Experimental evidence for the functional relevance of anion-π interactions

Ryan E. Dawson,¹ ⁵ Andreas Hennig,¹ ⁵ Dominik P. Weimann,² Daniel Emery,¹ Velayutham Ravikumar,¹ Javier Montenegro,¹ Tosihide Takeuchi,¹ Sandro Gabutti,³ Marcel Mayor,³ ⁴ Jiri Mareda,¹ Christoph A. Schalley² & Stefan Matile¹*

¹Department of Organic Chemistry, University of Geneva, Geneva, Switzerland. ²Institut für Chemie und Biochemie der Freien Universität, Berlin, Germany. ³Department of Chemistry, University of Basel, Basel, Switzerland. ⁴Institute of Nanotechnology, Karlsruhe Institute of Technology, Germany. ⁵These two authors contributed equally to the study.

*To whom correspondence should be addressed. E-mail: stefan.matile@unige.ch

Supplementary Information

Table of Content

1. Materials and methods S2
2. Supplementary text S4
  2.1. Synthesis S4
    2.1.1. Synthesis of O-NDI rods S4
    2.1.2. Synthesis of monomeric NDIs S7
    2.1.3. Synthesis of O-NDI cyclophanes S12
    2.1.4. Synthesis of NDI hydrazones S12
  2.2. ESI-FTICR-MS-MS experiments S15
  2.3. Charge-transfer complex formation S16
  2.4. Anion transport S19
    2.4.1. Vesicle preparation S19
    2.4.2. Determination of transport activity with the HPTS assay S21
    2.4.3. Determination of transport activity with the CF assay S22
    2.4.4. Determination of transport activity with the lucigenin assay S23

SUPPLEMENTARY INFORMATION
doi: 10.1038/nchem657

nature chemistry | www.nature.com/naturechemistry

© 2010 Macmillan Publishers Limited. All rights reserved.
1. Materials and methods

As in refs. S1-S3, Supplementary Information. Briefly, reagents for synthesis were purchased from Fluka and Aldrich, amino acid derivatives from Novabiochem and Bachem, HATU from Applied Biosystems, buffers and salts of the best grade available from Fluka or Sigma-Aldrich and used as received. 8-Hydroxy-1,3,6-pyrenetrisulfonate (HPTS) was from Fluka. Egg yolk phosphatidylcholine (EYPC) and a Mini-Extruder used for vesicle preparation were from Avanti Polar Lipids. All reactions were performed under N₂ or argon atmosphere. Unless stated otherwise, column chromatography was carried out on silica gel 60 (Fluka, 40-63 μm). Analytical (TLC) and preparative thin layer chromatography (PTLC) was performed in silica gel 60 (Fluka, 0.2 mm) and silica gel GF (Analtech, 1000 μm), respectively. HPLC was performed using a Jasco HPLC system (PU-980, UV-970, FP-920), [α]²⁰⁺D values were recorded on a Jasco P-1030 Polarimeter, melting points (m.p.) on a heating table from Reichert (Austria). IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer (ATR, Golden Gate, unless stated otherwise) and are reported as wavenumbers ν in cm⁻¹ with band intensities indicated as s (strong), m (medium), w (weak). ESI-MS for the characterization of new compounds was performed on a Finnigan MAT SSQ 7000 instrument or a ESI API 150EX and are reported as mass-per-charge ratio m/z (intensity in %, [assignment]). Accurate mass determinations using ESI (HR ESI-MS) were performed on a
Sciex QSTAR Pulsar mass spectrometer. Anion binding studies were conducted with an Ionspec QFT-7 FT-ICR mass spectrometer (Varian Inc., Lake Forest, CA), equipped with a 7 T superconducting magnet and a Micromass Z-Spray electrospray ionization (ESI) source (Waters Co., Saint-Quentin, France). $^1$H and $^{13}$C spectra were recorded (as indicated) either on a Bruker 300 MHz, 400 MHz or 500 MHz spectrometer and are reported as chemical shifts (δ) in ppm relative to TMS (δ = 0). Spin multiplicities are reported as a singlet (s), doublet (d), triplet (t), quartet (q) and quintet (quint) with coupling constants (J) given in Hz, or multiplet (m). Broad peaks are marked as br. $^1$H and $^{13}$C resonances were assigned with the aid of additional information from 1D & 2D NMR spectra (H,H-COSY, DEPT 135, HSQC and HMBC). UV-Vis spectra were recorded on a JASCO V-650 spectrophotometer equipped with a stirrer and a temperature controller (25 ºC) and are reported as maximal absorption wavelength $\lambda$ in nm (extinction coefficient ε in mM$^{-1}$cm$^{-1}$).

Fluorescence measurements were performed with a FluoroMax-2 spectrofluorometer (Jobin-Yvon Spex) equipped with a stirrer and a temperature controller. All measurements were performed at 25 ºC.

**Abbreviations.** ACN: Acetonitrile; AcOH: Acetic acid; Alloc: Allyloxycarbonyl; AMFE: Anomalous mole fraction effect; Boc: $t$-Butoxycarbonyl; calcd: Calculated; Cbz: (Benzylxoy)carbonyl; CF: 5(6)-Carboxyfluorescein; DCM: Dichloromethane; DMAc: $N$,$N$-Dimethylacetamide, DMF: $N$,$N$-Dimethylformamide; DMSO: Dimethylsulfoxide; DTBP: Di-$t$-Bu-pyridine; EDC: $O$-(3-Dimethylaminopropyl)-$N$,$N$'-tetramethyluronium hexafluorophosphate; HBTU: $O$-(Benzotriazol-1-yl)-$N$,$N$,$N'$,$N''$-tetramethyluronium hexafluorophosphate; Hepes: $N$-(2-hydroxyethyl)piperazine-$N'$-(2-ethanesulfonic acid); HPTS: 8-hydroxy-1,3,6-pyrenetrisulfonate;
2. Supplementary text

2.1. Synthesis

2.1.1. Synthesis of O-NDI rods (fig. S1)

**Compound 2c.** This compound was prepared from the commercially available starting materials 2a and 2b as previously reported\(^1\).

**Compound 2d.** This compound was prepared from compound 2c as previously reported\(^1\).

**Compound 2e.** This compound was prepared from compound 2d and 2b as previously reported\(^1\).

**Compound 2f.** This compound was prepared from compound 2e as previously reported\(^1\).

**Compound 2h.** A solution of the amine 2f (220 mg, 0.35 mmol) and the anhydride 2g (69.8 mg, 0.70 mmol) was stirred in THF (10 ml) at rt under a nitrogen atmosphere and in the absence of
light. After 4 h the reaction mixture was evaporated to dryness and the resultant residue was purified by column chromatography (DCM/MeOH 9:1) to afford the acid 2h as a colorless semi-solid (247 mg, 97%). $R_f$ (DCM/MeOH 9:1): 0.31; $[\alpha]_D^{20} = -31.7$ (c = 0.18 M in MeOH); IR (neat): 3278 (m), 2982 (m), 2933 (m), 1721 (s), 1623 (s), 1532 (m), 1367 (m), 1318 (m), 1272 (m), 1249 (m), 1148 (s), 1019 (m), 1005 (m), 952 (m), 849 (m), 707 (m); $^1$H NMR (400 MHz, CD$_3$OD): 4.41-4.36 (m, 1H), 4.35-4.29 (m, 2H), 2.70-2.45 (m, 6H), 2.45-2.29 (m, 6H), 2.20-2.02 (m, 3H), 2.00-1.83 (m, 3H), 1.47 (s, 9H), 1.45 (s, 27H); $^{13}$C NMR (100 MHz, CD$_3$OD): 176.0 (s), 174.4 (s), 174.4 (s), 174.3 (s), 174.2 (s), 174.1 (s), 172.2 (s), 83.5 (s), 82.3 (s), 82.3 (s), 54.8 (t), 54.3 (d), 52.7 (d), 33.2 (t), 33.1 (t), 33.0 (t), 32.3 (t), 31.5 (t), 30.6 (t), 28.9 (q), 28.8 (q), 28.7 (t), 28.5 (t), 28.3 (t); MS (ESI, -ve): 729 (100, [M]-); HR-MS (ESI, -ve): Calcd for C$_{35}$H$_{58}$O$_{13}$N$_3$: 728.3975, found: 728.3904.

**Compound 2k.** To water (1.3 l), the anhydride 2i (6.51 g, 24.3 mmol) and 1 M KOH (130 ml) were added at rt. The solution was briefly stirred at rt before the pH was adjusted to 6.4 with 1 M H$_3$PO$_4$. To the resulting mixture the amine 2j (2.36 g, 12.2 mmol) was added and the pH was readjusted to 6.4 with 1 M H$_3$PO$_4$. The mixture was then stirred at reflux for 15 h. After the cooling to rt, AcOH was added until the product precipitated. The solid was filtered and dried under vacuum to give 2k (4.25 g, 76%) as a yellow solid. Analytical and spectroscopic data were as previously reported$^{32}$.

**NDI 2m.** This compound was prepared from compounds 2i and 2l as previously reported$^{31}$.

**O-NDI 2n.** The anhydride 2k (1.70 g, 3.83 mmol) and the amine 2m (204 mg, 0.36 mmol) were suspended in dry DMAc (40 ml) with 5 Å molecular sieves under an atmosphere of nitrogen.
The mixture was stirred at 135 °C for 22 h. The reaction mixture was then allowed to cool to rt and the solvent was removed in vacuo. The residue was suspended in DCM (100 ml) and subjected to centrifugation. The filtrate was collected and the extraction and centrifugation process with DCM was repeated a further two times (2 x 100 ml). The combined DCM extracts were washed with 1M KHSO₄ (2 x 100 ml), dried over Na₂SO₄ and evaporated to dryness. The residue was then suspended in MeOH (100 ml) and subjected to centrifugation before the supernatant was discarded. The step was repeated until the supernatant was colorless (5 times). The residue was then purified by column chromatography (DCM/MeOH 90:1) to afford the O-NDI 2n as a yellow solid (284 mg, 56%). Analytical and spectroscopic data were as previously reported.

