Rigid-Rod Anion-π Slides for Ion Hopping across Lipid Bilayers

Virginie Gorteau, Guillaume Bollot, Jiri Mareda and Stefan Matile*

Department of Organic Chemistry, University of Geneva, Geneva, Switzerland
stefan.matile@chiorg.unige.ch

Supporting Information

General. See ref. S1, Supporting Information.

Abbreviations. Boc: Butoxycarbonyl; DMAc: *N,N*-Dimethylacetamide; DMF: *N,N*-Dimethylformamide; DMSO: *N,N*-Dimethylsulfoxide; EYPC-LUVs: Egg yolk phosphatidylcholine large unilamellar vesicles; Gly: *L*-Glycine; HBTU: *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HEPES: 4-(2-Hydroxyethyl)-1-piperazine-ethanesulfonic acid; HPTS: 8-Hydroxy-1,3,6-pyrenetrisulfonate; NDI: Naphthalenediimide; TEA: Triethylamine; TFA: Trifluoroacetic acid; Z: benzyloxycarbonyl.

Compounds 1-3 and 8-12. These compounds were prepared following the procedures reported in ref S1 without change.

Compound 4. A solution of 3,6,9-trioxaundecanedioic acid **13** (66.6 mg, 0.3 mmol) in dry CH_2Cl_2 (2 ml) was cooled to 0 °C. Oxalyl chloride solution (2 M in CH_2Cl_2, 0.9 ml, 1.8 mmol) was added to the mixture, followed by one drop of dry DMF. After 5 minutes, the reaction mixture was allowed to warm up to room temperature and stirred for 1 hour. The solvent was evaporated and the resulting solid dissolved in dry CH_2Cl_2 that was subsequently evaporated. This process was repeated twice. The obtained crude acid chloride was used without further purification.[S2] N-Hydroxysuccinimide (76 mg, 0.66 mmol) was dissolved in toluene (2 ml) that was subsequently evaporated. This process was repeated 3 times. The dried N-hydroxysuccinimide was dissolved in dry THF (1 ml), and the solution was cooled to −10 °C. The previously synthesized acid chloride was added in CH_2Cl_2 (2 ml) and pyridine (53 μL, 0.66 mmol) were added. The reaction was allowed to warm up to room temperature and stirred for 2 hours. 37 μl of the obtained reaction mixture (0.1 mmol/ml, 3.70 μmol) was added slowly to a solution of **2** (10 mg, 7.36 μmol) and NEt_3 (4 μl) in dry DMF (150 μl) at 0°C. The reaction mixture was stirred for 10 minutes at 0°C and a further 20 minutes at room temperature. After evaporation of the solvents, the crude mixture was purified by PTLC (CH_2Cl_2/MeOH 10/1) to yield HPLC-pure (YMC-Pack SIL, CH_2Cl_2/MeOH 97/3, 2 ml/min, t_R = 9.86 min) **4** (7.5 mg, 35%) as a light pink solid. TLC (CH_2Cl_2/MeOH 10/1): R_f 0.30; ^{1}H NMR (300 MHz, $CDCl_3$/MeOD 6/1): δ 8.94 (s, 8 H), 8.87 (d, ^3J (H,H) = 7.6 Hz, 8 H), 8.84 (d, ^3J (H,H) = 7.9 Hz, 8 H), 4.50-4.35 (m, 8 H), 4.12-4.01 (m, 4 H), 3.84-3.78 (m, 4 H), 3.77-

3.59 (m, 20 H), 2.14 (s, 48 H), 1.41 (s, 18 H). MS (ESI, MeCN): *m/z (%)* 1453.4 (35) [M + 2H]$^{2+}$, 1475.9 (100) [M + Na + H]$^{2+}$.

