Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

A polyoxapolyaza macrobicyclic receptor for the recognition of zwitterions

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Experimental Section

General considerations All solvents and chemicals were commercially purchased reagent grade quality and used as supplied without further purification. (4-diethoxymethylphenyl)methanol and 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene were prepared according to literature methods.[1,2] Tetramethylammonium *p*-toluenesulfonate (NMe_4TsO) was prepared by the neutralization of HTsO with NMe_4OH in water, followed by recrystallization from acetone. NMR spectra used for characterization of products were recorded on a 400 MHz instrument. TMS was used as reference for the ^{1}H-NMR measurements in $CDCl_3$. Peak assignments are based on peak integration and multiplicity for 1D ^{1}H spectra and on 2D COSY, NOESY and HMQC experiments (Figs. S1–S8). Microanalyses were carried out by the ITQB Microanalytical Service.

Syntheses

Tripodal trialdehyde (3). To a solution of (4-diethoxymethylphenyl)methanol (2.40 g, 11.4 mmol) in dry DMSO (30 cm^3) was added NaH (60% dispersion in oil) (456 mg, 11.4 mmol) under nitrogen. The temperature was raised to 90 ºC and left stirring until bubbling ceased and a clear brown solution was obtained. Then 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene (1.640 g, 3.7 mmol) was added in one portion and the solution was left stirring at 90 ºC for 3 hours. The solution was allowed to cool to r.t., poured into water (80 cm^3) and extracted with $CHCl_3$ (5×50 cm^3). The organic portions were collected in an Erlenmeyer flask, dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The solid was dissolved in CH_2Cl_2 (25 cm^3) to which water (25 cm^3) and CF_3COOH 99% (25 cm^3) was added. The mixture was vigorously stirred overnight.

The mixture was transferred to a separating funnel and the aqueous phase was rejected. The organic portion was then washed with $NaHCO_3$ saturated solution (3×30 cm^3), dried over anhydrous sodium sulfate, filtered and evaporated to dryness to give the crude tripodal trialdehyde as a yellow viscous oil which solidified after drying in a vacuum line. The product was purified by dissolving a minimum amount in boiling ethyl acetate, and then n-hexane was added until the solution became turbid. After cooling the precipitate formed was filtered and dried in vacuum, yielding pure tripodal trialdehyde (**3**) (1.413 g, 61%). δ_H (400 MHz; $CDCl_3$; 298 K; TMS): 1.07 (t, ^3J (H,H) = 8.0 Hz, 9 H; bzCH_2CH_3), 2.73 (q, ^3J (H,H) = 8.0 Hz, 6 H; bzCH_2CH_3), 4.51 (s, 6 H; CH_2bz), 4.63 (6 H, s, CH_2*p*-xylyl), 7.47 (6 H, d, ^3J (H,H) = 8.0 Hz, *H2*/*H6* of *p*-xylyl), 7.79 (6 H, d, ^3J (H,H) = 8.0 Hz, *H3*/*H5* of *p*-xylyl) and 9.94 (3 H, s, *H*C=O) ppm; δ_C(100 MHz; $CDCl_3$; 298 K; Me_4Si) 16.7 ppm (bzCH_2CH_3), 23.0 (bzCH_2CH_3), 67.1

(*C*H_2bz), 77.5 (*C*H_2*p*-xylyl), 128.3 (C2/*C6* of *p*-xylyl), 130.0 (*C3*/*C5* of *p*-xylyl), 131.9 (*C2*/*C4*/*C6* of bz), 135.9 (*C4* of *p*-xylyl), 145.5 (*C1*/*C3*/*C5* of bz), 145.5 (*C1* of *p*-xylyl), 192.1 (*C*=O) ppm.

