Synthesis and Conformational Analysis of Linear and Cyclic Peptides Containing Sugar Amino Acids

Erich Graf von Roedern,† Elisabeth Lohof, Gerhard Hessler, Matthias Hoffmann, and Horst Kessler*

Contribution from the Institut für Organische Chemie und Biochemie, Technische Universität München, Lichtenbergstrasse 4, D-85747 Garching, Germany

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Abstract: Sugar amino acids (SAAs) were designed and synthesized as new non-peptide peptidomimetics utilizing carbohydrates as peptide building blocks. They represent sugar-like ring structures that carry an amino and a carboxylic functional group and have a specific conformational influence on the backbone of peptides due to their distinct substitution patterns in rigid pyranose sugar rings. Five different SAAs (SAA**1**α, SAA**1**β, SAA**2**, SAA**3**, and SAA**4**) have been synthesized that show the ability to constrain linear backbone conformations or distinct turn structures. Linear and cyclic peptides involving SAAs have been prepared in solution as well as by solid phase synthesis. SAA**1**α and SAA**2** were incorporated into two linear Leu-enkephalin analogs, replacing the natural Gly-Gly dipeptide. NMR studies provide evidence for the conformation-inducing effect of the carbohydrate moiety. SAA**2** and SAA**3** have been placed in cyclic hexapeptide analogs of somatostatin; SAA**4** was incorporated in a model peptide. The conformation of the cyclic peptides *cyclo*(-SAA**2**-Phe-D-Trp-Lys-Thr-), *cyclo*(-SAA**3**-Phe-D-Trp-Lys(Boc)-Thr(tBu)-), and *cyclo*(-SAA**4**-Ala-D-Pro-Ala-Ala-) have been analyzed by various NMR techniques in combination with distance geometry calculations and subsequent molecular dynamic simulations. The determined solution conformations were compared to representative idealized peptide backbones. SAA**2** and SAA**3** induce a β-turn structure while SAA**4** mimics a γ-turn. Both enkephalin analogs were not active in the guinea pig ileum assay. The somatostatin analog containing SAA**2** has an inhibition constant (IC_{50}) of 0.15 μM for the inhibition of the release of growth hormone.

In recent years the interest in a rational design of amino acid and peptide mimetics has steadily grown due to the pharmacological limitations of bioactive peptides. A large variety of modifications of peptide structures has been used for conformationally directed drug design to investigate the active peptide-receptor binding conformation.[1] Constrained peptidomimetics and cyclization of peptides remain of special interest to obtain a distinct, bioactive conformation, especially in the field of combinatorial synthesis for high throughput screening.[2] Carbohydrates present as an attractive option for non-peptide scaffolding as they contain well-defined and readily convertible substituents[3] with a rigid pyran ring.

Carbohydrates are frequently found in proteins as a result of enzyme-mediated glycosylation in post-translational modification processes.[4] Sugar amino acids (SAAs) in particular occur in nature as subunits of oligosaccharides (neuraminic acid), in cell walls of bacteria (muraminic acid), and in some antibiotics.[5] The syntheses of SAAs[6] so far concentrated on the use of SAA analogs as biopolymer building blocks[7,8] to mimic oligo- and polysaccharide structures via amide bond linkages.

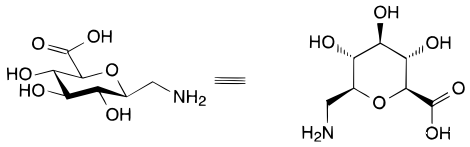

Figure 1. Example of a sugar amino acid (SAA**2**).

Two years ago we reported one example of a sugar amino acid as a new type of peptidomimetic (Figure 1).[9,10] The novel SAA was successfully incorporated into a cyclic peptide with the β-turn motif of the somatostatin containing tetrapeptide Phe-

* Author to whom correspondence should be addressed. Tel.: ++49-89-289 13301. Fax: ++49-89-289 13210. E-mail: kessler@joshua.org.chemie.tu-muenchen.de.

† Present address: Max Planck Institut für Biochemie, Am Klopferspitz 18 a, D-82152 Martinsried, Germany.

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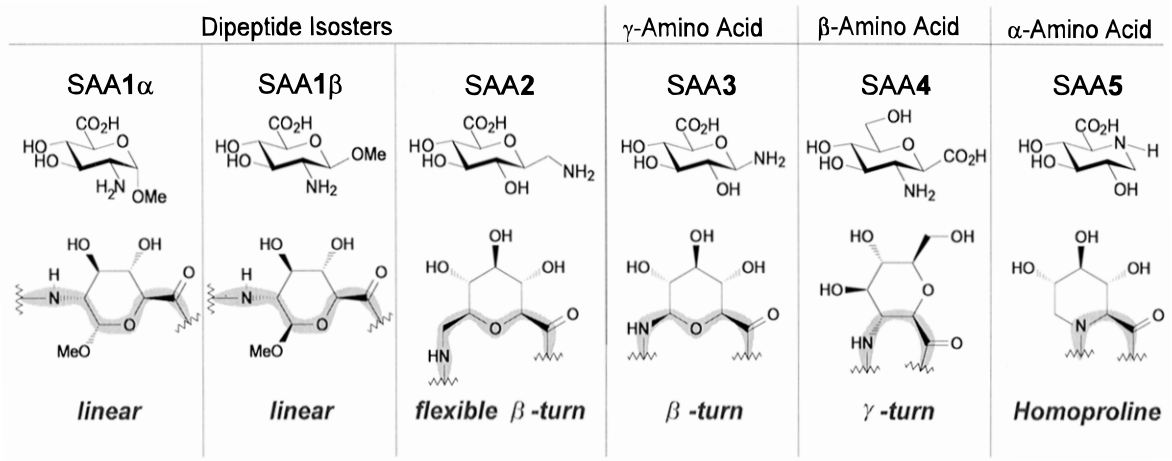

Figure 2. Structures of sugar amino acids (SAAs) discussed in this work.

D-Trp-Lys-Thr. The conformational analysis clearly typified two β-turns. We herein report a systematic approach using several sugar amino acids (SAAs) as a new class of building blocks for peptide scaffolds and conformational restrained peptidomimetics.[9,10] SAAs unit the functional groups of amino acids with the rigidity of pyranoid ring structures.[11] Figure 2 shows a construction kit for predetermined constrained local conformations in synthetic peptides containing a series of six SAAs;[10] these units offer possibilities as mimetic structures for both amino acids and dipeptide isosteres. The syntheses of all SAAs were performed using readily available starting materials. SAA compounds of type **1**α,[6] **3**,[12] and **5**[13] have already been synthesized by other groups, although they have not been used as structural units in peptides.

The SAAs shown in Figure 2 contain a six-membered ring with all substituents in equatorial positions, except for the methoxy group in SAA**1**α. Therefore, the chair conformation is very stable and rigid, consequently allowing a prediction on the conformational restriction introduced to peptides. As will be demonstrated, SAA**1**α and SAA**1**β constrain a linear peptide conformation, whereas the others are turn mimetics. Thereby the turn diameter is decreasing from SAA**2** to SAA**5** (Figure 2). SAA**2** and SAA**3** serve as β-turn mimetics and SAA**4** as a γ-turn mimetic, and SAA**5** can be regarded as a homoproline derivative (or hydroxylated pipecolic acid).

In order to explore the effect of the dipeptide isosteres SAA**1**α and SAA**2** on the conformation of linear peptides by NMR spectroscopy, we synthesized the Leu-enkephalin analogs H-Tyr-SAA**1**α-Phe-Leu-OMe (**19**) and H-Tyr-SAA**2**-Phe-Leu-OMe (**20**) in which the SAAs replace the Gly-Gly dipeptide of the natural sequence H-Tyr-Gly-Gly-Phe-Leu-OH (Figure 3).

The dipeptide Gly-Gly serves as a spacer in enkephalin between the messenger amino acid Tyr which is essential for

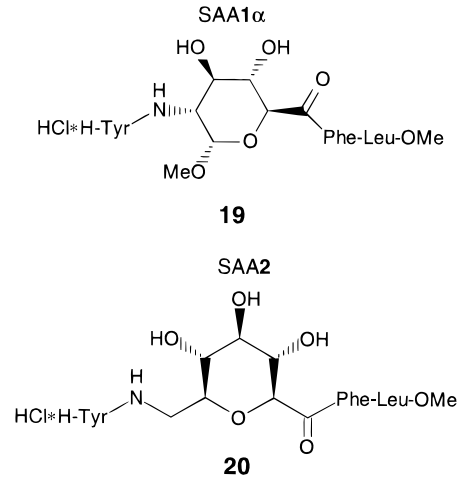

Figure 3. SAA**1**α and SAA**2** incorporated into enkephalin anlogs **19** and **20**.

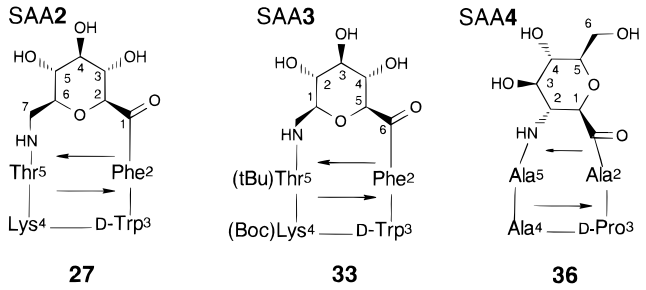

Figure 4. SAA**2**, SAA**3** and SAA**4** incorporated in cyclic peptides **26**, **33**, and **36**.

the activity and the address sequence Phe-Leu responsible for the selectivity.[14] The conformational influence of SAA**2**, SAA**3**, and SAA**4** on the peptide backbone of three cyclic peptides (Figure 4) was investigated in more detail by NMR spectroscopy, distance geometry, and subsequent molecular dynamic calculations.

The highly active somatostatin cyclic hexapeptide *cyclo*(-Phe-Pro-Phe-D-Trp-Lys-Thr-)[15] was used as a classical peptide for design of peptidomimetics,[16] since the solution structure revealed two β-turns.[17] The sequence Phe-D-Trp-Lys-Thr remained in

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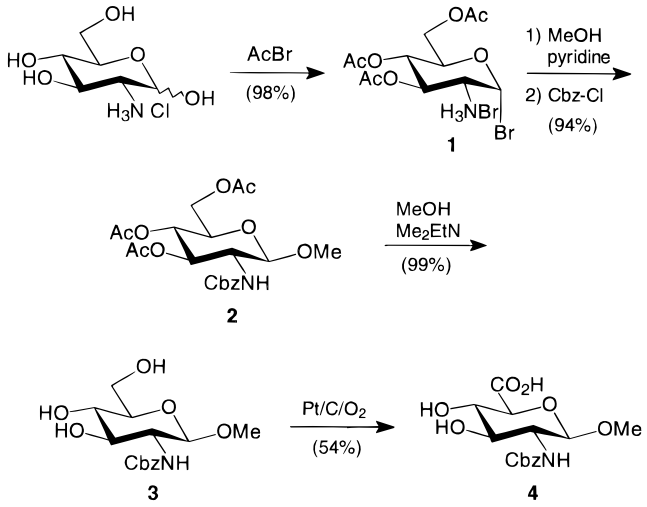
Scheme 1. Synthesis of the Protected Building Block SAA**1**β



a βII′-turn being part of many other active somatostatin analogs.[18] In *cyclo*(-SAA**2**-Phe-D-Trp-Lys-Thr-) and *cyclo*(-SAA**3**-Phe-D-Trp-Lys-Thr-) the SAAs replaced the two neighboring amino acids Phe-Pro to investigate the resulting turn pattern. SAA**4** was incorporated in a model peptide of the sequence *cyclo*(-SAA**4**-Ala-D-Pro-Ala-Ala-).

