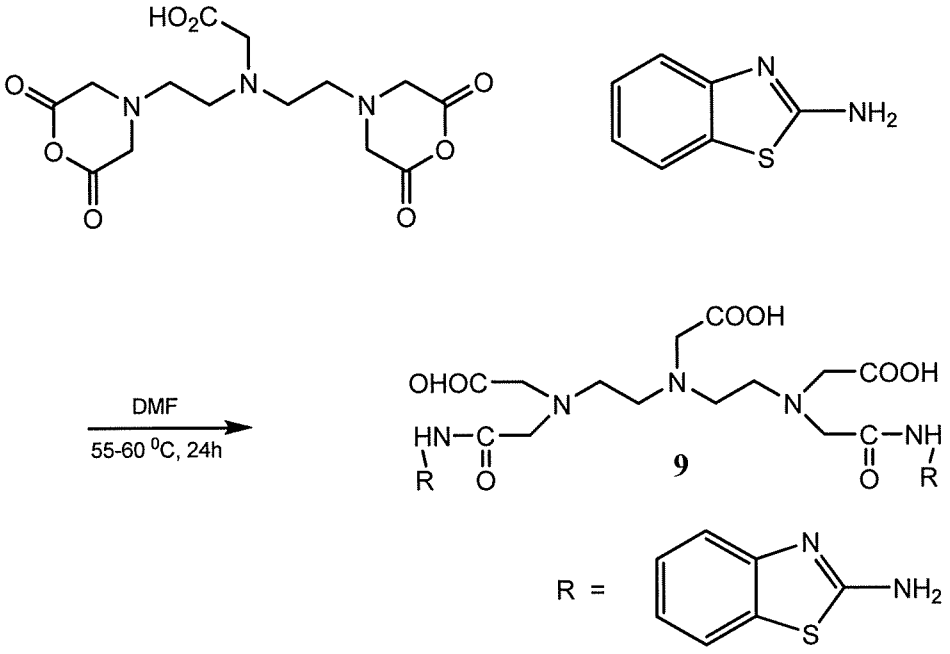
Synthesis of ligand

The synthesis of dendritic ligand employed a convergent method to couple core and diamine. To the solution of 2-Aminobenzothiazole (0.84 g, 5.59 mmol) in dry DMF(20 mL) was added DTPA anhydride (1.0 g, 2.8 mmol) and stirred for 24 h at 55- 60^{0}C. After completion of the reaction, the solution was purified by recrystalisation and the solvent was evaporated to dryness under reduced pressure to get yellow crystals. The yield of the compound is 90% .The Scheme is given bellow.



Scheme 2-9-14 Synthesis of Ligand compound 9

^{1}H-NMR and ^{13}C-NMR spectral analysis of the above compound **9** was shown in Fig. 2-9-17 and 2-9-18. ^{1}H-NMR spectrum of it showed different chemical shift values at exhibited regions 8.27-8.14 ppm, 7.93-7.01 ppm, 6.98-6.96 ppm, 4.23 ppm, 3.95-3.39 ppm, 2.96-2.07 ppm and 1.94-1.02 ppm with expected multiplicity confirmed its structure. ^{13}C-NMR showed distinctive singelets at 171.31 indicates the presence of amide C=O group, the other aliphatic carbons observed in the expected region.

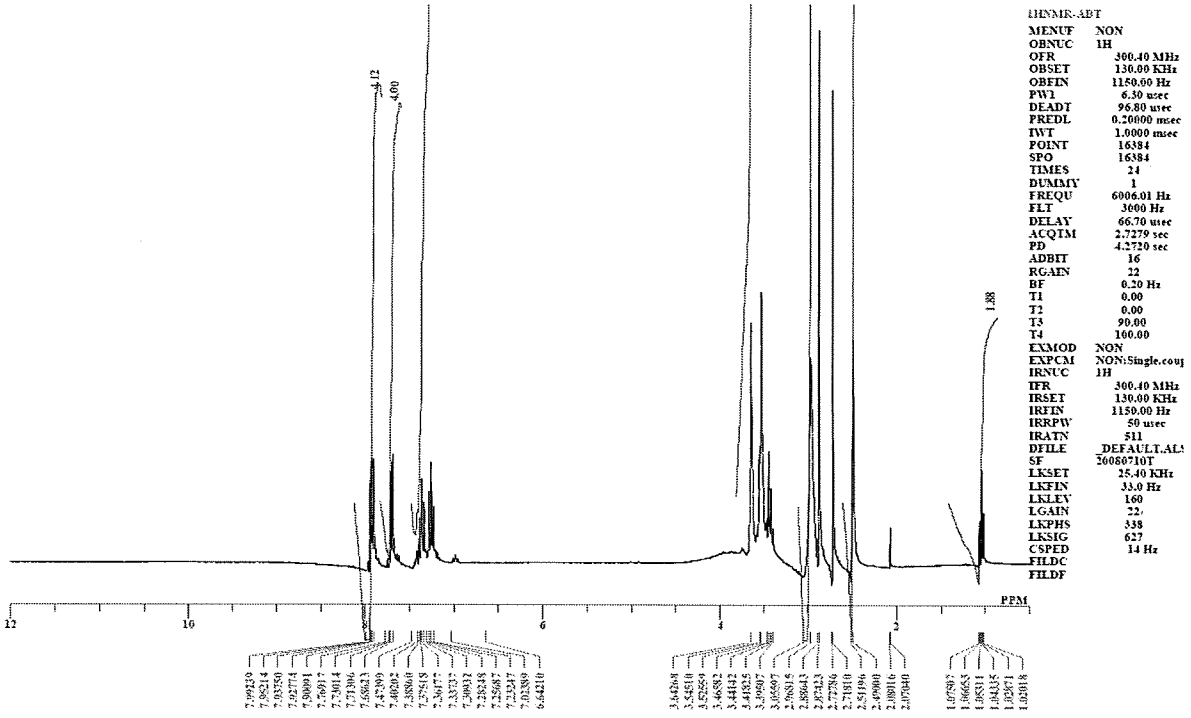


Fig. 2-9-17 ^{1}H-NMRspectrum of Ligand compound 9

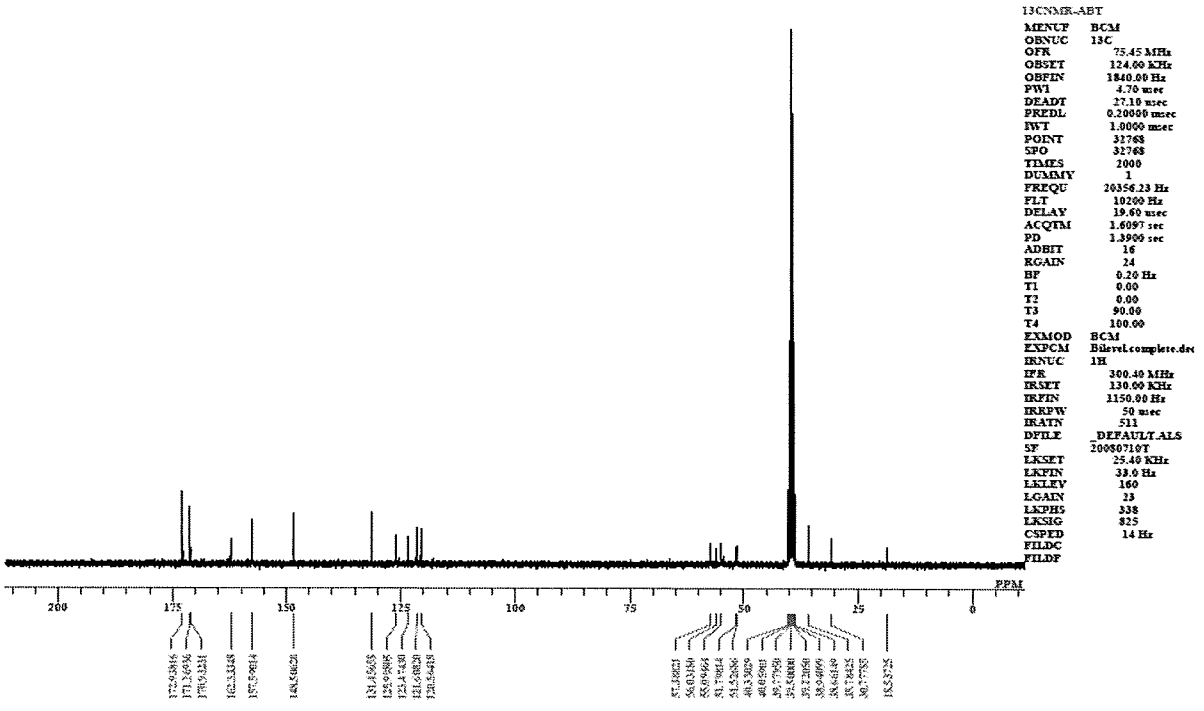
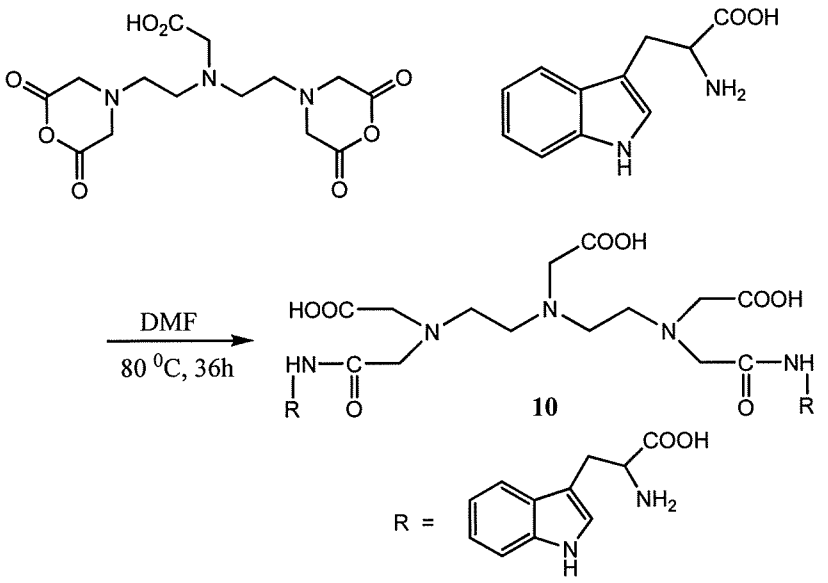


