DALTON
FULL PAPER

Novel optically-active bis(amino acid) ligands and their complexation with gadolinium

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A condensation reaction of glyoxal with (*S*)-histidine and (*S*)-aspartic acid yielded a new optically-active bis(amino acid) ligand, *N*-[(*S*)-1-carboxy-2-(imidazol-4-yl)ethyl]-*N*′-[(*S*)-1,2-dicarboxyethyl]ethylenediamine. Two bis(amino acid) ligands with picolyl (pyridylmethyl) groups were synthesized by condensation reactions of glyoxal with the picolyl derivative of (*S*)-histidine and that of (*S*)-aspartic acid: the obtained ligands are *N,N*′-bis(2-pyridylmethyl)-*N*-[(*S*)-1-carboxy-2-(imidazol-4-yl)ethyl]-*N*′-[(*S*)-1,2-dicarboxyethyl]ethylenediamine and *N,N*′-bis(2-pyridylmethyl)-*N,N*′-bis[(*S*)-1-carboxy-2-(imidazol-4-yl)ethyl]ethylenediamine. The protonation constants of these ligands were determined by potentiometry, and the corresponding protonation sites were located on the basis of ^{1}H NMR spectra obtained at different pD values. The formation constants of the Gd^{3+} complexes were determined by potentiometric titrations, and the NMR relaxivities r_1 and r_2 by the measurements of the NMR relaxation times.

Introduction

Synthesis of new lanthanide metal chelates is one of the most attractive research fields in biomedical and bioinorganic chemistry, because of their possible applications based on their magnetic and optical properties. One of the important applications is the use of gadolinium(III) chelates as contrast enhancers in magnetic resonance imaging (MRI).[1–4] Gadolinium(III) complexes currently used for routine clinical diagnosis are gadolinium(III) diethylenetriaminepentaacetate (DTPA), its bis(methyl amide) derivative (Gd–DTPABMA) and macrocyclic Gd^{3+} chelates, Gd–DOTA and Gd–HP-DO3A (DOTA = 1,4,7,10-tetraazacyclododecane-*N,N*′,*N*″,*N*‴-tetraacetic acid; HP-DO3A = 10-(hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid). Extensive efforts have been made to develop MRI agents that have specific functions such as tissue selectivity; as a part of such efforts, a variety of derivatives of DTPA, DOTA and related ligands have been synthesized, and their complexation with Gd^{3+} ions has been studied.[4] It has been reported that, when a chiral centre is introduced to DTPA or DOTA, the resulting optically-active Gd^{3+} complexes have pharmacodynamics different from that of the parent Gd^{3+} complexes and some of them show tissue selectivity.[5–8] In this paper, we report that a new type of acyclic ligand that has two chiral centres has been synthesized from optically pure (*S*)-aspartic acid, (*S*)-histidine and their picolyl (pyridylmethyl) derivatives: the ligands obtained are *N*-[(*S*)-1-carboxy-2-(imidazol-4-yl)ethyl]-*N*′-[(*S*)-1,2-dicarboxyethyl]ethylenediamine [Scheme 1; abbreviated as H_3(**L1**) with acidic protons], *N,N*′-bis(2-pyridylmethyl)-*N*-[(*S*)-1-carboxy-2-(imidazol-4-yl)-ethyl]-*N*′-[(*S*)-1,2-dicarboxyethyl]ethylenediamine [H_3(**L2**) in Scheme 1] and *N,N*′-bis(2-pyridylmethyl)-*N,N*′-bis[(*S*)-1-carboxy-2-(imidazol-4-yl)ethyl]ethylenediamine [H_2(**L3**) in Scheme 1]. These ligands form non-ionic and cationic gadolinium(III) chelates. Their stability constants have been determined by potentiometric titrations. The NMR relaxivities also have been determined at 300 MHz.

Experimental

Materials

All chemicals used (including amino acid esters) were commercially available and used without further purification.

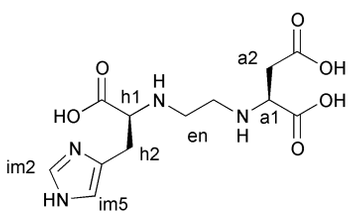
Syntheses

***N*-[(*S*)-1-Carboxy-2-(imidazol-4-yl)ethyl]-*N*′-[(*S*)-1,2-dicarboxyethyl]ethylenediamine H_3(L1).** The synthetic method reported previously for the precursor[9] (**1** in Scheme 2) of H_3(**L1**) was modified to enhance the yield, as follows. To a mixture of (*S*)-histidine methyl ester dihydrochloride (2.42 g, 10.0 mmol) and (*S*)-aspartic acid α,β-dimethyl ester hydrochloride (2.96 g, 15.0 mmol) in methanol (80 cm^3), a 40% solution of glyoxal in water (1.74 g, 1.37 ml, 12.0 mmol) was added dropwise at 0 °C. After the resulting mixture was stirred for 1 h, solid Na_2CO_3 (0.69 g, 5.0 mmol) was added, and stirring was continued until the solid was dissolved. Then solid $NaBH_3CN$ (1.50 g, 24.0 mmol) was added, and the solution was stirred for 5 h during which the temperature was raised gradually to room temperature. The reaction mixture was poured over chloroform and washed with a saturated Na_2CO_3 solution. The organic phase was separated and dried over anhydrous Na_2SO_4, and the solvent was removed by evaporation at a temperature below 30 °C. The trimethyl ester **1** of H_3(**L1**) was isolated by chromatography on silica gel with a $CHCl_3$/CH_3OH (15/1) eluent (yield: 1.14 g, 3.20 mmol, 32%).

