DOI: 10.1002/ejoc.200900460

Synthesis and Biological Evaluation of Novel Gramicidin S Analogues

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Keywords: Antibiotics / Peptidomimetics / Sugar amino acid / Conformation analysis

The synthesis of three new analogues of the cyclic cationic antimicrobial peptide Gramicidin S is described. These derivatives contain a modified turn region in which the ^{D}Phe-Pro motif has been replaced by a constrained furanoid sugar amino acid or a flexible linear aminoethoxy acetic acid moiety. Structural analysis revealed conformational changes in the modified turn region compared to GS. The biological profile of these compounds however resembles that of Gramicidin S and previously described analogues.

Gramicidin S (GS **1**) is a cationic antimicrobial peptide[1] (CAP) that was first isolated from *Bacillus brevis* by Gause and Brazhnikova.[2] GS is active against a wide range of bacteria, both Gram-positive and Gram-negative strains, and as such represents an attractive lead compound for the development of new antibiotic strategies. Unfortunately, GS itself is highly hemolytic, and its clinical use is therefore limited to topical applications.[2] Both bacterial and hemolytical activities are rooted in its molecular mode of action.[1a] GS acts on the cell membrane and disrupts the integrity of the lipid bilayer, with cell lysis as a result. Here lies the difficulty in GS-inspired research towards new antibiotic strategies: although highly different in nature and function, the bacterial cell is not that different from an erythrocyte from the point of view of the cell membrane. That GS is at all considered as an attractive starting point is based on the same molecular mode of action: unlike most other antibiotic compound classes GS does not target a specific molecule (protein, DNA or RNA) within the bacterial cell and thus the development of resistance towards GS and related CAPs is commonly thought to be less likely.[3] In this respect, an interesting finding underscoring the mode of action of GS is that enantiomeric GS is equally active as the natural compound.[4] Clearly, such would not be the case when a specific molecule, for instance an integrated membrane protein, would be the main target of GS.

Research on GS analogues with improved medicinal properties is mainly aimed at the development of analogues that have bactericidal properties inherent to GS, while not being active against erythrocytes. In practice, this entails the development of compounds able to distinguish between a mammalian cell membrane and that of a bacterial cell. This is a daunting task, firstly because, as said before, there is not much difference between the two types of lipid bilayers (and not much information on this issue is available). And secondly, there is little known about the exact mode of action by which GS inserts into a lipid bilayer.[5] Contemporary research on new and improved GS analogues therefore takes a three-pronged approach, namely: (1) the design and synthesis of new GS derivatives, (2) the in-depth analysis of the secondary structure these compounds may adopt in (aqueous) solution, and (3) the assessment of these compounds on their activities on both bacterial strains and erythrocytes. Recent years have further witnessed approaches to study the conformational behavior of GS in the context of membrane fractions, but no conclusive results have emerged from these studies yet.[6] Current state-of-the-art dictates that, whereas the conformational behavior of GS[7] (and that of quite a few synthetic analogues)[8] in aqueous solution is known in detail, it is not known whether, and to what extent, this behavior holds true for the conformational behavior (and therefore mode of action) of GS in lipid membranes. X-ray diffraction studies on GS assemblies further give conflicting results,[7d] and in any

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case it is debatable whether such artificial assemblies reflect those possibly found in lipid bilayers as pore entities. The approach taken in this study is therefore as outlined above, namely: (1) modify GS at specific positions, (2) evaluate the structure in solution, and (3) measure their biological activities.

At the basis of the study presented here is the solution structure of GS itself, together with that of a number of modified structures that have recently appeared in the literature.[7,8] GS is a cyclic decapeptide with sequence (Pro-Val-Orn-Leu-^{D}Phe-)$_2$ and has C_2 symmetry. In aqueous solution, and as measured by proton NMR,[7b] it adopts an antiparallel β-sheet with the two Val-Orn-Leu stretches aligning as two β-strands and the two ^{D}Phe-Pro dipeptides as two type II' β-turn. The cyclic β-hairpin structure is stabilized (see Figure 1) by four interstrand hydrogen bonds and the molecule adopts a slight twist.[7c] This overall conformational assembly gives GS its amphiphilic nature that is thought to be the basis for its lytic properties.[9] Here the two basic ornithine residues point to one side of the molecule and the four hydrophobic Val/Leu residues are situated at the opposite side. GS analogues of varying nature have been reported, including extended homologues,[9] derivatives featuring different α-amino acids[10] and derivatives incorporating amino acid analogues.[11] Modifications may be thus brought to both β-strand and β-turn regions.

With respect to the latter, of specific interest is the recently reported structure of **2a**,[12] in which one of the two β-turn dipeptides is replaced by a so-called sugar amino acid (SAA) dipeptide isoster **3a**.[13] SAAs have attracted some interest in recent years due to their ease of access, the conformational control they may give to a target peptide, their (putative) protease stability and the number of functional groups that are inherent to the parent carbohydrate and that opens ways for further functionalization.[14] Rather surprisingly, the secondary structure of **2a** and **2b**, that contains one copy of SAA **3a** and **3b** respectively, deviate from GS in that the leucine carbonyl adjacent to the SAA amine is flipped (in comparison to GS) out of the ring, and is not involved in hydrogen bonding.[14] Rather, there appears to be a hydrogen bond between the SAA-NH and one of the oxygen functionalities (C3-OH) attached to the SAA furan core. As a result, the solution structures of **2a** and **2b** deviate from that of GS in that, although the β-sheet character is to a large extent intact, one of the β-turn regions is drastically altered and the overall molecule has a much more pronounced twist around the β-strand. Interestingly, both bacterial and hemolytical activities of GS and **2b** (R = Bn) are comparable and a tentative conclusion from these studies may be that some conformational freedom in the amphiphilic structure is allowed, and possibly may be capitalized upon in the development of species-specific GS analogues.

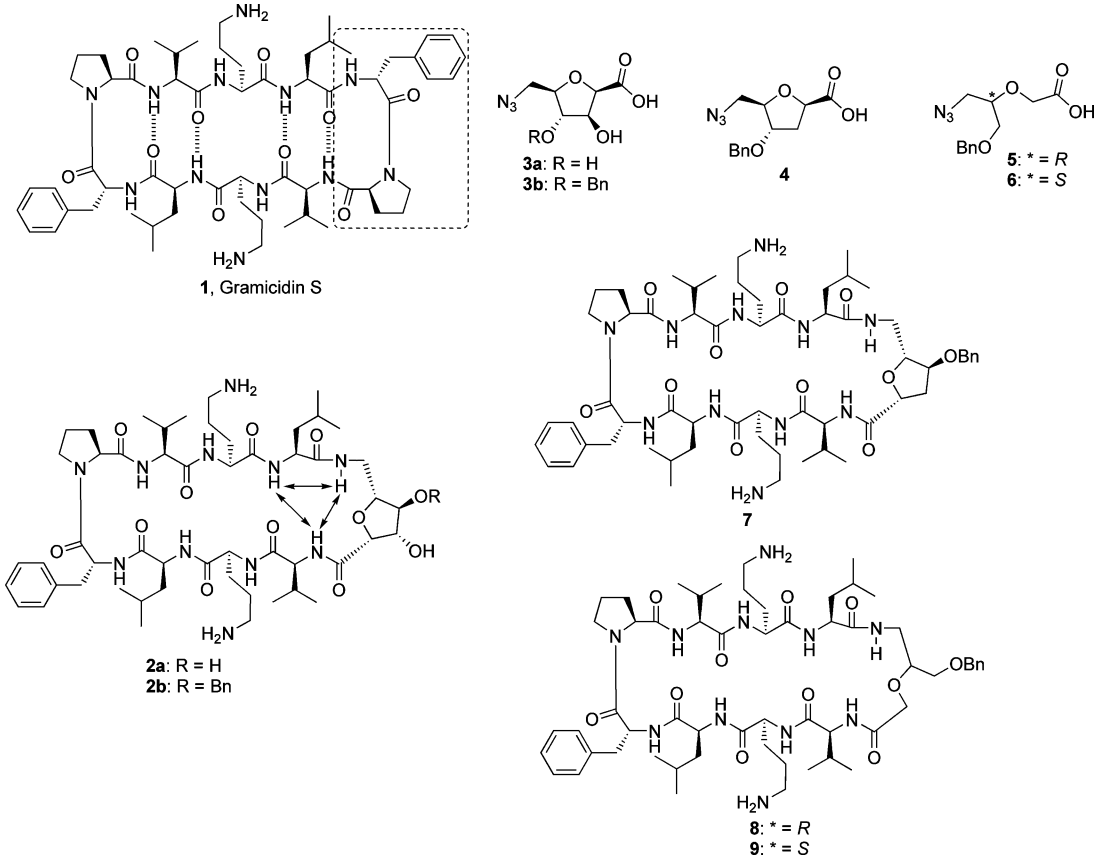


Figure 1. Gramicidin S and analogues thereof in which the ^{D}Phe-Pro motif (highlighted in GS) has been replaced by different dipeptide isosters.

