Fig. 2-8-02ー2-8-04 に示したように、3 つのサンプルはいずれも XRD 反射を示し、状態としては結晶である。それぞれの XRD パターンを見てみると、ルート B で合成した図 A の DTPA-DETA-D2-4Glc(OH) の XRD パターンは、図 B、図 C の加水分解生成物とは全く違ったパターンを示した。A と、加水分解生成物 (B,C) は全く異なる結晶構造を有することから、100% の確証はないものの、おそらくは同じ構造の化合物ではないと推定できる。

一方、加水分解生成物 B と C は、良く似たパターンを示し、反射の位置はほぼ一致していた。反面、反射の相対強度は異なり、二種の結晶の比率の異なる混合物のように見える。すなわち、図に示した[a 群のパターンを与える結晶]と、[b 群のパターンを与える結晶]の混合物が存在すると考えることができそうである。少なくとも結晶化した生成物に限定すれば、極度に複雑な混合物ではなく、数種の化合物からなる、比較的単純な混合物であると予想された。

今回はガドリニウムを含まない配位子のみの分析であったが、NMR 分析が不可能なガドリニウム錯体についても XRD による分析が有用な情報を与える可能性がある。

また、NaOMe や NaOEt などで加水分解を行うと好結果が得られることが判明したので、その生成物についても検討を行う。結晶化しなかった部分についても別途方法を考えて検討を行ってゆきたい。

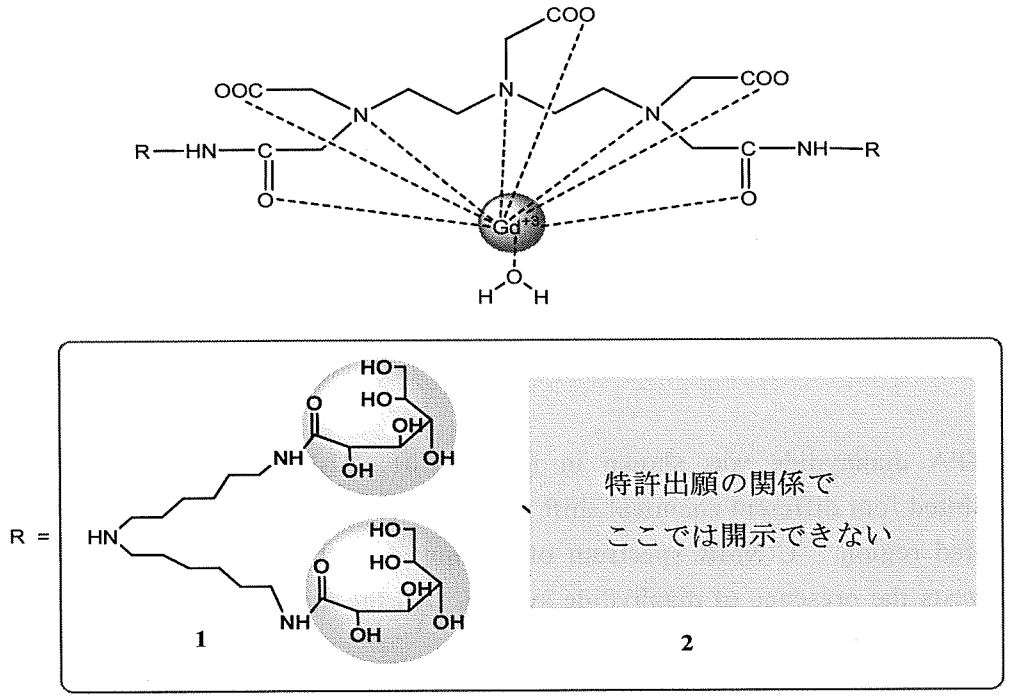
2-9 Design and Synthesis of New Potential M.R.I. contrast agents

Arigala Uma Ravi Sankar

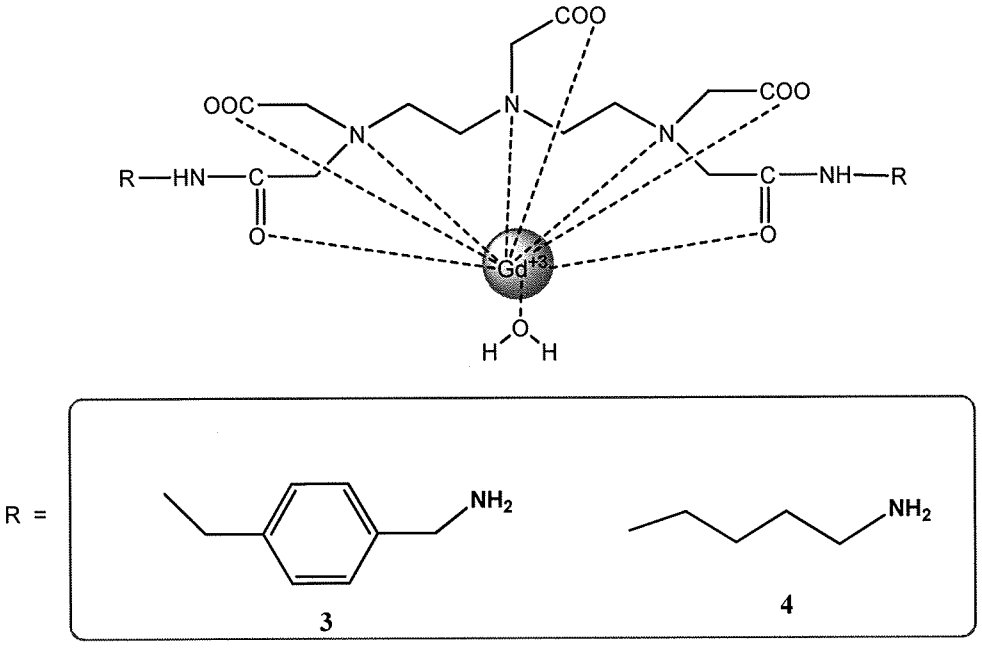
2-9-1 Synthesis of New Gd-DTPA-Sugar Frame Work

2-9-1-1 Introduction

Over the past two decades Magnetic Resonance Image (MRI) has become a very powerful tool of diagnostic medicine. The use of paramagnetic metal complexes as image enhancement agent aloe imaging that, for several important applications, is otherwise unobtainable.[1,2] Since the Gd(III) ion, with a $4f^7$ electronic configuration has a S= 7/2 ground state, it is particularly attractive as a imaging agent. Paramagnetic materials have been investigated as MRI contrast agents (CAs). These materials enhance the contrast of the image indirectly by lowering the magnetic relaxation time of water protons in the surrounding tissues.[3] The most frequently used CAs are stable gadolinium(III) complexes with hydrophilic poly(aminocarboxylate) ligands resulting in rapid extracellular distribution and renal elimination. Gd(III) is preferred because of its favorable magnetic properties. Depending on the density of the ligand one or more water molecules might be directly coordinated to the paramagnetic center. Gd complexes with amphiphilic properties have previously been prepared and evaluated as blood-pool and liver imaging agents. Long chain amides and esters of Gd-DTPA are the most common.[4] As we now, the glycoside groups have a specific target and combine with asialoglycoprotein receptor (ASGPR) on the surface of hepatocyte. Also, the glycoside groups, which were introduced into DTPA, can improve the water-solubility of contrast agent. So in this work, DTPA was used as a core and glycoside was used as a biofunctional group to prepare a series of dendritic Gd-complexes for novel MRI contrast agents. To overcome the defects of MRI contrast agents. I have been synthesized a novel complexes containing four sugar and two sugar groups for MR image by using different linker to connect the MR imaging moiety with biofunctional group by sugars.

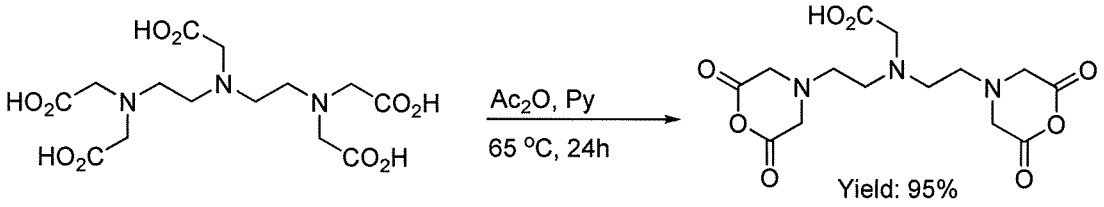


Scheme 2-9-01 Synthesis of Target molecules



Scheme 2-9-02 Synthesis of Target molecules

2-9-1-2 Results and Discussion



Scheme 2-9-03 Synthesis of DTPA dianhydride

^{1}H-NMR of DTPA dianhydide was shown in Fig. 2-9-01. ^{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 region. ^{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.

