SUPPORTING INFORMATION

Quantification of Cooperativity in the Self-Assembly of H-bonded Rosettes

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General Experimental

The chemicals were bought from commercial suppliers and used without further purifications unless stated otherwise. Solvents were either distilled before use or used as obtained. For chromatography, automatic chromatography systems CombiFlash R_f^+ and CombiFlash R_f^+ Lumen (with UV light detection at 254 nm and 280 nm and evaporative light scattering detector for Lumen) with pre-packed puriFlash columns from Interchim (silica, 25 μm) with a loading of mixtures on Celite were used. The microwave used was Biotage Initiator$^+$. The reactions were monitored by LSMS Waters Acquity H-class UPLC coupled with a single quadrupole Waters SQD2 with the conditions as follows: UPLC Column (see below), solvent A: Water + 0.1% formic acid; solvent B: acetonitrile od THF (see below) + 0.1% formic acid; gradient and flow rate (see below); column temperature of 40 °C, the signal was monitored at 254 nm and 280 nm.

Column

Col3: ACQUITY UPLC HSS T3 Column, 100Å, 1.8 μm, 2.1 mm X 50 mm

Methods

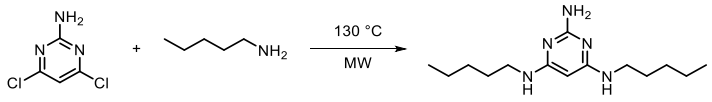
MeCN-FAST: Gradient: 0 – 2 minutes 5% – 100%B + 1 minute 100%B
Flow rate: 0.6 ml/min

MeCN-SLOW: Gradient: 0 – 4 minutes 5% – 100%B + 1 minute 100%B
Flow rate: 0.6 ml/min

^{1}H and ^{13}C NMR spectra were recorded on Bruker 400 MHz Avance III HD SmartProbe Spectrometers at 400 MHz for ^{1}H and 101 MHz for ^{13}C or on a Bruker 500 MHz Avance TCI CryoProbe Spectrometer and Bruker 500 MHz AVIII HD SmartProbe Spectrometer at 500 MHz for ^{1}H and 126 MHz for ^{13}C. The variable temperature NMR spectra were measured on a Bruker 500 MHz Avance III HD SmartProbe Spectrometer at 500 MHz for ^{1}H. All chemicals shift are quoted in ppm and were referenced to the residual peaks of used solvents: $CDCl_3$ (^{1}H: 7.26 ppm; ^{13}C: 77.00 ppm), CD_3OD (^{1}H: 3.31 ppm; ^{13}C: 49.00 ppm) or d^6-DMSO (^{1}H: 2.50 ppm; ^{13}C: 39.52 ppm). Coupling constants *J* are stated in Hz. FT-IR spectra were measured on a Bruker Alpha spectrometer. HR-MS spectra were obtained on a Waters Xevo G2-S, Waters Vion IMS Qtof or Waters LCT Premier by electrospray-ionisation of samples. Melting points were recorder on a Mettler-Toledo MP90 system. Elemental analysis was performed by the Microanalysis facility at the Department of Chemistry at the University of Cambridge.

Synthesis

Synthesis of P



4,6-Dichloropyrimidin-2-amine (210 mg, 1.28 mmol) was flushed with nitrogen in a MW vial. Amylamine (2.5 mL, 21.4 mmol) was added and the mixture was heated at microwave at 130 °C for 14 hours. The remaining amylamine was removed under reduced pressure and the residue was loaded on Celite. A combiflash of the residue on silica (EtOAc/MeOH: MeOH 0%→5%→10%) provided the title compound (210 mg, 62% yield) as a slightly-pale-brown solid.

^{1}H NMR ($CDCl_3$, 400 MHz, 298 K): δ 4.81 (s, 1H), 4.45 – 4.41 (m, 4H), 3.14 (dd, J = 13.0, 7.0 Hz, 4H), 1.62 – 1.54 (m, 4H), 1.40 – 1.30 (m, 8H), 0.92 – 0.89 (m, 6H).
^{13}C NMR ($CDCl_3$, 101 MHz, 298 K): δ 164.3, 162.3, 71.9, 41.7, 29.1, 29.1, 22.4, 14.0.

HR-MS (ESI): Calculated for $C_{14}H_{28}N_5$ $[M+H]^+$ 266.2345, found: 266.2348 (Δ = 1.1 ppm)

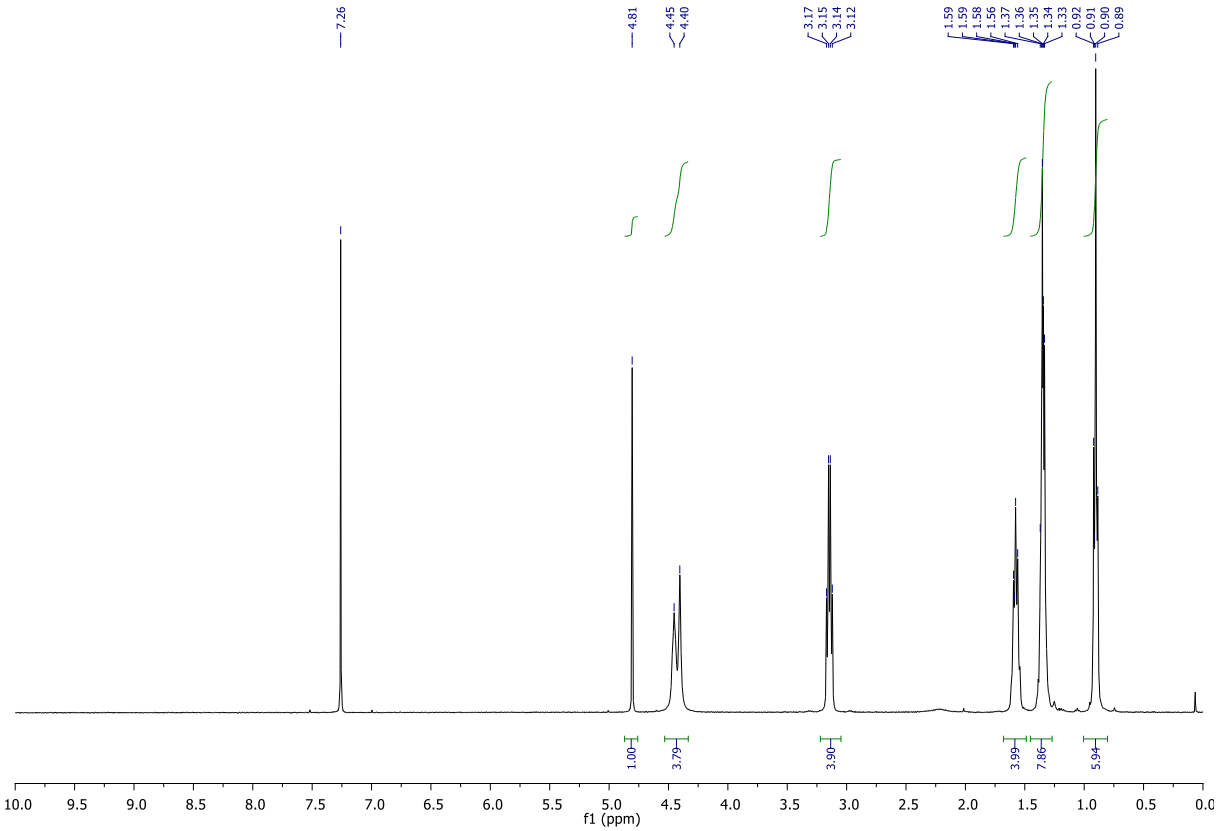
FT-IR (thin film): 3310, 2955, 2928, 2858, 1580, 1433, 1368 cm^{-1}.

MP: 85 – 87 °C

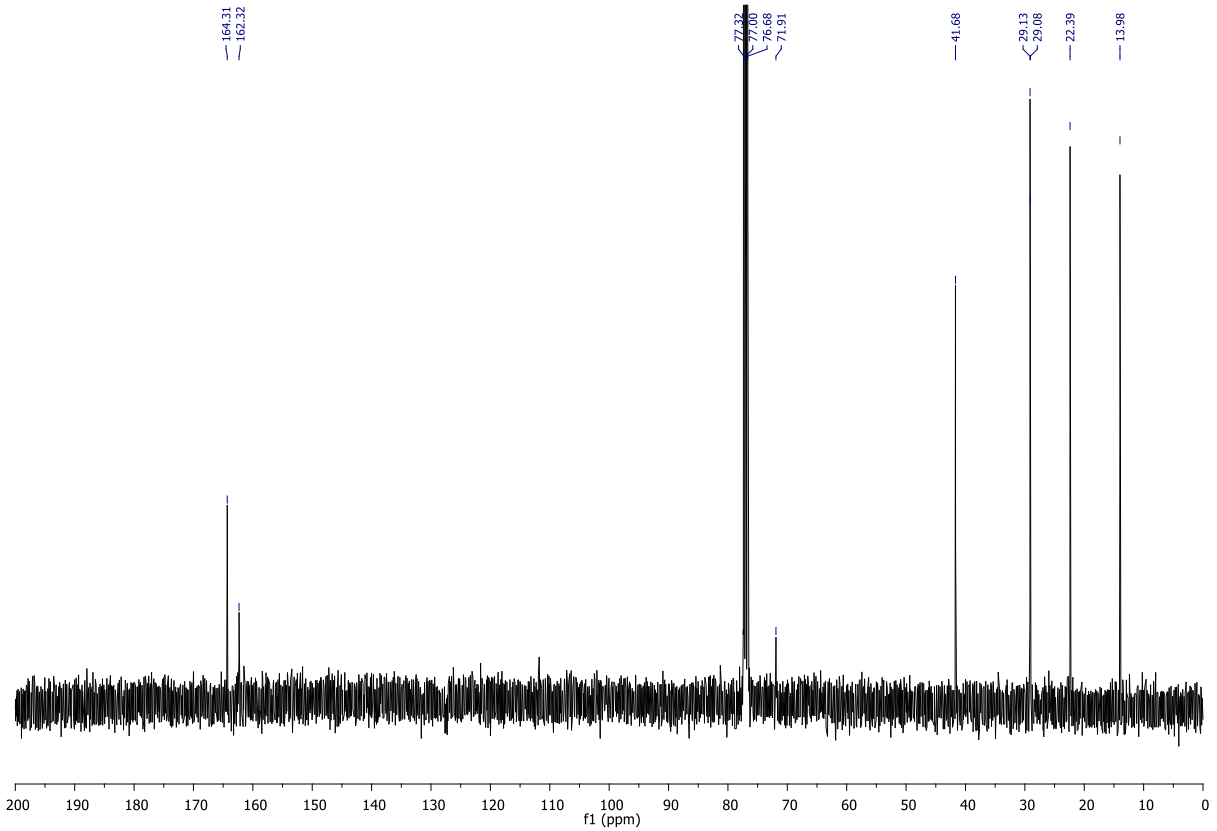
EA: Required for $C_{14}H_{27}N_5$: C 63.36, H 10.25, N 26.39; found: C 63.28, H 10.34, N 26.04.

LCMS Method: Col3-MeCN-SLOW

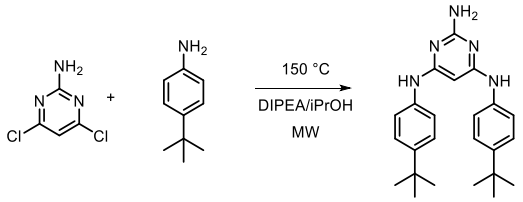
^{1}H NMR



^{13}C NMR



Synthesis of P'



4,6-Dichloropyrimidin-2-amine (178 mg, 1.06 mmol) was flushed with nitrogen in a MW vial. *N*,*N*-diisopropylethylamine (0.5 mL, 2.48 mmol), isopropyl alcohol (1.0 mL) and 4-*tert*butylaniline (0.6 mL, 562 mg, 3.77 mmol) were added and the mixture was heated in microwave at 150 °C for 1 hours. Then, additional 4-*tert*-butylaniline (0.5 µL, 467 mg, 3.14 mmol) was added under nitrogen atmosphere and the mixture was heated in microwave at 170 °C for 10 hours. A saturated solution of $NaHCO_3$ (20 mL) and water (20 mL) were added and the mixture was extracted with CH_2Cl_2/MeOH (10:1; 2 × 50 mL) and CH_2Cl_2 (2 × 50 mL). The organic phase was dried over $MgSO_4$, evaporated and loaded to Celite. A combiflash of the residue on silica (CH_2Cl_2/MeOH: MeOH 0%→10%) provided the title compound (294 mg, 71% yield) as a pale-pink solid.

^{1}H NMR (d^6-DMSO, 400 MHz, 298 K): δ 8.52 (s, 2H), 7.44 (d, J = 8.5 Hz, 4H), 7.25 (d, J = 8.5 Hz, 4H), 5.80 (s, 2H), 5.50 (s, 1H), 1.26 (s, 18H).

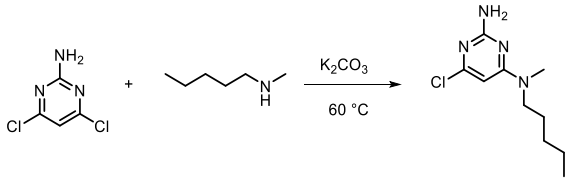
MP: 281 – 284 °C

EA: Required for $C_{24}H_{31}N_5$: C 74.00, H 8.02, N 17.98; found: C 73.75, H 8.14, N 17.66.

LCMS Method: Col3-MeCN-SLOW

The observed data was in agreement with the reported values.[1]

Synthesis of bP-Cl



A mixture of 4,6-dichloropyrimidin-2-amine (344 mg, 2.06 mmol), *N*-methylpentylamine (0.4 mL, 290 mg, 2.9 mmol), K_2CO_3 (430 mg, 3.11 mmol) and *t*BuOH (15 mL) were heated at 60 °C for 3 days. The mixture was evaporated and water (20 mL) was added. The mixture was then sonicated for 20 minutes. Solid was collected by suction and dried overnight in vacuum oven at 40 °C to provide the title compound (347 mg, 75% yield) as a yellowish solid.

