Synthesis of β-D-galactopyranoside-presenting glycoclusters, investigation of their interactions with *Pseudomonas aeruginosa* lectin A (PA-IL) and evaluation of their anti-adhesion potential

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Abstract: *Pseudomonas aeruginosa* is an opportunistic human pathogen associated with cystic fibrosis. This bacterium produces, among other virulence factors, a soluble D-galactose-specific lectin PA-IL (LecA). PA-IL plays an important role in the adhesion to the host cells and is also cytotoxic. Therefore, this protein is an interesting therapeutic target, suitable for inhibition by carbohydrate-based compounds. In the current work, β-D-galactopyranoside-containing tri- and tetravalent glycoclusters were synthesized. Methyl gallate and pentaerythritol equipped with propargyl groups were chosen as multivalent scaffolds and the galactoclusters were built from the above-mentioned cores by coupling ethylene or tetraethylene glycol-bridges and peracetylated propargyl β-D-galactosides using 1,3-dipolar azide-alkyne cycloaddition. The interaction between galactoside derivatives and PA-IL was investigated by several biophysical methods, including hemagglutination inhibition assay, isothermal titration calorimetry, analytical ultracentrifugation, and surface plasmon resonance. Their ability to inhibit adhesion of *P. aeruginosa* to bronchial cells was determined by *ex vivo* assay. The newly synthesized multivalent galactoclusters proved to be significantly better ligands than simple D-galactose for lectin PA-IL and as a result, two representatives of the dendrimers were able to decrease adhesion of *P. aeruginosa* to bronchial cells to approx. 32 % and 42 %, respectively.

Keywords: *Pseudomonas aeruginosa*; cystic fibrosis; lectin; D-galactosides; multivalency

Supplementary data

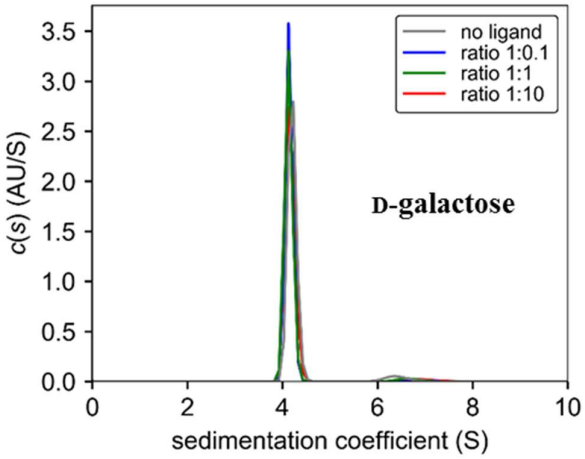


Figure S1. Continuous c(s) distributions of PA-IL samples obtained in the absence and presence of D-galactose. The distributions were obtained at different protein to ligand ratios (see the legend).

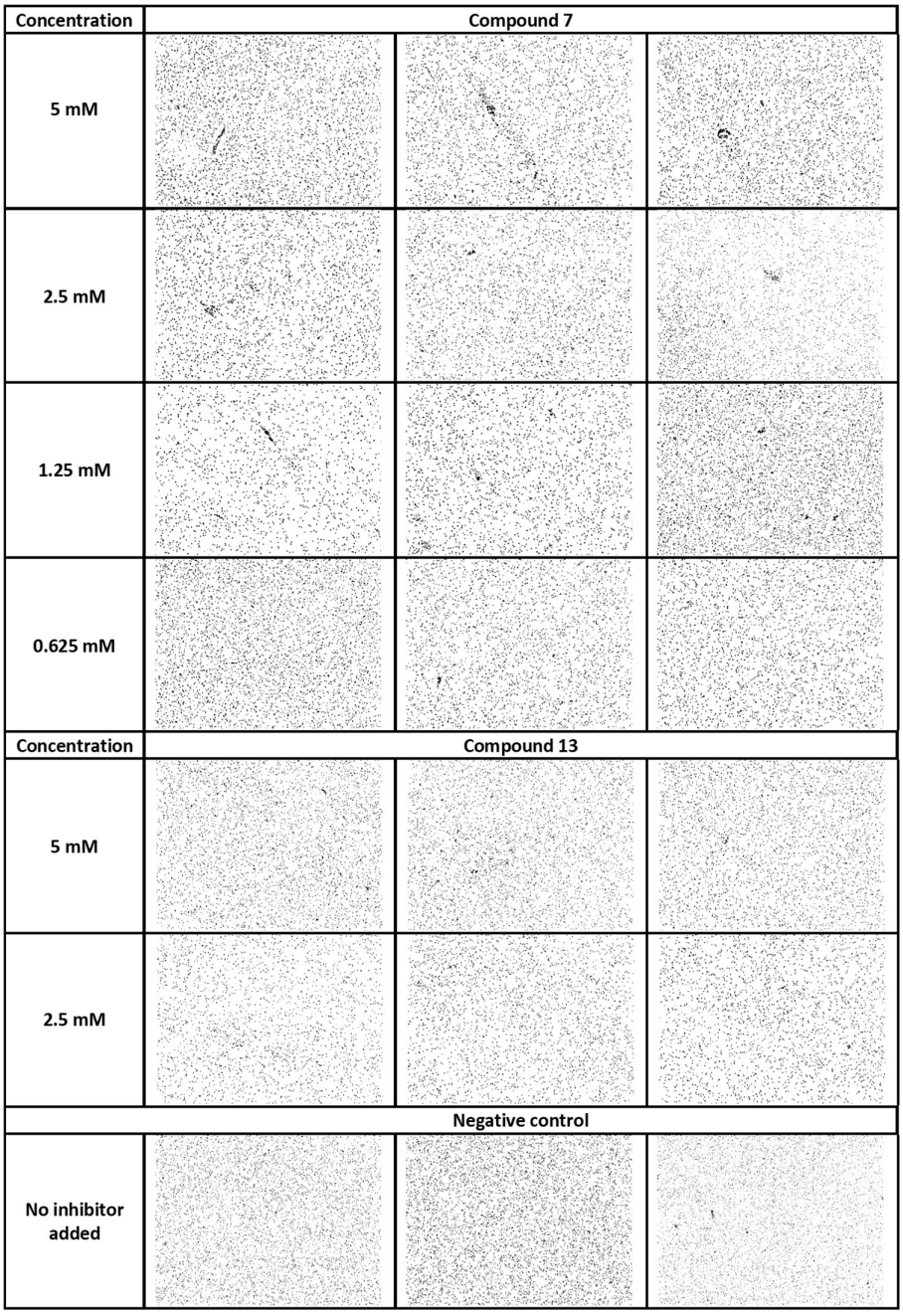


Figure S2. Cross-linking of *Pseudomonas aeruginosa* cells with D-galactopyranoside-presenting inhibitors. The *P. aeruginosa* cells with no added inhibitors were used as a control. Magnification 100x, phase contrast, background subtraction in GIMP, triplicates. Results for compounds **7** and **13**.