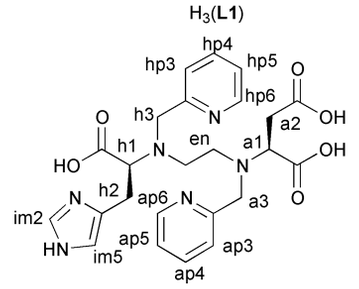
This triester (1.09 g, 3.06 mmol) was allowed to hydrolyze with $LiOH·H_2O$ (0.385 g, 9.18 mmol) in 50% methanol (80 cm^3) for 3.5 h. Addition of 1 M HCl (9.2 cm^3) to the solution gave H_3(**L1**) as a colourless solid, which was washed with a small amount of methanol and diethyl ether and dried *in vacuo* at 40 °C for several hours (yield 0.44 g, 1.38 mmol) (Found: C, 45.12; H, 5.81; N, 17.40. $C_{12}H_{18}H_4O_6·0.3H_2O$ requires C, 45.08; H, 5.86; N, 17.52%); mp >200 °C. $[a]_D^{29}$: +33 (deg dm^{-1} g^{-1} cm^3, 1 M HCl). IR: ν_{max}/cm^{-1} 1589 (CO_2H). ^{1}H NMR (D_2O,

pD = 5.82): δ 2.70 (dd, 1H, J = 17.6, 8.5), 2.84 (dd, 1H, J = 17.6, 3.8), 3.11 (m, 1H), 3.20 (d, 2H, J = 6.1), 3.30 (m, 3H), 3.66 (t, 1H, J = 6.1), 3.84 (dd, 1H, J = 8.5, 3.8), 7.32 (s, 1H), 8.56 (d, 1H, J = 1.5). FAB MS: m/z 313 ([M − H]$^-$).

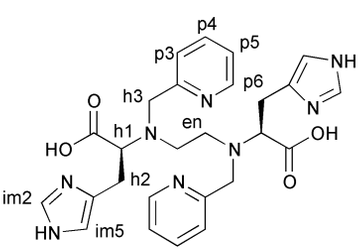
***N*-(2-Pyridylmethyl)-(*S*)-aspartic acid α,β-dimethyl ester dihydrochloride 2.** A mixture of (*S*)-aspartic acid α,β-dimethyl ester hydrochloride (9.88 g, 50.0 mmol) and 2-pyridinecarbaldehyde (5.35 g, 50.0 mmol) in methanol (100 cm^3) was stirred for 2 h at 0 °C. To the resulting solution, a methanol solution of $NaBH_3CN$ (3.49 g, 55.5 mmol in 10 cm^3) was added, and the mixture was stirred for 10 h. Any solid formed was removed by filtration, and the solvent of the filtrate was removed by evaporation under vacuum. The residue was dissolved in dichloromethane and washed with a saturated $NaHCO_3$ solution. The organic phase was separated and dried over anhydrous Na_2SO_4, and the solvent was evaporated under vacuum. The residue was dissolved in a small amount of methanol. Addition of 4 M HCl in ethyl acetate (KOKUSAN Chem. Co. Ltd.) (30 cm^3) to this solution gave a colourless solid, which was recrystallized from ethanol and washed with cold ethanol and dichloromethane (yield 9.33 g, 28.7 mmol, 57%); mp. 155–157 °C (Found: C, 43.95; H, 5.65; N, 8.62. $C_{12}H_{16}N_2O_4$·2HCl requires C, 44.32; H, 5.58; N, 8.66%). $[\alpha]_D^{24}$: +0.8 (deg dm^{-1} g^{-1} cm^3, CH_3OH). IR: ν_{max}/cm^{-1} 1749 (CO). ^{1}H NMR (D_2O, pD = 1.18): δ 3.25 (d, 2H, J = 5.4), 3.75 (s, 3H), 3.86 (s, 3H), 4.51 (t, 1H, J = 5.4), 4.66 (d, 1H, J = 15.0), 4.75 (d, 1H, J = 15.0), 8.00 (ddd, 1H, J = 8.0, 5.9, 1.3), 8.08 (d, 1H, J = 8.0), 8.53 (td, 1H, J = 8.0, 1.6), 8.82 (dd, 1H, J = 5.9, 0.9). FAB MS: m/z 253 ([M + H]$^+$).



H_3(**L1**)



H_3(**L2**)

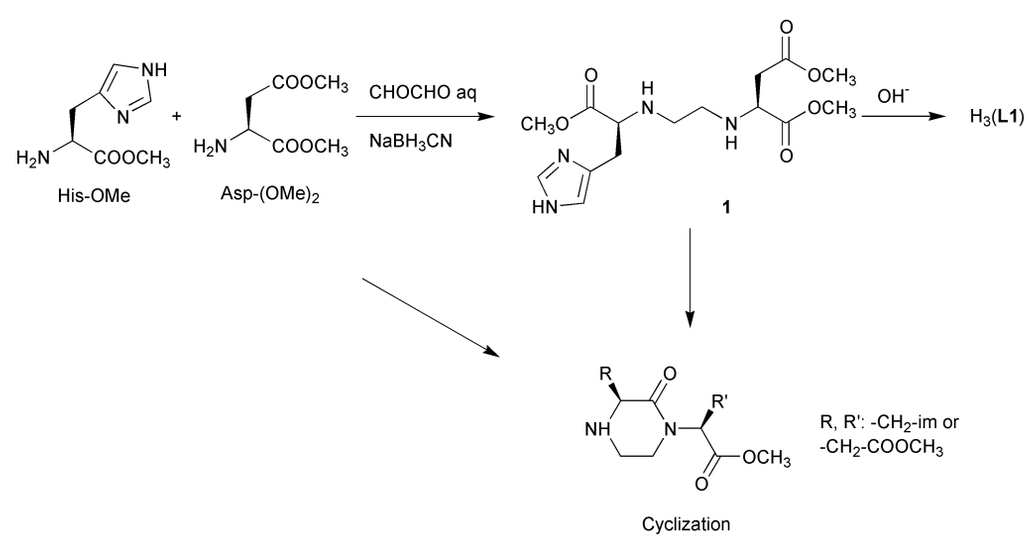


H_2(**L3**)