btpN_4O_3. A boiling solution of the tripodal trialdehyde (**3**) (174 mg, 0.29 mmol) in MeCN (20 cm^3) was quickly added to a refluxing solution of tren (44 mg, 0.29 mmol) in MeCN (500 cm^3) under nitrogen and left stirring for 1 hour. The solution was concentrated to a third of its volume and MeOH (350 cm^3) was added. To this solid $NaBH_4$ (320 mg, 8.46 mmol) was added in small portions to avoid excessive foaming. After the addition was completed, the mixture was left under stirring at r.t. for 1 hour, and under reflux for 2 hours. The solution was evaporated under vacuum to dryness, then water (20 cm^3) was added. The solution was made strongly basic with 6 M KOH and extracted with $CHCl_3$ (3×50 cm^3). The organic portions were collected in an Erlenmeyer flask, dried over anhydrous sodium sulfate, filtered off, evaporated to dryness to give the crude btpN_4O_3 (177 mg). The product was purified by flash column chromatography using $CHCl_3$/MeOH/NH_3(aq.) (90:9:1) as eluent, followed by recrystallization from MeCN to yield pure btpN_4O_3 (80 mg, 40%); mp 173 ºC dec; δ_H (400 MHz; $CDCl_3$; 298 K; TMS): 1.11 (9 H, t, ^3J (H,H) = 8.0 Hz, bzCH_2CH_3), 1.19 (3H, br s, N–*H*), 2.44 (6 H, t, ^3J (H,H) = 4.0 Hz, NCH_2CH_2NH), 2.52 (6 H, t, ^3J (H,H) = 4.0 Hz, NCH_2CH_2NH), 3.09 (6 H, q, ^3J (H,H) = 8.0 Hz, bzCH_2CH_3), 3.34 (6 H, s, NHCH_2*p*-xylyl), 4.34 (6 H, s, *p*-xylylCH_2O), 4.75 (6 H, s, OCH_2bz), 6.64 (6 H, d, ^3J (H,H) = 8.0 Hz, *H3*/*H5* of *p*-xylyl), 6.78 (6 H, d, ^3J (H,H) = 8.0 Hz, *H2*/*H6* of *p*-xylyl); δ_C (100 MHz; $CDCl_3$; 298 K; Me_4Si) 16.9 (bzCH_2CH_3), 22.7 (bzCH_2CH_3), 48.2 (NCH_2CH_2NH), 55.1 (NCH_2CH_2NH), 55.5 (NHCH_2*p*-xylyl), 65.2 (OCH_2bz), 69.7 (*p*-xylylCH_2O), 128.3 (*C3*/*C5* of *p*-xylyl), 128.4 (*C2*/*C6* of *p*-xylyl), 131.8 (*C2*/*C4*/*C6* of bz), 137.3 (*C4* of *p*-xylyl), 139.7 (*C1* of *p*-xylyl), 145.9 (*C1*/*C3*/*C5* of bz) ppm; m/z (ESI–MS;MeOH) 705.1 $[M+H]^+$.; elemental analysis calcd (%) for $C_{45}H_{60}N_4O_3$: C 76.67, H 8.58, N 7.95; found: C 77.00, H 8.59, N 8.04.

Crystals of btpN_4O_3·MeCN. The cryptand btpN_4O_3 (5.0 mg, 7.1 μmol) was dissolved in the minimum amount of hot MeCN then the mixture was allowed to slowly cool to r.t. Single colourless crystals suitable for X-ray crystallographic determination were obtained overnight.

Potentiometric measurements

Reagents and solutions. All solutions were prepared in water/methanol (50:50 v/v) mixed solvent. A stock solution of the receptor was prepared at *ca*. 2.0×10^{-3} M. Solutions of the

substrates were prepared at 0.025 M and the concentrations were checked by titration with standard 0.1 M NMe_4OH solutions. Carbonate-free solutions of the titrant NMe_4OH were obtained at *ca.* 0.1 M by treating freshly prepared silver oxide with a 500 cm^3 solution of NMe_4I, under nitrogen atmosphere and in appropriate glass apparatus according to the method of Schwarzenbach and Biederman,[3] to which 500 cm^3 of methanol was added. These solutions were discarded every time carbonate concentration was about 0.5% of the total amount of base. The titrant solutions were standardized (tested by Gran's method).[4]

Equipment and working conditions. The equipment used was described before.[5] The ionic strength of the experimental solutions was kept at 0.10±0.01 M with NMe_4TsO, and the temperature was maintained at 298.2±0.1 K. Atmospheric CO_2 was excluded from the titration cell during experiments by passing purified nitrogen across the top of the experimental solution. The glass electrode was pre-treated by soaking it in the water–methanol (50:50 v/v) solution over a period of 2 days, in order to prevent erratic responses.

Measurements. The $[H^+]$ of the solutions was determined by the measurement of the electromotive force of the cell, $E = E^{o\prime} + Q \log [H^+] + E_j$. The term pH is defined as $-\log [H^+]$. $E^{o\prime}$, Q, E_j and K_w were determined by titration of a solution of known hydrogen-ion concentration at the same ionic strength, using the acid pH range of the titration. The liquid-junction potential, E_j, was found to be negligible under the experimental conditions used. The value of K_w was determined from data obtained in the alkaline range of the titration, considering $E^{o\prime}$ and Q valid for the entire pH range and found to be equal to $10^{-13.91}$ in our experimental conditions. Before and after each set of titrations the glass electrode was calibrated by titration of 1.00×10^{-3} M standard HCl solution with standard NMe_4OH. The potentiometric equilibrium measurements were carried out using 20.00 cm^3 of $\approx 2.00\times10^{-3}$ M of the receptor stock solution diluted to a final volume of 40.00 cm^3, in the absence of substrates, then in the presence of each amino acid at 1:3 R:A ratios (R = receptor and A = amino acid). In each titration 85 to 140 points were collected, and a minimum of two titration curves were performed. Care has been taken to maintain unaltered the methanol–water ratio in measured solution. The exact concentration of the receptors was obtained by determination of the excess of acid present in a mixture of the receptors and a known amount of standard *p*-toluenesulfonic acid by titration with standard NMe_4OH solution.

