COMMUNICATIONS

- [9] a) R. Irie, Y. Ito, T. Katsuki, Synlett 1991, 265; b) N. S. Finney, P. J. Pospisil, S. Chang, M. Palucki, R. G. Konsler, K. B. Hansen, E. N. Jacobsen, Angew. Chem. 1997, 109, 1798; Angew. Chem. Int. Ed. Engl. **1997** 36 1720.
- [10] It should be pointed out that the role of spin contamination in Kohn-Sham theory is not clear. For a more extensive discussion see: W. Koch, M. C. Holthausen, A Chemist's Guide to Density Functional Theory, Wiley-VCH, Weinheim, 2000.
- [11] These would also be similar to the "bent" conformation proposed for chromium-salen complexes: K. M. Ryan, C. Bousquet, D. G. Gilheany, Tetrahedron Lett. 1999, 40, 3613.
- [12] Further evidence for the electronic nature of this effect comes from preliminary B3LYP/3-21G* calculations of the catalyst bearing an axial OCl- ligand. As in the case of trimethylamine N-oxide, the ligation of hypochlorite leads to highly nonplanar conformations. Similarly, inspection of the calculated structure of a Cl--ligated Mnsalen model catalyst depicted in ref. [7b] reveals a strong tendency for a nonplanar conformation.
- [13] K. Miura, T. Katsuki, Synlett 1999, 783.
- [14] For examples of such calculations, see: a) J. N. Harvey, M. Aschi, H. Schwarz, W. Koch, Theor. Chem. Acc. 1998, 99, 95; b) S. Mitchell, M. Blitz, P. Siegbahn, M. Svensson, J. Chem. Phys. 1994, 100, 423; compare also: c) D. Schröder, S. Shaik, H. Schwarz, Acc. Chem. Res. 2000, 33, 139.

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

Hans-Dieter Arndt, Andrea Knoll, and Ulrich Koert*

Biological ion channels are key molecules for cellular regulation and communication. They couple (bio)molecular events to electric signals.^[1] This property of natural poreforming 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 NH₄⁺-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.

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



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

D.L-dipeptide

The selectivity filters of biological K⁺ and Ca²⁺ 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





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/Et₂O (7:1), Figure 2). After elongation with a succinate building block, the resulting diester 10 was connected at both termini with the α -peptide $\mathbf{11}^{[11]}$ 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 nм (Figure 1 a, b). Probably, a bottleneck conformation permits only protons to pass.^[17]

^[*] Prof. Dr. U. Koert, Dipl.-Chem. H.-D. Arndt, Dr. A. Knoll Institut für Chemie der Humboldt-Universität zu Berlin Hessische Strasse 1-2, 10115 Berlin (Germany) Fax: (+49) 30-2093-7266 E-mail: koert@chemie.hu-berlin.de

^[**] Financial support by the Fonds der Chemischen Industrie (FCI), the Volkswagen Foundation, the Pinguin Foundation, and Schering AG is gratefully acknowledged. H.-D.A. thanks the FCI for a PhD fellowship. We thank Dr. B. Ziemer (Humboldt-Universität zu Berlin) for the X-ray structure analysis and Dr. P. Franke (Freie Universität Berlin) for MALDI-TOF mass spectra.

COMMUNICATIONS



Scheme 2. Synthesis of 2: a) 1. TMSN₃ (1.1 equiv), 2 mol% [Cr(N₃)(salen)],^[12] 20 mol % *i*PrOH, Et₂O (2M), 0°C, 36 h, 94%; 2.0.1% TFA in MeOH, 20 °C, 1 h, >99%; b) *t*BuOCOCH₂Br, *n*Bu₄Br, 12 m NaOH/ toluene, 20° C, 24 h, 95%; c) TFA/CH₂Cl₂ (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, NEt₃, PivCl, 0°C, 30 min, then 8, NEt₃, 0°C \rightarrow 20°C, 1 h, 77%; f) pNBnO-Succ, EDC, HOBt, EtNiPr₂, CH₂Cl₂/DMF (10:1), $0^{\circ}C \rightarrow 20^{\circ}C$, 12 h, 90%; g) 11, HATU, HOAt, EtNiPr₂, CH₂Cl₂/DMF (3:1), $0^{\circ}C \rightarrow 20^{\circ}C$, 6 h; h) LiOH, THF/H₂O 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-tetramethyluroniumhexa $fluorophosphate,\ HOAt=7-aza-1-hydroxy-1H-benzotriazole,\ HOBt=1$ hydroxy-1*H*-benzotriazole monohydrate, H_2 Salen = (*R*,*R*)-*N*,*N'*-bis(3,5-ditert-butylsalicylidene)cyclohexane-1,2-diamine, Piv = pivaloyl (COCMe₃), pNBn = 4-nitrobenzyl, Succ = succinyl (COCH₂CH₂CO₂H), TFA = trifluoroacetic acid.