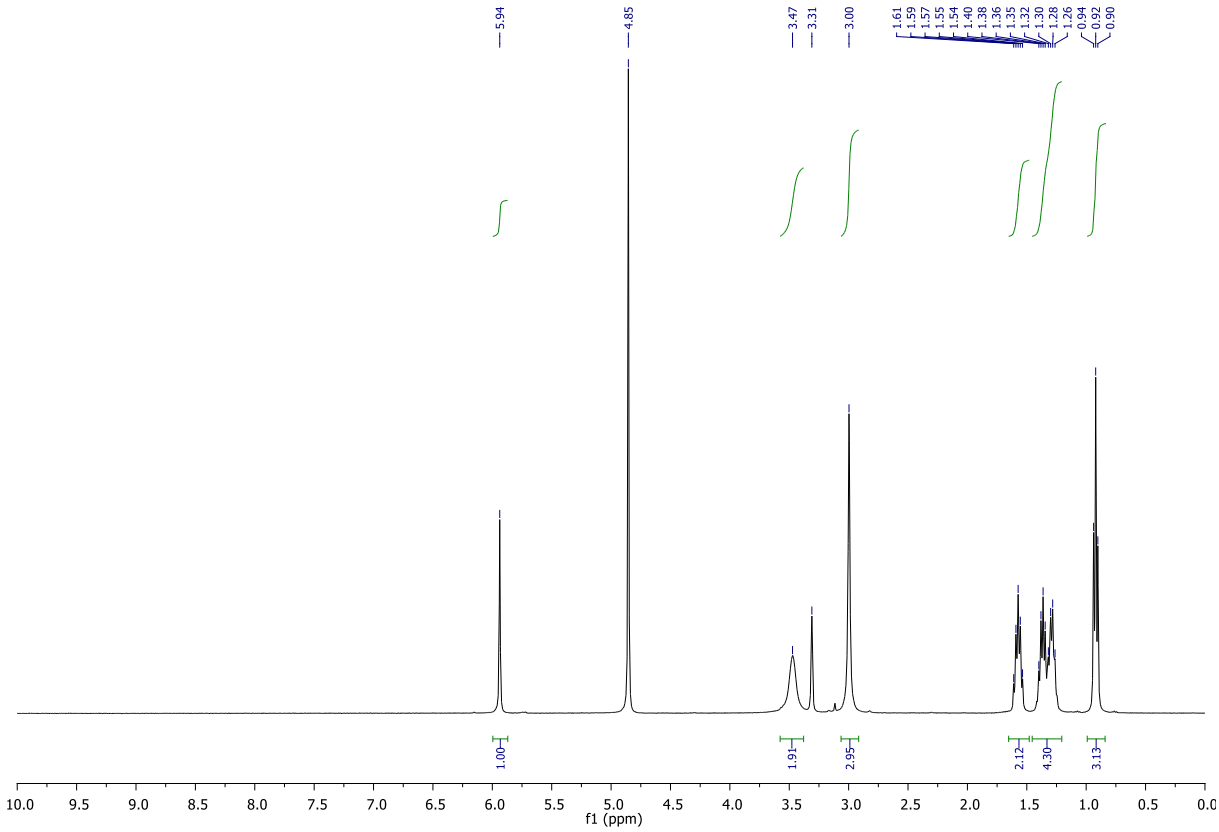
^{1}H NMR (CD_3OD, 400 MHz, 298 K): δ 5.94 (s, 1H), 3.47 (s, 2H), 3.00 (s, 3H), 1.61 – 1.54 (m, 2H), 1.42 – 1.25 (m, 4H), 0.92 (t, J = 7.0 Hz, 3H).
^{13}C NMR (CD_3OD, 101 MHz, 298 K): δ 164.9, 163.9, 160.0, 92.3, 50.3, 35.9, 30.1, 28.0, 23.6, 14.4.

HR-MS (ESI): Required for $C_{10}H_{18}N_4Cl$ $[M+H]^+$ 229.1220, found: 229.1218 (Δ = 0.9 ppm).

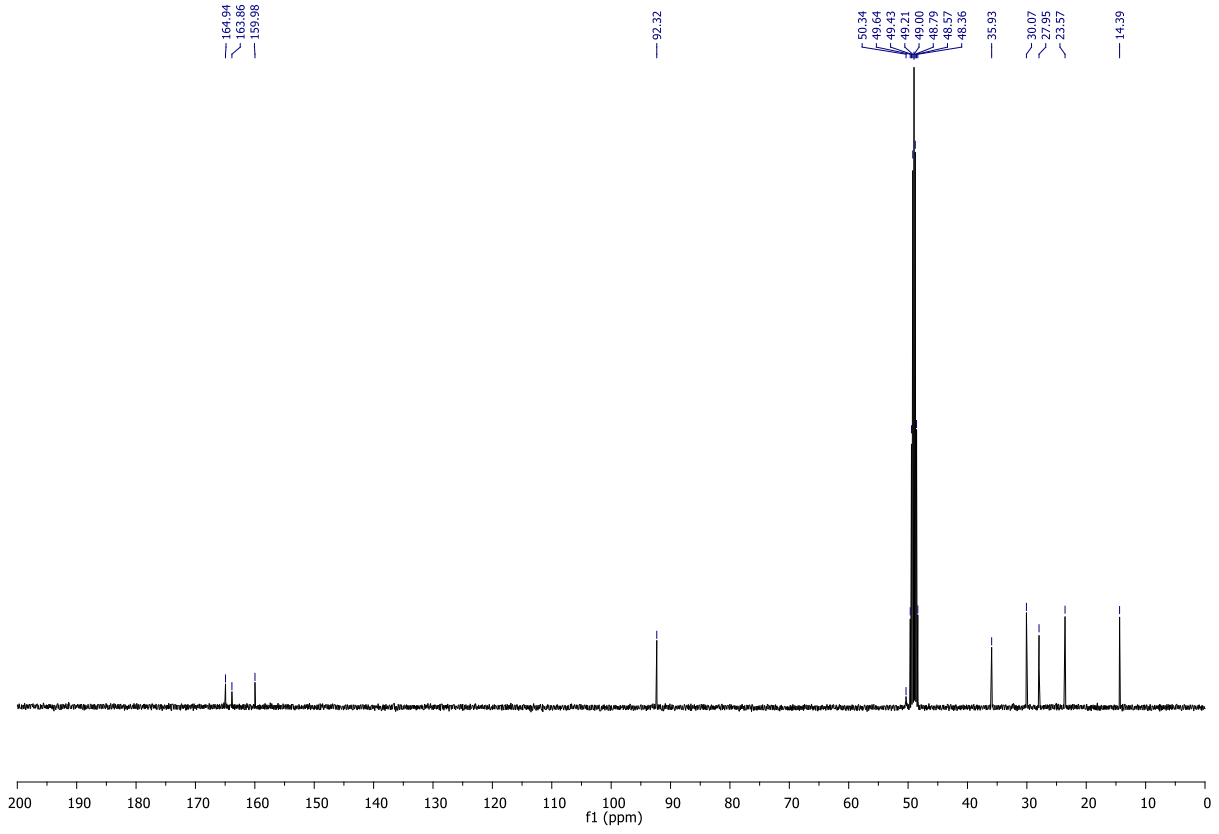
FT-IR (thin film): 3304, 3204, 2955, 2930, 2859, 2406, 1575, 1498, 1465, 1405, 1367, 974, 785 cm^{-1}.

MP: 96 – 98 °C

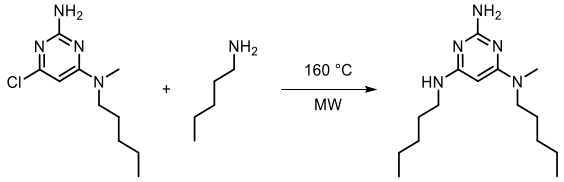
^{1}H NMR



^{13}C NMR



Synthesis of bP



bP-Cl (198 mg, 1.89 mmol) was flushed with nitrogen in a MW vial. *n*-Pentylamine (1 mL, 0.75 g, 8.6 mmol) was added and the mixture was heated in microwave at 160 °C for 10 hours. A saturated solution of $NaHCO_3$ (20 mL) was added and the mixture was extracted with CH_2Cl_2 (4 × 50 mL). The organic phase was dried over $MgSO_4$, evaporated and loaded to Celite. A combiflash of the residue on silica (CH_2Cl_2/MeOH: MeOH 0%→10%) provided the title compound (178 mg, 84% yield) as reddish oil.

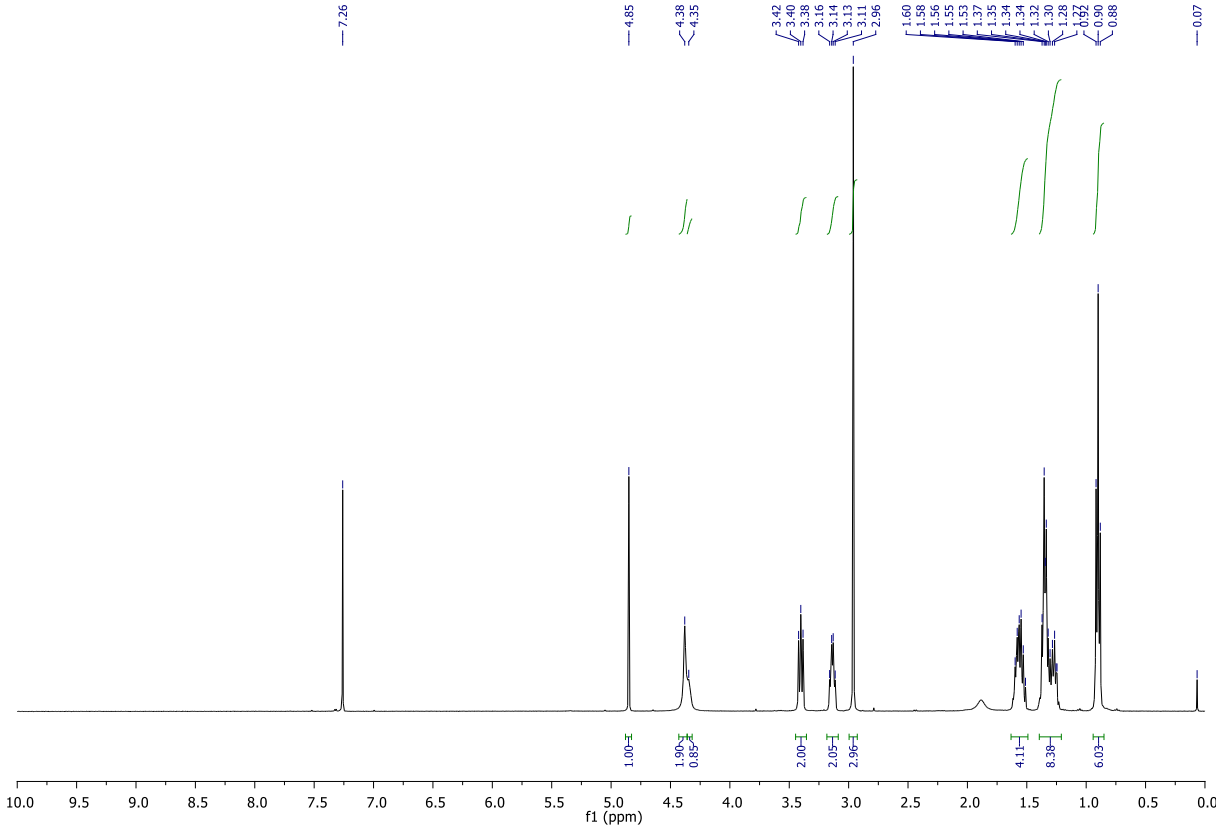
^{1}H NMR ($CDCl_3$, 400 MHz, 298 K): δ 4.85 (s, 1H), 4.38 (s, 2H), 4.35 (s, 1H), 3.42 – 3.38 (m, 2H), 3.14 (dd, J = 12.0, 7.0 Hz, 2H), 2.96 (s, 3H), 1.62 – 1.51 (m, 4H), 1.39 – 1.23 (m, 8H), 0.90 (t, J = 7.0 Hz, 6H).
^{13}C NMR ($CDCl_3$, 101 MHz, 298 K): δ 164.1, 163.9, 162.2, 72.0, 49.2, 41.7, 35.4, 29.2, 29.1, 29.1, 27.0, 22.5, 22.4, 14.1, 14.0.

HR-MS (ESI): Required for $C_{15}H_{30}N_5$ $[M+H]^+$ 280.2501, found: 280.2502 (Δ = 0.4 ppm).

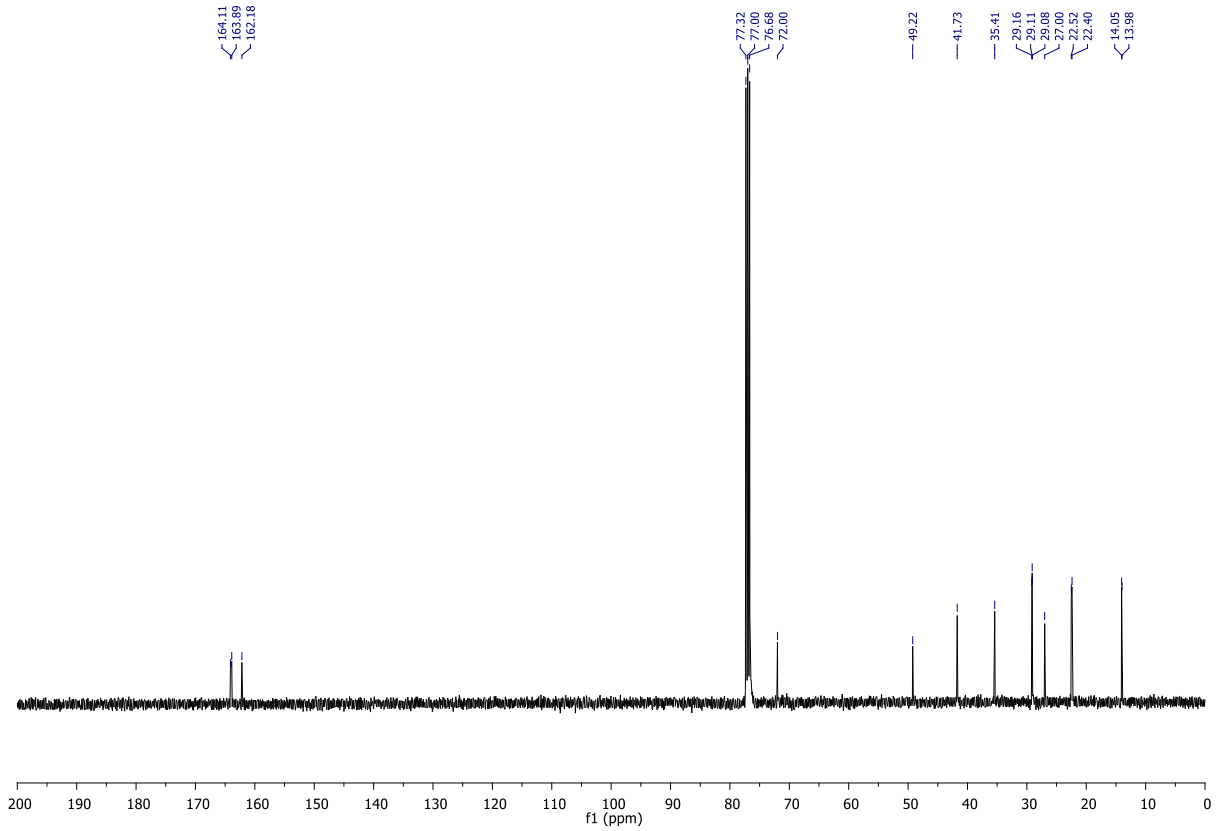
FT-IR (thin film): 3315, 2954, 2927, 2857, 1558, 1507, 1434, 1400, 1360, 1196 cm^{-1}.

LCMS Method: Col3-MeCN-SLOW/Col3-MeCN-FAST

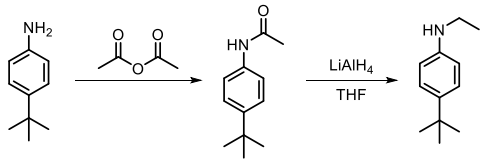
^{1}H NMR



^{13}C NMR



Synthesis of bTBA



4-*tert*-Butylaniline (0.60 mL, 0.56 g, 3.77 mmol) and acetic anhydride (2.0 mL, 2.2 g, 21.6 mmol) were left stirring for 3 hours at RT. A saturated solution of NH_4Cl (20 mL) was then added with cooling to 0 °C. The mixture was extracted with (3 × 50 mL). The collected organic phase was dried over $MgSO_4$ and the solvents were removed under reduced pressure to provide after drying in vacuum oven overnight the title product (724 mg, *quantitative*) as a white crystalline solid.

^{1}H NMR ($CDCl_3$, 400 MHz, 298 K): δ 7.40 (d, J = 8.5 Hz, 2H), 7.33 (d, J = 8.5 Hz, 2H), 7.11 (bs, 1H), 2.17 (s, 3H), 1.30 (s, 9H).