**O-NDI 2o.** This compound was prepared from compound 2n as previously reported.

**O-NDI 2s.** This compound was prepared from the mixture of compounds 2p, 2q and 2r as previously reported.

**O-NDI 2t.** A solution of the peptide 2h (88.8 mg, 0.121 mmol), HATU (45.6 mg, 0.121 mmol) and TEA (57.6 µl, 0.408 mmol) in freshly distilled DMF (1.5 ml) was stirred under an atmosphere of nitrogen for 15 min at rt. 0.5 ml of the peptide solution was then added to a solution of the O-NDI 2s (20 mg, 0.014 mmol) and TEA (5.8 µl, 0.040 mmol) in DMF (0.5 ml). After 1.5 h a further 0.5 ml of the peptide solution was added to the reaction mixture. After a further 1.5 h the final 0.5 ml portion of the peptide solution was added to the reaction mixture. After a total reaction time of 4
h the reaction mixture was evaporated to dryness and the residue was purified by PTLC (DCM/MeOH 15:1) to afford the O-NDI 2t (8.9 mg, 30% (36% based on recovered starting material)) as a yellow solid. Mp: >230 °C; HPLC (YMC-Pack SIL 10 x 250 mm, DCM/MeOH 9:1, 2 ml/min): Rt = 6.8 min; UV/vis (CHCl₃): 382 (41.0), 361 (32.0); IR (DCM): 1712 (s), 1676 (s), 1604 (w), 1582 (m), 1520 (m), 1451 (m), 1369 (m), 1335 (s), 1154 (m), 846 (m); ¹H NMR (400 MHz, CDCl₃): 8.97-8.91 (m, 4H), 8.89-8.83 (m, 4H), 8.80-8.74 (m, 4H), 7.82-7.73 (m, 3H), 7.65-7.58 (m, 2H), 7.46-7.39 (m, 3H), 7.36-7.30 (m, 2H), 7.29-7.18 (m, 2H), 6.74 (t, 3J(H,H) = 4.0 Hz, 1H), 6.60-6.55 (m, 1H), 5.30-5.23 (m, 1H), 4.52-4.20 (m, 9H), 4.00-3.91 (m, 1H), 3.81-3.60 (m, 8H) 2.75-1.90 (m, 40H), 1.47-1.39 (m, 36H); MS (ESI, -ve): 2252 (100, [M + OAc]⁻), 2193 (100, [M⁻]).

O-NDI 2. A solution of the O-NDI 2t (7.9 mg, 0.0036 mmol) in TFA (1 ml) was stirred for 75 min at rt. The solvent was removed in vacuo and the residue was suspended in n-hexane (7.5 ml). The suspension was centrifuged and the supernatant discarded. Centrifugation another two times with n-hexane (2 × 7.5 ml) afforded the O-NDI 2 as a light brown solid (7.1 mg, quant.). ¹H NMR (400 MHz, CDCl₃/CD₃OD (6:1) + 1% TFA-d): 8.92-8.85 (m, 4H), 8.83-8.75 (m, 4H), 8.70 (dd, 3J(H,H) = 7.6 Hz, 3J(H,H) = 18.8 Hz, 4H), 7.71 (d, 3J(H,H) = 7.2 Hz, 2H), 7.55 (d, 3J(H,H) = 7.2 Hz, 2H), 7.32 (t, 3J(H,H) = 7.2 Hz, 2H), 7.25-7.20 (m, 2H), 4.40-4.30 (m, 7H), 4.16 (t, 3J(H,H) = 7.2 Hz, 1H), 3.81-3.50 (m, 10H), 2.60-1.88 (m, 40H); MS (ESI, -ve): 1968 ([M⁻]).

2.1.2. Synthesis of monomeric NDIs (fig. S2)

NDI 3. NDA 3a (300 mg, 1.12 mmol) and 2,4,6-trimethylaniline 13 (454 mg, 3.36 mmol) were added to dimethylacetamide (50 ml) and heated to 135 °C for 16 h. After cooling of the reaction
mixture, the solvent was removed in vacuo, and the residue was vigorously stirred with CHCl₃ (100 ml). Insoluble material was removed by filtration and the organic phase was washed with H₂O (2 x 50 ml) and 1 M KHSO₄ (2 x 50 ml). The organic phase was dried over Na₂SO₄ and the solvent removed by rotary evaporation. Purification by recrystallization from MeOH/CHCl₃ 5:1 gave pure 3 (196 mg, 35%) as a colorless solid. Mp: >300 °C; Rᵣ (CH₂Cl₂): 0.32; ¹H NMR (400 MHz, CDCl₃): 8.93 (s, 4H), 7.13 (s, 4H), 2.44 (s, 6H), 2.17 (s, 12H); ¹³C NMR (125 MHz, CDCl₃): 162.3 (s), 139.1 (s), 134.9 (s), 131.5 (d), 130.4 (s), 129.6 (d), 127.6 (s), 126.9 (s), 21.2 (q), 17.7 (q); IR (neat): 3373 (w), 3271 (w), 2918 (w), 1713 (m), 1676 (s), 1608 (w), 1578 (m), 1486 (w), 1449 (m), 1438 (m), 1339 (s), 1305 (m), 1198 (m); MS (ESI, MeOH/CHCl₃ 9:1): 504 (100, [M + H]+).

**NDI 4.** NDA 3a (300 mg, 1.12 mmol) and aniline 4a (336 mg, 3.36 mmol) were added to dimethylacetamide (30 ml) and heated to 135 °C for 20 h. After cooling of the reaction mixture, the solvent was removed in vacuo, and the residue was vigorously stirred with CHCl₃ (100 ml). Insoluble material was removed by filtration and the organic phase was washed with H₂O (2 x 50 ml) and 1 M KHSO₄ (2 x 50 ml). The organic phase was dried over Na₂SO₄ and the solvent removed by rotary evaporation. Purification by flash column chromatography on silica gel (CH₂Cl₂/MeOH 99.5:0.5) gave pure 4 (81 mg, 17%) as a colorless solid. Mp: >300 °C; Rᵣ (CH₂Cl₂/MeOH 99:1): 0.40; ¹H NMR (400 MHz, CDCl₃): 8.86 (s, 4H), 7.63-7.52 (m, 6H), 7.37-7.34 (m, 4H); ¹³C NMR (125 MHz, CDCl₃/TFA-d 7:3): 165.0 (s), 133.3 (s), 133.0 (d), 130.7 (d), 130.5 (d), 128.3 (d), 127.5 (s), 127.0 (s); IR (neat): 3086 (w), 3058 (w), 1710 (m), 1663 (s), 1580 (m), 1489 (m), 1447 (m), 1344 (s), 1243 (s), 1196 (s); MS (ESI, MeOH/CHCl₃ 9:1): 419 (100, [M + H]+).
**Br,Br-NDA 12.** This compound was obtained as a mixture of bromination products following previously reported procedures\(^{S3}\).

**Br,Br-NDI 14.** A mixture of NDI bromination products including *Br,Br*-NDA 12 (1.0 g, \(<2.36\) mmol) and 2,4,6-trimethylaniline 13 (1.32 ml, 9.44 mmol) in AcOH (50 ml) was heated at 80 °C for 12 h. The reaction mixture was filtered and the remaining precipitate was washed with AcOH (5 ml). The crude product was purified by silica gel chromatography (CH\(_2\)Cl\(_2\) / petroleum ether 7:3, \(R_f\) (EtOAc / petroleum ether 3:2): 0.35) to give 14 (460 mg, \(>28\)% as a yellow solid. Mp: >220 °C; IR: 2916 (m), 1716 (s), 1672 (s), 1561 (m), 1412 (s), 1224 (s), 886 (m); \(^1\)H NMR (400 MHz, CDCl\(_3\)): 9.16 (s, 2H), 7.13 (s, 4H) 2.43 (s, 6H), 2.15 (s, 12 H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): 160.3 (s), 160.2 (s), 139.6 (d), 139.4 (s), 134.8 (s), 130.2 (s), 129.7 (d), 128.6 (s), 128.6 (s), 125.7 (s), 124.5 (s), 21.2 (q), 17.8 (q); MS (ESI, +ve): 661/663 (100, [M + H\(^+\)]).

**CN,CN-NDI 5.** A mixture of 14 (100 mg, 0.15 mmol) and CuCN (136 mg, 1.52 mmol) in NMP (10 ml) was stirred at 100 °C for 5 h. The reaction mixture was concentrated in vacuo and purified by a silica gel chromatography (CH\(_2\)Cl\(_2)/\text{MeOH}\) 99:1; \(R_f\) (CH\(_2\)Cl\(_2\)): 0.20) to give 5 (52 mg, 63%) as a yellow solid. Mp: >220 °C; IR: 3388 (m), 2918 (m), 2234 (m), 1719 (s), 1675 (s), 1590 (s), 1434 (s), 1232 (s), 923 (m); \(^1\)H NMR (400 MHz, CD\(_2\)Cl\(_2\)): 9.18 (s, 2H), 7.15 (s, 4H) 2.44 (s, 6H), 2.14 (s, 12 H); \(^{13}\)C NMR (125 MHz, CD\(_2\)Cl\(_2\)): 160.1 (s), 159.7 (s), 136.8 (s), 135.4 (s), 130.0 (d), 129.8 (s), 129.2 (s), 128.7 (s), 127.8 (s), 117.6 (s), 115.9 (s), 21.2 (q), 17.8 (q); MS (ESI, +ve): 575 (7, [M + NH\(_4\)]\(^+\)), 553 (100, [M + H\(^+\)]).

**Br,Br-NDI 15.** *Br,Br*-NDA 12 (1.00 g, 2.34 mmol, mixture of brominated products) was suspended in AcOH (20 ml) with NH\(_4\)OAc (3.62 g, 47.0 mmol) and stirred at reflux for 1 h. The
reaction mixture was allowed to cool to rt and the precipitate was filtered and washed with AcOH (50 ml) and diethyl ether (100 ml) to afford 15 (0.77 g, 77%) as a mixture of its brominated isomers. MS (ESI, +ve): 423 (100, [M(15, diBr) + H]⁺), 345 (47, [M(monoBr) + H]⁺), 265 (71, [M(noBr) + H]⁺).