Fig. 2-9-18 ^{13}C-NMR of Ligand compound 9

Synthesis of ligand

The synthesis of dendritic ligand employed a convergent method to couple core and diamine. To the solution of L-triptophan (1.14 g, 5.59 mmol) in dry DMF (20 mL) was added DTPA anhydride (1.0 g, 2.8 mmol) and stirred for 36 h at 80^{0}C. After completion of the reaction, the solution was purified, and the solvent was evaporated to dryness under reduced pressure to get yellow crystals. The yield of the compound is 90% .The Scheme is given bellow.



Scheme 2-9-15 Synthesis of Ligand compound 10

^{1}H-NMR, ^{13}C-NMR and mass spectral analysis of the above compound **10** were shown in Fig. 2-9-19, 2-9-20 and 2-9-21. ^{1}H-NMR spectrum of it showed different chemical shift values at exhibited regions 8.23-8.14 ppm, 7.95-7.02 ppm, 6.99-6.94ppm, 4.52-4.47 ppm, 3.95-3.04 ppm, 2.96-2.33 ppm, 1.90-1.06 ppm with expected multiplicity confirmed its structure. ^{13}C-NMR showed distinctive singelets at 171.31 indicates the presence of amide C=O group, the other aliphatic carbons observed in the expected region. Mass peak at m/z 766 (M +H).

