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Scaling the amphiphilic character and antimicrobial activity of Gramicidin S by dihydroxylation or ketal formation

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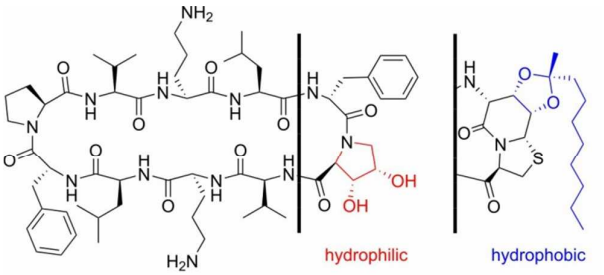


Gramicidin S analogs by dihydroxylation and ketal formation

Abstract: The acid lability of aliphatic ketals, which often serve as protection groups for 1,2-diols, is influenced by their local structural environment. The acetonide of the protected amino acid *cis*-dihydroxyproline (Dyp) is a typical protecting group cleavable by traces of TFA. The tricyclic acetonide of the dipeptide D-Hot=Tap is resistant to TFA and thus can serve as a bioorthogonal modification of bioactive peptides. With the aim of improving antimicrobial activity and hemolytic properties, we use these reactivity differences to scale the membrane affinity of the decapeptide Gramicidin S *cyclo*(D-Phe-Pro-Val-Orn-Leu-)$_2$ (**GS**). The *cis*-dihydroxylated amino acids are used to increase the polarity of GS or obversely decrease the polarity by stereoselective ketal formation with an aliphatic ketone. While Dyp (GS mimetic **15**) has only minimal influence on the biological properties of **GS**, D-Hot=Tap at the position of D-Phe1-Pro2 eradicates the biological activity (GS mimetic **16**). The acid-stable ketals **17**-**19** are bioorthogonal modifications which reconstitute the biological activity

of **GS**. We describe an improved synthesis of orthogonally protected Fmoc-Dyp-acetonide (**9**) and of several Fmoc-D-Hot=Tap-ketals for solid-phase peptide synthesis.

Introduction: Gramicidin S (**GS**) is a potent antimicrobial peptide which was first isolated from *Aneurinibacillus migulanus* (formerly *Bacillus brevis*) in the 1940s by GAUSE and BRAZHNIKOVA.[1,2] The cyclic decapeptide with the primary structure *cyclo*(D-Phe-Pro-Val-Orn-Leu-)$_2$ assumes a C_2-symmetric antiparallel β-sheet with two hydrogen bonded pairs of amino acids (Val/Leu) flanking the two central Orn residues which are not involved in hydrogen bonds.[3] Two D-Phe-Pro II´ β-turns cap this secondary structure on both sides.[4] Its amphiphilic character with the aliphatic Val und Leu sidechains oriented to one side and the cationic Orn sidechains to the opposite direction forms the basis for the antimicrobial activity of **GS** towards a wide range of Gram-positive and Gram-negative bacteria by interacting with the lipid bilayer, lowering its integrity, ultimately causing cell death.[5-8] Bacterial resistance develops slower against antibiotics targeting the cell membrane than against other modes of action but the unwanted hemolytic activity restricts the use of **GS** beyond topical applications.[9,10] In spite of the many efforts and numerous GS analogs which were synthesized in the past 40 years, it was not possible to separate and suppress this unspecific membrane activity in a structure-based approach. Several investigations report ring contractions, ring expansions, mutations in the β-sheet and in the β-turn region, natural or non-natural amino acids and their influence on the antimicrobial and hemolytic activity of **GS**.[10-20] Here, we describe a novel approach to the problem of unspecific membrane affinity of GS: We systematically scale its membrane affinity by linking the peptide with different types of membrane anchors in a modular way. We introduce Dyp at the position of Pro as smallest possible dihydroxylation of **GS**. Dyp only minimally alters the bioactivity of **GS** and its cis-diol group is a useful bioorthogonal linker for the subsequent ligation to boronic esters or benzoboroxoles in aqueous solution by dynamic covalent chemistry.[21] A further mutation of **GS** described here is the dihydroxylated dipeptide D-Hot=Tap (D-Hydroxythreonine=Thiaproline with the "=" representing the two covalent ring connections) which allows the attachment of a number of different functionalities because it can form an exceptionally stable ketal. This manuscript focuses on alkyl chains of varying length to keep the structure activity relationship as simple as possible. The unique properties of the bicyclic β-turn mimic D-Hot=Tap were described previously by our group.[22] The D,L-dipeptide has its diol function at the former position of the D-Phe residue and is, in contrast to Dyp, able to

form ketals that are remarkably stable under physiological conditions. This offers a novel way of introducing functionality to D-Hot=Tap modified GS derivatives used as templates (Figure 1) and gives access to several unique GS analogs modified with alkyl ketones. They show different biological profiles, encompassing antimicrobial and hemolytic activity, by simple manipulation of polarity and amphiphilic character.

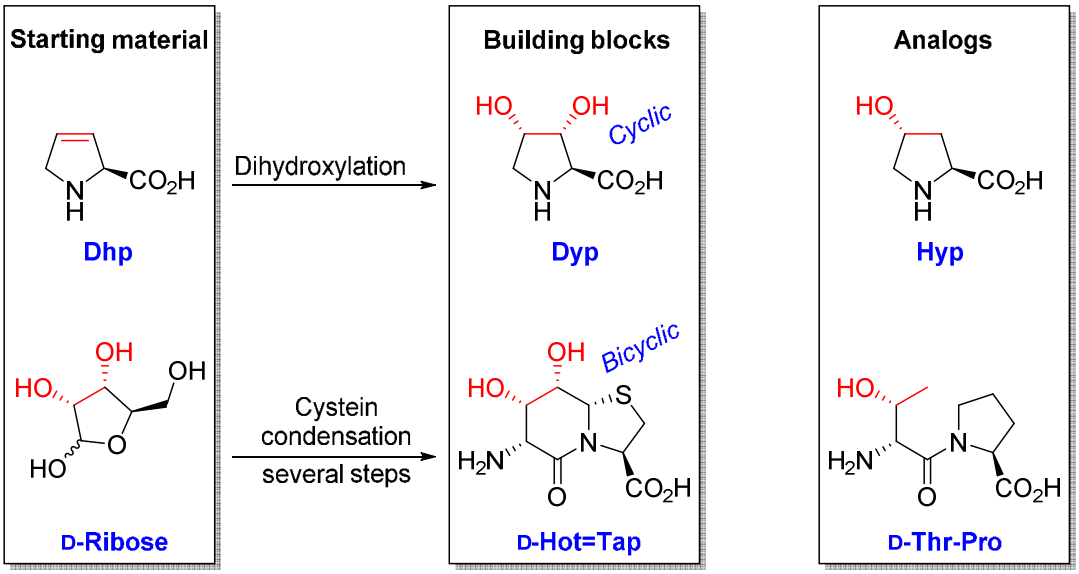
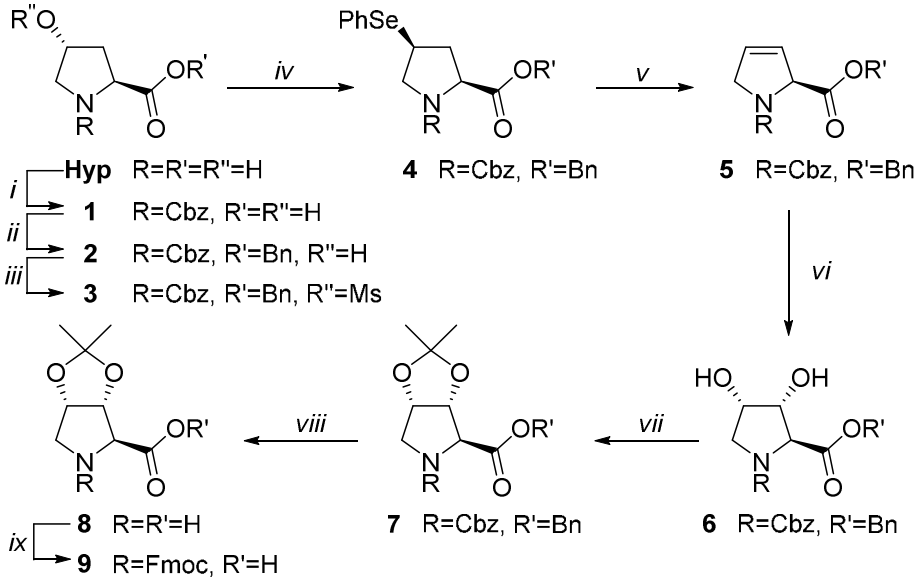


Figure 1. Two different types of dihydroxylation are shown here. *Cis*-dihydroxylation of dehydroproline (Dhp) yields dihydroxyproline (Dyp). The bicyclic D-Hot=Tap is obtained in several steps from the pentose D-ribose. Hydroxyproline (Hyp) and the dipeptide D-Thr-Pro are shown as monohydroxylated structural analogs of Dyp and D-Hot=Tap, respectively.

Results and Discussion: The synthesis of Fmoc-Dyp-acetonide was performed according to literature procedures and optimized as shown in Scheme 1.[23-25] Commercially available Hyp was protected and activated in four steps to **4**, followed by regioselective elimination to the protected Dhp derivative **5**.

Scheme 1. Dyp-building block synthesis[a]

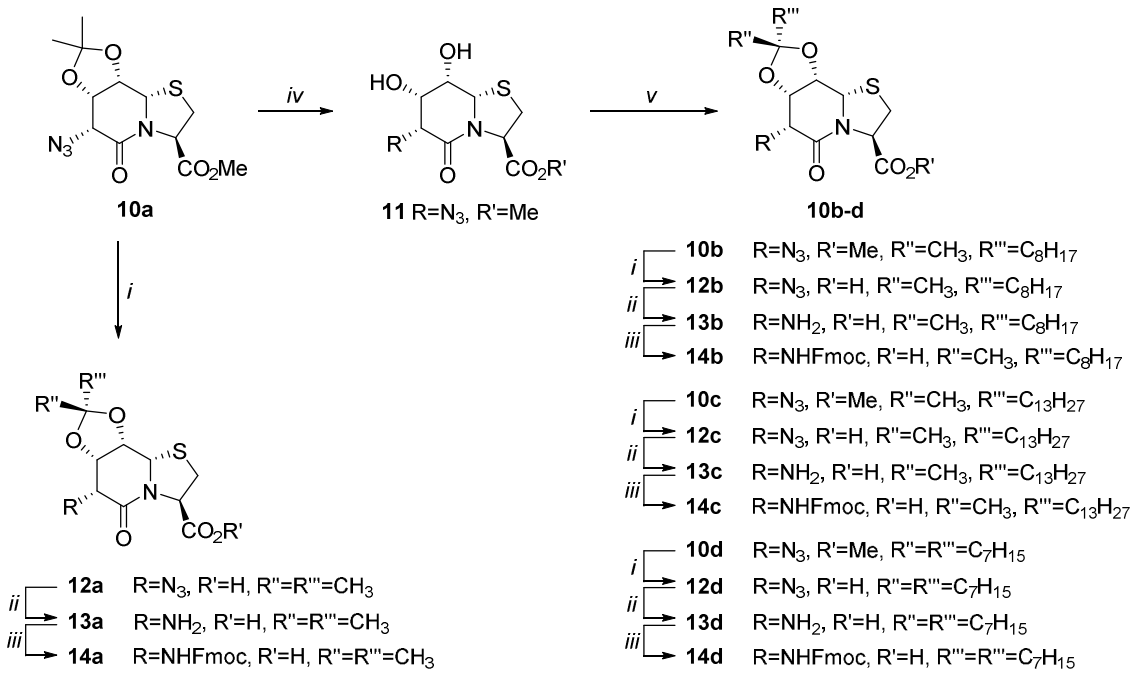


[a] Reagents and conditions: (i) CbzCl, 1 N NaOH/THF 1:1, 16 h, RT; (ii) BnBr, K_2CO_3, NaI, DMF, 16 h, RT; (iii) MsCl, NEt_3, DMAP, DCM, 2 h, RT; (iv) Ph_2Se_2, $NaBH_4$, *tert*-butanol, 3 h, 80 °C; (v) H_2O_2, pyridine, DCM, 5 h, RT; (vi) K_2OsO_4•$2H_2O$, NMO•H_2O, *tert*-butanol/H_2O 7:3, 21 h, RT; (vii) PTSA, 2,2-dimethoxypropane, 4 h, RT; (viii) H_2 (10 bar), Pd/C, EtOAc, RT; (ix) FmocCl, 10% Na_2CO_{3aq}/1,4-dioxane 1:1, 16 h, RT.

Diastereoselective dihydroxylation and reprotection yielded Fmoc-Dyp-acetonide (**9**) in an overall yield of 30% over nine steps which could be directly used in solid-phase peptide synthesis as described below.

Ketals are expected to easily hydrolyze under acidic conditions, but the tricyclic ring system of the O7,O8-acetonide-protected N_3-D-Hot=Tap-OMe **10a** is surprisingly stable against acids, necessitating an alternative protecting group for the synthesis of oligomeric D-Hot=Tap peptides.[22] In the present approach, this acid stability was made a virtue of necessity to install alkyl chains of different lengths and topology as shown in Scheme 2. The dipeptide precursor **10a** was first deprotected by recurrent treatment with pure TFA to obtain diol **11** which was reketalized with 2-decanone, 2-pentadecanone, and 8-pentadecanone to obtain the ketals **10b-d**, respectively. Surprisingly, the formations of ketals **10b** and **10c** yielded a single diastereomer. These three azides were transformed to Fmoc-protected dipeptide building blocks **14b-d**, suitable for SPPS, in a sequence of reduction, saponification and acylation with FmocCl. The dimethylketal **10a** was transformed into the Fmoc-protected dipeptide **14a** using the same synthetic transformations.

