

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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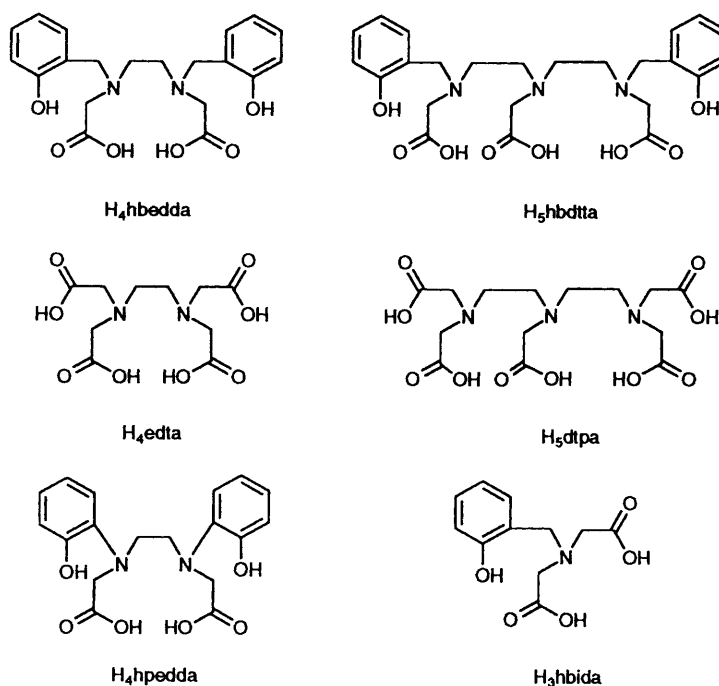
The ligand *N,N'*-bis(2-hydroxybenzyl)diethylenetriamine-*N,N',N''*-triacetic acid (H_5L) has been synthesised and the protonation constants for L determined by potentiometric methods in 0.5 mol dm⁻³ NaNO₃, and spectrophotometric methods in 0.5 and 0.1 mol dm⁻³ NaCl, all at 25 °C. The sites of protonation have been inferred from ¹H NMR studies in D₂O. 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 NaNO₃, 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 use⁴ 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 show¹ 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 larger⁹ than Fe^{III}, so that while $H_4hbedda$ gives six-coordination 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 (NaBH₄, EtOH) to generate the diamine **II** (89%). Subsequent reaction of the diamine **II** with BH₃·thf (thf = tetrahydrofuran) cleanly generated **III** (85%). Alkylation of **III** to yield the final product was performed as a modification of a previously reported procedure¹⁰ [BrCH₂CO₂Bu^t, NaHCO₃, 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 ¹³C NMR spectra were obtained using a Varian 300XL instrument. Chemical shifts (δ) are reported in ppm relative to tetramethylsilane, sodium 3-(trimethylsilyl)tetrauteriopropionate (tsp) or CD₃CN (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



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 = $0.05 \text{ mol dm}^{-3} \text{ NEt}_3\text{-CH}_3\text{CO}_2\text{H}$, pH 5.5, solvent B = methanol) at $1.0 \text{ cm}^3 \text{ min}^{-1}$ was used for all analyses.

