Synthesis of Diarylethenes by Cycloaddition & GABA-Amides and Photoswitchable $GABA_A$-Receptor Ligands

Dissertation

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The experimental part of this work was carried out between January 2010 and September 2013 under the supervision of Prof. Dr. Burkhard König at the Institute of Organic Chemistry, University of Regensburg.

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„Das Runde muss ins Eckige“
Sepp Herberger

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Chapter 1

General Introduction

1.1 Photochromism

Photochromism is defined as the reversible conversion of a compound between two states **A** and **B** by irradiation with light.[1] A photochromic system can be converted by irradiation with light (h*ϑ_1) from state **A** to state **B**. The reverse reaction from **B** to **A** can be induced by irradiation with light of a different wavelength (h*ϑ_2). The two photoisomers have different chemical and physical properties like different absorption wavelengths resulting in different colours. Figure 1 shows the UV-Vis absorption spectra of the conversion of a photochromic diarylethene derivative from state **A** to state **B**.

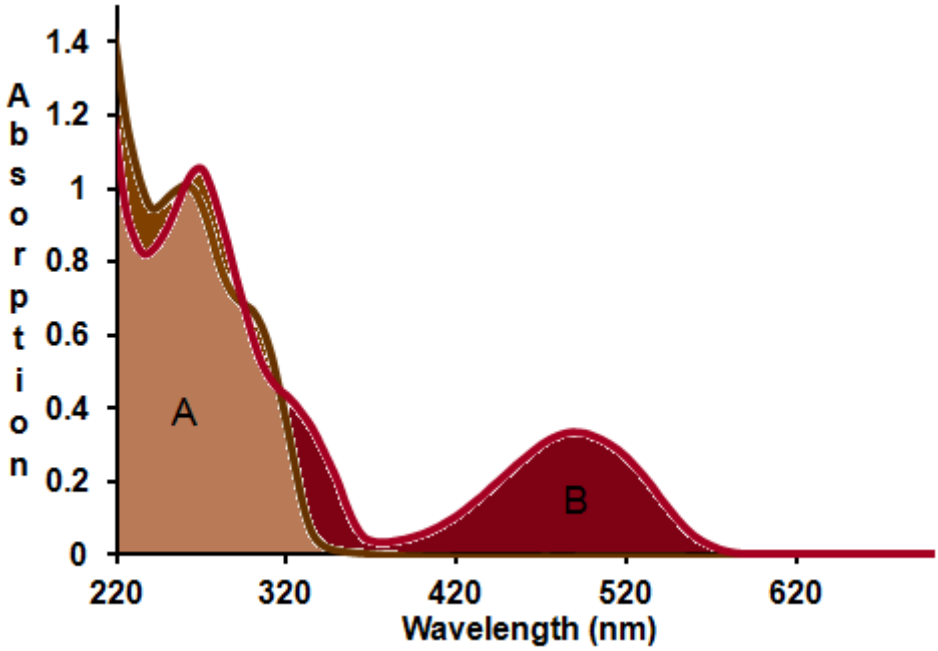
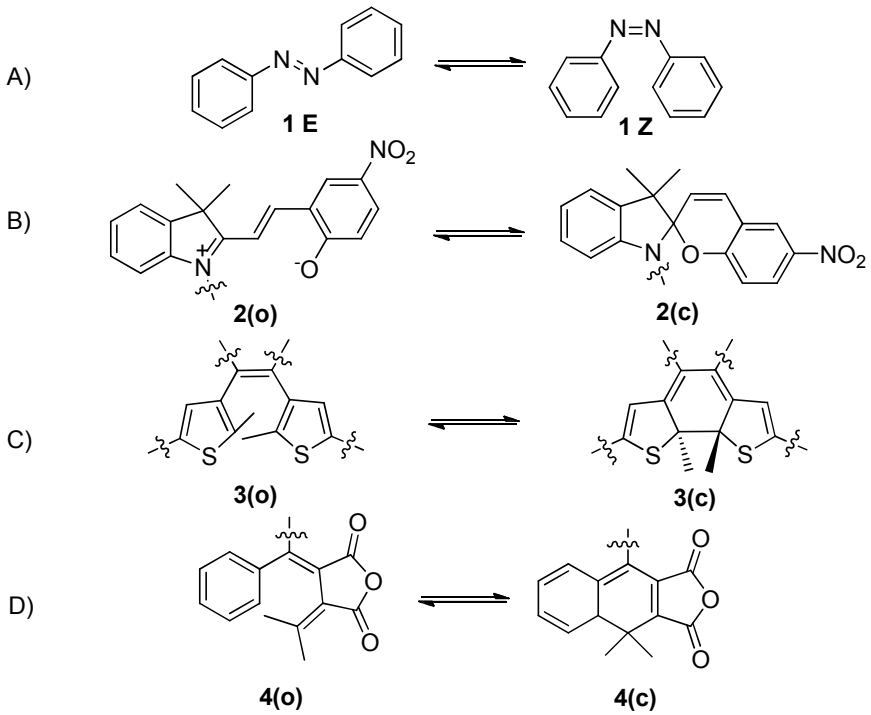


Figure 1. UV-Vis absorption spectra of a photoisomerization reaction of a diarylethene with open state **A** and closed state **B**.

1.2 Molecular Switches

Molecular switches are photochromic compounds that can be toggled between two different isomers by an external stimuli like light, heat or electrons.[2] Reversibly photoswitchable compounds are interesting for applications in optoelectronic data storage, as active parts of organic polymers with switchable refractive indices or as photochromic moieties used to

control biological processes.[3,4] There are different classes of reversibly photswitchable compounds known like the azobenzenes,[5] spiropyrans,[6] dihydropyrans,[7] fulgides and diarylethenes (Scheme 1).[2, 3, 8]



Scheme 1. Different examples of photo switches. A) Azobenzenes; B) Dihydropyrans; C) Diarylethenes; D) Fulgides.

Many of this photoswitches find applications in different fields and areas, but the use of some of these compounds is limited by their chemical resistance, thermal stability and difficulties during synthesis of functionalized derivatives.[2]

1.3 Diarylethenes

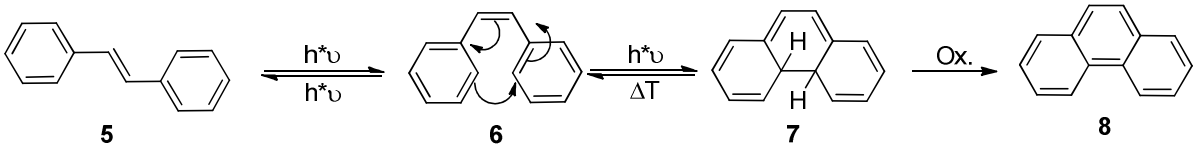
1.3.1 Photo Characteristics

One promising class of molecular switches are the diarylethenes because they exhibit high stability in both isomers, high photostationary states, high quantum yields and many of them have a high fatigue resistance.[3]

1.3.2 Stilbene

The simplest sample of a diarylethene is stilbene **6** (Scheme 2).[3] The ring open isomer **6** can rotate around the sigma bonds which are connecting the central double bound with the phenyl-groups making it flexible. According to the Woodward-Hoffmann-rules,[9] UV-irradiation induces a conrotatory 6-∏-electrocyclic reaction generating the conjugated

dihydrophenanthrene **7**. The closed isomer is more rigid than the open isomer and almost planar to the conjugated ∏-system.[3]



Scheme 2. Isomerization of stilbene and oxidation of dihydrophenanthrene.

Beside the 6-∏-cyclization reaction, cis-stilbene undergoes an UV induced cis/trans isomerization reaction to trans-stilbene. Moreover, the closed isomer **7** is thermally not stable and reisomerizes immediately back to the open isomer at room temperature. Furthermore dihydrophenanthrene **7** can easily be oxidized to phenanthrene by oxygen or the oxidation may even occour spontaneously.[3]

1.3.3 Dithienylethenes

One popular class of diarylethenes are the dithienylethenes which exhibit a configuration that provides them promising photochromic properties (Figure 2).

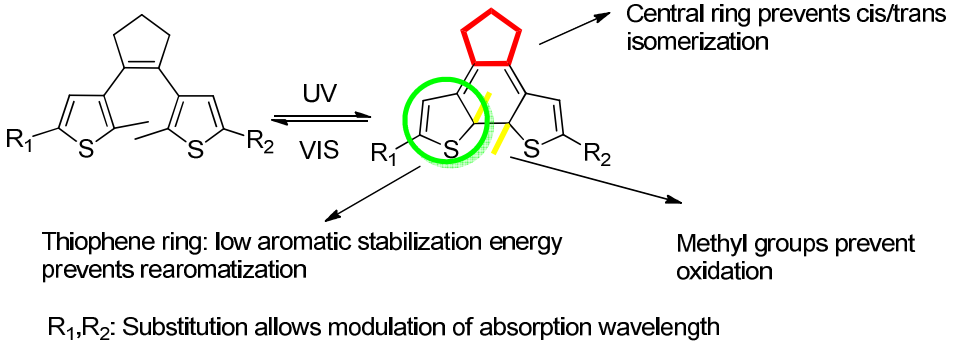


Figure 1. Configuration of a typical used dithienylethene.

The central ring stabilizes the cis-conformation and prevents the cis/trans isomerization reaction. In many cases thiophenes are used as aryl unit because they have a small aromatic stabilization energy and this lead to thermally stable photochromic diarylethenes.[2]

Table 1. Aromatic stabilization energy of different aryl groups.[1]

Aryl group	E_{Ar} [kJ/Mol]
Phenyl	115.9
Pyrrolyl	57.8
Furyl	38.1
Thienyl	19.7

The 2-position of the thiophenes is mostly substituted with methyl groups to prevent the oxidation of the closed isomer. Substitution of the 5-position with different substituents offers

the possibility to adjust the absorption wavelength of the conjugated ∏-system and to insert different anchor groups.

1.3.4 Diarylethenes in this Thesis

Diarylethenes are promising photochromic compounds and exhibit advanced photochemical properties. To get a better accessibility to this compound class and in order enlarge the scope of synthetic routes, a main part of this thesis is focused on the development of new synthetic routes. Chapter 2 and 3 deal with cycloaddition routes to the synthesis of reversibly photoswitchable diarylethenes. Chapter 5 of this thesis demonstrates the use of diarylethenes for biological applications and a diarylethene unit was used to synthesize a photoswitchable ligand with potential effect on the activation of the $GABA_A$-receptor. Such a ligand could be a helpful tool to explore the functions of this ionic channel more detailed.

1.4 References

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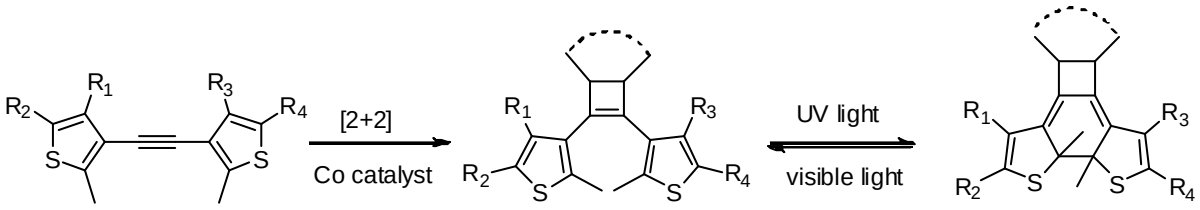
Chapter 2

Synthesis and Photoisomerization of Diarylcyclobutenes



Symmetrically and unsymmetrically substituted diarylcyclobutenes are synthesized in 20 – 70 % yield from alkyne precursors via cobalt-catalyzed [2+2] cycloadditions. The reactions proceed under mild conditions and provide access to differently substituted diarylethene derivatives. All the diarylcyclobutene products undergo reversible photoisomerization upon irradiation with UV/Vis light. The ring-closed isomers show different thermal stabilities towards reisomerization with half-lives ranging from 9 to 300 hours.

The results described in this chapter are published:

Raster, P.; Weiß, S.; Hilt, G.; König, B. *Synthesis* **2011**, 905.

Stefan Weiss synthesized norbornene derivative **6**.

2.1 Introduction

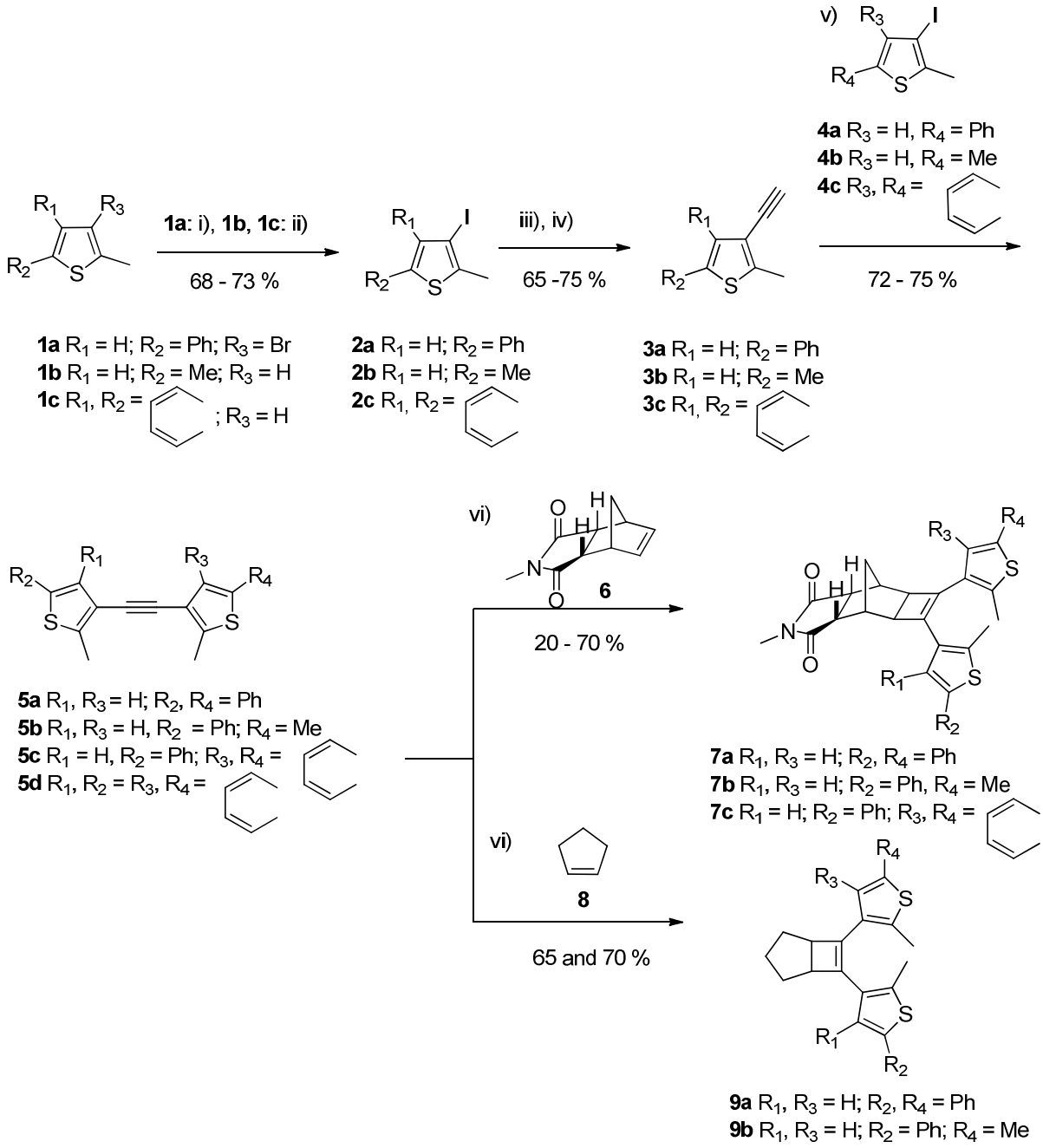
Reversibly photoswitchable compounds are interesting for applications in optoelectronic data storage, as active parts of organic polymers with switchable refractive indices,[1] or as photochromic moieties used to control biological processes.[2] One recent example is a diarylethene based photoswitchable inhibitor for the enzyme human carboanhydrase.[3] With some diarylethenes both isomers show high fatigue resistance and good thermal stability, which is important for a variety of applications. However, the synthesis of derivatives of higher complexity is challenging, as known routes are either not versatile enough, or do not tolerate certain functional groups.[4] The most convenient routes for diarylethene synthesis were reported by Feringa and Irie.[5,6] The key step in the Feringa route is a McMurry coupling reaction using the corresponding diarylketone precursors to afford a five-membered ring system.[7] The Irie route uses octaflourocyclopentene as the starting material, which is substituted with the appropriate lithiated aryl compounds giving fluorinated cyclopentenes.[8] Our goal was to establish a new route for the synthesis of symmetrically and unsymmetrically substituted diarylethenes. The described approach uses diarylalkynes, obtained via Sonogashira coupling, and a cobalt-catalyzed [2+2]-cycloaddition reaction to give the photochromic diarylethenes. This reaction sequence leads to diarylethenes bearing a cyclobutene moiety,[9-16] a group of compounds not widely studied so far and allows the simple preparation of symmetrically and unsymetrically substituted derivatives. The photochromic properties of the new compounds were investigated.

2.2 Results and Discussion

2.2.1 Synthesis

Symmetrically and unsymmetrically substituted diarylethenes **7a–c**, **9a** and **9b**, with a central cyclobutene moiety, were prepared as shown in Scheme 1. Thiophene derivatives **1a-c** were converted into iodoarenes **2a-c** by bromine–iodine exchange using n-butyllithium and iodine (for **1a**), or aromatic substitution with iodine and iodic acid (for **1b** and **1c**). Both reactions yielded the desired products in about 70 % yield. Palladium-catalyzed Sonogashira coupling of compounds **2** with ethynyltrimethylsilane, followed by cleavage of the trimethylsilyl protecting group with potassium carbonate gave alkynes **3** in good yields. Using a second Sonogashira cross-coupling reaction, terminal alkynes **3** were reacted with thiophenes **4** to yield diarylalkynes **5** as precursors for the subsequent cobalt-catalyzed [2+2] cycloaddition. This reaction was carried out under very mild conditions using the cobalt catalyst previously reported by Hilt.[17-19] At room temperature, zinc was used as the reducing agent to generate the active catalytic cobalt(I) species. Maleimide derivative **6** and cyclopentene **8** were used

as the alkene components in the cycloaddition reaction. The yield of the reaction strongly depends on the nature of the diarylalkyne and its aryl substitution pattern.



Scheme 1. i) n-BuLi, Et_2O, I_2; ii) I_2, HIO_3, H_2O, AcOH, CCl_4; iii) $Me_3SiC{\equiv}CH$, $Pd(PPh_3)_2Cl_2$,Ph_3P, CuI, Et_3N, THF; iv) K_2CO_3, MeOH; v) $Pd(PPh_3)_2Cl_2$, Ph_3P, CuI, Et_3N, THF; vi) $Co(dppp)Br_2$, ZnI_2, Zn, CH_2Cl_2.

Cycloaddition reactions of alkenes **6** and **8** with diarylalkynes **5a** and **5b** gave the corresponding products **7a**, **7b**, **9a** and **9b** in yields of 65-70 %, while benzothienyl substituted diarylalkynes **5c** and **5d** each reacted sluggishly, and only product **7c** was isolated in a poor 20 % yield.

2.2.2 Photoisomerization and Photobleaching of 7a-c, 9a and 9b

All the synthesized diarylethenes underwent reversible photochromic ring-closing reactions in dichloromethane on irradiation with UV light (320 nm), and ring-opening in the presence of visible light. The absorption properties and thermal stabilities of the ring-closed forms of compounds **7a–c**, **9a** and **9b** are summarized in Table 1.

Table 1. Photophysical Data of Compounds **7a–c**, **9a** and **9b.**

entry	λ_{max}(nm) (ϵ 10^4) (open form)	λ_{max}(nm) (closed form)	Thermal stability at r.t. [$t_{1/2}$ (h)]
7a	287 (3.3)	534	8.9
7b	288 (1.5)	495	301.3
7c	265 (4.1)	490	133.0
9a	287 (4.1)	534	8.9
9b	288 (3.3)	495	300.1

The ring-closed diarylethenes **7** and **9** can switch back to the ring-open isomers in the presence of visible light, and undergo a slow thermal cycloreversion reaction in the dark. The thermal ring-opening of related four membered ring systems has been described in the literature.[9] The half-lives of the ring-closed isomers depend on the aromatic stabilization energies gained upon conversion into the ring-open isomer, and therefore on the thiophene substitution pattern.[20] The thermal stabilities range from $t_{1/2}$ = 8.9 h for compound **7a** to $t_{1/2}$ = 300 h for the unsymmetrically substituted derivative **7b**. The photoisomerization reactions were performed several times and a loss of about 50 % of the initial absorption intensity due to photodecomposition was observed after 10 irradiation cycles. Figure 1 (left) shows the changes in the absorption intensity of compound **7a**, at 312 nm, over 10 photoisomerization cycles.

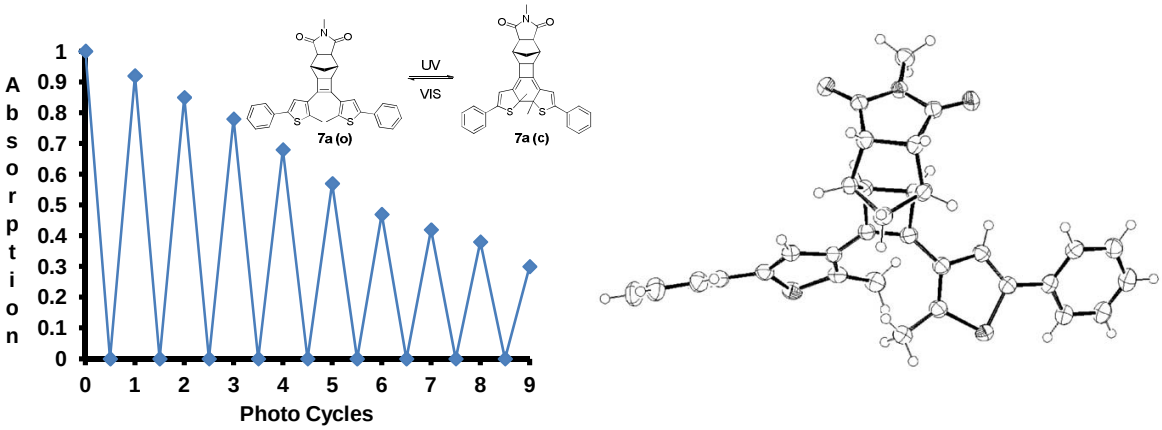


Figure 1. Left: Photoisomerization and bleaching of compound **7a** as monitored by changes in the absorption at 520 nm. Right: ORTEP representation of the X-ray crystal structure of compound **7a**.

The structure of compound **7a** was determined by X-ray crystal structure analysis (Figure 1, right). The conjugated thiophene rings are twisted due to steric interactions between the methyl substituents. The cyclobutene ring of the [2.2.1]bicycloheptene unit is exo configured, while the imide group adopts an endo orientation.

2.3 Conclusion

In summary, photoisomerizable diarylcyclobutenes were obtained from diarylalkynes via a cobalt-catalyzed [2+2] cycloaddition reaction. The efficiency of the ringclosing reaction depends on the structure of the diarylalkyne precursor, and product yields of up to 70 % were obtained. The products undergo reversible photochromic reactions in dichloromethane by alternating irradiation with UV-light and visible light. A specific advantage of the presented synthetic route is the simpler access to diarylethenes bearing two different aryl substituents.

