Carbohydrate-Based Mimetics in Drug Design:
Sugar Amino Acids as Structural Templates and
Key Residues of Bioactive Peptidomimetics

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From November 1998 to November 2001, the work for this PhD thesis has been carried out in the working group of Prof. Dr. Horst Kessler at the “Institut für Organische Chemie und Biochemie der Technischen Universität München”.

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dedicated to my parents and Christian
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1 Incentive

The impressive development of molecular biology, biophysical methodology, and computer based molecular modeling and design in the last two decades has caused considerable change in the approach to target and pharmacological lead identification. Unfortunately, the “de novo design” of new biologically active compounds, based on structure activity relationships, has failed to meet expectations. Therefore, in the 90’s combinatorial chemistry paired with robot aided high-throughput screening for certain biological activities and thus lead identification, has emerged as the method of choice. However, it soon became obvious that generating tremendous libraries of millions of randomly chosen compounds, followed by high-throughput screening has also major draw backs.

As a consequence, the combination of rational design and combinatorial chemistry has gained more and more interest. Now, the design of smaller, rationally designed libraries has become the state of the art.

Carbohydrates as well as peptides and proteins are essential biopolymers of life. They are involved in complex biological processes such as catalysis and highly selective molecular recognition. In order to perform these functions, the correct folding of the biopolymers creating the active site is crucial, since any kind of interaction is observed only if the reactive groups are positioned in the correct spatial orientation to each other. Thus, the development of small, easy-to-functionalize building blocks and oligomers with backbones of discrete and predictable folding patterns (“foldamers”) is required in order to design and develop molecules with useful biological functions. Nevertheless, for a successful application, their structural properties have to be scrutinized.

SAAs are sugar moieties containing at least one amino as well as at least one carboxyl group. Thus, they may be considered as monomeric building blocks of chimeras between carbohydrates and proteins. They allow among other modifications the
replacement of the naturally occurring, but synthetically difficult to generate, O-/N-glycosidic bonds by peptidic bonds. This not only increases the enzymatic stability, but facilitates the assembly of large, diverse oligomer carbohydrate or peptidomimetic libraries by solid phase techniques as well. Their use as structural templates in peptides, is also a very attractive application of SAAs.

The aim of this work was therefore the development of new SAA building blocks suitable for rational combinatorial synthesis and spatial screening. To meet our demands the SAAs had to full fill a number of requirements:

- They had to be obtainable by a fast, easy to scale up, high yielding and cheap synthetic route.
- They had to have secondary structure inducing properties
- They should introduce chiral diversity
- They ought to be compatible to solid phase synthetic techniques
- and were supposed to enhance enzymatic stability.

The basic structural properties of these new SAA building blocks, the folding patterns and secondary structures induced, were to be investigated by synthesis and structural analysis of several SAA oligomers. Finally, the insights gained were then intended to be applied and evaluated in biologically active systems by implementation of the SAA building blocks in small rational combinatorial libraries of biological active peptides.
2 State of the Art: Sugar Amino Acids, and β-Peptides in Drug Design

2.1 The Concept of Sugar Amino Acids in Drug Design

Among the major classes of biomolecules carbohydrates, allow almost unlimited structural variations due to their chiral diversity and high density of easy to functionalize groups. The molecular diversity of carbohydrates offers a valuable tool for drug discovery in the areas of biologically important oligosaccharides, glyco-conjugates and molecular scaffolds by investigating their structural and functional impact. Chimeras of the three big classes of biopolymers, that is, nucleic acids, proteins and carbohydrates, have attracted great interest as both functional, and structural analogues in recent years, also because of their potential application in drug design. However, Sugar Amino Acids\(^\text{a} \) (SAAs) and their oligomers, which bridge carbohydrates and proteins, have only recently been investigated.\(^{3, 5, 14-22} \) SAAs are sugar moieties containing at least one amino as well as at least one carboxyl group (Figure 1).

Besides their immediate intrinsic different pharmacological properties, SAAs can be used as building blocks for the preparation of modified analogs of biologically

\(^{a} \) The term "sugar amino acid", SAA, as a functional, succinct classification term, was introduced by our group and is widely accepted, although a plethora of terms have been proposed in the literature for compounds derived from SAAs. These include saccharide-peptide hybrids, glycosaminic acids and glycotides,\(^{1} \) peptidosaccharides,\(^{2} \) saccharopeptides,\(^{3} \) amide-linked carbohydrates, tetrahydro-furan (pyran) amino acids and carboproteinoids,\(^{4-7} \) although the latter compounds most often do not have a peptoid functionality (IUPAC definition). The term saccharopeptides has also been used to describe oligosaccharides in which the glycosidic linkage has been replaced by an amide bond.\(^{8, 9} \) Some publications use the term “sugar amino acids” for glycosylated amino acids,\(^{10, 11} \) for a disaccharide based on an amino- and carboxyl cyclopropyl-carbohydrate derivative,\(^{12} \) or in one case even for conjugates based on the Michael addition of C-terminally protected amino acids to 2,3-dideoxy-hex-2-enopyranos-4-uloses.\(^{13} \) In some cases the SAAs are linked to each other, in other cases to amino acids. Herein the term SAA is used for compounds with two immediate linkages of the amino and carboxy functionalities to the carbohydrate frame. Further elongations with one connectivity, that is a carbon chain, are considered glycosylated amino acids or glycopeptides.
active peptides and/or oligosaccharides. The difference in ring size allows modification of the conformations of the peptides and carbohydrates.

SAAs can as well be used as starting compounds for different oligomers. They are potential pharmaceutical compounds, are valuable for the synthesis of natural products or analogs, and also as building blocks in drug design and drug research.

Figure 1: Sugar amino acids as structural scaffolds, as carbohydrate mimetics, and as peptide mimetics.

In the following the state of the art of SAA synthesis, with emphasis on furanoid SAAs, their incorporation in peptidic or saccharidic structures, and their potential use in medicinal chemistry is described.
2.2 Naturally Occurring Sugar Amino Acids

Some sugar amino acids can be found in nature largely as construction elements. The most prominent and abundant example is sialic acid often located peripherally on glycoproteins. This family of natural SAAs consists of \( N \)- and \( O \)-acyl derivatives of neuraminic acid \( 1 \) (Figure 2). The main substituents on nitrogen are the \( N \)-acetyl and \( N \)-glycosyl groups. Glycosaminuronic acids (2-5) are more common in form of their derivatives. For instance 2-acetamido-2-deoxy-glucuronic acid is found in bacterial cell walls\[^{23}\] and 2-acetamido-2-deoxygalacturonic acid is one component of bacterial Vi antigen of \textit{Escherichia coli}.\[^{24}\] Derivatives of glucosaminuronic acid were also detected in the cancomycin family of antibiotics similar to vancomycin.\[^{25}\]

Interestingly, natural SAAs can be found in nucleoside antibiotics.\[^{26}\] Two different 3-amino-3-deoxy uronic acids, derivatives of 3-amino-3-deoxy-\( D \)-gulopyranuronic acid and 3-amino-3,4-dideoxy-\( D \)-xylohexopyranuronic acid, were found in ezomycin A \( 6 \).\[^{26, 27}\] 4-Amino-4-deoxy-glucuronic acid \( 5 \), can be found in gougerotin,\[^{28-32}\] a antibiotic from \textit{Streptomyces} bacteria, as the carbohydrate residue of the nucleoside.

The naturally occurring furanoid SAA (+)-hydantocidin \( 7 \) (Figure 2), which represent a spirohydanthion derivative,\[^{33-35}\] exhibits herbicidal activity.

Siastatin B \( 8 \) (Figure 2) is among the class of SAAs, in which the nitrogen is located within the pyranoid ring structure. This inhibitor for both \( \beta \)-glucuronidase and \( N \)-acytyleuraminidase was isolated from a \textit{Streptomyces} culture.\[^{36}\]
### Naturally Occurring Sugar Amino Acids

#### Glycosaminuronic Acids

<table>
<thead>
<tr>
<th>Glycosaminuronic Acid</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>R⁴</th>
<th>R⁵</th>
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<tr>
<td>Glucosaminuronic Acid</td>
<td>NH₂</td>
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<tr>
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<tr>
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<td>H</td>
<td>OH</td>
<td>NH₂</td>
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#### Figure 2: Naturally occurring sugar amino acids.

![Diagram](image-url)
2.3 Synthesis of individual SAA building blocks

The synthesis of sugar amino acids is accomplished starting from commercially available, or easy accessible monosaccharides, i.e., glucose, glucosamine, diacetone glucose, galactose, etc. The amino functionality of the SAA is usually introduced as an azide, cyanide or nitromethane equivalent, followed by subsequent reduction. The carboxylic function is introduced directly as CO$_2$, or as a hydrolyzable cyanide, by a Wittig reaction and subsequent oxidation or by selective oxidation of a primary alcohol.

2.3.1 Furanoid SAAs

2.3.1.1 $\alpha$-SAAs

Several derivatives with an $\alpha$-amino acid moiety at the anomeric position of the sugar were synthesized by Fleet et al. including glucose,$^{[37]}$ rhamnose,$^{[38]}$ galactose,$^{[39]}$ and mannose$^{[6]}$ (Figure 3) derivatives. The various routes developed by Fleet’s group and Dondoni’s group have recently been summarized.$^{[11]}$

![Figure 3: Some of Fleet’s $\alpha$-SAAs.](image-url)
Fleet’s azide precursors and N-protected SAAs are stable against epimerization; however, all free SAAs (also the pyranoid derivatives) and their respective esters equilibrate to a mixture of α- and β-anomers in solution.

This class of SAAs has also been employed as precursors to five- and six-membered spiroheterocyclic derivatives of carbohydrates such as the rhamnose functionality, required for enhanced activity analogs of hydantocidin. The naturally occurring (+)-hydantocidin (see Figure 2) exhibits herbicidal activity. Those spirodiketopiperazine derivatives are considered as potential inhibitors of carbohydrate processing enzymes and thus might be useful in elucidating the biosynthesis of the cell walls of mycobacteria.

2.3.1.2 β- and γ-SAAs

Fleet et al. published the synthesis of several azide precursors to β- and γ-SAAs shown in Figure 4.

![Figure 4](image)

**Figure 4:** Some of Fleet’s large collection of azides, used as β- and γ-SAA precursors.

They synthesized the β-SAA precursor 19 and the γ-SAA precursors 20 and 21 via 22, which was obtained by the route described in Scheme 2. Key step of the
synthesis of 22 was the hydrolysis of the side-chain acetonide of 24 and methanolysis of the lactone with intramolecular displacement of the triflate at C-2 by 5-OH. Side-chain hydrolysis of the acetonide of 22 afforded the γ-SAA precursor 21, subsequent periodate cleavage, followed by immediate cyanoborohydride reduction of the resulting aldehyde in acetic acid yielded the γ-SAA precursor 20. Overall yields for 20 and 21, starting from D-glycero-D-gulo-heptono-1,4-lactone 23, were 10 % and 13 %, respectively. Sodium borohydride reduction of the ester function in 22, followed by a series of protection deprotection steps and subsequent oxidation of the diol moiety with sodium periodate in the presence of catalytic amounts of ruthenium(III) chloride led to the β-SAA precursor 19 in an overall yield starting from 23 of about 3%.

Scheme 2: Synthesis of protected γ-SAA precursor 22.
2.3.1.3 d-SAAs

As dipeptide isosteres for the incorporation into peptide based drugs Le Merrer and co-workers synthesized 25 and 28 (Figure 5).\[^{47}\] The benzylated derivatives were designed as mimics for hydrophobic, the unprotected SAAs as mimics for hydrophilic amino acids (see also chapter 2.7).

\[\text{Figure 5: Some of Le Merrer’s, Fleet’s and Charkraborty’s furanoid } \delta\text{-SAAs.}\]

Key step of their synthesis was the one-pot silica gel assisted azidolysis followed by \(O\)-ring closure of the bis-epoxides 32 and 33 (Scheme 3) to yield 34 and 35 respectively. Sodium dichromate oxidation of the primary hydroxyl group, treatment with an excess of diazomethane, followed by a one pot conversion of the respective azidoesters by hydrogenolysis in presence of di-\text{-}t \text{-} tert\text{-}butyldicarbonate, yielded the N-Boc protected, fully benzylated SAA methylesters of 25 and 28.
Synthesis of individual SAA building blocks

Scheme 3: Key step of Le Merrer’s synthesis of 25 and 28.

SAA 25 was also synthesized by Chakraborty’s and Fleet’s groups, using different reaction pathways.\textsuperscript{[48-51]}

Furthermore, also for the use as dipeptide isosteres, Chakraborty et al. synthesized several new $\delta$-SAAs (Figure 5).\textsuperscript{[48, 49]} The key step of their synthesis of SAA 25 and SAA 26 followed a different reaction path, in which an intramolecular 5-\textit{exo} opening of the terminal aziridine ring (Scheme 4) of the hexose-derived substrate 36 (with the respective stereochemistry) by the $\gamma$-benzyloxy oxygen with concomitant debenzylolation occurred during pyridinium dichromate (PDC) oxidation of the primary hydroxyl group with complete stereocontrol.

Scheme 4: Synthesis of 25 and 26 starting from 36. The stereochemistry of 36 determines, if the synthesis results in 25 or 26.\textsuperscript{[49]}
At about the same time Fleet et al. synthesized a wide range of different δ-SAAAs including also 25, to study the influence of the SAA’s stereochemistry and that of the hydroxyl protecting groups on the secondary structure of their linear homo-oligomers.\textsuperscript{[19, 20, 50-54]} A few representative examples are shown in Figure 5.

### 2.3.2 Pyranoid SAAs

The most obvious approach for the synthesis of pyranoid SAAs is the oxidation of amino sugars for example glucosamine. Thus, Heyns and Paulsen described the first synthesis of the SAA, glucosaminuronic acid, in 1955 by catalytic oxidation of the primary hydroxyl group,\textsuperscript{[55]} in an effort to elucidate the structure of bacterial cell wall components and synthesize analogues. The oxidation of the amino sugar was also used by Paulsen et al. in the early synthesis of D-galactosaminuronic acid to confirm the structure of isolated components of bacterial Vi antigen.\textsuperscript{[24]} Since then a large number of pyranoid α-, β-, γ-, δ-, and e-SAAs have been synthesized using various, sometimes very elegant methods (Table 1).\textsuperscript{[18, 21, 22, 56]}
Table 1: Some pyranoid SAAs and references for their synthesis.

<table>
<thead>
<tr>
<th>a-SAs</th>
<th>β-SAs</th>
<th>γ-SAs</th>
<th>d-SAs</th>
<th>e-SAs</th>
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<td><img src="image3" alt="γ-SAs" /></td>
<td><img src="image4" alt="d-SAs" /></td>
<td><img src="image5" alt="e-SAs" /></td>
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References:
- 37 [11, 57, 58]
- 38 [22]
- 39 [22]
- 40 [60]
- 41 [65]
- 42 [68]
- 43 [14, 22, 59]
- 44 [61, 62]
- 45 [66]
- 46 [69]
- 47 [55]
- 48 [63, 64]
- 49 [67]
2.4 Carbohydrates and SAAs as Scaffolds

Carbohydrates represent an attractive source of readily available, stereochemically defined scaffolds as they contain well-defined and readily convertible substituents with a rigid pyran ring or the more flexible furan ring.\cite{70-73} The functional pharmacophoric groups can thus be presented in a distinct arrangement.

The genesis of carbohydrate privileged structures was first described by Hirschmann and co-workers.\cite{74} A somatostatin agonist was discovered which contained a deoxyglucose nucleus carrying the key amino acid side chains of a cyclic hexapeptide agonist (Figure 6).\cite{70} Successively, a NK-1 receptor antagonist was identified by modification of the deoxyglucose-based somatostatin agonist.\cite{75,76}

\[ \text{Veber-peptide or L-363 301} \]

\[ \text{somatostatin agonist IC}_{50} = 5 \mu M \]

\[ \text{NK-1 receptor antagonist IC}_{50} = 60 \text{ nM} \]

**Figure 6: Glucose-based scaffold for the cyclic hexapeptide.**

The tetrasubstituted xylofuranose 53 was synthesized by Papageorgiou et al. as a potential nonpeptide mimic of somatostatin (Figure 7).\cite{77} The scaffold was designed based on molecular dynamics simulations and the results from Hirschmann et al.\cite{70} The biological activity of the mixture of the $\alpha$ and $\beta$ anomers in a ratio of 2:3 revealed a rather low IC$_{50} = 16 \mu M$. They did show a similar conformational behavior as the Hirschmann scaffolds.
Sugar amino acids in particular are also ideal peptidomimetic scaffolds, as they may function as structural pharmacophores depending on their substituents in addition to the imperative amino and carboxyl function.\cite{14} Smith III et al. reported the design of an inhibitor of mammalian ribonucleotide reductase (mRR) \textbf{54} based on the bound conformation of the heptapeptide N-AcFTLDADF \textbf{55} using a pyranoid SAA scaffold (Figure 8).\cite{78} This SAA was employed to mimic a $\beta$-turn present in the peptidic precursor and to carry, via ether linkage, the pharmacophores, Leu and Asp side chains, present in the $i+1$ and $i+2$ positions of the turn, respectively. The tetrahydropyran-based mimetic \textbf{54} was found to inhibit mRR, though considerably less well than the peptidic N-AcFTLDADF ($K_i$ of 400-500 $\mu$M for \textbf{54} vs. $K_i$ of 15-20 $\mu$M for \textbf{55}).
2.5 Carbohydrate Mimetics

SAAs have been used as analogs of biopolymer building blocks to mimic oligo- and polysaccharide structures via amide bond linkages.\[79, 80\] In fact, the assembly of synthetic polysaccharide libraries in solution or on solid-phase is difficult in spite of the recent progresses using chemical and enzymatic techniques.\[81, 82\] Taking advantage of the well-established chemistry of peptides many homooligomers from SAAs and hybrid sequences containing natural amino acids (AAs), carbohydrates and SAAs have been synthesized. The resultant oligomers represent useful drug candidates, since they may overcome the problems associated with oligosaccharide and peptide libraries such as the susceptibility towards glycosidases due to the altered peptide backbone, and such as their resistance to many proteases due to their resemblance to carbohydrates.

2.5.1 Linear Oligomers

The first oligomers were synthesized in solution by Fuchs and Lehmann, although they only characterized the individual products by mass spectroscopy.\[83-86\] The first dimers were synthesized from D-glucosaminuronic acid and D-mannosaminuronic acid by coupling with DCC by Tsuchida et al. in 1976.\[87\]

More recently oligomers were synthesized both in solution\[3, 5, 16\] and on solid phase,\[17, 88\] and have been proposed to mimic oligosaccharides\[4\] and oligonucleotides (so called GNA, Glucopyranosyl Nucleic Amide) (Figure 9).\[89, 90\] The aim of GNA development was to improve the properties of the presently most useful class of antisense agents, the phosphorothioates. Hence, to find more stable agents, less toxic and more selective binders than the phosphorothioates. After the discovery, that peptide nucleic acid (PNA) is a selective binder of DNA and RNA, the idea of replacing the phosphodiester linkages with amide bonds was advisable. Following this idea Goodnow et al. presented oligomers of 43 (Gum) as novel antisense agents with
the nucleobases attached via N-glycosidic linkage at the anomeric center.\textsuperscript{[89, 90]} The GNAs showed similar selectivity and binding affinities as DNA and RNA.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{https://example.com/figure9.png}
\caption{Oligomers of Gum; $R=\text{Me,}^{[91]} R=\text{Bn}^{[88]}$ and with nucleobases.\textsuperscript{[89, 90]}}
\end{figure}

Wessel and co-workers, who first introduced in 1995 the synthesis of amide-linked oligomers in solution (Figure 9), used a [2+2] block synthesis.\textsuperscript{[3]} In their synthetic protocol benzyloxycarbonyl (Z) was employed as amine protecting group and carboxylic acids were protected as tert-butyl ester. The two monomer building blocks were coupled via mixed anhydrides and the so obtained dimer after tert-butyl deprotection was activated via 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT). No protection of the hydroxyl groups was needed.

Fügedi et al. presented several oligomers, which were evaluated in glycosidase inhibitor assays with moderate activities (Figure 10).\textsuperscript{[91-94]}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{https://example.com/figure10.png}
\caption{Oligomers by P. Fügedi’s group.\textsuperscript{[92, 94]}}
\end{figure}
In the field of carbohydrate mimetics sialic acid analogs have received particular attention. Sabesan reported the peptide linked sialoside 56 mimicking an $\alpha$-D-NeuAc(2→6)$\beta$-D-Gal unit which is found in numerous glycoproteins and glycolipids, and as well as a receptor ligand for influenza virus hemagglutinin and as a substrate for neuraminidase (Figure 11). The inhibitory activity of 56 was not reported.

Figure 11: SAA-galactose conjugate 56 with an amide linkage replacing the natural ether linkage.

Gervay et al. described the synthesis of amino acid conjugates to $N$-acetylneuraminic acid and later that of amide-linked dimeric sequences in solution. Oligomer 57 (Figure 12) however was synthesized using solid-phase techniques. To determine whether it adopted the same conformation as its glycosyl-linked cognate, its solution structure was analyzed. Although the conformational preferences of these structure were not completely resolved and were dependent on chain-length, amide proton NH/ND exchange rates determined by NMR and circular dichroism spectra can be interpreted as evidence for a preferred secondary structure.

Figure 12: Amide-linked sialooligomers by Gervay et al.
SAA oligomers were sulphated as reported by the Ichikawa group (Figure 13) to mimic and replace natural sulphated polysaccharides such as dextran sulfate and heparin, well-known inhibitors of HIV replication, in order to overcome the poor absorption, instability and anticoagulant activity of their precursors. A sulphated tetrameric analogue linked via the C-1 β-carboxylate and the C-2 amino groups fully blocked syncytium<sup>b</sup> formation caused by HIV infection to CD4 cell at 50 µM concentration<sup>5</sup> and the sulphated β-1,6 amide-linked analogue showed micromolar activity in the protection of MT2 cells from HIV infection.<sup>16</sup>

![Chemical structures](image)

**Figure 13:** SAA oligomer building blocks and general structures of the oligomers by Ichikawa et al. (R=H, SO₃Na)<sup>5,16</sup>

Hybrid molecules with alternating SAAs and β-amino acids were also designed (Figure 14).<sup>98</sup> The objective was to create another class of non-natural peptide or carbohydrate molecules and to screen them in an *in vitro* assay system involving highly metastatic tumor cell lines. An inhibitory activity towards cell adhesion (Chemotaxis) and invasion was observed with an IC₅₀ = 10 µM.

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<sup>b</sup> *syncytium*: Multinucleated masses produced by the fusion of many cells; often associated with viral infections.
The work of van Boom et al. introduced a new aspect to the synthesis of SAA oligomers. For the first time glycosylated SAAs or disaccharide SAAs were used to assemble the branched oligosaccharide mimetic 58 as a mimetic of the phytoalexin elicitor branched α-methyl heptaglucoside 59 (Figure 15). In 58 four amide bonds replace the β-1,6-acetal linkages of the pentasaccharide backbone of 59. The building blocks for oligomer 58 were readily accessible by glycal chemistry. They attributed the lack of phytoalexin-elicitor activity shown by the β-1,6-glucuronosylamide oligomer to the reduced flexibility of the amide bond with respect to the native acetal bond or to a different molecular structure and hydrogen-bonding potential.

With the aim of designing artificial glycoclusters with specific functions, Nishimura and co-workers reported the synthesis of poly(SAAs) 60 able to self-assemble to form stable monomolecular layers (Figure 16). Polymerization of 1-O-
dodecyl-Gum, derived from the readily available D-glucofuranurono-6,3-lactone, was accomplished using diphenylphosphoryl azide (DPPA).

![Figure 16: Poly(SAAs) able to self-assemble.](image)

### 2.5.2 Cyclic SAA-Oligomers

Recently, mixed cyclic oligomers containing SAAs have been proposed by van Boom et al.\cite{101} and by us as potential molecules for host guest chemistry (Figure 17).\cite{102, 103} 2D NMR data suggests preferred secondary conformations for oligomers 61\cite{101} and 62.\cite{102, 103}

![Figure 17: Mixed cyclic oligomers 61\cite{101} and 62.\cite{102}](image)
The parallel solid-phase synthesis of cyclic sugar amino acid/amino acid hybrid molecules containing furanoid SAAs was carried out by van Boom et al. using Boc N-protection strategy and BOP/DIPEA as coupling reagents.\textsuperscript{[101]} The first amino acid was anchored on an oxime resin in order to employ acid catalyzed cyclization and cleavage from the resin.

In recent work, we introduced cyclic homooligomers of SAAs as novel cyclo-dextrin-like artificial receptors (Figure 18).\textsuperscript{[59]} This idea was based upon the assumption that a cyclic array of carbohydrate moieties and amino acid functional groups may lead to exquisite specificity of recognition and catalysis.