MP: 172 – 174 °C

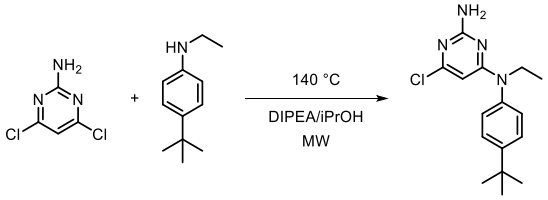
The observed data was in agreement with the reported values.[2]

This compound (499 mg, 2.61 mmol) was flushed with N_2 and THF (10 mL) was added and the mixture was cooled to 0 °C. A solution of $LiAlH_4$ (1.0 M in THF, 6 mL, 6 mmol) was added dropwise at 0 °C. After 1 hour, the cooling bath was removed and the mixture was left stirring overnight at RT. Then, a saturated solution of $NaHCO_3$ (10 mL) was added at 0 °C and the mixture was extracted with CH_2Cl_2 (4 × 50 mL). The organic phase was dried over $MgSO_4$ and evaporated to produce the title product (407 mg, 88% yield) as reddish oil.

^{1}H NMR ($CDCl_3$, 400 MHz, 298 K): δ 7.23 – 7.19 (m, 2H), 6.60 – 6.56 (m, 2H), 3.42 (bs, 1H), 3.15 (q, J = 7.0 Hz, 2H), 1.28 (s, 9H), 1.25 (t, J = 7.0 Hz, 3H).

The observed data was in agreement with the reported values.[3]

Synthesis of bP'-Cl



4,6-Dichloropyrimidin-2-amine (229 mg, 1.40 mmol) was flushed with nitrogen in a MW vial. **bTBA** (585 g, 3.31 mmol), isopropyl alcohol (1.5 mL), DIPEA (1 mL) were added and the mixture was heated at microwave at 140 °C for 44 hours. A saturated solution of $NaHCO_3$ (30 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 50 mL). The organic phase was dried over $MgSO_4$, evaporated and loaded to Celite. A combiflash of the residue on silica (CH_2Cl_2/MeOH: MeOH 0%→5%) provided the title compound (363 mg, 85% yield) as a pale-yellowish solid.

^{1}H NMR ($CDCl_3$/CD_3OD, 400 MHz, 298 K): δ 7.48 (d, *J* = 8.5 Hz, 2H), 7.10 (d, *J* = 8.5 Hz, 2H), 5.45 (s, 1H), 3.89 (q, *J* = 7.0 Hz, 2H), 1.35 (s, 9H), 1.15 (t, *J* = 7.0 Hz, 3H). (*referenced to methanol*)
^{13}C NMR ($CDCl_3$/CD_3OD, 101 MHz, 298 K): *δ* 164.7, 163.4, 159.0, 151.7, 140.5, 128.5, 127.7, 94.4, 45.6, 35.3, 31.7, 13.2. (*referenced to methanol*)

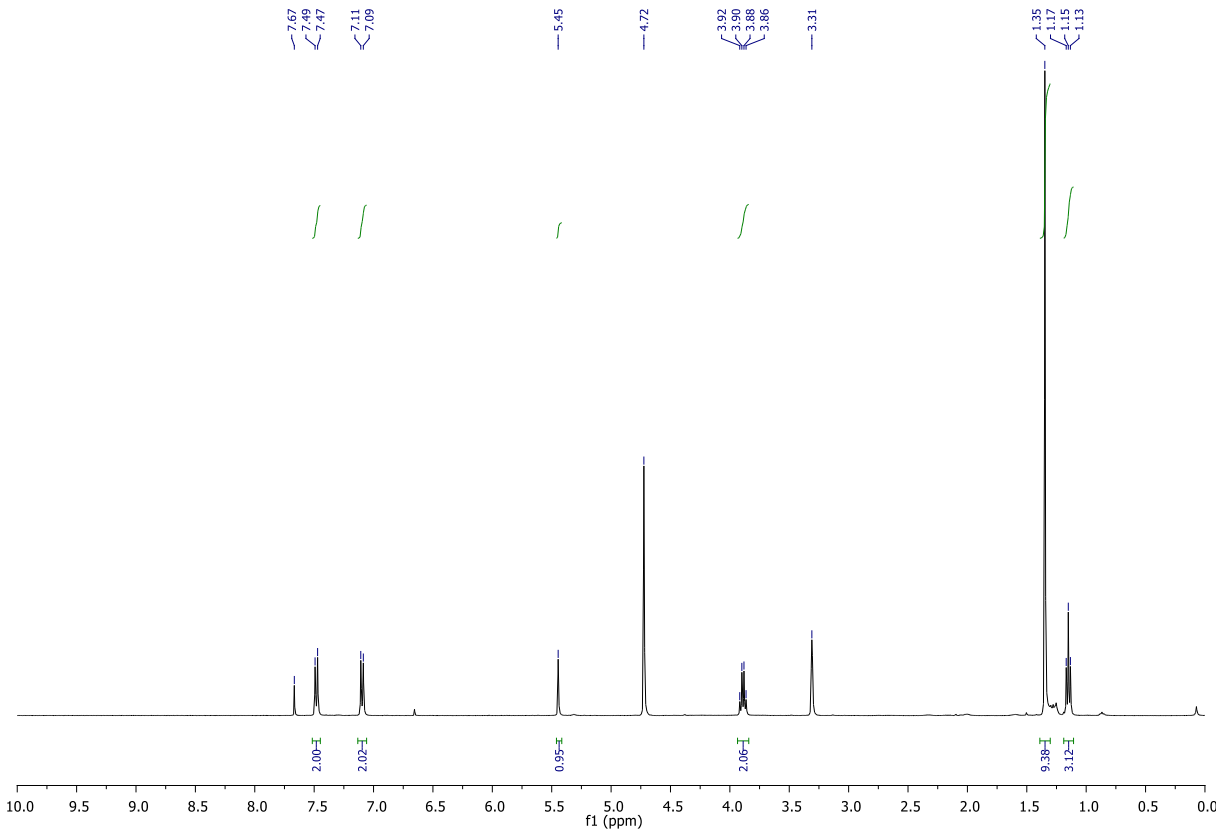
HR-MS (ESI): Required for $C_{16}H_{21}N_4Cl$ $[M+H]^+$ 305.1533, found: 305.1533 (Δ = 0.0 ppm).

FT-IR (thin film): 3314, 2920, 2851, 1651, 1546, 1519, 1260, 1111 cm^{-1}.

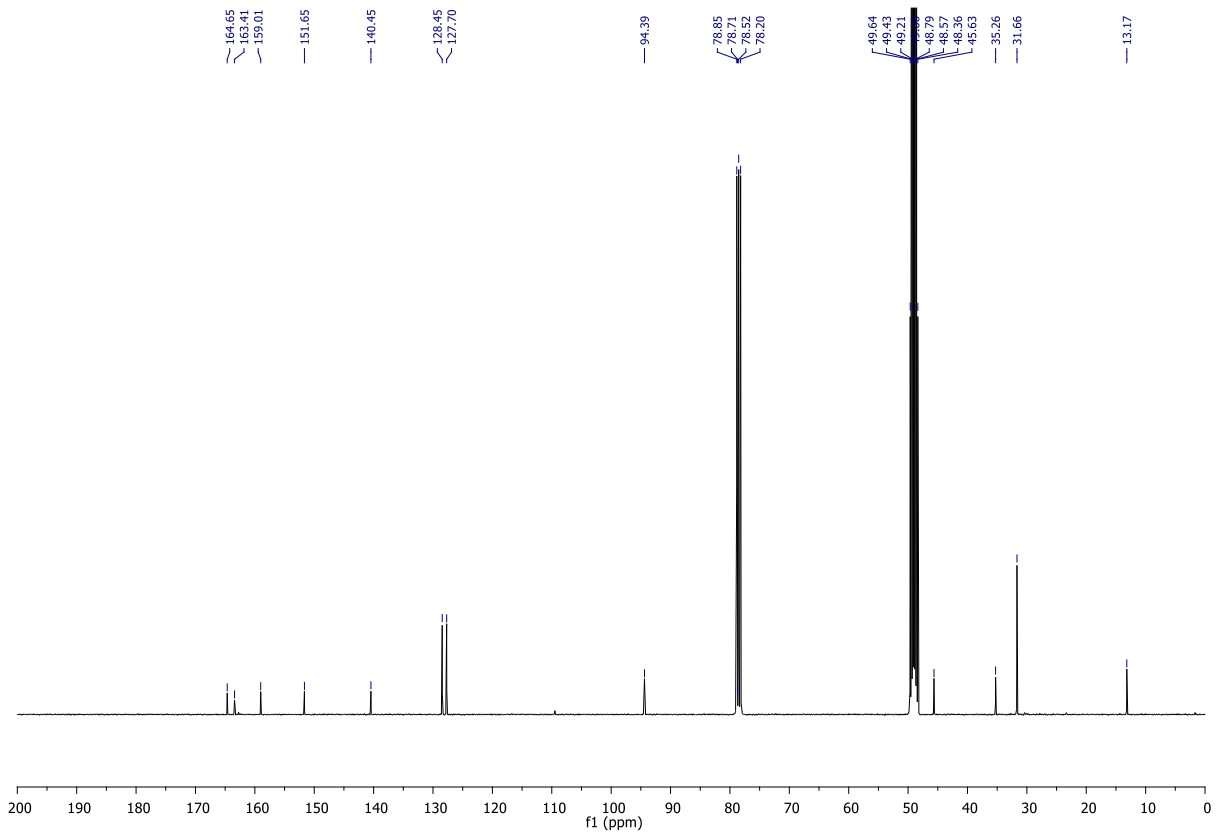
MP: 198 – 202 °C

LCMS Method: Col3-MeCN-SLOW

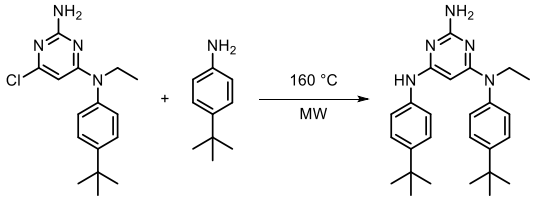
^{1}H NMR



^{13}C NMR



Synthesis of bP'



bP'-Cl (83 mg, 0.272 mmol) was flushed with nitrogen in a MW vial. 4-*tert*-Butylaniline (0.6 mL, 0.56 g, 3.8 mmol) was added and the mixture was heated in microwave at 160 °C for 2 hours. A saturated solution of $NaHCO_3$ (5 mL) was added and the mixture was extracted with CH_2Cl_2/MeOH (20:1, 2 × 50 mL) and CH_2Cl_2 (2 × 50 mL). The organic phase was dried over $MgSO_4$, evaporated and loaded to Celite. A combiflash of the residue on silica (CH_2Cl_2/MeOH: MeOH 0%→10%) provided the title compound (75 mg, 66% yield) as a pale purple solid.

^{1}H NMR ($CDCl_3$, 400 MHz, 298 K): δ 7.42 – 7.38 (m, 2H), 7.19 – 7.16 (m, 2H), 7.13 – 7.10 (m, 2H), 7.00 – 6.98 (m, 2H), 6.15 (s, 1H), 5.06 (s, 1H), 4.57 (s, 2H), 3.92 (d, J = 7.0 Hz, 2H), 1.33 (s, 9H), 1.24 (s, 9H), 1.17 (t, J = 7.0 Hz, 3H).
^{13}C NMR ($CDCl_3$, 101 MHz, 298 K): δ 164.4, 162.6, 160.9, 149.6, 145.6, 141.0, 137.0, 127.9, 126.3, 125.6, 120.5, 77.2, 44.0, 34.5, 34.2, 31.4, 31.3, 13.6.

HR-MS (ESI): Required for $C_{26}H_{36}N_5$ $[M+H]^+$ 418.2971, found: 418.2962 (Δ = 2.2 ppm).

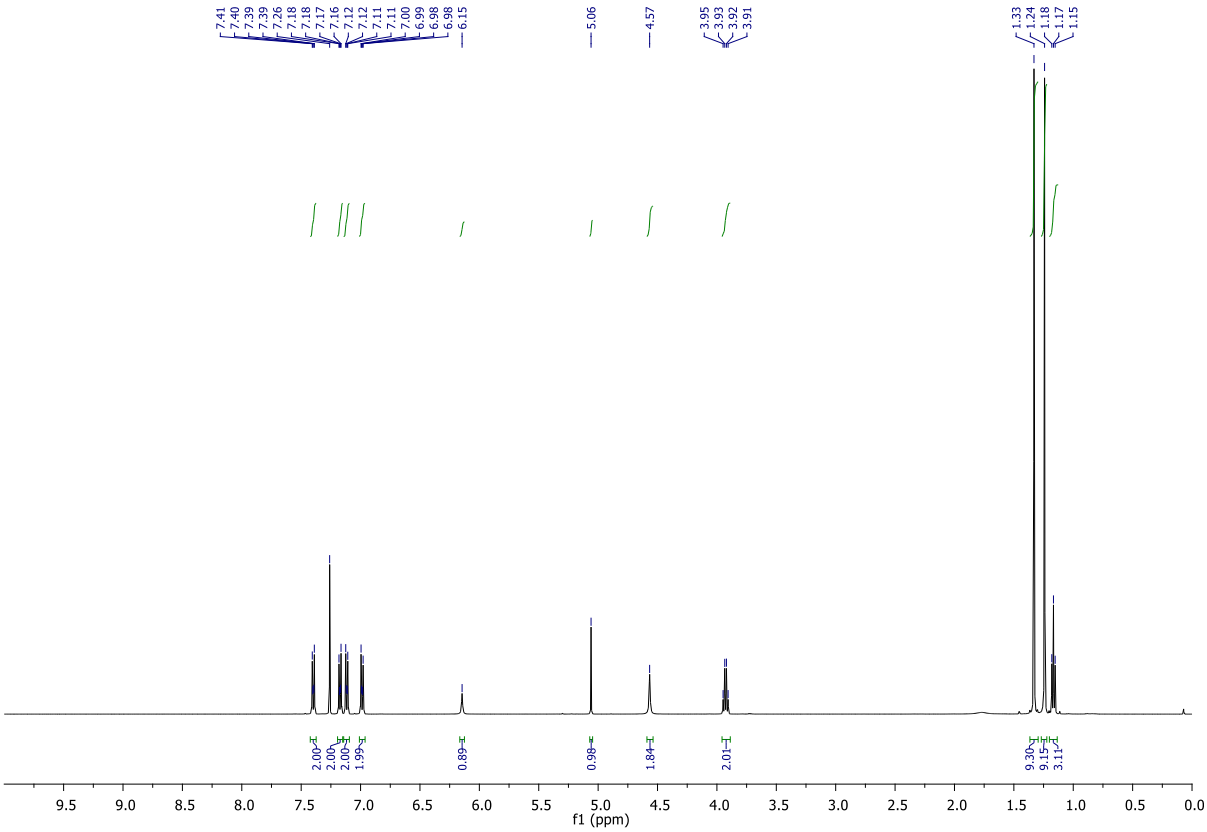
FT-IR (thin film): 3456, 3343, 3229, 2953, 2918, 2850, 1629, 1573, 1540, 1429, 1359, 1256, 1229, 1018, 791 cm^{-1}.

