Toward Biomimetic Ion Channels Formed by Rigid-Rod Molecules: Length-Dependent Ion-Transport Activity of Substituted Oligo(*p*-Phenylene)s

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Polyene macrolide antibiotics, e.g. amphotericin B (AmB **1**, Figure 1), are unique examples of nonpeptide natural products which form ion channels in biomembranes.[1,2] Three structural subunits are essential for the formation of "barrel stave"-type AmB aggregates: the mycosamine group (Figure 1A) "anchors" AmB **1** at the bilayer/water interface, the hydrophobic polyene rods (Figure 1B) allow cooperative van der Waals forces with components of cell membranes, and the polyols (Figure 1C) function as ionophoric "relays". While numerous alternative "anchors" and "relays" have been explored by means of synthetic, nonpeptide ion channel models,[3] specific hydrophobic interactions have not received equal attention, even though they apparently give rise to the cell membrane specificity of AmB **1**. Here, as a first step toward artificial ion channels which specifically recognize (bio)membranes by their thickness, we report syntheses and activities of substituted oligo(*p*-phenylene)s **2**−**4** (Figure 2).

On the basis of the findings by Ourisson and co-workers,[4] we hypothesized that the ion transport activity of substituted rigid-rod molecules[5,6] would be maximized if their length matches the thickness of lipid bilayers. Shape and length of the rigid-rod skeletons should further control their organization in lipid bilayers, and the attachment of "relays" along a rigid-rod skeleton may result in versatile, artificial ion channels. Oligo(*p*-phenylene)s[7] were selected as model rigid-rod molecules because of the immense synthetic possibilities to modify the hydrophobic skeleton, as well as their luminescence, conductivity, and conformational flexibility along the long molecular axis.

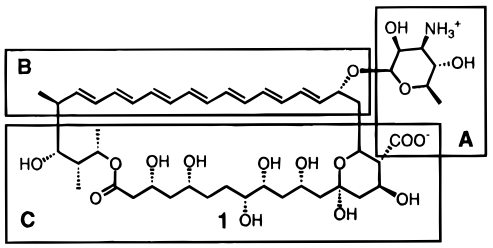

Figure 1. Amphotericin B (AmB **1**), composed of mycosamine ("anchoring") (A), polyene ("rigid-rod") (B), and polyol ("relay") subunits (C).

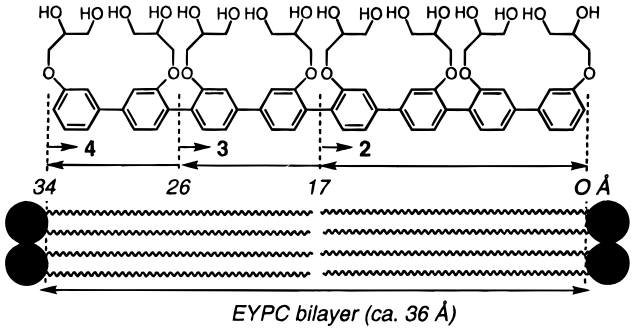

Figure 2. In-scale planar structures of membrane-bound oligo(*p*-phenylene)s **2**−**4**.

Syntheses of oligomers **2**−**4** are shown in the Scheme 1. Chelation-controlled lithiation of 3,3′-bianisole **5** with the sterically demanding *t*-BuLi followed by iodination gave **6** and **7** as major products in a mixture of regioisomers. Oxidative coupling of **6** with *n*-BuLi/$CuCl_2$ furnished tetramer **8**.[7d,e] For the preparation of oligomers **9** and **10**, dimer **6** was converted into boronic acid **11**. Suzuki coupling[8] of **11** and bisiodo derivative **7** afforded hexamer **9**. Regioselectively iodinated tetramer **12** was elongated identically to give **10**. The methyl groups of oligomers **8**−**10** were removed, and the resulting oligophenols **13**−**15** were converted into acetonides **16**−**18** with racemic tosylate **19**. Acid-catalyzed deprotection yielded polyols **2**−**4**.

