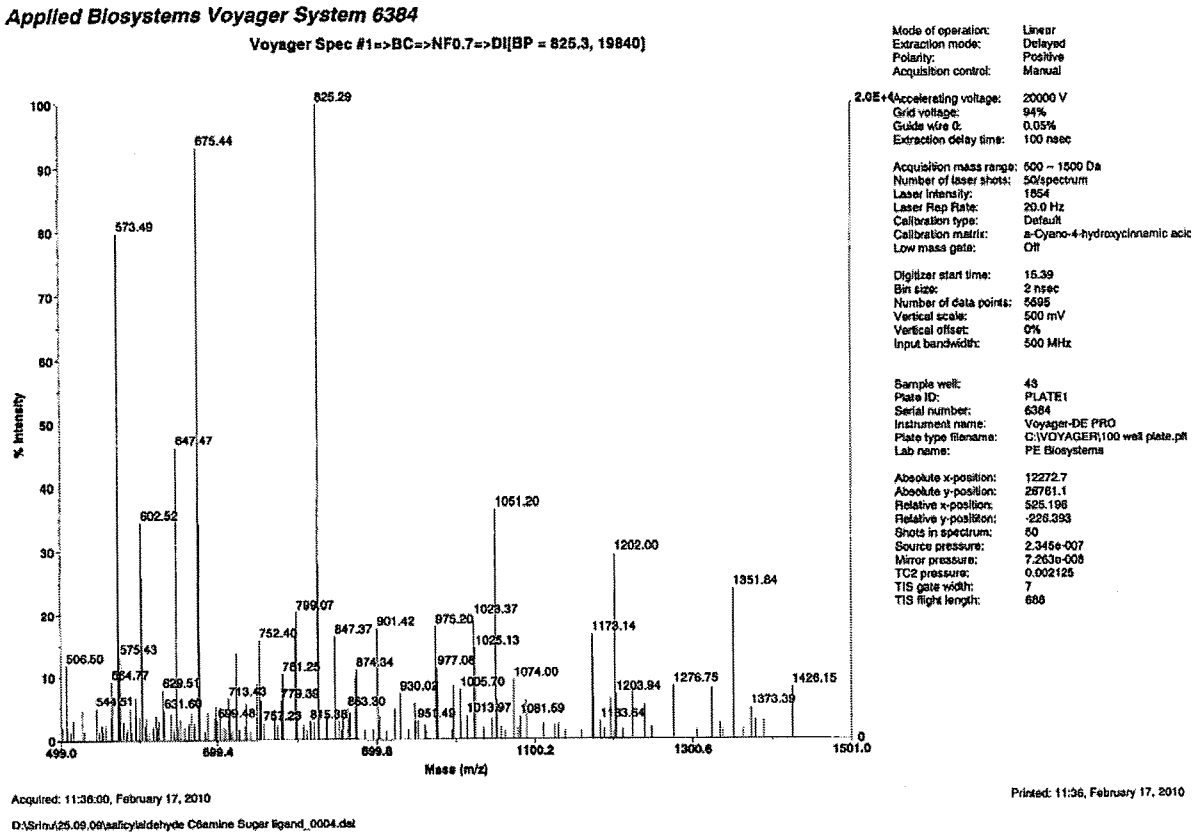


Fig. 2-10-8 Mass Spectra of DTPA-HMTA-C6-D2-imine-Glc(OH) Ligand

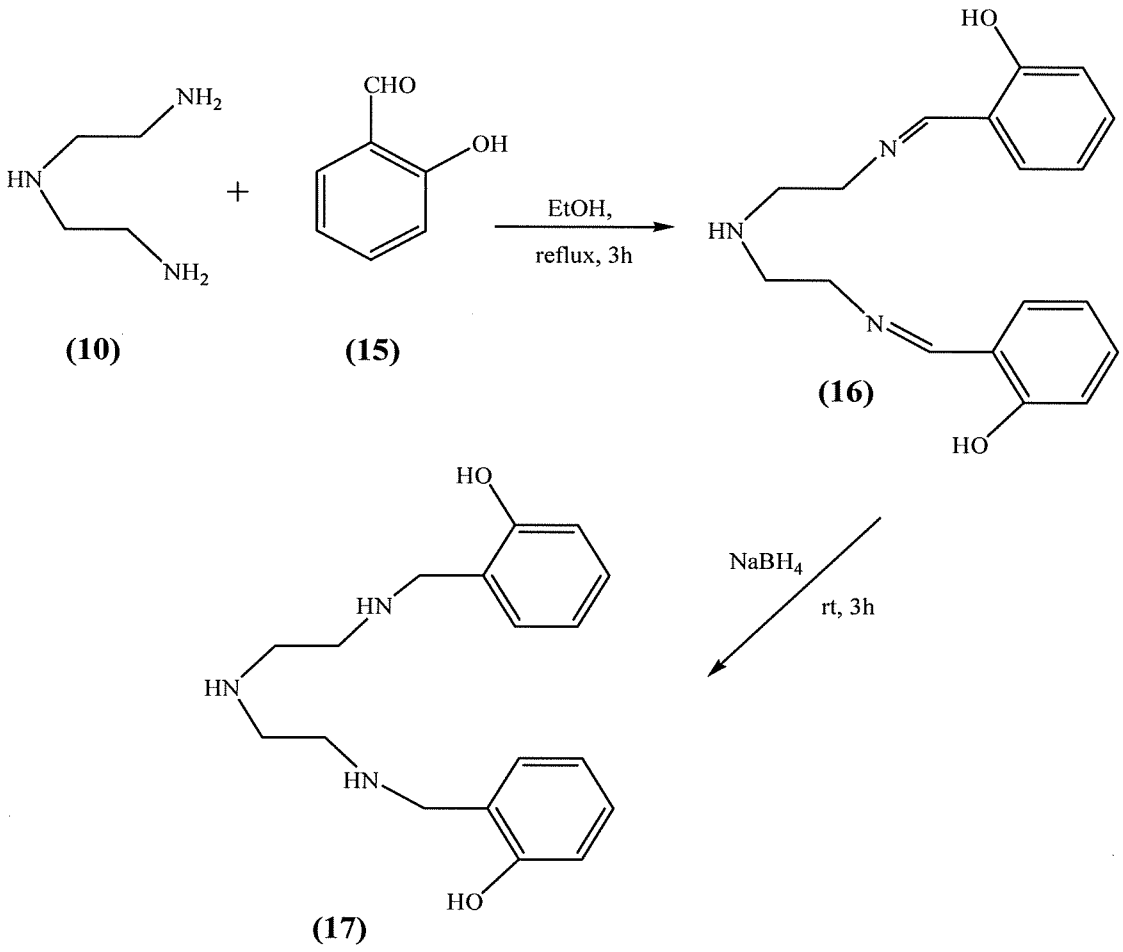
$$\textbf{(14)} \quad \xrightarrow[\text{water, pyridine}]{65^{\circ}C,\ 24\ h} \quad \textbf{Complex not formed}$$

Scheme 2-10-10 Synthesis of Gd-DTPA-HMTA-C6-D2-imino-Glc(OH) complex

2-10-6 Synthesis of Gd-DTPA-C2-D2-amine benzylphenol Complex

2-10-6-1 Synthesis of Gd-DTPA-C2-D2-amine benzylphenol Terminal

Bis-(2-aminoethyl)amine and 2-hydroxybenzaldehyde were dissolved in ethanol and the solution was refluxed for 3 h [8-10]. After completion of the reaction the reaction mixture was cooled to rt then sodium borohydride ($NaBH_4$) was added to the reaction mixture and stirred for 3h at rt. After completion of the reaction the reaction mixture was poured in to cold water and extracted with MDC, solvent was evaporated, a crude product was washed with hexane and then dried under vacuum for 3 h then the gummy crude was used for next step. Yield: 80%



