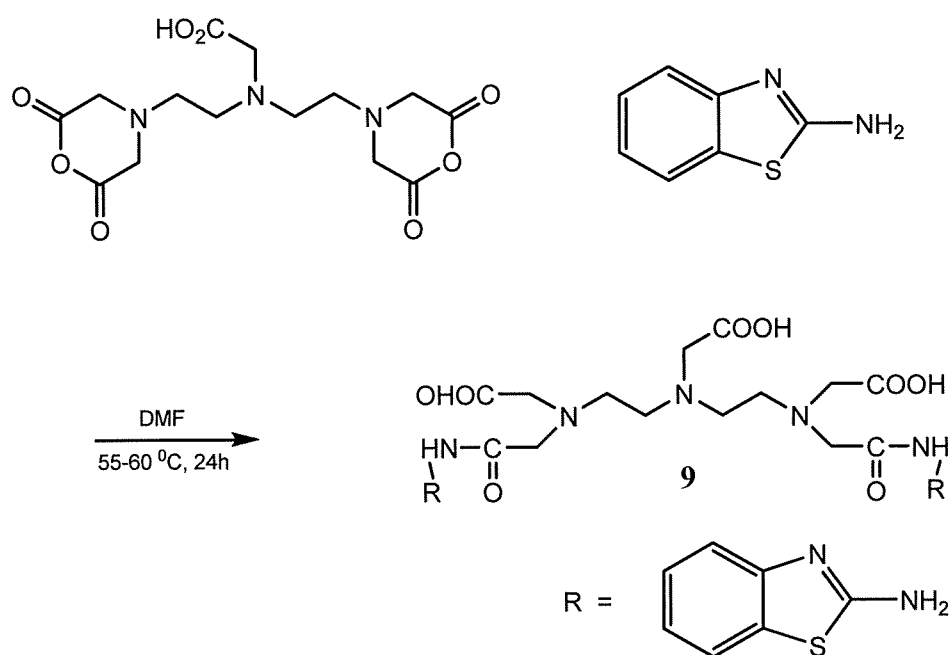


Synthesis of ligand

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^oC. After completion of the reaction, the solution was purified by recrystallisation 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

¹H-NMR and ¹³C-NMR spectral analysis of the above compound **9** was shown in Fig. 2-9-17 and 2-9-18. ¹H-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. ¹³C-NMR showed distinctive singlets at 171.31 indicates the presence of amide C=O group, the other aliphatic carbons observed in the expected region.

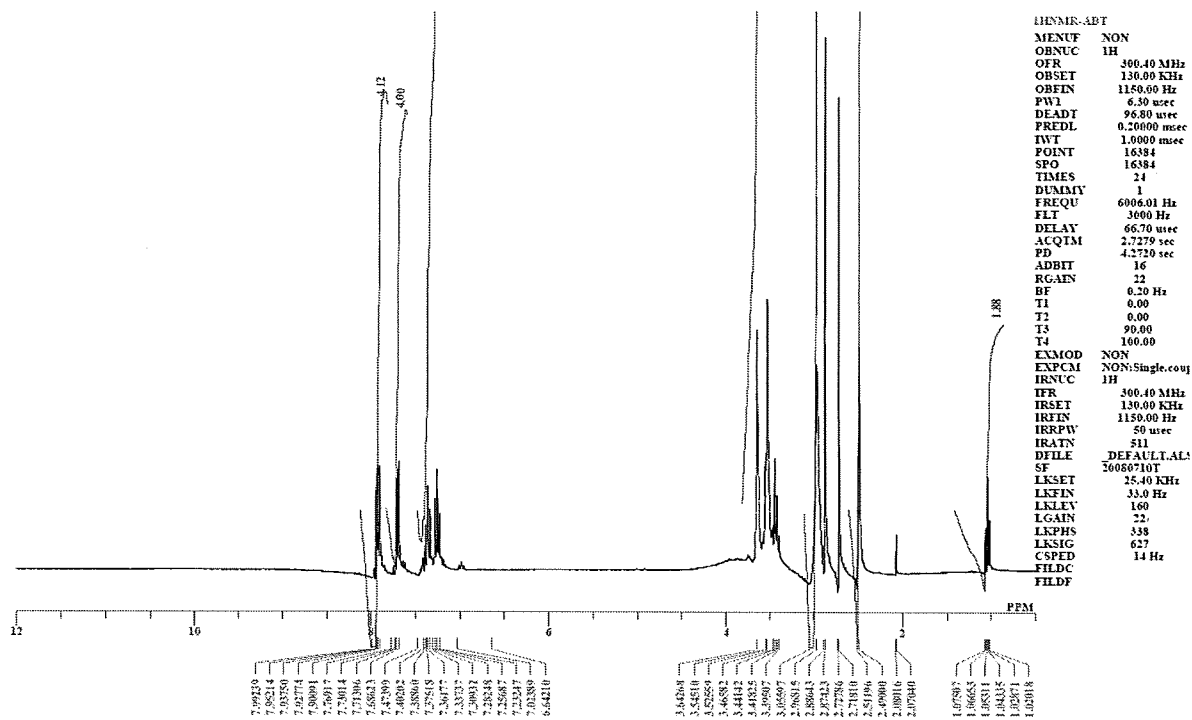


Fig. 2-9-17 ¹H-NMR spectrum of Ligand compound 9

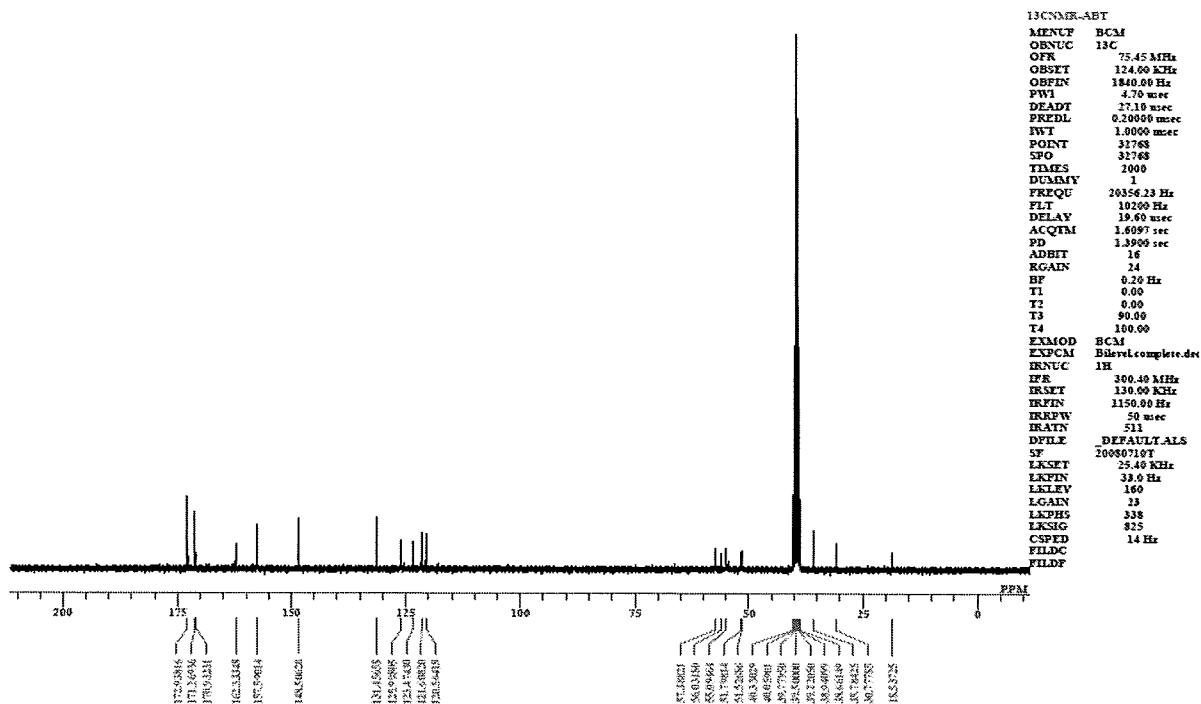
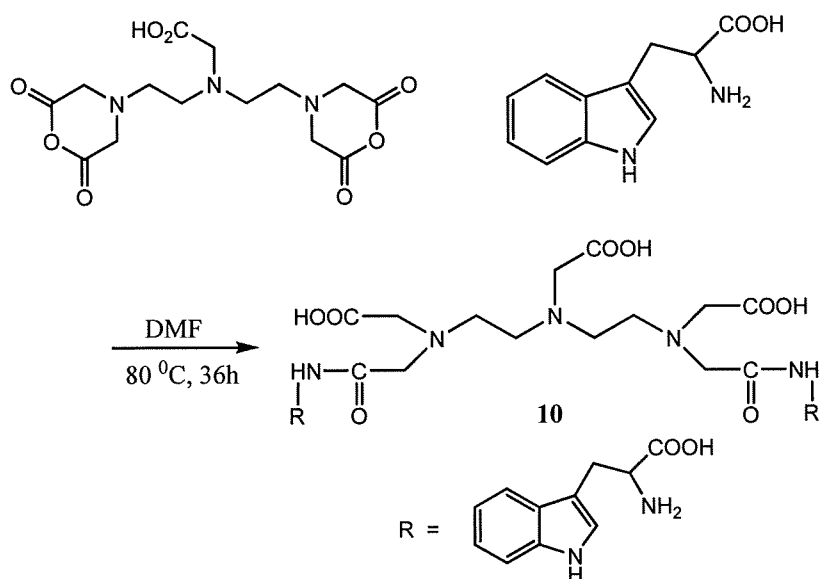


Fig. 2-9-18 ¹³C-NMR of Ligand compound 9

Synthesis of ligand

The synthesis of dendritic ligand employed a convergent method to couple core and diamine. To the solution of L-tryptophan (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°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 below.



