ARTICLE

OBC
www.rsc.org/obc

Synthesis and functional studies of THF–gramicidin hybrid ion channels †

Andrea Vescovi,[b] Andrea Knoll[b] and Ulrich Koert*[a]

[a] *Fachbereich Chemie, Philipps Universität, D-35032 Marburg, Germany. E-mail: koert@chemie.uni-marburg.de; Fax: +49 6421282 5677; Tel: +49 6421 282 6970*

[b] *Institut für Chemie, Humboldt-Universität Berlin, Brook-Taylor-Str. 2, D-12489 Berlin, Germany*

Received 21st March 2003, Accepted 23rd June 2003
First published as an Advance Article on the web 11th July 2003

THF–gramicidin hybrids **2**–**4** with the L-THF amino acid **1** in positions 11 and 12 and compounds **5**–**8** with the D-THF amino acid **ent-1** in positions 10 and 11 were synthesized and their ion channel properties were studied by single-channel-current analysis. The replacement of positions 11 and 12 by the L-THF amino acid **1** gave a strongly reduced channel performance. In contrast, replacement of positions 10 and 11 by the D-THF amino acid **ent-1** gave rise to new and interesting channel properties. For the permeability ratios, the ion selectivity shifts from Eisenman I towards Eisenman III selectivity and the channels display ms-dynamics. Most remarkable is the asymmetric compound **8**, which inserts selectively into a DPhPC membrane and displays voltage-directed gating dynamics.

Introduction

Biological ion channels transport cations or anions through the phospholipid bilayer of biological membranes.[1] The channel transport is a passive process driven by a concentration gradient or a membrane potential. The control of biochannels (opening/closing) occurs *via* ligand gating, voltage gating, mechanosensitive response or temporary covalent modification. Recent progress in X-ray crystallography has given much insight into the structure and function of biological potassium channels and chloride channels.[2] Genetically encoded channel malfunction can cause channelopathies leading to cardiovascular, pulmonary or neuronal diseases.[3]

The design, synthesis and functional analysis of synthetic ion channels contributes to the understanding of channel function and control.[4] A long term objective for synthetic ion channels is their implantation into biological systems.[5] Another possible application lies in the area of sensing and nanoelectric devices.[6]

As part of an ongoing project on synthetic ion channels we focus on several objectives:

– the correlation of the channel function with structural data for the channel-active conformation;[7]

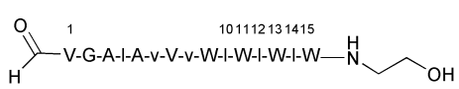
– the use of molecular switches for channel gating;[8]

– ion-selectivity;[9]

– implantation of synthetic channels into biological systems;[5]

– the control of transversal asymmetry for channel insertion and function.[10]

The naturally occuring, ion channel-active gramicidin A (gA) is a suitable lead structure for the design of synthetic channels.[11] gA is a pentadecapeptide with an *N*-formyl and an aminoethanol *C*-terminus.



gramicidin A (gA)

Its alternative sequence of L- and D-amino acids favours β-helical secondary structures.[12] In a membrane, two gA monomers form a head-to-head hydrogen-bridged dimer, which acts as an ion channel for monovalent cations. The structure of the channel-active conformation was determined as a right-handed single-stranded β-helix with 6.3 residues per turn by solid state-NMR.[12]

A covalent linkage of the two *N*-termini *via* a succinyl bridge blocks the dissociation of the channel-active dimer and opens the way for studying unimolecular channels.[13] When synthetic building blocks are coupled with the gA-motif, hybrid channels result which show new functionality (photoswitching with azobenzene,[14] ion selectivity with cyclohexyl ether amino acids[9]).

The tetrahydrofuran amino acids (THF amino acids) **1** and **ent-1** insert an ether oxygen atom into the channel architecture.[15–17] The gA-channel entrance is formed by the first turn of the β-helix which consists of D-Leu10-Trp11-D-Leu12-Trp13-D-Leu14-Trp15. This is the place where the water molecules of the cation hydration shell are removed until two water molecules per cation remain for the single-file region of the channel interior.[18] The exchange of two amino acids of the entrance region for a THF amino acid leads to an alternation of the available cation binding sites (amide⟶ether oxygen). Two possible modifications of the gA-entrance sequence **I** were chosen for closer examination (Scheme 1):

– replacement of Trp11-D-Leu12 with the L-THF amino acid **1** resulting in the sequence **II**;

– replacement of D-Leu10-Trp11 with the D-THF amino acid **ent-1** resulting in the sequence **III**.

Both replacements occur with retention of stereochemical information at the α carbon atoms of the amino acids. Here, we report on the synthesis and functional studies of the following series of THF–gA hybrids. The replacement by the L-THF amino acid **1** results in the target compounds **2**–**4** (Fig. 1).

The replacement by the D-THF amino acid **ent-1** leads to the target structures **5**–**8** (Fig. 2).

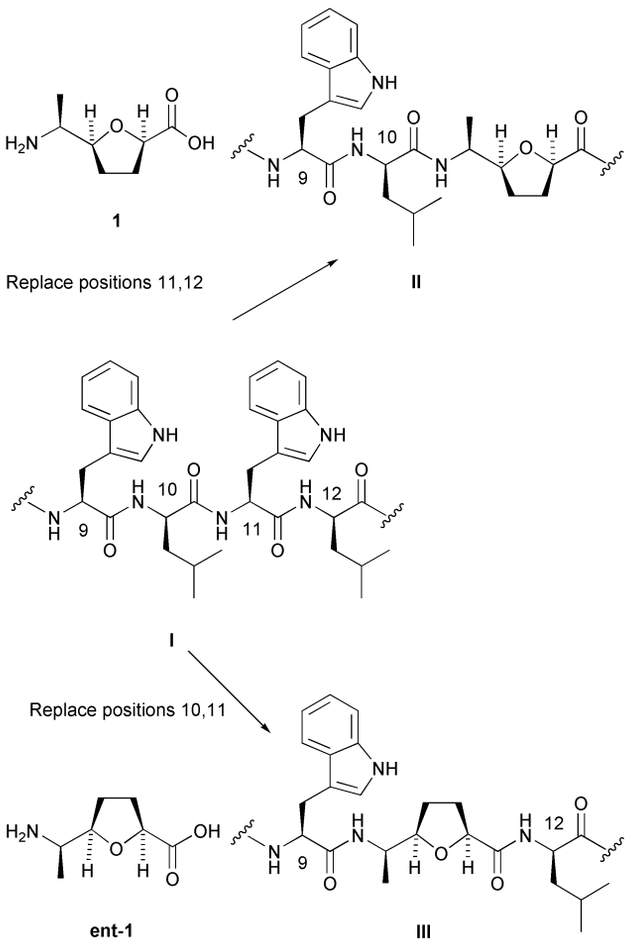
Results and discussion

The stereoselective synthesis of the THF amino acids **1** and **ent-1** used L- and D-alanine as enantiopure starting materials.[15] The synthetic route is exemplified in Scheme 2 for the preparation of compound **13**, the *N*-Boc-protected form of **ent-1**.

Reduction of *N*-Boc-D-alanine methyl ester **9** gave the aldehyde **10**, which was allowed to react with buten-1-yl lithium cuprate to produce the alcohol **11** (diastereoselectivity 20 : 1,

† Electronic supplementary information (ESI) available: HPLC-traces and ^{1}H-NMR spectra for the THF–gA-hybrids and Table S1: conductances of compounds **5**, **6**, **7** and gramicidin A for monovalent cations. See http://www.rsc.org/suppdata/ob/b3/b303249n/

DOI: 10.1039/b303249n



Scheme 1

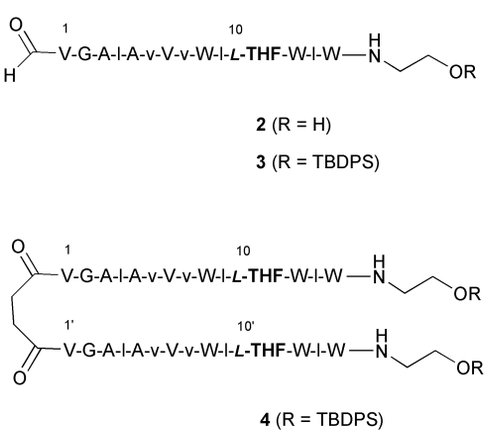


Fig. 1

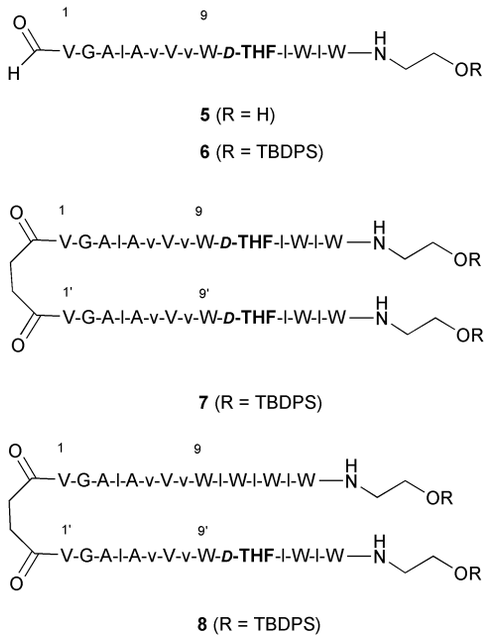
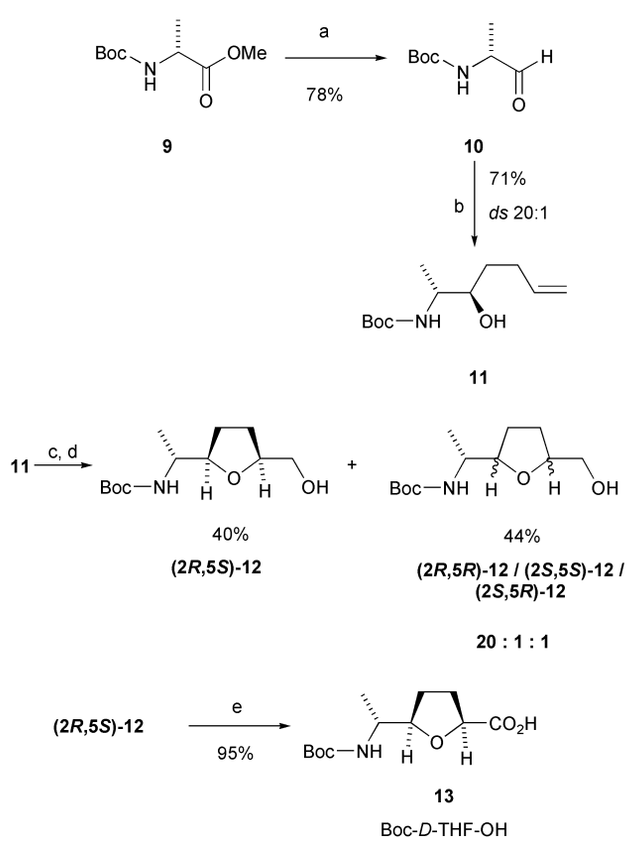


Fig. 2

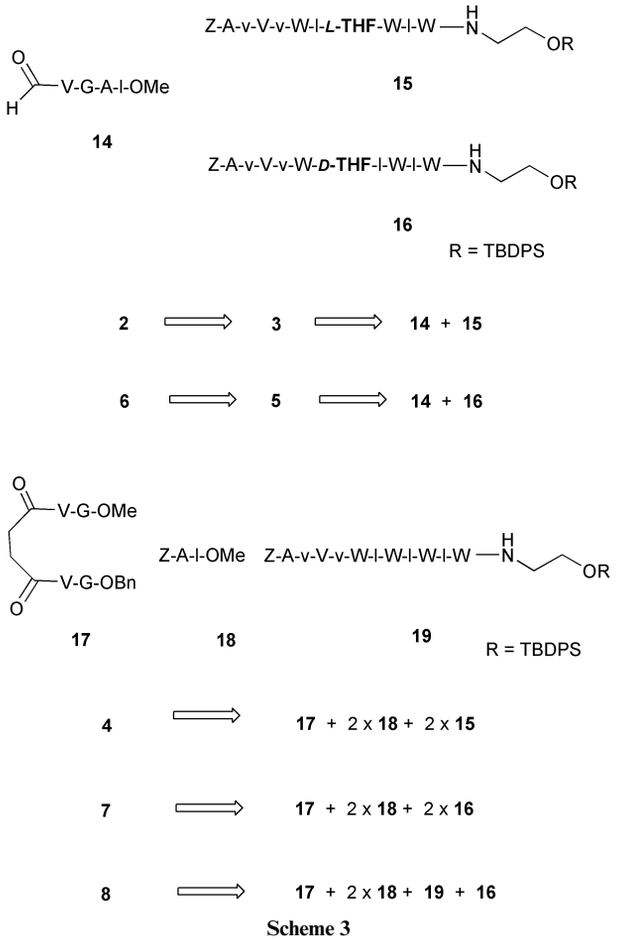


Scheme 2 (a) DIBAL-H (2.0 eq.), CH_2Cl_2–*n*-hexane 30 : 1, −60 °C; (b) (i) lithiation of 1-bromo-3-butene (4.5 eq.): *t*-BuLi (9.9 eq.), Et_2O–*n*-hexane 1 : 1, −78 °C; (ii) cuprate-addition: 1-lithium-3-butene (4.5 eq.), $CuBr{\cdot}SMe_2$ (2.2 eq.), Et_2O–*n*-hexane 2 : 1, −40 °C⟶−80 °C; (c) MCPBA (2.0 eq.), CH_2Cl_2, 0 °C⟶rt; (d) CSA (0.04 eq.), CH_2Cl_2, rt; (e) iodobenzene diacetate (2.2 eq.), 2,2,6,6-tetramethylpiperidin-1-yloxyl free radical (0.05 eq.), CH_2Cl_2, rt.

chelation control). Epoxidation of the terminal double bond and a subsequent acid-mediated intramolecular epoxide opening gave, after chromatographic separation, the D-*cis*-THF alcohol (2*R*,5*S*)-**12** and the corresponding *trans*-THF alcohol together with the minor stereoisomers from the cuprate addition. A one-step oxidation of (2*R*,5*S*)-**12** led to the desired *N*-Boc-protected D-*cis*-THF amino acid **13** (Scheme 2). The synthesis of the target peptides was accomplished by fragment coupling in solution. Although protocols for the solid-phase synthesis of gA are available,[19] we chose the fragment-coupling strategy[20] for the following reasons: higher purity, larger quantities, modular approach with predictable diversity.

The retrosynthetic analysis of the target peptides is summarized in Scheme 3. All four monomers **2**, **3**, **5**, and **6** share the common tetramer **14**. Thus, disconnection between D-Leu4 and Ala5 leads to the decamer **15** containing the L-THF amino acid **1** and the decamer **16** which includes the D-THF amino acid **ent-1**.

The covalently linked dimers **4**, **7**, and **8** were assembled from the two building blocks **17** and **18**, as well as the decamer **15**, the decamer **16** and the undecamer **19**. The use of the tetramer clamp **17** results in final Gly–Ala coupling steps, which in our hands proved to be more efficient than the previously reported



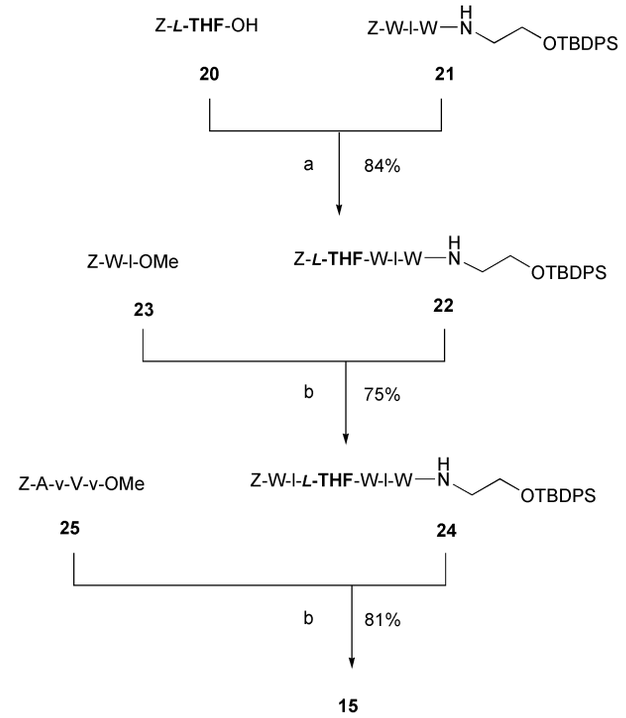
Scheme 3

succinyl–Val or tartrate–Val couplings for the synthesis of covalently linked gA dimers.[13,20] The lipophilic TBDPS-group at the aminoethanol terminus increases the solubility in organic solvents and eases chromatographic purification. Z/OMe protections were used throughout the synthesis due to their mild deprotection conditions and the possibility of leaving the Trp side chain unprotected.

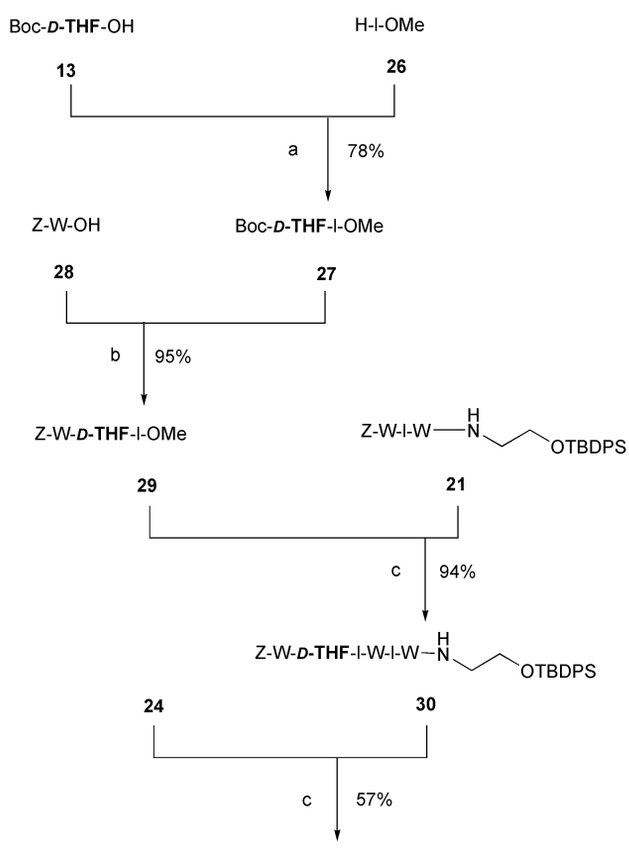
The synthesis of the decamer **15** containing the L-THF **1** utilizes HBTU–HOBt couplings in CH_2Cl_2 or CH_2Cl_2–DMF to assemble the peptide sequence (Scheme 4). Reaction of Z-L-THF-OH **20** with the ethanolamine-tagged tripeptide **21**[20] resulted in the tetramer **22**. A detailed discussion of the proper choice of deprotection conditions (H_2, Pd/C and LiOH–THF–MeOH) is given in our earlier work on minigramicidins.[20] Coupling of **22** with the dipeptide **23**[20] gave the hexamer **24**, which was further elaborated using the tetramer **25**[20] to yield the desired decamer **15**.