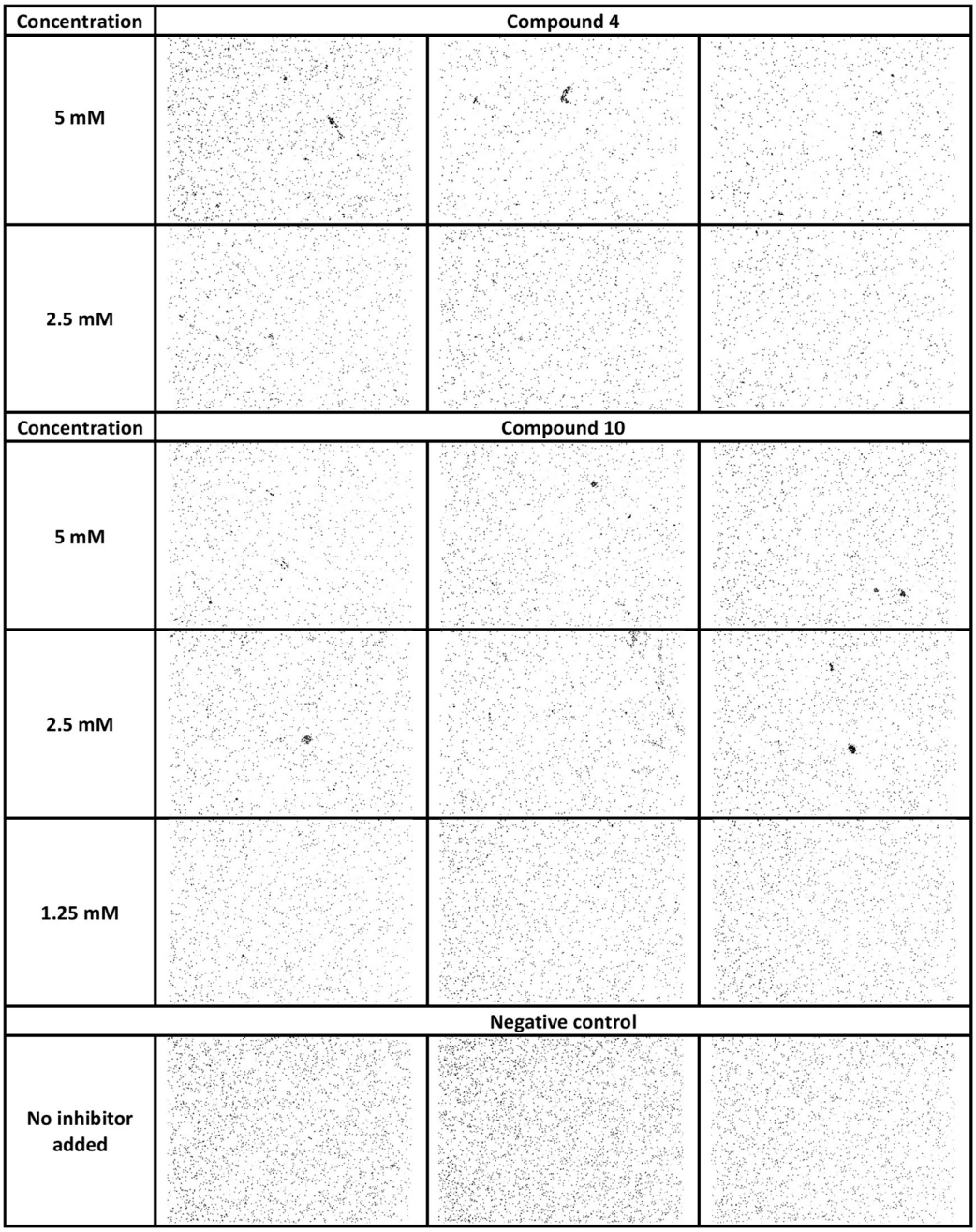
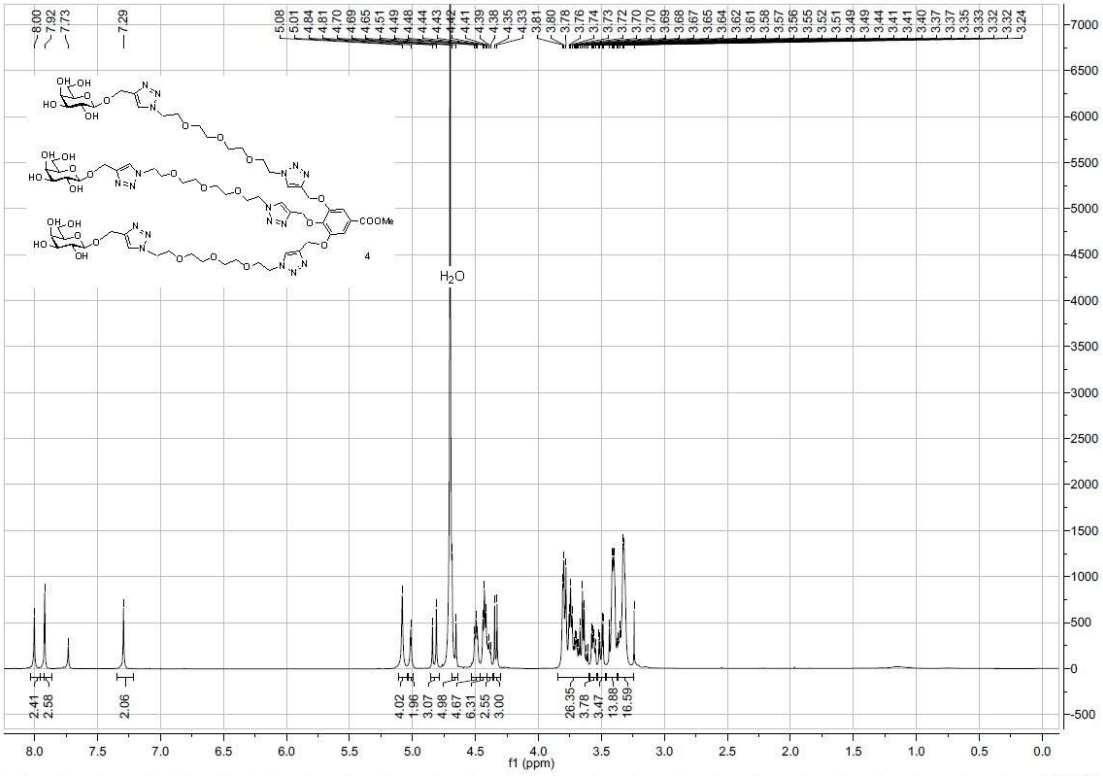


