Electronic Supplementary Material (ESI) for RSC Advances

Effect of the Second Coordination Sphere on New Contrast Agents Based on Cyclodextrin Scaffold for MRI Signal

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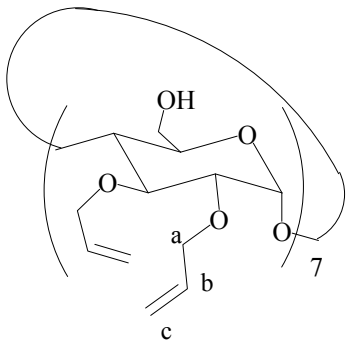
Supporting Information available

1. General

All solvents and reagents were purchased from commercial sources and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) on a plate of silica gel 60 F_{254} (E. Merck, Darmstadt, Germany) and detection by charring with sulfuric acid. Column chromatographies were performed on silica gel 60 (0.063-0.200 mm, E. Merck). ^{1}H (300 MHz), ^{13}C (75.5 MHz) and NMR spectra were recorded on Bruker AVANCE 300. Chemical shifts in NMR spectra are reported in parts per million from TMS. IR spectra were recorded on a Perkin-Elmer IRFT 1650 spectrometer. MALDI-TOF/MS experiments were performed with an Autoflex III (Bruker Daltonics, Bremen, Germany) equipped with a Nd:YAG laser emitting at 355 nm. ESI-MS data were acquired using a HCT Ultra Ion Trap mass spectrometer (Bruker Daltonics, Bremen, Germany) or using a LCT Premier XE (Waters, Manchester, UK) for the complexes. Accurate mass measurements (HR-MS) were realized using a Synapt G2 HDMS (Waters, Manchester, UK) equipped with a lockspray electrospray (ESI) source. Experiments were achieved in positive or negative ion mode using protonated or deprotonated molecule of bombesine as internal reference (m/z 1619.8229 and 1617.8073 respectively).

2. Experimental procedures for the synthesis

Heptakis-(2,3-*O*-diallyl)-β-cyclodextrin 5



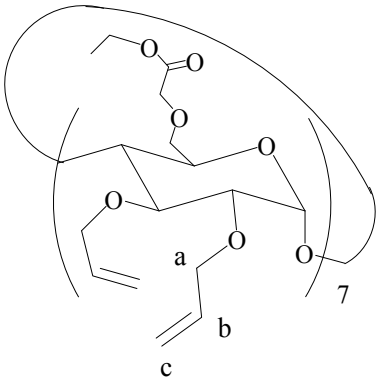
Step 1. Synthesis of heptakis-(6-*O*-*tert*-butyldimethylsilyl-2,3-*O*-diallyl)-β-cyclodextrin Under nitrogen atmosphere sodium hydride (2.43 g, 0.06 mol, dispersed in mineral oil 60%) was added to a solution of heptakis-(6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin **4** (4.4 g, 2.2 mmol) in 90 mL of anhydrous tetrahydrofuran THF at 0°C, and the mixture was stirred during 3 hours. A solution of allyl iodide (12.2 mL, 0.13 mol) was then added dropwise at room temperature during 1 hour and the mixture was stirred for 24 h at room temperature. Methanol

was then added to degrade the excess of sodium hydride. The solvents were evaporated under reduced pressure. The crude product was purified by chromatography on silica gel column (dichloromethane-cyclohexane; 6/4, v/v) to give a white powder (2.7 g, 46% yield). Analytical data were in accordance with the literature.[1]

Step 2.

Ammonium fluoride (0.86 g, 23 mmol) was added at room temperature to a solution of heptakis-(6-*O*-*tert*-butyldimethylsilyl-2,3-*O*-diallyl)-β-cyclodextrin (2.51 g, 1 mmol) in 70 mL of methanol. The mixture was stirred at 75°C for 24 hours. Further ammonium fluoride (0.86 g, 23 mmol) was then added, and the mixture was stirred at 75°C for 24 hours. The solvent was evaporated under reduced pressure and the residue was dissolved in 100 mL of methylene chloride. The white precipitate obtained was then filtered off and it was eliminated. The solvent from the residual filtrate was evaporated under reduced pressure to give the desired compound as a white powder (1.6 g, 90% yield). m.p. >260 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.02 - 5.81 (m, 14 H, H-b), 5.28 - 5.05 (m, 35 H, H-c, H-1), 4.44 - 4.11 (m, 28 H, H-a), 3.93 - 3.52 (m, 35 H, H-6, H-5, H-4, H-3), 3.34 - 3.30 (dd, *J* = 9 Hz, *J* = 3 Hz, 7 H, H-2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 136.0, 135.2 (C-b), 116.9, 115.8 (C-c), 98.6 (C-1), 79.8, 78.9, 77.9 (C-4, C-2, C-3), 74.3 (C-5), 72.5, 72.2 (C-a), 61.4 (C-6). IR ν_{max} (cm^{-1}): 3393, 2922, 1694, 1017. LRMS (ESI-MS+): m/z 1717 $[M+Na]^+$. HRMS (ESI-MS+): m/z Calcd for $C_{84}H_{126}O_{35}Na$: 1717.7977. Found: 1717.7950. Anal. Calcd for $C_{84}H_{126}O_{35}$: C, 59.49; H, 7.49. Found: C, 57.24; H, 7.45.

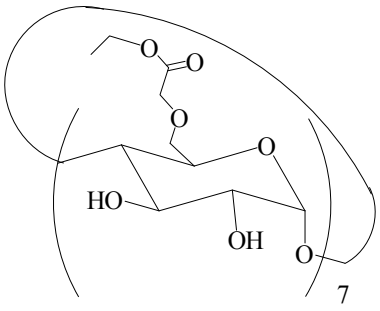
Heptakis-(6-*O*-ethoxycarboxymethyl-2,3-*O*-diallyl)-β-cyclodextrin 6



[1] H. H. Baer, Y. Shen, F. S. Gonzalez, A. V. Berenguel and J. I. Garcia, *Carbohydr. Res.*, 1992, **235**, 129.

Under nitrogen atmosphere heptakis-(2,3-*O*-diallyl)-β-cyclodextrin **5** (874 mg, 0.516 mmol) was dissolved in 72 mL of dry CH_2Cl_2 and ethyl diazoacetate (590 mg, 5.165 mmol) was added. A solution of HBF_4 (54% ethereal) was diluted in methylene chloride (39.4 µL dissolved in 3.94 mL of CH_2Cl_2) and it was then added. Bubbles of N_2 were observed. The reaction mixture was stirred at room temperature for 24 hours. 7 ML of 5% aqueous $NaHCO_3$ was added and the mixture was stirred for 5 min. The organic phase was separated, dried over Na_2SO_4, and it was evaporated under reduce pressure. The residue was purified on silica gel column chromatography (dichloromethane-methanol; 180/1 to 140/1, v/v) to give a white oil (431 mg, 36% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.02 - 5.83 (m, 14 H, H-b), 5.27 - 5.04 (m, 35 H, H-c, H-1), 4.47 - 3.99 (m, 70 H, -C<u>H</u>$_2$-CO-, -O-C<u>H</u>$_2$-CH$_3$, H-6, H-a), 3.78 - 3.67 (m, 21 H,H-5, H-4, H-3), 3.35 - 3.31 (dd, *J* = 9 Hz, *J* = 3 Hz, 7 H, H-2), 1.24 (t, *J* = 6 Hz, 21 H, CH$_2$-C<u>H</u>$_3$,).^{13}C NMR (75 MHz, $CDCl_3$) δ: 169.2 (C=O), 135.3, 134.4 (C-b), 115.8, 114.6 (C-c), 97.8 (C-1), 78.8, 78.2, 78.1 (C-4, C-2, C-3), 73.4 (C-5), 71.1 (O<u>C</u>H$_2$CO), 69.9, 69.6 (C-a), 67.6 (C-6), 59.53 (O<u>C</u>H$_2$CH$_3$), 13.2 (O-CH$_2$-<u>C</u>H$_3$). IR ν_{max} (cm^{-1}): 2905, 1749, 1204, 1144, 1026, 1020, 918, 855. LRMS (ESI-MS+): m/z 2320 $[M+Na]^+$. HRMS (MALDI-MS+): m/z Calcd for $C_{112}H_{168}O_{49}Na$: 2320.0546. Found: 2320.0630. Anal. Calcd for $C_{112}H_{168}O_{49}$: C, 58.52; H, 7.37. Found: C, 57.82; H, 7.60.

Heptakis-(6-*O*-ethoxycarboxymethyl)-β-cyclodextrin 7



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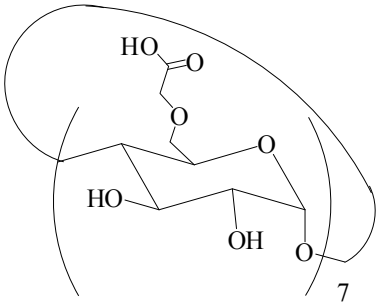
Under nitrogen atmosphere heptakis-(6-*O*-ethoxycarboxymethyl-2,3-*O*-diallyl)-β-cyclodextrin **6** (100 mg, 0.043 mmol) was dissolved in 5 mL of glacial acetic acid and tetrakis(triphenylphosphine)palladium(0) (237 mg, 0.204 mmol) was added. The reaction mixture was stirred at 80°C for 17 hours. The catalyst was then precipitated in 15 mL of water and filtered off through celite. The crude product was passed through an ion-exchange column (Dowex® 50WX8-100), and the obtained product was then lyophilized. The obtained solid was dissolved in 50 mL of methylene chloride and polystyrene-bound diethanolamine (PS-DEAM® 1.7 mmol/g, 500 mg) was added. The mixture was stirred at room temperature

for 24 hours and was filtered. The filtrate was evaporated under reduce pressure to give a white powder (52 mg, 70% yield). m.p. >260 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.95 (s, 7 H, H-1), 4.16 - 3.48 (m, 56 H, H-5, H-4, H-3, H-2, H-6, -C$\underline{H}_2$OCO), 1.25 (t, 21H, -OCH$_2$C$\underline{H}_3$). IR ν_{max} (cm^{-1}): 3402, 2918, 1744, 1207, 1020, 856. LRMS (ESI-MS+): m/z 1759 $[M+Na]^+$. HRMS (ESI-MS+): m/z Calcd for $C_{70}H_{112}O_{49}Na$: 1759.6170. Found: 1759.6143. Anal. Calcd for $C_{70}H_{112}O_{49}$: C, 48.39; H, 6.50. Found: C, 47.48; H, 6.15.

Heptakis-(6-*O*-carboxymethyl)-β-cyclodextrin 9



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Heptakis-(6-*O*-ethoxycarboxymethyl)-β-cyclodextrin **8** (118 mg, 0.068 mmol) was dissolved in methanol (5 mL) and a solution of NaOH (7.3 mL, 2.72 mmol) was added. The reaction mixture was stirred for 18 hours at room temperature. Methanol was evaporated, and the solution was filtered on Dowex 50WX8-100 column (H^+). Lyophilization of the resulting solution gave the desired product as a white powder (75 mg, 72% yield). m.p. >260 °C; ^{1}H NMR (300 MHz, D_2O) δ: 5.02 (d, J = 3 Hz, 7 H, H-1), 4.24 - 4.09 (dd, J_{gem} = 15 Hz, 14 H, C$\underline{H}_2$CO), 3.93 - 3.80 (m, 28 H, H-5, H-4, H-6), 3.64 - 3.57 (m, 14 H, H-3, H-2). ^{13}C NMR (75 MHz, D_2O) δ: 173.9 (C=O), 101.8 (C-1), 81.2 (C-4), 72.9 (C-2 or C-3), 71.8 (C-2 or C-3), 70.4 (C-5), 69.6 (OCH$_2$CO), 68.7 (C-6). IR ν_{max} (cm^{-1}): 3366, 2930, 1744, 1208, 1234, 1143, 1023, 946, 856. LRMS (ESI-MS-): m/z 1539 $[M-H]^-$, 769.3 $[M-2H]^{2-}$, 512.5 $[M-3H]^{3-}$. Anal. Calcd for $C_{56}H_{84}O_{49}$: C, 43.64; H, 5.49. Found: C, 40.74; H, 5.50.

3. General procedure for the preparation of Lanthanides Gd(III), Eu(III) and La(III) complexes 1a, b, c and 2a, b, c

A solution of lanthanide chloride hexahydrate $LnCl_3.6H_2O$ (0.497 ml, 30.67 mM) was added in three portions to a solution of heptakis-(6-*O*-carboxymethyl)-β-cyclodextrin **9** (0.013 mmol, 7.67 mM) in water. The pH was adjusted to 7.0 after each addition using a 1 M sodium hydroxide solution. The reaction mixture was stirred at room temperature for 18 hours. The pH was then adjusted to 8.5. The lanthanide residual was precipitated, centrifuged and filtered through a 0.2 µm membrane. The resulting solution was lyophilized to give the desired product.

Heptakis(6-*O*-carboxymethyl-2,3-di-*O*-methyl)-β-cyclodextrin Gadolinium 1a was isolated as a white solid. IR ν_{max} (cm^{-1}): 3392, 1589, 1324, 1019. LRMS (ESI-MS-): m/z 1890 $[M+Gd-4H]^-$, 944 $[M+Gd-5H]^{2-}$, 629 $[M+Gd-6H]^{3-}$, 471 $[M+Gd-7H]^{4-}$. HRMS (ESI-MS-): m/z Calcd for $C_{70}H_{108}O_{49}Gd$: 1890.5200. Found: 1890.5232.

Heptakis(6-*O*-carboxymethyl-2,3-di-*O*-methyl)-β-cyclodextrin Europium 1b was isolated as a white solid. IR ν_{max} (cm^{-1}): 3421, 1589, 1424, 1015. LRMS (ESI-MS-): m/z 1883 $[M+Eu-4H]^-$, 941 $[M+Eu-5H]^{2-}$, 627 $[M+Eu-6H]^{3-}$, 470 $[M+Eu-7H]^{4-}$. HRMS (ESI-MS-): m/z Calcd for $C_{70}H_{108}O_{49}Eu$: 1883.5157. Found: 1883.5087.

