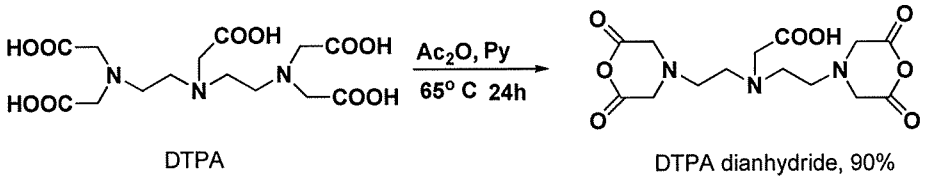
refluxed for 24h. After completion of the reaction excess gadolinium was removed by treating with Chelex 100 resin (Na form 100-200, 2.8 g). After removal of excess gadolinium water was removed under reduced pressure and the resulted dried compound was dissolved in 30 ml of ion exchange water and to this 20 ml of 1N NaOH solution was added and stirred for 2 h at room temperature to hydrolyse the protected acetyl groups. The resulted solution was then treated with DOWEX 50W-X8 ion exchange resin(H form 100-200, 2.5 g). After completion of treatment, resin was filtered off and filtrate was evaporated to give the Gd-DTPA-HMTA-Glc(OH) in 0.85g (80%) of yield.

2-11-4 Synthesis of Gd-DTPA-E complex

In my experiment, the new Gd-DTPA-E was synthesized by the reaction of DTPA-E with equimolar amount of gadolinium in water which was in turn prepared from DTPA di anhydride and ethylene diamine.

2-11-4-1 Synthesis of DTPA dianhydride

DTPA dianhydride was prepared according to the previously established procedure (1). DTPA was added to a mixture of acetic anhydride and pyridine and stirred thoroughly over a period of 24h at 65°C under inert conditions. After the completion of reaction mixture was filtered and washed thoroughly with acetic anhydride and acetonitrile. This was dride over suffident peiod of time under vacuum and the yield of the product was 90%. Scheme 2-11-08 depicted the preparation route of DTPA dianhydride.



DTPA

DTPA dianhydride, 90%

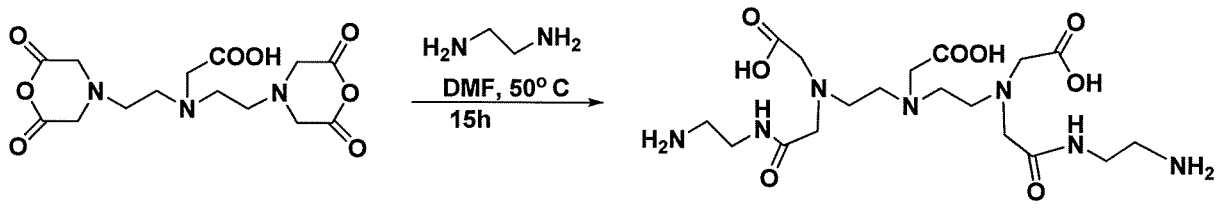
Scheme 2-11-08 Synthesis of DTPA dianhydride

^{1}H-NMR and ^{13}C-NMR spectra of DTPA dianhydride were shown in Fig.2-11-10, 2-11-11 respectively. From the IR spectrum, we can find the peakes at 1820cm^{-1} (C=O stretch symmetric), 1774cm^{-1} (C=O asymmetric stetching) which are charecteristics of cyclo-dianhydride. ^{1}H-NMR spectrum of DTPA dianhydride exhibited four different chemical shift values at 4.0ppm, 3.6ppm, 3.5ppm and 3.2ppm with expected coupling. ^{13}C-NMR spectrum of it showed distinct singlets at 171.65 and 165.67 also suggests the presence of di-anhydride system. All the above furnished information has confirmed the structure of dianhydride system.

2-11-4-2 Synthesis of DTPA-E

To a solution of DTPA di anhydride in DMF was added excess amount of ethylene diamine and the reaction mixture was thoroughly stirred at 50°C for a period of 15h. After completion of the reaction

DMF was removed under reduced pressure and the excess of E was removed by washing repeatedly with chloroform.



