Pergamon

PII: S0277-5387(97)00029-6

Polyhedron Vol. 16, No. 16, pp. 2755–2759, 1997
© 1997 Elsevier Science Ltd
All rights reserved. Printed in Great Britain
0277-5387/97 $17.00+0.00

Synthesis, characterization and relaxivity of amphiphilic chelates of DTPA derivatives with Gd^{III}, Yb^{III} and Mn^{II}

Xia Zhao,[a] Renxi Zhuo,[a]* Zhengrong Lu[a] and Wuyang Liu[b]

[a] Department of Chemistry, Wuhan University, Wuhan 430072, P.R. China

[b] Institute of Physics in Wuhan, Academia Sinica, Wuhan 430072, P.R. China

(Received 22 November 1996; accepted 14 January 1997)

Abstract—Some novel amphiphilic ligands were synthesized by the reaction of diethylenetriaminepentaacetic acid (DTPA) dianhydride and octadecylester of tyrosine or phenylalanine. Their paramagnetic Gd^{III}, Yb^{III} and Mn^{II} metal complexes were also synthesized and characterized. The amphipathic metal complexes were inserted into the membrane of liposomal vesicles and the liposomes bearing metal complexes were obtained. The paramagnetic metal labeled liposomes show high spin-lattice relaxivity (R_1) on the surrounding water protons. © 1997 Elsevier Science Ltd

Keywords: amphiphilic ligand; DTPA derivative; paramagnetic metal complex; MRI contrast agent; relaxivity; liposome.

Paramagnetic compounds have been utilized as contrast agents to enhance the contrast of images in magnetic resonance imaging (MRI) [1]. At present, two gadolinium complexes Gd-DTPA [2] and Gd-DOTA [3] (DTPA is diethylenetriaminepentaacetic acid, DOTA is 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid) are routinely used as contrast agents in humans, but have limited potentials because of their non-specificity. The design and development of novel organ-specific contrast agents has attracted much attention, and a variety of organ-specific agents are currently being investigated. Our research program mainly involves liver-specific MRI contrast agents. Some efforts were made to achieve liver-specificity by chemically modifying Gd-DTPA with hydrophobic alkyl (e.g. stearylamine) [4,5] or aromatic groups [6] to form new amphiphilic gadolinium-DTPA derivatives. However, it is reported that stearylamine was found to be neurotoxic [7]. In this report, we use stearylesters of L-tyrosine and L-phenylalamine for modifying DTPA, and attempt to obtain novel amphiphilic paramagnetic DTPA metal complexes containing both hydrophobic alkyl group (stearylester) and aromatic groups (phenol and phenyl), which are expected to be liver specific. The modified DTPA ligands were synthesized from the reaction of DTPA dianhydride with the stearylesters of L-tyrosine and L-phenylalanine respectively. Six paramagnetic Gd^{III}, Yb^{III}, Mn^{II} complexes of the ligands were prepared and characterized. The amphiphilic complexes were then incorporated into the membrane of liposomal vesicles, and the paramagnetic labeled liposomes showed high proton relaxivity.

EXPERIMENTAL

All the chemicals were commercially available and all solvents were dried and distilled prior to use. DTPA dianhydride was prepared according to Sosnovsky *et al.* [8]. The hydrochlorides of tyrosine octadecylester ($TyrOC_{18}H_{37}\cdot HCl$) and phenylalanine octadecylester ($PheOC_{18}H_{37}\cdot HCl$) were prepared according to Penney *et al.* [9]. The anhydrous metal acetates were prepared according to the procedure of Witt *et al.* [10].

The elemental analyses were performed on a Carlo Erba 1106 elemental analyzer. The ^{1}H-NMR spectra were recorded on an FX-90Q spectrometer. The IR

* Author to whom correspondence should be addressed.

