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A Synthetic Transmembrane Polyether Model Active in Lipid Bilayers

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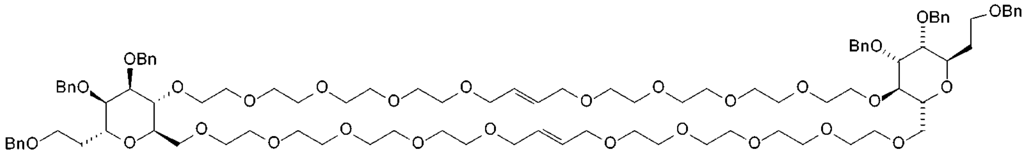
ABSTRACT



1d

A model polyether-based ion channel-like compound was rationally designed and synthesized. Macromolecules 1c–f were incorporated into phospholipid vesicles and shown to facilitate the transmembrane sodium transport.

The recently reported[1] crystal structure of the potassium ion (K^+) channel resolves some aspects of channel selectivity and establishes the physical principles underlying selective K^+ conduction. Remarkably, the crystal structure reveals that main chain atoms create a structurally constrained stack of oxygen atoms that tightly coordinate K^+ ions but not smaller Na^+ ions. However, to fully understand the molecular mechanism of these complex processes, model studies with carefully designed synthetic analogues will be essential.[2] Our interest was to determine, based on Na^+ transport rate, if flexible polyoxyethylene systems are able to stand on a well-defined organization in lipid bilayers.

It was anticipated that the incorporation of the flexible molecule **1d** into a lipid bilayer would result in the adoption of an extended conformation of its ion-conducting (polyether) segments,[3] having a length (∼32 Å)[4] which nearly matches the thickness of the PC membrane.

The concept of our approach for the synthesis of **1d** employing Ru-catalyzed[5,6] olefin metathesis is outlined in Scheme 1. In concentrated substrate solutions,[7] acyclic diene metathesis (ADM) of precursor **4**[8,9] may proceed[10] to afford

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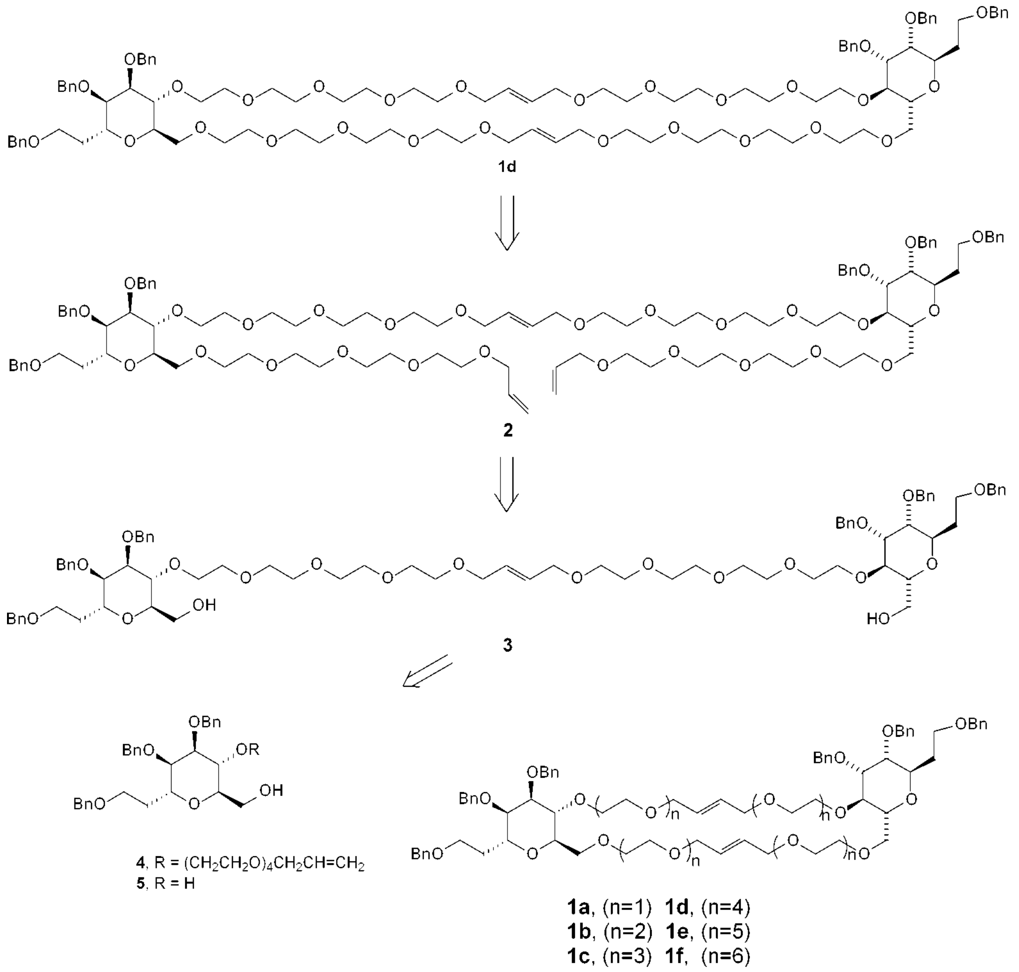
(7) Conditions of concentrated substrate solutions for ADM: 0.1 M in CH_2Cl_2, 2 mol % of ruthenium carbene $[(PCy_3)_2RuCHPh]Cl_2$.

