Chinese Journal of Chemistry, **2004**, *22*, 502—504 **Note**

Synthesis and Characterization of *α*-Hexadecyl-DOTA and its Gd(III) Chelate

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Synthesis and characterization of the ligand, 10-(*α*-hexadecylcarboxymethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (H_4L), and its Gd(III) chelate are described. Protonation constants for H_4L ($\lg K_i^H$ =10.52, 9.45, 4.74, 4.10) and the stability constant for GdL^- ($\lg K_{GdL^-}$ =24.50) were determined by potentiometric titrations. The results obtained show that the ligand still maintains the strong chelating properties of the parent DOTA (1,4,7,10-tetraazacyclododecane-*N*,*N*',*N*''*N*'''-tetraacetic acid) after introduction of a linear chain hexadecyl group at the acetic side chain of DOTA, and its basicity is not significantly altered.

Keywords Gd(III) chelate, macrocycle complex, TA derivative, stability

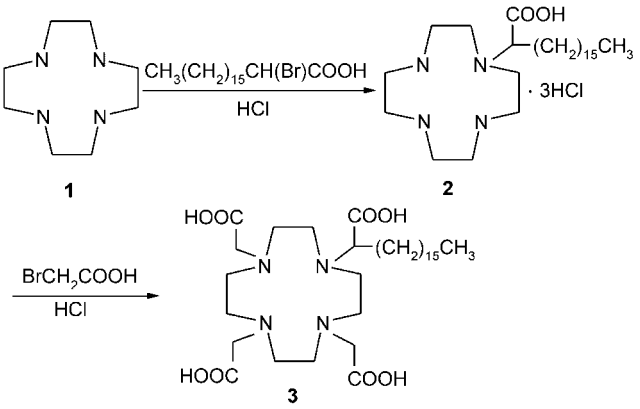
Introduction

Recent interest in polyazamacrocyclic paramagnetic Gd(III) chelates largely results form their clinical application for magnetic resonance imaging (MRI) contrast agents. The ligand DOTA (1,4,7,10-tetraazacyclododecane-*N*,*N*',*N*'',*N*'''-tetraacetic acid) forms one of the most thermodynamically stable and kinetically inert complexes with the trivalent lanthanide cations of any known chelate. These properties make Gd-DOTA one of the most effective and the safest MRI contrast agents available. However, Gd-DOTA, like Gd-DTPA (diethylenetriaminepentaacetic acid-gadolinium chelate), is a nonspecific extracellular MRI contrast agent that distributes throughout all extracellular space before being excreted through the kidneys. Current interest in searching organ or tissue specific contrast agents has led to the synthesis and application of the Gd(III) chelates of DOTA derivatives with lipophilic group both in acetic side chain and the cyclododecane backbone.[1-3]

Liposomes made by phospholipid or fatty acids are extensively used as carrier of medicine. After intravenous injection, these liposomes could be highly concentrated in liver and spleen that have matured reticuloendothelial system *in vivo*, and have good affinity to liver or hepatic targeting.[4]

The paper describes in detail the synthesis and characterization of the ligand, 10-(*α*-hexadecylcarboxymethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (H_4L, **3**) and its Gd(III) chelate. The synthetic pathway for the ligand is shown in Scheme 1.

Scheme 1 Synthetic pathway for the ligand



Experimental

General

Evaporation of solvents was performed on an RE 52-99 rotary evaporator at aspirator pressure. Potentiometric experiments were operated on a ZD-2 potentiometric titration apparatus. Melting points were determined with a WRS-1 digital melting point apparatus, and melting points are uncorrected. ^{1}H NMR spectra were measured on an AC-80 NMR spectrometer using TMS as internal standard and D_2O as solvent, coupling constants (*J*) are reported in Hz. IR spectra were recorded on a Nicolet-550 IR spectrometer in KBr. Mass spectra were obtained by a GC/MS HP-5000 spectrometer (EI, 70 eV). Elemental analysis was performed at a PE- 2400 elemental analyzer.

