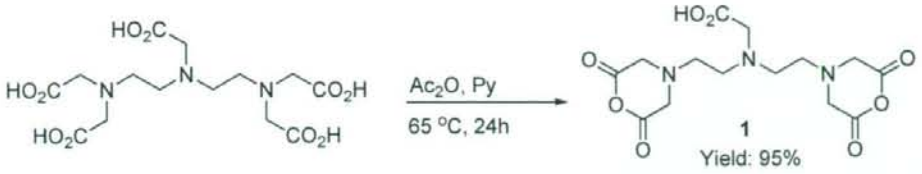
2-4-2 Results and Discussion



Scheme 2-4-05 Synthesis of Target molecule

IR, ^{1}H-NMR and ^{13}C-NMR spectra of DTPA dianhydide were shown in fig.2-4-2-01 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 3.7 ppm, 3.3 ppm, 2.7 ppm and 2.5 ppm with expected coupling. ^{13}C-NMR spectrum of it showed distinct singlets at 171.65 and 165.67 also suggests the presence of dianhydide system. All the above furnished information has confirmed the structure of dianhydride system.

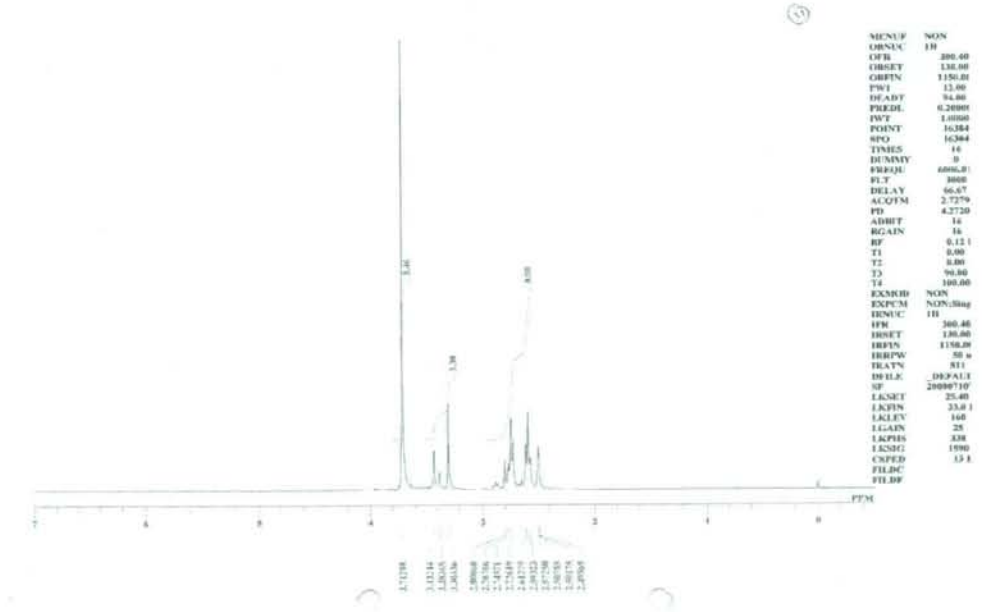
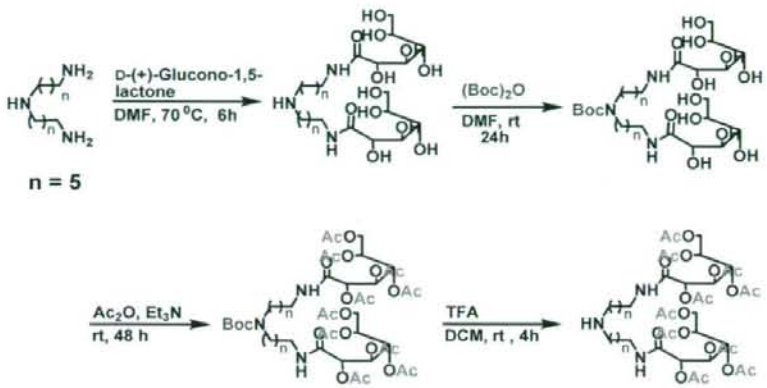


Fig. 2-4-01 ^{1}H-NMR of DTPA dianhydride

Synthesis of terminal

To a solution of D-(+)-Glucono-1, 5-lactone in dry DMF was added hexaethelene triamine at 70^0C. After 6 h of stirring, the mixture was left in the refrigerator for an overnight. To the solution of the mixture was added (Boc) $_2$O at 0^oC then stirred for 24h at room temperature. The mixture was added into Et_3N and Ac_2O at 0^oC then stirred for 2days at room temperature. After completion of the reaction, the solution was purified, and the solvent was evaporated to dryness under reduced pressure to get a brown crystal. Trifloroacetic acid was added into solution of the crystal in CH_2Cl_2, and the mixture was allowed to react for 4h at room temperature. After the completion of the reaction, the solution was purified. And then, the solvent was removed by rota-evaporator under reduced pressure. At last, the product isolation by recycle GPC gave terminal in 65 %, yield. The Scheme is given bellow.



Scheme 2-4-06 Synthesis of Terminal

^{1}H-NMR, ^{13}C-NMR and mass spectral analysis of the above compound was shown in Fig. 2-4-02 respectively. ^{1}H-NMR spectrum of it showed different chemical shift values at exhibited regions 5.66-5.06 ppm, 4.34-4.10 ppm, 3.23 ppm, 2.96 ppm, 2.20-204 ppm and 1.67-1.32 ppm with expected multiplicity confirmed its structure.