Scheme 1

***N*-(2,2-Dimethoxyethyl)-*N*-(2-pyridylmethyl)-(*S*)-histidine methyl ester 4.** *N*-(2-Pyridylmethyl)-(*S*)-histidine methyl ester trihydrochloride **3** was synthesized by the method reported in the literature.[10] To a mixture of this ester (6.00 g, 16.2 mmol) with 45% glyoxal dimethyl acetal (11.26 g, 48.7 mmol) and K_2CO_3 (2.24 g, 16.2 mmol) in methanol (50 cm^3), $NaBH_3CN$ (3.05 g, 48.7 mmol) was added slowly with stirring at 0 °C. Stirring was continued for 5 h at 35 °C. The reaction mixture was poured over chloroform, and washed with saturated $NaHCO_3$ solution. The chloroform phase was separated and dried over anhydrous Na_2SO_4, and the solvent was removed by evaporation. From the residue, the product **4** was isolated by the use of a silica gel column with a $CHCl_3/CH_3OH$ (45/1) eluent (yield 4.79 g, 13.7 mmol, 85%). IR: ν_{max}/cm^{-1} 1732 (CO). ^{1}H NMR ($CDCl_3$): δ 2.68 (dd, 1H, J = 14.4, 5.2), 2.93 (dd, 1H, J = 14.4, 5.2), 3.00 (dd, 1H, J = 15.4, 12.0), 3.05 (s, 3H), 3.06 (s, 3H), 3.13 (dd, 1H, J = 15.4, 3.2), 3.79 (s, 3H), 3.82 (dd, 1H, J = 12.0, 3.2), 3.95 (d, 1H, J = 14.9), 4.02 (t, 1H, J = 5.2), 4.21 (d, 1H, J = 14.9), 6.86 (s, 1H), 7.21 (d, 1H, J = 7.7), 7.26 (dd, 1H, J = 7.7, 4.1), 7.63 (s, 1H), 7.68 (td, 1H, J = 7.7, 1.7), 8.65 (d, 1H, J = 4.1). HRFAB MS: found m/z 349.1873; $C_{17}H_{25}N_4O_4$ ([M + H]$^+$) requires 349.1875.

***N*,*N*′-Bis(2-pyridylmethyl)-*N*-[(*S*)-1-carboxy-2-(imidazol-4-yl)ethyl]-*N*′-[(*S*)-1,2-dicarboxyethyl]ethylenediamine H_3(L2).** To **4** (4.79 g, 13.7 mmol) in dichloromethane (1 cm^3) was added 25% HBr/acetic acid (20 cm^3). The resulting oil was stirred for 15 min, washed with copious amounts of diethyl ether and dissolved in chloroform. The resulting solution was washed with 5% $NaHCO_3$ solution and water, and dried over anhydrous Na_2SO_4. The organic solvent was removed by evaporation under vacuum. The residue, which was crude **5**, was dissolved in methanol (100 cm^3) together with the dihydrochloride **2** (5.24 g, 16.1 mmol) and K_2CO_3 (1.13 g, 8.17 mmol). To this solution, a



Scheme 2

methanol solution (10 cm^3) of $NaBH_3CN$ (1.03 g, 16.4 mmol) was added slowly at 0 °C. The reaction mixture was stirred for 10 h during which the temperature was raised gradually to room temperature, and then poured over chloroform. The solution was washed with a 5% $NaHCO_3$ solution. The organic phase was separated and dried over anhydrous Na_2SO_4, and the organic solvent was removed by evaporation under vacuum. From the obtained residue, the triester of H_3(**L2**) was isolated by chromatography on silica gel with a chloroform/methanol eluent (30/1 to 5/1) (yield 1.77 g, 3.28 mmol). The triester was allowed to hydrolyze with $LiOH·H_2O$ (0.55 g, 13.1 mmol) in 50% methanol (60 cm^3) for 10 h. The resulting solution was neutralized with 1 M HCl (13.1 cm^3) and the solvent was removed by evaporation under vacuum. The product was purified by the use of DIAION HP20 (Mitsubishi Chem. Co.; eluents were water/methanol mixtures with an increasing proportion of methanol) and Sephadex LH20 columns (eluent, methanol). Addition of acetone to the eluate gave the product H_3(**L2**) as a colourless solid, which was separated by filtration and dried *in vacuo* at 40 °C for several hours (yield 0.95 g, 1.56 mmol) (Found: C, 52.43; H, 6.38; N. 14.03. $C_{24}H_{28}N_6O_6·0.85CH_3COCH_3·3.4H_2O$ requires C, 52.52; H, 6.62; N, 13.84%: the quantity of acetone was determined by the integrated intensities of the ^{1}H NMR signals); mp 124–127 °C. $[a]_D^{29}$: −69 (deg dm^{-1} g^{-1} cm^3, 1 M HCl). IR: ν_{max}/cm^{-1} 1705.0, 1629.7, 1593.1. ^{1}H NMR (D_2O, pD = 4.78): δ 2.70 (dd, 1H, J = 17.1, 9.0), 2.88 (dd, 1H, J = 17.1, 5.1), 3.2–3.0 (overlapped, 4H), 3.33 (m, 2H), 3.52 (dd, 1H, J = 8.5, 6.1), 3.97 (dd, 1H, J = 9.0, 5.1), 4.21 (d, 1H, J = 16.6), 4.26 (d, 1H, J = 16.6), 4.43 (d, 1H, J = 15.6), 4.48 (d, 1H, J = 15.6), 7.16 (s, 1H), 7.51 (ddd, 1H, J = 7.8, 5.5, 1.2) 7.58 (d, 1H, J = 7.8), 7.83 (d, 1H, J = 7.8), 7.84 (overlapped, 1H), 8.02 (td, 1H, J = 7.8, 1.5), 8.35 (td, 1H, J = 7.8, 1.5), 8.39 (d, 1H, J = 5.5), 8.56 (d, 1H, J = 1.2), 8.67 (dt, 1H, J = 4.9, 1.5). FAB MS: m/z 497.2 ($[M + H]^+$), 495.2 ($[M - H]^+$).