Heptakis(6-*O*-carboxymethyl-2,3-di-*O*-methyl)-β-cyclodextrin Lanthane 1c was isolated as a white solid. The ^1H and ^{13}C NMR analyses showed a clear spectrum with significant shifts of the carbon atoms around the carboxylate functions proving the complexation of the lanthanide with the seven ligands. The (-O-$\underline{CH_2}$-CO) proton and carbon signals shifted respectively of 0.3 and 1.4 ppm. The carbon on position 6 shifted of 1.75 ppm. ^{13}C NMR spectrum showed only one signal for (C-6) and for the (-O-$\underline{CH_2}$-CO). ^1H NMR (300 MHz, D_2O) δ: 5.28 (s, 7 H, H-1), 4.13 - 3.89 (m, 14 H, OC$\underline{H_2}$CO), 3.75 - 3.62 (m, 35 H, H-6a, H-5, H-4, H-6b, H-3), 3.54 (s, 21 H, CH_3), 3.46 (s, 21 H, CH_3), 3.30 (dd, J = 3 Hz, J = 9 Hz, 7 H, H-2). ^{13}C NMR (75 MHz, D_2O) δ: 97.1 (C-1), 80.7 (C-2), 79.8 (C-3), 77.5 (C-4), 70.5 (C-5), 71.2 (-O$\underline{C}H_2$CO-), 69.6 (C-6), 59.4 (CH_3), 57.9 (CH_3). IR ν_{max} (cm^{-1}): 3321, 1563, 1389,1031. LRMS (ESI-MS-): -): m/z 1871 $[M+La-4H]^-$, 935 $[M+La-5H]^{2-}$, 623 $[M+La-6H]^{3-}$467 $[M+La-7H]^{4-}$. HRMS (ESI-MS-): m/z Calcd for $C_{70}H_{108}O_{49}La$: 1871.5023. Found: 1871.5056.

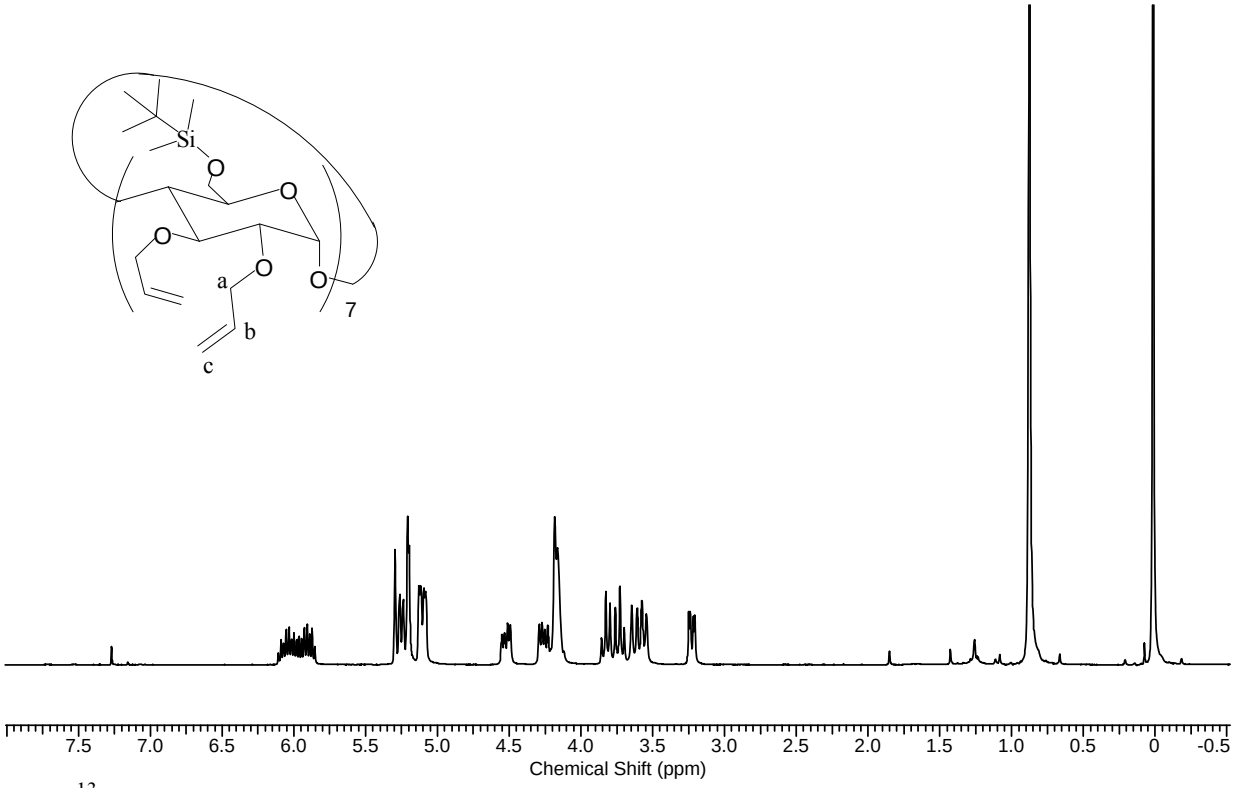
Heptakis-(6-*O*-carboxymethyl)-β-cyclodextrin Gadolinium 2a was isolated as a white-yellow solid. IR ν_{max} (cm^{-1}): 3366, 2936, 1744, 1559, 1139, 1040. LRMS (ESI-MS-): m/z 563 $[M+Gd-6H]^{3-}$, 422 $[M+Gd-7H]^{4-}$. HRMS (ESI-MS-): m/z Calcd for $C_{56}H_{77}O_{49}Gd^{-}$: 422.8198. Found: 422.8178.

Heptakis-(6-*O*-carboxymethyl)-β-cyclodextrin Europium 2b was isolated as a white-yellow solid. IR ν_{max} (cm^{-1}): 3326, 2936, 1748, 1559, 1208, 1139, 1021. LRMS (ESI-MS-): 843 $[M+Eu-5H]^{2-}$, m/z 561 $[M+Eu-6H]^{3-}$. HRMS (ESI-MS-): m/z Calcd for $C_{56}H_{79}O_{49}Eu$ $[M-2H]^{2-}$: 843.1481. Found: 843.1444.

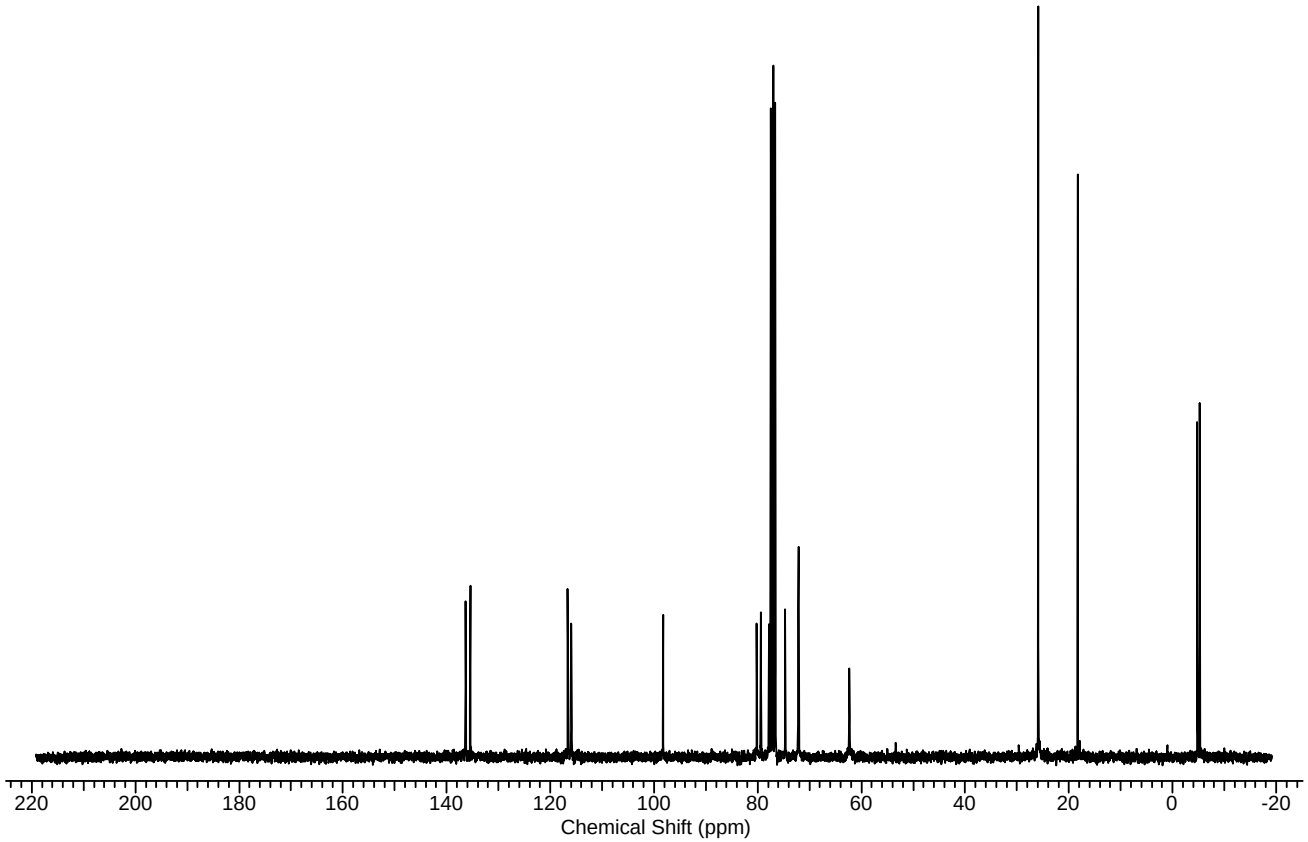
Heptakis-(6-*O*-carboxymethyl)-ß-cyclodextrin Lanthane 2c was isolated as a white-yellow solid. The ^{1}H and ^{13}C NMR analysis showed a clear spectrum after complexation with significant shifts of the carbon atoms around the carboxylate functions proving the complexation of the lanthanide with the seven ligands. In this case, the (-O-$\underline{CH_2}$-CO) proton and carbon signals shifted respectively of 0.36 and 1.2 ppm. The carbonyl functions shifted of 3.9 ppm. ^{13}C NMR spectrum showed only one signal for (C=O) at 177.8 ppm. ^{1}H NMR (300 MHz, D_2O) δ: 4.97 (s, 7 H, H-1), 3.84 -3.79 (m, 28 H, $C\underline{H_2}CO$, H-6), 3.67 - 3.49 (m, 28 H, H-5, H-4, H-3, H-2). ^{13}C NMR (75 MHz, D_2O) δ: 177.8 (C=O), 101.8 (C-1), 80.9 (C-2), 72.9 (C-4 or C-3), 71.8 (C-4 or C-3), 70.3 (C-5), 69.5 (O-$\underline{C}H_2$-CO), 68.8 (C-6). IR ν_{max} (cm^{-1}): 3318, 1587, 1250, 1087, 1011. LRMS (ESI-MS-): m/z 1675 $[M+La-4H]^{-}$, 837 $[M+La-5H]^{2-}$, 558 $[M+La-6H]^{3-}$, 418 $[M+La-7H]^{4-}$. HRMS (ESI-MS-): m/z Calcd for $C_{56}H_{80}O_{49}La$: 1675.2832 Found: 1675.2860.

4. Routine NMR analysis

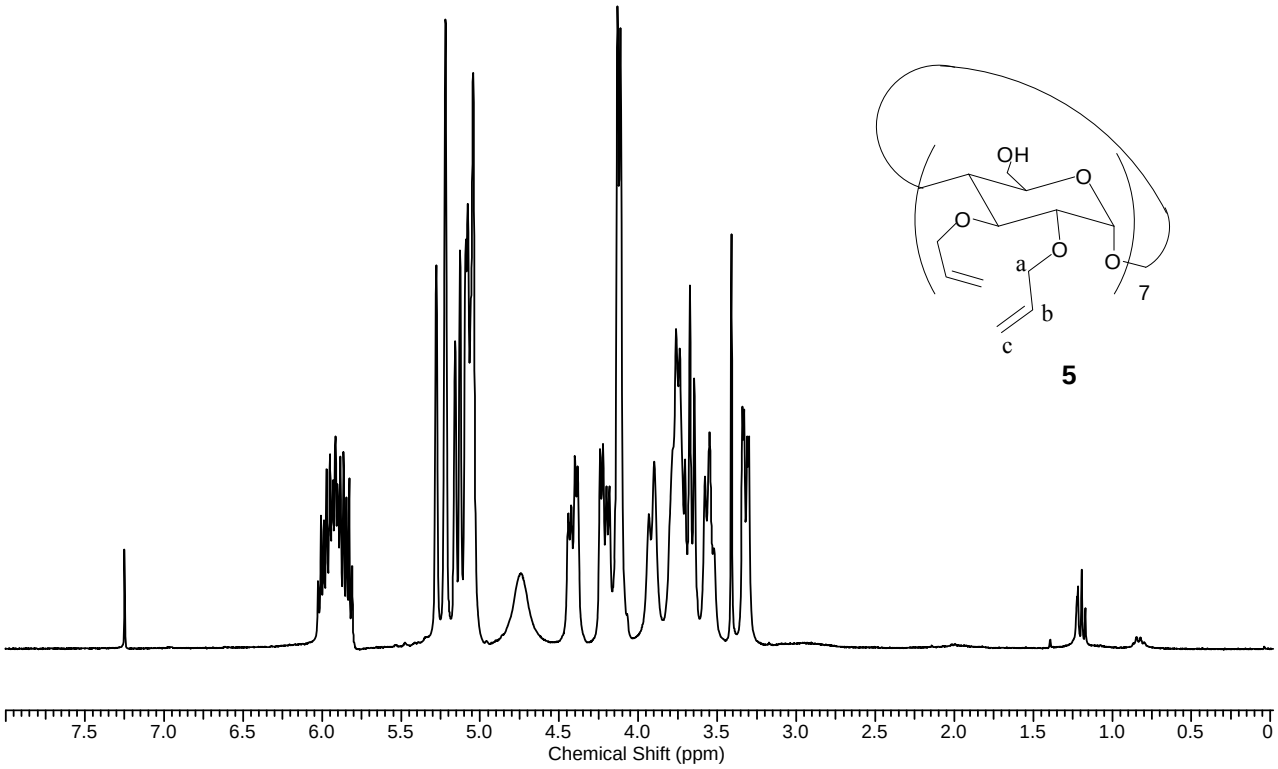
4.1.1 ^{1}H NMR spectrum of **intermediate 4/5** ($CDCl_3$, 300 MHz)