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All organic and inorganic reagents purchased from commerce were chemically pure or analytically pure, and used directly without purification. Silica gel (200—300) for column chromatography was the product of Qingdao Ocean Chemical Plant. 2-Bromosteric acid and 1,4,7,10-tetraazacyclododecane were prepared according to the reported method.[5,6]

Synthesis of 1-(α-hexadecylcarboxymethyl)-1,4,7,10-tetraazacyclododecane tris(hydrochloride) (2)

A solution of **1** (34.4 g, 0.2 mol) and 2-bromosteric acid (36.3 g, 0.1 mol) in dimethylformamide (200 mL) was stirred at 50 ℃ for 48 h. The resulting solution was concentrated in vacuum, and the residue was suspended in water (200 mL). The aqueous phase was washed with CH_2Cl_2 (2×100 mL) and acidified with concentrated HCl to give an amorphous precipitate. The precipitate was first dissolved in H_2O (200 mL) and neutralized by addition of NaOH (1.0 mol•L^{-1}) and then loaded onto a column of silica gel (200—300). The column was eluted first with water and then with NH_3•H_2O (4.0 mol•L^{-1}). The alkaline solution (2.0 L) was collected and evaporated in vacuum to dryness, and the residue was treated with HCl (6.0 mol•L^{-1}) in EtOH. The precipitate obtained was crystallized from EtOH to give **2** (24.3 g, 43%) as a white solid, m.p. 223—226 ℃. MS (70 eV) *m/z* (%): 561 (M^+, 35), 171 (46), 46 (100). Anal. calcd for $C_{26}H_{54}Cl_3N_4O_2$: C 55.64, H 9.72, N 9.98; found C 55.27, H 10.26, N 9.82.

Synthesis of 10-(α-hexadecylcarboxymethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (3)

Compound **2** (56.4 g, 0.1 mol) was first suspended in H_2O (200 mL). After addition of Na_2CO_3 solution (2.0 mol•L^{-1}, 100 mL), bromoacetic acid (55.6 g, 0.4 mol) dissolved in Na_2CO_3 solution (2.0 mol•L^{-1}, 100 mL) was dropped into the reaction mixture and the resultant mixture was refluxed for 24 h. During this time, pH of 10 was maintained by continuous addition of Na_2CO_3 solution (2.0 mol•L^{-1}, 100 mL). After being cooled to room temperature, the reaction mixture was loaded onto a column of silica gel (200—300). The column was eluted first with water and then with NH_3•H_2O (2.5 mol•L^{-1}). The alkaline solution containing the product was evaporated in vacuum to dryness. The residue was dissolved in H_2O (300 mL) and then the pH of the solution was adjusted to 3.2 by addition of concentrated HCl. The precipitate obtained was filtered and washed with water ad dried in vacuum. The white solid (45.9 g, 73%) obtained was **3**, m.p. 175—178 ℃. ^{1}H NMR (D_2O/TMS) δ: 0.86 (t, J=8 Hz, 3H), 1.10—1.30 (m, 30H), 2.80—3.42 (m, 16H), 3.62 (s, 6H), 4.00—4.20 (m, 1H); IR (KBr) ν: 3430, 2980, 1745, 1640, 1348 cm^{-1}; MS (70 eV) *m/z* (%): 629 (M^+, 32), 168 (43), 59 (100). Anal. calcd for $C_{32}H_{60}N_4O_8$: C 61.10, H 9.63, N 8.91; found C 60.82, H 9.89, N 8.80.

Synthesis of monosodium [10-(α-hexadecylcarboxymethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetato(4-)]gadolinate (—)[7]

Compound **3** (31.5 g, 0.05 mol) was suspended in water (200 mL) and dissolved by addition of NaOH (1.0 mol • L^{-1}, 100 mL). After addition of Gd_2O_3 (9.1 g, 0.025 mol), the suspension was stirred at 70 ℃ for 2 h. The reaction solution was filtered at room temperature, and then concentrated in vacuum to dryness. The residue was collected and washed with EtOH∶H_2O (90∶10, V∶V), and dried in vacuum at 50 ℃ to yield NaGdL (14.1 g, 70%) as a white solid, m.p.＞250 ℃. IR (KBr) ν: 2985, 1645, 1560, 1335 cm^{-1}. MS (70 eV) *m/z* (%): 805 (M^+, 32), 339 (36), 168 (42), 58 (100). Anal. calcd for $C_{32}H_{56}Gd$•N_4NaO_8: C 47.73, H 7.02, N 6.96; found C 47.66, H 7.12, N 6.85.