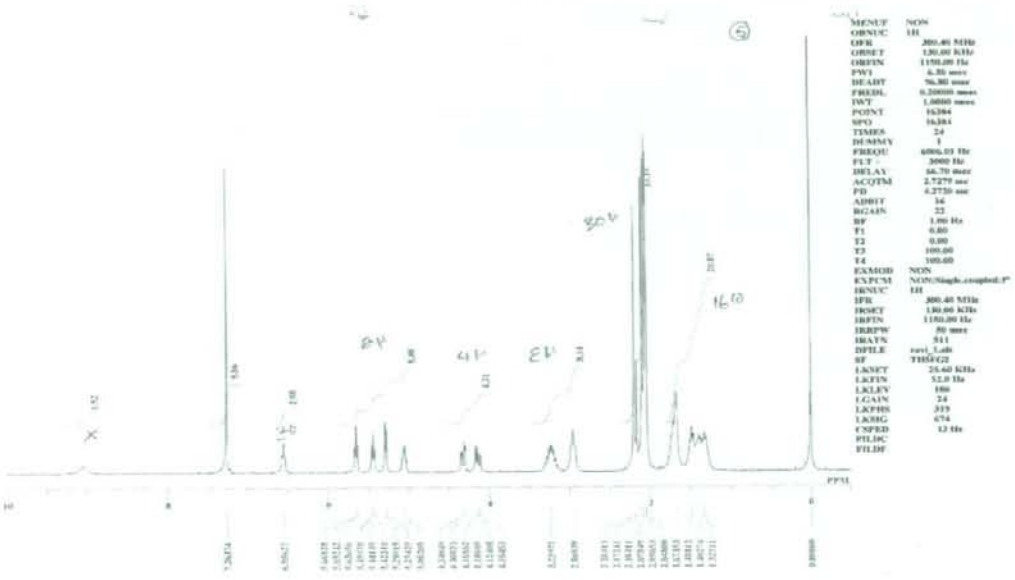
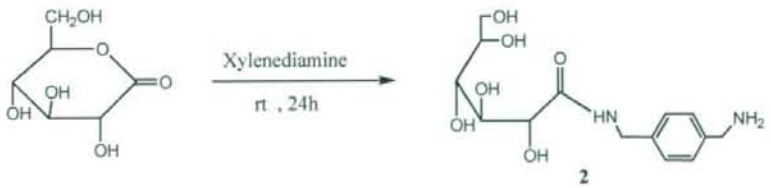


Fig. 2-4-02 ^{1}H-NMR of Terminal

Synthesis of terminal

To a solution of D-(+)–Glucono-1,5-lactone (1g, 7.3mmol) in dry DMF(20mL) was added xylenediamine (1.3g, 7.3 mmol) ,then stirred for 24h at room temperature. After completion of the reaction, the solution was purified, and the solvent was evaporated to dryness under reduced pressure to get a yellow crystals. The yield of the compound is 90% .The Scheme is given bellow.



Scheme 2-4-07 Synthesis of Terminal

^{1}H-NMR, ^{13}C-NMR and mass spectral analysis of the above compound was shown in fig 2-4-03 respectively. ^{1}H-NMR spectrum of the above compound it showed different chemical shift values at exhibited regions 8.10-8.05 ppm, 7.2-7.1 ppm, 4.50-4.05 ppm, 3.95-3.32 ppm, 2.88 ppm, 2.73 ppm and 2.50 ppm with expected multiplicity confirmed its structure.

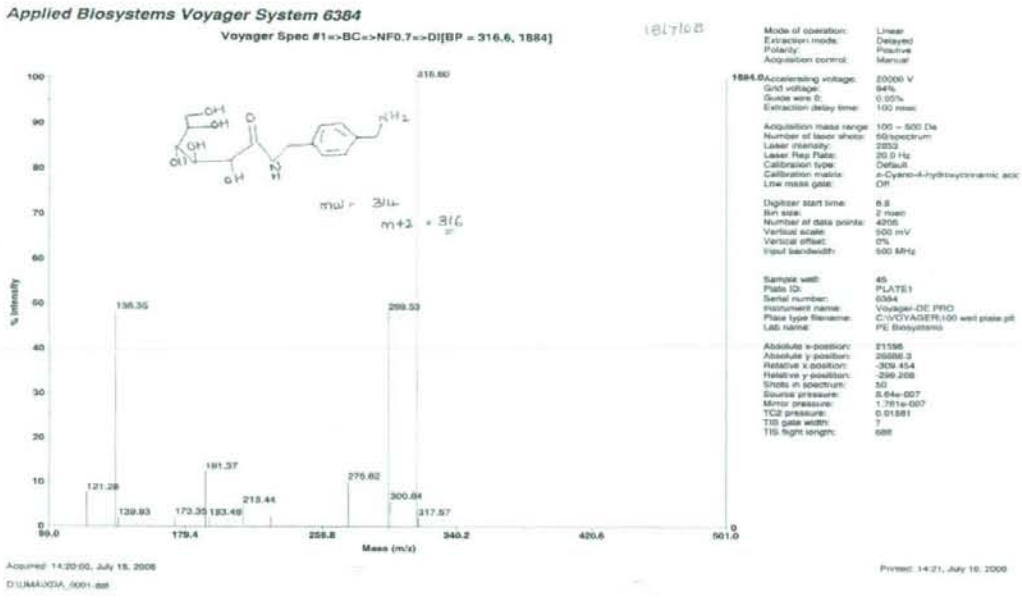
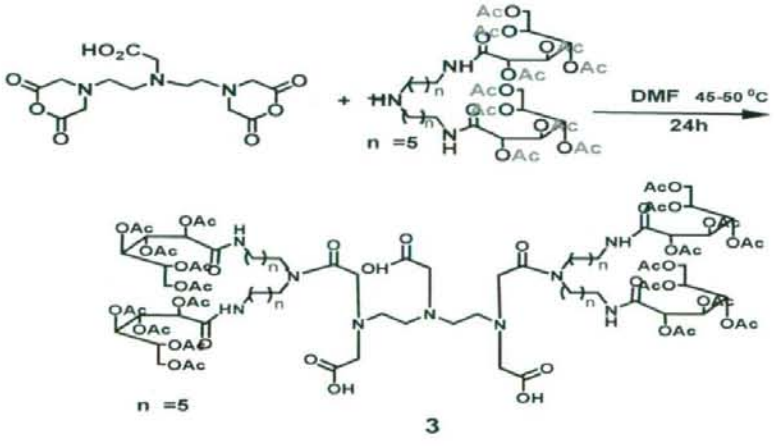


