SUPPORTING INFORMATION

Synthesis and Assessment of Glycosaminoglycan Priming Activity of Cluster-xylosides for Potential Use as Proteoglycan Mimetics

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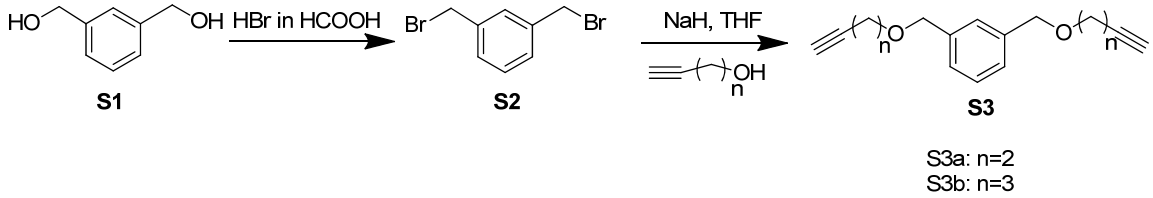
Experimental Sections:

Anhydrous solvents were purchased and used directly or dried over standard drying agents and freshly distilled prior to use. Reactions were monitored by TLC on silica gel 60 F-254 with detection by Von's reagent. Intermediate compounds were purified by flash chromatography columns using silica gel 60 (230–400 mesh) and were run under medium pressure at 5–7 psi. Final products were purified by high performance liquid chromatography (HPLC) on reverse phase C18 column (VYDAC 2.2 cm×25 cm) with solvent A (25 mM formic acid in water) and solvent B (95% Acetonitrile) at a flow rate of 5 ml/min in a linear gradient over 120 min starting with 0% B. All synthetic compounds were characterized by NMR using a Varian Mercury 400 MHz spectrometer. The compounds were also confirmed for their final structures by electrospray ionization mass spectrometry (ESI-MS) using a Finnigan LCQ mass spectrometer or a Bruker Q-Tof mass spectrometer in either positive or negative ion mode .

Scheme 1: Preparation of precursors carrying alkyne group for click-chemistry



Compound **S2**: 1, 3-Benzenedimethanol (3.618 mmol, 500 mg) was treated with 31 % HBr in acetic acid (5 ml). The reaction was stirred at room temperature for 2 hours. After addition of dichloromethane (10 ml), the organic solvent was washed with water, saturated bicarbonate solution, dried over Na_2SO_4 and evaporated. The crude material was purified by flash chromatography on silica gel to give the compound (**S2)** (900mg, 90% yield). ^{1}H NMR ($CDCl_3$): δ 7.42 (1H, s), 7.33 (3H, d, J = 1.2 Hz), 4.48 (4H, s)

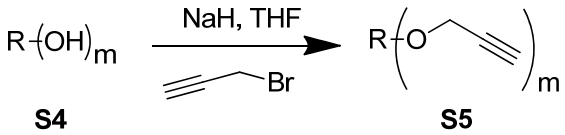
Compound **S3**: To an anhydrous THF solution (10 ml) of alcohol carrying an alkyne group (2 mmol) was added sodium hydride (3 mmol). The reaction mixture was stirred for 30 minutes at room temperature under argon atmosphere followed by the addition of (**S2)** (1 mmol). The mixture was stirred overnight at room temperature. THF solvent was evaporated. The crude material was dissolved in ethyl acetate, washed with water and then saturated sodium chloride solution, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography to give the compound (**S3**)

n	**S3**	Yield (%)	Reference
2	S3a	84%	N/A
3	S3b	97%	N/A

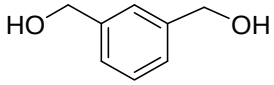
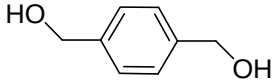
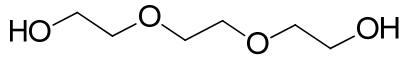
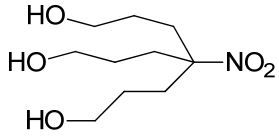
Compound **S3a**: ^{1}H NMR ($CDCl_3$) δ 7.34-7.25 (4H, m), 4.51 (4H, s), 3.58 (4H, t, J = 6.6 Hz), 2.51 (4H,dt, J = 6.8, 2.7 Hz), 2.00 (2H, t, J = 2.7 Hz)

Compound **S3b**: ^{1}H NMR ($CDCl_3$) δ 7.34-7.25 (4H, m), 4.51 (4H, s), 3.58 (4H, t, J = 6.3 Hz), 2.32 (4H,dt, J = 7.0, 2.7 Hz), 1.94 (2H, t, J = 2.7 Hz), 1.84 (4H, p, J = 6.3 Hz)

Scheme 2: Preparation of propargylated precursors for the synthesis of bis- and tris- xylosides



Compound **S5**: To an anhydrous THF solution (10 ml) of compound (**S4)** (1 mmol) was added sodium hydride ([m+1] mmol). The reaction mixture was stirred for 30 minutes at room temperature under argon atmosphere followed by addition of propargyl bromide ([m+1] mmol). The mixture was stirred overnight at room temperature. Solvent was evaporated. The crude material was dissolved in ethyl acetate and washed with water and saturated sodium chloride solution, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography to give the compound (**S5**)

S4	m	**S5**	Yield (%)	Reference*
	2	S5a	48%	N/A
	2	S5b	65%	[1]
	2	S5c	54%	[2]
	3	S5d	27%	N/A

* the procedure was slightly modified from the published reports

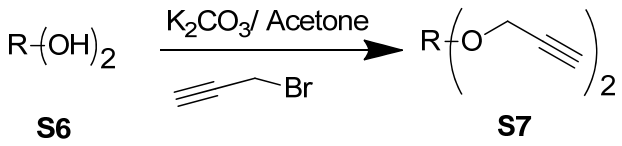
Compound **S5a**: ^{1}H NMR ($CDCl_3$) δ 7.39-7.30 (4H, m), 4.61 (4H, s), 4.18 (4H, d, J = 2.3 Hz), 2.47 (2H, t, J = 2.3 Hz)

Compound **S5b**: ^{1}H NMR ($CDCl_3$) δ 7.35 (4H, s), 4.61 (4H, s), 4.17 (4H, d, J = 2.3 Hz), 2.45 (2H, t, J = 2.3 Hz)

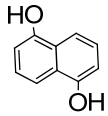
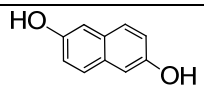
Compound **S5c**: ^{1}H NMR ($CDCl_3$) δ 4.12 (4H, d, J = 2.4 Hz), 3.61-3.58 (12H, m), 2.38 (2H, s)

Compound **S5d**: ^{1}H NMR ($CDCl_3$) δ 4.12 (6H, d, J = 2.4 Hz), 3.51 (6H, t, J = 5.9 Hz), 2.42 (3H, t, J = 2.4 Hz), 2.03-1.99 (6H, m), 1.55-1.48 (6H, m)

Scheme 3: Preparation of additional propargylated precursors



Compound **S7**: To the solution (10 ml) of compound (**S6)** (1 mmol) in acetone, was added potassium carbonate (3 mmol). The reaction mixture was stirred for 30 minutes at room temperature. Propargyl bromide (3 mmol) was then added and the mixture was stirred overnight. The reaction mixture was concentrated. The resulting crude material was dissolved in ethyl acetate, washed with water and saturated sodium chloride solution, dried over Na_2SO_4 and rotary evaporated under reduced pressure. The residue was purified by column chromatography to give the compound (**S7)**.

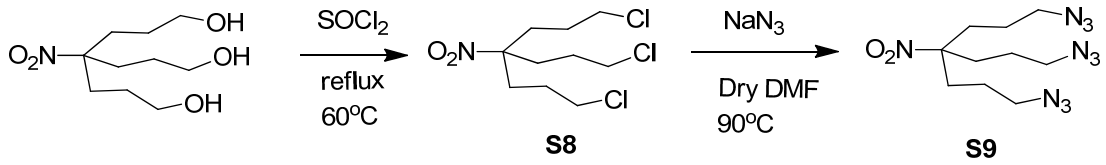
S6	**S7**	Yield (%)	Reference
	S7a	20%	[3]
	S7b	56%	[4]

***** The procedures were modified from the published reports

Compound **S7a**: ^{1}H NMR ($CDCl_3$) δ 7.89 (2H, d, *J* = 7.8), 7.38 (2H, t, *J* = 7.8), 6.97 (2H, d, *J* = 7.0), 4.88 (4H, s), 2.53 (2H, s)

Compound **S7b**: ^{1}H NMR ($CDCl_3$) δ 7.68 (2H, d, *J* = 8.6 Hz), 7.19 (4H, d, *J* = 12.1 Hz), 4.78 (4H, s), 2.54 (2H, s)

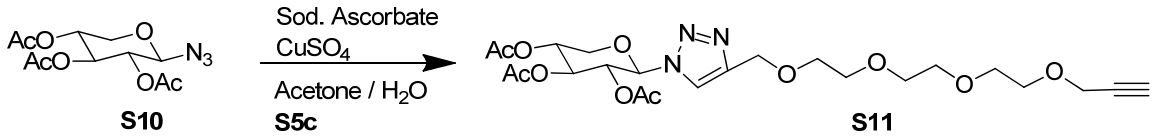
Scheme 4: Preparation of the precursor for the synthesis of tris-xyloside 9



Compound **S8**: Nitromethanetrispropanol (2.127 mmol) was dissolved in $SOCl_2$ (42.54 mmol). The reaction mixture was refluxed at 60 °C. After the completion of reaction, the reaction mixture was dissolved in ethyl acetate and washed with water, brine and then dried over Na_2SO_4. The final mass is 380 mg (62 %) and the crude product was used in the next step without any further purification. ^{1}H NMR ($CDCl_3$): δ 3.54 (6H, t, J = 6.4 Hz), 2.10-2.06 (6H, m), 1.74-1.67 (6H, m)

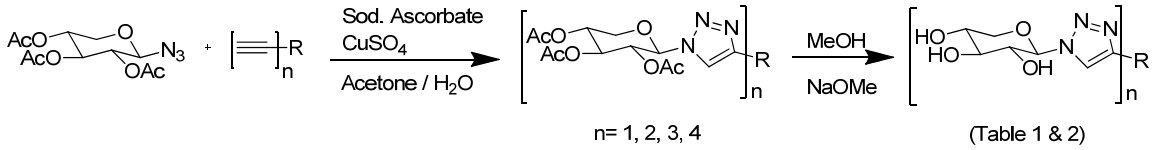
Compound **S9**: The compound (**S8)** (1.275 mmol) was dissolved in dry DMF (10 ml) followed by addition of sodium azide (6.38 mmol). The reaction was heated to 90 °C under stirring. After completion of the reaction as indicated by TLC, the reaction mixture was dissolved in ethyl acetate, washed with water and brine, and then dried over Na_2SO_4. The final mass is (270mg, 66%). ^{1}H NMR ($CDCl_3$): δ 3.28 (2H, t, J = 6.6 Hz), 1.95-1.91 (2H, m), 1.47-1.39 (2H, m)

Scheme 5: Synthesis of mono xylosides using click chemistry



Compound **S11**: To a solution of alkyne (**S5c)** (4.98 mmol) and xylosyl azide (**S10)** (1.66 mmol) in 8 ml of acetone and water (1:1) solvent mixture, sodium ascorbate (0.66 mmol) was added, followed by Cu_2SO_4. $5H_20$ (1.33 mmol) at room temperature, and the mixture was stirred until disappearance of compound (**S10)** (as indicated by TLC). At the end of the reaction, the reaction mixture was evaporated using rotary evaporator under reduced pressure to obtain the final product (360 mg, 41%).[5, 6] ^{1}H NMR (CD_3OD): δ 8.24 (1H, s), 6.02 (1H, d, *J* = 8.6 Hz), 5.58-5.47 (2H, m), 5.23-5.17 (1H, m), 4.64 (2H, s), 4.25 (1H, dd, *J* = 5.9, 11.3 Hz), 4.18 (2H, d, *J* = 2.3 Hz), 3.77 (1H, t, *J* = 10.9 Hz), 3.66-3.62 (12H, m), 2.83 (1H, t, *J* = 2.3 Hz), 2.04 (3H, s), 2.02 (3H, s), 1.83 (3H, s)

Scheme 6: Synthesis of cluster-xylosides using click chemistry[5, 6]



Various cluster-xylosides were synthesized using click chemistry and purified as described in the earlier section.

Xyloside **12***:* ^{1}H NMR (CD_3OD)*:* δ 8.60 (2H, s, triazolyl H), 8.34 (1H, s, Ar-H), 7.86 (2H, d, *J* = 7.8 Hz, Ar-H), 7.54 (1H, t, *J* = 7.8 Hz, Ar-H), 5.57 (2H, d, *J* = 9.0 Hz, H-1), 4.05 (2H, dd, *J* = 5.5, 11.1 Hz, H-5a), 3.96 (2H, t, *J* = 9.0 Hz, H-2), 3.68-3.75 (2H, m, H-4), 3.53 (2H, t, *J* = 9.0 Hz, H-3), 3.51 (2H, t, *J* = 10.9 Hz, H-5b);^{13}C NMR (CD_3OD)*:* δ 148.5, 132.4, 130.8, 126.6, 121.6, 90.5, 78.6, 74.0, 70.7, 69.9*;* ESI-MS*:* Calcd for $C_{20}H_{24}N_6O_8Na$*:* 499.1553 $[M+Na]^+$, found 498.9333.

Xyloside **13***:* ^{1}H NMR (CD_3OD)*:* δ 8.58 (1H, s, triazolyl H), 7.92 (1H, s, Ar-H), 5.57 (1H, d, *J* = 9.0 Hz, H-1), 4.05 (1H, dd, *J* = 5.5, 11.3 Hz, H-5a), 3.97(1H, t, *J* = 9.0 Hz, H-2), 3.69-3.75 (1H, m, H-4), 3.54 (1H, t, *J* = 9.0 Hz, H-3), 3.51 (1H, t, *J* = 10.9 Hz, H-5b)*;* ^{13}C NMR (CD_3OD)*:* δ 148.4, 131.9, 127.3, 121.5, 90.4, 78.6, 74.0, 70.7, 69.9*;* ESI-MS*:* Calcd for $C_{20}H_{25}N_6O_8$*:* 477.1734 $[M+H]^+$, found 476.8667.

Xyloside **14**: ^{1}H NMR (CD_3OD): δ 8.16 (2H, s, triazolyl H), 7.37 (1H, s, Ar-H), 7.33-7.28 (3H, m, Ar-H), 5.52 (2H, d, *J* = 9.4 Hz, H-1), 4.65 (4H, s), 4.58 (4H, s), 4.01(2H, dd, *J* = 5.5, 11.3 Hz, H-5a), 3.90 (2H, t, *J* = 9.0 Hz, H-2), 3.709-3.647 (2H, m, H-4), 3.50 (2H, t, *J* = 9.4 Hz, H-3), 3.47 (2H, t, *J* = 10.9 Hz, H-5b; ^{13}C NMR (CD_3OD): 144.8, 138.3, 128.4, 127.4, 127.3, 123.1, 89.0, 77.4, 72.7, 72.0, 69.5, 68.7, 62.8; ESI-MS: Calcd for $C_{24}H_{33}N_6O_{10}$: 565.2258 $[M+H]^+$, found 565.1189.

Xyloside **15**: ^{1}H NMR (CD_3OD): δ 8.14 (2H, s, triazolyl H), 7.34 (4H, s), 5.51 (2, d, *J* = 9.4 Hz, H-1), 4.65 (4H, s), 4.58 (4H, s), 4.01 (2H, dd, *J* = 5.1, 11.1 Hz, H-5a), 3.88 (2H, t, *J* = 9.4 Hz, H-2), 3.71-3.64 (2H, m, H-4), 3.49 (2H, t, *J* = 9.4 Hz, H-3), 3.46 (2H, t, *J* = 10.9 Hz, H-5b); ESI-MS: Calcd for $C_{24}H_{32}N_6O_{10}Na$: 587.2078 $[M+Na]^+$, found 587.1150.

