SUPPORTING INFORMATION

Addressing the Biochemical Foundations of a Glucose-Based "Trojan Horse"–Strategy to Boron Neutron Capture Therapy: From Chemical Synthesis to *In Vitro* Assessment

Jelena Matović,[1,ϕ] Juulia Järvinen,[2,ϕ] Helena C. Bland,[1] Iris K. Sokka,[1] Surachet Imlimthan,[1] Ruth Mateu Ferrando,[1] Kristiina M. Huttunen,[2] Juri Timonen,[2] Sirpa Peräniemi,[2] Olli Aitio,[3] Anu J. Airaksinen,[1,4] Mirkka Sarparanta,[1] Mikael P. Johansson,[1,5] Jarkko Rautio[2] and Filip S. Ekholm[1*]

[1] Department of Chemistry, University of Helsinki, Finland, P.O. Box 55, 00014 Helsinki, Finland

[2] School of Pharmacy, University of Eastern Finland, P.O. Box 1627, 70211 Kuopio, Finland

[3] Glykos Finland Ltd., Viikinkaari 6, 00790 Helsinki, Finland

[4] Turku PET Centre, Department of Chemistry, University of Turku, P.O. Box 52, 20521 Turku, Finland

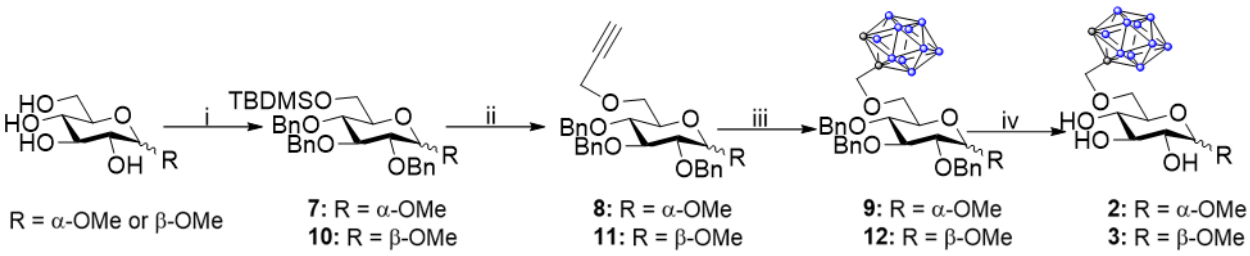
[5] Helsinki Institute of Sustainability Science, HELSUS, FI-00014 Helsinki, Finland