Synthesis of the SAA Building Blocks. Cbz-SAA**1**α-OH was synthesized as described by Heyns and Paulsen starting from the α-methyl glycoside in an overall yield of 37%.[6] The β-anomer Cbz-SAA**1**β-OH (**4**) was prepared from glucosamine which was transformed to glycosyl bromide **1** with acetyl bromide (Scheme 1).[19]

The β-methyl glycoside[20] was obtained by treatment of bromide **1** with methanol and pyridine and further protected by the benzyloxycarbonyl (Cbz) group. Deacetylation of **2** was achieved by methanolysis, and the resulting compound **3** was selectively oxidized at the free primary hydroxyl group with oxygen on a platinum catalyst in aqueous solution by the method of Heyns and Paulsen[6] in an overall yield of 49%.

The synthesis of SAA**2** has already been published by our group.[9] SAA**2** was obtained as Cbz-SAA**2**-OMe in an overall yield of 12% and adequately deprotected for further synthesis. The enantiomer of SAA**2** was prepared by Fuchs and Lehmann[7] in 11 steps starting from glucose.

H-SAA**3**-OMe (**8**) has already been described by Nitta et al.,[12] who prepared the azide from the unstable bromide (obtained from α/β acetate mixture) using NaN_3 in 53% yield for this step. Because of the unsatisfactory yields, we have improved the synthesis following the route outlined in Scheme 2. The glucuronolactone was converted to the methyl ester with methanol via base catalysis and was then acetylated by a mixture of acetic anhydride and sodium acetate.[21] Crystallization allowed an excellent separation of the β-acetate **6** from the α-anomer. Other acetylation methods, i.e., acetic anhydride/pyridine or acetic anhydride/perchloric acid also provide the acetylated glucuronolactone along with α-acetate. The β-azide **7** was obtained from **6** with tin tetrachloride and trimethylsilyl azide[22] in an overall yield of 43%. Catalytic reduction at low temperature provided H-SAA**3**-OMe (**8**), which was used without further purification.

The synthesis of Fmoc-SAA**4**-(tri-*O*-benzyl)-OH, **15** (Scheme 3), followed a procedure published for the stereoselective *C*-glycosidation of 2-acetamido-2-deoxy-D-glucose.[23] With D-glucosamine as the starting material, the partially benzylated sugar **9** was obtained in two steps according to a procedure of Fletcher and Inch.[24] The amino function was subsequently protected by Cbz-Cl to obtain **10** in 90% yield. Chlorination of the anomeric hydroxyl group provided the α-chloro compound which was treated with tributyltin lithium to afford **11** in 79% yield.[23] The generation of the glycosyl dianion **12** was accomplished in two separate temperature steps: first, deprotonation of the urethane nitrogen at −78 °C using 1 equiv of BuLi; second, transmetalation at −55 °C using 1.2 equiv of BuLi. The dianion **12** was visualized by a deep red color of the solution and was subsequently trapped by carbon dioxide to afford **13** in 83% yield. For the application of SAA**4** in solid phase peptide synthesis, **13** was transformed into the Fmoc derivative **15**. TFA/thioanisole[25] or catalytic hydrogenolysis on Pd/C[26] were not selective for removal of the Cbz group in **13**. The best result for cleaving the Cbz group was obtained by using trimethylsilyl iodide in CH_3CN.[27] However, the C^7-*O*-benzyl ether of **13** was cleaved to some extend. While the amount of side product was temperature independent, the yield was optimized by varying the reaction time. The crude reaction mixture was treated with Fmoc−ONSu[28] to afford **15** in 48% yield.

The synthesis of SAA**5** has been published by Fleet et al.[13] As part of our ongoing study this azasugar will be incorporated into peptides to compare its conformational and pharmaceutical influences as a proline and pipecolic acid surrogate.

Synthesis of the SAA Peptides. The enkephalin analogs **19** and **20** (Figure 3) were assembled starting from dipeptide H-Phe-Leu-OMe, which was coupled with the monoprotected SAAs using standard peptide solution protocol with EDCl·HCl (1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride) and HOBt (1-hydroxybenzotriazole) as coupling reagents in an overall yield of 34% **19** and 50% **20**. Protection of the hydroxyl groups of SAA**1**α and SAA**2** was not necessary. In these syntheses the chemical behavior of the SAAs was similar to that of unprotected threonine.

Cyclo(-SAA**2**-Phe-D-Trp-Lys-Thr-) (**27**) was synthesized using THF and DMF as a solvent mixture in the coupling reactions. EDCl·HCl and HOBt were used as coupling agents, and the cyclization step was activated with TBTU (2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate). By using such strong activation, some β-elimination in SAA**2**

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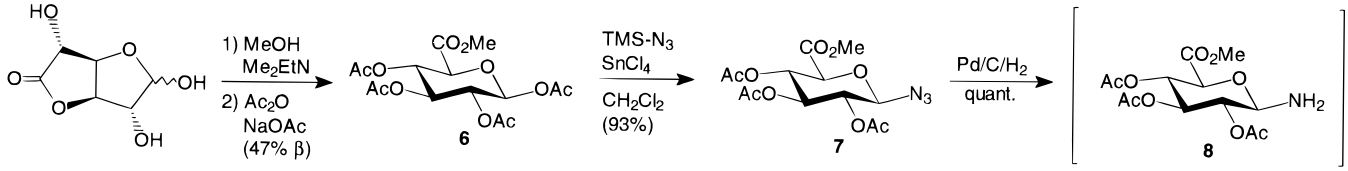
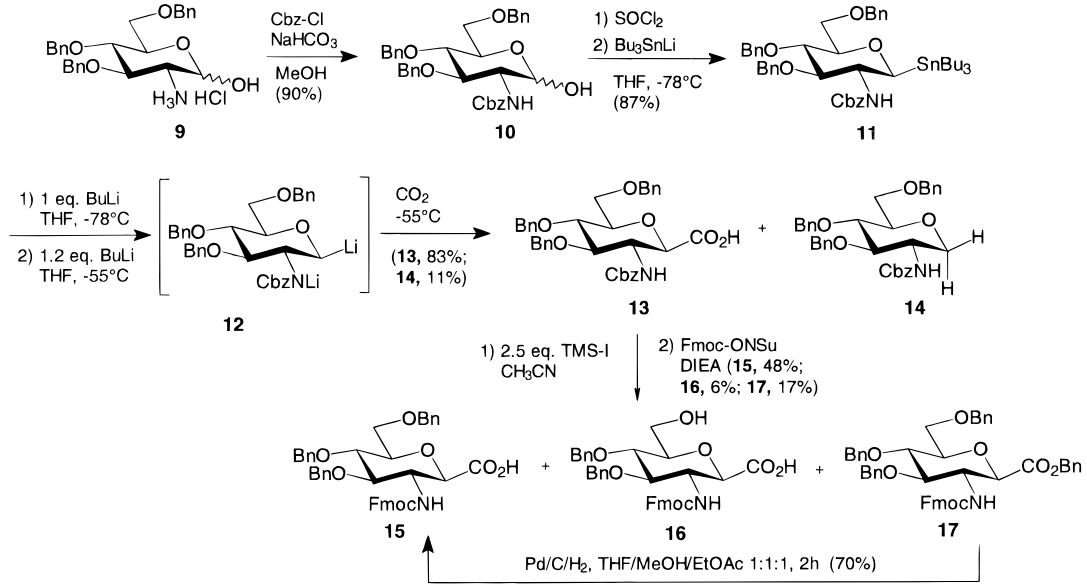
Scheme 2. Synthesis of the Protected Building Block SAA**3**



Scheme 3. Synthesis of the Protected Building Block SAA**4**



wasobserved as TBTU reacted with the unprotected sugar hydroxyl groups.

Cyclo(-SAA**3**-Phe-D-Trp-Lys(Boc)-Thr(tBu)-) (**33**) was also synthesized in solution. Contrary to the C-glycosidic SAA**2,** the free amine of SAA**3** was unstable due to epimerization. Therefore, hydrogenation of the azide **7** on Pd/C was performed in THF since anomerization is known to occur preferably in protic solvents. After isolation of compound **8**, the amine was immediately coupled with IIDQ (1-(isobutoxycarbonyl)-2-isobutoxy-1,2-dihydroquinoline).[29] Coupling with EDCl·HCl in THF gave more side products than IIDQ, because of the low nucleophilicity of the anomeric amine. Further couplings were performed with standard peptide coupling procedures (see Experimental Section) and by adding H_2O as a scavenger for the hydroxyl groups during cyclization with TBTU.

The peptide *cyclo*(-SAA**4**-Ala-D-Pro-Ala-Ala-) (**36**) was synthesized by solid phase synthesis. The pentapeptide analog H-Ala-SAA**4**(tri-*O*-benzyl)-Ala-D-Pro-Ala-OH (**34**) was assembled on the 2-chlorotritylchloride resin using Fmoc-chemistry and cleaved from the resin by HOAc/trifluoroethanol in CH_2Cl_2. Compound **15** was used in 1.1 equiv and all other amino acids in 1.7 equiv relative to the determined loading of the resin. The subsequent cyclization was performed at high dilution using TBTU as a coupling agent. The protecting groups were removed by hydrogenolysis in the presence of Pd/C.

Conformational Analysis. The ^{1}H NMR spectrum in DMSO-d_6 of the linear peptide **19** shows large ^3J(H,H) coupling constants around 10 Hz between the ring protons of SAA**1**α, and this implies that the pyranoid ring of SAA**1**α is in the predicted $^4C_1$ chair conformation. In Figure 5 the dihedral angles of SAA**1**α are compared to those of the backbone of a dipeptide with a *cis* amide bond. The ω_i angle of SAA**1**α is fixed to −60°, whereas the ψ_i and φ_{i+1} angles are about 180°. The dihedral angles φ_i and ψ_{i+1} display no conformational restriction. Therefore the peptidomimetic SAA**1**α can be used to replace two adjacent amino acids introducing conformational constraints at the ψ_i, ω_i, and φ_{i+1} angle. These restrictions result in an extended peptide conformation. In agreement with a linear conformation, ROE cross signals were only found between adjacent amino acids.

Analysis of the ^3J(H,H) coupling constants in peptide **20** indicates that the pyranoid ring of SAA**2** is in the all-equatorial chair conformation, fixing the backbone angles ω_i and φ_{i+1} around 180°. The φ_i and both ψ angles are not restricted, which allows SAA**2** to form loop structures. The homonuclear ^3J(H,H) coupling constants between the methylene protons of C^7 and the H^6 in **20** were 1.0 and 8.5 Hz, respectively. This indicates a preferred conformation about the C^6-C^7 bond in **20** with one of the methylene protons oriented antiperiplanar to H^6 ($\chi_1 = -60°$ or $\chi_1 = -180°$). The assignment of the two diastereotopic protons was performed using the distance information of a ROESY spectrum (see Experimental Section). The ROE cross signals between aromatic protons of the tyrosine side chain and H^2 of SAA**2** show a distance of 3.8 and 3.9 Å. Obviously SAA**2** induces a bent structure, without acting as a rigid β-turn mimetic. Presuming a *trans* amide bond between Tyr and SAA**2**, such short distances are only possible if H^7(*pro-S*) is antiperiplanar to H^6 ($\psi_i = -60°$). In the case of the other rotamer ($\psi_i = 180°$), the distance would be larger than 6.5 Å. This turn structure is also in agreement with Newman's 1,5-repulsion theory.[30] The other possible rotamer would result in a strong sterical repulsion between the NH- and HO- substituent of SAA**2** (Figure 5).