Xyloside **16**: ^{1}H NMR (CD_3OD): δ 7.91 (2H, s, triazolyl H), 7.22 (1H,s , Ar-H), 7.21 (2H, d, *J* = 7.0 Hz, Ar-H), 7.29 (1H, t, *J* = 7.0 Hz, Ar-H), 5.46 (2H, d, *J* = 9.0 Hz, H-1), 4.51 (4H, s), 3.99 (2H, dd, *J* = 5.5, 11.3 Hz, H-5a), 3.86 (2H, t, *J* = 9.0 Hz, H-2), 3.74 (4H, t, *J* = 6.6 Hz), 3.67-3.63 (2H, m), 3.49 (2H, t, *J* = 9.0 Hz, H-3), 3.45 (4H, t, *J* = 10.9 Hz, H-5b), 3.00 (4H, t, *J* = 6.6 Hz,); ESI-MS: Calcd for $C_{26}H_{36}N_6O_{10}Na$: 615.2391 $[M+Na]^+$, found 615.1550.

Xyloside **17**: ^{1}H NMR (CD_3OD): δ 7.83 (2H, s, triazolyl H), 7.34-7.25 (4H, m), 5.44 (2H, d, *J* = 9.0 Hz, H-1), 4.51 (4H, s), 3.99 (2H, dd, *J* = 5.5, 11.3 Hz, H-5a), 3.83 (2H, t, *J* = 9.0 Hz, H-2), 3.70-3.64 (2H, m), 3.54-3.42 (8H, m), 2.81 (4H, t, *J* = 7.8 Hz), 1.99-1.92 (4H, m); ESI-MS: Calcd for $C_{28}H_{40}N_6O_{10}Na$: 643.2704 $[M+Na]^+$, found 643.2550.

Xyloside **18**: ^{1}H NMR (CD_3OD): δ 8.33 (2H, s, triazolyl H), 7.81 (2H, d, *J* = 8.6 Hz, Ar-H), 7.35 (2H, t, *J* = 7.8 Hz, Ar-H), 7.10 (2H, d, *J* = 7.8 Hz, Ar-H), 5.55 (2H, d, *J* = 9.0 Hz, H-1), 5.35 (4H, s), 4.02 (2H, dd, *J* = 5.5, 11.3 Hz, H-5a), 3.93 (2H, t, *J* = 9.0 Hz, H-2), 3.74-3.65 (2H, m, H-4), 3.50 (2H, t, *J* = 9.0 Hz, H-3), 3.48 (2H, t, *J* = 11.3 Hz, H-5b); ^{13}C NMR (CD_3OD): δ 155.2, 145.1, 128.1, 126.3, 124.6, 115.9, 107.2, 90.3, 78.6, 73.9, 70.7, 69.9, 62.7; ESI-MS: Calcd for $C_{26}H_{29}N_6O_{10}$: 585.1945 $[M-H]^-$, found 585.0000.

Xyloside **19**: ^{1}H NMR (CD_3OD): δ 8.32 (2H, s, triazolyl H), 7.73 (2H, d, *J* = 9.0 Hz, Ar-H), 7.39 (2H, s, Ar-H), 7.18 (2H, d, *J* = 9.0 Hz, Ar-H), 5.55 (2H, d, *J* = 9.0 Hz, H-1), 5.29

(4H, s), 4.01 (2H, dd, J = 5.5, 11.3 Hz, H-5a), 3.91 (2H, t, J = 9.4 Hz, H-2), 3.70-3.64 (2H, m, H-4), 3.49 (2H, t, J = 9.4 Hz, H-3), 3.48 (2H, t, J = 10.94 Hz, H-5b); ^{13}C NMR (CD_3OD): δ 156.4, 145.1, 131.4, 129.6, 124.7, 120.2, 108.6, 90.2, 78.7, 73.9, 70.7, 69.9, 62.4; ESI-MS: Calcd for $C_{26}H_{29}N_6O_{10}$: 585.1945 $[M-H]^-$, found 585.0667.

Xyloside **20**: ^{1}H NMR (CD_3OD): δ 8.22 (2H, s, triazolyl H), 5.73 (2H, s), 5.55 (2H, d, J = 9.4 Hz, H-1), 4.60 (4H, s), 4.12 (4H, s), 4.01 (2H, dd, J = 5.5, 11.3 Hz, H-5a), 3.91 (2H, t, J = 9.4 Hz, H-2), 3.67-3.53 (2H, m, H-4), 3.51-3.44 (4H, m, H-3, H-5b); ESI-MS: Calcd $C_{20}H_{30}N_6O_{10}Na$: 537.202 $[M+Na]^+$, found 537.151

Table S1. Sulfation density analysis of xyloside-primed GAG chains. GAG chains primed by cluster-xylosides in pgsA-745 cell lines at 100 μM were analyzed using anion exchange HPLC; the bound GAG chains were eluted with a linear gradient of 0.2 M to 1 M NaCl over 80 minutes at a flow rate of 1 ml/min and elution times indicate charge density of primed GAG chains.

Xylosides	**Elution times of primed GAG chains (min)**
Mono-xyloside **1**	44.5
Mono-xyloside **2**	40.8
Bis-xyloside **3b**	49.8
Bis-xyloside **4**	50.2
Bis-xyloside **5**	50.8
Bis-xyloside **6**	51.5
Bis-xyloside **7b**	50.6
Tris-xyloside **8a**	46.0
Tris-xyloside **9**	47.2
Tetrakis-xyloside **10a**	48.9
Tetrakis-xyloside **11a**	49.7

Figure Legends

Figure S1. Comparison of GAG priming activity of protected bis-xylosides and deprotected bis-xylosides. The priming ability of protected bis-xylosides (**3a**, **7a**) and deprotected bis-xylosides (**3b**, **7b**) were examined using xylosyl transferase deficient CHO cells (pgsA-745). 400,000 cells were seeded in wells of 6-well plates and treated with xyloside (**3a**, **3b**, **7a** or **7b)** at 100 μM and 250 μM concentrations in the presence of 100 μCi of *D*-[6-^{3}H]-glucosamine. The medium was removed from the well at 24 hours, GAG chains were purified and quantified as described in the method section.

Figure S2. Comparison of the priming ability of GAG chains by bis-xylosides at various concentrations. The priming ability of the bis-xylosides (**3b** and **7b**)were examined using xylosyl transferase deficient CHO cells (pgsA-745). 400,000 cells were seeded in wells of 6-well plates and treated with bis-xylosides at 100 μM, 300 μM, 600 μM and 1 mM concentrations in the presence of 100 μCi of *D*-[6-^{3}H]-glucosamine. The medium was removed from the well at 24 hours, GAG chains were purified and quantified as described in the method section.

Figure S3. Comparison of GAG priming activity of protected cluster-xylosides and deprotected cluster-xylosides. The priming ability of protected and deprotected tris and tetrakis-xylosides was examined using xylosyl transferase deficient CHO cells (pgsA-745). 400,000 cells were seeded in wells of 6-well plates and treated with cluster xylosides at 100 μM in the presence of 100 μCi of *D*-[6-^{3}H]-glucosamine. The medium was removed from the well at 24 hours, GAG chains were purified and quantified as described in the method section.

Figure S4. Priming activity of bis-xylosides in the pgs A-745 cell line at 1 mM. CHO cells were treated with bis- xylosides at 1 mM in the presence of ^{3}H-GlcNH$_2$ (100 μCi) as described in the method section. The GAG chains were purified by anion exchange chromatography and quantitated using a liquid scintillation counter. The results were average of two independent experiments that were carried out in duplicate.

Figure S5. Calibration of size exclusion column with polystyrene sulfonate standards. Polystyrene sulfonate standards of various molecular weights "I (3600 Da), II (6530 Da), III (14900 Da), IV (32900 Da), V (63900 Da), VI (152000)" were analyzed by size exclusion chromatography. The migration times of the various polystyrene sulfonate species were plotted against the molecular weight to obtain a calibration curve. The migration time of GAG chains primed by cluster-xylosides were compared to the calibration curve to determine the molecular weight of primed GAG chains.

Figure S6. Effect of various hydrophobic moieties on the DEAE elution profiles of GAG chains. GAG chains primed by various cluster-xylosides eluted from an analytical anion-exchange column (7.5 mm x 7.5 cm) using linear gradient of 1 M NaCl solution. GAG samplers were diluted five-fold prior to analysis by DEAE-HPLC. The variations in the elution profiles and migration times indicate differences in both sulfation pattern and extent of sulfation of the primed GAG chains. The elution profiles are representative of at least two independent experiments.

Figure S7. Structural analysis of GAG chains primed by bis-xyloside (3b) at 100 μM. PgsA-745 cells were incubated with the bis-xyloside (**3b**) for 24 hours. The primed GAG chains were then purified as described in the method section. The HS/CS composition of the primed GAG chains was determined by digesting the GAG chains

with heparitinase I/II/III or chondroitinase ABC enzymes. The reaction mixture was incubated at 37 °C for 2 hours, the solution was then loaded on to two tandem G3000 SWXL columns (7.8 mm x 30 cm) and analyzed with the aid of an inline radiometric detector using phosphate buffer as an eluent. The HS/CS composition was determined based on the percentage area of undigested and digested GAG peak. **A**: The elution profile of GAG chains without heparitinase I/ II/ III or chondroitinase ABC treatment. **B**: The elution profile of GAG chains after treatment with heparitinase I/ II/ III enzyme. **C**: The elution profile of GAG chains after treatment with chondroitinase ABC enzyme. **D**: The elution profile of GAG chains after treatment with heparitinase I/ II/ III and chondroitinase ABC enzyme.

Figure S8. GAG priming activity was determined by additional bis-xylosides. **A**: Priming activity of bis-xylosides (**12** and **13)**. **B**: priming analysis of bis-xylosides (**14**, **15**, **16** and **17)**. **C**: Priming activity of bis-xylosides (**18** and **19).** The priming ability of bis-xylosides were examined using xylosyl transferase deficient CHO cells (pgsA-745). 400,000 cells were seeded in wells of 6-well plates and treated with bis-xylosides at 100 μM in the presence of 100 μCi of *D*-[6-^{3}H]-glucosamine. The medium was removed from the well at 24 hours, GAG chains were purified and quantified as described in the method section.

Figure S9. Disaccharide profiles of xyloside-primed CS chains. GAG chains, which were primed by cluster-xylosides (**3b**, **8a** and **10a**) at 100 μM, were treated with chondroitinase ABC and the resulting disaccharides were analyzed by SAX-HPLC. I: ΔUA-GalNAc; and II: ΔUA-GalNAc4S

Figure S10. Periodate oxidation-alkaline elimination of GAG chains. GAG chains, which were primed by mono-xyloside **2** and bis-xyloside **7** at 100 μM, were subjected to periodate oxidation-alkaline elimination as described in the method section. **A**: The elution profiles of GAG chains, primed by mono-xyloside **2**, before (gray trace) and after (black trace) treatments are shown. **B**: The elution profiles of GAG chains primed by bis-xyloside **7**, before (gray trace) and after (black trace) treatments are shown. This data showed that only GAG chains primed by bis-xylosides can be broken by oxidation-elimination treatments whereas GAG chains primed by mono-xylosides cannot be cleaved and thus, demonstrated the bidirectional GAG priming ability of bis-xylosides.

Figure S11. **Ozonolysis of GAG chains**. GAG chains, which were primed by bis-xyloside **20** at 100 μM, were subjected to ozonolysis. A: Schematic representation that shows the action of ozone on double bond. Bis-xyloside **20** that carries a double bond is susceptible to ozone treatment resulting in the cleavage of two GAG chains that are assembled on the same scaffold. B: The elution profiles of GAG chains primed by bis-xyloside **20** before (black trace) and after ozonolysis (gray trace) are shown and the data shown here demonstrate the bidirectional priming ability of bis-xyloside **20**.

Figure S1

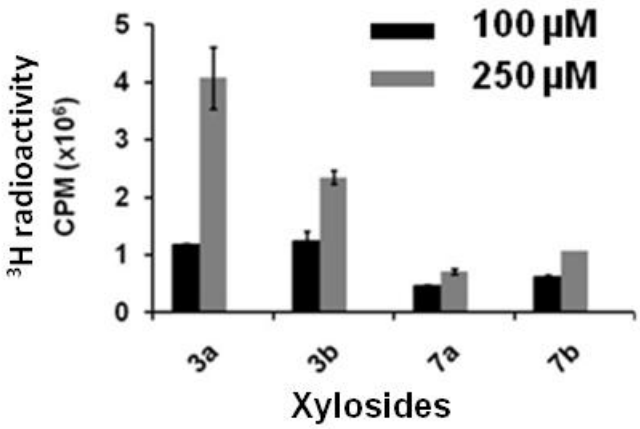

100 µM
250 µM
^{3}H radioactivity
CPM (x10^6)
0
1
2
3
4
5
3a
3b
7a
7b
Xylosides

Figure S2

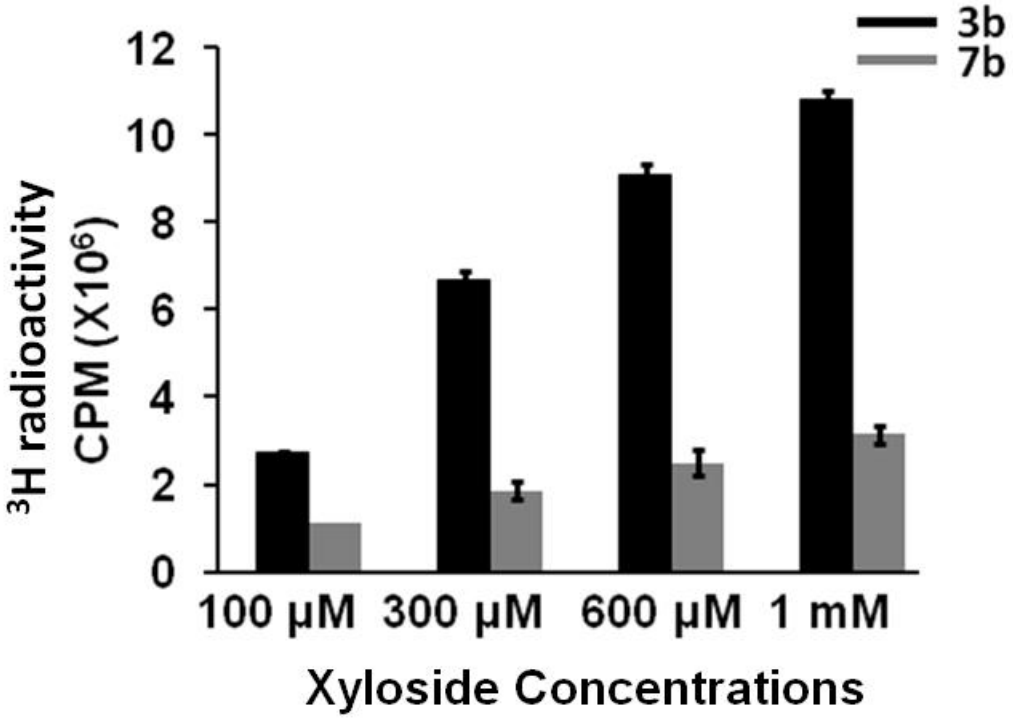

3b
7b
12
10
8
6
4
2
0
^{3}H radioactivity
CPM (X10^6)
100 µM
300 µM
600 µM
1 mM
Xyloside Concentrations