In contrast, compound **2** formed well-defined channels with a remarkable selectivity among monovalent cations: Cs⁺ and NH₄⁺ were conducted well, but K⁺ single-channel events were at the detection limit (Figure 1 d – f). For Na⁺ and Li⁺, singlechannel events could no longer be resolved any more. The analysis of the permeability ratios revealed a pronounced Eisenman-I selectivity:^[1b] H⁺ \gg NH₄⁺ \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,



Figure 1. Representative current traces of compounds **2**–**4** in planar soyabean 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 ($c(\mathbf{3}, \mathbf{4}) = 500$ nM, U = +100 mV; in 1M HCl); c) **2**, H⁺; d) **2**, K⁺; e) **2**, Cs⁺; f) **2**, NH₄⁺ ($c(\mathbf{2}) = 10$ pM, U = +200 mV; in 1M MCl, M⁺ = H⁺, K⁺, Cs⁺, NH₄⁺); g) amplitude histogram for NH₄⁺-channels of compound **2** at 120 mV (A = amplitude, N = number of results). Experimental details can be found in the Supporting Information.

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 1g). 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 NH₄⁺-selective ion

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

Table 1. Permeability ratios P and conductivity figures A of 2 and gA.							
M^+	$P_{\rm rel}({\rm gA})^{[{\rm a}]}$	$P_{\rm rel}(2)^{[a]}$	$\Lambda(\mathrm{gA})^{[\mathrm{b}]}$	$arLambda_1(2)^{[b]}$	$arLambda_2(2)^{[b]}$	$\Lambda_{\rm rel}({\rm gA})^{[c]}$	$\Lambda_{\rm rel}(2)^{[c]}$
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	-
\mathbf{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)
\mathbf{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 μ MCl solutions relative to a 1 μ NH₄Cl 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 NH₄⁺ conductivity. The second conductivity level is given in parentheses.

Angew. Chem. Int. Ed. 2001, 40, No. 11 © WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2001 1433-7851/01/4011-2077 \$ 17.50+.50/0

COMMUNICATIONS

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]

Received: December 18, 2000 [Z16287]

- a) E. Neher, B. Sakmann, *Nature* 1976, 260, 779-802; b) B. Hille, *Ionic Channels of Excitable Membranes*, 2nd ed., Sinauer, Sunderland, 1992.
- [2] a) B. A. Cornell, V. L. B. Braach-Maksvytis, L. G. King, P. D. J. Osman, B. Raguse, L. Wieczorek, R. J. Pace, *Nature* 1997, *387*, 580–583;
 b) M. Akeson, D. Branton, J. J. Kasianowicz, E. Brandin, D. W. Deamer, *Biophys. J.* 1999, *77*, 3227–3233;
 c) H. Bayley, C. R. Martin, *Chem. Rev.* 2000, *100*, 2575–2594.
- [3] Reviews on artificial ion channels: a) Y. Kobuke in Advances in Supramolecular Chemistry, Vol. 4 (Ed.: G. W. Gokel), JAI, Greenwich, 1997, pp. 163–210; b) N. Voyer, Top. Curr. Chem. 1996, 184, 1– 37; c) G. W. Gokel, O. Murillo, Acc. Chem. Res. 1996, 29, 425–432; recent contributions: d) B. Baumeister, N. Sakai, S. Matile, Angew. Chem. 2000, 112, 2031–2034; Angew. Chem. Int. Ed. 2000, 39, 1955– 1958; e) N. Yoshino, A. Satake, Y. Kobuke, Angew. Chem. 2001, 113, 471–473; Angew. Chem. Int. Ed. 2001, 40, 457–459; f) ref. [10], and references therein.
- [4] a) Y. Tanaka, Y. Kobuke, M. Sokabe, Angew. Chem. 1995, 107, 717–719; Angew. Chem. Int. Ed. Engl. 1995, 34, 693–694; b) T. M. Fyles, D. Loock, W. F. van Straaten-Nijenhuis, X. Zhou, J. Org. Chem. 1996, 61, 8866–8874; c) M. M. Tedesco, B. Ghebremariam, N. Sakai, S. Matile, Angew. Chem. 1999, 111, 523–526; Angew. Chem. Int. Ed. 1999, 38, 540–543; d) S. Das, U. D. Lengweiler, D. Seebach, R. N. Reusch, Proc. Natl. Acad. Sci. USA 1997, 94, 9075–9079; e) T. Renkes, H. J. Schäfer, P. M. Siemens, E. Neumann, Angew. Chem. 2000, 112, 2566–2570; Angew. Chem. Int. Ed. 2000, 39, 2512–2516.
- [5] a) C. J. Stankovic, S. H. Heinemann, S. L. Schreiber, *Biochim. Biophys. Acta* 1991, *1061*, 163–170; b) G. A. Woolley, V. Zunic, J. Karanicolas, A. S. I. Jaikaran, A. V. Starostin, *Biophys. J.* 1997, *73*, 2465–2475; c) T. M. Fyles, D. Loock, X. Zhou, *J. Am. Chem. Soc.* 1998, *120*, 2997–3003.