The conformational analyses of the cyclic peptides were carried out using the side chain protected *cyclo*(-SAA**2**-Phe-D-Trp-Lys(Cbz)-Thr-) (**26**), *cyclo*(-SAA**3**-Phe-D-Trp-Lys(Cbz)-Thr(tBu)-) (**33**), and *cyclo*(-SAA**4**-Ala-D-Pro-Ala-Ala-) (**36**). The

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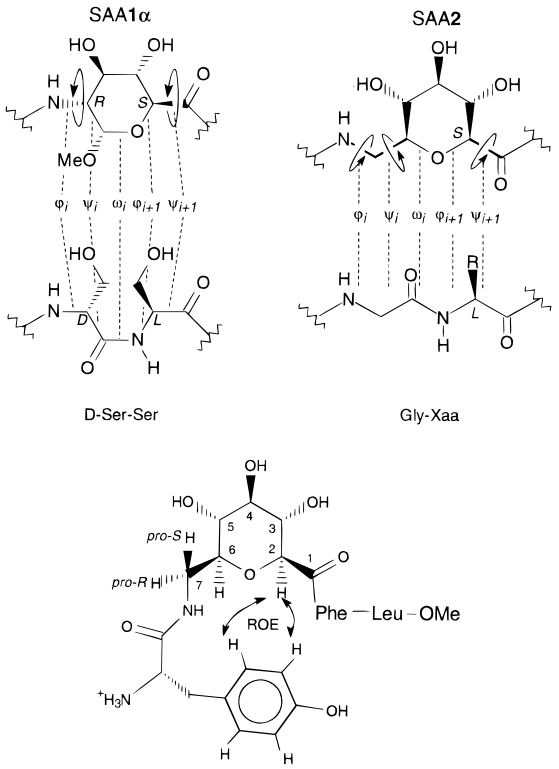

Figure 5. Comparison of SAA**1**α with the natural dipeptide D-Ser-Ser and SAA**2** with Gly-Xaa and characteristic long-range NOE effects in the linear peptide **20**.

hydroxyl groups of the sugar moiety were unprotected in all cases. The conformational analyses were based on NMR spectroscopy in DMSO-d_6 at 300 K: Interproton distances were calculated from NOESY and ROESY cross peak volumes, homonuclear proton coupling constants were obtained from 1D spectra or P.E. COSY spectra, and temperature dependence of the amide protons has been measured by 1D spectra in the range 300−340 K.

The starting geometries for the MD simulations were obtained by distance geometry (DG) with a modified version[31] of the DISGEO[32] program using proton−proton distances and $^3J(H^N,H^\alpha)$ and $^3J(H^\alpha,H^\beta)$ proton−proton coupling constants.[33] The following distance restraint MD simulations (rMD) were performed in explicit DMSO solvent[34] with the CVFF[35] force field of the Discover[36] program. The last 150 ps of the rMD simulation were collected and analyzed. The depicted structures were obtained by averaging over the last 50 ps and energy-minimized by 300 steps steepest descent (for details, see Experimental Section). In order to compare the rMD calculations with experimental data, distances were calculated by $\langle r^{-3}\rangle$ averaging over the trajectory; coupling constants were obtained from averaging the J values of each individual conformation over the trajectory. Moreover radial distribution functions (radf)[37] were calculated to judge solvent accessibility of the amide protons.

Cyclo(-SAA**2**^{1}-Phe^2-D-Trp^3-Lys^4(Cbz)-Thr^5-) (**26**) shows only one conformer in the ^{1}H NMR spectrum. Large ^3J(H,H) coupling constants (approximately 9 Hz) between the carbohydrate protons indicated a $^4C_1$ analog chair conformation. The backbone amide bonds of **26** are all *trans* configurated as no strong $H^\alpha-H^\alpha$ NOE were detectable.

Figure 6 shows the averaged and minimized structure of **26** consistent with a pseudo-β/βII′-turn arrangement with D-Trp in the $i+1$ position of the distorted βII′-turn and the dipeptide isostere SAA**2** in the i and $i+1$ positions of a pseudo-β-turn as indicated by the backbone dihedral angles in Table 1. As expected, radial distribution functions indicated solvent accessibility only for amide protons with large negative temperature coefficients. The analysis of the trajectory revealed large fluctuations for the φ-angles of D-Trp and Phe. This flexibility was most likely to cause large deviations between the experimental and calculated $^3J(H^N,H^\alpha)$ coupling constants (Phe $^3J(H^N,H^\alpha) = 7.2$ Hz (exp) and 10.1 Hz (calcd), D-Trp $^3J(H^N,H^\alpha) = 5.9$ Hz (exp) and 9.2 Hz (calcd)), especially since the Karplus curve has a steep slope for the relevant coupling constants. For the side chain of Lys (diastereotopic assignment according to Wüthrich et al.[38]), the Pachler equations[39] predicted a population of 51% for the preferential χ_1 angle of −60°, whereas according to the Pachler equations no predominant side chain orientation exists for D-Trp and Phe in solution. The synclinal orientation of the Thr$^5H^\alpha-H^\beta$ protons was confirmed by the $H^\alpha-H^\beta$ coupling constant of 4.6 Hz and the ROEs between Thr$^5H^\alpha-H^\beta$ and Thr$^5H^\beta-$SAA**2**H^N.

The ^{1}H NMR spectrum of *cyclo*(-SAA**3**^{1}-Phe^2-D-Trp^3-Lys^4-(Boc)-Thr^5(tBu)-) (**33**) in DMSO-d_6 at 300 K showed three conformations with ratios of 80:15:5. The ROESY spectrum proved them to be different conformers of the same molecule by exchange peaks. Only the major conformation could be completely assigned. The large ^3J(H,H) coupling constants (approximately 9 Hz) between the carbohydrate protons were characteristic for a $^4C_1$ analog chair conformation.

Figure 7 shows the averaged backbone conformation of **33** with a βII′/pseudo-β-turn arrangement. D-Trp occupies the $i+1$ position of a distorted βII′-turn. SAA**3** is acting as a β-turn mimetic (Table 2). The corresponding hydrogen bond between Thr carbonyl oxygen and Phe amide proton is present to a degree of 13% during the rMD simulation. Only the ROE between D-Trp$^3H^\alpha$ and D-Trp$^3H^2$ is significantly violated due to flexibility which was indicated by the homonuclear $H^\alpha-H^\beta$ coupling constant and the fact that the D-Trp$^3H^\alpha$-D-Trp$^3H^2$ and D-Trp$^3H^\alpha$-D-Trp$^3H^4$ ROEs cannot be met by a single side chain conformer. Both Thr and Phe had a flexible side chain as indicated by the $^3J(H^\alpha,H^\beta)$ coupling constants (Thr $^3J(H^\alpha,H^\beta) = 6.5$ Hz, Phe $^3J(H^\alpha,H^\beta) = 7.8^t$ and 5.6^h Hz). As in the case of **26** only the Lys side chain occupied a preferred rotamer. According to the Pachler equations, $\chi_1 = -60°$ was populated to 81%. Similar to compound **26**, large fluctuations were observed for the φ-angles of D-Trp and Phe during the simulation. For all amide protons radial distribution functions were calculated from the 150 ps rMD trajectory. They agree well with the temperature coefficients.

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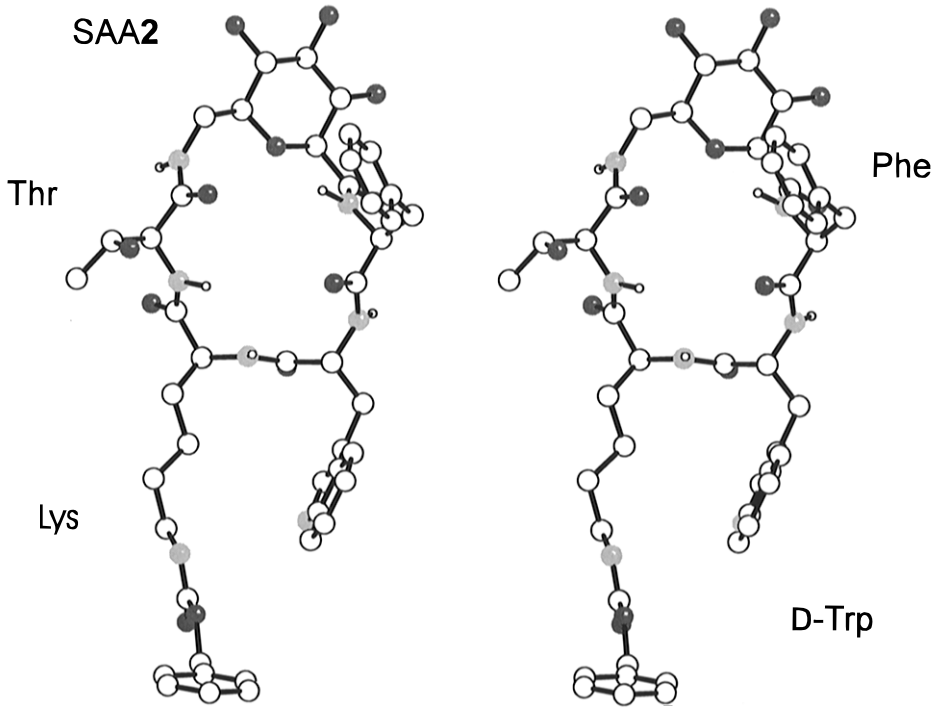

Figure 6. Stereoplot of *cyclo*(-SAA**2**-Phe-D-Trp-Lys(Cbz)-Thr-) (**26**) obtained by averaging the last 150 ps of the rMD tarjectory and subsequent energy minimization by 300 steps steepest descent. The depicted orientation of the side chains of D-Trp and Phe is arbitrary due to their flexibilty.

Table 1. Backbone Dihedral Angles for **26**

	φ	ψ	ω
SAA**2**	111 (ThrCO−H^N−C^7−C^6)	−63 (H^N-C^7−C^6−O)	-177 (C^7−C^6−O−C^2)
	177 (C^6−O−C^2−CO)	−26 (O−C^2−CO−PheH^N)	172 (C^2−CO−H^N-Phe$C\alpha$)
Phe	−122	105	−173
D-Trp	95	−112	−177
Lys	−108	−37	−173
Thr	−123	126	175

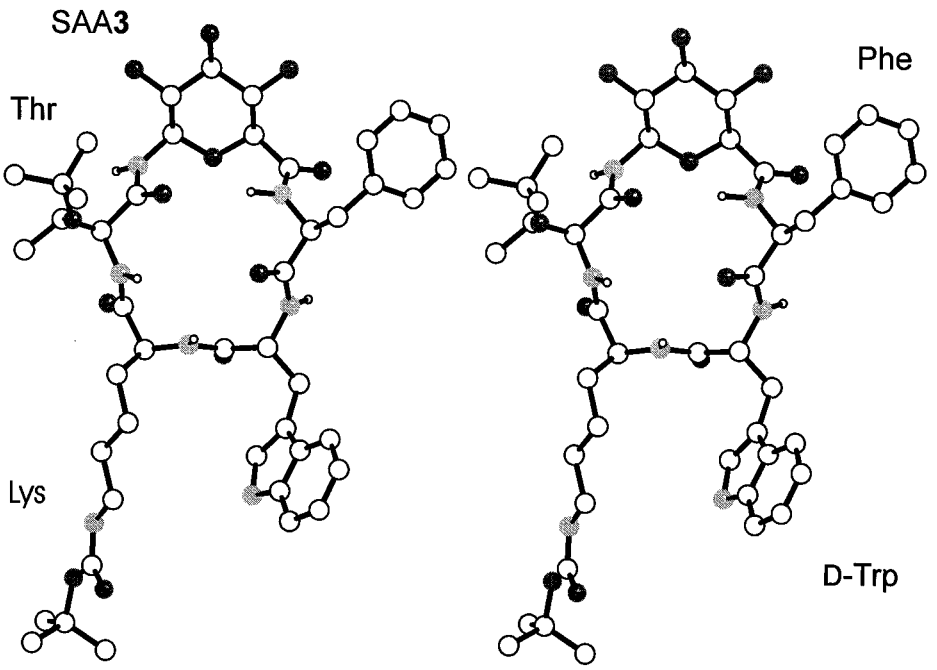

Figure 7. Stereoplot of *cyclo*(-SAA**3**-Phe-D-Trp-Lys(Boc)-Thr(tBu)-) (**33**) obtained by averaging the last 150 ps of the rMD tarjectory and subsequent energy minimization by 300 steps steepest descent. The depicted orientation of the side chains of D-Trp and Phe is arbitrary due to their flexibility.