By exploiting standard solid and solution phase coupling procedures linear and cyclic homooligomers containing Gum were synthesized.\textsuperscript{[59]} High yields and very short coupling times for the oligomerization and cyclization of sequences containing 2, 3, 4 and 6 units were achieved. The conformational preferences in aqueous solution of the cyclic derivatives and their applications as potential host molecules were described. Taking into consideration the trans configuration of the amide bonds and the $^4C_1$ conformation of the pyranoid ring, confirmed via coupling constants and ROE data, two low energy structures were found for the SAA unit which differ in the relative orientation (\textit{syn} or \textit{anti}) of the C-H$^5$ and C=O bonds. Stereoviews of the all-\textit{syn} and all-\textit{anti} conformations of the cyclic trimer are depicted in Figure 18. The molecular structure of the cyclic oligomers in the all-\textit{syn} conformation generates a hydrophilic exterior surface and a nonpolar interior cavity which resemble the cyclo-dextrin molecular shape. Indeed, the complexation of the cyclic hexamer with two model guest molecules ($p$-nitrophenol, benzoic acid) was proved from titration studies using NMR the spectroscopic parameters: chemical shifts, longitudinal relaxations ($T_1$) and diffusion coefficients. All of them showed different values for host and guest molecules measured independently and in the presence of each other.
2.5.3 **Glucosidase Inhibitors**

Sugar mimics in which the ring oxygen has been replaced by nitrogen have gained considerable interest as inhibitors of glycosidase, enzymes which are involved in numerous biological processes. The nitrogen substitution renders the compounds metabolically inert, but does not prevent their recognition by glycosidases and other carbohydrate-recognizing proteins. They inhibit glycosidases by mimicking the pyranosyl and furanosyl moiety of the corresponding substrates. The realization that amino-sugar glycosidase inhibitors might have enormous therapeutic potential in many disease or protective mechanisms by altering the glycosylation or catabolism of glycoproteins, or by blocking the recognition of specific sugars, has led to a tremendous interest and demand of these compounds. Thus, glycosidase inhibitors are potential antiviral, anticancer and antidiabetic drugs. As the extraction and isolation of naturally occurring glycosidase inhibitors from often rather scarce sources is both time-consuming and costly, many natural products and analogues have been synthesized.\[104-106\]

Figure 18: Stereoviews of the all-syn and all-anti conformations of the cyclic trimer.
Many of them, may be considered as SAAs, like the examples shown in Figure 19. The analogue 63 of deoxymannonojirimycin was isolated from Lonchocapus seciceus, and has been shown to be a potent and specific inhibitor for both a glucoprotein\(^c\)-processing mannosidase and a bovine \(\alpha\)-L-fucoside.\(^{[107]}\)

\((2S,3R,4R,5S)-3,4,5\text{-Trihydroxy}p\text{ipecolic acid}\) 64 was isolated from Raphia racemosa\(^{[108]}\) as a glucuronidase and iduronidase inhibitor.\(^{[109]}\) Siastatin B (8) is a potent neuraminidase inhibitor, and was first isolated in 1974 from Clostridium perfringens,\(^{[36]}\) its absolute configuration was proven by total synthesis.\(^{[110]}\) The inhibitory activity of those isolated natural SAAs, sparked extensive research in that field and many synthetic derivatives were synthesized.\(^{[111, 112]}\) Using 63 as a lead structure Ichikawa et al. developed the potent (\(K_i= 79\) nM) \(\beta\text{-glucuronidase inhibitor}\) 68.\(^{[113]}\)

\begin{align*}
\text{63} & \quad \text{64} \quad \text{65} \quad \text{66} \\
\text{67} & \quad \text{8} \quad \text{68}
\end{align*}

**Figure 28:** Some of the SAA, which are glucosidase inhibitors; their names and references: 63 (2S,3R,4R,5R)-3,4,5-\text{Trihydroxy}p\text{ipecolic acid},\(^{[107]}\) 64 (2S,3R,4R,5S)-3,4,5-\text{Trihydroxy}p\text{ipecolic acid},\(^{[108, 109, 112]}\) 65 (2R,3R,4R,5S)-3,4,5-\text{Trihydroxy}p\text{ipecolic acid},\(^{[112]}\) 66 (2S,4S,5S)-3,4-\text{dihydroxy}p\text{ipecolic acid},\(^{[112]}\) 67 bulgencinine,\(^{[112]}\) 8 siastatin B,\(^{[36, 110]}\) 68 (3S,4R,5R)-4,5-\text{dihydroxy}3-\text{piperidinecarboxylic acid}.\(^{[113]}\)

Among the many ring nitrogen containing glycosidase inhibitors, most are synthesized by reductive cyclic amination. The hydrogenation of pyridine ring is seriously hampered by the lack of crucial stereoselective hydrogenation.\(^{[104-106]}\)

\(^c\) Glucoproteins: Glycoproteins with a molecular weight of approximately 620,000 to 680,000
2.6 Peptidomimetics

2.6.1 Carbohydrate-Based Peptidomimetics

Since proteins tend to exert their biological activity through only small regions of their folded surfaces, their functions could in principle be reproduced in much smaller designer molecules that retain these crucial surfaces. Their are many options for modifications, such as steric constraints, cyclization, and/or replacement of the peptidic backbone or part of it to stabilize the bioactive conformation and fine tune bioavailability. Carbohydrates in general and SAA in particular can provide just that. SAAs, their mixed linear and cyclic oligomers, and homooligomers can adopt robust secondary turn structures or helices and thus may allow one to mimic structural elements of natural biopolymers. They can be used as substitutes for single amino acids or as dipeptide isosters. If used as replacement of hydrophobic residues, the sugar can also be functionalized with hydrophobic side chains (e.g. they may be benzylated), however if hydrophilic residues are replaced, or if solubility should be improved the sugar hydroxyl groups are unprotected or functionalized with hydrophilic residues.

2.6.1.1 Linear SAA Homooligomers and Their Structures

For the peracetylated tetramers of SAA 25 (Figure 5) as well as for the SAAs 29 and 30 Fleet et al. observed a repeating \( \beta \)-turn like bond structure by a combination of solution NMR and IR techniques.\[^7, 20, 51, 114]\] All of the oligomers adopt a repeating 10-membered hydrogen-bonded ring structure. These results show, that protecting groups and substitution patterns of the hydroxyl groups in the sugar ring do not significantly influence the secondary structure of their homooligomers.

Based on their solution NMR studies Fleet et al. propose a left-handed helical structure for the octamer of 31 (Figure 5).\[^{51, 53, 115}]\]
The tetramer of 26 shows no secondary structure. The NMR signals are not dispersed. For all three amide protons the chemical shifts are indistinguishable, while for the oligomers above with defined secondary structures dispersed chemical shifts were observed. Chakraborty and co-workers investigated several protected and unprotected oligomers of 26. The unprotected octamer shows a strong positive band in its circular dichroism (CD) spectra in MeOH and TFE, which might hint at a possible presence of a distinct secondary structure. However, the $^1$H-NMR spectra in various polar solvents, did not show dispersed chemical shifts for the amide protons.

2.6.1.2 Turn Mimetics and Model Peptides Containing SAAs

We explored the conformational influence of a large number of SAAs on the peptide backbone by incorporation of SAA 43, 47, 48 and 69-73 respectively into several different model peptides as well as in biologically active peptides. 

Figure 20: Superposition of 74 (black) and an idealized $\beta^{II'}/\beta^{II'}$-turn arrangement (gray); superposition of 75 (black) and an idealized $\beta^{II'}/\beta^{II'}$-turn arrangement (gray); superposition of 76 (black) and an idealized $\beta^{II'}/\gamma$-turn arrangement (gray).
The cyclic and linear peptides were investigated by NMR spectroscopy, distance geometry, and subsequent MD calculations to determine the potential of the turn-inducing and stabilizing potential of SAAs as both local and global constraints. As can be seen in Figure 20 the SAAs can adopt defined turn arrangements in cyclic peptides.

This resulted, in a SAA construction kit for predetermined constrained local conformations in synthetic peptides containing a series of SAAs (Figure 21).\[18, 21, 22\]

These units offer possibilities as mimetic structures for both, amino acids and dipeptide isosters.

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**Figure 21:** Extended SAA construction kit.
SAAs 43 and 69-72 induce β-turns independent of the substitution pattern of the sugar ring while SAA 73 mimics a γ-turn.

The first non-cyclic, peptidic β-hairpin structure containing a SAA, proven by CD and NMR spectroscopy, was recently reported by van Boom et al. They used SAA 77, to stabilize a β-turn in the polypeptide chain AcKKYTVSI-SAA 77-KKITVSI (Figure 22).[122]

Figure 22: Relevant long range and turn region NOEs of 78. [122]
2.6.2 ß-Peptides

ß-peptides are oligomers formed of ß-amino acids. ß-Amino acids are not unknown in nature. Some simple ß-amino acids are found in the metabolism of mammals. More complicated and highly active ones are found for instance in ß-lactam-antibiotics, in macrocyclic peptides from marine organisms, in microorganisms and in the anticancer agent paclitaxel (taxol).

The structure of several polydisperse poly-ß-amino acids, the so called nylon-3 derivatives, have been studied in both the solid state and in solution. However, there do exist quite controversial reports on whether poly(α-isobutyl-L-asparatate) forms a helical or a ß-sheet structure. Until 1995, no structure of a ß- or γ-peptide was known. However Gellman et al. concluded in 1994 from IR studies of ß-di-peptides, that compact and specific folding patterns will be most likely adopted by ß-peptides, when intramolecular hydrogen bonds are unfavorable between nearest neighbor amide groups on the polymer backbone. It was assumed that the extra methylene group adds additional degrees of rotational freedom, thus making the formation of a stable secondary structure less favourable than for the analogous α-peptide. However, further work of Seebach’s and Gellman’s groups demonstrated the opposit: Already a ß-hexapeptide can form a stable helical structure in solution. α-Peptides only form distinct stable secondary structures in solution, when they consist of at least 15-20 amino acids.
2.6.2.1 Helices

Up to now four different types of β-peptide-helices are known.\cite{136} The 14-helix, also called \(3_1\) helix\cite{137-139} the 12/10/12 helix,\cite{139, 140} and the 12-helix, also called \(2.5_1\) helix.\cite{141-145}

2.6.2.1.1 The 14-Helix

The 14-helix (also called \(3_1\) helix\textsuperscript{d}) depicted in Figure 23 was discovered almost simultaneously by Gellman’s and Seebach’s groups.\cite{137, 138} Short oligomers of the β-amino acid trans-2-aminocyclohexanecarboxylic acid (trans-ACHC)\cite{137} and the hexa-β-peptide TFA·H-(\(β^3\)-hVal-\(β^3\)-hAla-\(β^3\)-hLeu-\(β^3\)-hVal-\(β^3\)-hAla-\(β^3\)-hLeu)-OH\textsuperscript{e} 79 already form that helix.\cite{149} It is characterized by a 14-membered ring hydrogen bond between an amide proton and a carbonyl. The side chains of the β-amino acids \(i\) and \(i+3\) reside above each other in close proximity on the outside of the helix.

The helicity and the dipole of the homologous L-β-peptides are inverse to that of the \(α\)-helix formed by the corresponding \(α\)-peptides (Figure 23). Only three β-amino acids are needed per full turn of a 14-helix, while 3.6 a-amino acids are required for a single winding of the a-helix. The pitch off an a-helix is 5.4 Å wide and its diameter 4.3 Å, whereas the pitch of the 14-helix is only 5.0 Å wide, however the diameter is 4.7 Å.

\textsuperscript{d} Crystallographic nomenclature according to ref.\cite{146}.

\textsuperscript{e} The notation β-HXaa for a homolog of the \(α\)-amino acid Xaa was introduced by Ondetti and co-workers\cite{147} and has been used and refined by Seebach and co-workers to \(β^2\) and \(β^3\)-HXaa, were the numbers indicate the position of the side chains in the β-amino acids.\cite{148}
Figure 23: Comparison of the side and top views of the α-helix (3.613-helix) formed by α-L-peptides (left) and the 14-helix (3_{14}-helix) formed by the homologous β-peptides (right).\textsuperscript{138, 149} From the side view the opposite dipoles, and opposite helicities are distinctly visible. The top view clearly reveals the different diameters and the different relative arrangements of the side chains.
There does also exist a right-handed 14-helix formed for instance by the \( \beta^2 \)-peptide \( \text{CF}_3\text{CO}_2\text{H-H-(}\beta^2\text{-hVal-} \beta^2\text{-hAla-} \beta^2\text{-hLeu-} \beta^2\text{-hVal-} \beta^2\text{-hAla-} \beta^2\text{-hLeu)-OH} \) 80 with all-R-configuration. The CD spectra of 80 is almost an exact mirror image of the one of 79 (Figure 14). The CD pattern is characteristic for the presence of an left- or right-handed 14-helix.

![Figure 14: CD-spectra of the \( \beta^2 \)-hexapeptide 79 (- - -) forming a left-handed 14-helix and of the \( \beta^2 \)-hexapeptide 80 (— ) forming a right-handed 14-helix in methanol (concentration 2·10^{-4} M, molar ellipticity \([\theta]\) in 10 deg cm^2 mol^{-1}).](image)

2.6.2.1.2 The 12/10/12-Helix

Seebach and co-workers discovered this new type of \( \beta \)-peptide helix in 1997.\(^{150} \) \( \beta \)-hexapeptides constructed from alternating \( \beta^2 \)- and \( \beta^3 \)-amino acid building blocks as the hexapeptide 81 (Figure 25) were designed to form a 14- but they formed a 12/10/12-helix, which consists of a central 10-membered and two terminal 12-membered hydrogen-bonded rings (Figure 25). The C=O and N-H bonds point alternatively up and down along the axis of the helix, thus the net dipole is almost zero. As in the 14-helix, side chains of the \( \beta \)-amino acids \( i \) and \( i+3 \) reside above each other.
Figure 25: Side and top view of the low energy model of β-peptide 81, obtained by a long, 10 ns MD simulation without any experimental restraints in vacuo at 50 K using the AMBER* molecular model and force field. All carbon-bound hydrogens have been omitted for clarity. The figures were generated by MolMol\cite{151} and raytraced by POV-Ray\cite{140}).

The 12/10/12-helix shows a new type of CD pattern with a strong maximum at 205 nm, different from that of the 14-helix, with a maximum at 200 nm and a minimum at 215 nm (Figure 26).
Figure 26: Circular dichroism (CD) spectra of β-peptides 81 and 82. Comparison of the typical CD pattern assigned to the 12/10/12 helix (—— 81), and to the 14-helix (- - - 82).

Seebach and co-workers argued that hydrophobic interactions of the aliphatic side chains as well as hindrance of solvent accessibility were the reasons why the 12/10/12 helix rather than the 14-helix is formed in alternating β²/β³-peptides.

The major difference between those two helices is the polarity: The 12/10/12 helix has almost no resulting dipole moment of the molecule, while the 14-helix has one with the positive end at the C- and the negative at the N-terminus. The 14-helix consists of only one type of 14-membered turn whereas in the 12/10/12 helix we have two different turns, the central 10-membered- and the two terminal 12-membered turns.

2.6.2.1.3 The 12-Helix

Gellman and his group reported β-peptides with a different secondary structure, a left-handed 12-helix (also called 2.5₁-helix) (Figure 27).[^141] They synthesized a series
of oligomers of (R,R)-trans-2-aminocyclopentanecarboxylic acid (trans-ACPC). The two longest oligomers, a hexamer 83 and the octamer, have been examined by X-ray crystallography\textsuperscript{[145]} and NMR spectroscopy in pyridine.\textsuperscript{[141, 144]} The CD-spectra of hexamer 83 show a maximum at about 204 nm, zero crossing at about 214 nm and a minimum at 221 nm.

![Model of the 12-helix and structure of the hexapeptide 83.][1]

Both crystal structures show that the β-peptide backbone adopts a regular helix that is defined by a series of interwoven 12-membered ring hydrogen bonds. Each hydrogen bond links an amide proton to a carbonyl oxygen three residues towards the N-terminus. The resulting dipole of this helix, has the same direction as that of the a-helix of a-peptides. CD data suggest that the conformational preference of trans-ACPC oligomers in methanol is strongly length-dependent, which implies that 12-helix formation is a cooperative process, as seen for the a-helix formed by conventional peptides.

\textbf{Figure 27: Model of the 12-helix and structure of the hexapeptide 83.}\textsuperscript{[141]}
Recently Gellman and co-workers succeeded in designing water-soluble \( \beta \)-peptides forming the 12-helix.\(^{[142, 143]} \) Hexameric \( \beta \)-peptides 84a-c (Figure 28) contains N-sulfonylated trans-3-aminopyrrolidine-4-carboxylic acid (S-APC). The S-APC residue represent Gellman’s group’s general strategy for introducing specific side chains at defined positions along the surface of 12-helical \( \beta \)-peptides.\(^{[142]} \)

### 2.6.2.2 The \( \beta \)-sheet structure

The first \( \beta \)-peptide sheet-type structure was discovered by Seebach and co-workers\(^{[138]} \) for the fully protected tripeptide Boc-\( \beta \)-hVal-\( \beta \)-hAla-\( \beta \)-hLeu-OMe. Only recently they succeeded in obtaining an X-ray structure of the N-deprotected derivative 85, which adopts also a sheet-type structure (Figure 29).\(^{[152]} \)
It is different from the known pleated sheet of α-peptides, where C=O and N-H bonds point up and down alternatively. The β-peptide pleated sheet is polar, since all carbonyls point in one direction, while all N-H bonds point in the opposite direction. The peptides are linked by a 14-membered H-bonded ring.

2.6.2.3 Tube-like Structures

The β³-HAla-derived cyclic oligomers 86-88 form supramolecular, tubular structures similar to that of certain cyclo-α-peptides, as was determined by powder diffraction experiments (Figure 30). Those 16-membered rings are linked by four non-linear C=O···H-N intermolecular H-bonds.
Figure 30: X-ray and molecular structure of the cyclic oligomers 86-88\cite{153}
2.7 Biologically Active Peptides Containing SAA Building Blocks

After verifying the conformational influence of the SAAs in cyclic peptides as compared to the backbone of model peptides our group, as well as several other groups focused the attention on the synthesis, conformational and biological evaluation relative to biologically active peptides. SAAs were used as turn mimetics and local constraints, but also to improve pharmacokinetics, to introduce a radioactive label for imaging or to glycosylate a peptide. The opioid, integrin and somatostatin receptors were some of the pharmacologically interesting targets, which were chosen to investigate. Two structurally cyclic peptides, such as the "Veber-Hirschmann" peptide cyclo(-Phe-Pro-Phe-D-Trp-Lys-Thr-) and our cyclo(-Arg-Gly-Asp-D-Phe-Val-) peptide as well as linear LH-RH analogs, were selected as a platform to determine the pharmacological potential of SAA scaffolds. Soon other groups started using SAAs in various biologically active peptides as well.

2.7.1 Somatostatin Analogues

Somatostatin is a 14-residue cyclic peptide hormone formed in the hypothalamus. Like its precursor somatostatin-28 it plays an important role in a large number of physiological actions. For instance it inhibits the release of growth hormone (GH), and plays a role in the inhibition of insulin secretion. Somatostatin analogues were shown to inhibit tumor cell growth and induce apoptosis.

The first SAA introduced into a bioactive peptide was glucosyluronic acid methylamine (Gum) (Chapter 2.6.1.2, Figure 21). We introduced it as a dipeptide isoster into a cyclic hexapeptide of the “minimal” somatostatin sequence cyclo[SAA-Phε-D-Trp-Lys-Thr-]. By various NMR techniques and subsequent distance geometry calculations and molecular dynamic simulation it was shown, that SAA 43 induces a β-turn. (Figure 20)

The growth hormone release was inhibited with an IC50 value in the sub-µM range.
2.7.2 SAAs in enkephalin analogs

In order to explore the effect of the dipeptide isosters SAA 47 and SAA 43 on the conformation of linear peptides by NMR spectroscopy we synthesized the Leu-enkephalin analogs H-Tyr-SAA 47-Phe-Leu-OMe (89) and H-Tyr-SAA 43-Phe-Leu-OMe (90) (Figure 31). The SAAs replace the Gly-Gly dipeptide of the natural sequence H-Tyr-Gly-Gly-Phe-Leu-OH, where Gly-Gly serves as a spacer in enkephalin between the messenger amino acid Tyr which is essential for the activity and the address sequence Phe-Leu responsible for the selectivity.

![Chemical structures](image)

**Figure 31:** Leu-enkephalin analogues, in which Gly-Gly is replaced by SAAs.

Due to the functionality of the SAAs, these building blocks can be synthesized as Fmoc-derivatives and then be employed under standard SPPS conditions using the Fmoc strategy and TBTU/HOBt as a coupling reagents. The two enkephalin analogs 89 and 90 showed no biological activity in the guinea pig ileum assay (GPI).
However, when Chakraborty et al. incorporated their furanoid SAAs \textsuperscript{25, 26, 27} and dideoxy-\textsuperscript{27} analogously into Leu-enkephalin, the analgesic activity of the resulting compounds \textsuperscript{91-94} (Figure 31) was in the same range as that of Leu-enkephalin methyl ester.\textsuperscript{[48, 49]} The N-terminally Boc protected analogues of \textsuperscript{91-94} showed comparable activities as the unprotected ones. For synthesis of their peptidomimetic compounds they employed standard solution Boc strategy, using EDCI and HOBt as coupling agents.\textsuperscript{[172]} Extensive CD and NMR studies, followed by constrained molecular dynamics simulations revealed, that the Boc-protected \textsuperscript{91} and Boc-protected \textsuperscript{93} have folded conformations composed of an unusual nine-membered pseudo $\beta$-turn-like structure with a strong intramolecular H-bond between LeuNH $\rightarrow$ sugarC3-OH. This turn moves the two aromatic rings of Tyr and Phe in close proximity, a prerequisite for biological activities of all opioid peptides. From analysis of the 3-D structures of \textsuperscript{91-94}, they concluded, that a cis-$\beta$-hydroxycarboxyl moiety anchored on a five-membered ring was the essential structural motif, whose presence in some of these analogues was responsible for their folded conformations.

The group of Toth used SAA \textsuperscript{72} (Figure 21) to “glycosylate” Leu- and Met-enkephalins via amide bond linkage to the C-terminal Leu- or Met respectively (Figure 32).\textsuperscript{[157]}

They constructed the peptides \textsuperscript{95-98} on solid phase, using Fmoc-strategy and HBTU/HOBt/DIEA as coupling reagents. The peracetylated SAA \textsuperscript{72} (Figure 21) azide precursor was reduced on resin by treatment with a mixture of triethylamine and propane-1,3-dithiol to generate the free amine \textit{in situ}. They thereby used the azide as a
amine protecting group instead of the Fmoc-protected SAA 72. Pharmacological evaluation, using a GPI and mouse vas deferens (MVD) assay revealed, that 95 was 3 times more potent in the GPI assay and 40 times more potent in the MVD assay than Leu-enkephalinamide at inhibiting electrically stimulated muscle contractions.

### 2.7.3 Protein:Farnesyltransferase

Inhibition of **Protein:Farnesyl Transferase** (PFT) should be effective to combat colon and pancreatic carcinomas.\[173\] Ras protein association with the plasma membrane is initiated by post-translational farnesylation of the cysteine unit of the CAAX box (C, cysteine; A, any aliphatic amino acid; X, serine or methionine) in the pre-Ras protein by PFT, and that is essential for Ras function.\[174\] Continuously switched on phosphorylation cascade is caused by the activated GTP-bound state, in which onco-genic Ras proteins are locked. Van Boom et al. used SAA 77 and SAA 99 (Figure 33) as dipeptide isoster to replace AA. The resulting compounds 100 and 101 were both less active than for instance CAAX based inhibitors containing 4-aminobenzoic acid as AA. However both compounds show a distinct inhibitory effect – the “2,6-cis” isomer, the one containing SAA 77 is about twice as active as the “2,6-trans” isomer, containing SAA 99 (Figure 33).\[154\]

\[
\begin{align*}
\text{SAA} & = \text{SAA} 77, 2,6-\text{cis} \\
\text{IC}_{50} &= 764 \text{ mM} \\
\text{100} & = \text{SAA} 99, 2,6-\text{trans} \\
\text{IC}_{50} &= 214 \text{ mM}
\end{align*}
\]

**Figure 33:** Structures and \(\text{IC}_{50}\) values of PFT inhibitors by van Boom et al.\[154\]
2.7.4 Integrin-Ligands

Integrins are located at the cell surface of a number of different cell types. Several play a major role in cell-matrix interaction as well as in angiogenesis, which is important for tumorgenesis. This invoked a pharmaceutical interest in $\alpha_v\beta_3$-antagonists, to block tumor induced neo-angiogenesis.\cite{160, 175-179} The recognition sequence of many integrin ligands has been shown to consist of the three amino acids arginine (R), glycine (G), and aspartic acid (D), the “RGD”-motif. In our group SAAs have been used in cyclic RGD analogues as turn mimetics,\cite{117} as well as “glycosylating agent” to improve the pharmacokinetic properties of the compounds\cite{117, 155} and for tumor imaging to introduce the radio-nuclides.\cite{156} In the earliest attempts to modify cyclic RGD-peptides with carbohydrates impaired the biological activity of the RGD-compounds.\cite{180, 181} In more recent work\cite{117} the cyclic pentapeptide cyclo(-Arg-Gly-Asp-D-Phe-Val-) \textbf{102}, which binds selectively $\alpha_v\beta_3$-integrins, was chosen as a lead structure.\cite{160, 175-179} In \textbf{102} the backbone conformation of the residues D-Phe-Val resembles a $\beta$II'-turn, thus forcing the RGD-sequence, which acts as pharmacophore, into a kinked, $\alpha_v\beta_3$-selective conformation. Fully benzylated Gum \textbf{43}, (both the $\alpha$- and the $\beta$-anomer) has been shown to induce the type of turn necessary for the biological activity,\cite{15, 18, 21} and also had the aromatic functionality required for enhanced activity. Therefore we used both anomers to replace D-Phe-Val in \textbf{102} to give \textbf{103} and \textbf{104}, which exhibit a relatively high $\alpha_v\beta_3$ activity of IC$_{50}$=150 nM (\textbf{103}) and 25 nM (\textbf{104}). However \textbf{104} showed also a high activity against the $\alpha_{\text{IIb}}\beta_3$-receptor (IC$_{50}$=13.4 nM). The loss of selectivity of \textbf{104} compared to that of the $\alpha$-SAA-peptide \textbf{103} can be explained by the higher flexibility of $\beta$-SAA-peptide \textbf{104}. Because of that flexibility a kinked as well as a stretched flexibility of $\beta$-SAA-peptide \textbf{104}. Because of that flexibility a kinked as well as a stretched conformation can be realized in solution. The compound is therefore able to re-adjust its conformation, matching the steric demands of both the $\alpha_{\text{IIb}}\beta_3$- and $\alpha_v\beta_3$-receptor pockets: high activity with a loss of selectivity is the consequence (Figure 34). This is confirmed by their NMR-based structural analysis and subsequent MD-simulations.
Figure 34: The RGD-conformations of the α-(103) and β-compounds (104) are compared to typical representatives of αvβ3- and αIIbβ3-antagonist structures, to the lead peptide cyclo(-RGDFV-) and the compound cyclo(-D-Abu-NMeArg-Gly-Asp-Mamb-) (Abu = A-aminobutyric acid, Mamb=meta-(aminomethyl)benzoic acid). View along the pharmacophoric RGD-moiety is directed parallel to the ring plane of the cyclic peptide, as indicated by black arrows.

