ^4J = 2.4Hz; phenyl-$\underline{H}^5$), 6.45 (d; 1H; ^4J = 2.2Hz; phenyl-$\underline{H}^3$), 7.05 (d; 1H; ^3J = 8.5Hz; phenyl-$\underline{H}^6$), 6.84/7.98 (AA'BB'; 4H; ^3J = 9.0Hz; phenyl-$\underline{H}$)

7-(2,4-Dimethoxyphenyl)-8-(4-methoxyphenyl)-8-oxooctanoic acid methyl-[3-(pentylsulfanyl)propyl]amide (**96**)



Preparation from 2-(2,4-dimethoxyphenyl)-1-(4-methoxyphenyl)ethan-1-one **93a** (5.2mmol) and 6-bromohexanoic acid methyl-[3-(pentylsulfanyl)propyl]amide **19** (6.3mmol). The crude product was purified by column chromatography (SiO_2; DCM/ethyl acetate 5:1, v/v).

Slighly yellow oil; yield 88%

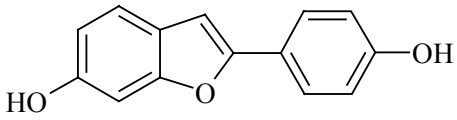
$C_{32}H_{47}NO_5S$ (557.79)

IR: ν (cm^{-1}) = 1671 (s; C=O), 1643 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.90 (t; 3H; ^3J = 7.0Hz; -C$\underline{H}_3$), 1.18-1.41 (m; 8H; -(C$\underline{H}_2$)$_2$-CH$_3$, -CH-CH$_2$-(C$\underline{H}_2$)$_2$-), 1.52-1.86 (m; 7H; -CH-C$\underline{H}_a$H$_b$-, -CO-CH$_2$-C$\underline{H}_2$-, -C$\underline{H}_2$-CH$_2$-S-, -N-CH$_2$-C$\underline{H}_2$-CH$_2$-S-), 2.01-2.13 (m; 1H; -CH-CH$_a$$\underline{H}_b$-), 2.21-2.32 (m; 2H; -CO-CH$_2$-), 2.49 (t; 4H; ^3J = 7.3Hz; -C$\underline{H}_2$-S-C$\underline{H}_2$-), 2.89/2.96 (2x s; 3H; -N-C$\underline{H}_3$), 3.36 (t; 1H; ^3J = 7.5Hz; -N-C$\underline{H}_a$H$_b$-), 3.42 (t; 1H; ^3J = 7.3Hz; -N-CH$_a$$\underline{H}_b$-), 3.74 (s; 3H; -O-C$\underline{H}_3$), 3.81 (s; 3H; -O-C$\underline{H}_3$), 3.89 (s; 3H; -O-C$\underline{H}_3$), 4.91 (t; 1H; ^3J = 7.1Hz; -C$\underline{H}$-CO-), 6.38 (dd; 1H; ^3J = 8.2Hz; ^4J = 2.5Hz; phenyl-$\underline{H}^5$), 6.44 (d; 1H; ^4J = 2.5Hz; phenyl-$\underline{H}^3$), 7.04 (d; 1H; ^3J = 8.5Hz; phenyl-$\underline{H}^6$), 6.83/7.96 (AA'BB'; 4H; ^3J = 9.0Hz; phenyl-$\underline{H}$)

2.4.1.3 Demethylation and Cyclisation to 6-Hydroxy-2-(4-hydroxyphenyl)benzo[b]furans

6-Hydroxy-2-(4-hydroxyphenyl)benzo[b]furan (**97a**)



Preparation from 2-(2,4-dimethoxyphenyl)-1-(4-methoxyphenyl)ethan-1-one **93a** (5.2mmol) following the general demethylation procedure (cf. section E.2.2.6). The reaction mixture was stirred at room temperature for 24h.

The crude product was chromatographed (SiO_2; DCM/ethyl acetate 9:1, v/v) and subsequently recrystallised from ethyl acetate/DCM.

Colourless solid; yield: 30%

Melting point: 239-240°C

$C_{14}H_{10}O_3$ (226.23)

IR: ν (cm^{-1}) = 3283 (w, br; O-H)

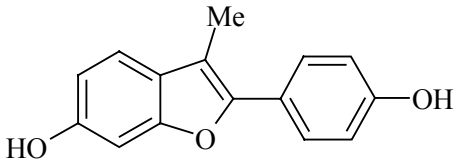
Analysis: Calculated: C: 74.33 H 4.46

Found: C: 74.02 H 4.34

MS: m/z (%) = 226 (100; $M^{+\bullet}$), 113 (20; $M^{2+\bullet}$)

^{1}H-NMR (DMSO-d_6): δ (ppm) = 6.72 (dd; 1H; ^{3}J = 8.4Hz; ^{4}J = 2.0Hz; phenyl-$\underline{H}^5$), 6.85/7.65 (AA'BB'; 4H; ^{3}J = 8.6Hz; phenyl-$\underline{H}$), 6.92 (d; 1H; ^{4}J = 1.9Hz; phenyl-$\underline{H}^7$), 7.01 (d; 1H; ^{4}J = 0.8Hz; furan-$\underline{H}^3$), 7.35 (d; 1H; ^{3}J = 8.5Hz; phenyl-$\underline{H}^4$), 9.49 (s; 1H; -O$\underline{H}$), 9.74 (s; 1H; -O$\underline{H}$)

6-Hydroxy-2-(4-hydroxyphenyl)-3-methylbenzo[b]furan (**97b**)



Preparation from 2-(2,4-dimethoxyphenyl)-1-(4-methoxyphenyl)propan-1-one **93b** (2.3mmol) following the general demethylation procedure (cf. section E.2.2.6). The reaction mixture was stirred at room temperature for 24h.

The crude product was chromatographed (SiO_2; DCM/ethyl acetate 9:1, v/v) and subsequently recrystallised from DCM.

Colourless crystals; yield: 25%

Melting point: 190-191°C

$C_{15}H_{12}O_3$ (240.26)

IR: ν (cm^{-1}) = 3267 (w, br; O-H)

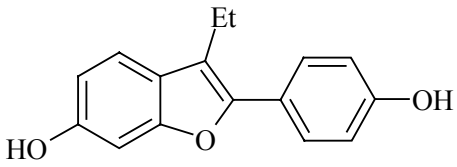
Analysis: Calculated: C: 74.99 H 5.03

Found: C: 74.65 H 4.76

MS: m/z (%) = 240 (100; $M^{+\bullet}$), 120 (6; $M^{2+\bullet}$)

^{1}H-NMR (DMSO-d_6): δ (ppm) = 2.33 (s; 3H; -C$\underline{H}_3$), 6.73 (dd; 1H; ^{3}J = 8.4Hz; ^{4}J = 2.0Hz; phenyl-$\underline{H}^5$), 6.88 (d; 1H; ^{4}J = 2.0Hz; phenyl-$\underline{H}^7$), 6.89/7.55 (AA'BB'; 4H; ^{3}J = 8.6Hz; phenyl-$\underline{H}$), 7.34 (d; 1H; ^{3}J = 8.4Hz; phenyl-$\underline{H}^4$), 9.47 (s; 1H; -O$\underline{H}$), 9.71 (s; 1H; -O$\underline{H}$)

3-Ethyl-6-hydroxy-2-(4-hydroxyphenyl)benzo[b]furan (**97c**)



Preparation from 2-(2,4-dimethoxyphenyl)-1-(4-methoxyphenyl)butan-1-one **93c** (5.8mmol) following the general demethylation procedure (cf. section E.2.2.6). The reaction mixture was stirred at room temperature for 24h.

The crude product was chromatographed (SiO_2; DCM/ethyl acetate 9:1, v/v) and subsequently recrystallised from DCM.

Colourless crystals; yield: 29%

Melting point: 132-133°C

$C_{16}H_{14}O_3$ (254.29)

IR: ν (cm^{-1}) = 3286 (w, br; O-H)

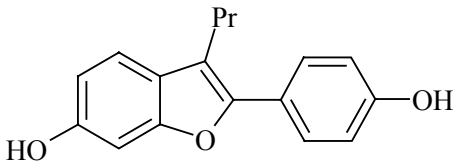
Analysis: Calculated: C: 75.57 H 5.55

Found: C: 75.38 H 5.20

MS: m/z (%) = 254 (100; $M^{+\bullet}$), 239 (57; $[M\text{-}CH_3]^{+\bullet}$),127 (8; $M^{2+\bullet}$)

^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.25 (t; 3H; ^{3}J = 7.4Hz; -C<u>H</u>$_3$), 2.79 (q; 2H; ^{3}J = 7.5Hz; -C<u>H</u>$_2$-), 6.73 (dd; 1H; ^{3}J = 8.4Hz; ^{4}J = 2.0Hz; phenyl-<u>H</u>^{5}), 6.87 (d; 1H; ^{4}J = 2.0Hz; phenyl-<u>H</u>^{7}), 6.89/7.50 (AA'BB'; 4H; ^{3}J = 8.6Hz; phenyl-<u>H</u>), 7.38 (d; 1H; ^{3}J = 8.2Hz; phenyl-<u>H</u>^{4}), 9.47 (s; 1H; -O<u>H</u>), 9.73 (s; 1H; -O<u>H</u>)

6-Hydroxy-2-(4-hydroxyphenyl)-3-propylbenzo[b]furan (**97d**)



Preparation from 2-(2,4-dimethoxyphenyl)-1-(4-methoxyphenyl)pentan-1-one **93d** (2.0mmol) following the general demethylation procedure (cf. section E.2.2.6). The reaction mixture was stirred at room temperature for 24h.

The crude product was chromatographed (SiO_2; DCM/ethyl acetate 9:1, v/v) and subsequently recrystallised from DCM.

Colourless crystals; yield: 20%

Melting point: 153-154°C

$C_{17}H_{16}O_3$ (268.31)

IR: ν (cm^{-1}) = 3185 (w, br; O-H)

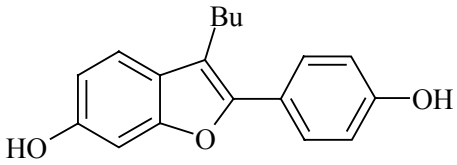
Analysis: Calculated: C: 76.10 H 6.01

Found: C: 75.90 H 5.88

MS: m/z (%) = 268 (68; $M^{+\bullet}$), 239 (100; $[M\text{-}CH_2CH_3]^{+\bullet}$)

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.96 (t; 3H; ^3J = 7.3Hz; -C<u>H</u>$_3$), 1.67 (sex; 2H; ^3J = 7.5Hz; -C<u>H</u>$_2$-CH$_3$), 2.75 (t; 2H; ^3J = 7.5Hz; -C<u>H</u>$_2$-CH$_2$-), 6.72 (dd; 1H; ^3J = 8.4Hz; ^4J = 2.0Hz; phenyl-<u>H</u>^{5}), 6.87 (d; 1H; ^4J = 2.0Hz; phenyl-<u>H</u>^{7}), 6.89/7.52 (AA'BB'; 4H; ^3J = 8.8Hz; phenyl-<u>H</u>), 7.36 (d; 1H; ^3J = 8.2Hz; phenyl-<u>H</u>^{4}), 9.46 (s; 1H; -O<u>H</u>), 9.72 (s; 1H; -O<u>H</u>)

3-Butyl-6-hydroxy-2-(4-hydroxyphenyl)benzo[b]furan (**97e**)



Preparation from 2-(2,4-dimethoxyphenyl)-1-(4-methoxyphenyl)hexan-1-one **93e** (2.04mmol) following the general demethylation procedure (cf. section E.2.2.6). The reaction mixture was stirred at room temperature for 24h.

The crude product was chromatographed (SiO_2; DCM/ethyl acetate 9:1, v/v) and subsequently recrystallised from DCM.

Colourless crystals; yield: 24%

Melting point: 169°C

$C_{18}H_{18}O_3$ (282.34)

IR: ν (cm^{-1}) = 3171 (w, br; O-H)

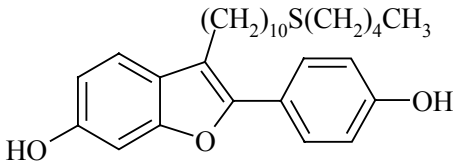
Analysis: Calculated: C: 76.57 H 6.43

Found: C: 75.30 H 6.25

MS: m/z (%) = 282 (100; $M^{+\bullet}$), 239 (87; $[M\text{-}(CH_2)_2CH_3]^{+\bullet}$)

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.90 (t; 3H; ^{3}J = 7.3Hz; -C$\underline{H}_3$), 1.38 (sex; 2H; ^{3}J = 7.4Hz; -C$\underline{H}_2$-CH$_3$), 1.62 (quin; 2H; ^{3}J = 7.8Hz; -C$\underline{H}_2$-CH$_2$-CH$_3$), 2.77 (t; 2H; ^{3}J = 7.5Hz; -C$\underline{H}_2$-CH$_2$-CH$_2$-), 6.72 (dd; 1H; ^{3}J = 8.3Hz; ^{4}J = 2.0Hz; phenyl-$\underline{H}^5$), 6.87 (d; 1H; ^{4}J = 2.0Hz; phenyl-$\underline{H}^7$), 6.89/7.51 (AA'BB'; 4H; ^{3}J = 8.6Hz; phenyl-$\underline{H}$), 7.36 (d; 1H; ^{3}J = 8.5Hz; phenyl-$\underline{H}^4$), 9.47 (s; 1H; -O$\underline{H}$), 9.73 (s; 1H; -O$\underline{H}$)

6-Hydroxy-2-(4-hydroxyphenyl)-3-[10-(pentylsulfanyl)decyl]benzo[b]furan (**98**)



Preparation from 2-(2,4-dimethoxyphenyl)-1-(4-methoxyphenyl)-12-(pentylsulfanyl)dodecan-1-one **94** (3.8mmol) following the general demethylation procedure (cf. section E.2.2.6). The reaction mixture was stirred at room temperature for 24h.

The crude product was chromatographed (SiO_2; DCM/ethyl acetate 13:1, v/v) and subsequently recrystallised from DCM.

Colourless crystals; yield: 37%

Melting point: 82-83°C

$C_{29}H_{40}O_3S$ (468.70)

IR: ν (cm^{-1}) = 3352 (w, br; O-H)

MS: m/z (%) = 468 (100; $M^{+\bullet}$), 366 (8; $[M\text{-}(CH_2)_5S]^{+\bullet}$), 239 (40; $[M\text{-}(CH_2)_9SC_5H_{11}]^{+\bullet}$)

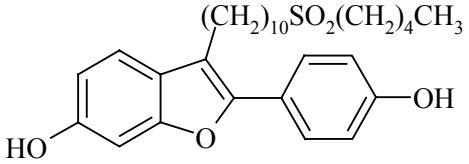
HRMS: Calculated for $C_{29}H_{40}O_3S$: 468.2698

Found: 468.2697 ± 0.0005

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.85 (t; 3H; ^{3}J = 7.0Hz; -C$\underline{H}_3$), 1.22-1.36 (m; 16H; -(C$\underline{H}_2$)$_6$--(C$\underline{H}_2$)$_2$-CH$_3$), 1.43-1.54 (m; 4H; -C$\underline{H}_2$-CH$_2$-S-CH$_2$-C$\underline{H}_2$-), 1.63 (quin; 2H; ^{3}J = 7.1Hz; -CH$_2$-C$\underline{H}_2$-(CH$_2$)$_8$-), 2.44 (t; 4H; ^{3}J = 7.3Hz; -C$\underline{H}_2$-S-C$\underline{H}_2$-), 2.76 (t; 2H; ^{3}J = 7.5Hz; -C$\underline{H}_2$-(CH$_2$)$_9$-), 6.72 (dd; 1H; ^{3}J = 8.4Hz; ^{4}J = 2.0Hz; phenyl-$\underline{H}^5$), 6.87 (phenyl-$\underline{H}^7$; merged in AA'BB'-

system), 6.88/7.50 (AA'BB'; 4H; ^3J = 8.8Hz; phenyl-H), 7.34 (d; 1H; ^3J = 8.2Hz; phenyl-H^4), 9.44 (s; 1H; -OH), 9.70 (s; 1H; -OH)

6-Hydroxy-2-(4-hydroxyphenyl)-3-[10-(pentylsulfonyl)decyl]benzo[b]furan (**99**)



Preparation from 2-(2,4-dimethoxyphenyl)-1-(4-methoxyphenyl)-12-(pentylsulfonyl)dodecan-1-one **95** (5.0mmol) following the general demethylation procedure (cf. section E.2.2.6). The reaction mixture was stirred at room temperature for 24h.

The crude product was chromatographed (SiO_2; DCM/ethyl acetate 9:1, v/v) and subsequently recrystallised from DCM.

Colourless crystals; yield: 32%

Melting point: 71-72°C

$C_{29}H_{40}O_5S$ (500.69)

IR: ν (cm^{-1}) = 3364 (w, br; O-H)

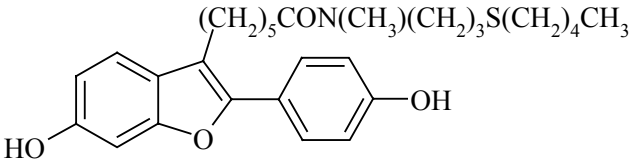
MS: m/z (%) = 500 (54; $M^{+\bullet}$), 239 (100; $[M-(CH_2)_9SO_2C_5H_{11}]^{+\bullet}$)

HRMS: Calculated for $C_{29}H_{40}O_5S$: 500.2596

Found: 500.2589 ± 0.0003

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.87 (t; 3H; ^3J = 7.0Hz; -CH$_3$), 1.23-1.40 (m; 16H; -(CH$_2$)$_6$--(CH$_2$)$_2$-CH$_3$), 1.61-1.71 (m; 6H; -CH$_2$-CH$_2$-S-CH$_2$-CH$_2$-, -CH$_2$-CH$_2$-(CH$_2$)$_8$-), 2.77 (t; 2H; ^3J = 7.5Hz; -CH$_2$-(CH$_2$)$_9$-), 3.02 (t; 4H; ^3J = 8.0Hz; -CH$_2$-SO$_2$-CH$_2$-), 6.72 (dd; 1H; ^3J = 8.4Hz; ^4J = 2.0Hz; phenyl-H^5), 6.86 (phenyl-H^7; merged in AA'BB'-system), 6.88/7.50 (AA'BB'; 4H; ^3J = 8.6Hz; phenyl-H), 7.34 (d; 1H; ^3J = 8.2Hz; phenyl-H^4), 9.45 (s; 1H; -OH), 9.71 (s; 1H; -OH)

6-Hydroxy-2-(4-hydroxyphenyl)-3-{5-{N-methyl-N-[3-(pentylsulfanyl)propyl]carbamoyl}-pentyl}benzo[b]furan (**100**)



Preparation from 7-(2,4-dimethoxyphenyl)-8-(4-methoxyphenyl)-8-oxooctanoic acid methyl-[3-(pentylsulfanyl)propyl]amide **96** (4.6mmol) following the general demethylation procedure (cf. section E.2.2.6). The reaction mixture was stirred at room temperature for 24h.
The crude product was chromatographed (SiO_2; DCM/ethyl acetate 3:1, v/v).

Yellow oil; yield: 53%

$C_{29}H_{39}NO_4S$ (497.69)

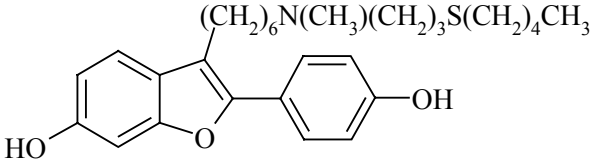
IR: ν (cm^{-1}) = 3400-2600 (w, br; -OH), 1611 (s; C=O)

MS: m/z (%) = 497 (100; $M^{+\bullet}$), 393 (16; $[M\text{-}HSC_5H_{11}]^{+\bullet}$), 239 (53; $[M\text{-}(CH_2)_4CON(CH_3)(CH_2)_3SC_5H_{11}]^{+\bullet}$), 104 (11; $HSC_5H_{11}]^{+\bullet}$)

HRMS: Calculated for $C_{29}H_{39}NO_4S$: 497.2600
Found: 497.2599 ± 0.0005

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.80-0.87 (m; 3H; -C<u>H</u>$_3$), 1.21-1.76 (m; 14H; -C<u>H</u>$_2$-CH$_2$-S-CH$_2$-(C<u>H</u>$_2$)$_3$-, -(C<u>H</u>$_2$)$_3$-CH$_2$-CO-), 2.21-2.30 (m; 2H; -CO-C<u>H</u>$_2$-), 2.37-2.48 (m; 4H; -C<u>H</u>$_2$-S-C<u>H</u>$_2$-), 2.76 (t; 2H; ^{3}J = 7.5Hz; -C<u>H</u>$_2$-(CH$_2$)$_4$-), 2.76/2.89 (2x s; 3H; -N-C<u>H</u>$_3$), 3.29 (-N-C<u>H</u>$_2$; merged in H_2O-signal), 6.72 (dd; 1H; ^{3}J = 8.5Hz; ^{4}J = 1.9Hz; phenyl-<u>H</u>^{5}), 6.88 (phenyl-<u>H</u>^{7}; merged in AA'BB'-system), 6.88/7.50 (AA'BB'; 4H; ^{3}J = 8.5Hz; phenyl-<u>H</u>), 7.35 (d; 1H; ^{3}J = 8.5Hz; phenyl-<u>H</u>^{4}), 9.45 (s; 1H; -O<u>H</u>), 9.71 (s; 1H; -O<u>H</u>)

6-Hydroxy-2-(4-hydroxyphenyl)-3-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-benzo[b]furan (**101**)



Preparation from 3-[6-hexanoic acid methyl-[3-(pentylsulfanyl)propyl]amido]-6-hydroxy-2-(4-hydroxyphenyl)benzo[b]furan **100** (2.2mmol) as described for compound **4a**, using 3eq of $LiAlH_4$. The crude product was chromatographed (SiO_2; MeOH/ethyl acetate 1:9, v/v).

Colourless solid; yield: 71%

$C_{29}H_{41}NO_3S$ (483.71)

Melting point: 46-47°C

IR: ν (cm^{-1}) = 3400-2600 (w, br; -OH)

MS: m/z (%) = 483 (53; $M^{+\bullet}$), 412 (11; $[M\text{-}HSC_5H_{11}]^{+\bullet}$), 352 (100; $[M\text{-}(CH_2{=})CH_2SC_5H_{11}]^{+\bullet}$), 308 (10; $[M\text{-}HN(CH_3)(CH_2)_3SC_5H_{11}]^{+\bullet}$), 239 (22; $[M\text{-}(CH_2)_5N(CH_3)(CH_2)_3SC_5H_{11}]^{+\bullet}$), 188 (45; $[CH_2{=}N(CH_3)\text{-}(CH_2)_3SC_5H_{11}]^{+\bullet}$), 176 (25; $[H_2N(CH_3)(CH_2)_3SC_5H_{11}]^{+\bullet}$),

HRMS: Calculated for $C_{29}H_{41}NO_3S$: 483.2807

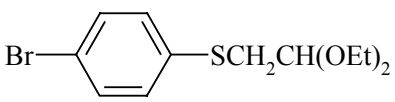
Found: 483.2803 ± 0.0003

^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.83 (t; 3H; ^3J = 7.0Hz; -C<u>H</u>$_3$), 1.21-1.38 (m; 10H; -(C<u>H</u>$_2$)$_2$-CH$_3$, -(C<u>H</u>$_2$)$_3$-CH$_2$-N-), 1.48 (quin; 2H; ^3J = 7.1Hz; -C<u>H</u>$_2$-CH$_2$-S-), 1.55-1.68 (m; 4H; -C<u>H</u>$_2$-(CH$_2$)$_4$-N-, -S-CH$_2$-C<u>H</u>$_2$-CH$_2$-N-), 2.08 (s; 3H; -N-C<u>H</u>$_3$), 2.22 (t; 2H; ^3J = 6.1Hz; -N-C<u>H</u>$_2$-), 2.32 (t; 2H; ^3J = 6.7Hz; -N-C<u>H</u>$_2$-), 2.44 (t; 2H; ^3J = 7.3Hz; -S-C<u>H</u>$_2$-), 2.46 (t; 2H; ^3J = 7.4Hz; -S-C<u>H</u>$_2$-), 2.76 (t; 2H; ^3J = 7.5Hz; -C<u>H</u>$_2$-(CH$_2$)$_5$-), 3.29 (-N-C<u>H</u>$_2$; merged in H_2O-signal), 6.72 (dd; 1H; ^3J = 8.4Hz; ^4J = 2.0Hz; phenyl-<u>H</u>^{5}), 6.88 (phenyl-<u>H</u>^{7}; merged in AA'BB'-system), 6.88/7.50 (AA'BB'; 4H; ^3J = 8.8Hz; phenyl-<u>H</u>), 7.34 (d; 1H; ^3J = 8.5Hz; phenyl-<u>H</u>^{4}), 9.46 (s; 1H; -O<u>H</u>), 9.72 (s; 1H; -O<u>H</u>)

2.4.2 New Synthesis of 5-Hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene

2.4.2.1 Synthesis of the Bromobenzene Precursor

1-Bromo-4-(2,2-diethoxyethylsulfanyl)benzene (**102**)



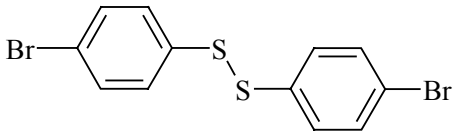
A mixture of 4-bromobenzenethiol (60.0mmol), bromoacetaldehyde diethyl acetal (55.0mmol) and anhydrous potassium carbonate (60.0mmol) were dissolved in dry acetone (80ml) and stirred at room temperature overnight. The reaction mixture was filtered, the solid washed with acetone and the combined filtrates concentrated *in vacuo*. The residue was diluted with water (100ml) and extracted with diethyl ether (3x100ml). The combined etheral extracts were washed with 0.5M KOH, water and brine, dried over Na_2SO_4 and concentrated. The desired compound was separated from the bis(4-bromophenyl)disulfane by-product **103** and purified by column chromatography (SiO_2; DCM/petroleum ether 40-60 1:3→1:0, v/v).

Yellow oil; yield: 71%

$C_{12}H_{17}BrO_2S$ (305.23)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.19 (t; 6H; ^{3}J = 7.0Hz; -CH_2-C$\underline{H}_3$), 3.10 (d; 2H; ^{3}J = 5.5Hz; -S-C$\underline{H}_2$-), 3.53 (dq; 2H; ^{2}J = 9.3Hz, ^{3}J = 7.1Hz; -O-C$\underline{H}_2$), 3.67 (dq; 2H; ^{2}J = 9.3Hz, ^{3}J = 7.1Hz; -O-C$\underline{H}_2$), 4.63 (t; 1H; ^{3}J = 5.5Hz; -CH_2-C$\underline{H}$-), 7.24/7.39 (AA'BB'; 4H; ^{3}J = 8.6Hz; phenyl-$\underline{H}$)

Bis(4-bromophenyl)disulfane (**103**)



By-product from the preparation of compound **102**. Isolated from the crude product mixture by column chromatography (SiO_2; DCM/petroleum ether 40-60 1:3, v/v).

Colourless solid; yield: 18%

Melting point: 88-90°C

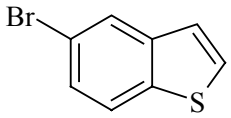
$C_{12}H_8Br_2S_2$ (376.12)

MS: m/z (%) = 378 (55; $M^{+\bullet}$[only ^{81}Br]), 376 (100; $M^{+\bullet}$), 374 (49; $M^{+\bullet}$[only ^{79}Br]), 297 (8; $[M-^{79}Br]^{+\bullet}$), 295 (8; $[M-^{81}Br]^{+\bullet}$), 189 (48; $^{81}BrC_6H_4S^{+\bullet}$), 187 (46; $^{79}BrC_6H_4S^{+\bullet}$), 108 (61; $C_6H_4S^{+\bullet}$)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.33/7.43 (AA'BB'; 4H; ^{3}J = 8.7Hz; phenyl-$\underline{H}$)

2.4.2.2 Cyclisation to 5-Bromobenzo[b]thiophene

5-Bromobenzo[b]thiophene (**104**)

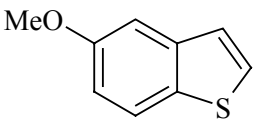


Under nitrogen, polyphosphoric acid (24g) was added to anhydrous chlorobenzene (300ml) and heated to gentle reflux. 1-Bromo-4-(2,2-diethoxy-ethylsulfanyl)-benzene **102** was added slowly over 1h and the mixture refluxed for 24h with vigorous stirring. The reaction mixture was allowed to cool to ambient temperature and the organic phase was separated from the PPA. Residual PPA was decomposed with water (300ml) and the resulting aqueous phase extracted with DCM (2x 100ml). The combined organic extracts were dried over Na_2SO_4. Finally the solvent was evaporated.
Chromatographic purification (SiO_2; DCM/petroleum ether 40-60 1:5, v/v) of the brown crude product afforded the product as a colourless oil, that solidified upon cooling overnight.

Colourless solid; yield: 81%
Melting point: 46-47°C
C_8H_5BrS (213.09)
^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.26 (d; 1H; ^3J = 6.1Hz; thiophene-$\underline{H}^3$), 7.43 (dd; 1H; ^3J = 8.6Hz; ^4J = 1.8Hz; phenyl-$\underline{H}^6$), 7.47 (d; 1H; ^3J = 5.5Hz; thiophene-$\underline{H}^2$), 7.73 (d; 1H; ^3J = 8.5Hz; phenyl-$\underline{H}^7$), 7.96 (d; 1H; ^4J = 1.8Hz; phenyl-$\underline{H}^4$)

2.4.2.3 Copper Catalysed Nucleophilic Aromatic Substitution

5-Methoxybenzo[b]thiophene (**105**)



Under nitrogen at room temperature, solid sodium methoxide (47.2mmol) was added to a solution of 5-bromobenzo[b]thiophene **104** (31.4mmol) in DMF (20ml) and methanol (8ml). The temperature was raised to 110°C and solid copper(I) bromide (3.1mmol) was added. Af-

ter 2h the reaction was stopped by cooling to ambient temperature. The reaction mixture was then poured into water (100ml) and the aqueous phase extracted with DCM (3x75ml). The combined organic layers were dried over sodium sulphate and the solvent was evaporated.
The crude product was purified by column chromatography (SiO_2; DCM/petroleum ether 40-60 1:3, v/v).