The decamer **16** containing the D-THF **ent-1** was synthesized starting with the coupling of Boc-D-THF-OH **13** and H-D-Leu-OMe **26** to the dimer **27** (Scheme 5). Addition of Z-Trp-OH **28** led to **29**. The latter was coupled with the ethanolamine-tagged tripeptide **21** to produce the hexamer **30**. The conjugation of **24** and **30** completed the assembly of the decamer **16**.

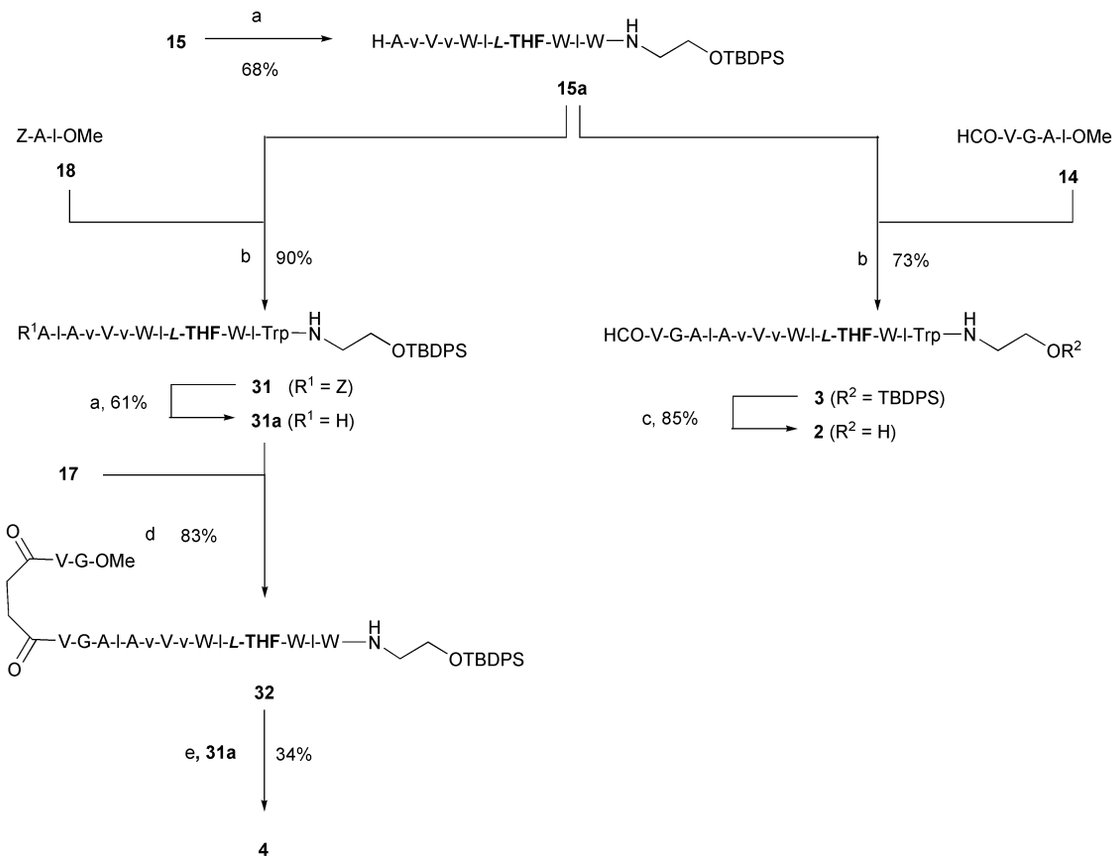
To proceed towards the L-THF–gA hybrids **2**, **3**, and **4** (Scheme 6), the decamer **15** was *N*-deprotected by hydrogenolysis and the resulting amine **15a** was coupled with the *N*-formyl tetramer **14** to yield compound **3** in 73% yield. Cleavage of the TBDPS-group in **3** with 5% HF in CH_3CN gave **2**. Treatment of the methyl ester **18** with LiOH resulted in the corresponding carboxylic acid, which was coupled with the amine **15a** to give the dodecamer **31**. After hydrogenolytic clevage of the Z-group the amine **31a** was obtained and subsequently conjugated with the the tetrameric clamp **17** to produce compound **32**. Hydrolysis of the methyl ester in **32** and



Scheme 4 (a) *Z*-group cleavage: Pd/C, H_2 (1 bar), MeOH, rt; coupling: EDC (1.5 eq.), HOBt (3.0 eq.), $(i\text{-Pr})_2$EtN (3.0 eq.), CH_2Cl_2, 0 °C⟶rt; (b) methyl ester deprotection: 2.5 eq LiOH, THF–H_2O 3 : 1, 0 °C; Z-group cleavage: Pd/C, H_2 (1 bar), MeOH, 40 °C; coupling: HBTU (1.5 eq.), HOBt (3.0 eq.), $(i\text{-Pr})_2$EtN (3.0 eq.), CH_2Cl_2–DMF 5 : 1, −15 °C.



Scheme 5 (a) EDC (1.5 eq.), HOBt (3.0 eq.), $(i\text{-Pr})_2$EtN (3.0 eq.), CH_2Cl_2, 0 °C⟶rt; (b) *Boc*-group cleavage: TFA, CH_2Cl_2, rt; coupling: EDC (1.5 eq.), HOBt (3.0 eq.), $(i\text{-Pr})_2$EtN (3.0 eq.), CH_2Cl_2, 0 °C⟶rt; (c) methyl ester deprotection: 2.5 eq LiOH, THF–H_2O 3 : 1, 0 °C; *Z*-group cleavage: Pd/C, H_2 (1 bar), MeOH, 40 °C; coupling: HBTU (1.5 eq.), HOBt (3.0 eq.), $(i\text{-Pr})_2$EtN (3.0 eq.), CH_2Cl_2–DMF 5 : 1, −15 °C.



Scheme 6 (a) Pd/C, H_2 (1 bar), EtOH, 50 °C; (b) methyl ester deprotection: 2.5 eq LiOH, THF–H_2O 3 : 1, 0 °C; coupling: HBTU (1.5 eq.), HOBt (3.0 eq.), (*i*-Pr)$_2$EtN (3.0 eq.), CH_2Cl_2–DMF 5 : 1, −15 °C; (c) HF (5% in CH_3CN), CH_2Cl_2–DMF 10 : 1, rt; (d) benzyl ester deprotection: Pd/C, H_2 (1 bar), MeOH, rt; coupling: HBTU (1.5 eq.), HOBt (3.0 eq.), (*i*-Pr)$_2$EtN (3.0 eq.), CH_2Cl_2–DMF 5 : 1, −15 °C; (e) methyl ester deprotection: 2.5 eq LiOH, THF–H_2O 3 : 1, 0 °C; coupling: HATU (1.5 eq.), HOAt (3.0 eq.), (*i*-Pr)$_2$EtN (3.0 eq.), CH_2Cl_2–DMF 5 : 1, −15 °C.

coupling of the resulting carboxylic acid with the amine **31a** led to the symmetrical bis-L-THF channel hybrid **4**.

The decamer **16** was converted into the free amine **16a** and coupled with the carboxylic acid prepared from the tetrameric methyl ester **14** (Scheme 7). The desired monomeric D-THF–gA hybrid **6** could be obtained in 87% yield. Deprotection of the silyl ether led to compound **5**. In order to get to the linked dimers containing the D-THF amino acid **ent-1**, the methyl ester **18** was transformed into the corresponding carboxylic acid and coupled with the amine **16a** to yield the dodecamer **33** and its *Z*-deprotected form **33a**, respectively. The benzyl ester **17** could be converted into the free acid and coupled with the amine **33a** to produce compound **34**. Hydrolysis of the methyl ester in **34** and subsequently coupling with a second molecule of **33a** gave the symmetrically bis-D-THF channel hybrid **7**. After deprotection of the methyl ester in **35**,[20] the coupling of the resulting carboxylic acid with the amine **33a** produced the unsymmetrical mono-D-THF channel hybrid **8**.

All gA-hybrid channels **2**–**8** were purified by RP-HPLC and characterized by ^{1}H-NMR and MS (see experimental section and electronic supplementary information (ESI)†). The characterisation of ion channels by single-channel currents is a single-molecule technique and has to fulfil high-purity criteria in order to avoid misinterpretation of channel-active impurities. The advanced synthetic intermediates of Schemes 6 and 7 were all tested routinely for ion channel activity. HPLC-separation was additionally monitored by single-channel current analysis.

For the functional analysis of the ion channels, planar phospholipid bilayers (black-lipid membranes, Fig. 3)[1] were used. If not stated otherwise, soybean lecithin was the phospholipid of choice. The channel formers were added from a methanol stock solution to the *cis* and/or the *trans* side of the measurement chamber. Details are given in the experimental section.

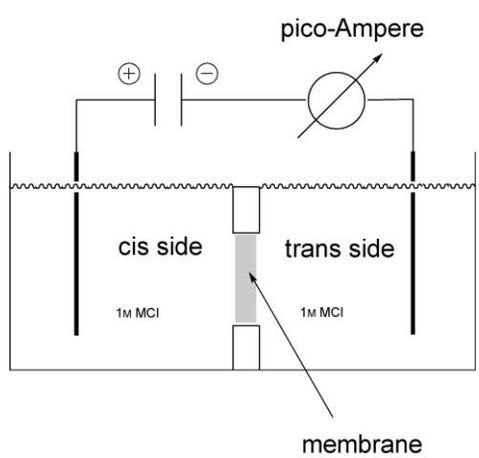


Fig. 3 Experimental setup for single-channel measurements in planar phospholipid bilayers.

The replacement of Trp11-D-Leu12 of gA with the L-THF amino acid **1** resulted in a considerable change in the ion channel properties. Selected traces are shown in the upper part of Fig. 4. For the monomeric hybrids **2** and **3** it was necessary to add the compounds in high concentrations from the *cis* and the *trans* side to see any channels at all. Conductivities were reduced to 10% compared with gA. The observation of single channel currents was restricted to Cs^+. For compounds **2** and **3** multiple noisy events were found. The covalently linked dimer **4** showed better resolved channel signals at lower concentrations (Fig. 4c). Here, it was sufficient to add the compound from the *cis* side only. The channel conductivity of **4** was still low and allowed the detection of Cs^+ currents only. Thus, the use of the L-THF-amino acid **1** in positions 11 and 12 of gA reduces the transport efficiency of the gA channel strongly.

Scheme 7 (a) Pd/C, H_2 (1 bar), EtOH, 50 °C; (b) methyl ester deprotection: 2.5 eq LiOH, THF–H_2O 3 : 1, 0 °C; coupling: HBTU (1.5 eq.), HOBt (3.0 eq.), (*i*-Pr)$_2$EtN (3.0 eq.), CH_2Cl_2–DMF 5 : 1, −15 °C; (c) HF (5% in CH_3CN), CH_2Cl_2–DMF 10 : 1, rt; (d) Pd/C, H_2 (1 bar), EtOH, 50 °C; coupling: HBTU (1.5 eq.), HOBt (3.0 eq.), (*i*-Pr)$_2$EtN (3.0 eq.), CH_2Cl_2–DMF 5 : 1, −15 °C; (e) methyl ester deprotection: 2.5 eq LiOH, THF–H_2O 3 : 1, 0 °C; coupling: HATU (1.5 eq.), HOAt (3.0 eq.), (*i*-Pr)$_2$EtN (3.0 eq.), CH_2Cl_2–DMF 5 : 1, −15 °C.

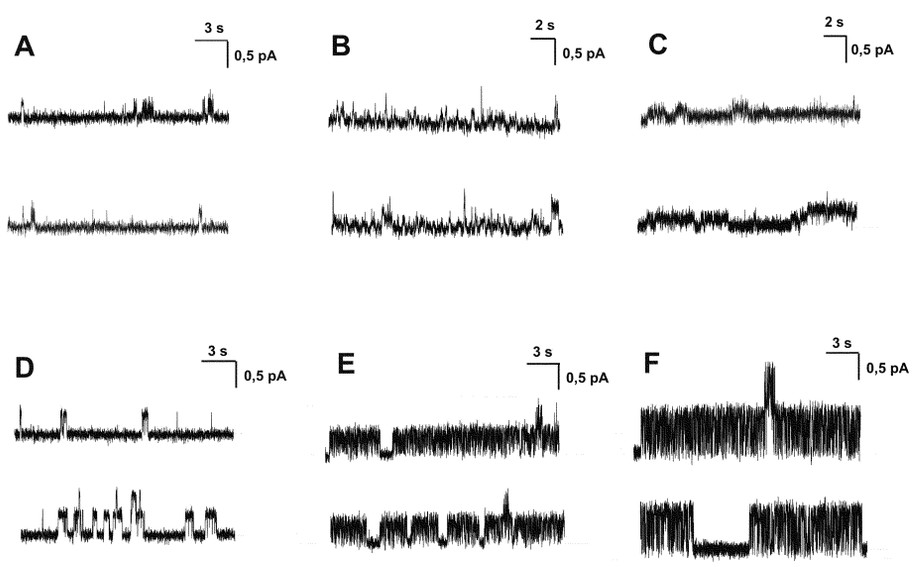


Fig. 4 Channel traces of THF–gA-hybrids. The upper part shows the results from the replacement of positions 11 and 12 by the L-THF amino acid **1**(A: **2**, B: **3**, C: **4**); the lower part shows the results from the replacement of positions 10 and 11 by the D-THF amino acid **ent-1** (D: **5**, E: **6**, F: **7**) in asolectin (1 M CsCl, +170mV).

The effect of the replacement of D-Leu10-Trp11 with the D-THF amino acid **ent-1** was examined next. The monomeric D-THF hybrids **5** and **6** as well as the symmetric, dimeric D-THF-gA hybrid **7** show single channel behavior for Cs^+, Rb^+, K^+ and Na^+ ions (Table 1). The conductivities were much lower for **5**, **6**, and **7** than for gA but the channels were uniform and well defined.

Representative traces are shown in the lower part of Fig. 4. The conductivity series $Cs^+ > Rb^+ > K^+ > Na^+$ is similiar to that for gA (see electronic supplementary information (ESI)†).

Table 1 Single-channel conductances of compounds **7**, **8** and succ(gA-TBDPS)$_2$ for different cations

Compound	Conductance/pS		
	1 M CsCl	1 M KCl	1 M NaCl
8	5.7	2.2	0.6
7	7.0	1.8	0.6
Succ(gA-TBDPS)$_2$	19.1	14.0	7.7

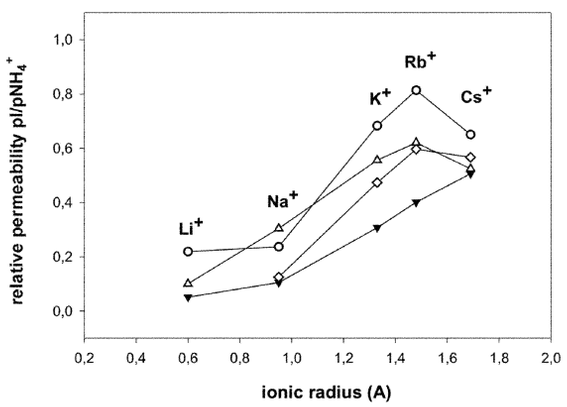


Fig. 5 Relative permeabilities of compounds **5**, **6**, **7** and gA for monovalent cations in relation to the ionic radius of the cation; —○— **6**; —Δ— **7**; —◆— **5**; —▼— gA.

The permeability ratios relative to NH_4^+ reveal a tendency from an Eisenman I towards an Eisenman III selectivity with $Rb^+ > Cs^+$ (see Fig. 5). This behavior is most pronounced for compound **6** and indicates the presence of the donor ether oxygen as a new binding site in the channel.

The single channel events for **6** and **7** display dynamic behavior in the millisecond range (openings and closings: 10–20 ms). This occurs with the monomeric compound **6** and the dimeric compound **7**. Therefore, in the case of **6**, the dimer formation and dissociation cannot be the reason for this type of channel opening and closing. Its cause is the presence of the THF amino **ent-1**, which leads to a type of conformational gating.

The asymmetric compound **8** in its extended β-helical conformation can be schematically described as a cylinder-type structure with a gramicidin (grami) end and a THF end. In Figs. 6–8 the THF end is depicted in red and the grami end in blue. If compound **8** is added from the *cis* side to the membrane there are two modes of insertion possible: a *THF-end-first* mode and a *grami-end-first* mode. If the insertion occurs randomly, two types of channel, A and B, should be possible. Let us assume that both channels are different in functionality, so that both are distinguishable by their current traces. In channel type A the cations enter the channel through the grami end and leave it through the THF end. In channel B the cations enter the channel through the THF end and leave it through the grami end. The entrance and the exit site are determined by the membrane potential. Independent of the membrane potential one expects the occurence of both channel types, A and B, if the insertion occurs randomly (Fig. 6). If the insertion occurs selectively, one type of channel either A or B is possible (Fig. 7). A positive potential at the *cis* side should lead to the observation of channel type A (Fig. 7A). After change of the potential the channel type B should be observed (Fig. 7B).