1,9-Bis(2-hydroxyphenyl)-4-oxo-2,5,8-triaza-nonane-1,8-diene

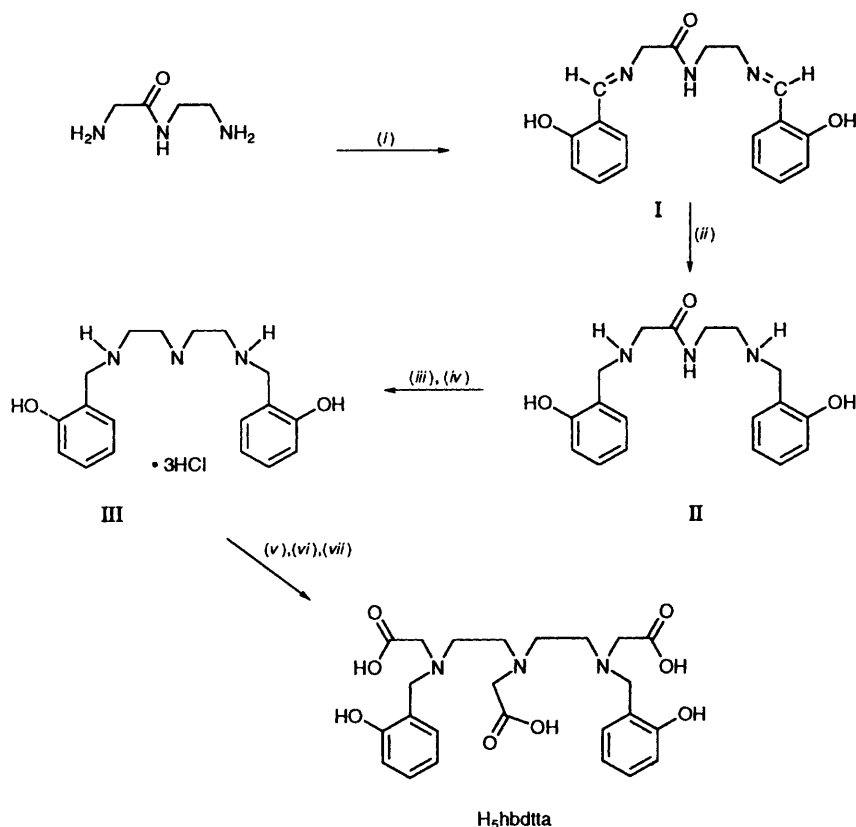
I. Salicylaldehyde (9.28 g, 76 mmol) was added directly to a solution of *N*-(2-aminoethyl)glycinamide (4.44 g, 38 mmol) in 100% ethanol (50 cm^3). After *ca.* 5 h the product precipitated and the suspension was stirred for an additional hour. The yellow solid was collected and dried at 0.1 mmHg for 24 h to give the diimine (11.86 g, 96%) (Found: C, 66.35; H, 5.90; N, 12.90. $\text{C}_{18}\text{H}_{19}\text{N}_3\text{O}_3$ requires C, 66.40; H, 5.90; N, 12.90%). $\delta_{\text{H}}(\text{CDCl}_3)$ 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, NHCH_2), 3.63 (q, 2 H, J 12.0, 6.0, CH_2N); $\delta_{\text{C}}(\text{CDCl}_3)$ 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-triaza-nonane 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 ($3 \times 100 \text{ cm}^3$). The combined organic extracts were washed with 5% NaHCO_3 ($2 \times 200 \text{ cm}^3$) 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. $\text{C}_{18}\text{H}_{23}\text{N}_3\text{O}_3$ requires C, 65.60; H, 7.05; N, 12.75%). $\delta_{\text{H}}[(\text{CD}_3)_2\text{SO}]$ 7.92 (br t, 1 H, NH), 7.06 (m, 4 H, aryl), 6.72 (m, 4 H, aryl), 3.89 (s, 2 H, CH_2NH), 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); $\delta_{\text{C}}(\text{CDCl}_3)$ 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-triaza-nonane trihydrochloride III. The amide **II** (7.0 g, 21.3 mmol) was dissolved in thf (100 cm^3), cooled (ice bath), and $1 \text{ mol dm}^{-3} \text{ BH}_3\text{-thf}$ (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. $\text{C}_{18}\text{H}_{25}\text{N}_3\text{O}_2\cdot 3\text{HCl}$ requires C, 50.90; H, 6.65; N, 9.90%). $\delta_{\text{H}}(\text{D}_2\text{O}, \text{pH } 1)$ 7.04–6.97 (m, 4 H, aryl), 4.34 (s, 4 H, CH_2NH), 3.57 (m, 8 H, CH_2CH_2); $\delta_{\text{C}}(\text{D}_2\text{O}, \text{pH } 1, \text{tsp})$ 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''-tri-acetic acid (H₅hbdtta). 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 ($3 \times 100 \text{ cm}^3$). 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 $3 \text{ mol dm}^{-3} \text{ NH}_4\text{OH}$ (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, $\text{CH}_3\text{CO}_2\text{H}$ form, 1.6×30 cm), and eluted from the column with a 0.0–1.5 $\text{mol dm}^{-3} \text{ CH}_3\text{CO}_2\text{H}$ gradient (2 dm^3). The eluent was collected in 88×150 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. $\text{C}_{24}\text{H}_{31}\text{N}_3\text{O}_8$ requires C, 58.85; H, 6.40; N, 8.60%). $\delta_{\text{H}}(\text{D}_2\text{O}, \text{pH } 12)$ 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 H₅hbdtta. (i) Salicylaldehyde, ethanol; (ii) NaBH₄, ethanol; (iii) BH₃-thf; (iv) HCl(g), ethanol; (v) BrCH₂CO₂Bu^t, NaHCO₃, dmf; (vi) trifluoroacetic acid; (vii) cation-, anion-exchange chromatography (see text)

