Supporting Information

Binding of Calix[4]pyrroles to Pyridine *N*-oxides Probed with Surface Plasmon Resonance

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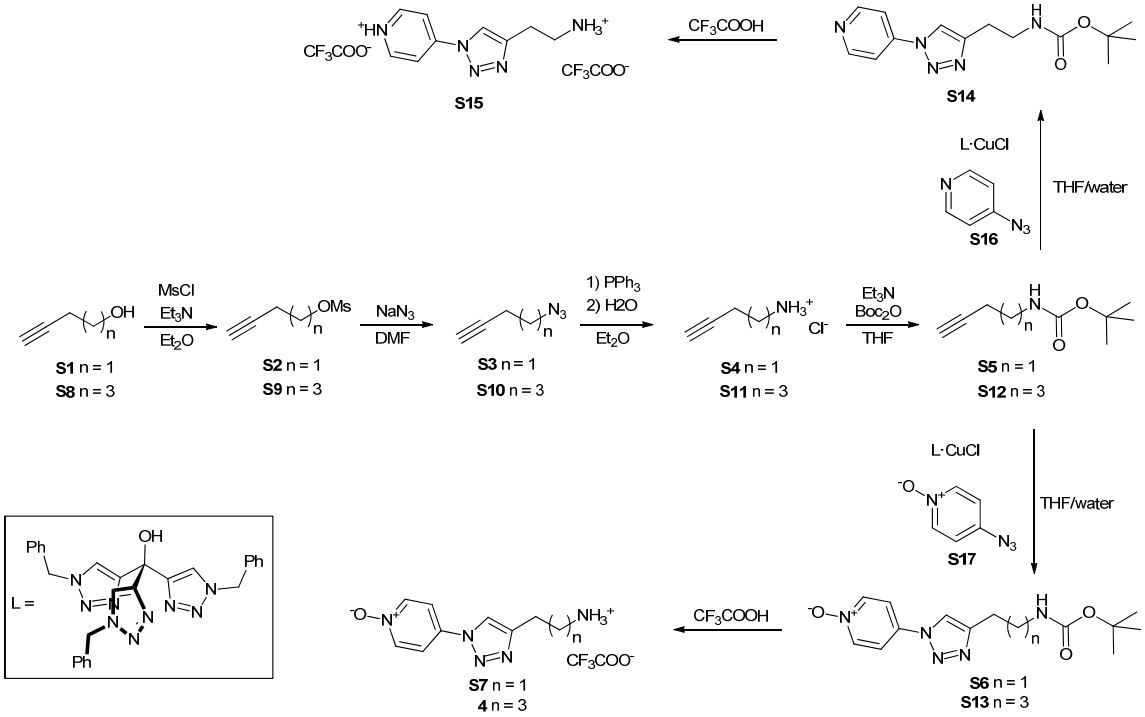
General Information and Instrumentation

Calixpyrrole **1** was prepared as previously described.[1] Unless otherwise stated, reagents and solvents were obtained from commercial suppliers and used without further purification. Routine ^{1}H NMR spectra were recorded on a Bruker Avance 300 (300 MHz for ^{1}H NMR), Bruker Avance 400 (400 MHz for ^{1}H NMR) or a Bruker Avance 500 (500 MHz for ^{1}H NMR) ultrashield spectrometer. The deuterated solvents (Aldrich) used are indicated in the experimental part; chemical shifts are given in ppm. For $CDCl_3$ the peaks were referenced relative to the solvent residual peak δH = 7.26 ppm and δC = 77.0 ppm. For D_2O the peaks were referenced relative to the solvent residual peak δH = 4.79 ppm. For CD_3OD the peaks were referenced relative to the solvent residual peak δH = 3.31 ppm. Deuterated borate buffer was prepared as follows. A solution of NaOH (80 mg) in D_2O (0.7 ml) is added to boric acid (470 mg). This is diluted to 3 mL with D_2O. The D_2O is removed in vacuo and the resultant white powder is diluted again in D_2O. This is repeated a further 2 times in an attempt to exchange all protons for deuteriums. Finally the solution is evaporated to dryness and the resultant white powder is dissolved in 10 mL D_2O to make a stock solution that is diluted 20 × before use. All NMR *J* values are given in Hz and are uncorrected. High resolution mass spectra were obtained on a Bruker Autoflex MALDI-TOF Mass Spectrometer. Mass spectra were recorded on a Waters LCT Premier ESI-TOF spectrometer, in an Agilent 1100 LC/MSD ESI-Quadrupole or in a Waters GTC spectrometer. Flash column chromatography was performed with Silica gel Scharlab60. Isothermal titration calorimetry experiments (ITC) were performed using a MicroCal VP-ITC Microcalorimeter. Surface plasmon resonance (SPR) analysis was performed using the Biacore 3000 system and a CM5 sensor chip. The kinetic and thermodynamic parameters were calculated using Biaeval 4.1 software. SPR data analysis was performed by using either the simple 1:1 Langmuir binding model or the 1:1 heterogeneous ligand model.

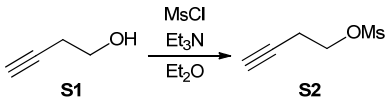
Synthesis

The immobilizable ligands **4**, **S7** and **S15** were prepared according to **Scheme S 1**.



Scheme S 1. Synthetic approach to immobilizable ligands.

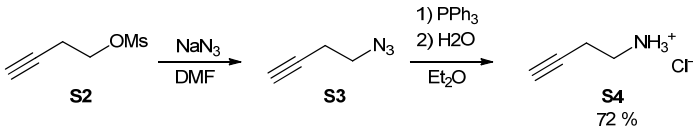
But-3-yn-1-ol methanesulfonate **S2**[2]



Methane sulfonyl chloride (4.6 mL, 59.5 mmol, 1.5 equiv.) is added dropwise under Ar to a stirred solution of but-3-yn-1-ol **S1** (3 mL, 39.6 mmol, 1 equiv.) and Et_3N (8.3 mL, 59.5 mmol, 1.5 equiv.) in dry Et_2O (40 mL, stirred over 4Å molecular sieves for an hour and then transferred by cannula to the alcohol) at 0 °C. The reaction is allowed to come to RT and stirs for a further 4 h. The reaction mixture is diluted with water (20ml) and the aqueous and organic layers are separated. The organic layer is washed with water 2× and then dried over Na_2SO_4, filtered and concentrated in vacuo to give 4.18 g of but-3-yn-1-yl methanesulfonate **S2** as a light yellow liquid, 71% yield.

^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC) δ (ppm) 2.07 (t, J = 2.7, 1H), 2.66 (td, J = 6.7, 2.7, 2H), 3.06 (s, 3H), 4.31 (t, J = 6.7, 2H).
^{1}H NMR spectrum of this compound is in agreement with that reported in the literature.[3]

But-3-yn-1-amine · HCl **S42**

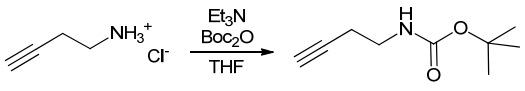


S2 **S3** **S4**
72 %

Sodium azide (4.28 g, 65.8 mmol, 2.5 equiv.) is added in batches over one minute to a well-stirred turbid solution of but-3-yn-1-yl methanesulfonate **S2** (3.9 g, 26.3 mmol, 1 equiv.) in dry DMF (25 mL, DMF prepared by stirring for an hour over 4Å molecular sieves) under Ar at RT in a schlenk flask. The reaction is heated to 70 °C and stirred for 4 h. A blast shield is placed in front of the reaction during this time. The reaction mixture is poured into water (20 ml) and the resulting solution is extracted 3x with Et_2O (do *not* use chlorinated solvents with NaN_3). The ether extracts are combined, washed with water, dried over Na_2SO_4, filtered and concentrated. The solvent removal is not pursued to completion due to the volatility of the product. This mixture of Et_2O, DMF, and but-3-yn-1-azide **S3** is taken directly to the reduction step.
A solution of but-3-yn-1-azide **S3** from above in Et_2O (100 mL, degassed by the passage of Ar gas) is cooled to 0 °C under Ar atmosphere. Triphenylphosphine (6.9 g, 26.3 mmol, 1 equiv.) is added and the reaction stirs at 0 °C for two hours. Water (4.1 mL) is added to the mixture and the reaction mixture stirs a further 20 h at RT. The reaction mix is poured into 10 % $HCl_{(aq)}$ (25 mL). The mixture is shaken and the aqueous and ether layers are separated. The aqueous layer is washed with DCM 3× and then concentrated to dryness under high vacuum, to give 2.0 g of but-3-yn-1-amine · HCl **S4** as an off-white solid, 72 % yield.

^{1}H NMR (300 MHz, D_2O, 25 ºC) δ (ppm) 2.54 (t, *J* = 2.6, 1H), 2.66 (td, *J* = 6.5, 2.7, 2H), 3.21 (t, *J* = 6.5, 3H).
^{1}H NMR spectrum of this compound is in agreement with that reported in the literature.3

tert-butyl but-3-yn-1-carbamate **S5**



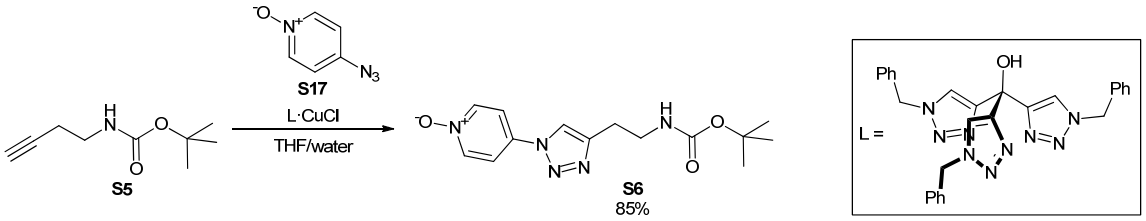
S4 **S5**
88%

Triethylamine (2.0 mL, 14.6 mmol, 2 equiv.) is added to a stirred mixture of ammonium salt **S4** (0.50 g, 7.28 mmol, 1 equiv.) and di-tert-butyl dicarbonate (1.58 g, 7.24 mmol, 1 equiv.) in dry THF (36 mL) under Ar atmosphere at RT. The mixture stirs at RT for 4 h after which time the reaction mixture is partitioned between a biphasic mixture of water (100 mL) and DCM (100 mL). The aqueous layer is washed with DCM 3×; the organic fractions are combined, dried over Na_2SO_4, filtered and reduced in vacuo to give the crude product as a yellow oil. This is purified by silica gel column chromatography (1:9 EtOAc:Hexane, Rf prod. = 0.3, stains blue with vanillin) to give 1.09 g of the pure product as a colorless oil, 88% yield.

^{1}H NMR (500 MHz, $CDCl_3$, 25 ºC) δ (ppm) 1.45 (s, 9H), 1.99 (t, *J* = 2.6, 1H), 2.38 (td, *J* = 6.5, 2.7, 2H), 3.28 (q, *J* = 6.5, 2H) 4.84 (broad s, 1H).