Scheme 2-9-15 Synthesis of Ligand compound 10

¹H-NMR, ¹³C-NMR and mass spectral analysis of the above compound **10** were shown in Fig. 2-9-19, 2-9-20 and 2-9-21. ¹H-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. ¹³C-NMR showed distinctive singlets 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).

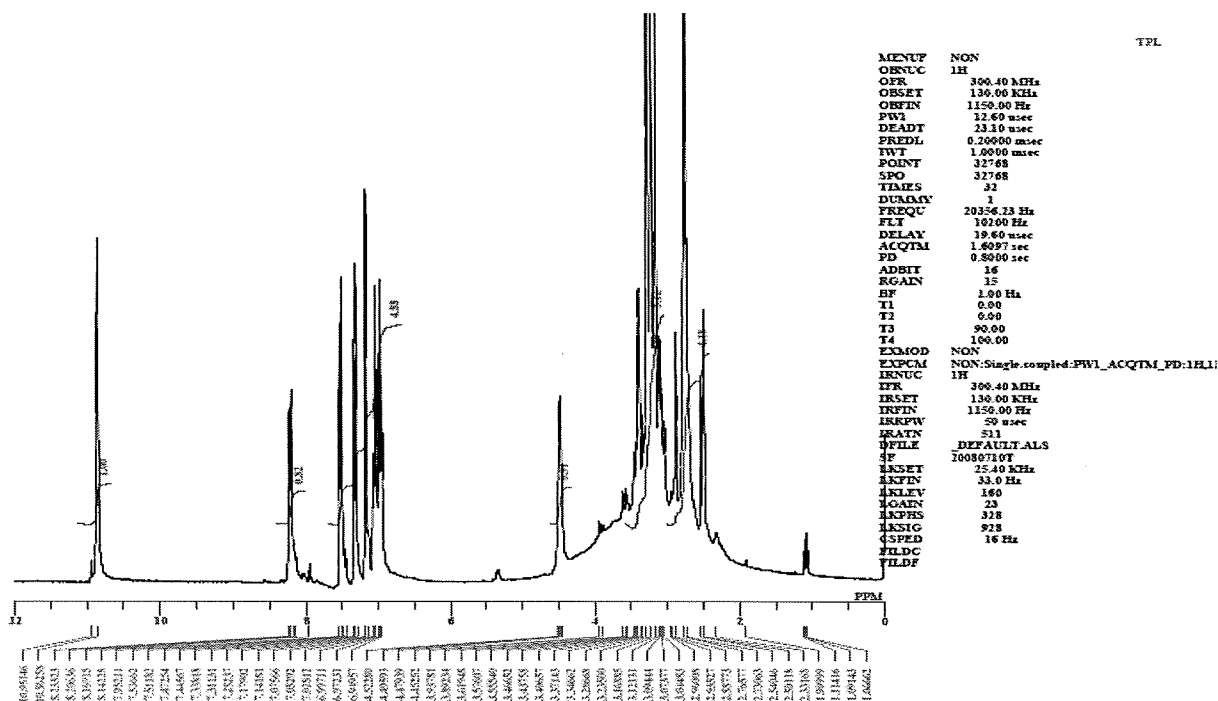


Fig. 2-9-19 ^1H -NMR spectrum of Ligand compound 10

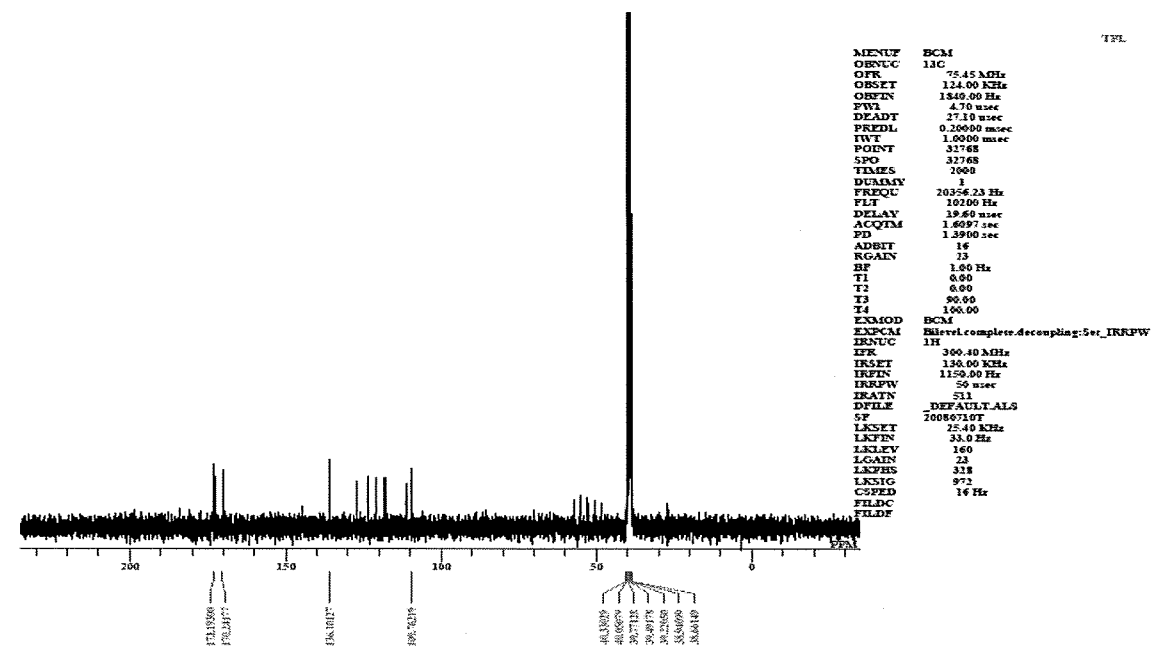
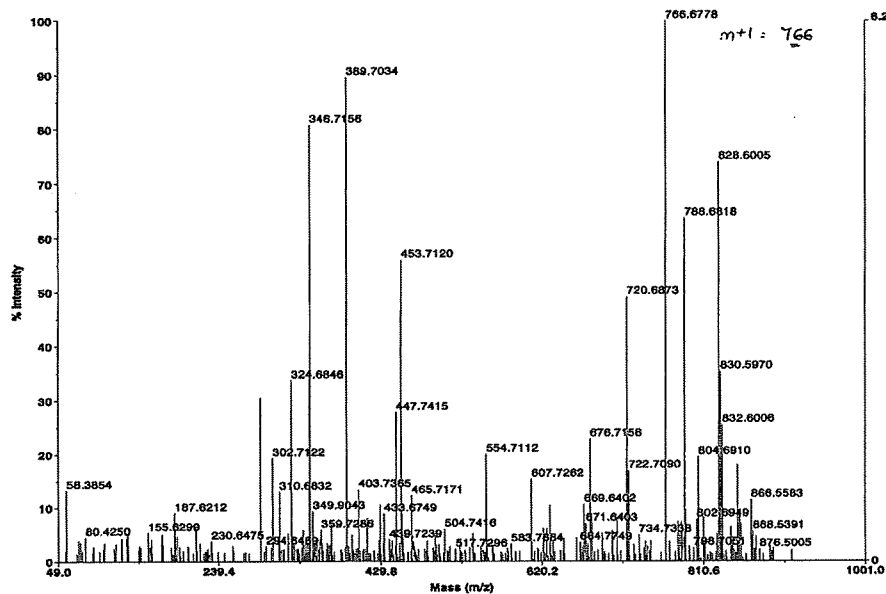


