Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Chloride Transport Activities of *trans*- and *cis*- Amide-Linked Bisureas

Eun Bit Park and Kyu-Sung Jeong*

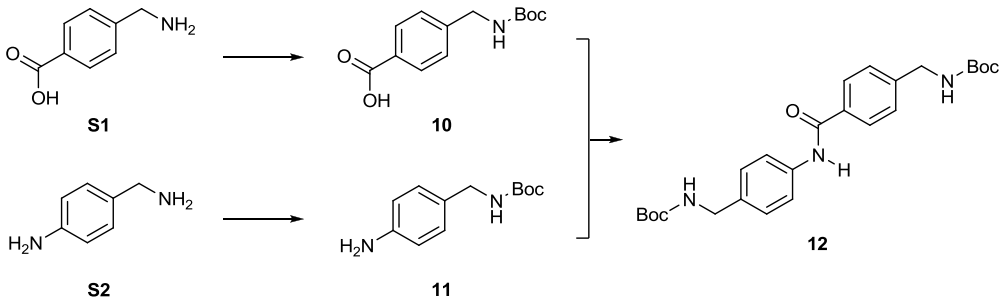
Department of Chemistry, Yonsei University, Seoul, 120-749, Korea

E-mail: ksjeong@yonsei.ac.kr; Fax: +82-2-364-7050; Tel: +82-2-2123-2643

Contents

1. Syntheses and characterization

General: All chemicals were purchased from commercial suppliers and used without further purification unless otherwise specified. Air sensitive reactions were carried out under nitrogen (N_2). Triethylamine (Et_3N) and tetrahydrofuran (THF) were purchased as anhydrous grade. Dichloromethane (CH_2Cl_2) was purified by drying over calcium hydride (CaH_2), followed by distillation. *n*-Hexane, ethyl acetate (EtOAc) and acetone were distilled. Thin layer chromatography (TLC) was performed on Merck (silica gel 60, F-254, 0.25 mm). Silica gel 60 (230–400 mesh, Merck) was used for column chromatography. Melting points were determined with a Barnstead Electrothermal (IA9100) apparatus. NMR spectra were measured by using Bruker DRX 400, Avance II instruments, and chemical shifts were reported using residual protonated solvent peaks (for ^{1}H NMR spectra, DMSO-d_6 2.50 ppm; $CDCl_3$ 7.26 ppm; acetone-d_6 2.05 ppm and for ^{13}C NMR spectra, DMSO-d_6 39.52 ppm; acetone-d_6 29.8 ppm, 206.3 ppm). FT-IR spectra were measured by using a Nicolet Impact-400 FT-IR spectrometer. MALDI-TOF mass spectrometric measurements were performed on a Bruker (LRF20), and GC-mass spectrometric measurements were carried on Agilent 7890A GC spectrometer. The elemental analysis data were obtained from *the Yonsei Center for Research Facilities* at Yonsei University and *the Organic chemistry Research Center* at Sogang University. Fluorescence spectra were measured with HITACHI-F4500. To satisfy biocompatible conditions for the transport experiments, phosphate buffer solution at pH = 7.2 was prepared using a Thermo Scientific Orion DUAL STAR^{TM} pH Meter (9617BNWP).



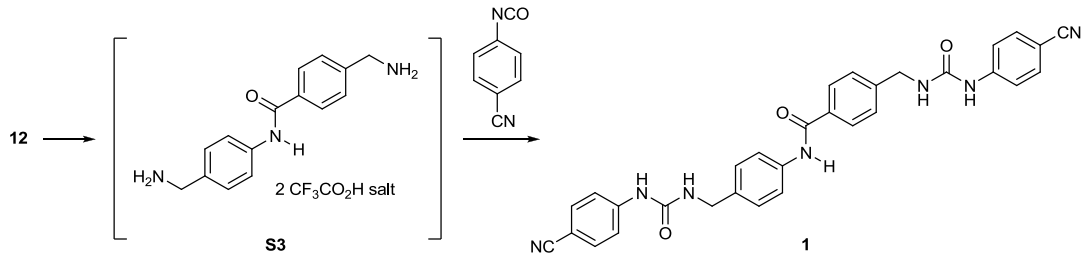
10: 4-(Aminomethyl)benzoic acid (**S1**, 3.0 g, 19.8 mmol) was added to a solution of Et_3N (30 mL, 3 equiv) in MeOH (65 mL, 0.3 M). After stirring for 30 min at room temperature, di-*tert*-butyl dicarbonate (4.5 mL, 19.8 mmol, 1 equiv) was added dropwise and the solution was

heated at reflux for 2 h. The reaction mixture was concentrated and the residue was dissolved in EtOAc. The organic solution was washed with brine and saturated NH_4Cl solution, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography (silica gel, MeOH/CH_2Cl_2 = (v/v) 1:18) to afford **10** (3.6 g, 14.4 mmol, 73 %) as a white solid. Mp 168-169 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 7.89 (d, 2H, ArH), 7.51 (t, 1H, NH), 7.33 (d, 2H, ArH), 4.18 (d, 2H, benzylic H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 167.6, 156.9, 146.7, 130.6, 130.0, 128.0, 79.1, 44.5, 28.6; IR (thin film) υ 3358 (NH), 1680 (C=O) cm^{-1}; GC-MS *m/z* calcd for $C_{13}H_{17}NO_4$ $[M]^+$ 251.1, found $[M]^+$ 251.1.

11: Di-*tert*-butyl dicarbonate (5.64 mL, 24.5 mmol, 1 equiv) was added dropwise to a solution of 4-aminobenzylamine (**S2**, 3.0 g, 24.5 mmol) in CH_2Cl_2 (80 mL, 0.3 M) and stirred for 2 h at room temperature. The reaction mixture was concentrated and the residue was dissolved in CH_2Cl_2. The organic solution was washed with brine and saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography (silica gel, EtOAc/CH_2Cl_2 = (v/v) 1:4) to afford **11** (5.4 g, 23.5 mmol, 96 %) as a yellow solid. Mp 74-75 ℃; ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 6.99 (d, 2H, ArH), 6.59 (d, 2H, ArH), 6.19 (t, 1H, NH), 4.53 (s, 2H, NH), 4.09 (d, 2H, benzylic H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 156.7, 148.2, 129.2, 129.0, 115.0, 78.5, 44.5, 28.7; IR (thin film) υ 3359 (NH), 1712 (C=O) cm^{-1}; GC-MS *m/z* calcd for $C_{12}H_{18}N_2O_2$ $[M]^+$ 222.1, found $[M]^+$ 222.1.

12: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI•HCl, 0.8 g, 5 mmol, 1.1 equiv) was treated with Et_3N (0.7 mL, 5 mmol, 1.1 equiv) in CH_2Cl_2 (30 mL, 0.3 M). After stirring for 30 min at room temperature, compound **10** (1.13 g, 4.5 mmol), 1-hydroxybenzotriazole (HOBt, 0.33 g, 0.3 mmol, 1.1 equiv) and **11** (1.0 g, 4.5 mmol, 1 equiv) were orderly added and the solution was stirred for additional 5 h at room temperature. The reaction mixture was filtered through Celite, concentrated, and the residue was dissolved in EtOAc. The organic solution was washed with brine and saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography (silica gel, EtOAc/CH_2Cl_2 = (v/v) 1:8) to afford **12** (1.5 g, 3.2 mmol, 72 %) as an ivory solid. Mp 188-189 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 10.17 (s, 1H,

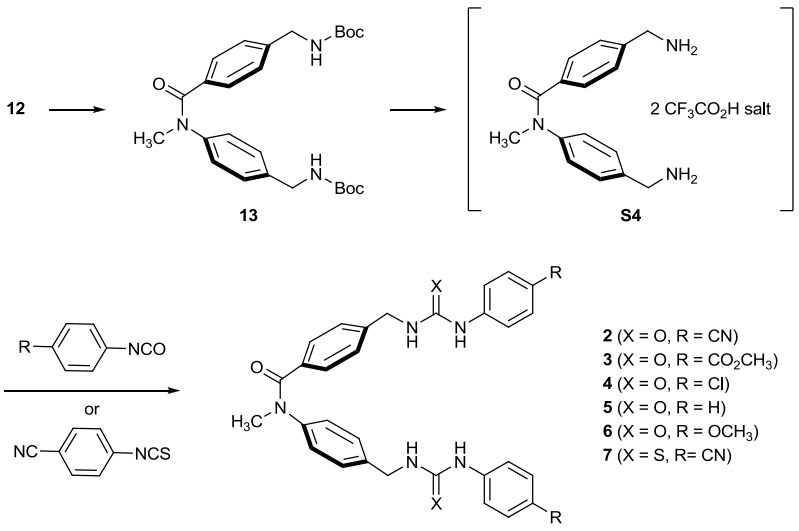
NH), 7.9 (d, 2H, ArH), 7.69 (d, 2H, ArH), 7.50 (t, 2H, NH), 7.36 (d, 2H, ArH), 7.20 (d, 2H, ArH), 4.19 (d, 2H, benzylic H), 4.08 (d, 2H, benzylic H), 1.40 (s, 18H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 167.2, 155.9, 145.4, 129.4, 129.2, 126.9, 78.0, 43.2, 28.3; IR (thin film) υ 3359 (NH), 1688 (C=O) cm^{-1}; MALDI-TOF *m/z* calcd for $C_{25}H_{33}N_3O_5$ $[M+Na]^+$ 478.2, found $[M]^+$ 478.3; elemental analysis cald (%) for $C_{25}H_{33}N_3O_5$: C 66.0, H 7.3, N 9.2; found C 66.2, H 7.6, N 9.3.



