

# Synthesis, Antimicrobial and Mutagenic Activity of a New Class of D-Xylopyranosides

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## 1. General methods

Commercial xylose (Fluka) was used. All reactions were monitored by a thin-layer chromatography (TLC), using pre-coated silica gel 60 F<sub>254</sub> plates (100–200 mesh). Detection of spots was carried out by spraying 5% solution of H<sub>2</sub>SO<sub>4</sub> in methanol and charring. Purification of products was performed using FLASH (Interchim PuriFlash 450) chromatography, 30 µm silica gel as a stationary phase. The structures of all products were determined with the <sup>1</sup>H, <sup>13</sup>C, and 2D (HSQC, COSY) NMR, Bruker AVANCE III, at 400 or 500 MHz and 100 or 125 MHz for <sup>1</sup>H, <sup>13</sup>C, respectively, in CDCl<sub>3</sub> or D<sub>2</sub>O as solvents. Mass spectrometry analysis was done using Bruker Biflex III MALDI-TOF-MS spectrometer (with 2,5-dihydroxybenzoic acid (DHB) matrix) and HRMS was performed using electrospray ionization with time-of-flight mass analysis (Agilent Technologies 6550 iFunnel Q-TOF). Optical rotation was measured with 343 PerkinElmer polarimeter.

**Table S1.** Reaction yields and solvents used for the quaternization reaction at 70 °C.

No.	Substrate	Amine	Solvent	Product	Yield [%]
1	2	pyridine	-	<b>4a</b>	67
2		trimethylamine	ethanol	<b>4b</b>	87
3		<i>N,N</i> -dimethylhexylamine	acetonitrile	<b>4c</b>	72
4		<i>N,N</i> -dimethyloctylamine	acetonitrile	<b>4d</b>	70
5	3	pyridine	-	<b>5a</b>	75
6		trimethylamine	ethanol	<b>5b</b>	55
7		<i>N,N</i> -dimethylhexylamine	acetonitrile	<b>5c</b>	68
8		<i>N,N</i> -dimethyloctylamine	acetonitrile	<b>5d</b>	70

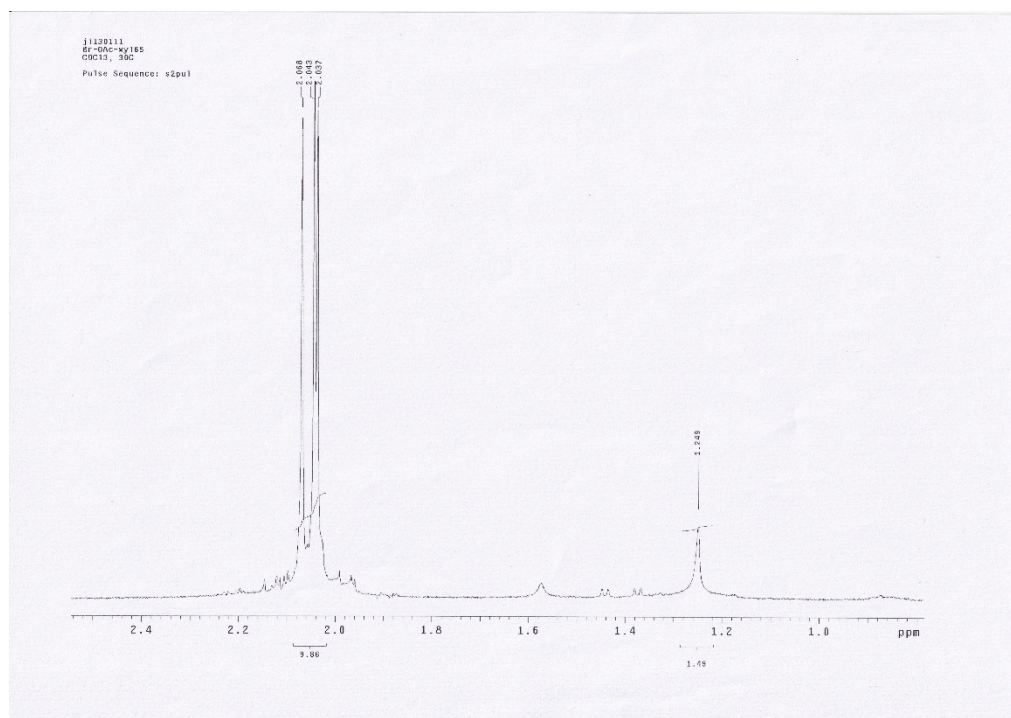
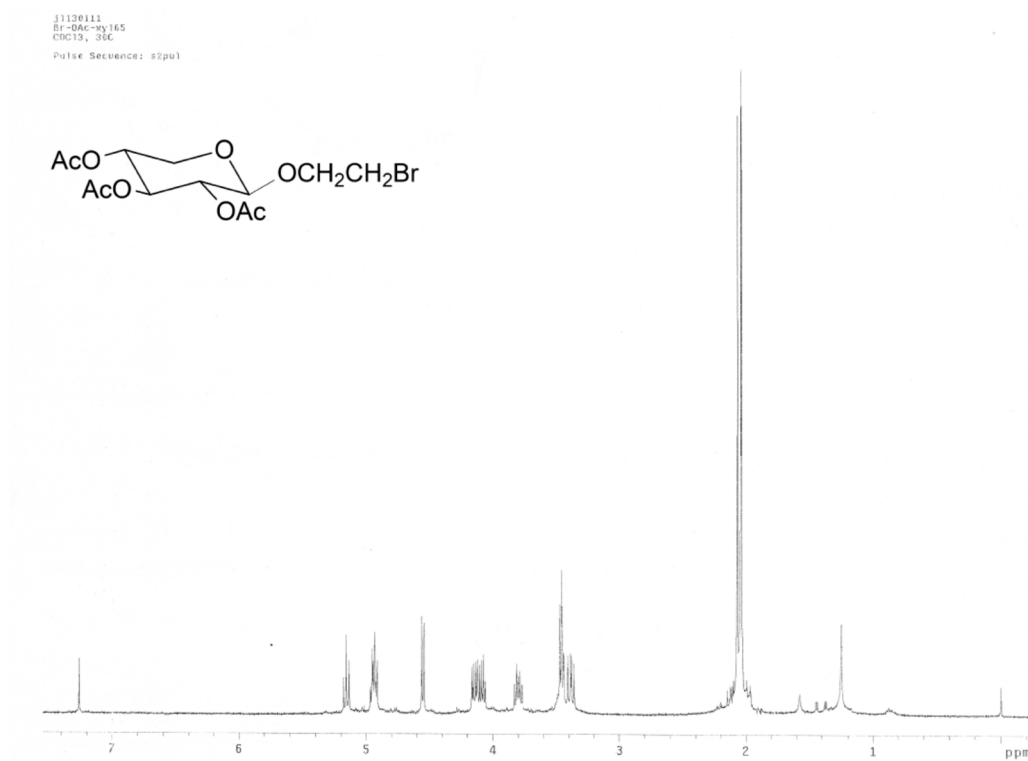
## 2. Antimicrobial Activity

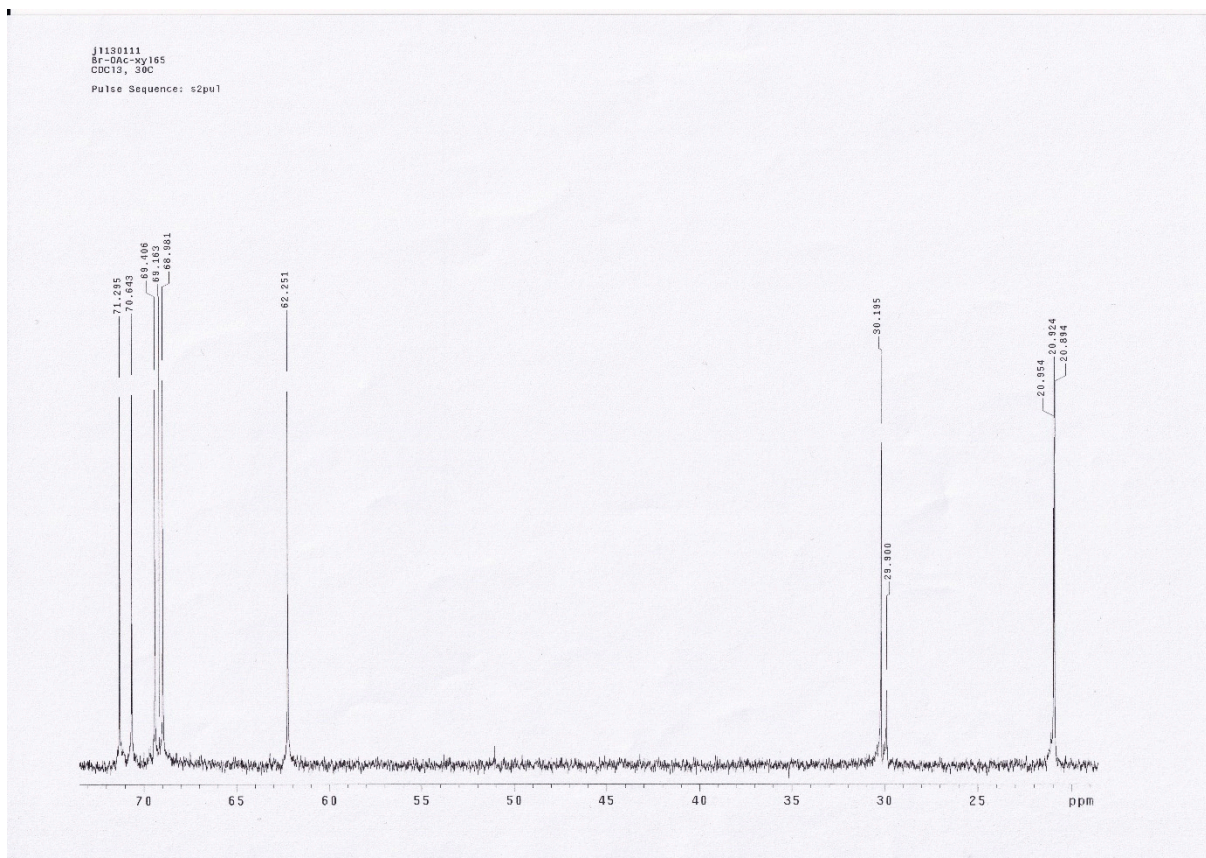
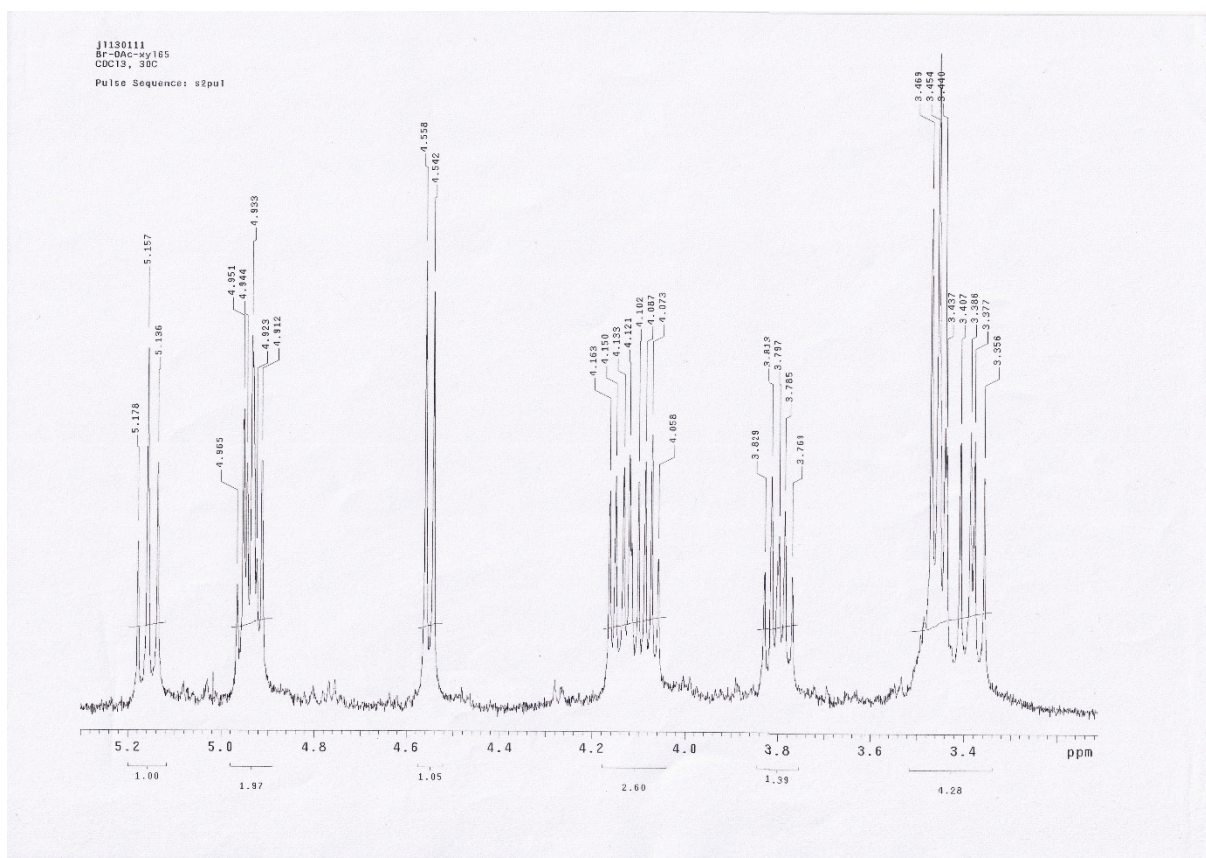
The preliminary assessment of antimicrobial potential of the synthesized compounds was performed against two reference strains of bacteria: *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* K12 and also two reference strains of pathogenic yeasts, *Candida albicans* ATCC10231 and *Candida glabrata* DSMZ11226. Moreover, 20 clinical isolates of *S. aureus* collected from patients with skin and soft tissue infections (*n* = 8) and derived from subclinical bovine mastitis milk samples (*n* = 12) were used for evaluation of anti-staphylococcal activity of two compounds that were found to be most promising in preliminary

test. Four of these strains were classified as MRSA (Methicillin-Resistant *Staphylococcus aureus*); they were *mecA* positive and did not exhibit susceptibility to methicillin. The MICs (Minimum Inhibitory Concentration) values for all compound were determined by CLSI (Clinical and Laboratory Standards Institute) recommended microdilution methods with minor modifications [1,2]. Briefly, the 8192 mg/mL (8192 µg/mL) solutions of the synthesized compounds were prepared in Mueller-Hinton Broth 2 - cation adjusted (MHB2) for evaluation of antibacterial activity and in RPMI medium in the case of evaluation of the antifungal activity. Subsequently, the serial, two-fold dilutions of the tested agents (over the range of concentrations from 8192 to 16 µg/mL) were prepared in 96-well microtitration plates in the final volume of 100 µL of the appropriate medium. The pure bacterial cultures of (both the reference and clinical strains) were grown on the Mueller-Hinton Agar (MHA) for 18–24 h at 37 °C and yeasts strains were cultivated on Sabouraud Dextrose Agar (SDA) in the same conditions. A loop of a pure culture of each strain tested (three to five colonies separately growing on agar medium) was suspended in the sterile PBS (phosphate buffered saline, pH 7.4 at 25 °C, purchased from Sigma) solution to get an optical density  $OD_{600} = 0.13$  (for bacteria - equal to the cells concentration approximately  $1 \times 10^8$  CFU/mL) or  $OD_{660} = 0.1$  (for yeasts – equal to the cells concentration approximately  $1 \times 10^6$  CFU/mL). The obtained suspensions of the cells were next diluted 1:100 (*v/v*) in the MHB2 medium (bacteria) and 1:50 in RPMI medium (yeasts). One hundred µL of the cells' suspension was finally loaded into the wells of plates prepared in advance, which contained 100 µL of two-fold dilutions of the tested agents. The positive growth control of each strain (both bacteria and yeasts) was performed in the wells without the tested substances. The negative control containing only the media was included in each assay. The plates were incubated for 24 h at 37 °C. Following the incubation period, determination of the MIC values of the tested agents was carried out by measuring the absorbance at 531 nm using a Victor3 microplate reader (Perkin Elmer, Inc., Waltham, MA, USA). The lowest concentration of the agent causing inhibition of growth equal to or higher than 90% ( $MIC_{90}$ ) of growth control was taken as the MIC value. Each test was repeated three times.

### 3. Mutagenic Assay—the Ames Test

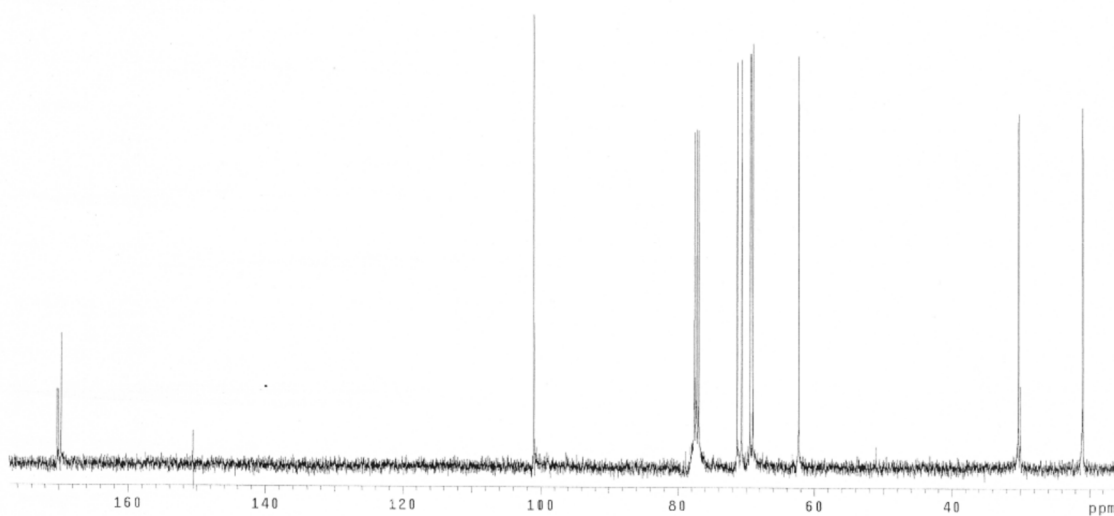
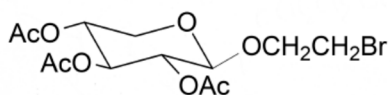
*Salmonella typhimurium* TA98 strain was kindly given by Dr. T. Nohmi, Division of Genetics and Mutagenesis, National Institute of Hygienic Sciences, Tokyo, Japan. *Salmonella* cultures were prepared by inoculation from stock cultures into nutrient broth No. 2 (Oxoid, Hampshire, UK) and incubation for 12 h at 37 °C. Next, 100 µL of the bacterial culture (approximately  $1-2 \times 10^8$  bacteria/ tube), 50 µL of the investigated compound at different concentrations (final concentrations used in the study were 2000, 500, 100, 20 and 4 µg/plate, diluted in DMSO), and 500 µL of a buffer solution (0.1 mM, pH=7.4) were mixed and preincubated for 30 min at 37 °C. Then, 2 mL of the top agar containing *histidine/biotin solution* were added to the incubation mixture. The mixture was then poured onto the surface of glucose minimal agar plates. After 48 h revertant colonies were counted. Negative (DMSO) and positive (4-Nitro-*o*-phenylenediamine, 4-NPD, 10 µg/plate, Sigma-Aldrich, St Louis, MO, USA) controls were performed in parallel in each experiment. Experiments were performed in triplicate [3,4]. The mutagenic index (MI) was calculated for each concentration tested, this being the average number of revertants per plate with the test compound divided by the average number of revertants per plate with the negative (solvent) control. A sample was considered mutagenic when a dose-response relationship was detected and at least 2-fold increase in the number of mutants ( $MI \geq 2$ ) was observed with at least one concentration [5,6].