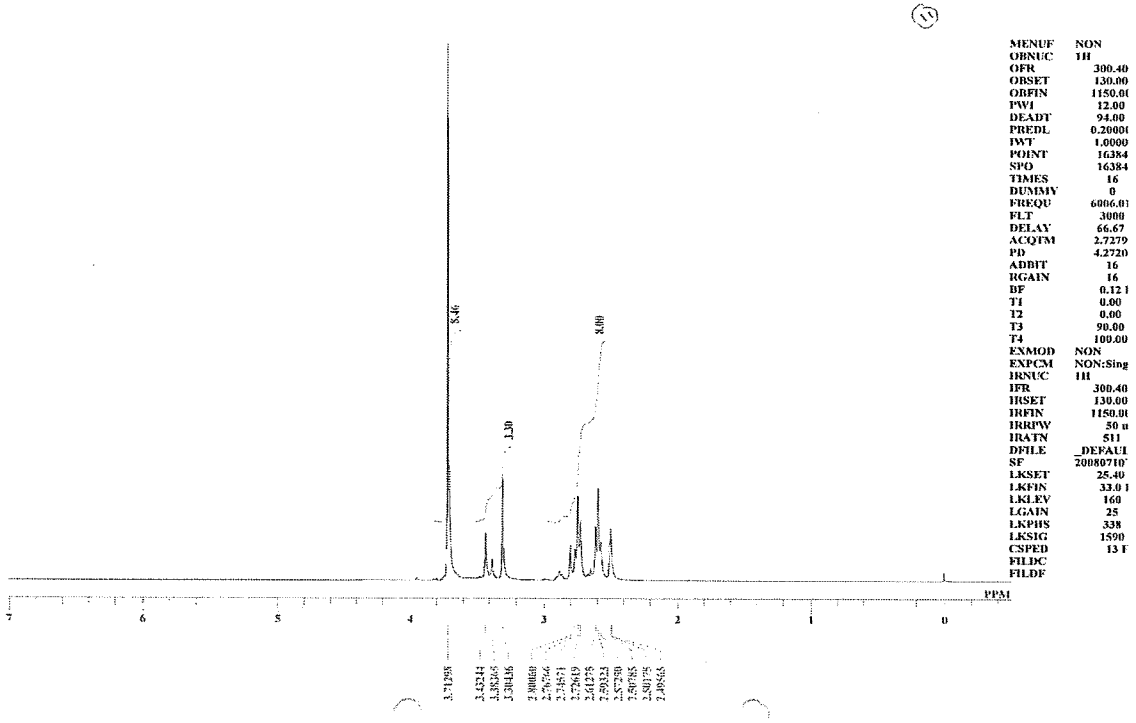


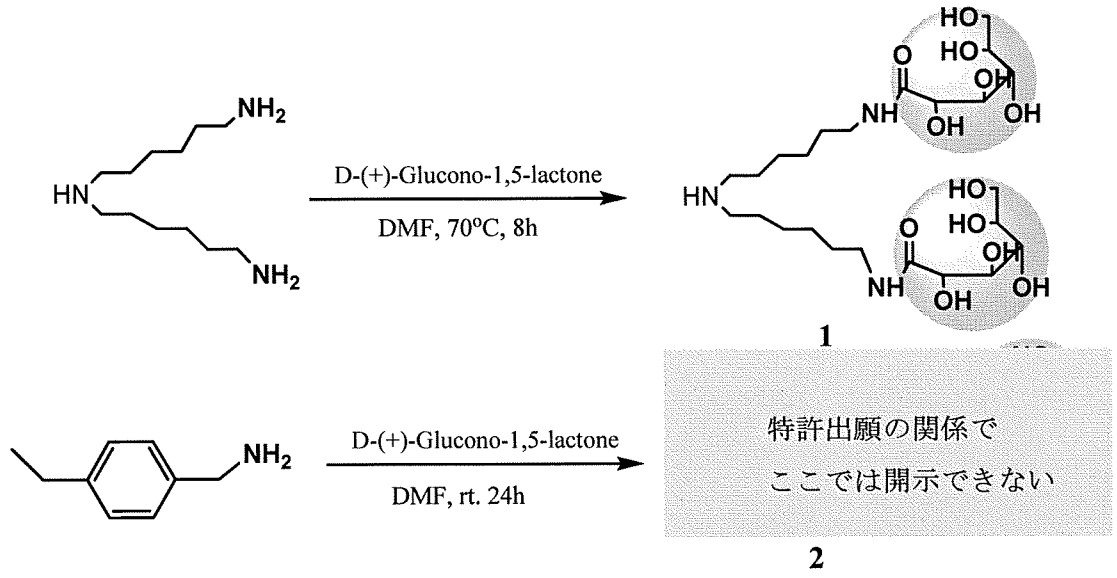
Fig. 2-9-01 ^{1}H-NMR of DTPA dianhydride

Synthesis of terminal 1

To a solution of D-(+)–glucono-1,5-lactone (1.0 g, 7.3 mmol) in dry DMF(20 mL) was added hexaethylene triamine (1.3 g, 7.3 mmol), then stirred for 8 h at 70^0C. 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%.

Synthesis of terminal 2

To a solution of D-(+)–glucono-1,5-lactone (1.0 g, 7.3 mmol) in dry DMF(20 mL) was added xylylenediamine (1.3 g, 7.3 mmol), then stirred for 24 h at room temperature. After completion of the reaction, the solvent was removed under reduced pressure and purified by column chromatography by using mixture of chloroform and methanol as eluents. to get a yellow crystals. The yield of the compound is 90% .The Scheme is given bellow



Scheme 2-9-04 Synthesis of Terminals 1 and 2

^{1}H-NMR, ^{13}C-NMR and mass spectral analysis of the above compounds 1 and 2 were shown in Fig. 2-9-02 and 2-9-03 and 2-9-04. ^{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.