To improve pharmacokinetic properties Val was substituted by Lys, which was glycosylated with different SAAs via an amide bond. Activity and selectivity was sometimes even enhanced. Furthermore via the amine function of the SAAs
\(^{18}\)F-labeling is possible.\(^{156, 183}\) In \textit{in vivo} studies using \(\alpha_\text{v}\beta_3\)-positive and -negative murine and xenotransplanted human tumors receptor-specific binding of the radio-labeled 105 yielded high tumor:background ratios. First imaging results, using a small animal positron emission tomograph suggest 105 is suitable for noninvasive determination of the \(\alpha_\text{v}\beta_3\) integrin status and therapy monitoring.

**Figure 35:** Structure of 105 and transaxial small animal PET images of nude mice bearing human melanoma xenografts, treated with 105.
3 Results and Discussion

3.1 Synthesis of Two New Furanoid SAAs [22, 118-121, 184, 185]

In contrast to most other SAAs so far described, synthesis of f-SAA 106 and f-SAA 107 is simple and direct (Scheme 5). Both were synthesized via the azides 109 and 110.

Scheme 5: Synthesis of Fmoc-protected f-SAA 106 and f-SAA 107: a) Tf$_2$O, py, -10 °C, CH$_2$Cl$_2$, 98 %; b) NaN$_3$, Bu$_4$NCl (cat), 50°C, DMF, 70 %; c) conc. HOAc, 3h, 65 °C, 94 %; d) NaIO$_4$, 5h, 10 °C, MeOH; e) KMnO$_4$, 50% HOAc, rt, 89 % over two steps; f) H$_2$, Pd/C, FmocCl, NaHCO$_3$, pH 8-9, MeOH, THF, rt, 76-90 %; g) NaOCl, TEMPO (cat), KBr, CH$_2$Cl$_2$, sat. aq NaHCO$_3$, Bu$_4$NCl, 62%.
The synthesis of the azide precursor 109, which has been reported before,\textsuperscript{[1, 186-190]} has been optimized. However, protected or unprotected f-SAA 106 has not so far been reported in the literature. The key step of the synthesis of f-SAA 106 is the azidolysis of the triflate activated diacetone glucose 108 (Scheme 5). Elimination occurs as a major side-reaction (Table 2).\textsuperscript{[188-190]}

**Table 2:** Reaction conditions, reagents and yields from the literature for the conversion of 108 to 109.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Reagents</th>
<th>Conditions</th>
<th>Yield</th>
<th>Author (et al.)/ Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF</td>
<td>NaN(_3)</td>
<td>50°C, 3.5 h</td>
<td>48 %</td>
<td>L. Daley\textsuperscript{[188]}</td>
</tr>
<tr>
<td>HMPT</td>
<td>NaN(_3)</td>
<td></td>
<td>60 %</td>
<td>J. M. G. Fernàndez\textsuperscript{[190]}</td>
</tr>
<tr>
<td>DMF</td>
<td>guanidinium-azide</td>
<td>25°C, 6 h</td>
<td>61 %</td>
<td>H. H. Baer\textsuperscript{[189]}</td>
</tr>
<tr>
<td>DMF</td>
<td>NaN(_3), cat. Bu(_4)NCl</td>
<td>50°C, 5 h</td>
<td>70 %</td>
<td>this work\textsuperscript{[118-121]}</td>
</tr>
<tr>
<td>THF</td>
<td>Me(_3)SiN(_3), Bu(_4)NF</td>
<td>50°C, Ar, 12 h</td>
<td>exclusive elimination</td>
<td>this work\textsuperscript{[119]}</td>
</tr>
</tbody>
</table>

This may be attributed to the poor solubility of azides in organic solvents. Considerably higher yields of 70 % were achieved and expensive\textsuperscript{[189]} or toxic\textsuperscript{[190]} reagents were avoided, by the simple addition of catalytic amounts of the phase-transfer-catalyst Bu\(_4\)NCl. The Bu\(_4\)NCl renders the NaN\(_3\) significantly more soluble. Thus under inversion of configuration of C\textsuperscript{3}, at 50°C in DMF, the triflate-ester 108 is converted to the azide 109 in 70 % yield. Efforts to further enhance substitution yields and suppress elimination more sufficiently by using the “naked” azide-anion generated *in situ* from trimethylsilylazide and t-Bu\(_4\)NF failed: Elimination took place almost exclusively - 109 could not be detected.

The exocyclic hydroxyl groups of 109 were quantitatively and selectively deprotected to 110 using concentrated acetic acid.\textsuperscript{[186]} To obtain Fmoc-protected f-SAA 106 the diol 110 is oxidatively cleaved using sodium periodate, followed by potassium permanganate oxidation to yield the acid 111.\textsuperscript{[186]} In an one-pot reaction the azide 111 was reduced and simultaneously Fmoc-protected to yield about 70 % of the Fmoc-protected f-SAA 106, suitable for solid phase synthesis. Because the acetonide
is fused to a second five-membered ring, it is stable against conventional acids like pure trifluoroacetic acid or 1N hydrochloric acid. The overall yield of f-SAA 106 starting from diacetone glucose was 44%.

For the preparation of f-SAA 107 the azide 110 is first reduced and Fmoc-protected in a similar one-pot reaction as for the conversion of the acid 111 to Fmoc-protected f-SAA 106, followed by selective oxidation of the primary alcohol with 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO), sodium hypochlorite and potassium bromide. To prevent decarboxylation during TEMPO oxidation, it is crucial to keep the pH between 8.5 and 9.5, and the temperature below 0 °C.

3.2 Foldamers\textsuperscript{[22, 118, 119, 184]}

Carbohydrates as well as peptides and proteins are essential biopolymers of life. They are involved in complex biological processes such as catalysis and highly selective molecular recognition. In order to perform those functions the correct folding of the biopolymers creating the active site is crucial, since any kind of interaction is observed only, if the reactive groups are positioned in the correct spatial orientation to each other. Thus the development of small, easy-to-functionalize building blocks and oligomers with backbones of discrete and predictable folding patterns (“foldamers”)\textsuperscript{[137]} is required in order to design and develop molecules with useful biological functions. Chimeras of the three big classes of biopolymers, that is, nucleic acids, proteins and carbohydrates, have attracted great interest as both functional, and structural analogues in recent years, also because of their potential application in drug design. However, sugar amino acids (SAAs) and their oligomers, which bridge carbohydrates and proteins, have only recently been investigated (See chapter 2.6.2).\textsuperscript{[3, 5, 14-21, 56]}

The new furanoid β- and γ-SAA building blocks f-SAA 106 and f-SAA 107 will be presented as analogues to β- and γ-peptides, as structural templates aiming at new structures for peptidomimetic drug design, and as potential foldamers.\textsuperscript{[184, 191]}

The linear mixed oligomer Fmoc–[f-SAA\textsuperscript{106}–β-hGly\textsubscript{3}–OH 113 and the cyclic
mixed oligomer cyclo\{-f-SAA106-β-hGly-\}_3 114 consist of the f-SAA 106 alternating with β-hGly\(^f\) (β-alanine), thus forming hybrids between SAAs and β-peptides. The linear mixed ?-hexapeptide 115 is built up of alternating f-SAA 107 and ?-amino butyric acid (GABA) units. β-hGly and GABA were used as amino acid counterparts, because they represent, likewise to f-SAA 106 and f-SAA 107, β- and γ-amino acids respectively. They are completely unsubstituted, and do not form secondary structures. Thus secondary structure is induced exclusively from the incorporated SAA.

3.2.1 Solid Phase Synthesis of the Linear Mixed SAA-AA-Oligomers 113 and 115

The mixed SAA oligomers 113 and 115 were synthesized using Fmoc strategy as shown in Scheme 6. The f-SAAs 106 and 107 were alternately coupled with β-homo-glycine or GABA respectively to form oligomers 113 and 115. Tritylchloropolystyrene (TCP) resin was employed as solid support. Two equivalents of the f-SAA derivative, whereas three equivalents of β-homo-glycine or GABA were used. Stoichiometric quantities, that is 2 or 3 equivalents of [O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] (HATU) and 20 or 30 equivalents of 2,4,6-collidine as base were used as coupling agent.\(^{[170, 192, 193]}\) After cleavage from the resin with hexafluoro isopropanol (20 % in dichloromethane, 3*10 min),\(^{[194]}\) rp-HPLC purification yielded 71.3 mg of the linear oligomer 113 (41 % yield) and 10 mg (4.7 % yield) of 115 in over 99 % HPLC-purity.

\(^{1}\) The notation β-HXaa for a homolog of the α-amino acid Xaa was introduced by Ondetti and co-workers\(^{[147]}\) and has been used and refined by Seebach and co-workers to β\(^2\)- and β\(^3\)-HXaa, were the numbers indicate the position of the side chains in the β-amino acids.\(^{[148]}\)
**Scheme 6:** Synthesis of the linear oligomer 113: a) HATU (2 equiv), 2,4,6-collidine (20 equiv), 1 (2 equiv), rt, NMP; b) i) washing: NMP (5 × 3 min), ii) Fmoc deprotection: 20 % piperidine (pip) in DMF (2 × 10 min), iii) washing: NMP (5 × 3 min); c) HATU (3 equiv), 2,4,6-collidine (30 equiv), ß-homo-glycine (3 equiv), rt, NMP; d) i) washing: NMP (3 × 3 min), CH$_2$Cl$_2$ (1 × 3 min), ii) vacuo overnight, iii) cleavage: 20 % HFIP in CH$_2$Cl$_2$ (3 × 10 min).
3.2.2 Synthesis of the Cyclic Mixed [f-SAA106-β-hGly], Oligomer 114 in Solution

After Fmoc-deprotection 113 was cyclized using 1 equivalent of HATU and 10 equivalents of 2,4,6-collidine at high dilution (0.4 mM), after 24 h an additional 0.1 equivalents of HATU and 1 equivalent of 2,4,6-collidine were added to afford 114 in quantitative yield (Scheme 7) in a purity >95 % after preparative HPLC.

Scheme 7: Cyclisation of the Fmoc-deprotected linear 113 to the cyclic 114: a) Fmoc deprotection: 20 % pip in DMF (2 × 10 min), rp-HPLC purification; b) 0.4 mM in DMF, HATU (1.1 equiv, added in intervals), 2,4,6-collidine (11 equiv), 25 h, rp-HPLC purification, HPLC purity >95 %.
3.2.3 Structural Analysis

3.2.3.1 CD Spectroscopy

CD spectroscopy has been used successfully to deduce the secondary structure of peptides and proteins consisting of \( \alpha \)-amino acids in solution. Recently Gellman’s and Seebach’s groups expanded the usage of CD spectroscopy to prove secondary structures of \( \beta \)- and \( \gamma \)-peptides.\[^{136, 138, 141, 143, 150, 195-200}\]

CD data for linear 113 in MeCN and MeCN-water solutions at various temperatures (Figures 36 a-d) reveal a distinctive secondary structure of the linear oligomer 113. In order to allow comparison of the different CD curves of the mixed oligomers 113-115, and the monomeric f-SAAs 106 and 107, each curve was divided by the number of amide bonds present.

Comparison of the CD curves of Fmoc-protected f-SAA 106 with the curves of the linear mixed oligomer 113 (Figure 36a) shows, that the major part of the observed molar ellipticity \( T \) per residue is a result of cooperative effects due to the orientation of the backbone amide chromophores caused by a secondary structure and not a result of a single chromophore within the f-SAA 106 residues. The CD signature of 113 (Figure 36a) comprises a strong minimum at about 188 nm, a zero crossing at about 193 nm, and a strong maximum at about 202-203 nm and a weak minimum at about 239 nm.

As expected for secondary structural effects the maxima of the CD curves decrease with increasing temperature (Figure 36b).

A random structure would show the same average spectrum at all different temperatures. When changing the solvent from the relatively unpolar solvent MeCN to a one to one mixture of MeCN and water, the molar ellipticity for 113 decreases to almost half of the value of pure MeCN (Figure 36c). This may be due to the fact that the secondary structure the curve corresponds to is less populated in more polar solvents. As the CD signature of 113 in MeCN and MeCN-water solutions at temperatures between 20 and 75 °C is independent from concentration between 0.5 mM and 0.125 mM, it seems unlikely that aggregation occurs under the chosen conditions.
**Figure 36:** a) CD spectra (molar ellipticity per residue in deg cm$^{-2}$ dmol$^{-1}$) of the linear Fmoc-[f-SAA$^{106}$-β-hGly]$_n$-OH 113 and of Fmoc-protected f-SAA$^{106}$ in CH$_3$CN at 20°C at 0.125 mM (113), and 0.5 mM (106). b) CD data for the linear Fmoc-[f-SAA$^{106}$-β-hGly]$_n$-OH 113 at 0.125 mM in CH$_3$CN at 20, 50 and 75°C. c) CD data for the linear Fmoc-[f-SAA$^{106}$-β-hGly]$_n$-OH 113 at 0.125 mM in CH$_3$CN and CH$_3$CN-water. d) CD spectra of the linear Fmoc-[SAA$^{106}$-β-hGly]$_n$-OH 113 and its cyclic analogue cyclo-[f-SAA$^{106}$-β-hGly]$_n$ 114 at 0.125 mM and 20°C in CH$_3$CN.
The CD signature of 113 (strong minimum at 188 nm, a zero crossing at about 193 nm, strong maximum at 202-203 nm, weak minimum at 239 nm) differs from the so far known CD patterns of β-peptides, which are associated with β-peptide structures. The CD pattern of the 12/10/12-helix shows a strong maximum at 205 nm, while the CD pattern of the 2.51- or 12-helix has a maximum at about 204 nm, zero crossing at about 214 nm and a minimum at 221 nm. The 31- or 14-helix finally exhibits a maximum at 200 nm and a minimum at 215 nm. However, the influence of the orientation of the different chromophores to the CD pattern is not known and rationalized. Especially in the mixed 12/10/12-helix the contribution of the different turns to the overall CD pattern is not known. Furthermore, it is not possible to differentiate between the contribution of the f-SAA residues’ chiral centers and the contribution of the amide chromophores to the overall molar ellipticity of the linear oligomer 113. Thus from the CD spectra of 113 only the presence of a stable secondary structure can be concluded. None of the known β-peptide helices can be excluded.

The typical CD signature of 113 is lost upon cyclisation to 114 (Figure 36d). This implies a drastic change of the orientation of the backbone amide chromophores upon cyclisation. However, the maximum is still at about 202 nm and the minimum at about 188 nm.

Due to low solubility of 115 in MeCN no CD spectra were recorded in this solvent. CD spectra of 115 in MeOH did not provide any useful structural information. The spectra were not very intense, and almost temperature independent. This suggests that there exists no stable secondary structure of 115 in MeOH.
3.2.3.2 NMR Analysis and MD-Calculations

Two-dimensional NMR data was obtained for 113 in MeCN and DMSO at 300 K. All \(^1\)H resonances of each of the residue’s spin systems were assigned by means of total correlation spectroscopy (TOCSY)\(^{[201]}\) and correlation spectroscopy (COSY).\(^{[202]}\) The backbone resonances were assigned using sequential \(C_\beta, C’, H_\alpha\) and \(H^N\) chemical shift information derived from HMQC\(^{[203]}\) and HMBC\(^{[204]}\) experiments as outlined in the experimental section. All the carbon signals were assigned, with the exception of the quaternary Fmoc-carbons, the quaternary carbons of the acetonide protecting groups of the f-SAA 106 residues, and the terminal carbon of the carboxylate function. Stereospecific assignment, utilizing NOESY\(^{[205]}\) cross-peak patterns, was possible for all the prochiral \(H_\alpha\) and \(H_\beta\) protons of the \(\beta\)-homoglycine residues and for the prochiral methyl groups of all f-SAA 106 residues, with the exception of the terminal \(H_\alpha\) and \(H_\beta\) resonances of \(\beta\)-hGly 6.

The secondary structure of 113 in MeCN is defined by 24 inter-residue NOE contacts comprised of a total set of 76 NOE restraints deduced from a NOESY experiment (see experimental section).

A first restrained conformational search, based on those 76 NOE cross peaks, was performed by utilizing standard simulated annealing protocols with the X-PLOR package,\(^{[206]}\) yielding an ensemble of 10 structures with good convergence. The structure which best fulfilled the experimental restraints was then used as a starting structure for a 150 picosecond simulated dynamics run. The MD calculation was carried out with the program Discover,\(^{[207]}\) using the CVFF forcefield, for further refinement. The refinement was performed in an explicit, all-atom MeCN solvent box developed especially for this purpose by Georg Voll in our group. This resulted in the 12/10/12 helical structure of 113, depicted in Figure 37. The averaged structure was

\(^{9}\) The assignment of the MeCN spectra of 113 and subsequent distance geometry calculations were carried out by Vincent Truffault. The MD structural refinement was done by Georg Voll. All NMR spectra were recorded by myself on 500 MHz and 600 MHz spectrometers in MeCN, DMSO and pyridine. The assignment and structural calculations for the oligomers 113-115 in DMSO were as well performed by myself.
finally used as a starting structure for a further unrestrained dynamics simulation, and remained stable in the CVFF forcefield.

**Figure 37**: Stereo view of oligomer 113’s average structure (deduced by a 150 picosecond restrained molecular dynamics simulation in an explicit, all-atom MeCN solvent box). Top view and side view of the formed right handed 12/10/12-helix of 113, consisting of a central 10-membered and two terminal 12-membered H-bonded rings, and with C=O and N-H bonds pointing alternatively up and down along the axis of the helix are shown. All four H-bonds formed are depicted.
The deduced conformation is right-handed helical, with the helix consisting of a central 10-membered and two terminal 12-membered H-bonded rings, and with C=O and N-H bonds pointing alternatively up and down along the axis of the helix. Four hydrogen bonds are formed, which are shown schematically in Figure 38.

![Schematic drawing of oligomer 113 with the hydrogen bonds formed in its MeCN solution structure of a 12/10/12 helix.]

We have carefully checked for evidence of all other possible helix types, which β-peptides may adopt as predicted by quantum mechanical calculations.[208-210] All distinctive cross peaks for the other possible helix types 10/10/10, 12/12/12, and 14/14/14, as well as for the most likely 10/12/10 helix (the counterpart of the observed 12/10/12 helix), were missing in the NOESY spectrum. All other characteristic distances, which would lead to strong signals for the mentioned helices, are represented by only weak signals in the spectrum. We therefore conclude that 113 assumes plain 12/10/12 helical conformation in MeCN, in competition with a slightly unfolded helix (see experimental section).

This is the first occasion of this type of helix found for SAA-homooligomers or short SAA–β-amino acid conjugates. So far, a similar helix has only been found by Seebach and co-workers for pure β-peptides.[150] The substitution pattern of these β-peptides, which have been shown to form this “mixed 12/10/12 helix”, is very different from that of oligomer 113. Oligomer 113 (when considered as a β-peptide) consists of alternating (S,S)-disubstituted β-(Sugar) amino acids\(^h\) and the

\(^h\) Here, only the substitution along the resulting peptidic backbone is considered
unsubstituted β-hGlys, denoted [SS–U]$_3$, according to the nomenclature of Hofmann and Günther\textsuperscript{[209]} (depicted in Table 3). Seebach’s β-peptide however shows the very different substitution pattern of [B–A]$_3$, where the β-amino acid residues are alternately (S)-mono-substituted in the α- or in the β-position.

**Table 3: Nomenclature of Substitution Patterns of β-Peptides.**

<table>
<thead>
<tr>
<th>R$_1$</th>
<th>R$_2$</th>
<th>R$_3$</th>
<th>R$_4$</th>
<th>Seebach$^I$</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>–β-hGly–</td>
<td>–U–</td>
</tr>
<tr>
<td>R≠H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>-(S)β$^2$-hX$_{aa}$–</td>
<td>–A–</td>
</tr>
<tr>
<td>H</td>
<td>H</td>
<td>R≠H</td>
<td>H</td>
<td>-(S)β$^3$-hX$_{aa}$–</td>
<td>–B–</td>
</tr>
<tr>
<td>H</td>
<td>R≠H</td>
<td>R≠H</td>
<td>H</td>
<td>-(R,S)β$^{2,3}$-hX$_{aa}$–</td>
<td>–RS–</td>
</tr>
<tr>
<td>R≠H</td>
<td>H</td>
<td>R≠H</td>
<td>H</td>
<td>-(S,S)β$^{2,3}$-hX$_{aa}$–</td>
<td>–SS–</td>
</tr>
</tbody>
</table>

The quantum-mechanical investigation of β-peptides by Möhle and coworkers\textsuperscript{[208]} reveals the helix types 10/10/10, 12/12/12, 14/14/14, 10/12/10 and 12/10/12 as stable conformations for various types of β-peptides (either with one or two different amino acids in the repeat unit), with the most stable conformation depending on the substitution pattern of the amino acid. Interestingly, for an alternating [BA]-substitution pattern, helix type 12/10/12 is predicted to be the most stable type in non-polar solvents. This may be ascribed to the fact that in the 12/10/12-helix, the carbonyl functions alternately point towards the N-terminus and the C-terminus,

$^I$ The notation β-hXaa for a homologue of the α-amino acid Xaa was introduced by Ondetti and co-workers,\textsuperscript{[147]} and has been used and refined by Seebach and co-workers to β$^2$- and β$^3$-hXaa, where the numbers indicate the position of the side chains in the β-amino acids.\textsuperscript{[148]}
resulting in a low overall dipole momentum of the helix. For the [BA]-substitution pattern, the spatial constrains imposed by the \( \beta \)-substituent of the first amino acid in the sequence leads to a lower energy of a 12/10/12 helix in respect to a 10/12/10 helix, which also has a low overall dipole momentum. Furthermore, it has been shown that substitution in \( \beta \)-position has a considerably higher structural influence than substitution in \( \alpha \)-position.\(^{[210]} \) Therefore, in a [SS]-substituted \( \beta \)-amino acid, the influence of the \( \beta \)-substituent will dominate over the influence of the \( \alpha \)-substituent. On the other hand, an unsubstituted \( \beta \)-amino acid lacks a \( \beta \)-substituent with its dominating structural influence. Thus, the [SS]-substituted f-SAA 106 in 113 rather acts like a [B]-substituted \( \beta \)-amino acid than an [A]-substituted one, whereas the unsubstituted \( \beta \)-hGly in 113 behaves like an [A]-substituted \( \beta \)-amino acid. In consequence the oligomer 113’s substitution pattern of [SS–U] should induce similar secondary structures as the [AB] substitution pattern of Seebach’s “mixed” \( \beta \)-peptides. This would explain why oligomer 3 forms the 12/10/12-helical structure in MeCN.

NMR structural analysis of oligomer 113 in DMSO revealed, that no longer a 12/10/12 helix is formed exclusively. This observation is in agreement with the results from the CD-spectra and quantum mechanical calculations, which reveal that helices with a large permanent dipole moment become favored in polar solvents over helices with a small dipole moment (such as the 12/10/12 helix).\(^{[208-210]} \)

Similar to the procedure often used to deduce the sugar ring conformations of DNA or RNAs, the conformation of the f-SAA 106 residues in the linear oligomer 113 in DMSO was deduced (Figure 41), utilizing the information contained in the coupling constants \( J(H_1H_2) \) of 5.3 Hz, \( J(H_3H_4) \) of 10 Hz, and \( J(NH_3) \) of 8.9 Hz. For that task the refined Karplus equation of Haasnoot et al. was used (Equation 1).\(^{[211]} \) In their equation the relationship between vicinal NMR proton-proton coupling constants and the pseudorotational properties of the furanoid sugar ring are taken into account. It also allows an accurate correction for the effects of electronegativity and orientation of substituents on \( J(HH) \).
\[ J(H^AH^B) = P_1 \cos^2 F_{AB} + P_2 \cos F_{AB} + P_3 + S \sum_i \{ P_4 + P_5 \cos^2 (?_i F_{AB} + P_6 + ?_i) \} \]

**Equation 1:** Haasnoot et al.’s refined Karplus equation for furanoid sugar rings,\[211\] which was used to determine the conformation of f-SAA 106 in oligomer 113 in DMSO. \( P_{1-6} \) are empirical determined parameters, their value depends on whether a \( H^A\text{C–CH}^B \) or a \( H^A\text{HC–CH}^B \) fragment is analyzed; \( F_{AB} \) is the proton–proton torsion angle, \(?_i \) is either +1 or –1, depending on the orientation of the substituent \( S_i \), \(?_i \) is the difference in the Huggins electronegativity of substituent \( S_i \) to hydrogen.