^{1}H NMR spectra of this compound are in agreement with those reported in the literature.[4]

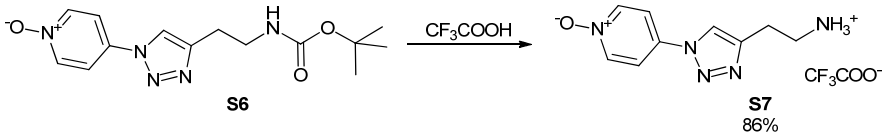
Pyridine *N*-oxide **S6**



Alkyne **S5** (105 mg, 0.62 mmol, 2.3 equiv.), 4-azido-pyridine-N-oxide **S17** (37 mg, 0.27 mmol, 1 equiv.) and L·CuCl[5] (8 mg, 0.013 mmol, 5 mol%) are all added to a schlenk tube. A stir bar is added and the system is purged three times with Ar. Degassed THF (1 mL) is added followed by degassed water (4mL). The reaction mixture is heated to 50 °C where the temperature is held for 2h. The reaction is cooled, the THF in the reaction mixture is evaporated and the resultant aqueous suspension is extracted with EtOAc (5 mL. 3×). The organic extracts are combined, dried over Na_2SO_4, filtered and concentrated in vacuo to give the crude product as a yellow residue. Silica gel column chromatography (gradient of DCM to 15% MeOH in DCM, Rf product = 0.35 for eluent of 10% MeOH in DCM) gives 137 mg of the pure product as a yellow solid, 85% yield.

Rf = 0.35 (1:9 MeOH:DCM, silica plate)
^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC) δ (ppm) 1.39 (s, 9H), 2.99 (t, *J* = 6.6, 2H), 3.51 (q, *J* = 6.6, 2H) 5.05 (broad s, 1H), 7.68-7.78 (broad m, 2H), 7.92 (s, 1H), 8.27 (broad s, 2H).
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$, 25 ºC) δ (ppm) 26.3 (CH_2), 28.3 (CH_3), 39.4 (CH_2), 79.4 (C), 116.4 (CH), 119.0 (CH), 133.5 (C), 140.6 (CH), 147.2 (C), 156.0 (C).
HR-MS (ESI+ve) m/z calcd for $C_{14}H_{19}N_5NaO_3$ ($[M+Na]^+$) 328.1380, found 328.1387.
IR $\tilde{\nu}$ (cm^{-1}) 846 s, 1203 s, 1502 s, 1548 s, 1711 vs, 3029 broad m, 3204 broad m
m.p. 194-195 °C.

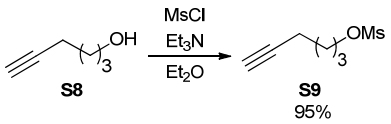
Pyridine *N*-oxide **S7**



Boc-protected amine **S6** (10 mg, 0.03 mmol) is dissolved in trifluoroacetic acid (1 mL). The solution is swirled for about one minute by hand. The trifluoroacetic acid is removed in vacuo to give a colorless glass. DCM (1 mL) is added to the flask and the mixture is sonicated producing a white precipitate. The precipitate is isolated and washed with DCM (1 mL, 2×) to give pure product **S7** as a white solid, 86% yield.

^{1}H NMR (400 MHz, D_2O, 25 ºC) δ (ppm) 3.24 (t, *J* = 7.2, 2H), 3.42 (t, *J* = 7.2, 2H), 8.11 (d, *J* = 7.5, 2H) 8.54 (d, J = 7.4, 2H), 8.57 (s, 1H).
^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$, 25 ºC) δ (ppm) 22.8 (CH_2), 38.6 (CH_3), 116.3 (q, *J* = 291.8, C),118.0 (CH), 122.2 (CH), 137.7 (C), 140.7 (CH), 144.6 (C), 162.9 (q, *J* = 35.5, C)
HR-MS (ESI+ve) m/z calcd for $C_9H_{12}N_5O$ ($[M–CF_3CO_2]^+$) 206.1036, found 206.1036.
IR $\tilde{\nu}$ (cm^{-1}) 830 s, 1121 s, 1502 s, 1681 broad s, 2914 broad m
m.p. 185 °C (decomposition).

Hex-5-yn-1-ol methanesulfonate **S9**[6]



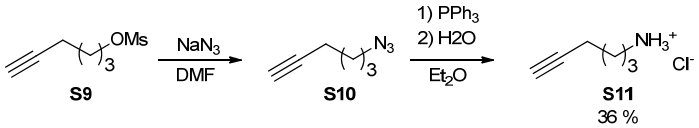
S8 **S9** 95%

Methane sulfonyl chloride (2.7 mL, 34.6 mmol, 1.5 equiv.) is added dropwise under Ar to a stirred solution of hex-5-yn-1-ol **S8** (2.5 mL, 23.1 mmol, 1 equiv.) and Et_3N (4.8 mL, 34.6 mmol, 1.5 equiv.) in dry Et_2O (30 mL, stirred over 4Å molecular sieves for an hour and then transferred by cannula to the alcohol) at 0 °C. The reaction is allowed to come to RT and stirs for a further 4 h. The reaction mixture is diluted with water (20ml) and the aqueous and organic layers are separated. The organic layer is washed with water 2× and then dried over Na_2SO_4, filtered and concentrated in vacuo to give 4.715 g of a light yellow liquid. This liquid is 82% hex-5-yn-1-ol methanesulfonate **S9** by weight (the rest being Et_2O); therefore, the material contains 3.866 g of **S9**, 95% yield.

^{1}H NMR (500 MHz, $CDCl_3$, 25 ºC) δ (ppm) 1.61–1.70 (m, 2H), 1.85–1.93 (m, 2H), 1.97 (t, *J* = 2.7, 1H), 2.26 (td, *J* = 6.9, 2.6, 2H), 3.01 (s, 3H), 4.26 (t, *J* = 6.3, 2H).

^{1}H NMR spectrum of this compound is in agreement with that reported in the literature with the exception of the td at 2.26 ppm which has been mistakenly labelled a dd in the literature.[7]

Hex-5-yn-1-amine · HCl **S112**



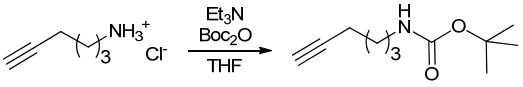
S9 **S10** **S11** 36 %

Sodium azide (3.77 g, 58.0 mmol, 2.5 equiv.) is added in batches over one minute to a well-stirred turbid solution of Hex-5-yn-1-yl methanesulfonate **S9** (4.09 g, 23.2 mmol, 1 equiv.) in dry DMF (23 mL, DMF prepared by stirring for an hour over 4Å molecular sieves) under Ar at RT in a schlenk flask. The reaction is heated to 70 °C and stirred for 4 h. A blast shield is placed in front of the reaction during this time. The reaction mixture is poured into water (20 ml) and the resulting solution is extracted 3x with Et_2O (do *not* use chlorinated solvents with NaN_3). The ether extracts are combined, washed

with water, dried over Na_2SO_4, filtered and concentrated. The solvent removal is not pursued to completion due to the volatility of the product. This mixture of Et_2O, DMF, and but-3-yn-1-azide **S10** is taken directly to the reduction step.
A solution of hex-5-yn-1-azide **S10** from above in Et_2O (93 mL, degassed by the passage of Ar gas) is cooled to 0 °C under Ar atmosphere. Triphenylphosphine (6.09 g, 23.2 mmol, 1 equiv.) is added and the reaction stirs at 0 °C for two hours. Water (4 mL, degassed by the passage of Ar gas) is added to the mixture and the reaction mixture stirs a further 20 h at RT. The reaction mix is poured into 10 % $HCl_{(aq)}$ (20 mL). The mixture is shaken and the aqueous and ether layers are separated. The aqueous layer is washed with DCM 3× and then concentrated to dryness under high vacuum, to give 1.13 g of hex-5-yn-1-amine HCl **S11** as a white solid, 36 % yield.

^{1}H NMR (300 MHz, D_2O, 25 ºC) δ (ppm) 1.57-1.68 (m, 2H), 1.74-1.86 (m, 2H), 2.30 (td, *J* = 6.9, 2.7, 2H), 2.41 (t, *J* = 2.7, 1H), 3.06 (t, *J* = 7.5, 2H).
^{1}H NMR (300 MHz, CD_3OD, 25 ºC) δ (ppm) 1.53-1.66 (m, 2H), 1.72-1.85 (m, 2H), 2.23-2.29 (m, 3H), 2.96 (t, *J* = 7.6, 2H).
^{1}H NMR spectrum of this compound is in agreement with that reported in the literature.[8]

tert-butyl hex-5-yn-1-carbamate **S12**



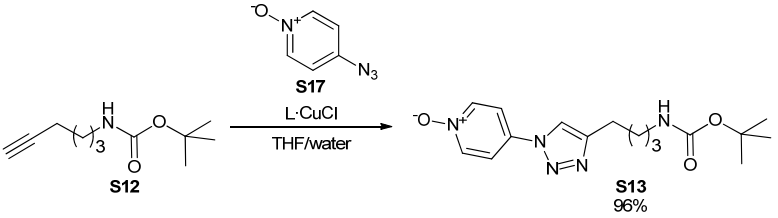
S11 → **S12** 81%

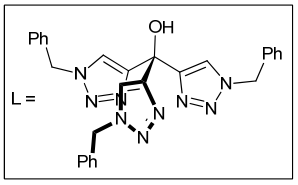
Triethylamine (1.1 mL, 7.63 mmol, 2 equiv.) is added to a stirred mixture of ammonium salt **S11** (0.51 g, 3.82 mmol, 1 equiv.) and di-tert-butyl dicarbonate (1.26 g, 5.77 mmol, 1.5 equiv.) in dry THF (20 mL) under Ar atmosphere at RT. The mixture stirs at RT for 4 h after which time the reaction mixture is partitioned between a biphasic mixture of water (100 mL) and DCM (100 mL). The aqueous layer is washed with DCM 3×; the organic fractions are combined, dried over Na_2SO_4, filtered and reduced in vacuo to give the crude product as a yellow oil. This is purified by silica gel column chromatography (1:9 EtOAc:Hexane, Rf prod. = 0.27, stains blue with vanillin) to give 0.61 g of the pure product **S12** as a colorless oil, 81% yield.

^{1}H NMR (500 MHz, $CDCl_3$, 25 ºC) δ (ppm) 1.44 (s, 9H), 1.52-1.63 (m, 4H), 1.95 (t, *J* = 2.6, 1H), 2.21 (td, *J* = 6.8, 2.6, 2H), 3.14 (q, *J* = 6.4, 2H) 4.52 (broad s, 1H).

^{1}H NMR spectra of this compound are in agreement with those reported in the literature.[9]

Pyridine *N*-oxide **S13**



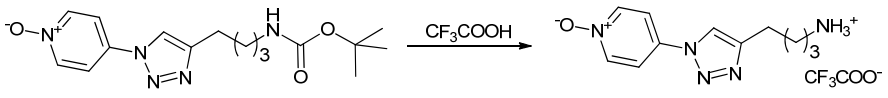


Alkyne **S12** (217 mg, 1.10 mmol, 2 equiv.), 4-azido-pyridine-N-oxide **S17** (75 mg, 0.55 mmol, 1 equiv.) and L·CuCl[5] (17 mg, 0.03 mmol, 5 mol%) are all added to a schlenk tube. A stir bar is added and the system is purged three times with Ar. Degassed THF (1 mL) is added followed by degassed water (4mL). The reaction mixture is heated to 50 °C where the temperature is held for 2h. The THF in the reaction mixture is evaporated and the resultant aqueous suspension is extracted with EtOAc (5 mL 3×). The organic extracts are combined, dried over Na_2SO_4, filtered and concentrated in vacuo to give the crude product as a yellow residue. Silica gel column chromatography (gradient of DCM to 15% MeOH in DCM, Rf product = 0.4 for eluent of 10% MeOH in DCM) gives 176 mg of the pure product as a yellow solid, 96% yield.

