ESI

Structure and stability of hexadentate complexes of ligands based on AAZTA for efficient PET labelling with gallium-68

Bradley P. Waldron, David Parker*, Carsten Burchardt, Dmitry S. Yufit, Melanie Zimny and Frank Roesch

1. General

Chemicals were purchased from commercial suppliers (Acros, Aldrich, Fluka, Merck) and were used without further purification unless otherwise stated. Solvents were dried using an appropriate drying agent when required (CH_3CN over CaH_2, CH_3OH and ethanol over $Mg(OR)_2$ and THF over Na/benzophenone). Unless otherwise mentioned, reactions were carried out under an argon atmosphere and the reaction flasks were pre-dried under reduced pressure. Ultra pure de-ionised water (<18 MΩ cm^{-1}) was used throughout. All glassware was washed with acid solution and rinsed with de-ionized, distilled water.

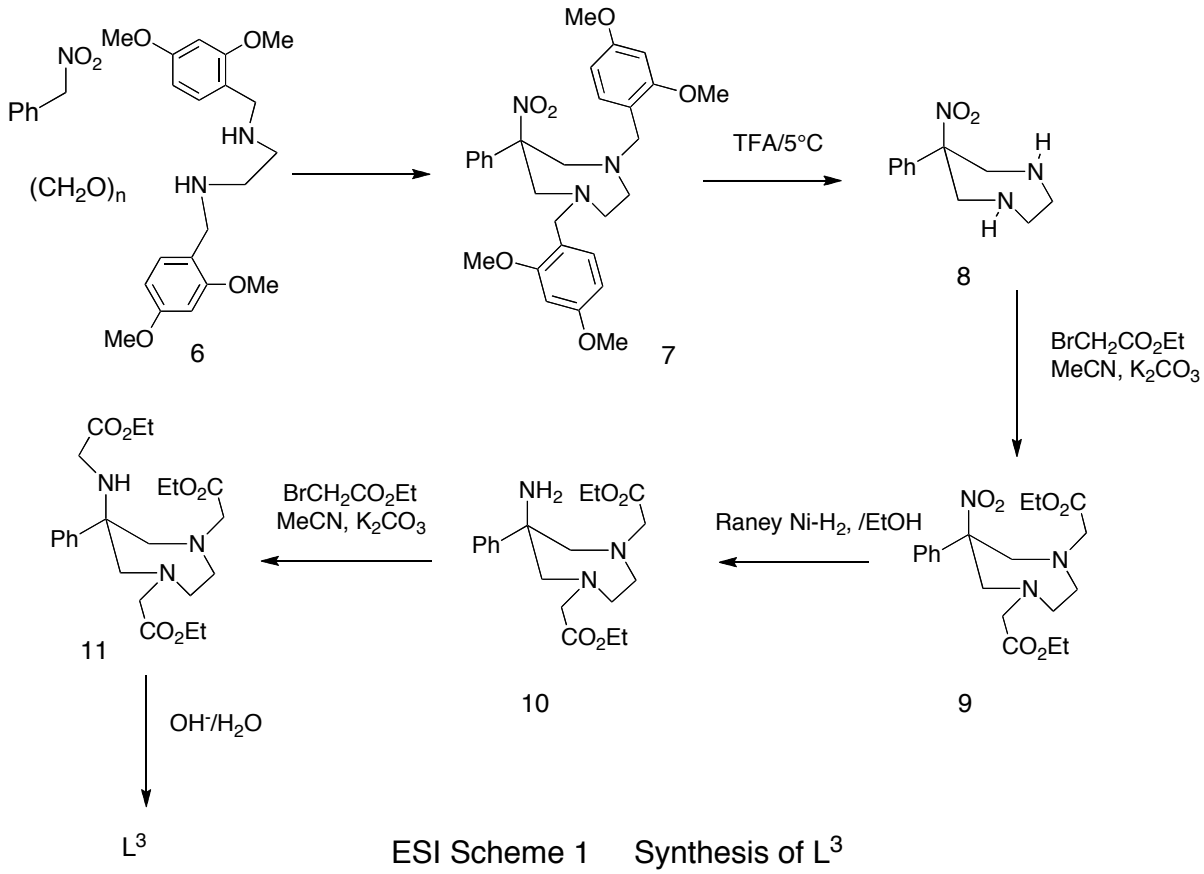
Spectroscopy

^{1}H, ^{13}C spectra were recorded in commercially available deuteriated solvents on a Varian Mercury-200 (^{1}H at 199.97 MHz, ^{13}C at 50.29 MHz), Varian Mercury-400 or Bruker Avance-400 (^{1}H at 399.96 MHz, ^{13}C at 100.57 MHz), Varian Inova-500 (^{1}H at 499.77 MHz, ^{13}C at 125.67 MHz) or Varian VNMRS-700 (^{1}H at 699.73 MHz) spectrometer. Chemical shifts are in ppm with coupling constants in Hz. Electrospray mass spectra were recorded on

a Waters Micromass LCT or Thermo-Finnigan LTQ FT instrument operating in positive or negative ion mode as stated, with methanol as the carrier solvent. Accurate mass spectra were recorded using the Thermo-Finnigan LTQ FT mass spectrometer. LC-MS analyses were performed on a Waters system comprising a 3100 Mass Detector and a 2998 Photodiode array detector.

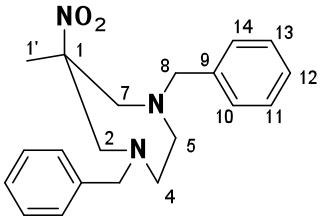
Ligand synthesis and characterization

The syntheses of L^1 and L^2 were undertaken using minor variations to the method that was published in 2011, during the course of this work [6], and L^4 was made using an adaptation to the route defined for L^3 (*ESI* Scheme 1).



ESI Scheme 1 Synthesis of L^3

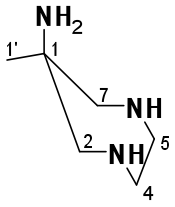
1,4-Dibenzyl-6-methyl-6-nitro-1,4-diazepine 1 [5a]



A solution of N,N'-dibenzylethylenediamine (2.000 g, 18.4 mmol) and *para*-formaldehyde (0.750 g, 25.0 mmol) in absolute ethanol (40 mL) was boiled under reflux under an atmosphere of argon for 2 h. Nitroethane (0.94 g, 12.5 mmol) was added dropwise over 20 min, and the mixture boiled under reflux overnight under argon. The solvent was removed under reduced pressure, and the resulting oil re-dissolved in chloroform (20 mL), filtered, and washed successively with aqueous potassium carbonate solution (2 x 20 mL, 0.1 M) and water (20 mL), dried over $MgSO_4$, filtered and solvent removed under reduced pressure. Purification by silica gel column chromatography (dichloromethane) afforded an off-white solid (2.20 g, 78 %). R_f = 0.40 (SiO_2, dichloromethane). m.p. = 49.5 – 50.5 °C. δ_H ($CDCl_3$, 400 MHz) 1.34 (3H, s, $H^{1'}$); 2.60 (4H, m, $H^{4,5}$); 2.95 (2H, d, J 13, $H^{2/7}$); 3.59 (2H, d, J 13, $H^{2/7}$); 3.64 (2H, d, J 13, H^{8}); 3.78 (2H, d, J 13, H^{8}); 7.26-7.33 (10H, m, $H^{10,14}$). m/z (ES+): 340.2017 $[M + H]^+$; $C_{20}H_{26}N_3O_2$ requires 340.2025.

The structure of **1** was confirmed by single crystal X-ray diffraction: $C_{20}H_{25}N_3O_2$, M_r = 339.43, monoclinic (C2/c); a = 32.4876 (13) Å, b = 6.0545 (2) Å, c = 20.2683 (8) Å, V = 3710.1(2) Å^3,α = 90.00°, β = 111.467° (10), γ = 90.00 °, Z = 8; μ = 0.085 mm^{-1}, $D_{calc.}$ = 1.219 mg.mm^{-3}, T 120(2) K; 4452 independent reflections (R_{int} = 0.0342), R_1 = 0.0482 , ωR_2 = 0.1252 ($I > 2\sigma(I)$).

6-Methyl-1,4-diazepan-6-amine, 2 [5c]

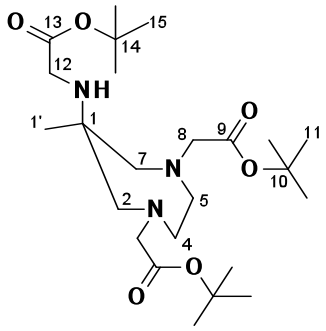


A catalytic amount of $Pd(OH)_2/C$ was added to a solution of the protected tri-amine **1** (0.10 g, 0.29 mmol) in methanol (20 mL), and the mixture agitated under an atmosphere of hydrogen for 48 h using a Parr hydrogenator (30 psi H_2). Thin layer chromatography (SiO_2, dichloromethane) was used to confirm complete reduction of the nitro group and cleavage of the benzyl N-sbstituents. Argon was bubbled through the solution for 15 min to purge remaining hydrogen, and the $Pd(OH)_2/C$ removed using a Celite® filter. The solvent was removed under reduced pressure to afford a pale yellow oil.

(0.031 g, 81 %) R_f = 0.10 (CH_2Cl_2 / MeOH 19% / NH_3 1%, SiO_2). δ_H ($CDCl_3$, 200 MHz) 1.03 (3H, s, $H^{1'}$); 1.98 (4H, br. s, NH and NH_2); 2.68 (4H, br. s, $H^{2,7}$); 2.92 (4H, m, $H^{4,5}$). m/z (ES+): 129.1264 $[M+H]^+$;$C_6H_{16}N_3$ requires 129.1266.