***N,N′*-Bis(2-pyridylmethyl)-*N,N′*-bis[(*S*)-1-carboxy-2-(imidazol-4-yl)ethyl]ethylenediamine H_2(L3).** To a mixture of crude **5** (1.36 g, *ca.* 4.5 mmol) with the trihydrochloride **3** (3.32 g, 9.0 mmol) and K_2CO_3 (1.24 g, 9.0 mmol) in methanol (50 cm^3), $NaBH_3CN$ (0.57 g, 9.0 mmol) was added slowly at 0 °C. This reaction mixture was stirred for 10 h during which the temperature was raised gradually to room temperature, poured over chloroform, and washed with a 5% $NaHCO_3$ solution. The organic phase was separated and dried over anhydrous Na_2SO_4, and the solvent was removed by evaporation under vacuum. From the residue the diester of H_2(**L3**) was isolated by the use of a Sephadex LH20 column with methanol as eluent (yield 1.62 g, 2.96 mmol). This diester was allowed to hydrolyze with $LiOH·H_2O$ (0.31 g, 7.39 mmol) in 50% methanol (40 cm^3) for 9 h. The solution was neutralized with 1 M HCl (7.39 cm^3) and the solvent was removed by evaporation under vacuum. From the residue, the product was isolated by the use of DIAION HP20 (eluents were water/methanol mixtures with an increasing proportion of methanol) and Sephadex LH20 columns (eluent, methanol). Addition of acetone to the eluate gave H_2(**L3**) as a colourless solid (1.06 g, 1.60 mmol) (Found: C, 52.88; H, 6.67; N, 16.83. $C_{26}H_{30}N_8O_4·1.05CH_3COCH_3·4.5H_2O$ requires C, 53.00; H, 6.91; N, 16.96%: the quantity of acetone was determined by the integrated intensities of the ^{1}H NMR signals). $[a]_D^{29}$: −60 (deg dm^{-1} g^{-1} cm^3, 1M HCl). IR: ν_{max}/cm^{-1} 1705.0, 1627.8, 1581.5. ^{1}H NMR (D_2O, pD = 7.13): δ 2.61 (m, 2H), 2.88 (m, 2H), 2.93 (dd, 2H, J = 15.4, 8.8), 3.06 (dd, 2H, J = 15.4, 6.3), 3.52 (dd, 2H, J = 8.8, 6.3), 3.81 (d, 2H, J = 14.9), 4.05 (d, 2H, J = 14.9) 7.02 (s, 2H), 7.23 (d, 2H, J = 7.7), 7.36 (dd, 2H, J = 7.7, 4.9), 7.78 (td, 2H, J = 7.7, 1.7), 8.31 (d, 2H, J = 1.2), 8.40 (d, 2H, J = 4.9). FAB MS: m/z 519.3 ($[M + H]^+$), 517.2 ($[M - H]^-$).

Potentiometric titrations

Potentiometric titrations were carried out by the use of a piston-type buret in an aqueous KCl medium at an ionic strength of 0.1 M and a controlled temperature of 25 °C under a nitrogen atmosphere; the ligands studied may have a significant interaction with K^+, but the protonation and complex-formation constants obtained can be compared with the corresponding constants of similar ligands determined in the same electrolyte media. The $-\log[H^+]$ values were determined by a Beckman Phi-72 pH meter equipped with Ross glass and reference electrodes. The experimental procedures including the calibration of the electrodes have been reported previously.[11] Sample solutions were acidified with HCl (0.1 mol dm^{-3}) to $-\log[H^+] \approx 2.5$, and titrated up to $-\log[H^+] \approx 11$. The number of data points collected in each titration was approximately 120 for H_3(**L1**) and 140 for the other ligands. The acid dissociation constants and the exact concentration of the ligands in the sample solutions were calculated by the use of the program PKAS.[12] A 0.01 mol dm^{-3} stock solution of Gd^{3+} was prepared from gadolinium chloride (Johnson Matthey) and standardized by edta titration. Titrations for the determination of the formation constants were carried out with 0.1 mmol of ligand in an initial volume of 50 cm^3, and the [L]/[Gd] ratios were 1.1–1.2 : 1 so that the formation of hydroxides was suppressed. A slow equilibrium was observed for 3–4 points in the unbuffered region of the Gd–**L2** and Gd–**L3** reaction systems; the longest time required for the equilibrium was 90 min for Gd–**L2**. Except for these points, equilibrium was reached in 5 min and each titration could be performed during the time in which the drift of the measuring system was tolerable. Approximately 90 data points were collected in a $-\log[H^+]$ range of 3–10 for each titration. In the Gd–**L1** system, precipitation occurred at $-\log[H^+] > 8$ even when [L]/[Gd] = 1.2. Titration data at pH < 7.5 (approximately 40 data points) were used for the calculation of the formation constants so that any effect of precipitation was avoided. Tentatively gadolinium hydroxide Gd(OH) was included in the simulation of the titration curves of Gd–**L1** by assuming $\log[GdH_{-1}][H]/[Gd] = -8.5$,[13] but this species was not detectable for the system of [L]/[Gd] = 1.2. Therefore, no hydroxides were included in the final calculation. The program BEST was used for the calculation of the formation constants.[12] Averages for two or three titrations are shown in Table 1.