Calculation of equilibrium constants. Overall protonation constants, β_i^H, of ligands and anions, were calculated by fitting the potentiometric data obtained for all the performed titrations in the same experimental conditions with the HYPERQUAD program.[6] All these constants were taken as fixed values to obtain the equilibrium constants of the new species from the experimental data corresponding to all the titrations at 1:3 R:A ratio, also using the HYPERQUAD program. The initial computations were obtained in the form of overall stability constants, $\beta_{H_hL_lA_a}$ values, $\beta_{H_hL_lA_a} = [H_hL_lA_a]/[H]^h[L]^l[A]^a$. The errors quoted are the standard deviations of the overall association constants given directly by the program for the input data, which include all the experimental points of all titration curves. The HYSS program[7] was used to calculate the concentration of equilibrium species from the calculated constants from which distribution diagrams were plotted. The species considered in a particular model were those that could be justified by the principles of supramolecular chemistry.

Crystallography. The X-ray data of $btpN_4O_3$ was collected at 150(2) K using graphite monochromatized Mo-Kα radiation (λ = 0.71073 Å). The crystal was positioned at 35 mm from the CCD and the spots were measured using a counting time of 60 s. Data reduction including a multi-scan absorption correction was carried out using the SAINT-NT software package. The structure was solved by a combination of direct methods with subsequent difference Fourier syntheses and refined by full matrix least squares on F^2 using the SHELX-97 suite.[8] The C−H hydrogen atoms were inserted at geometrical positions while the positions of the hydrogen atoms of the N−H secondary amine groups were taken from the last difference Fourier Maps. Anisotropic thermal parameters were used for all non-hydrogen atoms while the C−H and N−H hydrogen atoms were refined with isotropic parameters equivalent 1.2 times those of the atom to which they are attached. Molecular diagram was drawn with PyMOL software package.[9] Crystal data and refinement details are summarized in Table S1.

Table S1. Crystal data and refinement details of [btpN_4O_3 CH_3CN].

Empirical Formula	$C_{47}H_{63}N_5O_3$
M_w	746.02
Crystal System	Triclinic
Space group	$P\bar{1}$
a / [Å]	11.9682(3)
b / Å	13.5050(3)
c / Å	14.0716(4)
α / º	91.8620(10)
β /º	92.1060(10)
γ /º	11.1350(10)
V /Å^3	2117.42(9)
Z	2
D_c /Mg m^{-3}	1.170
μ /mm^{-1}	0.073
Reflections collected	51940
Unique reflections, [R_{int}]	11371 [0.0308]
Final R indices	
R_1, wR_2, [I>2σI]	0.0495, 0.1276, [8954]
R_1, wR$_2$ (all data)	0.0652, 0.1387

Table S2 Overall (β_i^H) and stepwise protonation (K_i^H) constants of $btpN_4O_3$ and of the studied amino acids in MeOH/H_2O.[a]

Equilibrium[b]	$btpN_4O_3$	gly^-	$bala^-$	tau^-	$gaba^-$	amp^{2-}	aep^{2-}
				$\log \beta_i^H$ [c]			
$L + H \rightleftharpoons HL$	8.76(1)	9.32(1)	9.68(1)	8.57(1)	9.95(1)	10.05(1)	10.90(1)
$L + 2\ H \rightleftharpoons H_2L$	17.01(1)	12.23(1)	13.81(1)	9.94(1)	14.67(1)	16.24(1)	17.92(1)
$L + 3\ H \rightleftharpoons H_3L$	23.27(1)	–	–	–	–	17.65(1)	19.84(1)
				$\log K_i^H$			
$L + H \rightleftharpoons HL$	8.65	9.32	9.68	8.57	9.95	10.05	10.90
$HL + H \rightleftharpoons H_2L$	8.25	2.91	4.13	1.37	4.72	6.18	7.02
$H_2L + H \rightleftharpoons H_3L$	6.26	–	–	–	–	1.42	1.92

[a] T = (298.2±0.1) K; I = (0.10±0.01) M in NMe_4TsO. [b] L denote in general the $btpN_4O_3$ and the amino acids in their deprotonated form, charges where omitted for simplicity. [c] Values in parenthesis are standard deviations in the last significant figures.

Table S3 Overall ($\log \beta_{H_hL_lA_a}$) and stepwise ($\log K_{H_hL_lA_a}$) association constants for the indicated equilibria between the $H_nbtpN_4O_3^{n+}$ receptor and Hgly, Hbala, Htau, Hgaba, H_2amp and H_2aep substrates in MeOH/H_2O.[a],[b]

