Side-chain hydrophobicity controls the activity of proton channel forming rigid rod-shaped polyols

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Increased activity, facile incorporation into lipid bilayers and intact active structure and transport selectivity are the consequences of modifications of the side-chain hydrophobicity of a proton channel-forming octa(*p*-phenylene).

Rigid-rod molecule **1**[1,2] represents a promising new class of non-peptide ion channel models[3–5] with unique properties, but unfortunately it does not incorporate well into lipid bilayers (Fig. 1). Although the incorporation of artificial ion channels into lipid bilayers is a general problem, it is often bypassed by using high temperatures or constructing the entire supramolecular assay system in the presence of the test sample for every new experiment. Rational design strategies to tune partition without changing desired properties of a parent model have not been established so far, in spite of the fact that poor incorporation may prevent potential biological studies and pharmaceutical applications. Here we present our efforts to solve this problem by increasing the hydrophobicity of lateral side-chains attached to oligo(*p*-phenylene)s without destructive effects on active structure or transport selectivity.

In addition to the general aim to increase the lipophilicity of octamer **1**, the following considerations were taken into account for the design of the new side-chains in **2** and **3** (Fig. 1). We anticipated that length and flexibility of the propylene spacer in polyol **2** could facilitate the arrangement of the diols to form the transmembrane hydrogen-bonded chain[6] essential for proton selectivity.[2] It further may enlarge the ionophoric tube between the rigid-rod scaffold and 'proton wire'. The additional propyl group at the terminus of each side-chain in **3** possibly covers the hydrophilic 'proton wire' with an external lipophilic layer.

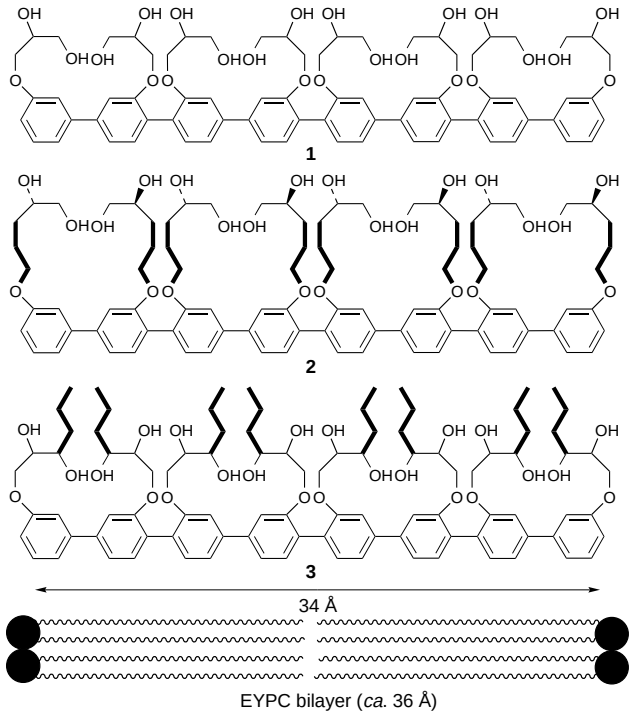
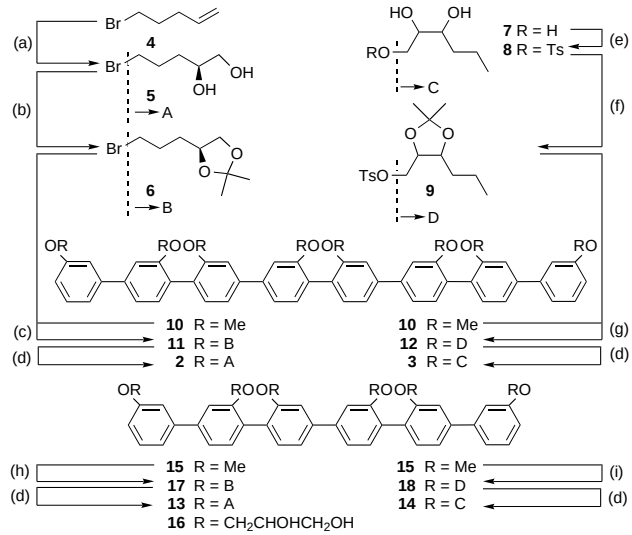


Fig. 1 In-scale planar structures of membrane-bound octa(*p*-phenylene)s **1**–**3**. Structural modifications of the side-chains are emphasized with bold lines.

