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Cyclohexylether δ-Amino Acids: New Leads for Selectivity Filters in Ion Channels**

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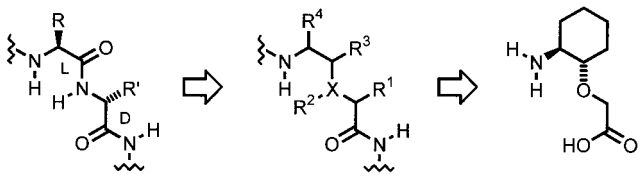
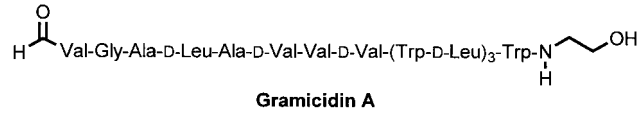
Biological ion channels are key molecules for cellular regulation and communication. They couple (bio)molecular events to electric signals.[1] This property of natural pore-forming substances has been utilized in engineering biosensors.[2] In order to use synthetic channel structures[3] as sensors or implants in biological systems, they have to meet requirements in two areas: ion selectivity[3e, 4] and gating.[5] We report here on novel oligomers made from δ-amino acids that led to H^+- and NH_4^+-selective ion channels.

On substituting the central amide bond of a dipeptide, a δ-amino acid is obtained (Scheme 1).[6] Besides offering structural diversity at four positions, a δ-amino acid allows incorporation of a heteroatom in the continuous backbone.[7] If one chooses an oxygen and constricts the degrees of conformational freedom with a cyclohexane ring, the ether amino acid (AA) **1** results.

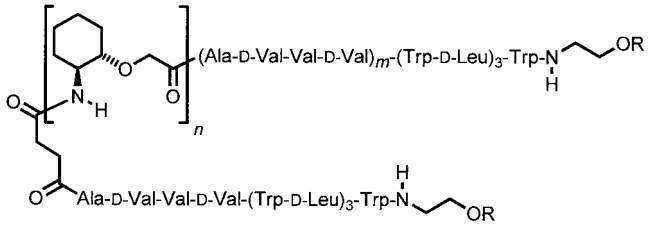


Scheme 1. From D,L-dipeptides to the stereoequivalent δ-amino acid **1**, a new building block for cation channels.

The selectivity filters of biological K^+ and Ca^{2+} channels are lined with backbone atoms.[8a,b] In the ion channel active, but weakly selective, $\beta^{6.3}$-helical dimer of the D,L-peptide gramicidin A (gA) only backbone amides are exposed towards the



Gramicidin A



2 (n = 2, m = 1)
3 (n = 4, m = 1)
4 (n = 6, m = 0)

R = tBuPh$_2$Si

interior.[8c] The incorporation of δ-AA **1** with its ether oxygens should offer additional binding sites for a cation in the lumen.[9] Our target compounds **2**–**4** were chosen by combining di-, tetra-, and hexameric δ-AA segments with functionally important sequences from gA to yield structures with the approximate total length of the gA dimer.[10, 11]

The synthesis of the compounds incorporating δ-AA **1** starts from cyclohexene epoxide **5**, which was transformed to the azido alcohol according to the method of Jacobsen and coworkers[12] (94% yield, 93% *ee* under optimized conditions, Scheme 2). Alkylation with *tert*-butylbromoacetate using phase-transfer catalysis[13] gave masked δ-AA monomer **6** (95%; R = tBu),[14] which was homodimerized to **9** via a mixed anhydride (R = COCMe$_3$; formed from **7** (step e in Scheme 2)). Dimer **9** could be obtained isomerically pure by crystallization (n-hexane/Et$_2$O (7:1), Figure 2). After elongation with a succinate building block, the resulting diester **10** was connected at both termini with the α-peptide **11**[11] to yield the target compound **2**. Compounds **3** and **4** were similarly synthesized (see Supporting Information).[14]

The compounds **2**–**4** were then examined for their ion channel forming activity.[15, 16] The compounds with tetra- and hexameric δ-peptide units, **3** and **4**, did not form detectable cation channels. But they induced short-lived proton channels when applied in concentrations above 100 nM (Figure 1a, b). Probably, a bottleneck conformation permits only protons to pass.[17]

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Supporting information for this article is available on the WWW under http://www.angewandte.com or from the author.

