DOI: 10.1002/ejoc.200600068

Tetrahydropyran-Amino Acids: Novel Building Blocks for Gramicidin-Hybrid Ion Channels

Sabine Schröder,[a] Anna K. Schrey,[b] Andrea Knoll,[a] Philipp Reiß,[c] Burkhard Ziemer,[a] and Ulrich Koert*[c]

Keywords: THP amino acid / Stereoselective synthesis / Peptide / Gramicidin A / Ion channel

The stereoselective synthesis of a *cis*-2,6-disubstituted tetrahydropyran bearing a δ-amino acid has been achieved starting from *N*-Boc-leucinal. The THP amino acid was incorporated into peptide sequences and the structural consequences were studied by X-ray crystallography and NMR analysis. Single-channel current measurements showed that the THP amino acid is a suitable substitute for positions 11 and 12 of the gramicidin ion channel. The resulting hybrid ion channel revealed Eisenman I ion selectivity and an ion-dependence of the channel dwell time.

Introduction

The channel-mediated transport of ions through the phospholipid bilayer of cellular membranes is an important biological process.[1] Parallel to the progress in the structural biology of ion channels,[2] synthetic chemists address ion channels as target functions.[3] Gramicidin A (**gA**) is a well suited lead structure for the design of synthetic ion channels.[4] **gA** is a naturally occurring pentadecapeptide with 15 amino acids in length (Scheme 1). Its alternating sequence of D- and L-amino acids favours the formation of a β-helical secondary structure. In the membrane **gA** forms a head-to-head dimer of two right-handed single-stranded β-helices, which is the accepted ion-channel active structure.[5,6] When synthetic building blocks are coupled with the **gA**-motif, hybrid channels result which show new functionality (photomodulation with hemithioindigo[3e] and azobenzene,[7] ion selectivity with cyclohexyl ether amino acids[8] and aza-crown ethers[9]). Tetrahydrofuran amino acids (THF amino acids) were extensively explored as building blocks for gramicidin hybrid ion channels.[10] The exchange of the THF ring by a tetrahydropyran (THP) leads to THP-amino acids of type **1**. Here we present a synthetic route to THP-amino acids and gramicidin hybrid channels containing this novel ion-channel building block motif. As one example, the synthesis of the hybrid channel **4** is described, Awhere Trp11-D-Leu12 of the **gA** sequence are replaced (**2** → **3**) by the THP amino acid **1**. Positions 11 and 12 were chosen because they are part of the channel entrance where the partial desolvation of the conducted ion takes place. The THP oxygen offers an additional binding site for the cation and can assist the partial desolvation process.

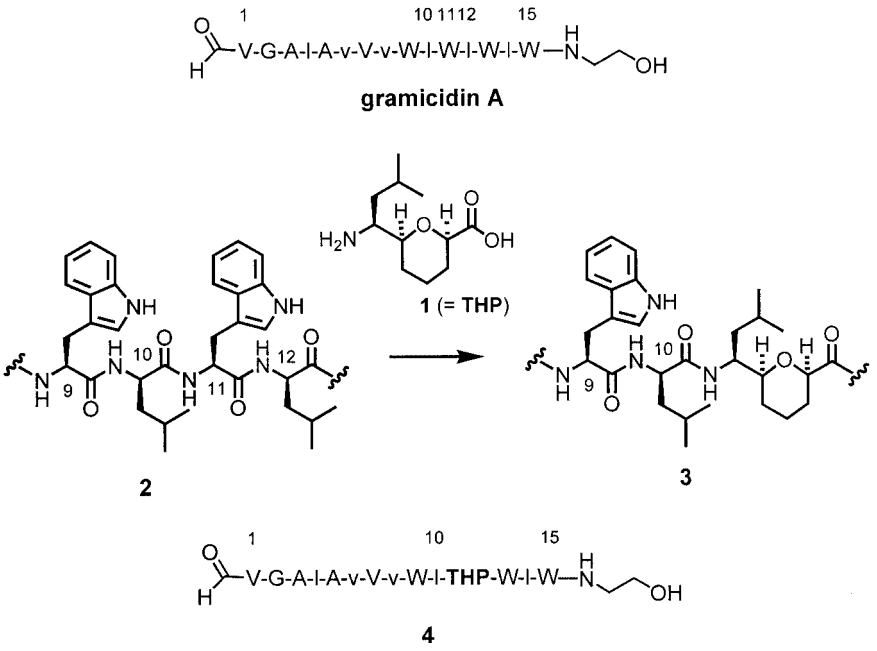
Results and Discussion

The synthesis of the *N*-Fmoc-protected THP amino acid **10** started with N-Boc L-leucinal **5** (Scheme 2).[11] A chelation-controlled[12] reaction of **5** with ethereal 4-pentenylmagnesium bromide to yield alcohol **6** gave the best diastereoselectivity (> 98:2), when the aldehyde was dissolved in CH_2Cl_2 and precomplexed with magnesium bromide. Epoxidation of the terminal double bond in **6** followed by an acid-catalyzed intramolecular epoxide opening gave the *trans* THP alcohol **7** and the *cis* THP alcohol **8** in nearly equal amounts.[10c] Both epimers could be separated by silica gel chromatography. Compound **8** was subjected to a Boc → Fmoc exchange to produce **9**, which was oxidized to the *N*-Fmoc-protected THP amino acid **10** by a two step procedure (Swern[13] followed by Pinnick[14]).

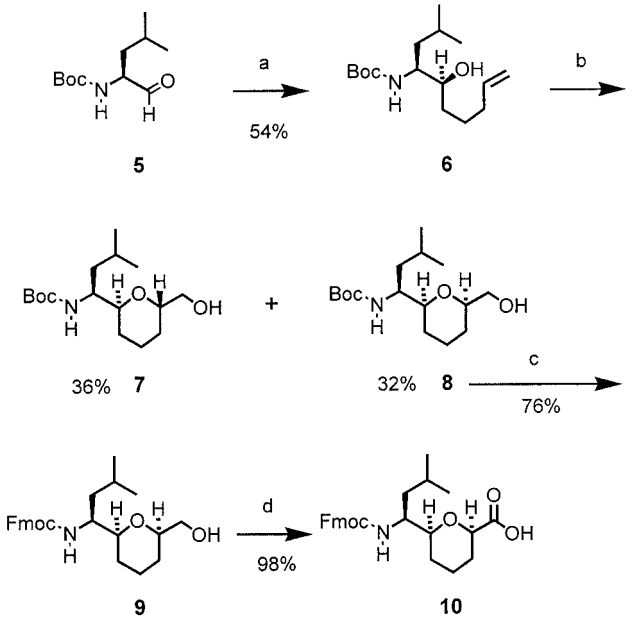
The *N*-Boc-protected THP amino acid **11** was accessible from the alcohol **8** (Scheme 3, A). From **11**, the THP amide **12** was prepared. The relative configuration of **12** was unambiguously proven by X-ray crystal-structural analysis (Scheme 3, B), which secured the stereochemical assignments of compounds **6**, **7**, and **8**.

The hexapeptide **17** was chosen as a model compound for the studies concerning the solution structure of peptides containing the THP amino acid building block. A segment coupling strategy[15] was applied for the synthesis of **17**

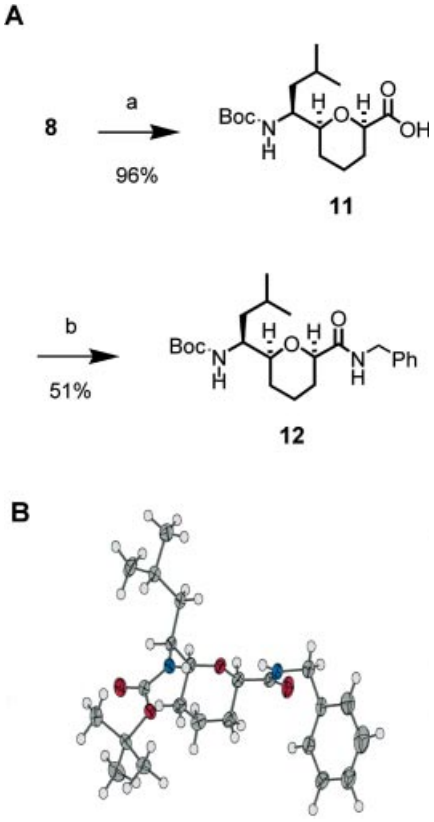
[a] Institut für Chemie, Humboldt-Universität zu Berlin, Brook-Taylor-Strasse 2, 12489 Berlin
[b] Forschungsinstitut für Molekulare Pharmakologie, Robert-Rössle-Straße 10, 13125 Berlin
[c] Fachbereich Chemie, Philipps-Universität Marburg, Hans-Meerwein-Strasse, 35032 Marburg
E-mail: koert@chemie.uni-marburg.de
Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.



Scheme 1. Generation of the THP-amino-acid gramicidin A hybrid **4** by replacement of positions 11,12 in **gA** with the THP amino acid **1** (**2** → **3**).

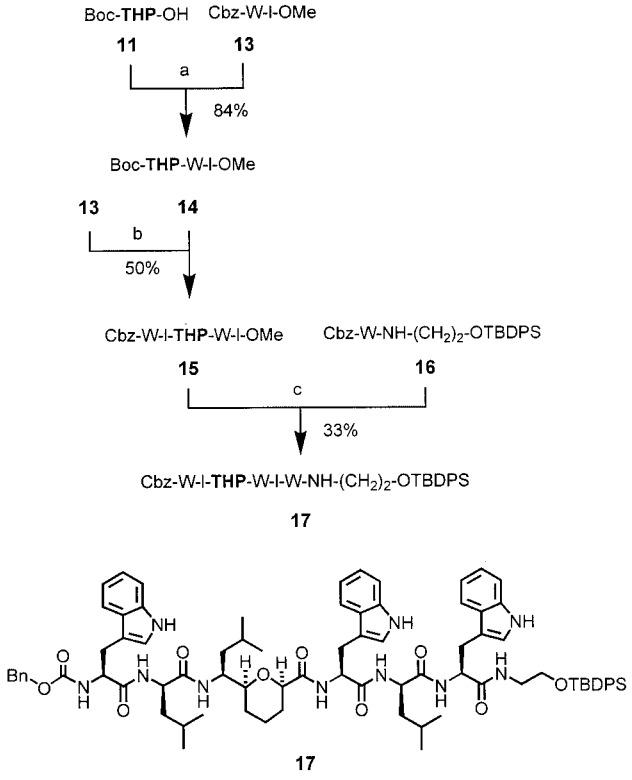


Scheme 2. Synthesis of the *N*-Fmoc-protected THP amino acid **10**. a) $H_2C{=}CH(CH_2)_2CH_2MgBr$, $MgBr_2$, Et_2O/CH_2Cl_2, –50 °C; b) i: *meta*-chloroperbenzoic acid, CH_2Cl_2, 20 °C; ii: camphor sulfonic acid, CH_2Cl_2, 20 °C; c) TFA, CH_2Cl_2, 20 °C; ii: Fmoc-succinimide, $NaHCO_3$, CH_3CN/H_2O, 20 °C; d) i: $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, –60 → 0 °C; ii: $NaClO_2$, NaH_2PO_4, amylene, *t*BuOH/H_2O, 20 °C.