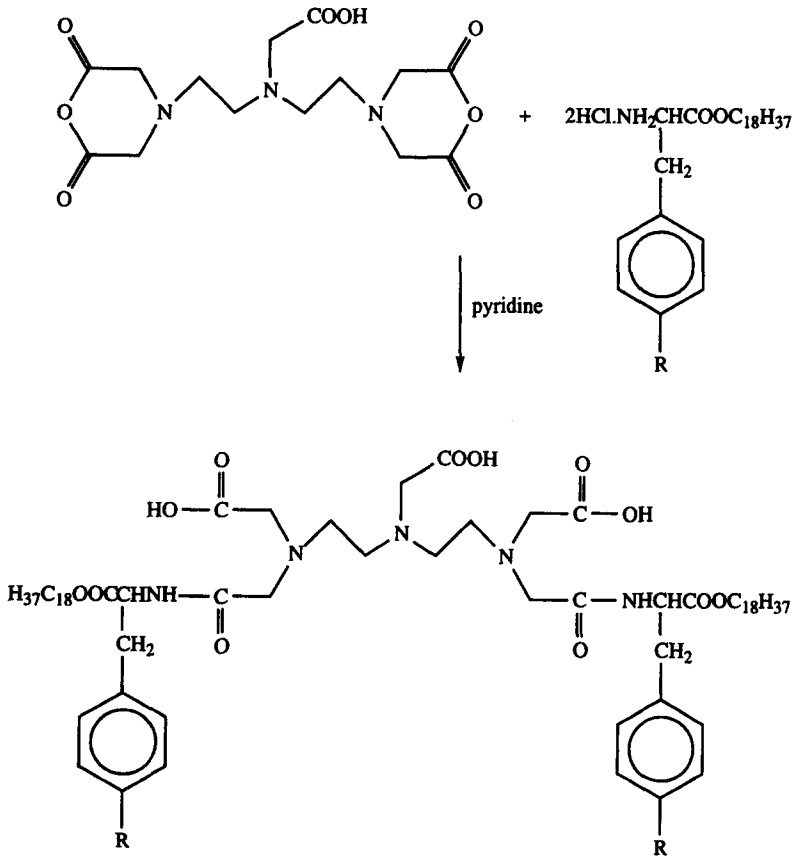
spectra were obtained on a Nicolet-5DX FT-IR spectrophotometer.

Diethylenetriamine-*N*,*N*″-di(acetyl-L-tyrosine octadecylester)-*N*,*N*′,*N*″-triacetic acid (DTPA-BTO): DTPA dianhydride 0.357 g (1.0 mmol), Tyr-$OC_{18}H_{37}\cdot HCl$ 0.939 g (2.0 mmol) were dissolved in 20 cm^3 dry pyridine and stirred at 60°C for 4 h. After removal of the solvent, the solid residue was washed with iced water and dried *in vacuo*, then recrystallized from methanol. Yield: 0.65 g (53%), m.p. = 157 ~ 160°C; Found: C: 66.5, H: 9.0, N: 5.3: Calc. for $C_{68}H_{113}N_5O_{14}$: C: 66.7, H: 9.2, N: 5.7%. ^{1}H-NMR (ppm 90 MHz, DMSO-*d*6): 8.34 (m, 2H, NHC=O), 6.90 and 6.64 (2d, 8H, Ph), 4.30 (m, 4H, CH_2OC=O), 3.92 (m, 12H, CHNHC=O+N-CH_2C=O), 3.20 (m, 8H, NCH_2CH_2N), 2.84 (m, 4H, CH_2Ph), 1.70 ~ 1.24 (m, 64H, $(CH_2)_{16}$), 0.84 (t, 6H, CH_3); IR (KBr): 2920 and 2851 (aliphatic CH_2), 1738 (acid, ester C=O), 1652 (amide C=O), 1516 and 1615 (Ph, C_6H_4), 1397 (C—N).

Diethylenetriamine-*N*, *N*″-di(acetyl-L-phenylalanine octadecylester)-*N*,*N*′,*N*″-triacetic acid (DTPA-BPO). The preparation method was the same as DTPA-BTO. Yield: 0.72 g (60%), m.p. = 134 ~ 136°C; Found: C: 68.4, H: 9.4, N: 5.9; Calc. for $C_{68}H_{113}N_5O_{12}$: C: 68.5, H: 9.5, N: 5.9%. ^{1}H-NMR (ppm, 90 MHz, $CDCl_3$): 7.70 (m, 2H, NHC=O), 7.06 (d, 10H, Ph), 4.70 (m, 4H, CH_2OCO), 3.96 (m, 12H, CHNHC=O+NCH_2C=O), 3.40 (m, 8H, NCH_2CH_2N), 3.05 (m, 6H, CH_2Ph), 1.56 ~ 1.23 (m, 64H, $(CH_2)_{16}$), 0.85 (t, 6H, CH_3); IR (KBr): 2923 and 2853 (aliphatic CH_2), 1744 (acid, ester, C=O), 1641 (amide, C=O), 1526 and 1580 (Ph, C_6H_5), 1366 (C—N).