Fig. 2-4-03 Mass spectrum of Terminal

Synthesis of ligand with four sugars

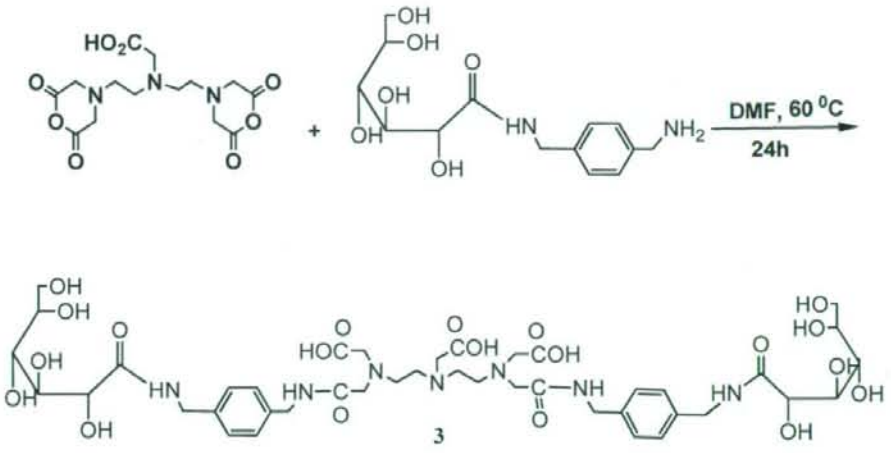
The synthesis of dendritic ligands employed a convergent method to couple core and glycoside branch. To the solution of terminal in DMF was added DTPA anhydride and stirred for 24h at 45-50^{0}C. After the completion of the reaction and evaporation of the solvent gave a series of ligand with four sugars .The Scheme is given bellow.



Scheme 2-4-08 Synthesis of Ligand

Synthesis of ligand with Two sugars

The synthesis of dendritic ligand employed a convergent method to couple core and glycoside branch. To the solution of terminal in DMF was added DTPA anhydride and stirred for 24h at 60°C. After the completion of the reaction and evaporation of the solvent gave a series of ligand with two sugars .The Scheme is the preparation route to compound.



Scheme 2-4-09 Synthesis of Ligand

^{1}H-NMR and mass spectral analysis of the above compound was shown in fig2-4-04 respectively. ^{1}H-NMR spectrum of it showed different chemical shift values at exhibited regions 8.29-8.02 ppm, 7.95-7.02 ppm, 4.28-4.06 ppm, 3.96-3.3.15 ppm and 2.89-2.50 ppm with expected multiplicity confirmed its structure.

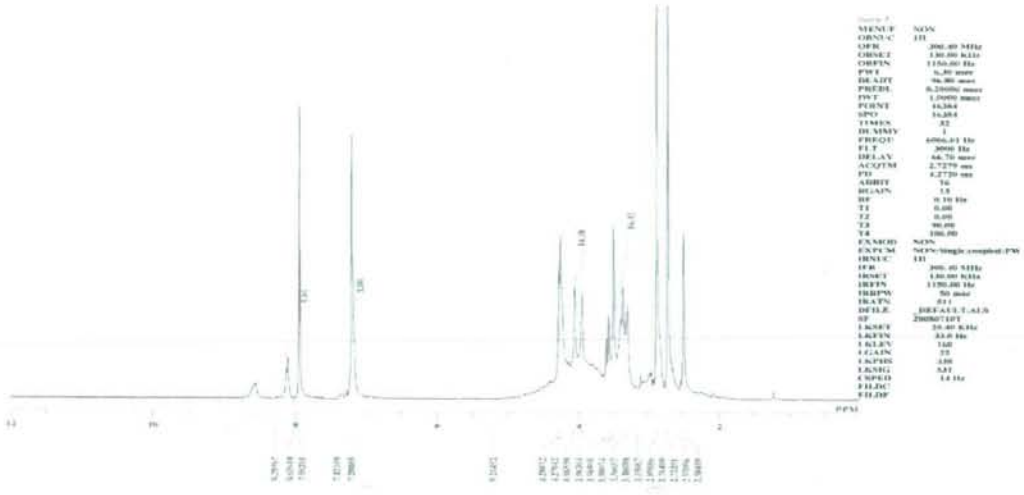
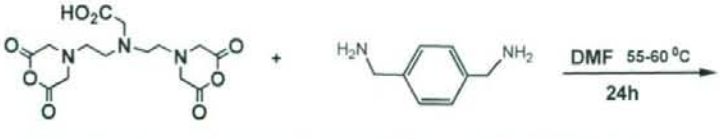


Fig. 2-4-04 ^{1}H-NMRspectrum of Ligand

Synthesis of ligand with free amines

The synthesis of dendritic ligand employed a convergent method to couple core and di amine. To the solution of xylenediamine (0.76g, 5.8mmol) in dry DMF(20mL) was added DTPA anhydride(1g, 2.8mmol) and stirred for 24h at55- 60^{0}C. After completion of the reaction, the solution was purified, and the solvent was evaporated to dryness under reduced pressure to get a yellow crystals. The yield of the compound is 90% .The Scheme is given bellow.



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Scheme 2-4-10 Synthesis of Ligand

^{1}H-NMR and mass spectral analysis of the above compound was shown in Fig. 2-4-05 respectively. ^{1}H-NMR spectrum of it showed different chemical shift values at exhibited regions 8.68-8.06 ppm, 7.10-7.11 ppm, 4.23 ppm, 3.26-3.30 ppm, 2.27 ppm with expected multiplicity confirmed its structure.

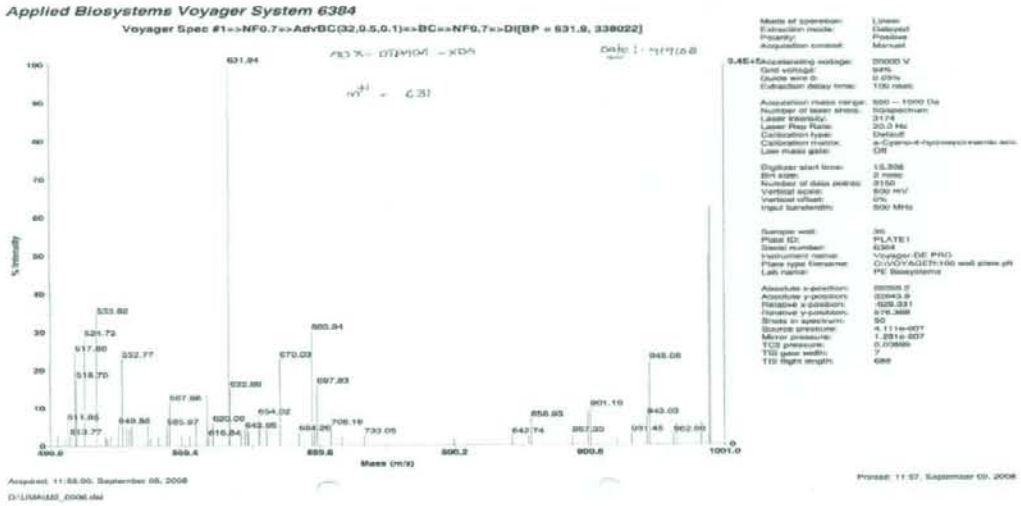
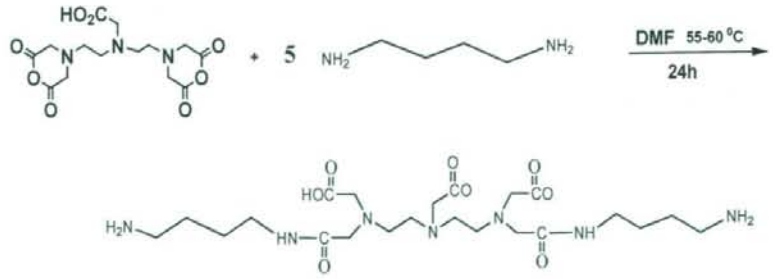