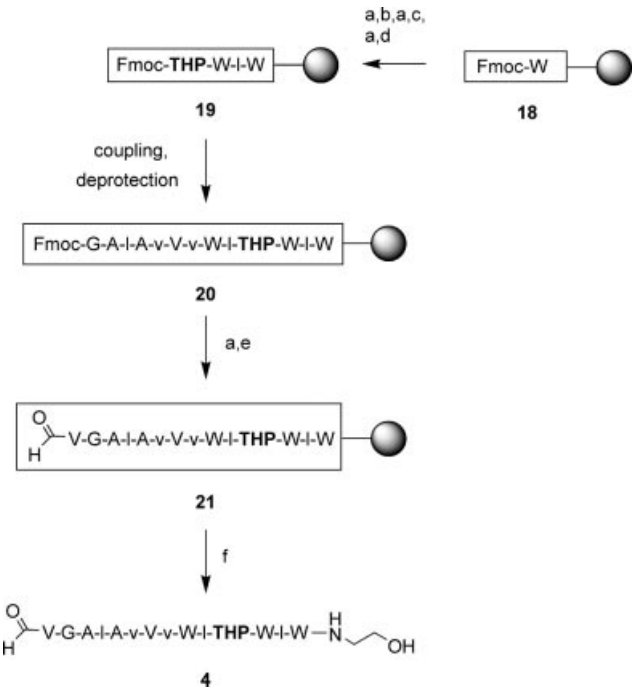


Scheme 3. A. Synthesis of THP-amino acid derivatives: a) i: $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, –60 → 0 °C; ii: $NaClO_2$, NaH_2PO_4, amylene, *t*BuOH/H_2O, 20 °C; b) benzylamine; HBtU, HOBt, DIEA, DMF/CH_2Cl_2, 20 °C. B: solid state conformation of **12** obtained by X-ray crystal-structural analysis.

(Scheme 4). Coupling of the Boc-protected THP acid **11** with the dipeptide **13**[15] provided the tripeptide **14**. The latter was elaborated to the pentapeptide **15**, whose coupling with the tryptophan derivative **16**[15] led to the desired hexapeptide **17**.



Scheme 4. Synthesis of the hexapeptide **17** by segment coupling in solution. a) i: **13**, H_2, Pd/C, MeOH, 20 °C; ii: EDC, HOBt, Et_3N, CH_2Cl_2, 20 °C; b) i: **13**, LiOH, THF/H_2O, 20 °C; ii: **14**, TFA, CH_2Cl_2, 20 °C; iii: HOBt, EDC, DIEA, HBtU, CH_2Cl_2, 20 °C; c) i: **16**, H_2, Pd/C, MeOH, 20 °C; ii: **15**, LiOH, THF/H_2O, 20 °C; iii: HOBt, DIEA, HBtU, CH_2Cl_2, 20 °C.



Scheme 5. Solid-phase synthesis of $^{11–12\text{-THP}}$gA (**4**). a) piperidine, DMF, b) Fmoc-l, HOBt, DIEA, HBtU, DMF, c) Fmoc-W, HOBt, DIEA, HBtU, DMF, d) **10**, HOBt, DIEA, HBtU, DMF, e) *N*-formyl-V, HOBt, DIEA, HBtU, DMF, f) ethanolamine, DMF, 55 °C.

The 11,12-THP-gA hybrid (**4**) was synthesized by solid phase synthesis on Wang resin using Fmoc-*N*-protection and HBtU-HOBt coupling conditions (Scheme 5). Starting from Wang resin PS-PHB-Trp-Fmoc **18**, the Fmoc-THP acid **10** was introduced in the third coupling step to yield **19**. *N*-Formyl-L-valine was used in the final coupling step (**20** → **21**). The cleavage of the peptide was carried out with 10% ethanolamine in DMF at 55 °C for 48 h. Chromatographic purification gave the 11,12-THP-gA hybrid (**4**) in 43% yield.

The solution structures of the THP-amide **12** and the model THP-peptide **17** were investigated by NMR spectroscopy combined with MD-calculation (details are given in the Exp. Sect.).

THP-Benzylamide 12

The NMR-data were collected with **12**, while the calculations were done with a replacement of Boc by Ac on the corresponding benzylamide **22** (Figure 1, A). The structure calculation afforded 100 structures, none of them violating the upper boundary of the distance restraints by more than 0.13 Å. Figure 1 (B) shows an ensemble of the 20 energy-lowest structures, fitted on the heavy atoms of the central THP amino-acid building block. The closest-to-average coordinates structure is highlighted. The mean rms deviation of the heavy-atom fit excluding the benzyl group was 0.035 ± 0.027 Å.

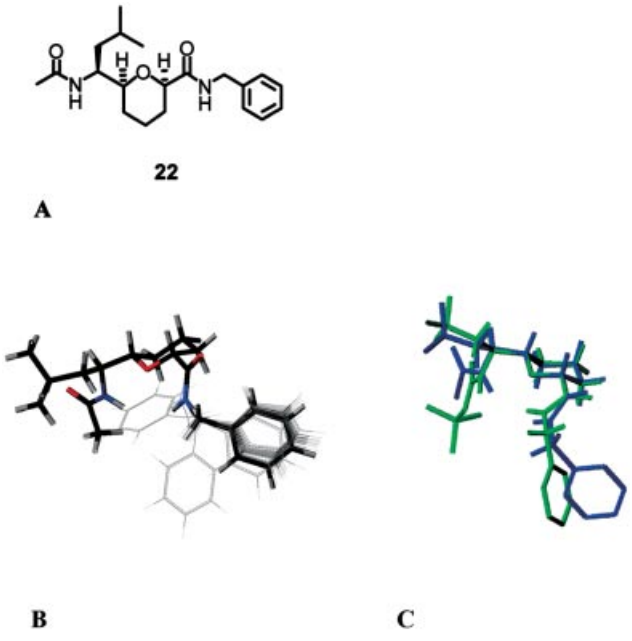


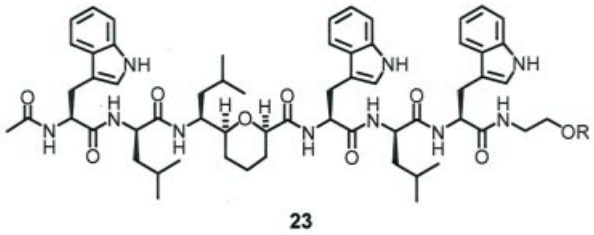
Figure 1. **A**: Formula structure of **22**. **B** Solution structure of **22**: 20 lowest-energy conformations from NMR calculations. Bold: Structure closest to the average coordinates. **C** Superposition between solution (blue) and X-ray structure (green).

The THP moiety including the isobutyl side chain as well as the acetamide group converged very well. Deviations are found in the benzylamide group because of the lack of structure-defining distance restraints. Nevertheless, in 18 of

20 structures, a similar orientation of the aromatic moiety can be found. The calculated solution structures are in good agreement with the X-ray structure of **12**. A superposition of the solution and the X-ray structures is shown in Figure 1 (C). The rms deviation between both structures including the orientation of the isobutyl group lies with 0.35 Å in the range of the experimental error of the X-ray structure (0.822 Å). The planes of the amide groups differ slightly in their orientation towards the central THP ring, which might be due to the different N-terminal side chains and the lack of structure-determining restraints for the benzamide moiety. None of the structures shows any propensity to form a hydrogen-bonded β-turn structure. The amide protons are pointing towards each other. The dihedral angle between the α and β protons is about 60 deg (gauche$^+$). In contrast, in a similar THF amide with a methyl instead of a isobutyl side chain which is known to form a hydrogen-bounded β-turn structure in solid state as well as in solution,[10c] those protons form a dihedral angle in the range 180 deg (*trans*). In the case of the THF-amide, hydrogen-bond formation might override the steric hindrance between the THF ring and the less bulky methyl side chain. In case of the bulkier THP ring and isobutyl side chain, it does not. The C-terminal carboxamides in both the THP and the THF structures exhibit similar orientations.

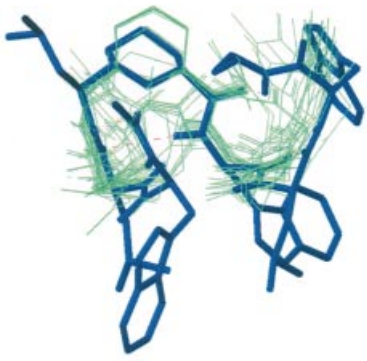
Model THP Peptide 17

The NMR spectroscopic data were collected with **17** while for practical reasons the calculations were done with compound **23**, where the CBz group was replaced by an acetyl group (Figure 2, A). The THP-containing peptide **23** forms a complex, hydrogen-bonded structure. The temperature dependence of the amide proton shifts is linear, suggesting a stable secondary structure over the whole temperature range. The corresponding temperature coefficients (see Supporting Information, Table S1a) imply hydrogen bonding of the amide protons at THP3, Trp4, and EAN7 ($\Delta\Delta T < 6$ -ppb/K) and partial hydrogen bonding for the amide protons at Trp1 ad Trp7 (6 -ppb/K $< \Delta\Delta T < 9$ -ppb/K), respectively. In the NMR structure calculations, 5 of 100 structures violated the restraint boundaries given in the methods section. Figure 2 (B) shows the ensemble of the 20 energy-lowest, unviolated structures. The structure closest

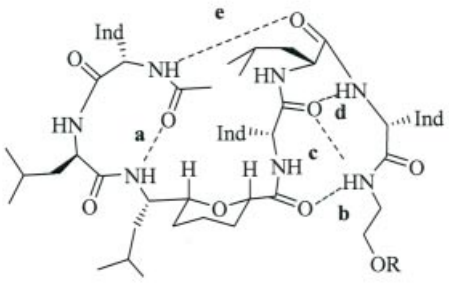


23

A



B



a: 3THP-NH /ACE-O
b: 7EA-NH / 3THP-O
c: 7EA-NH / 4Trp-O
d: 6Trp-NH / 4Trp-O
e: 1Trp-NH / D-5Leu-O

C

Figure 2. **A**: Formula structure of compound **23**. **B** 20 lowest-energy conformations of **23** from NMR calculations. Bold: Structure closest to the average coordinates. **C** Schematic representation of the multiturn hydrogen-bonding pattern.

to the average coordinates is highlighted. In the other structures, the side chains are omitted for clarity. The structures were fitted on N, Cα, Cβ, Cζ, C of the THP moiety and the backbone atoms of the adjacent Leu3 and Trp4 residues with a mean rms deviation of 0.510 ± 0.117 Å. For the whole backbone, a mean rmsd deviation of 1.153 ± 0.215 Å is found for this superposition.