Fig. 2-9-20 ^{13}C -NMR of Ligand compound 10

Applied Biosystems Voyager System 6384

Voyager Spec #1=>BC=>NF0.7=>DI[BP = 766.7, 61527]



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 Accelerating voltage: 20000 V
 Grid voltage: 75%
 Mirror voltage ratio: 1.12
 Guide wire 0: 0.002%
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 Number of laser shots: 50/spectrum
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 Laser Rep Rate: 3.0 Hz
 Calibration type: Default
 Calibration matrix: a-Cyano-4-hydroxycinnamic aci
 Low mass gate: Off
 Timed ion selector: Off
 Digitizer start time: 7.206
 Bin size: 0.5 nsec
 Number of data points: 49251
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 Vertical offset: 0.65%
 Input bandwidth 0: 500 MHz

Sample well: 24
 Plate ID: PLATE1
 Serial number: 6384
 Instrument name: Voyager-DE PRO
 Plate type filename: C:\VOYAGER\100 well plate.ph
 Lab name: PE Biosystems
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 Absolute y-position: 38485
 Relative x-position: -136.539
 Relative y-position: -682.502
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 TC2 pressure: 0.005041
 TIS gate width: 7
 TIS right length: 688

solvent: water

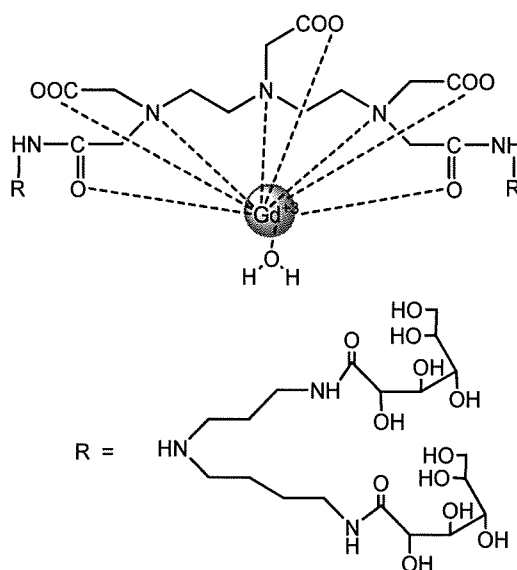
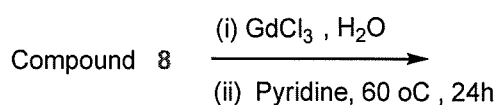
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Printed: 17:53, November 02, 2001

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

Synthesis of Gd complex

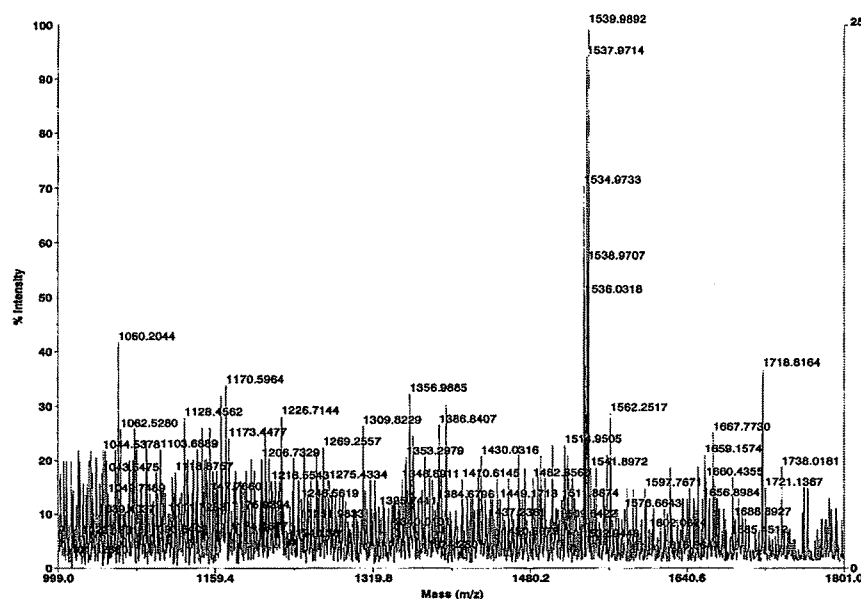
To a solution of ligand in water was added triethylamine and pyridine and the mixture was stirred thoroughly. To this $GdCl_3 \cdot 6H_2O$ was added slowly and the reaction was kept at $60^\circ C$ and stirred for 24 h. After completion of the reaction water was removed under vacuum and the crude product was dissolved in water and the excess of Gd was removed by using Chelex resin 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

Applied Biosystems Voyager System 6384

Voyager Spec #1=>BC=>NF0.7[BP = 1540.0, 258]



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Extraction mode:	Delayed
Polarity:	Positive
Acquisition control:	Manual
Accelerating voltage:	20000 V
Grid voltage:	75%
Mirror voltage ratio:	1.12
Guide wire Ø:	0.002%
Extraction delay time:	125 nsec
Acquisition mass range:	1000 -- 1800 Da
Number of laser shots:	50/spectrum
Laser intensity:	2089
Laser Flap Rate:	3.0 Hz
Calibration type:	Default
Calibration matrix:	α-Cyano-4-hydroxycinnamic acid
Low mass gate:	Off
Timed ion selector:	Off
Digitizer start time:	31.8305
Bin size:	0.5 nsec
Number of data points:	21674
Vertical scale Ø:	500 mV
Vertical offset:	0.65%
Input bandwidth Ø:	500 MHz
Sample well:	26
Plate ID:	PLATE1
Serial number:	6384
Instrument name:	Voyager-DE PRO
Plate type filename:	C:\VOYAGER\100 well plate.pt
Lab name:	PE Biosystems
Absolute x-position:	27920.8
Absolute y-position:	36675.2
Relative x-position:	933.254
Relative y-position:	-472.297
Shots in spectrum:	50
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TCC pressure:	0.006169
TIS gate width:	7
TIS flight length:	888

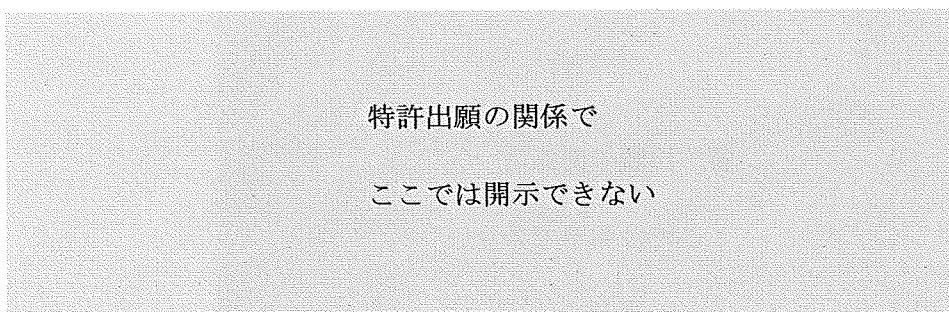
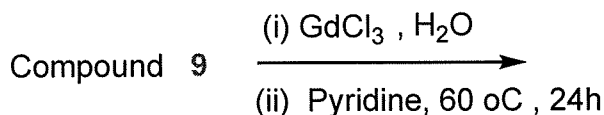
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Printed: 15:57, September 01, 2009

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

Synthesis of Gd complex

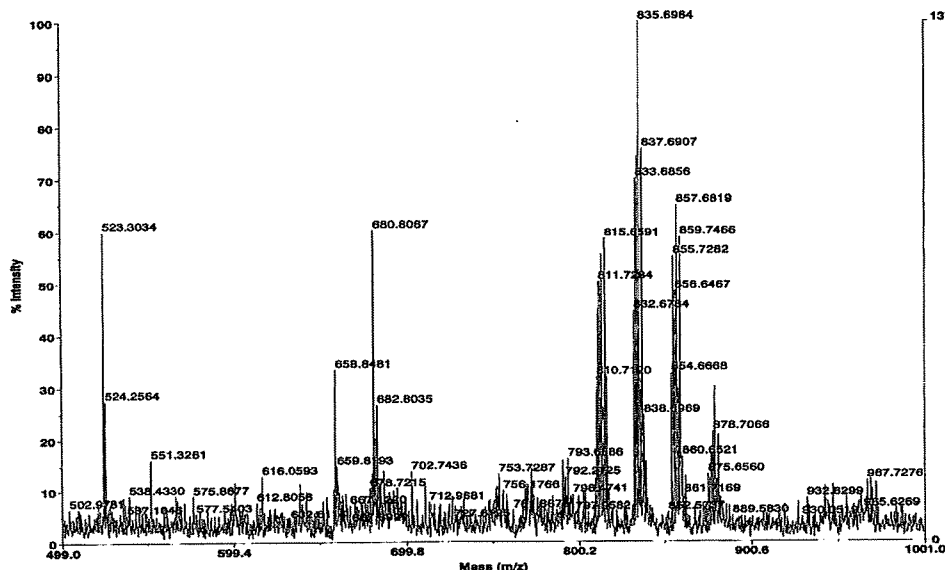
To a solution of ligand in water was added triethylamine and pyridine and the mixture was stirred thoroughly. To this $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$ was added slowly and the reaction was kept at 60°C and stirred for 24 h. After completion of the reaction water was removed under vacuum and the crude product was dissolved in water and the excess of Gd was removed by using Chelex resin 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).