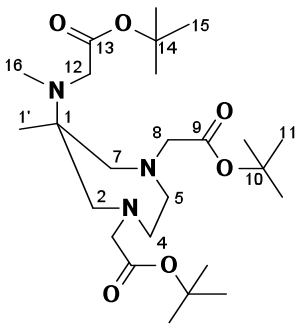
[Note: Compound 1 readily reacts with carbon dioxide in the atmosphere and should therefore be stored at < 0°C under an atmosphere of argon; or alternatively as the hydrochloride salt. As a consequence of this instability, compound **2** was always handled under argon, and subsequent reactions were carried out under an inert atmosphere.]

tert-Butyl 2,2'-(6-(2-tert-butoxy-2-oxoethylamino)-6-methyl-1,4-diazepane-1,4-diyl)diacetate, 3 [6]



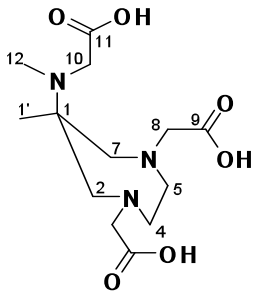
Tert-butyl-bromoacetate (0.113 g, 0.58 mmol) was added to a solution of **2** (0.030 g, 0.23 mmol) and potassium carbonate (0.25 g, 0.70 mmol) in acetonitrile (25 mL), and the mixture stirred for 24 h at 298 K under argon. The reaction was monitored by TLC, to follow formation of the unwanted tetra-alkylated by-product, **3**. The solvent was removed under reduced pressure, and the resulting oil re-dissolved in chloroform (25 mL) and washed successively with aqueous potassium carbonate solution (2 x 25 mL, 0.1 M) and water (25 mL), dried over $MgSO_4$, filtered and solvent removed under reduced pressure. Purification by silica gel column chromatography (hexane → 25 % ethyl acetate) afforded a pale yellow oil (0.06 g, 22 %). R_f = 0.20 (SiO_2; hexane:ethyl acetate; 65:35). δ_H ($CDCl_3$, 700 MHz) 0.85 (3H, s, $H^{1'}$); 1.37 (27H, m, $H^{11,15}$); 2.54 (2H, d, J 14, $H^{2,7}$) 2.59 (2H, d, J 14, $H^{2,7}$); 2.72 (4H, m, $H^{4,5}$); 3.18 (2H, s, H^{12}); 3.22 (4H, s, H^8). δ_C (176MHz, $CDCl_3$) 22.79 ($C^{1'}$); 28.20 ($C^{11/15}$); 28.35 ($C^{11/15}$); 45.13 (C^{12}); 56.05 (C^1); 57.35 ($C^{2,7}$); 61.99 (C^8); 64.81 ($C^{4,5}$); 80.77 ($C^{10/14}$); 80.89 ($C^{10/14}$); 171.06 ($C^{9/13}$); 171.97 ($C^{9/13}$). m/z (ES+): 472.3380 $[M + H]^+$; $C_{24}H_{46}N_3O_6$ requires 472.3378.

tert-Butyl 2,2'-(6-((2-tert-butoxy-2-oxoethyl)(methyl)amino)-6-methyl-1,4-diazepane-1,4-diyl)diacetate, 4 [6]



Iodomethane (0.030 g, 0.21 mmol) was added to a solution of **3** (0.100 g, 0.21 mmol) and potassium carbonate (0.029 g, 0.21 mmol) in anhydrous dichloromethane cooled in an ice-bath. After 15 min the mixture was allowed to warm to room temperature, and left for a further 5 h. The solvent was removed under reduced pressure and the resulting oil re-dissolved in chloroform (20 mL), filtered, and washed successively with aqueous potassium carbonate solution (2 x 20 mL, 0.1 M) and water (20 mL), dried over $MgSO_4$, filtered and solvent removed under reduced pressure. Purification by silica gel column chromatography (hexane → 20 % ethyl acetate) afforded a pale yellow oil (0.042 g, 41 %). R_F = 0.30 (SiO_2, hexane:ethyl acetate; 80:20). δ_H ($CDCl_3$, 700 MHz) 1.05 (3H, s, $H^{1'}$); 1.42 (27H, m, $H^{11,15}$); 2.30 (3H, s, H^{16}); 2.55 (2H, d, J 14, $H^{2,7}$); 2.68 (2H, m, $H^{4/5}$); 2.79 (2H, m, $H^{4/5}$); 2.93 (2H, d, J 14, $H^{2,7}$); 3.24 (2H, d, J 17, H^{8}); 3.27 (2H, d, J 17, H^{8}); 3.41 (2H, s, H^{12}). δ_C (176MHz, $CDCl_3$) 23.75 ($C^{1'}$); 28.11 (C^{15}); 28.31 (C^{11}); 37.30 (C^{16}); 54.26 (C^{12}); 58.98 ($C^{4,5}$); 60.70 (C^{1}); 62.34 (C^{8}); 63.55 ($C^{2,7}$); 80.20 (C^{14}); 80.73 (C^{10}); 170.87 (C^{9}); 171.99 (C^{13}). m/z (ES+): 486.3540 $[M + H]^+$; $C_{25}H_{48}N_3O_6$ requires 486.3543.

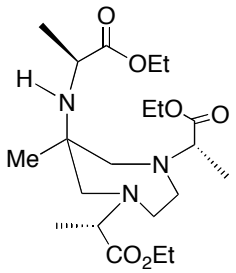
2,2'-(6-((Carboxymethyl)(methyl)amino)-6-methyl-1,4-diazepane-1,4-diyl)diacetic acid, H_3L^1



The triester,**4** (0.150 g, 0.31 mmol) was dissolved in trifluoroacetic acid / dichloromethane (1:1, 2 mL) and left to stir for 2 days at room temperature. The solvent was removed under reduced pressure, the residue re-dissolved in dichloromethane and evaporated. This procedure was repeated twice, and

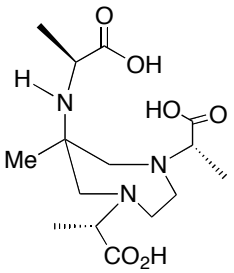
then with methanol. The resulting solid was dissolved in water (15 mL) and washed with dichloromethane (15 mL). Removal of solvent under reduced pressure afforded, after drying the bis-trifluoroacetic acid salt of the title compound, as a white solid (0.081 g, 83 %). δ_H (D_2O, pD = 2.05, 700 MHz) 1.22 (3H, s, $H^{1'}$); 2.91 (3H, s, H^{12}); 3.25 (2H, m, $H^{4,5}$); 3.31 (2H, m, $H^{4,5}$); 3.39 (2H, d, J 16, $H^{2,7}$); 3.52 (2H, d, J 16, $H^{2,7}$); 3.75 (4H, s, H^{8}); 3.90 (2H, s, H^{10});
δ_c (D_2O, pD = 2.05, 176 MHz): 15.43 ($C^{1'}$); 38.149 (C^{12}); 53.36 (C^{1}); 53.64 (C^{12}); 58.12 ($C^{4/5}$); 58.50 (^{8}); 67.42 ($^{2/7}$); 168.99 (C^{9}); 171.48 (C^{11}). *m/z* (ES+): 318.1677 $[M + H]^+$; $C_{13}H_{24}N_3O_6$ requires 318.1665. Found: C, 37.3; H, 4.53; N, 7.59%; $[C_{13}H_{25}N_3O_6].2CF_3COO^-$ requires: C 37.4; H, 4.62; N, 7.70%.

(2*S*,2'*S*)-Diethyl 2,2'-(6-((*S*)-1-ethoxy-1-oxopropan-2-ylamino)-6-methyl-1,4-diazepane-1,4-diyl)dipropanoate, **5** [6]



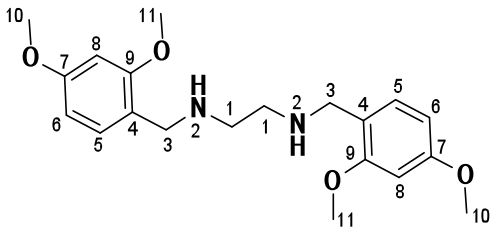
Ethyl (2*R*)-2-{[(trifluoromethyl)sulfonyl]oxy}propanoate (0.98 g, 3.9 mmol) was added to a solution of potassium carbonate (0.54 g, 3.9 mmol) and **2** (0.10 g, 0.78 mmol) in acetonitrile (20 mL), and the reaction mixture stirred at 308 K for 18 h. The solvent was removed under reduced pressure and the resulting oil re-dissolved in chloroform (30 mL) and washed successively with aqueous potassium carbonate solution (2 x 30 mL, 0.1 M) and water (30 mL), dried over $MgSO_4$, filtered and solvent removed under reduced pressure. Purification by silica gel column chromatography (hexane:ethyl acetate, 95:5) afforded the title compound as a colourless oil (0.11 g, 34%). R_f = 0.40 (hexane : ethyl acetate; 65 : 35). δ_H ($CDCl_3$, 600 MHz): 0.88 (3H, s, $H^{1'}$); 1.25 (18H, m, $H^{9,14,19}$ & $H^{12,17,22}$); 2.46 (2H, t, J 15, $H^{2/7}$); 2.55 (1H, m, $H^{4/5}$); 2.63 (1H, m, $H^{4/5}$); 2.69 (2H, t, J 15, $H^{2/7}$); 2.73 (1H, m, $H^{4/5}$); 2.84 (1H, m, $H^{4/5}$); 3.34 (2H, m, $H^{8,13}$); 3.45 (1H, q, J 7, H^{18}); 4.12 (6H, m, $H^{11,16,21}$). δ_C($CDCl_3$, 150 MHz): 14.40 ($C^{9/14/19}$ or $C^{12/17/22}$); 14.43 ($C^{9/14/19}$ or $C^{12/17/22}$); 15.24 ($C^{9/14/19}$ or $C^{12/17/22}$); 15.70 ($C^{9/14/19}$ or $C^{12/17/22}$); 16.52 ($C^{9/14/19}$ or $C^{12/17/22}$); 21.68 ($C^{9/14/19}$ or $C^{12/17/22}$); 20.34 ($C^{1'}$); 50.34 (C^{18}); 55.33 ($C^{4/5}$); 56.40 ($C^{4/5}$); 59.96 ($C^{11/16/21}$); 60.08 ($C^{11/16/21}$); 60.59 ($C^{11/16/21}$); 61.85 ($C^{2/7}$); 63.81 ($C^{8/13}$); 63.95 ($C^{8/13}$); 64.07 ($C^{2/7}$); 65.80 (C^{1}); 173.56 ($C^{10/15}$); 173.59 ($C^{10/15}$); 177.39 (C^{20}). (*m/z*) (ES+): 430.2926 $[M + H]^+$; $C_{21}H_{40}N_3O_6$ requires 430.2917.

(2S,2'*S*)-2,2'-(6-((*S*)-1-Carboxyethylamino)-6-methyl-1,4-diazepane-1,4-diyl)dipropanoic acid, L_2 [6]



Sodium hydroxide (0.003 g, 0.072 mmol) dissolved in water (0.5 mL) was added to a solution of the tri-ester **5** (0.010 g, 0.023 mmol) in THF (0.5 mL), and the mixture stirred at 298 K. The reaction was monitored using LC-ESMS, to follow ester cleavage. Once complete, the solvent was removed by lyophilisation. Water (5 mL) was added and removed by lyophilisation, and the procedure repeated two more times. The resulting solid was washed with ice-cold dichloromethane (0.5 mL), and dried *in vacuo* to afford the tris-hydrated tris-sodium salt of the title compound as a white solid (0.009 g, 92 %). δ_H ($CDCl_3$, 600 MHz): 0.84 (3H, s, $H^{1'}$); 1.04 (3H, d, J 7, H^9); 1.06 (3H, d, J 7, H^{15}); 1.08 (3H, d, J 7, H^{12}); 2.22 (1H, d, J 15, H^2); 2.27 (1H, d, J 15, H^7); 2.59 (1H, m, $H^{4,5}$); 2.74 (1H, d, J 15, H^7); 2.78 (1H, d, J 15, H^2); 2.98 (1H, q, J 7, H^8); 3.06 (1H, q, J 7, H^{11}); 3.16 (1H, q, J 7, H^{14}). δ_C ($CDCl_3$, 150 MHz): 15.64 (C^9); 16.25 (C^{15}); 21.26 (C^{12}); 22.09 ($C^{1'}$); 52.33 (C^{14}); 55.91 (C^1); 57.29 ($C^{4/5}$); 57.45 ($C^{4/5}$); 59.70 (C^2); 60.29 (C^7); 66.50 (C^8); 66.75 (C^{11}); 181.22 (C^{10}); 181.25 (C^{13}); 184.42 (C^{16}). *m/z* (ES+): 368.1807 $[M + Na]^+$; $C_{15}H_{27}N_3NaO_6$ requires 368.1798. Found:C, 38.6; H, 6.77; N, 8.91%. $[C_{15}H_{24}N_3Na_3O_6].3H_2O$ requires: C, 38.7; H, 6.50; N, 9.13%.