[ϕ] Equal contributions

Contact: filip.ekholm@helsinki.fi

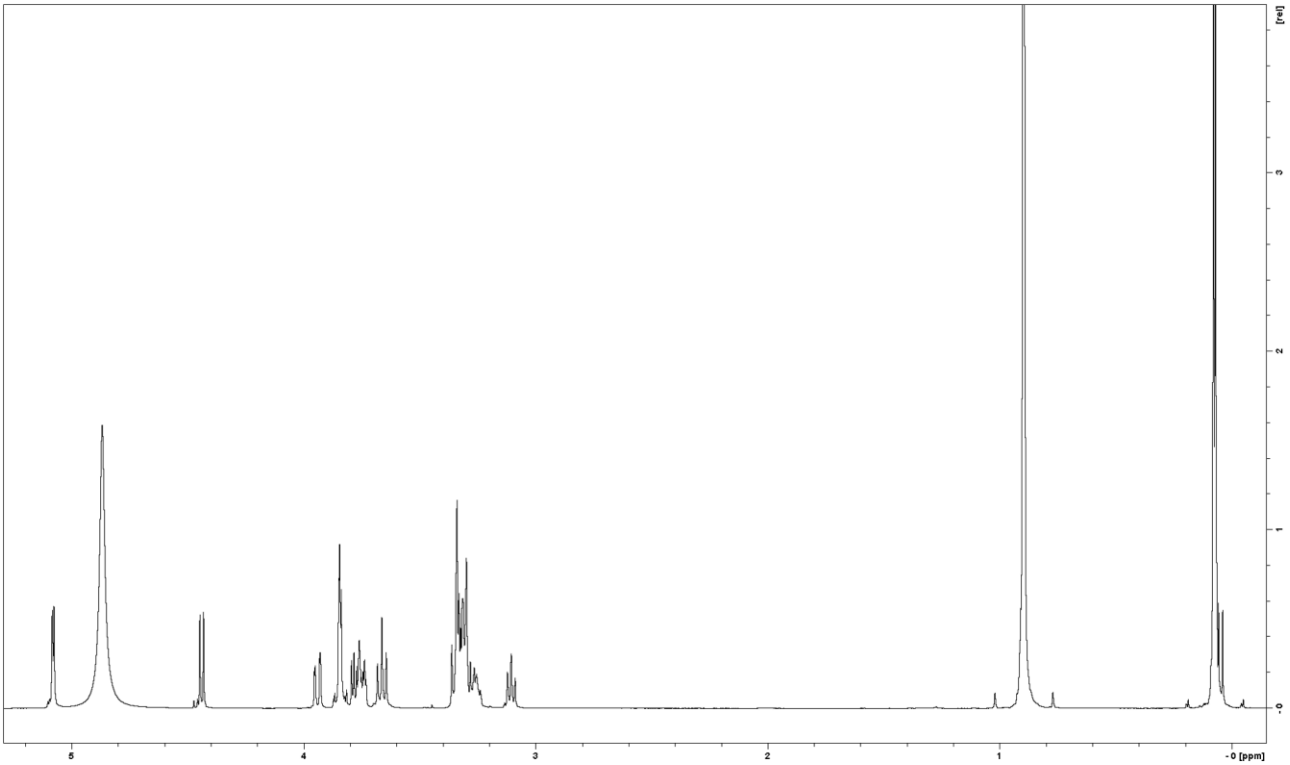
Table of contents

1. Additional synthetic routes

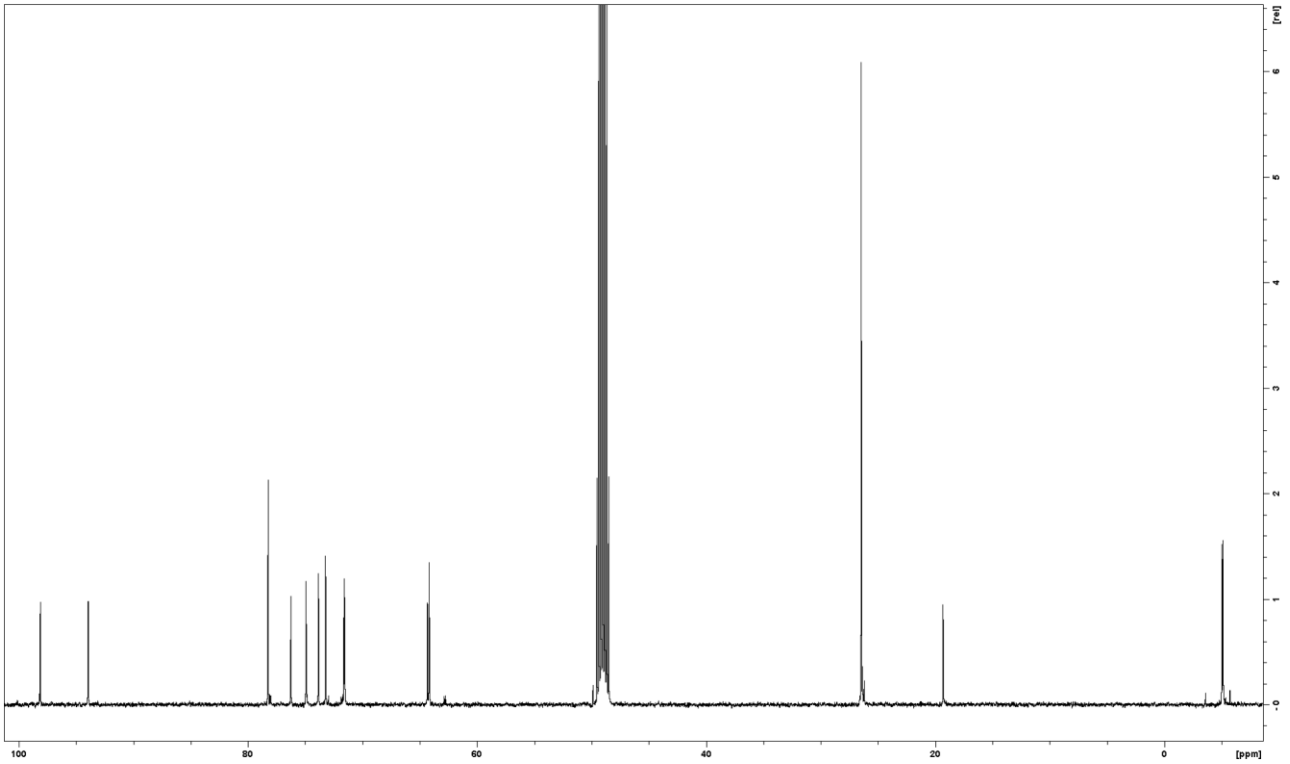


Supplementary scheme 1. Overview of reactions of Methyl α- and β-D-glucopyranosides: i) 1) TBDMSCl, imidazole, DMF, rt, 24 h, 80 % (α-OMe); 83 % (β-OMe); 2) BnBr, NaH, DMF, rt, 4 h, 78 % (**7**); 80 % (**10**). ii) 1) HF-pyridine, THF, rt, 18 h, 82 % (α-OMe); 89 % (β-OMe); 2) propargyl bromide, NaH, DMF, rt, 15 h, 89 % (**8**); 95 % (**11**); iii) a) $B_{10}H_{14}$, acetonitrile, 60 °C, 1 h: b) **8** or **11** toluene, 80 °C, 22 h, 53 % (**9**); 66 % (**12**); iv) H_2, 10 % Pd/C, EtOAc:MeOH 7:1, 3–5 bar, rt, 4–6 h, 72 % (**2**); 81 % (**3**).