Scheme 2-9-17 Synthesis of Complex

Applied Biosystems Voyager System 6384

Voyager Spec #1=>BC=>NF0.7[BP = 835.7, 1371]



Mode of operation:	Reflector
Extraction mode:	Delayed
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Acquisition control:	Manual
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Grid voltage:	75%
Mirror voltage ratio:	1.12
Guide wire 0:	0.002%
Extraction delay time:	125 nsec
Acquisition mass range:	500 - 1000 Da
Number of laser shots:	50/spectrum
Laser intensity:	1843
Laser Rep Rate:	3.0 Hz
Calibration type:	Default
Calibration matrix:	a-Cyano-4-hydroxycinnamic acid
Low mass gate:	Off
Timed ion selector:	Off
Digitizer start time:	22.423
Bin size:	0.5 nsec
Number of data points:	16465
Vertical scale 0:	500 mV
Vertical offset:	0.65%
Input bandwidth 0:	500 MHz
Sample well:	44
Plate ID:	PLATE1
Serial number:	6384
Instrument name:	Voyager-DE PRO
Plate type filename:	C:\VOYAGER\100 well plate.plt
Lab name:	PE Biosystems
Absolute x-position:	16346.8
Absolute y-position:	26826.7
Relative x-position:	-480.555
Relative y-position:	-360.847
Shot in spectrum:	50
Source pressure:	2.213e-007
Mirror pressure:	9.213e-008
TC2 pressure:	0.004007
TIS gate width:	7
TIS flight length:	888

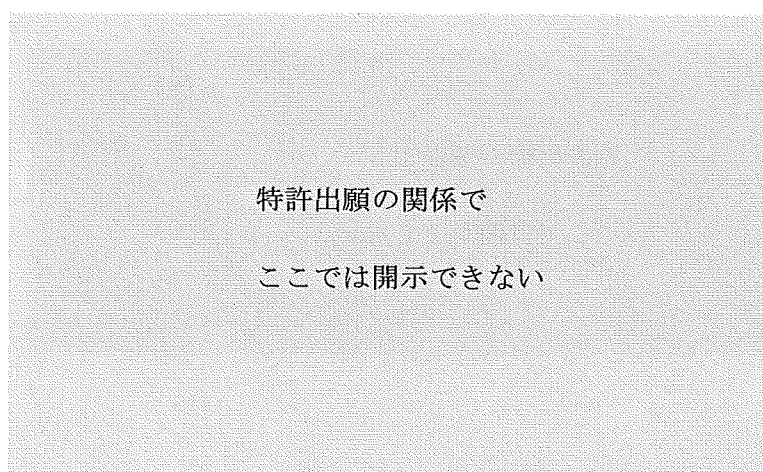
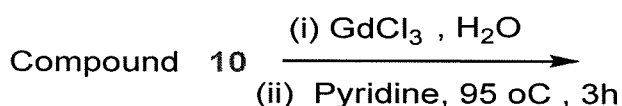
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Printed: 18:13, June 25, 2009

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 $95^\circ C$ 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 resin 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

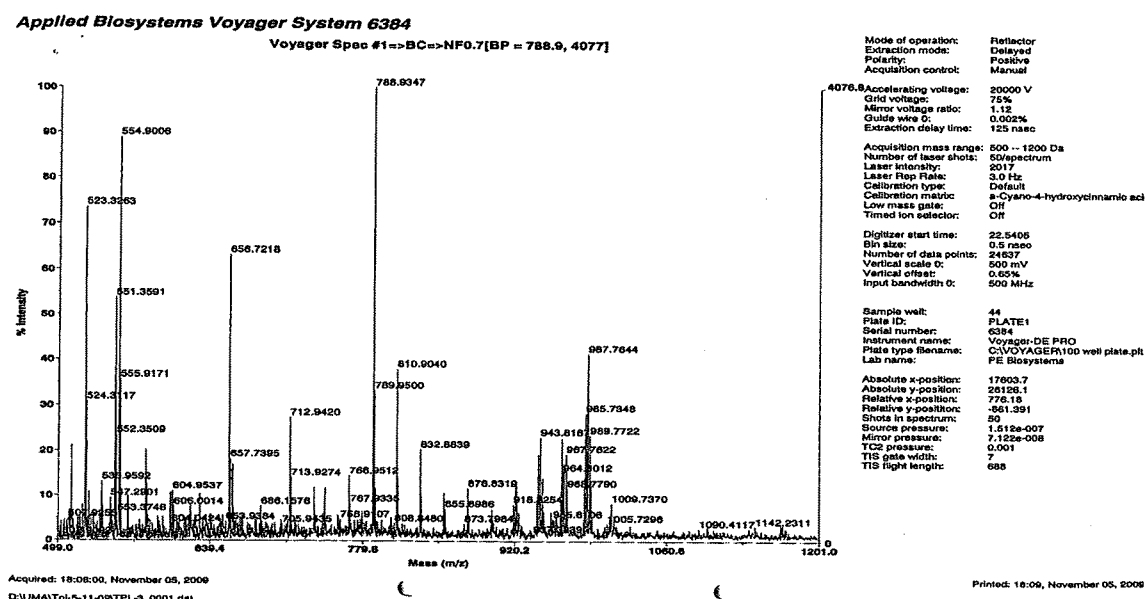


Fig. 2-9-24 Mass spectrum of Complex shown in Scheme 2-9-18

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°C. After the completion of the reaction, the precipitate was washed 3 times by acetic anhydride (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=COH), 1816 (O=COC=O), 1761 (O=COH)

¹H-NMR

δ (ppm) = 3.71 (s, -CO-CH₂-CO-X 4)
=3.30 (s, -CH₂COOH)
=2.72 (t, ²J_{CH} = 6.58 Hz, COCH₂NCH₂CH₂N X 2)
=2.59 (t t, ²J_{CH} = 12.0 Hz, COCH₂NCH₂CH₂N X2)

¹³C-NMR

δ (ppm)= 171.9 (O=COH)
=165.7 (O=COC=O)
=54.5 (NCH₂COOH)
=52.1 (CH₂O=COC=O)
=50.7, 51.7 (NCH₂CH₂ 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°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°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 (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-tryptophan (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°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 (1.5 g, 1.96 mmol), yield, 80%.

2-9-3 References and notes

1. R.B. Lauffer, *Chem. Rrv.* **1987**, 87, 901.
2. M.F. Tweedle, *In Lanthanide Probes in Life, Chemical, and Earth Sciences*; J.C.G. Bunzli, G.R. Choppin, Eds.; Elsevier: Amsterdam, **1989**; Chapter 5.
3. P. Caravan, J. J. McMurry, R. B. Lauffer, *Chem. Rrv.* **1999**, 99, 2293.
4. G. W. Kabalka, M. A. Davis, T. H. Moss, E. Buonocore, K. Hubner, E. Holmberg, K. Maruyama, L. Haung, *Magn, Reson, Med*, **1991**, 19, 406.
5. P.A. Rinck, *Magnetic Resonance in Medicine*; Black well Scientific Publications: Oxford, UK, **1993**.
6. A. E. Merbach, E. Toth, *The Chemistry of Contrast Agents in Medicinal Magnetic Resonance Imaging*; John Willy: Chichester, UK, 2001.
7. D. E. Reichert, J. S. Lewis, C. J. Anderson, *Coord, Chem, Rev*, **1999**, 184, 3.
8. L. Thunus, R. Lejeune, *Angew. Coord, Chem, Rev*, **1999**, 184, 125.
9. H-J. Weinmann, W. Ebert, B. Misselwitz, H. Schmitt-Willich, *Eur. J. Radiol* **2003**, 46, 33.
10. H.C. Schwickert, T.P.L. Roberts, A. Mühler, M. Stiskal, F. Demsar and R.C. Brasch. *Eur. J. Radiol.* **1995**, 20, 144.
11. S. Aime, M. Botta, L. Frullano, S.G. Crich, G.B. Giovenzana, R. Pagliarin, G. Palmisano and M. Sisti. *Chem. Eur. J.* **1999**, 5, 1253.
12. W.-H. Li, S.E. Fraser and T.J. Meade. *J. Am. Chem. Soc.* **1999**, 121, 1413.
13. G.A. Lemieux, K.J. Yarema, C.L. Jacobs and C.R. Bertozzi. *J. Am. Chem. Soc.* **1999**, 121, 4278.
14. Y.H. Jang, M. Blanco, S. Dasgupta, D.A. Keire, J.E. Shively and W.A. Goddard, III. *J. Am. Chem. Soc.* **1999**, 121, 6142.
15. M.S. Konings, W.C. Dow, D.B. Love, K.N. Raymond, S.C. Quay and S.M. Rocklage. *Inorg. Chem.* **1990**, 29, 1488.