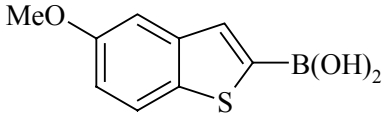
Colourless solid; yield: 59%
Melting point: 39-40°C
C_9H_8OS (164.22)

Analysis:	Calculated:	C: 65.83	H 4.91
	Found:	C: 65.67	H 4.61

^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.87 (s; 3H; -O-C$\underline{H}_3$), 7.00 (dd; 1H; ^{3}J = 8.8Hz; ^{4}J = 2.4Hz; phenyl-$\underline{H}^6$), 7.24-7.28 (m; 2H; thiophene-$\underline{H}^3$, phenyl-$\underline{H}^4$), 7.43 (d; 1H; ^{3}J = 5.3Hz; thiophene-$\underline{H}^2$), 7.73 (d; 1H; ^{3}J = 8.8Hz; phenyl-$\underline{H}^7$)

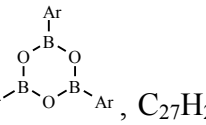
2.4.2.4 Synthesis of 5-Methoxybenzo[b]thiophene 2-Boronic Acid

5-Methoxybenzo[b]thiophene 2-boronic acid (**106**)



Under nitrogen atmosphere, n-BuLi (1.6M in hexane fraction, 20.09mmol) in dry THF (10ml) was cooled to –60°C and a solution of 5-methoxybenzo[b]thiophene **105** (18.27mmol) in dry THF (25ml) was added dropwise. After stirring for 30min, trimethyl borate (20.09mmol) was added and the reaction allowed to gradually come to room temperature within 1.5h. The reaction mixture was hydrolysed with 1N HCl (50ml) and the resulting aqueous phase extracted with ethyl acetate (2x 50ml). The combined organic extracts were dried over Na_2SO_4. Evaporation of the solvent *in vacuo* resulted in the formation of a trimeric cyclic boric acid anhydride that was sufficiently pure without further purification.

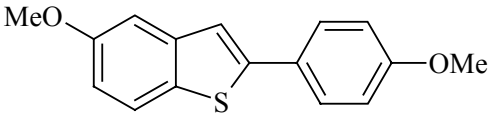
Light yellow solid; yield: 82%
Melting point: 166-169°C
$C_9H_9BO_3S$ (208.04)

MS: m/z (%) = 570 (66; $M^{+\bullet}$[ , $C_{27}H_{21}B_3O_6S_3$]), 285 (45; $M^{2+\bullet}$), 190 (35; $[Ar\text{-}BO]^{+\bullet}$), 164 (59; $[Ar]^{+\bullet}$)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.82 (s; 3H; -O-C<u>H</u>$_3$), 7.01 (dd; 1H; ^3J = 8.8Hz; ^4J = 2.5Hz; phenyl-<u>H</u>^{6}), 7.40 (d; 1H; ^4J = 2.4Hz; phenyl-<u>H</u>^{4}), 7.83 (d; 1H; ^3J = 8.8Hz; phenyl-<u>H</u>^{7}), 7.87 (s; 1H; thiophene-<u>H</u>^{3})

2.4.2.5 Suzuki Coupling Reaction

5-Methoxy-2-(4-methoxyphenyl)benzo[b]thiophene (**107**)



In a 10ml reaction flask were placed 5-methoxybenzo[b]thiophene 2-boronic acid anhydride **106** (1.3mmol), 4-bromoanisole (3.9mmol), anhydrous sodium carbonate (11.7mmol), tetrabutylammonium bromide (3.9mmol), palladium acetate (0.005mmol), water (8ml) and a magnetic stirrer bar. The flask was sealed with a septum and placed into an oil bath preheated to 150°C for five minutes. After the reaction mixture was allowed to cool to room temperature, the flask was opened and the content was transferred into a separating funnel using each ethyl acetate (50ml) and water (50ml). The layers were separated and the aqueous layer extracted again with ethyl acetate (50ml). The combined organic extracts were filtered to remove any residual palladium(0), washed with brine and dried over Na_2SO_4. Finally, the solvent was removed under reduced pressure and the crude product purified by column chromatography (SiO_2; DCM/petroleum ether 1:3, v/v).

Colourless solid; yield: 56%

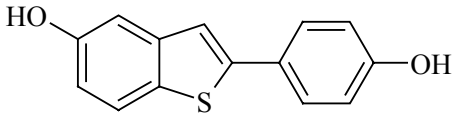
Melting point: 173-176°C

$C_{16}H_{14}O_2S$ (270.35)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.85 (s; 3H; -O-C<u>H</u>$_3$), 3.87 (s; 3H; -O-C<u>H</u>$_3$), 6.94 (dd; 1H; ^3J = 8.8Hz; ^4J = 2.5Hz; phenyl-<u>H</u>^{6}), 6.95/7.63 (AA'BB'; 4H; ^3J = 8.9Hz; phenyl-<u>H</u>), 7.21 (d; 1H; ^4J = 2.5Hz; phenyl-<u>H</u>^{4}), 7.35 (s; 1H; thiophene-<u>H</u>^{3}), 7.66 (d; 1H; ^3J = 8.8Hz; phenyl-<u>H</u>^{7})

2.4.2 6 Demethylation of the Hydroxy Protecting Groups

5-Hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene (**108**)



5-Methoxy-2-(4-methoxyphenyl)benzo[b]thiophene **107** (2.1mmol) was demethylated with boron tribromide following the general procedure in section E2.2.6. The reaction mixture was stirred at room temperature for 24h.

The crude product was chromatographed (SiO_2; DCM/ethyl acetate 9:1, v/v) and recrystallised from a mixture of DCM and ethyl acetate.

Colourless solid; yield: 65%

Melting point: > 250°C (dec.)

$C_{14}H_{10}O_2S$ (242.29)

IR: ν (cm^{-1}) = 3376 (w, br; -OH)

Analysis: Calculated: C: 69.40 H 4.16

Found: C: 69.43 H 4.37

^{1}H-NMR (DMSO-d_6): δ (ppm) = 6.80 (dd; 1H; ^3J = 8.6Hz; ^4J = 2.3Hz; phenyl-$\underline{H}^6$), 6.85/7.55 (AA'BB'; 4H; ^3J = 8.6Hz; phenyl-$\underline{H}$), 7.12 (d; 1H; ^4J = 2.3Hz; phenyl-$\underline{H}^4$), 7.48 (s; 1H; thiophene-$\underline{H}^3$), 7.67 (d; 1H; ^3J = 8.6Hz; phenyl-$\underline{H}^7$), 9.41 (s; 1H; -O-$\underline{H}$), 9.78 (s; 1H; -O-$\underline{H}$)

2.5 Benzopyran(one)s

2.5.1 1-Benzopyran-2-ones

2.5.1.1 Synthesis of Side Chain Precursors

Methyl 11-bromoundecanoate (**110**)

Preparation from 11-bromoundecanoic acid (75.4mmol) in the same way as described for the esterification of compound **3a** (cf. section E2.1.1). The crude product was sufficiently pure without additional purification.

Colourless oil; yield 91%

$C_{12}H_{23}O_2Br$ (279.22)

IR: ν (cm^{-1}) = 1741 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.29-1.47 (m; 12H; -(C<u>H</u>$_2$)$_6$-), 1.62 (quin; 2H; ^{3}J = 7.3Hz; -C<u>H</u>$_2$-CH$_2$-CO-), 1.85 (quin; 2H; ^{3}J = 7.1Hz; -C<u>H</u>$_2$-CH$_2$-Br), 2.30 (t; 2H; ^{3}J = 7.5Hz; -C<u>H</u>$_2$-CO-), 3.41 (t; 2H; ^{3}J = 6.7Hz; -C<u>H</u>$_2$-Br), 3.67 (s; 3H; -O-C<u>H</u>$_3$)

Methyl 11-(pentylsulfanyl)undecanoate (**111**)

Preparation from methyl 11-bromoundecanoate **110** (73.8mmol), following the procedure described for the synthesis of compound **14** (cf. section E2.2.1.1.2). Purification was achieved by column chromatography (SiO_2; DCM/petroleum ether 40-60 1:3, v/v).

Colourless oil; yield 90%

$C_{17}H_{34}O_2S$ (302.52)

IR: ν (cm^{-1}) = 1742 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.90 (t; 3H; ^{3}J = 7.0Hz;-(CH$_2$)$_4$-C<u>H</u>$_3$), 1.28-1.42 (m; 16H; -(C<u>H</u>$_2$)$_6$-, -(C<u>H</u>$_2$)$_2$-), 1.52-1.67 (m; 6H; -C<u>H</u>$_2$-CH$_2$-CO-, -C<u>H</u>$_2$-CH$_2$-S-CH$_2$-C<u>H</u>$_2$-), 2.30 (t; 2H; ^{3}J = 7.5Hz; -C<u>H</u>$_2$-CO-), 2.50 (t; 4H; ^{3}J = 7.3Hz; -C<u>H</u>$_2$-S-C<u>H</u>$_2$-), 3.67 (s; 3H; -O-C<u>H</u>$_3$)

11-(Pentylsulfanyl)undecanoic acid (**112**)

Preparation from methyl 11-bromoundecanoate **111** (71.3mmol), following the procedure described for the synthesis of compound **15** (cf. section E2.2.1.1.2). The crude product was sufficiently pure without additional purification.

Colourless solid; yield 95%

$C_{16}H_{32}O_2S$ (288.49)

Melting point: 43°C

IR: ν (cm^{-1}) = 3400-2500 (w, br; COOH), 1710 (s; C=O)

Analysis: Calculated: C: 66.61 H 11.18

Found: C: 66.72 H 11.44

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.90 (t; 3H; ^{3}J = 7.0Hz;-$(CH_2)_4$-C$\underline{H}_3$), 1.24-1.41 (m; 16H; -(C$\underline{H}_2$)$_6$-, -(C$\underline{H}_2$)$_2$-), 1.52-1.68 (m; 6H; -C$\underline{H}_2$-CH_2-CO-, -C$\underline{H}_2$-CH_2-S-CH_2-C$\underline{H}_2$-), 2.35 (t; 2H; ^{3}J = 7.5Hz; -C$\underline{H}_2$-CO-), 2.50 (t; 4H; ^{3}J = 7.3Hz; -C$\underline{H}_2$-S-C$\underline{H}_2$-)

7-Bromoheptanoic acid (**113**)

7-Bromoheptanitrile (78.9mmol) was heated with conc. HBr (48%; 45ml) at 95°C for 24h. The reaction mixture was cooled to ambient temperature, diluted with water (100ml) and extracted with diethyl ether (3x100ml). The combined organic extracts were basified with 1N NaOH (150ml) and the layers separated. The aqueous layer was reacidified with HCl and extracted again with diethyl ether (3x100ml). The resulting three organic phases were combined and washed with brine. After drying over Na_2SO_4 the solvent was evaporated. The resulting product required no additional purification.

Colourless solid; yield 96%

$C_7H_{13}BrO_2$ (209.08)

Melting point: 28-29°C

IR: ν (cm^{-1}) = 3500-2500 (w, br; COOH), 1697 (s; C=O)

Analysis: Calculated: C: 40.21 H 6.27

Found: C: 40.56 H 5.94

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.32-1.52 (m; 4H; -(C$\underline{H}_2$)$_2$-), 1.66 (quin; 2H; ^{3}J = 7.3Hz; -C$\underline{H}_2$-CH_2-CO-), 1.87 (quin; 2H; ^{3}J = 7.1Hz; -C$\underline{H}_2$-CH_2-Br-), 2.37 (t; 2H; ^{3}J = 7.4Hz; -C$\underline{H}_2$-CO-), 3.41 (t; 2H; ^{3}J = 6.7Hz; -C$\underline{H}_2$-Br)

11-(Pentylsulfanyl)undecanoic acid chloride (**114**)

Preparation from 11-(pentylsulfanyl)undecanoic acid **112** (25.3mmol) following the general procedure in section E2.2.1.2.1.

Colourless oil; yield 99%

$C_{16}H_{31}ClOS$ (306.94)

IR: ν (cm^{-1}) = 1798 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.90 (t; 3H; ^{3}J = 7.0Hz;-$(CH_2)_4$-C$\underline{H}_3$), 1.24-1.41 (m; 16H; -(C$\underline{H}_2)_6$-, -(C$\underline{H}_2)_2$-), 1.52-1.63 (m; 4H; -C$\underline{H}_2$-CH_2-S-CH_2-C$\underline{H}_2$-), 1.71 (t; 2H; ^{3}J = 7.2Hz; -C$\underline{H}_2$-CH_2-CO-), 2.50 (t; 4H; ^{3}J = 7.4Hz; -C$\underline{H}_2$-S-C$\underline{H}_2$-), 2.88 (t; 2H; ^{3}J = 7.4Hz; -C$\underline{H}_2$-CO)

7-Bromoheptanoic acid chloride (**115**)

Preparation from 7-bromoheptanoic acid **113** (31.3mmol) following the general procedure in section E2.2.1.2.1.

Colourless oil; yield 96%

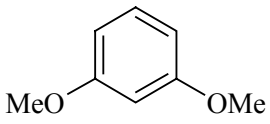
$C_7H_{12}BrClO$ (227.53)

IR: ν (cm^{-1}) = 1794 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.33-1.53 (m; 4H; -(C$\underline{H}_2)_2$-), 1.73 (quin; 2H; ^{3}J = 7.3Hz; -C$\underline{H}_2$-CH_2-CO-), 1.87 (quin; 2H; ^{3}J = 6.7Hz; -C$\underline{H}_2$-CH_2-Br-), 2.91 (t; 2H; ^{3}J = 7.3Hz; -C$\underline{H}_2$-CO-), 3.41 (t; 2H; ^{3}J = 6.7Hz; -C$\underline{H}_2$-Br)

2.5.1.2 Synthesis of *ortho*-Hydroxylated Phenylketones

1,3-Dimethoxybenzene (**116**)



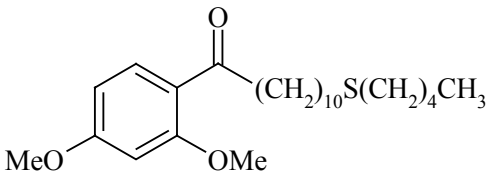
Under nitrogen, 1,3-dihydroxybenzene (250mmol) was dissolved into 10% NaOH solution (625mmol) and with water-cooling dimethylsulfate (500mmol) added dropwise. The reaction mixture was boiled for 30min to drive the methylation to completion and to decompose excess dimethylsulfate. After cooling to room temperature, the aqueous mixture was extracted with diethyl ether (3x150ml). The combined organic extracts washed with aqueous NaOH, water and brine.The solvent was dried over Na_2SO_4 and evaporated.

Colourless liquid; yield: 87%

$C_8H_{10}O_2$ (138.17)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.78 (s; 6H; -O-C<u>H</u>$_3$), 6.46-6.53 (m; 3H; phenyl-<u>H</u>^{2}, phenyl-<u>H</u>^{4}, phenyl-<u>H</u>^{6}), 7.18 (t; 1H; ^{3}J = 8.2Hz; phenyl-<u>H</u>^{5})

1-(2,4-Dimethoxyphenyl)-11-(pentylsulfanyl)undecan-1-one (**117**)



Under nitrogen, anhydrous aluminium chloride (50.0mmol) and 1,3-dimethoxybenzene **116** (125mmol) were dissolved in dry dichloroethane (150ml) and cooled by means of an ice-water bath. Then, 11-(pentylsulfanyl)undecanoic acid chloride **114** (25.0mmol) in dry DCE (50ml) was added dropwise and the resulting mixture stirred for additional 30min at this temperture. The reaction mixture was poured into an ice-cold mixture of water/conc. HCl (1:1, v/v; 200ml) and with vigorous stirring heated to 60°C for 15min. The clear mixture was cooled to ambient temperature and the layers were separated. The aqueous layer was extracted twice with ethyl acetate (2x100ml). The combined organic layers were washed with 2N HCl and brine. The solvent was dried over Na_2SO_4 and removed under reduced pressure.

The crude product was submitted to purification by column chromatography (SiO_2; ethyl acetae/petroleum ether 40-60 1:15, v/v), whereupon the excess 1,3-dimethoxybenzene **116** was regained in good quality almost completely.

Colourless solid; yield: 71%

Melting point: 37-39°C

$C_{24}H_{40}O_3S$ (408.64)

IR: ν (cm^{-1}) = 1656 (s; C=O)

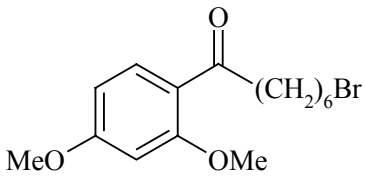
Analysis: Calculated: C: 70.54 H 9.87

Found: C: 70.40 H 10.08

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.90 (t; 3H; ^{3}J = 7.0Hz;-$(CH_2)_4$-C<u>H</u>$_3$), 1.28-1.42 (m; 16H; -$(C\underline{H}_2)_6$-, -$(C\underline{H}_2)_2$-), 1.52-1.70 (m; 6H; -C<u>H</u>$_2$-CH_2-S-CH_2-C<u>H</u>$_2$-, -C<u>H</u>$_2$-CH_2-CO-), 2.50 (t; 4H; ^{3}J = 7.4Hz; -C<u>H</u>$_2$-S-C<u>H</u>$_2$-), 2.92 (t; 2H; ^{3}J = 7.4Hz; -C<u>H</u>$_2$-CO), 3.85 (s; 3H; -O-C<u>H</u>$_3$), 3.88 (s; 3H; -O-C<u>H</u>$_3$), 6.45

(d; 1H; ^{4}J = 2.5Hz; phenyl-H^3), 6.52 (dd; 1H; ^{3}J = 8.8Hz; ^{4}J = 2.5Hz; phenyl-H^5), 7.78 (d; 1H; ^{3}J = 8.8Hz; phenyl-H^6)

7-Bromo-1-(2,4-dimethoxyphenyl)heptan-1-one (**118**)



Preparation form 7-bromoheptanoic acid chloride **115** (30.0mmol) and 1,3-dimethoxybenzene **116** (150mmol) as described for compound **117**. The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 1:10, v/v) and recrystallisation from EtOH (99%).

Colourless needles; yield: 93%

Melting point: 43-44°C

$C_{15}H_{21}BrO_3$ (329.23)

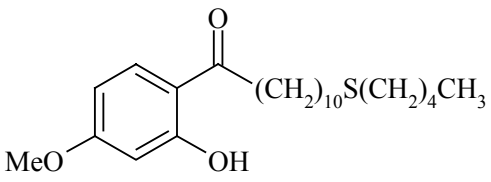
IR: ν (cm^{-1}) = 1655 (s; C=O)

Analysis: Calculated: C: 54.72 H 6.43

Found: C: 54.80 H 6.32

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.31-1.52 (m; 4H; -(CH$_2$)$_2$-), 1.68 (quin; 2H; ^{3}J = 7.4Hz; -CH$_2$-CH$_2$-CO-), 1.87 (quin; 2H; ^{3}J = 7.1Hz; -CH$_2$-CH$_2$-Br-), 2.94 (t; 2H; ^{3}J = 7.4Hz; -CH$_2$-CO-), 3.41 (t; 2H; ^{3}J = 6.9Hz; -CH$_2$-Br), 3.86 (s; 3H; -O-CH$_3$), 3.89 (s; 3H; -O-CH$_3$), 6.46 (d; 1H; ^{4}J = 2.2Hz; phenyl-H^3), 6.53 (dd; 1H; ^{3}J = 8.8Hz; ^{4}J = 2.5Hz; phenyl-H^5), 7.79 (d; 1H; ^{3}J = 8.5Hz; phenyl-H^6)

1-(2-Hydroxy-4-methoxyphenyl)-11-(pentylsulfanyl)undecan-1-one (**119**)



Preparation from 1-(2,4-dimethoxyphenyl)-11-(pentylsulfanyl)undecan-1-one **117** (5.6mmol) following the general demethylation procedure (cf. section E2.2.6). The reaction mixture was stirred at 0°C for 30min before it was hydrolysed with $NaHCO_3$.
The crude orange solid (100%) was sufficiently pure without additional purification. Nevertheless it was recrystallised from EtOH (99%).

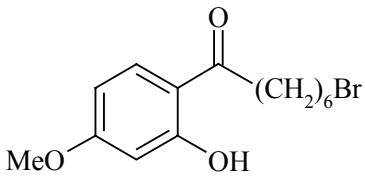
Colourless needles; yield: 83%
Melting point: 51°C
$C_{23}H_{38}O_3S$ (315.21)

IR: ν (cm^{-1}) = 1631 (s; C=O)

Analysis: Calculated: C: 70.00 H 9.71
Found: C: 69.73 H 9.61

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.90 (t; 3H; ^3J = 7.1Hz;-$(CH_2)_4$-C<u>H</u>$_3$), 1.29-1.42 (m; 16H; -$(C\underline{H}_2)_6$-, -$(C\underline{H}_2)_2$-), 1.52-1.63 (m; 4H; -C<u>H</u>$_2$-CH_2-S-CH_2-C<u>H</u>$_2$-), 1.72 (quin; 2H; ^3J = 7.3Hz; -C<u>H</u>$_2$-CH_2-CO-), 2.50 (t; 4H; ^3J = 7.3Hz; -C<u>H</u>$_2$-S-C<u>H</u>$_2$-), 2.89 (t; 2H; ^3J = 7.5Hz; -C<u>H</u>$_2$-CO), 3.84 (s; 3H; -O-C<u>H</u>$_3$), 6.42-6.46 (m; 2H; phenyl-<u>H</u>^{3}, phenyl-<u>H</u>^{5}), 7.66 (d; 1H; ^3J = 9.0Hz; phenyl-<u>H</u>^{6}), 12.87 (s; 1H; -O<u>H</u>)

7-Bromo-1-(2-hydroxy-4-methoxyphenyl)heptan-1-one (**120**)



Preparation from 7-bromo-1-(2,4-dimethoxyphenyl)heptan-1-one **118** (20.0mmol) following the general demethylation procedure (cf. section E2.2.6). The reaction mixture was stirred at 0°C for 30min before it was hydrolysed with $NaHCO_3$.
The crude orange solid (100%) was sufficiently pure without additional purification. Nevertheless it was recrystallised from EtOH (99%).

Colourless (slightly red) crystals; yield: 91%
Melting point: 52-53°C
$C_{14}H_{19}BrO_3$ (315.21)

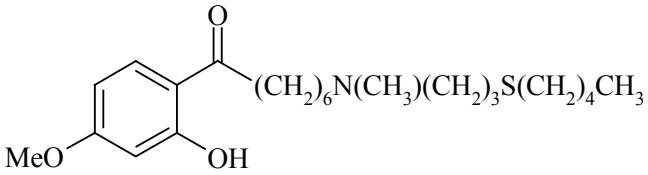
IR: ν (cm^{-1}) = 1620 (s; C=O)

Analysis: Calculated: C: 53.35 H 6.08

Found: C: 53.34 H 6.36

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.36-1.55 (m; 4H; -($C\underline{H}_2$)$_2$-), 1.75 (quin; 2H; ^3J = 7.4Hz; -$C\underline{H}_2$-CH_2-CO-), 1.88 (quin; 2H; ^3J = 6.8Hz; -$C\underline{H}_2$-CH_2-Br-), 2.91 (t; 2H; ^3J = 7.4Hz; -$C\underline{H}_2$-CO-), 3.42 (t; 2H; ^3J = 6.8Hz; -$C\underline{H}_2$-Br), 3.85 (s; 3H; -O-$C\underline{H}_3$), 6.43-6.46 (m; 2H; phenyl-$\underline{H}^3$, phenyl-$\underline{H}^5$), 7.66 (d; 1H; ^3J = 9.0Hz; phenyl-$\underline{H}^6$), 12.87 (s; 1H; -O$\underline{H}$)

1-(2-Hydroxy-4-methoxyphenyl)-7-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}heptan-1-one (**121**)



Preparation from 7-bromo-1-(2-hydroxy-4-methoxyphenyl)heptan-1-one **120** (15.5mmol), N-methyl-3-(pentylsulfanyl)propylamine **16** (15.5mmol) and triethylamine (15.5mmol). Purification of the crude product was achieved by column chromatography (SiO_2; ethyl acetate/MeOH 5:1, v/v).

Yellow oil; yield: 59%

$C_{23}H_{39}NO_3S$ (409.63)

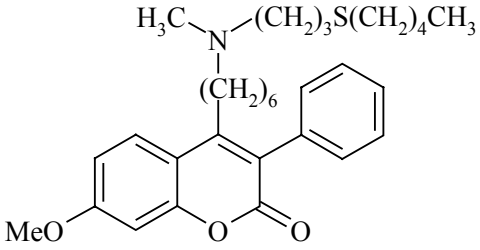
IR: ν (cm^{-1}) = 1628 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^3J = 7.1Hz; -(CH_2)$_4$-$C\underline{H}_3$), 1.29-1.63 (m; 12H; -($C\underline{H}_2$)$_3$-CH_3, -($C\underline{H}_2$)$_3$-), 1.74 (quin; 2H; ^3J = 7.5Hz; -N-CH_2-$C\underline{H}_2$-CH_2-S-), 1.81 (quin; 2H; ^3J = 7.5Hz; -CO-CH_2-$C\underline{H}_2$-), 2.29 (s; 3H; -N-$C\underline{H}_3$), 2.43 (t; 2H; ^3J = 7.5Hz; -N-$C\underline{H}_2$-), 2.49-2.55 (m; 6H; -$C\underline{H}_2$-N-, -$C\underline{H}_2$-S-$C\underline{H}_2$-), 2.90 (t; 2H; ^3J = 7.3Hz; -CO-$C\underline{H}_2$-), 3.84 (s; 3H; -O-$C\underline{H}_3$), 6.42-6.46 (m; 2H; phenyl-$\underline{H}^3$, phenyl-$\underline{H}^5$), 7.66 (d; 1H; ^3J = 9.0Hz; phenyl-$\underline{H}^6$), 12.89 (s; 1H; -O$\underline{H}$)

2.5.1.3 Formation of the Benzopyranone Core

Under nitrogen atmosphere, CDI (2eq) was added to a DMF solution of the respective phenylacetic acid (2eq) and stirred for 15min until the CO_2 evolution ceased. Then, the respective *ortho*-hydroxyphenylketone (1eq) in DMF was added, followed by anhydrous K_2CO_3 (5eq) and DMAP (0.2eq). The mixture was heated to 85°C for 6h. At ambient temperature the black reaction mixture was diluted with water and extracted with three portions of ethyl acetate. The combined organic phases were washed with 1N HCl, 5% $NaHCO_3$ solution, water and brine. The solvent was dried over Na_2SO_4 removed *in vacuo*.

7-Methoxy-4-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-3-phenyl-2H-1-benzopyran-2-one (**122**)



Preparation from 1-(2-hydroxy-4-methoxyphenyl)-7-{N-methyl-N-[3-(pentylsulfanyl)-propyl]amino}heptan-1-one **121** (4.2mmol) and phenylacetic acid (8.4mmol). The black crude product was purified by column chromatography (SiO_2; DCM/MeOH 25:1, v/v).

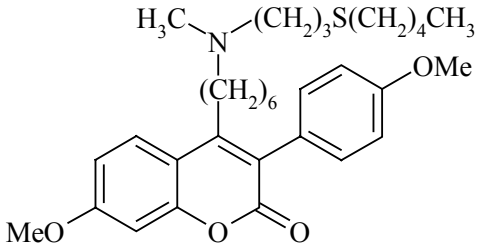
Red oil; yield: 70%

$C_{31}H_{43}NO_3S$ (509.75)

IR: ν (cm^{-1}) = 1714 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^3J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.14-1.44 (m; 10H; -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_3$-), 1.49-1.63 (m; 4H; -N-CH_2-C<u>H</u>$_2$-, -C<u>H</u>$_2$-CH_2-S-), 1.75 (quin; 2H; ^3J = 7.3Hz; -N-CH_2-C<u>H</u>$_2$-CH_2-S-), 2.20 (s; 3H; -N-C<u>H</u>$_3$), 2.27 (t; 2H; ^3J = 7.4Hz; -N-C<u>H</u>$_2$-), 2.42 (t; 2H; ^3J = 7.3Hz; -N-C<u>H</u>$_2$-), 2.50 (t; 2H; ^3J = 7.3Hz; -S-C<u>H</u>$_2$-), 2.52 (t; 2H; ^3J = 7.3Hz; -S-C<u>H</u>$_2$-), 2.60 (t; 2H; ^3J = 8.1Hz; =C-C<u>H</u>$_2$-), 3.89 (s; 3H; -O-C<u>H</u>$_3$), 6.86-6.92 (m; 2H; phenyl-<u>H</u>^{6}, phenyl-<u>H</u>^{8}), 7.26-7.29 (m; 2H; phenyl-<u>H</u>), 7.36-7.48 (m; 3H; phenyl-<u>H</u>), 7.55 (d; 1H; ^3J = 8.5Hz; phenyl-<u>H</u>^{5})

7-Methoxy-3-(4-methoxyphenyl)-4-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-2H-1-benzopyran-2-one (**123**)



Preparation from 1-(2-hydroxy-4-methoxyphenyl)-7-{N-methyl-N-[3-(pentylsulfanyl)-propyl]amino}heptan-1-one **121** (4.9mmol) and 4-methoxyphenylacetic acid (9.8mmol). The brown crude product was purified twice by column chromatography (SiO_2; DCM/MeOH 25:1 and 50:1, v/v), whereupon 23% of the starting material **121** was recovered.

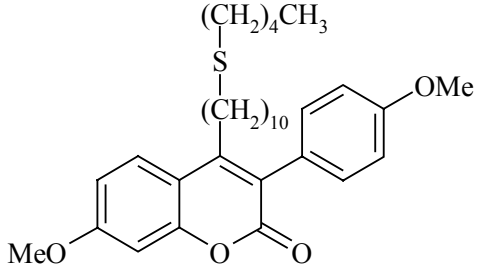
Yellow oil; yield: 50%

$C_{32}H_{45}NO_4S$ (539.78)

IR: ν (cm^{-1}) = 1713 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^3J = 7.1Hz; -$(CH_2)_4$-C$\underline{H}_3$), 1.14-1.62 (m; 14H; -(C$\underline{H}_2$)$_3$-CH_3, -(C$\underline{H}_2$)$_4$-), 1.76 (quin; 2H; ^3J = 7.2Hz; -N-CH_2-C$\underline{H}_2$-CH_2-S-), 2.23 (s; 3H; -N-C$\underline{H}_3$), 2.30 (t; 2H; ^3J = 7.1Hz; -N-C$\underline{H}_2$-), 2.44 (t; 2H; ^3J = 7.1Hz; -N-C$\underline{H}_2$-), 2.50 (t; 2H; ^3J = 7.5Hz; -S-C$\underline{H}_2$-), 2.52 (t; 2H; ^3J = 7.5Hz; -S-C$\underline{H}_2$-), 2.62 (t; 2H; ^3J = 8.1Hz; =C-C$\underline{H}_2$-), 3.86 (s; 3H; -O-C$\underline{H}_3$), 3.89 (s; 3H; -O-C$\underline{H}_3$), 6.86 (d; 1H; ^4J = 2.5Hz; phenyl-$\underline{H}^8$), 6.89 (dd; 1H; ^3J = 8.6Hz, ^4J = 2.5Hz; phenyl-$\underline{H}^6$), 6.98/7.20 (AA'BB'; 4H; ^3J = 8.8Hz; phenyl-$\underline{H}$), 7.54 (d; 1H; ^3J = 8.8Hz; phenyl-$\underline{H}^5$)

7-Methoxy-3-(4-methoxyphenyl)-4-[10-(pentylsulfanyl)decyl)-2H-1-benzopyran-2-one (**124**)



Preparation from 1-(2-hydroxy-4-methoxyphenyl)-11-(pentylsulfanyl)undecan-1-one **119** (6.3mmol) and 4-methoxyphenylacetic acid (12.6mmol). The red crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:10, v/v), whereupon 34% of the starting material **119** was recovered.