MP: 180 – 182 °C

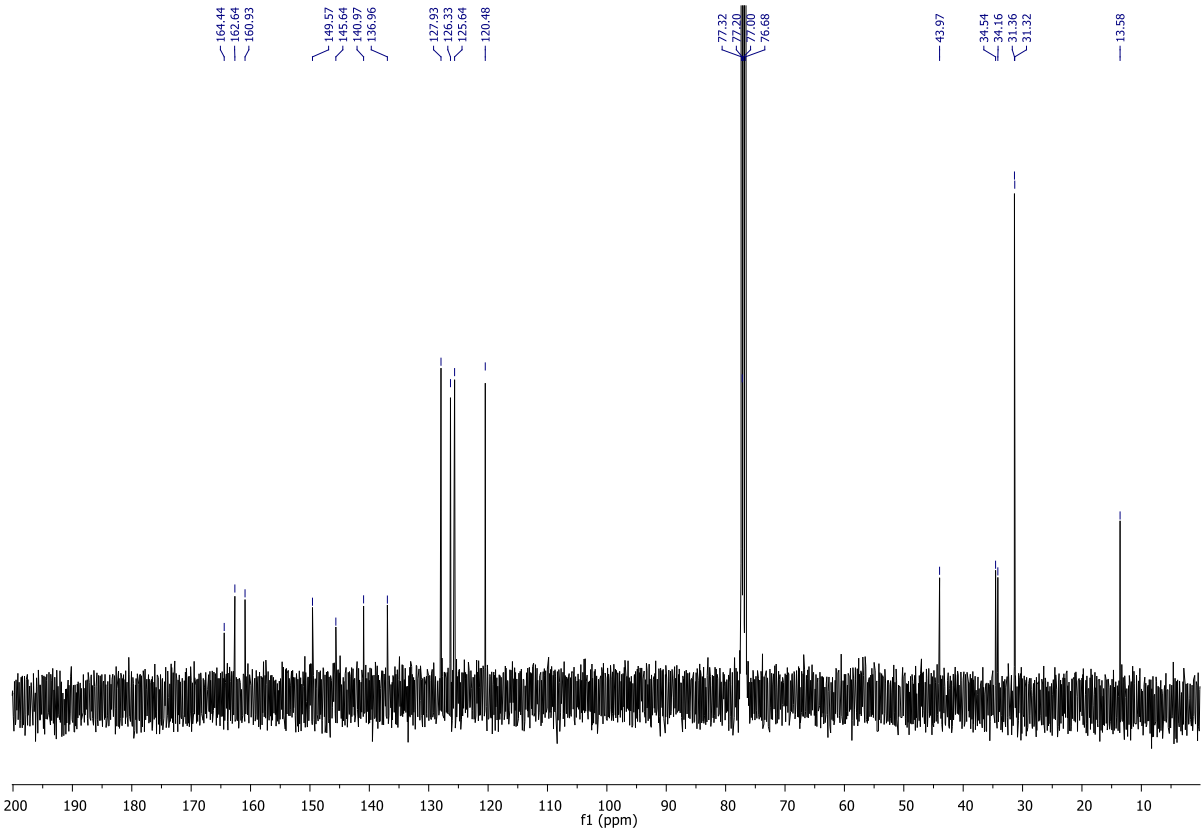
EA: Required for $C_{26}H_{35}N_5$: C 74.78, H 8.45, N 16.77; found: C 74.62, H 8.53, N 16.32.

LCMS Method: Col3-MeCN-FAST

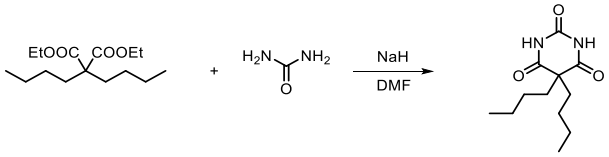
^{1}H NMR



^{13}C NMR



Synthesis of B



NaH (60% in mineral oil, 140 mg, 3.5 mmol) was flushed with nitrogen and DMF (6 mL) was added. The mixture was cooled to 0 °C and urea (418 mg, 7.0 mmol) was added. After 2 hours of stirring, diethyl dibutylmalonate (0.5 mL, 473 mg, 1.73 mmol) was added dropwise at 0 °C. The mixture was stirred overnight at RT and a saturated solution of NH_4Cl (10 mL) was added with cooling to 0 °C. Water (15 mL) was added and the mixture was extracted with CH_2Cl_2/MeOH (10:1, 40 mL) CH_2Cl_2 (2 × 50 mL). The collected organic phase was dried over $MgSO_4$ and the solvents were removed under reduced pressure (60 °C, <20 mBar). A combliflash of the residue on silica (Celite loading, PE/DCM, DCM: 0%→100%) provided after drying in vacuum oven overnight the title product (135 mg, 32% yield) as a white crystalline solid.

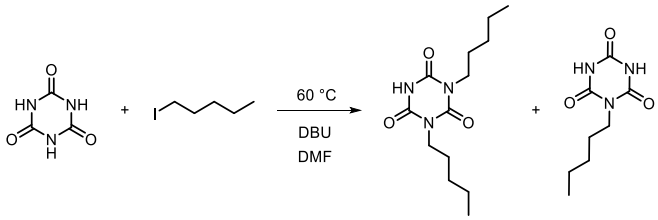
^{1}H NMR (CD_3OD, 400 MHz, 298 K): δ 1.92 – 1.88 (m, 4H), 1.33 – 1.24 (m, 4H), 1.20 – 1.12 (m, 4H), 0.88 (t, J = 7.5 Hz, 6H).

MP: 154 – 155 °C

EA: Required for $C_{12}H_{20}N_2O_3$: C 59.87, H 8.39, N 11.66; found: C 59.13, H 8.25, N 11.38.

The observed data was in agreement with the reported values.[4]

Synthesis of bC and C



Cyanuric acid (4.2 g, 32.5 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.57 mL, 0.58 g, 3.8 mmol) were flushed with N_2. DMF (10 mL) and 1-iodopentane (0.5 mL, 0.79 g, 3.96 mmol) were added and the mixture was heated at 60 °C for 5 days. The solvents was then removed under reduced pressure and a combiflash of the residue on silica (Celite loading, CH_2Cl_2/MeOH, MeOH: 0%→5%) provided the title products **bC** (164 mg, 15% yield) and **C** (99 mg, 13% yield) as white crystalline solids.

Compound **bC:**
^{1}H NMR (CD_3OD, 400 MHz, 298 K): δ 3.78 – 3.75 (m, 4H), 1.66 – 1.58 (m, 4H), 1.42 – 1.27 (m, 8H), 0.92 (t, J = 7.0 Hz, 6H).
^{13}C NMR (CD_3OD, 101 MHz, 298 K): δ 151.7, 150.5, 49.6, 43.0, 29.9, 28.5, 23.4, 14.3.

HR-MS (ESI): Required for $C_{13}H_{24}N_3O_3$ $[M+H]^+$ 270.1818, found: 270.1812 (Δ = 2.2 ppm).

FT-IR (thin film): 3218, 3116, 2955, 2926, 2871, 2859, 1741, 1660, 1482, 1444, 1379 cm^{-1}.

MP: 98 – 101 °C

EA: Required for $C_{13}H_{23}N_3O_3$: C 57.97, H 8.61, N 15.60; found: C 58.87, H 8.64, N 14.32.

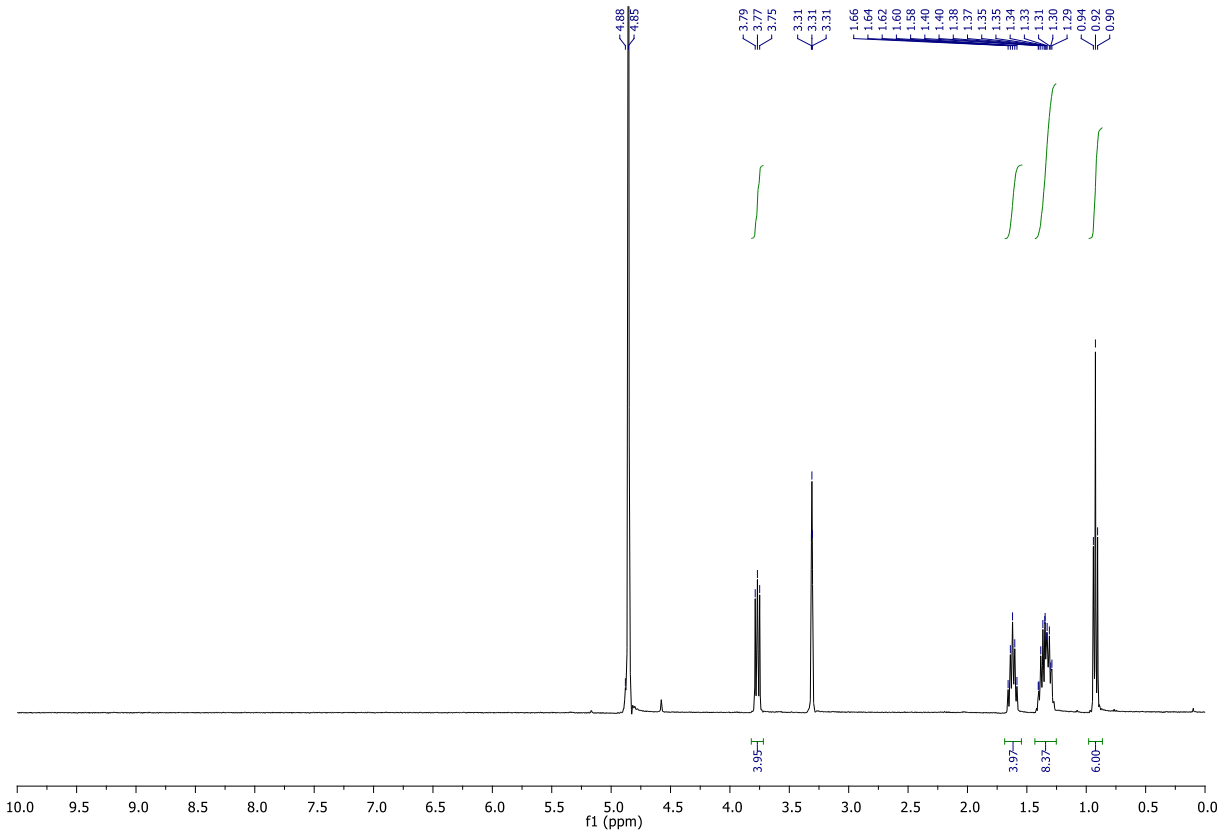
Compound **C:**
^{1}H NMR (CD_3OD, 400 MHz, 298 K): δ 3.79 – 3.75 (m, 2H), 1.66 – 1.58 (m, 2H), 1.42 – 1.27 (m, 4H), 0.92 (t, J = 7.0 Hz, 3H).

MP: 229 – 232 °C

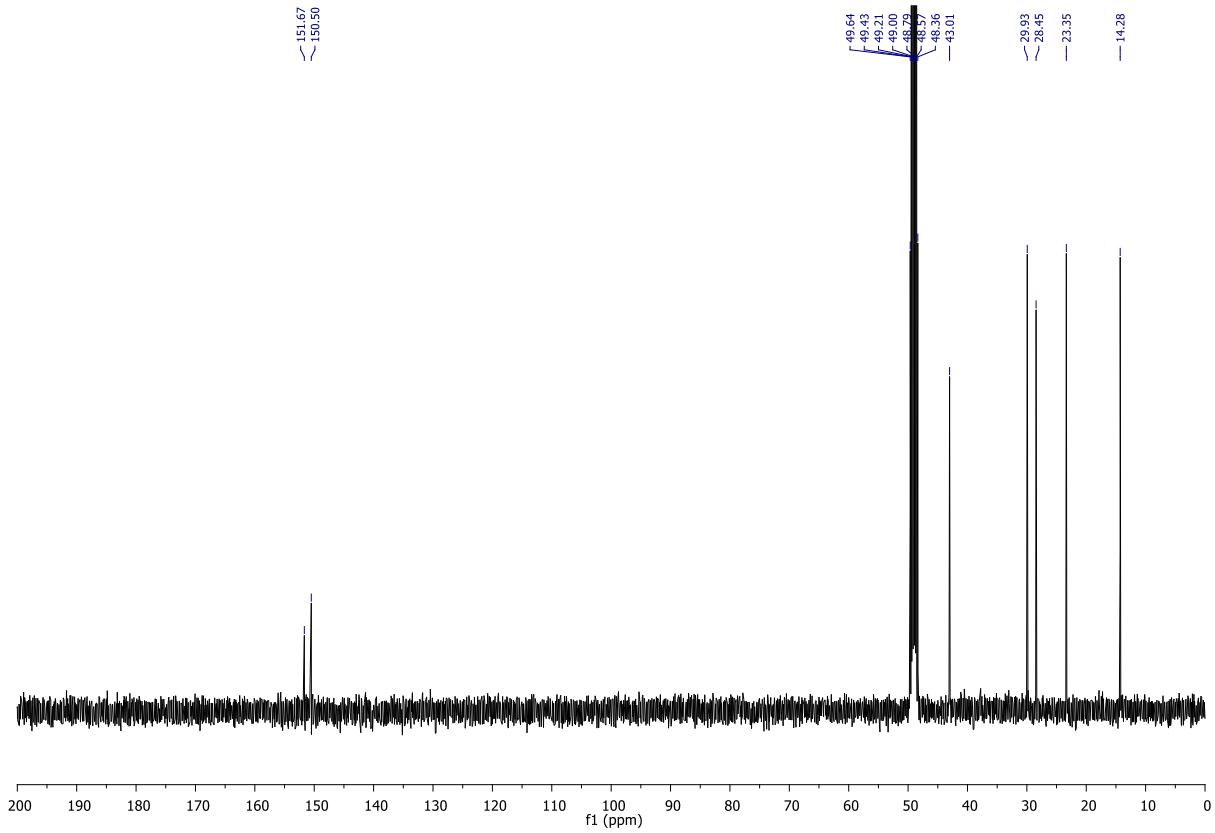
EA: Required for $C_8H_{13}N_3O_3$: C 48.23, H 6.58, N 21.09; found: C 48.27, H 6.55, N 20.92.

The observed data was in agreement with the reported values.[5]

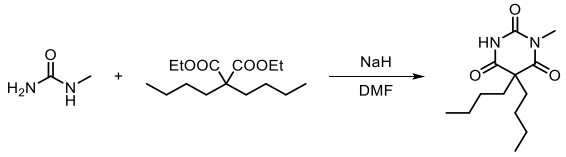
bC: ^{1}H NMR



bC: ^{13}C NMR



Synthesis of bB



NaH (60% in mineral oil, 266 mg, 6.65 mmol) was flushed with nitrogen and DMF (10 mL) was added. The mixture was cooled to 0 °C and *N*-methylurea (1.25 g, 16.9 mmol) was added. After 3 hours of stirring, diethyl butylmalonate (0.55 mL, 541 mg, 1.99 mmol) was added at 0 °C. The mixture was stirred overnight at 60 °C. Then a saturated solution of NH_4Cl (15 mL) was added at 0 °C. Water (20 mL) was added and the mixture was extracted with dichloromethane (4 × 50 mL). The collected organic phase was dried over $MgSO_4$ and the solvents were removed under reduced pressure. A combliflash of residue on silica (Celite loading, PE/CH_2Cl_2, CH_2Cl_2: 0%→100%) provided the title product (473 mg, 97% yield) as a white solid.

^{1}H NMR ($CDCl_3$, 400 MHz, 298 K): δ 8.04 (s, 1H), 3.31 (s, 3H), 2.03 – 1.92 (m, 4H), 1.31 – 1.22 (m, 4H), 1.19 – 0.99 (m, 4H), 0.85 (t, J = 7.5 Hz, 6H).
^{13}C NMR (CD_3OD, 101 MHz, 298 K): δ 174.6, 174.0, 151.7, 57.6, 40.4, 28.2, 27.7, 23.7, 14.0.