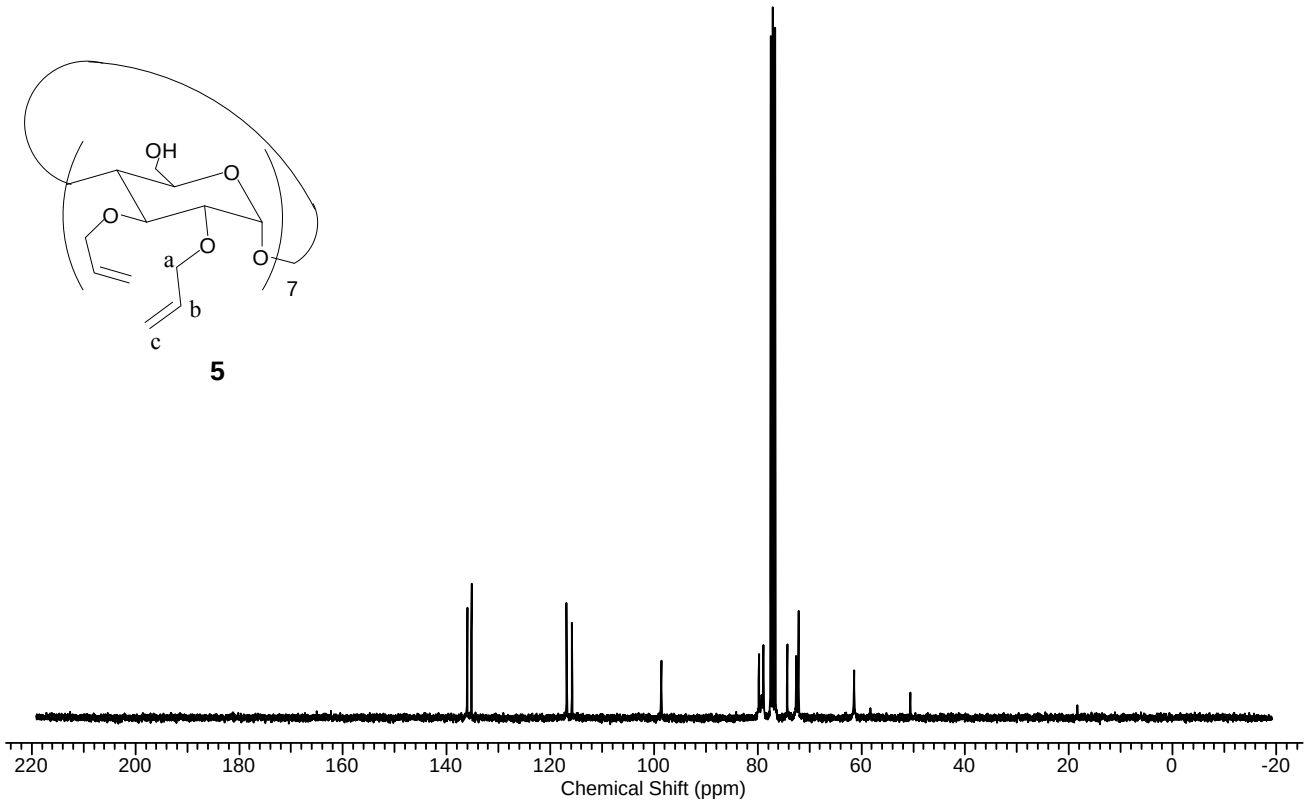
4.1.2 ^{13}C NMR spectrum of **intermediate 4/5** ($CDCl_3$, 75 MHz)



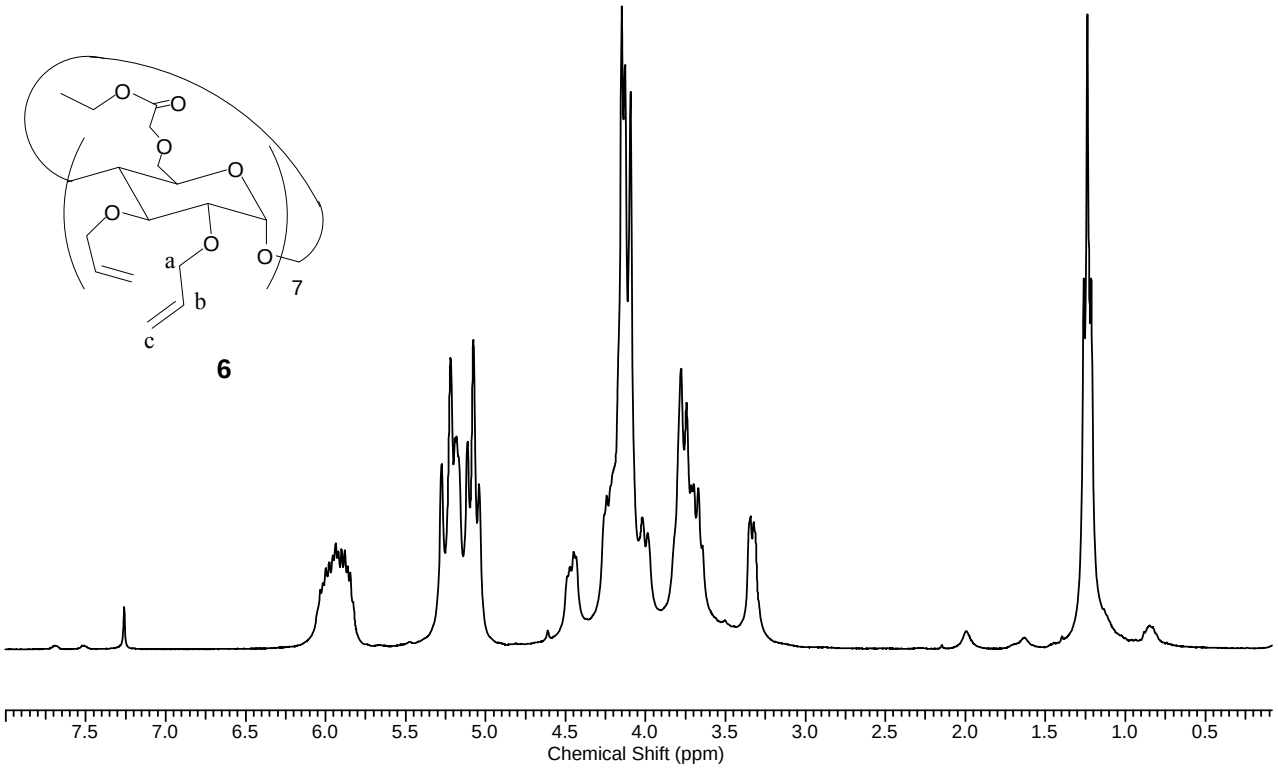
4.2.1 ^{1}H NMR spectrum of **5** ($CDCl_3$, 300 MHz)



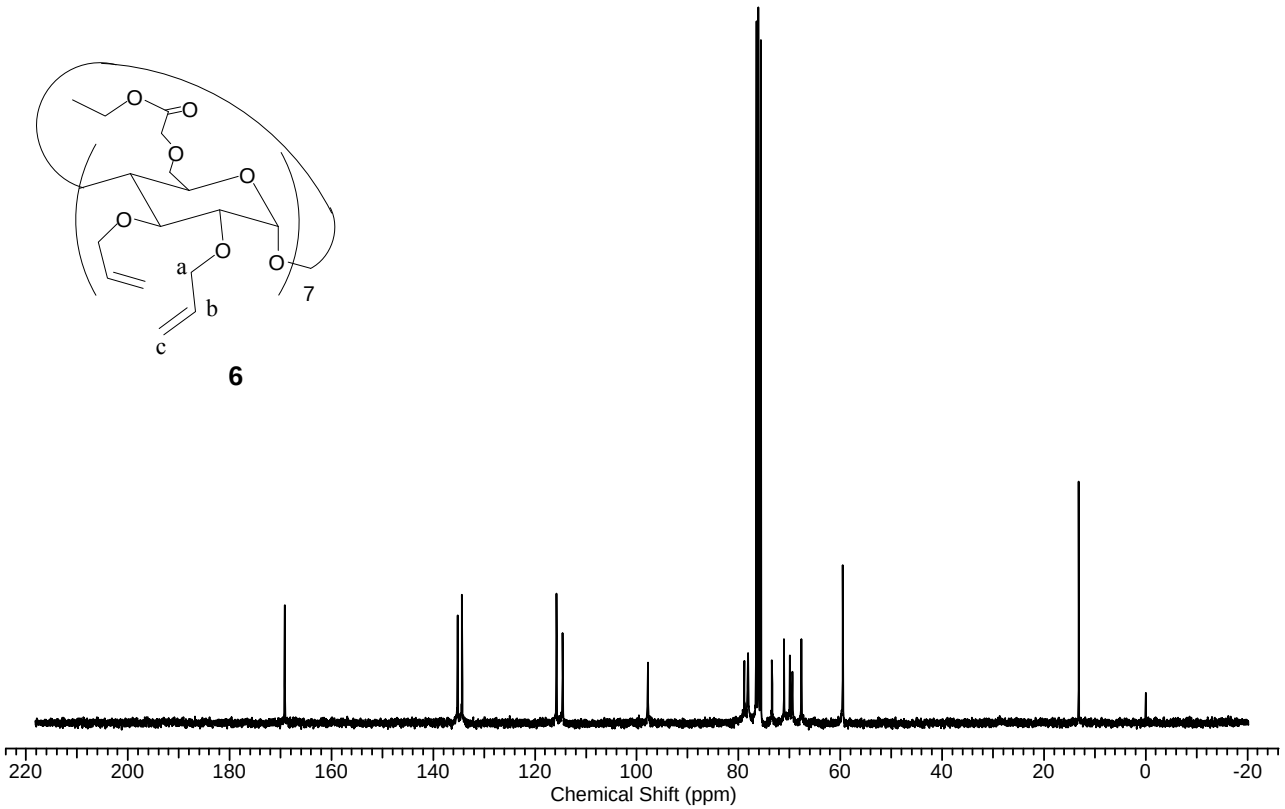
4.2.2 ^{13}C NMR spectrum of **5** ($CDCl_3$, 75 MHz)



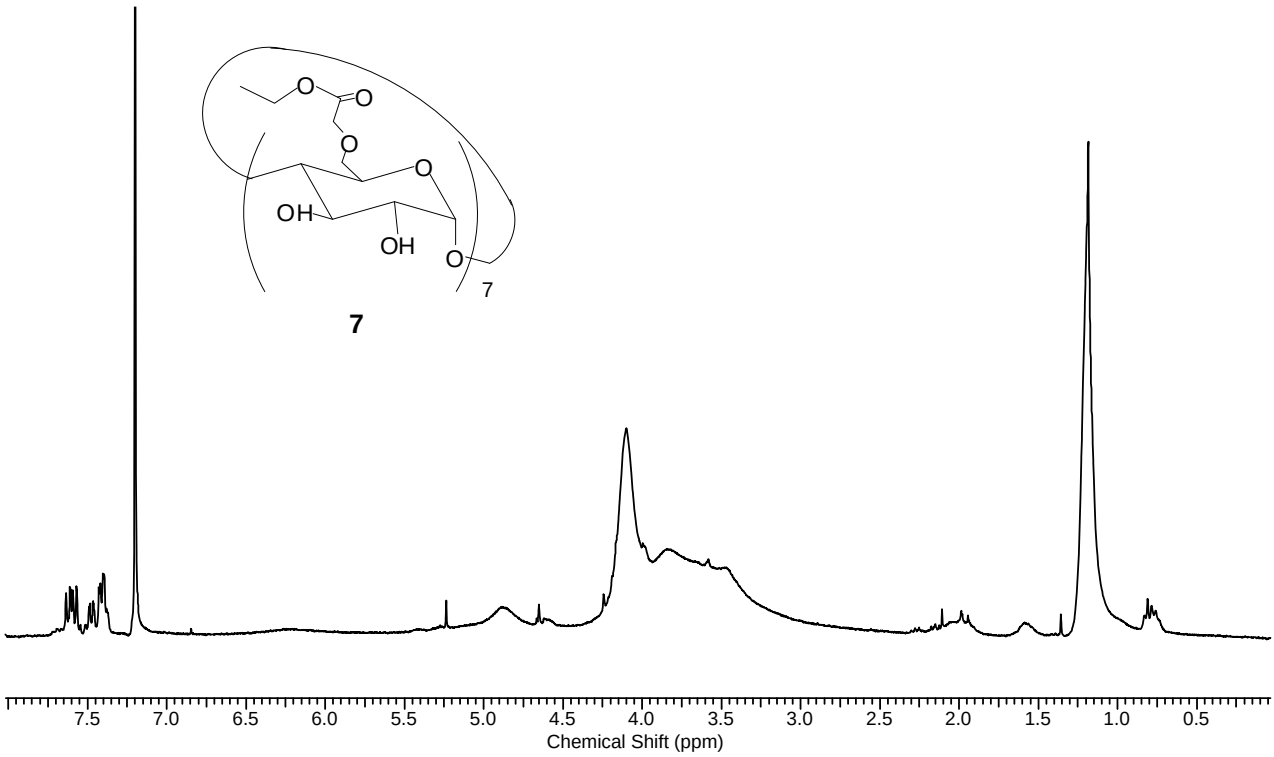
4.3.1 ^{1}H NMR spectrum of **6** ($CDCl_3$, 300 MHz)



