"Aplosspan:" a bilayer-length, ion-selective ionophore that functions in phospholipid bilayers†

Wei Wang,[a] Ruiqiong Li[a] and George W. Gokel*[b]

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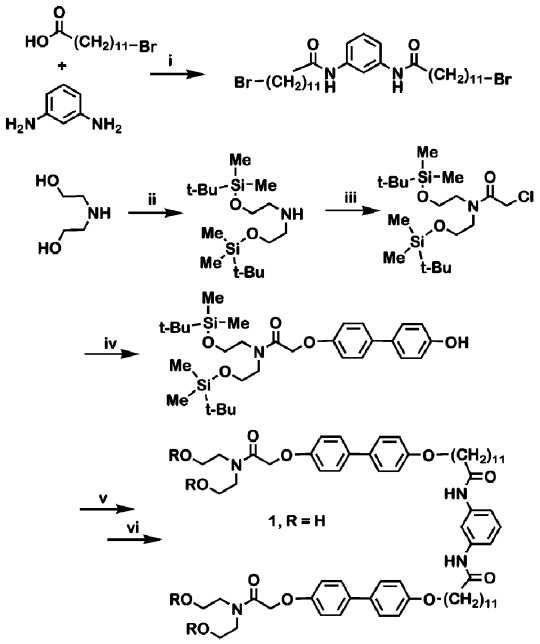
A structurally simple, novel, membrane-active ionophore has been designed, prepared, characterized, and shown to conduct Na^+, Cl^-, and carboxyfluorescein anions, probably as a dimer, across liposomal bilayers.

Synthetic ion channels, developed originally as biomimetic entities[1] that it was hoped would mimic natural channel function, have proved themselves to be more than simply models.[2] In fact, synthetic ion channels function in liposomal and planar phospholipid bilayers and even in vital cells.[3] They exhibit the classic open–close behavior characteristic of protein channels and natural pores. In many cases, they show proton, cation, or anion selectivity and even rectification.[4] It seemed clear that for a channel that will span a bilayer, three minimum elements are required: (1) the channel must be of sufficient length, (2) a polar central relay element must be present, and (3) polar headgroups are required to make the overall assembly amphiphilic. We note that these are necessary conditions, but their presence does not guarantee either a membrane-spanning conformation or channel function.

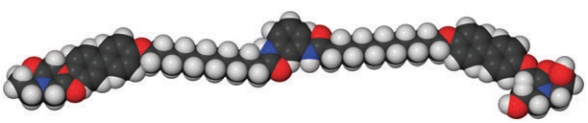
A few relatively simple membrane-spanning transporters or membrane disruptors have been reported.[5] Recent reports describe compounds that do not obviously meet these conditions, but are clearly membrane active.[6] Despite confirmed function, it is sometimes unclear how the transporters function as a conductance pore.[7] We therefore considered the question of how simple a functional, potentially membrane-spanning amphiphile could be. There is no fossil or other record from which any early channel structure can be inferred. The earliest channels must have been simple, poorly selective, and only marginally rectifying. It is unknown if the earliest channels were even peptides. It should be noted that Fyles and coworkers have also considered the issue of extremely simple, membrane-active compounds, although their structures were designed as leaflet-spanning amphiphiles rather than membrane-spanning bolaamphiphiles.[8]

Simple primordial channels must have existed or the evolution of boundary membranes would have been inimical to survival. Using the three criteria noted above, we designed compounds that incorporated synthetically accessible subunits having the size and polarity thought to be required to transport ions. The first example of the family was prepared, characterized and assayed for ion transport activity. Such simple structures that are membrane-active are sometimes referred to by the term "disruptor." This name is pejorative and the simple structures discussed here form functional channels. We suggest the name *aplosspan* from the Greek $\alpha\pi\lambda\ddot{o}\sigma$ (simple) + span for simple, membrane-spanning structures that mediate ion transport.



