Supplementary Material (ESI) for Chemical Communications

Modular Synthesis of Multivalent Glycoarchitectures and their Unique Selectin Binding Behavior

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Supporting Information

1. General Information

^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AC 250 (250 and 67.5 MHz for ^{1}H and ^{13}C, respectively), ECX 400 (400 MHz and 100 MHz for ^{1}H and ^{13}C, respectively) as well as Delta JEOL Eclipse 500 (500 and 125 MHz for ^{1}H and ^{13}C respectively) spectrometers at 25 ^{o}C. AMX 500 and ECX 400 were used to record high resolution ^{13}C NMR. The spectra were calibrated using the residual solvent signal as internal standard. FT-IR spectra were recorded on a Nicolet 5 SXC FT-IR spectrometer operating from 4000-400 cm^{-1} as film on potassium bromide plates from Aldrich. ESI-MS spectra samples were measured on an Agilent 6210 ESI-TOF, Agilent Technologies, Santa Clara, CA, USA. Solvent flow rate was adjusted to 4 µL/min, Spray voltage set to 4.000 V. Drying gas flow rate was set up to 15 psi (1 bar). All other parameters were adjusted for a maximum abundance of the relative $[M+H]^{+}$.

2. Materials

Reactions requiring dry or oxygen-free conditions were carried out under argon (Schlenk conditions). All reagents and solvents were purchased from commercial suppliers and used without further purification. Dry solvents were used as obtained by Acros or Aldrich in crown cap bottles over molecular sieve and under inert atmosphere (H_2O ≤0.005%). All other solvents were reagent grade and used as received. TLC was performed on aluminium-backed silica gel plates (60 F_{254}, 0.2 mm, Merck), detection was effected by charring with 10% sulphuric acid in ethanol, followed by heat treatment. Flash chromatography was performed on silica gel 60 Å (230-400 mesh, particle size 0.035- 0.070 mm, Acros). Dialysis was performed in benzoylated cellulose dialyse tubes (SIGMA-ALDRICH, (MWCO = 500

$g \cdot mol^{-1}$, 1000 $g \cdot mol^{-1}$). The purification time was between 24 and 48 h and the solvent was changed after every 4 hours. Azido-galactose moieties **3 a, b** were synthesized, following a literature procedure reported by Joosten and Pieters, *via* a nucleophilic substitution of the corresponding bromide with sodium azide.[1]

Reference

[1] J. A. F. Joosten, V. Loimaranta, C. C. M. Appeldoorn, S. Haataja, F. A. El Maate, R. M. J. Liskamp, J. Finne and R. J. Pieters, *J. Med. Chem.*, 2004, **47**, 6499.

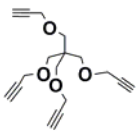
General procedure for the synthesis of alkyne derivates

Synthesis of hyperbranched polyglycerol propargylethers (**2**)

To a stirred solution of polyglycerol (8.26 g, 110 mmol OH groups) in 80 ml anhydrous DMF, NaH (5.81 g, 242 mmol, 2.2 eq., 95 %) was added. After stirring for 3 h at 0 ^{o}C, and later at room temperature, the mixture was cooled down again to 0 °C and propargyl bromide (22.00 g, 185 mmol, 1.7 eq.) was added slowly *via* syringe over a 20 minute period. The color of the solution changed to brown and a precipitation was observed. The ice bath was removed and after stirring for 16 hours at room temperature the reaction was quenched with water. Following, extraction with ethyl acetate (3 x 60 ml), the combined organic layers were concentrated *in vacuo* and the crude product was purified by dialysis in chloroform (48 h) to obtain a light brown viscous oil in 87 % chemical yield.

^{1}H NMR (500 MHz, $CDCl_3$): δ(ppm) = 4.29 - 4.14 ($OC\underline{H}_2C{\equiv}CH$), 3.84 - 3.52 (HPG backbone), 2.45 ($C{\equiv}C\underline{H}$), 1.40 ($C\underline{H}_2$ - HPG starter unit), 0.80 ($C\underline{H}_3$ -HPG starter unit); $^{13}C\{^{1}H\}$ NMR (125 MHz, $CDCl_3$): δ(ppm) = 80.5 - 57.5 (HPG backbone and alkyne groups), 30.3 ($C{\equiv}\underline{C}H$). IR (KBr): ν(cm^{-1}) = 3441 (ν-H_2O), 3289 (ν-$C{\equiv}\underline{C}H$), 2922, 2871 (ν-CH_3, ν-CH_2), 2114 (ν-C≡CH).

Tetrakis(2-propynyloxyymethyl)methane (**6**)



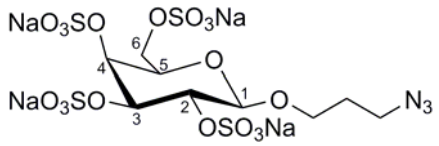
According to the general procedure tetrakis(2-propynyloxymethyl)methane (**6**) was obtained as white needles in 74 % chemical yield, after purification by flash column chromatography on silica gel eluting with ethyl acetate/hexane (2:8 v/v), followed by recrystallization in methanol.

^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) = 4.10 (d, ^{3}J = 2.4 Hz, 8H, HCCC$\underline{H}_2$), 3.51 (s, 8H, C(C$\underline{H}_2$)$_4$), 2.40 (t, ^{3}J = 2.3 Hz, 4H, $\underline{H}$CCCH$_2$); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ(ppm) = 80.0 (HC$\underline{C}$CH$_2$), 74.1 (H$\underline{C}$CCH$_2$), 68.9 (C($\underline{C}$H$_2$)$_4$), 58.6 (HCC$\underline{C}$H$_2$), 44.8 ($\underline{C}$(CH$_2$)$_4$); ESI-MS: Exact mass calculated for $C_{17}H_{20}O_4$ [M+Na]$^{+}$: 311.1361; found 311.1359.

General procedure for "de-O-acetylation" (3b)

Acetylated galactose (1 mmol) **3a** was dissolved in a mixture of dry MeOH/dry dichloromethane (1:3.33) and a solution of 30-% methanolic sodium methoxide (~ 0.08 ml) was added. The reaction mixture was stirred at room temperature for 24 h. At the end, water was added for entire solubilisation of desired compound and the solution was neutralized by addition of ion-exchange resin (Dowex-H^{+} 50 WX2 400) until pH 7, filtered, and the solvent was removed *in vacuo*. The residue was then lyophilized to give the deprotected compound in 90 % yield.