General procedure of preparation of the metal complexes [M(DTPA-BTO), M(DTPA-BPO), M = Gd^{III}, Yb^{III}, Mn^{II}]: 1.0 mmol metal acetate in 1.0 cm^3 H_2O was added dropwise to a solution of 1.0 mmol ligand in 20 cm^3 pyridine and the resulting solution was stirred at 40°C for 1.0 h. Then the solution was evaporated to dryness. The resulting solid was washed with iced water and dried *in vacuo*.

Preparation of liposome: the liposome was obtained according to the reverse phase evaporation method [11]. Egg lecithin, cholesterol and metal complex were mixed in the molar ratios of 1:1:1. The mixture (90 μmol total lipid) was dissolved in chloroform then the clear solution was taken to dryness. The



R = H DTPA-BPO
R = OH DTPA-BTO

lipid was redissolved in 6 cm^3 of diethyl ether, then 1/10 dilution of phosphate buffered saline 2 cm^3 was added and the mixture was sonicated in a bath-type sonicator (CQ-50) under N_2 at 20°C for 4 ~ 6 min. The organic solvent was removed by a rotary evaporator and liposomes were given. The size of the liposomal vesicles was measured by electron microscopy using a JEM-100CXII. The proton longitudinal relaxation time (T_1) was determined on a WP-80 spectrometer operating at 80.13 MHz using the inverse recovery technique at 23.0 ± 1.0°C.

RESULTS AND DISCUSSION

The ligands diethylenetriamine-*N*,*N*″-di(acetyl-L-phenylalanine octadecylester)-*N*,*N*′,*N*″-triacetic acid (DTPA-BPO) and diethylenetriamine-*N*,*N*″-di(acetyl-L-tyrosine octadecylester)-*N*,*N*′,*N*″-triacetic acid (DTPA-BTO) were synthesized from DTPA dianhydride with L-phenylalanine octadecylester hydrochloride and L-tyrosine octadecylester hydrochloride respectively in pyridine. Pyridine was used as solvent and as the scavenger of hydrochloric acid as well.

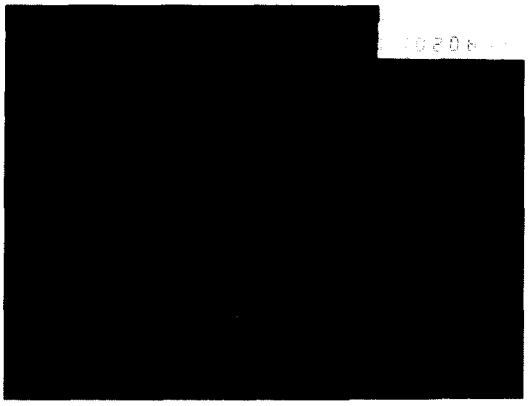
The metal complexes were prepared by reacting the ligand DTPA-BPO and DTPA-BTO with stoichiometric amounts of the metal acetate $Gd(OAc)_3$, $Yb(OAc)_3$ and $Mn(OAc)_2$ respectively in high yield. The Gd^{III} and Yb^{III} complexes are neutral non-ionic chelates, and the Mn^{II} complexes are ionic chelates. The complexes are soluble in ether, chloroform and ethanol, and insoluble in water. All the complexes are off-white solids with no clear melting point before decomposition. The C, H, N elemental data, yields and the characteristic infrared absorption data are listed in Table 1.

The infrared spectra of the complexes clearly showed the difference in coordination state of the metal complexes. The free ligands DTPA-BPO and DTPA-BTO have four different types of C=O group, ester, carboxylic acid, amide and carboxylate (zwitterions), and there are four (or three) corresponding C=O absorptions in their infrared spectra (as shown in Table 1). However, in the spectra of the metal complexes, it is observed that the spectra of these carboxyl C=O depends on the metal, that is, the coordination state of the metals. There are only two C=O absorptions in the infrared spectra of Gd^{III} and Yb^{III} complexes, and three C=O absorptions in those of Mn^{II} complexes. In the spectra of all the Gd^{III}, Yb^{III} and Mn^{II} complexes, the absorption of ester C=O in the amino acid moieties are the same as compared with those of the free ligands, suggesting that these ester C=O do not coordinate to the metal. The other absorption of C=O in the lanthanide Gd^{III} and Yb^{III} complexes is the strong broad absorption in the range of 1620 ~ 1630 cm^{-1} including the absorptions of the amide and carboxylate, indicating both the amide groups and the carboxylate groups coordinate to the Gd^{III} and Yb^{III}. In the spectra of Mn^{II} complexes, the C=O absorption of amide groups shift to lower wave number, and the C=O absorption of carboxylate groups is 1593 cm^{-1}, different from those of the free ligand, suggesting that the carboxylate groups coordinate to the Mn^{II} atom, and the amide group do not or only weakly coordinate to the Mn^{II} atom.