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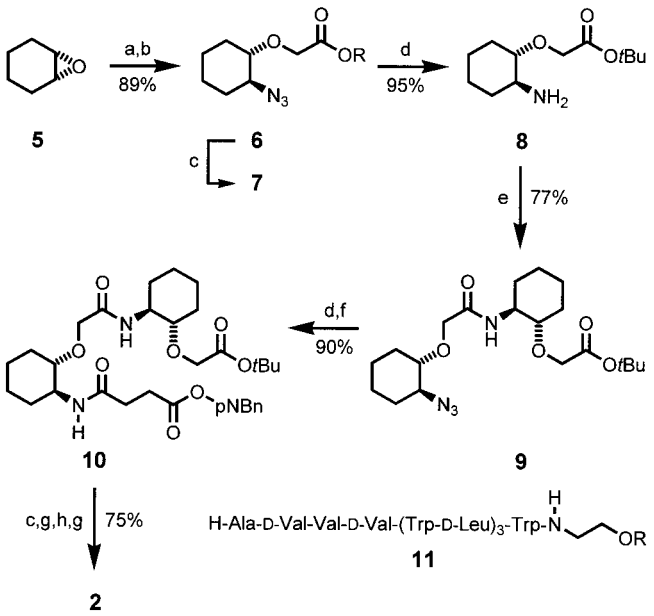

Scheme 2. Synthesis of **2**: a) 1. $TMSN_3$ (1.1 equiv), 2 mol% $[Cr(N_3)(salen)]$,[12] 20 mol% *i*PrOH, Et_2O (2 M), 0 °C, 36 h, 94%; 2. 0.1% TFA in MeOH, 20 °C, 1 h, >99%; b) *t*BuOCOCH$_2$Br, *n*Bu$_4$Br, 12 M NaOH/toluene, 20 °C, 24 h, 95%; c) TFA/CH_2Cl_2 (2:1), 20 °C, 1 h, >99%; d) MeOH, cat. Pd/C (5%), H_2 (1 bar), 2 h, >99%; e) **7** (R = H) in DMF, NEt_3, PivCl, 0 °C, 30 min, then **8**, NEt_3, 0 °C → 20 °C, 1 h, 77%; f) *p*NBnO-Succ, EDC, HOBt, EtN*i*Pr$_2$, CH_2Cl_2/DMF (10:1), 0 °C → 20 °C, 12 h, 90%; g) **11**, HATU, HOAt, EtN*i*Pr$_2$, CH_2Cl_2/DMF (3:1), 0 °C → 20 °C, 6 h; h) LiOH, THF/H_2O 3:1, 0 °C, 1 h. DMF = *N*,*N*-dimethylformamide, EDC = 3-(3-dimethyl-aminopropyl)-1-ethylcarbodiimide hydrochloride, HATU = *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HOAt = 7-aza-1-hydroxy-1*H*-benzotriazole, HOBt = 1-hydroxy-1*H*-benzotriazole monohydrate, H_2Salen = (*R*,*R*)-*N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene)cyclohexane-1,2-diamine, Piv = pivaloyl ($COCMe_3$), *p*NBn = 4-nitrobenzyl, Succ = succinyl ($COCH_2CH_2CO_2H$), TFA = trifluoroacetic acid.