10.1021/ol0063555 CCC: $19.00

Scheme 1



the dimer **3** (20%) (the reaction was not completed and starting material, **4**, was recovered). After introduction of the polyether chain, we obtained compound **2**, which could be further cyclized to give **1d**[11] (82%) by ring closing metathesis (RCM) conducted under high dilution conditions.[12]

The primary objective of this work was established, unequivocally, whether a simple lipophilic polyether such as **1d** could, in fact, mimic the essential functional features of natural transport processes. It was anticipated that the inner macroring would embed in the membrane and the two terminal oxacyclic rings would be near the bilayer surfaces.[13] This suggests that **1d** may not use the arene residues as relays but possibly as membrane anchors. If the arenes remain outside the membrane, they may interact with the polar headgroup residues. Such interactions are known[14] and could stabilize the extended conformation of **1d**, as shown in

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(8) Compound **4** was prepared from tri-*O*-acetyl-D-glucal by the following ten-step sequence of reactions: (a) $CH_2{=}CHCH_2SiMe_3$ (1.5 equiv), $TiCl_4$ (1.0 equiv), CH_2Cl_2 (0.2 M), −78 °C, 2 h; (b) NaOMe (1.0 equiv), MeOH (0.5 M), 25 °C, 30 min; (c) $Me_2C(OMe)_2$ (1.5 equiv), $POCl_3$ (cat.), CH_2Cl_2 (0.6 M), 25 °C, 12 h; (d) NMO (1.0 equiv), OsO_4 (cat.), H_2O/THF (1/1) (0.2 M), 25 °C, 12 h, 74% over four steps; (e) $NaIO_4$ (1.5 equiv), MeOH: H_2O (8:1), 0 °C, 1 h, then $NaBH_4$ (2.0 equiv), 0 °C, 1 h; (f) BnBr (2.0 equiv), NaH (1.5 equiv), THF (0.5 M), 25 °C, 12 h; (g) NMO (1.0 equiv), OsO_4 (cat.), THF/H_2O (1/1), 25 °C, 24 h, 66% over three steps; (h) BnBr (4.0 equiv), NaH (3.0 equiv), THF, 25 °C, 12 h; (i) CSA (0.2 equiv), CH_2Cl_2: MeOH (1: 1), 0 °C, 5 h; (j) ^{t}BuOK (1.7 equiv), THF (0.1 M), $CH_2{=}CHCH_2(OCH_2CH_2)_n$ OTs (n = 4) (1.5 equiv), 1 h.

