Design of Ligands containing the *o*-Hydroxybenzyl Group. Metal-complexing Properties of *N,N″*-Bis(2-hydroxybenzyl)-diethylenetriamine-*N,N′,N″*-triacetic Acid

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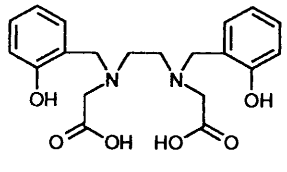
The ligand *N,N″*-bis(2-hydroxybenzyl)diethylenetriamine-*N,N′,N″*-triacetic acid (H_5L) has been synthesised and the protonation constants for L determined by potentiometric methods in 0.5 mol dm^{-3} $NaNO_3$, and spectrophotometric methods in 0.5 and 0.1 mol dm^{-3} NaCl, all at 25 °C. The sites of protonation have been inferred from ^1H NMR studies in D_2O. The complex formation constants of Ca^{II}, Zn^{II} Cd^{II}, Cu^{II}, Pb^{II} and Bi^{III} have been determined at 25 °C by potentiometric methods in 0.5 mol dm^{-3} $NaNO_3$, and spectrophotometric methods in 0.5 mol dm^{-3} NaCl. The results show that at biological pH the hydroxybenzyl groups tend to remain protonated, and over most of the pH range protonated complexes dominate, with fully deprotonated complexes occurring only at pH values above 9 or 10. The ligand L is, compared to some of its analogues, effectively a weak complexing agent. This is rationalised in terms of the six-membered chelate rings formed in the complex, which include the hydroxybenzyl group. The six-membered chelate rings destabilize complexes of the larger metal ions with which the octadentate ligand should prefer to co-ordinate.

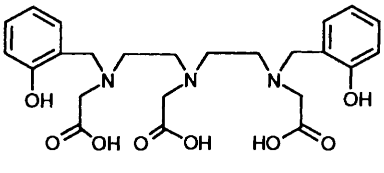
Ligand design for complexation of metal ions in biomedical applications has become of great importance over the last few years.[1-3] An interest here is the development of ligands that will complex both Pb^{II} and Bi^{III} strongly for use[4] in cancer therapy, based on complexes of radionuclides of these metals attached to monoclonal antibodies. Ligands containing the hydroxybenzyl donor group such as *N,N′*-bis(2-hydroxybenzyl)ethylenediamine-*N,N′*-diacetic acid (H_4hbedda) have proved to form exceptionally strong complexes of metal ions such as Fe^{III}, Ga^{III} and In^{III}.[5-8] This relates to the high basicity of the hydroxybenzyl group compared to the carboxylate group present in ligands such as ethylenediamine-*N,N,N′,N′*-tetraacetic acid (H_4edta) which show much less discrimination toward highly charged metal ions than H_4hbedda. More highly charged metal ions generally show[1] stronger Lewis acidity towards more basic negatively charged oxygen donors, so that the hydroxybenzyl group increases complex stability of more highly charged metal ions, and also enhances selectivity for more highly charged metal ions relative to less highly charged metal ions. In this paper we consider the hydroxybenzyl group and factors that control selectivity for metal ions in ligands containing the hydroxybenzyl group, as well as the synthesis and metal-ion complexing properties of the ligand *N,N″*-bis-(2-hydroxybenzyl)diethylenetriamine-*N,N′,N″*-triacetic acid (H_5hbdtta) which is an analogue of the familiar ligand diethylenetriamine-*N,N,N′,N″,N″*-pentaacetic acid (H_5dtpa). Just as H_4hbedda is derived from edta by replacement of two carboxylates with hydroxybenzyl groups, so H_5hbdtta is derived from H_5dtpa by replacement of two carboxylates with two hydroxybenzyl groups. The metal ions Pb^{II} and Bi^{III} are much larger[9] than Fe^{III}, so that while H_4hbedda gives six-co-ordination to metal ions such as Fe^{III}, H_5hbdtta provides eight co-ordination sites to the larger Pb^{II} and Bi^{III} ions. We report here the complex formation constants of H_5hbdtta with Bi^{III} and Pb^{II}, plus the toxic metal ion Cd^{II}. To allow for evaluation of how well H_5hbdtta will select against metal ions present in the body, complex formation constants of Ca^{II}, Zn^{II} and Cu^{II} are also reported.

Experimental

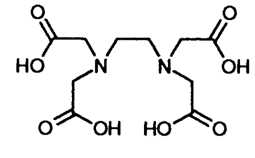
Synthesis of N,N′-*Bis*(2-*hydroxybenzyl*)*diethylenetriamine*-N,N′,N″-*triacetic Acid* (H_5hbdtta).—The synthesis of H_5hbdtta was carried out as outlined in Scheme 1. Synthesis was initiated by direct formation of the bis(salicylaldimine) (**I** in Scheme 1) derivative of the *N*-2-aminoethylamide of glycine (96%). The diimine was converted stepwise to the triamine trihydrochloride **III** by first reducing the imine double bond ($NaBH_4$, EtOH) to generate the diamine **II** (89%). Subsequent reaction of the diamine **II** with BH_3–thf (thf = tetrahydrofuran) cleanly generated **III** (85%). Alkylation of **III** to yield the final product was performed as a modification of a previously reported procedure[10] [$BrCH_2CO_2Bu^t$, $NaHCO_3$, dimethylformamide (dmf)] to eliminate generation of potentially troublesome *o*-alkylation products. The *tert*-butyl ester was cleaved (trifluoroacetic acid), and after complete removal of the reagent *via* cation-exchange chromatography the crude product was isolated by anion-exchange chromatography to provide analytically pure H_5hbdtta for further study (36.7%). Anhydrous solvents (thf, dioxane and dmf) were used throughout the synthesis. Proton and ^{13}C NMR spectra were obtained using a Varian 300XL instrument. Chemical shifts (δ) are reported in ppm relative to tetramethylsilane, sodium 3-(trimethylsilyl)tetradeuteriopropionate (tsp) or CD_3CN (δ 1.30). Proton chemical shifts are annotated as follows: ppm [multiplicity, integral coupling constant (Hz)]. Chemical ionization (CI) and fast-atom bombardment (FAB) mass spectra were obtained on Finnigan 3000 and JEOL JMS-SX102 instruments, respectively. Elemental analyses were performed by Atlantic Microlabs (Atlanta, Georgia). Analytical