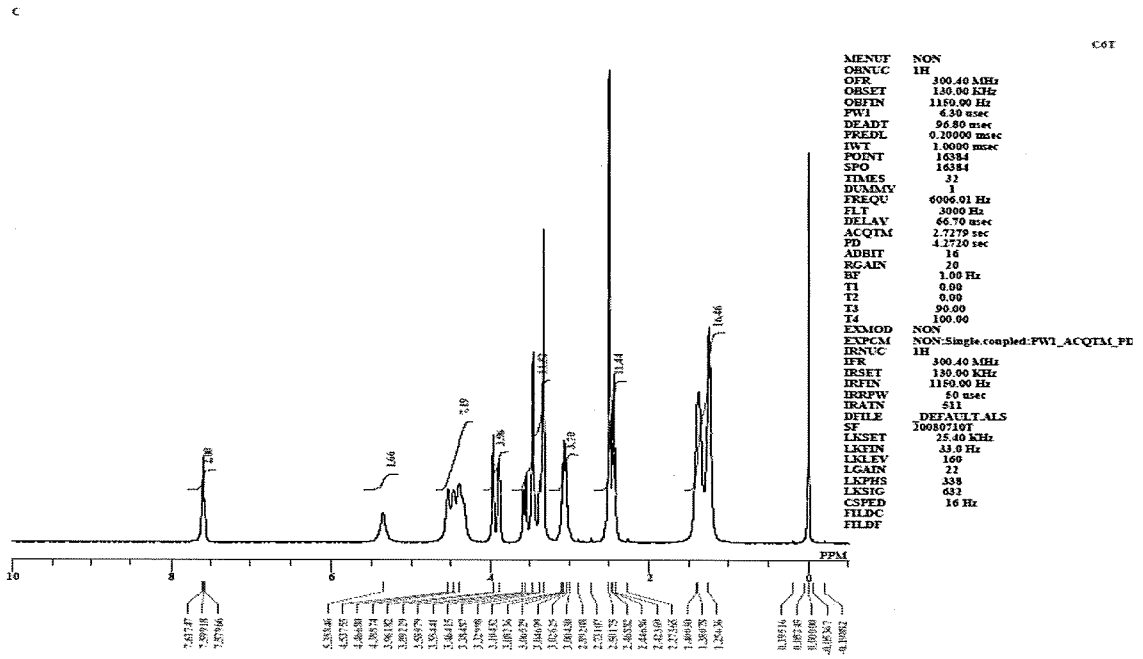


Fig. 2-9-02 ^{1}H-NMR of Terminal 1

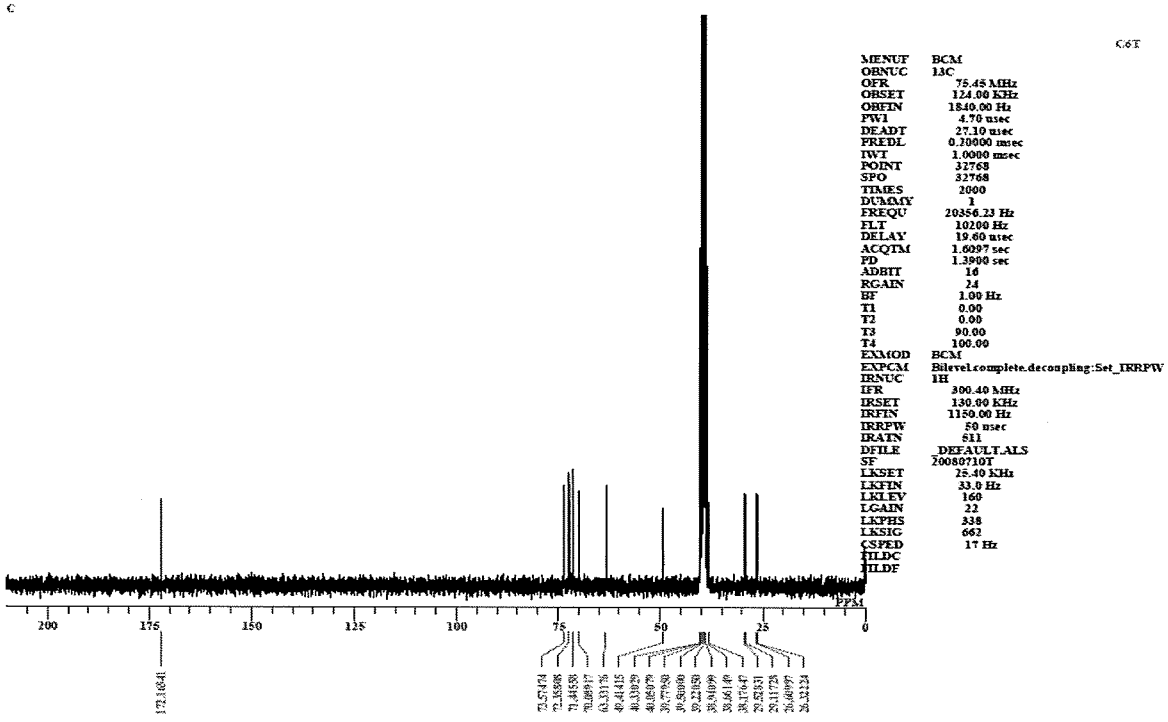


Fig. 2-9-03 ^{13}C-NMR of Terminal 1

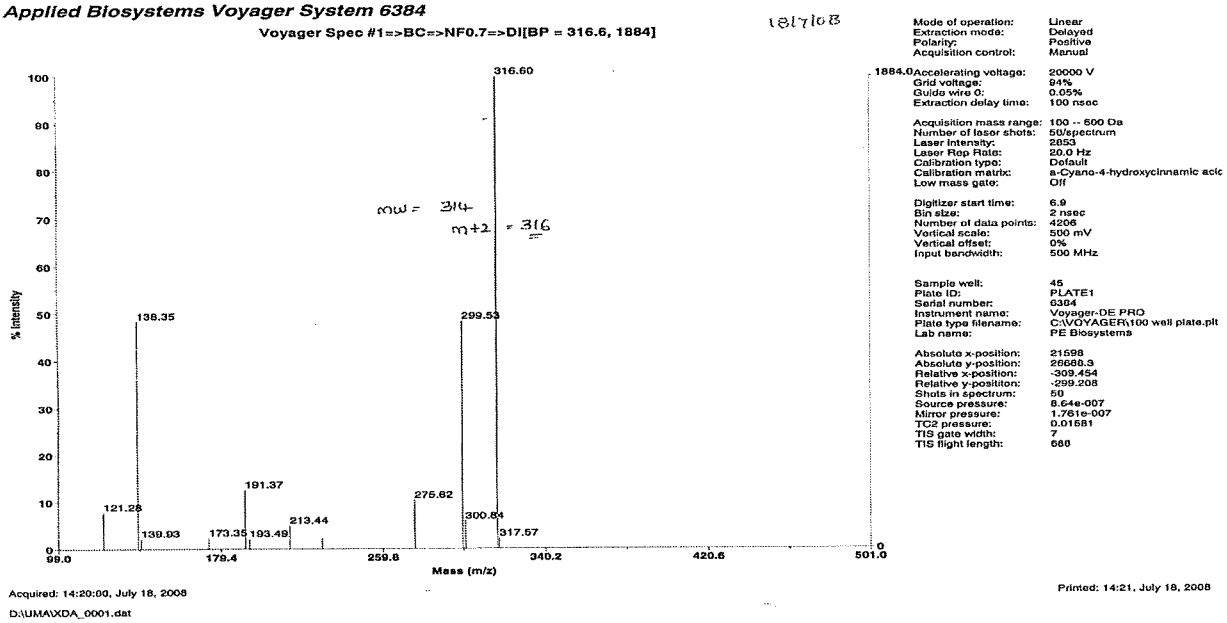


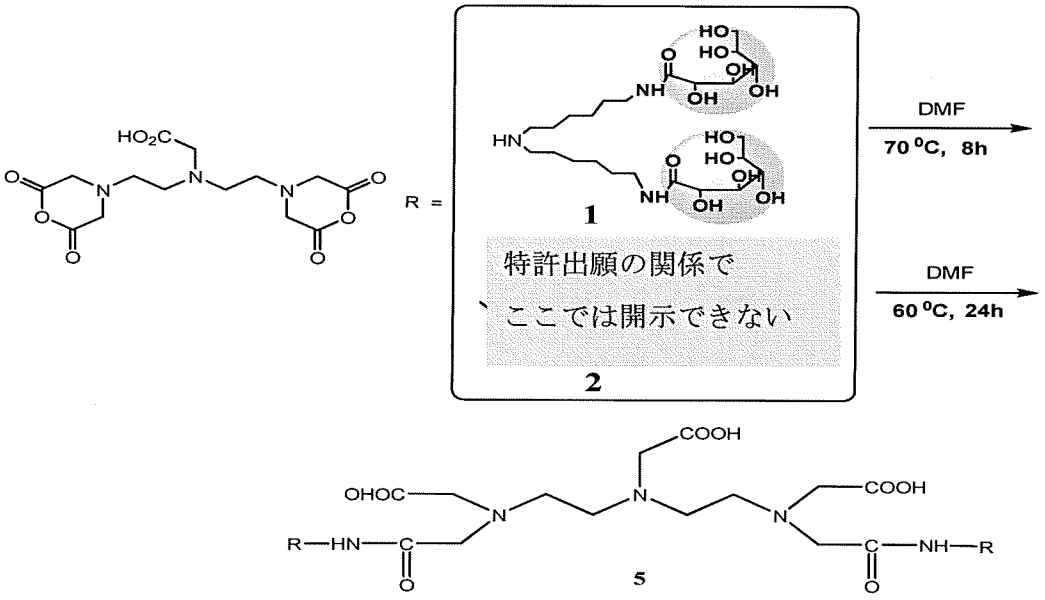
Fig. 2-9-04 Mass spectrum of Terminal 2

Synthesis of ligand with Four sugars

The synthesis of dendritic ligand employed a convergent method to couple core and glycoside branch. To the solution of compound 1 in DMF was added DTPA anhydride and stirred for 8 h at 70°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.

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 24 h 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 given bellow.



Scheme 2-9-05　Synthesis of Ligands 5

^{1}H-NMR spectral analysis of the above compound **5** was shown in Fig. 2-9-05. ^{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.

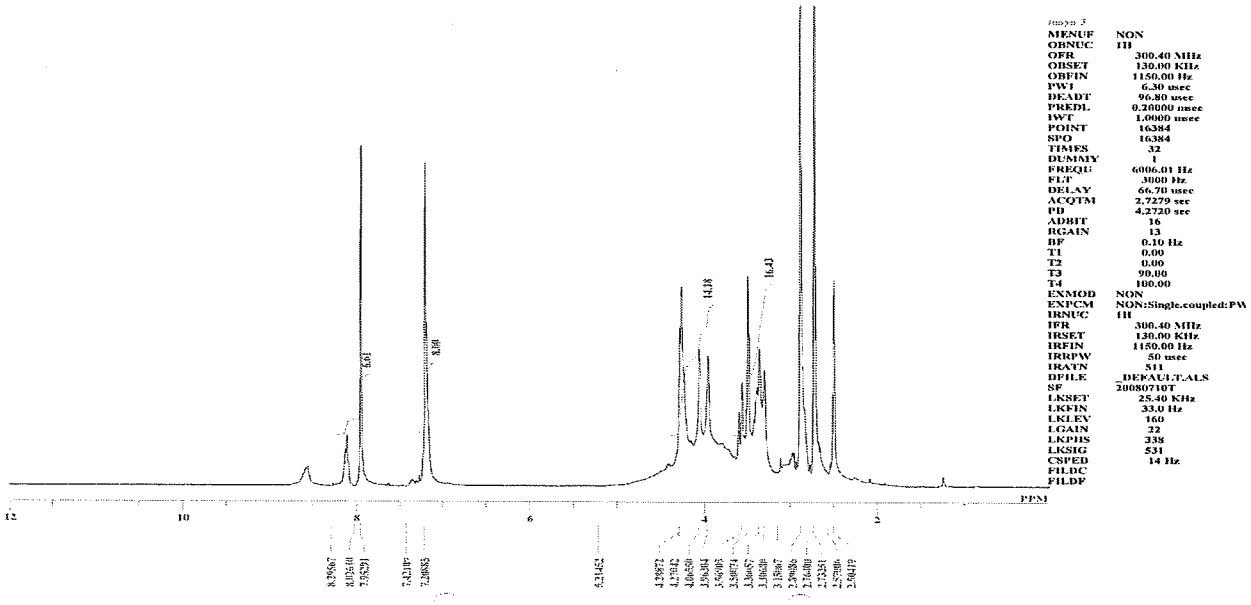
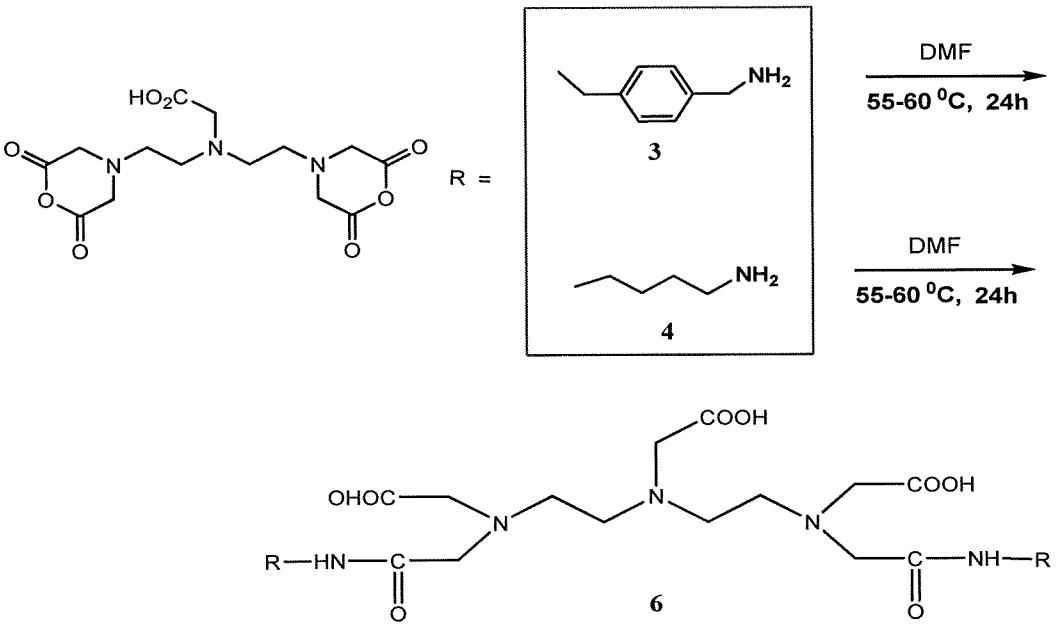