Rf = 0.40 (1:9 MeOH:DCM, silica plate)
^{1}H NMR (500 MHz, $CDCl_3$, 55 ºC) δ (ppm) 1.42 (s, 9H), 1.58 (p, *J* = 7.6, 2H), 1.76 (p, *J* = 7.5, 2H), 2.83 (t, *J* = 7.3, 2H), 3.15 (q, *J* = 6.7, 2H) 4.60 (broad s, 1H), 7.80 (broad s, 2H), 7.85 (s, 1H), 8.11 (broad s, 2H).
^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$, 55 ºC) δ (ppm) 24.9 (CH2), 26.1 (CH_2), 28.4 (CH_3), 29.4 CH2), 40.3 (CH_2), 79.2 (C), 115.7 (CH), 118.7 (CH) 134.8 (C), 142.1 (CH), 149.9 (C), 156.1 (C).
HR-MS (ESI+ve) m/z calcd for $C_{16}H_{23}N_5NaO_3$ ($[M+Na]^+$) 356.1693, found 356.1696.
IR $\tilde{\nu}$ (cm^{-1}) 775 s, 1221 s, 1503 s, 1684 s, 2976 m, 3355 m
m.p. 109-110 °C.

NMR spectra of this compound are measured at 55 °C to coalesce signals that result from dynamic processes.

Pyridine *N*-oxide **4**



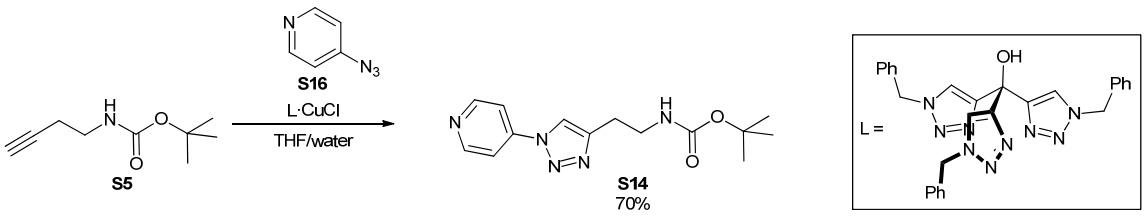
S13 **4** 60%

Boc-protected amine **S13** (34 mg, 0.10 mmol) is dissolved in trifluoroacetic acid (2 mL). The solution is swirled for about one minute by hand. The trifluoroacetic acid is removed in vacuo to give a colorless glass. DCM (1 mL) is added to the flask and the mixture is sonicated, eventually giving a pale yellow solution. A white solid precipitates from this solution over several minutes. The precipitate is isolated and washed with DCM (1 mL, 2×) to give pure product **4** as a white solid, 60% yield.

^{1}H NMR (400 MHz, D_2O, 25 ºC) δ (ppm) 1.65-1.89 (m, 4H), (t, *J* = 7.2, 2H), 2.87 (t, *J* = 7.0, 2H), 3.05 (d, *J* = 7.1, 2H) 8.06 (d, J = 6.9, 2H), 8.43 (s, 1H), 8.51 (d, J = 6.9, 2H).
^{13}C{^{1}H} NMR (75 MHz, D_2O, 25 ºC) δ (ppm) 23.8 (CH_2), 25.2 (CH2), 26.0 (CH2), 39.1 (CH_2), 116.4 (q, *J* = 292.6, C), 117.7 (CH), 121.2 (CH), 137.8 (C), 140.6 (CH), 149.2 (C), 162.9 (q, *J* = 35.1, C).
HR-MS (ESI+ve) m/z calcd for $C_{11}H_{16}N_5O$ ($[M–CF_3CO_2]^+$) 234.1349, found 234.1352.
IR $\tilde{\nu}$ (cm^{-1}) 1119 s, 1183 s, 1503 s. 1692 broad s, 2949 broad m

m.p. 155-158 °C.

Pyridine **S14**

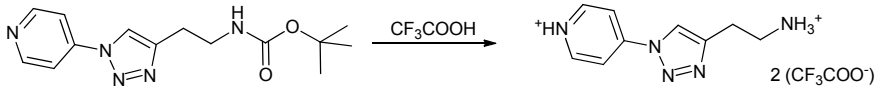


Alkyne **S5** (321 mg, 1.90 mmol, 2.3 equiv.), 4-azido-pyridine **S16** (99 mg, 0.83 mmol, 1 equiv.) and L·CuCl[5] (26 mg, 0.043 mmol, 5 mol%) are all added to a schlenk tube. A stir bar is added and the system is purged three times with Ar. Degassed THF (1 mL) is added followed by degassed water (5mL). The reaction mixture is heated to 50 °C where the temperature is held for 2h. The THF in the reaction mixture is evaporated and the resultant aqueous suspension is extracted with EtOAc (5 mL, 3×). The organic extracts are combined, dried over Na_2SO_4, filtered and concentrated in vacuo to give the crude product as a yellow residue. Silica gel column chromatography (gradient of 3 % MeOH in DCM to 20% MeOH in DCM, Rf product = 0.60 for eluent of 10% MeOH in DCM) gives a yellow solid that is washed with Et_2O (3 mL) giving a 137 mg of the pure product **S14** as a pale blue solid, 70% yield.

Rf = 0.60 (1:9 MeOH:DCM, silica plate)
^{1}H NMR (Note that the proton spectrum experiences extensive broadening in all solvents and even at elevated temperatures. For this reason, and because this compound is an intermediate en-route to the final amino-pyridine **S15**, not all of the peaks are described here) (500 MHz, $CDCl_3$, 55 ºC) δ (ppm) 1.41 (s, 9H), 3.00 (t, J = 6.6, 2H), 3.54 (q, J = 6.6, 2H), 5.00 (broad s, 1H).
^{13}C{^{1}H} NMR (Note that extensive-signal broadening in all solvents and even at elevated temperatures was unavoidable. For this reason, and because this compound is an intermediate en-route to the final amino-pyridine **S15**, the assignment of the type of carbon is not attempted) (125 MHz, $CDCl_3$, 55 ºC) δ (ppm) 26.3, 28.3, 39.5, 79.4, 116.4, 118.9, 143.0, 146.9, 151.5, 156.0
HR-MS (ESI+ve) m/z calcd for $C_{14}H_{19}N_5NaO_2$ ($[M+Na]^+$) 312.1431, found 312.1426.
IR $\tilde{\nu}$ (cm^{-1}) 1164 s, 1283 s, 1548 s, 1595 s, 1682 s, 3088 m, 3245 m
m.p. 138-140 °C.

NMR spectra of this compound are measured at 55 °C to coalesce signals that result from dynamic processes.

Pyridine *N*-oxide **S15**

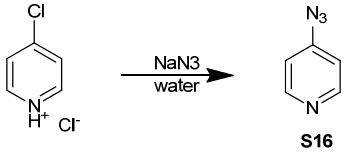


S14 **S15** X%

Boc-protected amine **S14** (18 mg, 0.06 mmol) is dissolved in trifluoroacetic acid (1.5 mL). The solution is stirred for 30 min. The trifluoroacetic acid is removed in vacuo to give a colorless glass. The residue is partitioned between DCM (1 mL) and water (1mL). The water layer is separated and washed with DCM 4×. The water fraction is concentrated in vacuo to give 24 mg of the product **S15** as a colorless glass, 92% yield.

^{1}H NMR (500 MHz, D_2O, 25 ºC) δ (ppm) 3.29 (t, J = 7.3, 2H), 3.46 (t, J = 7.2, 2H), 8.52 (d, J = 7.2, 2H) 8.77 (s, 1H), 9.01 (broad s, 2H).
^{13}C{^{1}H} NMR (125 MHz, D_2O, 25 ºC) δ (ppm) 22.8 (CH_2), 38.4 (CH_2), 116.6 (q, J = 290.1, C), 116.9 (CH), 122.6 (CH), 143.8 (CH), 145.4 (C), 149.1 (C), 162.7 (q, J = 35.9, C).
MS (ESI+ve) 190.1 ([M–CF_3CO_2H-CF_3CO_2]$^+$)
IR $\tilde{\nu}$ (cm^{-1}) 1119 s, 1183 s, 1633 broad s, 1672 s, 3106 broad m

4-azido-pyridine **S16**[10]

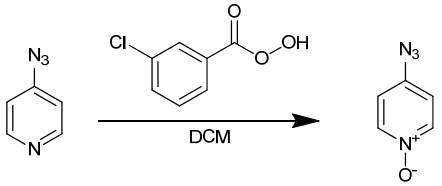


S16
57%

4-Chloro pyridinium chloride (2.49 g, 16.6 mmol, 1 equiv.) is added in small batches over about 3 min to a stirred solution of sodium azide (2.374 g, 36.5 mmol, 2.2 equiv.) in water (16.6 mL). The reaction is refluxed for 36 hours protected from light, open to air and with a blast shield. The reaction is cooled to RT and extracted with EtOAc (3×, no chlorinated solvents with NaN3!). The extracts are combined, dried over Na_2SO_4, filtered and concentrated to give 1.13 g of 4-azido-pyridine **S16** as an orange liquid.

^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC) δ (ppm) 6.94 (d, J = 6.5, 2H), 8.52 (d, J = 6.4, 2H).
^{1}H NMR spectrum of this compound is in agreement with that reported in the literature.[10]

4-azido-pyridine-*N*-oxide **S17**



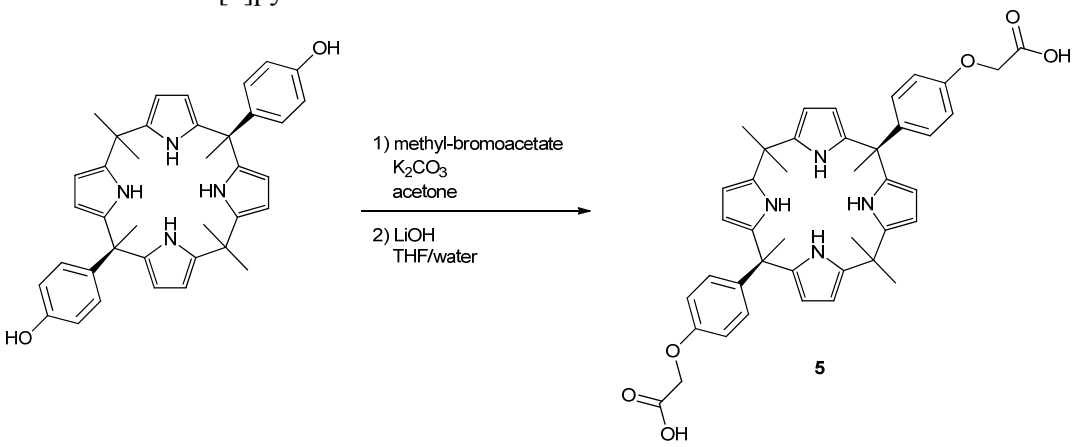
S16 **S17**
76%

Meta-chloro-peroxybenzoic acid (1.74 g, 7.74 mmol, 1 equiv.) is added in batches over 1 min to a stirred solution of 4-azido-pyridine **S16** (0.93 g, 7.74 mmol, 1 equiv.) in DCM (40 mL) under Ar at 0 °C. The addition is effected by simply removing the stopper of the flask and adding each batch of the peroxyacid quickly before re-stoppering the flask. When the addition is complete, the reaction comes to RT and stirs at this temperature for 13 hours.

