Click inspired Synthesis of Hexa and Octadecavalent Peripheral Galactosylated Glycodendrimer and their Possible Therapeutic Applications

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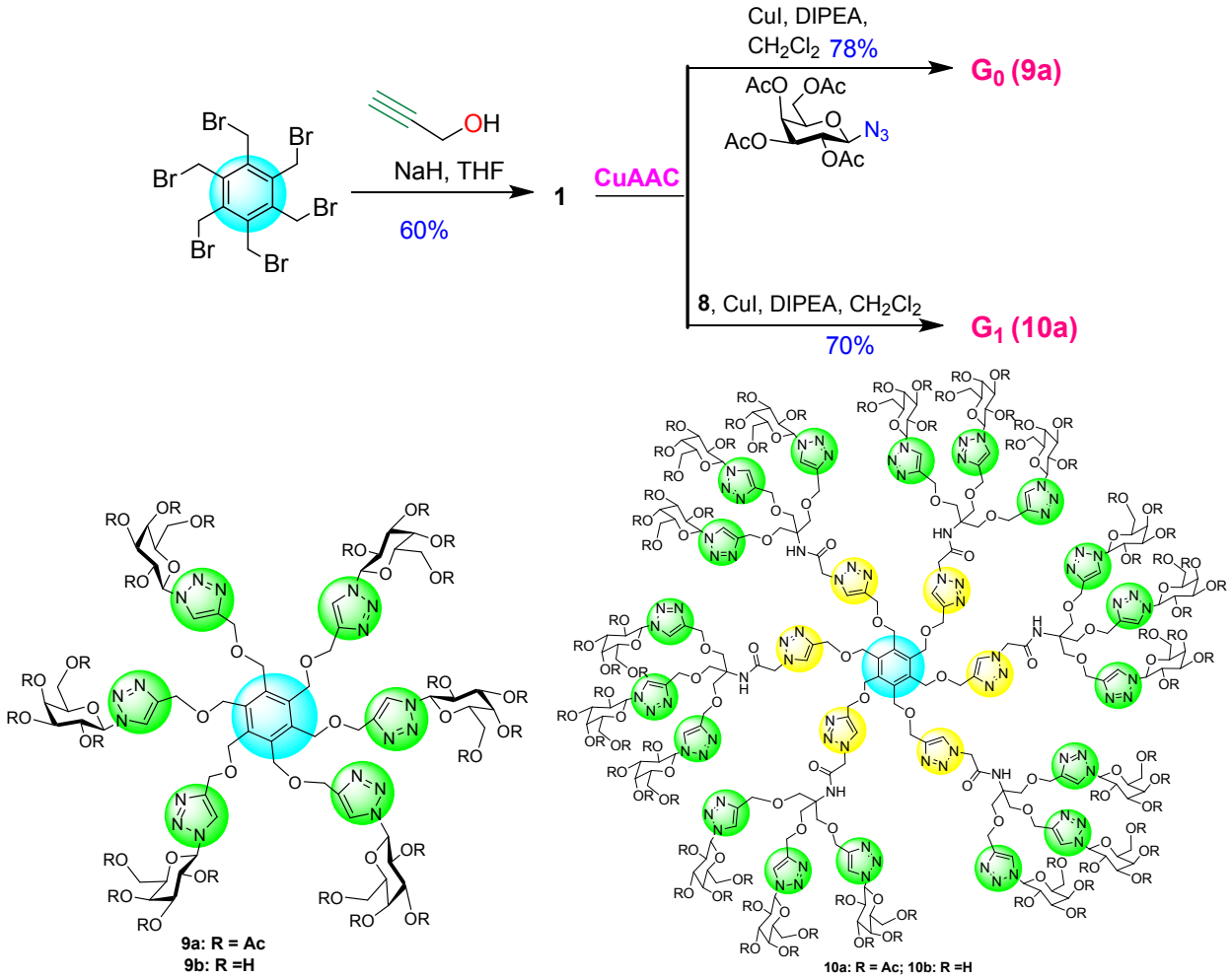
Experimental Section

1. General

All reagents and solvents were used are pure analytical grade. All the reactions were carried out under argon atmosphere (except such reactions where water was used as solvent). Thin-layer chromatography (TLC) was accomplished on 60 F254 Silica gel pre-coated aluminum plates and visualized under UV lamp Violet lamp (λ max = 254 nm) or iodine vapor or charring after 5% H_2SO_4-MeOH solution spray or alkaline $KMNO_4$ (for alkyne) as color reagent. Column chromatography (SiO_2) was performed on silica gel (230-400 mesh, Merck) by using distilled *n*-hexane, ethyl acetate and commercially available dichloromethane and methanol. Solvents were evaporated under reduced pressure <50^{0}C. Mass spectra were recorded by electron spray ionization mass spectrometry (ESI-MS); MALDI-TOF/TOF mass spectra were obtained from ABSCIEX TOF/TOFTM 5800 mass spectrometer. IR spectra were recorded as Nujol mulls in KBr pellets. ^{1}H and ^{13}C NMR were recorded at 500 MHz (^{1}H) and 125 MHz (^{13}C), respectively. Chemical shifts were given in ppm downfield from internal TMS; *J* values in Hz. Single-crystal X-ray data collected on CCD-diffractometer.



Scheme S1. Overall synthetic plan for the 0th generation and 1st generation glycodendrimers

2. Synthetic procedure and characterization of dendrons and glycodenrimers

Synthesis of 1,2,3,4,5,6-hexakis((propargyloxy)methyl)benzene (1).

To a stirring solution of propargyl alcohol (455 µl, 7.9 mmol) in anhydrous THF (10 mL), NaH (0.377 g, 15.7 mmol) was added slowly under an inert atmosphere at 0°C followed by addition of hexakis(bromomethyl)benzene (0.5 g, 0.79 mmol). The reaction was continued for 12 h at the same reaction condition. After completion of the reaction (monitored by TLC), the reaction mixture was quenched by adding methanol and the solvent was evaporated under reduced pressure and resulted residue was extracted with ethyl acetate (2 x 75 mL), washed with water (2 x 20 mL). The organic layer was separated, dried over anhydrous Na_2SO_4 and evaporated under vacuo. Crude mass thus obtained was subjected to column chromatography to afford compound **1** in pure form as light yellow crystalline solid. Yield (0.229 g, 60%); R_f = 0.5 (30% ethyl acetate/*n*-hexane), m.p. = 112-114 °C; MS: m/z = 487 $(M+H)^+$; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.79 (s, 12H, OCH_2Ar), 4.24-4.23 (m, 12H, OCH_2-alkyne), 2.50-2.49 (m, 6H, C(sp)H alkyne); ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.0, 80.0, 74.8, 65.1, 57.6 ppm; IR (KBr): ν_{max} 3288.52, 3238.43, 2925.01, 2886.41, 2857.13, 2130.02, 2107.60,1621.80, 1440.88, 1342.66, 1083.98 cm^{-1}.

Synthesis of 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl azide (2).[1]

Per-*O*-acetylated D-galactose (5.0g, 12.8 mmol) was taken in anhydrous dichloromethane and temperature was maintained at 0˚C. HBr (33%) in acetic acid (12 mL) was added slowly under the anhydrous condition in the reaction mixture and it was stirred for 3-4 h at (0 to 10˚C). After completion of the reaction (monitored by TLC), the mixture was diluted with DCM and neutralized by sodium bicarbonate and extracted with dichloromethane. The organic layer was collected and dried over anhydrous sodium sulfate and evaporated to obtain per-*O*-acetylated galactose bromide. Furthermore, anomeric bromide (4.0 g, 9.7 mmol) as the crude product was dissolved in anhydrous DMF followed by reaction with sodium azide (1.89 g, 29.2 mmol) at 80˚C for 5 h. After completion of the reaction (monitored by TLC), the solvent was evaporated and extracted with EtOAc and washed with cold water two times, following evaporation of organic layer to obtain the crude mass. Then after, column chromatography was performed to afford the pure galactosylated azide. White crystalline solid, yield (2.68 g, 74%); R_f = 0.5 (40% ethyl acetate/*n*-hexane);

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.417-5.413 (m, 1H, H_4), 5.16-5.12 (m, 1H, H_2), 5.05-5.02 (m, 1H, H_3), 4.61 (dd, J = 8.5, J = 2.0, 1H, H_1), 4.17-4.15 (m, 2H, $H_{6a,b}$), 4.03-4.00 (m, 1H, H_5), 2.17-2.12 (m, 3H, $COCH_3$), 2.09-2.04 (m, 6H, $COCH_3$), 1.99-1.98 (m, 3H, $COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1, 169.8, 169.7, 169.1, 88.02, 72.6, 70.5, 67.9, 66.7, 61.0, 20.4, 20.3 and 20.2 ppm.

***N*-(*tert*-Butyloxycarbonyl)tris(hydroxymethyl)aminomethane (4)**.[2]

A Suspension of tris(hydroxymethyl)aminomethane (10 g, 82.5 mmol) was made with 1:1 mixture of *t*-BuOH-MeOH (150 mL).Then after, a mixture of *t*-BuOH (100 mL) and di-*tert*butyl dicarbonate (23.4g, 107.3 mmol) was added to the above suspension and reaction mixture was stirred for 24 h at room temperature. The solvent was evaporated under low pressure to obtain a residue and refined by precipitation with cold ethyl acetate. The Precipitate was filtered by Buchner funnel to afford the refined product as white solid. Yield: (16.6 g, 91%); R_f = 0.3 (5% methanol/DCM); ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.70 (s, 1H, CH_3), 4.55 (s, 3H, OH), 3.46 (s, 6H, CqCH_2O), 1.31 (s, 9H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 155.3, 78.2, 60.7, 60.4, 28.4 ppm.

***N*-(*tert*-Butyloxycarbonyl)tris[(propargyloxy)methyl]aminomethane (5).[3]**

Compound **4** (5.0 g, 22.5 mmol) was dissolved in dry DMF (50.0 mL) and stirred at 0˚C with propargyl bromide (7.7 mL, 64.7 mmol). Finely Powdered KOH (5.7 g, 101.6 mmol) was added portion wise in the reaction mixture over a period of time 15 min. The reaction was heated at 35˚C and stirred under Argon ambient for 24 h. After completion of the reaction (monitored by TLC), ethyl acetate was added to the mixture and washed three times with water (3x60 mL), the organic layer was collected and dried over anhydrous sodium sulfate, and evaporated to dryness to obtain the crude product. Further, the crude compound was purified by column chromatography (SiO_2, 10% EtOAc in *n*-hexane) to afford compound **5**. Yellow oil, yield: (4.85 g, 64%); R_f = 0.6 (15% ethyl acetate/*n*-hexane);^{1}H NMR (500 MHz, $CDCl_3$): δ 4.92 (s, 1H, NH), 4.15 (d, J = 3.0 Hz, 6H, OCH_2-alkyne), 3.78 (s, 6H, CqCH_2O), 2.45 (m, 3H, C(sp)-*H*), 1.42 (s, 9H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 154.5, 79.4, 74.5, 68.7 58.4, 57.9, 28.2 ppm.

2-Chloroacetamide-tris[(propargyloxy)methyl]aminomethane (6).[3]

Compound **5** (3.0 g, 8.94 mmol) was dissolved in anhydrous DCM (35 mL) and cooled at 0˚C. Trifluoroacetic acid (12 mL, 156.8 mmol) was added dropwise using a syringe and stirred for 3h at r.t. The reaction mixture was then co-evaporated with toluene (4 x 15.0 mL), the residue obtained from the evaporation without purification was taken in R.B. already containing anhydrous dichloromethane (15 mL) and cooled at 0˚C. DIPEA (4.0 mL, 22.9 mmol) was added to the R.B. followed by addition of chloroacetyl chloride (1.77 mL, 22.3 mmol) solution in DCM (30 mL), the reaction mixture was stirred for the next 15 h under Argon atmosphere at room temperature. The resulting solution was washed with 0.5M hydrochloric acid and water (4 x 60.0 mL), the non-aqueous layer was collected and evaporated to obtain the crude product. The resulting crude was purified by column chromatography to afford as light yellow Oil. Yield : (1.70 g, 61%); R_f = 0.4 (30% ethyl acetate/n-hexane); ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.80 (s, 1H, NH), 4.16 (d, *J* = 2.0 Hz, 6H, OCH_2alkyne), 3.94 (s, 2H, $ClCH_2$), 3.82 (s, 6H, $CqCH_2O$), 2.42 (m, 3H, C(sp)-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.9, 79.4, 74.9, 68.1, 59.6, 58.7, 42.9 ppm.

Synthesis of chlorine functionalized first generation dendron 7: Compound **6** (0.2 g, 0.64 mmol) and galactose azides (0.84 g, 2.24 mmol) were dissolved in dichloromethane. CuI (0.11, 0.57 mmol) and DIPEA (101µL, 0.577 mmol) were added to the reaction mixture and stirred at r.t. for the 12h, the disappearance of the compound **6** (monitored by TLC) inferred the completion of the reaction. Furthermore, the reaction mixture was passed through cellite, obtained filtrate was taken in separating funnel, then after DCM (30 mL) was mixed and washed with water (2 x 30 mL). The organic layer was collected and dried over anhydrous sodium sulfate, the solvent was evaporated to give a residue which was refined by Flash column chromatography (2% methanol/DCM) to obtain compound **7** as Yellow solid. Yield: (0.71 g, 78%); R_f = 0.4 (3% methanol/DCM); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.83 (s, 3H, Triazolyl-H), 6.83 (s, 1H, NH), 5.88 (d, *J* = 9.5 Hz, 3H, H_4), 5.58-5.51 (m, 6H, H_2, H_3), 5.26-5.24 (m, 3H, H_1), 4.61 (d, *J* = 8.0 Hz, 6H, $OCH_2CH{=}CH$), 4.27-4.24 (m, 3H, H_{6a}), 4.15 (d, *J* = 5.5 Hz, 6H, $CqCH_2O$), 3.94 (s, 2H, $ClCH_2$), 3.82 (d, *J* = 9.5 Hz, 3H, H_{6b}), 3.75 (d, *J* = 9.5 Hz, 3H, H_5), 2.19 (s, 9H, $COCH_3$), 2.01 (s, 9H, $COCH_3$), 1.98 (s, 9H, $COCH_3$), 1.80 (s, 9H, $COCH_3$); ^{13}C NMR (125 MHz,

$CDCl_3$): δ 170.4, 170.1, 169.9, 169.0, 165.8, 145.4, 121.6, 86.1, 73.9, 70.8, 68.7, 67.9, 66.9, 64.6, 61.1, 60.0, 43.0, 20.7, 20.5, 20.2 ppm.

Synthesis of azide functionalised first generation dendron 8:

Compound **7** (0.3 g, 0.21 mmol) was dissolved in Dry DMF (3.0 mL) in an R.B., then NaN_3 (40 mg, 0.625 mmol) was added to the reaction mixture and stirred for 12 h at r.t., after completion of the reaction (monitored by TLC) solvent was evaporated in continuation to that ethyl acetate (30 mL) was added to the mixture and taken up in a separating funnel, washed with water (3 x 15 mL) followed by brine solution. Further, the organic layer was collected and dried over anhydrous sodium sulfate and reduced under high vacuum to obtain the crude compound which was further subjected to column chromatography to afford compound **8** as yellow solid. Yield (0.265 g, 88%); R_f = 0.5 (5% Methanol/DCM); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.87 (s, 3H, Triazolyl-H), 6.62 (s, 1H, NH), 5.91 (d, *J* = 9.5 Hz, 3H, H_4), 5.61 (d, *J* = 9.5 Hz, 3H, H_2), 5.56 (d, *J* = 3.0 Hz, 3H, H_3), 5.30-5.27 (m, 3H, H_1), 4.67-4.61 (m, 6H, OCH_2CH=CH), 4.29-4.27 (m, 3H, H_{6a}), 4.19 (d, *J* = 7.0 Hz, 6H, $CqCH_2O$), 3.87-3.86 (m, 3H, H_{6b}), 3.84 (s, 2H, $ClCH_2$), 3.77 (d, *J* = 9.5 Hz, 3H, H_5), 2.22 (s, 9H, $COCH_3$), 2.05 (s, 9H, $COCH_3$), 2.01 (s, 9H, $COCH_3$), 1.83 (s, 9H, $COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.3, 170.0, 169.8, 169.0, 166.7, 145.3, 121.5, 86.0, 73.8, 70.7, 68.8, 67.8, 66.8, 64.5, 61.0, 59.8, 52.6, 20.6, 20.4 and 20.1 ppm. IR (KBr): ν_{max} 3395.50, 3145.80, 2928.33, 2111.58, 1755.66, 1674.53, 1529.27, 1459.94, 1432.22, 1371.78 cm^{-1}.

Synthesis of glycodendrimer 9a:

2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl azide (184 mg, 0.493 mmol) was dissolved in dry DCM, synthesized core compound **1** (30 mg, 6.17x10^{-2} mmol) was added to the solution, CuI (0.21 mg, 0.111 mmol) and DIPEA (32.0 µL, 0.185 mmol) was added to the reaction mixture and stirred under argon atmosphere for 12h. When the reaction shows the complete disappearance (monitored by TLC) of the compound **1,** the mixture was passed through cellite to remove the metal, 20 mL of DCM was added to the obtained filtrate and taken in separating funnel, the organic layer was washed with water (2 x 20 mL). Organics were dried over anhydrous Na_2SO_4 and evaporated to afford crude product. Purification of

the compound was done by flash column chromatography in (2% methanol/DCM) to obtain the compound **9a** as yellow solid. Yield: (131 mg, 78%); R_f = 0.4 (5% methanol/DCM); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.87 (s, 6H, Triazolyl-H), 5.85 (d, *J* = 9.5 Hz, 6H, H_4), 5.56-5.44 (m, 12H, H_2, H_3), 5.22-5.19 (m, 6H, H_1), 4.56-4.48 (m, 24H, H_{6a}, H_{6b}, OCH_2CH=CH), 4.28-4.2 0 (m, 6H, H_5), 4.04-3.99 (m, 12H, OCH_2Ar), 2.11 (s, 18H, 6 x $COCH_3$), 1.93-1.90 (m, 36H, 12 x $COCH_3$), 1.74 (s, 18H, 6 x $COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.3, 170.1, 169.8, 168.9, 145.3, 137.9, 122.2, 85.9, 73.7, 70.9, 67.9, 66.9, 63.7, 61.0, 60.3, 54.7, 42.9, 21.0, 20.6, 20.5 and 20.2 ppm. IR (KBr): ν_{max} 3479.91, 3146.72, 2934.72, 1755.54, 1639.17, 1434.53, 1371.90 cm^{-1}, MALDI-TOF MS: m/z $C_{114}H_{144}N_{18}O_{60}Na^+$, calculated = 2748.8696; found = 2748.9348 $(M+Na)^+$.