2-Bromoethyl 2',3',4'-tri-O-acetyl- $\beta$ -D-xylopyranoside (2) $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):

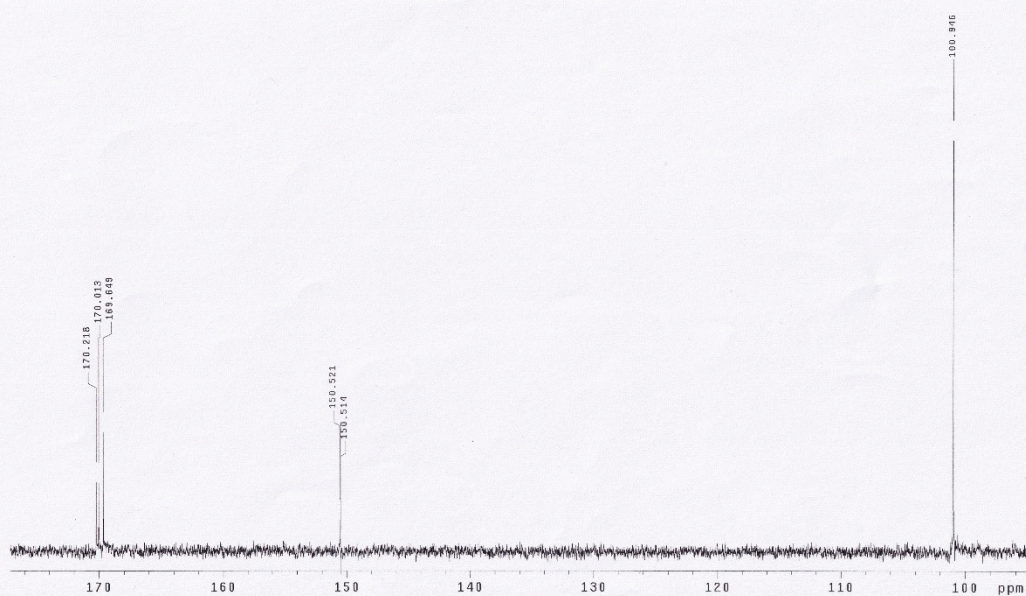


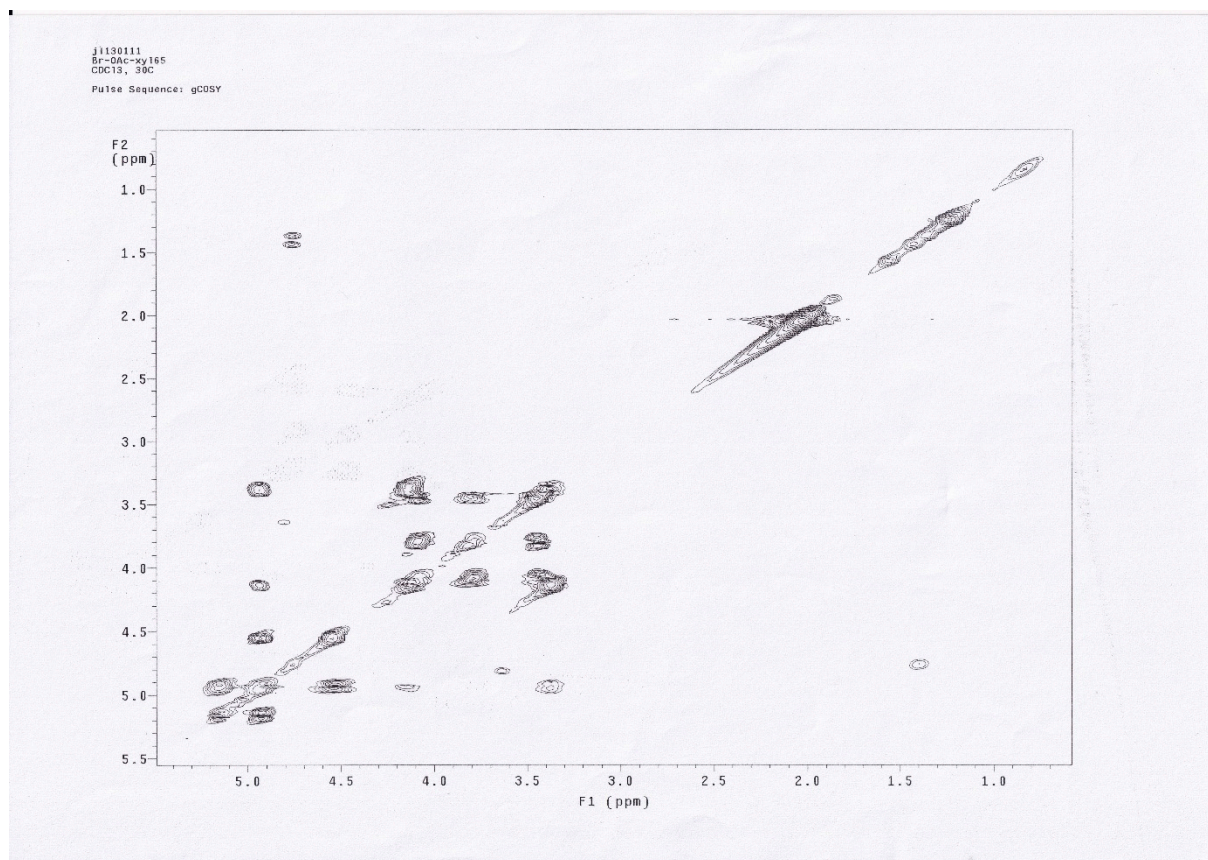
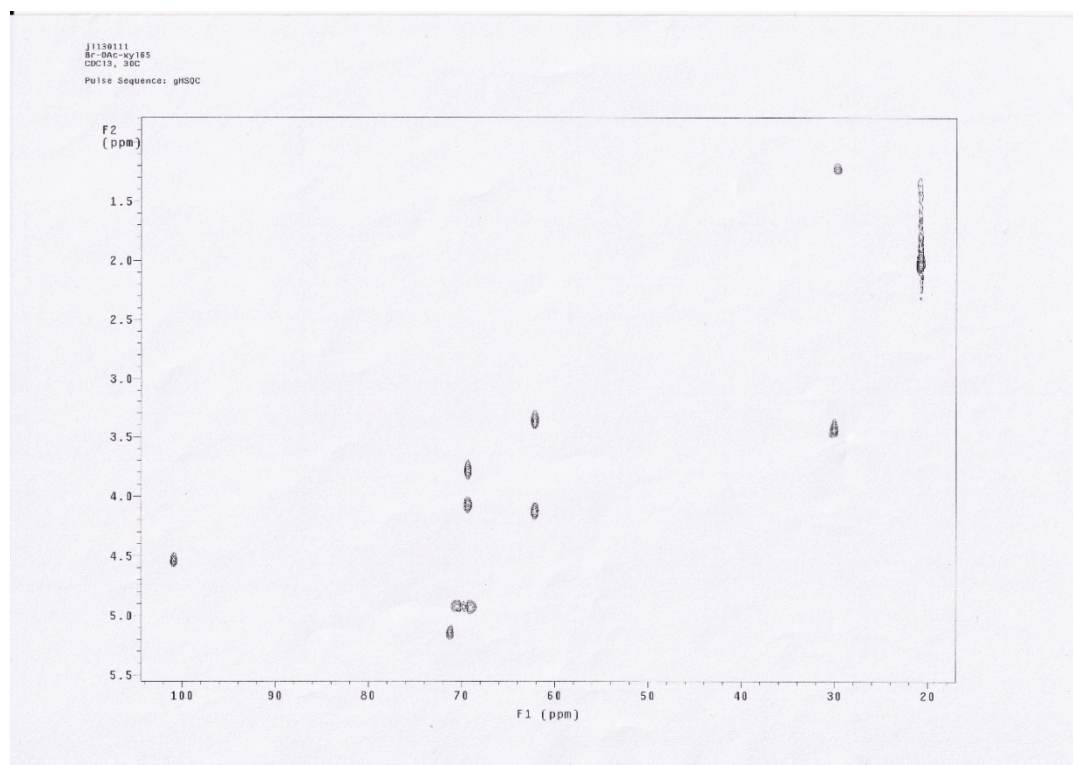
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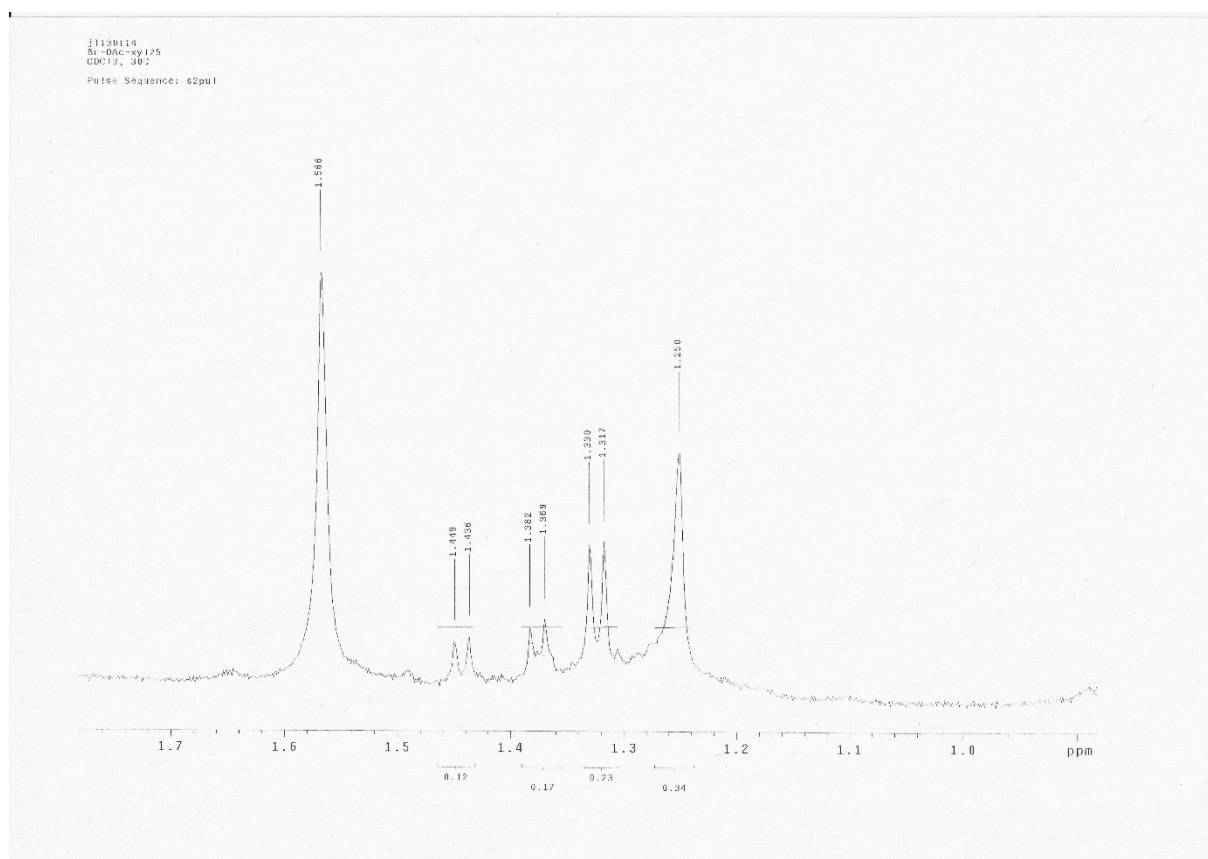
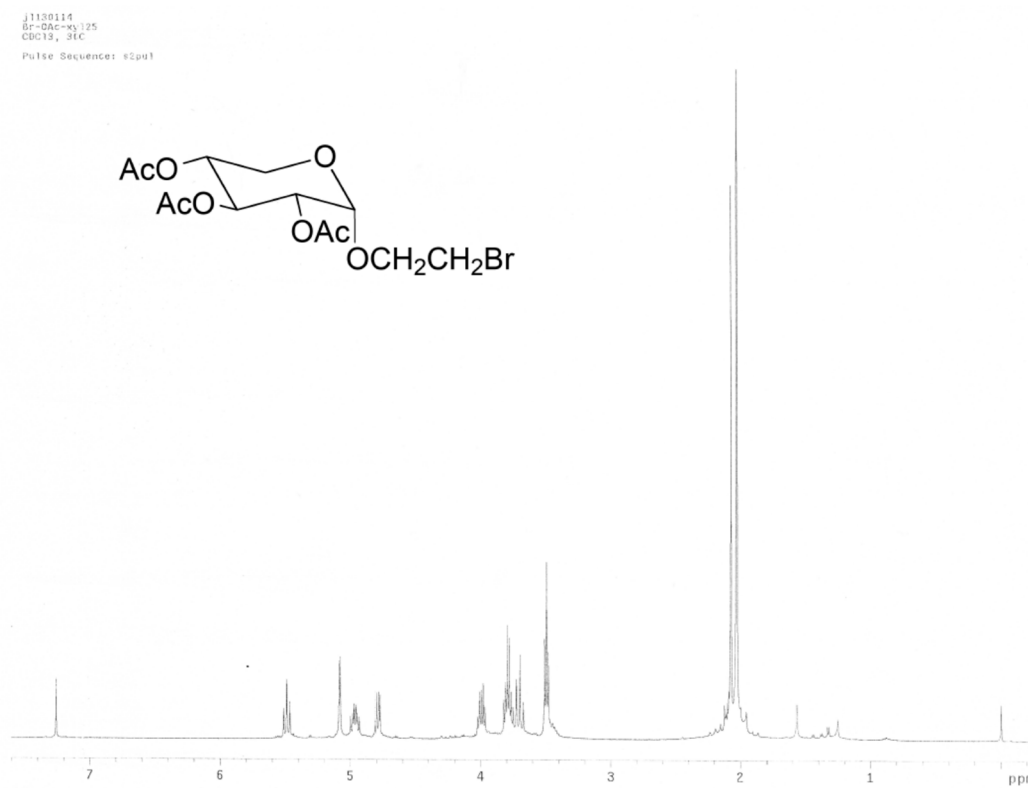
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 $\text{CDCl}_3$ , 31°C  
Pulse Sequence: s2pu1

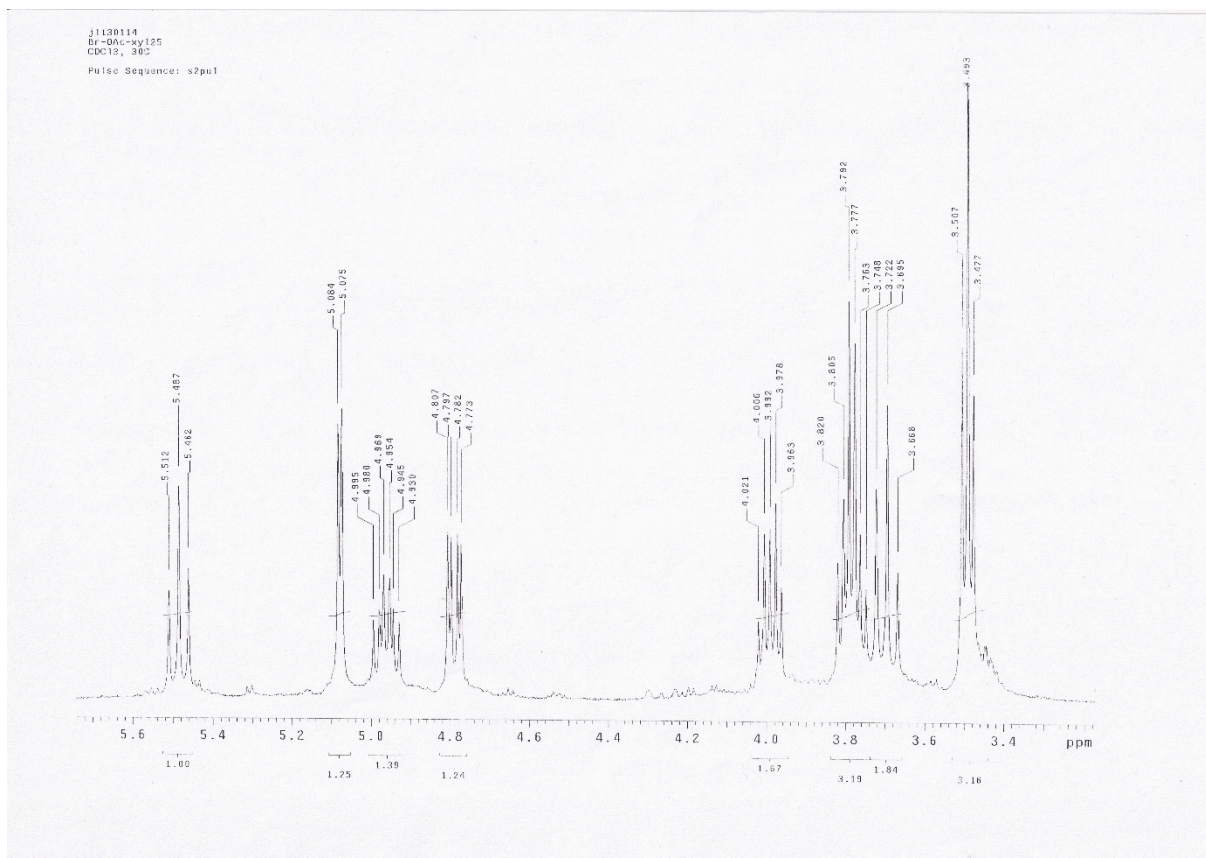
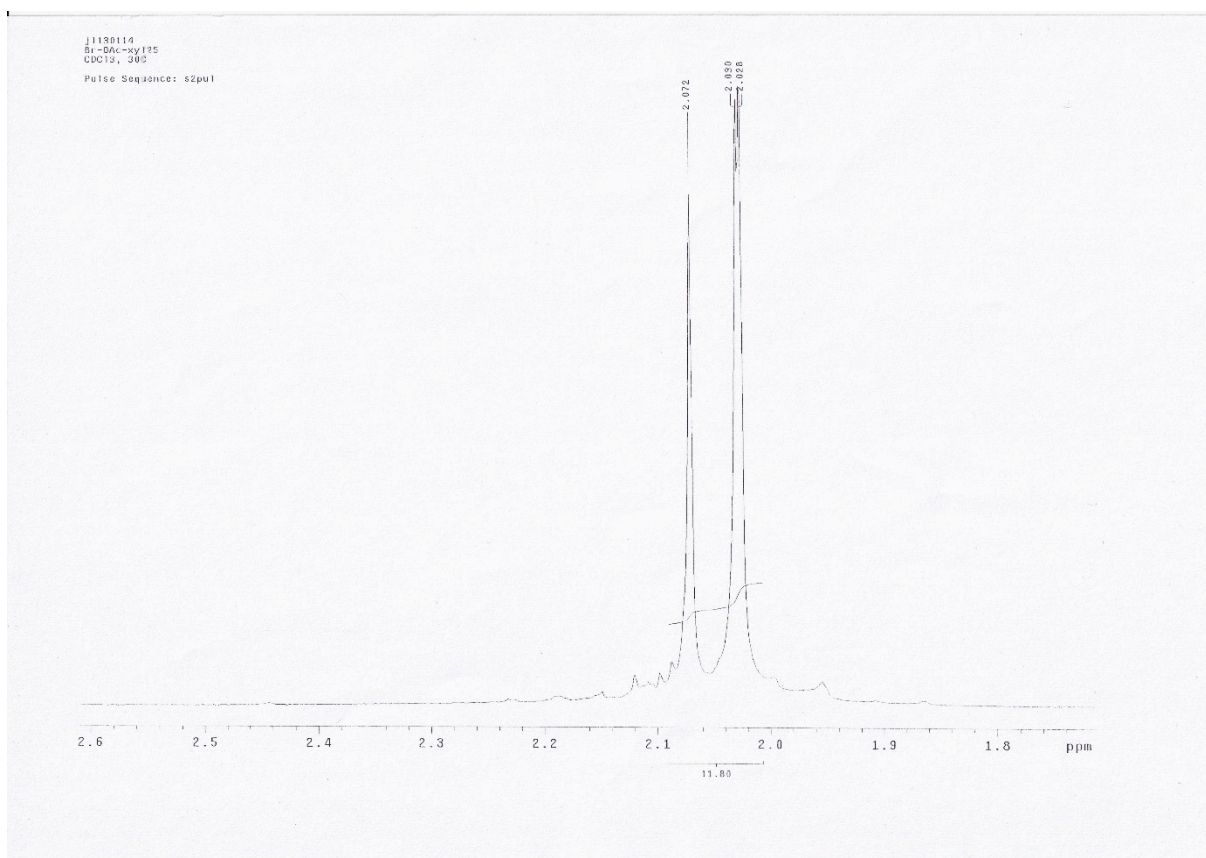


J1130111  
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 $\text{CDCl}_3$ , 30°C  
Pulse Sequence: s2pu1