Equilibrium[c]	gly^-	$bala^-$	tau^-	$gaba^-$	amp^{2-}	aep^{2-}
			$\log \beta_{H_hL_lA_a}$			
$5\ H + L + A \rightleftharpoons H_5LA$	–	–	–	–	42.29(5)	43.89(3)
$4\ H + L + A \rightleftharpoons H_4LA$	34.51(2)	35.00(3)	34.51(3)	34.85(1)	36.50 (5)	37.81(3)
$3\ H + L + A \rightleftharpoons H_3LA$	28.41(3)	28.72(3)	28.12(3)	28.68(1)	29.81(5)	30.62(6)
$2\ H + L + A \rightleftharpoons H_2LA$	20.18(3)	20.65(3)	19.73(4)	20.53(1)	21.46(5)	22.29(3)
$H + L + A \rightleftharpoons HLA$	11.57(3)	11.89(2)	11.45(3)	11.86(1)	12.63(7)	13.34 (3)
			$\log K_{H_hL_lA_a}$			
$H_3L + H_2A \rightleftharpoons H_5LA$	–	–	–	–	2.83(5)	2.69(3)
$H_2L + H_2A \rightleftharpoons H_4LA$	–	–	–	–	–	2.87(3)
$H_3L + HA \rightleftharpoons H_4LA$	1.92(2)	2.02(3)	2.67(3)	1.63(2)	3.21 (5)	–
$H_2L + HA \rightleftharpoons H_3LA$	2.09(3)	2.00(3)	2.55(3)	1.73(2)	2.79(5)	2.72(6)
$HL + HA \rightleftharpoons H_2LA$	2.11(3)	2.19(3)	2.41(4)	1.83(2)	2.69(5)	2.64(3)
$L + HA \rightleftharpoons HLA$	2.26(3)	2.19(2)	–	1.91(1)	2.62(7)	2.44(3)
$HL + A \rightleftharpoons HLA$	–	–	2.69(3)	–	–	–

[a] T = (298.2±0.1) K; I = (0.10±0.01) M in NMe_4TsO. [b] Values in parenthesis are standard deviations in the last significant figures. [c] L denote in general the $btpN_4O_3$ compound and A the amino acids in their deprotonated form, Charges where omitted for simplicity.

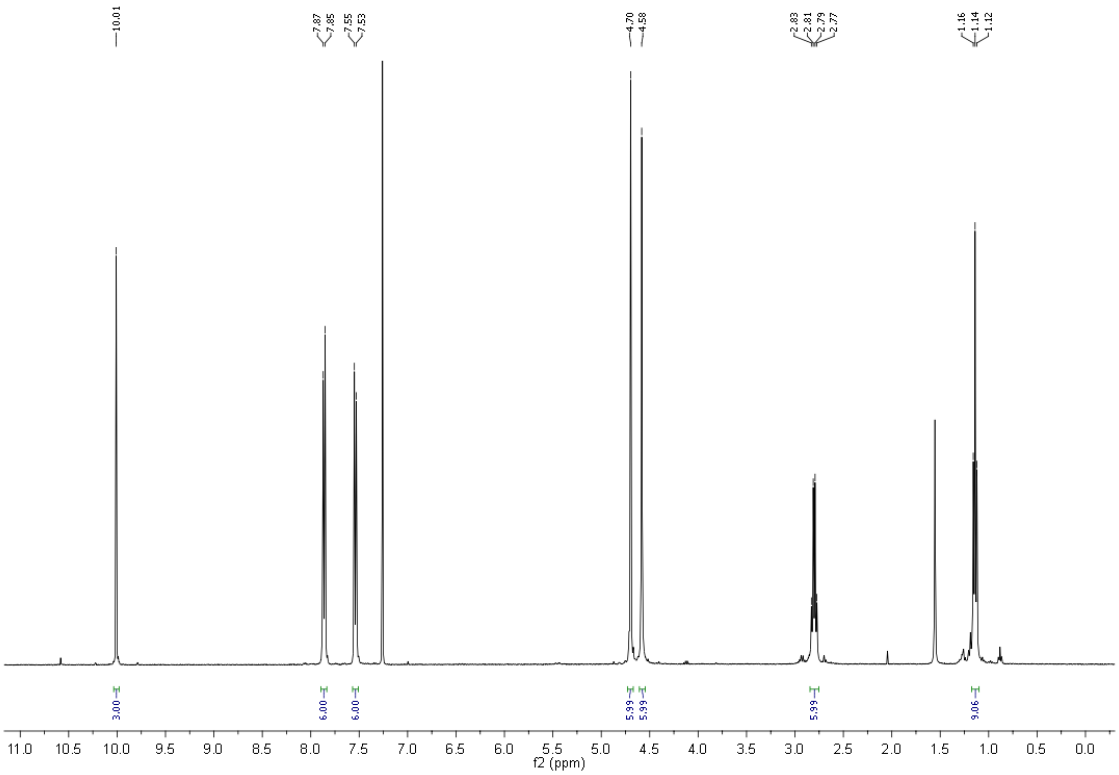

Fig. S1 ^{1}H NMR spectrum of the tripodal trialdehyde (**3**) in $CDCl_3$.