The THP residue forms a sharp kink in the peptide chain. The central ring is found in two different orientations, with torsion angles between Hα and Hβ of about 120 deg or –120 deg, respectively. This finding, however, has to be taken with care, because the ring orientation is mainly defined by the coupling constant between the two protons which has been found to be about 3 Hz by *J*-sensitive 2D NMR experiments and thus not unambiguous. Nevertheless, both orientations of the THP ring result in similar orientations of the adjacent peptide chains which exhibit a hydrogen-bonded turn pattern. A schematic representation of this pattern is shown in Figure 2 (C). The amide proton of THP3 is involved in a hydrogen bond to the terminal acetamide oxygen, resulting in a type II β-turn formed by Trp1 and Leu2. This is in good agreement with the temperature coefficients for the amide proton of THP3 (low) and Leu2 (high), the latter being accessible by the solvent. This hydrogen bond forces a reorientation of the amide plane between Leu2 and THP3 with the carbonyl oxygen of Leu2 (instead of the THP3 amide proton as in **12** and **22**) pointing in the direction of the THP ring. This reorientation enables an interaction of this oxygen to the amide proton of Trp4.

The amide protons of residues 6 and 7 are involved in fluctuating H-bonds to the carbonyl oxygens of THP3 and Trp4, resulting of a suite of β- and γ-turns. The finding that only the amide proton of the ethanolamine moiety and not that of Trp6 can be involved in a β-turn-forming hydrogen bond which is geometrically more favoured than that of a γ-turn is in good agreement with the lower temperature coefficient of the latter in contrast to the former proton shift.

In addition, the N-terminal and the C-terminal turn structures exhibit a tendency for a crosslink formed by a hydrogen bond between the amide proton of Trp1 and the carbonyl oxygen of Leu5. This crosslink explains the medium temperature coefficient for the proton shift which indicates its participation in a weak H-bond.

Taken together, a structural ensemble with a decent convergence could be calculated for **23**. In spite of the uncertainities in the THP region, the overall structure of the peptide can be considered as valid.

Ion-Channel Activity of $^{11–12\text{-THP}}$-gA (4)

Single-channel current measurements in planar lipid bilayers were done in asolectine to characterize the ion-channel activity of $^{11–12\text{-THP}}$-gA (**4**).[10a]

Compound **4** displayed single-channel activity for monovalent cations (Figure 3). Compared to gA, compound **4** showed single-channel currents with about 50% reduced conductivity. The observed ion selectivity exhibits Eisenman I selectivity for compound **4** ($Cs^+ > K^+ > Na^+$) follows the order known for gA.[16] Long dwell times in the order of seconds were observed for NH_4^+, while burst-type events dominated for Cs^+. This interesting dwell time behavior is not known for gA and shows the ability of the THP to modulate the ion channel functionality.

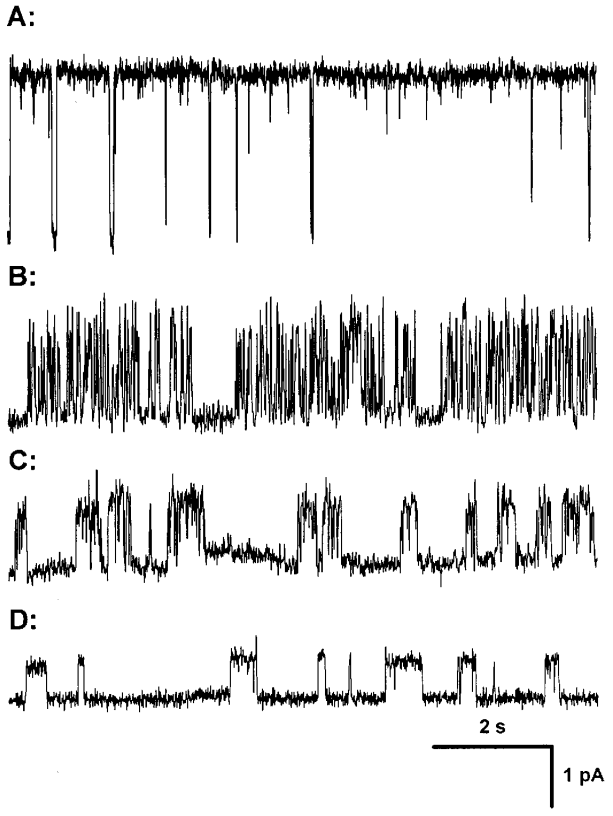


Figure 3. Single-channel current traces for ^{11,12}-THP gA (**4**) in asolectine at 100 mV. A: 1 M NH_4Cl, B: 1 M CsCl, C: 1 M KCl, D: 1 M NaCl.

Conclusions

The synthesis of the enantiomerically pure, novel THP amino acid **1** was achieved using L-leucine as a chiral-pool source. The structural properties of **1** as a dipeptide isoster were studied with compound **12** and the model peptide **17** by a combination of X-ray crystallography and solution-structure-determination via NMR spectroscopy. The functional analysis of the THP-gramicidin hybrid **4** shows, that **1** is a suitable substitute for positions 11 and 12 of gA. The solid-phase-synthesis as described for **4** should allow the broad study of substitutions at variable positions by synthetic building blocks and their functional consequences with respect to ion-channel activity.

Experimental Section

General: All reactions sensitive to air or moisture were conducted in flame-dried glassware under an atmosphere of dry Argon. THF and Et_2O were distilled from purple sodium benzophenone.

CH_2Cl_2, toluene, hexanes, pyridine, and Et_3N were distilled under Ar from CaH_2. All starting materials and reagents were used as received unless noted otherwise. PE: light petroleum, boiling range 40–60 °C. MTBE: Methyl *tert*-butyl ether. Thin-layer chromatography (TLC) was performed on glass-supported Merck silica gel 60 F_{254} plates. Spots were visualized by UV light and by heat staining with 2% molybdophosphoric acid in ethanol. Flash column chromatography (FCC) was performed on Merck silica gel 60 (63–200 μm). Melting points were determined from pulverized material in glass capillaries and are uncorrected. ^1H and ^{13}C NMR spectra were obtained on Bruker DPX 300, DRX 400, DRX 500, or AMX 600 spectrometers, respectively. All resonances are referenced to residual solvent signals. Optical rotations: Perkin–Elmer spectrophotopolarimeter 241, cuvette path length 10 cm. $CHCl_3$ for spectroscopy was filtered through basic aluminium oxide before use. MS: Finigan MAT 95 (EI: 70 eV. FAB), MSI Concept 1H (ESI), Finnigan LTQ FT (ESI). Elemental analyses: Leco CHNS 932 Analysator (microanalytical facility, HU Berlin).

Boc-L-leucinal (5): Oxalyl chloride (5.35 mL, 0.062 mol) was dissolved in CH_2Cl_2 (100 mL) at –60 °C. DMSO (8.5 mL, 0.13 mol), dissolved in CH_2Cl_2 (50 mL), was added and stirred for 5 min. Then Boc-L-leucinol (9 g, 0.041 mol), dissolved in CH_2Cl_2 (50 mL), was added and stirred for 15 min. DIEA (33.7 mL, 0.242 mol) was added and the reaction mixture was warmed to 0 °C and stirred for 30 min. Then, saturated $NaHCO_3$ solution (200 mL) was added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (100 mL) and the combined organic layers were washed with 0.1 M citric acid (100 mL) and with saturated NaCl solution (100 mL). The solvent was removed and the crude product used directly for the next reaction. To check enantiomeric purity a small amount was purified by column chromatography and the optical rotation was measured. $[\alpha]_D$ = –48.2 (c = 0.76, MeOH, T = 24 °C); ref.[11b] $[\alpha]_D$ = –48.8 (c = 1.07, MeOH).

(4*S*,5*S*)-4-(*tert*-Butoxycarbonylamino)-2-methyl-9-decen-5-ol (6): Magnesium turnings (0.80 g, 32.9 mmol) were covered with Et_2O (5 mL). Dibromoethane (6.2 g, 33 mmol, 2.84 mL) in Et_2O (5 mL) was added dropwise. A two-layer system with $MgBr_2$ was formed. Magnesium turnings (0.73 g, 30.1 mmol) were combined with Et_2O (10 mL) and 5-bromopent-1-ene (4.47 g, 30 mmol, 3.55 mL) in Et_2O (5 mL) was added dropwise. The reaction mixture was refluxed. Boc-leucinal (**5**) (3.08 g, 14.3 mmol) dissolved in CH_2Cl_2 (50 mL) was added dropwise to the $MgBr_2$ system and cooled to –60 °C. The Grignard solution was transferred via cannula in a dropping funnel and added dropwise to the reaction mixture while maintaining a maximum temperature of –50 °C. After 2.5 h saturated NH_4Cl solution (100 mL) was added dropwise and the reaction mixture was warmed to room temperature. The aqueous layer was extracted with MTBE (100 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (80 mL), with saturated NaCl solution (80 mL), dried with $MgSO_4$, and the solvent was removed in vacuo. After purification by column chromatography (MTBE/PE, 1:6 → 1:1) 2.2 g (54%) of alcohol **6** was obtained as a colourless oil. R_f = 0.44 (acetone/CH_2Cl_2, 1:20). $[\alpha]_D$ = –24.3 (c = 3.18, $CHCl_3$, T = 24 °C). ^1H NMR (300 MHz, $CDCl_3$): δ = 0.89 (d, J = 6.4 Hz, 6 H, 1/1^i-H_3), 1.19–1.30 (m, 1 H, 3-H_A), 1.41 (s, 9 H, Boc-H_3), 1.37–1.49 (m, 5 H, 8-H_2, 6-H_A, 3-H_B, 7-H_A), 1.58–1.67 (m, 2 H, 2-H, 6-H_B), 2.04–2.06 (m, 1 H, 7-H_B), 3.44–3.52 (m, 1 H, 5-H), 3.55–3.61 (m, 1 H, 4-H), 4.53–4.61 (m, 1 H, NH), 4.90–5.00 (m, 2 H, 10-H_2), 5.76 (ddt, J = 16.9/10.1/6.7 Hz, 1 H, 9-H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 22.1, 23.2 (C-1/1^i), 24.8 (C-2), 24.9 (C-6), 28.3 (Boc-CH_3), 32.2 (C-8), 33.7 (C-7), 41.6 (C-3), 52.4 (C-4), 73.9 (C-5), 79.6 (Cq), 114.7 (C-10), 138.6 (C-9), 160.7 (Boc-C=O) ppm. HR-MS calcd. 285.23039 found 285.2304.