Figure S3. Cross-linking of *Pseudomonas aeruginosa* cells with D-galactopyranoside-presenting inhibitors. The *P. aeruginosa* cells with no added inhibitors were used as a control. Magnification 100x, phase contrast, background subtraction in GIMP, triplicates. Results for compounds **4** and **10**.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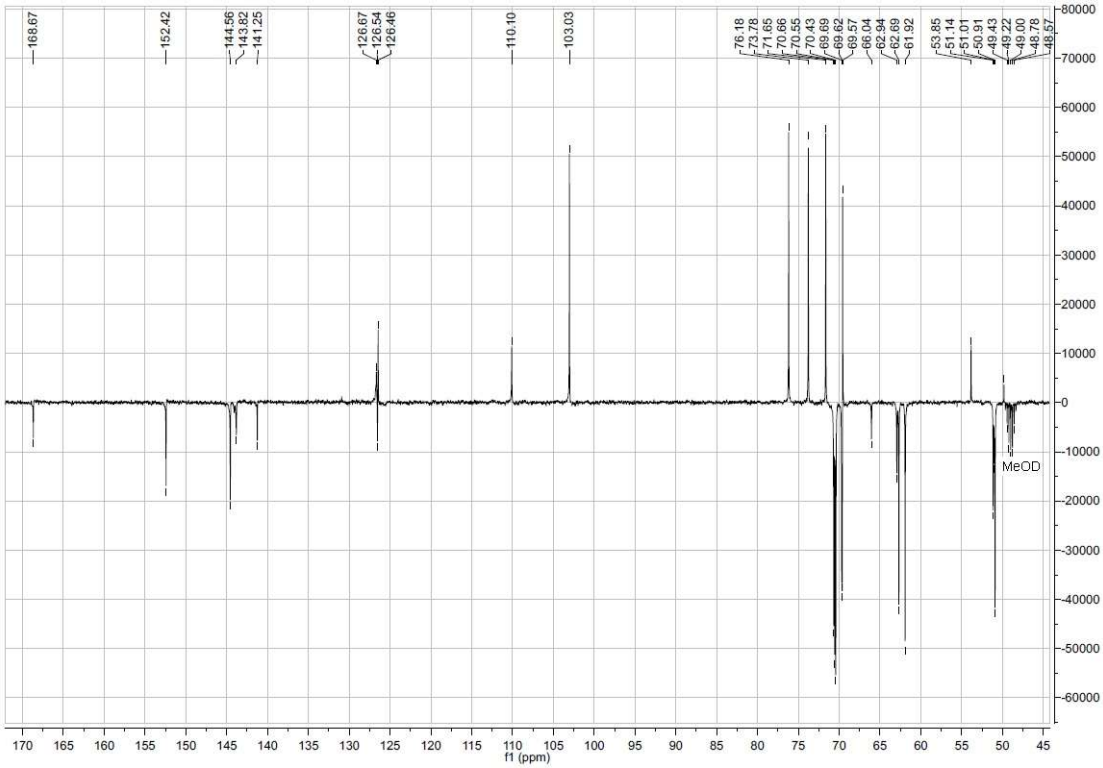
7.29
5.08
5.01
4.84
4.81
4.70
4.69
4.65
4.51
4.49
4.48
4.44
4.43
4.42
4.41
4.39
4.38
4.35
4.33
3.81
3.80
3.78
3.76
3.74
3.73
3.72
3.70
3.70
3.69
3.68
3.67
3.65
3.64
3.62
3.61
3.58
3.57
3.56
3.55
3.52
3.51
3.49
3.49
3.44
3.41
3.41
3.40
3.37
3.37
3.35
3.33
3.32
3.32
3.24
COOMe
4
H2O
2.41
2.58
2.06
4.02
1.96
3.07
4.98
4.67
6.31
2.55
3.00
26.35
3.78
3.47
13.88
16.59
f1 (ppm)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141.25
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126.54
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76.18
73.78
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70.66
70.55
70.43
69.69
69.62
69.57
66.04
62.94
62.69
61.92
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49.00
48.78
48.57
MeOD
f1 (ppm)