4.3.2 ^{13}C NMR spectrum of **6** ($CDCl_3$, 75 MHz)



4.4.1 ^{1}H NMR spectrum of **7** ($CDCl_3$, 300 MHz)



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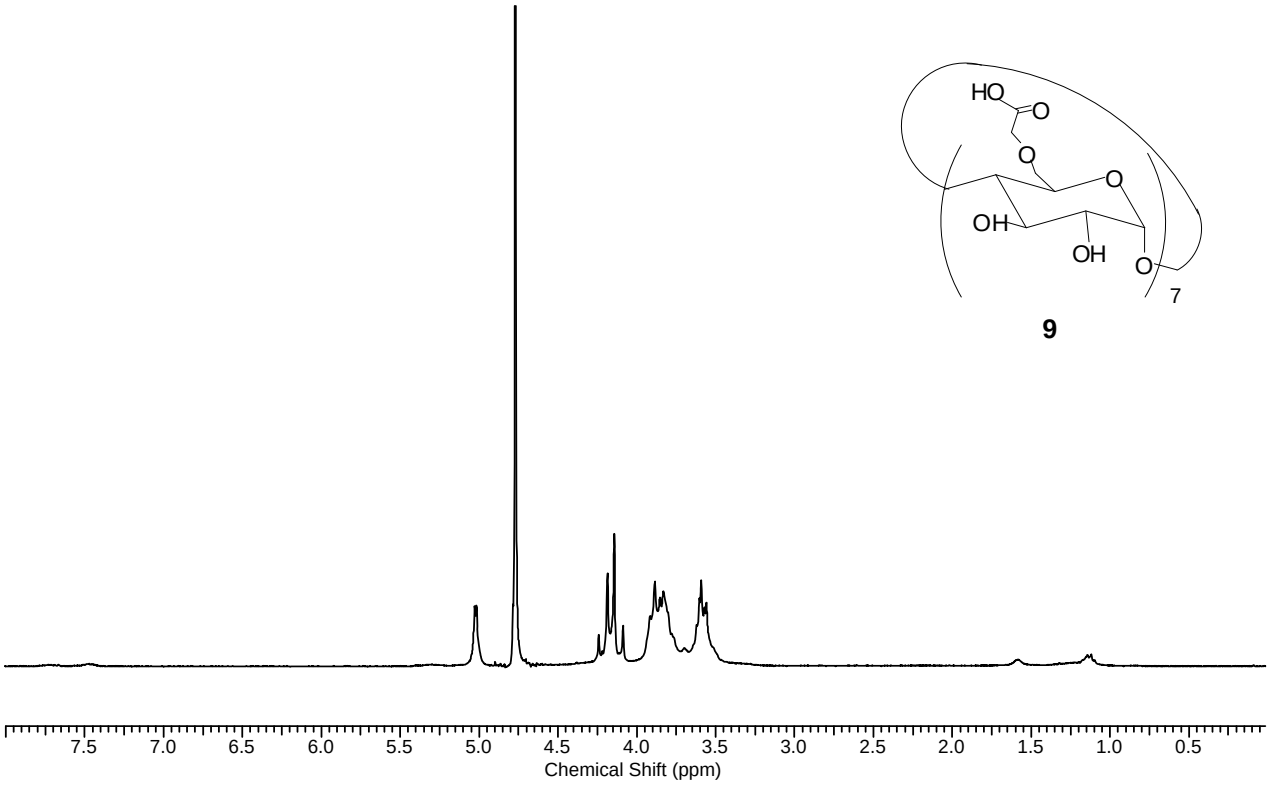
4.5.1 ^{1}H NMR spectrum of **9** (D_2O, 300 MHz)



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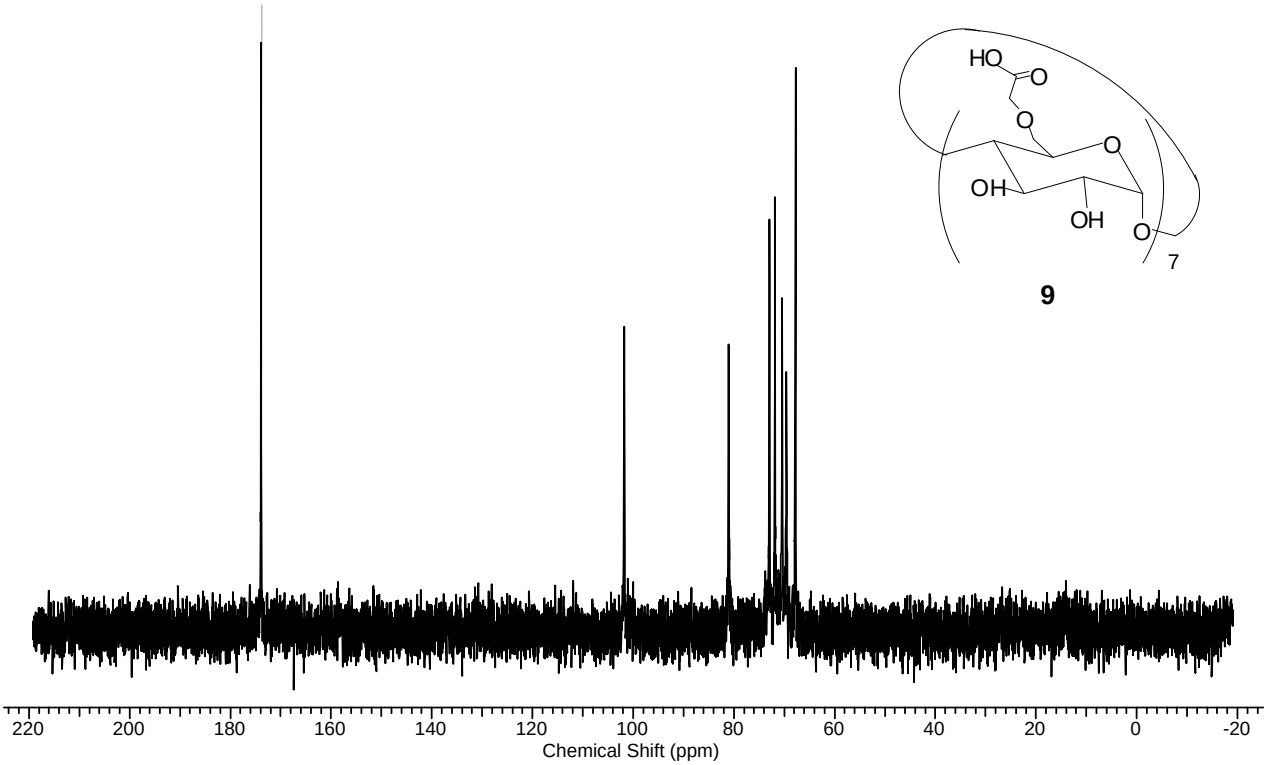
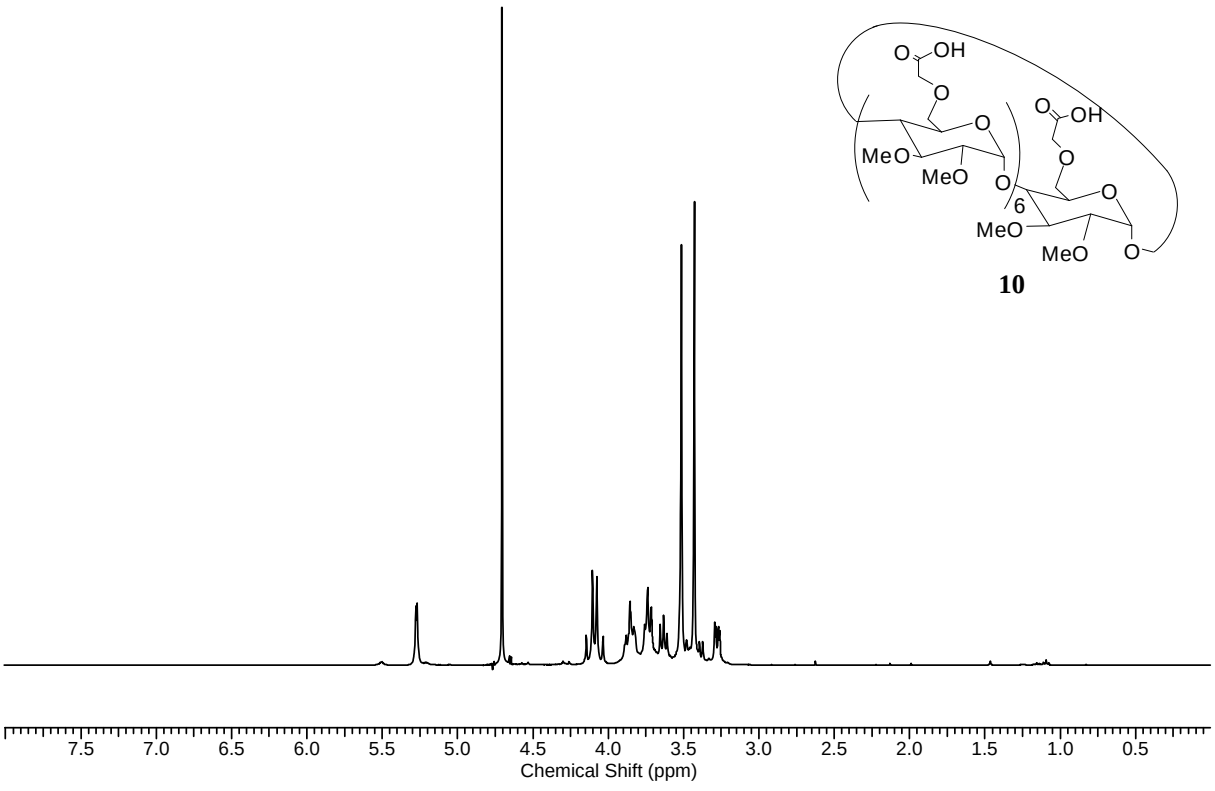
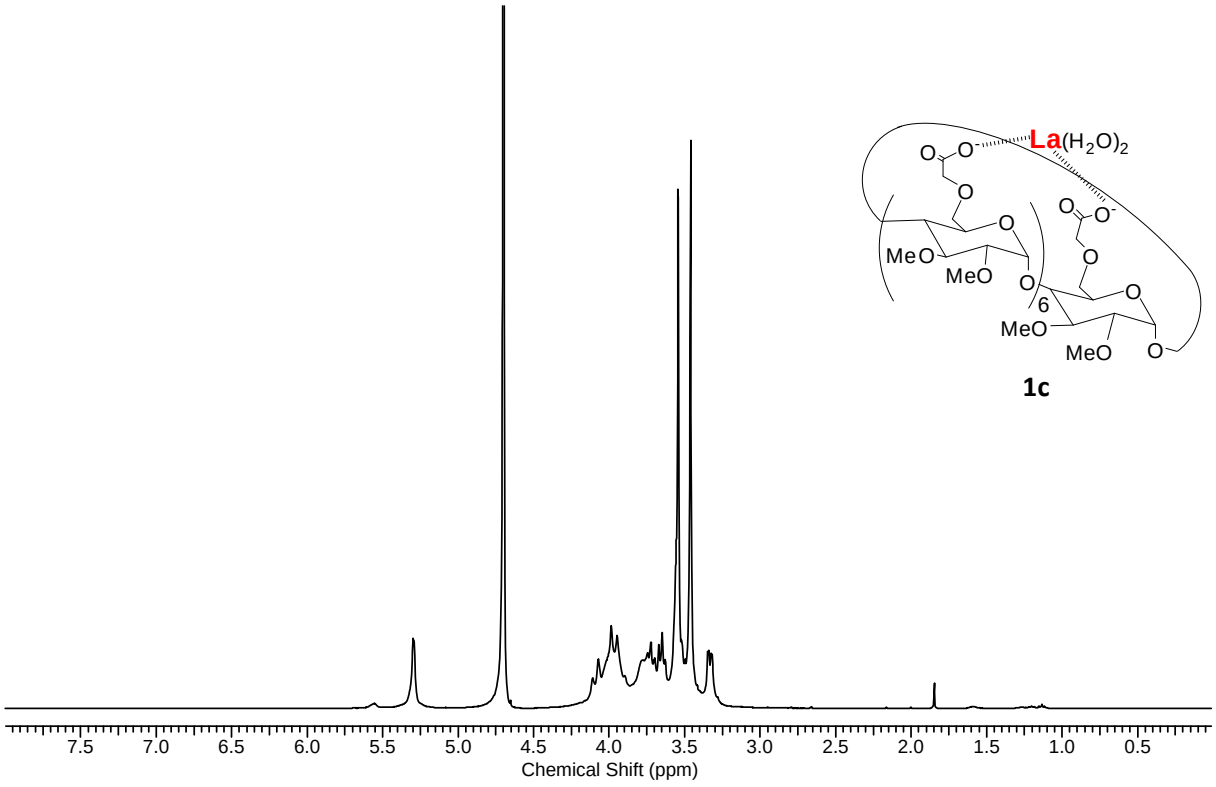
4.5.2 ^{13}C NMR spectrum of **9** (D_2O, 75 MHz)