(2*S*,6*S*,1′*S*)-2-[1′-(*tert*-Butoxycarbonylamino)-3′-methylbutyl]-6-(hydroxymethyl)tetrahydropyran (7) and (2*S*,6*R*,1′*S*)-2-[1′-(*tert*-Butoxycarbonylamino)-3′-methylbutyl]-6-(hydroxymethyl)tetrahydropyran (8): The alcohol **6** (3.27 g, 0.011 mol) was dissolved in CH_2Cl_2 (70 mL). mCPBA (3.9 g, 0.022 mol) dissolved in CH_2Cl_2 (20 mL) was added to the solution. The reaction mixture was stirred for 12 h at 20 °C. A saturated Na_2SO_3 solution (50 mL) was added to the mixture and the layers were separated. The organic layer was washed with saturated $NaHCO_3$ solution (100 mL), with saturated NaCl solution (100 mL) and dried with $MgSO_4$. The filtered solution was concentrated to 50 mL and camphorsulfonic acid (400 mg) was added. The reaction mixture was stirred for 2 h at 20 °C and subsequently quenched with saturated $NaHCO_3$ solution (50 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (100 mL). The combined organic layers were washed with saturated NaCl solution (50 mL) and dried with $MgSO_4$. The solvent was removed in vacuo. The purification by column chromatography yielded 1.1 g (32%) of the *cis* product **8** and 1.25 g (36%) of *trans* product **7** as colourless foams.

***cis*-THP Alcohol 8:** R_f = 0.37 (ethyl acetate/hexane, 1:1). $[\alpha]_D$ = –29.4 (c = 4.9, acetone, T = 25 °C). ^1H NMR (300 MHz, CD_3OD): δ = 1.05 (d, J = 6.6 Hz, 6 H, CH_3), 1.36–1.41 (m, 2 H, 5-H_2), 1.31–1.35 (m, 1 H, 2′-H_A), 1.53 (s, 9 H, Boc-H_3), 1.45–1.57 (m, 2 H, 3-H_2), 1.59–1.63 (m, 1 H, 2′-H_B), 1.67–1.73 (m, 1 H, 3′-H), 1.95–1.99 (m, 2 H, 4-H_2), 3.38–3.41 (m, 1 H, 2-H), 3.41–3.49 (m, 1 H, 6-H), 3.59 (d, J = 8.5 Hz, 2 H, CH_2OH), 3.65–3.68 (m, 1 H, 1′-H) ppm. OH and NH not detectable due to H/D exchange. ^{13}C NMR (75.5 MHz, CD_3Cl): δ = 22.3, 23.6 (CH_3), 22.6 (C-4), 24.7 (C-3′), 26.9 (C-5), 27.9 (C-3), 28.3 (Boc-CH_3), 41.9 (C-2′), 52.4 (C-1′), 66.2 (CH_2OH), 78.2 (C-6), 78.9 (Cq), 79.1 (C-2), 156.1 (Boc-C=O) ppm. HR-MS: calcd. 300.217483 found 300.21749.

***trans*-THP Alcohol 7:** R_f = 0.28 (ethyl acetate/hexane, 1:1). $[\alpha]_D$ = –14.5, $[\alpha]_{578}$ = –15.0, $[\alpha]_{546}$ = –16, $[\alpha]_{436}$ = –8.4, $[\alpha]_{365}$ = –44.0 (c = 4.1, acetone, T = 26 °C). ^1H NMR (300 MHz, CD_3OD): δ = 1.01 (d, J = 6.7 Hz, 6 H, CH_3), 1.32–1.33 (m, 2 H, 5-H_2), 1.35–1.44 (m, 2 H, 2′-H_2), 1.46–1.56 (m, 2 H, 3-H_2), 1.53 (s, 9 H, Boc-CH_3), 1.58–1.63 (m, 2 H, 4-H_2), 1.68–1.73 (m, 1 H, 3′-H), 3.50–3.69 (m, 1 H, 2-H), 3.78 (d, J = 7.1 Hz, 2 H, CH_2OH), 3.81–3.83 (m, 1 H, 1′-H), 3.93–3.97 (m, 1 H, 6-H) ppm. OH and NH not detectable due to H/D exchange. ^{13}C NMR (75.5 MHz, CD_3Cl): δ = 18.5 (C-4), 23.4, 24.7 (CH_3), 25.3 (C-5), 26.9 (C-3′), 27.2 (C-3), 28.4 (Boc), 41.8 (C-2′), 50.6 (C-1′), 62.03(CH_2OH), 72.5 (C-6), 72.8 (C-2), 79.1 (C_q), 156.3 (Boc-C=O) ppm. HR-MS calcd. 300.217483 found 300.21748.

(2*S*,6*R*,1′*S*)-2-[1′-(*tert*-Fluorenylmethoxycarbonylamino)-3′-methylbutyl]-6-(hydroxymethyl)tetrahydropyran (9): The *cis*-Boc-THP alcohol **8** (301 mg, 1.3 mmol) was dissolved in CH_2Cl_2 (7 mL). TFA (1.75 mL) was added and the reaction mixture was stirred for 1.5 h at room temperature. The solvent was removed by codistillation with toluene in vacuo. The residue was dissolved in acetonitrile/water (10 mL, 1:1.6) and $NaHCO_3$ (453.6 g, 5.4 mmol) was added. Fmoc-succinimide (606 mg, 1.8 mmol) was added to the reaction mixture which was stirred overnight. The acetonitrile was removed in vacuo. The residue was partitioned between MTBE (50 mL) and H_2O (10 mL). The layers were separated and the aqueous layer was extracted with MTBE (50 mL). The combined organic layers were washed with 0.1 M HCl (10 mL), with saturated NaCl (50 mL) and dried with Na_2SO_4. The solvent was removed in vacuo. Purification by column chromatography yielded 311 mg (76%) of **9** as a colourless foam. R_f = 0.33 (MTBE/PE, 2:1).

$[\alpha]_D$ = –26.0, $[\alpha]_{578}$ = –26.7, $[\alpha]_{546}$ = –30.2, $[\alpha]_{436}$ = –50.2, $[\alpha]_{365}$ = –76.0 (*c* = 0.824,MeOH, *T* = 24 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (d, *J* = 6.4 Hz, 3 H, CH_3), 0.89 (d, *J* = 6.4 Hz, 3 H, CH_3), 1.04–1.15 (m, 1 H, 5-H_A), 1.18–1.31 (m, 3 H, 3-H_A, 2′-H_A, 5-H_B), 1.39–1.45 (m, 1 H, 3-H_B), 1.45–1.63 (m, 2 H, 2′-H_B, 3′-H), 1.78–1.89 (m, 2 H, 4-H_2), 1.97 (br. s, 1 H, OH), 3.32 (d, *J* = 10.9 Hz, 1 H, 2-H), 3.37–3.59 (m, 3 H, CH_2OH, 6-H), 3.61–3.74 (m, 1 H, 1′-H), 4.20 (t, *J* = 6.6 Hz, 1 H, Fmoc), 4.44 (d, *J* = 6.8 Hz, 2 H, Fmoc), 4.80 (d, *J* = 9.8 Hz, 1 H, NH), 7.29 (t, *J* = 7.4 Hz, 2 H, Ar), 7.38 (t, *J* = 7.4 Hz, 2 H, Ar), 7.58 (dd, *J* = 7.2/3.0 Hz, 2 H, Ar), 7.74 (d, *J* = 7.5 Hz, 2 H, Ar) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 22.2, 23.3 (CH_3), 22.6 (C-4), 24.6 (C-3′), 26.9 (C-5), 27.9 (C-3), 41.9 (C-2′), 47.5 (*C*HCH$_2$O), 52.6 (C-1′), 66.1 (*C*H$_2$O Fmoc), 66.3 (*C*H$_2$OH), 78.3 (C-6), 79.1 (C-2), 119.9, 124.9, 126.9, 127.6, 141.4, 143.9 (Ar), 156.5 (Fmoc-C=O) ppm. HR-MS calcd. 423.2410 found 423.24093.