The solution is concentrated in vacuo giving a pale yellow solid. This solid is purified on silica (eluent = DCM for 15 min then a gradient of DCM to 10% MeOH in DCM over 15 min) to give 800 mg of pure material as an orange solid, 76% yield.

Rf = 0.3 (5% MeOH in DCM, silica plate)
^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC) δ (ppm) 6.92 (d, *J* = 7.6, 2H), 8.14 (d, *J* = 7.6, 2H).
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$, 25 ºC) δ (ppm) 116.47 (CH), 138.77 (C), 140.21 (CH).
NMR spectra of this compound are in agreement with that reported in the literature.[11]

"Two-wall" calix[4]pyrrole **5**



Methyl-bromoacetate (0.16 mL, 1.77 mmol, 6.9 equiv) is added to a suspension of "two-wall" *para*-hydroxy-phenyl calixpyrrole (150 mg, 0.26 mmol, 1 equiv.) and K_2CO_3 (228 mg, 1.65 mmol, 6.5 equiv.) in dry acetone (50 mL). This mixture is refluxed for 5 days. After cooling the solution is filtered to remove excess K_2CO_3, and the acetone is removed in vacuo to give an orange oil. This oil is dissolved in dichloromethane and washed with water. The organic phase is separated, dried with $MgSO_4$ and the solvent is removed in vacuo affording an oil which is triturated with ethanol affording the methyester as a white powder which is collected by filtration and dried under high vacuum (180 mg). This material is added to a flask followed by a 1:1 mixture of THF and water. This makes a turbid mixture. LiOH (24 mg, 1 mmol, 4 equiv.) is added to this mixture causing the turbidity to disappear and resulting in a clear solution. The THF is evaporated under reduced pressure and the aqueous solution is acidified with $HCl_{(aq)}$ (1M) forming a precipitate. This mixture is extracted with ethylacetate 3×, the organic layers are combined, dried over $MgSO_4$ and evaporated under reduced pressure to give the desired product as a light brown powder.

^{1}H NMR (400 MHz, deuterated borate buffer, 25 ºC) δ (ppm) 1.53 (s, 6H), 1.59 (s, 6H), 1.88 (s, 6H), 4.46 (s, 4H), 5.72 (d, *J* = 3.3, 4H), 5.98 (d, *J* = 3.3, 4H), 6.86 (d, *J* = 8.9, 4H), 7.00 (d, *J* = 8.9, 2H).
HR-MS (ESI-ve) m/z calcd for $C_{42}H_{42}N_4O_6$ ($[M-2H]^{2-}$) 349.1558, found 349.1561.
IR $\tilde{\nu}$ (cm^{-1}) 1142 s, 1598 s, 1713 s, 3255 broad m
m.p. 133.135 °C.

Surface Plasmon Resonance Studies

Surface plasmon resonance (SPR) analysis was performed using the Biacore 3000 system and a CM5 sensor chip. All biosensor assays were performed using borate buffer as a running buffer (7 g NaOH and 47 g boric acid disolved in 1 L H_2O, The resultant solution is diluted 20X with H_2O before use, pH = 8.2).

Loading: The chip consists of four channels that were loaded as described in the results and discussion section of the manuscript. Briefly, the carboxymethylated dextran matrix was activated *via* the passage of EDC·HCl and NHS (0.2 M and 0.05 M, respectively, in water). Then, a 1 mM solution of *N*-oxide **4**, **S7**, or control pyridine **S15** in PBS was passed over the surface. Finally, any remaining un-reacted active ester groups were consumed by the passage of a water solution of ethanolamine. Each step of the activation process and indeed the activation process as a whole was followed by monitoring the signal change (in response units, RU).

Regeneration: The sensor chip surface was regenerated after each experiment by passing sodium dodecyl sulphate (SDS, 100 mM) over the surface for 2 min. This simple procedure reliably returned the original signal response seen before the binding experiment started.

Data analysis: The kinetic and thermodynamic parameters were calculated using Biaeval 4.1 software. Analysis was performed by using either the simple 1:1 Langmuir binding model or the 1:1 heterogeneous ligand model. This was done by following the thorough instructions provided in the BIAevaluation software handbook. A short synopsis of the steps required is given below.

Firstly the optimum buffer solution, flow rate, and regeneration conditions had to be determined. This was done by thoughtful variation. Essentially, different buffers and flow rates were tried until the association and dissociation curves fit reasonably to a 1:1 binding model. In this case, only borate buffer gave satisfactory results. On the other hand, the flow rate could be varied extensively and still give data that reasonably matched the theoretical fits. The regeneration conditions are very important to ensure that each experiment starts with the same, analyte-free surface. We spent a considerable time trying many different regeneration agents to find a method that was very rapid and reproducibly returned the surface to the pre-injection state. Several methods worked sufficiently well, however, sodium dodecyl sulphate (SDS) significantly outperformed all other regeneration agents.

Once these conditions had been determined, several experiments were performed that each comprised an association phase, a dissociation phase and a regeneration phase. These experiments were performed using different concentrations of analyte, from blank buffer to the concentrations described in the main manuscript and Figure S1. At each concentration, the analyte solution was simultaneously passed through the channel of interest (the channel displaying the immobilized ligand) and the control channel (the channel displaying immobilized ethanolamine).

Once all the experiments had been performed, the data collected for the working channel was corrected for non-binding signal response. The first step was to subtract the data obtained from the simultaneous injection through ethanolamine control channel 1 from the data obtained using the working channel. This is done as even small changes in concentration of the analyte are enough to affect the response of the SPR. Thus, for higher concentrations of analyte in the buffer solution, the machine´s response departs further from the baseline represented by the passage of blank, running buffer. By subtracting control channel 1, this non-binding response is eliminated. Then, the data obtained from the

blank buffer injection through the working channel was subtracted from each experiment to account for any baseline drift that may occur. In our case the baseline drift was minimal and this final step was not essential. Finally, the data was X- and Y-transformed such that the baseline and injection of each experiment were identical. It should be noted that, if the regeneration process is efficient, only slight Y-transformation should be needed to ensure a uniform baseline.

Once the data has been treated as explained, the process of fitting could begin. The BIAevaluation software allows one to separately fit the association or dissociation processes or simultaneously fit the whole association/dissociation process. For the work shown in this paper, both the binding and release processes were important and so we fit the association and dissociation processes simultaneously. The experiments that are relevant are selected for fitting (i.e. data obtained with several different concentrations of analyte) and assigned an injection-start point, an association period, an injection stop point and a dissociation period. These will be identical for each experiment and so it is important that the data has been properly X- and Y-transformed. Then, the type of fit must be identified.

At first we fit the data with a 1:1 Langmuir model. When performing this fit, several parameters can be fixed (i.e. the concentration of the analyte), told to vary but to give one value that will be identical for all the experiments (fit globally) or told to vary and give a value that is different for each experiment (fit locally). It is even possible to fit the calculated k_{on} and k_{off} values locally. This will obviously give a better fit but will mean that each experiment (which uses different concentrations of analyte but in theory represents the same binding process) has a different association and dissociation rate constant, a situation that is far from ideal. As a consequence, when given this freedom, the program will give values of K_a that also vary. For the experiments in this paper, the concentrations were fixed, the k_{on} and k_{off} values were fit globally and the R_{max} value was fit locally. R_{max} represents the calculated maximum change in response should all the available ligand immobilized on the surface be bound to the analyte. Ideally, one should fit all the non-concentration parameters globally, however, in our case local fitting of R_{max} helped to achieve a better fit. Then, when all the parameters have been set, the program can be told to fit the data and will give a plot similar to those shown in Figure S1 and a table that contains the calculated values.

The process of fitting to the 1:1 heterogeneous ligand model is very similar. The only difference is that there are now two sets of k_{on}, k_{off} and R_{max} values for the processes happening at the two different ligand sites. Again these values can either be fit locally or globally. In this case, all k_{on} and k_{off} values were fit globally, however, as before, the R_{max} for both processes were fit locally.

By following these steps and the BIAevaluation software handbook version 3.0, we determined the kinetic and thermodynamic values that are reported in this paper.