Sulfatation of galactose: 2, 3, 4, 6-Tetra-*O*-sulfonyl-*ß*-D-galactopyranoside (3c)



To a solution of 2, 3, 4, 6-tetra-hydroxy-*ß*-D-galactopyranoside (**3b**) (100 mg, 0.380 mmol) in 10 ml dry DMF, sulfur trioxide/Py complex (363 mg, 2.28 mmol) was added, and the mixture was stirred over 6 h at 60 ^{o}C and 18 h at room temperature. After removal of the solvent, residue was redissolved in distilled water and 1M NaOH solution was added until a pH of 11 was reached. Concentration in vacuo gave the crude product, which was further purified by dialysis in water (MWCO: 500). After evaporation of the solvent **3c** were obtained as pale yellow solid in 90 % chemical yield. ^{1}H NMR (400 MHz, CD_3OD): δ(ppm) = 5.12 (d, ^{3}J = 3.1 Hz, 1H, H-4), 4.76 (d, ^{3}J = 7.7 Hz, 1H, H-2), 4.61 (dd, ^{3}J = 3.1 Hz, J = 9.9 Hz, 1H, H-3), 4.42 (dd, ^{3}J = 7.6 Hz, J = 9.9 Hz, 1H, H-1), 4.36 (dd, ^{3}J = 3.5 Hz, J = 11.2 Hz, 1H, H-6a), 4.28 (dd, ^{3}J = 8.4 Hz, J =11.2 Hz, 1H, H-6b), 4.20 (dd, ^{3}J = 3.6 Hz, J = 8.7 Hz, 1H, H-5), 4.04-4.08 (td, ^{3}J = 5.8 Hz, J = 10.8 Hz, 1H, OC$\underline{H}$HCH$_2$CH$_2$N$_3$), 3.82-3.87 (td, ^{3}J = 6.4 Hz, J = 10.5 Hz, 1H, OCH$\underline{H}$CH$_2$CH$_2$N$_3$), 3.56 (t, 2H, OCH$_2$CH$_2$C$\underline{H}_2$N$_3$), 1.95-1.98 (m, 2H, OCH$_2$C$\underline{H}_2$CH$_2$N$_3$); ^{13}C{^{1}H} NMR (100 MHz, CD_3OD): δ(ppm) = 101.3 (C-1), 75.8, 75.7, 75.1, 72.2 (C-2, C-3, C-4, and C-5), 67.9 (O$\underline{C}$H$_2$CH$_2$CH$_2$N$_3$), 67.8 (C-6), 48.1

($OCH_2CH_2\underline{C}H_2N_3$), 28.5 ($OCH_2\underline{C}H_2CH_2N_3$); ESI-MS (negative mode): Exact mass calculated for $C_9H_{13}O_{18}N_3S_4Na_3$ $[M]^-$: 647.8775; found 647.8932.

General procedure for the click reaction catalyzed by Cu(I)

A solution of poly(propargylated)cluster (1 mmol) and azidopropylene galactose **3 a-c** (1.5 eq per propargyl) were suspended in a 1:1 mixture of water and tetrahydrofuran (4 ml). Sodium ascorbate (10 mol-%) was added, followed by copper(II) sulfate pentahydrate (5-10 mol-%). The heterogeneous mixture was stirred vigorously overnight (12-24 h), at which point it cleared and TLC analysis indicated complete consumption of the reactants. Extracted with ethyl acetate, mixture was washed with NH_4Cl, water and brine. After removing the solvents *in vacuo* the residue was purified by dialysis or flash column chromatography (in case of PE-derivatives).

Compound 4a

Product was obtained according to the general click procedure as (pale) crystals in 89 % chemical yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ(ppm) = 7.65 (C=C$\underline{H}$), 5.36 - 4.11 (Carbohydrate peaks and O$\underline{C}H_2$ from HPG), 3.84 - 3.45 (HPG backbone), 2.13 - 1.96 (m, $OCH_2\underline{C}H_2CH_2N$ (Gal), 4 Ac), 1.27 ($C\underline{H}_2$-HPG starter unit), 0.85 ($C\underline{H}_3$-HPG starter unit); $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ(ppm) = 170.3, 170.2, 170.0, 169.6 (4 C=O), 145.3 (C=CH), 123.0 (C=$\underline{C}$H), 101.2 (C-1, Gal), 77.5 – 61.2 (PG backbone, and from Gal: C-2, C-3, C-4 and C-5, ($O\underline{C}H_2CH_2CH_2N$), C-6), 46.7 ($OCH_2CH_2\underline{C}H_2N$), 29.7 ($OCH_2\underline{C}H_2CH_2N$), 20.9, 20.7, 20.5, 20.4 (4 Ac). IR (KBr): ν(cm^{-1}) = 2922 (ν-CH_3, ν-CH_2), 1370, 1215, 1059, no peak at 3288 and 2114 (ν-C≡CH), and at 2104 (ν-N_3), respectively.

Compound 4b

Product was obtained as colorless crystals in 92 % chemical yield. ^{1}H NMR (500 MHz, D_2O): δ(ppm) = 8.06 (C=C$\underline{H}$), 4.93 - 3.9 (Carbohydrate peaks and O$\underline{C}H_2$ from HPG), 3.76 – 3.55 (HPG backbone), 2.17 (s, $OCH_2\underline{C}H_2CH_2N$ (Gal)), 1.19 (m, $C\underline{H}_2$-HPG starter unit), 0.79 ($C\underline{H}_3$-HPG starter unit); $^{13}C\{^1H\}$ NMR (125 MHz, D_2O): δ(ppm) = 144.3 ($\underline{C}$=CH), 123.5 (C=$\underline{C}$H), 100.5 (C-1, Gal), 77.7 – 60.3 (HPG backbone, and from Gal: C-2, C-3, C-4, C-5, ($O\underline{C}H_2CH_2CH_2N$) and C-6), 47.7 ($OCH_2CH_2\underline{C}H_2N$), 29.7 ($OCH_2\underline{C}H_2CH_2N$). IR (KBr): ν($cm^{-1}$) = 3441 (ν-$H_2O$), 2922 (ν-$CH_3$, ν-$CH_2$), no peak at 3288 and 2114 (ν-C≡CH), 2104 (ν-N_3).