(2*R*,6*S*,1′*S*)-6-[1′-(Fluorenylmethoxycarbonylamino)-3′-methylbutyl]tetrahydropyran-2-carboxylic Acid (10): Oxalyl chloride (0.08 mL, 0.94 mmol) was dissolved in CH_2Cl_2 (5 mL) at –60 °C and DMSO (0.12 mL, 1.88 mmol) dissolved in CH_2Cl_2 (1 mL) was added dropwise. After 5 min Fmoc-THP alcohol **9** (200 mg, 0.47 mmol) dissolved in CH_2Cl_2 (3 mL) was added dropwise and stirred for 15 min. Then NEt_3 (0.65 mL, 4.7 mmol) was added dropwise. After 30 min, the reaction mixture was warmed to 0 °C and saturated $NaHCO_3$ solution (10 mL) was added dropwise. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (20 mL). The combined organic layers were washed with 0.1 M citric acid (20 mL), with saturated NaCl solution (20 mL) and dried with $MgSO_4$. The solvent was removed in vacuo to yield 199 mg of the corresponding aldehyde, which was dissolved in *tert*-butyl alcohol (10 mL) and in amylene (2.5 mL, 23.5 mmol). $NaH_2PO_4 \cdot 2H_2O$ (592 mg, 3.8 mmol) and $NaClO_2$ (425 mg, 4.7 mmol), dissolved in a small amount of water, were added to the reaction mixture, which was stirred for 12 h at room temperature. The *tert*-butyl alcohol was removed in vacuo, the residue was covered with MTBE (50 mL), 2 M HCl was added to the mixture (10 mL) and the aqueous layer was extracted with MTBE (50 mL). The combined organic layers were concentrated, K_2CO_3 solution (1.2 g/50 mL water) was added and the aqueous layer was extracted with MTBE (50 mL). The aqueous layer was covered with ethyl acetate, 2 N HCl was added and the layers were separated. The aqueous layer was extracted with ethyl acetate (50 mL) three times. The combined organic layers were washed with saturated NaCl solution (50 mL), dried with Na_2SO_4 and the solvent was removed in vacuo to yield 202 mg (98%) of the acid **10** as a colourless foam. R_f = 0.25 ($CHCl_3$/CH_3OH/AcOH, 200:10:1). $[\alpha]_D$ = –12.4 (*c* = 0.432, MeOH, *T* = 24 °C). ^{1}H NMR (300 MHz, CD_3OH): δ = 0.87 (d, *J* = 6.2 Hz, 3 H, CH_3), 0.88 (d, *J* = 6.2 Hz, 3 H, CH_3), 1.14–1.41 (m, 4 H, 2′-H_A, 5-H_2, 3-H_A), 1.41–1.52 (m, 2 H, 2′-H_B, 3′-H), 1.84–1.92 (m, 3 H, 4-H_2, 3-H_B), 3.30–3.31 (m, 1 H, 6-H), 3.59–3.62 (m, 1 H, 1′-H), 3.97 (d, *J* = 11.7 Hz, 1 H, 2-H), 4.20 (t, *J* = 6.2 Hz, 1 H, Fmoc), 4.39 (dd, *J* = 10.6/6.4 Hz, 1 H, Fmoc), 4.54 (dd, *J* = 10.6/6.4 Hz, 1 H, Fmoc), 4.59 (d, *J* = 11.3 Hz, 1 H, NH), 7.27–7.40 (m, 4 H, Ar), 7.67 (d, *J* = 7.5 Hz, 2 H, Ar), 7.79 (d, *J* = 7.5 Hz, 2 H, Ar) ppm. ^{13}C NMR (75.5 MHz, CD_3OD): δ = 22.3, 23.5 (CH_3), 24.1 (C-4), 25.9 (C-3′), 28.6 (C-3), 30.3 (C-5), 42.5 (C-2′), 48.9 (Fmoc), 53.7 (C-1′), 67.0 (Fmoc), 77.3 (C-2), 80.9 (C-6), 120.9, 126.1/126.3, 128.1, 128.5, 142.8, 145.4 (Ar), 157.6 (Fmoc-C=O), 175.0 (COOH) ppm. HR-MS calcd. 437.2202 found [M + Na^+] 460.20996.

(2*R*,6*S*,1′*S*)-6-[1′-(*tert*-Butoxycarbonylamino)-3′-methylbutyl]tetrahydropyran-2-carboxylic Acid (11): Oxalyl chloride (1.8 mL, 20.5 mmol) was dissolved in CH_2Cl_2 (10 mL) at –60 °C and DMSO (2.6 mL, 41 mmol) dissolved in CH_2Cl_2 (10 mL) was added dropwise. After 5 min, Boc-THP-alcohol **8** (3.1 g, 10.29 mmol) dissolved in CH_2Cl_2 (20 mL) was added dropwise and the mixture was stirred for 15 min. Then NEt_3 (14.25 mL, 103 mmol) was added dropwise. After 30 min the reaction mixture was warmed to 0 °C and saturated $NaHCO_3$ solution (30 mL) was added dropwise. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (50 mL). The combined organic layers were washed with 0.1 M citric acid (50 mL), with saturated NaCl solution (50 mL) and dried with $MgSO_4$. The solvent was removed in vacuo.The residue was dissolved in *tert*-butyl alcohol (50 mL) and in amylene (100 mL, 500 mmol). $NaH_2PO_4 \cdot 2H_2O$ (12.5 g, 80 mmol) and $NaClO_2$ (9.04 g, 100 mmol), dissolved in a small amount of water, were added to the reaction mixture and stirred for 12 h at room temperature. The *tert*-butyl alcohol was removed in vacuo, the residue was covered with MTBE (100 mL), 2 M HCl was added to the mixture and the aqueous layer was extracted with MTBE (100 mL). The combined organic layers were concentrated, K_2CO_3 solution (1.2 g/50 mL water) was added and the aqueous layer was extracted with MTBE. The aqueous layer was covered with ethyl acetate, 2 M HCl was added and the layers were separated. The aqueous layer was extracted with ethyl acetate (100 mL) three times. The combined organic layers were washed with saturated NaCl solution (100 mL), dried with $MgSO_4$ and the solvent was removed to yield 3.04 g (96%) of acid **11** as a colourless foam. R_f = 0.20 ($CHCl_3$/MeOH/AcOH, 200:10:1) $[\alpha]_D$ = –23.4, $[\alpha]_{578}$ = –24.1, $[\alpha]_{548}$ = –275, $[\alpha]_{436}$ = –47.5, $[\alpha]_{365}$ = –75.6 (*c* = 1.5, MeOH, *T* = 25 °C). ^{1}H NMR (300 MHz, CD_3OH): δ = 0.80 (d, *J* = 6.4 Hz, 6 H, CH_3), 1.08–1.15 (m, 1 H, 2′-H_A), 1.16–1.22 (m, 1 H, 3-H_A), 1.25–1.30 (m, 2 H, 5-H_2), 1.38–1.39 (m, 1 H, 3-H_B), 1.42–1.45 (m, 1 H, 2′-H_B), 1.45–1.57 (m, 1 H, 3′-H), 1.47 (s, 9 H, Boc-H_3), 1.80–1.84 (m, 2 H, 4-H_2), 3.26–3.31 (m, 1 H, 6-H), 3.46–3.51 (m, 1 H, 1^i-H), 3.86–3.91 (dd, 1 H, *J* = 2.3 Hz, 11.7 Hz, 2-H), 5.08 (d, *J* = 5.0 Hz, 1 H, NH) ppm. ^{13}C NMR (75.5 MHz, CD_3OD): δ = 22.4, 23.7 (CH_3), 24.1 (C-4), 28.6 (C-3), 28.8 (C-3′), 29.2 (Boc-CH_3), 30.2 (C-5), 42.6 (C-2′), 53.1 (C-1′), 77.2 (C-6), 79.2 (C_q), 80.9 (C-2), 157.0 (Boc-C=O), 176.2 (COOH) ppm. HR-MS calcd. 315.20457 found 316.212398 [MH^+].

(2*R*,6*S*,1′*S*)-*N*-Benzyl-6-[1′-(*tert*-butoxycarbonylamino)-3′-methylbutyl]tetrahydropyran-2-carboxamide (12): The Boc-protected THP amino acid **11** (50 mg, 0.158 mmol) and benzylamine (51 mg, 0.474 mmol) were dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C. HOBt (61 mg, 0.395 mmol), dissolved in DMF (0.2 mL), was added to the mixture. Subsequently, DIEA (0.07 mL, 0.395 mmol) was added and stirred for 5 min. HBtU (90 mg, 0.237 mmol) was added and the mixture was stirred for 2 h. Ethyl acetate/toluene (20 mL, 1:1) was added to reaction mixture. 0.1 M HCl (5 mL) was added and the layers were separated. The aqueous layer was extracted with acetic acid/toluene (10 mL, 1:1). The combined organic layers were washed with saturated $NaHCO_3$ solution (20 mL), with saturated NaCl solution (20 mL), dried with Na_2SO_4 and the solvent was removed. The purification by column chromatography (MTBE/PE, 1:3 → 1:2) yielded 33 mg (51%) of the carboxamide **12**. R_f = 0.14 (MTBE/PE, 1:1). m.p. 112 °C. $[\alpha]_D$ = –14.3, $[\alpha]_{578}$ = –15.1, $[\alpha]_{546}$ = –16.6, $[\alpha]_{436}$ = –27.1, $[\alpha]_{365}$ = –30.3 (*c* = 1.026, MeOH, *T* = 23 °C). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.87 (d, *J* = 6.4 Hz, 6 H, CH_3), 1.26 (m, 1 H, 2′-H_A), 1.32 (m, 1 H, 2′-H_B), 1.33 (m, 1 H, 3-H_{ax}), 1.34 (m, 1 H, 5-H_{ax}), 1.37 (s, 9 H, Boc-H_3), 1.55 (m, 1 H, 5-H_{eq}), 1.56 (m, 1 H, 4-H_{ax}), 1.64 (m, 1 H, 3′-H), 1.95 (m, 1 H, 4-H_{eq}), 2.14 (m, 1 H, 3-H_{eq}), 3.34 (m, 1 H, 6-H), 3.67 (m, 1 H, 1′-H), 3.86 (dd, *J* = 11.6/ 2.1 Hz, 1 H, 2-H), 4.41 (d, *J* = 9.5 Hz, 1 H, THP-NH), 4.47 (m, 2 H, CH_2NH), 6.91 (br. s, 1 H, Bn-NH), 7.23–7.32 (m, 5 H, Ar) ppm. ^{13}C NMR (75.47 MHz,

DMSO): δ = 22.6, 24.0 (CH_3), 24.34 (C-4), 25.8 (C-3′), 29.1 (Boc-CH_3), 30.9 (C-3), 42.6 (C-2′), 43.6 (CH_2NH), 53.5 (C-1′), 79.1 (C-2), 79.8 (C_q), 81.6 (C-6), 128.5, 128.8, 129.5, 140.1 (Ar), 157.0 (Boc-C=O), 189.6 (THP-C=O) ppm. HR-MS calcd. 404.26750 found 404.26750.

X-ray Structure Determination of 12: Compound **12** was measured on a four-circle diffractometer. The crystal-structure analysis was performed with the program package SHELXL-97.2. Crystal data: $C_{23}H_{36}N_2O_4$, M = 404.54, monoclinic, $P21/c$, a = 9.789(2), b = 25.448(6), c = 10.088(2) Å, β = 110.13 (2)°, V = 2359.4(9) Å^3, Z = 4, $D_{calcd.}$ = 1.139 g cm^{-1}, (0.48 × 0.40 × 0.16) mm^3, 15453 measured reflections, 4253 independent (R_{int} = 0.0766), R_1 = 0.0968, wR_2 = 0.0821 (all data).