The first three terms describe the dependence of the vicinal coupling in a given H–C–C–H fragment on the proton-proton torsion angle \( F \). The remaining terms account for the dependence of \( J(HH) \) on electronegative substituents, \( S_i \), and (as the orientation of the substituent relative to the coupling protons plays an important role in this effect) these terms are also dependent on \( F \). This term must be applied to all the non-hydrogen substituents on the central carbons of the H–C–C–H fragment under study, hence the summation sign. The magnitude of the correction due to each substituent \( S_i \) is correlated with the difference in the Huggins electronegativity between the substituent and hydrogen \((?_i = ?_i^{\text{substituent}} - ?_H)\). The relevant \(?_i \) used were: \(?_C = 0.4, \ ?_O = 1.3, \ ?_N = 0.85\). In addition this primary (a) \(?_i \) values are influenced by \( \beta \)-substituents. As electronegative \( \beta \)-substituents demonstrate an opposite behavior compared with that of \( \alpha \)-substituents \( (S_i) \), one can consider the influence of a \( \beta \)-substituent as moderating the electronegativity effect of an \( \alpha \)-substituent. This is expressed by a formula where the electronegativity of an \( \alpha \)-substituent \( S_i \) is defined by equation 2:

\[ \sum_i \text{group} = \sum_i^{\alpha-\text{substituent}} - P_7 \sum_i^{\beta-\text{substituent}} \]

**Equation 2:** Influence of the electronegativity of the group \( S_i^{\text{group}} \) attached to the fragment H–C–C–H as defined by Haasnoot et al.\[211\] The summation is over all the substituents \( j \) attached to the \( \alpha \)-substituent. \(?_i^{\text{group}} \) is the difference in electronegativity of the whole substituent group to a hydrogen; \(?_i^{\alpha-\text{substituent}} \) and \(?_j^{\beta-\text{substituent}} \) are the difference in electronegativity for the \( \alpha \)- or each of the \( \beta \)-substituents respectively; \( P_7 \) is an empirically derived constant by Haasnoot et al.\[211\]
? \, ?^\text{group}_i \) is used in Equation 1, when a more refined calculation is desired. Here \(? \, ?^\text{group}_i\) was used.

The change of the position of a substituent \(S_i\) with respect to its geminal proton causes a change in the sign of the angle \(F\) in the electronegativity term in Equation 1. The influence of this relative orientation of the substituent \(S_i\) was incorporated into Equation 1 by means of \(?_i\). According to the orientation of the substituent \(S_i\), \(?_i\) becomes +1 or –1 as depicted in Figure 39.

![Figure 39: Definition of “positive” (\(?_i = +1\)) and “negative” (\(?_i = -1\)) substituents and application to \(H^1H^2\) of f-SAA 106.](image_url)

Projecting the \(H^A–C^1–C^2–H^B\) fragment along the vector \(C^1–C^2\) (Figure 39) the orientation of a substituent \(S\) on \(C^1\) is defined as being positive \(?_i = +1\), when the projected valency angle between \(H^A\) and \(S\) amounts to approximately +120°, counting clockwise from \(H^A\). The orientation of the substituent is negative \(?_i = -1\), when this angle amounts to 240°.

Application of the Equations 1 and 2, the definition of \(?_i\) depicted in Figure 39 and the \(?_i\)s listed above, resulted in the modified Karplus curves for \(^3J(H^1H^2)\) and \(^3J(H^3H^4)\) of f-SAA 106 shown in Figure 40.
The concept of pseudorotation was introduced by Kilpatrick et al.\textsuperscript{[212]} in their discussion of the “indefiniteness” of the cyclopentane ring. The starting point for defining the geometry of 5-membered rings is cyclopentane. It has been shown\textsuperscript{[213]} that the relationship between the five endocyclic torsion angles ($F_0$-$F_4$) can be described by the simple cosine function in Equation 3.

$$F_j = F_m \cos(P + 4j\nu/5), \quad j = 0-4;$$

$F_m$ = puckering amplitude

$P$ = phase angle of pseudorotation

\textbf{Equation 3: Relation between the endocyclic proton–proton torsion angle $F_j$, the puckering amplitude $F_m$ and the phase angle of pseudorotation $P$, as well as the definition of the different endocyclic torsion angles in heterocyclic 5-membered rings $F_j$.}
The application of the concept of pseudorotation and empirical correlations between the parameters governing the conformation of furanosides fragments in solid state, resulted in Equations 4a-c, interrelating the proton–proton torsion angles $F_{A,B}$ and the pseudorotation parameters $F_m$ and $P$ for $\alpha$-D-ribose (and several others not listed here).\[214\] f-SAA 106 is a derivative of ribofuranoic acid, hence Equations 4a-c are applicable.

\[
\begin{align*}
F_{1,2} &= 3.3^\circ + 1.102 F_m\cos(P-144^\circ) \\
F_{2,3} &= 0.2^\circ + 1.090 F_m\cos P \\
F_{3,4} &= -124.9^\circ + 1.095 F_m\cos(P+144^\circ)
\end{align*}
\]

Equation 4: Relation between the proton–proton torsion angles $F_{A,B}$ and the pseudorotation parameters $F_m$ and $P$ for $\alpha$-D-ribose.

When using the concept of pseudorotation, the conformation of the furanoid sugar ring is fully accounted for by the puckering amplitude $F_m$ and the phase angle of pseudorotation $P$. It therefore was sufficient to determine only the two proton-proton torsion angles $F_{1,2}$ and $F_{3,4}$, in order to deduce the complete ring conformation of f-SAA 106 in oligomer 113 in DMSO shown in Figure 41. For f-SAA 106 in the linear oligomer 113 in DMSO $F_m = 38^\circ$, and $P = 21.8^\circ$ are obtained, when applying the Equations 1-4 in the above described manner. This corresponds to a C$^3$-endo conformation, when using DNA/RNA nomenclature.
Figure 41: Stereoviews of the conformation of the f-SAA 106 residues of the linear oligomer 113 in DMSO. In DNA/RNA nomenclature this corresponds to a C3'-endo conformation. Pseudorotational parameters deduced: puckering amplitude $F_m = 38^\circ$, phase angle of pseudorotation $P = 21.8^\circ$.

The coupling constants $^3J(H^\beta H^N)$ and $^3J(H^\beta' H^N)$ of the $\beta$-hGlys were also measured. For the $H^\beta$ they are about 5.8 Hz, for the $H^\beta'$s around 6.7 Hz. This implies a gauche conformation. The chemical shifts of $H^\beta$ and $H^\beta'$ of the same $\beta$-hGly differ by about 0.1 ppm. However, for all three $\beta$-hGly residues the different $H^\beta$s and $H^\beta'$s have almost identical chemical shifts. The $H^N$ chemical shifts, on the other hand, are very well dispersed (Figure 42).
However, due to large overlaps of the sugar and alkyl protons in the NOESY spectra recorded in DMSO [d₆], the integration of several cross peaks is not possible, thus preventing reliable 3D structure modeling. Nonetheless these results show, that 113 also forms an ordered structure in DMSO, with a periodical structural motif, since all the β-hGlys and f-SAA 106 residues have coupling constants in the same range, and similar chemical shifts.
NMR studies for 115 and 114 at 500 or 600 MHz in DMSO and/or pyridine were performed. Full sequential assignment of 115 and 114 was achieved similar to the above described procedure for 113. NOESY and ROESY spectra were recorded to obtain 3D structural information. The amide protons of the linear oligomer 115 have different chemical shifts (Figure 43). However due to large overlap of several NOEs no reliable structural calculation is possible.

![Figure 43: Amide bond region of the TOCSY of 115 measured at 600 MHz, and 300 K in [D₅] pyridine.](image)
The cyclic oligomer 114 exhibits apparent $C_3$ symmetry on the NMR chemical shift time scale (Figure 44), and mean J-coupling constants of 7 Hz between the $H^N$s and the $H^3$s of the f-SAA 106 residues (as opposed to the well differentiated residues in the linear oligomer 113 in DMSO).

**Figure 44:** Amide bond region of the TOCSY spectra of the linear 113 and the cyclic 114 measured at 600 MHz, and 296 K in $[d_6]$ DMSO.
3.2.4 Conclusions

The straightforward syntheses in a few steps from diacetone glucose of two new furanoid sugar amino acids is described, making them easily accessible and therefore to useful building blocks for combinatorial syntheses. f-SAA 106 resembles a β-amino acid, while f-SAA 107 resembles a ϖ-amino acid. Thus, f-SAA 106 was used in conjunction with β-homoglycine for the synthesis of the mixed, linear and cyclic oligomers 113 and 114. For the synthesis of linear oligomer 115 f-SAA 107 was used with GABA as amino acid counterpart. 113 and 115 were assembled successfully using standard solid-phase peptide synthesis.

The linear Fmoc–[f-SAA106–β-hGly]3–OH 113 exhibited 12/10/12 helical secondary structure in MeCN as proven by NMR studies, and subsequent molecular dynamics calculations. This is remarkable, since 113 contains β-homoglycine, which is known to destabilize helices.[149] Stable secondary structures were only reported for β-peptides consisting almost exclusively of substituted β-amino acids. It is obvious that the unsubstituted β-homoglycin is much more flexible (compare Gly substitution of L-α-amino acids in α-peptides). Hence, in short oligomeric sequences f-SAA 106 very strongly induces the secondary structural element of a 12/10/12-helix. We can not confirm Seebach’s hypothesis, that the driving force of the 12/10/12-helix formation is the hydrophobic interaction between the side chains of the i and i+3 residues.[140] In our 12/10/12-helix forming oligomer 113 either i or i+3 is a β-hGly residue and therefore has no side chains. However, these hydrophobic interactions surely stabilize Seebach’s 12/10/12 helical conformations for his “mixed” β-peptides. The 12/10/12 preferred helical conformation of 113 is lost in the polar solvent DMSO. In DMSO 113 also adopts a secondary structure with a periodic structural motif.


Another remarkable feature is, that cyclo[–f-SAA106–β-hGly–]3 114 is well soluble in organic solvents, such as MeCN, DMSO and MeOH. Cyclic β-peptides prepared by Seebach et al. exhibited low solubility in classical, pure organic
Thus, 1D NMR studies were either not possible or [d$_8$]-THF solutions containing more than 10 equivalents of anhydrous LiCl had to be used.

Cyclic oligomer 114 exhibits a $C_3$ symmetric conformation on the NMR chemical shift time scale. However, our preliminary NMR results do not yet give evidence of clearly distinct conformation, since the observed NMR signals are averaged, and similar to those of the monomeric subunits.

The new compounds presented here are useful as new structural templates, and might also be of interest for host-guest chemistry.
3.3 Somatostatin Analogues Containing f-SAA 106 and f-SAA 107 as Structure-Inducing Templates

3.3.1 Somatostatin – General Introduction

Somatostatin (Figure 45) is a cyclic peptide hormone, which was first isolated from ovine hypothalamus in 1973, while searching for the growth hormone of the pituitary gland. Due to its central role in the regulation of growth hormone secretion, it is often referred to as somatropin release-inhibiting factor (SRIF) or growth hormone (GH) release-inhibiting factor. Native somatostatin occurs in two biologically active forms, somatostatin-14 (SST-14 or SRIF-14) and a 28-residue peptide with an additional 14 amino acids at the N-terminus of SST-14, somatostatin-28 (SST-28 or SRIF-28).

\[
\begin{align*}
R & \text{-} \text{Ala}^1 \text{-Gly}^2 \text{-Cys}^3 \text{-Lys}^4 \text{-Asn}^5 \text{-Phe}^6 \text{-Phe}^7 \text{-Trp}^8 \\
& \text{HO-} \text{Cys}^{14} \text{-Ser}^{13} \text{-Thr}^{11} \text{-Phe}^{11} \text{-Thr}^{10} \text{-Lys}^9
\end{align*}
\]

SST 14: R= H
SST 28: R= HSer-Ala-Asn-Ser-Aln-Pro-Ala-Met-Ala-Pro-Arg-Lys-

Figure 45: Bioactive forms of native somatostatin: somatostatin-14 (SST 14 or SRIF-14) and somatostatin-28 (SST 28 or SRIF-28).

Both of them are derived from the polypeptide precursor prosomatostatin. Somatostatin is synthesized in neuronal and endokine cells in various body-tissues.

3.3.1.1 Physiological Activity of Somatostatin

It was shown that somatostatin not only inhibits growth hormone (GH) release, but acts on a variety of physiological functions in various areas of the body. All its actions are inhibitory in nature. Somatostatin inhibits virtually every endocrine and
exocrine secretion, such as insulin, glucagon, gastrin, secretin and pepsin secretion. It inhibits the gastrointestinal absorption of ions, nutrients and minerals, inhibits cell proliferation and modulates vascular contraction. Motoric, sensory, behavioral, cognitive and autonomic effects as well as intestinal motility are influenced. These pleiotropic effects can be resolved in three cellular processes, that are modulated by somatostatin: neurotransmission, secretion, and cell proliferation (Figure 46).

**Figure 46:** *Cellular processes influenced by somatostatin (SST 14 and SST 28).*

Because of its wide range of physiological functions, somatostatin may play an important role in the treatment of numerous human diseases, such as diabetes, cancer and in all diseases based on endocrine and gastrointestinal malfunction. This therapeutic potential was recognized years ago. However, the clinical use of somatostatin itself has been hampered, because of its very short half-life in circulation of about one minute and because of its lack of selectivity. So obtaining potent, selective and stable analogues that could be useful clinically proved to be very difficult.
3.3.1.2 Non-peptidic Somatostatin Analogues

The pharmacological limitations of somatostatin analogues, such as their poor oral bioavailability or too short half-life, are the main reasons for the steadily growing interest in a rational design of nonpeptide mimetics of somatostatin.

Hirschmann et al. designed peptidomimetics of somatostatin employing a β-D-glucose scaffold 52 (Chapter 2.7.1, Figure 6).\textsuperscript{[70, 74]} The SAA 43 containing somatostatin analogue (Chapter 2.7.1) is also considered as a nonpeptidic mimetic of somatostatin.

Damour et al. synthesized a series of 3-functionalized proline and β-lactam derivatives bearing an aryl group, such as a phenyl or a 3-indolyl, either in position 3 of the proline moiety (116–120) or on the 3-methyl chain of the β-lactam skeleton (121, 122) (Figure 47).\textsuperscript{[221]}

![Figure 47: Structures of non-peptidic analogues of SST based on 3-functionalized proline (116–120) and β-lactam (121, 122).](image)

The enzymatic stability of β-peptides makes them an interesting alternative to natural peptides in medicinal chemistry.\textsuperscript{[222]} Seebach et al. used β-peptides as somatostatin mimetics. Their β-dipeptide 123 shows high selectivity to SSTR 4.\textsuperscript{[223, 224]}
Figure 48: Seebach’s β-dipeptide 123 somatostatin analogue, selective on SSTR 4 with a $K_D$ of 74 nM.

Until 1998 there were no reports in the literature of the successful development of a selective, competitive somatostatin receptor ligand of nonpeptidic origin. The first potent nonpeptidic agonists to selectively bind at the SSTR 2 receptor (in the nanomolar range) were reported by the Merck group. They described studies utilizing direct screening, which resulted in the discovery of first lead from which a new class of small nonpeptide analogues of somatostatin was derived. Among them two analogues, 124 and 125 (Figure 49), exhibited low nanomolar binding to human SSTR2, functional potency and high selectivity (> 1000-fold) toward the other receptor subtypes. 125 has even higher affinity for SSTR2 than somatostatin.

Figure 49: Structure of 124 and 125.

Ankersen et al. initiated a screening program to identify new nonpeptide chemical entities with affinities for somatostatin receptor subtypes. Because four amino acid residues, Phe7, Trp8, Lys9 and Thr10, which comprise a β-turn, are thought to be
necessary for biological activity, they based their screening on a scaffold containing two aromatic groups and a basic group. Two aromatic groups, one of them heteroaromatic, were postulated to mimic Trp\textsuperscript{8} and Phe\textsuperscript{7} residues of SRIF. Lys\textsuperscript{9} was mimicked by primary amino groups, as well as by all sorts of nitrogen-containing functional groups. The most potent compound of this series, 126 (Figure 50) binds to the SSTR4 receptor with an affinity of 6 nM and at least a 100-fold selectivity for SSTR4 over the other four somatostatin receptor subtypes. In a functional assay, 126 showed full somatostatin receptor agonism (95% of SRIF) with an EC\textsubscript{50} of 2 nM.

![Figure 50: Structure of non-peptidic SST analogue 126.](image)

The lack of somatostatin analogues, both peptide and nonpeptide, with selectivity for all five receptor subtypes has impeded progress in understanding the functions associated with these receptors. The use of combinatorial chemistry seemed to be the fastest way to identify nonpeptide ligands selective for each of somatostatin receptor subtypes. Recently, the Merck group\textsuperscript{[228]} used an integrated approach of combinatorial chemistry and high-throughput receptor-binding techniques to rapidly identify subtype-selective compounds. Nonpeptide agonists of each of the five somatostatin receptors were identified in combinatorial libraries constructed on the basis of molecular modeling of the known peptide analogues (Figure 51). These nonpeptide, small-molecule analogues of somatostatin may be useful in the development of orally active chemotherapeutic agents capable of crossing the blood–brain barrier.
3.3.1.3 Somatostatin Receptors

Somatostatin acts via a family of high-affinity plasma membrane receptors termed SST receptors or SSTR.\textsuperscript{[229-232]} Beginning in 1992, the structures of the SSTRs were elucidated by molecular cloning.\textsuperscript{[233, 234]} Five individual subtypes were rapidly identified and shown to consist of a family of heptahelical G protein-coupled receptors (GPCR).\textsuperscript{[229, 230, 232, 234-238]} Human SST receptors (hSSTR) are encoded by a family of five nonallelic genes located on separate chromosomes. Four of the genes are intronless, the exception being SSTR 2, which gives rise to spliced variants SSTR 2A and SSTR 2B, which differ only in the length of the cytoplasmic C-tail.\textsuperscript{[229, 230]} There are thus six putative SSTR subtypes of closely related size, each displaying a seven trans-membrane domain (TM) topology. All SSTR-isoforms that have been cloned so far from humans as well as from other species possess a highly conserved sequence motif YANSCANP1/VLY in the VIIth TM, which serves as a signature sequence for this receptor family.\textsuperscript{[229, 230]} Overall, there is 39-57% sequence identity among the various members of this family, with SSTR 1 and SSTR 4 showing the highest
sequence identity. The individual subtypes display a remarkable degree of structural conservation across species. Thus there is 94–98% sequence identity between the human, rat, and mouse isoforms of SSTR 1.[220]

The receptors can be further divided into two subfamilies: SSTR 2, 3, and 5 react with octapeptide and hexapeptide SRIF analogues and belong to one subclass; SSTR 1 and 4 react poorly with these compounds and form the second subclass.

SSTRs are located in various densities throughout our body (Figure 52). They are widely expressed in many tissues, frequently as multiple subtypes that coexist in the same cell.

SSTRs are found in the brain, gut, pituitary, endocrine and exocrine pancreas, adrenals, thyroid, kidneys, gastrointestinal tract, and immune cells.[220, 229, 230, 232, 239-241]

The deduction of the specific functional roles of the different SSTRs is under continuous and extensive investigation. SSTR 2 has been proposed to play a major role in inhibiting growth hormone regulation, glucagon and gastric acid secretion by somatostatin and its analogues.[230] Receptor SSTR 5 has been found to have a limited role in growth hormone regulation, but instead may have a selective function in controlling insulin secretion.[230, 242]
3.3.2 Somatostatin Analogues and Cancer

Various isoforms of the SSTRs are overexpressed on a large number of tumor cells, such as AtT-20 pituitary tumor cells, hamster insulinoma and Rin m5F islet tumor cells, AR42J, and MiaPaCa pancreatic tumor cells and in human breast cancer, neuroblastoma, glioma, and leukemic and myeloma cell lines.\cite{220, 229, 230, 232}

Some researchers claim, that SSTR 2 and 5 mediate the antiproliferative effects of somatostatin and its analogues on tumor growth,\cite{243-245}, other postulate, that SSTR 1, 2, 4 and 5 induce cell cycle arrest, while SSTR 3 triggers apoptosis accompanied by activation of p53 and the pro-apoptotic protein Bax,\cite{239} again other authors suggest, that only equipotent binding to all five SSTRs, causes the desired high antitumor activity of somatostatin analogues.\cite{246} Therefore two major classes of somatostatin analogues have been developed: The receptor selective ones and the universal ones.

3.3.2.1 Chemotherapy

For some neoplasms, such as testicular cancer, cytotoxic chemotherapy is very effective. However, one of the major problems of cancer treatment is the development of resistance to chemotherapy.\cite{247} Furthermore conventional chemotherapeutic drugs, have shown limited efficacy and considerable toxicity in the first place.\cite{248} The efficacy of cytotoxic chemotherapy in the treatment of many common neoplasms such as those of the lung, breast, prostate, bowel, pancreas, liver, and kidney is limited. Cure of macroscopic metastatic disease is exceedingly rare, and palliation of symptoms of metastatic neoplasms by cytotoxic chemotherapy is problematic, since the toxicity of the treatment often outweighs any improvement in quality of life resulting from the temporary decrease in tumor burden.\cite{249-252} Because of this situation, a lot of effort is put into the development of novel cytotoxic agents and innovative noncytotoxic approaches.
Amongst the various anti-cancer agents, increasing attention is being paid to somatostatin analogues. The aim is to find somatostatin analogues, which induce cell cycle arrest, but do not retain the hormonal activity of somatostatin.

### 3.3.2.2 Somatostatin Analogues in Clinical Use

The increasing attention directed to somatostatin analogues, such as octreotide, is largely due to the antineoplastic (that is mainly antiproliferative) activity of these compounds in a variety of experimental models in vitro and in vivo and to the clinical experience with somatostatin analogues in the treatment of conditions like acromegaly and GEP tumors. These analogues have been shown to be well tolerated compared to other antineoplastic therapeutics currently in use. However thus far, only three octapeptide somatostatin analogues are in clinical studies and/or use. They are octreotide (SMS 201,995, sandostatin), lanreotide (BIM 23,014, somatuline) and vapreotid (RC-160, octastatin).

Besides being used in cancer treatment and palliation, radio labeled somatostatin analogues are employed for the localization of primary and metastatic tumors expressing somatostatin receptors. The so-called “somatostatin receptor scintigraphy” is indeed the most important clinical diagnostic investigation for patients with suspected neuroendocrine tumors. Targeted radiotherapy, which is in clinical trials, represents the obvious extension of somatostatin scintigraphy.

### 3.3.2.3 Apoptosis

In multicellular organisms, homeostasis is maintained through a balance between cell proliferation and cell death. Although much is known about the control of cell proliferation, considerable less is known about the control of cell death. Physiological cell death occurs primarily through an evolutionarily conserved programmed form of cell suicide termed apoptosis (Greek: apo = off, from; ptosis = falling). The basic machinery to carry out apoptosis appears to be present in essentially
all mammalian cells at all times, but the activation of the suicide program is regulated
by a wide range of regulatory stimuli that originate from both the intracellular and the
extracellular milieu.\cite{257-262} Malfunction of apoptosis leads to severe diseases, such as
cancer, viral infections and Alzheimer’s disease. Treatments designed to specifically
alter the apoptotic threshold may have the potential to change the natural progression
of some of these diseases.

Recent research has demonstrated that Kéri’s somatostatin analog TT232 (135)
is capable of inducing apoptosis selectively in certain tumor cell lines, without the
hormonal bioactivity of native somatostatin.\cite{165, 247, 263, 264}

3.3.2.4 Concept, Strategy and Design of SAA Containing Somatostatin
Analogues

3.3.2.4.1 Structure Activity Relationship Considerations

Extensive structure activity relationship studies of somatostatin revealed that
the essential pharmacophore of somatostatin is Phe\(^7\)-Trp\(^8\)-Lys\(^9\)-Thr\(^{10}\).\cite{265, 266} According to those studies Lys\(^9\) and Trp\(^8\) are assumed to be absolutely crucial for
activity, while moderate substitutions of Phe\(^7\) and Thr\(^{10}\) are tolerated. Further
structure-activity relationship studies of somatostatin revealed that the structural
reduction retaining the cyclic nature of somatostatin led to a \(\beta\)-turn about the native
Phe\(^7\)-Trp\(^8\)-Lys\(^9\)-Thr\(^{10}\)-sequence of somatostatin.\cite{159, 167, 267-273} Hence, replacement of
Trp\(^8\) with D-Trp stabilizes the \(\beta\)-turn of the postulated active conformation of
somatostatin. Activity is thus increased, while at the same time enzymatic stability is
enhanced.\cite{274, 275} D-Trp\(^8\) SST-14\cite{274} 136 (Table 4) was the first analogue with
significantly higher potency than somatostatin itself. Ever after D-Trp\(^8\) substitution has
been considered critical for the potency of somatostatin analogues. It therefore has
been retained in almost all somatostatin analogues synthesized thereafter (Tabel 4).

The “Veber-Hirschmann” peptide cyclo[–Phe–Pro–Phe\(^7\)–D-Trp\(^8\)–Lys\(^9\)–Thr\(^{10}\)–] 50 showed similar activity as somatostatin (Figure 6; chapter 2.7.1).\cite{158, 276-278} However, its clinical development has been halted due to steatorrhea caused as a side
effect. However replacement of the Phe-Pro bridge with Phe-Ala(NMe) resulted in Seglitid \[137\], which is in clinical trials.\[279\] In 1994 the incorporation of glucosyluronic acid methylamine (Gum) \[43\] as a dipeptide isoster replacing the Phe–Pro bridge in the sequence of \[50\] (See chapter 2.7.1) was introduced by our group.