Fig. 2-4-05 Mass spectrum of Ligand

Synthesis of ligand with free amines

The synthesis of dendritic ligand employed a convergent method to couple core and diamine. To the solution of 1,4-Diaminobutane (1.4g, 14.0 mmol) in dry DMF(20mL) was added DTPA anhydride(1g, 2.8mmol) and stirred for 24h at 55-60^{0}C. After completion of the reaction, the solution was purified, and the solvent was evaporated to dryness under reduced pressure to get a yellow crystals. The yield of the compound is 90% .The Scheme is given bellow.



3

Scheme 2-4-11 Synthesis of Ligand

^{1}H- NMR, and mass spectral analysis of the above compound was shown in fig 2-4-06 respectively ^{1}H-NMR spectra of it showed different chemical shift values at exhibited regions

5.42 ppm, 4.69-4.45 ppm, 3.78-3.03 ppm, 2.92-2.73 ppm and 1.91-1.41 ppm with expected multiplicity confirmed its structure.

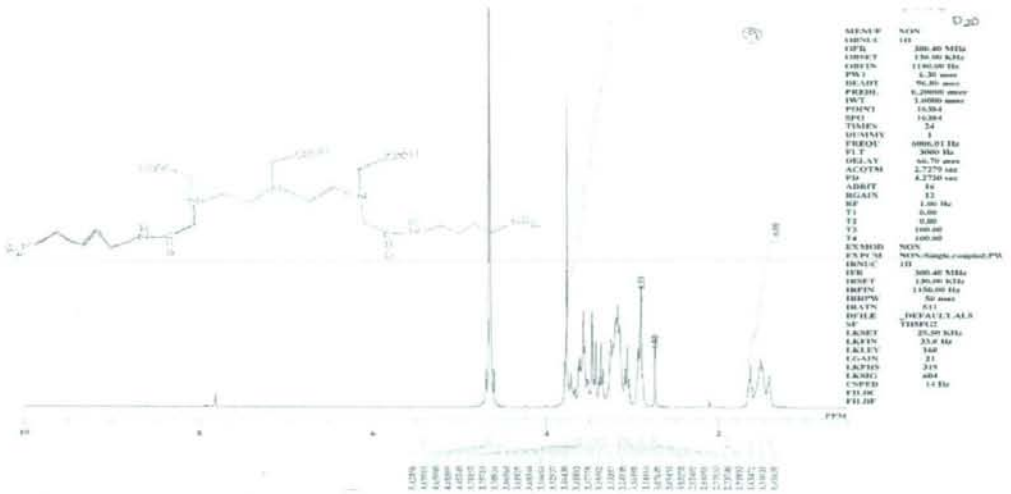
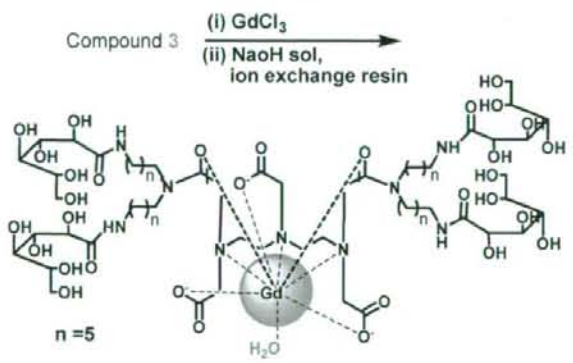


Fig. 2-4-06 ^{1}H-NMRspectrum of Ligand

Synthesis of Gd complex

To a solution of ligand in water was added triethylamine and pyridine and the mixture was stirred thoroughly. To this $GdCl_3.6H_2O$ was added slowly and the reaction was kept at 90°C and stirred for 24h. After completion of the reaction water was removed under vacuum and the crude product was dissolved in water and the excess of Gd was removed by using chelex resign and after removal of excess Gd resin was filtered off and then the protected glucoside hydroxyl groups were deprotected under alkaline condition. After completion of hydrolysis it was treated with DOWEX 50W-X8 ion exchange resin and after the completion of the reaction,, the solvent was removed by rota-evaporator under reduced pressure then dried.



