Synthesis and Complexation Behaviour of an Effective Octadentate Complexone 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetrakis[methylene(methylphosphinic acid)]

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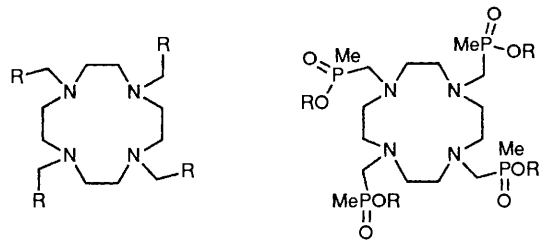
The title ligand and a *C*-functionalised analogue have been prepared; the ligand is very strongly basic [pK_a LH^+, $LH^{2+} > 14$ (298 K)] and forms remarkably stable complexes with yttrium and calcium.

The ligand 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra-acetic acid **1a** (dota) is well known to form thermodynamically stable complexes with a wide range of divalent and trivalent metal ions.[1] In particular it forms the most stable complexes known (in aqueous solution) with calcium and the rare earths[2] and lanthanoids.[3] A *C*-functionalised analogue has been linked to monoclonal antibodies and may be radiolabelled with ^{90}Y to form a kinetically stable complex *in vivo*.[4,5] The gadolinium complex of dota is an effective paramagnetic contrast agent for magnetic resonance imaging.[6] Both these applications are dependent upon the respective complexes being resistant to acid (or cation) catalysed decomplexation. Using alkylphosphinic acid donors instead of carboxylates, protonation on oxygen in both the ligand and the monoanionic complexes will occur under more strongly acidic conditions.[7] In addition, a phosphinic acid oxygen is a better σ donor than a carboxylate for cations of high charge density. Given the relative ease of structural variation of the aminoalkylphosphinic acids, a new and versatile class of complexing agents may be expected.

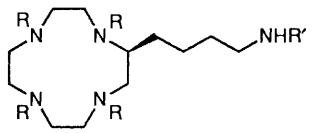
Reaction of 1,4,7,10-tetraazacyclododecane with paraformaldehyde and $MeP(OEt)_2$ in tetrahydrofuran (THF) yielded the tetraester **2a** as a mixture of the four possible diastereoisomers, at least two of which may be observed by ^{31}P NMR spectroscopy [δ_P ($CDCl_3$) major 51.9, 51.8, 51.6]. Acid hydrolysis (6 mol dm^{-3} HCl; 18 h; 125 °C) yielded the tetraacid **2b**,† as the hydrochloride salt. Analysis of pH-metric titration data yielded protonation constants of 8.12 and 3.66

† *Selected data*: **2a** *m/z* (DCI) 653 ($M^+ + 1$), 652 (M^+), 533; δ_C ($CDCl_3$) 13.44 (d, J_{CP} 91 Hz), 16.42 (d, J_{CCOP} 5 Hz), 54.18 (CH_2N ring), 54.30 (d, J_{CP} 110 Hz, CH_2P), 59.82 (CH_2O, d, J_{CP} 6 Hz, PMe).
2b *m/z* (FAB) 540 (M^+); δ_P (D_2O, pD 14) 39.2; δ_C (D_2O, pD 1) 14.86 (J_{CP} 94), 50.70 (CH_2N), 51.64 (J_{CP} 118).
[**2b**·**Y**]$^-$ *m/z* (FAB) 626, 625; δ_P (D_2O, pD 6) 44.4; δ_c (D_2O) 51.41 (d, J_{CP} 96 Hz, CH_2P); 46.80, 46.65 (CH_2N); 11.20 (d, J_{CP} 98 Hz); δ_H (pD 6.5) 1.33 (12H, d, J_{PCH} 14 Hz), 2.25 (4 H, d, *J* 13.3 Hz, ring CH), 2.45 (8H, dd + dd, CH_2P), 3.35 (12 H, m, NCH + NCH_2).
3 *m/z* (DCI) 829 ($M^+ + 1$); δ_P ($CDCl_3$) 52.3–50.4 (m); δ_C ($CDCl_3$) 164.8 (CONH); 132.5 (PhCCO); 128.3, 125.5, 124.7 (Ph); 60.5 (br, CH_2O); 59.2, 58.2, 58.1, 58.0, 56.8, 56.3, 55.9, 55.0, 54.7, 51.7, 51.0, 49.7 (CH_2N of diastereoisomers); 40.89 (CH_2NHCO); 31.0, 29.6, 29.5, 26.4 (CH_2C of diastereoisomers); 19.2 (CH_3CH_2, d, J_{CCOP} 5 Hz); 16.1 and 15.9 (d + d, J_{CP} 91 Hz, CH_3P-diastereoisomers).
5 *m/z* (FAB) 763 ($M^+ + 1$); δ_H (D_2O, pD 1) 6.64 (2H, s), 4.0–2.60 (27H, m), 2.26 (2H, t, *J* 7.2 Hz, CH_2CO), 1.8–1.0 (6H, br, CH_2C), 1.21 (12H, J_{CHP} 14 Hz).