Scheme 2-10-11 Synthesis of C2-D2-ethylamino methylphenol Terminal

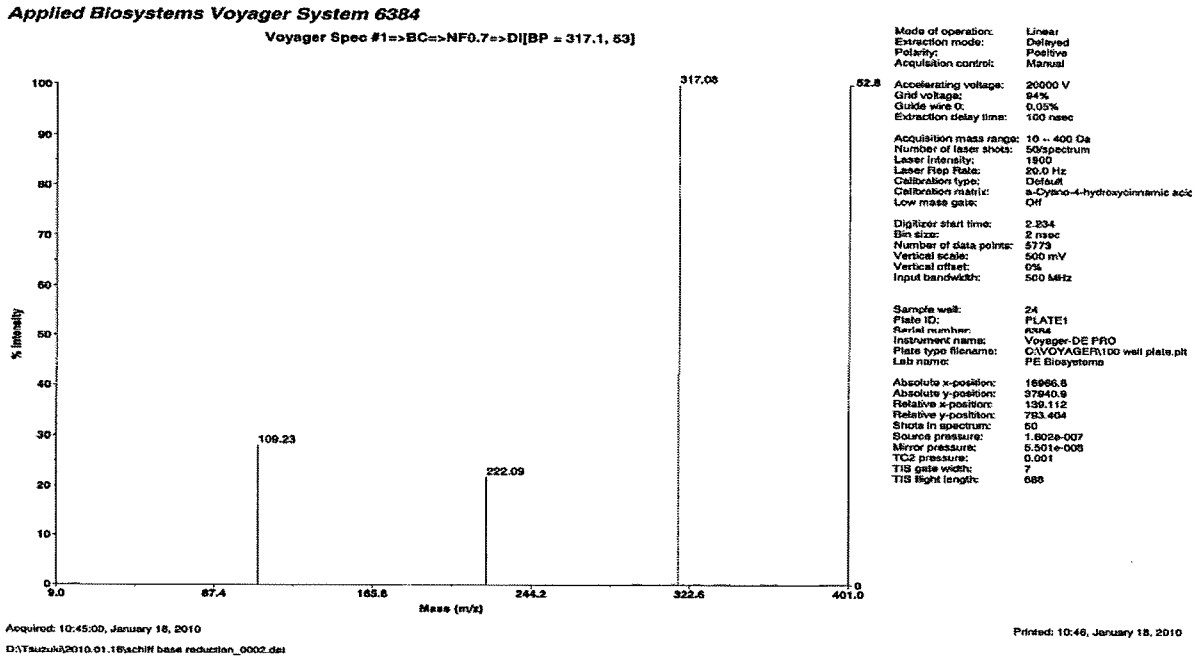
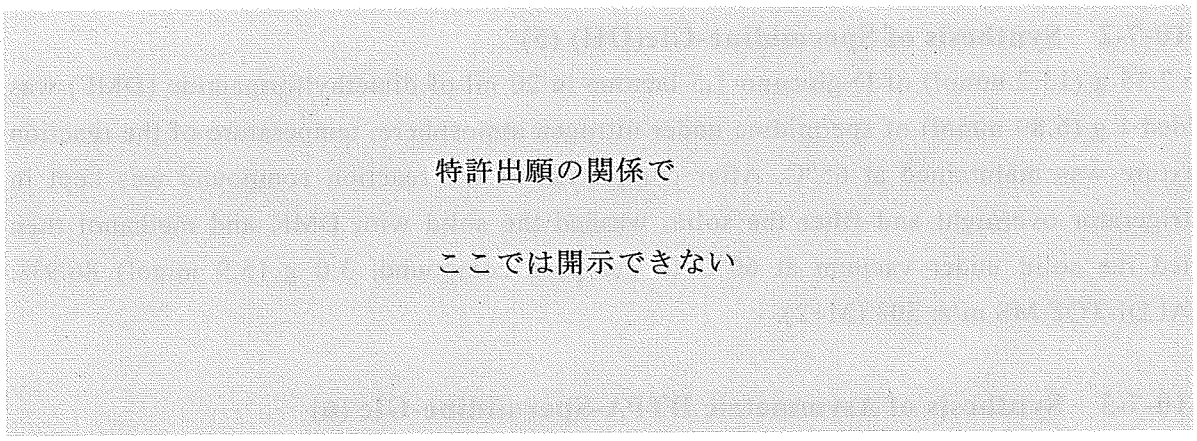


Fig. 2-10-9 Mass Spectra of C2-D2-ethylamino-methylphenol Terminal

2-10-6-2 Synthesis of DTPA-C2-D2-ethylamino-methylphenol Ligand

To a solution of DTPA dianhydride in DMF was added C2-D2-ethylaminomethylphenol terminal in quantitative ratio and pyridine was added, 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 continued stirring for 10 min separate the solid washed with ethyl acetate thoroughly to remove the traces of DMF and finally dried to get the pure compound. The compound was insoluble in water so we can't proceed further reaction for Gd complex.

(17) $\xrightarrow[60^{\circ}C,\ 24\ h]{DMSO,\ pyridine}$



(18)

Scheme 2-10-12 Synthesis of DTPA-C2-D2-ethylamino methylphenol Ligand

2-10-7 Experimental

D-Glucono-1,5-lactone, DMF, DTPA were procured from Wako Pure Chemical Industries Limited and Spermidine, HMPA procured from Sigma Aldrich chemicals. IR spectra were recorded on FTIR-spectrometer, using reflection on silicon. ^{1}H and ^{13}C NMR spectra were recorded on JEOL EX300 (300 MHz). Mass measurements were performed on VOYAGER-Deporimerix.

2-10-7-1 Synthesis of DTPA dianhydride (2)

DTPA (3.0 g, 7.6 mmol) was added to a mixture of acetic anhydride (3.0 ml, 31.8 mmol) and pyridine (4.5 ml, 53.6 mmol) and the reaction mixture was stirred for 24 h at 65°C. After Completion of the reaction filter off the solid, and washed solid repeatedly with acetic anhydride (20 ml) and acetonitrile (20 ml). Half white solid obtained was dried under vacuume at 60°C to get 2.45 g (6.94 mmol), 90%.

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

^{1}H-NMR

δ (ppm) = 3.70 (s, -CO-$\underline{CH_2}$-CO- X 4)

=3.29 (s, -$\underline{CH_2}$COOH)

=2.73 (t, $^{2}J_{CH}$ 12.1 = Hz, $COCH_2N\underline{CH_2}CH_2N$ X 2)

=2.58 (t, $^{2}J_{CH}$ 12.0 = Hz, $COCH_2NCH_2\underline{CH_2}N$ X2)

^{13}C-NMR

δ (ppm)= 171.9 ($\underline{O{=}C}OH$)

=165.7 ($\underline{O{=}C}OC{=}O$)

=54.5 ($N\underline{C}H_2COOH$)

=52.1 ($\underline{C}H_2O{=}COC{=}O$)

=50.7, 51.7 (NCH_2CH_2 X 2)

2-10-7-2 Synthesis of Spermidine-Glc(OH) (5)

To 2.35 g (13.2 mmol) of D-glucono-1,5-lactone in 20 ml of dimethylformamide (DMF) was added 1 g (6.89 mmol) of spermidine under nitrogen atmosphere, temperature of the reaction mixture was maintained at 65°C. After completion of the reaction compound was kept in refrigerator overnight and filter the solid, washed the solid with DMF, and methanol then dried the solid under vacuum at 60°C to get pure compound. 3.0 g (5.9 mmol) 86.9%. MALDI-TOF MS m/z: 503 (M+2).

2-10-7-3 Synthesis of Asymmetric DTPA-Spermidine-Glc (6)

To a solution of (1.0 g, 2.80 mmol) of DTPA dianhydride in DMF was added (1.4 g, 2.80 mmol) of Spermidine-Glc(OH) and the reaction mixture was stirred over a period of 24 h under nitrogen atmosphere. After completion of the reaction reaction mixture poured in to ethyl acetate under stirring and stirred for 10 min. Filter the solid washed with methanol and dried the compound under vacuum for 3 h, 1.4 g (1.65 mmol) 83%. MALDI-TOF MS m/z: 860 (M+2)

2-10-7-4 Synthesis of Asymmetric Gd-DTPA-Spermidine-Glc(OH) (7)

To a solution of DTPA-Spermidine-Glc(OH) (1.0 g, 1.16 mmol) in water was added 0.75 g (11.6 mmol) of pyridine was added and stirred 10 min. To this 0.432 g (1.16 mmol) of gadolinium chloride was added and the temperature of the reaction was kept constant at 65°C for 24h. After completion of the reaction excess gadolinium was removed by treating with Chelex 100 resin (Na form 100-200, 2.8 g). After removal of excess gadolinium, resin was filtered and filtrate was evaporated to give the Gd-DTPA-Spermidine-Glc(OH) in 0.9 g (0.85 mmol) 73% of yield. MALDI-TOF MS m/z: 1031 (M-H_2O).

2-10-7-5 Synthesis of DTPA-C2-D2-amine Ligand (9)

The synthesis of DTPA ligand employed a convergent method to couple core and bis

(2-aminoethyl)amine. Excess of bis(2-aminoethyl)amine was added to DTPA dianhydride (0.8 g, 2.24 mmol) and stirred for 24 h at 60 °C. After the completion of the reaction ethyl acetate was added to the reaction mixture and stirred for 30 min the compound was isolated, filtered the solid and washed with ethyl acetate and dried the compound under vacuum for 3 h. yield 1.0 g (1.79 mmol) 80%. MALDI-TOF MS m/z: 565 M+2.

2-10-7-6 Synthesis of Gd-DTPA-C2-D2-amine complex (10)

To a solution of Gd-DTPA-C2-D2-Amine (0.5 g, 0.88 mmol) in water was added 0.70 g (8.8 mmol) of pyridine was added and stirred. To this well stirred solution (0.329 g, 0.88 mmol) of gadolinium chloride was added and the temperature of the reaction was kept constant at 65°C and stirred for 24 h. After completion of the reaction excess gadolinium was removed by treating with Chelex 100 resin (Na form 100-200, 2.8 g). After completion of treatment, resin was filtered off and filtrate was evaporated to obtain Gd-DTPA-C2-D2-Amine 0.5 g (0.68 mmol) 76.6% of yield. MALDI-TOF MS m/z: 717 (M^+-H_2O).