4 H, aryl), 3.61 (s, 4 H, HOC₆H₄CH₂NCH₂CO₂), 3.11 (s, 4 H, HOC₆H₄CH₂N), 3.01 (s, 2 H, NCH₂CO₂), 2.61 (s, 8 H, CH₂CH₂); δ_c (D₂O, pH 13, CD₃CN) 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 NaNO₃ 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(NO₃)₃ had to be prepared in 0.5 mol dm⁻³ HNO₃ to avoid hydrolysis. Titrations were carried out in a cell thermostatted to 25 °C, with nitrogen bubbled through the solution to exclude CO₂. 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, MLH₂ 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 H₅hbdtta 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 NaNO₃, 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 NaNO₃. At one point it was thought that the ligand might, like H₅dtpa, 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 H₅hbdtta 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 K₁. 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⁻, [MLH₂]⁻) 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}-H₅hbdtta 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 H₅hbdtta determined in this work^a

pK _n	Equilibrium	log K	Metal	Equilibrium	log K
pK ₁	H ⁺ + L ⁵⁻ ⇌ HL ⁴⁻	12.46 ^b	Pb ^{II}	M + L ⇌ ML	17.09(6)
		12.51 ^c		ML + H ⁺ ⇌ MLH	9.72(6)
pK ₂	HL ⁴⁻ + H ⁺ ⇌ H ₂ L ³⁻	11.24 ^b		MLH + H ⁺ ⇌ MLH ₂	8.36(9)
		11.90 ^c		MLH ₂ + H ⁺ ⇌ MLH ₃	5.51(9)
pK ₃	H ₂ L ³⁻ + H ⁺ ⇌ H ₃ L ²⁻	9.27 ^b	Bi ^{III}	MLH ₃ + H ⁺ ⇌ MLH ₄	2.96(16)
		9.43 ^c		M + L ⇌ ML	27.76(4)
pK ₄	H ₃ L ²⁻ + H ⁺ ⇌ H ₄ L ⁻	7.42		ML + H ⁺ ⇌ MLH	8.11(5)
		7.37 ^c			7.95(5) ^b
pK ₅	H ₄ L ⁻ + H ⁺ ⇌ H ₅ L	4.22		MLH + H ⁺ ⇌ MLH ₂	7.19(5)
pK ₆	H ₅ L + H ⁺ ⇌ H ₆ L ⁺	2.46			7.0(1) ^b
Metal				MLH ₂ + H ⁺ ⇌ MLH ₃	4.88(6)
Ca ^{II}	M + L ⇌ ML	7.94(9)			4.60(5) ^b
	ML + H ⁺ ⇌ MLH	10.68(8)		MLH ₃ + H ⁺ ⇌ MLH ₄	3.77(6)
	MLH + H ⁺ ⇌ MLH ₂	8.84(9)		ML + OH ⇌ MLOH	3.8(1) ^b
Zn ^{II}	M + L ⇌ ML	16.04(6)	Cu ^{II}	M + L ⇌ ML	21.9(1)
	ML + H ⁺ ⇌ MLH	10.90(5)		ML + H ⁺ ⇌ MLH	10.6(1) ^d
	MLH + H ⁺ ⇌ MLH ₂	8.31(4)		MLH + H ⁺ ⇌ MLH ₂	7.4(1) ^d
	MLH ₂ + H ⁺ ⇌ MLH ₃	5.28(4)		MLH ₂ + H ⁺ ⇌ MLH ₃	4.2(1) ^d
	MLH ₃ + H ⁺ ⇌ MLH ₄	3.96(5)		MLH ₃ + H ⁺ ⇌ MLH ₄	2.0(1) ^d
Cd ^{II}	M + L ⇌ ML	19.76(7)			
	ML + H ⁺ ⇌ MLH	9.39(6)			
	MLH + H ⁺ ⇌ MLH ₂	7.35(5)			
	MLH ₂ + H ⁺ ⇌ MLH ₃	4.37(5)			