SPR Studies with "4-wall" receptor 1

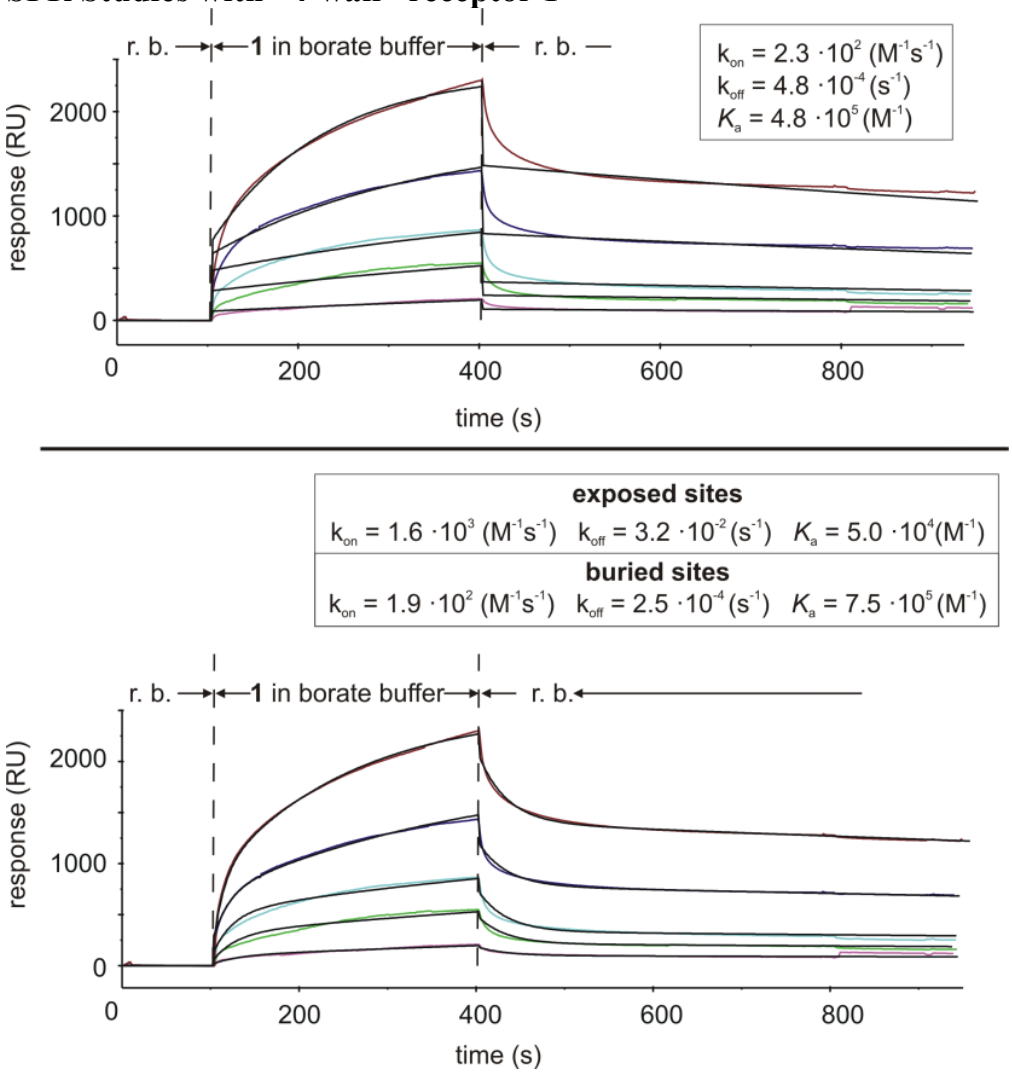


Figure S 1. Response of channel 3 (corrected for non-specific binding and bulk effect by subtraction of channel 1 and baseline drift by subtraction of injection of blank-buffer in channel) to the passage (20 µL/min) of different concentrations of calix[4]pyrrole **1** in borate buffer (2.1 µM, 4.2 µM, 8.3 µM, 16.6 µM, 33.2 µM). r. b. = running buffer = borate buffer. Black lines represent the theoretical binding curves obtained by fitting the data to a 1:1 binding model (top, Langmuir binding in Biaevaluation software version 4.1, Biacore AB) or a 1:1 binding model considering two distinct ligands (bottom, heterogeneous ligands model in Biaevaluation software version 4.1, Biacore AB). The SPR angle change is reported as response units (RU), where 1000 RU correspond to an angle change of ~ 0.1º.

Thermodynamics of surface-supported binding process

To investigate the thermodynamic properties of the binding process on the surface, we performed SPR studies similar to those shown in Figure S 1 at different temperatures and then inserted the obtained binding constants into the van´t Hoff equation. Specifically, by plotting the natural log of the binding constant against the inverse of the temperature, ΔH and ΔS could be extracted from the slope and y intercept of the linear regression line using the integrated van´t Hoff equation (equation 1). This returned values of ΔH = -9.1 kcal/mol and $T\Delta S$ = -1.1 at 298 K for the binding of receptor **1** to the *N*-oxide derivative immobilized to the surface of channel 3.

Table S 1. Binding constants and the natural log of those binding constants for the complex formed between "four-wall" calixpyrrole **1** and the *N*-oxide immobilized to the surface of channel 3 at different temperatures.

T (K)	1/T×10^3	K_a (M^{-1})	Ln(Ka)
290	3.45	$1.2\ 10^6$	14.01
295	3.39	$9.0 \cdot 10^5$	13.71
303	3.30	$7.2 \cdot 10^5$	13.49
308	3.25	$4.4 \cdot 10^5$	13.00

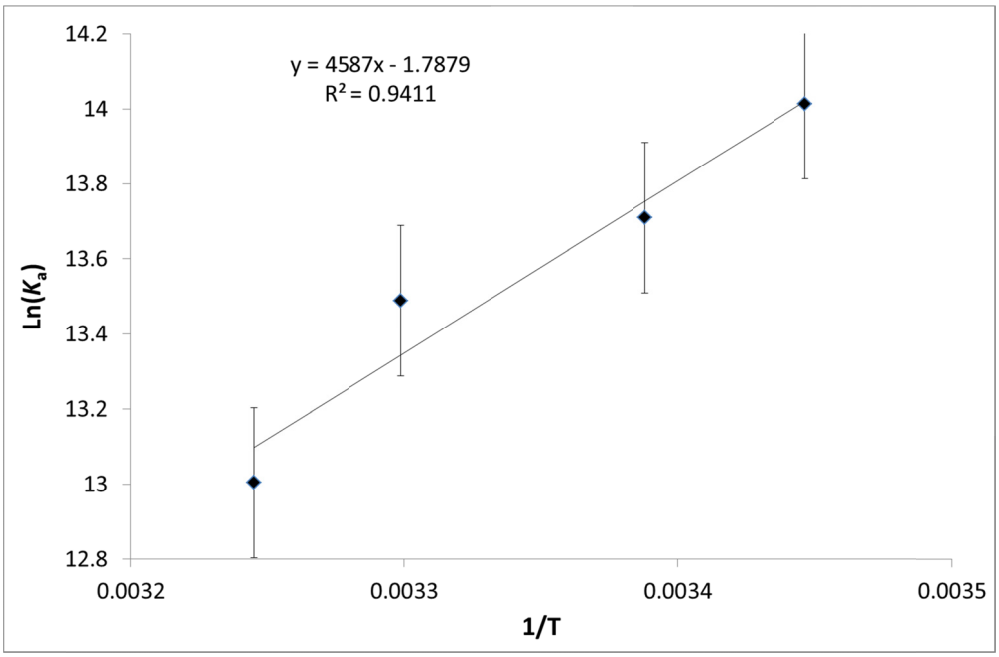


Figure S 2 Plot of Ln(K_a) vs. 1/T for the complex formed between "four-wall" calixpyrrole **1** and the *N*-oxide derivative immobilised to the surface of channel 3 at different temperatures. The equation shown defines the linear regression line obtained from the data fit and was used to determine ΔH and ΔS for the binding processes using the slope, y intercept and the integrated van't Hoff equation (equation 1). Error bars are derived from the propagation of a 20% of error in the calculated binding constant value.

(Equation 1) $\text{Ln}(K_a) = -(\Delta H/\text{R})\ 1/\text{T} + \Delta\text{S/R}$.

ITC Titrations (thermodynamic study)

Both titrations were carried out on a Microcal VP-ITC microcalorimeter, at 298 K, in borate buffer (7 g NaOH and 47 g boric acid disolved in 1 L H_2O, The resultant solution is diluted 20× with H_2O before use, pH = 8.2). The association constant between receptor **1** and pyridine *N*-oxide **3** was determined by monitoring the heat released by the system as incremental amounts of the *N*-oxide **3** were added. The values of the association constant K_a and the enthalpy of binding ΔH were calculated using the Origin 7 software package which uses least-squares minimization to obtain globally optimized parameters as described in Wiseman *et al*.[12] In all cases the data fit well to a simple 1:1 binding model.

Specifically, the association constant was determined using either a 0.4 or 1.5 mM solution of **1** in borate buffer at 298 K, and adding aliquots of a solution of pyridine *N*-oxide **3**, approximately 10 times more concentrated, also in borate buffer. The association constant (Ka), $T\Delta S$ and ΔH values for the binding process were determined by averaging the values from the two titrations.

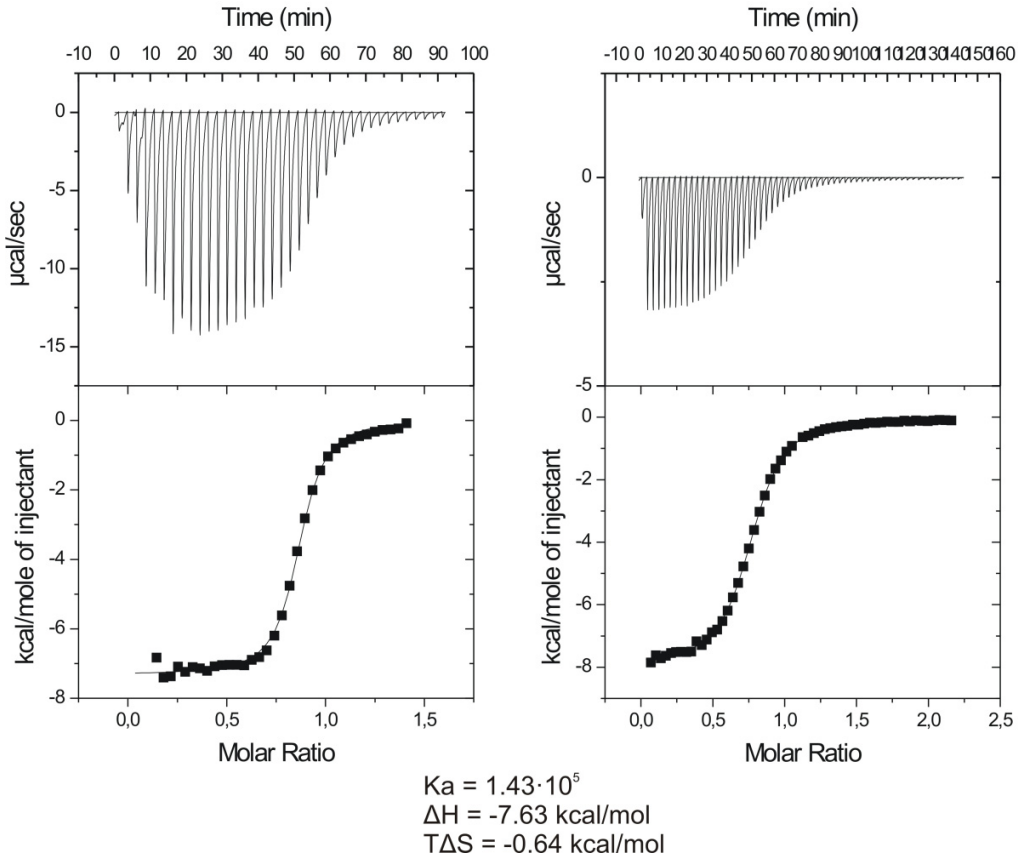


Figure S 3. Top: Raw data for the ITC titration of pyridine *N*-oxide **3** into calix[4]pyrrole receptor **1** in borate buffer. Bottom: Binding isotherm of the calorimetric titration data shown on top. The enthalpy of binding for each injection is plotted against the molar ratio guest/host in the cell. The continuous black line represents the least squares fit of the data to a 1:1 binding model. left) [**1**] = 1.5 mM right) [**1**] = 0.4 mM.

NMR study (kinetic study)

To characterize the kinetic stability of the N-oxide⊂**1** complexes, we performed 2D EXSY experiments at 298 K in deuterated borate buffer. The buffer was prepared as follows. A solution of NaOH (80 mg) in D_2O (0.7 ml) is added to boric acid (470 mg). This is diluted to 3 mL with D_2O. The D_2O is removed in vacuo and the resultant white powder is diluted again in D_2O. This is repeated a further 2 times in an attempt to exchange all protons for deuteriums. Finally the solution is evaporated to dryness and the resultant white powder is dissolved in 10 mL D_2O to make a stock solution that is diluted 20 × before use.

These experiments were performed using simple pyridine *N*-oxide **3** (Figure S 5) or the pyridyl *N*-oxide derivative **S6** described in this paper (Figure S 4). EXSY spectra of the complexes were obtained with both excess *N*-oxide and excess receptor **1**. Based on the integration of the diagonal and the cross peaks due to the chemical exchange of free and bound guest or host, we calculated the exchange rate constant using the EXSYCalc software developed by MestReC. The value for k_{off} was independent of the *N*-oxide used and was determined to be 1.4 s^{-1}.