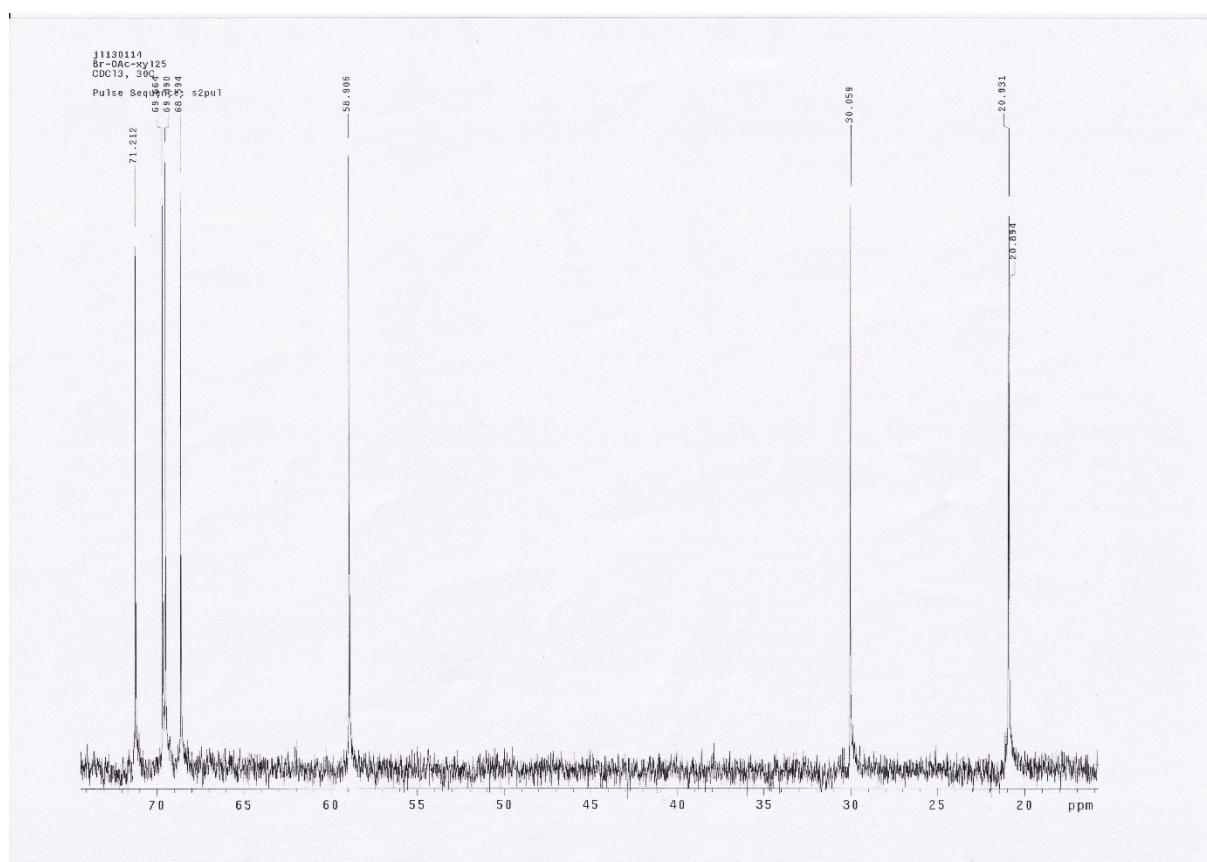
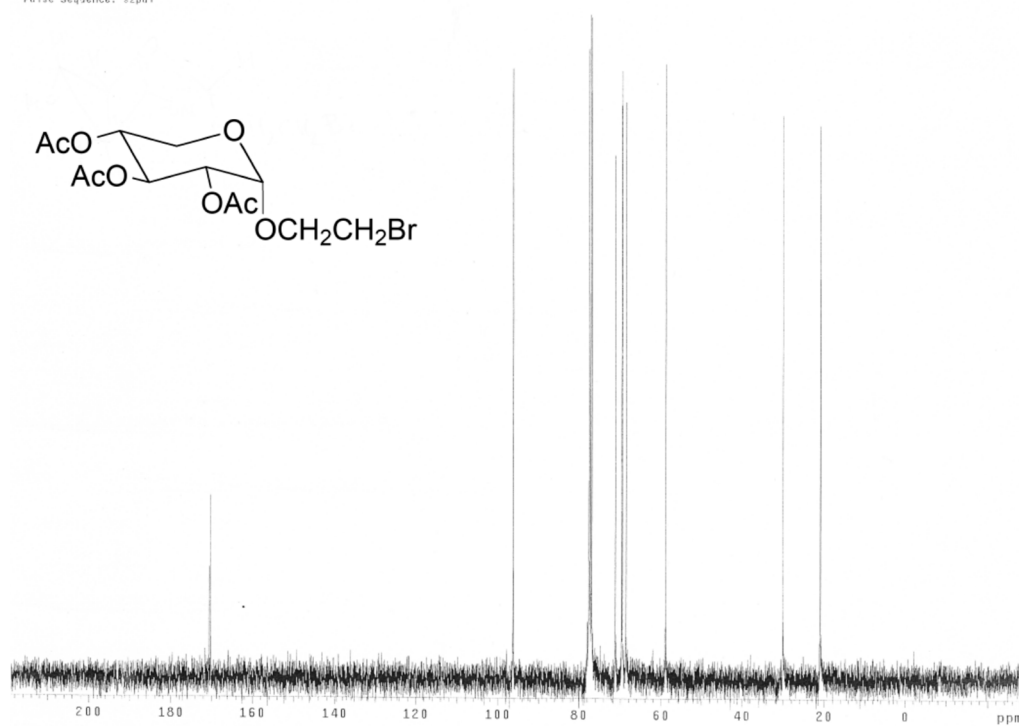
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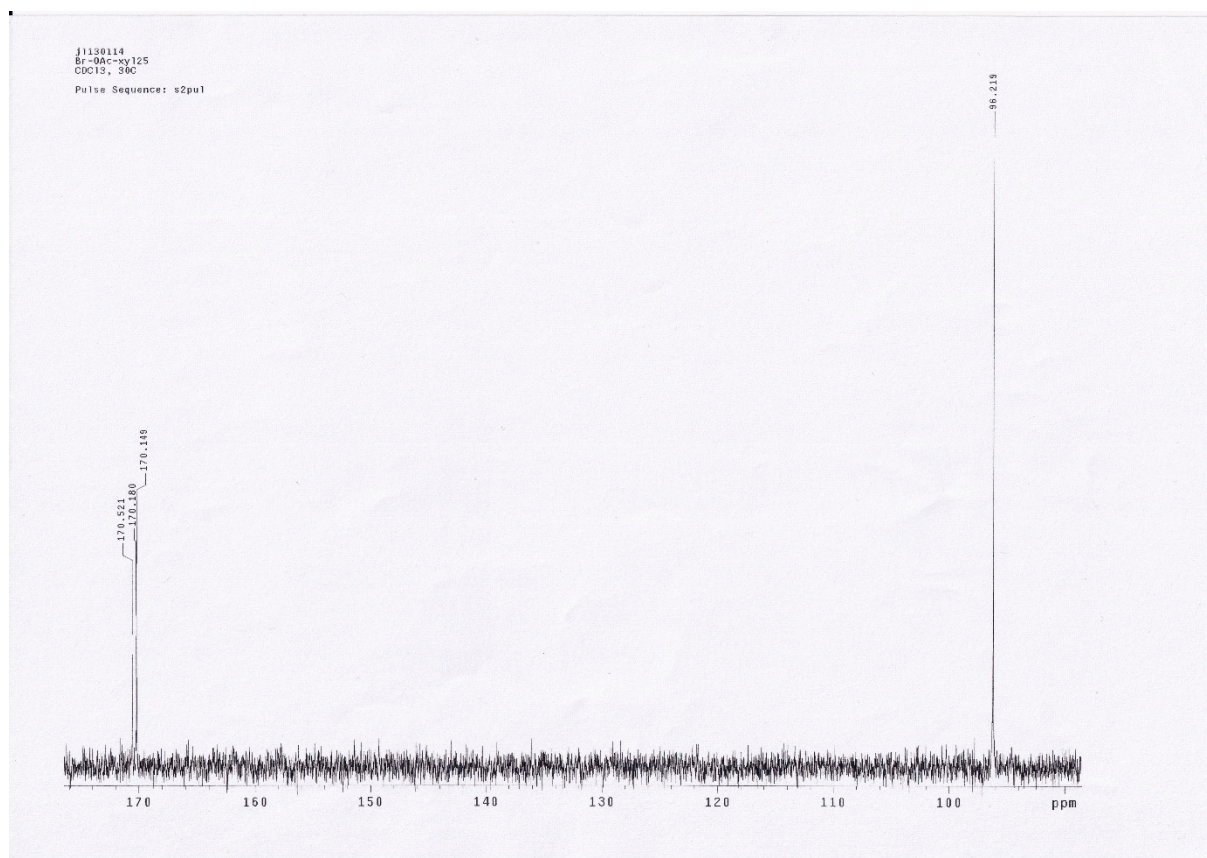
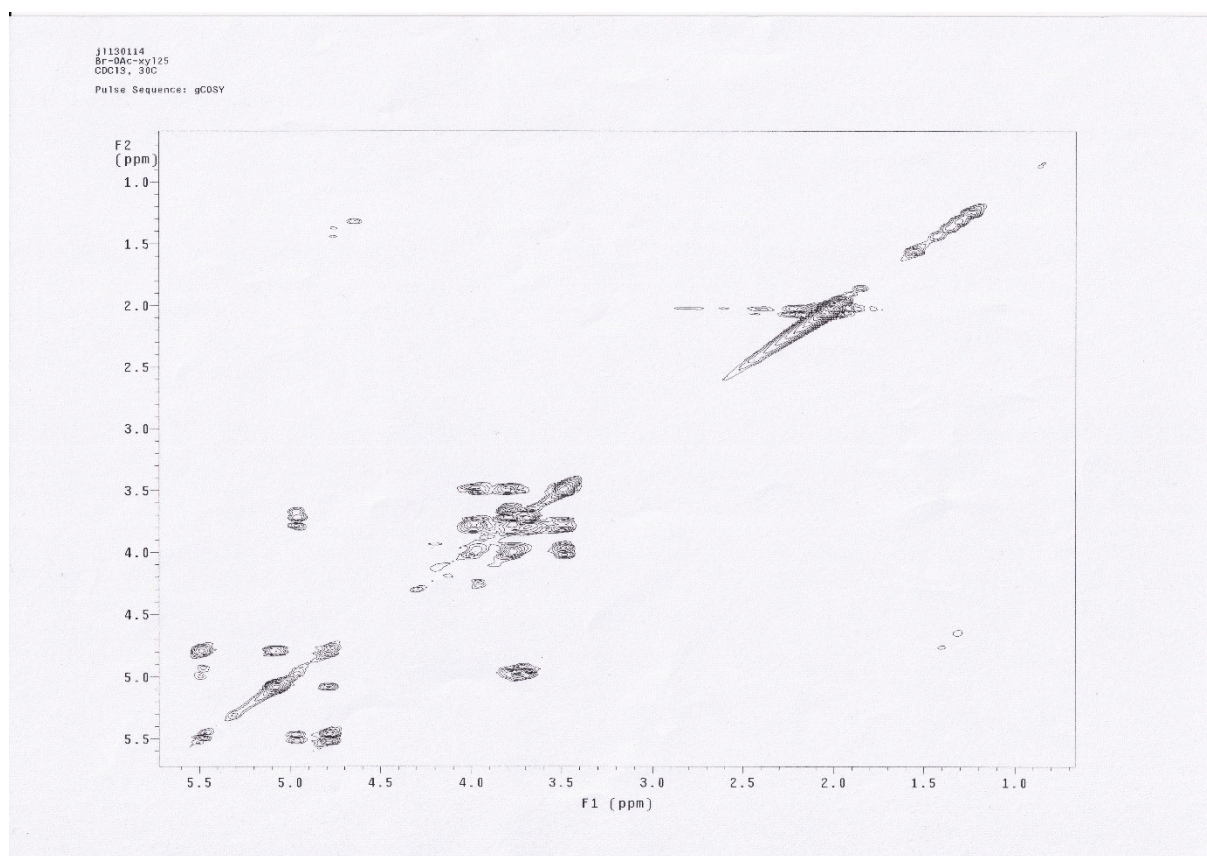
2-Bromoethyl 2',3',4'-tri-O-acetyl- $\alpha$ -D-xylopyranoside (**3**) $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):

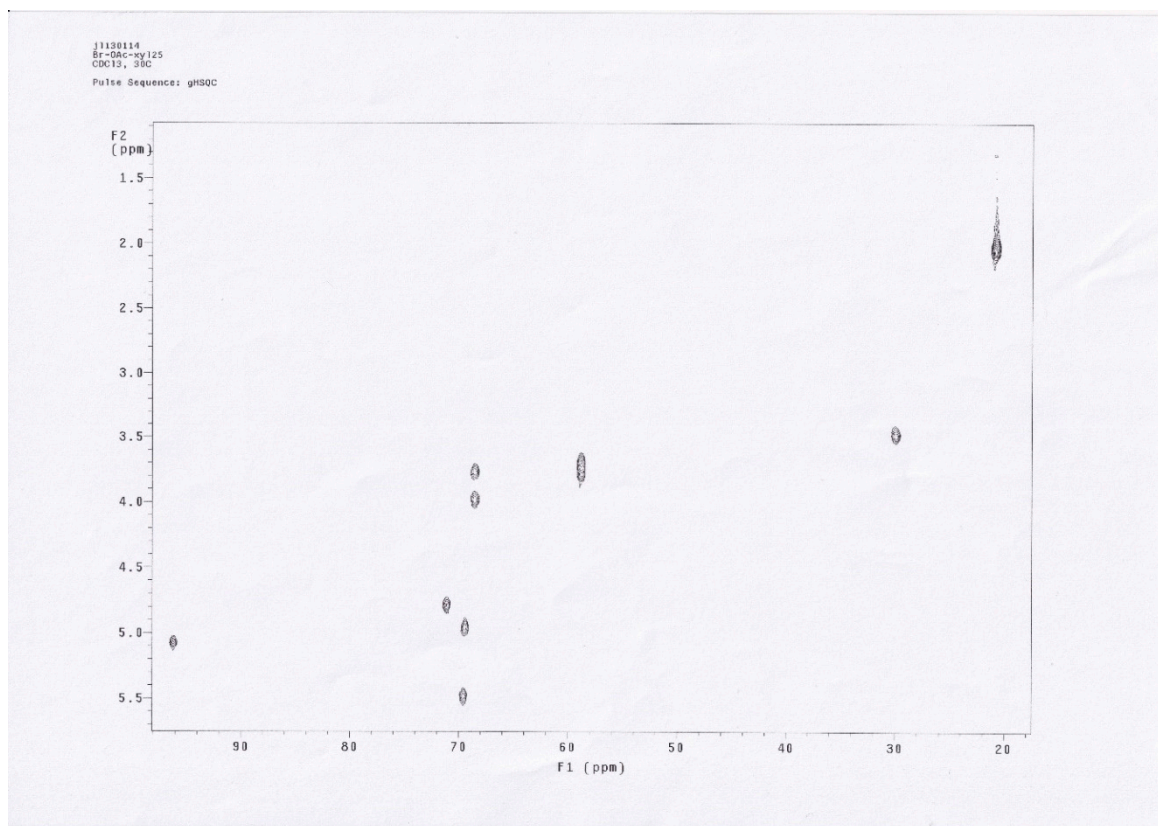


$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):

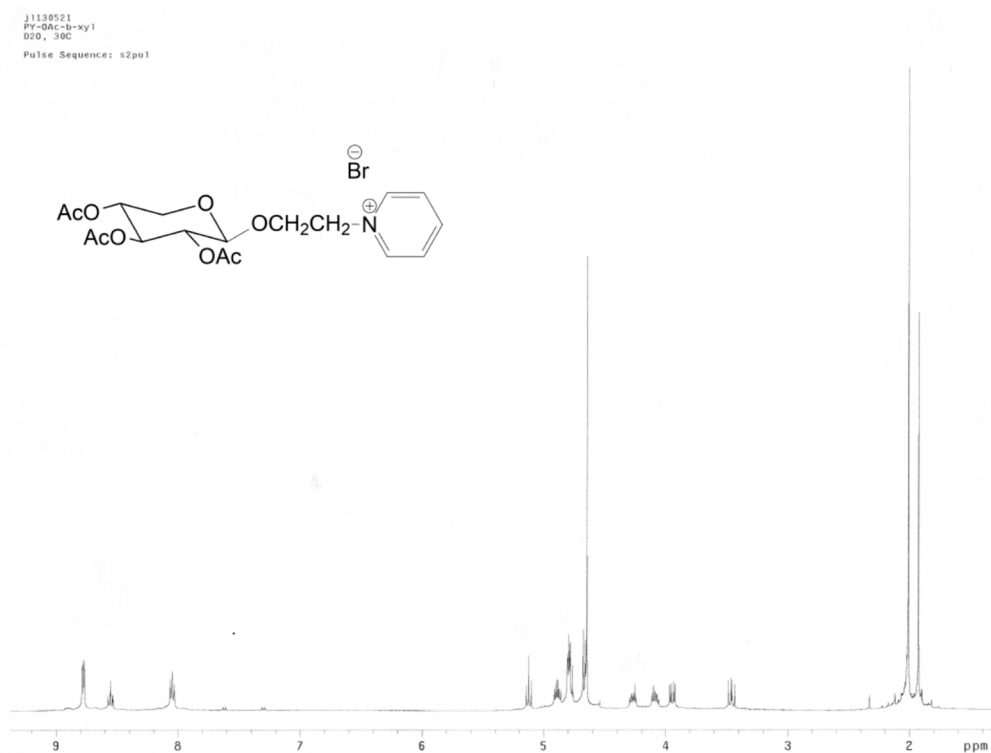
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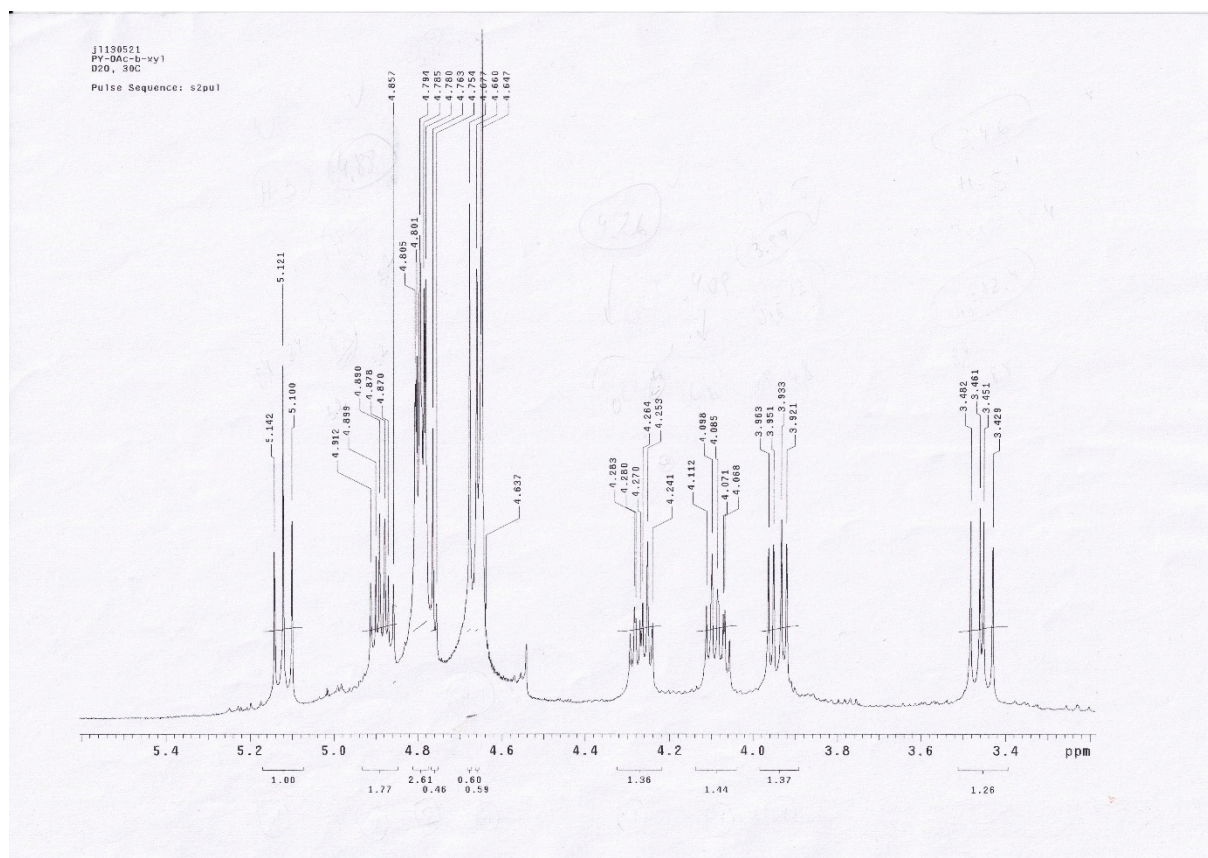
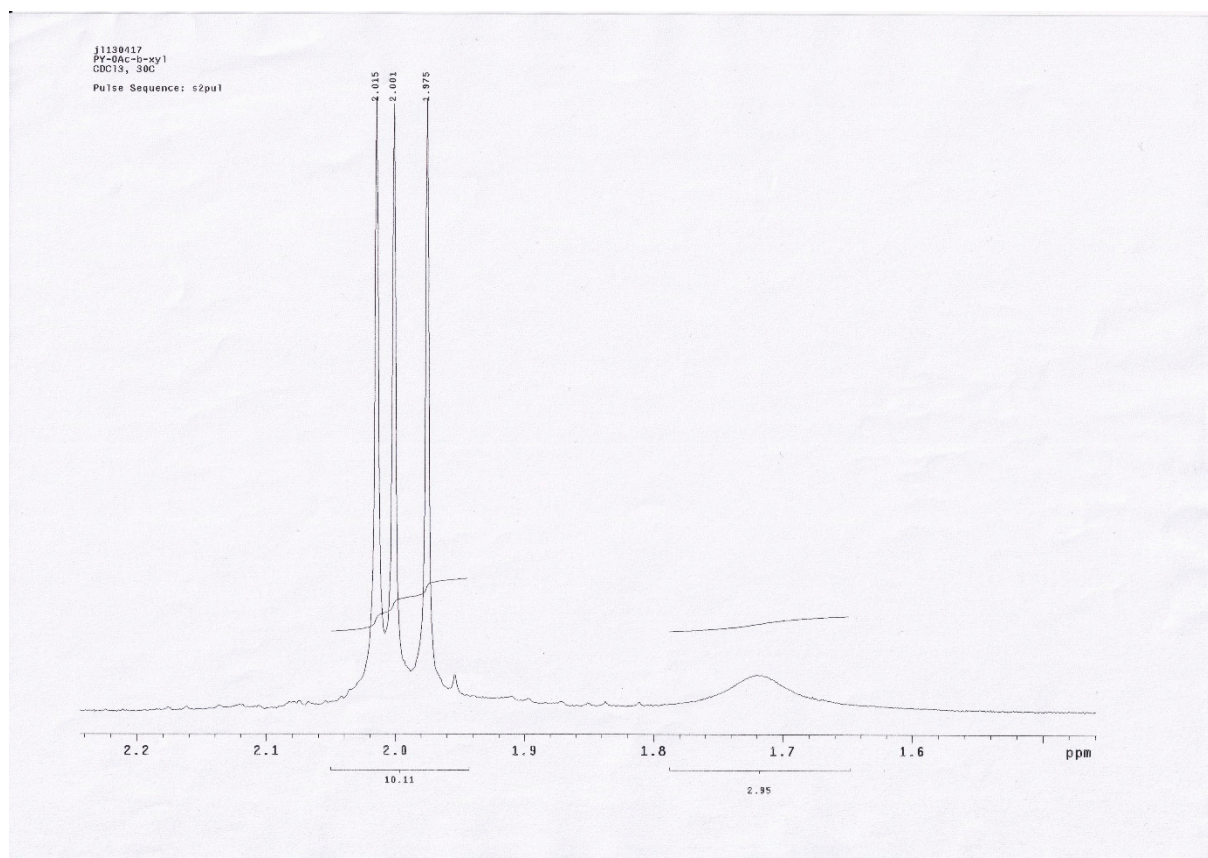


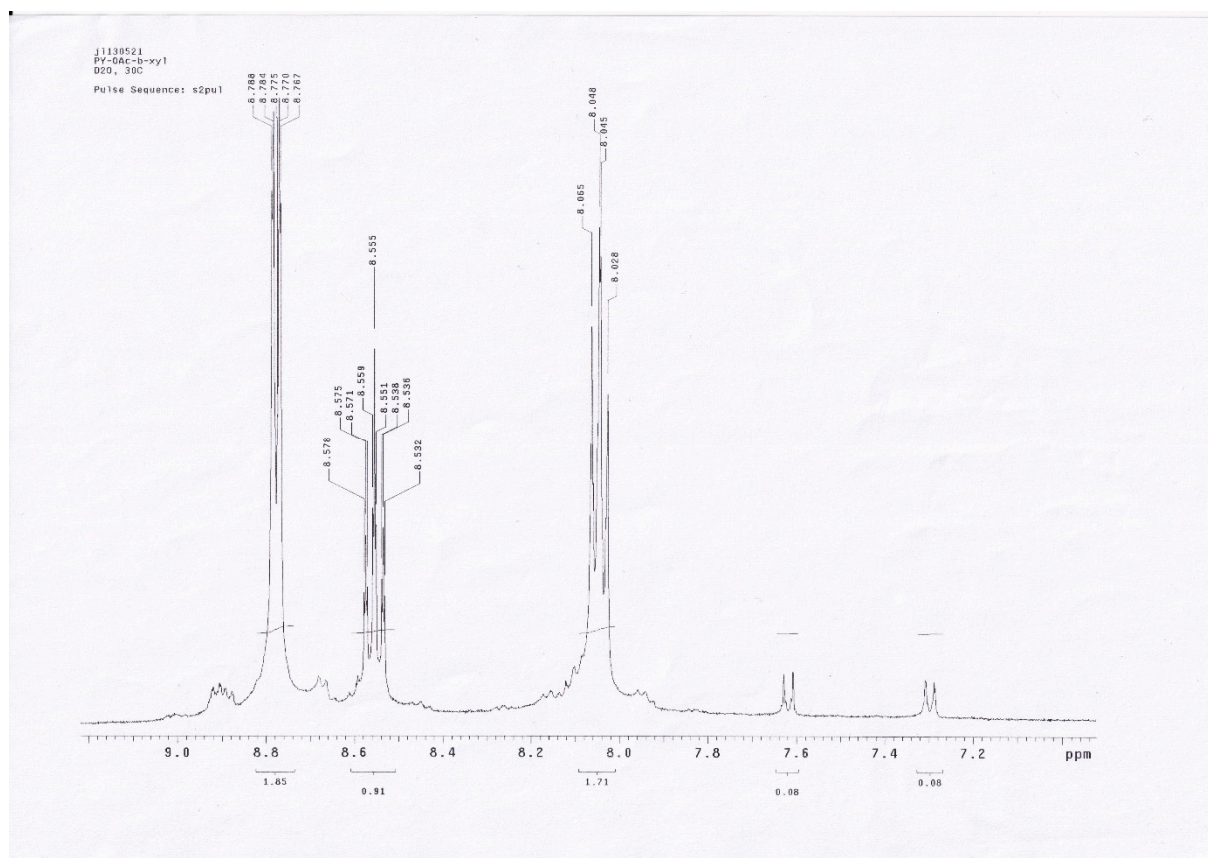
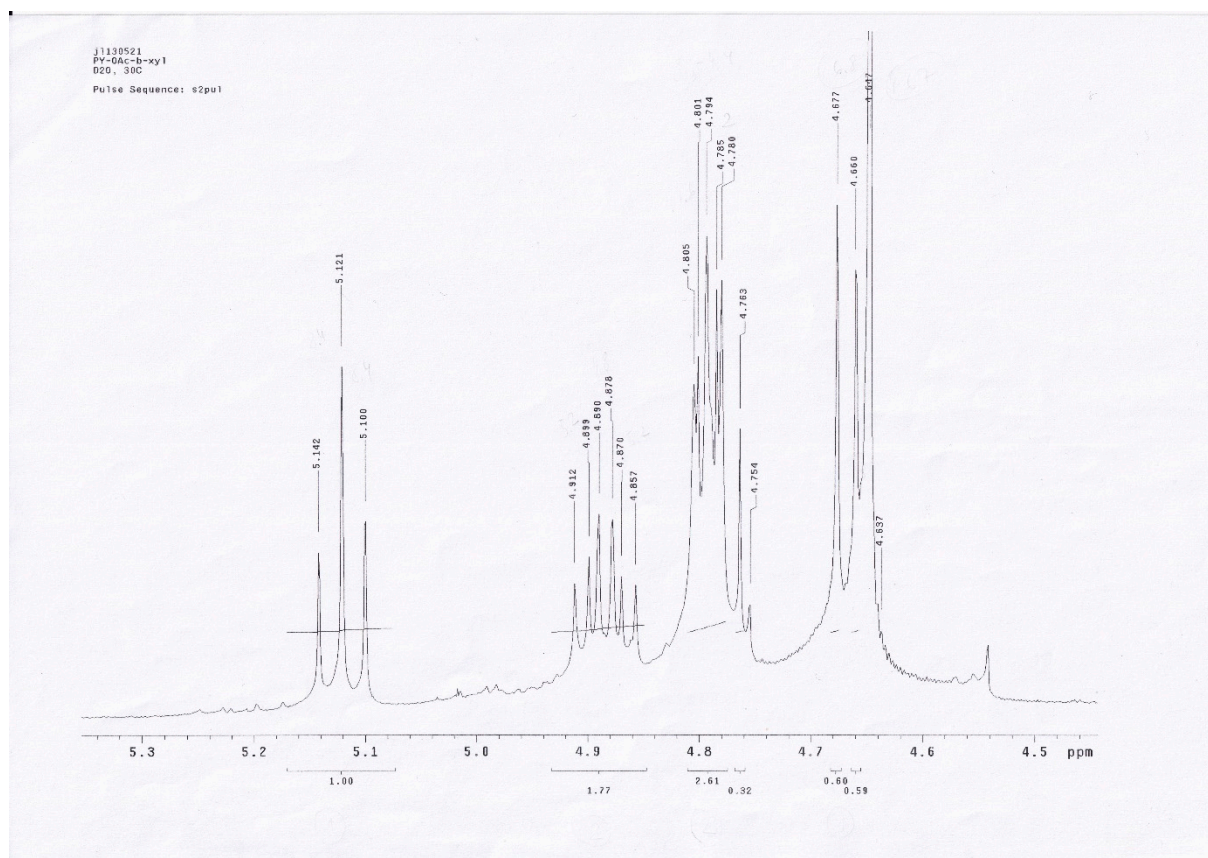
 $^1\text{H}$ - $^1\text{H}$  COSY NMR:

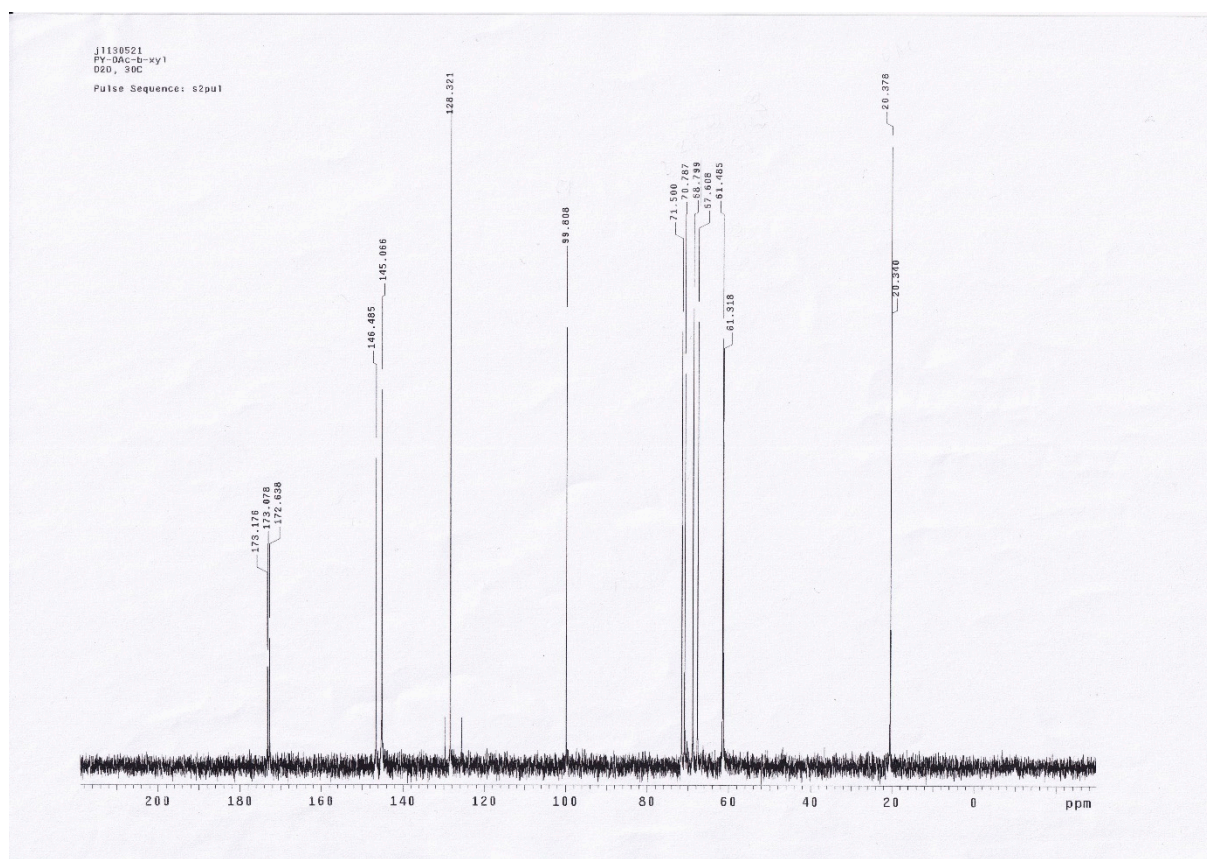
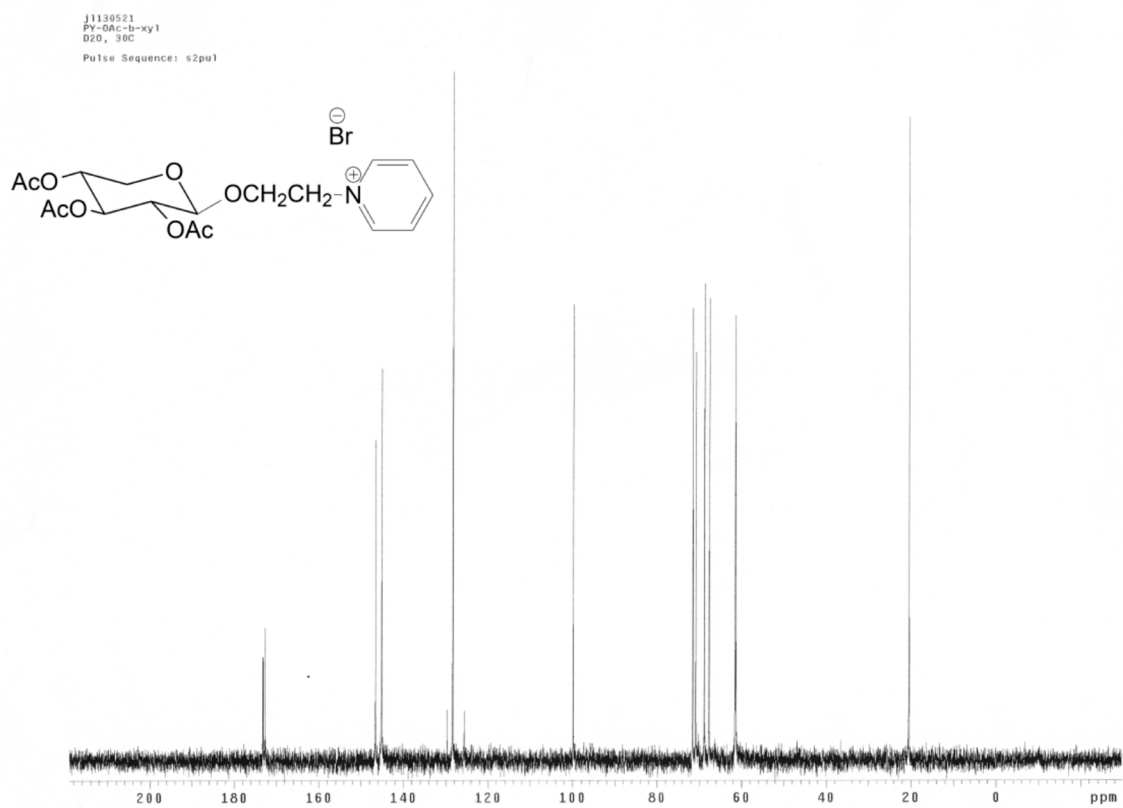
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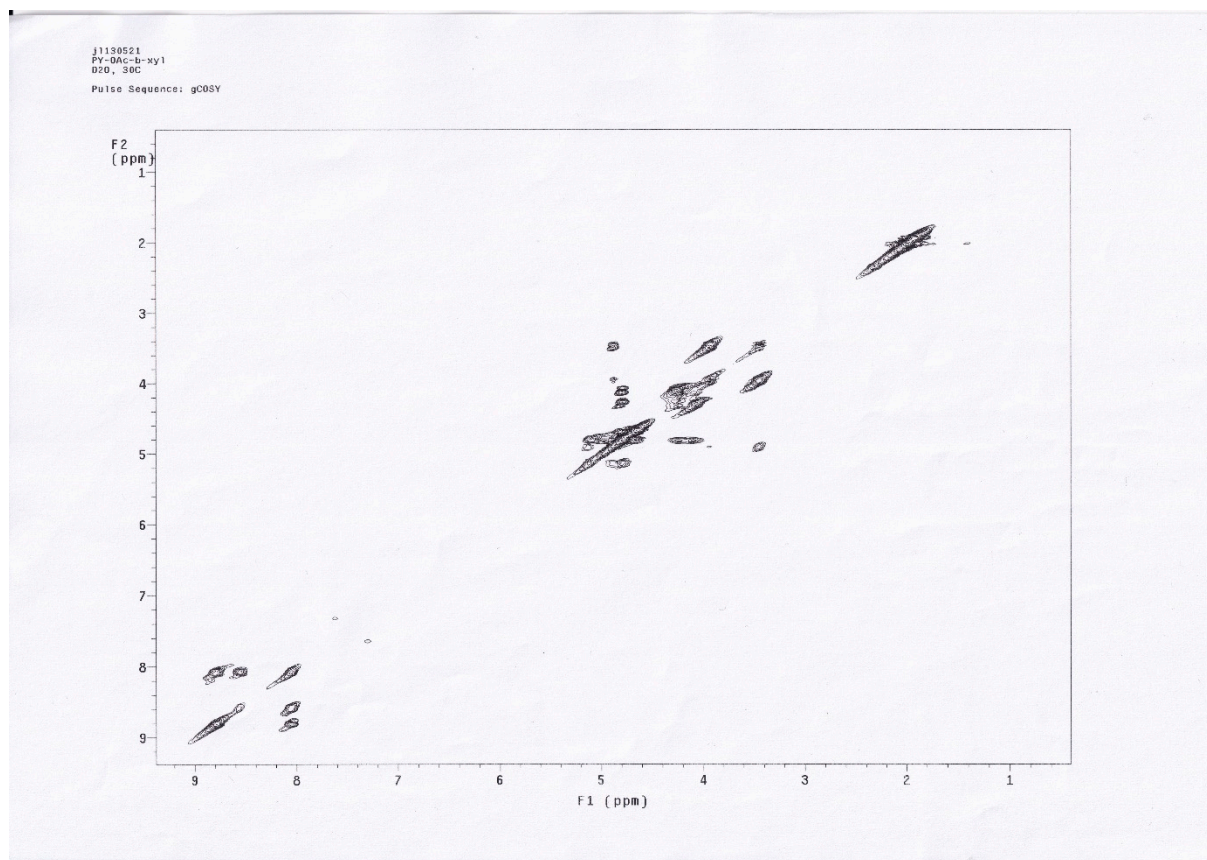
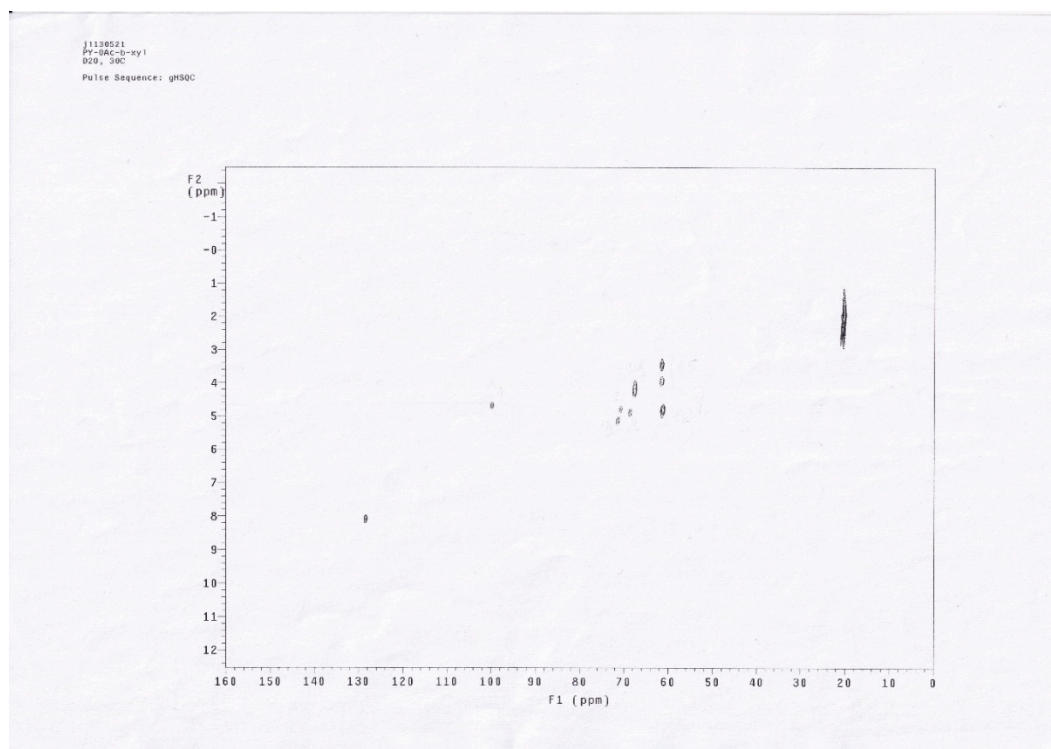
*N*-[2-(2',3',4'-tri-*O*-acetyl- $\beta$ -D-xylopyranosyloxy)ethyl]pyridinium bromide (**4a**)  
 $^1\text{H}$  NMR (500 MHz,  $\text{D}_2\text{O}$ ):





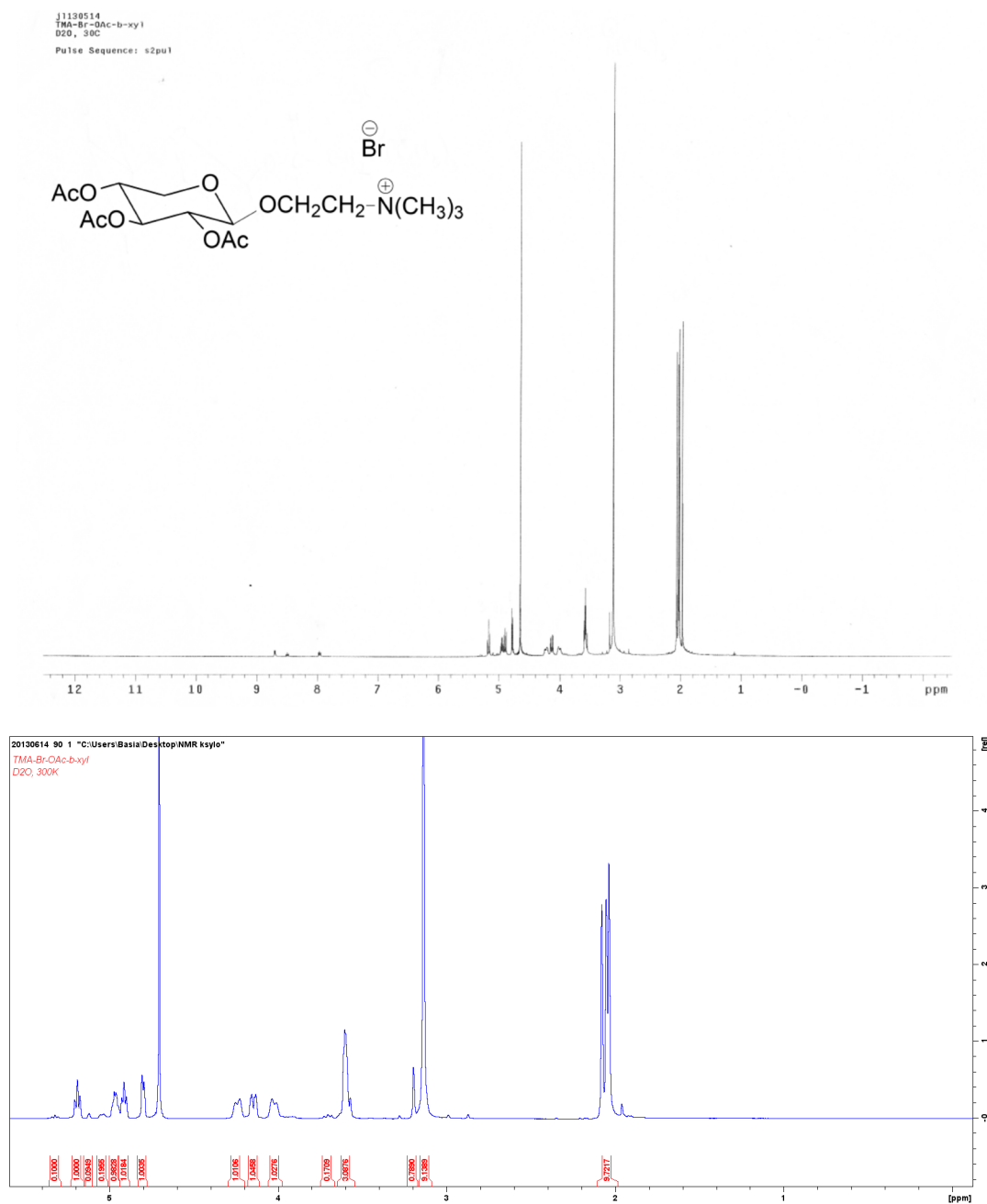


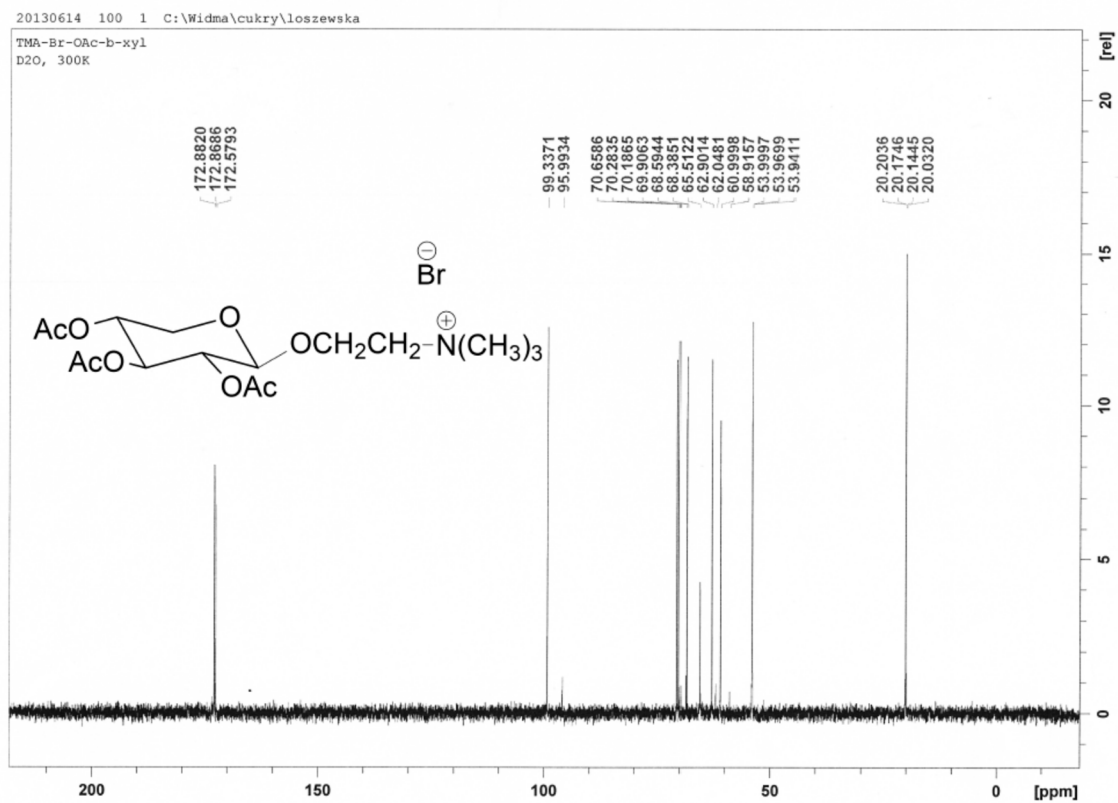
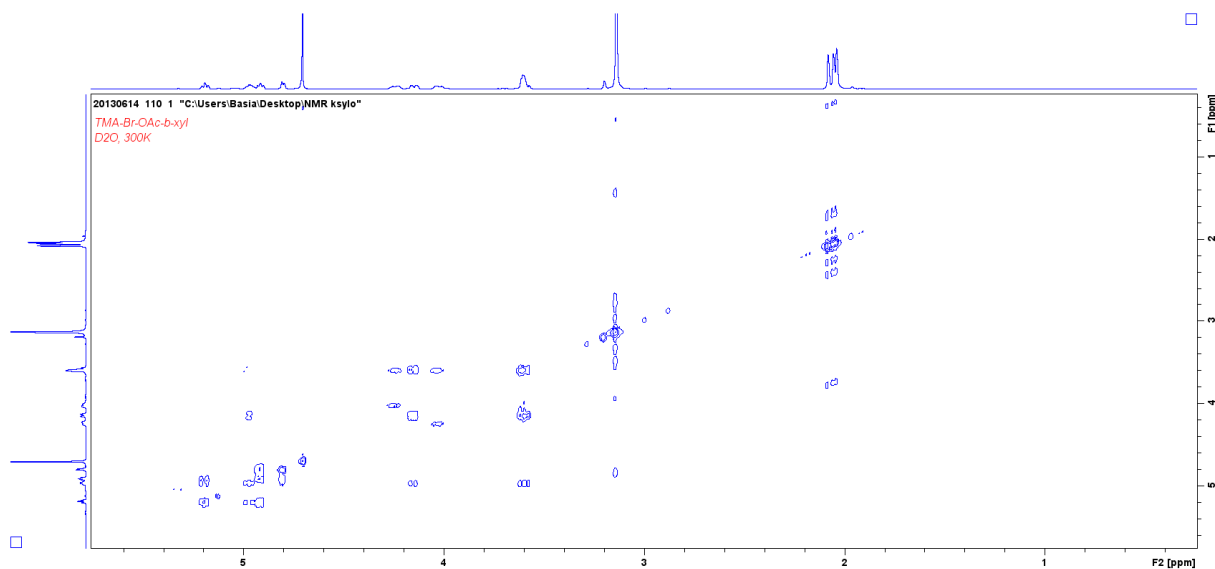
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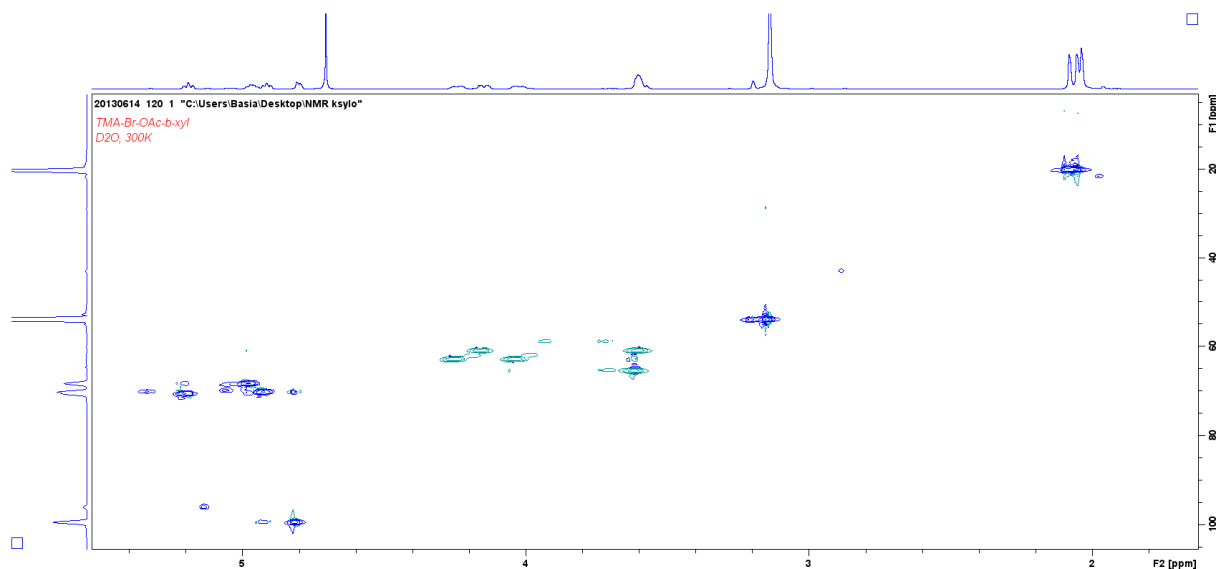
$^1\text{H}$ - $^1\text{H}$  COSY NMR: $^1\text{H}$ - $^{13}\text{C}$  HSQC NMR:

*N*-[2-(2',3',4'-tri-*O*-acetyl- $\beta$ -D-xylopyranosyloxy)ethyl] ]-*N,N,N*-trimethylammonium bromide (**4b**)

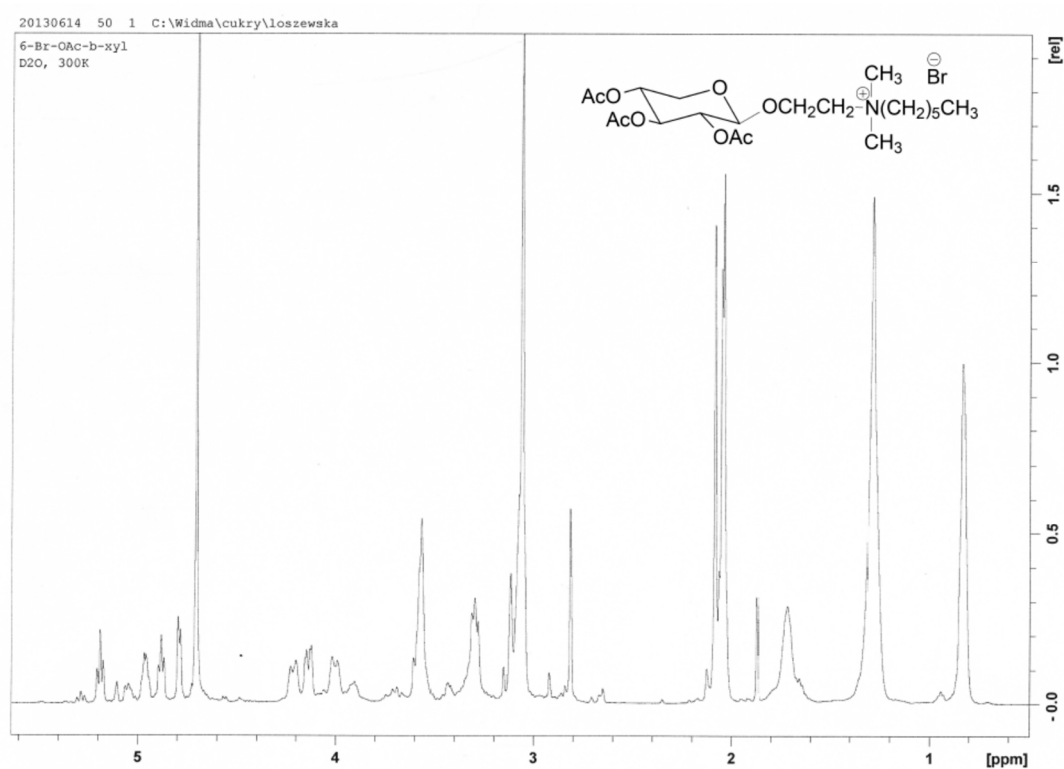
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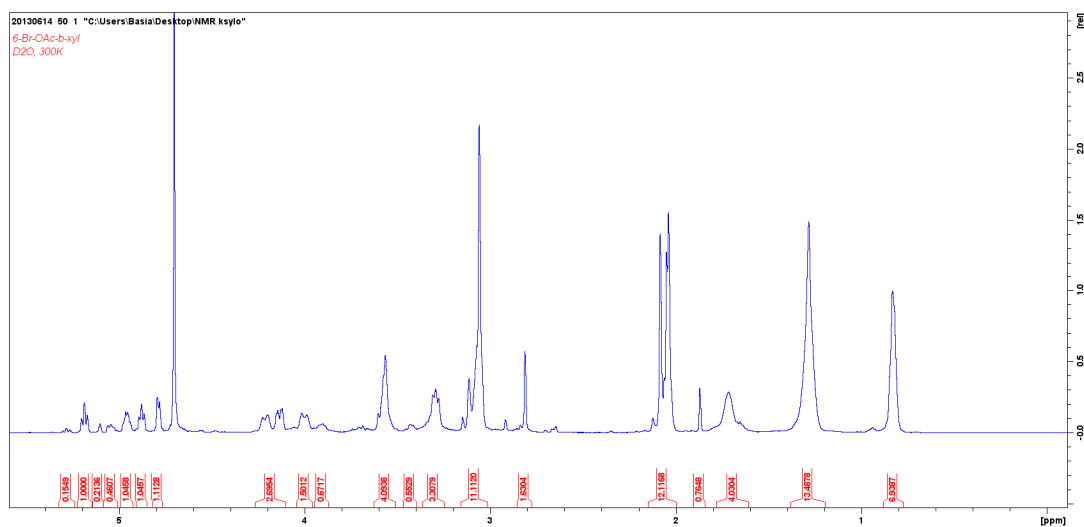
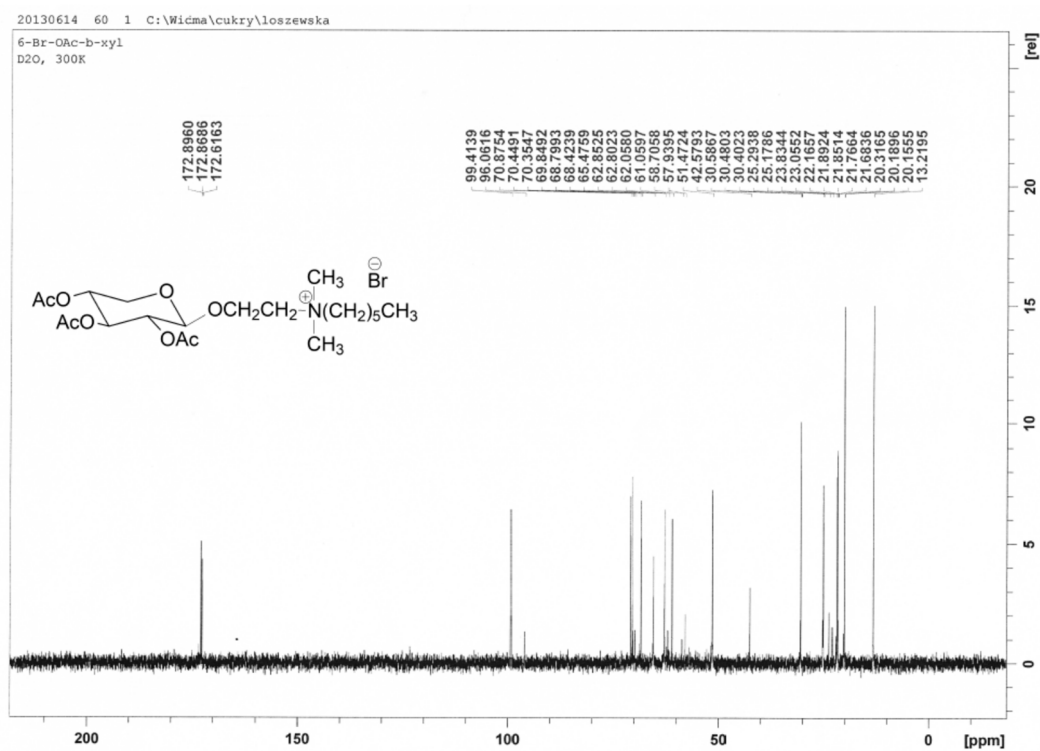


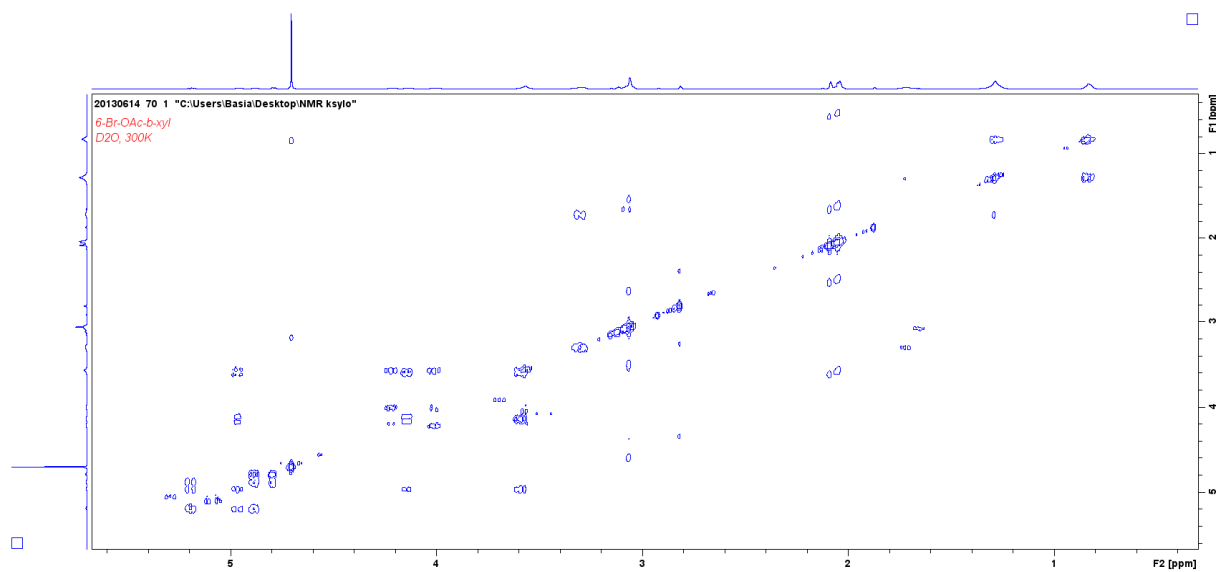
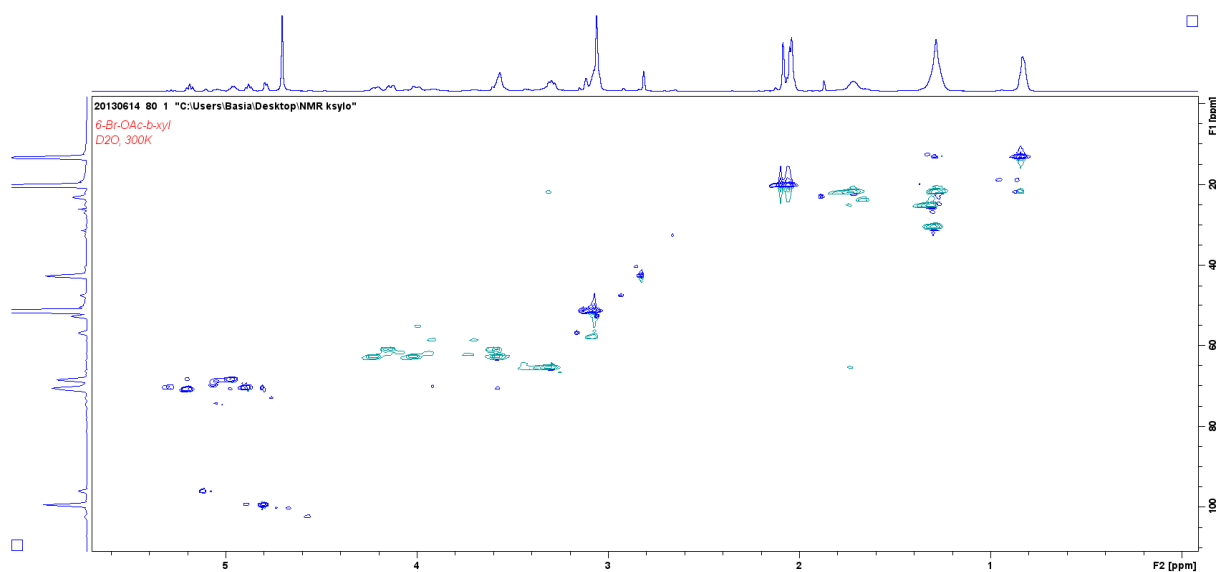
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$^1\text{H}$ - $^{13}\text{C}$  HSQC NMR:

*N*-[2-(2',3',4'-tri-*O*-acetyl- $\beta$ -D-xylopyranosyloxy)ethyl]-*N*-hexyl-*N,N*-dimethylammonium bromide (**4c**)