Scheme 2-11-09 Synthesis of DTPA-E

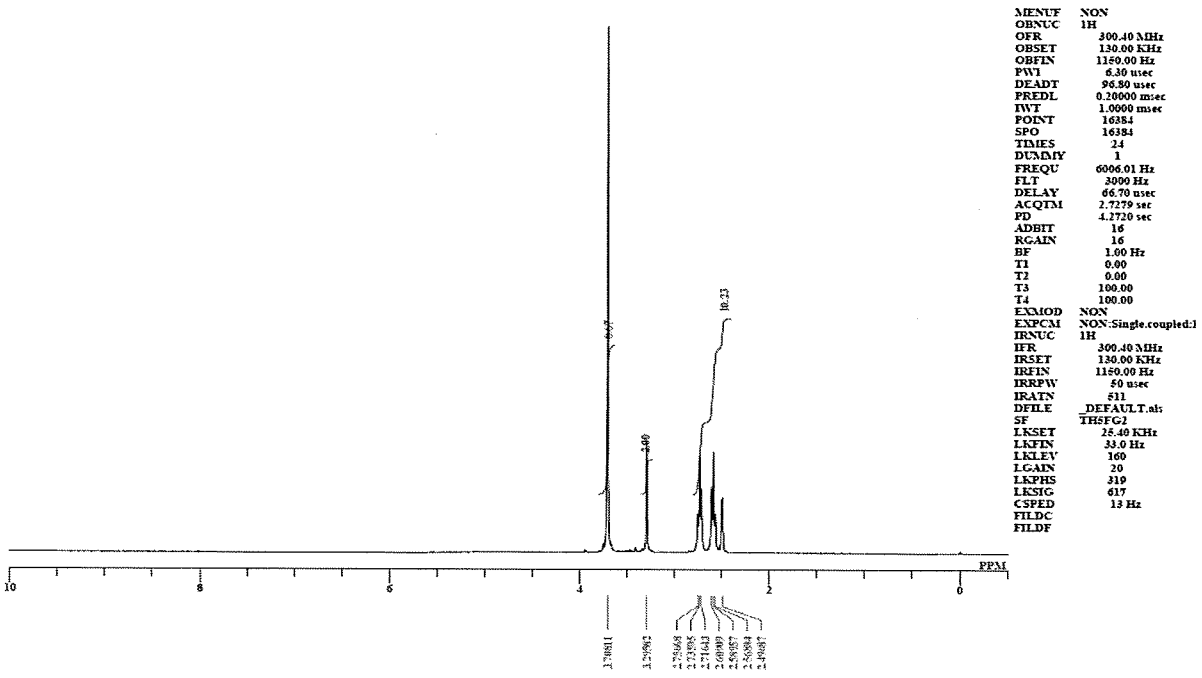


Fig. 2-11-10 ^{1}H-NMR of DTPA di-anhydride

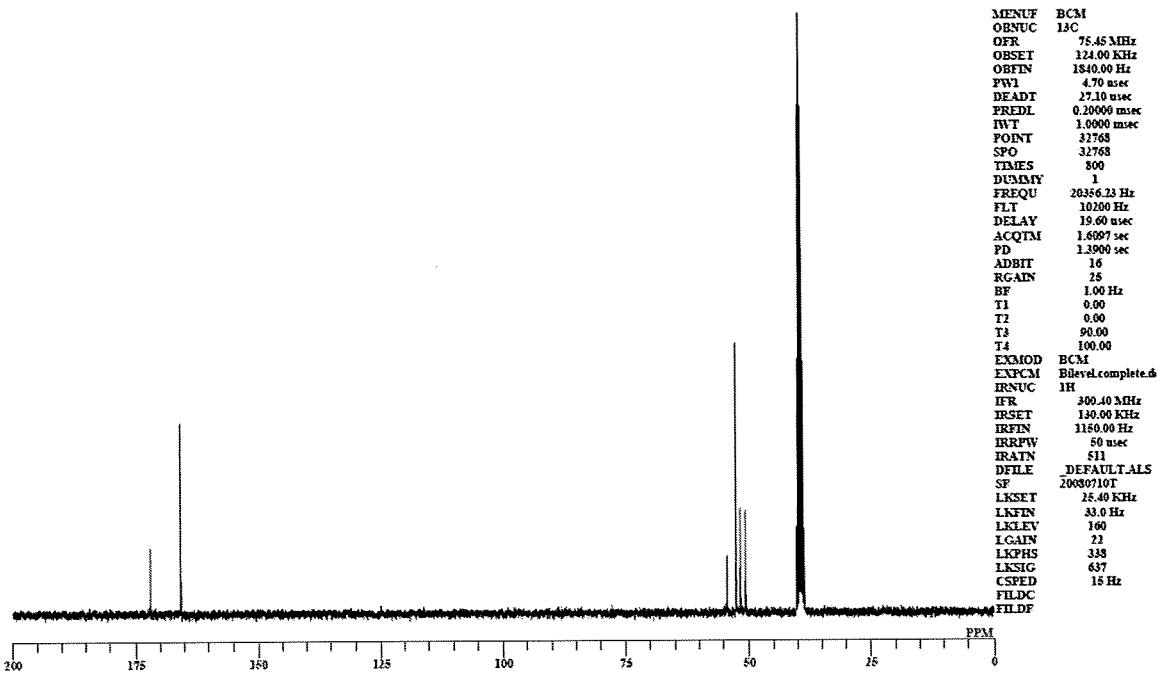


Fig. 2-11-11 ^{13}C-NMR of DTPA di-anhydride

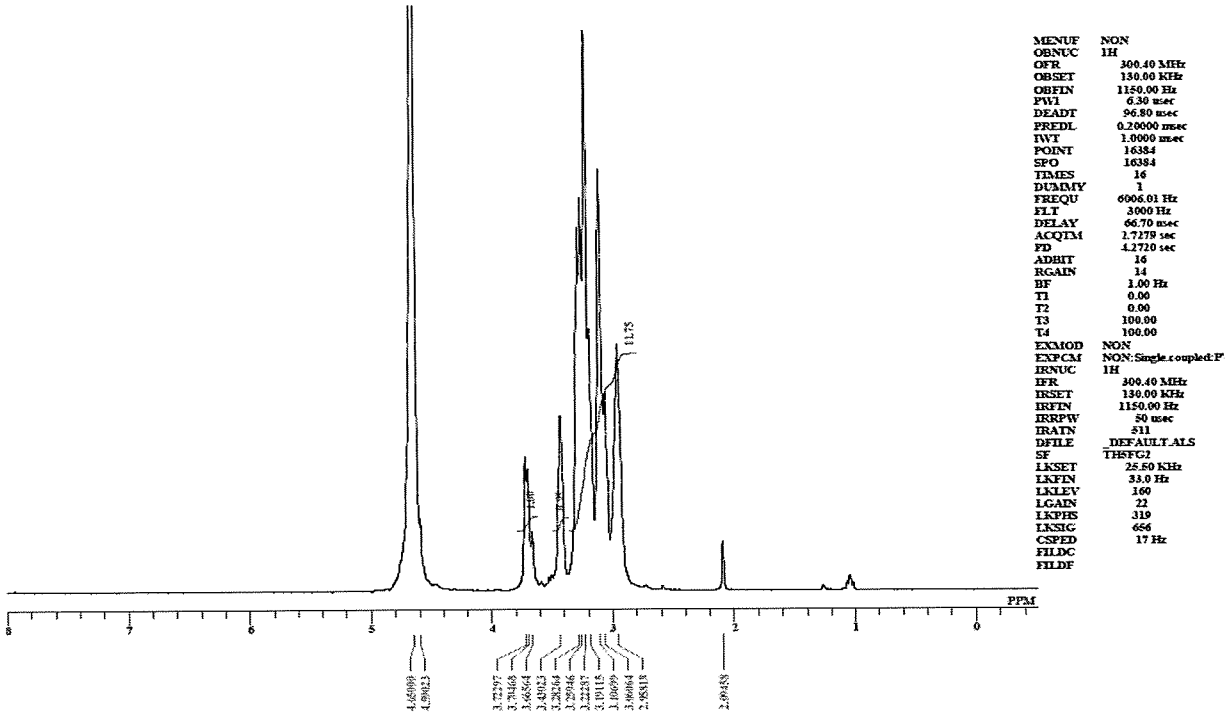
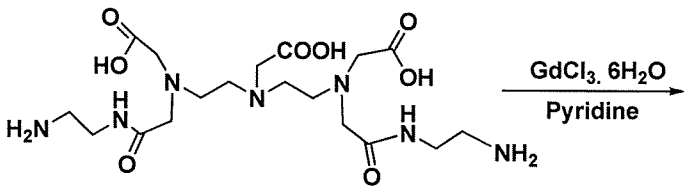


Fig. 2-11-12 ^{1}H-NMR of DTPA-E

2-11-4-3 Synthesis of Gd-DTPA-E

To a solution of DTPA-E in ion exchange water was added pyridine, gadolinium chloride and stirred the reaction at 50°C for 24h. After completion of the reaction water was removed and the resulted crude product was washed with ether repeatedly and dried.



特許出願の関係で

ここでは開示できない

Scheme 2-11-10 Synthesis of Gd-DTPA-E

2-11-4-4 Synthesis of DTPA dianhydride

DTPA (3.0g, 7.6 mmol) was added to a mixture of acetic anhydride (3.0 ml, 31.8 mmol) and pyridine (4.5 ml, 53.6 mmol) and the reaction mixture was thoroughly stirred for 24h at 65° C. After that reaction mixture was washed repeatedly with acetic anhydride (20ml) and acetonitrile (20ml). White solid obtained was dried strongly to get 2.45g (6.94 mmol), 90%.

IR (KBr): ν (cm^{-1}) ; 3445(O=C<u>OH</u>), 1816 (O=COC=O), 1761 (<u>O=C</u>OH)

^{1}H-NMR

δ (ppm) = 3.70 (s, -CO-<u>CH_2</u>-CO- X 4)

=3.29 (s, -<u>CH_2</u>COOH)

=2.73 (t, $COCH_2N$<u>CH_2</u>CH_2N X 2)

=2.58 (t, $COCH_2NCH_2$<u>CH_2</u>N X2)

^{13}C-NMR

δ (ppm)= 171.9 (<u>O=C</u>OH)

=165.7 (<u>O=C</u>OC=O)

=54.5 (N<u>C</u>H_2COOH)

=52.1 (<u>C</u>H_2O=COC=O)

=50.7, 51.7 (NCH_2CH_2 X 2)

2-11-4-5 Synthesis of DTPA-E

To a solution of DTPA dianhydride 1.0g (2.8 mmol) in DMF was added 0.94g (14.0 mmol) of ethylene diamine and the reaction mixture was stirred at 50°C over a period of 15h. After completion of reaction DMF was removed under reduced pressure and the crude product was washed with chloroform to remove the excess of E. This was recrystallised from *i*-propanol to yield 1.02g (78%) as pale while compound.

^{1}H-NMR
δ (ppm) = 3.71 (s, N$\underline{CH_2}$C=ONH X 2)
= 3.43 (s, NCH_2COOH X 3)
= 3.39-2.90 (m)

2-11-4-6 Synthesis of Gd-DTPA-E

To a solution of DTPA-E 0.8g (1.60 mmol) in ion exchange water was added 1.30g (16.0 mmol) of pyridine was added and stirred thoroughly. To this solution 0.62g (1.60 mmol) of gadolinium chloride was added and the temperature of the reaction was kept constant at 50°C and refluxed for 24h. After completion of the reaction excess gadolinium was removed by treating with Chelex 100 resin (Na form 100-200, 2.8 g). After removal of excess gadolinium resin was filtered off and the filtrate was rota-evaporated under reduced pressure and the crude complex was washed with 2-propanol to get 0.82g (75%) of pale yellow compound.

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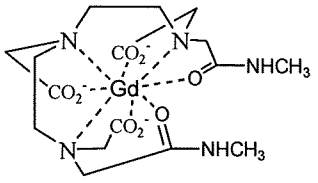
2-12 Synthesis of DTPA amido ester ligands

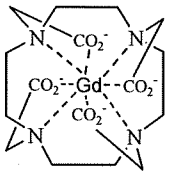
Yu Gang

2-12-1 Introduction

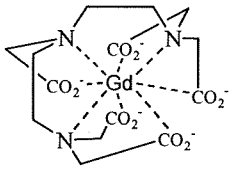
Magnetic resonance imaging (MRI), which is based on the principles of nuclear magnetic resonance (NMR) where a spectroscopic technique is used by scientists to obtain microscopic chemical and physical information about molecules, is safe and efficient technique for human in clinical diagnosis. Polyaminopolycarboxylic complexes of gadolinium ion are the most widely used contrast agents in MRI because the paramagnetic lanthanide Gd(Ⅲ) can increase locally the longitudinal relaxation rate of surrounding tissue water, highlighting the intensity of specific tissue areas in T_1 weighted images.[1] However, now, contrast agents (Fig. 2-12-01) in clinical currently being used suffer from several defects, for example, they are not tissue-specific, rapidly excreted and their synthesis required a complex, tedious and expensive process, and or they may provoke allergic reactions in the recipient. Therefore, a novel target-specific MRI contrast agent with favorable properties needs to be developed.



Gd(DTPA-BMA)
(BMA=bis(methylamide))



Gd(DOTA)
(DOTA=1,4,7,10-tetraazacyclo-dodecane-1,4,7,10-tetraacetate)



Gd(DTPA)
(DTPA=diethylenetriamine-pentaacetic acid)

Fig. 2-12-01 Currently being used MRI contrast agents

In our lab, a novel Gd-DTPA derivative for MRI contrast agent has been prepared (Fig. 2-12-02) that displayed a good property on relaxivity and specific *in vivo* distribution, [2, 3] but the disadvantage of this contrast agent is that it cannot be excrete from body in time because its molecular size is too big or its affinity to materials in blood vessel is too high. The other disadvantage is that its synthesis required a complex, tedious and expensive process. These defects limited the application of this novel MRI contrast agent.

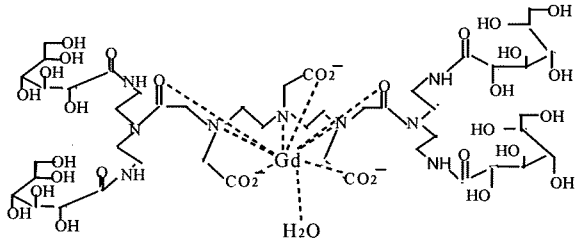


Fig. 2-12-02 The chemical structure of Gd-DTPA-D1

To overcome these disadvantages, Gd-DTPA derivatives have been prepared by using amino acid to react with DTPA anhydride or using amino alcohol as a linker to react with sugar to obtain amino ester.

2-12-2 Results and discussion

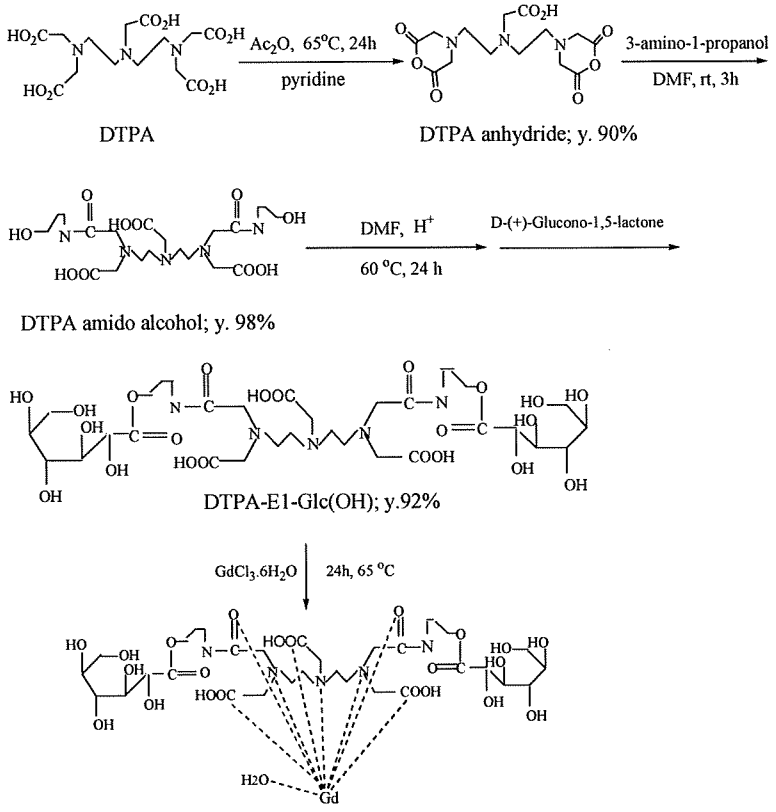
In my experiments, three Gd-DTPA derivatives have been prepared, which are Gd-DTPA-bis(amido sugar),Gd-DTPA-amino acids and Gd-DTPA-ME sugar.