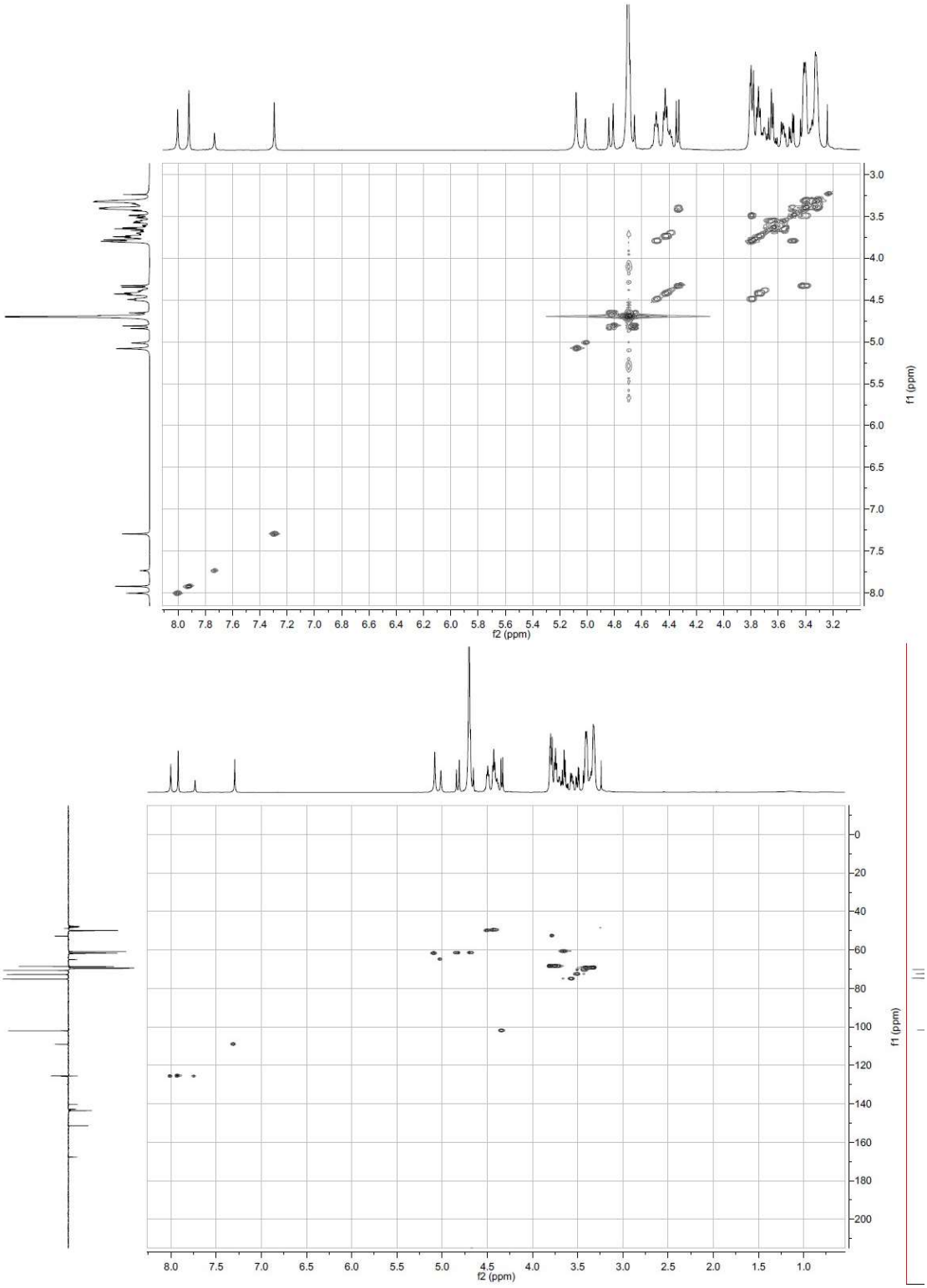
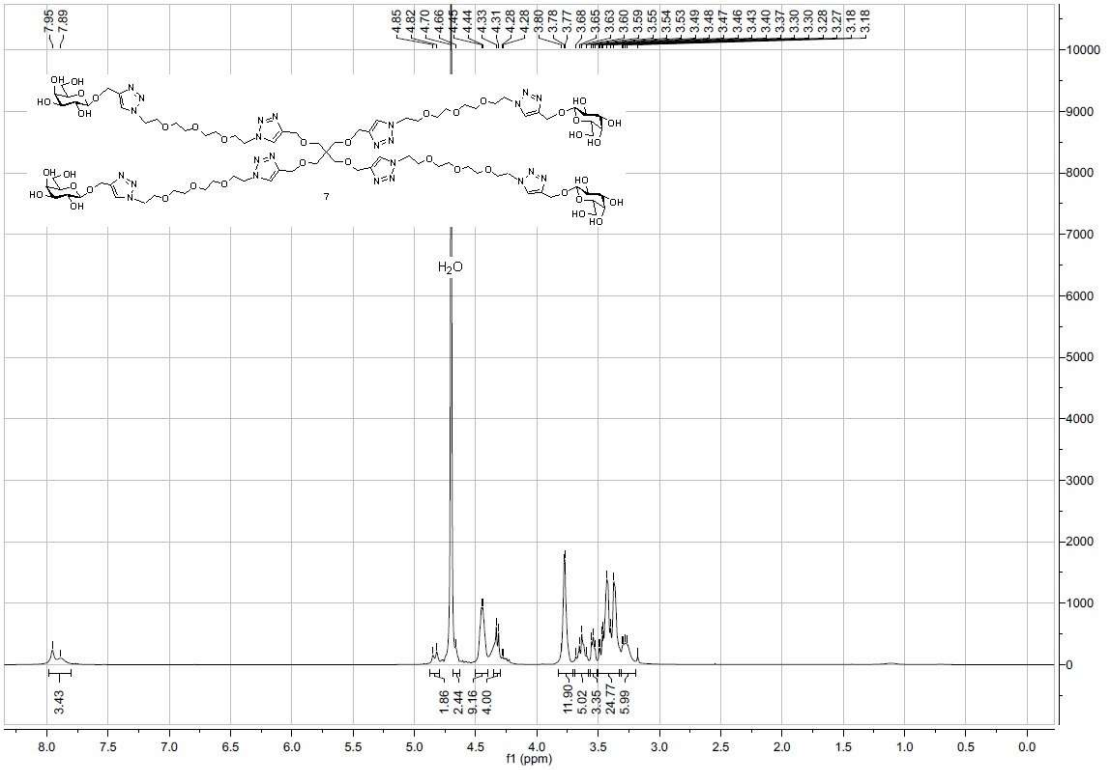