The addition of compound **8** from the *cis* side to a diphytanoyl phosphatidyl choline (DPhPC) membrane with 1 M CsCl and a membrane potential of +160 mV at the *cis* side gave rise to only one type of channel with a very long dwell time (type 1, Fig. 8A). Change of the potential to −160 mV gave rise to a second type of channel (type 2, Fig. 8B) with a long overall dwell time but with the same short closings and openings that

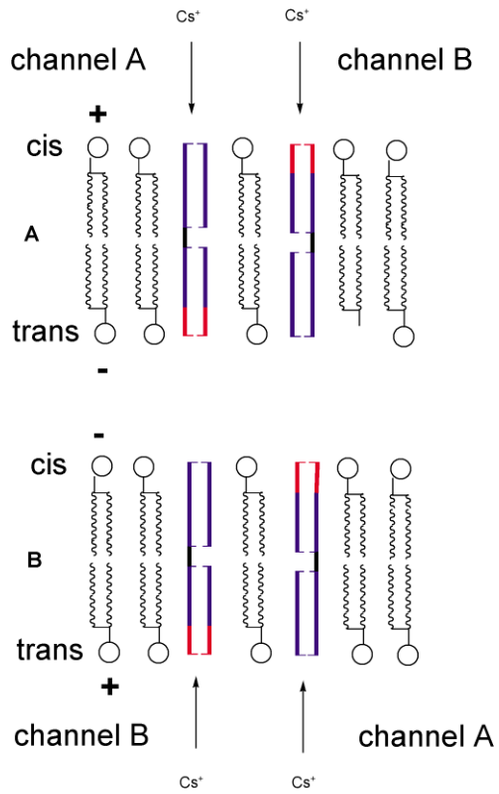


Fig. 6 Two channel types A and B resulting from random insertion of **8** into a membrane.

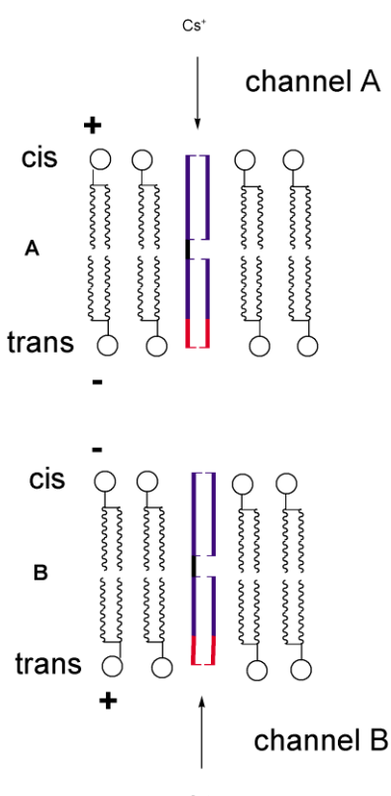


Fig. 7 One channel type either A or B resulting from selective insertion of **8** into a membrane.

were observed for compounds **6** and **7**. Switching the potential back to +160 mV gave rise to the first channel type. This behavior indicates a selective insertion of compound **8** into the membrane. A control experiment to support this hypothesis was done by the addition of compound **8** from the *trans* side. This time, at a membrane potential of +160 mV, the type 2

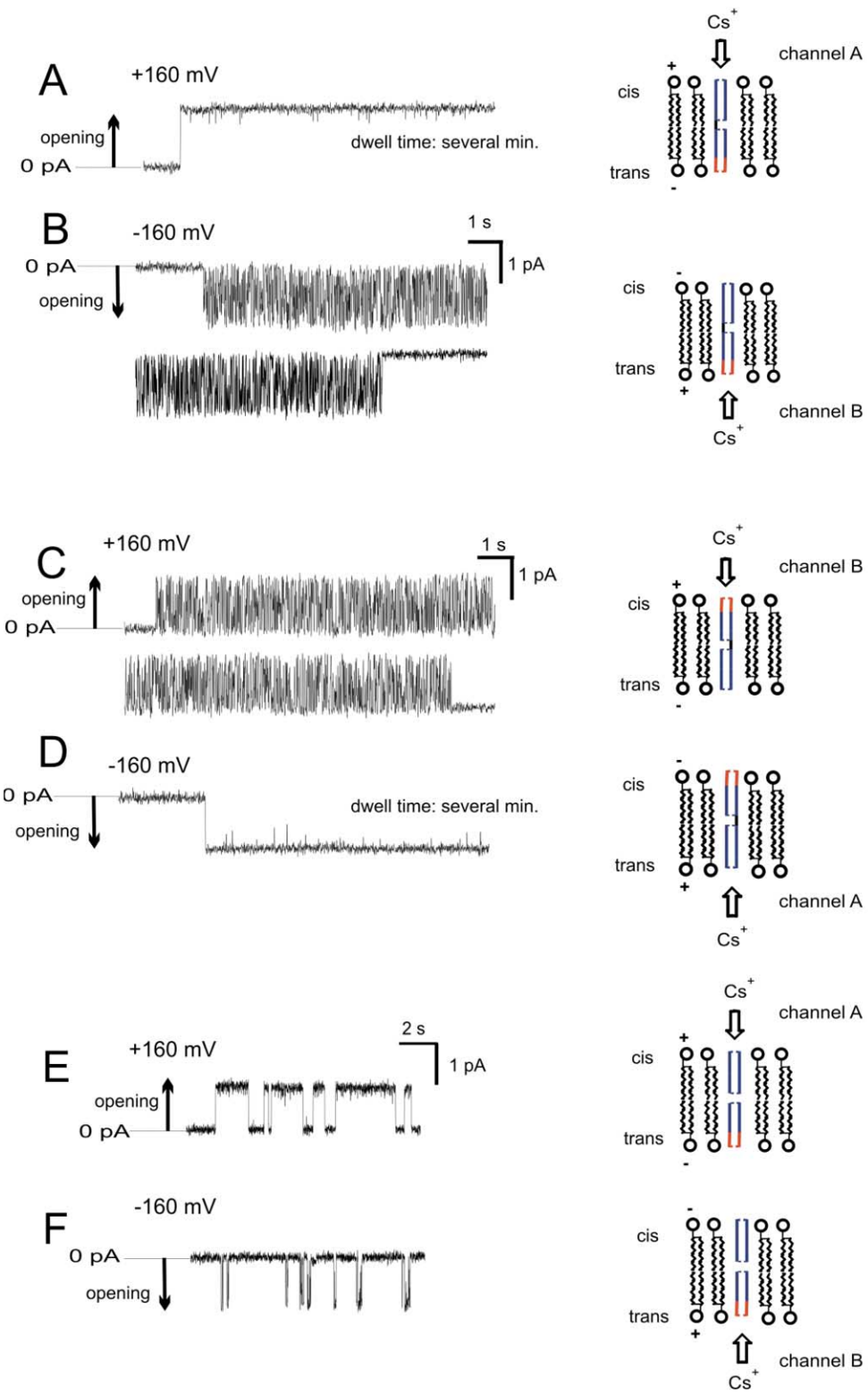


Fig. 8 Selective insertion of compound **8** into the phospholipid bilayer. A and B: addition of **8** from the *cis* side; C and D: addition of **8** from the *trans* side, E and F: addition of gA from the *cis* side and **5** from the *trans* side. Current traces were recorded with 1 M CsCl and DPhPC membranes.

channel (Fig. 8C) and at −160 mV the type 1 channel (Fig. 8D) were observed.

Having proven that a selective insertion had occurred, the question of a *THF-first* or a *grami-first* insertion was addressed next. Towards this end, the monomer compound **5** was added to the *trans* side and gA to the *cis* side. By this token, a predictable *trans* orientation of the THF end and a *cis* orientation of the grami end were accomplished. Mixed **5**–gA channels were formed that showed single channel behavior as depicted in Figs. 7 E and F. Because of the lack of covalent succinyl linker, the dwell times were shorter, but the characteristics in the channel dynamics observed in Figs. 7A–D were found again. If the cations enter the channel from the grami end, stable grami-type channels are formed (Fig. 8E). If the cations enter from the THF end, channels with ms-dynamics are formed (Fig. 8F). It has to be noted, that after 30 min of experiments no transversal mixing was noticeable. The TBDPS-deprotected compounds **5** and gA gave reproducible mixed channels, while their protected counterparts **6** and gA-TBDPS caused trans-bilayer motion (flip/flop) problems due to their higher

lipophilicity resulting in the gA-TBDPS dimer as the major detectable species.

The asymmetric orientation leads to a potential-dependent function, which can be compared with the voltage gating of biological channels. The ms-dynamics (short closings and openings) occur if a positive potential is applied at the THF end. A type of potential-driven conformational switching (**36**⟶**37**) around the THF building block in the gA sequence may cause this behavior. As depicted in Fig. 9 the positive potential next to the THF end may lead a switch into a partial β-turn,[15] channel-inactive form of **37**.

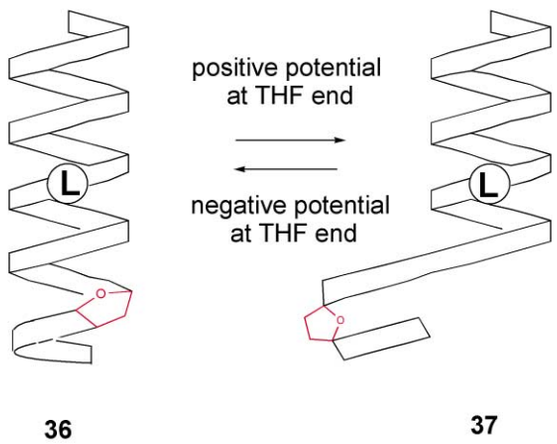


Fig. 9 Schematic representation of a potential-driven conformational switch from an active conformation **36** into an inactive conformation **37**.

The voltage dependence of the gating dynamics for compound **8** was studied over a broader potential range between +200 mV and −200 mV (Fig. 10). Due to the low conductivities in the range between +50 mV and −50 mV no data could be collected in this region. The data show that, for negative potentials, the long lived channel of type A dominates. In the positive range of channel type B a decrease in the dwell time with an increase of the potential is observed.

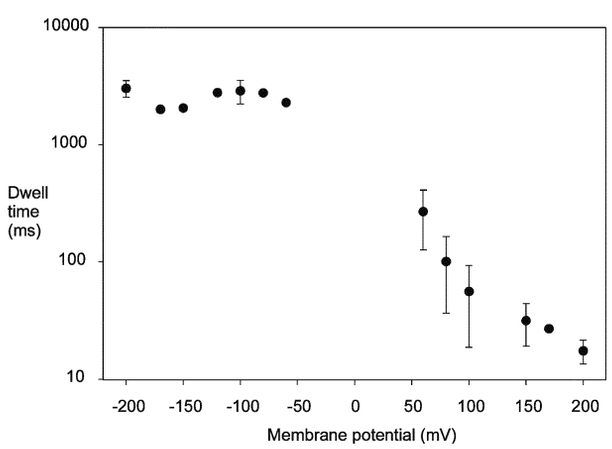


Fig. 10 Voltage dependence of the dwell time for **8** in DPhPC and 1 M CsCl.

No significant difference between positive and negative potential was observed for the conductivity of **8**. There is a rectifying effect on the channel-gating dynamics, but not on the channel conductivities. Single-channel currents for Cs^+, K^+ and Na^+ are summarized in Table 1. Succ(gA-TBDPS)$_2$[20] was used as a reference compound. The conductivities of **7** and **8** are of comparable size and smaller than for succ(gA-TBDPS)$_2$, with an increased selectivity for Cs^+ *versus* K^+ and Na^+.

For a detailed understanding of the potential-dependent function of **8** it will be necessary to perform a detailed structural NMR-analysis of compound **8** in a membrane-like environment with different electrical potentials. This is a challenging problem which hopefully can be solved. Data from other examples of potential-dependent channel tranport by Matile[4f] and Kobuke[4h] will be useful for comparison. The structural reasons for the selective insertion of **8** into the membrane also remain to be clarified.

Conclusion

In summary, it was found that THF amino acids are valuable building blocks to modulate the ion channel activity of gA. The choice of the THF amino acid and the positions of replacement are crucial. The replacement of positions 11 and 12 by the L-THF amino acid **1** leads to compounds **2**, **3**, and **4** with a drastically reduced channel performance. In contrast, replacement of positions 10 and 11 by the D-THF amino acid **ent-1** gave rise to componds **5**–**8** with new and interesting channel properties. The ion selectivity shifts from Eisenman I towards Eisenman III selectivity and the channels display ms-dynamics. Most remarkable is the asymmetric compound **8**, which inserts selectively into a DPhPC membrane and displays voltage-dependent gating dynamics.

Experimental

Analytical techniques

Single channel measurement. Planar lipid membranes were prepared by painting a solution of soybean lecithin (40%, Avanti Polar Lipids, Alabaster, AL) or diphytanoylphosphatidylcholin (DPhPC, Avanti Polar Lipids, Alabaster, AL) in *n*-decane (25 mg ml^{-1}) over the aperture of a polystyrene cuvette with an inner diameter of 0.15 mm. All experiments were performed at ambient temperature. The used electrolyte solutions at a concentration of 1 M each were unbuffered. Probes of monomolecular channels, dissolved in methanol, were added to the *trans* or *cis* side (containing the measuring electrode) of the cuvette. Probes of bimolecular channels, dissolved in methanol, were added to both *trans* and *cis* sides of the cuvette. Current detection and recording was performed with a patch-clamp amplifier Axopatch 200B, a Digidata A/D converter and pClamp6 software (Axon Instruments, Foster City, MA). The acquisition frequency was 5 kHz. The data were filtered with a digital filter at 50 Hz for further analysis.

NMR-spectroscopy. NMR spectroscopy was carried out on Bruker AMX300, DPX600, AMX500 and AX400 instruments. All resonances are referenced to residual solvent signals ($CDCl_3$: 7.25 ppm, DMSO-d_6: 2.49 ppm).

HPLC. HPLC was carried out on a Rainin Dynamax HPLC-System, DA-Detector. Column: Jupiter C5 250 × 4 mm. Method 1 (M1): 65%⟶85% B in 25 min, A: H_2O; B: acetonitrile–isopropanol 2 : 1; 0.7 ml min^{-1}, 30 °C. Method 2 (M2): 60%⟶100% B in 20 min, A: H_2O + 0.1% TFA; B: acetonitrile–isopropanol 2 : 1 + 0.1% TFA; 0.7 ml min^{-1}, 30 °C. Method 3 (M3) 55%⟶65% B in 20 min, A: H_2O; B: acetonitrile–isopropanol 2 : 1; 0.7 ml min^{-1}, 30 °C. Method 4 (M4): 70%⟶100% B in 25 min, A: H_2O + 0.1% TFA; B: acetonitrile–isopropanol 2 : 1 + 0.1% TFA; 0.7 ml min^{-1}, 30 °C.

General procedures

The term "aqueous work-up" refers to an extraction of the organic layer with 1 M $NaHSO_4$, sat. $NaHCO_3$ and brine consecutively (a third of the volume of the organic layer each), followed by drying over $MgSO_4$ and evaporation. Thin layer chromatography (TLC) and flash column chromatography (FCC) were performed on silica gel (Merck Si60, 40–60 μm). Columns for FCC were slurry-packed. TLC-spots were visual-

ised by UV-light and by staining with 2% molybdophosphoric acid in EtOH or 2% *p*-methoxy-benzaldehyde in EtOH followed by 20% H_2SO_4 and heating. Optical rotations are given in 10^{-1} deg cm^2 g^{-1}

Methyl ester deprotection **(GP 1).** The methyl ester was dissolved in THF (0.05 M) and cooled to 0 °C. 2.5 eq. LiOH·H_2O was added as an aqueous solution (1 : 3 v/v H_2O–THF) and stirred until complete conversion. The mixture was then neutralised with a 2 M HCl solution, THF was evaporated and the residue was partitioned between EtOAc (0.01 M) and 1 M $NaHSO_4$. The aqueous layer was extracted with EtOAc, the collected organic fractions were washed with brine and dried over $MgSO_4$. After evaporation the residue was dried to constant weight (0.01 Torr). The crude carboxylic acid proved to be sufficiently pure (TLC, NMR, HPLC).

Z-Group cleavage and benzyl ester deprotection **(GP 2 A+B).** The *Z*-protected compound (or benzyl ester) was dissolved in the indicated solvent (0.02 M) and 10 mol% (2 mol% for benzyl ester deprotection) Pd from Pd/C (5%, Degussa E101 NO/W) was added. The reaction mixture was then degassed, purged with H_2 and hydrogenated under 1 bar at the given temperature. (A): the mixture was vigorously stirred until complete conversion (TLC). The catalyst was then filtered off on Celite® 512 (2 cm). After evaporation and coevaporation with MeOH–toluene the residue was dried to constant weight (0.01 Torr). The crude amine obtained was sufficiently pure. (B): After stirring for 1.5 h the reaction was stopped and the catalyst filtered off on Celite® 512 (2 cm). The fractions were collected, the mixture was reduced to the initial volume and hydrogenated a second time under the same conditions for 2.5 h. After filtration on Celite®, evaporation and coevaporation with MeOH–toluene the residue was dried to constant weight (0.01 Torr). In several cases the crude amines had to be further purified by FCC.

EDC–HOBt-mediated coupling **(GP 3).** The materials were dissolved in CH_2Cl_2 (indicated concentration) and cooled to 0 °C. EDC, HOBt, and diisopropylethylamine (1.5 eq, 3.0 eq and 3.0 eq respectively, relative to the carboxylic acid) were then added consecutively. After 30 min at 0 °C the reaction mixture was stirred at room temperature until conversion was complete.

HBTU–HOBt-mediated coupling **(GP 4).** The materials were dissolved in CH_2Cl_2–DMF 5 : 1 (indicated concentration) and cooled to −15 °C. HBTU, HOBt, and diisopropylethylamine (1.5 eq, 3.0 eq and 3.0 eq respectively, relative to the carboxylic acid) were then added consecutively. After 2 h at −15 °C the reaction mixture was stirred at room temperature until conversion was complete.

HATU–HOAt-mediated coupling **(GP 5).** The materials were dissolved in CH_2Cl_2–DMF 5 : 1 (indicated concentration) and cooled to −15 °C. HATU, HOAt, and diisopropylethylamine (1.5 eq, 3.0 eq and 3.0 eq respectively, relative to the carboxylic acid) were then added consecutively. After 2 h at −15 °C the reaction mixture was stirred at room temperature for 22 h.

Work-up of preparative-HPLC acidic fractions **(GP 6).** The collected acidic HPLC-fractions were neutralised with $NaHCO_3$ (pH paper) and reduced (50%) by evaporation. The obtained mixture was then diluted with $CHCl_3$ (same volume) and after phase separation, the aqueous layer was extracted four times with $CHCl_3$–*i*-PrOH 5 : 1. The collected organic fractions were dried over Na_2SO_4 and after evaporation the obtained pure compound was dried to constant weight.