CCDC-291471 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Boc-THP-Trp-D-Leu-OMe (14): Cbz-W-l-OMe (**13**) (296 mg, 0.63 mmol) was dissolved in CH_3OH (5 mL) and Pd/C (200 mg) was added. The reaction mixture was degassed, flushed with argon and hydrogen was bubbled through the solution/mixture. Pd/C was separated by filtration through Celite and the filtrate was concentrated. The residue was dissolved together with the *N*-Boc-THP amino acid **11** (100 mg, 0.31 mmol) in CH_2Cl_2 (2 mL). EDC (89 mg, 0.465 mmol) and HOBt (71 mg, 0.465 mmol) were added. After addition of NEt_3 (0.2 mL, 1.6 mmol) the reaction mixture was stirred for 12 h at room temperature; 0.1 M citric acid (2 mL) was added and the layers were separated. The aqueous layer was extracted with ethyl acetate/toluene (1:1, 20 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (10 mL), with saturated NaCl solution (10 mL), dried with Na_2SO_4 and the solvent was removed. The purification by column chromatography (cyclohexane/ethyl acetate, 1:2) yielded 168 mg of tripeptide **14** (84%). R_f = 0.54 ($CHCl_3$/CH_3OH/AcOH, 100:10:1). $[\alpha]_D$ = –19.7, $[\alpha]_{578}$ = –20.8, $[\alpha]_{546}$ = –24.6, $[\alpha]_{436}$ = –47.8, $[\alpha]_{365}$ = –89.0 (c = 0.456, CH_2Cl_2, T = 25 °C). ^{1}H NMR (600 MHz, CD_3OH): δ = 0.70–0.86 (m, 6 H, ^{3}Leu-δ-H_3), 0.96–1.07 (m, 4 H, ^{1}THP-γ-H_2, ^{1}THP-δ-H_2), 1.09–1.51 (m, 10 H, ^{1}THP-β^i-H_2, ^{3}Leu-γ-H, ^{1}THP-ε-H_A, ^{1}THP-δ^i-H_3), 1.41 (s, 9 H, Boc-H_3), 1.63–1.98 (m, 2 H, ^{1}THP-γ^i-H, ^{1}THP-ε-H_B), 3.10–3.32 (m, 2 H, ^{2}Trp-β-H_2), 3.40 (s, 3 H, O-CH_3), 3.50–3.61 (m, 3 H, ^{3}Leu-β-H_2, ^{1}THP-β-H), 3.68 (dd, J = 11.7/ 2.3 Hz, ^{1}THP-ζ-H), 4.36–4.47 (m, 2 H, ^{1}THP-α-H, ^{3}Leu-α-H), 4.64–4.73 (m, 1 H, ^{2}Trp-α-H), 6.31 (d, J = 7.9 Hz, 2 H, THP-NH, ^{2}Leu-NH), 6.92 (d, J = 7.5 Hz, 1 H, ^{3}Trp-NH), 7.00–7.15 (m, 3 H, Ar), 7.28 (d, J = 7.9 Hz, 1 H, Ar), 7.60 (d, J = 7.5 Hz, 1 H, Ar), 8.15 (s, 1 H, indole-NH) ppm. HR-MS calcd. 628.3836 found 628.3836.

Cbz-Trp-D-Leu-*cis*-THP-Trp-D-Leu-OMe (15): Dipeptide **13** (200 mg, 0.43 mmol) was dissolved in THF (3 mL) and LiOH (45 mg, 1 mmol) dissolved in water (1 mL) was added. The reaction mixture was stirred at 20 °C until TLC control showed complete turnover. 0.1 M HCl was added to adjust pH = 2 and the layers were separated. The aqueous layer was extracted with ethyl acetate (20 mL). The combined organic layers were washed with saturated NaCl solution (10 mL), dried with Na_2SO_4 and the solvent removed. Boc-protected THP-tripeptide **14** (100 mg, 0.16 mmol) was dissolved in CH_2Cl_2 (4 mL) and TFA (1.5 mL) was added. The reaction mixture was stirred for 1.5 h at room temperature. The solvent was removed in vacuo, residual TFA was removed by aceotrope distillation with toluene in vacuo. The Boc-deprotected THP-tripeptide was dissolved together with the corresponding acid prepared from **13** (108 mg, 0.24 mmol) in CH_2Cl_2 (2 mL) and cooled to 0 °C. HOBt (49 mg, 0.31 mmol), EDC (43 mg, 0.225 mmol) and DIEA (0.047 mL, 0.297 mmol) were added and the mixture was stirred for 12 h at room temperature. HBtU (15 mg, 0.039 mmol) was added and stirred for 2 h. 0.1 M HCl (2 mL) was added to the reaction mixture and the solvent was removed. The aqueous layer was extracted with ethyl acetate/toluene (1:1, 20 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (10 mL), with saturated NaCl solution (10 mL), and dried with $MgSO_4$. The solvent was removed. Purification by column chromatography ($CHCl_3$/CH_3OH, 40:1) yielded 71 mg (50%) of pentapeptide **15**. R_f = 0.53 ($CHCl_3$/MeOH, 10:1). ^{1}H NMR (600 MHz, DMSO): δ = 0.63 (d, J = 6.5 Hz, 3 H, ^{3}THP-δ^i-H_3), 0.64 (m, 1 H, ^{3}THP-δ-H_A), 0.70 (d, J = 6.1 Hz, 3 H, Leu–δ–H_3), 0.73 (d, J = 6.6 Hz, 3 H, Leu-δ-H_3), 0.74 (m, 1 H, ^{3}THP-δ-H_B), 0.76 (d, J = 6.5 Hz, 3 H, ^{3}THP-δ^i-H_3), 0.77 (m, 1 H, ^{5}Leu-γ-H), 0.78 (d, J = 6.1 Hz, 3 H, Leu-δ-H_3), 0.80 (m, 1 H, ^{2}Leu-γ-H), 0.82 (d, J = 6.6 Hz, 3 H, Leu-δ-H_3), 1.13 (m, 2 H, ^{3}THP-γ-H_2), 1.17 (m, 1 H, ^{3}THP-β^i-H_A), 1.18 (m, 1 H, ^{3}THP-γ^i-H), 1.19 (m, 1 H, ^{3}THP-ε-H_{eq}), 1.29 (m, 1 H, ^{2}Leu-β-H_A), 1.34 (m, 1 H, ^{3}THP-β^i-H_B), 1.43 (m, 1 H, ^{2}Leu-β-H_B), 1.47 (m, 1 H, ^{5}Leu-β-H_A), 1.54 (m, 1 H, ^{5}Leu-β-H_B), 1.66 (m, 1 H, ^{3}THP-ε-H_{ax}), 2.93 (m, 1 H, ^{1}Trp-β-H_A), 3.05 (m, 1 H, ^{1}Trp-β-H_B), 3.09 (m, 2 H, ^{4}Trp-β-H_2), 3.27 (m, 1 H, ^{3}THP-β-H), 3.58 (s, 3 H, O-CH_3), 3.66 (m, 1 H, ^{3}THP-ζ-H), 3.86 (m, 1 H, ^{3}THP-α-H), 4.15 (m, 1 H, ^{2}Leu-α-H), 4.29 (m, 1 H, ^{5}Leu-α-H), 4.38 (dd, J = 15.1/7.8 Hz, 1 H, ^{1}Trp-α-H), 4.68 (dd, J = 14.4/8.4 Hz, 1 H, ^{4}Trp-α-H), 4.91 (d, J = 12.8 Hz, 1 H, Z-CH_A), 4.98 (d, J = 12.8 Hz, 1 H, Z-CH_B), 7.02 (m, 6 H, Ar), 7.27 (m, 7 H, Ar), 7.41 (d, J = 9.5 Hz, 1 H, ^{3}THP-NH), 7.47 (d, J = 8.3 Hz, 1 H, ^{4}Trp-NH), 7.57 (d, J = 8.0 Hz, 1 H, Ar), 7.64 (d, J = 8.1 Hz, 1 H, Ar), 7.68 (d, J = 7.6 Hz, 1 H, ^{1}Trp-NH), 8.28 (d, J = 7.9 Hz, 1 H, ^{2}Leu-NH), 8.58 (d, J = 7.9 Hz, 1 H, ^{5}Leu-NH), 10.79 (s, 1 H, indole-NH), 10.81 (s, 1 H, indole-NH) ppm. HR-MS calcd. 961.5313 found 962.5393 [M + H].

Cbz-Trp-D-Leu-*cis*-THP-Trp-D-Leu-Trp-NH-$(CH_2)_2$OTBDPS (17): Cbz-Trp-NH-$(CH_2)_2$-OTBDPS **16** (50 mg, 0.8 mmol) was dissolved in CH_3OH (3 mL) and Pd/C (49 mg) was added under argon. Hydrogen was bubbled through the reaction mixture. After 1 h Pd/C was filtered off via Celite and the filtrate concentrated in vacuo. Methyl ester **15** (50 mg, 0.052 mmol) was dissolved in THF (3 mL) and LiOH (26 mg/1 mL water, 0.624 mmol) was added. The reaction mixture was stirred and monitored by TLC. Upon completion, 0.1 M HCl (4 mL) was added to adjust pH = 2. Ethyl acetate (20 mL) was added and the layers were separated. The aqueous layer was extracted with ethyl acetate (10 mL). The combined organic layers were washed with saturated NaCl solution (20 mL), dried with Na_2SO_4 and the solvent was removed in vacuo. The free acid resulting from **15** (49 mg, 0.067 mmol) and the Cbz-deprotected amine resulting from **16** (32 mg, 0.067 mmol) were dissolved in CH_2Cl_2 (5 mL) at 0 °C and HOBt (27 mg, 0.17 mmol) was added. DIEA (0.029 mL, 0.17 mmol) was added and the mixture was stirred for 5 min. After addition of HBtU (32 mg, 0.0832 mmol) the reaction mixture was stirred for 4 h. Ethyl acetate (20 mL) and 0.1 M HCl (10 mL) were added and the layers were separated. The aqueous layer was extracted with ethyl acetate (10 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (20 mL), with saturated NaCl solution (20 mL), and dried with Na_2SO_4. The solvent was removed. Purification by column chromatography (CH_3OH in $CHCl_3$ 0.5%–1.25%) gave 24 mg (33%) of hexapeptide **17** as a colourless solid. R_f = 0.53 (CH_3OH/$CHCl_3$, 1:10). $[\alpha]_D$ = +12.6, $[\alpha]_{578}$ = +13.2, $[\alpha]_{546}$ = +15.2, $[\alpha]_{436}$ = +27.9, $[\alpha]_{365}$ = +32.2 (c = 0.348, CH_2Cl_2, T = 25 °C). ^{1}H NMR (600 MHz, $CDCl_3$/CD_3OH, 3:1): δ = 0.21 (m, 1 H, ^{5}Leu-γ-H), 0.48 (m, 6 H, ^{5}Leu-δ-H_3), 0.64 (m, 1 H, ^{2}Leu-γ-H), 0.84 (m,