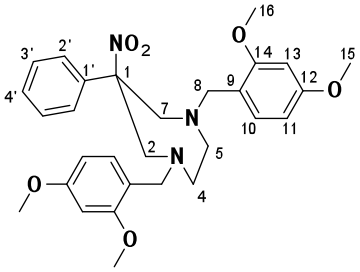
N^1,N^2-Bis(2,4-dimethoxybenzyl)ethane-1,2-diamine, 6



Under an atmosphere of argon, 2,4-dimethoxybenzaldehyde (3.00 g, 18 mmol) was dissolved in dry methanol (100 mL) containing 3 Å sieves. The mixture was stirred at room temperature for 30 min before N,N'-ethylenediamine (0.59 g, 9.9 mmol) was added, and the mixture left to stir at room temperature overnight. The methanol was removed under reduced

pressure and chloroform (200 mL) added, before the molecular sieves were removed by filtration. Removal of the chloroform under reduced pressure yielded the diimine as a yellow solid. The di-imine formed during this reaction is sensitive to water and was used in the next step without further purification and characterisation. Under argon, sodium borohydride (2.70 g, 71 mmol) was added gradually over 30 minutes to a solution of the di-imine dissolved in dry methanol (40 mL). The solution was left to stir overnight at room temperature. The methanol was removed under reduced pressure and the solid re-dissolved in chloroform and washed successively with aqueous sodium hydroxide solution (0.1 M) and water. Removal of the chloroform under reduced pressure and subsequent drying *in vacuo* yielded a golden yellow oil that crystallized overnight to give a dark yellow solid. (3.03 g, 93 %) m.p. 56°C. δ_H **(**$CDCl_3$, 500 MHz): 2.72 (4H, s, H^1); 3.75 (4H, s, H^3); 3.78 (6H, s, H^{11}); 3.82 (6H, s, H^{10}); 6.44 (4H, m, $H^{5,6}$); 7.12 (1H, d, J 8, H^8). δ_C ($CDCl_3$, 125 MHz): 48.71 (C^1); 48.83 (C^3); 55.55 (C^{11}); 55.68 (C^{10}), 98.71 ($C^{5/6}$); 103.72 ($C^{5/6}$); 121.32 (C^4); 130.56 (C^8); 158.84 (C^9); 160.29 (C^7). *m/z*: (ES+) 361.2134 $[M + H]^+$; $C_{20}H_{29}N_2O_4$ requires 361.2127.

1,4-Bis(2,4-dimethoxybenzyl)-6-nitro-6-phenyl-1,4-diazepane, **7**

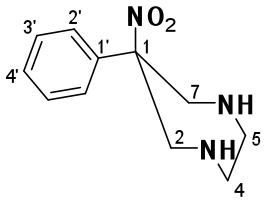


Nitromethylbenzene (0.32 g, 2.3 mmol) was added dropwise over 30 min to a solution of the N,N'-bis(2,4-dimethoxybenzyl)ethane-1,2-diamine **6** (0.83 g, 2.3 mmol) and paraformaldehyde (0.24 g, 8 mmol) in ethanol/toluene (50/50: 30 mL), and the reaction mixture stirred at 313 K overnight. The solvent was removed under reduced pressure and the resulting oil re-dissolved in chloroform (30 mL) and washed successively with aqueous potassium carbonate solution (2 x 30 mL, 0.1 M) and water (50 mL), dried over magnesium sulphate, filtered and the solvent removed under reduced pressure. Purification by silica gel column chromatography (hexane → 15 % ethyl acetate) yielded, after drying *in vacuo*, a white solid (0.134 g, 11 %). R_f = 0.55 (hexane : ethyl acetate; 65 : 35), m.p. 67 °C. δ_H ($CDCl_3$, 700 MHz): 2.85 (4H, m, $H^{4,5}$); 3.52 (2H, d, J 14, H^8); 3.73 (2H, d, J 14, $H^{2,7}$); 3.76 (6H, s, H^{16}); 3.77 (2H, d, J 14, $H^{2,7}$); 3.78 (6H, s, H^{15}) 3.85 (2H, d, J 14, H^8); 6.42 (4H, m, $H^{11,13}$); 7.17 (2H, d, J 9 , H^{10}); 7.28 (3H, m, $H^{3',4'}$); 7.37 (2H, t, J 4, $H^{2'}$). δ_C ($CDCl_3$, 176

MHz): 55.33 (C^{16}); 55.33 (C^{15}) 56.79 ($C^{2,7}$); 57.94 ($C^{4,5}$); 64.77 (C^{8}); 97.98 ($C^{1'}$); 98.38 (C^{11}); 104.00 (C^{13}); 119.62 (C^{9}); 124.87 ($C^{2'}$); 128.20 ($C^{4'}$); 128.55 ($C^{3'}$); 131.12 (C^{10}); 140.04 (C^{1}); 158.71 (C^{16}); 159.91 (C^{15}). *m/z*: (ES+) 522.2603 $[M + H]^+$; $C_{29}H_{36}N_3O_6$ requires 522.2604.

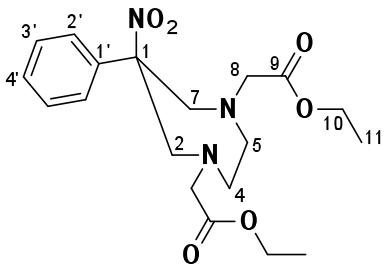
The structure of **7** was confirmed by single crystal X-ray diffraction: $C_{29}H_{35}N_3O_6$, M_r = 521.60, monoclinic ($P2_1/n$); a = 8.8932(2) Å, b = 19.1927(5) Å, c = 15.3402(3) Å, V = 2616.02(11) $Å^3$, β = 92.411(2)°, Z = 4, μ = 0.093 mm^{-1}, $D_{calc.}$ = 1.324 mg/mm^3, T 120(2) K; 7279 independent reflections (R_{int} = 0.0449), R_1 = 0.0478, ωR2 = 0.1084 (I > 2σ (I)).

6-Nitro-6-phenyl-1,4-diazepane, **8.**



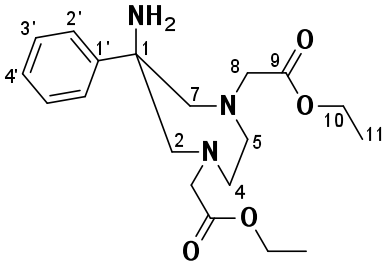
Trifluoroacetic acid (2 mL) was added to a solution of **7** (0.15 g, 0.29 mmol) in dichloromethane (2 mL), and the mixture stirred at room temperature. The reaction was monitored using LC-ESMS, to follow removal of the dimethoxy-benzyl substituents. Once complete, the solvent was removed by lyophilisation. Excess trifluoroacetic acid was removed through repeated addition and removal of dichloromethane (3 x 10 mL) and subsequently methanol (3 x 10 mL). Filtration of a methanol solution (20 mL), using a 0.45 µm syringe filter, and subsequent removal of the methanol under reduced pressure yielded a very palebrown oil. The oil was re-dissolved in chloroform : isopropyl alcohol (80 : 20, 20 mL) and washed successively with aqueous sodium hydroxide (2 x 20 mL, 0.5 M) and water (10 mL), dried over $MgSO_4$, filtered and solvent removed under reduced pressure to yield a pale yellow oil, (0.029 g, 45 %). δ_H (CD_3OD, 600 MHz): 1.44 (4H, m, $H^{4,5}$); 3.71 (2H, d, J 15, $H^{2,7}$); 4.30 (2H, d, J 15 $H^{2,7}$); 7.35 (1H, t, $H^{4'}$); 7.38 (2H, t, $H^{3'}$); 7.42 (2H, d, $H^{2'}$). δ_C (CD_3OD, 150 MHz): 45.78 ($C^{4,5}$); 53.89 ($C^{2,7}$); 94.44 ($C^{1'}$); 124.88 ($C^{4'}$); 124.96 ($C^{3'}$); 128.23 ($C^{2'}$); 135.16 (C^{1}). *m/z* (ES+): 222.1240 $[M + H]^+$; $C_{11}H_{16}N_3O_2$ requires 222.1243.

Diethyl 2,2'-(6-nitro-6-phenyl-1,4-diazepane-1,4-diyl)diacetate, **9**



Ethyl bromoacetate (0.061 g, 0.52 mmol) was added dropwise to a stirred solution of potassium carbonate (0.072 g, 0.52 mmol) and **22** (0.029 g, 0.13 mmol) in acetonitrile (10 mL). The reaction mixture was stirred at 308 K for 24 hours, and solvent removed under reduced pressure. The resulting oil was re-dissolved in chloroform (20 mL) and washed successively with aqueous potassium carbonate solution (2 x 20 mL, 0.1 M) and water (20 mL), dried over $MgSO_4$, filtered and solvent removed under reduced pressure. Purification by silica gel column chromatography (hexane → 20 % ethyl acetate) yielded a yellow oil (0.034 g, 66%). R_f = 0.27 (hexane : ethyl acetate, 65 : 35). δ_H ($CDCl_3$, 700 MHz): 1.26 (6H, t, J 7, H^{11}); 2.96 (2H, m, $H^{4,5}$); 3.04 (2H, m, $H^{4,5}$); 3.49 (2H, d, J 18, H^8); 3.64 (2H, d, J 15, $H^{2,7}$); 3.67 (2H, d, J 18, H^8); 3.97 (2H, d, J 15, $H^{2,7}$); 7.24 (2H, d, J 8, $H^{2'}$); 7.32 (2H, m, $H^{3',4'}$). δ_C ($CDCl_3$, 175 MHz): 14.26 (C^{11}); 55.85 ($C^{4,5}$); 59.41 (C^8); 60.40 (C^{10})62.70 ($C^{2,7}$); 97.76 ($C^{1'}$); 124.63 ($C^{2'}$); 128.62 ($C^{4'}$); 128.84 ($C^{3'}$); 138.41 (C^1); 171.53 (C^9). *m/z* (ES+): 394.1968 $[M + H]^+$; $C_{19}H_{28}N_3O_6$ requires 394.1978.