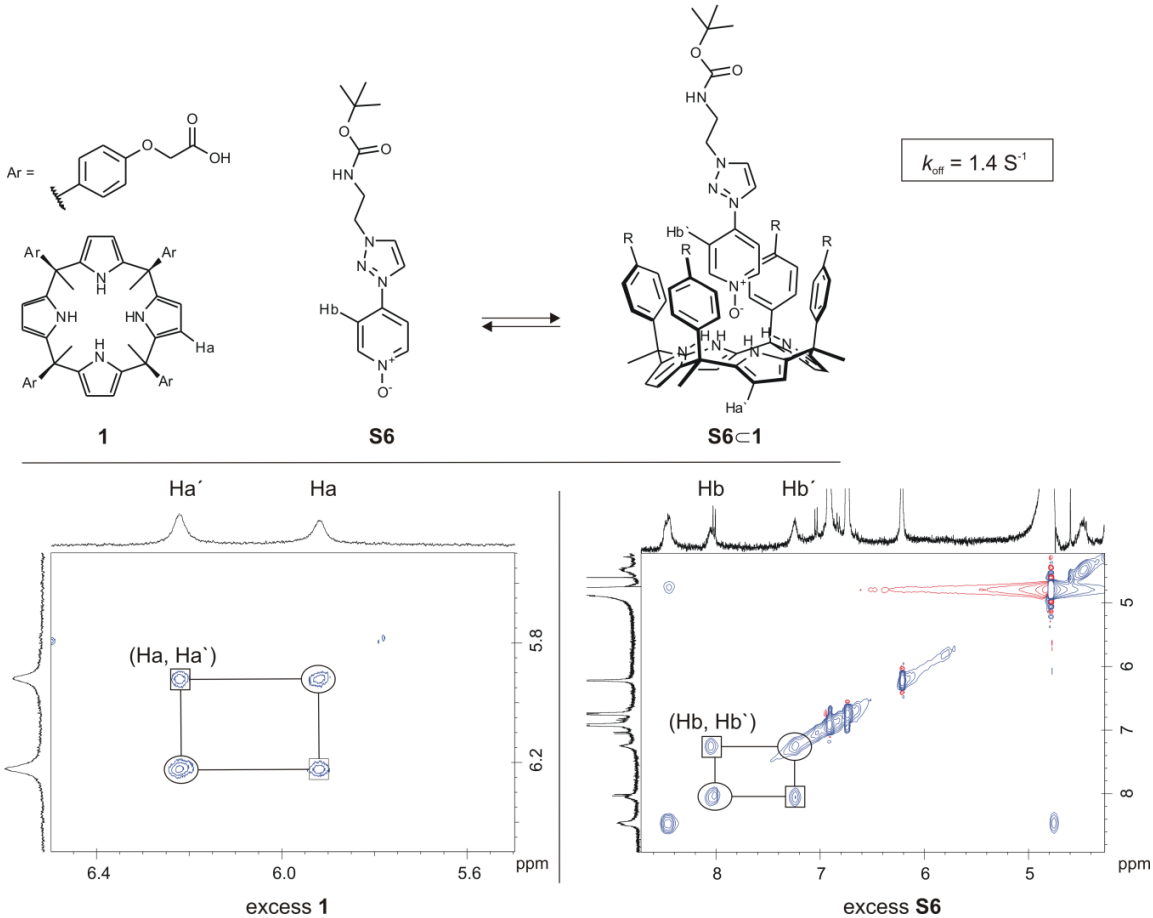


Figure S 4. Selected regions of the 2D EXSY spectra of complex **S6**⊂**1** in deuterated borate buffer in the presence of excess receptor **1** (left) and excess *N*-oxide **S6** (right).

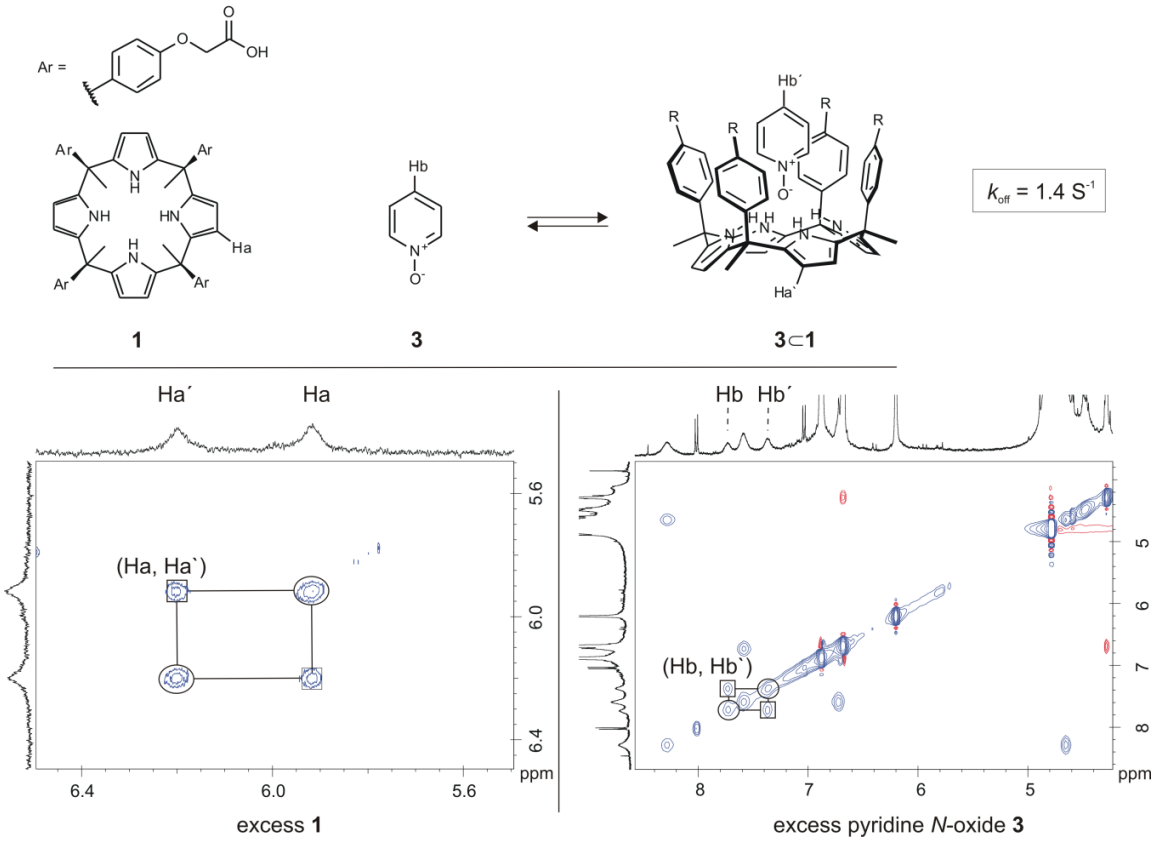


Figure S 5. Selected regions of the 2D EXSY spectra of complex **3⊂1** in deuterated borate buffer in the presence of excess receptor **1** (left) and excess *N*-oxide **3** (right).

Spectral Data for Novel Compounds.

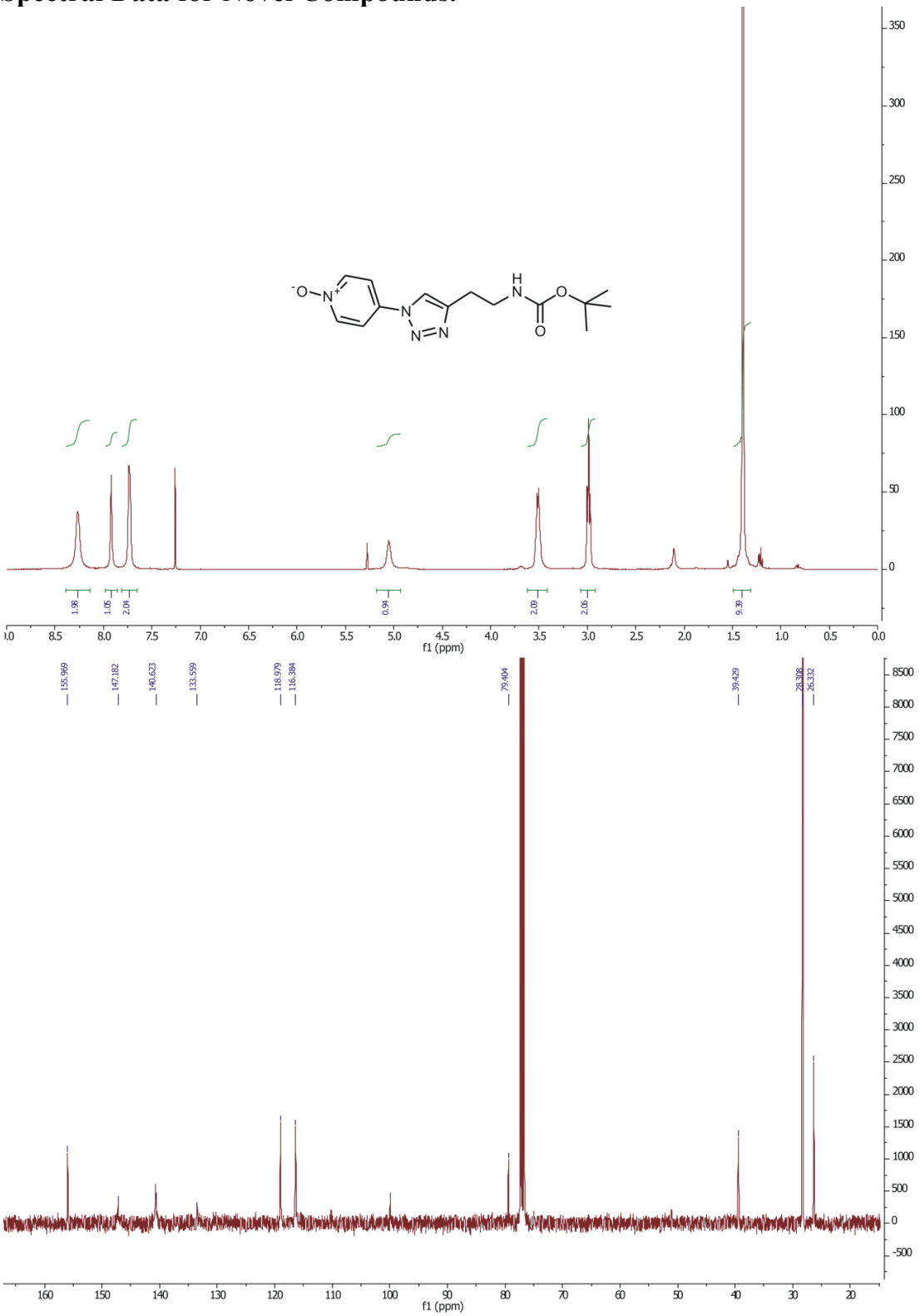


Figure S 6. ^{1}H (top) and ^{13}C (bottom) NMR spectra ($CDCl_3$, 25°C) for N-oxide **S6**.