Scheme 2. D-Hot=Tap-building block synthesis[a]



[a]Reagents and conditions; (i) Me_3SnOH, DCE, 80 °C; (ii) H_2 (1 bar), Pd/C, MeOH, RT; (iii) Fmoc-OSu, DIPEA, 1,4-dioxane/H_2O 4:1; (iv) 95% TFA_{aq}; (v) dimethylketal, CSA.

The configuration of the quaternary carbon of the asymmetric ketals **10b** and **10c** was determined by NMR spectroscopy. The crystal structure of **10a** was used as a template for the configurational assignment (Figure 2). The pro*R* methyl group of **10a** is close to the ring protons H7 and H8 of the valerolactam, while the pro*S* methyl group is closer to H3 on the other ring face. ROE (rotating frame NOE) contacts differentiate the diastereotopic methyl groups in solution. The low-field methyl shows ROE contacts to H7 and H8 while the methyl group at higher field shows a ROE contact to H3. The rigid 5,6,5-tricyclic ring system of all D-Hot=Tap ketals shows this ROE pattern. The stereocenter at the ketals **10b** and **10c** is *S*-configurated in both cases.

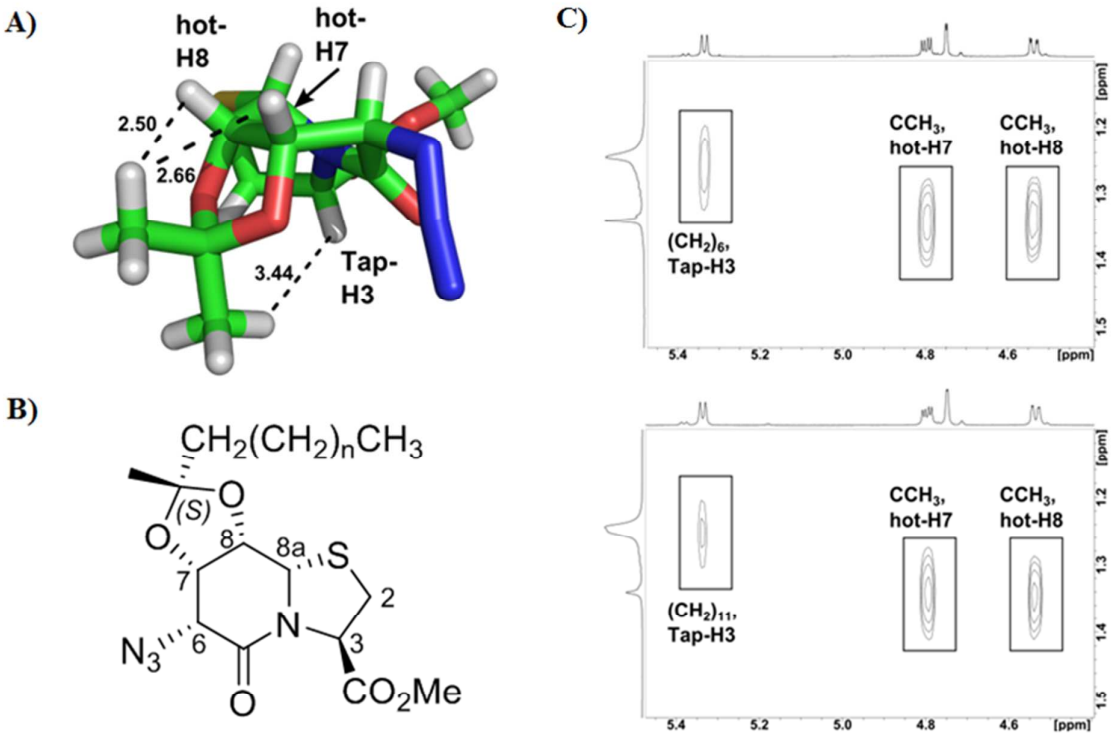
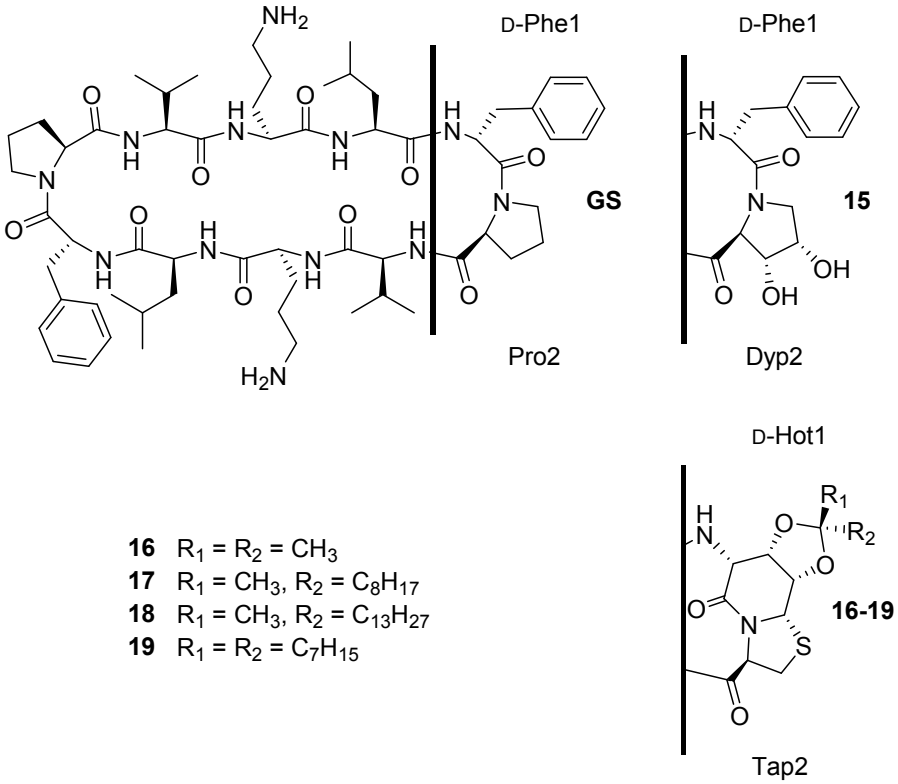


Figure 2: Determination of the configuration at the ketal stereocentre of **10b** and **10c**. (A) Crystal structure of **10a**. The distances important for the NMR-based analysis of asymmetric ketals are highlighted. (B) Stereoconfiguration and atom numbering of **10b** and **10c**. (C) Expansion from the ROESY spectrum (500 MHz, 300 K, $CDCl_3$) of **10b** (upper right) and **10c** (lower right). The methyl group shows ROE contacts to H7 and H8 while the methylene groups are close to H3.

The synthesis of the linear precursors of the GS analogs was performed by standard peptide chemistry on 2-CTC-resin with Fmoc-protected building blocks. The resin was treated with 1% TFA in DCM to cleave the peptide from the resin without fragmentation of the Boc-protecting group on the side chain of Orn. The crude peptide was cyclized using HATU/HOAt and DIPEA in DMF under high dilution conditions to favor the intramolecular condensation. The Boc protecting group on the side chain of Orn was removed with TFA/DCM (1:6) and the final purification was done by semi-preparative RP-HPLC to obtain the GS analogs **15-19** (Figure 3).



16 $R_1 = R_2 = CH_3$
17 $R_1 = CH_3$, $R_2 = C_8H_{17}$
18 $R_1 = CH_3$, $R_2 = C_{13}H_{27}$
19 $R_1 = R_2 = C_7H_{15}$

Figure 3: The parent peptide Gramicidin S (**GS**) and the five analogs containing the dihydroxylated amino acid Dyp (**15**) or the turn mimetic D-Hot=Tap (**16-19**).

The complete homo- and heteronuclear assignment of **15** was achieved by TOCSY, HSQC, HMBC, and ROESY spectra (600 MHz, MeOH-*d*3, 300K). The ^{1}H NMR spectrum of **15** shows a decapeptide signal set in the ^{1}H NMR spectrum caused by the loss of C_2-symmetry. The NH resonances and all other signals of the two Val-Orn-Leu-D-Phe tetrapeptide sequences appear as pairs of signals with only minor chemical shift differences. The close to identical coupling constants and NOE pattern of both molecular halves yield twice as much spectroscopic information for **15** compared to the parent peptide **GS** and confirmed that the dihydroxylation has a negligible influence on the overall structure. Because the NH temperature coefficients and the $^3J_{NH\text{-}H\alpha}$ coupling constants of the two pentapeptide moieties of **15** were unmeasurably different in the ^{1}H NMR, we discuss only one value per two amino acids in the following. The temperature coefficients of the NH protons of Val, Orn, Leu and D-Phe were -3.1, -7.5, -3.1 and -8.9 ppb K^{-1}. The small temperature dependence of Val and Leu confirm the expected intramolecular hydrogen-bonded pair Leu and Val as described for **GS**.[16] The $^3J_{NH\text{-}\alpha CH}$ coupling constants for Val, Orn Leu and D-Phe were 9.2, 9.5, 9.5 and 3.6 Hz, respectively. The large values occurring for the extended β-sheet structure and the small values occurring for D-Phe at the i+1 position of the β-turn. Comparison with

temperature coefficients and coupling constants of wild type **GS** indicated a similar structure. Only the puckering of the pyrrolidine ring of Dyp differs to natural **GS**. In contrast to the pyrrolidine ring of Pro, which prefers the Cγ-*endo*-pucker, the Cγ-*exo*-pucker is favored by Hydroxy- and Dihydroxyproline in the single amino acid or dipeptides.[26] Interestingly, conformational analysis of the Dyp in **15** showed a C_β-*exo*-pucker. The coupling constant $^3J_{\alpha CH\text{-}\beta CH}$ of < 1 Hz indicates a dihedral angle of about 90°. $^3J_{\gamma CH\text{-}\delta CH}$ of 7.9 and 8.3 Hz suggests a dihedral angle of approximately 30° and 150° between the γ- and the diastereotopic δ-protons. This leads to C_β-*exo*-pucker as single possible ring conformation (Figure 4).

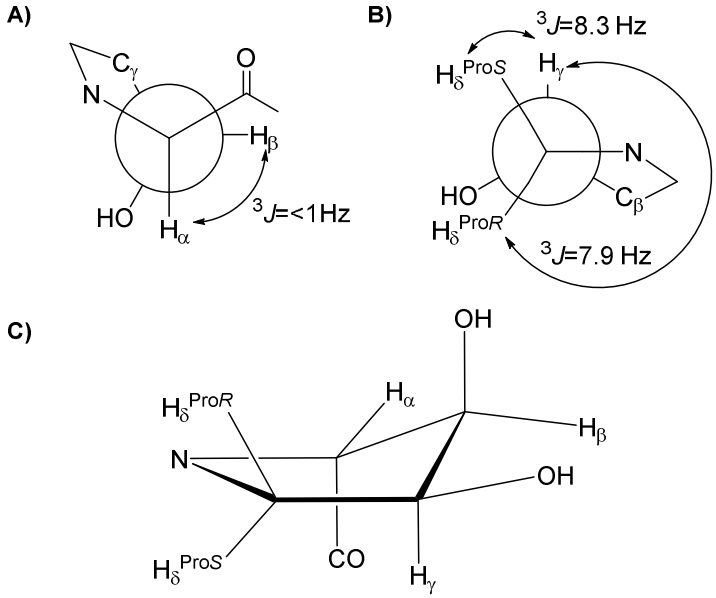


Figure 4. (A) The preferred rotamer along αC-βC bond explains the small coupling constant of < 1 Hz. (B) Rotamer along γC-δC axis, with coupling constant of 7.9 and 8.3 Hz. (C) Proposed Cβ-*exo* ring conformation of the pyrrolidine ring of Dyp.

In contrast to **15**, conformational analysis of the D-Hot=Tap modified GS analog **16** indicated greater influence of substituting the β-turn on the overall structure. The coupling constants of Val (10.0 and 7.9 Hz), Orn (9.0 and 9.6 Hz) and Leu (7.5 and 8.2 Hz) differ slightly from wild type **GS**, but indicate a similar extended β-sheet structure. Nevertheless, temperature coefficients partly differ significantly from wild type **GS**. The biggest difference showed Val3, Orn9 and Leu10 with values of -8.5, -2.0 and -7.0 ppb K^{-1}, in comparison to wild type **GS** with values of -2.5, -7.3 and -2.9 ppb K^{-1}. This is caused by a higher rigidity of the bicyclic ring system of the D-Hot=Tap building block in **16**, which results in a different twist of the β-sheet and a consequential change of solvent exposure of the amino acids as consequence (Figure 5).