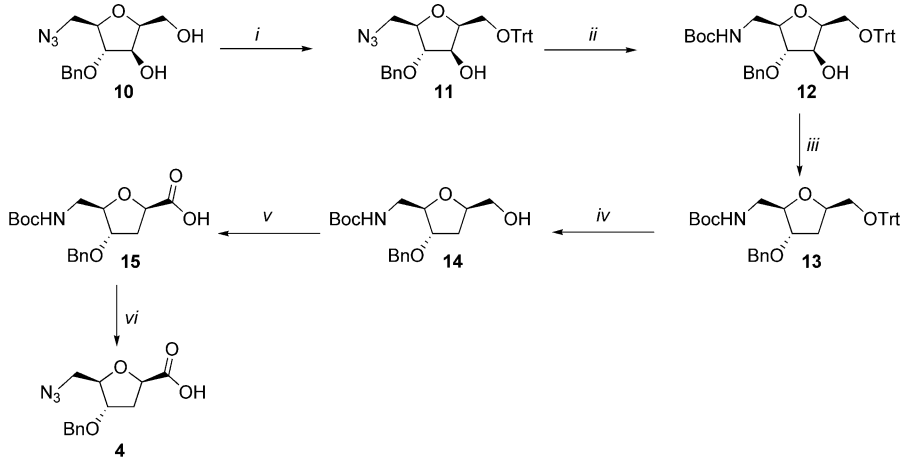
Incidentally, the hydrophobic nature of the turn region is much more important: earlier studies proved that the analogous compound **2a**, without a benzyl group at a position corresponding to DPhe in the GS structure, has no activity at all, even though its solution structure is highly similar to that of **2b**.[12,14]

The subject of this paper is to further study to what extent the SAA design in **3** is amenable to modification. Three analogues are presented here, of which the first (compound **7**, see Figure 1) lacks the C3-OH group involved in hydrogen bonding and the other two (**8** and **9**, see Figure 1) are rendered more flexible through the incorporation of linear dipeptide isosters. The synthesis of these molecules, and the SAA building blocks on which they are based, are presented. Further, their conformational behavior in solution and their bactericidal/hemolytical activities are discussed, taking into consideration the data known on GS and analogues **2a** and **2b**.

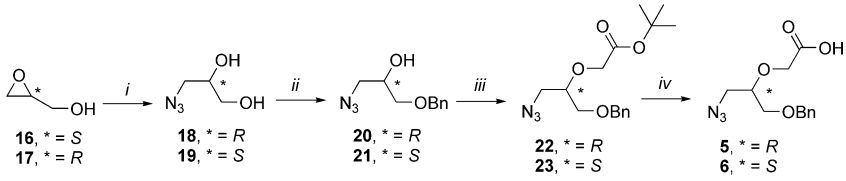
The synthesis of SAA **4** commenced from the known intermediate **10**[14] of which the primary alcohol was selectively protected using trityl chloride in pyridine to give compound **11** in 68% yield (Scheme 1). Conversion of the azide group in **11** into the more compatible Boc-protective group yielded compound **12** in 93% yield. Next, Barton deoxygenation using thiocarbonyldiimidazole, AIBN and Bu_3SnH gave deoxygenated compound **13** in 74% yield. Deprotection of the tritylated alcohol under slightly acidic conditions (**13** → **14**, 70%) and oxidation of the primary alcohol to the carboxylic acid under classical TEMPO conditions (**14** → **15**, 80%) followed by Boc removal and diazotransfer using freshly prepared triflic azide afforded SAA **4** in 77% yield.

Azidoethoxy acetic acid (AAA) **5** was synthesized as follows (Scheme 2). Commercially available (*S*)-glycidol **16** was selectively azidolyzed with NaN_3 in wet *t*BuOH under Lewis acid catalysis according to the literature procedure,[15] affording diol **18** in 49% yield. Tin-ketal formation followed by selective alkylation towards the primary benzyl ether yielded **20** in 62% yield. Alkylation using *tert*-butyl bromoacetate (**20** → **22**, 64% yield) and acidic deprotection resulted in formation of **5** in 72% yield. The stereoisomeric **6** was obtained following the same sequence of reactions starting from (*R*)-glycidol in comparable overall yield.

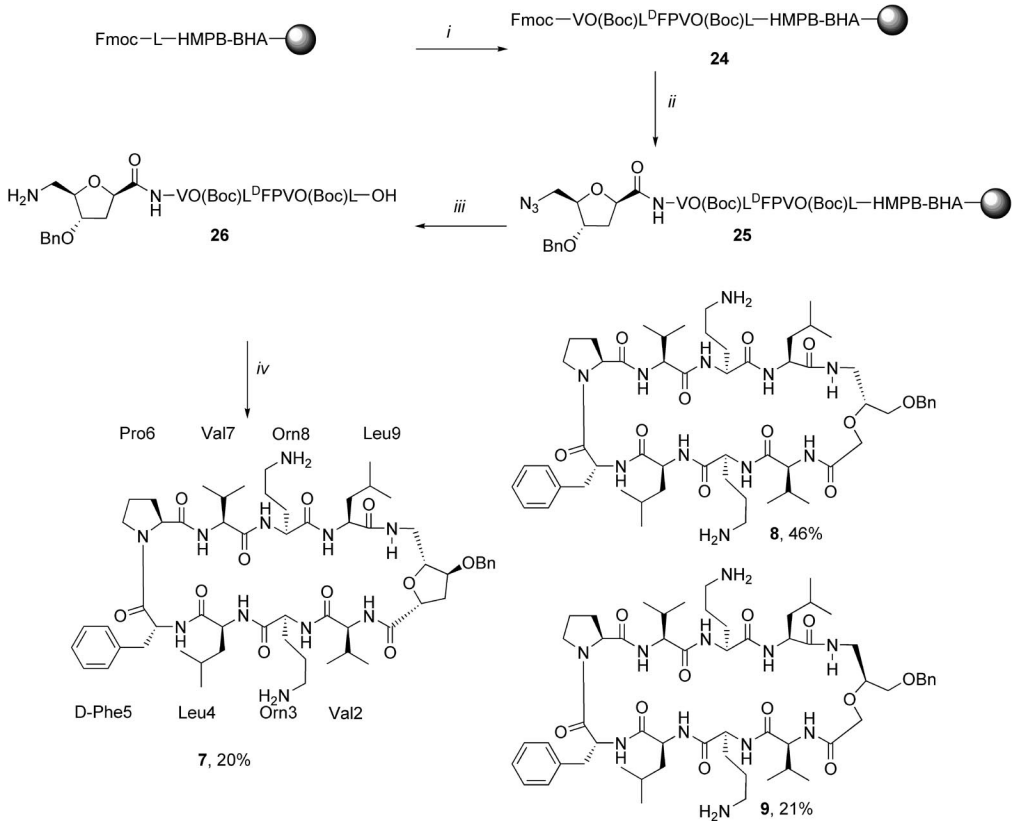
The synthesis of GS analogues **7**–**9** is exemplified by the synthesis of **7** and is depicted in Scheme 3. Commercially available polystyrene based resin, preloaded with Fmoc-Leu, attached via the hyper acid labile benzhydrilamine/4-hydroxymethyl-3-(methoxyphenoxy)butanoic acid linker was elongated with ornithine, valine, proline, D-phenylalanine, leucine, ornithine and valine using repetitive cycles of standard Fmoc-based SPPS as depicted in Scheme 3. Coupling of SAA **4** was performed with a 1.5-fold excess of



Scheme 1. Reagents and conditions: (*i*) TrtCl (1.5 equiv.), pyridine, 16 h, 0 °C, 68%. (*ii*) a: PMe_3 (1 M soln); toluene, 2 equiv., 0 °C, THF, 1 h; then H_2O 2 equiv., 6 h, b: Boc_2O (1.5 equiv.), DiPEA (2 equiv.) 16 h, 93% over two steps. (*iii*) a: 1,1-thiocarbodiimidazole (4 equiv.), 90 °C, toluene, 5 h, b: AIBN (0.2 equiv.), Bu_3SnH (4 equiv.), 80 °C, toluene, 4 h, 74% over two steps. (*iv*) *p*TsOH (0.5 equiv.), 40 °C, 10% MeOH/DCM 6 h, 70%. (*v*) TEMPO (cat.), KBr (0.1 equiv.), NaOCl (2 equiv.), ACN, 80%. (*vi*) a: 50% TFA/DCM, b: TfN_3 (5 equiv.), $CuSO_4$ (0.1 equiv.), K_2CO_3 (3 equiv.), MeOH/H_2O, 77% over two steps.



Scheme 2. Reagents and conditions: (*i*) NaN_3 (1.2 equiv.) $LiBF_4$ (0.2 equiv.), *t*BuOH/H_2O (5:1), 49% **18**; 44% **19**; (*ii*) a: Bu_2SnO (1.5 equiv.) 100 °C, toluene, 3 h, b: BnBr (2 equiv.) 100 °C, toluene, 16 h, 62% **20**; 28% **21**; (*iii*) NaH (60% suspension in mineral oil, 1.2 equiv.), *tert*-butyl bromoacetate (2 equiv.), 0 °C to room temp. DMF, 16 h, 64% **22**; 40% **23**; (*iv*) TFA/DCM/TIS, 50:50:1. 72% **5**; 86% **6**.