S3: Trifluoroacetic acid (70 µL, 0.85 mmol, 5 equiv) was added to a solution of compound **12** (0.08 g, 0.17 mmol) in CH_2Cl_2 (2.8 mL, 0.06 M) and stirred for 12 h at room temperature. The reaction mixture was concentrated and washed repeatedly with CH_2Cl_2 and toluene to afford **S3** (0.16 mmol, 92 %) as a yellow solid. The crude product was directly used for the next reaction without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.85 (s, 1H, NH), 8.13 (s, 6H, NH), 7.83 (d, 2H, ArH), 7.56 (d, 2H, ArH), 7.43 (d, 2H, ArH), 7.26 (d, 2H, ArH), 4.34 (br, 2H, benzylic H). 4.21 (br, 2H, benzylic H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 165.7, 152.3, 142.8, 133.6, 131.1, 129.5, 126.3, 44.1.

1: Anhydrous Et_3N (0.17 mL, 1.2 mmol, 5 equiv) was added to a solution of compound **12** (0.12 g, 0.24 mmol) in CH_2Cl_2 (1.5 mL, 0.16 M) and stirred for 1 h at room temperature (Solution A). A CH_2Cl_2 (2.0 mL, 0.27 M) solution of 4-aminobenzonitrile (62 mg, 0.53 mmol, 2.2 equiv) and anhydrous Et_3N (17 µL, 0.11 mmol, 0.44 equiv) was added dropwise to a solution of triphosgene (65 mg, 0.23 mmol, 0.88 equiv) in CH_2Cl_2 (1.0 mL, 0.23 M) at 0 °C (Solution B). After stirring for 30 min at room temperature, solution B (0.18 M in CH_2Cl_2) was added dropwise to a solution A without further purification and the solution was stirred for 6 h at room temperature. The reaction mixture was filtered and the filter cake was washed repeatedly with CH_2Cl_2. The crude product was purified by re-crystallizing from a solution in EtOAc, affording **1** (0.18 g, 0.3 mmol, 56 %) as a white solid. Mp 274-276 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 9.48 (s, 1H, NH), 8.65 (s, 1H, NH), 8.54 (s, 1H, NH), 7.94 (d, 2H,

ArH), 7.78 (d, 2H, ArH), 7.71 (m, 4H, ArH), 7.61 (m, 4H, ArH), 7.47 (d, 2H, ArH), 7.33 (d, 2H, ArH), 6.62 (t, 1H, NH), 6.44 (t, 1H, NH), 4.50 (d, 2H, benzylic H), 4.41 (d, 2H, benzylic H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 164.3, 153.1, 143.2, 141.5, 137.1, 133.2, 132.1, 131.7, 128.1, 127.8, 122.2, 118.3, 117.9, 108.1, 43.5; IR (thin film) υ 3346 (NH), 1655 (C=O) cm^{-1}; MALDI-TOF *m/z* calcd for $C_{31}H_{25}N_7O_3$ $[M+Na]^+$ 566.2, found $[M]^+$ 566.3; elemental analysis cald (%) for $C_{31}H_{25}N_7O_3$: C 68.5, H 4.6, N 18.0; found C 68.4, H 4.7, N 18.3.



13: Potassium hydroxide (0.09 g, 1.65 mmol, 3.75 equiv) was added to a solution of compound **12** (0.2 g, 0.44 mmol) in acetone (10 mL, 0.04 M) and the solution was heated at reflux for 30 min. After addition of methyl iodide (MeI, 41 µL, 0.66 mmol, 1.5 equiv), the solution was kept heated at reflux and stirred for additional 1 h. The reaction mixture was filtered through Celite, concentrated, and the residue was dissolved in CH_2Cl_2. The organic solution was washed with brine and saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography (silica gel, EtOAc/*n*-hexane = (v/v) 1:1) to afford **13** (0.2 mg, 0.43 mmol, 97 %) as an ivory solid. Mp 64-66 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 7.33 (t, 2H, NH), 7.21 (d, 2H, ArH), 7.07 (m, 6H, ArH), 4.05 (d, 4H, benzylic H), 3.38 (s, 3H), 1.37 (s, 18H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 170.4, 156.8, 144.8, 142.7, 139.4, 136.0, 129.6, 128.7, 127.8, 127.1, 78.9, 44.3, 44.1, 38.6, 28.6; IR (thin film) υ 3414 (NH), 1714 (C=O) cm^{-1}; MALDI-TOF *m/z* calcd for $C_{26}H_{35}N_3O_5$ $[M+Na]^+$ 492.3, found $[M+Na]^+$ 492.5; elemental analysis cald (%) for $C_{26}H_{35}N_3O_5$: C 66.5, H 7.5, N 8.9; found C 66.1, H 7.9, N 8.8.

S4: Compound **S4** was prepared from compound **13** instead of **12**, following the procedure described for the syntheses of compound **S3**. A yellow solid (0.1 g, 0.38 mmol, 89 %). The crude product was directly used for the next reaction without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 7.37 (s, 6H, NH), 7.24 (d, 2H, ArH), 7.12 (m, 6H, ArH), 4.24 (d, 4H, benzylic H), 3.41 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 169.8, 137.2, 136.1, 134.6, 133.8, 133.2, 130.4, 129.2, 128.5, 127.1, 126.3, 125.5, 43.2, 42.7, 37.0.

2: Compound **2** was prepared from compound **S4** instead of **S3**, following the procedure described for the syntheses of compound **1**. The crude product was purified by flash column chromatography (silica gel, EtOAc/CH_2Cl_2 = (v/v) 2:1) to afford **7** (84 mg, 0.15 mmol, 71 %) as a white solid. Mp 176-177 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 8.58 (d, 2H, NH), 7.65 (m, 4H, ArH), 7.60 (d, 4H, ArH), 7.27 (d, 2H, ArH), 7.22 (d, 2H, ArH), 7.12 (m, 4H, ArH), 6.46 (t, 2H, NH), 4.33 (d, 4H, benzylic H), 3.38 (s, 3H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 169.2, 154.6, 142.4, 141.3, 137.6, 134.5, 134.0, 132.1, 127.2, 126.7, 118.6, 118.2, 108.3, 43.1, 39.2; IR (thin film) υ 3362 (NH), 2222 (C=N), 1700 (C=O) cm^{-1}; MALDI-TOF *m/z* calcd for $C_{32}H_{27}N_7O_3$ $[M+H]^+$ 558.2, found $[M+H]^+$ 558.2; elemental analysis cald (%) for $C_{32}H_{27}N_7O_3$: C 68.9, H 4.9, N 17.6; found C 68.5, H 4.8, N 17.4.

3: Compound **3** was prepared from compound **S4** instead of **S3** and the corresponding *para*-substituted anilines (methyl 4-aminobenzoate) instead of 4-aminobenzonitrile, following the procedure described for the syntheses of compound **1**. The crude product was purified by flash column chromatography (silica gel, EtOAc/CH_2Cl_2 = (v/v) 1:3) to afford **6** (72 mg, 0.2 mmol, 67 %) as a white solid. Mp 144-146 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.14 (d, 2H, NH), 7.82 (d, 4H, ArH), 7.51 (d, 4H, ArH), 7.24 (d, 2H, ArH), 7.19 (d, 2H, ArH), 7.14 (m, 4H, ArH), 6.89 (t, 2H, NH), 4.23 (d, 4H, benzylic H), 3.80 (s, 6H), 3.38 (s, 3H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 167.0, 155.8, 146.1, 145.0, 142.6, 139.4, 136.2, 131.3, 129.7, 128.9, 128.0, 127.3, 123.8, 118.0, 52.0, 43.6, 43.5, 38.7; IR (thin film) υ 3369 (NH), 1702 (NH) cm^{-1}; MALDI-TOF *m/z* calcd for $C_{34}H_{33}N_5O_7$ $[M+H]^+$ 624.2, found $[M+H]^+$ 624.2; elemental analysis cald (%) for $C_{34}H_{33}N_5O_7$: C 65.4, H 5.3, N 11.2; found C 65.1, H 5.3, N 11.5.