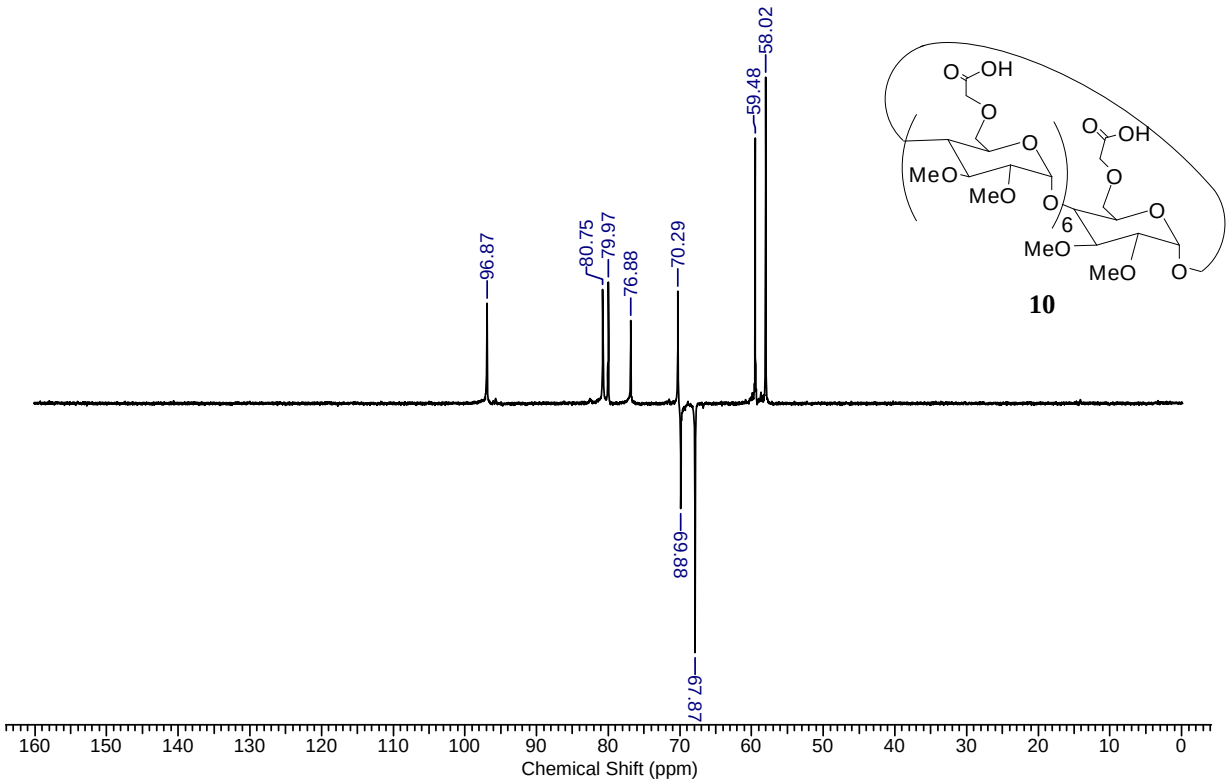
4.6.1 ^{1}H NMR spectrum of **10** (D_2O, 300 MHz)



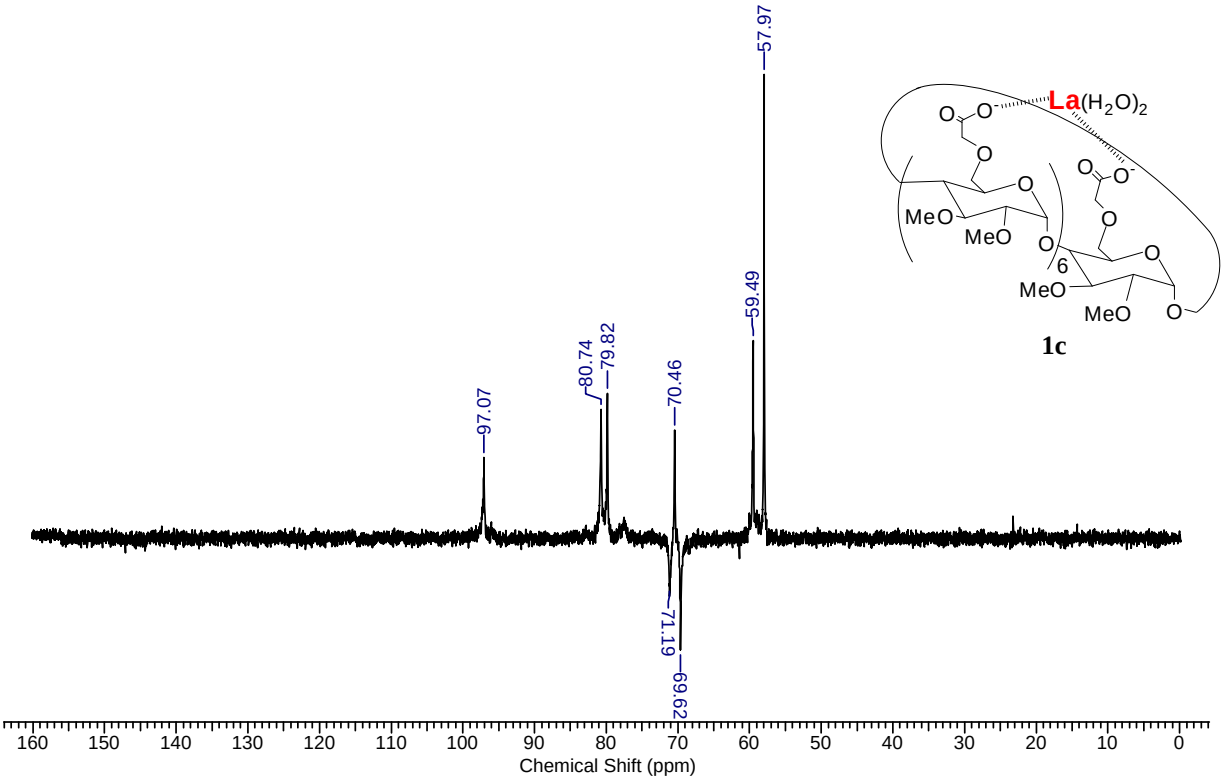
4.6.2 ^{1}H NMR spectrum of **1c** (D_2O, 300 MHz)



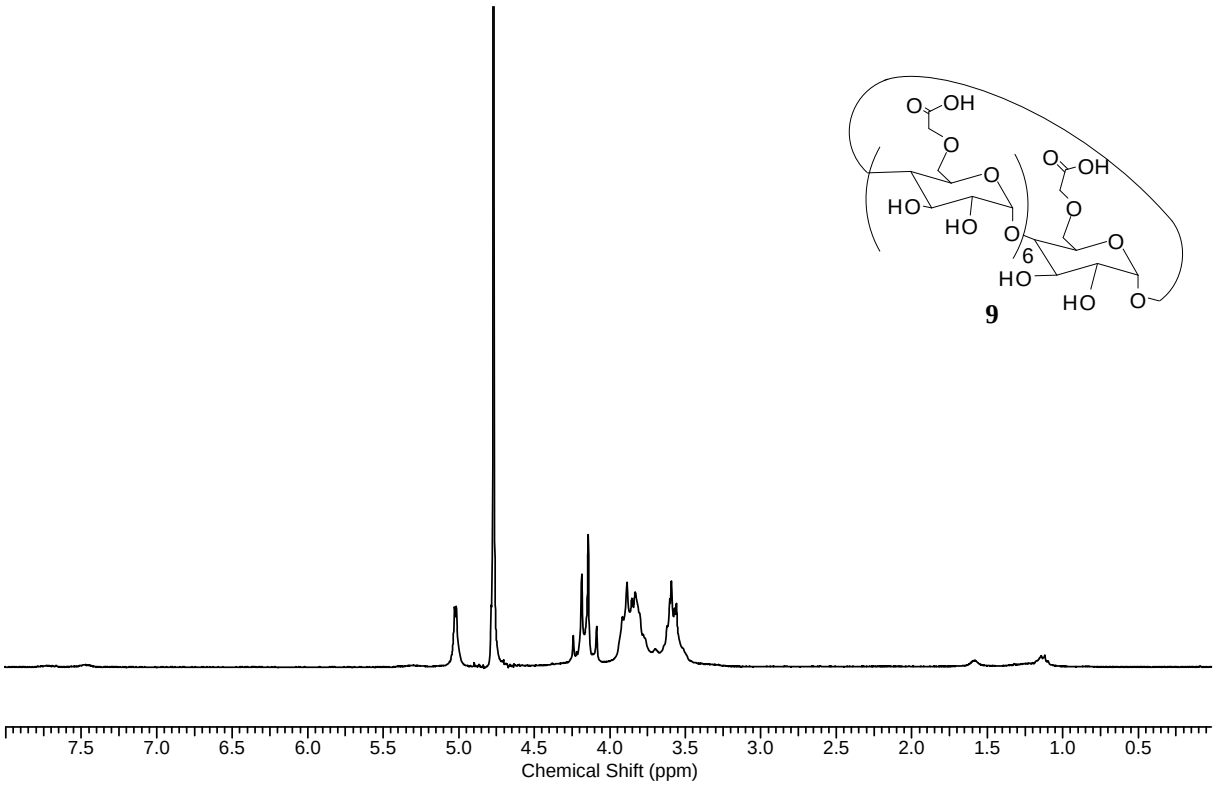
4.7.1 ^{13}C NMR Dept 135 spectrum of **10** (D_2O, 75 MHz)



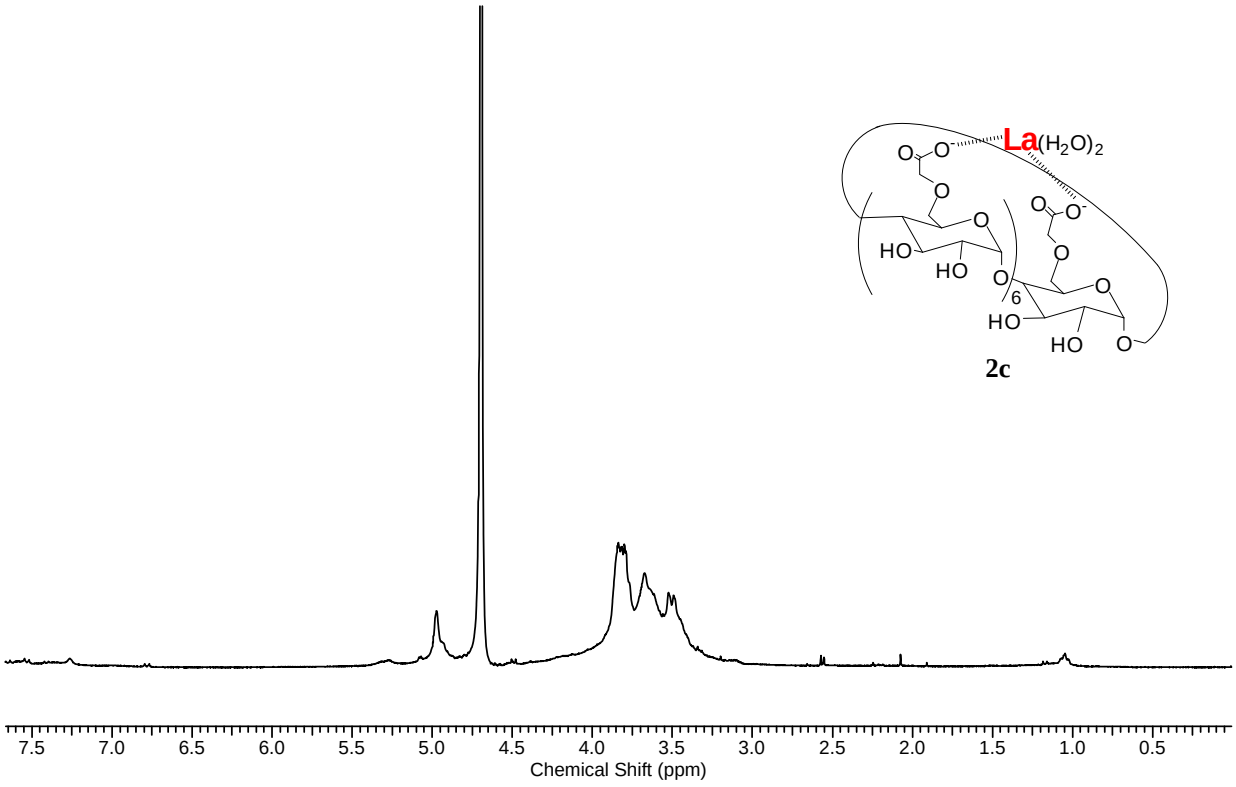
4.7.2 ^{13}C NMR Dept 135 spectrum of **1c** (D_2O, 75 MHz)



4.8.1 ^{1}H NMR spectrum of **9** (D_2O, 300 MHz)