Scheme 3. Reagents and conditions: (i) 8 cycles of a: 20% pip/NMP, 3 × 5 min; b: Fmoc-AA-OH (5 equiv.), HCTU (5 equiv.), DiPEA (10 equiv.) 90 min. (*ii*) a: 20% pip/NMP, 3 × 5 min. b: **4**, **5** or **6** (1.5 equiv.), HCTU (1.5 equiv.), DiPEA (3 equiv.), 16 h. (*iii*) a: PMe_3 (1 M in 9:1 THF/H_2O, 25 equiv.), 16 h. b: 1% TFA/DCM. (*iv*) a: PyBOP (5 equiv.), HOBt (5 equiv.), DiPEA (10 equiv.), 16 h. b: 50% TFA/DCM, 1 h.

SAA with respect to the resin-loading and the coupling time was extended to 16 h affording **25**. On-resin Staudinger reduction using excess PMe_3 in wet THF[16] followed by acidic cleavage using 1% TFA/DCM yielded linear protected nonamer **26**. Cyclization under dilute conditions (10 mM in DMF) using PyBOP/HOBt and DiPEA and acidic deprotection (50% TFA/DCM) afforded GS analogue **7** in 20% after HPLC purification. Analogues **8** and **9** were prepared in a similar fashion.

Structural analysis of the newly synthesized analogues was performed by extensive 1D and 2D NMR experiments. From the ^{1}H NMR, all the NH-coupling constants could be determined and compared to those obtained from native GS (**1**) and **2b** (Figure 2, A). The presence of a β-turn conformation around D-Phe5 was evidenced in all analogues by its small coupling constant[17] ($^3J_{NH\text{-}H\alpha} < 4$ Hz) and a negative value for the chemical shift perturbation of the α-proton of the D-Phe and Pro residues.[18]

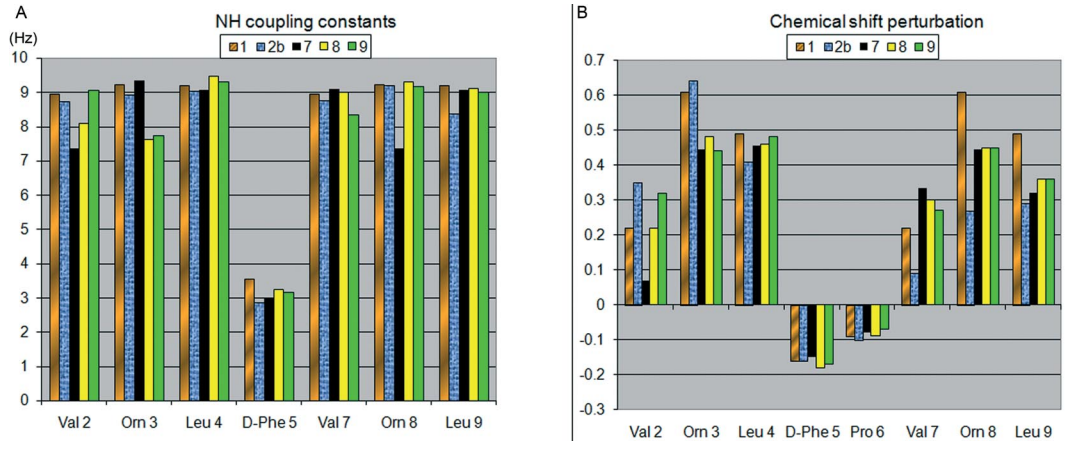


Figure 2. A: $^3J_{NH\text{-}H\alpha}$ of GS, **2b** and **7**–**9**; B: chemical shift perturbation of GS, **2b** and **7**–**9**.

(Figure 2, B). For **7**, the remaining coupling constants are similar to both native GS and **2b**, indicating a comparable overall β-sheet structure[19] (8.5 Hz < $^3J_{NH\text{-}H\alpha}$ < 9 Hz), except Val2 and Orn8 which are less evident. The coupling constants and chemical shift perturbation of **8** and **9** indicate a similar structure of these two analogues.

A close inspection of the amide region in the NOE spectrum of **7** (see Figure 3) reveals, besides numerous sequential NOEs, several interstrand NOEs indicating the presence of a β-sheet character in this analogue. Interestingly, NH-NH NOE cross correlations were found between the amide protons of SAA and Leu9 (A, in Figure 3), the SAA and the Val2 amide protons (B in Figure 3) and between the NH protons of Val2 and Leu9 similar to those found for **2a** and **2b** which is indicative for the same altered orientation of the SAA amide proton. These results clearly indicate that a hydrogen bond between the SAA amide and the hydroxy group is not the determining factor for the observed re-orientation of the Leu - SAA amide bond in **2a** and **2b**. A stabilizing effect of this hydrogen bond can of course not be ruled out. It does, however, implicate the involvement of conformational restrictions imposed by the furanoid ring as major factor in the amide re-orientation. This conclusion is substantiated by results obtained from flexible analogues **8** and **9** where the NOE spectra lack the NOE cross correlation signals between the AAA and Val2 amide protons.

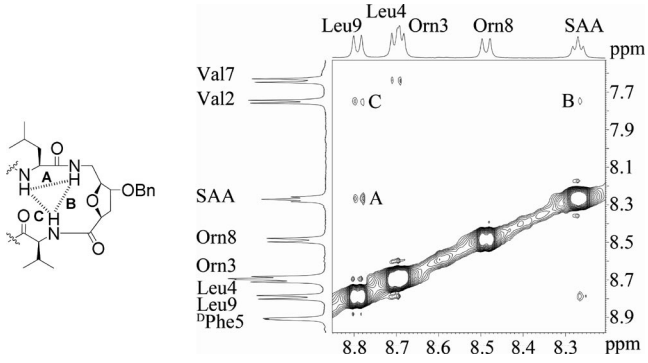


Figure 3. Turn region of **7** and part of the amide region of the NOE spectrum of **7**.

Next, the newly synthesized analogues were assessed for their antibacterial activity and compared to GS and **2b**. The results are depicted in Table 1. Both the Gram-negative bacterial strains used in this assay, *E. coli* and *P. aeruginosa*, were largely unaffected by the presence of any of the peptides. Compound **8** was most effective against *E. coli* with a minimal inhibitory concentration (MIC) of 16 μg/mL. The GS analogues **7**, **8** and **9** are potent against gram-positive strains with MIC values between 8 and 32 μg/mL. Compound **8** is about as potent as GS, **7** and **9** are about half as potent.

The hemolytic activity of the GS analogues was determined and the results are plotted in Figure 4. Analogues **7** and **8** are about as toxic towards human erythrocytes as GS and **2b** both showing 100% hemolysis at 125 μM peptide concentration. Compound **9** shows a reduced hemolytical activity with only 90% lysis at a peptide concentration of 500 μM.

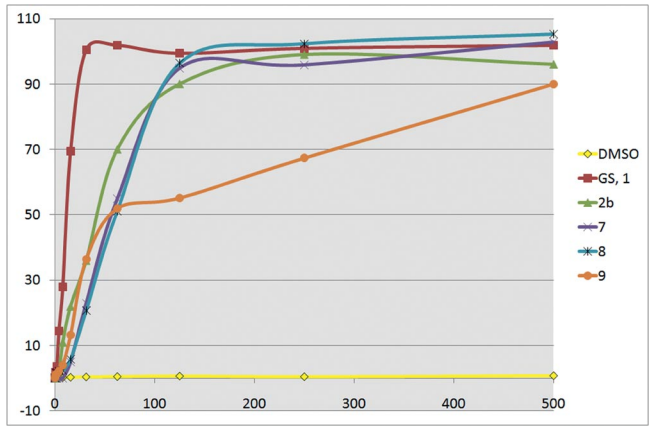


Figure 4. Hemolytical activity of GS and analogues thereof.

Conclusions

Three novel dipeptide isosters were successfully synthesized and used to replace the D-Phe/Pro turn motif of Gramicidin S. A cyclic SAA was designed without a hydroxy group on C3 that could possibly function as a hydrogen-bond-accepting moiety that could stabilize the re-orientated backbone conformation found in GS analogue **2a** and **2b**. Two stereoisomeric acyclic dipeptide isosters were also synthesized and incorporated into GS. All three GS analogues were analyzed by means of 1D- and 2D-NMR experiments to investigate their structural conformation in organic solvent. In the case of **7**, containing the cyclic SAA **4**, the backbone showed a similar re-orientation as was found in **2a** and **2b**. The backbone conformations of the acyclic dipeptide isoster containing GS analogues **8** and **9** were not distorted and resembled the conformation found in native GS.

Table 1. MIC values for **1**, **2b** and **7**–**9**.

	Gram pos. *Staph. aureus*	Gram pos. *Staph. epidermis*	Gram pos. *Entrerococ. faecalis*	Gram neg. *E. coli*	Gram neg. *P. auruginosa*	Gram pos. *Bacillus ereus*
1	8	8	8	>64	>64	8
2b	16	8	32	32	>64	8
7	16	16	32	64	>64	16
8	8	8	16	16	64	8
9	16	8	32	32	>64	8

The biological evaluation revealed that **7** was similarly active as **2b** and slightly less active compared to GS in both the bacterial and hemolytical assays. Replacing the β-turn motif in GS with a flexible dipeptide isoster resulted in analogues with a similar (**8**) or slightly decreased (**9**) activity towards both bacterial strains as well as erythrocytes depending on the stereochemistry of the aromatic moiety in the dipeptide isoster. Compound **8** shows a slight reduction in toxicity against human erythrocytes compared to GS. However, a similar decrease in potency was observed in the antibacterial assay. The stereoisomeric analogue **9** possessed reduced bactericidal potency combined with a marked decrease in hemolytical activity. These findings indicate that the distorted backbone conformation of GS, imposed by the cyclic nature of furanoid SAAs **2b** and **4**, is a determining factor in the decreased antibacterial activity. However, the presence of a linear flexible turn moiety as in compounds **8** and **9** is more detrimental with respect to the antibacterial activity. Although the precise correlation between the increased conformational freedom in the modified turn region in **8** and **9** and the decreased biological activity remains unidentified, these results demonstrate the potential of peptide isosters as elements to replace secondary structural elements in biologically active peptides in an attempt to optimize the behavior in biological surroundings.