CN,CN-NDI 6. A mixture of 15 (666 mg, 1.57 mmol) and CuCN were suspended in NMP (120 ml) and stirred at 100 °C for 1 h. The solvent was then removed in vacuo and the residue was washed with 10% NH₄OH (6 × 200 ml). The obtained crude 16, a brown solid, was carried over to the next step without further purification. The crude 16, boronic acid 17 (1.15 g, 9.4 mmol), Cu(OAc)₂ (1.14 g, 6.3 mmol) and TEA (1.3 ml, 9.4 mmol) were suspended in DMAc (125 ml) containing 4 Å molecular sieves. The reaction mixture was purged with oxygen and then stirred at 55 °C for 2 d. Further equivalents of the boronic acid 17 (1.15 g, 9.4 mmol), Cu(OAc)₂ (1.14 g, 6.3 mmol) and TEA (1.3 ml, 9.4 mmol) were then added with DMAc (25 ml). After a total reaction time of 5 d, the reaction mixture was evaporated to dryness and the residue was filtered through a pad of silica and flushed with 25:1 DCM/MeOH (500 ml). The collected filtrate was concentrated, absorbed to silica and then purified by column chromatography (DCM and then DCM/acetone 100:1 and finally 50:1). Fractions containing the desired product were concentrated, absorbed to silica and purified again by column chromatography (DCM/acetone 50:1) to afford the NDI 6 (31 mg, 4.2%) as a pale yellow solid. Mp: >230 °C; Rf (DCM/MeOH 100:1): 0.11; UV/vis (DMSO): 393 (9.0), 360 (11.0), 261 (41.0); IR (neat): 2231 (w), 1717 (m), 1672 (s), 1587 (w), 1486 (m), 1438 (m), 1377 (w), 1330 (s), 1243 (s), 1197 (w), 1162 (w), 1138 (w), 950 (m), 914 (m), 845 (m), 794 (m), 723 (s), 691 (m), 654, (m); ¹H NMR (400 MHz, CDCl₃): 9.25 (s, 2H), 7.67-7.61 (m, 6H), 7.38-7.33 (m, 4H); ¹³C NMR (75 MHz, CDCl₃/TFA-d₁): 161.9 (s), 161.2 (s), 137.2 (d), 132.5 (s), 131.1 (d), 130.7 (d), 129.9 (s), 128.6 (s), 128.1 (d), 114.3 (s); MS (ESI, -ve): 468 (100, [M]⁻);
HR-MS (ESI, -ve): Calcd for C$_{28}$H$_{12}$O$_4$N$_4$: 468.0864, found: 468.0883.

**NDI 18.** NDA 3a (500 mg, 1.86 mmol) and 2,6-dimethylaniline 18a (685 mg, 5.66 mmol) were added to acetic acid (10 ml) and heated under microwave irradiation at 140 °C for 20 min. After cooling of the reaction mixture, CHCl$_3$ (100 ml) was added, the suspension was filtered and the remaining precipitate was washed twice with CHCl$_3$ (50 ml). The combined organic phases were washed with H$_2$O (2 x 50 ml), sat NaHCO$_3$ (2 x 50 ml) and 1 M KHSO$_4$ (2 x 50 ml), dried over Na$_2$SO$_4$. The solvent was removed by rotary evaporation to give pure 18 (125 mg, 14%) as a colorless solid. Mp: >300 °C; $R_f$ (CH$_2$Cl$_2$/MeOH 99:1): 0.47; IR (neat): 3376 (w), 3016 (w), 2983 (w), 2926 (w), 2860 (w), 1709 (s), 1666 (s), 1580 (m), 1469 (m), 1448 (m), 1336 (s), 1243 (s), 1194 (s); $^1$H NMR (400 MHz, CDCl$_3$): 8.90 (s, 4H), 7.37-7.42 (m, 2H), 7.30-7.32 (m, 4H), 2.18 (s, 12H); $^{13}$C NMR (125 MHz, CDCl$_3$/TFA-d$_7$:3): 164.2 (s), 135.7 (s), 133.3 (d), 131.6 (s), 130.7 (d), 129.6 (d), 127.9 (s), 127.0 (s), 17.3 (q); MS (ESI, MeOH/CHCl$_3$ 9:1): 475 (100, [M + H]$^+$).

**NDI 19.** This compound was prepared from NDA 3a (500 mg, 1.86 mmol) and 3,5-dimethylaniline 19a (685 mg, 5.66 mmol) as described for regioisomer 18. The reaction was not optimized because 19 was inactive in transport experiments (see below).

**O,O-NTE 20a.** This compound was obtained following previously reported procedures (NTE, 1,4,5,8-Naphthalenetetraester)$^{33}$.

**O,O-NDI 20.** O,O-NTE 20a (337 mg, 0.67 mmol) was suspended in 10 ml 1 M KOH in i-propanol and heated for 18 h at 85 °C. After cooling of the reaction mixture, the solvent was removed in vacuo and acetic acid (12 ml) and 2,4,6-trimethylaniline 13 (270 mg, 2.0 mmol) were
added to the crude product. The reaction mixture was heated to 120 °C for 24 h. After cooling of
the reaction mixture, CHCl₃ (50 ml) were added and the organic phase was washed with H₂O (2 x
20 ml) and sat. NaHCO₃ (2 x 20 ml). The combined organic phases were dried with MgSO₄ and the
solvent was removed by rotary evaporation. The crude product was purified by flash column
chromatography on silica gel (CH₂Cl₂/MeOH 99.5:0.5) to afford 20 (364 mg, 92%) as a yellow
solid. Mp: >300 °C; Rᵣ(CH₂Cl₂): 0.32; ¹H NMR (400 MHz, CDCl₃): 8.62 (s, 2H), 7.10 (s, 4H),
4.54 (q, ³J = 6.9 Hz, 4H), 2.41 (s, 6H), 2.16 (s, 12H), 1.65 (t, ³J = 6.9 Hz, 6H); ¹³C NMR (125 MHz,
CDCl₃): 161.9 (s), 160.5 (s), 138.7 (s), 135.0 (s), 130.9 (s), 129.5 (d), 127.6 (s), 120.3 (d), 111.3 (s),
66.5 (t), 21.2 (q), 17.9 (q), 14.8 (q); IR (neat): 2990 (w), 2943 (w), 2911 (w), 1707 (s), 1668 (s),
1574 (s), 1478 (m), 1436 (s), 1408 (m), 1378 (m), 1355 (m), 1331 (s), 1307 (s), 1217 (s), 1206 (s).

2.1.3. Synthesis of O-NDI cyclophanes

O-NDI 8 was prepared following literature procedures⁵⁵.

2.1.4. Synthesis of NDI hydrazones (fig. S3)

NDIs 9a and 9b. A mixture of NDA 3a (707 mg, 2.64 mmol) and EDC (200 mg, 1 mmol) in
DMF (5 ml) was stirred for 5 min at 120 °C under an atmosphere of argon. The amine 4a (155 mg,
1.67 mmol) was added and the mixture was stirred for a further 2 h. The solvent was removed in
vacuo and the residue was then purified by column chromatography (DCM/MeOH 100:1, then 5:1
(+ 3% AcOH) to afford a brown solid (1.5 g) that contained residues of AcOH. A portion of the
 crude product (1.3 g) was suspended in AcOH (2 ml) with NH₄OAc (1.87 g, 24.3 mmol) and stirred
at reflux for 1 h. The reaction mixture was allowed to cool to rt and the precipitate was filtered and washed with AcOH (10 ml) and diethyl ether (50 ml) to afford a 4:1 mixture of the NDIs 9a and 9b as a peach solid (212 mg, 36% of 9a, 5% of 9b (based on 1H NMR of 9a relative to 9b)); 1H NMR (400 MHz, d6-DMSO): 12.16 (s, 1H, 9a), 12.11 (s, 2H, 9b), 8.66 (dd, 3J(H,H) = 7.6 Hz, 3J(H,H) = 12.8 Hz, 4H, 9a), 8.60 (s, 4H, 9b), 7.58-7.42 (m, 5H, 9a); MS (ESI, +ve): 703 (38, [2M(9a) + NH4]+), 360 (53, [M(9b) + NH4]+), 343 (100, [M(9a) + H]+).

NDI 9. Under an atmosphere of nitrogen, the imide mixture 9a and 9b (50 mg, 0.122 mmol of 9a, 0.031 mmol of 9b), the boronic acid 9c (123 mg, 0.822 mmol), Cu(OAc)2 (100 mg, 0.548 mmol) and TEA (0.11 ml, 0.822 mmol) were suspended in DMAc containing 4 Å molecular sieves. The reaction mixture was purged with oxygen and then stirred at 55 °C for 22 h. The reaction mixture was then evaporated to dryness and the residue was purified by column chromatography (DCM then DCM/acetone 20:1). Fractions containing the desired product were concentrated, absorbed to silica and purified again by column chromatography (DCM/acetone 20:1) to afford 9 (47 mg, 86%) as a pale yellow solid. Mp: >230 ºC; Rf (DCM/acetone 4:1): 0.76; UV/vis (DMSO): 382 (21.0), 362 (21.0), 258 (19.0); IR (neat): 3063 (w), 1705 (m), 1580 (m), 1490 (w), 1448 (w), 1345 (m), 1241 (s), 1197 (s), 978 (m), 887 (w), 829 (m), 788 (w), 768 (s), 732 (s), 707 (m); 1H NMR (400 MHz, d6-DMSO): 10.12 (s, 1H), 8.74 (s, 4H), 8.12 (d, 3J(H,H) = 8.4 Hz, 2H), 7.74 (d, 3J(H,H) = 8.4 Hz, 2H), 7.60-7.47 (m, 5H); 13C NMR (100 MHz, d6-DMSO): 192.7 (d), 163.0 (s), 162.9 (s), 141.0 (s), 136.1 (s), 135.6 (s), 130.6 (d), 130.5 (d), 130.2 (d), 129.1 (d), 128.6 (d), 127.2 (s), 126.9 (s), 126.7 (s); MS (ESI, +ve): 447 (100, [M + H]+); HR-MS (ESI, +ve): Calcd for C33H39O11N4+: 689.2429, found: 689.2469.

Hydrazones 10, 11, 21-23. Hydrazines/hydrazides 10a, 11c or 21a-23a (7.5 mM) in DMSO
(50 µl) and HCl (0.5 M) in water (2 µl) were added to 9 (5 mM) in DMSO (75 µl). After incubation at 60 °C for 2 h, the mixture was cooled to rt, and NaOH (0.5 M) in water (2 µl) was added. In-situ prepared hydrazones 10, 11 and 21-23 could be used without further work-up and purification.