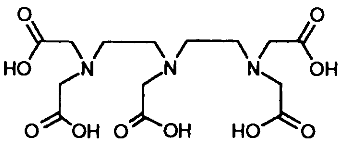
H$_4$hbedda



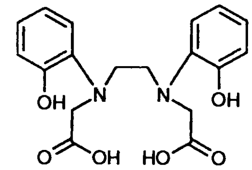
H$_5$hbdtta



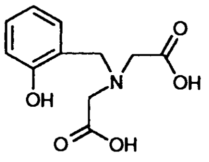
H$_4$edta



H$_5$dtpa



H$_4$hpedda



H$_3$hbida

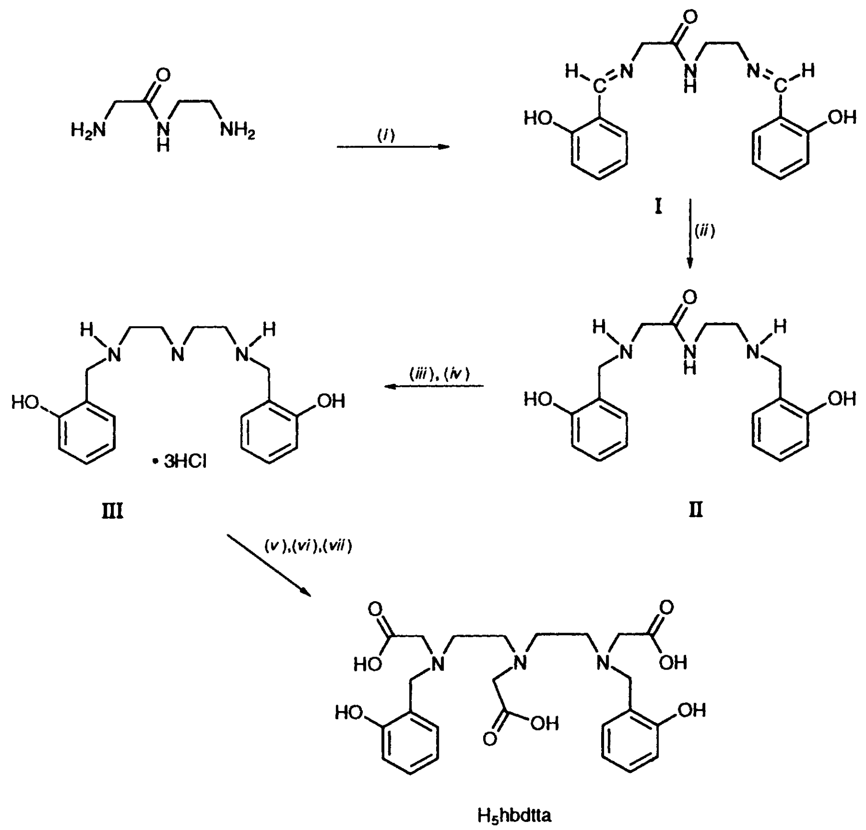
HPLC was performed using a Beckman gradient system equipped with model 114M pumps controlled by System Gold software with a model 165 dual wavelength detector set at 254 and 280 nm. An Altex reversed phase column (5 mm particles, 4.6 × 250 mm) and a binary gradient of 0.0–100% B over 25 min (solvent A = 0.05 mol dm^{-3} NEt_3–CH_3CO_2H, pH 5.5, solvent B = methanol) at 1.0 cm^3 min^{-1} was used for all analyses.

1,9-*Bis*(2-*hydroxyphenyl*)-4-*oxo*-2,5,8-*triazanona*-1,8-*diene* **I**. Salicylaldehyde (9.28 g, 76 mmol) was added directly to a solution of *N*-(2-aminoethyl)glycinamide (4.44 g, 38 mmol) in 100% ethanol (50 cm^3). After *ca.* 5 h the product precipitated and the suspension was stirred for an additional hour. The yellow solid was collected and dried at 0.1 mmHg for 24 h to give the diimine (11.86 g, 96%) (Found: C, 66.35; H, 5.90; N, 12.90. $C_{18}H_{19}N_3O_3$ requires C, 66.40; H, 5.90; N, 12.90%). δ_H($CDCl_3$) 8.32 (s, 1 H, HC=N), 8.31 (s, 1 H, HC=N), 7.38–7.16 (m, 4 H, aryl), 6.93–6.80 (m, 4 H, aryl), 6.40 (br t, 1 H, NH), 4.28 (s, 2 H, CH_2CO), 3.73 (q, 2 H, *J* 12.0, 6.0, NHC*H*$_2$), 3.63 (q, 2 H, *J* 12.0, 6.0, C*H*$_2$N); δ_C($CDCl_3$) 168.72, 168.46, 160.67, 160.36, 132.65, 132.05, 131.69, 131.26, 118.70, 118.34, 116.68, 116.55, 62.47, 58.21, 39.75; *m*/*z* (CI, NH_3) 326 (M^+ + 1).