2-12-2-1 Synthesis of Gd-DTPA-bis(amido sugar)

The ligand DTPA-bis(amido sugar) was obtained by reaction between D-(+)Glucono-1,5 lactone and DTPA-bis(amido alcohol) which can be prepared by reaction between DTPA bis(anhydride) and 3-amino-1-propanol. The Scheme 2-12-01 is the preparation route to Gd-DTPA-bis(amido sugar).

The structure of ligands can be identified by IR, ^{1}H-NMR and MS. The IR, ^{1}H-NMR and MS spectra of ligands were shown in Fig.2-12-03~Fig.2-12-05. From the ^{1}H-NMR spectra, the chemical shift appeared in the regions of 4.3~4.7ppm because of the existence of sugars. In the IR spectra of the ligands, the peaks at 1735 cm^{-1} and 1664cm^{-1} were attributed to C=O (ester).

Further, the evidence for the existence of ligands was obtained from the mass spectrum. From the mass spectra, the peaks at m/z 508, 686, 864 can be found. The peak at m/z 508 is hydrolyzed ligand DTPA-bis(amido alcohol) without sugar; the peak at m/z 686 is DTPA derivate containing one sugar; the peak at m/z 864 is DTPA monohydrolyzed containing two sugars. Also, from the mass spectrum of Gd-DTPA-bis(amido sugar) (Fig.2-12-06), we can find the seven peaks from m/z 663~669, 840~846, 1036~1042, which are the evidence for the existence of Gd-DTPA derivative.



Scheme 2-12-01 The preparation route to Gd-DTPA-bis(amido sugar)

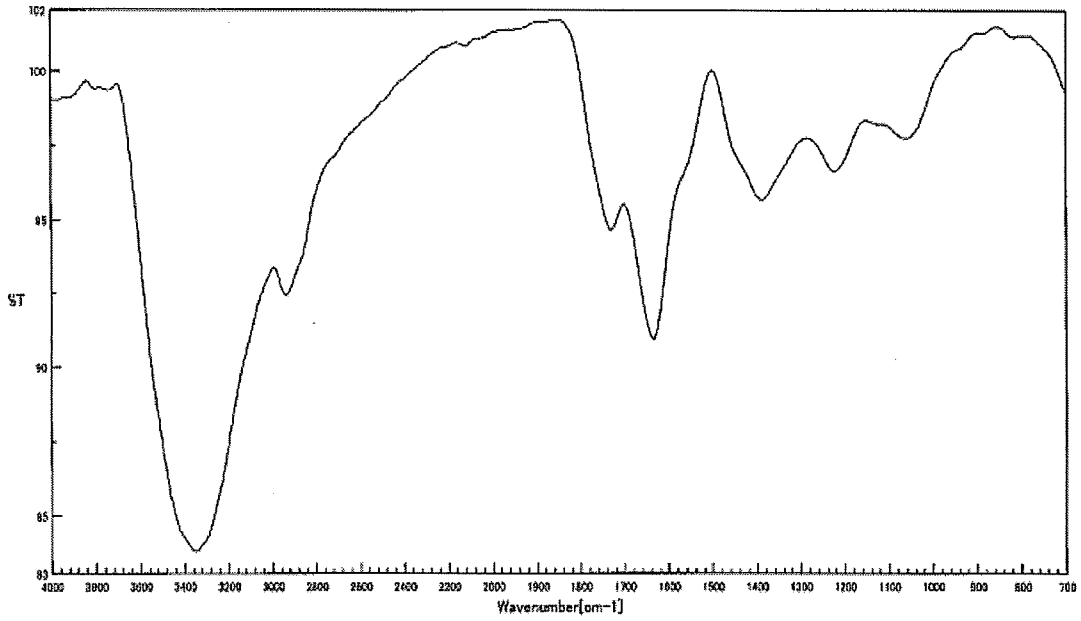


Fig.2-12-03 The IR spectrum of DTPA-bis(amido sugar)

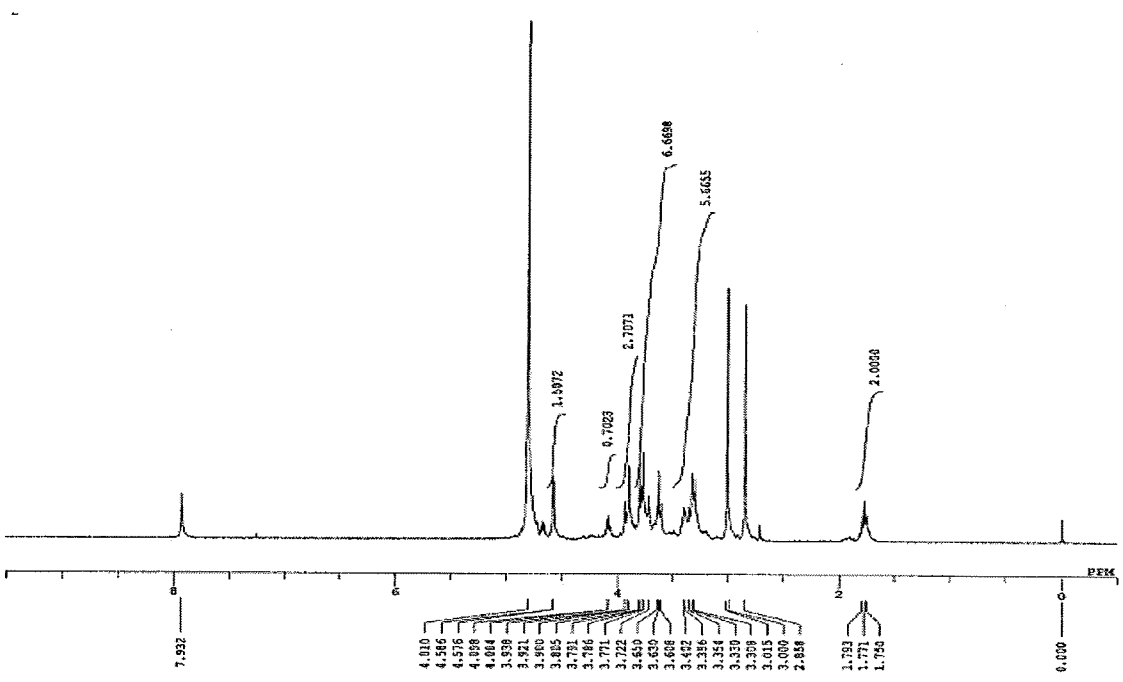


Fig.2-12-04 The ^{1}H-NMR spectrum of DTPA-bis(amido sugar)

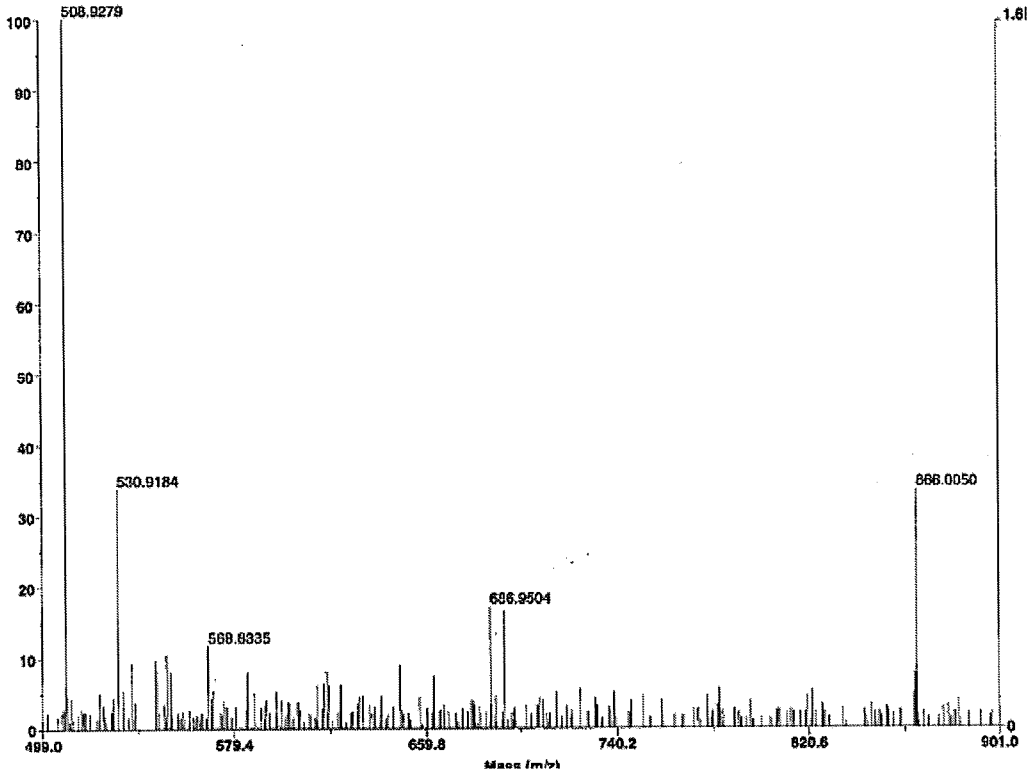


Fig.2-12-05 The MS spectrum of DTPA-bis(amido sugar)