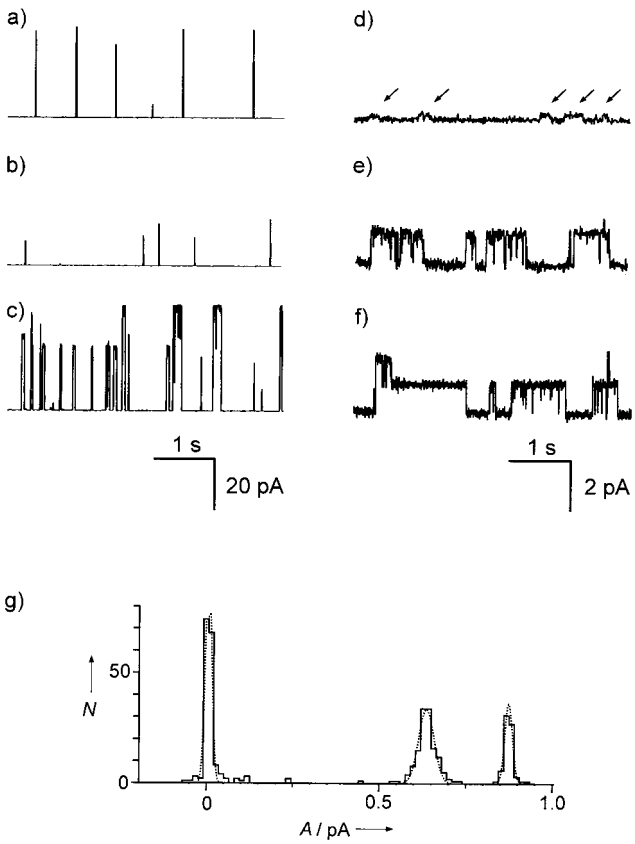

Figure 1. Representative current traces of compounds **2**–**4** in planar soybean lecithin lipid bilayers: a) **4**, conductivity $\Lambda = 355/198$ pS, dwell time $\tau = 3.7$ ms; b) **3**, $\Lambda = 255/107$ ps, $\tau = 8$ ms (c(**3**, **4**) = 500 nM, $U = +100$ mV; in 1 M HCl); c) **2**, H^+; d) **2**, K^+; e) **2**, Cs^+; f) **2**, NH_4^+ (c(**2**) = 10 pM, $U = +200$ mV; in 1 M MCl, $M^+ = H^+$, K^+, Cs^+, NH_4^+); g) amplitude histogram for NH_4^+-channels of compound **2** at 120 mV (A = amplitude, N = number of results). Experimental details can be found in the Supporting Information.

In contrast, compound **2** formed well-defined channels with a remarkable selectivity among monovalent cations: Cs^+ and NH_4^+ were conducted well, but K^+ single-channel events were at the detection limit (Figure 1 d–f). For Na^+ and Li^+, single-channel events could no longer be resolved any more. The analysis of the permeability ratios revealed a pronounced Eisenman-I selectivity:[1b] $H^+ \gg NH_4^+ \gg Cs^+ > Rb^+ > K^+ > Na^+ \approx Li^+$ (Table 1). The selectivity (P_{rel}, Λ_{rel}) of **2** is three to four times higher than that of gA. The absolute conductance is decreased by the incorporated δ-AAs, which indicates a narrowed pore or a reinforced binding.[18] The mean dwell times of the cation channels of **2** are between 200 and 350 ms, but the proton-channel dwell time is only 8 ms (Figure 1 c).[5b, 19]

With the exception of Cs^+, two levels of conductance were found (Table 1, Figure 1 g). Asymmetric compounds such as **2**–**4** may adopt at least two orientations within the membrane, with respect to the membrane normal. A maximum of two detectable conductance levels supports the assumptions that a) unimolecular channels are present and b) the δ-AAs do indeed influence the ions pathway.

These results introduce the new δ-AA **1** as a novel lead structure in the synthesis of H^+- and NH_4^+-selective ion

Table 1. Permeability ratios P and conductivity figures Λ of **2** and gA.