Experimental Section

General: PE with a boiling range of 40–60 °C was used. THF and Et_2O were distilled from $LiAlH_4$ prior to use. DCM was distilled from CaH_2 prior to use. All other solvents used under anhydrous conditions were stored over molecular sieves (4 Å) except for methanol, which was stored over molecular sieves (3 Å). Solvents used for work-up and column chromatography were of technical grade and distilled before use. Unless stated otherwise, solvents were removed by rotary evaporation under reduced pressure at 40 °C. Reactions were monitored by TLC-analysis using DC-fertigfolien (Schleicher & Schuell, F1500, LS254) with detection by spraying with 20% H_2SO_4 in EtOH, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g/L) in 10% sulfuric acid or by spraying with a solution of ninhydrin (3 g/L) in EtOH/AcOH (20:1 v/v), followed by charring at about 150 °C. Column chromatography was performed on Fluka silica gel (0.04–0.063 mm). For LC/MS analysis, an JASCO HPLC-system (detection simultaneously at 214 and 254 nm) equipped with an analytical C_{18} column (4.6 mm × 250 mm, 5 μ particle size) in combination with buffers A: H_2O, B: MeCN and C: 0.5% aq. TFA and coupled to a mass instrument with a custom-made Electronspray Interface (ESI) was used. For reversed-phase HPLC purification of the final compounds, an automated HPLC system supplied with a semi-preperative C_{18} column (10.0 mm × 250 mm, 5μ particle size) was used. The applied buffers were A: H_2O, B: MeCN and C: 1.0% aq. TFA. High resolution mass spectra were recorded by direct injection (2 μL of a 2 μM solution in water/acetonitrile; 50:50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150–2000) and dioctyl phthalate (m/z = 391.28428) as a "lock mass".[20] The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). ^{1}H- and ^{13}C-NMR spectra were measured on a Joel JNM-FX-200 (200/50 MHz). Chemical shifts are given in ppm (δ) relative to TMS (δ = 0 ppm) or MeOD (δ = 3.30 ppm) and coupling constants are given in Hz. Optical rotations were measured on a propol automatic polarimeter.

General Procedure for Peptide Synthesis. (a) Stepwise Elongation: Fmoc-Leu-HMPB-BHA resin **6** (196 mg, 0.51 mmol/g, 0.1 mmol) was submitted to seven cycles of Fmoc solid-phase synthesis with the appropriate commercially available amino acid building blocks Fmoc-Orn(Boc)-OH, Fmoc-Val-OH, Fmoc-Pro-OH, Fmoc-^{D}Phe-OH, as follows: a) deprotection with piperidine/NMP (1:4, v/v, 5 mL, 15 min); b) washing with NMP (5 mL, 3× , 3 min); c) coupling of the appropriate Fmoc amino acid (5 equiv., 0. 5 mmol) in the presence of HCTU (5 equiv., 0.5 mmol, 206 mg) and D*i*PEA (10 equiv., 1 mmol, 162 μL) which was preactivated for 2 min in NMP (5 mL) and shaken for 90 min; d) washing with NMP (5 mL, 3× , 3 min). Couplings were monitored for completion by the Kaiser test.[21] Finally, the *N*-terminal amine was liberated by Fmoc-deprotection with piperidine/NMP (1:4, v/v, 5 mL, 15 min) followed by washing with NMP (5 mL, 3× , 3 min). Coupling of SAA **4** was performed as follows: To the resin bound peptide, a preactivated solution of SAA **4** (1.5 equiv. 44 mg, 0.150 mmol), HCTU (1.5 equiv., 62 mg, 0.150 mmol) and D*i*PEA (3.0 equiv., 74 μL, 0.45 mmol) in NMP (3 mL) was added and the resulting suspension was shaken for 16 h. The resin was finally washed with NMP (5 mL, 3× , 3 min) to give the azide protected linear nonapeptide.

(b) On-Resin Staudinger Reduction: The resin-bound azide is treated with a pre-mixed cocktail of H_2O (0.5 mL) and PMe_3 (3.5 mL, 1 M in THF) and shaken for 16 h. The resin is washed with methanol (4 mL, 3× , 3 min) and DMF (4 mL, 3× , 3 min.)

(c) Cleavage from the Resin: Resin-bound nonapeptide is repeatedly treated with 1% TFA/DCM (4 mL). The filtrate was coevapporated with toluene (3×) and used as such in the cyclization step.

(d) Cyclization: The linear nonapeptide was taken up in DMF (5 mL) and added dropwise over the course of one hour to a solution of benzotriazol-1-yloxy-tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) (5 equiv., 270 mg, 0.5 mmol), HOBt (5 equiv., 67 mg, 0.5 mmol) and DiPEA (15 equiv., 254 μL, 1.5 mmol) in DMF (70 mL) and stirred for 16 h. The solvent was removed in vacuo and the resulting mixture was used without further purification in the deprotection step.

(e) Deprotection: The crude cyclised peptide was treated with 50% TFA/DCM (10 mL) for 1 h before it was concentrated and purified by HPLC purification.

(2S,3*R*,4*S*,5*R*)-5-(Azidomethyl)-4-(benzyloxy)-2-(trityloxymethyl)-tetrahydrofuran-3-ol (11): Diol **10** (1.11 g, 2.13 mmol) was dissolved in pyridine (11 mL) and cooled to 0 °C. Trityl chloride (0.89 g, 3.2 mmol, 1.5 equiv.) was added at 0 °C. The reaction mixture was stirred for 18 h and when TLC analysis (3:20 v/v EtOAc in PE) indicated complete conversion of the diol into a higher running product, the reaction mixture was evaporated to dryness. The residue was taken up in EtOAc, washed with satd. aq. $NaHCO_3$ and Brine, dried with Na_2SO_4, filtered and the solvents evaporated. Purification was performed by column chromatography (mixture of eluents: EtOAc/PE, 0:1 → 3:20 v/v) and afforded compound **11** (0.75 g, 1.44 mmol, 68% yield) as a yellow syrup. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.82–7.11 (m, 20 H, H_{Ar}), 4.69 (d, J = 11.8 Hz, 1 H, CH_2Ph), 4.57 (d, J = 11.8 Hz, 1 H, CH_2Ph), 4.30 (m, 1 H, C*H*OH), 4.17 (d, J = 4.9 Hz, 1 H, C*H*OTrt), 4.04 (d, J = 3.7 Hz, 1 H, C*H*OTrt), 3.84–3.76 (m, 1 H, C*H*OPh), 3.60–3.34 (m,

3 H, CH_2N$_3$, OCHCCH$_2$OTrt, OCHCH$_2$N$_3$), 3.18 (d, J = 5.7 Hz, 1 H, CH_2N$_3$)2.36 (s, 1 H, OH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 147.94 (C_qAr), 142.05 (C_qAr), 133.04 (CH_{Ar}), 132.55 (CH_{Ar}), 132.46 (CH_{Ar}), 132.38 (CH_{Ar}), 132.23 (CH_{Ar}), 131.75 (CH_{Ar}), 131.68 (CH_{Ar}), 91.88 (C_qTrt), 91.21 (CH$_2$OBn), 86.59 (OCCH$_2$-OTrt), 84.62 (OCCH$_2$N$_3$), 81.06 (CHOH), 76.37 (OCH$_2$Ph), 66.84 (CH$_2$OTrt), 57.52 (CH$_2$N$_3$) ppm.

***tert*-Butyl [(2*R*,3*S*,4*R*,5*S*)-3-(Benzyloxy)-4-hydroxy-5-(trityloxymethyl)tetrahydrofuran-2-yl]methylcarbamate (12):** Tritylated **11** (5.54 g, 10.64 mmol) was co-evaporated with toluene (2×), re-dissolved in THF (55 mL), cooled to 0 °C under argon and PMe_3 [21.3 mL (1 M solution in toluene), 2 equiv.] was added. The reaction mixture was stirred for 1 h and then H_2O (0.38 mL, 21.3 mmol, 2 equiv.) was added, after which stirring was continued for another 6 h. After that, the reaction mixture was concentrated, co-evaporated with toluene (2×), re-dissolved in toluene (50 mL) and cooled to 0 °C and DiPEA (2.75 g, 3.5 mL, 21.3 mmol, 2 equiv.) and Boc_2O (3.5 g, 15.96 mmol, 1.5 equiv.) were added. The reaction was stirring for 12 h at room temp., EtOAc was added and the organic layer was washed with satd. aq. $NaHCO_3$, NH_4Cl and brine, dried with Na_2SO_4, filtered and concentrated. Protected amine **12** was isolated by column chromatography (eluents: 1:20 → 3:20 v/v EtOAc in PE) (5.89 g, 9.9 mmol, 93% yield) as a white foam. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.68–7.01 (m, 20 H, H_{Ar}), 4.98 (br. s, 1 H, NHBoc), 4.64 (d, J = 2.0 Hz, 2 H, CH_2Ph), 4.32 (dd, J = 0.7, 4.6 Hz, 1 H, CHOH), 4.21–4.05 (m, 1 H, CH_2OTrt), 3.97 (m, 1 H, CH_2OTrt), 3.77 (dd, J = 1.6, 3.7 Hz, 1 H, CHOPh), 3.61–3.25 (m, 4 H, OCHCH$_2$OTrt, OCHCH$_2$NHBoc, CH_2NHBoc), 1.42 (s, 9 H, 3× CH$_3$) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 155.96 (C=O), 143.31 (C_qAr), 137.49 (C_qAr), 128.35 (CH_{Ar}), 128.30 (CH_{Ar}), 127.83 (CH_{Ar}), 127.65 (CH_{Ar}), 127.51 (CH_{Ar}), 127.05 (CH_{Ar}), 87.17 (C_qTrt), 86.60 (CHOBn), 81.81 (OCHCH$_2$NHBoc), 79.60 (OCHCH$_2$OTrt), 79.10 (C_qBoc), 76.51 (CHOH), 71.60 (CH_2Ph), 62.32 (CH_2OTrt), 42.84 (CH_2NHBoc), 28.23 (CH_3Boc) ppm.

