Synthesis and Functional Studies of a Membrane-Bound THF-Gramicidin Cation Channel**

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Channel-mediated transport of ions through lipid bilayers is a particularly important molecular function.[1] Synthetic ion channels can contribute to the understanding of the active conformations of biological ion channels.[2, 3] As a result of the ability of tetrahydrofurans (THFs) to complex cations, a channel approach based on oligo-THFs[4] and oligo-THF-amino acids[5] was developed. Herein we report on the synthesis of a functional cation channel with a biomimetic channel entrance and exit where THF-amino acids are incorporated as building blocks.

Our biomimetic concept originates at gramicidin A, an ion channel active pentadecapeptide with the sequence HCO-L-Val_1-Gly_2-L-Ala_3-D-Leu_4-L-Ala_5-D-Val_6-L-Val_7-D-Val_8-L-Trp_9-D-Leu_{10}-L-Trp_{11}-D-Leu_{12}-L-Trp_{13}-D-Leu_{14}-L-Trp_{15}-$CONHCH_2$-CH_2OH.[6] The active conformation of the gramicidin channel in lipid bilayers is a H-bonded head-to-head dimer consisting of two right-handed, single-stranded $\beta^{6,3}$-helices.[7] The helix turns forming the channel entrance and exit each contain four L-tryptophan residues. It is proposed that the indole side chains of the tryptophan groups contribute to the stabilization of the channel structure by H-bonding to the polar lipid head groups and through electrostatic dipole interactions with the surface potential of the membrane.[8]

Our goal is to construct a unimolecular ion channel that would have the advantage of better structural characterization and functional control relative to a self-assembled channel consisting of numerous subunits. THF-gramicidin hybrids **1** and **2** were chosen as target compounds.

Compound **1** contains the heptapetide part (L-Trp-D-Leu)$_3$-L-Trp, a tetra-THF-peptide part, a linker derived from tartaric acid, and the pentadecapeptide known from gramicidin A. The (*S*,*S*)-tartrate unit has been used successfully for a covalently linked head-to-head gramicidin dimer.[9] Both ends of **1** are terminated by ethanolamine units protected by lipophilic *tert*-butyldiphenylsilyl (TBDPS) groups. Compound **2** possesses free ethanolamine end groups. An INSIGHT/DISCOVER generated structural model of **2** is shown in Figure 1. In this structure the four THF-amino acids continue the gramicidin β-helix.

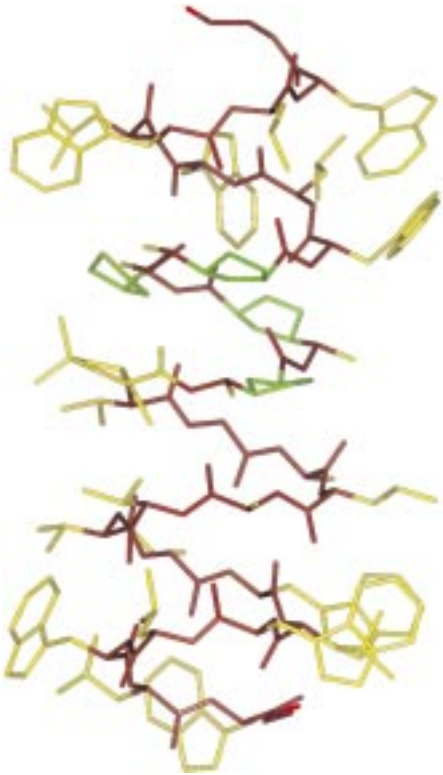
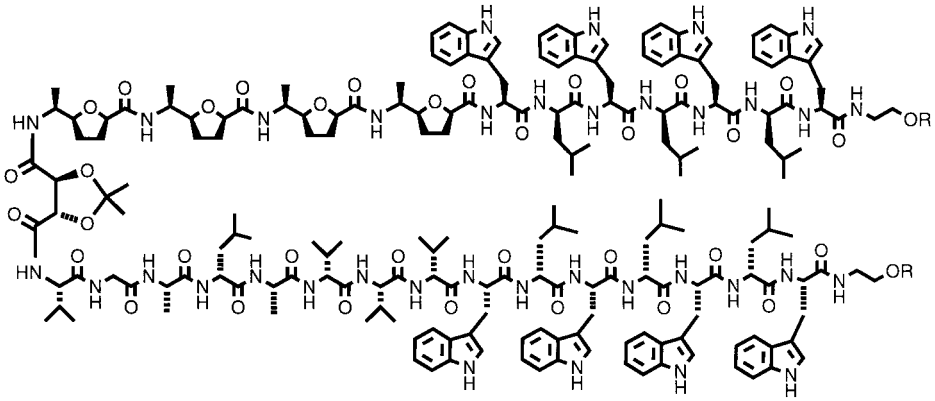


Figure 1. Structural model (INSIGHT/DISCOVER) of the ion channel formed by compound **2**. Peptide backbone is shown in brown, side chains and tartrate linker in yellow, THF rings in green.