(9) **4**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32−7.24 (m, 15H, aromatic), 5.89 (dddd, J = 5.0, 5.0, 10.4, 17.0 Hz, 1H, CH=CH$_2$), 5.15 (d, J = 10.4 Hz, 1H, CH=CH_2), 5.25 (dd, J = 1.6, 17.0 Hz, 1H, CH=CH_2), 4.63 (d, J = 12.3 Hz, 1H, CH_2Ph), 4.60 (d, J = 12.3 Hz, 1H, CH_2Ph), 4.52 (d, J = 12.0 Hz, 1H, CH_2Ph), 4.50 (d, J = 12.0 Hz, 1H, CH_2Ph), 4.43 (d, J = 12.0 Hz, 1H, CH_2Ph), 4.41 (d, J = 12.0 Hz, 1H, CH_2Ph), 4.18 (ddd, J = 3.0, 5.0, 8.0 Hz, 1H, CH), 3.99 (br d, J = 6.0 Hz, 2H, OCH_2CH=CH$_2$), 3.95 (ddd, J = 4.0, 4.0, 11.4 Hz, 1H, OCH_2CH$_2$O), 3.79 (ddd, J = 5.0, 6.5, 11.4 Hz, 1H, CH_2OH), 3.74 (ddd, J = 4.4, 4.4, 11.4 Hz, 1H, OCH_2CH$_2$O), 3.71 (dd, J = 8.0, 9.6 Hz, 1H, CH), 3.69 (dd, J = 8.9, 11.4 Hz, 1H, CH_2OH), 3.68 (dd, J = 3.0, 9.6 Hz, 1H, CH), 3.63−3.59 (m, 11H, CH, OCH_2CH$_2$O), 3.59−3.56 (m, 4H, OCH_2CH$_2$O), 3.48 (ddd, J = 5.0, 8.0, 8.9 Hz, 1H, CH), 3.45 (br dd, J = 5.1, 7.2 Hz, 2H, CH_2OBn), 2.60 (br s, 1H, OH), 1.84 (dddd, J = 5.0, 5.0, 9.5, 14.2 Hz, 1H, CH_2CH$_2$OBn), 1.68 (dddd, J = 2.0, 5.3, 7.3, 14.2 Hz, 1H, CH_2CH$_2$OBn). ^{13}C NMR (125 MHz, $CDCl_3$) δ [138.3, 138.2, 138.1 (d, 3 × C, aromatic)], 134.8 (d, CH=CH$_2$), [128.4, 128.4, 128.3, 127.9, 127.7, 127.6, 127.5 (d, 7 × C, aromatic], 117.1 (t, CH=CH$_2$), [78.8, 76.9, 76.0, 73.6 (d, 4 × C, CH)], 73.1 (t, CH$_2$Ph), 72.2 (t, OCH$_2$CH=CH$_2$), 71.9 (t, CH$_2$Ph), 71.9 (d, CH), 71.8 (t, CH$_2$Ph), [70.8, 70.6, 70.6, 70.5, 69.4 (t, 5 × C, OCH$_2$CH$_2$O], 66.6 (t, CH$_2$OBn), 62.9 (t, CH$_2$OH), 29.3 (t, CH$_2$CH$_2$OBn). $[\alpha]_D$ = +2.3° (c 1.2, $CHCl_3$); MALDI-TOF-MS (2,5-dihydroxybenzoic acid) m/z 718.6, 734.6 ($[M + Na]^+$ requires 717.86, ($[M + K]^+$ requires 733.86).

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Table 1. Intravesicular ^{23}Na Line Widths as a Function of Ionophore Concentration (μM)[a]

		concn					
		0	10	20	40	80	160
1a	$\Delta\nu_{1/2}$	9.00	9.02	9.00	9.01	9.04	9.15
	k (s^{-1})	0	0.06 ± 0.05	0.01 ± 0.01	0.04 ± 0.03	0.11 ± 0.06	0.46 ± 0.09
1b	$\Delta\nu_{1/2}$	9.00	9.00	9.10	9.22	9.54	9.82
	k (s^{-1})	0	0.01 ± 0.01	0.31 ± 0.03	0.70 ± 0.31	1.71 ± 0.04	2.56 ± 0.07
1c	$\Delta\nu_{1/2}$	9.00	9.32	9.54	9.90	10.41	11.32
	k (s^{-1})	0	0.99 ± 0.05	1.70 ± 0.04	2.83 ± 0.01	4.42 ± 0.02	7.29 ± 0.02
1d	$\Delta\nu_{1/2}$	9.00	9.32	9.78	10.64	11.55	12.32
	k (s^{-1})	0	1.02 ± 0.01	2.44 ± 0.04	5.15 ± 0.05	8.01 ± 0.02	10.44 ± 0.01
1e	$\Delta\nu_{1/2}$	9.00	9.05	9.12	10.21	10.76	11.01
	k (s^{-1})	0	0.16 ± 0.02	0.39 ± 0.01	3.81 ± 0.01	5.53 ± 0.02	6.32 ± 0.01
1f	$\Delta\nu_{1/2}$	9.00	9.12	9.31	9.58	10.16	10.73
	k (s^{-1})	0	0.38 ± 0.02	0.97 ± 0.02	1.83 ± 0.03	3.63 ± 0.01	5.42 ± 0.01

[a] $\Delta\nu_{1/2}$ (Hz): average for $n = 3$ where n is the number of experiments.

