Synthesis and Use of Tetrahydrofuran Amino Acids and Reductive Deoxygenation of Alcohols

Dissertation

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Michael Dobmeier

aus Weiden i. d. Opf.

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	Prof. Dr. Burkhard König	(1st referee)
	Prof. Dr. Oliver Reiser	(2nd referee)
	Prof. Dr. Arno Pfitzner	(examiner)

Meiner Familie

Danke für Alles!

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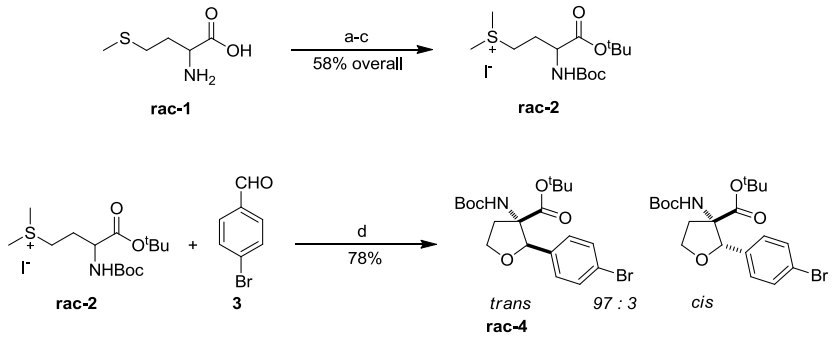
1 Chemistry of tetrahydrofuran amino acids – a short introduction

This chapter provides to the reader a comprehensive overview about the chemistry of the amino acid 3-amino-2-(4-bromophenyl)tetrahydrofuran-3-carboxylic acid, which is one representative from the group of tetrahydrofuran amino acids.

1.1 3-Amino-2-(4-bromophenyl)tetrahydrofuran-3-carboxylic acid

Preparation and properties. The four step synthesis of the cyclic unnatural amino acid 3-amino-2-(4-bromophenyl)tetrahydrofuran-3-carboxylic acid was reported the first time by König et al. in 2007.[1] The synthesis illustrated in Scheme 1, starts from cheap and commercial available racemic methionine **rac-1** with N-Boc protection, subsequent protection of the carboxylic group via Steglich-type esterification and alkylation of the side chain using methyl iodide obtaining sulfonium salt **rac-2**. The crucial step of the reaction sequence is initiated by treatment with KOH to abstract the acidic proton in α-position generating an ester enolate. This reacts with 4-bromobenzaldehyde in an aldol-type reaction, which is followed by an intramolecular nucleophilic substitution reaction employing dimethylsulfide as leaving group to build up the tetrahydrofuran amino acid **rac-4**.



Scheme 1. Synthetic pathway towards unnatural tetrahydrofuran amino acid **rac-4** obtained as racemic mixture. *Reaction conditions:* (a) Boc_2O, NaOH, 1,4-dioxane/water, RT, 3 h. (b) ^{t}BuOH, DCC, DMAP, DCM, RT, 12 h. (c) MeI, in the dark, RT, 3 d. (d) KOH, dry MeCN, -6 °C, 2-4 h.

During the reaction two new stereocenters are formed, leading to in total 4 possible stereoisomers. The reaction proceeds with a broad range of aromatic aldehydes, e.g. 4-nitrobenzaldehyde, 4-methoxybenzaldehyde, benzaldehyde, 4-methylbenzaldehyde, 4-cyanobenzaldehyde, 3-bromobenzaldyde among others in a highly diastereoselective manner (trans/cis ratio up to 97:3) with moderate to good yields (35-78%) depending on the aldehyde used. Best results were obtained with aldehyde **3**, giving 78% yield and a trans-selectivity of 97:3. Figure 1 shows the proposed reaction mechanism and a comparison of the formation of the trans- and cis- isomers. The high sterical demanding ^{t}Bu-ester leads to the preferred formation of trans-isomers and high diastereoselectivity.

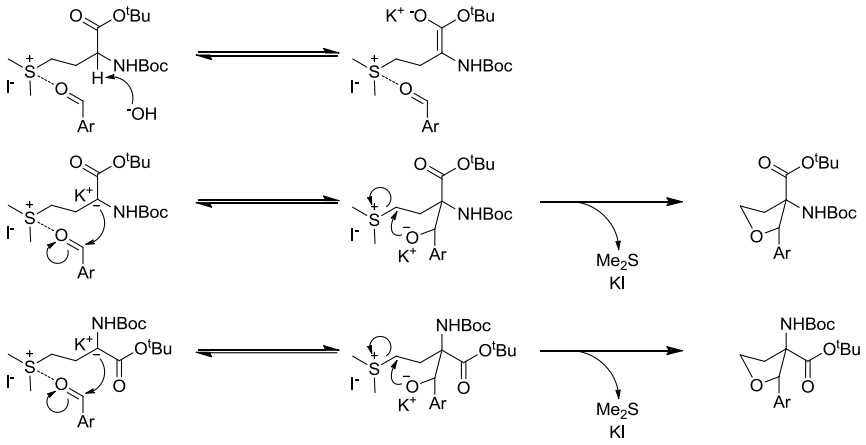


Figure 1. Proposed reaction mechanism for the formation of C$^{\alpha}$-tetrasubstituted tetrahydrofuran amino acids.

Incorporated in small peptides, interesting properties of compound **rac-4** were revealed as this class of compounds is able to stabilize secondary structures in short peptides. Therefore the R,S,S-isomer of Boc-TAA-Ala-NHBn dipeptide adopts a β-turn type I conformation, whereas the S,R,R-isomer does not. The elongated R,S,S-isomer of the Ac-Gly-TAA-Ala-NHBn tripeptide showed in the solid state as well as in solution a conformation of two consecutive β-turn type III structures, which are stabilized by i+3 -> i intramolecular hydrogen bonds (Figure 2).

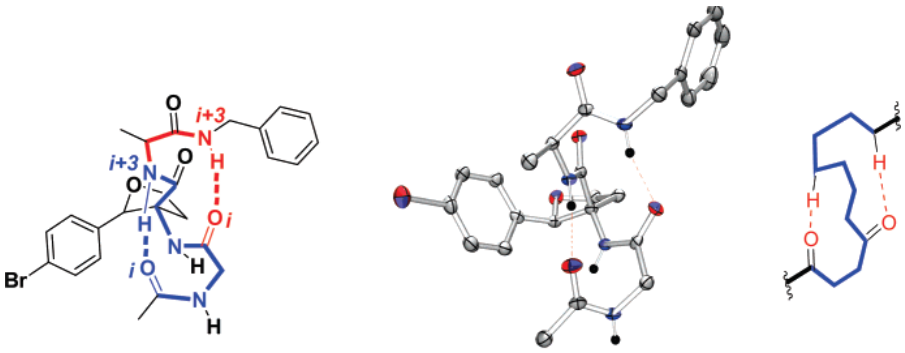
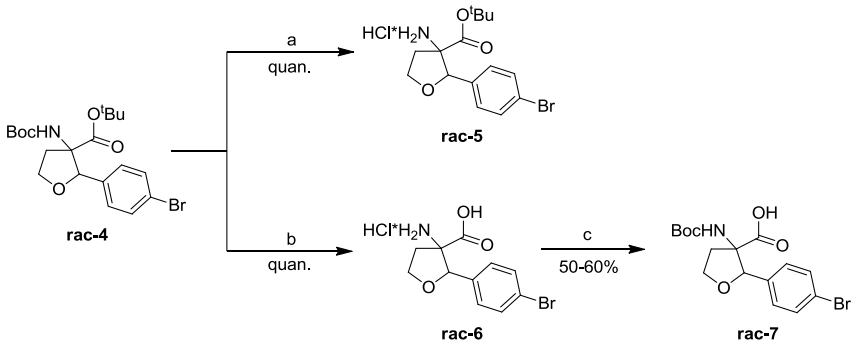


Figure 2. Structure (left) and X-ray diffraction analysis (center) of Ac-Gly-(R,S)-TAA-(S)-Ala-NHBn, which exhibits two consecutive β-turns. They are each stabilized by an intramolecular i+3 -> i hydrogen bond (dashed lines), where only amide hydrogen atoms are drawn. The backbone structure of the tripeptide is shown at the right side. (Graphics are depicted from literature: Maity, P. PhD Thesis, Universität Regensburg, Regensburg, **2008**).

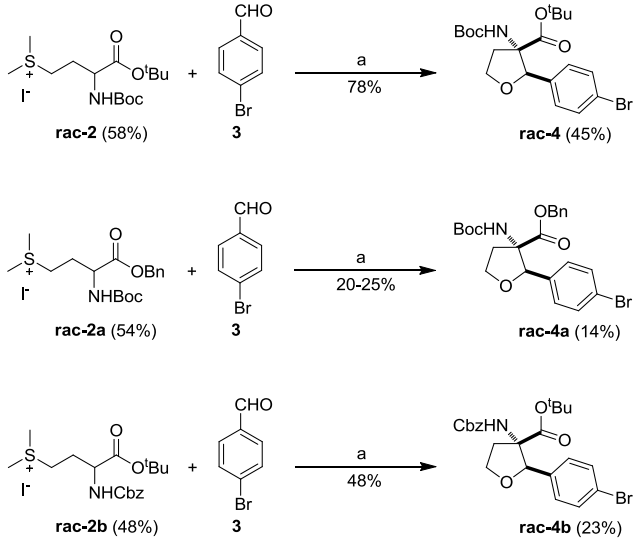
Protecting group strategy. First investigations focused on the optimization of the protecting group strategy since the combination of Boc-/^{t}Bu-ester is not fully orthogonal in the reported synthesis. On the one hand, the selective removal of the Boc-group in the presence of the ^{t}Bu-ester using HCl saturated diethyl ether was feasible. But on the other hand, there was no way to cleave the ^{t}Bu-ester and at the same time leaving the Boc-group unharmed. To obtain the N-terminally protected amino acid **rac-7** it was necessary to remove both groups followed by the reprotection of the amine. This

procedure gave **rac-7**, which is the most interesting building block for peptide syntheses in a maximum yield of only 50-60% (Scheme 2).



Scheme 2. Deprotection of **rac-4**. *Reaction conditions:* (a) HCl sat. Et_2O, DCM, RT, 3 h. (b) 6M HCl, MeOH, reflux, 8 h. (c) Boc_2O, 1.25M aq. NaOH, 1,4-dioxane, 0 °C, 3.5 h.

In scheme 3, a comparison between Boc-/^{t}Bu-ester strategy and the two alternative approaches Boc-/Bn-ester[2] and Cbz-/^{t}Bu-ester[3,4] is shown. These strategies were examined in detail as they are each fully orthogonal protection groups and in addition stable to the strongly basic conditions, which were needed during the key step of the tetrahydrofuran amino acid synthesis.



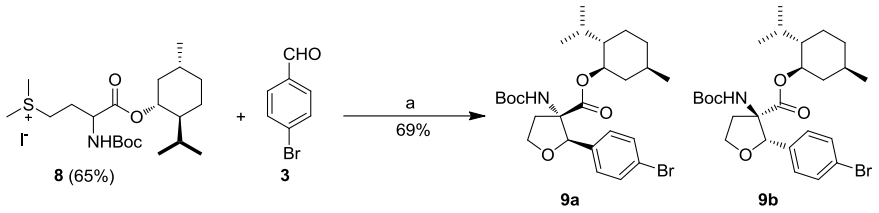
Scheme 3. Comparison of different protection group strategies in the synthesis of tetrahydrofuran amino acids. *Reaction conditions:* (a) KOH (or CsOH), dry MeCN, -6 °C, 2-6 h.

The overall yields, which are given in brackets for the three step synthesis of the sulfonium salts were, still in a comparable range. Moreover the high diastereoselective character (*trans/cis* = 97:3) of the original reaction was retained in both cases and the desired products were formed as racemic mixture of the trans-isomers. Unfortunately the yields of the cyclization dropped drastically whereby these alternative protection group strategies became quite unattractive indicated also by the 4-step

overall yields. In addition some problems during the deprotection occurred: The cleavage of the Cbz-group via palladium on charcoal catalyzed hydrogenation reaction was accompanied by a ten-time faster unwanted reduction of the bromoarene substituent. This site reaction was also partly observed during the reductive deprotection of the Bn-ester. Saponification of the Bn-ester with KOH in boiling ethanol for 24 h achieved the free acid in poor 24% yield. A third attempt to remove the Bn-group using a flavine-mediated photocatalytic reduction[5] in MeCN/H_2O showed no conversion at all. Finally it must be admitted that the combination Boc-/^{t}Bu-ester is in spite of the partial restriction the best protection group strategy available so far.

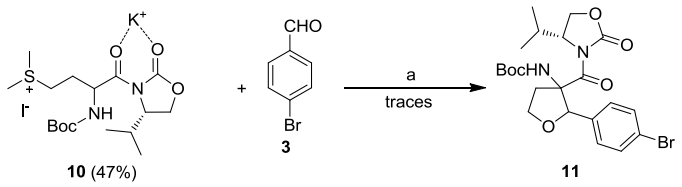
Enantioselective reaction pathway. The second problem of the reported synthesis which needed to be investigated more precisely concerned the enantioselective differentiation of the two trans-isomers representing the major product. Therefore three different approaches were examined in detail: chiral menthol ester (Scheme 4),[4,6] chiral auxiliary (Scheme 5)[7] and the implementation of chiral phase transfer catalysts (Figure 3, Scheme 6).[8]

In the first attempt, the ^{t}Bu-ester was replaced by a chiral ester employing (-)menthol during esterification, which finally lead to the sulfonium salt derivative **8**. The cyclisation reaction with **3** delivered the trans-product in good yield (69%) and high diastereoselectivity but no enantioselectivity was observed and the products **9a** and **9b** were obtained again as 1:1 mixture. Since the two trans-isomers are now no longer enantiomers, but diastereomers, they could be separated by standard column chromatography for the first time. The deprotection of the Boc-group was easy possible using HCl-saturated diethyl ether whereas the deprotection of the ester group needed some more efforts. Finally cleavage with KOH in water/methanol under microwave irradiation gave the free acid in acceptable 55% yield.



Scheme 4. Cyclisation reaction analogous to reported procedure using menthol ester derivative **8** to obtain the two trans-isomers of Boc-TAA-Omenthol **9a** and **9b** (1:1). *Reaction conditions:* (a) KOH, dry MeCN, -6 °C, 4-7 h.

As a second alternative, the chiral auxiliary (S)-4-isopropyloxazolidin-2-one was used. This was reacted with Boc-(S)-Met-OH applying NEt_3 and pivaloyl chloride to end up with compound **10**. The possibility to form a chelat complex with cations can fix the conformation of the ester during cyclisation and may induce an access of one enantiomer. Unfortunately the reaction delivered only traces of the desired product **11**. Modifications of the reaction conditions, like increased reaction times up to 24 h, different base (LDA) or different solvent (THF) were also not successful.



Scheme 5. Cyclisation reaction performed with sulfonium salt derivative **10** carrying the chiral auxiliary (S)-4-isopropyloxazolidin-2-one. *Reaction conditions:* (a) KOH, dry MeCN, -6 °C, 6-24 h or LDA, THF, -78 °C, 6-24 h.

In the last approach an attempt was made to prepare the substrates **rac-2** and **3** of the original reaction, using toluene as non-polar solvent and compounds **12-14** (figure 3) as chiral catalysts in a phase-transfer reaction.

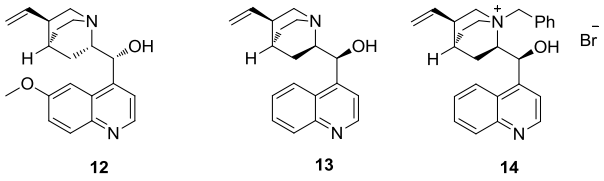
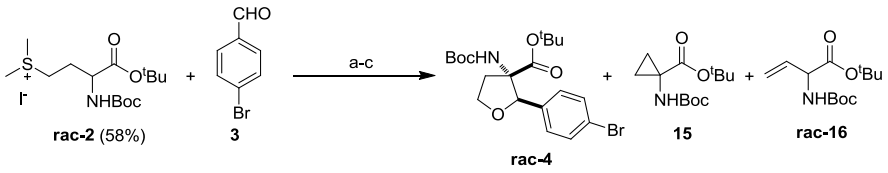


Figure 3. Chiral phase transfer catalyst **12-14**.

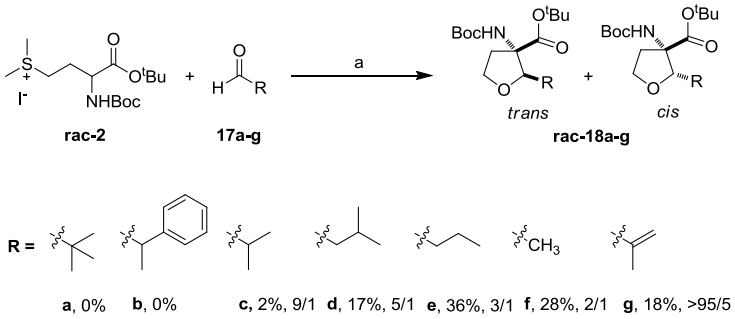
The key-step reaction was carried out in each case with 25 mol% of catalyst. The reaction temperature was first kept at -5 °C for 6 h and then allowed to warm up to room temperature for additional 12 h. Surprisingly all reactions didn't lead to the expected tetrahydrofuran amino acid but in all cases major amounts of cyclopropan amino acid **15** and elimination product **rac-16** were formed. Compound **15** is obtained through intramolecular cyclisation of **rac-2** when not enough aldehyde **3** is present.



Scheme 6. Cyclisation reaction between **rac-2** and **3** under phase transfer catalysis with chiral catalysts. *Reaction conditions:* (a) Compound **12** (25 mol%), KOH, toluene, -5 °C for 6 h to RT, 18 h. (b) Compound **13** (25 mol%), KOH, MeCN, -5 °C for 6 h to RT, 18 h. (c) Compound **14** (25 mol%), KOH, toluene, -5 °C for 6 h to RT, 18 h.

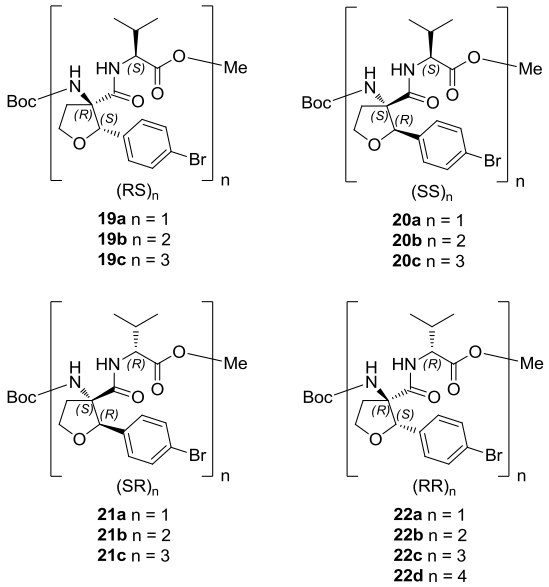
In summary the menthol ester method delivered at least separable diastereomers whereas the chiral auxiliary (S)-4-isopropyloxazolidin-2-one and the chiral phase transfer catalysts failed completely. An enhancement of a single enantiomer could not be observed in all three cases.

Aliphatic side chains. Since the reaction performed well with a broad scope of aromatic aldehydes, a series of aliphatic aldehydes was tested to extend the range of available side chains at the second stereocenter.[9] In contrast to aromatic aldehydes, aliphatic ones are able to undergo undesired aldol side reactions under the strong basic conditions. Therefore pivalaldehyde **17a**, which has no hydrogen atom in α-position was tested first, but unfortunately no product was obtained. Then n-butyraldehyde **17e** was used, giving 16% of corresponding product **18e.** Optimization of the reaction conditions showed that CsOH is the best base for aliphatic aldehyde whereas KOH was the best choice for aromatic ones. Afterwards some more aldehydes were screened: 2-phenylpropanal **17b**, isobutyraldehyde **17c**, 3-methylbutanl **17d**, acetaldehyde **17f** or methacrylaldehyde **17g**. In summary the target cyclic amino acids with aliphatic side chain were obtained in low to moderate yields and sometimes good diastereoselectivity (Scheme 7). But in general the selectivity was lower than in the case of aromatic aldehydes.



Scheme 7. Cyclisation reaction with various aliphatic aldehydes receiving tetrahydrofuran amino acids. *Reaction conditions:* (a) CsOH, dry MeCN, -6 °C, 4-7 h.

Right- and left-handed helical peptides.[10] C^{α}-tetrasubstituted α-amino acids like the tetrahydrofuran amino acid **rac-4** are interesting compounds to rigidify peptide backbones or to prepare peptides with defined secondary structures. The turn inducing properties of one stereoisomer, which were discovered in the first investigations suggest extending the investigations regarding the conformation of TAA containing peptides. Compound **rac-4** was used to prepare a small library of in total 13 different peptides covering di-, tetra-, hexa- and octapeptides with alternating sequences of TAA **rac-4** and (S)- or (R)-valine (scheme 8). The structures were characterized by X-ray diffraction analysis in the solid state and NMR and circular dichroism spectroscopy in solution. The measurements proved that all-S-backbone-configured peptides **20b** and **20c** $(SS)_{2\text{-}3}$ form right-handed 3_{10}-helices, while the all-R-configured peptides **22b**, **22c** and **22d** $(RR)_{2\text{-}4}$ form left-handed 3_{10}-helices in the solid state and solution. In figure 4, the crystal structure of peptide **22d** is exemplary compared to an α-helix indicating close proximity of corresponding residues. Thus **rac-4** is applicable for the synthesis of short peptidomimetics with stable secondary structures in solution.



Scheme 8. Overview about TAA-containing isomeric di-, tetra- hexa- and octapeptides which were synthesized and their conformation investigated in detail.

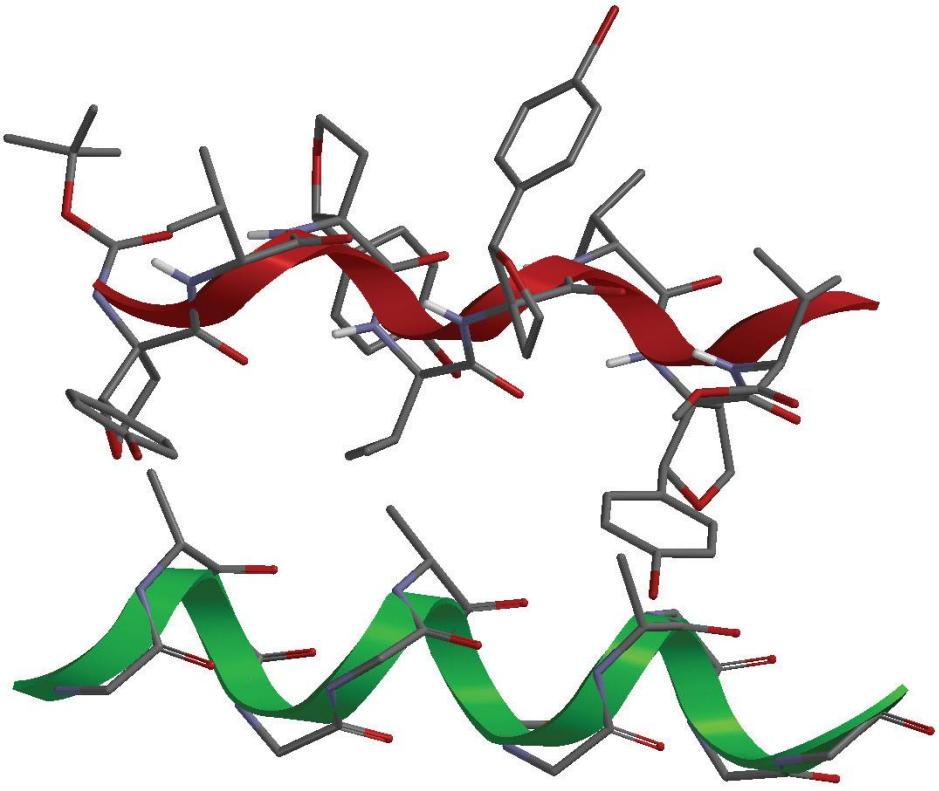
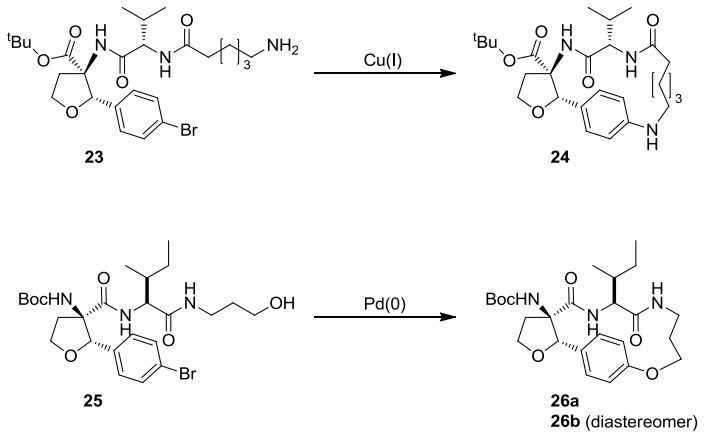


Figure 4. Comparison between the crystal structure of octapeptide **22d** (red ribbon, top) and of an ideal α-helix (green ribbon, bottom, side chains exemplary indicated by alanine residues). The i, i+3, i+6 residues of **22d** are in close proximity to the i, i+1 and i+7 residues of the natural α-helical peptide, which make them suitable as scaffolds and peptidomimetics (Graphic is depicted from literature: Grauer, A. PhD Thesis, Universität Regensburg, Regensburg, **2009**).

Cyclopeptides. The bromoarene substituent of compound **rac-4** enabled further synthetic side chain modifications by transition metal-catalyzed reactions, which were examined in an additional study.[11] At first stage the bromo substituent was replaced by a broad range of aliphatic and aromatic amines, which was mediated by a homogeneous copper(I) catalyzed N-arylation reaction. After optimization of the reaction conditions intramolecular N-arylation reactions were performed to build up cyclic peptides like compound **24**, but unfortunately only small amounts were obtained. In contrast palladium(0) catalyzed O-arylation reaction delivered peptides **26a** and **26b** in an enantiomerically pure form in reasonable yields of 40%, respectively 19%.



Scheme 9. Application of metal-catalyzed reactions like N-arylation with Cu(I) (top) or O-arylation by Pd(0) (bottom) for the synthesis of TAA-containing cyclic peptide mimics **24** or **26a,b**.

Conformational study. Based on the results of the previous synthesized $(TAA\text{-}Val)_n$ (n = 1-4) peptides, which adopted 3_{10}-helical right- or left-handed structures, it was interesting to clarify in an additional conformational study if a single TAA building block is sufficient to induce helical structures in short peptides.[12] As target peptide sequence -Arg-Lys-**Trp**-Gln-Lys-**Thr**-Gly-His-Ala-**Val**- which represents the active side of smooth muscle myosin light chain kinase (smMLCK) was chosen. Advantages of this choice are, that smMLCK is a well-known protein, the binding side has an α-helical structures and the binding amino acids are also known. Furthermore it binds to Calmodulin, which is also well-known and allows examining the synthesized peptide derivative in a binding assay for biological activity. Peptides **27a** and **28a** bearing the turn inducing TAA-building block at the end were synthesized. For comparison also the diastereomers **27b** and **28b** containing S,R-TAA, which does not introduce turns into the structure as well as the peptide Gly-His-Ala-Val were prepared. Investigations using NMR-techniques and circular dichroism spectroscopy revealed that the peptide Gly-His-Ala-Val is too short to adopt a helical structure. So it was excluded that a helical structure is already present in the peptide itself and not due to the influence of the TAA-building block. The results for **27b** and **28b** showed as expected also no defined helical structure. The spectroscopic measurements of peptides **27a** and **27b** showed similar results like in the case of **27b** and **28b**. This, proved that no helical structures either 3_{10}-helic or α-helic was present. In consequence it was

concluded that the induced turn at the beginning of the peptide is not determining the conformation of the subsequent peptide chain.

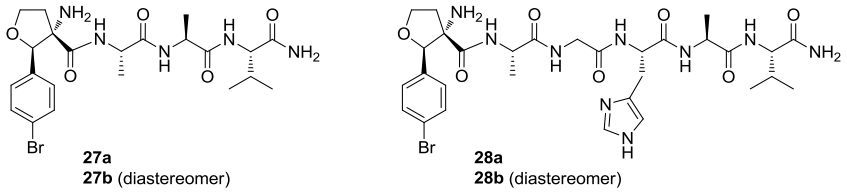


Figure 5. Synthesized peptide **27a,b** and **28a,b** in this study.

1.2 References

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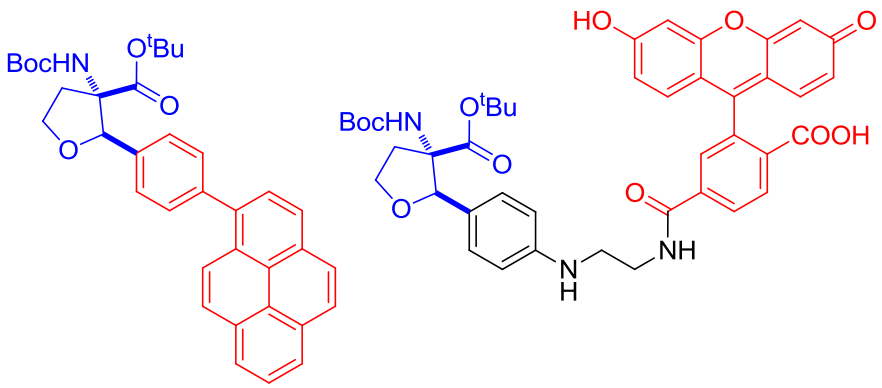
2 Pyrene labeled C^{α}-tetrasubstituted α-amino acids as building blocks for fluorescent peptidomimetics

In this chapter the synthesis of pyrene and carboxyfluorescein labeled C^{α} tetrasubstituted amino acids (TAAs) is described. They can be incorporated into peptides to rigidify the structure and at the same time introducing a fluorescent label. The fluorescent dye can be coupled to the TAA before or after its incorporation into a peptide sequence using a Suzuki-type C-C bond formation. Thus these building blocks combine two properties which are often of high interest in the preparation of peptide analogues.



All compounds which are described in this chapter were synthesized by Michael Dobmeier with the following exceptions: Compounds **rac-3** and **12a** were prepared by Prantik Maity and compounds **16a**/**16b** were synthesized by Muruganantham Rajendran. Prantik Maity contributed the crystal structures for compounds **rac-3** and **18a**/**18b**. Michael Dobmeier did all UV/Vis and fluorescence measurements.

2.1 Introduction

The biological activity of peptides and proteins is based on their conformation which is related to the secondary structures of the amino acid sequence.[1-3] In this context the major drawback of small natural peptides is the conformational flexibility as well as the biological and chemical instability, which may hamper the investigation of biological processes or to perform structural studies. Therefore the rational design and synthesis of peptides and peptidomimetics[4,5] with defined structural properties[6,7] gained much attention by chemists and biologists in the recent past. To stabilize or mimic the conformation of peptides many different approaches exist.[8] One very successful strategy is the disubstitution in α-position of amino acids[9] resulting in a conformational constrain, which enables this class of Cα-tetrasubstituted α-amino acids[10-14] (TAAs) to induce stable β-turn-[15] and helical structures.[16-18] Our group recently reported the synthesis of the Cα-tetrasubstituted tetrahydrofuran amino acid **rac-4** with a broad scope of aromatic side chains. As part of a peptide sequence they initiate two consecutive β-turns and thus leading to stable secondary structure in small peptides.[19] Further we described their use in the preparation of stable right- and left-handed peptide helices,[20] the synthesis of cyclopeptides[21] and the synthesis of derivatives with aliphatic side chains.[22] One of the most powerful and versatile tool for the analysis of peptide and protein conformations, visualization of biopolymers[23-25] and studies on intracellular processes[26] as well as intermolecular interactions[27-30] is emission spectroscopy. Since only two of the proteinogenic amino acids are fluorescent (Trp and Tyr), it is often necessary to attach emitting dyes via side chain functionalities to peptide sequences,[31,32] e.g. the attachment of 1-vinylpyrrole-2-carbaldeyhdes to the ε-amino group of lysine residues by formation of Schiff bases.[33] However, side chain functionalities are responsible for many molecular interactions in proteins and amino acid labeling may alter or prohibit such interactions. Therefore many synthetic fluorescent amino acids have been developed.[34-38] Some representative examples are depicted in Figure 1 including 3-[2-(8-quinolinyl)benzoxazol-5-yl]alanine derivatives (Fig. 1, a),[39] phthalimide based amino acids (Fig. 1, b),[40] different substituted coumaryl analogues (Fig. 1, c)[41] or acridin-9(10H)-one moieties (Fig. 1, d).[42] Suhartono et al. described the linkage of pyrene and other aromatics by Pd-catalyzed Heck-reaction to β,γ-unsaturated and protected amino acids, which were obtained via known side chain transformation of methionine (Fig. 1, e).[43] Finally an example of an anthracene-based bis-armed amino acid is shown (Fig. 1, f).[44] Transition metal-catalyzed reactions, like Suzuki-,[45] Heck-[46] or Sonogashira-[47] couplings are well-established in organic chemistry and highly interesting for the modification of peptides, proteins and other biomolecules since they are usually regarded as bio-orthogonal reactions. In consequence new catalysts or ligands for mild reaction conditions in aqueous buffered solution at room temperature were developed in the last two decades making organopalladium reactions compatible to the thermo sensitive and hydrophilic nature of peptides and proteins.[48-51] Today 4-iodo-phenylalanine is frequently used, which can be incorporated into target peptides and proteins by solution phase chemistry, solid phase peptide synthesis or through genetically encoding.[52]

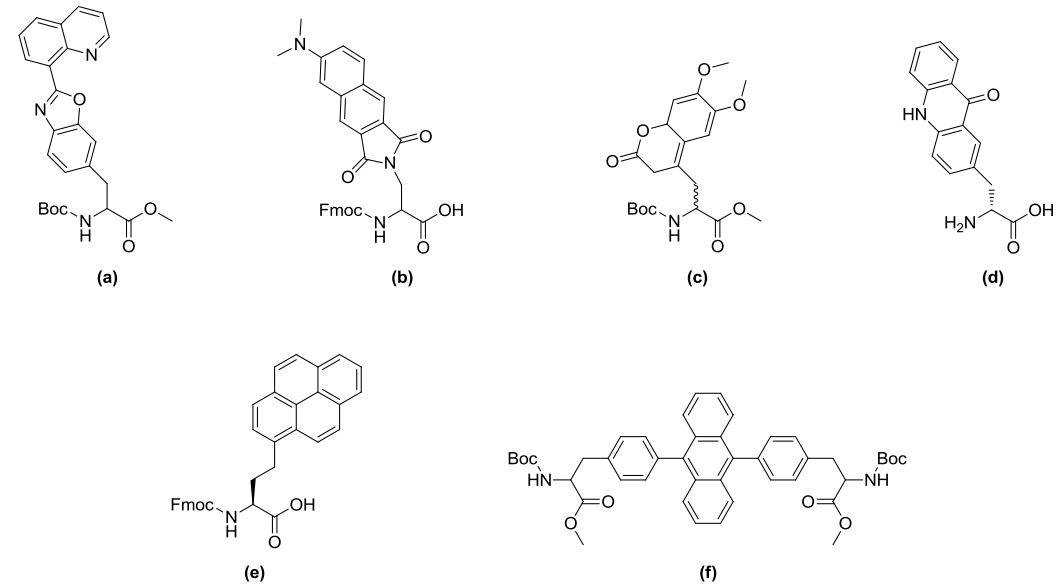


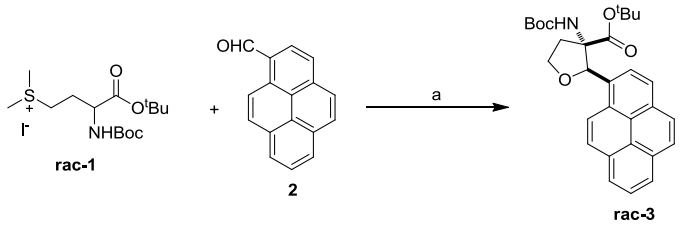
Figure 1. Structures of selected synthetic fluorescent amino acids reported in literature.

We now report several synthetic unnatural TAAs derived from **rac-4** bearing fluorescent dyes such as pyrene or carboxyfluorescein moieties. Moreover, we show the incorporation of a fluorescent TAA building block into a short peptide and the post-functionalization of its bromoarene moiety by a pyrene substituent in the peptide via metal-catalyzed bond formation. These amino acids favorably combine two properties: They stabilize a secondary peptide structure in solution and show interesting absorption and emission in the visible part of the spectrum.