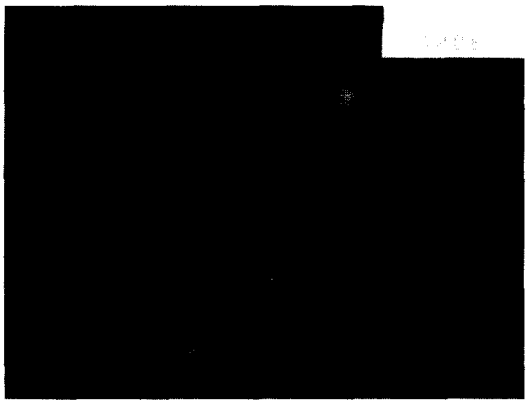
These metal complexes are not soluble in water, but they can be emulsified in warm water. The amphiphilic nature of these complexes is ideal for incorporating them into the lamellar membrane of liposomes, which has been reported as effective drug carriers for the liver [12]. The complexes were mixed with egg lecithin and cholesterol in the molar ratios of 1 : 1 : 1, respectively, and the paramagnetic metal labeled liposomes were prepared according to the reverse phase evap-

Table 1. The C, H, N elemental analysis data and the characteristic infrared absorption data of the complexes

Compounds	Yield (%)	Elemental analysis, found (calc.)			Infrared spectra		
		C%	H%	N%	C=O (ester)	C=O (amide)	C=O (carboxylate)
Gd(DTPA-BPO)·$5H_2O$	71	56.9 (56.9)	8.5 (8.4)	4.6 (4.9)	1743 (m)		1623 (s.br)
Yb(DTPA-BPO)·$4H_2O$	75	56.6 (56.9)	8.4 (8.2)	4.5 (4.9)	1741 (m)		1624 (s.br)
Mn(DTPA-BPO)·$4H_2O$	70	62.1 (62.0)	9.1 (9.0)	5.0 (5.3)	1744 (m)	1638 (s)	1593 (s)
Gd(DTPA-BTO)·$4H_2O$	72	56.4 (56.3)	8.4 (8.1)	4.3 (4.8)	1740 (m)		1626 (s.br)
Yb(DTPA-BTO)·$3H_2O$	72	56.7 (56.4)	8.4 (8.0)	4.4 (4.8)	1741 (m)		1627 (s.br)
Mn(DTPA-BTO)·$3H_2O$	68	61.1 (61.3)	9.0 (8.8)	4.9 (5.3)	1742 (m)	1615 (s)	1593 (s)



(a)



(b)

Fig. 1. Electron micrographs of liposomes prepared with (a) cholesterol: phospholipid: Gd^{3+}—DTPA—BPO (1:1:1); (b) cholesterol: phospholipid: Gd^{3+}—DTPA—BTO (1:1:1). Magnification $\times 10^4$.