Figure 1. Cation flux would be enhanced by organization within the bilayer, i.e., by forging a defined conduit. Indeed, this appears to be what is observed for bacteriorhodopsin,[15] which is rigidified by an α-helical structure and ionophore **1d** may be anchored at the membrane surface as well.[16]

The five homologues of **1d**, compounds **1a**−**c** and **1e**,**f**,[17] were synthesized and studied by ^{23}Na NMR techniques, using the dynamic (equilibrium) NMR method, presented initially by Riddell and Hayer[18] and recently extended by Hinton et al.[19]

Lecithin large unilamellar (LUV) vesicles were prepared by the dialytic detergent removal method of Reynolds[20] (20 mM PC, 20% volume entrapment, [Na$^+$] = 200 mM). Dysprosium (external solution, 5 mM, tripolyphosphate) was added to create a 10−15 ppm shift difference of ^{23}Na inside from ^{23}Na outside. Incorporation of **1a**−**f** was accomplished by microliter injection of the appropriate stock solution (2 mM) at 25 °C. Final concentrations were typically 0−160 μM. The transport rate [$K = \pi(\nu - \nu_0)$, where ν_0 is the line width at concentration 0 μM, before addition of ionophore] increases with the increasing concentration of ionophores. This experiment shows that differences in flux rates correlate directly to differences in the length of the polyoxyethylene chains and with the amount of compound inserted into the bilayer. As shown in Table 1, compounds **1c**−**f** were found to facilitate the transmembrane transport of sodium cations

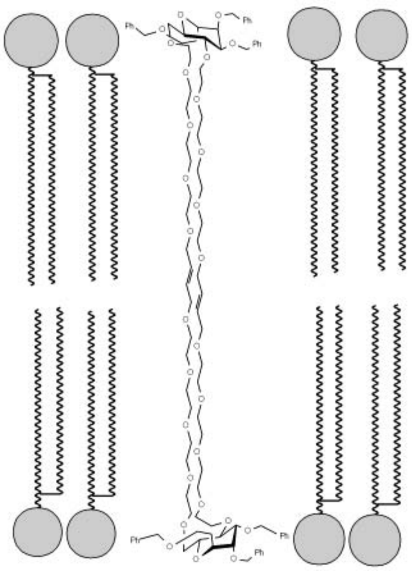

Figure 1. Postulated channel conformation for **1d** in a phospholipid bilayer.

