Rigid Push−Pull Oligo(*p*-Phenylene) Rods: Depolarization of Bilayer Membranes with Negative Membrane Potential

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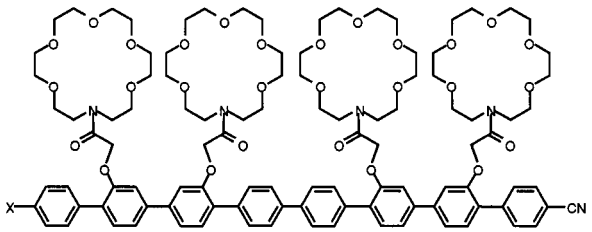
In light of steadily emerging multi-drug-resistant bacteria,[1] the molecular mechanism of natural antibiotics is of high interest for the development of new routes toward antimicrobials that can be hoped to cause minimal microbial resistance.[2] Many natural antibiotics are cationic, α-helical peptides that presumably act by recognizing and depolarizing anionic, highly polarized bacterial cell membranes.[2] The alignment of their dipole moment with negative membrane potentials in particular has been proposed to be critical for their specificity.[2,3] To study this central aspect of the molecular recognition mechanism of natural antibiotics, one would need the development of novel functional biomimetics[3,4] *with permanently oriented dipole moments that can be systematically varied in magnitude without significant modification of the global structure of the synthetic model.* As an example of the usefulness of rigid-rod molecules to address bioorganic topics of current concern,[5] we here report the design, synthesis, and evaluation of push−pull rigid-rod ionophore **1** that is, in clear contrast to octi(*p*-phenylene) **2** with nearly identical structure but



1: X = OMe; **2**: X = CN

without permanently fixed dipole moment, capable of depolarizing bilayer membranes with negative membrane potential (Figure 1).

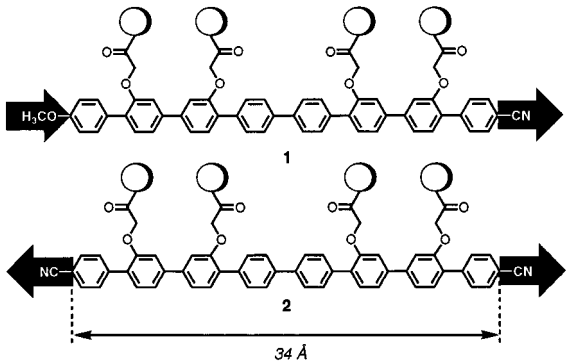
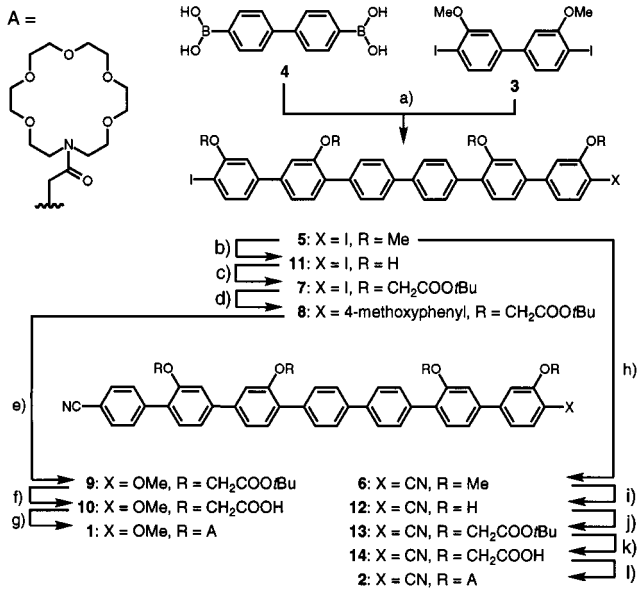


Figure 1. Structure of push−pull (**1**) and pull-pull (**2**) rigid-rod ionophores; →, permanently fixed dipole moments; ○, 18-azacrown-6.