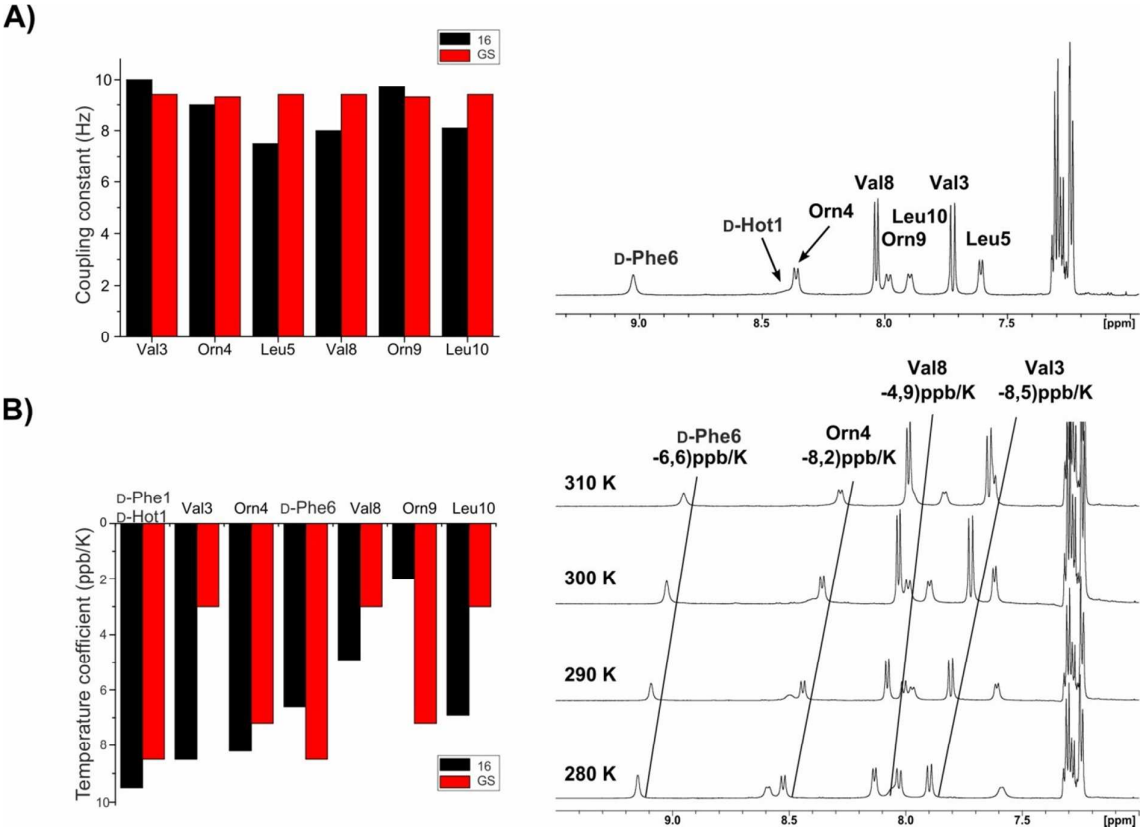


Figure 5: Structural relevant data from GS analog **16** compared to wild type **GS**. (A) J_{NH}-α_{CH} coupling constants of β-sheet amide protons of **16** and wild type **GS** and the corresponding expansion from ^{1}H NMR spectrum (600 MHz, 300 K, MeOH-*d*3) of **16**. (B) Temperature dependence of amide protons in **16** and wild type **GS** as well as the corresponding expansion from the ^{1}H NMR spectrum of **16** in the temperature range between 280 and 310K.

CD studies of compound **15-19** were carried out in methanol from 195 nm to 260 nm to investigate the relationship between structure and activity. As we expected, CD spectra of Dyp modified GS analog **15** showed an identical behavior to wild type **GS**. CD spectra of compound **16-19** showed, beside significantly lower intensities, a trough around 214 nm, similar to wild type **GS**. Nevertheless, the minimum at 206 nm is not as distinct in the spectra of **16-19**. It was reported, that the origin of the through at 206 nm was the II´ β-turn and β-sheet structure of **GS**.[27,28] Therefore, these findings can be explained with the substitution of the whole β-turn with the D-Hot=Tap building block in **16-19**.[29] This is consistent with the NMR data and our proposed twisted β-sheet structure of compounds **16-19** (Figure 6).

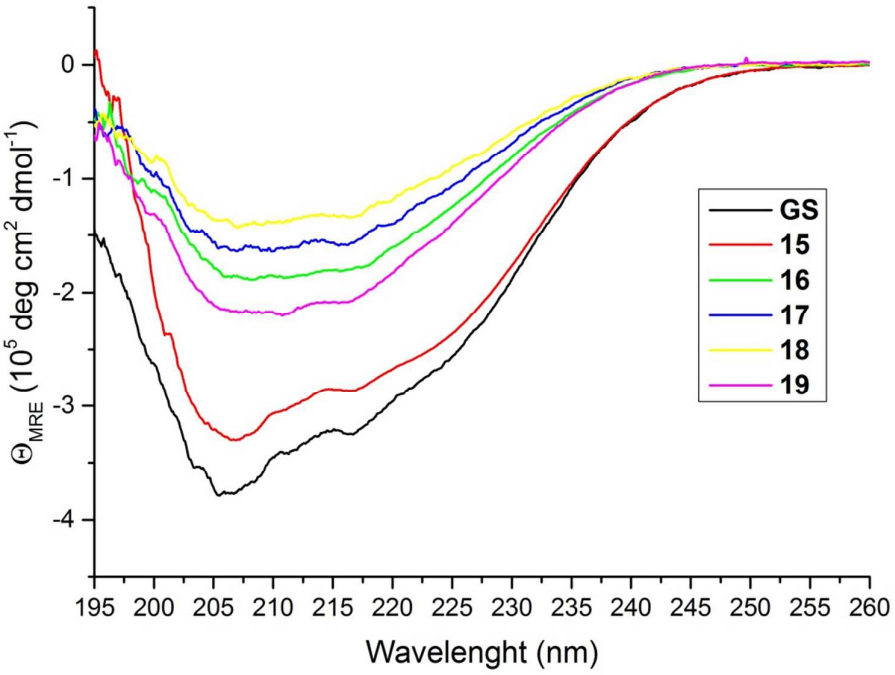


Figure 6: CD spectra of GS analogs **15** (red), **16** (green), **17** (blue), **18** (yellow), **19** (purple) and wild type **GS** (black) in methanol from 195 nm to 260 nm at 27°C.

All peptides were tested against several Gram-positive and -negative bacteria (minimum inhibitory concentration) and investigated on their hemolytic activity (HC_{90}) as described earlier.[8,30] The antimicrobial activity of the Dyp modified GS analog **15** is slightly lower. Nevertheless the selectivity index (HC_{90}/MIC) is higher for most bacteria tested. This may be explained by the increased polarity of the two additional hydroxy groups and the altered amphiphilic character of the GS derivative, both properties essential for the antimicrobial activity.[5] Interestingly, the dimethylketal modified GS analog **16** was inactive and has no hemolytic activity. Enhanced polarity and decreased amphiphilic character, as well as polar residues at the D-Phe position are supposed to cause these findings.[31,32] GS analog **18** modified with a C13-alkyl chain, as well as analog **19** modified with two C7-alkyl-chains restored the activity and showed a drastic increase of hemolytic activity and a lower activity against gram-positive bacteria tested, as well as no activity against the Gram-negative bacteria *E. coli*. For **18**, this can be explained with the length of the C13 alkyl chain, which enhanced the hemolytic activity, lowering the selectivity index. The two shortened alkyl chains in **19** have almost the same effect caused by the orientation of one alkyl chain to the polar residues, lowering the amphiphilic character. Nevertheless, GS analog **17**, modified with a C8-alkyl chain, showed comparable activities against all Gram-positive bacteria, as well as slightly

lowered activity against all Gram-negative bacteria. Even hemolytic activity was significantly lowered yielding an improved selectivity index compared to **GS**. The antimicrobial and hemolytic activity of all compounds is summarized in Table 1.

Table 1: Minimum inhibitory concentration [µg/mL] with parenthesized selectivity index (HC_{90}/MIC) against several bacteria and concentration causing 90% hemolysis (HC_{90}, µg/mL) of **GS** and analogs **15-19**.

	A[a]	B[a]	C[b]	D[b]	E[b]	HC_{90}
GS	32 (0.38)	64 (0.19)	4 (3.00)	2 (6.00)	8 (1.50)	12
15	64 (1.39)	128 (0.66)	4 (21.25)	16 (5.31)	32 (2.66)	85
16	>256 (0.78)	>256 (0.78)	256 (0.78)	128 (1.56)	>256 (0.78)	>200
17	64 (0.59)	128 (0.30)	4 (9.50)	4 (9.50)	8 (4.75)	38
18	>256 (0.02)	64 (0.09)	16 (0.38)	8 (0.75)	16 (0.38)	6
19	>256 (0.02)	128 (0.04)	8 (0.63)	8 (0.63)	8 (0.63)	5

[a] Gram-negative bacteria. [b] Gram-positive bacteria. A: *E. coli*, B: *P. aeruginosa*, C: *S. aureus*, D: *S. epidermidis*, E: *E. faecalis.*

Conclusion: The synthesis of precursors of dihydroxylated amino acids and their incorporation in the macrocyclic peptide **GS** are presented here. The dipeptide D-Hot=Tap offered the opportunity to incorporate one or two aliphatic chains by the formation of a ketal of extraordinary stability against acidolysis. We observed that the dihydroxylation leads to an enhanced biological profile of GS derivative **15** with slightly decreased antimicrobial activity and concurrent significant decrease of hemolytic activity, yielding an increasedselectivity index. We described a straightforward way to manipulate the polarity and amphiphilic character of **GS** analogs by formation of different stable ketals via D-Hot=Tap modification, restoring as well as enhancing the selectivity index of those GS analogs. Dihydroxylation of **GS** created the opportunity to synthesize different analogs with increased biological profiles by ketalisation with various aliphatic ketones of different alkyl chain lengths and topology.

Experimental Section:

General information. Materials and reagents were of commercially available grade and used without additional purification. Compound **10a** was synthesized according to our former publication.[22] Solvents were used after distillation. Column chromatography was performed by using *Merck* silica gel (0.040-0.063 mm 230-400 *mesh*) TLC was performed on Merck silica gel 60 F_{254} aluminum plates with visualization with UV, ninhydrin or permanganate. ^{1}H, ^{13}C, TOCSY, COSY, HSQC, HMBC and ROESY spectra were recorded on Bruker AV II 300, Bruker DRX-500, Bruker AV III 500 or Bruker AV II 600. Chemical shifts are reported in ppm using the signal of solvent (DMSO-d_6: ^{1}H: 2.50 ppm, ^{13}C: 39.52 ppm; $CDCl_3$: ^{1}H: 7.26 ppm, ^{13}C: 77.16; MeOH-d_3: ^{1}H: 3.31 ppm, ^{13}C: 49.00 ppm) as reference. Diastereotopic

groups are differentiated by l (low field) and h (high field) signal. HSQC spectra were recorded from 0 to 100 ppm in the ^{13}C dimension. Mass spectra (ESI+) were acquired on a *Thermo Fisher Scientific LTQ-FT* with an ion trap detector. Analytical HPLC was performed on a *Thermo Scientific Dionex UltiMate 3000* system with a *ACE UltraCore 2.5 SuperC18* column (150 x 2.1 mm, 2.5 µm, 95 Å) with an diode array detector. As eluent with a flow rate of 0.45 mL/min were used the following solvents: H_2O + 0.1% TFA, B: ACN + 0.085% TFA. Semi preparative HPLC was performed on a *Thermo Scientific Dionex UltiMate 3000* with a *Macherey-Nagel VP Nucleodur C18 Gravity* column with a flow rate of 15.0 mL/min or an *ACE 5 SuperC18* column with a flow rate of 7.0 mL/min. CD spectra were acquired on a *JASCO J-810* with an *HELMA 110-QS* quartz cuvette (path length 0.1 cm), a scanning rate of 10 nm/min, data pitch of 0.5 response of 2 s and a temperature of 300K

Resin loading: 2-Chlortritylchlorid resin (1.42 mmol/g, 200-400 mesh) was loaded by adding Fmoc-Leu-OH (1.0 eq) and DIPEA (6.0 eq) in DMF and stirring for 1 h. After washing three times with DMF, the resin was treated twice with DCM/MeOH/DIPEA (80:15:5) for respectively 30 min. The resin was washed three times with DMF, MeOH and DCM. The loading was determined by UV-Vis spectroscopy at 289 and 300 nm after cleavage of protecting group with 20% piperidine in DMF for 20 min using the following equation.

$$B\left[\frac{\text{mmol}}{\text{g}}\right] = \frac{E \cdot V[\text{mL}]}{\varepsilon\left[\frac{\text{mL}}{\text{mmol} \cdot \text{cm}}\right] \cdot d[\text{cm}] \cdot m_{resin}[\text{g}]}$$

B = Resin loading, E = Extinction, V = Total volume, ε = extinctioncoefficient, d = layer thickness, m_{resin} = resin mass.

Manual peptide synthesis: The manual peptide synthesis was performed using the following protocol.

1) Stirring in DMF for 20 min
2) 2 x cleavage of the Fmoc protection group with 25% piperidine in DMF for 10 min
3) 3 x washing with DMF, MeOH and DCM
4) Coupling with Fmoc-AA-OH (3.0 eq), HOBt (3.0 eq), HBTU (3.0 eq) and DIPEA (8.0 eq) in DMF for 60 min
5) 3 x washing with DMF, MeOH and DCM

Steps 2-5 were performed as long as the peptide were fully assembled

Automated peptide synthesis: Peptides were synthesized by a microwave assisted peptide synthesizer (*Liberty Blue, CEM*) using the following cycles of deprotection and coupling.

1) Fmoc deprotection: T = 50 °C, $P_{microwave}$ = 30 W, t = 210 s with 20% piperidine in DMF, 3.00 mL/deprotection
2) AA coupling: T = 50 °C, $P_{microwave}$ = 30 W, t = 600 s with Fmoc-AA-OH (0.2 M in DMF, 5.0 eq, 2.5 mL/coupling), DIC (0.5 M in DMF, 5.00 eq, 1.0 mL/coupling) and Oxyma (1.0 M in DMF, 5.0 eq, 0.5 mL/coupling)

Resin cleavage: Resin cleavage was performed with 1% TFA in DCM to keep the sidechain protecting groups intact. Coevaporation with toluene and lyophilisation from H2O/MeCN

(4:1) afforded the sidechain protected, linear peptides as white solid and was used without further purification.

Peptide cyclisation: To a solution of HATU (3.0 eq) and HOAt (3.0 eq) in DMF (90 mL) was added DIPEA (15.0 eq). After cooling to 0 °C the linear peptide dissolved in DMF (10 mL) was added dropwise over 1 h. After stirring overnight at RT, the solvent was removed under reduced pressure.

Final deprotection: The cyclized peptide was in dissolved in DCM (6 mL) and TFA (1 mL) was added. After stirring 1 h at RT, the volume was halved by removing the solvent under reduced pressure. Toluene (10 mL) was added and the solvent removed under reduced pressure. Purification by semi-preparative RP-HPLC and lyophilisation gave the GS analogs as white solid (Purity >95% in all cases).