Scheme 2-4-12 Synthesis of Ligand

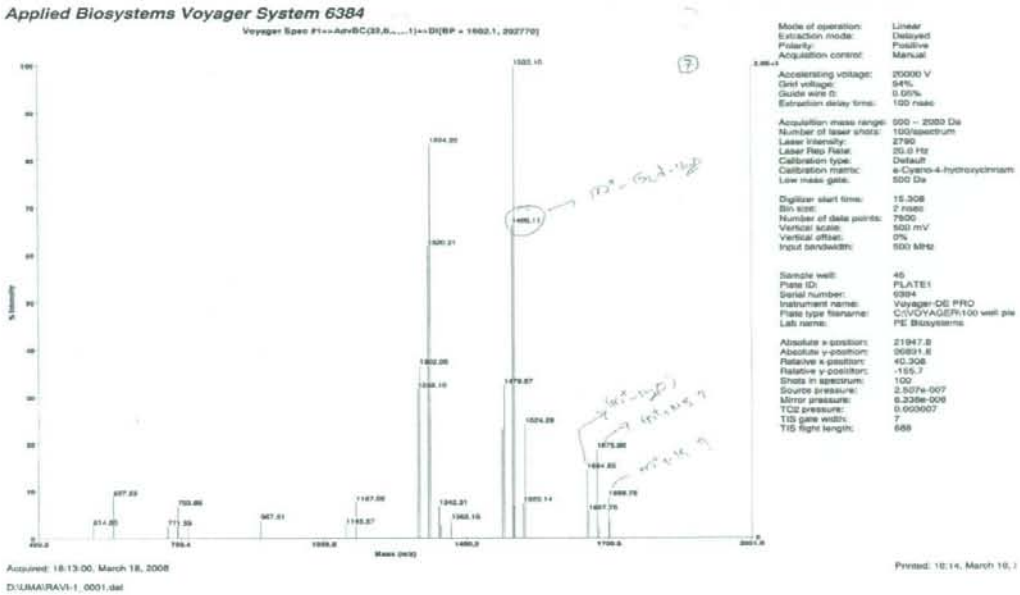


Fig. 2-4-07 Mass spectrum of Complex

Synthesis of Gd complex

To a solution of ligand in water was added triethylamine and pyridine and the mixture was stirred thoroughly. To this $GdCl_3.6H_2O$ was added slowly and the reaction was kept at 55-60°C and stirred for 24h. After completion of the reaction water was removed under vacuum and the crude product was dissolved in water and the excess of Gd was removed by using chelex resign and after removal of excess Gd resin was filtered off and after the completion of the reaction, the solvent was removed by rota-evaporator under reduced pressure then dried. The yield of the compound is 90%.

Compound 3 $\xrightarrow[\text{(ii) Pyridine , } H_2O]{\text{(i) } GdCl_3}$

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Scheme 2-4-13 Synthesis of Ligand

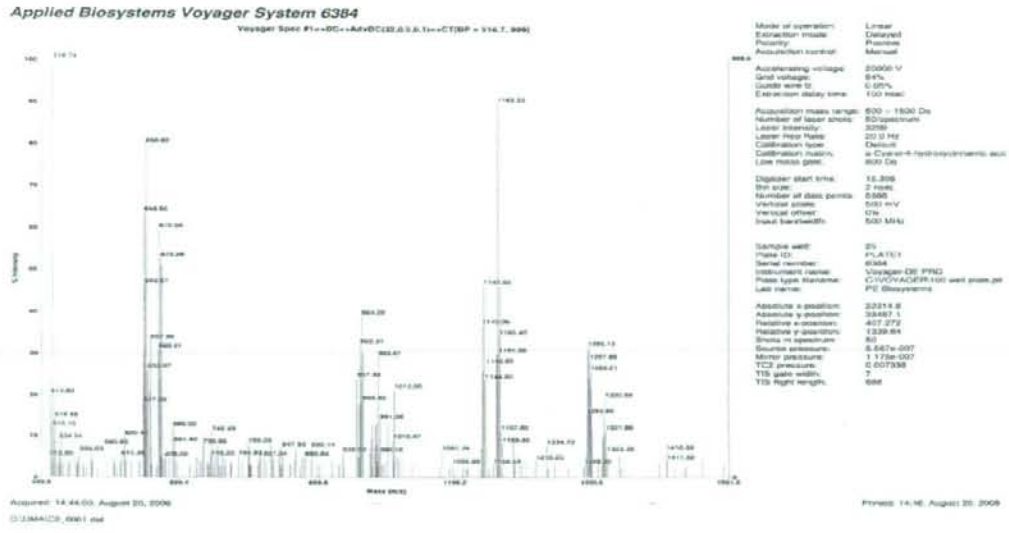

Fig. 2-4-08 Mass spectrum of Complex

Synthesis of Gd complex

To a solution of ligand in water was added triethylamine and pyridine and the mixture was stirred thoroughly. To this $GdCl_3.6H_2O$ was added slowly and the reaction was kept at 60°C and stirred for 24h. After completion of the reaction water was removed under vacuum and the crude product was dissolved in water and the excess of Gd was removed by using chelex resign and after removal of excess Gd resin was filtered off and after the completion of the reaction, the solvent was removed by rota-evaporator under reduced pressure then dried. The yield of the compound is 90%.

Compound 3 $\xrightarrow[\text{(ii) Pyridine , } H_2O]{\text{(i) } GdCl_3}$

特許出願の関係で

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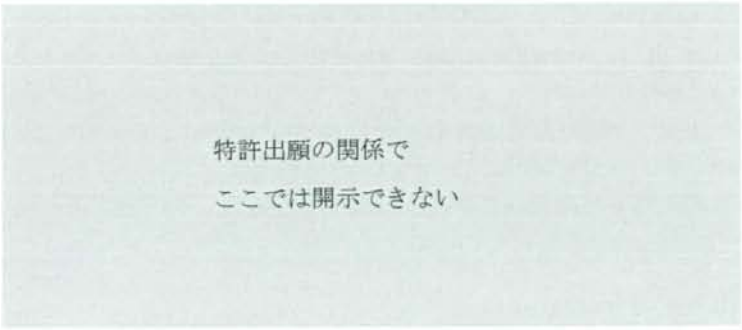
Scheme 2-4-14 Synthesis of Complex

Synthesis of Gd complex

To a solution of ligand in water was added triethylamine and pyridine and the mixture was stirred thoroughly. To this $GdCl_3.6H_2O$ was added slowly and the reaction was kept at 55-60°C and stirred for 24h. After completion of the reaction water was removed under vacuum and the crude product was dissolved in water and the excess of Gd was removed by using chelex resign and after removal of excess Gd resin was filtered off and after the completion of the reaction, the solvent was removed by rota-evaporator under reduced pressure then dried. The yield of the compound is 90%.

Compound 3 $\xrightarrow[\text{(ii) Pyridine , } H_2O]{\text{(i) } GdCl_3}$



Scheme 2-4-15 Synthesis of Complex

Following intensive investigations on a wide variety of carbohydrate-modified dendritic sugar frame work, the feasibility of their metal complexes as new potential candidates for MRI contrast media are now in progress.

2-4-3 Experimental

2-4-3-1 Preparation of DTPA di-anhydride

The DTPA anhydride was synthesized according to previously reported procedure.[16] DTPA (5.0g, 12.7mmol) was added with stirring to a mixture of acetic anhydride(5.2g, 51mmol) and pyridine (7.5mL) for 24h at 65 ^{0}C. After the completion of the reaction, the precipitate was washed 3times by acid anhydride (25mL) and acetonitrile (25mL) .The precipitate was dried in vacuum for 1h. The product is white powder. yield, 4.8g, (11.45mmol), 95%.