Determination of protonation constants and stability constant

Protonation constants for **3** were determined by potentiometric titration at 25 ℃ and μ=0.1 mol•L^{-1} KCl. The ligand **3** solution (0.001 mol•L^{-1}, 20 mL) was titrated with a standard solution of KOH (0.1 mol•L^{-1}) added by means of 2 mL piston buret. Stability constant of GdL^- was determined at 25 ℃ by potentiometry in the presence of DTPA as a reference ligand of known stability constant (lg $K_{Gd\text{-}DTPA}$=22.52). The experiments were performed at [**3**]=0.001 mol•L^{-1} with ratio [Gd^{3+}]∶[**3**]∶[DTPA] of 1∶1∶1. Protonation constants for **3** and stability constant of GdL^- were otained by the reported methods.[8]

Results and discussion

Synthesis

Ligand **3** was synthesized by alkylation of 1,4,7,10-tetraazacyclododecane with 2-bromostearic acid and then with bromoacetic acid (Scheme 1). The gadolinium chelate was prepared by using Gd_2O_3. Gd^{3+} was coordinated by oxygen atom of carboxyl group of the ligand in which the frequency of antisymmetric stretching vibration of carboxyl group shifted from 1745 cm^{-1} at H_4L to 1645 cm^{-1} at GdL^- and nitrogen atom of the ligand in which the frequency of C—N vibration shifted from 1640 cm^{-1} at H_4L to 1560 cm^{-1} at GdL^-.

Protonation constants of 3

The protonation constants of **3** calculated from the potentiometric titration curves are given in Table 1. In the protonation constants, lg K_1^H of the ligand is slightly lower than that of DOTA, and the others are similar to those of DOTA, indicating that the substitution of a hydrogen atom on an acetic side chain at DOTA with linear chain hexadecyl group does not substantially alter the basicity of the protonation sites.

Table 1 Protonation constants for **3**, DOTA and DTPA[a,b]

	$\lg K_1^H$	$\lg K_2^H$	$\lg K_3^H$	$\lg K_4^H$	$\lg K_5^H$
3	10.52 (0.03)	9.45 (0.03)	4.74 (0.02)	4.10 (0.04)	
DOTA[c]	11.14	9.69	4.85	3.95	
DTPA[d]	10.34	8.59	4.25	2.71	2.18

[a] Values obtained at 25 ℃ and μ=0.1 mol•L^{-1} KCl. [b] Values in parentheses are estimated standard deviations. [c] From Ref. 2. [d] From Ref. 9.

Stability constant of GdL^-

The potentiometric determination of the stability constant of the Gd(III) chelate is difficult due to both the extremely high value ($\lg K>20$) and the slow kinetics that characterize the formation of the chelate. These problems were solved by a competition method with DTPA and allowing the reaction mixture to reach the equilibrium conditions at 60 ℃ before measuring at 25 ℃. The competition method according to the following overall equilibrium with Y=DTPA is shown as follows:

$$GdY^{2-} + H_4L + H^- \rightleftharpoons GdY^- + H_5Y$$

Potentiometric data on competition experiments with reference ligand (DTPA) were analyzed, and the results are presented in Table 2. The conditional stability constant of GdL^- was calculated on the basis of the protonation constants of **3** (Table 1) under physiologically relevant conditions (pH=7.4, μ=0.1 mol•L^{-1} KCl).

According to Table 2, the stability constant and conditional stability constant of GdL^- are only slightly lower than that of Gd-DOTA and significantly higher than those of Gd(III) chelates of DTPA and DOTA-amide and DOTA-ester conjugates.[9,10] The results indicate that the introduction of hexadecyl residue on an acetic side chain at DOTA does not alter the strong chelating properties of the parent DOTA.

Table 2 Stability constants of GdL^-, Gd-DOTA and Gd-DTPA[a,b]

	X=**3**	X=DOTA[d]	X=DTPA[e]
$\lg K_{GdX}$	24.50 (0.18)	25.30	22.52
$\lg K'_{GdX}$ [c]	18.60	19.00	18.40

[a] Values obtained at 25 ℃ and μ=0.1 mol•L^{-1} KCl. [b] Values in parentheses are the estimated standard deviations. [c] Conditional stability constants at pH 7.4 (physiologically relevant conditions). [d] From Ref. 2. [e] From Ref. 9.

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