- [6] β-Peptides: a) D. Seebach, J. L. Matthews, *Chem. Commun.* 1997, 21, 2015–2022; b) S. H. Gellman, *Acc. Chem. Res.* 1998, 31, 173–180; γ-peptides: c) T. Hintermann, K. Gademann, B. Jaun, D. Seebach, *Helv. Chim. Acta* 1998, 81, 983–1002; d) S. Hanessian, X. L. Luo, R. Schaum, S. Michnick, *J. Am. Chem. Soc.* 1998, 120, 8569–8570.
- [7] As opposed to their β- and γ-counterparts, δ-peptides should generally interact easily with the "natural" α-peptide world. In essence, the α-peptides form a subset of all possible δ-peptides. Examples for δ-peptides can be found in: a) U. Koert, J. Prakt. Chem. 2000, 342, 325-333; b) F. Machetti, A. Ferrali, G. Menchi, E. G. Occhiato, A. Guarna, Org. Lett. 2000, 2, 3987-3990, and references therein.
- [8] a) D. Doyle, J. M. Cabral, R. A. Pfuetzner, A. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait, R. MacKinnon, *Science* **1998**, *280*, 69–77; b) C. Toyoshima, M. Nakasako, H. Nomura, H. Ogawa, *Nature* **2000**, *405*, 647–655; c) R. R. Ketchem, B. Roux, T. A. Cross, *J. Biomol. NMR* **1996**, *8*, 1–14.
- [9] Modifications of the sequence and terminal groups of gramicidin A are well studied: a) P. Läuger, Angew. Chem. 1985, 97, 939-959; Angew. Chem. Int. Ed. Engl. 1985, 24, 905-925; b) C. J. Stankovic, S. L. Schreiber, Chemtracts: Org. Chem. 1991, 4, 1-19; c) R. E. Koeppe II, O. S. Andersen, Annu. Rev. Biophys. Biomol. Struct. 1996, 25, 231-258; d) V. Borisenko, D. C. Burns, Z. Zhang, G. A. Woolley, J. Am. Chem. Soc. 2000, 122, 6364-6370. The state of research in the field is presented in Gramicidin and Related Ion Channel-Forming Peptides (Eds.: D. J. Chadwick, G. Cardew), Wiley, Chichester, 1999.
- [10] THF-δ-AAs give functional ion channels: A. Schrey, A. Vescovi, A. Knoll, C. Rickert, U. Koert, Angew. Chem. 2000, 112, 928–931; Angew. Chem. Int. Ed. 2000, 39, 900–902.
- [11] Short minigramicidins from the terminal 11-mer of gA form ion channels dependent on membrane thickness: H.-D. Arndt, A. Knoll, U. Koert, *ChemBioChem* 2001, 2, 221–223.
- [12] S. E. Schaus, J. F. Larrow, E. N. Jacobsen, J. Org. Chem. 1997, 62, 4197–4199.
- [13] M. Pietraszkiewicz, J. Jurczak, Tetrahedron 1984, 40, 2967–2970.
- [14] All new compounds were characterized by NMR spectroscopy, HPLC, elemental analysis, or HR-EI/HR-MALDI-TOF/ESI mass spectrometry (see Supporting Information). For ion-channel analysis all compounds were purified by repetitive semipreparative HPLC (C8, CH₃CN/*i*PrOH/H₂O).
- [15] P. Mueller, D. Rudin, Nature 1968, 217, 713-719.
- [16] We could not find evidence for anion conductivity. Variation of the anion (Cl⁻/SO₄²⁻) did not affect the reversal potentials.
- [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) \approx NH₄⁺ (150 pm) < Cs⁺ (169 pm).
- [19] gA proton channels are long lived: S. Cukierman, E. P. Quigley, D. S. Crumrine, *Biophys. J.* 1997, 73, 2489–2502.
- [20] Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-154390. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).