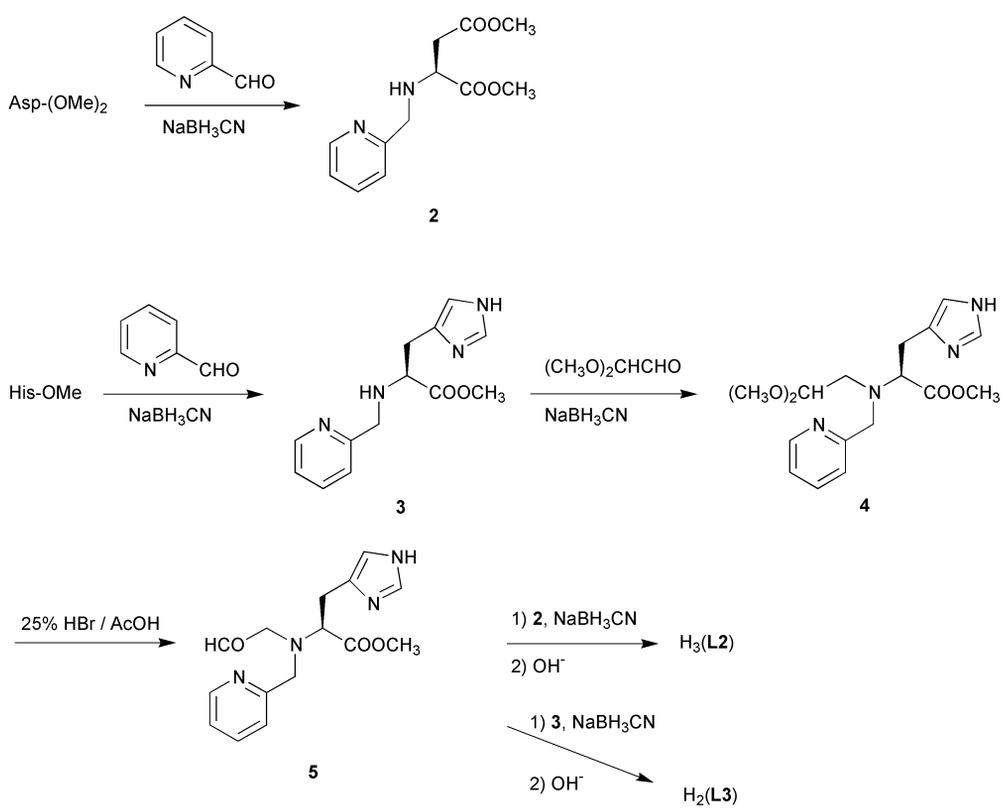
Spectroscopic measurements

The ^{1}H NMR spectra were recorded on JEOL LA-300, JEOL GX-400 or Bruker AM-250 spectrometers at an ambient temperature of *ca.* 25 °C. The internal reference was sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) or sodium 3-trimethylsilylpropionate-2,2,3,3-d_4 (TSP-d_4). The pH value of each sample solution was measured with a long-stem combination electrode (Aldrich or Horiba), which was calibrated with aqueous standard buffers (Fisher or Nacalai). The measured pH values were converted to pD values by the relation $pD = pH_{measd} + 0.44$.[14] A minimum quantity of a dilute KOD solution or dilute DCl was used for adjusting pD.

The NMR relaxation times T_1 and T_2 of water protons were determined with a Varian Gemini-300 spectrometer for aqueous solutions containing the Gd^{3+} complexes at a resonance frequency of 300 MHz and a probe temperature of 22 °C. Approximately 12 mm^3 of a sample solution was placed in the inner tube of a double-walled NMR sample tube (Wilmad WGS-5BL) and the outer tube was filled with $CDCl_3$ (50 mm high), which was used for external locking. The 180°–τ–90° pulse sequence technique was employed for T_1 measurements, and the Carr–Purcell–Meiboom–Gill method for T_2.[15] A stock solution was prepared from a standardized Gd^{3+} solution and precisely weighed ligand in the concentration ratio [L]/[Gd] = 1.1 : 1; the ligands were used in excess so that any dissociation of the complexes was suppressed. The pH of the stock solution was adjusted to a desired value by the use of TRIS

Table 1 Logarithmic successive protonation constants, $\log[LH_n]/[LH_{n-1}][H]$, of $\mathbf{L1}^{3-}$, $\mathbf{L2}^{3-}$ and $\mathbf{L3}^{2-}$, and the logarithmic overall formation constants, $\log[MLH_r]/[M][L][H]^r$, and the NMR relaxivities, r_1 and r_2, of the gadolinium(III) complexes

	$\mathbf{L1}^{3-}$	$\mathbf{L2}^{3-}$	$\mathbf{L3}^{2-}$
$\log[LH_n]/[LH_{n-1}][H]$ (0.1 mol dm^{-3} KCl, 25 °C)			
LH	9.96 (±0.08)	8.66 (±0.04)	8.37 (±0.03)
LH_2	7.24 (±0.05)	7.43 (±0.02)	7.41 (±0.02)
LH_3	4.71 (±0.07)	5.11 (±0.01)	5.88 (±0.02)
LH_4	2.76 (±0.04)	3.74 (±0.02)	4.65 (±0.01)
LH_5	—	2.85 (±0.02)	3.10 (±0.02)
$\log[MLH_r]/[M][L][H]^r$ (0.1 mol dm^{-3} KCl, 25 °C)			
ML	10.96 (±0.07)	13.52 (±0.07)	10.88 (±0.05)
MLH	17.22 (±0.03)	20.09 (±0.05)	17.56 (±0.07)
MLH_2	—	22.70 (±0.09)	23.46 (±0.05)
MLH_3	—	—	26.22 (±0.03)
MLH_{-1}	—	1.94 (±0.27)	0.32 (±0.25)
MLH_{-2}	—	—	−9.83 (±0.08)
Relaxivities/mmol^{-1} dm^3 s^{-1} (300 MHz and 22 °C)			
r_1	—	8.9 (pH = 9.0)	8.9 (pH = 8.5)
	—	9.4 (pH = 7.4)	9.9 (pH = 7.4)
r_2	—	10.8 (pH = 9.0)	11.4 (pH = 8.5)
	—	11.9 (pH = 7.4)	12.6 (pH = 7.4)



Scheme 3

($(HOCH_2)_3CNH_2$) buffer (0.1 M). The sample solutions were prepared from the stock solution in the concentration range 0.2–2 mmol dm^{-3} per Gd^{3+}.