Figure S4. ^{1}H, ^{13}C NMR, COSY and HSQC spectra of compound **4.**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4.44
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4.31
4.28
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3.80
3.78
3.77
3.68
3.65
3.63
3.60
3.59
3.55
3.54
3.53
3.49
3.48
3.47
3.46
3.43
3.40
3.37
3.30
3.30
3.28
3.27
3.18
3.18
7
H2O
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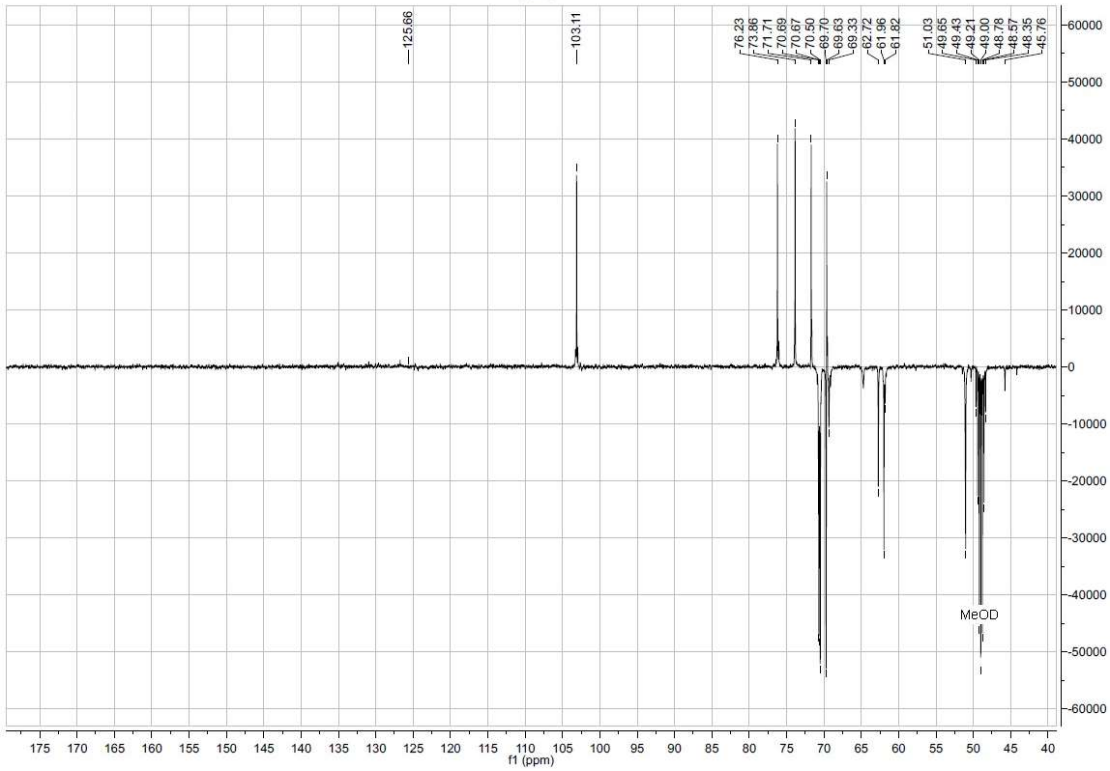
5.02
3.35
24.77
5.99
f1 (ppm)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76.23
73.86
71.71
70.69
70.67
70.50
69.70
69.63
69.33
62.72
61.96
61.82
51.03
49.65
49.43
49.21
49.00
48.78
48.57
48.35
45.76
MeOD
f1 (ppm)

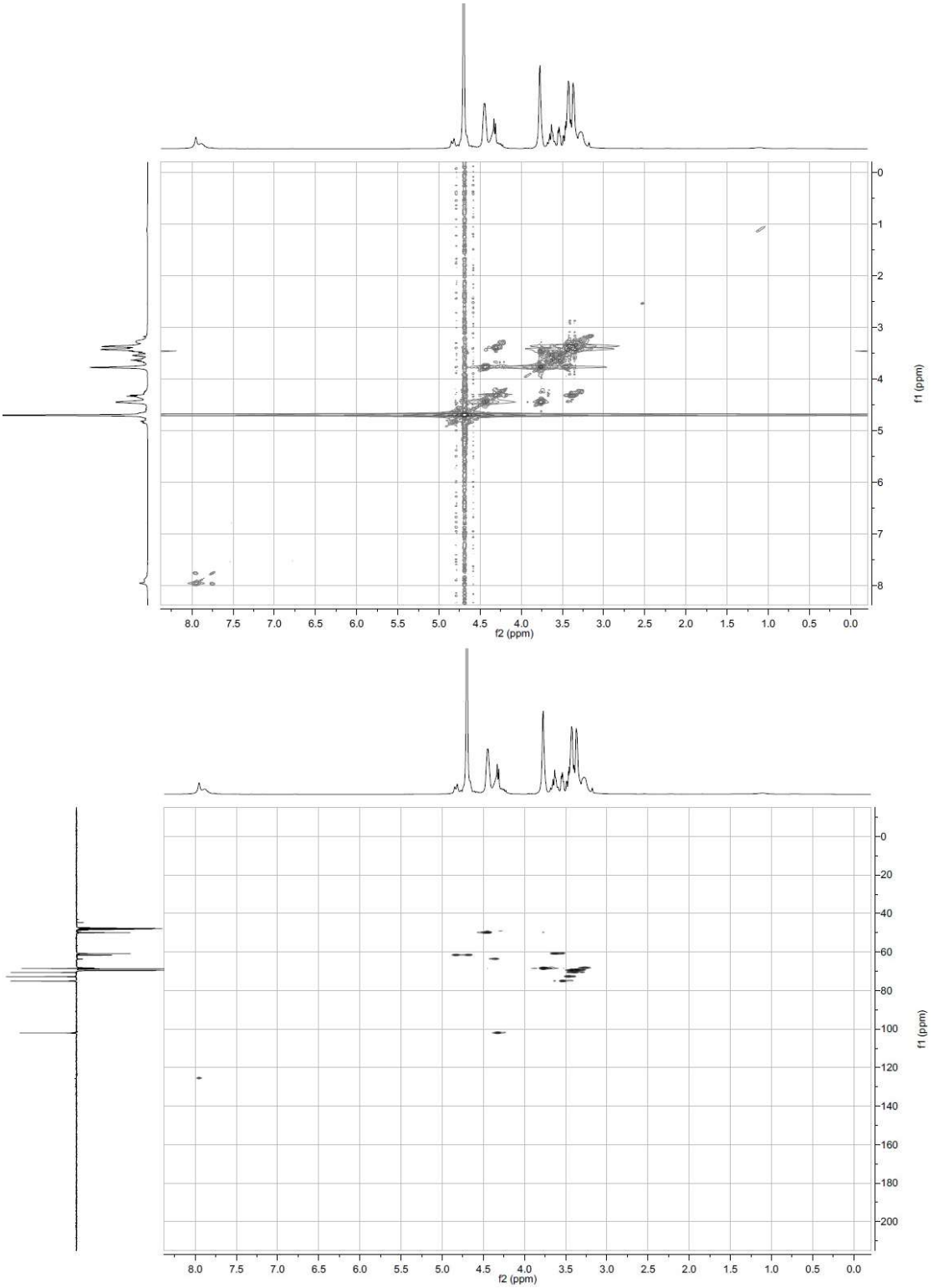
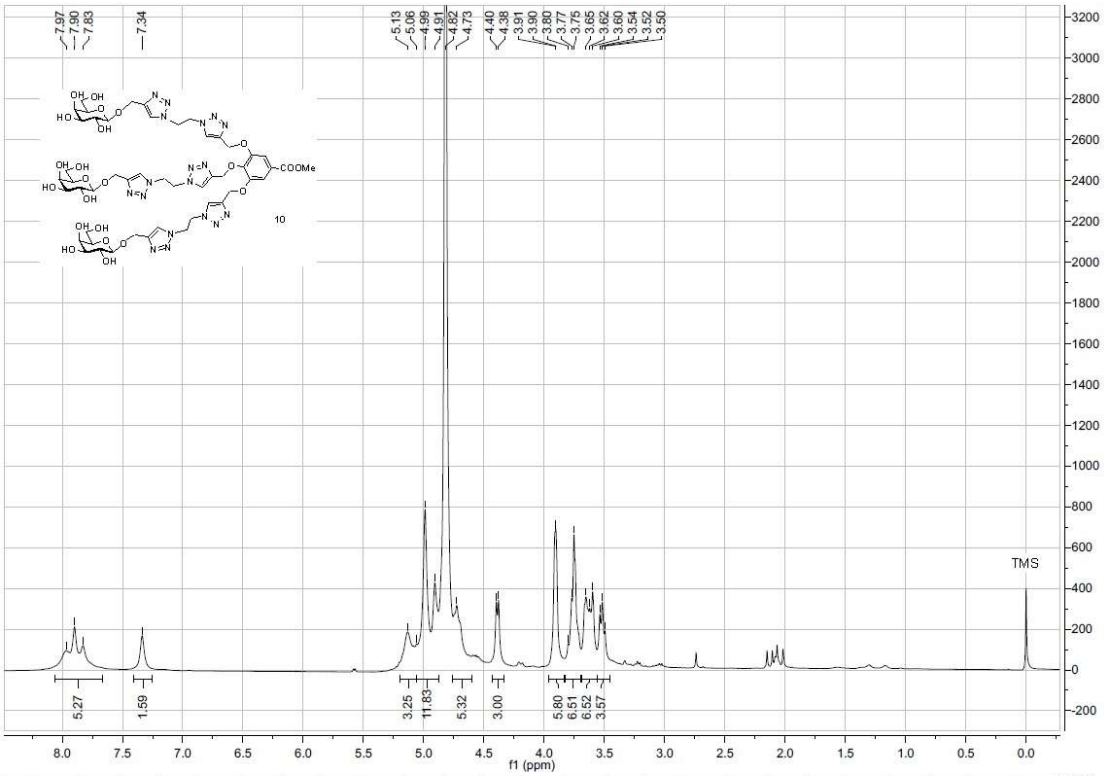