2-4-3-2 Synthesis of Terminal

To a solution of D-(+)-Glucono-1,5-lactone in dry DMF was added hexaethelene triamine at 70^{0}C. After 6 h of stirring, the mixture was left in the refrigerator for an overnight. To the solution of the mixture was added $(Boc)_2O$ at 0^{o}C then stirred for 24h at room temperature. The mixture was added into Et_3N and Ac_2O at 0^{o}C then stirred for 2days at room temperature. After completion of the reaction, the solution was purified, and the solvent was evaporated to dryness under reduced pressure to get a brown crystal. Trifloroacetic acid was added into solution of the crystal in CH_2Cl_2, and the mixture was allowed to react for 4h at room temperature. After the completion of the reaction, the solution was purified. And then, the solvent was removed by rota-evaporator under reduced pressure. At last, the product isolation by recycle GPC gave terminal in 65 %, yield.

2-4-3-3 Synthesis of Terminal

To a solution of D-(+)–Glucono-1,5-lactone (1g, 7.3mmol) in dry DMF(20mL) was added xylenediamine (1.3g, 7.3 mmol) ,then stirred for 24h at room temperature. After completion of the reaction, the solution was purified, and the solvent was evaporated to dryness under reduced pressure to get a yellow crystals. The yield of the compound is 90%.

2-4-3-4 Synthesis of ligand with four sugars

The synthesis of dendritic ligands employed a convergent method to couple core and glycoside branch. To the solution of terminal (2g, 1.75mmol) in DMSO(25mL) was added DTPA anhydride(0.6g, 0.87mmol) and Pyridine(0.5mL) stirred for 24h at 60^{0}C. After the completion of the reaction and evaporation of the solvent gave a series of ligand with four sugars. The yield of the compound is 70%.

2-4-3-5 Synthesis of ligand withTwo sugars

The synthesis of dendritic ligand employed a convergent method to couple core and glycoside branch. To the solution of terminal (1.41g, 4.49mmol) in DMF was added DTPA anhydride (0.80g, 2.24mmol) and stirred for 24h at 50-60^{0}C. After the completion of the reaction and evaporation of the solvent gave a series of ligand with two sugar .The yield of the compound is 95%.

2-4-3-6 Synthesis of ligand with free amines

The synthesis of dendritic ligand employed a convergent method to couple core and di amine. To the solution of xylenediamine (0.76g, 5.8mmol) in dry DMF(20mL) was added DTPA anhydride(1g, 2.8mmol) and stirred for 24h at55- 60^{0}C. After completion of the reaction, the solution was purified, and the solvent was evaporated to dryness under reduced pressure to get a yellow crystals. The yield of the compound is 95%.

2-4-3-7 Synthesis of ligand with free amines

The synthesis of dendritic ligand employed a convergent method to couple core and diamine. To the solution of 1,4-Diaminobutane (1.4g, 14.0 mmol) in dry DMF(20mL) was added DTPA anhydride(1g, 2.8mmol) and stirred for 24h at 55-60^{0}C. After completion of the reaction, the solution was purified, and the solvent was evaporated to dryness under reduced pressure to get a yellow crystals. The yield of the compound is 90%.

2-4-4 References and notes

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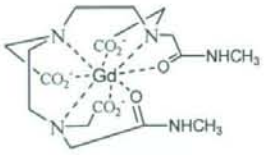
2-5 Synthesis of DTPA amido ester ligands

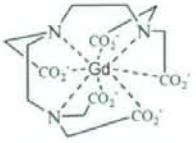
2-5-1 Introduction

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. 2-5-01) 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. 2-5-01 Currently being used MRI contrast agents

In our lab, a novel Gd-DTPA derivative for MRI contrast agent has been prepared (Fig. 2-5-02) 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.

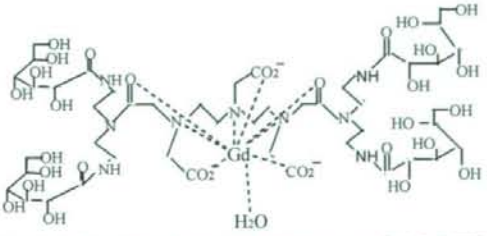


Fig. 2-5-02 The chemical structure of Gd-DTPA-D1

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.

2-5-2 Results and discussion

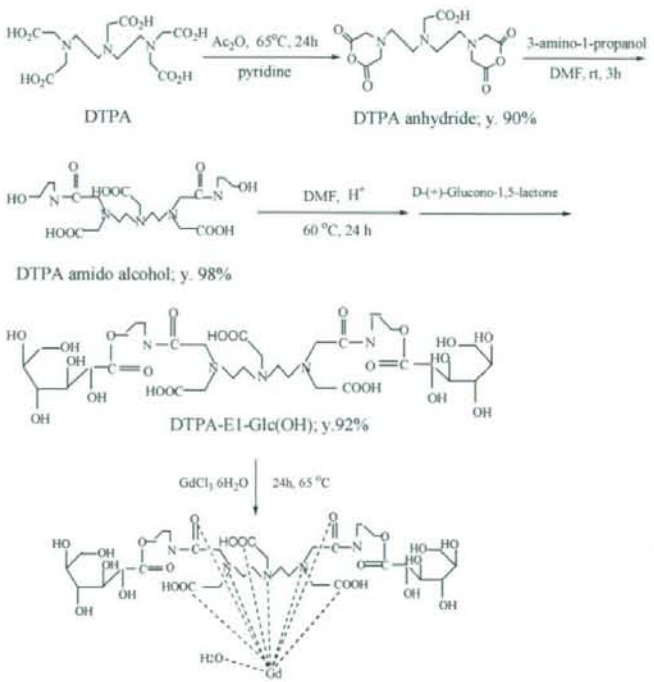
In my experiments, three Gd-DTPA derivatives have been prepared, which are Gd-DTPA-bis(amido sugar),Gd-DTPA-amino acids and Gd-DTPA-ME sugar.