Experimental procedures and compound characterisation

(2*R*)-2-*N*-*t*-Butoxycarbonyl-aminopropanal 10. 29.5 ml of DIBAL-H (1 M in *n*-hexane, 29.5 mmol) was added dropwise at −60 °C to a solution of *N*-Boc-D-alanine methyl ester **9** in CH_2Cl_2 (0.1 M, 150 ml). After 1.5 h the reaction was quenched with *i*-PrOH (15 ml), warmed to room temperature and the resulting mixture was stirred for 3 h with K-Na-tartrate (30 ml, 1 M). The layers were then separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 ml). The collected organic layers were washed with brine and dried over $MgSO_4$. After evaporation and FCC (220 g, CH_2Cl_2–acetone 25 : 1) 2.0 g (11.6 mmol, 78%) of pure aldehyde **10** was obtained. R_f = 0.68 (CH_2Cl_2–acetone 20 : 1); $\nu_{max}(CHCl_3)/cm^{-1}$ 3687, 3620, 3441, 3013, 2979, 1734, 1707, 1501, 1451, 1393, 1369, 1236, 1166, 1048, 878, 668; δ_H(75 MHz; $CDCl_3$; Me_4Si) 1.27 (3 H, d, *J* 7.5, Ala 3-H_3), 1.39 (9H, s, 3 × *t*-Bu-H_3), 4.09–4.24 (1H, m, 2-H), 4.94–5.11 (1H, m, NH), 9.50 (1H, s, C*H*O); δ_C (300 MHz; $CDCl_3$; Me_4Si) 14.8 (3-CH_3), 28.3 (*t*-Bu-CH_3), 65.4 (2-CH), 199.7 (1-CHO); $[\alpha]_D$ = −33.1, $[\alpha]_{365}$ = −174.0, (*c* = 1.06, $CHCl_3$, 25 °C).

(2*R*)-2-*N*-*t*-Butoxycarbonylamino-6-hepten-3-ol 11. 22 ml of a 1.7 M solution of *t*-BuLi in *n*-hexane were added at −30 °C to 30 ml dry Et_2O. The mixture was then cooled to −78 °C and 1-bromo-3-butene (1.7 ml, 17.1 mmol) was added dropwise under continuous stirring. In a second flask CuBr·SMe_2 (1.77 g, 8.6 mmol) was dried under vacuum (0.01 Torr) and successively suspended in 50 ml dry Et_2O. After cooling to −78 °C, the metallation product 1-lithium-3-butene (52 ml Et_2O–*n*-hexane 1 : 1 mixture) was transfered into the suspension *via* cannula within 1 min. The mixture was warmed to −40 °C and after 5 min a colour change from yellow to dark green indicated the formation of the cuprate. After cooling to −80 °C a solution of the aldehyde **10** (654 mg, 3.8 mmol) in Et_2O (20 ml) was added dropwise and the resulting mixture was stirred for 1.5 h. The reaction was quenched at room temperature by addition of 50 ml of half sat. NH_4Cl. After phase separation the organic layer was extracted with aqueous NH_3 (3 × 50 ml), washed with sat. NH_4Cl (2 × 30 ml), brine and dried over anhydrous $MgSO_4$. Evaporation followed by FCC (100 g, CH_2Cl_2–acetone 20 : 1) gave 614 mg (2.7 mmol, 71%) of the chromatographically inseparable diastereomeric mixture (**11** *syn*/*anti* 20 : 1, estimated by ^{1}H NMR upon integration of the C-1 CH_3 signals) as a colourless oil. R_f = 0.32 (CH_2Cl_2–acetone 20 : 1); $\nu_{max}(CHCl_3)/cm^{-1}$ 3442, 3016, 2980, 2935, 1702, 1502, 1453, 1393, 1368, 1236, 1166, 917; *m*/*z* (EI) 229.1678 (M^+. $C_{12}H_{23}NO_3$ requires 229.1678).

(2*S*,5*R*,1′*R*)-2-(1′-*t*-Butoxycarbonylamino-ethyl)-5-hydroxymethyl-tetrahydrofuran (2*S*,5*R*)-12. The diastereomeric alcohol mixture **11** 614 mg (2.70 mmol) was dissolved in 40 ml CH_2Cl_2 and cooled to 0 °C. A solution of MCPBA (*m*-chloroperbenzoic acid) in CH_2Cl_2 (20 ml, 0.26 M) was then added dropwise and the reaction was stirred overnight at room temperature. After dilution with CH_2Cl_2 (20 ml) the mixture was washed with sat. Na_2SO_3 (2 × 20 ml), brine (2 × 15 ml) and dried over anhydrous Na_2SO_4. The clear solution obtained was reduced to 30 ml and stirred for 3 h with 24 mg (0.10 mmol) of CSA (camphorsulfonic acid). CH_2Cl_2 (30 ml) was then added and the mixture was washed with sat. $NaHCO_3$, brine and dried over $MgSO_4$. After evaporation and FCC (90 g MTBE–PE 3 : 1) 250 mg of the *cis*-THF-alcohol **2*S*,5*R*-12** (1.02 mmol, 40% over two steps) were obtained as a colourless oil as well as 275 mg of the inseparable diastereomeric mixture **(2*S*,5*S*)-12**, **(2*R*,5*R*)-12** and **(2*R*,2*S*)-12** (20 : 1 : 1 respectively). R_f = 0.38 (MTBE–PE 3 : 1); $\nu_{max}(CHCl_3)/cm^{-1}$ 3439, 3009, 2980, 2877, 1699, 1510, 1455, 1368, 1336, 1238, 1165, 1100, 1076, 884; δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.08 (3H, d, *J* 6.7, 2′-H_3), 1.42 (9H, s, 3 × *t*-Bu-H_3), 1.67–1.73 (1H, m, 3-*H*H), 1.82–2.01 (3 H, m, 4-H_2 and 3-H*H*), 2.15 (1H, s, OH), 3.45 (1H, dd, *J* 10.6 and 7.0, C*H*HOH), 3.65–3.73 (2H, m, CH*H*OH and 2-H), 3.99–4.03 (1H, m, 5-H), 4.62 (1H, d, *J* 8.4, NH); δ_C (75 MHz; $CDCl_3$; Me_4Si) 18.1 (2′-CH_3), 26.3 (3-CH_2), 28.3 (*t*-Bu-CH_3), 28.8 (4-CH_2), 50.3 (1′-CH), 64.0 (CH_2OH), 79.4 (*t*-Bu-*C*$(CH_3)_3$),

80.3 (5-CH), 83.4 (2-CH), 156.6 (C=O, Boc); $[\alpha]_D$ = + 3.0, $[\alpha]_{436}$ = +3.9 (*c* = 1.10, $CHCl_3$, 25 °C); *m/z* (FAB) 246.1711 (M + H^+. $C_{12}H_{24}NO_4$ requires 246.1705).

(5*S*,2*R*,1′*R*)-5-(1′-*t*-Butoxycarbonylamino-ethyl)-tetrahydrofuran-2-carboxylic acid (Boc-D-THF-OH, 13). 280 mg (1.13 mmol) of the alcohol **2***S*,5*R*-**12** were dissolved in 15 ml CH_2Cl_2. Two drops of H_2O, 800 mg (2.48 mmol) of iodobenzene diacetate (IBDA) and 9.0 mg (0.06 mmol) of TEMPO were added and the mixture was vigorously stirred for 3 h at room temperature. After dilution with 40 ml CH_2Cl_2 and phase separation, the organic layer was washed with sat. $Na_2S_2O_3$ (4 × 15 ml) until reduction of the residual IBDA was complete (KI/starch paper). The organic layer was then extracted with sat. $NaHCO_3$ (4 × 10 ml) and after phase separation the collected aqueous fractions were acidified with 2 M HCl to pH = 3 (pH paper) and extracted with CH_2Cl_2 (4 × 30 ml). The organic layer was washed with brine and dried with $MgSO_4$. After evaporation and drying to constant weight (0.01 Torr) 278 mg (1.07 mmol, 95%) of the pure carboxylic acid **13** was obtained. R_f = 0.31 ($CHCl_3$–MeOH–HCO_2H 200 : 10 : 1); $\nu_{max}(CHCl_3)/cm^{-1}$ 3442, 3100, 2975, 2932, 2877, 1748, 1691, 1600, 1510, 1430, 1395, 1380, 1343, 1244, 1164, 1086, 840, 770; δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.18 (3H, d, *J* 6.8, 2′-H_3), 1.44 (9H, s, 3 × *t*-Bu-H_3), 1.66 (1H, dddd, *J* 12.3, 10.4, 9.1 and 8.1, 3-H*H*′), 1.93 (1H, dddd, *J* 12.5, 7.8, 6.2 and 3.3, 3-*H*H), 2.09 (1H, dddd, *J* 12.7, 8.1, 4.0 and 3.3, 4-*H*H) 2.30 (1H, dddd, *J* 12.7, 10.4, 9.0 and 7.8, 4-H*H*′), 3.65 (1H, dq, *J* 6.8 and 3.2, 1′-H), 3.98 (1 H, ddd, *J* 9.1, 6.2 and 3.3, 5-H), 4.42 (1H, dd, *J* 9.0 and 4.0, 2-H); δ_C (75 MHz; $CDCl_3$; Me_4Si) 19.5 (2′-CH), 25.5 (4-CH_2), 28.6 (*t*-Bu-CH_3), 85.8 (5-CH), 158.9 (C=O, Boc). $[\alpha]_D$ = + 14.0, $[\alpha]_{365}$ = +43.8 (*c* = 1.80 in EtOH, 25 °C); *m/z* (FAB) 260.1511 (M + H^+. $C_{12}H_{22}NO_5$ requires 260.1498).

Z-L-THF-Trp-Leu-Trp-2-(*t*-butyldiphenylsilyloxy)-ethylamide 22. 1.70 g (1.85 mmol) tripeptide **21** were *N*-deprotected according to GP 2 (MeOH, rt, 2 h; R_f = 0.38 in $CHCl_3$–MeOH–NH_3(aq.) 100 : 10 : 1). The crude product and 390 mg (1.33 mmol) of carboxylic acid **20** were then combined and coupled according to GP 3 (0.03 M, 3 h). After aqueous work-up, FCC (120 g, 3% MeOH in $CHCl_3$) gave 1.19 g (1.12 mmol, 84%) of ethylamide **22** as a colourless foam. R_f = 0.49 ($CHCl_3$–MeOH–HCO_2H 100 : 10 : 5); δ_H (300 MHz; $CDCl_3$; Me_4Si) 0.60 (3H, d, *J* 6.0, Leu δ-H_3), 0.64 (3H, d, *J* 6.4, Leu δ-H_3), 0.97 (12H, s, 2″-H_3, and 3 × *t*-Bu-H_3), 1.08–1.19 (2H, m, Leu β-H_2), 1.20–1.40 (2H, m, Leu γ-H and THF 3- or 4-C*H*H), 1.58–1.77 (1H, m, THF 3- or 4-C*H*H), 1.83–2.00 (2H, m, THF 3- or 4-CH*H*′), 2.90 (1H, dd, *J* 13.9 and 10.2, Trp β-*H*H), 3.05–3.21 (3H, m, 1 × Trp β-*H*H and 2 × Trp β-H*H*′), 3.28 (2H, t, *J* 7.4, 1-H_2), 3.60 (2H, t, *J* 6.4, 2-H_2), 3.62–3.75 (2H, m, THF 1′-CH and THF 5-H), 4.09–4.27 (2H, m, THF 2-H and Leu α-H), 4.37–4.57 (2H, m, 2 × Trp α-H), 4.94 (1H, d, *J* 12.4, Z-*H*H), 5.05 (1H, d, *J* 12.4, Z-*H*H), 6.87–6.95 (2H, m, 2 × ar. CH), 6.98–7.08 (3H, m, 3 × ar. CH), 7.12 (1H, s, ar. CH), 7.22–7.34 (7H, m, 7 × ar. CH), 7.36–7.45 (6H, m, 6 × ar. CH), 7.48–7.69 (7H, m, THF NH and 6 × ar. CH), 7.93–8.05 (2H, m, Leu NH and AE NH), 8.15 (1H, d, *J* 8.7, Trp NH), 8.35 (1H, d, *J* 7.2, Trp NH), 10.78 (1H, s, indole NH), 10.80 (1H, s, indole NH); $[\alpha]_D$ = + 38.3, $[\alpha]_{365}$ = + 134.2 (*c* = 1.2, $CHCl_3$, 20 °C); HPLC: 12.8 min (M1); *m/z* (FAB) 1060.5379 (M + H^+. $C_{61}H_{74}N_7O_8Si$ requires 1060.5368).

Z-Trp-D-Leu-L-THF-Trp-D-Leu-Trp-2-(*t*-butyldiphenylsilyloxy)-ethylamide 24. 627 mg (1.35 mmol) of dipeptide **23** were *C*-deprotected according to GP 1 and 1.06 g (1.00 mmol) of peptide **22** were *N*-deprotected according to GP 2 (MeOH, 40 °C, 3 h, R_f = 0.26 in $CHCl_3$–MeOH–NH_3 (aq.) 100 : 10 : 1). The crude products were combined and coupled according to GP 4 (0.02 M, 1 h). After aqueous work-up, FCC (123 g 3% MeOH in $CHCl_3$⟶5% MeOH in $CHCl_3$ + 0.5% HCO_2H) gave 1.02 g (0.75 mmol, 75%) of peptide **24** as a colourless foam. R_f = 0.49 ($CHCl_3$–MeOH–NH_3 (aq.) 100 : 10 : 1); δ_H (400 MHz; DMSO-d_6; Me_4Si) (ppm): 0.63 (3H, d, *J* 6.3, Leu δ-H_3), 0.67 (d, *J* 6.6, 3H, Leu δ-H_3), 0.80 (d, *J* 5.9, 3H, Leu δ-H_3), 0.83 (3H, d, *J* 5.9, Leu δ-H_3), 0.94–1.03 (12H, m, THF C-2′-H_3 and 3 × *t*-Bu-H_3), 1.03–1.12 (1H, m, Leu γ-H), 1.13–1.22 (2H, m, Leu β-H_2), 1.24–1.36 (1H, m, THF 4-*H*H), 1.39–1.54 (3H, m, Leu β-H_2 and Leu γ-H), 1.64–1.74 (1H, m, THF 3-*H*H), 1.85–1.94 (2H, m, THF 4-H*H*′ and THF 3-H*H*′), 2.65 (1H, dd, *J* 8.8 and 14.0, Trp β-*H*H), 2.91 (1H, dd, *J* 10.2 and 14.0, Trp β-*H*H), 3.05–3.21 (4H, m, Trp β-*H*H and 3 × Trp β-H*H*′), 3.21–3.28 (2H, m, AE 2-H_2), 3.61 (2H, t, *J* 6.6, AE 1-H_2), 3.67–3.75 (1H, m, THF 5-H), 3.87 (1H, dt, *J* 2.3 and 7.0, THF 1′-H), 4.12 (1H, t, *J* 5.5, THF 2-H), 4.19–4.27 (1H, m, Leu α-H), 4.37–4.53 (4H, m, Leu α-H and 3 × Trp α-H), 4.94 (1H, d, *J* 12.4, Z-*H*H), 5.05 (1H, d, *J* 12.4, Z-*H*H), 6.89–6.97 (3H, m, 3 × ar. CH), 6.99–7.06 (3H, m, 3 × ar. CH), 7.08 (1H, s, ar. CH), 7.14 (2H, d, *J* 15.6, 2 × ar. CH), 7.26–7.33 (8H, m, 8 × ar. CH), 7.38–7.48 (7H, m, Trp NH and 6 × ar. CH), 7.53–7.65 (7H, m, 7 × ar. CH), 7.98 (1H, t, *J* 5.8, AE NH), 8.06 (1H, d, *J* 7.9, Leu NH), 8.09–8.15 (2H, m, Leu NH and Trp NH), 8.18 (1H, d, *J* 8.6, THF NH), 8.38 (1H, d, *J* 7.3, Trp NH), 10.73 (1H, s, indole NH), 10.79 (2H, s, indole NH); $[\alpha]_D$ = + 19.5, $[\alpha]_{365}$ = + 90.8 (*c* = 1.3, $CHCl_3$, T = 20 °C); HPLC: 14.4 min (M1); *m/z* (ESI) 1381.8 (M + Na^+. $C_{78}H_{94}N_{10}NaO_{10}Si$ requires 1381.7).