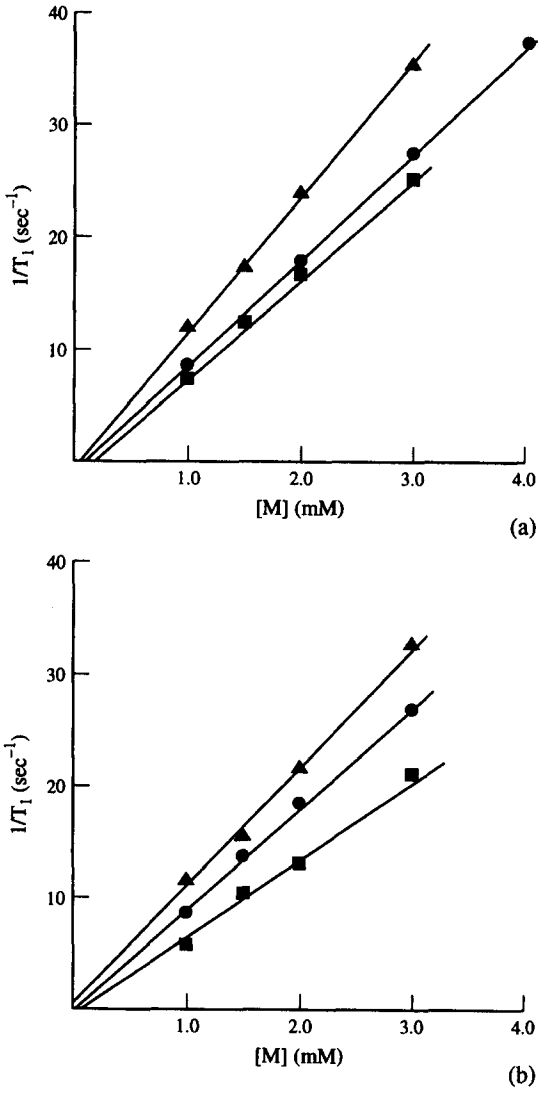


Fig. 2. Water proton $1/T_1$ values for liposomal vesicles bearing (a) Gd—DTPA—BPO (▲); Mn—DTPA—BPO (●) and Yb—DTPA—BPO (■); (b) Gd—DTPA—BTO (▲), Mn—DTPA—BTO (●) and Yb—DTPA—BTO (■).

oration method. Figure 1 shows the electron micrographs of the liposomes containing the Gd^{III} complexes. The size of the liposomal vesicles is less than about 400 nm.

Figure 2 shows the paramagnetic relaxation effects of liposomes containing the metal complexes on the surrounding water protons. The straight lines have been plotted as a function of the water proton spin-lattice relaxation rates ($1/T_1$) *vs* the concentration of metals. The relaxivity of the labeled liposomes can be obtained from the slope of the corresponding line. The relaxivities (R_1) of the Gd^{III}, Yb^{III} and Mn^{II} complexes of DTPA-BPO are 12.11, 8.46 and 9.23 mM^{-1} s^{-1} respectively, and those of the Gd^{III}, Yb^{III} and Mn^{II} complexes of DTPA-BTO are 10.86, 6.55, 9.09 mM^{-1} s^{-1} respectively. These data indicate that all the complexes containing liposomes have strong relaxation effects on the protons in surrounding water molecules. The metal complexes of DTPA-BPO have higher relaxivities than the corresponding metal complexes of DTPA-BTO. The ytterbium complexes are less effective than the corresponding Gd^{II} and Mn^{II} complexes.

In conclusion, the amphiphilic paramagnetic chelates of Gd^{I}, Yb^{III}, and Mn^{II} with DIPA-BPO and DTPA-BTO have been prepared and characterized. The amphiphilic nature of these complexes made them easily incorporated into the lamella of the membrane of liposomes. The metal complexes and the labeled liposomes showed high water proton spin-lattice relaxation rates ($1/T_1$). The further investigation including imaging, biological and toxicological assays are on-going.

REFERENCES

1. Lauffer, R. B., *Chem. Rev.*, 1987, **87,** 901.
2. Simon, J. H. and Szumowski, J, *Radiology*, 1989, **172,** 717.
3. Magerstagt, M., Gansow, O. A., Brechbiel, M. W., Colcher, D., Baltzer, L., Knop, R. H., Girton, M. E. and Naegele, M., *Magn. Reson. Med.*, 1986, **3,** 808.
4. Kabalka, G. W., Davis, M. A., Moss, T. H., Buonocore, E., Hubner, K., Holmberg, E., Maruyama, K. and Huang, L., *Magn. Reson. Med.*, 1991, **19,** 406.
5. Grant, C. W. M., Karlik, S. and Florio, E., *Magn. Reson. Med.*, 1989, **11,** 236.
6. Cavagna, F., Dapra, M., Maggioni, F., de Haen, C., and Felder, E., *Magn. Reson. Med.*, 1991, **22,** 333.
7. Adams, D. H., Joyce, G., Richardson, V. J., Ryman, B. E. and Widniewski, H. M., *J. Neurol. Sci.*, 1977, **31,** 173.
8. Sosnovsky, G., Li, S. W. and Rao, N. U. M., *Z. Naturforsch.*, 1985, **40b,** 1558.
9. Penney, C. L., Shah, P. and Landi, S., *J. Org. Chem.*, 1985, **50,** 1457.
10. Witt, J. R. and Onstott, E. I., *J. Inorg. Nucl. Chem.*, 1962, **24,** 637.
11. Szoka, F., Olson, F., Heath, T., Vail, W., Mayhew, E. and Papahadjopoulos, D., *Biochim. Biophys. Acta*, 1980, **601,** 559.
12. Unger, E. C., MacDougall, P., Cullis, P. and Tilcock, C., *Magn. Reson. Imaging*, 1989, **7,** 417.