Biological Studies: Minimum inhibitory concentration was tested by double dilution of studied peptides in the Mueller-Hinton broth as described earlier.[8] Exponentially growing control strains of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228 and *Enterococcus faecalis* ATCC 29212 were used for inoculation of the 96-well microtiter plates to reach $5x10^5$ colony forming units/mL. After incubation of plates for 22 h at 37°C bacterial growth was examined as respiration activity, when the redox indicator resazurin (20 µL of an aqueous 80 µM solution per well) was added and incubated for another 2 h at 37 °C. Positive values of the differences in the absorbance of resorufin at 570 nm and resazurin at 600 nm indicated bacterial growth and allowed the determination of the lowest peptide concentrations that inhibit bacterial growth. All results were obtained from two independent experiments, and each was performed in triplicate.

The hemolytic activity of all peptides was investigated using human erythrocytes as described earlier.[30] Human red blood cells in citrate-phosphate-dextrose buffer was obtained from the blood transfusion department of the municipal hospital in Karlsruhe, washed twice in isotonic Tris-HCl wash-buffer (pH 7.6, RT), and then diluted to 0.5 % of haematocrit in Tris-HCl reaction-buffer (pH 7.6, 37 °C). Studied peptides were diluted 1:1 in Tris-HCl reaction-buffer to an end volume of 200 µL. Then the 200 µL of erythrocyte suspension were added to start the haemolytic reaction (37 °C, paused agitation, 30 minutes). After centrifugation, the haemoglobin concentration in supernatant was characterized in a spectrophotometer at 540 nm. The haemolytic concentrations (HC_{90}) were calculated as the percentage haemolysis compared to 100% induced by 0.1% Triton X-100 in two independent experiments.

(2*S*,4*R*)-1-((Benzyloxy)carbonyl)-4-hydroxypyrrolidine-2-carboxylic acid (1)[33]

Hydroxyproline (6.56 g, 50.0 mmol, 1.0 eq) was dissolved in 1 n NaOH (50 mL) and THF (50 mL). Then a solution of Cbz-Cl (8.92 mL, 62.5 mmol, 1.25 eq) in 1 n NaOH (50 mL) and THF (50 mL) was added dropwise at 0 °C about 1.5 h). The mixture was stirred overnight at RT. After dilution with H_2O (50 mL), the solution was washed with EtOAc (3 x 125 mL). 2 n HCl was added to the aqueous layer until pH = 3. The aqueous layer was extracted with EtOAc (4 x 125 mL). The combined organic layers were washed with brine (100 mL), dried over $MgSO_4$. The solvent was removed under reduced pressure **1** was obtained as white foam (12.2 g, 46.0 mmol, 92%). ^{1}H NMR (300 MHz, 300 K, DMSO-d_6): δ 7.37-7.32 (m, 5H, H_{arom}), 5.13-5.04 (m, 3H, OH, $PhCH_2O$), 4.31-4.18 (m, 2H, Hα, Hγ), 3.50-3.36 (m, 2H, Hδ), 2.23-2.11 (m, 1H, Hβ), 2.00-1.87 (m, 1H, Hβ); ^{13}C NMR (75 MHz, 300 K, DMSO-d_6): δ 173.9, 173.5 (CO_2H), 154.2, 153.9 (NCO_2), 136.8, 136.8, 128.4, 128.2, 127.8, 127.6, 127.4, 127.0

($C_{arom.}$), 68.5, 67.7 (Cγ), 66.0 (Ph*C*H_2O-), 59.0, 57.4 (Cα), 55.0, 54.5 (Cδ), 37.9 (Cβ). HRMS (ESI-ICR) m/z: [M + Na]+ Calcd for $C_{13}H_{15}NO_5Na$ 288.0842; Found 288.0844.

Dibenzyl (2*S*,4*R*)-4-hydroxypyrrolidine-1,2-dicarboxylate (2)[34]

1 (12.2 g, 46.0 mmol, 1.0 eq) was dissolved in DMF (90 mL) and K_2CO_3 (12.7 g, 92.0 mmol, 2.0 eq), NaI (689 mg, 4.60 mmol, 0.1 eq) and BnBr (16.4 mL, 138 mmol, 3.0 eq) were added. The solution was stirred at RT for 16 h. After dilution with H_2O (250 mL), the aqueous layer was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with H_2O (2 x 100 mL), brine (100 mL), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude product was purified by column chromatography over silica gel (DCM→DCM/MeOH 20:1). **2** was obtained as pale yellow oil (12.8 g, 36.0 mmol, 78%). ^{1}H NMR (500 MHz, 300 K, DMSO-d_6): δ 7.37-7.24 (m, 10H, H_{arom}), 5.18-4.99 (m, 5H, OH, PhCH_2O-), 4.41 (t, ^3J = 8.0 Hz, 1H, Hα$_{c/t}$), 4.37 (t, ^3J = 8.0 Hz, 1H, Hα$_{c/t}$), 4.29 (bs, 1H, Hγ), 3.53-3.46 (m, 1H, Hδ), 3.42-3.37 (m, 1H, Hδ), 2.25-2.14 (m, 1H, Hβ), 2.02-1.90 (m, 1H, Hβ); ^{13}C NMR (125 MHz, 300 K, DMSO-d_6): δ 172.3, 171.9 (CO_2H), 154.3, 153.7 (NCO_2), 136.7, 136.6, 135.9, 135.7 (C_{arom}), 128.4, 128.3, 128.0, 128.0, 127.8, 127.8, 127.7, 127.6, 127.5, 127.3 (C_{arom}), 68.5, 67.7 (Cγ), 66.1, 66.0, 65.9 (PhCH_2O-), 58.0, 57.5 (Cα), 55.0, 54.5 (Cδ), 37.8 (Cβ). HRMS (ESI-ICR) m/z: [M + Na]+ Calcd for $C_{20}H_{21}NO_5Na$ 378.1312; found 378.1313.

Dibenzyl (2*S*,4*R*)-4-((methylsulfonyl)oxy)pyrrolidine-1,2-dicarboxylate (3)

2 (12.4 g, 35.0 mmol, 1.0 eq) was dissolved in DCM (130 mL). After cooling to 0 °C, DMAP (1.28 g, 10.5 mmol, 0.3 eq), NEt_3 (6.70 mL, 48.8 mmol, 1.4 eq) and MsCl (3.78 mL, 48.8 mmol, 1.4 eq) were added. The solution was stirred at RT for 2 h. H_2O (25 mL) was added and the layers separated. The aqueous layer was extracted with DCM (3 x 30 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, filtered oder the solvent removed under reduced pressure**. 3** was obtained as yellow, viscous oil (15.5 g, 31.4 mmol, 90%). ^{1}H NMR (500 MHz, 300 K, DMSO-d_6): δ 7.39- 7.26 (m, 10 H, H_{arom}), 5.30 (bs, 1H, Hγ), 5.20-5.00 PhCH_2O-), 4.50 (t, ^3J = 8.07 Hz,1H, Hα$_{cis/trans}$), 4.45 (t, ^3J = 8.07 Hz, 1H, Hα$_{cis/trans}$), 3.79- 3.75 (m, 1H, Hδ), 3.73- 3.68 (m, 1H, Hδ), 3.25 (s, 3H, CH_3), 2.66-2.58 (m, 1H, Hβ), 2.35-2.25 (m, 1H, Hβ). ^{13}C NMR (125 MHz, 300 K, DMSO-d_6): δ 171.4, 171.0 ($CHCO_2$), 153.8, 153.4 ($NHCO_2$), 136.4, 136.2, 135.7, 135.5 ($C_{arom.}$), 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.5, 127.4 ($C_{arom.}$), 79.2, 78.6 (Cγ), 66.5, 66.5, 66.3, 66.2 (PhCH_2O-), 57.3, 56.9 (Cα), 52.8, 52.4 (Cδ), 37.7 (-SCH_3), 36.6, 35.6 (Cβ). HRMS (ESI-ICR) m/z: [M + Na]+: Calcd for $C_{21}H_{23}NO_7SNa$ 456.1087; Ffound 456.1087.

Dibenzyl (2*S*,4*S*)-4-(phenylselanyl)pyrrolidine-1,2-dicarboxylate (4)[24]

Ph_2Se_2 (6.55 g, 21.0 mmol, 0.6 eq) was dissolved in *tert*-butanol (100 mL). NaBH4 (1.63 g, 43.0 mmol, 1.2 eq) was added in several portions. The mixture was refluxed until the yellow solution became colorless. Then, **3** (15.2 g, 35.0 mmol, 1.0 eq) in *tert*-butanol (120 mL) was added and the solution was refluxed for 3 h. EtOAc (400 mL) was added and the organic layer was washed with H_2O (3 x 500 mL). The organic layer was washed with brine (200 mL), dried over MgSO4, dried and the solvent removed under reduced pressure. The crude product was purified by column chromatography over silica gel (DCM→DCM/MeOH 100:1). **4** was obtained as pale yellow oil (13.7 g, 27.7 mmol, 79%). ^{1}H NMR (500 MHz, 300 K, DMSO-d_6): δ 7.53-7.24 (m, 15H, H_{arom}), 5.20-4.99 (m, 6H, PhCH_2O), 4.47 (t, ^3J = 7.38 Hz, 1H, Hα$_{c/t}$), 4.41 (t, ^3J = 7.38 Hz, 1H, Hα$_{c/t}$), 4.00-3.85 (m, 2H, Hγ, Hδ), 3.36-3.29 (m, 1H, Hδ), 2.82-2.73 (m, 1H, Hβ), 2.00-1.90 (m, 1H, Hβ). ^{13}C NMR (125 MHz, 300 K, DMSO-d_6): δ 171.5, 171.1 ($CHCO_2$), 153.6, 153.2 (NCO_2), 136.6, 136.4, 135.7, 135.5, 133.6, 129.3, 128.4,

128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.5, 127.3 ($C_{arom.}$), 66.3, 66.2, 66.2, 66.0 ($PhCH_2O$-), 58.3, 58.8 (Cα), 52.6, 53.2 (Cδ), 36.3, 37.3 (Cβ), 36.5, 35.2 (Cγ), HRMS (ESI-ICR) m/z: [M + Na]+ Calcd for $C_{26}H_{25}NO_4SeNa$ 518.0843; Found 518.0839.

Dibenzyl (*S*)-2,5-dihydro-1H-pyrrole-1,2-dicarboxylate (5)[24]

4 (13.7 g, 27.7 mmol, 1.0 eq) was dissolved in DCM (210 mL). Pyridine (3.13 mL, 38.8 mmol, 1.4 eq) was added and the solution cooled to 0 °C. After addition of H_2O_2 (50 wt% in H_2O, 3.93 mL, 69.3 mmol, 2.5 eq) the mixture was stirred at RT for 5 h. The solvent was removed under reduced pressure und the residue resolved in EtOAc (100 mL). The organic layer was washed with 0.1 N HCl (50 mL), sat. $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude product was purified by column chromatography over silica gel (DCM→DCM/MeOH 100:1) **5**was obtained as colorless oil (7.70 g, 22.8 mmol, 82%). ^{1}H NMR (500 MHz, 300 K, DMSO-d_6): δ 7.39-7.26 (m, 10 H, H_{arom}), 6.14-6.09 (m, 1H, Hβ), 5.89-5.84 (m, 1H, Hγ), 5.16-5.03 (m, 5H, Hα, PhCH_2O), 4.24-4.18 (m, 2H, Hδ). ^{13}C NMR (125 MHz, 300 K, DMSO-d_6): δ 169.7, 169.4 (CHCO_2), 153.5, 153.1 (NCO_2), 136.7, 136.5, 135.7, 135.6 ($C_{arom.}$), 129.8, 129.4 (Cβ), 128.4, 128.3, 128.1, 128.0, 127.8, 127.7, 127.7, 127.6, 127.4, 127.3 ($C_{arom.}$), 124.6, 124.3 (Cγ), 66.4, 66.2 (PhCH_2O-), 66.2, 66.2 (Cα), 66.1, 65.9 (PHCH_2O-), 53.8, 53.28 (Cδ). HRMS (ESI-ICR) m/z: [M + Na]+ Calcd for $C_{20}H_{19}NO_4Na$ 360.1206; Found 360.1204.

Dibenzyl (2*S*,3*R*,4*S*)-3,4-dihydroxypyrrolidine-1,2-dicarboxylate (6)[25]

5 (2.00 g, 5.93 mmol, 1.0 eq) was dissolved in *tert*-butanol (70 mL) and H_2O (30 mL). Then NMO•H_2O (800 mg, 5.93 mmol, 1.0 eq) and K_2OsO_4•$2H_2O$ (44.0 mg, 120 µmol, 2 mol%) was added, the mixture was stirred at RT for 21 h. 5% Na_2SO_{3aq} (50 mL) was added and the solution stirred for additional 30 min. The aqueous layer was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with 0.1 N HCl (100 mL) and brine (100 mL), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude product was purified by column chromatography over silica gel (DCM/MeOH 20:1) **6** was obtained as pale yellow, viscous oil (1.56g, 4.23 mmol, 71%). ^{1}H NMR (500 MHz, 300 K, DMSO-d_6): δ 7.29-7.23 (m, 10H, H_{arom}), 5.56 (d, ^3J = 5.9 Hz, 1H, O*H*), 5.17-4.98 (m, 5H, O*H*, PhCH_2O-), 4.09-4.01 (m, 3H, Hα,Hβ,Hγ), 3.56-3.50 (m, 1H, Hδ), 3.34-3.32 (m, 1H, Hδ). ^{13}C NMR (125 MHz, 300 K, DMSO-d_6): δ 171.2, 170.7 ($CHCO_2$), 154.2, 153.7 (NCO_2), 136.7, 136.5, 135.9, 135.7, 128.4, 128.3, 127.8, 127.6, 127.5, 127.3 ($C_{arom.}$), 75.5, 74.6 (Cγ), 69.9, 69.2 (Cβ), 66.2, 66.1, 66.1, 66.0 ($PhCH_2O$-), 64.6, 64.1 (Cα), 51.2, 50.8 (Cδ). HRMS (ESI-ICR) m/z: [M + Na]+ Calcd for $C_{20}H_{21}NO_6Na$ 394.1261; Found 394.1259.