Fig. 2-9-05　^{1}H-NMRspectrum of Ligand 5 (R= 2)

Synthesis of ligand with free amines

The synthesis of dendritic ligand employed a convergent method to couple core and diamine. To the solution of xylyienediamine (0.76 g, 5.8 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, 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 2-9-06 Synthesis of Ligands

Mass and ^{1}H-NMR spectral analysis of the above compounds 6 (R= 3) were shown in Fig. 2-9-06 and compounds 6 (R= 4) were shown in Fig. 2-9-07. ^{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.

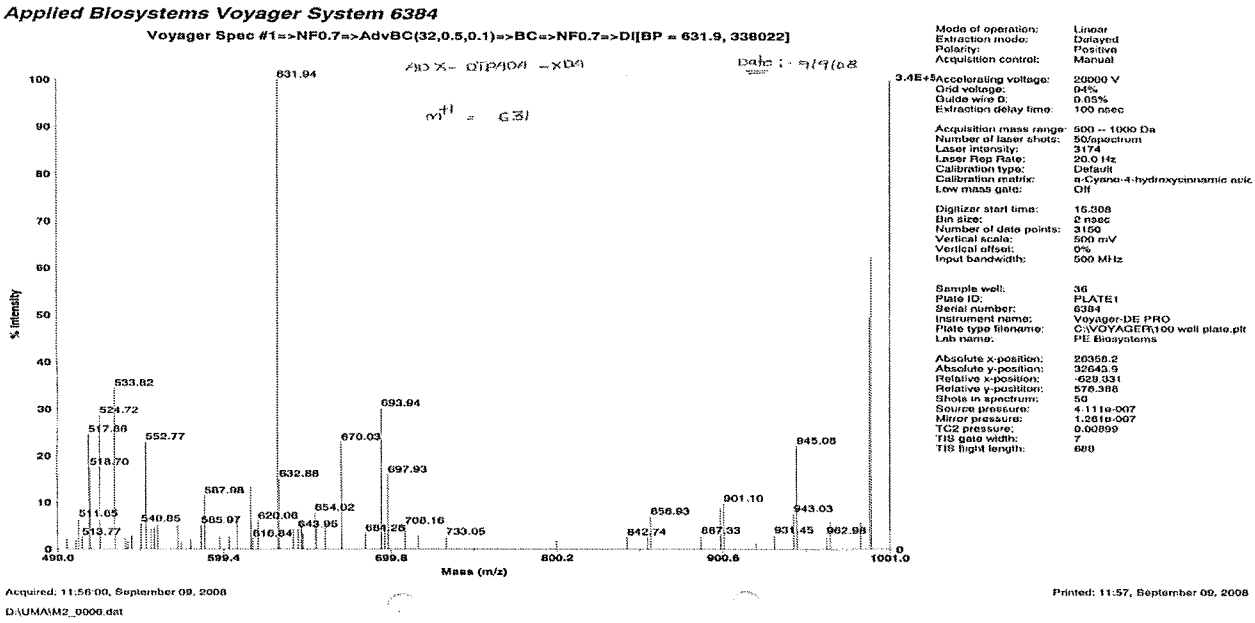


Fig. 2-9-06 Mass spectrum of Ligand 6 (R= 3)

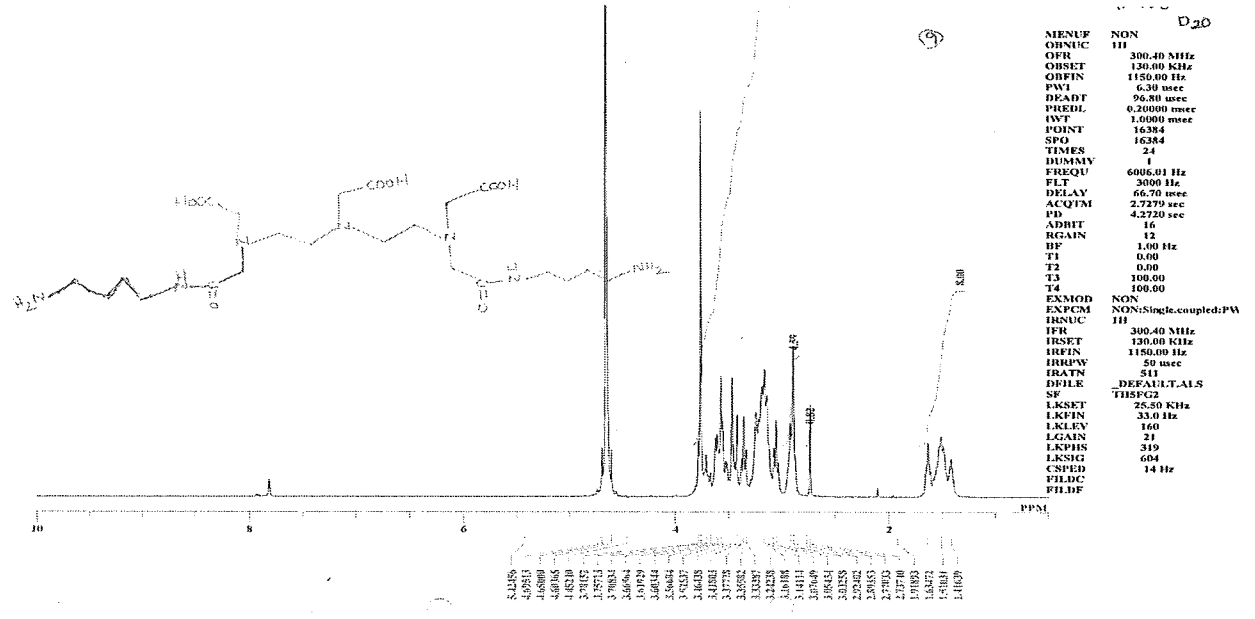
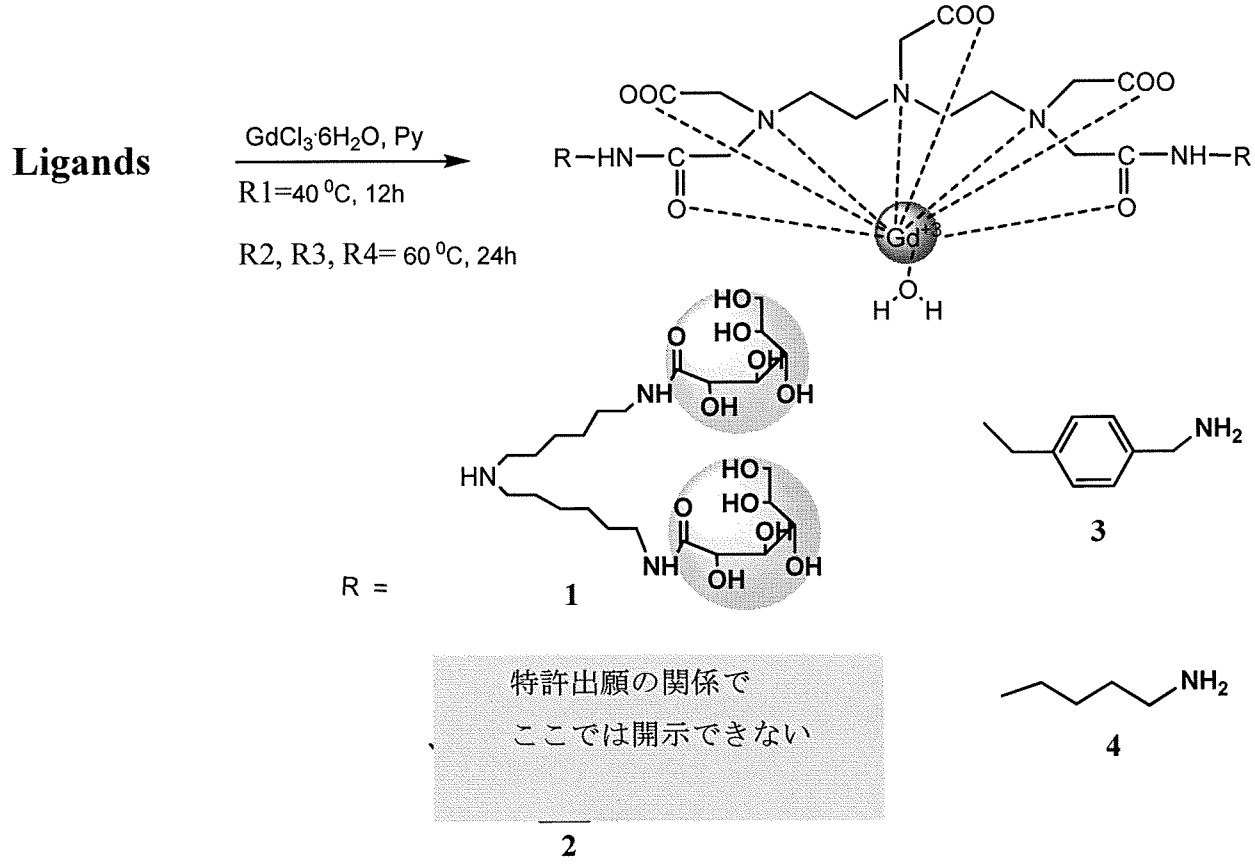


Fig. 2-9-07 ^{1}H-NMRspectrum of Ligand 6 (R= 4)

Synthesis of Gd Complexes

To a solution of ligands **5 and 6** 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 40°C and stirred for 12 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.