Colourless solid; yield: 53%

Melting point: 43°C

$C_{32}H_{44}O_4S$ (524.76)

IR: ν (cm^{-1}) = 1703 (s; C=O)

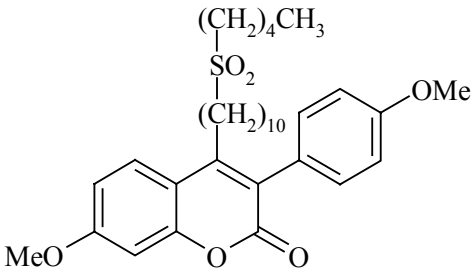
Analysis: Calculated: C: 73.24 H 8.45

Found: C: 73.38 H 8.31

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.90 (t; 3H; ^3J = 7.0Hz; -$(CH_2)_4$-C$\underline{H}_3$), 1.19-1.41 (m; 16H; -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_6$-), 1.48-1.63 (m; 6H; =C-CH_2-C$\underline{H}_2$-, -C$\underline{H}_2$-CH_2-S-CH_2-C$\underline{H}_2$-), 2.50 (t; 4H; ^3J = 7.4Hz; -C$\underline{H}_2$-S-C$\underline{H}_2$-), 2.61 (t; 2H; ^3J = 8.1Hz; =C-C$\underline{H}_2$-), 3.85 (s; 3H; -O-C$\underline{H}_3$), 3.89 (s; 3H; -O-C$\underline{H}_3$), 6.86 (d; 1H; ^4J = 2.2Hz; phenyl-$\underline{H}^8$), 6.89 (dd; 1H; ^3J = 8.6Hz, ^4J = 2.5Hz; phenyl-$\underline{H}^6$), 6.98/7.20 (AA'BB'; 4H; ^3J = 8.8Hz; phenyl-$\underline{H}$), 7.55 (d; 1H; ^3J = 8.5Hz; phenyl-$\underline{H}^5$)

2.5.1.4 Oxidation of the Side Chain Sulfur

7-Methoxy-3-(4-methoxyphenyl)-4-[10-(pentylsulfonyl)decyl]-2H-1-benzopyran-2-one (**125**)



Preparation from 7-methoxy-3-(4-methoxyphenyl)-4-[10-(pentylsulfanyl)decyl]-1-benzopyran-2-one **124** (6.3mmol) following the general procedure in section E2.2.2.4. After addition of m-CPBA the reaction mixture was stirred for 30min at room temperature. The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 40-60 1:2, v/v).

Colourless solid; yield: 95%

Melting point: 89-90°C

$C_{32}H_{44}O_6S$ (556.76)

IR: ν (cm^{-1}) = 1714 (s; C=O)

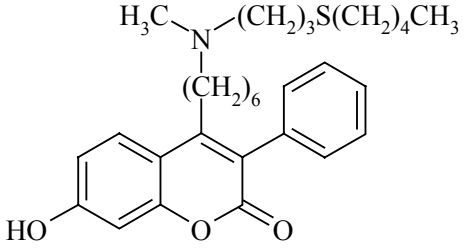
Analysis: Calculated: C: 69.03 H 7.97

Found: C: 68.94 H 8.45

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.92 (t; 3H; ^{3}J = 7.0Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.19-1.56 (m; 18H; -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_7$-), 1.77-1.89 (m; 4H;-C<u>H</u>$_2$-CH_2-SO_2-CH_2-C<u>H</u>$_2$-), 2.62 (t; 2H; ^{3}J = 8.1Hz; =C-C<u>H</u>$_2$-), 2.91-2.96 (m; 4H;-C<u>H</u>$_2$-SO_2-C<u>H</u>$_2$-), 3.85 (s; 3H; -O-C<u>H</u>$_3$), 3.89 (s; 3H; -O-C<u>H</u>$_3$), 6.86 (d; 1H; ^{4}J = 2.2Hz; phenyl-<u>H</u>^{8}), 6.89 (dd; 1H; ^{3}J = 8.6Hz, ^{4}J = 2.6Hz; phenyl-<u>H</u>6), 6.98/7.20 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 7.55 (d; 1H; ^{3}J = 8.5Hz; phenyl-<u>H</u>^{5})

2.5.1.5 Demethylation of the Hydroxy Protecting Groups

7-Hydroxy-4-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-3-phenyl-2H-1-benzopyran-2-one (**126**)



Preparation from 7-methoxy-4-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-3-phenyl-1-benzopyran-2-one **122** (2.3mmol) following the general procedure in section E2.2.6. The reaction mixture was stirred at room temperature for 7h. The crude product was purified by column chromatography (SiO_2; MeOH/ethyl acetate 1:5, v/v).

Yellow oil; yield: 90%

$C_{30}H_{41}O_3S$ (495.72)

IR: ν (cm^{-1}) = 3400-2600 (w, br; -OH), 1703 (s; C=O)

Analysis: Calculated: C: 72.69 H 8.34 N 2.83

Found: C: 71.67 H 8.35 N 2.48

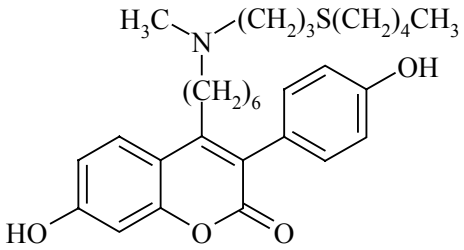
MS: m/z (%) = 495 (16; $M^{+\bullet}$), 364 (100; $[M-(CH_2)_2SC_5H_{11}]^{+\bullet}$), 244 (12; $[(CH_2)_5N(CH_3)(CH_2)_3SC_5H_{11}]^{+\bullet}$), 188 (21; $[CH_2{=}N(CH_3)(CH_2)_3S\text{-}C_5H_{11}]^{+\bullet}$)

HRMS: Calculated for $C_{30}H_{41}O_3S$: 495.2807

Found: 495.2805 ± 0.0004

^{1}H-NMR (MeOD-d_4): δ (ppm) = 0.90 (t; 3H; ^{3}J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.10-1.46 (m; 10H; -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_3$-), 1.49-1.61 (m; 4H; -N-CH_2-C<u>H</u>$_2$-, -C<u>H</u>$_2$-CH_2-S-), 1.75 (quin; 2H; ^{3}J = 7.5Hz; -N-CH_2-C<u>H</u>$_2$-CH_2-S-), 2.27 (s; 3H; -N-C<u>H</u>$_3$), 2.37 (t; 2H; ^{3}J = 8.0Hz; -N-C<u>H</u>$_2$-), 2.47-2.55 (m; 6H; -N-C<u>H</u>$_2$-, -C<u>H</u>$_2$-S-C<u>H</u>$_2$-), 2.63 (t; 2H; ^{3}J = 8.1Hz; =C-C<u>H</u>$_2$-), 6.72 (d; 1H; ^{4}J = 2.5Hz; phenyl-<u>H</u>^{8}), 6.83 (dd; 1H; ^{3}J = 8.8Hz, ^{4}J = 2.5Hz; phenyl-<u>H</u>^{6}), 7.24-7.27 (m; 2H; phenyl-<u>H</u>), 7.36-7.48 (m; 3H; phenyl-<u>H</u>), 7.63 (d; 1H; ^{3}J = 8.8Hz; phenyl-<u>H</u>^{5})

7-Hydroxy-3-(4-hydroxyphenyl)-4-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-2H-1-benzopyran-2-one (**127**)



Preparation from 7-methoxy-3-(4-methoxyphenyl)-4-{6-{N-methyl-N-[3-(pentylsulfanyl)-propyl]amino}hexyl}-1-benzopyran-2-one **123** (2.0mmol) following the general procedure in section E2.2.6. The reaction mixture was stirred at room temperature for 7h. The crude product was purified by column chromatography (SiO_2; MeOH/ethyl acetate 1:2, v/v).

Yellow solid; yield: 87%

Melting point: 89-91°C (dec.)

$C_{30}H_{41}O_4S$ (511.72)

IR: ν (cm^{-1}) = 3400-2600 (w, br; -OH), 1673 (s; C=O)

Analysis: Calculated: C: 70.42 H 8.08 N 2.74

Found: C: 69.22 H 8.19 N 2.43

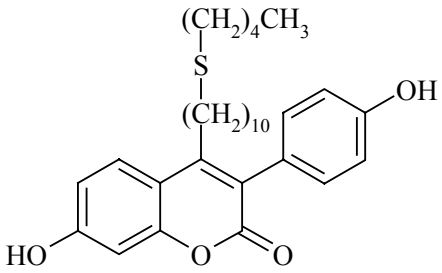
MS: m/z (%) = 511 (26; $M^{+\bullet}$), 380 (100; $[M-(CH_2)_2SC_5H_{11}]^{+\bullet}$), 244 (13; $[(CH_2)_5N(CH_3)(CH_2)_3SC_5H_{11}]^{+\bullet}$), 188 (23; $[CH_2{=}N(CH_3)(CH_2)_3S\text{-}C_5H_{11}]^{+\bullet}$)

HRMS: Calculated for $C_{30}H_{41}O_4S$: 511.2756

Found: 511.2756 ± 0.0002

^{1}H-NMR (MeOD-d_4): δ (ppm) = 0.90 (t; 3H; ^{3}J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.14-1.61 (m; 14H; -(C<u>H</u>$_2$)$_3$-CH_3, -(C<u>H</u>$_2$)$_4$-), 1.76 (quin; 2H; ^{3}J = 7.5Hz; -N-CH_2-C<u>H</u>$_2$-CH_2-S-), 2.28 (s; 3H; -N-C<u>H</u>$_3$), 2.38 (t; 2H; ^{3}J = 7.8Hz; -N-C<u>H</u>$_2$-), 2.47-2.57 (m; 6H; -N-C<u>H</u>$_2$-, -C<u>H</u>$_2$-S-C<u>H</u>$_2$-), 2.66 (t; 2H; ^{3}J = 7.8Hz; =C-C<u>H</u>$_2$-), 6.71 (d; 1H; ^{4}J = 2.5Hz; phenyl-<u>H</u>^{8}), 6.82 (dd; 1H; ^{3}J = 8.8Hz, ^{4}J = 2.5Hz; phenyl-<u>H</u>^{6}), 6.85/7.07 (AA'BB'; 4H; ^{3}J = 8.5Hz; phenyl-<u>H</u>), 7.61 (d; 1H; ^{3}J = 8.8Hz; phenyl-<u>H</u>^{5})

7-Hydroxy-3-(4-hydroxyphenyl)-4-[10-(pentylsulfanyl)decyl]-2H-1-benzopyran-2-one (**128**)



Preparation from 7-methoxy-3-(4-methoxyphenyl)-4-[10-(pentylsulfanyl)decyl]-1-benzopyran-2-one **124** (1.4mmol) following the general procedure in section E2.2.6. The reaction mixture was stirred at room temperature for 8h. The crude product was recrystallised from hot ethyl acetate.

Colourless solid; yield: 90%

Melting point: 157-159°C (dec.)

$C_{30}H_{40}O_4S$ (496.71)

IR: ν (cm^{-1}) = 3270 (w, br; -OH), 1677 (s; C=O)

Analysis: Calculated: C: 72.54 H 8.12

Found: C: 72.24 H 7.80

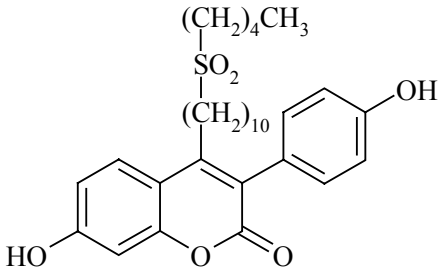
MS: m/z (%) = 496 (85; $M^{+\bullet}$), 425 (100; $[M\text{-}C_5H_{11}]^{+\bullet}$), 394 (41; $[M\text{-}S(CH_2)_5]^{+\bullet}$), 281 (62; $[M\text{-}(CH_2)_8SC_5H_{11}]^{+\bullet}$), 268 (31; $[M\text{-}CH{=}CH_2(CH_2)_7SC_5H_{11}]^{+\bullet}$), 239 (23; $[M\text{-}(CH_2)_9SC_5H_{11}\text{-}CO]^{+\bullet}$)

HRMS: Calculated for $C_{30}H_{40}O_4S$: 496.2641

Found: 496.2641 ± 0.0002

^{1}H-NMR (MeOD-d_4): δ (ppm) = 0.90 (t; 3H; ^{3}J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.19-1.41 (m; 16H; -(C<u>H</u>$_2$)$_2$-CH_3, -(C<u>H</u>$_2$)$_6$-), 1.47-1.61 (m; 6H; =C-CH_2-C<u>H</u>$_2$-, -C<u>H</u>$_2$-CH_2-S-CH_2-C<u>H</u>$_2$-), 2.48 (t; 4H; ^{3}J = 7.3Hz; -C<u>H</u>$_2$-S-C<u>H</u>$_2$-), 2.65 (t; 2H; ^{3}J = 8.1Hz; =C-C<u>H</u>$_2$-), 6.74 (d; 1H; ^{4}J = 2.2Hz; phenyl-<u>H</u>^{8}), 6.84 (dd; 1H; ^{3}J = 8.6Hz, ^{4}J = 2.5Hz; phenyl-<u>H</u>^{6}), 6.86/7.07 (AA'BB'; 4H; ^{3}J = 8.5Hz; phenyl-<u>H</u>), 7.63 (d; 1H; ^{3}J = 9.0Hz; phenyl-<u>H</u>^{5})

7-Hydroxy-3-(4-hydroxyphenyl)-4-[10-(pentylsulfonyl)decyl]-2H-1-benzopyran-2-one (**129**)



Preparation from 7-methoxy-3-(4-methoxyphenyl)-4-[10-(pentylsulfonyl)decyl]-1-benzopyran-2-one **125** (1.2mmol) following the general procedure in section E2.2.6. The reaction mixture was stirred at room temperature for 7h. The crude product was recrystallised from hot ethyl acetate.

Colourless solid; yield: 95%

Melting point: 165-167°C (dec.)

$C_{30}H_{40}O_6S$ (528.70)

IR: ν (cm^{-1}) = 3315 (w, br; -OH), 1678 (s; C=O)

Analysis: Calculated: C: 68.15 H 7.63

Found: C: 68.31 H 7.44

MS: m/z (%) = 528 (100; $M^{+\bullet}$), 394 (28; $[M\text{-}SO_2(CH_2)_5]^{+\bullet}$), 281 (47; $[M\text{-}(CH_2)_8SO_2C_5H_{11}]^{+\bullet}$), 268 (40; $[M\text{-}CH{=}CH_2(CH_2)_7SO_2C_5H_{11}]^{+\bullet}$), 239 (36; $[M\text{-}(CH_2)_9SO_2C_5H_{11}\text{-}CO]^{+\bullet}$)

HRMS: Calculated for $C_{30}H_{40}O_6S$: 528.2544

Found: 528.2544 ± 0.0004

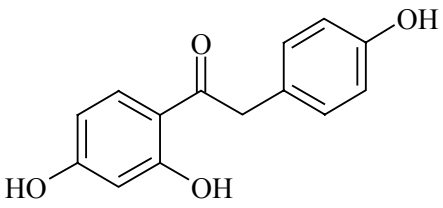
^{1}H-NMR (MeOD-d_4): δ (ppm) = 0.93 (t; 3H; ^{3}J = 7.0Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.18-1.57 (m; 18H; -(C<u>H</u>$_2$)$_2$-CH_3, -(C<u>H</u>$_2$)$_7$-), 1.72-1.84 (m; 4H; -C<u>H</u>$_2$-CH_2-SO_2-CH_2-C<u>H</u>$_2$-), 2.65 (t; 2H; ^{3}J = 8.1Hz; =C-C<u>H</u>$_2$-), 3.05 (t; 4H; ^{3}J = 8.0Hz; -C<u>H</u>$_2$-SO_2-C<u>H</u>$_2$-), 6.74 (d; 1H; ^{4}J = 2.5Hz; phenyl-<u>H</u>^{8}), 6.84 (dd; 1H; ^{3}J = 8.8Hz, ^{4}J = 2.5Hz; phenyl-<u>H</u>^{6}), 6.86/7.07 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 7.63 (d; 1H; ^{3}J = 8.8Hz; phenyl-<u>H</u>^{5})

2.5.2 1-Benzopyrans

2.5.2.1 Preparation from Isoflavanones

2.5.2.1.1 Synthesis from Isoflavanones with Methoxy Protecting Groups

1-(2,4-Dihydroxyphenyl)-2-(4-hydroxyphenyl)ethanone (**130**)



Under nitrogen, resorcinol (100mmol) and 4-hydroxyphenylacetic acid (95mmol) were dissolved into freshly distilled boron triflouride etherate (300mmol) and subsequently heated with stirring to 120°C. The resulting orange mixture was poured onto ice and water (500ml). The precipitated solid was washed with two portions of water and chloroform each and recrystallised from 33% EtOH.

Off-white crystals; yield: 84%

Melting point: 180-182°C

$C_{14}H_{12}O_4$ (244.25)

IR: ν (cm^{-1}) = 3517, 3448 (w, br; -OH),3315 (w; -OH), 1632 (s; C=O)

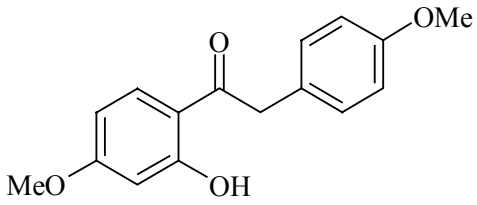
Analysis: Calculated: C: 68.84 H 4.95

C: 64.11 H 5.38 (for $C_{14}H_{12}O_4*H_2O$)

Found: C: 63.93 H 5.74

^{1}H-NMR (DMSO-d_6): δ (ppm) = 4.13 (s; 2H; -C<u>H</u>$_2$-CO-), 6.24 (d; 1H; ^{4}J = 2.3Hz; phenyl-<u>H</u>^{3}), 6.37 (dd; 1H; ^{3}J = 8.8Hz, ^{4}J = 2.3Hz; phenyl-<u>H</u>^{5}), 6.69/7.07 (AA'BB'; 4H; ^{3}J = 8.5Hz; phenyl-<u>H</u>), 7.93 (d; 1H; ^{3}J = 8.9Hz; phenyl-<u>H</u>^{6}), 9.28 (s; 1H; -OH), 10.66 (s; 1H; -OH), 12.60 (s; 1H; -OH)

1-(2-Hydroxy-4-methoxyphenyl)-2-(4-methoxyphenyl)ethanone (**131**)



At 0°C, to a solution of 1-(2,4-dihydroxyphenyl)-2-(4-hydroxyphenyl)ethanone **130** (15.5mmol) and MeOH (15.5mmol) in dry THF (60ml) were added triphenylphosphine (15.5mmol) and DIAD (15.5mmol) and the mixture warmed to room temperature over 2h. The solution was diluted with EtOAc (150ml), washed twice with water and brine (100ml) dried over Na_2SO_4 and concentrated under reduced pressure. Finally, the crude product was purified by column chromatography (SiO_2; EtOAc/petroleum ether 1:5, v/v).

Colourless solid; yield: 83%

Melting point: 98-100°C

$C_{16}H_{16}O_4$ (272.30)

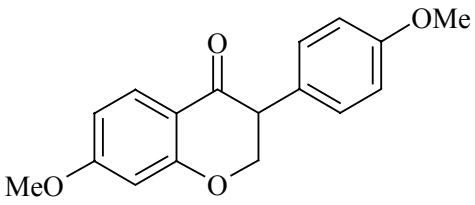
IR: ν (cm^{-1}) = 1633 (s; C=O)

Analysis: Calculated: C: 70.58 H 5.92

Found: C: 70.41 H 5.70

^{1}H-NMR (DMSO-d_6): δ (ppm) = 3.72 (s; 3H; -O-C<u>H</u>$_3$), 3.82 (s; 3H; -O-C<u>H</u>$_3$), 4.26 (s; 2H; -C<u>H</u>$_2$-CO-), 6.48 (d; 1H; ^{4}J = 2.5Hz; phenyl-<u>H</u>^{3}), 6.54 (dd; 1H; ^{3}J = 8.9Hz, ^{4}J = 2.5Hz; phenyl-<u>H</u>^{5}), 6.88/7.21 (AA'BB'; 4H; ^{3}J = 8.6Hz; phenyl-<u>H</u>), 8.02 (d; 1H; ^{3}J = 9.0Hz; phenyl-<u>H</u>^{6}), 12.58 (s; 1H; -OH)

2,3-Dihydro-7-methoxy-3-(4-methoxyphenyl)-4H-1-benzopyran-4-one (**132**)



To a solution of 1-(2-hydroxy-4-methoxyphenyl)-2-(4-methoxyphenyl)ethanone **131** (5.1mmol) and paraformaldehyde (10.2mmol) in EtOH (70ml) was added 40% aqueous dimethylamine (2.0ml) and heated under reflux for 5h. The mixture was concentrated and the remaining residue taken up in water (50ml). Upon acidification with 2N HCl a solid percipitated, that was collected by suction and washed with water.

Colourless solid; yield: 68%

Melting point: 125-127°C

$C_{17}H_{16}O_4$ (284.31)

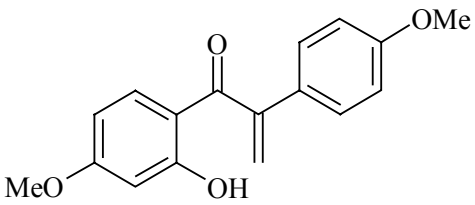
IR: ν (cm^{-1}) = 1674 (s; C=O)

Analysis: Calculated: C: 71.82 H 5.67

Found: C: 71.84 H 5.84

^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.79 (s; 3H; -O-C$\underline{H}_3$), 3.85 (s; 3H; -O-C$\underline{H}_3$), 3.88 (dd; 1H; ^3J = 8.0Hz, ^3J = 5.7Hz; -C$\underline{H}$-), 4.62 (dd; 2H; ^3J = 8.0Hz, ^3J = 5.7Hz; -C$\underline{H}_2$-), 6.44 (d; 1H; ^4J = 2.3Hz; phenyl-$\underline{H}^8$), 6.60 (dd; 1H; ^3J = 8.8Hz, ^4J = 2.4Hz; phenyl-$\underline{H}^6$), 6.88/7.20 (AA'BB'; 4H; ^3J = 8.7Hz; phenyl-$\underline{H}$), 7.89 (d; 1H; ^3J = 8.8Hz; phenyl-$\underline{H}^5$)

1-(2-Hydroxy-4-methoxyphenyl)-2-(4-methoxyphenyl)propenone (**133**)



Intermediate compound that was isolated as main product from the preparation of compound **132**, when only 1 equivalent of secondary amine was used. The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 1:5, v/v).

The compound was quantitatively converted into 2,3-dihydro-7-methoxy-3-(4-methoxy-phenyl)-4H-1-benzopyran-4-one **132** by refluxing an ethanolic solution with 4% aqueous Na_2CO_3 for 0.75h.

Colourless oil; yield: 57%

$C_{17}H_{16}O_4$ (284.31)

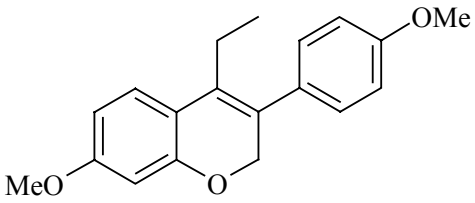
IR: ν (cm^{-1}) = 1625 (s; C=O)

MS: m/z (%) = 284 (68; $M^{+\bullet}$), 269 (9; $[M\text{-}CH_3]^{+\bullet}$), 151 (100; $[MeO(OH)C_6H_3CO]^{+\bullet}$), 133 (15; $[M\text{-}MeOC_6H_5C{=}CH_2]^{+\bullet}$)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.81 (s; 3H; -O-C$\underline{H}_3$), 3.84 (s; 3H; -O-C$\underline{H}_3$), 5.37 (s; 1H; =C$\underline{H}_a$H$_b$), 5.87 (s; 1H; =CH$_a\underline{H}_b$), 6.34 (dd; 1H; ^{3}J = 9.0Hz, ^{4}J = 2.5Hz; phenyl-$\underline{H}^5$), 6.47 (d; 1H; ^{4}J = 2.5Hz; phenyl-$\underline{H}^3$), 6.87/7.35 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-$\underline{H}$), 7.55 (d; 1H; ^{3}J = 9.0Hz; phenyl-$\underline{H}^5$), 12.67 (s; 1H; -OH)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 55.34 (CH_3), 55.67 (CH_3), 100.90 (CH), 107.69 (CH), 113.38 (C), 114.19 (2x CH), 115.66 (=CH_2), 127.75 (2x CH), 129.14 (C), 135.01 (CH), 146.46 (C=), 159.93 (C-O), 166.37 (C-O), 166.62 (C-O), 201.85 (C=O)

4-Ethyl-7-methoxy-3-(4-methoxyphenyl)-2H-1-benzopyran (**134**)



Mg turnings (3.5mmol) and ethylbromide (3.5mmol) in dry THF (6ml) were activated with iodine and subsequently stirred at 50°C for 1h, until all the Mg was converted into the Grin-gard reagent. 2,3-dihydro-7-methoxy-3-(4-methoxyphenyl)-1-benzopyran-4-one **132** was added dropwise and the reaction refluxed for 3h. The mixture was hydrolysed by the addition of an ammonium chloride solution and subsequently the aqueous phase extracted with ethyl acetate. The solvent was washed with water and removed under reduced pressure.

The intermediate tertiary alcohol was dissolved in EtOH (20ml) and heated to reflux for 1h with conc. HCl (0.2ml). The mixture was cooled to room temperature and poured into 5%

$NaHCO_3$ solution. This aqueous phase was extracted again with ethyl acetate (3x 50ml). The combined organic phases were washed with water and brine. After drying over Na_2SO_4 the solvent was evaporated.

The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 1:25, v/v).

Colourless solid; yield: 44%

Melting point: 76-78°C

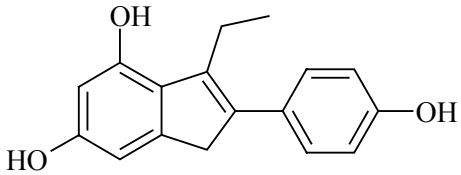
$C_{19}H_{20}O_3$ (296.37)

Analysis: Calculated: C: 77.00 H 6.80

Found: C: 75.45 H 6.49

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.06 (t; 3H; ^{3}J = 7.5Hz; -CH_3), 2.44 (q; 2H; ^{3}J = 7.4Hz; -CH_2-), 3.80 (s; 3H; -O-C$\underline{H}_3$), 3.83 (s; 3H; -O-C$\underline{H}_3$), 4.82 (s; 2H; -O-C$\underline{H}_2$-), 6.45 (d; 1H; ^{4}J = 2.6Hz; phenyl-$\underline{H}^8$), 6.52 (dd; 1H; ^{3}J = 8.5Hz, ^{4}J = 2.6Hz; phenyl-$\underline{H}^6$), 6.92/7.18 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-$\underline{H}$), 7.19 (d; 1H; ^{3}J = 8.5Hz; phenyl-$\underline{H}^5$)

4,6-Dihydroxy-3-ethyl-2-(4-hyroxyphenyl)-1H-indene (**135**)



Preparation from 4-ethyl-7-methoxy-3-(4-methoxyphenyl)-1-benzopyran **134** (0.47mmol) following the general procedure in section E2.2.6. The reaction mixture was stirred at room temperature for 3h. The crude product was purified by column chromatography (SiO_2; ethyl acetate/petroleum ether 1:2, v/v).

Red-brown solid; yield: 48%

Melting point: 180-182°C

$C_{17}H_{16}O_3$ (268.31)

IR: ν (cm^{-1}) = 3475, 3293 (w, br; -OH)

MS: m/z (%) = 286 (80; $M^{+\bullet}$), 253 (9; $[M\text{-}CH_3]^{+\bullet}$), 239 (100; $[M\text{-}CH_2CH_3]^{+\bullet}$)

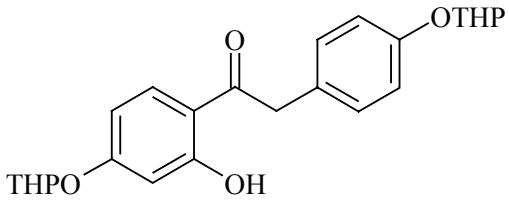
HRMS: Calculated for $C_{17}H_{16}O_3$: 268.1099
Found: 268.1102 ± 0.0002

^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.24 (t; 3H; ^{3}J = 7.3Hz; -CH_3), 2.67 (q; 2H; ^{3}J = 7.1Hz; -CH_2-), 3.49 (s; 2H; -C$\underline{H}_2$-), 6.20 (d; 1H; ^{4}J = 1.9Hz; phenyl-$\underline{H}^5$), 6.36 (d; 1H; ^{4}J = 1.9Hz; phenyl-$\underline{H}^7$), 6.78/7.20 (AA'BB'; 4H; ^{3}J = 8.6Hz; phenyl-$\underline{H}$), 8.99 (s; 1H; -OH), 9.16 (s; 1H; -OH), 9.37 (s; 1H; -OH)

^{13}C-NMR (DMSO-d_6): δ (ppm) = 14.84 (CH_3), 20.56 (CH_2), 40.93 (CH_2), 100.98 (CH), 102.83 (CH), 115.06 (2x CH), 123.97 (C), 128.43 (C), 128.59 (2x CH), 133.66 (C), 138.75 (CH), 145.76 (C), 151.62 (C-O), 155.63 (C-O), 155.85 (C-O)

2.5.2.1.2 Synthesis from Isoflavanones with THP-Ether Protecting Groups

1-[4-(Tetrahydro-2H-pyran2-yloxy)-2-hydroxyphenyl]-2-[4-(tetrahydro-2H-pyran-2-yloxy)-phenyl]ethanone (**136**)



A solution of 1-(2,4-dihydroxyphenyl)-2-(4-hydroxyphenyl)ethanone **130** (10.0mmol), DHP (100mmol) and 4-toluenesulfonic acid monohydrate (0.1mmol) were stirred at 0°C for 3h. The reaction mixture was diluted with diethyl ether (50ml), washed with 5% NaOH, water and brine (25ml each). The solvent was dried over Na_2SO_4 and evaporated. The resulting yellow oil was crystallised from n-hexane.