M^+	P_{rel}(gA)[a]	P_{rel}(**2**)[a]	Λ(gA)[b]	Λ_1(**2**)[b]	Λ_2(**2**)[b]	Λ_{rel}(gA)[c]	Λ_{rel}(**2**)[c]
Li^+	0.051 ± 0.001	0.035 ± 0.002	2.93 ± 0.07	–	–	0.07	–
Na^+	0.105 ± 0.003	0.036 ± 0.002	14.80 ± 0.08	–	–	0.34	–
K^+	0.248 ± 0.004	0.078 ± 0.008	26.0 ± 0.2	1.06 ± 0.11	0.66 ± 0.06	0.60	0.14 (0.12)
Rb^+	0.401 ± 0.012	0.122 ± 0.004	43.1 ± 0.3	2.38 ± 0.13	1.85 ± 0.11	1.00	0.32 (0.34)
Cs^+	0.507 ± 0.009	0.273 ± 0.025	43.6 ± 0.2	6.48 ± 0.11	–	1.01	0.87
NH_4^+	1	1	43.3 ± 0.2	7.43 ± 0.07	5.49 ± 0.09	1	1
H^+	3.99 ± 0.19	4.77 ± 0.18	561 ± 17	90 ± 15	67.2 ± 0.8	13.0	12.1 (12.2)

[a] Determined from the reversal potentials of 1 M MCl solutions relative to a 1 M NH_4Cl solution.[1b] This is the mean of all conductivity levels because sum currents were measured (see Supporting Information). [b] Conductivity [pS], determined from the slope of the current-voltage curve at $U = 0$ mV. For Cs^+ only one level was found. [c] Conductivity relative to NH_4^+ conductivity. The second conductivity level is given in parentheses.

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channels. Synthetic compound **2** shows a more than threefold increase in NH_4^+/K^+ selectivity compared to gA. The X-ray crystal structure analysis of dimer **9** (Figure 2) gives a hint of the role of δ-AA **1** in the channel:[20] The δ-peptide **9** adopts

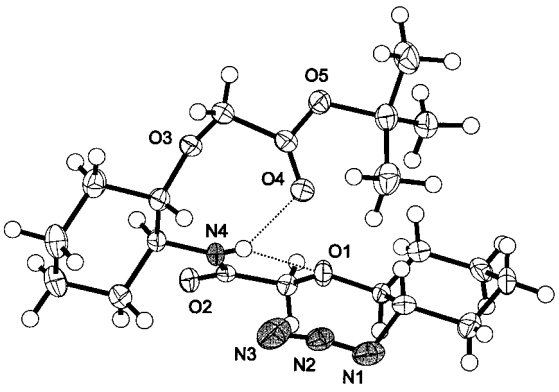

Figure 2. X-ray crystal structure analysis of δ-dipeptide **9** (ellipsoids with 50% probability). Selected distances [pm]: N4-O1 256.3(6), N4-O4 315.8(7). Please note the right-handed helical conformation (N1-O1-N4-O3-O5).

the conformation of a right-handed helix in the solid state. Thus, it should be ideally suited to propagate the right-handed $\beta^{6.3}$-helices of the D,L-peptide segments in their ion-conducting conformation.[8c, 11] The central bifurcated hydrogen bond has to open to allow ions to pass vertically in the plane of display. Such "gating" could account for the dwell times, which are short compared with known examples.[9b, 11, 19]

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[17] Distinct proton-channel activity was only observed at high concentrations, which could indicate that aggregates of these compounds form the active channels. This possibility is under active investigation.

[18] The bulk of the hydration shell of an ion is stripped off on entering the gA channel.[9] A comparison of the changed selectivities with the effective ionic radii[1b] reveals that particularly the smaller, harder cations pass the novel channel **2** more slowly (effective ionic radii are given in parentheses): Li^+ (60 pm) < Na^+ (95 pm) < K^+ (133 pm) < Rb^+ (148 pm) ≈ NH_4^+ (150 pm) < Cs^+ (169 pm).

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