1,9-*Bis*(2-*hydroxyphenyl*)-4-*oxo*-2,5,8-*triazanonane* **II**. The diimine **I** (10.0 g, 30.8 mmol) was suspended in 100% ethanol (100 cm^3) under argon and $NaBH_4$ (2.5 g, 65.8 mmol) was added in a single portion. The colourless solution that formed was stirred for 18 h, then poured into H_2O (100 cm^3) and extracted into CH_2Cl_2 (3 × 100 cm^3). The combined organic extracts were washed with 5% $NaHCO_3$ (2 × 200 cm^3) and saturated salt solution (100 cm^3). The CH_2Cl_2 solution was dried over Mg_2SO_4, filtered, and then concentrated to a thick oil, which slowly crystallized (0.02 g, 89%) (Found: C, 65.50; H, 6.90; N, 12.65. $C_{18}H_{23}N_3O_3$ requires C, 65.60; H, 7.05; N, 12.75%). δ_H[$(CD_3)_2SO$] 7.92 (br t, 1 H, NH), 7.06 (m, 4 H, aryl), 6.72 (m, 4 H, aryl), 3.89 (s, 2 H, C*H*$_2$NH), 3.34 (br q, 2 H, *J* 5.5), 3.20 (s, 2 H, CH_2CO), 2.71 (t, 2 H, *J* 11.0, 5.5); δ_C($CDCl_3$) 170.16, 156.78, 156.16, 128.19, 127.64, 127.41 (2 C), 117.93, 117.78, 114.91, 114.79, 50.41, 49.93, 46.79, 46.77, 37.23; *m*/*z* (CI, NH_3) 330 (M^+ + 1).

1,9-*Bis*(2-*hydroxyphenyl*)-2,5,8-*triazanonane trihydrochloride* **III**. The amide **II** (7.0 g, 21.3 mmol) was dissolved in thf (100 cm^3), cooled (ice bath), and 1 mol dm^{-3} BH_3–thf (130 cm^3) was injected. The solution was refluxed for 1 w, then cooled on an ice bath and methanol (50 cm^3) added. Removal of solvent on a rotary evaporator yielded a gummy solid which was taken up in 100% ethanol (150 cm^3) and saturated with HCl(g) while cooling. The saturated solution was refluxed for 6 h and then stirred at room temperature (r.t.) for 18 h. The suspension was held at 4 °C for 24 h, collected under an argon blanket and vacuum dried at 0.05 mmHg (*ca.* 6.66 Pa) (5.72 g, 85%) (Found: C, 50.75; H, 6.65; N, 9.80. $C_{18}H_{25}N_3O_2{\cdot}3HCl$ requires C, 50.90; H, 6.65; N, 9.90%). δ_H(D_2O, pH 1) 7.04–6.97 (m, 4 H, aryl), 4.34 (s, 4 H, C*H*$_2$NH), 3.57 (m, 8 H, CH_2CH_2); δ_C(D_2O, pH 1, tsp) 157.76, 134.51(2), 123.43, 119.58, 118.44, 50.14, 46.28, 45.67; *m*/*z* (CI, NH_3) 316 (M^+ + 1).

N,N″-*Bis*(2-*hydroxybenzyl*)*diethylenetriamine*-N,N′,N″-*triacetic acid* (H_5hbdtta). The compound $NaHCO_3$ (3.60 g, 42.8 mmol) was added to the triamine **III** (3.0 g, 7.07 mmol) in dmf (50 cm^3), and heated to *ca.* 80 °C, when *tert*-butyl bromoacetate (4.95 g, 25.4 mmol) was added. The mixture was heated under argon for 18 h, cooled to r.t., extracted into CH_2Cl_2 (100 cm^3) and washed with H_2O (3 × 100 cm^3). The organic phase was dried over $MgSO_4$, filtered, and evaporated on a rotary evaporator to a thick light brown oil. A mass spectrum (CI, NH_3) of the oil showed an M^+ + 1 signal at 658 without any indication of tetra- or penta-alkylated products. The oil was treated with trifluoroacetic acid (25 cm^3) for 18 h, after which time the acid was removed on a rotary evaporator. The residue was taken up in the minimum amount of H_2O and loaded onto a cation-exchange column (AG50WX8, 200–400 mesh, H^+ form, 2.6 × 30 cm). The column was washed until the eluent was neutral, and then the crude product was eluted with 3 mol dm^{-3} NH_4OH (1 dm^3). Removal of solvent on a rotary evaporator left a pinkish lavender solid, which was further purified on an anion-exchange column (AG1X8, 200–400 mesh, CH_3CO_2H form, 1.6 × 30 cm), and eluted from the column with a 0.0–1.5 mol dm^{-3} CH_3CO_2H gradient (2 dm^3). The eluent was collected in 88 18 × 150 mm test tubes and the pure product was found in tubes 33–56. The fractions were combined and concentrated to *ca.* 30 cm^3. The concentrate was lyophilized to leave a white solid which was dried at 100 °C and 0.01 mmHg (*ca.* 1.33 Pa) for 72 h (1.27 g, 36.7%). HPLC, $t_{\text{retention}}$ 14.99 min (Found: C, 58.85; H, 6.20; N, 8.30. $C_{24}H_{31}N_3O_8$ requires C, 58.85; H, 6.40; N, 8.60%). δ_H(D_2O, pH 12) 7.19 (dd, 2 H, *J* 7.0, 2.0, aryl), 7.10 (dd, 2 H, *J* 7.5, 2.0, aryl), 6.69–6.57 (m,



Scheme 1 Synthesis of H_5hbdtta. (*i*) Salicylaldehyde, ethanol; (*ii*) $NaBH_4$, ethanol; (*iii*) BH_3–thf; (*iv*) HCl(g), ethanol; (*v*) $BrCH_2CO_2Bu^t$, $NaHCO_3$, dmf; (*vi*) trifluoroacetic acid; (*vii*) cation-, anion-exchange chromatography (see text)

4 H, aryl), 3.61 (s, 4 H, $HOC_6H_4CH_2NC\mathit{H}_2CO_2$), 3.11 (s, 4 H, $HOC_6H_4C\mathit{H}_2N$), 3.01 (s, 2 H, NCH_2CO_2), 2.61 (s, 8 H, CH_2CH_2); δ_C(D_2O, pH 13, CD_3CN) 182.13(2) (C=O), 181.88 (C=O), 166.82, 166.71, 133.72, 131.62, 128.54, 121.36, 61.21, 60.98, 57.34, 53.75, 53.32; *m*/*z* (FAB, thioglycerol–glycerol) 490 ($M^+ + 1$).