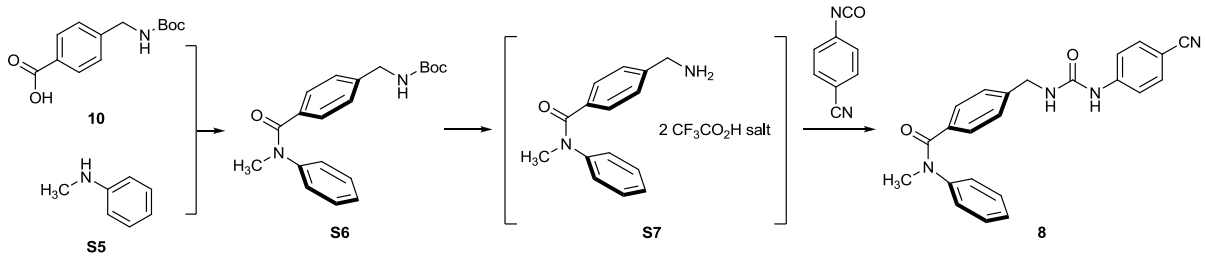
4: Compound **4** was prepared from compound **S4** instead of **S3** and the corresponding *para*-substituted anilines (4-chloroaniline) instead of 4-aminobenzonitrile, following the procedure

described for the syntheses of compound **1**. The crude product was purified by flash column chromatography (silica gel, EtOAc/CH_2Cl_2 = (v/v) 1:1) to afford **5** (47 mg, 0.08 mmol, 52 %) as a white solid. Mp 228-229 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.73 (d, 2H, NH), 7.40 (m, 4H, ArH), 7.24 (m, 6H, ArH), 7.18 (d, 2H, ArH), 7.12 (m, 4H, ArH), 6.65 (t, 2H, NH), 4.22 (d, 4H, benzylic H), 3.38 (s, 3H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 169.0, 153.7, 142.1, 138.6, 138.2, 133.7, 133.1, 128.6, 127.5, 127.1, 121.8, 44.7, 38.9; IR (thin film) υ 3366 (NH), 1700 (C=O) cm^{-1}; MALDI-TOF *m/z* calcd for $C_{30}H_{27}Cl_2N_5O_3$ $[M+Na]^+$ 598.1, found $[M+Na]^+$ 598.1; elemental analysis cald (%) for $C_{30}H_{27}N_5O_3$: C 62.5, H 4.7, N 12.3; found C 62.4, H 4.4, N 12.4.

5: Anhydrous Et_3N (70 µL, 0.5 mmol, 5 equiv) was added to a solution of compound **S4** (0.05 g, 0.1 mmol) in CH_2Cl_2 (1.0 mL, 0.1 M) and stirred for 1 h at room temperature. Phenylisocynate (26 µL, 0.22 mmol, 2.2 equiv) was added dropwise and stirred for additional 1 h at room temperature. The crude product was purified by flash column chromatography (silica gel, EtOAc/CH_2Cl_2 = (v/v) 1:6) to afford **5** (20 mg, 0.04 mmol, 49 %) as a white solid. Mp 125-126 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 170.6, 156.5, 144.9, 142.9, 141.4, 139.6, 136.1, 129.7, 129.5, 128.6, 127.9, 127.1, 122.5, 119.2, 43.5, 43.3, 38.6; IR (thin film) υ 3407 (NH), 1698 (C=O) cm^{-1}; MALDI-TOF *m/z* calcd for $C_{30}H_{29}N_5O_3$ $[M+H]^+$ 508.2, found $[M+H]^+$ 508.3; elemental analysis cald (%) for $C_{30}H_{29}N_5O_3$: C 71.0, H 5.8, N 13.8; found C 70.7, H 5.6, N 13.7.

6: Compound **6** was prepared from compound **S4** instead of **S3** and the corresponding *para*-substituted anilines (4-methoxynitrile) instead of 4-aminobenzonitrile, following the procedure described for the syntheses of compound **1**. The crude product was purified by flash column chromatography (silica gel, EtOAc/CH_2Cl_2 = (v/v) 1:2) to afford **6** (34 mg, 0.06 mmol, 76 %) as a white solid. Mp 114-115 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.36 (d, 2H, NH), 7.25 (m, 6H, ArH), 7.17 (d, 2H, ArH), 7.11 (d, 4H, ArH), 6.80 (d, 4H, ArH), 6.50 (t, 2H, NH), 4.21 (d, 4H, benzylic H), 3.68 (s, 6H), 3.38 (s, 3H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 168.9, 159.8, 154.2, 142.2, 138.0, 133.2, 132.9, 131.5, 127.1, 119.8, 114.1, 57.2, 43.2, 38.1; IR (thin film) υ 3394 (NH), 1683 (C=O) cm^{-1}; MALDI-TOF *m/z* calcd for $C_{32}H_{33}N_5O_5$ $[M+H]^+$ 568.2, found $[M+H]^+$ 568.2; elemental analysis cald (%) for $C_{32}H_{33}N_5O_5$: C 67.7, H 5.9, N 12.3; found C 67.4, H 6.1, N 12.0.

7: Anhydrous Et_3N (0.6 mL, 4.5 mmol, 5 equiv) was added to a solution of compound **S4** (0.45 g, 0.9 mmol) in CH_2Cl_2 (3.0 mL, 0.3 M) and stirred for 1 h at room temperature. 4-Cyanophenyl thiocyanate (0.14 g, 2 mmol, 2.2 equiv) was added dropwise and stirred for additional 8 h at room temperature. The crude product was purified by flash column chromatography (silica gel, EtOAc/CH_2Cl_2 = (v/v) 1:1) to afford **7** (0.17 g, 0.3 mmol, 75 %) as a white solid. Mp 140-141 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 9.48 (d, 2H, NH), 7.97 (t, 2H, NH), 7.84 (m, 4H, ArH), 7.69 (m, 4H, ArH), 7.30 (m, 4H, ArH), 7.22 (d, 2H, ArH), 7.15 (d, 2H, ArH), 4.83 (d, 4H, benzylic H), 3.38 (s, 3H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 182.4, 172.3, 143.8, 141.2, 137.8, 134.6, 134.2, 132.5, 131.2, 127.6, 127.2, 118.7, 107.2, 52.3, 38.4; IR (thin film) υ 3352 (NH), 2221 (C=N), 1246 (C=S) cm^{-1}; MALDI-TOF *m/z* calcd for $C_{32}H_{27}N_7OS_2$ $[M+H]^+$ 469.3, found $[M+H]^+$ 469.3; elemental analysis cald (%) for $C_{32}H_{27}N_7OS_2$: C 65.2, H 4.6, N 16.6, S 10.9; found C 65.5, H 4.9, N 16.3, S 10.8.

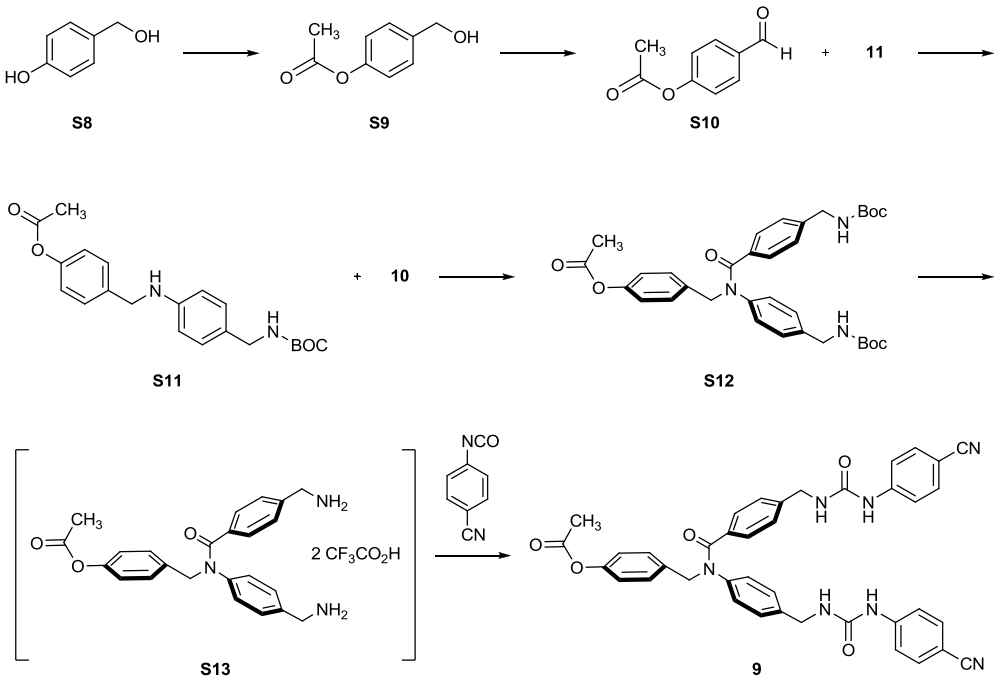


S6: Compound **10** (0.2 g, 8.0 mmol) was added to a solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI•HCl, 0.2 g, 1.2 mmol, 1.5 equiv) and 4-dimethylaminopyridine (4-DMAP, 9.7 mg, 0.8 mmol, 0.1 equiv) in DMF (40 mL, 0.2 M). After *N*-methyl aniline (**S5**, 0.3 mL, 1.2 mmol, 1.5 equiv) was additionally injected, the solution was stirred for 4 h at room temperature. The reaction mixture was filtered through Celite, concentrated, and the residue was dissolved in CH_2Cl_2. The organic solution was washed with brine and saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography (silica gel, diethyl ether/CH_2Cl_2 = (v/v) 1:3) to afford **S6** (0.2 g, 5.8 mmol, 73 %) as a white solid. Mp 121-122 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 7.26 (m, 4H, ArH), 7.14 (m, 5H, ArH), 6.43 (t, 1H, NH), 4.19 (d, 2H, benzylic H), 3.40 (s, 3H), 1.39 (s, 9 H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 170.4, 156.9, 146.3, 142.7, 136.0, 130.0, 129.7, 128.0, 127.2, 78.9, 44.3, 38.5, 28.6; IR (thin film) υ 3414 (NH), 1761 (C=O) cm^{-1}; GC-MS *m/z* calcd for $C_{20}H_{24}N_2O_3$

$[M+H]^+$ 341.2, found $[M+H]^+$ 341.2; elemental analysis cald (%) for $C_{20}H_{24}N_2O_3$: C 70.6, H 7.1, N 8.2; found C 70.6, H 7.5, N 8.2.