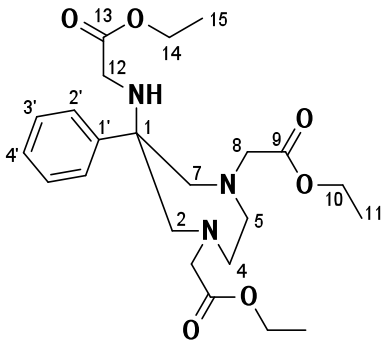
Diethyl 2,2'-(6-amino-6-phenyl-1,4-diazepane-1,4-diyl)diacetate, **10**



An aqueous slurry of Raney nickel (0.010 g) was washed with methanol (3 x 25 mL) and ethanol added (10 mL). The suspension was transferred into a solution of **9** (0.050 g, 0.13 mmol) in ethanol (25 mL). The flask was then evacuated and back-filled with hydrogen gas using four vacuum-purge cycles, and the mixture stirred at 298 K under an atmosphere of hydrogen. The reaction was monitored by TLC for formation of the primary amine. Once complete (~ 3 h), the solution was decanted, and the solid washed with methanol (3 x 20 mL). The washings and decanted solution were combined, removed under reduced pressure and re-

dissolved in methanol (25 mL). Remaining Raney nickel and insoluble by-products were removed by filtration through a base washed Celite® filter, and the solvent removed under reduced pressure. The resulting oil was re-dissolved in chloroform:isopropyl alcohol (80:20, 20mL) and washed successively with aqueous sodium hydroxide (2 x 20 mL, 0.5 M) and water (15 mL), dried over $MgSO_4$, filtered and solvent removed under reduced pressure to yield a colourless oil (0.014 g, 30 %). δ_H ($CDCl_3$, 700 MHz): 1.22 (6H, t+t, H^{11}); 2.12 (2H, br. s, NH_2) 2.63 (2H, d, J 14, $H^{2,7}$); 2.95 (4H, m, $H^{4,5}$); 3.30 (2H, d, J 14, $H^{2,7}$); 3.46 (4H, s, H^8); 4.10 (4H, q+q, J 7, H^{10}) 7.25 (2H, t, J 7, $H^{4'}$); 7.29 (1H, t, J 7, $H^{3'}$); 7.59 (2H, d, J 7, $H^{2'}$). δ_C ($CDCl_3$, 175 MHz): 14.25 (C^{11}); 56.90 ($C^{4,5}$); 58.15 (C^1); 60.28 (C^{10}); 60.82 (C^8); 69.25 ($C^{2,7}$); 125.19 ($C^{4'}$); 128.11 ($C^{3'}$); 128.44 ($C^{2'}$); 146.62 (C^1); 171.47 (C^9). *m/z* (ES+): 364.2235 $[M + H]^+$; $C_{19}H_{30}N_3O_4$ requires 364.2236.

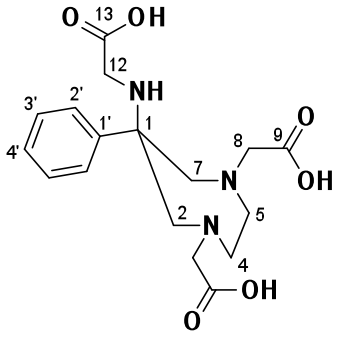
Diethyl 2,2'-(6-(2-ethoxy-2-oxoethylamino)-6-phenyl-1,4-diazepane-1,4-diyl)diacetate, **11**



Ethyl-bromoacetate (0.068 g, 0.41 mmol) was added to a solution of **10** (0.030 g, 0.083 mmol) and potassium carbonate (0.080 g, 0.58 mmol) in acetonitrile (20 mL), and the mixture stirred for 18 h at 313 K under an atmosphere of argon. The solvent was removed under reduced pressure and the resulting oil re-dissolved in chloroform (20 mL) and washed successively with aqueous potassium carbonate solution (2 x 15 mL, 0.1 M) and water (15 mL), dried over $MgSO_4$, filtered and solvent removed under reduced pressure. Purification by silica gel column chromatography (hexane → 15 % ethyl acetate) afforded the title compound as a pale yellow oil (0.016 g, 43 %). R_f = 0.35 (hexane : ethyl acetate; 65 : 35). δ_H ($CDCl_3$, 700 MHz): 1.24 (9H, t, J 7, $H^{11,15}$); 1.69 (1H, br. s, NH); 2.89 (2H, d, J 14, $H^{2,7}$); 2.94 (4H, s, $H^{4,5}$); 3.18 (2H, s, H^{12}); 3.34 (2H, d, J 14, $H^{2,7}$); 3.49 (4H, s, H^8); 4.13 (4H, q, J 7, H^{10}); 4.17 (2H, q, J 7, H^{14}) 7.19 (1H, t, J 8, $H^{4'}$); 7.29 (2H, t, J 8,$H^{3'}$); 7.50 (2H, d, J 8 , $H^{2'}$). δ_C ($CDCl_3$, 175 MHz): 14.16 ($C^{11/15}$); 14.27 ($C^{11/15}$); 44.85 (C^{12}); 55.23 (C^1); 56.51 ($C^{4,5}$); 60.39 (C^{10}); 60.57 (C^{12}); 60.74 (C^8); 65.51 ($C^{2,7}$); 126.50 ($C^{2'}$); 126.75 ($C^{4'}$); 128.33 ($C^{3'}$); 144.17 ($C^{1'}$);

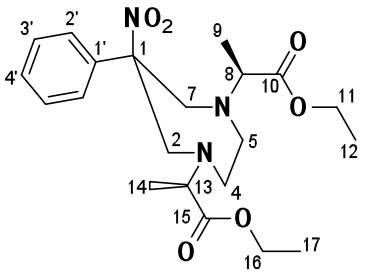
171.54 (C^{9}); 172.46 (C^{13}). *m/z* (ES+): 450.2602 $[M + H]^+$, 472.2423 $[M + Na]^+$; $C_{23}H_{36}N_3O_6$ requires 450.2604; $C_{23}H_{35}N_3NaO_6$ requires 472.2424.

2,2'-(6-(Carboxymethylamino)-6-phenyl-1,4-diazepane-1,4-diyl)diacetic acid, L^3



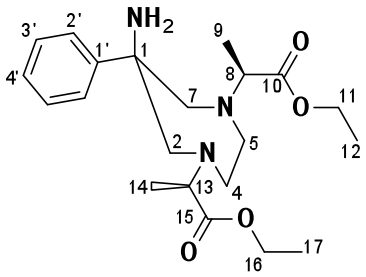
Sodium hydroxide (0.003 g, 0.069 mmol) dissolved in water (0.5 mL) was added to a solution of the triester **11** (0.010 g, 0.022 mmol) in THF (0.5 mL), and the mixture stirred at 298 K. The reaction was monitored using LC-ESI MS, to follow ester cleavage. Once complete, the solvent was removed by lyophilisation. Water (5 mL) was added and removed by lyophilisation, and the procedure repeated two more times. The resulting solid was washed with ice-cold dichloromethane (0.5 mL), and dried *in vacuo*, to afford the tetra-hydrated tri-sodium salt of the title compound as a white solid (0.010 g, 85 %). δ_H ($CDCl_3$, 600 MHz): 2.61 (2H, m, $H^{4,5}$); 2.71 (3H, s, H^{10}); 2.76 (2H, m, $H^{4,5}$); 2.88 (2H, d, J 14, $H^{2,7}$); 3.09 (2H, d, J 16, H^{8}); 3.16 (4H, d+d, $H^{2,7,8}$); 7.419 (1H, t, J 7, $H^{4'}$); 7.29 (2H, t, J 7, $H^{3'}$); 7.34 (2H, d, J 7, $H^{2'}$). δ_C ($CDCl_3$, 150 MHz): 46.32 (C^{10}); 56.72 ($C^{4,5}$); 61.99 (C^{1}); 63.51 (C^{8}); 65.09 ($C^{2,7}$); 126.73 ($C^{2'}$); 127.11 ($C^{4'}$); 128.51 ($C^{3'}$); 143.40 ($C^{1'}$); 179.23 (C^{11}); 179.35 (C^{9}). *m/z* (ES+): 366.1666 $[M + H]^+$; $C_{17}H_{24}N_3O_6$ requires 366.1665. Found: C, 40.7; H, 5.73; N, 8.27. $[C_{17}H_{20}N_3Na_3O_6].4H_2O$ requires: C, 40.6; H, 5.61; N, 8.35%.

(2S,2'S)-Diethyl 2,2'-(6-nitro-6-phenyl-1,4-diazepane-1,4-diyl)dipropanoate, **13**



Ethyl (2R)-2-{[(trifluoromethyl)sulfonyl]oxy}propanoate (0.20 g, 0.78 mmol) was added to a stirred solution of potassium carbonate (0.11 g, 0.78 mmol) and **8** (0.029 g, 0.13 mmol) in acetonitrile (15 mL). The reaction mixture was stirred at 308 K for 24h, and solvent removed under reduced pressure. The resulting oil was re-dissolved in chloroform (20 mL) and washed successively with aqueous potassium carbonate solution (2 x 20 mL, 0.1 M) and water (20 mL), dried over $MgSO_4$, filtered and solvent removed under reduced pressure. Purification by silica gel column chromatography (hexane: ethyl acetate, 95:5) afforded a colourless oil (0.042 g, 77 %). R_f = 0.54 (hexane : ethyl acetate; 65 : 35). δ_H ($CDCl_3$, 600 MHz): 1.15 (3H, d, J 7, H^9); 1.24 (6H, t+t, J 7, $H^{12,17}$); 1.31 (3H, d, J 7, H^{14}); 2.67 (2H, m, $H^{4,5}$); 2.94 (1H, m, H^5); 2.98 (1H, m, H^4); 3.48 (1H, q, J 7, H^{13}); 3.51 (1H, d, J 15, H^7); 3.59 (1H, q, J 7, H^8); 3.70 (1H, d, J 15, H^2); 3.89 (1H, d, J 15, H^2); 3.96 (1H, d, J 15, H^7); 4.12 (4H, q, J 15, $H^{11,16}$); 7.31 (5H, m, $H^{2',3',4'}$). δ_C ($CDCl_3$, 150 MHz): 14.38 ($C^{12,17}$); 16.16 (C^9); 16.34 (C^{14}); 53.11 (C^5); 55.24 (C^4); 60.26 ($C^{11/16}$); 60.38 ($C^{11/16}$); 63.38 (C^2); 63.48 (C^8); 63.87 (C^7); 63.93 (C^{13}); 98.14 ($C^{1'}$); 124.79 ($C^{2'}$); 128.45 ($C^{4'}$); 128.71 ($C^{3'}$); 139.39 (C^1); 173.21 ($C^{10,15}$); 173.56 ($C^{10,15}$). *m/z*: (ES+) 422.2301 $[M + H]^+$; $C_{21}H_{32}N_3O_6$ requires 422.2291.