2.2 Results and Discussion

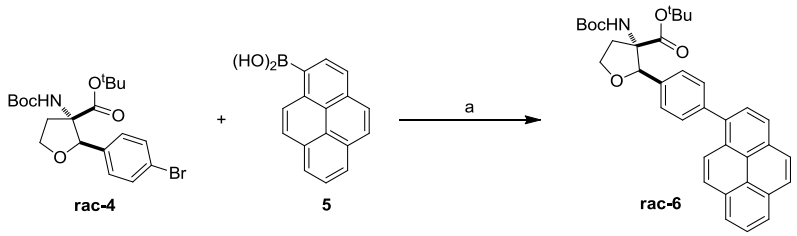
2.2.1 Synthesis of fluorescent TAA building blocks

The reported synthesis of the TAA **rac-4** starts from commercial available methionine with N-Boc protection followed by esterification with tert-butanol and finally methylation of the side-chain by treatment with methyl iodide to obtain compound **rac-1**. The key step of the reaction sequence consists of an aldol-type reaction of the methionine-derived sulfonium salt **rac-1** with an aromatic aldehyde and subsequently a cyclization through an intermolecular S_N2 reaction. Therefore a first derivative **rac-3** containing a fluorescent pyrene moiety was prepared by reacting pyrene aldehyde instead of 4-bromobenzaldehyde with the sulfonium salt **rac-1** under basic conditions in dry acetonitrile according to the known procedure (Scheme 1).[19]



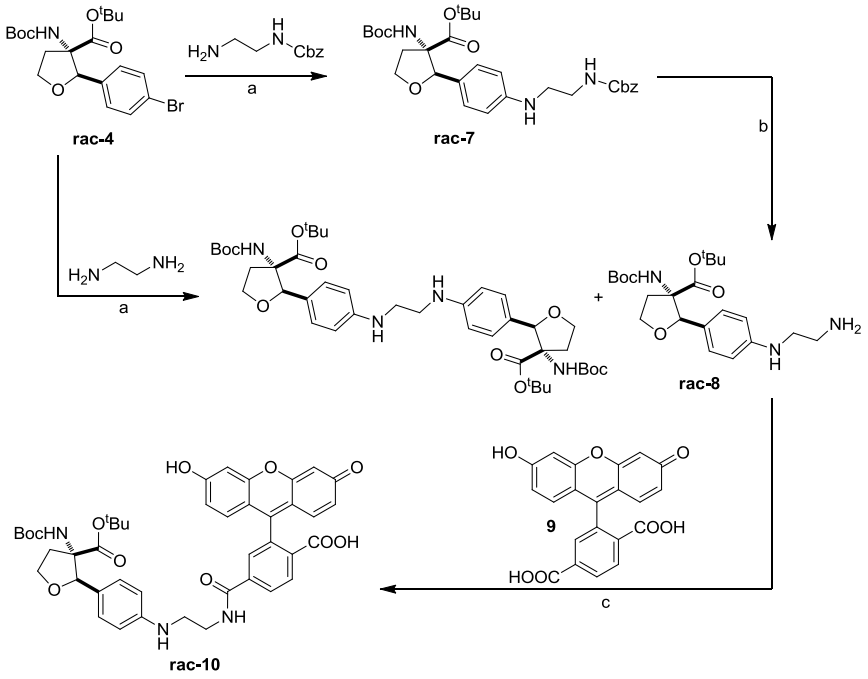
Scheme 1. Synthesis of modified TAA **rac-3**. *Reaction conditions:* (a) KOH, MeCN, 0 °C, 2-4 h.

The compound **rac-3** was obtained in a moderate yield of 56%, which is significantly lower than in the case of **rac-4** (78%) and with a diastereoselectivity of 20:1 (*trans/cis*), being also slightly worse compared to 97:3 (*trans/cis*) for **rac-4**. A possible explanation can be the increased steric demand of the pyrene moiety directly attached to the tetrahydrofuran ring. To avoid such decrease in yield and selectivity the fluorescent moiety was attached via a different strategy using a well-established Pd-catalyzed Suzuki coupling[45] of 1-pyrenyl boronic acid **5** to the brominated substituent of the previously reported compound **rac-4** (Scheme 2). The reaction was carried out in a mixture of DME and water (1:1), in the presence of K_2CO_3 as base and afforded compound **rac-6** as a fluorescent derivate in a yield of 63%.



Scheme 2. Suzuki reaction of TAA **rac-4** with 1-pyrenyl boronic acid **5**. *Reaction conditions:* (a) $Pd(PPh_3)_4$, K_2CO_3, DME/H_2O (1:1), 80 °C, 24 h.

In a third attempt a different dye should be connected to illustrate the scope of the concept. For the introduction of a carboxyfluorescein moiety we used a copper(I)-catalyzed N-arylation[53] to attach ethylene diamine as a linker group to **rac-4**, ending up with **rac-7** in quite low yields of 21%.[21] After quantitative deprotection of the cbz-group under standard conditions, **rac-8** was reacted in a peptide coupling with **9** using HOAt and HATU as coupling reagents and DIPEA as base to obtain the fluorescent labeled TAA derivative **rac-10** in 20% yield (Scheme 3). If the N-arylation was performed with the more reactive non-protected linker in twofold access the desired product **rac-8** was obtained in slightly higher yield (28%) although a small amount of dimerisation product (3-4%) leads to a loss of **rac-4**, the workup and purification was facilitated.

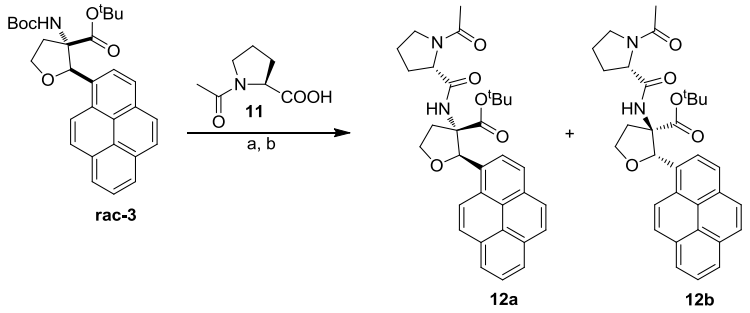


Scheme 3. Copper-catalyzed N-arylation of **rac-4**, if necessary deprotection and subsequent amide bond formation with dye **9** leading to fluorescent compound **rac-10**. *Reaction conditions:* (a) CuI, L-proline, K_2CO_3, dry DMSO, 100 °C, 48 h. (b) Pd/C, H_2, THF, RT, 24 h. (c) HATU, HOAt, DIPEA, DMF, 0 °C to RT, 24 h.

2.2.2 Fluorescent TAA building block in peptide synthesis

Next, the pyrene labeled TAA **rac-3** was incorporated into a short peptide to check if the sterical demanding residue affects the peptide coupling (Scheme 4). Compound **rac-3** was quantitative deprotected at the N-terminus with HCl saturated diethyl ether cleaving the Boc protecting group selectively and resulting in the hydrochloride salt of **rac-3**. This was immediately coupled with N-acetyl-L-proline **11** in the presence of HOAt and HATU as coupling reagent and DIPEA as base to

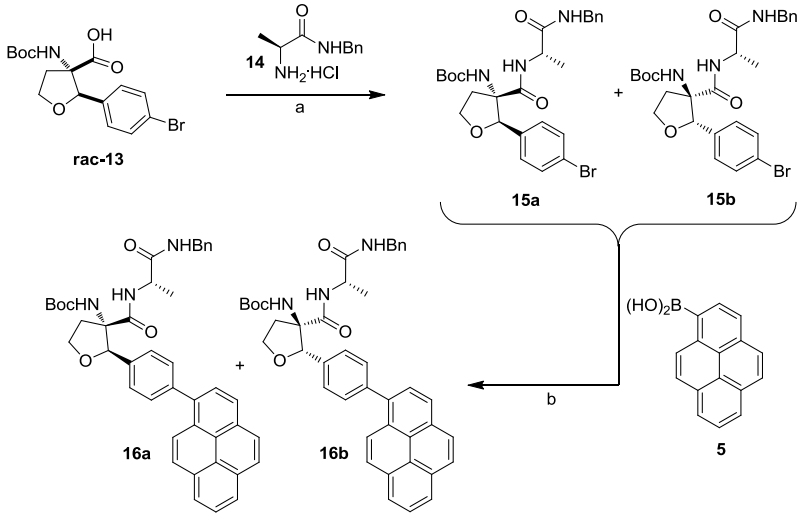
afford the two diastereomeric dipeptides **12a** and **12b** in good yield (70% for **12a**), which were separated by flash column chromatography on silica gel.



Scheme 4. Incorporation of **rac-3** into a short peptide. *Reaction conditions:* (a) HCl sat. Et_2O, DCM, RT, 3 h. (b) Compound **11**, HATU, HOAt, DIPEA, DCM, RT, 48 h.

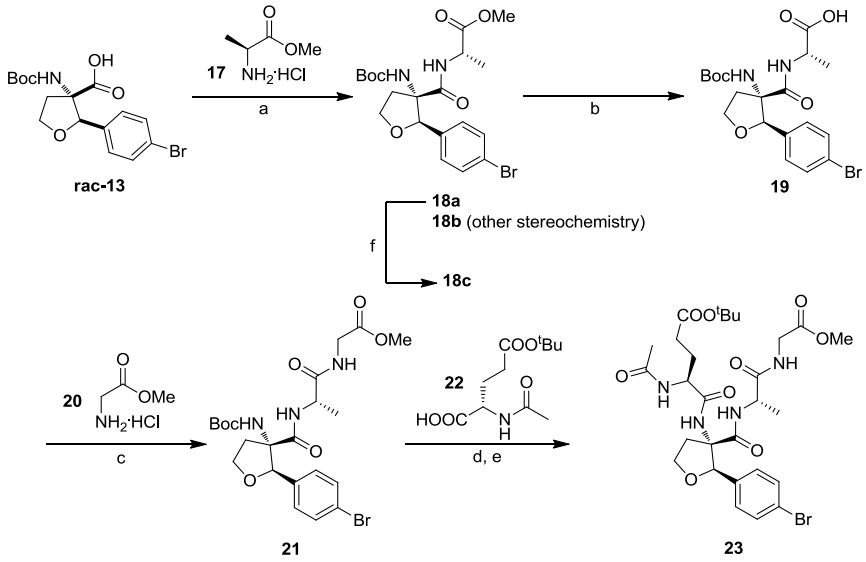
2.2.3 Post-functionalization of small peptides with fluorescent label

The synthetic strategy used for the preparation of **rac-6** is also suitable for the labeling of various small peptides after they have been assembled. Compound **15a** which already adopts a β-turn I conformation as known from the earlier investigations[19] can easily be synthesized by standard peptide coupling reaction of **rac-13** with the hydrochloride salt of L-alanine benzyl amide in 60% yield. Whereas **rac-13** is obtained from **rac-4** through complete deprotection and once more Boc-protection like already described. The mixture of **15a/b** is again reacted in a Suzuki coupling with **5** under identical conditions to receive **16a/b** in moderate yields of 54% (Scheme 5). The separation of these diastereomeric peptides can be done be standard column chromatography on silica gel.



Scheme 5. Functionalization of small TAA containing peptides **15a/b** with 1-pyrenyl boronic acid. *Reaction conditions:* (a) EDC, HOBt, DIPEA, DCM, RT, 24 h. (b) $Pd(PPh_3)_4$, K_2CO_3, DME/H_2O (1:1), 80 °C, 24 h.

The synthesis of tetrapeptide **23** shown in Scheme 6 starts with N-Boc protected racemic tetrahydrofuran amino acid **rac-13**, which was coupled with the hydrochloride salt of S-alanine methyl ester employing EDC, HOBt and DIPEA as coupling reagents to give the diastereomeric dipeptides **18a** (60%) and **18b**. After separation by column chromatography compound **18a** was deprotected at the C-terminus with 1 molar aqueous LiOH solution providing the free acid **19** quantitatively. Under these mild conditions no racemization on the α-carbon of alanine occurs. Compound **19** was then coupled using the same activation reagents with the hydrochloride salt of glycine methyl ester providing tripeptide **21** in high yield of 87%. Finally the N-terminal quantitative deprotection was realized with HCl saturated diethyl ether, followed by peptide coupling reaction with **22** using HOAt and HATU for activation resulting in 23% of compound **23**. As an additional example a fluorescent pyrene moiety was attached to compound **18a** by metal-catalyzed coupling reaction under phase transfer conditions using $Pd(OAc)_2$ as catalyst in the presence of Na_2CO_3 as base and TBAB as phase transfer catalyst. The product **18c** was obtained in a moderate to good yield of 57% which is in a comparable range like the other synthetic protocol used before.



Scheme 6. Synthesis of helical structured tetrapeptide **23** and exemplary labeling of the intermediate dipeptide **18a** with a pyrene moiety. *Reaction conditions:* (a) **17**, EDC, HOBt, DIPEA, DMF, RT, 24 h. (b) LiOH (1M), $MeCN/H_2O$ (4:1), RT, 24 h. (c) **20**, EDC, HOBt, DIPEA, DMF, RT, 24 h. (d) HCl sat. Et_2O, DCM, RT, 3 h. (e) Compound **22**, HATU, HOAt, DIPEA, DMF, RT, 24 h. (f) 1-pyrenyl boronic acid **5**, $Pd(OAc)_2$, TBAB, Na_2CO_3, DMF/H_2O (1:1), 100 °C, 20 h.

2.2.4 UV/Vis- and fluorescene measurements

The absorption and emission spectra for all synthesized compounds carrying a pyrenyl moiety (**rac-3**, **rac-6**, **12a**, **16a**, **16b** and **18c**) were recorded in a concentration range of $5x10^{-6}$ to $5x10^{-5}$ mol/L in MeOH solution and can be found in the supporting information (section 2.4.3, Figure 3). For the fluorescence measurements all compounds were excited at a wavelength of 343 nm. The spectra of **rac-3** and **rac-6** at $3.0x10^{-5}$ mol/L in MeOH are exemplary compared in Figure 2. The absorption spectra of **rac-3** shows maxima at λ (ε) = 343 nm (45900), 276 nm (46000) and 243 nm (66400). The absorption maxima for **rac-6** are located at λ (ε) = 343 nm (29900), 278 nm (39500) and 243 nm (42600) and are therefore quite identical indicating no red shift for **rac-6**. In both spectra intense bands with high molar extinction coefficients (log ε ≥ 4.2) at the lowest energy peak (243 nm) are present which are typically expected for π-π* transitions.[54] The fine structure of **rac-3** is more pronounced whereas in the case of **rac-6** the absorption bands are broadened.

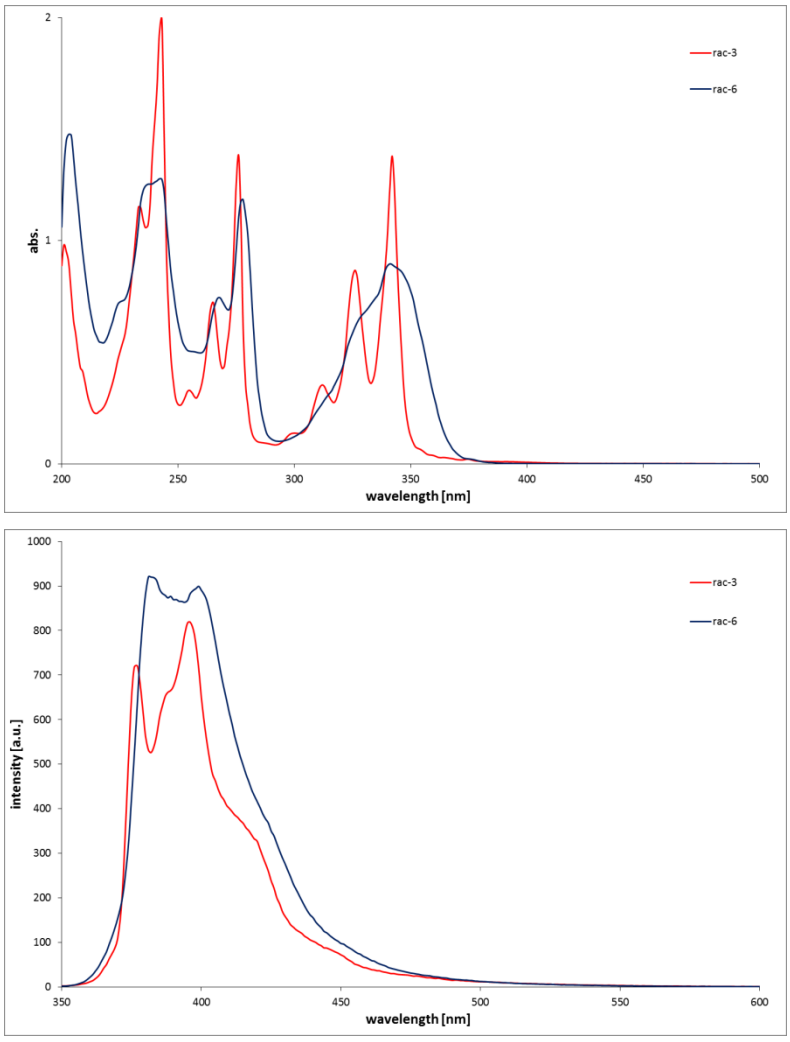


Figure 2. Comparison of the absorption (at top) and emission (below) spectra of compounds **rac-3** (red) and **rac-6** (blue) at a concentration of $3x10^{-5}$ mol/L dissolved in MeOH.

This behavior can be explained by the loss of vibrational structure of the pyrene caused by the conjugation of the additional phenyl ring, which is described for 1-phenylpyrene.[55-57]

The fluorescence spectra revealed emission maxima at 396 nm for **rac-3** and at 400 nm for **rac-6**. The observed slightly red-shift of the maximum as well as the loss of vibrational fine structure and stronger tailing of compound **rac-6** relative to **rac-3** can be explained by interaction of the phenyl group and the pyrene. In addition the fluorescence quantum yields for both compounds were determined using pyrene in cyclohexane (Φ_F = 0.58) as reference: Φ_F = 0.24±0.03 (**rac-3**) and Φ_F = 0.09±0.02 (**rac-6**). The quantum yield of **rac-6** is considerably lower than for **rac-3** since the conjugation of the phenyl group and the pyrene π electrons causes an increase in the non-radiative deactivation pathways.

2.3 Conclusion

In conclusion, we have prepared unnatural Cα-tetrasubstituted α-amino acids, which stabilize secondary peptide structures and bear a fluorescent pyrene or carboxyfluorescein moiety. The compounds are useful as peptidomimetics. To illustrate their synthetic feasibility the building blocks were incorporated in a short peptide. Furthermore we showed for different short peptides that the introduction of a fluorescent dye after the peptide synthesis is possible using the same metal-catalyzed reactions on the bromoarene substituent. The absorption and emission spectra of the prepared building blocks and peptides were investigated. For two building blocks the fluorescence quantum yields were determined and found significantly smaller than the parent chromophore pyrene.

2.4 Experimental Section

2.4.1 General methods and materials

Melting point. Melting points were determined on a Stanford Research System OptiMelt melting point apparatus 100 and are uncorrected.

IR spectra. IR spectra were recorded with a Bio-Rad FT-IR-FTS 155 spectrometer and a Bio-Rad Excalibur series FT-IR-spectrometer FTS 2000 MX using a Specac Golden Gate Mk II ATR accessory where stated.

NMR spectra. NMR spectra were recorded with Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz, T = 300 K), Bruker Avance 400 (^{1}H: 400.1 MHz, ^{13}C: 100.6 MHz, T = 300 K), and Bruker Avance 600 (^{1}H: 600.1 MHz, ^{13}C: 150.1 MHz, T = 300 K) instruments. The chemical shifts are reported in δ [ppm] relative to internal standards (solvent residual peak) or external standard (TMS). The spectra were analyzed by first order, the coupling constants J are given in Hertz [Hz]. Abbreviations of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, psq = pseudo quintet, dd = double doublet, dt = double triplet, ddd = double double doublet. Integration is determined as the relative number of atoms. Assignment of signals in ^{13}C-spectra was determined with DEPT-technique (pulse angle: 135°) and given as (+) for CH_3 or CH, (-) for CH_2 and ($C_{quat.}$) for quaternary C-Atoms. Error of reported values: chemical shift: 0.01 ppm for ^{1}H-NMR, 0.1 ppm for ^{13}C-NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum.

Mass spectra. MS spectra were recorded on a Varian CH-5 (EI), a Finnigan MAT 95 (CI), a ThermoQuest Finnigan TSQ 7000 LC/MS spectrometer and a Finnigan MAT TSQ 7000 (ESI) spectrometer for low resolution (LR-MS) and on a Finnigan MAT 95 (FAB) for high resolution (HR-MS). Xenon served as the ionization gas for FAB.

Absorption spectroscopy. Absorption spectra were recorded on a Varian Cary Bio 50 UV/VIS/NIR Spectrometer using a 1 cm quartz cell from Hellma and UV-grade solvents from Merck (Uvasol®). The temperature for all measurements was kept constant at 25 °C.

Fluorescence spectroscopy. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer using 1 cm quartz cells from Hellma and UV-grade solvents from Merck (Uvasol®) at a constant temperature of 25 °C.

Determination of fluorescence quantum yields. As reference agent pyrene (Φ_F = 0.58, $\lambda_{exc.}$ = 335 nm)[58] dissolved in cyclohexane at a concentration of $3.0*10^{-5}$ mol/L was used. The emission spectra for compounds **rac-3** and **rac-6** were recorded at three different combinations of emission and excitation slit widths (5 nm/5 nm, 5 nm/10 nm, 10 nm/5 nm), while PMT voltage was hold constant

at 600V (for both compounds) and 550V in the case of pyrene. The samples were dissolved in methanol at a concentration of 3.0*10-5 mol/L and excited with 343 nm. Then integrals $\int F$ of solvent-baseline corrected spectra were calculated and absorption spectra for all compounds were measured. According to the equation below, the fluorescence quantum yields Φ_F for each compound at all slit width combinations were calculated and finally given as mean ± s.d. of these three slit width combinations.

$$\Phi_{F,probe} = \frac{A^{335\,nm}_{ref.}}{A^{343\,nm}_{probe}} \times \frac{\int F_{probe}}{\int F_{ref.}} \times \left(\frac{n_{probe}}{n_{ref.}}\right)^2 \times \Phi_{F,ref.}$$

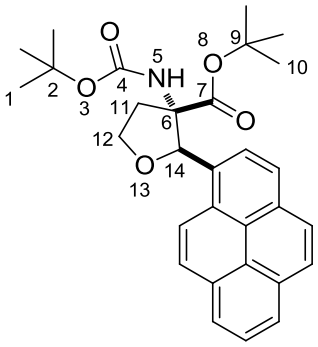
TLC analysis and column chromatography. TLC analyses were performed on silica gel coated alumina plates (Merck 60 F^{254} Silica gel, layer thickness 0.2 mm). Visualization was done by UV-light at 254 nm / 366 nm and/or through staining with ninhydrine in EtOH. For preparative column chromatography, Merck Geduran SI 60 (70-230 mesh) and Macherey-Nagel GmbH & Co. KG 60M (0.04-0.063 mm, 230-400 mesh) silica gels were used. For chromatography commercially available solvents of standard quality were used without further purification.

Solvents and reagents. Commercial solvents, reagents and starting materials were of analytical grade and purchased from Aldrich, Fluka, Merck or Arcos and used without further purification. All reactions were performed under an inert atmosphere of N_2 using standard Schlenk techniques if not otherwise stated. Unless stated otherwise, purification and drying of solvents used was done according to accepted general procedures.[59,60]

2.4.2 Syntheses

The sulfonium salt **rac-1**, the unnatural amino acid Boc-TAA-OtBu **rac-4**, respectively Boc-TAA-OH **rac-13**, compound **rac-7** as well as the diastereomeric peptides Boc-TAA-(S)-Ala-NHBn **15a/15b** and Boc-TAA-(S)-Ala-OMe **18a/18b** were synthesized according to literature known procedures.[19,21]

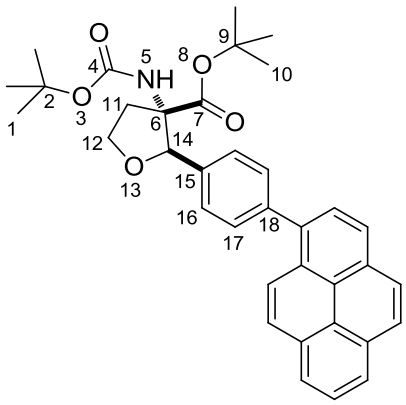
tert-Butyl-3-((tert-butoxycarbonyl)amino)-2-(pyren-1-yl)tetrahydrofuran-3-carboxylate (rac-3):
An oven or flame dried flask was cooled under a stream of nitrogen and charged with sulfonium iodide **rac-1** (447 mg, 1.0 mmol, 1.0 eq.) in 4 mL of dry acetonitrile. The colorless solution was cooled to 0 °C and powdered KOH (56 mg, 1.0 mmol, 1.0 eq.) was added and the reaction mixture was stirred for 15 min. Then pyrene-1-carbaldehyde (207 mg, 0.9 mmol, 0.9 eq.) was added and the mixture was stirred for another 2-4 h. After consumption of all the starting material, the reaction mixture was quenched by adding water (3 mL). The reaction mixture was diluted with diethyl ether (4 mL) and transferred to a separatory funnel. The layers were separated and the aqueous layer was extracted with diethyl ether (2 x 5 mL). Then the combined ether layers were washed with brine (5 mL), dried over $MgSO_4$ and the solvent was removed in vacuo. The crude product was then purified by flash column chromatography on silica gel eluting with petroleum ether/diethyl ether 17:3. The pure product was obtained as yellow solid in 56% yield (245 mg, 0.51 mmol).



R_f (PE/Et_2O = 17:3) = 0.18. – **Mp:** 159-161 °C. – **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 0.80 (s, 9H, 10), 1.58 (s, 9H, 1), 2.80-2.99 (m, 2H, 11), 4.44 (dd, $^3J_{H,H}$ = 8.7 Hz, $^3J_{H,H}$ = 16.2 Hz, 1H, 12), 4.55 (ddd, $^2J_{H,H}$ = 3.8 Hz, $^3J_{H,H}$ = 8.2 Hz, $^3J_{H,H}$ = 8.2 Hz, 1H, 12), 5.73 (bs, 1H, 5), 6.23 (bs, 1H, 14), 7.97-8.31 (m, 9H, Ar-CH). – **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 27.2 (+, 3C, CH_3), 28.5 (+, 3C, CH_3), 36.2 (-, 1C, CH_2), 68.3 (-, 1C, OCH_2), 71.0 ($C_{quat.}$, 1C, CCH_3), 80.2 ($C_{quat.}$, 1C, CNH), 82.4 ($C_{quat.}$, 1C, CCH_3), 82.5 (+, 1C, OCH), 123.0 (+, 1C, Ar-CH), 124.5 ($C_{quat.}$, 1C, Ar-C), 124.7 (+, 1C, Ar-CH), 124.8 ($C_{quat.}$, 1C, Ar-C), 125.0 (+, 1C, Ar-CH), 125.2 (+, 1C, Ar-CH), 125.3 (+, 1C, Ar-CH), 125.9 (+, 1C, Ar-CH), 127.4 (+, 1C, Ar-CH), 127.5 (+, 1C, Ar-CH), 127.5 (+, 1C, Ar-CH), 128.7 ($C_{quat.}$, 1C, Ar-C), 130.6 ($C_{quat.}$, 1C, Ar-C), 131.0 ($C_{quat.}$, 1C, Ar-C), 131.2 ($C_{quat.}$, 1C, Ar-C), 131.4 ($C_{quat.}$, 1C, Ar-C), 154.6 ($C_{quat.}$, 1C, CONH), 169.7 ($C_{quat.}$, 1 C, CO). – **MS** [ESI, CH_2Cl_2/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 488.3 (90) $[MH]^+$, 505.0 (100) $[MNH_4]^+$, 992.7 (100) $[2MNH_4]^+$. – **Anal.** calcd. (%) for $C_{30}H_{33}NO_5$ (487.59): C 73.90, H 6.82, N 2.87, found: C 73.80, H 7.17, N 2.58. – **IR** (KBr) [cm^{-1}]: ν = 3359, 2974, 2867, 2830, 1750, 1703, 1506, 1454. – **UV** (MeOH): λ (ε) = 343 (45900), 276 (46000), 243 (66400). – **MF:** $C_{30}H_{33}NO_5$. – **MW:** 487.59.

tert-Butyl-3-((tert-butoxycarbonyl)amino)-2-(4-(pyren-1-yl)phenyl)tetrahydrofuran-3-carboxylate (rac-6):

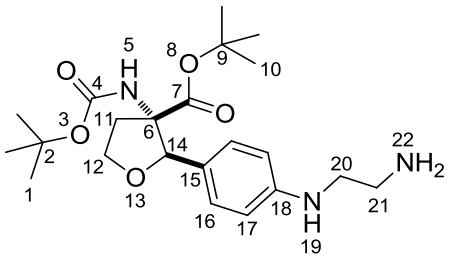
In a three-neck round bottom flask potassium carbonate (137 mg, 0.99 mmol, 3.3 eq.) was dissolved in 2.5 mL of water. The mixture was purged with nitrogen for 10 min. Under nitrogen with stirring compound **rac-4** (132 mg, 0.30 mmol), 1-pyrenyl boronic acid **5** (66 mg, 0.27 mmol, 0.9 eq.) and 2.5 mL of 1,2-dimethoxyethane were added. After 2 min the catalyst $Pd(PPh_3)_4$ (26 mg, 3.5% molar amount) was added and the mixture was stirred for 5 min. The resulting mixture was then heated to reflux in an oil bath at 70 °C for 24 hours. After cooling down to room temperature, water (5 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on silica gel eluting with petroleum ether/ethyl acetate 3:1. The pure product was obtained as yellow solid in 63% yield (96 mg, 0.17 mmol).



R_f (PE/EtOAc = 3:1) = 0.24. – **Mp:** 173.1-174.5 °C. – **^{1}H-NMR** (600 MHz, COSY, $CDCl_3$): δ = 1.26 (s, 9H, 10), 1.55 (s, 9H, 1), 2.65-2.80 (m, 1H, $11_{a/b}$), 2.81-2.92 (m, 1H, $11_{a/b}$), 4.27-4.38 (m, 1H, $12_{a/b}$), 4.44 (ddd, $^2J_{H,H}$ = 3.8 Hz, $^3J_{H,H}$ = 8.2 Hz, $^3J_{H,H}$ = 8.2 Hz, 1H, $12_{a/b}$), 5.22 (bs, 1H, 14), 5.77 (bs, 1H, 5), 7.57 (d, $^3J_{H,H}$ = 8.1 Hz, 2H, 16), 7.61 (d, $^3J_{H,H}$ = 8.2 Hz, 2H, 17), 7.92 (d, $^3J_{H,H}$ = 7.8 Hz, 1H, Ar-CH), 7.97-8.03 (m, 2H, Ar-CH), 8.06-8.10 (m, 2H, Ar-CH), 8.13-8.17 (m, 2H, Ar-CH), 8.17-8.21 (m, 2H, Ar-CH). – **^{13}C-NMR** (150 MHz, HSQC, HMBC, $CDCl_3$): δ = 27.5 (+, 3C, 10), 28.4 (+, 3C, 1), 36.0 (-, 1C, 11), 68.0 (-, 1C, 12), 69.9 ($C_{quat.}$, 1C, 6), 80.0 ($C_{quat.}$, 1C, 2), 82.3 ($C_{quat.}$, 1C, 9), 85.5 (+, 1C, 14), 124.6 (+, 1C, Ar-CH), 124.8 (+, 1C, Ar-CH), 124.8 ($C_{quat.}$, 1C, Ar-C), 124.9 ($C_{quat.}$, 1C, Ar-C), 125.0 (+, 1C, Ar-CH), 125.1 (+, 1C, Ar-CH), 126.0 (+, 1C, Ar-CH), 126.4 (+, 2C, 16), 127.3 (+, 1C, Ar-CH), 127.4 (+, 1C, Ar-CH), 127.4 (+, 1C, Ar-CH), 128.4 ($C_{quat.}$, 1C, Ar-C), 130.1 (+, 2C, 17), 130.5 ($C_{quat.}$, 1C, Ar-C), 130.9 ($C_{quat.}$, 1C, Ar-C), 131.4 ($C_{quat.}$, 1C, Ar-C), 136.7 ($C_{quat.}$, 1C, 15), 137.3 ($C_{quat.}$, 1C, Ar-C), 140.8 ($C_{quat.}$, 1C, 18), 154.5 ($C_{quat.}$, 1C, 4), 170.1 ($C_{quat.}$, 1C, 7). – **MS** (ESI, CH_2Cl_2/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 564.2 (100) $[MH]^+$, 581.3 (50) $[MNH_4]^+$, 605.3 (35) $[MH+MeCN]^+$, 1127.9 (50) $[2MH]^+$, 1144.9 (100) $[2MNH_4]^+$, 1149.8 (65) $[2MNa]^+$. – **HR-MS** (PI-LSIMS, MeOH/CH_2Cl_2/NBA): $[MH]^+$ calcd. for $C_{36}H_{37}NO_5$ 564.2750, found 564.27543. – **Anal.** calcd. (%) for $C_{36}H_{37}NO_5$ (563.68): C 76.71, H 6.62, N 2.48, found: C 76.36, H 6.78, N 2.40. – **IR** (neat) [cm^{-1}]: ν = 2977, 2883, 1699, 1604, 1488, 1364, 1253, 1157, 1067, 842, 720, 682. – **UV** (MeOH): λ (ε) = 343 (29900), 278 (39500), 243 (42600). – **MF:** $C_{36}H_{37}NO_5$. – **MW:** 563.68.

tert-Butyl 2-(4-((2-aminoethyl)amino)phenyl)-3-((tert-butoxycarbonyl)-amino)tetrahydrofuran-3-carboxylate (rac-8):

To a solution of compound **rac-7** (66 mg, 0.12 mmol) in 2 mL of THF, Pd/C (2.5 mg, 20% molar amount) as catalyst was added. Afterwards the mixture was stirred over night at room temperature under an atmosphere of H_2 (20 bar). The catalyst was filtered off, washed with THF and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on silica gel eluting with ethyl acetate/ethanol 1:1. The pure product was obtained as yellow solid in 97% yield (48 mg, 0.11 mmol).



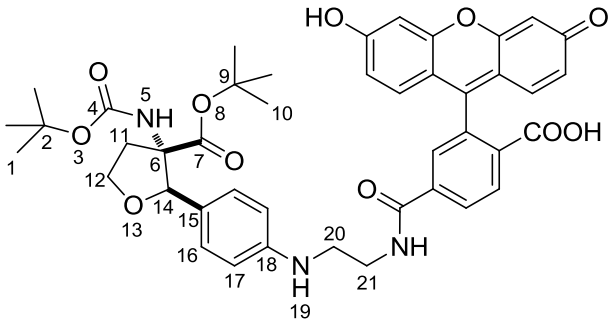
R_f (EtOAc/EtOH = 1:1) = 0.42. – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.15 (s, 9H, 10), 1.45 (s, 9H, 1), 2.43-2.57 (m, 1H, $11_{a/b}$), 2.69-2.83 (m, 1H, $11_{a/b}$), 3.23 (t, $^3J_{H,H}$ = 5.8 Hz, 2H, 21), 3.46 (dd, $^3J_{H,H}$ = 5.9 Hz, $^3J_{H,H}$ = 11.7 Hz, 2H, 20), 4.08 (dd, $^3J_{H,H}$ = 7.4 Hz, $^3J_{H,H}$ = 14.4 Hz, 1H, $12_{a/b}$), 4.27 (ddd, $^2J_{H,H}$ = 3.7 Hz, $^3J_{H,H}$ = 8.3 Hz, $^3J_{H,H}$ = 8.3 Hz, 1H, $12_{a/b}$), 4.78 (bs, 1H, 14), 5.44 (bs, 1H, 5), 6.32 (bs, 1H, 19), 6.51 (d, $^3J_{H,H}$ = 8.5 Hz, 2H, 16/17), 7.08 (d, $^3J_{H,H}$ = 8.5 Hz, 2H, 16/17). – **MS** [ESI, CH_2Cl_2/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 421.3 (100) $[MH]^+$, 462.3 (25) $[MH+MeCN]^+$. – **IR** (neat) [cm^{-1}]: ν = 3368, 2975, 2930, 1695, 1614, 1510, 1487, 1366, 1298, 1256, 1157, 1070, 1030, 934, 797, 734. – **MF:** $C_{22}H_{35}N_3O_5$. – **MW:** 421.53.

4-((2-((4-((2R,3S)-3-(tert-Butoxycarbonyl)-3-((tert-butoxycarbonyl)amino)-tetrahydrofuran-2-yl)phenyl)amino)ethyl)carbamoyl)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid (rac-10):

Under an atmosphere of nitrogen compound **9** (34 mg, 0.09 mmol) was dissolved in 0.6 mL of dry DMF and cooled to 0 °C in an ice bath. To this solution DIPEA (38 µL, 0.22 mmol, 2.5 eq.), HOAt (15 mg, 0.105 mmol, 1.2 eq.) and HATU (40 mg, 0.105 mmol, 1.2 eq.) were added. Afterwards compound **rac-8** (45 mg, 0.105 mmol, 1.2 eq.) was slowly added in several portions. After the addition was completed, the mixture was allowed to reach room temperature and stirred for 24 hours. The reaction was quenched with 2 mL of water and acidified with 0.5 mL of 1M KHSO4 solution and extracted with diethyl ether (3 x 5 mL). The combined organic layers were washed with brine (5 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on silica gel eluting with petroleum ether/ethyl acetate 1:2. The pure product was obtained as yellow solid in 20% yield (14 mg, 0.018 mmol).

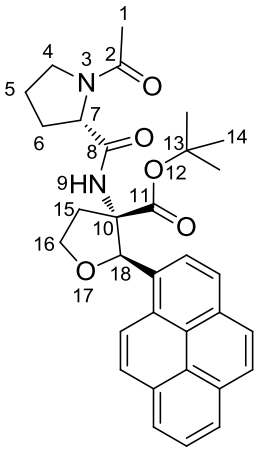
R_f (PE/EtOAc = 1:2) = 0.19. – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.20 (s, 9H, 10), 1.48 (s, 9H, 1), 2.44-2.56 (m, 1H, $11_{a/b}$), 2.67-2.80 (m, 1H, $11_{a/b}$), 3.23-3.35 (m, 2H, 21), 3.45-3.61 (m, 2H, 20), 4.06 (dd, $^3J_{H,H}$ = 7.2 Hz, $^3J_{H,H}$ = 14.0 Hz, 1H, $12_{a/b}$), 4.26 (ddd, $^2J_{H,H}$ = 3.6 Hz, $^3J_{H,H}$ = 8.1 Hz, $^3J_{H,H}$ = 8.1 Hz, 1H, $12_{a/b}$), 4.81 (bs, 1H, 14), 5.46 (bs, 1H, 5), 5.91 (s, 1H, Ar-CH), 6.04 (bs, 1H, 22), 6.31 (bs, 1H, 19), 6.40-6.48 (m, 2H, Ar-CH), 6.50 (d, $^3J_{H,H}$ = 8.4 Hz, 2H, 16/17), 6.80-6.95 (m, 2H, Ar-CH), 7.06 (d, $^3J_{H,H}$ = 8.4 Hz, 2H,

16/17), 7.19 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, Ar-CH), 8.01 (d, $^3J_{H,H}$ = 8.2 Hz, 1H, Ar-CH), 8.20-8.31 (m, 2H, Ar-CH). – **MS** [ESI, CH_2Cl_2/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 780.8 (100) $[MH]^+$, 821.8 (45) $[MH+MeCN]^+$. – **IR** (neat) [cm^{-1}]: ν = 3545, 3360, 2978, 2933, 1691, 1602, 1517, 1482, 1360, 1291, 1251, 1152, 1063, 1026, 931, 798, 743. – **MF:** $C_{43}H_{45}N_3O_{11}$. – **MW:** 779.83.