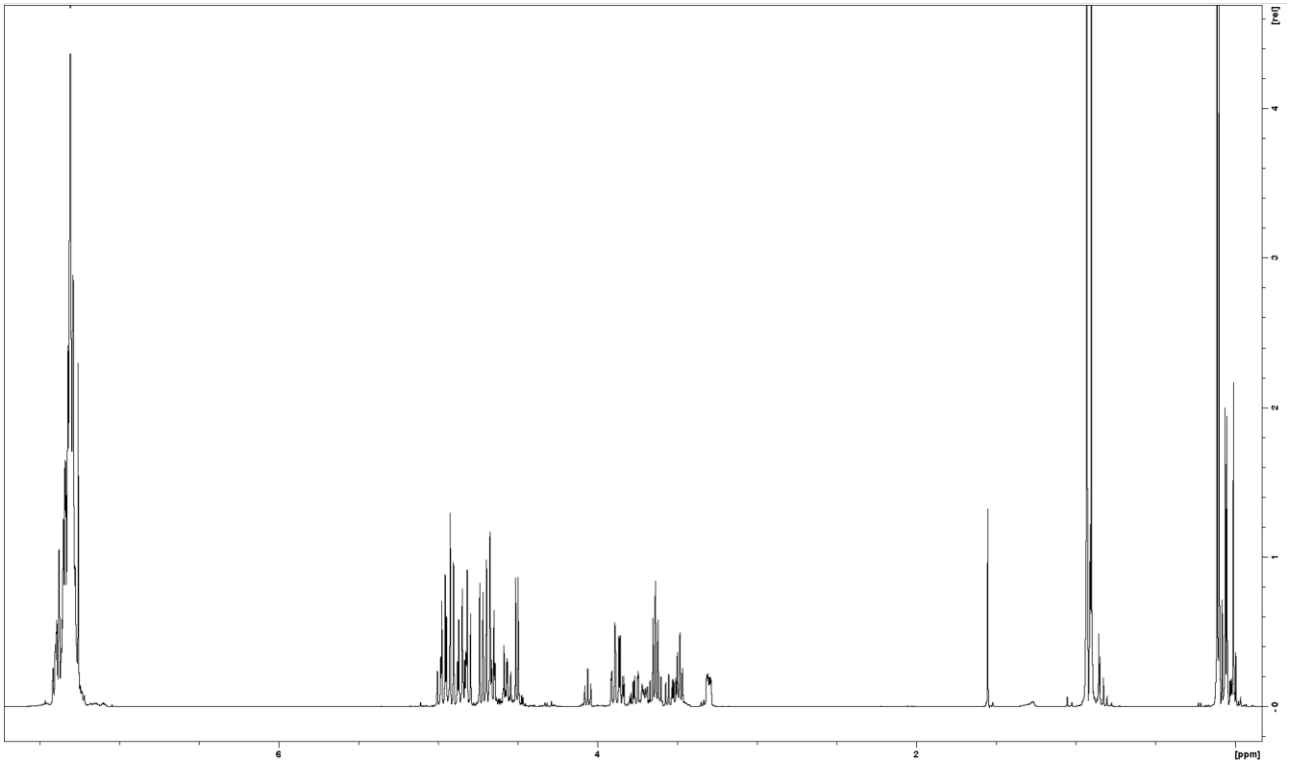
2. NMR spectra of synthesized compounds



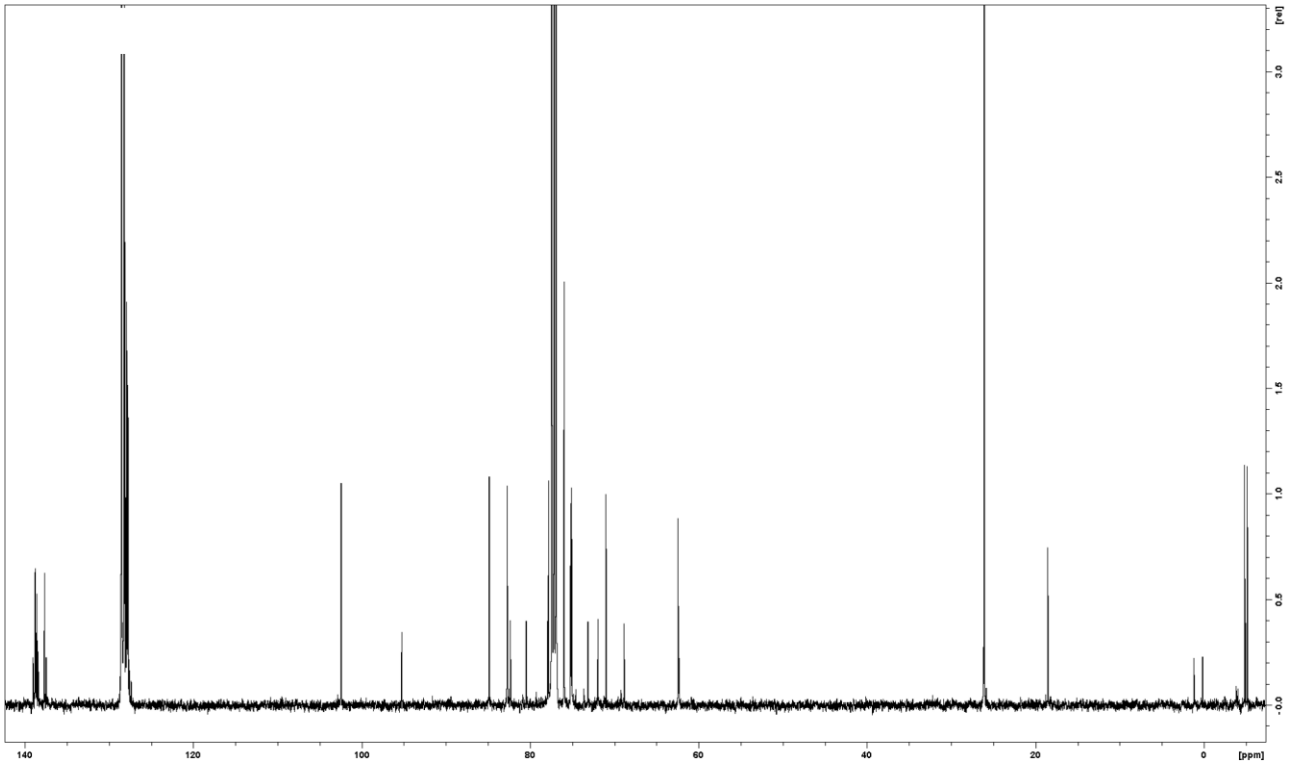
Supplementary figure 1. ^{1}H NMR spectrum of 6-*O*-(tert-butyldimethylsilyl)-D-glucopyranose (500.13 MHz, 23 °C, CD_3OD).