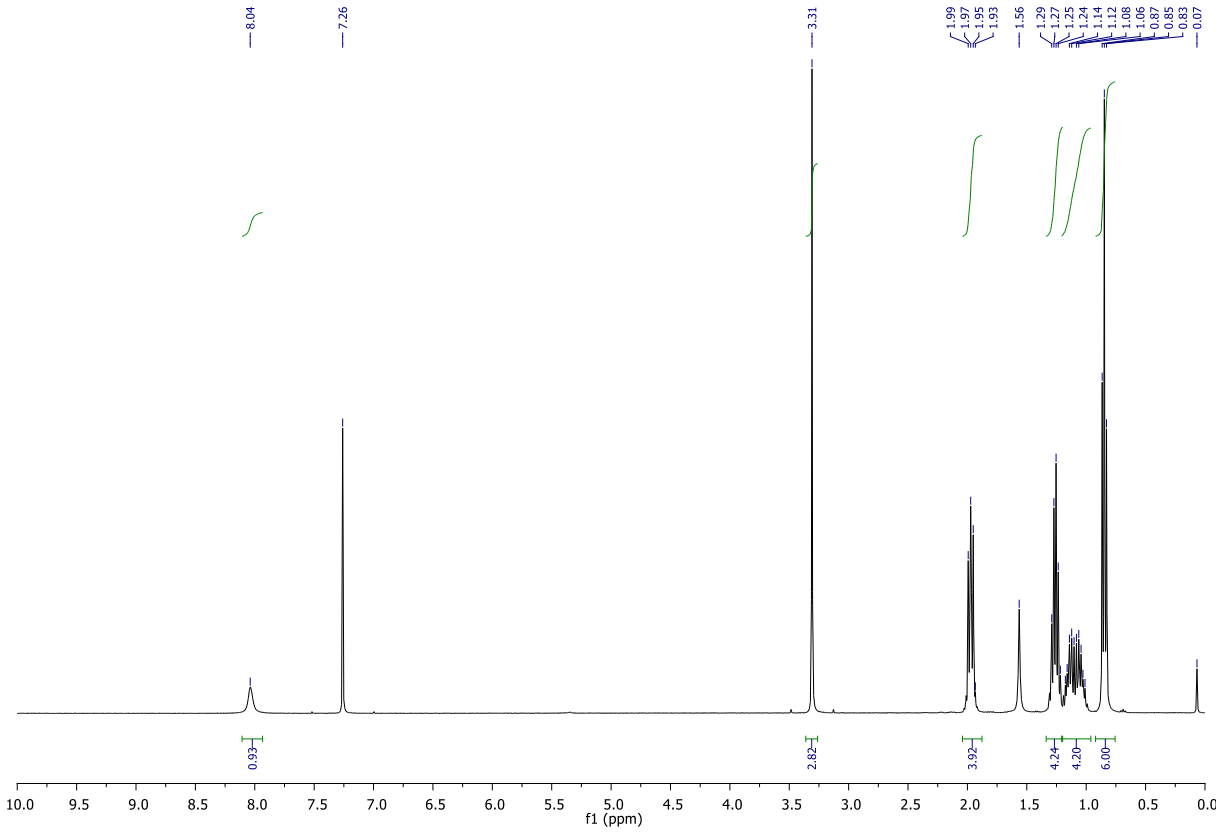
HR-MS (ESI): Required for $C_{13}H_{21}N_2O_3$ $[M–H]^-$ 253.1552, found: 253.1549 (Δ = 3.3 ppm).

FT-IR (thin film): 3237, 3127, 2959, 2873, 2862, 1751, 1710, 1683, 1442, 1383, 1358, 1267, 1190 cm^{-1}.

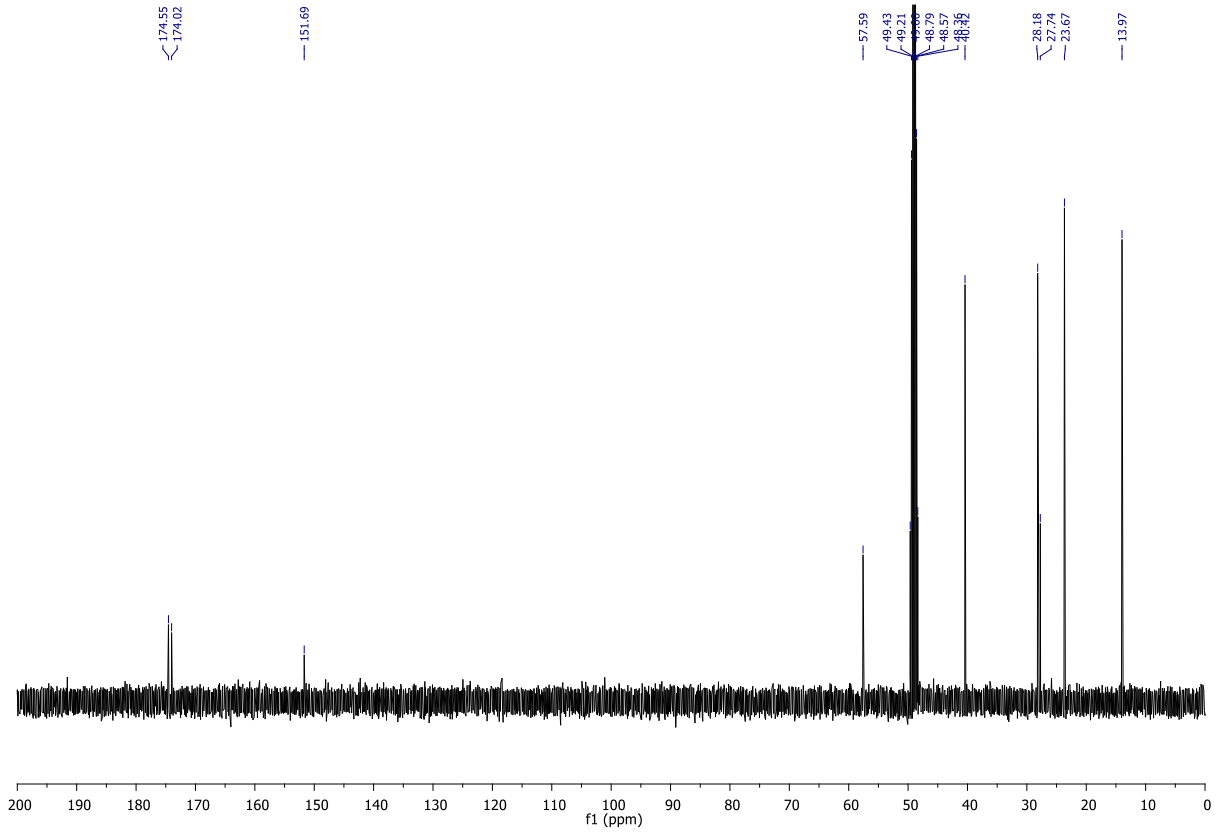
MP: 97 – 98 °C

EA: Required for $C_{13}H_{22}N_2O_3$: C 61.39, H 8.72, N 11.01; found: C 61.55, H 8.73, N 10.93.

^{1}H NMR



^{13}C NMR



NMR dilutions and titrations

General

The NMR titrations were recorded on a Bruker 400 MHz Avance III HD Smart Probe at 400 MHz and on a Spectrometer and 500 MHz AVIII HD SmartProbe Spectrometer at 500 MHz at 298 K with $CDCl_3$ as a solvent. A host solution with known concertation was prepared and a fraction of this solution (500/600 μL) was transferred into a NMR tube. Then, the guest solution was prepared by dissolving a known amount of guest in the host stock solution in a volumetric flask. A change of chemicals shifts of ^1H upon addition of aliquots of guest solution were followed. For dilution experiments, a solution with known concentration of a compound was prepared and of aliquots of this solution were added to a NMR tube containing only the solvent (500 μL) and the change of chemicals shifts of ^1H upon addition was followed. The observed changes of chemical shifts were analysed using a purpose-written fitting macro in Microsoft Excel. A dimerisation isotherm, 1:1 binding isotherm, different 1:2 binding isotherms (see below) and 3:3 binding isotherm were used to fit the experimental data. The experiments were measured at least two times on at least two different days with freshly prepared solutions in order to eliminate possible systematic errors. The results are stated as average values and errors are quoted as two times the standard deviation.

1:1 complexes

An iterative VBA macro was used to optimise the values of K, ∂_H and $\partial_{H\bullet G}$ to minimise the difference between the experimental values of ∂ and the values calculated using Equations S1-S4.

$[H\bullet G] = K\,[H]\,[G]$ (Equ. S1)
where [H] is the concentration of free host, [G] is the concentration of free guest and [H•G] is the concentration of complex.

$[G]_0 = [G] + [H\bullet G]$ (Equ. S2)
where $[G]_0$ is the total concentration of guest in the cuvette.

$[H]_0 = [H] + [H\bullet G]$ (Equ. S3)
where $[H]_0$ is the total concentration of guest in the cuvette.

$\partial = \partial_H\,[H]/[H]_0 + \partial_{H\bullet G}\,[H\bullet G]/[H]_0$ (Equ. S4)

For a given estimate of the association constant (K), an iterative cycle was used to solve for the concentrations of all species present in the cuvette at every point in the titration using Equations S1-S3. For a given estimate of the chemical shifts of the free and bound host (∂_H and $\partial_{H\bullet G}$), the chemical shift (∂) at every point in the titration was calculated using Equation S4.

2:1 complexes

An iterative VBA macro was used to optimise the values of K_1, K_2, $\square_H$, $\square_{H\bullet G}$ and $\square_{H\bullet GG}$ to minimise the difference between the experimental values of $\square$ and the values calculated using Equations S5-S4.

$[H•G] = K_1 [H] [G]$ (Equ. S5)
where [H] is the concentration of free host, [G] is the concentration of free guest and [H•G] is the concentration of the 1:1 complex.

$[H•GG] = K_1 K_2 [H] [G]^2$ (Equ. S6)
where [H•GG] is the concentration of the 2:1 complex.

$[G]_0 = [G] + [H•G] + 2[H•GG]$ (Equ. S7)

$[H]_0 = [H] + [H•G] + [H•GG]$ (Equ. S8)

$\partial = \partial_H [H]/[H]_0 + \partial_{H•G} [H•G]/[H]_0 + \partial_{H•GG} [H•GG]/[H]_0$ (Equ. S9)

For a given estimate of the association constants, an iterative cycle was used to solve for the concentrations of all species present in the cuvette at every point in the titration using Equations S5-S8. For a given estimate of the chemical shifts of the free and bound host, the chemical shift (∂) at every point in the titration was calculated using Equation S9. Three different methods of fitting were investigated.

a) Non-cooperative model using identical binding sites:
   $K_1 = K_2 = K$nc and the change in chemical shift for formation of the 1:1 complex is identical to the subsequent change in chemical for formation of the 1:2 complex
b) Non-cooperative model where K_1 and K_2 were fixed to be the appropriate K_{ref}
c) Cooperative isotherm where K_1 was fixed to be the appropriate K_{ref}

3:3 complexes

An iterative VBA macro was used to optimise the values of $K_{rosette}$, ∂_H and ∂_{bound} to minimise the difference between the experimental values of ∂ and the values calculated using Equations S10-S13.

$[rosette] = K [H]^3 [G]^3$ (Equ. S10)
where [rosette] is the concentration of fully assembled rosette.

$[G]_0 = [G] + 3[rosette]$ (Equ. S11)

$[H]_0 = [H] + 3[rosette]$ (Equ. S12)

$\partial = \partial_H [H]/[H]_0 + 3\partial_{bound} [rosette]/[H]_0$ (Equ. S13)

For a given estimate of the association constant, an iterative cycle was used to solve for the concentrations of all species present in the cuvette at every point in the titration using Equations S10-S2. For a given estimate of the chemical shifts, the chemical shift (∂) at every point in the titration was calculated using Equation S4.

Dilution experiments

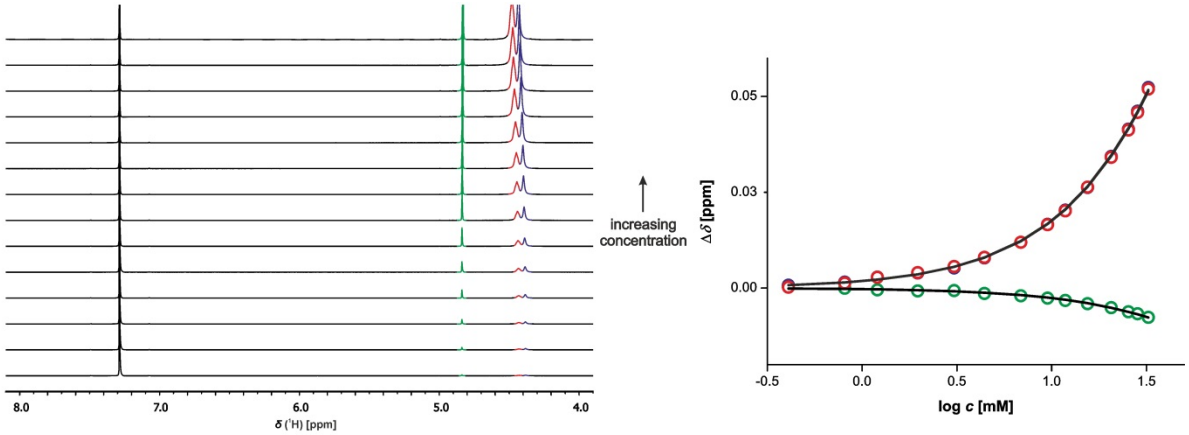


Figure S5. ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) dilution experiments for **P**. The ^{1}H NMR spectra are shown on the left (pyrimidine C-H signal green, N-H signals blue and red). On the right are shown the experimental data (circles) and calculated values (lines) based on a dimerisation isotherm.

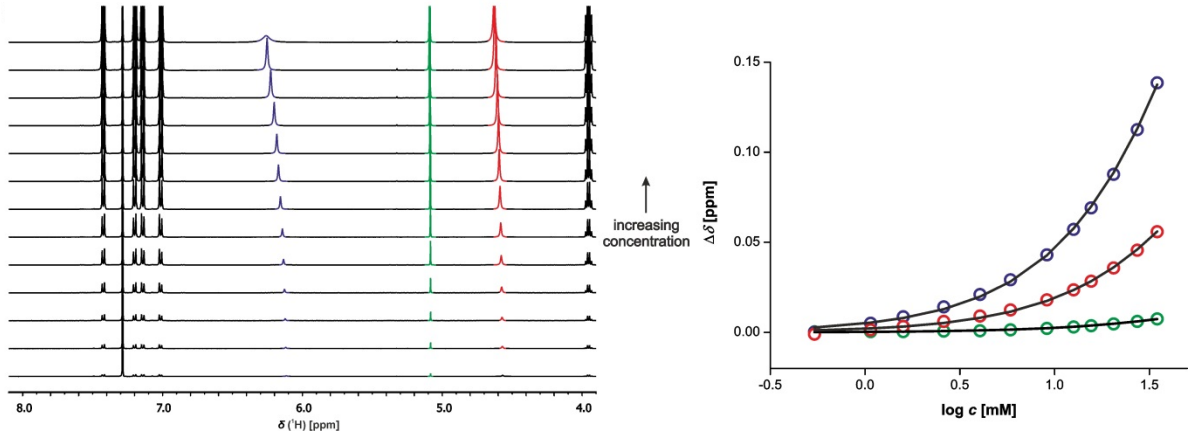


Figure S6. ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) dilution experiments for **bP'**. The ^{1}H NMR spectra are shown on the left (pyrimidine C-H signal green, N-H signals blue and red). On the right are shown the experimental data (circles) and calculated values (lines) based on a dimerisation isotherm.