Synthesis of first generation glycodendrimer 10a:

Compound **1** (12 mg, 2.47x10^{-2} mmol) and compound **8** (0.266 mg, 0.185 mmol) were dissolved in DCM. CuI (9 mg, 4.73 x 10^2 mmol) and DIPEA (13 μL, 7.74 x10^{-2} mmol) both were added to the solution and stirred at r.t. for 12 h, the disappearance of the core i.e. compound **1** (monitored by TLC) inferred the completion of the reaction. The reaction mixture was passed through cellite and obtained filtrates were taken in separating funnel, DCM (20 mL) was mixed and followed by washing with water (2x 30 mL). The organic layer was collected and dried over anhydrous sodium sulfate and evaporated to give residue **10a**, which was purified by flash column chromatography (2-9%, M-D). The product **10a** was obtained as off white solid. Yield : (157 mg, 70%); R_f = 0.50 (10% methanol/DCM); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.90 (br s, 18H, Peripheral triazolyl-H), 7.87 (br s, 6H, Inner triazolyl-H), 7.03 (br s, 6H, NH), 5.97-5.95 (m, 18H, H_4), 5.59-5.55 (m, 18H, H_2), 5.50 (br s, 18H, H_3), 5.30-5.25 (m, 18H, H_1), 4.55 (br s, 72H, H_{6a}, H_{6b}, OCH_2CH=CH), 4.29 (br s, 18H, H_5), 4.15-4.07 (m, 36H, CH_2C_q), 3.74-3.69 (m, 36H, CH_2CON, OCH_2Ar, OCH_2CH=CH), 2.15-2.12 (m, 54H, 18 x $COCH_3$), 1.96-1.95 (m, 108 H, 36 x $COCH_3$), 1.74 (s, 54H, 18 x $COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.4, 170.1, 169.9, 169.1, 165.3, 145.1, 139.3, 138.0, 125.7, 122.2, 114.0, 85.8, 73.7, 70.8, 68.7, 68.0, 67.0, 64.4, 61.1, 50.8, 40.5, 20.7, 20.6, 20.2 and 19.6 ppm. IR (KBr): ν_{max} 3454.46, 3144.32, 2925.33, 2854.91, 1755.21, 1636.08, 1552.67, 1463.37, 1432.7, 1371.64 cm^{-1};

MALDI-TOF MS: m/z $C_{372}H_{484}N_{78}O_{192}Na_3^+$ calculated = 9187.0296; found = 9187.0693 $(M+3Na+4H)^+$

"De-*O*-acetylation" (Zemplèn reaction) procedure for the synthesis of glycodendrimer 9b and 10b:

Glycoconjugate cluster **9a** or **10a** was dissolved in a mixture of dry Methanol: dry THF: dry DCM in ratio of 3.0:0.5:0.5 by fixing the cluster molarity 2.5 x 10^{-3}M. A freshly prepared solution of NaOMe (1M, 30-40 μL approx.) was added until the solution pH became 9-10. The reaction was stirred for 48 h at room temperature. After that, Milli-Q water was poured to solubilize the whole mixture and neutralized by ion exchange resin (Amberlite 120 H^+) till pH reaches in between 6-7, followed by filtration, and the solvent was evaporated under reduced pressure to afford the deprotected glycodendrimers **9b** and **10b**. The developed compound further characterized by the NMR, IR and MALDI-TOF MS only.

Physical data of compound 9b: White solid, yield (26 mg, 88%); ^{1}H NMR (500 MHz, D_2O): δ 8.21 (s, 6H, Triazolyl-H), 5.57 (d, *J* = 8.5 Hz, 6H, H_4), 4.40 (br s, 12H, $OCH_2CH=CH$), 4.30 (br s, 12H, H_2, H_3), 4.12-4.08 (m, 6H, H_1), 3.925-3.920 (m, 6H, H_{6a}), 3.83-3.80 (m, 6H, H_{6b}), 3.73-3.70 (m, 6H, H_5), 3.59-3.51(m, 12H, OCH_2Ar); ^{13}C NMR (125 MHz, D_2O): δ 146.7, 143.9, 137.7, 130.3, 125.7, 124.8, 88.1, 78.2, 73.0, 69.7, 68.5, 64.7, 62.7 and 60.7 ppm. IR (KBr): ν_{max} 3425.79, 2925.43, 2851.8, 1633.62, 1457.3, 1404.34, 1093.77 cm^{-1}; MALDI-TOF MS: for $C_{66}H_{100}N_{18}O_{37}^+$ Calculated = 1736.6492; found = 1736.1666 $(M+H_2O+2H)^+$.

Physical data of compound 10b: yellow solid, yield (28 mg, 84%); ^{1}H NMR (500 MHz, D_2O): δ 8.06 (br s, 18H, Peripheral triazolyl-H), 7.88 (br s, 6H, Inner triazolyl-H), 5.51-5.49 (m, 18H, H_4), 5.02 (br s, 12H, CH_2CON), 4.45-4.36 (m, 54H, H_1, H_2, H_3), 3.90 (br s, 18H, H_{6a}), 3.80 (br s, 18H, H_{6b}), 3.69-3.67 (m, 24H, $OCH_2CH=CH$, OCH_2Ar), 3.62-3.50 (m, 90H, H_5, $OCH_2CH=CH$, CH_2C_q); ^{13}C NMR (125 MHz, D_2O): δ 170.3, 147.6, 145.1, 133.2, 131.2, 130.1, 126.7, 126.6, 125.1, 109.5, 89.0, 79.2, 73.9, 70.6, 69.5, 68.5, 64.4, 61.7 and 61.2 ppm. IR (KBr): ν_{max} 3419.51, 2925.22, 1686.02, 1642.3, 1401.87, 1195.52, 1095.55 cm^{-1}.

Cytotoxicity evaluation of developed glycodendrimers:

Lactate dehydrogenase assay:

The cytotoxicity of dendrimers **9a** and **10a** were evaluated using SiHa cells by measuring lactate dehydrogenase (LDH) activity described earlier.[5] The said drugs were evaluated in concentration range 32, 64, 128, 256, 512, 1024 µg/mL. Briefly, the 48 hrs treated cells were spun at 100 g for 15 mins and as the supernatant, the growth medium was collected. The collected broth and the LDH reagent were incubated in the ratio of 2:1 for 30 mins followed by the absorbance reading at 500 nm. For positive control, we used 2% Triton X to treat the SiHa cells. The extent of percentage (%) cytotoxicity was calculated as follow

(Mean absorbance of Treated cells-Absorbance of Medium-Absorbance of Cell and Medium)/(Absorbance of Triton X treated Cell-Absorbance of Cell and Medium) × 100

Table S1: Lactate Dehydrogenase Cytotoxicity assay of glycodendrimers against SiHa Cells

Medium Absorbance (λ_{max} 500)	Cell + Medium Absorbance (λ_{max} 500)	Cell + 2% Triton X 100 Absorbance (λ_{max} 500)	Concentration (µg/ mL)	Glycodendrimers 10a and 9a treated Cells Absorbance (λ_{max} 500)	Amphotericin B treated Cells Absorbance (λ_{max} 500)	% Cytotoxicity by Amphotericin B
1.119±0.116	1.331±0.215	4.106±0.304	32	1.405±0.157/ 1.386±0.125	2.466±0.258	0.706
			64	1.453±0.163/ 1.411±0.157	2.617±0.247	6.096
			128	1.495±0.354/ 1.448±0.137	2.939±0.306	18.104
			256	1.574±0.315/ 1.496±0.213	3.477±0.281	38.031
			512	1.668±0.187/ 1.547±0.324	3.905±0.371	54.014
			1024	1.794±0.345/1.683±0.385	4.376±0.363	71.784

3. ^{1}H and ^{13}C NMR Spectrum of compounds (**1, 2, 4-10b**)