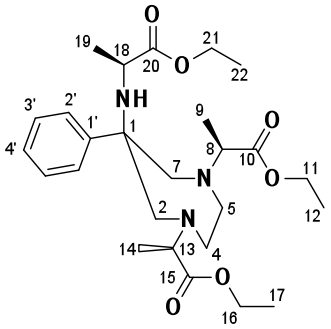
(2*S*,2′*S*)-Diethyl 2,2'-(6-amino-6-phenyl-1,4-diazepane-1,4-diyl)dipropanoate, **14**



An aqueous slurry of Raney nickel (0.010 g) was washed with methanol (3 x 25 mL) and ethanol added (10 mL). The suspension was transferred into a solution of **13** (0.050 g, 0.12 mmol) in ethanol (25 mL). The flask was evacuated and back-filled with hydrogen gas using four vacuum-purge cycles, and the mixture stirred at 298 K under an atmosphere of hydrogen. The reaction was monitored by TLC for formation of the primary amine. Once complete (~ 3 h), the solution was decanted, and the solid washed with methanol (3 x 20 mL). The washings and decanted solution were combined, removed under reduced pressure and re-dissolved in methanol (25 mL). Remaining Raney nickel and insoluble by-products were removed by filtration through a base washed Celite® filter, and the solvent was removed

under reduced pressure. The resulting oil was re-dissolved in chloroform: isopropyl alcohol (80 : 20, 20 mL) and washed successively with aqueous sodium hydroxide (2 x 20 mL, 0.5 M) and water (15 mL), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to yield a colourless oil (0.020 g, 44 %). δ_H ($CDCl_3$, 600 MHz): 1.23 (9H, m, $H^{12,17,9/14}$); 1.29 (3H, d, J 7, $H^{9/14}$); 2.13 (2H, br. s, NH_2); 2.65 (1H, d, J 14, H^2); 2.74 (2H, d+m, $H^{7+4/5}$); 2.82 (1H, m, $H^{4/5}$); 2.92 (1H, m, $H^{4/5}$); 3.00 (1H, m, $H^{4/5}$); 3.22 (2H, d+d, $H^{2,7}$); 3.47 (2H, q+q, $H^{8,13}$); 4.11 (4H, q+q, $H^{11,16}$); 7.22 (1H, t, J 7, $H^{4'}$); 7.31 (2H, t, J 7, $H^{3'}$); 7.60 (2H, d, J 7, $H^{2'}$). δ_C ($CDCl_3$, 150 MHz): 14.35 ($C^{12,17}$); 14.38 ($C^{12,17}$); 15.27 (C^9); 16.37 (C^{14}); 52.94 (C^4); 55.31 (C^5); 57.60 (C^1); 60.19 ($C^{11/16}$); 60.23 ($C^{11/16}$); 65.97 (C^7); 63.48 (C^{13}); 63.87 (C^2); 63.93 (C^8); 125.25 ($C^{2'}$); 126.51 ($C^{4'}$); 128.15 ($C^{3'}$); 146.09 ($C^{1'}$); 173.59 ($C^{10,15}$); 173.68 ($C^{10,15}$). *m/z*: (ES+) 392.2567, $[M + H]^+$; $C_{21}H_{34}N_3O_4$ requires 392.2549.

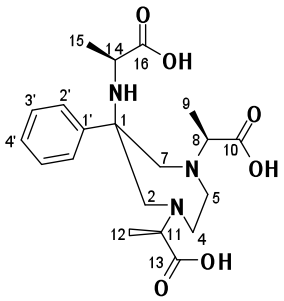
(2*S*,2'*S*)-Diethyl 2,2'-(6-((*S*)-1-ethoxy-1-oxopropan-2-ylamino)-6-phenyl-1,4-diazepane-1,4-diyl)dipropanoate , 15



Ethyl (2*R*)-2-{[(trifluoromethyl)sulfonyl]oxy}propanoate (0.096 g, 0.38 mmol) was added to a solution of **14** (0.050 g, 0.13 mmol) and potassium carbonate (0.050 g, 0.38 mmol) in acetonitrile (15 mL), and the reaction mixture stirred at 308 K for 18 h. The solvent was removed under reduced pressure and the resulting oil re-dissolved in chloroform (20 mL) and washed successively with aqueous potassium carbonate solution (2 x 15 mL, 0.1 M) and water (15 mL), dried over $MgSO_4$, filtered and solvent removed under reduced pressure. Purification by silica gel column chromatography (hexane : ethyl acetate, 95 : 5) afforded the title compound as a colourless oil (0.030 g, 47 %). R_f = 0.65 (hexane:ethyl acetate; 65:35). δ_H ($CDCl_3$, 600 MHz): 1.16 – 1.31 (19H, m, $H^{9,14,19,12,17,22}$); 1.61 (1H, br. s, NH); 2.64 (1H, m, H^5); 2.70 (1H, m, H^4); 2.81 (1H, m, H^4); 2.86 (1H, d, J 13, H^7); 2.90 (2H, m, $H^{2,5}$); 3.05 (1H, d, J 13, H^2); 3.13 (1H, q, J 7, H^{18}); 3.27 (1H, d, J 13, H^7); 3.39 (2H, q+q, $H^{8,13}$); 4.01 (2H, q, J 7, $H^{11/16/21}$); 4.09 (2H, q, J 7, $H^{11/16/21}$); 4.14 (2H, q, J 7, H^5); 7.18 (1H, t, J 8, $H^{4'}$); 7.27 (2H, t, J 8, $H^{3'}$); 7.56 (2H, d, J 8, $H^{2'}$). δ_C ($CDCl_3$, 150 MHz): 14.07 ($C^{9/12/14/17}$); 14.37 ($C^{9/12/14/17}$);

14.37 ($C^{9/12/14/17}$); 14.42 ($C^{9/12/14/17}$); 17.38 (C^{22}); 21.78 (C^{19}); 51.11 (C^{18}); 52.83 (C^{5}); 55.01 (C^{4}); 60.05 (C^{21}); 60.09 ($C^{11/16}$); 60.31 ($C^{11/16}$); 62.28 (C^{1}); 63.38 ($C^{8/13}$); 63.51 (C^{2}); 63.85 ($C^{28/13}$); 65.03 (C^{7}); 126.49 ($C^{4'}$); 127.19 ($C^{2'}$); 127.84 ($C^{3'}$); 145.61 ($C^{1'}$); 173.64 ($C^{10/15}$); 173.71 ($C^{10/15}$); 177.13 (C^{20}). *m/z*: (ES+) 492.3071 $[M + H]^+$; $C_{26}H_{42}N_3O_6$ requires 492.3074.

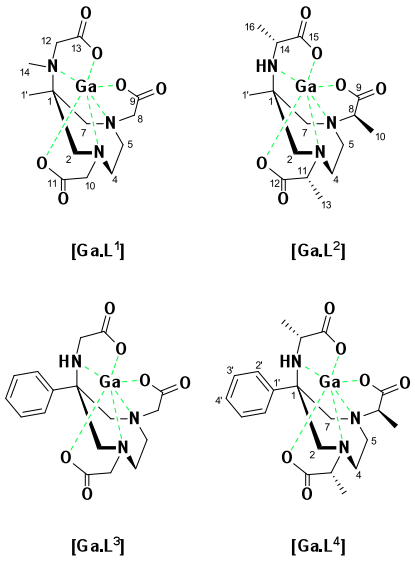
(2*S*,2'*S*)-2,2'-(6-((*S*)-1-Carboxyethylamino)-6-phenyl-1,4-diazepane-1,4-diyl)dipropanoic acid, L^4



Sodium hydroxide (0.003 g, 0.063 mmol) dissolved in water (0.5 mL) was added to a solution of the tri-ester **15** (0.010 g, 0.020 mmol) in THF (0.5 mL), and the mixture stirred at 298 K. The reaction was monitored using LC-ESMS, to follow ester cleavage. Once complete, the solvent was removed by lyophilisation. Water (5 mL) was added and removed by lyophilisation, and the protocol repeated two more times. The resulting solid was washed with ice cold dichloromethane (0.5 mL), and dried *in vacuo* to afford the tri-sodium salt as a white solid (0.091 g, 95 %). $\delta_H(CDCl_3$, 600 MHz): 0.87 (3H, d, J 7, H^{9}); 0.90 (3H, d, J 7, H^{15}); 1.09 (3H, d, J 7, H^{12}); 2.49 (1H, m, $H^{4/5}$); 2.60 (1H, m, $H^{4/5}$); 2.67 (2H, q, J 9, $H^{4,5}$); 2.73 (1H, q, J 7, H^{14}); 2.83 (1H, d, J 14, H^{7}); 2.96 (1H, q, J 7, H^{8}); 2.99 (1H, d, J 14, H^{2}); 3.10 (1H, d, J 14, H^{2}); 3.21 (1H, q, 7, H^{11}); 3.25 (1H, d, J 14, H^{7}); 7.14 (1H, t, J 8, $H^{4'}$); 7.23 (2H, t, J 8, $H^{3'}$); 7.37 (2H, d, J 8, $H^{2'}$). $\delta_C(CDCl_3$, 150 MHz): 14.87 (C^{12}); 15.70 (C^{9}); 21.35 (C^{15}); 53.50 (C^{14}); 54.70 ($C^{4/5}$); 56.07 ($C^{4/5}$); 61.37 (C^{7}); 62.08 (C^{1}); 62.18 (C^{2}); 66.58 (C^{8}); 67.07 (C^{11}); 126.92 ($C^{4'}$); 127.78 ($C^{2'}$); 127.96 ($C^{3'}$); 143.50 ($C^{1'}$); 181.65 ($C^{10/11}$); 181.94 ($C^{10/11}$); 183.84 (C^{16}). *m/z*: (ES+) 430.1972 $[M + Na]^+$; $C_{20}H_{29}N_3NaO_6$ requires 430.1964. Found: C, 45.4; H, 6.43; N, 7.77%. $[C_{20}H_{26}N_3Na_3O_6].3H_2O$ requires: C, 45.5; H, 6.12; N, 7.97%.

Syntheses of 'Cold' Gallium(III) Complexes of L^n (n = 1, 2, 3, 4)

[Ga.L^n]



The general procedure for the synthesis of cold gallium complexes is as follows. The ligand (0.1mM) was dissolved in methanol:water (1 : 2, 2 mL) and equilibrated at 333 K. Gallium nitrate hexahydrate (0.1mM) was added, and the pH adjusted to 4.5 using 0.10 M aqueous sodium hydroxide. After 1h, the solution was allowed to cool slowly to room temperature. Single crystals of the complexes suitable for single crystal X-ray diffraction formed from solution upon standing over about 36h. Crystalline material was isolated by filtration, and dried *in vacuo*. Typically, yields were 80%. In ^{1}H NMR assignments, 'Pureshift' ^{1}H NMR spectroscopy was used to assist signal resolution. The superscript '*' is used to denote proton resonances arising from protons attached to the same carbon atom, when the orientation (ax/eq) could not be assigned. ^{71}Ga NMR spectra were recorded in D_2O at 295K, and [Ga.NOTA] has values as follows: δ_{Ga} (pD 6.2, 182.9 MHz): +171 ($w_{1/2}$ 175 Hz). The narrow linewidth of [GaNOTA] is ascribed to the anisotropic electric field gradient at the nucleus associated with local C_3 symmetry, reducing line-broadening due to quadrupolar relaxation.