Syntheses of the octamers **2** and **3** are shown in Scheme 1. For **2**, bromide **4** was subjected to Sharpless asymmetric dihydroxylation,[7]‡ and the resulting diol **5** was converted to acetonide **6**. For **3**, the diastereomeric mixture of triol **7** was selectively tosylated, and protection of the resulting diol **8** afforded **9**. Williamson ether synthesis using **6**/**9** and the crude octaphenol prepared by treatment of **10** with BBr_3,[1] followed by deprotection of **11**/**12**, yielded the polyols **2**/**3**, respectively. The corresponding hexamers **13** and **14** were prepared identically from hexaanisole **15**.§

The interaction of the fluorophores **1**–**3** with lipid bilayers was examiend using uniformly sized (68 ± 3 nm) EYPC-SUVs labeled with either 2 mol% 5- or 12-DOXYL-PC.[8,2]¶ Quenching of *ca.* 60% of the red-shifted emission of **2** and **3** by 5-DOXYL-PC labeled EYPC-SUVs proved facile incorporation compared to **1** (Fig. 2). Nearly identical quenching of **1**–**3** by 5-DOXYL-PC and 12-DOXYL-PC (not shown) confirmed that variation of side-chain hydrophobicity does not disturb the preferred transmembrane orientation of **1**–**3**.

In sharp contrast to the membrane-spanning octamers **1**–**3** (34 Å), the side-chain structure governs the organization of the corresponding hexamers (26 Å) in EYPC-bilayers. Namely, **16** was found at the membrane/water interface (quenching by 12-DOXYL-PC < 5-DOXYL-PC),[2] **13** in transmembrane orientation [12-DOXYL-PC (67%) ≈ 5-DOXYL-PC (69%)], and **14** between the two leaflets of the lipid bilayer [12-DOXYL-PC (49%) > 5-DOXYL-PC (26%)].



Scheme 1 *Reagents and conditions*: (a) AD-mix-α, 71%; (b) 2,2-dimethoxypropane, H_2SO_4, 80%; (c) (1) BBr_3; (2) **6**, Cs_2CO_3, 66%; (d) TFA (quant.); (e) TsCl, pyridine, 65%; (f) 2,2-dimethoxypropane, H_2SO_4, 83%; (g) (1) BBr_3; (2) **9**, Cs_2CO_3, 27%; (h) see (c), 15%; (i) see (g), 27%

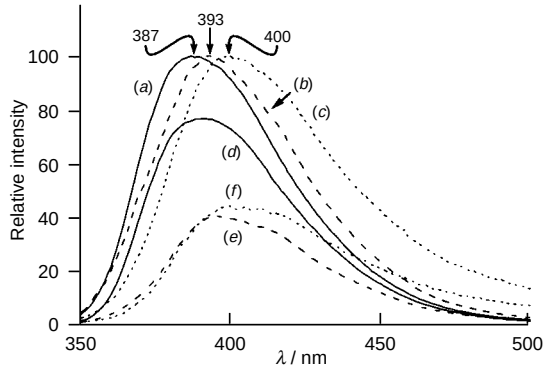


Fig. 2 Relative emission intensities [I/I (λ^{e}_{max}) × 100%, λ_{ex} = 328 nm) of 5 μM solutions of **1** (*a*), **2** (*b*), and **3** (*c*) with 0.5 mM of unlabeled EYPC-SUVs, and of **1** (*d*), **2** (*e*) and **3** (*f*) with 5-DOXYL-PC labeled EYPC-SUVs (100 mM KCl, 100 mM HEPES, pH 7.1)

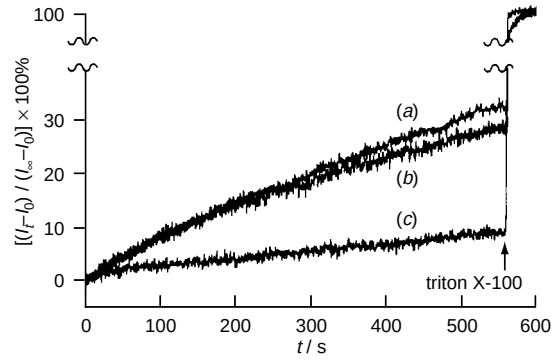


Fig. 3 Change in fluorescent intensity {[(I_t–I_0)/(I_∞–I_0)] × 100%, λ_{ex} = 460 nm, λ_{em} = 510 nm} of EYPC-SUV-entrapped HPTS (100 mM KCl, 100 mM HEPES, pH_{in} = 7.0, pH_{out} = 7.6) as a function of time after the addition of 10 nmoles of octa(*p*-phenylene)s **1** (*c*), **2** (*b*) and **3** (*a*) in 20 μl MeOH followed by 40 μl of 1.2% triton X-100¶