NMR titrations

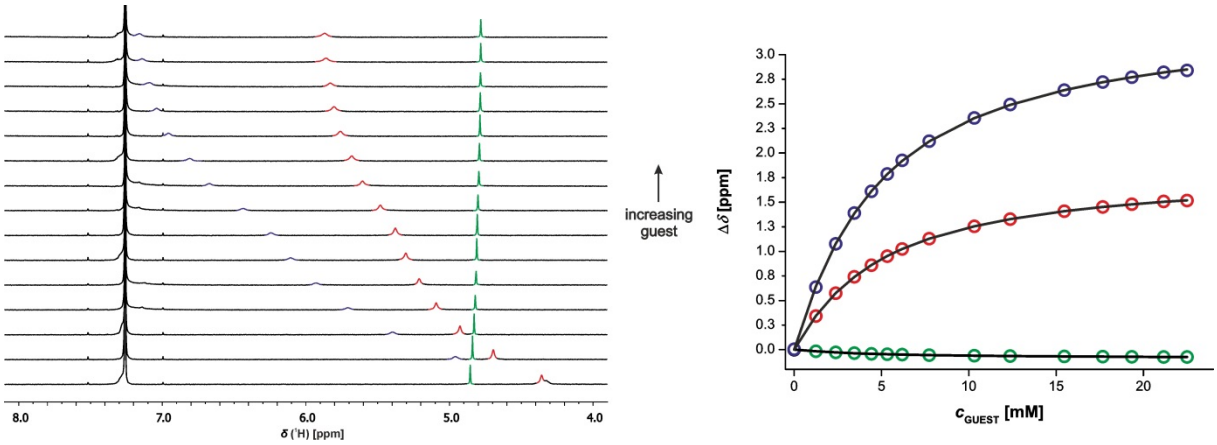


Figure S7. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) titration of **bB** into **bP** (1.4 mM). ^{1}H NMR spectrum is shown on the left (pyrimidine C-H signal green, N-H signals blue and red). On the right are shown the experimental data (circles) and calculated values (lines) based on a 1:1 binding isotherm.

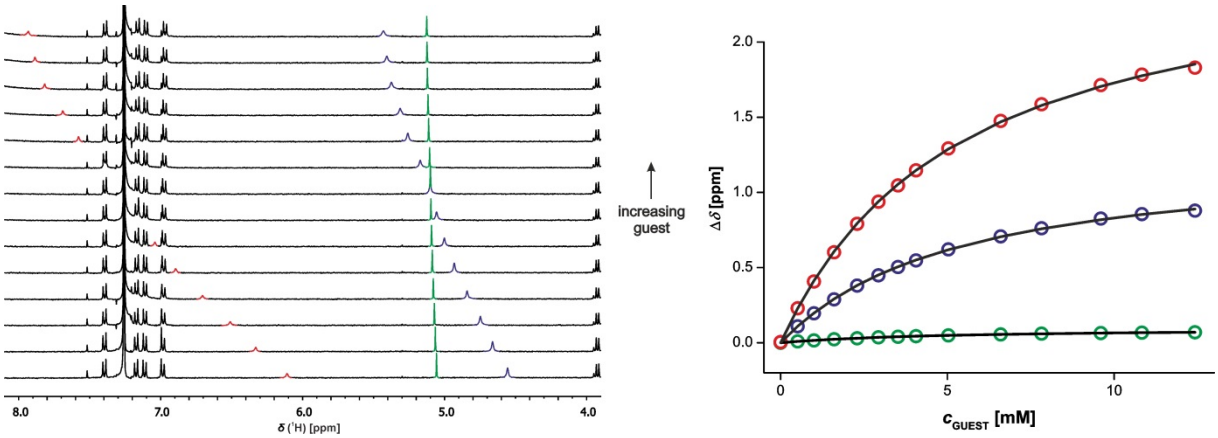


Figure S8. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) titration of **bB** into **bP'** (0.53 mM). ^{1}H NMR spectrum is shown on the left (pyrimidine C-H signal green, N-H signals blue and red). On the right are shown the experimental data (circles) and calculated values (lines) based on a 1:1 binding isotherm.

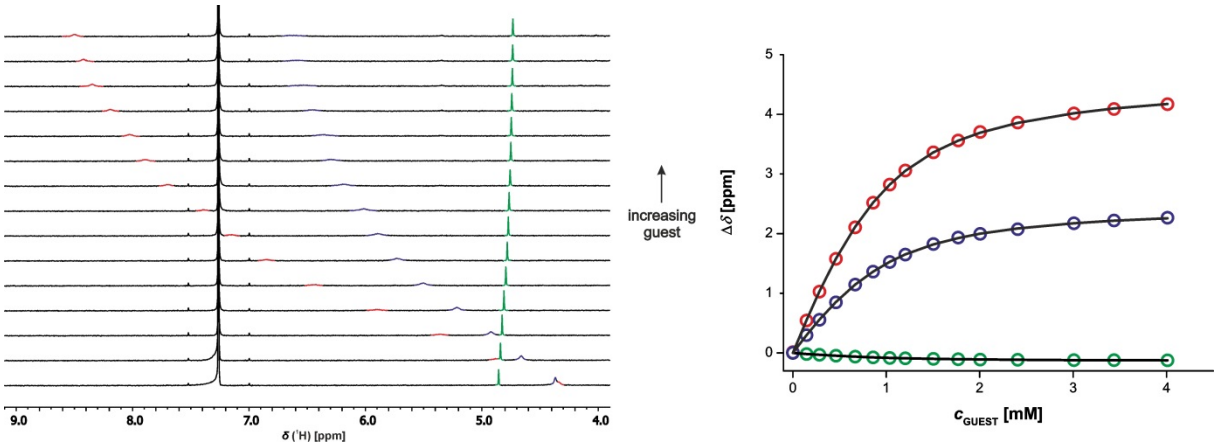


Figure S9. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) titration of **bC** into **bP** (0.86 mM). ^{1}H NMR spectrum is shown on the left (pyrimidine C-H signal green, N-H signals blue and red). On the right are shown the experimental data (circles) and calculated values (lines) based on a 1:1 binding isotherm.

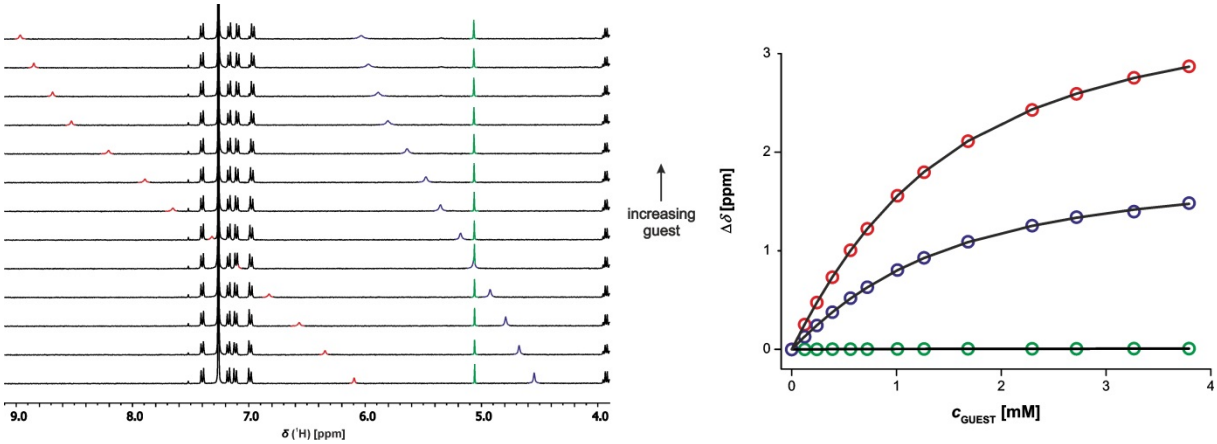


Figure S10. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) titration of **bC** into **bP'** (0.83 mM). ^{1}H NMR spectrum is shown on the left (pyrimidine C-H signal green, N-H signals blue and red). On the right are shown the experimental data (circles) and calculated values (lines) based on a 1:1 binding isotherm.

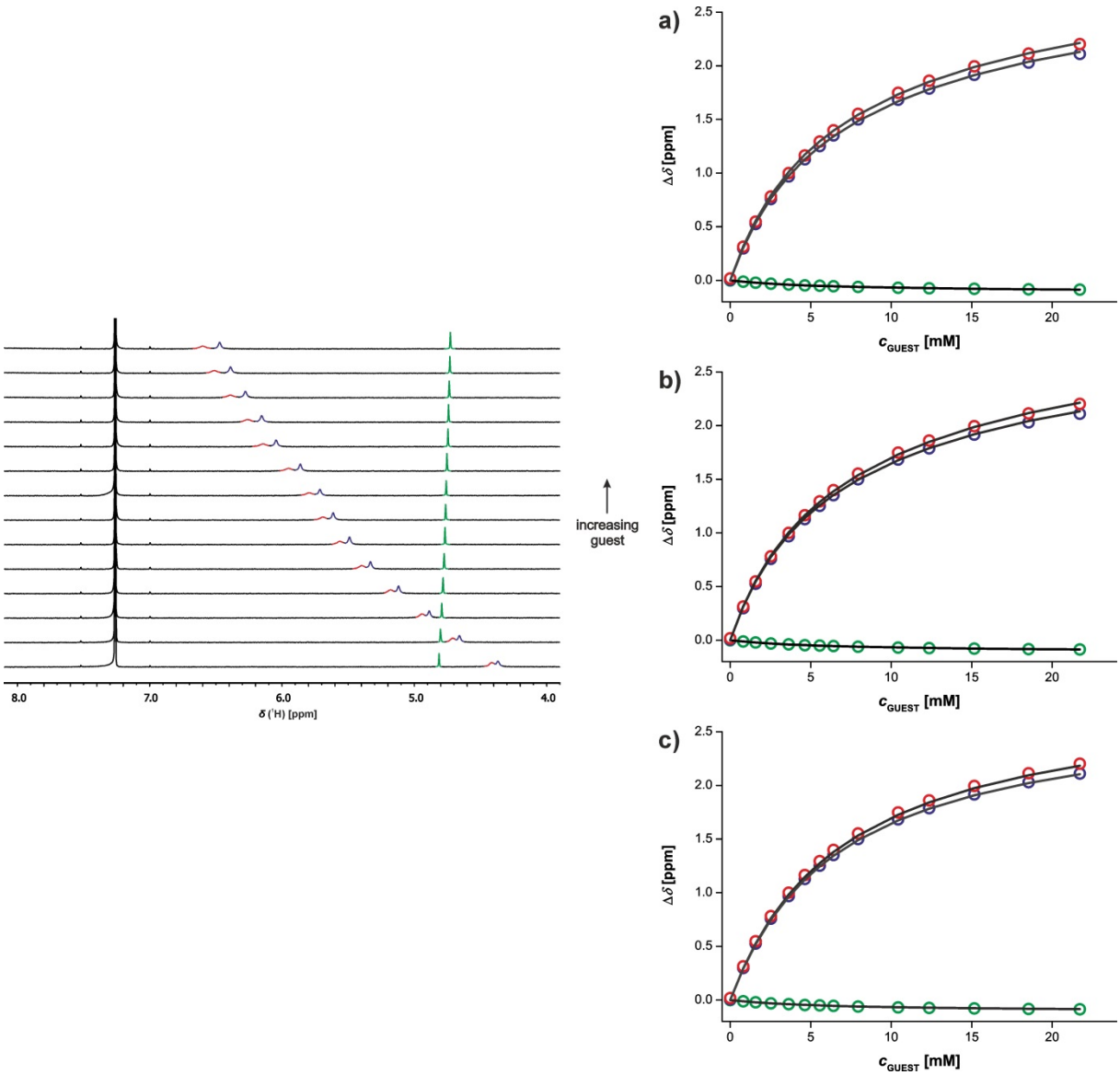


Figure S11. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) titration of **bB** into **P** (0.99 mM). ^{1}H NMR spectrum is shown on the left (pyrimidine C-H signal green, N-H signals blue and red). On the right are shown the experimental data (circles) and calculated values (lines) based on a 1:2 binding isotherms using (a) non-cooperative model using identical binding sites, (b) non-cooperative model where K_1 and K_2 were fixed to be the appropriate K_{ref} and (c) cooperative isotherm where K_1 was fixed to be the appropriate K_{ref}.

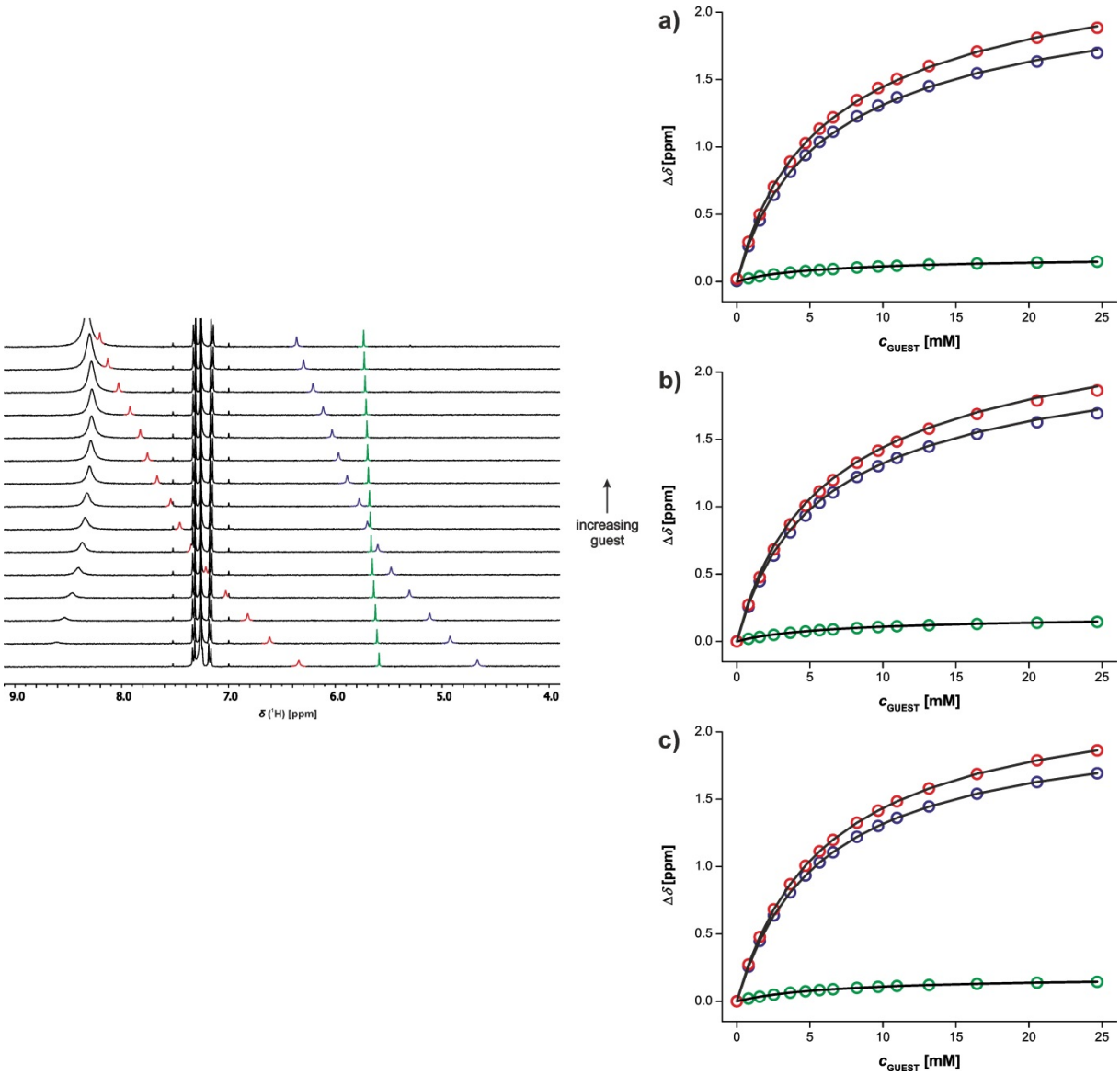


Figure S12. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) titration of **bB** into **P'** (0.53 mM). ^{1}H NMR spectrum is shown on the left (pyrimidine C-H signal green, N-H signals blue and red). On the right are shown the experimental data (circles) and calculated values (lines) based on a 1:2 binding isotherms using (a) non-cooperative model using identical binding sites, (b) non-cooperative model where K_1 and K_2 were fixed to be the appropriate K_{ref} and (c) cooperative isotherm where K_1 was fixed to be the appropriate K_{ref}.