Z-Ala-D-Val-Val-D-Val-Trp-D-Leu-L-THF-Trp-D-Leu-Trp-2-(*t*-butyldiphenylsilyloxy)-ethylamide 15. 480 mg (0.9 mmol) of tetrapeptide **25** were *C*-deprotected according to GP 2 and 800 mg (0.60 mmol) of peptide **24** were *N*-deprotected according to GP 1 (MeOH, 40 °C, 3 h; R_f = 0.12 in $CHCl_3$–MeOH–HCO_2H 100 : 10 : 1). The crude products were combined and coupled according to GP 4 (0.01 M, 2 h). After aqueous work-up, FCC (120 g, 3⟶4% MeOH in $CHCl_3$) gave 828 mg (0.48 mmol 81%) of product **15** as a colourless foam. R_f = 0.55 ($CHCl_3$–MeOH–HCO_2H 100 : 10 : 1); δ_H (500 MHz; DMSO-d_6; Me_4Si) 0.56–0.63 (9H, m, 2 × Leu δ-H_3 and Val γ-H_3), 0.64–0.67 (6H, m, Leu δ-H_3 and Val γ-H_3), 0.69 (3H, d, *J* 6.2, Leu δ-H_3), 0.77–0.87 (12H, m, 4 × Val γ-H_3), 0.99 (9H, s, 3 × *t*-Bu-H_3), 0.95–1.05 (4H, m, THF 2′-H_3 and Leu γ-H), 1.20 (3H, d, *J* 6.9, Ala β-H_3), 1.08–1.23 (3H, m, Leu β-H_2 and Leu γ-H), 1.28–1.39 (2H, m, THF 4-*H*H and THF 3-*H*H), 1.40–1.49 (2H, m, Leu β-H_2), 1.66–1.74 (1H, m, THF 4-H*H*′), 1.84–1.94 (2H, m, Val β-H and THF 3-H*H*′), 1.96–2.06 (2H, m, 2 × Val β-H), 2.89–3.01 (2H, m, 2 × Trp β-*H*H), 3.22–3.08 (4H, m, Trp β-*H*H and 3 × Trp β-H*H*′), 3.23–3.31 (2H, m, AE 2-H_2), 3.61 (2H, t, *J* 6.4, AE 1-H_2), 3.68–3.75 (1H, m, THF 5-H), 3.85–3.93 (1H, m, THF 1′-H), 4.09–4.19 (2H, m, THF 2-H and Ala α-H), 4.20–4.28 (3H, m, Val α-H and 2 × Leu α-H), 4.29–4.36 (2H, m, 2 × Val α-H), 4.41–4.47 (1H, m, Trp α-H), 4.51 (1H, dd, *J* 7.3 and 14.2, Trp α-H), 4.57 (1H, dd, *J* 7.7 and 14.1, Trp α-H), 4.98 (1H, d, *J* 13.0, Z-*H*H), 5.01 (1H, d, *J* 13.0, Z-H*H*′), 6.89–6.97 (3H, m, 3 × ar. CH), 6.99–7.06 (3H, m, 3 × ar. CH), 7.08 (1H, s, ar. CH), 7.15 (2H, d, *J* 7.1, 2 × ar. CH), 7.26–7.37 (8H, m, 8 × ar. CH), 7.38–7.48 (7H, m, 6 × ar. CH and Ala NH), 7.53–7.60 (3H, m, 3 × ar. CH), 7.62 (4H, d, *J* 6.6, 4 × ar. CH), 7.83 (1H, d, *J* 9.9, NH), 7.86 (1H, d, *J* 9.2, NH), 7.90 (1H, d, *J* 8.7, THF NH), 7.92 (1H, d, *J* 8.7, NH), 7.99 (1H, t, *J* 4.8, AE NH), 8.02 (1H, d, *J* 8.3, NH), 8.10–8.17 (2H, m, 2 × Trp NH), 8.23 (1H, d, *J* 8.3, NH), 8.46 (1H, d, *J* 7.1, Trp NH), 10.74 (1H, s, indole NH), 10.79 (2H, s, indole NH); HPLC: 16.6 min (M1); *m/z* (ESI) 1728.6 (M + H^+. $C_{96}H_{127}N_{14}O_{14}Si$ requires 1727.9).

H-Ala-D-Val-Val-D-Val-Trp-D-Leu-L-THF-Trp-D-Leu-Trp-2-(*t*-butyldiphenylsilyloxy)-ethylamide 15a. 400 mg (231 μmol) of peptide **15** were *N*-deprotected according to GP 2A (EtOH, 50 °C). After filtration, evaporation, FCC (20 g, $CHCl_3$–MeOH–NH_3 100 : 10 : 1) of the crude product gave 250 mg (157 μmol, 68%) of amine **15a**. R_f = 0.16 ($CHCl_3$–MeOH–HCO_2H

100 : 10 : 1). HPLC: 11.8 min (M2). *m/z* (ESI) 1615.6 (M + Na^+. $C_{88}H_{120}N_{14}NaO_{12}Si$ requires 1615.9).

HCO-Val-Gly-Ala-D-Leu-Ala-D-Val-Val-D-Val-Trp-D-Leu-L-THF-Trp-D-Leu-Trp-2-(*t*-butyldiphenylsilyloxy)-ethylamide 3. 55.0 mg (137 μmol) of tetrapeptide **14** were *C*-deprotected according to GP 1 (R_f = 0.26 in $CHCl_3$–MeOH–HCO_2H 100 : 10 : 1) and then coupled with 100 mg (62.7 μmol) of amine **15a** according to GP 4 (6.0 mM, 3 h). After aqueous work-up and FCC (12 g, $CHCl_3$–MeOH–HCO_2H 200 : 10 : 1) 90.0 mg (46.0 μmol, 73%) of peptide **3** were obtained as a colourless foam. Prior to single-channel measurement, purification of 10.0 mg of compound **3** by preparative HPLC (250 mm Rainin C 8, 60⟶99% B in 20 min, A: H_2O; B: acetonitrile–isopropanol 2 : 1; 10 ml min^{-1}) afforded 8.0 mg (80%) of purest product. R_f = 0.52 ($CHCl_3$–MeOH–HCO_2H 100 : 10 : 1); δ_H (400 MHz; DMSO-d_6; Me_4Si) 0.55–0.63 (9H, m, 3 × Leu δ-H_3), 0.64–0.71 (9H, m, 3 × Leu δ-H_3), 0.77–0.88 (24H, m, 8 × Val γ-H_3), 0.95–1.05 (13H, m, THF 2′-H_3, 3 × *t*-Bu-H_3 and Leu γ-H), 1.19 (3H, d, *J* 4.3, Ala β-H_3), 1.21 (3H, d, *J* 4.3, Ala β-H_3), 1.06–1.25 (4H, m, Leu γ-H, Leu β-H_2 and THF 4-*H*H), 1.27–1.37 (2H, m, THF 3-*H*H and Leu β-*H*H), 1.39–1.49 (3H, m, Leu β-H*H* and Leu β-H_2), 1.50–1.60 (1H, m, Val β-H), 1.65–1.75 (1H, m, THF 4-H*H*), 1.83–2.07 (5H, m, THF 3-H*H*, Leu γ-H and 3 × Val β-H), 2.86–3.02 (2H, m, Trp β-H_2), 3.07–3.20 (4H, m, 2 × Trp β-H_2), 3.21–3.31 (2H, m, AE 1-H_2), 3.60 (2H, t, *J* 6.4, AE 1-H_2), 3.67–3.76 (3H, m, Gly α-H_2 and THF 5-H), 3.89 (1H, dd, *J* 14.7 and 7.4, THF 1′-H), 4.12 (1H, t, *J* 5.8, Val α-H), 4.17–4.34 (9H, m, 3 × Val α-H, 3 × Leu α-H, 2 × Ala α-H and THF 2-H), 4.40–4.58 (3H, m, 3 × Trp α-H), 6.88–6.96 (3H, m, 3 × ar. CH), 6.98–7.05 (3H, m, 3 × ar. CH), 7.06 (1H, d, *J* 1.9, ar. CH), 7.12 (1H, d, *J* 1.9, ar. CH), 7.14 (1H, d, *J* 1.9, ar. CH), 7.26–7.32 (3H, m, 3 × ar. CH), 7.36–7.47 (6H, m, 6 × ar. CH), 7.51–7.64 (7H, m, 7 × ar. CH), 7.85 (1H, d, *J* 8.6, NH), 7.88–8.02 (7H, m, 7 × NH), 8.04 (1H, s, C*H*O), 8.09–8.26 (5H, m, 5 × NH), 8.31 (1H, t, *J* 5.6, AE NH), 8.46 (1H, d, *J* 7.0, Trp NH), 10.71 (1H, s, indole NH), 10.77 (2H, s, indole NH); HPLC: 14.3 min (M1); *m/z* (ESI) 1962.7 (M + H^+. $C_{105}H_{149}N_{18}O_{17}Si$ requires 1962.1).

^{11,12}[L-THF]-gA 2. 20.0 mg (10.2 μmol) of peptide **3** were dissolved in 20 ml CH_2Cl_2–DMF 10 : 1. Under stirring at rt 1.8 ml of a 5% solution of HF in CH_3CN were then added dropwise. After 45 min the reaction mixture was diluted with 20 ml CH_2Cl_2, carefully washed with sat. $NaHCO_3$ (2 × 10 ml) and brine (1 × 10 ml) and dried over Na_2SO_4. After evaporation, 20.0 mg of the crude peptide **2** were isolated and purified by preparative HPLC (250 mm Rainin C 8, 60%⟶99% B in 20 min, A: H_2O; B: acetonitrile–isopropanol 2 : 1; 10 ml min^{-1}). Pure product **2** (15.0 mg 8.70 μmol, 85%) was isolated as a colourless solid. R_f = 0.40 ($CHCl_3$–MeOH–HCO_2H; 100 : 10 : 1); δ_H (400 MHz; DMSO-d_6; Me_4Si) 0.56–0.64 (9H, m, 3 × Leu δ-H_3), 0.65–0.73 (9H, m, 3 × Leu δ-H_3), 0.77–0.88 (24H, m, 8 × Val γ-H_3), 0.99 (3H, d, *J* 6.6, THF 2′-H_3), 0.95–1.08 (2H, m, 2 × Leu γ-H), 1.11–1.28 (5H, m, Leu γ-H and 2 × Leu β-H_2), 1.19 (3H, d, *J* 7.6, Ala β-H_3), 1.21 (3H, d, *J* 7.6, Ala β-H_3), 1.28–1.41 (3H, m, Leu β-H_2 and THF 4-*H*H), 1.43–1.51 (1H, m, THF 3-*H*H), 1.52–1.61 (1H, m, Val β-H), 1.69–1.79 (1H, m, THF 4-H*H*), 1.86–2.08 (5H, m, THF 3-H*H*, Leu γ-H and 3 × Val β-H), 2.89 (1H, dd, *J* 14.5 and 9.9, Trp β-*H*H), 2.98 (1H, dd, *J* 14.0 and 8.4, Trp β-*H*H), 3.06–3.24 (6H, m, Trp β-*H*H, 3 × Trp β-H*H* and AE 2-H_2), 3.35–3.43 (1H, m, AE 1-*H*H), 3.66–3.80 (3H, m, Gly α-H_2 and THF 5-H), 3.90 (1H, dd, *J* 15.4 and 7.7, THF 1′H), 4.13–4.33 (10H, m, 4 × Val α-H, 3 × Leu α-H, 2 × Ala α-H and THF 2-H), 4.43 (1H, dt, *J* 4.9 and 9.1, Trp α-H), 4.51 (2H, dd, *J* 9.1 and 4.9, 2 × Trp α-H), 4.67–4.73 (1H, m, AE 1-H*H*), 6.88–6.97 (3H, m, 3 × ar. CH), 6.98–7.05 (3H, m, 3 × ar. CH), 7.08 (1H, d, *J* 1.9, ar. CH), 7.09 (1H, d, *J* 1.9, ar. CH), 7.16 (1H, d, *J* 1.9, ar. CH), 7.25–7.34 (3H, m, 3 × ar. CH), 7.52–7.62 (3H, m, 3 × ar. CH), 7.89 (1H, t, *J* 5.6, AE NH), 7.93–8.12 (8H, m, 7 × NH and C*H*O), 8.17 (1H, d, *J* 8.6, Trp NH), 8.22–8.31 (3H, m, 3 × NH), 8.35 (1H, t, *J* 5.8, Gly NH), 8.47 (1H, d, *J* 7.6, Trp NH), 10.73 (1H, s, indole NH), 10.79 (2H, s, indole NH); HPLC: 10.9 min (M3); *m/z* (ESI) 1745.9 (M + Na^+. $C_{89}H_{130}N_{18}NaO_{17}$ requires 1746.0).

Z-Ala-D-Leu-Ala-D-Val-Val-D-Val-Trp-D-Leu-L-THF-Trp-D-Leu-Trp-2-(*t*-butyldiphenylsilyloxy)-ethylamide 31. 43.0 mg (123 μmol) of dipeptide **18** were *C*-deprotected according to GP 1 and coupled with 60.0 mg (37.7 μmol) of amine **15a** according to GP 4 (6.0 mM, 2.5 h). After aqueous work-up and FCC (12 g, 3% MeOH in $CHCl_3$⟶4% MeOH in $CHCl_3$ + 0.4% HCO_2H) 65.0 mg (34.0 μmol, 90%) of peptide **31** were obtained as a colourless foam. R_f = 0.52 ($CHCl_3$–MeOH–HCO_2H 100 : 10 : 1); δ_H (500 MHz; DMSO-d_6; Me_4Si) 0.57–0.63 (9H, m, 3 × Leu δ-H_3), 0.65 (6H, d, *J* 6.4, 2 × Leu δ-H_3), 0.70 (3H, d, *J* 6.6, Leu δ-H_3), 0.76–0.86 (18H, m, 6 × Val γ-H_3), 0.95–1.01 (13H, m, 3 × *t*–Bu–H_3, THF 2′-H_3 and Leu γ-H), 1.08–1.16 (3H, m, Leu γ-H and Leu β-H_2), 1.17–1.23 (7H, m, 2 × Ala β-H_3 and THF 3-*H*H), 1.28–1.35 (2H, m, THF 4-*H*H and Leu γ-H), 1.42–1.49 (4H, m, Leu β-H_2), 1.51–1.59 (1H, m, Val β-H), 1.66–1.75 (1H, m, THF 4-H*H*), 1.84–1.94 (2H, m, Leu γ-H, and THF 3-H*H*), 1.95–2.05 (2H, m, Val β-H), 2.88–3.00 (2H, m, 2 × Trp β-*H*H), 3.07–3.21 (4H, m, Trp β-*H*H and 3 × Trp β-H*H*), 3.23–3.33 (2H, m, AE 2-H_2), 3.61 (2H, t, *J* 6.2, AE 1-H_2), 3.71 (1H, dd, *J* 15.1 and 7.1, THF 5-H), 3.89 (1H, dd, *J* 14.4 and 7.5, THF 1′-H), 4.07 (1H, t, *J* 7.2, Ala α-H), 4.14 (1H, t, *J* 5.6, THF 2-H), 4.19–4.28 (4H, m, Val α-H and 3 × Leu α-H), 4.29–4.35 (3H, m, Ala α-H and 2 × Val α-H), 4.41–4.47 (1H, m, Trp α-H), 4.45 (1H, dd, *J* 14.7 and 7.1, Trp α-H), 4.57 (1H, dd, *J* 14.3 and 7.0, Trp α-H), 4.99 (1H, d, *J* 12.4, Z-*H*H), 5.02 (1H, d, *J* 12.1, Z-H*H*), 6.88–6.96 (3H, m, 3 × ar. CH), 6.98–7.05 (3H, m, 3 × ar. CH), 7.07 (1H, d, *J* 1.8, ar. CH), 7.13 (1H, d, *J* 1.7, ar. CH), 7.16 (1H, d, *J* 1.8, ar. CH), 7.26–7.36 (8H, m, 8 × ar. CH), 7.39–7.47 (7H, m, 6 × ar. CH and Ala NH), 7.53–7.60 (3H, m, 3 × ar. CH), 7.61–7.65 (4H, m, 4 × ar. CH), 7.77 (1H, d, *J* 8.7, NH), 7.86 (1H, d, *J* 9.9, NH), 7.88–7.93 (2H, m, NH and THF NH), 7.96 (1H, d, *J* 7.1, NH), 7.99 (1H, t, *J* 6.0, AE NH), 8.02 (1H, d, *J* 8.5, NH), 8.09–8.17 (3H, m, NH and 2 × Trp NH), 8.23 (1H, d, *J* 8.3, NH), 8.46 (1H, d, *J* 7.1, Trp NH), 10.73 (1H, s, indole NH), 10.79 (2H, s, indole NH); HPLC: 10.7 min (M4); *m/z* (ESI) 1934.3 (M + Na^+. $C_{105}H_{142}N_{16}NaO_{16}Si$ requires 1934.0).

H-Ala-D-Leu-Ala-D-Val-Val-D-Val-Trp-D-Leu-L-THF-Trp-D-Leu-Trp-2-(*t*-butyldiphenylsilyloxy)-ethylamide 31a. 124 mg (64.9 μmol) of peptide **31** were *N*-deprotected according to GP 2A (EtOH, 50 °C). After filtration and evaporation FCC (15 g, $CHCl_3$–MeOH–NH_3(conc.) 100 : 10 : 1) gave 70.0 mg (39.4 μmol, 61%) of amine **31a**. R_f = 0.28 ($CHCl_3$–MeOH–NH_3(conc.) 100 : 10 : 1); HPLC: 7.2 min (M4). *m/z* (ESI) 1799.4 (M + Na^+. $C_{97}H_{136}N_{16}NaO_{14}Si$ requires 1800.0).

Succ-1-(1′-*t*-butyldiphenylsilyloxy-^{11,12}[L-THF]-gramicidin A)-4-(Val-Gly-OMe)-diamide 32. 30.0 mg (56.2 μmol) of benzyl ester **17** were *C*-deprotected according to GP 2A. The crude product was coupled with amine **31a** (30.0 mg, 17.0 μmol) according to GP 4 (3.0 mM, 3 h). After aqueous work-up FCC (5 g, 3% MeOH in $CHCl_3$⟶4% MeOH in $CHCl_3$ + 0.4% HCO_2H) gave 31.0 mg (14.1 μmol, 83%) of pure diamide **32** as a colourless solid. R_f = 0.42 ($CHCl_3$–MeOH–HCO_2H 100 : 10 : 1); δ_H (600 MHz; DMSO-d_6; Me_4Si) 0.52–0.71 (18H, m, 6 × Leu δ-H_3), 0.74–0.89 (30H, m, 10 × Val γ-H_3), 0.91–1.02 (13H, m, 3 × *t*-Bu-H_3, Leu γ-H and THF 2′-H_3), 1.03–1.13 (3H, m, Leu γ-H and Leu β-H_2), 1.18 (3H, d, *J* 6.8, Ala β-H_3), 1.19 (3H, d, *J* 6.8, Ala β-H_3), 1.16–1.23 (1H, m, THF 3-*H*H), 1.25–1.35 (2H, m, THF 4-*H*H and Leu γ-H), 1.41–1.51 (4H, m, 2 × Leu β-H_2), 1.52–1.59 (1H, m, Val β-H), 1.64–1.74 (1H, m, THF 4-H*H*), 1.82–2.07 (5H, m, 4 × Val β-H and THF 3-H*H*), 2.36–2.45 (4H, m, succ 2-H_2 and 3-H_2), 2.85–3.03 (2H, m, 2 × Trp β-*H*H), 3.04–3.21 (4H, m, Trp β-*H*H and 3 × Trp β-H*H*), 3.22–3.28

(2H, m, AE 2-H_2), 3.60 (3H, s, OCH_3) 3.55–3.63 (3H, m, THF 5-H and AE 1-H_2), 3.63–3.74 (4H, m, 2 × Gly α-H_2), 3.85–3.93 (1H, m, THF 1′-H), 4.03–4.33 (11H, m, 3 × Leu α-H, 5 × Val α-H, THF 2-H and 2 × Ala α-H), 4.37–4.60 (3H, m, 3 × Trp α-H), 6.87–6.95 (3H, m, ar. 3 × CH), 6.97–7.03 (3H, m, 3 × ar. CH), 7.05 (1H, d, *J* 1.9, ar. CH), 7.11 (1H, d, *J* 1.9, ar. CH), 7.14 (1H, d, *J* 1.5, ar. CH), 7.24–7.32 (3H, m, 3 × ar. CH), 7.35–7.45 (6H, m, 6 × ar. CH), 7.49–7.57 (3H, m, 3 × ar. CH), 7.58–7.64 (4H, m, 4 × ar. CH), 7.78 (1H, d, *J* 8.7, NH), 7.83–7.93 (6H, m, 6 × NH), 7.94–8.05 (4H, m, 4 × NH), 8.06–8.15 (2H, m, 2 × NH), 8.17–8.28 (2H, m, 2 × NH), 8.37 (1H, t, *J* 5.8, AE NH), 8.45 (1H, d, *J* 5.3, Trp NH), 10.71 (1H, s, indole NH), 10.76 (2H, s, indole NH); HPLC: 14.7 min (M4); *m*/*z* (ESI) 2226.0 (M + Na^+. $C_{116}H_{166}N_{20}NaO_{21}Si$ requires 2226.2).