Determination of Protonation and Formation Constants.—Formation constants were determined in 0.5 mol dm^{-3} $NaNO_3$ at 25 °C by standard potentiometric techniques described previously.[11] Stock solutions of the metal nitrates were prepared and standardized by usual methods, and used to prepare the solutions for the stability-constant studies. The stock solution of $Bi(NO_3)_3$ had to be prepared in 0.5 mol dm^{-3} HNO_3 to avoid hydrolysis. Titrations were carried out in a cell thermostatted to 25 °C, with nitrogen bubbled through the solution to exclude CO_2. The pH values were recorded on a Beckman PHI 72 pH meter. The glass electrode system was calibrated by measuring potential *versus* calculated pH in titrations with standard acid and base in the pH range 2–12. The potentiometric data were analysed using the program ESTA,[12] considering all probable species such as ML, MLH, MLH_2 and MLOH. The first two protonation constants of the ligand, corresponding to protonation of the hydroxybenzyl oxygens, are rather high, and therefore difficult to determine reliably by glass-electrode measurements alone. This is because the calculated values of the extent of protonation of the ligand are very sensitive to small errors in measured pH. A better approach is to record the UV/VIS spectra of the ligand as a function of pH. The UV/VIS spectra of the ligand solutions thus gives a more accurate measure of the extent of protonation of the ligand, and pH measurements are used only to indicate the pH values at which protonation occurred. The aromatic rings of H_5hbdtta give intense charge transfer bands at 233, 272 and 288 nm in the electronic spectra, which allow for accurate determination of pK_a values. Because the nitrate ion absorbs strongly in this region of the spectrum, the spectroscopic determination of the higher pK_a values of the ligand was carried out in 0.5 mol dm^{-3} NaCl rather than $NaNO_3$, which should not,[13,14] however, greatly alter the protonation constants, which were used for calculating the formation constants of the metal ions in 0.5 mol dm^{-3} $NaNO_3$. At one point it was thought that the ligand might, like H_5dtpa, complex the Na^+ ion strongly, and the protonation constants were determined in 0.1 mol dm^{-3} NaCl, but showed (Table 1) little sensitivity to the lower Na^+ concentration. The variation of the UV/VIS spectra of H_5hbdtta as a function of pH is shown in Fig. 1. The protonation constants were obtained from the variation of absorbance at the wavelengths of the peaks at 233, 272 and 288 nm, as a function of pH. A simple computer program was written that fitted calculated absorbance as a function of pH to the observed variation of absorbance with pH. There was some evidence from slow changes of the UV/VIS spectrum with time that at high pH the ligand underwent some kind of decomposition on standing for a few hours. For this reason fresh ligand solutions were prepared for all titrations. Analysis of the potentiometric data for the Cu^{II} complex with ESTA was unsatisfactory as the program indicated strong cross correlation between all of the constants determined. As an alternative, the variation of absorbance of the spectrum of the Cu^{II} complex at 275 nm as a function of pH was determined, which gave well defined inflections for the protonation–deprotonation equilibria of the complex. These constants were used as fixed values in ESTA to give a satisfactory value of log K_1. The latter problem with Cu^{II} appeared to be due to the low preferred co-ordination number of Cu^{II}, which left unco-ordinated groups available for protonation. This meant that protonated species of the complex ($[MLH]^{2-}$, $[MLH_2]^-$) were present up to high pH values, and the $[ML]^{3-}$ complex itself was formed only at high pH, where glass-electrode studies alone were unsatisfactory. In order to confirm the results of the potentiometric study of the Bi^{III}–H_5hbdtta system, the electronic spectrum of the Bi^{III} complex was recorded as a function of pH in the range 0–13, which allowed for the calculation of the formation constants shown in Table 1. The

Table 1 Protonation and formation constants of the ligand H_5hbdtta determined in this work[a]

pK_n	Equilibrium	log K
pK_1	$H^+ + L^{5-} \rightleftharpoons HL^{4-}$	12.46[b]
		12.51[c]
pK_2	$HL^{4-} + H^+ \rightleftharpoons H_2L^{3-}$	11.24[b]
		11.90[c]
pK_3	$H_2L^{3-} + H^+ \rightleftharpoons H_3L^{2-}$	9.27[b]
		9.43[c]
pK_4	$H_3L^{2-} + H^+ \rightleftharpoons H_4L^-$	7.42
		7.37[c]
pK_5	$H_4L^- + H^+ \rightleftharpoons H_5L$	4.22
pK_6	$H_5L + H^+ \rightleftharpoons H_6L^+$	2.46
Metal		
Ca^{II}	$M + L \rightleftharpoons ML$	7.94(9)
	$ML + H^+ \rightleftharpoons MLH$	10.68(8)
	$MLH + H^+ \rightleftharpoons MLH_2$	8.84(9)
Zn^{II}	$M + L \rightleftharpoons ML$	16.04(6)
	$ML + H^+ \rightleftharpoons MLH$	10.90(5)
	$MLH + H^+ \rightleftharpoons MLH_2$	8.31(4)
	$MLH_2 + H^+ \rightleftharpoons MLH_3$	5.28(4)
	$MLH_3 + H^+ \rightleftharpoons MLH_4$	3.96(5)
Cd^{II}	$M + L \rightleftharpoons ML$	19.76(7)
	$ML + H^+ \rightleftharpoons MLH$	9.39(6)
	$MLH + H^+ \rightleftharpoons MLH_2$	7.35(5)
	$MLH_2 + H^+ \rightleftharpoons MLH_3$	4.37(5)
Metal	Equilibrium	log K
Pb^{II}	$M + L \rightleftharpoons ML$	17.09(6)
	$ML + H^+ \rightleftharpoons MLH$	9.72(6)
	$MLH + H^+ \rightleftharpoons MLH_2$	8.36(9)
	$MLH_2 + H^+ \rightleftharpoons MLH_3$	5.51(9)
	$MLH_3 + H^+ \rightleftharpoons MLH_4$	2.96(16)
Bi^{III}	$M + L \rightleftharpoons ML$	27.76(4)
	$ML + H^+ \rightleftharpoons MLH$	8.11(5)
		7.95(5)[b]
	$MLH + H^+ \rightleftharpoons MLH_2$	7.19(5)
		7.0(1)[b]
	$MLH_2 + H^+ \rightleftharpoons MLH_3$	4.88(6)
		4.60(5)[b]
	$MLH_3 + H^+ \rightleftharpoons MLH_4$	3.77(6)
	$ML + OH \rightleftharpoons MLOH$	3.8(1)[b]
Cu^{II}	$M + L \rightleftharpoons ML$	21.9(1)
	$ML + H^+ \rightleftharpoons MLH$	10.6(1)[d]
	$MLH + H^+ \rightleftharpoons MLH_2$	7.4(1)[d]
	$MLH_2 + H^+ \rightleftharpoons MLH_3$	4.2(1)[d]
	$MLH_3 + H^+ \rightleftharpoons MLH_4$	2.0(1)[d]