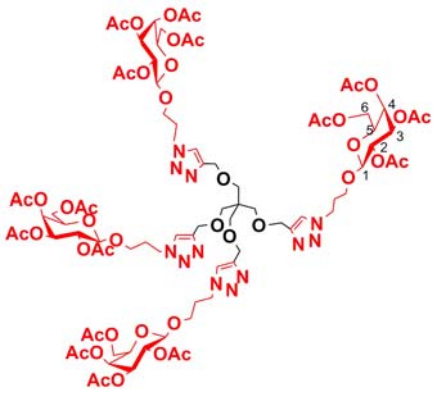
Compound 4c

Product was obtained as light brown crystals in 86 % chemical yield. ^{1}H NMR (500 MHz, D_2O): δ(ppm) = 8.28 (C=C<u>H</u>), 5.12 - 3.9 (Carbohydrate peaks and OC<u>H</u>$_2$ from HPG), 3.84 - 3.54 (HPG backbone and Carbohydrate peaks), 1.96 (m, $OCH_2C\underline{H}_2CH_2N$ (Gal)); ^{13}C{^{1}H} NMR (125 MHz, D_2O): δ(ppm) = 144.5 (<u>C</u>=CH), 126.7 (C=<u>C</u>H), 102.7 (C-1), 78.8 – 51.7 (HPG backbone, and from Gal: C-2, C-3, C-4, C-5, ($O\underline{C}H_2CH_2CH_2N$) and C-6), 45.6 ($OCH_2CH_2\underline{C}H_2N$), 30.9 ($OCH_2\underline{C}H_2CH_2N$); IR (KBr): ν(cm^{-1}) = 2922 (ν-CH_3, ν-CH_2), 1260 [S=O], 1148, 1056, 813 [C-O-S]; no peak at 3288 and 2114 left (ν-C≡CH), as well as at 2104 (ν-N_3). Sulfur content from elemental analysis for **4c**: 18.43 % S.

Table 1. Characterization of multivalent HPG 4a-c:
M_n of the Polymer core 3 kDa; DP_n (degree of polymerisation)~ 40; M_w/M_n = 1.18; DB (degree of branching) = 0.57.

Products	M_n of the polyglycerol derivates [Da]	Degree of functionalization [%]	Chemical yield [%]	DLS size distribution [nm]
1	3000	100	90	2
2	4330	88	87	6
4a	19430	88	89	-
4b	13540	88	92	7
4c	24400	88	86	10

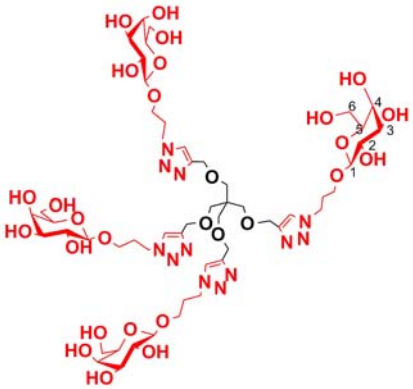
Tetramer (7a)



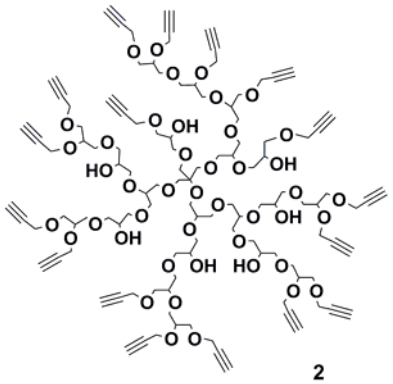
Compound was obtained as colorless foam in 85 % chemical yield after column chromatography purification (methanol:dichloromethane = 3 : 97). ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) = 7.57 (s, 4H, C=C<u>H</u>), 5.36 (d, ^{3}J = 2.5 Hz, 4H, H-3), 5.18 (dd, ^{3}J = 7.9 Hz,

J = 10.5 Hz, 4H, H-4), 5.00 (dd, ^3J = 3.4 Hz, J = 10.5 Hz, 4H, H-2), 4.51 (s, HC=CC$\underline{H}_2$O, 8H), 4.47 (d, ^3J = 7.9 Hz, 4H, H-1), 4.38 (m, 8H, H-6), 4.17 - 3.87 (m, 16H, H-5, OC$\underline{H}_2$CH$_2$CH$_2$N, OCH$_2$CH$_2$C$\underline{H}$HN), 3.50-3.44 (m, 12H, OCH$_2$CH$_2$CH$\underline{H}$N and C(C$\underline{H}_2$)$_4$), 2.16 (m, 2H, OCH$_2$C$\underline{H}_2$CH$_2$N), 1.95, 1.99, 2.06, 2.12 (s, 48 H, Ac); ^{13}C{^{1}H} NMR (400 MHz, CDCl$_3$): δ(ppm) = 170.2, 170.1, 169.9, 169.5 (4 Ac), 145.2 (C=CH), 122.6 (C=CH), 101.1 (C-1), 70.7 (C-3), 70.6 (C-2), 69.1 (C(CH$_2$)$_4$), 68.7 (C-5), 66.9 (C-4), 65.9 (OCH$_2$CH$_2$CH$_2$N), 64.8 (HC=CCH$_2$O), 61.1 (C-6), 46.5 (OCH$_2$CH$_2$CH$_2$N), 45.2 (C(CH$_2$)$_4$), 30.2 (OCH$_2$CH$_2$CH$_2$N), 20.8, 20.5, 20.4, 20.3 (4 Ac); ESI-MS Exact mass calculated for $C_{85}H_{120}N_{12}O_{44}$ [M+Na]$^+$: 2035.7413 ; found 2035.7395.