[Ga.L^1]:

δ_H (D_2O, pD 6.2, 600 MHz): 1.03 (3H, s, $H^{1'}$); 2.43 (3H, s, H^{14}); 3.12 (1H, m, $H^{4*/5*}$); 3.14 (1H, m, $H^{5*/4*}$); 3.20 (1H, d, J 16, H^{2ax}); 3.21 (1H, d, J 12, H^{12*}); 3.28 (1H, d, J 16, H^{10*}); 3.35 (1H, m, $H^{4/5}$); 3.41 (1H, m, $H^{5/4}$); 3.46 (1H, d, J 16, H^{7eq}); 3.57 (1H, d, J 16, H^{7ax}); 3.59 (1H, d, J 16, H^{2eq}); 3.71 (1H, d, J 18, H^{8*}); 3.77 (1H, d, J 16, H^{10}); 3.89 (1H, d, J 12, H^{12}); 3.91 (1H, d, J 18, H^8). δ_C (D_2O, pD 6.2, 151 MHz): 12.10 ($C^{1'}$); 37.96 (C^{14}); 53.01 (C^{14}); 58.22 ($C^{4/5}$); 59.80 (C^1); 60.41 (C^8); 61.31 (C^{10}); 63.57 (C^7); 64.00 (C^2); 174.10 & 174.67 & 174.82 ($C^{9,11,13}$). HRMS ES+ (*m/z*): found 384.0677 $[M + H]^+$; $C_{13}H_{21}N_3O_6Ga$ requires 384.0686. δ_{Ga}

(D_2O, pD 6.2, 182.9 MHz): +129 ($w_{1/2}$ 980 Hz). The structure of **[Ga.L^2]** was confirmed by single crystal X-ray diffraction: $C_{12}H_{20}N_3O_6.½H_2O$, M_r = 393.05, orthorhombic (Fdd2); a = 28.8536(7) Å, b = 27.2438(7) Å, c = 7.57981(19) Å, V = 5958.3(2) Å^3,α = 90.00°, β = 90.00°, γ = 90.00°, Z = 16; μ = 1.888 mm^{-1}, $D_{calc.}$ = 1.753 mg.mm^{-3}, T 120(2) K; 4319 independent reflections (R_{int} = 0.0417), R_1 = 0.0307, ωR_2 = 0.0657 ($I > 2\sigma(I)$). CCDC # 906036.

[Ga.L^2]:

δ_H (D_2O, pD 6.1, 600 MHz): 1.13 (3H, d, J 7, H$^{10/13/16}$); 1.21 (3H, s, H$^{1'}$); 1.36 (3H, d, J 7, H$^{10/13/16}$); 1.46 (3H, d, J 7, H$^{10/13/16}$); 2.83 (1H, d, H^{7ax}); 2.85 (1H, m, H$^{4*/5*}$); 3.07 (1H, m, H$^{4/5}$); 3.21 (1H, m, H$^{5*/4*}$); 3.42 (2H, m, H$^{5/4\ \&\ 7eq}$); 3.63 (1H, d, H^{2eq}); 3.78 – 3.84 (3H, m, H^{8,11,14}). δ_C (D_2O, pD 6.1, 151 MHz): 10.49 & 14.23 & 17.36 (C^{10,13,16}); 17.95 (C$^{1'}$); 41.15 (C$^{4/5}$); 46.68 (C$^{4/5}$); 49.66 (C^{14}); 56.94 (C^7); 60.64 (C^1); 64.77 (C^2); 64.44 & 66.38 (C^{8,11}); 176.96 & 178.42 & 179.07 (C^{9,12,15}). HRMS ES+ (*m/z*): found 412.1018 [M + H]$^+$; $C_{15}H_{25}N_3O_6Ga$ requires 412.0999. δ_{Ga} (D_2O, pD 6.2, 182.9 MHz): +123 ($w_{1/2}$ 1175 Hz). The structure of **[Ga.L^3]** was confirmed by single crystal X-ray diffraction: $C_{15}H_{24}N_3O_6.H_2O$, M_r = 430.11, monoclinic ($P2_1$); a = 7.58267(20) Å, b = 12.6657(3) Å, c = 9.2185(2) Å, V = 869.85(4) Å^3,α = 90.00°, β = 100.735(3)°, γ = 90.00°, Z = 2; μ = 1.627 mm^{-1}, $D_{calc.}$ = 1.642 mg.mm^{-3}, T 120(2) K; 4501 independent reflections (R_{int} = 0.0433), R_1 = 0.0322, ωR_2 = 0.0670 ($I > 2\sigma(I)$). CCDC # 906037.

[Ga.L^3]:

δ_H (D_2O, pD 6.2, 600 MHz): 3.21 (1H, d, J 12, H^{12*}) 3.29 (1H, m, H$^{4*/5*}$); 3.30 (1H, m, H$^{5*/4*}$); 3.32 (1H, d, J 16, H^{2ax});; 3.34 (1H, d, J 16, H^{10*}); 3.37 (1H, m, H$^{4/5}$); 3.54 (1H, m, H$^{5/4}$); 3.60 (1H, d, J 16, H^{7eq}); 3.67 (1H, d, J 16, H^{7ax}); 3.69 (1H, d, J 16, H^{2eq}); 3.75 (1H, d, J 17, H^{8*}); 3.88 (1H, d, J 16, H^{10}); 3.92 (1H, d, J 12, H^{12}); 4.01 (1H, d, J 17, H^8) 7.41 (5H, m, H$^{2',3',4'}$. HRMS ES+ (*m/z*): found 431.0599 [M + H]$^+$; $C_{17}H_{21}N_3O_6Ga$ requires 431.0608. The structure of **[Ga.L^6]** was confirmed by single crystal X-ray diffraction: $C_{17}H_{20}N_3O_6$, M_r = 432.08, triclinic (P-1); a = 10.6961(8) Å, b = 13.0778(12) Å, c = 12.8414(12) Å, V = 1652.8(2) Å^3,α = 116.076(9)°, β = 105.208(7)°, γ = 92.002(2)°, Z = 4; μ = 1.709 mm^{-1}, $D_{calc.}$ = 1.736 mg.mm^{-3}, T 120(2) K; 7571 independent reflections (R_{int} = 0.0881), R_1 = 0.0699, ωR_2 = 0.1553 ($I > 2\sigma(I)$). CCDC # 906038.

[Ga.L^4]:

δ_H (D_2O, pD 6.0, 600 MHz): 0.86 & 1.25 & 1.42 (9H, d, J 7, H^{10,13,16}); 2.94 (1H, m, H$^{4*/5*}$); 3.13 (1H, m, H$^{4*/5*}$); 3.33 (1H, m, H$^{5*/4*}$); 3.53 (1H, q, H^{14}); 3.56 (1H, m, H$^{5/4}$); 3.65 (1H, d, H$^{2*/7*}$); 3.75 (2H, d+d, H$^{7*/2*\ \&\ 7/2}$); 3.97 (2H, m, H$^{2/7\ \&\ 8*/11*}$); 4.17 (1H, d, H$^{8/11}$); 7.42 (5H, m, H$^{2',3',4'}$). δ_C (D_2O, pD 6.0, 151 MHz): 10.69 & 14.38 & 16.29 (C^{10,13,16}); 46.67 (C$^{4/5}$); 50.67

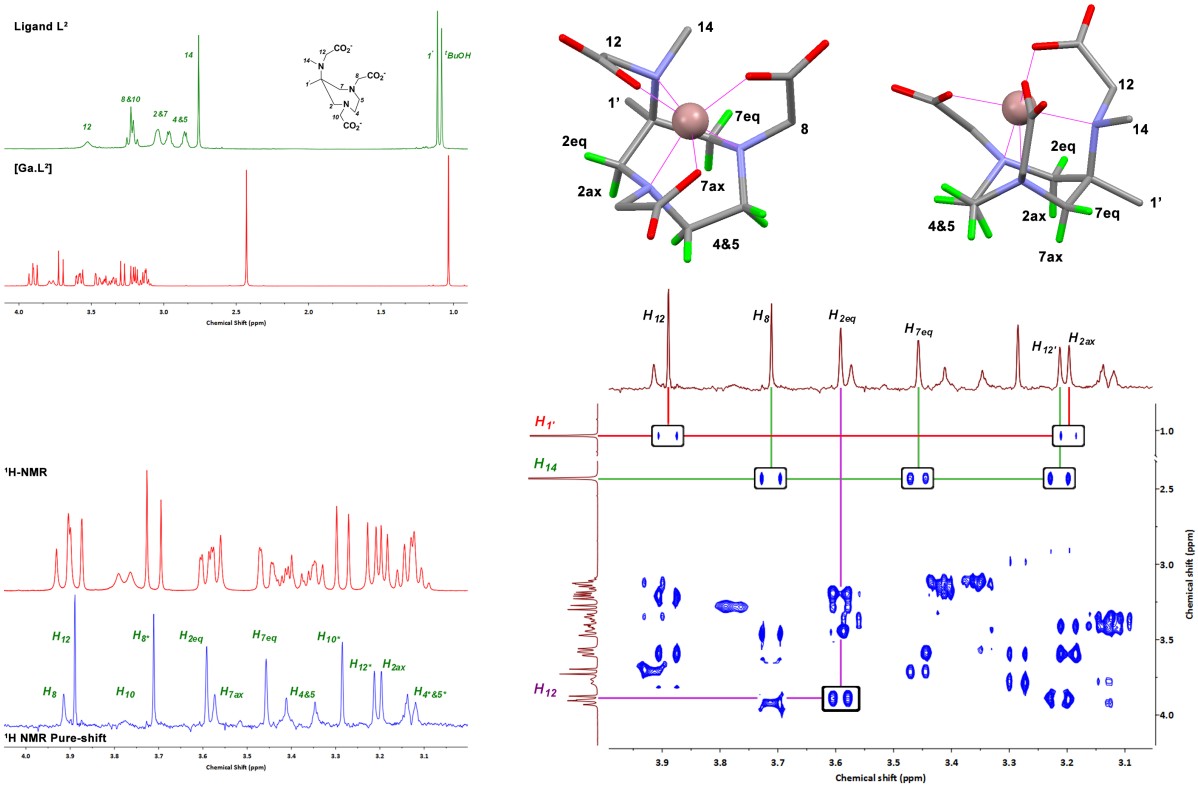
(C^{14}); 55.58 ($C^{2/7}$); 58.92 (C^{1}); 61.04 ($C^{4/5}$); 64.34 ($C^{2/7}$); 65.79 & 67.06 ($C^{8,11}$); 77.73 ($C^{1'}$); 126.47 & 129.32 & 129.96 ($C^{2',3',4'}$); 177.04 & 177.47 & 178.32 ($C^{9,12,15}$). δ_{Ga} (D_2O, pD 6.2, 182.9 MHz): +124 ($w_{1/2}$ 1815 Hz). HRMS ES+ (*m/z*): found 474.1149 $[M + H]^+$; $C_{20}H_{27}N_3O_6Ga$ requires 474.1156.

An example of the proton NMR spectra of the L^2 ligand and its Ga complex, illustrating assignments is shown in the Figure below.