1 R = *t*BuPh$_2$Si

2 R = H

The starting point of the synthesis of **1** were the L-alanine-derived[10] Boc-THF-amino acid building blocks **3** and **4** (Scheme 1). Both were connected via a dimer intermediate to THF-tetrapeptide **5**. Coupling of the acetonide-protected monomethyl tartrate (**6**) to **5** gave compound **7**. Hydrogenolysis of the benzyl ester of **7** afforded a carboxylic acid, which could be linked with the heptapeptide building block

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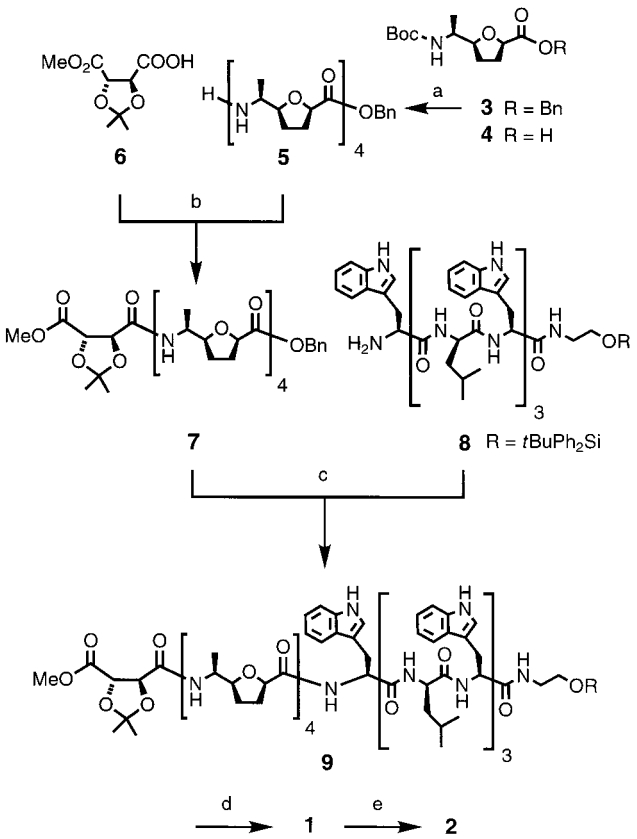

Scheme 1. Synthesis of **1** and **2**. a) 1. CF_3COOH (5% in CH_2Cl_2), 30 min; 2. **4**, HOBt, EDC, Et_3N, $CHCl_3$, 12 h, 80%; 3. dipeptide, Pd(10%)/C, H_2, CH_3OH:H_2O (5:1), 25 °C, 1 h or CF_3COOH (5% in CH_2Cl_2), 30 min; 4. N- and C-deprotected dipeptides, HOBt, EDC, Et_3N, $CHCl_3$, 12 h, 81%; 5. CF_3COOH (5% in CH_2Cl_2), 30 min; b) HOBt, EDC, Et_3N, $CHCl_3$, 12 h, 86%; c) 1. **7**, Pd(10%)/C, H_2, CH_3OH:H_2O (5:1), 25 °C, 3 h; 2. **8**, 2,6-lutidine, HATU, DMF, 0 → 25 °C, 6 h, 80%; d) 1. LiOH, THF:H_2O (2:1), 25 °C, 30 min; 2. pentadecapeptide buildig block, 2,6-lutidine, HATU, DMF, 0 → 25 °C, 4 h, 60%; e) nBu_4NF, THF, 25 °C, 2 h, 15% after column chromatography. HOBt = 1-hydroxybenzotriazole, EDC = *N*′-(3-dimethylaminopropyl)-*N*-ethyl-carbodiimide hydrochloride, HATU = *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetra-methyluronium hexafluorophosphate.

8[11] to yield compound **9**. After hydrolysis of the methyl ester of **9**, a peptide coupling with the pentadecapeptide L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-$CONHCH_2$-$CH_2OTBDPS$[11] afforded target compound **1** (MALDI-MS: found: $[M+Na]^+ = 4215$). A fluoride-mediated cleavage of the TBDPS groups gave compound **2** (MALDI-MS: found: $[M+Na]^+ = 3741$).

The ability of **1** and **2** to function as channels was studied using conductance measurements[12] on synthetic lipid bilayers (see Experimental Section). Compound **1** is incorporated into the membrane and acts as a functional cation channel. The single channel measurements (Figure 2a) display more than one conductance level. Level I with 15.4 pS and an average open time of 500 ms accounts for up to 30% of the channel events. Level II with 19.7 pS is found less often; its contribution to the overall current, however, is the highest as a result of its long open time of up to 15 s. The appearence of multiple channel levels suggests the existence of more than one channel-active conformation in the membrane. The