The 1D-proton spectrum of *cyclo*(-SAA**4**[1]-Ala[2]-D-Pro[3]-Ala[4]-Ala[5]-) (**36**) shows two conformations in a ratio of 80:20, which were both assigned. Surprisingly the second conformation does not result from a *cis* Pro bond as would be expected. Exchange peaks in the ROESY spectrum proved that the two sets of signals result from two conformers of the same compound. The major conformation formed an *all-trans* configuration around the amide bonds, since no strong Hα−Hα ROEs were observed.

Table 2. Backbone Dihedral Angles for the Major Conformation of **33**

	φ	ψ	ω
SAA**3**	53 (ThrCO−H^N−C1−O) −58 (O−C^5−CO−PheH^N)	178 (H^N−C1−O−C^5)	−174 (C^1−O−C^5−CO) 173 (C^5−CO−H^N−Phe$C\alpha$)
Phe	−139	118	−178
D-Trp	109	−117	179
Lys	−94	−54	179
Thr	−114	139	−168

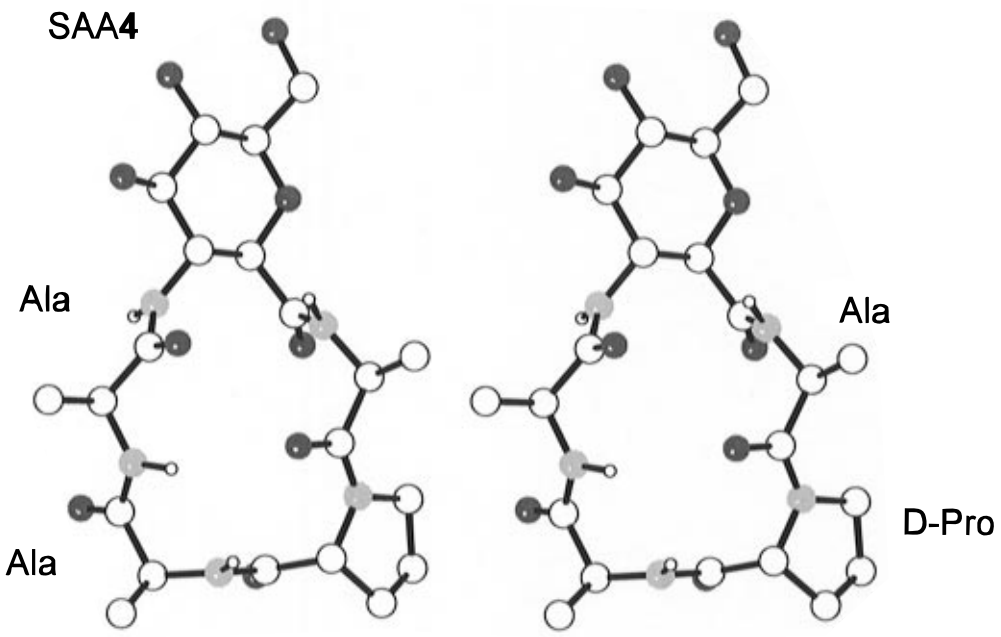

Figure 8. Stereoplot of *cyclo*(-SAA**4**-Ala-D-Pro-Ala-Ala-) (**36**) obtained by averaging the last 150 ps of the rMD tarjectory and subsequent energy minimization by 300 steps steepest descent.

Table 3. Backbone Dihedral Angles of the Major Conformation of **36**

	φ	ψ	ω
SAA**4**	114 (Ala^5CO−H^N−C^2−C^1)	−53 (H^N−C^2−C^1−CO) −63 (C^2−C^1−CO− Ala^2H^N)	−177 (C^1−CO−H^N− Ala^2C^α)
Ala^2	−85	136	178
D-Pro^3	76	−111	173
Ala^4	−86	−39	−178
Ala^5	−142	124	−178

The chemical shifts of the proline β- and γ-carbons indicated that the preceding amide bond is *trans*-configurated.[40] The distance of 273 pm for SAA**4**H^2 and SAA**4**H^4 (see Supporting Information) was also indicative for a $^4C_1$ chair conformation close to the corresponding value of β-D-glucose.

Figure 8 shows the averaged and minimized structure of the major conformation of **36** with a βII′/pseudo-γ-turn arrangement. D-Pro occupies the $i + 1$ position of a βII′-turn SAA**4** acting as a γ-turn mimetic (Table 3).

During the simulation a frequent switch between a βII′- and a γ-turn was observed for residues located in the β-turn region. Hence, hydrogen bonding occurred for both types of turns. This flexibility of the βII′-turn might cause the violation of the $^3J(Ala^5H^N,H^\alpha)$ coupling constant.[41]

This clearly shows that SAA**4** occupies the $i + 1$ position of the pseudo-γ-turn forming a hydrogen bond between the Ala^2 amide proton and the SAA**4** carbonyl populated to 38.8%.

In the minor conformation, the carbohydrate moiety exists in a $^4C_1$ chair conformation as confirmed by the large homonuclear coupling constants between the carbohydrate protons. Furthermore, the carbon chemical shifts of D-Pro^3 indicate a *trans* configuration at the preceding amide bond. No strong H^α−H^α NOEs were detectable for the second conformation. Due to the low population, no structural calculations were performed. Carbon chemical shifts of D-Pro^3 were very similar for both conformers, so no change of the local structure is expected. Available ROE data (Ala^5H^N−Ala^4H^N, 306 pm; Ala^4H^N−D-Pro^3, 231 pm; Ala^5H^N−D-Pro^3, 389 pm; Ala^4H^N−Ala^4H^α, 315 pm) and the small negative temperature coefficient Ala^5H^N are in agreement with a βII′-turn with D-Pro^3 in the $i + 1$ position. The experimental data indicate a different conformation for both conformers at SAA**4**H^N. The chemical shift changed drastically (from 7.99 ppm to 6.23 ppm). The change of the temperature coefficient for SAA**4**H^N from −6.5 ppb/K to −1.0 ppb/K indicated an internal orientation of this amide proton, which was confirmed by ROE data (shortening of the Ala^5H^N−SAA**4**H^N distance from 370 pm to 251 pm). The existence of such conformers suggests that the rotation about the bond between SAA**4**H^N and the SAA**4**C^1 is hindered.

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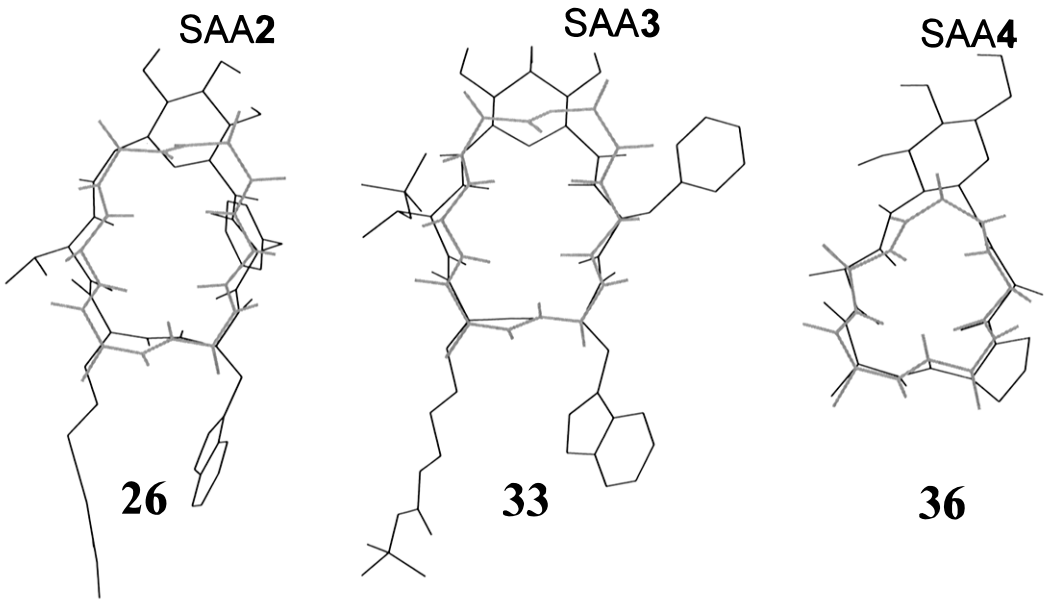

Figure 9. Superposition of **26** (black) and an idealized βII′/βII′-turn arrangement (gray); superposition of **33** (black) and an idealized βII′/βII′-turn arrangement (gray); and superposition of **36** (black) and an idealized βII′/γ-turn-arrangement (gray).

Biological Tests. The two enkephalin analogs **19** and **20** and the somatostatin analog **27** were tested for their biological activity. The enkephalin analogs **19** and **20** show no activity in the guinea pig ileum assay.[42] The somatostatin analog **27** has an inhibition constant (IC_{50}) of 0.15 μM in displacing the receptor-bound radioligand [^{125}I]Try^{11} somatostatin-14 in AtT20 cell membranes obtained from mice hypophyses. In fact, compound **27** is only 75 times less active than the highly potent somatostatin analog *cyclo*(-Phe-Pro-Phe-D-Trp-Lys-Thr-). This is particularly remarkable, since **27** does not contain the lipophilic residues on both sides of the active tetrapeptide sequence that are considered to be important for high somatostatin activity.[15,18]

Conclusion

The conformational analyses of the cyclic peptides presented here show that the replacement of the amino acids by SAAs introduced the proposed turn motifs combining the structural features of peptides and carbohydrates. As confirmed by the large ^3J(H,H) coupling constants, the carbohydrate ring remains in the $^4C_1$ conformation rendering a significant influence on the peptide backbone. Figure 9 shows the superpositions of the averaged and minimized structures of **26**, **33**, and **36** with cyclic peptide backbones consistent with idealized turn structures.[43] **26** and **33** were compared with the appropriate βII′/βII′-turn replacing the Phe-Pro residues of the cyclic hexapeptide *cyclo*(-Phe-Pro-Phe-D-Trp-Lys-Thr-).[18] The backbone dihedral angles and the temperature coefficients were in agreement with the corresponding data in the literature.[44] Peptide **36** containing SAA**4** was superimposed with the backbone of a cyclic pentapeptide with an idealized βII′/γ-turn arrangement. Although SAA**4** has one more backbone atom than a natural α-amino acid, the superposition showed that SAA**4** meets the geometric requirements to form a γ-turn.

Obviously the dipeptide isostere SAA**2** mimics a β-turn in peptide **26**. SAA**3** whose backbone is one atom shorter than that of a dipeptide isostere is suited as a β-turn mimetic in **33** just as well. In comparison to SAA**3** in **33** the carbohydrate moiety of SAA**2** in **26** is slightly out of plane of the β/βII′-turn arrangement. Apparently **26** and **33** form very similar backbone structures.