Dibenzyl (3a*R*,4*S*,6a*S*)-2,2-dimethyltetrahydro-5H-[1,3]dioxolo[4,5-c]pyrrole-4,5-dicarboxylate (7)

6 (3.80 g, 10.2 mmol, 1.0 eq) was dissolved in 2,2-dimethoxypropane (50 mL). After addition of PTSA (350 mg, 2.05 mmol, 0.2 eq) the mixture was stirred for 4 h at RT. Sat. $NaHCO_3$ (50 mL) was added and the mixture stirred for additional 15 min. The aqueous layer was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. **7** was obtained as pale yellow oil (4.10 g, 9.96 mmol, 97%). ^{1}H NMR (500 MHz, 300 K, DMSO-d_6): δ 7.39-7.24 (m, 10H, H_{arom}), 5.18-5.04 (m, 4H, PhCH_2O), 4.88-4.79 (m, 2H, Hβ,Hγ), 4.42 (s, 1H, Hα), 3.76-3.51 (m, 2H, Hδ), 1.36 (s, 3H, CH_3), 1.26 (s, 3H, CH_3). ^{13}C NMR (125 MHz, 300 K, DMSO-d_6): δ 169.4, 169.2 ($CHCO_2$), 154.5, 153.9 (NCO_2), 136.5, 136.4, 135.4,

135.3, 128.4, 128.4, 128.3, 128.2, 128.2, 127.9, 127.9, 127.8, 127.8, 127.3, 127.2 ($C_{arom.}$), 111.6 (-$C(CH_3)_2$), 82.7, 81.6 (Cβ), 79.0, 78.0 (Cγ), 66.7, 66.6, 66.4 ($PhCH_2O$-), 66.2, 65.9 (Cα), 52.0, 51.6 (Cδ), 26.5 (CH_3), 24.6 (CH_3). HRMS (ESI-ICR) m/z: [M + Na]+ Calcd for $C_{23}H_{25}NO_6Na$ 434.1574, Found 434.1574.

(3a*R*,4*S*,6a*S*)-2,2-Dimethyltetrahydro-4H-[1,3]dioxolo[4,5-c]pyrrole-4-carboxylic acid (8)

7 (4.10 g, 9.46 mmol) was dissolved in EtOAc (60 mL) and Pd/C (10 wt%, 410 mg) was added. After 19 h at RT with 9 bar H_2-atmosphere, MeOH (60 mL) was added and the suspension was filtered. The solvent was removed under reduced pressure. **8** was obtained as colorless solid (1.52 g, 7.76 mmol, 82%). ^{1}H NMR (500 MHz, 300 K, D_2O) δ 5.20 (d, ^3J = 5.8 Hz, 1H, Hβ), 5.12 (dd, ^3J = 4.5 Hz, ^3J = 5.8 Hz, 1H, Hγ), 4.34 (s, 1H, Hα), 3.67 (d, ^2J = 13.6 Hz, 1H, Hδ), 3.57 (dd, ^2J = 13.6 Hz, ^3J = 4.5 Hz, 1H, Hδ), 1.58 (s, 3H, CH_3), 1.42 (s, 3H, CH_3). ^{13}C NMR (125 MHz, 300 K, D_2O): δ 169.7 (CO_2H), 112.7 (CMe$_2$), 82.1 (Cβ), 77.9 (Cγ), 50.3 (Cδ), 66.9 (Cα), 24.9 (CH_3), 22.8 (CH_3), HRMS (ESI-ICR) m/z: [M + Na]+ Calcd for $C_8H_{13}NO_4Na$ 210.0737; Found 210.0738.

3a*R*,4*S*,6a*S*)-5-(((9H-Fluoren-9-yl)methoxy)carbonyl)-2,2-dimethyltetrahydro-4H-[1,3]dioxolo[4,5-c]pyrrole-4-carboxylic acid (9)[35]

8 (1.82 g, 8.12 mmol, 1.0 eq) was dissolved in 10% Na_2CO_{3aq} (12.5 mL) and FmocCl (2.31 g, 8.93 mmol, 1.1 eq) was added in 1,4-Dioxane (12.5 mL). After stirring for 16 h, the mixture was washed with Et_2O (3 x 25 mL). 1 N HCl was added until pH = 6 und extracted with EtOAc (6 x 25 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. **9** was obtained as pale yellow solid (2.80 g, 6.82 mmol, 84%). ^{1}H NMR (500 MHz, 300 K, DMSO-d_6): δ 7.90-7.28 (m, 8H, H_{arom}), 4.84-4.66 (m, 2H, Hβ, Hγ), 4.34-4.06 (m, 4H, Hα, CO_2CH_2CH), 3.57-3.51 (m, 2H, Hδ), 1.38 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 1.23 (s, 3H, CH_3), ^{13}C NMR (125 MHz, 300 K, DMSO-d_6): δ 170.3 (CHO_2), 154.8, 154.4 (NCO_2), 143.9, 143.7, 140.7, 140.6, 127.6 127.1, 125.5, 125.4, 125.2, 125.1, 120.1, 120.0, ($C_{arom.}$), 110.4, 110.3 (CMe_2), 84.1, 83.0 (Cβ), 78.8, 77.9 (Cγ), 67.9, 67.8, 66.4, 66.3 (CO_2CH_2CH), 52.1, 51.8 (Cδ), 46.6, 46.6 (Cα), 26.7, 24.6, 24.6 (CH_3). HRMS (ESI-ICR) m/z: [M + Na]+ Calcd for $C_{23}H_{23}NO_6Na$ 432.1418; Found 432.1416.

Methyl (3*R*,6*R*,7*S*,8*S*,8a*S*)-6-azido-7,8-dihydroxy-5-oxohexahydro-5H-thiazolo[3,2-a]pyridine-3-carboxylate (11)

10a (617 mg, 1.88 mmol, 1.0 eq) was dissolved in 95% TFA_{aq} (10 mL). After stirring for 1 h, the solvent was removed under reduced pressure. This procedure was repeated four times. The crude product was purified by column chromatography over silica gel (DCM/DCEtOAc 1:3). **11** was obtained as white solid (439 mg, 1.52 mmol, 81%).^{1}H NMR (500 MHz, 300 K, DMSO-d_6): δ 5.59-5.50 (m, 2H, 7-OH, 8-OH), 5.09 (dd, ^3J = 7.4 Hz, ^3J = 4.9 Hz, 1H, H3), 5.02 (d, ^3J = 4.3 Hz, 1H, H8a), 4.20-4.14 (m, 2H, H6, H7), 4.12-4.06 (m, 1H, H8), 3.68 (s, 3H, $COOCH_3$), 3.32 (dd, ^2J = 11.6 Hz, ^3J = 7.5 Hz, 1H, H2), 3.03 (dd, ^2J = 11.5 Hz, ^3J = 4.9 Hz, 1H, H2). ^{13}C NMR (125 MHz, 300 K, DMSO-d_6): δ 170.0 (COOCH$_3$), 164.9 (C5), 69.7 (C7), 66.4 (C8), 65.4 (C8a), 61.0 (C3), 60.4 (C6), 52.4 ($COOCH_3$), 30.9 (C2). HRMS (ESI-ICR) m/z: [M + Na]+ Calcd for $C_9H_{12}N_4O_5SNa$: 311.0421, Found; 311.0424.

Methyl (2*S*,3a*S*,4*R*,7*R*,9a*S*,9b*S*)-4-azido-2-methyl-2-octyl-5-oxohexahydro-5H-[1,3]dioxolo[4,5-c]thiazolo[3,2-a]pyridine-7-carboxylate (10b)

2-Dekanone (231 µL, 1.21 mmol, 5.0 eq) was dissolved in MeOH (5 mL) and $HC(OMe)_3$ (2.5 mL). After PPTS (30.5 mg, 121 µmol, 0.5 eq) was added, the solution was stirred

overnight at RT. The reaction was quenched by the addition of NEt_3 (40 µL) and the solvent was removed under reduced pressure. The residue was dissolved in toluene (3 mL) and added to a solution of the diol **11** (70.0 mg, 243 µmol, 1.0 eq) in MeOH (5 mL). After addition of CSA (28.1 mg, 121 µmol, 0.5 eq), the mixture was stirred at 65 °C for 2.5 h. The reaction was quenched by the addition of NEt_3 (50 µL) and the solvent removed under reduced pressure. The crude product was purified by column chromatography over silica gel (DCM/MeOH 60:1). The ketal **10b** was obtained as pale yellow solid (60.0 mg, 141 µmol, 58%). ^{1}H NMR (500 MHz, 300 K, $CDCl_3$): δ 5.34 (dd, ^3J = 6.6 Hz, ^3J = 0.9 Hz, 1H, H3), 4.80 (dd, ^3J = 7.7 Hz, ^3J = 3.6 Hz, 1H, H7), 4.75 (d, ^3J = 1.8 Hz, 1H, H8a), 4.54 (dd, ^3J = 7.7 Hz, ^3J = 1.8 Hz, 1H, H8), 3.78 (s, 3H, $COOCH_3$), 3.73 (d, ^3J = 3.5 Hz, 1H, H6), 3.38 (dd, ^2J = 11.2 Hz, ^3J = 6.5 Hz, 1H, H2^l), 3.25 (dd, ^2J = 11.2 Hz, ^3J = 1.0 Hz, 1H, H2^h), 1.69-1.62 (m, 2H, $CCH_2(CH_2)_6CH_3$), 1.34 (s, 3H, CCH_3), 1.32-1.19 (m, 12H, $CCH_2(CH_2)_6CH_3$), 0.88 (t, ^3J = 7.0 Hz, 3H, $CCH_2(CH_2)_6CH_3$). ^{13}C NMR (125 MHz, 300 K, $CDCl_3$): δ 169.5 ($COOCH_3$), 163.8 (C5), 111.8 (CCH_3), 76.5 (C8), 75.7 (C7), 61.8 (C3), 60.2 (C8a), 59.9 (C6), 53.1 ($COOCH_3$), 38.8 ($CCH_2(CH_2)_6CH_3$), 32.5 (C2), 31.9 ($CCH_2(CH_2)_6CH_3$), 29.3 ($CCH_2(CH_2)_6CH_3$), 24.2 (CCH_3), 22.9 ($CCH_2(CH_2)_6CH_3$), 22.6 ($CCH_2(CH_2)_6CH_3$), 14.0 ($CCH_2(CH_2)_6CH_3$). HRMS (ESI-ICR) m/z: [M + Na]+ Calcd for $C_{19}H_{30}N_4O_5SNa$ 449.1829; Found 449.1835.

Methyl (2*S*,3a*S*,4*R*,7*R*,9a*S*,9b*S*)-4-azido-2-methyl-5-oxo-2-tridecylhexahydro-5H-[1,3]dioxolo[4,5-c]thiazolo[3,2-a]pyridine-7-carboxylate (10c)

2-Pentadekanone (589 mg, 2.60 mmol, 3.0 eq) was dissolved in MeOH (7 mL) and $HC(OMe)_3$ (3.5 mL). After PPTS (65.4 mg, 260 µmol, 0.3 eq) was added, the solution was stirred 5 h at RT. The reaction was quenched by the addition of NEt_3 (60 µL) and the solvent was removed under reduced pressure. The residue was dissolved in Toluene (6 mL) and added to a solution of the diol **11** (250 mg, 867 µmol, 1.0 eq) in MeOH (9 mL). After addition of CSA (60.4 mg, 260 µmol, 0.3 eq), the mixture was stirred at 65 °C for 2.5 h. The reaction was quenched by the addition of NEt_3 (50 µL) and the solvent removed under reduced pressure. The crude product was purified by column chromatography over silica gel (DCM/MeOH 70:1 → 50:1). The ketal **10c** was obtained as white solid (241 mg, 485 µmol, 55%). ^{1}H NMR (500 MHz, 300 K, $CDCl_3$): δ 5.34 (d, ^3J = 6.3 Hz, 1H, H3), 4.80 (dd, ^3J = 7.6 Hz, ^3J = 3.5 Hz, 1H, H7), 4.75 (d, ^3J = 1.4 Hz, 1H, H8a), 4.54 (dd, ^3J = 7.7 Hz, ^3J = 1.6 Hz, 1H, H8), 3.78 (s, 3H, $COOCH_3$), 3.73 (d, ^3J = 3.5 Hz, 1H, H6), 3.38 (dd, ^2J = 11.2 Hz, ^3J = 6.5 Hz, 1H, H2^l), 3.24 (d, ^2J = 11.2 Hz, 1H, H2^h), 1.69-1.62 (m, 2H, $CCH_2(CH_2)_{11}CH_3$), 1.34 (s, 3H, CCH_3), 1.32-1.20 (m, 22H, $CCH_2(CH_2)_{11}CH_3$), 0.88 (t, ^3J = 6.9 Hz, 3H, $CCH_2(CH_2)_{11}CH_3$). ^{13}C NMR (125 MHz, 300 K, $CDCl_3$): δ 169.5 ($COOCH_3$), 163.9 (C5), 111.9 (CCH_3), 76.5 (C8), 75.7 (C7), 61.8 (C3), 60.2 (C8a), 59.9 (C6), 53.0 ($COOCH_3$), 38.8 ($CCH_2(CH_2)_{11}CH_3$), 32.4 (C2), 31.8 ($CCH_2(CH_2)_{11}CH_3$), 29.6 ($CCH_2(CH_2)_{11}CH_3$), 24.2 (CCH_3), 22.6 ($CCH_2(CH_2)_{11}CH_3$), 14.1 ($CCH_2(CH_2)_{11}CH_3$). HRMS (ESI-ICR) m/z: [M + Na]+ Calcd for $C_{24}H_{40}N_4O_5SNa$ 519.2612; Found 519.2611.

