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Rigid Oligonaphthalenediimide Rods as Transmembrane Anion−π Slides

Virginie Gorteau, Guillaume Bollot, Jiri Mareda, Alejandro Perez-Velasco, and Stefan Matile*

Department of Organic Chemistry, University of Geneva, Geneva, Switzerland

Received September 12, 2006; E-mail: stefan.matile@chiorg.unige.ch

We report the design, synthesis, and evaluation of π-acidic, shape-persistent oligo-(*p*-phenylene)-*N*,*N*-naphthalenediimide (O-NDI) rods **1**−**3** that can transport anions across lipid bilayer membranes with a rare halide VI selectivity ($Cl^- > F^- > Br^- > I^-$)[1] and a substantial anomalous mole fraction effect (AMFE, Figure 1 and Scheme 1).[2] Dynamic cation−π interactions have been confirmed theoretically[3] and experimentally[4] to provide access to ion channels/transporters with the biologically relevant Eisenman IV cation selectivity topology.[5] This experimental support for π-basic rigid *p*-oligophenyl rods as functional scaffolds[4] suggested that electron-deficient rigid O-NDI rods[6] could give the complementary anion−π slides (Figure 1). The development of strategies to design synthetic anion channels/transporters[7,8] beyond ion pairing and hydrogen bonding is of quite general interest considering the importance of anion channels in diseases such as cystic fibrosis.[1,2,8] Anion−π interactions are appealing for this purpose because they are theoretically attractive,[9] poorly explored in solution,[10] absent in ion channel proteins,[1,2,8] and unexplored in the context of synthetic ion channels and pores.[7,8]

NDI, a compact, organizable, colorizable, and functionalizable organic *n*-semiconductor[6,11] was considered as an ideal module for the creation of transmembrane anion−π slides (Figure 1). Our high-level DFT calculations[12] for model NDI **4** revealed a global quadrupole moment $Q_{zz} = +19.4$ B (Buckinghams) that promised anion−π interactions beyond hexafluorobenzene ($Q_{zz} = +9.6$ B)[13] and cation−π interactions with the complementary model pyrene **5** ($Q_{zz} = -13.8$ B). Comparison with rigid *p*-oligophenyl rods[14] suggested that the alignment of three NDI acceptors separated by phenyl spacers would afford rods with appropriate length ($l = 32.6$ Å, Figure 1B) for hydrophobic matching with common lipid bilayer membranes.

Rigid O-NDI rods **1**−**3** were readily accessible from the commercially available dianhydride **6** (Scheme 1). Reaction with excess diamine **7** gave the central NDI module **8**. Unlikely to affect the fixed phenyl−NDI torsion angle of $\omega \approx 90°$, reduction of the number of methyls in diamine **8** was nevertheless found to be undesirable because of increasingly poor solubility of higher rods (not shown). The terminal module **9** was prepared by reaction of monoamine **10** with excess dianhydride **6** under controlled pH. Coupling of the central diamine **8** with two terminal diacids **9** yielded the desired rigid O-NDI scaffold **11**. *Z*-Removal and elongation of diamine **12** with Boc-Gly-OH gave target rod **1**. Mild Boc-deprotection produced the asymmetric ammonium salt **2** in up to 64% conversion yield, together with 30% of the fully deprotected, symmetric diammonium salt **3**.