2.4 Experimental

2.4.1 General Experimental Conditions

The following compounds were prepared according to literature methods: 3-bromo-2-methyl-5-phenylthiophene (**1a**),[21] 2-methyl- benzo[b]thiophene (**1c**),[22] 3-iodo-2-methyl-5-phenylthiophene (**2a**),[20] 3-iodo-2-methylbenzo[b]thiophene (**2c**)[21] and 1,2-bis(2- methyl-5-phenylthiophene-3-yl)ethyne (**5a**).[22] All other reagents were obtained from commercial sources. Unless otherwise noted, solvents (analytical grade) were purchased from commercial suppliers and used without further purification. Melting points were obtained using a Lambda Photometrics Optimelt MPA100 apparatus (Lambda Photometrics, Harpenden, UK), and are not corrected. IR spectra were obtained using a Varian Biorad FT-IR Excalibur FTS 3000 spectrometer. 1H NMR spectra were recorded at 300 MHz on a Bruker Avance 300 spectrometer, or at 600 MHz on a Bruker Avance III Br600 with a cryogenic probe head (Bruker, Karlsruhe, Germany). 13C NMR spectra were recorded at 75 MHz on a Bruker Avance 300 spectrometer. The NMR spectra were recorded in $CDCl_3$ as solvent and chemical shifts are reported in ppm. UV/Vis spectra were recorded using a Varian Cary BIO 50 UV/Vis/NIR spectrophotometer (Varian Inc., CA, USA). Mass spectra were obtained using Finnigan SSQ 710A (EI), Finnigan MAT 95 (CI) or Finnigan MAT TSQ 7000 (Thermo FINNIGAN, USA) (ES/LC–MS) instrumentation. Thin layer chromatography (TLC) was performed on alumina plates coated with silica gel (Merck silica gel 60 F245, thickness 0.2 mm). Column chromatography was accomplished with Merck Geduran SI 60 silica gel as the stationary phase. Petroleum ether (PE) refers to the fraction boiling at 30–60 °C.

2.4.2 Synthetic Protocols and Analytical Data of 3c, 5b-d, 7a-c, 9a and 9b

3-Ethynyl-2-methylbenzo[b]thiophene (3c)

A mixture of THF–Et_3N (48 mL, 2:1) was degassed for 10 min and then 3-iodo-2-methylbenzo[b]thiophene **2c** (5.3 g, 19.3 mmol), TMS-acetylene (5.57 mL, 38.7 mmol), $Pd(PPh_3)_2Cl_2$ (54 mg, 0.4 mol%), Ph_3P (40 mg, 0.8 mol%) and CuI (29 mg, 0.6 mol%) were added under nitrogen. The resulting solution was heated at 65 °C for 12 h. The reaction mixture was cooled, Et_2O (40 mL) and H_2O (40 mL) were added and the organic layer was separated. Subsequently, the water layer was extracted with Et_2O (2 × 40 mL). Then the organic layers were combined, dried over $MgSO_4$ and the solvent was removed. The residue was dissolved in MeOH (220 mL), K_2CO_3 (2.97 g, 21.1 mmol) was added and the resulting solution was stirred for 2 h at r.t. The reaction mixture was filtered, the solvent was removed and the residue was purified by silica gel flash chromatography (PE; Rf = 0.6) and a white solid was obtained (70 %), mp 174-176 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 2.72 (s, 3H), 3.48 (s, 1H), 7.30-7.41 (m, 2H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.86 (d, *J* = 8.0 Hz, 1H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 15.4, 82.3, 111.5, 114.8, 122.0, 122.4, 124.5, 124.7, 137.4, 140.0, 146.6 ppm; **IR** (film): υ = 2952, 2854, 1458, 1377, 1122, 933, 815 cm^{-1}; **HRMS** (EI): calculated for $C_{11}H_8S$ 172.0347; found 172.0342.

Diarylalkynes 5b-d; General Procedure (GP 1)

A mixture of THF–Et_3N (24 mL, 2:1) was degassed for 10 min and then iodothiophene **4a–c** (10.0 mmol), acetylene **3a–c** (10.0 mmol), $Pd(PPh_3)_2Cl_2$ (27 mg, 0.4 mol%), Ph_3P (20 mg, 0.8 mol%) and CuI (15 mg, 0.6 mol%) were added under nitrogen. The resulting solution was heated at 65 °C for 12 hours. The reaction mixture was cooled, Et_2O (20 mL) and H_2O (20 mL) were added and the organic layer was separated. Subsequently, the water layer was extracted with Et_2O (2 × 20 mL) and then the organic layers were combined and dried over $MgSO_4$. The solvent was removed and the residue was purified by silica gel flash chromatography (PE/EE, 9.5:0.5 v/v).

3-((2,5-Dimethylthiophen-3-yl)ethynyl)-2-methyl-5-phenylthiophene (5b)

The compound was prepared according to GP 1 yielding 75 % of **5b** as a white solid, mp 161-163 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 2.41 (s, 3H), 2.52 (s, 3H), 2.58 (s, 3H), 6.68 (s, 1H), 7.22 (s, 1H), 7.24-7.30 (m, 2H), 7.34-7.40 (m, 2H), 7.52-7.57 (m, 1H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 14.5, 14.7, 15.2, 85.6, 86.7, 119.4, 121.1, 125.2, 125.6, 127.1, 127.5, 128.9, 134.0, 135.9, 140.2, 140.9, 142.2 ppm; **IR** (film): $\bar{\nu}$ = 2337, 1605, 1301, 748, 539, 496 cm^{-1}; **HRMS** (EI): calculated for $C_{19}H_{16}S_2$ 308.0693; found 308.0690.

2-Methyl-(3-((2-methyl-5-phenylthiophen-3-yl)ethynyl)benzo[b]thiophene (5c)

The compound was prepared according to GP 1 yielding 75 % of **5c** as a brown solid, mp 168-170 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 2.64 (s, 3H), 2.71 (s, 3H), 7.23-7.42 (m, 5H), 7.54-7.58 (m, 2H), 7.73 (d, *J* = 7.7 Hz, 1H), 7.86 (d, *J* = 7.4 Hz, 2H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 14.8, 15.4, 84.8, 89.1, 116.0, 120.9, 122.1, 122.4, 124.5, 124.7, 125.2, 125.6, 127.6, 129.0, 133.9, 137.5, 139.9, 140.4, 142.6, 144.4 ppm; **IR** (film): $\tilde{\nu}$ = 2911, 2336, 2197, 1595, 837, 681 cm^{-1}; **HRMS** (EI): calculated for $C_{22}H_{16}S_2$ 344.0693; found 344.0695.

1,2-Bis(2-methylbenzo[b]thiophen-3-yl)ethyne (5d)

The compound was prepared according to GP 1 yielding 50 % of **5d** as a yellow solid, mp 155-157 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 2.77 (s, 6H), 7.30-7.48 (m, 4H), 7.75 (d, *J* = 7.9 Hz, 2H), 7.93 (d, *J* = 7.6 Hz, 2H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 15.5, 87.7, 116.0, 122.1, 122.4, 124.5, 124.8, 137.6, 139.8, 144.5 ppm; **IR** (film): $\tilde{\nu}$ = 3053, 2360, 1740, 720, 620, 557 cm^{-1}; **HRMS** (EI): calculated for $C_{20}H_{14}S_2$ 318.0537; found 318.0539.

Diarylcyclobutenes 7a-c; General Procedure (GP 2)

In a Schlenk tube, [1,3-bis(diphenylphosphino)propante]cobalt(II)bromide [Co(dppp)Br_2] (64 mg, 0.1 mmol, 20 mol%), anhydrous ZnI_2 (64 mg, 0.2 mmol, 40 mol%) and Zn powder (13 mg, 0.2 mmol, 40 mol%) were dissolved in anhydrous DCM (2 mL) under nitrogen. The alkyne **5a-c** (0.5 mmol) and alkene **6** (0.5 mmol) were added and the mixture was stirred for 24 h at r.t. DCM (10 mL) and H_2O (10 mL) were then added to the reaction mixture and the organic layer was separated. Subsequently, the water layer was extracted with DCM (2 x 10 mL) and then the organic layers were combined and dried over $MgSO_4$. The solvent was removed and the residue was purified by silica gel flash chromatography.

2-Methyl-5,6-bis(2-methyl-5-phenylthiophen-3-yl)-3a,4,4a,6a,7,7a-hexahydro-1H-4,7-methanocyclobuta[f]isoindole-1,3(2H)-dione (7a)

The compound was prepared according to GP 2 yielding 70 % of **7a** as a purple solid after purification by column chromatography on silica gel (PE/EE, 1:1 v/v), mp 223-225 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 1.50 (d, *J* = 10.7 Hz, 1H), 2.11 (d, *J* = 10.7 Hz, 1H), 2.27 (s, 6H), 2.80-2.85 (m, 2H), 2.96 (s, 2H), 3.01 (s, 3H), 3.27-3.30 (m, 2H), 7.09 (s, 2H), 7.20-7.28 (m, 2H), 7.30-7.38 (m, 4H), 7.48-7.53 (m, 4H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 14.9, 24.4, 34.7, 37.5, 44.0, 48.1, 122.8, 125.4, 127.4, 128.9, 133.7, 134.0, 134.2, 136.7, 140.4, 177.8 ppm; **IR** (film): $\tilde{\nu}$ = 2353, 2132, 1533, 1442, 1291, 1030, 883, 773, 631, 537, 498 cm^{-1}; **HRMS** (EI): calculated for $C_{34}H_{29}NO_2S_2$ 547.1640; found 547.1648.

5-(2,5-Dimethylthiophen-3-yl)-2-methyl-6-(2-methyl-5-phenylthiophen-3-yl)-3a,4,4a,6a,7,7a-hexahydro-1H-4,7-methanocyclobuta[f]isoindole-1,3(2H)-dione (7b)

The compound was prepared according to GP 2 yielding 65 % of **7b** as purple oil after purification by column chromatography on silica gel (PE/EE, 1:1 v/v). **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 1.42 (d, *J* = 10.6 Hz, 1H), 2.02 (d, *J* = 10.6 Hz, 1H), 2.12 (s, 3H), 2.21 (s, 3H), 2.32 (s, 3H), 2.74 (s, 2H), 2.82-2.90 (m, 2H), 2.94 (s, 3H), 3.22-3.30 (m, 2H), 6.50 (s, 1H), 7.01 (s, 1H), 7.16-7.20 (m, 1H), 7.25-7.30 (m, 2H), 7.43-7.47 (m, 2H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ 14.6, 14.8, 15.1, 24.4, 34.6, 37.5, 43.9, 48.2, 77.3, 122.9, 125.1, 125.4, 127.3, 128.9, 132.5, 133.3, 133.9, 134.1, 134.7, 134.9, 136.0, 136.5, 140.2, 177.8 ppm; **IR** (film): $\tilde{\nu}$ = 2927, 2361, 2253, 2168, 1769, 1695, 1598, 1434, 1136, 997, 631 cm^{-1}; **HRMS** (EI): calculated for $C_{29}H_{27}NO_2S_2$ 485.1483; found 485.1489.

2-Methyl-5-(2-methyl-5-phenylthiophe-3-yl)-6-(2-methylbenzo[b]thiophen-3-yl)-3a,4,4a,6a,7,7a-hexahydro-1H-4,7-methanocyclobuta[f]isoindole-1,3(2H)-dione (7c)

The compound was prepared according to GP 2 yielding 20 % of **7c** as purple oil after purification by preparative HPLC. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 1.28 (d, *J* = 10.7 Hz, 1H), 1.54 (d, *J* = 10.7 Hz, 1H), 2.10 (s, 3H), 2.42 (s, 3H), 2.66 (d, *J* = 5.3 Hz, 1H), 3.01 (d, *J* = 5.4 Hz, 1H), 3.03-3.05 (m, 4H), 3.20-3.21 (m, 1H), 3.25 (dd, *J* = 5.4, 9.2 Hz, 1H), 3.34 (dd, *J* = 5.4, 9.2 Hz, 1H), 6.99 (s, 1H), 7.21-7.32 (m, 5H), 7.39-7.40 (m, 2H), 7.51-7.56 (m, 1H), 7.73-7.76 (m, 1H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 14.7, 15.5, 24.4, 34.9, 37.3, 37.8, 43.8, 45.3, 47.9, 48.3, 122.0, 122.4, 123.8, 124.3, 125.4, 127.3, 128.0, 128.8, 132.6, 133.6, 133.9, 137.1, 137.8, 137.9, 138.8, 139.3, 140.3, 177.6, 177.8 ppm; **IR** (film): $\tilde{\nu}$ = 3061, 2933, 2360, 2169, 1942, 1697, 1596, 1433, 1229, 1032, 831, 686, 420 cm^{-1}; **HRMS** (EI): calculated for $C_{32}H_{27}NO_2S_2$ 521.1483; found 521.1490.

Diarylcyclobutenes 9a and 9b; General Procedure (GP 3)

In a Schlenk tube, [1,3-bis(diphenylphosphino)propane]cobalt(II)bromide [Co(dppp)Br_2] (64 mg, 0.1 mmol, 20 mol%), anhydrous ZnI_2 (64 mg, 0.2 mmol, 40 mol%) and Zn powder (13 mg, 0.2 mmol, 40 mol%) were dissolved in anhydrous DCM (2 mL) under nitrogen. The alkyne **5a** or **5b** (0.5 mmol) and cyclopentene **8** (0.045 ml, 0.5 mmol) were added and the mixture was stirred for 24 h at r.t. DCM (10 mL) and H_2O (10 mL) were then added to the reaction mixture and the organic layer was separated. Subsequently, the water layer was extracted with DCM (2 x 10 mL) and then the organic layers were combined and dried over $MgSO_4$. The solvent was removed and the residue was purified.

6,7-Bis(2-methyl-5-phenylthiophen-3-yl)bicyclo[3.2.0]hept-6-ene (9a)

The compound was prepared according to GP 3 yielding 65 % of **9a** as purple oil after purification by preparative HPLC. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 1.35-1.47 (m, 2H), 1.74-1.88 (m, 4H), 2.29 (s, 6H), 3.94 (d, *J* = 6.5 Hz, 2H), 7.18 (s, 2H), 7.21-7.26 (m, 2H), 7.32-7.37 (m, 4H), 7.51-7.54 (m, 4H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 14.8, 23.4, 26.8, 46.9, 123.2, 125.4, 127.1, 128.8, 134.3, 134.9, 135.6, 135.8, 139.9 ppm; **IR** (film): $\tilde{\nu}$ = 2361, 2170, 1740,
The compound was prepared according to GP 3 yielding 65 % of **9b** as a orange oil after purification by column chromatography on silica gel (PE/EE, 9.75:0.25 v/v). **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 1.30-1.42 (m, 2H), 1.68-1.84 (m, 4H), 2.20 (s, 3H), 2.26 (s, 3H), 2.38 (s, 3H), 3.40-3.47 (m, 2H), 6.59 (s, 1H), 7.13 (s, 1H), 7.20-7.37 (m, 3H), 7.49-7.53 (m, 2H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 14.5, 14.7, 15.2, 23.3, 26.8, 30.3, 46.8, 77.0, 123.3, 125.3, 125.4, 127.1, 128.8, 133.6, 133.9, 134.4, 134.8, 135.3, 135.5, 135.7, 136.0, 139.7 ppm; **IR** (film): $\tilde{\nu}$ = 2360, 2339, 2207, 2171, 2116, 2032, 1740, 1669, 1437, 1374, 1220, 834 cm^{-1}; **HRMS** (EI): calculated for $C_{29}H_{26}S_2$ 376.1327; found 367.1319.

2.5 References

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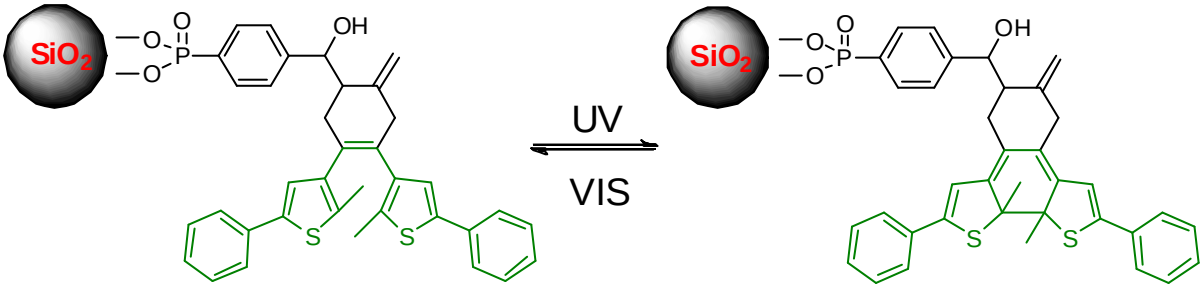
Chapter 3

Immobilization of Photoswitchable Diarylcyclohexenes Synthesized via Cobalt-Mediated Diels-Alder Reaction



(open) (closed)

Functionalized photoswitches in two steps – Photochromic dithienylcyclohexenes were prepared by a cobalt-mediated Diels-Alder reaction of internal alkynes with isoprenyl-pinacolboronic ester. The primary cycloaddition products were reacted *in situ* with different aldehydes introducing an additional functional group, which allows immobilization on cellulose or silica gel. The three-component one-pot reaction sequence provides the photochromic dithienylcyclohexenes in up to 67 % overall yield.

The results described in this chapter are published:

Raster, P.; Schmidt, A.; Rambow, M.; Kuzmanovic, N.; König, B.; Hilt, G. *Chem. Commun.* **2014**, accepted.

Natascha Kuzmanovic prepared the internal alkynes **1b-d**. Maxie Rambow synthesized diarycyclohexenes **5a** and **5c** by cobalt mediated Diels-Alder and allylboration reactions. Diarylcyclohexenes **5b** and **5d-g** were synthesized by Anastasia Schmidt.

3.1 Introduction

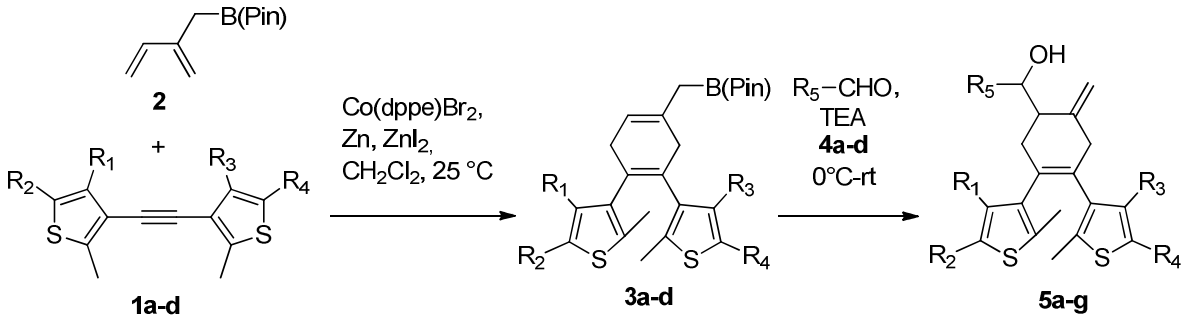
Photochromic compounds find increasing interest in material science[1-3] or as tools in molecular biology.[4-6] Recent applications are optical data storage,[7-9] materials with photoswitchable refractive indices[10-11] or conformation[12-13] and photochromic agonists or inhibitors that affect the physiological activity of ionic channels,[14] enzymes[15-16] and proteins.[17] Widely used are photochromic diarylethenes, which show in many cases high fatigue resistance, complete photoisomerization and slow thermal interconversion of the photoisomers.[18-19] However, the synthesis of diarylethenes, particular of unsymmetrically substituted compounds is laborious. Two main routes for the synthesis of diarylethenes were reported by Irie[20] and Feringa.[21] The Irie route uses volatile octafluorocyclopentene as starting material, which is reacted with the corresponding lithiated arenes. The Feringa route starts from a diarylketone precursor, which is ring-closed by a McMurry coupling reaction.[22] However, the synthesis does not tolerate certain functional groups like aldehydes and ketones. Therefore we explored cycloaddition routes[23-25] for the synthesis of photochromic diarylethenes and reported recently the cobalt catalyzed [2+2] cycloaddition reaction of diarylalkynes with cyclopentene or norbornene derivatives.[26] The method yields dithienylcyclobutenes, but their fatigue resistance is unfortunately low. Searching for more photostable cycloaddition products we investigated the cobalt-mediated Diels-Alder cycloaddition of dithienylacetylenes **1a-d** with boroprene **2** [= isoprenylpinacolboronic ester **2** (Pin = pinacole)][27] giving the dihydroaromatic intermediates **3a-d** (Table 1). The allylboronic ester reacts *in situ* with aldehydes **4a-d** affording photochromic diarylcyclohexenes **5a-g**. This last step of the synthesis allows the facile introduction of a variety of moieties or functional groups modifying the backbone of the photoswitch without altering its photophysical properties. The modification can be used to introduce specific labels, probes, binding sites or anchoring groups for immobilization. For the Diels-Alder-type [4+2] cycloaddition, an established catalyst system consisting of Co(dppe)Br_2, zinc iodide and zinc powder in dichloromethane was used.[28]

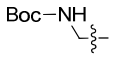
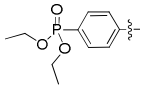
3.2 Results and Discussion

3.2.1 Synthesis

The catalyst converts a range of dithienylalkynes **1a-d** and boroprene **2** under mild reaction conditions into the dihydroaromatic dithienylethenes **3a-d**. The products of the cycloaddition reaction are subsequently reacted without isolation with different aldehydes **4a-d** yielding the desired diarylcyclohexenes **5a-g** in moderate to good overall yield. The results of this three-component one-pot reaction sequence are summarized in Table 1.

Table 1. Synthesis of functionalized dithienylethenes **5a-g** by [4+2] cycloaddtion.



entry	Alkyne	R^1	R^2	R^3	R^4	Aldehyde	R^5	Yield
1	**1a**	H	Ph	H	Ph	**4a**		**5a** (64%)
2	**1a**	H	Ph	H	Ph	**4b**		**5b** (29%)
3	**1a**	H	Ph	H	Ph	**4c**		**5c** (58%)
4	**1a**	H	Ph	H	Ph	**4d**		**5d** (67%)
5	**1b**	H	Cl	H	Cl	**4a**		**5e** (32 %)
6	**1c**			H	Ph	**4a**		**5f** (5 %)[a]
7	**1d**	H	Me	H	Ph	**4c**		**5g** (30 %)[b]

(a) The product could not be isolated and the yield determined by LC-MS was 5 %. (b) Compound **5g** was obtained as regioisomeric mixture **A** : **B** = 59 : 41 %.

3.2.2 Photoisomerization and Photobleaching of 5a-e and 5g

Compounds **5a-e** and **5g** photoisomerize reversibly in solution upon irradiation with light of 312 nm wavelength. This is exemplarily shown for compound **5d** in Figure 1 (left). The closed isomers are stable for days in the dark and isomerize quantitatively to the open forms upon irradiation with a 200 W tungsten light source that was passed through a 420 nm cut-off filter to eliminate shorter wavelengths. Diarylethenes **5a-e** and **5g** photodegrade in solution. After

10 photoisomerization cycles the absorption of the chromophore at 530 nm is reduced by about 60 % of the original vaule. This is exemplarily shown for compound **5d** in Figure 1 (right).