Colourless solid; yield: 69%
Melting point: 112-114°C
$C_{24}H_{28}O_6$ (412.48)

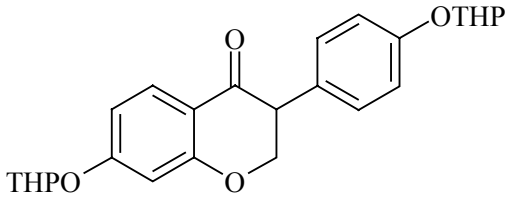
IR: ν (cm^{-1}) = 1633 (s; C=O)

Analysis: Calculated: C: 69.89 H 6.84
Found: C: 69.87 H 6.90

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.57-2.02 (m; 12H; -C$\underline{H}_2$-), 3.55-3.64 (m; 2H; -O-C$\underline{H}_2$-), 3.78-3.94 (m; 2H; -O-C$\underline{H}_2$-), 4.15 (s; 2H; -C$\underline{H}_2$-CO-), 5.39 (t; 1H; ^{3}J =

3.1Hz; -O-C<u>H</u>-O-), 5.48 (t; 1H; ^{3}J = 3.1Hz; -O-C<u>H</u>-O-), 6.55 (dd; 1H; ^{3}J = 8.9Hz, ^{4}J = 2.4Hz; phenyl-<u>H</u>^{5}), 6.62 (d; 1H; ^{4}J = 2.4Hz; phenyl-<u>H</u>^{3}), 7.02/7.17 (AA'BB'; 4H; ^{3}J = 8.6Hz; phenyl-<u>H</u>), 7.76 (d; 1H; ^{3}J = 8.9Hz; phenyl-<u>H</u>^{6}), 12.60 (s; 1H; -OH)

*2,3-Dihydro-7-(tetrahydro-2H-pyran2-yloxy)-3-[4-(tetrahydro-2H-pyran-2-yloxy)phenyl]-4*H*-1-benzopyran-4-one* (**137**)



To a solution of 1-[4-(tetrahydro-2H-pyran-2-yloxy)-2-hydroxyphenyl]-2-[4-(tetrahydro-2H-pyran-2-yloxy)phenyl]ethanone **136** (4.6mmol) and paraformaldehyde (9.2mmol) in EtOH (70ml) was added 40% aqueous dimethylamine (1.8ml) and heated at reflux for 5h. The solvent was reduced to about one third and set aside until crystallization of the product was complete. The solid was collected by suction, washed with 50% aqueous EtOH and dried over P_2O_5.

Colourless solid; yield: 77%

Melting point: 146-149°C

$C_{25}H_{28}O_6$ (412.48)

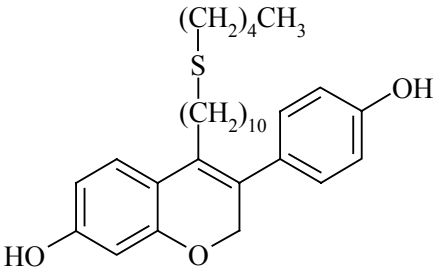
IR: ν (cm^{-1}) = 1676 (s; C=O)

Analysis: Calculated: C: 70.74 H 6.65

Found: C: 70.39 H 6.71

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.57-2.03 (m; 12H; -C<u>H</u>$_2$-), 3.56-3.67 (m; 2H; -O-C<u>H</u>$_2$-), 3.81-3.92 (m; 3H; -O-C<u>H</u>$_2$-, -C<u>H</u>-CO-), 4.59-4,63 (m; 2H; -C<u>H</u>-CH$_2$-CO-), 5.39 (t; 1H; ^{3}J = 3.1Hz; -O-C<u>H</u>-O-), 5.48 (t; 1H; ^{3}J = 3.1Hz; -O-C<u>H</u>-O-), 6.65 (d; 1H; ^{4}J = 2.3Hz; phenyl-<u>H</u>^{8}), 6.71 (dd; 1H; ^{3}J = 8.8Hz, ^{4}J = 2.3Hz; phenyl-<u>H</u>^{6}), 7.02/7.19 (AA'BB'; 4H; ^{3}J = 8.7Hz; phenyl-<u>H</u>), 7.89 (d; 1H; ^{3}J = 8.8Hz; phenyl-<u>H</u>^{5})

7-Hydroxy-3-(4-hydroxyphenyl)-4-[10-(pentylsulfanyl)decyl]-2H-1-benzopyran (**138**)



Preparation from 2,3-dihydro-7-(tetrahydro-2H-pyran2-yloxy)-3-[4-(tetrahydro-2H-pyran-2-yloxy)phenyl]-4H-1-benzopyran-4-one **137** (1.2mmol) and 1-bromo-10-(pentylsulfanyl)-decane **10** (1.2mmol) following the Gringard reaction procedure described for **134**.
The crude product was purified twice by column chromatography (SiO_2; EtOAc/petroleum ether 40-60 1:4 and 1:8, v/v).

Colourless solid; yield: 53%

Melting point: 51-51°C

$C_{30}H_{42}O_3S$ (482.72)

IR: ν (cm^{-1}) = 3340 (w, br; -OH)

Analysis: Calculated: C: 74.65 H 8.77

Found: C: 73.83 H 8.94

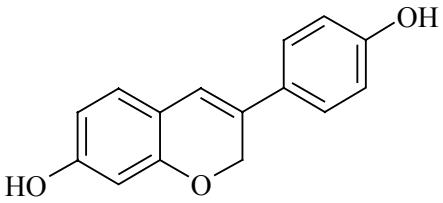
MS: m/z (%) = 482 (12; $M^{+\bullet}$), 239 (100; $[M-(CH_2)_{10}SC_5H_{11}]^{+\bullet}$)

HRMS: Calculated for $C_{30}H_{42}O_3S$: 482.2855

Found: 482.2851 ± 0.0004

^{1}H-NMR (MeOD-d_4): δ (ppm) = 0.91 (t; 3H; ^3J = 7.1Hz; -$(CH_2)_4$-C$\underline{H}_3$), 1.18-1.45 (m; 18H; -(C$\underline{H}_2$)$_2$-CH_3, -(C$\underline{H}_2$)$_7$-), 1.50-1.61 (m; 4H; -C$\underline{H}_2$-CH_2-S-CH_2-C$\underline{H}_2$-), 2.41 (t; 2H; ^3J = 7.5Hz; =C-C$\underline{H}_2$-), 2.48 (t; 4H; ^3J = 7.3Hz; -C$\underline{H}_2$-S-C$\underline{H}_2$-), 4.70 (s; 2H; -O-C$\underline{H}_2$-), 6.27 (d; 1H; ^4J = 2.5Hz; phenyl-$\underline{H}^8$), 6.38 (dd; 1H; ^3J = 8.5Hz, ^4J = 2.5Hz; phenyl-$\underline{H}^6$), 6.80/7.06 (AA'BB'; 4H; ^3J = 8.8Hz; phenyl-$\underline{H}$), 7.08 (d; 1H; ^3J = 8.5Hz; phenyl-$\underline{H}^5$)

7-Hydroxy-3-(4-hydroxyphenyl)-2H-1-benzopyran (**139**)



By-product from the preparation of compound **139**, isolated by column chromatography (SiO_2; EtOAc/petroleum ether 40-60 1:4, v/v) and recrystallised from DCM.

Slighly red solid; yield: 23%

Melting point: 209-212°C (dec.)

$C_{15}H_{12}O_3$ (240.26)

IR: ν (cm^{-1}) = 3395 (w, br; -OH)

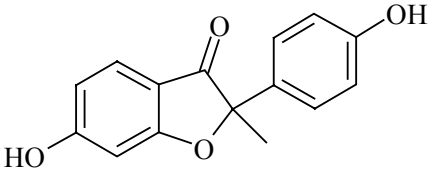
Analysis: Calculated: C: 74.99 H 5.05

Found: C: 74.61 H 4.97

^{1}H-NMR (DMSO-d_6): δ (ppm) = 5.02 (s; 2H; -O-C$\underline{H}_2$-), 6.24 (d; 1H; ^{4}J = 2.2Hz; phenyl-$\underline{H}^8$), 6.33 (dd; 1H; ^{3}J = 8.2Hz, ^{4}J = 2.2Hz; phenyl-$\underline{H}^6$), 6.77 (merged; pyran-$\underline{H}^4$), 6.77/7.33 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-$\underline{H}$), 6.94 (d; 1H; ^{3}J = 8.2Hz; phenyl-$\underline{H}^5$), 9.53 (s; 1H; -O$\underline{H}$), 9.58 (s; 1H; -O$\underline{H}$),

^{13}C-NMR (DMSO-d_6): δ (ppm) = 66.21 (CH_2), 102.23 (CH), 108.47 (CH), 114.69 (C), 115.37 (2x CH), 116.64 (CH), 125.55 (2x CH), 127.07 (C), 127.20 (C), 127.40 (CH), 153.64 (C-O), 156.96 (C-O), 157.96 (C-O)

6-Hydroxy-2-(4-hydroxyphenyl)-2-methylbenzofuran-3-one (**140**)



By-product from the preparation of compound **139**, isolated by column chromatography (SiO_2; EtOAc/petroleum ether 40-60 1:4, v/v) and recrystallised from EtOAc/petroleum ether 40-60.

Colourless solid; yield: 12%

Melting point: 121-123°C

$C_{15}H_{12}O_4$ (256.26)

IR: ν (cm^{-1}) = 3349 (w, br; -OH), 1673 (s; C=O)

Analysis: Calculated: C: 70.31 H 4.72

C: 67.07 H 5.63 (for $C_{15}H_{12}O_4$*0.75 EtOAc)

Found: C: 66.84 H 5.60

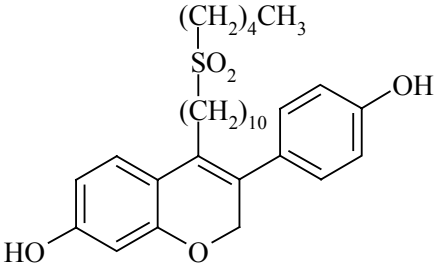
MS: m/z (%) = 256 (100; $M^{+\bullet}$), 241 (36; $[M\text{-}CH_3]^{+\bullet}$), 227 (24; $[M\text{-}CHO]^{+\bullet}$), 137 (46; $[M\text{-}119]^{+\bullet}$), 119 (46; $[M\text{-}137]^{+\bullet}$)

^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.67 (s; 3H; -C<u>H</u>$_3$), 6.57-6.60 (m; 2H; phenyl-<u>H</u>^{5}, phenyl-<u>H</u>^{7}), 6.74/7.20 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 7.46 (d; 1H; ^{3}J = 8.8Hz; phenyl-<u>H</u>^{4}), 9.53 (s; 1H; -O<u>H</u>), 11.00 (s; 1H; -O<u>H</u>)

^{1}H-NMR (MeOD-d_4): δ (ppm) = 1.71 (s; 3H; -C<u>H</u>$_3$), 6.52 (d; 1H; ^{4}J = 1.9Hz; phenyl-<u>H</u>^{7}), 6.59 (dd; 1H; ^{3}J = 8.5Hz, ^{4}J = 1.9Hz; phenyl-<u>H</u>^{5}), 6.74/7.27 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-<u>H</u>), 7.47 (d; 1H; ^{3}J = 8.5Hz; phenyl-<u>H</u>^{4})

^{13}C-NMR (MeOD-d_4): δ (ppm) = 22.91 (CH_3), 89.64 (C), 97.97 (CH), 110.40 (C), 112.08 (CH), 115.10 (2x CH), 125.95 (2x CH), 126.21 (CH), 128.54 (C), 157.17 (C-O), 167.33 (C-O), 172.93 (C-O), 198.36 (C=O)

7-Hydroxy-3-(4-hydroxyphenyl)-4-[10-(pentylsulfony)decyl]-2H-1-benzopyran (**141**)



Preparation from 7-hydroxy-3-(4-hydroxyphenyl)-4-[10-(pentylsulfanyl)decyl]-2H-1-benzopyran **138** (0.23mmol) following the general procedure in section E2.2.2.4. The reaction was carried out in dry THF and after addition of m-CPBA the reaction mixture was stirred for 30min at room temperature. The crude product was purified by column chromatography (SiO_2; ethyl acetate/DCM 1:10, v/v).

Colourless oil; yield: 76%

$C_{30}H_{42}O_5S$ (514.72)

IR: ν (cm^{-1}) = 3390 (w, br; -OH)

MS: m/z (%) = 514 (25; $M^{+\bullet}$), 239 (100; $[M-(CH_2)_{10}SO_2C_5H_{11}]^{+\bullet}$)

HRMS: Calculated for $C_{30}H_{42}O_5S$: 514.2753

Found: 514.2748 ± 0.0004

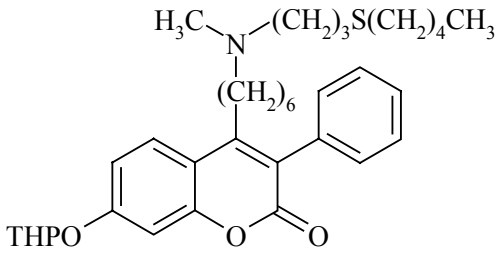
^{1}H-NMR (MeOD-d_4): δ (ppm) = 0.94 (t; 3H; ^{3}J = 7.1Hz; -$(CH_2)_4$-C<u>H</u>$_3$), 1.18-1.47 (m; 18H; -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_7$-), 1.71-1.84 (m; 4H;-C<u>H</u>$_2$-CH_2-SO_2-CH_2-C<u>H</u>$_2$-), 2.42 (t; 2H; ^{3}J = 7.5Hz; =C-C<u>H</u>$_2$-), 3.01-3.07 (m; 4H;-C<u>H</u>$_2$-SO_2-C<u>H</u>$_2$-), 4.70 (s; 2H; -O-C<u>H</u>$_2$), 6.27 (d; 1H; ^{4}J = 2.5Hz; phenyl-<u>H</u>^{8}), 6.38 (dd; 1H; ^{3}J = 8.8Hz, ^{4}J = 2.5Hz; phenyl-<u>H</u>^{6}), 6.80/7.06 (AA'BB'; 4H; ^{3}J = 8.5Hz; phenyl-<u>H</u>), 7.08 (d; 1H; ^{3}J = 8.5Hz; phenyl-<u>H</u>^{5})

2.5.2.2 Preparation from Coumarins

2.5.2.2.1 THP-Protection of the Phenolic Hydroxy Groups

A THF solution of the unprotected 1-benzopyran-2-one **126** or **127** (1eq), DHP (10eq) and *para*-toluenesulfonic acid monohydrate (1.1eq) was stirred at room temperature overnight. The mixture was diluted with EtOAc and washed with 5% $NaHCO_3$, water and brine. The solvent was dried over Na_2SO_4 and removed under reduced pressure.

7-(Tetrahydro-2H-pyran-2-yloxy)-4-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-3-phenyl-2H-1-benzopyran-2-one (**142**)



Preparation from 7-hydroxy-4-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-3-phenyl-2H-1-benzopyran-2-one **126** (1.01mmol). The crude yellow oil was chromatographed (SiO_2; DCM/MeOH 20:1, v/v).

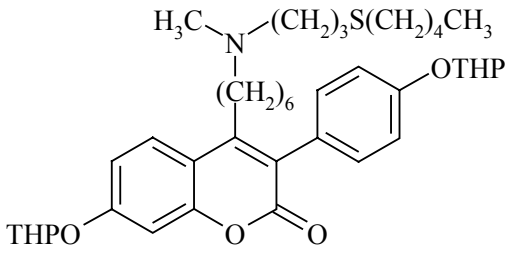
Yellow oil; yield: 80%

$C_{35}H_{49}NO_4S$ (579.84)

IR: ν (cm^{-1}) = 1718 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^{3}J = 7.1Hz; -$(CH_2)_4$-C$\underline{H}_3$), 1.16-1.93 (m; 22H; -$(C\underline{H}_2)_3$-CH_3, -$(C\underline{H}_2)_4$-CH_2-N-, -N-CH_2-C$\underline{H}_2$-CH_2-S-, -$(C\underline{H}_2)_3$-CHO-), 2.20 (s; 3H; -N-C$\underline{H}_3$), 2.28 (t; 2H; ^{3}J = 7.3Hz; -N-C$\underline{H}_2$-), 2.42 (t; 2H; ^{3}J = 7.3Hz; -N-C$\underline{H}_2$-), 2.50 (t; 2H; ^{3}J = 7.3Hz; -S-C$\underline{H}_2$-), 2.52 (t; 2H; ^{3}J = 7.3Hz; -S-C$\underline{H}_2$-), 2.60 (t; 2H; ^{3}J = 8.1Hz; =C-C$\underline{H}_2$-), 3.61-3.67/3.82-3.93 (m; 2H; -O-C$\underline{H}_2$-), 5.50 (t; 1H; ^{3}J = 3.0Hz; -O-CH-O-), 7.01 (dd; 1H; ^{3}J = 8.8Hz, ^{4}J = 2.5Hz; phenyl-$\underline{H}^6$), 7.08 (d; 1H; ^{4}J = 2.5Hz; phenyl-$\underline{H}^8$), 7.25-7.28 (m; 2H; phenyl-$\underline{H}$), 7.36-7.48 (m; 3H; phenyl-$\underline{H}$), 7.55 (d; 1H; ^{3}J = 8.8Hz; phenyl-$\underline{H}^5$)

7-(Tetrahydro-2H-pyran-2-yloxy)-3-[4-(tetrahydro-2H-pyran-2-yloxy)phenyl]-4-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-2H-1-benzopyran-2-one (**143**)



Preparation from 7-hydroxy-3-(4-hydroxyphenyl)-4-{6-{N-methyl-N-[3-(pentylsulfanyl)-propyl]amino}hexyl}-2H-1-benzopyran-2-one **127** (0.98mmol). The crude yellow oil was chromatographed (SiO_2; DCM/MeOH 15:1, v/v).

Yellow oil; yield: 75%

$C_{40}H_{57}NO_6S$ (679.96)

IR: ν (cm^{-1}) = 1717 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^{3}J = 7.1Hz; -$(CH_2)_4$-C$\underline{H}_3$), 1.16-1.94 (m; 28H; -$(C\underline{H}_2)_3$-CH_3, -$(C\underline{H}_2)_4$-CH_2-N-, -N-CH_2-C$\underline{H}_2$-CH_2-S-, 2x -$(C\underline{H}_2)_3$-CHO-), 2.23 (s; 3H; -N-C$\underline{H}_3$), 2.32 (t; 2H; ^{3}J = 7.3Hz; -N-C$\underline{H}_2$-), 2.49-2.54 (m; 6H; -N-C$\underline{H}_2$-, -C$\underline{H}_2$-S-C$\underline{H}_2$-), 2.62 (t; 2H; ^{3}J = 7.8Hz; =C-C$\underline{H}_2$-), 3.62-3.67 (m; 2H; -O-C$\underline{H}_2$-), 3.82-3.93 (m; 2H; -O-C$\underline{H}_2$-), 5.45 (t; 1H; ^{3}J = 3.0Hz; -O-CH-O-), 5.50 (t; 1H; ^{3}J = 3.0Hz; -O-CH-O-), 7.00 (dd; 1H; ^{3}J = 8.8Hz, ^{4}J = 2.5Hz; phenyl-$\underline{H}^6$), 7.07 (d; 1H; ^{4}J =

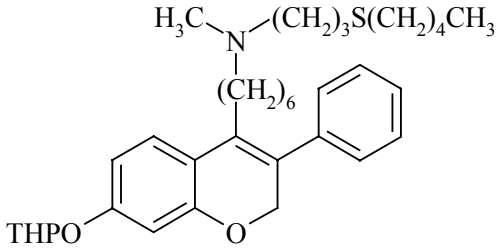
2.5Hz; phenyl-H^8), 7.12/7.18 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-H), 7.54 (d; 1H; ^{3}J = 8.8Hz; phenyl-H^5)

2.5.2.2.2 Reduction and Recyclisation

The THP-protected 1-benzopyran-2-ones **142** and **143** (1eq) were reduced with $LiAlH_4$ within 30min stirring at room temperature, according to the procedure used for preparation of compound **4a**.

The resulting crude diols (1eq) and triphenylphosphine (1.5eq) were dissolved in dry THF (8ml) and DIAD (1.5eq) was added at room temperature. The reaction was kept strirring for 24h. The mixture was diluted with EtOAc and washed with water and brine. The solvent was dried over Na_2SO_4 and removed *in vacuo*.

7-(Tetrahydro-2H-pyran-2-yloxy)-4-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-3-phenyl-2H-1-benzopyran (**144**)



Preparation from 7-(tetrahydro-2H-pyran-2-yloxy)-4-{6-{N-methyl-N-[3-(pentylsulfanyl)-propyl]amino}hexyl}-3-phenyl-2H-1-benzopyran-2-one **142** (0.77mmol). The crude product was purified by column chromatography (SiO_2; DCM/MeOH 15:1, v/v).

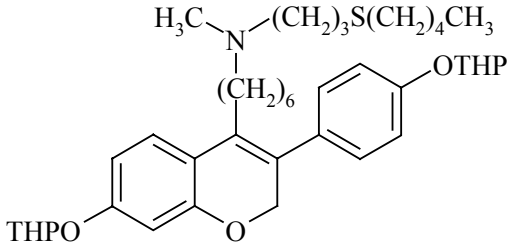
Colourless oil; yield: 63%

$C_{35}H_{51}NO_3S$ (565.86)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^{3}J = 7.1Hz; -(CH$_2$)$_4$-CH$_3$), 1.15-2.06 (m; 22H; -(CH$_2$)$_3$-CH$_3$, -(CH$_2$)$_4$-CH$_2$-N-, -N-CH$_2$-CH$_2$-CH$_2$-S-, -(CH$_2$)$_3$-CHO-), 2.24 (s; 3H; -N-CH$_3$), 2.32 (t; 2H; ^{3}J = 7.3Hz; -N-CH$_2$-), 2.40 (t; 2H; ^{3}J = 7.3Hz; -N-CH$_2$-), 2.47-2.55 (m; 6H; -CH$_2$-S-CH$_2$-, =C-CH$_2$-), 3.58-3.65/3.88-3.96 (m; 2H; -O-CH$_2$-), 4.83 (s; 2H; -O-CH$_2$), 5.41 (t; 1H; ^{3}J = 3.2Hz; -O-CH-O-), 6.62 (d; 1H; ^{4}J = 2.5Hz; phenyl-H^8), 6.66 (dd; 1H; ^{3}J = 8.5Hz, ^{4}J = 2.5Hz; phenyl-H^6), 7.16 (d; 1H; ^{3}J = 8.8Hz;

phenyl-H^5), 7.22-7.32 (m; 3H; phenyl-H), 7.38-7.44 (m; 2H; phenyl-H)

7-(Tetrahydro-2H-pyran-2-yloxy)-3-[4-(tetrahydro-2H-pyran-2-yloxy)phenyl]-4-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-2H-1-benzopyran (**145**)



Preparation from 7-(tetrahydro-2H-pyran-2-yloxy)-3-[4-(tetrahydro-2H-pyran-2-yloxy)-phenyl]-4-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-2H-1-benzopyran-2-one **143** (0.74mmol). The crude product was purified by column chromatography (SiO_2; DCM/MeOH 20:1, v/v).

Colourless oil; yield: 61%

$C_{40}H_{59}NO_5S$ (665.97)

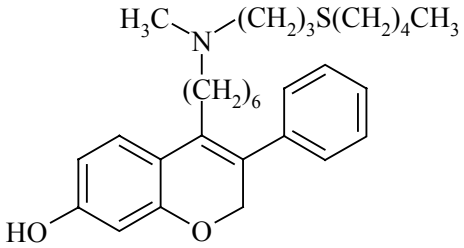
^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.89 (t; 3H; ^{3}J = 7.0Hz; -(CH$_2$)$_4$-CH$_3$), 1.15-2.06 (m; 28H; -(CH$_2$)$_3$-CH$_3$, -(CH$_2$)$_4$-CH$_2$-N-, -N-CH$_2$-CH$_2$-CH$_2$-S-, 2x -(CH$_2$)$_3$-CHO-), 2.27 (s; 3H; -N-CH$_3$), 2.38-2.56 (m; 6H; -CH$_2$-N-CH$_2$-, -CH$_2$-S-CH$_2$-, =C-CH$_2$-), 3.60-3.66 (m; 2H; -O-CH$_2$-), 3.83-4.00 (m; 2H; -O-CH$_2$-), 4.83 (s; 2H; -O-CH$_2$), 5.40 (t; 1H; ^{3}J = 3.2Hz; -O-CH-O-), 5.44 (t; 1H; ^{3}J = 3.2Hz; -O-CH-O-), 6.61 (d; 1H; ^{4}J = 2.5Hz; phenyl-H^8), 6.65 (dd; 1H; ^{3}J = 8.2Hz, ^{4}J = 2.5Hz; phenyl-H^6), 7.06/7.15 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-H), 7.14 (d; 1H; ^{3}J = 8.2Hz; phenyl-H^5)

2.5.2.2.3 Deprotection of the Phenolic Hydroxy Groups

The THP-protected 1-benzopyrans **144** and **145** were dissolved in MeOH (10ml). *para*-Toluenesulfonic acid (1.1eq) was added and the mixture stirred at room temperature for 1hr.

The mixture was diluted with EtOAc and washed with 5% $NaHCO_3$, water and brine The solvent was dried over Na_2SO_4 evaporated.

7-Hydroxy-4-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-3-phenyl-2H-1-benzopyran (**146**)



Preparation from 7-(tetrahydro-2H-pyran-2-yloxy)-4-{6-{N-methyl-N-[3-(pentylsulfanyl)-propyl]amino}hexyl}-3-phenyl-2H-1-benzopyran **144** (0.30mmol). The crude product was purified by column chromatography (SiO_2; DCM/MeOH 10:1, v/v).

Yellow oil; yield: 90%

$C_{30}H_{43}NO_2S$ (481.74)

IR: ν (cm^{-1}) = 3400-2600 (w, br; -OH)

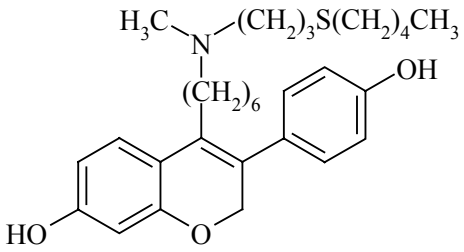
MS: m/z (%) = 481 (100; $M^{+\bullet}$), 350 (97; $[M\text{-}(CH_2)_2SC_5H_{11}]^{+\bullet}$), 188 (74; $[CH_2{=}N(CH_3)(CH_2)_3SC_5H_{11}]^{+\bullet}$)

HRMS: Calculated for $C_{30}H_{43}O_2S$: 481.3015

Found: 481.3012 ± 0.0002

^{1}H-NMR (MeOD-d_4): δ (ppm) = 0.90 (t; 3H; ^3J = 7.1Hz; -$(CH_2)_4$-C$\underline{H}_3$), 1.15-1.48 (m; 12H; -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_4$-CH_2-S-), 1.57 (quin; 2H; ^3J = 7.5Hz; -C$\underline{H}_2$-CH_2-S-), 1.77 (quin; 2H; ^3J = 7.4Hz; -N-CH_2-C$\underline{H}_2$-CH_2-S-), 2.30 (s; 3H; -N-C$\underline{H}_3$), 2.40 (t; 2H; ^3J = 7.8Hz; -N-C$\underline{H}_2$-), 2.44 (t; 2H; ^3J = 7.8Hz; -N-C$\underline{H}_2$-), 2.51 (t; 2H; ^3J = 7.3Hz; -S-C$\underline{H}_2$-), 2.52 (t; 2H; ^3J = 7.3Hz; -S-C$\underline{H}_2$-), 2.57 (t; 2H; ^3J = 7.8Hz; =C-C$\underline{H}_2$-), 4.74 (s; 2H; -O-CH_2-), 6.29 (d; 1H; ^4J = 2.5Hz; phenyl-$\underline{H}^8$), 6.40 (dd; 1H; ^3J = 8.5Hz, ^4J = 2.5Hz; phenyl-$\underline{H}^6$), 7.12 (d; 1H; ^3J = 8.5Hz; phenyl-$\underline{H}^5$), 7.23-7.32 (m; 3H; phenyl-$\underline{H}$), 7.37-7.42 (m; 2H; phenyl-$\underline{H}$)

7-Hydroxy-3-(4-hydroxyphenyl)-4-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-2H-1-benzopyran (**147**)



Preparation from 7-(tetrahydro-2H-pyran-2-yloxy)-3-[4-(tetrahydro-2H-pyran-2-yloxy)-phenyl]-4-{6-{N-methyl-N-[3-(pentylsulfanyl)propyl]amino}hexyl}-2H-1-benzopyran **145** (0.37mmol). The crude product was purified by column chromatography (SiO_2; DCM/MeOH 9:1, v/v).