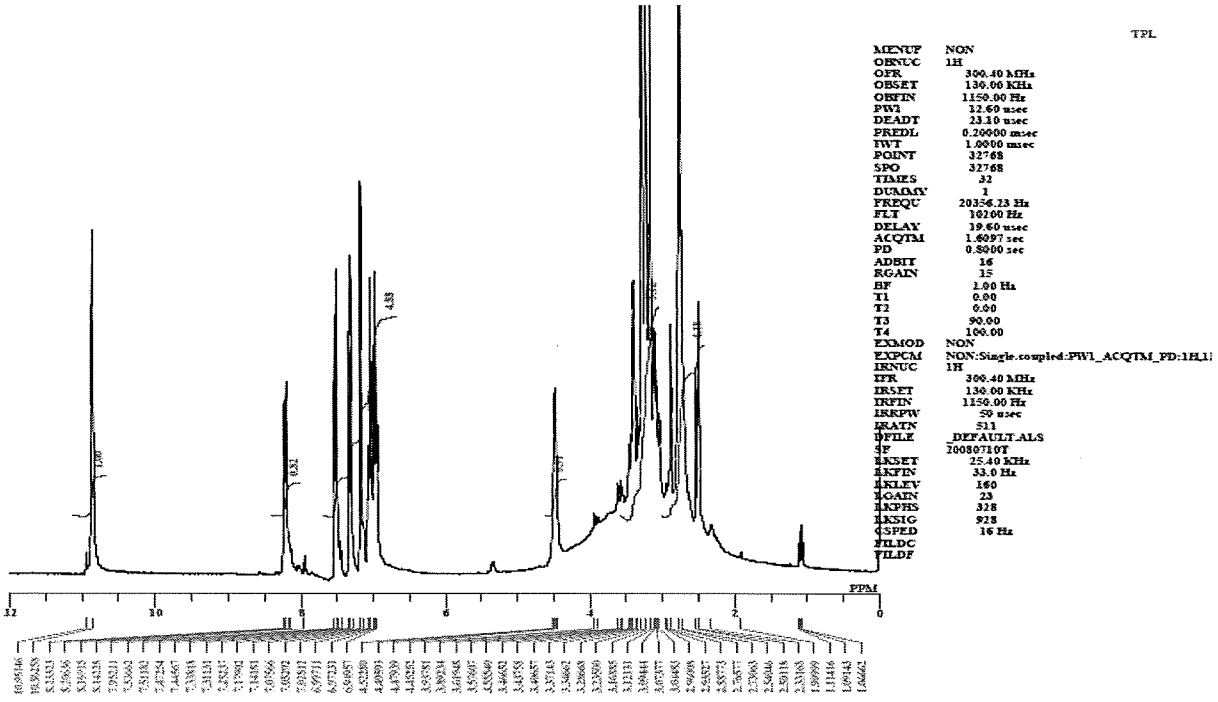


Fig. 2-9-19 ^{1}H-NMR spectrum of Ligand compound 10

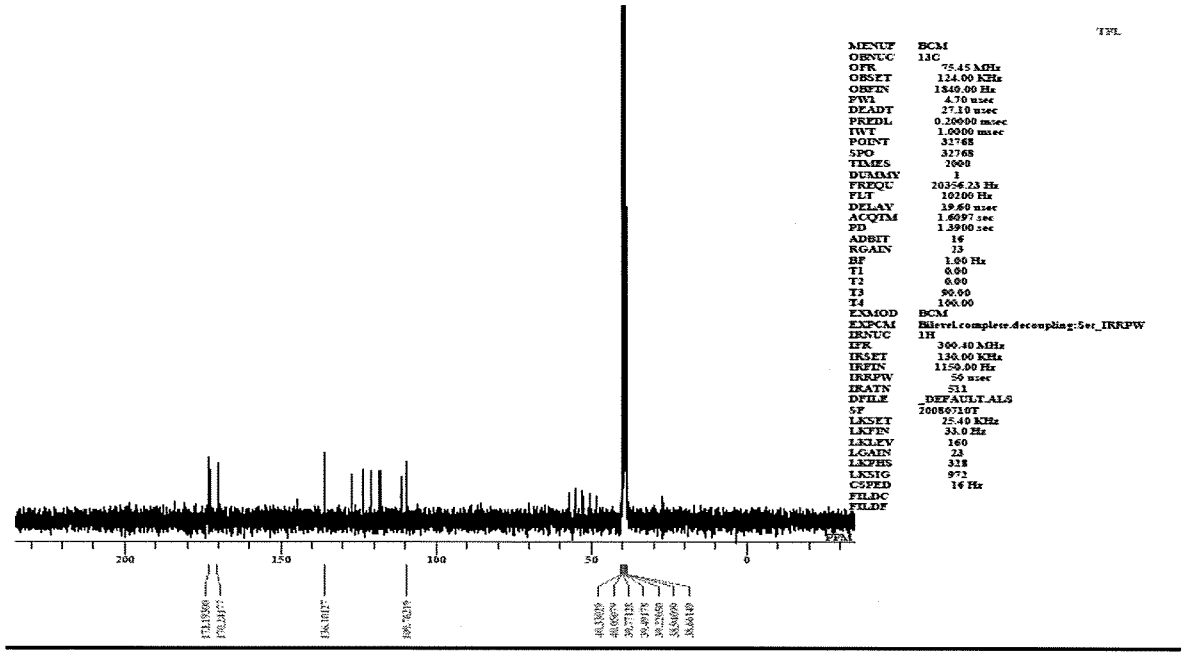


Fig. 2-9-20 ^{13}C-NMR of Ligand compound 10

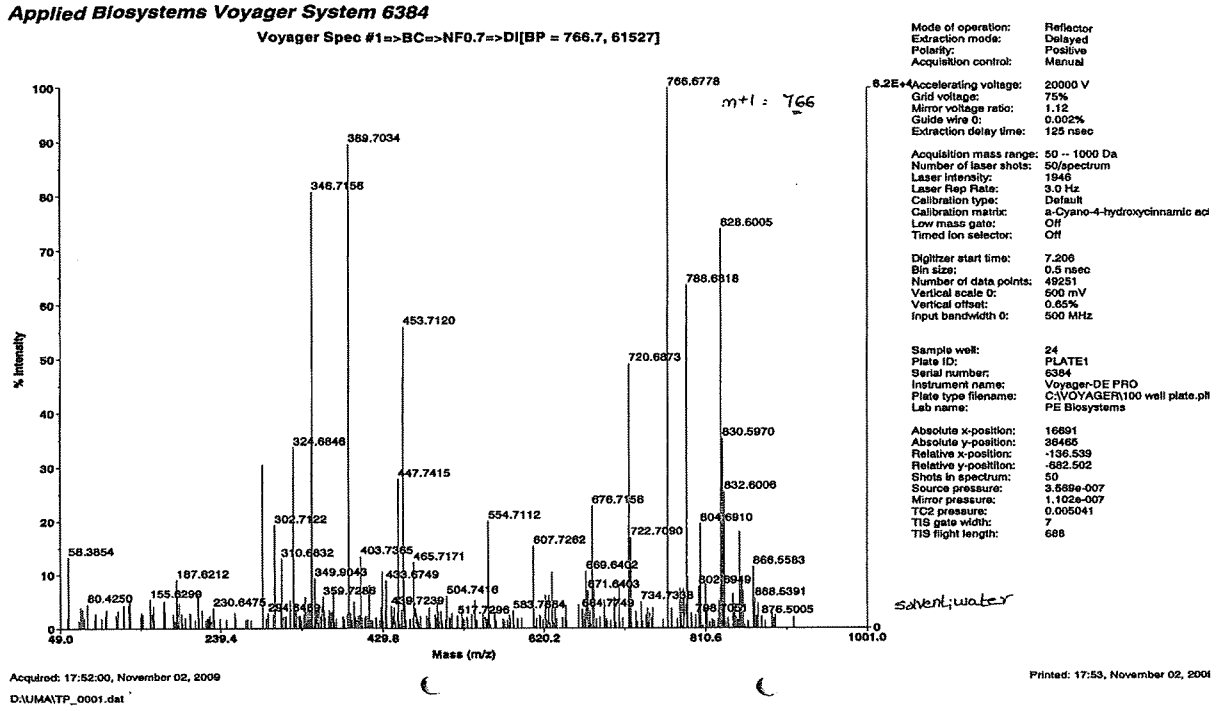
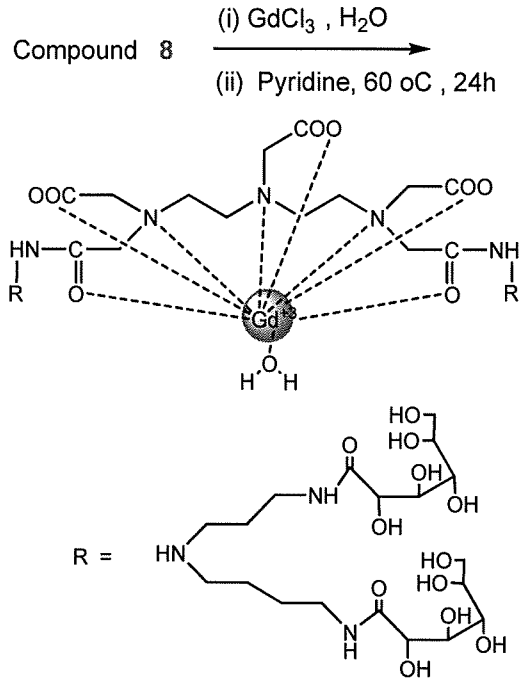


Fig. 2-9-21 Mass spectrum of Ligand compound 10

Synthesis of Gd complex

To a solution of ligand in water was added triethylamine and pyridine and the mixture was stirred thoroughly. To this $GdCl_3 \cdot 6H_2O$ was added slowly and the reaction was kept at 60°C and stirred for 24 h. 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. Mass peak at m/z 1532 (M^{+2}).



Scheme 2-9-16 Synthesis of Gd-complex

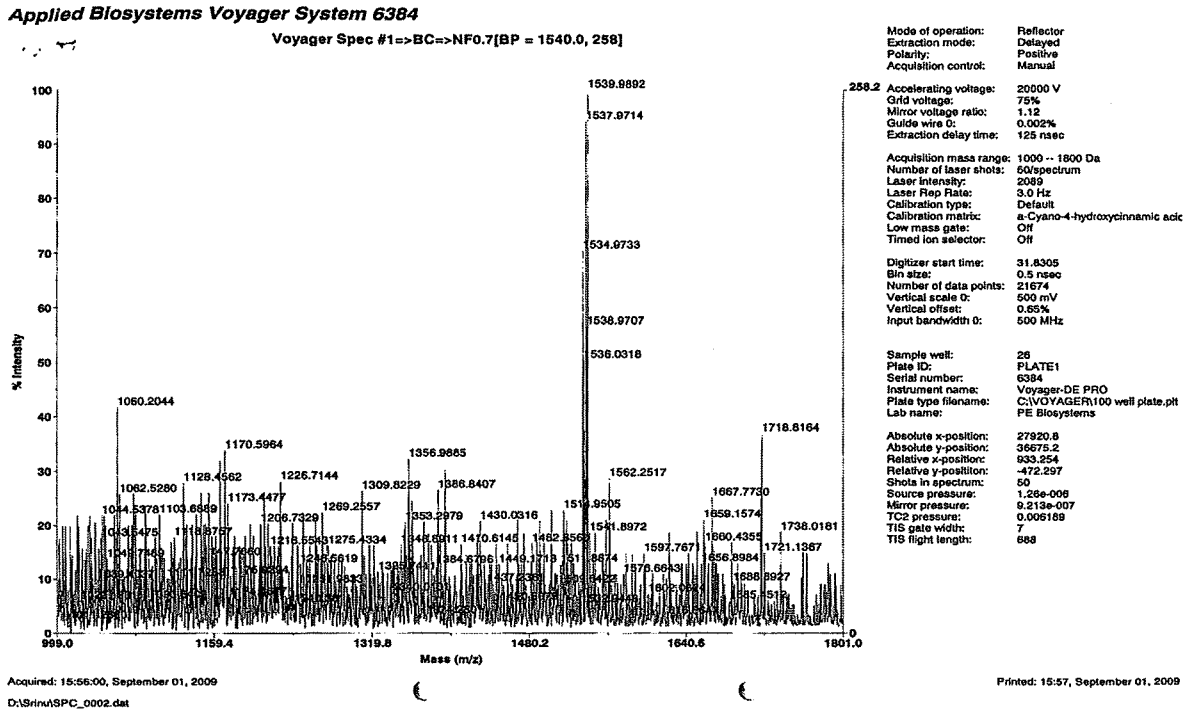
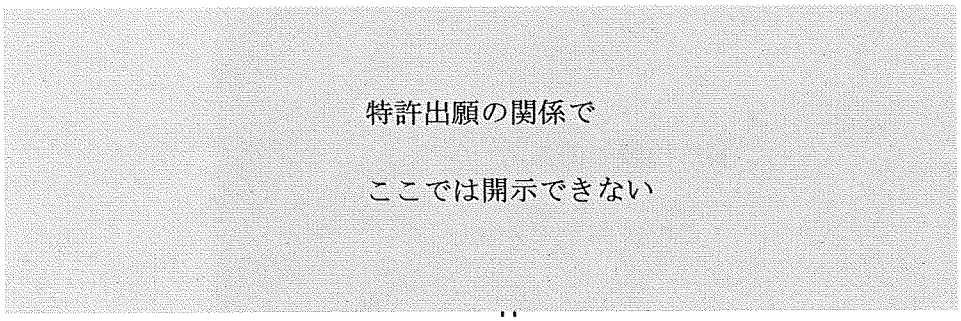


Fig. 2-9-22 Mass spectrum of Complex shown in Scheme 2-9-16

Synthesis of Gd complex

To a solution of ligand in water was added triethylamine and pyridine and the mixture was stirred thoroughly. To this $GdCl_3 \cdot 6H_2O$ was added slowly and the reaction was kept at 60°C and stirred for 24 h. 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%. Mass peak at m/z 832 (M +H).

Compound 9 $\xrightarrow[\text{(ii) Pyridine, 60 oC , 24h}]{\text{(i) } GdCl_3 \text{ , } H_2O}$