IR spectra, mass spectra, polarization degrees and melting points were obtained by the use of a Jasco FT/IR-420 spectrophotometer, a JEOL AX-500 spectrometer, a Jasco DIP-370 polarimeter and a Yanaco MP-13, respectively.

Results and discussion

Syntheses of ligands

Bis(amino acid) trimethyl ester **1** was synthesized by a condensation reaction of glyoxal with (*S*)-histidine methyl ester and (*S*)-aspartic acid α,β-dimethyl ester and by a successive reduction of the Schiff base-type product with $NaBH_3CN$ (Scheme 2).[9] This ester underwent self-cyclization to form lactams (Scheme 2) under the conditions reported previously.[6,9] In this work, the undesirable lactam formation was prevented by carrying out all operations of the synthesis and purification at temperatures below 30 °C, and the ester obtained was immediately converted to the corresponding acid. In this manner, pure H_3(**L1**) could be readily isolated in a reasonable yield.

The syntheses of *N,N′*-bis(pyridylmethyl) bis(amino acid)s, H_3(**L2**) and H_2(**L3**), were initially attempted by a method similar to that for H_3(**L1**). Although the total yields were almost 30%, separation of the products by chromatography was complicated and time-consuming. For this reason, an alternative synthetic route (shown in Scheme 3) was employed for H_3(**L2**)

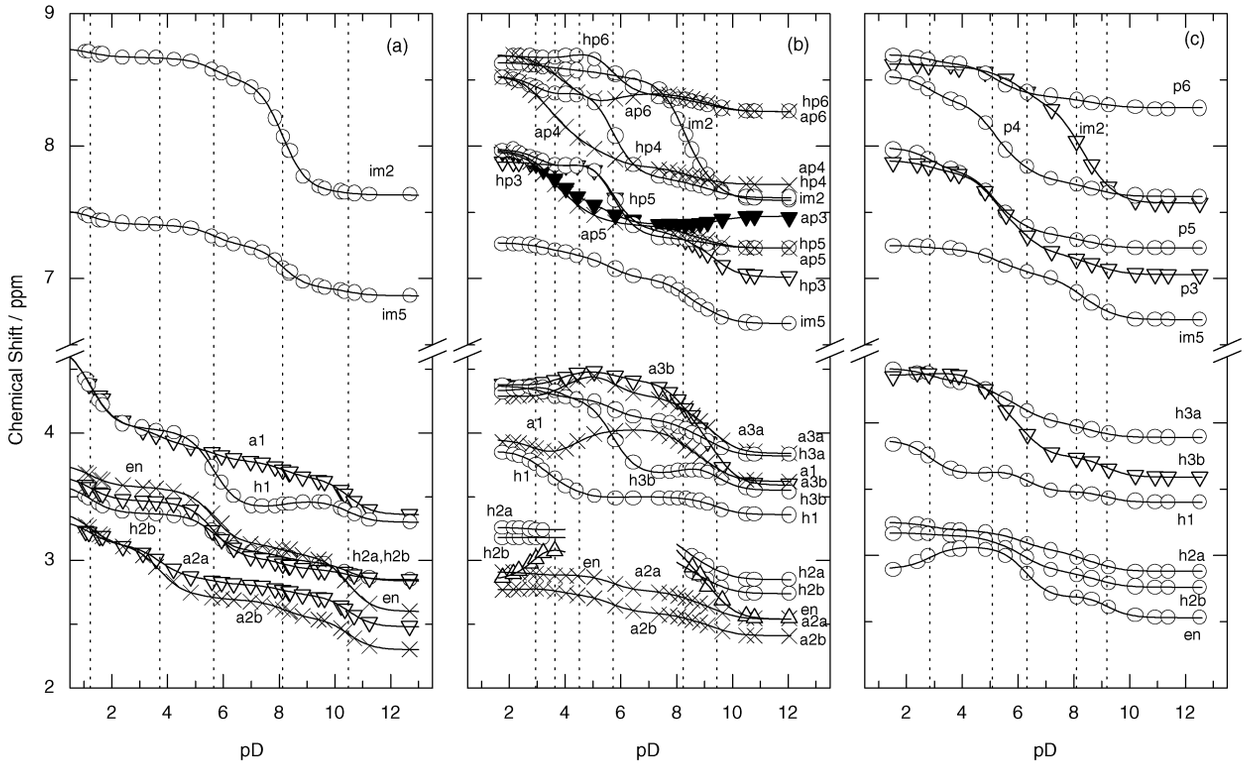


Fig. 1 ^{1}H NMR shifts observed for (a) $\mathbf{L1}^{3-}$, (b) $\mathbf{L2}^{3-}$ and (c) $\mathbf{L3}^{2-}$; for labeling see Scheme 1. The vertical broken lines show the pD values corresponding to the logarithmic protonation constants (in D_2O) determined by curve-simulation with eqn. (1).