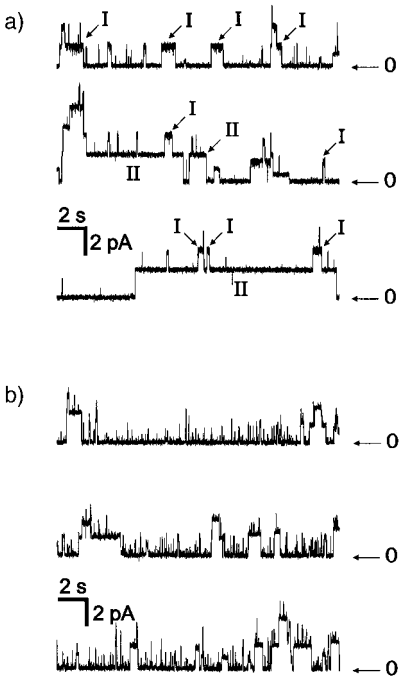

Figure 2. Typical single channel currents through soybean lecithin membranes in 1 M KCl at a membrane potential of +100 mV in the presence of 0.01 μM **1** (a) and **2** (b). The numbers indicate the closed channel (0), the conductance level with the highest frequency (I), and that with the highest open probability (II).

channels exhibit an Eisenman-I selectivity[13] towards monovalent cations ($NH_4^+ > Cs^+ > K^+ > Na^+$, Table 1). If the passage of the ion through the channel requires a partial loss of the hydration sphere, then a strong influence of the dehydration energy and the weak binding sites in the channel would lead to the observed order of Eisenman-I selectivity.[1c] Figure 3 shows the linear current–voltage relationships over the range of −100 mV to +100 mV for compound **1** with various cations.

Table 1. Conductance and relative permeabilities of **1** and **2** to various cations.

Electrolyte/channel	Conductance[a]	Permeability[b]
NH_4Cl/**1**	35.7	9.8
NH_4Cl/**2**	42.3	10.9
CsCl/**1**	30.5	4.7
CsCl/**2**	34.0	5.4
KCl/**1**	20.2	2.7
KCl/**2**	23.1	3.1
NaCl/**1**	9.5	1.0
NaCl/**2**	16.3	1.0

[a] All values in pS, corresponding to the regression slope of the current–voltage relationship for the highest single-channel conductance level. [b] Values relative to Na^+. Determined from the reversal potentials according to Goldmann, Hodgkin, and Katz. See ref. [1c].

The single channel measurements of compound **2** (Figure 2b) also exhibit multiple-conductance levels with Eisenman-I selectivity for monovalent cations, however, the open times are remarkably shorter. The removal of the lipophilic TBDPS groups results in a shortening of the channel dwell times.

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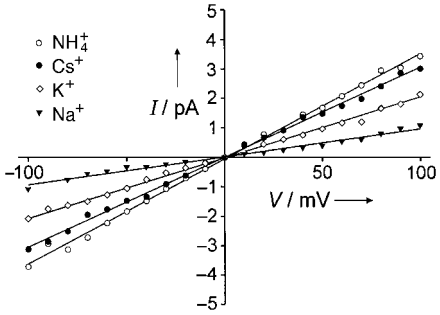

Figure 3. Current–voltage relationships for single channels of **1** in soybean lecithin membranes in the presence of various electrolytes. Only the highest conductance levels were considered.

The present work reports on the synthesis and functional characterization of a novel cation channel. The structural characterization of the active conformation of the THF-gramicidine hybrids in the membrane by isotope labeling and solid-phase NMR is currently under investigation and will be reported elsewhere.

Experimental Section

Planar lipid membranes were prepared by painting a solution of soybean lecithin (45% Avanti Polar Lipids) in *n*-decane (25 mg mL^{-1}) over a cuvette aperture with a diameter of 0.15 mm.[1c] The membrane surface was 0.01 to 0.02 mm^2 assuming a specific membrane capacity of 0.4 μF cm^{-2}. All experiments were performed at ambient temperature. The electrolyte solutions with a concentration of 1M each were unbuffered. The probes, dissolved in methanol, were added to the *trans* side, to give a final concentration of 0.01–0.03 μM. Current detection and recording was performed using a patch-clamp amplifier Axopatch 200, a DigiData A/D converter, and the pClamp 6 software (Axon Instruments). The aquisition frequency was 5 kHz. The data were filtered with an analogue filter at 100 Hz for further analysis.

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Simple Synthesis of Tetra-Acceptor-Substituted Alkenes by the Formal Dehydrodimerization of Malonates**

Torsten Linker* and Ursula Linker

Dedicated to Professor Bernd Giese on the occasion of his 60th birthday

Acceptor-substituted alkenes are important precursors for Diels–Alder reactions, Michael additions, or polymerizations. Due to their electronic properties, ethylenetetracarboxylates exhibit an especially high reactivity, but are difficult to prepare. Traditional syntheses start from halomalonates and sodium or mesoxalic acid,[1] whereas modern methods by the dimerization of malonates require an excess of oxidant or the use of expensive azo compounds.[2] Furthermore, disadvantages of all methodologies are the moderate yields. Herein we describe a simple new access to tetra-acceptor-substituted alkenes by the formal dehydrodimerization of malonates in only two steps. The synthesis is characterized by cheap reagents and high yields.

During the course of our investigations on transition metal mediated radical reactions,[3] we succeeded in the addition of dimethyl malonate (**1a**) to various alkenes. To further improve the yields and due to the mild conditions, we became

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