Figure S3

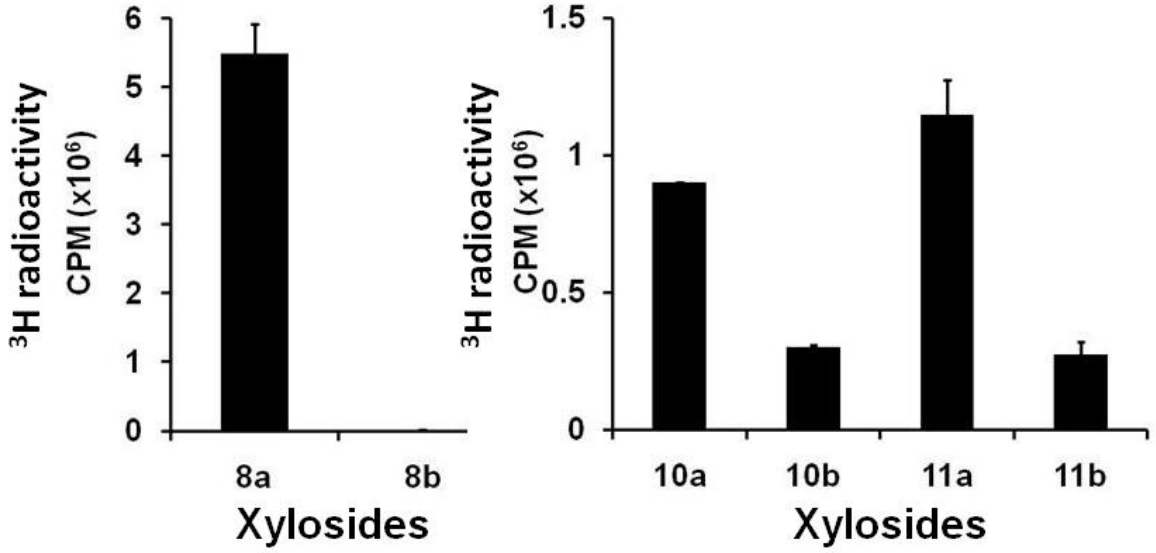
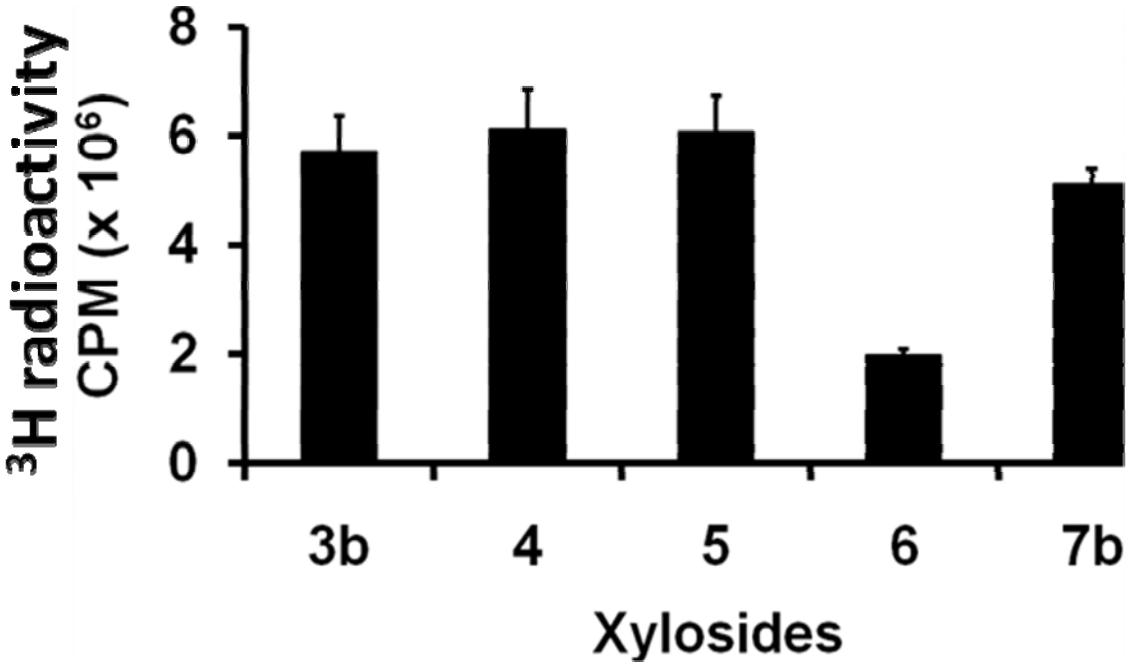

^{3}H radioactivity
CPM (x10^6)
6
5
4
3
2
1
0
8a
8b
Xylosides
^{3}H radioactivity
CPM (x10^6)
1.5
1
0.5
0
10a
10b
11a
11b
Xylosides

Figure S4



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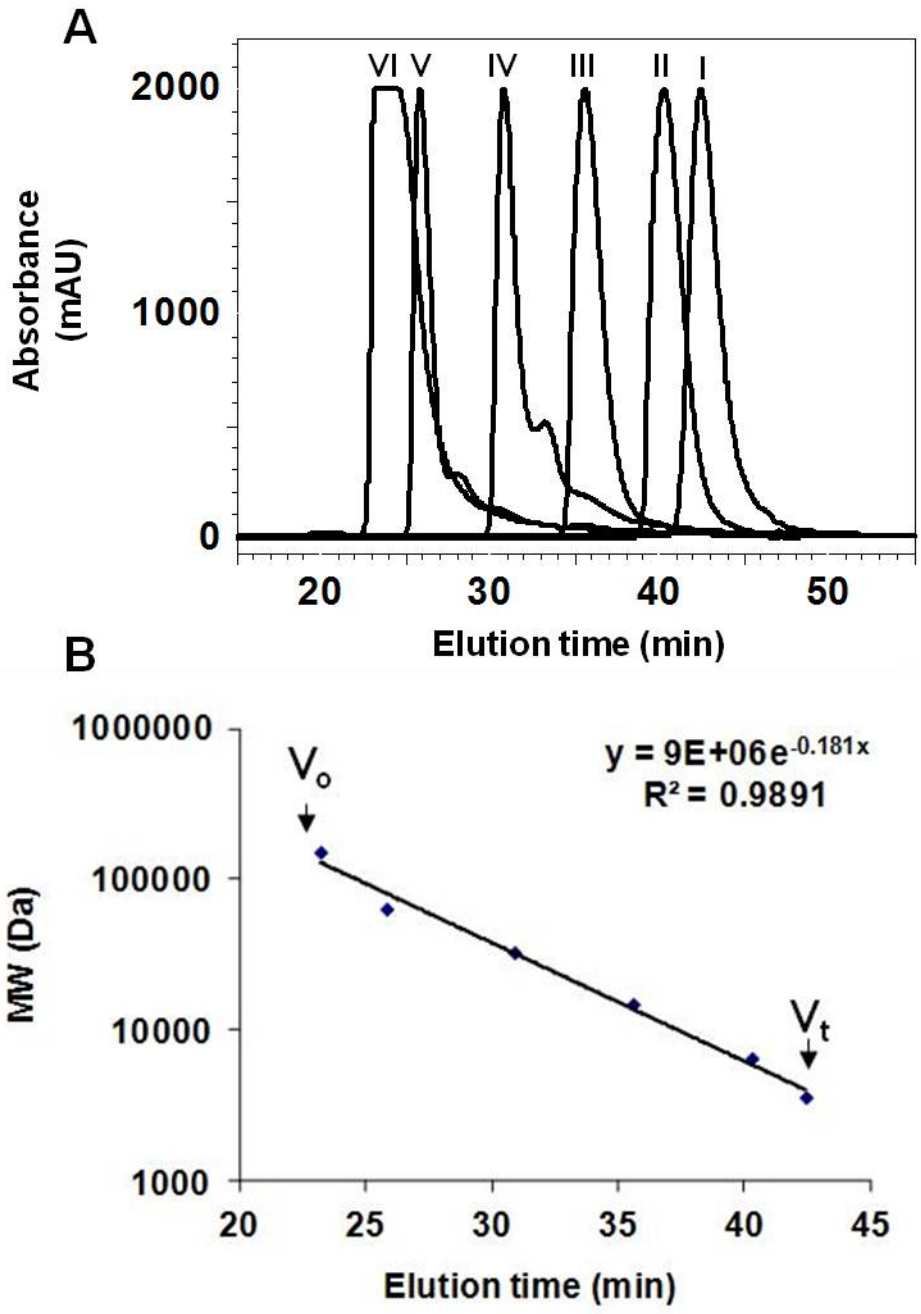

A
VI V IV III II I
2000
1000
0
Absorbance (mAU)
20 30 40 50
Elution time (min)
B
1000000
100000
10000
1000
MW (Da)
V_o
V_t
y = 9E+06e-0.181x
R² = 0.9891
20 25 30 35 40 45
Elution time (min)

Figure S6

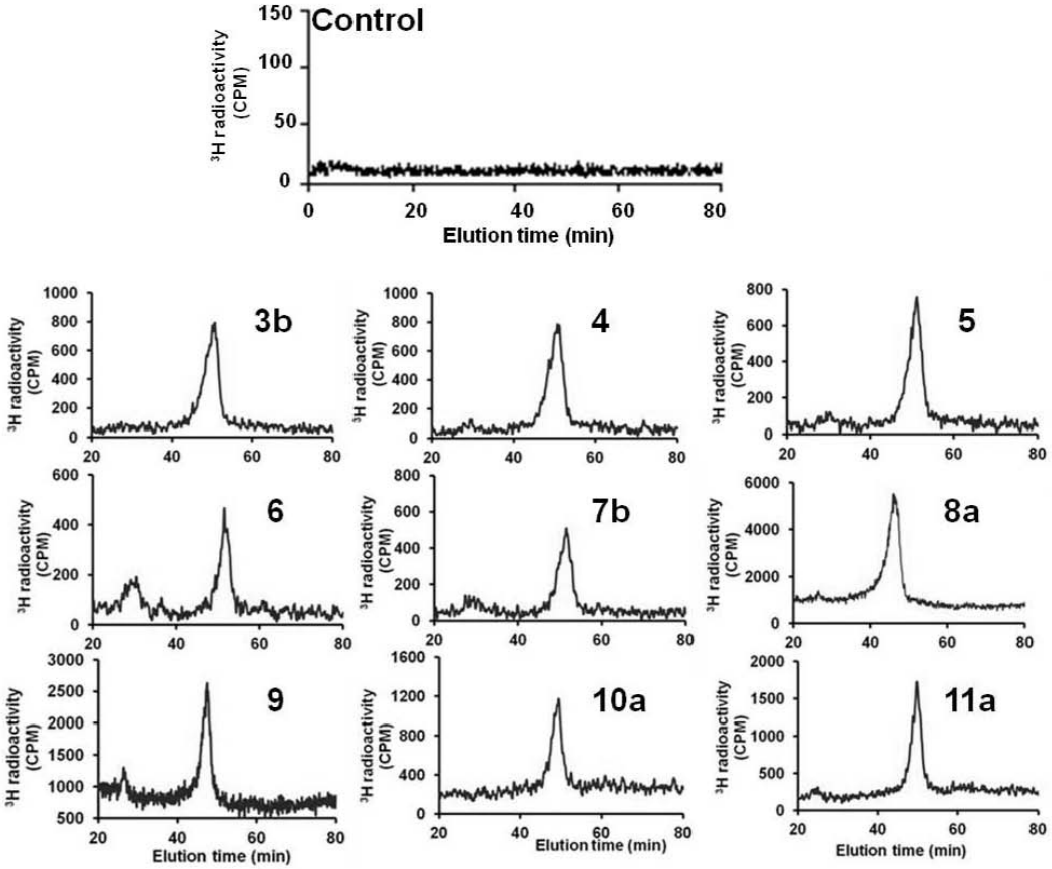

Control
3b
4
5
6
7b
8a
9
10a
11a
³H radioactivity (CPM)
Elution time (min)

Figure S7

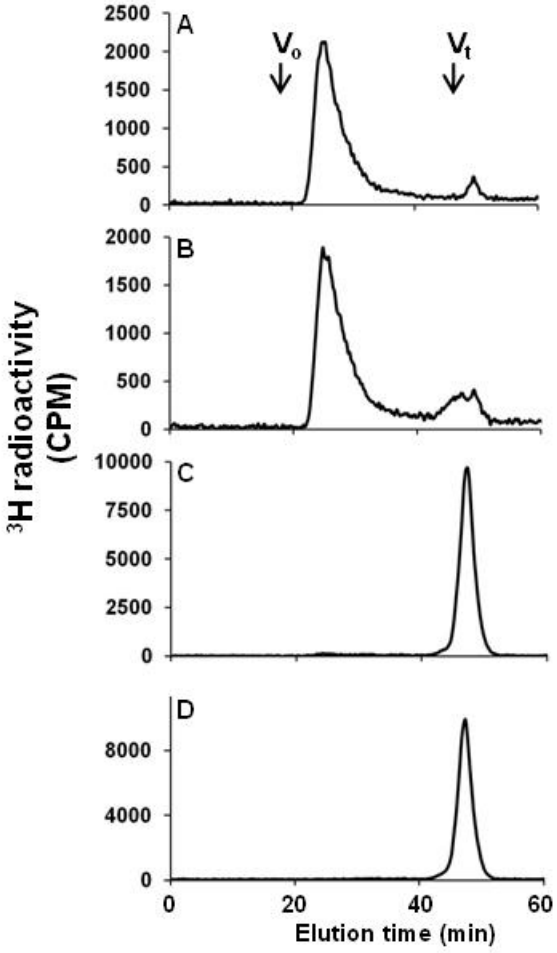

A
B
C
D
V_o
V_t
2500
2000
1500
1000
500
0
10000
7500
5000
8000
4000
0
20
40
60
^{3}H radioactivity (CPM)
Elution time (min)

Figure S8

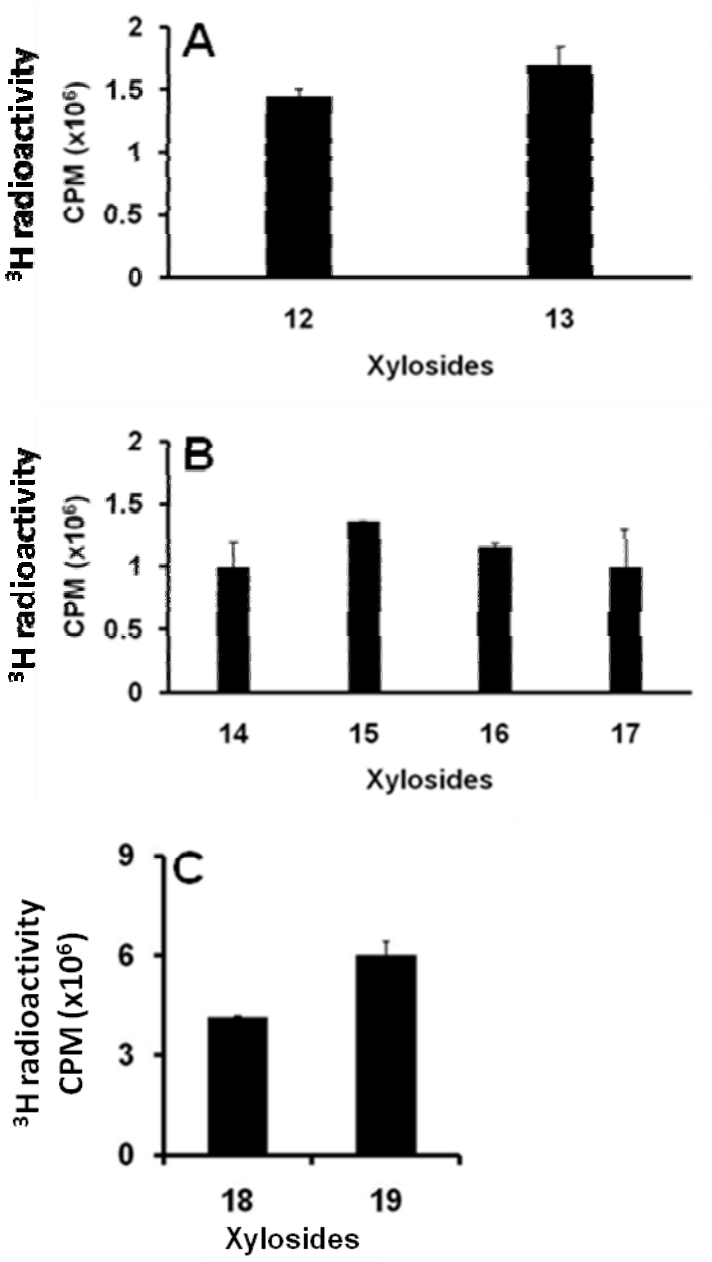

A
^{3}H radioactivity
CPM (x10^6)
2
1.5
1
0.5
0
12
13
Xylosides
B
^{3}H radioactivity
CPM (x10^6)
2
1.5
1
0.5
0
14
15
16
17
Xylosides
C
^{3}H radioactivity
CPM (x10^6)
9
6
3
0
18
19
Xylosides