Scheme 2-9-17 Synthesis of Complex

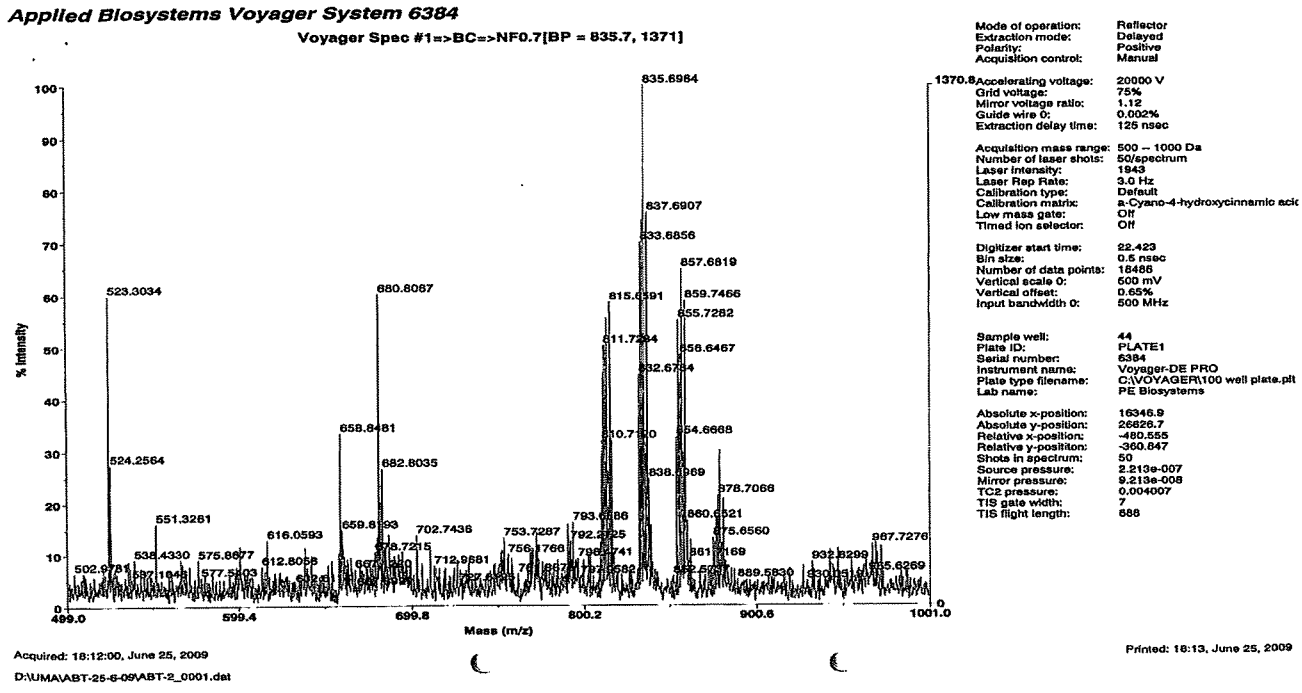
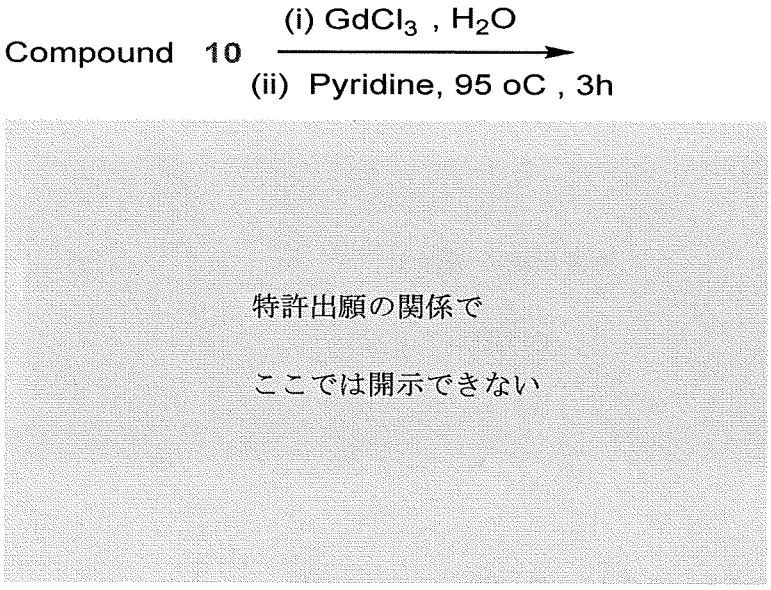


Fig. 2-9-23 Mass spectrum of Complex shown in scheme 2-9-17

Synthesis of Gd complex

To a solution of ligand in water was added triethylamine and pyridine and the mixture was stirred thoroughly. To this $GdCl_3 \cdot 6H_2O$ was added slowly and the reaction was kept at 95°C and stirred for 3 h. 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%. Mass peak at m/z 920 ($M-H_2O+Na$).



Scheme 2-9-18 Synthesis of Complex

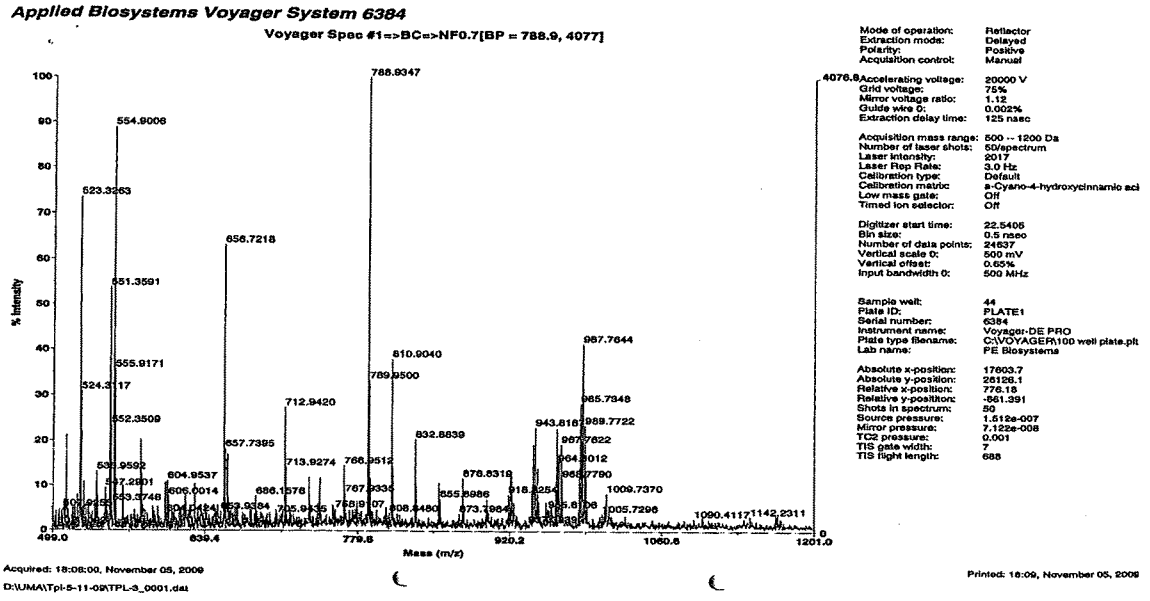


Fig. 2-9-24 Mass spectrum of Complex shown in Scheme 2-9-18

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.

2-9-2-3 Experimental

Preparation of DTPA di-anhydride

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

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) = 3.71 (s, -CO-<u>CH_2</u>-CO-X 4)

=3.30 (s, -<u>CH_2</u>COOH)

=2.72 (t, $^2J_{CH}$ = 6.58 Hz, $COCH_2N\underline{CH_2}CH_2N$ X 2)

=2.59 (t t, $^2J_{CH}$= 12.0 Hz, $COCH_2NCH_2\underline{CH_2}N$ 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)

Synthesis of Terminal

To a solution of D-(+)–glucono-1,5-lactone (1.0 g, 5.61 mmol) in dry DMF (20 mL) was added spermidine (0.44 mL, 2.80 mmol), then stirred for 24 h at room temperature. After completion of the reaction, the solution was purified, and the solvent was evaporated to dryness under reduced pressure to get yellow crystals. The yield of the compound is (4.1 g, 8.18 mmol), yield, 90%.

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 (1.4 g, 2.79 mmol) in DMSO (25 mL) was added DTPA anhydride (0.5 g, 1.39 mmol) and Pyridine (0.5 mL) stirred for 24 h at 60^{0}C. After the completion of the reaction and evaporation of the solvent gave a series of ligand compound 8 with four sugars. The yield of the compound is (3.0 g, 2.23 mmol),yield, 80%.

Synthesis of ligand

The synthesis of dendritic ligand employed a convergent method to couple core and terminal To the solution of 2-aminobenzothiazole (0.84 g, 5.59 mmol) in dry DMF (20 mL) was added DTPA anhydride (1.0 g, 2.8 mmol) and stirred for 24 h at 60^0C. After completion of the reaction, the solution was purified, and the solvent was evaporated to dryness under reduced pressure to get yellow crystals. The yield of the compound is (1.6 g, 2.51 mmol), yield, 90%.

Synthesis of ligand

The synthesis of dendritic ligand employed a convergent method to couple core and terminal. To the solution of L-triptophan (1.14 g, 5.59 mmol) in dry DMF (20 mL) was added DTPA anhydride (1.0 g, 2.8 mmol) and stirred for 36 h at 80^0C. After completion of the reaction, the solution was purified, and the solvent was evaporated to dryness under reduced pressure to get yellow crystals. The yield of the compound is (1.5 g, 1.96 mmol), yield, 80%.

2-9-3 References and notes

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2-10 Synthesis of Gd-DTPA-complexes

Kambam Srinivasulu

Introduction: The role of the Contrast Agents (CAs) is to enhance the MRI signal by shortening the relaxation times of water protons in those tissues in which they distribute. Generally, the most investigated paramagnetic CAs are lanthanide complexes, with particular emphasis on the corresponding Gd(III)-chelates. Mn(II) and Fe(III) salts have also been investigated as paramagnetic metals, but they often become weakly chelated and dissociate spontaneously under *invivo* conditions [1-3]. As mentioned, the paramagnetic metal of choice in clinical practice is generally Gd(III), but free Gd(III) is toxic *in vitro* as well as *in vivo,* and the use of Gd(III) chelates becomes mandatory in biomedical applications to reduce its toxicity. Gd(III) remains the optimal paramagnetic ion because of its high electronic spin (S=7/2), relatively long electronic relaxation time, high magnetic moment and relatively labile hydration sphere for water exchange. The first generation of Gd(III) chelates was derived from linear or macrocyclic polyaminopolycarboxylates such as diethylenetriamine pentaaceticacid ([DTPA-Gd(H_2O)]2-)or 4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid ([DOTA-Gd(H_2O)]-), respectively [4-5]. Gd-DTPA, patented as Magnevist by Schering (Germany), was the first CA approved for clinical use. Gd-DOTA (Dotarem, Guerbet, France), Gd-DTPA-BMA (Omniscan, Amersham, Great Britain), Gd-HP-DO3A (Prohance, Bracco, Italy), Gd-DTPA-BMEA (Optimark, Mallinkrodt, USA) and Gd- DO3A-Butriol (Gadovist, Schering, Germany) followed as other Gd based CAs commonly used in clinical practice. All of them present similar pharmacokinetic properties and renal elimination rates. The modification of both linear and macrocyclic parental structures, Gd-DTPA and Gd-DOTA, is currently found to be an essential part of the investigations generating new CAs with improved magnetic properties. In this respect, new Gd-based CAs must exhibit sufficiently high thermodynamic and kinetic stabilities as important determinants for their use in MRI diagnosis. In addition, the CAs must have improved molecular relaxivity properties, r_1 or r_2 (s^{-1} mM^{-1}), defined as the longitudinal or transversal relaxation rates of the water protons in a 1 mM aqueous solution of the Gd(III)-chelate.

