研究简报

α-十四烷基-DOTA 及其钆(Ⅲ)螯合物的合成与表征

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Synthesis and Characterization of α-Tetradecyl-DOTA and Its Gd(Ⅲ) Chelate

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Synthesis and characterization of the ligand, [10-(α-tetradecylcarboxymethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, H_4L] and its Gd(Ⅲ) chelate are described. Protonation constants for H_4L($\lg K_i^H$ = 10.62, 9.50, 4.74, 4.12) and the stability constant for GdL^- ($\lg K_{GdL^-}$ = 24.60) were determined by potentiometric titrations. The results obtained show that the basicity of the ligand is not significantly altered and the ligand still maintains the strong chelating properties of the parent DOTA after introduction of a linear chain tetradecyl group at the acetic side chain of DOTA.

Keywords: **Gd(Ⅲ) chelate** **DOTA derivative** **stability**

0 Introduction

Several types of paramagnetic Gd(Ⅲ) chelate have been proposed for use as contrast-enhancing agents in magnetic resonance imaging (MRI). Gd-DTPA (diethylenetriaminepentaacetic acid-gadolinium chelate) is the first of those agents to be approved for use in humans and the standard to which newer agents are compared. 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, is a nonspecific extracellular MRI contrast agent which distributes throughout all extracellular space before being excreted through the kidneys. Current interest in searching organ or tissue specific contrast agents has led to synthesize and apply the Gd(Ⅲ) chelates of DOTA derivatives with lipophilic group

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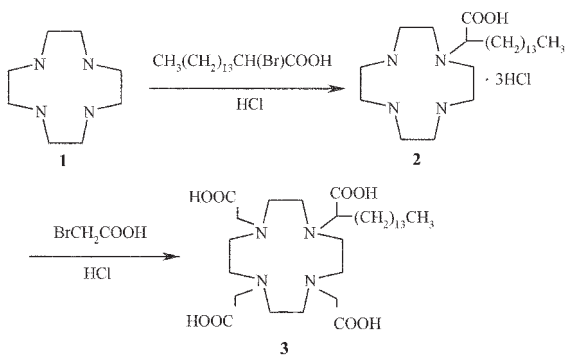
both in acetic side chain and the cyclododecane backbone[1~3].

Liposomes made by phospholipid or fatty acids are extensively used as carrier of medicine. After intravenous injection, these liposomes could highly concentrated in liver and spleen that have matured reticuloendothelial system *in vivo*, and have good affinity to liver or hepatic targeting[4]. So introduction of a linear long chain alkyl group at DOTA might obtain a new paramagnetic Gd(Ⅲ) chelate that would enter hepatocytes and be excreted by bile and could be useful for the liver MRI.

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



Scheme 1 Synthetic pathway for the ligand

1 Experimental Section

1.1 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 a 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 GC/MS HP-5000 (EI, 70 eV). Elemental analysis was performed at a PE-2400 elemental analyzer.

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-Bromopalmitic acid and 1,4,7,10-tetraazacyclododecane (**1**) were prepared according to known procedure[5,6].

1.2 Synthesis of l-(α-tetradecylcarboxymethyl)-1,4,7,10-tetraazacyclododecane tris(hydrochloride) (2)

A solution of **1** (34.4 g, 0.2 mol) and 2-bromopalmitic acid (33.5 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 first was dissolved in H_2O (200 mL) and neutralized by addition of NaOH (1.0 mol·L^{-1}) and then was loaded onto a column of silica gel (200~300). The column was eluted first with water and then with $NH_3 \cdot H_2O$ (4.0 mol·L^{-1}). The alkaline solution (2.0 L) was collected and evaporated in vacuum to dryness, the residue was treated with HCl (6.0 mol·L^{-1}) in EtOH. The precipitate obtained was crystallized from EtOH to give **2** (24.1 g, 45.0 %) as a white solid, m.p. 221~224 ℃. Anal. Calcd (found) for $C_{24}H_{53}Cl_3N_4O_2$: C, 53.76(53.57) %; H, 9.98(10.16) %; N, 10.45(10.32) %.

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

2 (53.6 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 then 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}). After cooling to room temperature, the reaction mixture was loaded onto a column of silica gel (200~300). The column was elut-

ed first with water and then with $NH_3 \cdot H_2O$ (2.5 mol·L^{-1}). The alkaline solution containing the product was evaporated in vacuum to dryness. The residue was dissolved in water (300 mL) and then the pH of the solution was adjusted to 3.2 by addition of concentrated HCl. The precipitate was filtered and washed with water and dried in vacuum.The white solid(45.0g, 75.0 %) obtained was **3**. m.p. 170~173 ℃. ^{1}H NMR (D_2O/TMS) δ: 0.84(t, J=8 Hz, 3H, -CH_3), 1.18(s, 26H, -CH_2-), 3.26(s, 16H, -NCH_2-), 3.62(s, 6H, -CH_2COO), 4.20(t, J=7Hz, 1H, -CHCOOH). IR(KBr, cm^{-1}): 3435 (ν_{O-H}),2982 (ν_{C-H}),1742 (ν_{-CO-}),1643 (ν_{C-N}),1350 (ν_{CH_2COOH}), 1110 (ν_{C-N}).MS(70 eV) m / z(%): 601(M^+, 30), 442(27), 168(38), 59(100). Anal. Calcd (found) for $C_{30}H_{56}N_4O_8$: C, 59.96(59.82)%; H, 9.41(9.59)%; N, 9.32(9.10)%.

1.4 Synthesis of monosodium [10-(α-tetradecyl carboxymethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triaceteto(4-)] gadolinate(-) [7]

3 (30.0 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(V_{EtOH}:V_{H_2O} = 90:10), and dried in vacuum at 50 ℃ to yield NaGdL as a white solid(14.2 g, 73.2%). m.p. > 250 ℃. MS (70 eV) m / z(%): 777(M^+, 27), 439(34), 168(43), 58 (100). Anal. Calcd (found) for $C_{30}H_{52}GdN_4NaO_8$: C, 46.36(46.23)%; H, 6.76(6.82)%; N, 7.21(7.15)%.

1.5 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 ℃ and 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 C_3=0.001 mol·L^{-1} with ratio $C_{Gd^{3+}}$:C_3:C_{DTPA} = 1:1:1. Protonation constants for **3** and stability constant of GdL^- were obtained by methods reported previously[8].

2 Results and Discussion

2.1 Synthesis

The synthetic scheme was shown in Scheme 1. The ligand (**3**) was synthesized by alkylation of 1,4,7, 10-tetraazacyclododecane first with 2-bromopalmitic acid and then with bromoacetic acid. The gadolinium chelate was prepared by using Gd_2O_3.

2.2 The 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, the others are similar to those of DOTA. Together with the values for DOTA, this indicates that the substitution of a hydrogen atom on acetic side chain at DOTA with linear chain tetradecyl 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	10.62(0.03)	9.50(0.02)	4.74(0.04)	4.12(0.03)	
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].

2.3 The 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:

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

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** (see 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 found for Gd-DOTA and significantly higher than those reported for other Gd(Ⅲ) chelates of DTPA and DOTA-amide and -ester conjugates [9,10]. Comparison between the $\lg K_{GdL^-}$ and $\lg K_{Gd\text{-}DOTA}$ clearly indicates that the ligand has maintained the strong chelating properties of the parent DOTA.

Table 2 Stability Constants for GdL^-, Gd-DOTA and Gd-DTPA[a,b]

	X=**3**	X=DOTA[d]	X=DTPA[e]
$\lg K_{GdX}$	24.60(0.20)	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 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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