Succ-(^{11,12}[L-THF]-gA-TBDPS)$_2$ 4. 15.0 mg (6.8 μmol) of methyl ester **32** were *C*-deprotected according to GP 1 (R_f = 0.20 in $CHCl_3$–MeOH–HCO_2H 100 : 10 : 1; HPLC: 7.2 min M4). 12.0 mg of the crude carboxylic acid were combined with 12.0 mg (6.8 μmol) of amine **31a** and coupled according to GP 5 (1.0 mM, 12 h). After aqueous work-up 24.0 mg of crude diamide **4** were obtained and purified by preparative HPLC (250 mm Rainin C 4, 60%⟶100% B in 25 min, A: H_2O + 0.1% TFA; B: acetonitrile–isopropanol 2 : 1 + 0.1% TFA; 14 ml min^{-1}). Treatment of the collected fractions according to GP 6 gave 9.0 mg (2.3 μmol, 34%) of pure diamide **4** as a colourless solid. δ_H (500 MHz; DMSO-d_6; Me_4Si) 0.55–0.73 (30H, m, 10 × Leu δ-H_3), 0.75–0.91 (54H, m, 16 × Val γ-H_3 and 2 × Leu δ-H_3), 0.92–1.05 (26H, m, 6 × *t*-Bu-H_3, 2 × THF 2′-H_3 and 2 × Leu γ-H), 1.05–1.39 (20 H, m, 4 × Ala β-H_3 and 4 × Leu β-H_2), 1.40–1.59 (6H, m, 2 × Leu γ-H, 2 × Val β-H and 2 × THF 3- or 4-H*H*), 1.61–1.78 (4H, m, 2 × Leu β-H_2), 1.85–2.08 (12H, m, 2 × THF 3- or 4-*H*H, 2 × THF 3- or 4-H*H*, 6 × Val β-H and 2 × Leu γ-H), 2.26–2.32 (2H, m, 2 × THF 3- or 4-*H*H), 2.36–2.45 (4H, m, succ 2-H_2 and 3-H_2), 2.88–3.05 (6H, m, 6 × Trp β-*H*H), 3.08–3.29 (6H, m, 6 × Trp β-H*H*), 3.42 (4H, t, *J* 5.3, 2 × AE 2-H_2), 3.56–3.63 (6H, m, 2 × AE 1-H_2 and 2 × Gly αC*H*H), 3.66–3.76 (2H, m, 2 × Leu α-H), 3.86–3.95 (4H, m, 2 × THF 1′-H and 2 × Val α-H), 4.05–4.15 (6H, m, 2 × Val α-H, 2 × Gly α-H*H* and 2 × Leu α-H), 4.18–4.34 (12H, m, 4 × Val α-H, 4 × Ala α-H, 2 × Leu α-H and 2 × THF 5-H), 4.39–4.56 (6H, m, 6 × Trp α-H), 4.56–4.62 (2H, m, 2 × THF 2-H), 6.89–6.97 (6H, m, 6 × ar. CH), 6.99–7.05 (6H, m, 6 × ar. CH), 7.07 (2H, s, 2 × ar. CH), 7.15 (4H, d, *J* 12.8, 4 × ar. CH), 7.30 (6H, t, *J* 7.7, 6 × ar. CH), 7.38–7.48 (12H, m, 12 × ar. CH), 7.52–7.66 (14H, m, 14 × ar. CH), 7.90 (16H, m, 16 × NH), 8.15–8.21 (6H, m, 6 × NH), 8.24–8.36 (6H, m, 6 × NH), 8.49–8.56 (2H, m, 2 × NH), 10.72 (2H, s, indole NH), 10.80 (4H, s, indole NH); HPLC: 18.6 min (M4); *m*/*z* (ESI) 1999.9 (M + 2Na^+. $C_{212}H_{298}N_{36}Na_2O_{34}Si_2$ requires 1997.1).

Boc-D-THF-D-Leu-OMe 27. 289 mg (1.15 mmol) of carboxylic acid **5** were combined with 616 mg (3.40 mmol) of D-Leucine hydrochloride and coupled according to GP 3 (0.04 M 2 h). After aqueous work-up FCC (35g, 1% MeOH in $CHCl_3$) gave 346 mg (0.90 mmol, 78%) of pure methyl ester **27** as a colourless foam. R_f = 0.57 ($CHCl_3$–MeOH 20 : 1); δ_H (300 MHz; DMSO-d_6; Me_4Si) 0.84 (3H, d, *J* 6.4, Leu δ-H_3), 0.91 (3H, d, *J* 6.4, Leu δ-H_3), 0.99 (3H, d, *J* 5.5, THF 2′-H_3), 1.29–1.39 (1H, m, Leu β-*H*H), 1.40 (9H, s, 3 × *t*-Bu-H_3), 1.54 (1H, ddd, *J* 13.2, 9.9 and 3.6, THF 4-*H*H), 1.61–1.71 (1H, m, Leu γ-H), 1.83–1.92 (2H, m, Leu β-H*H* and THF 3-*H*H), 2.00–2.09 (1H, m, THF 3-H*H*), 2.11–2.18 (1H, m, THF 4-H*H*), 3.64 (3H, s, OCH_3), 3.65–3.71 (2H, m, THF 1′-H and Leu α-H), 4.34 (1H, d, *J* 8.2, THF 5-H), 4.39 (1H, ddd, *J* 11.5, 8.4 and 3.5 THF 2-H), 7.06 (1H, d, *J* 8.0, THF NH), 8.68 (1H, d, *J* 8.6, Leu NH); $[\alpha]_D$ = −9.3, $[\alpha]_{365}$ = −42.1 (*c* = 1.0, $CHCl_3$, 20 °C); HPLC: 7.9 min (M1); *m*/*z* (FAB) 387.2491 (M + H^+. $C_{19}H_{35}N_2O_6$ requires 387.2495).

Z-Trp-D-THF-D-Leu-OMe 29. 216 mg (0.56 mmol) of methyl ester **27** were dissolved in a solution of CF_3CO_2H in CH_2Cl_2 (20 ml, 20%) and stirred 20 min. at room temperature. The reaction mixture was then diluted with CH_2Cl_2 (30 ml), washed carefully with sat. $NaHCO_3$ (10 ml) and brine (20 ml) and dried over $MgSO_4$. After evaporation, 160 mg of crude amine (R_f = 0.28 in $CHCl_3$–MeOH–HCO_2H 100 : 10 : 1) were obtained and directly coupled with 379 mg (1.12 mmol) Z-Trp-OH according to GP 3 (0.02 M, 2 h). After aqueous work-up and FCC (7 g, 3% MeOH in $CHCl_3$) 322 mg (0.53 mmol, 95%) of pure peptide **29** were obtained as a colourless foam. R_f = 0.50 ($CHCl_3$–MeOH–HCO_2H 100 : 10 : 1); δ_H (500 MHz; DMSO-d_6; Me_4Si) 0.80 (3H, d, *J* 6.4, Leu δ-H_3), 0.87 (3H, d, *J* 6.4, Leu δ-H_3), 0.98 (3H, d, *J* 6.9, THF 2′-H_3), 1.38–1.48 (1H, m, THF 4-H*H*), 1.52 (1H, ddd, *J* 13.3, 9.6 and 3.9, THF 3-H*H*), 1.57–1.67 (1H, m, Leu γ-H), 1.86–1.95 (2H, m, THF 3-*H*H and Leu β-H*H*), 2.04–2.13 (2H, m, THF 4-*H*H and Leu β-H*H*), 2.96 (1H, dd, *J* 13.9 and 8.8, Trp β-H*H*), 3.31 (1H, dd, *J* 14.4 and 4.8, Trp β-*H*H), 3.60 (3H, s, OCH_3), 3.76–3.83 (1H, m, Leu α-H), 3.91–3.99 (1H, m, THF C-1′-H), 4.25–4.32 (1H, m, THF 5-H), 4.32–4.41 (2H, m, Trp α-H and THF 2-H), 4.97 (2H, s, Z-H_2), 6.97 (1H, t, *J* 7.5, ar. CH), 7.01–7.09 (2H, m, 2 × ar.CH), 7.10–7.21 (1H, m, ar. CH), 7.22–7.27 (2H, m, 2 × ar.CH), 7.27–7.37 (4H, m, 4 × ar. CH), 7.61 (1H, d, *J* 8.0, THF NH), 8.16 (1H, d, *J* 8.5, Trp NH), 8.68 (1H, d, *J* 8.0, Leu NH), 10.84 (1H, s, indole NH); $[\alpha]_D$ = −35.8, $[\alpha]_{365}$ = −133.2 (*c* = 1.0, $CHCl_3$, 20 °C); HPLC: 6.6 min (M1). *m*/*z* (FAB) 607.3139 (M + H^+. $C_{33}H_{43}N_4O_7$ requires 607.3132).

Z-Trp-D-THF-D-Leu-Trp-D-Leu-Trp-2-(*t*-butyldiphenylsilyloxy)-ethylamide 30. 150 mg (0.25 mmol) of peptide **29** were *C*-deprotected according to GP 1 (R_f = 0.28 in $CHCl_3$–MeOH–HCO_2H), as well as 1.20 g (1.30 mmol) of tripeptide **21** were *N*-deprotected according to GP 2A (MeOH, rt, 2 h; R_f = 0.38 in $CHCl_3$–MeOH–NH_3(aq.) 100 : 10 : 1). The crude carboxylic acid and a portion (1/4) of the crude amine were collected and coupled according to GP 4 (8.0 mM, 3 h). After aqueous work-up and FCC (40 g, 3% MeOH in $CHCl_3$) 319 mg (23.5 mmol, 94%) of peptide **30** were isolated as a colourless foam. R_f = 0.65 ($CHCl_3$–MeOH–HCO_2H 200 : 10 : 1); δ_H (400 MHz; DMSO-d_6; Me_4Si) 0.59 (3H, d, *J* 6.6, Leu δ-H_3), 0.65 (3H, d, *J* 6.3, Leu δ-H_3), 0.66(3H, d, *J* 6.6, Leu δ-H_3), 0.70 (3H, d, *J* 6.3, Leu d-H_3), 0.84 (3H, d, *J* 6.6, THF 2′-H_3), 0.97 (9H, s, 3 × *t*–Bu–H_3), 0.96–1.03 (1H, m, Leu γ-H), 1.11–1.21 (3H, m, THF 4-*H*H and Leu β-H_2), 1.22–1.36 (2H, m, THF 3*H*H and Leu γ-H), 1.48–1.58 (1H, m, THF 3-H*H*), 1.68–1.78 (1H, m, THF 4-H*H*), 1.88–2.05 (2H, m, Leu β-H_2), 2.83–2.99 (3H, m, 3 × Trp β-*H*H), 3.05–3.20 (3H, m, 3 × Trp β-H*H*), 3.20–3.29 (2H, m, AE 2-H_2), 3.62 (2H, t, *J* 6.6, AE 1-H_2), 3.65 (1H, dd, *J* 14.9 and 8.9, THF 5-H), 3.81 (1H, sextet, *J* 7.0, THF 1′-H), 4.15–4.31 (4H, m, 2 × Leu α-H, Trp α-H and THF 2-H), 4.47 (1H, dt, *J* 4.4 and 9.1, Trp α-H), 4.53 (1H, dt, *J* 5.6 and 8.5, Trp α-H), 4.92 (1H, d, *J* 13.5, Z-*H*H), 4.96 (1H, d, *J* 13.2, Z-H*H*), 6.89–6.96 (3H, m, 3 × ar. CH), 6.98–7.09 (6H, m, 5 × ar. CH and Trp NH), 7.11–7.14 (1H, m, ar. CH), 7.19–7.24 (2H, m, 2 × ar. CH), 7.25–7.34 (6H, m, 6 × ar. CH), 7.37–7.47 (6H, m, 6 × ar. CH), 7.51–7.60 (3H, m, 3 × ar. CH), 7.59–7.65 (4H, m, 4 × ar. CH), 7.83 (1H, d, *J* 7.6, Leu NH), 8.02, (1H, d, *J* 8.6, THF NH), 8.04 (1H, t, *J* 5.5, AE NH), 8.13 (1H, d, *J* 7.9, Trp NH), 8.20 (1H, d, *J* 8.3, Trp NH), 8.24 (1H, d, *J* 7.6, Leu NH), 10.73 (1H, d, *J* 1.9, indole NH), 10.76–10.83 (2H, m, indole NH); $[\alpha]_D$ = −19.8, $[\alpha]_{365}$ = −70.8 (*c* = 1.1, $CHCl_3$, 20 °C); HPLC: 13.2 (M1); *m*/*z* (ESI) 1381.9 (M + Na^+. $C_{78}H_{94}N_{10}NaO_{10}Si$ requires 1381.7).

Z-Ala-D-Val-Val-D-Val-Trp-D-THF-D-Leu-Trp-D-Leu-Trp-2-(*t*-butyldiphenylsilyloxy)-ethylamide 16. 319 mg (0.23 mmol) of peptide **30** were *N*-deprotected according to GP 2A (EtOH, 50 °C, 2h; R_f = 0.18 in $CHCl_3$–MeOH–HCO_2H 200 : 10 : 1) and 369 mg (0.70 mmol) of tetrapeptide **24** were *C*-deprotected according to GP 1. The crude amine and a portion (1/4) of the crude saponification product were then collected and coupled

according to GP 4 (3.0 mM, 3 h). After aqueous work-up, FCC (20 g, 3% MeOH in $CHCl_3$⟶5% MeOH in $CHCl_3$ + 0.5% HCO_2H) gave 231 mg (0.13 mmol, 57%) of pure ethylamide **16** as a colourless foam. R_f = 0.28 ($CHCl_3$–MeOH 20 : 1); δ_H(400 MHz; DMSO-d_6; Me_4Si) 0.38 (3H, d, *J* 6.6, Leu δ-H_3), 0.55 (3H, d, *J* 6.6, Leu δ-H_3), 0.57 (3H, d, *J* 6.6, Leu δ-H_3), 0.64 (6H, d, *J* 5.9, Leu δ-H_3 and Val γ-H_3), 0.72 (3H, d, *J* 6.3, Val γ-H_3), 0.74–0.83 (12H, m, 4 × Val γ-H_3), 0.86 (3H, d, *J* 6.9, THF 2′-H_3), 0.98 (9H, s, 3 × *t*-Bu-H_3), 0.95–1.05 (1H, m, Leu γ-H), 1.09–1.21 (3H, m, THF 4-*H*H and Leu β-H_2), 1.19 (3H, d, *J* 7.3, Ala β-H_3), 1.22–1.35 (2H, m, THF 4-H*H*, and Val β-H), 1.45–1.55 (1H, m, THF 3-*H*H), 1.63 (1H, sextet, *J* 7.2, Leu γ-H), 1.69–1.79 (1H, m, THF 4-H*H*), 1.90–2.11 (4H, m, 2 × Val β-H and Leu β-H_2), 2.79 (1H, dd, *J* 14.2 and 10.1, Trp β-*H*H), 2.84–2.95 (2H, m, 2 × Trp β-*H*H), 3.13–3.24 (2H, m, 2 × Trp β-H*H*), 3.25–3.31 (3H, m, AE 2-H_2 and Trp β-H*H*), 3.64 (2H, t, *J* 6.4, AE 1-H_2), 3.64–3.74 (1H, m, THF 5-H), 3.82 (1H, t, *J* 7.1, Leu α-H), 3.92 (1H, sextet, *J* 8.3, THF 1′-H), 4.13 (1H, dt, *J* 14.5 and 7.3, Ala α-H), 4.17–4.27 (3H, m, Leu α-H, Val α-H and THF 2-H), 4.28–4.36 (2H, m, 2 × Val α-H), 4.38–4.51 (2H, m, 2 × Trp α-H), 4.52–4.61 (1H, m, Trp α-H), 4.98 (1H, d, *J* 12.9, Z-*H*H), 5.02 (1H, d, *J* 12.9, Z-H*H*), 7.26–7.35 (11H, m, Ala NH and 10 × ar. CH), 7.36–7.46 (10H, m, 10 × ar. CH), 7.47–7.58 (5H, m, 5 × ar. CH), 7.59–7.64 (6H, m, THF NH and 5 × ar. CH), 7.66 (1H, d, *J* 8.9, Val NH), 7.68 (1H, d, *J* 7.3, NH), 7.82 (1H, d, *J* 6.9, Leu NH), 7.92 (1H, d, *J* 8.9, Val NH), 8.05 (1H, t, *J* 5.8, AE NH), 8.13 (1H, d, *J* 6.9, NH), 8.24 (1H, d, *J* 8.3, Trp NH), 8.26–8.30 (2H, m, Trp NH), 10.71–10.76 (2H, m, indole NH), 10.80 (1H, d, *J* 1.3, indole NH); HPLC: 10.9 min (250 × 4 mm Jupiter C 5, 70⟶100% B in 25 min, A: H_2O; B: acetonitrile–isopropanol 2 : 1; 0.7 ml min^{-1}); *m*/*z* (ESI) 1749.9 (M + Na^+. $C_{96}H_{126}N_{14}O_{14}Si$ requires 1749.9).