2-10-7-7 HMTA-imino-Glc(OH) Terminal (12) &(13): It was prepared in two steps

Step 1

HMTA (1.0 g, 4.6 mmol) and 2-hydroxybenzaldehyde (0.56 g, 4.6 mmol) were dissolved in ethanol and the solution was refluxed for 3 h. After completion of the reaction solvent was evaporated, a crude product was washed with hexane and then water dried under vacuum for 3 h to obtain 2-((E)-(6-(6-aminohexylamino)hexylimino)methyl)phenol gummy crude was used for next step. Yield 1.2 g (3.76 mmol) 81%.

Step 2

2-((E)-(6-(6-aminohexylamino)hexylimino)methyl)phenol (1 g, 3.1 mmol) was dissolved in 30 ml of DMF and D-glucono-1,5-lactone (0.55 g, 3.1 mmol) was added to the solution was stirred for 24 h. After completion of the reaction poured the reaction mixture in to ethyl acetate under stirring continued stirring for 10 min then filter the solid washed with ethyl acetate and methanol and dried the solid to obtained pure compound. Yield 1.0 g (2.0 mmol) 66.6%.

2-10-7-8 Synthesis of DTPA-HMTA-C6-D2-imino-Glc(OH) Ligand (14)

To a solution of DTPA dianhydride (0.30 g, 0.840 mmol) in DMSO was added HMTA-C6-D2-imino-Glc(OH) terminal (0.83 g, 1.68 mmol) and pyridine (0.13 g, 1.68 mmol) the mixture was stirred for 24 h at 60°C and after the completion of the reaction, the reaction mixture was poured in to ethyl acetate under stirring and continued stirring for 10 min separate the solid washed with ethyl acetate thoroughly to remove the traces of DMSO and finally dried to get the pure compound. Yield 0.9 g (0.66 mmol) 79.2%. MALDI-TOF MS m/z: 1351 M+.

2-10-7-9 Synthesis of C2-D2-ethylamino methylphenol Terminal (17)

Bis(2-aminoethyl)amine (1.0 g, 9.7 mmol) and 2-hydroxybenzaldehyde (2.36 g, 19.3 mmol) were dissolved in ethanol and the solution was refluxed for 3 h. Reaction was monitored by TLC. After completion of the reaction cool to rt. then sodium borohydride ($NaBH_4$) (1.18 g, 3.8 mmol) was added to the reaction mixture and stirred for 3 h at rt. After completion of the reaction reaction mixture was poured in to cold water and extracted with MDC, solvent was removed under reduced pressure the product was washed with hexane and then dried under vacuum for 3 h to obtained gummy crude was used for next step. Yield 2.44 g (7.7 mmol) 80%., MALDI-TOF MS m/z: 317 M+2

2-10-7-10 Synthesis of DTPA-C2-D2-ethylamino methylphenol Ligand (18)

To a solution of DTPA dianhydride (0.5 g, 1.40 mmol) in DMSO was added C2-D2-amino methylphenol terminal (0.88 g, 2.79 mmol) and pyridine (0.22 g, 2.78 mmol) was added, 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 continued stirring for 10 min separate the solid washed with ethyl acetate thoroughly to remove the traces of DMSO and finally dried to get the pure compound. Ligand has not soluble in water so we can't proceed further reaction for Gd complex. Yield 1.1 g (1.14 mmol) 79.7%.

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2-11 Synthesis of Gd-DTPA-HMTA-A-2Glc(OH) and Gd-DTPA-E

Bitragunta Siva Kumar

Introduction: Magnetic Resonance Imaging (MRI), at present, is a well established safe and effective imaging technique in clinical diagnosis [1]. Use of some contrast agents (CA) effectively increases the contrast efficiency. Therefore sheer investigation of these contrast agents is more essential. Among these contrast agents stable, water soluble gadolinium (III)complexes have the ideal properties such as higher water relaxivity, chemical stability and low toxicity [2]. Presently Gd-Dota, Gd-Dtpa-BMA, Gd-HP-DO3A,Gd-DTPA-BMEA and Gd-DO3A-Butriol are using as standard contrast agents for clinical practice [3]. Though these candidates are using widely for the clinical practice yet they didn't fulfill their role. So it is very necessary to design new candidates of this family in order to overcome the drawbacks of the existing agents. To be a good candidate of this class, CA should have considerably high molecular weight [4], rigid structure, which can restrict the fast rotation of the molecule and consequently offer slow tumbling motion [5-10]. More over the presence of hydrophilic groups in these molecules increase their water solubility and the presence of hydroxyl and amino groups can easily recognize the protein molecules *in vivo* and can adhere to them strongly.

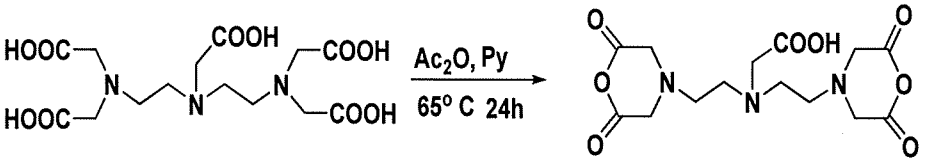
In response to the above conclusion we have rationally designed two novel contrast agents exhibiting high relaxivity values when compared to the existing standards

Result and Discussion

In my experiment, the new asymmetric Gd-DTPA-HMTA-A-2Glc(OH) was synthesized by the reaction of DTPA di-anhydride and C_6 ligand which was prepared from D-Glucono-1,5-Lactone and hexamethylene triamine followed by Boc protection of secondary amine and protection of all glycoside hydroxyl groups and deprotection of Boc.

2-11-1 Synthesis of DTPA dianhydride

DTPA dianhydride was prepared according to the previously established procedure [11]. DTPA was added to a mixture of acetic anhydride and pyridine and stirred thoroughly over a period of 24hrs 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 2-11-1 depicted the preparation route of DTPA dianhydride.



DTPA DTPA dianhydride, 90%

Scheme 2-11-01 Synthesis of DTPA di-anhydride

IR,^{1}H-NMR and ^{13}C-NMR spectra of DTPA dianhydride were shown in Fig.2-11-01, 2-11-02 respectively. From the IR spectrum, we can find the peaks at 1820cm^{-1} (C=O stretch symmetric), 1774cm^{-1} (C=O asymmetric stretching) which are characteristics of cyclo-dianhydride. ^{1}H-NMR spectrum of DTPA dianhydride exhibited four different chemical shift values at 4.0ppm, 3.6ppm, 3.5ppm and 3.2ppm with expected coupling. ^{13}C-NMR spectrum of it showed distinct singlets at 171.65 and 165.67 also suggests the presence of dianhydride system. All the above furnished information has confirmed the structure of dianhydride system.