### Table 4: Structure of somatostatin and some of its peptidic analogues.

<table>
<thead>
<tr>
<th>name</th>
<th>structure</th>
</tr>
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</table>
| Somatostatin-14 (SRIF-14, SST-14) | H-Ala–Gly–Cys–Lys–Asn–Phe–DTrp \[8\]  
                             | HO–Cys\[14\]–Ser\[13\]–Thr\[12\]–Phe\[11\]–Thr\[10\]–Lys \[9\] |
                             | Arg–Lys–Ala–Gly–Cys–Lys–Asn–Phe–DTrp \[8\]  
                             | HO–Cys–Ser–Thr–Phe–Thr–Lys |
| D-Trp\[8\] SST-14\[265\] | H-Ala–Gly–Cys–Lys–Asn–Phe–DTrp \[8\]  
                             | HO–Cys\[14\]–Ser\[13\]–Thr\[12\]–Phe\[11\]–Thr\[10\]–Lys \[9\] |
| Octreotide\[269\] (Sandostatin, SMS 201-995) | H–DPhe–Cys–Phe–DTrp  
                             | Thr(ol)–Cys–Thr–Lys |
| Vapreotid (RC-160) | H–DPhe–Cys–Phe–DTrp  
                             | H2N–Trp–Cys–Val–Lys |
| Seglitid\[279\] (MK678) | (NMe)Ala–Tyr–DTrp \[8\]  
                             | Phe–Val–Lys |
| Lanreotid\[280, 281\] (BIM23014) | H–DβNal–Cys–Tyr–DTrp  
                             | HO–Thr–Cys–Val–Lys |
| Veber-Peptid\[158, 276-278\] (L-363,301) | Pro–Phe–DTrp  
                             | Phe–Thr–Lys |
                             | H2N–Thr–Cys–Lys |

Hirschmann et al.’s β-D-glucose scaffold derived somatostatin analogue 52 showed moderate activities, which demonstrated (See also 2.7.1, Figure 6)\[70, 74\], that the peptide backbone is not a crucial part of the pharmacophore, but serves as a
scaffold to support the side-chains of Phe$^7$, Trp$^8$, Lys$^9$ and Phe$^{11}$ in the required spatial arrangement. This is also supported by several nonpeptidic, highly receptor selective and active compounds, found in a combinatorial approach using high-throughput screening techniques (See also Chapter 3.3.1.2).[225-228, 266, 283-285]

3.3.2.4.2 Stability - Cleavage Sites of Native Somatostatin

One of the major drawbacks of the therapeutic use of somatostatin itself is the low half life in the body of about one minute. For native somatostatin SST 14, at least 5 sites of enzymatic cleavage are known (Figure 53).[286] Trypsin-cleavage between Trp$^8$ and Lys$^9$, causes complete loss of activity, as well as cleavage between Phe$^6$ and Phe$^7$ and cleavage between Thr$^{10}$ and Phe$^{11}$. Aminopeptidase attack at the N-terminus is less threatening, since the resulting one or two amino acid shorter peptides retain activity completely.

\[
\begin{array}{c}
H-Ala^1-Gly^2-Cys^3-Lys^4-Asn^5-Phe^6-Phe^7-Trp^8-HO-Cys^{14}-Ser^{13}-Thr^{11}-Phe^{11}-Thr^{10}-Lys^9 \\
SST 14
\end{array}
\]

Figure 53: Enzymatic cleavage sites of Somatostatin 14.

3.3.2.4.3 Design of Apoptosis Inducing Somatostatin Analogues

The ultimate goal is a drug, which induces apoptosis selectively on tumor cells, without damaging healthy surrounding tissue, and without somatostatin’s hormonal activities; a drug, which is orally available, stable in circulation with a long half life.

For that aim several parameters, such as the enzymatic stability and the optimum presentation of the pharmacophore, that is in the right spatial orientation,
have to be optimized. The pharmacophore might be optimized itself, since nature only has 21 natural amino acids to choose from, while chemist can use any unnatural amino acid as well.

To design such a drug, we used TT-232 \(135\) (Table 4) as a lead structure, since this is the only compound known so far to selectively induce apoptosis on tumor cells, without any of somatostatins hormonal activities. As a second lead structure we used the Veber-Hirschmann-peptide \(50\) (Table 4).

To optimize the enzymatic stability and to introduce at the same time a spatial constraint, we replaced the Cys-Cys-disulfide bridge of TT-232 \(135\) and the Phe-Pro bridge of the Veberpeptide \(50\) by the SAAs \(f\)-SAA \(106\) and \(f\)-SAA \(107\) (Figure 54). The SAAs are not recognized by peptidases. Thereby the lethal cleavage sites in native somatostatin (Figure 53) between Phe\(^6\) and Phe\(^7\) and between Thr\(^{10}\) and Phe\(^{11}\) are “blocked”. The resulting compounds should show considerable longer half life in circulation.

\[
\begin{align*}
\text{H} & \text{DPhe} \quad \text{Cys} & \text{Tyr} & \text{DTrp} \\
\text{H}_2\text{N} & \text{Thr} \quad \text{Cys} & \quad \text{Lys} \\
135 & \text{TT-232} \\
\end{align*}
\]

\[
\begin{align*}
\text{Pro} & \quad \text{Phe} & \quad \text{DTrp} \\
\quad & \quad \text{Thr} & \quad \text{Lys} \\
50 & \text{Veberpeptide} \\
\end{align*}
\]

\[
\begin{align*}
\text{SAA} & \quad \text{Tyr} & \quad \text{DTrp} & \quad \text{(L-Trp)} \\
\text{SAA} & \quad \text{Lys} \\
138-141 & \text{TT-232} \\
\end{align*}
\]

\[
\begin{align*}
\text{SAA} & \quad \text{Phe} & \quad \text{DTrp} & \quad \text{(L-Trp)} \\
\quad & \quad \text{Thr} & \quad \text{Lys} \\
\quad & \text{(Thr(OTrt))} & \text{(Phe)} \\
142-147 & \text{Veberpeptide} \\
\end{align*}
\]

**Figure 54:** Design of somatostatin analogues; SAA = \(f\)-SAA \(106\).

\(f\)-SAA \(106\) and \(107\) were especially designed as structure inducing templates, which are cheaper and faster to synthesize than the previously described SAAs and turn-mimetics.\(^{18, 21}\) (Their synthesis is described in chapter 2.3.2.5). At the Phe\(^7\) position of the natural sequence always Phe and Tyr and in the Thr\(^{10}\) position Thr,
Thr(OTrt) or Phe were used, since for those two amino acids moderate substitutions are tolerated. The aim of modifying those positions is to gain more selectivity and activity. D- and L-Trp were placed in the Trp\textsuperscript{8} position. This resulted in a family of four TT-232 derived and six Veber-Hirschmann peptide derived compounds.

Antitumor activity tests (Chapter 3.3.2.6) showed only activity for Veber-Hirschmann peptide derived compounds cyclo[–Tyr–D-Trp–Lys–Thr(OTrt)–f-SAA\textsuperscript{106–}142 and cyclo[–Tyr–Trp–Lys–Thr(OTrt)–f-SAA\textsuperscript{106–}] 143, with a Thr(OTrt) in the Thr\textsuperscript{10} position. Therefore a big aromatic residue in that position seemed to be crucial for antitumor-activity. Based on this results a second larger library was designed (Figure 55).

\textbf{Figure 55:} Design of second library, based on cyclo[–Tyr–Trp–Lys–Thr(OTrt)–f-SAA\textsuperscript{106–}] 142 and cyclo[–Tyr–D-Trp–Lys–Thr(OTrt)–f-Saa\textsuperscript{106–}] 143. Color code: Trp\textsuperscript{8} position red, Lys\textsuperscript{9} position orange, Thr\textsuperscript{10} position blue, and f-SAA bridge green.

In a first strategy it was planned to introduce a large number of different aromatic side chains in the Thr\textsuperscript{10} position, by putting an aspartic acid (either as a a- or a β-amino acid) in that position and than couple it with different aromatic amines on solid phase (Figure 56). This strategy was designed to rapidly introduce a large diversity to the library during the solid phase assembly of the compounds.
However, all of the various coupling reagents and conditions applied did not result in satisfactory yields, to make this a rapid, successful and general way to introduce diversity into solid phase libraries (for the different synthetic strategies tried out see Chapter 2.3.2.5 and 4). Therefore the strategy to insert an aromatic residue in the Thr¹⁰ position was changed: Ready made aromatic building blocks were used for the assembly of the compounds. Various unnatural aromatic amino acids such as 1- and 2-naphthylalanine (1-Nal, 2-Nal), biphenylalanine (Bip), benzophenon-alanine (Bpa), pentafluorophenyl-alanine (Phe(F₅)), etc. as well as benzylethers of the natural amino acids Tyr and Thr were integrated into the sequence (Figure 57).

Diversity was also introduced, by replacing the amino acids of the pharmacophore Trp⁸ and Lys⁹ by several unnatural amino acids. Trp⁸ was substituted with 1-Nal, and D- and L-benzothienylalanine (Bta) in the otherwise unmodified sequence. Lys⁹ was replaced by norleucine, to test, if the amino functionality is crucial for activity or if only the alkyl residue is responsible for recognition. Since the incorporation of Bta in the Trp⁸ position improved activity tremendously, several derivatives of D- and L-Bta containing analogues with various aromatic side chains in the Thr¹⁰ position were synthesized as well.
Figure 57: Variations of **143**: variations of the aromatic residue in the Thr_{10} position, variations of the f-SAA residue, variations of the Trp_{8} position and variation of Lys_{9}. Not all possible combinations were synthesized.

### 3.3.2.5 Synthesis of SAA Containing Somatostatin Analogues on Solid Phase

The peptides were assembled on solid phase on tritylchloride-polystyrene-(TCP)-resin. Standard Fmoc-protocol was employed, using 2 equivalents of the amino acid (or 1.5 eq. of the f-SAA), 2 equivalents of HATU ([O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate]) and 2 equivalents of HOAt (1-Hydroxy-7-azabenzotriazol) as coupling reagents and 2,4,6-collidine (20 eq.) as base.\cite{170, 192, 193} The first amino acid anchored to the resin was either Fmoc-Tyr-OH, Fmoc-Phe-OH or Fmoc-D-Asp-Dmab (aspartic acid, used as a β-amino acid, the acid side chain is built into the backbone) respectively. Sidechain- unprotected tyrosine and tryptophane were used. This proved to be even beneficially to coupling but especially to cyclization yields, compared to the use of t-Bu-protected tyrosine and Boc-protected tryptophane. Lysine was orthogonally protected with the hydrazine labile ivDde-protecting group (1-(4,4-dimethyl-2,6-dioxo-cyclo-hexylidene)3-methylbutyl-).\cite{287} After final Fmoc-deprotection the linear peptides were cleaved from the resin with
hexafluoro isopropanol (20 % in dichloromethane, 3*20 min), before being cyclized using DPPA (DiphenylPhosPhoryl Azide) and NaHCO$_3$ in DMF (0.1 mM). Deprotection with 3% hydrazine in DMF led to the crude cyclic peptides. rp-HPLC purification yielded the cyclic compounds 138-161 in over 99 % HPLC-purity and good overall yields between 20 and 41 %.

For the coupling of the deprotected side chain carboxylic function with various amines on bead, a protocol were the coupling reagents and the amine were added in small portions over several hours, proved most effective. Different coupling reagents and conditions were tested, with HATU/HOAt/2,4,6-collidine working the best. However, the yields were always low, especially when coupling aromatic amines (as is to be expected, due to their lower nucleophily). Therefore this strategy, proved not an effective way to introduce diversity in combinatorial libraries. Most often the yields were too low, to provided enough material for the biological test. The same is true for esterification on bead of f-SAA 107.

### 3.3.2.6 Antitumor Activity of the f-SAA Containing Compounds

#### 3.3.2.6.1 Antitumor Activity of the 1st Library on Multidrug-Resistant and Drug-Sensitive Tumorcells

Antitumor activity of the TFA salts of the compounds 138-147 was tested on drug sensitive rat hepatoma carcinoma cell line Clone 2 and multidrug-resistant subclone Clone 2(10x80)T1 for the first library of 10 compounds (Figure 58). The concentration of compounds 138-147, which reduced the viability of the treated cells by 50% compared to controls (IC$_{50}$ values), were determined from at least 2 independent experiments for each compound tested, using the XTT assay.

---

1 Tests were performed by our collaborators Gyorgy Kéri and Richard Schwab from the Semmelweis Medicinal University, Budapest, Hungary and Aniko Venetianer from the Hungarian Academy of Sciences, Szeged Temesvari krt., Hungary.
Figure 58: Structures and activities of compounds 138-147. Cell lines used for IC₅₀-determination: MDR: multidrug resistant hepatoma cell line clone 2(10x80)T1;[291] DS: drug sensitive hepatoma cell line clone 2,[290] was internal reference for all tests: MDR: 9 ± 0.5 μM; DS: 10 ± 0.7 μM.
Compound 142 showed an IC$_{50}$ of 75µM for the drug sensitive, and of 47µM for the MDR cell line. Compound 143 containing D-Trp, was even more active with an IC$_{50}$ of 31µM for the drug sensitive and of 25µM for the drug resistant cell line. 135 was used as internal reference, with IC$_{50}$ values of 10µM for the drug sensitive clone and 9µM for the MDR one. Compounds 146 and 147, with Thr in the Thr$^{10}$ position, and compounds 144 and 145 with the comparably small aromatic amino acid Phe in the 10 position, show IC$_{50}$ values over 120 µM, while compounds 142 and 143, with the big aromatic trityl ether in the corresponding position, show high activities.

3.3.2.6.2 Antitumor Activity Tests of the 2nd Library

Compounds 148 - 161 (Figure 59) of the second library were tested on two cell-lines, A431$^{[294]k}$ (an epidermoid cancer) and Panc-1$^{l}$ (a well differentiated pancreatic adenocarcinoma), both of human origin.

k Reference for A-431 in the “American Type Culture Collection” is found under http://phage.atcc.org/cgi-bin/searchengine/longview.cgi?view=ce,663682,CRL-661555&text=a-663431

l Reference for Panc-1 in the “American Type Culture Collection” is found under http://phage.atcc.org/cgi-bin/searchengine/longview.cgi?view=ce,609764,CRL-1469&text=panc-1
Figure 59: Structures of compounds 148-161 tested on human cancer cell lines A431\textsuperscript{k} (epidermoid cancer) and Panc-1\textsuperscript{l} (pancreatic adenocarcinoma).
Each compound was tested under 4 conditions: 6 h (to exclude necrosis\textsuperscript{m}) and 48 h to see inhibition of proliferation and apoptosis. High ratio between 48/6 h inhibition shows little necrotic, but pronounced apoptotic activity of the tested compound. The results are summarized in Table 5.

Table 5: Apoptotic activity of compounds 148-161 (Figure 58).

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC\textsubscript{50} [µM]*</th>
<th>Necrosis**</th>
</tr>
</thead>
<tbody>
<tr>
<td>148</td>
<td>= 10</td>
<td>non</td>
</tr>
<tr>
<td>149</td>
<td>~ 50</td>
<td>some</td>
</tr>
<tr>
<td>150</td>
<td>~ 60</td>
<td>some</td>
</tr>
<tr>
<td>151</td>
<td>&gt; 100</td>
<td>yes</td>
</tr>
<tr>
<td>152</td>
<td>&gt; 100</td>
<td>yes</td>
</tr>
<tr>
<td>153</td>
<td>~ 100</td>
<td>almost non</td>
</tr>
<tr>
<td>154</td>
<td>&gt; 100</td>
<td></td>
</tr>
<tr>
<td>155</td>
<td>~ 110</td>
<td>some</td>
</tr>
<tr>
<td>156</td>
<td>~ 50</td>
<td>some</td>
</tr>
<tr>
<td>157</td>
<td>&gt; 2</td>
<td></td>
</tr>
<tr>
<td>158</td>
<td>= 35</td>
<td>almost non</td>
</tr>
<tr>
<td>159</td>
<td>~ 38</td>
<td>non</td>
</tr>
<tr>
<td>160</td>
<td>40\textsubscript{Panc-1}</td>
<td>some</td>
</tr>
<tr>
<td></td>
<td>50\textsubscript{A431}</td>
<td></td>
</tr>
<tr>
<td>161</td>
<td>40\textsubscript{Panc-1}</td>
<td>some</td>
</tr>
<tr>
<td></td>
<td>55\textsubscript{A431}</td>
<td></td>
</tr>
</tbody>
</table>

\* Cell lines used for IC\textsubscript{50} determination: A431\textsuperscript{[294]} (an epidermoid cancer) and Panc-1 (a well differentiated pancreatic adenocarcinoma), both of human origin. Only when the IC\textsubscript{50} values were not the same for both cell lines, different IC\textsubscript{50} values are given with the respective corresponding cell line noted in the superscript index.

** The quantity of necrosis detected as number of cell deaths after 6 h of incubation.

Compound 148, where the Lys\textsuperscript{9} side chain of the parent compound 143 is replaced by norleucine, shows the highest apoptotic activity of all compounds tested so far. With its IC\textsubscript{50} value of 10 µM for the pancreatic cancer cell line panc-1 and for the epidermoid cancer cell line A431, it presumably is considerable more active than 143 and TT232 135. It shows no unspecific cytotoxic activity (no necrosis), which is

\textsuperscript{m} nekrosis: Late Latin (originating from Greek nekr\texttextsuperscript{O}sis): nekros dead body

necrosis: non-specific, non-selective cell death, caused by outer stress
crucial for its potential use as a anti-tumor drug. These results show that lysine in the Lys\textsuperscript{9} position is by no means essential for inducing apoptosis in cancer cells. The removal of the charged amine in the side chain even results in higher apoptotic and antiproliferative activity on cancer cells. Substitution of Trp\textsuperscript{8}, the other amino acid, which has so far been considered as a crucial part of the pharmacophore, resulted in compounds 158-161. All of these compounds show similar activities (all IC\textsubscript{50}s \textasciitilde 40 \(\mu\text{M}\)) as the parent compound 143. Variation of the aromatic side chain in the Thr\textsuperscript{10} position, demonstrated, that this position is very sensitive to structural variations. The “wrong” aromatic side chain either causes complete loss of antiproliferative and apoptotic activity or might cause unspecific cytotoxicity (that is induce necrosis). Substitution of the Thr(OT\textsubscript{trt}) side chain of 143 with the side chain of biphenyl alanine (Bip) (compound 151) for instance, caused an necrotic IC\textsubscript{50} of about 50 \(\mu\text{M}\) in the epidermoid cancer cell line A431. However, substitution of the Thr(OT\textsubscript{trt}) with the – under physiological conditions much more stable – benzylated tyrosine (compare 158 and 160) enhanced the apoptotic and antiproliferative activity slightly. 158’s apoptotic and antiproliferative IC\textsubscript{50} value of 35 \(\mu\text{M}\) (for both A431 and Panc-1) is about the same as that of 143 and is in the same order as that of TT232 135.
3.3.2.7 Conclusions and Outlook

The studies presented here demonstrate that a big aromatic residue in the Thr\textsuperscript{10} position seems to be imperative for high antiproliferative and apoptotic activity. Compounds 142 and 143 with IC\textsubscript{50} values in the low µM range are very promising leads for potential chemotherapeutic drugs against multidrug-resistant hepatoma carcinoma. The results obtained from preliminary tests using epidermoid and pancreatic human tumor cell lines suggest, that removal of the charged amine functionality, that is replacement of Lys\textsuperscript{9} with Nle as in 148, enhances apoptotic and antiproliferative activity considerably. 148 is presumably more active than TT232 135, the only other compound known to show apoptotic and antiproliferative activity against carcinoma cell lines. Replacement of the Trp\textsuperscript{8} with D-Bta (D-Benzothienylalanin) resulting in compound 159, with L-Bta resulting in compound 160 or with 2-naphtyl alanine (2-Nal) resulting in compound 161 leads to equal or slightly enhanced antiproliferative and apoptotic activity. Therefore the paradigm, that the sequence Lys\textsuperscript{9}–Trp\textsuperscript{8} is the essential pharmacophore for antitumor activity, is not true.

158 is a very interesting drug candidate against epidermoid, pancreatic and most probably several other tumors. Its IC\textsubscript{50} is in the same low µM range as that of 143 and TT232 135. The replacement of Thr(OTrt) of the 143 parent compound with Tyr(OBn), and the introduction of the unnatural amino acid Bta in the Trp\textsuperscript{8} position should make it considerable more stable under physiological conditions than 143. However, it might still be optimized by substituting Lys\textsuperscript{9} with Nle. This would even make the synthesis easier and cheaper, since no longer an orthogonal protecting group of the amine side chain is needed.

The nonproteinogenic properties of SAAs should make the compounds physiologically more stable, as does the introduction of Nle (since the peptidase trypsin recognizes the peptide-sequence Trp–Lys), and Bta or 2-Nal and other non-natural amino acids.

Preliminary studies suggest that our f-SAA 106 containing compounds selectively exert their apoptotic and antiproliferative activity against cancer cells. We know, that the apoptosis induced by the compounds 142 and 143 is mediated via a so
called tumor marker\(^n\). Therefore only tumor cells should receive the apoptotic signals induced by our compounds and thereby programmed suicidal cell death is triggered exclusively for them.

In vivo studies in several animal models as well as studies to determine the toxicological profile of the compounds 143, 148, and 158 are planned.

\(^n\) Tumor markers are biomolecules exclusively present in tumor cells and sometimes in embryotic cells.
3.3.3 Neurogenic Inflammation – Inflammatory Diseases

A lot of the diseases common in the well developed countries are based on inflammatory processes. In all those processes neurogenic inflammation plays an important part.

3.3.3.1 General Introduction

In all inflammatory processes occurring in the body neurogenic components, such as certain neuropeptides are involved. Neurogenic inflammation consists of a vicious cycle: The inflammation replicates itself, generating chronic inflammation and pain. Neuropeptides released due to inflammation cause yet again inflammation. The exact mechanism of those inflammatory processes is not yet fully understood. However we do know, that neurogenic inflammation is a major cause of many diseases. These include allergic inflammations of mucous membranes and airways, such as asthma, bronchitis, rhinitis and hay fever as well as arthritis, allergic conjunctivitis, urticaria,[sup]o[/sup] inflammations of the gastrointestinal system, such as colitis and inflammatory diseases of the skin, such as psoriasis.[sup]p[/sup] This list is far from exhaustive.

To date there is no drug on the market, that reliably inhibits neurogenic inflammation,[sup]295[/sup] thereby providing a possibility of an efficient treatment of the pathological pictures of the above listed diseases. This results in the misery of chronic pain, which extremely effects the quality of life of these patients. Classic non-steroidal anti-inflammatory drugs like for instance salicylate, amidopyridine, phenylbutazone, flufenamic acid or indomethacin do not inhibit neurogenic inflammation at all.

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[o] Urtica: latin for stinging-nettle; urticaria: an allergic disorder marked by raised edematous patches of skin or mucous membrane and usually intense itching and caused by contact with a specific precipitating factor (as a food, drug, or inhalant) either externally or internally
[p] from Greek psOrian to have the itch, Psoriasis: a chronic skin disease characterized by circumscribed red patches covered with white scales
Steroids do inhibit neurogenic inflammation, but only in very high doses, that cause considerable toxic side effects. Opiates alone proved to be effective. However, they cannot be used due to their tremendous side effects.²⁹⁶,²⁹⁷

Pretreatment with somatostatin prevented experimentally induced neurogenic inflammation. Nonetheless, it is of no therapeutic use due to its extremely short half life ($t_{1/2} < 1$ min) in the body and its lack of selectivity.

It has been shown that somatostatin can be found in the peripheral endings of capsaicin-sensitive primary afferent neurons (CSPAN) and is liberated upon stimulation. Capsaicin (8-methyl-N-vanillyl-6-nonene amide), the pungent substance of red pepper, selectively stimulates or, in high doses, degenerates a subgroup of primary afferent neurons (small dark nerve cells). Because of this property, this subpopulation of neurons is called “capsaicin-sensitive primary afferent neurons” (CSPAN) (Figure 60).²⁹⁸–³⁰⁰ CSPAN form about one half of the nerve cell population of sensory ganglions. This group includes the C-polymodal nociceptors⁹ amounting to about 60 to 70% of C-afferentation of the skin as well as the perivascular chemoceptive interoceptors⁵ of the mucous membranes (conjunctiva, airways, urogenital system, etc.) and visceral organs (heart, kidney, stomach etc.), which can be excited by chemical painstimuli (bradykinin,⁶ acids, capsaicin). A common property of these nociceptive afferents is that when stimulated, they release tachykinins (TKs)¹ (substance P (SP), neurokinin A), calcitonin gene-related peptide (CGRP)³⁰⁰ and

³
cinese: Latin for to harm;

Nociceptors are peripheral high threshold receptors for pain. All of them are free nerve endings. There are several distinct types of nociceptors:

- mechano-nociceptors - generally stimulus specific (intense pressure, pinching); mediate fast/first pain, via AS (small diameter, myelinated) afferent fibres.
- polymodal nociceptors - respond to many different forms of noxious stimuli: chemicals, intense heat, etc.; mediate slow/second pain, via C (small diameter, unmyelinated) afferent fibres
- ‘Silent’ nociceptors - respond only after tissue damage (or sensitization)

⁵ interoceptors: receptors for stimuli from within the body itself.