[a] H_5L = H_5hbdtta. All work carried out at 25.0 ± 0.1 °C. Values obtained from potentiometric techniques with ionic strength of 0.5 mol dm^{-3} $NaNO_3$ unless otherwise stated. [b] Obtained from UV/VIS spectra, ionic strength 0.5 mol dm^{-3} NaCl. [c] Obtained from UV/VIS spectra, ionic strength 0.1 mol dm^{-3} NaCl. [d] Obtained from UV/VIS spectra, ionic strength 0.5 mol dm^{-3} $NaNO_3$.

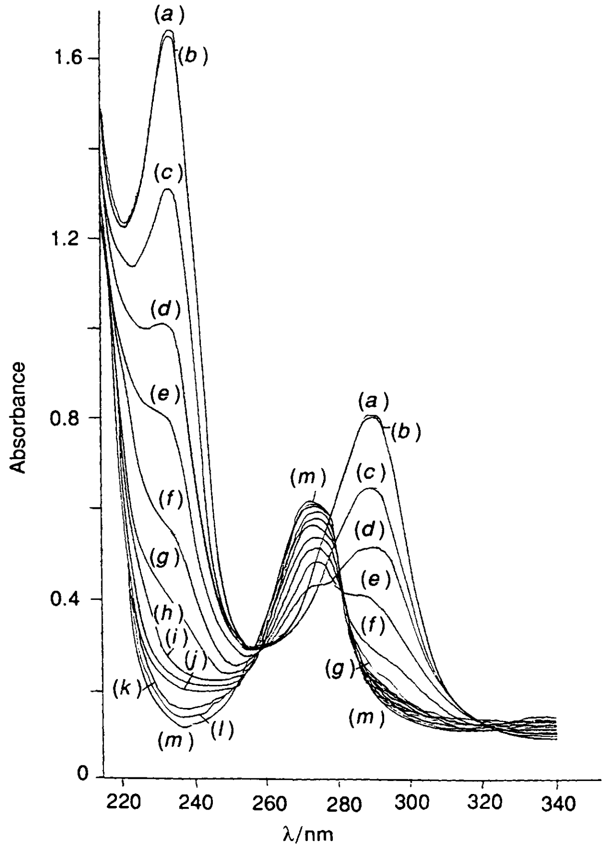


Fig. 1 The variation of the electronic spectrum of H_5hbdtta (2×10^{-4} mol dm^{-3}) as a function of pH, in 0.5 mol dm^{-3} NaCl. The pH values are (*a*) 13.03, (*b*) 12.76, (*c*) 12.42, (*d*) 11.88; (*e*) 11.52, (*f*) 11.05, (*g*) 10.52, (*h*) 10.18, (*i*) 9.16, (*j*) 8.07, (*k*) 6.56, (*l*) 1.71, (*m*) 0.32

agreement between the potentiometric and UV/VIS studies is reasonable considering the different media, and the fact that Bi^{III} forms moderately stable complexes with chloride ion. The electronic spectrum of the Bi^{III} complex reveals a $[MLOH]^{4-}$ complex above pH 10, which was not seen in the potentiometric titrations which were terminated at pH 10.

NMR Studies.—Because of the large number of protonation constants of the ligand, an NMR study of the protonation of the ligand was undertaken in order to elucidate the protonation scheme more completely, and provide confirmation of the glass electrode results. The NMR spectra of H_5hbdtta in D_2O were recorded on a Bruker 200 MHz NMR spectrometer as a function of pD. The pD of the solution was varied using DCl and NaOD.