Figure S5. ^{1}H, ^{13}C NMR, COSY and HSQC spectra of compound **7.**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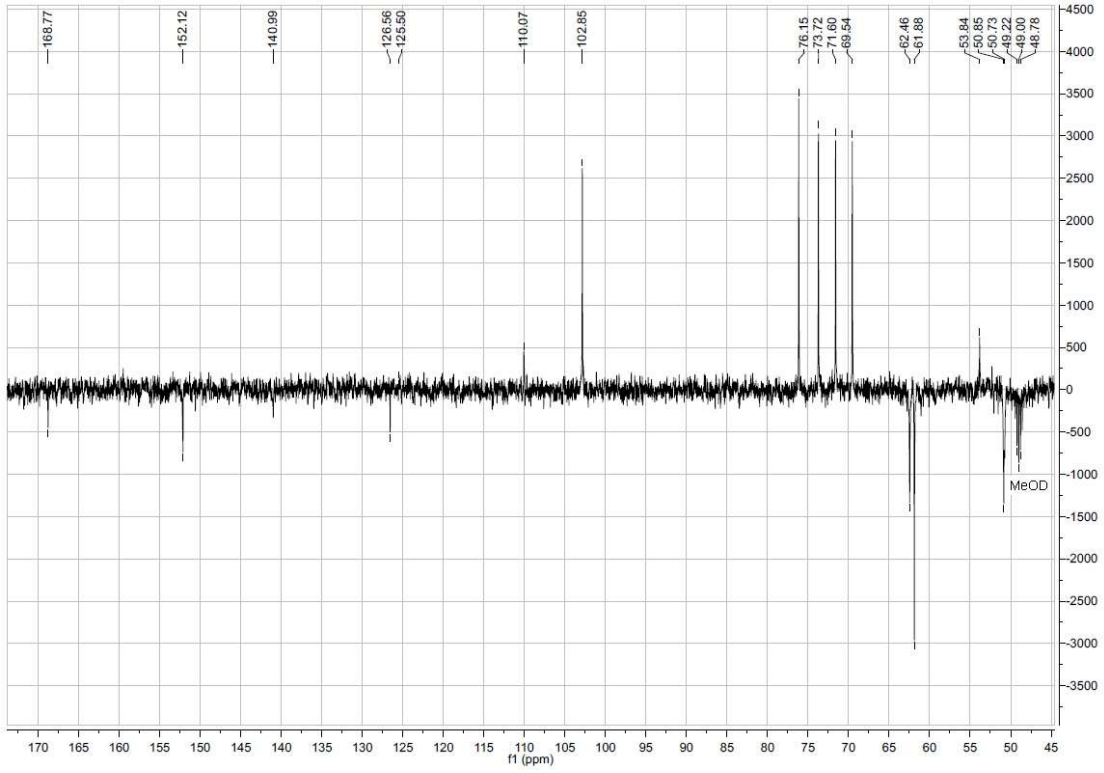
7.90
7.83
7.34
5.13
5.06
4.99
4.91
4.82
4.73
4.40
4.38
3.91
3.90
3.80
3.77
3.75
3.65
3.62
3.60
3.54
3.52
3.50
COOMe
10
TMS
5.27
1.59
3.25
11.83
5.32
3.00
5.80
6.51
6.52
3.57
f1 (ppm)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126.56
125.50
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50.85
50.73
49.22
49.00
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MeOD
f1 (ppm)