2-5-2-1 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 Scheme2-5-01 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.2-5-03~Fig.2-5-05. 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.2-5-06), 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 2-5-01 The preparation route to Gd-DTPA-bis(amido sugar)

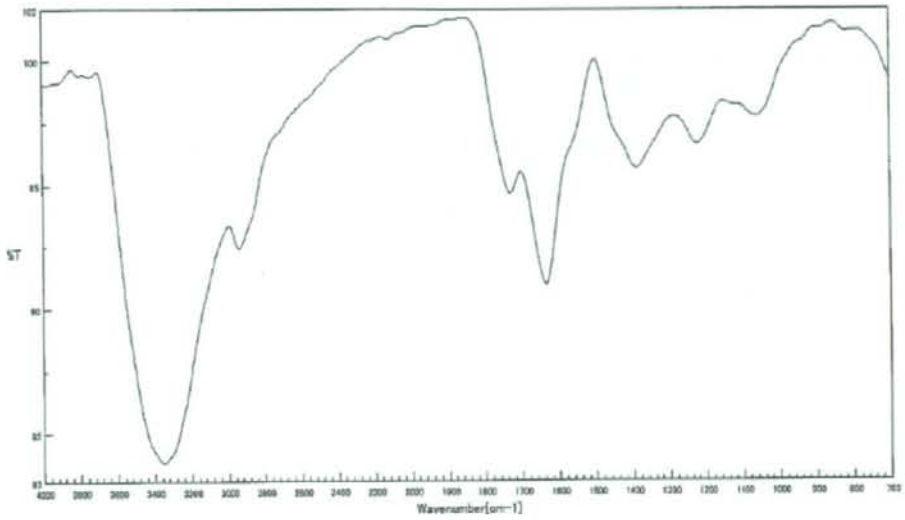


Fig.2-5-03 The IR spectrum of DTPA-bis(amido sugar)

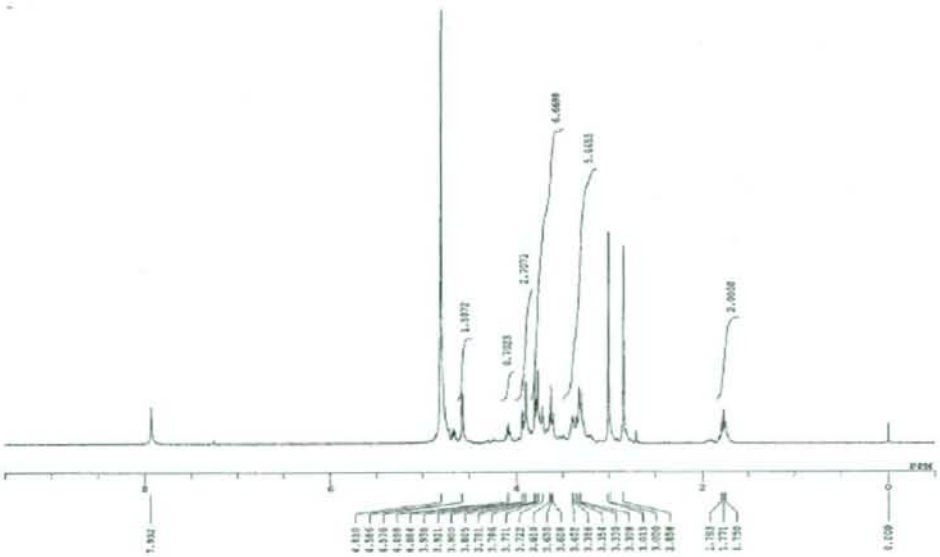


Fig.2-5-04 The ^{1}H-NMR spectrum of DTPA-bis(amido sugar)

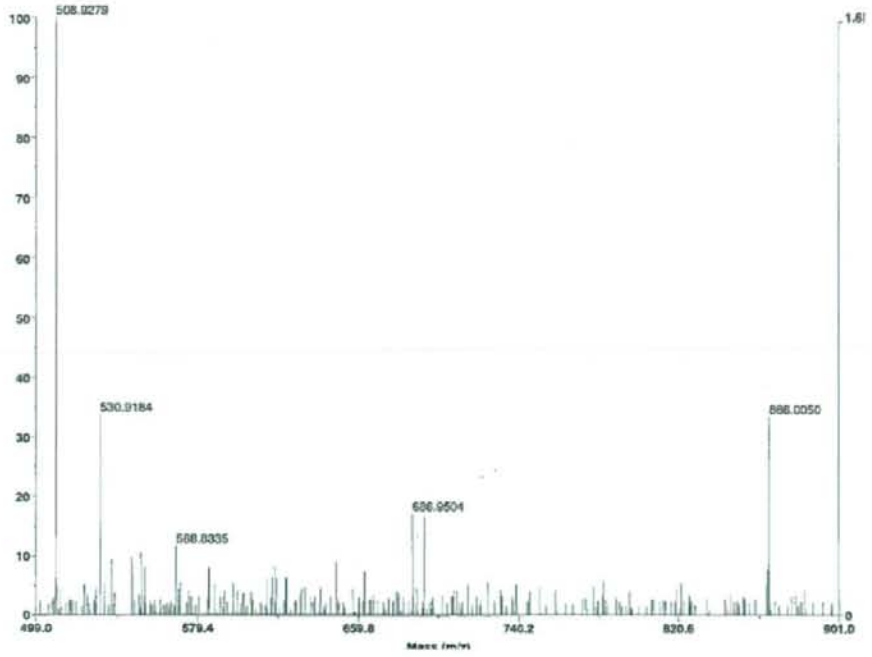


Fig.2-5-05 The MS spectrum of DTPA-bis(amido sugar)