The initial evaluation of ion transport activities was made with uniformly sized, small unilamellar vesicles (SUVs) composed of fresh egg yolk phosphatidylcholine (EYPC). The thickness of the hydrophobic part of EYPC bilayers, which consist of ∼70% 16:0 and 18:1 PC, is thought to be nearly equal to that of pure di-18:1 PC bilayers (36 Å).[9] In contrast to tetramer **2** (17 Å) and hexamer **3** (26 Å), octamer **4** (34 Å) should thus be able to span this bilayer subunit (Figure 2). EYPC-SUVs containing entrapped HPTS (8-hydroxypyrene-1,3,6-trisulfonic acid) were prepared by the dialytic detergent removal technique (100 mM HEPES, 100 mM NaCl, 0.05 mM KCl, pH 7.0).[10] Increase of extravesicular pH from 7.0 to 7.6 led to negligible changes in the emission of the intravesicular HPTS.[11] The presence of 20 μM tetramer **2** had a minor effect on the intravesicular pH compared to the negative control

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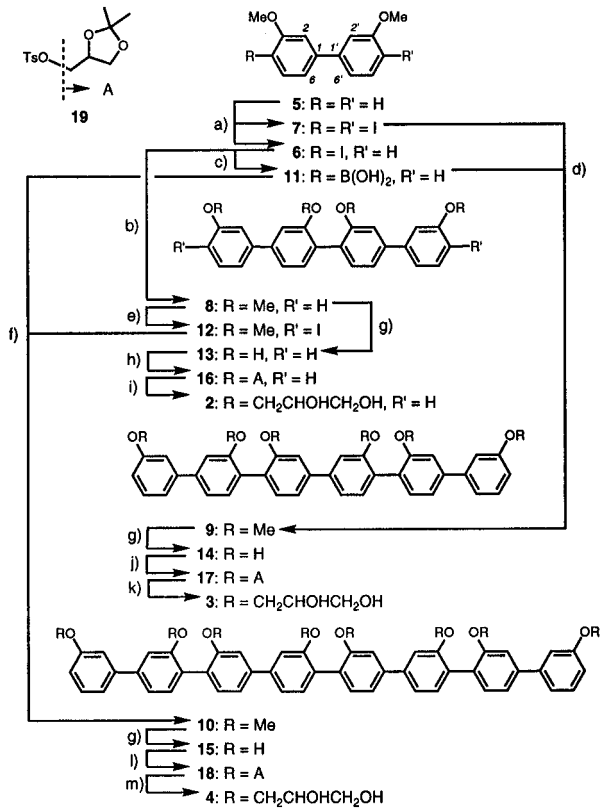
Scheme 1[a]



[a] (a) (1) *t*-BuLi; (2) I_2, Et_2O. Yields: **7**, 4%; **6**, 34%. (b) *n*-BuLi, $CuCl_2$. Yield: 64%. (c) (1) *n*-BuLi; (2) B(O-*i*-Pr)$_3$; (3) HCl. Yield: 75%. (d) Pd(PPh$_3$)$_4$, Na_2CO_3. Yield: 77%. (e) (1) *t*-BuLi; (2) I_2. Yield: 29%. (f) See d. Yield: 66%. (g) BBr_3. (h) **19**, Cs_2CO_3. Yield: 82% from **8**. (i) TFA. Yield: 85%. (j) See h. Yield: 21% from **9**. (k) TFA. Yield: 88%. (l) See h. Yield: 25% from **10**. (m) TFA. Yield: 92%.

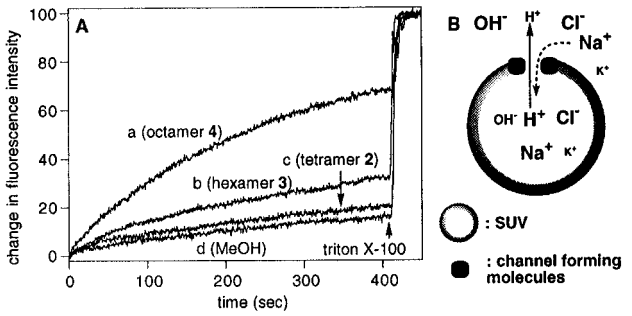


Figure 3. (A) Change in fluorescent intensity ($(I_t - I_0)/(I_\infty - I_0)\times100\%$) of vesicle-entrapped HPTS as a function of time after the addition of 40 nmoles of octamer **4** (curve a), hexamer **3** (curve b), tetramer **2** (curve c), and 40 μL of MeOH (curve d, negative control) followed by 40 μL of 1.2% triton X-100 ($pH_{in} = 7.0$, $pH_{out} = 7.6$); excitation at 460 nm, emission at 510 nm. (B) Schematic representation of the transport experiments.