Figure S9

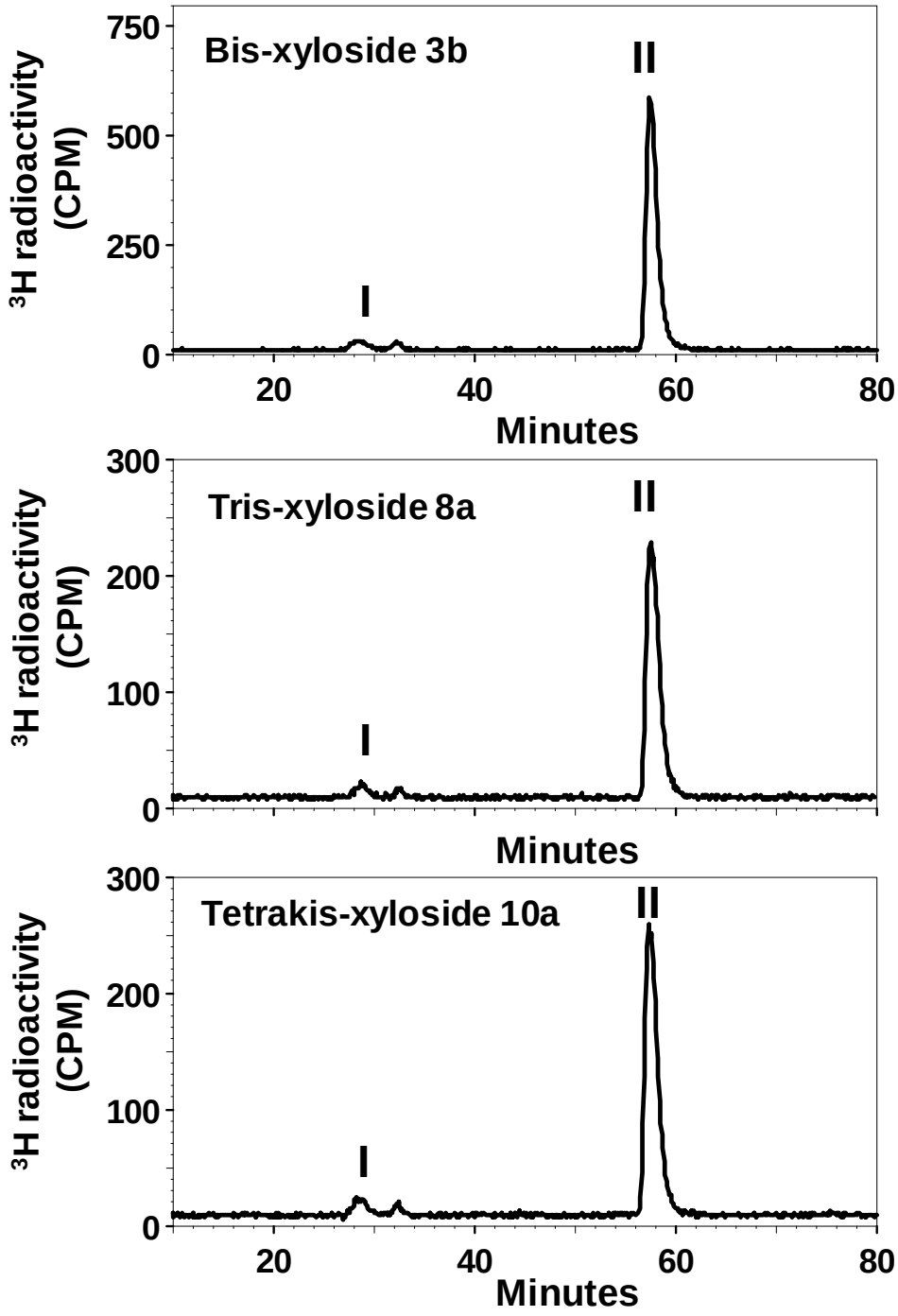

Bis-xyloside 3b
II
I
750
500
250
0
^{3}H radioactivity (CPM)
20
40
60
80
Minutes
Tris-xyloside 8a
II
I
300
200
100
0
^{3}H radioactivity (CPM)
Minutes
Tetrakis-xyloside 10a
II
I
300
200
100
0
^{3}H radioactivity (CPM)
20
40
60
80
Minutes

Figure S10

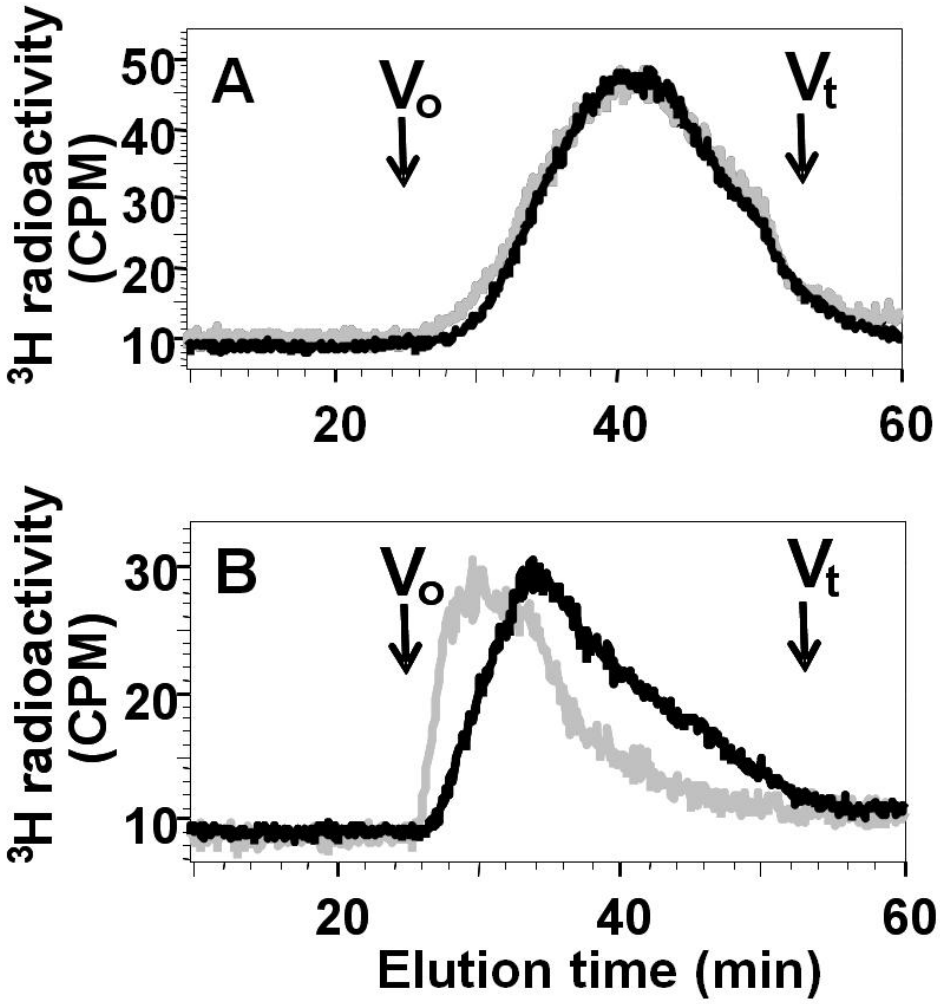
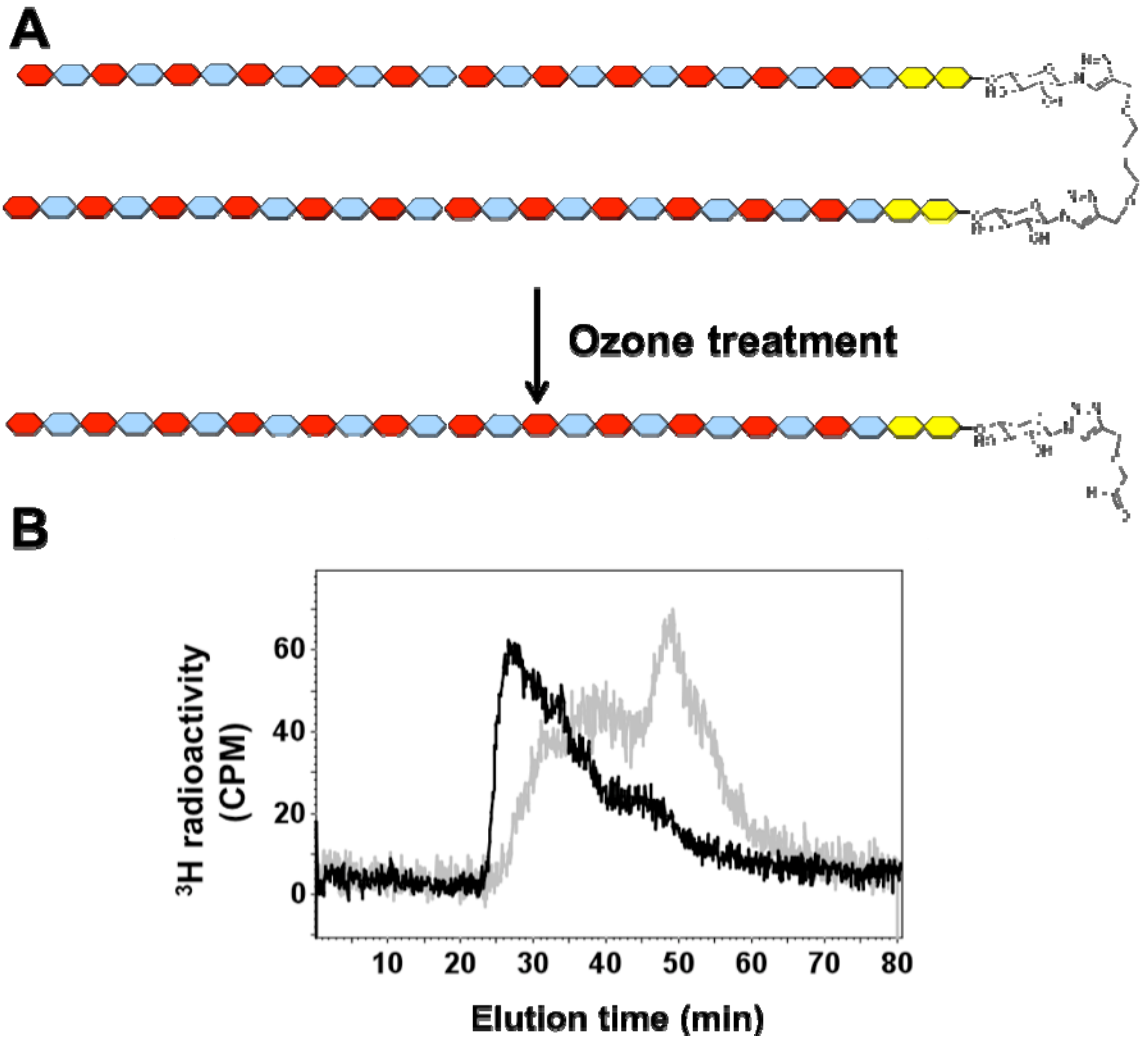
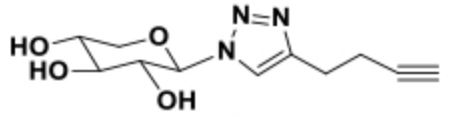

A
V_o
V_t
^{3}H radioactivity (CPM)
50
40
30
20
10
20
40
60
B
V_o
V_t
^{3}H radioactivity (CPM)
30
20
10
20
40
60
Elution time (min)

Figure S11


A
Ozone treatment
B
^{3}H radioactivity (CPM)
0
20
40
60
10
20
30
40
50
60
70
80
Elution time (min)

Reference

1. Lemercier, G., Gendreizig, S., Kindermann, M., and Johnsson, K. (2007) Inducing and sensing protein--protein interactions in living cells by selective cross-linking, *Angew Chem Int Ed Engl 46*, 4281-4284.
2. McPhee, M. M., and Kerwin, S. M. (2001) Synthesis, DNA cleavage, and cytotoxicity of a series of bis(propargylic) sulfone crown ethers, *Bioorganic & Medicinal Chemistry 9*, 2809-2818.
3. Venugopalan, B., and Balasubramanian, K. K. (1985) Studies of Claisen rearrangement of bispropargyl ethers. Synthesis of naphthodipyrans, naphthodifurans and naphthofuropyrans, *Heterocycles 23*, 81-92.
4. Whitlock, B. J., Jarvi, E. T., and Whitlock, H. W. (1981) Preparation and characterization of 1,8,19,26-tetraoxa[8.8](2,6)naphthalenophane-3,5,21,23-tetrayne and related donut-shaped cyclophanes, *Journal of Organic Chemistry 46*, 1832-1835.
5. Tornoe Christian, W., Christensen, C., and Meldal, M. (2002) Peptidotriazoles on solid phase: [1,2,3]-triazoles by regiospecific copper(i)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides, *The Journal of organic chemistry 67*, 3057-3064.
6. Meldal, M., and Tornoe Christian, W. (2008) Cu-catalyzed azide-alkyne cycloaddition, *Chemical reviews 108*, 2952-3015.



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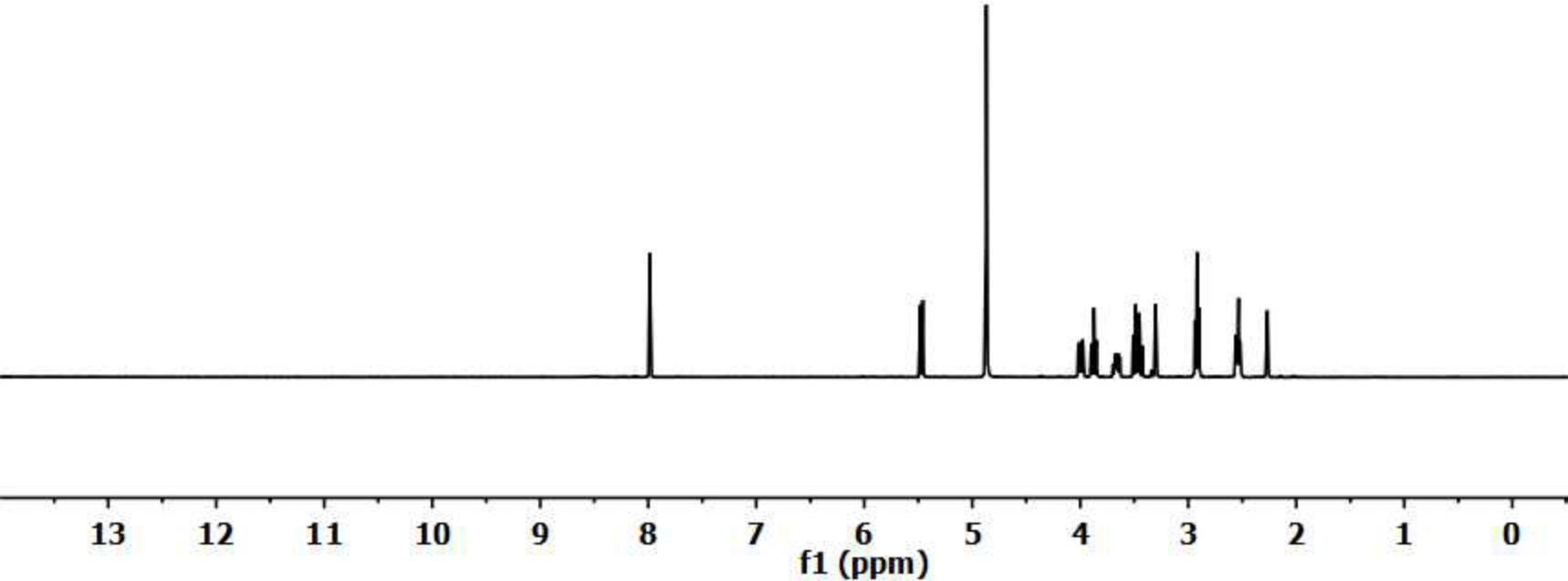