Compound 5. A solution of **4** (6.0 mg) and TFA (1 ml) in CH_2Cl_2 (1 ml) was stirred for one hour at room temperature and was then concentrated *in vacuo*. Impurities were removed by solid-liquid extraction (2x ether, 2x hexane) to yield HPLC-pure (YMC-Pack ODS-A, 250 x 10 mm, MeCN + 1% TFA, 2 ml/min, t_R = 11.39 min) **5** (4.4 mg, 79%) as a brown solid. *Caution:* Dimeric O-NDIs in general were poorly soluble, bolaamphiphile **5** in particular was essentially insoluble in most common solvents. Best solubility was observed in mixtures of chloroform and trifluoroacetic acid. Similar observations with higher O-NDI rods have been reported previously.[S3] UV-vis (CH_2Cl_2/10% TFA): λ [nm] (ε [mM^{-1}cm^{-1}]) 359 (143), 380 (188); ^{1}H NMR (300 MHz, CDCl$_3$/10% TFA-d): δ 9.06 (s, 8 H), 9.02-8.84 (m, 16 H), 4.67-4.49 (m, 8 H), 4.31-4.19 (m, 4 H), 4.13-4.01 (m, 4 H), 3.97-3.82 (m, 20 H), 2.17 (s, 48 H). MS (MALDI, dithranol): found 2703.88; calcd for $C_{148}H_{119}N_{20}O_{33}$, 2703.82.

Ion Transport Measurements. With only a few exceptions, ion transport experiments were either following or adapting the methods described in ref. S1. A brief summary follows:

Stock Solutions. The following stock solutions were prepared: O-NDI monomer **1** (in DMSO), O-NDI monomer **2** (in DMSO), O-NDI monomer **3** (in DMSO), O-NDI dimer **4** (in DMSO) and O-NDI dimer **5** (in DMSO). pH was adjusted spectroscopically following a previously described method,[S4] final concentrations were confirmed by UV-vis spectroscopy.

EYPC-LUVs ⊃ HPTS. Stock solutions of large unilamellar vesicles composed of egg yolk phosphatidylcholine loaded with HPTS were prepared by freeze-thaw-extrusion following the previously described method.[S5] Final conditions: ~2.5 mM EYPC; inside: 1 mM HPTS, 10 mM HEPES, 100 mM NaCl or NaBr, pH 7.0, outside: 10 mM HEPES, 100 mM NaCl or NaBr, pH 7.0.

Hill Plots. EYPC-LUVs ⊃ HPTS (25 µl) were added to gently stirred, thermostated buffer (1980 µl, 10 mM HEPES, 100 mM NaCl (pH = 7.0) in a fluorescence cuvette (t = 0 sec). The time course of HPTS fluorescence emission intensity, F_t, was monitored at λ_{em} = 510 nm (λ_{ex}^1 = 450 nm, pH-control: λex^2 = 405 nm) during the addition of 0.5 M NaOHaq (20 µl) at t = 100 s, rods (20 µl **1-5** in DMSO at different concentration 0-2 mM, final concentrations 0-20 µM, Fig. 4) at t = 200 s, and gramicidin A (20 µl of 20 µM in DMSO) at the end of every experiment (t = 400s). Fluorescence time courses were normalized to fractional emission intensity I^n using equation [S1]

$$I^n = (F_t - F_0) / (F_\infty - F_0) \qquad\qquad [S1],$$

where $F_0 = F_t$ at slide addition, $F_\infty = F_t$ at saturation after complete leakage. The baseline (DMSO only, no rod) I^n_0 was then subtracted from I^n to give I [S2],

$$I = I^n - I^n_0 \qquad\qquad [S2].$$

The obtained I was further normalized into fractional HPTS emission I_F using equation [S3]

$$I_\text{F} = I \, / \, I_\text{MAX} \hspace{6cm} \text{[S3],}$$

where I_MAX is a reference emission for the varied parameter of interest, *e.g.*, the activity in NaCl before the addition of gramicidin A.

The term fractional activity Y is used to compare fractional HPTS emissions I_F at a given time, usually directly before the addition of gramicidin A, i.e., 200 s after the start of the transport experiment.