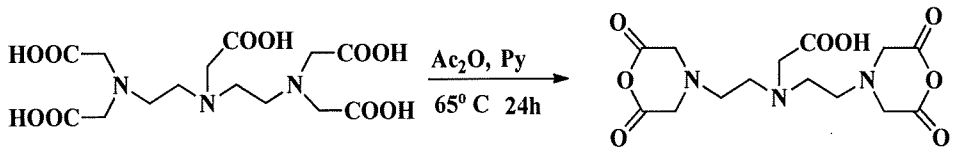
2-10-1 Synthesis of Gd-DTPA-Spermidine

2-10-1-1 Result and Discussion

In the present experiment, the new asymmetric Gd-DTPA-Spermidine-Glc(OH) was synthesized by the reaction of DTPA dianhydride with DTPA-Spermidine-Glc terminal to form ligand which was further reacted with $GdCl_3 \cdot 6H_2O$ to form Gd-DTPA-Spermidine-Glc(OH). Spermidine-Glc(OH) terminal was prepared by D-glucono-1,5-lactone and Spermidine.

2-10-1-2 Synthesis of DTPA dianhydride

DTPA dianhydride was prepared according to the previously established procedure [6]. DTPA was added to a mixture of acetic anhydride and pyridine and stirred thoroughly over a period of 24 h at 65°C under inert conditions. After the completion of reaction mixture was filtered and washed thoroughly with acetic anhydride and acetonitrile. This was dried over sufficient period of time under vacuum and the yield of the product was 90%. Scheme 2-10-1 depicted the preparation route of DTPA dianhydride.



DTPA DTPA dianhydride, 90%

(1) **(2)**

Scheme 2-10-1 Synthesis of DTPA dianhydride

^{1}H-NMR and ^{13}C-NMR spectra of DTPA dianhydride were shown in Fig.2-10-1, 2-10-2 respectively. From the IR spectrum, we can find the peaks at 1820 cm^{-1} (C=O stretch symmetric), 1774 cm^{-1} (C=O asymmetric stretching) which are characteristics of cyclo-dianhydride. ^{1}H-NMR spectrum of DTPA dianhydride $COCH_2N\underline{CH_2}CH_2N$ X 2 chemical shifts showed at 2.73 (t, $^{2}J_{CH}$ 12.1 = Hz,), and $COCH_2NCH_2\underline{CH_2}N$ X2 at 2.58 (t, $^{2}J_{CH}$ 12 = Hz,) respectively. ^{13}C-NMR spectrum of it showed distinct singlets at 171.65 and 165.67 also suggests the presence of dianhydride system. All the above furnished information has confirmed the structure of dianhydride system.

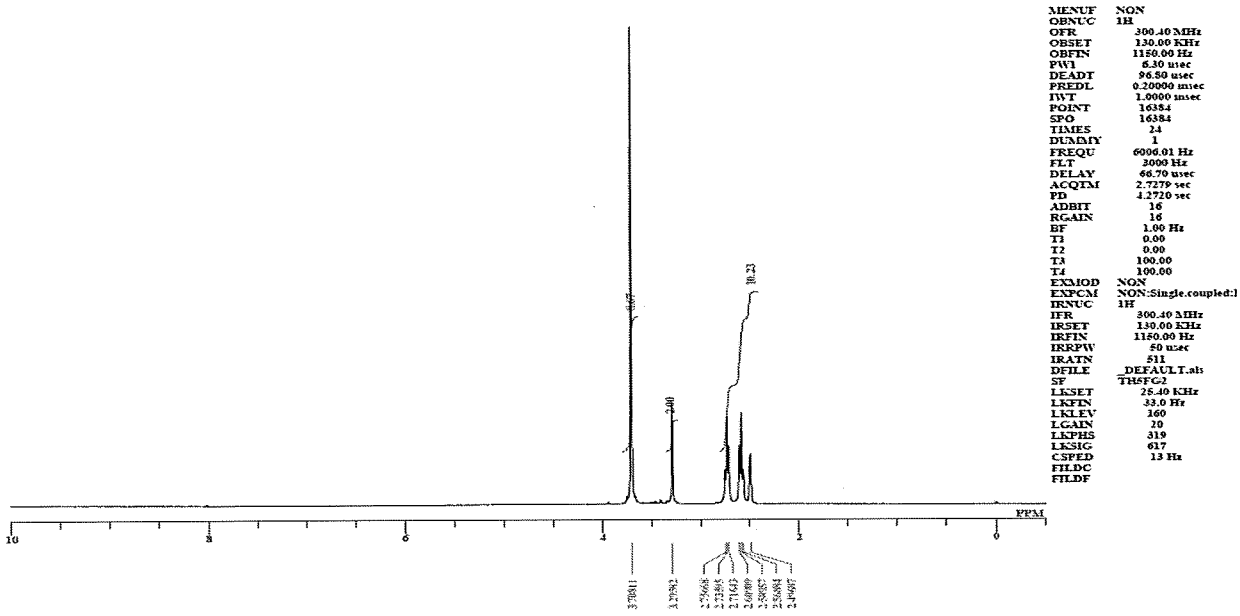


Fig. 2-10-1 ^{1}H-NMR of DTPA dianhydride

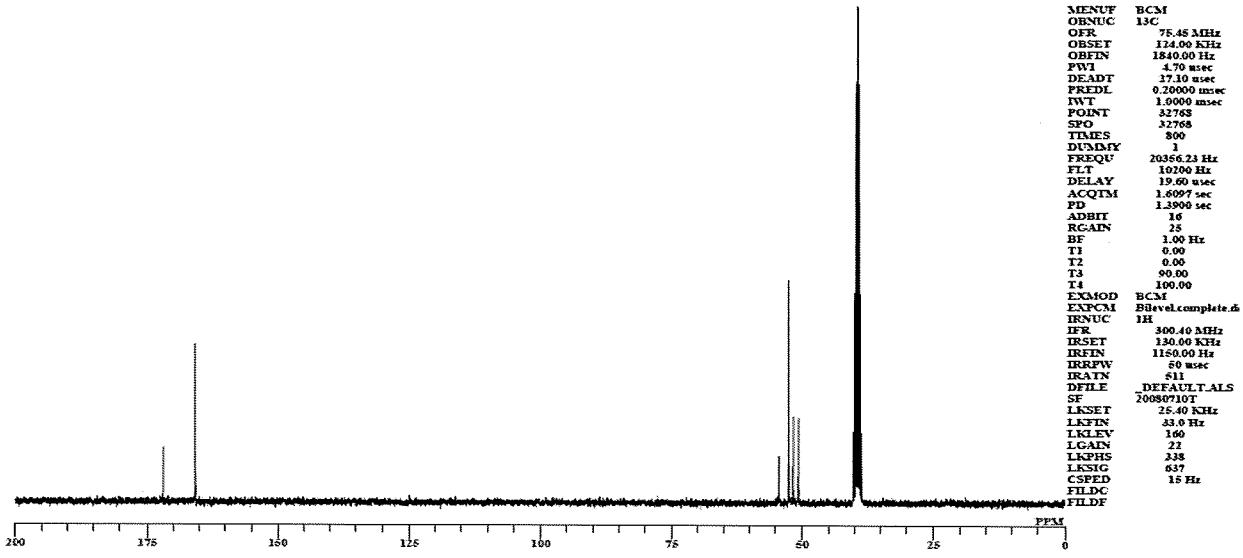
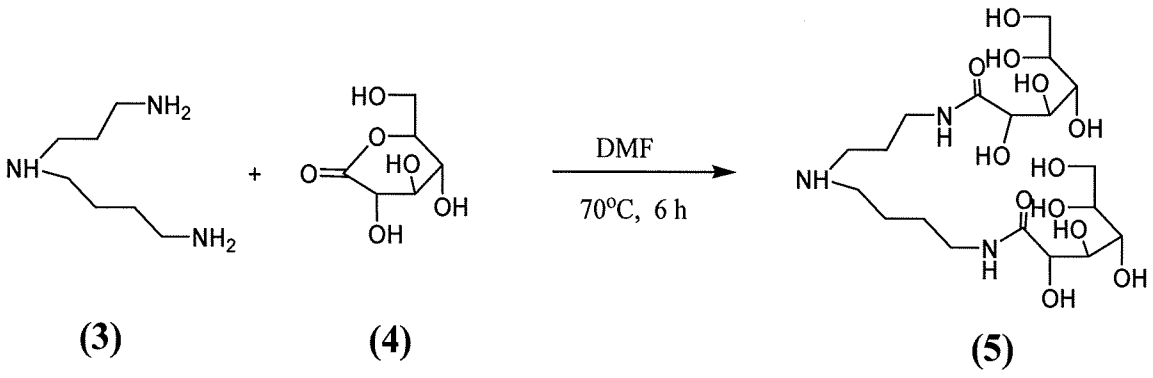