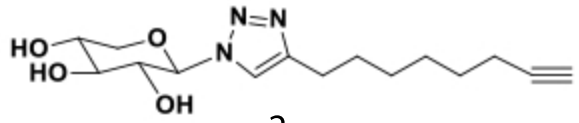

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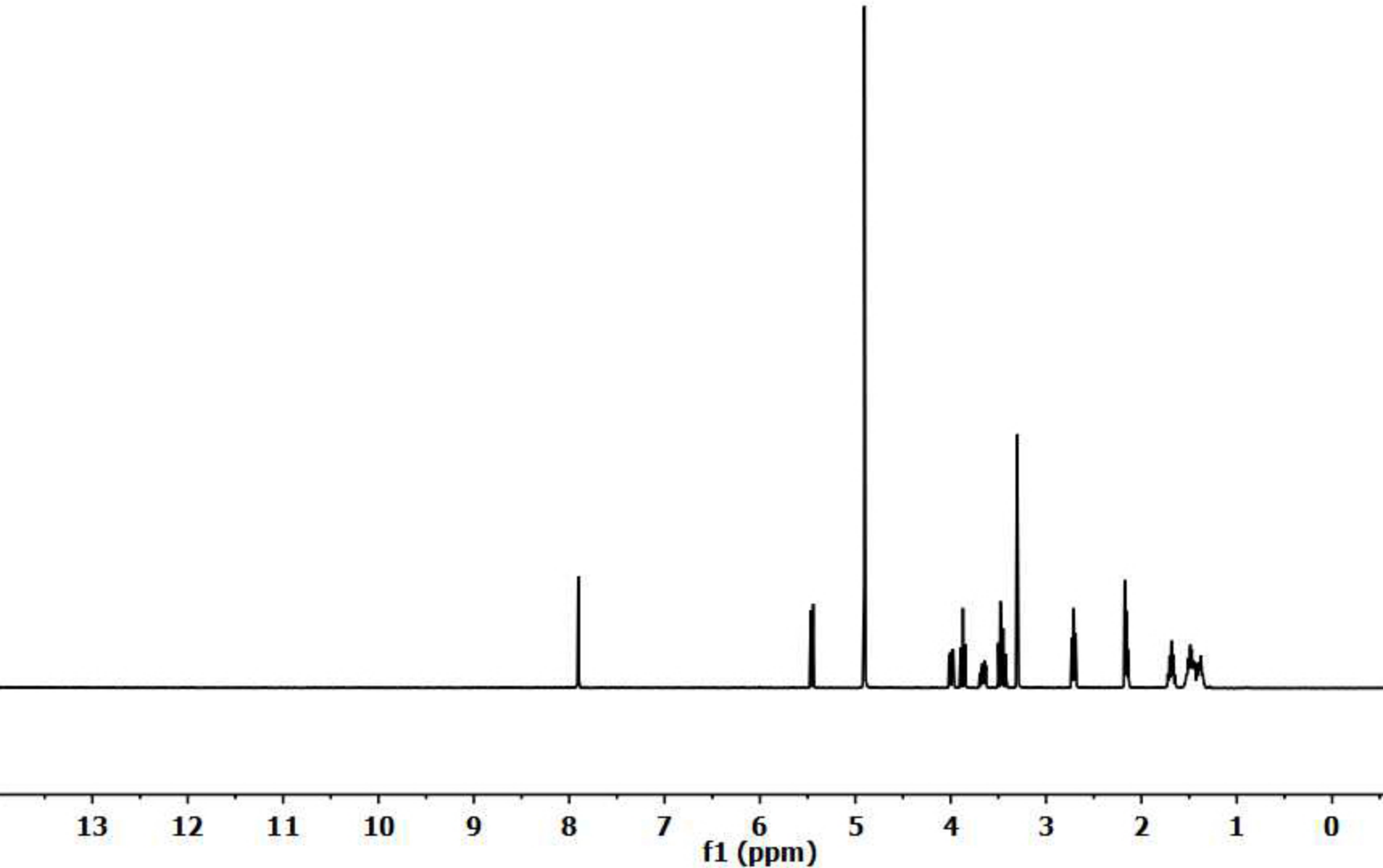

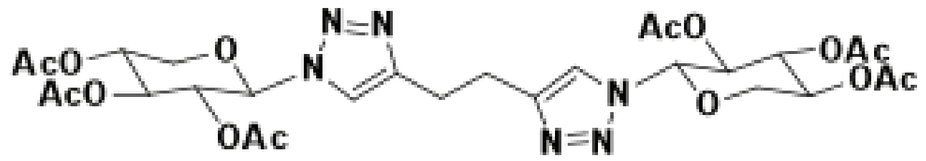

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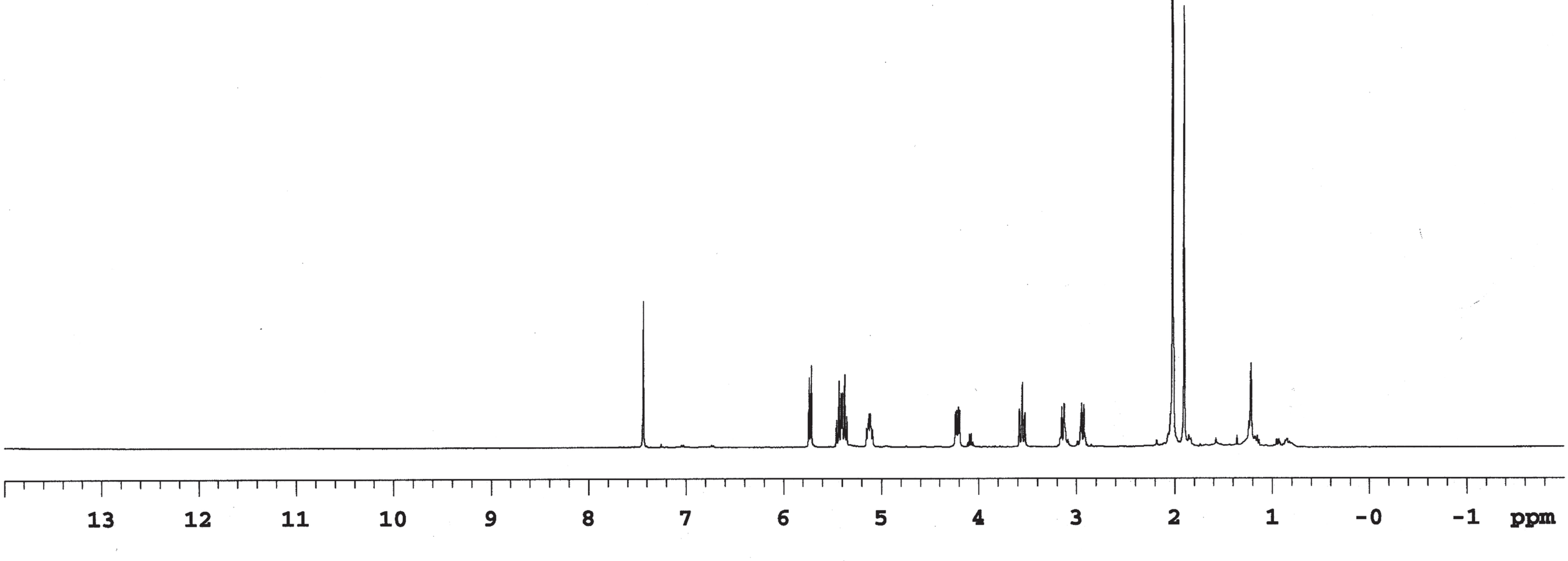

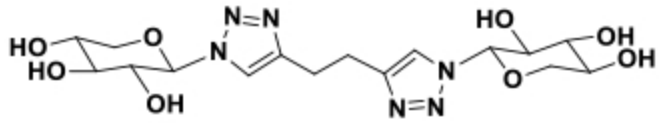

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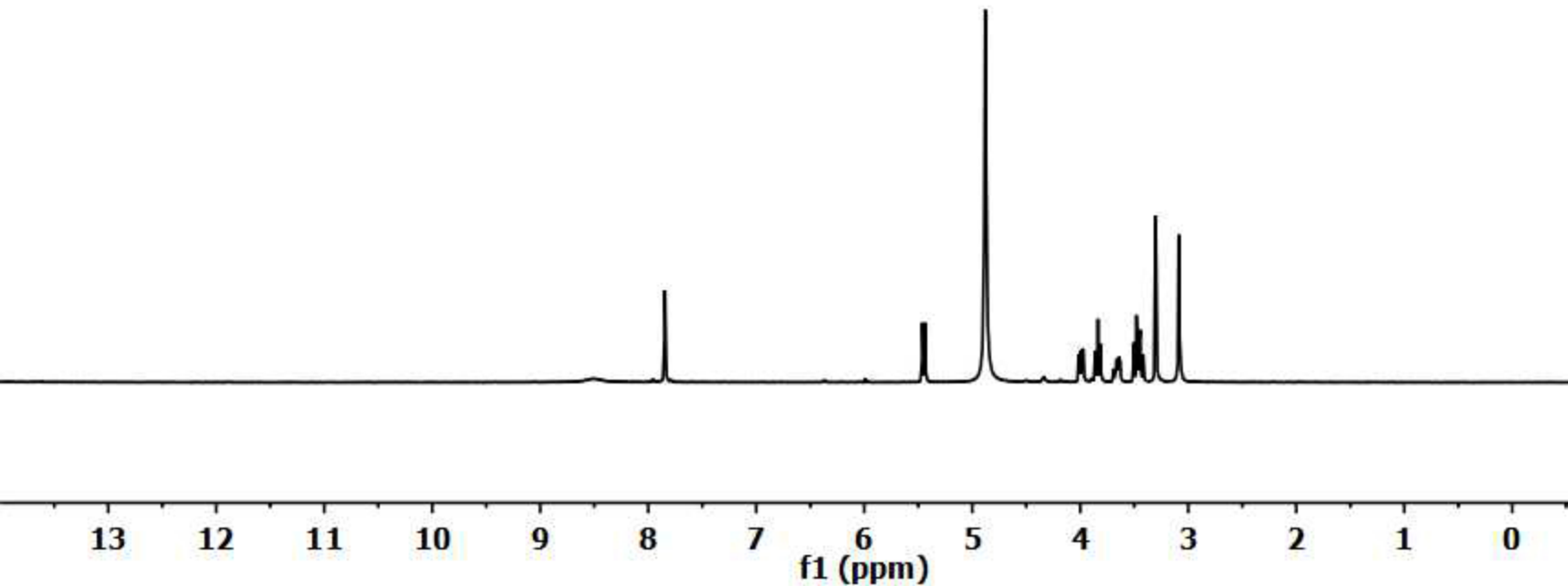

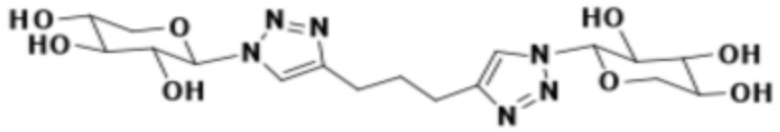

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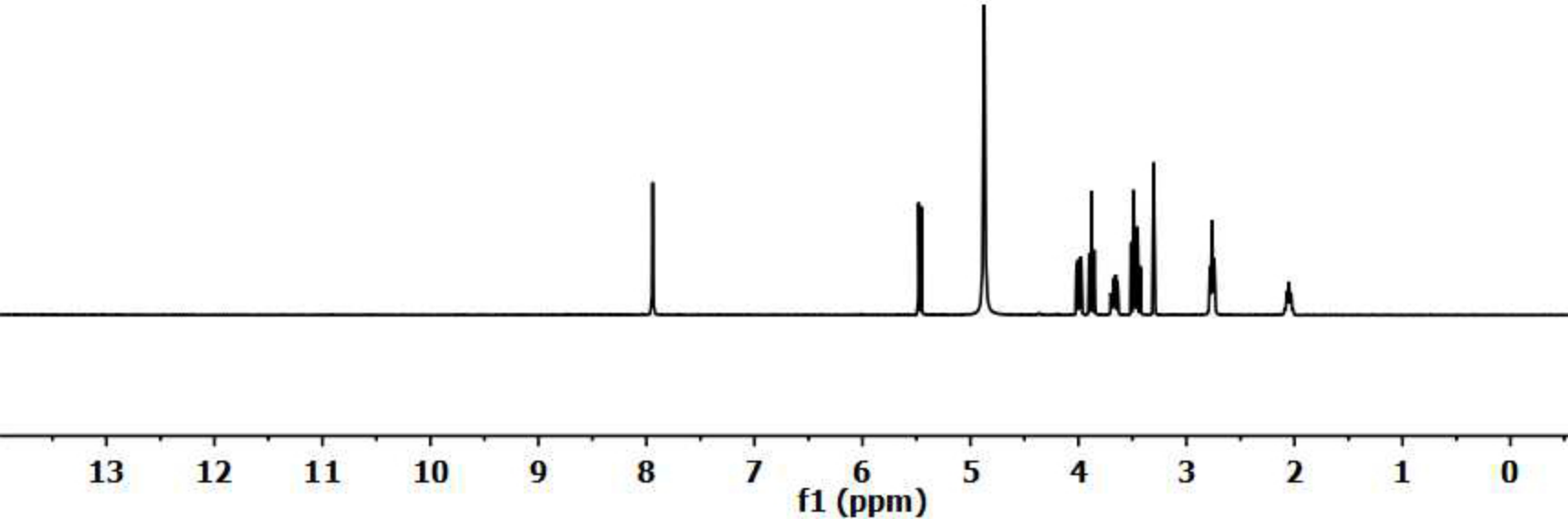

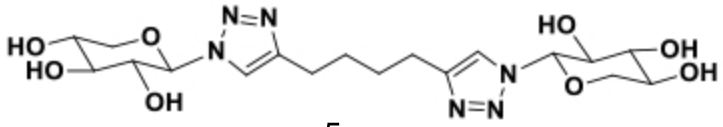

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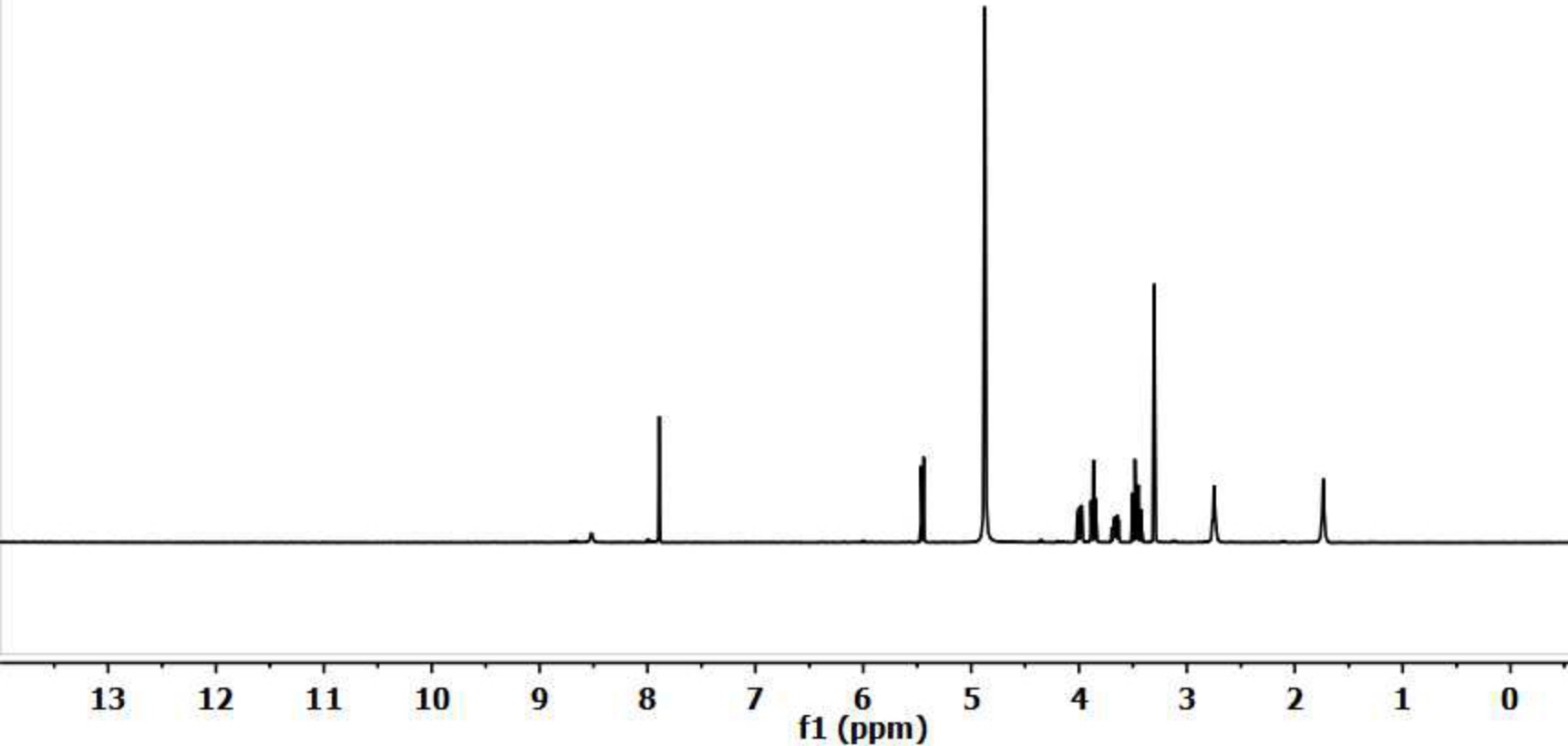