S7: Compound **S7** was prepared from compound **S6** instead of **12**, following the procedure described for the syntheses of compound **S3**. A yellow solid (91 mg, 0.38 mmol, 85 %). The crude product was directly used for the next reaction without further purification. ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 7.41 (d, 2H, ArH), 7.36 (d, 2H, ArH), 7.24 (d, 2H, ArH), 7.15 (d, 2H, ArH), 7.06 (s, 1H, ArH), 6.91 (s, 3H, NH), 4.23 (d, 2H, benzylic H), 3.40 (s, 3H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 169.6, 149.9, 140.1, 136.4, 132.2, 129.6, 128.8, 127.1, 126.2, 43.6, 38.2.

8: Compound **8** was prepared from compound **S7** instead of **S4**, following the procedure described for the preparation of compound **2**. The crude product was purified by flash column chromatography (silica gel, EtOAc/CH_2Cl_2 = (v/v) 1:2) to afford **8** (28 mg, 0.07 mmol, 61 %) as a white solid. Mp 208-210 ℃; ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 8.54 (s, 1H, NH), 7.67 (d, 2H, ArH), 7.60 (d, 2H, ArH), 7.26 (m, 4H, ArH), 7.16 (m, 5H, ArH), 6.42 (t, 1H, NH), 4.35 (d, 2H, benzylic H), 3.40 (s, 3H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 170.6, 155.6, 146.2, 145.9, 142.5, 136.1, 133.9, 130.1, 129.7, 127.3, 120.0, 118.7, 104.6, 43.6, 38.6; IR (thin film) υ 3353 (NH), 2221 (C=N), 1703 (C=O) cm^{-1}; GC-MS *m/z* calcd for $C_{23}H_{20}N_4O_2$ $[M+H]^+$ 385.2, found $[M+H]^+$ 385.3; elemental analysis cald (%) for $C_{23}H_{20}N_4O_2$: C 71.9, H 5.2, N 14.6; found C 71.6, H 5.1, N 14.4.



S9: To a solution of 4-hydroxybenzyl alcohol (**S8**, 2.0 g, 16 mmol) in anhydrous tetrahydrofuran (50 mL, 0.3 M) under nitrogen (N_2), anhydrous Et_3N (2.2 mL, 18 mmol, 1.1 equiv) was added dropwise and the solution was evacuated under vacuum and filled with N_2. Acetyl chloride (1.3 mL, 18 mmol, 1.1 equiv) was additionally injected dropwise for 30 min at 0 ℃. After stirring for 3 h at room temperature, the reaction mixture was filtered through Celite, concentrated, and the residue was dissolved in EtOAc. The organic solution was washed with brine and saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography (silica gel, EtOAc/CH_2Cl_2 = (v/v) 1:2) to afford **S9** (1.9 g, 12 mmol, 73 %) as a colorless oily liquid. ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 7.39 (d, 2H, ArH), 7.10 (d, 2H, ArH), 4.63 (d, 2H, benzylic H), 4.47 (s, 1H, OH), 2.25 (s, 3H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 169.9, 150.8, 140.9, 128.4, 122.4, 64.1, 21.0; IR (thin film) υ 1754 (C=O) cm^{-1}; GC-MS *m/z* calcd for $C_9H_{10}O_3$ $[M+H]^+$ 167.1, found $[M+H]^+$ 167.2.

S10: Compound **S9** (0.5 g, 3 mmol) was added to a solution of pyridinium chlorochromate (PCC, 0.93 g, 4.5 mmol, 1.5 equiv) in anhydrous CH_2Cl_2 (30 mL, 0.1 M). After stirring for 4 h at room temperature, the reaction mixture was filtered through Celite, concentrated, and the residue was dissolved in EtOAc. The organic solution was washed with brine and saturated

$NaHCO_3$ solution, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography (silica gel, EtOAc/CH_2Cl_2 = (v/v) 1:6) to afford **S10** (0.41 g, 2.5 mmol, 84 %) as a colorless oily liquid. ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 10.02 (s, 1H), 7.97 (d, 2H, ArH), 7.35 (d, 2H, ArH), 2.29 (s, 3H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 191.9, 169.4, 156.4, 135.1, 132.0, 123.5, 21.0; IR (thin film) υ 1758 (C=O) cm^{-1}; GC-MS *m/z* calcd for $C_9H_8O_3$ $[M+H]^+$ 165.0, found $[M+H]^+$ 165.1.

S11: Compound **S10** (0.12 mg, 0.8 mmol) and sodium cyanoborohydride ($NaBH_3CN$, 80 mg, 1.2 mmol, 1.5 equiv) were added orderly to a solution of compound **11** (0.24 mg, 1.2 mmol, 1.5 equiv) in anhydrous MeOH (25 mL, 0.32 M). After stirring 16 h at room temperature, the reaction mixture was filtered through Celite, concentrated, and the residue was dissolved in CH_2Cl_2. The organic solution was washed with brine and saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography (silica gel, EtOAc/*n*-hexane = (v/v) 1:2) to afford **S11** (0.2 g, 0.56 mmol, 72 %) as an ivory solid. Mp 85-86 ℃; ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 7.39 (d, 2H, ArH), 7.06 (d, 2H, ArH), 7.04 (d, 2H, ArH), 6.60 (d, 2H, ArH), 6.21 (t, 1H, NH), 5.44 (t, 1H, NH), 4.32 (d, 2H, benzylic H), 4.12 (d, 2H, benzylic H), 2.24 (s, 3H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 169.8, 156.7, 150.5, 148.5, 138.5, 129.2, 128.9, 128.7, 122.4, 113.3, 78.5, 47.5, 44.4, 28.7, 21.0; IR (thin film) υ 3407 (NH), 1698 (C=O) cm^{-1}; GC-MS *m/z* calcd for $C_{26}H_{35}N_3O_5$ $[M+H]^+$ 371.2, found $[M+H]^+$ 371.2.

S12: Compound **S12** was prepared from compound **S11** instead of *N*-methyl aniline (**S5**), following the procedure described for the preparation of compound **S6**. The crude product was purified by flash column chromatography (silica gel, EtOAc/CH_2Cl_2 = (v/v) 1:1) to afford **S12** (0.16 g, 0.22 mmol, 69 %) as a white solid. Mp ℃; ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 7.37 (d, 2H, ArH), 7.32 (d, 2H, ArH), 7.05 (m, 8H, ArH), 6.47 (t, 1H, NH), 6.43 (t, 1H, NH), 5.13 (s, 2H, benzylic H), 4.17 (d, 2H, benzylic H), 4.14 (d, 2H, benzylic H), 2.23 (s, 3H), 1.41 (s, 18H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 170.6, 169.7, 156.9, 151.0, 143.1, 142.8, 139.5, 136.3, 135.8, 130.0, 129.8, 128.5, 127.2, 122.5, 78.9, 53.6, 44.3, 44.0, 28.6, 21.0; IR (thin film) υ 3449 (NH), 1715 (C=O); MALDI-TOF *m/z* calcd for $C_{34}H_{41}N_3O_7$ $[M+H]^+$ 604.3, found $[M]^+$ 604.4; elemental analysis cald (%) for $C_{34}H_{41}N_3O_7$: C 67.6, H 6.9, N 7.0; found C 67.6, H 6.6, N 7.2.

S13: Compound **S13** was prepared from compound **S12** instead of **12**, following the procedure described for the syntheses of compound **S3**. A yellow solid (0.38 mmol, 91 %). The crude product was directly used for the next reaction without further purification. ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 7.61 (d, 2H, ArH), 7.55 (d, 2H, ArH), 7.31 (d, 2H, ArH), 7.27 (d, 2H, ArH), 7.04 (m, 4H, ArH), 6.21 (s, 3H, NH), 6.15 (s, 3H, NH), 5.11 (s, 2H, benzyic H), 4.32 (s, 2H, benzylic H), 4.29 (s, 2H, benzylic H), 2.26 (s, 3H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 170.6, 169.2, 155.3, 150.6, 145.2, 144.4, 138.7, 135.2, 134.6, 133.1, 128.2, 127.4, 126.0, 125.2, 120.4, 51.2, 44.7, 44.1, 20.5.

9: Compound **9** was prepared from compound **S13** instead of **S4**, following the procedure described for the preparation of compound **2**. The crude product was purified by flash column chromatography (silica gel, EtOAc/CH_2Cl_2 = (v/v) 2:1) to afford **9** (34 mg, 0.06 mmol%, 49%) as a white solid. Mp 165-166 ℃; ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 8.58 (d, 2H, NH), 7.62 (m, 8H, ArH), 7.34 (m, 4H, ArH), 7.13 (d, 4H, ArH), 7.03 (d, 4H, ArH), 6.48 (t, 1H, NH), 6.43 (t, 1H, NH), 5.12 (s, 2H, benzylic H), 4.34 (d, 2H, benzylic H), 4.30 (d, 2H, benzylic H), 2.23 (s, 3H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 170.2, 169.7, 156.9, 150.7, 145.1, 143.2, 138.2, 136.9, 136.5, 135.2, 129.1, 128.4, 122.6, 118.4, 117.8, 118.6, 52.4, 44.2, 21.0; IR (thin film) υ 3352 (NH), 2221 (C=N), 1703 (C=O) cm^{-1}; MALDI-TOF *m/z* calcd for $C_{26}H_{35}N_3O_5$ $[M+H]^+$ 692.3, found $[M+H]^+$ 692.5; elemental analysis cald (%) for $C_{40}H_{33}N_7O_5$: C 69.5, H 4.8, N 14.2; found C 69.7, H 5.1, N 13.9.