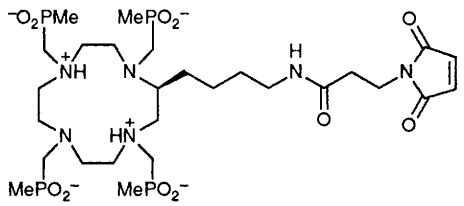


1 a; R = CO_2H
b; R = PO_3H_2

2 a; R = Et
b; R = H



3 R = $CH_2P(Me)O_2Et$, R′ = COPh
4 R = $CH_2P(Me)O_2^-$, R′ = H



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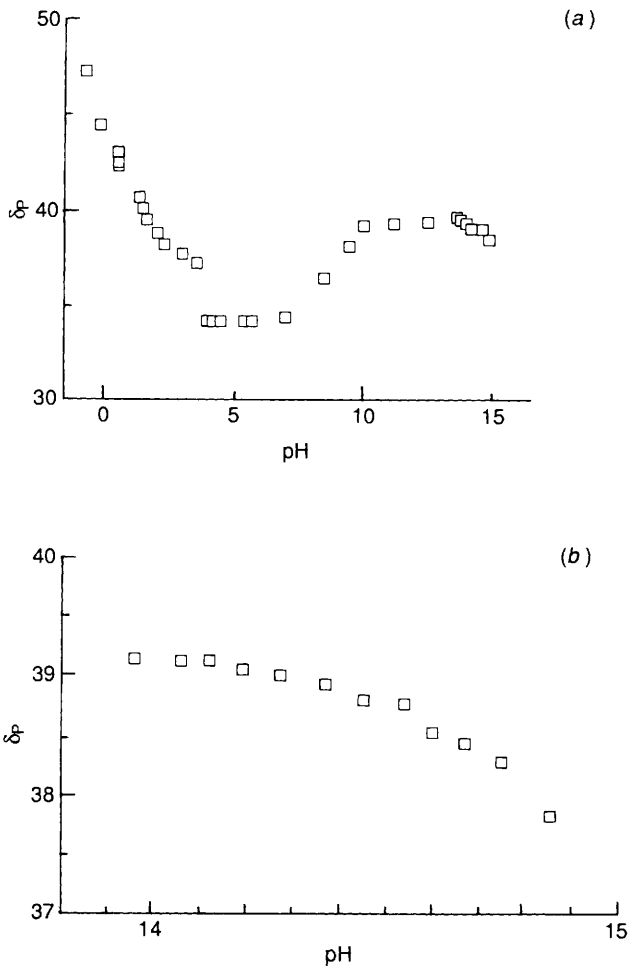

Fig. 1 Variation of ^{31}P NMR shift of **2b** with pH: (*a*) at 298 K; (*b*) at 333 K (Me_4NOH)