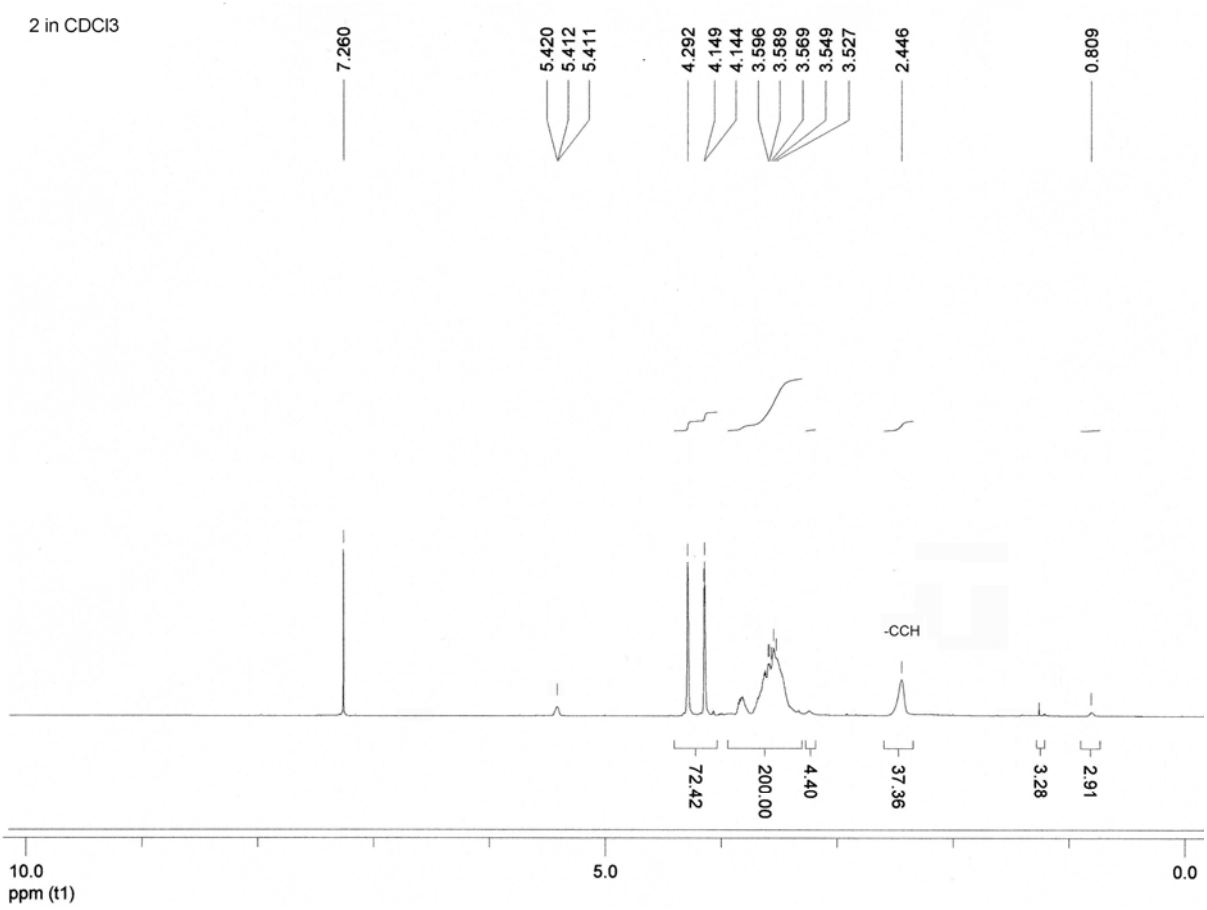
Tetramer (7b)



Compound obtained as colorless crystals in 94 % chemical yield. ^{1}H NMR (400 MHz, D$_2$O): δ(ppm) = 7.92 (s, 4H, C=CH), 4.47 (m, H-3, H-4, HC=CCH$_2$O, 16H), 4.30 (d, ^3J = 7.8 Hz, 4H, H-1), 3.88 (d, ^3J = 3.2 Hz, 4H, H-2), 3.83-3.49 (m, 28H, H-6, OCH$_2$CH$_2$CH$_2$N, H-5, OCH$_2$CH$_2$CH$_2$N), 3.33 (s, 8H, C(CH$_2$)$_4$), 2.13 (m, 8H, OCH$_2$CH$_2$CH$_2$N); ^{13}C{^{1}H} NMR (400 MHz, D$_2$O): δ(ppm) = 144.0 (C=CH), 125.1 (C=CH), 102.8 (C-1), 75.1 (C-3), 72.7 (C-2), 70.7 (C(CH$_2$)$_4$), 68.6 (C-5), 67.8 (C-4), 66.2 (OCH$_2$CH$_2$CH$_2$N), 63.5 (HC=CCH$_2$O), 60.9 (C-6), 47.0 (OCH$_2$CH$_2$CH$_2$N), 44.5 (C(CH$_2$)$_4$), 29.6 (OCH$_2$CH$_2$CH$_2$N); ESI-MS Exact mass calculated for $C_{53}H_{88}N_{12}O_{28}$ [M+Na]$^+$: 1363.5831; found 1363.5829.