(2R,3S)-tert-Butyl 3-((S)-1-acetylpyrrolidine-2-carboxamido)-2-(pyren-1-yl)tetrahydrofuran-3-carboxylate (12a):

Compound **rac-3** (100mg, 0.21 mmol) was dissolved in 3 mL of CH_2Cl_2. To this solution 2 mL HCl saturated diethyl ether solution (9.5 mL/mmol Boc) was added and stirred for 20 min at room temperature. The solvent was evaporated by *vacuo* and the resulting light yellow solid was dissolved in 3 mL of dry CH_2Cl_2 followed by N-acetylated L-proline (39 mg, 0.25 mmol), HOAt (16.7 mg, 0.12 mmol), HBTU (95 mg, 0.25 mmol) and DIPEA (133 mg, 1.25 mmol). The reaction was stirred at room temperature for 2 days, quenched with 2 mL of 1M $KHSO_4$ solution, diluted with 4 mL of EtOAc and transferred to a separatory funnel. The aqueous layer was extracted with EtOAc (2 x 3 mL). The combined EtOAc layers were washed with 3 mL of brine solution, dried over $MgSO_4$ and the solvent was removed in *vacuo*. The crude product was purified by flash column chromatography on silica gel using 40-45% ethyl acetate in dichloromethane as eluent. The pure product was obtained with a yield of 75% (40 mg, 0.076 mmol).

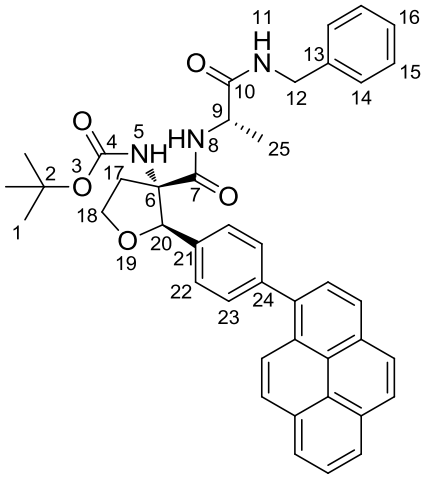


R_f (EtOAc/DCM = 1:1) = 0.20. – **Mp:** 159-161 °C. – **^{1}H-NMR** (400 MHz, COSY, $CDCl_3$): δ = 0.62 (s, 9H, 14), 1.93-2.05 (m, 2H, $6_{a/b}$+$5_{a/b}$), 2.12 (s, 3H, 1), 2.14-2.24 (m, 1H, $5_{a/b}$), 2.37-2.43 (m, 1H, $6_{a/b}$), 2.67 (ddd, $^2J_{H,H}$ = 2.6 Hz, $^3J_{H,H}$ = 6.7 Hz, $^3J_{H,H}$ = 12.9 Hz, 1H, $15_{a/b}$), 3.00 (ddd, $^2J_{H,H}$ = 8.4 Hz, $^3J_{H,H}$ = 9.7 Hz, $^3J_{H,H}$ = 12.9 Hz, 1H, $15_{a/b}$), 3.47 (ddd, $^2J_{H,H}$ = 6.8 Hz, $^3J_{H,H}$ = 9.4 Hz, $^3J_{H,H}$ = 9.5 Hz, 1H, $4_{a/b}$), 3.63-3.70 (m, 1H, $4_{a/b}$), 4.40 (ddd, $^2J_{H,H}$ = 6.7 Hz, $^3J_{H,H}$ = 8.3 Hz, $^3J_{H,H}$ = 9.7 Hz, 1H, $16_{a/b}$), 4.54 (ddd, $^2J_{H,H}$ = 2.6 Hz, $^3J_{H,H}$ = 8.2 Hz, $^3J_{H,H}$ = 8.2 Hz, 1H, $16_{a/b}$), 6.29 (bs, 1H, 18), 7.95-8.34 (m, 9H, Ar-CH). – **^{13}C-NMR** (100 MHz, HSQC, HMBC, $CDCl_3$): δ = 22.5 (+, 1C, 1), 25.1 (-, 1C, 5), 26.9 (+, 3C, 14), 28.0 (-, 1C, 6), 36.4 (-, 1C, 15), 48.5 (-, 1C, 4), 60.2 (+, 1C, 7), 68.5 (-, 1C, 16), 71.5 ($C_{quat.}$, 1C, 13), 82.1 ($C_{quat.}$, 1C, 10), 83.1 (+, 1C, 18), 123.5 (+, 1C, Ar-CH), 124.5 ($C_{quat.}$, 1C, Ar-C), 124.6 (+, 1C, Ar-CH), 124.7 ($C_{quat.}$, 1C, Ar-C), 125.0 (+, 1C, Ar-CH), 125.2 (+, 1C, Ar-CH), 125.3 (+, 1C, Ar-CH), 125.8 (+, 1C, Ar-CH), 127.4 (+, 1C, Ar-CH), 127.4 (+, 1C, Ar-CH), 127.5 (+, 1C, Ar-CH), 128.7 ($C_{quat.}$, 1C, Ar-C), 130.6 ($C_{quat.}$, 1C, Ar-C), 131.0 ($C_{quat.}$, 1C, Ar-C), 131.3 ($C_{quat.}$, 1C, Ar-C), 131.6 ($C_{quat.}$, 1C, Ar-C), 169.0 ($C_{quat.}$, 1C, CO), 170.9 ($C_{quat.}$, 1C, CO), 171.0 ($C_{quat.}$, 1C, CO). – **MS** [ESI, CH_2Cl_2/MeOH + 10

mmol/l NH_4OAc): m/z (%) = 527.2 (100) $[MH]^+$, 544.2 (20) $[MNH_4]^+$, 568.3 (10) $[MH+MeCN]^+$, 1053.7 (60) $[2MH]^+$, 1070.7 (20) $[2MNH_4]^+$. – **Anal.** calcd. (%) for $C_{32}H_{34}N_2O_5$ (526.62): C 72.98, H 6.51, N 5.32, found: C 72.70, H 6.77, N 5.30. – **IR** (KBr) [cm^{-1}]: ν = 3258, 3223, 3049, 2976, 2889, 1923, 1730, 1685, 1618, 1550, 1452, 1430. – **UV** (MeOH): λ (ε) = 343 (30600), 276 (32000), 243 (45400). – **MF:** $C_{32}H_{34}N_2O_5$. – **MW:** 526.62.

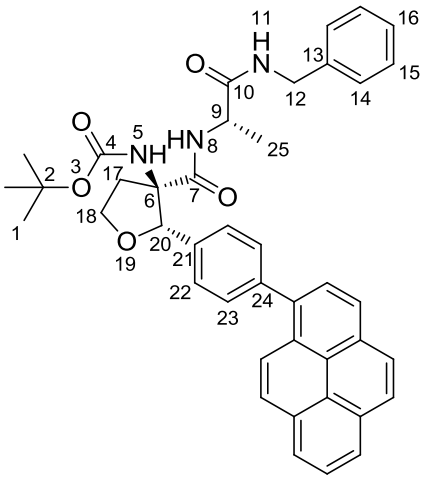
tert-Butyl (3-(((*S*)-1-(benzylamino)-1-oxopropan-2-yl)carbamoyl)-2-(4-(pyren-1-yl)phenyl)tetrahydrofuran-3-yl)carbamate(16):

In a three-neck round bottom flask potassium carbonate (80 mg, 0.58 mmol, 3.3 eq.) was dissolved in 1.5 mL of water. The mixture was purged with nitrogen for 10 min. Under nitrogen with stirring a mixture of dipeptides **15a** and **15b** (89 mg, 0.17 mmol), 1-pyrenyl boronic acid (38 mg, 0.15 mmol, 0.9 eq.) and 1.5 mL of 1,2-dimethoxyethane were added. After 2 min the catalyst $Pd(PPh_3)_4$ (15 mg, 3.5% molar amount) was added and the mixture was stirred for 5 min. The resulting mixture was then heated to reflux in an oil bath at 70 °C for 24 hours. After cooling down to room temperature, water (5 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on silica gel using 20-25% EtOAc in hexanes as eluent. The pure products were obtained with an overall yield of 54% (54 mg, 0.081 mmol) as light yellow **16a** and colorless solid **16b**.



16a: R_f (PE/EtOAc = 3:1) = 0.22. – **Mp:** 110 °C. – **^{1}H-NMR** (400 MHz, COSY, $CDCl_3$): δ = 1.06 (d, $^3J_{H,H}$ = 7.0 Hz, 3H, 25), 1.51 (s, 9H, 1), 2.45-2.62 (m, 1H, $17_{a/b}$), 2.74-2.99 (m, 1H, $17_{a/b}$), 4.15-4.25 (m, 1H, 9), 4.35-4.46 (m, 4H, 12+18), 5.57 (bs, 1H, 20), 6.20 (bs, 1H, 5), 6.27 (bs, 1H, 11), 6.69 (d, $^3J_{H,H}$ = 7.3 Hz, 1H, 8), 7.14-7.26 (m, 5H, 14-16), 7.51-7.59 (m, 4H, 22+23), 7.88-8.22 (m, 9H, Ar-CH). – **^{13}C-NMR** (100 MHz, HSQC, HMBC, $CDCl_3$): δ = 17.6 (+, 1C, 25), 28.4 (+, 3C, 1), 36.1 (-, 1C, 17), 43.6 (-, 1C, 12), 49.1 (+, 1C, 9), 66.7 (-, 1C, 18), 67.8 ($C_{quat.}$, 1C, 6), 80.2 ($C_{quat.}$, 1C, 2), 81.5 (+, 1C, 20), 124.6 (+, 1C, Ar-CH), 124.9 (+, 1C, Ar-CH), 124.9 ($C_{quat.}$, 1C, Ar-C), 125.0 ($C_{quat.}$, 1C, Ar-C), 125.2 (+, 1C, Ar-CH), 125.3 (+, 1C, Ar-CH), 126.0 (+, 1C, Ar-CH), 127.4 (+, 2C, Ar-CH), 127.4 (+, 1C, Ar-CH), 127.5 (+, 1C, Ar-CH), 127.5 (+, 1C, Ar-CH), 127.5 (+, 2C, Ar-CH), 127.6 (+, 2C, Ar-CH), 128.4 ($C_{quat.}$, 1C, Ar-C), 128.7 (+, 1C, Ar-CH), 128.7 (+, 1C, Ar-CH), 130.3 (+, 2C, Ar-CH), 130.6 ($C_{quat.}$, 1C, Ar-C), 130.9 ($C_{quat.}$, 1C, Ar-C), 131.5 ($C_{quat.}$, 1C, Ar-C), 136.1 ($C_{quat.}$, 1C, Ar-C), 137.1 ($C_{quat.}$, 1C, Ar-C), 137.7 ($C_{quat.}$, 1C, Ar-C), 140.5 ($C_{quat.}$, 1C, Ar-C), 154.3 ($C_{quat.}$, 1C, 4), 171.4 ($C_{quat.}$, 1C, 7), 171.4 ($C_{quat.}$, 1C, 10). – **MS** [ESI, CH_2Cl_2/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 668.3 (100) $[MH]^+$, 690.4 (20) $[MNa]^+$, 1336.0 (60) $[2MH]^+$. – **HR-MS** (PI-LSIMS, MeOH/CH_2Cl_2/NBA): $[MH]^+$ calcd. for

$C_{42}H_{41}N_3O_5$ 667.3046, found 667.3039. – **IR** (neat) [cm^{-1}]: ν = 3309, 3043, 2977, 2927, 1693, 1645, 1497, 1366, 1158, 1074, 843, 722. – **UV** (MeOH): λ (ε) = 343 (23600), 278 (30200), 243 (34000). – **MF:** $C_{42}H_{41}N_3O_5$. – **MW:** 667.79.

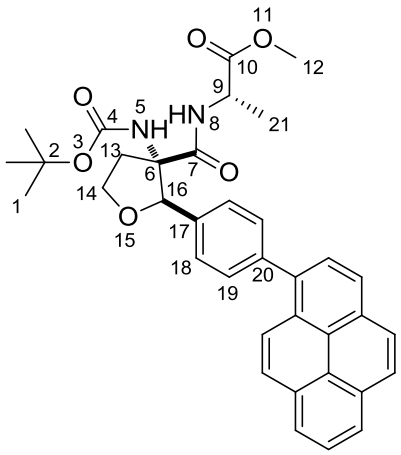


16b: $\mathbf{R_f}$ (PE/EtOAc = 3:1) = 0.26. – **Mp:** 124 °C. – **^{1}H-NMR** (400 MHz, COSY, $CDCl_3$): δ = 1.18 (d, $^3J_{H,H}$ = 7.2 Hz, 3H, 25), 1.43 (s, 9H, 1), 2.45-2.60 (m, 1H, $17_{a/b}$), 2.94-3.08 (m, 1H, $17_{a/b}$), 4.09-4.20 (m, 1H, 9), 4.21-4.37 (m, 3H, $12+18_{a/b}$), 4.47 (ddd, $^2J_{H,H}$ = 4.3 Hz, $^3J_{H,H}$ = 8.6 Hz, $^3J_{H,H}$ = 8.7 Hz, 1H, $18_{a/b}$), 5.17 (bs, 1H, 20), 5.78 (bs, 1H, 5), 6.34 (d, $^3J_{H,H}$ = 6.3 Hz, 1H, 8), 6.56 (bs, 1H, 11), 7.03-7.21 (m, 5H, 14-16), 7.50 (d, $^3J_{H,H}$ = 8.2 Hz, 2H, 22), 7.55 (d, $^3J_{H,H}$ = 8.2 Hz, 2H, 23), 7.83-8.24 (m, 9H, Ar-CH). – **^{13}C-NMR** (100 MHz, HSQC, HMBC, $CDCl_3$): δ = 18.1 (+, 1C, 25), 28.3 (+, 3C, 1), 36.5 (-, 1C, 17), 43.4 (-, 1C, 12), 49.4 (+, 1C, 9), 67.6 (-, 1C, 18), 69.2 ($C_{quat.}$, 1C, 6), 81.3 ($C_{quat.}$, 1C, 2), 85.1 (+, 1C, 20), 124.7 (+, 1C, Ar-CH), 124.9 (+, 1C, Ar-CH), 124.9 ($C_{quat.}$, 1C, Ar-C), 125.0 ($C_{quat.}$, 1C, Ar-C), 125.3 (+, 1C, Ar-CH), 126.1 (+, 1C, Ar-CH), 126.1 (+, 1C, Ar-CH), 127.2 (+, 2C, Ar-CH), 127.4 (+, 1C, Ar-CH), 127.5 (+, 1C, Ar-CH), 127.6 (+, 1C, Ar-CH), 127.7 (+, 2C, Ar-CH), 127.7 (+, 2C, Ar-CH), 128.4 ($C_{quat.}$, 1C, Ar-C), 128.5 (+, 1C, Ar-CH), 128.6 (+, 1C, Ar-CH), 130.5 (+, 2C, Ar-CH), 130.7 ($C_{quat.}$, 1C, Ar-C), 130.9 ($C_{quat.}$, 1C, Ar-C), 131.5 ($C_{quat.}$, 1C, Ar-C), 135.7 ($C_{quat.}$, 1C, Ar-C), 136.9 ($C_{quat.}$, 1C, Ar-C), 138.1 ($C_{quat.}$, 1C, Ar-C), 141.2 ($C_{quat.}$, 1C, Ar-C), 155.3 ($C_{quat.}$, 1C, 4), 170.5 ($C_{quat.}$, 1C, CO), 171.7 ($C_{quat.}$, 1C, CO). – **MS** [ESI, CH_2Cl_2/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 668.3 (100) $[MH]^+$, 685.3 (40) $[MNH_4]^+$, 690.3 (20) $[MNa]^+$, 1336.0 (60) $[2MH]^+$. – **HR-MS** (PI-LSIMS, MeOH/CH_2Cl_2/NBA): $[MH]^+$ calcd. for $C_{42}H_{41}N_3O_5$ 667.3046, found 667.3048. – **IR** (neat) [cm^{-1}]: ν= 3428, 3042, 2976, 2930, 1649, 1603, 1554, 1366, 1155, 1072, 844, 723. – **UV** (MeOH): λ (ε) = 343 (27800), 278 (37200), 243 (41200). – **MF:** $C_{42}H_{41}N_3O_5$. – **MW:** 667.79.

(*S*)-Methyl 2-((2*R*,3*S*)-3-((tert-butoxycarbonyl)amino)-2-(4-(pyren-1-yl)phenyl)tetrahydrofuran-3-carboxamido)propanoate (18c):

In a 10 mL Schlenk flask were placed compound **18a** (50 mg, 0.11 mmol), 1-pyrenyl boronic acid **5** (31 mg, 0.13 mmol, 1.2 eq.), Na_2CO_3 (45 mg, 0.42 mmol, 3.8 eq.) $Pd(OAc)_2$ (2 mg, 6 µmol), tetrabutyl ammonium bromide (34 mg, 0.11 mmol, 1.0 eq.) and 1 mL a water / DMF (1:1) mixture. The flask was sealed with a septum and placed into an oil bath preheated to 100°C. The reaction mixture was stirred at this temperature for 20 h. Then the mixture was allowed to cool to room temperature, water and diethyl ether (5 mL of each) were added. The phases were separated and the aqueous phase was extracted three times with 5 mL of diethyl ether. The combined organic phases were dried

over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on silica gel eluting with petroleum ether/ethyl acetate 3:1. The pure product was obtained as yellow solid in 57% yield (37 mg, 0.06 mmol).



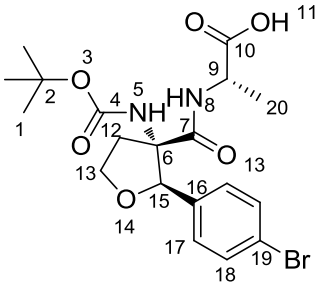
R_f (PE/EtOAc = 3:1) = 0.28. – **Mp:** 168.3-179.7 °C. – **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 0.89 (d, $^3J_{H,H}$ = 7.0 Hz, 3H, 21), 1.52 (s, 9H, 1), 2.64-2.81 (m, 1H, $13_{a/b}$), 2.83-2.92 (m, 1H, $13_{a/b}$), 3.67 (s, 3H, 12), 4.03-4.10 (m, 1H, 9), 4.25-4.37 (m, 1H, $14_{a/b}$), 4.40-4.47 (m, 1H, $14_{a/b}$), 5.20 (bs, 1H, 16), 5.75 (bs, 1H, 5), 6.03 (d, $^3J_{H,H}$ = 8.2 Hz, 1H, 8), 7.58 (d, $^3J_{H,H}$ = 8.0 Hz, 2H, 18), 7.64 (d, $^3J_{H,H}$ = 8.1 Hz, 2H, 19), 7.90-7.93 (m, 1H, Ar-CH), 7.97-8.04 (m, 2H, Ar-CH), 8.06-8.11 (m, 2H, Ar-CH), 8.13-8.17 (m, 2H, Ar-CH), 8.17-8.21 (m, 2H, Ar-CH). – **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 17.8 (+, 1C, 21), 28.3 (+, 3C, 1), 36.4 (-, 1C, 13), 48.9 (+, 1C, 9), 52.5 (+, 1C, 12), 68.1 (-, 1C, 14), 69.9 ($C_{quat.}$, 1C, 6), 80.2 ($C_{quat.}$, 1C, 2), 86.3 (+, 1C, 16), 124.6 (+, 1C, Ar-CH), 124.8 (+, 1C, Ar-CH), 124.8 ($C_{quat.}$, 1C, Ar-C), 124.9 ($C_{quat.}$, 1C, Ar-C), 125.0 (+, 1C, Ar-CH), 125.1 (+, 1C, Ar-CH), 126.0 (+, 1C, Ar-CH), 126.6 (+, 2C, 18), 127.2 (+, 1C, Ar-CH), 127.4 (+, 1C, Ar-CH), 127.4 (+, 1C, Ar-CH), 128.4 ($C_{quat.}$, 1C, Ar-C), 130.2 (+, 2C, 19), 130.5 ($C_{quat.}$, 1C, Ar-C), 130.9 ($C_{quat.}$, 1C, Ar-C), 131.4 ($C_{quat.}$, 1C, Ar-C), 136.7 ($C_{quat.}$, 1C, 15), 137.3 ($C_{quat.}$, 1C, Ar-C), 141.2 ($C_{quat.}$, 1C, 20), 154.7 ($C_{quat.}$, 1C, 4), 170.2 ($C_{quat.}$, 1C, 7), 172.9 ($C_{quat.}$, 1C, 10). – **MS** [ESI, CH_2Cl_2/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 593.7 (100) $[MH]^+$, 610.7 (30) $[MNH_4]^+$, 634.7 (40) $[MH+MeCN]^+$, 1186.4 (20) $[2MH]^+$. – **IR** (neat) [cm^{-1}]: ν = 3325, 2977, 2888, 1696, 1599, 1488, 1354, 1251, 1150, 1061, 842, 722, 687. – **UV** (MeOH): λ (ε) = 343 (30500), 278 (43200), 243 (47400). – **MF:** $C_{36}H_{36}N_2O_6$. – **MW:** 592.68.

(*S*)-2-((2R,3S)-2-(4-Bromophenyl)-3-(tert-butoxycarbonylamino)tetrahydro-furan-3-carbox-amido)propanoic acid (19):

Compound **18a** (150mg, 0.32 mmol) was dissolved in 8 mL of an acetonitrile/water mixture (4:1). Under stirring a 1M aqueous LiOH solution (0.35 mL, 0.35 mmol, 1.1 eq.) was added drop by drop. The mixture was stirred over night at room temperature. After acidification with 1M aqueous $KHSO_4$ solution, the mixture was extracted with dichloromethane (3 x 10 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure to give the product as colorless solid in analytical pure form and quantitative yield (145 mg, 0.32 mmol).

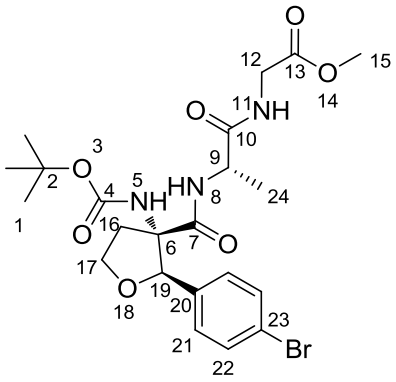
Mp: 59.6-60.9 °C. – **^{1}H-NMR** (300 MHz, MeOH-d^4): δ= 0.80 (d, $^3J_{H,H}$ = 7.1 Hz, 3H, 20), 1.45 (s, 9H, 1), 2.14-2.28 (m, 1H, $12_{a/b}$), 2.97-3.12 (m, 1H, $12_{a/b}$), 3.98 (dd, $^3J_{H,H}$ = 8.5 Hz, $^3J_{H,H}$ = 16.2 Hz, 2H, 13), 4.31 (dt, $^3J_{H,H}$ = 3.3 Hz, $^3J_{H,H}$ = 8.4 Hz, 1H, 9), 4.88 (s, 1H, 15), 7.27 (d, $^3J_{H,H}$ = 8.4 Hz, 2H, 17), 7.44 (d, $^3J_{H,H}$ = 8.5 Hz, 2H, 18). – **^{13}C-NMR** (75 MHz, MeOH-d^4): δ = 18.1 (+, 1C, 20), 28.7 (+, 3C, 1), 36.7 (-, 1C, 12), 49.4 (+, 1C, 9), 68.6 (-, 1C, 13), 71.6 ($C_{quat.}$, 1C, 6), 81.4 ($C_{quat.}$, 1C, 2), 87.3 (+, 1C, 15), 123.1 ($C_{quat.}$, 1C,

19), 130.1 (+, 2C, 17), 132.1 (+, 2C, 18), 138.5 ($C_{quat.}$, 1C, 16), 157.1 ($C_{quat.}$, 1C, 4), 163.0 ($C_{quat.}$, 1C, 7), 175.5 ($C_{quat.}$, 1C, 10). – **MS** [ESI, CH_2Cl_2/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 456.9 (100) $[MH]^+$, 497.9 (5) $[MH+MeCN]^+$. – **IR** (neat) [cm^{-1}]: = 2984, 2936, 1704, 1663, 1509, 1487, 1451, 1392, 1366, 1250, 1160, 1070, 1011, 847, 794, 587. – **MF:** $C_{19}H_{25}BrN_2O_6$. – **MW:** 457.32.



Methyl 2-((S)-2-((2R,3S)-2-(4-bromophenyl)-3-(tert-butoxycarbonylamino)-tetrahydro-furan-3-carboxamido)propanamido)acetate (21):

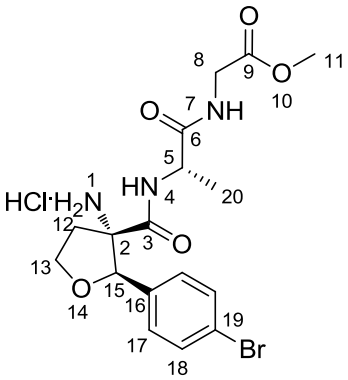
Under an atmosphere of nitrogen compound **19** (145 mg, 0.32 mmol) was dissolved in 2 mL of dry DMF and cooled to 0 °C in an ice bath. To this solution DIPEA (135 µL, 0.79 mmol, 2.5 eq.), HOBt (60 mg, 0.44 mmol, 1.4 eq.) and EDC (67 µL, 0.38 mmol, 1.2 eq.) were added. Afterwards the hydrochloride salt of glycine methylester 20 (48 mg, 0.38 mmol, 1.2 eq.) was slowly added in several portions. After the addition was completed, the mixture was allowed to reach room temperature and stirred for 24 hours. The reaction was quenched with 2 mL of water and acidified with 1.5 mL of 1M $KHSO_4$ solution and extracted with diethyl ether (3 x 5 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on silica gel eluting with petroleum ether/ethyl acetate 4:6.. The pure product was obtained as colorless solid in 84% yield (140 mg, 0.26 mmol).



R_f (PE/EtOAc = 4:6) = 0.22. – **Mp:** 162.6-164.5 °C. – **^{1}H-NMR** (600 MHz, COSY, ROESY, $CDCl_3$): δ = 1.08 (d, $^3J_{H,H}$ = 5.5 Hz, 3H, 24), 1.44 (s, 9H, 1), 2.47-2.57 (m, 1H, $16_{a/b}$), 2.76-2.85 (m, 1H, $16_{a/b}$), 3.69 (s, 3H, 15), 3.73-3.80 (m, 1H, 12), 3.84-3.90 (m, 1H, 12), 4.04-4.11 (m, 1H, 9), 4.15-4.22 (m, 1H, $17_{a/b}$), 4.34 (dt, $^3J_{H,H}$ = 3.9 Hz, $^3J_{H,H}$ =8.7 Hz, 1H, $17_{a/b}$), 5.09 (bs, 1H, 19), 6.07 (bs, 1H, 5), 6.36 (d, $^3J_{H,H}$ = 4.7 Hz, 1H, 8), 6.51-6.56 (m, 1H, 11), 7.19 (d, $^3J_{H,H}$ = 8.4 Hz, 2H, 21), 7.40 (d, $^3J_{H,H}$ = 8.4 Hz, 2H, 22). – **^{13}C-NMR** (150 MHz, HSQC, HMBC, $CDCl_3$): δ = 17.4 (+, 1C, 24), 28.3 (+, 3C, 1), 36.1 (-, 1C, 16), 41.0 (-, 1C, 12), 48.8 (+, 1C, 9), 52.2 (+, 1C, 15), 67.1 (-, 1C, 17), 68.7 ($C_{quat.}$, 1C, 6), 81.0 ($C_{quat.}$, 1C, 2), 83.2 (+, 1C, 19), 121.9 ($C_{quat.}$, 1C, 23), 127.5 (+, 2C, 21), 131.3 (+, 2C, 22), 135.9 ($C_{quat.}$, 1C, 20), 155.1 ($C_{quat.}$, 1C, 4), 169.8 ($C_{quat.}$, 1C, 13), 170.5 ($C_{quat.}$, 1C, 7), 171.9 ($C_{quat.}$, 1C, 10). – **MS** [ESI, CH_2Cl_2/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 471.9 (15) $[MH–C_4H_8]^+$, 527.9 (90) $[MH]^+$, 545.0 (100) $[MNH_4]^+$. – **Anal.** calcd. (%) for $C_{22}H_{30}BrN_3O_7$ (528.39): C 50.01, H 5.72, N 7.95, found: C 49.13, H 5.87, N 7.81. – **IR** (neat) [cm^{-1}]: ν= 3328, 2976, 1748, 1669, 1648, 1544, 1498, 1452, 1366, 1252, 1207, 1158, 1069, 1003, 872, 797, 647. – **MF:** $C_{22}H_{30}BrN_3O_7$. – **MW:** 528.39.

Methyl 2-((*S*)-2-((2R,3S)-3-amino-2-(4-bromophenyl)tetrahydrofuran-3-carboxamido)propanamido)acetate hydrochloride(21b):

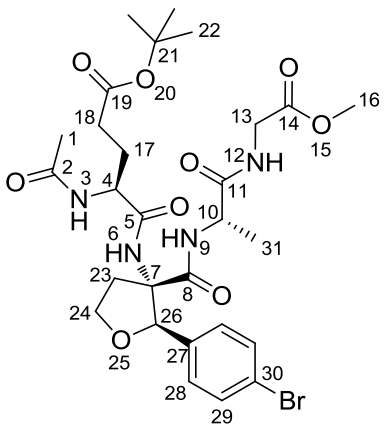
Compound **21** (100 mg, 0.19 mmol) was dissolved under ice bath cooling at 0 °C in CH_2Cl_2. Under vigorous stirring 1.3 mL of ice-cold HCl saturated diethyl ether (7 mL/mmol Boc) was added and the mixture was stirred for 3 hours. The solvent was removed under reduced pressure to give the HCl salt of the product as a white to yellow solid in quantitative yield (88 mg, 0.19 mmol).



Mp: > 180 °C. – **^{1}H-NMR** (300 MHz, MeOH-d^4): δ = 1.26 (d, $^3J_{H,H}$ = 7.0 Hz, 3H, 20), 2.38-2.49 (m, 1H, $12_{a/b}$), 2.82-2.94 (m, 1H, $12_{a/b}$), 3.75 (s, 3H, 11), 3.86-4.02 (m, 2H, 8), 4.06-4.25 (m, 2H, $5+13_{a/b}$), 4.51 (dt, $^3J_{H,H}$ = 2.9 Hz , $^3J_{H,H}$ = 9.0 Hz, 1H, $13_{a/b}$), 4.98 (s, 1H, 15), 7.26 (d, $^3J_{H,H}$ = 8.4 Hz, 2H, 17), 7.49 (d, $^3J_{H,H}$ = 8.5 Hz, 2H, 18). – **^{13}C-NMR** (75 MHz, MeOH-d^4): δ = 19.2 (+, 1C, 20), 36.0 (-, 1C, 12), 41.9 (-, 1C, 8), 50.4 (+, 1,C, 5), 52.6 (+, 1C, 11), 67.6 (-, 1C, 13), 69.4 ($C_{quat.}$, 1C, 2), 86.2 (+, 1C, 15), 123.9 ($C_{quat.}$, 1C, 19), 129.4 (+, 2C, 17), 132.7 (+, 2C, 18), 135.1 ($C_{quat.}$, 1C, 16), 168.2 ($C_{quat.}$, 1C, 9), 171.4 ($C_{quat.}$, 1C, 3), 173.9 ($C_{quat.}$, 1C, 6). – **MS** [ESI, CH_2Cl_2/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 427.9 (100) $[MH]^+$, 468.9 (12) $[MH+MeCN]^+$. – **IR** (neat) [cm^{-1}]: ν = 3310, 2943, 1741, 1659, 1517, 1443, 1214, 1076, 1009, 814, 595. – **MF:** $C_{17}H_{22}BrN_3O_5$ x HCl. – **MW:** 464.74.

(*S*)-tert-Butyl 4-acetamido-5-(((2R,3*S*)-2-(4-bromophenyl)-3-(((*S*)-1-((2-methoxy-2-oxoethyl)amino)-1-oxopropan-2-yl)carbamoyl)tetrahydrofuran-3-yl)amino)-5-oxopentanoate (23):

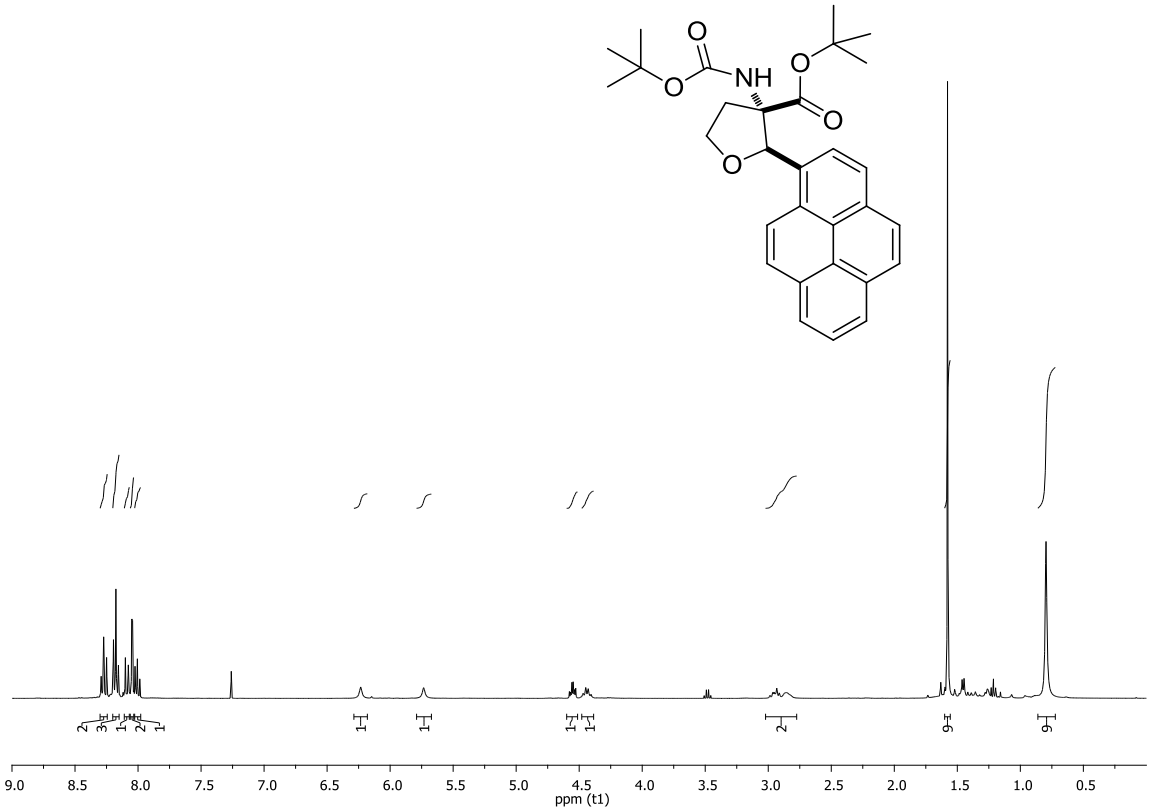
Under an atmosphere of nitrogen N-acetylated L-glutamic acid 22 (55 mg, 0.22 mmol) was dissolved in 1.3 mL of dry DMF and cooled to 0 °C in an ice bath. To this solution DIPEA (96 µL, 0.56 mmol, 2.5 eq.), HOAt (37 mg, 0.27 mmol, 1.2 eq.) and HATU (102 mg, 0.27 mmol, 1.2 eq.) were added. Afterwards compound **21b** (88 mg, 0.19 mmol, 0.9 eq.) was slowly added in several portions. After the addition was completed, the mixture was allowed to reach room temperature and stirred for 24 hours. The reaction was quenched with 2 mL of water and acidified with 1.5 mL of 1M $KHSO_4$ solution and extracted with diethyl ether (3 x 5 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. A first purification of the crude product was done by flash column chromatography on silica gel eluting with petroleum ether/ethyl acetate 2:8. The final purification was achieved by preparative HPLC using a Knauer HPLC system with a Phenomenex Luna 10 C18(2) 250x21.x mm 10 micron column. The detection wavelength was at 220 nm, column temperature 25 °C, injection volume 200 µL, solvent A: H_2O [0.0059 % TFA w/w], solvent B: MeCN. Gradient 0 min 30% B, 30 min 60% B, 40 min 95% B, 50 min 95% B; flow rate 11 mL/min. Eluation time: 15.1 min. The pure product was obtained as white solid in a yield of 3% (3.5 mg, 5 µmol).