16. C. Sun, P. Wirsching and K. D. Janda, *Bioorg. Med. Chem.* **2003**, 11, 1761.
17. S. W. A. Blish, A. H. M. S. Chowdhury, M. Mcpartlin, T. J. Scowen, R.A. Bulman, *Polyhedron*, **1995**, 14, 567.

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 *in vivo* 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 pentaacetic acid ([DTPA-Gd(H₂O)]²⁻) or 4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid ([DOTA-Gd(H₂O)]⁻), 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.

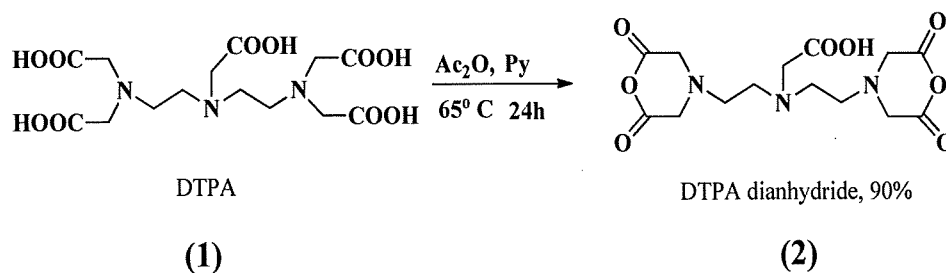
2-10-1 Synthesis of Gd-DTPA-Spermidine

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.



Scheme 2-10-1 Synthesis of DTPA dianhydride

$^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ 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} ($\text{C}=\text{O}$ stretch symmetric), 1774 cm^{-1} ($\text{C}=\text{O}$ asymmetric stretching) which are characteristics of cyclo-dianhydride. $^1\text{H-NMR}$ spectrum of DTPA dianhydride $\text{COCH}_2\text{NCH}_2\text{CH}_2\text{N} \times 2$ chemical shifts showed at 2.73 (t, $^2J_{\text{CH}} 12.1 = \text{Hz}$), and $\text{COCH}_2\text{NCH}_2\text{CH}_2\text{N} \times 2$ at 2.58 (t, $^2J_{\text{CH}} 12 = \text{Hz}$) respectively. $^{13}\text{C-NMR}$ 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.

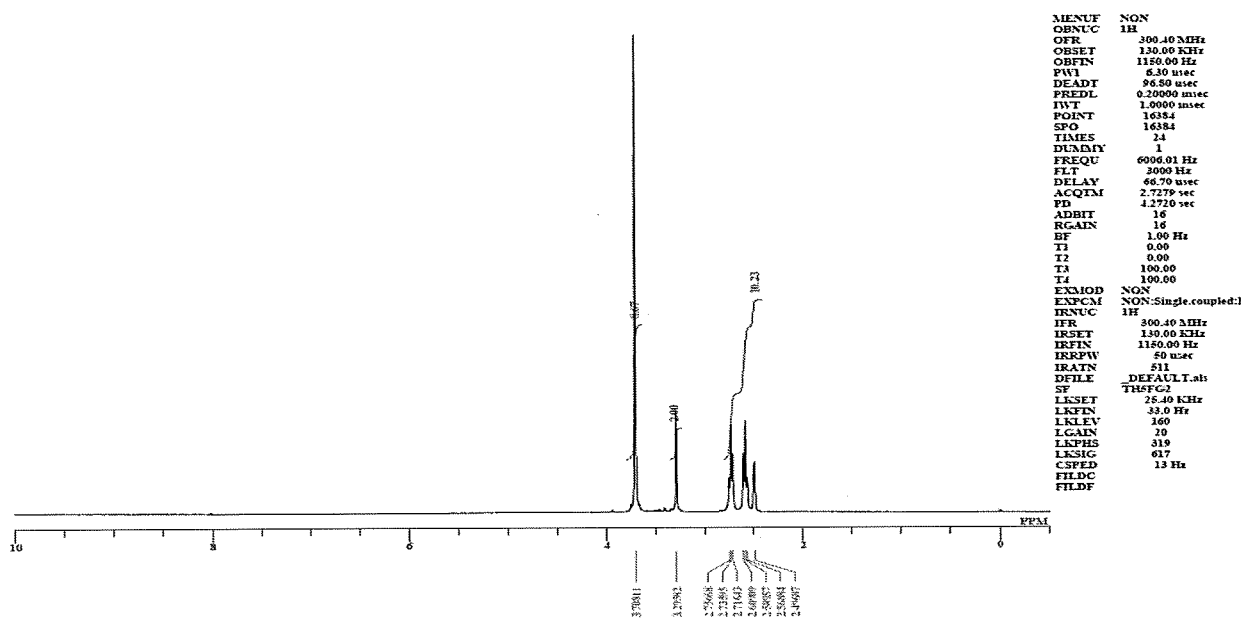


Fig. 2-10-1 $^1\text{H-NMR}$ of DTPA dianhydride

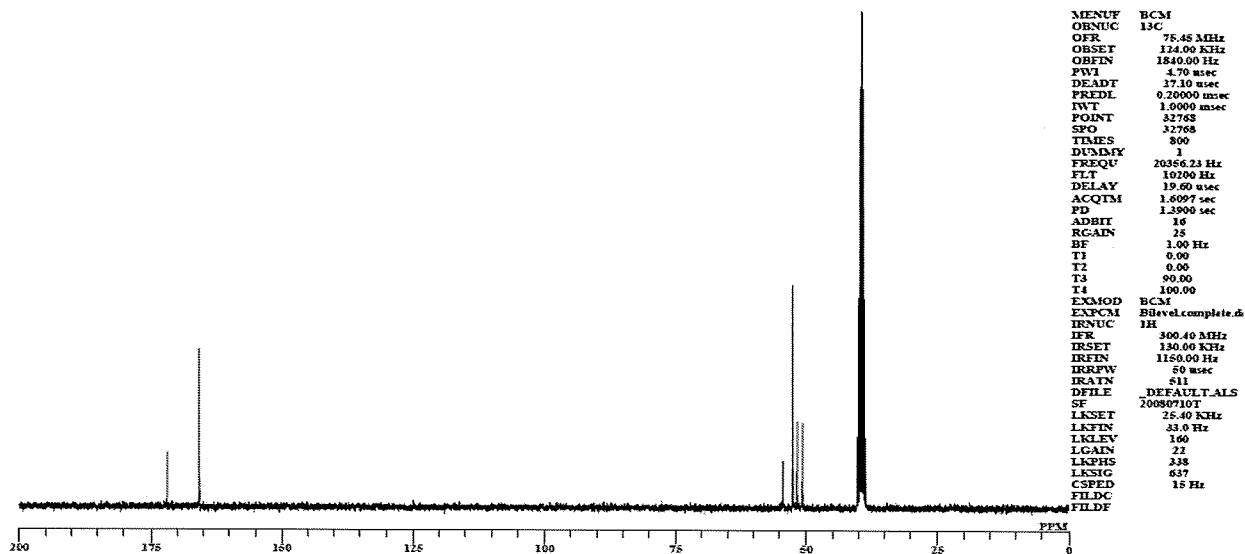
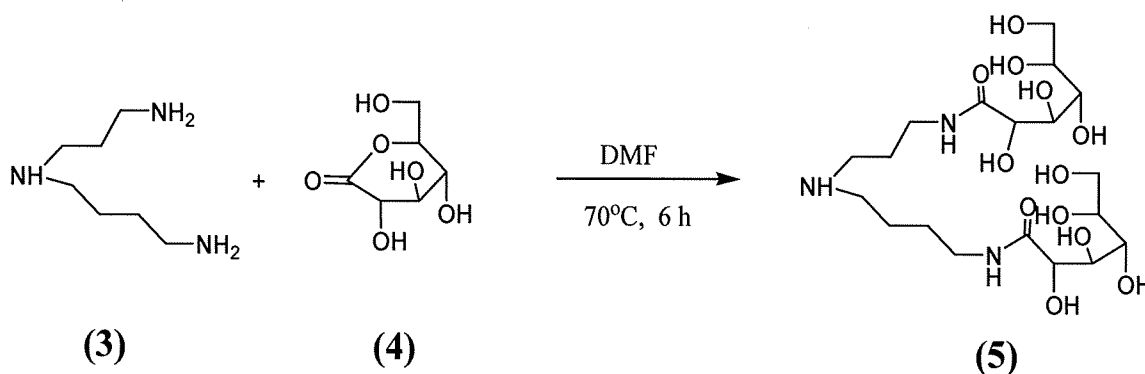