Table 1 Protonation and binding constants for **1b** [298 K; H_2O; *I* 0.1 mol dm^{-3} (Me_4NNO_3)][d,c]

pK_1	pK_2	pK_3	pK_4	$\log K_{MgL}$	$\log K_{MgLH}$
> 14.5[a] (11.4)[b]	> 14[a] (10.0)[b]	8.12 (6.70)[b]	3.66	13.0	8.06
$\log K_{CaL}$	$\log K_{CaLH}$	$\log K_{YL}$		$\log K_{NaL}$	
18.1	6.52	25.1		3.90	

[a] Determined using ^{31}P NMR. [b] Determined using ^{31}P NMR in the presence of Na^+. [c] Determined by pH-metric titration followed by data analysis with SCOGS2 then SUPERQUAD. [d] In comparison **1a** gives pK_a values of 12.09, 9.68, 4.55 and 4.13 [298 K; *I* 0.1 mol dm^{-3} (Me_4NNO_3)],[1] and **1b** gives 13.7, 12.2, 9.28, 8.09, 6.12, and 5.22.[8]

(Table 1), consistent with successive protonation on nitrogen and oxygen. Measurement of the ^{31}P NMR shift of **2b** as a function of pH (Fig. 1*a*) revealed that further ligand protonations were occurring both above pH 14 and below pH 2.5. Even measurements at 60 °C [*I* > 5 mol dm^{-3} (Me_4NNO_3)] failed to pinpoint accurately the upper pK_a values (Fig. 1*b*). Using NaOH instead of Me_4NOH to adjust the base concentration, observable pK_a values of 11.4 and 10.0 were defined. Given that **2b** forms a weak complex with sodium (log K_{ML} = 3.90, Table 1), such a depression of the ligand protonations was expected. These protonation values may be compared with those obtained with the analogous tetrakis(phosphonic acid) **1b**, for which values of 13.7 and 12.2 were measured [298 K, *I* 0.1 mol dm^{-3} (Me_4NNO_3)] reducing to 10.9 and 9.2 in the presence of sodium.[8]

Stability constants (log K_{ML}) for complexation with Y^{3+}, Ca^{2+} and Mg^{2+} have also been measured (Table 1), each of which is slightly higher than those reported with **1a** (dota) (log K_{ML} = 24.9, 17.2 and 11.9 respectively[1,2]). The yttrium complex $[Y\cdot\mathbf{2b}]^-$ exhibits an invariant ^{31}P NMR shift in the pH range 12–2 and monoprotonation occurs only at pH 1.15 (*cf.* 3.08 for $[Y\cdot\mathbf{1a}]^-$). The formation and dissociation of this yttrium complex has been studied using ^{90}Y radiolabelled complex; rapid complexation (monitored by anion-exchange HPLC) occurs above pH 6.5 and radiolabelling yields of ≥98% may be obtained at ligand concentrations of 5 μmol dm^{-3} (37 °C, 30 min). The complex [^{90}Y·**2b**] clears rapidly from the body of injected mice and no measurable value was obtained for ^{90}Y in the femur (at 24 and 48 h post injection) consistent with the *in vivo* stability of the complex with respect to ^{90}Y loss (free ^{90}Y accumulates in the bone). Dissociation of ^{90}Y from the complex was monitored in the pH range 1–2.7, following established methods.[4] The rates observed (Fig. 2) were comparable with those obtained with [^{90}Y·dota] under identical conditions.[4]

The yttrium complex of **2b** was formed as a *single* diastereoisomer [δ_P (D_2O, pD 6) 44.4] in which the configuration at each of the four stereogenic centres at phosphorus is