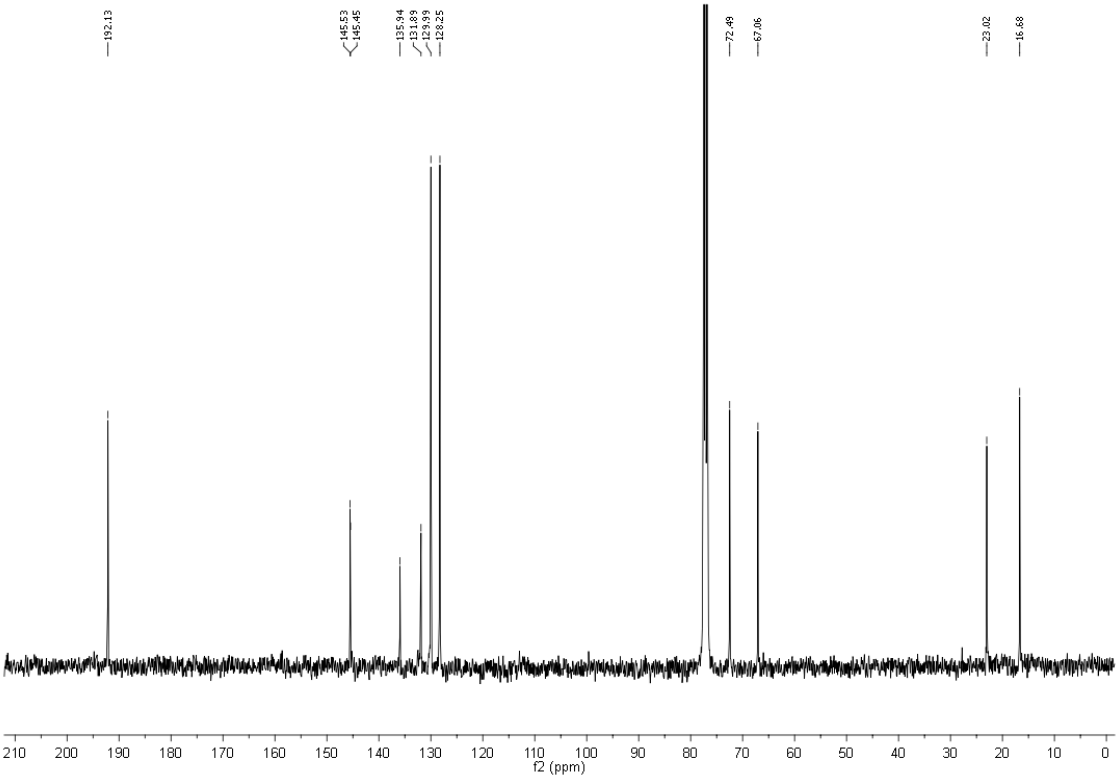

Fig. S2 ^{13}C NMR spectrum of the tripodal trialdehyde (**3**) in $CDCl_3$.

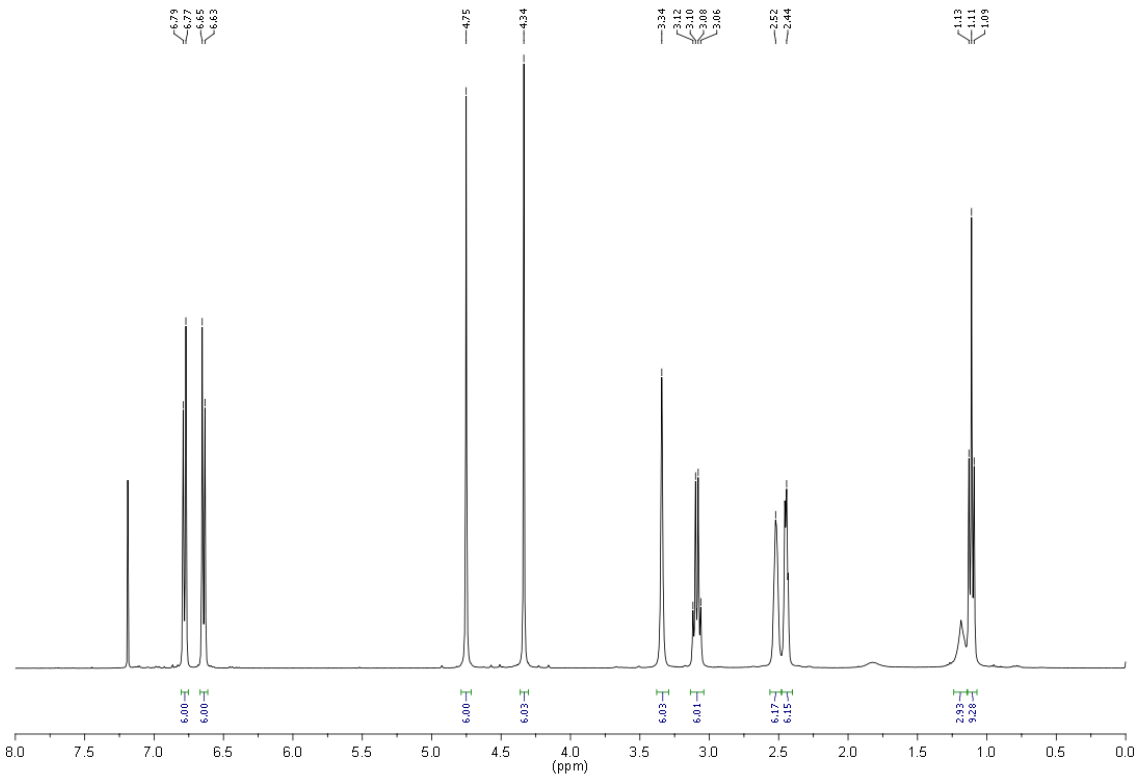

Fig. S3 ^{1}H NMR spectrum btpN_4O_3 in $CDCl_3$.