2. Binding studies

Job's plot: $CDCl_3$ solvent was filtered through basic alumina prior to use. Stock solutions of compounds **2**–**8** (6.0×10^{-3} M) and tetrabutylammonium chloride (TBA^+Cl^-, 6.0×10^{-3} M) in 10% (v/v) DMSO-d_6/$CDCl_3$ saturated by water (< 0.1%) was prepared separately at 24 ± 1 ℃. Keeping the total volume of 500 μL, aliquots of two stock solutions were added to an NMR tube in the following ratio; compound : tetrabutylammonium chloride = 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. Job's plots were constructed by plotting chemical shifts against mol fractions of the compound.

^{1}H NMR titrations: $CDCl_3$ solvent was filtered through basic alumina prior to use. Each stock solution of compounds **2**–**8** (1.0×10^{-3} M) in 10% (v/v) DMSO-d_6/$CDCl_3$ saturated by water (< 0.1%) was prepared at 24 ± 1 ℃. Using this solution as a solvent, a guest stock solution of tetrabutylammonium chloride (TBA^+Cl^-) was prepared to maintain the concentration of the compound constant during the experiments. In addition, each guest stock solution was prepared with diverse range of concentrations (10–80 × 10^{-3} M) upon the binding affinities of corresponding compound. In order to conduct the experiment, a 500 μL of the host stock solution was transferred to a NMR tube, and an initial spectrum was taken to determine the chemical shift of free host. Aliquots of the guest solution (at first 10 μL and finally 200 μL) were added to the receptor stock solution and the spectrum was recorded after each addition. The association constants (K_a, M^{-1}) was determined by non-linear least square fitting of the titration curve[S1], plotting the chemical shift changes of the urea NH signals against the concentrations (or equivalents) of the added chloride ion. Titration experiments were at least duplicated, and errors in the association constants were found to be less than 10% in all cases.

[S1] (*a*) K. A. Connors, *Binding Constants*, John Wiley & Son; New York, 1987; (*b*) R. S. Macomber, *J. Chem. Educ.* 1992, **69**, 375− 378; (*c*) K.-J. Chang, Y.-J. An, H. Uh and K.-S. Jeong, *J. Org. Chem.*, 2004, **69**, 6556–6563; (*d*) P. Thordarson, *Chem. Soc. Rev.*, 2011, **40**, 1305− 1323.

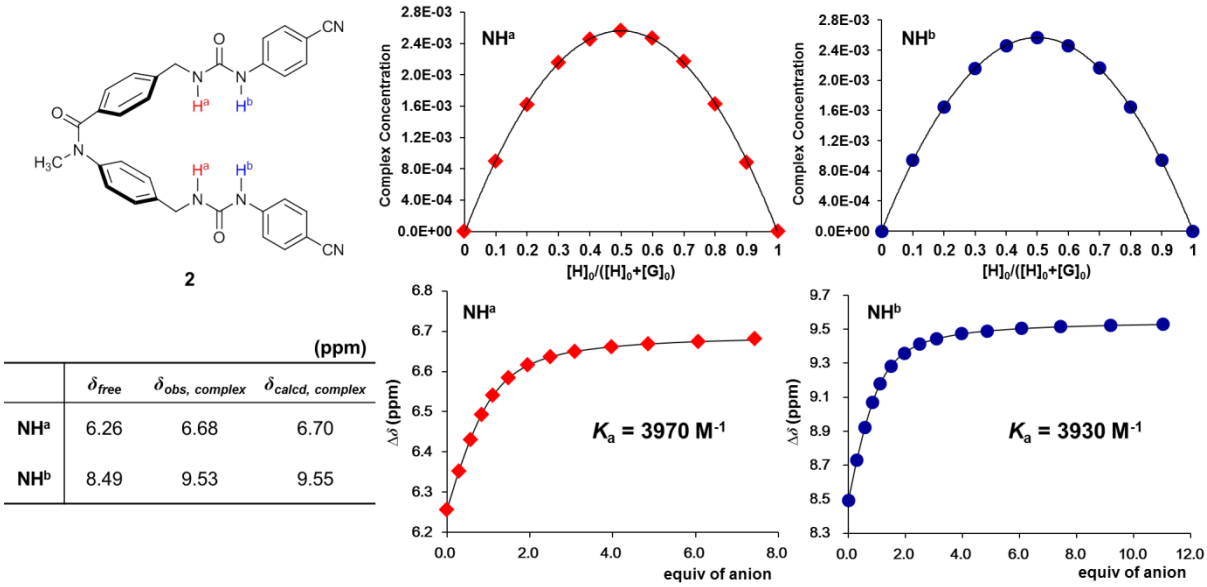


Figure S1. Job's plots (right-top), chemical shifts (left-bottom) and titration curves (right-bottom, dots: experimental values, lines: theoretical values) of **2** (K_a = 3950 ± 20 M^{-1})

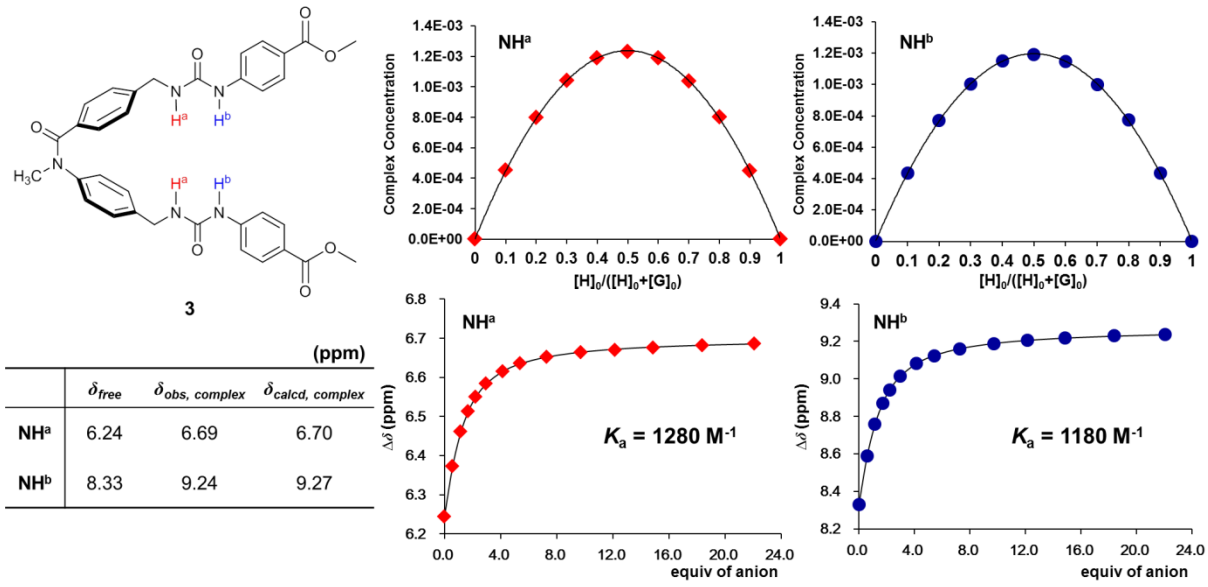
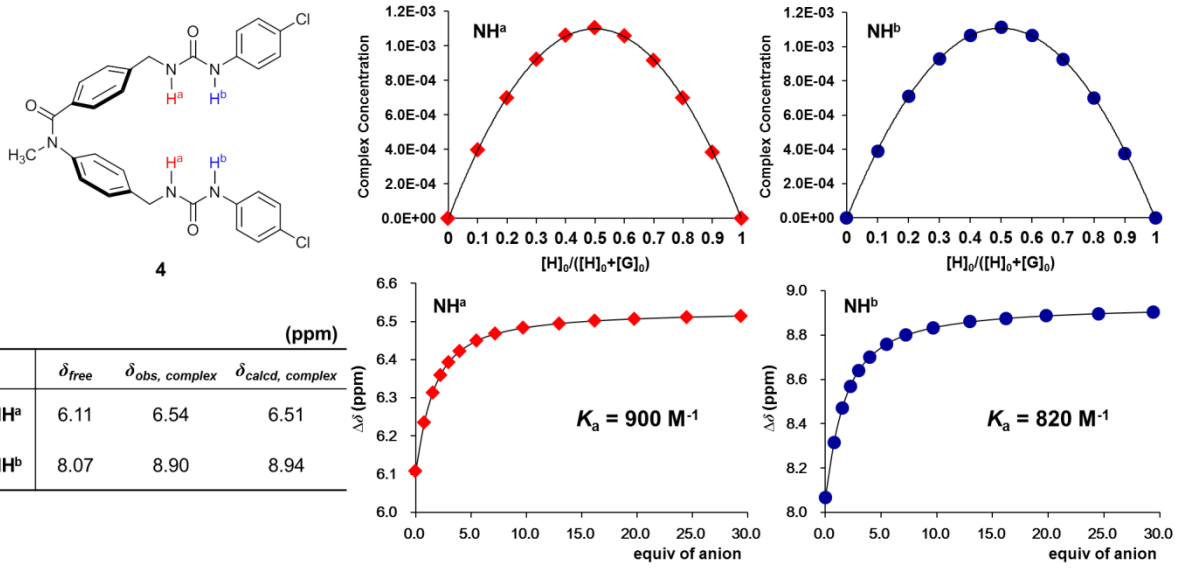