Fig. 2-10-2 ^{13}C -NMR of DTPA dianhydride

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

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

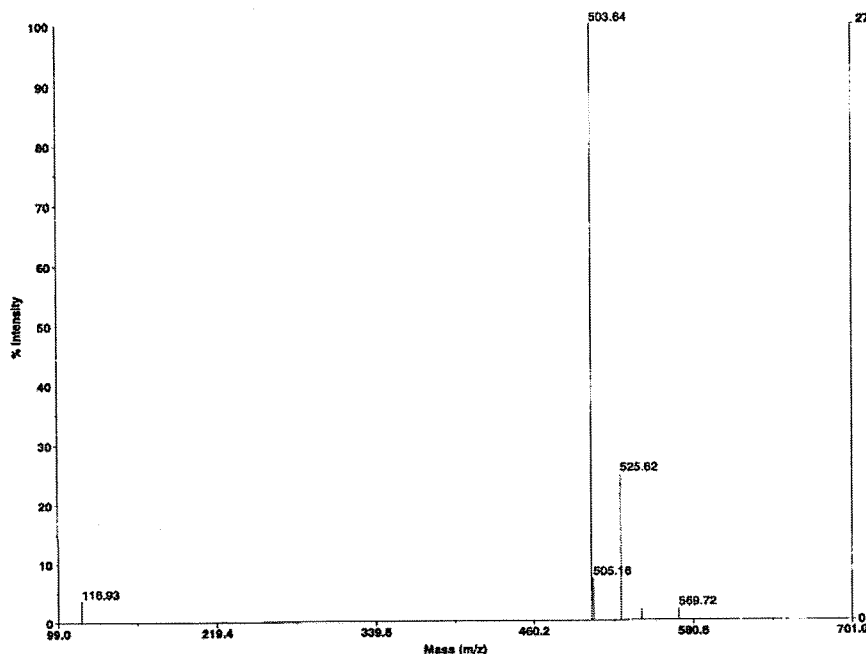
To a solution of D-glucono-1,5-lactone in adequate amount of DMF was added spermidine (N-(3-aminopropyl)butane-1,4-diamine) under inert conditions and the temperature of the reaction mixture was maintained at 70°C over a period of six hours. After completion of the reaction, products were left in refrigerator 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)

Applied Biosystems Voyager System 6384

Voyager Spec #1=>BC=>NF0.7=>D[BP = 503.6, 2724]



Mode of operation: Linear
 Extraction mode: Delayed
 Polarity: Positive
 Acquisition control: Manual
 Accelerating voltage: 20000 V
 Grid voltage: 94%
 Guide wire G: 0.03%
 Extraction delay time: 100 nsec
 Acquisition mass range: 100 - 700 Da
 Number of laser shots: 50/spectrum
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 Laser Rep Rate: 20.0 Hz
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 Calibration matrix: a-Cyano-4-hydroxycinnamic acid
 Low mass gate: Off
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 Vertical offset: 0%
 Input bandwidth: 600 MHz
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 Serial number: 6384
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 Absolute y-position: 31662.3
 Relative x-position: 735.229
 Relative y-position: -205.151
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 Mirror pressure: 8.776e-008
 TCO pressure: 0.003615
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 TIS flight length: 688

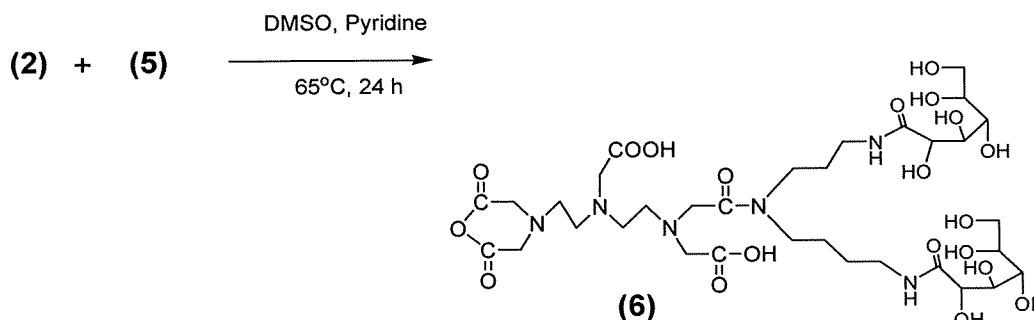
Acquired: 17:58:00, November 24, 2009
 D:\Tsuzyukh\2009.11.24\GC4-3_0002.d

Printed: 17:56, November 24, 2009

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

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

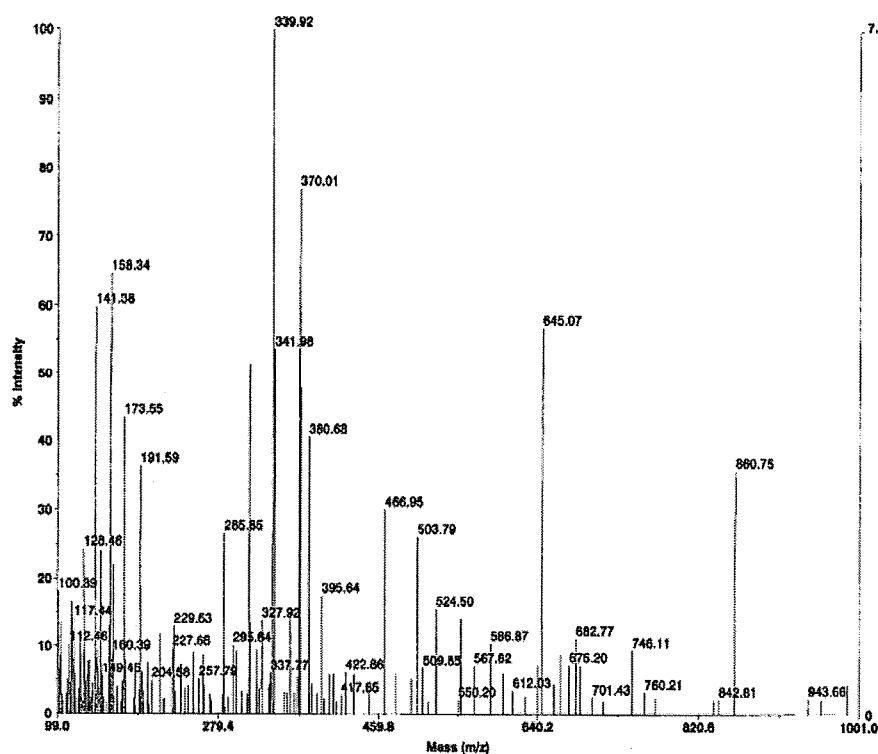
To a solution of DTPA dianhydride in DMSO and was added terminal, pyridine in quantitative ratio and the mixture was stirred for 24 h at 65°C and after the completion of the reaction, reaction mixture was poured in to ethyl acetate under stirring and continued stirring for 10 min separated the solid 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)

Applied Biosystems Voyager System 6384

Voyager Spec #1=>BC=>NF0.7=>DI[BP = 339.9, 697534]



Mode of operation: Linear
 Extraction mode: Delayed
 Polarity: Positive
 Acquisition control: Manual

Accelerating voltage: 20000 V
 Grid voltage: 94%
 Guide wire O: 0.05%
 Extraction delay time: 100 nsec

Acquisition mass range: 100 - 1000 Da
 Number of laser shots: 50/spectrum
 Laser intensity: 2414
 Laser Rep Rate: 20.0 Hz
 Calibration type: Default
 Calibration matrix: a-Cyano-4-hydroxycinnamic acid
 Low mass gate: Off

Digitizer start time: 6.936
 Bin size: 2 nsec
 Number of data points: 7394
 Vertical scale: 500 mV
 Vertical offset: 0%
 Input bandwidth: 500 MHz