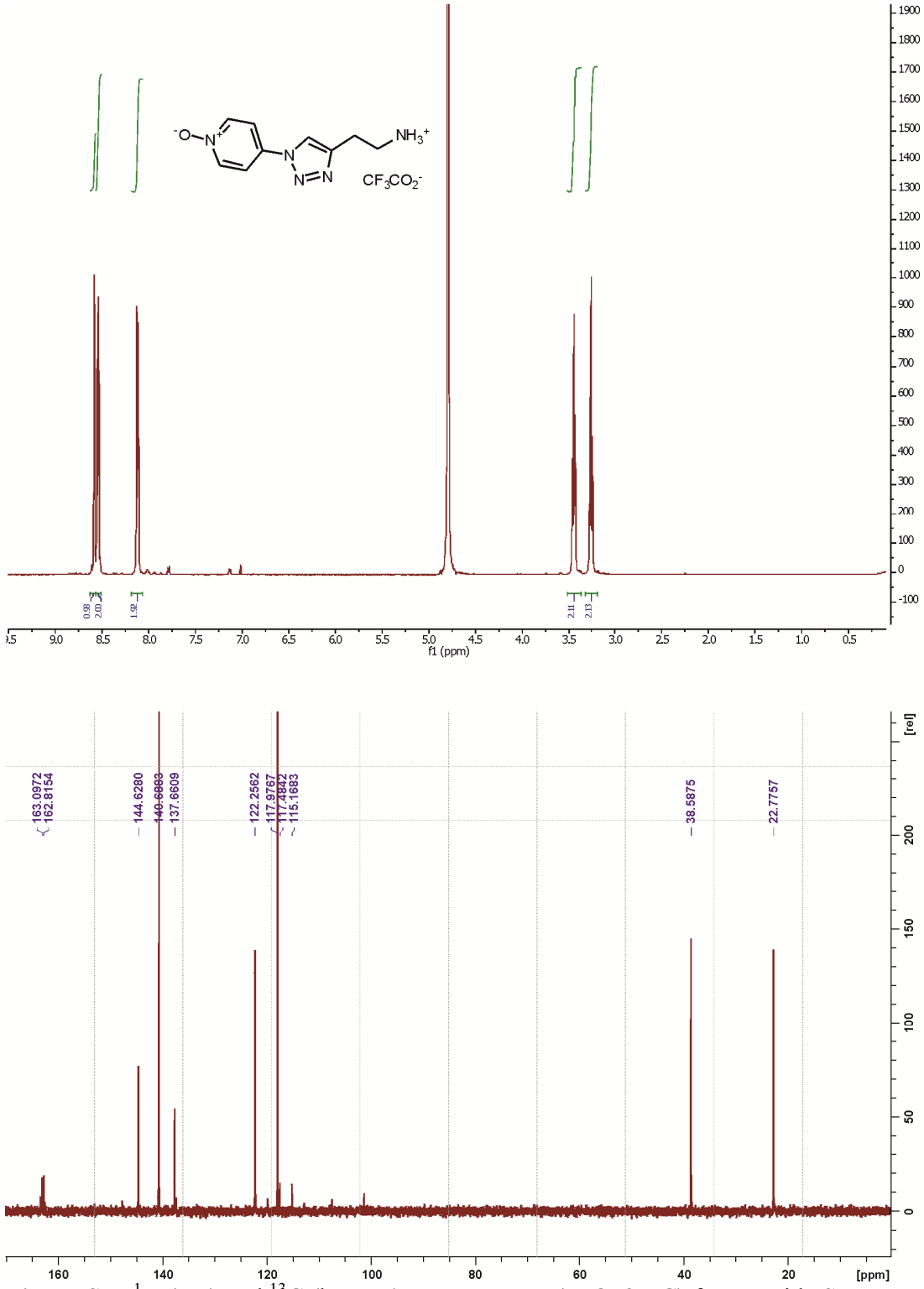


Figure S 7. ^{1}H (top) and ^{13}C (bottom) NMR spectra (D_2O, 25°C) for N-oxide **S7**.

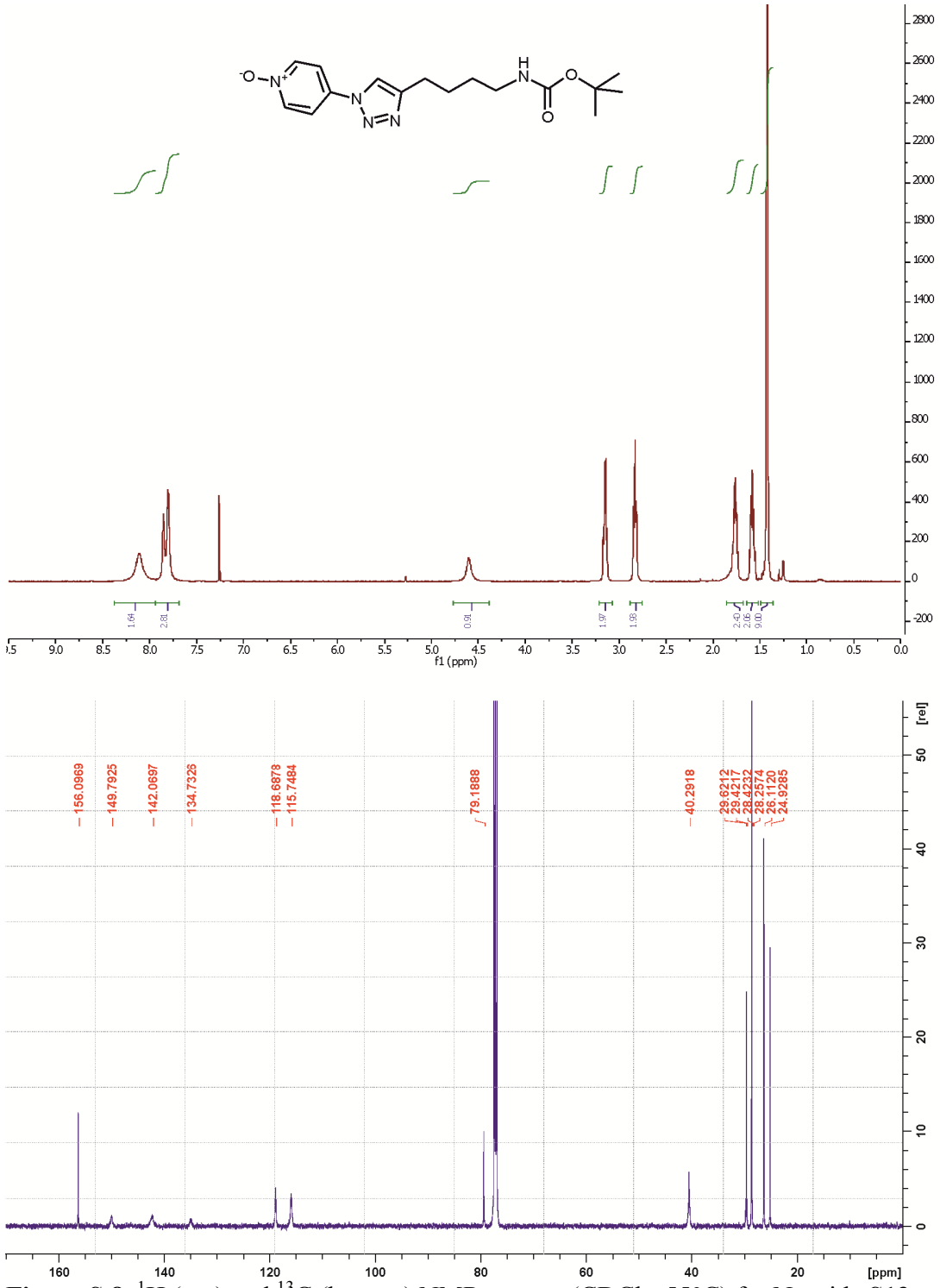


Figure S 8. ^{1}H (top) and ^{13}C (bottom) NMR spectra ($CDCl_3$, 55°C) for N-oxide **S13**.

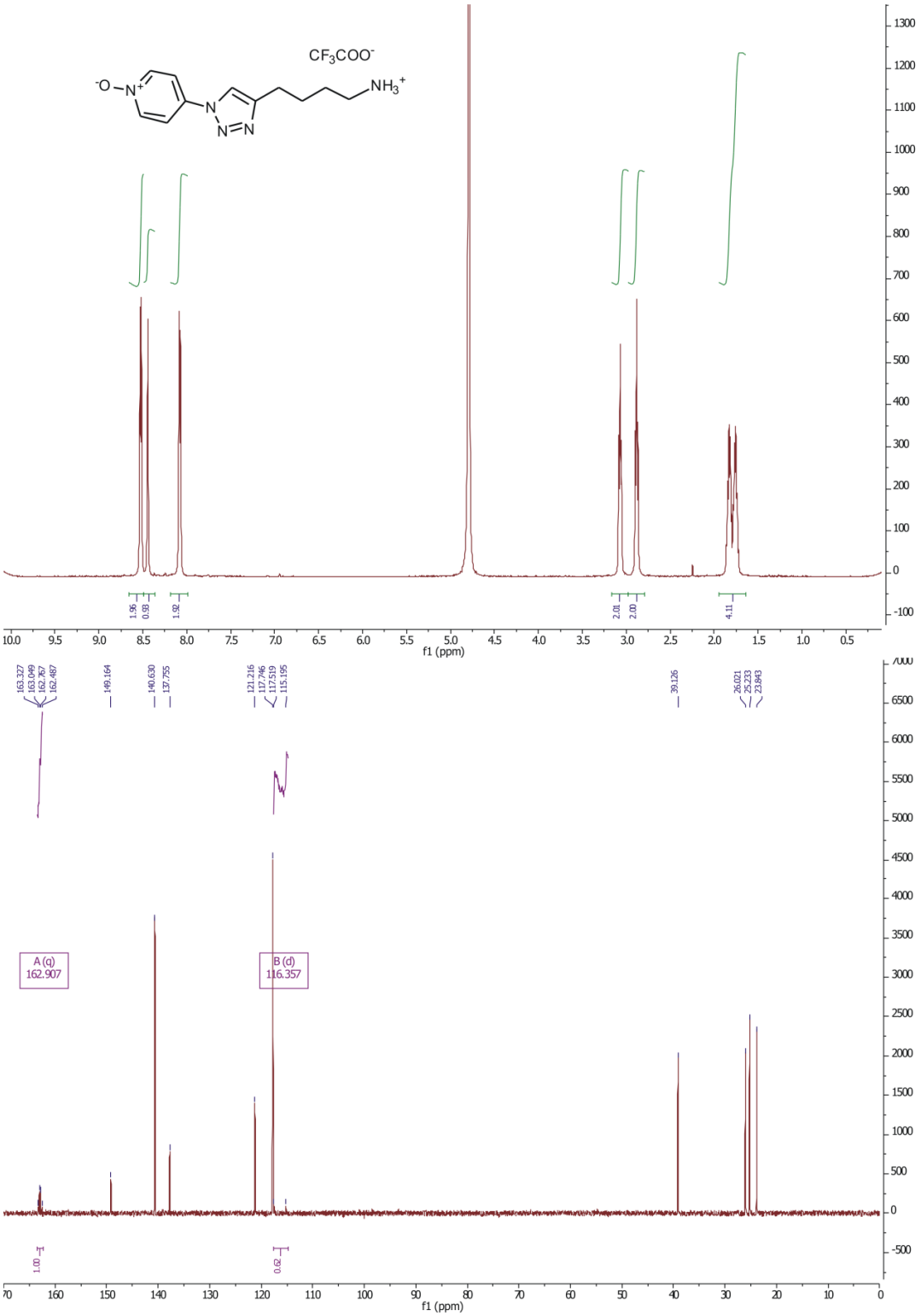


Figure S 9. ^{1}H (top) and ^{13}C (bottom) NMR spectra (D_2O, 25°C) for N-oxide **4**.