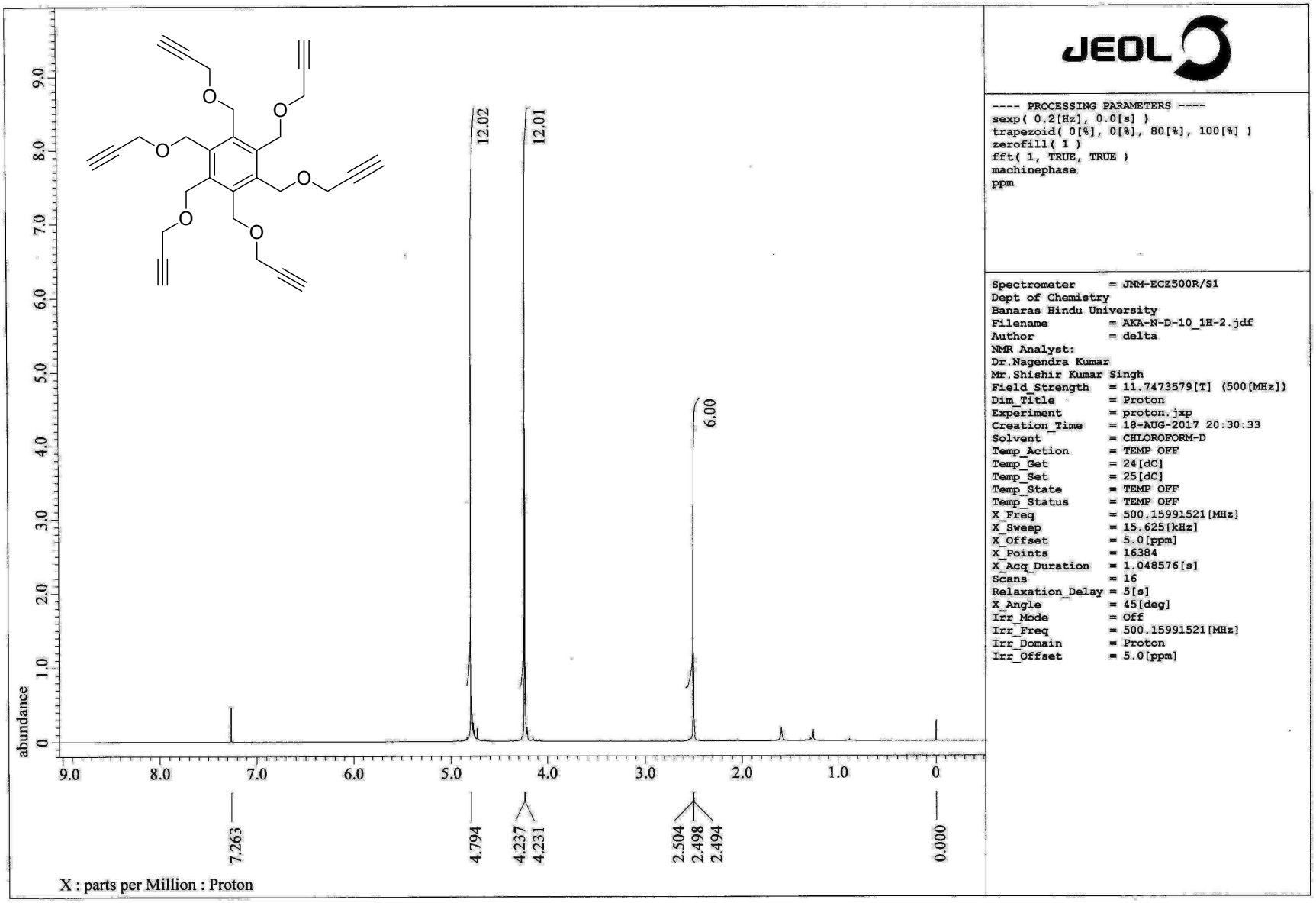


Figure S1. ^{1}H NMR (500 MHz, $CDCl_3$) of compound **1**

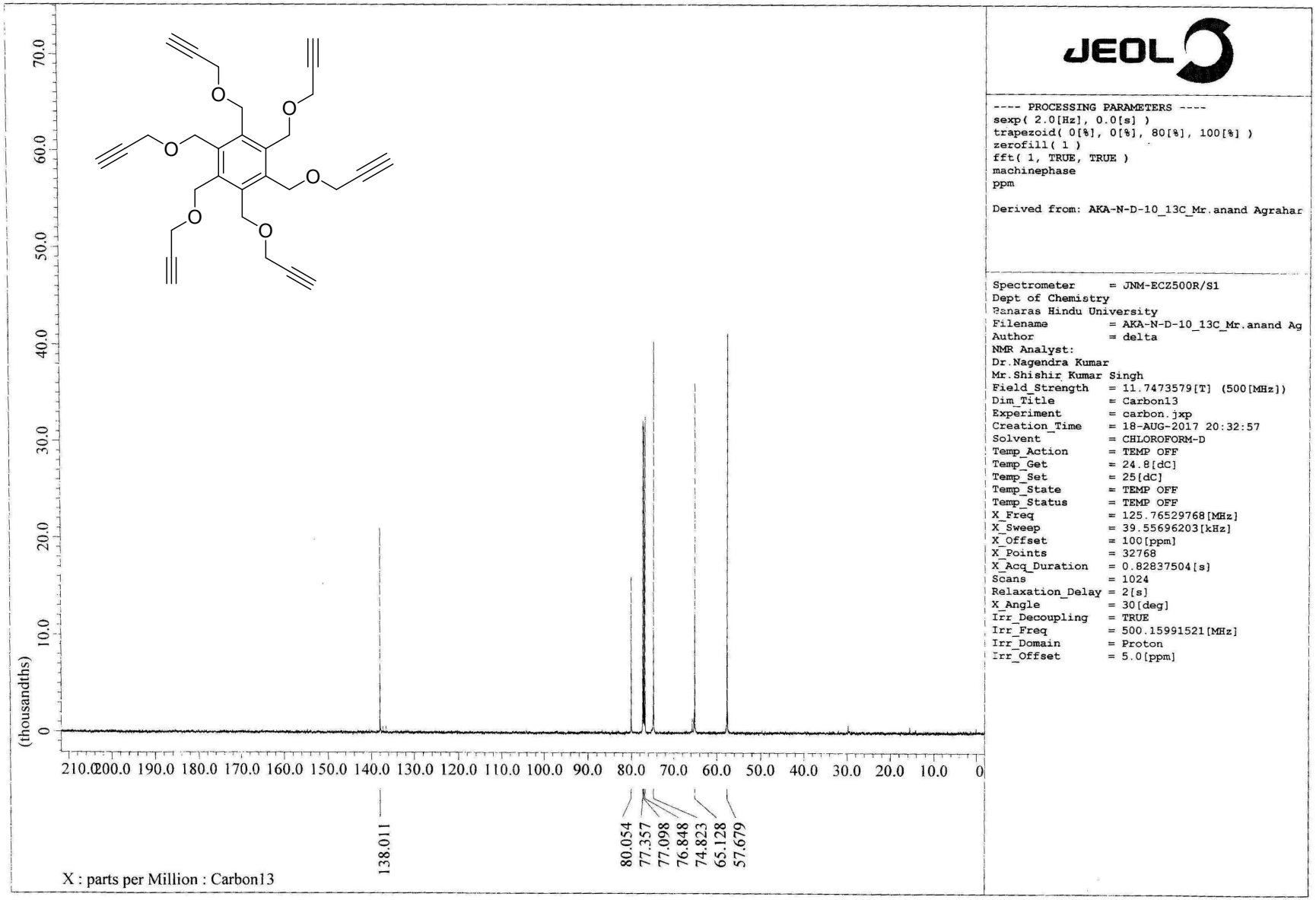


Figure S2. ^{13}C NMR (125 MHz, $CDCl_3$) of compound **1**

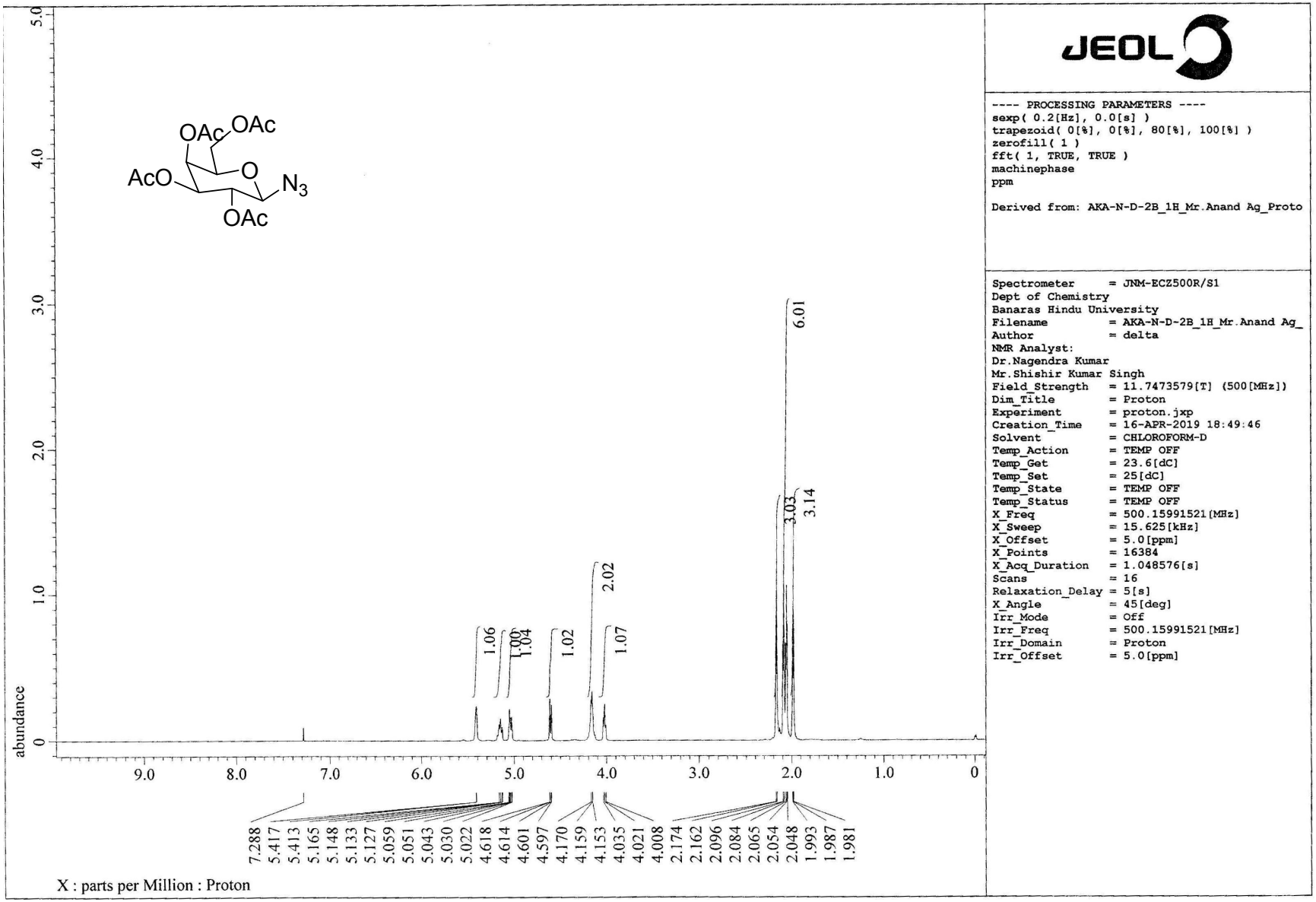


Figure S3. ^{1}H NMR (500 MHz, $CDCl_3$) of compound **2**

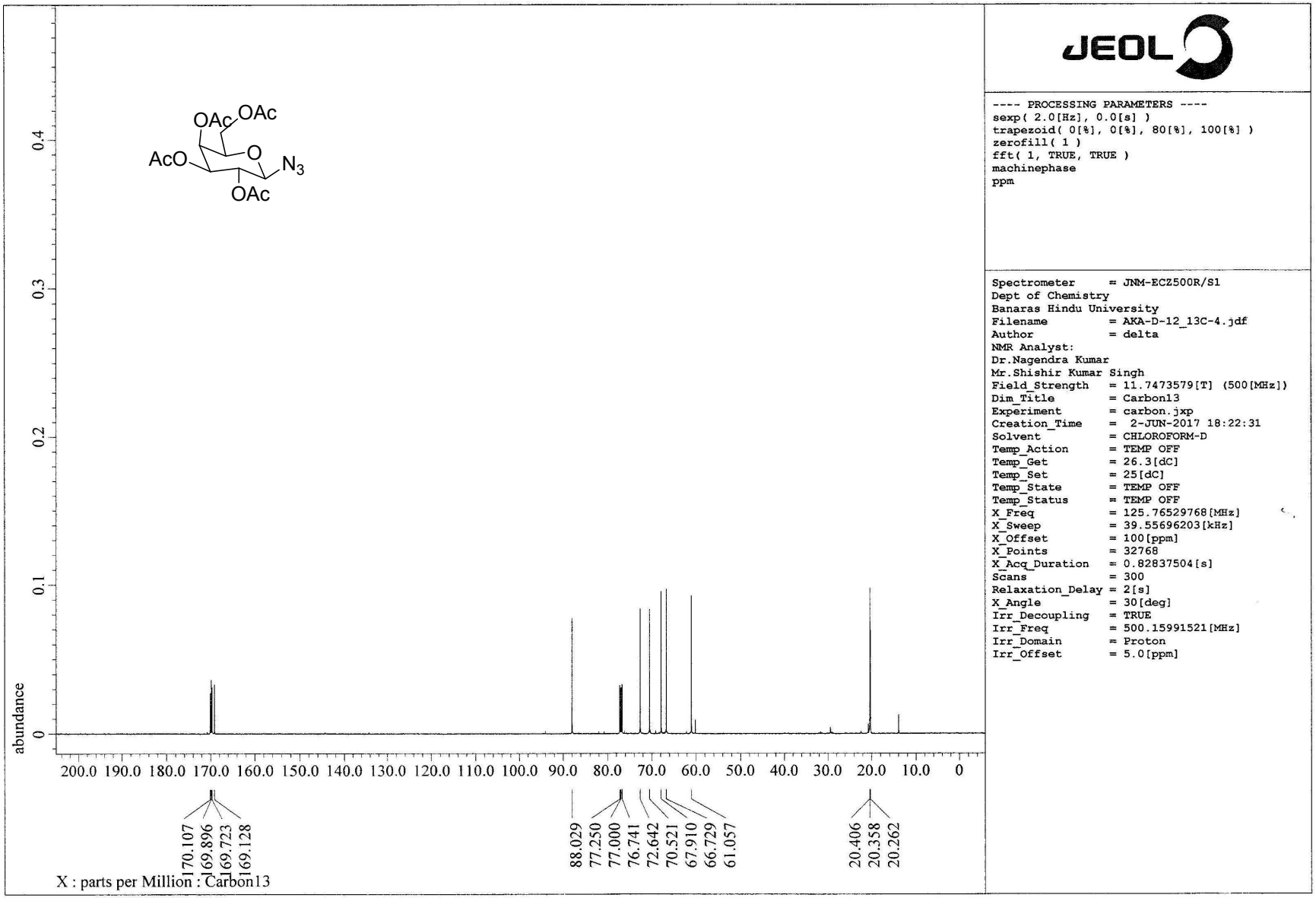


Figure S4. ^{13}C NMR (125 MHz, $CDCl_3$) of compound **2**

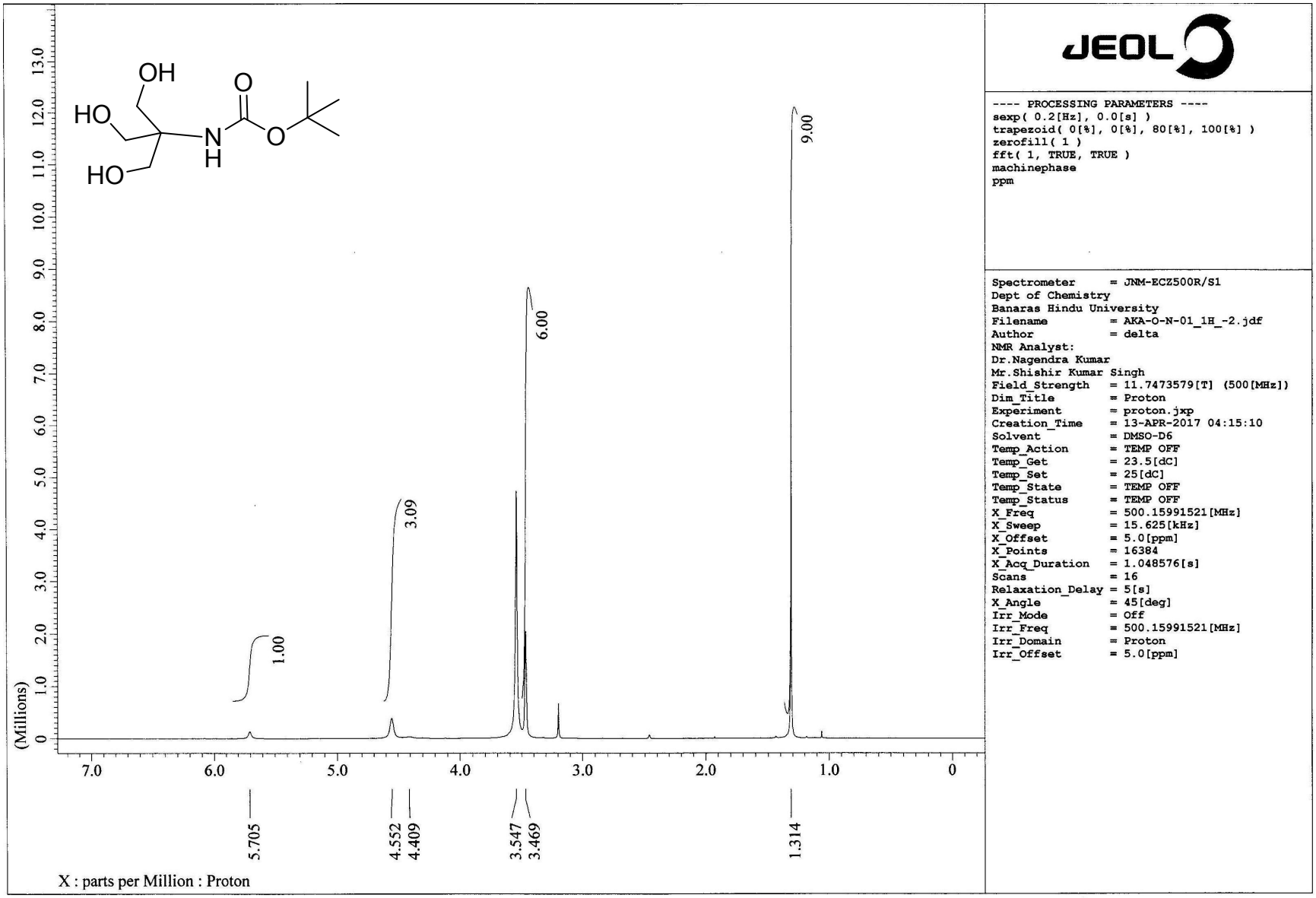


Figure S5. ^{1}H NMR (500 MHz, DMSO-d_6) of compound **4**

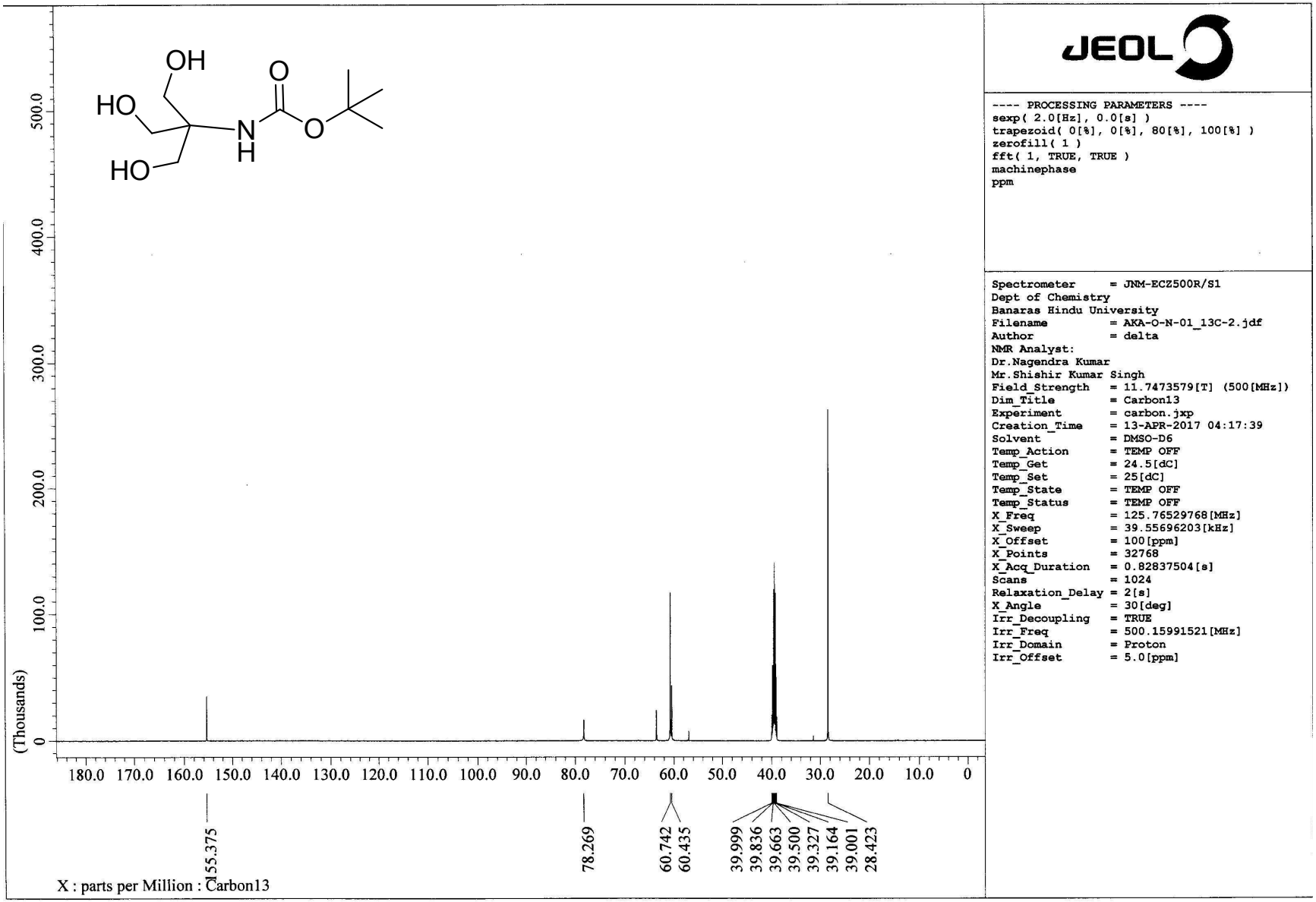


Figure S6. ^{13}C NMR (125 MHz, DMSO-d_6) of compound **4**