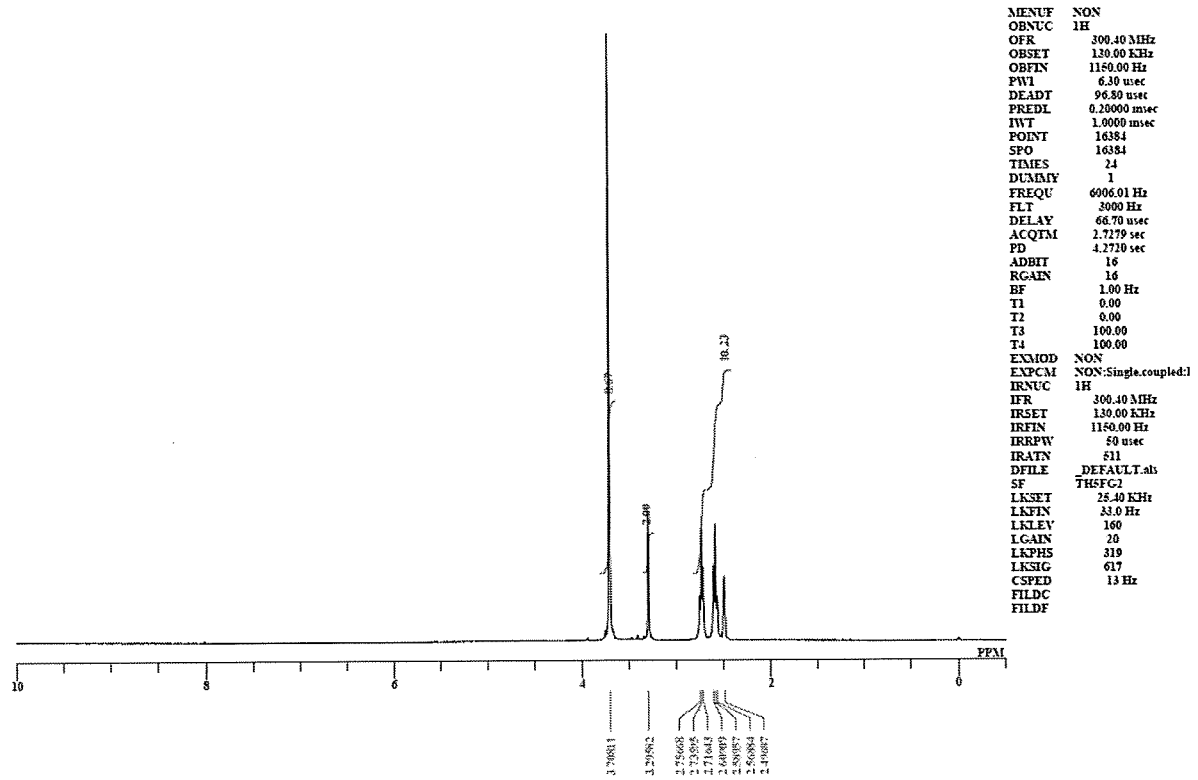


Fig. 2-11-01 ^{1}H-NMR of DTPA dianhydride

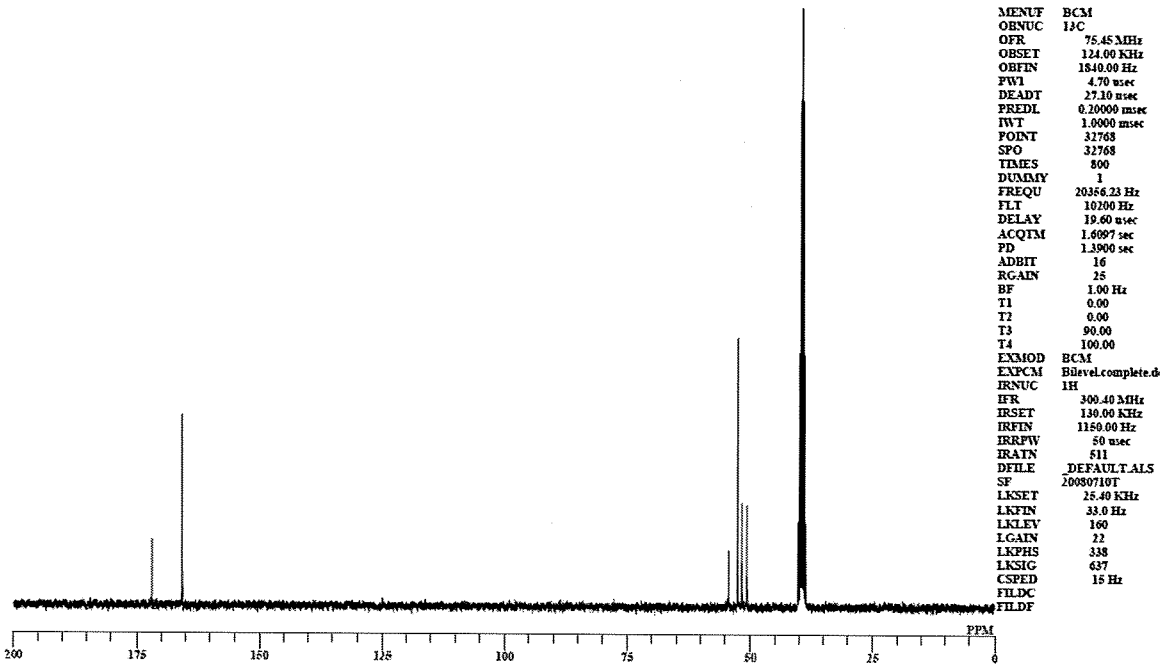
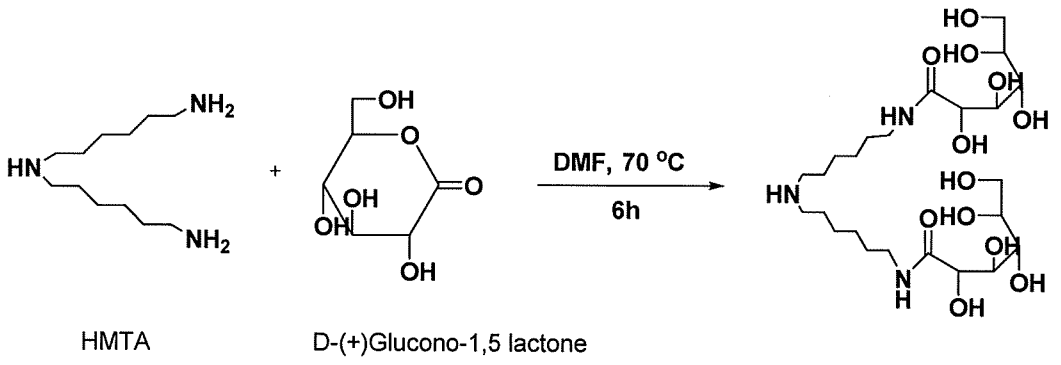


Fig. 2-11-02 ^{13}C-NMR of DTPA dianhydride

2-11-2 Synthesis of DTPA-HMTA-Glc(OAc)

2-11-2-1 Synthesis of HMTA-Glc(OH)

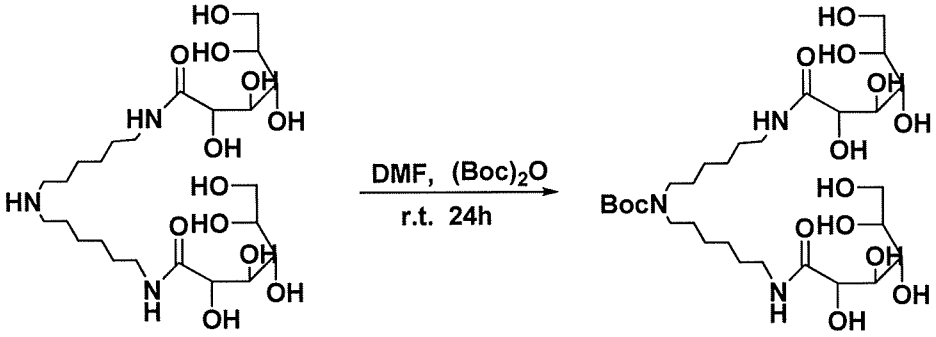
To a solution of D-(+)Glucono-1,5-lactone in adequate amount of DMF was added hexa- methylene triamine under inert conditions and the temperature of the reaction mixture was maintained at 70°C over a period of six hours. After ensuring the completion of reaction, product was left in refrigerator overnight and taken directly without further purification. Preparation of the title compound is depicted in Scheme 2-11-02



Scheme 2-11-02 Synthesis of HMTA-Glc(OH)

2-11-2-2 Synthesis of Boc protected HMTA-Glc(OH)

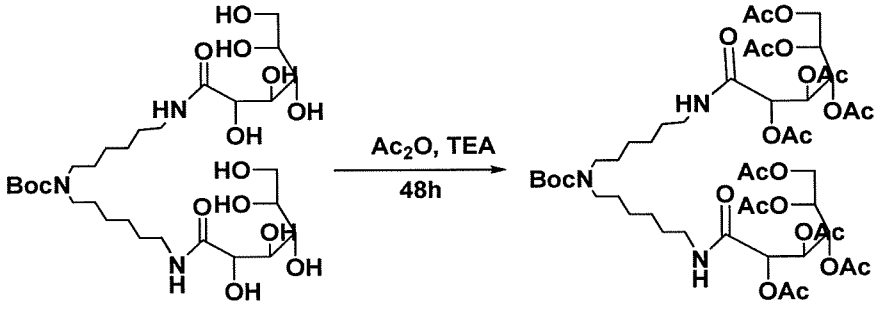
To a solution of DMF containing the above preparation was added Boc under cold conditions very carefully and the reaction mixture was subjected to through stirring under inert and dry condition then the reaction mixture was brought to room temperature and stirring was continued for a period of 24h. After completion of reaction the product was taken to next step without further purification.



Scheme 2-11-03 Synthesis of Boc protected HMTA-Glc(OH)

2-11-2-3 Synthesis of Boc protected HMTA-Glc(OAc)

To the above Boc protected compound under chilled condition triethyl amine and acetic anhydride was added carefully with continues stirring and after one hour the reaction mixture was brought to room temperature and stirred thoroughly for 48h. After the completion of reaction mixture was extracted in to ethylacetate and washed thoroughly with sat.$NaHCO_3$ and brine solution and the organic layer was rotaevaporated and purified by column chromatography by using step gradient mixtures of chloroform and methanol. Schematic procedure is summarized in Scheme 2-11-04.