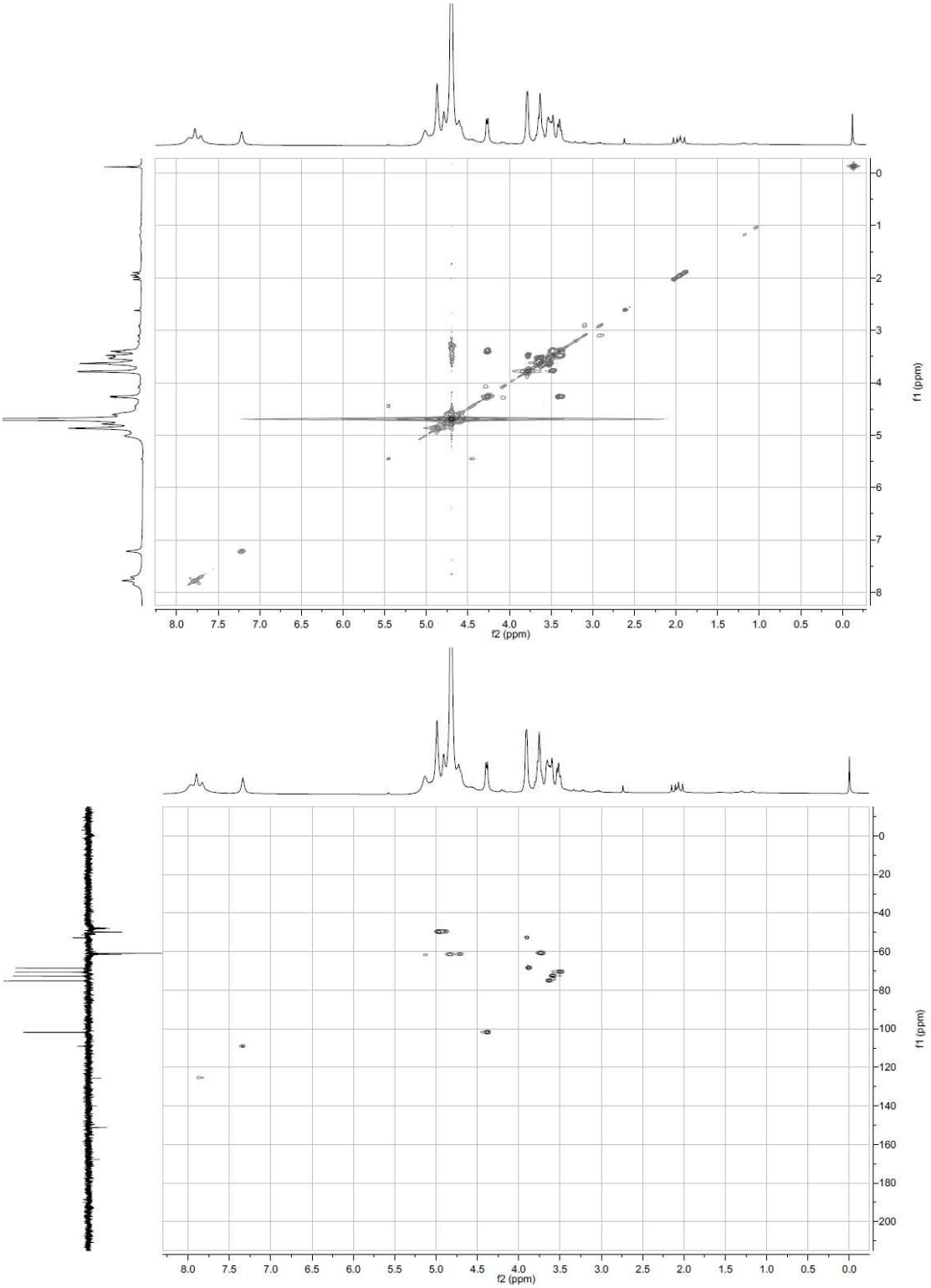
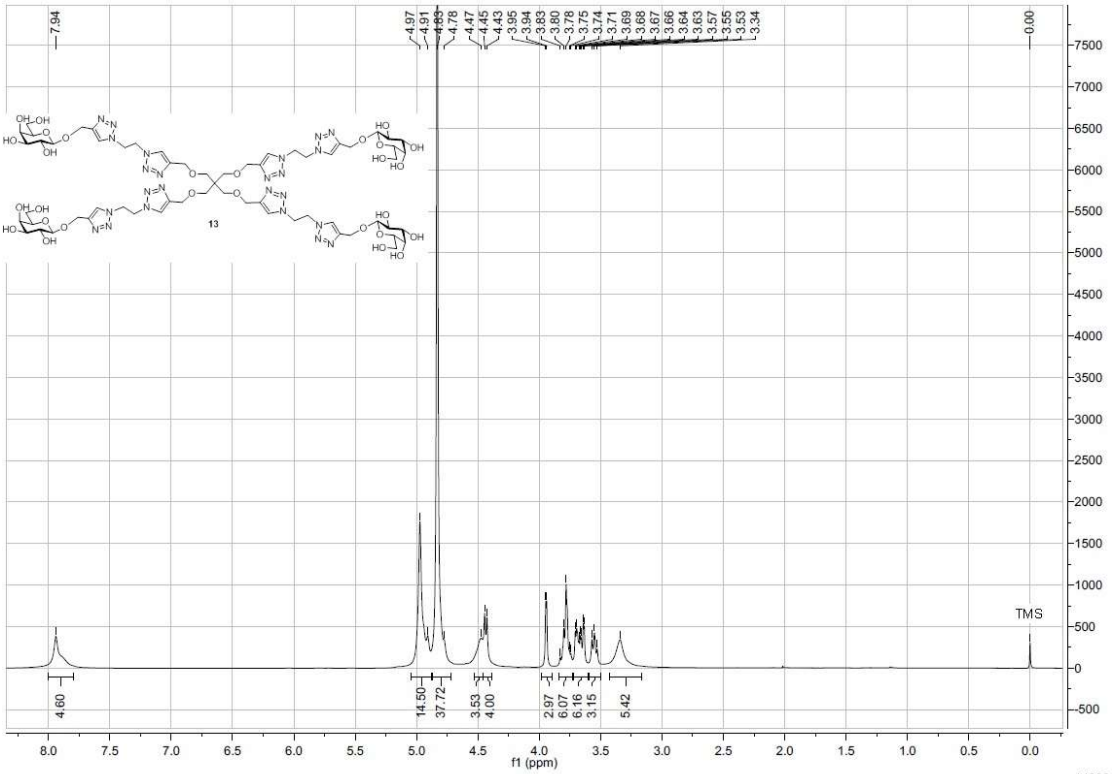