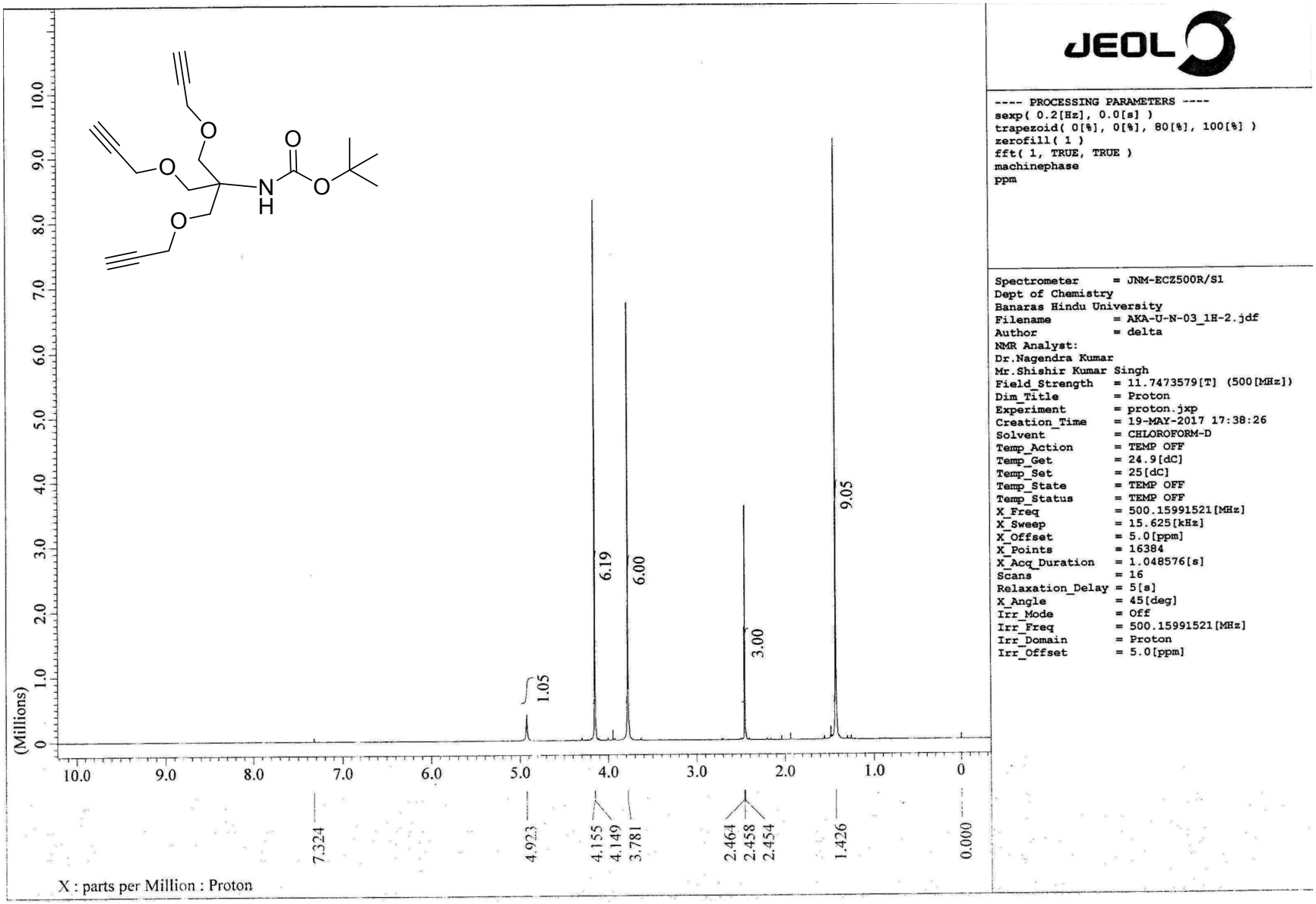


Figure S7. ^{1}H NMR (500 MHz, $CDCl_3$) of compound **5**

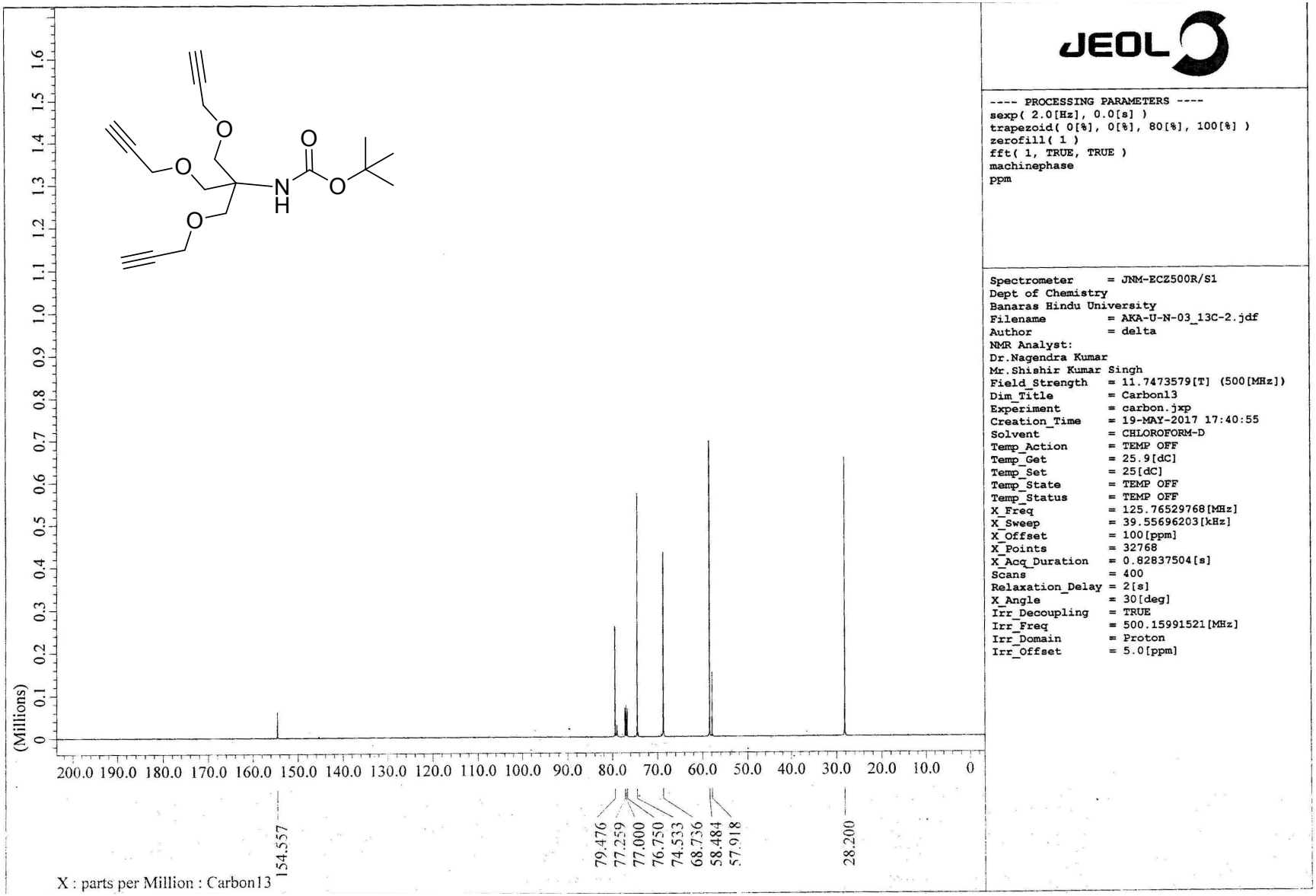


Figure S8. ^{13}C NMR (125 MHz, $CDCl_3$) of compound **5**

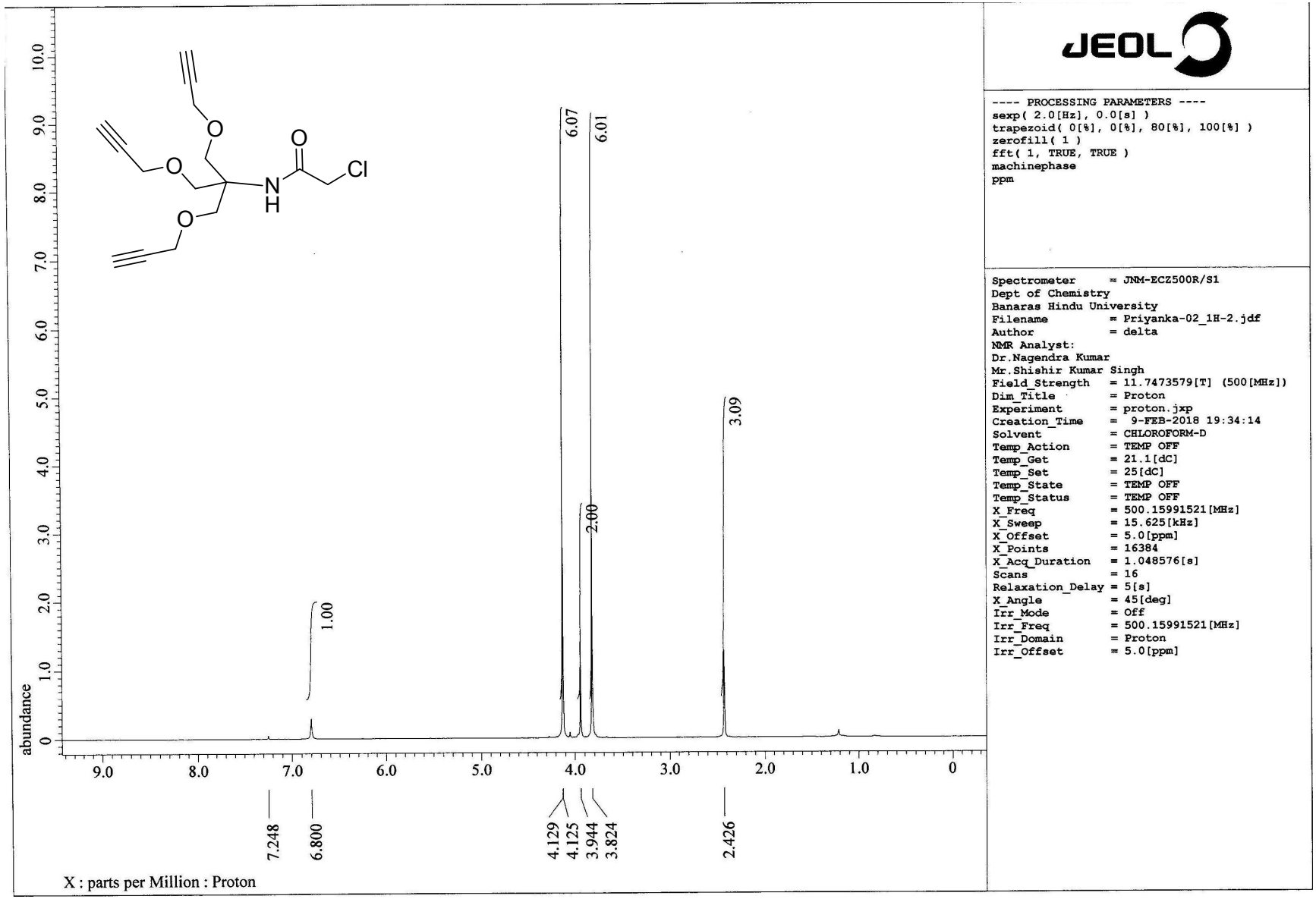


Figure S9. ^{1}H NMR (500 MHz, $CDCl_3$) of compound **6**

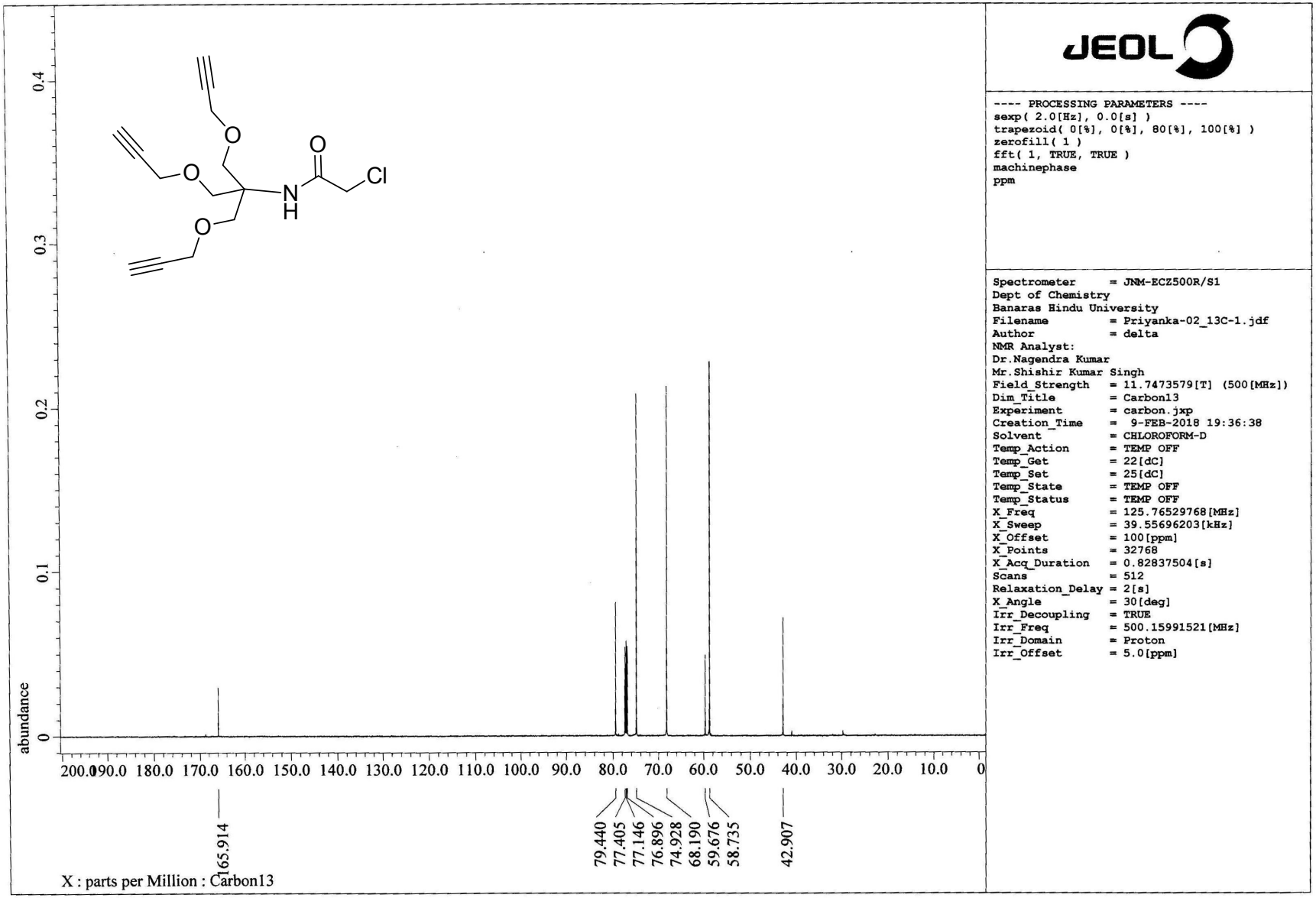


Figure S10. ^{13}C NMR (125 MHz, $CDCl_3$) of compound **6**

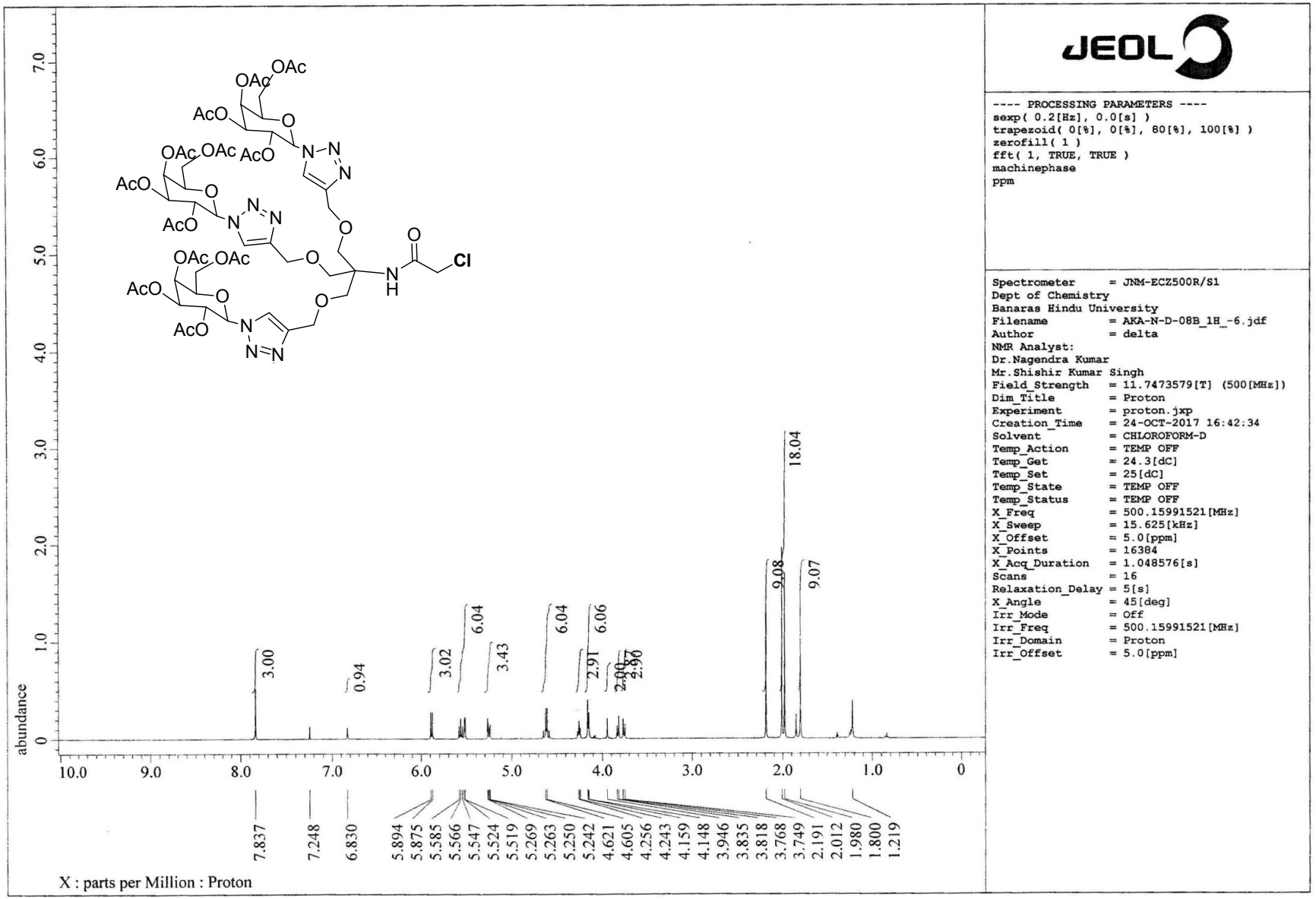


Figure S11. ^{1}H NMR (500 MHz, $CDCl_3$) of compound **7**

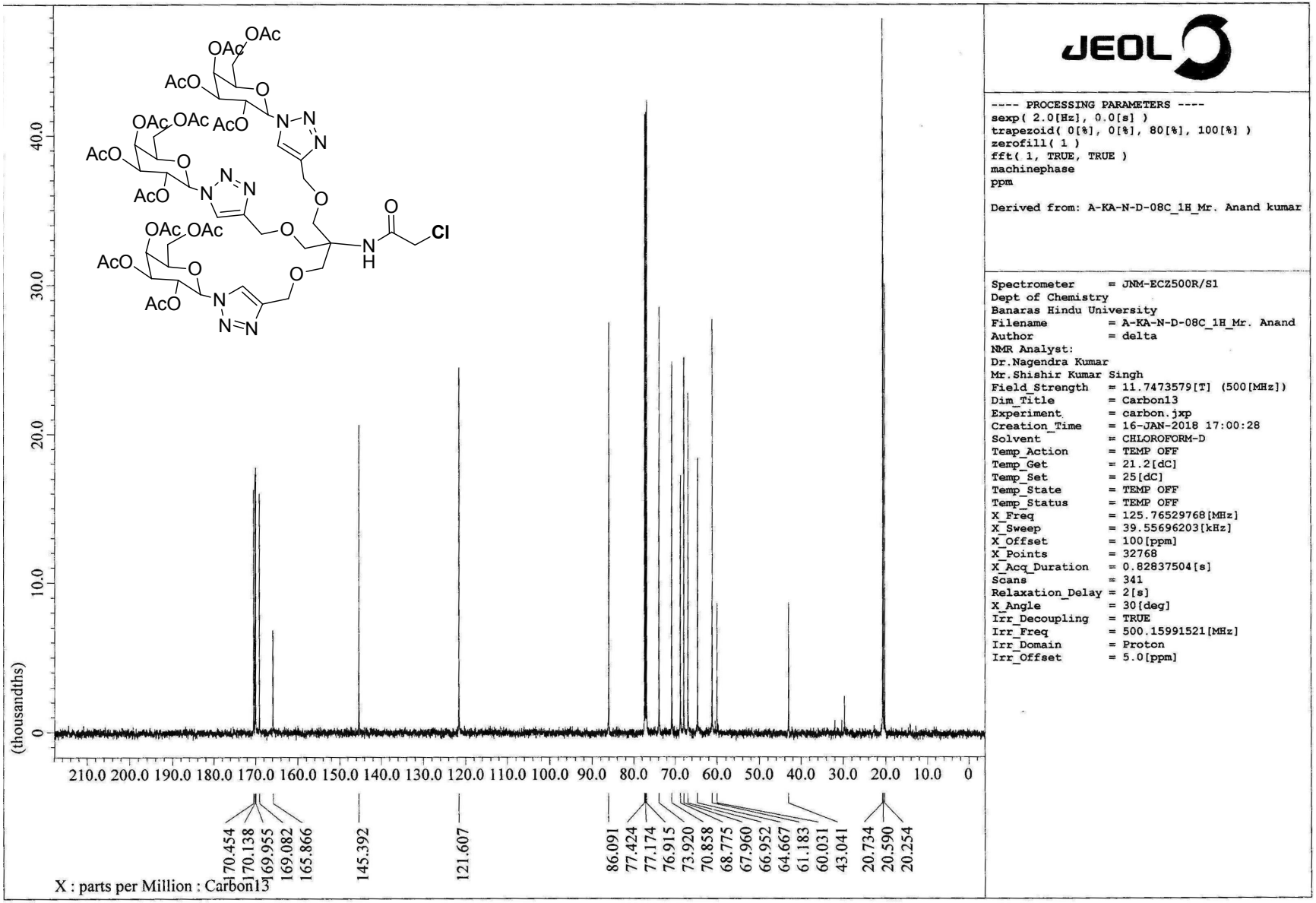


Figure S12. ^{13}C NMR (125 MHz, $CDCl_3$) of compound **7**

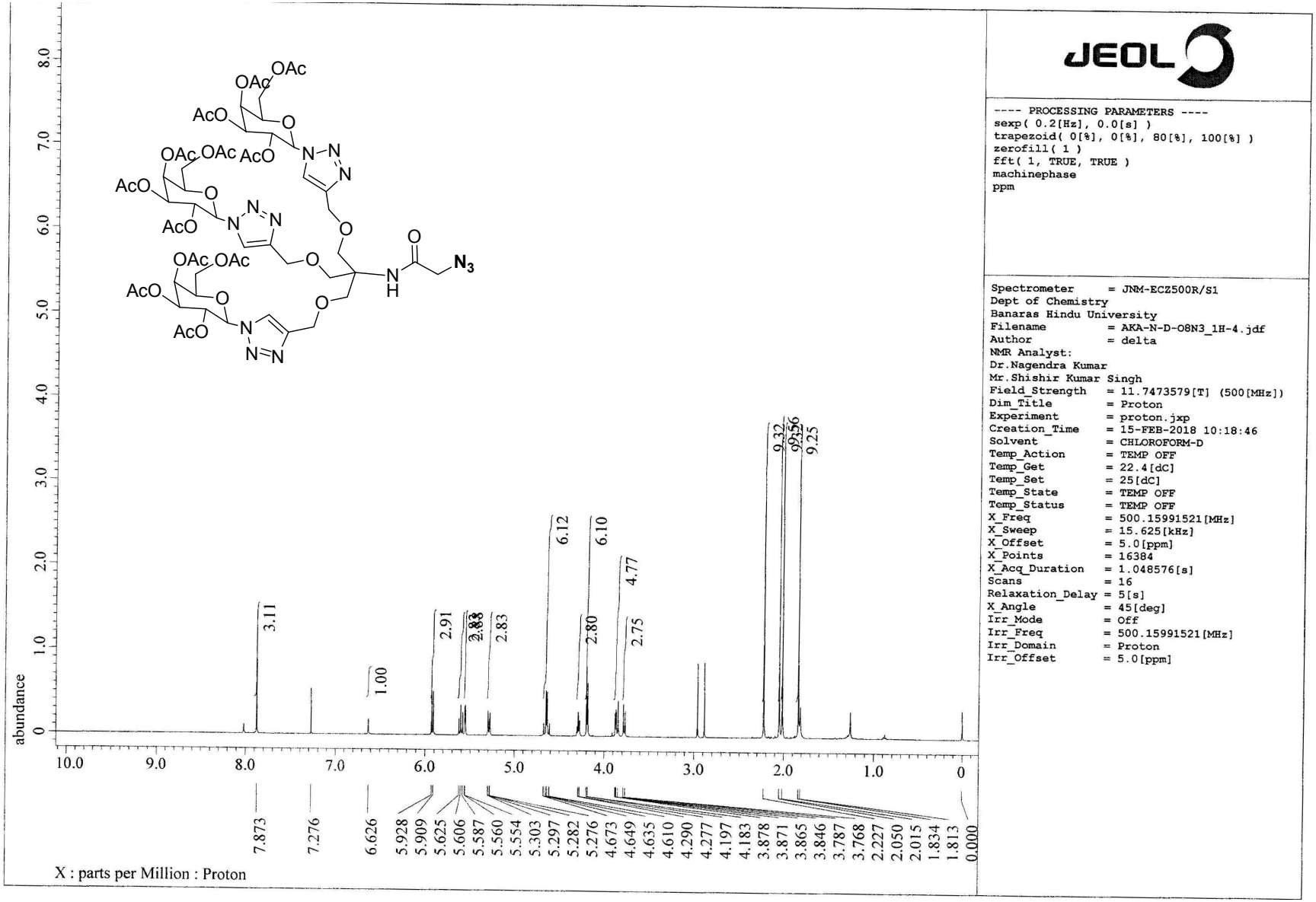