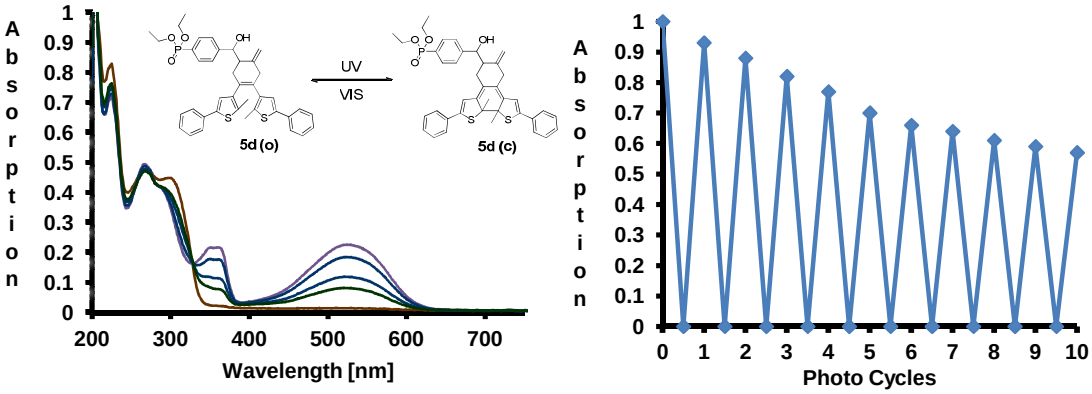
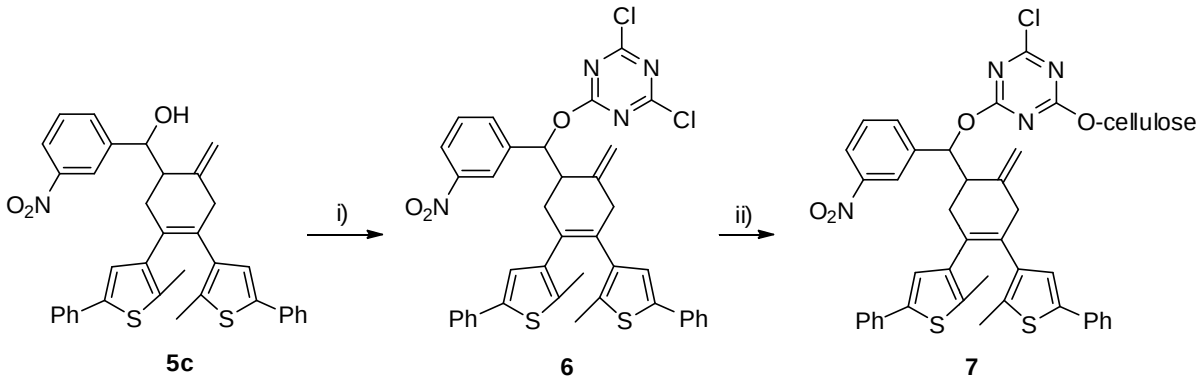


Figure 1. Left: UV/vis absorption spectrum of compound **5d** (c = $3.0 \cdot 10^{-5}$ mmol/L in MeOH). Irradiation of diarylcyclohexene **5d** with UV-light (312 nm) in MeOH. Irradiation times were 15, 30, 45 and 60 s. Right: Decreasing absorption at 530 nm of diarylethene **5d** in MeOH during repeated photoisomerization.

3.2.3 Immobilization

The reaction sequence yields a secondary hydroxyl group, which can be used for facile subsequent modifications and surface immobilization of the chromophores without affecting their photophysical properties. Reactive dye **6** was obtained from diarylcyclohexene **5c** and cyanuric chloride under basic conditions (Scheme 1).



Scheme 1. Immobilization of diarylethene **5c** on cellulose. i) cyanuric chloride, K_2CO_3, THF; ii) cellulose, K_2CO_3, DMF.

The sensitive compound was isolated by flash column chromatography. For the immobilization of **6** a cellulose sheet was heated for 12 hours at 60 °C with the crude reactive dye and potassium carbonate in DMF (Scheme 1). To remove not immobilized chromophore molecules, the cellulose sheet was washed with dichloromethane, acetone and ethanol until

no dye could be detected in the rinsing solution. After drying, the cellulose sheet **7** showed visible photochromic properties upon irradiation with UV-light and visible light for several cycles (Figure 2).

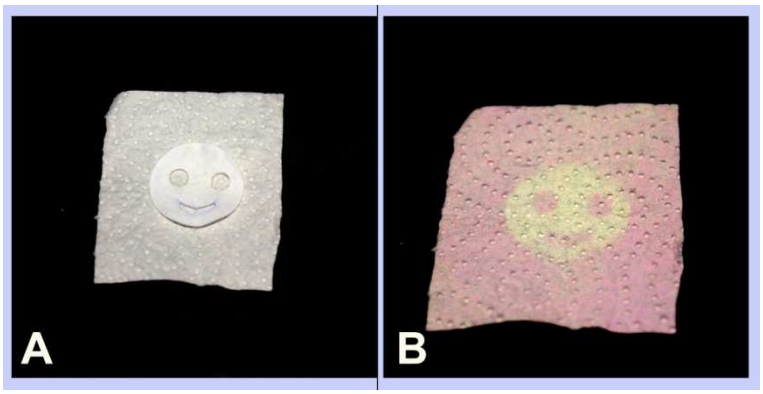


Figure 2. A photochromic cellulose sheet. Irradiation with low intensity UV-light (312 nm) converts the sheet from A to B; day light irradiation converts it back from B to A.

The average dye loading of the cellulose sheet was determined gravimetrically to be $3.0 \pm 0.8 \cdot 10^{-4}$ $mmol_{dye}/mg_{cellulose}$. This corresponds to about 5 % conversion of the cellulose primary hydroxyl groups. Dithienlyethene **5d** bearing a phosphonate ester was used to prepare a photochromic pigment on SiO_2. Silica gel powder (0.04−0.063 mm) was incubated with a solution of diarylcyclohexene **5d** in dichloromethane for 12 h. The reaction mixture was filtered and washed with dichloromethane and ethanol until no coloration of the rinsing solvent was detected after irradiation with UV-light. The dye loading of the SiO_2 was determined by elemental analysis to be $2.2 \cdot 10^{-5}$ $mmol_{dye}/mg_{silicagel}$. The functionalized SiO_2 powder is reversible photochromic and showed a broad absorption at 522 nm after UV-irradiation (Figure 3). Suspending the coated silica gel in water with poly-2-ethyloxazoline polymer (200.000 g/mol) gave a photochromic paint. Unfortunately, the immobilized chromophores bleach more quickly than in solution and after three photocycles the relative absorption of the closed isomer decreased to less than 50 %. The immobilization of compound **5d** on TiO_2 (P-25) semiconductor using the same protocol gave particles that did not show photochromic behaviour upon irradiation with UV-light.

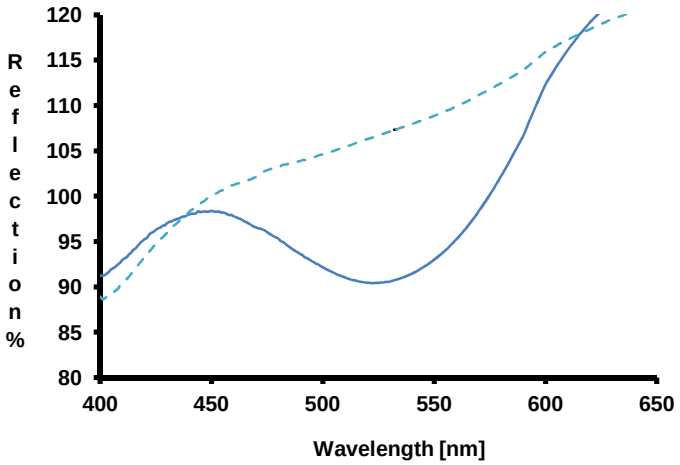


Figure 3. Solid state UV-spectra changes of SiO_2 with immobilized compound **5d** upon irradiation with UV-light of 312 nm and visible light. Dashed line: Spectrum before UV-irradiation. Solid line: Spectrum after 30 s UV-irradiation.

3.3 Conclusion

In conclusion, we have developed a new cycloaddition route for the synthesis of reversible photochromic dithienylcyclohexenes in a three-component one-pot reaction. The chromophores were generated via a cobalt-mediated Diels-Alder reaction of internal dithienylalkynes with isoprenylpinacolboronic ester under mild reaction conditions followed by an allylboration with various aldehydes. The aldehydes allow the facile introduction of different functional groups without affecting the chromophore. Applications of the functionalized dithienylethenes were demonstrated by immobilizing the chromophore onto cellulose and silica gel powder retaining their photochromic properties. The reported reaction sequence facilitates the preparation of functionalized photochromic dithienylethenes and may thereby increase their availability for photonic applications.

3.4 Experimental

3.4.1 General Experimental Conditions

The following compounds were prepared according to literature methods: 1,2-bis(2-methyl-5-phenylthiophene-3-yl)ethyne (**1a**),[26] 2-methyl-3-((2-methyl-5-phenylthiophen-3-yl)ethynyl)-benzo[b]thiophene (**1c**),[26] 3-((2,5-dimethylthiophen-3-yl)ethynyl)-2-methyl-5-phenylthiophene (**1d**),[26] 4,4,5,5-tetramethyl-2-(2-methylenebut-3-en-1-yl)-1,3,2-dioxaborolane (**2**).[27] All other reagents were obtained from commercial sources. IR spectra were obtained using an IFS 200 Interferometer or an Alpha-P FT-IR-spectrometer both manufactured by Bruker Physics. ^{1}H NMR spectra were recorded either on an AV-300 (300 MHz) or DRX-500 (500 MHz) both manufactured by Bruker Physics. ^{13}C NMR spectra were recorded on either an AV-300 (75

MHz or an DRX-500 (150 MHz) both manufactured by Bruker Physics. The NMR spectra were recorded in $CDCl_3$ or MeOD as solvent and chemical shifts are reported in ppm. GC/MS spectra were recorded utilizing an Agilent 6890 GC-System coupled with a Hewlett Packard 5973 Mass Selective Detector. Ionization was accomplished by electron ionization (EI) at an energy of 70 eV. The detected ion masses (m/z) are reported in u corresponding to the intensity of the signals as a percentage of the most intense signal. High-resolution mass spectra (HRMS) were recorded as electron ionization spectra (EI/HRMS) at an energy of 70 eV on a Finnigan MAT 95S Mass Spectrometer or as electron spray ionization (ESI/HRMS) on a Micromass VG AutoSpec Mass Spectrometer. The detected ion masses (m/z) are given in u. Thin layer chromatography (TLC) was carried out on prefabricated plates (silica gel 60, F254 with fluorescence indicator) manufactured by Merck. Flash chromatography (FC) was carried out on silica gel 60 (40 – 64 µm, 230 – 400 mesh ASTM) purchased from Macherey-Nagel. Petroleum ether (PE) refers to the fraction boiling at 30-60 °C.

3.4.2 Synthetic Protocols and Analytical Data of 1b, 4c and 5a-g

1,2-Bis(5-chloro-2-methylthiophen-3-yl)ethyne (1b)

A mixture of NEt_3 (10 mL) and THF (20 mL) was degassed for 10 min and finally 5-chloro-3-iodo-2-methylthiophene (2.97 g, 11.5 mmol), 5-chloro-3-ethynyl-2-methylthiophene (1.20 g, 7.7 mmol), bis-triphenylphosphinpalladium(II)-chlorid (110 mg, 2 mol %), triphenylphosphine (80 mg, 4 mol %) and CuI (60 mg, 4 mol %) were added under nitrogen and the solution was refluxed for 24 h at 60 °C. The solution was allowed to warm up, the organic phase was separated, washed with water (2 x 50 ml) and dried over $MgSO_4$. The solvent was evaporated and the precipitate was purified by silica gel chromatography (PE) and a white solid was obtained. Yield: (2.0 g, 64 %), mp 64-66 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 2.37 (s, 6H), 6.80 (s, 2H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 14.5, 77.2, 115.1, 125.8, 127.9, 141.8 ppm; **HRMS** (EI): calculated for $C_{12}H_8Cl_2S_2$ 285.9445; found: 285.9443.

Diethyl(4-formylphenyl)phosphonate (4c)

A mixture of triethyl phosphite (1.35 g, 7.4 mmol), 4-bromobenzaldehyde (1.36 g, 7.4 mmol) and $NiBr_2$ (35 mg, 0.16 mmol) was heated for 4 h at 170 °C. The reaction mixture was finally purified by flash column chromatography on silica gel (PE/EE, 9:1 v/v), followed by vacuo short path distillation. A colorless oil was obtained. Yield: (1.03 g, 33 %). Analytics is in good agreement to literature reported values.[29]

Diarylcyclohexenes 5a-g; General Procedure (GP 1)

Anhydrous zinc iodide (2.0 eq.), zinc powder (2.0 eq.) and Co(dppe)Br_2 (1.0 eq.) were suspended in anhydrous dichloromethane (concentration of Co(dppe)Br_2: 0.2 M) under an

argon atmosphere in a flame dried Schlenk tube fitted with a teflon screwcap. The internal alkyne **1a-d** (1.0 eq.) and 4,4,5,5-tetramethyl-2-(2-methylenebut-3-enyl)-1,3,2-dioxaborolane (= boroprene, 3.0-5.0 eq.) were added and the mixture was stirred at room temperature until complete conversion of the starting materials was indicated by TLC. The reaction mixture was cooled to 0 °C, the aldehyde **4a-d** (4.0–10.0 eq.) was added and the mixture was stirred at 0 °C. The progress of the reaction was monitored by TLC. Triethanolamine (1.2 eq. based on the dioxoborolane) was added at room temperature. After stirring for 1 h the reaction mixture was adsorbed on a small amount of silica gel and purified by column chromatography to afford the desired compound.

(*E*)-1-(3,4-Bis(2-methyl-5-phenylthiophen-3-yl)-6-methylencyclohex-3-en-1-yl)but-2-en-1-ol (5a)

The compound was prepared according to GP 1 yielding 64 % of **5a** as brownish solid after purification by column chromatography on silica gel (Pentane/Et_2O, 1:1 v/v). **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 1.80 (dd, *J* = 1.5, 6.4 Hz, 3H), 1.85 (d, *J* = 2.0 Hz, 1H), 2.01 (s, 3H), 2.04 (s, 3H), 2.53-2.69 m (2H), 3.14-3.33 (m, 2H), 4.29 (t, *J* = 8.9 Hz, 1H), 5.12 (d, *J* = 7.6 Hz, 2H), 5.55 (ddd, *J* = 1.5, 8.3, 15.2 Hz, 1H), 5.84 (qd, *J* = 6.4, 12.9 Hz, 1H), 6.90 (s, 1H), 7.00 (s, 1H), 7.20-7.24 (m, 2H), 7.30-7.35 (m, 4H), 7.43-7.49 (m, 4H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 14.3, 14.4, 17.9, 35.1, 37.3, 48.2, 72.9, 111.7, 124.1, 124.2, 125.3, 127.0, 128.8, 129.8, 130.2, 132.3, 134.1, 134.1, 134.5, 139.4, 139.6, 139.8, 144.7 ppm; **IR** (KBr): $\tilde{\nu}$ = 3434, 3062, 3022, 2911, 2243, 1947, 1871, 1798, 1651, 1598, 1499, 1437, 1376, 1304, 1200, 1159, 1116, 1076, 1004, 965, 902, 840, 754, 728, 689 cm^{-1}; **HRMS** (EI): calculated for $C_{33}H_{32}OS_2$: 508.1895; found: 589.1898.

***tert*-Butyl (2-(3,4-bis(2-methyl-5-phenylthiophen-3-yl)-6-methylenecyclohex-3-en-1-yl)-2-hydroxyehtyl)carbamate (5b)**

The compound was prepared according to GP 1 yielding 25 % of **5b** as purple solid after purification by column chromatography on silica gel (Pentane/Et_2O, 1:1 v/v). **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 1.47 (m, 9H), 2.02 (s, 6H), 2.46-2.84 (m, 4H), 3.11-3.33 (m, 3H), 3.62-3.69 (m, 1H), 3.99 (t, *J* = 7.1 Hz, 1H), 5.02-5.11 (m, 2H), 5.12 (d, *J* = 0.9 Hz, 1H), 6.99 (s, 1H), 7.17-7.25 (m, 3H), 7.28-7.36 (m, 4H), 7.44-7.58 (m, 4H) ppm; **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.5 (2H), 28.5, 35.2, 38.1, 44.7, 45.6, 71.0, 79.8, 111.8, 124.1, 124.8, 125.4 (2C), 127.0 (2C), 128.9 (2C), 129.9, 130.4, 134.2, 134.5, 134.6 (2C), 139.5, 139.7 (2C), 139.8, 144.6, 156.7 ppm; **IR** (film): $\tilde{\nu}$ = 3426, 3065, 2972, 2971, 2857, 1701, 1599, 1503, 1442, 1397, 1367, 1251, 1166, 1094, 1033, 898, 846, 756, 692 cm^{-1}; **HRMS** (EI): calculated for $C_{36}H_{39}NO_3S_2$ + Na^+ 620.2264; found 620.2261.

(3,4-Bis(2-methyl-5-phenylthiophen-3-yl)-6-methylencyclohex-3-enyl)(3-nitrophenyl)-methanol (5c)

The compound was prepared according to GP 1 yielding 58 % of **5c** as purple solid after purification by column chromatography on silica gel (Pentane/Et_2O, 1:1 v/v). **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 2.02 (s, 3H), 2.09 (s, 3H), 2.53-2.62 (m, 2H), 2.79-2.85 (m, 1H), 3.23-3.42 (m, 2H), 4.99 (d, *J* = 9.8 Hz, 1H), 5.25 (s, 2H), 6.83 (s, 1H), 7.04 (s, 1H), 7.19-7.24 (m, 2H), 7.25-7.43 (m, 5H), 7.48-7.58 (m, 4H), 7.74 (d, *J* = 7.7 Hz, 1H), 8.19 (ddd, *J* = 1.0, 2.2, 8.2 Hz, 1H), 8.32-8.34 (m, 1H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 14.4, 14.5, 34.8, 37.1, 50.2, 73.3, 113.1, 122.3, 123.2, 123.6, 123.8, 125.2, 125.3, 127.1, 128.8, 129.2, 129.5, 130.4, 133.3, 134.1, 134.2, 139.2, 139.3, 139.9, 143.8, 144.4, 148.4 ppm; **IR** (KBr): $\tilde{\nu}$ = 3533, 3064, 2912, 2248, 1947, 1646, 1597, 1527, 1499, 1470, 1437, 1346, 1199, 1147, 1028, 971, 901, 841, 809, 756, 729, 687 cm^{-1}; **HRMS** (EI): calculated for $C_{36}H_{31}NO_3S_2$ 589.1745; found: 589.1756.

Diethyl(4-((3,4-bis(2-methyl-5-phenylthiophen-3-yl)-6-methylenecyclohex-3-en-1-yl)-(hydroxyl)methyl)phenyl)phosphonate (5d)

The compound was prepared according to GP 1 yielding 67 % of **5d** as pink-coloured oil after purification by column chromatography on silica gel (EE). **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 1.29 (dt, *J* = 7.1, 2.0 Hz, 6H), 1.98 (s, 3H), 2.07 (s, 3H), 2.05-2.09 (m, 1H), 2.50-2.54 (m, 1H), 2.56 (s, 1H), 2.81-2.85 (m, 1H), 3.24 (d, *J* = 19.9 Hz, 1H), 3.43 (d, *J* = 19.8 Hz, 1H), 4.01-4.17 (m, 4H), 4.90 (d, *J* = 9.9 Hz, 1H), 5.21 (s, 2H), 6.79 (s, 1H), 7.02 (s, 1H), 7.19-7.24 (m, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 7.7 Hz, 2H), 7.39 (d, *J* = 8.2, 1.0Hz, 2H), 7.48 (d, *J* = 8.3 Hz, 2H), 7.53 (dd, *J* = 8.1, 3.9 Hz, 2H), 7.83 (dd, *J* = 13.1, 8.1 Hz, 2H) ppm; **^{13}C NMR** (125 Mhz, $CDCl_3$): δ = 14.4, 14.5, 16.3 (2C), 34.9, 37.2, 50.0, 62.1, 62.2, 73.7, 112.5, 123.9, 125.2, 125.3, 126.99, 127.0 (2C), 127.4, 128.8 (2C), 129.4, 130.2, 132.1, 134.0, 134.30 (2C), 134.4, 139.3, 139.4, 139.6, 139.8, 144.3, 146.8; ppm; **^{31}P NMR** (121 MHz, $CDCl_3$): δ = 17.8 ppm; **IR** (film): $\tilde{\nu}$ = 3374, 2979, 2918, 1600, 1499, 1440, 1347, 1233, 1160, 1130, 1022, 961, 757, 693 cm^{-1}; **HRMS** (ESI): calculated for $C_{40}H_{41}O_4PS_2+H^+$ 681.2257; found: 681.2253.

(*E*)-1-(3,4-Bis(5-chloro-2-methylthiophen-3-yl)-6-methylenecyclohex-3-en-1-yl)but-2-en-1-ol (5e)

The compound was prepared according to GP 1 yielding 32 % of **5d** as brownish solid after purification by column chromatography on silica gel (Pentane/Et_2O, 1:1 v/v). **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 1.77 (dd, *J* = 6.5, 1.6 Hz, 3H), 1.92 (s, 3H), 1.95 (s, 3H), 2.26-2.29 (m, 1H), 2.55-2.58 (m, 2H), 3.15 (d, *J* = 19.7 Hz, 1H), 4.17 (t, *J* = 8.6 Hz, 1H), 5.04-5.08 (m, 2H), 5.50 (ddd, *J* = 15.2, 8.3, 1.7 Hz, 1H), 5.72-5.81 (m, 1H), 6.42 (s, 1H), 6.52 (s, 1H) ppm; **^{13}C**

NMR (126 MHz, $CDCl_3$): δ = 14.2, 14.3, 18.0, 35.1, 37.3, 48.1, 73.0, 112.1, 125.2, 125.3, 126.7, 126.9, 129.8, 130.2, 130.5, 132.3, 133.0, 133.1, 137.9, 138.2, 144.3 ppm; **IR** (film): $\tilde{\nu}$ = 2920, 2240, 1950, 1854, 1800, 1670, 1601, 1499, 1420, 1380, 1340, 1210, 1120, 1090, 970, 910, 829, 730, 680; 680 cm^{-1}; **HRMS** (EI): calculated for $C_{21}H_{22}OS_2Cl_2$ 424.0489; found 424.0487.