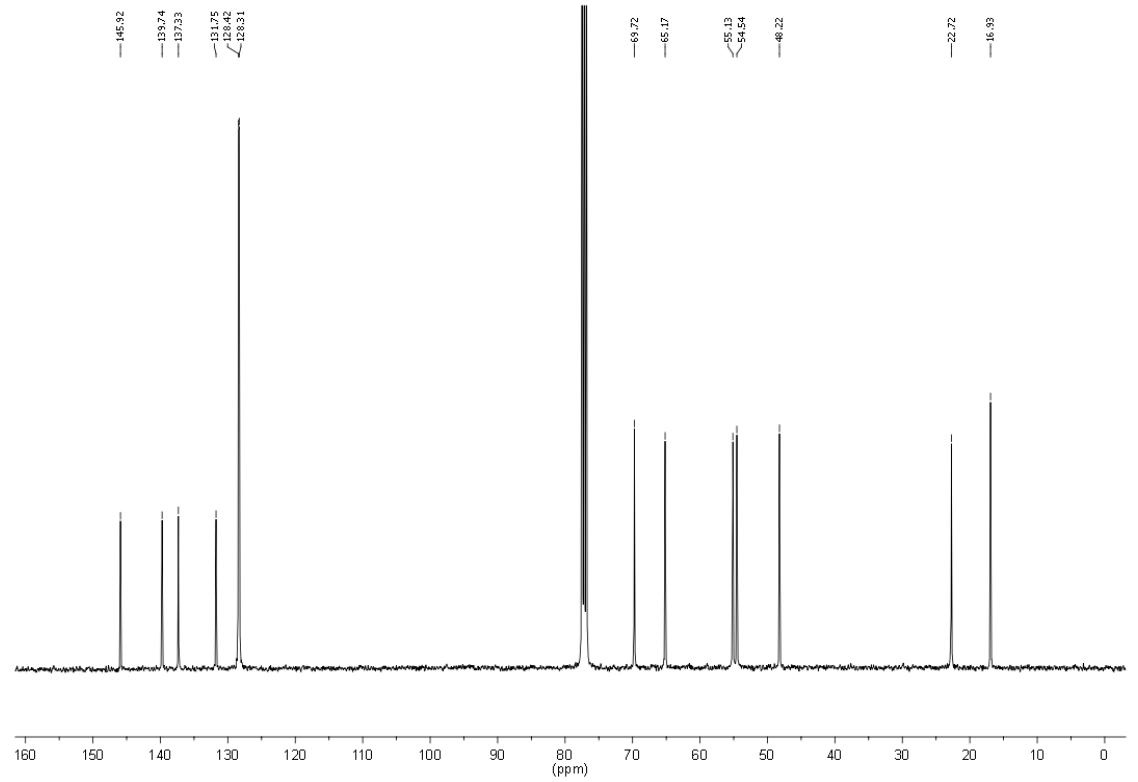

Fig. S4 ^{13}C NMR spectrum of btpN_4O_3 in $CDCl_3$.