***tert*-Butyl [(2*R*,3*S*,5*R*)-3-(Benzyloxy)-5-(trityloxymethyl)tetrahydrofuran-2-yl]methylcarbamate (13):** Protected amine (**12**) (0.18 g, 0.3 mmol) was co-evaporated with toluene (1×), 1,1-thiocarbonyldiimidazole (0.23 g, 1.2 mmol, 4 equiv.) was added, the reaction mixture was heated to 90 °C for 5 h using an oil bath. EtOAc was added and the organic layer was washed with demi water (3×), dried with Na_2SO_4, filtered and the solvents evaporated. The residue was co-evaporated with toluene (2×), re-dissolved in toluene and AIBN (9.8 mg, 0.06 mmol, 0.2 equiv.) was added. Argon was bubbled through the flask for 15 min at 80 °C and then Bu_3SnH (0.27 g, 0.244 mL, 0.92 mmol, 4 equiv.) was added. TLC analysis indicated completion of the reaction after 4 h and the mixture was concentrated. The residue was purified by column chromatography (eluents: 1:20 → 2:10 v/v EtOAc in PE) yielding compound **13** (0.13 g, 0.22 mmol, 74%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.58–7.11 (m, 20 H, H_{Ar}), 4.92–4.75 (br. s, 1 H, NHBoc), 4.52 (s, 2 H, CH_2Ph), 4.34 (m, 1 H, CH_2OTrt), 4.23–3.87 (m, 2 H, CH_2OTrt, CHOBn), 3.37 (m, 1 H, OCHCH$_2$NHBoc), 3.17 (m, 3 H, OCHCH$_2$OTrt, CH_2NHBoc), 1.95–1.77 (m, 2 H, CCH_2CHOBn) 1.42 (s, 9 H, CH_3Boc) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 154.64 (C=O), 143.83 (C_qAr), 137.81 (C_qAr), 128.56 (CH_{Ar}), 128.30 (CH_{Ar}), 127.64 (CH_{Ar}), 127.56 (CH_{Ar}), 127.49 (CH_{Ar}), 126.84 (CH_{Ar}), 86.39 (C_qTrt), 82.94 (CHOBn), 80.66 (OCHCH$_2$HBoc), 79.12 (C_qBoc), 77.77 (OCHCH$_2$OTrt), 71.18 (CH_2OTrt), 65.78 (CH_2Ph), 43.23 (CH_2NHBoc), 34.67 (CH$_2$CHOBn), 28.23 (CH_3Boc) ppm.

***tert*-Butyl [(2*R*,3*S*,5*R*)-3-(Benzyloxy)-5-(hydroxymethyl)tetrahydrofuran-2-yl]methylcarbamate (14):** De-oxygenated (**13**) (9.9 g, 17 mmol) was diluted in a mixture of DCM/MeOH (9:1 v/v, 106 mL), heated up to 40 °C, *p*TsOH (10.2 mmol, 1.94 g) was added and the system was heated under reflux for 6 h. When TLC analysis (1:5 v/v EtOAc in PE) indicated completion, satd. aq. $NaHCO_3$ and brine were added, the aqueous layer was washed with diethyl ether (5×) and the organic layer was dried with $MgSO_4$, filtered and the solvents evaporated. Purification by silica gel column (eluents: 2:5 → 3:5 v/v EtOAc in PE) yielded **14** (4 g, 11.9 mmol, 70%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.39–7.28 (m, 5 H, H_{Ar}), 4.84 (br. s, 1 H, NHBoc), 4.52 (s, 2 H, CH_2Ph), 4.29 (m, 1 H, CH_2OH), 4.21–4.08 (m, 1 H, CH_2OH), 4.08–3.86 (m, 3 H, CHOBn, OCHCH$_2$NHBoc, CH_2NHBoc), 2.03–1.97 (m, 2 H, CCH_2CHOBn), 1.44 (s, 9 H, CH_3Boc) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 156.43 (C=O), 137.66 (C_qPh), 128.17–127.35 (CH_{Ar}), 83.53 (CHOBn), 81.13 (OCHCH$_2$NHBoc), 79.37 (OCHCH$_2$OH), 79.20 (C_qBoc) 70.94 (CH_2OBn), 63.03 (CH_2OH), 43.26 (CH_2NHBoc), 32.37 (CCH$_2$COBn), 28.09 (CH_3Boc) ppm. MS: m/z = 338.2 [M + H]$^+$, 282.1 [M – tBu + H]$^+$, 238.1 [M – Boc + H]$^+$.

(2*S*,4*S*,5*R*)-4-(Benzyloxy)-5-[(*tert*-butoxycarbonylamino)methyl]tetrahydrofuran-2-carboxylic Acid (15): Alcohol (**14**) (2.4 g, 7 mmol) was diluted in a solution of TEMPO in CH_3CN (18.2 mg in 14 mL) and KBr in satd. $NaHCO_3$ (88 mg, 0.7 mmol in 18.2 mL) and cooled to 0 °C. 87 mL of a solution of NaOCl (31 mL), $NaHCO_3$ (19.6 mL) and NaCl (36.4 mL) was slowly added over a 1 h period, after which there was no more change of color with extra addition of the solution. TLC analysis (4:5 v/v EtOAc in PE) indicated completion of the reaction and the reaction mixture was quenched with MeOH, after which it was washed with DCM and the aqueous layer was acidified with 1 M HCl solution to pH 6, washed with DCM (4×), dried with $MgSO_4$, filtered and the solvents evaporated. Compound **15** was retrieved as a yellow solid (1.96 g, 5.6 mmol, 80%). ^{1}H NMR ($CDCl_3$): δ = 7.27–7.09 (m, 5 H, H_{Ar}), 5.56 (br. s, 1 H, NHBoc), 4.69 (m, 1 H, CHCO$_2$H), 4.61, (s, 2 H, CH_2Ph), 4.14 (m, 1 H, CHOBn), 3.91 (m, 1 H, OCHCH$_2$NHBoc), 3.22 (CH_2NHBoc), 2.44 (CCH_2CHOBn), 2.07 (CCH_2CHOBn), 1.41 (s, 9 H, CH_3Boc) ppm. ^{13}C NMR ($CDCl_3$): δ = 175.22 (CO_2H), 156.84 (C=O$_{Boc}$), 137.25 (C_qPh), 128.70–127.30 (CH_{Ar}), 84.61, (OCHCH$_2$NHBoc), 79.60 (CHCO$_2$H), 76.05 (CHOBn), 70.96 (CH_2Ph), 42.61 (CH_2NHBoc), 35.81 (CCH$_2$COBn), 27.96 (CH_3Boc) ppm. MS: m/z = 352.1 [M + H]$^+$, 252.1 [M – Boc + H]$^+$.

(2*R*,4*S*,5*R*)-5-(Azidomethyl)-4-(benzyloxy)tetrahydrofuran-2-carboxylic Acid (4): Boc-protected amine **15** (175 mg, 0.5 mmol) was treated with 50% TFA/DCM (2 mL) for 30 min before it was concentrated. Residual traces of TFA were removed by repeated coevapporation with toluene (5 × 2 mL) to yield the crude TFA-salt which was dissolved in H_2O (1.6 mL). To this solution K_2CO_3 (207 mg, 1.5 mmol, 3 equiv.) was added, followed by a solution of $CuSO_4$ (8 mg, 0.05 mmol, 0.1 equiv.) in H_2O (0.5 mL) and MeOH (3.2 mL). To this homogeneous solution, freshly prepared TfN_3 (5 mmol) in DCM (7 mL) was added and the reaction was allowed to stir for 16 h before the reaction was diluted with DCM (10 mL) and H_2O (10 mL) and the aqueous phase washed with DCM (2 × 5 mL). The aqueous phase was acidified to pH = 2 by addition of satd. aq. citric acid and extracted with DCM (3 × 5 mL) to yield the title compound (107 mg, 0.39 mmol, 77%) as colorless oil. ^{1}H NMR (200 MHz, MeOD): δ = 10.09 (s, 1 H, CO_2H), 7.44–7.17 (m, 5 H), 4.71 (t, J = 7.9 Hz, 1 H, CHCO$_2$H), 4.54 (d, J = 5.4 Hz, 2 H, CH_2Ph), 4.26–4.16 (m, 1 H, CHOBn), 4.10–4.00 (m, 1 H, OCHCH$_2$N$_3$), 3.46 (dd, J = 1.4, 4.7 Hz, 2 H, CH$_2$N$_3$), 2.46 (ddd, J = 3.4, 7.4, 13.4 Hz, 1 H, CCH_2CHOBn), 2.23 (ddd, J = 5.1, 7.2, 8.4 Hz, 1 H, CCH_2CHOBn) ppm. ^{13}C NMR (50 MHz, MeOD): δ

= 176.10 (CO_2H), 137.16 (C_qPh), 128.54 (CH_{Ar}), 128.05 (CH_{Ar}), 127.70 (CH_{Ar}), 83.70 (O*C*HCH$_2$NHBoc), 79.60 (*C*HCO$_2$H), 76.41 (CHOBn), 71.67 (CH_2Ph), 52.38 (CH_2N_3), 35.91 (CH_3Boc) ppm. MS: *m*/*z* = 555.2 $[2M + H]^+$, 277.1 $[M + H]^+$, 250.1 $[M - N_2 + H]^+$.