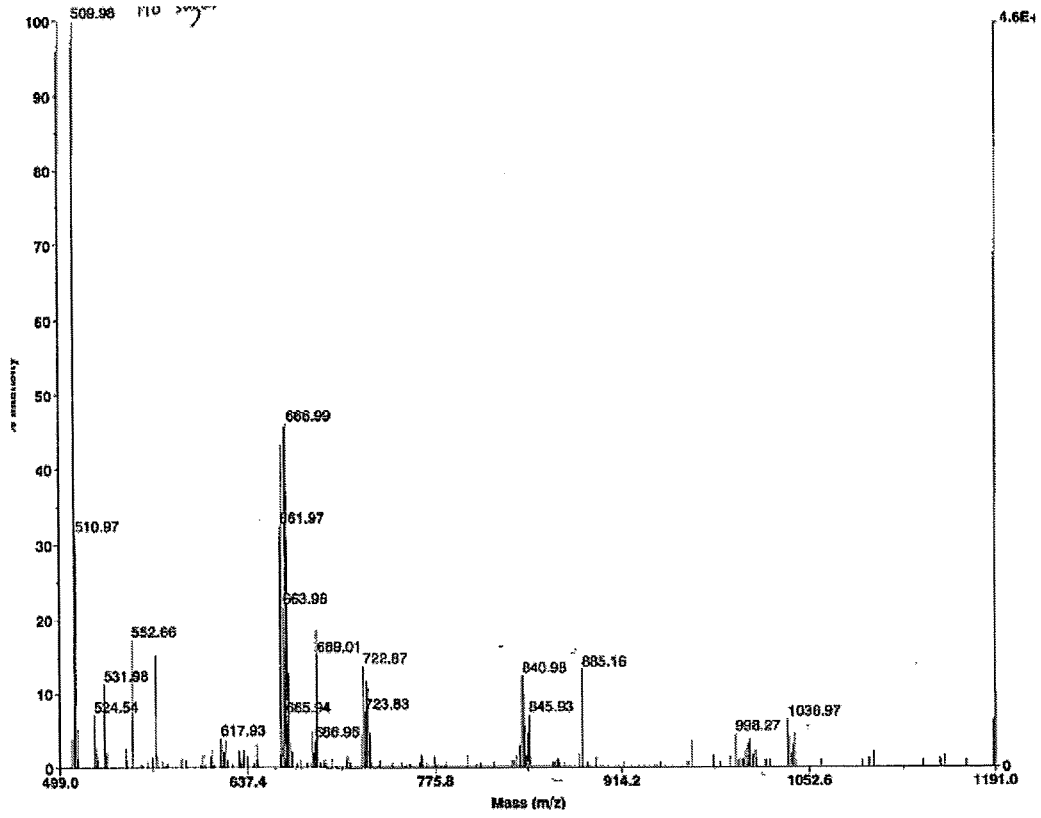
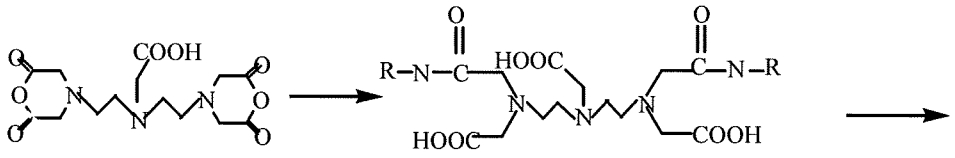


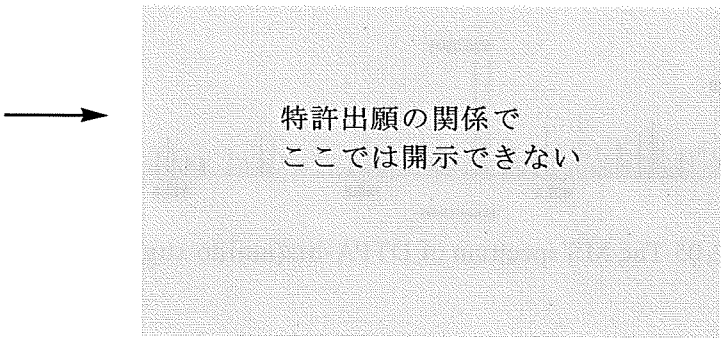
Fig.2-12-06 The MS spectrum of Gd-DTPA-bis(amido sugar)

2-12-2-2 Synthesis of Gd-DTPA amino acid

To obtain small molecular sized Gd-DTPA derivatives, Gd-DTPA amino acids can be prepared by reaction between DTPA and a series of amino acid, for example, L-Valine, L-Serine and L-Leucine. The Scheme 2-12-02 is the preparation route to Gd-DTPA amino acides.



y. 85~95%



R_1=CH(COOH)CH_2OH; R_2=CH(COOH)$CH(CH_3)_2$; R_3=CH(COOH)$CH_2CH(CH_3)_2$

Scheme 2-12-02 The preparation route to Gd-DTPA amino acids

The evidence for the existence of ligands and Gd derivatives were obtained from the mass spectrum. From the mass spectra of Fig. 2-12-07~2-12-09, the peaks at m/z 592, 568 and 620 can be found which are peaks of DTPA-L-Valine, DTPA-L-Serine and DTPA-L-Leucine, respectively. From the mass spectra of Fig. 2-12-10~2-12-12, we can find the seven peaks at m/z 743~749, 719~726 and 772~779, which are the evidence for the existence of Gd-DTPA L-Valine, Gd-DTPA-L-Serine and Gd-DTPA-L-Leucine, respectively. However, from the Fig. 2-12-13~2-12-15, when free Gd has be removed, we can find the peaks at m/z 743~749, 719~726 and 772~779 disappeared because not only free Gd but also Gd of Gd derivatives have be removed, which indicate the stability of Gd-DTPA derivatives is low. So, it is necessary to improve the stability of Gd derivatives.