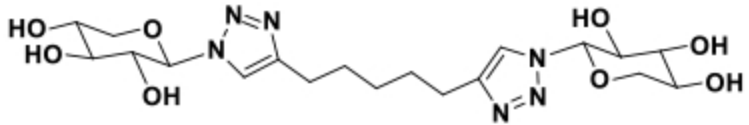

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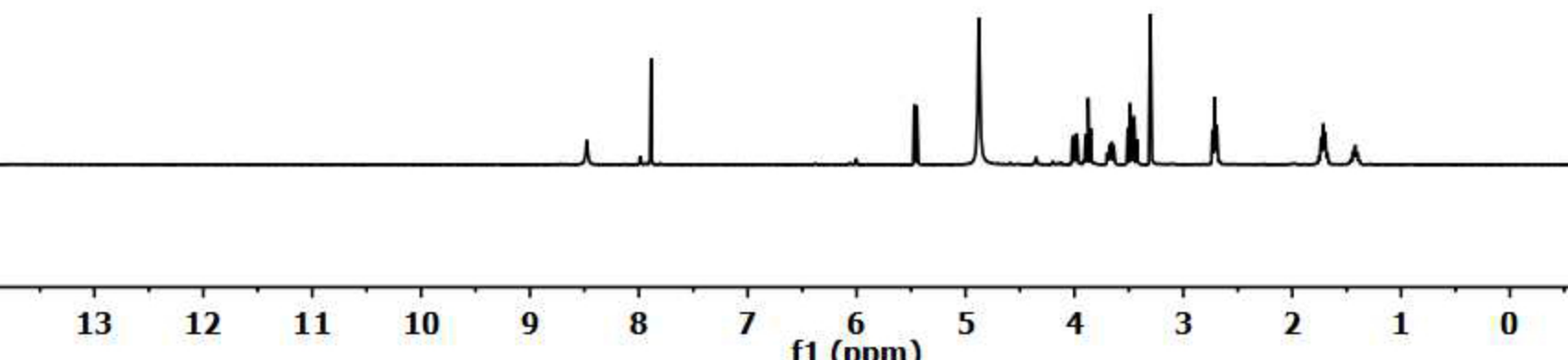

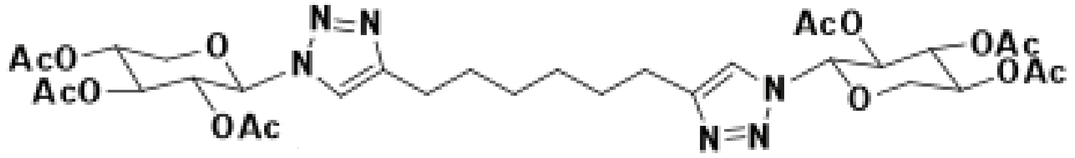

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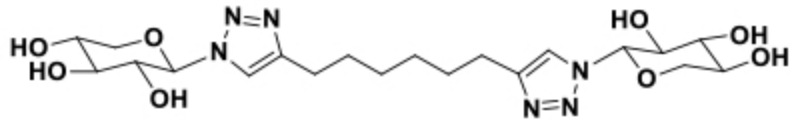

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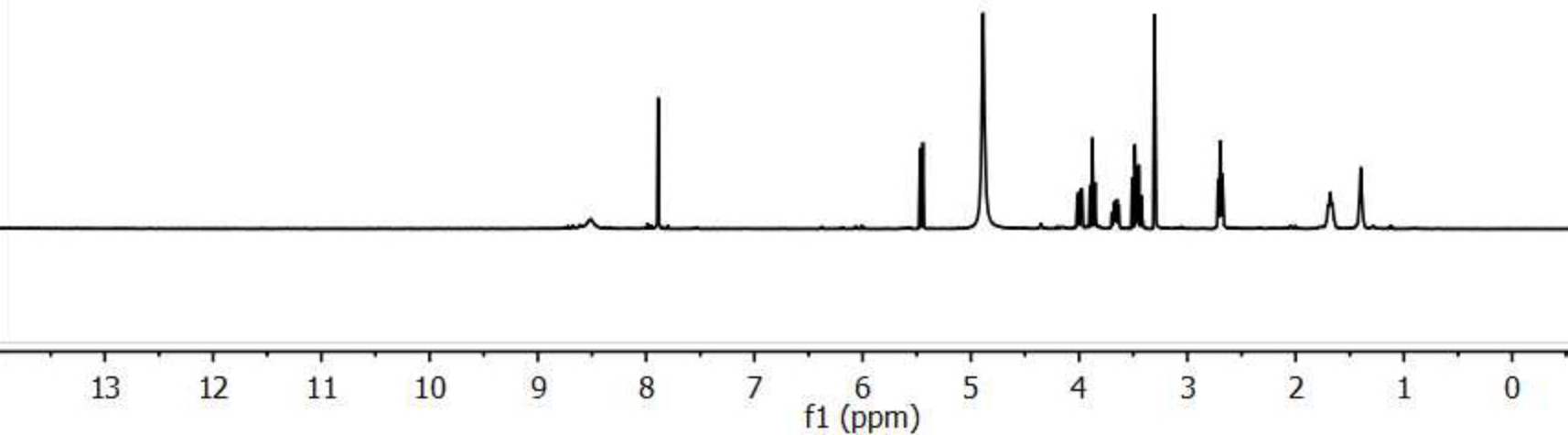

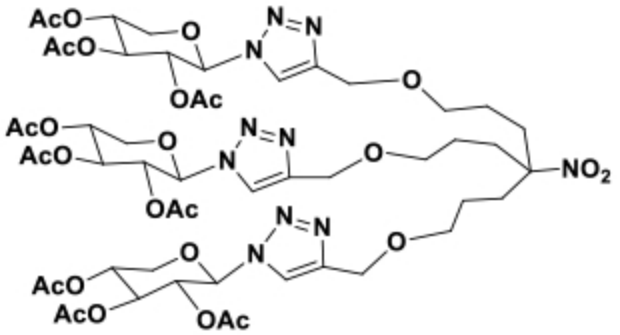

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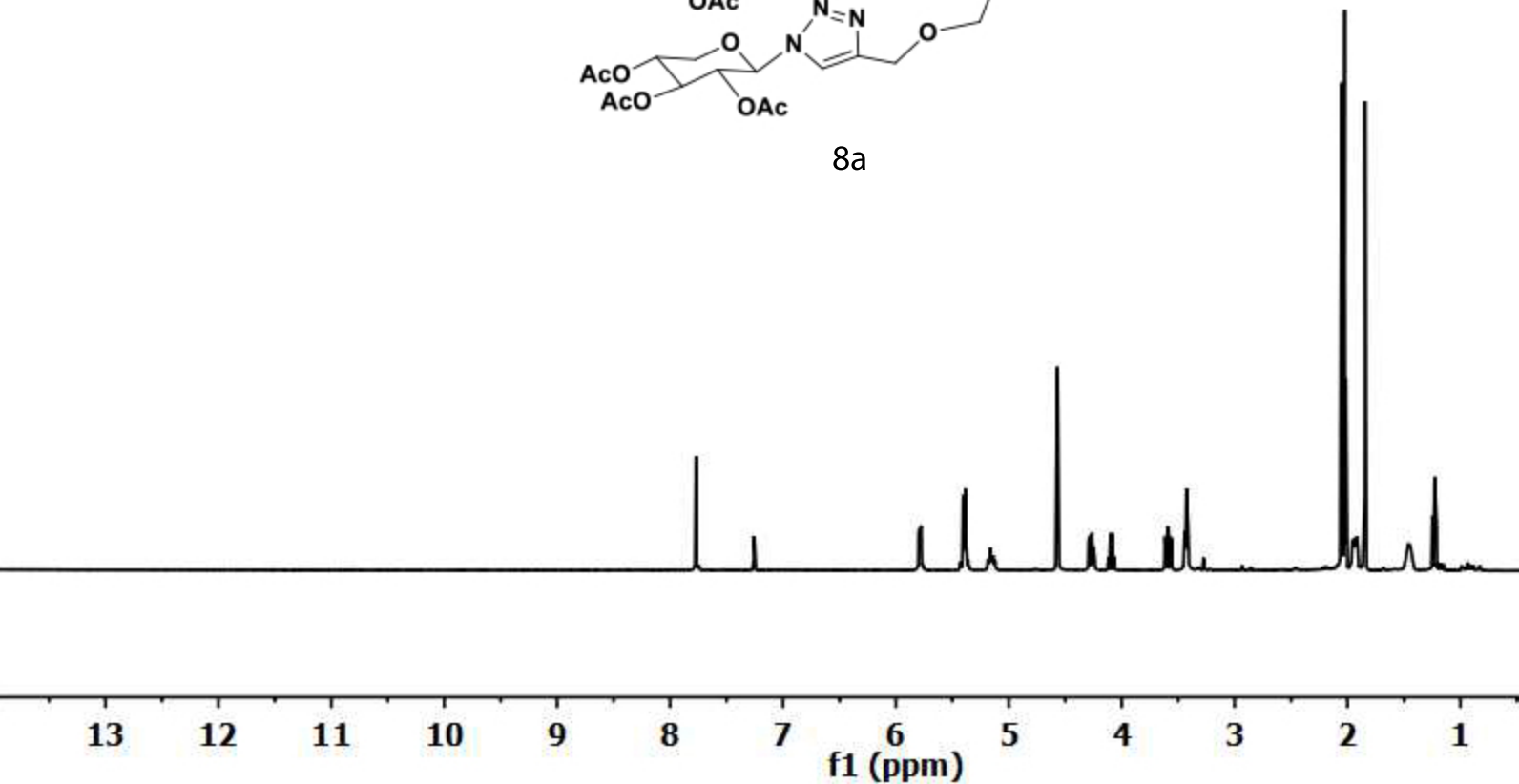
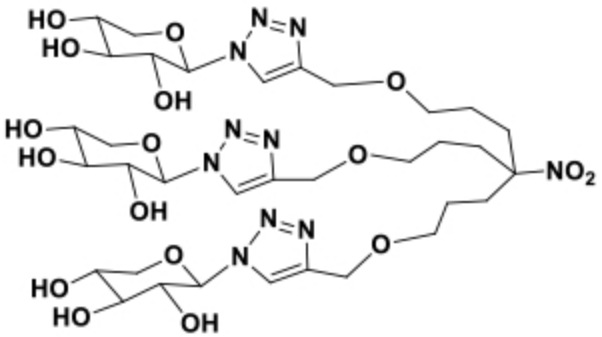
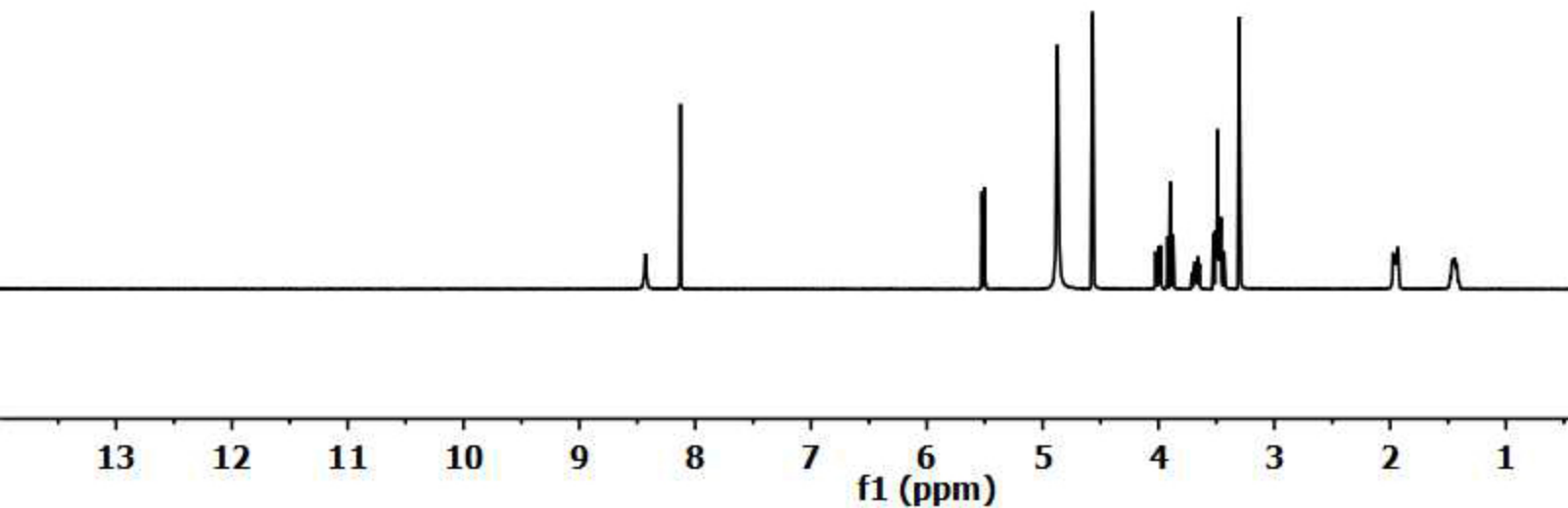
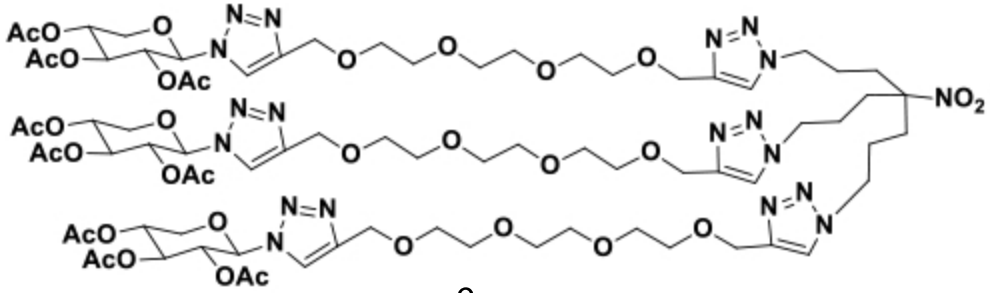



HO
HO
OH
O
N
N=N
O
HO
HO
OH
O
N
N=N
O
NO2
HO
HO
OH
O
N
N=N
O

8b


13
12
11
10
9
8
7
6
5
4
3
2
1
f1 (ppm)



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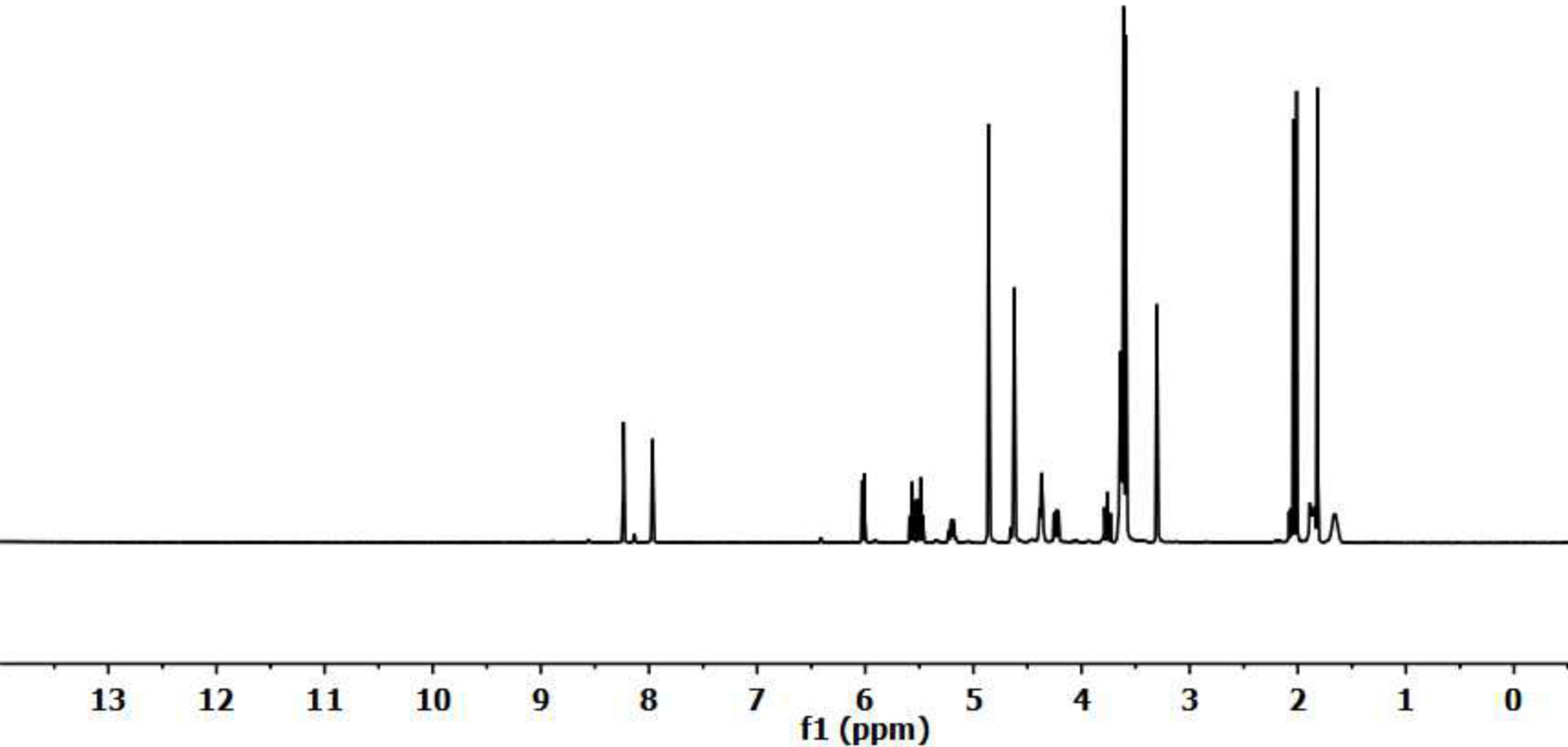