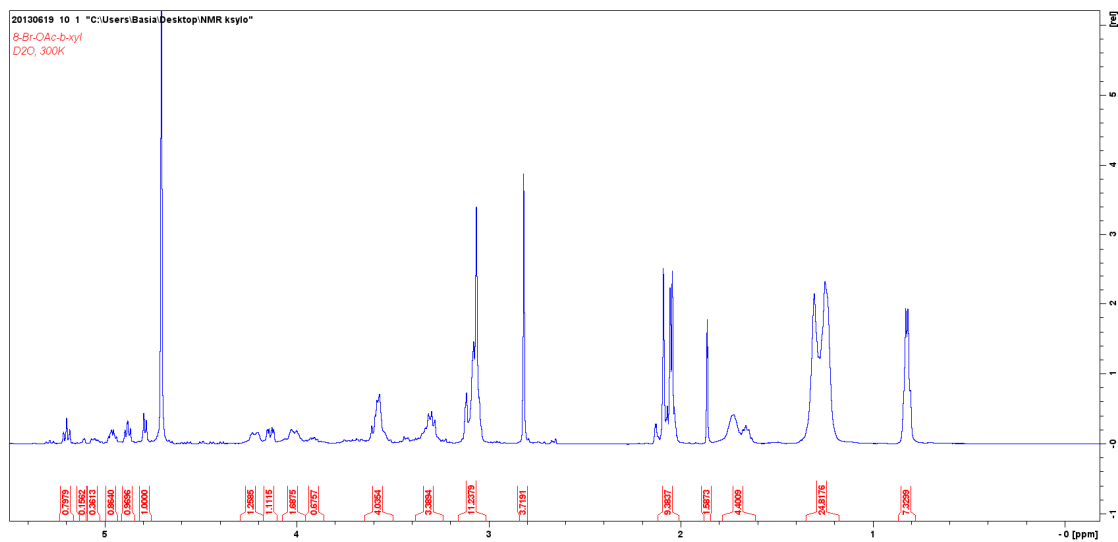
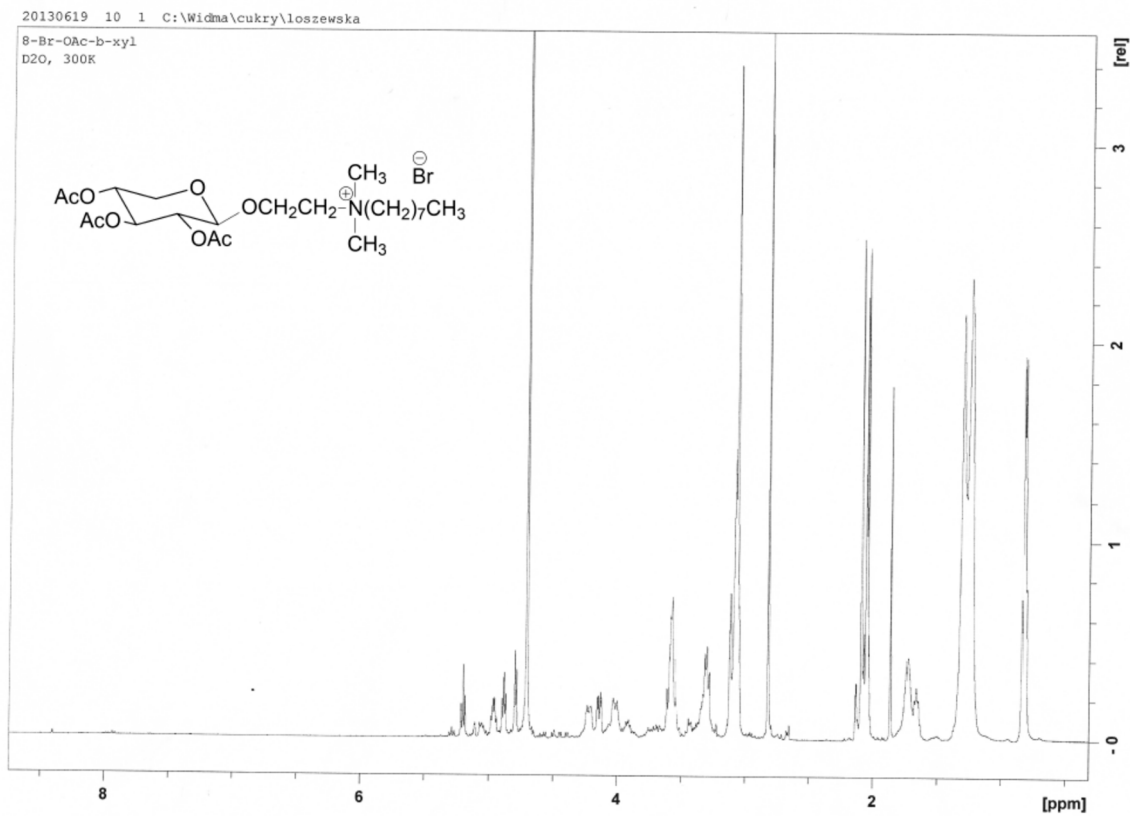
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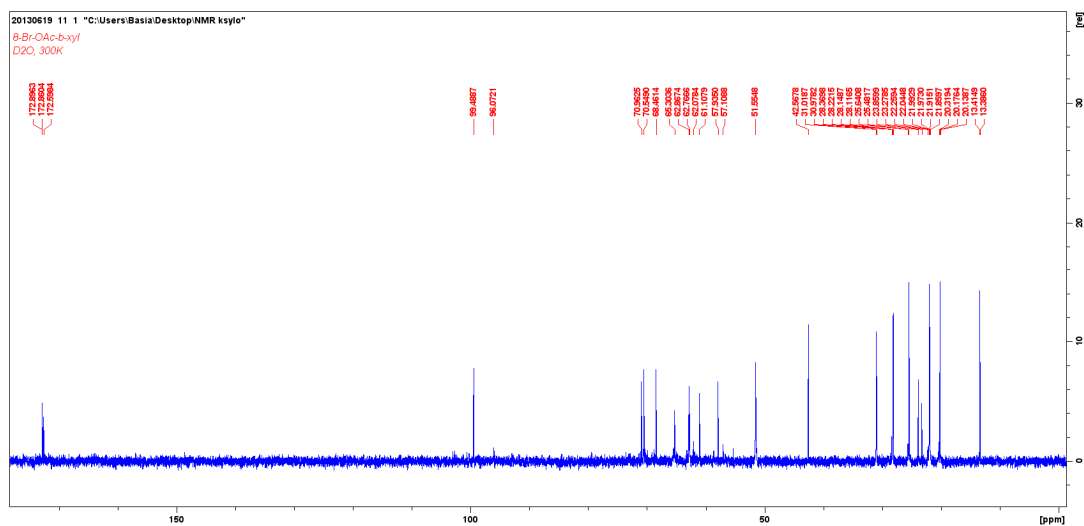
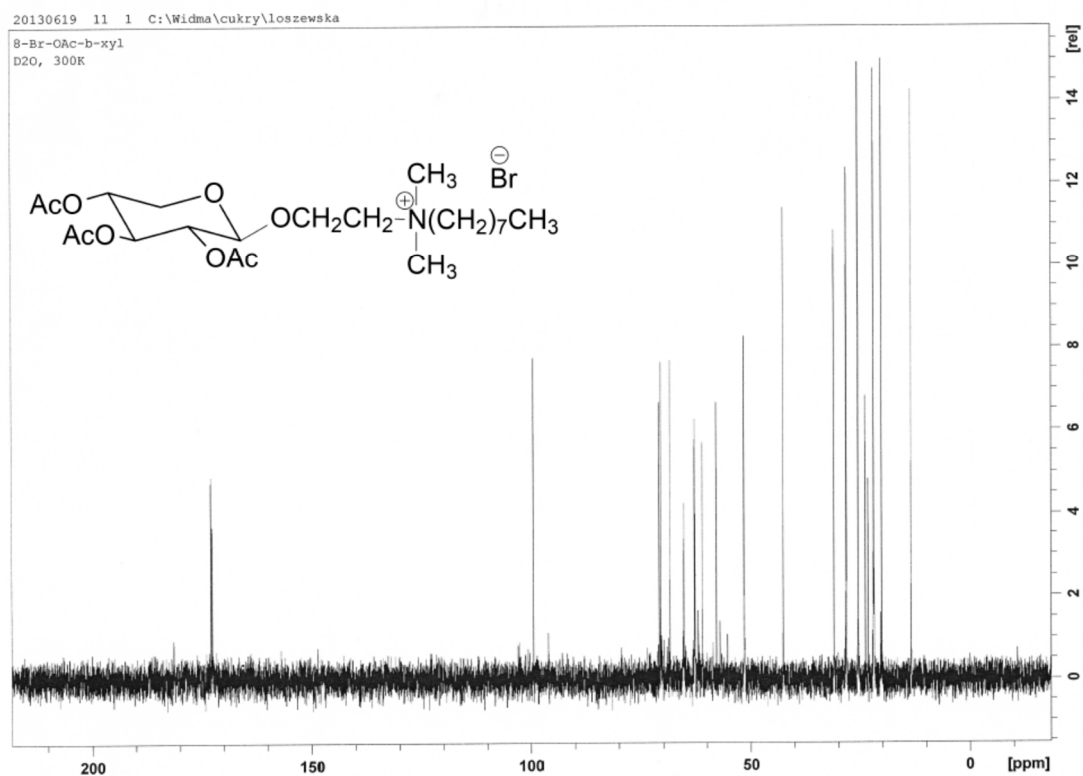
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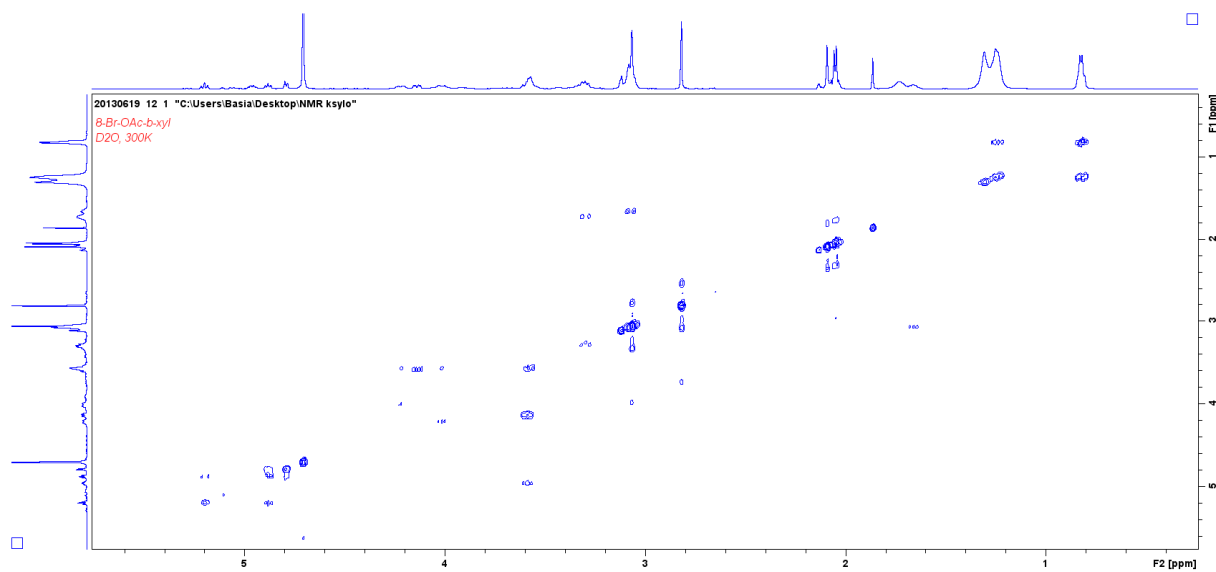
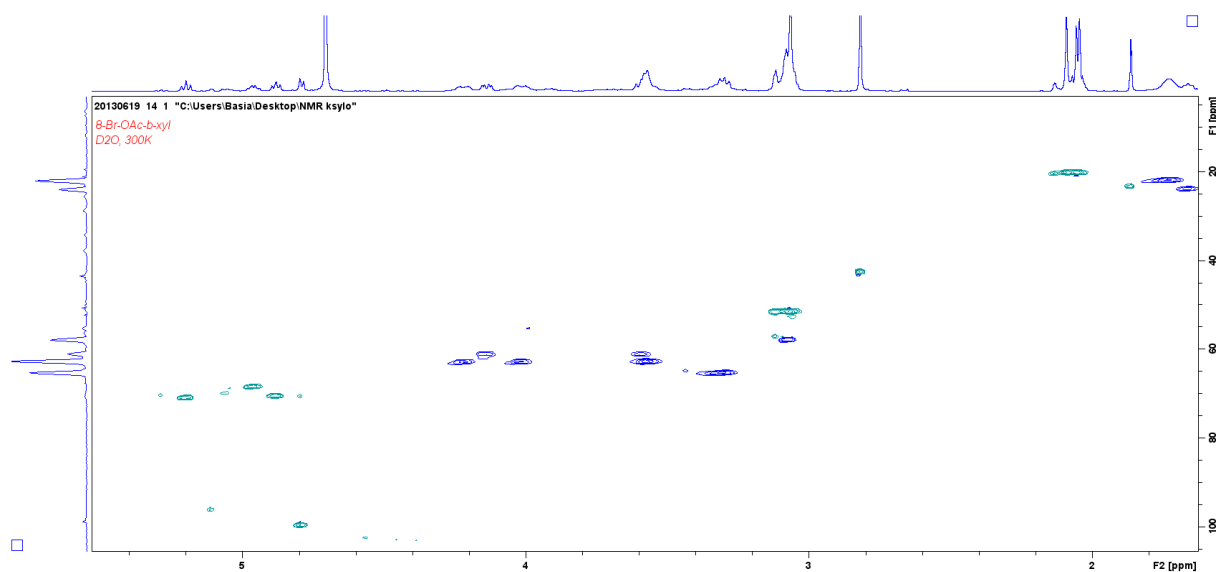
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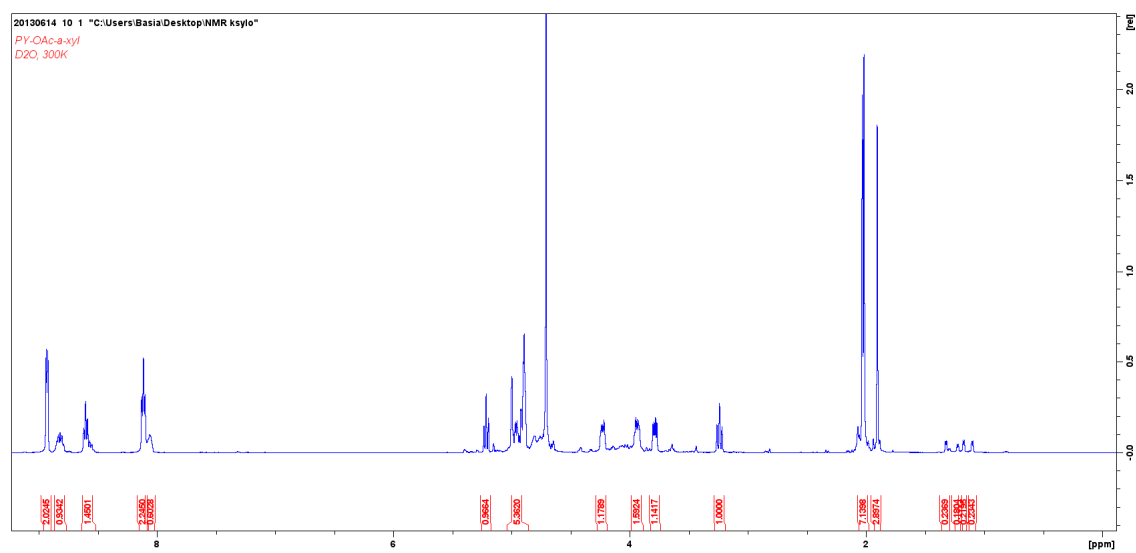
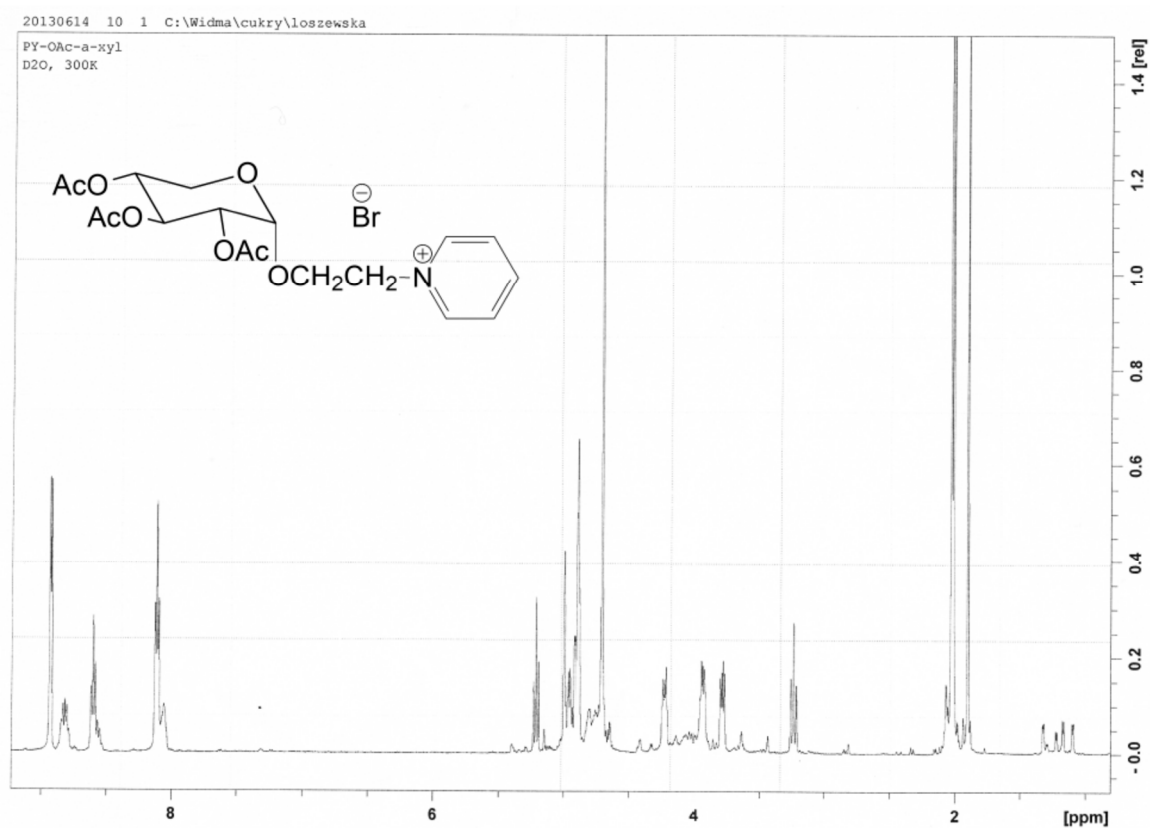
*N*-[2-(2',3',4'-tri-*O*-acetyl- $\beta$ -D-xylopyranosyloxy)ethyl]-*N,N*-dimethyl-*N*-octylammonium bromide (**4d**)

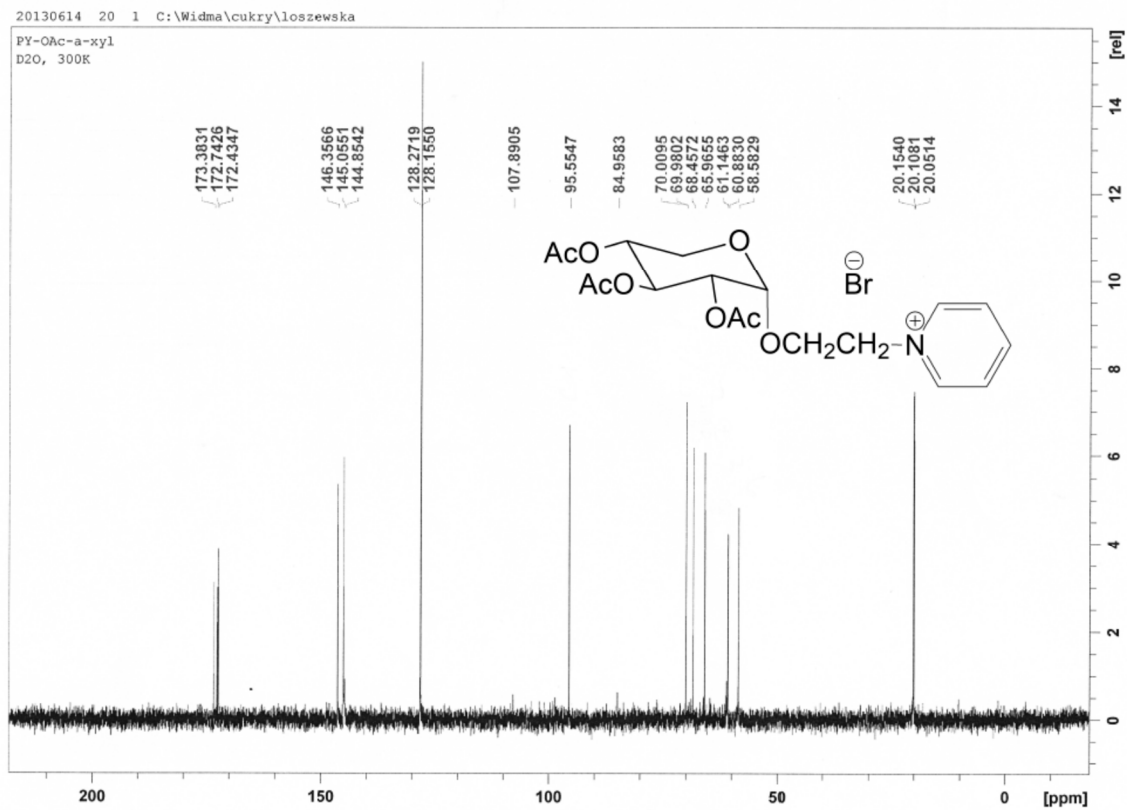
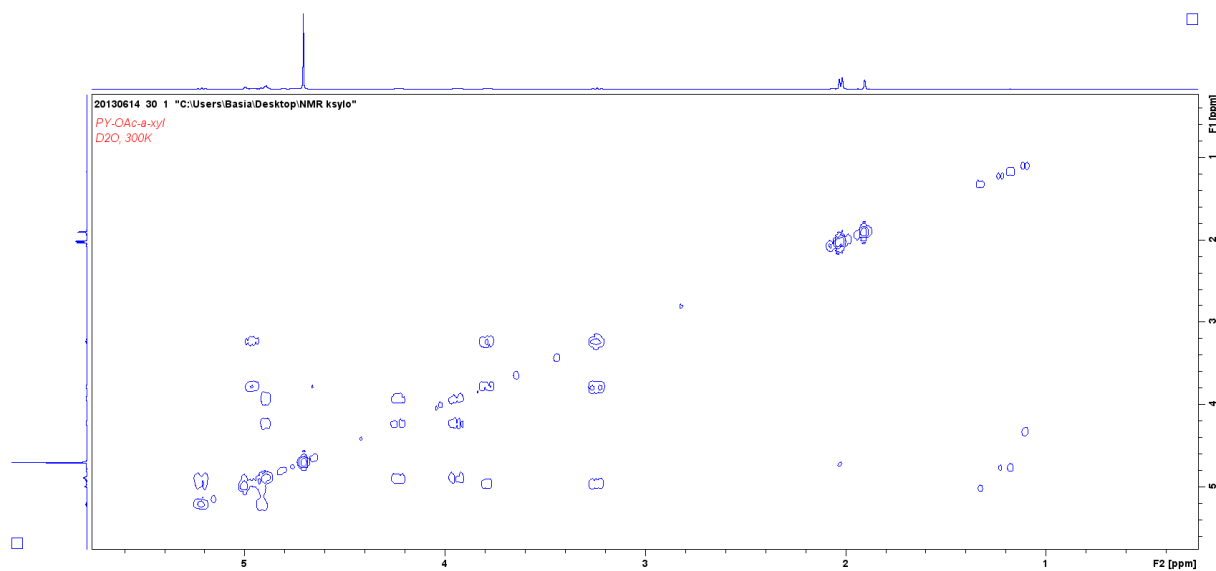
$^1\text{H}$  NMR (500 MHz,  $\text{D}_2\text{O}$ ):

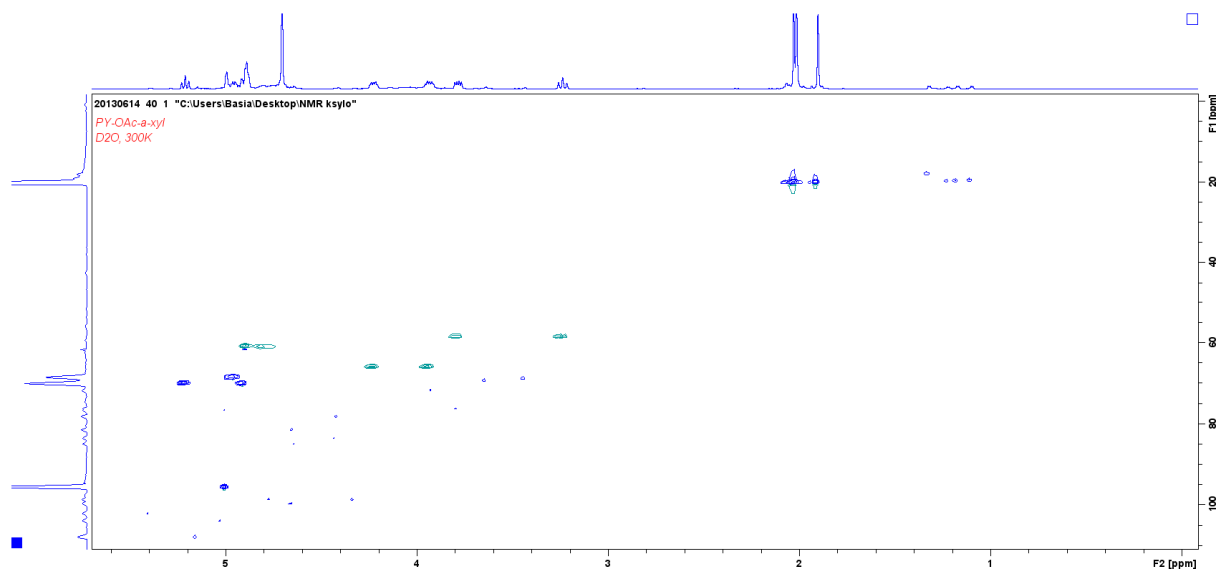


$^{13}\text{C}$  NMR (125 MHz,  $\text{D}_2\text{O}$ ):

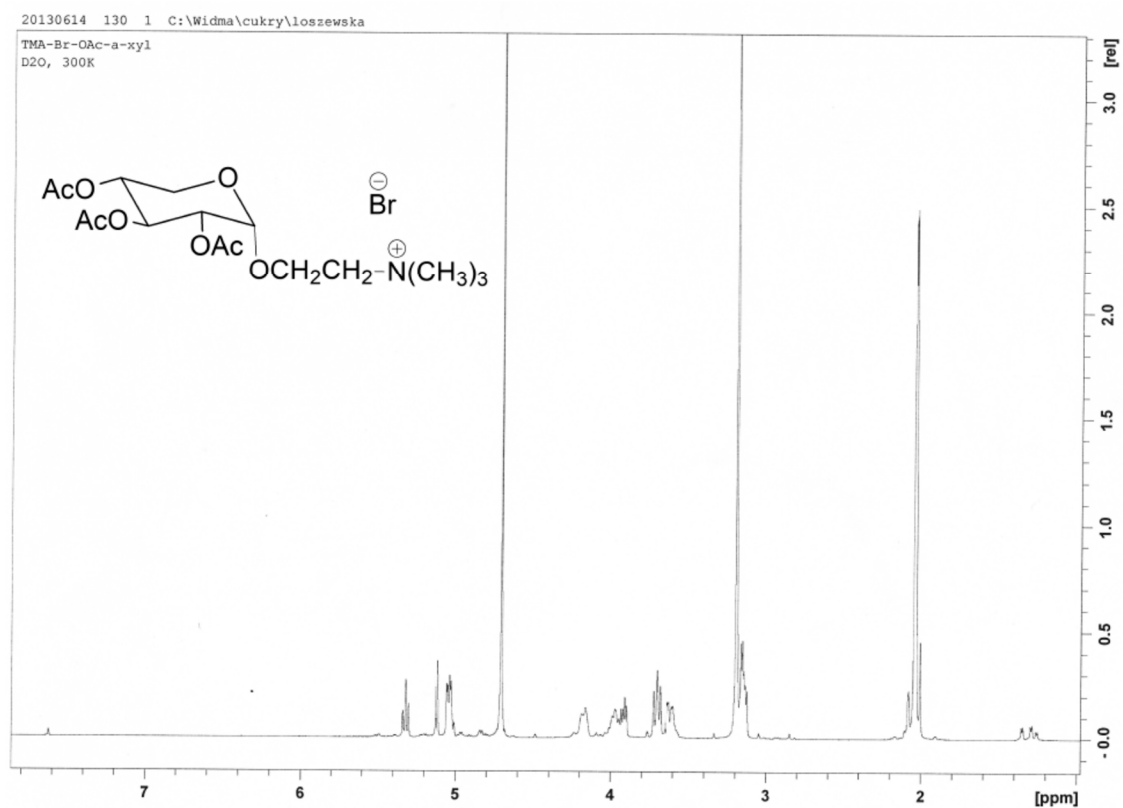
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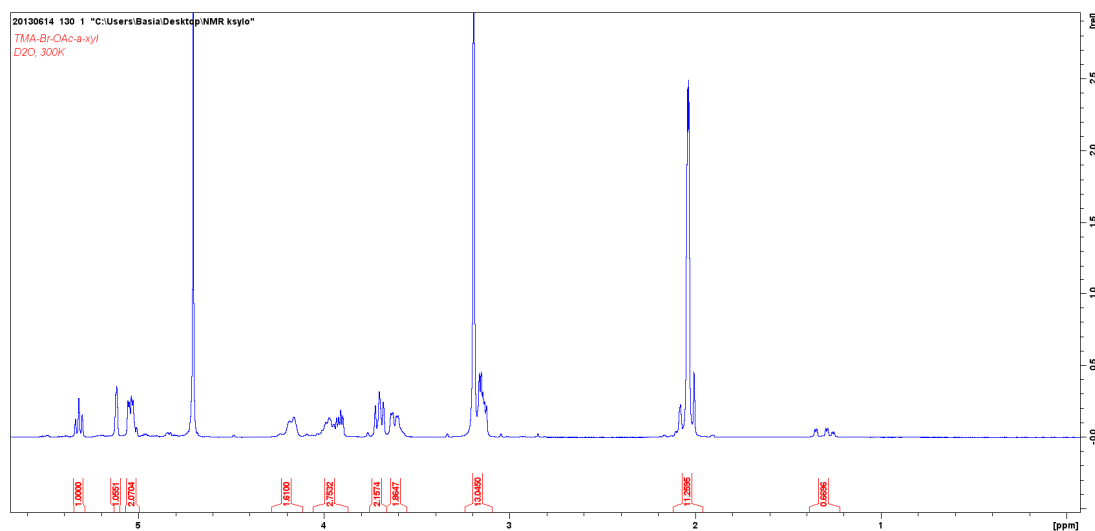
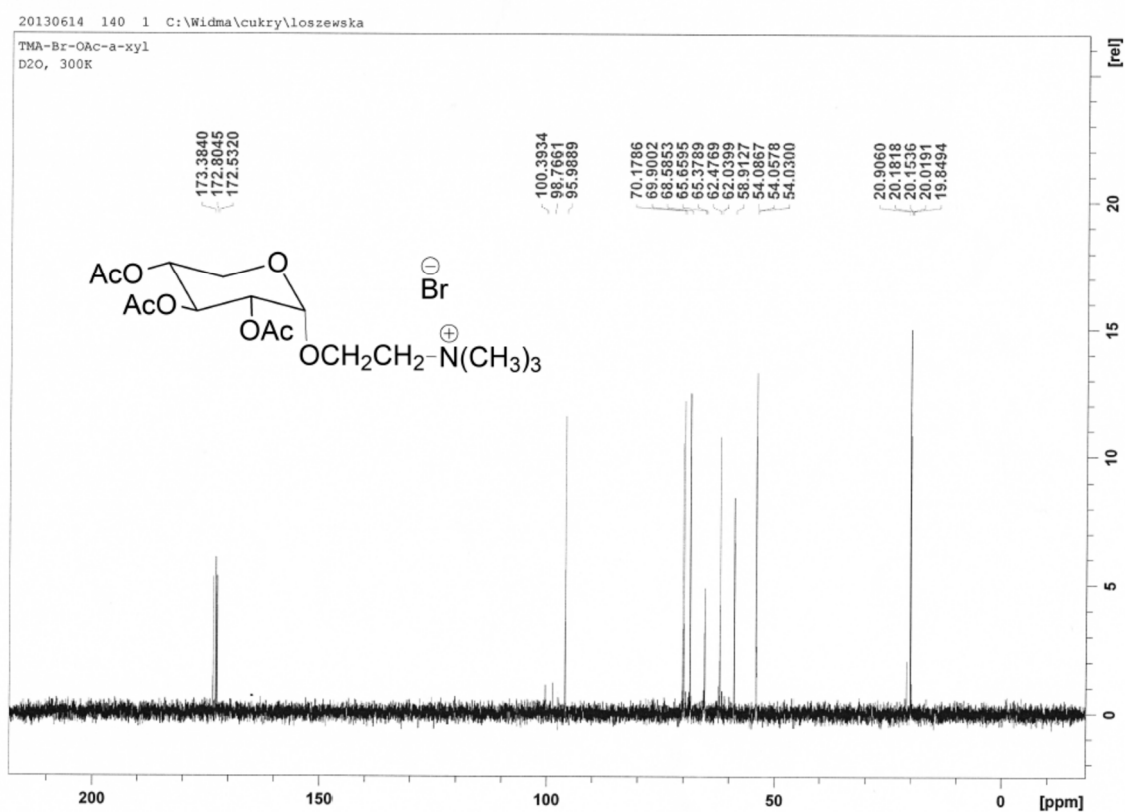
<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O):