**Compound 11b.** HBTU (1082 mg, 2.85 mmol) and DIEA (2.44 ml, 14.25 mmol) were sequentially added to a solution of Boc-Gly (500 mg, 2.85 mmol) in CH$_2$Cl$_2$ (25 ml) under N$_2$. The solution was stirred for 5 min and a solution of tert-butylcarbazate (377 mg, 2.85 mmol) in DCM (5 ml) was added dropwise. The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was poured into a separation funnel containing CH$_2$Cl$_2$ (15 ml) and KHSO$_4$ 1 M (35 ml). The aqueous phase was extracted with CH$_2$Cl$_2$ (2 x 15 ml). The organic layer was washed with HCl 1 M (35 ml), NaHCO$_3$ saturated aqueous solution (35 ml), water (35 ml) and brine (35 ml) and dried over Na$_2$SO$_4$. After filtration and removal of solvent under vacuum, the residue was purified by silica gel column chromatography (CH$_2$Cl$_2$/MeOH, 9:1) to give 11b (742 mg, 90%) as a colorless solid. Mp: 49-50 ºC; IR (neat): 3285 (m), 2979 (w), 1684 (s), 1140 (s); $^1$H NMR (400 MHz, CDCl$_3$): 8.69 (s, 1H), 7.47-6.80 (m, 1H), 5.78-5.30 (m, 1H), 4.01-3.90 (m, 2H), 1.48 (s, 9H), 1.47 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$): 169.8 (s), 156.3 (s), 155.6 (s), 81.7 (s), 42.7 (t), 28.3 (q), 28.2 (q); MS (ESI, MeOH): 602 (30, [2M+Na]$^+$), 597 (12, [2M+H$_2$O]$^+$), 312 (83, [M+Na]$^+$), 307 (43, [M$^+$+H$_2$O]$^+$), 290 (36, [M+H]$^+$), 256 (43), 234 (45), 178 (100); HRMS: Calculated for C$_{12}$H$_{23}$N$_3$O$_5$Na: 312.1529; found: 312.1527.

**Compound 11c.** HCl (1 M in Et$_2$O, 10 ml) was added to a solution of 11b (250 mg, 0.86 mmol) in CH$_2$Cl$_2$ (5 ml) under N$_2$. The reaction mixture was stirred under reflux for 12 h. The resulting suspension was then sonicated for 10 min. and stirred again under reflux for 8 h. Solvent was removed under vacuum and the white solid formed was dissolved in MeOH (0.5 ml) and Et$_2$O
(20 ml) was added to obtain a white precipitate. The resulting suspension was centrifuged for 20 minutes and the solvent was discharged. The precipitate was washed with Et₂O (2 x 15 ml) and dried under high vacuum to afford the 11c (119 mg, 86% (hydrochloric salt)) as a colorless solid.

Mp: 199-200 ºC; IR (neat): 3005 (s), 2936 (s), 2849 (s), 2661 (s), 1708 (s); ¹H NMR (400 MHz, D₂O): 3.93 (s, 2H); ¹³C NMR (100 MHz, D₂O): 165.9 (s), 39.3 (t); MS (ESI, MeOH): 178 (89, [2M+H]^+), 134 (59), 99 (100), 90 (49, [M+H]^+), 55 (71).

2.2. ESI-FTICR-MS-MS Experiments

All gas-phase experiments described herein were conducted with an Ionspec QFT-7 FT-ICR mass spectrometer (Varian Inc., Lake Forest, CA), equipped with a 7 T superconducting magnet and a Micromass Z-Spray electrospray ionization (ESI) source (Waters Co., Saint-Quentin, France). The samples were introduced into the source as 50 µM solutions of NDI and the corresponding anion in acetonitrile at flow rates of 1-2 µL/min. A constant spray and highest intensities were achieved with a capillary voltage of 3000-3800 V – depending on the used NDI and anion – at a source temperature of 40ºC. The parameters for sample cone (20-45 V) and extractor cone voltage (8-10 V) were optimized for maximum intensities of the desired complexes (see fig. S4 for an example). Multiple scans (20-50) were recorded and averaged for each spectrum in order to improve the signal-to-noise ratio. After accumulation and transfer into the instrument’s FTICR analyzer cell, the ions were detected by a standard excitation and detection sequence. The results of these measurements are summarized in table S1 (see fig. S4 and S5 for example spectra). These relatively simple MS experiments unravel a much higher ability to bind anions for the macrocyclic NDI dimer 8 - also towards weakly coordinating anions - as well as the ability to form dimeric complexes for both receptors (3 and 8).
The formation of dimers was used for gas-phase competition experiments with NDI monomers 3-5 and 20. Since chloride proved to show the highest affinity to 3, this anion was used for the competition experiments. To determine a ranking of binding strengths, equimolar solutions of each pair of NDIs with 1 eq. NEt₄Cl were electrosprayed and the corresponding heterodimer was isolated and fragmented by irradiation with a 25 W IR laser (Fig. 3 and figs. S6 and S7). Although these experiments are not at all trivial to perform because of the rather low parent ion intensities, a clear order of binding strengths to chloride can be extracted from the data: 5 >> 20 > 4 > 3 (Table 1, entries 1-3). A direct comparison of 4 and 5 was not possible, because the intensity of the corresponding heterodimer was too low for the tandem MS experiment. We therefore chose 20 as a "relay" and compared both 4 and 5 separately with 20. Comparison with transport data was possible only for 3-5, because 20 was not reliably accessible for solution studies due to solubility problems. The anion affinity of 20 was high despite comparably low active-site decrowding and π-acidity. This behavior is counter-intuitive given the electron-donating character of the two ethoxy substituents. However, the polarizability of the substituents is a second effect that needs to be taken into account. It may even overcompensate the electron-donating effects.

Different to the more qualitative results from simple day-to-day ESI MS measurements, intensities found in tandem ESI MS relate to true gas phase experiments and can thus be used directly to determine anion affinity sequences quantitativelyS6,S7. These results from tandem ESI MS were thus of highest importance because they secured direct experimental evidence for anion binding to minimalist NDI models, as well as their anion selectivity.

2.3. Charge-transfer complex formation

In general, determination of anion binding in solution proved to be challenging owing to the
overall very low solubility of NDIs preventing an extensive solvent screening in NMR and the presumably very low binding constants, which call for very high concentrations. Solvents capable of solubilizing NDIs sufficiently were either anion coordinating (in case of chloroform, a hydrogen bond donor; strong shifts of the chloroform signal occur in the presence of TBAX) or could be expected to efficiently shield the π-acidic surface (in case of toluene/acetonitrile). Therefore, the anyway very low binding affinity would be further diminished. On the other hand, it was not obvious if significant shifts could be expected theoretically upon anions binding to NDIs. They should be much smaller than with H-bonding anion complexes (ring current effects?). Pertinent CT literature does not report NMR titrations.

ITC was also not feasible because the very low binding constants call for very high concentrations. The resulting very high heat of dilution during addition of tetrabutylammonium salts exceeds any expected heat of binding by far. In fact the instruments detector was saturated in an attempt to determine binding constants with ITC. Reduction of the concentrations to reduce the heat of dilution also reduces the heat of binding and thus does not solve the problem. Ultimately, UV proved to be the only way to investigate anion binding by minimalist NDIs in solution.

Titrations were carried out in mixtures of acetonitrile, freshly distilled dry THF and chloroform. Tetrabutylammonium iodide (TBAI) was recrystallized from 1 mM Na$_2$S$_2$O$_3$ prior to use to remove traces of iodine. Background spectra with tetrabutylammonium halides were recorded before each measurement. For core-unsubstituted NDIs, only the combination of TBAI in acetonitrile afforded significant changes in absorption spectra (see Fig. 3e for TBAI with NDI 3).

The position of the band is in qualitative agreement with the Mulliken correlation reported by Kochi and co-workers$^{S8}$, i.e. the CT band of tetracyanopyrazine ($E_{\text{red}} \approx -0.2$ V) with iodide has been reported around 570 nm. Since core-unsubstituted NDIs have a significantly lower reduction potential ($E_{\text{red}} \approx -0.4$ to $-0.5$ V) the CT band is expected to lie at higher energy, which is in fact the
case ($\lambda_{\text{max}} \approx 470$ nm). In further agreement with the Mulliken correlation, CT bands with bromide or chloride are presumably too far blue-shifted to be observed owing to the overlap with the very strong NDI absorption (note that the absorbance is 9(!) for 300 $\mu$M NDI and $\varepsilon = 30$ mM$^{-1}$cm$^{-1}$).

Dicyano NDI 5 with tetrabutylammonium bromide gave charge transfer band at $\lambda_{\text{max}} \approx 510$ nm (fig. S8). This suggested a reduction potential of around $+0.1$ to $+0.3$ V (note that the CT absorption is very broad and weak), when interpolated with the Mulliken correlation reported by Kochi$^S8$, which is in agreement with a reported reduction potential of $+0.08$ V vs. SCE for dicyano-naphthalenedioclylimide by Wasielewski$^S9$.

As can be seen from Figure 3e, it was not possible to get full titration curves (solubility for TBAI is ca. 1.3 M) and therefore a conventional nonlinear curve fitting was not possible. Therefore, the Rose-Drago method$^{S10}$, which was also applied by Kochi and co-workers, has been tried$^{S8}$. The most general form of the Rose-Drago equation can be seen in (S1), in which the only unknowns are the extinction of the complex $\varepsilon_c$ and the binding constant $K_a$. The observed absorbance $A_{\text{obs}}$ is measured and the extinctions $\varepsilon_g$, $\varepsilon_h$ and concentrations $[G]_0$, $[H]_0$ of the binding partners G and H are determined independently.

$$\frac{1}{K_a} = \frac{A_{\text{obs}} - \varepsilon_h [H]_0 - \varepsilon_g [G]_0}{\varepsilon_c - \varepsilon_h - \varepsilon_g} - \left( [H]_0 + [G]_0 \right) + \frac{\varepsilon_c - \varepsilon_h - \varepsilon_g}{A_{\text{obs}} - \varepsilon_h [H]_0 - \varepsilon_g [G]_0} [H]_0 [G]_0 \quad (S1)$$

The idea of Rose and Drago was to set-up a graph which displays $1/K_a$ for all possible $\varepsilon_c$. If this is done for more than one combination of $[G]_0$ and $[H]_0$ the line graphs should intersect at the true values of $1/K_a$ and $\varepsilon_c$. This is the graphical version of setting up systems of linear equations for all individual $[G]_{0,n}$ and $[H]_{0,n}$ and solving them. For the analytical version, an Excel spreadsheet has been developed, which provides $\varepsilon_c$ and $1/K_a$ for all combinations investigated. The range of values
for the extinction coefficient obtained was in qualitative agreement with that of typical charge transfer bands (500-5000 M⁻¹ cm⁻¹), while $K_a \sim 0.2$-0.4 M⁻¹ was as low as expected for monomeric NDIs and weakly binding anions such as $\Gamma$ and $Br^-$ ($K_a = 1/K_D$, $K_D$ = dissociation constant).