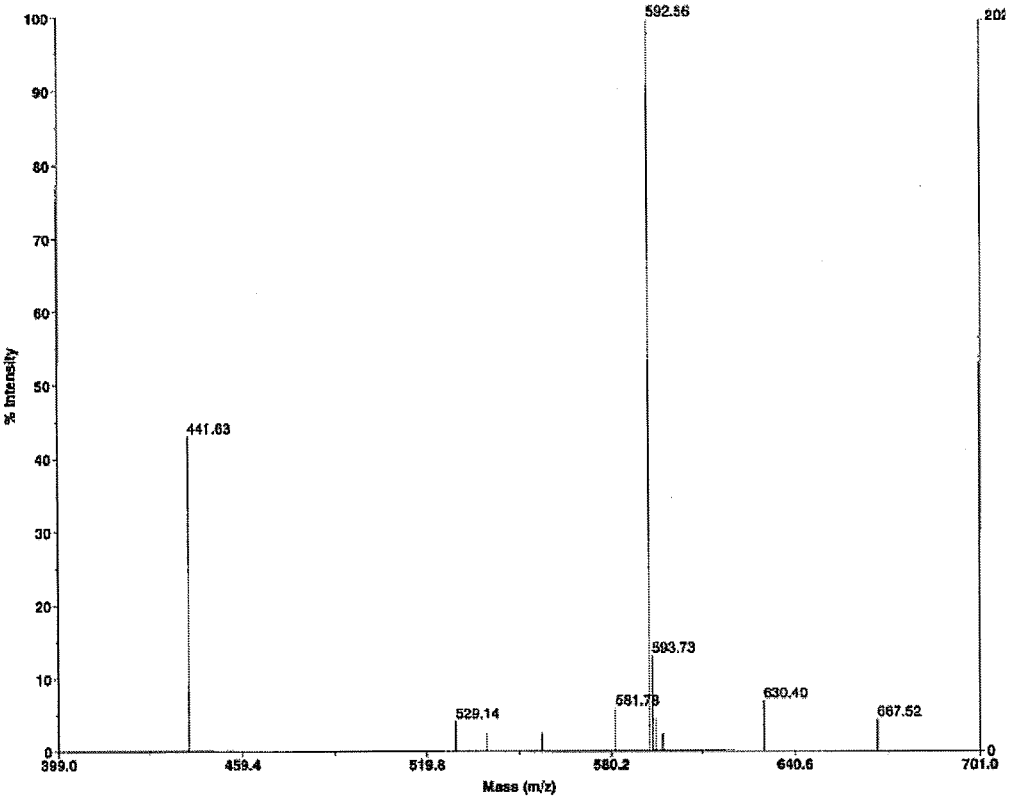


Fig.2-12-07 The MS spectrum of DTPA-L-Valine

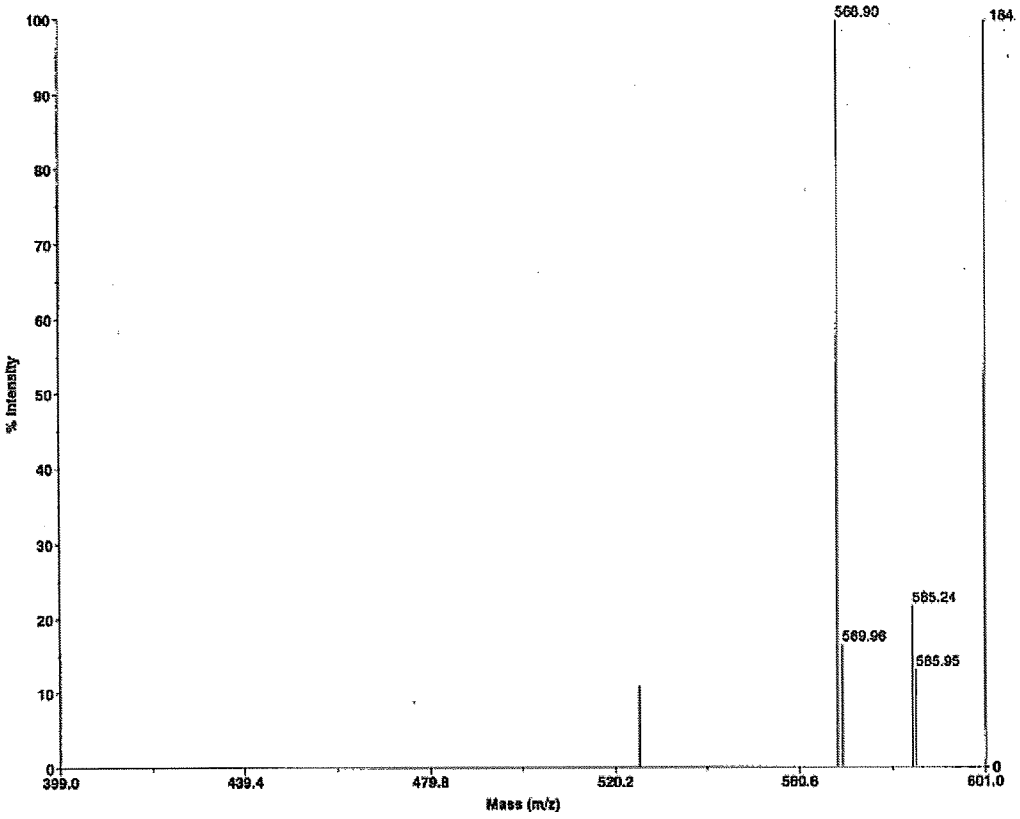


Fig.2-12-08 The MS spectrum of DTPA-L-Serine

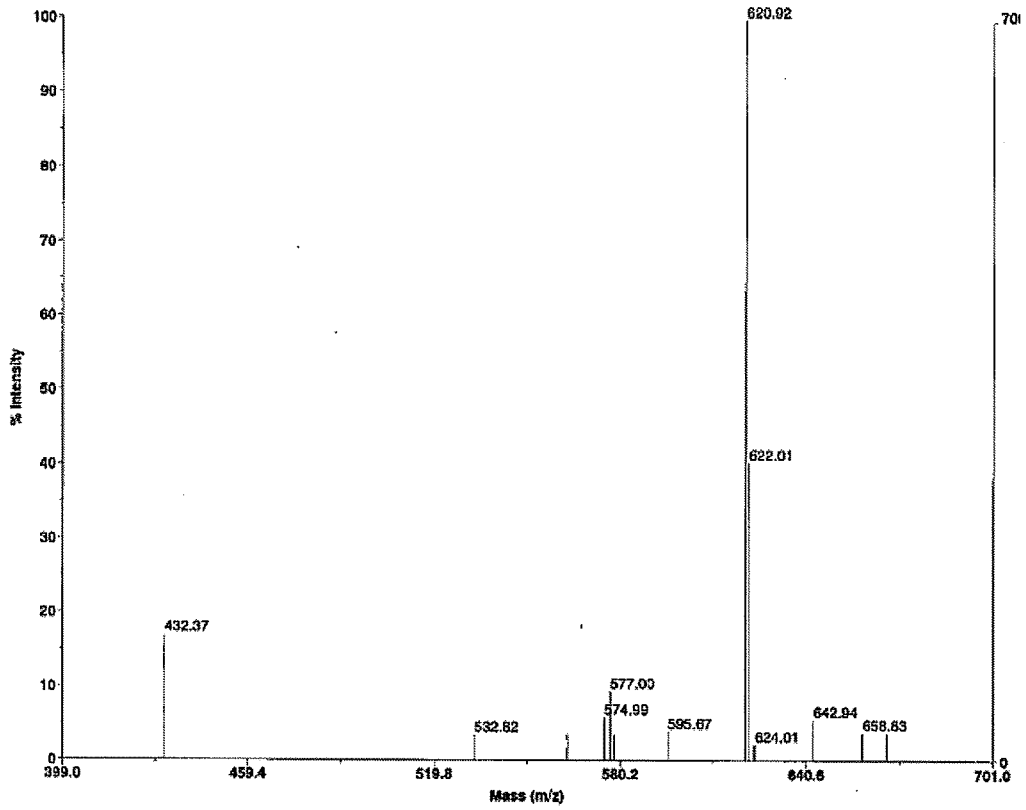


Fig.2-12-09 The MS spectrum of DTPA-L-Leucine

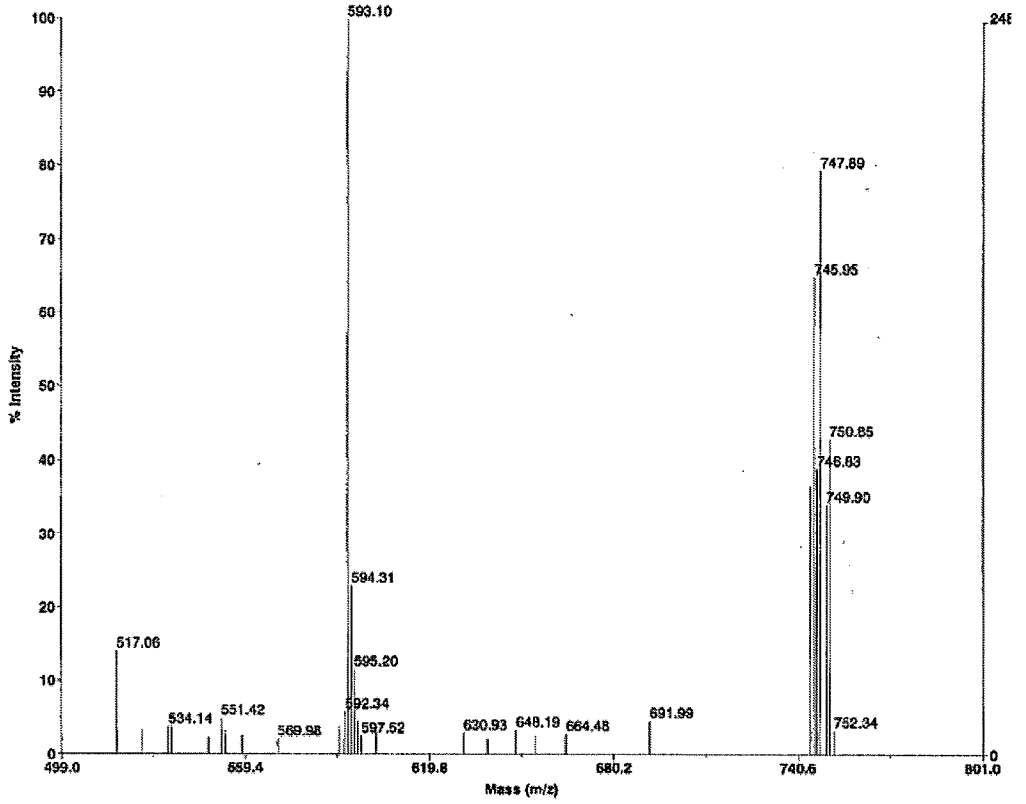


Fig.2-12-10 The MS spectrum of Gd-DTPA-L-Valine

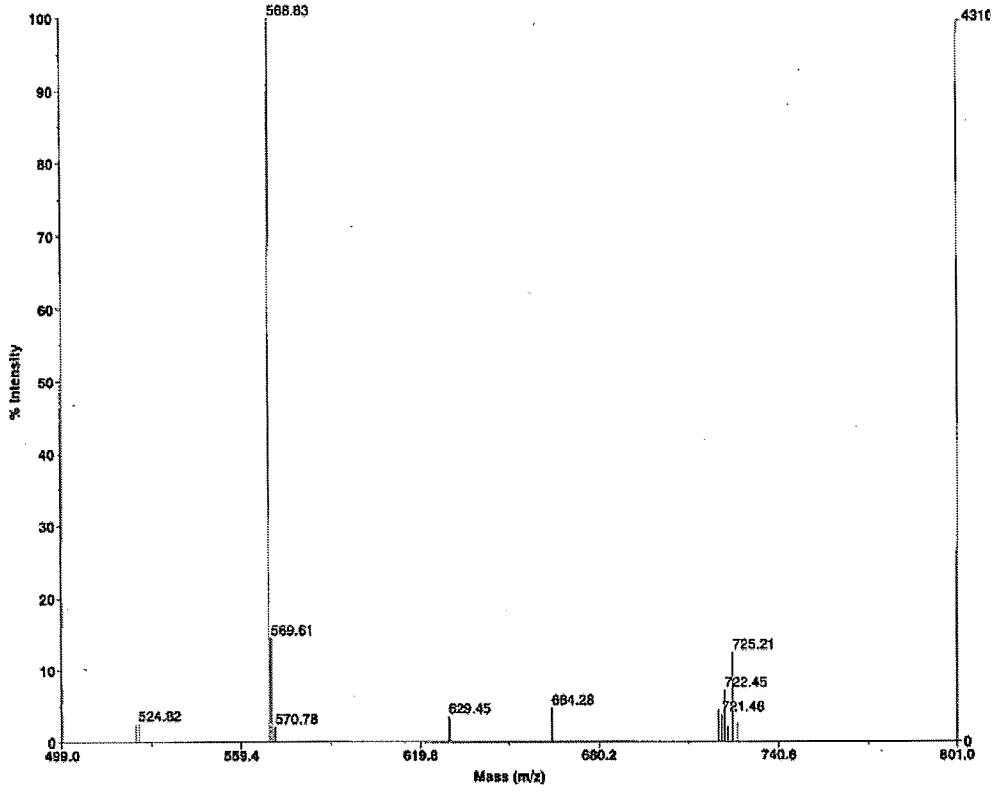


Fig.2-12-11 The MS spectrum of Gd-DTPA-L-Serine

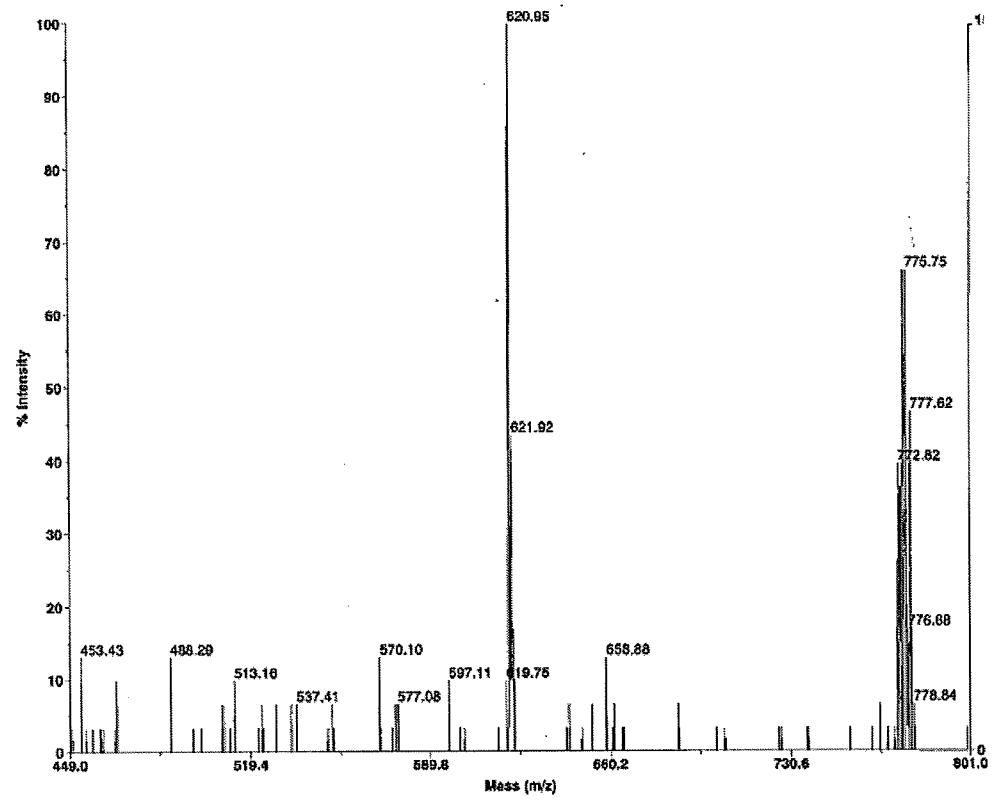


Fig.2-12-12 The MS spectrum of Gd-DTPA-L-Leucine

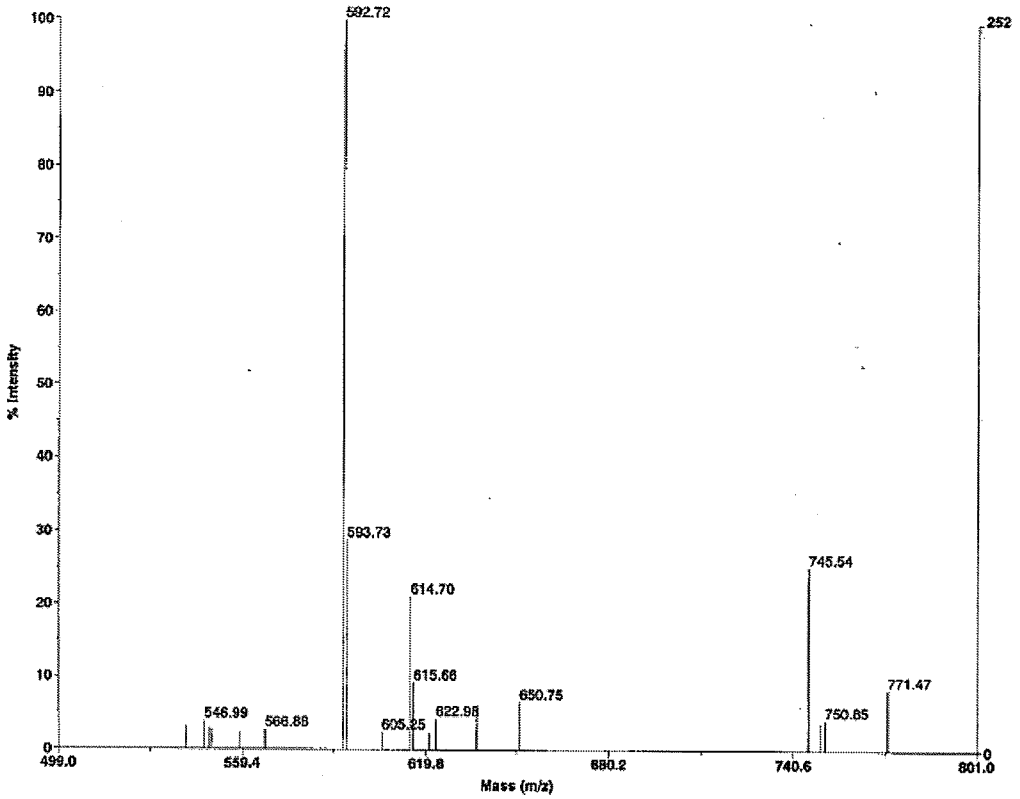


Fig.2-12-13 The MS spectrum of Gd-DTPA-L-Valine (Free Gd be removed)

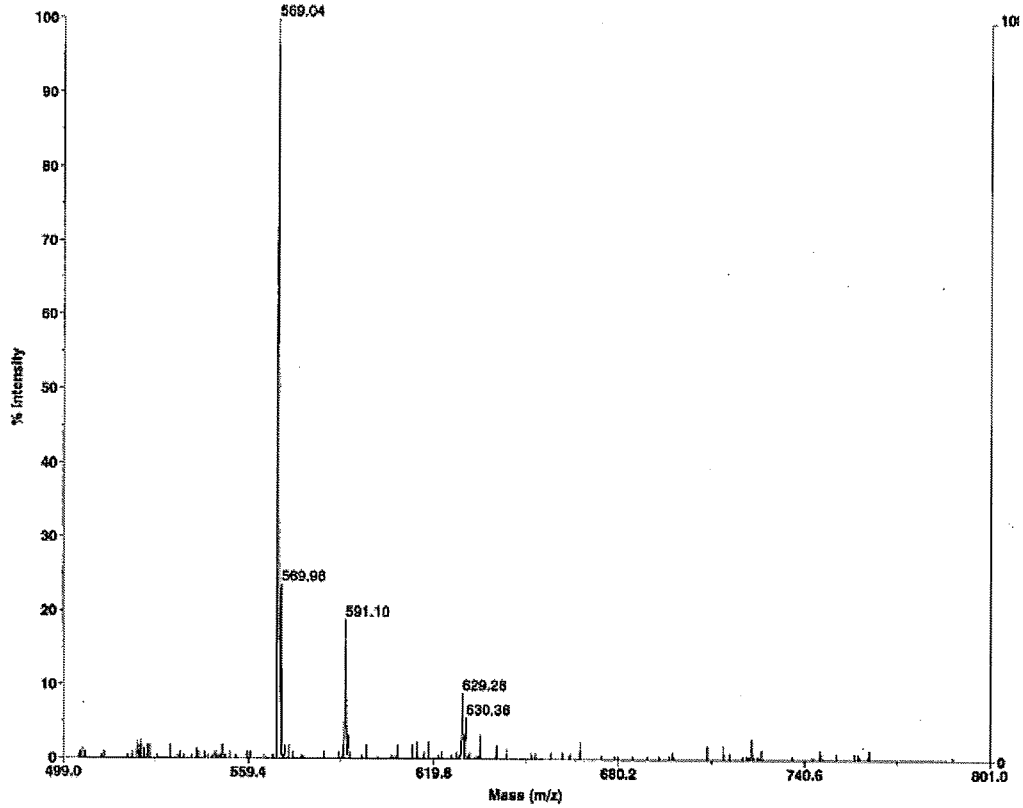


Fig.2-12-14 The MS spectrum of Gd-DTPA-L-Serine (Free Gd be removed)

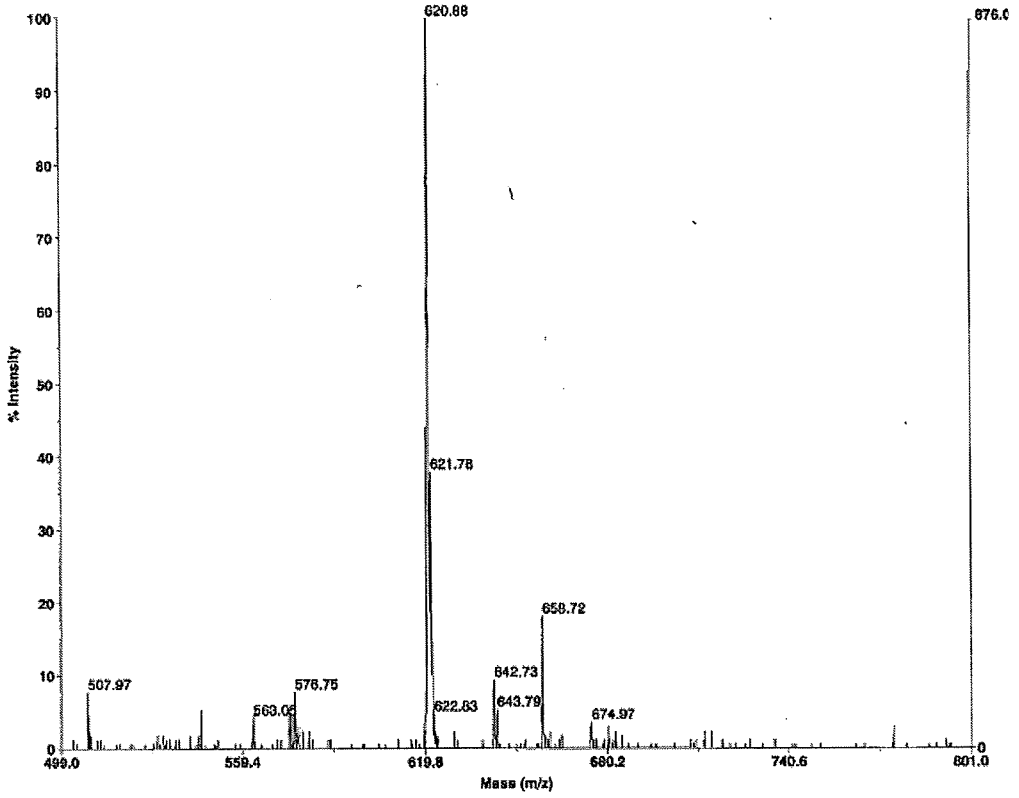
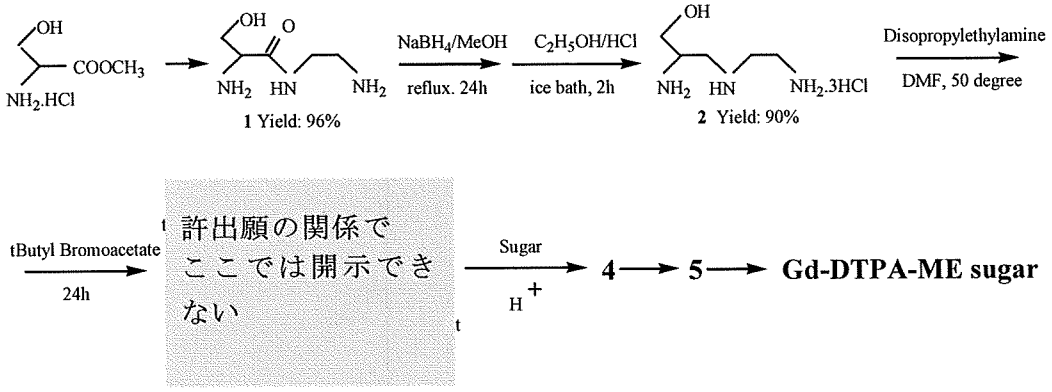


Fig.2-12-15 The MS spectrum of Gd-DTPA-L-Leucine (Free Gd be removed)

2-12-2-3 Synthesis of Gd-DTPA-ME sugar

According to some references, [4, 5] we have prepared a novel ligand for MRI contrast agent to improve the stability of Gd-DTPA derivative. The Scheme 2-12-03 is the preparation route to Gd-DTPA-ME sugar.



Scheme 2-12-03 The preparation route to Gd-DTPA-ME sugar

The Complex 1, Complex2 and Complex3 were synthesized according to previously reported procedures. [4,5] Complex 3 react with sugar for 24 h at 60℃ to obtain complex 4. Concentrated HCl was added and the solution was stirred overnight at room temperature to get complex 5.At last, complex 5 react with $GdCl_3.6H_2O$ at 60℃ for 24 h to obtain Gd-DTPA-ME (exact structure of complex 4~6 can not be disclosed).

The structure of complexes can be identified by MS and ^{1}H-NMR. The MS and ^{1}H- NMR spectra of complexes were shown in Fig.2-12-16~Fig.2-12-21.