Scheme 2-11-04 Synthesis of Boc-HMTA-Glc(OAc)

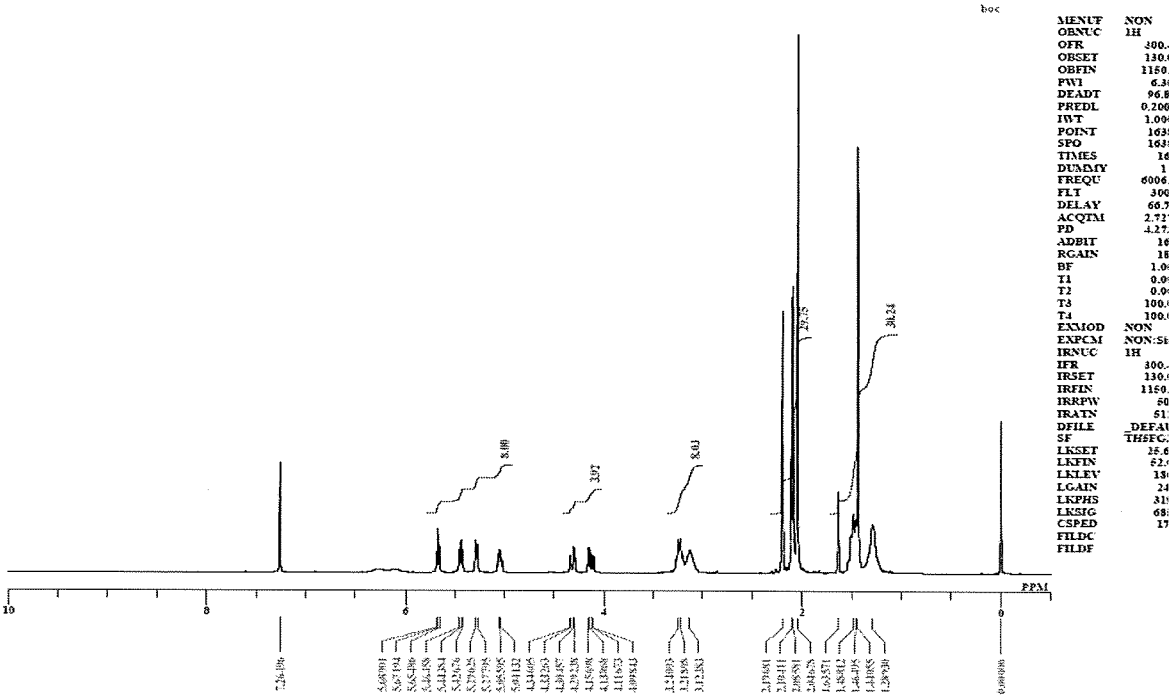
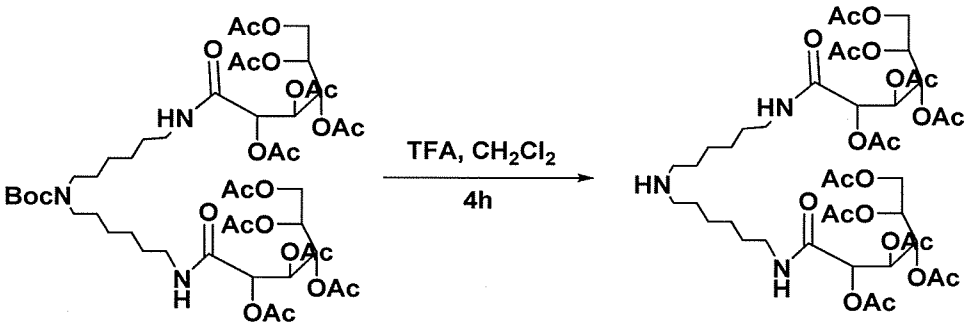


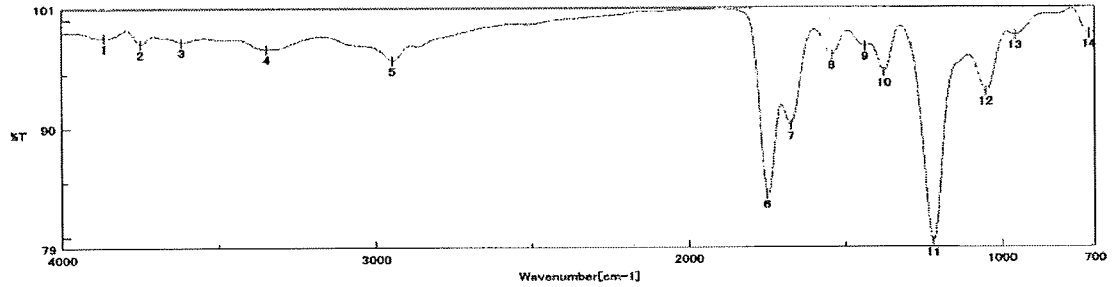
Fig. 2-11-03 ^{1}H-NMR spectrum of Boc-HMTA-Glc(OAc)

2-11-2-4 Synthesis of HMTA-Glc(OAc)

To a solution of above compound in dichloromethane was added triflouro acetic acid at 5° C carefully and the reaction was kept at room temperature for a period of 4h. After ensuring the completion of the reaction, reaction mixture was washed with and the organic phase was concentrated on rotaevaporation and this crude product was dissolved in ethyl acetate and washed successively with adequate amount of sat.$NaHCO_3$, water and finally with brine solution. Organic phase was concentrated under vacuum and the resulted crude product was purified by column chromatography using step gradient mixtures of chloroform and methanol as eluents.



Scheme 2-11-05 Synthesis of HMTA-Glc(OAc)



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アポダイゼーション Cosine
スキャンスピード 2 mm/sec

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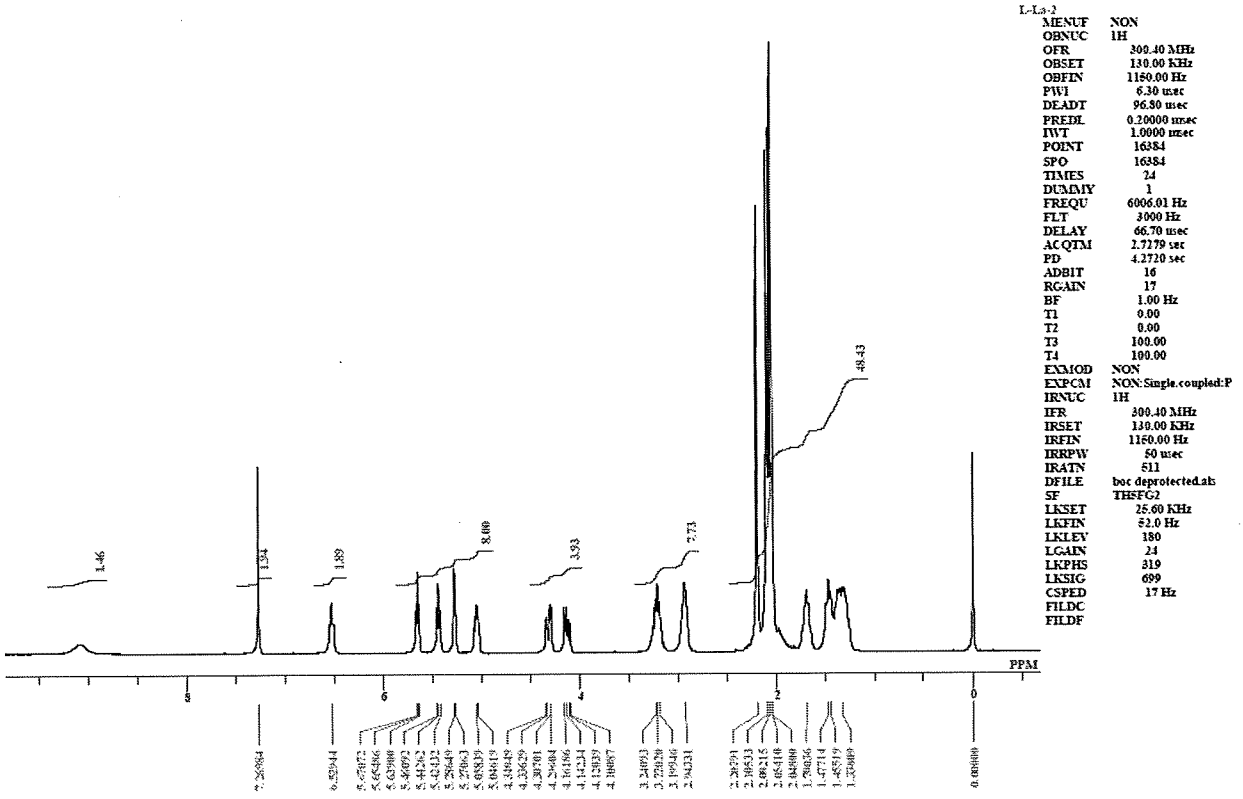
5: 2946.7, 96.1942	6: 1761.05, 83.8842	7: 1673.91, 90.1954	8: 1642.77, 96.6548
9: 1434.78, 97.4634	10: 1373.07, 95.0326	11: 1218.79, 79.4036	12: 1049.09, 93.2933
13: 956.52, 98.439	14: 717.39, 98.562		

) Terminal IR 5/03. (Measured as liquid film from CH_2Cl_2).