(exteroceptors: receptors for stimuli outside the body)

⁶ a kinin (kinin: polypeptide hormones that are formed locally in the tissues and cause dilation of blood vessels and contraction of smooth muscle), which is a linear nonapeptide and is formed locally in injured tissue, acts in vasodilation (widening of the lumen of blood vessels) of small arterioles. It is considered to play a part in inflammatory processes.

¹ A family of biologically active peptides sharing a common conserved C-terminal sequence, -Phe-X-Gly-Leu-Met-NH2, where X is either an aromatic or a branched aliphatic amino acid
Somatostatin from their peripheral endings. (Substance P is a neuropeptide of the sequence H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂. SP is widely distributed in the brain, spinal cord, and peripheral nervous system, and acts across nerve synapses to produce prolonged postsynaptic excitation.)

**Figure 60:** Schematic drawing of the functional anatomy of the capsaicin-sensitive primary afferent neuron (CSPAN). Sensory neuropeptides (TKs - substance P (SP) and neurokinin A - and CGRP) are synthesized in the perikaryon and transported to both peripheral (1, 2, 3) and central terminals (4) of the CSPANs. Environmental stimuli (mechanical, chemical, thermal) induce the release of sensory neuropeptides (like SP, NKA, and CGRP) from the same nerve terminal at which they activate afferent discharge.
During inflammation or tissue damage an overall up-regulation of the synthesis of sensory neuropeptides by the dorsal root ganglion (DRG) neurons and increased release from central endings is caused (Figure 61). Increased afferent activity by peripheral stimuli and increased nerve growth factor (NGF) production by target tissues are mechanisms inducing changes in the expression of sensory neuropeptides by CSPANs. There is also evidence for increased transport of sensory neuropeptides. Decreased immunostaining for sensory neuropeptides in peripheral tissues is observed. This is due to tissue damage as well as due to increased release of sensory neuropeptides contributing to initiation and maintenance of the inflammatory process (neurogenic inflammation).

**Figure 61:** Changes occurring in CSPANs during inflammation/ tissue damage.

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*dorsal root:* the one of the two roots of a spinal nerve that passes dorsally to the spinal cord and consists of sensory fibres

*ganglion:* a mass of nerve tissue containing nerve cells external to the brain or spinal cord
The TKs (especially substance P) induce plasma extravasation and neurogenic inflammation, whereas CGRP gives mainly rise to vasodilation of the arterioles and enhancement of microcirculation.

Thus, the capsaicin-sensitive peptidergic sensory nerve endings and terminal varicosities equally provide both a nociceptive afferent function as well as an efferent function eliciting a local tissue response. They play an important role in the signaling of neuropathic or inflammatory as well as hot stimulus- or irritant-induced pains.[301]

To conclude: CSPANs synthesize and utilize neuropeptides and tachykinins such as substance P (SP) as transmitters. We know that SP plays an important role in the neurogenic inflammatory process, but the exact mechanism is not yet completely understood. However, we do know, that the inhibition of mechanical, chemical and thermal induced SP and CGRP release, prevents the inflammatory process and pain otherwise caused.

3.3.3.2 Biological Test on Anti-inflammatory Activity of Compounds 142-145

Since pretreatment with somatostatin prevented experimentally induced neurogenic inflammation, we tested the above described and synthesized somatostatin analogues 142-145 (c.f. chapter 3.3.2.5) from the first library on their potential to prevent and to cure neurogenic inflammation. We do know, as described above, that the inhibition of mechanical, chemical and thermal induced substance P (SP) and CGRP release, prevents the inflammatory process and pain otherwise caused. We therefore screened for the ability of our compounds to inhibit the release of substance P and CGRP in vitro. The release of sensory peptides from the tissue of rat tracheas in an organ bath was induced by electric (40 V, 0,1 ms, 10 Hz, 120s) and chemical (10^{-7}M of capsaicin) stimulation, in the presence and in the absence of the tested compounds.

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\* extrasvitation: escape of a fluid from a (blood) vessel into surrounding tissue
\* vasodilation: widening of the lumen of blood vessels
\* varicosity: part of the ending of neurons

Tests were performed by our collaborators Gyorgy Kéri and Jozsef Nemeth from the Department of Medicinal Chemistry, Semmelweis Medicinal University, Budapest, Hungary
compound. The concentration of SP, CGRP and somatostatin were determined by using a specific radioimmunoassay developed by Kéri and co-workers.\cite{302, 303} For compounds 144 and 145 no substance P release inhibition could be detected in our test set up. However, compound 142 inhibited substance P release with 37.02 % (**significance\textsuperscript{2}), compound 143 with 19.1 % (*significance). The anti-neurogenic inflammation activity of 142 and 143 is in the same range as the activity of TT232 135. Non of the analogues of TT232 135 tested (or any other compound ever tested) had remotely the same activity as 142 or 143.

3.3.3.3 Conclusions

Whilst acute pain has undoubted survival value, the misery of chronic pain caused by neurogenic inflammation and may be e.g. associated with arthritis or terminal cancer, is less easy to comprehend. The compounds 142 and 143 are very interesting lead compounds for drugs against asthma, arthritis, allergic inflammations, hay fever, conjunctivitis and all other, often chronic, diseases, where inflammation processes are involved. The analogues 142 and 143 diminish inflammations of both neurogenic and non-neurogenic origin with simultaneous exertion of an analgetic effect. They should be considerable more stable than somatostatin or TT232 135 the only other compound known to induce an equally strong anti-inflammatory effect by substance P release inhibition. There is evidence that 142 and 143 induces no endocrine effects, which would appear as severe side effects during the anti-inflammatory or analgetic treatment, and seems to be more effective with considerable less side effects than any drug on the market against inflammatory processes (such as cortisone).

Interestingly the good antitumor analogues are also the best inhibitors of neurogenic inflammation. We therefore will test the promising lead compounds 148 and 158 for the anti-cancer indication form the second library, also on their ability to inhibit substance P release and thereby neurogenic inflammation. Compound 158 would be especially interesting, since it is chemically more stable then the trityl ether containing compounds, and its activity should be in the same order.

\textsuperscript{2} **significance: the error is bellow 0.001; *significance: the error is bellow 0.05
4 Summary

A straightforward synthesis of the two new furanoid sugar amino acids f-SAA 106 and f-SAA 107 in high yields in a few steps from glucose was developed. The described new synthetic pathway allows an easy access to f-SAA 106 and f-SAA 107, making them useful building blocks and structural templates for drug design and combinatorial syntheses. Their synthesis starts from readily available diacetone glucose, and is faster, easier to scale up and cheaper, than the syntheses of other SAAs so far known.

f-SAA 106’s and f-SAA 107’s structural properties have been studied. Linear and cyclic mixed oligomers of f-SAA 106 and f-SAA 107 were synthesized and solution structures extensively investigated by circular dichroism (CD) and 2D-NMR spectroscopy. The linear oligomer Fmoc–[f-SAAA106–β-hGly]3–OH 113 exhibited 12/10/12 helical secondary structure in MeCN as proven by NMR, and subsequent molecular dynamics calculations. It is very remarkable that 113 forms a stable secondary structure, since stable secondary structures were only reported for β-peptides consisting almost exclusively of α- or β-substituted β-amino acids. The unsubstituted β-homoglycin is obviously much more flexible than substituted β-amino acids. f-SAA 106 therefore very strongly induces turn elements in short oligomeric sequences.

Furthermore the insights gained on the structure inducing properties of the f-SAAS 106 and 107, were applied to biologically active systems. A first library of 12 somatostatin analogues was designed under consideration of structure activity relationships. After biological testing (antiproliferation and selective induction of apoptosis on tumor cells) the identified lead structures were further optimized, in a second, larger library. From those libraries several promising drug candidates against both cancer and inflammatory diseases could be extracted. 10 analogues have IC₅₀ values in the low µM-range. 142, 143, 148 and 158 possess antiproliferative and apoptotic activity against several different multidrug-resistant and/or drug-sensitive carcinoma cell lines. Some of these compounds are more active than TT232 135, the only other compound
known to show apoptotic and antiproliferative activity against multi drugresistant carcinoma cell lines. Preliminary studies suggest that our compounds selectively exert their apoptotic and antiproliferative activity against cancer cells.

The compounds 142 and 143 are also interesting lead compounds for drugs against asthma, arthritis, allergic inflammations, hay fever, conjunctivitis and all the other, often chronic, diseases, where inflammation processes are involved. The analogues 142 and 143 diminish inflammations of both neurogenic and non-neurogenic origin with simultaneous exertion of an analgetic effect. There is evidence that 142 and 143 induces no endocrine effects, which would appear as severe side effects during the anti-inflammatory or analgetic treatment, and seems to be more effective with considerable less side effects than any drug on the market against inflammatory processes (such as cortisone).

The nonproteinogenic properties of f-SAAs 106 and 107 should make the compounds physiologically more stable than the purely peptidic TT232 (135), the only other compound known to comparably induce either apoptosis on multidrug resistant tumor cells or to prohibit inflammation by substance P release inhibition.
5 Experimental Section

**General:** Solvents for moisture sensitive reactions were distilled and dried according to standard procedures. All other solvents were distilled before use. Pd/C was donated by Degussa, Frankfurt/M., Germany. Flash column chromatography (FC) was performed with solvents indicated on silica gel 60, 230 - 400 mesh (Merck KGaA, Darmstadt). For solid-phase synthesis TCP resin (tritylchloropolystyrene-resin) from PepChem Goldammer & Clausen, H-ß-hGly-2-ClTrt resin and Fmoc-ß-hGly-OH from Novabiochem, HATU from Perseptive Biosystems and Fmoc-GABA-OH (Fmoc-4-aminobutyric acid) from Neosystems were used. All reactions were monitored by thin-layer chromatography with 0.25-mm precoated silica gel 60 F<sub>254</sub> aluminium plates (Merck KGaA, Darmstadt). Melting points were obtained on a Büchi-Tottoli apparatus and are uncorrected. RP-HPLC analysis and semiscale preparations were carried out on a Waters (high pressure pump 510, multi-wavelength detector 490E, chromatography workstation Maxima 820), a Beckman (high pressure pump 110B, gradient mixer, controller 420, UV detector Uvicord from Knauer), or an Amersham Pharmacia Biotech (Äkta Basic 10/100, autosampler A-900) facility. RP-HPLC preparative separations were carried out on a Beckman System Gold (high pressure pump module 126, UV detector 166). C<sub>18</sub>-columns were used. As solvents, solvent A: H<sub>2</sub>O + 0.1% CF<sub>3</sub>COOH, and B: CH<sub>3</sub>CN + 0.1% CF<sub>3</sub>COOH with UV detection at 220 and 254 nm, were used. <sup>1</sup>H, and <sup>13</sup>C, and 2D NMR spectra were recorded on either Bruker - AC 250, Bruker DMX-500 or Bruker DMX-600 spectrometers. Proton chemical shifts are reported in ppm relative to residual CHCl<sub>3</sub> (δ = 7.24), DMSO (δ = 2.49) or pyridine (δ=7.19, 7.55, 8.71). Multiplicities are given (obtained from 1D spectra) as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). <sup>13</sup>C chemical shifts are reported relative to CDCl<sub>3</sub> (δ = 77.0), [D<sub>6</sub>] DMSO- (δ = 39.5) and [D<sub>3</sub>] pyridine (δ= 123.5, 135.5, 149.9). Data was processed on a Bruker X32 workstation using the UXNMR-software. Assignment of proton and carbon signals was achieved by HMQC,<sup>203</sup> COSY,<sup>202</sup> TOCSY<sup>201</sup> and HMBC<sup>204</sup> experiments. Coupling constants were determined whenever possible from the corresponding 1D-spectrum, and in some cases from different COSY experiments. HPLC-ESI mass
spectra were recorded on a Finnigan NCQ-ESI with HPLC conjunction LCQ (HPLC-system Hewlett Packard HP 1100, Nucleosil 100 5C18). IR spectra were recorded on a Perkin-Elmer 257 spectrophotometer. High-resolution mass spectra were performed by the Department of Chemistry of the Ludwigs Maximilians University of Munich on Finnigan MAT 95Q, using fast ion bombardment with Cs\(^+\) ions and \(m\)-nitrobenzylalcohol (matrix).

**General procedure for reduction and simultaneous Fmoc protection of azides (GP 1):** A stirred solution of the azide in MeOH/H\(_2\)O 2:1 (0.15 M) is adjusted to pH 8 with saturated NaHCO\(_3\) solution. A solution of Fmoc-Cl (1.1 equiv) in THF (0.16 M) is added to this solution, followed by the addition of the catalyst Pd/C (Degussa E 101 w/w 10 %, wet 49.7 % H\(_2\)O, 1g cat per g azide). The suspension is flushed several times with H\(_2\). The reaction in generally completed after 18-24 h (TLC control). Solvents are removed under reduced pressure. The residue is suspended in water, and the pH is adjusted to 8-9 with saturated NaHCO\(_3\) solution, and the aqueous phase is extracted with AcOEt (3x). The combined organic phases are washed with NaHCO\(_3\). The aqueous phase is adjusted to pH 1 with 1N HCl and extracted with AcOEt (3x). The organic phase is washed with sat. aqueous NaCl solution, dried (MgSO\(_4\)) and concentrated under reduced pressure.

**General procedure for anchoring of the first Fmoc-protected amino acid on TCP resin (GP 2):** The unloaded dry TCP resin in a syringe (exact weight known), completed with a frit, was swelled in NMP (30 min). The resin was filtered off, before a solution (~ 0.125 M) of 1.2 equiv of Fmoc-protected amino acid (with respect of the theoretical capacity of the TCP resin) and 2.5 equiv DIPEA (with respect to the quantity of Fmoc-protected amino acid used) in DCM (abs.) was added. After shaking for 1h at rt the capping solution (20% DIPEA in MeOH) is added. After 15 min the resin is filtered of, and the resin is washed with DCM (3 × 3 min), DMF (3 × 3 min),
and MeOH (3 × 3 min), and dried overnight under vacuo. Subsequently the exact weight of the dried resin was determined, and the loading of the resin was calculated:

\[
c[_{\text{mol/g}}] = \frac{(m_{\text{total}} - m_{\text{resin}})}{(M_{\text{Xaa}} - 36.461) \times m_{\text{total}}}
\]

cloading

\(m_{\text{resin}}\) mass of resin before loading

\(m_{\text{total}}\) mass of loaded resin

\(M_{\text{Xaa}}\) molar weight of the Fmoc-protected amino acid (Xaa)

**General procedure for solid-phase peptide synthesis (GP 3)**

**General procedure for the solid phase synthesis of the oligomers 3 and 4 (GP 3a):**
The Fmoc-protecting group of the amino acid attached to the resin is removed by treating the resin with a 20% piperidine solution in DMF (2 × 10 min). The resin is filtered off and washed with NMP (5 × 3 min), before a solution of the next Fmoc-protected amino acid (2 equiv Fmoc-SAA-OH, but 3 equiv Fmoc-β-hGly-OH or Fmoc-GABA-OH), HATU\[^{[192, 193]}\] (2 equiv for SAA coupling, 3 equiv for other amino acids), and 2,4,6-collidine (20 equiv/30 equiv) in NMP is added. After 3-48 h reaction is complete (monitoring by ESI-HPLC-MS). The resin is washed with NMP (5 × 3 min), prior to the subsequent Fmoc-deprotection and coupling steps. After coupling of the last amino acid, the resin is washed with NMP (3 × 3 min), CH₂Cl₂ (1 × 3 min), and dried overnight under vacuo. The oligomers are cleaved from the dry resin using 20% HFIP solution in CH₂Cl₂ (3 × 10 min). The crude peptides were purified via RP-HPLC.

**General Procedure for solid phase peptide synthesis (GP 3b):**
The preloaded resin was swelled for 30 min in NMP. The Fmoc-protecting group of the amino acid attached to the resin is removed by treating the resin with a 20% piperidine solution in DMF (3 ×10 min). The resin is filtered off and washed with NMP (5 × 3 min), before a solution of the next Fmoc-protected amino acid (3 equiv),
or of the Fmoc-protected f-SAA (1.5 equiv), HATU and HOAt\textsuperscript{192, 193} (1.5 equiv each for SAA coupling, 3 equiv each for other amino acids), and 2,4,6-collidine (15 equiv/30 equiv) in NMP (for coupling with Fmoc-protected f-SAA DMF, was used as solvent) is added. After 2-3 h reaction is complete (monitoring by ESI-HPLC-MS). The resin is washed with NMP (5 × 3 min), prior to the subsequent Fmoc-deprotection and coupling steps. After coupling of the last amino acid, and subsequent Fmoc-deprotection, the resin is washed with NMP (3 × 3 min), CH\textsubscript{2}Cl\textsubscript{2} (1 × 3 min), and dried overnight in vacuo. The compounds are cleaved from the dry resin using 20 % HFIP solution in CH\textsubscript{2}Cl\textsubscript{2} (3 × 10 min).\textsuperscript{194} The crude peptides were purified via RP-HPLC. In all cases peptide (HPLC) purity was > 99 %.

**General procedure for cyclization with DPPA/NaHCO\textsubscript{3} (GP 4):**

The Fmoc-deprotected linear peptide is dissolved in DMF (0.1 mM), and DPPA (3 equiv) and NaHCO\textsubscript{3} (11 equiv) are added.\textsuperscript{288, 289} After 12 h reaction is usually complete. After side chain deprotection (c. f. GP 5) the cyclic peptides were precipitated with Et\textsubscript{2}O and purified via RP-HPLC, and finally lyophilized from water or dioxane.

**General procedure for ivDde deprotection (GP 5):**

The peptide is dissolved in 3 % hydrazine/DMF solution, stirred for 10-15 min, and the solvent is evaporated. This procedure is repeated 3 times.
1,2:5,6-Di-O-isopropylidene-3-O-triflyl-a-D-glucofuranose (108): Triflic anhydride (54.2 g, 0.19209 mol) was slowly added to a stirred solution of diacetoneglucose (25 g, 0.96 mol), and pyridine (30.39 g, 0.384 mol) in CH$_2$Cl$_2$ (1 L) in a three-necked flask at −10°C (acetone-ice bath). Pyridinium triflate salt precipitated, and the solution turned brown. The reaction was complete after 1.5 h (TLC control: AcOEt/hexane 2:1). The reaction mixture was poured onto ice water (1 L). The aq phase was extracted with CH$_2$Cl$_2$ (4×). The organic phases were dried (MgSO$_4$), and repeatedly coevaporated with toluene to remove pyridine. The brown residue was extracted with hexane (3×). Evaporation of hexane yielded the desired product as white crystals (36.88 g, 98 %). $R_f = 0.61$ (AcOEt/hexane 2:1); m.p. and $^1$H NMR were in agreement with ref. [304, 305]

3-Azido-3-deoxy-1,2:5,6-di-O-isopropylidene-a-D-allofuranose (109): A solution of 108 (37.1 g, 0.0945 mol) in DMF (200 mL) was added slowly to a solution of NaN$_3$ (12.3 g, 0.189 mol), Bu$_4$NCl (catalytic, ~0.1 g) in DMF (1.5 L) at 50 °C. The reaction was complete after 5 h of stirring at 50 °C (TLC control: AcOEt/hexane 2:1). DMF was removed under reduced pressure, and the residue was dissolved in AcOEt. The organic phase was washed with H$_2$O (2×). The aqueous phase was re-extracted with AcOEt (2×) until TLC showed no traces of 109. The combined organic phases were dried (MgSO$_4$) and evaporated to yield a syrup of crude 109 and elimination by-product. ($^1$H NMR gave a ratio of 109:elimin. of 7:3). The crude product 109 was purified by FC (AcOEt/hexane 1:3) to yield 109 (18.2 g, 70 %) as a colorless liquid. $R_f = 0.55$ (AcOEt/hexane 1:3); $^1$H NMR of both 109 and of the elimination product were in agreement with ref. [189].
Experimental Section

3-Azido-3-deoxy-1,2-O-isopropylidene-a-D-allofuranose (110): For oxidation,[186] 109 (16 g, 0.056 mol) was dissolved in AcOH (77%, 38 mL) and stirred under reflux for 3 h. After evaporation of the organic solvent, crude 109 was purified by FC (AcOEt/hexane 2:1) to give 109 as white crystals (12.9 g, 94%). M.p. and 1H NMR were in agreement with ref. [306].

3-Azido-3-deoxy-1,2-O-isopropylidene-a-D-ribofuranose aldehyde: NaIO₄ (8.4 g, 0.036 mmol) was added gradually to a cooled solution (10°C) of 110 (8 g 0.0327 mol) in MeOH (60 mL) and H₂O (100 mL).[186] The mixture was stirred for 5 h. The inorganic salts were precipitated by the addition of MeOH (150 mL), filtered off, and washed several times with MeOH. The combined organic phases were removed under vacuum to yield the aldehyde as a light yellow syrup. The crude aldehyde was used without further purification for the oxidation to 111. 1H NMR (250 MHz, CDCl₃/MeOD, 298 K): δ=1.35 (s, CH₃), 1.55 (s, CH₃), 3.65 (dd, J₃,₄=4.72, J₂,₃=4.37 Hz, H₃), 4.1 (d, J= 4.7 Hz, H⁴), 4.7 (dd, J₁,₂=3.7, J₂,₃=4.5 Hz, H²), 5.9 (d, J₁,₂=3.8 Hz, H¹), 9.7 (br. s, H⁵).

3-Azido-3-deoxy-1,2-O-isopropylidene-a-D-ribofuranose acid (111): KMnO₄ (6.7 g, 42 mmol) was added slowly to a stirred solution of the above described aldehyde in HOAc (50 %, 150 mL) resulting in a purple solution.[186] After 12 h reaction was
complete. The solution was adjusted to pH 1 with conc. HCl, excess KMnO₄ was removed with Na₂SO₃. The solution was extracted with CHCl₃ (3x). The organic phase was dried (MgSO₄) and concentrated under reduced pressure. Recrystallization from AcOEт/hexane afforded 111 (4.29 g, 1.87 mmol, 89 % over two steps) as white crystals.