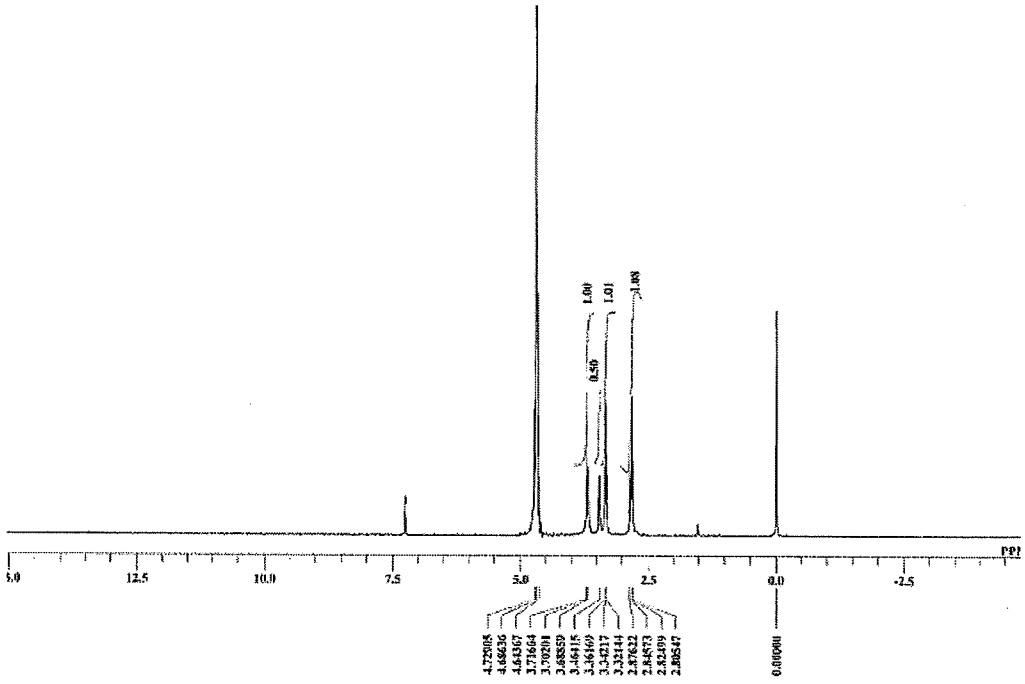


Fig.2-12-16 The ^{1}H-NMR spectrum of Complex1

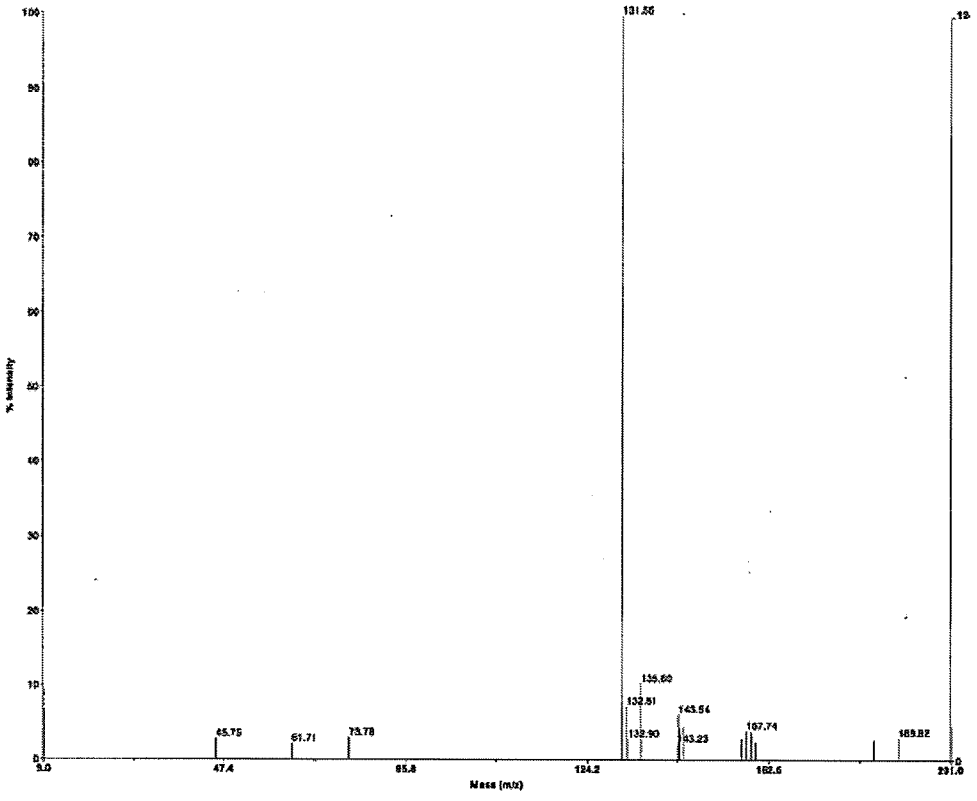


Fig.2-12-17 The MS spectrum of Complex 2

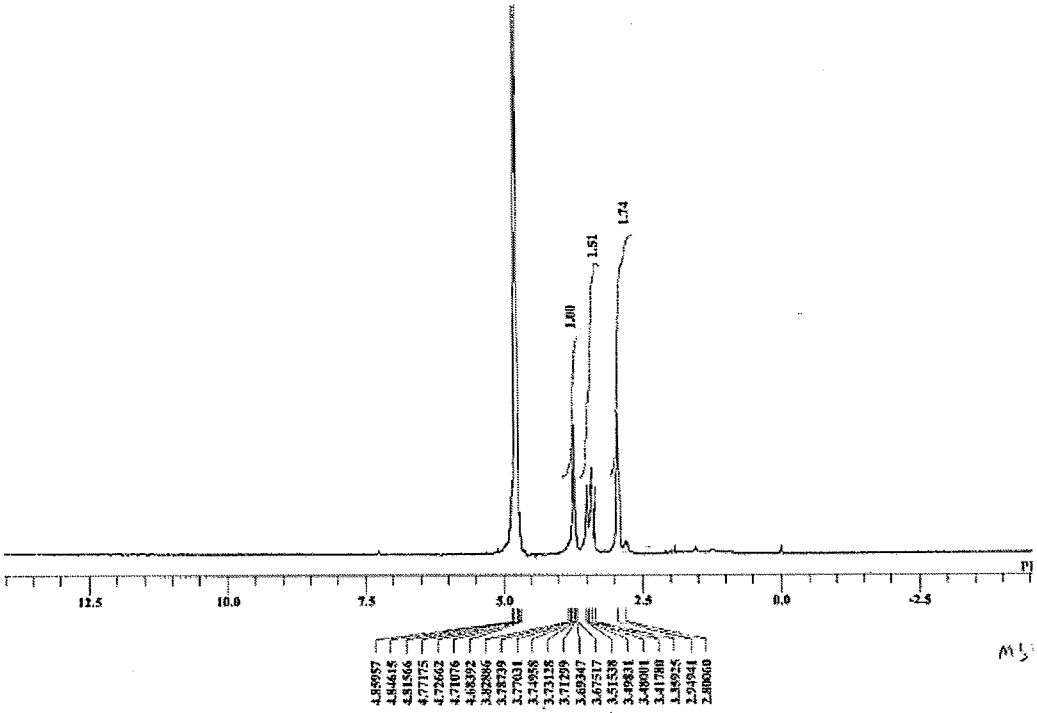


Fig.2-12-18 The ^{1}H-NMR spectrum of Complex 2

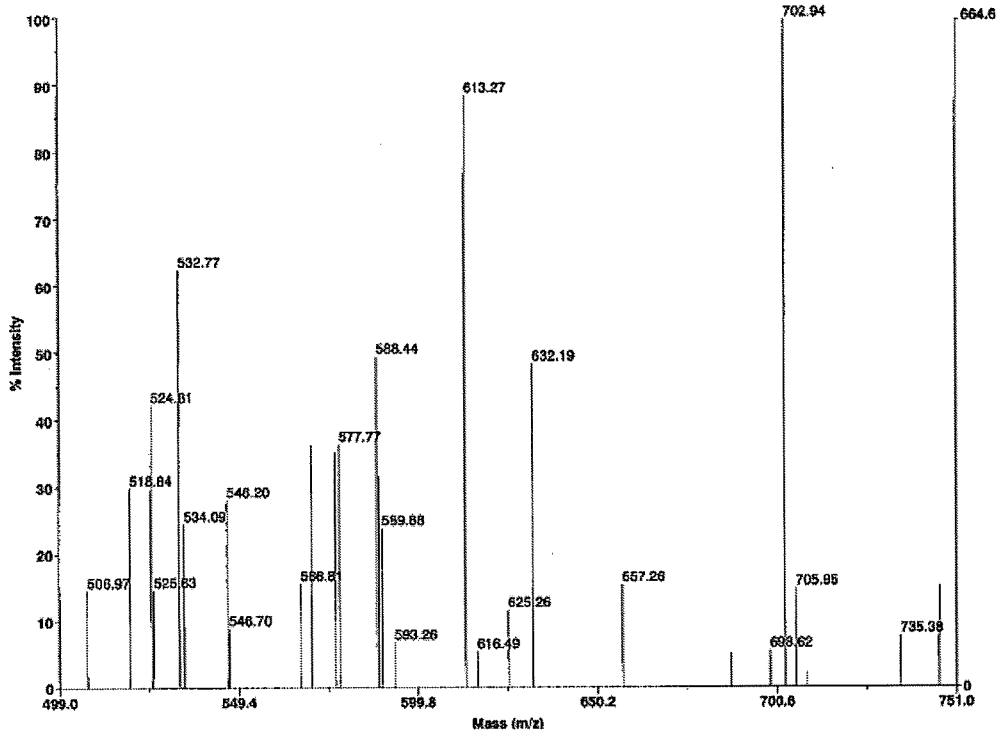


Fig.2-12-19 The MS spectrum of Complex 3

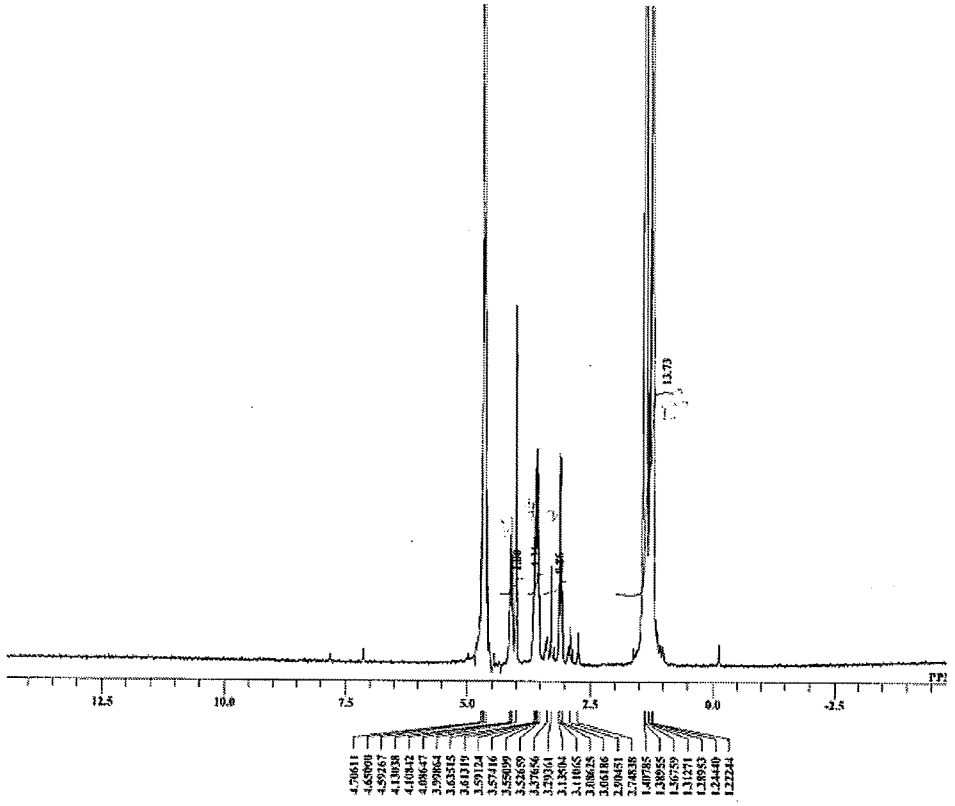


Fig.2-12-20 The ^{1}H-NMR spectrum of Complex 3

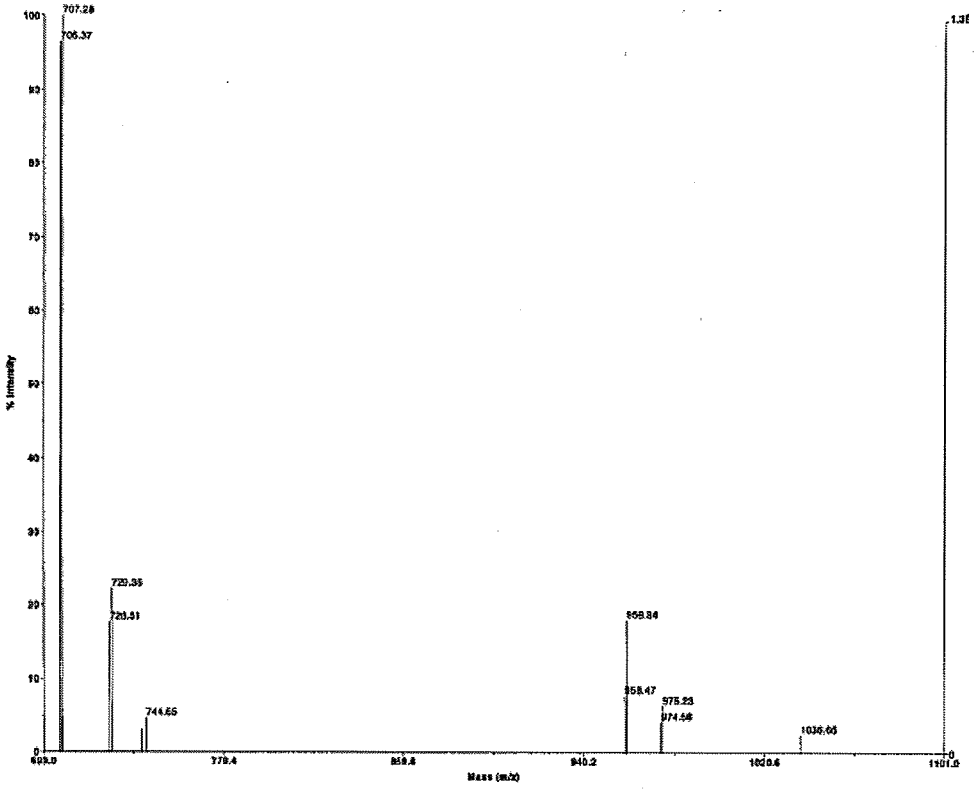


Fig.2-12-21 The MS spectrum of Complex 4

2-12-2-4 Physical property of Gd-DTPA-bis(amido sugar)

The MR image intensity in ^{1}H-NMR signal of water protons linked with Gd(III) is dependent on nuclear relaxation times.[6] They have a good correlation to the relaxation rate of the protons. The parameters of relaxation times for Gd-DTPA-bis(amido sugar) was examined. The relaxivity vs. temperature profile is shown in Fig.2-12-22.

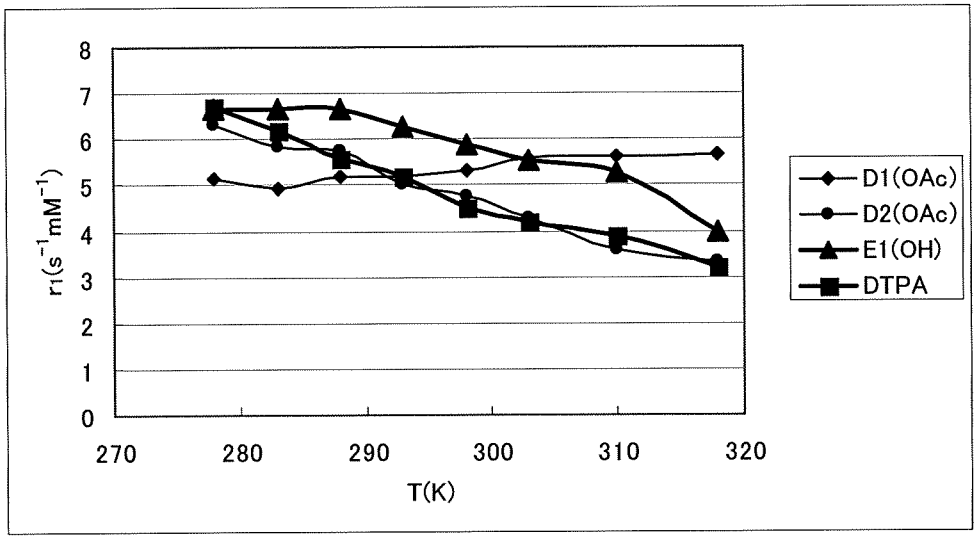


Fig.2-12-22 Relaxivity of the Gd- DTPA-bis(amido sugar) vs. temperature profile.

These results show that relaxivity of Gd-DTPA-bis(amido sugar) decreased as temperature risen, that is same with the change of relaxivity of Gd-DTPA. However, at 310K, we can find the relaxivity of Gd-DTPA-bis(amido sugar) is higher than Gd-DTPA which indicate Gd-DTPA-bis(amido sugar) may better than Gd-DTPA as MRI contrast agents.

2-12-3 Conclusions

We have successfully synthesized a new gadolinium conjugated compound by a simple two-step reaction. This complex can be obtained by using DTPA-bis(amido sugar) as the ligand, and using gadolinium(III) as the paramagnetic centers. From the relaxivity of Gd-DTPA-bis(amido sugar), we think these paramagnetic polymetallic species has a potential as MRI contrast agents.

Also, we have prepared a series of Gd-DTPA amino acids. However, the stability of Gd derivatives is low, which limit the application.

To modify DTPA, a novel contrast agent also has been prepared. The feasibility of this metal complex as new potential candidates for MRI contrast agent is now in progress. The results will be published in future elsewhere. According to the chemical structure of Gd-DTPA-ME sugar, we hope this complex has a potential as MRI contrast agents.

2-12-4 Experimental