(3-(2,5-Dimethylthiophen-3-yl)-4-(2-methyl-5-phenylthiophen-3-yl)-6-methylene-cyclohex-3-en-1-yl)(3-nitrophenyl)methanol (5g A) and (4-(2,5-dimethylthiophen-3-yl)-3-(2-methyl-5-phenylthiophen-3-yl)-6-methylenecyclohex-3-en-1-yl)(3-nitrophenyl)-methanol (5g B)

The compound was prepared according to GP 1 yielding 30 % of **5g** as brownish solid after purification by column chromatography on silica gel (Pentane/Et_2O, 7:3 v/v). **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 1.92 (s, 3H), 1.99 (s, 3H), 2.00 (s, 3H), 2.03 (dd, *J* = 17.2, 2.1 Hz, 2H), 2.07 (s, 3H), 2.29 (s, 3H), 2.36 (s, 3H), 2.46-2.75 (m, 4H), 2.75-2.81 (m, 2H), 3.20 (t, 2H, *J* = 19.5 Hz), 3.39 (t, *J* = 19.7 Hz, 2H), 4.95 (d, *J* = 9.7 Hz, 2H), 5.21 (s, 2H), 5.22 (s, 2H), 6.25 (d, *J* = 0.9 Hz, 1H), 6.44 (d, *J* = 0.9 Hz, 1H), 6.78 (s, 1H), 6.98 (s, 1H), 7.19-7.26 (m, 2H), 7.30-7.37 (m, 4H), 7.39-7.42 (m, 2H), 7.47-7.51 (m, 2H), 7.52-7.57 (m, 2H), 7.72 (t, *J* = 6.6 Hz, 2H), 8.16-8.20 (m, 2H), 8.32-8.28 (m, 2H) ppm; **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.3, 14.4, 14.5, 14.6, 15.2, 15.3, 34.9, 37.2, 37.3, 50.5 (2C), 73.4, 113.1 (2C), 122.4, 122.5, 123.2, 123.9, 124.1, 125.3, 125.4, 125.7, 125.9, 127.2, 128.6, 128.9, 129.6 (2C), 129.7, 129.8, 130.9, 131.9, 132.2, 133.4, 133.5, 134.2, 134.4, 134.5, 134.6, 135.5, 135.6, 137.9, 138.0, 139.5, 139.6, 139.7, 144.2 (2C), 144.5, 148.5, 148.6 ppm; **IR** (film): $\tilde{\nu}$ = 3543, 3069, 2917, 2857, 1698, 1651, 1598, 1530, 1500, 1440, 1349, 1314, 1205, 1145, 1088, 1047, 901, 814, 758, 691; **HRMS** (ESI): calculated for $C_{31}H_{29}NO_3S_2$ 550.1481; found 550.1482. Regioisomeric ratio: **5g A** : **5g B** = 59:41 %.

3.4.3 Immobilization

Diarylcyclohexene **5d** (100 mg, 0.15 mmol) was dissolved in dichloromethane (20 mL) and silica gel (100 mg, 0.04 – 0.063 mm) was added. The mixture was stirred over night at r.t. and then the silica gel was filtered off, washed with DCM (4 x 5 mL) and EtOH (4 x 5 mL) until the rinsing solution showed no UV-absorption after irradiation with UV-light (312 nm). After drying, the amount of immobilized compound **7** was determined by elementary analysis to be $2.2 \cdot 10^{-5}$ $mmol_{dye}/mg_{silicagel}$.

Preparation of a photoswitchable paint

Poly-2-ethyloxazoline (100 mg, Aquazol 200: molecular weight = 200.000 g/mol) was dissolved in water (2.0 mL), the coated silica gel (100 mg) was suspended and after stirring a jellylike gel was obtained.

Preparation of a photoswitchable cellulose sheet

The diarylcyclohexene **5c** (100 mg, 0.15 mmol) was dissolved in dry THF (20 mL) under nitrogen atmosphere. Then $K_2(CO_3)$ (56 mg, 0.4 mmol) and cycanuric chloride (27 mg, 0.15 mmol) were added and the mixture was stirred over night at r.t. Finally the solvent was evaporated and the residue was purified by silicagel column chromatography (PE/EE, 9:1 v/v). A red oil (90 mg) was obtained which contained traces of unidentified by-products. Finally the roughly purified reactive dye was dissolved in dry DMF (20 mL) under nitrogen atmosphere. Then $K_2(CO_3)$ (38 mg, 0.28 mmol) and a cellulose tissue were added to the mixture and the solution was heated to 60 °C over night. The cellulose tissue was washed with DCM, EtOH and aceton until no coloration of the rinsing solution was detected after irradiation with UV-light.

Table 2. Immobilization of diarylcyclohexene **5c** on cellulose.

Cellulose sheet before immobilization [mg]	Cellulose-sheet after immobilization [mg]	Immobilized dye [mg]	Immobilized dye [mmol]	Dye[mmol]/cellulose [mg]
176	198	22	$3.7\cdot10^{-2}$	$2.1\cdot10^{-4}$
170	208	38	$6.5\cdot10^{-2}$	$3.8\cdot10^{-4}$
195	230	35	$5.9\cdot10^{-2}$	$3.0\cdot10^{-4}$

Midpoint: $3.0\cdot10^{-4}$ $mmol_{dye}/mg_{cellulose}$

Standard aberration: $0.8\cdot10^{-4}$ $mmol_{dye}/mg_{cellulose}$

Immobilization (%) = 5.2 (Assumption: only the primary hydroxyl group is reactive)

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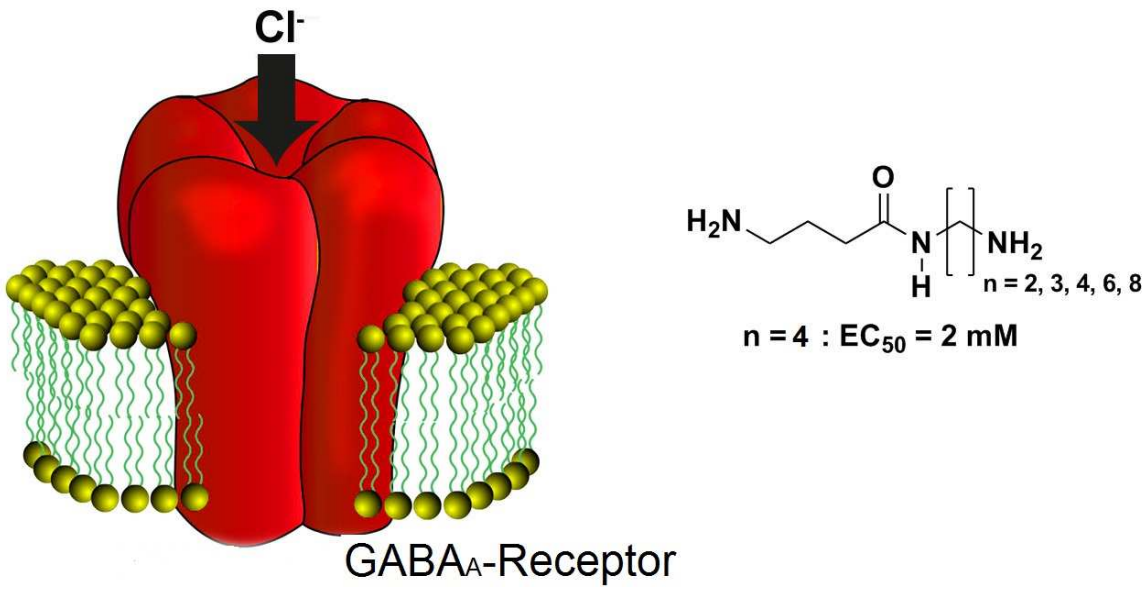
Chapter 4

New GABA-Amides Activating $GABA_A$



We have prepared a series of new and some literature reported GABA-amides and determined their effect on the activation of $GABA_A$-receptors expressed in CHO cells. Special attention was paid to the purification of the target compounds to remove even traces of GABA contaminations, which may arise from deprotection steps in the synthesis. GABA-amides were previously reported to be partial, full or superagonists. In our hands, these compounds were not able to activate $GABA_A$-receptor channels at whole cell patch-clamp recordings. New GABA-amides, however, gave moderate activation responses with a clear structure activity relationship suggesting some of these compounds as promising molecular tools for the functional analysis of $GABA_A$-receptors.

The results of this chapter are published:

Raster, P.; Späth, A.; Bultakova, S.; Gorostiza, P.; König, B.; Bregestovski, P. *Beilstein J. Org. Chem.* **2013**, *9*, 406.

Dr. Andreas Späth synthesized and characterized Boc-Pyrrolidone **1** and GABA-amide hydrochlorides **4a** and **7c**. Svetlana Bultakova performed the transient transfection of CHO-cells and the electrophysiological recording.

4.1 Introduction

The γ-aminobutyric acid (GABA) is the major inhibitory amino acid transmitter of the central nervous system (CNS) of vertebrates (Figure 1).

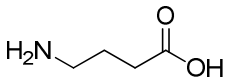


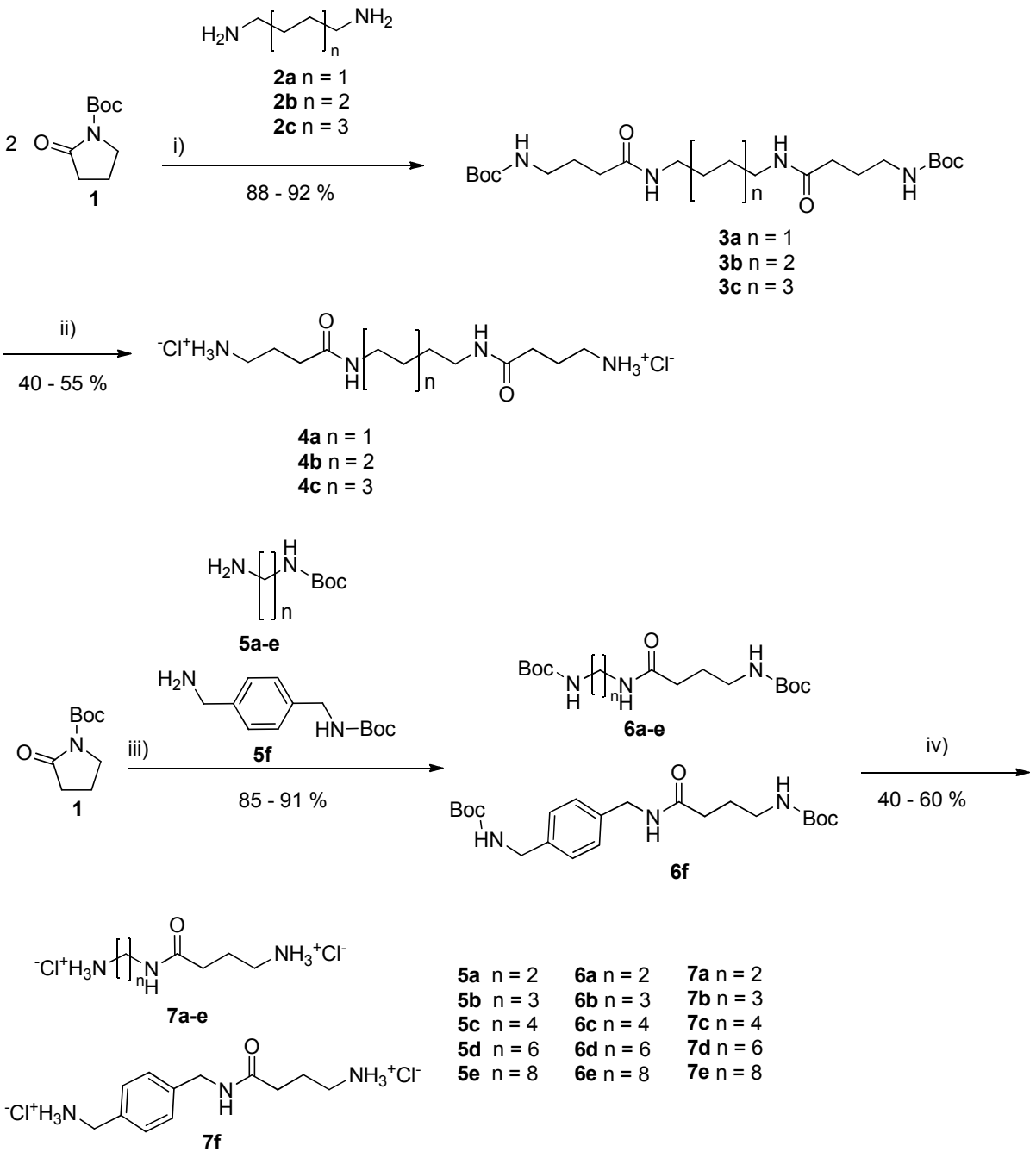
Figure 1. The neurotransmitter GABA.

It plays an important role in a variety of physiological functions, including motion control, vision, relaxation, sleep and many other brain functions.[1-3] In vertebrates GABA activates specific receptors of several classes: $GABA_A$, $GABA_B$ and $GABA_C$.[4] $GABA_A$-receptors are transmembrane heterooligomeric proteins, forming Cl^- selective channels and are composed of five subunits: two α, two β and one γ subunit.[5] Being highly expressed in the peripheral and central nervous system $GABA_A$-receptors represent a key therapeutic target for benzodiazepines, barbiturates, neurosteroids and general anesthetics.[6-8] Therefore, in spite of a large variety of existing agonists, antagonists and modulators of GABA-receptors, there is a high interest in the development of new drugs which can interact with these targets. Many compounds of different substance classes are known to modulate the activity of $GABA_A$-receptors.[9-12] One substance group that has been less explored is GABA-amides. Compounds **4a**, **4b**, **4c, 7a** (as triflate salt) and **7c** are previously reported to potently activate the $GABA_A$-channels being "partial, full or superagonists". However, in their work the authors studied the activity of GABA-amides on $GABA_A$-receptors using chloride flux assays on synaptoneurosomes. Such preparations can contain many damaged cells leading to highly variable intracellular Cl^- distribution in different cells. In our study we used the patch-clamp technique, which is much more reliable and informative in comparison to the approach based on chloride flux assays. Like Carlier et al.,[13] we noticed that in the course of the GABA-amide synthesis, GABA impurities are generated in the deprotection step. However, in contrast to Carlier et al.,[13] who used a modification of Sallers`s procedure[14] (detection limit < 0.1 wt%) to check the purity of the compounds, we have developed a improved purification procedure and used a more sensitive HPLC-MS analysis to ensure that the GABA-amide products do not contain any detectable amount of GABA (detection limit < 0.002 wt%). Our observations may help to give a more complete picture on the ability of GABA-amides to activate $GABA_A$-receptors.

4.2 Results and Discussion

4.2.1 Synthesis

Boc-Pyrrolidone **1** was nucleophilic ring-opened by diamines **2a–c** or the mono Boc-protected diamines **5a-f** in THF (Scheme 1).



Scheme 1. Synthesis of GABA-amide hydrochlorides **4a-c** and **6a-f**. i) THF, reflux; ii) MeOH, HCl (5 %); iii) THF, reflux; iv) MeOH, HCl (5 %).

To cleave the Boc-protecting groups, the compounds were deprotected in dilute HCl 5 % ($EtOH/H_2O$). It was observed that the use of more concentrated HCl can cause the cleavage of the amide-bonds and the release of free GABA. To remove any impurities of GABA, which

may falsify testing results, all substances were carefully purified: In a first purification step the GABA-amide hydrochlorides were dissolved in MeOH and precipitated by slow addition of Et_2O. The precipitates were centrifuged and recrystallized several times from MeOH until no traces of GABA could be detected by HPLC-MS analysis. To determine the sensitivity of the analysis method three stock solutions of GABA were prepared (10^{-3}, 10^{-5} and 10^{-7} mol/L) and 1 µL was injected in the HPLC coupled mass spectrometer. On the basis of the obtained mass spectra a GABA detection limit of 0.14 pmol (14 pg) was determined.

4.2.2 Electrophysiological Recording

By examining the chloride Cl^- uptake elicited by different $GABA_A$-receptor agonists, Carlier et al. demonstrated that compounds with general structure **4a-c** were capable to stimulate Cl^- uptake with different efficacy.[13] Moreover, the authors described compound **4b** as "superagonist", because it induced maximal uptake about 50% higher than that achieved by GABA. Surprisingly, EC_{50} value for this compound was more than 30-fold higher than for GABA, i.e., at 733 µM and 14.3 µM, respectively. The investigations in this study were performed using a standard $^{36}Cl^-$-flux assay in mouse brain synaptoneurosomes. This technique does not allow a comparative analysis on one cell. Moreover, $^{36}Cl^-$-flux assays were carried out using a 15-s incubation time. As GABA-receptors usually exhibit strong desensitization at long exposures to agonists, we decided to re-examine these results using patch-clamp recordings and fast perfusion technique for the application of the tested compounds. The activity of synthesized compounds was tested using CHO cells transiently expressing $GABA_A$-receptors in the configuration (α_1-GFP+ β_2 + γ_{2Long}). To analyze the functional properties of the compounds we performed monitoring of ionic currents using whole-cell patch-clamp techniques. First, concentration-response curves for GABA were obtained and its EC_{50} was determined. Then we applied different concentrations of the studied compounds and estimated the minimal concentration that induced currents and, if possible, their EC_{50}. The EC_{50} for GABA varied in different cells from 4 µM to 15 µM with a mean of 9.5 ± 0.3 µM (n = 10) (Figure 2).

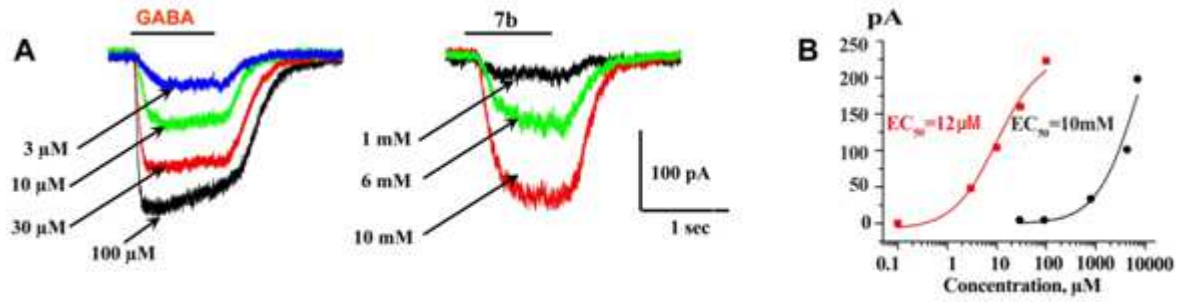


Figure 2. Effect of **7b** on $GABA_A$-receptor activation. (A) Superimposed traces of whole-cell currents induced by rapid application of GABA (left) or compound **7b** (right) in CHO cells transfected with α_1-GFP/β_2/γ_{2L} combination of $GABA_A$-receptor subunits; (B) Concentration dependecies of GABA (closed squares) and **7b** (closed circles). EC_{50}s were 12 µM and 10 mM for GABA and **7b**, respectively.

Surprisingly, in contrast to previously described observations, our purified compounds **4a-c** were not able to activate $GABA_A$-receptors in concentrations up to 10 mM. Figure 3 illustrates this for the compound **4c**.

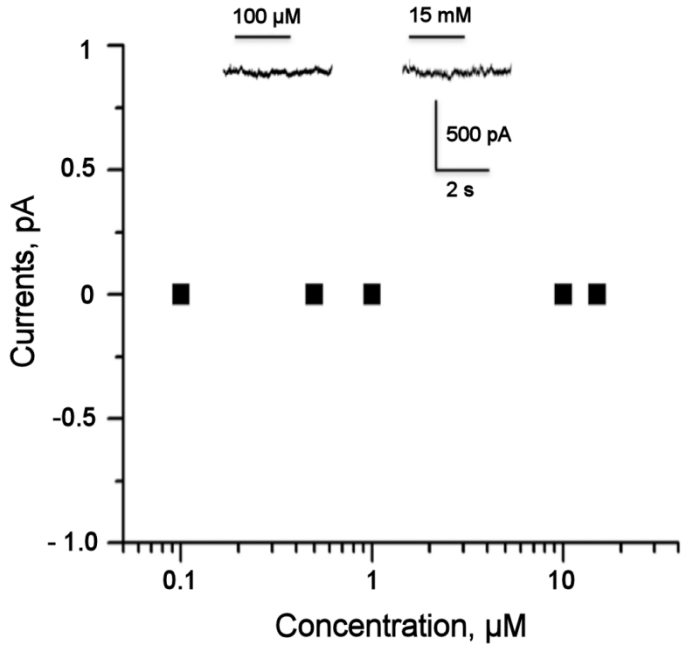


Figure 3. Absence of $GABA_A$-receptor activation on application of **4c**. Top traces: Examples of whole-cell currents on rapid application of **4c** on CHO cells transfected with an α_1-GFP/β_2/γ_{2L} combination of $GABA_A$-receptor subunits. Note that the compound was not able to induce currents even at a concetration of 15 mM.

Similar results were obtained also for compounds **4a** and **4b**: on application of 10 mM, the changes in the current were 0 pA, (x = 4 for each compound). In contrast, all studied

compounds from series **7a-e** were capable to induce ionic currents with different efficacy (Figure 4).

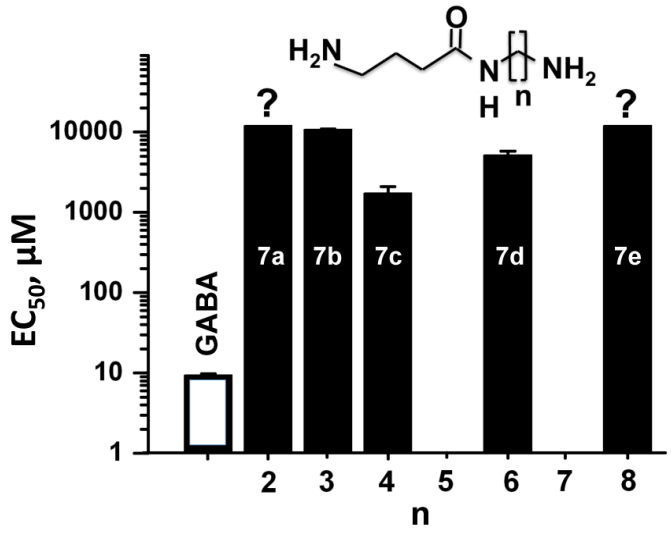


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Figure 4. Variation of EC_{50} values with the number n of methylene units separating the amide and the ammonium group in compounds **7a-e**. Each column represents results from 3-7 cells. The mark [?] on the top of bars indicates that for the EC_{50}s for these compounds were not determined precisely.

Thus, compound **7a** (n = 2) activated currents at 2.6 mM with an amplitude of 10-30 pA (x = 4). The compound **7b** (n = 3) activated GABA-receptors more strongly and at concentrations of 10 mM induced currents comparable to those for GABA 30-100 µM (Figure 2). The EC_{50} for the compound **7b** is 1080 ± 140 µM (x = 4). The efficacy of the compounds is significantly weaker than for GABA. The number of -CH_2-units between the amide nitrogen atom and the ammonium moiety of compounds **7** affects the efficacy significantly, reaching a maximum with compound **7c** (n = 4). The EC_{50} for compound **7c** is 1750 ± 330 µm (x = 7). Compound **7d** (*n* = 6) also effectively activated $GABA_A$-receptors with an activation threshold of about 100 µM. At concentrations of 10 mM it caused currents similar of those induced by saturated GABA concentrations (30-100 µM) with an EC_{50} of about 5 mM (Figure 5A-C). The EC_{50} for compound **7d** is 5250 ± 560 µM (*x* = 6). Kinetics of desensitization and the current-voltage dependencies (Figure 5D) of the studied compounds are similar to those for GABA. Compound **7e** (n = 8) weakly activated currents at 5 mM with an amplitude of 10-20 pA (x = 3).