Scheme 2-9-07 Synthesis of Complexes

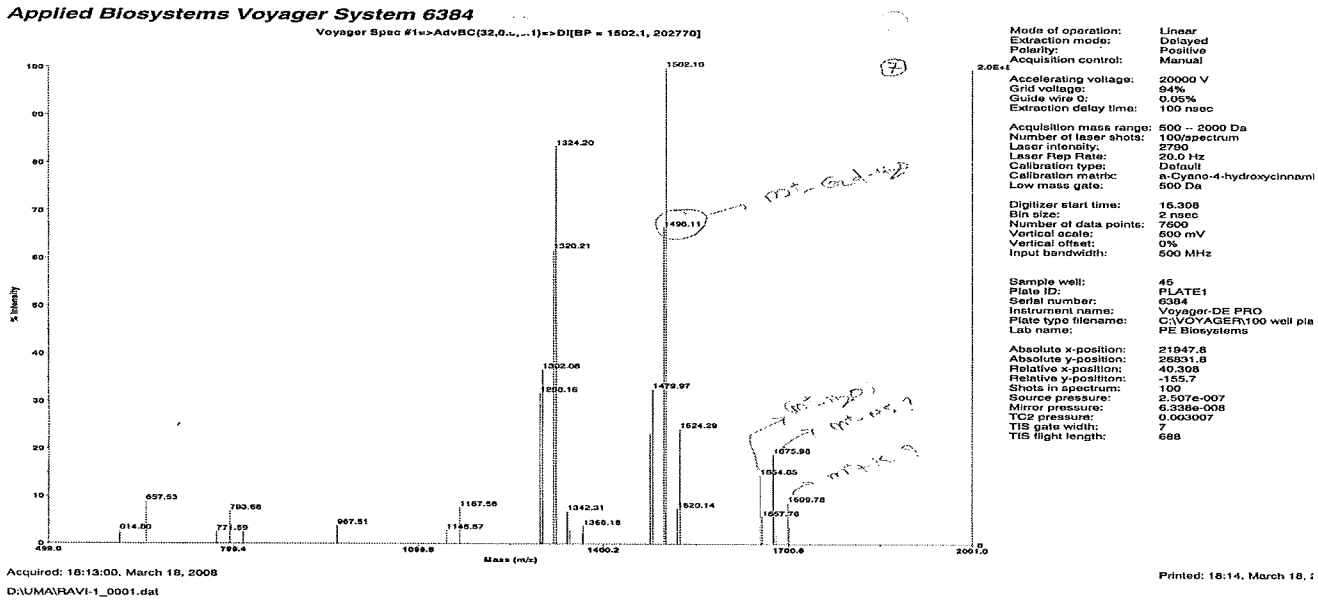


Fig. 2-9-08 Mass spectrum of Complex R1

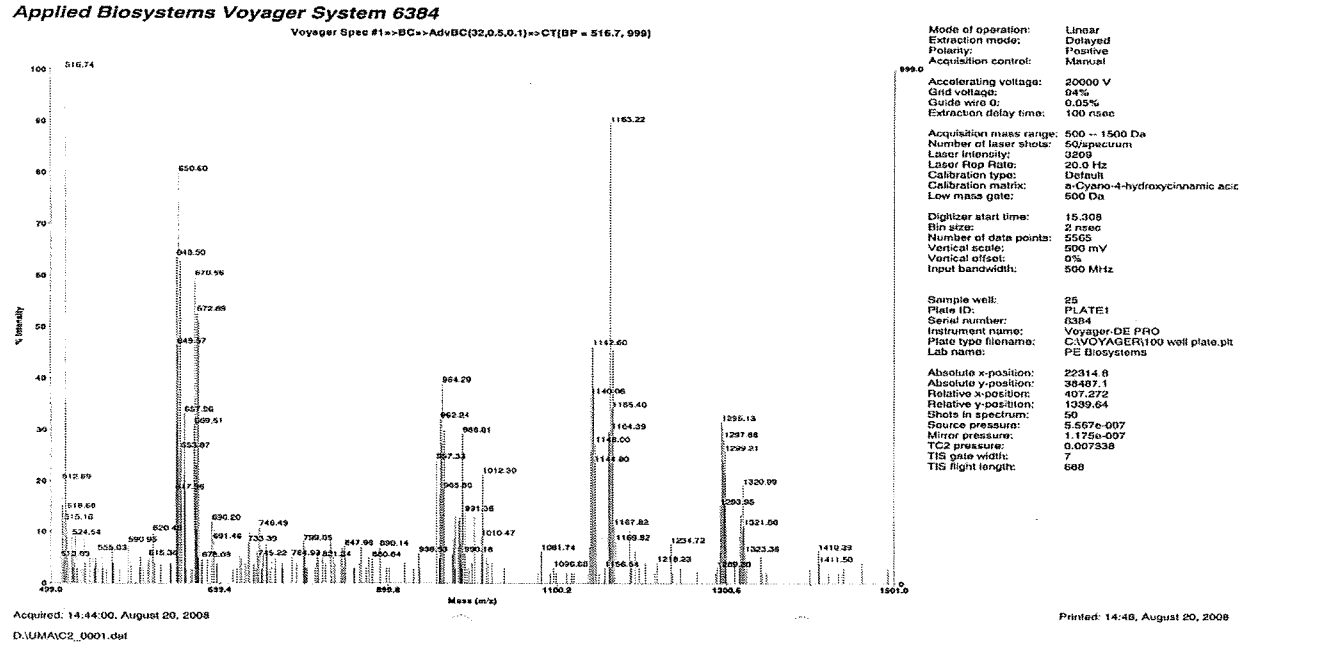


Fig. 2-9-09 Mass spectrum of Complex R2

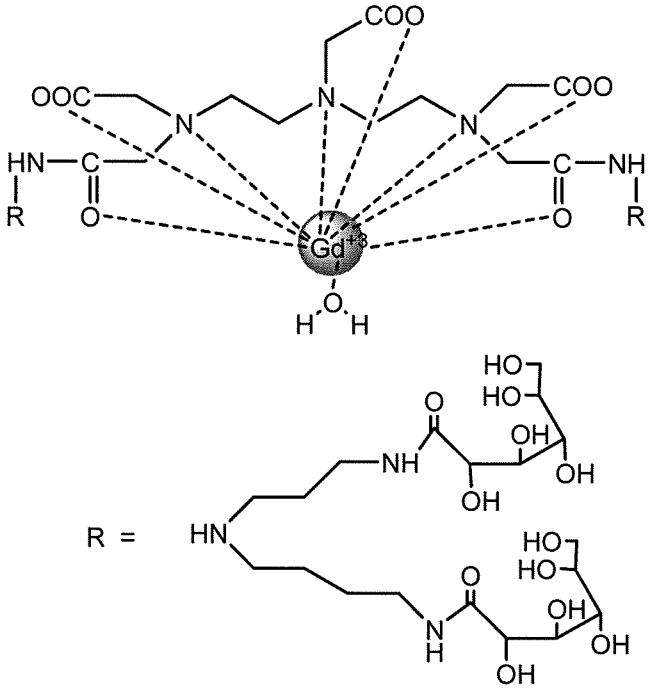
2-9-2 Design and Synthesis of New Potential M.R.I. contrast agents

2-9-2-1 Introduction

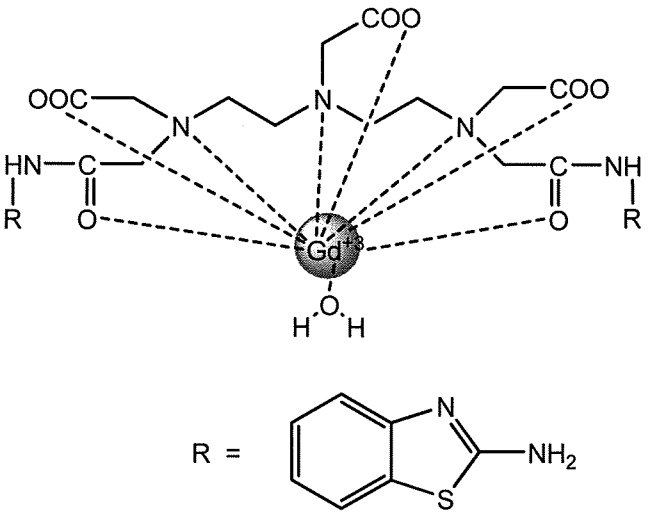
Magnetic resonance imaging (MRI) is a powerful, noninvasive, and widely applied diagnostic technique which allows to obtain images of the inside of the humanbody.[1, 5] Nowadays, more than one-third of the MRI scans are performed by administration of a contrast agent, usually a gadolinium complex.[3,6] Gadolinium(III) ion is able, due to its favorable paramagnetic properties, to increase the relaxation rate of the surrounding water protons, making the region of interest brighter than the background.