Results and Discussion

The protonation constants and complex formation constants for H_5hbdtta with a variety of metal ions are given in Table 1, and the protonation constants of H_5hbdtta and H_4hbedda are compared in Table 2. The values of the protonation constant for H_5hbdtta measured here are reasonable in relation to those obtained for the similar H_4hbedda, and also the analogues H_4edta and H_5dtpa containing only carboxylate groups in addition to the nitrogen donors. The variation of ^1H NMR shifts for aminocarboxylate ligands has[16] proved useful in assigning protonation sites on the ligands. The variation of the ^1H resonances for H_5hbdtta in D_2O as a function of apparent pH is seen in Fig. 2, where apparent pH in D_2O is the pH measured using a glass electrode previously standardized in H_2O. Protonation constants measured by this technique are usually[16] about 0.4 log units higher than values obtained by conventional techniques in H_2O. The shifts of the aromatic protons as pD changes in the pH range above 10 are larger than those below pH 10 which is consistent with the first two protonation constants above pH 10 referring to protonation of the hydroxybenzyl groups. Potentiometry indicates that three protons are added between pH 10 and 4, and it seems possible that these three protons add to the nitrogen donors of the ligand. However, the protons of the ethylene bridges of the ligand are only significantly shifted between pH 6 and 10, which suggests that the protonation constant at 4.22 may be protonation of a carboxylate. In support of this, the ^1H NMR resonances for the acetate methylene groups show a shift at about pH 4. There is little change in the resonances of the benzyl methylene groups at pH *ca.* 10, where potentiometry indicates a proton is added, but there is a large shift between pH 8 and 6, with little variation again between 6 and 4. The shifts of the methylene protons of the benzyl groups are in accord with the first proton adding to the central nitrogen donor of the three nitrogens, which is consistent with the two outer nitrogens being rendered less accessible to protonation by hydrogen-bonding with the hydroxyls of the benzyl groups. The small shifts of the benzyl methylene groups between pH 4 and 6 are consistent with the protonation constant at pH 4.22 referring to protonation of a carboxylate rather than a nitrogen donor. The ^1H NMR shifts below pH 4 are small for all the resonances observed, so it is not clear whether the protonation constant at 2.46 refers to protonation of the third nitrogen donor, or a carboxylate group. The inferred protonation scheme for H_5hbdtta is shown in Scheme 2.

Table 2 A comparison of stability constants of the hydroxybenzyl-containing ligands H_4hbedda and H_5hbdtta and their analogues H_4edta and H_5dtpa, which contain only carboxylate groups

log *K*[a]	H_4hbedda	H_5hbdtta	H_4edta	H_5dtpa
$H^+ + L \rightleftharpoons HL$	12.53	12.46	10.23	9.9
$HL + H^+ \rightleftharpoons H_2L$	11.00	11.24	6.14	8.32
$H_2L + H^+ \rightleftharpoons H_3L$	8.38	9.27	2.69	4.10
$H_3L + H^+ \rightleftharpoons H_4L$	4.68	7.42	2.00	2.7
$H_4L + H^+ \rightleftharpoons H_5L$	2.5	4.22	1.5	2.1
$H_5L + H^+ \rightleftharpoons H_6L$		2.46		1.5
$Ca^{2+} + L \rightleftharpoons CaL$	9.29	7.94	10.61	10.75
$CaL + H^+ \rightleftharpoons CaHL$	8.69	10.68	3.18	6.11
$Cu^{2+} + L \rightleftharpoons CuL$	21.38	21.9	18.70	21.38
$CuL + H^+ \rightleftharpoons CuHL$	8.63	10.6	3.0	4.81
$Zn^{2+} + L \rightleftharpoons ZnL$	18.37	16.04	16.44	18.29
$Cd^{2+} + L \rightleftharpoons CdL$	17.52	19.76	16.36	19.0
$Pb^{2+} + L \rightleftharpoons PbL$	18.24	17.09	17.88	18.66
$Bi^{3+} + L \rightleftharpoons BiL$	—	27.76	27.8	35.6
$Fe^{3+} + L \rightleftharpoons FeL$	39.01	30.44[b]	25.1	28.0

[a] Constants for H_5hbdtta are from this work, other values from ref. 14. Constants for H_5hbdtta at ionic strength 0.5, other ligands at ionic strength 0.1 mol dm^{-3}. H_4hbedda = H_4L, H_5hbdtta = H_5L, H_4edta = H_4L and H_5dtpa = H_5L. [b] Ref. 15.

Table 2 shows that the ligand H_5hbdtta does not produce complexes of significantly greater thermodynamic stability than does H_4hbedda itself. In fact, even H_4edta, which lacks the highly basic hydroxybenzyl groups, complexes most metal ions at least as well as H_5hbdtta. The stability of the H_5hbdtta complex with Bi^{III} is a particular disappointment, being much lower than that for H_5dtpa. One should attempt to understand why this should be so. An important factor is the size of the chelate rings. The ligand H_5hbdtta, containing the hydroxybenzyl group, forms six-membered chelate rings, compared to the five-membered chelate rings formed with the carboxylate group. The importance of chelate-ring size in ligand design has been outlined previously.[1,17] Briefly, smaller metal ions co-ordinate with less steric strain when forming six-membered chelate rings while larger metal ions co-ordinate with less steric