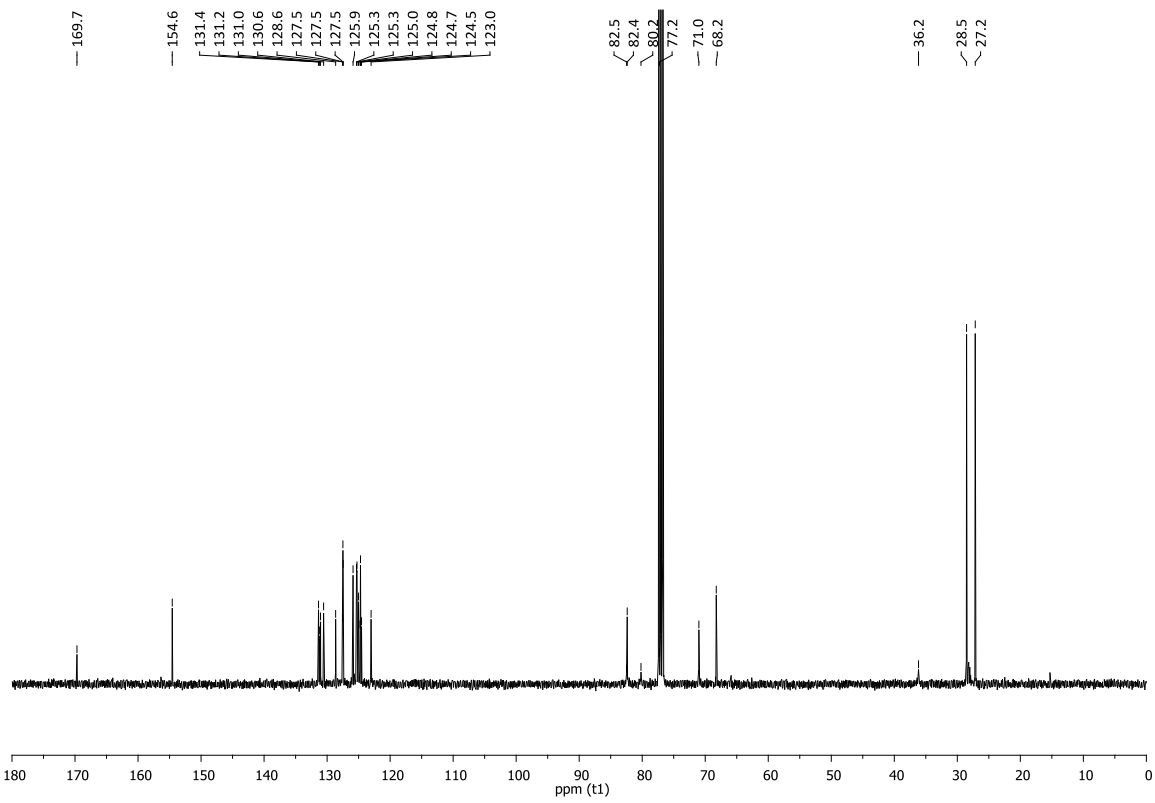
R_f (PE/EtOAc= 2:8) = 0.27. – **^{1}H-NMR** (600 MHz, COSY, ROESY, DMSO-d^6): δ = 0.80 (d, $^3J_{H,H}$ = 7.2 Hz, 3H, 31), 1.39 (s, 9H, 22), 1.71-1.79 (m, 1H, $17_{a/b}$), 1.88 (s, 3H, 1), 1.89-1.96 (m, 1H, $17_{a/b}$), 2.00-2.06 (m, 1H, $23_{a/b}$), 2.21-2.31 (m, 2H, 18), 2.79-2.88 (m, 1H, $23_{a/b}$), 3.58 (s, 3H, 16), 3.70-3.78 (m, 3H, 10+13.), 3.83-3.88 (m, 1H, $24_{a/b}$), 4.17-4.25 (m, 2H, 4+$24_{a/b}$), 5.06 (s, 1H, 26), 7.22 (d, $^3J_{H,H}$ = 8.5 Hz, 2H, 28), 7.29 (d $^3J_{H,H}$ = 7.2 Hz, 1H, 9), 7.46 (d $^3J_{H,H}$ = 8.5 Hz, 2H, 29), 7.73 (t, $^3J_{H,H}$ = 6.0 Hz, 1H, 12), 8,15 (d, $^3J_{H,H}$ = 6.6 Hz, 1H, 3), 8.79 (s 1H, 6). – **^{13}C-NMR** (150 MHz, HSQC, HMBC, DMSO-d^6): δ = 16.9 (+, 1C, 31), 22.4 (+, 1C, 1), 26.2 (-, 1C, 17), 27.7 (+, 3C, 22), 31.4 (-, 1C, 18), 34.7 (-, 1C, 23), 40.4 (-, 1C, 13), 48.0 (+, 1C, 10), 51.6 (+, 1C, 16), 52.8 (+, 1C, 4), 67.1 (-, 1C, 24), 69.8 ($C_{quat.}$, 1C, 7), 79.7 ($C_{quat.}$, 1C, 21), 84.3 (+, 1C, 26), 120.9 ($C_{quat.}$, 1C, 30), 128.7 (+, 2C, 28), 130.4 (+, 2C, 29), 137.7 ($C_{quat.}$, 1C, 27), 168.0 ($C_{quat.}$, 1C, 8), 169.7 ($C_{quat.}$, 1C, 14), 170.1 ($C_{quat.}$, 1C, 2), 171.6 ($C_{quat.}$, 1C, 19), 172.2 ($C_{quat.}$, 1C, 11),173.0 ($C_{quat.}$, 1C, 5). – **MS** [ESI, CH_2Cl_2/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 599.3 (10) $[MH-C_4H_8]^+$, 657.3 (20) $[MH]^+$, 679.3 (100) $[MNa]^+$. – **HR-MS** (PI-LSIMS, MeOH/CH_2Cl_2/NBA): $[MH]^+$ calcd. for $C_{28}H_{39}BrN_4O_9$ 655.1979, found 655.19925. – **IR** (neat) [cm^{-1}]: ν= 3295, 2979, 1727, 1650, 1534, 1447, 1368, 1202, 1155, 1075, 1011, 838, 799, 650, 600. – **MF:** $C_{28}H_{39}BrN_4O_9$. – **MW:** 655.53.

2.4.3 Supporting Information

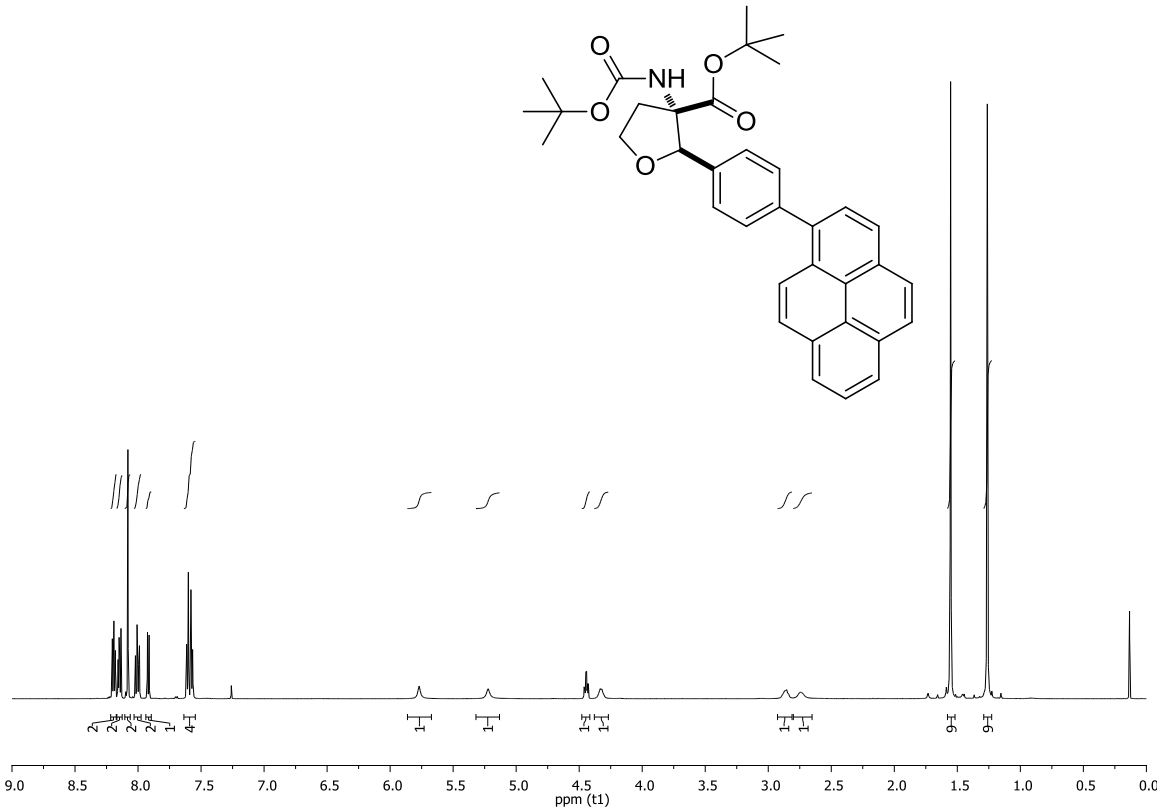
^{1}H-NMR spectra of compound **rac-3** (400 MHz, $CDCl_3$):



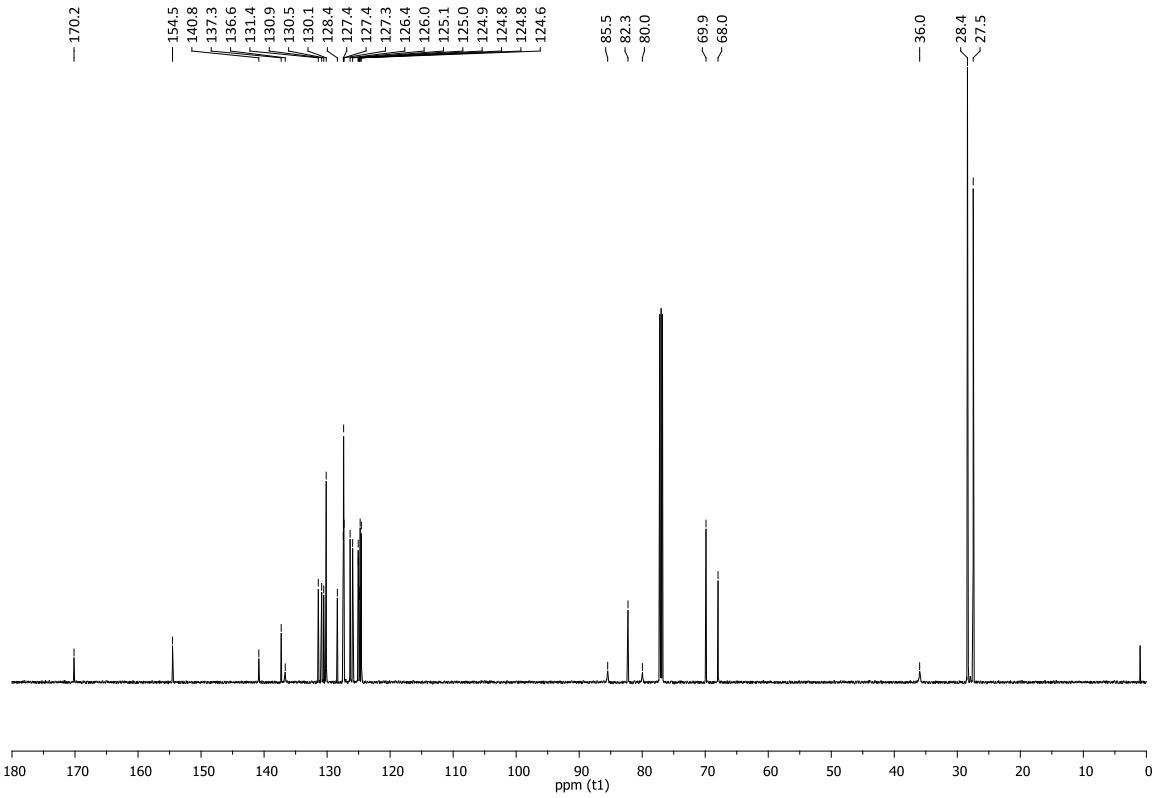
^{13}C-NMR spectra of compound **rac-3** (100 MHz, $CDCl_3$):



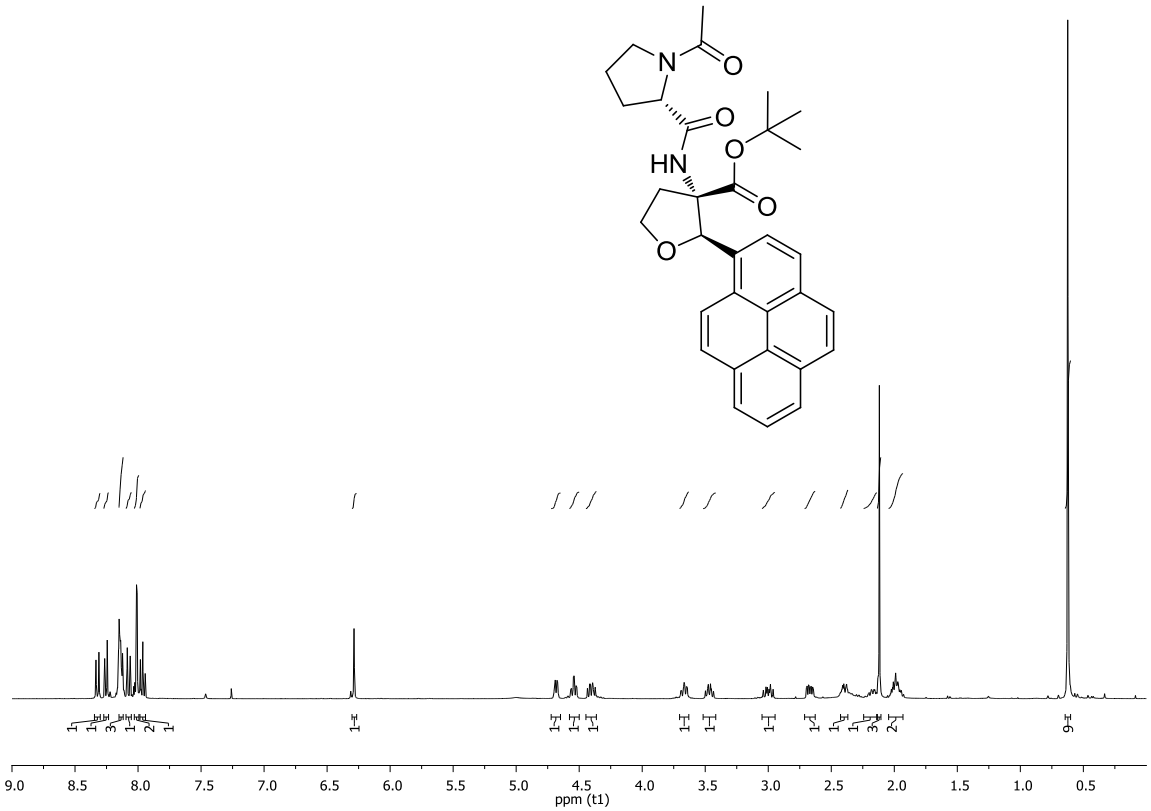
^{1}H-NMR spectra of compound **rac-6** (600 MHz, $CDCl_3$):



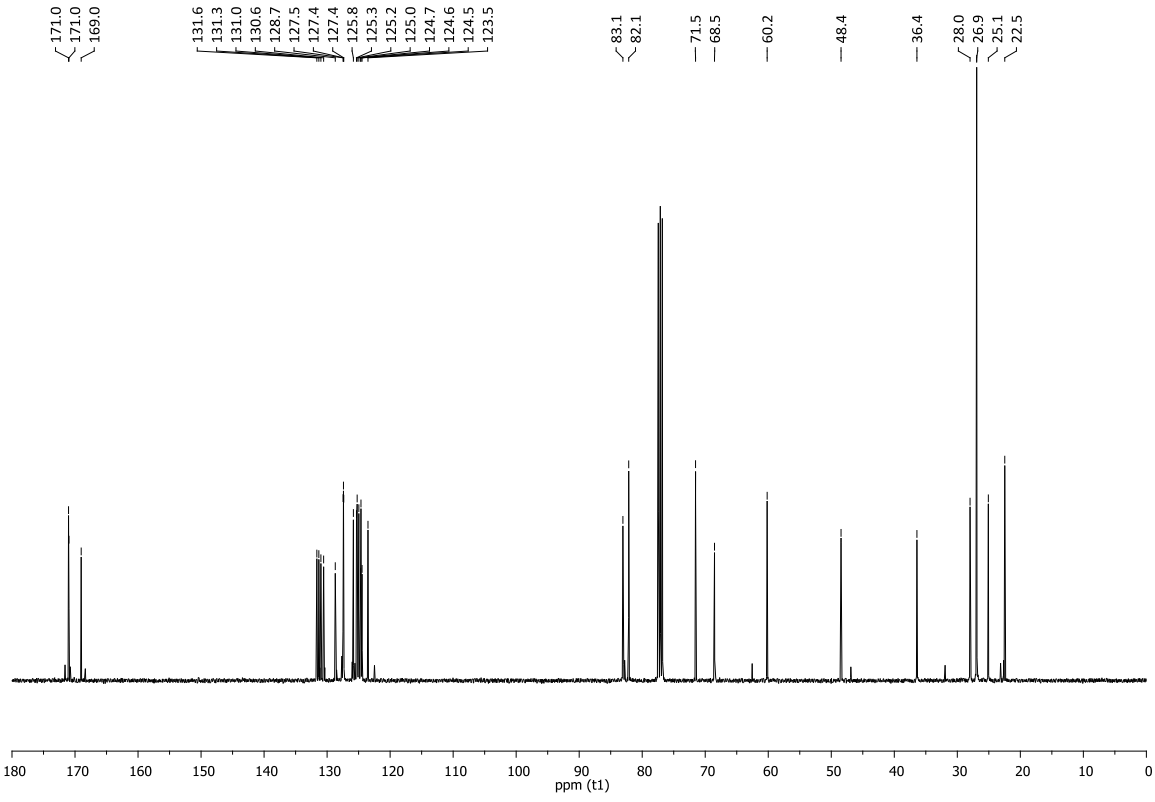
^{13}C-NMR spectra of compound **rac-6** (150 MHz, $CDCl_3$):



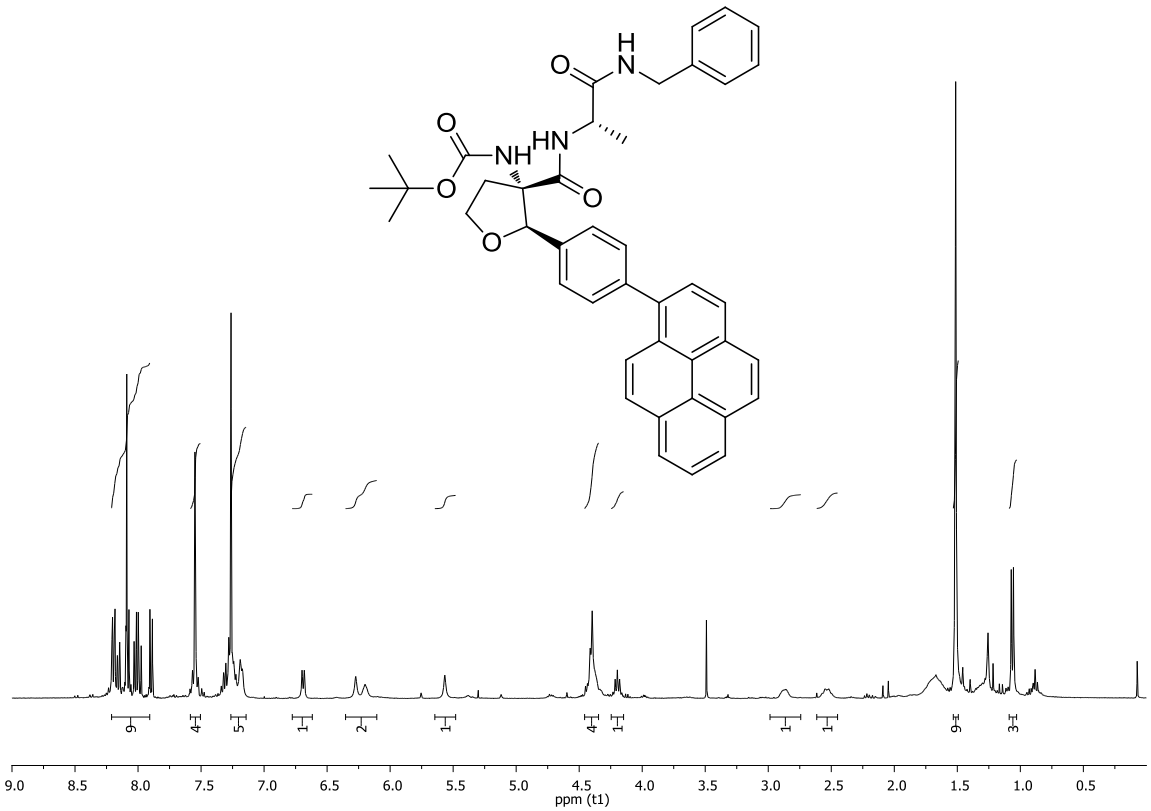
^{1}H-NMR spectra of compound **12a** (400 MHz, $CDCl_3$):



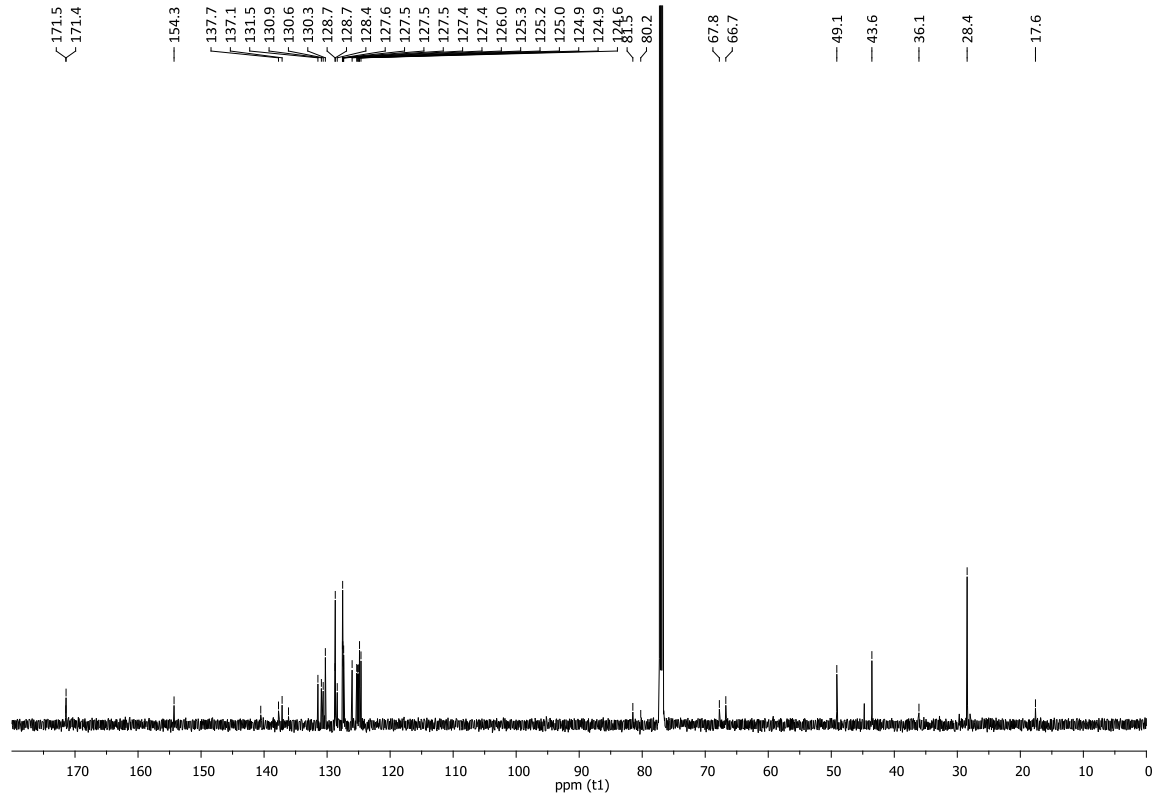
^{13}C-NMR spectra of compound **12a** (100 MHz, $CDCl_3$):



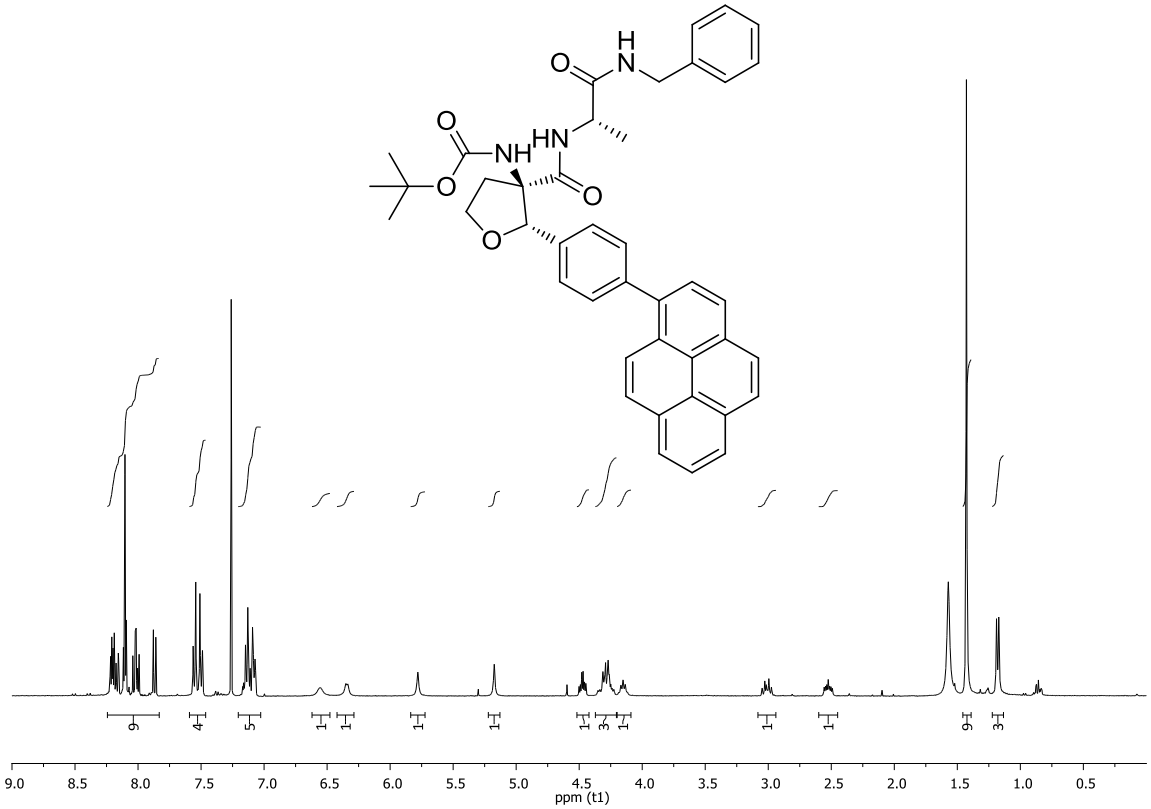
^{1}H-NMR spectra of compound **16a** (400 MHz, $CDCl_3$):



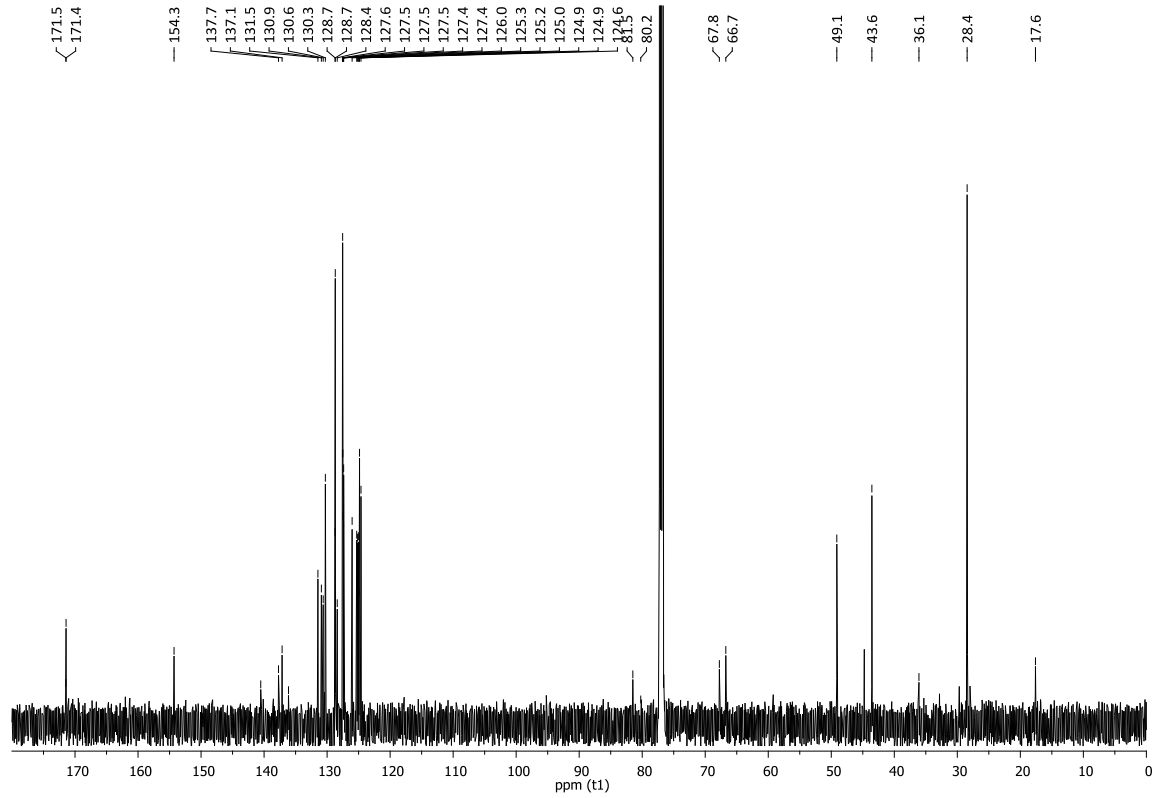
^{13}C-NMR spectra of compound **16a** (100 MHz, $CDCl_3$):



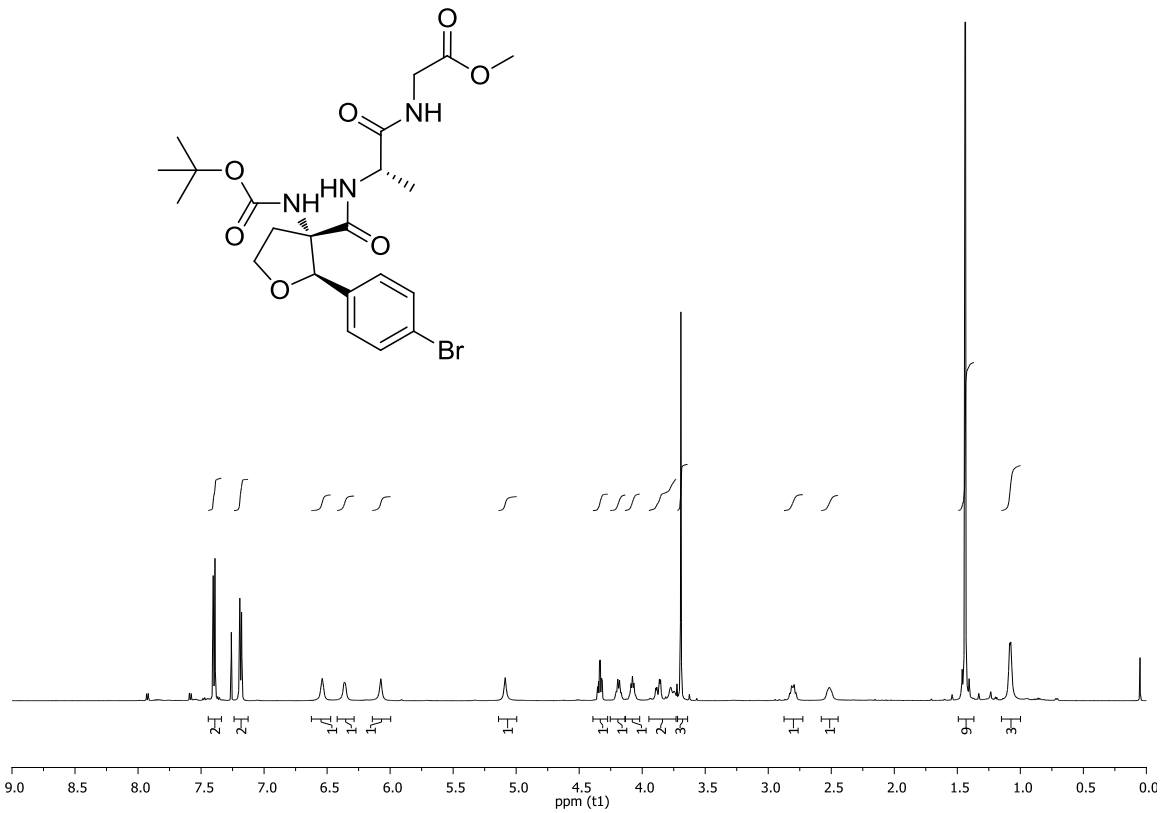
^{1}H-NMR spectra of compound **16b** (400 MHz, $CDCl_3$):



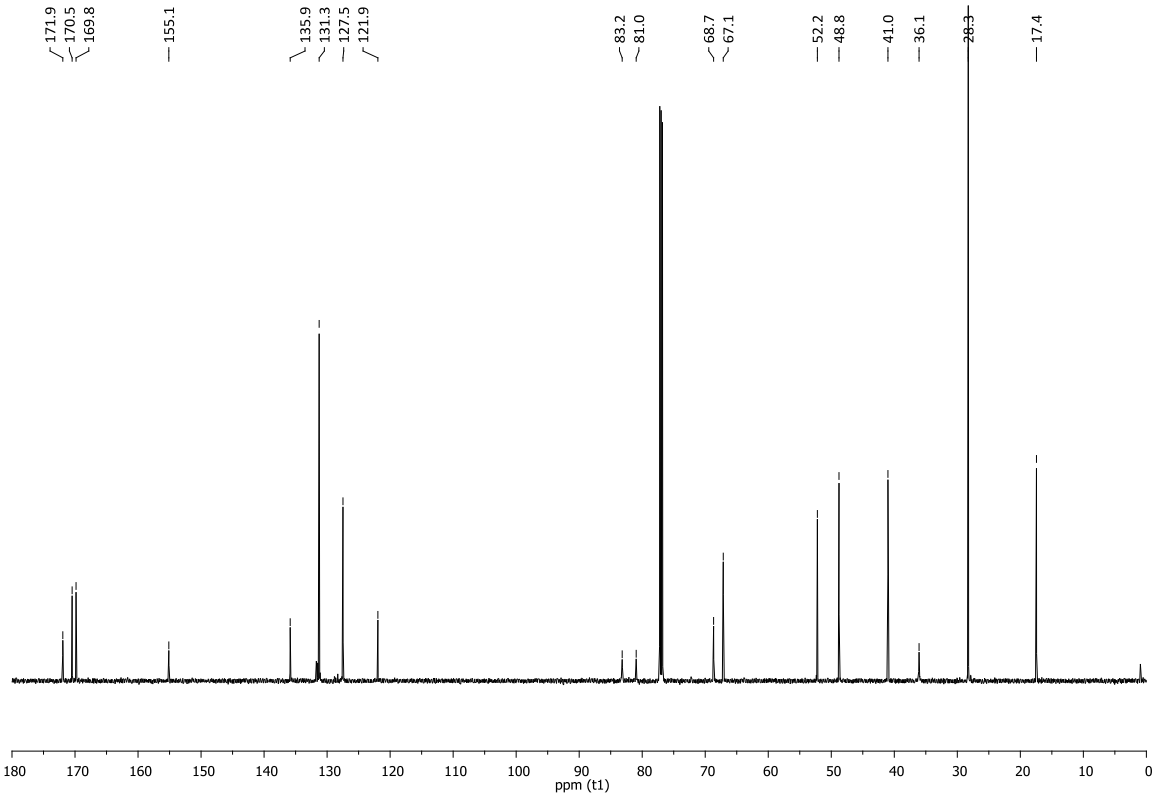
^{13}C-NMR spectra of compound **16b** (100 MHz, $CDCl_3$):



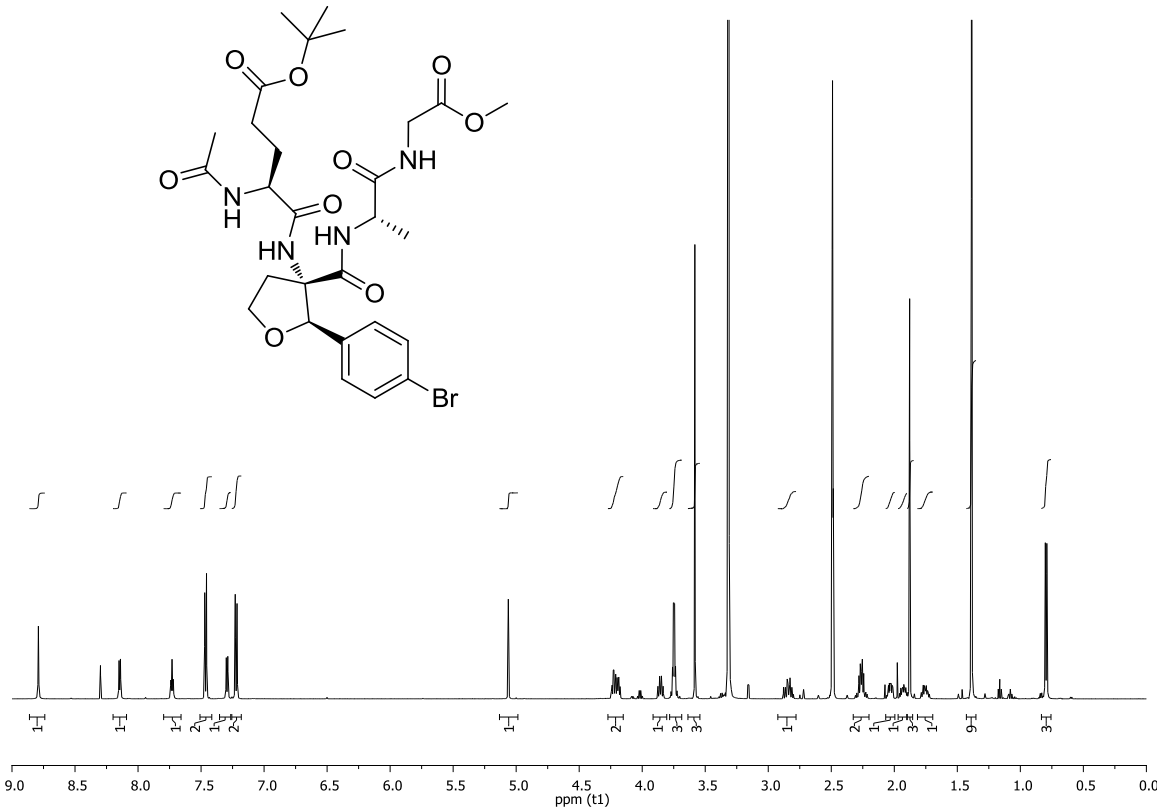
^{1}H-NMR spectra of compound **21** (600 MHz, $CDCl_3$):