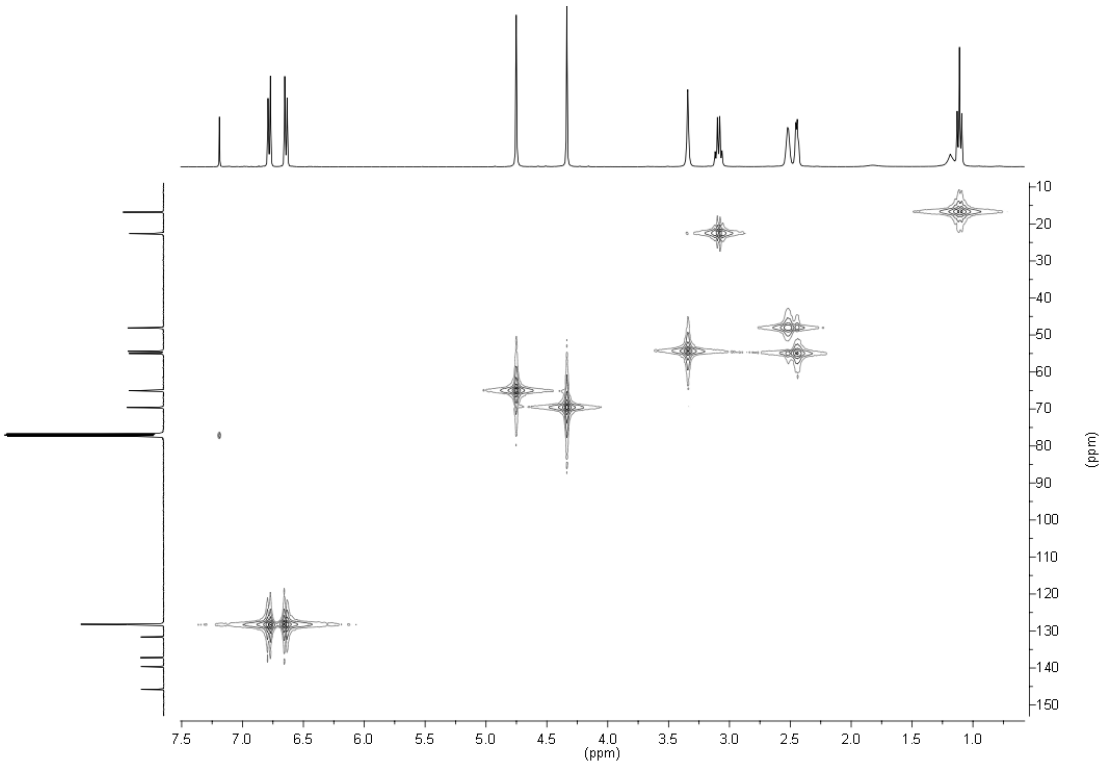

Fig. S5 HMQC spectrum of $btpN_4O_3$ in $CDCl_3$.

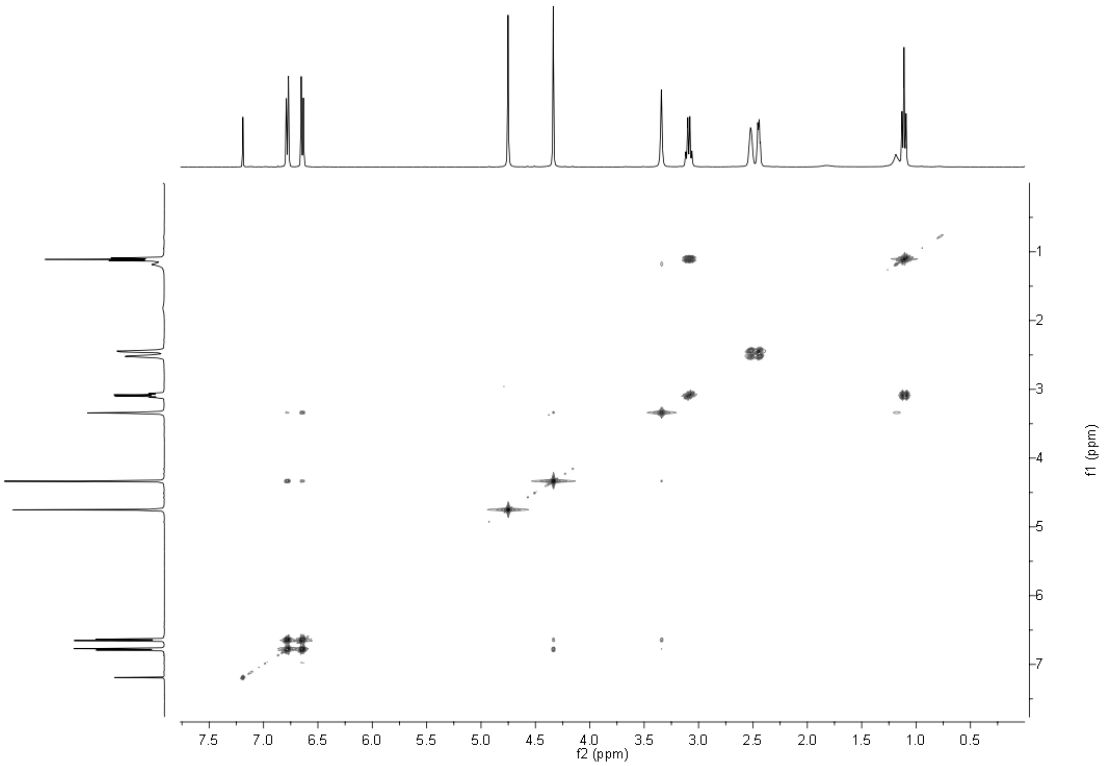

Fig. S6 COSY spectrum of $btpN_4O_3$ in $CDCl_3$.