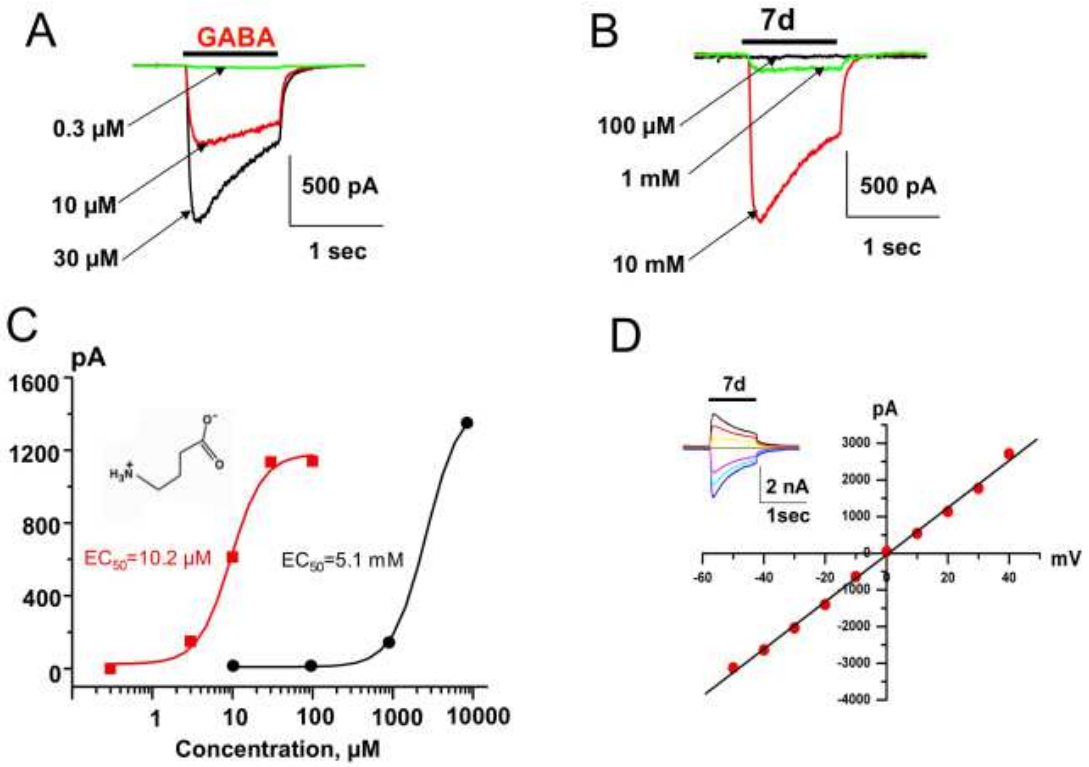

Figure 5. Effect of **7d** on $GABA_A$-Receptor activation. (A, B) Superimposed traces of whole-cell currents induced by rapid application of GABA (A) of the compound **7d** (B) in CHO cells transfected with an α_1-GFP/β_2/γ_{2L} combination of $GABA_A$-receptor subunits; (C) Concentration dependencies obtained on application of GABA (red closed square) and **7d** (black closed circles). EC_{50} values were 10.2 µM and 5.1 mM for GABA and **7d**, respectively; (D) Current-voltage relations for responses induced by compound **7d**. Insert: examples of traces at different membrane potentials. Scales: 2 nA and 1s.

4.3 Conclusion

Our data suggest that compounds **4a-c** are not capable of activating $GABA_A$-receptors. Compounds **7a-e** are able to simulate these receptors and show a distinct structure-activity correlation. The compounds may become useful as molecular tools for the functional analysis of $GABA_A$-receptors.

4.4 Experimental

4.4.1 General Experimental Conditions

The following compounds were prepared according to literature methods: tert-butyl-2-oxopyrrolidine-1-carboxylate **(1)**,[15] tert-butyl (2-aminoethyl)carbamate **(5a)**,[16] tert-butyl (3-

aminopropyl)carbamate **(5b)**,[17] tert-butyl (4-aminobutyl)carbamate **(5c)**,[18] tert-butyl (6-aminohexyl)carbamate **(5d)**,[17] tert-butyl (8-aminooctyl)carbamate **(5e)**,[17] tert-butyl-4-(aminomethyl)-benzylcarbamate **(5f)**.[19] All other reagents were obtained from commercial sources. Unless otherwise noted, solvents (analytical grade) were purchased from commercial suppliers and used without further purification. Melting points were obtained using a Lambda Photometrics Optimelt MPA 100 apparatus (Lambda Photometrics Harpenden, UK), and are not corrected. IR spectra were obtained using a Varian 3000 spectrometer. ^{1}H-NMR spectra were recorded at 300 MHz on a Bruker Avance 300 spectrometer. ^{13}C-NMR spectra were recorded at 75 MHz on a Bruker Avance 300 spectrometer. The NMR spectra were recorded in $CDCl_3$, DMSO or MeOD as solvent and chemical shifts are reported in ppm. Mass spectra were obtained using Finnigan SSQ 710A (EI), Finnigan MAT 95 (CI) or Finnigan MAT TSQ 7000 (Thermo FINNIGA, USA) (ES/LS-MS)] instrumentation. Thin layer chromatography (TLC) was performed on alumina plates coated with silica gel (Merck silica gel 60 F_{245}, thickness 0.2 mm). Column chromatography was accomplished with Merck Geduran SI 60 silica gel as the stationary phase. Petroleum ether (PE) refers to the fraction boiling at 30-60 °C.

4.4.2 Synthetic Protocols and Analytical Data of 3a-c, 4a-c, 6a-f and 7a-f

Boc-protected GABA-amides 3a-c; General Procedure (GP 1)

Boc-pyrrolidone **1** (5 mmol, 0.86 g) was dissolved in abs. THF (5 mL) and the diamines **2a–c** (2.5 mmol) were added. The solution was stirred under reflux and nitrogen atmosphere until the diamines were completely converted as monitored by TLC. The solvent was evaporated, the crude product was purified by flash chromatography (EE) and white solids were obtained.

Di-*tert*-butyl ((butane-1,4-diylbis(azanediyl))bis(4-oxobutane-4,1-diyl))dicarbamate (3a)

The compound was prepared according to GP 1 yielding 92 % (1.05 g) of the target product. Analytics is in good agreement to literature reported values.[13]

Di-*tert*-butyl ((hexane-1,6-diylbis(azanediyl))bis(4-oxobutane-4,1-diyl))dicarbamate (3b)

The compound was prepared according to GP 1 yielding 90 % (1.09 g) of the target product. Analytics is in good in agreement to literature reported values.[13]

Di-*tert*-butyl ((octane-1,8-diylbis(azanediyl))bis(4-oxobutane-4,1-diyl))dicarbamate (3c)

The compound was prepared according to GP 1 yielding 88 % (1.10 g) of the target product.

Diamines 4a-c; General Procedure (GP 2)

The Boc protected diamines **3a-c** (1 mmol) were dissolved in EtOH (10 mL), a HCl solution (5 %, 20 ml) was added over a period of 2 – 3 h, and the reaction progress was monitored by

TLC. A second portion of aqueous HCl (0.5 M, 100 mL) was added if necessary, and the solution was stirred until the deprotection was completed. EtOH was evaporated and the remaining H_2O was removed by lyophilisation. The product was recrystallised from MeOH twice. Drying in vacuo afforded the deprotected amine hydrochlorides as colorless, white solids. Analytics is in good agreement to literature reported values.[13]

***N,N´*-(Butane-1,4-diyl)bis(4-aminobutanamide) dihydrochloride (4a)**

The compound was prepared according to GP 2 yielding 48 % (168 mg) of the target compound. Analytics is in good agreement to literature reported values.[13]

***N,N´*-(Hexane-1,6-diyl)bis(4-aminobutanamide) dihydrochloride (4b)**

The compound was prepared according to GP 2 yielding 55 % (179 mg) of the target compound. Analytics is in good agreement to literature reported values.[13]

***N,N´*-(Octane-1,8-diyl)bis(4-aminobutanamide) dihydrochloride (4c)**

The compound was prepared according to GP 2 yielding 40 % (154 mg) of the target compound. [13]

Boc protected GABA-amides 6a-f; General Procedure (GP 3)

N-Boc-pyrrolidone **1** (1.85 g, 10 mmol) and the mono Boc-protected amines **(5a-f)** (15 mmol) were dissolved in THF (10 mL) and the solution was refluxed for 2 d. The solvent was evaporated and the residue was dissolved in dichloromethane (30 mL). The organic layer was washed with H_2O (20 mL) and brine (20 mL), dried over $MgSO_4$ and the solvent was evaporated. The raw material was purified by column chromatography (EE/PE, 1:1 v/v → 3 : 1) to give colorless solids.

4-(*tert*-Butyloxycarbonyl-amino)-N-(2-(*tert*-butyloxycarbonyl-amino)ethyl)butanamide (6a)

The compound was prepared according to GP 3 yielding 91 % (3.10 g) of the target compound. Analytics is in good agreement to literature reported values.[13]

4-(*tert*-Butyloxycarbonyl-amino)-N-(3-(*tert*-butyloxycarbonyl-amino)propyl)butanamide (6b)

The compound was prepared according to GP 3 yielding 88 % (3.15 g) of **6b** as a white solid, mp 80-82 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 1.27-1.30 (m, 4H), 1.40-1.57 (m, 14H), 1.64-1.87 (m, 4H), 2.18 (t, *J* = 6.8 Hz, 2H), 3.04-3.26 (m, 6H), 4.47 (s, 1H), 4.80 (s, 1H), 6.20 (s, 1H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 26.8, 27.5, 27.6, 28.8, 30.4, 34.4, 40.8, 41.4, 79.4,

79.6, 155.8, 155.9, 172.5 ppm; **IR** (film): $\bar{\nu}$ = 3430, 3230, 3080, 3050, 2940, 2970, 2870, 2803, 2744, 2635, 2383, 2340, 2032, 1779, 1682, 1611, 1539, 1482, 1386, 1349, 1310, 1284, 1260, 1213, 1152, 1068, 1019, 976 cm^{-1}; **HRMS** (EI)**:** calculated. for $C_{17}H_{33}N_3O_5$ 359.2420; found 359.2425.

4-(*tert*-Butyloxycarbonyl-amino)-N-(4-(*tert*-butyloxycarbonyl-amino)butyl)butanamide (6c)

The compound was prepared according to GP 3 yielding 86 % (3.06 g) of the target compound. Analytics is in good agreement to literature reported values.[13]

4-(*tert*-Butyloxycarbonyl-amino)-N-(6-(*tert*-butyloxycarbonyl-amino)hexyl)butanamide (6d)

The compound was prepared according to GP 3 yielding 90 % (3.21 g) of **6d** as a white solid, mp 82-84 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 1.27-1.31 (m, 4H), 1.40-1.55 (m, 20H), 1.66 – 1.85 (m, 4H), 2.20 (t, *J* = 6.9 Hz, 2H), 3.04-3.28 (m, 6H), 4.50 (s, 1H), 4.77 (s, 1H), 6.18 (s, 1H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 26.8, 27.5, 27.7, 28.8, 30.4, 30.9, 34.5, 40.4, 40.9, 41.3, 41.5, 79.5, 79.6, 155.8 155.9, 172.6 ppm; **IR** (film): $\bar{\nu}$ = 3437, 3231, 3085, 3053, 2944, 2972, 2874, 2803, 2744, 2629, 2585, 2383, 2335, 2032, 1779, 1682, 1611, 1539, 1482, 1386, 1349, 1310, 1284, 1263, 1213, 1152, 1068, 1019, 976, 909 cm^{-1}; **HRMS** (ESI): calculated for $C_{20}H_{39}N_3O_5$ 401.2890; found 401.2895.

4-(*tert*-Butyloxycarbonyl-amino)-N-(8-(*tert*-butyloxycarbonyl-amino)octyl)butanamide (6e)

The compound was prepared according to GP 3 yielding 85 % (3.65 g) of **6e** as a white solid, mp 92-94 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 1.41-1.51 (m, 26H), 1.75-1.85 (m, 4H), 2.16-2.23 (d, *J* = 6.9 Hz, 2H), 3.05-3.26 (m, 8H), 4.52 (s, 1H), 4.77 (s, 1H), 6.16 (s, 1H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 27.5, 27.7, 28.3, 28.4, 29.3, 29.4, 26.7, 26.8, 26.4, 26.2, 39.2, 39.5, 40.3, 79.5, 79.6, 155.9, 155.8, 172.6 ppm; **IR** (film): $\bar{\nu}$ = 3353, 2970, 2936, 2868, 1682, 1640, 1517, 1446, 1388, 1364, 1298, 1270, 1249, 1162, 1102, 999, 984, 870 cm^{-1}; **HRMS** (EI): calculated for $C_{22}H_{43}N_3O_5$ 429.3203; found 429.3200.

4-(*tert*-Butyloxycarbonyl-amino)-N-(4-(*tert*-butyloxycarbonyl)amino)benzyl)butanamide (6f)

The compound was prepared according to GP 3 yielding 88 % (2.96 g) of **6f** as a white solid, mp 124–126 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.41-1.43 (s, 9H), 1.44-1.46 (s, 9H), 1.76-1.88 (q, *J* = 6.7 Hz, 2H), 2.20-2.28 (t, *J* = 7.1 Hz, 4H), 3.10–3.20 (m, 2H), 4.24-4.30 (d, *J* = 5.9 Hz, 2H), 4.38-4.44 (d, *J* = 5.8 Hz, 4H), 4.79 (s, 1H), 4.85 (s, 1H), 6.45 (s, 1H), 7.23 (m,

4H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 26.8, 27.4, 27.6, 27.7, 28.8, 34.4, 40.1, 43.9, 44.8, 80.0, 80.2, 128.4, 128.7, 138.79, 140.0, 158.6, 175.4, 175.5 ppm; **IR** (film): $\tilde{\nu}$ = 3340, 2973, 2936, 2882, 1678, 1643, 1531, 1463, 1445, 1389, 1364, 1266, 1208, 1100, 1052, 1027, 990, 897, 874, 837, 804 cm^{-1}; **HRMS** (EI): calculated for $C_{22}H_{35}N_3O_5$ 421.2577; found 421.2576.

Diamines (7a–f); General Procedure (GP 4)

The Boc-protected diamines **6a-f** (1 mmol) were dissolved in EtOH (10 mL). Aqueous HCl (0.5 %, 20 mL) was slowly added, and stirring was continued over night. EtOH was evaporated, and the remaining H_2O was removed by lyophilisation. The residue was dissolved in warm EtOH (30 mL), the product was precipitated by careful addition of Et_2O and then centrifuged, and the supernatant was decanted off. Recrystallisation from MeOH and drying in vacuo afforded the deprotected amine hydrochlorides as colourless solids.

4-Amino-*N*-(2-aminoethyl)butanamide dihydrochloride (7a)

The compound was prepared according to GP 4 yielding 60 % (130 mg) of **7a** as a white solid, mp 144-146 °C. **^{1}H NMR** (MeOD, 300 MHz): δ = 1.85-1.99 (q, 2H), 2.38–2.47 (t, 2H, 7.2 Hz), 2.90 (t, *J* = 7.0 Hz, 2H), 3.08 (t, *J* = 7.3 Hz, 2H), 3.48 (t, *J* = 7.0 Hz, 2H) ppm; Amine and amid protons are not detected due exchange effects with the solvent (5H); **^{13}C NMR** (DMSO, 75 MHz): δ = 24.5, 24.7, 54.8, 113.2, 130.3, 174.4 ppm; **IR** (film): $\tilde{\nu}$ = 3430, 3251, 3085, 3053, 2944, 2383, 2335, 2032, 1779, 1682, 1611, 1539, 1482, 1386, 1349, 1213, 1152, 1068, 1019, 976, 909 cm^{-1}; **HRMS** (EI): calculated for $C_6H_{15}N_3O$ 145.1215; found 145.1217.

4-Amino-*N*-(3-aminopropyl)butanamide dihydrochloride (7b)

The compound was prepared according to GP 4 yielding 45 % (104 mg) of **7b** as a white solid, mp 140-142°C. **^{1}H NMR** (D_2O, 300 MHz): δ = 1.72-1.91 (m, 4H), 2.28 (t, *J* = 7.2 Hz, 2H), 2.90 (m, 4H), 3.20 (t, *J* = 7.0 Hz, 2H) ppm; Amine and amid protons are not detected due exchange effects with the solvent (5H); **^{13}C NMR** (DMSO, 75 MHz): δ = 24.5, 33.8, 37.1, 54.2, 110.2, 124.4, 174.4 ppm; **IR** (film): $\tilde{\nu}$ = 3430, 3231, 3075, 3053, 2972, 2940, 2874, 2803, 2744, 2629, 1779, 1682, 1611, 1534, 1492, 1386, 1340, 1310, 1284, 1263, 1019, 976, 909 cm^{-1}; **HRMS** (EI): calculated for $C_7H_{17}N_3O$ 159.1371; found 159.1374.

4-Amino-*N*-(4-aminobutyl)butanamide dihydrochloride (7c)

The compound was prepared according to GP 4 yielding 55 % (138 mg) of **7c**.[13]

4-Amino-*N*-(6-aminohexyl)butanamide dihydrochloride (7d)

The compound was prepared according to GP 4 yielding 50 % (147 mg) of **7d** as a white solid, mp 147-150 °C; **^{1}H NMR** (MeOD, 300 MHz) δ = 1.34-1.48 (m, 4H), 1.49-1.60 (m, 2H), 1.62-1.74 (m, 2H), 1.87-1.99 (q, *J* = 7.3 Hz, 2H), 2.34-2.41 (t, *J* = 7.2 Hz, 2H), 2.87-3.01 (m, 4H), 3.16-3.23 (t, *J* = 7.0 Hz, 2H); amine and amid protons are not detected due exchange effects with the solvent (5 H); **^{13}C NMR** (DMSO, 75 MHz): δ = 24.5, 24.7, 30.5, 32.3, 33.8, 37.1, 48.2, 110.2, 130.3, 174.5 ppm; **IR** (film): $\tilde{\nu}$ = 3437, 3231, 3085, 3053, 2972, 2944, 2874, 2803, 2744, 2629, 2585, 2383, 2335, 2032, 1779, 1682, 1611, 1539, 1482, 1386, 1349, 1310, 1284, 1263, 1213, 1152, 1068, 1019, 976, 909 cm^{-1}; **HRMS** (EI): calculated for $C_{10}H_{23}N_3O$ 201.1841; found 201.1845.

4-Amino-*N*-(8-aminooctyl)butanamide dihydrochloride (7e)

The compound was prepared according to GP 4 yielding 40 % (120 mg) of **7e** as white solid, mp 136-138 °C. **^{1}H NMR** (MeOD, 300 MHz) δ = 1.30-1.45 (m, 8H), 1.46-1.58 (m, 2H), 1.59-1.72 (m, 2H), 1.86-1.98 (q, *J* = 7.3 Hz, 2H), 2.34-2.40 (t, *J* = 7.1 Hz, 2H), 2.87-3.00 (m, 4H), 3.14-3.22 (t, *J* = 7.1 Hz, 2H) ppm; amine and amid protons are not detected due exchange effects with the solvent (5 H); **^{13}C NMR**: δ = 24.6, 27.4, 27.9, 28.6, 30.0, 30.1, 33.8, 40.8, 49.1, 113.4, 127.8, 171.2 ppm; **IR** (film): $\tilde{\nu}$ = 3347, 3329, 3126, 3080, 3046, 2973, 2941, 2892, 2862, 2813, 2740, 2380, 2175, 2020, 1681, 1611, 1540, 1484, 1384, 1349, 1311, 1284, 1262, 1240, 1197, 1152, 1065, 1011, 976, 939, 893, 839 cm^{-1}; **HRMS** (EI): calculated for $C_{12}H_{27}N_3O$ 229.2154; found 229.2160.

4-Amino-*N*-(4-(aminomethyl)benzyl)butanamide dihydrochloride (7f)

The compound was prepared according to GP 4 yielding 50 % (168 mg) of **7f** as a white solid, mp 251-253 °C. **^{1}H NMR** (MeOD, 300 MHz): δ = 1.89-2.01 (q, 2H, *J* = 7.3 Hz, 2H), 2.38-2.46 (t, *J* = 7.1 Hz, 2H), 2.94-3.01 (t, 2H, *J* = 7.4 Hz), 4.10 (s, 2H), 4.39 (s, 2H), 7.34–7.46 (m, 4H) ppm; Amin and amid protons are not detected due exchange effects with the solvent (5H); **^{13}C NMR** (DMSO, 75 MHz): δ = 24.5, 33.7, 40.5, 43.8, 44.2, 114,1, 125,3 129.3, 130.3, 133.4, 141.3, 174.5 ppm; **IR** (film): $\tilde{\nu}$ = 3340, 3279, 2972, 2939, 2880, 2361, 2174, 2048, 1678, 1624, 1536, 1505, 1463, 1423, 1388, 1364, 1299, 1266, 1248, 1161, 1099, 1053, 1027, 989, 896, 875, 836 cm^{-1}; **HRMS** (EI): calculated for $C_{12}H_{19}N_3O$ 221.1528; found 221.1530.

4.4.3 Cell Culture and Transient Transfection

Chinese Hamster Ovary (CHO-K1) cells were obtained from the American Type Tissue Culture Collection (ATCC, Molsheim, France) and maintained as previously described.[20,21] One day before the transfection, cells were plated on the coverslips (12-14 mm in diameter), which were placed inside 35 mm cell culture dishes with 2 ml of medium. CHO cells were

transfected with a total 3 µg cDNA of α1-GFP + β2 + $\gamma2_{Long}$ (ratio 1:1:1) using the Lipofectamine 2000 transfection protocol (Life Technology, USA). Three hours after the initial exposure of the cells to the cDNAs, a fresh cDNA-containing solution replaced the old one. Electrophysiological recordings were performed from fluorescent cells 48-72 hours after transfection.

4.4.4 Biology - Electrophysiological Recording

Whole-cell recordings were conducted on CHO-K1 cells at room temperature (20-25 °C) using an EPC-9 amplifier (HEKA Elektronik, Germany). Cells were continuously superfused with external solution containing (mM): NaCl 140, $CaCl_2$ 2, KCl 2.8, $MgCl_2$ 1, HEPES 20, glucose 10; pH 7.3; 320-330 mOsm. The patch pipette solution contained (mM): CsCl 140, $MgCl_2$ 2, MgATP 2, NaGTP 0.4, HEPES/CsOH 10, BAPTA/KOH 20; pH 7.3; 290 mOsm. Pipettes were pulled from borosilicate glass capillaries (Harvard Apparatus Ltd, USA) and had resistances of 5-8 MOhms. For rapid replacement of solutions, a system of two parallel rectangular tubes, 100 µm in diameter, located at a distance of 40-50 µm from the tested cell, was used. The movement of tubes was controlled by a computer-driven fast exchange system (SF 77A Perfusion Fast-Step, Warner, USA). As measured by open tip electrode controls (1/10 NaCl), in this system a 20-80% solution exchange time was within 2-3 ms. Cells with a low input resistance (<150 MOhms) were excluded from analysis. For quantitative estimations of agonist action, dose-response relationships were fitted by the equation:

$$I = I_{max} / (1 + (EC_{50} / [C])^n)$$

I is the current amplitude induced by the agonist at concentration [C], I_{max} is the maximum response of the cell, n is the Hill coefficient and EC_{50} is the concentration for which a half-maximum response is induced. Concentration-response relationships were constructed using at least four points.