and H_2(**L3**). *N*-Pyridylmethyl-amino acid esters, **2** and **3**, were obtained by a reductive *N*-alkylation of appropriate amino acid ester hydrochlorides with pyridylcarbaldehyde in the presence of $NaBH_3CN$ in methanol. These reactions gave only a little amount of the by-products, *i.e.*, *N,N*-bis(pyridylmethyl) derivatives, and pure products **2** and **3** in their hydrochloride forms could be readily obtained by recrystallization from ethanol. A reductive *N*-alkylation of **3** with $(CH_3O)_2CHCHO$ in the presence of $NaBH_3CN$ in methanol gave acetal derivative **4**, which could be used for the next synthetic steps without chromatographical purification when $(CH_3O)_2CHCHO$ was used in more than five-fold excess. It has been reported that N^{α}-(diethoxyethyl)-(*S*)-histidine is obtained when (*S*)-histidine and an equimolar amount of bromoacetaldehyde diethyl acetal were refluxed in the presence of potassium carbonate.[16] This method, however, requires a long reaction time, and the purification process needs ion-exchange or styrene-gel chromatography. When compared with this reported method, our method in which amino acid ester is used as a starting material is very convenient, because the product can be readily purified by chromatography on silica gel with appropriate organic eluents. Treatment of **4** with 25% HBr/acetic acid gave **5**, which was immediately used for the next coupling reactions without any purification because this formyl derivative was unstable. Reductive *N*-alkylations of **2** and **3** with **5** in the presence of $NaBH_3CN$ yielded the ester derivatives of H_3(**L2**) and H_2(**L3**), respectively, which were purified by chromatography on silica gel. The hydrolyses of the ester derivatives with LiOH, followed by neutralization with HCl, gave H_3(**L2**) and H_2(**L3**), which could be readily purified by chromatography on DIAION HP20 with mixtures of water and methanol as eluents in which the proportion of methanol was gradually increased. Addition of acetone to the eluates gave the pure products as colourless solids.

NMR and protonation sites

Since the ligands have a number of potential protonation sites, the order of the local basicities has been determined by ^{1}H NMR. The NMR shifts observed for the ligands are shown in Fig. 1 as functions of pD: the labeling of the protons is given in Scheme 1. Protonation at a donor atom leads to a decrease in the electron density of a CH_n group bonded to the protonated donor atom, and consequently the ^{1}H NMR signal of the CH_n protons shifts downfield.[17] The chemical shift $\delta(i)$ of the *i*-th proton signal is given by a function of pD as follows:

$$\delta(i) = [\delta_0(i) + \Sigma\, \delta_j(i)\cdot\beta_j\cdot 10^{-pD\cdot j}]/[1 + \Sigma\beta_j\cdot 10^{-pD\cdot j}] \quad (1)$$

where $\delta_0(i)$ is the chemical shift of the *i*-th proton signal of the species in which acidic protons are completely dissociated, $\delta_j(i)$ is the chemical shift of the *i*-th proton in the *j*-protonated species, and β_j is the *j*-th overall protonation constant, $K_{D1}K_{D2}\cdots K_{Dj}$, in D_2O. The solid lines in Fig. 1 show the best fits of eqn. (1), and the vertical broken lines show the pD values corresponding to the logarithmic K_{Dj} values determined; these values are higher than the corresponding log K_j values determined by potentiometry owing to the difference between the dissociation constants in D_2O and H_2O media as reported for common weak acids.[18] The three ligands have a number of protonation sites between which proton exchange can occur. In fact, some proton signals show a reverse shift upon protonation, which is caused by a decrease in proton population of the neighbouring donor atom due to internal redistribution of acidic protons.[19] For these ligands, therefore, the protonation constants determined by potentiometry are not definitely related to microscopic protonation processes at specific donor atoms. The major protonation site at each protonation step, however, can be assigned to the donor atom whose neighbouring CH protons show the largest change in the NMR shift at the protonation step. Fig. 1 shows that commonly in the three compounds, the first protonation occurs at one of the nitrogen atoms in the ethylenediamine units (probably at the nitrogen atom of the aspartic moiety in $\mathbf{L1}^{3-}$ and $\mathbf{L2}^{3-}$); this atom has the highest basicity. The second protonation occurs mainly at the imidazole nitrogen atom, and the third at the second ethylenediamine nitrogen atom.

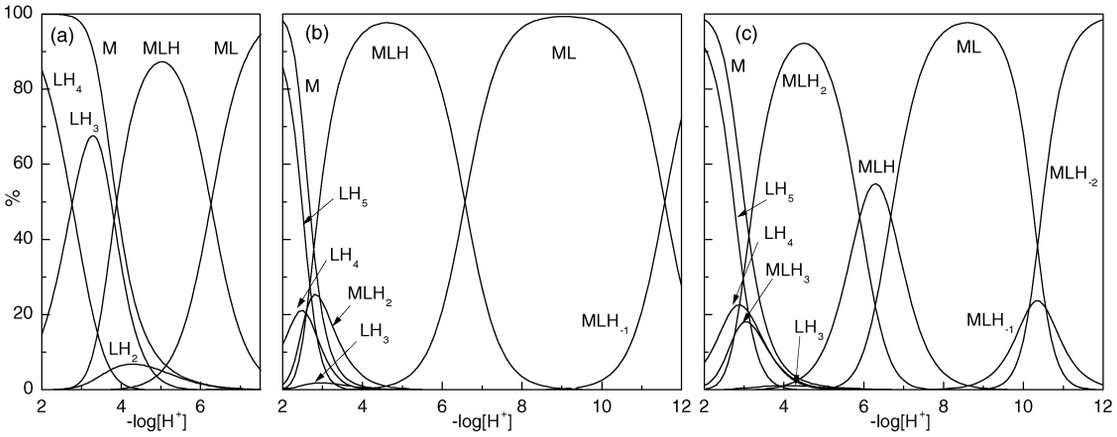


Fig. 2 Species distribution diagrams calculated for (a) Gd–**L1**, (b) Gd–**L2** and (c) Gd–**L3** with concentration [Gd] = [L] = 1 mmol dm^{-3}.