Fig.2-11-04 IR spectrum of HMTA-Glc(OAc)



L-La-2

MENUF	NON
OBNUC	1H
OFR	300.40 MHz
OBSET	130.00 KHz
OBFIN	1150.00 Hz
PWI	6.30 usec
DEADT	96.80 usec
PREDL	0.20000 msec
IWT	1.0000 msec
POINT	16384
SPO	16384
TIMES	24
DUMMY	1
FREQU	6006.01 Hz
FLT	3000 Hz
DELAY	66.70 usec
ACQTM	2.7279 sec
PD	4.2720 sec
ADBIT	16
RGAIN	17
BF	1.00 Hz
T1	0.00
T2	0.00
T3	100.00
T4	100.00
EXMOD	NON
EXPCM	NON:Single.coupled:P
IRNUC	1H
IFR	300.40 MHz
IRSET	130.00 KHz
IRFIN	1150.00 Hz
IRRPW	50 usec
IRATN	511
DFILE	boc deprotected.als
SF	THSFG2
LKSET	25.60 KHz
LKFIN	52.0 Hz
LKLEV	180
LGAIN	24
LKPHS	319
LKSIG	699
CSPED	17 Hz
FILDC	
FILDF	

Fig. 2-11-05 ^{1}H-NMR Spectrum of HMTA-Glc(OAc)

IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral analysis of the above compound has confirmed its structure. ^{1}H-NMR spectrum of it showed different chemical shift values at exhibited regions 5.67-5.06ppm, 4.34-4.10ppm, 3.22ppm, 2.94ppm, 2.20-204ppm and 1.70-1.33ppm with expected multiplicity.

^{13}C-NMR of it showed distinctive singlets at 170.65-166.26 indicates the presence of amide C=O group all other aliphatic carbons observed between 71.78-20.48ppm. In addition to these data mass peak at m/z 998 suggests the formation of expected compound.

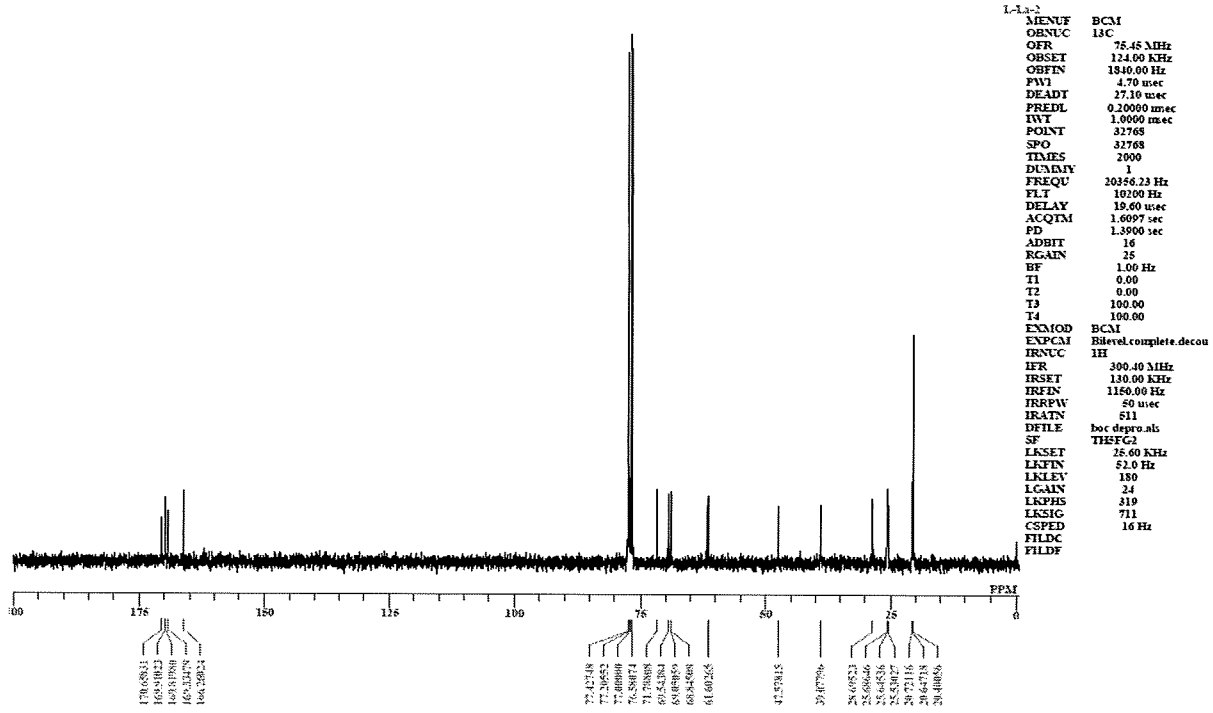
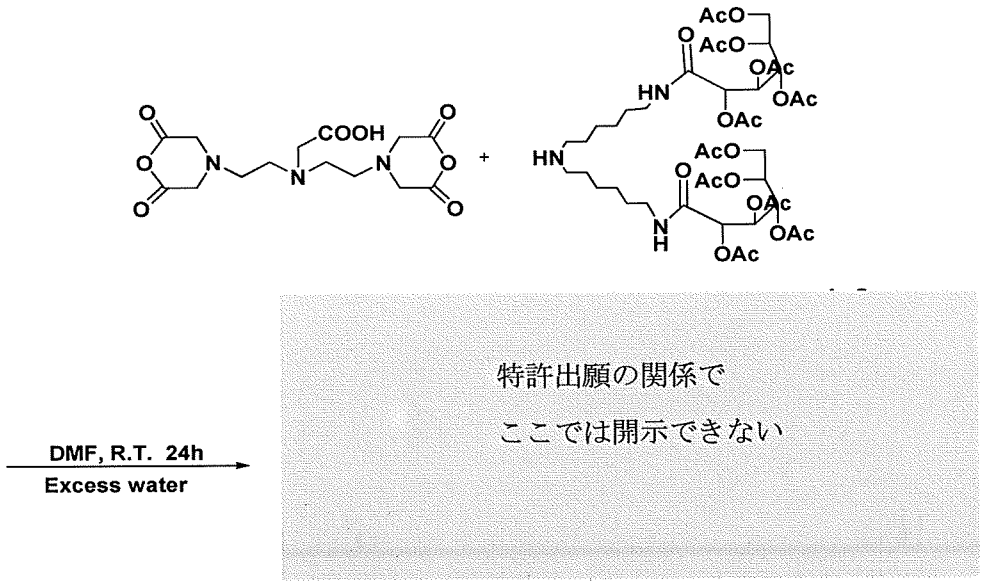


Fig. 2-11-06 ^{13}C-NMR Spectrum of HMTA-Glc(OAc)

2-11-2-5 Synthesis of DTPA-HMTA-Glc(OAc)

To a solution of DTPA di-anhydride in DMF was added terminal in quantitative ratio and the mixture was refluxed for 24h at 60°C and after the completion of the reaction DMF was removed under vacuum and the crude product was extracted into ethyl acetate and washed with water thoroughly to remove the traces of DMF and finally dried to get the pure compound. During treatment with excess of water the second unreacted anhydride ring was opened in to a dicarboxylic system.



Scheme 2-11-06 Synthesis of DTPA-HTMA-Glc(OAc)

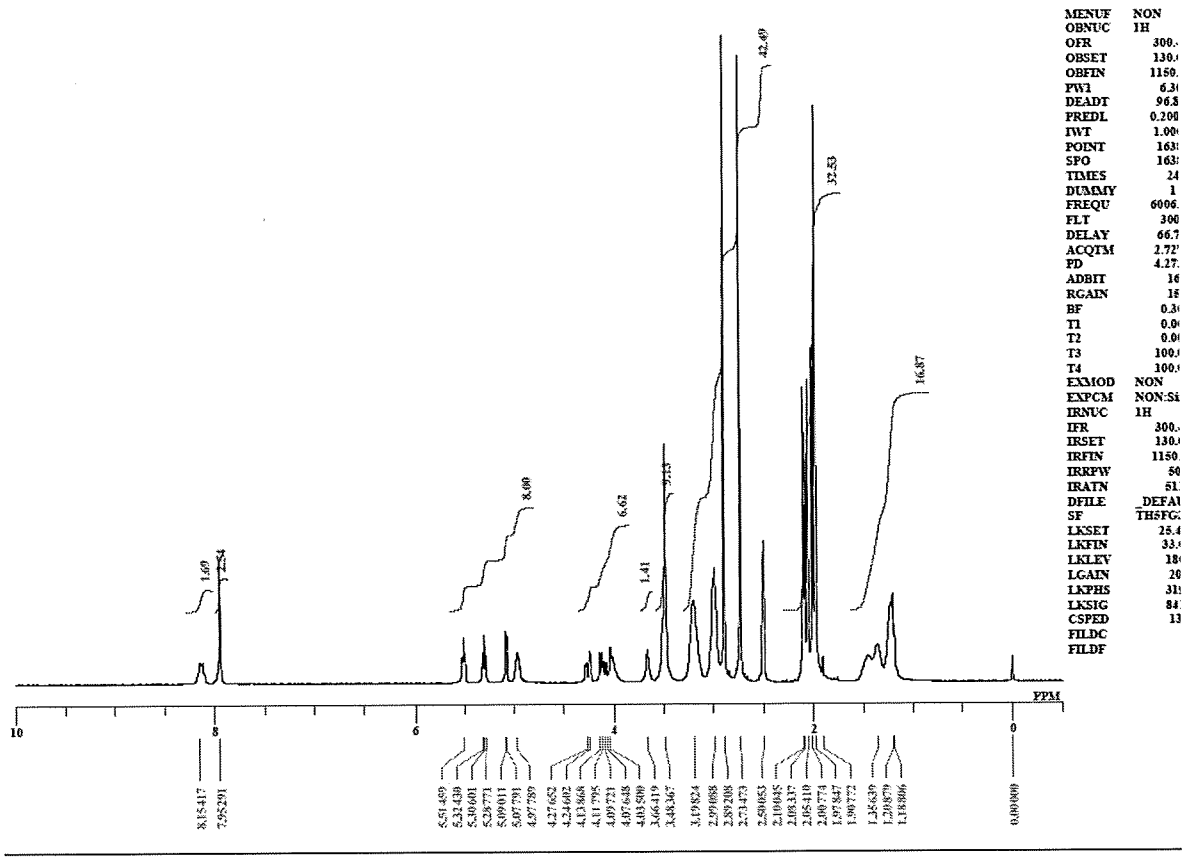


Fig. 2-11-07 ^{1}H-NMR of DTPA-HMTA-Glc(OAc)