Methyl (3a*S*,4*R*,7*R*,9a*S*,9b*S*)-4-azido-2,2-diheptyl-5-oxohexahydro-5H-[1,3]dioxolo[4,5-c]thiazolo[3,2-a]pyridine-7-carboxylate (10d)

8-Pentadekanone (274 mg, 1.21 mmol, 5.0 eq) was dissolved in MeOH (5 mL) and $HC(OMe)_3$ (2.5 mL). After PPTS (30.5 mg, 121 µmol, 0.5 eq) was added, the solution was stirred overnight at RT. The reaction was quenched by the addition of NEt_3 (50 µL) and the solvent was removed under reduced pressure. The residue was dissolved in Toluene (3 mL) and added to a solution of the diol **11** (70.0 mg, 243 µmol, 1.0 eq) in MeOH (5 mL). After addition of CSA (28.1 mg, 121 µmol, 0.5 eq), the mixture was stirred at 65 °C for 2.5 h. The reaction was quenched by the addition of NEt_3 (50 µL) and the solved removed under reduced pressure. The crude product was purified by column chromatography over silica gel

(DCM/MeOH 50:1). The ketal **10d** was obtained as white solid (80.4 mg, 162 µmol, 67%). ^{1}H NMR (500 MHz, 300 K, $CDCl_3$): δ 5.34 (d, ^3J = 6.3 Hz, 1H, H3), 4.80 (dd, ^3J = 7.6 Hz, ^3J = 3.5 Hz, 1H, H7), 4.75 (d, ^3J = 1.4 Hz, 1H, H8a), 4.54 (dd, ^3J = 7.7 Hz, ^3J = 1.6 Hz, 1H, H8), 3.78 (s, 3H, $COOCH_3$), 3.73 (d, ^3J = 3.5 Hz, 1H, H6), 3.38 (dd, ^2J = 11.2 Hz, ^3J = 6.5 Hz, 1H, $H2^l$), 3.24 (d, ^2J = 11.2 Hz, 1H, $H2^h$), 1.69-1.62 (m, 2H, $CCH_2(CH_2)_{11}CH_3$), 1.34 (s, 3H, CCH_3), 1.32-1.20 (m, 22H, $CCH_2(CH_2)_{11}CH_3$), 0.88 (t, ^3J = 6.9 Hz, 3H, $CCH_2(CH_2)_{11}CH_3$). ^{13}C NMR (125 MHz, 300 K, $CDCl_3$): δ 169.5 ($COOCH_3$), 163.9 (C5), 111.9 (CCH_3), 76.5 (C8), 75.7 (C7), 61.8 (C3), 60.2 (C8a), 59.9 (C6), 53.0 ($COOCH_3$), 38.8 ($CCH_2(CH_2)_{11}CH_3$), 32.4 (C2), 31.8 ($CCH_2(CH_2)_{11}CH_3$), 29.6 ($CCH_2(CH_2)_{11}CH_3$), 24.2 (CCH_3), 22.6 ($CCH_2(CH_2)_{11}CH_3$), 14.1 ($CCH_2(CH_2)_{11}CH_3$). HRMS (ESI-ICR) m/z: [M + Na]+ Calcd for $C_{24}H_{40}N_4O_5SNa$ 519.2612; Found 519.2611.

(3a*S*,4*R*,7*R*,9a*S*,9b*S*)-4-Azido-2,2-dimethyl-5-oxohexahydro-5H-[1,3]dioxolo[4,5-c]thiazolo[3,2-a]pyridine-7-carboxylic acid (12a)

To a solution of **10a** (215 mg, 655 µmol, 1.0 eq) in DCE (10 mL) was added Me_3SnOH (592 mg, 3.28 mmol, 5.0 eq). The mixture was stirred at 80°C until complete conversion. Then, EtOAc was added and the organic layer was washed with 0.2 N HCl and brine. The organic layer was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. **12a** was obtained as pale yellow solid (201 mg, 639 µmol, 98%). ^{1}H NMR (500 MHz, 300 K, DMSO-d_6): δ 13.15 (bs, 1H, COOH), 5.15 (dd, ^3J = 6.2 Hz, ^3J = 1.5 Hz, 1H, H3), 4.94 (d, ^3J = 1.9 Hz, 1H, H8a), 4.76 (dd, ^3J = 7.6 Hz, ^3J = 3.4 Hz, 1H, H7), 4.51 (dd, ^3J = 7.5 Hz, ^3J = 1.9 Hz, 1H, H8), 4.24 (d, ^3J = 3.4 Hz, 1H, H6), 3.19 (dd, ^2J = 11.2 Hz, ^3J = 6.2 Hz, 1H, H2), 3.15 (dd, ^2J = 11.2 Hz, ^3J = 1.6 Hz, 1H, H2), 1.30 (s, 3H, CCH_3), 1.28 (s, 3H, CCH_3). ^{13}C NMR (125 MHz, 300 K, DMSO-d_6): δ 170.6 (COOH), 163.8 (C5), 109.2 ($C(CH_3)_2$), 76.9 (C8), 76.0 (C7), 61.7 (C3), 59.7 (C8a), 59.4 (C6), 32.3 (C2), 26.1 (CCH_3), 24.2 (CCH_3), HRMS (ESI-ICR) m/z: [M - H]- Calcd for $C_{11}H_{13}N_4O_5S$ 313.0612; Found 313.0614.

(3a*S*,4*R*,7*R*,9a*S*,9b*S*)-4-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-2,2-dimethyl-5-oxohexahydro-5H-[1,3]dioxolo[4,5-c]thiazolo[3,2-a]pyridine-7-carboxylic acid (14a)

Pd/C (10 wt%) was added to a solution of **12a** (135 mg, 430 µmol, 1.0 eq) in MeOH (15 mL). After stirring overnight under H_2-atmosphere, the suspension was filtered and the solvent was removed under reduced pressure. The residue was dissolved in 1,4-Dioxane/H_2O (4:1, 10 mL) and Fmoc-OSu (189 mg, 559 µmol, 1.3 eq) and DIPEA (293 µL, 1.72 mmol, 4.0 eq) were added at 0°C. The mixture was stirred at RT for 4.5 h. After the solvent was removed under reduced pressure, the crude **13a** was dissolved in EtOAc (20 mL) and 0.5 N HCl (5 mL) was added. The aqueous layer was separated and extracted with EtOAc (20 mL) three times. The combined organic layers were dried over $MgSO_4$, filtrated and the solvent removed under reduced pressure. The crude product was purified by column chromatography over silica gel (DCM/MeOH 5:1). **14a** was obtained as pale yellow solid (88.0 mg, 172 µmol, 40%). ^{1}H NMR (500 MHz, 300 K, DMSO-d_6): δ 7.89 (d, ^3J = 7.5 Hz, 2H, Fmoc-$H_{arom.}$), 7.83-7.79 (m, 2H, Fmoc-$H_{arom.}$), 7.42 (t, ^3J = 7.5 Hz, 2H, Fmoc-$H_{arom.}$), 7.33 (t, ^3J = 7.4 Hz, 2H Fmoc-$H_{arom.}$), 7.14 (d, ^3J = 8.1 Hz, 1H, Fmoc-NH), 5.07 (s, 1H, H8a), 5.02-4.96 (m, 1H, H3), 4.65 (dd, ^3J = 7.4 Hz, ^3J = 2.9 Hz, 1H, H7), 4.55-4.46 (m, 2H, H6, H8), 4.34-4.21 (m, 3H, Fmoc-CH, Fmoc-CH_2), 3.18-3.10 (m, 2H, H2), 1.28 (s, 3H, CCH_3), 1.27 (s, 3H, CCH_3). ^{13}C NMR (125 MHz, 300 K, DMSO-d_6): δ 172.8 (COOH), 164.7 (C5), 143.8 (Fmoc-$C_{arom.,q}$), 143.7 (Fmoc-$C_{arom.,q}$), 140.7 (Fmoc-$C_{arom.,q}$), 127.6 (Fmoc-$C_{arom.}$), 127.1 (Fmoc-$C_{arom.}$), 127.0 (Fmoc-$C_{arom.}$), 125.6 (Fmoc-$C_{arom.}$), 125.5 (Fmoc-$C_{arom.}$), 120.0 (Fmoc-$C_{arom.}$), 108.6 ($C(CH_3)_2$), 76.8 (C8), 74.8 (C7), 66.0 (Fmoc-CH_2), 62.5 (C3), 59.8 (C8a), 53.5 (C6), 46.5 (Fmoc-CH), 32.5 (C2), 26.2 (CCH_3), 24.2 (CCH_3). HRMS (ESI-ICR) m/z: [M + Na]+ Calcd for $C_{26}H_{26}N_2O_7SNa$ 533.1353; fFound 533.1346.

(2*S*,3a*S*,4*R*,7*R*,9a*S*,9b*S*)-4-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-2-methyl-2-octyl-5-oxohexahydro-5H-[1,3]dioxolo[4,5-c]thiazolo[3,2-a]pyridine-7-carboxylic acid (14b)

To a solution of corresponding ketal **10b** (60.0 mg, 141 µmol, 1.0 eq) in DCE (10 mL) was added Me_3SnOH (114 mg, 633 µmol, 4.5 eq). The mixture was stirred at 80°C until complete conversion. Then, EtOAc was added and the organic layer was washed with 0.2 N HCl and brine. The organic layer was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude **12b** was dissolved in MeOH (10 mL) was added Pd/C (10 wt%). After stirring overnight under H_2-atmosphere, the suspension was filtered and the solvent was removed under reduced pressure. The crude **13b** was dissolved in 1,4-Dioxane/H_2O (4:1, 7.5 mL) and Fmoc-OSu (61.7 mg, 183 µmol, 1.3 eq) and DIPEA (95.9 µL, 564 µmol, 4.0 eq) were added at 0°C. The mixture was stirred at RT overnight. The solution was diluted with EtOAc (20 mL) and 0.2 N HCl (7 mL) was added. The aqueous layer was separated and extracted with EtOAc (10 mL) three times. The combined organic layers were dried over $MgSO_4$, filtrated and the solvent removed under reduced pressure. The crude product was purified by column chromatography over silica gel (DCM/MeOH 10:1). The Fmoc protected build block **14b** was obtained as pale yellow solid (58.3 mg, 96.0 µmol, 68%). ^{1}H NMR (500 MHz, 300 K, $CDCl_3$): δ 7.76 (d, ^3J = 7.5 Hz, 2H, Fmoc-$H_{arom.}$), 7.65-7.61 (m, 2H, Fmoc-$H_{arom.}$), 7.40 (t, ^3J = 7.5 Hz, 2H, Fmoc-$H_{arom.}$), 7.34-7.29 (m, 2H, Fmoc-$H_{arom.}$), 5.91 (d, ^3J = 7.2 Hz, 1H, Fmoc-NH), 5.29 (d, ^3J = 6.4 Hz, 1H, H3), 4.87 (d, ^3J = 1.5 Hz, 1H, H8a), 4.84 (dd, ^3J = 7.6 Hz, ^3J = 3.4 Hz, 1H, H7), 4.56 (dd, ^3J = 7.6 Hz, ^3J = 1.8 Hz, 1H, H8), 4.53 (dd, ^3J = 7.3 Hz, ^3J = 3.3 Hz, 1H, H6), 4.48-4.34 (m, 2H, Fmoc-CH_2), 4.25 (t, ^3J = 7.2 Hz, 1H, Fmoc-CH), 3.40 (dd, ^2J = 11.2 Hz, ^3J = 6.6 Hz, 1H, H2^t), 3.32 (d, ^2J = 11.1 Hz, 1H, H2^h), 1.69-1.54 (m, 2H, $CCH_2(CH_2)_6CH_3$), 1.32 (s, 3H, CCH_3), 1.30-1.15 (m, 12H, $CCH_2(CH_2)_5CH_3$), 0.89 (t, ^3J = 6.9 Hz, 3H, $CCH_2(CH_2)_5CH_3$). ^{13}C NMR (125 MHz, 300 K, $CDCl_3$): δ 171.9 (COOH), 166.0 (C5), 156.5 (Fmoc-C=O), 143.9 (Fmoc-$C_{arom.,q}$), 143.8 (Fmoc-$C_{arom.,q}$), 141.4 (Fmoc-$C_{arom.,q}$), 127.9 (Fmoc-$C_{arom.}$), 127.3 (Fmoc-$C_{arom.}$), 127.2 (Fmoc-$C_{arom.}$), 125.4 (Fmoc-$C_{arom.}$), 125.3 (Fmoc-$C_{arom.}$), 120.1 (Fmoc-$C_{arom.}$), 111.5 (CCH_3), 76.8 (C8), 74.1 (C7), 67.8 (Fmoc-CH_2), 62.1 (C3), 60.7 (C8a), 54.0 (C6), 47.2 (Fmoc-CH), 39.0 ($CCH_2(CH_2)_5CH_3$), 32.2 (C2), 32.0 ($CCH_2(CH_2)_5CH_3$), 29.8 ($CCH_2(CH_2)_5CH_3$), 29.6 ($CCH_2(CH_2)_5CH_3$), 29.4 ($CCH_2(CH_2)_5CH_3$), 23.3 (CCH_3), 22.8 ($CCH_2(CH_2)_5CH_3$), 22.7 ($CCH_2(CH_2)_5CH_3$), 14.3 ($CCH_2(CH_2)_5CH_3$). HRMS (ESI-ICR) m/z: [M + Na]+ Calcd for $C_{33}H_{40}N_2O_7SNa$ 631.2448; Found 631.2464.