The ion transport activity of polyol **1** was originally assessed in comparison with the structurally related antifungal polyol amphotericin B (AmB).[1] Both mediated intravesicular pH changes with comparable exchange rates (**1** > AmB) when added to EYPC-SUVs having entrapped pH sensitive fluorophore HPTS and a transmembrane pH gradient.[1] The K^+/Na^+ > H^+ selectivity of AmB was shown by accelerated internal pH changes in the presence of a selective H^+ carrier,[9,1] while similar enhancements induced by the presence of the K^+ carrier valinomycin demonstrated proton selectivity for **1**.[2]

For direct comparison with incorporation efficiencies of polyols **1**–**3** (Fig. 2), we conducted the activity measurements summarized above under the conditions used for fluorescence quenching, *i.e.* reduced polyol and increased lipid concentrations compared to previous reports.[1,2] Under these conditions, the transport activity of **1** without additional valinomycin is nearly identical with the negative control ($k = 9.0 \times 10^{-6}$ s^{-1}, Fig. 3).‖ Improved incorporation (Fig. 2) resulted in significantly increased ion flux rates for octamer **2** ($k = 2.2 \times 10^{-4}$ s^{-1}) and **3** ($k = 2.4 \times 10^{-4}$ s^{-1}, Fig. 3). For all octamers (**1**–**3**), accelerated internal pH changes in the presence of 12 nM valinomycin were observed as reported before for polyol **1** (not shown).[2] Thus, the difference in side-chain lipophilicity of **2** and **3** does not significantly alter the proton selectivity previously observed for **1**.‖

These results demonstrate that the incorporation of proton channel forming rigid-rod octa(*p*-phenylene)s into lipid bilayers can be precisely tuned without significant disturbance of active structure and transport selectivity. The erratic interactions of identically modified hexa(*p*-phenylene)s with lipid bilayers further corroborate the importance of the length of the rigid-rod scaffold for controlled, transmembrane binding of substituted oligo(*p*-phenylene)s. Ion channel formation of octamers **1**–**3**, but not hexamer **16** in planar lipid bilayers supports these conclusions and will be reported in due course.

We thank NIH (GM56147-01), the donors of the Petroleum Research Fund, administered by the American Chemical Society, Suntory Institute for Bioorganic Research (SUNBOR Grant), and Georgetown University for support of this work. Both authors thank Dr Naomi Sakai for invaluable discussions and experimental advise.

Notes and References

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‡ The stereochemistry of the side-chain is with all likelihood irrelevant for the transport activity of rigid-rod polyols.[2] However, judging from the outcome of osmium-catalyzed asymmetric dihydroxylation of terminal olefins, formation of the *S*-enantiomer **5** in 78–97% ee can be expected using AD-mix-α.[7]

§ All final products were purified by reverse-phase HPLC and gave satisfactory spectroscopic data.

¶ Abbreviations: AmB: amphotericin B; 5-DOXYL-PC: 1-palmitoyl-2-stearoyl(5-DOXYL)-*sn*-glycero-3-phosphocholine; 12-DOXYL-PC: 1-palmitoyl-2-stearoyl(12-DOXYL)-*sn*-glycero-3-phosphocholine; EYPC: egg yolk phosphatidylcholine; HPTS: 8-hydroxypyrene-1,3,6-trisulfonic acid; SUV: small unilamellar vesicle.

‖ In all experiments conducted with HPTS, the intensity change was confirmed to be the consequence of internal pH change by simultaneous measurement of the time course of emission intensity at 510 nm due to excitation at 460 nm as well as 405 nm. The contribution of the negative control was eliminated for the calculation of the initial first-order rate constants. The effect of valinomycin further corroborates the absence of HPTS-leakage.[2]

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Received in Corvallis, OR, USA, 9th December 1997; 7/08841H