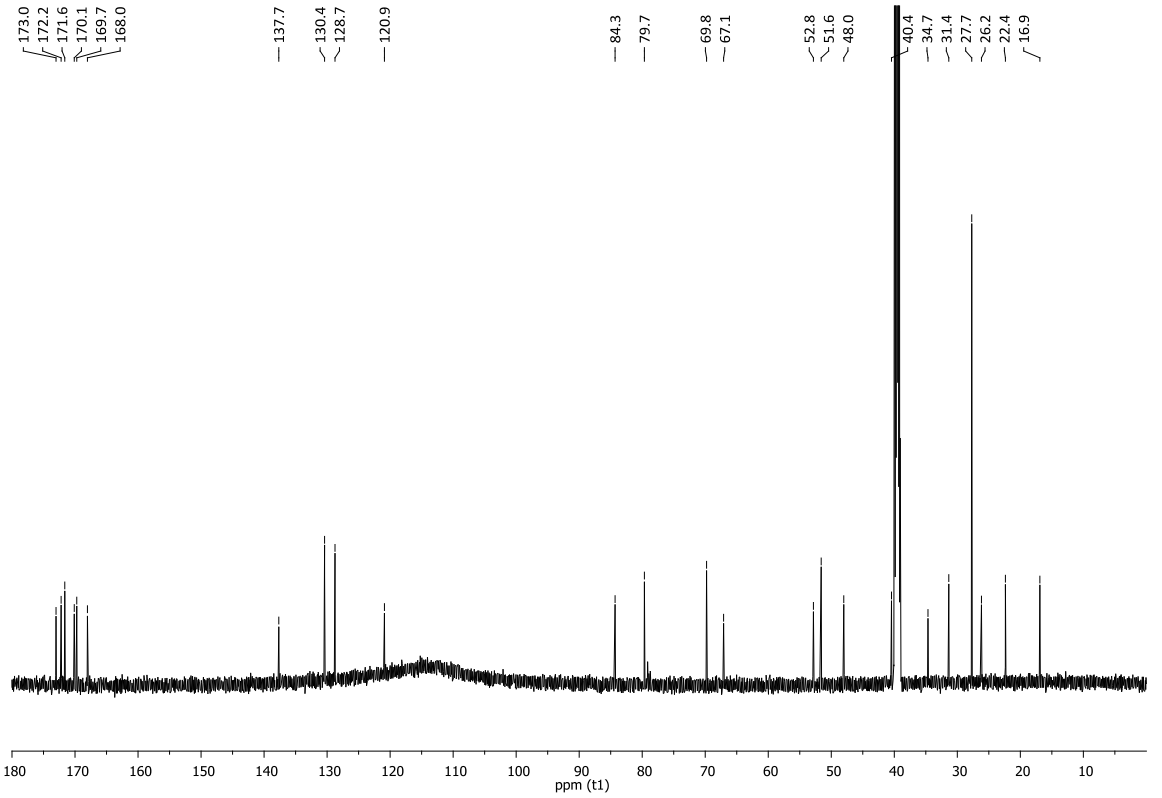
^{13}C-NMR spectra of compound **21** (150 MHz, $CDCl_3$):



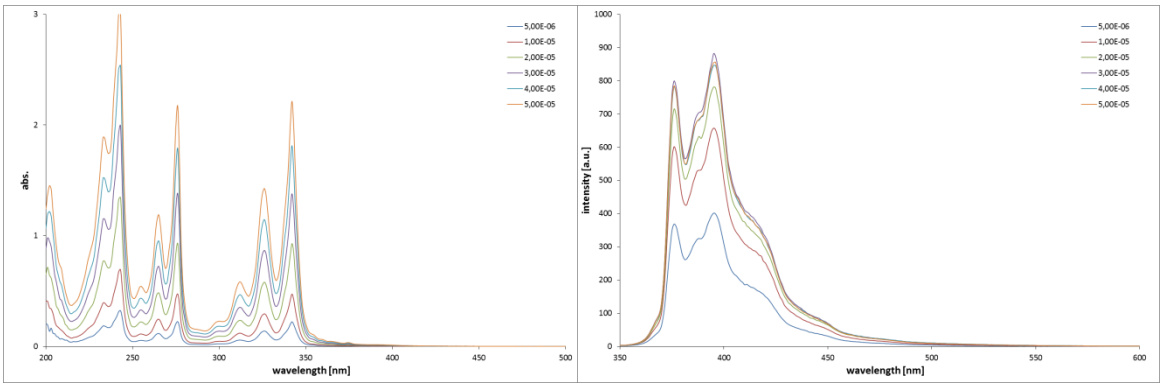
^{1}H-NMR spectra of compound **23** (600 MHz, DMSO-d^{6}):



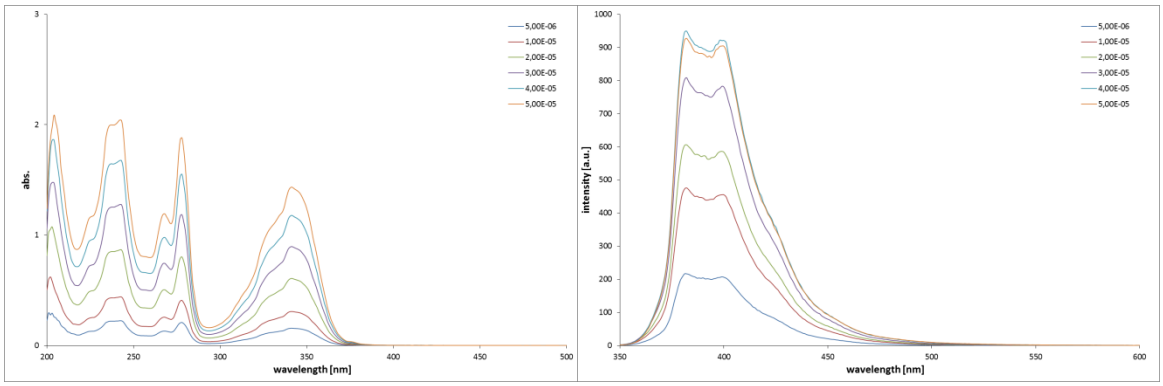
^{13}C-NMR spectra of compound **23** (150 MHz, DMSO-d^{6}):



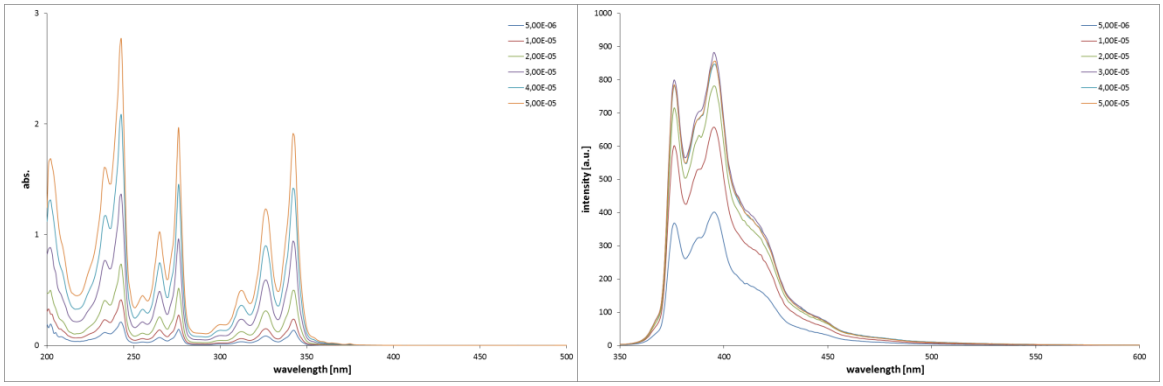
UV/Vis- and fluorescence spectra:



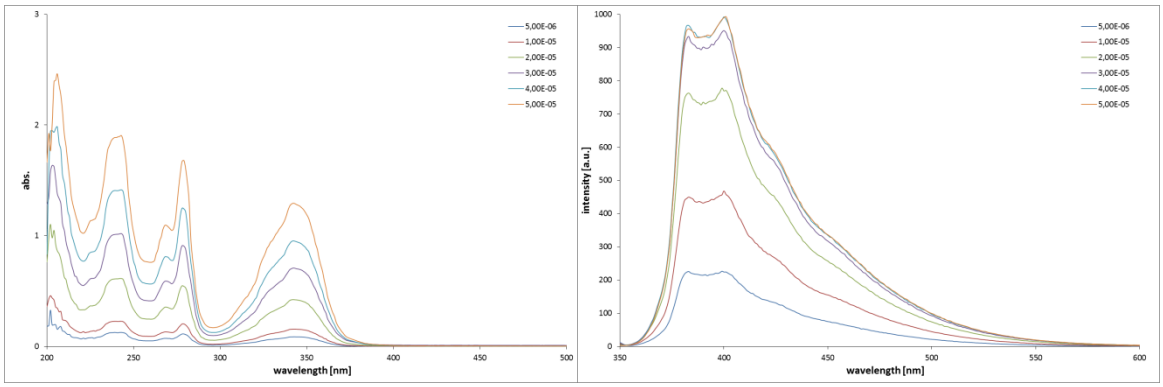
a)



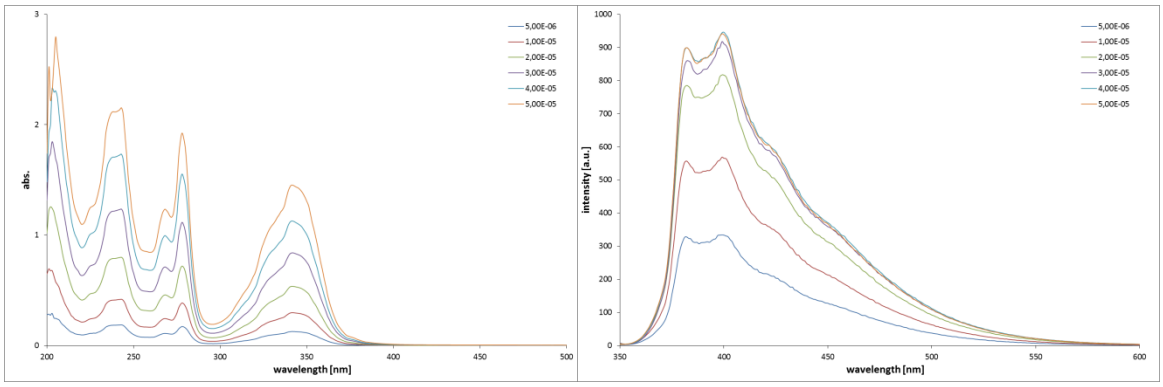
b)



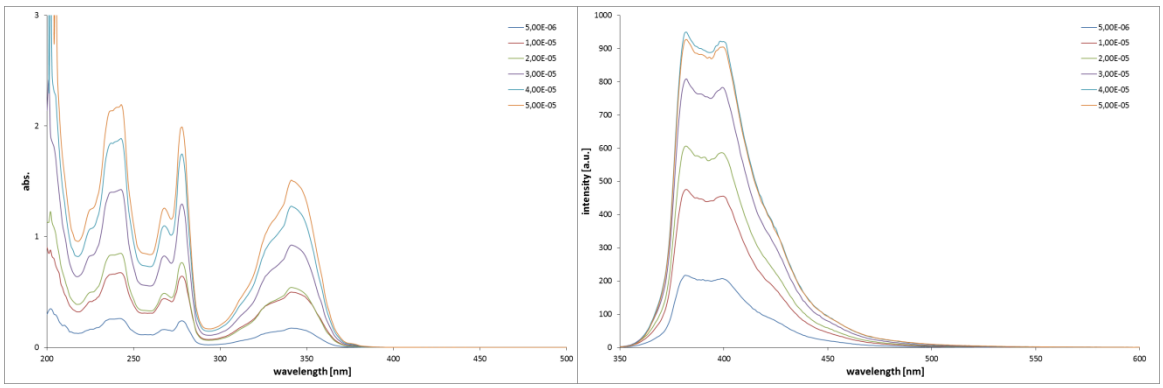
c)



d)



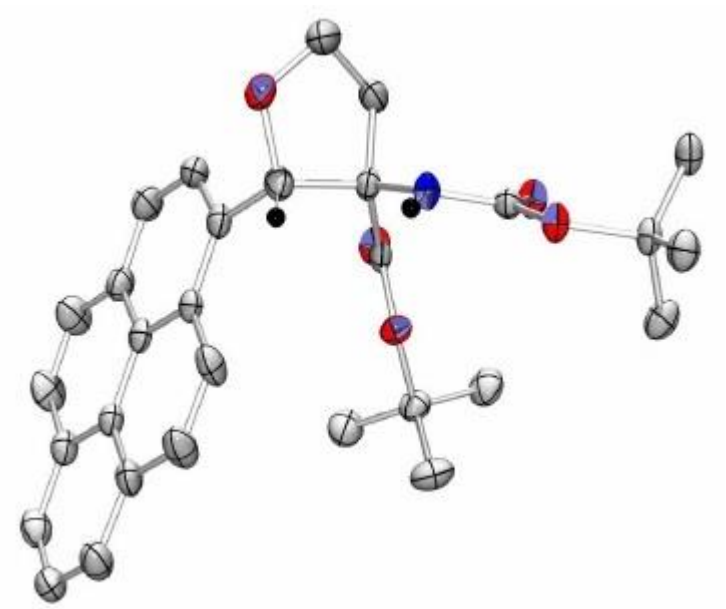
e)

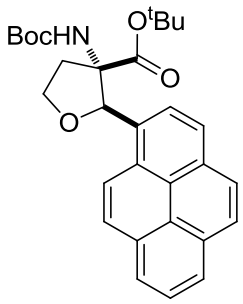


f)

Figure 3. Absorption (left) and emission (right) spectra of pyrene labeled compounds in the concentration range of $5x10^{-6}$ to $5x10^{-5}$ mol/L dissolved in MeOH. Excitation wavelength for emission was 343 nm. (a) Compound **rac-3** (b) Compound **rac-6** (c) Compound **12a** (d) Compound **16a** (e) Compound **16b** (f) Compound **18c**.

Crystal structure analysis of compound **rac-3**:

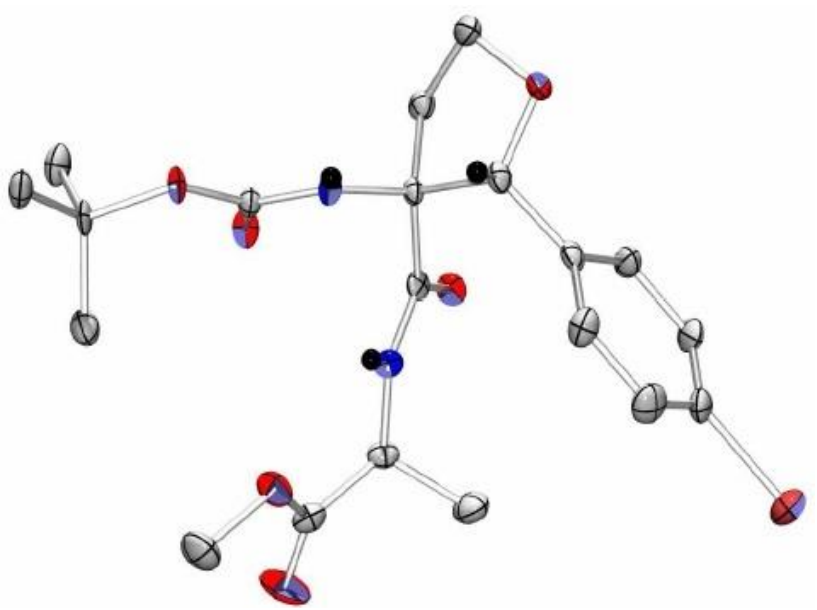


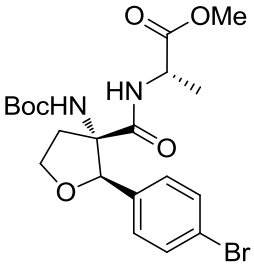


Crystal Data

Empirical formula	$C_{30}H_{33}NO_5$	
Formula weight	487.57	
Crystal size	0.410 x 0.060 x 0.020 mm	
Crystal description	flat needle	
Crystal colour	colourless	
Crystal system	Monoclinic	
Space group	P 21/c	
Unit cell dimensions	a = 5.88530(10) Å	α = 90º
	b = 20.9715(3) Å	β = 91.4300(10)º
	c = 20.6745(3) Å	γ = 90º
Volume	2550.93(7) Å^3	
Z, Calculated density	4, 1.270 Mg/m^3	
Absorption coefficient	0.692 mm^{-1}	
F(000)	1040	

Crystal structure analysis of compound **18a**:

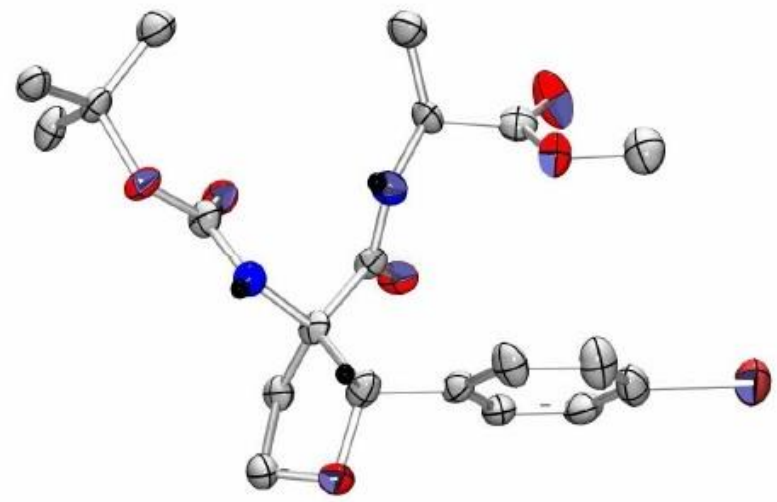


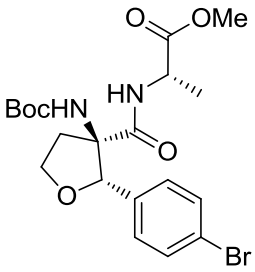


Crystal Data

Empirical formula	$C_{20}H_{27}BrN_2O_6$	
Formula weight	471.34	
Crystal size	0.340 x 0.085 x 0.024 mm	
Crystal description	thin plate	
Crystal colour	colourless	
Crystal system	Monoclinic	
Space group	P 21	
Unit cell dimensions	a = 10.7567(4) Å	α = 90º
	b = 6.1367(3) Å	β = 102.512(3)º
	c = 16.4151(6) Å	γ = 90º
Volume	1057.84(8) $Å^3$	
Z, Calculated density	2, 1.477 Mg/m^3	
Absorption coefficient	1.981 mm^{-1}	
F(000)	486	

Crystal structure analysis of compound **18b**:





Crystal Data

Empirical formula	$C_{20}H_{27}BrN_2O_6$	
Formula weight	471.34	
Crystal size	0.270 x 0.140 x 0.140 mm	
Crystal description	needle	
Crystal colour	colourless	
Crystal system	Monoclinic	
Space group	P 21	
Unit cell dimensions	a = 10.7776(14) Å	α = 90º
	b = 6.1129(13) Å	β = 91.201(13)º
	c = 16.069(3) Å	γ = 90º
Volume	1058.4(3) $Å^3$	
Z, Calculated density	2, 1.456 Mg/m^3	
Absorption coefficient	2.937 mm^{-1}	
F (000)	488	

2.5 References

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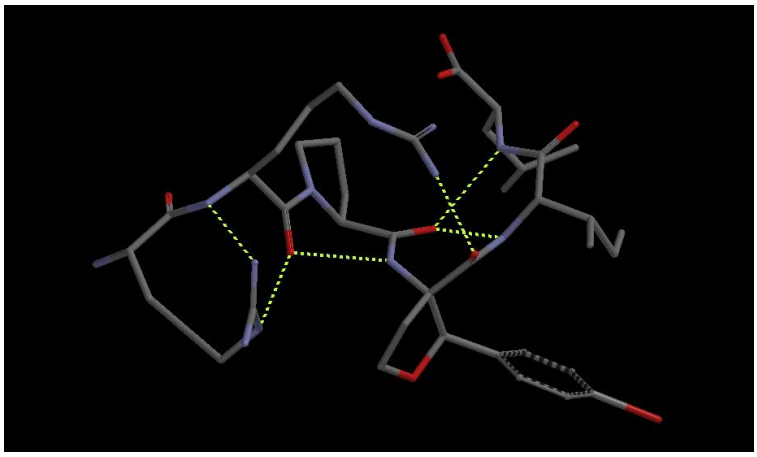
3 Synthesis of new NTS2 selective NT(8-13) peptide analogues by the incorporation of a C^{α}-tetrasubstituted amino acid by SPPS

This chapter describes the synthesis of a small peptide library incorporating HCl*H-TAA-OH **1** by solid phase supported peptide synthesis into the lead structure NT(8-13). In one series **1** is used as scaffold for Tyr^{11}-Ile^{12} and in a second series it replaces only Tyr^{11} leading to new NT(8-13) peptide mimetics. Biological investigations employing a radioligand binding assay were performed, revealing selectivity towards hNTS2.



All compounds which are described in this chapter were synthesized by Michael Dobmeier. The radioligand binding studies and the data analysis of the primary data were performed by Dr. Harald Hübner, Department of Chemistry and Pharmacy Emil Fischer Center, Friedrich Alexander University, Erlangen.[1]

3.1 Introduction

The tridecapeptide pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH is known as the neuropeptide neurotensin,[2,3] which is located and produced in the gastrointestinal tract, the central nervous system and the brain.[4] Acting as a neuromodulator, a wide range of biological functions is mediated by the binding of neurotensin to three different neurotensin receptors which are known so far. The neurotensin receptor 1 (NTS1)[5,6] and also the neurotensin receptor 2 (NTS2)[7,8] belong to the class A of G-protein coupled receptors (GPCRs),[9] which are responsible for most of the biological effects related with neurotensin. Receptor subtype 3 (NTS3)[10] is part of the Vps10p family of sorting receptors[11] and consists only of one single transmembrane domain. Besides neurotensin NTS3 can bind various other ligands, e.g. lipoprotein lipase or apo lipoprotein A, but binding of neurotensin is inhibiting the binding of all other ligands. NTS3 is probably involved in the neurotensin signaling.[12] The different physiological effects, which are associated with NTS1 are analgesic and antipsychotic properties as well as the control of dopamine-mediated neuroleptic effects leading to the opinion that NTS1 play a major role in psychiatric and neurological diseases,[13] e.g. in the pathogenesis of Parkinson's Disease[14] or schizophrenia.[15] Moreover NTS1 stimulation is regarded to be involved in the promotion of cancer growth.[16-20] NTS2 is related with hypothermia,[21] antipsychotic properties[22] and the promotion of μ-opioid-independent antinociception[23-25] as an important part in the modulation of tonic pain sensitivity.[26,27] In the past early investigations proofed that the C-terminal fragment NT(8-13) is representing the pharmacological active part[28,29] of Neurotensin and became therefore the most applied lead structure for the development of NTS1 respectively NTS2 selective ligands as therapeutic agents or for the use in imaging. Recently the group of Grisshammer was able to co-crystalize a mutant of NTS1 in the active bound state with NT(8-13)[30] providing valuable information about the binding mode and the bioactive conformation as basis for a rational design of new neurotensin peptide analogues. This was the first report of a crystal structure of NTS1 or NTS2. Therefore structure-activity-relationship studies of the last two decades were less rationally guided. In the beginning investigations mainly focused towards the NTS1, but with revealing negative aspects like promotion of cancer cell growth the studies today are mainly focussing on NTS2 selective ligands. These ligands are seen as highly interesting candidate for drug development since they show activity in the modulation of tonic pain sensitivity. The huge range of structural modification of the lead structure NT(8-13) include D-amino acid scans,[31,32] alanine scans,[33] homo-β-amino acid scans,[34] backbone modifications introducing conformational constraints,[35] side chain modifications,[36] N-terminal modifications,[37,38] C-terminal modifications,[39] peptide-peptoid hybrids[40] (N-homotyrosine), incorporation of proline derivatives,[41] modifications for metabolically stability[42] (Nα-methylarginine). Further modifications are containing ^{18}F substituents[43,44] for PET imaging or radioisotopes for tumors treatment.[45-47]

We recently reported the synthesis of the Cα-tetrasubstituted tetrahydrofuran amino acid (TAA) **1** and investigated their stereochemical properties. Incorporated in small peptides, the dipeptide

mimetic **1** induces two consecutive β turns leading to the formation of stable secondary structures[48] and stable peptide helices.[49] The bromo substituent was used for synthetic modifications to prepare cyclic peptides,[50] attach fluorescent labels (see Chapter 2) or further modifications of the side chain.[51]

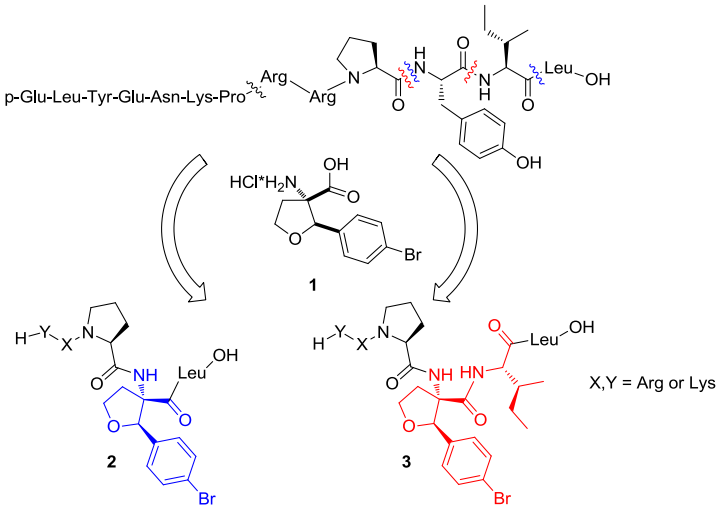


Figure 1. Incorporation of compound **1** as modification of NT(8-13) leading to the target structures **2** and **3**.

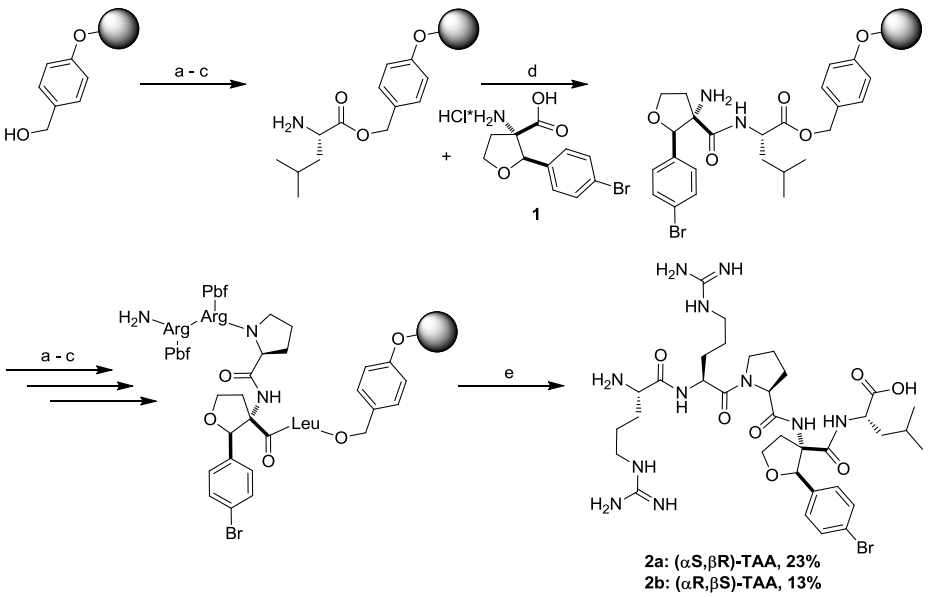
Herein we report a structure-activity-relationship study, based on a small peptide library of new NTS ligands analogous to the lead structure NT(8-13) depicted in Figure 1. In this approach either Tyr[11]-Ile[12] is replaced by the TAA building block **1** (blue) leading to peptides of type **2** or only the tryrosine residue is exchanged (red) resulting in compounds of type **3**. The new compounds are expected to show NTS2 selective properties in biological testing.

3.2 Results and Discussion

3.2.1 Syntheses

TAA building block. The unnatural amino acid building block HCl*H-TAA-OH **1** was prepared according to a literature known procedure, which was developed in our laboratory.[48] The five-step synthesis started from commercial available methionine with N-Boc protection, esterification with tert-butanol and methylation of the side-chain by treatment with methyl iodide. The key step was an aldol-type reaction of the ester enolate formed under strong basic conditions with 4-bromobenzaldehyde followed by an intramolecular nucleophilic substitution to build up the tetrahydrofurane core. The deprotonation of the α-carbon is accompanied by a loss of stereochemical information. Together with the non-stereospecific substitution reaction this resulted in the formation of in total four stereoisomers (trans/cis-ratio: 97:3). Final deprotection with 6.0 M hydrochloric acid gave compound **1** in a good overall yield of 54%. The building block was used as racemic mixture of the two trans-isomers (αS,βR) respectively (αR,βS) in the peptide synthesis.

Peptide synthesis. The target peptides **2** and **3** were synthesized by solid phase supported peptide synthesis (SPPS) applying a standard protocol for Fmoc-strategy.[52,53] Commercial available Wang resin and Fmoc protected α-amino acids: Fmoc-Leu-OH, Fmoc-Ile-OH, Fmoc-Pro-OH, Fmoc-Lys(Boc)-OH and Fmoc-Arg(Pbf)-OH were used.



Scheme 2. Synthesis of target compounds **2a** and **2b**. *Reaction conditions:* (a) Fmoc-AA-OH, TBTU, HOBt, DIPEA, DMF, RT, 3.5 h (2x). (b) Ac_2O, DIPEA, DMF, RT, 1 h. (c) piperidine/DMF (40:60, v/v), RT, 5 min followed by piperidine/DMF (20:80,v/v), RT, 10 min. (d) TBTU, HOBt, DIPEA, DMF, RT, 3.5 h (2x). (e) TFA/TIS/water (90:5:5, v/v), RT, 3 h respectively 30 min.

The coupling reaction was enabled by the presence of TBTU, HOBt and DIPEA as activation reagents and performed twice to increase the yield of the coupling products. After every amino acid (except for **1**) an acylation reaction using Ac_2O and DIPEA in DMF was carried out to cap unreacted amino groups and prevent complex product mixtures, which complicate the purification. Cleavage of the Fmoc group required treatment with pyridine in DMF twice. Protection of **1** was not necessary as known from earlier investigations: Due to the high steric demand of the Cα-tetrasubstituted amino acids, homo coupling of building block **1** is entirely excluded.[49,54,55] Final cleavage from the resin was performed with a mixture TFA/TIS/water, employing TIS as cation scavenger to prevent alkylation of nucleophilic functional groups in the peptides during side-chain deprotection.[56-58] As a consequence of the racemic compound **1** the peptides of type **2** were obtained as couples of two diasteromers. The purification and separation was achieved by preparative HPLC giving eight pentapeptides **2a-h** with moderate yields. Peptides **2a** and **2b** are exemplary presented in Scheme 1 and for **2c-h** see Figure 2.

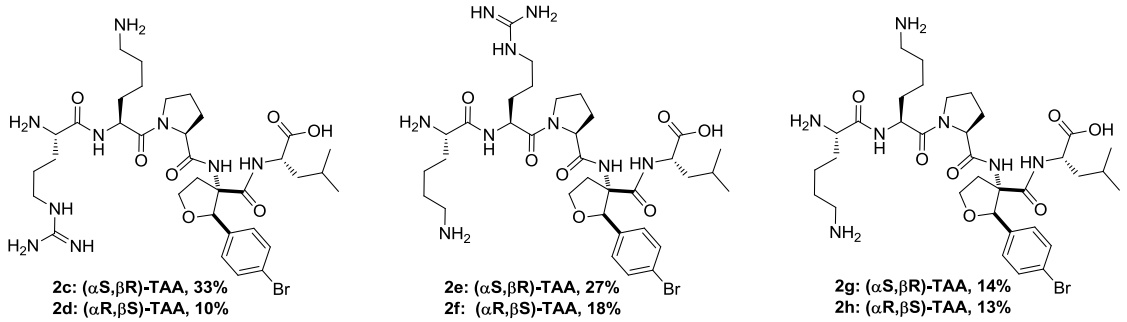


Figure 2. Structures and yields of the synthesized pentapeptides **2c-h**.

The preparation of type **3** peptides only replacing the Tyr moiety by **1** was done as described above. Purification by preparative chromatography did not allow to separate the two diastereomers completely. With the diasteromeric peptide mixtures **3ad-gh** (Figure 3) in our hands we decided to continue with the pharmacological investigations and postponed the separation and identification of the stereochemically pure peptides. The preliminary stereochemical assignment of (αS,βR) or (αR,βS) for type **2** and type **3** peptides is based on the chromatography retention time in comparison with previous investigations.[48,49,54] For a final proof of the assignment the stereocontrolled synthesis of at least one pair of peptides as described below or a crystal structure of one target molecules is necessary. Two dimensional NMR investigations and variable temperature ^{1}H-NMR in an appropriate solvent like DMSO-d^6 leads to very complex spectra and does not allow an assignment. In summary the use of compound **1** as racemic mixtures allows a very fast, flexible and convenient synthesis of a small peptide library in a non-stereocontrolled fashion. These stereoisomeric mixtures were used for an initial pharmacological investigation.

Besides target peptides **2** and **3** two more hexapeptides **4a** and **4b** (Figure 3) were synthesized via SPPS using commercial available Fmoc-(S)-Phe(4-Br)-OH or Fmoc-(R)-Phe(4-Br)-OH. Those compounds serve as reference compounds and should indicate the effect of the Br-substituent and

the conformational rigidification of the peptide back-bone on the biological activity. Moreover they show, which stereochemistry in α-position of the TAA is preferred for high activity.

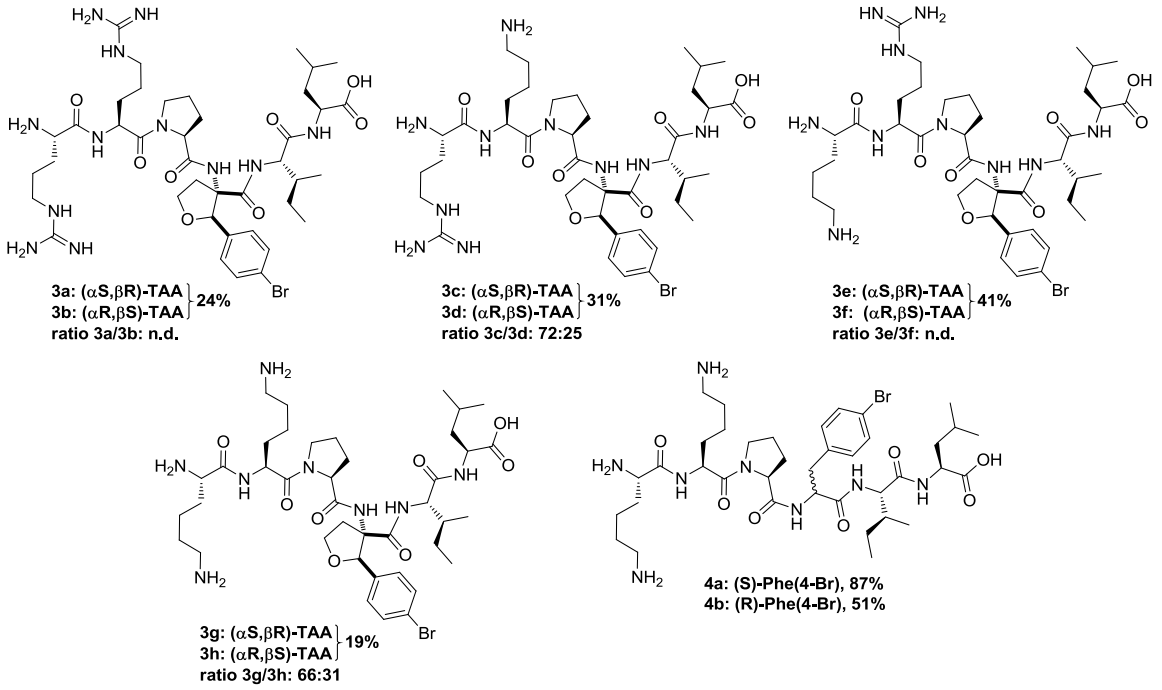
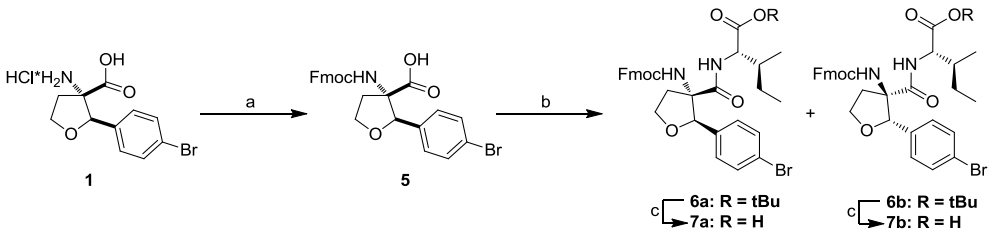


Figure 3. Structures and yields of synthesized hexapeptides **3ab-gh** and compounds **4a** and **4b**.

Stereoselective synthesis. To have a direct synthetic access to single stereoisomers, which show interesting activity in the initial testing, we investigated the crucial steps of an alternative reaction route using a similar strategy, which we applied several times in solution peptide synthesis, illustrated in Scheme 2.