Slighly red solid; yield: 81%

Melting point: 45-47°C

$C_{30}H_{43}NO_3S$ (497.74)

IR: ν (cm^{-1}) = 3400-2600 (w, br; -OH)

MS: m/z (%) = 497 (91; $M^{+\bullet}$), 366 (87; $[M-(CH_2)_2SC_5H_{11}]^{+\bullet}$), 188 (90; $[CH_2=N(CH_3)(CH_2)_3SC_5H_{11}]^{+\bullet}$)

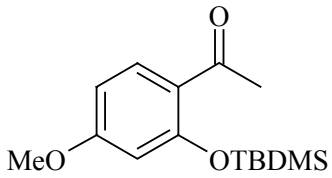
HRMS: Calculated for $C_{30}H_{43}O_3S$: 497.2964

Found: 497.2965 ± 0.0003

^{1}H-NMR (MeOD-d_4): δ (ppm) = 0.91 (t; 3H; ^3J = 7.0Hz; -$(CH_2)_4$-C$\underline{H}_3$), 1.15-1.48 (m; 12H; -$(C\underline{H}_2)_2$-CH_3, -$(C\underline{H}_2)_4$-CH_2-S-), 1.57 (quin; 2H; ^3J = 7.2Hz; -C$\underline{H}_2$-CH_2-S-), 1.78 (quin; 2H; ^3J = 7.5Hz; -N-CH_2-C$\underline{H}_2$-CH_2-S-), 2.34 (s; 3H; -N-C$\underline{H}_3$), 2.42-2.55 (m; 8H; -C$\underline{H}_2$-N-C$\underline{H}_2$-, -C$\underline{H}_2$-S-C$\underline{H}_2$-), 2.62 (t; 2H; ^3J = 7.8Hz; =C-C$\underline{H}_2$-), 4.70 (s; 2H; -O-CH_2-), 6.27 (d; 1H; ^4J = 2.5Hz; phenyl-$\underline{H}^8$), 6.37 (dd; 1H; ^3J = 8.2Hz, ^4J = 2.5Hz; phenyl-$\underline{H}^6$), 6.80/7.07 (AA'BB'; 4H; ^3J = 8.5Hz; phenyl-$\underline{H}$), 7.09 (d; 1H; ^3J = 8.2Hz; phenyl-$\underline{H}^5$)

2.5.3 Synthesis of 2-Phenyl-Substituted 1-Benzopyran-4-ones

2-(tert-*Butyldimethylsiloxy)-4-methoxyacetophenone* (**148**)



At 0°C, TBDMSCl (3.3mmol) in dry DCM (8ml) was added to a solution of 2-hydroxy-4-methoxyacetophenone (3.0mmol), triethylamine (4.5mmol) and DMAP (0.30mmol) in dry DCM (10ml). The mixture was stirred at this temperature for 0.5h and then at room temperature overnight (12h). The mixture was poured onto ice-water and the aequeous phase was extracted with DCM (3x20ml). Then, the combined organic extracts were washed with water and brine (20ml each). The solvent was dried over Na_2SO_4 and evaporated.
Purification of the crude product was achieved by column chromatography (neutral Al_2O_3; EtOAc/petroleum ether 40-60 1:9, v/v).

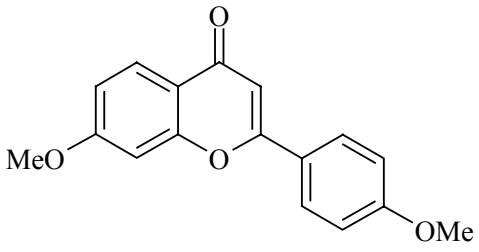
Colourless oil; yield: 82%

$C_{15}H_{24}O_3Si$ (280.45)

IR: ν (cm^{-1}) = 1668 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.30 (s; 6H; -Si-C$\underline{H}_3$), 1.01 (s; 9H; -Si-C(-C$\underline{H}_3$)), 2.57 (s; 3H; -CO-C$\underline{H}_3$), 3.84 (s; 3H; -O-C$\underline{H}_3$), 6.37 (d; 1H; ^{4}J = 2.4Hz; phenyl-$\underline{H}^3$), 6.54 (dd; 1H; ^{3}J = 8.8Hz, ^{4}J = 2.4Hz; phenyl-$\underline{H}^5$), 7.71 (d; 1H; ^{3}J = 8.8Hz; phenyl-$\underline{H}^6$)

7-Methoxy-2-(4-methoxyphenyl)-4H-1-benzopyran-4-one (**149**)



Under nitrogen at –78°C, 2-(*tert*-butyldimethylsiloxy)-4-methoxyacetophenone **148** (2.5mmol) in dry THF (10ml) was added dropwise to a solution of LDA (5.3 mmol) in dry

THF (10ml) and the resulting solution stirred at –25°C for 1h. The reaction mixture was cooled to –78°C again and anisic acid chloride (2.6mmol) in dry THF (10ml) added. After stirring for 3h with gradually warming to –20°C the mixture was partitioned between water and ethyl acetate (50ml each) and brought to pH5 with 2N HCl. The organic layer was separated, washed with water and brine, dried over Na_2SO_4. The solvent was removed under reduced pressure to give a viscous residue of 1-[2-(*tert*-butyldimethylsiloxy)-4-methoxyphenyl]-3-(4-methoxyphenyl)-propan-1,3-dione.

The crude dione was mixed with 0.5% H_2SO_4 in glacial acetic acid (10ml) and heated at 90°C for 0.5h. After cooling, the mixture was poured into sat. sodium bicarbonate solution and the aqueous phase extracted with chloroform (3x20ml). The combinded organic extracts were washed with sat. $NaHCO_3$, water and brine. The solvent was over Na_2SO_4 dried and evaporated.

The resulting red oil was purified by column chromatography (SiO_2; EtOAc/petroleum ether 40-60 1:1, v/v) and finally recrystallised from EtOAc.

Grey needles; yield: 75%

Melting point: 145-146°C; (lit: 146-148°C [Ismail and Aziem, 2001])

$C_{17}H_{14}O_4$ (282.30)

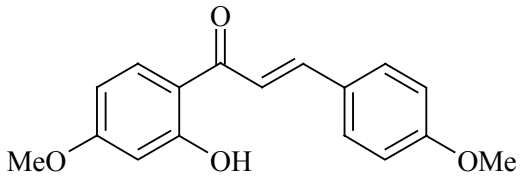
IR: ν (cm^{-1}) = 1642 (s; C=O)

Analysis: Calculated: C: 72.33 H 5.00

Found: C: 72.07 H 5.02

^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.89 (s; 3H; -O-CH_3), 3.93 (s; 3H; -O-CH_3), 6.69 (s; 1H; -CO-CH-), 6.95-7.00 (m; 2H; phenyl-$\underline{H}^6$, phenyl-$\underline{H}^8$), 7.02/8.13 (AA'BB'; 4H; ^{3}J = 9.0Hz; phenyl-$\underline{H}$), 7.87 (d; 1H; ^{3}J = 8.9Hz; phenyl-$\underline{H}^5$)

1-(2-Hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)propenone (**150**)



A ethanolic solution (50ml) of 2-hydroxy-4-methoxyacetophenone (6.0mmol) and anisaldehye (12.0mmol) was added with aqueous KOH (9g) and stirred at room temperature for 24h.

With ice-cooling the mixture was acidified with conc. HCl. Upon dilution with water precipitation occurred. The collected precipitate was washed with water and recrystallised from EtOH.

Yellow needles; yield: 58%

Melting point: 103-105°C

$C_{17}H_{16}O_4$ (284.31)

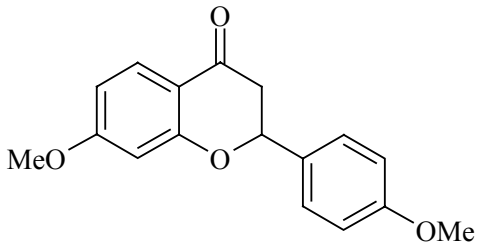
IR: ν (cm^{-1}) = 1626 (s; C=O)

Analysis: Calculated: C: 71.82 H 5.67

Found: C: 71.77 H 5.40

^{1}H-NMR (DMSO-d_6): δ (ppm) = 3.84 (s; 3H; -O-CH_3), 3.85 (s; 3H; -O-CH_3), 6.52 (s; 1H; ^{4}J = 2.4Hz; phenyl-H^3), 6.57 (dd; 1H; ^{3}J = 8.8Hz; ^{4}J = 2.5Hz; phenyl-H^5), 7.04/7.90 (AA'BB'; 4H; ^{3}J = 8.8Hz; phenyl-H), 7.81 (d; 1H; ^{3}J = 15.4Hz; =CH-), 7.90 (d; 1H; ^{3}J = 15.4Hz; =CH-), 8.28 (d; 1H; ^{3}J = 9.0Hz; phenyl-H^6)

2,3-Dihydro-7-methoxy-2-(4-methoxyphenyl)-4H-1-benzopyran-4-one (**151**)



An ethanolic solution (15ml) of chalcone **150** (1.0mmol) was either refluxed with excess 10% H_2SO_4 or triethylamine (1.0mmol) overnight. The reaction mixture was neutralized by pouring into sat. $NaHCO_3$ solution or aqueous HCl, respectively, and the product extracted with ethyl acetate from the aqueous phase. The combined organic phase were washed with water and brine. The solvent was dried over Na_2SO_4 and removed *in vacuo*.

The product was separated from the starting material by column chromatography (SiO_2; toluene/diethyl ether 25:1, v/v).

Yellow solid; yield: 60%

Melting point: 77-79°C

$C_{17}H_{16}O_4$ (284.31)

IR: ν (cm^{-1}) = 1671 (s; C=O)

^{1}H-NMR (DMSO-d_6): δ (ppm) = 2.69 (dd; 1H; ^{2}J = 16.8Hz; ^{3}J = 3.0Hz; -CO-C$\underline{H}_a$H$_b$-), 3.20 (dd; 1H; ^{2}J = 16.8Hz; ^{3}J = 12.9Hz; -CO-CH$_a\underline{H}_b$-), 3.84 (s; 3H; -O-CH$_3$), 3.85 (s; 3H; -O-CH$_3$), 5.57 (dd; 1H; ^{3}J = 12.9Hz; ^{3}J = 3.0Hz; -CH$_2$-C$\underline{H}$-), 6.61 (d; 1H; ^{4}J = 2.4Hz; phenyl-$\underline{H}^8$), 6.66 (dd; 1H; ^{3}J = 8.7Hz; ^{4}J = 2.4Hz; phenyl-$\underline{H}^6$), 6.98/7.47 (AA'BB'; 4H; ^{3}J = 8.7Hz; phenyl-$\underline{H}$), 7.72 (d; 1H; ^{3}J = 8.7Hz; phenyl-$\underline{H}^5$)

3 Biological and Pharmacological Methods

3.1 Radiometric Binding Assay

3.1.1 With Calf Uterus Cytosol

3.1.1.1 Preparation of the Cytosol

The calf uteri excised from freshly butchered animals were placed in ice-cold physiological NaCl-solution and packed on ice during transportation.

All of the following procedures were carried out at +4°C. The uteri were freed from fat tissue, parametrium and perimetrium, cut up endways and washed with 0.9% NaCl-solution to get rid of any residual blood and mucus. With scissors, they were cut into small pieces, covered with TED-Mo-buffer (1ml per 1g of uterus) and homogenized by means of an Ultraturrax (three to five times for about 10 sec). To remove coarse tissue components the homogenate was centrifuged at 6000 x g for 15 minutes. The resulting supernatant was centrifuged once more at 105000 x g for 100 minutes. The clear cytosol was obtained by gentle pipetting (to avoid contamination with fat floating on the surface of the cytosol) and stored at –70°C immediately, if not needed for the experiment.

The protein concentration of the cytosol was determined according to the method of Bradford (cf. section E3.3.3.3) and was usually about 20mg/ml. For the experiment the cytosol was diluted with Tris-buffer (pH 7.5) to a final concentration of 5mg/ml.

3.1.1.2 Preparation of the DCC Suspension

A suspension of 8g of charcoal (Norit A) and 100ml of Tris-buffer (pH 7.4) was stirred at 0°C for 4 hours and equilibrated at +4°C overnight. On the next day any charcoal particles on the surface were removed by suction. Then, 80mg of dextran 60 was added and stirred at 0°C for another 15 minutes. The resulting DCC suspension is stored at +4°C.

3.1.1.3 The Binding Experiment with Calf Uterus Cytosol

Each compound was tested twice (independently) in six different concentrations. For each concentration the experiment was performed in triplicate. For each experiment 17ß-estradiol was used as standard and 4,4'-hexestrol (RBA = 77 ± 16; n = 20) as reference to guarantee identical conditions for independent experiments.
The binding assay was performed according to scheme E1 using 1.5ml Eppendorf cups, that are constantly kept in an ice-bath during pipetting and work-up:

solutions	**background [µl]**	**control [µl]**	**sample [µl]**
Tris-buffer (pH 7.5)	200	300	n[a]
inhibitor	----	----	300 - n[a]
cytosol	100	100	100
17ß-estradiol[b], $2x10^{-5}$ mol/l	100	----	----
[^{3}H]-17ß-estradiol[b], $1x10^{-8}$ mol/l[c]	100	100	100

Scheme E1: Pipetting scheme for binding assay with cytosol

[a] n is a definite volume in µl to make the total volume 500µl
[b] solution in Tris-buffer
[c] giving a final concentration of 2nM of [^{3}H]-17ß-estradiol at a total volume of 500µl

All the reaction vessels were vortexed and incubated 18 – 20hr at +4°C with gentle shaking. Excess of ligands and unbound [^{3}H]-17ß-estradiol were removed by DCC treatment, following the recommendation of EORTC [1973]. 500µl of this DCC suspension was added to each vial, incubated at +4°C for 2 hours and finally centrifuged at +4°C at 1500 x g for 10 minutes. 100µl of the supernatant was transferred into a scintillation vial supplemented with 3ml of scintillation liquid and counted for radioactivity.

3.1.1.4 Determination of Relative Binding Affinities

The radioactivity of the receptor bound [^{3}H]-17ß-estradiol was counted with a liquid scintillation counter. For the determination of the maximum radioactivity the mean value for the background radioactivity was subtracted from the radioactivity measured in the control experiment and set to 100%:
For each concentration the average of the three experiments was calculated, reduced by the background radioactivity and put in relation to the maximum radioactivity to obtain values for the bound radioactivity. Bound radioactivity was plotted against the logarithm of the respective concentrations and thereof the IC_{50}-value determined.
For estimation of the relative binding affinities (RBA) the IC_{50}-values were compared with that of the physiological ligand 17β-estradiol. By definition the RBA-value for 17β-estradiol is set to100.

$$RBA = \frac{IC_{50}\ [17\beta\text{-estradiol}]}{IC_{50}\ [\text{test compound}]} \times 100$$

3.1.1 With Recombinant Receptor Proteins

3.1.2.1 Preparation of the Receptor Proteins

The recombinant full-length human receptor proteins ERα and ERβ were delivered as a solution in ER binding buffer. In order to prevent many freeze and thaw circles the whole protein solution was aliquoted into 1.5ml Eppendorf cups in portions of 10µl and stored at –80°C.
Prior to use an aliquots of ERα (1800nM) and ERβ (3500nM), respectively, were diluted up to 1ml with ER binding buffer by gentle pipetting (1:100, v/v). Another dilution step (gentle pipetting, no vortexing to prevent protein aggregation!!) with ER binding buffer (1:20, v/v) provided the receptor concentrations that were used in the binding assay (ERα: 0.9nM, ERβ: 1.75nM).

3.1.2.2 Preparation of the HAP Slurry

10g of HAP was mixed vigorously with 60mL of HAP equilibration buffer. After at least 10 minutes the supernatant was decanted and the process repeated ten more times using 60mL of HAP equilibration buffer. The HAP slurry was equilibrated at +4°C overnight before adjusted to 50% (v/v) with HAP equilibration buffer. At +4°C the slurry is stable for several month.

3.1.2.3 The Binding Experiment with the Recombinant Receptors

Each compound was tested twice (independently) in six different concentrations. For each concentration the experiment was performed in triplicate. For each experiment 17ß-estradiol was used as standard and 4,4'-hexestrol (RBA = 20 ± 5; n = 20) as reference to guarantee identical conditions for independent experiments.
The binding assay was performed according to scheme E2 using 1.5ml Eppendorf cups, that are constantly kept in an ice-bath during pipetting and work-up:

solutions	**background [µl]**	**control [µl]**	**sample [µl]**
ER binding buffer	----	50	n[a]
inhibitor	----	----	50 - n[a]
receptor protein[b]	100	100	100
17ß-estradiol[c], $2x10^{-5}$ mol/l	50	----	----
[^{3}H]-17ß-estradiol[c], $8x10^{-9}$ mol/l[d]	50	50	50

Scheme E2: Pipetting scheme for binding assay with recombinant receptor proteins

[a] n is definite volume in µl to make the total volume 200µl
[b] giving final protein concentrations of 0.45nM for ERα and 0.875nM for ERβ at a total volume of 200µl
[c] solution in ER binding buffer
[d] giving a final concentration of 2nM of [^{3}H]-17ß-estradiol at a total volume of 200µl

All the reaction vessels were vortexed and incubated 18 – 20hr at +4°C with gentle shaking. To bind the ligand-receptor-complex 100µl of HAP slurry was added to each vial and vortexed three times within 15 minutes. After the addition of 1ml of ER wash buffer the vial were vortexed and centrifuged in a microcentrifuge at 3000 x g at +4°C for 10min and the supernatant discarded. This washing step was repeated twice. After the last wash the HAP

pellet was resuspended in 400µl of EtOH supplemented with 3ml of scintillation fluid and counted for tritium activity.

3.1.2.4 Determination of Relative Binding Affinities

For determination of the relative binding affinities to both recombinant receptors ERα and ERβ compare section E3.1.1.4

3.2 Proliferation Assay with Human Mammary Carcinoma Cell Lines

3.2.1 Human Breast Cancer Cell Lines

For the determination of antiproliferative activity two different human breast cancer cell lines were used:

- The hormone-dependent MCF-7 cell line was applied to demonstrate an estrogen receptor mediated action of test compounds.
- The hormone-independent MDA-MB-231 cell line was applied to determine non-specific, cytostatic or cytotoxic effects of test compounds.

3.2.2 Preparation of Cell Medium and Stripped FCS

3.2.2.1 Preparation of Cell Medium

The MCF-7 cell line was grown in phenol red containing MEM Eagles's medium supplemented with 2.2g of sodium bicarbonate, 110mg of sodium pyruvate and 50mg of gentamycin per liter of deionised water. The medium was sterilized through a 0.2µm membrane filter and stored at +4°C. Prior to usage 10vol% of sterile FCS or ct-FCS (charcoal-treated FCS; cf. section E3.2.2.2) was added to the medium.
The MDA-MB-231 cell line was cultivated in phenol red containing McCoy's 5A medium supplemented with 2.2g of sodium bicarbonate, 72.8mg of L-glutamine and 50mg of gentamycin per liter of deionized water. The medium was sterilized through a 0.2µm membrane filter and stored at +4°C. Prior to usage 5vol% of sterile FCS was added to the medium.

3.2.2.2 Preparation of Stripped Fetal Calf Serum

For the experiment with MCF-7 cells (cf. section E3.2.5.1) the FCS was replaced steroid-depleted FCS (ct-FCS):
A suspension of 5.0g of charcoal (Norit A) and 100ml of Tris-buffer (pH 7.4) was stirred at 0°C for 4 hours and equilibrated at +4°C overnight. At the next day any charcoal particles on the surface were removed by suction. Then, 50mg of dextran 60 was added and stirred at 0°C for another 15 minutes. This charcoal suspension was split into two equal parts and centrifuged at 1500 x g at 0 – 4°C for 15min. The supernatant was discarded. One charcoal pellet was added to 250ml of FCS, stirred at 0°C for 3 hours and finally centrifuged at 6000 x g at +4°C for 15min. The supernatant serum was decanted onto the second charcoal pellet. It was incubated with stirring at 56°C for 1h to reach complete inactivation of the serum, centrifuged again at 6000 x g for 15min and filtered through a 0.2µm membrane filter for sterilisation. The ct-FCS was stored at –20°C

3.2.3 Freezing and Thawing of Cells

Both cell lines should be stored in liquid nitrogen in a storage medium containing 10% of sterile DMSO as cryoprotectant, plus 80% of EMEM and 10% of FCS for the MCF-7 cells or 85% of McCoy's medium and 5% of FCS for the MDA-MB-231 cells.
Confluently grown cells were trypsinated (cf. section E3.2.4) and suspended in 5ml of storage medium per 75cm^2 culture flask. One milliliter of this cell suspension was pipetted into sterile plastic pasteur pipettes, sealed by melting and put on ice. After 30 minutes the ampoules were frozen in a cryostat to a final temperature of –140°C with a cooling rate of 1.5°C per minute. Alternatively, the ampoules can be frozen in an upright position in a polystyrene box, which is placed at –70°C overnight or until they have frozen to bellow –50°C, and then they are immediately transferred to liquid nitrogen for rapid cooling. The ampoules are stored in liquid nitrogen at –196°C.
In general, when viable cells are frozen the formation of ice crystals inside and outside the cells is a critical issue. Ice crystal formation in the extracellular matrix may cause mechanical damage to the cell membrane, whereas intracellular ice crystals, that are exclusively formed of water, can lead to a dehydration of the cell and create osmotic pressure within the cell. This effect can also be responsible for cell damage or cell death. This can be avoided by adding a

cryoprotective such as DMSO in 7-10% final concentration to the storage medium. Additionally, the cells must be frozen very slowly between the temperature range of 0 to –50°C and rapidly to the final temperature of -140°C.
Cells, that are removed from liquid nitrogen storage must be thawed rapidly to ensure maximum survival. First, the ampoules were plunged into a beaker with water at 37°C until they were completely defrosted, followed by 70% aqueous EtOH for 5 minutes for sterilisation. With a broad canula the cell suspension was transferred into a sterile centrifuge tube, using a second canula for pressure compensation. After addition of 10ml of the respective medium the cell suspension was centrifuged at 100xg for 10 minutes and the supernatant liquid decanted. The cell pellet was redissolved in fresh medium and transferred into 75 cm^2 culture flasks.

3.2.4 Cultivation of Cell Lines

Both cell lines grow as monolayer in 75 cm^2 culture flasks in a humidified, 5% CO_2-containing atmosphere at 37°C. Depending on cell density and growth the respective medium was replaced after 3-7 days.
Cell adhesion to specially treated culture ware occurs because extracellular proteins, so called glycoproteins or proteoglycans, are bound to the cell surface. The simplest way to remove adherent cells is to cut those adhesion molecules by the addition of proteolytic enzymes, often in combination with divalent cation chelators, leaving the cells themselves intact and free to float in the medium.
This was accomplished by treatment with 5ml of a trypsine/EDTA solution after the culture medium had been removed by suction. After an incubation period of one minute, when the cells begin to detach from the flask surface, the trypsine/EDTA solution was removed together with any dead cell material. The remaining intact cells were taken up in new culture medium to give a single-cell suspension. This suspension was diluted (15-20 fold) and used either in cell assays or transferred to other culture flasks, where they started to grow as monolayer within few hours again.

3.2.5 Determination of Antiproliferative Activity in a Microculture Assay

3.2.5.1 Cell Plating and Addition of Test Compounds

Both cell lines were plated shortly before confluence. The old medium was removed, the cells trypsinated and a single-cell suspension prepared (cf. section E3.2.4) with either 10% ct-FCS supplemented EMEM for the MCF-7 cell line or 5% FCS supplemented McCoy's 5A medium for the MDA-MB-231 cell line. The cells were plated (100µl/well) in 96-well microtitration plates at an approximate density of 15 (MDA-MB-231) or 20 cells (MCF-7) per microscopic field (320-fold magnification). After 48 hours the medium was carefully removed by suction and replaced by fresh medium (200µl/well) containing different concentrations of test compounds (16 or 24 wells/plate), added as a 1000-fold concentrated DMF based stock solution. All experiments with MCF-7 cells were performed in the presence of 17β-estradiol at a final concentration of 1nM. Control wells (16/plate) contained pure medium with 0.1vol% of DMF (MDA-MB-231) or 0.1vol% of DMF with additional 1nM E2 (MCF-7). DMF at this concentration has no influence on the growth characteristics of both cell lines [Bernhardt et al., 1992].
After drug addition the initial cell density was determined by addition of glutardialdehyde solution for fixation (cf section E3.2.5.2) to one untreated plate. This plate was stored with PBS at +4°C until staining.

3.2.5.2 Fixation and Determination of the Cell Density

After an incubation for 4 (MDA-MB-231) to 10 days (MCF-7) or until the control wells had reached confluency, the medium was carefully shaken off and each of the 96 wells treated with 100µl of glutardialdehyde solution (1% in PBS) for 25 minutes. The fixative was decanted. The cells were either stored under PBS (200µl/well) in the refrigerator or stained with 100µl of an 0.02% aqueous solution of crystal violet for 25min. After decanting, adherent dye was removed by repeated washings (three times) with water and incubation with water (20min) after the last washing step. Subsequently, 150µl of 70% EtOH was added and the plates gently shaken at ambient temperature for 2 – 3 hours.
The optical density of each well was measured at 578 nm using an microplate autoreader. The evaluation of the data and the calculation of significance by Wilcoxon's U-test [Mann and Whitney, 1947] were performed by a computer program [Birnböck, 1988].

Antiproliferative effects of the tested drugs were expressed as corrected T/C values for each group according to:

$$T/C_{corr.}\ [\%] = [(T^* - C_0) / (C^* - C_0)] \times 100$$

T^*: Mean absorbance of treated cells
C_0: Mean absorbance of untreated cells
C^*: Mean absorbance of cell in the control group

3.3 Luziferase Assay

3.3.1 The MCF-7/2a Cell Line

The MCF-7/2a cell line is an estrogen receptor positive MCF-7 subcell line, that was stably transfected with the luciferase reporter plasmid 'EREwtc luc' and applied to determine estrogenic and antiestrogenic activities of test compounds *in vitro*.

3.3.2 Cultivation of the MCF-7/2a Cell Line

The MCF-7/2a cell line was cultivated in Dulbecco's modified eagle medium (DMEM) without phenol red, supplemented with 5vol% sterile FCS (25ml), 5.3ml of a sterile PBS-solution containing streptomycin sulphate (10mg/ml) and penicillin-G (6mg/ml) and 5.0ml of a solution of L-glutamine (29.2mg/ml in sterile PBS) per 500ml of medium.
Since the MCF-7/2a cells carry the gene for neomycin resistance the cells were grown in the presence of neomycin (Geneticin®, G-418; 175µg/ml medium added from a 35mg/ml solution in sterile PBS).
The incubation condition for the MCF-7/2a cell line was equivalent to those described for the MCF-7 or MDA-MB-231 cells (cf. section E3.2.4). They grow as monolayer in 75 cm^2 culture flasks in a humidified, 5% CO_2-containing atmosphere at 37°C.
Shortly before the cells had grown confluent, the medium was removed and the cells were washed with 10ml of PBS. Then, the cells were treated with 2.5ml of a trypsin/EDTA solution. After a short incubation period the trypsin was removed by suction together with any dead cell material, the remaining intact cells taken up in 10ml of fresh medium and seed as diluted (1:20) single-cell suspension into a new cultured flasks.

3.3.3 Determination of Estrogenic and Antiestrogenic Activity in MCF-7/2a Cells

3.3.3.1 Cell Plating and Addition of Test Compounds

Nine days before the start of the experiment, the cells were cultivated with medium supplemented with 5vol% of sterile ct-FCS. At the start of the experiment 0.5ml of a single-cell suspension was added to 2.0ml of fresh culture medium in six-well plates. The plates were incubated for at least 20-24h or until the cells reached a density of about 40%.
The luciferase assay was performed in triplicate for each drug concentration in two independent experiments. 5µl of a 500-fold concentrated DMF based stock solution of test compound was added alone for the determination of agonistic effects or in combination with E2 at a final concentration of 1nM for the determination of an antagonistic drug action. Additionally, a negative and a positive control experiment was performed for each assay. For the positive control experiment 5µl of an E2 stock solution at a final concentration of 1nM with or without 5µl of additional DMF (p.a.) was added to 3 wells and for the negative control experiment 5 or 10µl of DMF (p.a.) was added to 3 wells, depending on the determination of agonistic or antagonistic effects.

3.3.3.2 Cell Harvest and Luminescence Measurement

After an incubation period of 50h, the culture medium was removed and the cells washed twice with PBS (2ml/well). “Cell lysis buffer” as part of Promega’s luciferase-assay kit was diluted with water (1:5) and added to each well (175µl/well). After incubating for 20min at room temperature the cells were collected with a rubber policeman, transferred into 1.5ml Eppendorf cups and centrifuged at 6000 x g for 10min. The cell pellets were discarded and the supernatant either stored at –20°C or used for the luminescence measurement.
The luciferase activity was assayed using the Promega’s luciferase-assay kit according to the manufactor’s protocol. The luciferase substrate and the appropriate buffer are usually stored at –70°C. After defrosting they were mixed and equilibrated at ambient temperature. 30µl of the above cell extract were transferred into polystyrene tubes and the luminescence measured in a luminometer after automatic injection of 100µl of freshly prepared assay solution. Luminescence was integrated over 10s and the result given in relative light unit (RLU). RLU can be converted into the amount of expressed luciferase according to:

log [mass luciferase in pg] = (log [RLU] - 5.1607) / 0.866325

This linear correlation between luminescence (RLU/10s) and the amount of luciferase was calculated for triplicated samples from *Photinus pyralis* in the range from 0.03fg to 100pg of enzyme with 3000RLU referring to 10fg luciferase. The background was approximately 250RLU/10s [Koop, 1992].

3.3.3.3 Bradford's Protein Assay

The results of the luminescence measurement were corrected for the total protein content of each sample, which was quantified by Bradford's protein assay [1976].
5µl of each cell extract and 95µl of deionised water were pipetted in polystyrene cuvettes followed by the addition of 1ml of Bradford reagent (5-fold concentrate diluted with water). After 10min at room temperature the absorbance of each samples was measured in UV-spectrophotometer at 595nm over 3s.
For each assay a calibration curve was determined using a HSA-solution in a concentration of 1.0µg/µl. Increasing amounts of this HSA-solution (1µg, 2µg, 4µg, 6µg, 8µg and 10µg) were added to 5µl of cell lysis buffer, whose protein content had to be taken into account, and filled up with deionised water to the final volume of 100µl. After adding 1ml of Bradford reagent the samples were measured against 5µl of cell lysis buffer in 95µl of water as described above. Protein contents up to 15mg per sample show a linear correlation between absorbance and protein concentration.
Luciferase activity was given as percent ratio of RLU to total protein concentration. 17β-Estradiol at a concentration of 1nM was used as reference estrogen (=100%). IC_{50}-values were calculated from dose-response curves.

3.4 Mouse Uterus Weight Test

The uterus weight test provides information on estrogenic (uterotrophic) and antiestrogenic (antiuterotrophic) activity *in vivo* by the determination of the uterus growth in immature mice.

3.4.1 Uterotrophic Test

Immature female NMRI-mice at the age of about 18 to 20 days and with an average body weight of approximately 10g were randomly divided into groups of at least 6 (test group) to 10 animals (control group).