Further evidence for the CT character of the new absorption band of NDI 3 with TBAI was found when THF with 20% ACN was used instead of pure ACN (fig. S9). The less polar solvent presumably destabilized the polar ground state to a larger extent than the less polar excited state, which lead overall to a smaller energy gap and thus a bathochromic shift. This observation was common for polar ground and unpolar excited states\textsuperscript{S11}. The solvent exchange experiments also suggested that a small shoulder, which appears with bromide in ACN or THF could be attributed to the corresponding CT band (fig. S9). Multiple, very strong bands were observed for dicyano NDI 5 with iodide and for core-unsubstituted NDIs with fluoride (fig. S10). In the former case, reduction of dicyano NDI 5 (+0.08 V \textit{vs.} SCE for dicyano-naphthalenedioctylimide\textsuperscript{S9}) by iodide (+0.29 V \textit{vs.} SCE) was perhaps possible\textsuperscript{S12,S13}, while in the latter a case the formation of a Meisenheimer complex, i.e. nucleophilic addition, might be responsible for the appearance of the absorption bands\textsuperscript{S13}.

2.4. Anion transport

2.4.1. Vesicle preparation

\textbf{EYPC-LUVs $\supset$ HPTS\textsuperscript{S1,S2,S14}.} A thin lipid film was prepared by evaporating a solution of 25 mg EYPC in 1 ml MeOH/CHCl₃ (1:1) on a rotary evaporator (40 °C) and then \textit{in vacuo} overnight. After hydration (> 30 min) with 1.0 ml buffer (10 mM Hepes, 100 mM NaCl, 1 mM HPTS, pH 7.0), the resulting suspension was subjected to >5 freeze-thaw cycles (liquid N₂, 40 °C water bath), and
>15 times extruded through a polycarbonate membrane (pore size 100 nm). Extravesicular components were removed by size exclusion chromatography (Sephadex G-50, Sigma-Aldrich) with 10 mM Hepes, 100 mM NaCl, pH 7.0. Final conditions: ~2.5 mM EYPC; inside: 10 mM Hepes, 100 mM NaCl, 1 mM HPTS, pH 7.0; outside: 10 mM Hepes, 100 mM NaCl, pH 7.0.

**EYPC-LUVs ⊆ CF^{S14,S15}.** A thin lipid film was prepared by evaporating a solution of 25 mg EYPC (25 mg) in CHCl₃/MeOH (1:1, 1 ml) on a rotary evaporator (40 °C) and then overnight in vacuo. After hydration (> 30 min) with 1 ml buffer (1 ml; 10 mM Hepes, 10 mM NaCl, 50 mM CF, pH 7.0), the resulting suspension was subjected to >5 freeze-thaw cycles (liquid N₂; 40 °C, water bath), and >15 times extruded through a polycarbonate membrane (pore size 100 nm). Extravesicular components were removed by size exclusion chromatography (Sephadex G-50) with 10 mM Hepes, 100 mM NaCl, pH 7.0. Final conditions: ~2.5 mM EYPC; inside: 10 mM Hepes, 10 mM NaCl, 50 mM CF, pH 7.0; outside: 10 mM Hepes, 100 mM NaCl, pH 7.0.

**EYPC-LUVs ⊆ LG^{S16-S19}.** A thin lipid film was prepared by evaporating a solution of 25 mg EYPC (25 mg) in CHCl₃/MeOH (1:1, 1 ml) on a rotary evaporator (40 °C) and then overnight in vacuo. After hydration (> 30 min) with buffer (1 ml; 1 mM LG, 10 mM Hepes, 100 mM NaNO₃, pH 7.0), the resulting suspension was subjected to >5 freeze-thaw cycles (liquid N₂; 40 °C, water bath), and >15 times extruded through a polycarbonate membrane (pore size 100 nm). Extravesicular components were removed by size exclusion chromatography (Sephadex G-50) with 10 mM Hepes, 100 mM NaNO₃, pH 7.0. Final conditions: ~2.5 mM EYPC; inside: 10 mM Hepes, 100 mM NaNO₃, 1 mM LG, pH 7.0; outside: 10 mM Hepes, 100 mM NaNO₃, pH 7.0.
2.4.2. Determination of transport activity with the HPTS assay

To 1950 µl gently stirred, thermostated buffer (10 mM Hepes, 100 mM NaCl, pH 7.0) in a disposable plastic cuvette, 25 µl EYPC-LUVs ⊆ HPTS were added. The time-dependent change in fluorescence intensity ($\lambda_{em} = 510$ nm) was monitored at two excitation wavelengths simultaneously ($\lambda_{t,450}: \lambda_{exc} = 450$ nm, $I_{t,405}: \lambda_{exc} = 405$ nm) during the addition of base (20 µl 0.5 M NaOH) at $t = 50$ s, NDI (see table S2 for details) at $t = 100$ s, and 20 µl 100 µM gramicidin A in DMSO at $t = 300$ s. Time courses of fluorescence intensity $I_t$ were obtained by first, ratiometric analysis ($R = I_{t,450} / I_{t,405}$) and second, normalization according to equation S2,

$$I_t = \frac{(R_t - R_0)}{(R_\infty - R_0)}$$  \hspace{1cm} (S2),

where $R_0 = R_t$ before addition of transporter and $R_\infty = R_t$ after addition of gramicidin A (fig. S11). $I_t$ at 300 s just before addition of gramicidin A was defined as transmembrane activity $Y$, and analyzed with the Hill equation S3 to give effective concentration $EC_{50}$ and the Hill coefficient $n$,

$$Y = Y_\infty + \frac{(Y_0 - Y_\infty)}{(1 + c / EC_{50})^n}$$  \hspace{1cm} (S3),

where $Y_0$ is $Y$ in absence of NDI, $Y_\infty$ is $Y$ with excess NDI, and $c$ is the NDI concentration.

Complete results for all NDIs are shown in table S3.

Comments beyond the ones made in the manuscript: In general, Hill coefficients $n > 1$ demonstrate the presence of unstable supramolecules, $n \leq 1$ demonstrate that of active monomers or stable supramolecules\(^{14}\). Saturation behavior with $n < 1$ suggested precipitation of the hydrophobic NDIs 5 and 8 at high concentrations after addition of highly concentrated stock solutions (table S2).
before reaching the membrane. $n > 1$ suggested that charged NDIs 2, 10, 11, 22 and 23 act cooperatively as unstable supramolecules. $n \sim 1$ suggested that neutral NDIs 3, 4, 6 and 9 act as supramolecules that are stable but soluble enough to avoid precipitation at high concentration, or as monomers. The change from $n \sim 1$ to $n > 1$ with the addition of charges demonstrated that charge repulsion gives destabilized active suprastructures. In the case of extreme solubility / delivery problems (18-20) or inactivity even at high concentrations (23, 6 in CF assay), dose response curves could not be completed and $EC_{50}$ and n not determined (n.a. in table S3). Inactivity of trianionic 23 but not singly charged 10, 11 and 18-20 suggested that more than one charge per NDI is needed to either fully destabilize the active superstructure or inhibit the (auto-mediated) transmembrane translocation needed to form the dimeric bundles (Fig. 1d).

**2.4.3. Determination of transport activity with the CF assay**

EYPC-LUVs ⊏ CF (25 μl, inside: 10 mM Hepes, 10 mM NaCl, 50 mM CF, pH 7.0) were added to 1975 μl gently stirred, thermostated buffer (10 mM Hepes, 100 mM NaCl or NaClO 4, pH 7.0) in a disposable plastic cuvette. The time-dependent changes in fluorescence intensity $I_t$ ($\lambda_{\text{exc}} = 492$ nm, $\lambda_{\text{em}} = 517$ nm) were monitored during the addition of NDIs (see table S2 for stock solutions) at $t = 0$ min, and addition of triton X-100 (40 μl 1.2 % aq) at the end of every experiment. Time courses of $I_t$ were normalized to fractional intensities $I_f$ using equation S4,

$$I_f = (I_t - I_0) / (I_\infty - I_0)$$  \hspace{1cm} (S4),

where $I_0 = I_t$ before pore addition and $I_\infty = I_t$ after lysis (fig. S12). $I_t$ at 200 s after the start of the experiment just before lysis was defined as transmembrane activity $Y$, and Hill analysis (eq S3)
was applied to determine EC_{50} and \( n \) (table S3).

Comments: Inactivation of active NDIs 4, 5, and 9 by external chloride to perchlorate exchange suggested that activity in this case originates from CF/anion antiport rather than from leakage, although other explanations are conceivable (inactivation of delivery/active structure by perchlorate, fig. S12). \( EC_{50} \) (HPTS) < \( EC_{50} \) (CF) for 2 and 6 indicated that the organization of the active suprastructures is high enough to exclude non-specific CF transport without losses in specific anion transport. The appearance of nitrate selectivity with \( EC_{50} \) (HPTS) < \( EC_{50} \) (CF) supports the supramolecular origin of nitrate recognition.

2.4.4. Determination of transport activity with the lucigenin assay

EYPC-LUVs \( \supset \) LG^{S16-S20} (25 \( \mu \)l, inside: 1 mM LG, 10 mM Hepes, 100 mM NaNO\_3, pH 7.0) were added to 1975 \( \mu \)l gently stirred, thermostated buffer (10 mM Hepes, 100 mM NaCl, pH 7.0) in a disposable plastic cuvette. The time-dependent changes in fluorescence intensity \( I_t \) (\( \lambda_{ex} = 372 \) nm, \( \lambda_{em} = 504 \) nm) were monitored during the addition of NDIs (see table S2 for stock solutions) at \( t = 50 \) s and triton X-100 (40 \( \mu \)l 1.2 \% aq) at the end of every experiment. Time courses of \( I_t \) were normalized following refs. S12-S15. \( I_t / I_0 \), were calibrated for \( I_0 = I_t \) before pore addition (fig. S13, left). Addition of triton-X 100 and calibration to the final fluorescence intensity \( I_\infty = I_t \) after lysis introduces undesirable uncertainties as discussed previously^{S13}. \( EC_{50} \) and \( n \) were determined by Hill analysis of dose response curves as described above (fig. S13, right).