Sample well: 23
 Plate ID: PLATE1
 Serial number: 6384
 Instrument name: Voyager-DE PRO
 Plate type filename: C:\VOYAGER\100 well plate.pht
 Lab name: PE Biosystems

Absolute x-position: 13900.1
 Absolute y-position: 36826.5
 Relative x-position: 2152.6
 Relative y-position: -321.015
 Shots in spectrum: 50
 Source pressure: 5.501e-007
 Mirror pressure: 1.03e-007
 TCD pressure: 0.001244
 TIS gate width: 7
 TIS flight length: 688

Acquired: 10:27:00, February 24, 2010

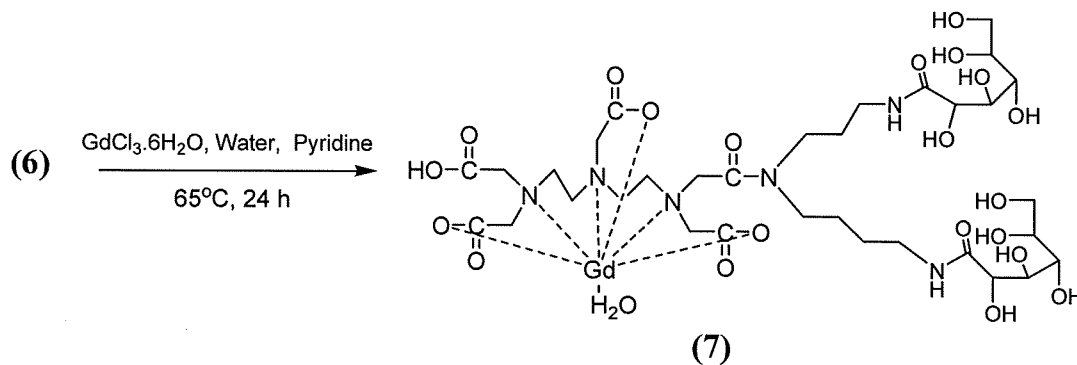
Printed: 10:27, February 24, 2010

D:\Srnul\C3C4one Ligand1_0001.dat

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

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

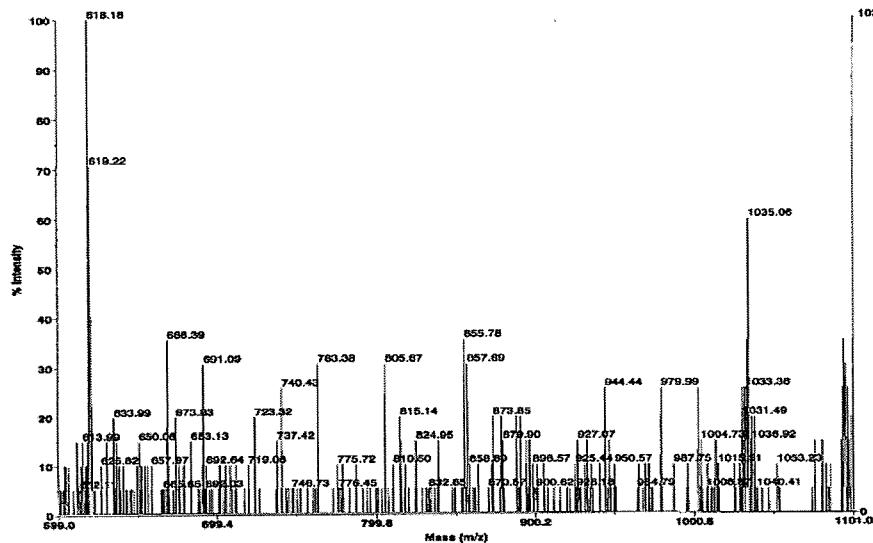
To a solution of above prepared ligand (6) in water pyridine was added and the mixture was stirred. To this $GdCl_3 \cdot 6H_2O$ was added and the reaction was kept at $65^\circ C$ and stirred for 24 h. After completion of the reaction water was removed under vacuum and the crude product was dissolved in water and the excess of Gd was removed by using Chelex resin 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)

Applied Biosystems Voyager System 6384

Voyager Spec #1->BC=>NF0.7[BP = 618.1, 102]



Mode of operation: Linear
 Extraction mode: Delayed
 Polarity: Positive
 Acquisition control: Manual
 Accelerating voltage: 20000 V
 Grid voltage: 94%
 Guide wire 0: 0.05%
 Extraction delay time: 100 nsec
 Acquisition mass range: 600 - 1100 Da
 Number of laser shots: 50/spectrum
 Laser intensity: 2058
 Laser Rep Rate: 20.0 Hz
 Calibration type: Default
 Calibration matrix: α-Dyano-4-hydroxycinnamic acid
 Low mass gate: 500 Da
 Digitizer start time: 16.848
 Rin extra: 2 nsec
 Number of data points: 2585
 Vertical scale: 500 mV
 Vertical offset: 0%
 Input bandwidth: 500 MHz
 Sample well: 24
 Plate ID: PLATE1
 Serial number: 6384
 Instrument name: Voyager-DE PRO
 Plate type filename: C:\VOYAGER\100 well plate.plt
 Lab name: PE Bioystems
 Absolute x-position: 16410.1
 Absolute y-position: 37498.8
 Relative x-position: -417.41
 Relative y-position: 347.310
 Shots in spectrum: 50
 Source pressure: 1.49e-007
 Mirror pressure: 7.371e-008
 TIC2 pressure: 0.001355
 TIS gate width: 7
 TIS flight length: 688

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Printed: 11:28, February 25, 2010

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

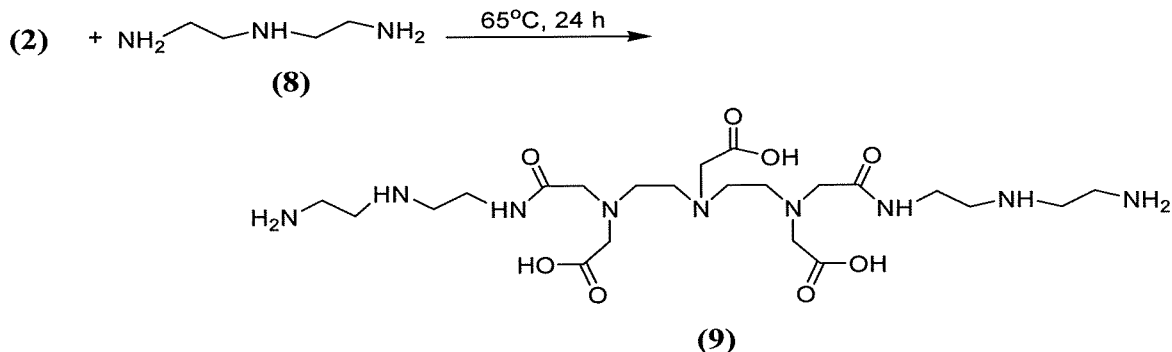
2-10-4 Synthesis of Gd-DTPA-C2-D2-Amine

2-10-4-1 Result and Discussion

In 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

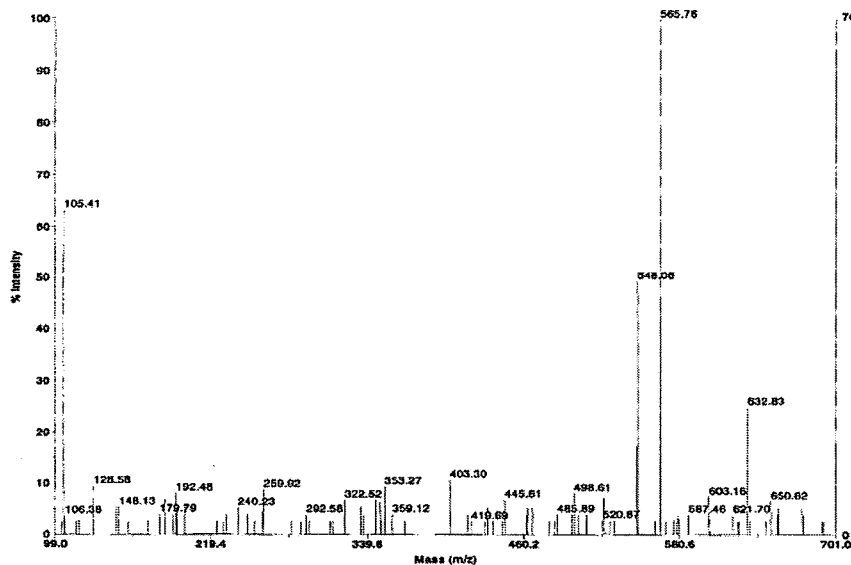
To a solution of DTPA dianhydride in excess of bis(2-aminoethyl)amine and the mixture was stirred for 24 h at 60°C [7]. After the completion of the reaction ethyl acetate was added and stirred for 10 min then filter the solid and dried the compound under vacuum obtain pure compound.