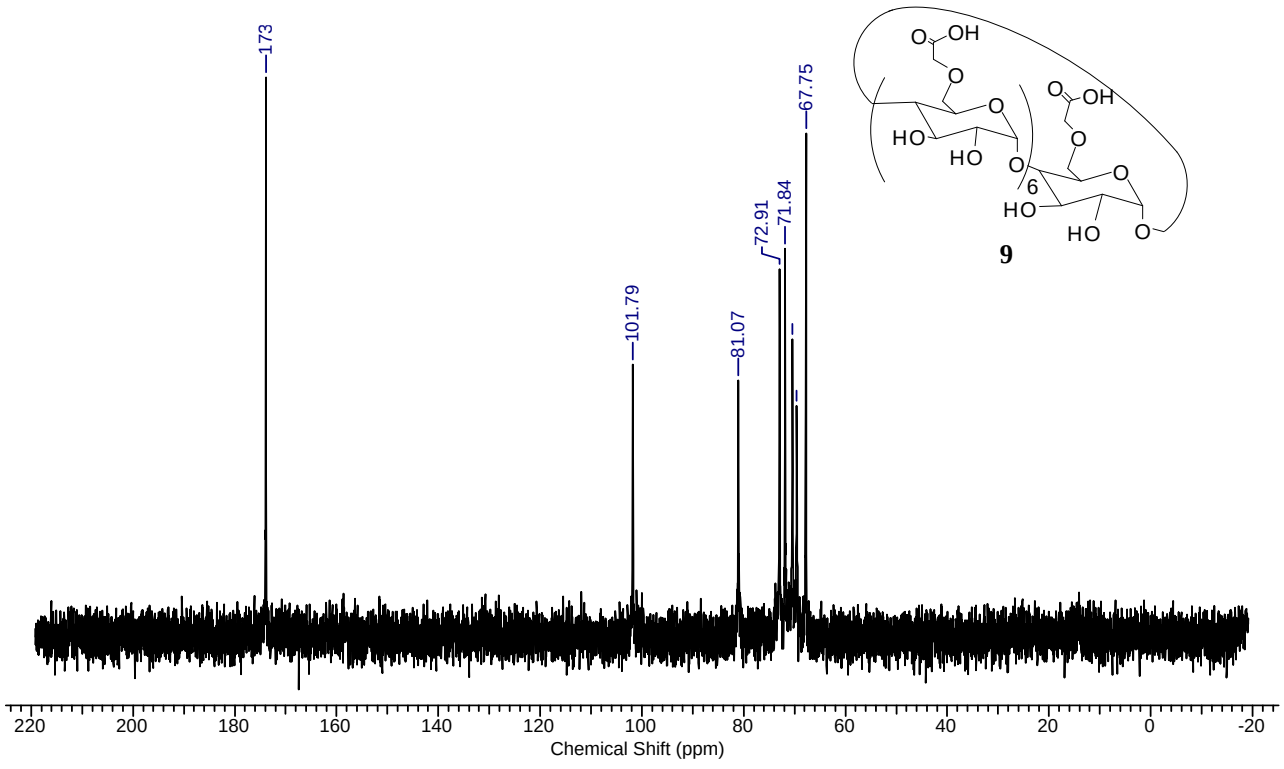


4.8.2 ^{1}H NMR spectrum of **2c** (D_2O, 300 MHz)



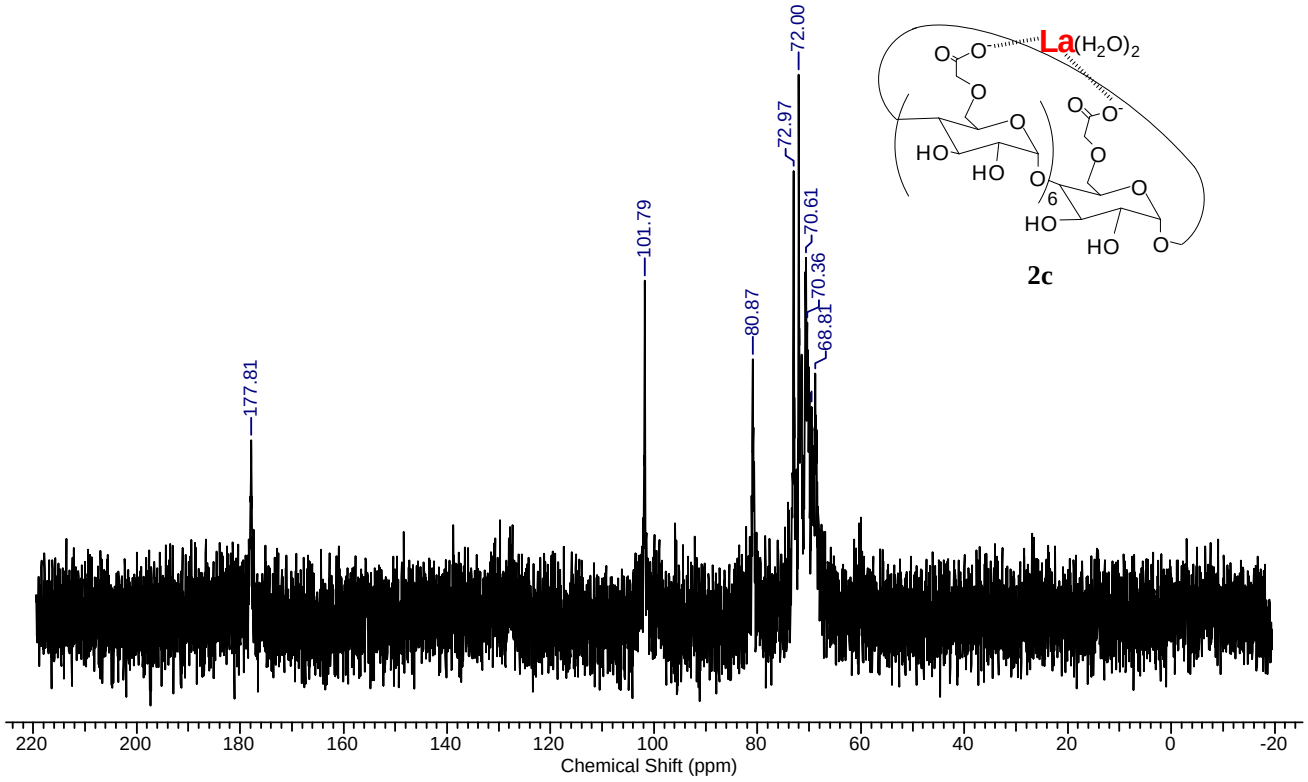
2c

4.9.1 ^{13}C NMR spectrum of **9** (D_2O, 75 MHz)



9

4.9.2 ^{13}C NMR spectrum of **2c** (D_2O, 75 MHz)



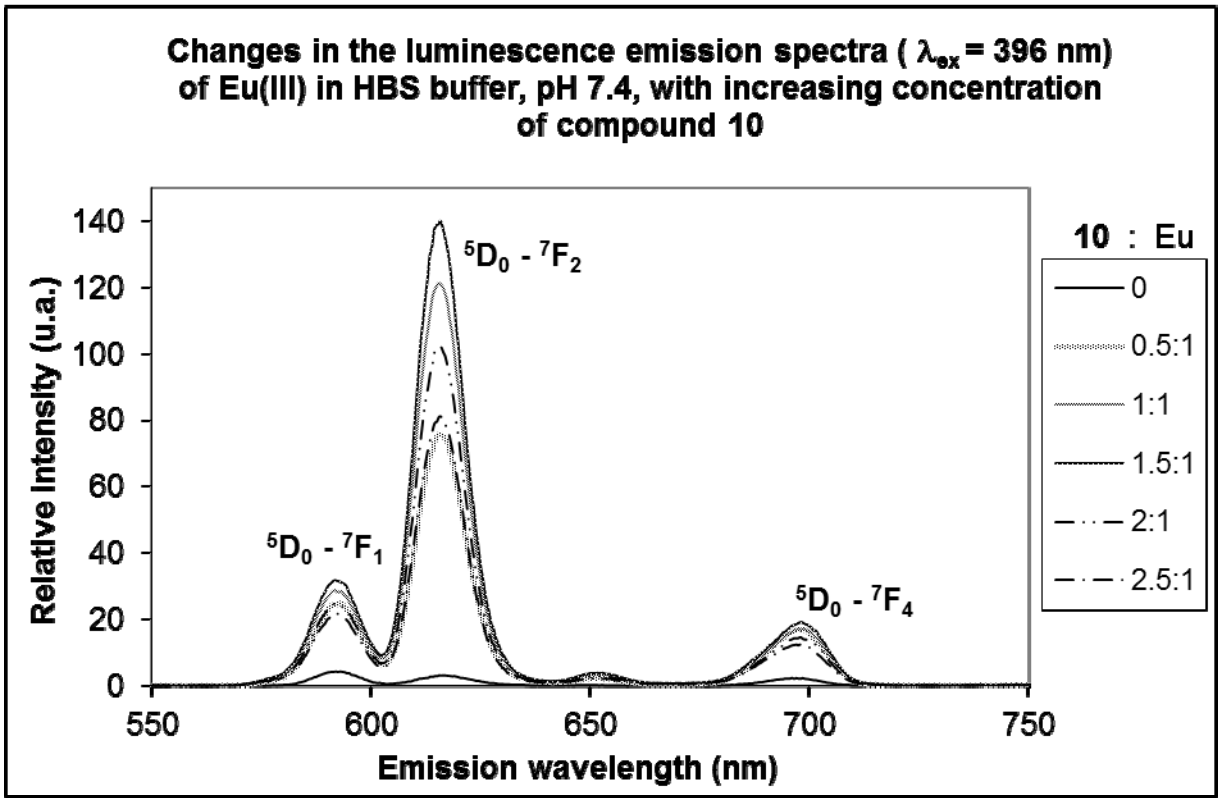
5. Luminescence analysis

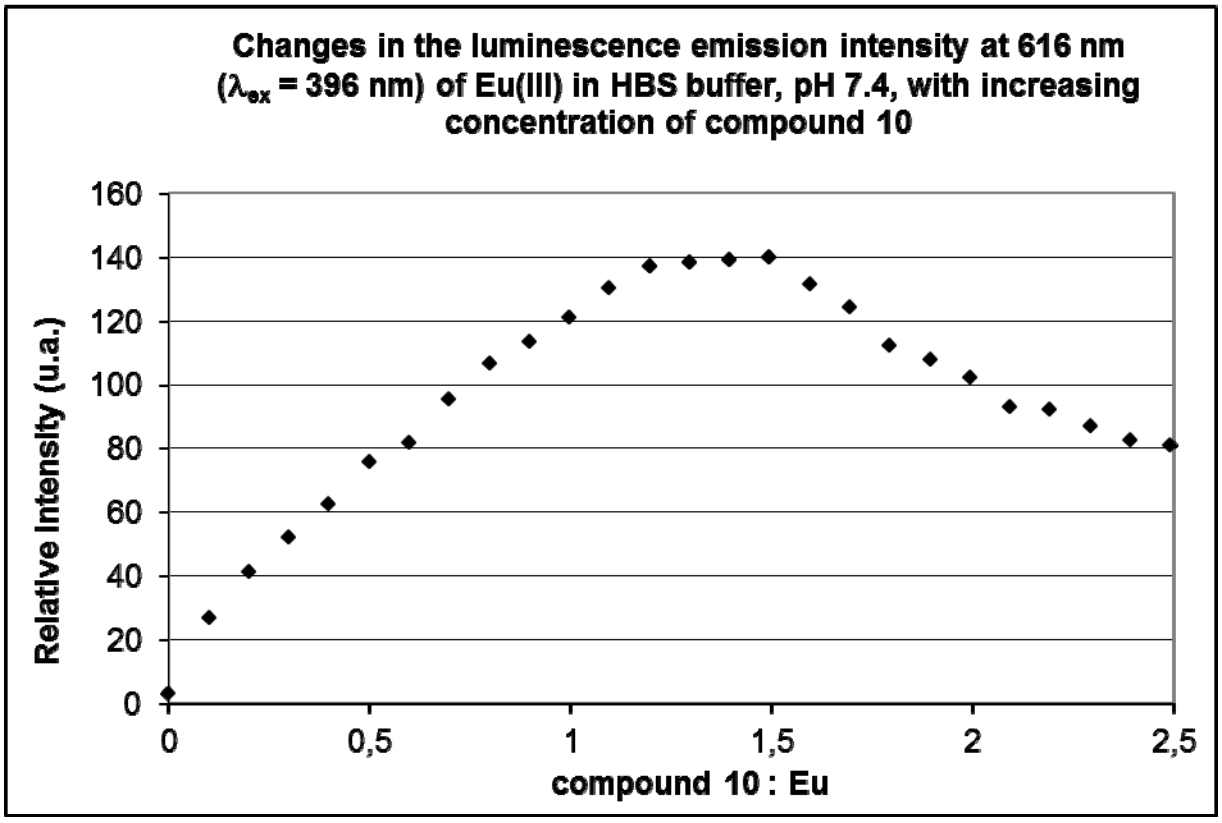
All chemicals were reagent-grade or higher. Milli-Q water was used for the solutions. Stock solutions were prepared by dissolving 99.9% $EuCl_3.6H_2O$ from Sigma-Aldrich in HBS buffer adjusting pH to 7.4.