Scheme 1

For simplicity of design and synthesis, the hydroxyl groups of diethanolamine were chosen as headgroups, and biphenyl and alkyl chain units composed the hydrophobic tunnels.[9] Matile, Sakai, and their coworkers have used arenes extensively and successfully in their family of "rigid rod" channels.[10] Acetylated 1,3-diaminobenzene was chosen as the central relay. The amide residues should serve the water-organizing role[11] of the central relay. Moreover, Kobuke and coworkers successfully incorporated a *bis*(aminomethyl)benzene unit in a synthetic channel that showed rectification properties.[4] The compound that was prepared and studied is shown below as **1** (space-filling model) along with its preparation (see Scheme 1).



[a] *Department of Chemistry, Washington University, St. Louis, MO 63130, USA*
[b] *Departments of Chemistry & Biochemistry and Biology, Center for Nanoscience, University of Missouri – Saint Louis, One University Boulevard, Saint Louis, MO 63121, USA. E-mail: gokelg@umsl.edu; Tel: +1 314-516-5321*

† Electronic supplementary information (ESI) available: Carboxyfluorescein release, Hill plot of compound **1**, cation *vs.* anion selectivity, synthesis of **1**, sodium release experiments. See DOI: 10.1039/b816819a

The preparation of **1** is shown in Scheme 1. 12-Bromododecanoic acid was converted to the acid chloride ($(ClCO)_2$) and then coupled with 1,3-phenylenediamine (84%) to afford $Br(CH_2)_{11}CONHC_6H_4NHCO(CH_2)_{11}Br$ (i). Diethanolamine's hydroxyl groups were protected (ii, *t*-butyldimethylsilyl ethers, 74%) and *N*-acylated (iii, $ClCH_2COCl$, CH_2Cl_2, 74%). 4,4′-Dihydroxybiphenyl was coupled to $ClCH_2CON(CH_2CH_2OTBDMS)_2$ (iv, Na_2CO_3, KI, Δ, PrCN, 55%) to give $HOC_6H_4C_6H_4OCH_2CON(CH_2CH_2OTBDMS)_2$. The synthesis was completed by reaction of the biphenol fragment with the 1,3-diamide (v, Na_2CO_3, KI, Δ, PrCN, 19%) followed by hydroxyl group deprotection (vi, HCl, EtOH, 93%). Tetraol **1** is a slightly brown solid, mp ~189 °C (*dec*).[12] Full experimental and spectral details are included in the ESI.†

It seemed possible that either cations or anions or both could be transported by **1**. The activity of **1** was surveyed by recording ion release from liposomes. This method is more convenient and less time consuming than using the planar bilayer voltage clamp (BLM) method. The latter method was also used as described below because "this is the sole method to unequivocally establish the presence of channel activity, in contrast to detergent effects that might be expected for amphiphilic compounds".[8] Initially, sodium ion release from dioleolylphosphatidylcholine (DOPC, 0.4 mM) vesicles mediated by **1** was assayed by use of a sodium ion selective electrode (ISE). The vesicles (200 nm) were suspended in a sodium-free buffer consisting of 750 mM choline chloride and 15 mM HEPES at pH = 7.0. The internal buffer was 750 mM NaCl and 15 mM HEPES at pH = 7.0. Compound **1** was dissolved in DMSO and added to an aqueous suspension of liposomes. Sodium cation release was monitored by using a sodium ISE; vesicle preparation and ISE methodology have been reported.[13] Cation release from vesicles showed predictable behavior, increasing proportionally over a nine-fold concentration range (Fig. 1).

Membrane permeability[14] was further assayed by carboxyfluorescein (CF) release (fluorescence dequenching) from DOPC liposomes (see ESI†). The log–log plot of $v/V_m - v$ (ordinate) *vs.* concentration gave a straight-line relationship ($r^2 = 0.89$) with a slope of 1.5 for the CF transport data for **1**. This Hill plot,[15] although evaluated over a limited (6-fold) concentration range, suggests that more than one monomer is involved in the conducting pores. We note that transport activities assayed by ISE or by fluorescence methods are not directly comparable owing to differences in the analytical methods and conditions.[16]