References (as in main text)

5. (a) S. Aime, L. Calabi, C. Cavalloti, E. Gianolio, G. B. Giovenzana, P. Losi, A. Maiocchi, G. Palmisano and M. Sisti, *Inorg. Chem*. 2004, **43**, 7588; (b) S. Aime, G. Bombieri, C. Cavollotti, G. B. Giovenzana, D. Imperio and N. Marchini, *Inorg. Chim. Acta*, 2008, **361**, 1534; (c) Z. Baranyai, F. Uggeri, G. B. Giovenzana, A. Benyei, E. Brucher and S. Aime, *Chem. Eur. J.* 2009, **15**, 1696 ; (d) E. Elemento, D. Parker, S. Aime, E. Gianolio and L. Lattuada, *Org. Biomol. Chem*. 2009, **7**, 1120.

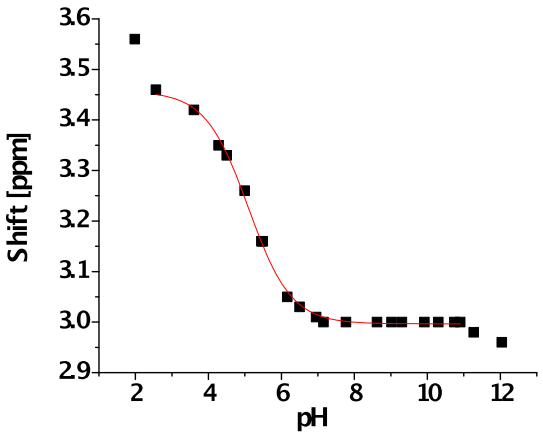
6 . L. Tei, G. Gugliotta, M. Fekete, F. K. Kalman and M. Botta, *Dalton Trans*. 2011, **40**, 2025.



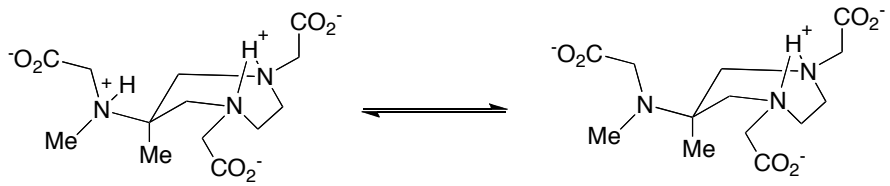
2. 1-H NMR titrations for L^1 (295K, D_2O)

The following plots reveal the variation in chemical shift for the L^1 ligand resonances as pH is varied. In assessing these changes, the following equation has been used: pD = pH (meter reading) + 0.41: P. K. Glasoe and F. A. Long, *J. Phys. Chem*. 1960, **64**, 188. The plots show the fit (line) to the experimental data using standard iterative least squares minimisation methods.

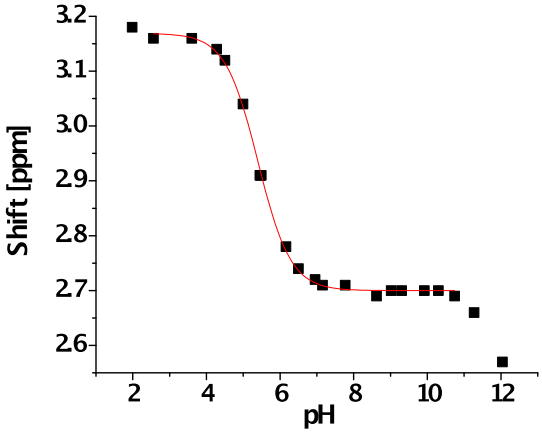
The mean protonation constant in D_2O is 5.70(±0.1) for the equilibrium shown and 11.7(±0.15) for the first pK_a value, in each case adjusted for the pD/pH change.



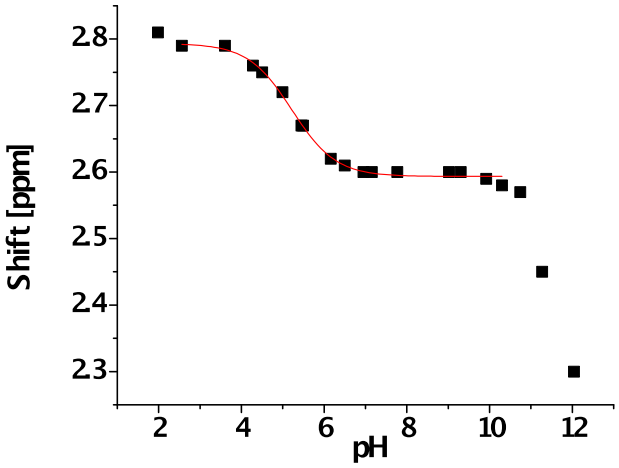
a) Variation with measured pH (meter reading) of the ^{1}H NMR shift of the endocyclic $NCH_2CO_2^-$ protons in D_2O (295K).



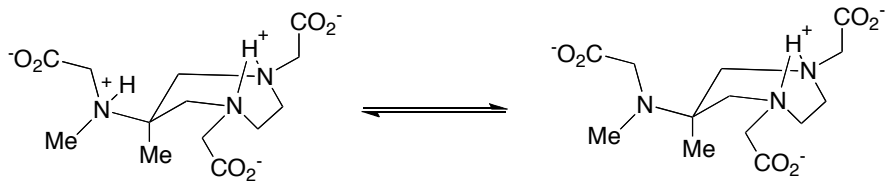
$[H_2L^1]^-$ $[HL^1]^{2-}$



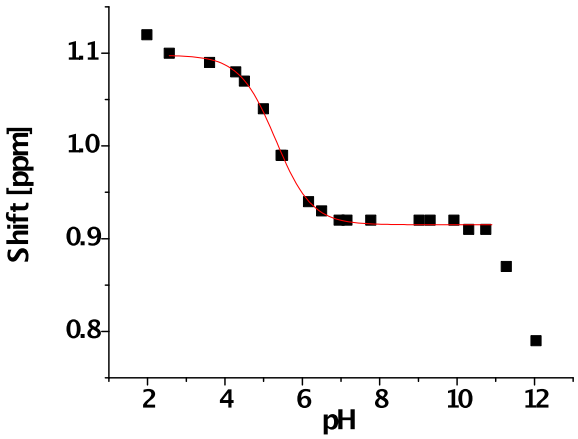
b) Variation with measured pH (meter reading) of the mean ^{1}H NMR shift of the endocyclic NCH_2CH_2N protons in D_2O (295K).



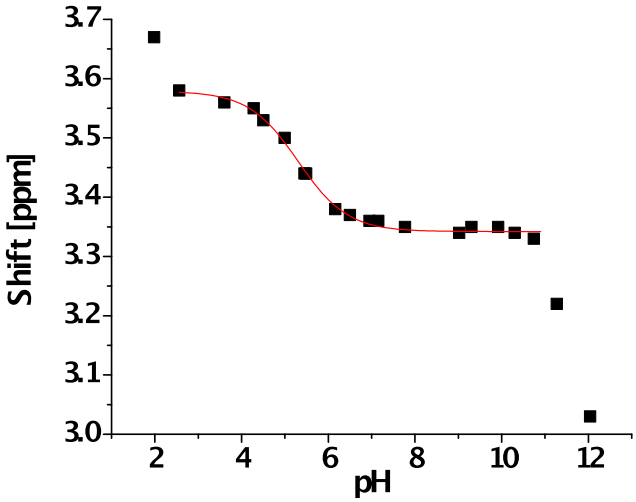
c) Variation with measured pH (meter reading) of the ^{1}H NMR shift of the N-Me singlet in D_2O (295K).



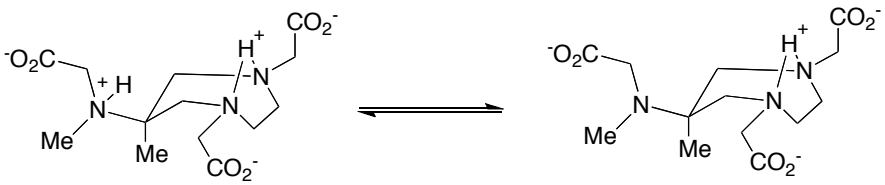
$[H_2L^1]^-$ $[HL^1]^{2-}$



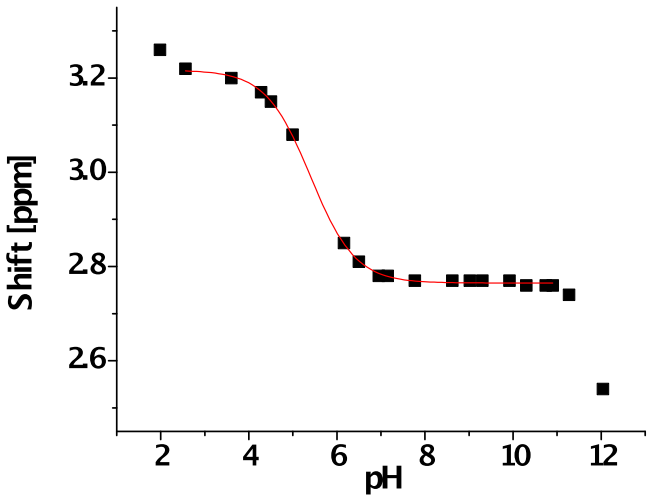
d) Variation with measured pH (meter reading) of the ^{1}H NMR shift of the C-Me singlet in D_2O (295K).



e) Variation with measured pH (meter reading) of the mean ^{1}H NMR shift of the exocyclic NCH_2 protons in D_2O (295K).



$[H_2L^1]^-$ $[HL^1]^{2-}$



f) Variation with measured pH (meter reading) of the ^{1}H NMR shift of the MeCC**H**$_2$N protons in D_2O (295K).

3. **Single Crystal X-ray Crystallography**

The X-ray single crystal data for all compounds have been collected at 120.0K on a Bruker SMART 6000 (**ESI_1**) and an Agilent Gemini S-Ultra (all other compounds) diffractometers (graphite monochromators, λMoKα, λ=0.70073Å, ω-scans of various widths) equipped with Cryostream (Oxford Cryosystems) open-flow nitrogen cryostats. All structures were solved by direct method and refined by full-matrix least squares on F^2 for all data using Olex2 [1] and SHELXTL [2] software. All non-hydrogen atoms were refined with anisotropic displacement parameters, H-atoms in all structures, except [Ga.L^3], were located on the difference map and refined isotropically. During the refinement H atoms of the water molecule tended to move along the direction of the hydrogen bonds (as it often happens in case of strong hydrogen bonds) so O-H distances have been restrained by DFIX. The H atoms in the structure [Ga.L^3] were placed into the calculated positions and refined in standard "riding" mode. Crystallographic data and parameters of structures refinements are given in Table ESI 1. Crystallographic data for the structure have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC-906036-906040.