![f-SAA 106](image)

3-Amino-3-deoxy-N-9-fluorenlymethoxycarbonyl-1,2-isopropylidene-a-D-ribofurananoic acid (f-SAA 106): According to GP 1, azide 9 (1 g, 4.36 mmol) was reduced to the amine and simultaneously Fmoc-protected to yield f-SAA 106 (1.4 g, 3.29 mmol, 76 %) as a colorless syrup which later crystallized. ¹H NMR (500 MHz, [D₆] DMSO, 300 K): δ=1.26 (s, 3H, CH₃), 1.46 (s, 3H, CH₃), 4.07 (m, H₃), 4.22 (m, 1H, Fmoc-CH), 4.25 (m, 1H, H¹), 4.30 (m, 2H, CH₂Fmoc), 4.60 (t, J=4.0, 1H, H²), 5.84 (d, J=3.4, 1H, H¹), 7.32 (m, arom H), 7.40 (m, arom H), 7.63 (m, H₃), 7.72 (m, arom H), 7.87 (d, J=7.3 Hz, 2H, arom H); ¹³C NMR (125MHz, [D₆] DMSO, 300 K): δ=26.06 (CH₃), 26.29 (CH₃), 46.30 (CHFmoc), 56.25 (C³), 65.61 (CH₂Fmoc), 75.36 (C⁴), 78.01 (C²), 104.17 (C¹), 111.63 (Cisoprop.), 119.75 (C arom), 124.89 (C arom), 127.17 (C arom), 143.32 (C⁵); FAB-HRMS calcd for C₂₃H₂₃NO₇Na [M+Na]⁺ 448.1372, found 448.1366;

![112](image)

3-Amino-3-deoxy-N-9-fluorenlymethoxycarbonyl-1,2-isopropylidene-a-D-allofuranose (112): According to GP 1, azide 110 (2 g, 8.31 mmol) was reduced to the amine and Fmoc-protected. FC (AcOEт/hexane 1:1 ->1) afforded 112 (3.3 g, 7.48 mmol, 92 %) as a white powder. ¹H NMR (500 MHz, CDCl₃, 300 K): δ=1.35 (s, 3H, CH₃), 1.55 (s, 3H, CH₃), 2.12 (s, 0.8H, OH), 3.60-4.65 (m, 13H, H², H³, H⁴, H⁵,
3-Amino-3-deoxy-N-9-fluorenylmethoxycarbonyl-1,2-isopropylidene-α-D-allofuranonic acid (f-SAA 107): The diol 112 and TEMPO (1 mg, 0.064 mmol, 0.011 equiv) was suspended in CH₂Cl₂ (1.8 mL) at 0°C, a solution of KBr (14.5 mg, 0.064 mmol, 0.11 equiv) and tBu₄NCl (8.9 mg) in sat. aq NaHCO₃ (1.2 mL) was added slowly. To this a mixture of NaOCl (13%, 1.5 mL), sat. aq NaCl (1.32 mL) and sat. aq NaHCO₃ (0.7 mL) was added dropwise over a period of 30 min. The reaction mixture was stirred overnight, diluted with AcOEt (2 mL). The organic phase was extracted twice with brine. The aq phase was adjusted to pH 2 with diluted HCl (1 N) and extracted with AcOEt. Removal of the combined organic phases under reduced pressure afforded f-SAA 107 as a colorless syrup (0.17 g, 62%). ¹H NMR (500 MHz, [D₆] DMSO, 300 K): δ=1.25 (s, 3H, CH₃), 1.47 (s, 3H, CH₃), 4.05-4.30 (m, 6H, H³, H⁴, H⁵, CH₂Fmoc, CHFmoc), 4.55 (br. s, 1H, H¹), 5.73 (br. s, 1H, H¹); 13C NMR (125 MHz, [D₆] DMSO, 300 K): δ=23.97 (CH₃), 24.39 (CH₃), 45.19 (CHFmoc), 51.88 (C⁵), 63.70 (C⁴), 67.18 (C⁵), 75.30 (C²), 76.98 (CH₂Fmoc), 101.73 (C¹), 111.35 (Cisoprop), 117.20 (Carom), 122.39 (Carom), 124.14 (Carom), 124.51 (Carom), 143.80 (C⁶); FAB-HRMS calcd for C₂₄H₂₇NO₇Na [M+Na]⁺ 478.1478, found 478.14167; tᵣ=15.71 (HPLC-MS, 10-90%B in 20min).
**Fmoc-[f-SAA106-β-hGly],-OH (113):** In a syringe (10 mL), completed with a frit, preloaded H-β-hGly-2-CITrt resin (399.5 mg, 0.44 mmol g⁻¹, 0.17578 mmol) was swelled for 45 min in NMP. According to **GP 3a**, Fmoc-protected f-SAA 106 (0.1494 g, 0.35156 mmol, 2 equiv) and Fmoc-β-hGly (0.1642 g, 0.5273 mmol, 3 equiv) were coupled alternately to the hexamer. Cleavage yielded the crude 113 as a redish syrup, which was purified by RP-HPLC (40.5-54%B in 30 min) to yield 113 (71.3 mg, 41 %) as a white, fluffy solid. ¹H NMR (600 MHz, [D₆] DMSO, 296 K): δ=1.223 (f-SAA106, CH₃), 1.242 (f-SAA106, CH₃), 1.264 (f-SAA106, CH₃), 1.279 (f-SAA106, CH₃), 1.386 (f-SAA106 3, CH₃), 1.413 (f-SAA106, CH₃), 2.220 (β-hGly 4, Hᵃ), 2.263 (β-hGly 2, Hᵃ), 2.304 (β-hGly 4, Hᵃ), 2.318 (β-hGly 2, Hᵇ), 2.378 (β-hGly 6, Hᵇ), 3.192 (β-hGly 4, J(Hⁿ/Hᵇ)=6.1, Hᵇ), 3.203 (β-hGly 2, J(Hⁿ/Hᵇ)=5.8, Hᵇ), 3.204 (β-hGly 6, J(Hⁿ/Hᵇ)=5.7, Hᵇ), 3.298 (β-hGly 6, J(Hⁿ/Hᵇ)=6.7, Hᵇ), 3.328 (β-hGly 4, J(Hⁿ/Hᵇ)=6.4, Hᵇ), 4.020 (f-SAA106 1, J(H¹/H²)=10.0, J(Hⁿ/H³)=8.9, H³), 4.066 (f-SAA106 3, J(H³/H⁴)=10.4, H⁴), 4.071 (f-SAA106 5, J(H¹/H²)=10.0, H⁴), 4.200 (f-SAA106 1, J(H³/H⁴)=10.0, H⁴), 4.219 (CH²⁻Fmoc), 4.256 (f-SAA106 5, J(H³/H⁴)=10.0, J(Hⁿ/H³)=8.8, H³), 4.296 (CH²⁻Fmoc), 4.335 (f-SAA106 3, J(H¹/H²)=10.4, J(Hⁿ/H³)=9.1, H³), 4.583 (f-SAA106 1, J(H¹/H²)=5.3, H²), 4.565 (f-SAA106 3, J(H¹/H²)=5.3, H²), 4.576 (f-SAA106 5, J(H¹/H²)=5.3, H²), 5.85 (f-SAA106, J(H¹/H²)=5.3, H¹), 7.317 (Fmoc), 7.401 (Fmoc), 7.549 (f-SAA106 1, J(Hⁿ/H³)=8.9, H³), 7.731 (Fmoc), 7.813 (β-hGly 4, J(Hⁿ/Hᵇ)=6.1, J(Hⁿ/Hᵇ)=6.4, Hⁿ), 7.837 (β-hGly 2, J(Hⁿ/Hᵇ)=5.8, J(Hⁿ/Hᵇ)=6.8, Hⁿ), 7.885 (Fmoc), 7.905 (β-hGly 6, J(Hⁿ/Hᵇ)=5.7, J(Hⁿ/Hᵇ)=6.7, Hⁿ), 8.050 (f-SAA106 5, J(Hⁿ/H³)=8.8, Hⁿ), 8.098 (f-SAA106 3, J(Hⁿ/H³)=9.1, H³), 12.212 (β-hGly 6, COOH); Temperature coefficients [−Δδ/ΔT]: ¹H NMR (500 MHz, [D₃] CD₃CN, 295 - 320 K, ΔT= 5 K) f-SAA106 1 Hⁿ: 4.92; β-hGly 2 Hⁿ: 2.48; f-SAA106 3 Hⁿ: 7.90; β-hGly 4 Hⁿ: 4.17;
f-SAA106 5 HN: 9.25; β-hGly 6 HN: 1.95; 13C NMR (150 MHz, [D6] DMSO, 296 K):
d=22.179 (f-SAA106, CH3), 23.012 (f-SAA106 3, CH3), 26.207 (f-SAA106, CH3), 26.393 (f-SAA106, CH3), 28.708 (f-SAA106, CH3), 29.588 (f-SAA106, CH3), 33.266 (β-hGly 6, Cß), 34.6 (β-hGly, Cß), 34.7 (β-hGly 2+4, Cß), 35.2 (β-hGly, Cß), 46.383 (Fmoc, CH), 54.388 (f-SAA106 5, C3), 54.560 (f-SAA106 3, C3), 56.726 (f-SAA106 1, C3), 65.662 (Fmoc, CH2), 76.360 (f-SAA106 1, C4), 76.660 (f-SAA106 3+5, C4), 78.575 (f-SAA106, C3), 104.103 (f-SAA106, C1), 119.871 (Fmoc, CHarom), 125.04 (Fmoc, CHarom), 126.967 (Fmoc, CHarom), 127.226 (Fmoc, CHarom) 155.429 (Fmoc, NCOO), 168.149 (f-SAA106 1, C5), 168.297 (f-SAA106 5, C5), 168.515 (f-SAA106 3, C5), 170.431 (β-hGly 4, CO), 170.590 (β-hGly 2, CO); 15N NMR (60 MHz, [D6] DMSO, 296 K): d=78.46 (f-SAA106 1, NH), 110.86 (β-hGly 4, NH), 111.53 (f-SAA106 5, NH), 111.84 (β-hGly 6, NH), 111.86 (f-SAA106 3, NH), 111.88 (β-hGly 2, NH); tR=13.65 (HPLC-ESI-MS, 30-90%B in 20min); ESI-MS: 1053.2 [M-H+2Na]+, 1047.2 [M+K]+, 1031.3 [M+Na]+, 1009.1 [M+H]+; FAB-HRMS calcd for C49H60N6O18Na [M+Na]+ 1031.3861, found 1031.3852

NMR and structure determination of 113: All NMR spectra were acquired at 300 K in MeCN on a Bruker DMX500 spectrometer, processed and analyzed using the X-WINNMR [‘X-WINNMR V3.0’, Bruker, Karlsruhe] and AURELIA [‘AURELIA V2.8.11’, Bruker, Karlsruhe] software, respectively. As described previously, sequential assignment was done using information on the intra-residual and sequential Cß, C’, Hα and HN chemical shifts taken from COSY, TOCSY, HMQC and HMBC experiments. The COSY, HMQC and HMBC were all recorded with 8192 (t2) * 512 (t1) complex points using 8, 16 and 64 scans per increment, respectively. A mixing time of 75 ms and a data size of 2048 * 512 in t2 and t1, respectively, were used for the TOCSY experiment. NOESY [205] experiments using four mixing times (100, 200, 400, 600 ms) were measured and distance data were derived from the NOESY spectra (8192 (t2) * 512 (t1) complex points and 16 scans per increment). A mixing time of 400 ms was necessary to achieve adequate cross-peak intensities. At this mixing time, most of the NOE build up curves were still in the linear region. However, some of the integrated 76 NOESY cross-peaks were adjusted manually to account for spin diffusion. On the derived distances, a tolerance of ± 10 % was applied to obtain upper and lower bound distance restraints
for structural calculations. Allowances for the use of pseudo-atoms were added only for the terminal $H^\alpha$ and $H^\beta$ spin groups of $\beta$-hGly 6, which could not be stereospecifically assigned. A first restrained conformational search was performed with the X-PLOR package$^{[206]}$, using standard simulated annealing protocols, yielding an ensemble of 10 structures with good convergence. This ensemble already clearly showed a 12/10/12-helical conformation. The structure which best fulfilled the experimental restraints was then used as a starting structure for a 150 picosecond simulated dynamics run in the CVFF forcefield, using Discover, version 2.98$^{[207]}$. Since to our knowledge no explicit, all-atom acetonitrile solvent box is available for this program package, we equilibrated a solvent box of $31.3 \times 31.9 \times 31.5 \, \text{Å}^3$, containing 360 $\text{CH}_3\text{CN}$ molecules, for this purpose. The dynamics run was then performed in a solvent box of $47 \times 47 \times 47 \, \text{Å}^3$, containing 1039 $\text{CH}_3\text{CN}$ molecules, and using the equilibrated box as a template. During simulation time, 1500 frames were collected in the trajectory. Restraint violations all remained below 0.1 Å, except for one contact. Averaging of the heavy atom coordinates over the 150 picosecond trajectory, and subsequent 300 steps of steepest descent minimization yielded the structure of 113, as described in the main section. Trajectory population statistics for the hydrogen bonds are shown in Table 6.

Table 6: Trajectory population statistics for hydrogen bonds

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Fmoc CO – f-SAA106 3 HN</td>
<td>3.77</td>
<td>2.94</td>
<td>142.5</td>
<td>52.2</td>
</tr>
<tr>
<td>f-SAA106 3 CO – $\beta$-hGly2 HN</td>
<td>3.70</td>
<td>2.86</td>
<td>142.6</td>
<td>41.0</td>
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<tr>
<td>$\beta$-hGly2 CO – f-SAA106 5 HN</td>
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<td>2.34</td>
<td>158.0</td>
<td>85.5</td>
</tr>
<tr>
<td>f-SAA106 5 CO – $\beta$-hGly4 HN</td>
<td>3.52</td>
<td>2.62</td>
<td>149.9</td>
<td>65.5</td>
</tr>
</tbody>
</table>

To test the stability of the average minimized structure under unrestrained conditions, it was used as the starting structure for a further free dynamics simulation of
150 picoseconds duration in explicit solvent (CH$_3$CN Box, dimensions 43 x 43 x 43 Å$^3$, 779 CH$_3$CN molecules). During this simulation, 5 restraints were violated by more than 0.1 Å. The violated NOEs indicate an occasional, partial unwinding of the helix, which can be explained by the fact that acetonitrile is not a completely unpolar solvent.

**Fmoc-[f-SAA107–GABA]$_3$–OH (115):** In a syringe (10 mL), completed with a frit, TCP resin (1.0550 g, ~0.95 mmol g$^{-1}$) was swelled in NMP (30 min). After filtering off the resin, a solution of Fmoc-GABA (0.3274 g, 1.002 mmol, 1 equiv) and (iPr)$_2$EtN (0.51 mL, 3.004 mmol, 3 equiv) in CH$_2$Cl$_2$ (7.5 mL) was added. After 2h the resin was filtered off, washed with CH$_2$Cl$_2$ (3×3 min), DMF (3×3 min) and MeOH (3×3 min), before dried under reduced pressure overnight. The resin’s loading was 0.591 mmol g$^{-1}$, according to gravimetric measurements. In a syringe (10 mL) the GABA loaded resin (0.3164 g, 0.591 mmol g$^{-1}$, 0.187 mmol) was swelled for 30 min in NMP. According to **GP 3a** Fmoc-protected f-SAA 107 (0.1703 g, 0.3798 mmol, 2 equiv) and Fmoc-GABA (0.1825 g, 0.5610 mmol, 3 equiv) were coupled alternately. Purification by RP-HPLC (36-50 % B in 30 min) yielded 115 (10 mg, 4.7 %). $^1$H NMR (600 MHz, [D5] pyridine, 300 K): d=1.181 (f-SAA107 3, CH$_3$), 1.195 (f-SAA107 3, CH$_3$), 1.199 (f-SAA107 5, CH$_3$), 1.218 (f-SAA107 5, CH$_3$), 1.243 (f-SAA107 1, CH$_3$), 1.301 (f-SAA107 1, CH$_3$), 2.097 (GABA 6, H$^\alpha$), 2.113 (GABA 4, H$^\delta$), 2.127 (GABA 2, H$^\delta$), 2.499 (GABA 2, H$^\beta$), 2.592 (GABA 4, H$^\beta$), 2.607 (GABA 6, H$^\beta$), 3.414 (GABA 4, H$^\beta$), 3.431 (GABA 2, H$^\beta$), 3.525 (GABA 6, H$^\beta$), 3.686 (GABA 2+4, H$^\gamma$), 3.728 (GABA 6, H$^\gamma$), 4.035 (Fmoc, CH), 4.272 (Fmoc, CH$_2$), 4.715 (f-SAA107 3, H$^\delta$), 4.774 (f-SAA107 5, H$^\delta$), 4.833 (f-SAA107 1, H$^\delta$), 4.992 (f-SAA107 1, H$^\beta$), 4.998 (f-SAA107 3, H$^\beta$), 4.999 (f-SAA107 3, H$^\delta$), 5.018 (f-SAA107 5, H$^\delta$), 5.031 (f-SAA107 5, H$^\delta$), 5.100 (d, J=10.1, f-SAA107 1, H$^\beta$), 5.233
(f-SAA107 1, H\text{3}), 5.391 (f-SAA107 3, H\text{3}), 5.47 (f-SAA107 5, H\text{3}), 5.897 (d, J=2.6 Hz, f-SAA107 3, H\text{1}), 5.938 (d, J=2.5, f-SAA107 5, H\text{1}), 5.985 (br. s, f-SAA107 1, H\text{1}), 7.223 (m, Fmoc, H\text{arom}), 7.359 (m, Fmoc, H\text{arom}), 7.658 (m, Fmoc, H\text{arom}), 7.806 (t, J=5.9, Fmoc, H\text{arom}), 8.611 (GABA 4, H\text{N}), 8.633 (GABA 2, H\text{N}), 8.69 (GABA 6, H\text{N}), 9.255 (d, J=9.4, f-SAA107 3, H\text{N}), 9.289 (J=9.5, f-SAA107 1, H\text{N}), 9.303 (J=9.6 Hz, f-SAA107 5, H\text{N}); $^{13}$C NMR (150MHz, [D5] pyridine, 300 K): d=22.94 (f-SAA107 5, CH\text{3}), 23.6 (f-SAA107 1, CH\text{3}), 25.7 (GABA, C\text{ß}), 26.14 (f-SAA107 5, CH\text{3}), 30.13 (f-SAA107 1, CH\text{3}), 31.78 (f-SAA107 3, CH\text{3}), 31.91 (GABA 6, C\text{a}), 33.3 (GABA 2, C\text{a}), 33.35 (GABA 4, C\text{a}), 38.49 (GABA 4, C\text{i}), 38.7 (GABA 2, C\text{i}), 39.15 (GABA 6, C\text{i}), 47.196 (Fmoc, CH\text{2}), 50.15 (f-SAA107 3, C\text{i}), 50.3 (f-SAA107 5, C\text{i}), 52.5 (f-SAA107 1, C\text{i}), 61.139 (Fmoc, CH), 70.4 (f-SAA107, C\text{arom}), 80 (f-SAA107 1+5, C\text{arom}), 80.1 (f-SAA107 3, C\text{arom}), 81 (f-SAA107, C\text{arom}), 104.3 (f-SAA107 3+5, C\text{arom}), 104.4 (f-SAA107 1, C\text{arom}), 111 (f-SAA107, C\text{isoprop}), 119.917 (Fmoc, C\text{arom}), 125.410 (Fmoc, C\text{arom}), 127.063 (Fmoc, C\text{arom}), 127.647 (Fmoc, C\text{arom}), 171.3 (f-SAA107, CO), 173 (GABA 2, CO), 173.2 (GABA 4+6, CO); t_R=17.06 min(30-60% B in 30min); ESI-MS: 1185 [M-H+2Na]^+, [M-H+2K]^+, 1179.2 [M+K]^+, 1163.4 [M+Na]^+, 1141.1 [M+H]^+, 1083.1, 1025.1, 967.1, 179.1; HRMS: m/z calcd for C_{54}H_{73}N_{6}O_{21} (M+H): 1141.4829.

**cyclo[–f-SAA106–ß-hGly–]_{3} (114):** 113 (8.9 mg) was dissolved in 20 % piperidine in DMF (300 µL). After 30 min deprotection was complete. Purification by RP-HPLC (20-80 % B in 30 min) afforded deprotected 113 as a white fluffy powder (6.5 mg, quantitative). Deprotected 113 (3.9 mg, 4.96×10^{-3} mMol) was dissolved in DMF
(11 mL), a solution of HATU$^{[192, 193]}$ (1 mL of a solution of 18.8 mg HATU in 10 mL DMF) and 2,4,6-collidine (6.5 µL) was added. After 24 h reaction was not complete. Additional HATU solution (100 µL of a solution of 18.8 mg HATU in 10 mL DMF) and 2,4,6-collidine (1 µL) were added. After further 2 h the reaction was complete (HPLC control). Purification by RP-HPLC (20-80 % B in 30 min) afforded 5 as a white fluffy solid (3.8 mg, quant.). $^1$H NMR (500 MHz, [D$_6$] DMSO, 300 K): d=1.29 (s, 9H, 3×CH$_3$), 1.46 (s, 9H, 3×CH$_3$), 2.18 (m, 3H, 3×H$^a$), 2.29 (m, 3H, 3×H$^a$), 3.19 (m, 3H, 3×H$^b$), 3.42 (m, 3H, 3×H$^b$), 4.20 (m, 6H, 3×H$^3$+3×H$^4$), 4.64 (dd, $J_{H2-H1} = 3.26$, $J_{H2-H3} = 4.24$, 3H, 3×H$^2$), 5.82 (d, J=3.3, 3H, 3×H$^1$), 7.67 (t, J=6.0, 3×Hgly-H$^N$), 8.03 (d, J=7.0 Hz, 3×f-SAA1-H$^N$); $^{13}$C NMR (125 MHz, [D$_6$] DMSO, 300 K, HMQC-experiment): d=26.23 (CH$_3$), 26.46 (CH$_3$), 35.13 (C$^\alpha$), 35.37 (C$^\alpha$), 54.83 (C$^3$), 76.63 (C$^4$), 78.98 (C$^2$), 104.05 (C$^1$); $t_R = 20$ min (15-85% B in 30min); ESI-MS: 921.3 [M+TFA+K]$^+$, 905.3 [M+TFA+Na]$^+$, 807.4 [M+K]$^+$, 791.5 [M+Na]$^+$, 769.3 [M+H]$^+$.

**Synthesis of the first library of somatostatin analogues 138-141 and 142-147:**

*Loading of the TCP resin with Fmoc-Phe-OH:*

For the syntheses of

cyclo[–Phe–Trp–Lys–f-SAA106–] 138,
cyclo[–Phe–DTrp–Lys–f-SAA106–] 139,
cyclo[–Phe–Trp–Lys–Phe–f-SAA106–] 144,
cyclo[–Phe–DTrp–Lys–Phe–f-SAA106–] 145:

According to GP 2, TCP resin (2.008 g) was loaded with Fmoc-Phe-OH (933.6 mg, 2.4098 mmol) and DIPEA (1.05 mL, 6.025 mmol) in 16 mL DCM. The loading was $c = 0.677$ mol/g resin.

*Loading of the TCP resin with Fmoc-Tyr-OH:*

For the syntheses of

cyclo[–Tyr–Trp–Lys–f-SAA106–] 140,
cyclo[–Tyr–DTrp–Lys–f-SAA106–] 141,
cyclo[–Tyr–Trp–Lys–Thr(OTrt)–f-SAA106–] 142,
\( \text{cyclo[-Tyr-} \delta \text{Trp-} \text{Lys-} \text{Thr(OTrt)-f-SAA}106-] 143, \)
\( \text{cyclo[-Tyr-} \text{Trp-} \text{Lys-} \text{Thr-f-SAA}106-] 146, \)
\( \text{cyclo[-Tyr-} \delta \text{Trp-} \text{Lys-} \text{Thr-f-SAA}106-] 147: \)

Similar to GP 2 (instead of DIPEA 2,4,6-collidine was used as base), TCP resin (1.300 g) was loaded with Fmoc-Tyr-OH (629 mg, 1.56 mmol) and 2,4,6-collidine (2.77 mL) in 10 mL DCM. The loading was \( c = 0.477 \) mmol/g resin.

**Synthesis of 138 and 139:** According to GP 3b, 138 and 139 were synthesized parallel in the same syringe ((2 mL), 137 mg of the Fmoc-Phe-OH loaded TCP resin). Coupling was verified by a sample cleavage of the depeptide Fmoc-f-SAA 106–Phe-OH: ESI-MS: 1205.6 [2M-H+Na+K]⁺; 1167.2 [2M+Na]⁺; 1144.9 [2M+H]⁺; 611.4 [M+K]⁺; 595.4 [M+Na]⁺; 573.3 [M+H]⁺; \( t_R=25.04 \) min (anal. HPLC, 20-80%B in 30 min). The first coupling was done with Fmoc-protected f-SAA 106 (60.8 mg), HOAt (18.9 mg), HATU (53 mg) and 2,4,6-collidine (184 µL). Subsequently Fmoc-Lys(ivDde)-OH (133 mg) (HOAt (31.6 mg), HATU (88.2 mg), 2,4,6-collidine (307 µL)) was coupled. The resin was split into two equal parts – one for the synthesis of 138, one for the synthesis of 139. Coupling with Fmoc-L-Trp-OH, or Fmoc-D-Trp-OH (59.4 mg of L-, or D-Trp respectively) (HOAt (18.9 mg), HATU (52.9 mg), 2,4,6-collidine (184 µL)) respectively, and subsequent washing Fmoc-deprotection and cleavage steps (GP 3b) yielded the linear, ivDde-protected precursors of compounds 138 and 139, characterized by HPLC-MS:

\( \text{H}_2 \text{N-Trp–} \text{Lys(ivDde)-} \text{f-SAA}106–\text{Phe-OH} \) (precursor to 138): 909.5 [M+K]⁺; 893.5 [M+Na]⁺; 871.5 [M+H]⁺. 813.5; [M-acetone +H]⁺; \( t_R=11.41 \) min (HPLC-MS, 30-90%B in 15min), \( t_R=14.41 \) min (anal. HPLC, 30-90%B in 15min).

The precursors to 138 and 139 were cyclized according to GP 4 (DPPA (37.9 µL), NaHCO$_3$ (25 mg), DMF (12 mL)) to yield the protected cyclic precursors:


ivDde-deprotection according to GP 5, purification via rp-HPLC (semipreparative; gradient: 35-55% B in 30 min (138), and 20-60%B in 30 min (139), respectively; (B=90% MeCN, 10% H$_2$O, + 0.1%TFA)), and subsequently lyophilization yielded the compounds 138 (10 mg, 33 %) and 139 (10.7 mg, 36 %) as white, fluffy powder.


139: ESI-MS: 1445.2 [2M+TFA+K]$^+$; 1331.4 [2M+K]$^+$; 1315.3 [2M+Na]$^+$; 1293.3 [2M+H]$^+$; 799.1 [M+TFA+K]$^+$; 669.3 [M+Na]$^+$; 647.2 [M+H]$^+$; 589.3 [M-acetone+H]$^+$; $t_R=5.74$ min (HPLC-MS, 30-70%B in 15min; B=MeCN+0.1%TFA).
Synthesis of 140 and 141: According to GP 3b, 140 and 141 were synthesized parallel in the same syringe (2 mL), 190 mg of the Fmoc-Tyr-OH loaded TCP resin. The first coupling was done with Fmoc-protected f-SAA 106 (58 mg), HOAt (18.5 mg), HATU (52 mg) and 2,4,6-collidine (180 µL). Coupling was verified by a sample cleavage: Some beads were fished out, and the dipeptide Fmoc-f-SAA 106–Tyr-OH cleaved from those beads in an Eppendorf cap according to GP 3b. Characterization: ESI-MS: 1237.6 [2M-H+Na+K]⁺; 1221.4 [2M-H+2Na]⁺; 1215.4 [2M+K]⁺; 1199.2 [2M+Na]⁺; 921.6 [(3M+2K)/2]²⁺; 913.7 [(3M+Na+K)/2]²⁺; 633.4 [M-H+2Na]⁺; 627.4 [M+K]⁺; 611.4 [M+Na]⁺; 589.3 [M+H]⁺; t_R = 21.68 min (anal. HPLC, 20-80%B in 30 min). According to GP 3b Fmoc-Lys(ivDde)-OH (130 mg) (HOAt (31 mg), HATU (86 mg), 2,4,6-collidine (300 µL)) was coupled. The resin was split into two equal parts – one for the synthesis of 140, one for the synthesis of 141. Coupling with Fmoc-L-Trp-OH, or Fmoc-D-Trp-OH (58 mg of L-, or D-Trp respectively) (HOAt (18.5 mg), HATU (52 mg), 2,4,6-collidine (180 µL)) respectively, and subsequent washing Fmoc-deprotection and cleavage steps (GP 3b) yielded the linear, ivDde-protected precursors of compounds 140 and 141. The precursors to 140 and 141 were cyclizied according to GP 4 (DPPA (38 µL), NaHCO₃ (25 mg), DMF (12 mL)) to yield the protected cyclic precursors:

\[
\text{cyclo}\left[\text{Trp–Lys(ivDde)–f-SAA 106–Tyr–}\right] \quad \text{(precursor of 140): ESI-MS: 1759.9 [2M+Na]⁺; 906.7 [M+K]⁺; 891.6 [M+Na]⁺; 869.6 [M+H]⁺; 811.6 [M-acetone+H]⁺; t_R=11.89 min (anal. HPLC, 30-90%B, 30 min).}
\]

\[
\text{cyclo}\left[\text{D-Trp–Lys(ivDde)–f-SAA 106–Tyr–}\right] \quad \text{(precursor of 141): ESI-MS: 906.7 [M+K]⁺; 891.6 [M+Na]⁺; 869.6 [M+H]⁺; 811.6 [M-acetone+H]⁺; t_R=11.74 min (anal. HPLC, 30-90%B, 30 min).}
\]

ivDde-deprotection according to GP 5, purification via rp-HPLC (semipreparative; gradient: 20-60% B in 30 min (140), and 25-60% B in 30 min (141), respectively; (B=90% MeCN, 10% H₂O, + 0.1%TFA)), and subsequently lyophilization yielded the compounds 140 and 141 as white, fluffy powder.
140: ESI-MS: 799.2 [M+TFA+Na]; 685.4 [M+Na]; 663.2 [M+H]; 605.3 [M-acetone+H]; \( t_R = 15.46 \) min (anal. HPLC, 20-60%B in 15min; B=MeCN+0.1%TFA).