Egg yolk phosphatidylcholine large unilamellar vesicles (EYPC LUVs) loaded with the pH-sensitive fluorescent probe 8-hydroxy-1,3,6-pyrenetrisulfonate (HPTS) and exposed to a pH gradient were used to evaluate the activity of rigid O-NDI rods **1**−**3**. In this assay, transport activity is reported as velocity of pH gradient collapse and can imply facilitated cation (H^+/M^{n+}) or anion exchange (OH^-/A^{n-}).[4,15] Consistent with transmembrane rod orientation, the overall quite poor activities of rigid O-NDI rods in the HPTS assay were best with one charged and one uncharged terminus and worst with two charged termini (**2** > **1** > **3**). Replacement of the extravesicular NaCl with isoosmolar KCl, RbCl, and CsCl did not much change the apparent activity of rigid O-NDI rod **1** (Figure 2A). The changes provoked by external anion exchange were clearly stronger (Figure 2B). Sensitivity to external anion and insensitivity to external cation exchange indicated that rigid O-NDI rod **1** operates by OH^-/A^{n-} rather than H^+/M^{n+} exchange, that is, anion selectivity. Recent direct comparison suggested that relative activities obtained by external ion exchange in HPTS-loaded vesicles may relate directly to permeability ratios from Goldman−Hodgkin−Katz analysis of planar bilayer conductance experiments.[15]

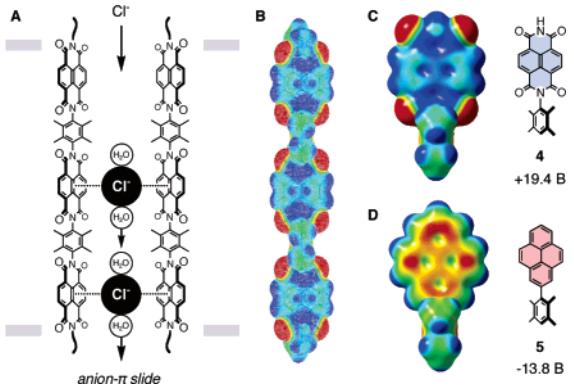
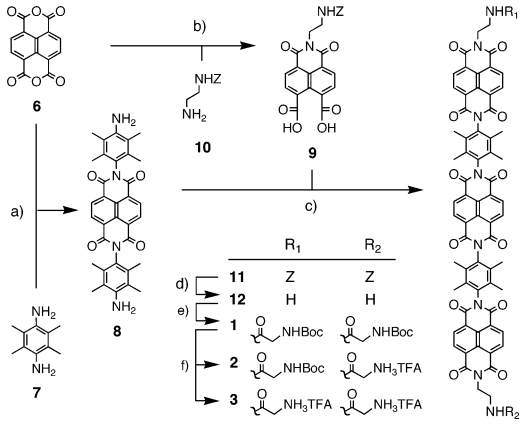


Figure 1. The concept of anion−π slide in lipid bilayers (A) with DFT-computed electrostatic potential maps (mesh surface) for rigid O-NDI rod **1** (B) and solid surfaces for the model NDI **4** (C) compared to the complementary model pyrene **5** (D); red: electron-rich, blue: electron-poor.

Scheme 1 [a]



[a] Conditions: (a) *N*,*N*-Dimethylacetamide, 135 °C, 12 h, 90%; (b) (1) H_2O, pH 6.4, reflux; (2) AcOH; 88%; (c) *N*,*N*-dimethylacetamide, 135 °C, 12 h, 57%; (d) TFA, 50 °C, 2 h, 61%; (e) Boc-Gly-OH, HBTU, TEA, DMF/DMSO 1:1, rt, 2 h, 54%; (f) 2% TFA, CH_2Cl_2, rt, 50 min, 64% **2**, 30% **3** (conversion yield).