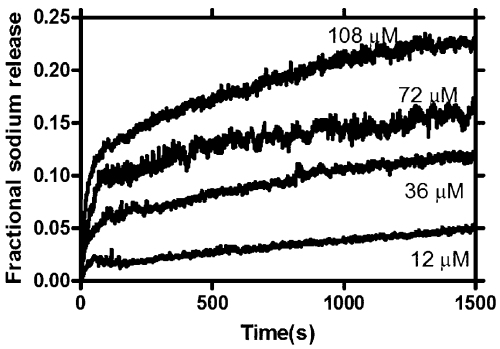


Fig. 1 Fractional sodium release from liposomes (DOPC, 0.4 mM) mediated by **1**. External buffer (750 mM choline chloride, 15 mM HEPES, pH = 7.0), internal buffer (750 mM NaCl, 15 mM HEPES, pH = 7.0).

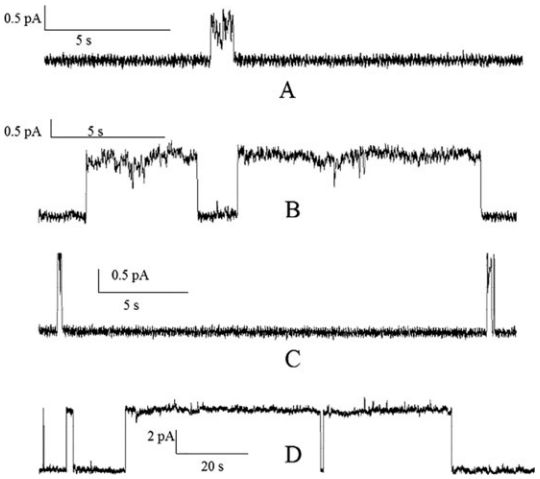


Fig. 2 Current–time records showing the channel gating behavior of **1** (asolectin, 1 μM) (A) gating current 0.80 pA, applied voltage 60 mV, 3 min into experiments; (B) gating current 1.44 pA, applied voltage 60 mV, 9 min into experiments; (C) gating current 1.86 pA, applied voltage 70 mV, 25 min; (D) gating current 5.5 pA, applied voltage 40 mV, 2 h. Buffer: 450 mM KCl, 10 mM HEPES, pH = 7.0.

Channel behavior was confirmed for **1** by a planar bilayer conductance study. Compound **1** (1 μM) was added to the cis chamber in a planar bilayer voltage clamp apparatus. The buffer was 450 mM KCl, 10 mM HEPES, pH = 7 and the bilayer was formed from asolectin (soybean). Traces obtained during the experiments are shown in Fig. 2. Ion selectivity experiments were conducted using an asymmetric buffer solution (*trans*: 450 mM KCl, 10 mM HEPES, pH = 7; *cis*: 3 M KCl, 10 mM HEPES, pH = 7). A current–voltage (*I–V*) plot revealed a V_r (reversal membrane potential) value of −14.95 mV. The Goldman–Hodgin–Katz equation was used to calculate a selectivity of $P(K^+)/P(Cl^-) = 2.3$, indicating that **1** is a modestly selective cation channel (experimental details in ESI†).

The planar bilayer conductance data revealed two facts. First, the channels or pores formed from **1** exhibited classic open–close behaviour. Second, the initial conductance was ~13 picoSiemens (pS, trace A). However, during the next several hours, the conductance pore gradually enlarged, suggesting a higher aggregation state and larger pore. After 2 h, the conductance had increased from 0.013 nS (13 pS) by about tenfold to ~0.14 nS. Using the Hille equation, we calculated the corrected Hille diameter,[17] $d_{Hille} > 2.6$ Å and enlarged over time to $d_{Hille} > 9.3$ Å. A dimeric or trimeric pore of diameter 2.6 Å that enjoys some flexibility is of sufficient size to pass Na^+ (ionic diameter: 1.9 Å) or K^+ (2.7 Å) ions if they are not fully solvated. Zhou *et al.*[18] have calculated the hydrated radii of Na^+ and K^+ to be 5.98 Å and 5.50 Å, respectively, and they would presumably be too large to be transported in that form. The calculated hydrated diameter of Cl^- is 6.48 Å from the same source and the ionic diameter is