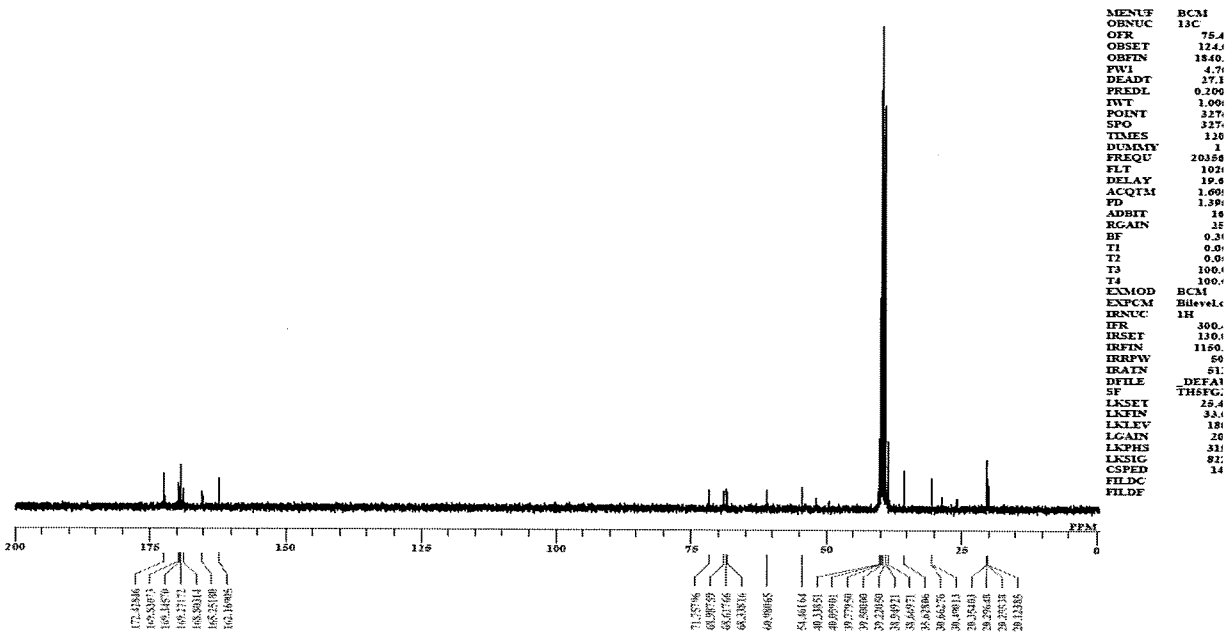


Fig. 2-11-08 ^{13}C-NMR of DTPA-HMTA-Glc(OAc)

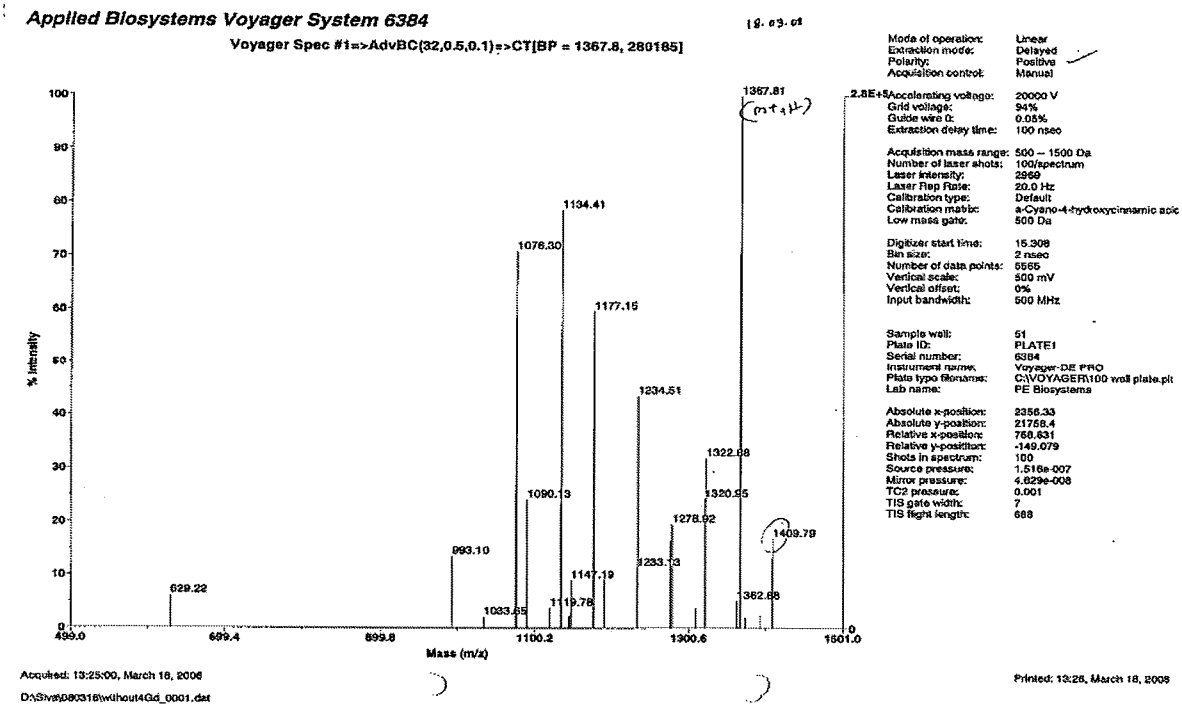
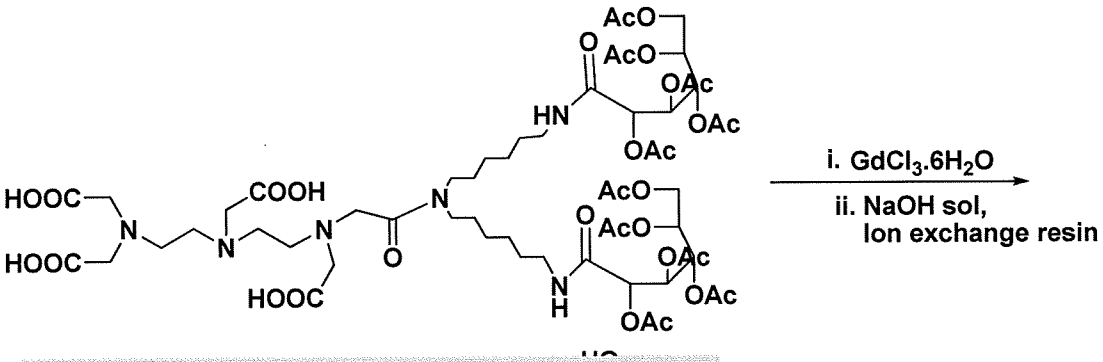


Fig. 2-11-09 Mass spectrum of DTPA-HMTA-Glc(OAc)

2-11-2-6 Synthesis of Gd-DTPA-HMTA-Glc(OAc)

To a solution of above prepared 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 glycoside hydroxyl groups were deprotected under alkaline condition. After completion of hydrolysis it was treated with DOWEX 50W-X8 ion exchange resin and after completion of ion exchange resin was filtered and the filtrate was concentrated under vacuum.



特許出願の関係で

ここでは開示できない

Scheme 2-11-07 Synthesis of Gd-DTPA-HMTA-Glc(OAc)

2-11-3 Experimental

D-(+) Glucono-1,5 lactone, DMF, DTPA were procured from Wako Pure Chemical Industries, LTD. HETA was procured from Aldrich chemicals LTD. IR spectra were recorded on FTIR-spectrometer, using reflection on silicon. ^{1}H and ^{13}C NMR spectra were recorded on JEOL EX300 (300 MHz). Mass measurements were performed on VOYAGER-Deporimerix.

2-11-3-1 Synthesis of DTPA dianhydride

DTPA (3.0g, 7.6 mmol) was added to a mixture of acetic anhydride (3.0 ml, 31.8 mmol) and pyridine (4.5 ml, 53.6 mmol) and the reaction mixture was thoroughly stirred for 24h at 65° C. After that reaction

mixture was washed repeatedly with acetic anhydride (20ml) and acetonitrile (20ml). White solid obtained was dried strongly to get 2.45g (6.94 mmol), 90%.