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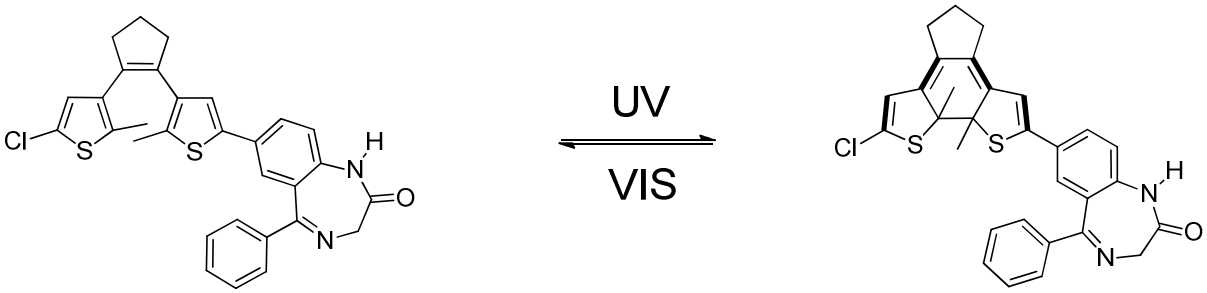
Chapter 5

Photoswitchable $GABA_A$-Receptor Ligands



We have synthesized a series of photoswitchable benzodiazepine derivatives and the effect on $GABA_A$-receptor activation was determined by whole cell patch-clamp recording and by two electrode voltage clamp in Xenopus oocytes. Two compounds showed moderate potentiation of the GABA induced current, but the observed extent was different in the two systems. UV-irradiation experiments gave no interpretable results, because of the insufficient solubility of the compounds. Therefore, a more polar and better water soluble benzodiazepine derivative was synthesized and the effect of $GABA_A$-receptor activation was determined. The test results to this point are inconsistent and additional experiments are ongoing.

Natascha Kuzmanovic synthesized diarylethene derivatives **9** and **10** and performed the Suzuki coupling reactions for the synthesis of benzodiazepine derivatives **2-4** and **21**. Svetlana Bultakova and Galina Maleeva (Marseille, France) transiently transfected CHO-cells and did the electrophysiological recording. Xenopus surgery, expression of recombinant rat $\alpha_1\beta_2\gamma_2$ $GABA_A$-receptors in Xenopus oocytes and functional characterization of these $GABA_A$-receptors were performed by Simon Middendorp and Roland Baur.

5.1 Introduction

$GABA_A$-receptors are transmembrane heterooligomeric proteins forming chloride (Cl^-) selective channels and are composed of five subunits: two α, two β and one γ subunit.[1] Highly expressed in the peripheral and central nervous system, $GABA_A$-receptors represent a key therapeutic target for benzodiazepines, barbiturates, neurosteroids and general anesthetics.[2-4] Binding of the endogenous neurotransmitter GABA induces channel opening and causes hyperpolarizing of postsynaptic neurons. Dysfunctions are related with several diseases like insomnia, schizophrenia, epilepsy and anxiety disorder.[5] To explore the functions of the $GABA_A$-receptor more detailed and to get a more complete picture about the role of this target, it would be helpful to have the possibility to activate single ionic channels. Up to now, different concepts like electronic stimulation, exposure to ultrasonic waves or irradiation with light are reported to trigger single ionic channels.[6-8] Maybe, the most promising method to stimulate the activity of ionic channels is the use of light, as the activation can be spatially and temporally resolved.[9] However, ionic channels are normally not light sensitive and cannot be activated directly by light. To stimulate ionic channels indirect, light sensitive compounds like “caged compounds” bearing photolabile protecting groups or photochromic ligands have been used.[9] Caged compounds release a biologic active compound after irradiation with light. The limitation of these compounds is that the release process is irreversible and cannot be repeated. In contrast, photochromic compounds can be reversibly switched between two different photoisomers, which may exhibit different biological activity. Typically, the switching process can be repeated several times and therefore, photoswitchable compounds seem to be promising candidates for the synthesis of light sensitive ligands for biological targets like the $GABA_A$-receptor. Recently, Trauner and coworkers reported the synthesis of a photoswitchable ligand for the $GABA_A$-receptor.[8] As photoswitchable unit they used an azobenzene derivative. The use of this ligand was limited because of the thermal instability of the (Z)-isomer, which immediately switched back to the more stable E-(isomer) as soon as the light source was switched off (Figure 1).

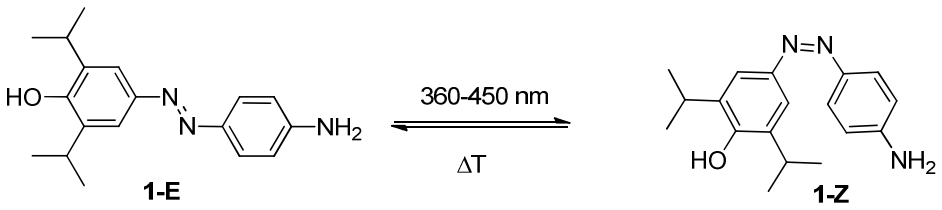


Figure 1. A photoswitchable ligand for the $GABA_A$-receptor. The azopropofol derivative **1-Z** is thermally not stable and switches immediately back to **1-E** as soon the light source is switched off.

In our approach towards light sensitive ligands for the $GABA_A$-receptor, we combined a diarylethene as photoswitchable unit with a benzodiazepine derivative as pharmacophore. Diarylethenes are a widely used photochromic class of substances, which show in many cases high fatigue resistance, complete photoisomerization and slow thermal interconversion of the photoisomers.[10] Benzodiazepines are an important compound class among the broad variety of tranquilizers and can modulate the effect of the endogenous neurotransmitter GABA.[11] Moreover, the structure-activity relationship of benzodiazepines is well studied and it is known that electron withdrawing substituents at the 2-position of the annulated benzene ring amplify the effect of benzodiazepines.[12]

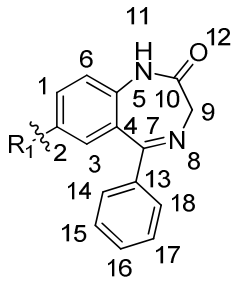


Figure 2. Structure-activity relationship of benzodiazepines. In general, the substitution of the 2-position of the annulated benzene ring by electron withdrawing groups ($R_1 = NO_2 > Br > Cl > F = H$) amplifies the activity of benzodiazepines.

In a first series, we synthesized three benzodiazepine derivatives which were substituted by a diarylethene photoswitch at the 1 and 2 position (Figure 3).

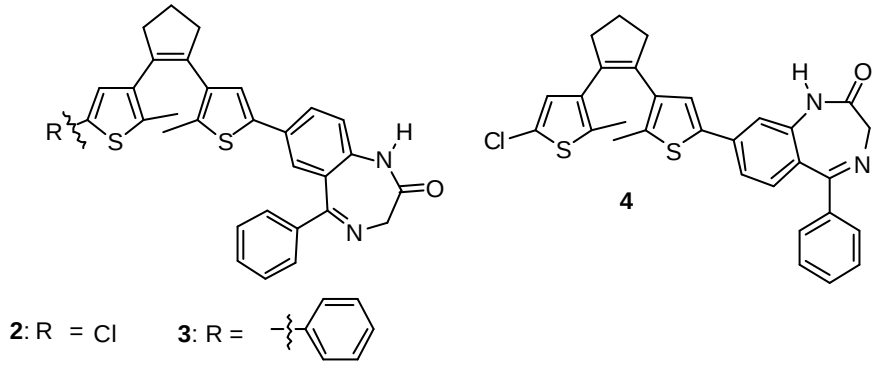


Figure 3. Photoswitchable benzodiazepine derivatives **2-4**.

In the open form **2(o)**, the pharmacophore is electronically separated from the electron withdrawing chloro substituent whereas in the closed form **2(c)**, the pharmacophore is in direct conjugation to the halogen atom. This is exemplarily shown for compound **2** in Figure 4. Furthermore, the closed isomer **2(c)** is more rigid, less flexible and sterically more hindered than the open isomer **2(o)** and therefore, we expected different activities of the photoisomers.

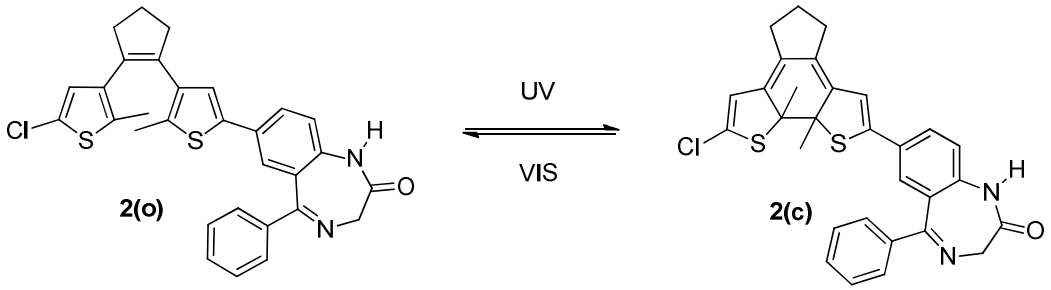
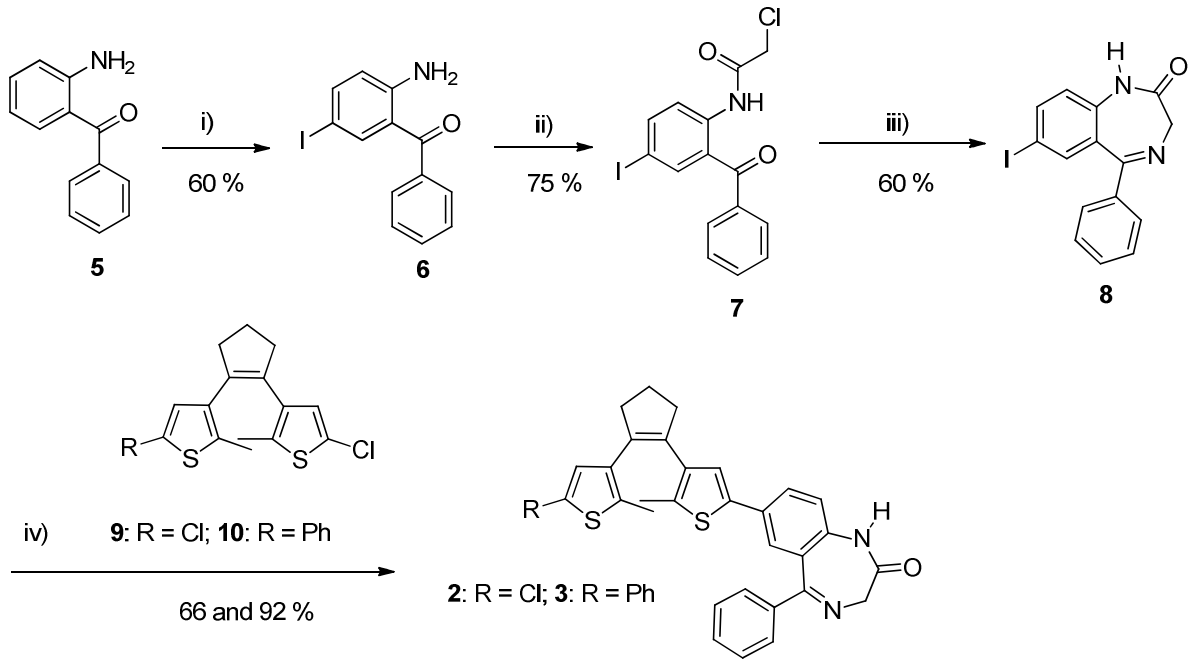


Figure 4. Photoisomerization of benzodiazepine derivative **2(o)** with UV-light. Reisomerization of **2(c)** is induced by irradiation with visible light.

5.2 Results and Discussion

5.2.1 Synthesis of Photoswitchable Benzodiazepines 2, 3 and 4

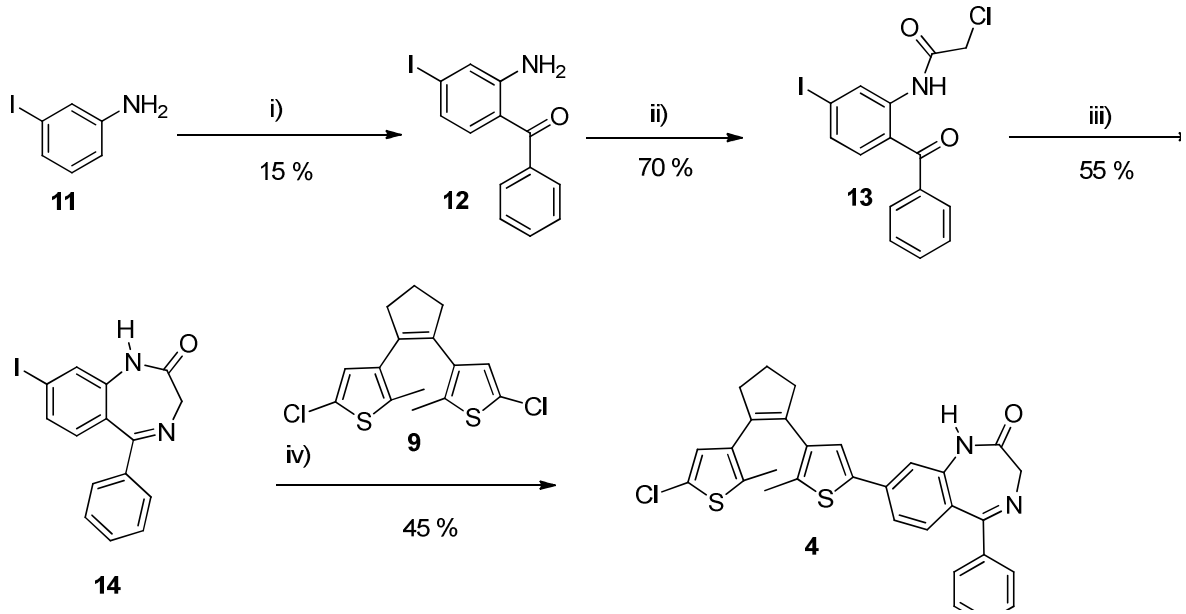
The synthesis of benzodiazepine derivatives **2** and **3** is illustrated in Scheme 1.



Scheme 1. Synthesis of photoswitchable benzodiazepine derivatives **2** and **3**. i) NaCl, $NaIO_4$, KI, AcOH, H_2O; ii) $Cl(C{=}O)CH_2Cl$, $CHCl_3$, NEt_3; iii) NH_4OAc, HMTM, EtOH; iv) 1.) **9** or **10**, *n*-BuLi, THF, -80° C 2.) $B(OCH_3)_3$ 3.) **8**, $(NH_4)_2CO_3$, $Pd(dppf)Cl_2$, THF.

In a first step, 2-aminobenzophenone **5** was iodinated in an aromatic substitution reaction using iodine, $NaIO_4$ and NaCl; the target compound **6** was obtained in 60 % yield. Secondly, compound **6** was acylated under basic conditions and gave compound **7** in 75 % yield. Next, compound **7** was ring closed with HMTM and $NH_4(OAc)$ affording benzodiazepine derivative **8** in 60 % yield. Finally, benzodiazepine derivative **8** was converted in a Suzuki reaction with DTE derivatives **10** and **9** resulting in the cross coupling products **3** in satisfying 92 % yield. Compound **2** was obtained in moderate 66 % yield. The reason for the smaller yield of

compound **2** was the occurance of disubstitution and dehalogenation products, which were generated under the reaction conditions of the halogen lithium exchange. Apart from the first reaction step, the synthesis of benzodiazepine derivative **4** follows the previously described reaction route (Scheme 2). Compound **4** could not be completely purified and contained small amounts of the dehalogenated byproduct.



Scheme 2. Synthesis of benzodiazepine derivative **4**. i) BCl_3, Bn-CN, $AlCl_3$, $C_2H_4Cl_2$; ii) $Cl(C{=}O)CH_2Cl$, $CHCl_3$, $N(Et)_3$; iii) NH_4OAc, HMTM, EtOH; iv) 1.) DTE **9**, *n*-BuLi, THF, -80°C 2.) $B(OCH_3)_3$, -80°C. 3.) $(NH_4)_2CO_3$, $Pd(dppf)Cl_2$, THF, -80°C.

Aminobenzophenone derivative **12** was synthesized by a Friedel-Crafts acylation reaction of 3-iodoaniline **11** with benzonitrile. The ortho acylation was performed in the presence of BCl_3 and $AlCl_3$. The six membered transition state directs the reaction site of aniline-derivative **11** ortho to the electrophilic center of benzonitrile (Figure 5).[13]

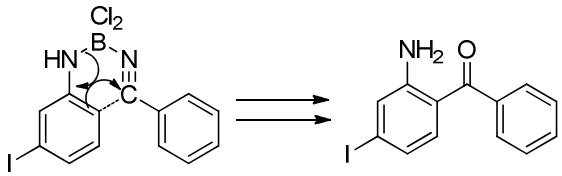


Figure 5. Boron trichloride-catalyzed Friedel-Crafts-Acylation reaction of 3-iodoaniline and benzonitrile.

5.2.2 Photoisomerization and Photobleaching of Benzodiazepine Derivatives 2, 3 and 4

Compounds **2-4** photoisomerize in DMSO by irradiation with UV-light of 312 nm wavelength. This is exemplarily shown for compound **2** in Figure 6 (left). The closed diarylethenes isomerize quantitatively to the open form upon irradiation with a 200 W tungsten light source

that was passed through a 420 nm cut-off filter to eliminate higher energy light. Benzodiazepine derivatives **2**-**4** photodegrade in solution. After 7 photoisomerization cycles, the absorption of the chromophore is reduced by half indicating about 50 % decomposition of the chromophore (Figure 6, right).

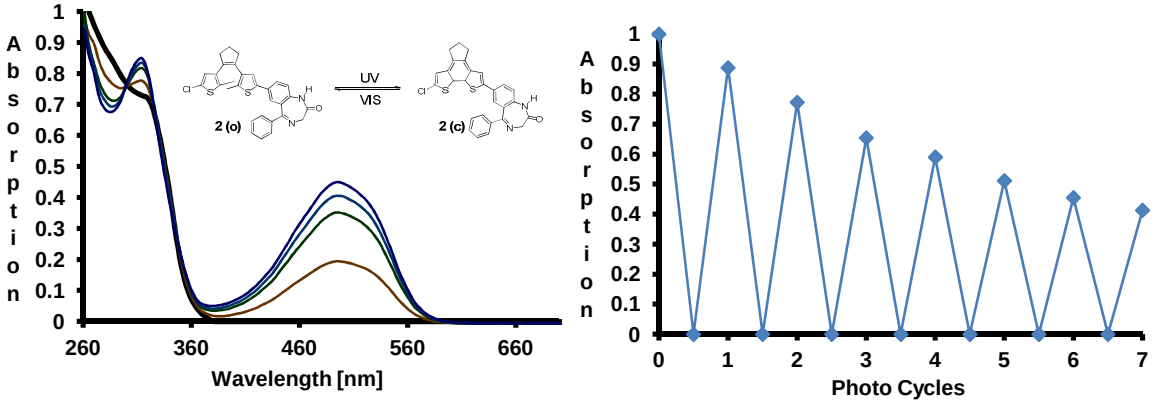


Figure 6. Irradiation of benzodiazepine **2** with UV-light (312 nm) in DMSO. Left: UV absorption spectrum of compound **2** (c = $3.0 \cdot 10^{-5}$ mmol/L in DMSO). Irradiation times were 15, 30, 45 and 60 s. Right: Decreasing absorption at 530 nm of a solution of benzodiazepine derivative **2** in DMSO during photoisomerization.

5.2.3 Electrophysiological Recording and Testing of Benzodiazepines 2,3 and 4 – Results from the Group of Prof. P. Bregestovski

The activity of the synthesized compounds was tested by patch-clamp recording using CHO-cells transiently expressing $GABA_A$-receptors in the configuration α_1-GFP/β/γ_{2L}.The heterologously expressed $GABA_A$-receptors were exposed to GABA at concentrations from 3-10 µM in combination with compounds **2-4** (20-36 µM). The solubility of the compounds in aqueous buffer (1 % DMSO) was limited and the test solution showed scattering of laser light indicating that the compounds were not completely dissolved and aggregated. Therefore, the results are preliminary and qualitative illustrating only trends. The UV-irradiation experiments were not fully reproducible. Higher concentrations of DMSO are cell toxic and could therefore not be used. Compound **2** (36 µM) amplified the effect of GABA (3 µM) by about 60 ± 11% (n [number of tested cells] = 7) (Figure 7), whereas compound **3** (20 µM) was weaker and potentiated GABA (10 µM)-induced currents only by about 20 ± 7 % (n = 7).

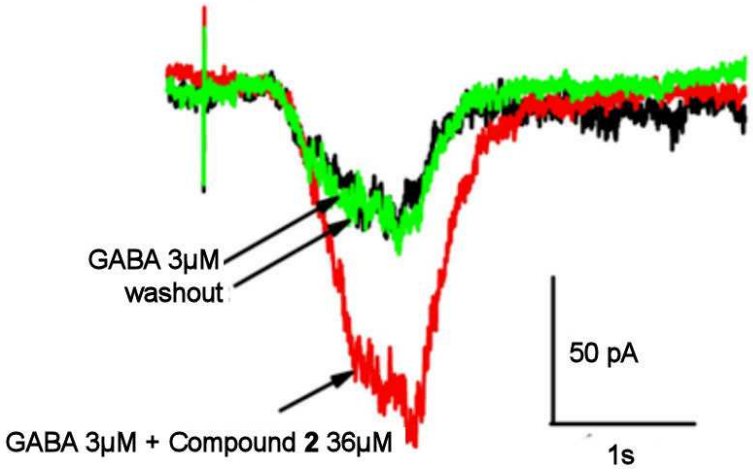


Figure 7. Effect of benzodiazepine derivative **2** on $GABA_A$-receptor activation. Superimposed traces of whole-cell currents induced by rapid application of GABA (3 µM) in combination with benzodiazepine derivative **2** (36 µM) in CHO cells transfected with α_1-GFP/β_2/γ_{2L} combination of $GABA_A$-receptor subunits.

In contrast, compound **4** (27 µM) reduced GABA (10 µM)-induced currents by about 24 ± 5% (n=6). Figure 8 summarizes the test results.