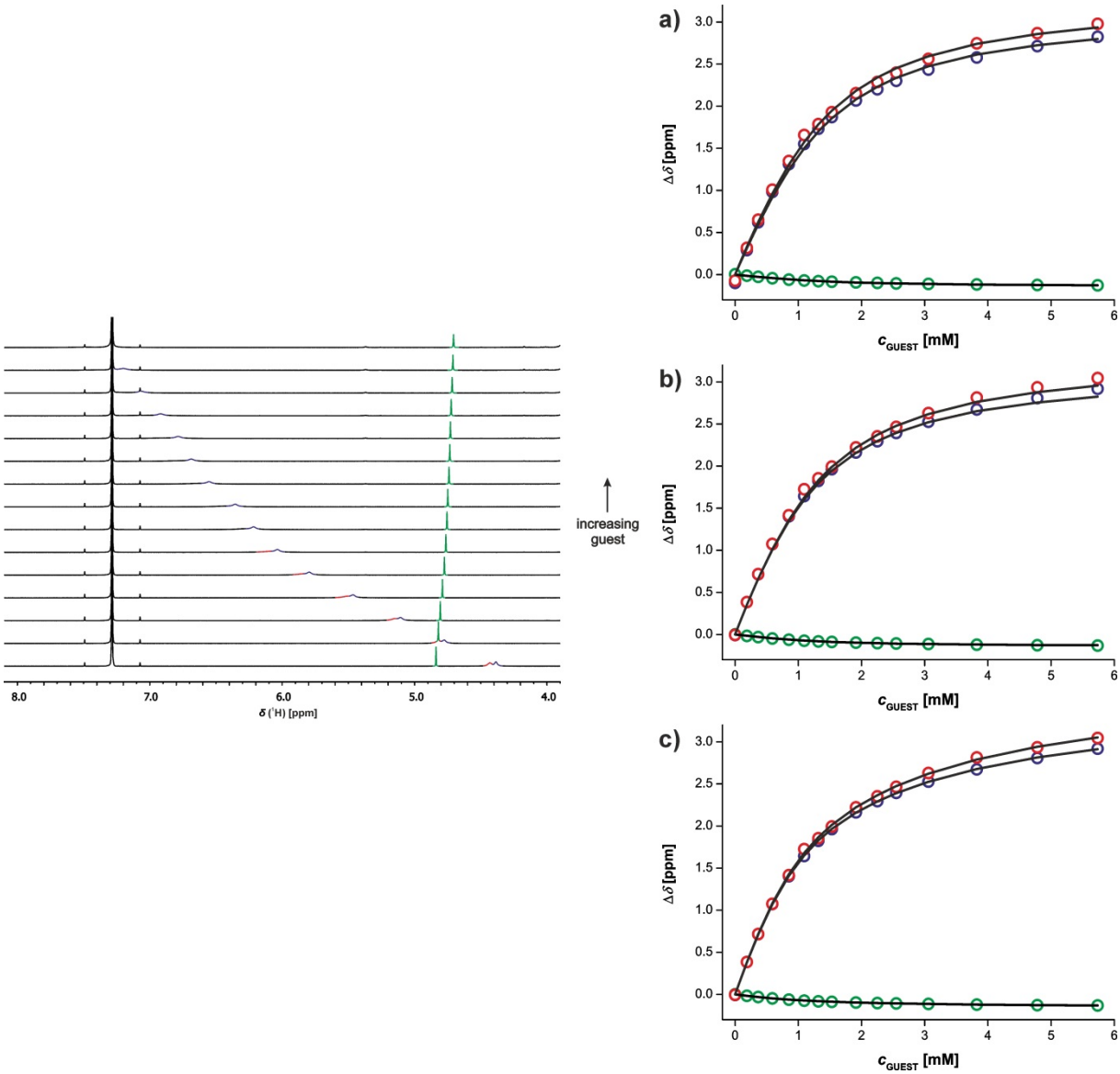


Figure S13. ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) titration of **bC** into **P** (0.77 mM). ^{1}H NMR spectrum is shown on the left (pyrimidine C-H signal green, N-H signals blue and red). On the right are shown the experimental data (circles) and calculated values (lines) based on a 1:2 binding isotherms using (a) non-cooperative model using identical binding sites, (b) non-cooperative model where K_1 and K_2 were fixed to be the appropriate K_{ref} and (c) cooperative isotherm where K_1 was fixed to be the appropriate K_{ref}.

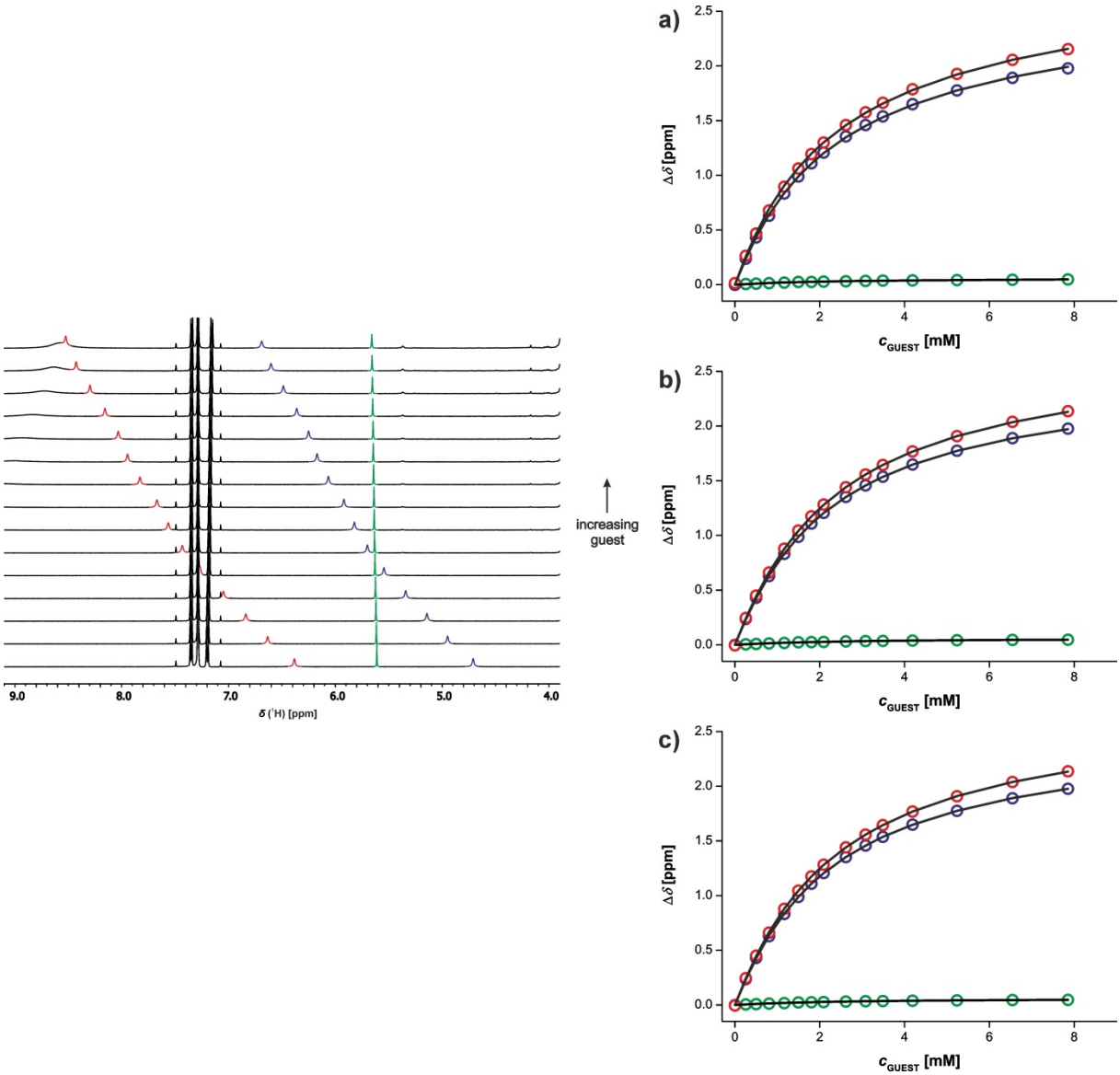


Figure S14. ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) titration of **bC** into **P'** (0.65 mM). ^{1}H NMR spectrum is shown on the left (pyrimidine C-H signal green, N-H signals blue and red). On the right are shown the experimental data (circles) and calculated values (lines) based on a 1:2 binding isotherms using (a) non-cooperative model using identical binding sites, (b) non-cooperative model where K_1 and K_2 were fixed to be the appropriate K_{ref} and (c) cooperative isotherm where K_1 was fixed to be the appropriate K_{ref}.

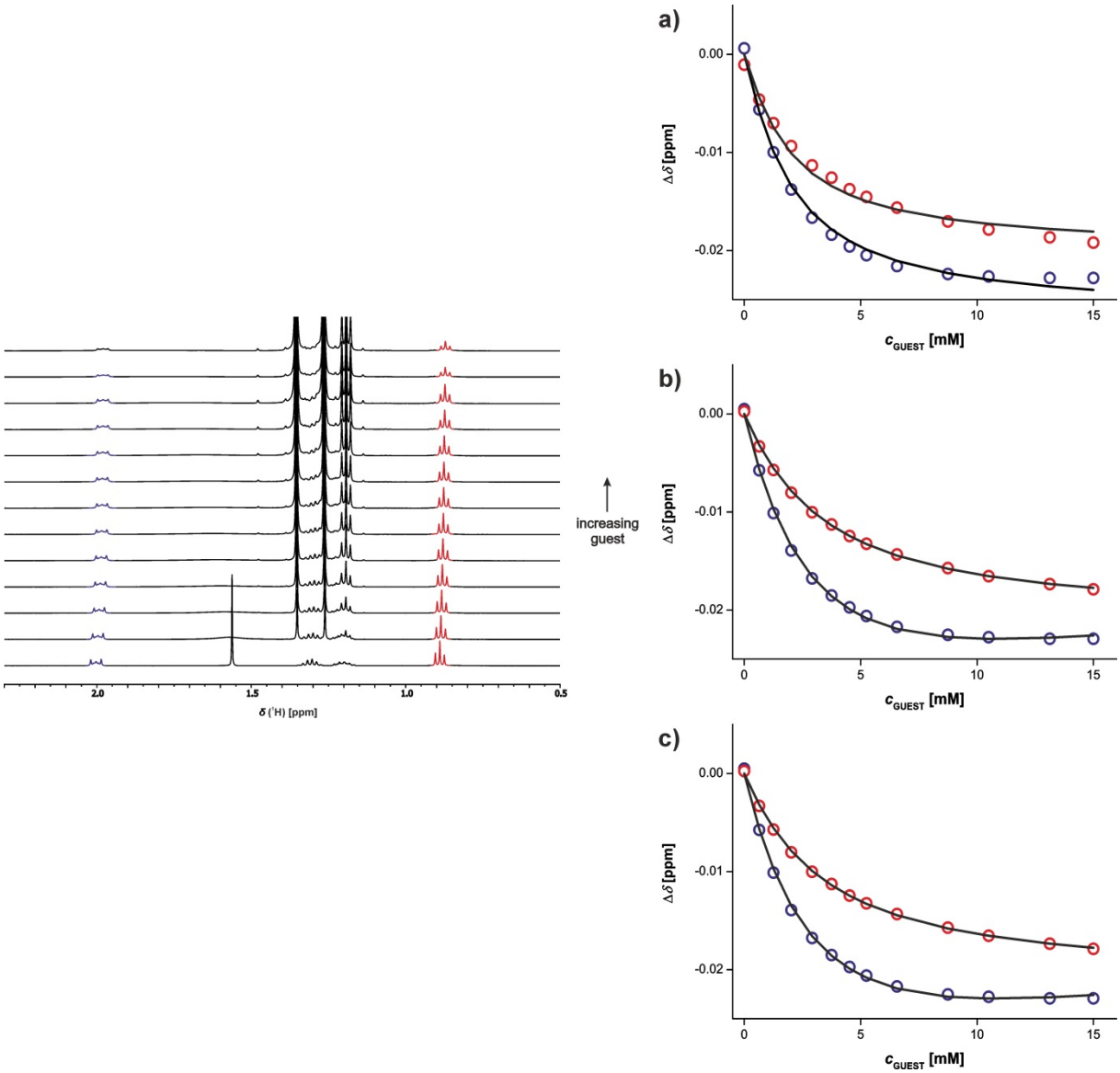


Figure S15. ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) titration of **bP'** into **B** (0.90 mM). ^{1}H NMR spectrum is shown on the left (CH_3 signal red, $(C{=}O)_2C(CH_2)_2$ signal blue). On the right are shown the experimental data (circles) and calculated values (lines) based on a 1:2 binding isotherms using (a) non-cooperative model using identical binding sites, (b) non-cooperative model where K_1 and K_2 were fixed to be the appropriate K_{ref} and (c) cooperative isotherm where K_1 was fixed to be the appropriate K_{ref}.

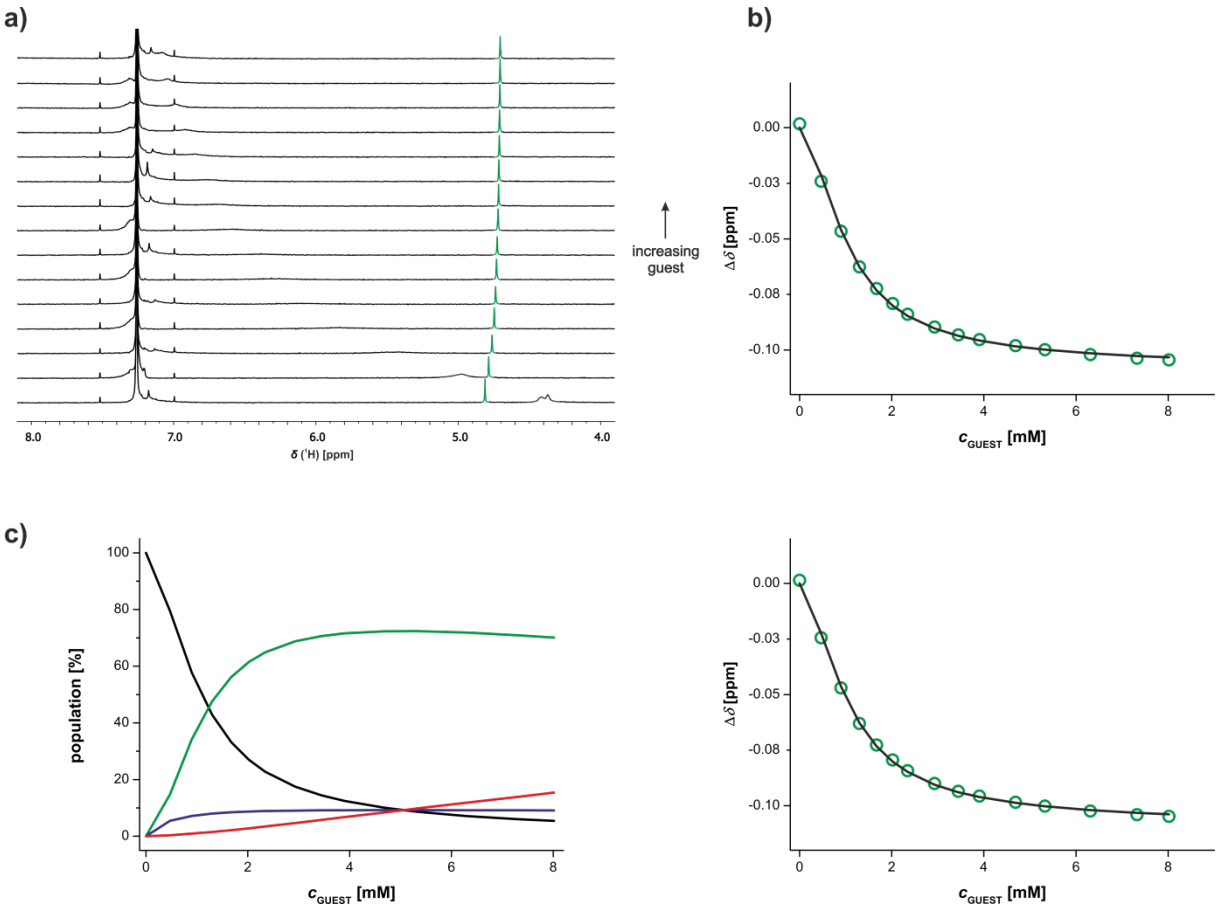


Figure S16. Titration of **B** into **P** (0.88 mM). (a) ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectrum (pyrimidine C-H signal green). (b) Fitting with an all-or-nothing 3:3 binding isotherm: experimental data (circles) and calculated values (line). (c) Fitting with a 3:3 binding isotherm allowing opening to 1:2 complexes. Fitting is on the right and the distribution diagram on the left (**P** black, **P**$_3$•**B**$_3$ green, **P**•**B** blue and **P**•**B**$_2$ red).