The rigid-rod molecules **1** and **2** are composed of three subunits. Octi(*p*-phenylene) scaffolds were selected because they adapt transmembrane orientation in hydrophobically matching bilayer membranes (Figure 1).[5b,c,f,g] The terminal cyano and methoxy groups were chosen as π acceptors ($\mu \approx 3.9$ D) and donors ($\mu \approx 1.3$ D),[6] respectively, to control magnitude and orientation of the permanently fixed dipole moments in **1** and **2**. 18-Azacrown-6 was placed as a lateral side chain because its capacity as ion-transporting "relays" is well established.[4]

Scheme 1[a]



[a] (a) $Pd(PPh_3)_4$, Na_2CO_3, 10%; (b) BBr_3; (c) *tert*-butyl bromoacetate, Cs_2CO_3, 46% from **5**; (d) *p*-methoxyphenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, 40% (conversion yield, 54%; dimethoxy octamer, 26%); (e) *p*-cyanophenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, 34%; (f) TFA/CH_2Cl_2; (g) 18-azacrown-6, PyBOP, DIPEA, 57% from **9**; (h) see (e) 35% (conversion yield: 50%); (i) BBr_3; (j) see (c), 42% from **6**; (k) TFA/CH_2Cl_2; (l) see (g), 42% from **13**.[7]

The synthesis of **1** and **2** was more difficult than expected (Scheme 1). In contrast to that of shorter oligo(*p*-phenylene)s,[5h]

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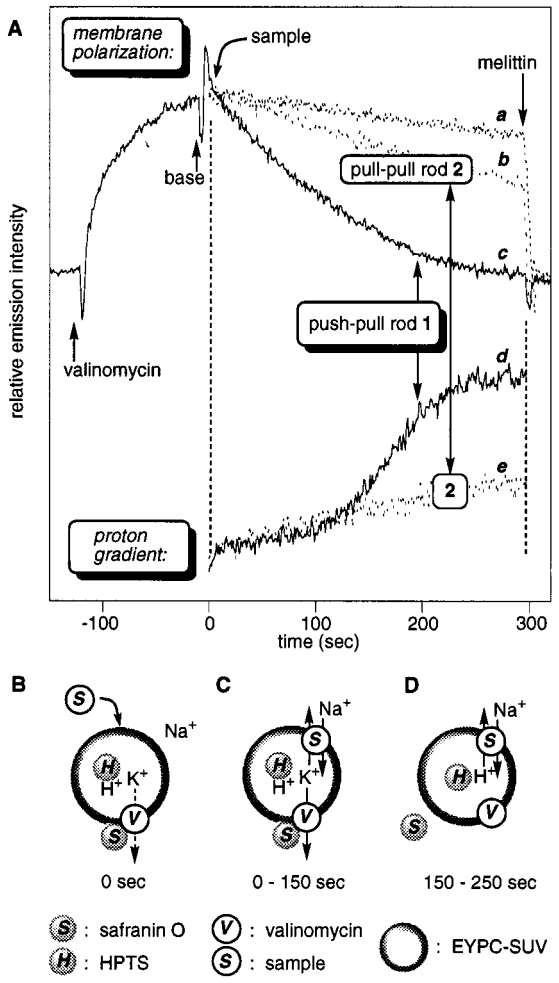

Figure 2. Activity of push−pull (**1**) and pull−pull (**2**) rods in polarized EYPC−SUVs. (A) *Representative* curves for *simultaneously detected* changes of membrane potential (emission of external safranin O at 581 nm; λ_{ex} 522 nm, curves a−c) and pH gradient (emission of internal HPTS at 510 nm; λ_{ex} 450 nm, curves d and e) during the addition of valinomycin (6 nM), base (NaOH, Δ*p*H 1), sample (curve b, e, 10 μM **2**; curve c, d, 10 μM **1**; curve a, negative control) and melittin as a function of time. All curves were normalized to 100% at 0 s and 0% at 320 s; in curves d and e, the negative control curves were subtracted after normalization.[7] (B−D) Schematic representation for ion flux mediated by push−pull rod **1**. Addition of **1** to doubly labeled, polarized SUVs with low internal pH (B) resulted in membrane depolarization by K^+/Na^+ antiport (C) followed by H^+ gradient collapse presumably due to H^+/Na^+ antiport (D); less likely transport mechanisms are not shown for clarity.