Figure S6. ^{1}H, ^{13}C NMR, COSY and HSQC spectra of compound **10.**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4.97
4.91
4.83
4.78
4.47
4.45
4.43
3.95
3.94
3.83
3.80
3.78
3.75
3.74
3.71
3.69
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3.67
3.66
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3.57
3.55
3.53
3.34
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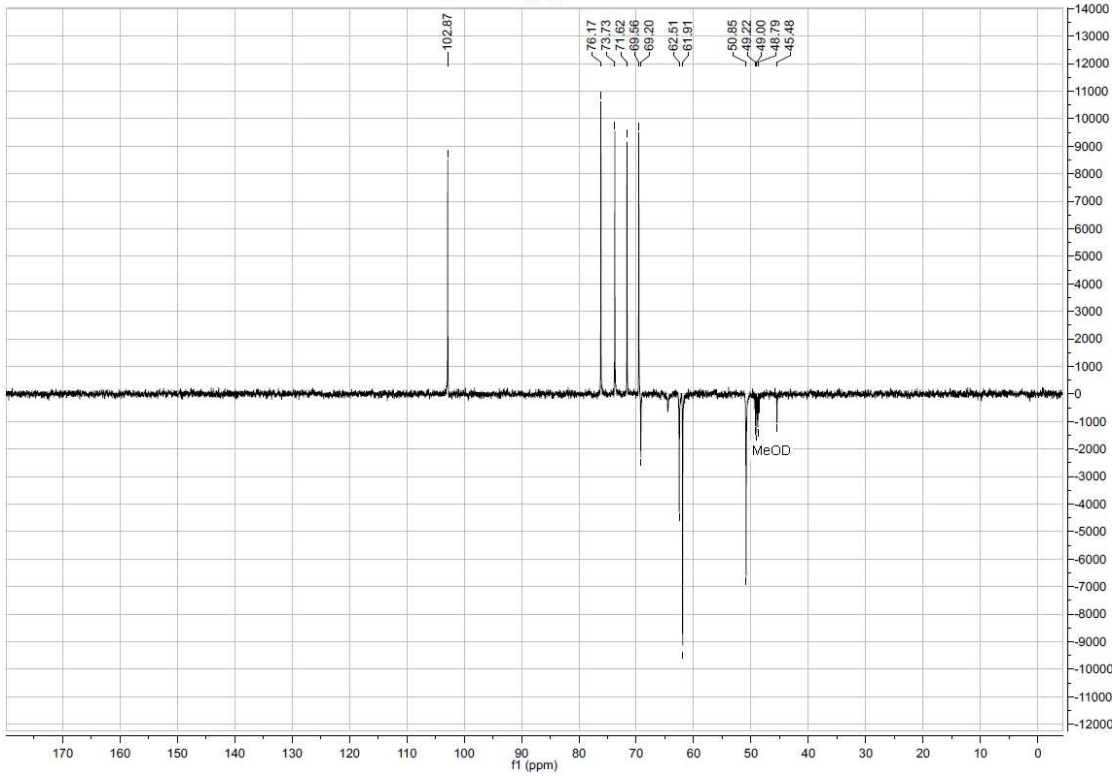
6.07
6.16
3.15
5.42
f1 (ppm)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61.91
50.85
49.22
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45.48
MeOD
f1 (ppm)

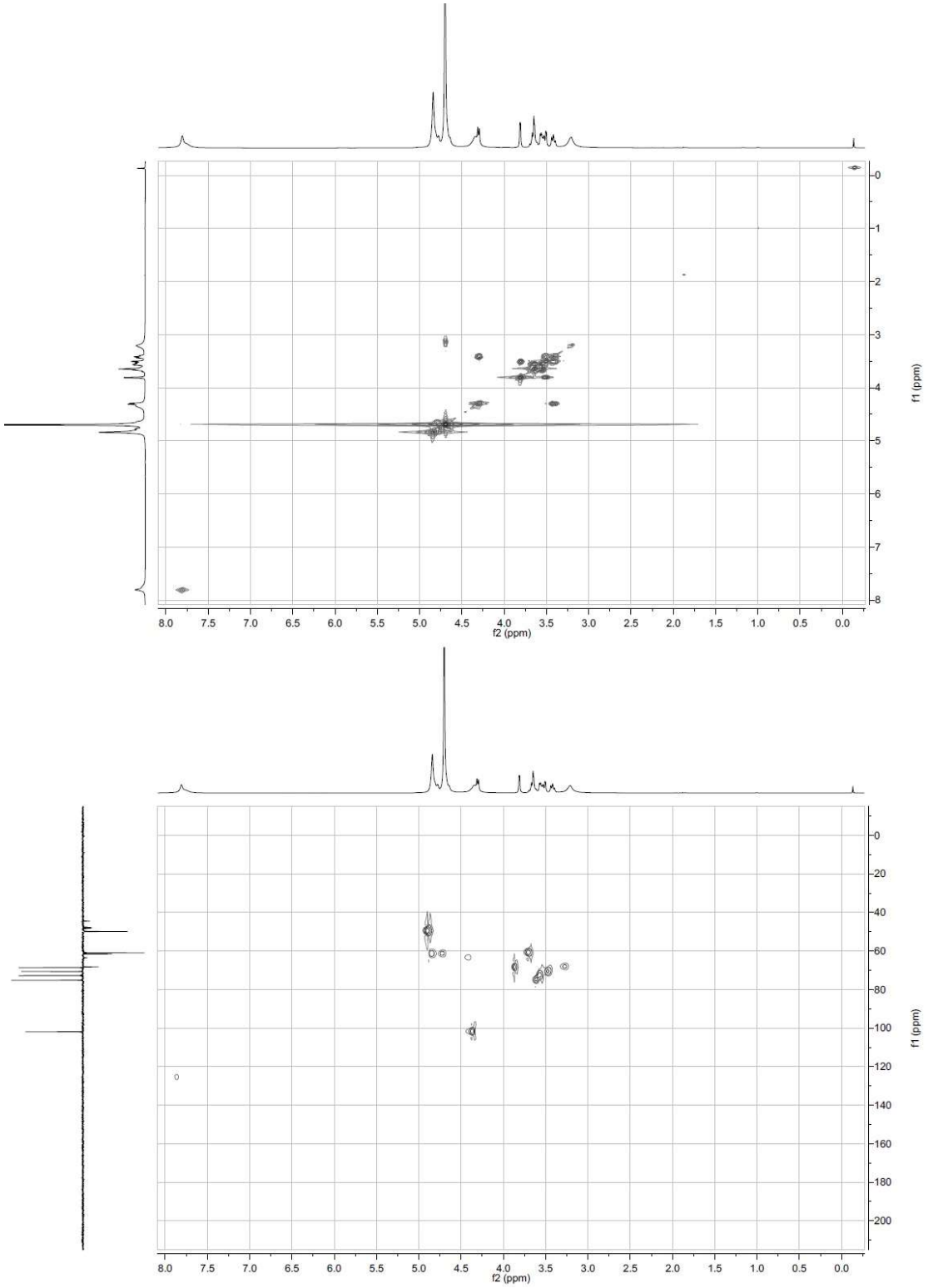

Figure S7. ^{1}H, ^{13}C NMR, COSY and HSQC spectra of compound **13.**