Figure S13. ^{1}H NMR (500 MHz, $CDCl_3$) of compound **8**

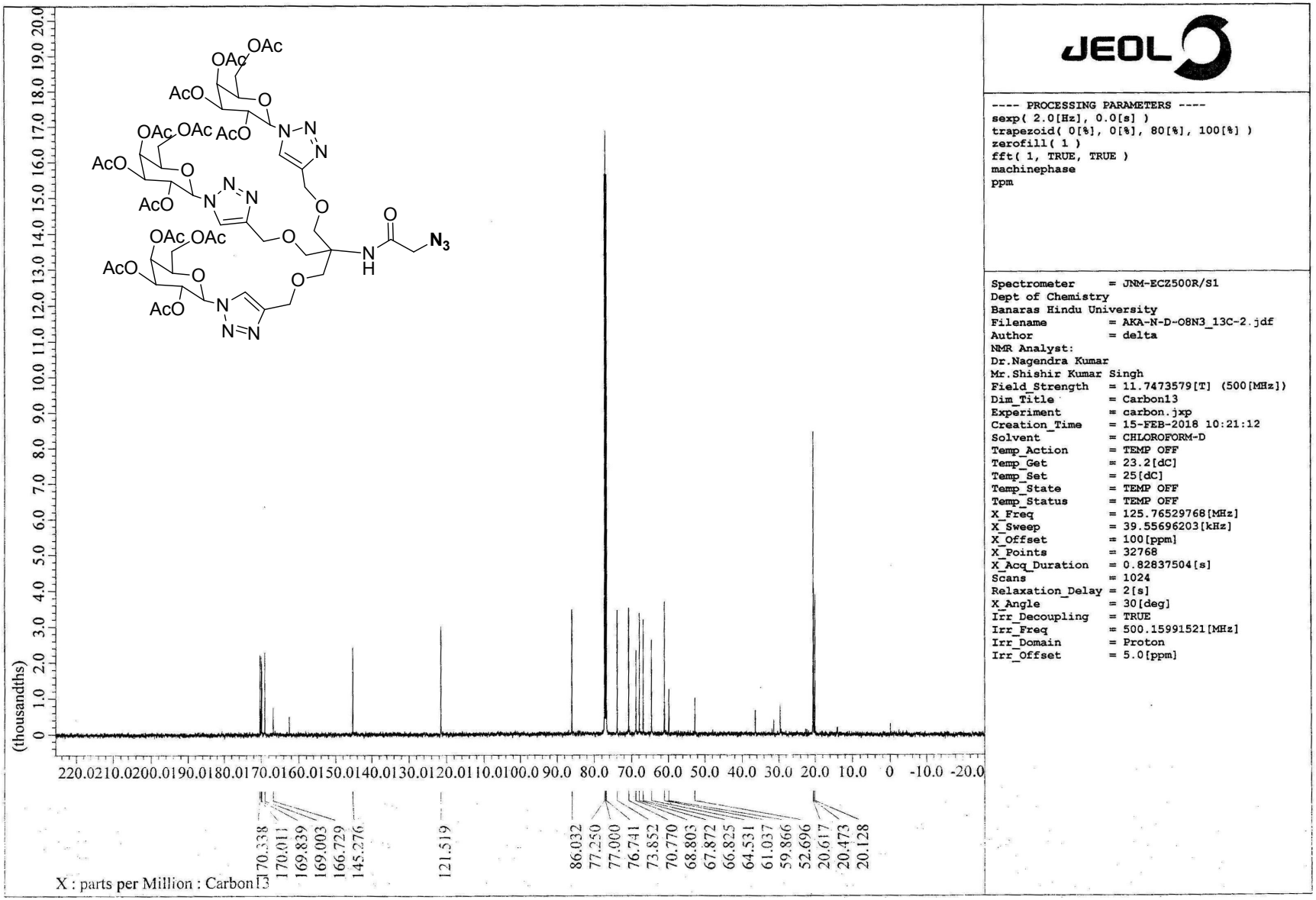


Figure S14. ^{13}C NMR (125 MHz, $CDCl_3$) of compound **8**

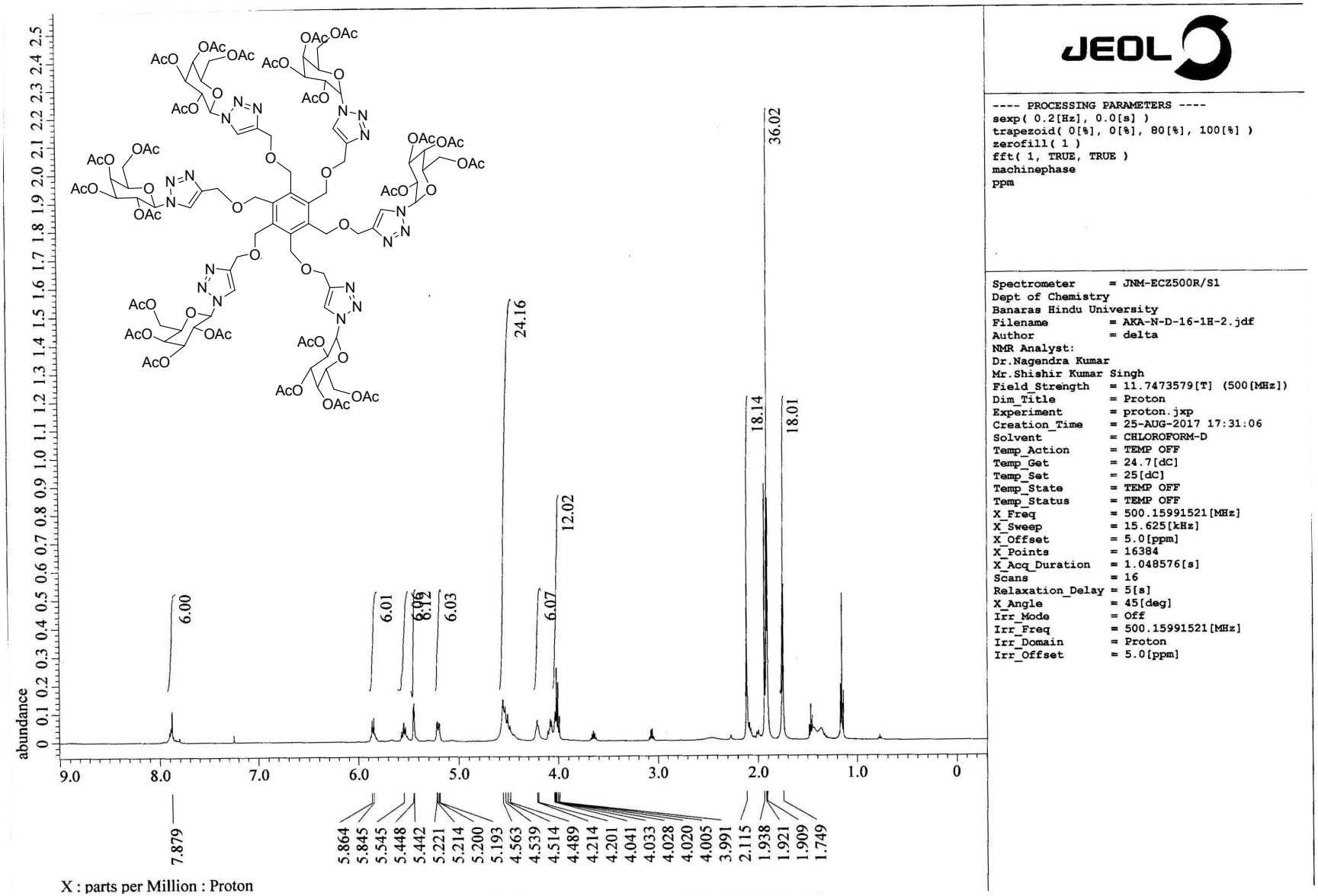


Figure S15. ^{1}H NMR (500 MHz, $CDCl_3$) of compound **9a**

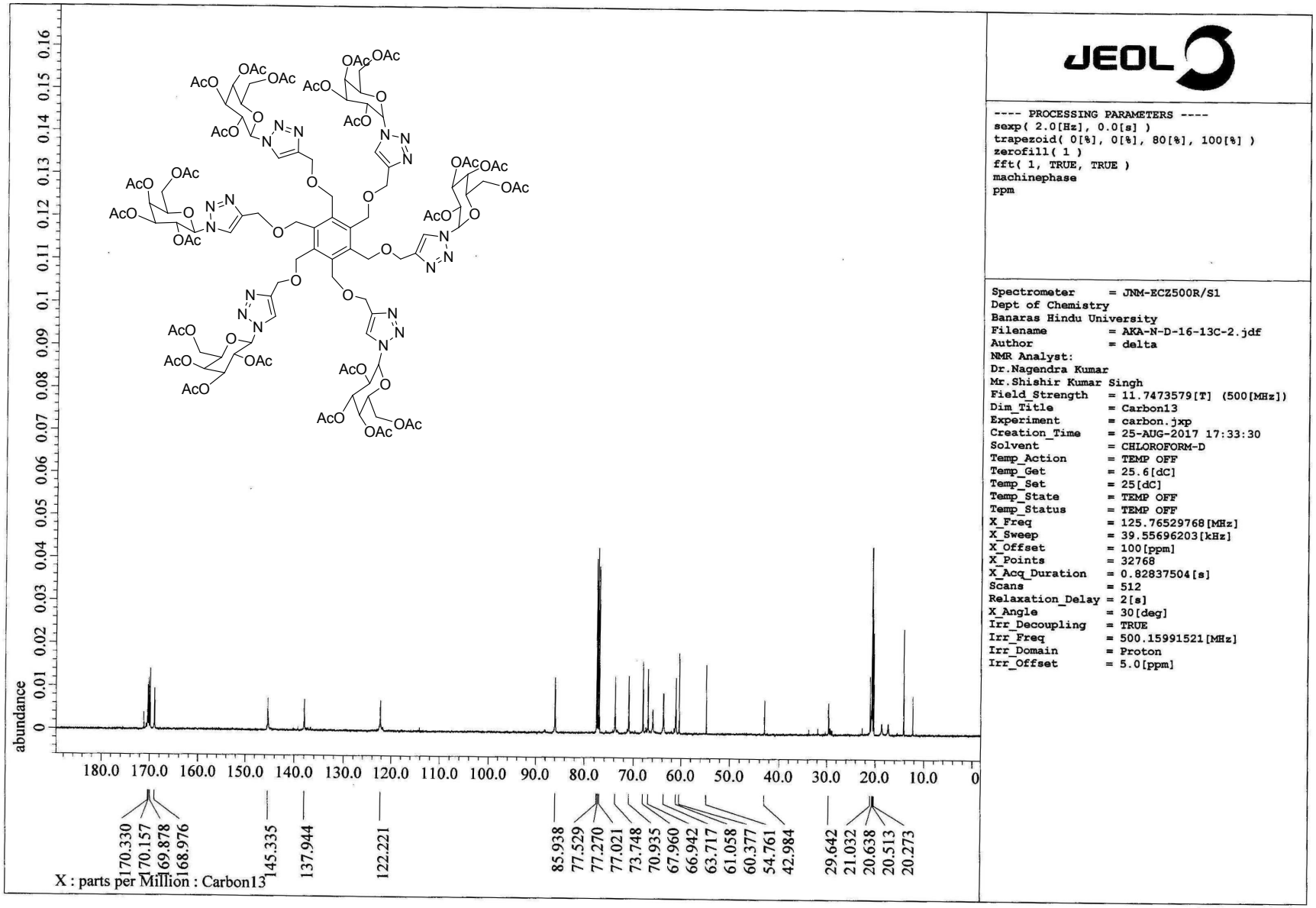


Figure S16. ^{13}C NMR (125 MHz, $CDCl_3$) of compound **9a**

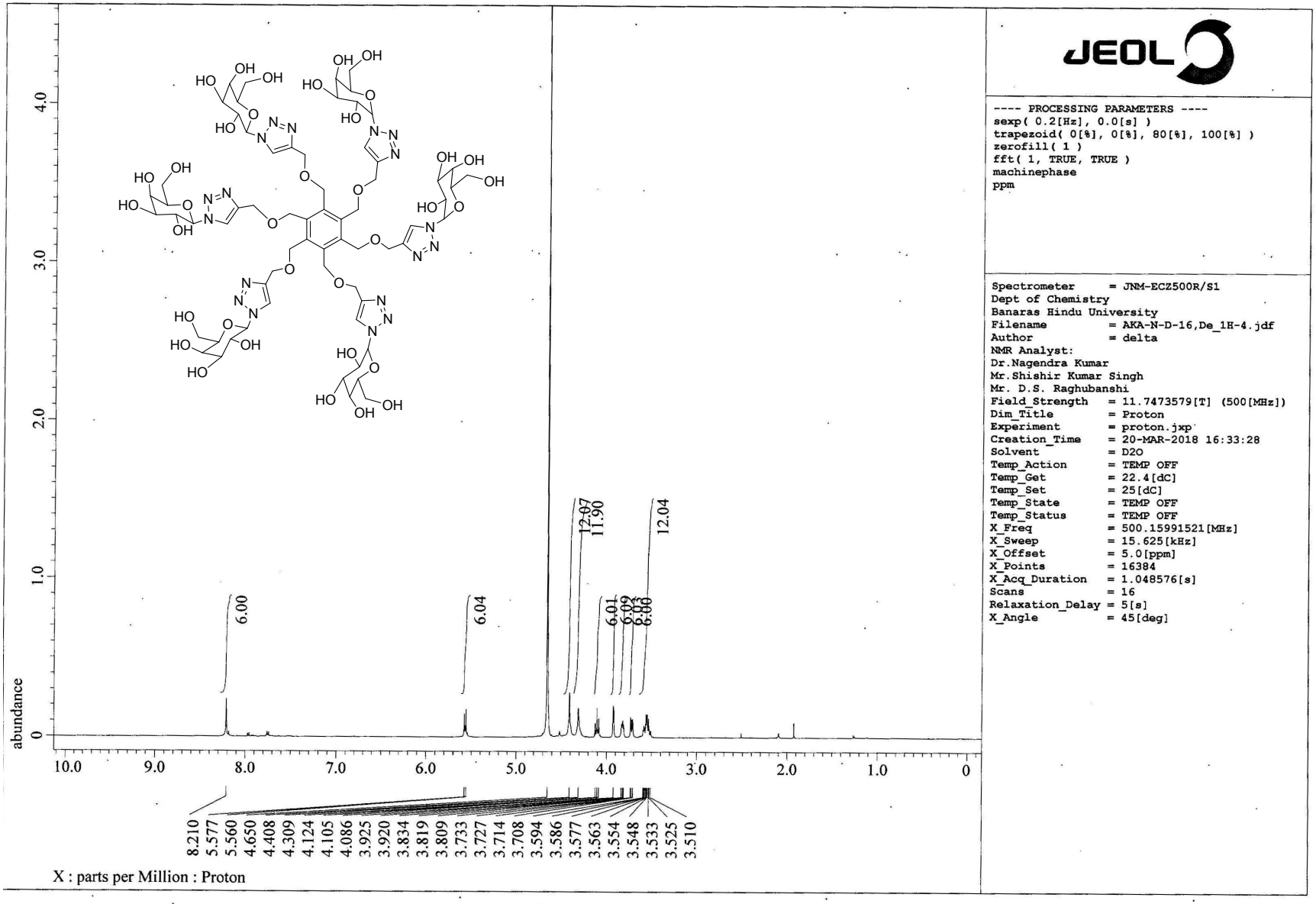


Figure S17. ^{1}H NMR (500 MHz, D_2O) of compound **9b**

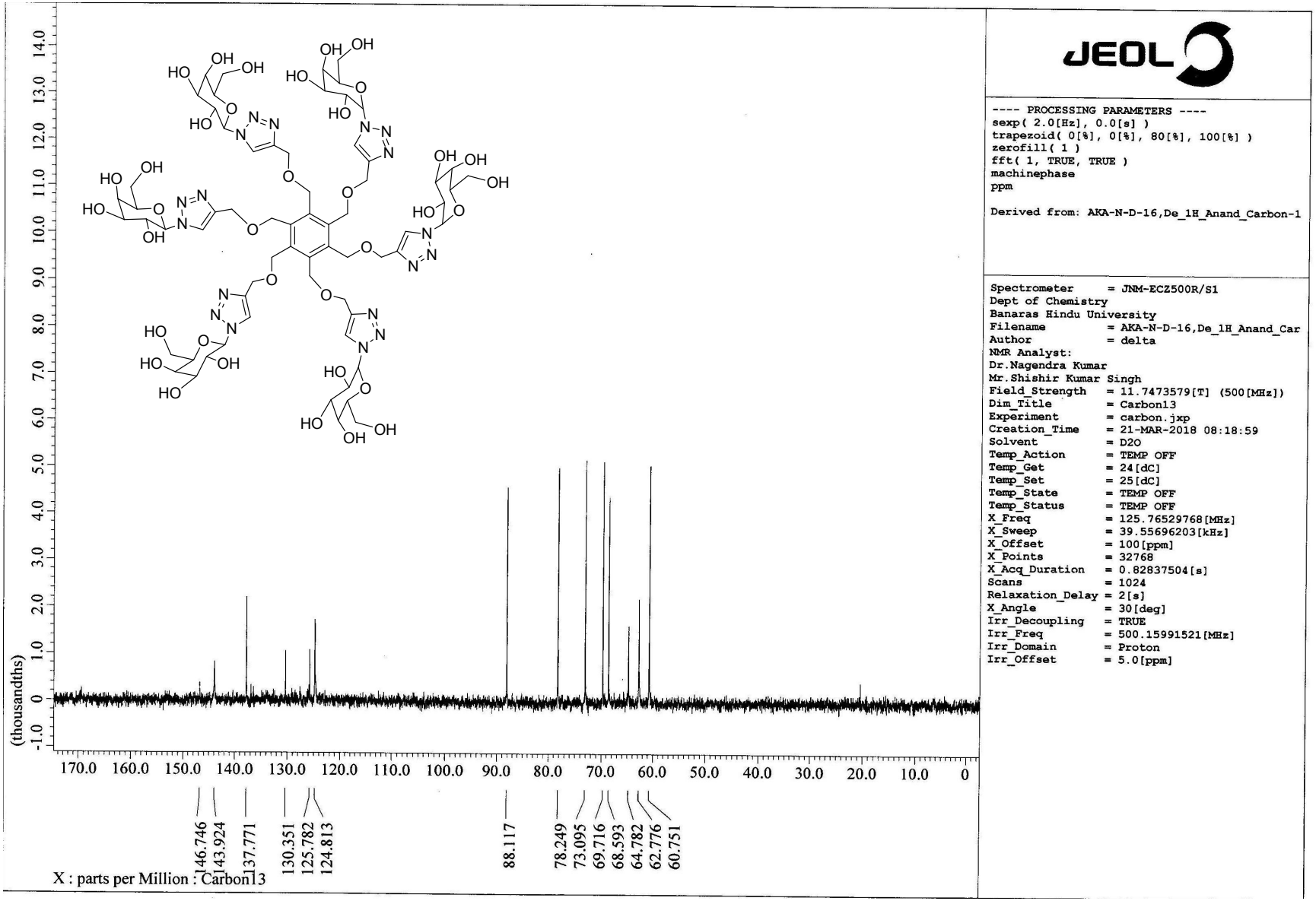


Figure S18. ^{13}C NMR (125 MHz, D_2O) of compound **9b**

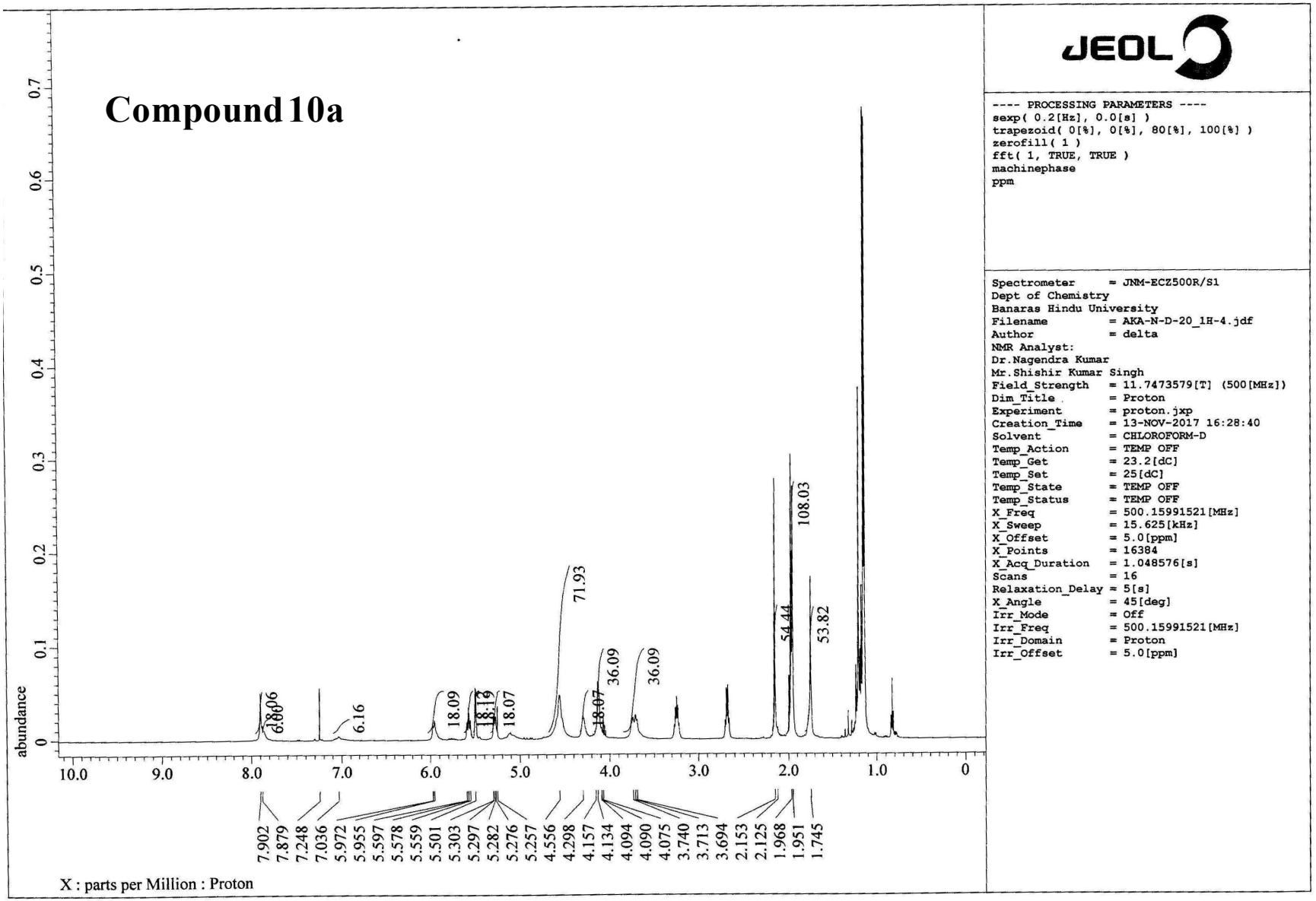