iodination of the termini of substituted sexi(*p*-phenylene)s is hampered by poor solubility and regioselectivity. However, Suzuki coupling of an excess of diiodo dimer **3**[5e,h] with diboronic acid **4** provided direct access to the key intermediate **5** in 10% yield. Coupling of diiodo hexamer **5** with *p*-cyanophenylboronic acid gave octamer **6**, which was readily converted into a pull−pull ionophore **2** following our previously established protocols,[5a,h] but the intermediate heptamer needed to prepare octi(*p*-phenylene)s with different termini was not observed. This problem was solved by attaching the "solubilizing" *tert*-butyl glycolate side chain[5a] on the hexamer stage. Indeed, Suzuki coupling of the diiodo tetraester **7** with *p*-methoxyphenylboronic acid gave heptamer **8** in excellent 54% conversion yield and subsequent coupling of **8** with *p*-cyanophenylboronic acid yielded octamer **9**. Cleavage of ester **9** with TFA and coupling of acid **10** with 18-azacrown-6 gave ionophore **1**.[7]

The capacity of rigid-rod ionophores **1** and **2** to depolarize polarized small unilamellar vesicles (SUVs) composed of egg yolk phosphatidylcholine (EYPC) was assessed by double-channel fluorescence kinetics using the membrane potential sensitive safranin O (excitation: 522 nm, emission: 581 nm)[8] as an extravesicular probe and the pH sensitive 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS, excitation: 450 nm, emission: 510 nm)[5a,c,f−h] as an intravesicular fluorescence probe. Uniformly sized EYPC−SUVs in saline phosphate buffer, pH 6.4, with internal HPTS and KCl (100 mM) and external safranin O (60 nM), KCl (36 μM), and NaCl (100 mM) were prepared by the dialytic detergent removal method described before.[5c,g,7] External addition of the selective K^+ carrier valinomycin established an inside negative membrane potential that is, according to Nernst′ equation,[7] comparable to that of bacteria (−200 mV).[2c] The assay system was completed by the application of a pH gradient, and both membrane potential (Figure 2A, curves a−c) and intravesicular pH (Figure 2A, curves d, e) were subsequently monitored by changes of the emission intensity of safranin O and HPTS, respectively. The presence of 10 μM ionophore **2** affected neither membrane potential nor proton gradient significantly (Figure 2A, curves b and e). In clear contrast, push−pull rod **1** rapidly depolarized the EYPC−SUVs (Figure 2C and A, curve c) and, *after depolarization*, caused pH gradient collapse (Figure 2D and A, curve d).

Control experiments showed that both ionophores have identical activity to mediate intravesicular pH changes in unpolarized EYPC- and anionic EYPG-SUVs (EYPG = egg yolk phosphatidylglycerol).[7] The increased activity of push−pull rod **1** in polarized SUVs can thus be *unambiguously* attributed to its permanently fixed dipole moment. Although other explanations are possible,[3b] the presence of a push−pull scaffold is likely to increase transmembrane binding to polarized bilayers due to favorable alignment of the fixed dipole with the membrane potential[3c] that results in the recognition of polarized membranes. The delayed pH gradient collapse (Figure 2D and A, curve d vs e) further demonstrated that push−pull rod **1** acts with $M^+ > H^+$ selectivity and not by lysis.

In summary, we have shown that a permanent dipole moment along a hydrophobically matching rigid-rod scaffold as in **1** is sufficient for recognition and depolarization of polarized bilayer membranes. These findings imply the promise of push−pull rods as potential antimicrobials.

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Supporting Information Available: Experimental procedures, vesicle preparation, and flux assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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