12 H, ^{2}Leu-δ-H_3, ^{3}THP-δ^{i}-H_3), 0.87 (m, 1 H, ^{5}Leu-β-H_A), 0.94 (s, 9 H, *tert*-butyl-H_3), 1.06 (m, 1 H, ^{3}THP-γ-H_A), 1.09 (m, 1 H, ^{3}THP-ε-H_A), 1.21 (m, 1 H, ^{2}Leu-β-H_A), 1.30 (m, 1 H, ^{5}Leu-β-H_B), 1.34 (m, 1 H, ^{3}THP-δ-H_A), 1.37 (m, 1 H, ^{3}THP-β^{i}-H_A),1.46 (m, 1 H, ^{3}THP-ε-H_B), 1.56 (m, 1 H, ^{2}Leu-β-H_B), 1.69 (m, 1 H, ^{3}THP-γ^{i}-H), 1.76 (m, 1 H, ^{3}THP-δ-H_B), 1.77 (m, 1 H, ^{3}THP-γ-H_B), 1.79 (m, 1 H, ^{3}THP-β^{i}-H_B), 2.83 (m, 1 H, ^{4}Trp-β-H_A), 3.01 (m, 1 H, ^{4}Trp-β-H_B), 3.08 (m, 1 H, ^{6}Trp-β-H_A), 3.12 (m, 1 H, ^{1}Trp-β-H_A), 3.15 (m, 2 H, ^{7}EAN-α-H_2), 3.17 (m, 1 H, ^{1}Trp-β-H_B), 3.19 (m, 1 H, ^{6}Trp-β-H_B), 3.26 (m, 1 H, ^{3}THP-β-H), 3.31 (m, 1 H, EAN-β-H_A), 3.44 (m, 1 H, EAN-β-H_B), 3.80 (m, 1 H, ^{3}THP-ζ-H), 3.96 (m, 1 H, ^{3}THP-α-H), 4.21 (m, 1 H, ^{5}Leu-α-H), 4.27 (m, 1 H, ^{2}Leu-α-H), 4.47 (m, 1 H, ^{4}Trp-α-H), 4.51 (m, 1 H, ^{1}Trp-α-H), 4.52 (m, 1 H, ^{6}Trp-α-H), 6.67 (m, 1 H, indole-δ-H), 6.85 (d, J = 5.4 Hz, 1 H, ^{1}Trp-NH), 6.86 (d, J = 5.6 Hz, 1 H, EAN-NH), 6.86 (m, 1 H, indole-H), 7.03 (m, 1 H, indole-H), 7.02–7.15 (m, 9 H, Ar), 7.19–7.25 (m, 8 H, Ar), 7.36–7.39 (m, 3 H, Ar), 7.45–7.47 (m, 2 H, Ar), 7.52–7.54 (m, 5 H, Ar) 7.57 (m, 1 H, ^{3}THP-NH), 7.67 (d, J = 7.9 Hz, 1 H, ^{5}Leu-NH), 7.77 (d, J = 7.9 Hz, 1 H, ^{6}Trp-NH), 7.81 (d, J = 5.6 Hz, 1 H, ^{4}Trp-NH), 8.28 (d, J = 7.4 Hz, 1 H, ^{2}Leu-NH), 9.39 (s, 1 H, indole-NH), 9.79 (s, 1 H, indole-NH), 9.84 (s, 1 H, indole-NH) ppm– HR-MS calcd. [M + Na] 1437.73 found [M + Na] 1437.36.

form-V-G-A-l-A-v-V-v-W-l-THP-W-l-W-NH-CH_2-CH_2-OH (4): The Wang resin PS-PHB-Trp-Fmoc (0.63 mmol/g) from RAPP Polymere GmbH was swelled in DMF. The deprotection of the Fmoc-group was performed with 25% piperidine in DMF. The Fmoc-protected amino acids (4 equiv.) and the THP amino acid (2 × 2 equiv.) were coupled with 2.5 equiv. HOBt, 1.5 equiv. HBtU and 2.5 equiv. DIEA in 1 mL DMF. The coupling time was 2:15 h. The coupling of the THP amino acid building block **10** took 2 × 2 h for complete reaction. After the coupling of the THP amino acid the free amino groups were capped with 0.1 mL Ac_2O, 0.04 mL NEt_3 in 1 mL DMF for 20 min. Formylated valine was used as the final amino acid. After the last coupling, the resin was washed with DMF, CH_2Cl_2, 30% CH_3COOH and MeOH. The cleavage of the peptide was carried out with 10% ethanolamine in DMF at 55 °C in 48 h. The resin was filtered and washed with MeOH, CH_2Cl_2 and TFE. The filtrate was concentrated in vacuo to an approximate volume of 2 mL and the product was precipitated with H_2O. The precipitate was centrifuged (4 °C, 14000 rpm, 2 h). The supernatant was decanted and the residue dissolved in MeOH, concentrated in vacuo and precipitated by addition of H_2O. The suspension was frozen in liquid nitrogen and lyophilised. The purification was performed by column chromatography ($CHCl_3$/MeOH/HCOOH, 100:3:7–100:5:7) and gave 24 mg (43%) of $^{11–12}$-THPgA (**4**). R_f = 0.28 ($CHCl_3$/CH_3OH/HCOOH, 100:7:7). ^{1}H NMR (600 MHz, $CDCl_3$/CD_3OH, 1:1): δ = 0.67–0.89 (m, 43 H, 2× ^{10}Leu-δ-H_3, 2× ^{14}Leu-δ-H_3, 8× Val-γ-H_3, ^{10}Leu-γ-H, 2× ^{4}Leu-δ-H_3), 0.90 (m, 6 H, $^{11–12}$THP-δ^{i}-H_3), 0.93 (m, 1 H, ^{14}Leu-γ-H), 1.05 (m, 1 H, ^{14}Leu-β-H_A), 1.22 (m, 2 H, $^{11–12}$THP-ε-H_A, $^{11–12}$THP-γ-H_A), 1.24 (m, 1 H, ^{10}Leu-β-H_A), 1.28 (m, 1 H, ^{14}Leu-β-H_B), 1.34 (m, 1 H, ^{4}Leu-γ-H), 1.35 (m, 3 H, ^{5}Ala-β-H_3), 1.39 (m, 3 H, ^{3}Ala-β-H_3), 1.46 (m, 3 H, $^{11–12}$THP-β^{i}-H_2, $^{11–12}$THP-γ-H_B), 1.47 (m, 1 H, $^{11–12}$THP-δ-H_A), 1.52 (m, 1 H, ^{10}Leu-β-H_B), 1.56 (m, 1 H, ^{4}Leu-β-H_A), 1.58 (m, 1 H, $^{11–12}$THP-γ^{i}-H), 1.63 (m, 1 H, ^{4}Leu-β-H_B), 1.78 (m, 1 H, $^{11–12}$THP-δ-H_B), 1.82 (m, 1 H, $^{11–12}$THP-ε-H_B), 2.07 (m, 1 H, Val-β-H), 2.10 (m, 2 H, ^{1}Val-β-H, Val-β-H), 2.11 (m, 1 H, Val-β-H), 3.15 (m, 1 H, ^{13}Trp-β-H_A), 3.17 (m, 3 H, ^{9}Trp-β-H_2, ^{15}Trp-β-H_A), 3.20 (m, 1 H, ^{13}Trp-β-H_B), 3.25 (m, 2 H, EAN-α-H_2), 3.37 (m, 1 H, ^{15}Trp-β-H_B), 3.41 (m, 1 H, $^{11–12}$THP-β-H), 3.45 (m, 1 H, EAN-β-H_A), 3.54 (m, 1 H, EAN-β-H_B), 3.76 (m, 1 H, ^{2}Gly-α-H_A), 3.82 (m, 1 H, $^{11–12}$THP-ζ-H), 3.95 (m, 1 H, ^{2}Gly-α-H_B), 4.00 (m, 1 H, $^{11–12}$THP-α-H), 4.09 (m, 1 H, ^{14}Leu-α-H), 4.14 (m, 1 H, Val-α-H), 4.15 (m, 1 H, ^{1}Val-α-H), 4.20 (m, 1 H, Val-α-H), 4.22 (m, 1 H, Val-α-H), 4.26 (m, 1 H, ^{4}Leu-α-H), 4.27 (m, 1 H, ^{10}Leu-α-H), 4.34 (m, 1 H, ^{3}Ala-α-H), 4.36 (m, 1 H, ^{5}Ala-α-H), 4.55 (m, 1 H, ^{9}Trp-α-H), 4.56 (m, 1 H, ^{15}Trp-α-H), 4.64 (m, 1 H, ^{13}Trp-α-H), 7.00–7.15 (m, 11 H, Ar), 7.32–7.39 (m, 4 H, Ar), 7.48 (m, 1 H, EAN-NH), 7.68 (m, 1 H, $^{11–12}$THP-NH), 7.73 (m, 1 H, ^{13}Trp-NH), 7.76 (m, 1 H, Val-NH), 7.77 (m, 1 H, Val-NH), 7.83 (m, 1 H, ^{9}Trp-NH), 7.84 (m, 1 H, Val-NH), 7.85 (m, 1 H, ^{15}Trp-NH), 7.90 (m, 1 H, ^{14}Leu-NH), 7.92 (m, 1 H, ^{10}Leu-NH), 7.97 (m, 1 H, ^{5}Ala-NH), 8.02 (m, 2 H, ^{3}Ala-NH, ^{4}Leu-NH), 8.14 (m, 1 H, CHO), 8.21 (m, 1 H, ^{1}Val-NH), 8.35 (m, 1 H, ^{2}Gly-NH), 10.02 (s, 1 H, indole-NH), 10.13 (s, 1 H, indole-NH), 10.19 (s, 1 H, indole-NH) ppm. HR-MS calcd. 1779.04 found 1779.2.

NMR Spectroscopy and Peak Assignment for Restraint Generation: NMR experiments were performed with a solution of THP peptide **17** (4.6 mM in $CDCl_3$/[D_3]MeOH, 1:3) and with a solution of the THP amide **12** (1.4 mM in $CDCl_3$), respectively. H_2O proton resonance in the $CDCl_3$/MeOH mixture was presaturated for signal suppression. The spectra were processed using the UXNMR program; all resonances were calibrated to the residual $CHCl_3$ proton signal (δ = 7.24 ppm). The peak assignments and NOE peak volume calculations were done with SPARKY (www.cgl.ucsf.edu/home/sparky). For the intraresidue resonance assignments of **17**, DQF-COSY(500 MHz) and TOCSY (500 MHz) spectra were used. Sequential assignment was taken from the NOESY spectrum (500 MHz, 300 ms mixing time). The high mixing time was necessary to ensure a sufficient peak intensity. For **12**, DQF-COSY (600 MHz) and NOESY (600 MHz, 400 ms mixing time) spectra were used (resonances, coupling constants and temperature coefficients see Table S1a and S1b, NOE peak lists see Table S2a and S2b in the Supporting Information). NOESY peaks were integrated using the Gaussian fit method. Coupling constants were taken directly from the corresponding 1D proton spectra (500 MHz, presat. H_2O suppression). The temperature coefficients for the amide protons of THP were calculated from a suite of 8 proton spectra at different temperatures between 278 K and 313 K.