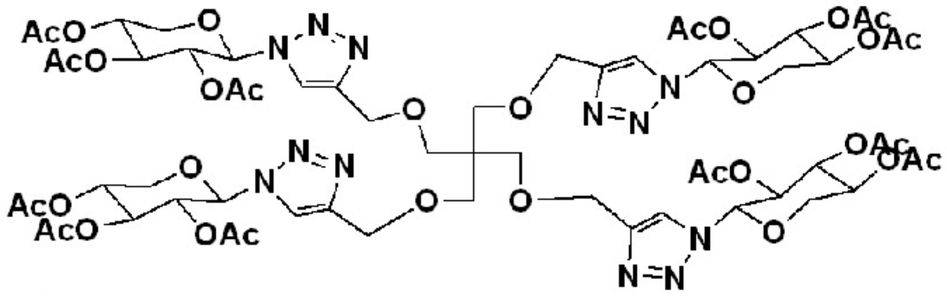

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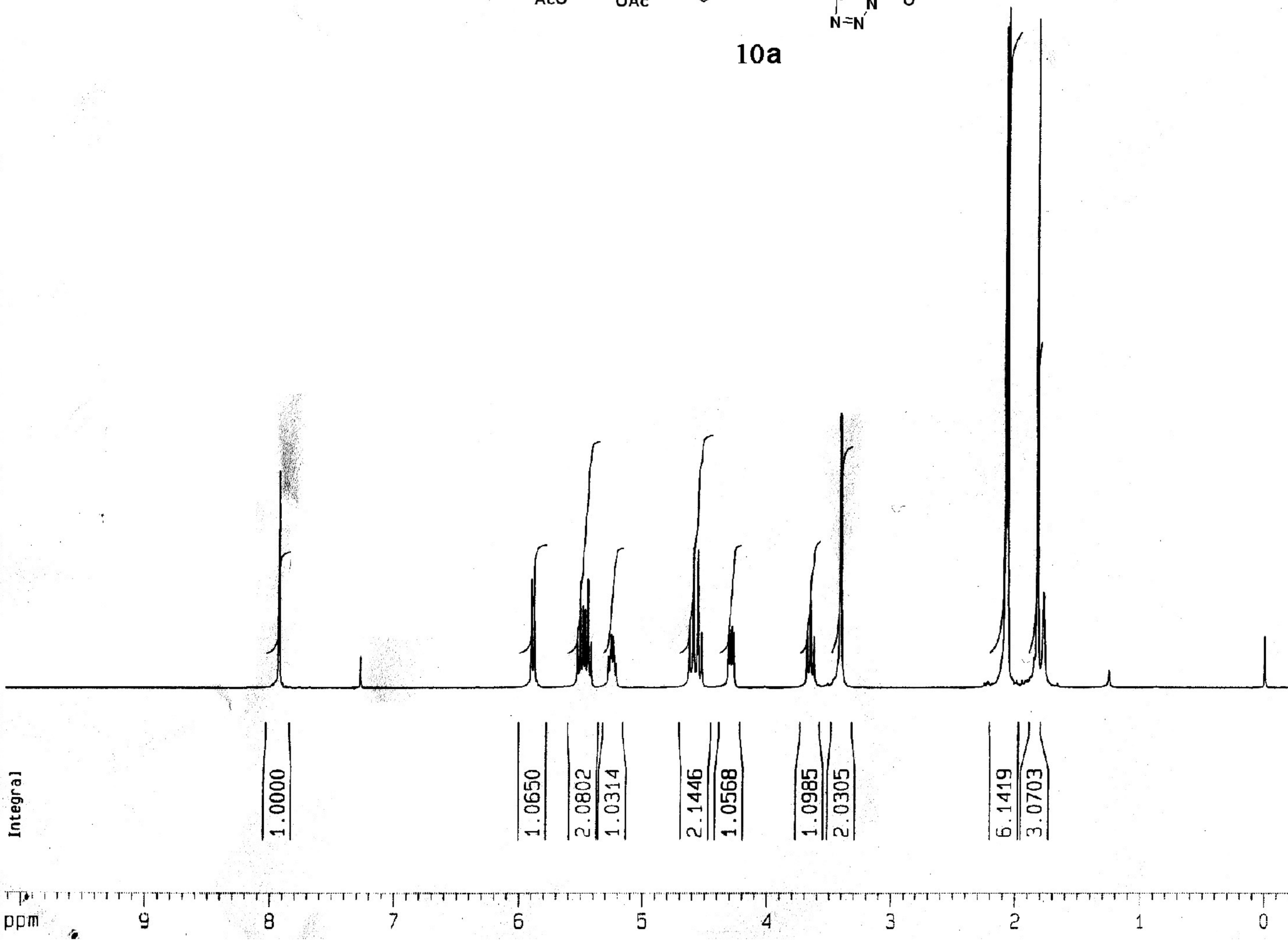
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Integral
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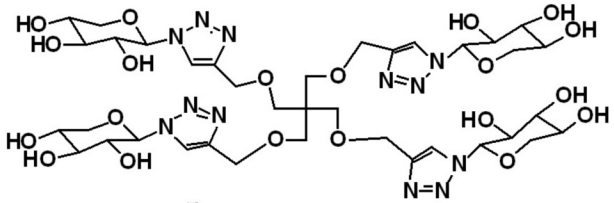
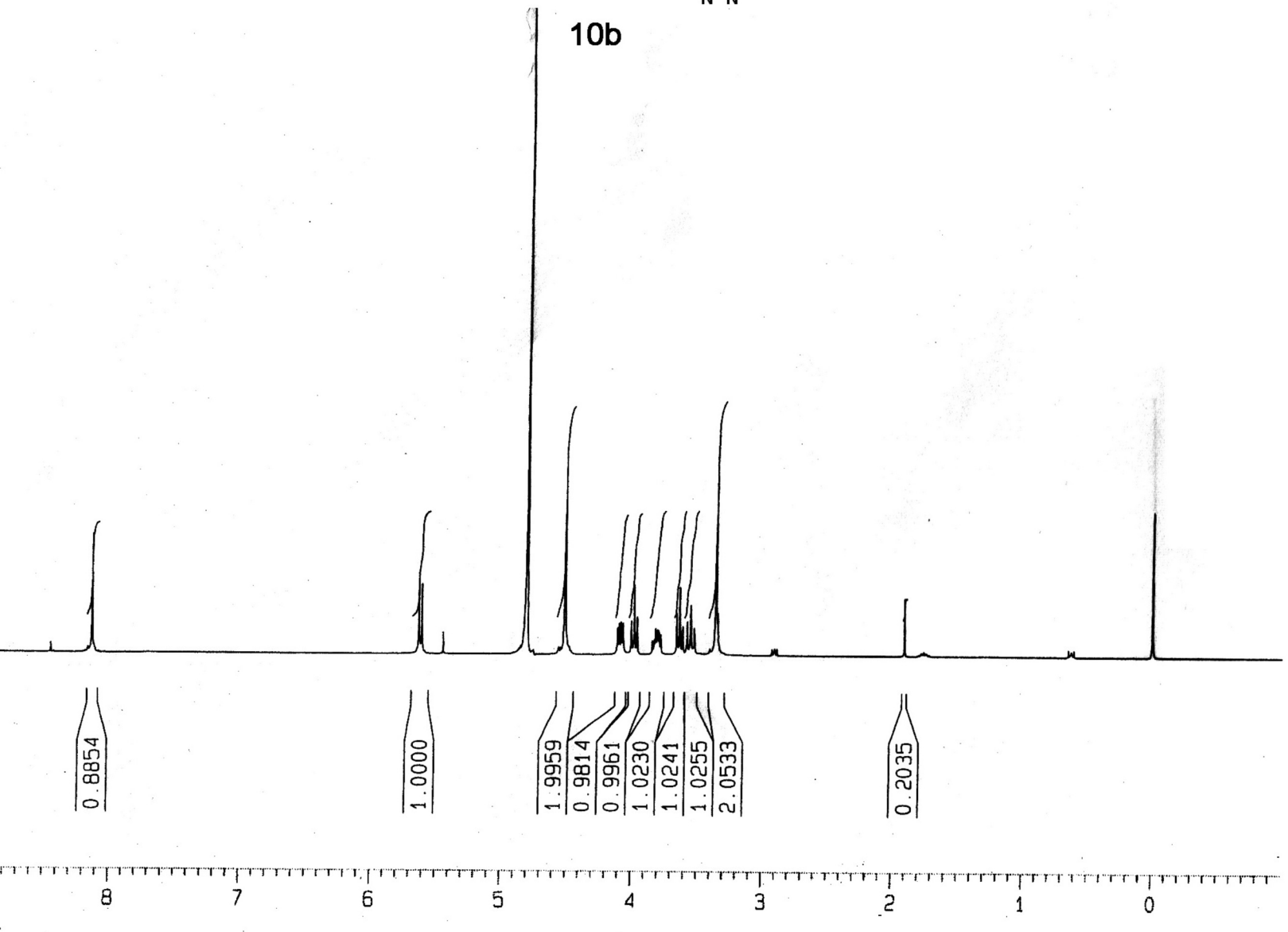
1.0650
2.0802
1.0314
2.1446
1.0568
1.0985
2.0305
6.1419
3.0703
9
8
7
6
5
4
3
2
1
0



10b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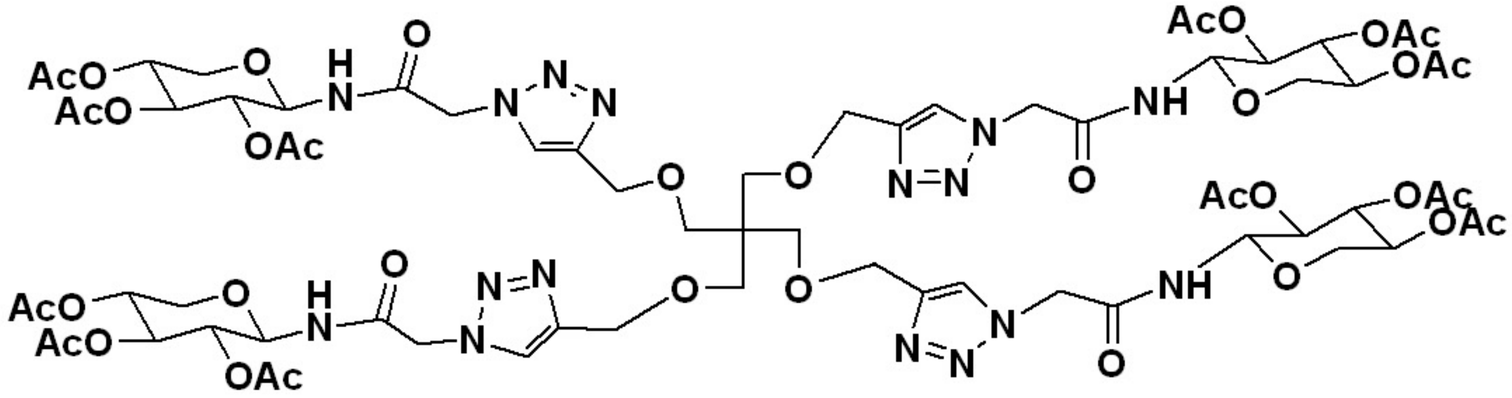
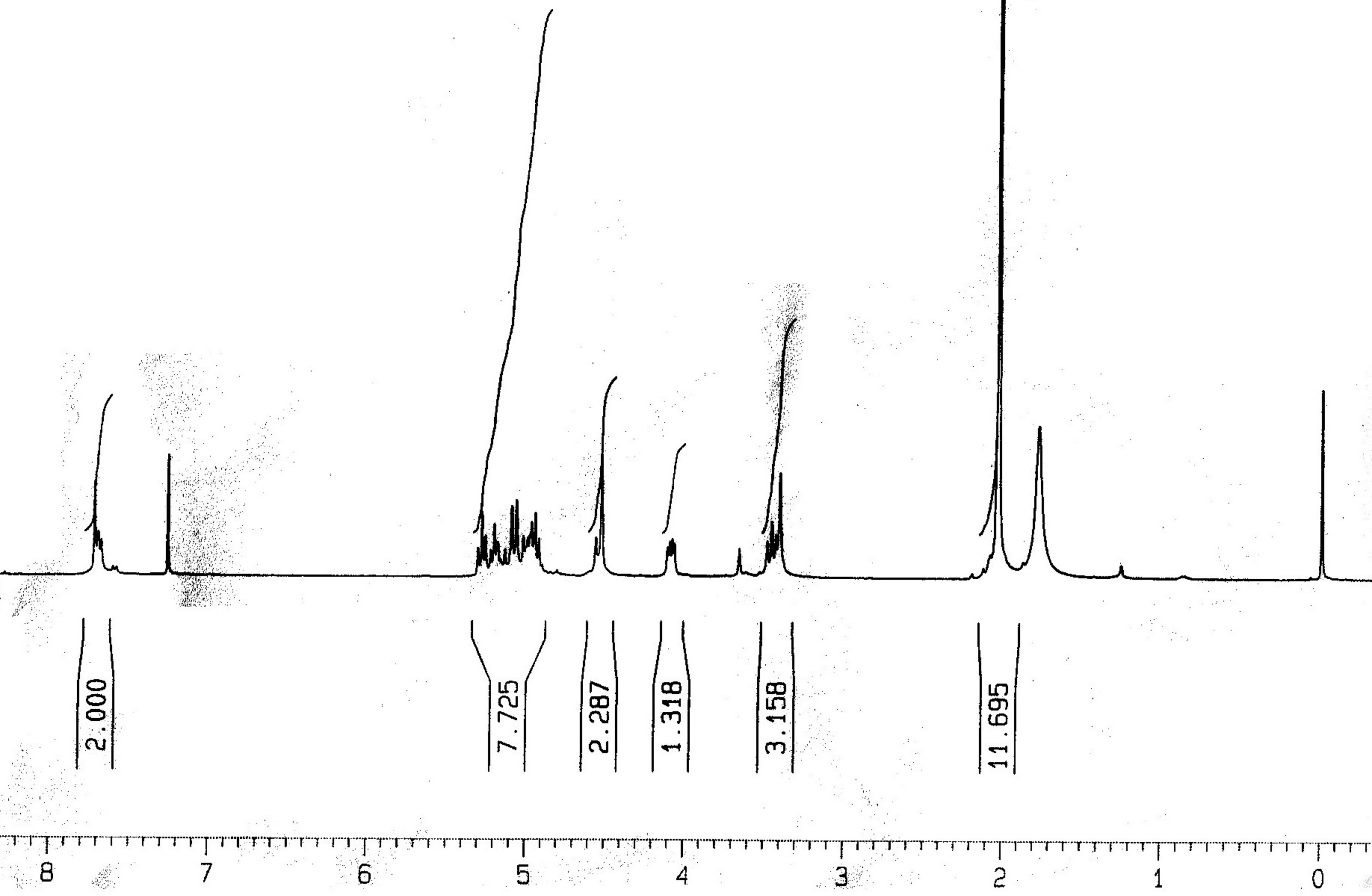
1.0000
1.9959
0.9814
0.9961
1.0230
1.0241
1.0255
2.0533
0.2035
8
7
6
5
4
3
2
1
0