^a H₅L = H₅hbdtta. All work carried out at 25.0 ± 0.1 °C. Values obtained from potentiometric techniques with ionic strength of 0.5 mol dm⁻³ NaNO₃ 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 NaNO₃.

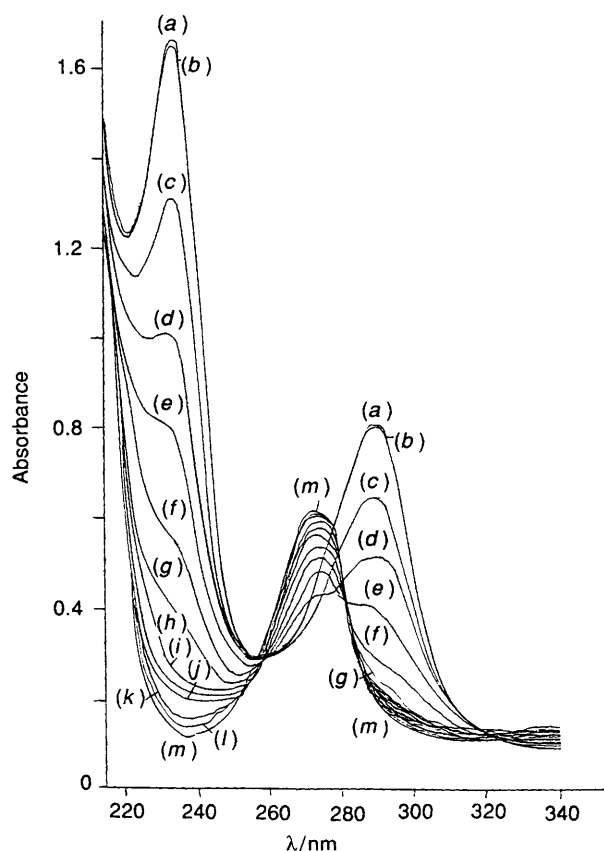


Fig. 1 The variation of the electronic spectrum of H₅hbdtta (2 × 10⁻⁴ mol dm⁻³) as a function of pH, in 0.5 mol dm⁻³ NaCl. The pH values are (a) 13.03, (b) 12.76, (c) 12.42, (d) 11.88; (e) 11.52, (f) 11.05, (g) 10.52, (h) 10.18, (i) 9.16, (j) 8.07, (k) 6.56, (l) 1.71, (m) 0.32

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 H₅hbdtta in D₂O 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 H₅hbdtta with a variety of metal ions are given in Table 1, and the protonation constants of H₅hbdtta and H₄hbedda are compared in Table 2. The values of the protonation constant for H₅hbdtta measured here are reasonable in relation to those obtained for the similar H₄hbedda, and also the analogues H₄edta and H₅dtpa containing only carboxylate groups in addition to the nitrogen donors. The variation of ¹H NMR shifts for aminocarboxylate ligands has¹⁶ proved useful in assigning protonation sites on the ligands. The variation of the ¹H resonances for H₅hbdtta in D₂O as a function of apparent pH is seen in Fig. 2, where apparent pH in D₂O is the pH measured using a glass electrode previously standardized in H₂O. Protonation constants measured by this technique are usually¹⁶ about 0.4 log units higher than values obtained by conventional techniques in H₂O. 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 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