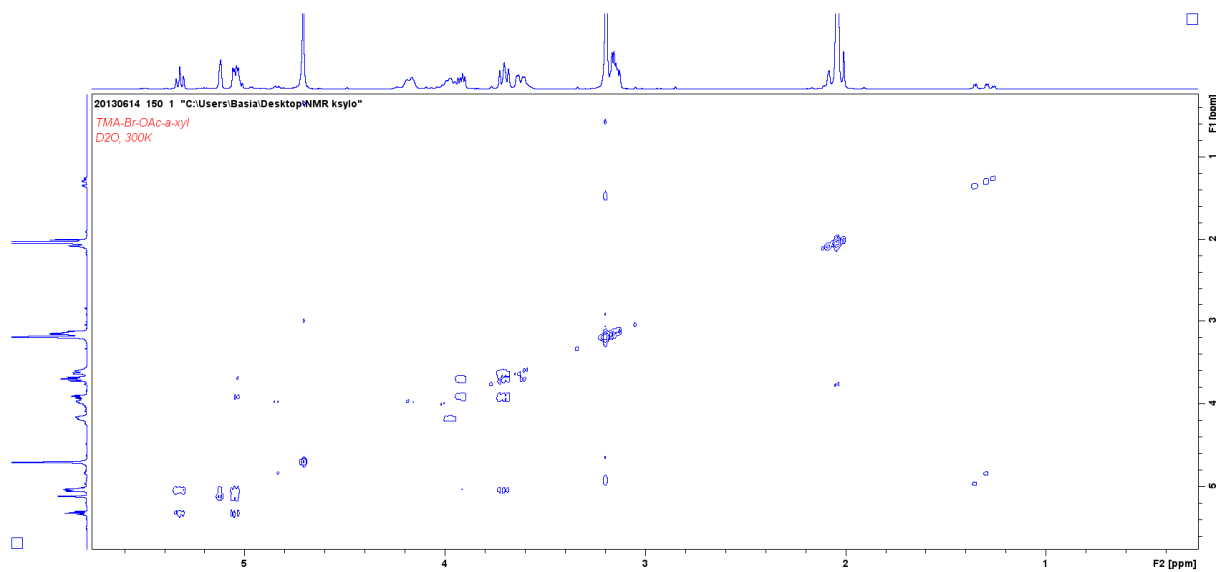
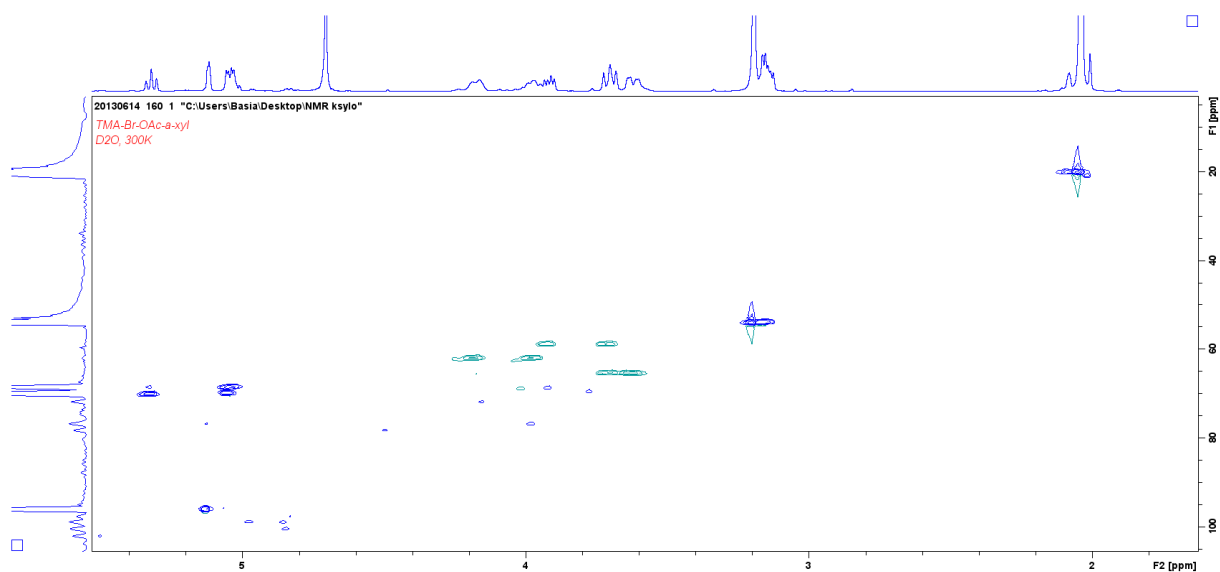
$^{13}\text{C}$  NMR (125 MHz,  $\text{D}_2\text{O}$ ): $^1\text{H}$ - $^1\text{H}$  COSY NMR:

$^1\text{H}$ - $^{13}\text{C}$  HSQC NMR:

*N*-[2-(2',3',4'-tri-*O*-acetyl- $\alpha$ -D-xylopyranosyloxy)ethyl] ]-*N,N,N*-trimethylammonium bromide (**5b**)

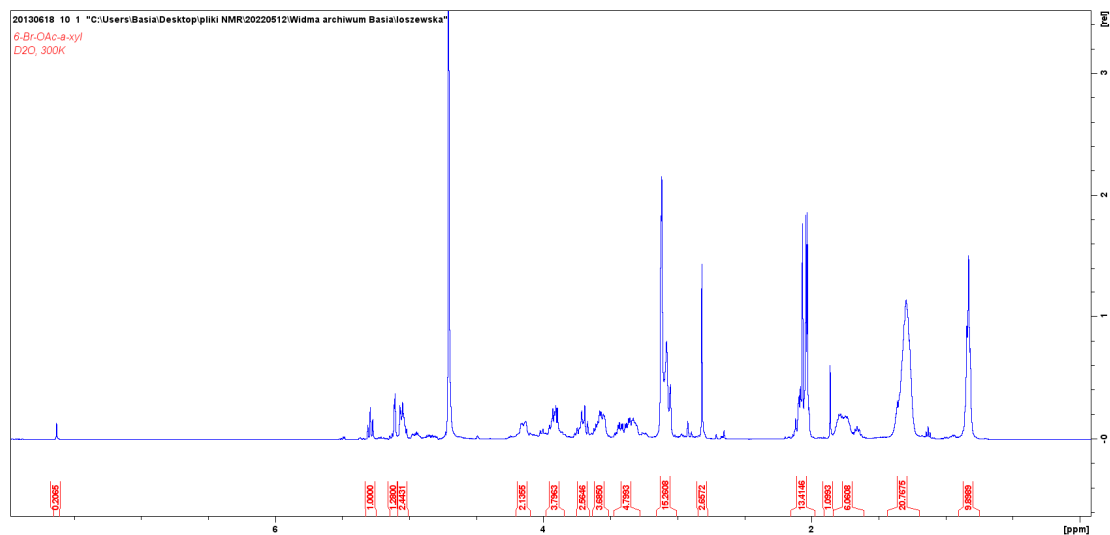
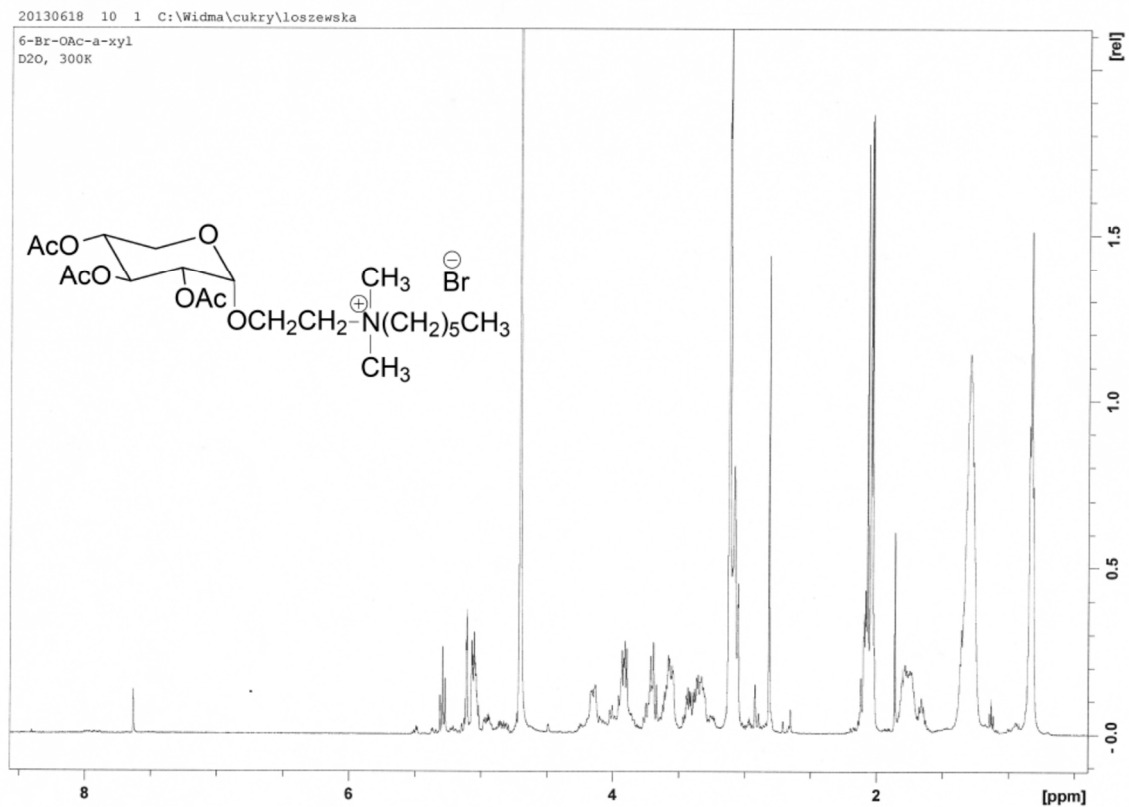
 $^1\text{H}$  NMR (500 MHz, D<sub>2</sub>O):

 $^{13}\text{C}$  NMR (125 MHz,  $\text{D}_2\text{O}$ ):

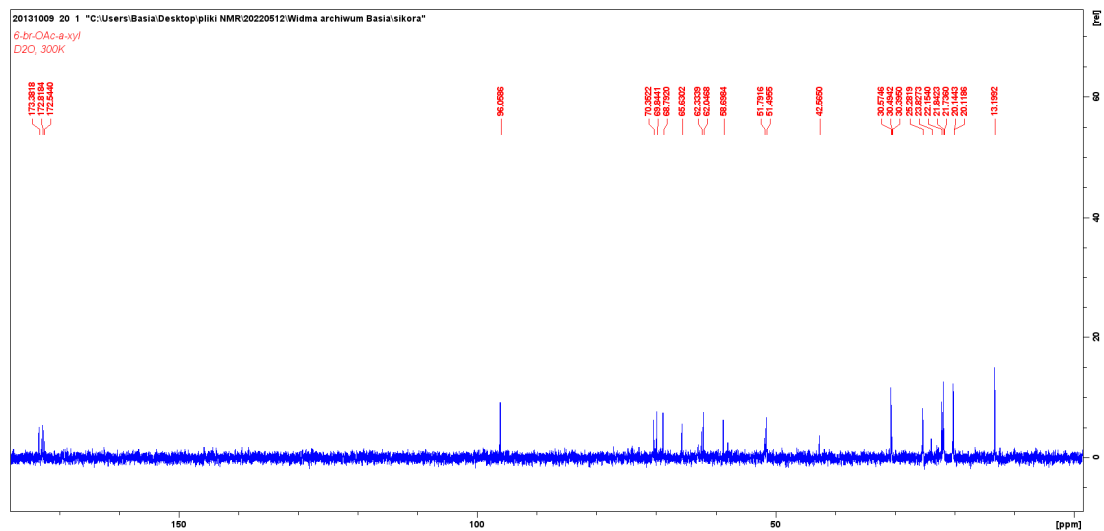
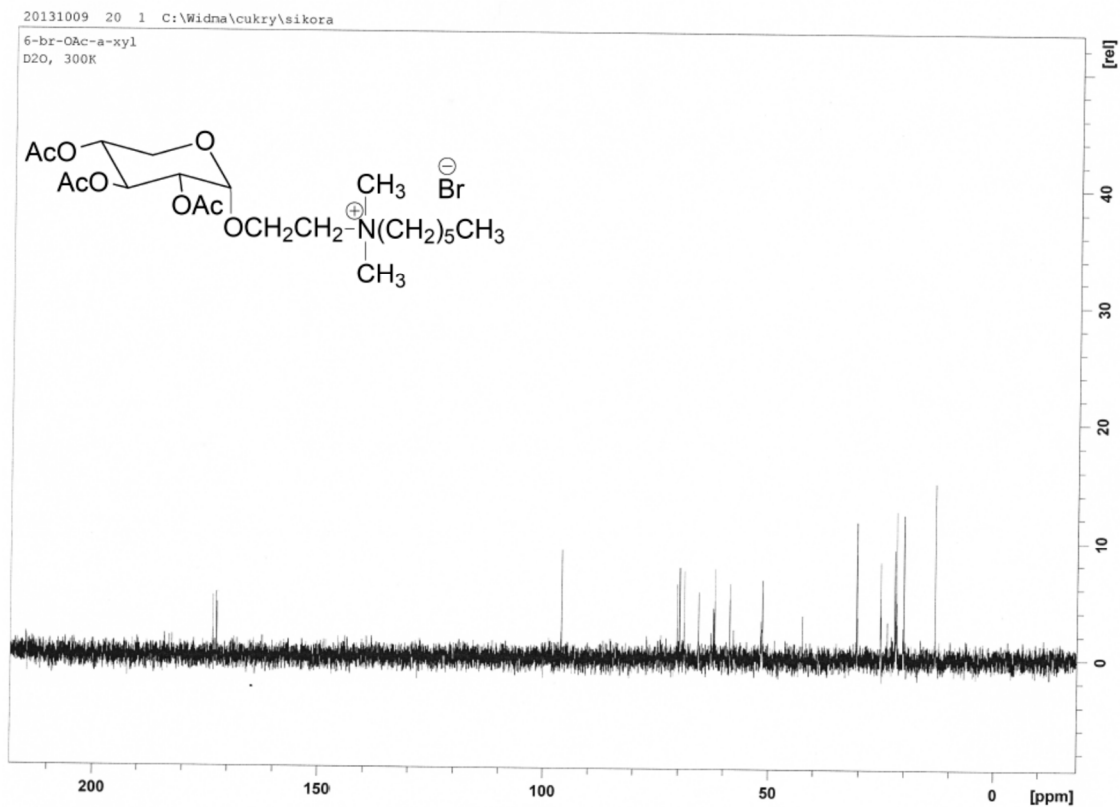
$^1\text{H}$ - $^1\text{H}$  COSY NMR: $^1\text{H}$ - $^{13}\text{C}$  HSQC NMR:

*N*-[2-(2',3',4'-tri-*O*-acetyl- $\alpha$ -D-xylopyranosyloxy)ethyl]-*N*-hexyl-*N,N*-dimethylammonium bromide (**5c**)

$^1\text{H}$  NMR (500 MHz,  $\text{D}_2\text{O}$ ):

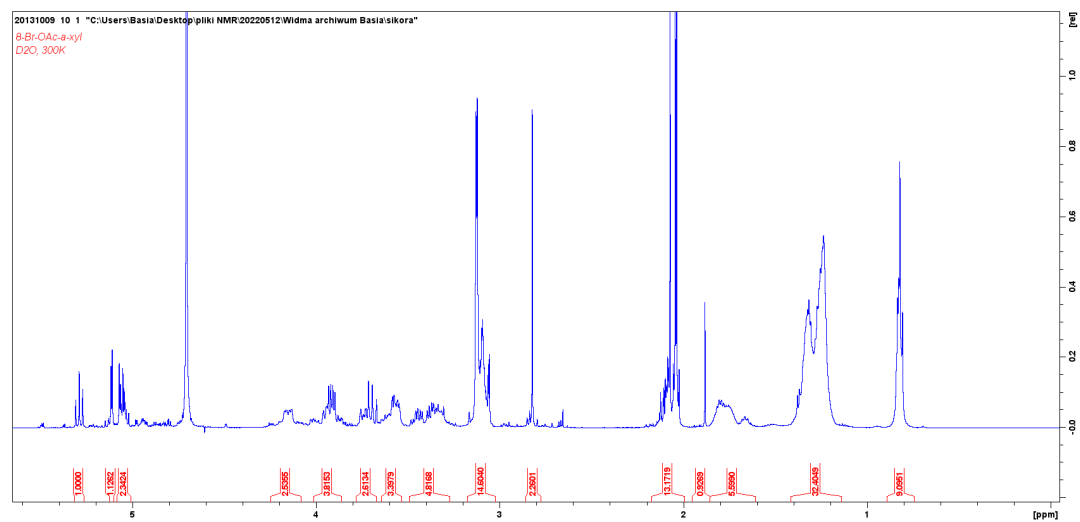
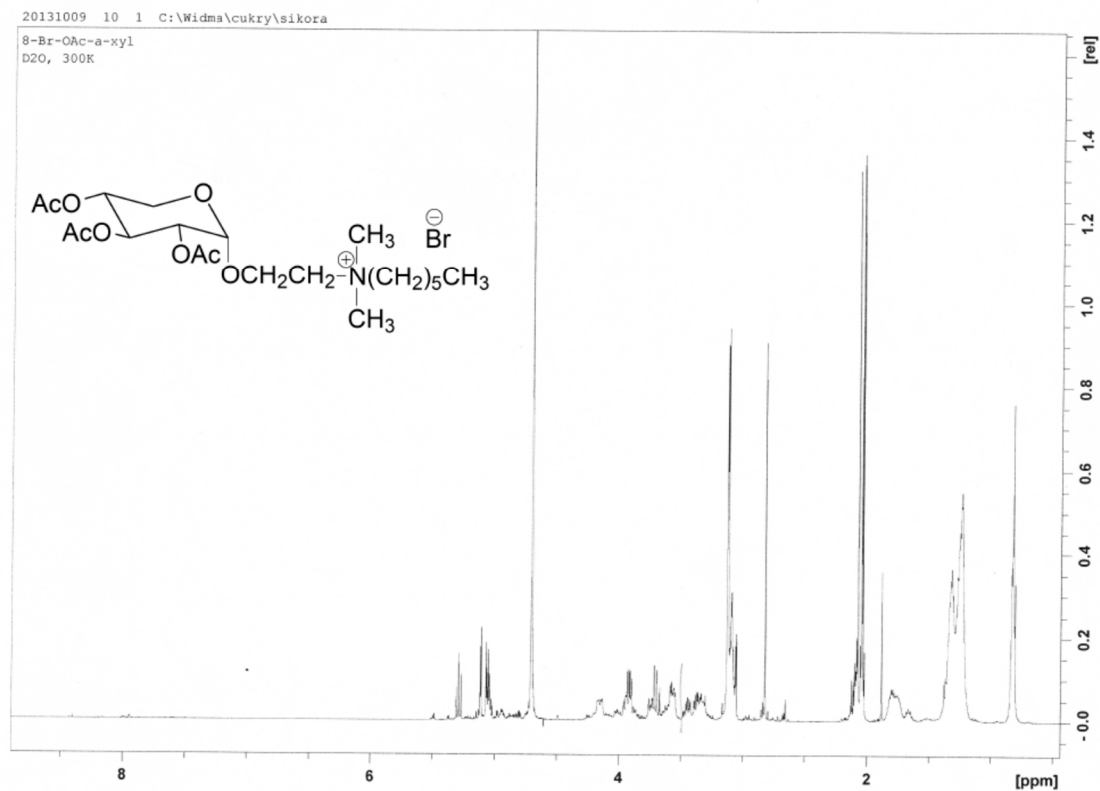


$^{13}\text{C}$  NMR (125 MHz,  $\text{D}_2\text{O}$ ):



*N*-[2-(2',3',4'-tri-*O*-acetyl- $\alpha$ -D-xylopyranosyloxy)ethyl]-*N,N*-dimethyl-*N*-octylammonium bromide (**5d**)

$^1\text{H}$  NMR (500 MHz,  $\text{D}_2\text{O}$ ):