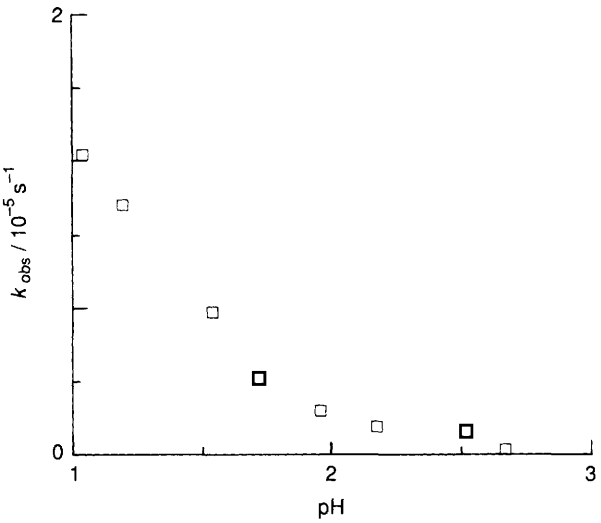

Fig. 2 Rate of yttrium dissociation from [^{90}Y·**2b**] (310 K, I 0.1 mol dm^{-3})

most likely to be the same, *i.e. RRRR* or *SSSS*. In this configuration the methyl groups are oriented away from the [12]N_4 ring plane. Resolution of this chiral complex is feasible with a suitable enantiomerically pure base.

In order to link **2b** to a protein or oligonucleotide a *C*-functionalised variant is required bearing pendent functionality. Reaction of 2-(4-benzamidobutyl)-1,4,7,10-tetraazacyclododecane[9] with $MeP(OEt)_2$ and $(CH_2O)_n$ in THF yielded **3** (30%). Acid hydrolysis and reaction with the heterobifunctional coupling agent *N*-succinimido-3-maleimidopropionate (Me_2SO, *N*-methylmorpholine; 3 h; 20 °C) yielded the maleimide **5**. This has been coupled to the tumour-localising antibody B72.3 using conventional methods,[10] and may be radiolabelled directly with ^{90}Y (pH 6.8; 15 min) to give labelling yields of 5 μCi per μg of antibody. ^{90}Y-based radioimmunotherapy using such a conjugate is being evaluated.

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References

1 R. Delgado and J. J. R. Frausto da Silva, *Talanta*, 1982, **29**, 815; H. Stetter, W. Frank and R. Mertens, *Tetrahedron*, 1981, **37**, 767.
2 J. P. L. Cox, K. J. Jankowski, R. Kataky, D. Parker, C. Walker, A. Harrison, N. R. A. Beeley, M. A. W. Eaton, A. T. Millican and K. Millar, *J. Chem. Soc., Chem. Commun.*, 1989, 797.
3 J. F. Desreux, E. Merciny and M. F. Loncin, *Inorg. Chem.*, 1981, **20**, 987; 1986, **25**, 2646.
4 C. J. Broan, J. P. L. Cox, A. S. Craig, R. Kataky, D. Parker, G. Ferguson, A. Harrison and A. M. Randall, *J. Chem. Soc., Perkin Trans. 2*, 1990, in the press.
5 See also: M. K. Moi, C. F. Meares and S. J. de Nardo, *J. Am. Chem. Soc.*, 1988, **110**, 6266.
6 R. B. Lauffler, *Chem. Rev.*, 1987, **87**, 901.
7 J. G. Dingwall, C. D. Campbell and E. K. Baylis, *J. Chem. Soc., Perkin Trans. 1*, 1984, 2845.
8 R. Delgado, L. C. Siegfried and T. A. Kaden, *Helv. Chim. Acta.*, 1990, **73**, 140; C. F. G. C. Geraldes, A. D. Sherry and W. P. Cacheris, *Inorg. Chem.*, 1989, **28**, 3336; I. M. Kabachnik, T. Y. Medved, F. I. Bel'skii and S. A. Pisareva, *Izv. Akad. Nauk. SSSR, Ser. Khim.*, 1984, 844.
9 J. P. L. Cox, A. S. Craig, I. M. Helps, K. J. Jankowski, D. Parker, M. A. W. Eaton, N. R. A. Beeley, A. T. Millican and K. Millar, *J. Chem. Soc., Perkin Trans. 1*, 1990, 2567.
10 J. R. Morphy, R. Kataky, D. Parker, A. Harrison, C. Walker, R. Alexander, M. A. W. Eaton and A. T. Millican, *J. Chem. Soc., Perkin Trans. 2*, 1990, 573.