2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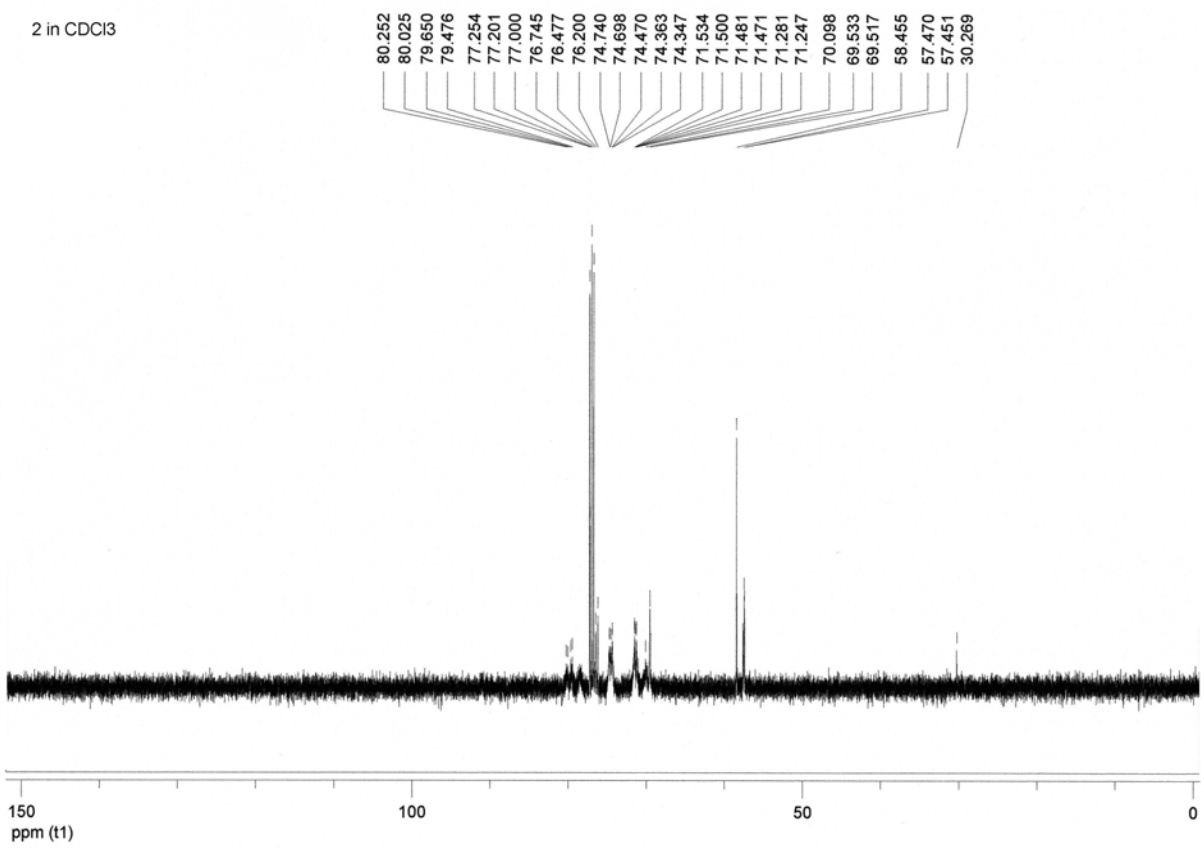
0.0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71.471
71.281
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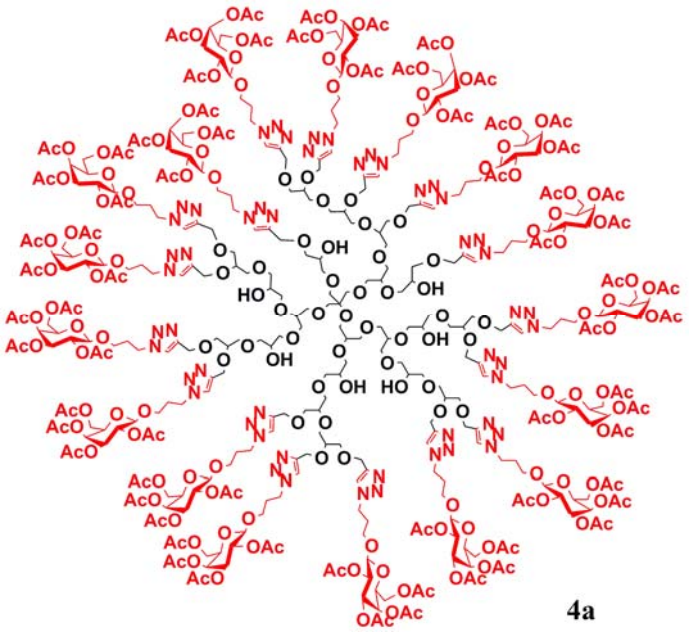
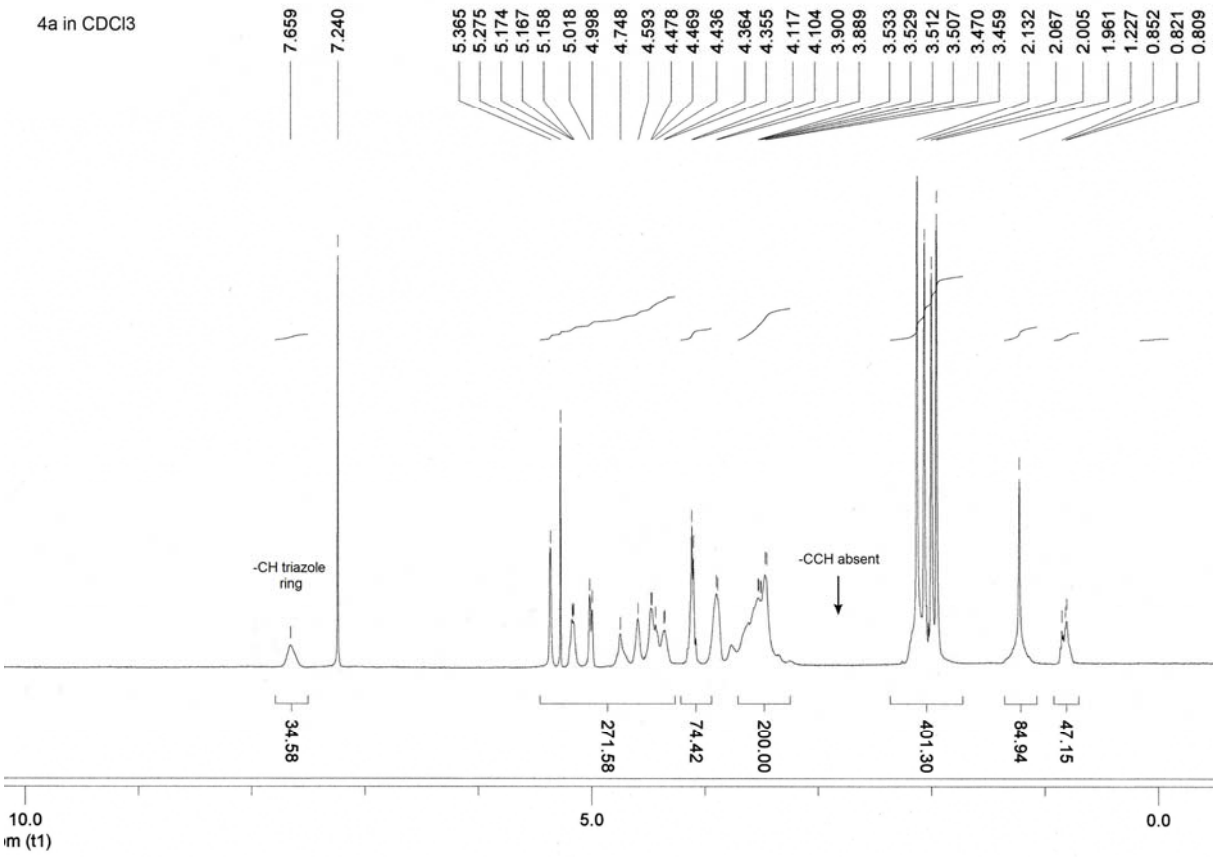
ppm (t1)