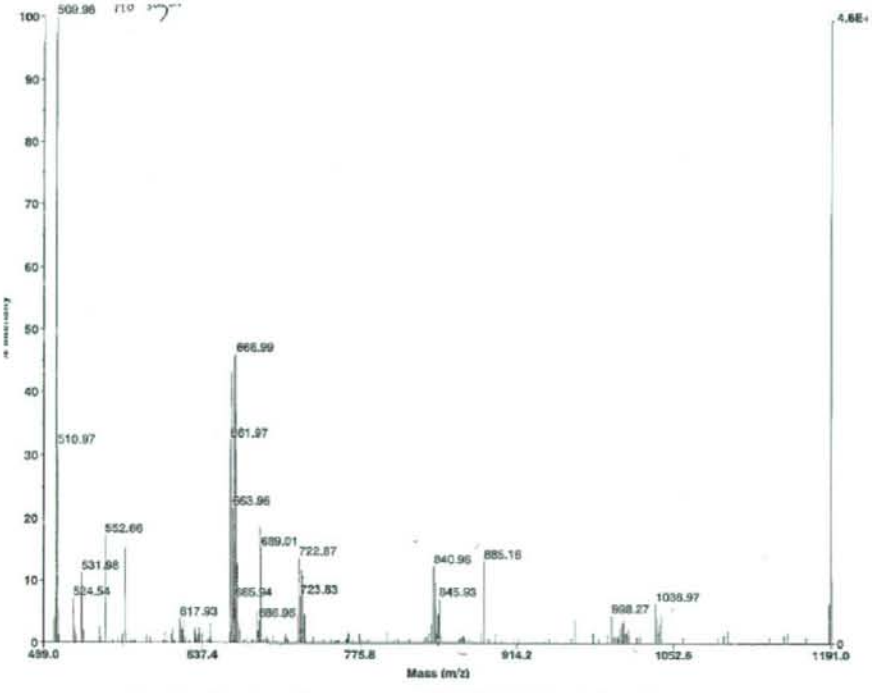
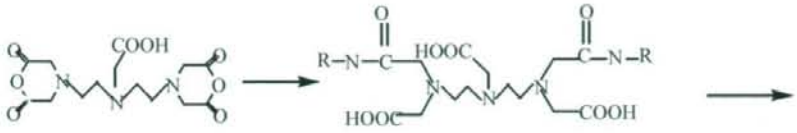


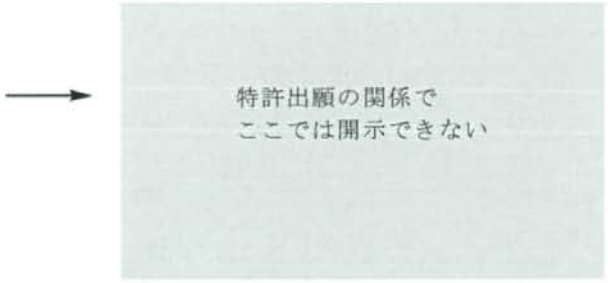
Fig.2-5-06 The MS spectrum of Gd-DTPA-bis(amido sugar)

2-5-2-2 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 Scheme2-5-02 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 2-5-02 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. 2-5-07~2-5-09, 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. 2-5-10~2-5-12, 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. 2-5-13~2-5-15, 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.

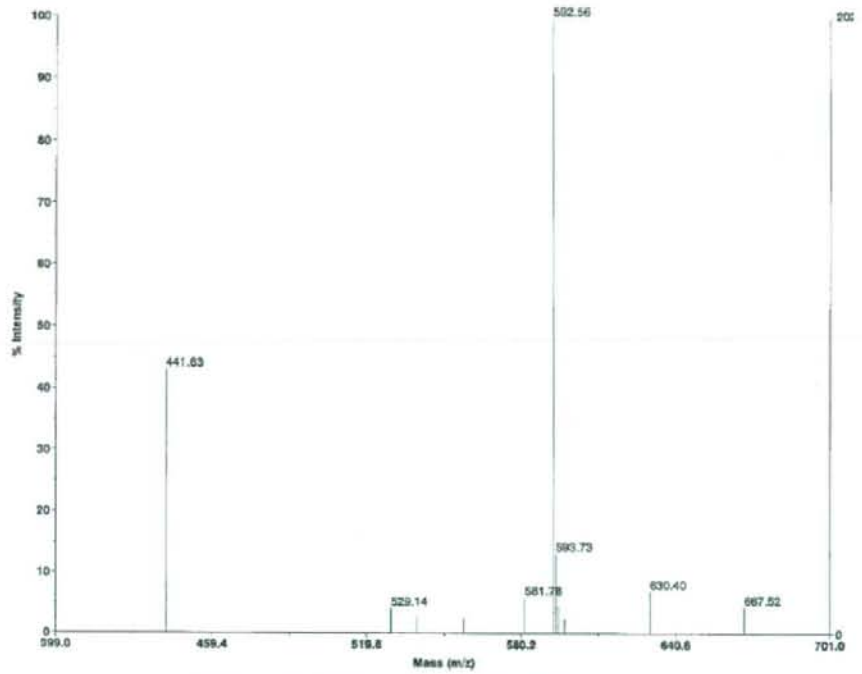


Fig.2-5-07 The MS spectrum of DTPA-L-Valine

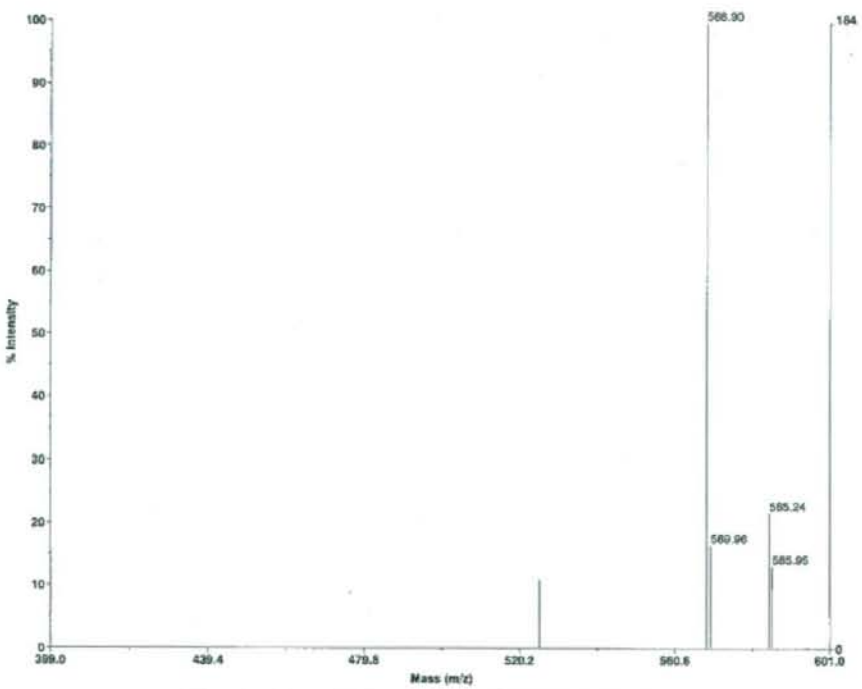


Fig.2-5-08 The MS spectrum of DTPA-L-Serine