The superposition of the cyclic SAA containing peptides with the idealized turns show that the SAA building blocks form the proposed turn structures. The SAAs of the peptide construction kit may thus become a tool for a rational design of peptide conformations. A main advantage of the SAAs is that the conformational restriction changes significantly while the structure of the sugar moiety is more or less preserved. The protocol developed also allows the use of SAAs in solid phase peptide syntheses as well as in combinatorial synthesis. Libraries may be composed of different SAAs alone or including other natural or unnatural amino acids. The hydroxyl groups of the SAA can be modified, e.g., by benzylation or other derivatives. Such modifications will change the physical and chemical properties without changing the backbone structure in the cyclic peptide. The protecting groups could even serve as mimics for additional peptidic structures. Moreover, the presented SAAs can also be used as building blocks of oligo- and polysaccharide analogs.[8]

Experimental Section[45]

General Methods. Solvents for moisture sensitive reactions were distilled and dried according to standard procedures. All other solvents were distilled before use. Pt/C and Pd/C were donated by Degussa, Frankfurt/M., Germany. Flash column chromatography (FC) was performed with indicated solvents on silica gel 60, 230−400 mesh (Merck KGaA, Darmstadt). All reactions were monitored by thin-layer chromatography with 0.25 mm precoated silica gel 60 plates with F_{254} indicator (Merck KGaA, Darmstadt). Melting points were obtained on a Büchi-Tottoli apparatus and are uncorrected. Optical rotation were determined with a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 257 spectrophotometer. FAB mass spectra were recorded on Varian MAT 311 A using NOBA or glycol matrices. Elemental analysis were performed on a Heraeus EA415-0 analyzer. RP-HPLC analysis were carried out on Beckman System Gold using a Nucleosil-7 C_{18} column; solvent A, H_2O + 0.1% CF_3COOH, and

(42) Biological test performed at the laboratories of Prof. P. Schiller, Clinical Research Institute of Montreal, Canada.

(43) The structures of the cyclic model peptides were assembled from five, respectively six alanines. The torsion angles of the idealizied turns were adjusted on the linear peptides which were subsequently cyclized and minimized by a few steps. The torsion angles were taken from the following: Richardson, J. S. *Adv. Protein Chem.* **1981**, *34*, 167−339.

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(45) Complete spectral details are available in the Supporting Information.

solvent B, CH_3CN + 0.1% CF_3COOH, with UV detection at 220 and 254 nm.

Proton signals were assigned by the combined use of TOCSY,[46] z-TOCSY,[47] DQF-COSY,[48] and/or P.E. COSY[49] spectra. Carbon shifts were obtained with HMQC[50] and HMQC-COSY[51] experiments. The HMQC experiment was performed with a BIRD-puls.[52] In addition, for peptide **36** a HMBC[53] spectrum with a low-pass *J* filter[54] was recorded and used for the assignment of the ^{13}C resonances. In this case, sequential assignment was performed with the HMBC spectrum whereas in the other cases the correct sequence was confirmed by a ROESY[55] with a pulsed spinlock[56] (**33**) and a NOESY[57] experiment (**26**).

Quantitative information on interproton distances was obtained from NOESY and ROESY spectra with mixing times of 120 and 150 ms, respectively. Integrals from the ROESY experiment were offset corrected.[58] For all distance calculations the isolated two-spin approximation was used. Interproton distances and homonuclear coupling constants were employed (for details, see Supporting Information) for structure calculation.

Structure Calculations. Structure calculations were performed using distance geometry to generate a starting structure for subsequent restrained MD refinements. For distance geometry (DG), a modified version of the DISGEO program using proton-proton distances and $^3J(H^N,H^\alpha)$ and $^3J(H^\alpha,H^\beta)$ homonuclear coupling constants as restraints using the Karplus equation. For the following MD simulations in explicit DMSO[59] the Discover program with the CVFF force field was used. It is especially parametrized for peptides and small organic molecules, but proved to be useful in simulations for saccharides.[60] Hence, it is an adequate choice for the mainly peptidic compounds investigated here.

Synthesis of SAA1β (3,4,6-Tri-*O*-acetyl-2-[*N*-(benzyloxycarbonyl)amino]-2-deoxy-1-*O*-methyl-β-D-glucopyranoside, 2). 3,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl bromide hydrobromide **1**[19] (123 g, 0.27 mol) was dissolved in dry MeOH (1 L) and pyridine (22 mL, 0.27 mol) added. After 6 h the solvent was evaporated and the residue dried in vacuo. $NaHCO_3$ (112 g, 1.12 mol) was dissolved in H_2O (1 L) and added to EtOAc (1.4 L). Benzyl chloroformate (100 mL, 300 mmol, 50% solution in toluene) was added under vigorous stirring. After the evolution of CO_2 stopped, the organic phase was separated, washed three times with 0.5 N HCl (100 mL) and three times with H_2O (100 mL), and dried ($MgSO_4$). The solvent was evaporated to yield 115 g (94%) as a colorless solid. ^1H NMR (250 MHz, DMSO-d_6, 300 K): δ 7.45 (d, 1H, H^N), 7.23−7.38 (m, 5H, C_6H_5), 4.77−5.15 (m, 4H, $PhCH_2$,H^3,H^4), 4.47 (d, 1H, H^1), 4.20 (dd, 1H, H^{6t}), 4.02 (dd, 1H, H^{6h}), 3,79 (ddd, 1H, H^5), 3.48 (ddd, 1H, H^2), 3.32 (s, 3H, CH_3), 1.84 + 1.97 + 2.02 (3 s, 9H, CH_3CO); $^3J(SAA1\beta H^1,H^2)$ = 8.3 Hz, $^3J(SAA1\beta H^N,H^2)$ = 9.0 Hz, $^3J(SAA1\beta H^2,H^3)$ = 9.0 Hz, $^3J(SAA1\beta H^3,H^4)$ = 9.0 Hz, $^3J(SAA1\beta H^4,H^5)$ = 9.0 Hz, $^3J(SAA1\beta H^5,H^{6a})$ = 4.7 Hz, $^3J(SAA1\beta H^5,H^{6b})$ = 1.9 Hz, $^3J(SAA1\beta H^{6a},H^{6b})$ = 12.4 Hz, $^3J(SAA1\beta H^N,H^2)$ = 9.0 Hz. Anal. Calcd for $C_{21}H_{27}NO_{10}$: C, 55.63; H, 6.00; N, 3.09. Found: C, 55.75; H, 6.00; N, 3.07.

Methyl 2-[*N*-(Benzyloxycarbonyl)amino]-2-deoxy-β-D-glucopyranoside (3). Acetate **2** (62 g, 0.14 mol) was dissolved in dry MeOH (400 mL), and dimethylethylamine (20 mL) was added. After 24 h the solvent was evaporated, and the residue was recrystallized from H_2O to yield 44 g (99%) as colorless needles. ^1H NMR (250 MHz, DMSO-d_6 + 10% D_2O, 300 K): δ 7.27−7.36 (m, 5H, C_6H_5), 4.98 (s, 2H, $PhCH_2$), 4.13 (d, 1H, H^1), 3.66 (dd, 1H, H^{6t}), 3.43 (dd, 1H, H^{6h}), 3.31 (s, 3H, CH_3), 3.31−3.02 (m, 4H, H^2,H^3,H^4;H^5); $^3J(SAA1\beta H^1,H^2)$ = 7.6 Hz, $^3J(SAA1\beta H^5,H^{6a})$ = 1.0 Hz, $^3J(SAA1\beta H^{6a},H^{6b})$ = 11.7 Hz. Anal. Calcd for $C_{15}H_{21}N_1O_7$: C, 55.04; H, 6.47; N, 4.28. Found: C, 55.00; H, 6.56; N, 4.31.

2-[*N*-(Benzyloxycarbonyl)amino]-1-*O*-methyl-2-deoxy-β-D-glucopyranuronic Acid (4). Compound **3** (10.0 g, 31 mmol) was dissolved in H_2O (100 mL) and stirred with 10% Pt/C (5.0 g, ca. 50% H_2O) at 90 °C under a stream of O_2. The gas was pumped through the closed apparatus and purified by passing through 4 N NaOH. The pH of the mixture was maintained between 7 and 8 by addition of 10% $NaHCO_3$. Catalyst (2 g) was added after 15 and 30 h. After 50 h the catalyst was removed by filtration. The filtrate was neutralized with ion exchange resin (20 g, Aldrich, Amberlyst 15, strongly acidic, H^+ form), stirred for 10 min, filtered, and concentrated in vacuo to 30 mL. The product crystallized at 4 °C, yielding 5.2 g (54%) as colorless needles. Mp: 142 °C dec. $[\alpha]^{20}_D$: −35.7° (*c* = 1.0, MeOH). ^1H NMR (250 MHz, DMSO-d_6 + 10% D_2O, 300 K): δ 7.37−7.31 (m, 5H, C_6H_5), 4.98 (s, 2H, $PhCH_2$), 4.61 (s, 1H, H^1), 3.76 (d, 1H, H^5), 3.45−3.35 (m, 3H, H^2,H^3,H^4), 3.23 (d, 1H, CH_3); $^3J(SAA1\beta H^1,H^2)$ = 2.1 Hz, $^3J(SAA1\beta H^4,H^5)$ = 9.2 Hz. FAB-MS: 342 $[M + H]^+$. Anal. Calcd for $C_{15}H_{19}NO_8$: C, 52.79; H, 5.61; N, 4.10. Found: C, 52.47; H, 5.68; N, 4.21.

Synthesis of SAA2 (7-Amino-2,6-anhydro-L-*glycero*-L-*gulo*-7-deoxyheptonic Acid, 5). Cbz-SAA2-OMe[9] (0.36 g, 1.0 mmol) was dissolved in MeOH (4 mL) and treated with 1 N NaOH (1.2 mL, 1.2 mmol). After 1 h, ion exchange resin (0.5 g, Aldrich, Amberlyst 15, strongly acidic, H^+ form) was added and stirred for 10 min. The resin was filtered off and washed with MeOH. 10% Pd/C catalyst (50 mg) was added to the filtrate, and the reaction mixture was stirred for 1 h under an hydrogen atmosphere. The catalyst was filtered off, and the solvent was removed in vacuo to yield 201 mg (97%) as colorless crystals. Mp: >250 °C dec. $[\alpha]^{20}_D$: −41.2° (*c* = 1, H_2O). FAB-MS: 230 ($[M + Na]^+$).

Synthesis of SAA3 (1,2,3,4-Tetra-*O*-acetyl-β-D-glucuronic Acid Methyl Ester, 6). Glucuronolactone (43 g, 240 mmol) was suspended in dry MeOH (1.5 L), and dimethylethylamine (0.5 mL) was added. The reaction mixture was stirred for 3 h until the glucuronolactone was dissolved. The solvent was evaporated and the foam used without purification. Acetic anhydride (210 mL, 2.2 mol) and sodium acetate (21 g, 260 mmol) were added, and the suspension was stirred for 8 days. The reaction mixture was poured onto 1 L ice water and stirred overnight. The β-acetate was separated by filtration, washed with water, and recrystallized from EtOAc/hexanes. The mother liquor was extracted three times with ether (200 mL), and the combined ether extracts were washed with saturated sodium bicarbonate solution and brine, dried ($MgSO_4$), and evaporated. The resulting solid was recrystallized from EtOAc/hexanes to yield 42.8 g (47%). R_f (EtOAc/hexanes, 1:1): 0.53; ^1H NMR (250 MHz, DMSO-d_6): δ 6.02 (d, *J* = 8.1 Hz, 1H, H^1), 5.51(dd, *J* = 9.4 Hz, 1H, H^3), 5.01 (dd, *J* = 9.6 Hz, 1H, H^4), 4.97 (dd, *J* = 8.2 Hz, 1H, H^2), 4.67 (d, *J* = 9.8 Hz, 1H, H^5), 3.63 (s, 3H, OCH_3), 2.08−1.97 (4s, 12H, CH_3CO).