Scheme 3. Stereoselective preparation of TAA containing and Fmoc protected building blocks **6a**/**6b** respectively **7a**/**7b** for SPPS. *Reaction conditions:* (a) Fmoc-Cl, Na_2CO_3, dioxane/water, RT, 2 h. (b) HCl*H-Ile-OtBu, EDC*HCl, HOBt, DIPEA, DCM, RT, 24 h. (c) TFA, DCM, RT, 1 h.[59]

Compound **1** was first Fmoc protected yielding compound **5** after optimization in a yield of 51%, which is considered good taking into account the significant steric hindrance of the starting material. Then **5** was coupled in the present of HOBt, EDC*HCl and DIPEA with the hydrochloride salt of isoleucine tert-butyl ester yielding the diastereomeric compounds **6a** and **6b**. The separation of both fully protected dipeptides was achieved by standard flash column chromatography on silica gel. The

separability depends on the protecting groups, the natural amino acid and the direction of the synthesis. Coupling the N-terminus of **1** with a natural amino acid leads to inseparable mixtures of dipeptides. Bulky protecting groups hamper the separation in general. In solution phase chemistry (e.g. see Chapter 2) Boc groups and Me-esters were used. To have the peptides compatible with Fmoc-strategy on SPPS Fmoc/TBDMS-ester and Fmoc/tert-butyl ester were tried. However, in the first case the separation was not successful, while with tert-butyl the separation gave only low yields. The stereochemistry of **6a**/**6b** was proven by 2-dimensional NMR experiments (NOESY) and variable temperature ^{1}H-NMR to determine the temperature coefficients of the NH-protons, which indicates the present or absence of hydrogen bonds. The chemical shifts of the NH-protons fit to literature know values.[54] The deprotection of the carboxylic ester groups leads to SPPS compatible building blocks **7a**/**7b** as single stereoisomers for the synthesis of type **3** peptides. The synthesis of single stereoisomers of hexapeptides **3a** or **3b** was not possible during the timeframe of this work.

3.2.2 Biological investigations

For the determination of binding affinities of compounds **2a-h**, **3ab-gh** and **4a**/**4b** towards NTS1 and NTS2 receptors a radioligand displacement assay was performed (Table 1). In the case of NTS1 the radioligand [^{3}H]neurotensin and stably transfected Chinese hamster ovary (CHO) cells expressing human NTS1 were used. Binding data for NTS2 receptor were collected employing human embryonic kidney (HEK293) cells which were transiently transfected with the pcDNA3.1 vector containing the hNTS2 gene against [^{3}H]NT(8-13) as radioactive reference agent.

The pentapeptides **2a-h** show K_i values in the range of 53-100 µM for NTS1 and 1-49 µM for NTS2 and hence can be regarded as inactive. This may be rationalized by the crystal structure of the activating NT(8-13) bound to NTS1, which was recently published by Grisshammer and co-workers.[30] The absence of Ile^{12} leads to an insufficient distance between the C-terminal carboxylic group and the bulky sterically demanding TAA. As a consequence the formation of hydrogen bonds between Leu^{13} of type **2** compounds and Y146 and R327 of the receptor is not possible or restricted leading to lower binding affinities. However interestingly all compounds showed binding selectivity towards NTS2, which is in accordance with the structural data available that indicate NTS2 is tolerating changes at position of Tyr^{11} more easily than NTS1.[60-62] Moreover a clear preference for one possible stereochemistry is present; according to our assignment (αS,βR) is superior to (αR,βS).

The binding data for the hexpeptides **3ab-gh** revealed K_i(NTS2) values in the nanomolar region (67-227 nM) and all compounds show a about 50fold selectivity over NTS1. From the results of compound **4a**/**4b** in comparison with **2g**/**2h** and **3gh** we concluded that the bromo substituent has no significant influence on the binding affinity or selectivity. The selectivity of **2h** (αR,βS) is identical to **4b** (R) and the K_i values of **3gh** and **4a**/**4b** are in a similar range taking in account that **3gh** is the average value of the active and the less active isomer. Comparison of **2g** with **4a** suggested that for the selectivity the rigidification and the right stereochemistry of the peptide back bone are crucial. Peptides **4a**/**4b** can adopt several conformations and also **2h** can still undergo some conformational

Table 1. Binding data of target compounds **2a-h**, **3ab**-**3dh** and **4a,b** at the human NTS1 and NTS2 receptor determined via radioligand binding assay relative to the reference agent NT(8-13).[63]

Compd	Sequence	K_i [nM][a]		SR[f]
		hNTS1[b]	hNTS2[c]	
NT(8-13)	H-Arg-Arg-Pro-Tyr-Ile-Leu-OH	0.24±0.024	1.2 ± 0.17[d]	0.20
2a	H-Arg-Arg-Pro-(αS,βR)-TAA-Leu-OH	72500 ± 38900[e]	1430 ± 440	51
2b	H-Arg-Arg-Pro-(αR,βS)-TAA-Leu-OH	91500 ± 4900	49300 ± 15300	1.9
2c	H-Arg-Lys-Pro-(αS,βR)-TAA-Leu-OH	>100000 ± 0[e]	5800 ± 2000	17
2d	H-Arg-Lys-Pro-(αR,βS)-TAA-Leu-OH	70500 ± 41700[e]	16000 ± 5600[e]	4.4
2e	H-Lys-Arg-Pro-(αS,βR)-TAA-Leu-OH	92300 ± 7700	11100 ± 870	8.3
2f	H-Lys-Arg-Pro-(αR,βS)-TAA-Leu-OH	53000 ± 2800[e]	28000 ± 21200[e]	1.9
2g	H-Lys-Lys-Pro-(αS,βR)-TAA-Leu-OH	95500 ± 6300[e]	2030 ± 290	47
2h	H-Lys-Lys-Pro-(αR,βS)-TAA-Leu-OH	67000 ± 46700[e]	26500 ± 7800[e]	2.5
3ab	H-Arg-Arg-Pro-(αS,βR)-TAA-Ile-Leu-OH H-Arg-Arg-Pro-(αR,βS)-TAA-Ile-Leu-OH	3500 ± 830	67 ± 12	52
3cd	H-Arg-Lys-Pro-(αS,βR)-TAA-Ile-Leu-OH H-Arg-Lys-Pro-(αR,βS)-TAA-Ile-Leu-OH	9900 ± 2970	147 ± 23	68
3ef	H-Lys-Arg-Pro-(αS,βR)-TAA-Ile-Leu-OH H-Lys-Arg-Pro-(αR,βS)-TAA-Ile-Leu-OH	5000 ± 750	110 ± 0[e]	46
3gh	H-Lys-Lys-Pro-(αS,βR)-TAA-Ile-Leu-OH H-Lys-Lys-Pro-(αR,βS)-TAA-Ile-Leu-OH	12600 ± 2470	227 ± 66	56
4a	H-Lys-Lys-Pro-(S)-Phe(4-Br)-Ile-Leu-OH	93 ± 22	69 ± 9	1.3
4b	H-Lys-Lys-Pro-(R)-Phe(4-Br)-Ile-Leu-OH	2170 ± 540	873 ± 265	2.5

[a] Values are the means ± SEM of 3-6 individual experiments, each done in triplicate. [b] Determined with [^{3}H]neurotensin and membranes from CHO cells stably expressing human NTS1. [c] Determined with [^{3}H]NT(8-13) and homogenates from HEK293 cells transiently expressing human NTS2. [d] K_D value. [e] Values are the means ± SD of two individual experiments, both done in triplicate. [f] Selectivity ratio: K_i(NTS1)/K_i(NTS2).

changes since the (αR,βS)-TAA building block is not inducing a rigid secondary structure element. Whereas (αS,βR)-TAA leads to two consecutive type I β-turns and consequently exerts a larger impact on the conformation of the peptide **2g** resulting in an increased selectivity.

3.3 Conclusion and Outlook

In conclusion we have reported the synthesis of a new class of NTS2 receptor selective ligands based on the structural modification of the lead structure NT(8-13) via SPPS. Compound **1** was introduced as scaffold for Tyr or Tyr-Ile, respectively, leading to a small library of penta- and hexapeptides. For selected peptides that showed interesting biological activity when tested as mixtures of stereoisomers the crucial steps of an alternative synthetic pathway were investigated. This stereoselective synthesis will lead to diastereomerically pure compounds.

The biological investigations by radioligand binding assay at hNTS1 or hNTS2 receptors revealed that target compounds **2a-h** have only very low affinity and were therefore not further investigated. The K_i values for compounds **3ab-dh** showed good binding affinities and increased selectivity towards the NTS2 receptor. The most promising compound **3ab** has a K_i(NTS2) value of 67 ± 12 nM and a selectivity ratio of 52. The most active and selective compound for NTS2 receptor binding reported so far has still a significant lower K_i(NTS2) = 2.8 ± 0.69 nM and a higher selectivity ratio of: 22000.

Ongoing investigations regarding the stereochemistry of the biological active isomer of compound **3ab** and concerning the sterical demand of the TAA building block at the binding site of the protein are in progress. The active isomer of **3ab** should have an estimated K_i(NTS2) of 33-45 nM and also a slightly higher selectivity towards NTS2. It would be favorable to resynthesize the compound using the indirect route with compounds **7a**/**7b** as it would at the same time clarify the assignment of the stereochemistry. Besides a molecular modeling study, the compounds **8a-d** are designed to examine the sterical demand of these new class of ligands. Finally compounds **9a-d** are very interesting candidates for potent and selective NTS2 receptor ligands representing a conformational rigidified tyrosine mimetic. Compared to the compounds of type **2** and **3** they allow the formation of an additional hydrogen bond at the binding site, which should significantly improve the K_i values.

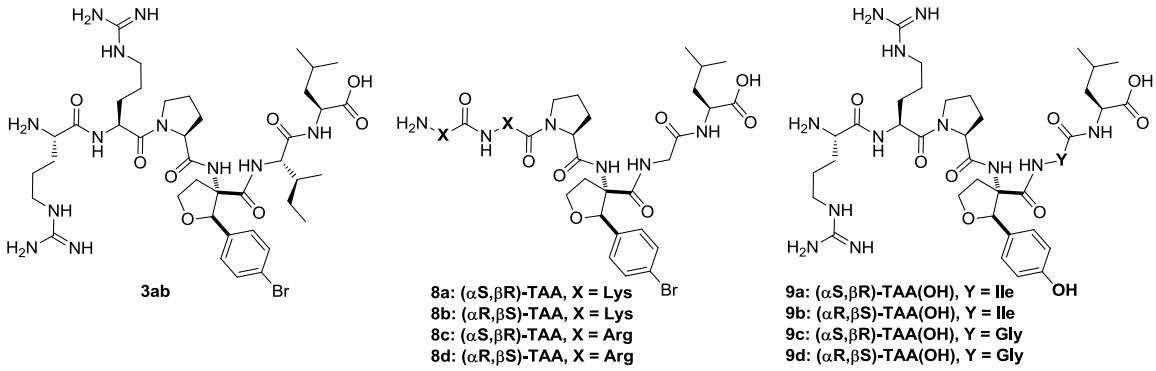


Figure 4. Structures of most favorable compounds **8a-d** and **9a-d** for ongoing investigations.

3.4 Experimental Section

3.4.1 General

Melting point. Melting points were determined on a Stanford Research System OptiMelt melting point apparatus 100 and are uncorrected.

IR spectra. IR spectra were recorded with a Bio-Rad FT-IR-FTS 155 spectrometer and a Bio-Rad Excalibur series FT-IR-spectrometer FTS 2000 MX using a Specac Golden Gate Mk II ATR accessory where stated.

NMR spectra. NMR spectra were recorded with Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz, T = 300 K), Bruker Avance 400 (^{1}H: 400.1 MHz, ^{13}C: 100.6 MHz, T = 300 K), and Bruker Avance 600 (^{1}H: 600.1 MHz, ^{13}C: 150.1 MHz, T = 300 K) instruments. The chemical shifts are reported in δ [ppm] relative to internal standards (solvent residual peak) or external standard (TMS). The spectra were analyzed by first order, the coupling constants are given in Hertz [Hz]. Abbreviations of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, dd = double doublet. Integration is determined as the relative number of atoms. Assignment of signals in ^{13}C-spectra was determined with DEPT-technique (pulse angle: 135°) and given as (+) for CH_3 or CH, (-) for CH_2 and ($C_{quat.}$) for quaternary C-Atoms. Error of reported values: chemical shift: 0.01 ppm for ^{1}H-NMR, 0.1 ppm for ^{13}C-NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum.

Mass spectra. MS spectra were recorded on a Finnigan MAT 95 (CI), a ThermoQuest Finnigan TSQ 7000 LC/MS spectrometer and a Finnigan MAT TSQ 7000 (ESI) spectrometer for low resolution (LR-MS) and on an Agilent Tech 6540 UHD Accurate Mass Q-TOF LC/MS for high resolution (HR-MS).

Analytical UHPLC. UHPLC/MSD-System (S3): Agilent 1290 Infinity Bin Pump G4220A, Agilent 1290 Infinity DAD G4212A, Agilent 1290 Infinity Sampler G4226A, Agilent 1290 TCC G1316C, MSD Agilent Quadrupol G6130A, MM-ESI-APCI Multimode-Source G1978B; software: OpenLAB CDS ChemStation Rev C.01.0435; column: Phenomenex Luna 3 um C18 (2) 100A, 150 mm x 2.00 mm, column temperature: 25 °C; UV detection at 220 nm, 230 nm and 254 nm; gradient: from 0 min 5% MeCN/H_2O (0.059% TFA), 20 min 33% MeCN/H_2O (0.059% TFA), 25 min 98% MeCN/H_2O (0.059% TFA); Flow rate: 0.3 mL/min, injection volume: 2 µL, sample concentration: 1.0 mg/mL.

Analytical HPLC. LC/MSD-System (S1): Agilent Technologies 1100 Bin Pump G1312A, 1100 DAD G1315B, 1100 Degasser G1379A, ALS G1329A, 1200 FC G1330B, 1100 COLCOM G1316A, MSD Agilent Quadrupol G6130A, MM-ESI-APCI Multimode-Source G1978B; software: LC/MSD Chemstation Rev. B.04.03; column: Phenomenex Luna 3 um C18 (2) 100A, 150 mm x 2.00 mm , column temperature: 25 °C; UV detection at 220 nm, 230 nm and 254 nm; gradient: linear from 5% MeCN/H_2O (0.059% TFA) to 95% MeCN/H_2O (0.059% TFA) within 30 min; Flow rate: 0.3 mL/min, injection volume: 3 µL,

sample concentration: 1.0 mg/mL. LC-System (S2): Agilent 1100/1 Bin Pump G1312A, DAD G1315B, Degasser G1379, ALS G1313A, COLCOM G1316A, FLD G1321A; software: ChemStation for LC 3D Systems Rev B.03.01. SR1; column: Phenomenex Luna 3 um C18 (2) 100A, 150 mm x 2.00 mm, column temperature: 25 °C, FLD-A, ELS; UV detection at 220 nm, 230 nm and 254 nm, fluorescence detection: zero-order; gradient: linear from 5% MeCN/H_2O (0.059% TFA) to 95% MeCN/H_2O (0.059% TFA) within 30 min; Flow rate: 0.3 mL/min, injection volume: 6 µL, sample concentration: 1.0 mg/mL.

Preparative HPLC. LC-System: Agilent 1100 Series PrepPump G1361A, PrepPump G1361A, AFC G1364A, MWD G1365B, ALS G1329A, ALS Therm G1330B, HPLC Column Chiller/Heater C030: Echo Therm Torrey Pines Scientific; software: ChemStation for LC 3D Systems Rev B03.02; column: Phenomenex Luna 10 um C18 (2) 100A, 250 mm x 21.2 mm, column temperature: 25 °C; UV detection at 220nm; solvents: MeCN (B) / H_2O (0.059% TFA) (A); Flow rate: 21.0 mL/min.

TLC analysis and column chromatography. TLC analyses were performed on silica gel coated alumina plates (Merck 60 F^{254} Silica gel, layer thickness 0.2 mm). Visualization was done by UV-light at 254 nm / 366 nm and/or through staining with ninhydrine in EtOH. For preparative column chromatography, Merck Geduran SI 60 (70-230 mesh) and Macherey-Nagel GmbH & Co. KG 60M (0.04-0.063 mm, 230-400 mesh) silica gels were used. For chromatography commercially available solvents of standard quality were used without further purification. Furthermore an automated flash column chromatography system Biotage Isolera One with 200-800 nm wavelength detector and spectra option was used.

Solvents and reagents. Commercial solvents, reagents and starting materials were of analytical grade and purchased from Aldrich, Fluka, Merck or Arcos and used without further purification. Iris Biotech GmbH supplied all Fmoc-protected amino acids, coupling reagents and resins. Unless stated otherwise, purification and drying of solvents used was done according to accepted general procedures.[64,65]

3.4.2 Syntheses

The unnatural amino acid HCl*H-TAA-OH **1** was prepared in a five-step synthesis according to a literature known procedure.[48]

General procedure (GP) for the preparation of target peptides via SPPS.

All peptides **2a-h**, **3ab-gf** and **4a**/**4b** were prepared manually using BD discardit II syringes, standard Fmoc chemistry and Wang resin (2% DVB, loading 1.0-1.1 mmol g^{-1}). Solvents and soluble reagents were removed in various washing steps by *vacuo*. As side-chain protection groups Boc and Pbf[66] were applied for Lys respectively Arg during peptide synthesis. Wang resin (50 mg, 50-55 µmol) was weighed in a syringe equipped with a frit and swollen in DCM (2 mL) for 60 min. Afterwards, the corresponding Fmoc protected amino acid (250 µmol, 5 eq.) was dissolved in 2 mL of DMF and the coupling reagents HOBt (250 µmol, 5 eq.), TBTU (245 µmol, 4.9 eq.) and DIPEA (500 µmol, 10 eq.) were added and stirred for 1 min. Then the reaction mixture was added to the resin and reacted for 3.5 h. The resin was washed with 2 mL of DMF, MeOH, DCM and DMF (5 times each). The coupling step and washings steps were repeated. Acylation with Ac_2O (250 µmol, 5 eq.) and DIPEA (500 µmol, 10 eq.) in 2 mL of DMF was done for 1 h to eliminate unreacted sites and the resin was washed like mentioned above. The removal of the Fmoc group was carried out with piperidine in DMF (40:60, v/v) for 5 min followed by (20:80, v/v) for 10 min and washed again with 2 mL DMF, MeOH, DCM, DMF (5 times each). The peptide coupling cycles consisting of coupling, acylation, deprotection and washing steps were repeated according to the desired peptide sequence. After coupling of HCl*H-TAA-OH the acylation and deprotection step was skipped. The final cleavage of the peptides from the resin and simultaneous side-chain deprotection was achieved by treatment of the peptidyl resin with 1.5 mL of a TFA/TIS/H_2O (90:5:5, v/v) mixture for 3 h followed by 1 mL for 30 min. The combined TFA solutions were filtered of from the resin, collected in a Falcon tube and reduced in volume to about 0.5 mL under vacuum. Then the peptide was precipitated with ice-chilled Et_2O and the suspension was centrifuged at -4 °C for 10 min. The solution was carefully taken off and the precipitate re-suspended in ice-chilled Et_2O and centrifuged again. To remove most of the scavengers this procedure of re-suspending/centrifuging was repeated five times. Finally the crude peptide was dried under vacuum. The purification was done either by preparative RP-HPLC or by automated flash column chromatography on RP-silica gel like stated below.

H-Arg-Arg-Pro-(αS,βR)-TAA-Leu-OH (2a):

Synthesized according to the GP. Yield: 4.6 mg, 23%; Purification by preparative HPLC. Gradient (t [min], % B): (0, 5), (20, 65), (22, 98), (32, 98), t_R = 10.974 min. – **HPLC** (analytical, S1): t_R = 12.344 min (DAD), Purity: 92%. – **LC/MS** (ESI, t_R = 12.478 min): m/z (%) = 404.7 (60) $[M+2H]^{2+}$, 808.3 (100) $[MH]^+$. – **HR-MS**: $[M+2H]^{2+}$ calcd. for $C_{34}H_{54}BrN_{11}O_7$ 404.6768, found 404.6770. – **MF:** $C_{34}H_{54}BrN_{11}O_7$. – **MW:** 808.77.

H-Arg-Arg-Pro-(αR,βS)-TAA-Leu-OH (2b):

Synthesized according to the GP. Yield: 2.6 mg, 13%; Purification by preparative HPLC. Gradient (t [min], % B): (0, 5), (20, 65), (22, 98), (32, 98), t_R = 10.590 min. – **HPLC** (analytical, S1): t_R = 12.038 min (DAD), Purity: 94%. – **LC/MS** (ESI, t_R = 12.185 min): m/z (%) = 404.7 (100) $[M+2H]^{2+}$, 808.3 (100) $[MH]^+$. – **HR-MS**: $[M+2H]^{2+}$ calcd. for $C_{34}H_{54}BrN_{11}O_7$ 404.6768, found 404.6775. – **MF:** $C_{34}H_{54}BrN_{11}O_7$. – **MW:** 808.77.

H-Arg-Lys-Pro-(αS,βR)-TAA-Leu-OH (2c):

Synthesized according to the GP. Yield: 6.4 mg, 33%; Purification by preparative HPLC. Gradient (t [min], % B): (0, 5), (20, 65), (22, 98), (32, 98), t_R = 10.938 min. – **HPLC** (analytical, S1): t_R = 12.162 min (DAD), Purity: 98%. – **LC/MS** (ESI, t_R = 12.297 min): m/z (%) = 390.7 (30) $[M+2H]^{2+}$, 780.3 (100) $[MH]^+$. – **HR-MS**: $[M+2H]^{2+}$ calcd. for $C_{34}H_{54}BrN_9O_7$ 390.6738, found 390.6738. – **MF:** $C_{34}H_{54}BrN_9O_7$. – **MW:** 780.75.

H-Arg-Lys-Pro-(αR,βS)-TAA-Leu-OH (2d):

Synthesized according to the GP. Yield: 1.9 mg, 10%; Purification by preparative HPLC. Gradient (t [min], % B): (0, 5), (20, 65), (22, 98), (32, 98), t_R = 10.573 min. – **HPLC** (analytical, S1): t_R = 11.831 min (DAD), Purity: 97%. – **LC/MS** (ESI, t_R = 11.969 min): m/z (%) = 390.7 (40) $[M+2H]^{2+}$, 780.3 (100) $[MH]^+$. – **HR-MS**: $[M+2H]^{2+}$ calcd. for $C_{34}H_{54}BrN_9O_7$ 390.6738, found 390.6740. – **MF:** $C_{34}H_{54}BrN_9O_7$. – **MW:** 780.75.

H-Lys-Arg-Pro-(αS,βR)-TAA-Leu-OH (2e):

Synthesized according to the GP. Yield: 5.4 mg, 27%; Purification by preparative HPLC. Gradient (t [min], % B): (0, 5), (20, 65), (22, 98), (32, 98), t_R = 10.883 min. – **HPLC** (analytical, S1): t_R = 12.118 min (DAD), Purity: 97%. – **LC/MS** (ESI, t_R = 12.302 min): m/z (%) = 390.7 (45) $[M+2H]^{2+}$, 780.3 (100) $[MH]^+$. – **HR-MS**: $[M+2H]^{2+}$ calcd. for $C_{34}H_{54}BrN_9O_7$ 390.6738, found 390.6739. – **MF:** $C_{34}H_{54}BrN_9O_7$. – **MW:** 780.75.

H-Lys-Arg-Pro-(αR,βS)-TAA-Leu-OH (2f):

Synthesized according to the GP. Yield: 3.6 mg, 18%; Purification by preparative HPLC. Gradient (t [min], % B): (0, 5), (20, 65), (22, 98), (32, 98), t_R = 10.561 min. – **HPLC** (analytical, S1): t_R = 11.863 min (DAD), Purity: 99%. – **LC/MS** (ESI, t_R = 12.009 min): m/z (%) = 390.7 (55) $[M+2H]^{2+}$, 780.3 (100) $[MH]^+$. – **HR-MS**: $[M+2H]^{2+}$ calcd. for $C_{34}H_{54}BrN_9O_7$ 390.6738, found 390.6747. – **MF:** $C_{34}H_{54}BrN_9O_7$. – **MW:** 780.75.

H-Lys-Lys-Pro-(αS,βR)-TAA-Leu-OH (2g):

Synthesized according to the GP. Yield: 2.7 mg, 14%; Purification by preparative HPLC. Gradient (t [min], % B): (0, 5), (20, 65), (22, 98), (32, 98), t_R = 10.615 min. – **HPLC** (analytical, S1): t_R = 11.951 min (DAD), Purity: 82%. – **LC/MS** (ESI, t_R = 12.103 min): m/z (%) = 376.7 (40) $[M+2H]^{2+}$, 752.3 (100)

$[MH]^+$. – **HR-MS**: $[M+2H]^{2+}$ calcd. for $C_{34}H_{54}BrN_7O_7$ 376.6707, found 376.6712. – **MF:** $C_{34}H_{54}BrN_7O_7$. – **MW:** 752.74.

H-Lys-Lys-Pro-(αR,βS)-TAA-Leu-OH (2h):

Synthesized according to the GP. Yield: 2.5 mg, 13%; Purification by preparative HPLC. Gradient (t [min], % B): (0, 5), (20, 65), (22, 98), (32, 98), t_R = 10.293 min. – **HPLC** (analytical, S1): t_R = 11.678 min (DAD), Purity: 99%. – **LC/MS** (ESI, t_R = 11.813 min): m/z (%) = 376.7 (50) $[M+2H]^{2+}$, 752.3 (100) $[MH]^+$. – **HR-MS**: $[M+2H]^{2+}$ calcd. for $C_{34}H_{54}BrN_7O_7$ 376.6707, found 376.6707. – **MF:** $C_{34}H_{54}BrN_7O_7$. – **MW:** 752.74.

H-Arg-Arg-Pro-(αS,βR)-TAA-Ile-Leu-OH (3a)/H-Arg-Arg-Pro-(αR,βS)-TAA-Ile-Leu-OH (3b) – (3ab):

Synthesized according to the GP. Yield: 11.1 mg, 24%; Purification by preparative HPLC. Gradient (t [min], % B): (0, 5), (10, 35), (13, 98), (23, 98), t_R = 8.940 min. – **HPLC** (analytical, S1): t_R = 12.841 min (DAD), Purity: 99%, Ratio **3a/3b**: n.d. – **LC/MS** (ESI, t_R = 12.967 min): m/z (%) = 461.2 (75) $[M+2H]^{2+}$, 921.4 (100) $[MH]^+$. – **HR-MS**: $[M+2H]^{2+}$ calcd. for $C_{40}H_{65}BrN_{12}O_8$ 461.2189, found 461.2192. – **MF:** $C_{40}H_{65}BrN_{12}O_8$. – **MW:** 921.92.

H-Arg-Lys-Pro-(αS,βR)-TAA-Ile-Leu-OH (3c)/H-Arg-Lys-Pro-(αR,βS)-TAA-Ile-Leu-OH (3d) – (3cd):

Synthesized according to the GP. Yield: 13.8 mg, 31%; Purification by preparative HPLC. Gradient (t [min], % B): (0, 5), (22, 71), (24, 98), (34, 98), t_R = 8.897 min. – **HPLC** (analytical, S1): t_R = 12.814 min (**3c**) / 12.714 min (**3d**) (DAD), Purity: 97%, Ratio **3c/3d**: 72/25. – **LC/MS** (ESI, t_R = 12.948 min / 12.830 min): m/z (%) = 447.2 (80) $[M+2H]^{2+}$, 893.4 (100) $[MH]^+$. – **HR-MS**: $[M+2H]^{2+}$ calcd. for $C_{40}H_{65}BrN_{10}O_8$ 447.2158, found 447.2164. – **MF:** $C_{40}H_{65}BrN_{10}O_8$. – **MW:** 893.90.

H-Lys-Arg-Pro-(αS,βR)-TAA-Ile-Leu-OH (3e)/H-Lys-Arg-Pro-(αR,βS)-TAA-Ile-Leu-OH (3f) – (3ef):

Synthesized according to the GP. Yield: 18.5 mg, 41%; Purification by preparative HPLC. Gradient (t [min], % B): (0, 5), (10, 35), (13, 98), (23, 98), t_R = 8.978 min. – **HPLC** (analytical, S1): t_R = 12.703 min (DAD), Purity: 99%, Ratio **3e/3f**: n.d. – **LC/MS** (ESI, t_R = 12.830 min): m/z (%) = 447.3 (50) $[M+2H]^{2+}$, 893.4 (100) $[MH]^+$. – **HR-MS:** $[M+2H]^{2+}$ calcd. for $C_{40}H_{65}BrN_{10}O_8$ 447.2158, found 447.2161. – **MF:** $C_{40}H_{65}BrN_{10}O_8$. – **MW:** 893.90.

H-Lys-Lys-Pro-(αS,βR)-TAA-Ile-Leu-OH (3g)/H-Lys-Lys-Pro-(αR,βS)-TAA-Ile-Leu-OH (3h) – (3gh):

Synthesized according to the GP. Yield: 8.2 mg, 19%; Purification by preparative HPLC. Gradient (t [min], % B): (0, 5), (22, 71), (24, 98), (34, 98), t_R = 8.660 min. – **HPLC** (analytical, S1): t_R = 12.614 min (**3g**) / 12.466 min (**3h**) (DAD), Purity: 97%, Ratio **3g/3h**: 66/31. – **LC/MS** (ESI, t_R = 12.765 min / 12.602 min): m/z (%) = 433.3 (55) $[M+2H]^{2+}$, 865.4 (100) $[MH]^+$. – **HR-MS**: $[M+2H]^{2+}$ calcd. for $C_{40}H_{65}BrN_8O_8$ 433.2127, found 433.2133. – **MF:** $C_{40}H_{65}BrN_8O_8$. – **MW:** 865.90.

H-Lys-Lys-Pro-(S)-Phe(4-Br)-Ile-Leu-OH (4a):

Synthesized according to the GP. Yield: 36.0 mg, 87%; Purification by automated flash column chromatography (12g KP-C18-HS). Solvents: MeCN (B) / H_2O (0.05% TFA (v,v)) (A); Flow rate: 12.0

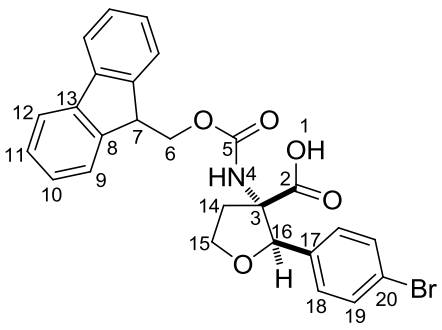
mL/min. Gradient: linear from 3% B to 97% B within 35 min; UV detection at 220 nm. – **HPLC** (analytical, S2): t_R = 12.513 min (DAD), t_R = 12.600 min (ELSD), Purity: 92%. – **LC/MS** (ESI, t_R = 1217-1.289 min): m/z (%) = 275.8 (30) $[M+3H]^{3+}$, 413.2 (100) $[M+2H]^{2+}$, 825.4 (5) $[MH]^+$. – **HR-MS:** $[M+2H]^{2+}$ calcd. for $C_{38}H_{63}BrN_8O_7$ 412.2074, found 412.2080. – **MF:** $C_{38}H_{63}BrN_8O_7$. – **MW:** 823.86.

H-Lys-Lys-Pro-(R)-Phe(4-Br)-Ile-Leu-OH (4b):

Synthesized according to the GP. Yield: 21.1 mg, 51%; Purification by automated flash column chromatography (12g KP-C18-HS). Solvents: MeCN (B) / H_2O (0.05% TFA (v,v)) (A); Flow rate: 12.0 mL/min. Gradient: linear from 3% B to 97% B within 35 min; UV detection at 220 nm. – **HPLC** (analytical, S2): t_R = 13.371 min (DAD), t_R = 13.464 min (ELSD) Purity: 86%. – **LC/MS** (ESI, t_R = 1.382-1.449 min): m/z (%) = 275.8 (60) $[M+3H]^{3+}$, 413.2 (100) $[M+2H]^{2+}$, 825.4 (5) $[MH]^+$. – **HR-MS:** $[M+2H]^{2+}$ calcd. for $C_{38}H_{63}BrN_8O_7$ 412.2074, found 412.2083. – **MF:** $C_{38}H_{63}BrN_8O_7$. – **MW:** 823.86.

3-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-2-(4-bromophenyl)tetrahydrofuran-3-carboxylic acid (5):

HCl*H-TAA-OH **1** (4.93 g, 15.3 mmol) was dissolved in a mixture of 40 mL aqueous Na_2CO_3 solution (10% w/w) and 20 mL of dioxane and cooled to 0 °C in an ice bath. Then a solution of Fmoc-Cl (3.96 g, 15.3 mmol, 1 eq.) in 30 mL of dioxane was added drop wise over 30 min. The reaction mixture was stirred for 2 h at RT. Then the reaction mixture was poured on 400 mL of water and extracted twice with 80 mL of Et_2O. The aqueous layer was cooled again to 0 °C and acidified with concentrated HCl to congo red paper. The white precipitate was extracted three times with 100 mL of EtOAc, the combined extracts were washed with water (50 mL), brine (50 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to receive the crude product as viscous yellow oil. The purification was done by automated flash column chromatography on silica gel applying a linear gradient of 22 % EtOAc / PE to 66% EtOAc / PE within 60 min and a flow rate of 50 mL min^{-1}. The product was obtained as colorless solid with a yield of 51% (3.96 g, 7.8 mmol).

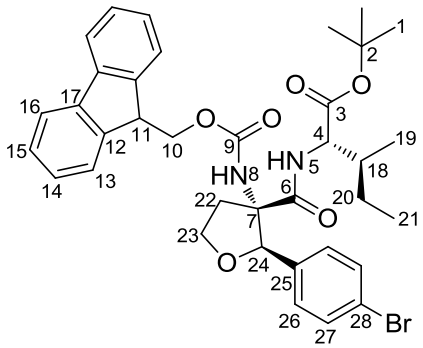


R_f (EtOAc/PE = 3:1) = 0.18. – **Mp:** 166.1 °C. – **^{1}H-NMR** (600 MHz, COSY, DMSO-d^6): δ = 2.21 (dd, $^3J_{H,H}$ = 4.4 Hz, $^3J_{H,H}$ = 12.0 Hz, 1H, 14a), 2.70 (dd, $^3J_{H,H}$ = 9.8 Hz, $^3J_{H,H}$ = 21.2 Hz, 1H, 14b), 3.80 (dd, $^3J_{H,H}$ = 8.1 Hz, $^3J_{H,H}$ = 15.9 Hz, 1H, 15a), 4.23 (pt, $^3J_{H,H}$ = 6.6Hz, 2H, 15b, 7), 4.33 (dt, $^3J_{H,H}$ = 10.3 Hz, $^3J_{H,H}$ = 16.9 Hz, 2H, 6), 5.00 (bs, 1H, 16), 7.25 (d, $^3J_{H,H}$ = 8.4 Hz, 2H, 18), 7.33 (q, $^3J_{H,H}$ = 7.2 Hz, 2H, 11), 7.41 (dd, $^3J_{H,H}$ = 7.2 Hz, $^3J_{H,H}$ = 11.7 Hz, 2H, 10), 7.48 (d, $^3J_{H,H}$ = 8.4 Hz, 2H, 19), 7.72 (dd, $^3J_{H,H}$ = 7.4 Hz, $^3J_{H,H}$ = 15.4 Hz, 2H, 9), 7.89 (d, $^3J_{H,H}$ = 7.1 Hz, 2H, 12), 8.07 (bs, 1H, 4), 12.29 (bs, 1H, 1) – **^{13}C-NMR** (150 MHz, HSQC, HMBC, DMSO-d^6): δ = 35.9 (-, 1C, 14), 47.2 (+, 1C, 7), 65.9 (-, 1C, 6), 67.8 (-, 1C, 15), 70.8 ($C_{quat.}$, 1C, 3), 85.4 (+, 1C, 16), 120.1 ($C_{quat.}$, 1C, 17), 121.1 (+, 2C, 12), 125.3

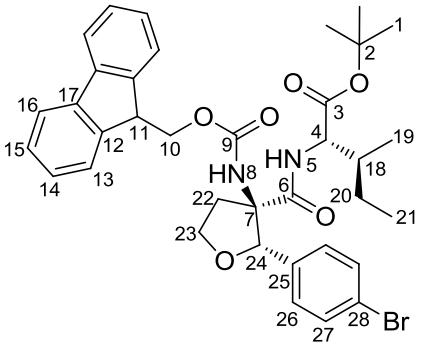
(+, 2C, 9), 127.1 (+, 2C, 11), 127.6 (+, 2C, 10), 129.0 (+, 2C, 18), 130.5 (+, 2C, 19), 137.9 ($C_{quat.}$, 1C, 20), 141.2 ($C_{quat.}$, 2C, 8), 144.1 ($C_{quat.}$, 1C, 13), 144.4 ($C_{quat.}$, 1C, 3), 155.9 ($C_{quat.}$, 1C, 5), 171.8 ($C_{quat.}$, 1C, 2) – **MS** (ESI, CH_2Cl_2/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 508.0 (30) $[MH]^+$, 549.0 (100) $[MH+MeCN]^+$, 1017.2 (25) $[2MH]^+$, 1034.3 (25) $[2MNH_4]^+$ – **HR-MS** (PI-LSIMS, MeOH/CH_2Cl_2/NBA): calcd. for $C_{26}H_{22}BrNO_5$ 508.0754, 510.0738, found: 508.0755, 510.0736. – **IR** (neat) [cm^{-1}]: $\tilde{\nu}$ = 3404, 3281, 3073, 2963, 2874, 2619, 1711, 1256, 1076, 1010, 736, 572, 528. – **MF:** $C_{26}H_{22}BrNO_5$. – **MW:** 508.36.