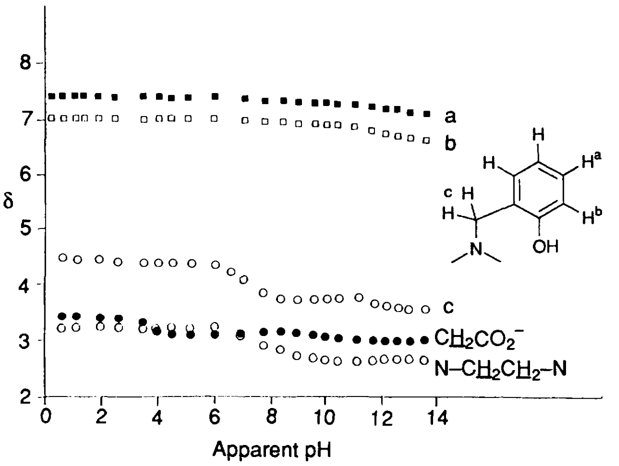
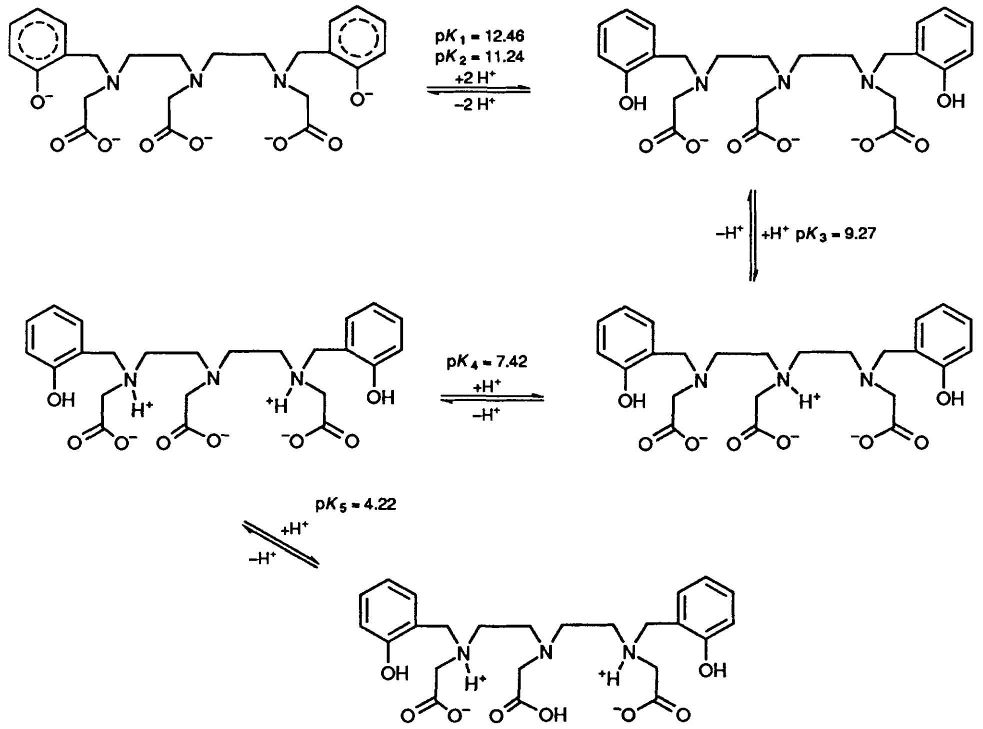


Fig. 2 ^1H NMR shifts for H_5hbdtta in D_2O as a function of apparent pH. To avoid cluttering only the shifts of representative peaks are shown



Scheme 2 Protonation scheme for H_5hbdtta

strain when forming five-membered chelate rings. Thus, the six-membered chelate ring formed by the hydroxybenzyl group and the nitrogen to which it is attached will strongly disfavour complexation of large metal ions. The ligand H_4hbedda is well suited for complexing the small Fe^{III} and Ga^{III} ions (ionic radii[9] about 0.55 Å).[5] Both Fe^{III} and Ga^{III} are very acidic, and so benefit from the greater basicity of the hydroxybenzyl oxygen compared to the less basic carboxylate, which raises log K_1 for the H_4hbedda complexes of Fe^{III} and Ga^{III} relative to the H_4edta complexes. As Fe^{III} and Ga^{III} are small ions they co-ordinate with low steric strain, forming six-membered chelate rings with H_4hbedda. As a result, H_4hbedda is an excellent ligand for co-ordinating small highly acidic metal ions such as Fe^{III}, and as seen in Table 2 log K_1 for the Fe^{III} complex of H_4hbedda is some 14 log units larger than for H_4edta. On the other hand, a large metal ion (Table 2) such as Ca^{II} shows a decrease in complex stability in passing from H_4edta to H_4hbedda, as the formation of six-membered chelate rings causes steric strain, and large metal ions of intermediate acidity such as Pb^{II} and Cd^{II} show only a small increase in log K_1 for H_4hbedda relative to H_4edta.

H_5hbdtta is what one might refer to as a contradiction in ligand design terms. The high basicity of the oxygen of the hydroxybenzyl groups favours co-ordination with small, highly acidic metal ions. On the other hand, as the ligand is octadentate, it prefers a large metal ion. These contradictory requirements mean that ultimately H_5hbdtta does not co-ordinate particularly well with any of the metal ions studied, whatever their size. The Bi^{III} result is somewhat surprising as Bi^{III}, with a log K_1 with hydroxide of 13.4,[14] is highly acidic, however the value of log K_1 with H_5hbdtta is not much higher than that with H_4edta, and is considerably lower than that with H_5dtpa. One must conclude that the large size of Bi^{III}, with an ionic radius of 1.03 Å,[9] is such that the steric strain caused by the presence of six-membered chelate rings in the H_5hbdtta complex cancels out any anticipated benefit from the more basic hydroxybenzyl oxygens.

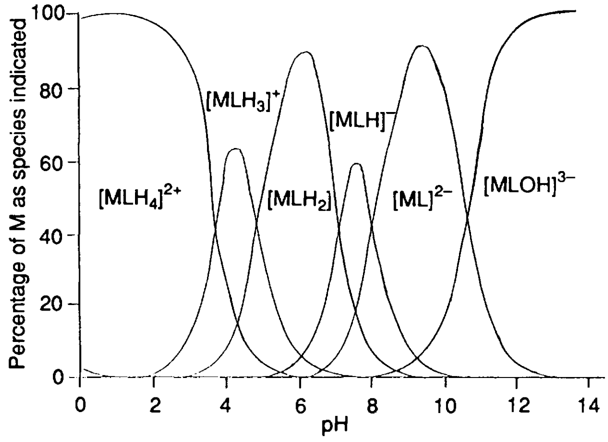


Fig. 3 Species distribution diagram as a function of pH for the complex of Bi^{III} with H_5hbdtta. The species distribution was calculated for 10^{-3} mol dm^{-3} total bismuth, and 2×10^{-3} mol dm^{-3} total H_5hbdtta. The constants used in the calculation are those reported in this paper in 0.5 mol dm^{-3} $NaNO_3$, except for the constant for the $[MLOH]^{3-}$ species, which was obtained by UV/VIS spectroscopy in 0.5 mol dm^{-3} NaCl

A further drawback of the hydroxybenzyl groups of H_5hbdtta, and also H_4hbedda, is the fact that the protons on these groups are tightly held, and increased log K_1 values for metal ions must be weighed against the energy expenditure necessary to deprotonate the ligand. As seen in Fig. 3, the dominant species of H_5hbdtta complexes of Bi^{III} at biological pH is the $[MLH_2]$ species. The magnitude of the protonation constants of the Bi–L complex suggest that in the $[MLH_2]$ complex the hydroxybenzyl oxygens may in fact still be protonated, and not contributing to complexation of bismuth at biological pH at all. The same arguments should apply to the other metal ions studied here as well, so that at biological pH the ligand H_5hbdtta is of limited use in complexing them.