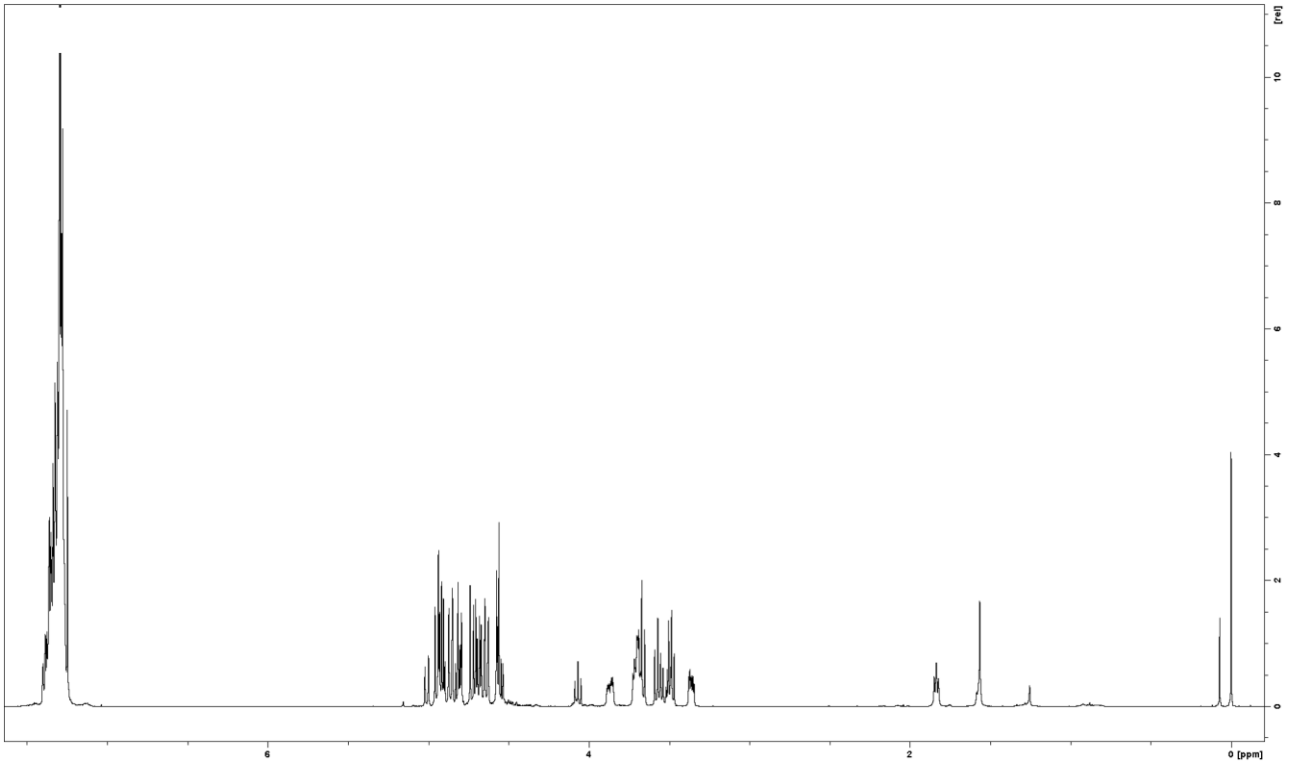
Supplementary figure 2. ^{13}C NMR spectrum of 6-*O*-(tert-butyldimethylsilyl)-D-glucopyranose (125.76 MHz, 23 °C, CD_3OD).