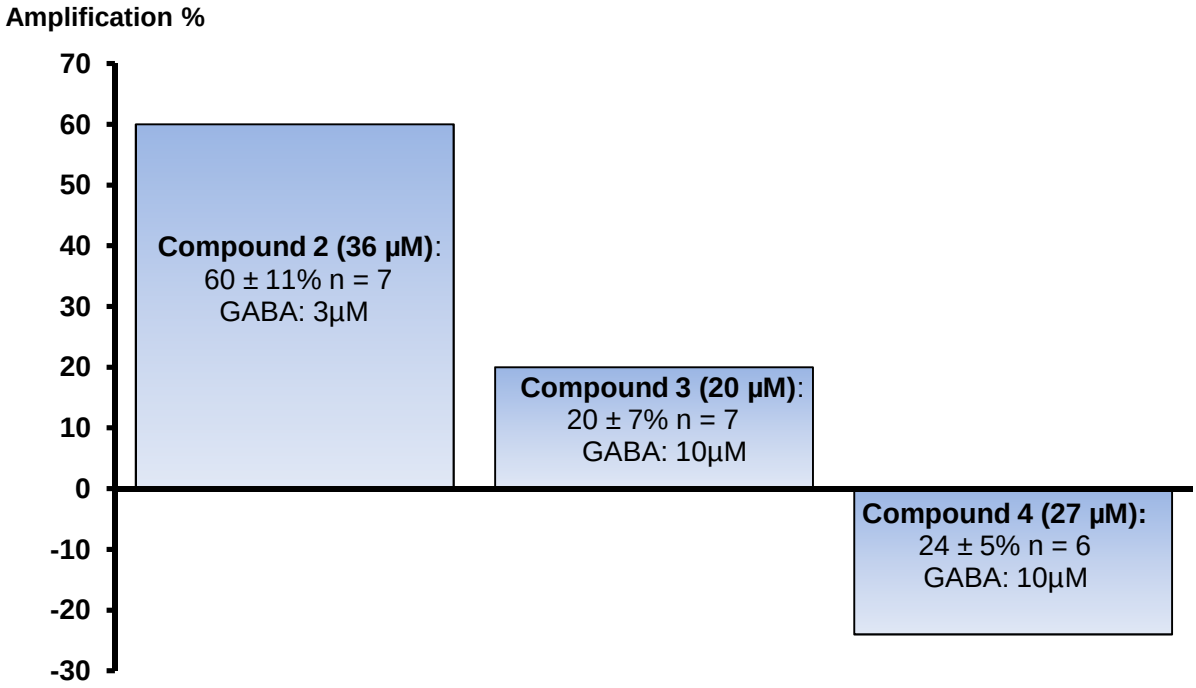
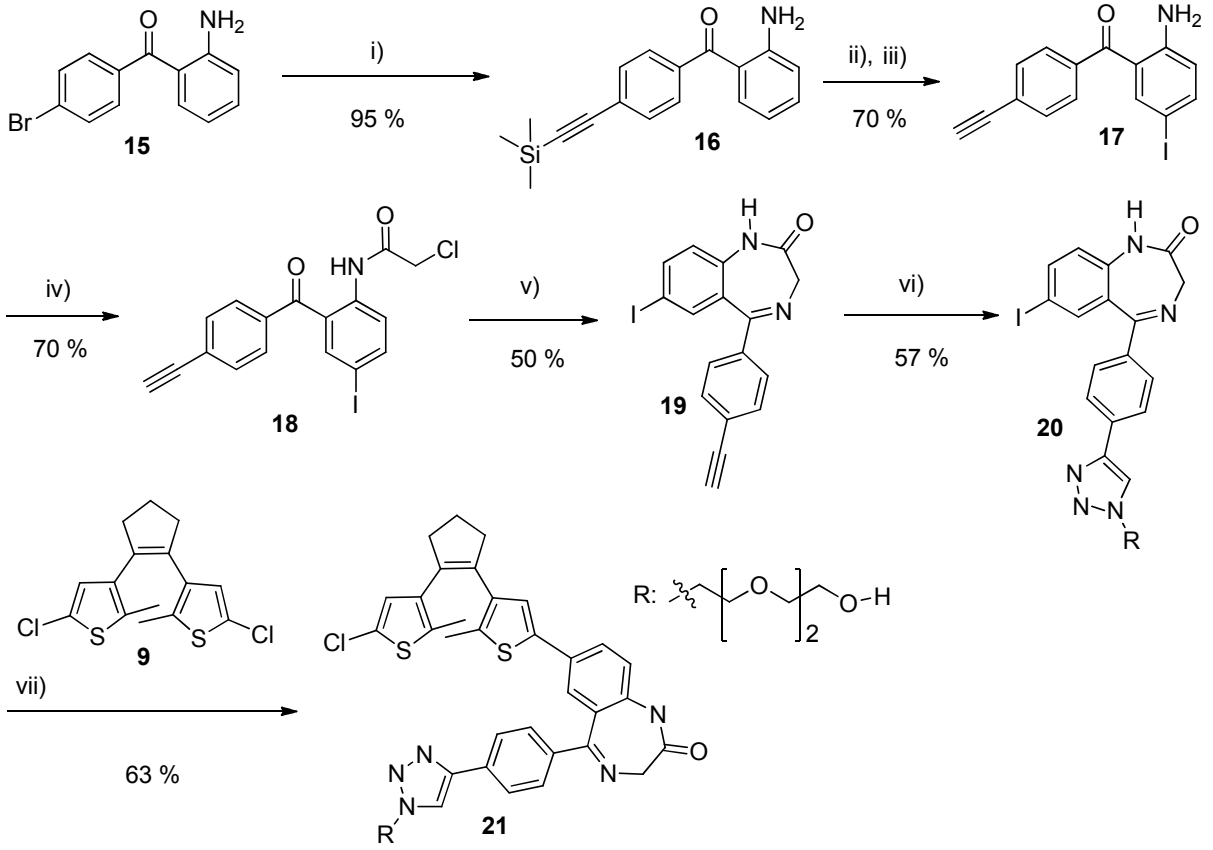


Figure 8. Effect of photoswitchable benzodiazepine derivatives **2-4** in combination with GABA on $GABA_A$-receptor activation relative to pure GABA.

5.2.4 Electrophysiological Recording and Testing of Benzodiazepine 2 – Results from the Group of Prof. Sigel

The effect of compound **2** on Cl^- currents elicited by GABA was investigated using electrophysiological techniques in $\alpha_1/\beta_2/\gamma_2$ $GABA_A$ receptors expressed in Xenopus oocytes. Compound **2** (10 µM) potentiated currents elicited by GABA (1 µM) by about 280 % (n = 1). 3 µM of the compound potentiated by 311 ± 51 % (mean ± SD, N = 5). The fact that 1 µM of the antagonist Ro15-1788 counteracted the potentiation indicates that compound **2** is a ligand of the benzodiazepine binding site. This was confirmed by displacement of [^{3}H]Ro15-1788 by compound **2** from $GABA_A$ receptor transiently transfected into HEK-cells with an K_i of 91 nM. This is surprising in view of the huge substituent replacing -Cl in diazepam. The above observations are in contrast to those made with the patch-clamp technique in another laboratory. The reasons for this discrepancy are not known. UV-irradiation experiments are still in progress.

5.2.5 Synthesis of Benzodiazepine 21



Scheme 3. Synthesis of benzodiazepine derivative **21**. i) TMS—≡, CuI, PPh_3, $Pd(Cl_2)(PPh_3)_2$, NEt_3, THF; ii) NaCl, $NaIO_4$, KI, AcOH, H_2O; iii) MeOH, K_2CO_3; iv) $Cl(C{=}O)CH_2Cl$, $CHCl_3$, NEt_3; v) NH_4OAc, HMTM, EtOH; vi) R-N_3, $CuSO_4$, sodium ascorbate; vii) 1.) **9**, *n*-BuLi, THF; 2.) $B(OCH_3)_3$; 3.) $(NH_4)_2CO_3$, **20**, $Pd(dppf)Cl_2$, THF, -80 °C.

Due to the insufficient solubility of compounds **2-4** in aqueous buffer, a better soluble derivative of benzodiazepine derivative **2** was synthesized (Scheme 3). The palladium-catalyzed Sonogashira reaction of (2-aminophenyl)(4-bromophenyl)-methanone **15** with TMS-acetylene gave coupling product **16** in excellent 95 % yield. Reaction steps ii-v follow the previously described synthesis. To improve the solubility, a PEG-chain was attached to compound **19** using standard click chemistry yielding compound **20** in 57 %. Suzuki coupling of precursor **20** with DTE **9** afforded finally the target compound **21** in 63 % yield.

5.2.6 Photoisomerization and Photobleaching of Benzodiazepine 21

Compound **21** photoisomerizes in DMSO by irradiation with light of 312 nm wavelength (Figure 9, left). The closed isomer isomerizes quantitatively to the open form upon irradiation with a 200 W tungsten light source that was passed through a 420 nm cut-off filter to eliminate shorter wavelengths. The compound photodegrades in solution. After 8 isomerization cycles the absorption of the chromophore at 520 nm is reduced by about 40 % (Figure 9, right).

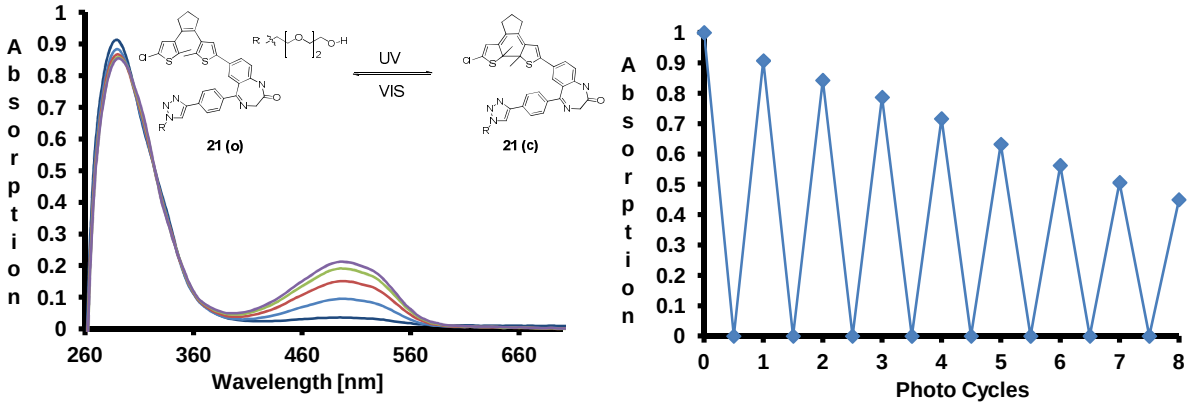


Figure 9. Irradiation of benzodiazepine derivative **21** with UV-light (312 nm) in DMSO. Left: UV-absorption spectrum of compound **21** (c = $3.0 \cdot 10^{-5}$ mmol/L in DMSO). Irradiation of diarylcyclopentene **21** with UV-light (312 nm) in DMSO. Irradiation times were 15, 30, 45 and 60 s. Right: Decreasing absorption at 530 nm of a solution of benzodiazepine derivative **21** in DMSO during photoisomerization.

5.2.7 Electrophysiological Recording and Testing of Benzodiazepine 21 – Results of the Group of Prof. Bregestovski

Compound **21** induced an inhibition of the GABA (5 µM) current. The inhibition was amplified by irradiation of the test solution with UV-light of 312 nm (Figure 10).

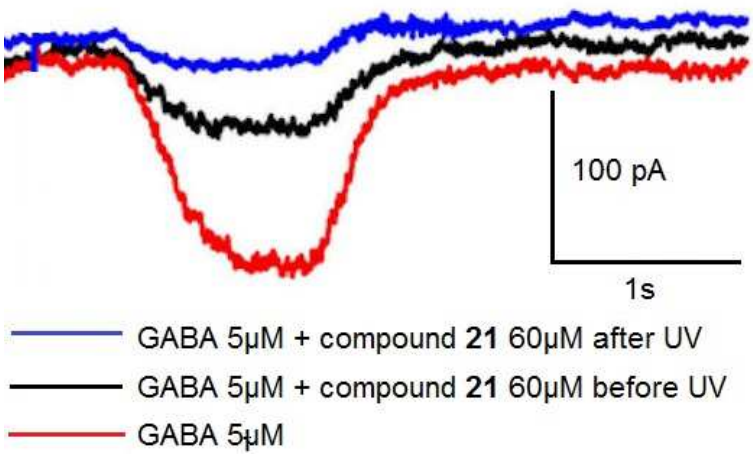


Figure 10. Effect of compound **21** on $GABA_A$-receptor activation. Superimposed traces of whole-cell currents induced by rapid application of GABA (5 µM) (red line); GABA in combination with compound **21** (60 µM) (black line); GABA (5 µM) in combination with **21** (60 µM) after irradiation with UV-light of 312 nm (blue line) in CHO cells transfected with α_1-GFP/β_2/γ_{2L} combination of $GABA_A$ receptor subunits.

Table 1 summarizes the results of the study on different cells and concentrations of compound **21**.

Table 1. Effect of compound **21** on $GABA_A$-receptor activation before and after 2 min UV-irradiation with UV-light of 312 nm.

Cell number	Concentration of 21	Inhibition of GABA induced current before irradiation %	Inhibition of GABA induced current after irradiation %
1	3 µM	0	11
2	10 µM	0	16
3	10 µM	6	18
4	60 µM	40	80

5.2.8 Electrophysiological Recording and Testing of Benzodiazepine 21 – Results of the Group of Prof. Sigel

Compound **21** was determined as positive allosteric modulator of GABA induced current and potentiated the effect of GABA by about 54 % (n = 1) whereas higher concentrations of compound **21** (10 µM) only potentiated the effect of GABA by about 45 % (n = 1). For the closed isomer of compound **21** at both concentrations a potentiation of 21 % (n = 1) was found. As the observed potentiation was low, the group concentrated on compound 2 (see above).

5.3 Conclusion

In a first series, three photoswitchable benzodiazepine derivatives were synthesized and their physiological activity was determined with patch-clamp recording in whole-cell configuration. Two compounds out of the first series showed moderate ampflification of GABA induced current, but the exact activity could not be determined because of the insufficent solubility of the compounds in the buffer. Therefore, a more polar and better water soluble benzodiazepine derivative was synthesized and the biological activity was determined. The test results are inconsistent and therefore no final conclusion on its physiological effect can be derived.

5.4 Experimental

5.4.1 General Experimental Conditions

The following compounds were prepared according to literature methods: 1,2-bis(5-chloro-2-methylthiophen-3-yl)cyclopent-1-ene (**9**),[14] 5-chloro-2-methyl-3-(2-(2-methyl-5-phenyl-thiophen-3-yl)cyclopent-1-en-1-yl)thiophene (**10**).[15] All other reagents were obtained from commercial sources. Unless otherwise noted, solvents (analytical grade) were purchased from commercial suppliers and used without further purification. Melting points were obtained using a Lambda Photometrics Optimelt MPA100 apparatus (Lambda Photometrics, Harpenden, UK) and are not corrected. IR spectra were obtained using a Varian Biorad FT-IR Excalibur FTS 3000 spectrometer. 1H NMR spectra were recorded at 300 MHz on a Bruker Avance 300 spectrometer, or at 600 MHz on a Bruker Avance III Br600 with a cryogenic probe head (Bruker, Karlsruhe, Germany). 13C NMR spectra were recorded at 75 MHz on a Bruker Avance 300 spectrometer. The NMR spectra were recorded in $CDCl_3$ as solvent and chemical shifts are reported in ppm. UV/Vis spectra were recorded using a Varian Cary BIO 50 UV/Vis/NIR spectrophotometer (Varian Inc., CA, USA). Mass spectra were obtained using Finnigan SSQ 710A (EI), Finnigan MAT 95 (CI) or Finnigan MAT TSQ 7000 (Thermo FINNIGAN, USA) (ES/LC– MS)] instrumentation. Thin layer chromatography (TLC) was performed on alumina plates coated with silica gel (Merck silica gel 60 F245, thickness 0.2 mm). Column chromatography was accomplished with Merck Geduran SI 60 silica gel as the stationary phase. Petroleum ether (PE) refers to the fraction boiling at 30-60 °C.

5.4.2 Synthetic protocols and analytical data of compounds 2-4, 6-8, 12-14 and 16-21

Photoswitchable benzodiazepine derivatives 2-4

General procedure for the Suzuki coupling (GP 1)

The DTE (1 eq) was dissolved in dry THF (3 mL) in a 10 mL capped vial under nitrogen atmosphere. After cooling to -80 °C, *n*-BuLi (2.0 M in hexane, 1.1 eq) was added dropwise

via a syringe and the resulting purple solution was stirred in the cold for 45 min. The reaction mixture was treated with trimethyl borate (1.5 eq) and the yellow solution was stirred at -80 °C for 30 min, the cooling bath was then removed and it was stirred at r.t. for further 60 min. The reaction was quenched with aqueous Na_2CO_3 (2.0 M, 1 mL) and a solution of the appropriate BDA (1 eq) and $Pd(dppf)Cl_2$ (0.03 eq) in THF (1 mL) was added to the reaction mixture. It was heated to 80 °C for 20 h by a heating block. After removal of the cap, the reaction mixture was diluted with EE (3 mL) and water (3 mL) and the phases were separated. The aqueous phase was extracted with EE (2 x 5 mL), the combined organic phases were dried over $MgSO_4$ and the solvents were removed under reduced pressure. Purification of the crude product was performed by flash column chromatography.

7-(4-(2-(5-Chloro-2-methylthiophen-3-yl)cyclopent-1-en-1-yl)-5-methylthiophen-2-yl)-5-phenyl-1H-benzo[e][1,4]diazepin-2(3H)-one (2)

Compound **2** was prepared from DTE **9** (56 mg, 0.17 mmol) and BDA **8** (62 mg, 0.17 mmol) following the general procedure GP 1 for Suzuki coupling. After flash column chromatography (PE/EE, 3:2 v/v), the compound was crystallized in EE and precipitated by the addition of hexane in the fridge. Finally, compound **2** was obtained as purple solid. Yield (420 mg, 66 %), mp 122-124 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 1.86 (s, 3H), 1.98 (s, 3H), 1.99-2.07 (m, 2H), 2.70-2.80 (m, 4H), 4.35 (s, 2H), 6.56 (s, 1H), 6.86 (s, 1H), 7.13 (d, *J* = 8.5 Hz, 1H), 7.36-7.49 (m, 4H), 7.56-7.62 (m, 3H), 8.94 (s, 1H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): 14.2, 14.5, 22.9, 38.3, 38.3, 56.7, 76.6, 121.6, 124.5, 125.1, 126.8, 127.5, 128.3, 128.6, 129.7, 130.0, 130.5, 133.2, 134.2, 135.0, 135.0, 135.3, 136.7, 137.4, 137.9, 139.11, 170.7, 171.7 ppm; **IR** (film): $\tilde{\nu}$ = 3270, 3056, 2950, 1680, 1635, 1590, 1565, 1500, 1440, 1380, 1159, 1100, 1078, 960, 740, 510 cm^{-1}; **HRMS** (ESI): calculated for $C_{30}H_{25}ClN_2OS_2$ 528.1097; found 528.1043.

7-(5-Methyl-4-(2-(2-methyl-5-phenylthiophen-3-yl)cyclopent-1-en-1-yl)thiophen-2-yl)-5-phenyl-1H-benzo[e][1,4]diazepin-2(3H)-one (3)

Compound **4** was prepared from DTE **10** (56 mg, 0.15 mmol) and BDA **8** (54 mg, 0.15 mmol) following the general procedure for GP 1 for Suzuki coupling. After flash chromatography (PE/EE, 1:1 to 5:7 v/v), compound **3** was obtained as purple foam. Yield (79 mg, 92 %), mp 92-93 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 1.96 (s, 3H), 2.00 (s, 3H), 2.03-2.11 (m, 2H), 2.79-2.83 (m, 4H), 4.34 (s, 2H), 6.89 (s, 1H), 6.99 (s, 1H), 7.08 (d, *J* = 8.6 Hz, 1H), 7.28-7.41 (m, 9H), 7.58 (dd, *J* = 2.1 Hz, 7.0 Hz, 2H), 7.62 (d, *J* = 2.1 Hz, 1H), 8.16 (s, 1H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 14.4, 14.5, 23.1, 38.4, 38.5, 58.9, 121.7, 123.9, 124.6, 125.3, 127.0, 127.5, 128.6, 128.8, 129.7, 130.0, 130.5, 134.3, 134.4, 134.5, 135.1, 135.2, 136.5, 137.0,

137.5, 137.7 , 139.1, 139.8, 170.7, 171.9 ppm; **IR** (film): $\tilde{\nu}$ = 1940, 1865, 1798, 1600, 1500, 1400, 1350, 1250, 1160, 920, 720 cm^{-1}; **HRMS** (ESI): calculated for $C_{36}H_{30}N_2OS_2$ 570.1794; found 570.1790.

8-(4-(2-(5-Chloro-2-methylthiophen-3-yl)cyclopent-1-en-1yl)-5-methylthiophen-2-yl)-5-phenyl-1H-benzo[e][1,4]diazepin-2(3H)-one (4)

Compound **4** was prepared from DTE **9** (50 mg, 0.15 mmol) and BDA **16** (54 mg, 0.15 mmol) following the general procedure GP 1 for Suzuki coupling. A mixture of compound **4** and the dehalogenated product which could not be separated was obtained as a purple foam. Yield (356 mg, 45 %).

(2-Amino-5-iodophenyl)(phenyl)methanone (6)

2-Aminophenylmethanone **5** (1.18 g, 6 mmol), $NaIO_4$ (1.51 g, 6 mmol), NaCl (0.70 g, 12 mmol) and KI (1.00 g, 6 mmol) were dissolved in AcOH-water (500 mL, 9:1) and stirred for 12 h at r.t. The compound was extracted with DCM (2 x 100 mL) and the organic layer was washed with water (2 x 100 mL) and conc. $NaHSO_3$ (100 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (PE/EE, 4:1 v/v) and a light red solid was obtained. Yield (1.15 g, 60 %). Analytical data are in accordance with literature reported values.[16]

***N*-(2-Benzoyl-4-iodophenyl)-2-chloroacetamide (7)**

Under nitrogen atmosphere, 2-chloroacetyl chloride (0.24 mL, 3.0 mmol) was added to a solution of (2-amino-5-iodophenyl)(phenyl)methanone (1.00 g, 3.0 mmol) and Et_3N (0.5 mL) in $CHCl_3$ (10 mL) at 0 °C. The mixture was allowed to warm up and stirred at r.t. for 12 h. The organic layer was washed with distilled water (2 x 50 mL) and the water layer was extracted with $CHCl_3$ (2 x 50 ml). The organic layers were combined, dried over Na_2SO_4 and filtered. The solvent was finally evaporated and the crude product was purified by column chromatography (PE/EE, 2:1 v/v) and a brown solid was obtained. Yield (0.9 g, 75 %), mp 135-137 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 4.20 (s, 2H), 7.49-7.76 (m, 5H), 7.84-7.92 (m, 2H), 8.45 (d, *J* = 8.5 Hz, 1 H), 11.48 (s, 1H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) : δ = 43.1, 86.1, 123.5, 126.2, 128.6, 130.1, 133.20, 137.6, 138.8, 141.4, 142.5, 165.4, 197.7 ppm; **IR** (film): $\tilde{\nu}$ = 3273, 3056, 2956, 1682, 1635, 1592, 1570, 1507, 1444, 1386, 1290, 1252, 1159, 1119, 1078, 967, 940, 888, 837, 801, 757, 740, 695, 649, 590, 516, 441 cm^{-1}; **HRMS** (ESI): calculated for $C_{15}H_{11}ClINO_2$ 398.9523; found 398.9520.

7-Iodo-5-phenyl-1H-benzo[e][1,4]diazepin-2(3H)-one (8)

To a solution of *N*-(2-benzoyl-5-iodophenyl)-2-chloroacetamide **7** (0.5 g, 1.3 mmol) in EtOH (30 mL), HMTM (387 mg, 2.8 mmol) and $NH_4(OAc)$ (216 mg, 2.8 mmol) were added and the reaction mixture was stirred under reflux for 12 h. The solvent was removed and the crude product was subjected to column chromatography (PE/EE, 2:1 v/v) and an orange solid was obtained. Yield (282 mg, 60 %). Analytical data are in accordance with literature reported values.[16]

(2-Amino-4-iodophenyl)(phenyl)methanone (12)

BCl_3 (DCM 1 M, 13.07 mmol, 13.07 mL) was added to a solution of 3-iodoaniline (2.5 g, 11.4 mmol) in 1,2-dichloroethane (9 mL) at 0 °C under nitrogen atmosphere. $AlCl_3$ (1.68 g, 13.07 mmol) and benzontitrile (1.16 mL, 11.4 mmol) were added to the reaction mixture and the solution was stirred under reflux at 80 °C for 20 h. After the reaction was completed, the reaction mixture was cooled to 0 °C and 2 M HCl (10 mL) was added. The mixture was stirred at 80 °C for 30 min and the product was extracted with DCM. The organic layer was washed with NaOH (1M) and brine, dried over $MgSO_4$ and concentrated in vacuo. The compound was purified by flash column chromatography (PE/EE, 4:1 v/v) to give 2-amino-4-iodophenyl)phenyl)methanone as a light red solid. Yield (553 mg, 15 %). Analytical data are in accordance to literature.[17]

***N*-(2-Benzoyl-5-iodophenyl)-2-chloroacetamide (13)**

2-Chloroacetyl chloride (0.12 mL, 1.5 mmol) was added to a solution of (2-amino-5-iodophenyl)(phenyl)methanone **12** (0.50 g, 1.5 mmol) and NEt_3 (0.25 mL) in $CHCl_3$ (10 mL) at 0 °C. The mixture was allowed to warm up and stirred at r.t. for 12 h. Distilled water (2 x 50 mL) was added and the the organic phase was separated. The water phase was extracted with $CHCl_3$ (2 x 10 ml) and the collected organic layers were combined, dried over Na_2SO_4, filtered and the solvent was removed in vacuo. The crude product was purified by flash chromatography (PE/EE, 2:1 v/v) and a yellow solid was obtained. Yield (470 mg, 70 %), mp 130-132 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 4.20 (s, 2H), 7.49-7.76 (m, 5H), 7.84-7.92 (m, 2H), 11.48 (s, 1H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 43.2, 86.2, 124.8, 126.2, 129.6, 130.1, 133.4, 137.6, 138.8, 141.4, 142.2, 165.4, 197.7 ppm; **IR** (film) : $\bar{\nu}$ = 3270, 3056, 2950, 1680, 1635, 1590, 1565, 1500, 1440, 1380, 1159, 1100, 1078, 960, 740, 510 cm^{-1}; **HRMS** (ESI): calculated for $C_{15}H_{11}ClINO_2$ 398.9523; found 398.9533.