D-(+)Glucono-1,5 lactone, DMF, DTPA, amino acids, serine methyl ester, 3-amino-1-propanol and another material were obtained from Wako Pure Chemical Industries, LTD. The DTPA-bis(anhydride) was synthesized according to previously reported procedures. [7] ^{1}H-NMR spectra were collected on JEOL EX300 (300MHz). IR spectra were measured on a FTIR-spectrometer, using reflection on silicon. Elemental analysis was performed on a FLASH EA1112. The MS measurement was performed on a VOYAGER-DE Porimerix.

2-12-4-1 Synthesis of DTPA-bis(amido sugar)

To a solution of DTPA anhydride 0.2500g (0.700mmol) in dry DMF (10mL), 3-amino-1-propanol 0.1051g (1.400mmol) was dropped into solution at room temperature. After 3 h of stirring, the solvent was evaporated to dryness under reduced pressure. The residue was dissolved in dry DMF (10mL) containing 1mL of ether saturated with hydrogen chloride, and the D-(+)Glucono-1,5 lactone 0.2469g (1.39mmol) was added and the reaction mixture was heated at 60 ℃ for 24 h. The solvent was removed under reduced pressure and the compound was washed with ethanol and ether and dried in vacuum 3 h. The residues were the product as a yellow crystal: yield, 0.5512 g (0.624 mmol), 95%.

IR (reflection on silicon): $\upsilon(cm^{-1})$=3320 (O-H), 1735 (O=C), 1648, 1543 (N-C), 1203, 1089(C-O-C)

^{1}H-NMR (300MHz, D_2O),

δ(ppm): = 1.79 (t, 4H; CH_2-CH_2×2)

= 3.32~3.41 (m, 12H; CH_2-N×6)

= 3.64 (s, 2H; CH_2-COOH)

= 3.73 (s, 4H; CH_2-COOH×2)

= 3.77 (s, 4H, CH_2-CO-NH×2)

= 3.87 (d, 4H, CH(OH)-OH)

= 3.92 (t, 4H; CH_2-O×2)

= 4.37 (t, 6H, CH(OH)-CH×6)

= 4.65 (d, 2H, CH(OH)-CO-O×2)

MS: 863

2-12-4-2 Synthesis of Gd-DTPA-bis(amido sugar)

DTPA-bis(amido sugar) (0.5562 g, 0.644 mmol) was dissolved in pyridine (10 mL), and the solution of $GdCl_3.6H_2O$ (0.2549g, 0.137mmol) in water (1 mL) was added. After 24 h of stirring at 65 ℃, the solvent was removed and the crude product was then refluxed in ethanol for 1 h. After cooling to room temperature, the complex was filtered off and dried in vacuum at room temperature. The product was a light yellow solid, yield: 0.6423g (0.618mmol), 96%.

Elem. Anal. Calcd.: (found): $C_{33}H_{57}N_5O_{22}.H_2O$: C, 36.34 (34.19), H, 5.78 (5.62), N, 6.69 (6.62).

IR (KBr): $\upsilon(cm^{-1})$=3361 (υOH), 1664 (O=C), 1538 (N-C=O), 1226 (C-N).