Comments: The \( EC_{50} = 0.75 \) \( \mu \)M obtained for NDI 6 was in excellent agreement with results from the CF assay, and demonstrated, according to pertinent reports in the literature^{S16-S19} and combined with inactivity in the CF assay, that NDI 6 mediates the influx of chloride into intact vesicles (table S3).
2.4.5. Determination of ion selectivity with the HPTS assay

EYPC-LUVs ⊇ HPTS (25 µl) prepared as described above were added to 1950 µl gently stirred, thermostated buffer (10 mM Hepes, 100 mM M⁺Cl⁻ (M⁺ = Li⁺, Na⁺, K⁺, Rb⁺, Cs⁺) or 100 mM Na⁺X⁻ (X⁻ = F⁻, OAc⁻, Cl⁻, NO₃⁻, Br⁻, I⁻, SCN⁻, ClO₄⁻, SO₄²⁻ (66 mM), pH 7.0) in a disposable plastic cuvette. The time-dependent change in fluorescence intensity was monitored and analyzed as described above to obtain the fractional transmembrane activity Y dependent on the externally added cation or anion (figs. S14-S24).

Data analysis was performed as described above and R₀ was defined as R just before addition of base. Fractional activities were plotted as a function of anion dehydration energies (figs. S14-S24). Sulfate is very negative, e.g. −1059 kJ/mol⁸²⁰ (note the break in the x-axis) and the absolute value seems quite correct (e.g. −1145 kJ/mol in another report)⁸²¹. Nitrate has been reported with two different positions in the series: (Br > NO₃ > I)⁸¹⁶ and (F > NO₃ > Cl)⁸²¹, whereby the former with −344 kJ/mol as a rough estimate fits nicely to our data.

Control experiments were carried out in which the addition of transporter and base has been reversed, i.e. NDI was added at t = 50 s, and base (20 µl 0.5 M NaOH) at t = 100 s (figs. S14-S24, ●; normal addition as in fig. S11: ○). It was apparent that the most active transporters (4, 5) respond much less significantly towards reversed addition, which suggested that in these cases membrane partitioning and X⁻/Cl⁻ exchange are complete before X⁻/OH⁻ exchange occurs. Overall, the NDIs were consistently more anion than cation selective (see manuscript text). Regarding cation selectivity, high activities were observed with lithium for the trimethylaryl derivatives 3 and 5, which may stem from binding to the π-basic mesityl substituents (similar for cyclophane 8).
addition, favorable interactions like C=O···M⁺ ion-dipole interactions may contribute to the observed increase in activity with Li⁺.

### 2.4.6. AMFE measurements with the HPTS assay

25 μl EYPC-LUVs ⊆ HPTS prepared as described above were added to a mixtures of a μl buffer A and b μl buffer B in a disposable plastic cuvette to give the indicated mole fractions x (figs. S25-S28). The time-dependent change in fluorescence intensity was monitored and analyzed as described above (final concentration as described for ion selectivity measurements) to obtain the fractional activity $Y$.

Two combinations of anions were selected for 4 and 5: ClO₄⁻/Cl⁻ and I⁻/Cl⁻. The more challenging combination of iodide and chloride showed a significant AMFE for NDI 5 only (fig. S26). In addition, the combination of perchlorate and chloride shows also a most pronounced AMFE for NDI 5, which further corroborates the importance of anion-π interactions.

### 2.5. Computational models

The geometries of the NDI-anion complexes were optimized at the PBE1PBE/6-311G** level of theory⁵²,⁵³ using the DFT methods of the Gaussian 03 program⁵⁴. Full geometry optimizations without any restriction led to series of $C_1$ minima listed in table S4. In addition, the NDI-anion complexes 3, 4 and 7 were optimized with the $C_s$-symmetry constraint. The interaction energies of the $C_1$ and $C_s$ complexes were computed with diffuse functions⁵⁵,⁵⁶ at the PBE1PBE/6-311++G**//PBE1PBE/6-311G** level of theory and corrected for the basis set superposition error (BSSE) using the counterpoise method⁵⁷. Separately, we undertook a comparative study for a
limited test-set of anion-NDI complexes to confirm that in our specific case the inclusion of diffuse functions would not modify the optimized geometries in a significant manner. To verify that the C\textsubscript{1}-symmetry structures correspond to minima, vibrational analysis was also performed with the PBE1PBE/6-311G** method. Table S4 lists, for each C\textsubscript{1} complex, the number of imaginary frequencies (NIMEG) issued from the frequency calculations, which for the minimum has to be null.

The relative energies $E_{\text{rel}}$ listed in the Table S4, indicate, for chlorides and bromides, that the C\textsubscript{1} complexes are systematically lower in energy when compared to conformers, which are restricted to the C\textsubscript{s}-symmetry point group. However, for the nitrate complexes the situation is quite different, as the structures optimized without any geometry restriction adopt quasi C\textsubscript{s} symmetric structure (table S4, entry 19 - 27). Consequently only a minute energy differences between C\textsubscript{s} and C\textsubscript{1} point group conformers are obtained together with very small variations in geometry parameters.

The computed BSSE-corrected binding energies of chloride anionic complexes decrease on one hand with the increasing steric hindrances, while on the other hand they increase with the higher $\pi$-acidity of the NDI surface (table S4). Not surprisingly, the affinity sequence for bromide 1:1 complexes is, like chloride, decreasing according to similar rules. Indeed, a poorer fit of the larger bromide anion into the confined binding site on the pyridinedione surface, results in binding energies which are systematically inferior to chloride by some 12 kJ mol\textsuperscript{-1}. The binding energies for nitrates compared to chlorides and bromides are still lower. Such weakening is also found for complexes 6 and 7, where the $\pi,\pi$-interactions between the flat nitrate anion and NDI are maximized. This suggests that $\pi,\pi$-interactions contribute to anion binding, not only in nitrates, but also in halides. As expected from experimental results, the anion binding by cyclophane 8 is stronger than in 3 and 4, but nevertheless remain inferior to cyano substituted 1:1 complexes.

In case of N-aryl substituted NDI-anion complexes 3 - 8, the position of the anion with respect
to the NDI core is controlled in a more or less subtle way. Due to the steric hindrance from the 
*ortho* substituent of the N-aryl group, the anion is displaced from the optimal pyridinedione binding 
site with the concomitant weakening of anion-π interactions. Thus the distance between the NDI 
plane and anion is increased (R₂, table S4). While the steric interference has a major negative 
impact on the binding and modifies the conformation of complexes, it is however partially 
compensated by stabilizing C–H • • •X⁻ interactions, witnessed by certain number of short 
contacts between anion and atoms in *ortho* position (dₓ, table S4). In addition to the subtle balance 
between these interactions, both the longitudinal (along N,N axes), as well as the transversal 
displacements of the anion, respectively d_long and d_transv in table S4, are also correlated with the 
orientation of aryl groups. In most cases the aryls are not perpendicular with respect to the NDI 
planes in C₁-symmetry complexes (figs. S29 and S30). Indeed, the dihedral angle controlling the 
orientation of the aryl groups is variable, depending on parameters such as the position of anion, its 
nature, as well as the type of the *ortho* substituent. Variations of anion location with respect to the 
π-surface of the NDI core as well as other conformational changes can also be appreciated in the 
optimized structures of chloride (fig. S29) and nitrate (fig. S30) anionic complexes.
Fig. S1. a) HBTU, TEA, DCM, 0 °C to rt, 3 h, 79% (ref. S1: 88%); b) piperidine, DMF, rt, 3 h, 95% (ref. S1: 85%); c) HBTU, TEA, DCM, 0 °C to rt, 3 h, 78% (ref. S1: 84%); d) piperidine, DMF, rt, 20 h, 68% (ref. S1: 70%); e) THF, rt, 4 h, 97%; f) H₂O, 1 M KOH, 1 M H₃PO₄, pH 6.4, reflux, 15 h, 76% (ref. S1: 88%); g) DMAc, 135 °C, 15 h, 90% (ref. S1: 90%); h) DMAc, 5 Å MS, 135 °C, 16 h, 56% (ref. S1: 57%); i) TFA, pentamethylbenzene, thioanisole, HBr in AcOH, rt, 1 h, quant. (ref. S1: 89%); j) 1.1 eq. Fmoc-Gly-OH, 1.1 eq. Boc-Gly-OH, HBTU, TEA, DMF, rt, 1 h, 85% (ref. S1: 60%); k) TFA/DCM 1:1, rt, 1 h, 94% (ref. S1: 95%); l) HATU, TEA, DMF, rt, 4 h, 30% (36% conversion); m) TFA, rt, 75 min, quant.
Fig. S2. a) DMAc, 135 °C, 16 h, 35%; b) DMAc, 135 °C, 20 h, 17%; c) dibromocyanuric acid, used as mixture of bromination products S3; d) AcOH, 80 °C, 12 h, >28%; e) CuCN, NMP, 100 °C, 5 h, 63%; f) NH₄OAc, AcOH, reflux, 1 h, 77%; g) CuCN, NMP, 100 °C; h) Cu(OAc)₂, TEA, DMAc, 4.2% (2 steps); i) AcOH, 140 °C, 20 min, MW, 14%; j) as in i; k) EtI, EtOH, K₂CO₃, 24% from 3a S3; l) 1. KOH, i-PrOH, 85 °C, 18 h, 1. KOH, i-PrOH, 85 °C, 18 h, 2. 13, AcOH, 120 °C, 24 h, 92% (2 steps).
Fig. S3. a) 1. 4a, EDC, DMF, 2h, 2. NH₄OAc, AcOH, reflux, 1 h, 36% (9a, 5% 9b); b) Cu(OAc)₂, TEA, DMAc, 55 °C, 22 h, 86%; c) 1. HCl, DMSO, 50 °C, 2 h; 2. NaOH, quant; d) NH₂-NHBoc, HBTU, DIEA, DCM, rt, 90%; e) 1 M HCl (Et₂O), DCM, reflux, 86%; f) 1. HCl, DMSO, 50 °C, 2 h; 2. NaOH, quant.
Fig. S4. ESI-MS of an equimolar solution of 3 and NEt₄Cl.