(*R*)-3-Azidopropane-1,2-diol (18): A solution of (*S*)(–)-glycidol **16** (1.26 mL, 20 mmol) in *t*BuOH (100 mL) was charged with a solution of $LiBF_4$ (375 mg, 4 mmol) in H_2O (4 mL) and NaN_3 (1.6 g, 25 mmol) and stirred for 5 h at 70 °C before the reaction mixture was concentrated. The residue was directly applied to a silica column and eluted with 5% MeOH/EtOAc to afford the title compound (1.15 g, 9.8 mmol, 49%) as colorless oil. ^{1}H NMR (200 MHz, MeOD): δ = 3.85–3.68 (m, 1 H, CC*H*OH), 3.53 (s, 1 H, OH), 3.34–3.29 (m, 4 H, CH_2N_3, CH_2OH) ppm. ^{13}C NMR (50 MHz, MeOD): δ = 71.98 (C*C*HOH), 64.20 (CH_2OH), 54.20 (CH_2N_3) ppm. $[\alpha]_D^{20}$ = +12.2 (*c* = 1.00, in MeOH)

(*S*)-3-Azidopropane-1,2-diol (19): Diol **19** was prepared similarly to **18** and obtained as colorless oil (1.06 g, 9.1 mmol, 44%). ^{1}H NMR (200 MHz, MeOD): δ = 3.91–3.70 (m, 1 H, CC*H*OH), 3.52 (s, 1 H, OH), 3.37 (m, 2 H, CH_2OH), 3.34–3.28 (m, 2 H, CH_2N_3) ppm. ^{13}C NMR (50 MHz, MeOD): δ = 72.05 (C*C*HOH), 64.29 (CH_2OH), 54.09 (CH_2N_3) ppm. $[\alpha]_D^{20}$ = –17.4 (*c* = 1.00, in MeOH)

(*R*)-1-Azido-3-(benzyloxy)propan-2-ol (20): Diol **17** (1.15 g, 9.8 mmol) was co-evaporated with toluene (3 × 25 mL) and dissolved in toluene (25 mL) before addition of Bu_2SnO (3.6 g, 15 mmol, 1.5 equiv.). The reaction mixture was stirred at 100 °C for 3 h, before it was concentrated. The residue was coevapporatd with toluene (3 × 25 mL) and redissolved in toluene (25 mL). To this solution, benzyl bromide (2.4 mL, 20 mmol 2 equiv.) was added and reaction stirred at 100 °C until TLC analysis (25% EtOAc/PE) indicated the formation of a higher running product. The reaction mixture was concentrated, the residue partitioned between EtOAc/H_2O and the aqueous phase extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with satd. aq. sodium hydrogen carbonate (25 mL) and brine (25 mL), dried with Na_2SO_4, filtered and concentrated. The residue was purified by silica column chromatography (0–8% EtOAc/PE) to yield the title compound (1.25 g, 6.0 mmol, 62%) as colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.59–7.13 (m, 5 H, H_{Ar}), 4.87 (br. s, 1 H, OH), 4.53 (s, 2 H, CH_2Ph), 3.99–3.78 (m, 1 H, C*H*OH), 3.55–3.44 (m, 2 H, CH_2OBn), 3.36–3.24 (m, 2 H, CH_2N_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 139.27 (C_qPh), 129.46 (CH_{Ar}), 128.86

Table 2. ^{1}H NMR + NOE (500 MHz, CD_3OH) of compound **7**.[a]

Atom 1	Chemical shift (m, *J* [Hz])	Atom 2	Atom 1	Chemical shift (m, *J*)	Atom 2
Val2 NH	7.75 (d, 7.4)	SAA βH (w) Leu9 NH (w)	Val7 NH	7.63 (d, 9.2)	Pro6 δH (m)
Val2 αH	4.01 (t, 7.6)		Val7 αH	4.28 (m)	
Val2 βH	2.20 (m)		Val7 βH	2.26 (m)	
Val2 γH	1.03 (d, 6.7)		Val7 γH	0.93 (d, 6.7)	
Val2 γH′	0.97 (d, 6.7)		Val7 γH′	0.92 (d, 6.7)	
Orn3 NH	8.68 (d, 7.4)	Val2 αH (s) Val2 βH (m) Val2 γH (w)	Orn8 NH	8.49 (d, 9.4)	Val7 αH (s) Val7 βH (m)
Orn3 αH	4.80 (m)		Orn8 αH	4.91 (m)	
Orn3 βH	2.02 (m)		Orn8 βH	1.62 (m)	
Orn3 βH′	2.02 (m)		Orn8 βH′	1.62 (m)	
Orn3 γH	1.75 (m)		Orn8 γH	1.50 (m)	
Orn3 γH′	1.75 (m)		Orn8 γH′	1.50 (m)	
Orn3 δH	3.04 (m)		Orn8 δH	2.90 (m)	
Orn3 δH′	2.93 (m)		Orn8 δH′	2.90 (m)	
Orn3 NH_2			Orn8 NH_2		
Leu4 NH	8.70 (d, 9.0)	Orn3 αH (s) Val7 NH (w)	Leu9 NH	8.79 (d, 9.1)	Orn8 αH (s)
Leu4 αH	4.64 (dd, 7.5, 15.8)		Leu9 αH	4.52 (m)	SAA αH′ (m)
Leu4 βH	1.55 (m)		Leu9 βH	1.62 (m)	SAA δH (w)
Leu4 βH′	1.55 (m)		Leu9 βH′	1.62 (m)	
Leu4 γH	1.42 (m)		Leu9 γH	1.47 (m)	
Leu4 δH	0.91 (m)		Leu9 δH	0.87 (m)	
Leu4 δH′	0.91 (m)		Leu9 δH′	0.86 (m)	
^{d}Phe5 NH	8.91 (d, 3.1)	Leu4 αH (s)			
^{d}Phe5 αH	4.55 (m)	Pro6 δH (s) Pro6 δH′(m)	SAA NH	8.27 (t, 6.2)	Leu9 αH (s) Val2 αH (s)
^{d}Phe5 βH	3.08 (m)		SAA αH	4.29 (m)	
^{d}Phe5 βH′	2.96 (m)		SAA αH′	4.09 (d, 5.2)	
^{d}Phe5 H_{Ar}	7.24–7.34 (m)		SAA βH	3.79 (dd, 6.3, 15.9)	
Pro6 αH	4.36 (d, 7.5)		SAA γH	3.07 (m)	
Pro6 βH	2.00 (m)		SAA δH	2.38 (dd, 5.2, 13.1)	
Pro6 βH′	2.00 (m)		SAA δH′	1.54 (m)	
Pro6 γH	1.66 (m)		SAA εH	4.44 (dd, 5.4, 11.2)	
Pro6 γH′	1.66 (m)		SAA CH_2Ph	4.54 (d, 4.8)	
Pro6 δH	3.73 (m)		SAA H_{Ar}	7.24–7.34 (m)	
Pro6 δH′	2.49 (m)				

[a] Columns 1 and 2 contain the interpretation of the ^{1}H NMR spectroscopy. Column 1: proton notation. Column 2: chemical shift in ppm. Between brackets are the multiplicity (s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet) and the coupling constant in Hertz. Column 3: NOE cross peaks. Intensities are given in terms of weak (w), medium (m) or strong (s).