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(11) **1d**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (m, 30H, aromatic), 5.77 (br s, 4H, $CH{=}CH$), 4.61 (d, $J = 12.4$ Hz, 2H, CH_2Ph), 4.56 (d, $J = 12.2$ Hz, 2H, CH_2Ph), 4.52 (br s, 4H, CH_2Ph), 4.41 (br s, 4H, CH_2Ph), 4.12 (ddd, $J = 4.0, 4.0, 8.1$ Hz, 2H, CH), 3.99 (br t, $J = 4.2$ Hz, 8H, $CH_2CH{=}CHCH_2$), 3.87 (ddd, $J = 4.5, 4.5, 15.4$ Hz, 2H, $CHOCH_2CH_2O$), 3.72−3.50 (m, 74H), 3.47 (m, 4H, CH_2OBn), 1.84 (m, 2H, CH_2CH_2OBn), 1.73 (m, 2H, CH_2CH_2OBn). ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.5 (q, aromatic), 138.4 (q, 2 × C, aromatic), 129.5 (d, 4 × C, $CH{=}CH$), [128.4, 128.3, 128.3, 127.9, 127.7, 127.7, 127.7, 127.6, 127.5 (d, aromatic)], [77.9, 75.9, 75.8, 73.4 (d, 4 × C, CH)], [73.1, 72.0, 71.5, 71.2 (t, 4 × C, CH_2)], 70.8 (d, 2 × C, CH), [70.8, 70.6, 70.6, 70.5, 70.5, 70.4, 69.5 (t, 7 ×C, CH_2)], 66.9 (t, 2 × C, CH_2OBn), 29.7 (t, 2 × C, CH_2CH_2OBn). HRMS *m*/*z* 1787.942885 ([M + Na]$^+$ requires 1787.942885). **2**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (m, 30H, aromatic), 5.80 (dddd, $J = 4.7, 5.7, 10.4, 16.4$ Hz, 2H, $CH{=}CH_2$), 5.74 (br s, 2H, $CH{=}CH$), 5.20 (d, $J = 10.4$ Hz, 4H, $CH{=}CH_2$), 4.58 (d, $J = 12.4$ Hz, 2H, CH_2Ph), 4.55 (d, $J = 12.4$ Hz, 2H, CH_2-Ph), 4.52 (br s, 4H, CH_2Ph), 4.40 (br s, 4H, CH_2Ph), 4.13 (m, 2H, CH), 4.0 (br s, 8H, $CH_2CH{=}CHCH_2$, $CH_2CH{=}CH_2$), 3.82 (m, 2H, $CHOCH_2CH_2O$), 3.67−3.55 (m, 74H), 3.53−3.35 (m, 4H, CH_2OBn), [1.96, 1.82 (m, 4H, CH_2CH_2OBn)]. ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.3 (q, aromatic), [128.4, 128.3, 128.3, 128.3, 127.9, 127.8, 127.7, 127.6 (d, aromatic)], 117.2 (t, 2xC, $CH_2CH{=}CH_2$), [77.3, 75.9, 75.9, 73.4 (d, 4 × C, CH)], [73.1, 72.3, 72.1, 71.5 (t, 4 × C, CH_2)], 70.9 (d, 2 × C, CH), [70.6, 70.6, 69.4 (t, 3 × C, CH_2)], 66.9 (t, 2 × C, CH_2CH_2OBn), 24.6 (t, 2 × C, CH_2CH_2OBn). FAB-MS (thioglycerol) *m*/*z* 1816 ([M + Na] $^+$). **3**: MALDI-TOF-MS (2, 5- Dihydroxybenzoic acid) *m*/*z* 1385.1, 1401.3 ([M + Na]$^+$ requires 1384.67, [M + K]$^+$ requires 1400.67).

(12) Conditions of dilute solution for RCM: 0.005 M in CH_2Cl_2, 20 mol % cat.

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across the lipid bilayer of the LUVs. Compounds **1a** and **1b** are inactive at any concentration, indicating that indiscriminate damage to bilayers does not occur. The observed **1a** < **1b** < **1c** < **1d** > **1e** > **1f** transport activity of the six oligomers thus arises with all likelihood from differences in length and not from unequal partition coeficients. Although various chain conformations and packings were considered, the above-reported results agree best with fully extended chains, extended normal to the layers with the ethylene oxide core in a planar zigzag conformation.[21]

In conclusion, we wish to emphasize that (1) the synthetic strategy to prepare compounds **1a−f** is simple and rapid and allows further molecular engineering of this type of compounds, (2) only compounds with a length greater than ∼26 Å (**1c**) are active in lipid bilayer, and (3) compounds longer than ∼38 Å are less active than **1d**. We believe that a length greater than that of the membrane hinders the transport in this case because the conformation is too flexible into the lipid bilayer for sodium transport. Applications of this strategy for the design of synthetic cell-surface receptor models[22] are on going and will be reported elsewhere.[23]

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(17) **1a**: HRMS *m/z* 1259.628308 ([M + Na]$^+$ requires 1259.628307). **1b**: HRMS *m/z* 1435.733167 ([M + Na]$^+$ requires 1435.733168). **1c**: MALDI-TOF-MS (2,5-dihydroxybenzoic acid) *m/z* 1613.3, 1629.5 ([M + Na]$^+$ requires 1612.96, [M + K]$^+$ requires 1628.96). **1e**: MALDI-TOF-MS (2,5-dihydroxybenzoic acid) *m/z* 1966.4, 1982.0 ([M + Na]$^+$ requires 1965.38, [M + K]$^+$ requires 1981.38). **1f**: MALDI-TOF-MS (2,5-dihydroxybenzoic acid) *m/z* 2142.0, 2158.3 ([M + Na]$^+$ requires 2141.59, [M + K]$^+$ requires 2157.70).

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(22) For a previous study of cell-surface receptors based on rigid-rod molecules, see: Ghebremariam, B.; Matile, S. *Tetrahedron Lett.* **1998**, *39*, 5335−5338.

(23) A further account of work in this area will appear in an issue of the *Israel Journal of Chemistry* honoring the Canadian chemist Professor Raymond U. Lemieux.