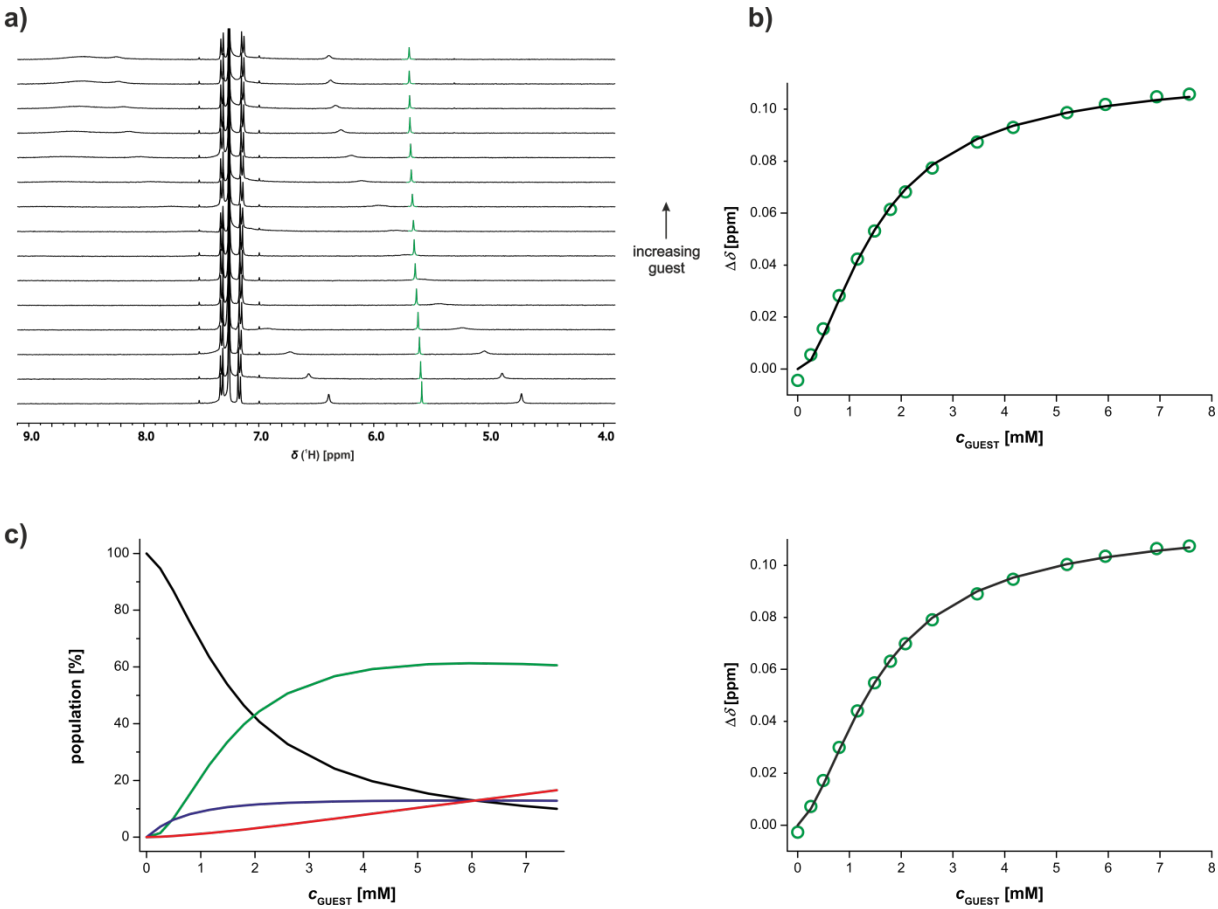


Figure S17. Titration of **B** into **P'** (1.1 mM). (a) ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectrum (pyrimidine C-H signal green). (b) Fitting with an all-or-nothing 3:3 binding isotherm: experimental data (circles) and calculated values (line). (c) Fitting with a 3:3 binding isotherm allowing opening to 1:2 complexes. Fitting is on the right and the distribution diagram on the left (**P'** black, **P'$_3$•B$_3$** green, **P'•B** blue and **P'•B$_2$** red).

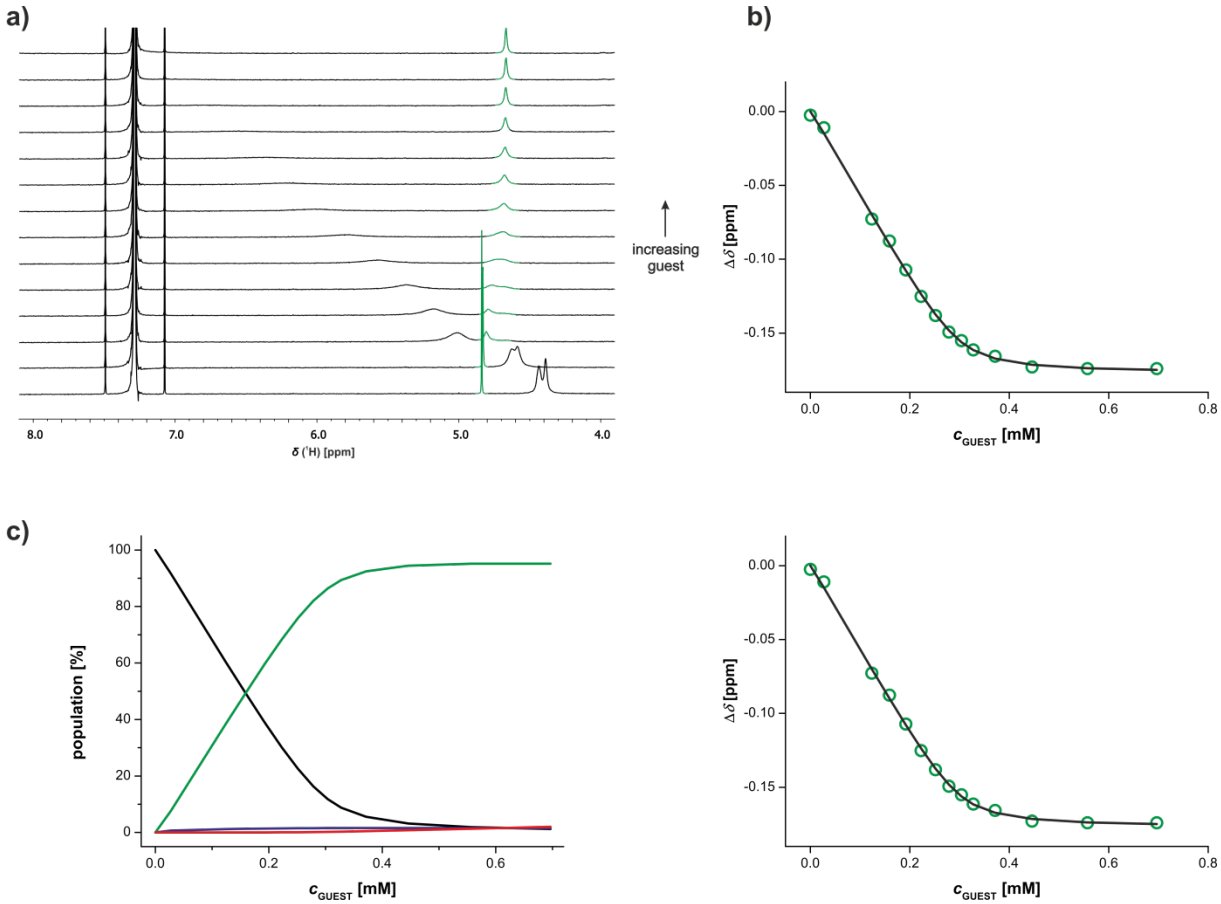


Figure S18. Titration of **C** into **P** (0.30 mM). (a) ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) spectrum (pyrimidine C-H signal green). (b) Fitting with an all-or-nothing 3:3 binding isotherm: experimental data (circles) and calculated values (line). The weight averaging was used to obtain the average chemical shift of pyrimidine C-H signal. (c) Fitting with a 3:3 binding isotherm allowing opening to 1:2 complexes. Fitting is on the right and the distribution diagram on the left (**P** black, **P$_3$•C$_3$** green, **P•C** blue and **P•C$_2$** red).

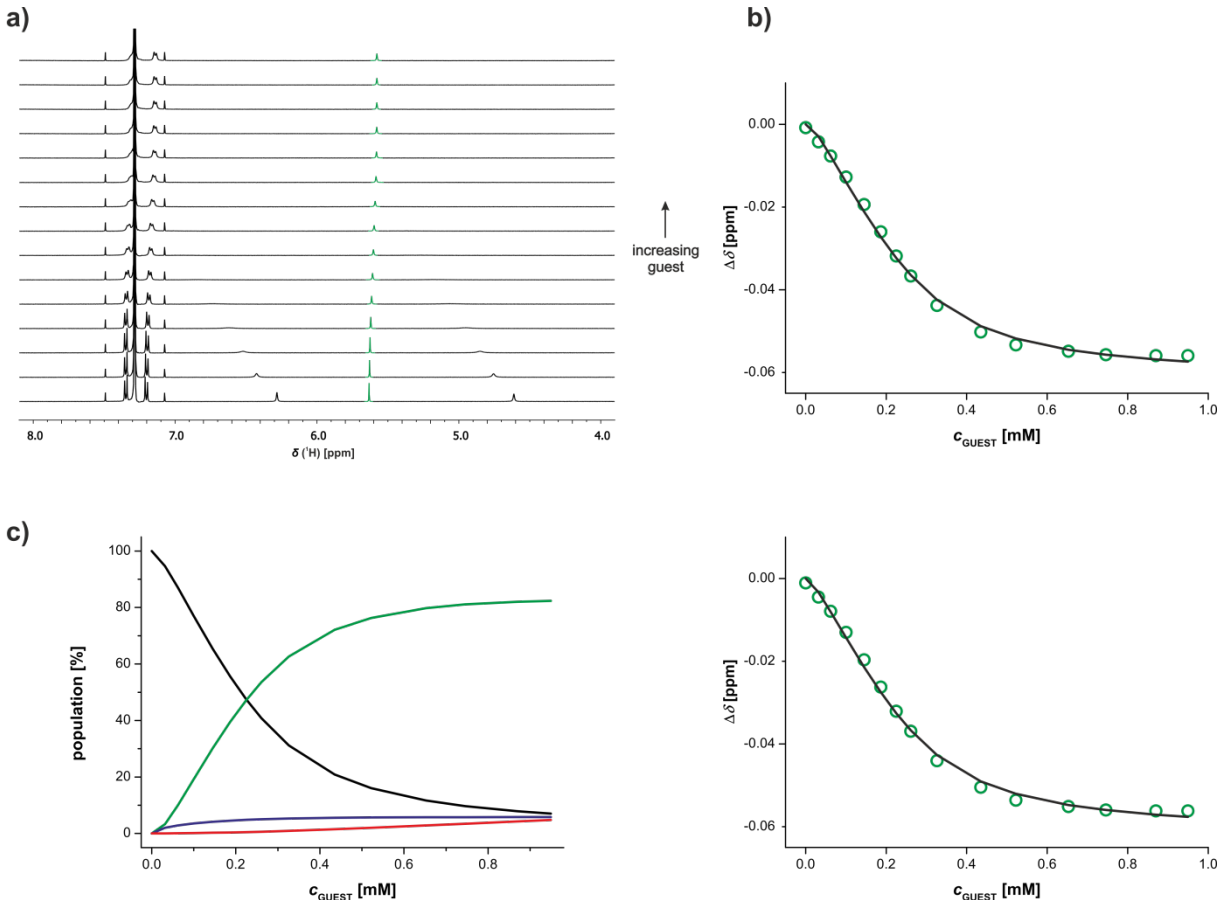
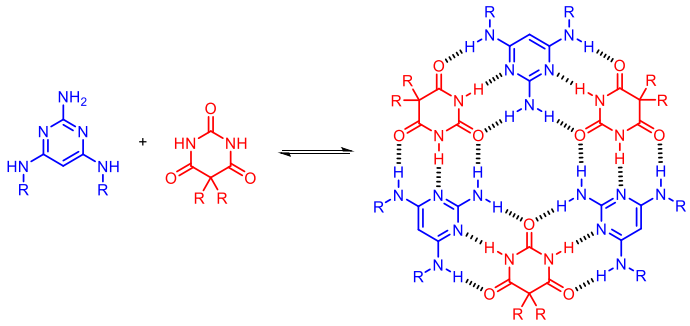


Figure S19. Titration of **C** into **P'** (0.26 mM). (a) ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) spectrum (pyrimidine C-H signal green). (b) Fitting with an all-or-nothing 3:3 binding isotherm: experimental data (circles) and calculated values (line). (c) Fitting with a 3:3 binding isotherm allowing opening to 1:2 complexes. Fitting is on the right and the distribution diagram on the left (**P'** black, **P'$_3$•C$_3$** green, **P'•C** blue and **P'•C$_2$** red).

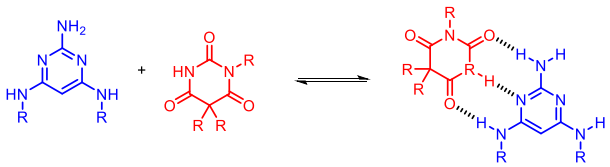
Statistical Factors



point group	C_{2v}	C_{2v}	D_{3h}
σ_{ext}	2	2	6
σ_{int}	1	1	1
σ	2	2	6

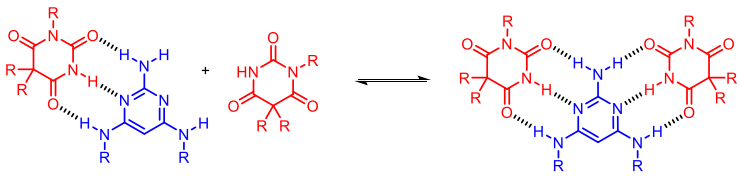
$$K_\sigma = \frac{2^3 \cdot 2^3}{6} = \frac{32}{3}$$

Figure S20. Determination of the statistical factor K_σ for the rosette assembly (R is a substituent). The statistical factor derived above for the barbiturates can also be applied to the cyanurates thanks to their analogous symmetry



point group	C_{2v}	C_{1v}	C_{1v}
σ_{ext}	2	1	1
σ_{int}	1	1	1
σ	2	1	1

$$K_\sigma = \frac{2 \cdot 1}{1} = 2$$



point group	C_{1v}	C_{1v}	C_{2v}
σ_{ext}	1	1	2
σ_{int}	1	1	1
σ	1	1	2

$$K_\sigma = \frac{1 \cdot 1}{2} = \frac{1}{2}$$

Figure S21. Determination of the statistical factors for the reference 1:2 systems. The statistical factors are identical for the corresponding system based on a barbiturate and blocked-pyrimidines. The statistical factor derived above for the barbiturates can also be applied to the cyanurates thanks to their analogous symmetry

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