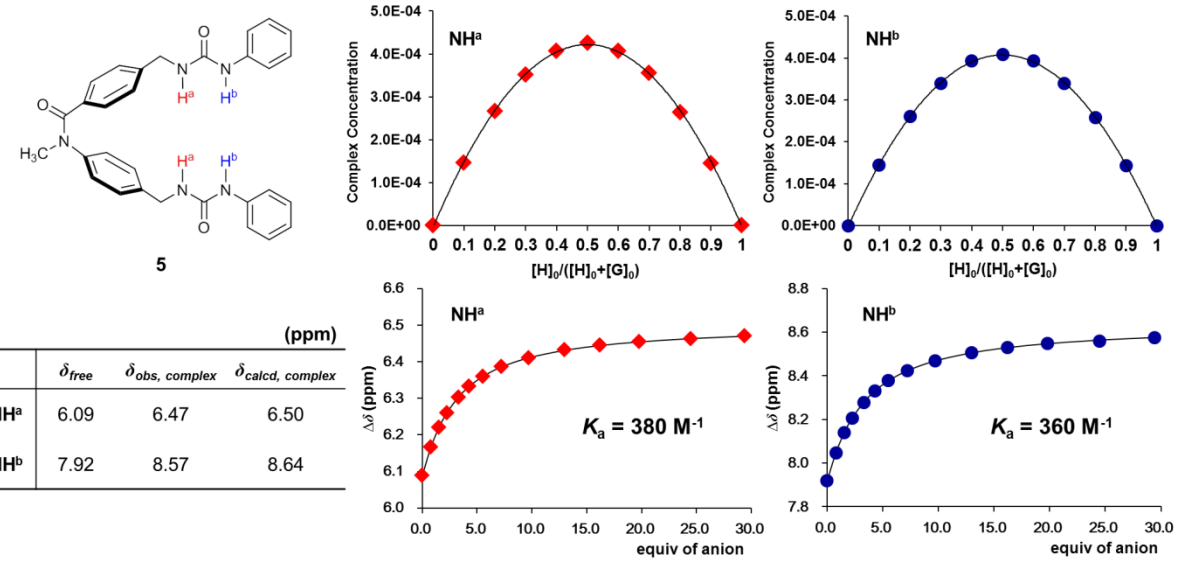
Figure S2. Job's plots (right-top), chemical shifts (left-bottom) and titration curves (right-bottom, dots: experimental values, lines: theoretical values) of **3** (K_a = 1230 ± 50 M^{-1})



(ppm)

	δ_{free}	$\delta_{obs,\ complex}$	$\delta_{calcd,\ complex}$
NH^a	6.11	6.54	6.51
NH^b	8.07	8.90	8.94

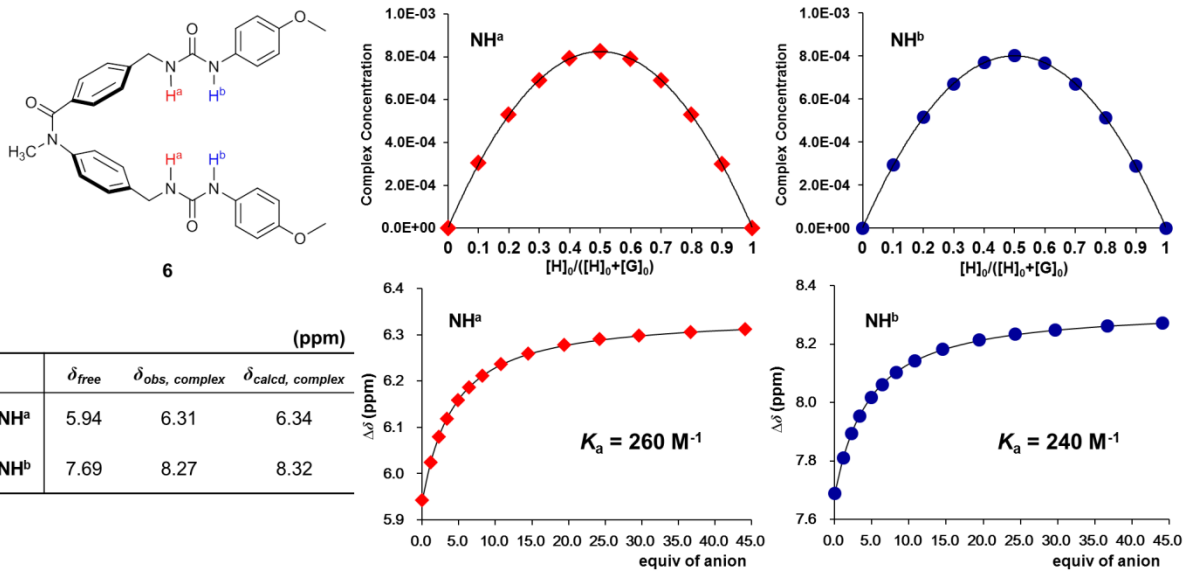
Figure S3. Job's plots (right-top), chemical shifts (left-bottom) and titration curves (right-bottom, dots: experimental values, lines: theoretical values) of **4** (K_a = 860 ± 40 M^{-1})



(ppm)

	δ_{free}	$\delta_{obs,\ complex}$	$\delta_{calcd,\ complex}$
NH^a	6.09	6.47	6.50
NH^b	7.92	8.57	8.64

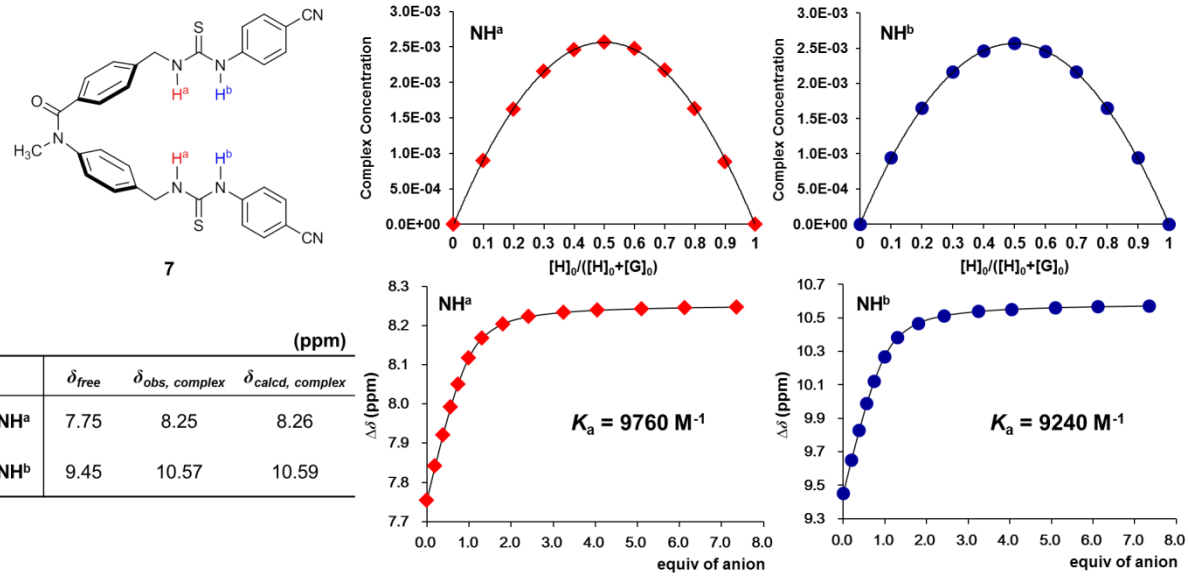
Figure S4. Job's plots (right-top), chemical shifts (left-bottom) and titration curves (right-bottom, dots: experimental values, lines: theoretical values) of **5** (K_a = 370 ± 10 M^{-1})



(ppm)

	δ_{free}	$\delta_{obs,\ complex}$	$\delta_{calcd,\ complex}$
NH^a	5.94	6.31	6.34
NH^b	7.69	8.27	8.32

Figure S5. Job's plots (right-top), chemical shifts (left-bottom) and titration curves (right-bottom, dots: experimental values, lines: theoretical values) of **6** (K_a = 250 ± 10 M^{-1})



(ppm)

	δ_{free}	$\delta_{obs,\ complex}$	$\delta_{calcd,\ complex}$
NH^a	7.75	8.25	8.26
NH^b	9.45	10.57	10.59

Figure S6. Job's plots (right-top), chemical shifts (left-bottom) and titration curves (right-bottom, dots: experimental values, lines: theoretical values) of **7** (K_a = 9500 ± 260 M^{-1})