Computational Procedure: All calculations were performed on a SGI Origin server (4x R12000). For practical reasons, both structures were calculated with an N-terminal acetamide group instead of a carbamate group (**12** → **22**, **17** → **23**). For **17**, the C-terminal TBDPS group was omitted. *Parameterisation of the THP, benzyl, and ethanolamine building blocks:* Energies and ESP charges were calculated using GAMESS (6-313G* basis set). The RESP fit was performed using a two-step procedure described by Bayley et al.[17] For all structures, standard AMBER atom types and standard AMBER parameters could be assigned.

Structure Calculations: Structures were calculated using AMBER6[18] (sander_classic, 2 processors) in a restrained 40 ps simulated annealing (SA) procedure (300 → 1500 → 500 → 0 K) in vacuo with subsequent restrained minimisation (for the exact conditions see Supporting Information). A total of 100 structures was sampled using the same start structure and different random seeds for every SA run. All stereogenic carbon atoms as well as amide bonds were constrained using torsional restraints (*fc* = 50 kcal/mol). The numbers of the used distance and *J* coupling restraints as well as the force constants (fc) are summarized in Table 1. The distances were calculated from the NOE peak volumina applying the r^6 relationship and using the distance between two geminal methylene protons (1.8 Å) as a reference. For the THP amide **12**, the ring protons at THP-Cε were used, for the THP peptide **17** the protons on Trp4-Cβ were evaluated. Because of the high NOESY

mixing time, all calculated distances had to be checked for consistence manually to avoid spin-diffusion artefacts. The lower boundary was set to $d-0.3$ Å, the upper boundary to $d+0.3$ Å. The boundaries for the J coupling constants were set to $J-0.5$ and $J+0.5$, respectively.

Table 1. Number of distance and *J* coupling restraints (*N*) and their force constants (*fc*) for the THP amide **12** and the THP peptide **17**.

	12		**17**	
	N	*fc*[kcal/mol]	*N*	*fc*[kcal/mol]
Distance restraints	24	50	23	100
Intraresidue			16	
Sequential			6	
Medium range			1	
J coupling restraints	–	–	8	10

The structure calculation proceeded in an iterative process, in which H-bond length restraints for amide protons which are known to be H-bonded from the temperature-dependent NMR experiments were added. The first run was performed without any H-bond restraints. After analysis of the resulting structures, several combinations of H-bonds were tested. The final H-bond length restraints are summarized in Table 2.

Table 2. H-bond length restraints for **17** applied in the last SA run.

N–H	C=O	Upper boundary [Å]
THP3	Ace	2.2
Trp4	Leu2	3.2
Trp6	THP3	3.2
	Trp4	3.2
EAN7	THP3	3.2
	Trp4	3.2
	Leu5	3.2

All structures with proper angle and dihedral geometries which did not exceed the upper boundary of the distance restraints by more than 0.1 Å (12) or 0.2 Å (17) and the upper and lower boundaries of the *J* coupling restraints by more than 0.2 Hz were considered as unviolated. From these structures, the final ensemble of 20 least-energy structures was chosen. The structures were fitted and averaged using the CARNAL module (AMBER7). The fitting procedure included the C-α atoms of Leu2 and Trp4, and the C-α, C-β, and C-ε atoms of the THF amino acid residue. Additionally, a H-bond analysis (distance: 3.5 Å; angle: 40 deg) was performed with CARNAL.

Single-Channel Conductance Measurements: The two compartments of a measuring chamber unit (cuvette: CP2A of polystyrene, bilayer chamber: BCH-22A, manufacturer: Warner Instruments) were filled with a salt solution (c = 1 mol/L, if not indicated differently) to the same hight. After inserting the electrodes (silver electrodes, coated with AgCl by electrolysis in 0.1 M HCl) a potentially existing offset was corrected. A membrane was generated over the cuvette aperture (ø = 200 or 150 μm) by painting. For this purpose, 1 μL of the lipid solution (25 mg/mL, in *n*-decane) was given onto a teflon-encased metal loop and painted over the aperture, until a membrane formed (detected by current flow between both chambers falling to 0 pA, optical evaluation by the typical interference). After measuring the capacity of the membrane (specific membrane capacity 0.4 μF/cm^2) a solution of the ion channel forming compound (c = $1\cdot10^{-4}$ to $1\cdot10^{-14}$, in MeOH) was given into both compartments. On the *cis* electrode voltages between +200 mV and –200 mV were applied; the *trans* electrode was virtually grounded. Thereupon current measurents were smoothed by a 1 kHz low-pass filter, amplified (Patch Clamp amplifier AXO Patch 200B, Axon Instruments) and digitized (5 kHz, DigiData A/D converter). Finaly, data was filtered by a Gauss filter with a low-pass frequency of 50 Hz.

Supporting Information (see also the footnote on the first page of this article): Table S1a, proton chemical shifts, coupling constants, and temperature coefficients for **17** in $CDCl_3$/[D_3]MeOH (3:1). Table S1b, proton chemical shifts for **12** in $CDCl_3$. Table S2, assignments and volume of the NOESY peaks for **17** in $CDCl_3$/[D_3]-MeOH (3:1). Graphic, C-atom numbering used for the NMR-based structure calculation.

Acknowledgments

Generous support by the Deutsche Forschungsgemeinschaft (FG 495) and the Fonds der Chemischen Industrie is greatfully acknowledged.

[1] B. Hille, *Ion channels of excitable membranes*, **2001**, Sinauer, Sunderland MA.
[2] a) D. Doyle, J. M. Cabral, R. A. Pfuetzner, A. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait, R. MacKinnon, *Science* **1998**, *280*, 69–77; b) R. MacKinnon, *Angew. Chem.* **2004**, *116*, 4363–4367; *Angew. Chem. Int. Ed.* **2004**, *43*, 4265–4277.
[3] a) S. Matile, A. Som, N. Sorde, *Tetrahedron* **2004**, *60*, 6405–6435; b) Y. Kobuke, in: *Advances in Supramolecular Chemistry*, vol. 4 (Ed.: G. W. Gokel), **1997**, JAI-Press, Greenwich, 163–210; c) G. W. Gokel, R. Ferdani, J. Liu, R. Pajewsky, H. Shabany, P. Uetrecht, *Chem. Eur. J.* **2001**, *7*, 33–39; d) N. Voyer, *Top. Curr. Chem.* **1996**, *184*, 1–37; e) T. Lougheed, V. Borisenko, T. Hennig, K. Rück-Braun, G. A. Woolley, *Org. Biomol. Chem.* **2004**, *2*, 2798–2801; f) U. Koert, L. Al-Momani, J. R. Pfeifer, *Synthesis* **2004**, 1129–1146.
[4] a) D. J. Chadwick, G. Cardew, *Gramicidin and related ion-channel forming peptides*, **1999**, Wiley, Chichester; b) R. E. Koeppe II, O. S. Andersen, *Annu. Rev. Biophys. Biomol. Struct.* **1996**, *25*, 231–258.
[5] R. R. Ketchem, W. Hu, T. A. Cross, *Science* **1993**, *261*, 1457–1460.
[6] a) R. E. Koeppe II, F. J. Sigworth, G. Szabo, D. W. Urry, A. Woolley, *Nat. Struct. Biol.* **1999**, *6*, 609; b) T. A. Cross, A. Arseniev, B. A. Cornell, J. H. Davis, J. A. Killian, R. E. Koeppe II, L. K. Nicholson, F. Separovic, B. A. Wallace, *Nat. Struct. Biol.* **1999**, *6*, 610–611; c) B. M. Burkhart, W. L. Duax, *Nat. Struct. Biol.* **1999**, *6*, 611–612.
[7] V. Borisenko, D. C. Burns, Z. Zhang, G. A. Woolley, *J. Am. Chem. Soc.* **2000**, *122*, 6364–6370.
[8] H.-D. Arndt, A. Knoll, U. Koert, *Angew. Chem.* **2001**, *113*, 2137–2140; *Angew. Chem. Int. Ed.* **2001**, *40*, 2076–2078.
[9] J. R. Pfeifer, P. Reiß, U. Koert, *Angew. Chem.* **2006**, *118*, 515–518; *Angew. Chem. Int. Ed.* **2006**, *45*, 501–504.
[10] a) A. Vescovi, A. Knoll, U. Koert, *Org. Biomol. Chem.* **2003**, *1*, 2983–2997; b) A. Schrey, A. Vescovi, A. Knoll, C. Rickert, U. Koert, *Angew. Chem.* **2000**, *112*, 928–931; *Angew. Chem. Int. Ed.* **2000**, *39*, 900–902; c) A. Schrey, F. Osterkamp, A. Straudi, C. Rickert, H. Wagner, U. Koert, B. Herrschaft, K. Harms, *Eur. J. Org. Chem.* **1999**, 2977–2990.
[11] a) J. Jurczak, A. Golebiowski, *Chem. Rev.* **1989**, *89*, 149–164; b) R. A. Ward, G. Procter, *Tetrahedron* **1995**, *51*, 12301–12318.
[12] R. M. Devant, H. E. Radunz, in: *Stereoselective Synthesis* (Houben-Weyl, Eds. G. Helmchen, R. W. Hoffmann, J. Mulzer, E. Schaumann), vol. E21, 1151, Thieme, Stuttgart, **1996**.
[13] A. J. Mancuso, S. L. Huang, D. Swern, *J. Org. Chem.* **1978**, *43*, 2480–2482.

[14] B. S. Bal, W. E. Childers, H. W. Pinnick, *Tetrahedron* **1981**, *37*, 2091–2096.
[15] H.-D. Arndt, A. Vescovi, A. Schrey, J. R. Pfeifer, U. Koert, *Tetrahedron* **2002**, *58*, 2789–2801.
[16] G. Eisenman, R. Horn, *J. Membr. Biol.* **1983**, *76*, 197–225.
[17] C. I. Bayley, P. Cieplak, W. D. Cornell, P. A. Kollman, *J. Phys. Chem.* **1993**, *97*, 10269–10280.
[18] W. D. Cornell, P. Cieplak, C. I. Bayly, I. R. Gould, K. M. Merz, D. M. Ferguson, D. C. Spellmeyer, T. Fox, J. W. Caldwell, P. A. Kollman, *J. Am. Chem. Soc.* **1995**, *117*, 5179–5197.
Received: January 27, 2006
Published Online: April 10, 2006