The test compound were pretreated with 5vol% of DMF (p.a.) and subsequently dissolved in olive oil. For stability reason these solutions were stored at –20°C and only defrosted for application. On three consecutive days 100µl of the drug solution was injected subcutaneously into each animal of the test group. Animals of the negative control group received the vehicle alone (5vol% of DMF in olive oil), whereas animals of the positive control group were treated with a daily dose of 17β-estradiol at a concentration of 0.01mg/kg body weight.

Twenty-four hours after the last injection the animals were killed by cervival dislocation and weighed. Uteri were excised, fixed in Bouin solution for 3 hours and freed from fat and connective tissue. Finally the uteri were washed with EtOH and dried at 100°C for 18h.

The relative uterus weight is the ratio of uterine dry weight (mg) to body weight of the respective animal (g), multiplied by 100.

The estrogenic effect of a test compound was calculated according to:

$$\text{Estrogenic effect [\%]} = \frac{(W_T - W_0)}{(W_S - W_0)} \times 100$$

W_T: Relative uterus weight of animals treated with test compound
W_0: Relative uterus weight of untreated animals
W_S: Relative uterus weight of animals treated with the standard

3.4.2 Antiuterotrophic Test

The antiuterotrophic test was performed in analogous manner to the uterotrophic test (cf. section E3.4.1) except that the daily injections for the test group contained increasing concentrations of test compound in combination with the 17β-estradiol standard (0.01mg/kg body weight).

$$\text{Antiestrogenic effect [\%]} = \left[1 - \frac{(W_{T,S} - W_0)}{(W_S - W_0)}\right] \times 100$$

$W_{T,S}$: Relative uterus weight of animals treated with a combination test compound and standard
W_0: Relative uterus weight of untreated animals
W_S: Relative uterus weight of animals treated with the standard alone

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G Appendix

The following report summarises the results of an independent project, that developed from June to September 2004 in the research group of Dr. Mary J. Meegan at the Trinity College Dublin in Ireland.

1 The Objectives of the Project

In this study we aimed to investigate specific structural requirements of drug-like molecules for optimum estrogen receptor binding. The study of novel potent drug candidates coupled with elucidation of the mode of action will advance our understanding of and ability to combat hormone-dependent breast cancer.

The main objective of this short-term project is to synthesise a library of structurally related fixed-ring, benzothiepins, analogues of previously synthesised compounds containing a modified tamoxifen-type triarylethylene pharmacophore with potential application as selective estrogen receptor modulators (partial antiestrogens).

We wish to investigate the tolerance of the estrogen receptor towards conformational restraint in heterocyclic structurally varied antagonist species and to define the optimum structural features for ER binding and antiproliferative activity. Therefore, a radiometric binding assay applying the recombinant full-length human ERα and ERβ should be set up. The synthesised compounds should be evaluated in terms of their affinity and selectivity for one of the two subtypes. We wonder if it is possible to express the ligand binding domains of ERα and ERβ in E. coli bacteria and to apply those proteins in the radiometric binding assay.

The biological activity of these estrogen receptor modulators should be fully characterised through extensive *in vitro* investigations.

2 Synthesis of SERMs Based on the Benzo[b]thiepine Scaffold

The main part of this project comprised the elaboration of a synthetic route to a new series of benzo[b]thiepine-based selective estrogen receptor modulators (SERMs). These compounds

are structurally related to previously synthesised fixed-ring analogues with a modified tamoxifen-like structure (cf. figure G1) [McCague et al., 1986 and 1988; Hughes, 2000].

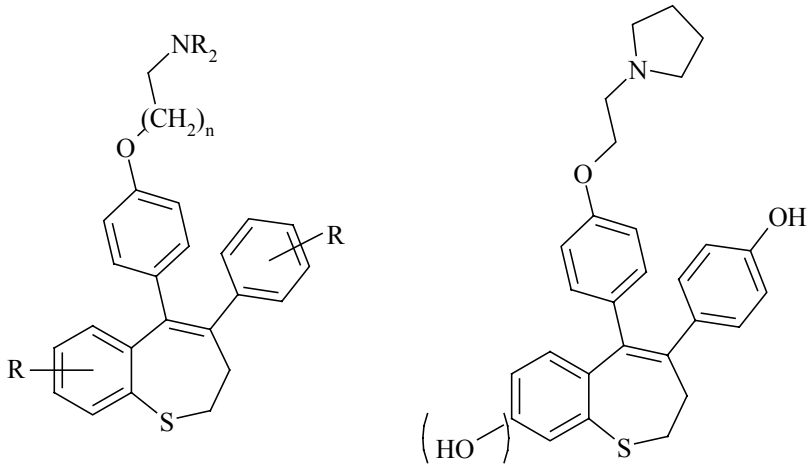


Figure G1: Structural skeleton and the synthetic prototype of benzo[b]thiepine-based SERMs

A hydroxy or methoxy group should be introduced into position 7 or 8 of the benzothiepine core and/or in position 3' or 4' of the arylring. Also a number of basic side chains with varying length (n = 1,2) and different substitution pattern at the nitrogen atom including open-chain structures such as two methyl or ethyl groups or cyclic structures such as pyrrolidine, piperidine or morpholine should be investigated. A synthetic prototype with a 4-hydroxyphenyl substituent in position 4 and a pyrrolidinylethyl side chain (n = 1) was chosen for the synthesis. Additionally, this compound should be substituted with a hydroxy or methoxy group in position 8 of the benzothiepine scaffold (cf. figure G1).

The synthesis of these triarylethylene-related compounds started from phenylsulfanylbutyric acid **1** or the 3-methoxy analogue **3**, respectively, which were prepared by a S_N2 reaction of the respective aromatic thiol with ethyl bromobutyrate and subsequent alkaline hydrolysis of the intermediate esters. In order to increase the nucleophilicity of the sulfur atom the thiols were deprotonated with K_2CO_3. The benzothiepine ring was constructed by cyclodehydration of **1** and **3** with polyphosphoric acid to give the benzothiepinones **4** and **5** (cf. figure G2) [Bindra et al., 1975; Traynelis and Love, 1961].

For the introduction of the basic side chain many different procedures are reported in the literature [Foster et al., 1985; McCague et al., 1986 and 1988; Renaud et al., 2003; Robertson and Katzenellenbogen, 1982; Runitz et al., 1982]. The first attempt was the preparation of the side chain **6** by nucleophilic substitution of 4-bromophenol and 2-chloroethylpyrrolidine prior to the introduction into the benzothiepine heterocycle by a Grignard reaction (cf. figure G2).

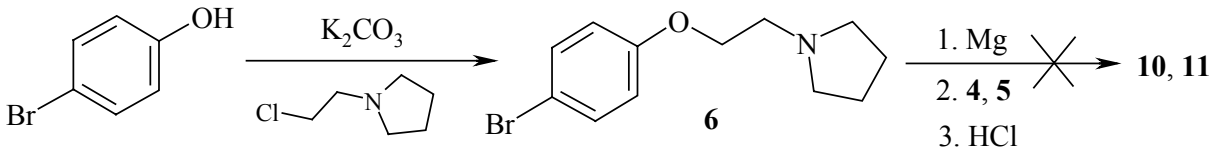


Figure G2: Grignard reaction to introduce the basic side chain

Unfortunately, this strategy was not successful, either because the Grignard reagent did not react with the carbonyl group of **4** or the Grignard reagent was not formed at all. The latter seemed to be very likely, because even at reflux temperature in THF and with the addition of iodine or ethylene bromide the turbidity usually going along with the formation of Grignard reagents was not observed.

The conversion of compound **6** into the Li derivative to introduce the complete side chain, was not tried. Instead, the THP-protected bromophenol **7** was lithiated with n-BuLi, reacted with the corresponding thiepinones **4** and **5**. The resulting tertiary alcohols were dehydrated under acidic conditions to give the free phenols **8** and **9** in good yield. The unconverted benzothiepinones could be recovered by column chromatography almost completely. A better conversion by this reaction might be achieved when an excess of the lithiated compound **7** is used. Finally, the side chain was completed by alkylation of **8** and **9** with 2-chloroethylpyrrolidine in the presence of K_2CO_3.

The action of pyridiniumhydrobromide perbromide ($PyHBr_3$) afforded the compounds **12** and **13** in good yield. Solid $PyHBr_3$ is a mild brominating agent and easier to handle than elemental bromine. The only drawback of the reaction with $PyHBr_3$ was the tedious separation of the pyridine from the desired product, which was finally achieved by column chromatography. Alternatively, in order to prevent this difficult purification step, a bromination utilizing NBS and catalytic amounts of AIBN was tried. The by-product of this reaction is succinimide, which is soluble in water and thus easy to separate. Concerning the expected bromination the reaction worked well and introduced a bromine atom in position 4 of the benzothiepine core, but probably due to the radical mechanism of this reaction the complete basic side chain was cleaved at the phenolic oxygen.

The brominated olefins **12** and **13** were used in a Suzuki reaction in order to introduce the third aryl ring. Following standard protocols [Hughes, 2000; Greenfield, 2003] with $Pd(PPh_3)_4$ as the catalyst, the desired triarylethylenes **14** and **15** were obtained in moderate yields after purification by column chromatography and recrystallisation. The recrystallisation process trapped 30% of DCM in both compounds as found by NMR and elemental analysis.

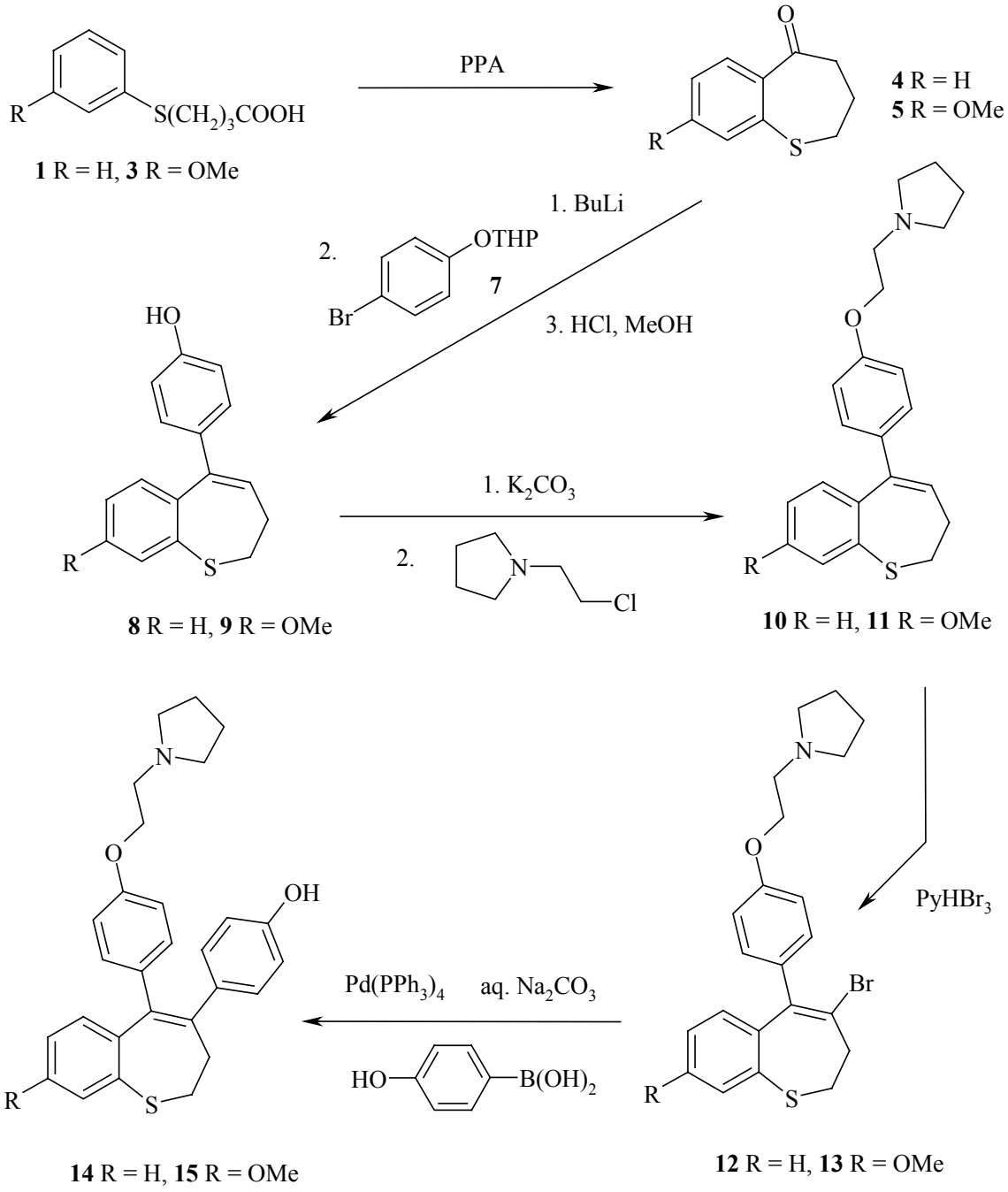


Figure G3: Synthetic pathway to benzothiepine-based antiestrogenes

The last step of the synthesis, the demethylation of compound **15** (cf. figure G4) turned out to be the most difficult reaction. Due to two quite similar aryl alkyl ether functions in the molecule a method was needed to cleave selectively only the methyl ether. Scientist with Eli Lilly [Jones et al., 1984] and recently Katzenellenbogen and coworkers [Stauffer et al., 2001] reported (partial) cleavage of the basic side chain using BBr_3, but the milder combination of $AlCl_3$ and EtSH proved to be successful on many occasions [Jones et al., 1984; Stauffer et al., 2001; Grese et al., 1997; Mortensen et al., 2001]. However, the action of this reagent resulted

in opening of the benzothiepine ring at the sulphur atom among the formation of other not identified by-products.

In the literature there are many procedure reported that gave good results with BBr_3 [Stauffer et al., 2001; Wallace et al., 2004; Kim et al., 2003]. Therefore, the demethylation with BBr_3 was attempted. An ecxcess of BBr_3 was added to the free base of substrate **15** at room temperature, which successfully cleaved the methoxy group and kept the side chain intact, but also lead to almost complete bromination in position 7 of the benzothiepine heterocycle. Performing the same reaction at 0°C lead only to the isolation of unconverted starting material, which must have precipitated prior to reaction with BBr_3. Finally, when the substrate **15** was added to a highly diluted solution of BBr_3 in DCM and the temperature kept at 0°C throughout the course of the reaction, only the desired biphenolic compound **16** and the unconverted starting material were obtained. Both compounds were separated by column chromatography. The demethylation leading to **16** was conducted just on a very small scale and has to be repeated with the remaining free base of compound **15** to yield larger quantities for the complete chemical characterisation.

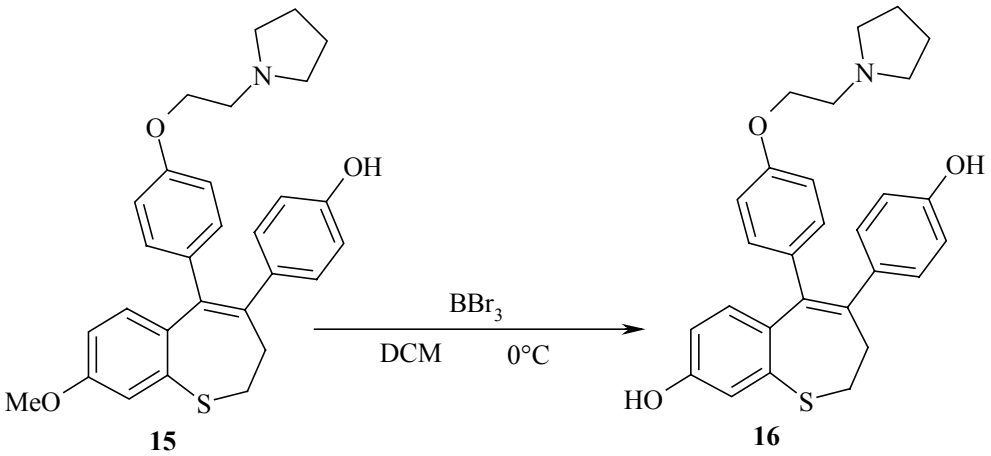


Figure G4: Deprotection of the phenolic methoxy group

The developed synthesis opens the door for the preparation of a library of various 4,5-diaryl-2,3-dihydro-benzo[b]thiepines, which provides the basis for a detailed investigation of the structure-activity relationship of this type of compounds in the context of ER modulation.

3 Biochemistry

3.1 Isolation and Purification of the Human ER LBD

The LBD incorporating the ligand binding site and the transcription activation function AF-2 is the essential part of the ER to study the interactions between ligand and receptor. There are studies reported in the literature that describe the expression of the LBD of ERα and ERβ in E. coli bacteria using a pET-15b vector (cf. figure G5) [Carlson et al., 1997; Henke et al., 2002]

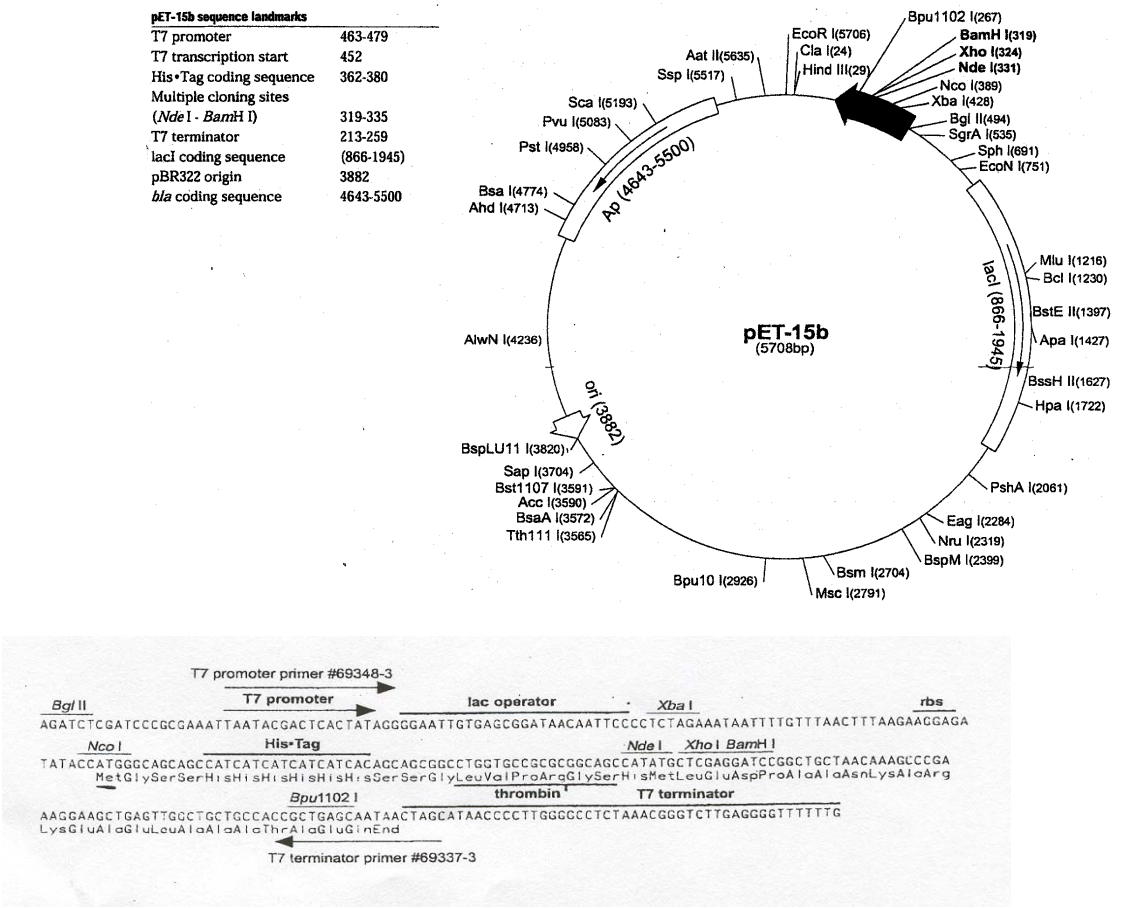


Figure G5: pET-15b vector and definite cloning/expression regions

The pET-15b vector possesses a T7 cloning/expression region (T7 promoter and T7 terminator) that is transcribed by the T7 RNA polymerase. Furthermore, it is equipped with a *lac* operator to regulate transcription, a N-terminal 6x-histidine purification tag sequence, a thrombin cleavage site and three restriction sites (*Nde* I, *Xho* I, *BamH* I) for the insertion of definite coding strands.

The DNA encoding the LBD (amino acids 256-505 for ERα and 204-454 for ERβ) of the human ERs were cloned into the cloning sites of the pET-15b vector. Both plasmids were kindly provided by Dr. David Lloyd, Department of Biology, Trinity College Dublin.

3.1.1 Protein Expression

The DNA constructs for the expression of the recombinant proteins were transformed into the E. coli bacteria BL21(DE3). The plasmids were incubated on ice with the E. coli cells to attach the DNA to the cell membrane, followed by shock-heating to reach uptake of the DNA into the cells. This method is not very efficient, but widely used to introduce some of the plasmids into the desired host cells. A separation from non-transformed cells was achieved by growing cell colonies on agarose-plates that were supplemented with ampicillin.

Ampicillin is an unstable antibiotic, which is rapidly depleted by transformed and, thus, resistant bacterial cells due to β-lactamase secretion. It was supplemented without exception to all of the following amplification steps, because transformed E. coli bacteria tend to liberate the foreign DNA strands during mitosis and, thus, loose the resistance towards ampicillin. Antibiotic conditions are necessary, because low concentrations of transformed cells can lead to the expression of only low levels of target protein. Low-level expression can also occur due to the toxicity or unstability of the recombinant proteins, but none of these aspects are known for the ER proteins.

The extremely high transcription rate initiated by the T7 promoter can only be efficiently regulated and repressed by high levels of the *lac* repressor protein. After the cell culture had been grown to an optimal cell concentration (OD_{600} = 0.5 - 0.7) the expression of the recombinant proteins was induced by the addition of IPTG, which binds to the *lac* operator and inactivates it. Once the repressor protein is inactivated, the RNA polymerase of E. coli can transcribe the sequences downstream from the promoter. The transcripts produced are subsequently translated into the recombinant proteins.

After the expression process the medium was removed and the cells taken up in lysis buffer. This concentrated cell suspension was frozen overnight to initiate the break-up of the cells by ice-crystal formation. Sonication of the defrosted cell suspension supported the break-up process and liberated the target protein.

The result of the protein expression was analysed by SDS-PAGE, which showed that both ER proteins (~29kDa) were expressed in high quantities and that no basal transcription of the desired proteins had occurred. The ERα protein was found almost completely in the soluble

fraction, whereas substantial quantities of ERβ were found in the insoluble fraction, probably due to the formation of insoluble inclusion bodies. The intermolecular association of hydrophobic domains during protein folding is believed to play a role in the formation of inclusion bodies. For proteins with many cysteine residues improper formation of disulfide bonds in the reducing environment of the E. coli cytoplasm may also contribute to incorrect folding and formation of inclusion bodies [Quiagen Ltd., 2001]

Insoluble inclusion bodies can be easily dissolved by protein denaturation, purified under these conditions and finally refolded to obtain the functionally active protein. However, the rate of recovery of active protein is often poor by this method. Alternatively, the change of the expression conditions leads to better results. The temperature at which the cells are grown often affect both the expression level and protein solubility. At lower temperatures the expression level can be reduced which leads to a higher amounts of soluble protein. Furthermore, the cells can be grown to a higher concentration before the induction and the expression period kept to a minimum. A lower concentration of IPTG (~0.005M final concentration) can also reduce the expression level [Quiagen Ltd., 2001].

3.1.2 Protein Purification

Immobilised-metal affinity chromatography was first applied in 1975 to purify proteins using the chelating ligand iminodiacetic acid (IDA, cf. figure G6) [Porath et al., 1975]. IDA has only three metal-chelating sites and cannot bind metal ions such as Zn^{2+}, Cu^{2+}, or Ni^{2+} very tightly. Weak binding leads to ion dissociation upon loading with strongly chelating proteins and peptides or during the washing procedure, which results on low yields, impure products and metal-ion contamination of isolated proteins [Quiagen Ltd., 2001].

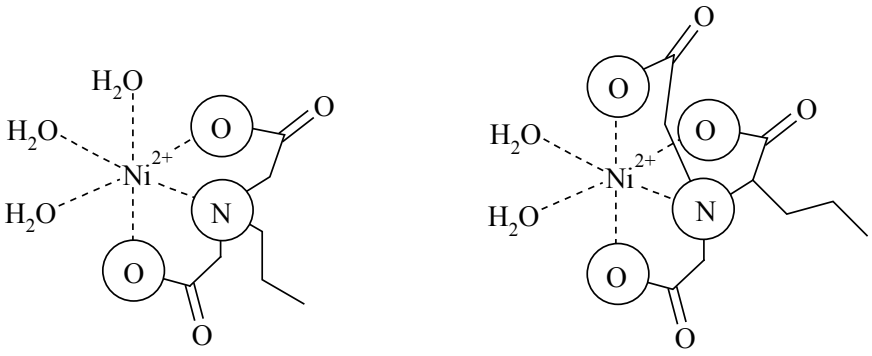


Figure G6: Comparison of Ni-IDA and Ni-NTA

Nitrilotriacetic acid (NTA, cf. figure G6) is a tetradentate chelating adsorbent that overcomes these problems. It binds metal ions more stably than IDA and retains the ions under a wide variety of conditions, especially under strong wash conditions. NTA occupies four of the six ligand binding sites in the coordination sphere of the metal ion, leaving two sites free for the interaction with the 6xHis affinity tag (cf. figure G7) [Porath et al., 1975].

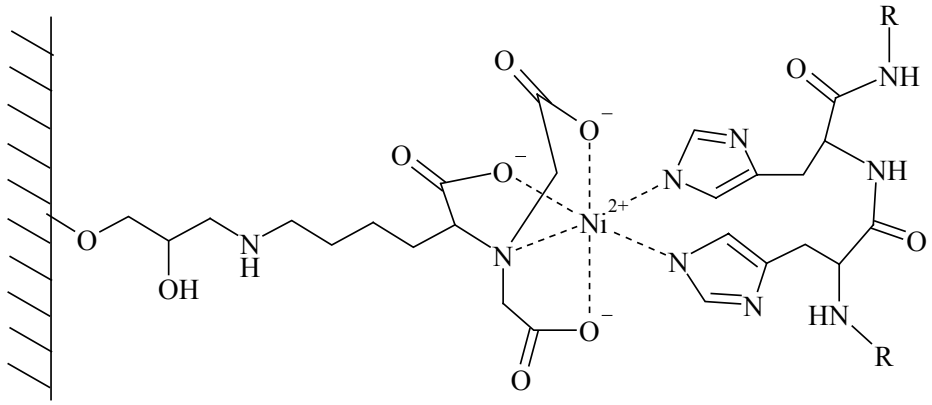


Figure G7: Interaction between the Ni-NTA matrix and residues in the 6xHis-tag

Therefore, 6xHis-tagged proteins are bound more tightly by NTA than by IDA matrices. This allows the purification of proteins from less than 1% of the total protein preparation to a homogeneity of more than 95% in just one step [Janknecht et al., 1991]

The 6xHis affinity tag comprises 6 consecutive histidine residues that can be placed at the C- or N-terminus of the protein of interest. It is small and uncharged at physiological pH, so that it does not interfere with secretion as well as with the structure and function of the recombinant protein. It is poorly immunogenic, which allows the recombinant protein to be used without prior removal of the tag as an antigen to generate antibodies against the protein of interest. However, removal of the 6xHis tag can also be carried out easily and efficiently [Quiagen Ltd., 2001]. If the pET15b vector has been used the cleavage is can be achieved by treatment with thrombin.

Ni-NTA agarose (Ni-NTA coupled to Sepharose® CL-6B) was used for the purification of 6xHis-tagged ER proteins. The imidazole rings of the histidine residues of the 6xHis tag bind to the nickel ions, that are immobilised by the NTA groups on the matrix. Imidazole itself can also bind to the nickel ions and interfere with histidine binding. At low imidazole concentrations (Ni-NTA wash buffer) the binding of dispersed histidine residues in non-tagged, background proteins is disrupted, while 6xHis-tagged proteins still bind strongly to the Ni-NTA matrix. At higher imidazole concentration (Ni-NTA elution buffer) even the 6xHis tag interactions are overcome and the purified protein of interest can be collected.

The binding of tagged proteins to Ni-NTA resins is not dependent on the protein conformation and is unaffected by most detergents and denaturants. The presence of low levels of β-mercaptoethanol in the lysis buffer prevents the co-purification of proteins that are co-expressed and may have formed disulfide bonds with the recombinant protein during cell lysis [Quiagen Ltd., 2001].

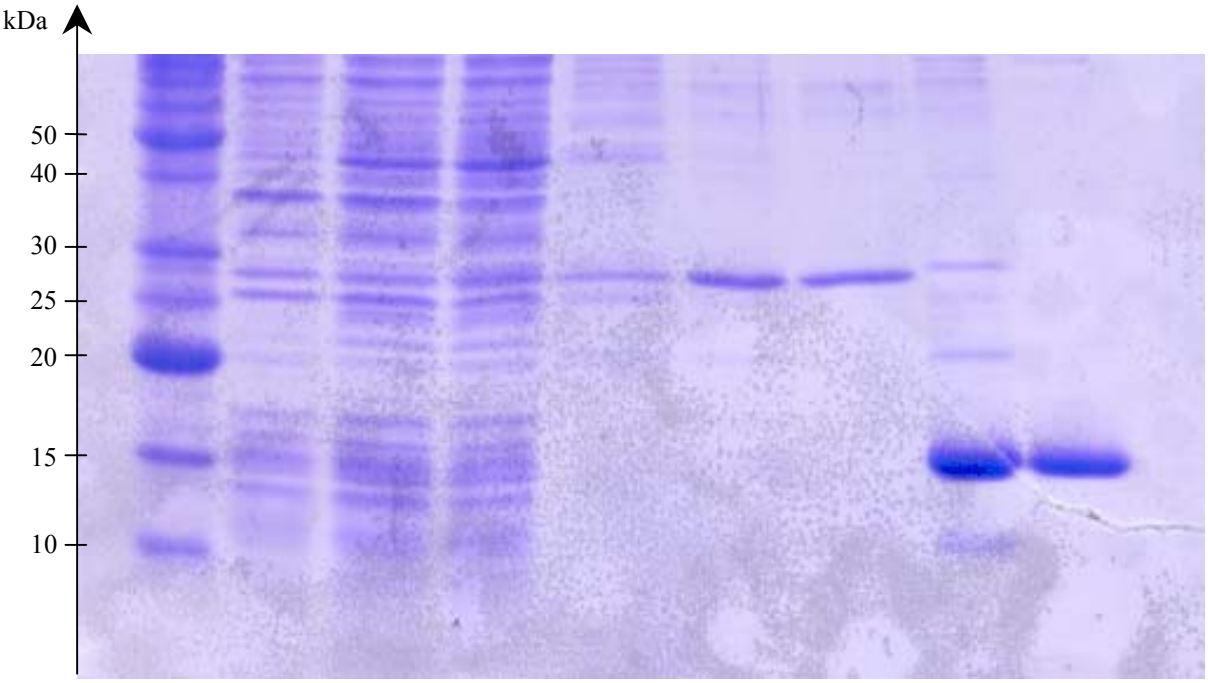


Figure G8: SDS gel after ERα purification over Ni-NTA resin

Columns 1-9 (from left to right): 1 protein ladder, 2-4 flow-through, 5 wash, 6-7 ERα, 8-9 other samples

Among other aspects the dependence on the planned application of the desired protein mainly decides whether to purify under native or denaturing conditions. For the radiometric binding assays (cf. section G3.2) the native state of the receptor protein should be maintained. Thus, both ER proteins were purified under native conditions and finally analysed by SDS-PAGE (cf. figure G8).