Figure S19. ^{1}H NMR (500 MHz, $CDCl_3$) of compound **10a**

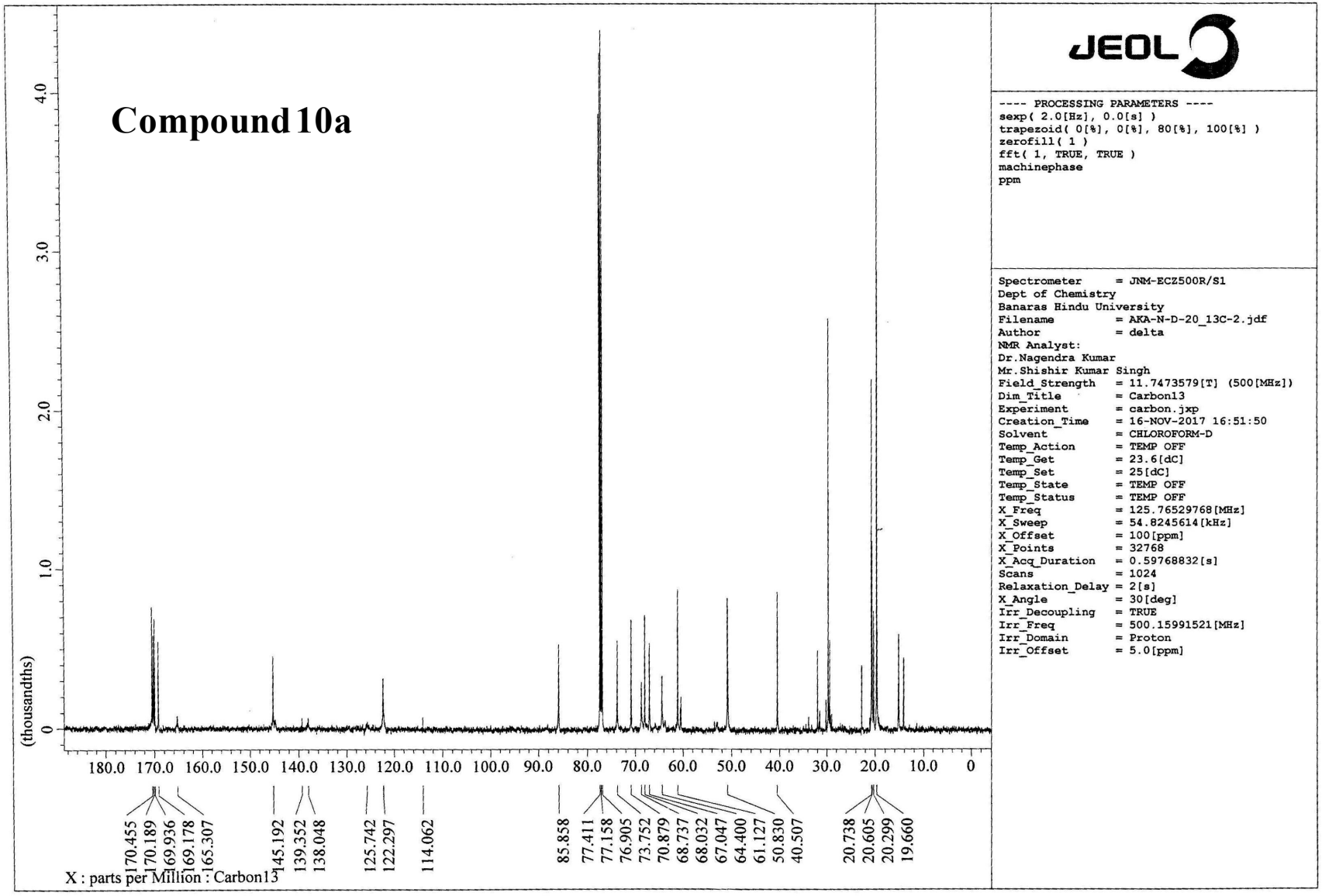


Figure S20. ^{13}C NMR (125 MHz, $CDCl_3$) of compound **10a**

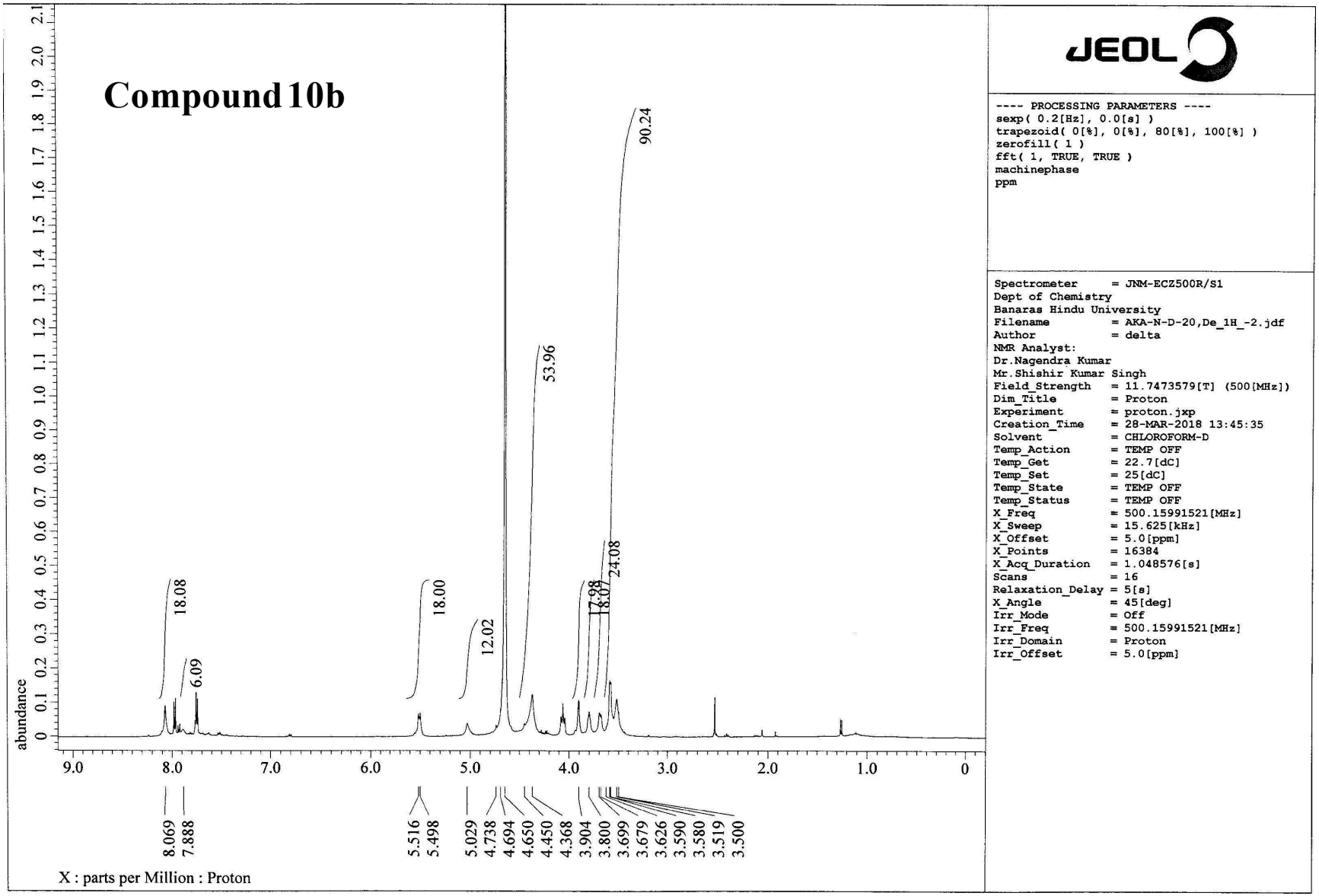


Figure S21. ^{1}H NMR (500 MHz, D_2O) of compound **10b**

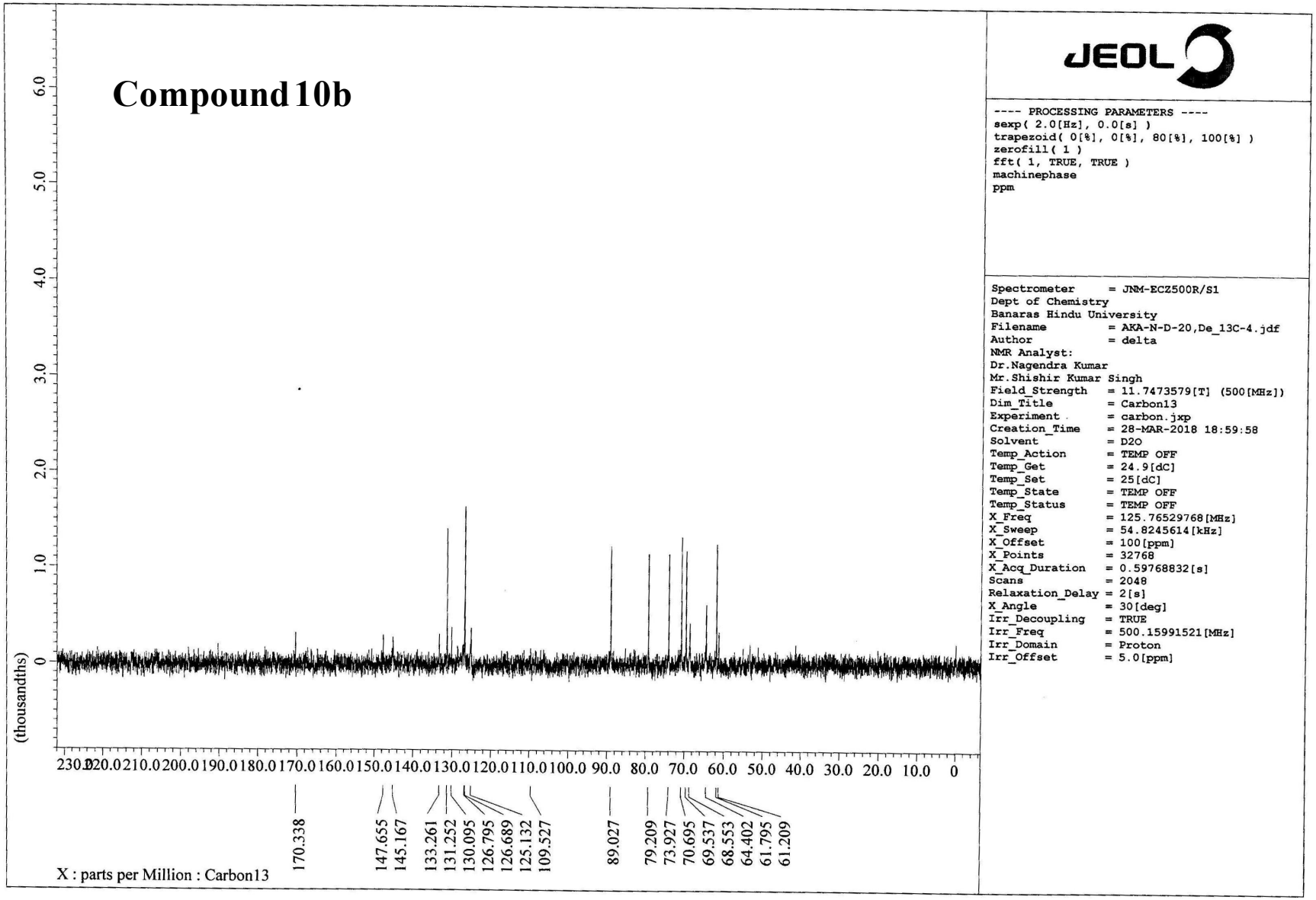


Figure S22. ^{13}C NMR (125 MHz, D_2O) of compound **10b**

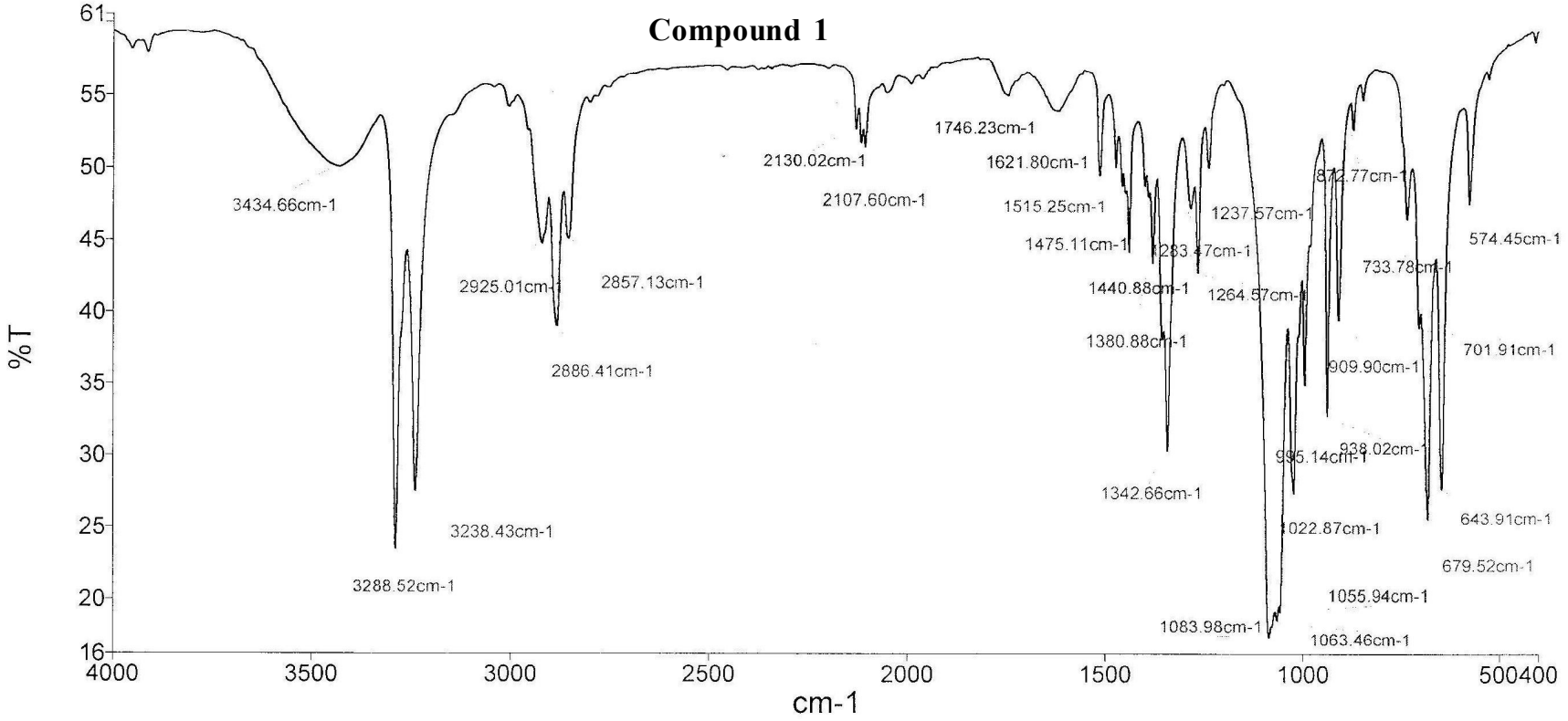


Figure S23. IR of compound **1**

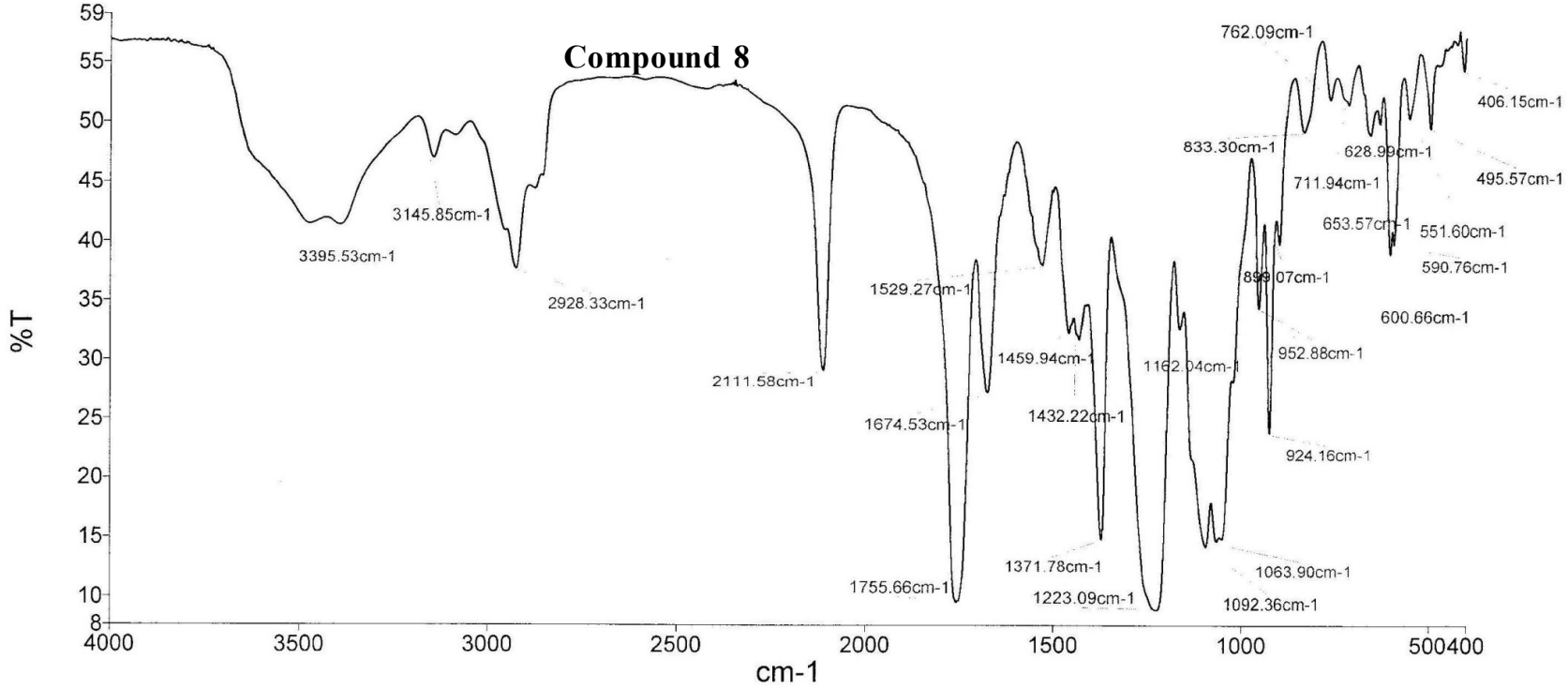


Figure S24. IR of compound **8**

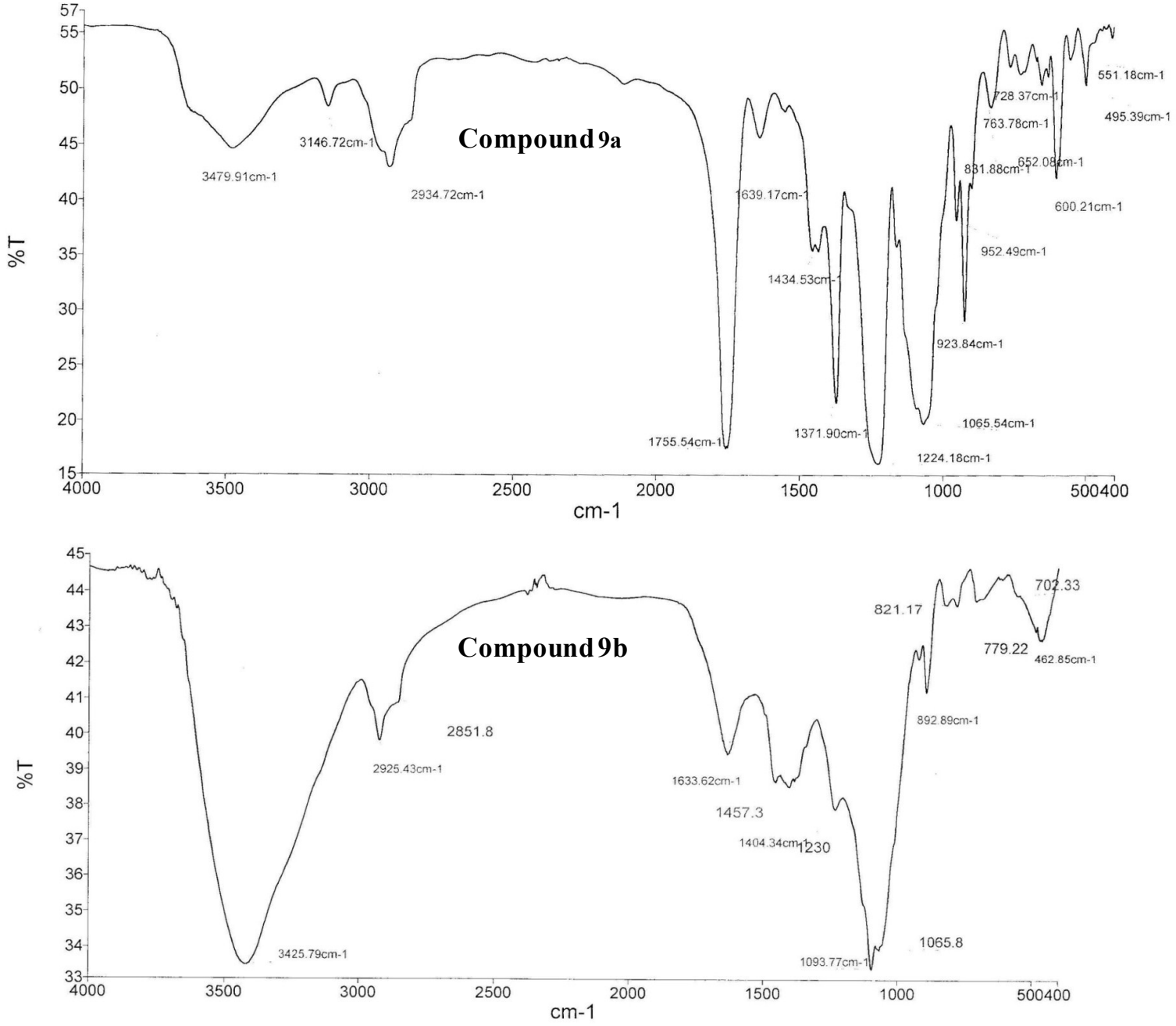


Figure S25. IR of compounds **9a** and **9b**