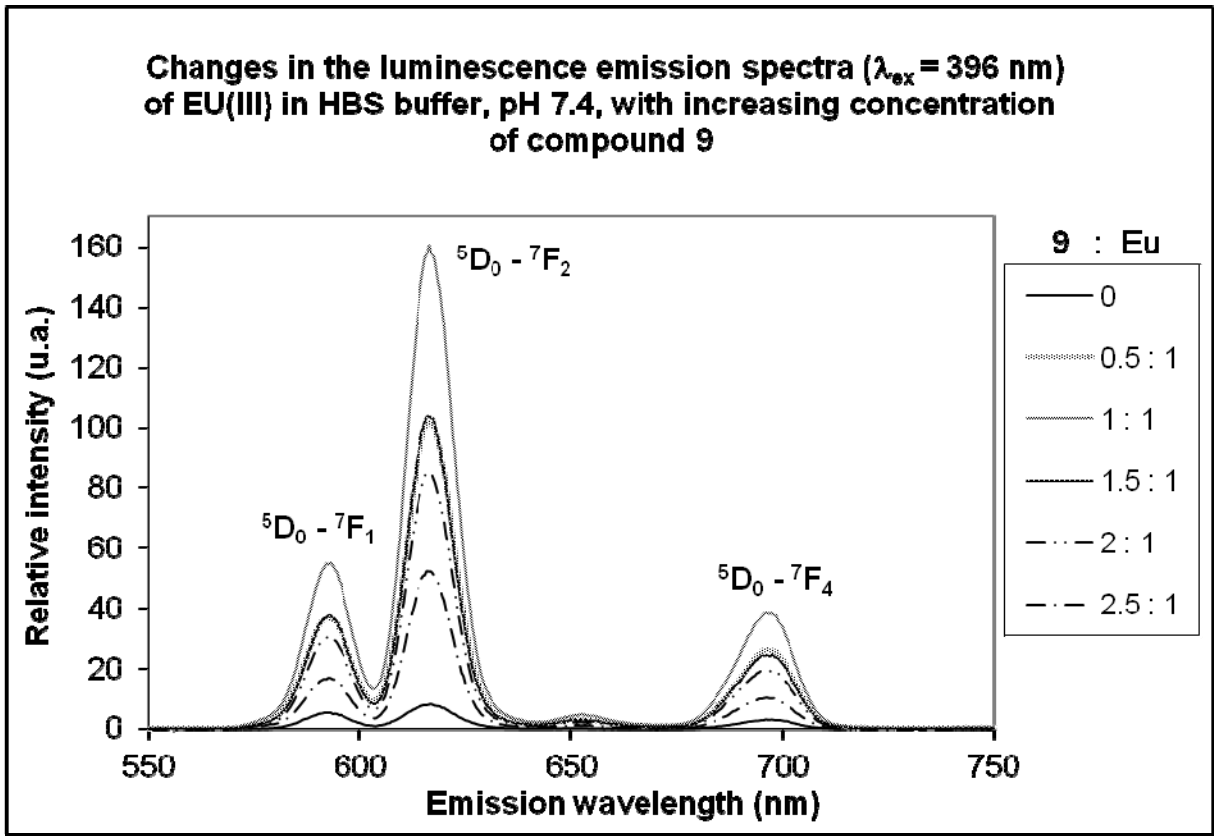
Spectrofluorimetric titrations were carried out by adding the ligands (10 µL aliquots of 29.90 mM solution) to a solution (1 mM) of $EuCl_3$ in HBS buffer (20 mM HEPES, 150 mM NaCl, pH 7.4). Overnight incubation time was sufficiently long to complete the complexation. 26 additions were carried out generating a set of 26 spectra for each batch of titration.

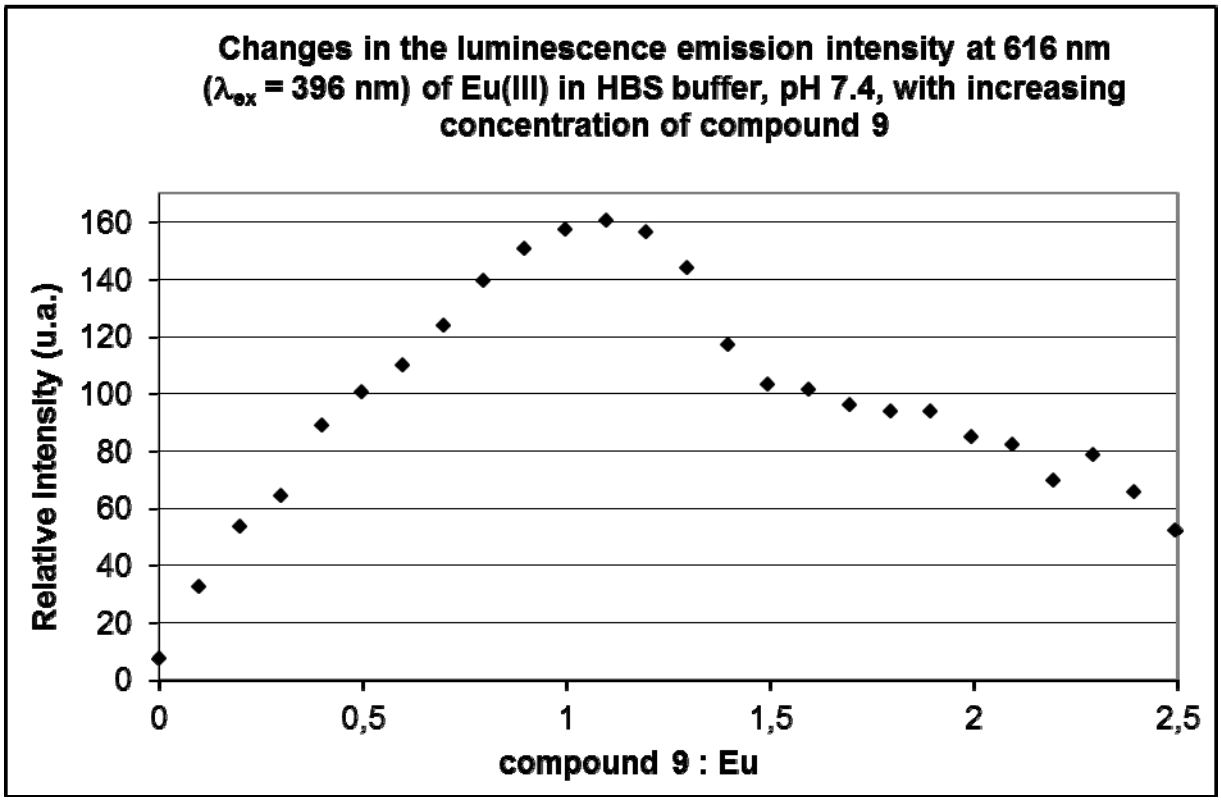
During the titration, the maximum intensity of the emission at 592 ($^5D_0$-$^7F_1$), 616 ($^5D_0$-$^7F_2$) and 698 nm ($^5D_0$-$^7F_4$) was monitored, the excitation wavelength being 396 nm for Eu(III). Corrections for dilutions were appropriately applied. Luminescence emission and phosphorescence spectra were recorded using a Varian Cary Eclipse Spectrofluorimeter equipped with a Xenon flash lamp source and a Hamamatsu R928 photomultiplier tube with quartz cell of 1 cm path length. A 5 ms total decay time, 0.1 ms delay time, 1 ms gate time,

one flash, with 10 nm excitation and emission slits were used for each measurement. Experiments were thermostated at 298K with a Peltier cell holder.









The spectra contain features originating from electronic transitions from the lowest excited state, $^5D_0$, to the ground-state manifold, ^7F_J (J = 0, 1, 2, 3…). By varying the ratio of **9** or **10**: Eu(III) from 0 to 2.5 to form **1b** or **2b** complexes respectively, the intensity of the total emission increased significantly. For the two complexes **1b** and **2b**, maximum of relative intensities were obtained for a ratio **9** or **10**: Eu(III) around 1:1. The relative intensities decreased with additional **9** and **10** aliquots.

The ratio of the integrated intensity of the $^5D_0$-$^7F_2$ transition ("hypersensitive transition") to the one of the $^5D_0$-$^7F_1$ (magnetic dipole transition, independent of the environment) is a measure for the symmetry of the coordination sphere (A.F. Kirby, F.S. Richardson, J. Phys. Chem 1983, 87, 2544). In a centrosymmetric environment, the above-mentioned ratio is < 1, while the distorsion of the symmetry around the metal center causes a ratio enhancement. The increase of this ratio indicated a perturbation of the primary coordination sphere of Eu(III) by **9** or **10**.

6. Hydration number[2]

Heptakis-(6-*O*-carboxymethyl)-β-cyclodextrin Europium **2b** or heptakis(6-*O*-carboxymethyl-2,3-di-*O*-methyl)-β-cyclodextrin Europium **1b** were diluted in H_2O or D_2O at equals concentrations of 9.45 mM (10 mM) and 10.2 mM (6.15 mM).

Measurements of the luminescence decay curves of the compounds studied were performed in H_2O and D_2O to evaluate the mean hydration state of these complexes in aqueous solution.

Lifetime measurements were recorded using a Varian Cary Eclipse spectrofluorimeter with quartz cell of 1 cm path length, operating in phosphorescence lifetime mode. In the case of compounds **1b** and **2b**, a 20 to 50 ms total decay time, 0.02 to 0.1 ms delay time, 0.05 to 0.4 ms gate time, one flash, with 10 to 20 nm excitation and emission slits were applied. Experiments were thermostated at 298K with a Peltier cell holder. Excitation and emission wavelength were fixed at 380 nm (394 nm) and 615 nm (614 nm) respectively. In all cases, the luminescence lifetimes of the pure complexes were monoexponential in both solvents at

[2] (a) Jr. Horrocks, W. DeW and D. R. Sudnick, *J. Am. Chem. Soc*., 1979, **101**, 334; (b) Jr. Horrocks, W. DeW and D. R. Sudnick, *Acc. Chem. Res.,* 1981, **14**, 384; (c) A. Beeby, I. M. Clarkson, R. S. Dickins, S. Faulkner, D. Parker, L. Royle, A. S. de Sousa, J. A. G. Williams and M. *J.* Woods, *Chem. Soc. Perkin Trans.,* 1999, *2*, 493; (d) R.M. Supkowski, W. DeW and Jr. Horrocks, *Inorg. Chim. Acta*, 2002, **340**, 44.

298 K. Table 1 summarizes the luminescence lifetimes of the pure compounds in H_2O and D_2O as well as the number of coordinated water molecules, q, of each compound. This number was calculated from the first decay rate constants of the excited state of the lanthanide compounds in H_2O and D_2O from the relation $q = A'\left(1/\tau_H - 1/\tau_D + f_{corr}\right)$ where either equals 1.2 ms^{-1} and the correction factor f_{corr} equals - 0.25 ms using the Parker relation or A'=1.11 ms^{-1} and f_{corr} = - 0.31 ms in case of Supkowski and Horrocks relation.

compounds	τ_H (ms)	τ_D (ms)	q^1	q^2
2b	0.33	1.33	2.43	2.18
1b	0.33	2.06	2.75	2.48

Table 1

Parker: $q^1 = 1.2 \times \left(1/\tau_H - 1/\tau_D - 0.25\right)$

Supkowski and Horrocks: $q^2 = 1.11 \times \left(1/\tau_H - 1/\tau_D - 0.31\right)$

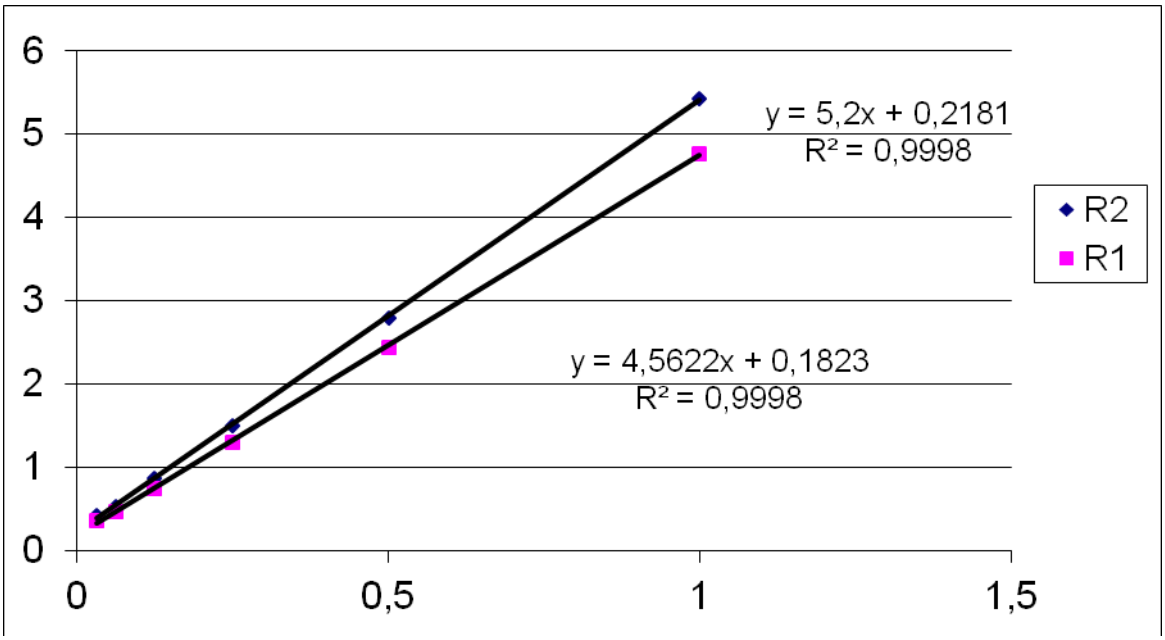
A hydration number q = 9 indicates that the nine coordination sites of the first coordination sphere of the lanthanide in solution are all occupied with molecules of water. Inversely, q = 0 indicates that no coordination site of the first coordination sphere of the Eu(III) is occupied with molecules of water. According to the values near 2, it seems reasonable to think that two water molecules are in the first coordination sphere and the seven carboxylate groups occupy a total of seven sites of coordination of the metal centre. As a typical hard acid cation, the interaction of Eu(III) with carboxylate functions in aqueous solution is expected to be ionic and strongly electrostatic.

7. MRI analysis

Heptakis-(6-*O*-carboxymethyl)-β-cyclodextrin gadolinium **2a** or heptakis(6-*O*-carboxymethyl-2,3-di-*O*-methyl)-β-cyclodextrin gadolinium **1a** were diluted (1, 0.5, 0.25, 0.125, 0.0625 mM) in TRIS buffer (10mM TRIS, 154 mM NaCl, pH 7.4). Compound **1a**/ guest and **2a**/guest were prepared in TRIS buffer. The guest was completely soluble in TRIS buffer after the addition of CA and stirred for 30 min. Water proton relaxation rates were measured on a minispec mq20 (Bruker, Germany) spectrometer operating at 20 MHz by plotting the curves of $1/\tau$ = f(concentrations). The temperature was kept at 37°C with a Julabo ED Heating Immersion Circulators (uncertainty ±0.1 °C).