3.6 Å. The conductance pathway of the ClC protein chloride transporter is thought to pass chloride ions in a chain bridged by dynamic (disordered) water molecules.[19]

This work demonstrates several important principles. First, exceptionally simple monomers form pores that are functional channels. Compound **1** was designed to be long enough to span the bilayer. It forms oligomeric pores that exhibit classic open–close behavior, although the aggregation state and pore size appear to increase over time. Nevertheless, this extremely simple, membrane-length compound is both functional and selective for K^+ over Cl^-. These compounds were designed to demonstrate that complex design criteria such as the incorporation of, for example, crown ethers[20] or guanine[21] residues, while potentially beneficial, may not be required for transport function. Compound **1** appears to fulfil the three criteria noted in the beginning of this report, although detailed evidence for bilayer conformation such as we reported for hydraphiles[20] is laborious to obtain. Our studies show that compounds that were designed to incorporate elements expected to impart specific properties should be compared with much simpler analogs to confirm that the design element is functioning as imagined.

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Notes and references

1 (*a*) I. Tabushi, Y. Kuroda and K. Yokota, *Tetrahedron Lett.*, 1982, **23**, 4601–4604; (*b*) V. E. Carmichael, P. J. Dutton, T. M. Fyles, T. D. James, J. A. Swan and M. Zojaji, *J. Am. Chem. Soc.*, 1989, **111**, 767–769; (*c*) A. Nakano, Q. Xie, J. V. Mallen, L. Echegoyen and G. W. Gokel, *J. Am. Chem. Soc.*, 1990, **112**, 1287–1289; (*d*) J. Canceill, L. Jullien, L. Lacombe and J.-M. Lehn, *Helv. Chim. Acta*, 1992, **75**, 791–812.

2 (*a*) G. W. Gokel and A. Mukhopadhyay, *Chem. Soc. Rev.*, 2001, **30**, 274–286; (*b*) G. W. Gokel, P. H. Schlesinger, N. K. Djedovic, R. Ferdani, E. C. Harder, J. Hu, W. M. Leevy, J. Pajewska, R. Pajewski and M. E. Weber, *Bioorg. Med. Chem.*, 2004, **12**, 1291–1304; (*c*) T. M. Fyles, *Chem. Soc. Rev.*, 2007, **36**, 335–347; (*d*) N. Sakai, J. Mareda and S. Matile, *Mol. BioSyst.*, 2007, **3**, 658–666; (*e*) A. L. Sisson, M. R. Shah, S. Bhosale and S. Matile, *Chem. Soc. Rev.*, 2006, **35**, 1269–1286.

3 (*a*) S. Fernandez-Lopez, H.-S. Kim, E. C. Choi, M. Delgado, J. R. Granja, A. Khasanov, K. Kraehenbuehl, G. Long, D. A. Weinberger, K. M. Wilcoxen and M. R. Ghadiri, *Nature*, 2001, **412**, 452–455; (*b*) E. Biron, F. Otis, J. C. Meillon, M. Robitaille, J. Lamothe, P. Van Hove, M. E. Cormier and N. Voyer, *Bioorg. Med. Chem.*, 2004, **12**, 1279–1290; (*c*) W. M. Leevy, S. T. Gammon, T. Levchenko, D. D. Daranciang, O. Murillo, V. Torchilin, D. Piwnica-Worms, J. E. Huettner and G. W. Gokel, *Org. Biomol. Chem.*, 2005, **3**, 3544–3550.

4 C. Goto, M. Yamamura, A. Satake and Y. Kobuke, *J. Am. Chem. Soc.*, 2001, **123**, 12152–12159.

5 (*a*) Y. Kobuke, K. Ueda and M. Sokabe, *J. Am. Chem. Soc.*, 1992, **114**, 7618–7622; (*b*) T. M. Fyles, C. Hu and R. Knoy, *Org. Lett.*, 2001, **3**, 1335–1337; (*c*) P. Bandyopadhyay, V. Janout, L. H. Zhang and S. L. Regen, *J. Am. Chem. Soc.*, 2001, **123**, 7691–7696; (*d*) L. M. Cameron, T. M. Fyles and C. W. Hu, *J. Org. Chem.*, 2002, **67**, 1548–1553; (*e*) A. P. Davis, *Molecules*, 2007, **12**, 2106–2122; (*f*) L. Ma, M. Melegari, M. Colombini and J. T. Davis, *J. Am. Chem. Soc.*, 2008, **130**, 2938–2939.