10.1021/ja0665747 CCC: $33.50

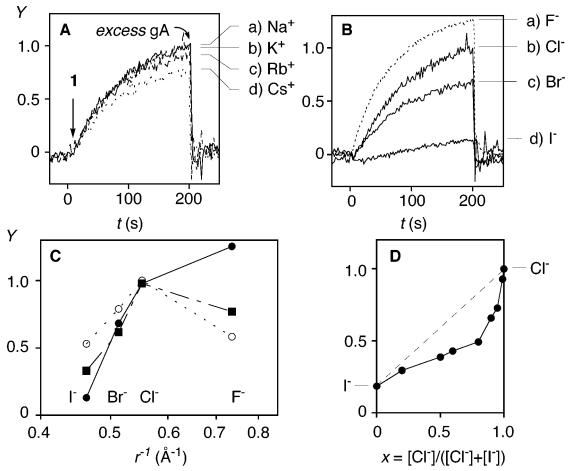


Figure 2. Anion/cation selectivity (A, B), anion selectivity topology (C), and mole fraction behavior (D) of rigid O-NDI rods **1** (A−D, ●■) and **2** (C, ○), with rods being added either after (A−D, ●○) or before the base pulse (C, ■). (A, B) Fractional HPTS emission Y (λ_{ex} = 450 nm, λ_{em} = 510 nm) as a function of time during addition of base (ΔpH 0.9) followed by **1** (1.5 μM) and excess gramicidin A (gA, for calibration only) to EYPC-LUVs⊃HPTS (10 mM HEPES, pH 7.0, 100 mM MX, A: X = Cl, M as indicated; B: M = Na, X as indicated. The baseline (same without **1**) was subtracted after calibration). (C, D) Fractional HPTS emission Y 200 s after beginning of an experiment as a function of the reciprocal anion radius (C) or the mole fraction x (D, expected: dashed line, found: solid line).

The halide VII sequence ($F^- > Cl^- > Br^- > I^-$) revealed in the selectivity topology of rigid O-NDI rod **1** is very unusual (Figure 2C, ●). Opposite to the common Hofmeister series (halide I), full compensation of the cost of dehydration by binding to the anion−π slide implied the existence of remarkably powerful anion−π interactions.[1] However, we observed that transmembrane $F^- \rightarrow Cl^-$ gradients applied by external $Cl^- \rightarrow F^-$ exchange caused a dramatic decrease of internal pH. Identical observations with external AcO^- and, less pronounced, SCN^- suggested the occurrence of passive AH influx with weak acids under these conditions. This implied that an unusually large effective pH gradient (rather than the ion selectivity of rigid O-NDI rod **1**) may at least, in part, account for the high activity found with external F^-. Addition of rigid O-NDI rod **1** to remove the HF-related pH gradient before application of the external base pulse caused indeed the expected drop from halide VII to halide VI selectivity ($Cl^- > F^- > Br^- > I^-$) (Figure 2C, ■). The magnitude of anion selectivity of rigid O-NDI rod **2** was reduced despite (and presumably because of) the presence of an ammonium cation at one terminus. The selectivity shifted from halide VII to a weaker halide V ($Cl^- > Br^- > F^- > I^-$) for rod addition after base pulse (Figure 2C, ○). These trends suggested that increasing proximity between transmembrane O-NDI rods could cause increasing selectivity but decreasing activity.

The existence of the multiple binding sites expected for a π slide was supported by a remarkably strong AMFE (Figure 2D). According to this classical test,[2] the underadditivity found for Cl^-/I^- mixtures suggested that occupation of one single site with the better binding Cl^- is insufficient for fast Cl^- transport. Occupation of multiple sites along the π slide is thus required for the high activity found with pure Cl^-. The classical biological answer to the dilemma of how to be fast and selective,[2,16] AMFE thus supported multi-Cl^- hopping along the π-acidic NDI modules of rigid rod **1** and disfavored the Gly-Boc termini as origin of activity and selectivity.

The rare halide VI sequence of neutral O-NDI rods, together with reduced selectivity and halide sequence but increased activity with one cationic rod terminus, were all in agreement with operational dynamic anion−π interactions; the AMFE confirmed the existence of multiple anion−π sites for transmembrane anion hopping, that is, anion−π slide **1** (Figure 1). However, these surprisingly consistent results should not distract from the fact that further studies are necessary to gain corroborative insights on the here introduced novel and complex system.

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Supporting Information Available: Experimental details and complete ref 12a. This material is available free of charge via the Internet at http://pubs.acs.org.

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