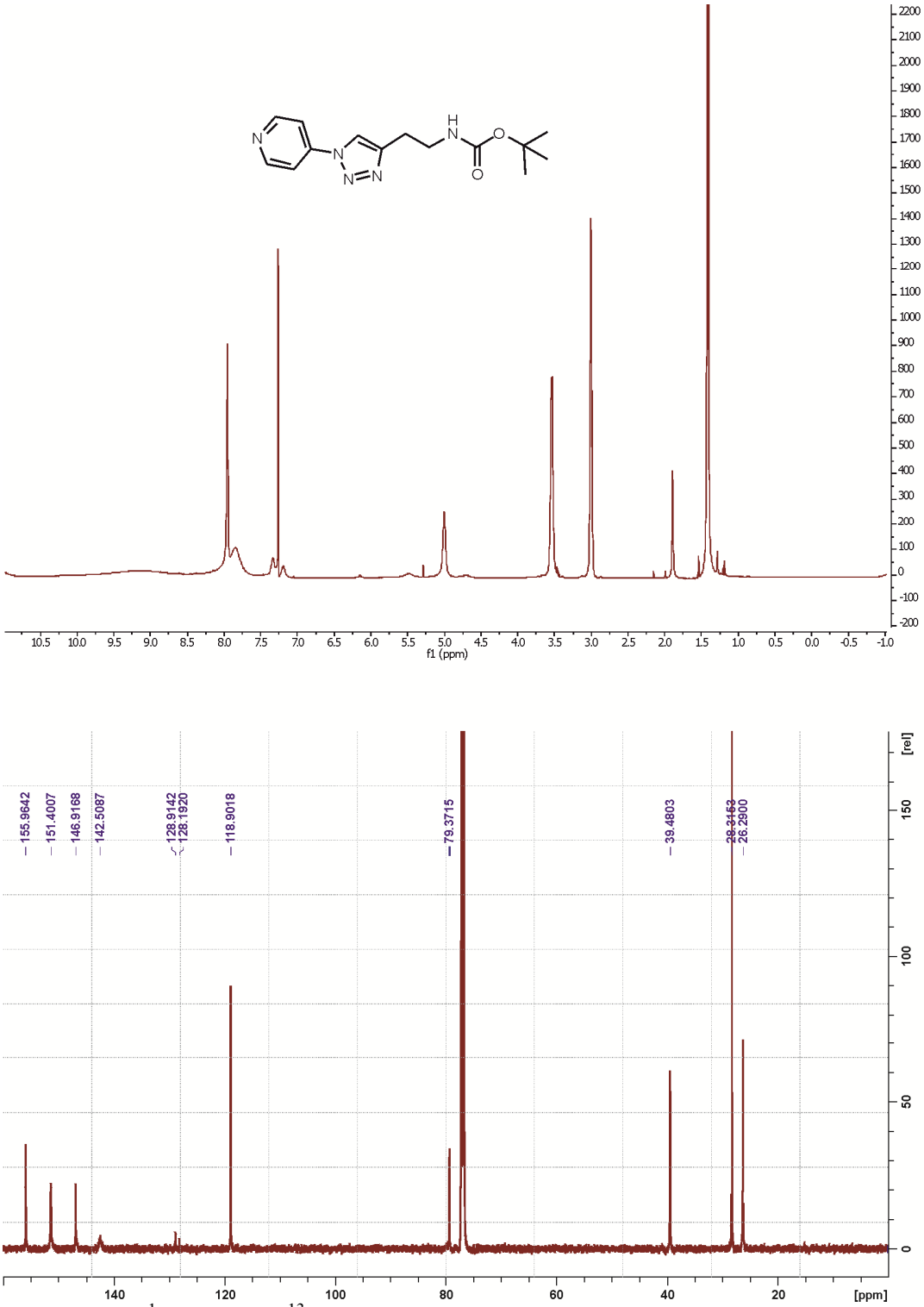


Figure S 10. ^{1}H (top) and ^{13}C (bottom) NMR spectra ($CDCl_3$, 55°C) for pyridine **S14**.

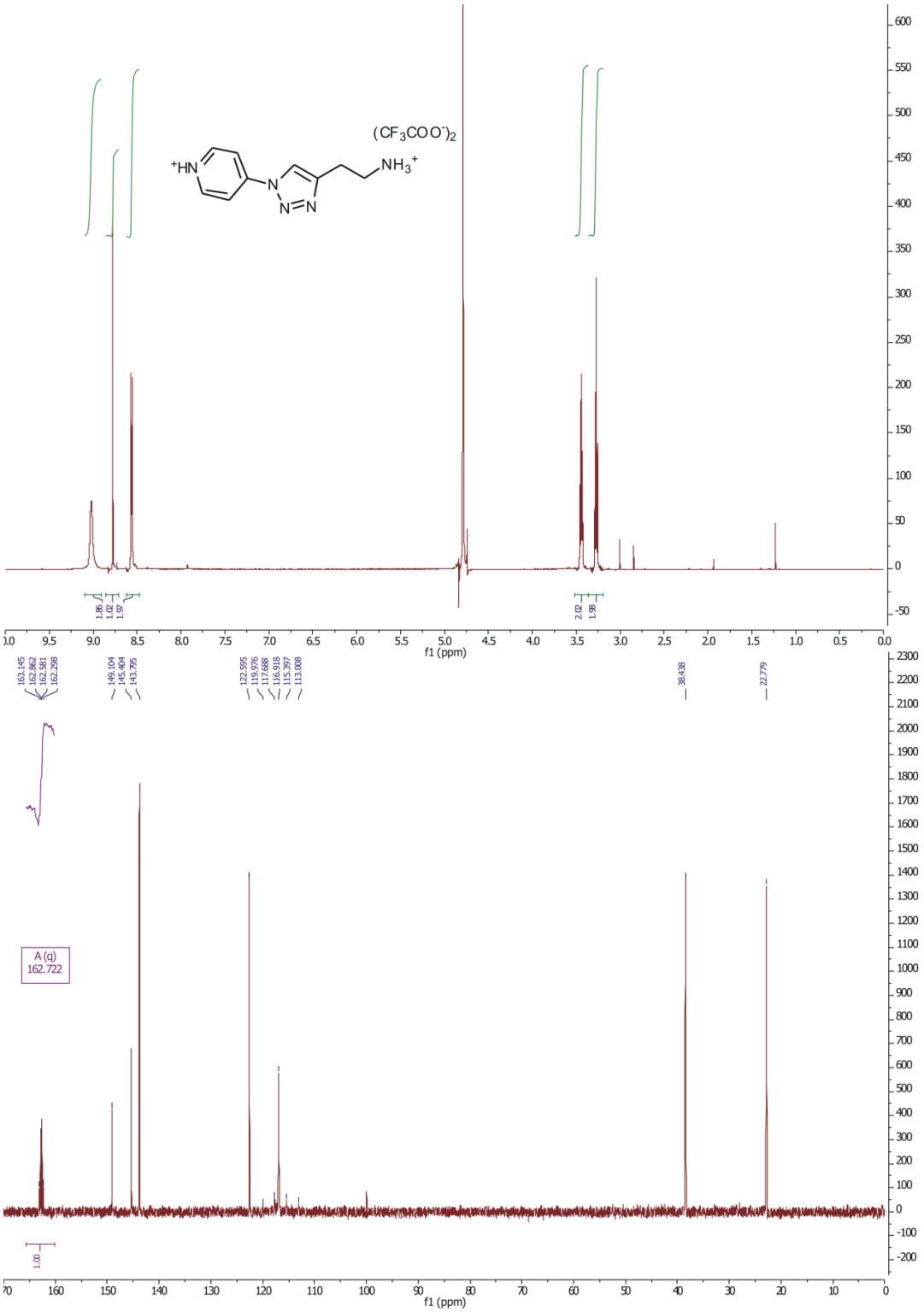


Figure S 11. ^{1}H (top) and ^{13}C (bottom) NMR spectra (D_2O, 25°C) for pyridine **S15**.

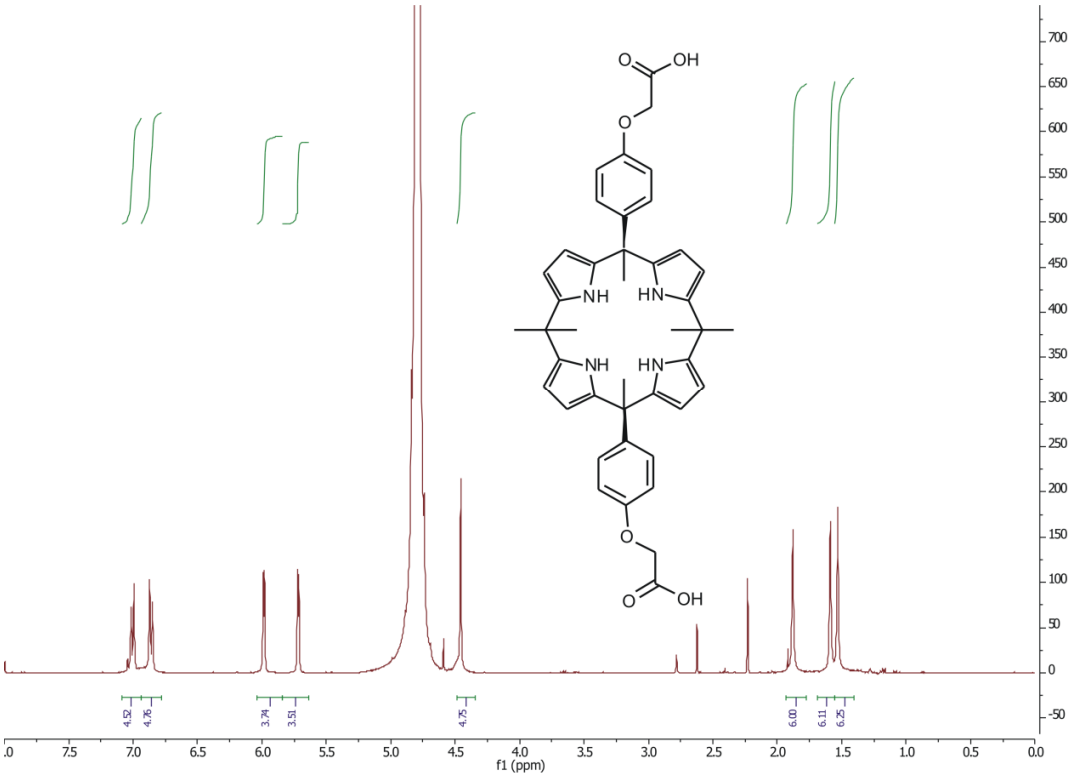


Figure S 12. [1]H NMR spectrum (deuterated borate buffer, 25°C) for "two wall" calixpyrrole **5**.

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