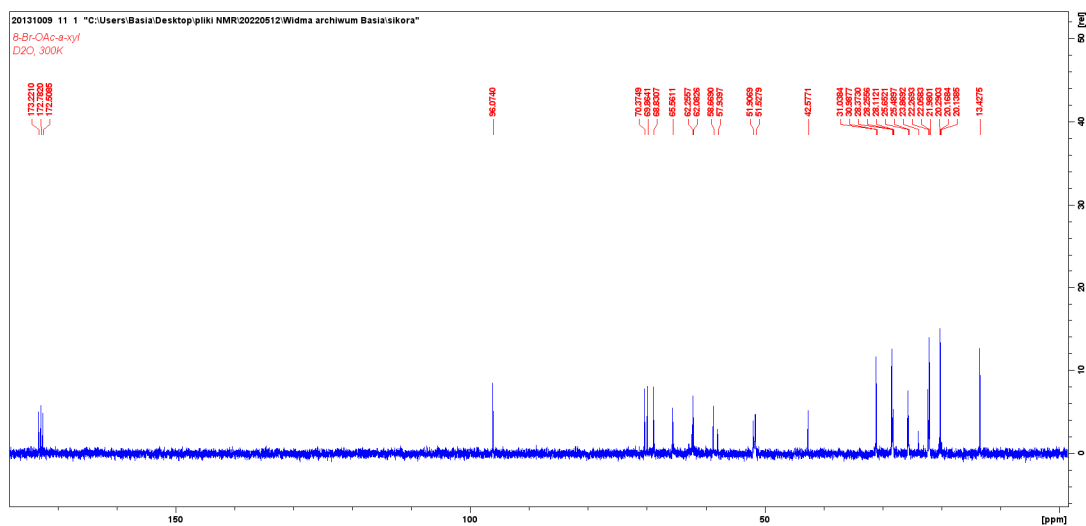


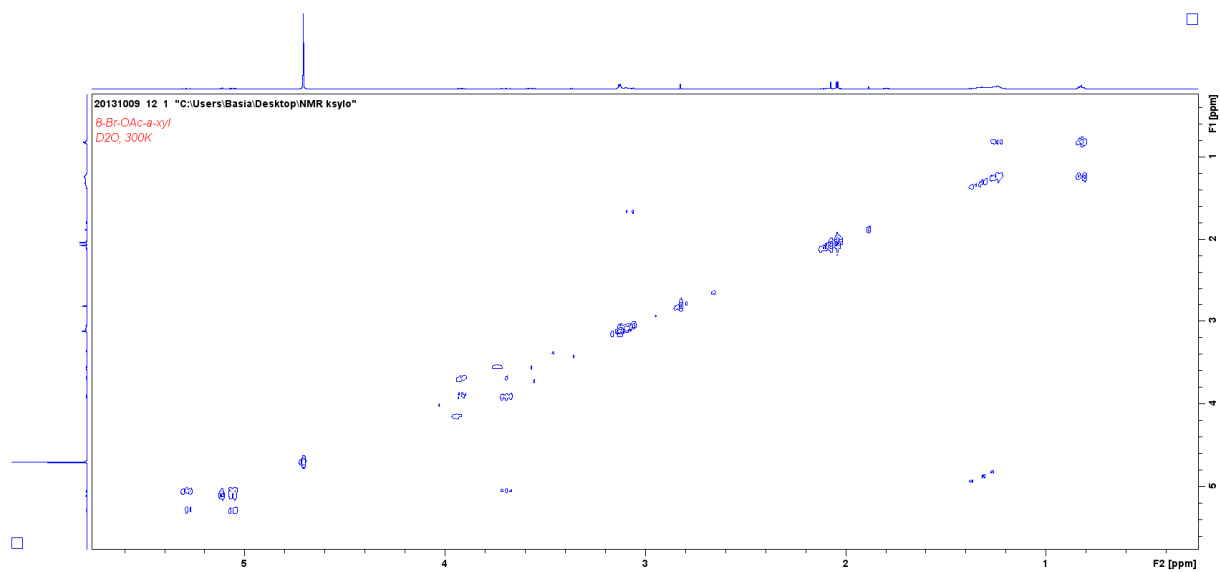
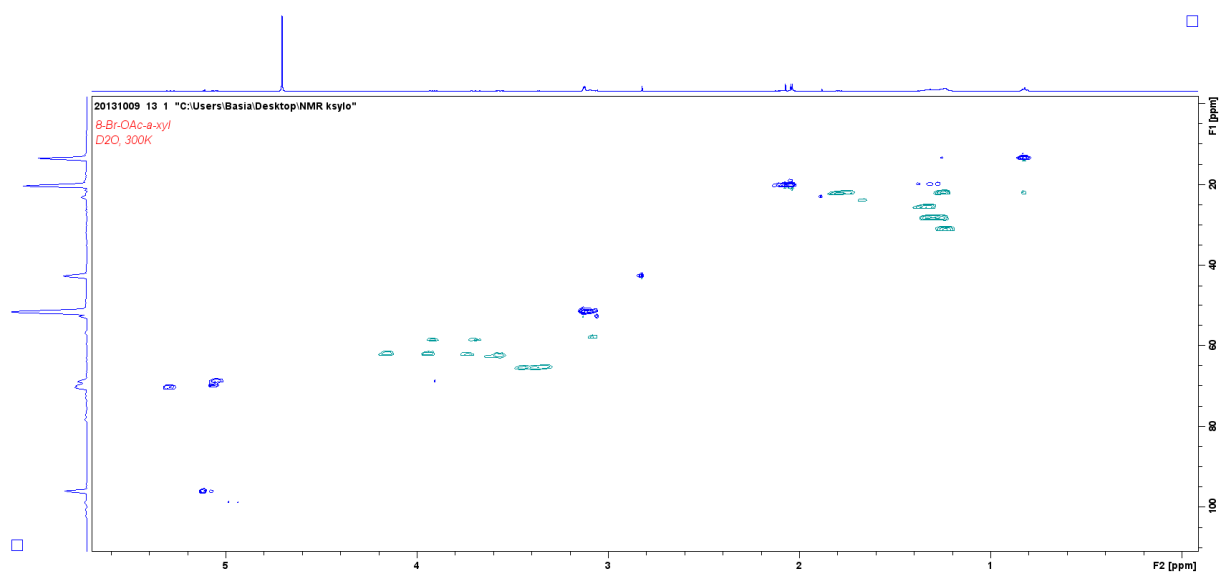
20131009 11 1 C:\Widma\cukry\sikora

8-Br-OAc-a-xyl  
D2O, 300K

Chemical structure of 8-bromo-2,3,4,6-tetra-O-acetyl-alpha-D-xylanose is shown. The structure features a furanose ring substituted with four acetoxy (OAc) groups and a side chain containing a quaternary ammonium cation (N<sup>+</sup>) and a bromide anion (Br<sup>-</sup>).

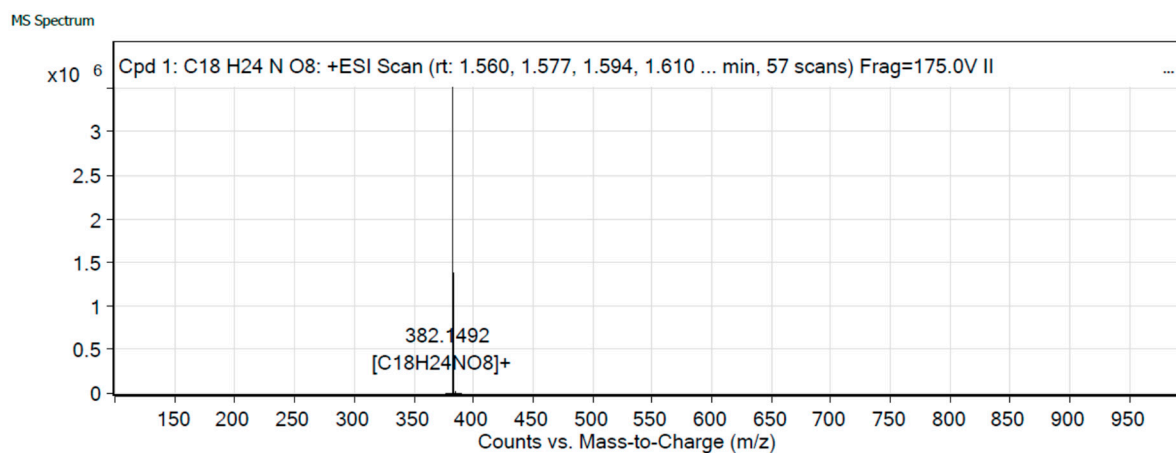
<sup>1</sup>H NMR spectrum (D<sub>2</sub>O, 300K) showing chemical shifts in ppm (x-axis, 0 to 200) and relative intensity (y-axis, 0 to 20). The spectrum displays several peaks, including a broad peak around 170 ppm, a sharp peak at approximately 100 ppm, a cluster of peaks between 50 and 70 ppm, and a large, complex multiplet between 1 and 4 ppm.



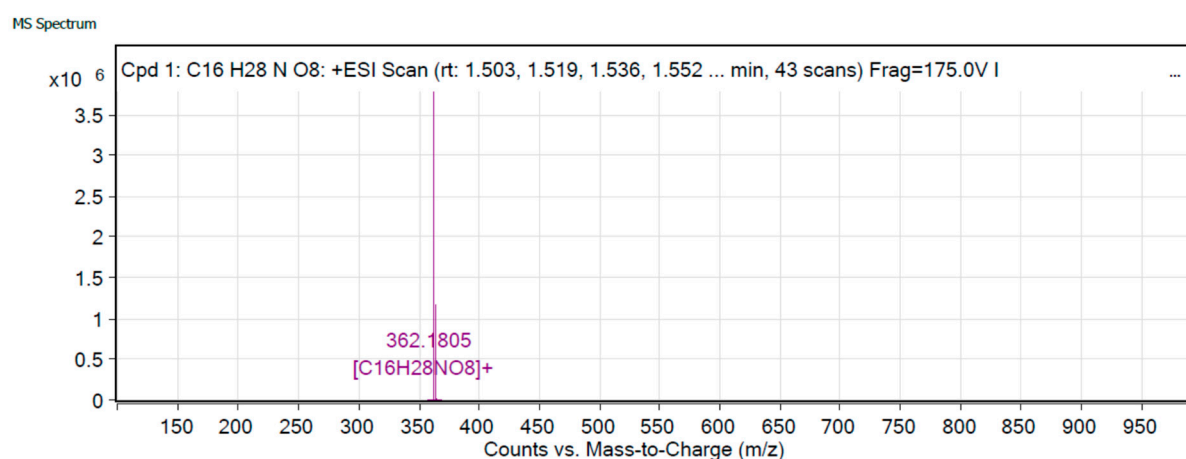
$^1\text{H}$ - $^1\text{H}$  COSY NMR: $^1\text{H}$ - $^{13}\text{C}$  HSQC NMR:

#### 4. MS SPECTRA

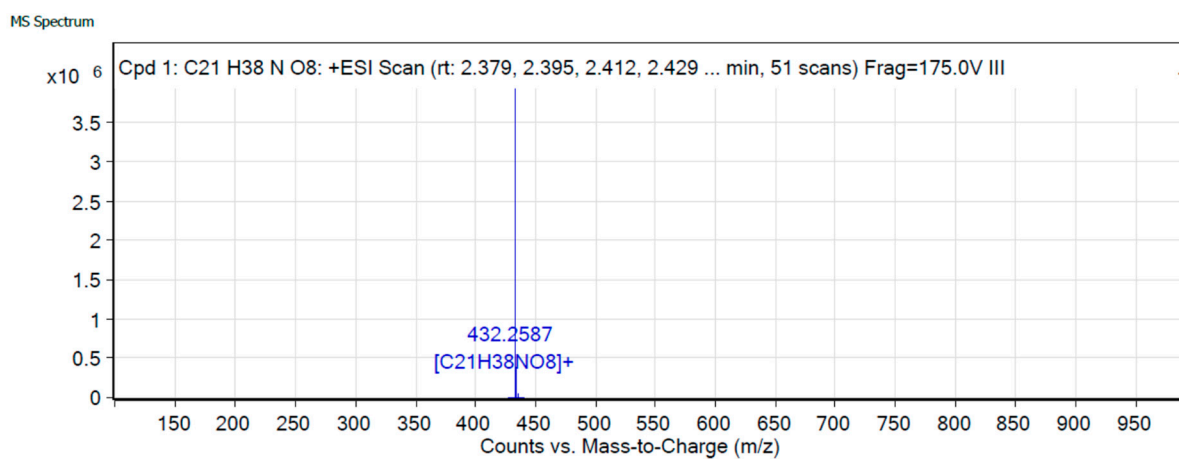
*N*-[2-(2',3',4'-tri-*O*-acetyl- $\beta$ -D-xylopyranosyloxy)ethyl]pyridinium bromide (**4a**) *m/z* 382.1492 ([M-Br]<sup>+</sup>)



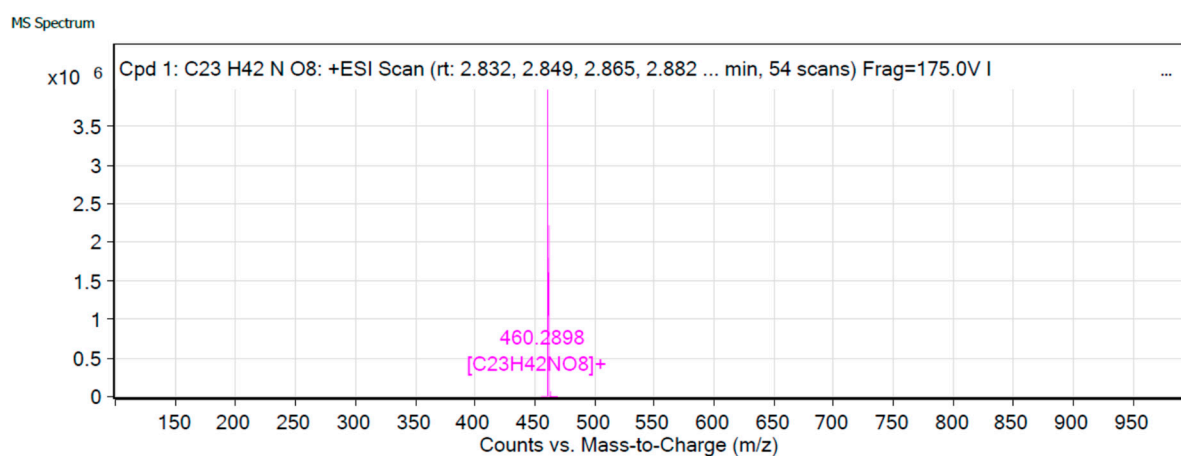
*N*-[2-(2',3',4'-tri-*O*-acetyl- $\beta$ -D-xylopyranosyloxy)ethyl] ]-*N,N,N*-trimethylammonium bromide (**4b**) *m/z* 362.1805 ([M-Br]<sup>+</sup>)



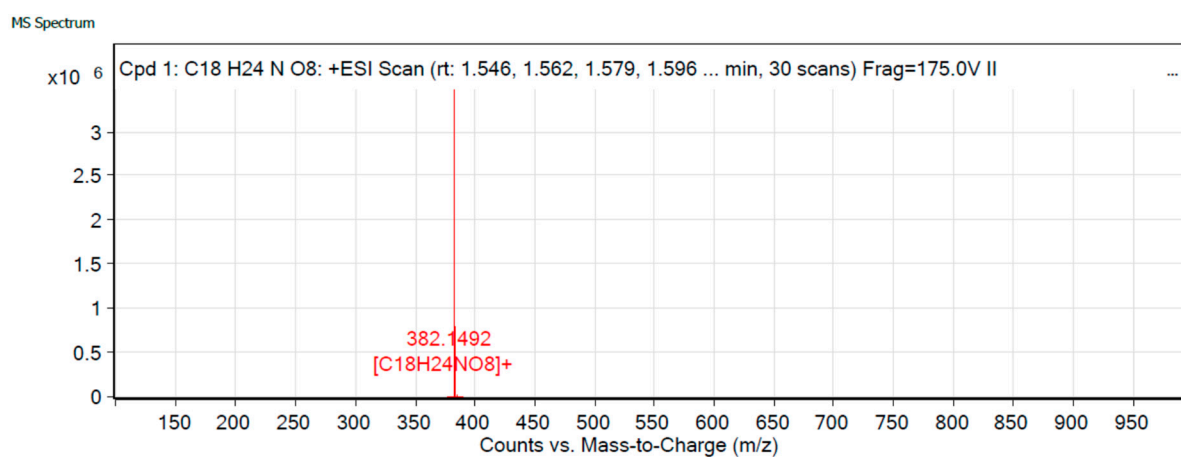
*N*-[2-(2',3',4'-tri-*O*-acetyl- $\beta$ -D-xylopyranosyloxy)ethyl]-*N*-hexyl-*N,N*-dimethylammonium bromide (**4c**) *m/z* 432.2587 ([M-Br]<sup>+</sup>)



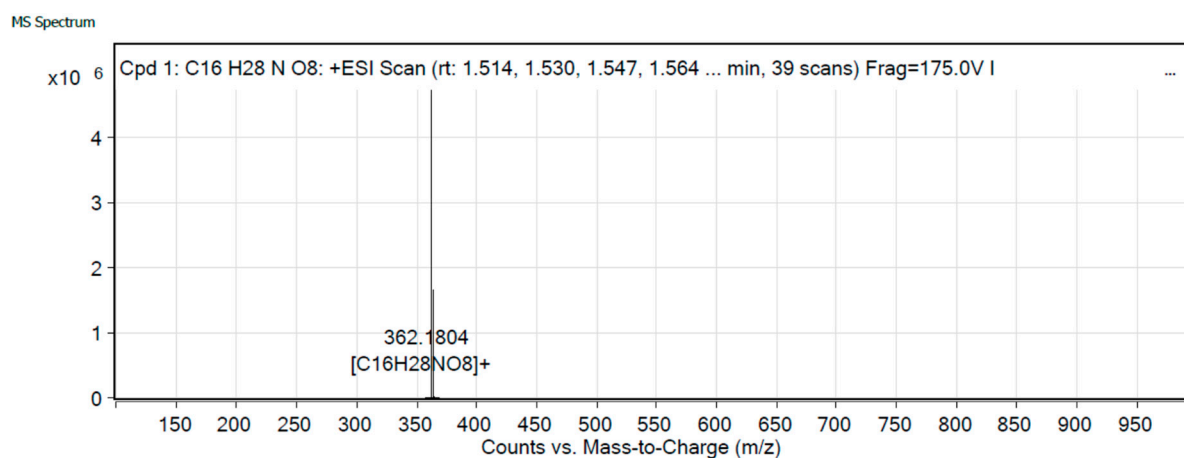
*N*-[2-(2',3',4'-tri-*O*-acetyl- $\beta$ -D-xylopyranosyloxy)ethyl]-*N,N*-dimethyl-*N*-octylammonium bromide (**4d**) *m/z* 460.2898 ([M-Br]<sup>+</sup>)



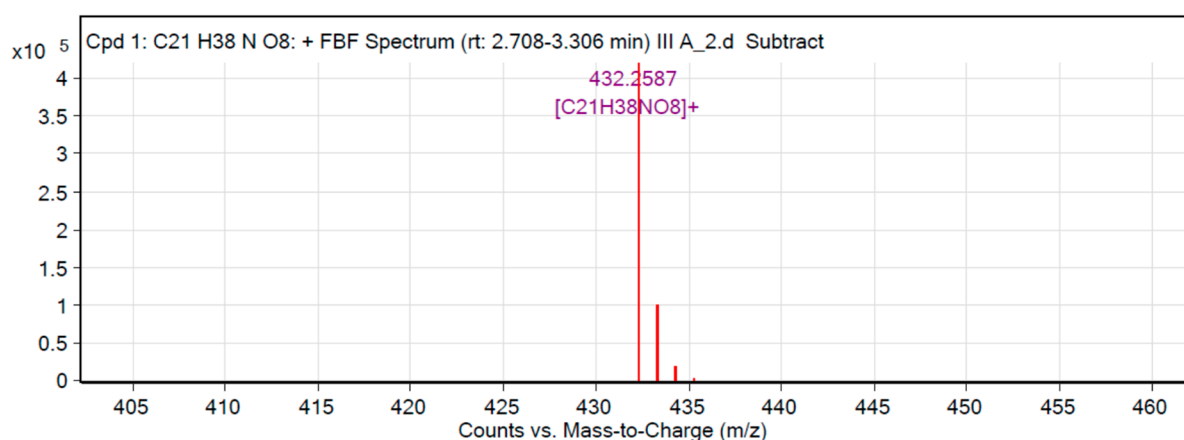
*N*-[2-(2',3',4'-tri-*O*-acetyl- $\alpha$ -D-xylopyranosyloxy)ethyl]pyridinium bromide (**5a**) *m/z* 382.1492 ([M-Br]<sup>+</sup>)



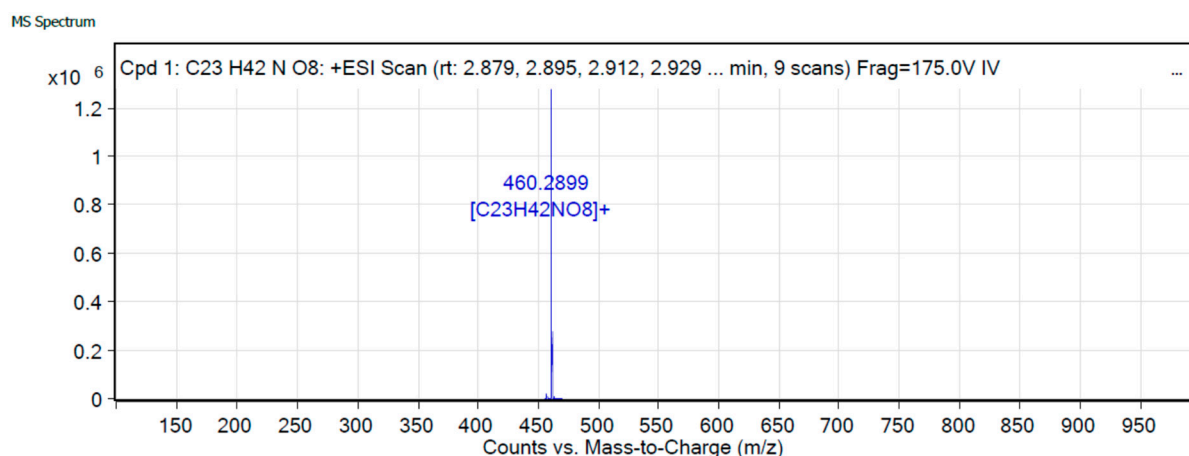
*N*-[2-(2',3',4'-tri-*O*-acetyl- $\alpha$ -D-xylopyranosyloxy)ethyl] ]-*N,N,N*-trimethylammonium bromide (**5b**) *m/z* 362.1804 ([M-Br]<sup>+</sup>)



*N*-[2-(2',3',4'-tri-*O*-acetyl- $\alpha$ -D-xylopyranosyloxy)ethyl]-*N*-hexyl-*N,N*-dimethylammonium bromide (**5c**) *m/z* 432.2587 ([M-Br]<sup>+</sup>)



*N*-[2-(2',3',4'-tri-*O*-acetyl- $\alpha$ -D-xylopyranosyloxy)ethyl]-*N,N*-dimethyl-*N*-octylammonium bromide (**5d**) *m/z* 460.2899 ([M-Br]<sup>+</sup>)



## References

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