(CH_{Ar}), 74.30 (CH_2OBn), 72.64 (CH_2Ph), 70.67 (CHOH), 54.82 (CH_2N_3) ppm. $[\alpha]_D^{20}$ = +17.0 (*c* = 1.00, in MeOH)

(*S*)-1-Azido-3-(benzyloxy)propan-2-ol (21): Benzyl ether **21** was prepared according to the procedure described for **20** to yield the product (1.87 g, 9 mmol, 28%) as colorless oil. ^{1}H NMR (400 MHz, MeOD): δ = 7.38–7.32 (m, 5 H, H_{Ar}), 5.13 (br. s, 1 H, OH), 4.53 (s, 2 H, CH_2Ph), 3.83–3.70 (m, 1 H, C*H*OH), 3.61 (dd, *J* = 5.2, 10.2 Hz, 2 H, CH_2OBn), 3.53–3.35 (m, 2 H, CH_2N_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 137.38 (C_qPh), 128.27 (CH_{Ar}), 127.70 (CH_{Ar}), 127.61 (CH_{Ar}), 127.29 (CH_{Ar}), 126.75 (CH_{Ar}), 73.26 (CH_2OBn), 71.18 (CH_2Ph), 69.39 (CHOH), 53.25 (CH_2N_3) ppm. $[\alpha]_D^{20}$ = –12.6 (*c* = 1.00, in MeOH)

(*R*)-*tert*-Butyl 2-[1-Azido-3-(benzyloxy)propan-2-yloxy]acetate (22): Alcohol **20** (1.15 g, 5.55 mol) was dissolved in DMF (25 mL) at 0 °C under argon before addition of NaH (266 mg, 6.67 mmol, 1.2 equiv. 60% susp. mineral oil). After the gas evolution ceased, *tert*-butyl bromoacetate (1.6 mL, 11.1 mmol, 2 equiv.) was added and the reaction mixture was allowed to stir at room temp. until TLC analysis (10% EtOAc/PE) indicated the formation of a higher running spot. The reaction was quenched by the addition of MeOH (5 mL) and partitioned between Et_2O (50 mL) and H_2O (100 mL). The aqueous phase was washed with Et_2O (2 × 25 mL) and the combined organic phase with satd. aq. sodium hydrogen carbonate (25 mL) and brine (25 mL), dried (Na_2SO_4), filtered and concentrated. The residue was applied to a silica column and eluted with 0–3% EtOAc/PE to afford the title compound (1.14 g, 3.56 mmol, 64%) as colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.29 (m, 5 H, H_{Ar}), 4.54 (s, 2 H, CH_2Ph), 4.15 (s, 2 H, OCH_2C=O), 3.80–3.66 (m, 1 H, CC*H*O), 3.66–3.57 (m, 2 H, CH_2OBn), 3.52–3.44 (m, 2 H, CH_2Ph), 1.47 (s, 9 H, CH_3tBu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 169.50 (C=O), 137.78 (C_qPh), 128.41 (CH_{Ar}), 127.76 (CH_{Ar}), 127.68 (CH_{Ar}), 81.71 (C*C*HO), 78.25 (CH_2CHOCH_2), 73.53 (CH_2Ph), 69.87 (CH_2OBn), 68.24 (O*C*H_2C=O), 52.15 (CH_2N_3), 28.04 (CH_3tBu) ppm. $[\alpha]_D^{20}$ = +7.2 (MeOH). MS: *m*/*z* = 344.0 $[M + Na]^+$, 339.0 $[M + NH_4]^+$, 322.0 $[M + H]^+$, 288.1 $[M - tBu + Na]^+$, 296.1 $[M - N_2 + H]^+$, 266.0 $[M - tBu + H]^+$, 238 $[M - tBu - N_2 + H]^+$.

(*S*)-*tert*-Butyl 2-[1-Azido-3-(benzyloxy)propan-2-yloxy]acetate (23): *tert*-Butyl ester **23** was prepared in a similar fashion as described for **22** resulting in the isolation of the target compound (153 mg, 0.48 mmol, 40%) as colorless oil. ^{1}HNMR (200 MHz, $CDCl_3$): δ = 7.32 (m, 5 H, H_{Ar}), 4.53 (s, 2 H, CH_2Ph), 4.14 (s, 2 H, OCH_2C=O), 3.78–3.65 (m, 1 H, CC*H*O), 3.65–3.55 (m, 2 H, CH_2OBn), 3.50–

Table 3. ^{1}H NMR + NOE (500 MHz, CD_3OH) of compound **8**.

Atom 1	Chemical shift (m, *J* [Hz])	Atom 2	Atom 1	Chemical shift (m, *J* [Hz])	Atom 2
Val2 NH	7.54 (d, 8.1)	OC*H*$_2$C=O (w) SAA αH (w) Leu9 βH (m)	Val7 NH	7.64 (9.2)	^{d}Phe5 αH (m) Pro6 αH (m) 3.72 Pro6 (m)
Val2 αH	4.15 (t, 7.7)		Val7 αH	4.25 (t, 8.4)	
Val2 βH	2.14 (m)		Val7 βH	2.25 (m)	
Val2 γH	0.98 (d, 6.7)		Val7 γH	0.93 (m)	
Val2 γH′	0.98 (d, 6.7)		Val7 γH′	0.88 (m)	
Orn3 NH	8.70 (d, 9.5)	Val2 NH (w) Val2 αH (s) Val7 βH (m)	Orn8 NH	8.54 (d, 9.3)	Val7 NH (s) Val7 βH (m)
Orn3 αH	4.84 (m)		Orn8 αH	4.83 (m)	
Orn3 βH	2.02 (m)		Orn8 βH	2.90 (m)	
Orn3 βH′	2.02 (m)		Orn8 βH′	1.70 (m)	
Orn3 γH	1.78 (m)		Orn8 γH	1.59 (m)	
Orn3 γH′	1.68 (m)		Orn8 γH′	1.49 (m)	
Orn3 δH	3.02 (m)		Orn8 δH		
Orn3 δH′	2.91 (m)		Orn8 δH′		
Orn3 NH_2			Orn8 NH_2		
Leu4 NH	8.65 (d, 9.1)	SAA NH (w) Val7 NH (w) Orn8 αH (s)	Leu9 NH	8.72 (d, 9.5)	Val2 NH (w) Val2 αH (s) Val2 βH (s)
Leu4 αH	4.55 (m)		Leu9 αH	4.64 (m)	
Leu4 βH	1.58 (m)		Leu9 βH	1.54 (m)	
Leu4 βH′	1.58 (m)		Leu9 βH′	1.54 (m)	
Leu4 γH			Leu9 γH	1.42 (m)	
Leu4 δH	0.88 (m)		Leu9 δH	0.89 (m)	
Leu4 δH′	0.87 (m)		Leu9 δH′	0.88 (m)	
^{d}Phe5 NH	8.95 (d, 3.2)	Leu9 NH (w) Leu9 αH (s)	SAA NH	8.03 (t, 6.1)	Leu9 αH (s) Val2 βH (w)
^{d}Phe5 αH	4.49 (m)	3.72 Pro6 (m) 2.46 Pro6 (m)	SAA αH	3.63 (m)	
			SAA αH′	3.55 (m)	
^{d}Phe5 βH	3.07 (dd, 4.8, 12.6)		SAA βH	3.29 (m)	
^{d}Phe5 βH′	2.94 (m)		OC*H*$_2$C=O	3.91 (d, 14.7)	
^{d}Phe5 H_{Ar}	7.31–7.23 (m)		SAA CH_2Ph	4.54 (d, 2.8)	
Pro6 αH	4.36 (d, 7.9)		SAA H_{Ar}	7.32–7.23 (m)	
Pro6 βH	2.00 (m)				
Pro6 βH′	2.00 (m)				
Pro6 γH	1.67 (m)				
Pro6 γH′	1.56 (m)				
Pro6 δH	3.72 (t, 8.2)				
Pro6 δH′	2.46 (m)				

3.41 (m, 2 H, CH_2Ph), 1.46 (s, 9 H, CH_3tBu) ppm. ^{13}CNMR (50 MHz, $CDCl_3$): δ = 169.30 (C=O), 137.65 (C_qPh), 128.22 (CH_{Ar}), 127.55 (CH_{Ar}), 127.47 (CH_{Ar}), 81.43 (C*C*HO), 78.07 (CH_2CHOCH_2), 73.30 (CH_2Ph), 69.69 (CH_2OBn), 68.03 (OCH_2C=O), 51.94 (CH_2N_3), 27.86 (CH_3tBu) ppm. $[\alpha]_D^{20}$ = –9.0 (*c* = 1.00, in MeOH). MS: *m*/*z* = 344.1 [M + Na]$^+$, 321.9 [M + H]$^+$, 296.1 [M – N_2 + H]$^+$, 265.9 [M – *t*Bu + H]$^+$.

(*R*)-2-[1-Azido-3-(benzyloxy)propan-2-yloxy]acetic Acid (5): *tert*-Butyl ester **22** (1.04 g, 3.2 mmol) was dissolved a mixture of DCM/TFA/TIS (10 mL, 1:1:0.05) and stirred for 1 h before TLC analysis (EtOAc/AcOH, 99:1) indicated complete conversion of the starting material into a lower running spot. The reaction mixture was concentrated and repeatedly evaporated with toluene (3 × 10 mL) and partitioned between DCM (10 mL) and satd. aq. sodium hydrogen carbonate (10 mL). The aqueous phase was washed with DCM (2 × 5 mL), acidified to pH = 2 with 1 M HCl and washed with DCM (3 × 10 mL). The combined organic phase was dried (Na_2SO_4), filtered and concentrated to afford the title compound (611 mg, 2.3 mmol, 72%) as colorless oil. ^{1}H NMR (200 MHz, MeOD): δ = 7.32 (m, 5 H, H_{Ar}), 4.50 (s, 2 H, CH_2Ph), 4.23 (s, 2 H, OCH_2C=O), 3.79–3.65 (m, 1 H, CC*H*O), 3.59 (d, *J* = 2.9 Hz, 1 H, CH_2OBn), 3.57 (d, *J* = 3.1 Hz, 1 H, CH_2OBn), 3.41 (t, *J* = 5.0 Hz, 2 H, CH_2N_3) ppm. ^{13}C NMR (50 MHz, MeOD): δ = 173.83 (CO_2H), 139.29 (C_qPh), 129.38 (CH_{Ar}), 128.83 (CH_{Ar}), 79.71 (C*C*HO), 74.36 (CH_2CHOCH_2), 70.95 (CH_2Ph), 68.24 (CH_2OBn), 52.76 (CH_2N_3) ppm. $[\alpha]_D^{20}$ = +13.6 (*c* = 1.00, in MeOH). MS: *m*/*z* = 283.3 [M + Na]$^+$, 266.1 [M + H]$^+$, 238.1 [M – N_2 + H]$^+$.