2,3,4-Tri-*O*-acetyl-1-azido-1-deoxy-D-glucuronic Acid Methyl Ester (7). Trimethylsilyl azide (15.5 mL, 190 mmol) was added to a stirred solution of acetate **6** (31 g, 82 mmol) in dry CH_2Cl_2 (450 mL)

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with $SnCl_4$ (4.0 mL, 29 mmol). The solution was stirred at room temperature for 3 h, then diluted with CH_2Cl_2 (300 mL), and washed three times with 10% K_2CO_3 solution (100 mL) and twice with brine (50 mL). After drying ($MgSO_4$), the solution was concentrated in vacuo and recrystallized from EtOAc/hexanes to yield 26.8 g (91%) as white solid. R_f (EtOAc/hexanes, 1:1): 0.60; ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.40 (dd, J = 9.6 Hz, 1H, H^3), 5.19 (d, J = 8.8 Hz, 1H, H^1), 5.05 (dd, J = 9.8 Hz, 1H, H^4), 4.87 (dd, J = 9.2 Hz, 1H, H^2), 4.57 (d, J = 9.9 Hz, 1H, H^5), 3.66 (s, 3H, OCH_3), 2.04−1.98 (3s, 9H, CH_3-CO); FAB-MS: 360 (3, $[M + H]^+$), 317 (66, $[M - N_3]^+$), 257 (23), 154 (100). Anal. Calcd for $C_{13}H_{17}N_3O_9$: C, 43.46; H, 4.77; N, 11.70. Found: C, 43.27; H, 4.77; N, 11.61.

Synthesis of SAA4 (2-[(Benzyloxycarbonyl)amino]-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucosamine, 10). Benzyl chloroformate (4.9 mL, 14 mmol, 50% solution in toluene) diluted in $CHCl_3$ (100 mL) was slowly added to a solution of **9**[24] (7.0 g, 14 mmol) and $NaHCO_3$ (6.0 g, 71 mmol) in MeOH (200 mL) at 0 °C. After 3 h additional $NaHCO_3$ (6.0 g, 71 mmol) and benzyl chloroformate (2.5 mL, 7.2 mmol) were added, and the suspension was stirred overnight at room temperature. The reaction mixture was concentrated, suspended in EtOAc (1 L), filtered, and concentrated again. The crude product was crystallized from EtOAc/hexanes to yield 8.03 g (90%) as colorless crystals. R_f (hexanes/acetone, 1:2): 0.39. Mp: 192−193 °C (hexanes/EtOAc). $[\alpha]^{20}_D$: +57.7 (c = 1.06, $CHCl_3$). ^{1}H NMR (250 MHz, DMSO-d_6): 7.42 (d, J = 9.2 Hz, 1H, H^N), 7.34−7.18 (m, 20H, H^{arom}), 6.75 (d, J = 4.0 Hz, 1H, HO), 5.12−4.95 (m, 3H, $PhCH_2$,H^1), 4.77−4.43 (m, 6 H, $PhCH_2$), 3.44−3.35 (m, 6H); ^{13}C NMR (76.7 MHz, DMSO-d_6): 156.2, 138.9, 138.4, 138.3, 137.2, 128.3−127.3, 91.3, 79.8, 78.7, 74.1, 74.0, 72.4, 69.9, 69.3, 65.3, 55.8. FAB-MS: 566 (4, $[M - H_2O + H]^+$). Anal. Calcd for $C_{35}H_{37}NO_7$: C, 72.02; H, 6.39; N, 2.40. Found: C, 72.14; H, 6.44; N, 2.41.

Tributyl[2-[(benzyloxycarbonyl)amino]-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl]stannane (11). Compound **10** (7.0 g, 12 mmol) was treated with $SOCl_2$ (100 mL) at room temperature for 30 min and concentrated to dryness, and the product was coevaporated with dry $CHCl_3$ (20 mL). The yellow solid was dissolved in dry THF (100 mL) and added within 20 min to a solution of Bu_3SnLi[23b,61] in THF (ca. 3 equiv) at −78 °C. The mixture was stirred for 1 h at −78 °C, quenched with saturated NH_4Cl solution (10 mL), warmed to room temperature, diluted with H_2O, and extracted twice with EtOAc (700 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to afford a yellow oil. Purification was achieved by FC (hexanes/EtOAc, gradient 1:0 to 4:1) to yield 8.10 g (79%) as colorless oil. R_f (hexanes/acetone, 3:1): 0.91. $[\alpha]^{20}_D$: −2.7 (c = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.64 (d, J = 8.0 Hz, 1H, H^N), 7.34−7.12 (m, 20H, H^{arom}), 5.06−4.44 (m, 8H, $PhCH_2$), 3.79 (m, 2H), 3.67−3.57 (m, 3H), 3.49−3.41 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): 170.6, 155.8, 138.5, 138.2, 138.1, 137.1, 128.3−127.2, 83.7, 78.0, 77.7, 74.2, 73.9, 72.3, 65.2, 54.5. FAB-MS (calcd for $C_{47}H_{63}NO_6{}^{120}Sn$): 801 (40, $[M - Bu + H]^+$), 711 (6), 291 (20, $[SnBu_3]^+$), 236 (40), 180 (100). Anal. Calcd for $C_{47}H_{63}NO_6Sn$: C, 65.89; H, 7.41; N, 1.63; Found: C, 66.12; H, 7.33; N, 1.65.

Conversion of 11 with BuLi and Addition of CO_2. Stannane **11** (8.1 g, 9.5 mmol) was dissolved in dry THF (60 mL). At −78 °C, BuLi (5.9 mL 1.6 M solution in hexanes, 9.5 mmol) was added within 10 min. The reaction mixture was then warmed to −55 °C, and BuLi (7.1 mL, 11 mmol) was added within 5 min while the color of the solution changed to deep red. CO_2 was pumped for 15 min through the reaction mixture which was quenched after 1 h with 10% $KHSO_4$ solution (50 mL) at −55 °C, warmed to room temperature, and extracted twice with EtOAc (500 mL). Purification was achieved by FC (hexanes/acetone, 1:0, 1:1, $CHCl_3$/MeOH/HOAc, 50:7:3) to afford **13** in 4.77 g (83%) as colorless solid, **11** in 0.46 g (5.7%) and **14** in 0.59 g (11%).

3-[(Benzyloxycarbonyl)amino]-2,6-anhydro-4,5,7-tri-*O*-benzyl-D-*glycero*-D-*gulo*-heptonic Acid (13). R_f ($CHCl_3$/MeOH, 1:3): 0.59. Mp: 155 °C. $[\alpha]^{20}_D$: +15.1 (c = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.64 (d, J = 8.0 Hz, 1H, H^N), 7.34−7.12 (m, 20H, H^{arom}), 5.06−4.44 (m, 8H, $PhCH_2$), 3.80−3.78 (m, 2H), 3.67−3.57 (m, 3H), 3.49−3.41 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): 170.6, 155.8, 138.5, 138.2, 138.1, 137.1, 128.3−127.2, 83.7, 78.0, 77.7, 74.2, 73.9, 72.3, 68.7, 65.2, 54.5. FAB-MS: 634 (28, $[M + Na]^+$), 517 (18), 182 (34), 147 (100). Anal. Calcd for $C_{36}H_{37}NO_8$: C, 70.69; H, 6.09; N, 2.29. Found: C, 70.71; H, 6.03; N, 2.33.

Treatment of 13 with Trimethylsilyl Iodide, followed by Fmoc−ONSu. Acid **13** (1.0 g, 1.6 mmol) was dissolved in dry CH_3CN (10 mL) at room temperature in a Falcon tube. Trimethylsilyl iodide (0.49 mL, 4.0 mmol) was added, and the reaction mixture was stirred for 12 min, treated with MeOH (100 μL), diluted with THF (10 mL), and treated with Fmoc−ONSu (720 mg, 2.0 mmol). The pH of the solution was maintained at 7−8 by addition of DIEA, and the completion of the reaction was followed by HPLC (30 → 90, B in A, 30 min: t_R(amine) = 20.4 vs t_R(urethane) = 26.4 min). After 14 and 24 h additional Fmoc−ONSu was added. Purification was achieved by FC (acetone/hexanes, 1:3, and acetone/hexanes (+0.1% TFA), gradient 1:2 to 2:1) to yield **15** in 546 mg (48%), **16** in 62 mg (6.3%), and **17** in 223 mg (17%).

3-[(9-Fluorenylmethoxycarbonyl)amino]-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-D-*glycero*-D-*gulo*-heptonic Acid (15). R_f ($CHCl_3$/MeOH, 1:3): 0.79. t_R 20.9 min (58 → 72, B in A, 30 min). $[\alpha]^{20}_D$: +13.8 (c = 0.92, THF). ^{1}H NMR (500.13 MHz, DMSO-d_6): δ 12.84 (s, 1 H, HOOC), 7.87 (d, J = 7.4 Hz, 2H), 7.71 (d, J = 7.4 Hz, 1H), 7.66−7.62 (m, 2H), 7.41−7.16 (m, 19H, H^{arom}), 4.71−4.54 (m, 6H, $PhCH_2$), 4.31 (dd, J = 6.7 Hz, J = 10.2 Hz, 1H), 4.25−4.16 (m, 2H, $PhCH_2$), 3.86 (d, J = 9.3 Hz, 1H, H^2), 3.77−3.60 (m, 4H, H^3,H^4,H^5,H^6), 3.49 (s, 2H, H^7). ^{13}C NMR (125 MHz, DMSO-d_6): 169.7, 155.7, 143.9, 143.7, 140.7, 138.4, 138.1, 138.0, 128.5−126.9, 125.2, 125.0, 120.1, 83.2 (C^4), 78.2 (C^2), 77.6 (C^5, C^6), 74.2, 74.0, 72.3, 68.8 (C^7), 65.7, 54.4 (C^3), 46.6 (C^H). FAB-MS: 722 (6, $[M + Na]^+$), 700 (24, $[M + 1]^+$), 478 (10), 179 (100). Anal. Calcd for $C_{36}H_{37}NO_8$: C, 73.80; H, 5.90; N, 2.00. Found: C, 73.70; H, 5.92; N, 2.01.

Cbz-SAA1α-Phe-Leu-OMe. General Procedure for Coupling of Peptide and SAA. In a representative experiment, H-Phe-Leu-OMe was dissolved in THF (5 mL) and cooled to 0 °C. Cbz-SAA**1**α-OH (0.68 g, 2.0 mmol), HOBt (0.3 g, 2.0 mmol), and EDCl·HCl (0.40 g, 2.1 mmol) were added. The pH was adjusted to 7 by dropwise addition of NMM (*N*-methylmorpholine). After 10 h the solvent was evaporated and the residue dissolved in EtOAc (100 mL). The solution was washed three times with 0.5 N HCl (20 mL) and three times with aqueous 5% $NaHCO_3$ (20 mL) and with H_2O (20 mL). The organic layer was dried ($MgSO_4$) and concentrated to yield 0.90 g (73%) as a colorless solid.