(2*S*,3*S*)-tert-Butyl-2-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-(4-bromophenyl)tetrahydrofuran-3-carboxamido)-3-methylpentanoate (6):

Under an atmosphere of nitrogen Fmoc-TAA-OH **5** (500 mg, 0.987 mmol) was dissolved in 8 mL of dry DCM (8 mL/mmol) and cooled to 0 °C in an ice bath. To this solution DIPEA (421 µL, 2.459 mmol, 2.5 eq.), HOBt (186 mg, 1.377 mmol, 1.4 eq.) and EDC*HCl (225 mg, 1.182 mmol, 1.2 eq) were added in this order. Afterwards the hydrochlorid salt of isoleucine tert-butyl ester (264 mg, 1.180 mmol, 1.2 eq.) was slowly added in several portions. After the addition was completed, the mixture was allowed to reach room temperature and stirred for 24 hours. The reaction was quenched with 5 mL of water and acidified with 10 mL of 1M $KHSO_4$ solution and extracted three times with 15 mL of DCM. The combined organic layers were washed with brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure. The purification was done by automated flash column chromatography on silica gel applying a linear gradient of 25 % Et_2O / n-pentane to 60% Et_2O / n-pentane within 45 min and a flow rate of 25 mL min^{-1}. The product was obtained with a moderate yield of 41% (137 mg, 0.20 mmol) for **6b** respectively of 34% (115 mg, 0.17 mmol) for **6a**.



6a: colorless to slightly yellow solid – $\mathbf{R_f}$ (Et_2O/n-pentane = 1:1) = 0.24. –**Mp**: 134.7 °C. –**^{1}H-NMR** (600 MHz, COSY, $CDCl_3$): δ = 0.81 (d, $^3J_{H,H}$ = 6.6 Hz, 3H, 19), 0.86-0.95 (m, 4 H, 21+20a), 1.10-1.17 (m, 1H, 20b), 1.38-1.42 (m, 1H, 18), 1.46 (s, 9H, 1), 2.37-2.48 (m, 1H, 22a), 2.92-3.00 (m, 1H, 22b), 3.97-4.03 (m, 1H, 4), 4.23-4.27 (m, 1H, 11), 4.31-4.38 (m, 2H, 23), 4.42-4.46 (m, 1H, 10), 4.53-4.58 (m, 1H, 10), 5.37 (bs, 1H, 24), 6.57 (s, 1H, 8), 6.82 (d, $^3J_{H,H}$ = 6.4 Hz, 1H, 5), 7.21 (d, $^3J_{H,H}$ = 7.9 Hz, 2H, 26), 7.31 (dd, $^3J_{H,H}$ = 6.8 Hz, $^3J_{H,H}$ = 13.8 Hz, 2H, 14), 7.38-7.42 (m, 4H, 15+27), 7.62 (d, $^3J_{H,H}$ = 7.4 Hz, 2H, 13), 7.78 (t, $^3J_{H,H}$ = 6.3 Hz, 2H, 16). – **^{13}C-NMR** (150 MHz, HSQC, HMBC, $CDCl_3$): δ = 11.4 (+, 1C, 21), 14.9 (+, 1C, 19), 24.6 (-, 1C, 20), 28.1 (+, 1C, 1), 36.4 (-, 1C, 22), 37.9 (+, 1C, 18), 47.4 (+, 1C, 11), 57.4 (+, 1C, 4), 66.4 (-, 1C, 23), 66.5 (-, 1C, 10), 67.2 ($C_{quat.}$, 1C, 7), 79.1 (+, 1C, 24), 82.3 ($C_{quat.}$, 1C, 2), 120.1 (+, 1C, 16), 121.4 ($C_{quat.}$, 1C, 28), 125.3 (+, 1C, 13), 126.8 (+, 1C, 26), 127.2 (+, 1C, 14), 127.7 (+, 1C, 15), 131.4 (+, 1C, 27), 136.3 ($C_{quat.}$, 1C, 25), 141.3 ($C_{quat.}$, 1C, 17), 143.6 ($C_{quat.}$, 1C, 12), 154.4 ($C_{quat.}$, 1C, 9), 170.5 ($C_{quat.}$, 1C, 3), 171.0 ($C_{quat.}$, 1C, 6). – **MS** (ESI, CH_2Cl_2/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 677.3 (100) $[MH]^+$, 696.4 (40) $[MNH_4]^+$. – **MF:** $C_{36}H_{41}BrN_2O_6$. – **MW:** 677.62.



6b: colorless viscous oil – $\mathbf{R_f}$ (Et_2O/n-pentane = 1:1) = 0.31 – **^{1}H-NMR** (600 MHz, COSY, $CDCl_3$): δ = 0.41 (d, $^3J_{H,H}$ = 6.7 Hz, 3H, 19), 0.76 (t, $^3J_{H,H}$ = 6.8 Hz, 3H, 21), 0.78- 0.82 (m, 1H, 20a), 1.04-1.09 (m, 1H, 20b), 1.36-1.40 (m, 1H, 18), 1.44 (s, 9H, 1), 2.51 (dd, $^3J_{H,H}$ = 8.6 Hz, $^3J_{H,H}$ = 21.3 Hz, 1H, 22a), 2.86-2.91 (m, 1H, 22b), 4.17 (dd, $^3J_{H,H}$ = 3.6 Hz, $^3J_{H,H}$ = 8.0 Hz, 1H, 4), 4.24 (t, $^3J_{H,H}$ = 6.7 Hz, 1H, 11), 4.31-4.38 (m, 2H, 23), 4.39-4.44 (m, 1H, 10), 4.49-4.55 (m, 1H, 10), 5.49 (bs, 1H, 24), 6.43 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 5), 6.66 (s, 1H, 8), 7.21 (d, $^3J_{H,H}$ = 7.9 Hz, 2H, 26), 7.31 (dd, $^3J_{H,H}$ = 6.8 Hz, $^3J_{H,H}$ = 13.8 Hz, 2H, 14), 7.38-7.42 (m, 4H, 15+27), 7.62 (d, $^3J_{H,H}$ = 7.4 Hz, 2H, 13), 7.78 (t, $^3J_{H,H}$ = 6.3 Hz, 2H, 16). – **^{13}C-NMR** (150 MHz, HSQC, HMBC, $CDCl_3$): δ = 11.5 (+, 1C, 21), 14.8 (+, 1C, 19), 24.8 (-, 1C, 20), 28.0 (+, 1C, 1), 36.4 (-, 1C, 22), 37.8 (+, 1C, 18), 47.3 (+, 1C, 11), 57.3 (+, 1C, 4), 66.3 (-, 1C, 23), 66.4 (-, 1C, 10), 67.0 ($C_{quat.}$, 1C, 7), 79.2 (+, 1C, 24), 82.2 ($C_{quat.}$, 1C, 2), 120.0 (+, 1C, 16), 121.4 ($C_{quat.}$, 1C, 28), 125.0 (+, 1C, 13), 126.7 (+, 1C, 26), 127.1 (+, 1C, 14), 127.8 (+, 1C, 15), 131.3 (+, 1C, 27), 136.2 ($C_{quat.}$, 1C, 25), 141.4 ($C_{quat.}$, 1C, 17), 143.6 ($C_{quat.}$, 1C, 12), 154.4 ($C_{quat.}$, 1C, 9), 170.4 ($C_{quat.}$, 1C, 3), 171.0 ($C_{quat.}$, 1C, 6). – **MS** (ESI, CH_2Cl_2/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 677.3 (100) $[MH]^+$, 696.4 (40) $[MNH_4]^+$. – **MF:** $C_{36}H_{41}BrN_2O_6$. – **MW:** 677.62.

3.4.3 Biological investigations

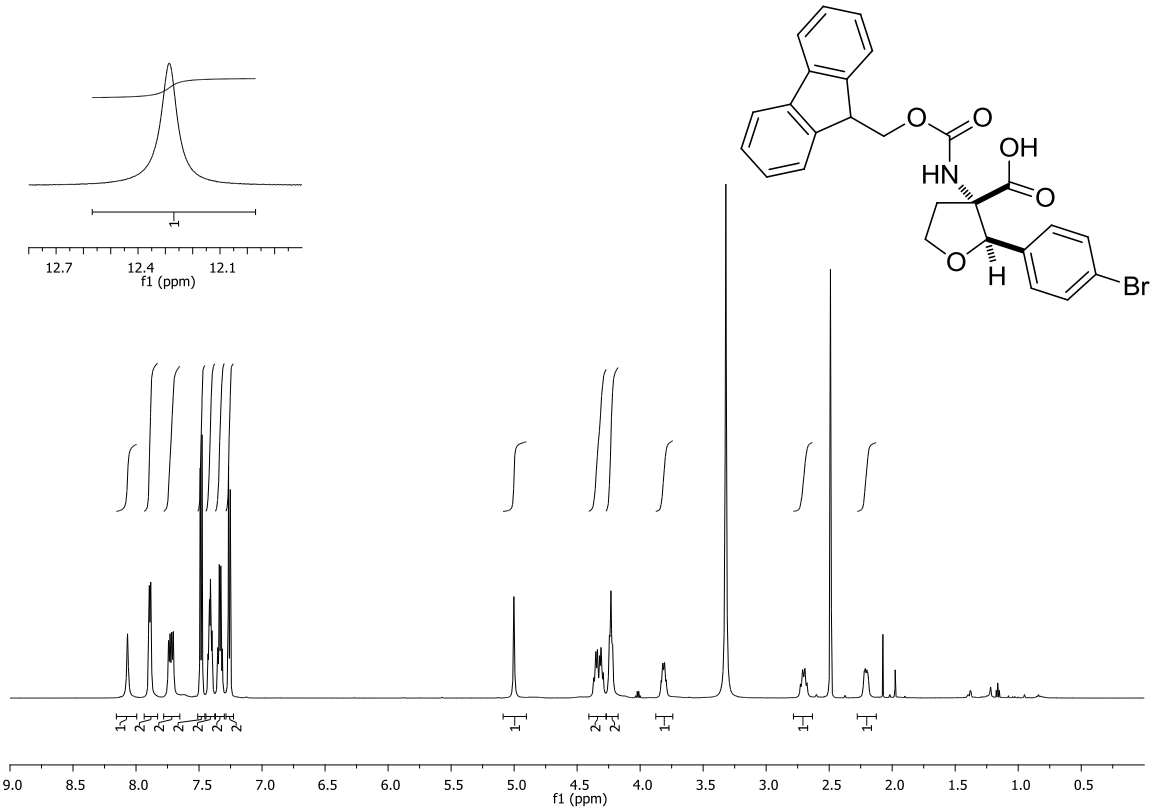
Receptor binding experiments. Receptor binding data were determined according to protocols as described previously.[67,68] In detail, NTS1 binding was measured using homogenates of membranes from CHO cells stably expressing human NTS1 at a final concentration of 1-2 µg per well, and the radioligand [^{3}H]neutrotensin (specific activity: 116 Ci mmol^{-1}; PerkinElmer, Rodgau, Germany) at a concentration of 0.50 nM. Specific binding of the radioligand was determined at K_D values of 0.37-0.96 nM and a B_{max} of 6170-9300 fmol(mg protein)$^{-1}$. Nonspecific binding was determined in the presence of 10 µM neurotensin. NTS2 binding assays were carried out by the calcium phosphate method, using homogenates of membranes from HEK293 cells, which were transiently transfected with the pcDNA3.1 vector containing the human NTS2 gene (Missouri S&T cDNA Resource Center (UMR), Rolla, MO, USA).[69] Membranes were incubated at a final concentration of 6-20 µg per well together with 0.50 nM [^{3}H]NT(8-13) (specific activity: 136 Ci mmol^{-1}); custom synthesis of [leucine-^{3}H]NT(8-13) by GE Healthcare, Freiburg, Germany) at K_D values in the range of 0.67-2.02 nM and a B_{max} value of 310-930 fmol(mg protein)$^{-1}$. Nonspecific binding was determined in the presence of 10µM NT(8-13), and the protein concentration was generally determined by the method of Lowry using bovine serum albumin as standard.[70]

Data analysis. Data analysis of the competition curves from the radioligand binding experiments was accomplished by nonlinear regression analysis using the algorithms in Prism 5.0 (GraphPad Software,

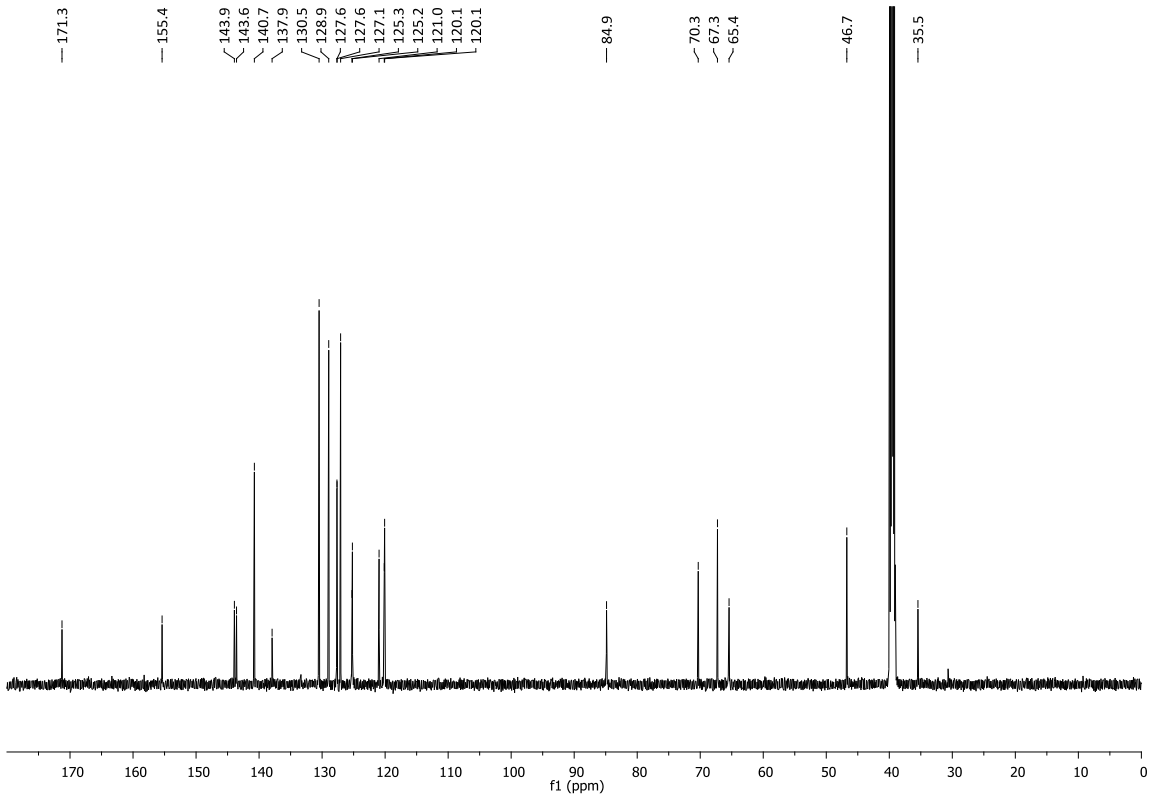
San Diego, CA, USA). EC_{50} values derived from the resulting dose-response curves were transformed into the corresponding K_i values using the equation of Cheng and Prusoff.[71]

3.4.4 Supporting Information – Copies of selected NMR – spectra

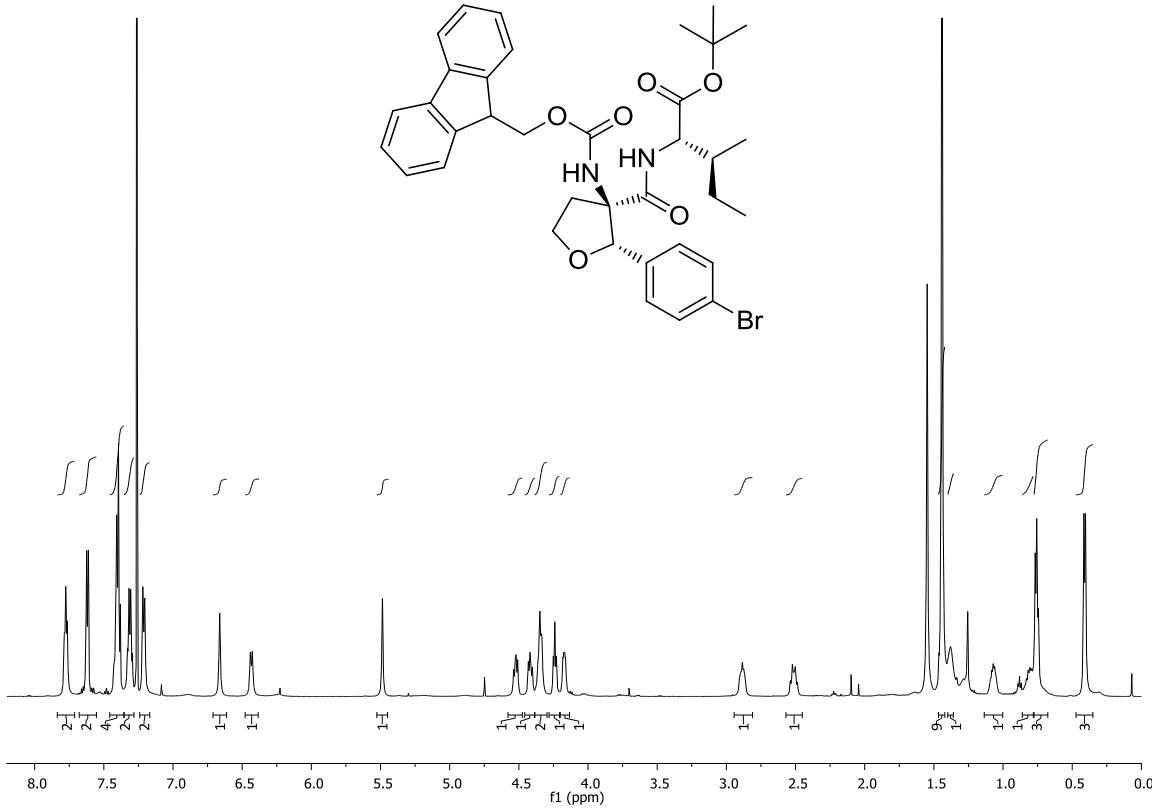
^{1}H-NMR spectra of compound **5** (600 MHz, DMSO-d^{6}):



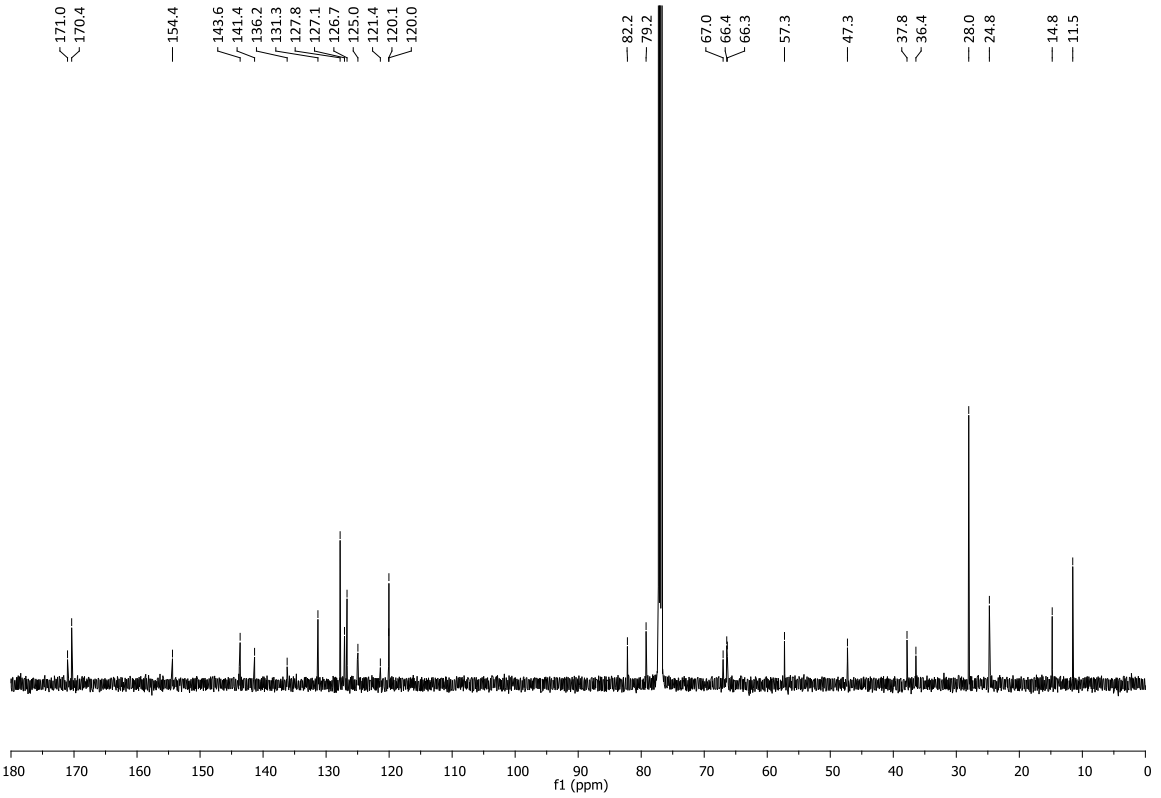
^{13}C-NMR spectra of compound **5** (150 MHz, DMSO-d^{6}):



^{1}H-NMR spectra of compound **6b** (600 MHz, $CDCl_3$):



^{13}C-NMR spectra of compound **6b** (150 MHz, $CDCl_3$):



3.5 References

1. The graphic on page 1 illustrates the most stable conformer (E = -826.0622 KJ/mol) of the peptide H-Arg-Arg-Pro-(αS,βR)-TAA-Ile-Leu-OH **3a** obtained by calculation with Spartan '06 v.112 performing a conformer distribution analysis with Molecular Mechanics MMFF94.
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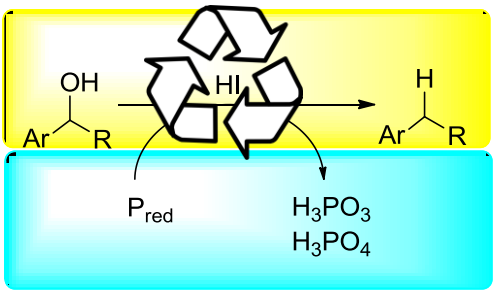
4 Reduction of benzylic alcohols and α-hydroxycarbonyl compounds by hydriodic acid in a biphasic reaction medium

The synthetic protocol for the reduction of alcohols to hydrocarbons by using hydriodic acid, first described by Kiliani more than 140 years ago, was improved to be more applicable to organic synthesis. Instead of a strongly acidic, aqueous solution, a biphasic toluene–water reaction medium was used, which allowed the conversion of primary, secondary and tertiary benzylic alcohols, in good yields and short reaction times, into the corresponding hydrocarbons. Red phosphorous was used as the stoichiometric reducing agent. Keto, ester, amide or ether groups are tolerated, and catalytic amounts of hydriodic acid (0.1 eq.) in the presence of 0.7 eq. phosphorous are sufficient to achieve conversion.



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4.1 Introduction

The reduction of hydroxy groups is a typical and important step in the synthesis of complex natural products or drugs.[1-4] Functional-group tolerance during this reduction step is essential since various other groups are usually present. A number of synthetic procedures have been developed, which allow selective reduction, but only a few one-step transformations are known, which use either titanium-(III)[5-8] or different metal complexes.[9-13] Most procedures require a sequence of steps, e.g., the conversion of hydroxy groups into a chloride or bromide substituent and subsequent catalytic reduction with H_2/Pt or the conversion into a tosylate and reduction with $LiAlH_4$. The most commonly applied method is the Barton–McCombie reaction,[14] due to its versatility and its very high functional group tolerance.[15-18] Although very general, the reaction has some drawbacks: The involved organotin hydrides are costly, highly toxic[19-21] and often difficult to separate from the reaction products. Furthermore, secondary alcohols give best results, while others may react less efficiently.

We have reinvestigated the long-known reduction of benzylic alcohols and α-hydroxycarbonyl compounds by hydriodic acid.[22-32] First described by Kiliani more than 140 years ago for the reduction of gluconic acid to hydrocarbons,[33,34] the method has been reported for a variety of alcohols, but typically proceeds in aqueous solution and requires an excess of HI or strong mineral acids such as phosphoric or sulfuric acid.[35-37]

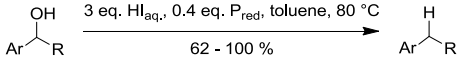
We describe a biphasic reaction medium consisting of toluene and aqueous hydriodic acid. The phase separation allows milder reaction conditions compared to the classic Kiliani protocol and is more applicable to organic synthesis.

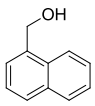
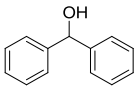
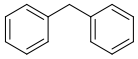
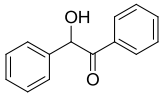
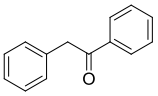
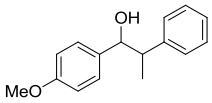
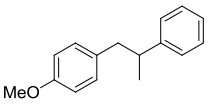
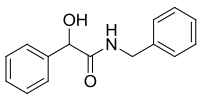
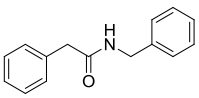
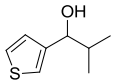
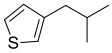
4.2 Results and Discussion

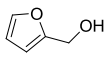
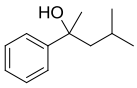
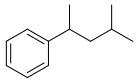
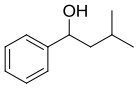
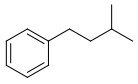
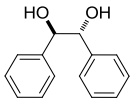
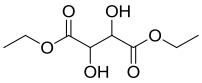
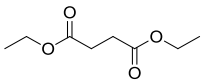
4.2.1 Deoxygenation of benzylic alcohols and α-hydroxycarbonyl compounds

Initial investigations focused on simple benzylic alcohols (Table 1, entries 1–3), which were converted in high to quantitative yields into the corresponding alkanes. Carbonyl groups or amides in a benzylic position (Table 1, entries 4 and 6) and aromatic hydroxy groups (Table 2, entry 7) or aromatic ethers (Table 1, entry 5) were not affected. Moreover, heterocycles such as thiophene (Table 1, entry 7) were stable under these conditions whereas furans (Table 1, entry 8) were decomposed due to ring opening. Benzylic alcohols were converted in good to high yields to alkanes with increasing reactivity in the order primary (2 h) < secondary (0.5–1 h) < tertiary alcohol (15–30 min); carbonyl groups and ethers were tolerated. Diethyl tartrate was converted into diethyl succinate under the reaction conditions given (Table 1, entry 12), but some of the material was lost due to ester hydrolysis.

Table 2. Reduction of benzylic alcohols and α-hydroxycarbonyl compounds to the corresponding alkanes.



Entry	Alcohol	Product[a]	Time [h]	Yield [%]
1*			2	70[b]
2*			0.5	96
3*			0.25	100
4*			1	80
5			0.5	92
6*			1	82
7			0.5	62[c]

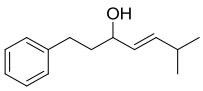
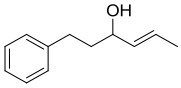
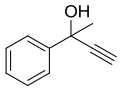
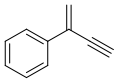
8*		decomposition	1	–
9			0.25	74[c]
10			0.5	49[c]
11*			0.5	78
12*			1.5	65

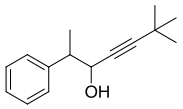
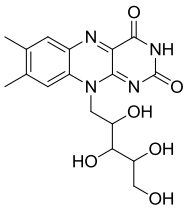
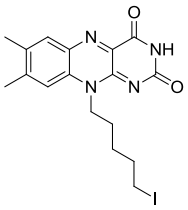
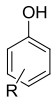
[a] All products are known compounds described in the literature. The identities have been proven by proton NMR and mass analysis, which match the literature data. [b] The corresponding iodo compound was identified as a byproduct. [c] The corresponding elimination product was obtained as a byproduct.

4.2.2 Deoxygenation of allylic and propargylic alcohols

Allylic alcohols are completely consumed, but the corresponding alkenes could not be isolated as pure products (Table 2). Mixtures of eliminiation and deoxygenation products, in some cases also rearangement of the deoxygenated product into the more highly substitued, thermodynamically more stable alkene occurred. Propargylic alcohols (Table 2, entry 3 and 4) showed elimination or decomposed. In the case of flavin (Table 2, entry 6), three hydroxy groups were reduced and one was converted into an iodo substituent.

Table 2. Alcohols showing incomplete or unselective reaction with hydriodic acid and red phosphorous.[a]

Entry	Alcohol	Product	Time [h]	Yield [%]
1		mixtures of several products	1	–
2		mixtures of several products	1	–
3*			1	traces

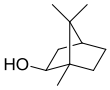
4		decomposition	1	–
5*		decomposition	1	–
6*			2	21
7*		no conversion	1	–

[a] 3.0 eq. $HI_{aq.}$, 0.4 eq. P_{red}.

4.2.3 Conversion of aliphatic alcohols without π-system in α-position

Alcohols other than those that were benzylic or α to carbonyl groups were not converted into the corresponding alkanes, and the reaction stopped at the iodoalkanes (Table 3). The reactivity follows the order of primary < secondary < tertiary alcohols, as expected for an S_N1 reaction. The reduction potential of the nonbenzylic iodoalkanes is not sufficient for reduction by hydriodic acid.

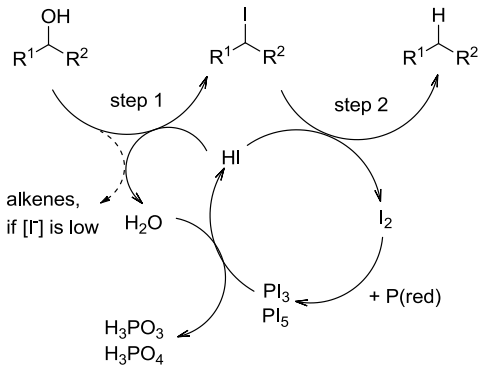
Table 3. Alcohols yielding alkyl iodides with hydriodic acid and red phosphorous.[a]

Entry	Alcohol	Product	Time [h]	Yield [%]
1*			8	98
2*			8	83[b]
3			20	81[c]

[a] 3.0 eq. $HI_{aq.}$, 0.4 eq. P_{red}. [b] Single isomer. [c] Products were analyzed by gas chromatography; chlorobenzene was used as an internal standard.

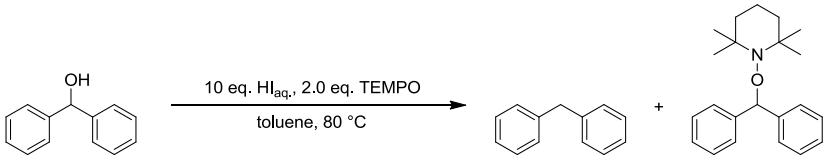
4.2.4 Mechanism of the deoxygenation with hydriodic acid

The mechanism of reduction by hydriodic acid consists of two steps (Scheme 1): The nucleophilic substitution of the hydroxy group by iodide and the subsequent reduction of the alkyl iodide by hydriodic acid. The iodine, generated in the second step, is recycled by reduction with red phosphorous regenerating hydriodic acid.



Scheme 4. Mechanism of the alcohol reduction and recycling of iodine.

The mechanistic details of the redox comproportionation of alkyl iodides and H–I have been strongly debated in the literature.[38-40] However, the required benzylic or α-carbonyl position for the redox comproportionation indicates an intermediate with mesomeric stabilization due to the adjacent π-system. In a trapping experiment, using HI without phosphorous, diphenylcarbinol as the substrate and TEMPO as a trapping agent for radical intermediates, the TEMPO adduct of diphenylcarbinol was detected by mass analysis (Scheme 2).

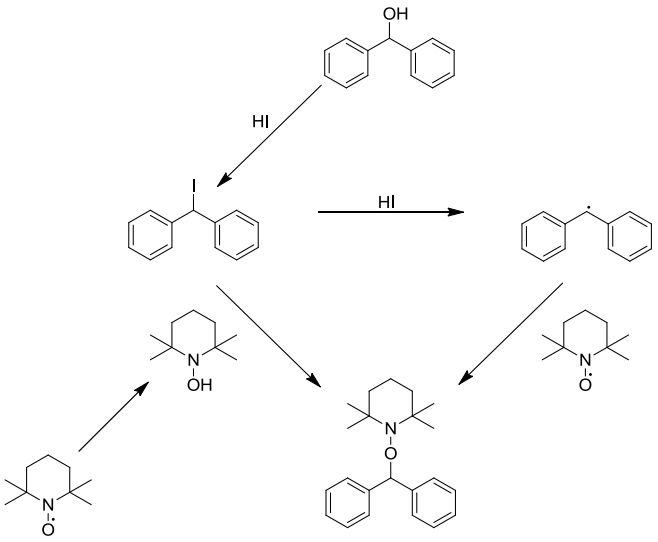


Scheme 2. Radical capture experiment with diphenylcarbinol and TEMPO.

This indicates a radical mechanism of the redox comproportionation. We suggest a stepwise reduction by single electron transfer (SET) accompanied by the oxidation of I^- to I_2. The iodine, generated in the second step, is recycled by reduction with red phosphorous, regenerating hydriodic acid. Admittedly, the above-mentioned TEMPO adduct could also be generated by nucleophilic substitution of the alkyl iodide with reduced TEMPO. At least this would be another proof for the first reaction step (Scheme 3).

Moreover the carbenium ion intermediate of the nucleophilic substitution (S_N1) in the first reaction step, gives a logical explanation for the reaction of aromatic hydroxy groups with HI (Table 2 entry 7).

In the case of a benzylic substrate, the positive charge is located in a p-orbital and can be stabilized by the mesomeric effect of the adjacent π-system. In contrast the positive charge of the carbenium ion intermediate of an aromatic substrate is located in the orthogonal sp^2-hybrid orbital and cannot benefit from the π-system. Therefore aromatic hydroxy groups do not react with hydriodic acid.



Scheme 3. Possible reaction pathways for the generation of the TEMPO adduct.

4.2.5 Deoxygenation with catalytic amounts of hydriodic acid

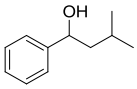
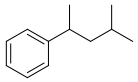
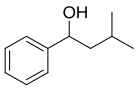
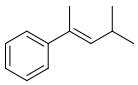
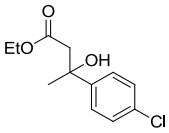
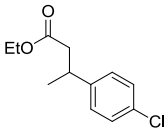
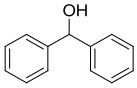
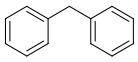
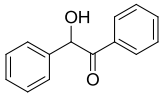
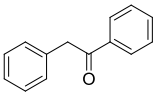
According to the redox equations of the reaction between iodine and red phosphorous, each mole of red phosphorous is able to reduce at least 1.5 mol of iodine:

$$3\ I_2 + 2\ P + 6\ H_2O \rightarrow 6\ HI + 2\ H_3PO_3$$

$$5\ I_2 + 2\ P + 8\ H_2O \rightarrow 10\ HI + 2\ H_3PO_4$$

Catalytic amounts of hydriodic acid are therefore sufficient[28] for the reduction of the hydroxy group (Table 4), when excess red phosphorous is added as a terminal reducing agent (Table 4, entry 1 and 3-6). However, depending on the amount of added hydriodic acid, the elimination of water may occur as an alternative reaction pathway (Table 4, entry 2). A low concentration of HI favors the elimination of water, while higher HI concentrations lead to substitution and reduction products (Table 4, entry 1). But for substrates without a methyl, methylene of methane group in α-position (Table 4, entry 4-6), even low concentration of HI were sufficient for deoxygenation. In these cases dehydration could not take place as alternative reaction pathway.

Table 4. Reduction of alcohols with catalytic amounts of hydriodic acid.

Entry	Alcohol	Product[a]	Time [h]	Yield [%]
1			0.25	74[a]
2			0.25	67[b]
3			0.5	82[a]
4*			0.5	92[b]
5*			0.25	98[b]
6*			0.5	74[b]

[a] 0.6 eq. $HI_{aq.}$, 0.4 eq. P_{red}. [b] 0.1 eq. $HI_{aq.}$, 0.7 eq. P_{red}.

4.3 Conclusion

Toluene and aqueous hydriodic acid are a suitable biphasic reaction mixture for the reduction of a range of benzylic alcohols. The two-phase system makes the Kiliani protocol more easily applicable to organic synthesis, as organic substrates and products dissolve in the organic phase and are separated from the mineral acids. The procedure allows the use of catalytic amounts of hydriodic acid and red phosphorous as the terminal reductant. In the case of alcohols having no activation by adjacent benzylic or carbonyl groups the reaction stops at the corresponding alkyl iodide. A quantitative mass-efficiency analysis[41] of the reaction in comparison to tosylation/$LiAlH_4$, Ti(III)-mediated and Barton–McCombie reduction revealed a better atom economy and mass efficiency.

4.4 Experimental Section

4.4.1 General

All reagents and solvents used were of analytical grade, purchased from commercial sources and used without further purification. Unless stated otherwise, purification and drying of the solvents used was performed according to accepted general procedures. All reactions were performed under an inert atmosphere of N_2 by using standard Schlenk techniques, if not otherwise stated. TLC analyses were performed on silica-gel-coated alumina plates (F^{254} silica gel, layer thickness 0.2 mm). Visualization was achieved by UV light at 254 nm/366 nm or through staining with ninhydrin or vanillin solutions. For preparative column chromatography, silica gels (70–230 mesh and 230–400 mesh) were used. For chromatography commercially available solvents of standard quality were used without further purification.

4.4.2 Syntheses

Representative experimental procedure:
The alcohol (1 mmol, 1 eq.) is dissolved in 4 mL of toluene. Red phosphorus (0.4 mmol, 0.4 eq.), followed by concentrated hydriodic acid (57% w/w, 3.0 mmol, 3 eq.) is added and the reaction mixture is heated to 80 °C for the stated time, allowed to cool to RT and quenched with $Na_2S_2O_3$ (10 mL; 10% w/w) solution. The aqueous phase is extracted with dichloromethane (3 × 10 mL), the combined organic phases are dried over $MgSO_4$ and filtered, and the solvent is removed. The crude product is purified by chromatography and spectroscopically characterized.