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

Applied Biosystems Voyager System 6384

Voyager Spec #1->BC->NF0.7->DI[BP = 565.8, 766]



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D:\Sku\DTPA-C2-Amine_0001.dat

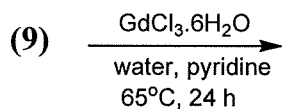
Mode of operation: Linear
Extraction mode: Delayed
Polarity: Positive
Acquisition control: Manual
Accelerating voltage: 20000 V
Grid voltage: 84%
Guide wire off
Extraction delay time: 100 nsec
Acquisition mass range: 100 - 700 Da
Number of laser shots: 50/spectrum
Laser intensity: 2307
Laser Rep Rate: 20.0 Hz
Calibration type: Default
Calibration matrix: a-Cyano-4-hydroxycinnamic acid
Low mass gate: Off
Digital start time: 6.896
On axis: 2 nsec
Number of data points: 6529
Vertical scale: 500 mV
Vertical offset: 0%
Input bandwidth: 500 MHz
Sample well: 22
Plate ID: PLATE1
Serial number: 6384
Instrument name: Voyager-DE PRO
Plate type filename: C:\VOYAGER\100 well plate.plt
Lab name: PE Biosystems
Absolute x-position: 6380.74
Absolute y-position: 07292
Relative x-position: -258.759
Relative y-position: 14.4491
Shots in spectrum: 50
Source pressure: 6.234e-007
Mirror pressure: 7.897e-006
TC2 pressure: 0.001
TIS gate width: 7
TIS right length: 669

Printed: 14:06, February 07, 2010

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 $65^\circ C$ 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 resin and after removal of excess Gd resin was filtered off and then the filtrate was concentrated under vacuum.



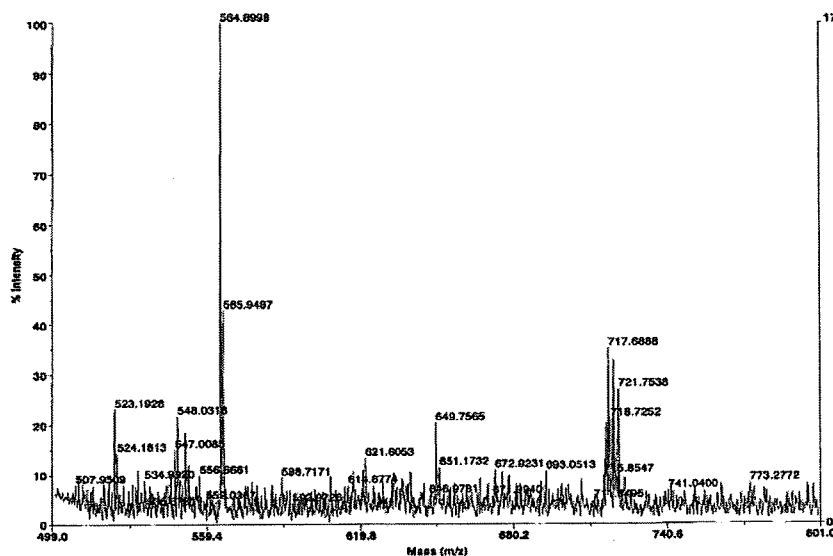
特許出願の関係で
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(10)

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

Applied Biosystems Voyager System 6384

Voyager Spec #1->BC->NF0.7[BP = 564.9, 1758]



Mode of operation: Reflector
 Extraction mode: Delayed
 Polarity: Positive
 Acquisition control: Menu
 Accelerating voltage: 20000 V
 Grid voltage: 75%
 Mirror voltage ratio: 1.12
 Guide wire 0: 0.002%
 Extraction delay time: 125 nsec
 Acquisition mass range: 500 - 800 Da
 Number of laser shots: 50/spectrum
 Laser intensity: 3232
 Laser Rep Rate: 3.0 Hz
 Calibration type: Default
 Calibration matrix: m-Cyano-4-hydroxycinnamic acid
 Low mass gate: 500 Da
 Timed ion selector: Off
 Digitizer start time: 22.5405
 Bin size: 0.5 msec
 Number of data points: 11895
 Vertical scale 0: 500 mV
 Vertical offset: 0.05%
 Input bandwidth 0: 500 MHz
 Sample well: 23
 Plate ID: PLATE1
 Serial number: 6384
 Instrument name: Voyager-DE PRO
 Plate type filename: C:\VOYAGER\100 well plate.pt
 Lab name: PE Biosystems
 Absolute x-position: 12539.7
 Absolute y-position: 38007.7
 Relative x-position: 752.259
 Relative y-position: 860.168
 Shots in spectrum: 50
 Source pressure: 1.49e-007
 Mirror pressure: 5.999e-008
 TCE pressure: 0.001
 TIS gate width: 7
 TIS light length: 688

Acquired: 16:21:00, February 08, 2010
 D:\SinauDTPA C2 Complex_0002.dat

Printed: 16:21, February 08, 2010

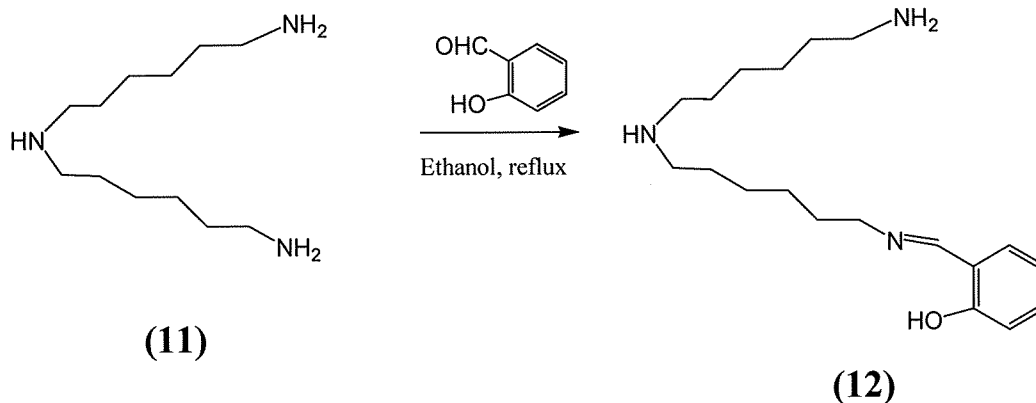
Fig. 2-10-7 Mass Spectra of DTPA-C2-D2-Amine complex

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

2-10-5-1 Synthesis of Terminal

Step 1

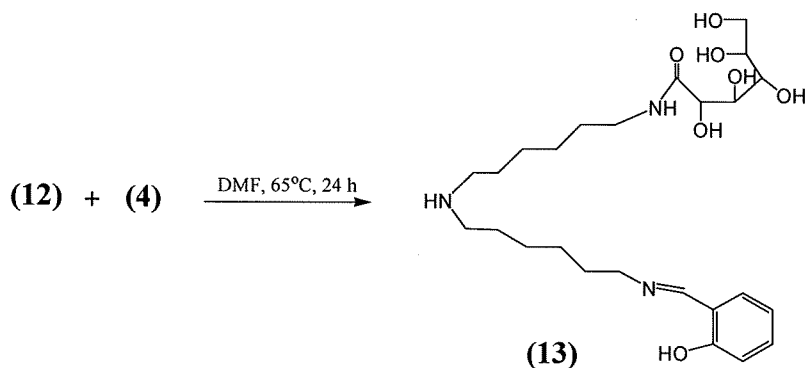
HMTA and 2-hydroxybenzaldehyde were dissolved in ethanol and the solution was refluxed for 3 h [8]. After completion of the reaction solvent was evaporated, the crude product was washed with hexane and then water dried under vacuum for 3 h to obtain gummy crude which was used for next step. Yield: 81%



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

Step 2

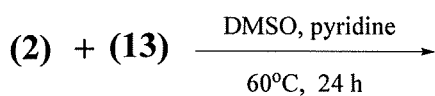
2-((E)-(6-(6-aminohexylamino)hexylimino)methyl)phenol were dissolved in 30 ml of DMF and D-glucono-1,5-lactone was added to the solution and then the mixture was stirred for 24 h. After completion of the reaction was poured in to ethyl acetate under stirring and continued for 10 min then filtered the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



特許出願の関係で

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(14)

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