Ion Selectivity. EYPC-LUVs ⊃ HPTS (25 μl) in NaX (X = Cl, Br) were added to gently stirred, thermostated buffer (1980 μl, 10 mM HEPES, 100 mM MCl (M = Na, K, Rb, Cs) or 100 mM NaX (X = F, Cl, Br, I, OAc, NO_3, SCN, ClO_4) or 67 mM Na_2SO_4, pH = 7.0) in a fluorescence cuvette (t = 0 sec). The time course of HPTS fluorescence emission intensity, F_t, was monitored at λ_em = 510 nm (λ_ex^1 = 450 nm, pH-control: λ_ex^2 = 405 nm) during the addition of NaOH (20 μl, 0.5 M) at t = 100 s, rods **1** (20 μl DMSO stock solution, 1.5 μM final concentration), **2** (1.0 μM), **3** (2.0 μM) or **4** (2.5 μM) at t = 200 s, and gramicidin A (0.2 μM) at the end of every experiment. For each situation, control experiments included reversal of addition (rods **1-4** at t = 100 s, NaOH at t = 200 s), and background curves without rods **1**, **2** and **4**. The fractional HPTS emissions Y were determined using equation [S1] - [S3] as described above.

Mole Fraction Behavior. EYPC-LUVs ⊃ HPTS (25 μl) in NaX (X = Cl, Br) were added to gently stirred, thermostated buffer (z x 1980 μl (0 < z < 1), 10 mM HEPES, 100 mM NaX, pH = 7.0 and (1-z) x 1980 μl, 10 mM HEPES, 100 mM NaY (X, Y = I, Br, Cl, ClO_4), pH = 7.0) in a fluorescence cuvette (t = 0 sec). The time course of HPTS fluorescence emission intensity, F_t, was monitored at λ_em = 510 nm (λ_ex^1 = 450 nm, pH-control: λ_ex^2 = 405 nm) during the addition of NaOH (20 μl, 0.5 M) at t = 100 s, **1** (20 μl stock solution, 1.5 μM final concentration) at t = 200 s, and gramicidin A (0.2 μM) at the end of every experiment. For each situation, control experiments included background curves without **1**. The fractional HPTS emissions Y were determined using equation [S1] - [S3] as described above.

HPTS Anion Selectivity Assay with Weakly Basic Anions (Full Text, Scheme 4). Ion selectivity HPTS assays with weakly basic anions X_B^- deserved particular attention (Scheme 4). The initial HPTS emission in vesicles with Cl^-/F^- gradients was much weaker than that with other Cl^-/X^- gradients (Fig. 5Ba). We interpreted this phenomenon with passive HF influx in response to external $Cl^- \rightarrow F^-$ exchange (Scheme 4b). Because of its comparably weak acidity (pK_a = 3.2), the neutral HF exists in sufficient amounts (besides excess F^-) to cause, driven by the Cl^-/X_B^- gradient, significant influx without the corresponding efflux of HCl ($pK_a \sim$ -7). The result is internal acidification, *i.e.*, the formation of a pH gradient to reduce the applied F^- gradient by HF symport (Scheme 4c). In the HPTS assay, this internal acidification was reported as drastic decrease in emission. This was observed for weak bases such as F^- and OAc^- but not for non-basic anions such as Br^-, I^- or ClO_4^- (Fig. 5a and e; SO_4^{2-} is special, see Scheme 5). Addition of rod **1** to HF-acidified vesicles naturally removed this HF-mediated pH gradient and made the HPTS emission return to normal before application of the base pulse (Fig. 5Ba and e).

The consequences of the internal acidification with weakly basic anions X_B^- on ion selectivity HPTS assays were dependent of the sequence of addition (Scheme 4). Internal excess of H^+ (Scheme 4c) is naturally equivalent to external excess of OH^- (Scheme 4d). A

base pulse will magnify this X_B^- mediated OH^- gradient (Scheme 4e). Residual internal Cl^- will disappear immediately in response to slide addition by rapid (and invisible) Cl^-/X_B^- exchange for the reasons elaborated above with non-basic anions X^- (Scheme 4f). In the final system for ion selectivity HPTS assays with weakly basic anions X_B^- (Scheme 4g), the effective pH gradient is larger than that with non-basic anions X^- (Scheme 3d). Activities found with weakly basic anions X_B^- will thus appear higher than they are in reality (Fig. 5A, a and e).