Fig. S5. ESI-MS of an equimolar solution of 8 and AgNO₃. The silver salt is advantageous here, because it helps to suppress signals that are caused by binding of background chloride.
Fig. S6. Laser-induced fragmentation of mass selected $4^{+}20^{+}\text{Cl}^{-}$ (a, b) and $5^{+}20^{+}\text{Cl}^{-}$ (c, d). (a) The heterodimer $4^{+}20^{+}\text{Cl}^{-}$ after mass-selection. (b) IRMPD experiment for $4^{+}20^{+}\text{Cl}^{-}$. The somewhat higher intensity of $20^{+}\text{Cl}^{-}$ indicates $20^{+}$ to bind slightly better to the chloride ion than $4^{+}$. (c) The heterodimer $5^{+}20^{+}\text{Cl}^{-}$ after mass-selection. (d) IRMPD experiment for $5^{+}20^{+}\text{Cl}^{-}$. No signal for $20^{+}\text{Cl}^{-}$ is observed, indicating $5^{+}$ to bind significantly better to the chloride ion than $20^{+}$ and thus also $4^{+}$. Interestingly, the $5^{-}$ anion-radical is also formed. This points to the ability of $5^{+}$ to even reduce the chloride ion in the gas phase.
**Fig. S7.** Laser-induced fragmentation of mass selected $5+6+\text{Cl}^-$. (a) The heterodimer after mass-selection. (b) IRMPD experiment with 250 ms laser pulse. (c) IRMPD experiment with 500 ms laser pulse. Stronger signals for $6+\text{Cl}^-$ than for $5+\text{Cl}^-$ are observed, indicating 6 to bind better to the chloride ion than 5.
**Fig. S8.** Changes in UV-VIS absorption of a solution of 150 μM NDI 5 in ACN/CHCl₃ 9:1 with varying amounts of TBABr (0-1 M).  

**Fig. S9.** Solvent dependence of the charge transfer bands: Absorption spectra of NDI 3 (300 μM) and TBAI (0.4 M, a, b) and TBABr (0.4 M, c, d) in THF / acetonitrile 82:18 (a), 92:8 (c) and acetonitrile (b, d).
Fig. S10. Absorption spectrum of 150 μM NDI 5 in ACN/CHCl$_3$ 9:1 with 0.5 M TBAI.

Fig. S11. Activity of NDI 5 in the HPTS assay. Ratiometric changes in fluorescence intensity of HPTS ($\lambda_{ex,1} = 405$ nm, $\lambda_{ex,2} = 450$ nm, $\lambda_{em} = 510$ nm) are shown during the addition of base (5 mM NaOH) at 50 s, 5 (0.03 to 90 μM final concentration) at 100 s and 1 μM gramicidin A at 300 s to EYPC-LUVs $\cap$ HPTS (1 mM HPTS, 10 mM Hepes, 100 mM NaCl, pH 7.0).
Fig. S12. Activity of NDIs 4 (b, e), 5 (a, d) and 9 (c, f) in the CF assay with external chloride (a-c) and perchlorate (d-f). Changes in fluorescence intensity of CF ($\lambda_{ex} = 492$ nm, $\lambda_{em} = 517$ nm) are shown during the addition of NDIs (20 μM final concentration) at 50 s and excess triton X-100 (40 μl 1.2% aq) at 250 s to EYPC-LUVs ⊆ CF (inside: 10 mM Hepes, 10 mM NaCl, 50 mM CF, pH 7.0; outside: 10 mM Hepes, NaCl (a-c) or NaClO₄ (d-f), pH 7.0).

Fig. S13. Activity of NDI 6 in the LG assay. Changes in fluorescence intensity of LG ($\lambda_{ex} = 372$ nm, $\lambda_{em} = 504$ nm) are shown after the addition of NDI 6 (20 μl in DMSO) at 50 s and excess triton X-100 (40 μl 1.2% aq) at 250 s to EYPC-LUVs ⊆ LG (1 mM lucigenin, 10 mM Hepes, 100 mM NaNO₃, pH 7.0, in 10 mM Hepes, 100 mM NaCl, pH 7.0).
**Fig. S14.** Fractional activity of O-NDI rod 2 (constant concentration, adjusted to $Y \sim 0.6$ with NaCl) in the HPTS assay in response to external cation exchange (left, MCl) and to external anion exchange (right, NaX), with NDI addition after base pulse (fig. S11).

**Fig. S15.** Fractional activity of NDI 3 (constant concentration at maximal $Y \sim 0.3$ with NaCl) in the HPTS assay in response to external cation exchange (left, MCl) and to external anion exchange (right, NaX), with NDI addition after (O, fig. S11) and before (●) base pulse.
**Fig. S16.** Fractional activity of NDI 4 (constant concentration adjusted to $Y \sim 0.5$ with NaCl) in the HPTS assay in response to external cation exchange (left, MCl) and to external anion exchange (right, NaX), with NDI addition after (○, fig. S11) and before (●) base pulse.

**Fig. S17.** Fractional activity of NDI 5 (constant concentration adjusted to $Y \sim 0.5$ with NaCl) in the HPTS assay in response to external cation exchange (left, MCl) and to external anion exchange (right, NaX), with NDI addition after (○, fig. S11) and before (●) base pulse.
**Fig. S18.** Fractional activity of NDI 6 (constant concentration adjusted to $Y \sim 0.5$ with NaCl) in the HPTS assay in response to external cation exchange (left, MCl) and to external anion exchange (right, NaX), with NDI addition after base pulse (fig. S11).

**Fig. S19.** Fractional activity of cyclic O-NDI 8 (constant concentration adjusted to the maximal accessible $Y \sim 0.2$ with NaCl) in the HPTS assay in response to external cation exchange (left, MCl) and to external anion exchange (right, NaX), with NDI addition after (○, fig. S11) and before (●) base pulse.
**Fig. S20.** Fractional activity of NDI 9 (constant concentration adjusted to $Y \sim 0.5$ with NaCl) in the HPTS assay in response to external cation exchange (left, MCl) and to external anion exchange (right, NaX), with NDI addition after base pulse (fig. S11).

**Fig. S21.** Fractional activity of NDI 10 (constant concentration adjusted to $Y \sim 0.5$ with NaCl) in the HPTS assay in response to external cation exchange (left, MCl) and to external anion exchange (right, NaX), with NDI addition after base pulse (fig. S11).
**Fig. S22.** Fractional activity of NDI 11 (constant concentration adjusted to \( Y \sim 0.5 \) with NaCl) in the HPTS assay in response to external cation exchange (left, MCl) and to external anion exchange (right, NaX), with NDI addition after base pulse (fig. S11).

**Fig. S23.** Fractional activity of NDI 21 (constant concentration adjusted to \( Y \sim 0.4 \) with NaCl) in the HPTS assay in response to external cation exchange (left, MCl) and to external anion exchange (right, NaX), with NDI addition after base pulse (fig. S11).
**Fig. S24.** Fractional activity of NDI 22 (constant concentration adjusted to \( Y \sim 0.3 \) with NaCl) in the HPTS assay in response to external cation exchange (left, MCl) and to external anion exchange (right, NaX), with NDI addition after base pulse (fig. S11).

**Fig. S25.** AMFE of NDI 4. Fractional activity of NDI 4 (constant concentration adjusted to \( Y \sim 0.4 \) with NaCl) in the HPTS assay in the presence of mixtures of external NaCl and NaI (left) and of mixtures of external NaCl and NaClO\(_4\) (right), with NDI addition after base pulse (fig. S11).
**Fig. S26.** AMFE of NDI 5. Fractional activity of NDI 5 (constant concentration adjusted to $Y \sim 0.4$ with NaCl) in the HPTS assay in the presence of mixtures of external NaCl and NaI (left) and of mixtures of external NaCl and NaClO$_4$ (right), with NDI addition after base pulse (fig. S11).

**Fig. S27.** AMFE of NDI 9. Fractional activity of NDI 9 (constant concentration adjusted to $Y \sim 0.4$ with NaCl) in the HPTS assay in the presence of mixtures of external NaCl and NaClO$_4$, with NDI addition after base pulse (fig. S11).
Fig. S28. AMFE of NDI 10. Fractional activity of NDI 10 (constant concentration adjusted to $Y \sim 0.4$ with NaCl) in the HPTS assay in the presence of mixtures of external NaCl and NaClO$_4$, with NDI addition after base pulse (fig. S11).
**Fig. S29.** PBE1PBE/6-311G**\(^*\)\)** optimized structures (\(C_1\)-symmetry) of anionic complexes 3–8 between NDI and chloride shown in top view of ball and stick (atom color coding, anion green) representations together with the longitudinal displacement of anions (in Å).
**Fig. S30.** PBE1PBE/6-311G** optimized structures (symmetry point group in parenthesis) of anionic complexes 3–8 between NDI and nitrate shown in top view of ball and stick (atom color coding) representations together with the longitudinal displacement of anions (in Å).
4. Supplementary tables

**Table S1.** Anion binding to naphthalene diimide systems (X = no complex observed).

<table>
<thead>
<tr>
<th>No.</th>
<th>Anion</th>
<th>NDI 3</th>
<th>NDI 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cl(^-)</td>
<td>3+Cl(^-) &amp; 3₂+Cl(^-)</td>
<td>8+Cl(^-) &amp; 8₂+Cl(^-)</td>
</tr>
<tr>
<td>2</td>
<td>Br(^-)</td>
<td>3+Br(^-) &amp; 3₂+Br(^-) &amp; 3+Cl(^-) &amp; 3₂+Cl(^-)</td>
<td>8+Br(^-) &amp; 8₂+Br(^-)</td>
</tr>
<tr>
<td>3</td>
<td>I(^-)</td>
<td>X</td>
<td>8+I(^-)</td>
</tr>
<tr>
<td>4</td>
<td>OTf(^-)</td>
<td>X</td>
<td>8+OTf(^-)</td>
</tr>
<tr>
<td>5</td>
<td>H(_2)PO(_4)(^-)</td>
<td>X</td>
<td>8+H(_2)PO(_4)(^-)</td>
</tr>
<tr>
<td>6</td>
<td>BF(_4)(^-)</td>
<td>X</td>
<td>8+BF(_4)(^-) (very weak)</td>
</tr>
<tr>
<td>7</td>
<td>BPh(_4)(^-)</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>8</td>
<td>PF(_6)(^-)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>9</td>
<td>CH(_3)COO(^-)</td>
<td>X</td>
<td>8+CH(_3)COO(^-) (very weak)</td>
</tr>
<tr>
<td>10</td>
<td>NO(_3)(^-)</td>
<td>3+NO(_3)(^-)</td>
<td>8+NO(_3)(^-) &amp; 8₂+NO(_3)(^-)</td>
</tr>
<tr>
<td>11</td>
<td>MnO(_4)(^-)</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>12</td>
<td>ClO(_4)(^-)</td>
<td>-</td>
<td>8+ClO(_4)(^-)</td>
</tr>
<tr>
<td>13</td>
<td>SO(_4)(^2-)</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>14</td>
<td>Cl(^-) &amp; I(^-)</td>
<td>-</td>
<td>8+Cl(^-) &amp; 8+I(^-) (~ 10:1) (+8₂+Cl(^-))</td>
</tr>
</tbody>
</table>
Table S2. Organic solvents used for membrane delivery.