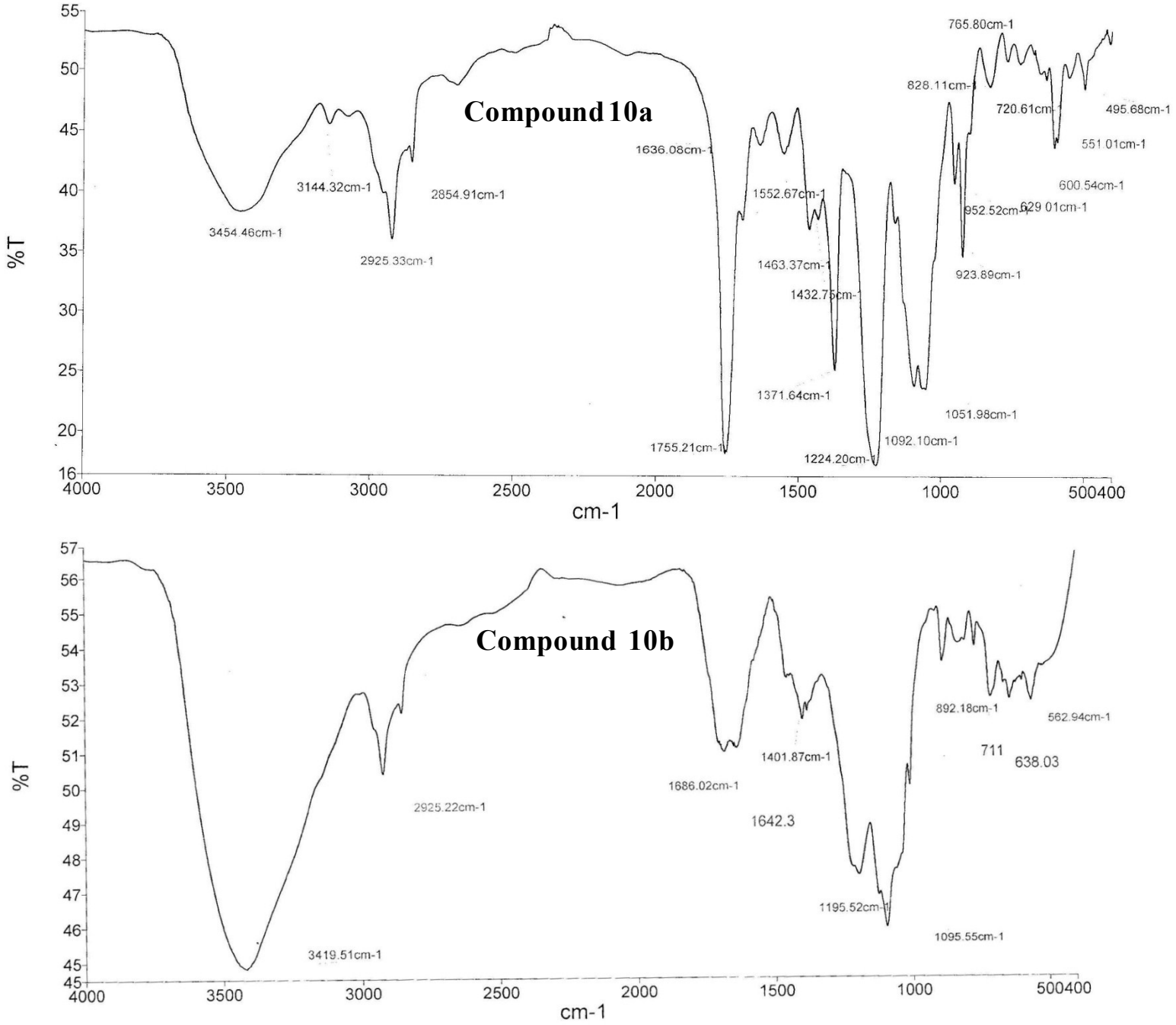


Figure S26. IR of compounds **10a** and **10b**

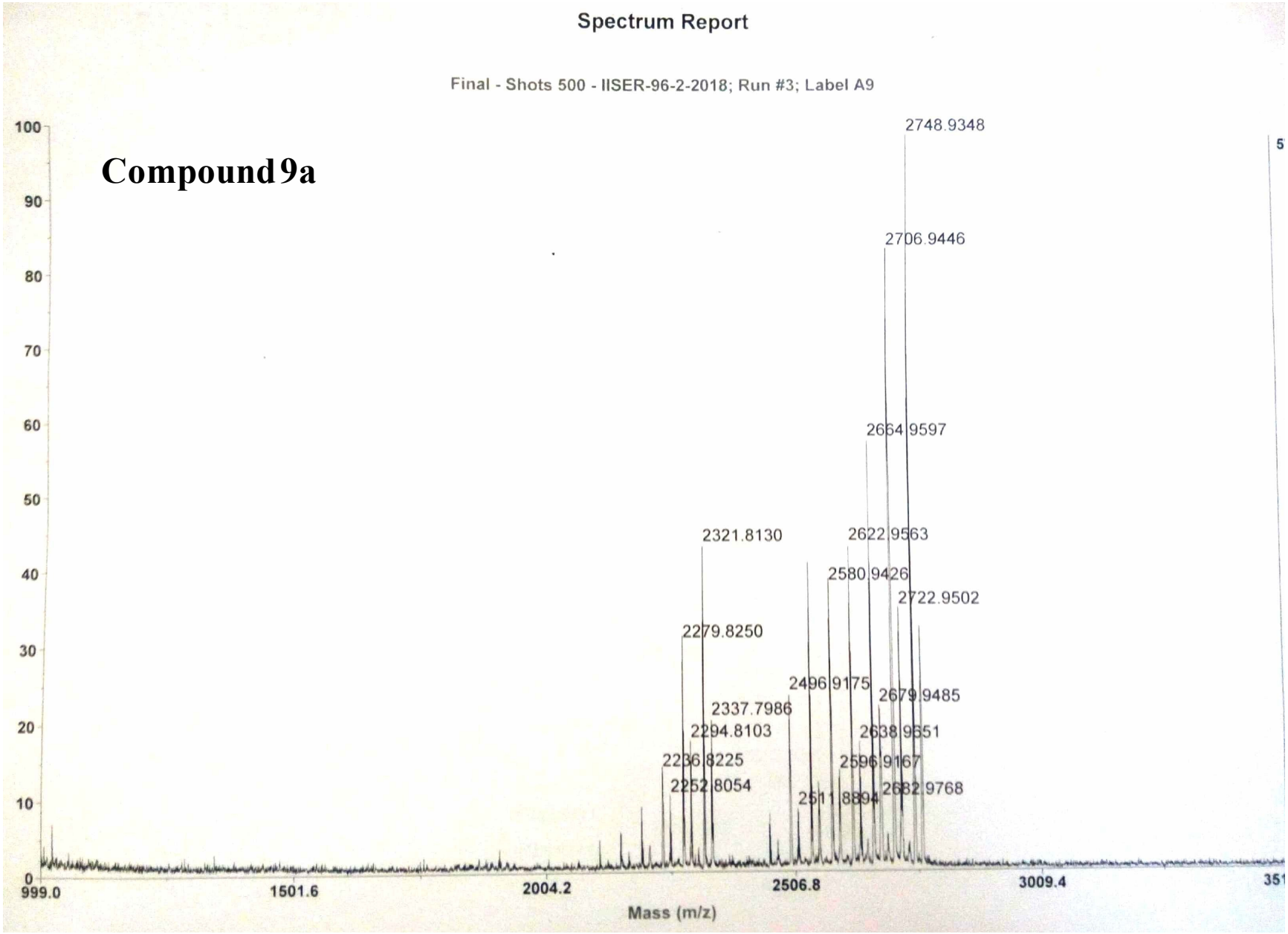


Figure S27. MALDI-TOF MS of compound **9a**

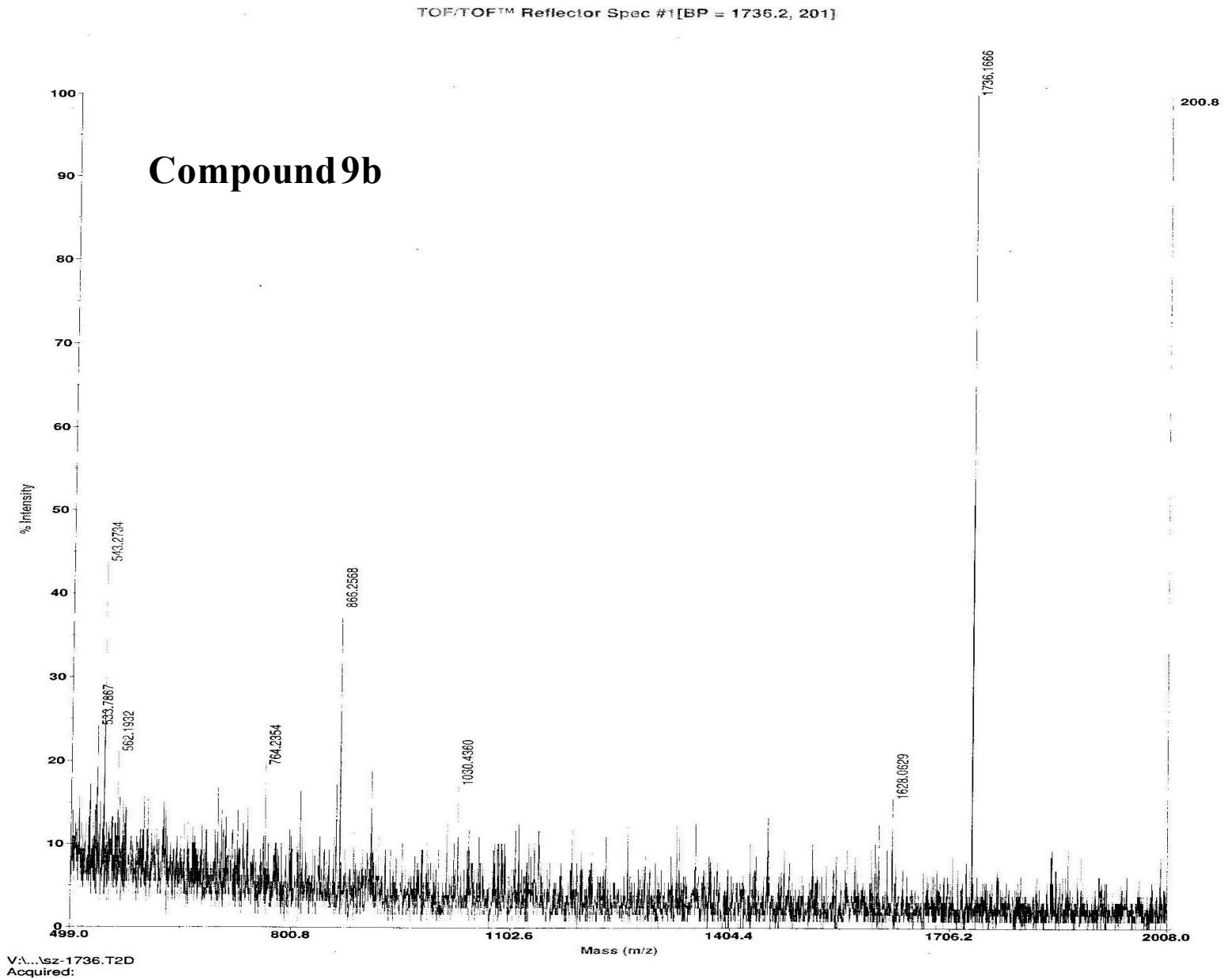


Figure S28. MALDI-TOF MS of compound **9b**

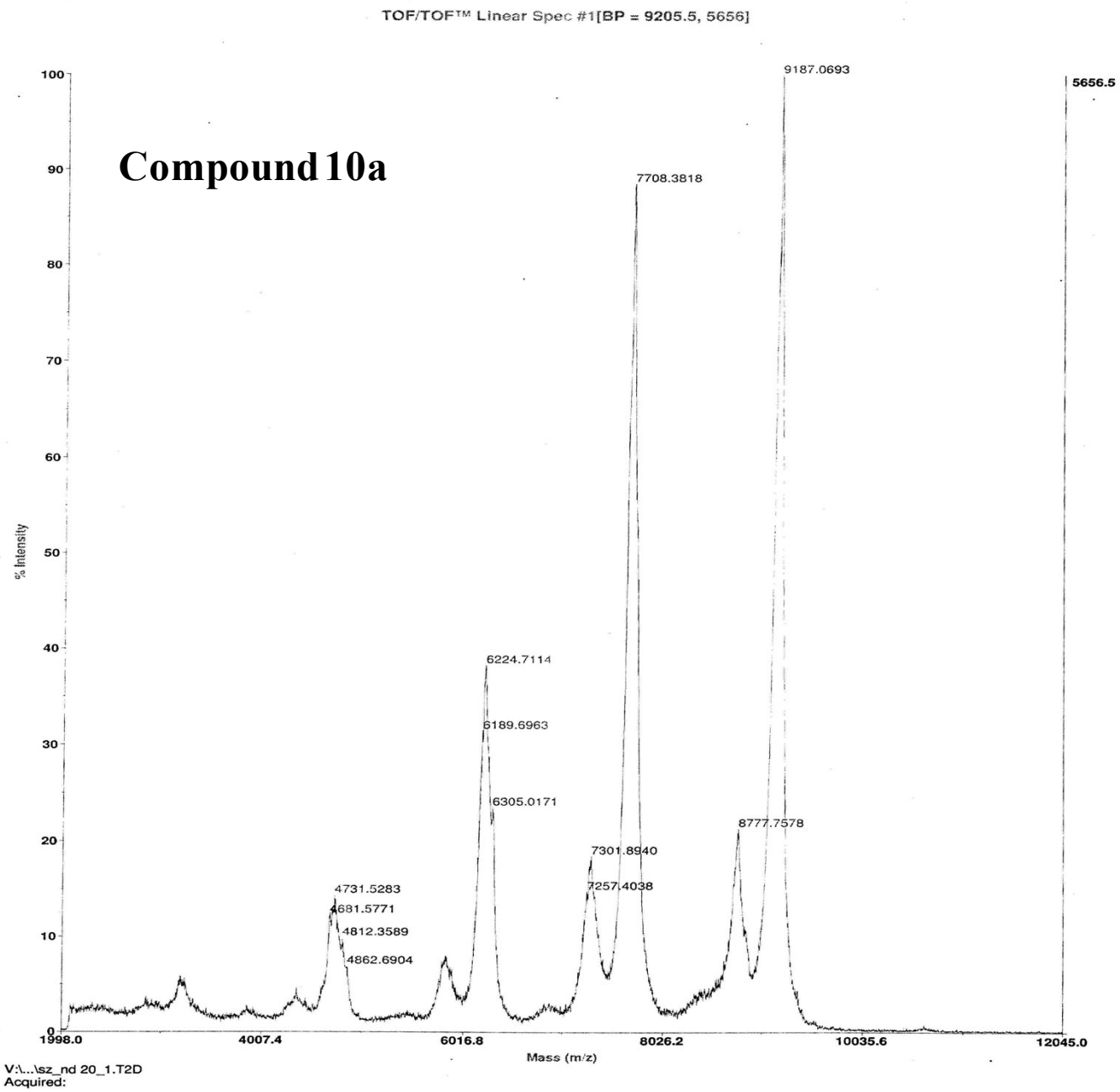


Figure S29. MALDI-TOF MS of compound **10a**

System

Temperature (°C):	25.0	Duration Used (s):	80
Count Rate (kcps):	5.1	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	11

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	1069	Peak 1:	1.762	63.0	0.2910
PdI:	1.000	Peak 2:	311.8	37.0	48.07
Intercept:	0.280	Peak 3:	0.000	0.0	0.000