Cbz-Tyr-SAA1α-Phe-Leu-OMe. General Procedure for Removal of Cbz Protecting Group. In a representative experiment Cbz-SAA**1**α-Phe-Leu-OMe (0.62 g, 1.0 mmol) was dissolved in MeOH (5 mL), 10% Pd/C catalyst (50 mg) was added, and the reaction mixture was stirred for 1 h under an H_2 atmosphere. The catalyst was filtered off, and the solvent was removed in vacuo. The residue was coupled with Cbz-Tyr-OH (0.32 g, 1.0 mmol) as mentioned above. After 10 h the solvent was evaporated, and the residue was chromatographed on silica gel ($CHCl_3$/MeOH, 12:1) to afford 0.41 g (53%) as a colorless solid. R_f ($CHCl_3$/MeOH, 12:1): 0.20.

H-Tyr-SAA1α-Phe-Leu-OMe·HCl (19). Cbz-Tyr-SAA**1**α-Phe-Leu-OMe (155 mg, 0.2 mmol) was hydrogenated as above. The catalyst was filtered off, a saturated solution of HCl in Et_2O (0.5 mL) was added, and the solvent was removed in vacuo to give 112 mg (94%) as a colorless solid. ^{1}H NMR (600 MHz, DMSO-d_6, 300 K): 9.25 (s, 1H, HO-Tyr), 8.49 (d, 1H, SAA1αH^N) 8.27 (d, 1H, LeuH^N), 8.17 (d, 1H, PheH^N), 7.97 (s, 3H, TyrH^N), 7.28−7.15 (m, 5H, C_6H_5), 7.13 (d, 2H, TyrH$^{3'}$), 6.68 (d, 2H, Tyr TyrH$^{2'}$), 5.23−5.10 (m, 2H, HO-SAA1α), 4.58 (m, 2H, PheH$^\alpha$,SAA1αH^1), 4.27 (ddd, 2H, LeuH$^\alpha$), 3.96 (ddd, 1H, TyrH$^\alpha$), 3.89 (d, 1H, SAA1αH^5), 3.72 (ddd, 1H, SAA1αH^2), 3.63 (s, 3H, LeuOMe), 3.57 (dd, 1H, SAA1αH^3), 3.38 (d, 1H, SAA1αH^4), 3.26 (s, 3H, SAA1αOMe), 3.08, 2.82 (m, 4H, PheH$^\beta$,TyrH$^\beta$), 1.70−1.45 (m, 3H, 2 LeuH$^\beta$,LeuH$^\gamma$), 0.85 + 0.90 (2d, 6H, 2 LeuH$^\delta$); ^3J(SAA1αH^N,H^1) = 8.3 Hz, ^3J(SAA1αH^1,H^2) = 10.1 Hz, ^3J(SAA1αH^4,H^5) = 9.7 Hz, ^3J(PheH^N,H$^\alpha$) = 7.5 Hz, ^3J(LeuH$^\gamma$,H$^\delta$) = 6.1 Hz. ^{13}C-NMR (150 MHz, DMSO-d_6, 300 K): δ 72.1 (C^4), 71.2 (C^5), 70.2 (C^3), 54.7 (SAA1αOMe), 53.5 (C^2), 53.4 (PheC$^\alpha$), 53.0 (TyrC$^\alpha$), 51.7 (LeuOMe), 50.3 (LeuC$^\alpha$), 39.4 (LeuC$^\beta$), 36.9 (TyrC$^\beta$), 36.0 (PheC$^\beta$), 23.9 (LeuC$^\gamma$), 22.5 (LeuC$^\delta$), 21.1 (LeuC$^\delta$). Anal. Calcd for $C_{32}H_{45}ClN_4O_{10}$: C, 56.42; H, 6.66; N, 8.22. Found: C, 56.44; H, 6.68; N, 8.21.

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Table 4. Proton and Carbon Chemical Shifts of **26** and Temperature Coefficients

$^1H/^{13}C$	SAA**2**		Phe		D-Trp		Lys		Thr	
H^N	7.62		7.38		8.51		8.54		7.26	
H^α/C^α	3.49 (H^2)	77.2 (C^2)	4.53	52.3	4.32	54.6	3.91	53.3	4.20	57.6
H^β/C^β	3.09 (H^3)	72.3 (C^3)	2.96/2.81	37.8	2.99/2.83	26.5	$1.60^{proS}/1.39^{proR}$	30.8	3.90	66.5
H^γ/C^γ	3.16 (H^4)	76.7 (C^4)			10.78 (H^N)		0.94	22.8	0.96	18.5
H^δ/C^δ	2.98 (H^5)	70.6 (C^5)	6.85−7.10	125.7−128.7	7.09 (H^2)	123.3 (C^2)	1.24	28.8	4.95 (OH)	
H^ϵ/C^ϵ	3.14 (H^6)	78.3 (C^6)			7.55 (H^4)	117.9 (C^4)	2.88	39.9		
H^7/C^7	3.28 ($H7^{proR}$)	39.6 (C^7)			7.02 (H^5)	117.7 (C^5)	4.98 (HN)			
	3.41 ($H7^{proS}$)				7.09 (H^6)	120.4 (C^6)	4.98 (CH_2(Cbz))	65.0 (CH_2(Cbz))		
					7.35 (H^7)	110.9 (C^7)		127 − 128 + 110.0 (C_6H_5(Cbz))		
$\Delta\delta/\Delta T$ [ppb/K] for the amide H	−6.8		−1.8		−9.8		−6.8		−0.5	

Cbz-SAA2-Phe-Leu-OMe. Cbz-SAA**2**-OMe (180 mg, 0.5 mmol) was dissolved in MeOH (5 mL) and 1 N NaOH (0.75 mL). After 2 h HOBt (75 mg, 0.5 mmol) was added, and the solvent was removed in vacuo. The Cbz group of Cbz-Phe-Leu-OMe (320 mg, 0.75 mmol) was removed as described above, and the deprotected compound was dissolved in THF (5 mL). This solution was given to the residue of the saponification and coupled with EDCI (see above). After 10 h the solvent was evaporated, and the residue was purified by FC ($CHCl_3$/MeOH, 9:1) to yield pure **30** in 220 mg (71%) as a colorless solid. FAB-MS: 638 $[M + Na]^+$.

H-Tyr-SAA2-Phe-Leu-OMe·HCl (20). The Cbz group of Cbz-SAA**2**-Phe-Leu-OMe (220 mg, 0.36 mmol) was removed and coupled with EDCI as above. The solvent was evaporated, and the residue was chromatographed on silica gel ($CHCl_3$/MeOH, 9:1). For the removal of the Boc group the purified peptide was treated with saturated HCl/Et_2O (5 mL) and MeSH (0.5 mL) for 1 h. The solvent was removed in vacuo to give 185 mg (75%) **20** as a colorless solid. FAB-MS: 645 $[M + H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ 9.37 (s, 1H, HO-Tyr), 8.43 (d, 1H, LeuH^N), 8.41 (dd, 1H, SAA**2**H^N), 8.06 (d, 1H, PheH^N), 8.04 (s, 3H, TyrH^N), 7.28−7.15 (m, 5H, C_6H_5), 7.02 (d, 2H, TyrH$^{3'}$), 6.72 (d, 2H, TyrH$^{3'}$), 5.14 (m, 3H, 3 HO-SAA2), 4.55 (ddd, 1H, PheH$^\alpha$), 4.30 (ddd, 2H, LeuH$^\alpha$), 3.93 (dd, 1H, TyrH$^\alpha$), 3.65 (dd, 1H, H^{7t}(*pro-R*)), 3.61 (s, 3H, LeuOMe), 3.57 (d, 1H, H^2), 3.19 (ddd, 1H, H^4), 3.16 (ddd, 1H, H^3), 3.11 (dd, 1H, PheH$^{\beta t}$), 3.03 (ddd, 1H, H^6), 2.98 (dd, 1H, H^{7h}(*pro-S*)), 2.95 (ddd, 1H, H^5), 2.91 (dd, 1H, TyrH$^{\beta t}$), 2.83 (dd, H, TyrH$^{\beta h}$), 2.82 (dd, 1H, PheH$^{\beta h}$), 1.65−1.50 (m, 3H, 2 LeuH$^\beta$,LeuH$^\gamma$), 0.83 + 0.88 (2d, 6H, 2 LeuH$^\delta$); ^3J(TyrH$^\alpha$,H$^{\beta t}$) = 7.0 Hz, ^3J(TyrH$^\alpha$,H$^{\beta h}$) = 7.0 Hz, ^3J(TyrH$^{\beta t}$,H$^{\beta h}$) = 14.0 Hz, ^3J(SAA2H^N,H^{7t}(*pro-R*)) = 6.2 Hz, ^3J(SAA2H^N,H^7(*pro-S*)) = 4.7 Hz, ^3J(SAA2H^{7t},H^{7h}) = 12.4 Hz, ^3J(SAA2H^{7t}(*pro-R*),H^6) = 1.0 Hz, ^3J(SAA2H^{7h}(*pro-S*),H^6) = 8.5 Hz, ^3J(SAA2H^4,H^3) = 8.8 Hz, ^3J(SAA2H^3,H^2) = 9.0 Hz, ^3J(PheH^N,H$^\alpha$) = 8.4 Hz, ^3J(PheH$^\alpha$,H$^{\beta t}$) = 9.0 Hz, ^3J(PheH$^\alpha$,H$^{\beta h}$) = 5.5 Hz, ^3J(PheH$^{\beta t}$,H$^{\beta h}$) = 13.3 Hz, ^3J(LeuH^N,H$^\alpha$) = 7.8 Hz. ^{13}C-NMR (125 MHz, DMSO-d_6, 300 K): δ = 130.0 (TyrC$^{2'}$), 125.3 + 127.7 + 128.7 (Phe^{arom}), 115.0 (TyrC$^{2'}$), 78.1 (C^2), 78.1 (C^6), 77.1 (C^4), 72.0 (C^3), 71.2 (C^5), 53.8 (PheC$^\alpha$), 53.5 (TyrC$^\alpha$), 51.7 (LeuOCH$_3$), 50.2 (LeuC$^\alpha$), 40.8 (C^7), 39.3 (LeuC$^\beta$), 37.1 (PheC$^\beta$), 36.2 (TyrC$^\beta$), 24.3 (LeuC$^\gamma$), 22.5 (LeuC$^{\delta a}$), 21.2 (LeuC$^{\delta b}$). Anal. Calcd for $C_{32}H_{45}ClN_4O_{10}$: C, 56.42; H, 6.66; N, 8.22. Found: C, 52.78; H, 5.96; N, 7.32.

Cbz-SAA2-Phe-D-Trp-OMe (22). Cbz-SAA**2**-OMe (0.72 g, 2.0 mmol) was dissolved in MeOH (10 mL) and 1 N NaOH (3.0 mL). After 2 h HOBt (300 mg, 2.0 mmol) was added, and the solvent was removed in vacuo. The Boc group of **21** (0.92 g, 1.84 mmol) was removed by adding HCl in ether (4 mL, saturated at 0 °C) to a stirred solution of the protected peptide with addition of MeSH (0.5 mL) as a scavenger. The solution was stirred at room temperature for 30 min and evaporated in vacuo. The deprotected compound was dissolved in THF (10 mL). This solution was added to the residue of the saponification and coupled as above. After 10 h the solvent was evaporated, and the residue was dissolved in EtOAc (100 mL). The solution was washed three times with 0.5 N HCl (20 mL) and three times with aqueous 5% $NaHCO_3$ (20 mL) and with H_2O (20 mL). The organic layer was dried ($MgSO_4$) and concentrated. The product was precipitated in Et_2O to yield 0.81 g (59%) as a colorless solid.