Formation constants of gadolinium(III) complexes

The logarithmic overall formation constants are listed in Table 1, and the species distribution diagrams calculated for [Gd] = [L] = 1 mmol dm^{-3} are shown in Fig. 2. The logarithmic formation constant of $[Gd(\mathbf{L1})]^{0}$ is much smaller than the value 17.32 reported for $[Gd(edta)]^{-}$,[20] although they have the identical number of donor atoms that potentially coordinate to a metal ion. The presence of a larger number of negatively charged oxygen atoms in $edta^{4-}$ is a major factor leading to the higher stability of the $edta^{4-}$ complex. The *N*-carboxyethyl and *N*-imidazolylethyl frameworks in H_3(**L1**) form a six-membered chelate ring, while all chelate rings formed in $[Gd(edta)]^{-}$ are five-membered. Normally a six-membered chelate ring is less stable than a similar five-membered ring.[21] This difference may be an additional reason why $[Gd(\mathbf{L1})]^{0}$ is less stable than $[Gd(edta)]^{-}$. The stability of $[Gd(\mathbf{L2})]^{0}$ is significantly higher than that of $[Gd(\mathbf{L1})]^{0}$. This is a result of the introduction of the picolyl groups. The replacement of the aspartate moiety by a histidinate moiety results in the lower stability of $[Gd(\mathbf{L3})]^{+}$. The ML species of the three complexes are readily protonated. The yield of Gd(**L3**)H_2 is much higher than that of Gd(**L3**)H. Since the six-membered chelate ring formed by an *N*-imidazolylethyl framework in $[Gd(\mathbf{L3})]^{+}$ is less stable than the other five-membered chelate rings, the imidazole nitrogen atoms are the most probable atoms that are protonated in Gd(**L3**)H and Gd(**L3**)H_2. The two imidazole nitrogen atoms are chemically equivalent, and they are almost simultaneously protonated so that the yield of Gd(**L3**)H_2 is higher than that of Gd(**L3**)H. In the MLH species of Gd–**L1** and Gd–**L2**, either the nitrogen atom of the imidazolylmethyl group or the oxygen atom of the carboxyethyl group is protonated; the former is more probable by comparison with Gd–**L3**.

NMR relaxivities

The ^{1}H NMR relaxation times, T_1 and T_2, of water molecules in the presence of Gd^{3+} complexes with the bis(amino acid)s were determined at a resonance frequency of 300 MHz, and the corresponding relaxivities, r_1 and r_2, were calculated by $T_n^{-1} = r_n[Gd] + T_{n0}^{-1}$ where $n = 1$ or 2, and $T_{n0} = T_1$ or T_2 at [Gd] = 0. The pH of the sample solutions of each Gd^{3+} complex was adjusted to the value at which the main species, ML, had the maximum yield (pH = 9.0 for Gd–**L2** and 8.5 for Gd–**L3**), because the relaxivities are dependent on the nature of the species formed. The relaxivities at physiological pH (7.4), which are important in clinical use, were also determined although the relaxivities at this pH were not intrinsic of a specific species but gave mean values for two or three coexisting species (see Fig. 2). The ligands were added in excess to the sample solutions so that any dissociation of the Gd complexes was suppressed because the presence of free Gd^{3+} ions resulted in a large error. The complex of **L1** was not subjected to relaxation measurements, because precipitation readily occurred at pH ≈ 7.4. The relaxivities obtained for the **L2** and **L3** complexes (shown in Table 1) are much larger than those determined for Gd^{3+}–DTPA under the same experimental conditions (r_1 = 4.7 $mmol^{-1}$ dm^3 s^{-1} and r_2 = 7.5 $mmol^{-1}$ dm^3 s^{-1}). X-Ray studies of Gd–DTPA complexes have shown that eight donor atoms of a DTPA molecule coordinate to the metal ion.[22,23] In aqueous solutions the ninth coordination site is occupied by a water molecule. Exchange of the coordinated water molecule with solvent water molecules is the major factor that governs the NMR relaxation process.[1,3] In the ML species of the Gd–**L2** and Gd–**L3** complexes, the central metal ion is coordinated to eight donor atoms from a ligand molecule, and the ninth coordination site is available for the coordination of a water molecule as in $[Gd(DTPA)(H_2O)]^{2-}$. The higher relaxivities of the **L2** and **L3** complexes suggest that water-exchange in these complexes is more rapid than in Gd–DTPA; the effective numbers of coordinated water molecules in the former complexes are larger than in the latter complex. The relaxivities at pH = 7.4 are significantly higher than the corresponding values at the pH values at which the main species have the maximum yields (Table 1). In the secondary species, MLH, that coexists with ML at pH = 7.4, one of the donor atoms is protonated and liberated from the metal ion, and consequently the number of coordinated water molecules increases and this results in the enhancement of the relaxivities.

Conclusion

In this work, novel synthetic routes have been established for a new family of ethylenediamine-based ligands consisting of two optically pure amino acid units. Combination of a variety of amino acids will give bis(amino acid) ligands that have different coordination properties and biological functions. Bis(amino acid) H_3(**L1**) consisting of histidine and aspartic acid units contains only six donor atoms, and consequently its gadolinium(III) complex has a low stability. Bis(amino acid)s with eight donor atoms (*i.e.*, the same number of donor atoms as in DTPA) can be obtained by the use of picolyl derivatives of amino acids. The resulting ligands, H_3(**L2**) and H_2(**L3**), form neutral and cationic Gd^{3+} complexes, $[Gd(\mathbf{L2})(H_2O)]^{0}$ and $[Gd(\mathbf{L3})(H_2O)]^{+}$, respectively, which show higher relaxivities than $[Gd(DTPA)(H_2O)]^{2-}$ although the stabilities of the former complexes are yet insufficient for use as MRI enhancers.

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