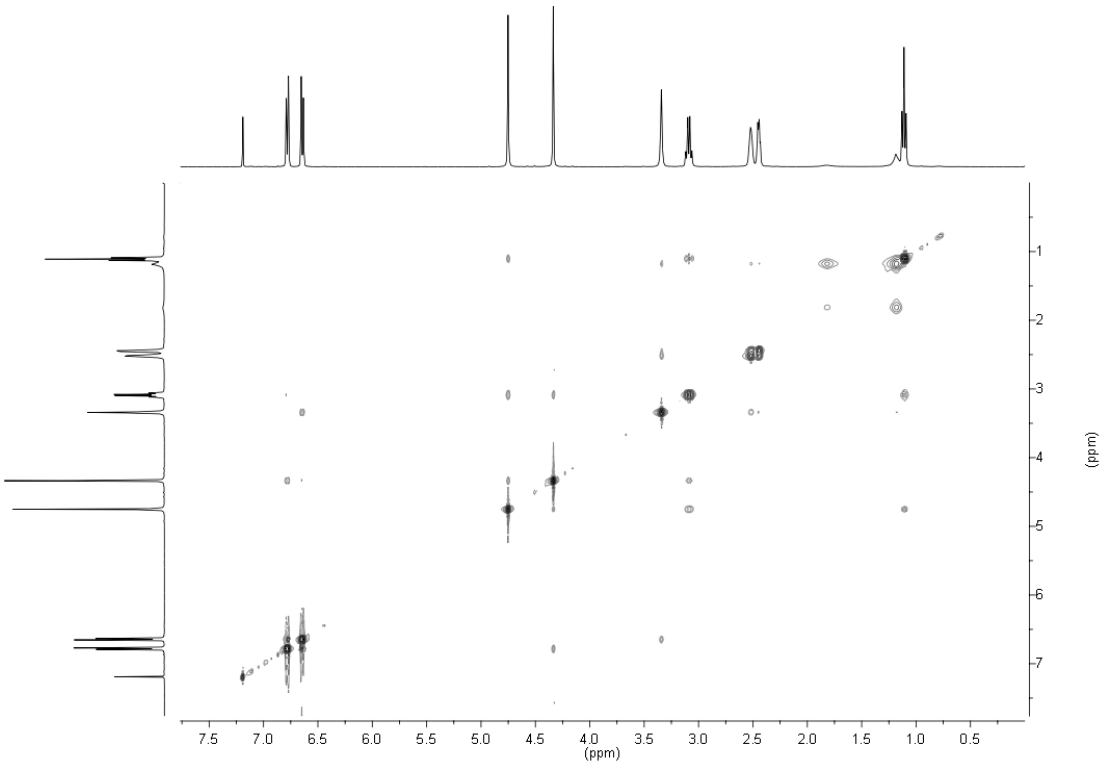

Fig. S7 NOESY spectrum of $btpN_4O_3$ in $CDCl_3$.

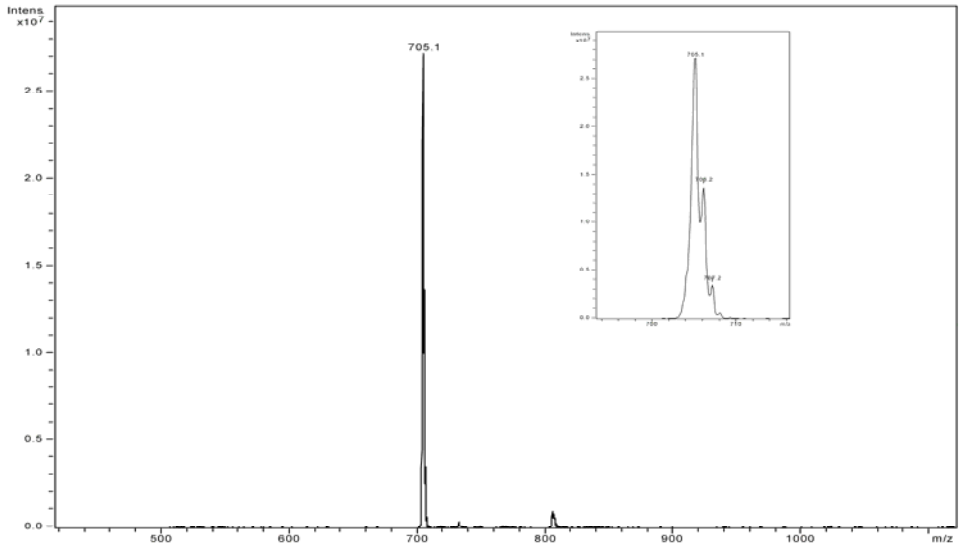

Fig. S8 ESI mass spectrum of $btpN_4O_3$ in MeOH.

References

1 P. Sarri, F. Venturi, F. Cuda and S. Roelens, *J. Org. Chem.*, 2004, **69**, 3654–3661.
2 K. J. Wallace, R. Hanes, E. V. Anslyn, J. Morey, K. V. Kilway and J. Siegel, *Synthesis*, 2005, 2080–2083.
3 G. Schwarzenbach and W. Biederman, *Helv. Chim. Acta*, 1948, **31**, 311–340.
4 F. J. Rossotti, H. J. Rossotti, *J. Chem. Educ.*, 1965, 42, 375–378.
5 P. Mateus, R. Delgado, P. Brandão, S. Carvalho and V. Félix, *Org. Biomol. Chem.*, 2009, **7**, 4661–4637.
6 P. Gans, A. Sabatini and A. Vacca, *Talanta*, 1996, **43**, 1739–1753.
7 L. Alderighi, P. Gans, A. Ienco, D. Peters, A. Sabatini and A. Vacca, *Coord. Chem. Rev.*, 1999, **184**, 311–318.
8 G. M. Sheldrick, *Acta Cryst*., 2008, **A64**, 112–122.
9 Schrodinger, LLC, The PyMOL Molecular Graphics System, Version 1.3r1. 2010.