The SDS gel shows, that the ERα protein was successfully purified to near homogeneity and that hardly any of the desired protein was lost due to improper attachment to the resin or during washing. Bands in the flow-through and wash fractions might also belong to either low-affinity background proteins with the same molecular weight as the receptor protein or receptor proteins that were insufficiently expressed and lack the His tag sequence. The SDS gel analysis after ERβ purification was identical.

Finally, the amount of isolated ER LBD was determined by means of Bradford's proteins assay. Expression of ERα from a 50ml culture scale yielded about 40µg/ml protein (240µg of

total protein). The yield of ERβ was much lower – as expected due to substantial quantities in the insoluble cell debris – and was difficult to determine with the Bradford assay to give a convincing value (~2µg/ml, 6µg of total protein).

3.2 Radiometric Binding Assay

3.2.1 Theoretical Background

The theoretical background of the radiometric binding assay is described in section B1.

3.2.2 Results and Discussion

Receptor binding is a prerequisite for drugs that mediate their effects via the estrogen receptor. For the determination of the binding affinity of the synthesised benzothiepines to the estrogen receptor a radiometric binding assay was set up using the full-length human receptors ERα and ERβ expressed as recombinant proteins in baculovirus infected insect cells. Furthermore it was investigated, if only the LBD of both receptor isoforms, that were expressed and purified at the Trinity College (cf. section G3.1), can be applied to this assay with the same HAP work-up procedure.

Two independent experiments were performed for the comparison of the binding assay with the full length receptor and the LBD. In experiment No.1 [^{3}H]-labelled E2 was used as tracer in a concentration of 2nM, unlabeled E2 as competitor in six concentrations ranging from 0.5nM to 20nM and the full length human ERα. In experiment No. 2 [^{3}H]-labelled E2 was used as tracer in a concentration of 2nM, unlabelled E2 as competitor in six concentrations ranging from 0.5nM to 20nM and the purified ERα protein, still carrying the N-terminal polyhistidine tag.

The results of these two experiments correlate well with each other, what is illustrated by the binding curves in figure G9. The IC_{50}-values for E2 calculated from both experiments is within acceptable experimental deviation to the theoretically expected value of 2nM.

On the basis of these results the radiometric binding assay with the full-length human estrogen receptor isoforms ERα and ERβ can be set up as standard assay at the Trinity College to characterise new compounds with respect to their affinity and selectivity for both ER subtypes.

Conc. of E2 [nM]	Bound radioactivity [%]	
	Ex No. 1 full-length ER	Ex No. 2 LBD
0.5	114.8	127.2
1	88.3	72.9
2	(88.3)	53.5
5	51.3	45.2
10	23.2	25.2
20	21.7	16.2
IC_{50} [nM]	5.2	3.1

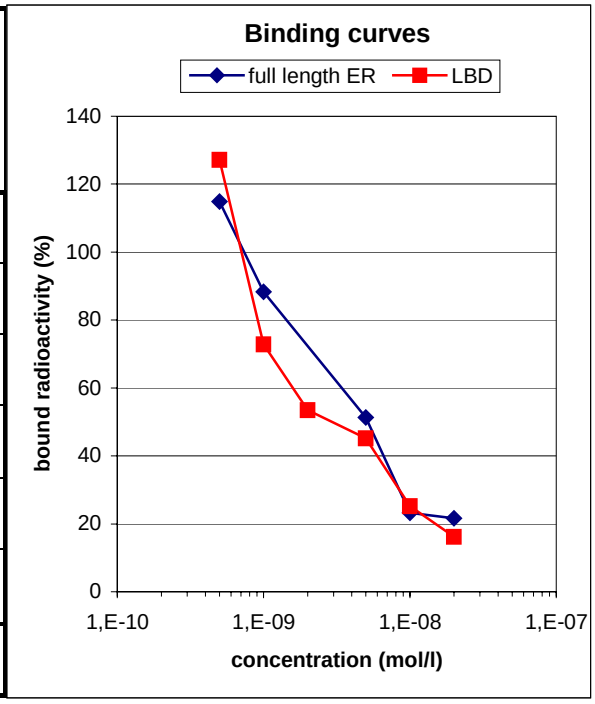


Figure G9: Comparison of the binding data

The first experiment with the purified human ER LBD showed that HAP work-up procedure can be used in an assay with this receptor construct. However, further trials have to be done to confirm this first result in terms of reliability and validity. Estradiol binding should be repeated and also different compounds, e.g. diethylstilbestrol, 4,4'-hexestrol, hydroxytamoxifen, ICI 182.780 with known RBA-values at the full-length receptor, should be tested. These experiments should answer the question, if the N-terminal His-tag sequence, that was not cleaved off for this first experiment, influences to binding assay and if this receptor construct produces reliable results for ER antagonist with a long functionalised side chain that protrudes form the binding cavity. Basically, higher RBA values can be expected from an assay utilizing the LBD instead of the full-length receptor, because the access of ligands to the active site is easier due to the reduced size of the protein.

The synthesised benzo[b]thiepines were not tested with the radiometric binding assay yet.

4 Conclusion

A new synthesis for compounds based on the benzo[b]thiepine scaffold, that is structurally related to the triarylethylene pharmacophore of tamoxifen, was developed. The synthetic route comprises a sequence of six to seven reactions. The Grignard reaction for the introduction of

the complete 4-(2-pyrrolidinylethoxy)phenyl side chain was not successful. Therefore it was introduced by two consecutive reactions. The final deprotection using $AlCl_3$/EtSH resulted in ring opening at the benzothiepine core. The demethylation was accomplished with BBr_3 when the reaction was performed in a highly diluted solution and the temperature was kept at 0°C. The successful application of this route was exemplified by the synthesis of 8H- and 8-methoxy substituted 2,3-dihydro-4-hydroxyphenyl-5-[4-(2-pyrrolidinylethoxy)phenyl]-benzo[b]thiepines.

The binding affinities and selectivities of the synthesised compounds will be determined following a radiometric binding protocol with the recombinant full-length human proteins ERα and ERβ. Investigations were started, whether the identical protocol can be used with the LBD of both receptor subtypes.

The LBDs of the human ERα and ERβ were expressed in the E.coli strain BL21(DE3) using a pET15b plasmid. A N-terminal extension that codes for a 6x-histidine purification tag and a thrombin cleavage site was encoded together with the sequences of ER LBDs. Expression was under the control of an IPTG inducible T7 promoter and the bacterially expressed receptors were purified readily to near-homogeneity over a Ni-NTA resin. Expression and purification of ERα afforded a good yield of the target protein, whereas the expression conditions for the ERβ need to be changed to prevent the formation of inclusion bodies that complicate purification under native conditions.

5 Experimental section

5.1 General aspects

5.1.1 Chemistry

Chemicals

The majority of the starting material was purchased from ALDRICH and LANCASTER.

Column - and thin-layer chromatography

Column chromatography (CC) and thin-layer chromatography (TLC) were performed with the following stationary phases:

TLC: MERCK 5554 TLC aluminium sheets, silica gel 60 F_{254}

CC: MERCK 9385 silica gel 60, 0.040 – 0.063 mm

Elemental analysis and mass spectrometry

Elemental analyses and mass spectrometry were carried out by the microanalytical laboratory of the Department of Chemistry at the University College Dublin.

Infrared spectroscopy

Infrared spectra (IR) were recorded on the FT-IR-spectrometer Spectrum One from PERKIN ELMER. The wave number ν is given in cm^{-1}. The following abbreviations are used to show the intensities of the bands: w = weak; m = moderate; s = strong; br = broad

Melting points

Melting points were determined by means of the melting point apparatus ELECTROTHER-MAL® and the values are uncorrected.

Nuclear resonance spectroscopy

^{1}H-NMR) and ^{13}C-NMR spectra were recorded on the DPX400 apparatus of BRUKER at 400.13 MHz or 100.61MHz, respectively, and standardised using the significant signal of the solvent. The chemical shift δ is given in ppm. The following abbreviations are used for the characterisation of the peaks: s = singlet; d = duplet; dd = duplet of duplet; t = triplet; q = quartet; quin = quintet; m = multiplet; br = broad signal; $^{(n)}$J = coupling constant over n bonds.

5.1.2 Biochemistry

(Bio)chemicals

Bench mark protein ladder (INVITROGEN)
Bovine Albumine (SIGMA)
Bradford Dye Reagent 1x (BIO-RAD LABORATORIES)
EcoScint (NATIONAL DIAGNOSTICS)
Hydroxylapatite Bio-Gel HTP-Gel (BIO-RAD LABORATORIES)
Human recombinant full length estrogen receptor α (INVITROGEN)
Human recombinant full length estrogen receptor β (INVITROGEN)
Ni-NTA agarose (QUIAGEN LTD.)
ProtoGel (NATIONAL DIAGNOSTICS)
Trizma Base (SIGMA)

17β-estradiol	1,3,5(10)-estratrien-3,17β-diol (SIGMA)

[2,4,6,7-³H]-17β-estradiol	[2,4,6,7-³H]-1,3,5(10)-estratrien-3,17β-diol (AMERSHAM BIOSCIENCES LTD.)
Tamoxifen	(Z)-1-[p-(2-dimethylammoniumethoxy)phenyl)-1,2-diphenyl-1-butencitrat (SIGMA)

Cell lines

Escherichia coli bacteria BL21(DE3)

Media, reagents and solutions

Ampicillin solution:	100mg/ml ampicillin in water sterile filtered and stored at –20°C
IPTG (1M):	238mg/ml IPTG in water sterile filtered and stored in aliquots at –20°C
LB agar:	LB medium containing 15g/l agar
LB medium:	10g/l tryptone 5g/l yeast extract 10g/l NaCl
Lysis buffer:	10mM Tris 300mM NaCl 10mM imidazol 10mM β-mercaptoethanol adjusted to pH 8.0 with hydrochloric acid
Ni-NTA elution buffer:	10mM Tris 300mM NaCl 250mM imidazol 10mM β-mercaptoethanol adjusted to pH 8.0 with hydrochloric acid
Ni-NTA wash buffer:	10mM Tris 300mM NaCl 10mM imidazol 10mM β-mercaptoethanol adjusted to pH 8.0 with hydrochloric acid
PAGE destaining solution:	10% AcOH 40% EtOH
PAGE separation gel (15%):	5.0ml ProtoGel

	2.5ml separation gel buffer
	2.4ml water
	0.1ml 10% w/v SDS
	7µl TEMED
	70µl 10% APS
PAGE stacking gel:	1.0ml ProtoGel
	1.5ml stacking gel buffer
	3.4ml water
	60µl 10% w/v SDS
	6µl TEMED
	60µl 10% APS
PAGE staining solution:	0.5% Coomassie Brilliant Blue R®-250
	10% AcOH
	40% EtOH
1xSDS buffer:	0.045M Tris
	10% glycerol
	1% SDS
	0.05M DTT
	adjusted to pH 6.8 with hydrochloric acid
4xSDS sample buffer:	4 times concentrated 1xSDS buffer supplemented with 0.04% bromophenol blue
Separation gel buffer:	1.5M Tris
	adjusted to pH 8.8 with hydrochloric acid
Stacking gel buffer:	0.5M Tris
	adjusted to pH 6.8 with hydrochloric acid

5.2 Chemical Methods and Analytical Data

5.2.1 Synthesis of 3,4-Dihydro-1-(2H)-benzo[b]thiepin-5-ones

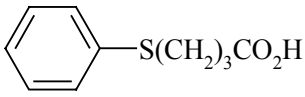
5.2.1.1 Synthesis of Substituted 4-Phenylsulfanylbutyric Acids

A mixture of the respective benzenethiol (100.0mmol), ethyl γ-bromobutyrate (105.0mmol) and potassium carbonate (120.0mmol) in DMF (150ml) was stirred at room temperature for 3h. Water (200ml) was added to the reaction mixture and the aqueous layer was extracted

with ethyl acetate (2x200ml). The organic layer was washed with water (100ml) and sat. NaCl (100ml) and dried over $MgSO_4$. Evaporation of the solvent under reduced pressure afforded the corresponding ethyl 4-phenylsulfanylbutyrates as colourless oils.

The crude intermediate was taken up in EtOH (100ml) and a solution of 4N NaOH (50ml) added. After refluxing for 3h the reaction mixture was concentrated, diluted with water (200ml) and extracted with ethyl acetate (2x100ml). The aqueous layer was acidified with 4N HCl, extracted with EtOAc (3x100ml) and washed with water (100ml) and sat. NaCl (100ml). Subsequent drying over $MgSO_4$ and evaporation of solvent *in vacuo* afforded the required product. The crystals that formed upon standing at rt were washed with petroleum ether.

4-Phenylsulfanylbutyric acid (**1**)



Colourless crystals; yield: 81%

Melting point: 66 – 69°C

$C_{10}H_{12}O_2S$ (196.26)

IR ($CHCl_3$): ν (cm^{-1}) = 3450 – 2300 (m, br; COOH), 1710 (s; C=O)

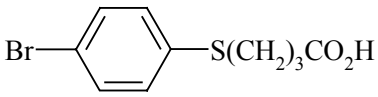
Analysis: Calculated: C: 61.20 H: 6.16

Found: C: 60.95 H: 5.98

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.99 (quin; 2H; ^3J = 7.0Hz; -CH_2-C<u>H</u>$_2$-CH_2-), 2.56 (t; 2H; ^3J = 7.3Hz; -CO-C<u>H</u>$_2$-), 3.01 (t; 2H; ^3J = 7.0Hz; -S-C<u>H</u>$_2$-), 7.19-7.38 (m; 5H; Phenyl-H)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 23.5 (CH_2), 32.1 (CH_2), 32.4 (CH_2), 125.7 (CH), 128.5 (2x CH), 129.0 (2x CH), 135.3 (C-S), 179.0 (C=O)

4-(4-Bromophenyl)sulfanylbutyric acid (**2**)



Colourless solid; yield: 92%

Melting point: 117 – 119°C

$C_{10}H_{11}BrO_2S$ (275.16)

IR ($CHCl_3$): ν (cm^{-1}) = 3450 – 2400 (m, br; COOH), 1710 (s; C=O)

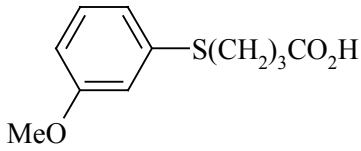
Analysis: Calculated: C: 43.65 H: 4.03

Found: C: 43.69 H: 3.80

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.96 (quin; 2H; ^{3}J = 7.1Hz; -CH_2-C<u>H</u>$_2$-CH_2-), 2.54 (t; 2H; ^{3}J = 7.0Hz; -CO-C<u>H</u>$_2$-), 2.98 (t; 2H; ^{3}J = 7.0Hz; -S-C<u>H</u>$_2$-), 7.22/7.42 (AA'BB'; 4H; ^{3}J = 8.4Hz; Phenyl-H)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 23.4 (CH_2), 32.0 (CH_2), 32.4 (CH_2), 119.6 (C-Br), 130.5 (2x CH), 131.6 (2x CH), 134.6 (C-S), 178.6 (C=O)

4-(3-Methoxyphenyl)sulfanylbutyric acid (**3**)



Colourless crystals; yield: 78%

Melting point: 41 – 44°C

$C_{11}H_{14}O_3S$ (226.29)

IR ($CHCl_3$): ν (cm^{-1}) = 3450 – 2300 (m, br; COOH), 1710 (s; C=O)

Analysis: Calculated: C: 58.39 H: 6.24

Found: C: 58.19 H: 6.12

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.99 (quin; 2H; ^{3}J = 7.0Hz; -CH_2-C<u>H</u>$_2$-CH_2-), 2.55 (t; 2H; ^{3}J = 7.3Hz; -CO-C<u>H</u>$_2$-), 3.01 (t; 2H; ^{3}J = 7.0Hz; -S-C<u>H</u>$_2$-), 3.82 (s; 3H; OC<u>H</u>$_3$), 6.75 (dd; 1H; ^{3}J = 8.0Hz, ^{4}J = 2.5Hz; H4), 6.91 (d; 1H; ^{4}J = 2.0Hz; H2), 6.94 (d; 1H; ^{3}J = 8.0Hz; H6), 7.22 (t; 1H; ^{3}J = 8.0Hz; H5)

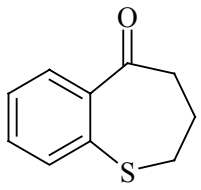
^{13}C-NMR ($CDCl_3$): δ (ppm) = 23.5 (CH_2), 32.1 (2x CH_2), 54.8 (CH_3), 111.3 (CH), 114.1 (CH), 120.9 (CH), 129.3 (CH), 136.7 (C-S), 159.4 (C-O), 178.8 (C=O)

5.2.1.2 Cyclodehydration to 3,4-Dihydro-1-(2H)-benzo[b]thiepin-5-ones

A mixture of the respective 4-phenylsulfanylbutyric acid (50.1mmol) and 100g of polyphosphoric acid were heated for 3h at 110°C whilst stirring with a big stirring bar as good as possible. The dark red syrup formed was poured onto 1 litre of ice-water and finally extracted

with ethyl acetate (3x250ml). The organic extracts were washed with water, 1N NaOH, water and sat. NaCl solution (1x250ml each). After drying over $MgSO_4$ the solvent was removed *in vacuo*.

3,4-Dihydro-1-(2H)-benzo[b]thiepin-5-one (***4***)



Preparation from 4-phenylsulfanylbutyric acid **1**. Purification was achieved by column chromatography (SiO_2; DCM / petroleum ether 40 – 60 1:1, v/v).

Yellow oil; yield: 77%

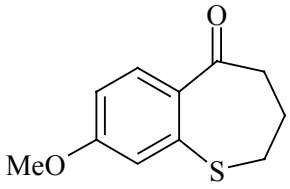
$C_{10}H_{10}OS$ (178.25)

IR (film): ν (cm^{-1}) = 1679 (s; C=O)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.30 (quin; 2H; ^3J = 6.8Hz; -CH_2-C$\underline{H}_2$-CH_2-), 3.01 (t; 2H; ^3J = 6.8Hz; -S-C$\underline{H}_2$-), 3.07 (t; 2H; ^3J = 6.8Hz; -CO-C$\underline{H}_2$-), 7.27 (t; 1H; ^3J = 7.5Hz; Phenyl-H), 7.35 (t; 1H; ^3J = 7.5Hz; Phenyl-H), 7.49 (d; 1H; ^3J = 7.5Hz; H9), 7.86 (d; 1H; ^3J = 8.0Hz; H6)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 29.5 (CH_2), 34.5 (CH_2), 39.7 (CH_2), 125.5 (CH), 129.7 (CH), 129.9 (CH), 130.5 (CH), 137.8 (C), 141.7 (C), 202.5 (C=O)

3,4-Dihydro-8-methoxy-1-(2H)-benzo[b]thiepin-5-one (***5***)



Preparation from 4-(3-methoxyphenyl)sulfanylbutyric acid **3**. Purifaction was achieved by column chromatography (SiO_2; DCM).

Yellow oil; yield: 73%

$C_{11}H_{12}O_2S$ (208.28)

IR (film): ν (cm^{-1}) = 1668 (s; C=O)

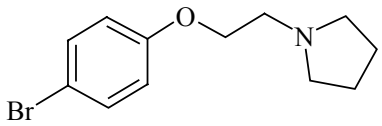
^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.26 (quin; 2H; ^{3}J = 6.8Hz; -CH_2-C<u>H</u>$_2$-CH_2-), 3.00 (t; 2H; ^{3}J = 6.8Hz; -S-C<u>H</u>$_2$-), 3.05 (t; 2H; ^{3}J = 6.8Hz; -CO-C<u>H</u>$_2$-), 3.85 (s; 3H; OC<u>H</u>$_3$), 6.86 (dd; 1H; ^{3}J = 8.8Hz, ^{4}J = 2.3Hz; H7), 6.97 (d; 1H; ^{4}J = 2.5Hz; H9), 7.87 (t; 1H; ^{3}J = 8.5Hz; H6)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 29.0 (CH_2), 34.7 (CH_2), 39.8 (CH_2), 55.1 (CH_3), 112.3 (CH), 113.7 (CH), 130.8 (C), 131.9 (CH), 144.4 (C-S), 160.8 (C-O), 200.9 (C=O)

5.2.2 Introduction of the Basic Side Chain

5.2.2.1 Synthesis of Alkylated or Protected Bromophenols

1-[2-(4-Bromophenoxy)ethyl]pyrrolidine (**6**)



Bromophenol (11.6mmol) and potassium carbonate (29.0mmol) in DMF (25ml) were heated at 100°C for 10min and subsequently 1-(2-chloroethyl)pyrrolidine hydrochloride (11.6mmol) was added in portions over 10min. The mixture was heated for another 3h. It was poured onto ice-water (75ml) and extracted with ethyl acetate (3x75ml). The organic layer was washed with 2N HCl (2x75ml). The aqueous layer was basified with 2N NaOH, and extracted again with ethyl acetate (3x75ml). The organic layer was washed with sat. NaCl and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The required compound was sufficiently pure without further purification.

Orange oil; yield: 82%

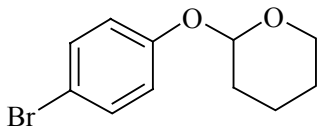
$C_{12}H_{16}BrON$ (270.17)

IR (film): ν (cm^{-1}) = 2786 (s; C-N)

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.82-1.83 (m; 4H; -N-CH_2-(C<u>H</u>$_2$)$_2$), 2.62-2.64 (m; 4H; -C<u>H</u>$_2$-N-C<u>H</u>$_2$-), 2.90 (t; 2H; ^{3}J = 5.7Hz; -C<u>H</u>$_2$-N-), 4.08 (t; 2H; ^{3}J = 6.0Hz; -O-C<u>H</u>$_2$-), 6.81/7.37 (AA'BB'; 4H; ^{3}J = 9.0Hz; Phenyl-H)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 23.0 (2x CH_2), 54.3 (CH_2-N), 54.5 (CH_2-N), 66.9 (CH_2-O), 112.4 (C-Br), 115.9 (CH), 131.7 (CH), 157.5 (C-O)

4-(Tetrahydro-2H-pyran-2-yloxy)bromobenzene (**7**)



To a solution of bromophenol (57.8mmol) in 3,4-dihydro-2H-pyran (15ml) were added 2 drops of 2N HCl. After stirring at room temperature for 3hr the solution was diluted with diethylether (100ml). The organic extracts were washed with 2N NaOH, water and brine (75ml each). After drying over $MgSO_4$ the solvent was evaporated and the remaining oil was crystallised from methanol at 4°C.

Colourless crystals; yield: 88%
Melting point: 52 – 55°C
$C_{11}H_{13}BrO_2$ (257.13)

Analysis:	Calculated:	C: 51.38	H: 5.10
	Found:	C: 51.32	H: 4.87

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.62-2.07 (m; 6H; -($C\underline{H}_2$)$_3$-), 3.59-3.64 (m; 2H; -O$C\underline{H}_2$-), 3.86-3.92 (m; 2H; -O$C\underline{H}_2$-), 5.39 (t; 1H; ^{3}J = 3.0Hz; -O$C\underline{H}$-), 6.96/7.39 (AA'BB'; 4H; ^{3}J = 8.8Hz; Phenyl-H)

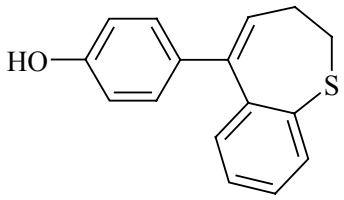
^{13}C-NMR ($CDCl_3$): δ (ppm) = 18.2 (CH_2), 24.7 (CH_2), 29.8 (CH_2), 61.6 (CH_2-O), 96.0 (CH-O), 113.4 (C-Br), 117.8 (2x CH), 137.8 (2x CH), 155.7 (C-O)

5.2.2.2 General Procedure for the Arylation to 5-Aryl-2,3-dihydrobenzo[b]thiepines

Under nitrogen atmosphere at –78°C, n-butyllithium (10ml, 2.5M in hexane) was added dropwise over 15min to a solution of 4-(tetrahydro-2H-pyran-2-yloxy)bromobenzene **7** (25.0mmol) in dry THF (40ml) and the resulting mixture stirred for 30 minutes. Then, a solution of the appropriate benzo[b]thiepine (25mmol) in dry THF (40ml) was added dropwise with a syringe. The reaction mixture was kept at this temperature for another 2hr and stirring continued overnight at room temperature. The pink-coloured mixture was partitioned between water (100ml) and ethyl acetate (100ml) and the layers separated. The aqueous layer was extracted with ethyl acetate (2x100ml). The combined organic layers were washed with brine and dried over $MgSO_4$. Evaporation of the solvent afforded the tertiary alcohol as a yellow oil.

For the dehydration the crude product was taken up in MeOH (100ml) and concentrated HCl (4ml) and stirred at 60 – 70°C for about 30 minutes. After cooling to room temperature the mixture was partitioned between ethyl acetate and water (100ml each). The layers were separated and the aqueous phase extracted with ethyl acetate (2x100ml). The combined organic phases were finally washed with sat. NaCl-solution, dried over $MgSO_4$ and concentrated.

2,3-Dihydro-5-(4-hydroxyphenyl)benzo[b]thiepine (**8**)



Preparation from 3,4-Dihydro-1-(2H)-benzo[b]thiepin-5-one **4** and 4-(tetrahydro-2H-pyran-2-yloxy)bromobenzene **7**. Purification of the crude product was achieved by column chromatography (SiO_2; ethyl acetate/hexane 1:10, v/v).

Yellow oil; yield: 60%

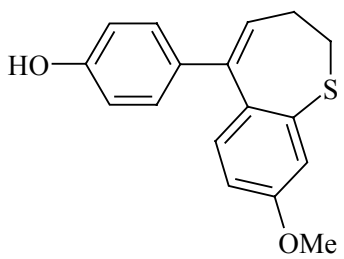
$C_{16}H_{14}OS$ (254.35)

IR ($CHCl_3$): ν (cm^{-1}) = 3364 (w, br; O-H), 1610 (s; C=C)

^{1}H-NMR (MeOD-d_4): δ (ppm) = 2.21 (q; 2H; ^{3}J = 6.5Hz; -CH-C<u>H</u>$_2$-), 3.43 (t; 2H; ^{3}J = 6.5Hz; -S-C<u>H</u>$_2$-), 6.46 (t; 1H; ^{3}J = 7.8Hz; -C<u>H</u>-CH$_2$-), 6.73/7.03 (AA'BB'; 4H; ^{3}J = 8.8Hz; Phenyl-H), 7.03 (1H merged; H9), 7.22 (dt; 1H; ^{3}J = 7.4Hz, ^{4}J = 1.8Hz; H7), 7.30 (dt; 1H; ^{3}J = 7.4Hz, ^{4}J = 1.4Hz; H8), 7.64 (dd; 1H; ^{3}J = 7.5Hz, ^{4}J = 1.5Hz; H6)

^{13}C-NMR (MeOD-d_4): δ (ppm) = 26.0 (CH_2), 42.8 (CH_2), 114.1 (2x CH), 126.1(CH), 126.7 (CH), 127.3 (CH), 128.3 (2x CH), 130.0 (CH), 133.0 (C), 134.0 (CH), 134.3 (C), 143.2 (C), 145.4 (C), 156.2 (C-O)

2,3-Dihydro-5-(4-hydroxyphenyl)-8-methoxybenzo[b]thiepine (**9**)



Preparation from 3,4-dihydro-8-methoxy-1-(2H)-benzo[b]thiepin-5-one **5** and 4-(tetrahydro-2H-pyran-2-yloxy)bromobenzene **7**. Purification of the red oil was achieved by column chromatography (SiO_2; ethyl acetate / petroleum ether 40 – 60 1:10, v/v).

Yellow oil; yield: 60%

$C_{17}H_{16}O_2S$ (284.37)

IR (film): ν (cm^{-1}) = 3390 (w, br; O-H), 1609 (s; C=C)

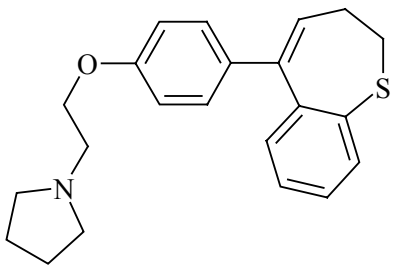
^{1}H-NMR (MeOD-d_4): δ (ppm) = 2.22 (q; 2H; ^{3}J = 6.9Hz; -CH-C<u>H</u>$_2$-), 3.42 (t; 2H; ^{3}J = 6.5Hz; -S-C<u>H</u>$_2$-), 3.82 (s; 3H; OC<u>H</u>$_3$), 6.38 (t; 1H; ^{3}J = 7.8Hz; -C<u>H</u>-CH_2-), 6.72/7.02 (AA'BB'; 4H; ^{3}J = 8.8Hz; Phenyl-H), 6.88 (dd; 1H; ^{3}J = 8.5Hz, ^{4}J = 2.5Hz; H7), 6.94 (d; 1H; ^{3}J = 8.5Hz; H6), 7.21 (d; 1H; ^{4}J = 2.5Hz; H9)

^{13}C-NMR (MeOD-d_4): δ (ppm) = 26.1 (CH_2), 42.7 (CH_2), 54.0 (CH_3), 113.2 (CH), 114.0 (2x CH), 118.5 (CH), 125.2 (CH), 128.3 (2x CH), 131.0 (CH), 133.3 (C), 135.5 (C), 137.3 (C), 143.0 (C), 156.2 (C-O), 158.0 (C-O)

5.2.2.3 General Procedure for the Alkylation of 5-Aryl-2,3-dihydrobenzo[b]thiepines

A mixture of a 2,3-dihydro-5-(4-hydroxyphenyl)benzo[b]thiepine (3.52mmol) and potassium carbonate (17.60mmol) in acetone (40ml) were heated for 1hr. Then, 1-(2-chloroethyl)-pyrrolidine hydrochloride (7.03mmol) was added in portions and the mixture refluxed for 24 hours. The resulting slightly brown solution was cooled to room temperature and filtered. The solid residue was washed thoroughly with acetone. The solvent of the combined filtrates was removed *in vacuo*.