H-Ala-D-Val-Val-D-Val-Trp-D-THF-D-Leu-Trp-D-Leu-Trp-2-(*t*-butyldiphenylsilyloxy)-ethylamide 16a. 210 mg (120 μmol) of peptide **16** were *N*-deprotected according to GP 2A (EtOH, 50 °C). After FCC (8 g; $CHCl_3$–MeOH 15 : 1⟶$CHCl_3$–MeOH–NH_3(conc.) 100 : 10 : 1) 131 mg (0.08 mmol, 69%) of the pure amine **16a** were obtained. R_f = 0.09 in $CHCl_3$–MeOH–HCO_2H 100 : 10 : 1; HPLC: 7.4 min (M4). *m*/*z* (ESI) 1616.1 (M + Na^+. $C_{88}H_{120}N_{14}NaO_{12}Si$ requires 1615.9).

CHO-Val-Gly-Ala-D-Leu-Ala-D-Val-Val-D-Val-Trp-D-THF-D-Leu-Trp-D-Leu-Trp-2-(*t*-butyldiphenylsilyloxy)-ethylamide 6. 217 mg (0.54 mmol) of tetrapeptide **14** were *C*-deprotected according to GP 1 (R_f = 0.21 in $CHCl_3$–MeOH–HCO_2H 100 : 10 : 1) and a portion (1/4) of the crude saponification product was coupled with amine **16a** (121 mg, 76.0 μmol) according to GP 4 (4.0 mM, 3 h). After aqueous work-up, FCC (14 g, $CHCl_3$–MeOH–HCO_2H 150 : 10 : 1) gave 130 mg (66.3 μmol, 87%) of the pure peptide **6** as a colourless solid. Prior to single-channel measurements, purification of 10.0 mg of compound **6** by preparative HPLC (250 mm Rainin C 8, 75⟶90% B in 25 min, A: H_2O; B: acetonitrile–isopropanol 2 : 1; 10 ml min^{-1}) afforded 9.0 mg (90%) of pure product. R_f = 0.36 ($CHCl_3$–MeOH–HCO_2H 100 : 10 : 1); δ_H (500 MHz; DMSO-d_6; Me_4Si) 0.21 (3H, d, *J* 6.4, Leu δ-H_3), 0.35 (3H, d, *J* 6.7, Leu δ-H_3), 0.38 (3H, d, *J* 6.4, Leu δ-H_3), 0.45 (6H, d, *J* 5.7, 2 × Leu δ-H_3), 0.50–0.73 (30H, m, Leu δ-H_3, THF 2′-H_3 and 8 × Val γ-H_3), 0.74–0.87 (11H, m, 3 × *t*-Bu-H_3 and 2 × Leu γ-H), 0.88–1.17 (11H, m, 2 × Ala β-H_3, 2 × Leu β-H_2 and Leu γ-H), 1.19–1.49 (4H, m, THF 4-*H*H, Val β-H and Leu β-H_2), 1.50–1.63 (1H, m, THF 3-*H*H), 1.70–1.91 (5H, m, THF 3-H*H*, THF 4-H*H* and 3 × Val β-H), 2.53–2.78 (3H, m, 3 × Trp β-*H*H), 2.91–3.13 (5H, m, 3 × Trp β-H*H* and AE 2-H_2), 3.45 (2H, t, *J* 6.2, AE 1-H_2), 3.49–3.60 (3H, m, Gly α-H_2 and Leu α-H), 3.61–3.80 (2H, m, Leu a-H and THF 1′-H), 3.95–4.17 (9H, m, 4 × Val α-H, THF 2-H and 5-H, Leu α-H and 2 × Ala α-H), 4.18–4.33 (2H, m, 2 × Trp α-H), 4.34–4.43 (1H, m, Trp α-H), 6.67–6.78 (3H, m, 3 × ar. CH), 6.79–7.00 (6H, m, 6 × ar. CH), 7.01–7.14 (3H, m, 3 × ar.CH), 7.15–7.28 (6H, m, 6 × ar.CH), 7.29–7.48 (9H, m, 7 × ar.CH, THF NH and NH), 7.51 (1H, d, *J* 5.8 Leu NH), 7.60–7.98 (8H, m, 8 × NH), 8.00–8.19 (5H, m, 3 × Trp NH, Gly NH and AE NH), 10.55 (2H, s, indole NH), 10.62 (1H, s, indole NH); HPLC: 7.6 min (250 × 4 mm Jupiter C 5, 65⟶75% B in 25 min, A: H_2O; B: acetonitrile–isopropanol 2 : 1; 0.7 ml min^{-1}); *m*/*z* (ESI): 1984.0 (M + Na^+. $C_{105}H_{148}N_{18}NaO_{17}Si$ requires 1984.1).

[10,11][D-THF]-gA 5. 30.0 mg (15.3 μmol) of peptide **6** were dissolved in 27 ml CH_2Cl_2. A solution of 5% HF in CH_3CN (2.7 ml) was then added dropwise and the mixture was stirred for 45 min at room temperature. After dilution with 30 ml CH_2Cl_2 the mixture was carefully washed with sat. $NaHCO_3$ (2 × 10 ml) and brine, and dried over $MgSO_4$. Evaporation gave 33.0 mg of the crude peptide **5**, which was purified by preparative HPLC (250 mm Rainin C 8, 60⟶75% B in 25 min, A: H_2O; B: acetonitrile–isopropanol 2 : 1; 10 ml min^{-1}). 22.0 mg (12.7 μmol, 83%) of pure product were obtained as a colourless solid. R_f = 0.16 ($CHCl_3$–MeOH–HCO_2H 100 : 10 : 1); δ_H (500 MHz; DMSO-d_6; Me_4Si) 0.33 (3H, d, *J* 6.8, Leu δ-H_3), 0.47 (3H, d, *J* 6.4, Leu δ-H_3), 0.51 (3H, d, *J* 6.8, Leu δ-H_3), 0.59 (6H, d, *J* 6.8, 2 × Leu δ-H_3), 0.63–0.87 (30H, m, 8 × Val γ-H_3, THF 2′-H_3 and Leu δ-H_3), 0.88–0.97 (2H, m, 2 × Leu γ-H), 1.04–1.29 (13H, m, 2 × Ala β-H_3, 3 × Leu β-H_2, and Leu γ-H), 1.35–1.62 (5H, m, Leu β-H_2, Leu γ-H, Val β-H and THF 4-*H*H), 1.65–1.77 (1H, m, THF 3-*H*H), 1.83–2.03 (5H, m, THF 4-H*H*, THF 3-H*H* and 3 × Val β-H), 2.64–2.90 (3H, m, 3 × Trp β-*H*H), 3.05–3.25 (5H, m, 3 × Trp β-H*H* and AE 1-H_2), 3.33 (1H, d, *J* 6.0, AE 2-*H*H), 3.37 (1H, d, *J* 6.4, AE 2-H*H*), 3.60–3.73 (3H, m, Gly α-H_2 and Leu α-H), 3.74–3.94 (2H, m, Leu α-H and THF 1′-H), 4.05–4.30 (9H, m, 4 × Val α-H, 2 × Ala α-H, Leu α-H, THF 2-H and 5-H), 4.31–4.44 (2H, m, 2 × Trp α-H), 4.46–4.57 (1H, m, Trp α-H), 4.63 (1H, t, *J* 5.7, OH), 6.82–6.92 (3H, m, 3 × ar. CH), 6.93–7.00 (3H, m, 3 × ar. CH), 7.01–7.08 (3H, m, 3 × ar. CH), 7.23 (3H, d, *J* 7.9, 3 × ar. CH), 7.47 (3H, dd, *J* 19.2 and 7.9, 3 × ar. CH), 7.53–7.62 (2H, m, 1H, NH and Leu NH), 7.68 (1H, d, *J* 7.9, Leu NH), 7.75–7.91 (4H, m, THF NH, AE NH and 2 × NH), 7.94 (1H, d, *J* 6.8, NH), 8.06 (2H, d, *J* 7.9, NH), 8.13–8.31 (5H, m, 3 × Trp NH, Gly NH and NH), 10.68 (1H, d, *J* 0.8, indole NH), 10.70 (1H, d, *J* 1.9, indole NH), 10.75 (1H, d, *J* 1.9, indole NH); HPLC: 6.8 min (250 × 4 mm Jupiter C 5, 65⟶75% B in 25 min, A: H_2O; B: acetonitrile–isopropanol 2 : 1; 0.7 ml min^{-1}.); *m*/*z* (ESI) 1745.9 (M + Na^+. $C_{89}H_{130}N_{18}NaO_{17}$ requires 1746.0).

Z-Ala-D-Leu-Ala-D-Val-Val-D-Val-Trp-D-THF-D-Leu-Trp-D-Leu-Trp-2-(*t*-butyldiphenylsilyloxy)-ethylamide 33. 210 mg (0.60 mmol) of dipeptide **18** were *C*-deprotected according to GP 1. A portion (1/2) of the crude saponification product was coupled with amine **16a** (220 mg 0.14 mmol) according to GP 4 (3.0 mM, 3 h) and after aqueous work-up FCC (20g, 4% MeOH in $CHCl_3$ + 0.4% HCO_2H) gave 215 mg (0.11 mmol, 79%) of pure peptide **33** as a colourless solid. R_f = 0.49 ($CHCl_3$–MeOH–NH_3(conc.) 100 : 10 : 1); δ_H (500 MHz; DMSO-d_6; Me_4Si) 0.40 (3H, d, *J* 6.6, Leu δ-H_3), 0.54 (3H, d, *J* 6.4, Leu δ-H_3), 0.58 (3H, d, *J* 6.4, Leu δ-H_3), 0.65 (6H, d, *J* 5.5, 2 × Leu δ-H_3), 0.70–0.82 (18H, m, Leu δ-H_3 and 5 × Val γ-H_3), 0.84 (3H, d, *J* 6.9, Val γ-H_3), 0.89 (3H, d, *J* 6.4, THF 2′-H_3), 0.98 (9H, s, 3 × *t*-Bu-H_3), 0.95–1.05 (1H, m, Leu γ-H), 1.19 (6H, d, *J* 7.1, 2 × Ala β-H_3), 1.09–1.22 (3H, m, THF 3-*H*H and Leu β-H_2), 1.23–1.36 (2H, m, THF 4-*H*H and Leu γ-H), 1.42–1.58 (4H, m, Val β-H, THF 3-H*H* and Leu β-H_2), 1.59–1.68 (1H, m, Leu γ-H), 1.71–1.80 (1H, m, THF 4-H*H*), 1.92–2.06 (4H, m, 2 × Val β-H and Leu β-H_2), 2.80 (1H, dd, *J* 13.9 and 10.8, Trp β-*H*H), 2.85–2.95 (2H, m, 2 × Trp β-H*H*), 3.14–3.24 (3H, m, 3 × Trp β-H*H*), 3.25–3.20 (2H, m, AE 2-H_2), 3.65 (2H, t, *J* 6.5, AE 1-H_2), 3.73 (1H, dd, *J* 14.8 and 7.5 THF 5-H), 3.84 (1H, t, *J* 7.2, Leu α-H), 3.93 (1H, dd, *J* 14.4 and 6.9, THF 1′-H), 4.02–4.09 (1H, m, Ala α-H), 4.17–4.33 (7H, m, 2 × Leu α-H, Ala α-H, THF 2-H and 3H and Val α-H), 4.41–4.51 (2H, m, 2 × Trp α-H), 4.54–4.60

(1H, m, Trp α-H), 4.99 (1H, d, *J* 12.6, Z-*H*H), 5.02 (1H, d, *J* 12.1, Z-H*H*), 6.90–6.97 (3H, m, 3 × ar. CH), 6.99–7.05 (3H, m, 3 × ar. CH), 7.08 (2H, s, 2 × ar. CH), 7.13 (1H, s, ar. CH), 7.27–7.37 (8H, m, 8 × ar. CH), 7.38–7.47 (7H, m, Ala NH and 6 × ar. CH), 7.51 (1H, d, *J* 7.6, ar. CH), 7.56 (2H, t, *J* 7.5, 2 × ar. CH), 7.56–7.60 (1H, m, NH), 7.61–7.67 (5H, m, 4 × ar. CH and THF NH), 7.71 (1H, d, *J* 7.3, NH), 7.85 (1H, d, *J* 6.9, Leu NH), 7.91 (1H, d, *J* 9.2, NH), 7.98 (1H, d, *J* 7.3, NH), 8.08 (1H, t, *J* 5.8, AE NH), 8.11–8.18 (2H, m, NH), 8.25–8.36 (3H, m, Trp NH), 10.73–10.78 (2H, m, indole NH), 10.82 (1H, s, indole NH); HPLC: 11.8 min (M4); *m*/*z* (ESI): 1933.8 ($M + Na^+$. $C_{105}H_{142}N_{16}NaO_{16}Si$ requires 1934.0).

H-Ala-D-Leu-Ala-D-Val-Val-D-Val-Trp-D-THF-D-Leu-Trp-D-Leu-Trp-2-(*t*-butyldiphenylsilyloxy)-ethylamide 33a. 200 mg (104 μmol) of peptide **33** were *N*-deprotected according to GP 2A (EtOH, 50 °C). After filtration and evaporation FCC (25 g, $CHCl_3$–MeOH–NH_3(conc.) 100 : 10 : 1) gave 125 mg (70.0 μmol, 67%) of amine **33a**. R_f = 0.15 ($CHCl_3$–MeOH–HCO_2H 100 : 10 : 1). HPLC: 9.9 min (250 × 4 mm Dynamax C8, 60⟶100% B in 25 min, A: H_2O + 0.1% TFA; B:acetonitrile–isopropanol 2 : 1 + 0.1% TFA; 0.7 ml min^{-1}). *m*/*z* (ESI): 1799.7 ($M + Na^+$. $C_{97}H_{136}N_{16}NaO_{14}Si$ requires 1800.0).

Succ-1-(1′-*t*-butyldiphenylsilyloxy-[10,11][D-THF]-gramicidin A)-4-(Val-Gly-OMe)-diamide 34. 23.5 mg (44.0 μmol) of benzyl ester **17** were *C*-deprotected according to GP 2A. The crude carboxylic acid and 40.0 mg (22.5 μmol) of amine **33a** were then collected and coupled according to GP 4 (1.0 mM, 3 h). After aqueous work-up FCC (4 g, 4% MeOH in $CHCl_3$⟶6% MeOH in $CHCl_3$ + 0.6% HCO_2H) gave 35.0 mg (16.0 mmol, 73%) of pure diamide **34** as a colourless solid. R_f = 0.35 ($CHCl_3$–MeOH–HCO_2H 100 : 10 : 1); δ_H (500 MHz; DMSO-d_6; Me_4Si) 0.40 (3H, d, *J* 6.6, Leu δ-H_3), 0.55 (3H, d, *J* 6.4, Leu δ-H_3), 0.58 (3H, d, *J* 6.4, Leu δ-H_3), 0.65 (6H, d, *J* 6.2, 2 × Leu δ-H_3), 0.72 (3H, d, *J* 6.6, Leu δ-H_3), 0.74–0.82 (15H, m, 5 × Val γ-H_3), 0.83–0.93 (18H, m, 5 × Val γ-H_3 and THF 2′-H_3), 0.98 (9H, s, 3 × *t*-Bu-H_3), 0.99–1.04 (1H, m, Leu γ-H), 1.08–1.34 (13H, m, 2 × Ala β-H_3, THF 3-H*H* and 4-H*H*, 2 × Leu β-H_2 and Leu γ-H), 1.41–1.60 (4H, m, Leu β-H_2, THF 3-*H*H and Val β-H), 1.61–1.69 (1H, m, Leu γ-H), 1.71–1.81 (1H, m, THF 4-*H*H), 1.91–2.07 (4H, m, 4 × Val β-H), 2.36–2.47 (4H, m, succ 3-H_2 and 4-H_2), 2.75–2.83 (1H, m, Trp β-*H*H), 2.85–2.96 (2H, m, 2 × Trp β-*H*H), 3.13–3.30 (5H, m, 3 × Trp βH*H* and AE 2-H_2), 3.62 (3H, s, OCH_3), 3.65 (2H, t, *J* 6.5, AE 1-H_2), 3.67–3.89 (6H, m, 2 × Gly α-H_2, THF 5-H and Leu αCH), 3.90–3.96 (1H, m, THF 1′-H), 4.06–4.34 (10H, m, 5 × Val α-H, 2 × Leu α-H, 2 × Ala α-H, and THF 2-H), 4.40–4.50 (2H, m, 2 × Trp α-H), 4.54–4.61 (1H, m, Trp α-H), 6.90–6.97 (3H, m, 3 × ar. CH), 6.99–7.05 (3H, m, 3 × ar. CH), 7.08 (2H, s, 2 × ar. CH), 7.13 (1H, d, *J* 1.4, ar. CH), 7.28–7.32 (3H, m, 3 × ar. CH), 7.38–7.47 (6H, m, 6 × ar. CH), 7.51 (1H, d, *J* 7.6, ar. CH), 7.56 (2H, t, *J* 7.1, ar. CH), 7.59–7.64 (5H, m, 4 × CH and NH), 7.65 (1H, d, *J* 8.5, THF NH), 7.71 (1H, d, *J* 6.7, NH), 7.83–7.93 (4H, m, Leu NH and 3 × NH), 7.96 (1H, d, *J* 8.9, NH), 8.05–8.11 (2H, m, 2 × NH), 8.12–8.17 (1H, m, NH), 8.20–8.37 (4H, m, 4 × NH), 8.39 (1H, t, *J* 5.84, Gly NH), 10.75 (1H, s, indole NH), 10.77 (1H, s, indole NH), 10.82 (1H, s, indole NH). HPLC: 9.8 min (M4); *m*/*z* (ESI): 2226.4 ($M + Na^+$. $C_{116}H_{166}N_{20}NaO_{21}Si$ requires 2226.2).