<table>
<thead>
<tr>
<th>NDI</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DMSO</td>
<td>DMSO</td>
<td>DMF</td>
<td>DMF</td>
<td>DMSO</td>
<td>DMAc</td>
<td>DMSO</td>
<td>DMSO</td>
<td>DMSO</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>20</td>
<td>60</td>
<td>20</td>
<td>20</td>
<td>60</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>none</td>
<td>none</td>
<td>stock solutions stored at 4 °C in the dark</td>
<td>stock solutions stored at 4 °C in the dark</td>
<td>none</td>
<td>stock solution was kept above 15 °C</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For high concentrations the derivatization solution (containing ca. 3% 0.5 M NaCl) was injected (cf. 2.1.4). Dilutions for the lower concentrations were made with neat DMSO.
Table S3. Activity of NDI transporters in HPTS and CF assay.<sup>a</sup>

<table>
<thead>
<tr>
<th>NDI</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (HPTS) (µM)</th>
<th>n (HPTS)</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (CF) (µM)</th>
<th>n (CF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22 ± 2</td>
<td>1.5 ± 0.1</td>
<td>95 ± 8</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>2</td>
<td>150 ± 20</td>
<td>1.0 ± 0.1</td>
<td>&gt;100</td>
<td>n.a.</td>
</tr>
<tr>
<td>3</td>
<td>27 ± 1</td>
<td>1.2 ± 0.1</td>
<td>17 ± 2</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>4</td>
<td>37 ± 2</td>
<td>0.72 ± 0.04</td>
<td>10 ± 1</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>5</td>
<td>0.33 ± 0.03</td>
<td>1.0 ± 0.1</td>
<td>&gt;100</td>
<td>n.a.</td>
</tr>
<tr>
<td>6</td>
<td>110 ± 20</td>
<td>0.49 ± 0.03</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>7</td>
<td>32 ± 1</td>
<td>1.3 ± 0.1</td>
<td>8.0 ± 0.7</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>8</td>
<td>7.8 ± 0.7</td>
<td>2.2 ± 0.3</td>
<td>4.5 ± 0.1</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>9</td>
<td>8.7 ± 1.4</td>
<td>1.2 ± 0.1</td>
<td>8.2 ± 0.2</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>10</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>11</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>12</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>13</td>
<td>6.1 ± 0.3</td>
<td>1.8 ± 0.2</td>
<td>7.0 ± 0.3</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>14</td>
<td>6.6 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td>2.1 ± 0.1</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td>15</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

<sup>a</sup>For original data, see figs. S11 and S12, for structures, see figs. S1-S3. n.a.: Not applicable; data not determinable, usually because precipitation after addition of more concentrated stock solutions caused precipitation (3, 6, 18-20).
Table S4. Energy and geometry data of NDI anion complexes computed at PBE1PBE/6-311++G**//PBE1PBE/6-311G** level of theory.

<table>
<thead>
<tr>
<th>Entry</th>
<th>NDI</th>
<th>Anion</th>
<th>Symmetry</th>
<th>$E_{\text{rel.}}$</th>
<th>$E_{\text{int.}}$</th>
<th>$R_e$</th>
<th>$d_x$</th>
<th>$d_{\text{long}}$</th>
<th>$d_{\text{transv.}}$</th>
<th>NIMEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>Cl</td>
<td>C$_s$</td>
<td>0.0</td>
<td>-76.2</td>
<td>2.98</td>
<td>3.50</td>
<td>1.71</td>
<td>0.00</td>
<td>N.A.</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>Cl</td>
<td>C$_1$</td>
<td>-3.8</td>
<td>-83.3</td>
<td>2.86</td>
<td>3.60</td>
<td>1.49</td>
<td>0.91</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>Cl</td>
<td>C$_s$</td>
<td>-87.5</td>
<td>2.96</td>
<td>2.62</td>
<td>1.21</td>
<td>0.00</td>
<td>N.A.</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Cl</td>
<td>C$_1$</td>
<td>-0.6</td>
<td>-92.1</td>
<td>2.85</td>
<td>2.65</td>
<td>1.12</td>
<td>0.93</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>Cl</td>
<td>C$_1$</td>
<td>-136.4</td>
<td>2.70</td>
<td>3.68</td>
<td>1.62</td>
<td>0.91</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>Cl</td>
<td>C$_1$</td>
<td>-142.7</td>
<td>2.63</td>
<td>2.84</td>
<td>1.36</td>
<td>0.94</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>Cl</td>
<td>C$_s$</td>
<td>-169.9</td>
<td>2.71</td>
<td>2.90</td>
<td>1.50</td>
<td>1.04</td>
<td>0</td>
<td>N.A.</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>Cl</td>
<td>C$_1$</td>
<td>-188.8</td>
<td>2.50</td>
<td>3.00</td>
<td>1.52</td>
<td>1.04</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>Cl</td>
<td>C$_1$</td>
<td>-121.0</td>
<td>3.01</td>
<td>2.48</td>
<td>1.71</td>
<td>0.90</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>Br</td>
<td>C$_s$</td>
<td>0.0</td>
<td>-65.3</td>
<td>3.19</td>
<td>3.66</td>
<td>1.90</td>
<td>0.00</td>
<td>N.A.</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>Br</td>
<td>C$_1$</td>
<td>-2.0</td>
<td>-72.0</td>
<td>3.06</td>
<td>3.75</td>
<td>1.50</td>
<td>0.93</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>Br</td>
<td>C$_s$</td>
<td>0.0</td>
<td>-76.2</td>
<td>3.14</td>
<td>2.77</td>
<td>1.27</td>
<td>0.00</td>
<td>N.A.</td>
</tr>
<tr>
<td>13</td>
<td>4</td>
<td>Br</td>
<td>C$_1$</td>
<td>-0.6</td>
<td>-79.9</td>
<td>3.05</td>
<td>2.79</td>
<td>1.18</td>
<td>0.88</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>5</td>
<td>Br</td>
<td>C$_1$</td>
<td>-123.1</td>
<td>2.88</td>
<td>3.82</td>
<td>1.70</td>
<td>0.88</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>Br</td>
<td>C$_1$</td>
<td>-129.8</td>
<td>2.80</td>
<td>2.96</td>
<td>1.40</td>
<td>0.89</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>7</td>
<td>Br</td>
<td>C$_s$</td>
<td>0.0</td>
<td>-156.5</td>
<td>2.89</td>
<td>3.01</td>
<td>1.53</td>
<td>0.00</td>
<td>N.A.</td>
</tr>
<tr>
<td>17</td>
<td>7</td>
<td>Br</td>
<td>C$_1$</td>
<td>-6.0</td>
<td>-173.7</td>
<td>2.69</td>
<td>3.08</td>
<td>1.54</td>
<td>1.01</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>8</td>
<td>Br</td>
<td>C$_1$</td>
<td>-104.6</td>
<td>3.18</td>
<td>2.64</td>
<td>1.75</td>
<td>1.07</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>3</td>
<td>NO$_3$</td>
<td>C$_s$</td>
<td>0.0</td>
<td>-69.5</td>
<td>2.97</td>
<td>4.39</td>
<td>3.51</td>
<td>0.00</td>
<td>N.A.</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td>NO$_3$</td>
<td>C$_1$</td>
<td>+0.4</td>
<td>-69.9</td>
<td>2.97</td>
<td>4.41</td>
<td>3.43</td>
<td>0.02</td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>4</td>
<td>NO$_3$</td>
<td>C$_s$</td>
<td>0.0</td>
<td>-69.1</td>
<td>2.87</td>
<td>2.91</td>
<td>1.77</td>
<td>0.00</td>
<td>N.A.</td>
</tr>
<tr>
<td>22</td>
<td>4</td>
<td>NO$_3$</td>
<td>C$_1$</td>
<td>+0.3</td>
<td>-69.5</td>
<td>2.87</td>
<td>2.90</td>
<td>1.80</td>
<td>0.04</td>
<td>0</td>
</tr>
<tr>
<td>23</td>
<td>5</td>
<td>NO$_3$</td>
<td>C$_1$</td>
<td>-110.9</td>
<td>2.86</td>
<td>4.43</td>
<td>3.39</td>
<td>0.02</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>6</td>
<td>NO$_3$</td>
<td>C$_1$</td>
<td>-110.1</td>
<td>2.79</td>
<td>3.01</td>
<td>1.93</td>
<td>0.12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>7</td>
<td>NO$_3$</td>
<td>C$_s$</td>
<td>0.0</td>
<td>-144.0</td>
<td>2.74</td>
<td>3.07</td>
<td>2.09</td>
<td>0.00</td>
<td>N.A.</td>
</tr>
<tr>
<td>26</td>
<td>7</td>
<td>NO$_3$</td>
<td>C$_1$</td>
<td>+0.5</td>
<td>-143.6</td>
<td>2.74</td>
<td>3.09</td>
<td>2.08</td>
<td>0.06</td>
<td>0</td>
</tr>
<tr>
<td>27</td>
<td>8</td>
<td>NO$_3$</td>
<td>C$_1$</td>
<td>-95.4</td>
<td>2.93</td>
<td>3.31</td>
<td>2.47</td>
<td>1.45</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$Relative energy of the C$_1$-symmetry complex compare to the associate C$_s$ complex in kJ/mol.  
$^b$Interaction energy in kJ/mol with BSSE correction for NDI anion complexes computed with the PBE1PBE/6-311++G** method on PBE1PBE/6-311G** optimized geometries.  
$^c$Equilibrium distance between anion and NDI plane in Å.  
$^d$Distance between anion (nitrogen atom for nitrate) and carbon in mesityl substituted NDI or hydrogen in phenyl substituted NDI in Å. Value in parenthesis
refer to the distance between anion and hydrogen in mesityl substituted NDI. ℓLongitudinal displacement of the anion relative to the nitrogen atom of the imide function in Å. ℓTransversal displacement of the anion relative to the center of the NDI in Å. ℓNumber of imaginary eigenvalue calculated at the PBE1PBE/6-311G** level.
5. Supplementary references