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

^{1}H-NMR

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

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

=2.73 (t, $COCH_2N\underline{CH_2}CH_2N$ X 2)

=2.58 (t, $COCH_2NCH_2\underline{CH_2}N$ 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)

2-11-3-2 Synthesis of HMTA-Glc(OH)

To 3.26 g of D-(+)Glucono-1,5- lactone in 20ml of dimethyle formamide (DMF) was added 2.0g (9.2 mmol) of hexamethylene triamine under nitrogen atmosphere. Temperature of the reaction mixture was maintained at 70° C and after completion of the reaction compound was kept in refrigerator overnight and used for next step without further purification.

2-11-3-3 Synthesis of Boc-HMTA-Glc(OAc)

To 5.26 g (9.20 mmol) of HMTA-Glc(OH) in 10ml of DMF was added 2.0 g (9.20 mmol) of $(Boc)_2O$ under cold conditions. Reaction mixture was thoroughly stirred for 24 which will leave a clear solution. To this clear solution under cold conditions 9.40 g (92.1 mmol) of acetic anhydride and 10ml of triethyamine were added slowly and the effective stirring was continued for 48h at room temperature. To this reaction mixture adequate amount of ethylacetate was added and washed thoroughly with Sat. $NaHCO_3$ and then with brine solution to remove the DMF. Organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. This crude compound was purified by column chromatography using chloroform and methanol as step gradients as eluents. Removal of solvent yields 9.26g (92%) of brown colored compound.

^{1}H-NMR

δ (ppm) = 8.12 (m, <u>NH</u>C=O X 2)

= 5.66 (t, C=OCH(OAc)<u>CH</u>(OAc) X 2)

= 5.43 (t, C=OCH(OAc)CH(OAc)<u>CH</u>(OAc) X 2)

= 5.27 (d, C=O<u>CH</u>(OAc) X 2)

= 5.05 (m, C=OCH(OAc)CH(OAc)CH(OAc)<u>CH</u>(OAc) X 2)

= 4.32 (dd, C=OCH(OAc)CH(OAc)CH(OAc)<u>CH</u>(OAc)C<u>H_a</u>H_b(OAc) X 2)

= 4.10 (dd, C=OCH(OAc)CH(OAc)CH(OAc)<u>CH</u>(OAc)CH_a<u>H_b</u>(OAc) X 2)

= 3.22 (m, -NH<u>CH_2</u>CH_2 X 2)

= 2.93 (t, N(Boc)<u>CH_2</u>CH_2 X 2)

= 2.20-2.04 (m, CH(O<u>Ac</u>) X 10)
= 1.71-1.41 (m, $NHCH_2(\underline{CH_2})_4CH_2NH$ X 4)
= 1.25 (s, $OCOC(\underline{CH_3})_3$)

2-11-3-4 Synthesis of HMTA-Glu(OAc)

To 10.43 g (9.56 mmol) of completely protected ligand in dichloromethane was added 8.52 ml (114.72 mmol) of trifluoroacetic acid(TFA) at 5°C carefully with constant stirring. Reaction mixture was allowed to stir over a period of 4h. After the completion of the reaction excess of TFA was quenched by washing the organic layer with water. Then the organic layer is evaporated under vacuum. This resulted crude product was dissolve in ethyl acetate and washed with Sat. $NaHCO_3$ to neutralize the organic layer (tested with pH paper). Later organic layer was concentrated under vacuum and finally crude product so obtained was purified by column chromatography by using chloroform and methanol step gradient mixtures as eluents. By removing the organic solvent under reduced pressure pale brown crystals was obtained in 86% (8.23g) of yield.

^{1}H-NMR
δ (ppm) = 6.52 (m, <u>NH</u>C=O X 2)
= 5.65 (t, C=OCH(OAc)<u>CH</u>(OAc) X 2)
= 5.44 (t, C=OCH(OAc)CH(OAc)<u>CH</u>(OAc) X 2)
= 5.27 (d, C=O<u>CH</u>(OAc) X 2)
= 5.05 (m, C=OCH(OAc)CH(OAc)CH(OAc)<u>CH</u>(OAc) X 2)
= 4.30 (dd, C=OCH(OAc)CH(OAc)CH(OAc)<u>CH</u>(OAc)<u>CH_aH_b</u>(OAc) X 2)
= 4.10 (dd, C=OCH(OAc)CH(OAc)CH(OAc)<u>CH</u>(OAc)CH_a<u>H_b</u>(OAc) X 2)
= 3.22 (m, -NH<u>CH_2</u>CH_2 X 2)
= 2.94 (m, NH<u>CH_2</u>CH_2 X 2)
= 2.20-2.04 (m, CH(O<u>Ac</u>) X 10)
= 1.71-1.33 (m, $NHCH_2(\underline{CH_2})_4CH_2NH$ X 4)
^{13}C-NMR
δ (ppm) = 170.69 (s, <u>C=O</u>NH)
= 71.78 (s, C=O<u>CH</u>(OAc) X 2)
= 69.54 (s, C=OCH(OAc)<u>CH</u>(OAc) X 2)
= 69.05 (s, C=OCH(OAc)CH(OAc)<u>CH</u>(OAc) X 2)
= 68.84 (s, C=OCH(OAc)CH(OAc)CH(OAc) <u>CH</u>(OAc) X 2)
= 61.60 (s, C=OCH(OAc)CH(OAc)CH(OAc) CH(OAc)<u>CH_2</u>(OAc) X 2)
= 47.57 (s, C=ONH<u>CH_2</u> X 2)
= 39.07 (s, NH<u>CH_2</u>CH_2 X 2)
= 28.69 (s, C=ONHCH$_2$<u>CH_2</u> X 2)
= 25.68 (s, NHCH$_2$<u>CH_2</u> X 2)
= 25.64 (s, C=ONHCH$_2$CH$_2$<u>CH_2</u> X 2)
= 25.53 (s, NHCH$_2$CH$_2$<u>CH_2</u> X 2)
= 20.72, 20.64, 20.40 (s, $(OC=OCH_3)_3$ X 10)

2-11-3-5 Synthesis of DTPA-HMTA-Glc(OAc)

To a solution of 0.5 g (1.40 mmol) of DTPA dianhydride in DMF was added 1.39 g (1.40 mmol) of HMTA-Glc(OAc) and the reaction mixture was stirred vigorously over a period of 24h under nitrogen atmosphere. After completion of the reaction solvent was removed under vacuum and washed with chloroform and then to that excess of water was added to initiate the opening of the second anhydride system. After completion of hydrolysis water was evaporated to get pale brown colour compound 1.65g (86%).

^{1}H-NMR
δ (ppm) = 8.15 (m, NHC=O X 2)
= 5.51 (t, C=OCH(OAc)CH(OAc) X 2)
= 5.30 (t, C=OCH(OAc)CH(OAc)CH(OAc) X 2)
= 5.08 (d, C=OCH(OAc) X 2)
= 4.97 (m, C=OCH(OAc)CH(OAc)CH(OAc)CH(OAc) X 2)
= 4.25 (dd, C=OCH(OAc)CH(OAc)CH(OAc)CH(OAc)C$H_a$$H_b$(OAc) X 2)
= 4.10 (dd, C=OCH(OAc)CH(OAc)CH(OAc)CH(OAc)C$H_a$$H_b$(OAc) X 2)
= 3.66 (s, NCH_2C=ON)
= 3.48 (s, NCH_2COOH X 4)
= 3.19 (m, -C=ON$CH_2$$CH_2$ X 4)
= 2.89 (m, NH$CH_2$$CH_2$ X 2)
= 2.50 (m, NHCH$_2$$CH_2$ X 2)
= 2.10-1.90 (m, CH(OAc) X 10)
= 1.35-1.18 (m, NHCH$_2$(CH_2)$_4$CH$_2$NH X 4)
^{13}C-NMR
δ (ppm) = 169.80 (s, C=ONH)
= 71.78 (s, C=OCH(OAc) X 2)
= 68.98 (s, C=OCH(OAc)CH(OAc) X 2)
= 68.61 (s, C=OCH(OAc)CH(OAc)CH(OAc) X 2)
= 68.33 (s, C=OCH(OAc)CH(OAc)CH(OAc) CH(OAc) X 2)
= 63.20 (s, NCH_2C=O)
= 60.98 (s, C=OCH(OAc)CH(OAc)CH(OAc) CH(OAc)CH_2(OAc) X 2)
= 54.46 (N$CH_2$$CH_2$N X 2)
= 52.40 (C=ONCH_2 X 4)
= 35.62 (s, C=ONHCH$_2$$CH_2$ X 4)
= 30.66 (s, C=ONHCH$_2$CH$_2$$CH_2$ X 2)
= 20.35, 20.29, 20.25, 20.13 (s, (OC=OCH_3)$_3$ X 10)

2-11-3-6 Synthesis of Gd-DTPA-HMTA-Glc(OAc)

To a solution of DTPA-HMTA-Glc(OAc) (1.30g, 0.95 mmol) in water was added 0.75g (9.5 mmol) of pyridine was added and stirred thoroughly. To this well stirred solution 0.353g (0.95 mmol) of gadolinium chloride was added and the temperature of the reaction was kept constant at 65°C and