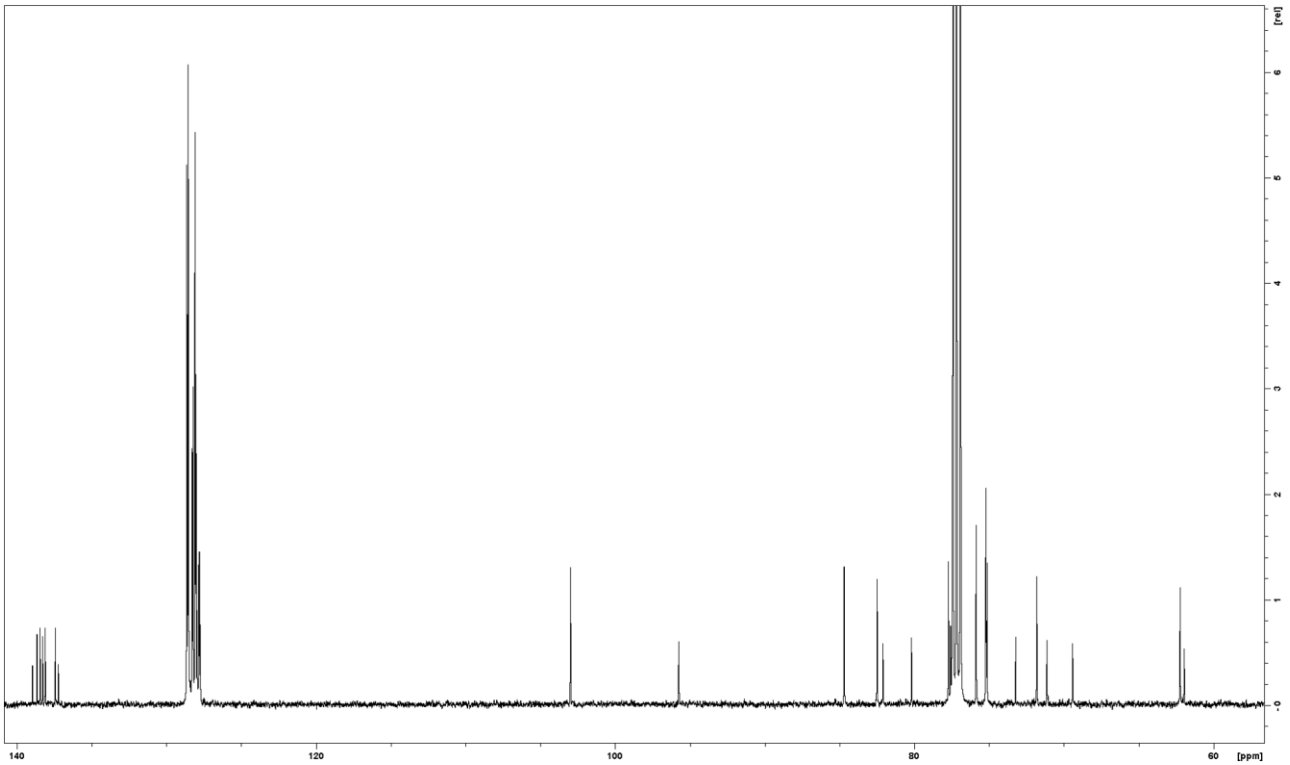
Supplementary figure 3. ^{1}H NMR spectrum of **4** (500.13 MHz, 23 °C, $CDCl_3$).