For catalytic reactions of 1 mmol of the respective alcohol the following amounts of hydriodic acid and P_{red} were used: (a) 0.6 mmol $HI_{aq.}$/0.4 mmol P_{red}, (b) 0.1 mmol $HI_{aq.}$/0.7 mmol P_{red}.

1-(4-Methoxyphenyl)-2-phenylpropan-1-ol (Table 1, entry 5):
The reaction was carried out under a dry nitrogen atmosphere by using standard Schlenk techniques. 1 mL of a solution of 4-bromo-1-methoxybenzene (0.62 mL, 5.0 mmol) in dry THF (10 mL) was added to Mg powder (0.12 g, 5.0 mmol). The Grignard reaction was initiated by the addition of iodine followed by sonication for several minutes. When the exothermic reaction started the rest of the 4-bromo-1-methoxybenzene solution was added through a septum by syringe over 15 min. After the addition, the reaction solution was heated under reflux for 1 h to complete the reaction. The reaction solution was allowed to cool to RT before 2-phenylpropionaldehyde (0.60 mL, 4.5 mmol) was added dropwise. To complete the reaction the solution was again heated under reflux for 2 h. The reaction

was quenched by the addition of HCl (2 M, 5 mL). The aqueous phase was extracted with diethyl ether (2 × 5 mL). The combined organic phases were washed with saturated $NaHCO_3$ (3 mL), H_2O (2 × 2.5 mL) and dried with $MgSO_4$. The solvent was removed with a rotary evaporator. The crude product was purified by flash chromatography (petroleum ether/ethyl acetate 4:1, staining with vanillin solution gave a blue spot). 1-(4-Methoxyphenyl)-2-phenylpropan-1-ol was isolated as yellow oil in 57% yield (0.62 g, 2.6 mmol). Analytical data were identical with literature.[42]

R_f (PE/EtOAc = 4:1) = 0.30. – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.34 (d, $^3J_{H,H}$ = 7.0 Hz, 3H, –CH_3), 3.09 (pq, $^3J_{H,H}$ = 6.9 Hz, 1H, –C*H*-Ph), 3.78 (s, 3H, –OCH_3), 4.76 (d, $^3J_{H,H}$ = 6.1 Hz, 1H, –C*H*-OH), 6.74-6.85 (m, 2H, –C*H*=C-OMe), 7.05-7.45 (m, 7H, –C_6H_4–/–C_6H_5). – **MS** (EI): m/z (%) = 137.1 (53) $[M–C_8H_9]^+$, 224.1 (2) $[M–H_2O]^+$, 242.1 (1) $[M]^+$. – **MF:** $C_{16}H_{18}O_2$. – **MW:** 242.31.

N-Benzyl-2-hydroxy-2-phenylacetamide (Table 1, entry 6):

The reaction was carried out under a dry nitrogen atmosphere by using standard Schlenk techniques. To a solution of rac-mandelic acid (2.00 g, 13.1 mmol) in 55 mL of dry THF first benzylamine (1.44 mL, 13.1 mmol) was added followed by N-hydroxysuccinimide (1.66 g, 14.5 mmol, 1.1 eq.). The reaction mixture was cooled to 0 °C and a solution of N,N-dicyclohexylcarbodiimide (2.98 g, 14.5 mmol, 1.1 eq.) in dry THF (20 mL) was added over 15 min. After stirring for additional 15 min at 0 °C, the cooling bath was removed, the reaction mixture allowed to warm up to RT and stirred overnight. After this time, the mixture was filtered and the dicyclohexylurea cake washed with THF (2 × 10 mL). The solvent was removed under reduced pressure, and the residue was dissolved in EtOAc (120 mL). The organic layer was washed with sat. aq. Na_2CO_3 (25 mL), H_2O (25 mL), aq. HCl (1 M, 25 mL), H_2O (25 mL), and brine (25 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography on silica gel eluting with PE/Et_2O 4:1. N-Benzyl-2-hydroxy-2-phenylacetamide was obtained as colorless solid in 75% yield (2.38 g, 9.9 mmol). Analytical data were identical with literature.[43]

R_f (PE/Et_2O = 4:1) = 0.20. – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 4.07 (d, $^3J_{H,H}$ = 2.8 Hz, 1H, –OH), 4.36 (dd, $^2J_{H,H}$ = 2.8 Hz, $^3J_{H,H}$ = 5.9 Hz, 2H, –CH_2-Ph), 4.99 (d, $^3J_{H,H}$ = 2.1 Hz, 1H, –C*H*-OH), 6.77 (s, 1H, –NH–), 7.16 (dd, $^4J_{H,H}$ = 1.8 Hz, $^3J_{H,H}$ = 7 Hz, 2H, –Ph), 7.25-7.38 (m, 8H, –Ph). – **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 43.4, 74.2, 126.8, 127.6, 128.6, 128.7, 128.8, 137.7, 139.5, 172.4. – **MS** (ESI): m/z (%) = 242.1 (10) $[MH]^+$, 283.1 (19) $[MH+MeCN]^+$, 483.2 (100) $[2MH]^+$. – **MF:** $C_{15}H_{15}NO_2$. – **MW:** 241.29.

rac-1,2-Diphenylethane-1,2-diol (Table 1, entry 11):

To a solution of $K_3[Fe(CN)_6]$ (19.75g, 60 mmol, 3 eq.) in 100 mL of H_2O and 100 mL of tBuOH, K_2CO_3 (8.29 g, 60 mmol, 3 eq.), trans-stilbene (3.60 g, 20 mmol) and $K_2[OsO_4]$ x $2H_2O$ (15 mg, 0.04 mmol, 0.002 eq.) were added in this order. The reaction was stirred at RT for 48 hours. Afterwards Na_2SO_3 (15.1 g, 120 mmol) were added and the reaction was stirred for additional 30 min. The phases were separated and the aqueous phase was extracted with EtOAc (3 x 30 mL). The combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE/EtOAc 4:1). Rac-1,2-diphenylethane was isolated as colorless to slightly yellow solid in 57% yield (2.44 g, 11.4 mmol). Analytical data were identical with literature.[44]

R_f (PE/EtOAc = 4:1) = 0.28. – **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 2.87 (bs, 2H, –OH), 4.74 (s, 2H, –C*H*-OH), 7.12-7.29 (m, 10H, –Ph). – **MS** (EI): m/z (%) = 77.0 (100) $[C_6H_5]^+$, 108.1 (71) $[C_7H_8O]^+$, 214.3 (38) $[M]^+$. – **MF:** $C_{14}H_{14}O_2$. – **MW:** 214.26.

(E)-6-Methyl-1-phenylhept-4-en-3-ol (Table 2, entry 1):

The reaction was carried out under dry nitrogen atmosphere by using standard Schlenk techniques. To a slurry of Mg powder (0.67 g, 28 mmol) in dry THF (4 mL), 2 mL of a solution of 2-phenyl-1-bromethane (3.0 mL, 28 mmol) in dry THF (10 mL) was added. The Grignard reaction was initiated by the addition of iodine followed by sonication for several minutes. When the exothermic reaction started the rest of the 2-phenyl-1-bromethane solution was added through a septum by syringe over 15 min. After the addition, the reaction solution was heated under reflux for 1 h to complete the reaction. The reaction solution was allowed to cool to RT before 4-methyl-2-pentenal (2.3 mL, 20 mmol) was added dropwise. To complete the reaction the solution was again heated under reflux for 1 h. The reaction was quenched by the addition of HCl (2 M, 25 mL). The aqueous phase was extracted with diethyl ether (3 × 15 mL). The combined organic phases were washed with saturated $NaHCO_3$ (15 mL) and H_2O (2 × 10 mL), and dried with $MgSO_4$. The solvent was removed with a rotary evaporator. The crude product was purified by flash chromatography (petroleum ether/ethyl acetate 4:1, staining with vanillin solution gave a blue spot). (E)-6-Methyl-1-phenylhept-4-en-3-ol was isolated as yellow oil in 74% yield (3.05 g, 14.9 mmol).

R_f (PE/EtOAc = 4:1) = 0.32. – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.00 (d, $^3J_{H,H}$ = 6.8 Hz, 6H, $–CH_3$), 1.46 (d, $^3J_{H,H}$ = 1.8 Hz, 1H, –OH), 1.72-1.97 (m, 2H, –C*H*$_2$-CHOH–), 2.21-2.39 (m, 1H, –C*H*-$(CH_3)_2$), 2.59-2.79 (m, 2H, –C*H*$_2$-Ph), 4.01-4.13 (m, 1H, –C*H*OH–), 5.44 (ddd, $^4J_{H,H}$ = 1.2 Hz, $^3J_{H,H}$ = 7.0 Hz, $^3J_{H,H}$ = 15.5 Hz, 1H, –CH=C*H*–), 5.63 (ddd, $^4J_{H,H}$ = 0.7 Hz, $^3J_{H,H}$ = 6.4 Hz, $^3J_{H,H}$ = 15.5 Hz, 1H, –C*H*=CH–), 7.14-7.33 (m, 5H, $–C_6H_5$). – **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 21.3, 22.4, 30.7, 31.8, 38.8, 72.6, 125.8, 128.4, 128.5, 129.7, 139.6. – **MS** (EI): m/z (%) = 91.1 (100) $[C_7H_7]^+$, 161.1 (81) $[M–C_3H_7]^+$, 186.1 (5) $[M–H_2O]^+$, 204.2 $[M]^+$. – **HR-MS**: $[M]^+$ calcd. for $C_{14}H_{20}O$ 204.1514, found 204.1511. – **MF:** $C_{14}H_{20}O$. – **MW:** 204.31.

(E)-1-Phenylhex-4-en-3-ol (Table 2, entry 2):

The reaction was carried out under a dry nitrogen atmosphere by using standard Schlenk techniques. A solution (1 mL) of 2-phenyl-1-bromethane (1.35 mL, 10.0 mmol) in dry THF (10 mL) was added to Mg powder (0.25 g, 10 mmol). The Grignard reaction was initiated by the addition of iodine followed by sonication for several minutes. When the exothermic reaction started the rest of the 2-phenyl-1-bromethane solution was added through a septum by syringe over 15 min. After the addition, the reaction solution was heated under reflux for 1 h to complete the reaction. The reaction solution was allowed to cool to RT before crotonaldehyde (0.74 mL, 9.0 mmol) was added dropwise. To complete the reaction the solution was again heated under reflux for 2.5 h. The reaction was quenched by the addition of HCl (2 M, 10 mL). The aqueous phase was extracted with diethyl ether (2 × 15 mL). The combined organic phases were washed with saturated $NaHCO_3$ (5 mL), H_2O (2 × 5 mL) and dried with $MgSO_4$. The solvent was removed with a rotary evaporator. (E)-1-Phenylhex-4-en-3-ol was obtained in 96% yield (1.53 g, 8.69 mmol) in analytical purity. Analytical data were identical with the literature.[45]

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.46 (d, $^3J_{H,H}$ = 1.8 Hz, 1H, –OH), 1.67 (dd, $^4J_{H,H}$ = 0.7 Hz, $^3J_{H,H}$ = 6.3 Hz, 3H, –CH_3), 1.72-1.97 (m, 2H, –C*H*$_2$-CHOH–), 2.56-2.73 (m, 2H, –C*H*$_2$-Ph), 4.02 (pq, $^3J_{H,H}$ = 6.7 Hz, 1H, –C*H*OH–), 5.48 (ddd, $^4J_{H,H}$ = 1.4 Hz, $^3J_{H,H}$ = 7.0 Hz, $^3J_{H,H}$ = 15.3 Hz, 1H, –CH=C*H*–), 5.63 (dq, $^3J_{H,H}$ = 6.2 Hz, $^3J_{H,H}$ = 15.3 Hz, 1H, –C*H*=CH–), 7.06-7.34 (m, 5H, –C_6H_5). – **MS** (EI): m/z (%) = 71.1 (100) $[C_4H_7O]^+$, 91.1 (67) $[C_7H_7]^+$, 105.1 (19) $[M–C_4H_7O]^+$, 176.1 (50) $[M]^+$. – **MF:** $C_{12}H_{16}O$. – **MW:** 176.25.

6,6-Dimethyl-2-phenylhept-4-yn-3-ol (Table 2, entry 4):

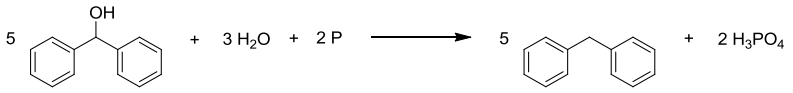
The reaction was carried out under a dry nitrogen atmosphere by using standard Schlenk techniques. The solution of 3,3-dimethyl-1-butyne (0.62 mL, 5 mmol) in dry THF (10 mL) was cooled to −78 °C. n-BuLi (1.6 M in hexane, 3.5 mL, 5.6 mmol) was added dropwise through a septum by syringe. The reaction mixture was allowed to warm to RT before the solution of 2-propionaldehyde (0.68 mL, 5 mmol) in dry THF (5 mL) was added dropwise through a septum by syringe. This solution was stirred for 4.5 h. The reaction was stopped by the addition of H_2O (10 mL). The aqueous phase was extracted with diethyl ether (3 × 15 mL), and the combined organic layers were dried with $MgSO_4$. The solvent was removed with a rotary evaporator. The crude product was purified by flash chromatography (petroleum ether/ethyl acetate 4:1, staining with vanillin solution gave a blue spot). 6,6-dimethyl-2-phenylhept-4-yn-3-ol was isolated as colorless oil in 46% yield (0.50 g, 2.3 mmol).

R_f (PE/EtOAc = 4:1) = 0.42. – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.17 (s, 9H, –C(CH_3)$_3$), 1.39 (d, $^3J_{H,H}$ = 7.1 Hz, 3H, –CH-C*H*$_3$), 1.64 (d, $^3J_{H,H}$ = 7.4 Hz, 1H, –OH), 3.03 (dd, $^3J_{H,H}$ = 5.4 Hz, $^3J_{H,H}$ = 7.4 Hz, 1H, –C*H*-Ph), 4.44 (dd, $^3J_{H,H}$ = 5.4 Hz, $^3J_{H,H}$ = 7.4 Hz, 1H, –C*H*-OH), 7.19-7.40 (m, 5H, –C_6H_5). – **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 16.3, 30.0, 31.0, 46.1, 55.0, 67.5, 67.8, 78.1, 95.5, 127.0, 128.2, 128.8, 141.9. – **MS** (EI): m/z (%) = 57.1 (36) $[C_4H_9]^+$, 99.1 (100), 105.1 (20) $[C_8H_{10}]^+$, 216.2 (7) $[M]^+$. – **MF:** $C_{15}H_{20}O$. – **MW:** 216.32.

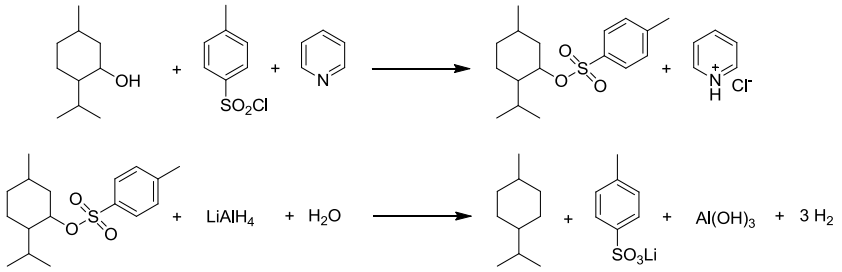
4.4.3 Supporting Information – Mass efficiency analysis of alternative alcohol deoxygenation methods

To allow a more quantitative comparison of the reduction method described herein with regard to environmental aspects and efficiency we determined the E factor which is defined in equation 1 of four alternative reactions for the synthetic step: $HI_{aq.}/P_{red}$ (Scheme 4), tosylation/$LiAlH_4$ (Scheme 5), Ti(III)-mediated (Scheme 6) and Barton-McCombie reduction (Scheme 7)

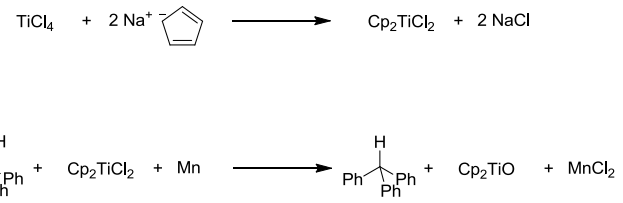
$$E = \frac{\text{Waste [kg]}}{\text{Product [kg]}} \qquad \textbf{Equation 1}$$



Scheme 4. Reduction of an alcohol by phosphorous (analogously to Table 1, entry 2).

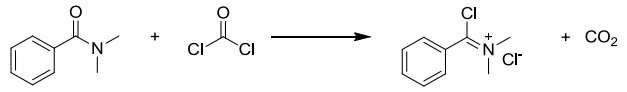


Scheme 5. Lithium aluminium hydride as reducing agent for alcohols.[46,47]

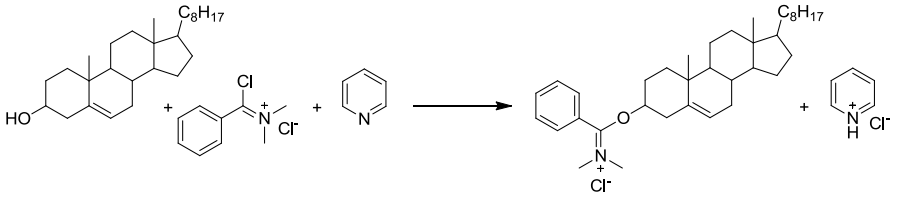


Scheme 6. Ti(III)-mediated reduction of alcohols.[5]

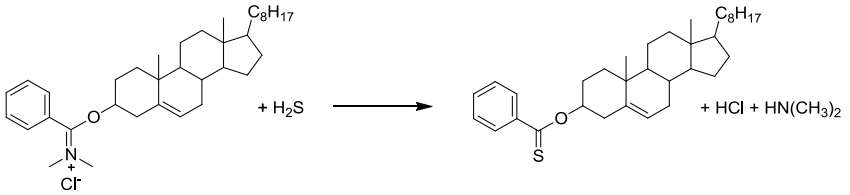
i)



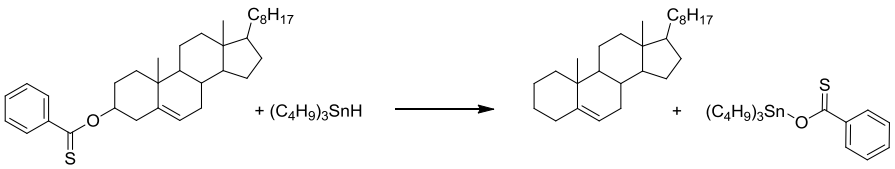
ii)



iii)



iv)



Scheme 7. Tributyl stannane as reducing agent in a synthesis sequence (i–iv) resulting in cholest-5-ene.[14]

The protocols were entered into the software EATOS[41] in order to determine the waste per kilogram of product. The result is shown in Table 5 and in Figure 1. Software-assisted mass balance is a tool to identify issues that require optimization.[48]

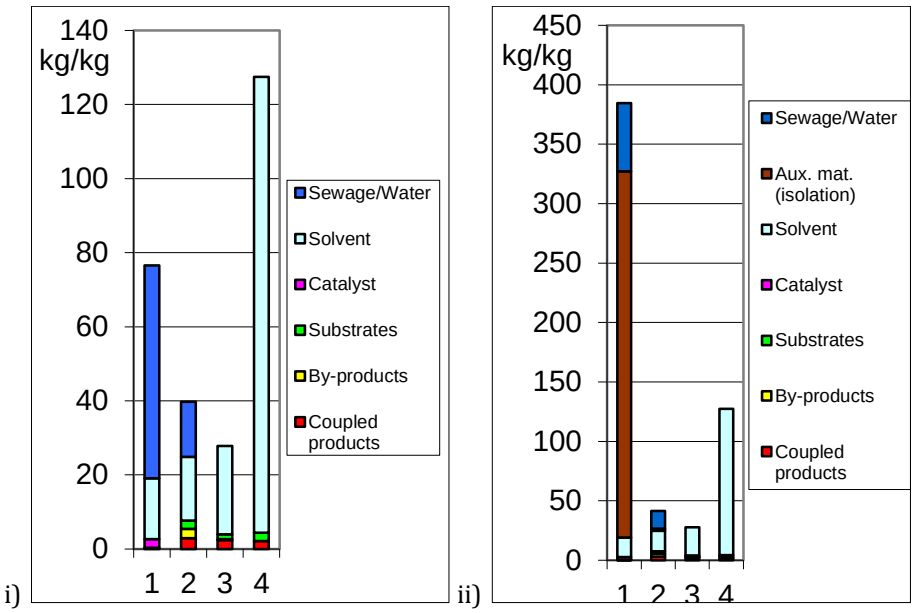


Figure 1. Environmental factor (E) of the deoxygenation methodologies shown in Schemes 4 to 7 numbered consecutively with 1 to 4. Whereas i) only shows substances used during reaction, ii) also illustrates those being applied during work-up. As more substance amounts are used altogether (reaction + isolation in case of ii)), the scaling is different.

Table 5. Reduction of alcohols with catalytic amounts of hydriodic acid.

Entry	1	2	3	4
i)				
Coupled products[a]	0.233	2.8862	2.4118	2.11
By-products[a]	0.0514	2.4997	0.2284	0
Substrates[a]	0	2.2933	1.3516	2.25
Catalyst[a]	2.376	0	0	0
Solvent[a]	16.4078	17.2271	23.7736	123
Sewage/Water[a]	57.4502	14.8146	0	0
ii)				
Coupled products[a]	0.233	2.8862	2.4118	2.11
By-products[a]	0.0514	2.4997	0.2284	0
Substrates[a]	0	2.2933	1.3516	2.25
Catalyst[a]	2.376	0	0	0
Solvent[a]	16.4078	17.2271	23.7736	123
Aux. mat. (isolation) [a]	308.0334	1.8085	0	0
Sewage/Water[a]	57.4502	14.8146	0	0
Substrates[b]	1.2843	8.6793	4.9919	6.13
Atom economy[c]	81	27	36	36

[a] Values shown in Figure 1. [b] Substrate amounts that were used for the reaction. [c] Atom economies of the synthesis sequences shown in Scheme 4 to 7.

The hydriodic acid catalyzed reduction (entry 1 in Figure 1 (i) and Table 5) is, except for sewage production, the most mass-efficient reaction. The low amount of substrate demand (entry 1 in Table 5[b]), which is due to the high atom economy (Table 5[c]), results in the formation of only low amounts of coupled products or byproducts compared to entries 2 to 4 (Figure 1 (i) and Table 5[a]). Additionally, some of the coupled products of entries 2 to 4 are supposed to be much more problematic with regard to waste treatment and recycling. In contrast, if hydriodic acid is not recycled it can easily be neutralized and disposed of in the sewage plant. The coupled product phosphoric acid will be easily flocculated in the treatment plant but will thereby contributing to the eutrophication problem. The catalytic use of iodine and the replacement of the stoichiometric reagent phosphorous, e.g., by an electrochemical method, are ways to improve the efficiency of the method. Another obvious optimization potential is the reduction of solvent and water during the work up.

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5 Summary

The first part of the thesis (chapter 1-3) describes the synthesis and use of tetrahydrofuran amino acids. In chapter 1 we summarize previous investigations followed by a more detailed description of the synthesis and conformational studies of the C^{α}-tetrasubstituted α-amino acid tert-butyl 2-(4-bromophenyl)-3-((tert-butoxycarbonyl)amino)tetra-hydrofuran-3-carboxylate, which is of importance for the following chapters.

Chapter 2 describes the synthesis of a small series of pyrene and carboxyfluorescein labeled unnatural tetrahydrofuran amino acid building blocks. They can be incorporated into peptides to rigidify the secondary structure and at the same time introducing a fluorescent label. The fluorescent dye can be coupled to the TAA before or after its incorporation into a peptide sequence using a Suzuki-type C-C bond formation on the bromoarene substituent, which illustrates the synthetic feasibility of these compounds. Thus these building blocks combine two properties which are often of high interest in the preparation of peptide analogues. The absorption and emission spectra of the prepared building blocks and peptides were investigated. Furthermore the fluorescence quantum yields of derivatives **rac-3** and **rac-6** were determined (Φ_F = 0.24±0.03 and Φ_F = 0.09±0.02, respectively). Compared to literature known values of pyrene (Φ_F = 0.58), the quantum yield is lower..

Chapter 3 reports the synthesis of a new class of NTS2 receptor selective ligands based on the structural modification of the lead structure NT(8-13) via solid phase supported peptide synthesis. The tetrahydrofuran amino acid HCl*H-TAA-OH was introduced as scaffold for Tyr^{11}-Ile^{12} or Tyr^{11}, respectively, leading to a small library of penta- and hexapeptides. For selected peptides that showed interesting biological activity when tested as mixtures of stereoisomers the crucial steps of an alternative synthetic pathway were investigated, allowing the synthesis of diastereomerically pure compounds. The biological investigations by radioligand binding assay at hNTS1 or hNTS2 receptors revealed that the prepared pentapeptides have only very low affinity and were therefore not further investigated. The K_i values for the hexapeptides showed good binding affinities and increased selectivity towards the NTS2 receptor. The most promising ligand possesses a K_i(NTS2) value of 67 ± 12 nM and a selectivity ratio of 52 in favour of the NTS2 receptor. However, the most active and selective compound for the NTS2 receptor reported so far shows a smaller K_i(NTS2) of 2.8 ± 0.69 nM and a superior selectivity ratio of 22000.

The second part of this thesis (Chapter 4) deals with the reductive deoxygenation of alcohols. The method for the reduction of alcohols to hydrocarbons by using hydriodic acid and red phosphorous presented in Chapter 4, was described for the first time by Kiliani more than 140 years ago for the deoxygenation of gluconic acid. We improved this method, applying a biphasic toluene – aqueous hydriodic acid reaction medium. This allowed the separation of organic substrates, which dissolve in toluene, from the strongly acidic aqueous phase leading to milder reaction conditions and therefore being more applicable to organic synthesis. A range of primary, secondary and tertiary benzylic alcohols as well as α-hydroxycarbonyl compounds are converted in good yields and functional groups like keto, ester, amide, or ether groups are tolerated. With an access of red phosphorous as the stoichiometric reducing agent, catalytic amounts of hydriodic acid are sufficient to achieve full conversion.

6 Zusammenfassung

Der erste Teil der vorliegenden Arbeit (Kapitel 1, 2 und 3) beschäftigt sich mit der Synthese und Anwendung von Tetrahydrofuran Aminosäuren. Kapitel 1 vermittelt dem Leser einen Überblick über die bisherigen Arbeiten auf diesem Gebiet. Eine detaillierte Beschreibung der Synthese und Konformationsuntersuchungen der C^{α}-tetrasubstituierten α-Aminosäure tert-butyl 2-(4-bromophenyl)-3-((tert-butoxycarbonyl)amino)tetra-hydrofuran-3-carboxylat schließt die Einleitung ab. Diese Aminosäure wird in den folgenden Kapitel erneut genutzt.

Kapitel 2 beschreibt die Synthese einer kleinen Serie unnatürlichen Tetrahydrofuran Aminosäuren-Bausteine, welche mit Pyren oder Carboxyfluorescein-Einheiten fluoreszenzmarkiert wurden. Diese können, eingebaut in Peptide, deren Sekundärstruktur stabilisieren und gleichzeitig eine fluoreszente Markierung einfügen. Dabei kann der fluoreszierende Farbstoff durch eine Pd-katalysierte C-C-Bindungsknüpfung am Bromoarene-Substituenten sowohl vor, als auch nach der Peptidsynthese an der Tetrahydrofuran Aminosäure eingeführt werden, welches die synthetischen Möglichkeiten dieser Verbindung aufzeigt. Damit vereinen diese Bausteine zwei Eigenschaften in Kombination, welche oftmals von besonderem Interesse in der Herstellung von Peptidmimetika sind. Die Absorptions- und Emissions-Eigenschaften der dargestellten Verbindungen wurden untersucht. Zusätzlich wurden die Fluoreszenzquantenausbeuten der Derivate **rac-3** und **rac-6** bestimmt (Φ_F = 0.24±0.03 bzw. Φ_F = 0.09±0.02) und mit literaturbekannten Werten von Pyren (Φ_F = 0.58) verglichen, wobei sich eine deutlich niedrige Quantenausbeute zeigte.

In Kapitel 3 wird über die Festphasenpeptidsynthese einer neuen Klasse von NTS2 rezeptorselektiven Liganden berichtet, die auf einer einzelnen strukturellen Änderung der Leitstruktur NT(8-13) basieren. Die Tetrahydrofuran Aminosäure HCl*H-TAA-OH wurde dabei als Ersatz für Tyr^{11}-Ile^{12} bzw. für Tyr^{11} eingebaut, wodurch eine kleine Bibliothek von Penta- und Hexapeptiden erhalten wurde. Um einen Zugang zu diastereomerenreinen Verbindungen zu haben, wurden für einige ausgewählte Peptide, welche in der biologischen Testung als Mischung von Stereoisomeren interessante Ergebnisse zeigten, die wesentlichen Schritte einer alternativen Syntheseroute untersucht. Die biologischen Untersuchungen mit Hilfe eines Bindungs-Assay unter Verwendung von radioaktivmarkierten Liganden an hNTS1 und hNTS2 Rezeptoren zeigten bei den Pentapeptiden nur geringe Aktivitäten, weshalb diese anschließend nicht weiter verfolgt wurden. Die Bindungskonstanten der Hexapeptide zeigten dagegen gute Bindungsaffinitäten und eine erhöhte Selektivität für den NTS2 Rezeptor. Verglichen mit dem aktivsten und selektivsten bekannten Liganden für diesen Rezeptor mit einem K_i(NTS2) = 2.8 ± 0.69 nM und einem Selektivitätsverhältnis von 22000, besitzt der beste TAA-Peptid Ligand nur einen K_i(NTS2) Wert von 67 ± 12 nM und ein Selektivitätsverhältnis von 52.

Der zweite Teil der Arbeit (Kapitel 4) beschäftigt sich mit der reduktiven Deoxygenierung von Alkoholen. Die in Kapitel 4 beschriebene Methode zur Reduktion von Alkoholen unter Verwendung von Iodwasserstoffsäure und rotem Phosphor wurde bereits vor mehr als 140 Jahren von Kiliani für die Deoxygenierung von Zuckersäuren, z.B. Gluconsäure beschrieben. Wir haben die Methode verbessert und ein zweiphasiges Reaktionsmedium aus Toluol und wässriger Iodwasserstoffsäure verwendet. Dadurch wird es ermöglicht, die in Toluol löslichen organischen Substrate von der stark sauren wässrigen Phase zu trennen und mildere Reaktionsbedingungen, welche sich besser zur organischen Synthese eigenen, einzustellen. Eine Reihe primärer, sekundärer und tertiärer benzylischer Alkohole sowie α-Hydroxycarbonyl-Verbindungen wurde in guten Ausbeuten unter Tolerierung von funktionellen Gruppen (Keto, Ester, Amide, und Ether) umgesetzt. Mit einem Überschuss an rotem Phosphor als stöchiometrisches Reduktionsmittel genügten katalytische Mengen an Iodwasserstoffsäure um vollständigen Umsatz zu erreichen.

7 Abbreviations

°C	degree Celsius
ε	molar extinction coefficients
λ	Wavelength
Φ_F	Fluorescence quantum yield
µL	Micro liter
µM	Micro molar
^{13}C-NMR	Carbon NMR
^{1}H-NMR	Proton NMR
AA	Amino acid
abs	absolute
Ac_2O	acetic anhydride
Ala	Alanine
Anal.	Elemental analysis
APCI	atmospheric pressure chemical ionization
aq.	aqueous
Arg	Arginine
Asn	Asparagine
ATR	attenuated total reflectance
Boc	t-butyloxycarbonyl
calcd	calculated
$CDCl_3$	deuterated chloroform
CHO	Chinese hamster ovary
CI	chemical ionization
Ci	Curie (1 Ci = $3.7*10^{10}$ Becquerel)
CuI	Copper(I)-iodide
Compd	compound
COSY	correlated spectroscopy
DAD	diode array detector
DCC	N,N'-dicyclohexylcarbodiimide
DCM	dichloromethane
DEPT	distortionless enhancement by polarization transfer
DIPEA	diisopropylethylamine
DMAP	4-(dimethylamino)-pyridine
DME	1,2-dimethoxyethan
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DMSO-d^6	deuterated dimethylsulfoxide
DVB	divinylbenzene
EATOS	Environmental Assessment Tool for Organic Syntheses
EC_{50}	concentration of 50% observed effect
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
EI	electron impact ionization
eq.	equivalent
ESI	electrospray ionization
Et_2O	diethylether
EtOAc	ethylacetate
EtOH	ethanol
eV	electron volts
FAB	Fast atom bombardment
FLD	fluorescence detector
Fmoc	fluorenylmethoxycarbonyl
FT	fourier transformed
Glu	Glutamic acid
Gly	Glycine
GP	general procedure
GPCRs	G-protein coupled receptors
h	hour(s)
H^+	Proton
HATU	2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HBTU	2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HCl	Hydrochloric acid

HEK293	human embryonic kidney
HI	Hydriodic acid
HMBC	heteronuclear multiple bond correlation
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
HPLC	High performance liquid chromatography
HR	high resolution
HSQC	heteronuclear single quantum coherence
Hz	Hertz
I	fluorescence intensity
Ile	Isoleucine
IR	Infrared spectroscopy
J	coupling constant
LC/MS	liquid chromatography/mass spectrometry
Leu	Leucine
$LiAlH_4$	Lithium aluminium hydride
LiOH	Lithium hydroxide
Lys	Lysine
K	Kelvin
K_2CO_3	Potassium carbonate
$KHSO_4$	Potassium hydrogen sulfate
KOH	Potassium hydroxide
M	Molecule / Molar
Me	methyl
MeCN	acetonitrile
MeOD	deuterated methanol, MeOH-d^4
MeOH	methanol
Met	Methionine
MF	molecular formula
$MgSO_4$	Magnesium sulfate
MHz	Mega hertz
min	minute(s)
mL	Milli liter
mm	Milli meter
mmol	Milli mole
Mp	melting point
MS	mass spectrometry
MW	molecular weight
n	refractive index
Na_2CO_3	Sodium carbonate
$NaHCO_3$	Sodium hydrogen carbonate
$Na_2S_2O_3$	Sodium thiosulfate
Na_2SO_3	Sodium sulfite
n.d.	not determined
nm	Nano meter
nM	Nano molar
NMR	nuclear magnetic resonance
NOE	nuclear overhauser effect
NOESY	nuclear overhauser enhancement spectroscopy
NT	Neurotensin
(h)NTSx	(human) Neurotensin receptor subtype x (x = 1-3)
Pbf	2,2,4,6,7-pentamethyldihydro-benzofuran-5-sulfonyl
Pd/C	Palladium on charcoal
$Pd(OAc)_2$	Palladium(II)-acetate
$Pd(PPh_3)_4$	Tetrakis(triphenylphosphine)-palladium(0)
PE	petroleum ether (hexanes, bp: 50-70 °C)
PET	positron emission tomography
pGlu	pyro Glutamic acid
Ph	phenyl
Phe	Phenylalanine
PMT	Photo multiplier tube
ppm	Parts per million
P_{red}	Red phosphorous
Pro	Proline
R_f	Retention factor
ROESY	rotating frame NOE spectroscopy
RP	Reversed phase
RT	room temperature
sat.	Saturated

SD	standard deviation
SEM	standard error of the mean
SET	single electron transfer
SPPS	solid phase (supported) peptide synthesis
SR	selectivity ratio
TAA	C^{α}-tetrasubstituted amino acid / Tetrahydrofuran amino acid
TBAB	Tetrabuthylammoniumbromide
TBDMS	tert-butyldimethylsilyl
TBTU	O-(benzotriazole-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate
tBu	tert.-butyl
tBuOH	tert-butanol
TEMPO	(2,2,6,6-tetramethyl-piperidin-1-yl)-oxyl
TFA	trifluoroacetic acid
THF	tetrahydrofurane
TIS	triisopropylsilane
TLC	thin layer chromatography
TMS	tetramethylsilane
t_R	retention time
Trp	Tryptophan
TOF	time of flight
Tyr	Tyrosine
UV	ultraviolet / UV-Vis spectroscopy
V	Volt
Vis	visible

8 Appendix

8.1 List of Publications

"Reduction of benzylic alcohols and α-hydroxycarbonyl compounds by hydriodic acid in a biphasic reaction medium" Dobmeier, M.; Herrmann, J. M.; Lenoir, D.; König, B. *Beilstein Journal of Organic Chemistry* **2012**, 8, 330-336.

"Antiproliferative and Erythroid Differentiation of Piperazine and Triphenyl Derivatives Against K-562 Human Chronic Myelogenous Leukemia" Saab, A. M.; Dobmeier, M.; König, B.; Fabri, E.; Finotti, A.; Borgatti, M.; Lampronti, I.; Bernardi, F.; Efferth, T.; Gambari, R. *Anticancer Research* **2013**, 33 (8), 3027-3032.

8.2 Curriculum Vitae

Personal Details

Name:	Michael Dobmeier
Date of birth:	17.02.1984
Place of birth:	Weiden i. d. Opf.
Nationality:	German
Status:	Single

Education:

02/2009 – 05/2014	PhD Thesis "Synthesis and Use of Tetrahydrofuran Amino Acids & Reductive Deoxygenation of Alcohols", Research group of Prof. Dr. Burkhard König, Institute of Organic Chemistry, University of Regensburg
04/2008 – 01/2009	Diploma Thesis "New Synthetic Receptors based on Tetrahydrofuran Amino Acids", Research group of Prof. Dr. Burkhard König, Institute of Organic Chemistry, University of Regensburg
10/2003 – 01/2009	Studies of Chemistry, University of Regensburg (Degree: Diploma in Chemistry)
06/2003	Abitur (A-levels)
09/1994 – 06/2003	Kepler-Gymnasium (grammar school), Weiden i. d. Opf.
09/1990 – 07/1994	Trautwein-Volksschule (primary school), Moosbach

Teaching Experience:

02/2009 – 07/2012	Graduate assistant in laboratory courses for chemistry, biology and biochemistry students in basic and advanced organic chemistry; supervisor of students during research projects and bachelor theses.

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