Result quality Refer to quality report

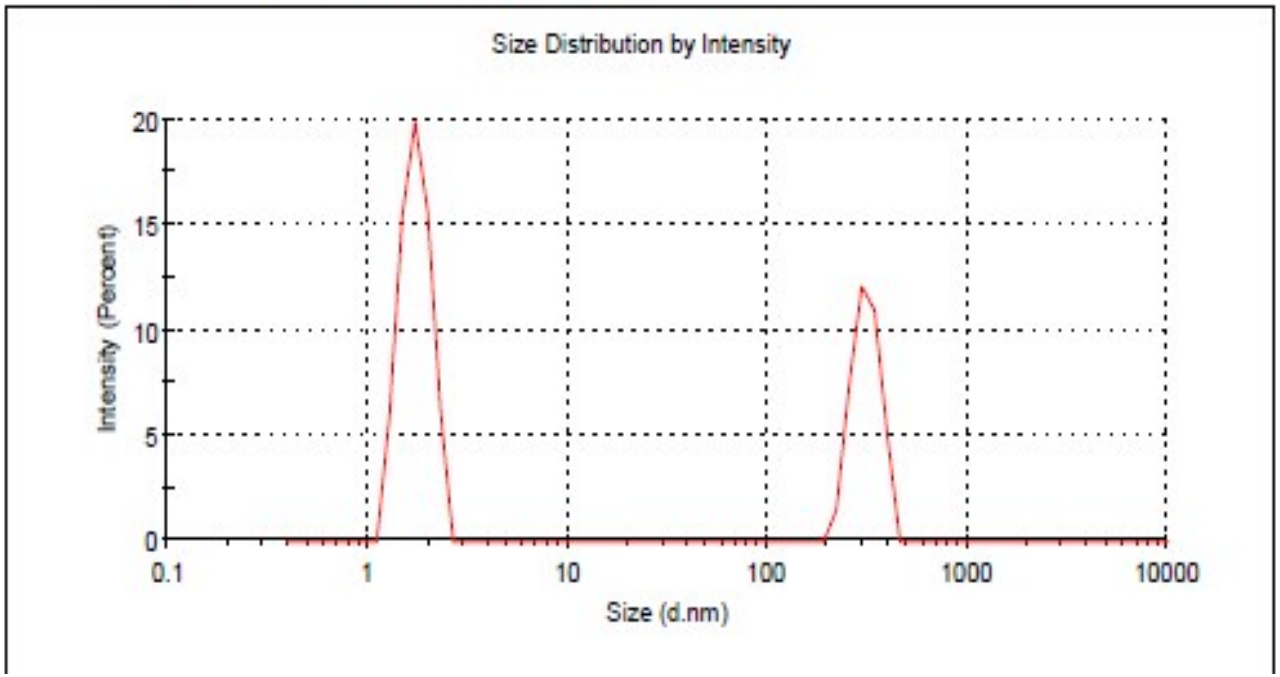


Figure S30: DLS curves of glycodendrimer **10a**

For DLS experiment, glycodendrimer **10a** was dissolved in in DMSO (1mg of dendrimer/ml) and then mixed with PBS (phosphate Buffered Saline) in the ratio 1:2, respectively. Solution was filtered by whatmann paper-1 and then after used for DLS analysis.

Gel Permiation chromatography;

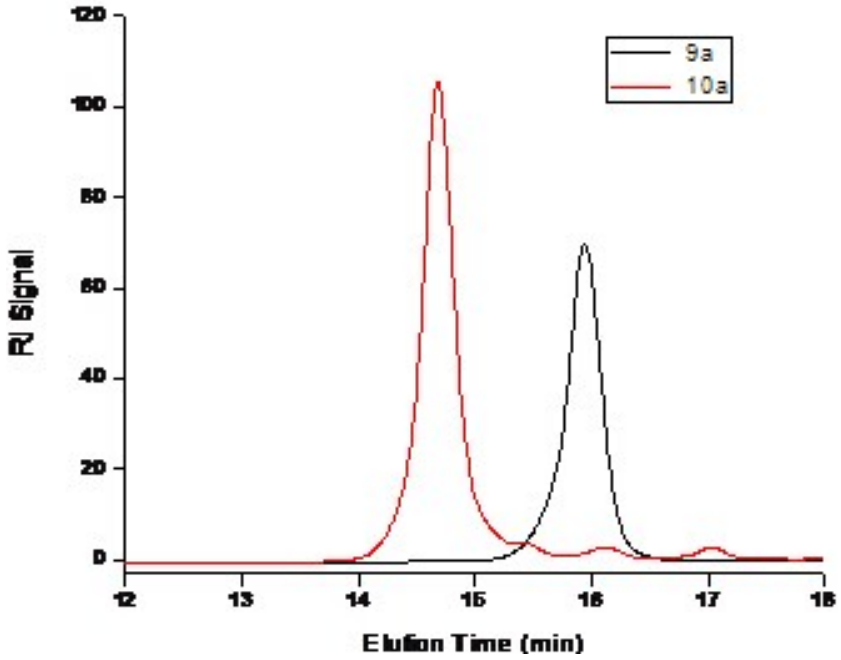


Figure S31: Gel permeation chromatogram of glycodendrimer **9a** and **10a** using THF as eluent.

9a: **Mn** = 2750; **PDI**= 1.03; **Mw** = 2835;

10a: **Mn** = 6,406; **PDI** = 1.01; **Mw** = 6,525;

8. Single Crystal X-Ray data and structure of compound 1.

X-ray data collection and structure refinement

Intensity data were collected on a Brüker APEX-II CCD diffractometer using a graphite monochromated Mo-Kα radiation (λ = 0.71073 Å) at 273 K. Data collections were performed using φ and ω scan. Olex2[1]was used as the graphical interface and the structures were solved with the ShelXT[2,3]structure solution program using intrinsic phasing. The models were refined with ShelXL[3]with full matrix least squares minimisation on F^2. All non-hydrogen atoms were refined anisotropically. The process has been validated through the IUCR site (International Union of Crystallography) and no A and B level of error was found. Hence the cystal solved is validated. Further information on the crystal structure (excluding structure factors) has been given in table 1(Supporting Information) and also deposited in the Cambridge Crystallographic Data Centre as supplementary publication number 1872402. Copies of the data can be obtained free of charge upon application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033. e-mail: deposit@ccdc.cam.ac.uk) or via internet.

1. O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard, H. Puschmann, A complete structure solution, refinement and analysis program. J. Appl. Cryst. 42 (2009) 339-341.
2. G. M. Sheldrick, SHELXTL-97, Program for X-ray Crystal Structure Solution; University of Göttingen: Göttingen, Germany, 1997.
3. G. M. Sheldrick, Crystal structure refinement with ShelXL. ActaCryst. C27 (2015) 3-8.

Procedure for crystallization of compound 1

For crystallization a mixture of ethyl acetate and hexane (3:7) has been used and kept in dark place at temperature 25 ^{o}C. The single crystal appeared after two days was isolated in its initial state of growth.

Table S3. Crystallographic refinement data for compound **1**

Compound 1	
Empirical Formula	$C_{30}H_{30}O_6$
Formula Weight	486.54
Crystal System	Triclinic
Space group	P -1
a (Å)	8.6582(4)
b (Å)	8.9482(4)
c (Å)	9.1287(4)
α (°), β (°) ,γ(°)	99.800(2), 94.649(2), 108.311(2)
V (Å^3)	654.76(5)
Z	1
Density (calc)	1.234
F(000)	258.0
μ (mm^{-1})	0.085
Crystal Size [mm]	0.1 x 0.7 x 0.6
Temperature (K)	273(2)
Radiation	0.71073
θ Min-Max [°]	2.860, 26.448
Limiting indices	*-10⇐h⇐10, -11⇐k⇐11, -11⇐l⇐11*
Tot.,UniqData, R(int)	15011, 2688, 0.0477
Obs. data [I > 2.0 σ(I)]	0.0466- 0.1128
Nref, Npar	2697, 163

R1, wR2, S	0.0555(2160), 0.1628(2683), 1.047
CCDC	1872402

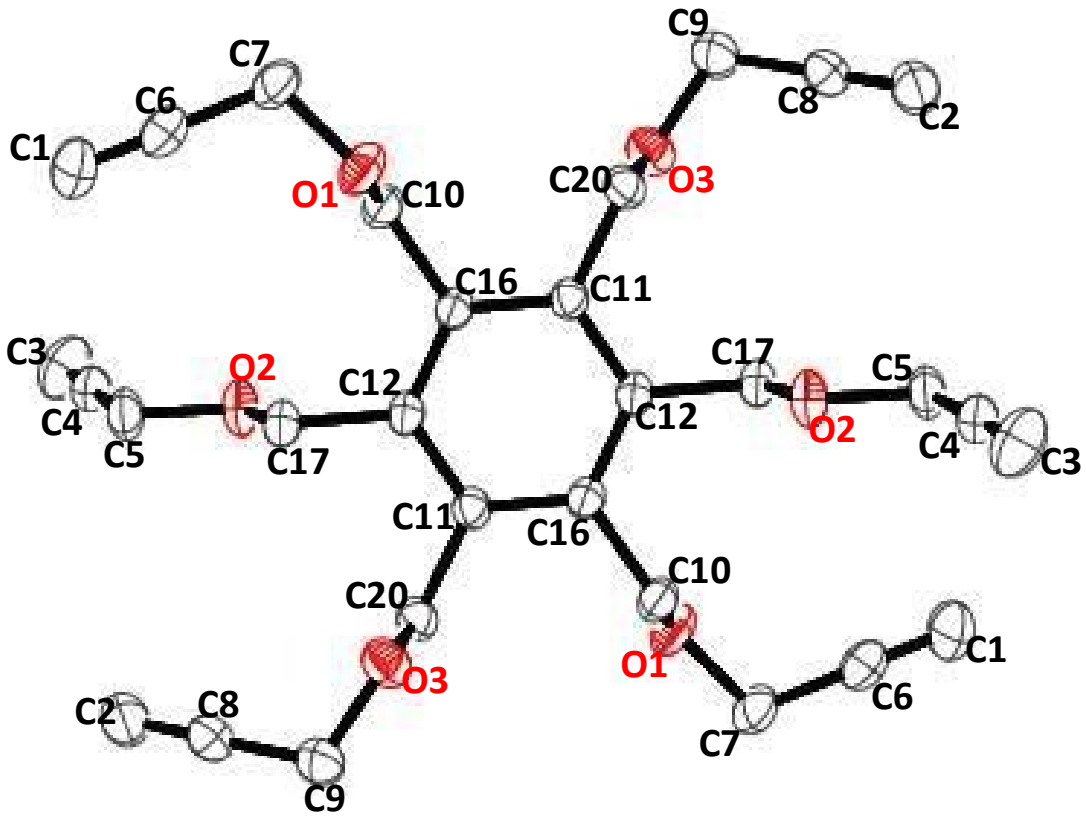


Figure S32. Molecular structure of compound **1**. Thermal ellipsoids of carbon, nitrogen and oxygen are set at 40% probability.

Table S4. **Bond angles of compound 1**

Number	Atom1	Atom2	Atom3	Angle
1.	C10	O1	C7	112.0(1)
2.	C17	O2	C5	111.4(1)
3.	C20	O3	C9	112.0(1)
4.	C11	C12	C17	119.6(1)
5.	C11	C12	C16	120.5(1)
6.	C17	C12	C16	120.0(1)
7.	C11	C16	C10	120.7(1)
8.	C11	C16	C12	119.7(1)
9.	C10	C16	C12	119.6(1)
10.	C12	C11	C16	119.8(1)
11.	C12	C11	C20	119.8(1)
12.	C16	C11	C20	120.4(1)
13.	O3	C20	C11	109.6(1)
14.	O3	C20	H20A	109.8
15.	O3	C20	H20B	109.7
16.	C11	C20	H20A	109.7
17.	C11	C20	H20B	109.7
18.	H20A	C20	H20B	108.2
19.	O2	C17	C12	107.9(1)
20.	O2	C17	H17A	110.1
21.	O2	C17	H17B	110.1

22.	C12	C17	H17A	110.1
23.	C12	C17	H17B	110.1
24.	H17A	C17	H17B	108.4
25.	O1	C10	C16	109.2(1)
26.	O1	C10	H10A	109.8
27.	O1	C10	H10B	109.8
28.	C16	C10	H10A	109.8
29.	C16	C10	H10B	109.8
30.	H10A	C10	H10B	108.3
31.	O2	C5	H5A	109.9
32.	O2	C5	H5B	109.9
33.	O2	C5	C4	108.8(1)
34.	H5A	C5	H5B	108.3
35.	H5A	C5	C4	109.9
36.	H5B	C5	C4	109.9
37.	C5	C4	C3	179.0(2)
38.	C7	C6	C1	178.5(2)
39.	O1	C7	C6	112.6(1)
40.	O1	C7	H7A	109.1
41.	O1	C7	H7B	109.1
42.	C6	C7	H7A	109.1
43.	C6	C7	H7B	109.1
44.	H7A	C7	H7B	107.8

45.	C6	C1	H1	180
46.	C9	C8	C2	178.9(2)
47.	O3	C9	C8	112.5(1)
48.	O3	C9	H9A	109.1
49.	O3	C9	H9B	109.1
50.	C8	C9	H9A	109.1
51.	C8	C9	H9B	109.1
52.	H9A	C9	H9B	107.9
53.	C4	C3	H3	180
54.	C8	C2	H2	180
55.	C10	O1	C7	112.0(1)
56.	C17	O2	C5	111.4(1)
57.	C20	O3	C9	112.0(1)
58.	C16	C12	C11	120.5(1)
59.	C16	C12	C17	120.0(1)
60.	C11	C12	C17	119.6(1)
61.	C12	C16	C11	119.7(1)
62.	C12	C16	C10	119.6(1)
63.	C11	C16	C10	120.7(1)
64.	C12	C11	C16	119.8(1)
65.	C12	C11	C20	119.8(1)
66.	C16	C11	C20	120.4(1)
67.	O3	C20	C11	109.6(1)

68.	O3	C20	H20A	109.8
69.	O3	C20	H20B	109.7
70.	C11	C20	H20A	109.7
71.	C11	C20	H20B	109.7
72.	H20A	C20	H20B	108.2
73.	O2	C17	C12	107.9(1)
74.	O2	C17	H17A	110.1
75.	O2	C17	H17B	110.1
76.	C12	C17	H17A	110.1
77.	C12	C17	H17B	110.1
78.	H17A	C17	H17B	108.4
79.	O1	C10	C16	109.2(1)
80.	O1	C10	H10A	109.8
81.	O1	C10	H10B	109.8
82.	C16	C10	H10A	109.8
83.	C16	C10	H10B	109.8
84.	H10A	C10	H10B	108.3
85.	O2	C5	H5A	109.9
86.	O2	C5	H5B	109.9
87.	O2	C5	C4	108.8(1)
88.	H5A	C5	H5B	108.3
89.	H5A	C5	C4	109.9
90.	H5B	C5	C4	109.9

91.	C5	C4	C3	179.0(2)
92.	C7	C6	C1	178.5(2)
93.	O1	C7	C6	112.6(1)
94.	O1	C7	H7A	109.1
95.	O1	C7	H7B	109.1
96.	C6	C7	H7A	109.1
97.	C6	C7	H7B	109.1
98.	H7A	C7	H7B	107.8
99.	C6	C1	H1	180
100.	C9	C8	C2	178.9(2)
101.	O3	C9	C8	112.5(1)
102.	O3	C9	H9A	109.1
103.	O3	C9	H9B	109.1
104.	C8	C9	H9A	109.1
105.	C8	C9	H9B	109.1
106.	H9A	C9	H9B	107.9
107.	C4	C3	H3	180
108.	C8	C2	H2	180

Table S5. **Bond lengths of compound 1**

Number	Atom1	Atom2	Cyclicity	Length
1.	O1	C10	acyclic	1.422(2)
2.	O1	C7	acyclic	1.428(2)
3.	O2	C17	acyclic	1.429(1)
4.	O2	C5	acyclic	1.415(2)
5.	O3	C20	acyclic	1.429(2)
6.	O3	C9	acyclic	1.432(2)
7.	C12	C11	cyclic	1.402(2)
8.	C12	C17	acyclic	1.512(2)
9.	C12	C16	cyclic	1.402(2)
10.	C16	C11	cyclic	1.402(2)
11.	C16	C10	acyclic	1.508(2)
12.	C16	C12	cyclic	1.402(2)
13.	C11	C20	acyclic	1.515(2)
14.	C20	H20A	acyclic	0.97
15.	C20	H20B	acyclic	0.97
16.	C17	H17A	acyclic	0.97
17.	C17	H17B	acyclic	0.97
18.	C10	H10A	acyclic	0.97
19.	C10	H10B	acyclic	0.97
20.	C5	H5A	acyclic	0.97
21.	C5	H5B	acyclic	0.97

22.	C5	C4	acyclic	1.454(2)
23.	C4	C3	acyclic	1.165(2)
24.	C6	C7	acyclic	1.466(2)
25.	C6	C1	acyclic	1.172(2)
26.	C7	H7A	acyclic	0.97
27.	C7	H7B	acyclic	0.97
28.	C1	H1	acyclic	0.93
29.	C8	C9	acyclic	1.453(3)
30.	C8	C2	acyclic	1.163(3)
31.	C9	H9A	acyclic	0.97
32.	C9	H9B	acyclic	0.97
33.	C3	H3	acyclic	0.93
34.	C2	H2	acyclic	0.93
35.	O1	C10	acyclic	1.422(2)
36.	O1	C7	acyclic	1.428(2)
37.	O2	C17	acyclic	1.429(1)
38.	O2	C5	acyclic	1.415(2)
39.	O3	C20	acyclic	1.429(2)
40.	O3	C9	acyclic	1.432(2)
41.	C12	C11	cyclic	1.402(2)
42.	C12	C17	acyclic	1.512(2)
43.	C16	C11	cyclic	1.402(2)
44.	C16	C10	acyclic	1.508(2)

45.	C11	C20	acyclic	1.515(2)
46.	C20	H20A	acyclic	0.97
47.	C20	H20B	acyclic	0.97
48.	C17	H17A	acyclic	0.97
49.	C17	H17B	acyclic	0.97
50.	C10	H10A	acyclic	0.97
51.	C10	H10B	acyclic	0.97
52.	C5	H5A	acyclic	0.97
53.	C5	H5B	acyclic	0.97
54.	C5	C4	acyclic	1.454(2)
55.	C4	C3	acyclic	1.165(2)
56.	C6	C7	acyclic	1.466(2)
57.	C6	C1	acyclic	1.172(2)
58.	C7	H7A	acyclic	0.97
59.	C7	H7B	acyclic	0.97
60.	C1	H1	acyclic	0.93
61.	C8	C9	acyclic	1.453(3)
62.	C8	C2	acyclic	1.163(3)
63.	C9	H9A	acyclic	0.97
64.	C9	H9B	acyclic	0.97
65.	C3	H3	acyclic	0.93
66.	C2	H2	acyclic	0.93

9. References:

1. K. B. Mishra, R. C. Mishra, and V. K. Tiwari, *RSC Adv.*, 2015, **5**, 51779-51789.
2. R. Das and B. Mukhopadhyay, *Tetrahedron Lett.*, 2016, **57**, 1775–1781.
3. Y. M. Chabre, C. Contino-Pépin, V. Placide, C. S. Tze and R. Roy, *J. Org. Chem.*, 2008, **73**, 5602–5605.
4. A. K. Singh, P. Prakash, R. Singh, N. Nandy, Z. Firdaus, M. Bansal, R. K. Singh, A. Srivastava, J. K. Roy, B. Mishra and R. K. Singh, *Front. Microbiol.*, 2017, **8**, 1–17