Boc-Lys(Cbz)-Thr-SAA2-Phe-D-Trp-OMe (24). Compound **23** (0.50 g, 1.0 mmol) was dissolved in MeOH (5 mL), THF (5 mL), and 1 N NaOH (1.5 mL). After 2 h HOBt (150 mg, 1.0 mmol) was added, and the solvent was removed in vacuo. The Cbz group of **22** (0.69 g, 1.0 mmol) was removed as described above, and the deprotected compound was dissolved in THF (8 mL) and DMF (2 mL). This solution was added to the residue of the saponification and coupled as above. After 10 h the solvent was evaporated, and the residue was chromatographed ($CHCl_3$/MeOH, 4:1) to give 0.74 g (73%) of **24** as a colorless solid.

***Cyclo*(-SAA2-Phe-D-Trp-Lys(Cbz)-Thr-) (26).** **24** (0.51 g, 0.5 mmol) was dissolved in MeOH (5 mL), THF (5 mL), and 1N NaOH (0.75 mL). After 4 h ion exchange resin (1 g, Aldrich, Amberlyst 15, strongly acidic, H^+ form) was added and stirred for 10 min. The resin was filtered off, and the filtrate was concentrated in vacuo. The residue was treated with saturated HCl/Et_2O (5 mL) and MeSH (0.5 mL) for 1 h. The solvent was removed in vacuo, and the residue was dissolved in DMF (0.5 L). HOBt (90 mg, 0.6 mmol), DIEA (0.43 mL), H_2O (0.5 mL), and TBTU (190 mg, 0.6 mmol) were added under stirring. After 1 h the solvent was removed in vacuo. The residue was chromatographed ($CHCl_3$/MeOH, 4:1) to give 246 mg (56%) of **26** as a colorless solid. FAB-MS: 887 $[M + H]^+$. Anal. Calcd for $C_{45}H_{55}N_7O_{12}$: C, 61.01; H, 6.26; N, 11.07. Found: C, 59.39; H, 6.14; N, 10.40.

***Cyclo*(-SAA2-Phe-D-Trp-Lys-Thr-) (27).** The Cbz group of **26** (30 mg, 0.033 mmol) was removed as described above. The product was lyophilized from H_2O/tBuOH (1:1) to give 24 mg (97%) of **27** as a colorless solid. FAB-MS: 753 $[M + H]^+$.

Cbz-Thr(tBu)-SAA3(tri-*O*-acetyl)-OMe (28). Azide **7** (3.7 g, 10.4 mmol) was dissolved in THF (140 mL), and 10% Pd/C (1 g) was added. After 10 min of ultrasonification the reaction mixture was hydrogenated on an ice bath for 2 h. The solution was reduced in vacuo. Cbz-Thr-(tBu)-OH was prepared from DCHA salt by dissolving Cbz-Thr(tBu) OH·DCHA (5.1 g, 10.4 mmol) in EtOAc (600 mL) and washing the organic phase four times with 1 N HCl and once with brine. After being dried with $MgSO_4$, the solvent was evaporated. The foam was dissolved in CH_2Cl_2 (30 mL), IIDQ (3.04 g) was added on an ice bath, and the reaction mixture was stirred overnight. The solvent was evaporated. FC (EtOAc/hexanes, 1:1) yielded 3.5 g (54%) as a white solid. R_f (EtOAc/hexanes, 2:1): 0.60.

Cbz-Lys(Boc)-Thr(tBu)-SAA3(tri-*O*-acetyl)-OMe (30). Compound **28** (1.9 g, 3.05 mmol) was dissolved in EtOAc (60 mL) and after addition of 10% Pd/C (900 mg) hydrogenated for 1 h. The solution was filtered through Celite. Cbz-Lys(Boc)-ONSu (1.45 g, 3.04 mmol) was dissolved in EtOAc (30 mL), and NMM was added to adjust the pH to 8. After being stirred overnight the solvent was evaporated and subjected to FC (EtOAc/hexanes, 1:1) to yield 2.2 g (85%). R_f (EtOAc/hexanes, 2:1): 0.33.

Table 5. Proton and Carbon Chemical Shifts of the Major Conformation of **33** and Temperature Coefficients

$^1H/^{13}C$	SAA**3**		Phe		D-Trp		Lys		Thr	
H^N	8.47		7.55		8.27		8.36		7.17	
H^α/C^α	4.37 (H^1)	81.0 (C^1)	4.35	53.4	4.42	54.2	3.86	53.9	4.00	54.0
H^β/C^β	3.60 (H^2)	70.6 (C^2)	2.98/2.87	35.8	2.96/2.82	27.0	$1.55^{proS}/1.37^{proR}$	31.0	3.87	66.3
H^γ/C^γ	3.18 (H^3)	76.4 (C^3)			10.75 (H^N)		0.9	22.8	1.04	19.0
H^δ/C^δ	3.30 (H^4)	72.4 (C^4)	6.93/7.07/7.12	129.0/127.8/125.8	7.00 (H^2)	121.5 (C^2)	1.22	29.0		
H^ϵ/C^ϵ	3.62 (H^5)	77.7 (C^5)			7.50 (H^4)	118.0 (C^4)	2.80	39.5		
					6.98 (H^5)	118.0 (C^5)	H^N: 6.74			
					7.07 (H^6)	120.7 (C^6)	Boc: 1.35	Boc: 22	*t*-Bu 1.12	
					7.35 (H^7)	111.2 (C^7)				
$\Delta\delta/\Delta T$ [ppb/K] for the amide H	−7.0		0.0		−5.9		−7.3		0.0	

Table 6. Proton and Carbon Chemical Shifts for the Major Conformation of **36** and Temperature Coefficients

$^1H/^{13}C$	SAA**4**		Ala^2		Pro^3		Ala^4		Ala^5	
H^N/CO	7.99	167.3	7.73	170.4		171.9	8.58	172.2	7.54	170.1
H^α/C^α	3.53(H^1)	79.7(C^1)	4.31	46.2	4.13	59.0	3.92	49.1	4.06	49.4
H^β/C^β	3.97(H^2)	51.5(C^2)	1.22	16.0	$1.98^{proR}/1.79^{proS}$	28.2	1.25	17.0	1.15	17.3
H^γ/C^γ	3.17(H^3)	75.4(C^3)			2.12/1.88	24.6				
H^δ/C^δ	3.25(H^4)	69.6(C^4)			3.65/3.51	46.2				
	3.12(H^5)	80.9(C^5)								
	3.65/3.49(H^6)	60.8(C^6)								
$\Delta\delta/\Delta T$ [ppb/K] for the amide H	−6.5		−5.2				−6.9		−0.1	

Cbz-Lys(Boc)-Thr(tBu)-SAA3-Phe-D-Trp-OMe (31). Cbz-Lys-(Boc)-Thr(tBu)-SAA**3**(tri-*O*-acetyl)-OMe (1.1 g, 1.3 mmol) was dissolved in THF (20 mL) and 1 N NaOH (5.5 mL) added. After 3 h Amberlyst 15 (1.7 g) was added and the suspension stirred for 15 min. The solid was removed by filtration. The solution was used without further purification. Cbz-Phe-D-Trp-OMe (**29**) (640 mg, 1.3 mmol) was dissolved in MeOH (20 mL), and after addition of 10% Pd/C (320 mg) the solution was hydrogenated for 1 h. The solvent was evaporated and the foam redissolved in THF (10 mL) and added to the Cbz-Lys-(Boc)-Thr(tBu)-SAA**3**-OH. The solution was cooled on an ice bath, and EDCl·HCl (290 mg, 1.5 mmol), HOBt (235 mg, 1.5 mmol), and NMM (380 μL) were added. The reaction mixture was stirred overnight. The solvent was evaporated and after redissolving EtOAc (200 mL) washed three times (50 mL each) with 1 N HCl, three times with saturated $NaHCO_3$, and once with brine. The organic phase was dried with $MgSO_4$ and the solvent removed by evaporation. FC ($CHCl_3$/MeOH, 15:1) gave 1.05 g of a white solid in 77% yield. R_f (CH_3CN/H_2O, 4:1): 0.71.

H-Lys(Boc)-Thr(tBu)-SAA3-Phe-D-Trp-OH (32). Methyl ester **31** (360 mg, 0.34 mmol) was dissolved in MeOH (5 mL), and THF (20 mL) and 1 N NaOH (0.5 mL) were added. After 3 h Amberlyst 15 (0.6 g) was added, and the mixture was stirred for 15 min. The resin was removed by filtration and the solvent evaporated. The foam was redisolved in MeOH (20 mL), and after addition of 10% Pd/C (200 mg) hydrogenated for 1 h. After evaporation of the solvent the foam was purified by preparative HPLC (35 → 55, B in A, 30 min, t_R 11.2 min), yielding 150 mg (48%). R_f (CH_3CN/H_2O, 4:1): 0.59.

***Cyclo*(-Lys(Boc)-Thr(tBu)-SAA3-Phe-D-Trp-) (33).** The linear peptide **32** (45 mg, 0.05 mmol) was dissolved in NMP (250 mL) and water (0.45 mL) added. The solution was stirred for 15 min and added dropwise to the coupling mixture of HOBt (38.3 g, 0.25 mmol), TBTU (48.2 g, 0.15 mmol), and DIEA (45 μL) in NMP (200 mL) over 1 h. The solvent was evaporated, and the peptide was purified by preparative HPLC (35 → 55, B in A, 30 min, t_R 20.2 min) to yield 10 mg (22%). FAB-MS: 894 $[M + H]^+$.

***Cyclo*(-SAA4-Ala-D-Pro-Ala-Ala-) (36).** Fmoc-Ala-OH (0.2 g, 0.7 mmol) was coupled to 2-chlorotrityl chloride resin (0.4 g, 1.25 mmol Cl^-/g resin) which was used to initiate the synthesis with Fmoc-D-Pro-OH (0.236 g, 0.7 mmol), Fmoc-Ala-OH (0.218 g, 0.7 mmol), and **15** (0.315 g, 0.46 mmol) for chain elongation; TBTU/HOBt was used for activation. The peptide was cleaved with CH_2Cl_2/trifluoroethanol/HOAc (8:1:1) to yield 265 mg (82%) of crude H-Ala-SAA**4**-(tri-*O*-benzyl)-Ala-D-Pro-Ala-OH (**34**), which was purified by preparative HPLC (40 → 70, B in A, 30 min, t_R 13.6 min). FAB-MS: 810 (14, $[M + Na]^+$); 788 (100, $[M + H]^+$).

34 (52.2 mg) was dissolved in NMP (100 mL) and added slowly to a solution of TBTU (70.2 mg), HOBt (58.2 mg), and DIEA (pH of solution was approximately 7.5) in NMP (300 mL). After 3 h at room temperature the solution was treated with H_2O (30 mL) and concentrated and the product purified by preparative HPLC (40 → 70, B in A, 30 min, t_R 20.7 min). FAB-MS for cyclo(SAA**4**-(tri-*O*-benzyl)-Ala-D-Pro-Ala-Ala) (**35**): 792 (3, $[M + Na]^+$).

The cyclic peptide was dissolved in H_2O/HOAc (30 mL, 1:1) and hydrogenated for 24 h in the presence of Pd/C (30 mg of 5% Pd/C). The product was purified by preparative HPLC (5 → 40, B in A, 30 min, t_R 12.5 min) to yield **36** in 22.9 mg (68%).

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Supporting Information Available: Comparisons between experimentally determined and simulated NOE-derived distances and homonuclear ^3J coupling constants for **26**, **33**, and **36;** details of structure calculation; synthesis and characterization of all precursor dipeptides; characterization of compounds **14**, **16**, and **17**; table with proton and carbon chemical shifts for the minor conformation of compound **36** and temperature coefficients (13 pages). See any current masthead page for ordering information and Internet access instructions.

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