Since the approval of the first gadolinium complex for human administration in 1988, several other analogues have reached the market.[7, 8] The contrast agents of first generation distribute in to the intravascular and interstitial space immediately after injection and in this context are called "non-specific agent". The medical need for tissue specific contrast agents has driven researchers to design and synthesize contrast agents of second generation able to visualize selectively the liver or the cardiovascular system, for instance.[9] The ultimate goal is a contrast agent that accumulates specifically in tumor cells, allowing an accurate diagnosis of the disease when it is still treatable.

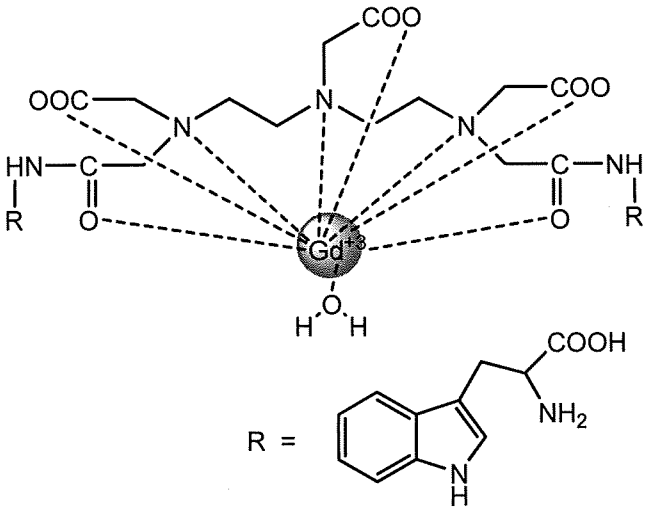
The most frequently used CAs are stable gadolinium(III) complexes with hydrophilic poly(aminocarboxylate) ligands resulting in rapid extracellular distribution and renal elimination. Gd(III) is preferred because of its favorable magnetic properties. Depending on the density of the ligand one or more water molecules might be directly coordinated to the paramagnetic center. Gd complexes with amphiphilic properties have previously been prepared and evaluated as blood-pool and liver imaging agents. Long chain amides and esters of Gd DTPA are the most common.[4] As we now, the glycoside groups have a specific target and combine with asialoglycoprotein receptor (ASGPR) on the surface of hepatocyte. Also, the glycoside groups, which were introduced into DTPA, can improve the water –solubility of contrast agent. So in this work, DTPA was used as a core and glycoside was used as a biofunctional group to prepare a series of dendritic Gd-complexes for novel MRI contrast agents. To overcome the defects of MRI contrast agents. I have been synthesized a novel complexes containing four sugar groups for MR image by using different linker to connect the MR imaging moiety with biofunctional group by sugars.



Scheme 2-9-08 Synthesis of Gd-DTPA-D2-SP-4Glc (OH)

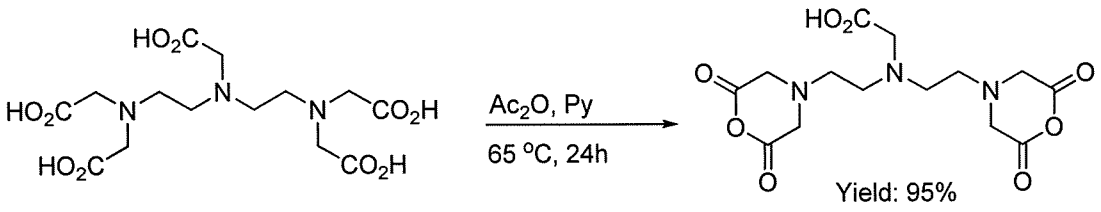


Scheme 2-9-09 Synthesis of Gd-DTPA-2-ABT



Scheme 2-9-10 Synthesis of Gd-DTPA-L-triptophan

2-9-2-2 Results and Discussion



Scheme 2-9-11 Synthesis of DTPA dianhydride

^{1}H-NMR and ^{13}C-NMR spectra of DTPA dianhydide were shown in Fig. 2-9-10 and 2-9-11. ^{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 regions. ^{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.

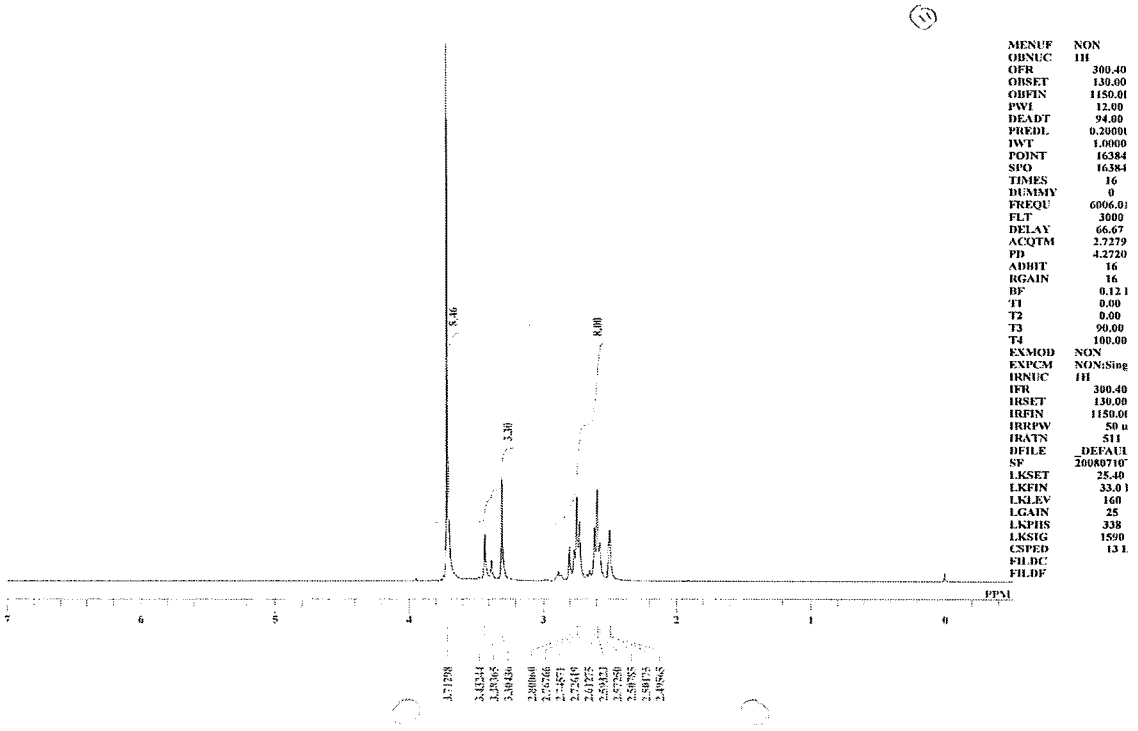


Fig. 2-9-10 ^{1}H-NMR of DTPA dianhydride

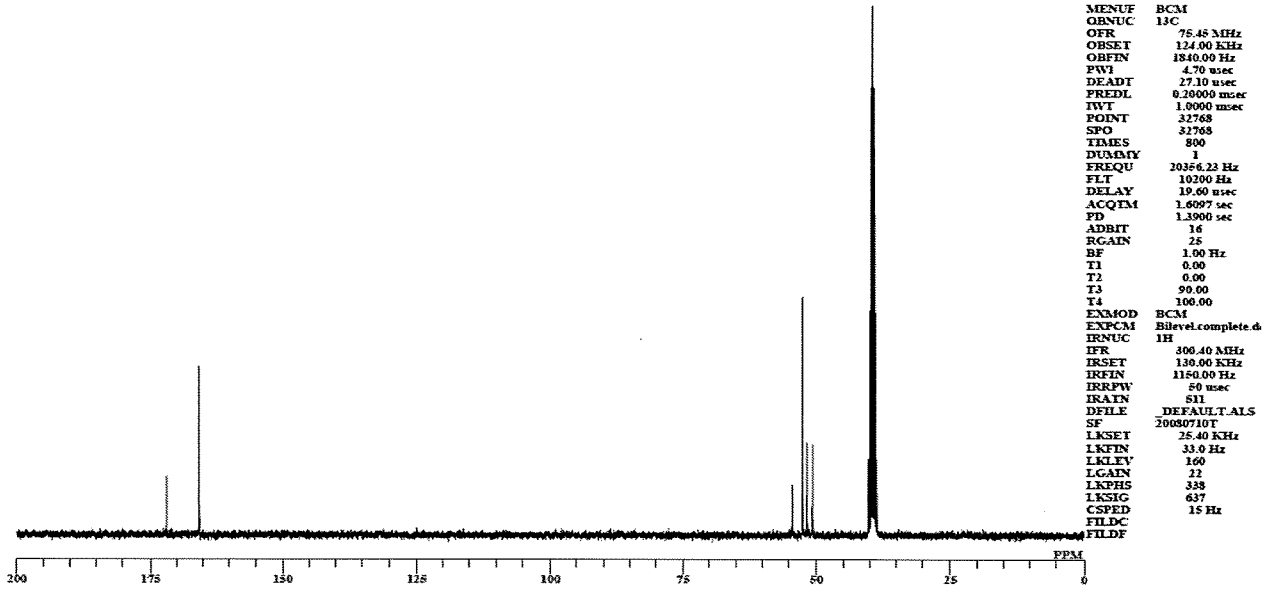
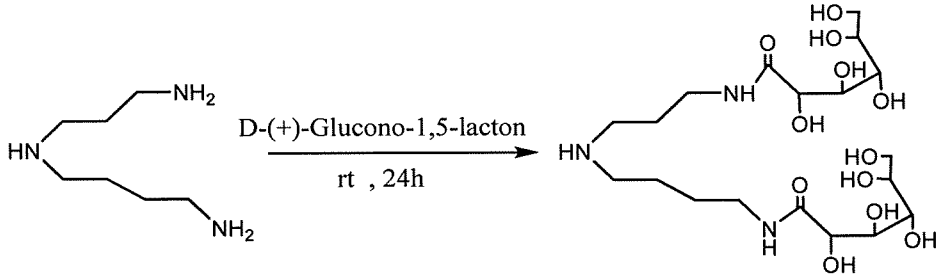