AcO
AcO
OAc
O
H
N
O
N
N
N
O
O
N
N
N
O
NH
AcO
OAc
OAc
O

11a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8
7
6
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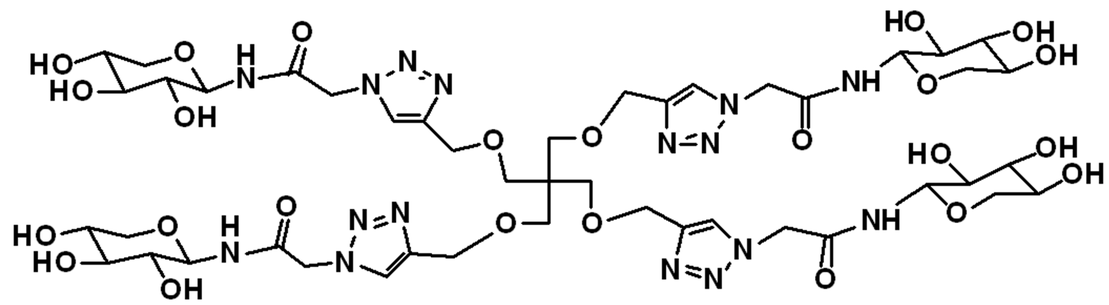

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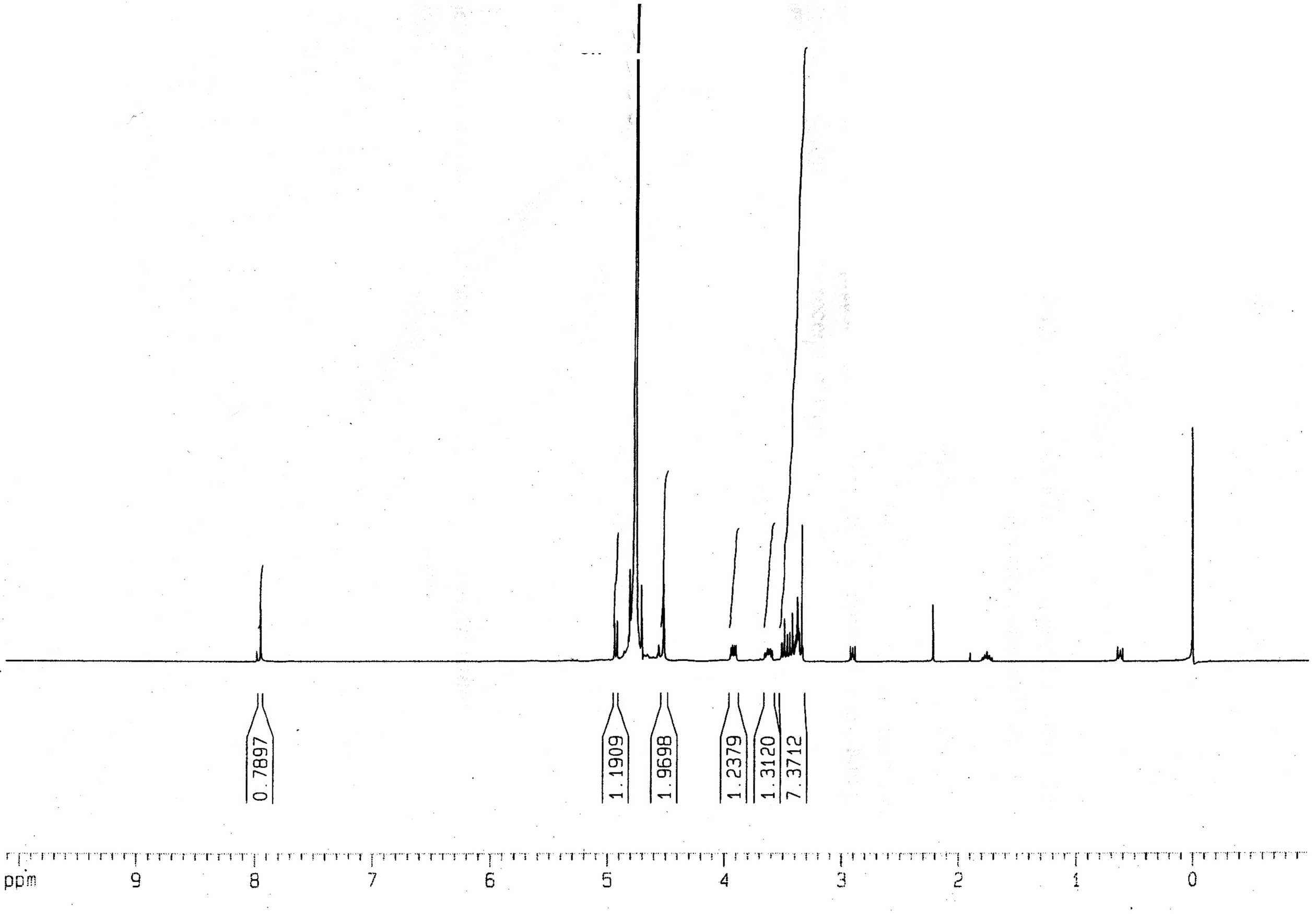

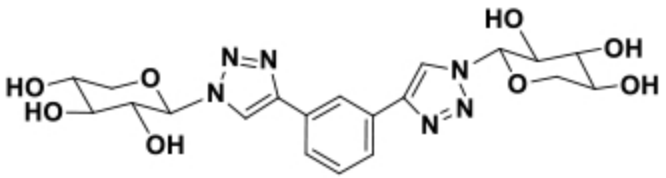

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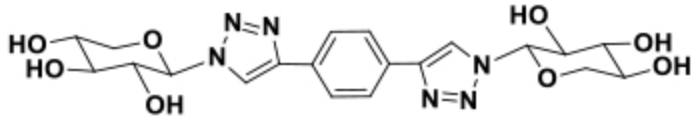

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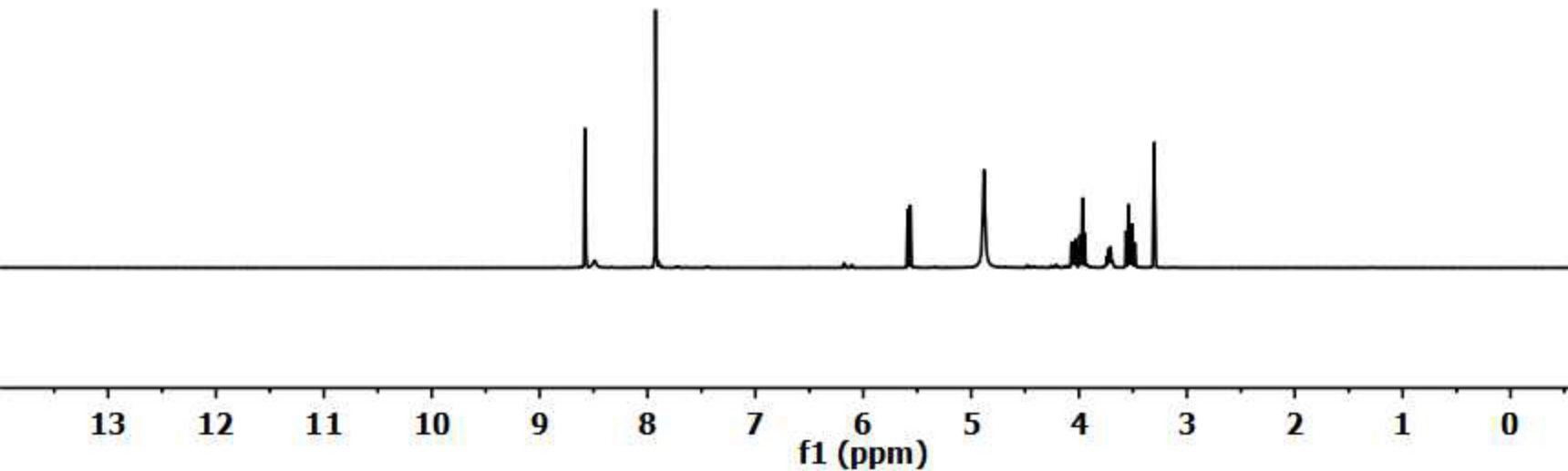

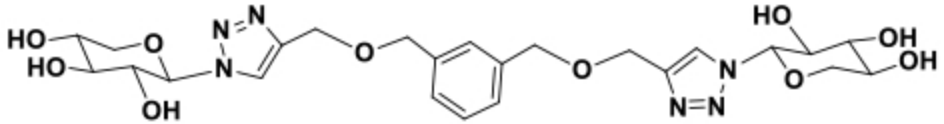

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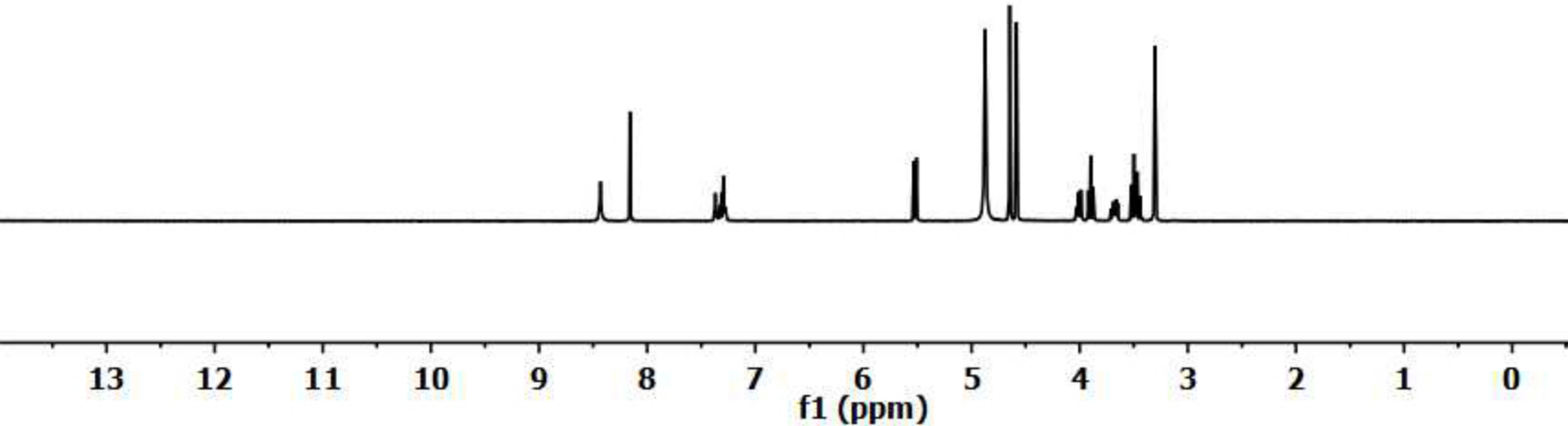

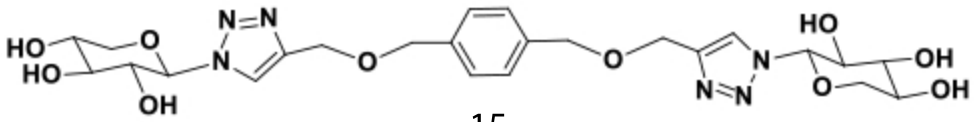

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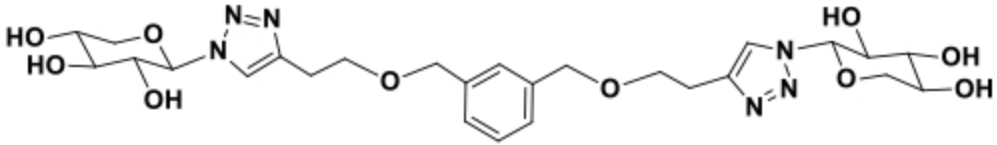

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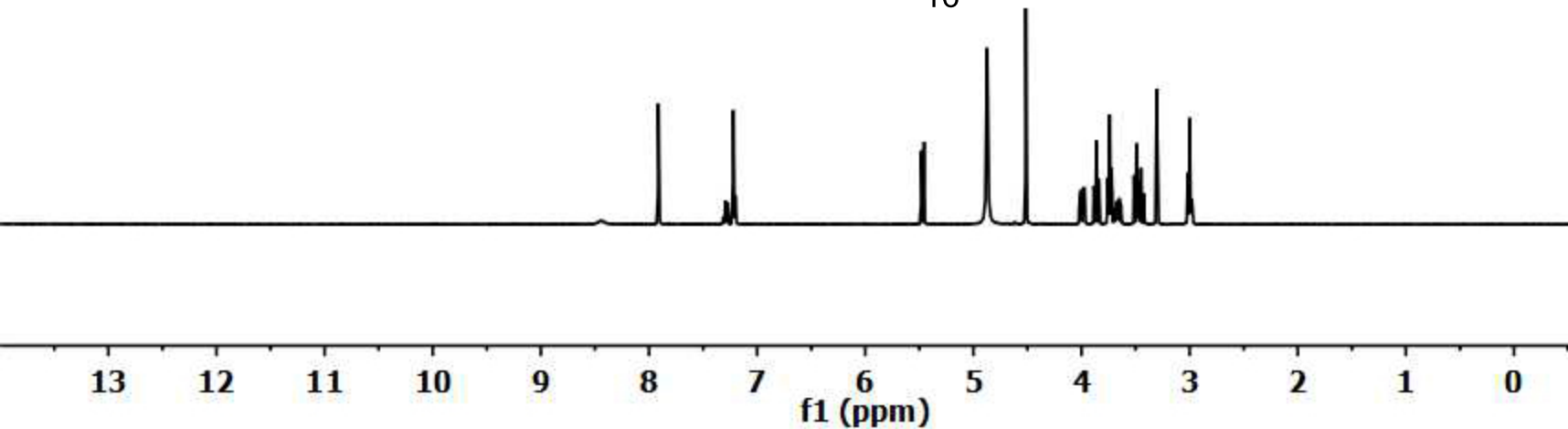

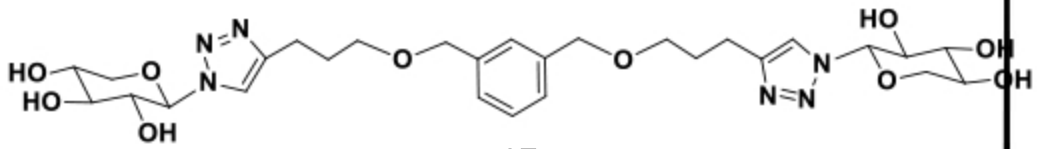

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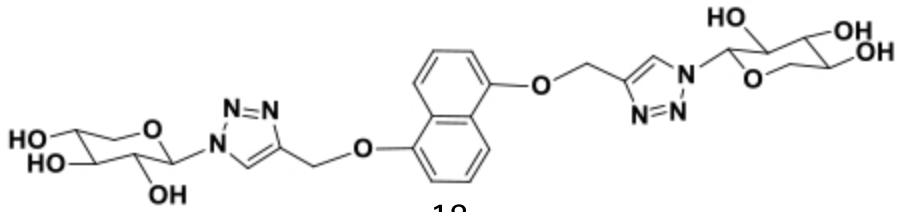

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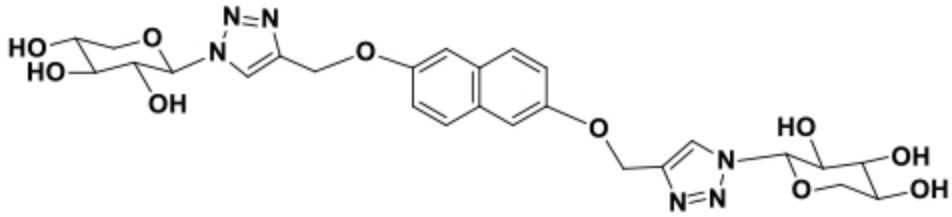

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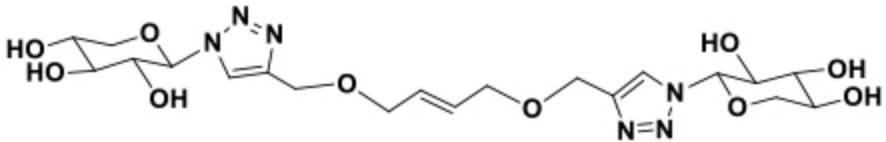

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