(2*S*,3a*S*,4*R*,7*R*,9a*S*,9b*S*)-4-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-2-methyl-5-oxo-2-tridecylhexahydro-5H-[1,3]dioxolo[4,5-c]thiazolo[3,2-a]pyridine-7-carboxylic acid (14c)

To a solution of corresponding ketal **10c** (240 mg, 483 µmol, 1.0 eq) in DCE (10 mL) was added Me_3SnOH (306 mg, 1.69 mmol, 3.5 eq). The mixture was stirred at 80°C until complete conversion. Then, EtOAc was added and the organic layer was washed with 0.2 N HCl and brine. The organic layer was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude **12c** was dissolved in MeOH (15 mL) was added Pd/C (10 wt%). After stirring overnight under H_2-atmosphere, the suspension was filtered and the solvent was removed under reduced pressure. The crude **13c** was dissolved in 1,4-Dioxane/H_2O (4:1, 10 mL) and Fmoc-OSu (212 mg, 628 µmol, 1.3 eq) and DIPEA (329 µL, 1.93 mmol, 4.0 eq) were added at 0°C. The mixture was stirred at RT for overnight. The solution was diluted with EtOAc (30 mL) and H_2O (5 mL) was added. The aqueous layer was separated, diluted with 1 n HCl to pH = 2 and extracted with EtOAc (10 mL) three times. The

combined organic layers were dried over $MgSO_4$, filtrated and the solvent removed under reduced pressure. The crude product was purified by column chromatography over silica gel (DCM/MeOH 10:1). The Fmoc protected build block **14c** was obtained as pale yellow solid (125mg, 184 µmol, 38%). ^{1}H NMR (500 MHz, 300 K, $CDCl_3$): δ 7.76 (d, ^3J = 7.5 Hz, 2H, Fmoc-$H_{arom.}$), 7.66-7.60 (m, 2H, Fmoc-$H_{arom.}$), 7.40 (t, ^3J = 7.4 Hz, 2H, Fmoc-$H_{arom.}$), 7.31 (t, ^3J = 7.3 Hz, 2H, Fmoc-$H_{arom.}$), 5.91 (d, ^3J = 6.2 Hz, 1H, Fmoc-NH), 5.28 (d, ^3J = 5.9 Hz, 1H, H3), 4.88 (s, 1H, H8a), 4.83 (dd, ^3J = 7.4 Hz, ^3J = 2.8 Hz, 1H, H7), 4.63-4.48 (m, 2H, H6, H8), 4.47-4.31 (m, 2H, Fmoc-CH_2), 4.24 (t, ^3J = 7.2 Hz, 1H, Fmoc-CH), 3.41-3.27 (m, 2H, H2), 1.69-1.54 (m, 2H, $CCH_2(CH_2)_{11}CH_3$), 1.31 (s, 3H, CCH_3), 1.31-1.17 (m, 22H, $CCH_2(CH_2)_{11}CH_3$), 0.87 (t, ^3J = 6.9 Hz, 3H, $CCH_2(CH_2)_{11}CH_3$). ^{13}C NMR (125 MHz, 300 K, $CDCl_3$: δ 172.1 (COOH), 166.0 (C5), 156.5 (Fmoc-C=O), 144.0 (Fmoc-$C_{arom.,q}$), 143.8 (Fmoc-$C_{arom.,q}$), 141.4 (Fmoc-$C_{arom.,q}$), 127.9 (Fmoc-$C_{arom.}$), 127.3 (Fmoc-$C_{arom.}$), 127.2 (Fmoc-$C_{arom.}$), 125.4 (Fmoc-$C_{arom.}$), 125.3 (Fmoc-$C_{arom.}$), 120.1 (Fmoc-$C_{arom.}$), 111.5 (CCH_3), 76.7 (C8), 74.1 (C7), 67.8 (Fmoc-CH_2), 62.2 (C3), 60.7 (C8a), 54.0 (C6), 47.2 (Fmoc-CH), 39.0 ($CCH_2(CH_2)_{11}CH_3$), 32.3 (C2), 32.1 ($CCH_2(CH_2)_{11}CH_3$), 29.9 ($CCH_2(CH_2)_{11}CH_3$), 29.8 ($CCH_2(CH_2)_{11}CH_3$), 29.7 ($CCH_2(CH_2)_{11}CH_3$), 29.5 ($CCH_2(CH_2)_{11}CH_3$), 23.3 (CCH_3), 22.8 ($CCH_2(CH_2)_{11}CH_3$), 22.7 ($CCH_2(CH_2)_{11}CH_3$), 14.3 ($CCH_2(CH_2)_{11}CH_3$). HRMS (ESI-ICR) m/z: [M + Na]+ Calcd for $C_{38}H_{50}N_2O_7SNa$ 701.3231; Found 701.3237.

(3a*S*,4*R*,7*R*,9a*S*,9b*S*)-4-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-2,2-diheptyl-5-oxohexahydro-5H-[1,3]dioxolo[4,5-c]thiazolo[3,2-a]pyridine-7-carboxylic acid (14d)

To a solution of corresponding ketal **10d** (71.6 mg, 144 µmol, 1.0 eq) in DCE (10 mL) was added Me_3SnOH (117 mg, 649 µmol, 4.5 eq). The mixture was stirred at 80°C until complete conversion. Then, EtOAc was added and the organic layer was washed with 0.2 N HCl and brine. The organic layer was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude **12d** was dissolved in MeOH (15 mL) was added Pd/C (10 wt%). After stirring overnight under H_2-atmosphere, the suspension was filtered and the solvent was removed under reduced pressure. The crude **13d** was dissolved in 1,4-Dioxane/H_2O (4:1, 7.5 mL) and Fmoc-OSu (63.1 mg, 187 µmol, 1.3 eq) and DIPEA (98.0 µL, 579 µmol, 4.0 eq) were added at 0°C. The mixture was stirred at RT for overnight. The solution was diluted with EtOAc (20 mL) and 0.2 N HCl (7 mL) was added. The aqueous layer was separated and extracted with EtOAc (10 mL) five times. The combined organic layers were dried over $MgSO_4$, filtrated and the solvent removed under reduced pressure. The crude product was purified by column chromatography over silica gel (DCM/MeOH 10:1). The Fmoc protected build block **14d** was obtained as white solid (71.4 mg, 105 µmol, 73%). ^{1}H NMR (500 MHz, 300 K, $CDCl_3$): δ 7.76 (d, 7.5 Hz, 2H, Fmoc-$H_{arom.}$), 7.67-7.61 (m, 2H, Fmoc-$H_{arom.}$), 7.40 (t, ^3J = 7.4 Hz, 2H, Fmoc-$H_{arom.}$), 7.32 (t, ^3J = 7.4 Hz, 2H, Fmoc-$H_{arom.}$), 5.91 (d, ^3J = 7.1 Hz, 1H, Fmoc-NH), 5.28 (d, ^3J = 6.4 Hz, 1H, H3), 4.86 (s, 1H, H8a), 4.80 (dd, ^3J = 7.5 Hz, ^3J = 3.2 Hz, 1H, H7), 4.58-4.49 (m, 2H, H6, H8), 4.47-4.33 (m, 2H, Fmoc-CH_2), 4.25 (t, ^3J = 7.2 Hz, 1H, Fmoc-CH), 3.39 (dd, ^2J = 11.1 Hz, ^3J = 6.7 Hz, 1H, H2^l), 3.31 (d, ^2J = 11.1 Hz, 1H, H2^h), 1.66-1.49 (m, 4H, $C(CH_2(CH_2)_5CH_3)_2$), 1.40-1.13 (m, 20H, $C(CH_2(CH_2)_5CH_3)_2$), 0.89 (t, ^3J = 6.9 Hz, 3H, $CCH_2(CH_2)_5CH_3$), 0.87 (t, ^3J = 6.9 Hz, 3H, $CCH_2(CH_2)_5CH_3$). ^{13}C NMR (125 MHz, 300 K, $CDCl_3$): δ 172.0 (COOH), 166.1 (C5), 156.5 (Fmoc-C=O), 144.0 (Fmoc-$C_{arom.,q}$), 143.8 (Fmoc-$C_{arom.,q}$), 141.4 (Fmoc-$C_{arom.,q}$), 127.9 (Fmoc-$C_{arom.}$), 127.3 (Fmoc-$C_{arom.}$), 127.2 (Fmoc-$C_{arom.}$), 125.4 (Fmoc-$C_{arom.}$), 125.3 (Fmoc-$C_{arom.}$), 120.1 (Fmoc-$C_{arom.}$), 113.7 ($C(CH_2(CH_2)_5CH_3)_2$), 77.1 (C8), 74.4 (C7), 67.8 (Fmoc-CH_2), 62.1 (C3), 60.8 (C8a), 54.2 (C6), 47.1 (Fmoc-CH), 36.3 ($CCH_2(CH_2)_5CH_3$), 36.0 ($CCH_2(CH_2)_5CH_3$), 32.2 (C2), 32.0 ($C(CH_2(CH_2)_5CH_3)_2$), 31.9 ($C(CH_2(CH_2)_5CH_3)_2$), 29.9 ($C(CH_2(CH_2)_5CH_3)_2$), 29.8 ($C(CH_2(CH_2)_5CH_3)_2$), 29.4 ($C(CH_2(CH_2)_5CH_3)_2$), 29.3 ($C(CH_2(CH_2)_5CH_3)_2$), 24.5 ($C(CH_2(CH_2)_5CH_3)_2$), 23.1 ($C(CH_2(CH_2)_5CH_3)_2$), 22.8 ($C(CH_2(CH_2)_5CH_3)_2$), 14.3

($CCH_2(CH_2)_5CH_3$), 14.2 ($CCH_2(CH_2)_5CH_3$). HRMS (ESI-ICR) m/z: [M + Na]+ Calcd for $C_{38}H_{50}N_2O_7SNa$ 701.3231; Found 701.3243.

***Cyclo*(D-Phe-Dyp-Val-Orn-Leu-D-Phe-Pro-Val-Orn-Leu-) (15)**

The peptide **15** was synthesized using automated peptide synthesis in 0.1 mM scale.[1]H NMR (600 MHz, 300 K, MeOH-d_3): δ 8.89 (d, ^3J = 3.5 Hz, 1H, D-Phe6-NH), 8.76 (d, ^3J = 3.7 Hz, 1H, D-Phe1-NH), 8.75 (d, ^3J = 9.5 Hz, 1H, Leu10-NH), 8.71 (d, ^3J = 9.5 Hz, 1H, Leu5-NH), 8.70 (d, ^3J = 9.5 Hz, 1H, Orn9-NH), 8.68 (d, ^3J = 9.4 Hz, 1H, Orn4-NH), 7.71 (d, ^3J = 9.2 Hz, 1H, Val3-NH), 7.70 (d, ^3J = 9.2 Hz, 1H, Val8-NH), 7.35-7.20 (m, 10H, HAr), 4.98-4.95 (m, 2H, Orn4-Hα, Orn9-Hα), 4.69-4.61 (m, 2H, Leu5-Hα, Leu10-Hα), 4.54-4.47 (m, 2H, D-Phe1-Hα, D-Phe6-Hα), 4.34 (dd, ^2J = 8.0, ^3J = 1.7 Hz, 1H, Pro7-Hα), 4.17 (ddd, ^3J = 8.0, ^3J = 8.0, ^3J = 4.0 Hz, 1H, Dyp2-Hβ), 4.15-4.12 (m, 2H, Val3-Hα, Val8-Hα), 4.11 (dd, ^3J = 4.0, ^3J = 0.8 Hz, 1H, Dyp2-Hβ), 4.03 (dd, ^2J = 10.0, ^3J = 7.9 Hz, 1H, Dyp2-Hδ), 3.77-3.67 (m, 1H, Pro7-Hδ), 3.12-2.98 (m, 5H, Orn4-Hδ, D-Phe1-Hβ, D-Phe6-Hβ, Orn9-Hδ), 2.96-2.82 (m, 3H, Orn4-Hδ, D-Phe6-Hβ, Orn8-Hδ), 2.66 (dd, ^2J = 10.0, ^3J = 8.3 Hz, 1H, Dyp2-Hδ), 2.52-2.42 (m, 1H, Pro7-Hδ), 2.33-2.22 (m, 2H, Val3-Hβ, Val8-Hβ), 2.10-1.94 (m, 3H, Orn4-Hβ, Pro7-Hβ, Orn9-Hβ), 1.80-1.66 (m, 6H, Orn4-Hγ, Pro7-Hβ, Pro7-Hγ, Orn9-Hγ), 1.65-1.57 (m, 3H, Orn4-Hβ, Pro7-Hγ, Orn9-Hβ), 1.56-1.46 (m, 4H, Leu5-Hβ, Leu5-Hγ, Leu10-Hβ, Leu10-Hγ), 1.45-1.36 (m, 2H, Leu5-Hβ, Leu10-Hβ), 0.97 (d, ^3J = 6.9 Hz, 3H, Val3-Hγ), 0.95 (d, ^3J = 6.9 Hz, 3H, Val8-Hγ), 0.91-0.85 (m, 18H, Val3-Hγ, Leu5-Hδ, Val8-Hγ, Leu10-Hδ). HRMS (ESI-ICR) m/z: [M + 2H]2+ Calcd for $C_{60}H_{92}N_{12}O_{12}H_2$ 587.3552, Found 587.3552.