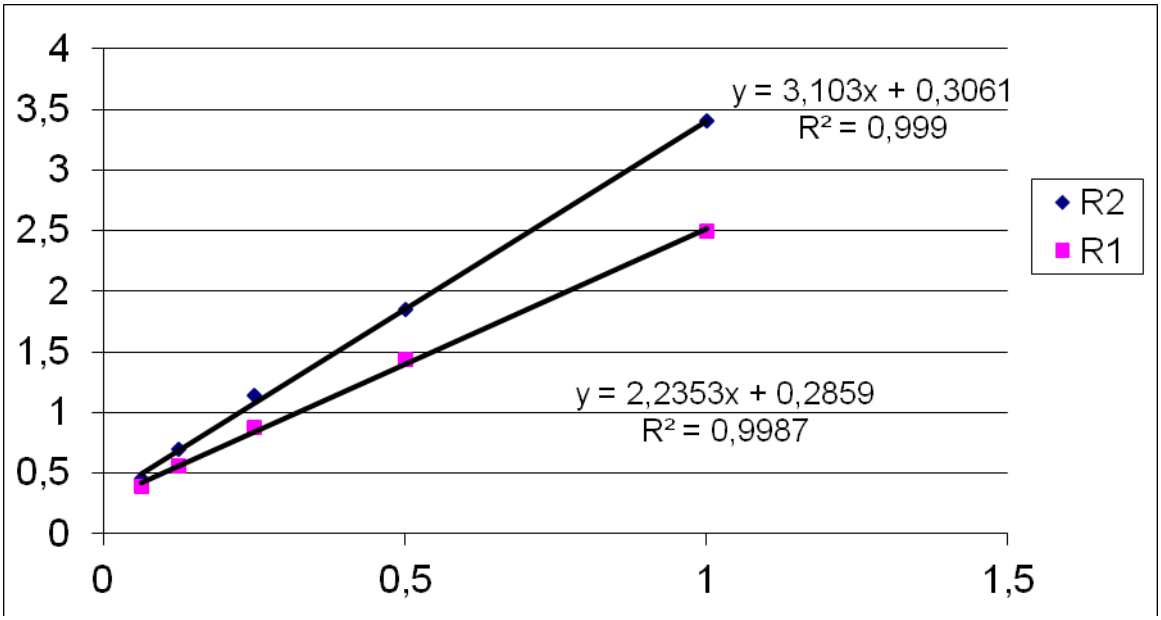
Water proton relaxation rates of aqueous solutions of compound **1a**

TRIS buffer (pH 7.4, 310 K, 0.47 T)



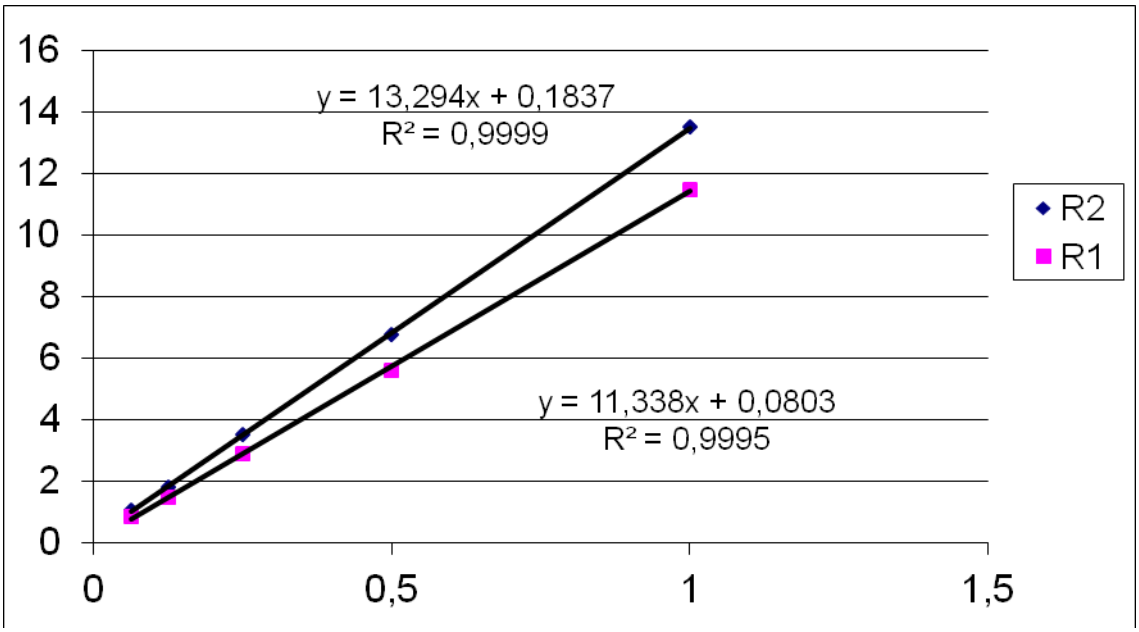
Water proton relaxation rates of aqueous solutions of compound **1a**

dioxane (pH 7.4, 310 K, 0.47 T)



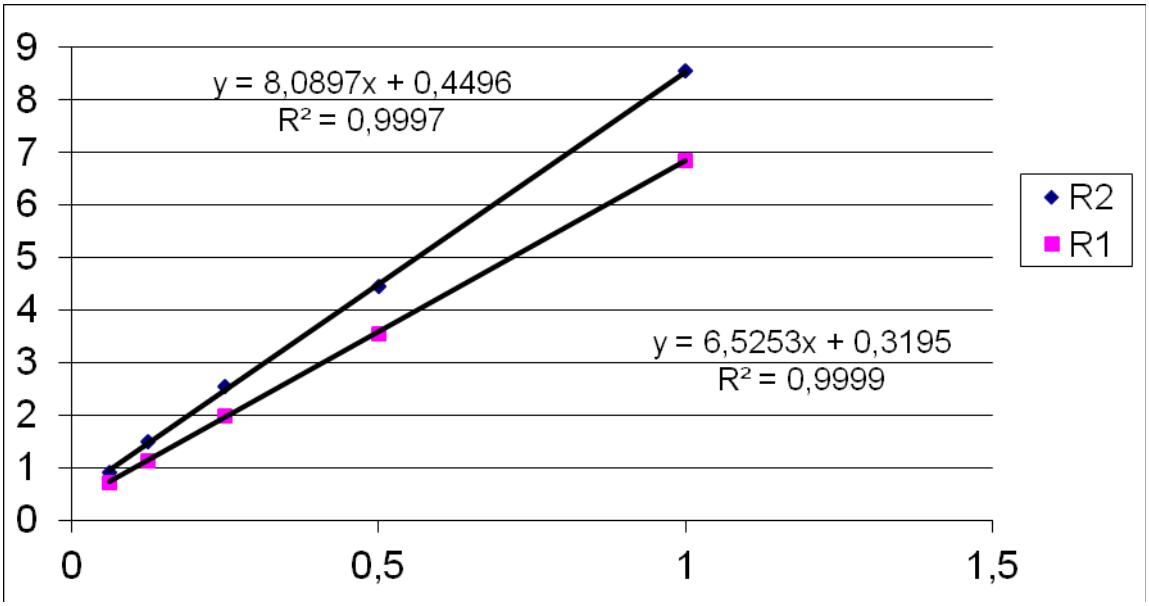
Water proton relaxation rates of aqueous solutions of compound **1a/guest**

TRIS buffer (pH 7.4, 310 K, 0.47T)



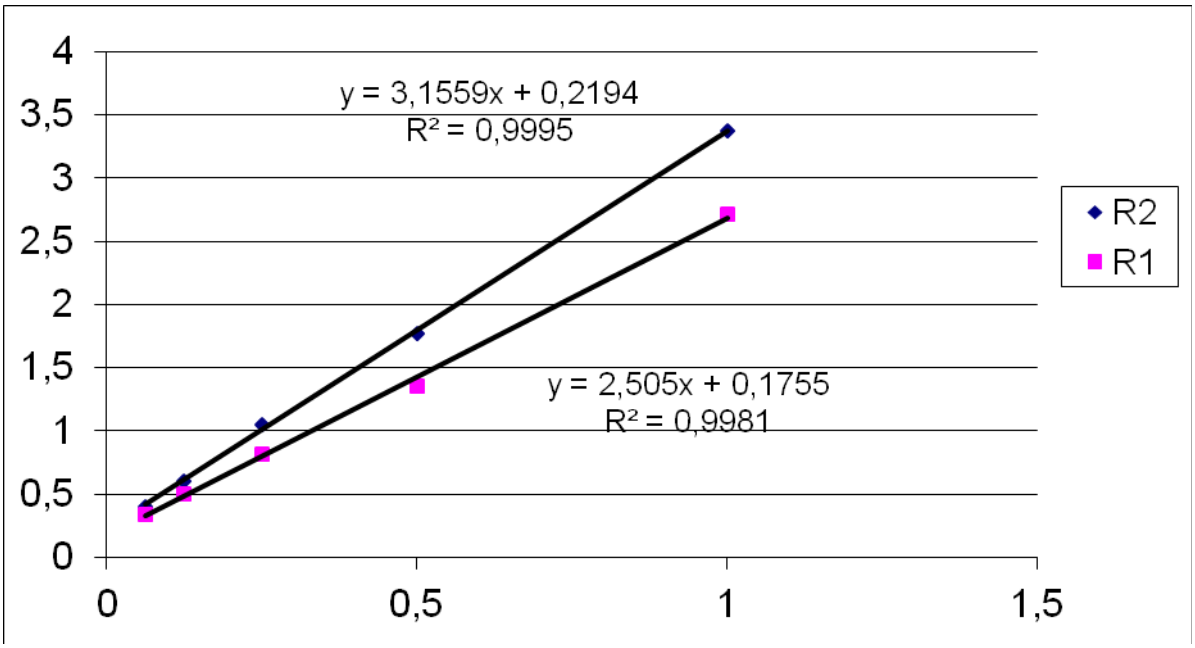
Water proton relaxation rates of aqueous solutions of compound **2a**

TRIS buffer (pH 7.4, 310 K, 0.47T)



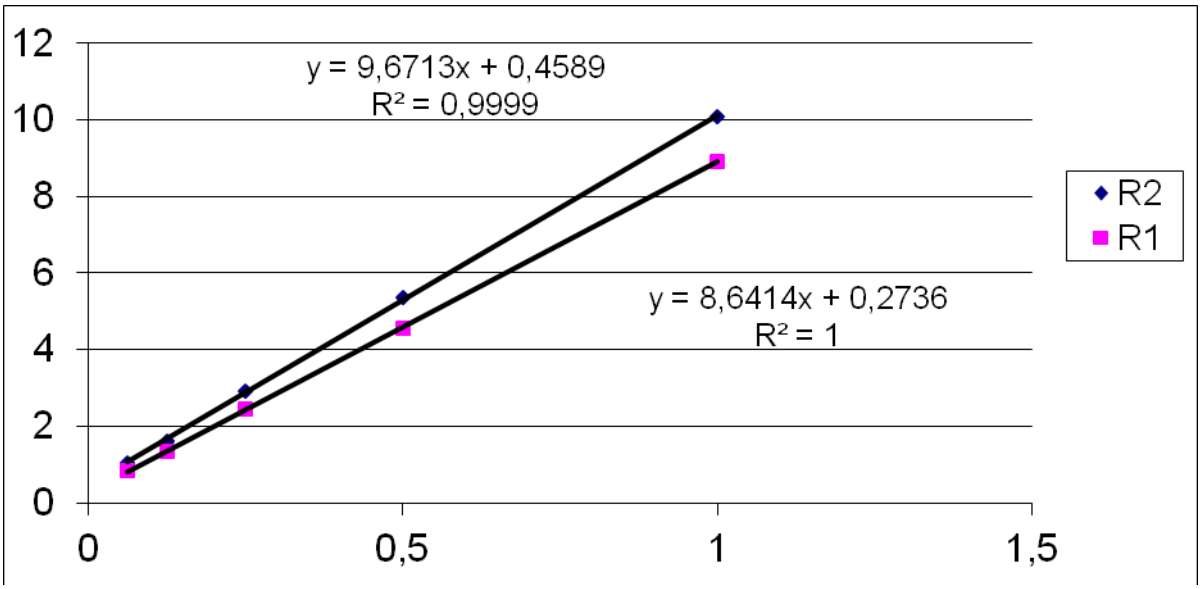
Water proton relaxation rates of aqueous solutions of compound **2a**

dioxane (pH 7.4, 310 K, 0.47 T)



Water proton relaxation rates of aqueous solutions of compound **2a/guest**

TRIS buffer (pH 7.4, 310 K, 0.47T)



MRI analysis in dioxane solution

Heptakis-(6-*O*-carboxymethyl)-β-cyclodextrin gadolinium **2a** (0.001 mmol, 1.67 mg) or heptakis(6-*O*-carboxymethyl-2,3-di-*O*-methyl)- β-cyclodextrin gadolinium **1a** (0.001 mmol, 1,89 mg) was added to a solution of dioxane (0.001 mmol, 1 mM) in Tris buffer (10 mM TRIS, 154 mM NaCl, pH 7.4). The mixture was diluted (1, 0.5, 0.25, 0.125, 0.0625 mM) in TRIS buffer. Water proton relaxation rates were then measured.

8. Titration of free metal in solution for complexes 1a and 2b

The amount of free residual Gd(III) was measured by complexometric titration with EDTA solution in presence of xylenol orange as indicator.

Preparation of buffer: Acetic acid (5.75 ml) was dissolved in water (700 ml) and pH was adjusted to 5.8 with NaOH. The volume of the solution was then adjusted to 1 liter with distilled water.

Preparation of indicator solution (Solution A): Xylenol orange (30 mg) was dissolved in acetic buffer at pH 5.8 (100 ml).

Titration procedure: Aliquots of **1a** (56.73 mg, 3.10^{-5}mol) or **2b** (57.78 mg, $3.4.10^{-5}$ mol) were dissolved in a mixture of acetic buffer (10 mL) and distilled water (20 mL) (solution B). A few drops of solution A were added until the solution B became violet. The solution B was then titrated with EDTA (0.001 M) using a burette in steps of 0.05 ml.