(*S*)-2-[1-Azido-3-(benzyloxy)propan-2-yloxy]acetic Acid (6): Following the same procedure as described for **5**. (*S*)-*tert*-Butyl ester **23** (150 mg, 0.47 mmol) yielded the title compound (100 mg, 0.40 mmol, 86%) as colorless oil. ^{1}H NMR (200 MHz, MeOD): δ = 7.24 (m 5 H, H_{Ar}), 4.43 (s, 2 H, CH_2Ph), 4.16 (s, 2 H, OCH_2C=O), 3.75–3.59 (m, 1 H, CC*H*O), 3.53 (d, *J* = 2.9 Hz, 1 H, CH_2OBn), 3.50 (d, *J* = 3.1 Hz, 1 H, CH_2OBn), 3.34 (t, *J* = 4.9 Hz, 2 H, CH_2N_3) ppm. ^{13}C NMR (50 MHz, MeOD): δ = 173.81 (CO_2H), 139.27 (C_qPh), 129.36 (CH_{Ar}), 128.82 (CH_{Ar}), 79.68 (C*C*HO), 74.34 (CH_2CHOCH_2), 70.93 (CH_2Ph), 68.22 (CH_2OBn), 52.74 (CH_2N_3) ppm. $[\alpha]_D^{20}$ = –16.8 (*c* = 1.00, in MeOH). MS: *m*/*z* = 266.1 [M + H]$^+$, 238.1 [M – N_2 + H]$^+$.

Compound **7** was prepared according to the general procedure on a 200-μmol scale; yield 54.5 mg, 40 μmol, 20%. For the ^{1}H NMR and NOE data see Table 2. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 174.50, 174.13, 173.68, 173.60, 173.41, 173.32, 173.12, 172.69, 172.64, 139.14, 136.86, 130.36, 129.66, 129.39, 128.77, 128.72, 128.48, 86.43, 81.72, 79.26, 71.98, 61.94, 60.24, 59.83, 55.86, 53.33, 53.04, 51.74, 47.83, 42.57, 42.06, 40.66, 40.61, 38.45, 37.30, 32.25, 31.91, 30.62, 30.44, 25.82, 25.61, 24.38, 23.25, 23.04, 22.80, 19.87, 19.58, 19.31, 18.78 ppm. HRMS calcd. for [$C_{59}H_{91}N_{11}O_{11}$ + H]$^+$ 1130.68713; found 1130.69908.

Table 4. ^{1}H NMR + NOE (500 MHz, CD_3OH) of compound **9**.

Atom 1	Chemical shift (m, *J* [Hz])	Atom 2	Atom 1	Chemical shift (m, *J* [Hz])	Atom 2
Val2 NH	7.95 (d, 8.7)		Val7 NH	7.63 (d, 9.1)	Pro6 αH (w) Pro6 δH (m)
Val2 αH	4.23 (m)		Val7 αH	4.28 (m)	
Val2 βH	2.14 (m)		Val7 βH	2.26 (m)	
Val2 γH	0.99 (d, 6.7)		Val7 γH	0.90 (d, 6.7)	
Val2 γH′	0.97 (d, 6.8)		Val7 γH′	0.89 (d, 6.8)	
Orn3 NH	8.73 (d, 7.7)	Val2 αH (s)	Orn8 NH	8.58 (d, 9.2)	Val7 αH (s) Val7 βH (w)
Orn3 αH	4.80 (m)		Orn8 αH	4.85 (m)	
Orn3 βH	2.02 (m)		Orn8 βH	1.76 (m)	
Orn3 βH′	2.02 (m)		Orn8 βH′	1.76 (m)	
Orn3 γH	1.77 (m)		Orn8 γH	1.55 (m)	
Orn3 γH′	1.65 (m)		Orn8 γH′	1.44 (m)	
Orn3 δH	3.00 (m)		Orn8 δH	2.81 (m)	
Orn3 δH′	2.89 (m)		Orn8 δH′	2.73 (m)	
Orn3 NH_2	7.90 (br. s)		Orn8 NH_2	7.77 (br. s)	
Leu4 NH	8.72 (d, 9.3)	Val7 NH (w)	Leu9 NH	8.66 (d, 9.0)	Val2 NH (m) Orn8 αH (m)
Leu4 αH	4.65 (m)		Leu9 αH	4.95 (m)	
Leu4 βH	1.53 (m)		Leu9 βH	1.64 (m)	
Leu4 βH′	1.53 (m)		Leu9 βH′	1.64 (m)	
Leu4 γH	1.43 (m)		Leu9 γH	1.53 (m)	
Leu4 δH	0.89 (m)		Leu9 δH	0.87 (m)	
Leu4 δH′	0.89 (m)		Leu9 δH′	0.87 (m)	
^{d}Phe5 NH	8.94 (d, 3.2)	Leu4 αH (s)	SAA NH	7.93 (m)	Leu9 αH (s)
^{d}Phe5 αH	4.51 (m)	Pro6 δH (s)	SAA αH	3.93 (m)	
^{d}Phe5 βH	3.08 (dd, 4.8, 12.5)		SAA αH′	3.77 (m)	
^{d}Phe5 βH′	2.94 (m)		SAA βH	3.02 (m)	
^{d}Phe5 H_{Ar}	7.36–7.23 (m)		OCH_2C=O	3.56 (m)	
Pro6 αH	4.37 (d, 7.8)		SAA CH_2Ph	4.54 (s)	
Pro6 βH	2.00 (m)		SAA H_{Ar}	7.36–7.23 (m)	
Pro6 βH′	2.00 (m)				
Pro6 γH	1.65 (m)				
Pro6 γH′	1.57 (m)				
Pro6 δH	3.71 (m)				
Pro6 δH′	2.46 (m)				

Compound **8** was prepared according to the general procedure on a 200-μmol scale; yield 123 mg, 91 μmol, 46%. For the ^{1}H NMR and NOE data see Table 3. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 174.34, 173.54, 173.51, 173.40, 173.00, 172.97, 172.73, 172.67, 172.43, 162.86, 162.63, 139.23, 136.86, 130.56, 130.36, 129.64, 129.36, 128.79, 128.75, 128.44, 117.05, 80.69, 74.35, 71.41, 70.32, 61.90, 59.86, 59.45, 55.86, 55.77, 53.27, 53.25, 52.74, 51.69, 47.80, 47.24, 42.27, 42.02, 40.73, 40.52, 40.44, 37.27, 32.27, 30.57, 30.40, 29.81, 27.27, 25.84, 25.65, 25.04, 24.63, 24.35, 23.44, 23.16, 23.08, 22.25, 19.81, 19.56, 19.02, 18.86 ppm. HRMS calcd. for $[C_{58}H_{91}N_{11}O_{11} + H]^+$ 1118.69723; found 1118.69896.

Compound **9** was prepared according to the general procedure on a 200-μmol scale; yield 56.1 mg, 41.7 μmol, 21%. For the ^{1}H NMR and NOE data see Table 4. ^{13}C NMR (151 MHz, CDCl3): δ = 174.32, 173.60, 173.54, 173.38, 173.05, 172.93, 172.68, 172.52, 172.11, 138.79, 136.85, 130.36, 129.66, 129.60, 129.08, 129.00, 128.48, 78.77, 74.17, 71.30, 69.89, 69.77, 61.92, 59.72, 59.67, 55.85, 53.52, 53.34, 52.83, 51.66, 49.85, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 47.83, 47.83, 42.03, 41.60, 40.68, 40.59, 32.51, 32.30, 30.61, 29.82, 25.85, 25.60, 24.36, 23.29, 23.23, 23.01, 22.35, 19.80, 19.50, 18.94, 18.79 ppm. HRMS calcd. for $[C_{58}H_{91}N_{11}O_{11} + H]^+$ 1118.69723; found 1118.69870.

Acknowledgments

We are very grateful for generous financial support by TNO Defense, Security and Safety, Rijswijk, the Netherlands. We thank Hans van der Elst for assistance with the LCMS analyses and HPLC purifications and Kees Erkelens and Fons Lefeber for assistance with the recording of NMR spectra.

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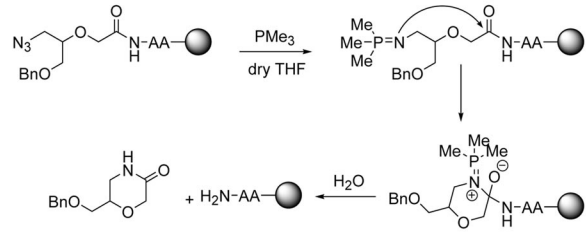

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Received: April 28, 2009
Published Online: July 21, 2009