8-Iodo-5-phenyl-1H-benzo[e][1,4]diazepin-2(3H)-one (14)

HMTM (310 mg, 2.2 mmol) and $NH_4(OAc)$ (216 mg, 2.8 mmol) were added to a solution of *N*-(2-benzoyl-5-iodophenyl)-2-chloroacetamide (400 mg, 1.0 mmol) in EtOH (24 mL) and the

reaction mixture was stirred under reflux for 12 h. The solvent was removed in vacuo and the crude product was subjected to column chromatography (PE/EE = 2:1, v/v) and an orange solid was obtained. Yield (200 mg, 55 %), mp 140-142 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 4.33 (s, 2H), 7.03 (d, *J* = 8.3 Hz, 2H), 7.34-7.57 (m, 6H), 9.15 s (1H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 43.1, 86.1, 123.5, 126.2, 128.6, 130.1, 133.2, 137.6, 138.8, 141.4, 142.6, 165.4, 197.7 ppm; **IR** (film): $\tilde{\nu}$ = 3181, 3085, 2964, 2122, 2057, 1902, 1676, 1587, 1553, 1499, 1476, 1444, 1376, 1321,1269, 1223, 1196, 1162, 1076, 1017, 951, 924, 870, 825, 776, 756, 726, 695, 650, 583, 562, 546, 503, 458, 438 cm^{-1}; **HRMS** (ESI): calculated for $C_{15}H_{11}IN_2O$ 361.9916; found 361.9910.

(2-Aminophenyl)(4-((trimethylsilyl)ethynyl)phenyl)methanone (16)

Under nitrogen atmosphere, the solvent THF-NEt_3 (48 mL, 2:1) was degassed for 15 min. 2-Aminophenyl(4-bromophenyl)methanone (500 mg, 1.8 mmol), TMS-acetylene (0.51 mL, 3.6 mmol), $PdCl_2(PPh_3)_2$ (64 mg, 5 mol%), CuI (34 mg, 10 mol%) and PPh_3 (48 mg, 10 mol%) were added and the solution was stirred under reflux for 12 h. Water (50 mL) was added and the organic layers were separated and extracted two times with Et_2O (2 x 50 ml). The organic layers were combined, dried over $MgSO_4$ and filtered. The solvent was evaporated and the product was purified by column chromatography (PE/EE = 2:1 v/v) and a black oil was obtained. Yield (500 mg, 95 %). **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 0.27 (s, 9H), 6.58-6.65 (m, 1H), 6.70-6.78 (m, 1H), 7.27-7.34 (m, 1H), 7.37-7.41 (m, 1H), 7.52-7.60 (m, 4H) ppm; **^{13}C NMR**: δ = 0.0, 115.9, 117.3, 118.2, 126.1, 129.2, 131.7, 132.0, 134.4, 134.5, 134.5, 139.7, 150.7, 198.3 ppm; **IR** (film): $\tilde{\nu}$ = 3200, 3040, 2950, 1670, 1630, 1590, 1570, 1500, 1442, 1380, 1280, 1070, 725, 580 cm^{-1}; **HRMS** (ESI): calculated for $C_{18}H_{19}NOSi$ 293.1253; found 293.1250.

(2-Amino-5-iodophenyl)(4-ethynylphenyl)methanone (17)

(2-Amino-5-iodophenyl((4-((trimethylsilyl)ethynyl)phenyl)methanone **16** (800 mg, 2.8 mmol), $NaIO_4$ (584 mg, 2.8 mmol), KI (456 mg, 2.8 mmol) and NaCl (160 mg, 2.8 mmol) were dissolved in a solution of AcOH-water (320 mL, 9:1) and stirred over night at r.t. for 24 h. The compound was extracted with DCM (3 x 100 mL), the organic layer was washed with 1 M NaOH (200 ml) and separated, dried over $MgSO_4$, filtered and evaporated. The crude product was dissolved in MeOH (50 mL), K_2CO_3 (3.5 g, 25.4 mmol) was added and the mixture was stirred overnight at r.t. DCM (200 mL) and water (200 ml) were added and the organic layer was separated, dried over $MgSO_4$, filtered and evaporated. The crude product was purified by column chromatography (PE/EE = 2:1 v/v) and an orange solid was obtained. Yield (480 mg, 70 %). **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 3.23 (s, 1H), 6.57 (d, *J* = 8.7 Hz, 1H), 7.46-7.67 (m, 6H) ppm; **^{13}C NMR** (75 Mhz): δ = 80.9, 82.5, 86.1, 123.6, 125.9, 127.1, 129.9,

132.7, 137.4, 138.8, 141.3, 142.9, 196.8 ppm; **HRMS** (ESI): calculated for $C_{15}H_{10}INO$ 346.9807; found 346.9800.

2-Chloro-*N*-(2-(4-ethynylbenzoyl)-4-iodophenyl)acetamide (18)

Under nitrogen atmosphere, (2-aminophenyl)(4-((trimethylsilyl)ethynyl)phenyl)methanone **17** (400 mg, 1.2 mmol) and Et_3N (0.11 ml) were dissolved in $CHCl_3$ (15 mL) and chloroacetyl chloride (0.11 mL, 1.3 mmol) was added dropwise to the solution at 0 °C. The solution was allowed to warm up and stirred at r.t. for 12 h. Water (20 mL) was added to the solution and the organic layer was separated. The water layer was extracted with DCM (2 x 20 mL) and the organic layers were combined, dried over $MgSO_4$, filtered and the solvent was evaporated to dryness. The product was purified by silica gel flash chromatography (PE/EE, 2:1 v/v) and a brown solid was obtained. Yield (355 mg, 70%), mp 160-162 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 3.29-3.30 (s, 1H), 4.19 (s, 2H), 7.57-7.71 (m, 4H), 7.82-7.84 (m, 1H), 7.86-7.91 (m, 1H), 8.39-8.43 (m, 1H), 11.43 (s, 1H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 43.1, 80.9, 82.5, 86.1, 123.6, 125.9, 127.1, 129.9, 132.3, 137.4, 138.8, 141.3, 142.9, 165.4, 196.8 ppm; **IR** (film): $\tilde{\nu}$ = 487, 521, 562, 599, 645, 665, 724, 780, 845, 900, 932, 964, 1015, 1082, 1111, 1155, 1175, 1235, 1250, 1289, 1309, 1384, 1403, 1508, 1567, 1590, 1633, 1689, 1946, 1990, 2026, 2111, 2168, 2952, 3116, 3289 cm^{-1}; **HRMS** (ESI): calculated for $C_{17}H_{11}ClINO_2$ 422.9523; found 422.9520.

5-(4-Ethynylphenyl)-7-iodo-1H-benzo[e][1,4]diazepin-2(3H)-one (19)

Under nitrogen atmosphere, 2-chloro-N-(2-(4-ethynylbenzoyl)-4-iodophenyl)acetamide **18** (320 mg, 0.8 mmol), $NH_4(OAc)$ (128 mg, 1.7 mmol) and HMTM (233 mg, 1.7 mmol) were dissolved in EtOH (100 mL) and stirred under reflux for 48 h. The solvent was evaporated to dryness and the precipitate was taken up in DCM (50 mL). The organic layer was washed with water (50 mL), dried over $MgSO_4$, filtered and evaporated to dryness. The product was purified by column chromatography (PE/EE, 2:1 v/v) and a brown solid was obtained. Yield: (154 mg, 50 %). **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 3.21 (s, 1H), 4.32 (s, 2H), 6.94 (d, *J* = 8.5 Hz, 1H), 7.49-7.56 (m, 4H), 7.58-7.61 (d, *J* = 7.6 Hz, 1H), 7.80 (dd, *J* = 10.5 , 8.5 Hz, 1H), 9.0 (s, 1H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 56.7, 81.5, 82.4, 119.8, 125.0, 127.6, 128.8, 131.7, 131.7, 132.0, 132.5, 138.4, 138.8, 168.6, 170.2 ppm; **IR** (film): $\tilde{\nu}$ = 3262, 3100, 2924, 2854, 2364, 2200, 2169, 1979, 1950, 1681, 1596, 1560, 1474, 1381, 1312, 1284, 1264, 1227, 1109 cm^{-1}; **HRMS** (ESI): calculated for $C_{17}H_{11}IN_2O$ 385.9916; found 385.9920.

5-(4-(1-(2-(2-(2-Hydroxyethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)phenyl)-7-iodo-1H-benzo[e][1,4]diazepin-2(3H)-one (20)

Under nitrogen atmosphere 5-(4-ethynylphenyl)-7-iodo-1H-benzo[e][1,4]diazepin-2(3H)-one **19** (100 mg, 0.26 mmol), 2-(2-(2-azidoethoxy)ethoxy)ethanol (59 mg, 0.34 mmol), $Cu(SO_4)$ x 5 H_2O (32 mg, 0.13 mmol) and sodium ascorbate (128 mg, 0.64 mmol) were dissolved in DMF (10 mL) and stirred for 24 h at r.t. EE (100 mL) was added and the organic layer was washed with brine (5 x 100 mL). The organic layers were combined, dried over $MgSO_4$, filtered and the solvent was removed in vacuo. The product was purified by flash column chromatography (EtOH) and a colorless oil was obtained. Yield: (63 mg, 57 %). **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 3.55-3.60 (m, 2H), 3.63-3.64 (m, 4H), 3.69-3.74 (m, 2H), 3.90-3.94 (m, 2H), 4.33 (s, 2H), 4.60 (t, *J* = 4.8, 9.8 Hz, 2H), 5.30 (s, 1H), 6.95 (d, *J* = 8.5 Hz, 1H), 7.54-7.60 (m, 2H), 7.65 (d, *J* = 1.98 Hz, 1H), 7.76 (dd, *J* = 2.0, 8.5 Hz, 1H), 7.84-7.90 (m, 2H), 8.05 (s, 1H), 9.20 (s, 1H) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 50.4, 61.7, 69.5, 70.2, 70.6, 72.5, 86.8, 121.7, 123.1, 125.6, 129.1, 130.2, 133.0, 138.2, 138.4, 139.7, 140.5, 146.7, 159.9, 169.2, 171.5 ppm; **IR** (film): $\tilde{\nu}$ = 3201, 3099, 2920, 2850, 2360, 1890, 1680, 1320, 1227, 1108, 830, 560, 470 cm^{-1}; **HRMS** (ESI): calculated for $C_{23}H_{24}IN_5O_4$ 561.0873; found 561.0870.

7-(4-(2-(5-Chloro-2-methylthiophen-3-yl)cyclopent-1-en-1-yl)-5-methylthiophen-2-yl)-5-(4-(1-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)phenyl)-1H-benzo[e][1,4]diazepin-2(3H)-one (21)

DTE **10** (36 mg, 0.11 mmol) was dissolved in dry THF (20 mL) under nitrogen atmosphere and cooled to -70 °C by a bath of dry ice in EtOH. *n*-BuLi (1.32 M in hexane, 91 µL, 0.12 mmol) was added dropwise via syringe and the resulting purple solution was stirred in the cold for 60 min and then it was treated with trimethyl borate (18 µL, 0.16 mmol). It was stirred for 30 min at -70 °C and after removal of the dry ice bath for further 60 min at r.t. The reaction was then quenched with aqueous Na_2CO_3 (2 M, 10 mL). After adding the BDA **20** (61 mg, 0.11 mmol), the reaction mixture was degassed by nitrogen bubbling for 15 min. $Pd(PPh_3)_2Cl_2$ (4 mg, 0.005 mmol) was added and the reaction was heated to 80 °C over-night. After cooling to r. t., the reaction mixture was diluted with DCM and water and the phases were separated. The aqueous phase was extracted with DCM (2 x 5 mL), the combined organic phases were dried over $MgSO_4$ and the solvents removed under reduced pressure. Purification of the crude product was performed by automated flash column chromatography (EtOH/DCM 9.8:0.2, → 9:1 v/v) followed by automated RP flash column chromatography (MeCN/water 0-100%) yielding compound **21** as purple oil. Yield: (53 mg, 66 %). **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 1.85 (s, 3), 1.95 (s, 3H), 2.00 (t, *J* = 7.3 Hz, 2H), 2.73 (dt, *J* = 7.3, 7.4 Hz, 4H), 3.45-3.78 (m, 8H), 3.92 (t, *J* = 4.9 Hz, 2H), 4.36 (s, 2H), 4.60 (t, *J* = 4.9 Hz, 2H), 6.55 (s, 1H), 6.86 (s, 1H), 7.15 (d, *J* = 8.5 Hz), 7.41 (d, *J* = 2.0 Hz, 1H), 7.52-

7.73 (m, 3H), 7.87 (d, *J* = 8.4 Hz, 2H), 8.04 (s, 1H), 9.06 (s, 1H) ppm; **^{13}C NMR** (75 Mhz, $CDCl_3$): δ = 14.2, 14.4, 22.9, 28.3, 38.4, 50.4, 56.8, 61.7, 69.5, 70.3, 70.7, 72.5, 121.6, 121.7, 124.4, 125.0, 125.5, 126.8, 127.4, 128.7, 130.0, 130.3, 132.8, 133.2, 134.2, 135.0, 135.3, 136.7, 137.4, 137.8, 138.6, 147.0, 170.2, 171.6 ppm; **HRMS** (ESI): calculated for $C_{38}H_{38}ClN_5O_4S_2$ 728.2127; found 728.2125.

5.4.3 Biology - Methods of the Working Group of Prof. Bregestovski

Cell culture, transient transfection and electrophysiological recording was done as reported in literature.[18]

5.4.4 Biology - Methods of the Group of Prof. Sigel

Xenopus surgery, expression of $GABA_A$-Receptors in Xenopus Oocytes, functional characterization of the $GABA_A$-Receptors was done as reported in literature.[8, 19, 20]

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Appendix

A1. Abbreviations

abs.	absolute
ATP	adenosintriphosphate
BAPTA	1,2-bis(o-aminophenoxy)ethane-N,N,N´,N´-tetraacetic acid
BDA	benzodiazepine
Bn	benzyl
Boc	tert-butoxycarbonyl
cDNA	complementary desoxyribonucleic acid
CHO	chinese hamster ovary
CNS	central nervous system
conc.	concentrated
cRNA	complementary ribonucleic acid
d	day
dppp	1,3-bis(diphenylphosphino)propan
dppe	1,2-bis(diphenylphosphino)ethane
dppf	1,1-bis(diphenylphosphino)ferrocen
DCM	dichloromethane
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DTE	dithienylethene
EC_{50}	agonist concentration that elicts 50 % of the max. response
EE	ethyl acetate
EI	electron ionization
eq	equivalent
ESI	electron spray ionization
Et_3N	triethylamine
EtOH	ethanol
eV	electron volt
GABA	gamma amino butyric acid
GC	gas chromatography
GP	general procedure
GTP	guanosine triphosphate

h	hour
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HMTM	hexamethylenetetramine
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectroscopy
IR	infrared spectroscopy
LC	liquid chromatography
MeOH	methanol
MHz	megahertz
MOHm	mega ohm
mp	melting point
MS	mass spectroscopy
n	number
NIR	near infrared spectroscopy
NMR	nuclear magnetic resonance
ORTEP	oak ridge thermal ellipsoid plot
Osm	osmole
pCMV	porcine cytomegalovirus
PE	petrolether
PEG	polyetheylene glycol
Pin	pinacole
ppm	parts per million
r.t.	room temperature
Rf	retention factor
RP	reverse phase
SI	supporting information
$t_{1/2}$	half-life time
TEA	triethanolamine
temp.	temperature
THF	tetrahydrofurane
TLC	thin layer chromatography
UV	ultraviolet
VIS	visible
wt	weight percent

A2. Curriculum Vitae

Personal data

Name	Peter Raster
Date of birth	25. September 1983
Place of birth	Deggendorf
Nationality	German
Marital status	Single
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Education

1990 – 1994	Grundschule St. Martin I, Deggendorf
1994 – 2003	Robert Koch Gymnasium, Deggendorf
2003	Abitur

Civilian service

2003 – 2004	Asklepios Klinik, Schaufling

Professional training

2004 – 2009	University of Regensburg
2009	Diplom (M. Sc.) in Chemistry
01/2010 – 09/2013	PhD work at the department of Organic Chemistry, University of Regensburg

A3. Summary in German and English

Summary in German

Kapitel 1 beschreibt die Synthese von symmetrischen und unsymmetrischen Diarylcyclobutenen in einer Kobalt-katalysierten [2+2] Cycloadditionsreaktion. Dazu wurden die entsprechenden Diarylalkinvorstufen mit Cyclopenten oder einem Norbornenderivat umgesetzt. Die Reaktion läuft unter milden Reaktionsbedingungen bei Raumtemperatur ab und die gewünschten Produkte konnten in 20-70 % Ausbeute isoliert werden. Die erhaltenen Diarylcyclobutene sind reversibel photoschaltbar und die geschlossenen Isomere zeigten Halbwertszeiten von 9 – 300 h.

Kapitel 2 behandelt die Synthese von photoschaltbaren Diarylcyclohexenen in einer Kobalt-katalysierten Diels-Alder Cycloadditionsreaktion. Dazu wurden verschiedene Diaryl-acetylene mit Boropren umgesetzt und eine dihydroaromatische Zwischenstufe mit einer Allylboronsäuregruppe generiert. Diese Zwischenstufe wurde dann *in situ* mit verschiedenen Aldehyden umgesetzt und photochrome Diarylcyclohexene erhalten. Die Synthesemethode ermöglicht die einfache Einführung von funktionellen Gruppen am Rückgrat des Photoschalters ohne den Chromophor zu beeinflussen. Eine Anwendung für die funktionalisierten Diarylethene wird durch die Immobilisierung der Farbstoffe auf Cellulose und Silikagel gezeigt.

Kapitel 3 beschreibt die Synthese, Reinigung und biologische Testung von neuen und zuvor literaturbekannten GABA-Amiden und die Bestimmung des Effekts auf die Aktivierung von $GABA_A$-Rezeptoren durch Patchclamp-Messungen in CHO-Zellen exprimierten $GABA_A$-Rezeptoren. Um Verfälschungen der Messungen durch GABA-Verunreinigung, die in der Synthese entstehen können, ausschließen zu können, wurde eine verbesserte Reinigungsmethode und HPLC-MS Bestimmungsmethode entwickelt. Wir konnten die in der Literatur beschriebenen Ergebnisse nicht bestätigen, aber die neu synthetisierten GABA-Amide zeigten moderate Aktivierung des $GABA_A$-Rezeptors und es war uns möglich eine eindeutige Struktur-Wirkungsbeziehung abzuleiten.

Kapitel 4 behandelt die Synthese von photoschaltbaren Liganden mit potentiellem Effekt auf die Aktivierung des $GABA_A$-Kanals. Dazu wurden in einer ersten Testreihe drei mit einem Diarylethenphotoschalter substituierte Benzodiazepinderivate synthetisiert und deren Effekt auf die Aktivierung des $GABA_A$-Kanals von zwei unabhängigen Forschungsgruppen durch Patch-Clamp Messungen an $GABA_A$-Rezeptoren untersucht. Zwei der synthetisierten Benzodiazepinderivate aus der ersten Serie zeigten moderate Verstärkung der durch GABA verursachten Signale, die genaue Aktivität konnte jedoch nicht bestimmt werden, da

die Substanzen eine zu geringe Wasserlöslichkeit hatten. Durchgeführte Bestrahlungsexperimente mit UV-Licht gaben auch kein eindeutiges Ergebnis und waren nicht reproduzierbar. Deshalb wurde ein besser wasserlösliches Benzodiazepin-Derivat synthetisiert und der Effekt auf den $GABA_A$-Rezeptor bestimmt. Die Meßergebnisse waren jedoch widersprüchlich und bis zum jetzigen Zeitpunkt kann keine eindeutige wissenschaftliche Aussage gemacht werden.

Summary in English

Chapter 1 describes the synthesis of symmetric and unsymmetric diarylcyclobutenes in a cobalt-catalyzed [2+2] cycloaddition reaction. Therefore, the appropriate diarylalkyne precursors were reacted with cyclopentene or a norbornene derivative. The reaction proceeds under mild reaction conditions at room temperature and the target compounds could be obtained in 20-70 % yield. The synthesized diarylcyclobutenes are reversibly photoswitchable and show different thermal stability towards reisomerization with half-lives ranging from 9 – 300 h.

Chapter 2 deals with the synthesis of photoswitchable diarylcyclohexenes in a cobalt catalyzed Diels-Alder cycloadditon reaction. Therefore different diarylacetylenes were reacted with boroprene and a dihydroaromatic intermediate with an allylboronic ester subunit was generated and then reacted *in sito* with different aldehydes and photochromic diarycyclohexenes were obtained. The synthesis allows the simple introduction of functional groups at the backbone of the photoswitch without affecting the chromophore. An application of the functionalized diarylethenes is the immobilization of the chromophore on cellulose and silica gel.

Chapter 3 describes the synthesis and purification of new and literature reported GABA-amides and the determination of their effect with patch-clamp recording on $GABA_A$-receptors which were expressed in CHO-cells. In order to exclude contaminations of GABA which could falsify test results, an improved purification method and sensitive HPLC-MS analysis was developed. We could not confirm the literature reported results, but the new GABA-amides showed moderate activation of the $GABA_A$-receptor and we were able to predict a clear structure activity relationship.

Chapter 4 describes the synthesis of photoswitchable ligands with potential effect on the activation of the $GABA_A$-receptor. Therefore, in a first approach, a series of benzodiazepine derivatives substituted with a diarylethene photoswitch were synthesized and their effect on $GABA_A$-receptor activation was determined by two independent research groups with patch

clamp recording. Two of the compounds out of the first series showed moderate amplification of GABA induced current, but the exact activity could not be determined because of the insufficient solubility of the compounds in aqueous solution. UV-irradiation experiments gave no clear results and were not reproducible. Therefore, a better water soluble benzodiazepine derivative was synthesized and the effect of $GABA_A$-receptor activation was determined. The test results were inconsistent and until now, no definite scientific conclusion can be drawn.