Fig. 2-9-11 ^{13}C-NMR of DTPA dianhydride

Synthesis of Terminal

To a solution of D-(+)–glucono-1,5-lactone (1.0 g, 5.6 mmol) in dry DMF (20 mL) was added spermidine (0.44 mL, 2.8 mmol), then stirred for 24 h at room temperature. After completion of the reaction, the solvent was removed under reduced pressure and purified by column chromatography by using mixture of chloroform and methanol as eluents. to get a yellow crystals. The yield of the compound is 90% .The Scheme is given bellow



7

Scheme 2-9-12 Synthesis of Terminal compound 7

^{1}H-NMR, ^{13}C-NMR and mass spectral analysis of the above compound **7** were shown in Fig. 2-9-12, 2-9-13 and 2-9-14. ^{1}H-NMR spectrum of it showed different chemical shift values at exhibited regions 7.72-7.61 ppm, 5.56 ppm, 4.97-4.03 ppm, 3.96-3.07 ppm, 2.73-2.14 ppm, and 1.64-1.38 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 502.

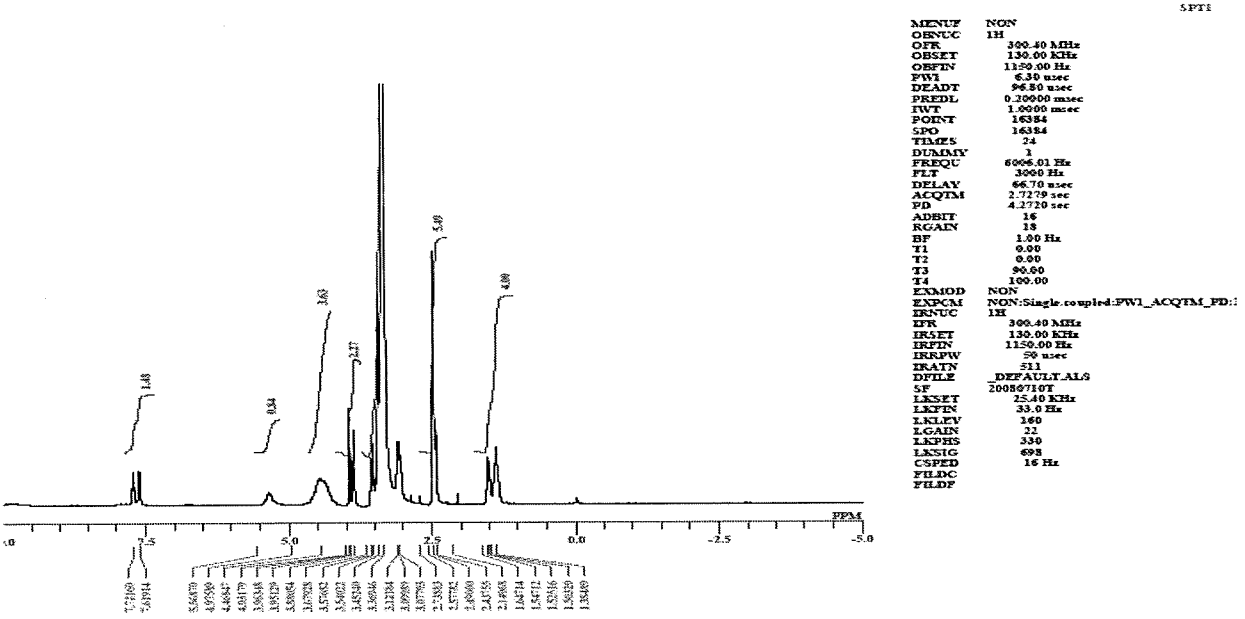


Fig. 2-9-12 ^{1}H-NMR of Terminal compound 7