6 (*a*) X. Li, B. Shen, X.-Q. Yao and D. Yang, *J. Am. Chem. Soc.*, 2007, **129**, 7264–7265; (*b*) S. D. Whitmarsh, A. P. Redmond, V. Sgarlata and A. P. Davis, *Chem. Commun.*, 2008, 3669–3671.

7 (*a*) R. Pajewski, R. Ferdani, J. Pajewska, N. Djedovic, P. H. Schlesinger and G. W. Gokel, *Org. Biomol. Chem.*, 2005, **3**, 619–625; (*b*) L. You and G. W. Gokel, *Chem.–Eur. J.*, 2008, **14**, 5861–5870.

8 T. M. Fyles, R. Knoy, K. Mullen and M. Sieffert, *Langmuir*, 2001, **17**, 6669–6674.

9 D. A. 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.

10 L. A. Weiss, N. Sakai, B. Ghebremariam, C. Ni and S. Matile, *J. Am. Chem. Soc.*, 1997, **119**, 12142–12149.

11 C. L. Murray, H. Shabany and G. W. Gokel, *Chem. Commun.*, 2000, 2371–2372.

12 ^{1}H-NMR (DMSO-d_6): 1.31–1.61 (36H, m), 2.29–2.32 (4H, m), 3.46–3.63 (16H, m), 4.01 (4H, m), 4.95 (4H, s), 6.97–7.02 (8H, m), 7.20–7.31 (3H, m), 7.51–7.56 (8H, m), 7.96 (1H, s), 9.88 (2H, s). ^{13}C-NMR: 25.20, 25.27, 36.42, 47.92, 49.49, 58.64, 58.78, 65.66, 67.51, 110.19, 113.97, 114.85, 114.96, 127.03, 127.256, 128.68 132.24, 132.53, 139.60, 157.43, 157.83, 167.71, 171.36. FAB-MS for silyl protected **1**, *m*/*z* calcd.: (M + Na) 1609.9912, found: 1609.9950.

13 M. E. Weber, P. H. Schlesinger and G. W. Gokel, *J. Am. Chem. Soc.*, 2005, **127**, 636–642.

14 E. E. Ambroggio, F. Separovic, J. H. Bowie, G. D. Fidelio and L. A. Bagatolli, *Biophys. J.*, 2005, **89**, 1874–1881.

15 I. Segel, in *Enzyme Kinetics. Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*, John Wiley & Sons, New York, 1975 (Wiley Classics Edition, 1993), pp. 371–375.

16 R. Ferdani, R. Li, R. Pajewski, J. Pajewska, R. K. Winter and G. W. Gokel, *Org. Biomol. Chem.*, 2007, **5**, 2423–2432.

17 (*a*) N. Sakai, Y. Kamikawa, M. Nishii, T. Matsuoka, T. Kato and S. Matile, *J. Am. Chem. Soc.*, 2006, **128**, 2218–2219; (*b*) O. S. Smart, J. Breed, G. R. Smith and M. S. P. Sansom, *Biophys. J.*, 1997, **72**, 1109–1126; (*c*) B. Hille, in *Ionic Channels of Excitable Membranes*, Sinauer, Sunderland, MA, 2nd edn, 1992, ch. 11, pp. 291–298.

18 J. Zhou, X. Lu, Y. Wang and J. Shi, *Fluid Phase Equilib.*, 2002, **194–197**, 257–270.

19 R. Dutzler, E. B. Campbell, M. Cadene, B. T. Chait and R. MacKinnon, *Nature*, 2002, **415**, 287–294.

20 G. W. Gokel, *Chem. Commun.*, 2000, 1–9.

21 L. Ma, M. Melegari, M. Colombini and J. T. Davis, *J. Am. Chem. Soc.*, 2008, **130**, 2938–2939.