The other ligand containing a hydroxybenzyl group that has been widely studied is (2-hydroxybenzyl)iminodiacetic acid (H_3hbida). A comparison of its log K_1 values with those of its analogue containing only carboxylate groups, nitrilotriacetic acid (H_3nta) illustrates the effect of the hydroxybenzyl groups on complex stability and metal-ion selectivity very well, as seen in Table 3. Table 3 shows clearly how large metal ions of low acidity such as Ba^{II} suffer a drop in complex stability with hbida. In contrast, very small highly acidic metal ions such as Fe^{III} show large increases in log K_1 when carboxylates are substituted by hydroxybenzyl groups.

In conclusion, ligands containing hydroxybenzyl groups are effective when used for small highly acidic metal ions. Ligands with high co-ordination numbers should thus be avoided, as these co-ordinate larger metal ions. Hydroxybenzyl groups do not appear to be effective even with highly acidic metal ions such as Bi^{III} if the metal ion is too large. A better approach for complexing large highly acidic metal ions may involve the use of hydroxyphenyl group as seen in the ligand *N,N'*-bis(2-hydroxyphenyl)ethylenediamine-*N,N'*-diacetic acid (H_4hpedda) reported[18] recently. Forming only five-membered chelate rings, this ligand shows[18] a distinct preference for larger metal ions such as Ca^{II} and Gd^{III} compared to the H_4hbedda complex, and an analogue of H_4hpedda with three nitrogens rather than two might prove to be ideal for complexing Bi^{III}.

Table 3 The difference in log K_1 (Δ log K_1) between complexes of H_3nta and H_3hbida in relation to metal-ion radius and metal-ion acidity[a]

	log K_1				
Metal ion[b]	nta	hbida	Δ log K_1	Ionic radius/Å	log K_1 (OH^-)
Ba^{II}	4.80	4.40	−0.40	1.36	0.6
Sr^{II}	4.97	4.99	+0.02	1.17	0.9
La^{III}	10.47	11.57	+1.10	1.03	5.3
Ca^{II}	6.37	6.74	+0.37	1.00	1.19
Mg^{II}	5.47	7.28	+1.81	0.72	2.62
Cu^{II}	12.94	16.11	+3.17	0.57	6.7
Fe^{III}	15.9	22.4	+6.5	0.55	11.8

[a] Formation-constant data from ref. 7, ionic radii from ref. 9. [b] Placed in order of decreasing ionic radius to show the response of complex stability to ionic radius when a five-membered chelate ring involving a carboxylate group is replaced with a six-membered chelate ring involving a hydroxybenzyl group. There is also a strong response to the acidity of the metal ion, indicated here by log $K_1(OH^-)$ for each metal ion where $M^{n+} + OH^- \rightleftharpoons MLOH^{(n-1)+}$.

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References

1 R. D. Hancock and A. E. Martell, *Chem. Rev.*, 1989, **89**, 1875.
2 S. Jurisson, D. Berning, W. Jia and D. Ma, *Chem. Rev.*, 1993, **93**, 1137.
3 R. A. Bulman, *Struct. Bonding* (*Berlin*), 1987, **76**, 91.
4 R. W. Kozak, T. A. Waldeman, R. W. Atcher and O. A. Gansow, *Trends Biotechnol.*, 1985, **4**, 259.
5 A. E. Martell, R. J. Motekaitis, I. Murase, L. F. Sala, R. Stoldt, C. Y. Ng, H. Rosenkrantz and J. J. Metterville, *Inorg. Chim. Acta*, 1987, **138**, 215.
6 F. l'Eplattenier, I. Murase and A. E. Martell, *J. Am. Chem. Soc.*, 1967, **89**, 837.
7 W. R. Harris and A. E. Martell, *Inorg. Chem.*, 1976, **15**, 713.
8 C. H. Taliaferro, R. J. Motekaitis and A. E. Martell, *Inorg. Chem.*, 1984, **23**, 249.
9 R. D. Shannon, *Acta Crystallogr., Sect. A*, 1976, **32**, 751.
10 M. W. Brechbiel and O. A. Gansow, *J. Chem. Soc., Perkin Trans. 1*, 1992, 1173.
11 A. S. de Sousa, G. J. B. Croft, C. A. Wagner, J. P. Michael and R. D. Hancock, *Inorg. Chem.*, 1991, **30**, 3525.
12 P. M. May, K. Murray and D. R. Williams, ESTA program, *Talanta*, 1985, **32**, 483.
13 G. Biedermann and L. G. Sillen, *Ark. Kemi*, 1953, **5**, 425.
14 A. E. Martell and R. M. Smith, *Critical Stability Constants*, Plenum, New York, 1974–1989, vols. 1–6.
15 A. E. Martell, personal communication.
16 C. F. G. C. Geraldes, A. D. Sherry, P. M. Marques, M. C. Alpoim and S. Cortes, *J. Chem. Soc., Perkin Trans. 2*, 1991, 137.
17 R. D. Hancock, *J. Chem. Educ.*, 1992, **69**, 615.
18 J. F. Gibson and O. J. Vaughan, *J. Chem. Soc., Dalton Trans.*, 1992, 1375; R. Ma and A. E. Martell, *Inorg. Chim. Acta*, 1993, **209**, 71.

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