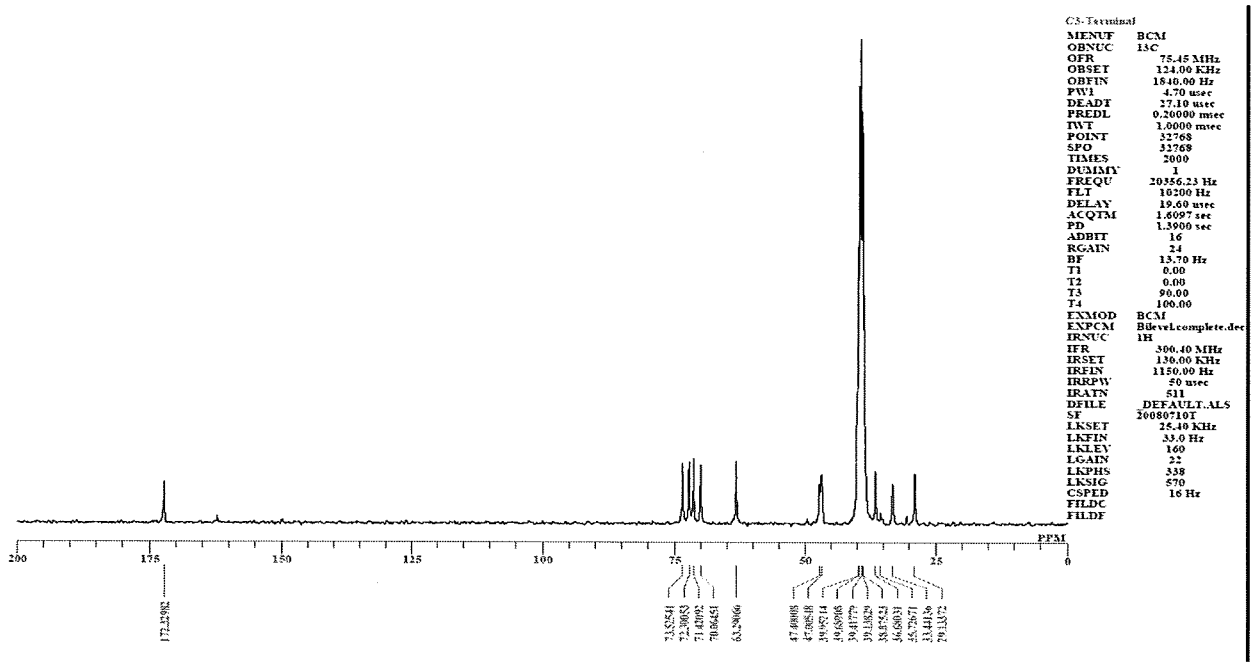


Fig. 2-9-13 ^{13}C-NMR of Terminal compound 7

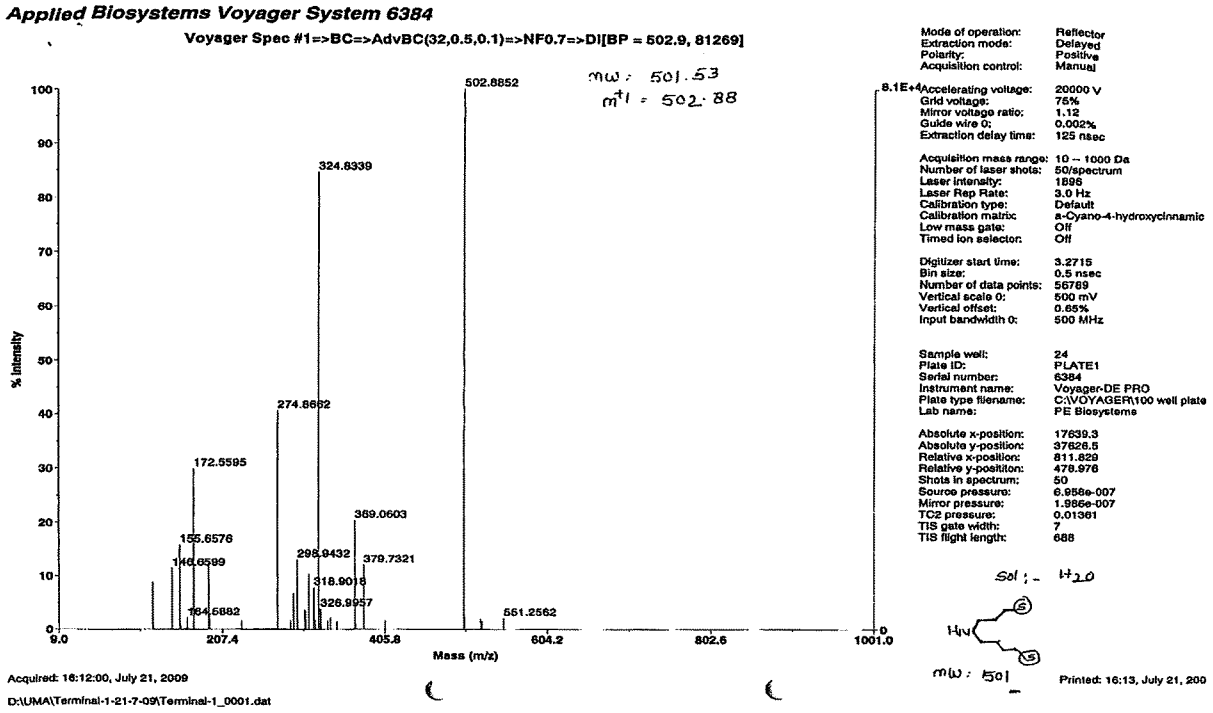
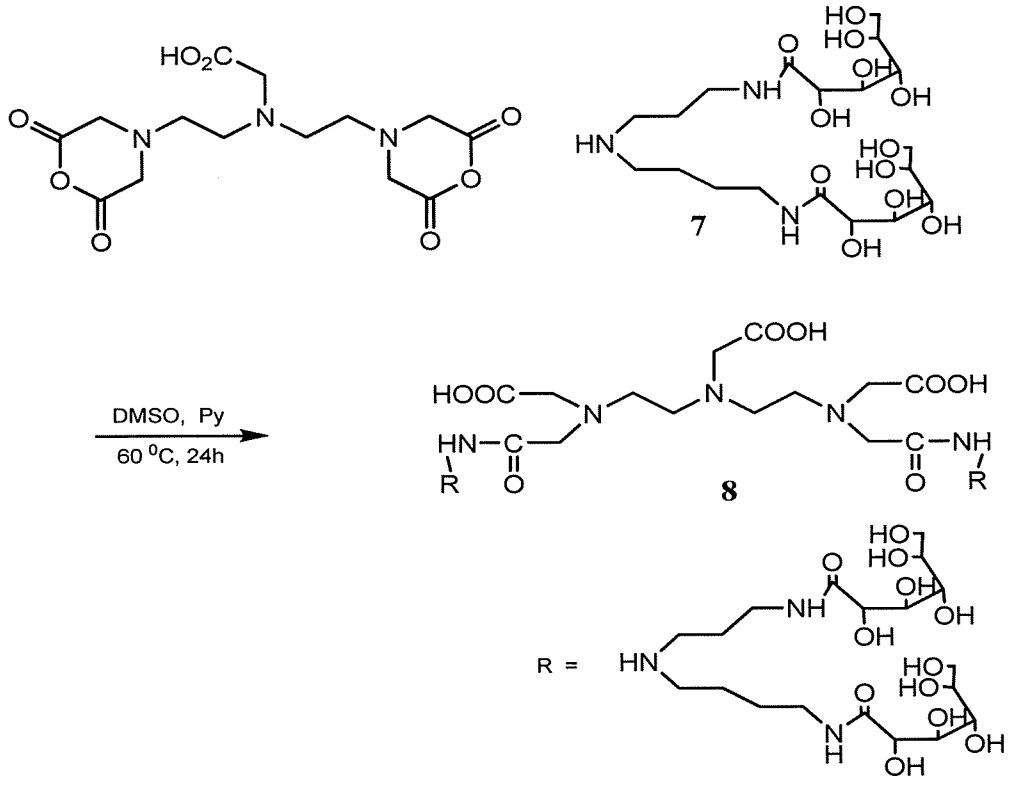


Fig. 2-9-14 Mass spectrum of Terminal compound 7

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 compound **7** in DMSO was added DTPA anhydride and stirred for 24 h at 60^{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 2-9-13 Synthesis of Ligand compound 8

^{1}H-NMR and ^{13}C-NMR spectral analysis of the above compound **8** was shown in Fig. 2-9-15 and 2-9-16. ^{1}H-NMR spectrum of it showed different chemical shift values at exhibited regions 7.72-7.61 ppm, 5.56 ppm, 4.97-4.03 ppm, 3.96-3.07 ppm, 2.73-2.14 ppm, and 1.64-1.38 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.

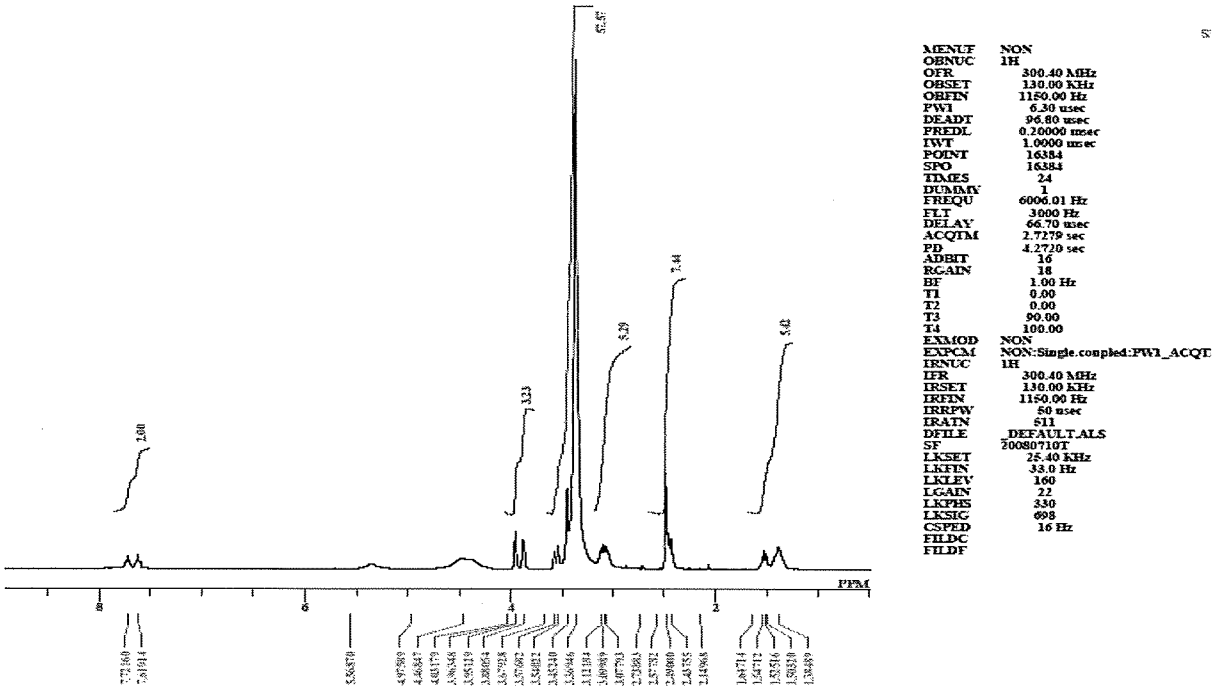


Fig. 2-9-15 ^{1}H-NMR spectrum of Ligand compound 8

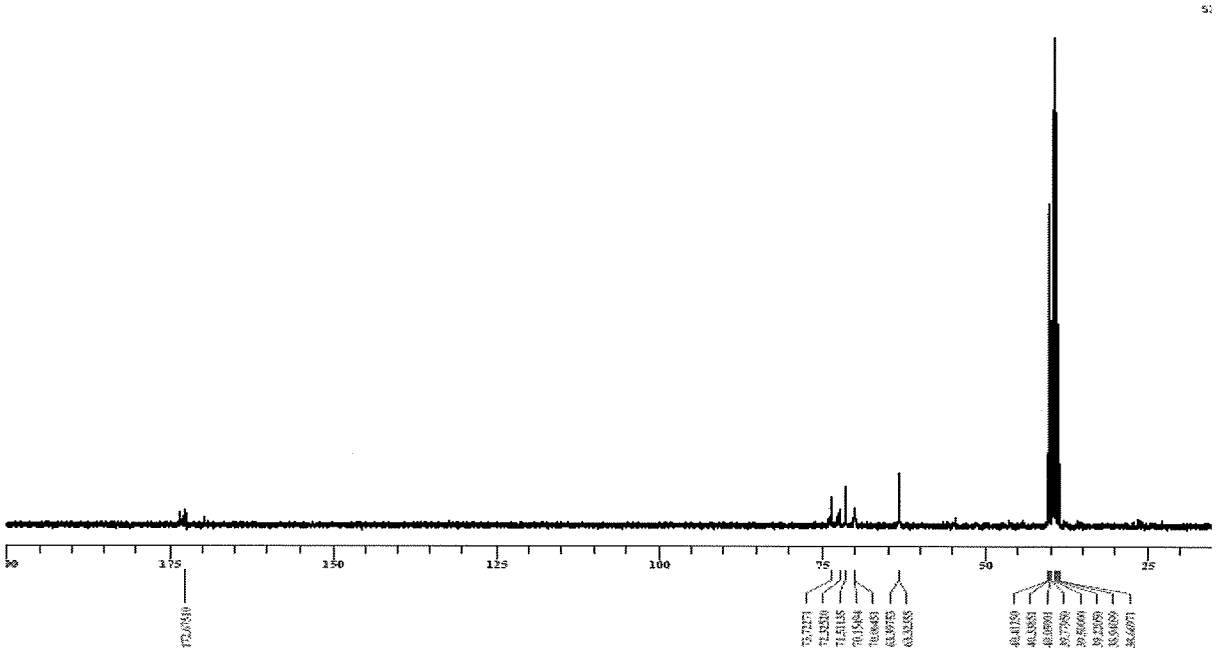


Fig. 2-9-16 ^{13}C-NMR of Ligand compound 8