Fig. 2-10-2 ^{13}C-NMR of DTPA dianhydride

2-10-2 Synthesis of Asymmetric DTPA-spermidine-Glc

2-10-2-1 Synthesis of Spermidine-Glc(OH)

To a solution of D-glucono-1,5-lactone in adequate amount of DMF was added spermidine (N-(3-aminopropyl)butane-1,4-diamine) under inert conditions and the temperature of the reaction mixture was maintained at 70°C over a period of six hours. After completion of the reaction, products were left in refrigerator overnight and then filtered the solid washed with DMF and dried under vacuum. Preparation of the title compound is depicted in Scheme 2-10-2



Scheme 2-10-2 Synthesis of Spermidine-Glc(OH)

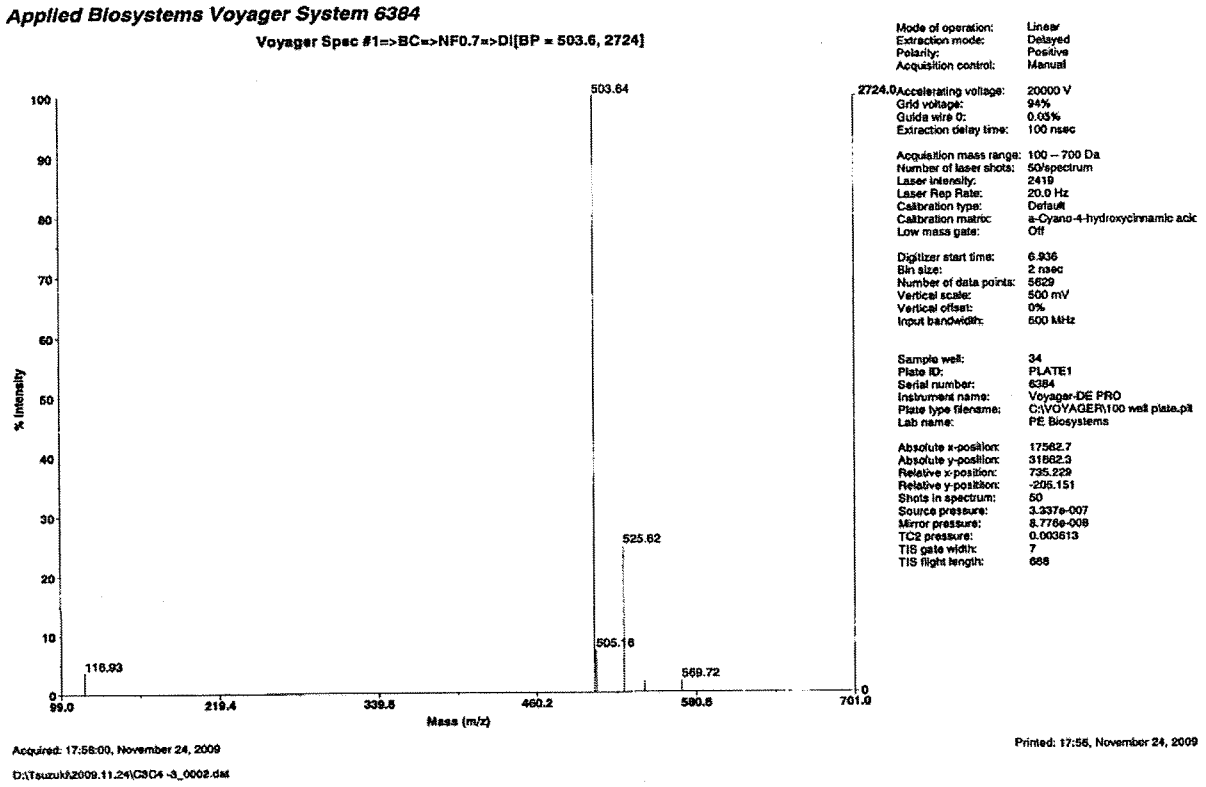
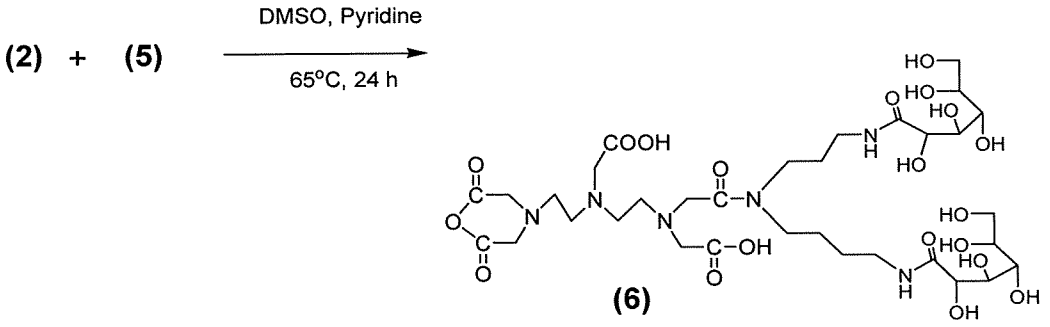


Fig. 2-10-3 Mass spectra of Spermidine-Glc(OH) Terminal

2-10-2-2 Synthesis of Asymmetric DTPA-Spermidine-Glc(OH)

To a solution of DTPA dianhydride in DMSO and was added terminal, pyridine in quantitative ratio and the mixture was stirred for 24 h at 65°C and after the completion of the reaction, reaction mixture was poured in to ethyl acetate under stirring and continued stirring for 10 min separated the solid washed with ethyl acetate thoroughly to remove the traces of DMSO and finally dried to get the pure compound.



Scheme 2-10-3 Synthesis of DTPA-Spermidine-Glc(OH)

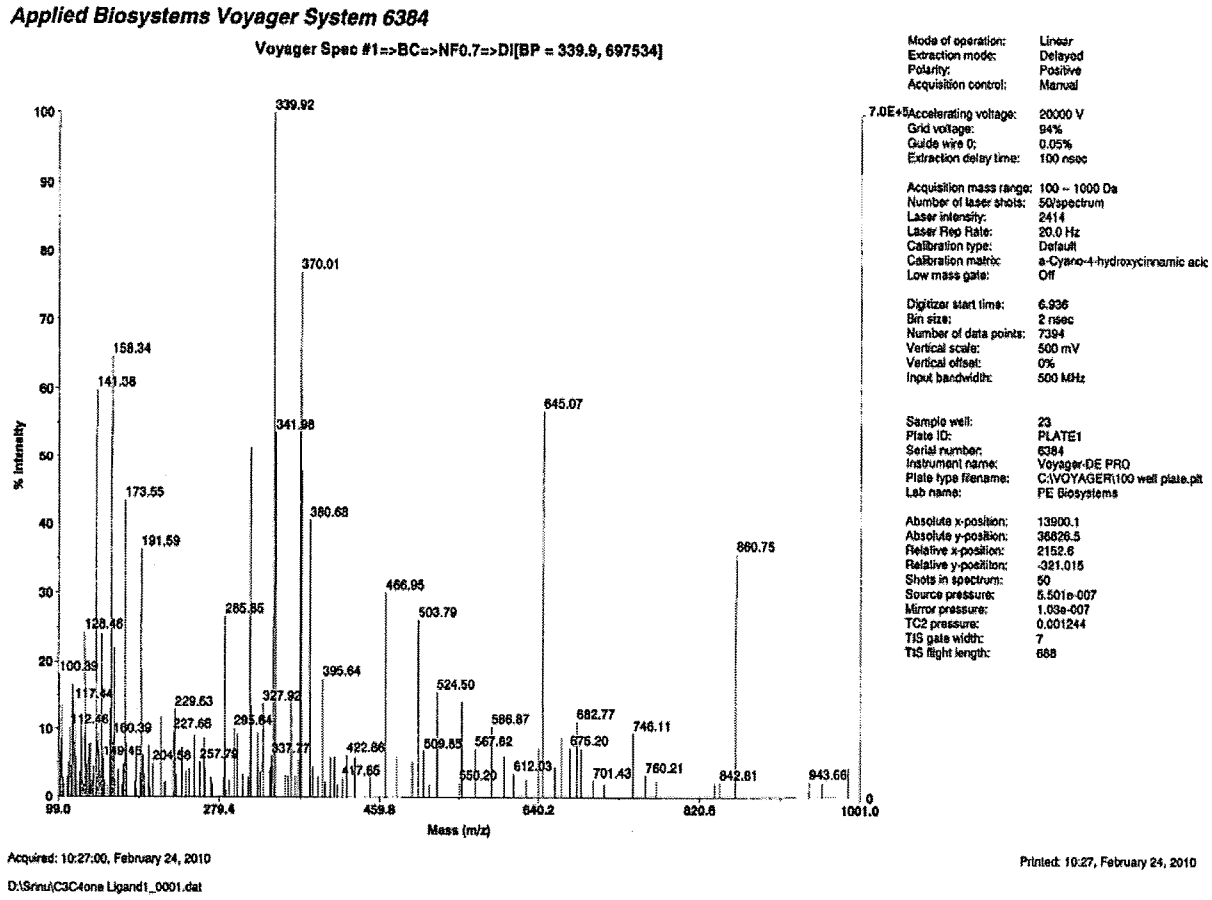
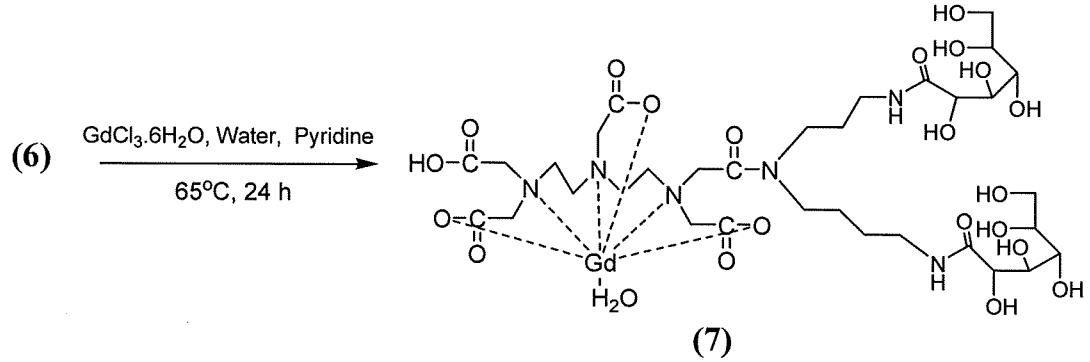