Succ-([10,11][D-THF]-gA-TBDPS)$_2$ 7. 39.0 mg (17.7 μmol) of methyl ester **34** were *C*-deprotected according to GP 1 (R_f = 0.20 in $CHCl_3$–MeOH–HCO_2H 100 : 10 : 1; HPLC: 9.8 min, 250 × 4 mm Jupiter C 5, 70⟶100% B in 25 min, A: H_2O + 0.1% TFA; B: acetonitrile–isopropanol 2 : 1 + 0.1% TFA; 0.7 ml min^{-1}). The crude carboxylic acid and 40.0 mg (22.5 μmol) of amine **33a** were collected and coupled according to GP 5 (1.0 mM, 12 h). After aqueous work-up 64.0 mg of crude diamide **7** were isolated and purified directly by preparative HPLC (250 mm Rainin C 4, 60⟶100% B in 25 min, A: H_2O + 0.1% TFA; B: acetonitrile–isopropanol 2 : 1 + 0.1% TFA; 14 ml min^{-1}). Treatment of the collected fractions according to GP 6 gave 36.0 mg (9.1 μmol, 61%) of pure diamide **7** as a colourless solid. δ_H(500 MHz; DMSO-d_6; Me_4Si) 0.40 (6H, d, *J* 6.6, 2 × Leu δ-H_3), 0.54 (6H, d, *J* 6.4, 2 × Leu δ-H_3), 0.58 (6H, d, *J* 6.4, 2 × Leu δ-H_3), 0.65 (12H, d, *J* 6.0, 4 × Leu δ-H_3), 0.72 (6H, d, *J* 6.19, 2 × Leu δ-H_3), 0.74–0.82 (30H, m, 10 × Val γ-H_3), 0.83–0.87 (18H, m, 6 × Val γ-H_3), 0.89 (6H, d, *J* 6.6, 2 × THF 2′-H_3), 0.98 (18H, s, 6 × *t*-Bu-H_3), 0.99–1.05 (2H, m, 2 × Leu γ-H), 1.09–1.38 (26H, m, 4 × Ala β-H_3, 4 × Leu β-H_2, 2 × THF 4- *H*H, 2 × THF 3-*H*H and 2 × Leu γ-H), 1.39–1.59 (8H, m, 2 × Leu β-H_2, 2 × THF 3-H*H* and 2 × Val β-H), 1.61–1.68 (2H, m, 2 × Leu γ-H), 1.72–1.80 (2H, m, 2 × THF 4-H*H*), 1.90–2.06 (6H, m, 6 × Val β-H), 2.36–2.47 (4H, m, succ 2-H_2 and 3-H_2), 2.76–2.84 (2H, m, 2 × Trp β-*H*H), 2.85–2.95 (4H, m, 4 × Trp β-*H*H), 3.14–3.31 (6H, m, 6 × Trp β-H*H*), 3.52 (2H, s, 2 × Gly α-*H*H), 3.61–3.68 (6H, m, 2 × AE 2-H_2 and 2 × Gly α-H*H*), 3.69–3.78 (4H, m, 2 × THF 5-H and 2 × Leu α-H), 3.81–3.88 (2H, m, 2 × Leu α-H), 3.89–3.97 (2H, m, 2 × THF 1′-H), 4.07–4.13 (2H, m, 2 × Val α-H), 4.15–4.33 (14H, m, 6 × Val α-H, 2 × THF 2-H, 4 × Ala α-H and 2 × Leu α-H), 4.39–4.51 (4H, m, 4 × Trp α-H), 4.54–4.61 (2H, m, 2 × Trp α-H), 6.90–6.97 (6H, m, 6 × ar. CH), 6.99–7.06 (6H, m, 6 × ar. CH), 7.08 (4H, s, 4 × ar. CH), 7.13 (2H, s, 2 × ar. CH), 7.30 (6H, d, *J* 8.0, 6 × ar. CH), 7.37–7.47 (12H, m, 12 × ar. CH), 7.51 (2H, d, *J* 7.8, 2 × ar. CH), 7.56 (4H, t, *J* 6.5, 4 × ar. CH), 7.59–7.67 (12H, m, 8 × ar. CH, NH and THF NH), 7.68–7.73 (2H, m, 2 × NH), 7.82–7.94 (8H, m, 6 × NH and 2 × Leu NH), 8.00–8.11 (6H, m, 4 × NH and 2 × AE NH), 8.12–8.18 (2H, m, 2 × NH), 8.21–8.39 (8H, m, 6 × Trp NH and 2 × Gly NH), 10.73–10.79 (4H, m, 4 × indole NH), 10.82 (2H, s, 2 × indole NH); HPLC: 18.1 min (M4); *m*/*z* (ESI): 1998.0 ($M + 2Na^+$. $C_{212}H_{298}N_{36}Na_2O_{34}Si$ requires 1997.1).

Succ-1-([10,11][D-THF-]-gA-TBDPS)-4-(gA-TBDPS) 8. 70.0 mg (29.6 μmol) of methyl ester **35** were *C*-deprotected according to GP 1. The crude carboxylic acid and 40.0 mg (22.5 μmol) of amine **33a** were collected and coupled according to GP 5 (1.0 mM, 12 h). After aqueous work-up 81.0 mg of crude diamide **8** were obtained and purified by preparative HPLC (250 mm Rainin C 4, 80⟶90% B in 10 min, then 90⟶100% B in 15 min. A: H_2O + 0.1% TFA; B: acetonitrile–isopropanol 2 : 1 + 0.1% TFA; 14 ml min^{-1}). Treatment of the collected fractions according to GP 6 gave 43.0 mg (10.5 μmol, 46%) of pure diamide **8** as a colourless solid. δ_H (500 MHz; DMSO-d_6; Me_4Si) 0.40 (3H, d, *J* 6.4, Leu δ-H_3), 0.49 (6H, d, *J* 6.4, 2 × Leu δ-H_3), 0.51–0.62 (15H, m, 5 × Leu δ-H_3), 0.61–0.68 (9H, m, 3 × Leu δ-H_3), 0.72 (3H, d, *J* 6.2, Leu d-H_3), 0.74–0.91 (54H, m, 2 × Leu δ-H_3 and 16 × Val γ-H_3), 0.92–0.95 (3H, m, THF 2′-H_3), 0.97 (9H, s, 3 × *t*-Bu-H_3), 0.98 (9H, s, 3 × *t*-Bu H_3), 1.09–1.32 (26H, m, 4 × Ala β-H_3, 5 × Leu β-H_2 and 4 × Leu γ-H), 1.42–1.59 (8H, m, 2 × Leu β-H_2, 2 × Val β-H, THF 4-*H*H and THF 3-*H*H), 1.61–1.67 (1H, m, Leu γ-H), 1.74–1.82 (1H, m, Leu γ-H), 1.90–2.05 (7H, m, 6 × Val β-H and Leu γ-H), 2.19 (1H, t, *J* 7.3, THF 3-*H*H), 2.25–2.32 (1H, m, THF 4-H*H*), 2.36–2.47 (4H, m, succ 2-H_2 and 3-H_2) 2.74–2.95 (6H, m, 3 × Trp β-H_2), 3.06–3.29 (8H, m, 3 × Trp β-H_2 and AE 2-*H*H), 3.35–3.43 (2H, m, AE 2-H*H*), 3.46–3.50 (2H, m, Trp β-H_2), 3.58–3.68 (6H, m, 2 × C-2 H_2 and Gly α-H_2), 3.69–3.75 (2H, m, 2 × Leu α-H), 3.82–3.88 (1H, m, Leu α-H), 3.90–3.95 (2H, m, THF 1′-H and Leu α-H), 4.05–4.16 (5H, m, 2 × Val α-H, Gly α-H_2 and Leu α-H), 4.17–4.34 (13H, m, 4 × Ala α-H, Leu α-H, THF 5-H and 2-H and 6 × Val α-H), 4.41–4.61 (7H, m, 7 × Trp α-H), 6.88–6.97 (6H, m, 6 × ar. CH), 6.99–7.05 (6H, m, 6 × ar. CH), 7.08 (6H, s, 6 × ar. CH), 7.13 (1H, s, ar. CH), 7.25–7.32 (7H, m, 7 × ar. CH), 7.36–7.47 (12H, 12 × ar. CH), 7.49–7.58 (7H, m, 7 × ar. CH), 7.59–7.63 (12H, m, 10 × ar. CH and 2 × NH), 7.64–7.67 (1H, m, THF NH), 7.68–7.82 (3H, m, NH), 7.85 (1H, d, *J* 5.7, Leu NH), 7.87–7.96 (7H, m, 7 × NH), 7.98 (1H, d, *J* 5.7, Leu NH), 8.01–8.18 (8H, m, 7 × NH and Trp NH), 8.21–8.31 (6H, m, Gly NH and 5 × Trp NH), 8.33 (1H, d, *J* 6.4, Trp NH), 10.71

(1H, s, indole NH), 10.76 (5H, s, 5 × indole NH), 10.82 (1H, s, indole NH). HPLC: 10.6 min (10 × 250 mm Rainin C 4, 80⟶90% B in 10 min, then 90⟶100% B in 15 min. A: H_2O + 0.1% TFA; B: acetonitrile–isopropanol 2 : 1 + 0.1% TFA; 3.3 ml min^{-1}); *m/z* (ESI) 2077.4 ($M + 2Na^+$. $C_{222}H_{308}N_{38}Na_2O_{34}Si_2$ requires 2076.2).

Abbreviations

Amino acid residues

For the schemes the common one letter code was used, where capital letters denote L- and small letters D-configuration. V = L-Val, G = Gly, A = L-Ala, l = D-Leu, v = D-Val, W = L-Trp.

Z: carboxy-benzyl; **TBDPS**: *t*-butyl-diphenyl-silyl; **HOBt**: 1-hydroxy-benzotriazole monohydrate; **EDC**: *N*-ethyl-*N*′-(3-dimethylamino-propyl)-carbodiimide hydrochloride; **HBTU**: *O*-(benzotriazol-1′-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate; **DIEA**: di-*iso*-propyl-ethylamine; **HATU**: *O*-(7′-aza-benzotriazol-1′-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate; **HOAt**: 1-hydroxy-7-aza-benzotriazole; **FCC**: flash column chromatography; **TFE**: trifluorethanol; **DMSO**: dimethylsulfoxide; **DMF**: dimethylformamide; **DIBAL-H**: diisobutyl aluminium hydride; **MCPBA**: *m*-chloroperbenzoic acid.

Acknowledgements

This work was supported by the Volkswagen Foundation, the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie and the Pinguin Foundation.

References

1 B. Hille, *Ionic Channels of Excitable Membranes*, 3rd edn., Sinauer, Sunderland, Massachusetts, 2001.

2 (*a*) Y. Jiang, A. Lee, J. Chen, M. Cadene, B. T. Chait and R. MacKinnon, *Nature*, 2002, **417**, 523–526; (*b*) Y. Jiang, A. Lee, J. Chen, M. Cadene, B. T. Chait and R. MacKinnon, *Nature*, 2002, **417**, 515–522; (*c*) R. Dutzler, E. B. Campbell, M. Cadene, B. T. Chait and R. MacKinnon, *Nature*, 2002, **415**, 287–294; (*d*) C. Toyoshima, M. Nakasako, H. Nomura and H. Ogawa, *Nature*, 2000, **405**, 647–655; (*e*) D. Doyle, J. M. Cabral, R. A. Pfuetzner, A. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait and R. MacKinnon, *Science*, 1998, **280**, 69–77; (*f*) S. J. Harrop, M. Z. DeMaere, D. Fairlie, T. Reztsova, S. M. Valenzuela, M. Mazzanti, R. Tonini, M. R. Qiu, L. Jankova, K. Warton, A. R. Bauskin, W. M. Wu, S. Pankhurst, T. J. Campbell, S. N. Breit, P. M. G. Curmi and M. G. Paul, *J. Biol. Chem.*, 2001, **276**, 44993–45000.

3 (*a*) D. M. Kullmann, *Brain*, 2002, **125**, 1177–1195; (*b*) S. G. Waxman, *Neurology*, 2001, **56**, 1621–1627; (*c*) B. Dworakowska and K. Dolowy, *Acta Biochim. Pol.*, 2000, **47**, 685.

4 Reviews on synthetic ion channels: (*a*) Y. Kobuke in *Advances in Supramolecular Chemistry,* ed. G. W. Gokel, JAI-Press, Greenwich, 1997, Vol. 4, pp. 163–210; (*b*) N. Voyer, *Top. Curr. Chem.*, 1996, **184**, 1–37; (*c*) G. W. Gokel and O. Murillo, *Acc. Chem. Res.*, 1996, **29**, 425–432; (*d*) G. W. Gokel and A. Mukhopadhyay, *Chem. Soc. Rev.*, 2001, **30**, 274–286; selected recent contributions; (*e*) B. Baumeister, N. Sakai and S. Matile, *Angew. Chem., Int. Ed.*, 2000, **39**, 1955–1958; (*f*) N. Sakai and S. Matile, *J. Am. Chem. Soc.*, 2002, **124**, 1184–1185; (*g*) N. Yoshino, A. Satake and Y. Kobuke, *Angew. Chem., Int. Ed.*, 2001, **40**, 457–59; (*h*) C. Goto, M. Yamamura, A. Satake and Y. Kobuke, *J. Am. Chem. Soc.*, 2001, **123**, 12152–12159; (*i*) L. M. Cameron, T. M. Fyles and C.-W. Hu, *J. Org. Chem.*, 2002, **67**, 1548–1553; (*j*) P. H. Schlesinger, R. Ferdani, J. Liu, J. Pajewska, R. Pajewski, M. Saito, H. Shabany and G. W. Gokel, *J. Am. Chem. Soc.*, 2002, **124**, 1848–1849; (*k*) A. J. Wright, S. E. Matthews, W. B. Fischer and P. D. Beer, *Chem. Eur. J.*, 2001, **7**, 3474–3481; (*l*) V. Sidorov, F. W. Kotch, G. Abdrakhmanova, R. Mizani and J. C. Fettinger, *J. Am. Chem. Soc.*, 2002, **124**, 2267–2278; (*m*) S. Futaki, M. Fukuda, M. Omote, K. Yamauchi, T. Yagami, M. Niwa and Y. Sugiura, *J. Am. Chem. Soc.*, 2001, **123**, 12128–12134.

5 P. Fidzinski, A. Knoll, R. Rosenthal, A. Schrey, A. Vescovi, U. Koert, M. Wiederholt and O. Strauß, *Chem. Biol.*, 2003, **10**, 35–43.

6 (*a*) B. A. Cornell, V. L. B. Braach-Maksvytis, L. G. King, P. D. J. Osman, B. Raguse, L. Wieczorek and R. J. Pace, *Nature*, 1997, **387**, 580–583; (*b*) M. Akeson, D. Branton, J. J. Kasianowicz, E. Brandin and D. W. Deamer, *Biophys. J.*, 1999, **77**, 3227–3233; (*c*) H. Bayley and C. R. Martin, *Chem. Rev.*, 2000, **100**, 2575–2594; (*d*) S. Terrettaz, W.-P. Ulrich, R. Guerrini, A. Verdini and H. Vogel, *Angew. Chem.*, 2001, **113**, 1788–1793.

7 H. D. Arndt, D. Bockelmann, A. Knoll, S. Lamberth, C. Griesinger and U. Koert, *Angew. Chem., Int. Ed.*, 2002, **41**, 4062–4065.

8 H.-G. Weinig, R. Krauss, M. Seydack, J. Bendig and U. Koert, *Chem. Eur. J.*, 2001, **7**, 2075–2088.

9 H.-D. Arndt, A. Knoll and U. Koert, *Angew. Chem., Int. Ed.*, 2001, **40**, 2076–2078.

10 U. Koert, A. Knoll, A. Vescovi, *Pure Appl. Chem.*, submitted for publication.

11 (*a*) R. E. Koeppe II and O. S. Andersen, *Annu. Rev. Biophys. Biomol. Struct.*, 1996, **25**, 231–258; (*b*) D. J. Chadwick, G. Cardew, *Gramicidin and Related Ion-Channel Forming Peptides*, Wiley, Chichester, 1999.

12 (*a*) R. R. Ketchem, W. Hu and T. A. Cross, *Science*, 1993, **261**, 1457–1460; (*b*) O. S. Andersen, H. J. Apell, E. Bamberg, D. D. Busath, R. E. Koeppe II, F. J. Sigworth, G. Szabo, D. W. Urry and A. Woolley, *Nat. Struct. Biol.*, 1999, **6**, 609; (*c*) T. A. Cross, A. Arseniev, B. A. Cornell, J. H. Davis, J. A. Killian, R. E. Koeppe II, L. K. Nicholson, F. Separovic and B. A. Wallace, *Nat. Struct. Biol.*, 1999, **6**, 610–611; (*d*) B. M. Burkhart and W. L. Duax, *Nat. Struct. Biol.*, 1999, **6**, 611–612; (*e*) B. A. Wallace, *J. Struct. Biol.*, 1998, **121**, 123–141.

13 (*a*) I. L. Barsukov, A. L. Lomize, A. S. Arseniev and V. F. Bystrov, *Biol. Membr.*, 1987, **4**, 171–193; (*b*) C. J. Stankovic and S. L. Schreiber, *Chemtracts: Org. Chem.*, 1991, **4**, 1–19 and references cited therein.

14 V. Borisenko, D. C. Burns, Z. Zhang and G. A. Woolley, *J. Am. Chem. Soc.*, 2000, **122**, 6364–6370.

15 A. Schrey, F. Osterkamp, A. Straudi, C. Rickert, H. Wagner, U. Koert, B. Herrschaft and K. Harms, *Eur. J. Org. Chem.*, 1999, 2977–2990.

16 U. Koert, *J. Prakt. Chem.*, 2000, **342**, 325–333.

17 A. Schrey, A. Vescovi, A. Knoll, C. Rickert and U. Koert, *Angew. Chem., Int. Ed.*, 2000, **39**, 900–902.

18 T. A. Cross, F. Tian, M. Cotten, J. Wang, F. Kovacs and R. Fu;, page 4 in ref. 11*b*.

19 (*a*) C. G. Fields, G. B. Fields, R. L. Noble and T. A. Cross, *Int. J. Pept. Protein Res.*, 1989, **33**, 298–303; (*b*) E. Suarez, E. D. Emmanuelle, G. Molle, R. Lazaro and P. Viallefont, *J. Pept. Sci.*, 1998, **4**, 371–377; (*c*) D. V. Greathouse, R. E. Koeppe II, L. L. Providence, S. Shobana and O. S. Andersen, *Methods Enzymol.*, 1999, **294**, 525–550.

20 The reference compound **succ(gA-TBDPS)$_2$** consitsts of two gA units with both *N*-termini linked by a succinyl moiety and a TBDPS group at the ethanolamine termini. H.-D. Arndt, A. Vescovi, A. Schrey, J. R. Pfeifer and U. Koert, *Tetrahedron*, 2002, **58**, 2789–2801.