The conclusion that the comparison of selectivity sequences with weak and non-basic anions X_B^- and X^- is intrinsically problematic did fortunately not apply for both sequences of addition. Slide addition before base pulse will rapidly dissipate all anion gradients (Scheme 4h) to end up with nonproductive and invisible X_B^-/X_B^- exchange (Scheme 4i). As mentioned above, this removal of the X_B^- induced pH gradient by slide addition before base pulse is very well visible in the HPTS assay as an increase of an unusually weak emission back to normal values (Fig. 5B, a and e). Application of the base pulse will then produce a final system (Scheme 4j) that lacks the additional pH gradient in the final system with weakly basic anions X_B^- obtained by base pulse before slide addition (Scheme 4g) and is identical with the final situation obtained with non-basic anions X^- (Scheme 3d). Results on ion selectivity with weakly and non-basic anions X_B^- and X^- obtained by slide addition before base pulse should thus be directly comparable (Fig. 5B), whereas results obtained by base pulse before slide addition may give activities for weakly basic anions A_B^- that appear higher than they are in reality (Fig. 5A). The concerned weakly basic anions X_B^- with proton carrier activity, here F^- and OAc^-, were readily identified by low initial emission intensity at the beginning of the HPTS assay in the presence of an Cl^-/X_B^- gradient. The presence of this HF-mediated pH gradient rather than anion selective transport may thus account for the high activity found when the O-NDI rod **1** was added after the base pulse (Fig. 5A, a and e). As communicated previously,[S1] the correct halide selectivity sequence of rod **1** is thus a halide VI ($Cl^- > F^- > Br^- > I^-$) rather than a halide VII ($F^- > Cl^- > Br^- > I^-$, Fig. 6, ■) for this reason.

Computational Methods. The geometries of all model structures and complexes included in this study were fully optimized with density functional theory (DFT) using Gaussian03 package.[S6] The functional PBE1PBE[S7] combined with 6-311++G** basis set was used for the geometry optimizations. Because the main objective of this study is to examine different aspects of interactions between anions and π-systems, we concentrated mainly on the geometries where the ion is located in the main symmetry plane. During the optimizations an appropriate symmetry element were imposed: typically for complexes with anions the C_s was used, while for structures without anion the C_{2v} and D_{2h} symmetries were imposed. The global quadrupole moments Q_{ZZ} in Buckinghams (1B = 3.336×10^{-40} C m^2) perpendicular to the NDI plan were computed at MP2/6-311G** level[S8,S9] *via* a single point calculations based on PBE1PBE/6-311++G** optimized geometries. The electrostatic potentials are mapped onto isosurface of the total electron density based on DFT calculations and were represented with GaussView program[S10] according to an energy scale corresponding to ±30 kcal mol^{-1}, where blue color-code is positive and red negative potential. The interaction energies were computed at the same level of theory and the correction for the basis set superposition error (BSSE) by using the classic counterpoise technique[S11] applied on geometries optimized at PBE1PBE/6-311++G** level.

Molecular dynamic simulations for assemblies of rigid O-NDI rods were carried out with *AMBER 9* package[S12] using *parm99* and *gaff* force fields.[S13,S14] At first, the *ChelpG*[S15] atomic charges for one NDI rod were calculated with PBE1PBE/6-31G* and applied on O-

NDI rods. Oligomers and in some cases anions were manually assembled using *xLEaP* module.[S16] Thus obtained bundles were submitted to starting minimization with 500 steepest descent iterations followed by 500 conjugate gradient iterations to avoid close contacts. Initial molecular dynamic simulations were performed *in vacuo* for 500 ps with special decreasing restraints to heat the system containing the chloride anions at 300 K° with a 1.0 fs time step. Then, the system was equilibrated in a simulation of 1000 ps where all atoms of the complexes were free to move. The final structures of assemblies were obtained after optimization from the average geometry of the last 50 ps using an RMS of 0.05 kcal mol^{-1} on the energy with an electrostatic cutoff of 50.0 Å.

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