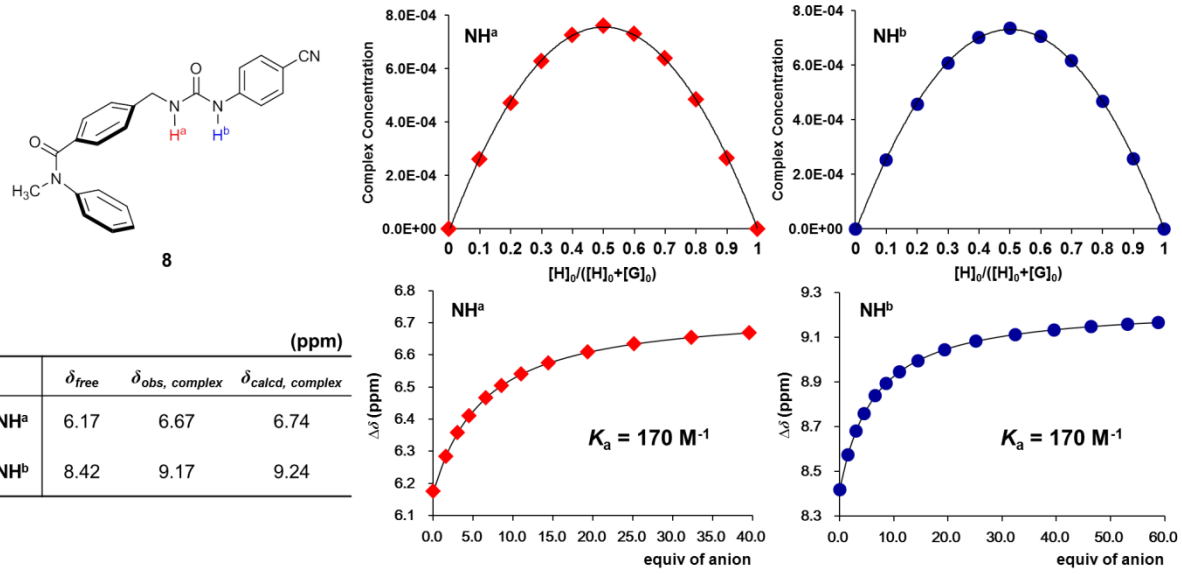


Figure S7. Job's plots (right-top), chemical shifts (left-bottom) and titration curves (right-bottom, dots: experimental values, lines: theoretical values) of **8** (K_a = 170 M^{-1})

3. Transport experiments

Preparation of POPC vesicles: A chloroform solution (10 mL) of 1-palmitoyl-2-oleoylphosphatidylcholine (POPC, 30 mg) was evaporated under reduced pressure and dried under high vacuum for 8 h to give a thin film. The lipid film was rehydrated by vortexing for 5 min with a 1 mL of an internal solution (200 mM $NaNO_3$, 1 mM Lucigenin and 10 mM phosphate buffer at pH = 7.2). The lipid suspension was then subjected to nine freeze–thaw cycles and was allowed to age for 1 h at room temperature. The suspension was extruded 23 times through a 200 nm polycarbonate membrane using extruder (Avanti, The Mini-Extruder set) to obtain unilamellar vesicles. Non-encapsulated lucigenin was removed by size exclusion chromatography (Sephadex G-50).

General procedure for transport experiments: The vesicles were diluted with a 40 mL of external solution (200 mM $NaNO_3$ and 10 mM phophate buffer at pH = 7.2) to a final lipid concentration of 1 mM. Each stock solutions of compounds **1**-**7** in dimethyl sulfoxide (DMSO, 8 mM) was prepared prior to the transport experiments. 2 mL of 1 mM POPC vesicles was tranferred to a cuvette and 30 μL of 2 M NaCl and 5 μL of the transport stock solution (8 mM, 2 mol% to lipid) were added to start the experiments. The chloride influx across vesicles was monitored by a decay in lucigenin fluorescence, which was measured at 372 nm emission upon excitation at 501 nm using fluorescence spectrophotometer. After 300 s, Triton X-100 was added to lyse the vesicle.

4. Hill analyses of chloride transport

To quantify the chloride transport activities, Hill analyses[S2] for the Cl^-/NO_3^- antiport assays was performed for various concentrations of compounds. The chloride influx (%) 300 s after the addition of transporter was plotted as a function of the transporter concentration and each data was fitted to the Hill equation using Origin 8.0:

$$y = V_{max}\frac{x^n}{(k^n + x^n)} = 100\ \% \frac{x^n}{(EC_{50}{}^n + x^n)}$$

where y is the chloride influx (%) at 300 s and x is the concentration of transporter (mol% to lipid). V_{max}, n and k are the parameters to be fitted. V_{max} was fixed to 100%, which is physically the maximum chloride influx possible. n is the Hill coefficient and k is the concentration of transporter required to obtain 50% chloride influx at 300 s.

Table S1. Overview of Hill analyses of compounds **2** and **7**.

Compound	$EC_{50}{}^a$ (mol%)	Error of EC_{50} (mol%)	n^b	Error of n
2	0.24	0.015	1.09	0.071
7	0.08	0.003	1.06	0.047

[a] Concentration of compounds (mol% to lipid) needed to achieve 50% chloride influx at 300 s. [b] Hill coefficient.

[S2] (*a*) A. V. Hill, *Biochem. J.*, 1913, **7**, 471– 480; (*b*) S. Bhosale and S. Matile, *Chirality*, 2006, **18**, 849– 856.

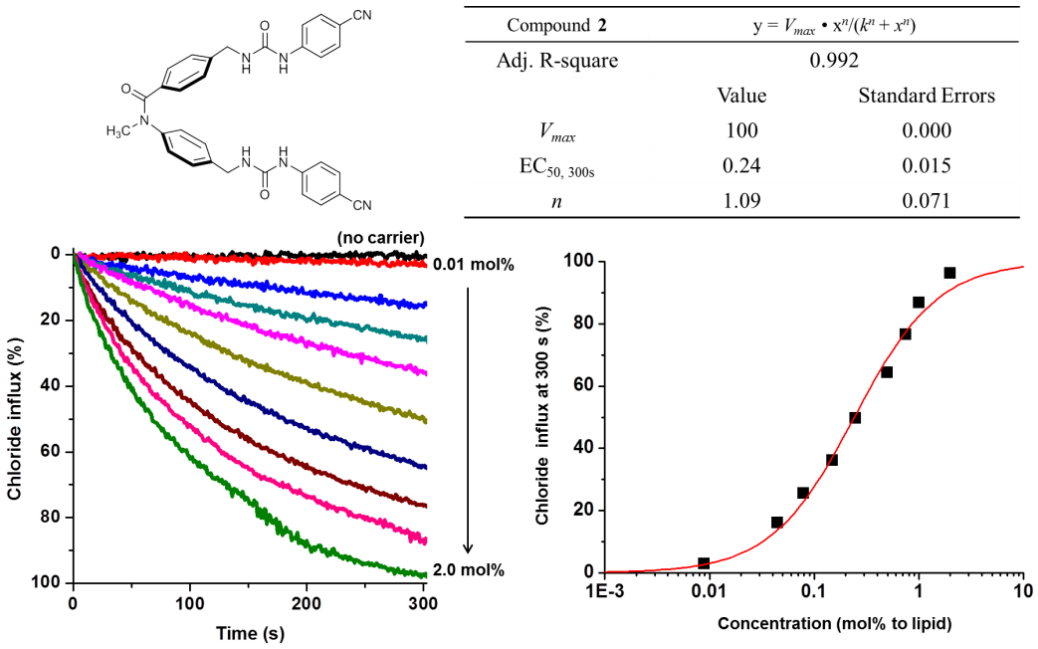


Figure S8. Chloride influx facilitated by various concentrations of **2** (0.01, 0.05, 0.08, 0.15, 0.25, 0.5, 0.75, 1.0 and 2.0 mol% to lipid, respectively) (left-bottom). Data points earned at 300s were fitted to the Hill equation using Origin 8.0 (right-bottom).

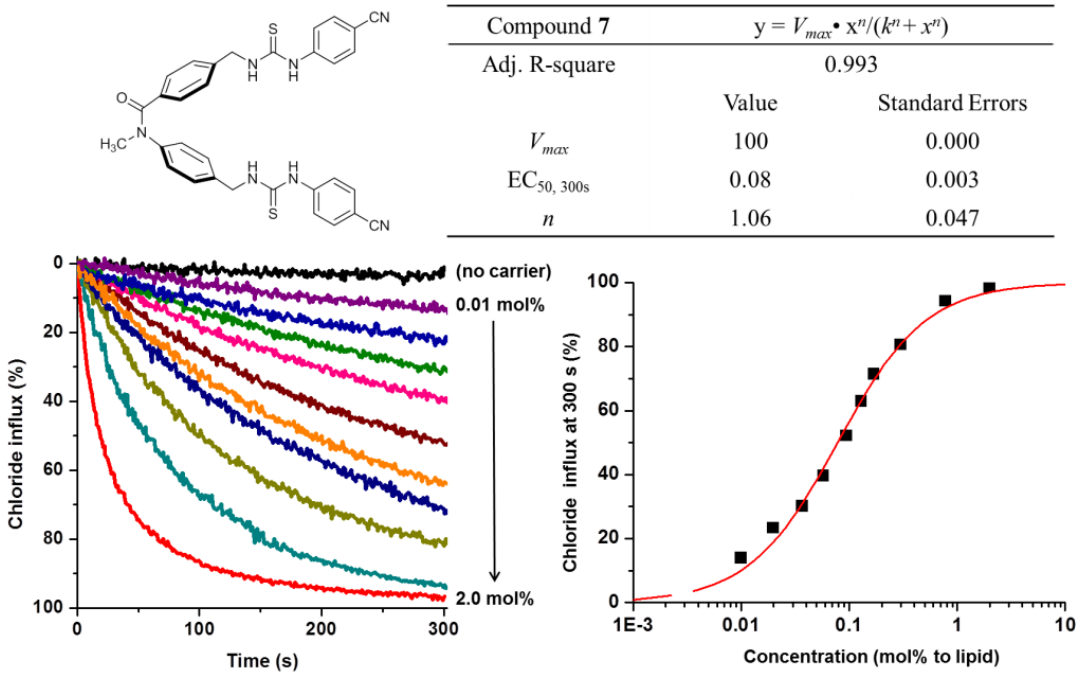


Figure S9. Chloride influx facilitated by various concentrations of **7** (0.01, 0.02, 0.04, 0.06, 0.1, 0.13, 0.17, 0.3, 0.8 and 2.0 mol% to lipid, respectively) (left-bottom). Data points earned at 300s were fitted to the Hill equation using Origin 8.0 (right-bottom).