Fig. 2-10-4 Mass Spectra of Asymmetric DTPA-Spermidine-Glc(OH)

2-10-2-3 Synthesis of Asymmetric Gd-DTPA-Spermidine-Glc(OH)

To a solution of above prepared ligand (**6**) in water pyridine was added and the mixture was stirred. To this $GdCl_3 \cdot 6H_2O$ was added and the reaction was kept at 65°C and stirred for 24 h. 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 and the filtrate was concentrated under vacuum.



Scheme 2-10-4 Synthesis of Gd-DTPA-Spermidine-Glc(OH)

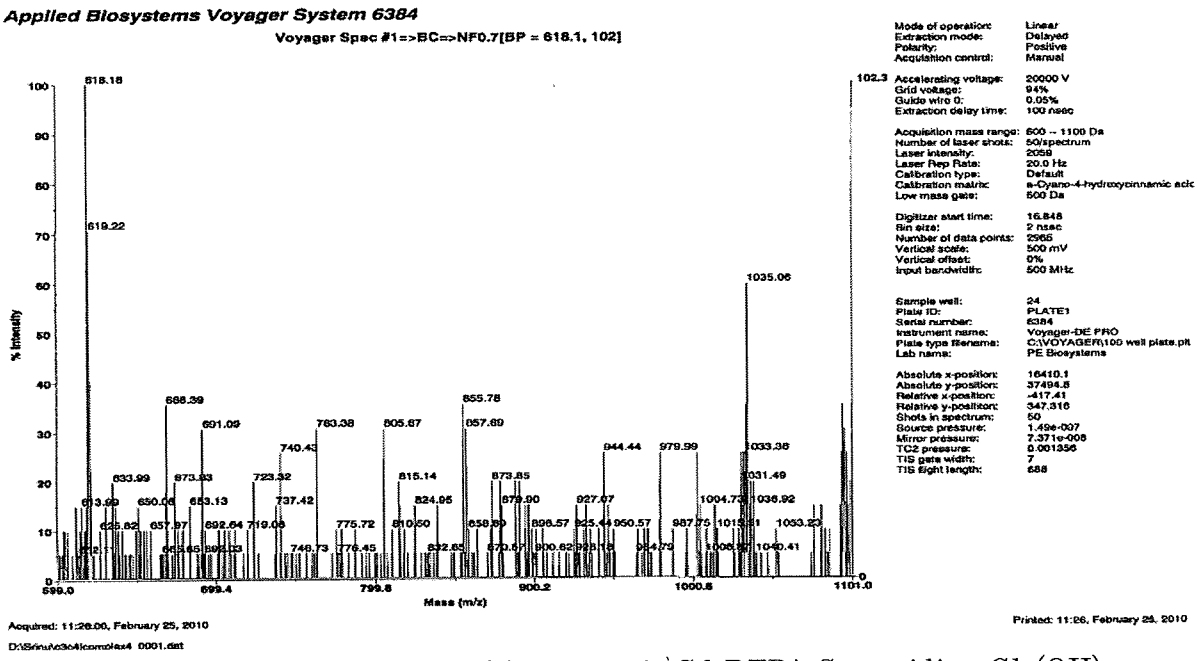


Fig. 2-10-5 Mass Spectra of Asymmetric Gd-DTPA-Spermidine-Glc(OH)

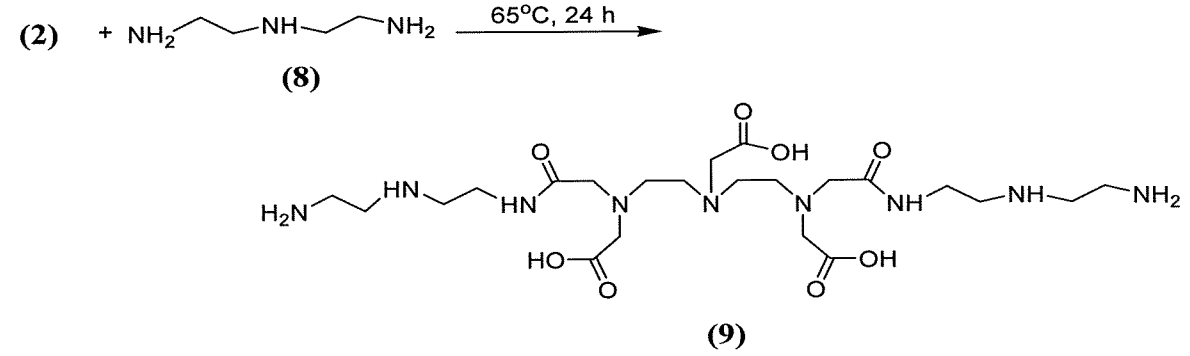
2-10-4 Synthesis of Gd-DTPA-C2-D2-Amine

2-10-4-1 Result and Discussion

I this experiment, the new Gd-DTPA-bis amine complex was synthesized by the reaction of DTPA dianhydride and bis(2-aminoethyl)amine to form ligand which was further reacted with $GdCl_3 \cdot 6H_2O$ to form Gd-DTPA-C2-D2 amine complex.

2-10-4-2 Synthesis of DTPA-C2-D2 amine Ligand

To a solution of DTPA dianhydride in excess of bis(2-aminoethyl)amine and the mixture was stirred for 24 h at 60°C [7]. After the completion of the reaction ethyl acetate was added and stirred for 10 min then filter the solid and dried the compound under vacuum obtain pure compound.



Scheme 2-10-5 Synthesis of DTPA-C2-D2-Amine ligand

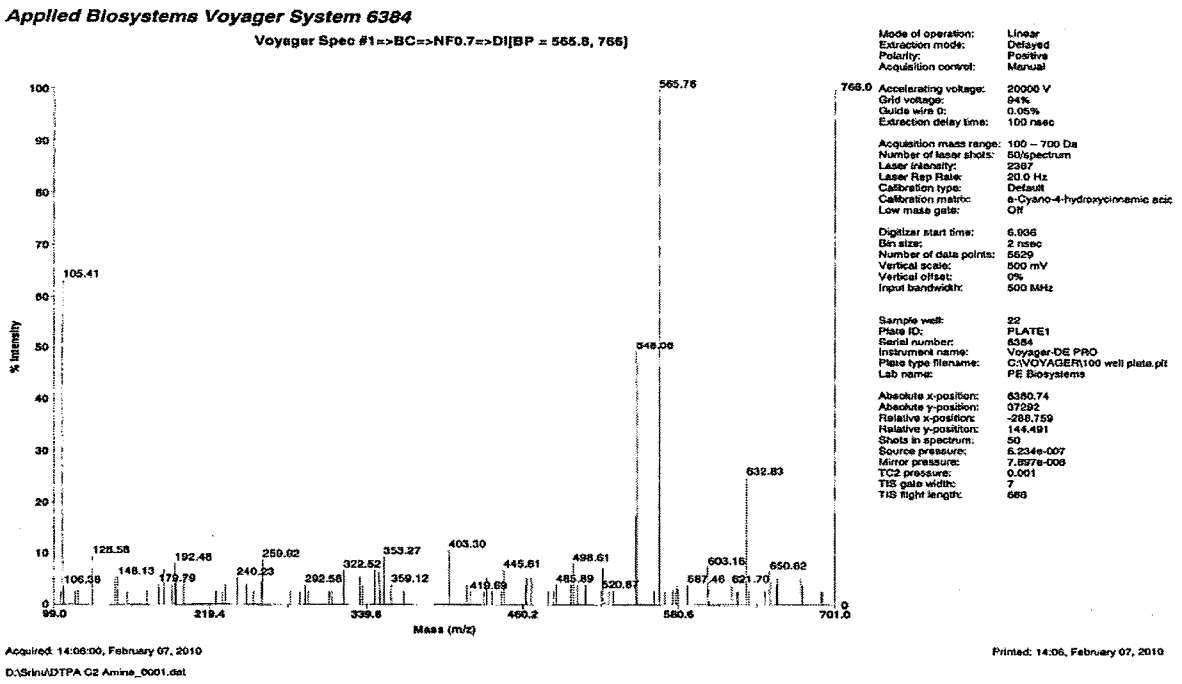


Fig. 2-10-6 Mass Spectra of DTPA-C2–D2-Amine ligand

2-10-4-3 Synthesis of Gd-DTPA-C2-D2 Amine Complex

To a solution of above prepared ligand in water was added pyridine and the mixture was stirred for 10 min. To this $GdCl_3 \cdot 6H_2O$ was added slowly and the reaction was kept at 65°C and stirred for 24 h. 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 filtrate was concentrated under vacuum.