***Cyclo*(D-Hot^p=Tap-Val-Orn-Leu-D-Phe-Pro-Val-Orn-Leu-) (16)**

The peptide **16** was synthesized using manual peptide synthesis in 0.1 mM scale. [1]H NMR (600 MHz, 300 K, MeOH-d_3): δ 9.02 (s_{br}, 1H, D-Phe6-NH), 8.40 (d, ^3J = 2.8 Hz, 1H, D-Hot^{p}1-NH), 8.36 (d, ^3J = 9.0 Hz, 1H, Orn4-NH), 8.03 (d, ^3J = 8.0 Hz, 1H, Val8-NH), 7.99 (d, ^3J = 9.7 Hz, 1H, Orn9-NH), 7.90 (d, ^3J = 8.1 Hz, 1H, Leu10-NH), 7.72 (d, ^3J = 10.0 Hz, 1H, Val3-NH), 7.62 (d, ^3J = 7.5 Hz, 1H, Leu5-NH), 7.33-7.21 (m, 5H, H_{Ar}), 5.20 (s, 1H, D-Hot^{p}1-H8a), 5.05-4.99 (m, 2H, D-Hot^{p}1-H6, Tap2-H3), 4.72-4.65 (m, 3H, D-Hot^{p}1-H7, D-Hot^{p}1-H8, Leu-10-Hα), 4.54 (ddd, ^3J = 10.9, ^3J = 8.9, ^3J = 4.1 Hz, 1H, Orn4-Hα), 4.50-4.40 (m, 3H, Leu5-Hα, D-Phe6-Hα, Orn9-Hα), 4.30 (dd, ^3J = 8.2, ^3J = 2.8 Hz, 1H, Pro7-Hα), 4.16 (t, ^3J = 10.2 Hz, 1H, Val3-Hα), 3.85 (dd, ^3J = 10.9, ^3J = 8.2 Hz, 1H, Val8-Hα), 3.72 (ddd, ^2J = 10.7, ^3J = 7.9, ^3J = 3.3 Hz, 1H, Pro6-Hδ), 3.50 (dd, ^2J = 11.9, ^3J = 7.7 Hz, 1H, Tap2-H2), 3.09 (d, ^2J = 12.1 Hz, 1H, Tap-H2), 3.06 (dd, ^3J = 12.8, ^3J = 5.6 Hz, 1H, D-Phe6-Hβ), 3.03-2.89 (m, 5H, Orn4-Hδ, D-Phe6-Hβ, Orn9-Hδ), 2.71-2.62 (m, 1H, Pro7-Hδ), 2.41-2.31 (m, 1H, Val8-Hβ), 2.10-1.95 (m, 3H, Val3-Hβ, Pro7-Hβ, Orn9-Hβ), 1.81-1.59 (m, 11H, Orn4-Hβ, Orn4-Hγ, Pro7-Hβ, Pro7-Hγ, Orn9-Hβ, Orn9-Hγ, Leu10-Hβ, Leu10-Hγ), 1.58-1.51 (m, 2H, Leu5-Hβ, Leu5-Hγ), 1.45-1.36 (m, 1H, Orn4-Hγ), 1.34 (s, 3H, CCH_3), 1.33 (s, 3H, CCH_3), 1.04 (d, ^3J = 7.0 Hz, 3H, Val8-Hγ), 1.01 (d, ^3J = 6.8 Hz, 3H, Val8-Hγ), 0.98 (d, ^3J = 6.5 Hz, 3H, Leu10-Hδ), 0.94 (d, ^3J = 6.5 Hz, 3H, Leu10-Hδ), 0.93-0.89 (m, 12H, Val3-Hγ, Leu5-Hδ). HRMS (ESI-ICR) m/z: [M + H]+ Calcd for $C_{57}H_{90}N_{12}O_{12}SH$ 1167.6595; Found 1167.6632.

***Cyclo*(D-Hot[(7*S*,8*S*)-*O*-(2(*S*)-decyliden)]=Tap-Val-Orn-Leu-D-Phe-Pro-Val-Orn-Leu–) (17)**

The peptide **17** was synthesized using automated peptide synthesis 0.1 mM scale.[1]H NMR (600 MHz, 300 K, MeOH-d_3): δ 9.00 (s_{br}, 1H, D-Phe6-NH), 8.39 (d, ^3J = 6.0 Hz, 1H, Orn4-NH), 8.03 (d, ^3J = 8.0 Hz, 1H, Val8-NH), 8.00 (s_{br}, 1H, Orn9-NH), 7.90 (s_{br}, 1H, Leu10-NH), 7.69 (d, ^3J = 10.0 Hz, 1H, Val3-NH), 7.62 (d, ^3J = 7.4 Hz, 1H, Leu5-NH), 7.33-7.22 (m, 5H,

H_{Ar}), 5.24 (s, 1H, D-Hot1-H8a), 5.01-4.99 (m, 1H, D-Hot1-H6), 4.93-4.91 (m, 1H, Tap2-H3), 4.67-4.64 (m, 2H, D-Hot1-H7, D-Hot1-H8), 4.57-4.38 (m, 5H, Orn4-Hα, Leu5-Hα, D-Phe6-Hα, Orn9-Hα, Leu10-Hα), 4.31 (dd, ^3J = 8.1, ^3J = 2.4 Hz, 1H, Pro7-Hα), 4.17 (t, ^3J = 10.1 Hz, 1H, Val3-Hα), 3.85 (dd, ^3J = 10.8, ^3J = 8.0 Hz, 1H, Val8-Hα), 3.75-3.68 (m, 1H, Pro7-Hδ), 3.49 (dd, ^3J = 11.8, ^3J = 7.7 Hz, 1H, Tap2-H2), 3.11 (d, ^3J = 11.8 Hz, 1H, Tap2-H2), 3.06 (dd, ^3J = 12.8, ^3J = 5.7 Hz, 1H, D-Phe6-Hβ), 3.00-2.85 (m, 5H, Orn4-Hδ, D-Phe6-Hβ, Orn9-Hδ), 2.68-2.60 (m, 1H, Pro7-Hδ), 2.40-2.30 (m, 1H, Val8-Hβ), 2.07-1.94 (m, 3H, Val3-Hβ, Pro7-Hβ, Orn9-Hβ), 1.80-1.46 (m, 16H, D-Hot1-CH_2(CH$_2$)$_6$CH$_3$, Orn4-Hβ, Orn4-Hγ, Leu5-Hβ, Leu5-Hγ, Pro7-Hβ, Pro7-Hγ, Orn9-Hβ, Orn9-Hγ, Leu10-Hβ, Leu10-Hγ), 1.33-1.23 (m, 18H, D-Hot1-CH$_2$(CH_2)$_6$CH$_3$, D-Hot1-CCH$_3$, Orn4-Hγ), 1.04 (d, ^3J = 6.9 Hz, Val8-Hγ), 1.02 (d, ^3J = 6.9 Hz, Val8-Hγ), 1.04 (d, ^3J = 6.9 Hz, Val8-Hγ), 0.98 (d, ^3J = 6.5 Hz, Leu10-Hδ), 0.95 (d, ^3J = 6.5 Hz, Leu10-Hδ), 0.92-0.86 (m, 15H, D-Hot1-CH$_2$(CH$_2$)$_6$CH_3, Val3-Hγ, Leu5-Hδ). HRMS (ESI-ICR) m/z: [M + 2H]2+ Calcd for $C_{64}H_{104}N_{12}O_{12}SH_2$ 633.3881; Found 633.3883.

Cyclo(D-Hot[(7_S_,8_S_)-_O_-(2(_S_)-pentadecyliden)]=Tap-Val-Orn-Leu-D-Phe-Pro-Val-Orn-Leu-) (18)

The peptide **18** was synthesized using automated peptide synthesis in 0.1 mM scale.^{1}H NMR (600 MHz, 300 K, MeOH-d_3): δ 9.02 (s_{br}, 1H, D-Phe-NH), 8.38 (d, ^3J = 8.9 Hz, 1H, Orn4-NH), 8.37 (s_{br}, 1H, D-Hot1-NH), 8.03 (d, ^3J = 8.0 Hz, 1H, Val8-NH), 7.99 (d, ^3J = 9.6 Hz, 1H, Orn9-NH), 7.89 (d, ^3J = 8.1 Hz, 1H, Leu10-NH), 7.69 (d, ^3J = 10.0 Hz, 1H, Val3-NH), 7.61 (d, ^3J = 7.4 Hz, 1H, Leu5-NH), 7.35-7.20 (m, 5H, H_{Ar}), 5.23 (s, 1H, D-Hot1-H8a), 4.99-4.92 (m, 2H, D-Hot1-H6, Tap2-H3), 4.70-4.60 (m, 3H, D-Hot1-H7, D-Hot1-H8, Leu10-Hα), 4.57-4.39 (m, 4H, Orn4-Hα, Leu5-Hα, D-Phe6-Hα, Orn9-Hα), 4.30 (dd, ^3J = 8.2, ^3J = 2.5 Hz, 1H, Pro7-Hα), 4.16 (t, ^3J = 10.2 Hz, Val3-Hα), 3.85 (dd, ^3J = 10.9, ^3J = 8.1 Hz, 1H, Val8-Hα), 3.74-3.68 (m, 1H, Pro7-Hδ), 3.49 (dd, ^3J = 11.9, ^3J = 7.7 Hz, 1H, Tap2-H2), 3.11 (d, ^3J = 12.0 Hz, 1H, Tap2-H2), 3.06 (dd, ^2J = 12.8, ^3J = 5.7 Hz, 1H, D-Phe6-Hβ), 3.00-2.85 (m, 5H, Orn4-Hδ, D-Phe6-Hβ, Orn9-Hδ), 2.76-2.59 (m, 1H, Pro7-Hδ), 2.40-2.30 (m, 1H, Val8-Hβ), 2.07-1.92 (m, 3H, Val3-Hβ, Pro7-Hβ, Orn9-Hβ), 1.80-1.57 (m, 12H, Orn4-Hβ, Pro7-Hβ, Pro7-Hγ, Orn9-Hβ, Orn9-Hγ, Leu10-Hβ, Leu10-Hγ), 1.56-1.47 (m, 6H, D-Hot1-CH_2(CH$_2$)$_{11}$CH$_3$, Orn4-Hγ, Leu5-Hβ, Leu5-Hγ), 1.40-1.20 (m, 26H, D-Hot1-CCH$_3$, D-Hot1-CH$_2$(CH_2)$_{11}$CH$_3$, Orn4-Hγ), 1.04 (d, ^3J = 6.9 Hz, 3H, Val8-Hγ), 1.01 (d, ^3J = 6.8 Hz, 3H, Val8-Hγ), 0.98 (d, ^3J = 6.5 Hz, 3H, Leu10-Hδ), 0.94 (d, ^3J = 6.6 Hz, 3H, Leu10-Hδ), 0.92-0.87 (m, 15H, D-Hot1-CH$_2$(CH$_2$)$_{11}$CH_3, Val3-Hγ, Leu5-Hδ). HRMS (ESI-ICR) m/z: [M + H]+ Calcd for $C_{69}H_{114}N_{12}O_{12}SH$ 1335.8473; Found 1335.8524.

Cyclo(D-Hot[(7_S_,8_S_)-_O_-(2(_S_)-8-pentadecyliden)]=Tap-Val-Orn-Leu-D-Phe-Pro-Val-Orn-Leu-) (19)

The peptide **19** was synthesized using automated peptide synthesis in 0.1 mM scale. ^{1}H NMR (600 MHz, 300 K, MeOH-d_3): δ 9.00 (s_{br}, 1H, D-Phe6-NH), 8.41 (s_{br}, 1H, Orn4-NH), 8.03 (d, ^3J = 8.1 Hz, 1H, Val8-NH), 7.98 (s_{br}, 1H, Orn9-NH), 7.92 (sbr, 1H, Leu10-NH), 7.66 (d, 3J = 10.1 Hz, 1H, Val3-NH), 7.61 (s_{br}, 1H, Leu5-NH), 7.34-7.21 (m, 5H, H_{Ar}), 5.01 (s, 1H, D-Hot1-H8a), 5.01-4.92 (m, 2H, D-Hot1-H6, Tap2-H3), 4.65-4.59 (m, 3H, D-Hot1-H7, D-Hot1-H8, Leu10-Hα), 4.57-4.39 (m, 4H, Orn4-Hα, Leu5-Hα, D-Phe6-Hα, Orn9-Hα), 4.31 (dd, ^3J = 8.7, ^3J = 2.6 Hz, 1H, Pro7-Hα), 4.17 (t, ^3J = 10.2 Hz, 1H, Val3-Hα), 3.85 (dd, ^3J = 10.8, ^3J = 8.3 Hz, 1H, Val8-Hα), 3.75-3.68 (m, 1H, Pro-Hδ), 3.49 (dd, ^2J = 11.9, ^3J = 7.7 Hz, 1H, Tap-H2), 3.12 (d, ^2J = 12.2 Hz, 1H, Tap2-H2), 3.06 (dd, ^2J = 12.8, ^3J = 5.7 Hz, 1H, D-Phe-Hβ), 3.00-2.85 (m, 5H, Orn4-Hδ, D-Phe6-Hβ, Orn9-Hδ), 2.69-2.59 (m, 1H, Pro7-Hδ), 2.40-2.30 (m, 1H, Val8-Hβ), 2.07-1.90 (m, 3H, Val3-Hβ, Pro7-Hβ, Orn9-Hβ), 1.80-1.35 (m, 20H, D-Hot1-(CH_2(CH$_2$)$_5$CH$_3$)$_2$, Orn4-Hβ, Orn4-Hγ, Leu5-Hβ, Leu5-Hγ, Pro7-Hβ, Pro7-Hγ, Orn9-Hγ, Leu10-Hβ, Leu10-Hγ), 1.30-1.20 (m, 20H, D-Hot1-(CH$_2$(CH_2)$_5$CH$_3$)$_2$), 1.03 (d,

^3J = 7.2 Hz, 3H, Val8-Hγ), 1.01 (d, ^3J = 7.1 Hz, 3H, Val8-Hγ), 0.99 (d, ^3J = 6.3 Hz, 3H, Leu10-Hδ), 0.95 (d, ^3J = 6.2 Hz, 3H, Leu10-Hδ), 0.92-0.86 (m, 12H, D-Hot1-($CH_2(CH_2)_5CH_3)_2$), Val3-Hγ, Leu5-Hδ). HRMS (ESI-ICR) m/z: [M + H]+ Calcd for $C_{69}H_{114}N_{12}O_{12}SH$ 1335.8473; Found 1335.8465.

Supporting Information. ^{1}H and ^{13}C NMR spectra of the reported compounds, 2D NMR spectra, VT studies, HPLC data.

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