4a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3.470
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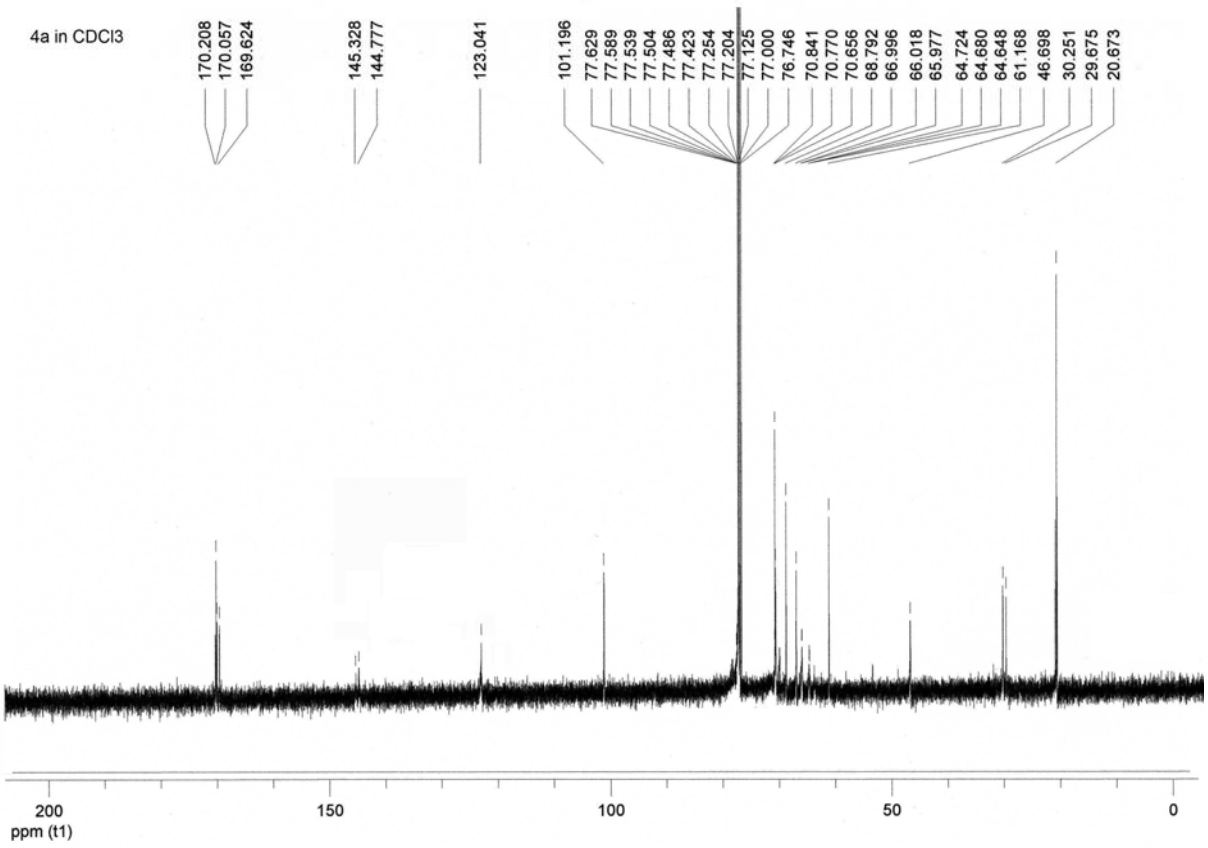
m (t1)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77.204
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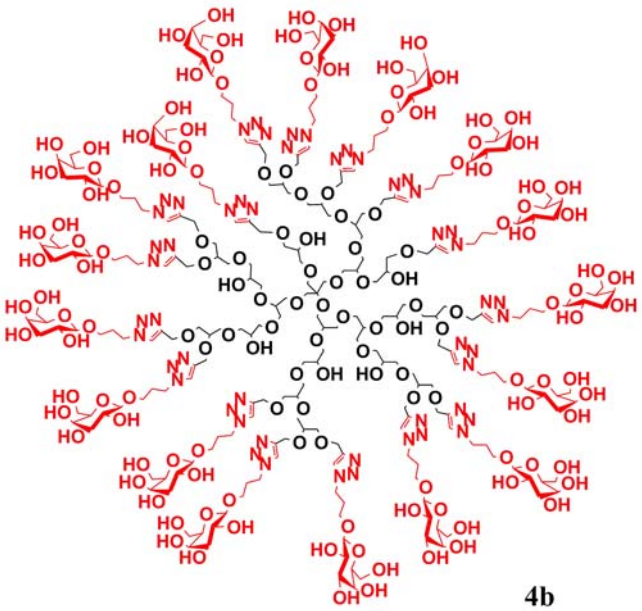
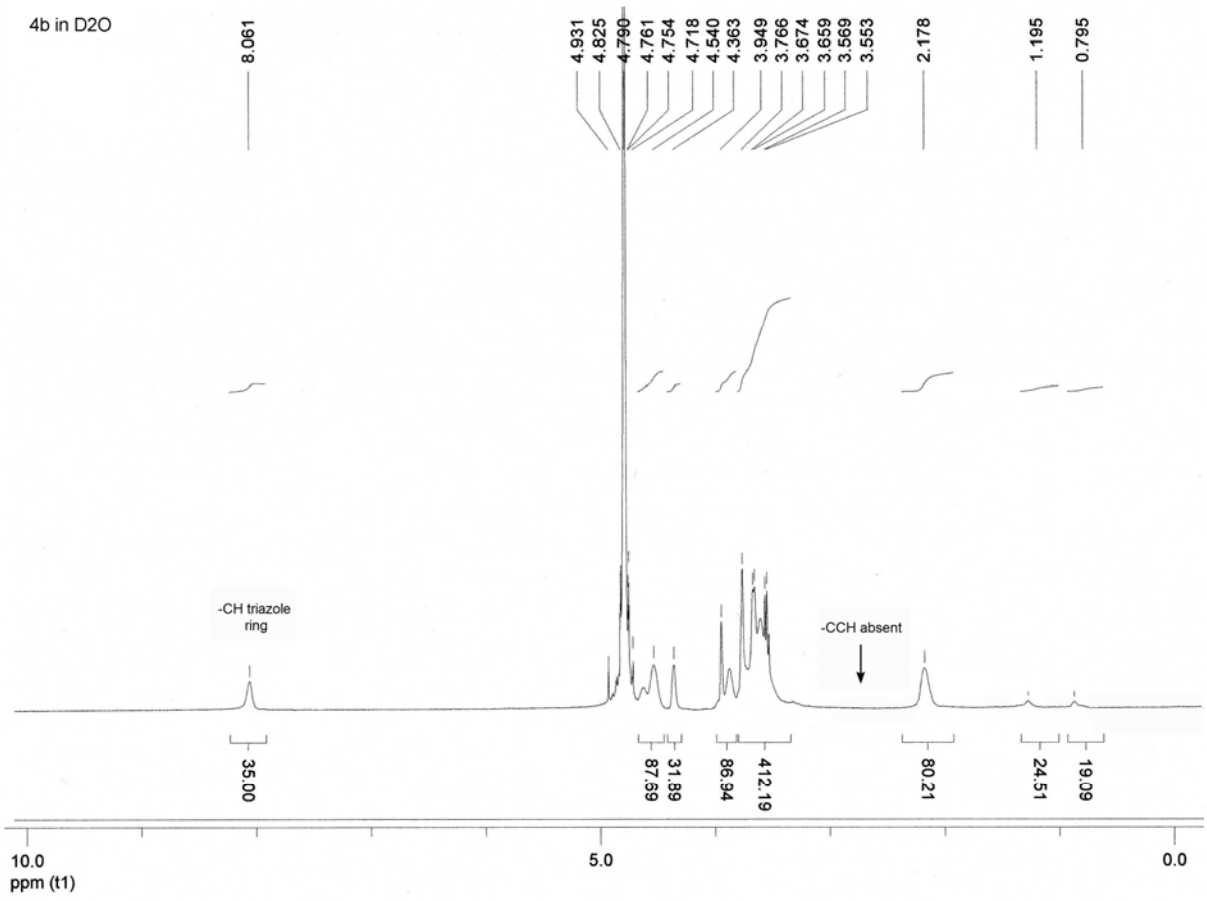