(Figure 3, curves c and d). Increased ion flux rates were observed with hexamer **3** ($k = 4.6 \times 10^{-4}$ s^{-1}) and octamer **4** ($k = 13.8 \times 10^{-4}$ s^{-1}, Figure 3, curves a and b). Under these conditions, octamer **4** transports ions across an EYPC bilayer 3.8 times faster than AmB **1** ($k = 3.6 \times 10^{-4}$ s^{-1}, Figure 4, curve b).[12]

The above method measures the change in intravesicular pH assuming HPTS leakage caused by the rigid-rod molecules **2**−**4** does not occur.[13] The observed rate of internal pH change is limited either by the slowest ion transport rate of facilitated H^+/M^+ or OH^-/Cl^- exchange (Figure 3B) or by the formation rate of the active suprastructures.[14] For instance, the 57-fold increase in ion flux in the presence of the proton carrier FCCP (carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone) indicates that proton efflux limits the rate of AmB mediated ion exchange (Figure 4, curves b and d).[1d] In contrast, FCCP did not significantly affect the activity of octamer **4**; proton transport is therefore not limiting the rate of intravesicular pH change (Figure 4, curves a and c).[13]

(12) The corresponding octamethoxy derivative **10** and octaphenol **15** were completely inactive; octaacetonide **18** had minor activity.

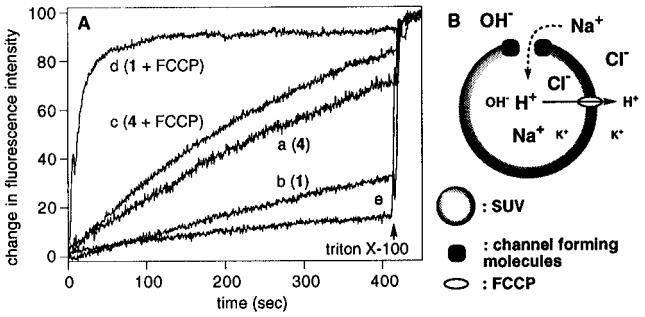


Figure 4. Same experiments (A) including schematic representation (B) as in Figure 3 for 20 μM octamer **4** and AmB **1** in the absence (curves a, b) and presence (curves c, d) of 10 μM FCCP (curve e: 40 μL of MeOH).

Although partition coefficients obtained by reverse-phase HPLC methods should be interpreted with caution,[15] the nearly identical retention times of **2** (6.86 min), **3** (6.73 min), and **4** (6.78 min) on a C_{18} column with methanol as the mobile phase should originate from roughly equal hydrophobic parameters. The observed **2** < **3** < **4** transport activity of the three oligomers thus arises with all likelihood from differences in length and not from unequal partition coefficients.

In conclusion, we have demonstrated that rigid-rod molecules carrying hydrophilic substituents facilitate ion transport across lipid bilayers. The ion flux rate mediated by octa(*p*-phenylene) **4**, having a length of 34 Å which nearly matches the hydrophobic part of EYPC bilayers, exceeds that of hexamer **3** (26 Å) by a factor of 3.0, while the tetramer **2** (17 Å) is almost inactive. The mechanism of transport remains to be elucidated, although the significant length dependence disfavors a mobile carrier mechanism, and the induction of membrane defects in a detergent-like manner is unlikely.[13] Vigorous studies on transport mechanism and active suprastructure of substituted rigid-rod oligomers are ongoing.

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Supporting Information Available: Experimental procedures and physical data of product(s), vesicle preparation, and HPTS-assay (7 pages). See any current masthead page for ordering and Internet access instructions.

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(13) Preliminary results show that the ion flux rate mediated by octamer **4** strongly increases in the presence of small amounts of the K^+ carrier valinomycin (e.g., 16-fold with 12 nM valinomycin). This effect implies that octamer **4** acts as a proton/cation exchanger with $H^+ > K^+$ selectivity and not as a detergent-like defect inducer; dye-release experiments fully corroborate the latter implication.

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