5. Mechanistic studies of chloride transport

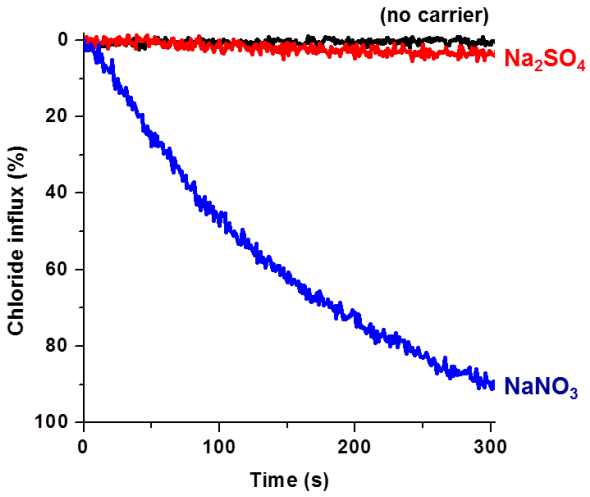


Figure S10. Comparision of chloride transport rates of **2** (2 mol% to lipid) into vesicles loaded with Na_2SO_4 (67 mM) or $NaNO_3$ (200 mM). The vesicles were suspended in a $NaNO_3$ solution (200 mM in 10 mM phosphate buffer at pH = 7.2).

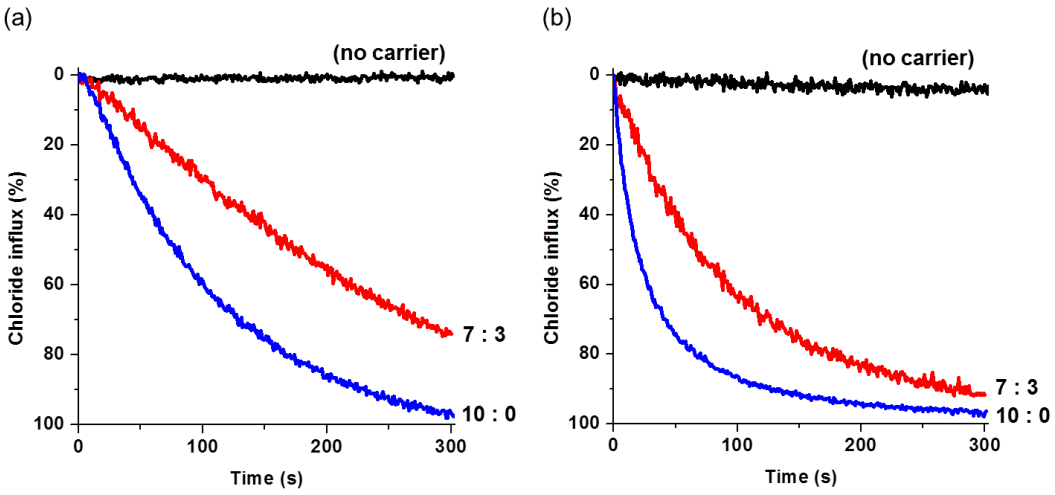


Figure S11. Comparison of chloride transport rates in vesicles composed of 10:0 and 7:3 molar ratios of POPC/cholesterol using (a) compound **2** and (b) compound **7** (2 mol% to lipid). Vesicles were loaded with $NaNO_3$ (200 mM), lucigenin (1 mM) and a phosphate buffer (10 mM, pH = 7.2) and suspended in a $NaNO_3$ solution (200 mM in 10 mM phosphate buffer at pH = 7.2).

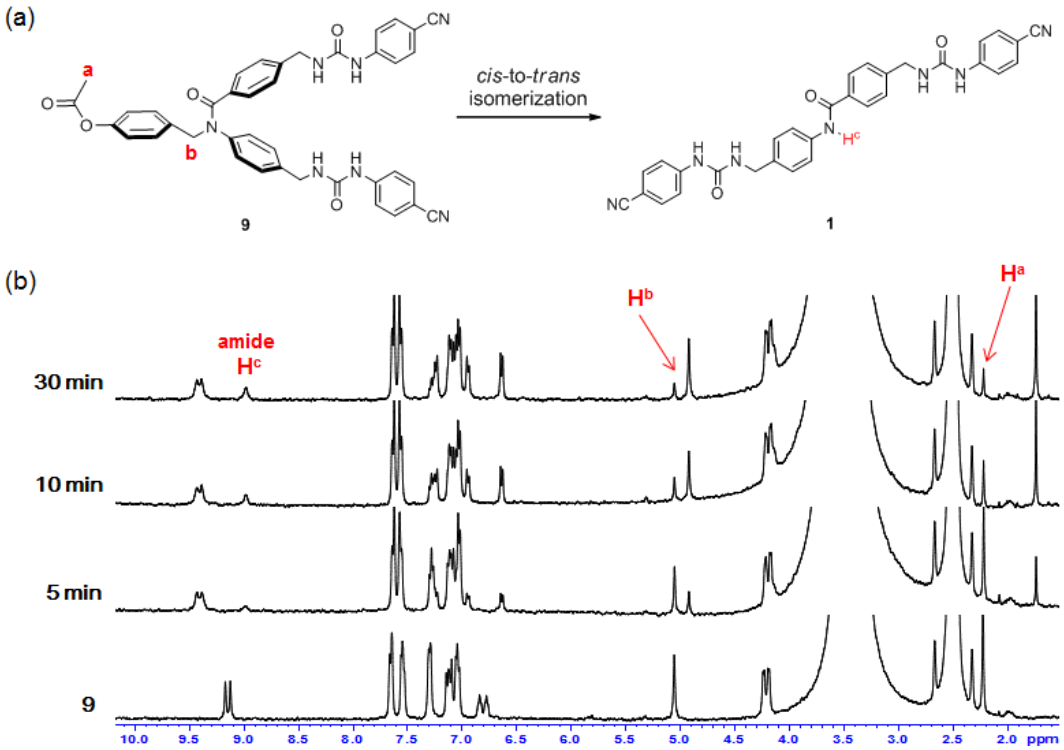


Figure S12. Time-dependent partial ^{1}H NMR spectral changes of **9** (1 mM in DMSO-d_6 at 24 ± 1 °C, 400 MHz) upon addition of hydrazine hydrate (10 equiv).

6. Stimuli-responsive chloride transport experiments

Hydrazine Method: Hydrazine hydrate (10 equiv) was injected to a solution of **9** in a 3:1 (v/v) DMSO : a phosphate buffer (pH = 7.2) and stirred for 10 min at 21 °C. A portion (5 μL) of the reaction mixture was added into a cuvette loaded with POPC vesicles (2 mL, lipid concentration : 1 mM). The vesicles contain a phosphate buffer (10 mM, pH = 7.2), lucigenin (1 mM) and $NaNO_3$ (200 mM), which were suspended in a phosphate buffer (10 mM, pH = 7.2) containing $NaNO_3$ (200 mM). A 30 μL of a NaCl solution in a phosphate buffer (10 mM, pH = 7.2) was injected to the cuvette solution to to start chloride transport by lucigenin assays. The chloride influx across vesicles was monitored by a decay in lucigenin fluorescence, which was measured at 501 nm emission upon excitation at 372 nm using fluorescence spectrophotometer. The inital reading was considered as 0% chloride transport and the reading after the addition of 10% Triton X-100 to the lipid solution at 300 s was considered as 100% transport possible. Transport experiments were repeated to **9** (14 μg, 1 mol% to lipid) and **1** (11 μg, 1 mol% to lipid).

Enzymatic Hydrolysis: 1 mg of PLE and 14 μg of **9** (1 mol% to lipid) were added to a 2 mL of POPC vesicles (1 mM) in a cuvette. The lipid solution contains a phosphate buffer (10 mM, pH = 7.2), lucigenin (1 mM) and $NaNO_3$ (200 mM), which were suspended in a phosphate buffer (10 mM, pH = 7.2) containing $NaNO_3$ (200 mM). After standing for 30 min at 21 $^{\circ}$C, a 30 μL of a NaCl solution in a phosphate buffer (10 mM, pH = 7.2) was injected to the cuvette solution to to start chloride transport by lucigenin assays. The chloride transport was analysed according to the precedure described above.