4b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3.674
3.659
3.569
3.553
2.178
1.195
0.795
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5.0
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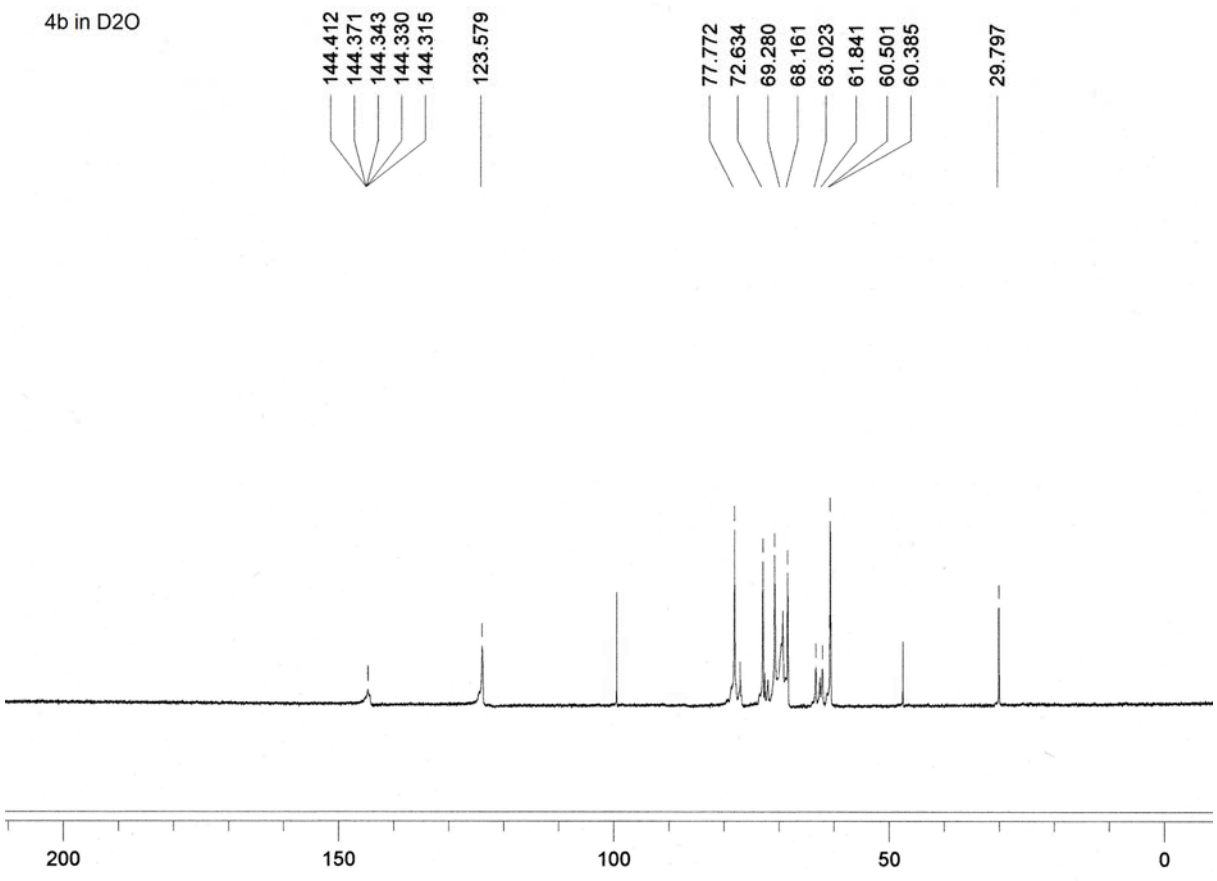
ppm (t1)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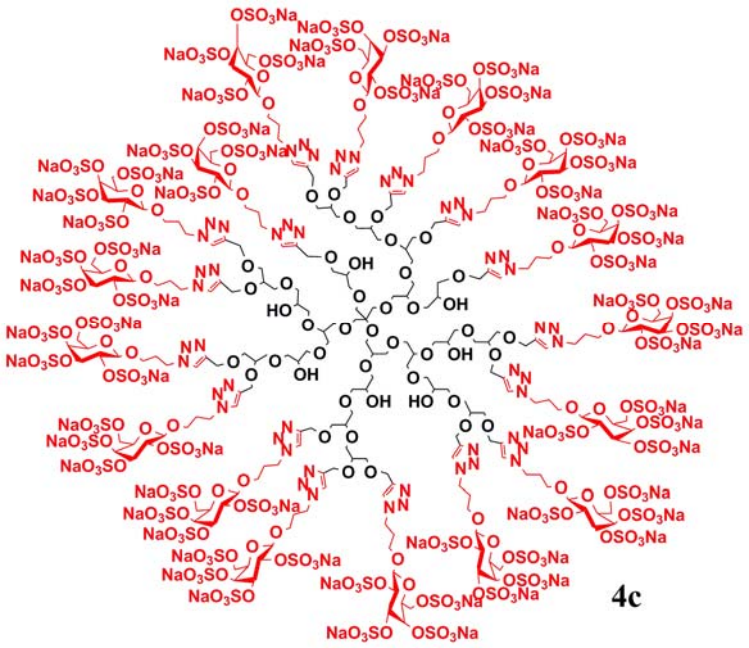
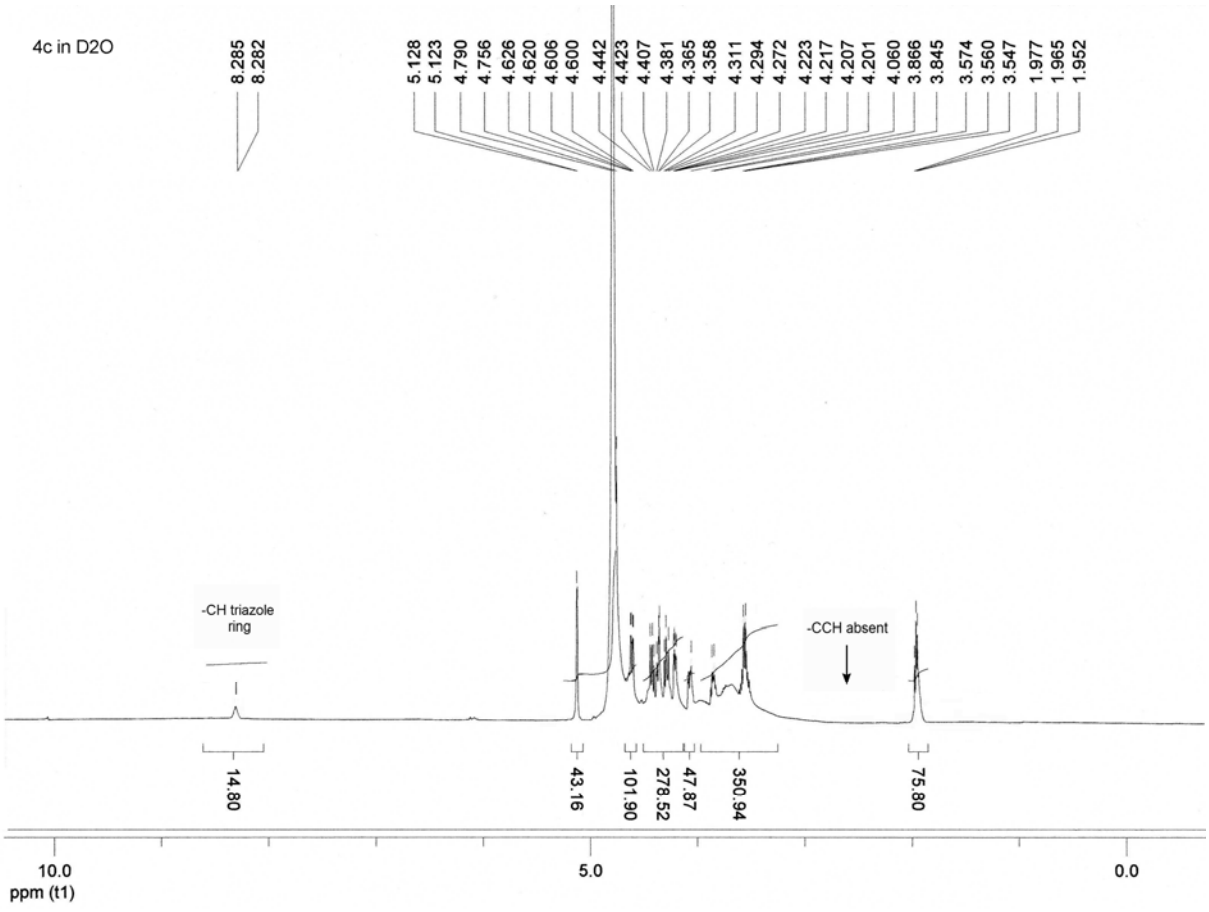
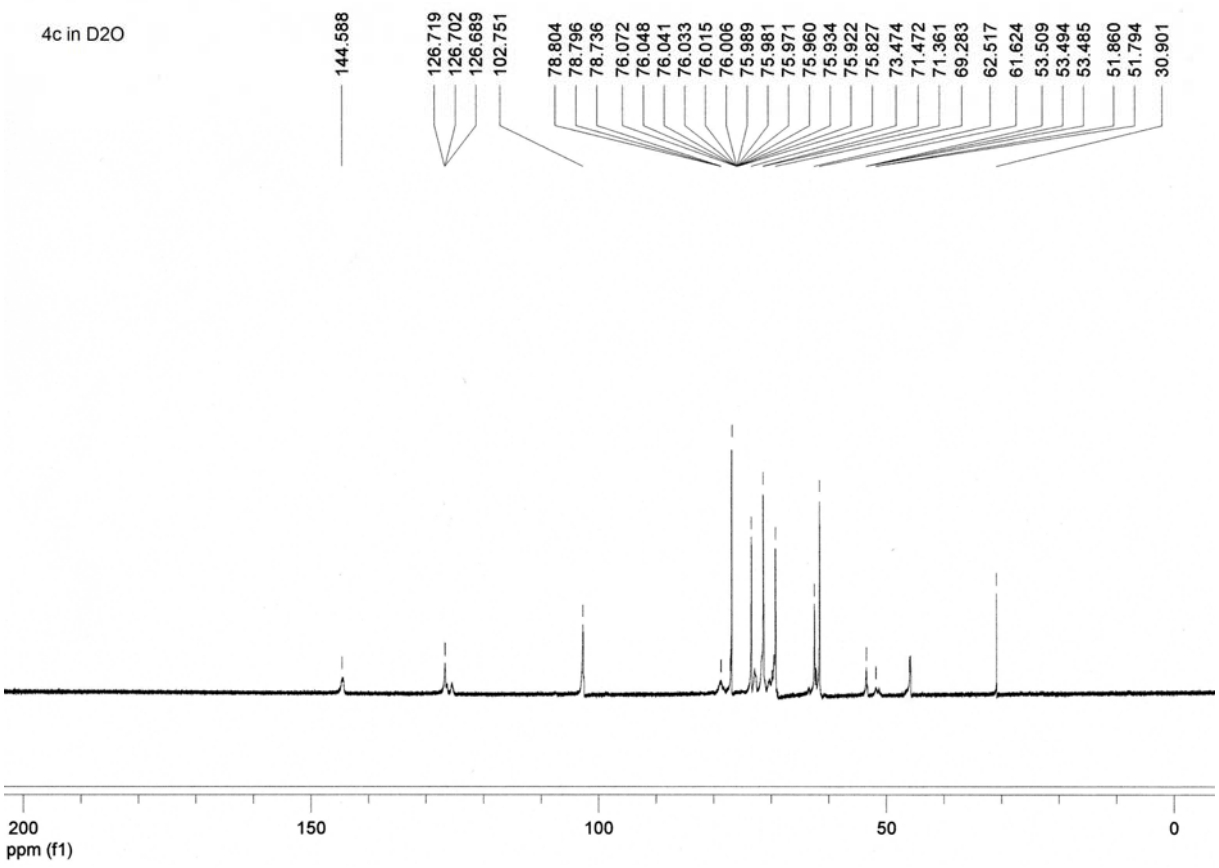
50
0



4c


4c in D2O
8.285
8.282
5.128
5.123
4.790
4.756
4.626
4.620
4.606
4.600
4.442
4.423
4.407
4.381
4.365
4.358
4.311
4.294
4.272
4.223
4.217
4.207
4.201
4.060
3.866
3.845
3.574
3.560
3.547
1.977
1.965
1.952
-CH triazole ring
-CCH absent
14.80
43.16
101.90
278.52
47.87
350.94
79.80
10.0
ppm (t1)
5.0
0.0


4c in D2O
144.588
126.719
126.702
126.689
102.751
78.804
78.796
78.736
76.072
76.048
76.041
76.033
76.015
76.006
75.989
75.981
75.971
75.960
75.934
75.922
75.827
73.474
71.472
71.361
69.283
62.517
61.624
53.509
53.494
53.485
51.860
51.794
30.901
200
150
100
50
0
ppm (f1)