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
$\text{H}^+ + \text{L} \rightleftharpoons \text{HL}$	12.53	12.46	10.23	9.9
$\text{HL} + \text{H}^+ \rightleftharpoons \text{H}_2\text{L}$	11.00	11.24	6.14	8.32
$\text{H}_2\text{L} + \text{H}^+ \rightleftharpoons \text{H}_3\text{L}$	8.38	9.27	2.69	4.10
$\text{H}_3\text{L} + \text{H}^+ \rightleftharpoons \text{H}_4\text{L}$	4.68	7.42	2.00	2.7
$\text{H}_4\text{L} + \text{H}^+ \rightleftharpoons \text{H}_5\text{L}$	2.5	4.22	1.5	2.1
$\text{H}_5\text{L} + \text{H}^+ \rightleftharpoons \text{H}_6\text{L}$	—	2.46	—	1.5
$\text{Ca}^{2+} + \text{L} \rightleftharpoons \text{CaL}$	9.29	7.94	10.61	10.75
$\text{CaL} + \text{H}^+ \rightleftharpoons \text{CaHL}$	8.69	10.68	3.18	6.11
$\text{Cu}^{2+} + \text{L} \rightleftharpoons \text{CuL}$	21.38	21.9	18.70	21.38
$\text{CuL} + \text{H}^+ \rightleftharpoons \text{CuHL}$	8.63	10.6	3.0	4.81
$\text{Zn}^{2+} + \text{L} \rightleftharpoons \text{ZnL}$	18.37	16.04	16.44	18.29
$\text{Cd}^{2+} + \text{L} \rightleftharpoons \text{CdL}$	17.52	19.76	16.36	19.0
$\text{Pb}^{2+} + \text{L} \rightleftharpoons \text{PbL}$	18.24	17.09	17.88	18.66
$\text{Bi}^{3+} + \text{L} \rightleftharpoons \text{BiL}$	—	27.76	27.8	35.6
$\text{Fe}^{3+} + \text{L} \rightleftharpoons \text{FeL}$	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. $\text{H}_4\text{hbedda} = \text{H}_4\text{L}$, $\text{H}_5\text{hbdtta} = \text{H}_5\text{L}$, $\text{H}_4\text{edta} = \text{H}_4\text{L}$ and $\text{H}_5\text{dtpa} = \text{H}_5\text{L}$. ^b Ref. 15.

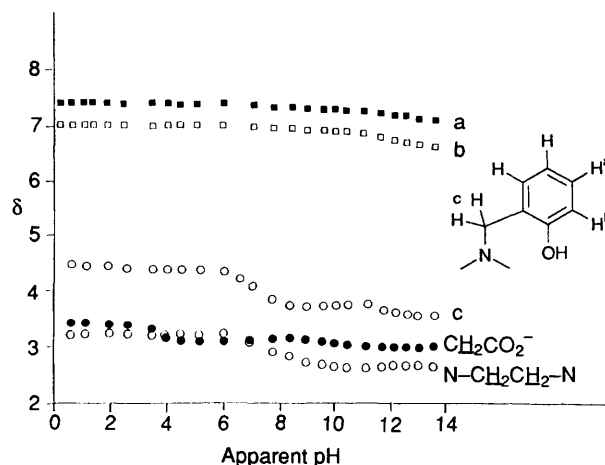
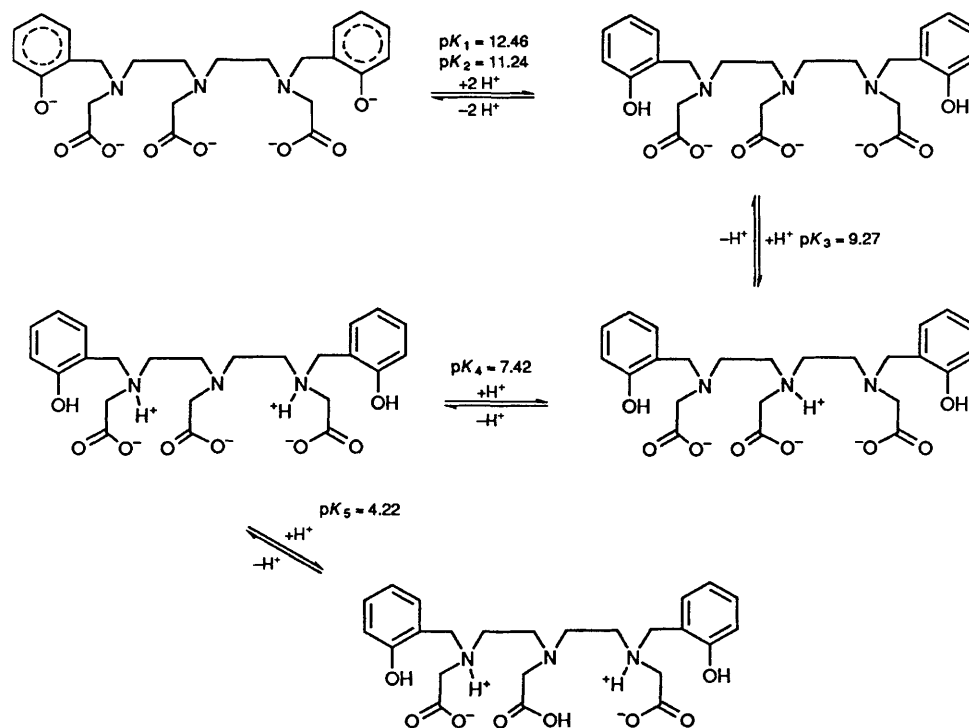