1.O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard and H. Puschmann, *J. Appl. Cryst*. 2009, **42**, 339-341.
2. G.M. Sheldrick, *Acta Cryst*. 2008,**A64**, 112-122

Table ESI 1 Crystal data and structure refinement for gallium complexes of L^{1-3} CCDC 906036-038 and of compounds 1 and 7 CCDC 906039-40 (synthesis section 1)

Compound	**[Ga.L^1]**	**[Ga.L^2]**	**[Ga.L^3]**	**[ESI_1]**	**[ESI_7]**
Empirical formula	$C_{13}H_{20}GaN_3O_6 \times 0.5H_2O$	$C_{15}H_{24}GaN_3O_6 \times H_2O$	$C_{17}H_{20}GaN_3O_6$	$C_{20}H_{25}N_3O_2$	$C_{29}H_{35}N_3O_6$
Formula weight	393.05	430.11	432.08	339.43	521.60
Crystal system	orthorhombic	monoclinic	triclinic	monoclinic	monoclinic
Space group	Fdd2	$P2_1$	P-1	C2/c	$P2_1/n$
a/Å	28.8536(7)	7.58267(20)	10.6961(8)	32.4876(13)	8.8932(2)
b/Å	27.2438(7)	12.6657(3)	13.0778(12)	6.0545(2)	19.1927(5)
c/Å	7.57981(19)	9.2185(2)	13.8414(12)	20.2683(8)	15.3402(3)
α/°	90.00	90.00	116.076(9)	90.00	90.00
β/°	90.00	100.735(3)	105.208(7)	111.467(1)	92.411(2)
γ/°	90.00	90.00	92.002(7)	90.00	90.00
Volume/$Å^3$	5958.3(2)	869.85(4)	1652.8(2)	3710.1(2)	2616.02(1)
Z	16	2	4	8	4
$\rho_{calc}mg/mm^3$	1.753	1.642	1.736	1.215	1.324
m/mm^{-1}	1.888	1.627	1.709	0.080	0.093
F(000)	3248.0	448.0	888.0	1456.0	1112.0
2Θ range for data collection	5.64 to 59.98°	5.46 to 57.98°	5.04 to 55°	2.7 to 56°	5.2 to 59°
Reflections collected	18337	12473	16836	17258	28554
Independent reflections	4319[R(int) = 0.0417]	4501[R(int) = 0.0433]	7571[R(int) = 0.0881]	4452[R(int) = 0.0342]	7279[R(int) = 0.0449]
Data/restraints/parameters	4319/1/297	4501/3/331	7571/0/487	4452/0/315	7279/0/483
Goodness-of-fit on F^2	1.056	1.044	1.063	1.030	1.038
Final R_1 indexes [I>=2σ (I)]	0.0307	0.0322	0.0699	0.0482	0.0478
Final wR_2 indexes [all data]	0.0674	0.0702	0.1864	0.1427	0.1184

4. **Radiochemistry and Micro-PET studies**

General

All chemicals used for the radiochemical experiments were procured from commercial suppliers in the high available purity. Purite® water used was filtered through a Millex®Millipore filter membrane (0.54 μm) prior to use. Care was taken to avoid any metal contamination, and standard safety protocols strictly adhered to. For radio-TLC analysis, Merck Silica F254 TLC plates were used, and the eluted plates radio-imaged using a flatbed Canberra Packard Instant Imager.

In this study a 350 MBq ^{68}Ga/^{68}Ge-generator based on titanium dioxide, produced by Cyclotron Co., Obninsk, Russian Federation, was used. The generator was eluted with 8 mL of 0.1 M HCl. Prior to radiolabelling, post-processing of the generator eluate was carried out using a cation exchange technique described elsewhere. [Zhernosekov et al, *J. Nucl. Med.* 2007, **48**, 1741] Using this approach the eluate volume was reduced to 400 μL, and the presence of metal impurities (^{68}Ge and non-radioactive metals) lowered in the protocol that takes less than 5 min to complete. Following post-processing, the fraction was diluted, such that 400 μL contained ~ 100 MBq of radioactivity (t = 0).

Standard radiolabelling protocols

The radiolabelling kinetics of the ligands was evaluated at 298, 323 and 368 K at pH 2.3, 3.3, 4.0, 5.3 and 6.8. Stock solutions of the ligands (1 mg/ml) were prepared in water. Radiolabelling experiments carried out at pH 4.0 and 5.3 were acetate buffered, and the experiments conducted at pH 6.8 were HEPES buffered. Various concentrations of the buffer solutions were evaluated for the lowest concentration that had sufficient buffering ability upon addition of the post-processed ^{68}Ga. The acetate 'buffers' were prepared by combining calculated amounts of acetic acid and sodium acetate to give a 0.2 M buffer solution, adding sufficient HCl to give the desired pH. The HEPES buffer was prepared by adjusting the pH of a 1.0 M sodium-HEPES solution to 7.0 using 1.0 M sodium hydroxide. Suitable dilution of the post-processed generator eluate was used to achieve

pH 2.3 and 3.3. Preparations of the radiolabelling solutions for the different conditions are described below.

pH 2.3:

The ligand (50 nmol) was added to 4.6 mL of water in a glass vial, and the mixture preheated at the desired temperature for 2 min. To this mixture the post-processed generator eluate (400 µL, ~ 100 MBq) was added and a timer started.

pH 3.3:

Followed a similar protocol to that described for 'pH 2.3', except 50 µL (~ 15MBq) of the post-processed generator eluate was used.

pH 4.0, 5.3 and 6.8:

The ligand (14 nmol) was added to 1 mL of the appropriate buffer solution in an Eppendorf® vial, and the mixture preheated for 2 min at the desired temperature. To this mixture the post-processed generator eluate (400 µL, ~ 100 MBq) was added and a timer started.

Radiolabelling solutions were continuously agitated using a rotary shaker with a temperature control function. At 1, 3, 5 and 10 min time intervals after addition of the post-processed ^{68}Ga; 1 µL fractions of the reaction mixture were withdrawn using an Eppendorf® pipette and spotted, in duplicate, on separate TLC plates. To ensure correct interpretation of the radiolabelling the duplicate TLC plates were eluted separately with a 0.5 M sodium citrate buffer solution (pH 4.0) and a 90 % MeOH in 10 % aq. NaCl solution. Using the citrate buffer, un-complexed ^{68}Ga forms a ^{68}Ga-citrate complex which elutes to the top of the TLC plate (R_f ~ 0.9), while the radiolabelled product remains on or close to the baseline. Using the 25 % EtOH saline solution the un-complexed ^{68}Ga remains on the baseline, whilst the radiolabelled product moves approximately half way up the TLC plate. Exact R_f values depend on the characteristics of the radiolabelled product, and were not recorded. From the eluted TLC plates it was possible to determine the ratio of complexed to un-complexed ^{68}Ga at the different time intervals. Each radiolabelling evaluation was repeated two more times. Control experiments, in which the ligand is not added, were conducted in parallel with the radiolabelling experiments.

No post-radiolabelling purification of the radiolabelled products was carried out because only ligands which showed quantitative radiolabelling were evaluated in the stability experiments.

Stability Studies

No post-radiolabelling purification of the radiolabelled products was carried out because only ligands which showed quantitative radiolabelling were evaluated in the stability experiments.

The stability of the radiolabelled complexes was assessed in the presence of DTPA, Fe(III) (each 5000 equivalents), *apo*-transferrin (130 equivalents) and newborn calf serum. The evaluations were carried out over 2 h at 310 K and pH 7.4 (1.0 M PBS), and the solutions agitated using a rotary shaker. All stability studies were performed twice and in duplicate for each ligand-radiolabelling pH combination of interest. Two control experiments where performed in each case; one in the absence of the ligand and the other in the absence of the challenge. These were used to establish the R_f values of the free ^{68}Ga, ^{68}Ga-*apo*-transferrin and ^{68}Ga-DTPA complexes, as well as any complexation of the free ^{68}Ga in the new-born calf serum. Stock solutions of DTPA, $FeCl_3$ (both 1.0 μM) and apo-transferrin (1 mg/ml) were prepared in 1.0 M PBS (pH 7.4) solution. The newborn calf serum was used as received.

The challenge solution (200 μL) was preheated at 310 K for 10 minutes in an Eppendorf® vial, and the radiolabelled complex (50 μL of the appropriate radiolabelling solution) added. At 15, 30, 60, 90 and 120 min time intervals following addition of the radioactivity, 2 μL fractions were withdrawn using an Eppendorf® pipette and spotted, in duplicate, on separate TLC plates. To ensure correct interpretation of the stability evaluation, the duplicate TLC plates were eluted separately with a 0.5 M citrate buffer solution (pH 4) and a 25 % EtOH in 5 % NaCl solution. From the eluted TLC plates it was possible to determine the amount of intact radiolabelled complex at the different time points.

NOTA challenge experiments

The conditions used for the NOTA-challenge experiments were selected so that they reflected the optimum conditions for radiolabelling of NOTA. The challenge was performed at 298 K and pH 4 (0.2 M citrate buffer) over the course of 10 minutes, with a 20 μM concentration of both NOTA and the ligand of interest. Under the conditions used each ligand (in isolation) would show quantitative radiolabelling within 1 min. Equimolar amounts (28 nmol from 1 mg/ml stock solutions) of NOTA and the ligand were added to 1 mL of a 0.2 M citrate buffer solution (pH 4), and the mixture left to equilibrate on a rotary shaker for 2 min at 298 K. The post-processed ^{68}Ga was added, and 1μL fractions of the radiolabelling solution spotted on a TLC plate at 1, 3, 5, 10 and 120 min time intervals. The TLC plates were eluted using a 0.5 M citrate solution (pH 4). With this mobile phase the un-complexed ^{68}Ga elutes to the top of the plate, the [^{68}Ga.NOTA] complex about a third of the way up (R_f ~ 0.35) and the radiolabelled ligand evaluated remains on, or close to, the base line. From the eluted TLC plates it was possible to determine the relative ratios of different complexes at each time interval. Control experiments were carried out in parallel in the absence of each and both ligands.

Full details of the labeling experiments and the time/pH dependence of complexation in the presence and absence of different additives will be reported elsewhere.

Micro-PET Studies

Animal imaging experiments were performed in Sprague Dawley rats (body weights 380 and 330 g, male) under isoflurane anesthesia (2% isoflurane, 98% oxygen). All experiments had previously been approved by the regional animal ethics committee and were conducted in accordance with the German Law for Animal Protection. (Tiergenehmigung Schmitt)

The complex **[^{68}Ga.L^{1}]** was prepared at pH 4 (0.2 M acetate buffer) as previously described. Prior to injection, the pH was adjusted to pH 7 and 0.8-0.9 mL of the labelling solution was injected into the tail vein by bolus injection. The injected dose was 23.0 and 23.3 MBq respectively.

µPET imaging was performed on a µPET Focus 120 small animal PET (Siemens/Concorde, Knoxville, USA). Animals were placed in head-first-supine position. After a 15 min transmission scan with an external ^{57}Co source, dynamic PET studies were acquired over 45 min in 2 D mode followed by a 15 min whole body scan.

For analysis the PET 'listmode' data were depicted into 14 - 20 frames with varying time frames and reconstructed using an OSEM algorithm. PMOD software (PMOD Technologies LTD.) was used to visualize the images.

An example is given below (40 minutes post-injection), showing the majority of the activity in the bladder (causing over-exposure) and the remainder almost exclusively in the kidneys.