141: ESI-MS: 1363.3 [2M+K]; 1347.1 [2M+Na]; 1325.2 [2M+H]; 685.4 [M+Na]; 663.3 [M+H]; 605.3 [M-acetone+H]; \( t_R = 20.19 \) min (anal. HPLC, 10-60%B in 15min; B=MeCN+0.1%TFA).

**Synthesis of 142 and 143:** According to GP 3b, **142** and **143** were synthesized parallel in the same syringe (2 mL, 165 mg of the Fmoc-Tyr-OH loaded TCP resin). The first coupling was done with Fmoc-protected f-SAA **106** (50.5 mg), HOAt (16 mg), HATU (45 mg) and 2,4,6-collidine (156 µL). Coupling was verified by a sample cleavage: Some beads were fished out, and the dipeptide Fmoc-f-SAA 106–Tyr-OH cleaved from those beads in an Eppendorf cap according to GP 3b. ESI-MS of that sample cleavage: 1237.6 [2M-H+Na+K]; 1221.4 [2M-H+2Na]; 1215.4 [2M +K]; 1199.2 [2M+Na]; 633.4 [M-H+2Na]; 627.4 [M+K]; 611.4 [M+Na]; 589.3 [M+H]. According to GP 3b Fmoc-Thr(OTrt)-OH (115 mg) (HOAt (27 mg), HATU (75 mg), 2,4,6-collidine (260 µL)), and Fmoc-Lys(ivDde)-OH (112.9 mg) (HOAt (27 mg), HATU (74.7 mg), 2,4,6-collidine (260 µL)) were coupled consecutively. The resin was split into two equal parts – one for the synthesis of **142**, one for the synthesis of **143**. Coupling with Fmoc-L-Trp-OH, or Fmoc-D-Trp-OH (50 mg of L-, or D-Trp respectively) (HOAt (16 mg), HATU (45 mg), 2,4,6-collidine (156 µL)) respectively, and subsequent washing Fmoc-deprotection and cleavage steps (GP 3b) yielded the
linear, ivDde-protected precursors of compounds **142** and **143**, characterized by HPLC-MS:

H–Trp–Lys(ivDde)–Thr(OTrt)–f-SAA 106–Tyr–OH (precursor of **142**):


H–D-Trp–Lys(ivDde)–Thr(OTrt)–f-SAA 106–Tyr–OH (precursor of **143**):


The precursors to **142** and **143** were cyclized according to GP 4 (DPPA (37.9 µL), NaHCO₃ (25 mg), DMF (12 mL)) to yield the protected cyclic precursors:

\(\textit{cyclo}[-\text{Trp–Lys(ivDde)–Thr(OTrt)–f-SAA 106–Tyr–}]\) (precursor of **142**):

ESI-MS: 1256.7 [M+H+2Na]⁺; 1250.7 [M +K]⁺; 1234.7 [M +Na]⁺; 1219.0 [M +Li]⁺; 970.5 [M-Trt+H]⁺; 912.6 [M-Trt-acetone+H]⁺; 243 [Trt]⁺; \(t_R=22.13\) min (HPLC-MS, 30-70%B in 15min).

\(\textit{cyclo}[-\text{D-Trp–Lys(ivDde)–Thr(OTrt)–f-SAA 106–Tyr–}]\) (precursor of **143**):

ESI-MS: 1256.7 [M+H+2Na]⁺; 1250.7 [M +K]⁺; 1234.8 [M +Na]⁺; 1218.8 [M +Li]⁺; 970.6 [M-Trt+H]⁺; 912.7 [M-Trt-acetone+H]⁺; 243 [Trt]⁺; \(t_R=22.28\) min (HPLC-MS, 30-70%B in 15min).

ivDde-deprotection according to GP 5, purification via rp-HPLC (semipreparative; gradient: 40-65%B in 30 min (**142**), and 50-65%B in 30 min (**143**), respectively; (B=90% MeCN, 10% H₂O, + 0.1%TFA)), and subsequently lyophilization yielded the compounds **142** (8.2 mg, 20%) and **143** (14 mg, 35%) as white, fluffy powder.

**142**: ESI-MS: 1044.5 [M+K]⁺; 1028.5 [M+Na]⁺; 1006.2 [M+H]⁺; 764.4 [M-Trt+H]⁺; 706.4 [M-Trt-acetone+H]⁺; 243.2 [Trt]⁺; \(t_R=13.94\) min (HPLC-MS, 30-70%B in 15min; B=MeCN+0.1%TFA).
**Experimental Section**

143: ESI-MS: 1044.5 [M+K]+; 1028.6 [M+Na]+; 1012.6 [M+Li]+; 764.4 [M-Trt+H]+; 706.4 [M-Trt-acetone+H]+; 243 [Trt]+; $t_R=14.35$ min (HPLC-MS, 30-70%B in 15min; B=MeCN +0.1%TFA).

Synthesis of 144 and 145: According to GP 3b, 144 and 145 were synthesized parallel in the same syringe (2 mL, 371.9 mg of the Fmoc-Phe-OH loaded TCP resin). The first coupling was done with Fmoc-protected f-SAA 106 (160.7 mg), HOAt (51 mg), HATU (144 mg) and 2,4,6-collidine (500 µL). Coupling was verified by a sample cleavage: Some beads were fished out, and the dipeptide Fmoc-f-SAA 106–Phe-OH cleaved from those beads in an Eppendorf cap according to GP 3b. ESI-MS of that sample cleavage: 1739.1 [3M+Na]+; 1716.9 [3M+H]+; 1205.5 [2M-H+Na+K]+; 1183.4[2M+K]+; 1167.4 [2M+Na]+; 1145.0 [2M+H]+; 897.5 [(3M+2K)/2]2+; 889.7 [(3M+Na+K)/2]2+; 617.4 [M-H+2Na]+; 611.3 [M+K]+; 595.3 [M+Na]+; 573.2 [M+H]+. According to GP 3b Fmoc-Phe-OH (244 mg) (HOAt (85.7 mg), HATU (239 mg), 2,4,6-collidine (835 µL)), and Fmoc-Lys(ivDde)-OH (362 mg) (HOAt (85.6 mg), HATU (239 mg), 2,4,6-collidine (835 µL)) were coupled consecutively. The resin was split into two equal parts – one for the synthesis of 144, one for the synthesis of 145. Coupling with Fmoc-L-Trp-OH, or Fmoc-D-Trp-OH (161 mg of L-, or D-Trp respectively) (HOAt (51 mg), HATU (144 mg), 2,4,6-collidine (501 µL)) respectively, and subsequent washing Fmoc-deprotection and cleavage steps (GP 3b) yielded the linear, ivDde-protected precursors of compounds 144 and 145, characterized by HPLC-MS:

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The precursors to 144 and 145 were cyclized according to GP 4 (DPPA (112 μL), NaHCO\(_3\) (73 mg), DMF (35 mL) respectively) to yield the protected cyclic precursors:

cyclo[–Trp–Lys(ivDde)–Phe–f-SAA 106–Phe–] (precursor of 144): ESI-MS: 1038.6 [M+K]+; 1022.6 [M+Na]+; 1006.8 [M+Li]+; 1000.6 [M+H]+; 942.6 [M-acetone+H]+; \( t_R = 19.99 \) min (HPLC-MS, 30-70%B in 15 min).

cyclo[–D-Trp–Lys(ivDde)–Phe–f-SAA 106–Phe–] (precursor of 145): ESI-MS: 1039.6 [M+K]+; 1022.6 [M+Na]+; 1006.6 [M+H]+; 942.6 [M-acetone+H]+; \( t_R = 20.43 \) min (HPLC-MS, 30-70%B in 15 min).

ivDde-deprotection according to GP 5, purification via rp-HPLC (semipreparative; gradient: 30-65%B in 30 min (144), and 30-45%B in 30 min (145), respectively; (B=90% MeCN, 10% H\(_2\)O, + 0.1%TFA)), and subsequently lyophilization yielded the compounds 144 (41.3 mg, 41 %) and 145 (35.5 mg, 36 %) as white, fluffy powder.

144: ESI-MS: 1739.4 [2M+TFA+K]+; 1625.4 [2M+K]+; 1609.3 [2M+Na]+; 1587.3 [2M+H]+; 946.2 [M+TFA+K]+; 930.2 [M+TFA+Na]+; 832.4 [M+K]+; 816.4 [M+Na]+; 794.3 [M+H]+; 736.3 [M-acetone+H]+; \( t_R = 13.76 \) min (anal. HPLC, 30-70%B in 30 min; B=MeCN +0.1%TFA).

**Synthesis of 146 and 147:** Syntheses analogous to the synthesis of compounds 142 and 143, except, that the crude cyclic peptides were stirred in TFA-MeCN solution for five minutes.

Characterization:


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**Synthesis of 148:** cyclo[–d-Trp–Nle–Thr(OTrt)–f-SAA 106–Tyr–]

148 was synthesized according to GP 3b (2 mL, 66.8 mg of the Fmoc-Tyr-OH loaded TCP resin). Coupling of the Fmoc-protected f-SAA 106 was verified by a sample cleavage: Some beads were fished out, and the dipeptide Fmoc-f-SAA 106-Tyr-OH cleaved from those beads in an Eppendorf cap according to GP 3b. ESI-MS of that sample cleavage: 1803.0 [3M+K]$^+$; 1786.9 [3M+Na]$^+$; 1237.3 [2M-H+Na+K]$^+$; 1221.4 [2M-H+2Na]$^+$; 1215.3 [2M+K]$^+$; 1199.1 [2M+Na]$^+$; 633.4 [M-H+2Na]$^+$; 627.4 [M+K]$^+$; 611.3 [M+Na]$^+$; 589.1 [M+H]$^+$. Coupling of the Fmoc-Thr(OTrt)-OH, was verified by a sample cleavage: Some beads were fished out, and the tripeptide Fmoc-Thr(OTrt)–f-SAA 106–Tyr-OH cleaved from those beads in an Eppendorf cap according to GP 3b. ESI-MS of that sample cleavage: 1885.3 [2M+Na]$^+$; 1863.0 [2M+H]$^+$; 976.4 [M-H+2Na]$^+$; 970.4 [M+K]$^+$; 954.4 [M+Na]$^+$; 932.4 [M+H]$^+$; 243.2 [Trt]$^+$. According to GP 3b Fmoc-Nle-OH, and Fmoc-d-Trp-OH were coupled consecutively. Subsequent cleavage from the resin (GP 3b), cyclization according to GP 4, and purification via RP-HPLC (semipreparative; gradient: 50-100%B in 30

**Synthesis of 149:** cyclo[–d-Trp–Lys–Thr(OTrt)–f-SAA 107–Phe–]  
149 was synthesized according to GP 3b (2 mL, 52.4 mg of the Fmoc-Phe-OH loaded TCP resin). Coupling of the Fmoc-protected f-SAA 107 was verified by a sample cleavage: Some beads were fished out, and the dipeptide Fmoc-f-SAA 107-Phe-OH cleaved from those beads in an Eppendorf cap according to GP 3b. ESI-MS of that sample cleavage: 1829.8 [3M+Na]+; 1227.2 [2M+Na]+; 1205.0 [2M+H]+; 663.4 [M-H+Na+K]+; 647.4 [M-H+2Na]+; 641.3 [M+K]+; 625.4 [M+Na]+; 603.2 [M+H]+; 551.3 [M-acetone+Li]+; 545.1 [M-acetone+H]+. According to GP 3b Fmoc-Thr(OTrt)-OH, Fmoc-Lys(ivDde)-OH, and Fmoc-d-Trp-OH were coupled consecutively. Subsequent cleavage from the resin (GP 3b), cyclization according to GP 4, the ivDde cyclic precursor cyclo[–d-Trp–Lys(ivDde)–Thr(OTrt)–f-SAA 107–Phe–]: ESI-MS: 1264.8 [M+K]+; 1248.9 [M+Na]+; 1226.5 [M+H]+; 1006.8 [M-Trt+Na]+; 984.6 [M-Trt+H]+; 926.7 [M-Trt-acetone+H]+; 243.2 [Trt]+. Subsequent ivDde deprotection according to GP 5, and purification via RP-HPLC (semipreparative; gradient: 50-65%B in 30 min), yielded the 149 as a white fluffy powder: ESI-MS: 1058.3 [M+K]+; 1042.5 [M+Na]+; 1020.2 [M+H]+; 800.6 [M-Trt+Na]+; 778.4 [M-Trt+H]+; 720.4 [M-Trt-acetone+H]+; 243.2 [Trt]+. \( t_R = 15.18 \text{ min} \) (HPLC-MS, 30-70%B in 15 min).
Synthesis of 150: \textit{cyclo}[-D-Trp–Lys–Thr(OTTrt)–f-SAA 107–Phe–]

150 was synthesized according to GP 3b (2 mL, 52.4 mg of the Fmoc-Phe-OH loaded TCP resin). Coupling of the Fmoc-protected f-SAA 107 was verified by a sample cleavage: Some beads were fished out, and the dipeptide Fmoc-f-SAA 107-Phe-OH cleaved from those beads in an Eppendorf cap according to GP 3b. ESI-MS of that sample cleavage: 1829.8 [3M+Na]$^+; 1227.2$ [2M+Na]$^+; 1205.0$ [2M+H]$^+; 663.4$ [M-H+Na+K]$^+; 647.4$ [M-H+2Na]$^+; 641.3$ [M+K]$^+; 625.4$ [M+Na]$^+; 603.2$ [M+H]$^+; 551.3$ [M-acetone+Li]$^+; 545.1$ [M-acetone+H]$^+$. According to GP 3b Fmoc-Bip-OH, Fmoc-Lys(ivDde)-OH, and Fmoc-D-Trp-OH were coupled consecutively. Subsequent cleavage from the resin (GP 3b), cyclization according to GP 4, ivDde-deprotection and purification via RP-HPLC (semipreparative; gradient: 50-65%B in 30 min), yielded 150 as a white fluffy powder: ESI-MS: 938.9 [M+K]$^+; 922.9$ [M+Na]$^+; 900.7$ [M+H]$^+; 842.7$ [M-acetone+H]$^+$. $t_R=13.10$ min (HPLC-MS, 30-70%B in 15 min).

Parallel Syntheses of compounds 151-155:

\textit{cyclo}[-D-Trp–Lys–Bip–f-SAA 106–Phe–] (151);
\textit{cyclo}[-D-Trp–Lys–Phe(F$_5$)–f-SAA 106–Phe–] (152);
\textit{cyclo}[-D-Trp–Lys–Bpa–f-SAA 106–Phe–] (153);
\textit{cyclo}[-D-Trp–Lys–1-Nal–f-SAA 106–Phe–] (154);
According to GP 2, TCP resin (2.008 g) was loaded with Fmoc-Phe-OH (933.6 mg, 2.4098 mmol) and DIPEA (1.05 mL, 6.025 mmol) in 16 mL DCM. The loading was c = 0.677 mol/g resin.

Each of the compounds 151-155 was prepared in a separate syringe (2 mL, 52.4 mg of the Fmoc-Phe-OH loaded TCP resin each), completed with a frit. The linear precursors of the compounds 151-155 were prepared according to GP 3b. However, if the same amino acid was to be coupled, only one coupling solution was prepared for all of the compounds. This solution was then divided into equal parts, and added to the different syringes. First coupling is performed with Fmoc-protected f-SAA 106 (113.5 mg, 1.5 equiv; HOAt (36.3 mg, 1.5 equiv); HATU (101.3 mg, 1.5 equiv); 2,4,6-collidine (353 µL, 15 equiv); dissolved in 1 mL DMF; 270.7 µL coupling solution per syringe). Exemplarily, some beads from the syringe for synthesis of 151 were fished out, the dipeptide Fmoc-f-SAA 106–Phe-OH cleaved according to GP 3b, and characterized by ESI-MS and HPLC: 1738.7 [3M+Na]+; 1716.8 [3M+H]+; 1205.4 [2M-H+Na+K]+; 1167.1 [2M+Na]+; 1144.9 [2M+H]+; 611.3 [M+K]+; 595.3 [M+Na]+; 573.2 [M+H]+; t_R=25.04 min (anal. HPLC, 20-80%B in 30 min). For the second coupling, a different unnatural amino acid was coupled for all of the different compounds 151-155, and therefore a different coupling solution was prepared for each of them: Each of the coupling solutions consisted of HATU (27 mg), HOAt (10 mg), 2,4,6-collidine (94 µL), and respectively one of the unnatural amino acids Fmoc-Bip-OH (151, 33.0 mg), Fmoc-Phe(F₅)-OH (152, 33.9 mg), Fmoc-Bpa-OH (153, 35.0 mg), Fmoc-1-Nal-OH (154, 31.0 mg), Fmoc-2-Nal-OH (155, 31.0 mg), in 300 µL NMP. Consecutive coupling of Fmoc-Lys(ivDde)-OH (204 mg, 2 equiv; HOAt (47 mg, 2 equiv); HATU (135 mg, 2 equiv); 2,4,6-collidine (470 µL, 20 equiv); dissolved in 1.5 mL DMF; 300 µL coupling solution per syringe), and Fmoc-D-Trp-OH (151.2 mg, 2 equiv; HOAt (47 mg, 2 equiv); HATU (135 mg, 2 equiv); 2,4,6-collidine (470 µL, 20 equiv); dissolved in 1.5 mL DMF; 300 µL coupling solution per syringe), subsequent Fmoc-deprotection and cleavage from the resin yielded the linear precursors of compounds 151-155. These were cyclized in individual flasks according to GP 4 (DPPA (23 µL), NaHCO₃ (14.9 mg), DMF (7.1 mL) each) to yield the protected cyclic precursors, exemplary ESI-MS of
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cyclo[(−D-Trp–Lys(ivDde)–Phe(F5)–f-SAA 106–Phe−] (precursor of 151): ESI-MS: 
1134.6 [M-H+2Na]+; 1128.6 [M+K]+; 1112.7 [M+Na]+; 1090.6 [M+H]+; 1032.6
[M-acetone+H]+. ivDde-deprotection according to GP 5, purification via rp-HPLC
(semipreparative; gradient: 45-63%B in 30 min (151), 30-70%B in 30 min (152),
45-65%B in 30 min (153, 155), 47-55%B in 30 min (154), respectively; (B=90%
MeCN, 10% H2O, + 0.1%TFA)), and subsequently lyophilization yielded the
compounds as white, fluffy powder.

**151**: ESI-MS: 1028.2 [M+TFA-H+2Na]+; 1022.4 [M+TFA+K]+; 1006.5
[M+TFA+Na]+; 908.4 [M+K]+; 892.6 [M+Na]+; 870.4 [M+H]+; 812.5
[M-acetone+H]+; \( t_r = 13.05 \) min (HPLC-MS, 30-70%B in 15min; B=MeCN
+0.1%TFA).

**152**: ESI-MS: 1806.4 [2M(1\textsuperscript{13}C)+K]+; 1805.4 [2M+K]+; 1790.3 [2M(1\textsuperscript{13}C)+Na]+;
1789.3 [2M+Na]+; 1768.2 [2M(1\textsuperscript{13}C)+H]+; 1767.2 [2M+H]+; 922.3 [M+K]+;
906.4[M+Na]+; 884.3 [M+H]+; 826.4 [M-acetone+H]+; \( t_r = 11.65 \) min (HPLC-MS,
30-70%B in 15min; B=MeCN +0.1%TFA).

**153**: ESI-MS: 1056.1 [M+TFA-H+2Na]+; 1050.3 [M+TFA+K]+; 1034.4
[M+TFA+Na]+; 936.5 [M+K]+; 920.6 [M+Na]+; 898.4 [M+H]+; 840.5
[M-acetone+H]+. \( t_r = 12.19 \) min (HPLC-MS, 30-70%B in 15min; B=MeCN
+0.1%TFA).

**154**: ESI-MS: 1839.6 [2M+TFA+K]+; 1709.6 [2M+Na]+; 1687.6 [2M+H]+; 1002.1
[M+TFA-H+2Na]+; 996.4 [M+TFA+K]+; 980.4 [M+TFA+Na]+; 882.5 [M+K]+; 866.6
[M+Na]+; 844.4 [M+H]+; 786.5 [M-acetone+H]+.

**155**: ESI-MS: 1840.7 [2M(1\textsuperscript{13}C)+TFA+K]+; 1710.6 [2M(1\textsuperscript{13}C)+Na]+; 1687.5
882.5 [M+K]+; 866.6 [M+Na]+; 844.4 [M+H]+; 786.5 [M-acetone+H]+. \( t_r = 3.75 \) min
(HPLC-MS, 30-70%B in 15min; B=MeCN +0.1%TFA).
Synthesis of 156: cyclo[–d-Trp–Lys–Thr(OBzl)–f-SAA 106–Tyr–]

156 was synthesized (2 mL, 66.8 mg of the Fmoc-Tyr-OH loaded TCP resin), according to GP 3b. Coupling of the Fmoc-protected f-SAA 106 was verified by a sample cleavage: Some beads were fished out, and the dipeptide Fmoc-f-SAA 106–Tyr-OH cleaved from those beads in an Eppendorf cap according to GP 3b. ESI-MS of that sample cleavage: 1803.0 [3M+K]+; 1786.9 [3M+Na]+; 1237.3 [2M-H+Na+K]+; 1221.4 [2M-H+2Na]+; 1215.3 [2M+K]+; 1199.1 [2M+Na]+; 633.4 [M-H+2Na]+; 627.4 [M+K]+; 611.3 [M+Na]+; 589.1 [M+H]+. According to GP 3b Fmoc-Thr(OBzl)-OH, Fmoc-Lys(ivDde)-OH, and Fmoc-d-Trp-OH were coupled consecutively. Subsequent cleavage from the resin (GP 3b), cyclization according to GP 4, ivDde deprotection according to GP 5, and purification via RP-HPLC (semipreparative; gradient: 35-50%B in 30 min), yielded the 156 as a white fluffy powder: ESI-MS: 892.2 [M+K]+; 876.5 [M+Na]+; 860.9 [M+Li]+; 854.4 [M+H]+; 796.3 [M-acetone+H]+; \( t_R = 8.82 \) min (HPLC-MS, 30-90%B in 15 min).

Synthesis of 157:

157 was synthesized (2 mL, 66.8 mg of the Fmoc-Tyr-OH loaded TCP resin), according to GP 3b. Coupling of the Fmoc-protected f-SAA 106 was verified by a sample cleavage: Some beads were fished out, and the dipeptide Fmoc-f-SAA 106–Tyr-OH cleaved from those beads in an Eppendorf cap according to GP 3b. ESI-MS of
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For the subsequent coupling of 2-nitro-3,4-dimethoxy-benzylamine several coupling reagents and conditions were tried out such as: conditions of GP 3b; PyBop (5 equiv), NMM (5 equiv) and amine (5 equiv) in NMP;[307] and pre-activation (3-4 h) with pyridine (1 equiv), and cyanarylfluoride (1 equiv) in DCM, followed by coupling with the amine (5 equiv) and 2,4,6-collidine (50 equiv) in NMP. Best coupling yields were obtained, when the acid on the resin was pre-activated with HATU/HOAt (0.6 equiv each, 30 equiv 2,4,6-collidine in DMF (0.5 mM)), after 30 min this solution was filtered, and the amine was dissolved in that same solution, and added again to the resin. After 2h coupling time, the resin was filtered off, washed, and the remaining carboxylic side chains again pre-activated with HATU/HOAt. This coupling cycle was repeated 3 times, before coupling according to GP 3b was continued. Fmoc-Lys(ivDde)-OH, and Fmoc-D-Trp-OH were coupled consecutively. Subsequent cleavage from the resin (GP 3b), cyclization according to GP 4, ivDde deprotection according to GP 5, and purification via RP-HPLC (semipreparative; gradient: 30-65%B in 30 min), yielded the 157 as a white fluffy powder: ESI-MS: 995.0 [M+Na]+; 978.6 [M+Li]+; 972.8 [M+H]+; 951.9 [M-acetone+K]+; 914.9 [M-acetone+H]++; $t_R$=5.0 min (HPLC-MS, 30-70%B in 15 min).
Synthesis of 158:

**Synthesis of 159:**

Synthesis of 160:

Synthesis of 161:

References


References