Fig. 2 ^1H NMR shifts for H_5hbdtta in D_2O as a function of apparent pH. To avoid cluttering only the shifts of representative peaks are shown



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 radii⁹ 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 .

$H_5hbdtda$ 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

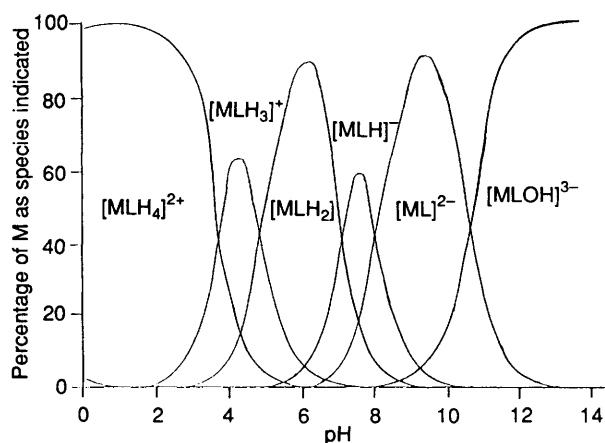


Fig. 3 Species distribution diagram as a function of pH for the complex of Bi^{III} with $H_5hbdtda$. The species distribution was calculated for 10^{-3} mol dm^{-3} total bismuth, and 2×10^{-3} mol dm^{-3} total $H_5hbdtda$. The constants used in the calculation are those reported in this paper in 0.5 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} $NaCl$.

octadentate, it prefers a large metal ion. These contradictory requirements mean that ultimately $H_5hbdtda$ 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 $H_5hbdtda$ 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 $H_5hbdtda$ complex cancels out any anticipated benefit from the more basic hydroxybenzyl oxygens.

A further drawback of the hydroxybenzyl groups of $H_5hbdtda$, 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 $H_5hbdtda$ 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 $H_5hbdtda$ 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 ($H_4hpdeda$) reported¹⁸ recently. Forming only five-membered chelate rings, this ligand shows¹⁸ a distinct preference for larger metal ions such as Ca^{II} and Gd^{III} compared to the $H_4hbedda$ complex, and an analogue of $H_4hpdeda$ with three nitrogens rather than two might prove to be ideal for complexing Bi^{III} .

Table 3 The difference in $\log K_1$ ($\Delta \log K_1$) between complexes of H_3nta and H_3hbida in relation to metal-ion radius and metal-ion acidity^a

Metal ion ^b	$\log K_1$		$\Delta \log K_1$	Ionic radius/Å	$\log K_1(OH^-)$
	nta	hbida			
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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