2,3-Dihydro-5-[4-(2-pyrrolidinylethoxy)phenyl]benzo[b]thiepine (**10**)



Preparation from 2,3-dihydro-5-(4-hydroxyphenyl)benzo[b]thiepine **8**. The crude product was purified by column chromatography (SiO_2; MeOH/DCM 1:25, v/v).

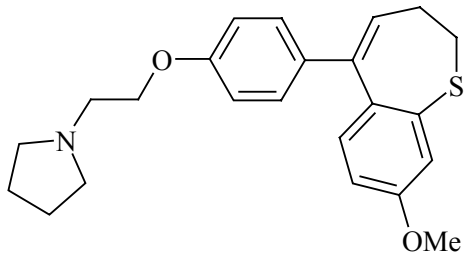
Yellow oil; yield: 66%

$C_{22}H_{25}NOS$ (351.51)

^{1}H-NMR (MeOD-d_4): δ (ppm) = 1.84-1.87 (m; 4H; -(C<u>H</u>$_2$)$_2$-C<u>H</u>$_2$-N-), 2.23 (q; 2H; ^{3}J = 7.0Hz; -CH-C<u>H</u>$_2$-), 2.70 (m; 4H; C<u>H</u>$_2$-N-C<u>H</u>$_2$-), 2.95 (t; 2H; ^{3}J = 5.8Hz; -N-C<u>H</u>$_2$-), 3.44 (t; 2H; ^{3}J = 6.5Hz; -S-C<u>H</u>$_2$-), 4.14 (t; 2H; ^{3}J = 5.5Hz; -O-C<u>H</u>$_2$-), 6.50 (t; 1H; ^{3}J = 7.8Hz; -C<u>H</u>-CH$_2$-), 6.89/7.13 (AA'BB'; 4H; ^{3}J = 8.8Hz; Phenyl-H), 7.01 (dd; 1H; ^{3}J = 7.5Hz, ^{4}J = 1.5Hz; H9), 7.24 (dt; 1H; ^{3}J = 7.5Hz, ^{4}J = 1.5Hz; H7), 7.31 (dt; 1H; ^{3}J = 7.5Hz, ^{4}J = 1.5Hz; H8), 7.65 (d; 1H; ^{3}J = 7.5Hz; H6)

^{13}C-NMR (MeOD-d_4): δ (ppm) = 22.3 (2x CH_2), 26.0 (CH_2), 42.7 (CH_2), 53.7 (2x CH_2-N), 54.0 (CH_3), 54.2 (CH_2-N), 65.7 (CH_2-O), 113.4 (2x CH), 126.8 (CH), 126.8 (CH), 127.3 (CH), 128.3 (2x CH), 130.0 (CH), 134.0 (CH), 134.4 (C), 142.9 (C), 145.2 (C), 157.8 (C-O)

2,3-Dihydro-8-methoxy-5-[4-(2-pyrrolidinylethoxy)phenyl]benzo[b]thiepine (**11**)



Preparation from 2,3-dihydro-5-(4-hydroxyphenyl)-8-methoxybenzo[b]thiepine **9**. The crude product was purified by column chromatography (SiO_2; MeOH/DCM 1:25, v/v).

Yellow oil; yield: 74%

$C_{23}H_{27}NO_2S$ (381.53)

^{1}H-NMR (MeOD-d_4): δ (ppm) = 1.84-1.88 (m; 4H; -(C<u>H</u>$_2$)$_2$-C<u>H</u>$_2$-N-), 2.25 (q; 2H; ^{3}J = 7.2Hz; -CH-C<u>H</u>$_2$-), 2.72 (m; 4H; C<u>H</u>$_2$-N-C<u>H</u>$_2$-), 2.97 (t; 2H; ^{3}J = 5.5Hz; -N-C<u>H</u>$_2$-), 3.44 (t; 2H; ^{3}J = 7.0Hz; -S-C<u>H</u>$_2$-), 3.85 (s; 3H; OC<u>H</u>$_3$), 4.14 (t; 2H; ^{3}J = 5.8Hz; -O-C<u>H</u>$_2$-), 6.42 (t; 1H; ^{3}J = 7.8Hz; -C<u>H</u>-CH$_2$-), 6.88 (1H merged; H7), 6.88/7.13 (AA'BB'; 4H; ^{3}J = 8.8Hz; Phenyl-H), 6.93 (d; 1H; ^{3}J = 8.5Hz, H6), 7.22 (d; 1H; ^{4}J = 2.5Hz, H9)

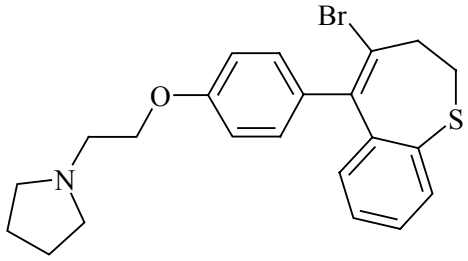
^{13}C-NMR (MeOD-d_4): δ (ppm) = 22.3 (2x CH_2), 26.2 (CH_2), 42.6 (CH_2), 53.7 (2x CH_2-N), 54.0 (CH_3), 54.1 (CH_2-N), 65.6 (CH_2-O), 113.2 (CH), 113.3 (2x CH), 118.5 (CH), 126.0 (CH), 128.3 (2x CH), 130.9 (CH), 134.7 (C), 135.6 (C), 137.1 (C), 142.7 (C), 157.7 (C-O), 158.1 (C-O)

5.2.3 Introduction of the Third Aromatic Ring

5.2.3.1 General Procedure for the Bromination to 4-Bromo-5-aryl-2,3-dihydrobenzo[b]thiepines

To a solution of the respective 5-aryl-2,3-dihydro-benzo[b]thiepines (2.11mmol) in dry dichloromethane (30ml) at -10°C was added pyridiniumbromide perbromide (2.11mmol) in small portions. $PyHBr_3$ was added at such a rate, that the reagent was completely dissolved before the next addition. The mixture attained room temperature and was stirred for 8 hours. A solution of sodium hydrogen carbonate (10%, 50ml) was added and the aqueous layer extracted with dichloromethane (3x50ml). The combined organic layers were washed with water and brine (50ml each). After drying over $MgSO_4$ the solvent was removed under reduced pressure.

4-Bromo-2,3-Dihydro-5-[4-(2-pyrrolidinylethoxy) phenyl]benzo[b]thiepine (**12**)



Preparation from 2,3-dihydro-5-[4-(2-pyrrolidinylethoxy)phenyl]benzo[b]thiepine **10**. The crude yellow liquid (containing one equivalent of pyridine) was purified by column chromatography (SiO_2; methanol/dichloromethane 1:25, v/v). Trials to evaporate the pyridine at elevated temperature (60-70°C) under reduced pressure resulted in formation of a black tar indicating decomposition of the desired product.

Yellow oil; yield: 82%

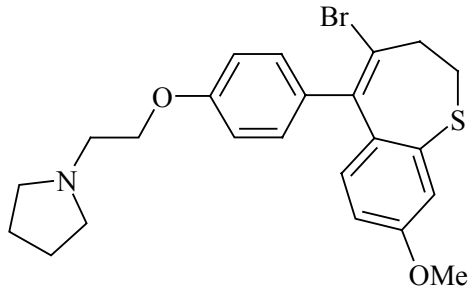
$C_{22}H_{24}BrNOS$ (430.40)

IR (film): ν (cm^{-1}) = 2787 (s; C-N), 1606 (s; C=C)

^{1}H-NMR (MeOD-d_4): δ (ppm) = 1.84-1.88 (m; 4H; -(C<u>H</u>$_2$)$_2$-CH$_2$-N-), 2.72 (m; 4H; C<u>H</u>$_2$-N-C<u>H</u>$_2$-), 2.80 (t; 2H; ^{3}J = 6.5Hz; -C<u>H</u>$_2$-CH$_2$-S-), 2.97 (t; 2H; ^{3}J = 5.5Hz; -N-C<u>H</u>$_2$-), 3.59 (t; 2H; ^{3}J = 6.5Hz; -S-C<u>H</u>$_2$-), 4.15 (t; 2H; ^{3}J = 5.5Hz; -O-C<u>H</u>$_2$-), 6.92/7.13 (AA'BB'; 4H; ^{3}J = 8.8Hz; Phenyl-H), 6.90-6.92 (m; 1H; H9), 7.20-7.27 (m; 2H; H7, H8), 7.61-7.64 (m; 1H; H6)

^{13}C-NMR (MeOD-d_4): δ (ppm) = 22.3 (2x CH$_2$), 37.6 (CH$_2$), 40.5 (CH$_2$), 53.7 (2x CH$_2$-N), 54.1 (CH$_2$-N), 65.7 (CH$_2$-O), 113.1 (2x CH), 122.4 (C-Br), 127.3 (CH), 127.3 (CH), 130.0 (CH), 130.3 (2x CH), 132.8 (C), 134.1 (C), 134.2 (CH), 141.2 (C), 146.0 (C), 157.7 (C-O)

4-Bromo-2,3-dihydro-8-methoxy-5-[4-(2-pyrrolidinylethoxy)phenyl]benzo[b]thiepine (**13**)



Preparation from 2,3-dihydro-8-methoxy-5-[4-(2-pyrrolidinylethoxy)phenyl]benzo[b]thiepine **11**. The crude yellow liquid (containing one equivalent of pyridine) was purified by column chromatography (SiO_2; methanol/dichloromethane 1:25, v/v).

Yellow oil; yield: 74%

$C_{23}H_{26}BrNO_2S$ (460.43)

IR (film): ν (cm^{-1}) = 2784 (s; C-N), 1606 (s; C=C)

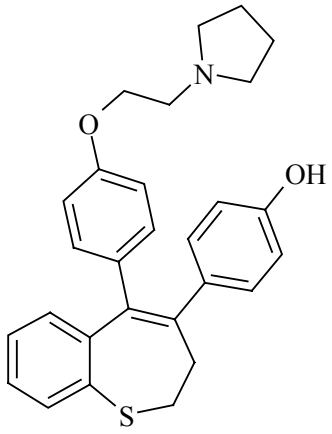
^{1}H-NMR (MeOD-d_4): δ (ppm) = 1.85-1.88 (m; 4H; -(C<u>H</u>$_2$)$_2$-CH$_2$-N-), 2.73 (m; 4H; C<u>H</u>$_2$-N-C<u>H</u>$_2$-), 2.82 (t; 2H; ^{3}J = 6.5Hz; -C<u>H</u>$_2$-CH$_2$-S-), 2.97 (t; 2H; ^{3}J = 5.5Hz; -N-C<u>H</u>$_2$-), 3.58 (t; 2H; ^{3}J = 6.5Hz; -S-C<u>H</u>$_2$-), 3.80 (s; 3H; OC<u>H</u>$_3$), 4.16 (t; 2H; ^{3}J = 5.5Hz; -O-C<u>H</u>$_2$-), 6.80-6.82 (m; 2H; H6, H7), 6.91/7.12 (AA'BB'; 4H; ^{3}J = 9.0Hz; Phenyl-H), 7.18-7-19 (m; 1H; H9)

^{13}C-NMR (MeOD-d_4): δ (ppm) = 22.3 (2x CH$_2$), 37.7 (CH$_2$), 40.2 (CH$_2$), 53.7 (2x CH$_2$-N), 54.0 (CH$_3$), 54.1 (CH$_2$-N), 65.6 (CH$_2$-O), 113.0 (2x CH), 113.4 (CH), 118.7 (CH), 121.2 (C-Br), 130.3 (2x CH), 131.1 (CH), 134.1 (C), 134.3 (C), 137.9 (C), 140.9 (C), 157.6 (C-O), 158.4 (C-O)

5.2.3.2 General Procedure for the Suzuki Reaction

$Pd(PPh_3)_4$ (0.035mmol) was added to a solution of the respective 5-aryl-4-bromo-benzo[b]thiepine (1.16mmol), 4-hydroxyphenylboronic acid (1.74mmol), and 2M Na_2CO_3 (5.80mmol) in THF (20ml) and the resulting mixture heated to reflux for 5-6 hours. After cooling, the mixture was partitioned between water and ethyl acetate (40ml each) and filtered to remove the black, insoluble residues of the palladium-catalyst. The layers were separated and the aqueous layer extracted with ethyl acetate (3x40ml). The combined organic layers were washed with water and brine (40ml each). After drying over $MgSO_4$ the solvent was removed *in vacuo*.

2,3-Dihydro-4-hydroxyphenyl-5-[4-(2-pyrrolidinylethoxy)phenyl]benzo[b]thiepine (**14**)



Preparation from 4-bromo-2,3-dihydro-5-[4-(2-pyrrolidinylethoxy)phenyl]benzo[b]thiepine **12**. The orange crude product was purified by column chromatography (SiO_2; MeOH/DCM 1:15, v/v) to separate the desired compound from a slightly more unpolar component, that is hardly visible on TLC due to overlapping. Staining the TLC plate with sublimating iodine revealed the product as dark-orange to brownish and the by-product as an orange spot. Finally, the isolated compound was recrystallised from MeOH (and few drops of DCM) at 4°C.

White solid; yield: 72%

Melting point: 178-180°C (dec.)

$C_{28}H_{29}NO_2S$ (443.61)

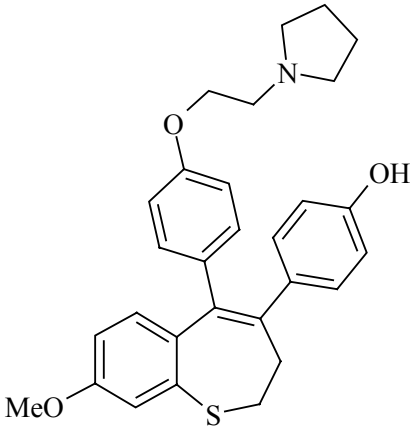
IR (KBr):	ν (cm^{-1}) = (w, br; -OH), (s; C=C)			
Analysis:	Calculated:	C: 75.81	H: 6.59	N: 3.16
	Cal. *0.3 CH_2Cl_2:	C: 72.46	H: 6.36	N: 2.99
	Found:	C: 72.15	H: 6.40	N: 2.86

HRMS: Calculated: 444.1997 for $[C_{28}H_{29}NO_2S+H]^+$
Found: 444.1999

^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.67-1.70 (m; 4H; -(C<u>H</u>$_2$)$_2$-C<u>H</u>$_2$-N-), 2.54-2.57 (m; 6H; -C<u>H</u>$_2$-C<u>H</u>$_2$-S-, -C<u>H</u>$_2$-N-C<u>H</u>$_2$-), 2.81 (t; 2H; ^{3}J = 5.6Hz; -N-C<u>H</u>$_2$-), 3.35 (t; 2H; ^{3}J = 6.0Hz; -S-C<u>H</u>$_2$-), 3.99 (t; 2H; ^{3}J = 5.5Hz; -O-C<u>H</u>$_2$-), 6.59 (d; 2H; ^{3}J = 8.5Hz; Phenyl-H), 6.68-6.72 (m; 4H; Phenyl-H), 7.00 (d; 2H; ^{3}J = 8.5Hz; Phenyl-H), 6.85 (dd; 1H; ^{3}J = 8.5Hz, ^{4}J = 1.5Hz; H9), 7.23 (dt; 1H; ^{3}J = 7.5Hz, ^{4}J = 1.8Hz; H7), 7.30 (dt; 1H; ^{3}J = 7.5Hz, ^{4}J = 1.3Hz; H8), 7.61 (dd; 1H; ^{3}J = 7.5Hz, ^{4}J = 1.5Hz; H6), 9.39 (s; 1H; -OH)

^{13}C-NMR (DMSO-d_6): δ (ppm) = 23.0 (2x CH_2), 34.3 (CH_2), 42.1 (CH_2), 54.0 (2x CH_2-N), 54.9 (CH_2-N), 66.0 (CH_2-O), 113.7 (2x CH), 114.9 (2x CH), 127.5 (CH), 128.3 (CH), 130.4 (2x CH), 130.9 (CH), 131.8 (2x CH), 132.0 (C), 133.2 (C), 134.4 (CH), 134.5 (C), 136.9 (C), 140.4 (C), 148.0 (C), 156.1 (C-O), 156.7 (C-O)

2,3-Dihydro-4-hydroxyphenyl-8-methoxy-5-[4-(2-pyrrolidinylethoxy)phenyl]benzo[b]thiepine (**15**)



Preparation from 4-bromo-2,3-dihydro-8-methoxy-5-[4-(2-pyrrolidinylethoxy)phenyl]-benzo[b]thiepine **13**. The dark-red crude product was purified by column chromatography (SiO_2; MeOH/DCM 1:15, v/v) to separate the desired compound from a slightly more unpolar component, that is hardly visible on TLC due to overlapping. Staining the TLC plate with sublimating iodine revealed the product as dark-green to black spot, whereas the by-product appears orange. A small fraction of the beige solid, isolated from chromatography, was dissolved in DCM, treated with a small volume of 6M HCl and set aside at 4°C to achieve crys-

tallisation of product as hydrochloride salt. Note that the data of the second ^{1}H-NMR and the ^{13}C-NMR data are those of the free base.

White solid; yield: 58%

Melting point: 246-249°C (dec.)

$C_{29}H_{31}NO_3S$*HCl (510.09)

IR (KBr): ν (cm^{-1}) = (w, br; -OH), (s; C=C)

Analysis:

Calculated:	C: 68.29	H: 6.32	N: 2.75
Cal. *0.3 CH_2Cl_2:	C: 65.70	H: 6.13	N: 2.63
Found:	C: 65.76	H: 6.20	N: 2.58

HRMS: Calculated: 474.2103 for $[C_{29}H_{31}NO_3S+H]^+$

Found: 474.2107

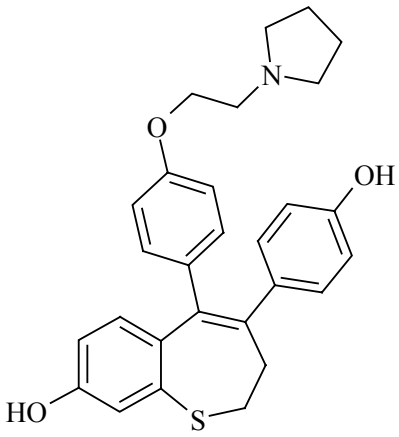
^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.92 (br; 4H; -(C<u>H</u>$_2$)$_2$-CH$_2$-N-), 2.55 (t; 2H; ^{3}J = 6.5Hz; -C<u>H</u>$_2$-CH$_2$-S-), 3.09 (br; 4H; -C<u>H</u>$_2$-N-C<u>H</u>$_2$-), 3.50 (m; 4H; -N-C<u>H</u>$_2$-, -S-C<u>H</u>$_2$-), 3.79 (s; 3H; -O-CH$_3$), 4.23 (t; 2H; ^{3}J = 4.8Hz; -O-C<u>H</u>$_2$-), 6.59 (d; 2H; ^{3}J = 8.5Hz; Phenyl-H), 6.74-6.76 (m; 5H; Phenyl-H), 6.89 (dd; 1H; ^{3}J = 8.5Hz, ^{4}J = 2.5Hz; H7), 6.99 (d; 2H; ^{3}J = 8.0Hz; Phenyl-H), 7.17 (d; 1H; ^{4}J = 2.5Hz; H9), 9.40 (s; 1H; -OH)

^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.68-1.71 (m; 4H; -(C<u>H</u>$_2$)$_2$-CH$_2$-N-), 2.52-2.57 (m; 6H; -C<u>H</u>$_2$-CH$_2$-S-, -C<u>H</u>$_2$-N-C<u>H</u>$_2$-), 2.81 (t; 2H; ^{3}J = 5.6Hz; -N-C<u>H</u>$_2$-), 3.35 (t; 2H; ^{3}J = 6.0Hz; -S-C<u>H</u>$_2$-), 3.78 (s; 3H; -O-CH$_3$), 3.98 (t; 2H; ^{3}J = 5.5Hz; -O-C<u>H</u>$_2$-), 6.58 (d; 2H; ^{3}J = 8.5Hz; Phenyl-H), 6.67-6.72 (m; 4H; Phenyl-H), 6.98 (d; 2H; ^{3}J = 8.5Hz; Phenyl-H), 6.76 (d; 1H; ^{3}J = 8.5Hz, H6), 6.88 (dd; 1H; ^{3}J = 8.5Hz, ^{4}J = 2.5Hz; H7), 7.16 (d; 1H; ^{4}J = 2.5Hz; H9), 9.32 (s; 1H; -OH)

^{13}C-NMR (DMSO-d_6): δ (ppm) = 23.0 (2x CH$_2$), 34.4 (CH$_2$), 41.9 (CH$_2$), 54.0 (2x CH$_2$-N), 54.9 (CH$_2$-N), 55.2 (CH$_3$), 66.1 (CH$_2$-O), 113.6 (2x CH), 114.4 (CH), 114.9 (2x CH), 118.7 (CH), 130.4 (2x CH), 131.8 (2x CH), 131.9 (CH), 132.2 (C), 134.4 (C), 134.6 (C), 136.7 (C), 139.4 (C), 140.0 (C), 156.0 (C-O), 156.6 (C-O), 157.7 (C-O)

5.2.4 Deprotection of the Phenolic Methoxy Groups

2,3-Dihydro-8-hydroxy-4-hydroxyphenyl-5-[4-(2-pyrrolidinylethoxy)phenyl]benzo[b]thiepine (**16**)



At 0°C, a solution of the free base of 2,3-dihydro-8-methoxy-4-hydroxyphenyl-5-[4-(2-pyrrolidinylethoxy)phenyl]benzo[b]thiepine **15** (52.8µmol) in dry DCM (5ml) was added dropwise to BBr_3 (0.26mmol, 1M solution in DCM) diluted with dry DCM (3ml). The reaction mixtures turns slightly red during addition and dark-red during the course of reaction going along with the formation of a dark precipitate. After stirring at 0°C for 10hr the mixture was quenched with 10% (w/w) $NaHCO_3$ (20ml). The dark precipitate was dissolved in a mixture of EtOAc/MeOH 10:1 (v/v) (20ml) with vigorous stirring. The aqueous phase was separated and extracted twice with the EtOAc/MeOH-mixture. The combined organic fractions were dried over $MgSO_4$ and concentrated *in vacuo*.

The crude product was purified by column chromatography (SiO_2; methanol / dichloromethane 1:7, v/v) to separate the desired compound from remaining starting material (24% beige solid) and a somewhat more unpolar component, that was only visible with long wave UV-light. Staining the TLC plate with sublimating iodine revealed the product as orange spot, whereas the unpolar starting material appeared darkgreen to black.

Slightly red solid; yield: 62%

$C_{28}H_{29}NO_3S$ (459.60)

^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.73 (s, br; 4H; -(C$\underline{H}_2$)$_2$-CH_2-N-), 2.53 (t; 2H; ^{3}J = 6.0Hz; -C$\underline{H}_2$-CH_2-S-), 2.71 (s, br; 4H; -C$\underline{H}_2$-N-C$\underline{H}_2$-), 2.95 (s, br; 2H; -C$\underline{H}_2$-N-), 3.31 (t; 2H; ^{3}J = 6.0Hz; -S-C$\underline{H}_2$-), 4.03 (t; 2H; ^{3}J = 5.1Hz; -O-C$\underline{H}_2$-), 6.57 (d; 2H; ^{3}J = 8.5Hz; Phenyl-H), 6.67-6.73 (m; 5H; Phenyl-

H), 6.64 (d; 1H; ^{3}J = 8.5Hz, H6), 6.97 (d; 2H; ^{3}J = 8.5Hz; Phenyl-H), 7.03 (d; 1H; ^{4}J = 2.5Hz; H9), 9.34 (s; 1H; -OH), 9.70 (s; 1H; -OH)

^{13}C-NMR (DMSO-d_6): δ (ppm) = 22.9 (2x CH_2), 34.5 (CH_2), 41.6 (CH_2), 53.9 (2x CH_2-N), 54.8 (CH_2-N), 65.5 (CH_2-O), 113.5 (2x CH), 114.9 (2x CH), 115.5 (CH), 120.4 (CH), 130.4 (2x CH), 131.8 (2x CH), 131.9 (CH), 132.4 (C), 134.1 (C), 135.0 (C), 136.9 (C), 138.2 (C), 138.9 (C), 155.9 (C-O), 156.1 (C-O), 156.4 (C-O)

5.3 Biochemical Protocols

5.3.1 Expression and Purification of ERα and ERβ LBD

5.3.1.1 Cell Transformation and Growth

One microlitre of the pET-15b plasmid was added – each separately – to 15µl of the E. coli bacteria BL21(DE3) in an Eppendorf cup and incubated on ice for 15 minutes. This DNA-cell-mixtures was heated at 37°C for three minutes, supplemented with 900µl LB medium, and incubated at 37°C for 1hr with constant shaking (180rpm). For the growth of cell colonies 75µl of the cell suspension were evenly spread on a petri-dish containing LB agar supplemented with amicillin. The petri-dish was incubated upside down at 37°C overnight.
For the amplification the cell culture 1ml of LB medium in sterile flask, supplemented with 1µl ampicillin, was inoculated and put in an incubator at 37°C overnight with constant shaking (180rpm). On the next day the transformed cells can either be used directly for protein expression (cf. section G5.3.1.2) or they can be harvested by centrifugation at 4000 x g at 4°C for 20 minutes, shock-frozen in a dry-ice/ethanol mixture and stored at –80°C.

5.3.1.2 Protein Expression and Determination of Protein Solubility

In a sterile tube 1ml of LB medium supplemented with 1µl ampicillin was inoculated with the transformed BL21-(DE3) cells and amplified at 37°C overnight with constant shaking (180rpm). For the inoculation the cells should not be thawed completely, but defrosted only a little on the surface. The overnight cell suspension was used completely to inoculate 50ml of LB medium supplemented with 50µl ampicillin. The resulting cell suspension was incubated at 37°C with vigorous shaking (300rpm) for approximately 2hrs until an OD_{600} of 0.5 – 0.7 was reached (measured against LB medium as background). Prior to induction 25µl sample of

the cell culture stored at –20°C until needed as non-induced control for SDS-PAGE. Finally, the protein expression was induced by adding 25µl of IPTG (0.5mM final concentration). The cell culture was incubated again at 37°C with constant shaking (180rpm). After app. 3 hours the cells were harvested by centrifugation at 4000 x g at 4°C for 20min.
For extraction of the protein the supernatant was discarded, the cell pellet resuspended in 5ml of lysis buffer and frozen at –20°C overnight. On the next day the lysate was thawed and sonicated 6 x 10s with 10s pauses at 200-300W. The samples were constantly kept on ice. To separate the soluble fraction from the insoluble material the lysate was centrifuged at 10000 x g at 4°C for 10min. The supernatant was decanted (soluble fraction) and the pellet of the cell mass was resuspended in 5ml PBS (insoluble fraction). Both solutions were stored at –20°C until they were subjected to the purification process (cf. section G5.3.1.3). Samples of these two fractions together with the non-induced control were analysed by means of SDS-PAGE (cf. section G5.3.1.4) to determine, if expression of the target protein had occurred and if the protein is soluble or not.

5.3.1.3 Protein Purification

The target protein proved to be soluble and was therefore purified by means of Ni-NTA affinity chromatography under native conditions. To set up the column (size: 10x1cm) a suspension of Ni-NTA agarose in 30% EtOH was gently shaken and poured into the column (5cm). The Ni-NTA agarose was washed for about 30min with Ni-NTA wash buffer to remove the entire EtOH. Then, the protein lysate was loaded onto the column. After the lysate was taken up by the agarose it was washed with 40ml of Ni-NTA wash buffer. Finally, the bound target protein was eluted with 20ml Ni-NTA elution buffer. To control the presence of protein in the collected fractions 4µl of sample solution were mixed with 40µl of Bradford reagent on a piece of parafilm by gently pipetting. The protein containing fraction were analysed by SDS-PAGE.

5.3.1.4 Gel Electrophoresis

5.3.1.4.1 Sample Preparation

The non-induced sample was defrosted and diluted with 25µl of deionised water and 12.5µl 4xSDS buffer. 20µl of the soluble fraction were mixed with 5µl 4xSDS-buffer and finally

10µl of the insoluble fraction with 3µl 4xSDS buffer. All the samples were heated at 95°C for about 5min to achieve denaturation of the proteins.

5.3.1.4.2 SDS-PAGE

The separation gel (15%) was prepared following a standard protocol. It was mixed by gently pipetting and filled between the electrophoresis glass plates up to four fifth. The gel was topped with a layer of isopropanol to create an even surface. After polymerisation the alcohol was removed and the remaining space between the glass plates was filled with stacking gel. Before polymerisation, the electrophoresis comb was put into the gel to create little spaces to be filled with 20µl of the prepared samples and 7µl of the protein ladder as reference [Laemmli, 1970]. Finally the electrophoresis chamber was equipped with the completely prepared glass plates, filled with 1xSDS buffer, closed and connected to a DC power supply. The gel was run at 150V for about 1.5hrs.
The protein bands were stained by means of the PAGE staining solution containing Coomassie blue for 2-4 hours and destained after some initial washings with tap water with PAGE destaining solution.

5.3.1.5 Bradford Protein Assay

The determination of the protein content or the total amount of purified protein, respectively, was done following the recommendations of Bradford [1976]. In polystyrol cuvets 20µl of protein solution were added to 480µl of Bradford dye reagent 1x and incubated at ambient temperature for 10min. Then, the extinction was measured by means of an UV-spectrophotometer at 600nm after 3s. The standard curve required to calculate the exact protein concentration was done in duplicate using a BSA-solution of 0.5mg/ml concentration.

5.3.2 Radiometric Binding Assay

For the radiometric binding assays two receptor sources were used. Either the recombinant full-length human receptor proteins ERα and ERβ or the LBDs of both receptor subtypes expressed and purified at the Trinity College Dublin (cf. section G3.1).The assay was performed according the procedure described in detail in section E3.1.1.

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