(9) $\xrightarrow[\text{water, pyridine}\ 65°C, 24\ h]{GdCl_3.6H_2O}$

特許出願の関係で
ここでは開示できない

(10)

Scheme 2-10-6 Synthesis of Gd-DTPA-C2-D2-Amine complex

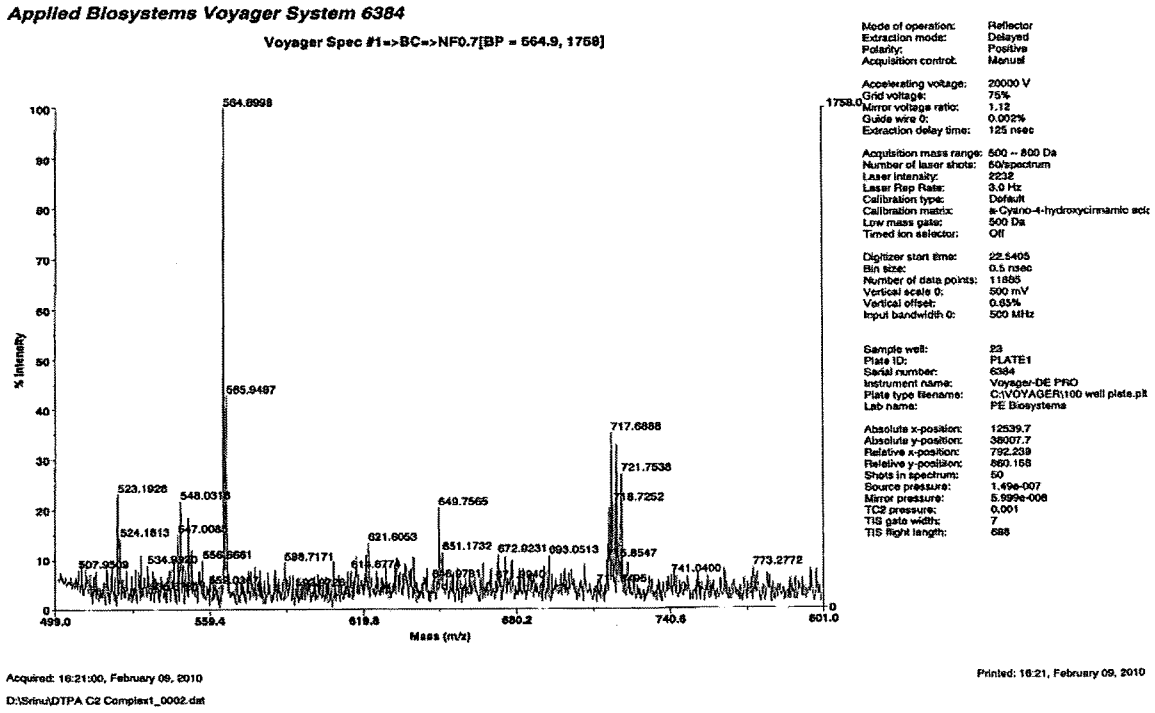


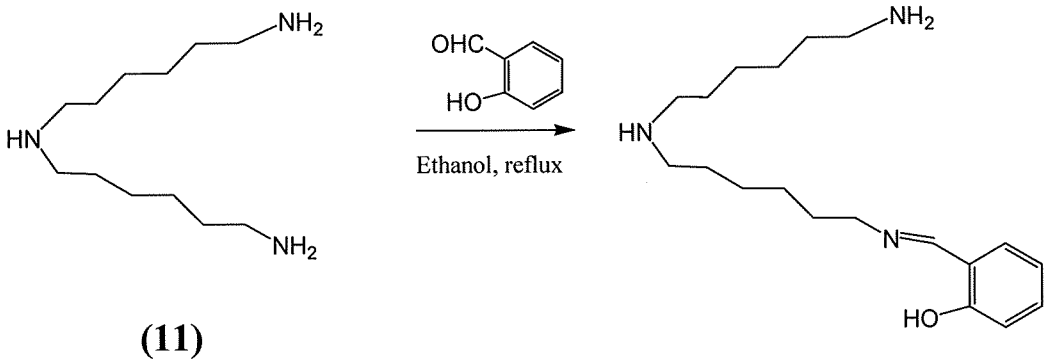
Fig. 2-10-7 Mass Spectra of DTPA-C2–D2-Amine complex

2-10-5 Synthesis of Gd-DTPA-HMTA-C6-D2-imino-Glc(OH) Complex

2-10-5-1 Synthesis of Terminal

Step 1

HMTA and 2-hydroxybenzaldehyde were dissolved in ethanol and the solution was refluxed for 3 h [8]. After completion of the reaction solvent was evaporated, the crude product was washed with hexane and then water dried under vacuum for 3 h to obtain gummy crude which was used for next step. Yield: 81%

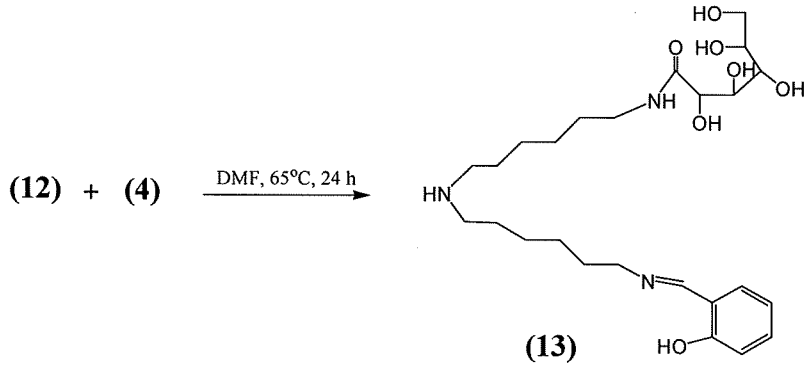


(11) **(12)**

Scheme 2-10-7 Synthesis of HMTA-C6-D2-imine

Step 2

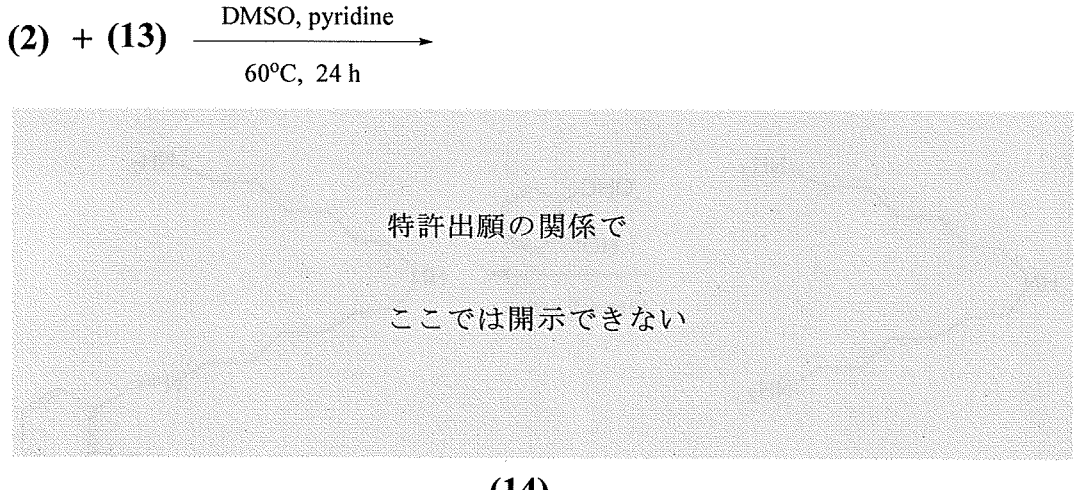
2-((E)-(6-(6-aminohexylamino)hexylimino)methyl)phenol were dissolved in 30 ml of DMF and D-glucono-1,5-lactone was added to the solution and then the mixture was stirred for 24 h. After completion of the reaction was poured in to ethyl acetate under stirring and continued for 10 min then filtered the solid washed with ethyl acetate and methanol and dried the solid to obtain pure compound.



Scheme 2-10-8 Synthesis of HMTA-C6-D2-imine-Glc(OH)

2-10-5-2 Synthesis of DTPA-HMTA-C6-D2-imine-Glc(OH) Ligand

To a solution of DTPA dianhydride in DMSO was added HMTA-C6-D2-imino-Glc(OH) terminal in quantitative ratio and the mixture was stirred for 24 h at 60°C and after the completion of the reaction, reaction mixture was poured in to ethyl acetate under stirring and stirring was continued for 10 min and then the solid was separated and washed with ethyl acetate thoroughly to remove the traces of DMSO and finally dried to get the pure compound.



(14)

Scheme 2-10-9 Synthesis of DTPA-HMTA-C6-D2-imino-Glc(OH) Ligand