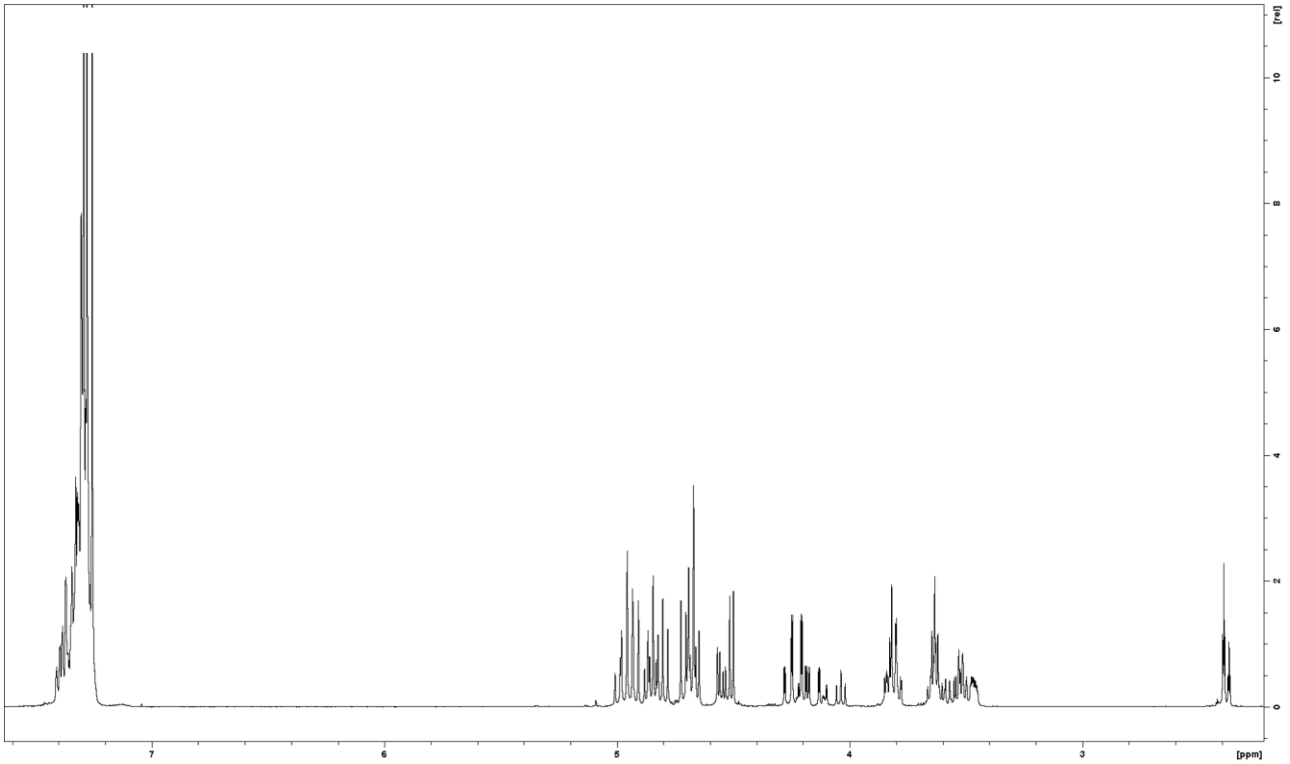
Supplementary figure 4. ^{13}C NMR spectrum of **4** (125.76 MHz, 23 °C, $CDCl_3$).



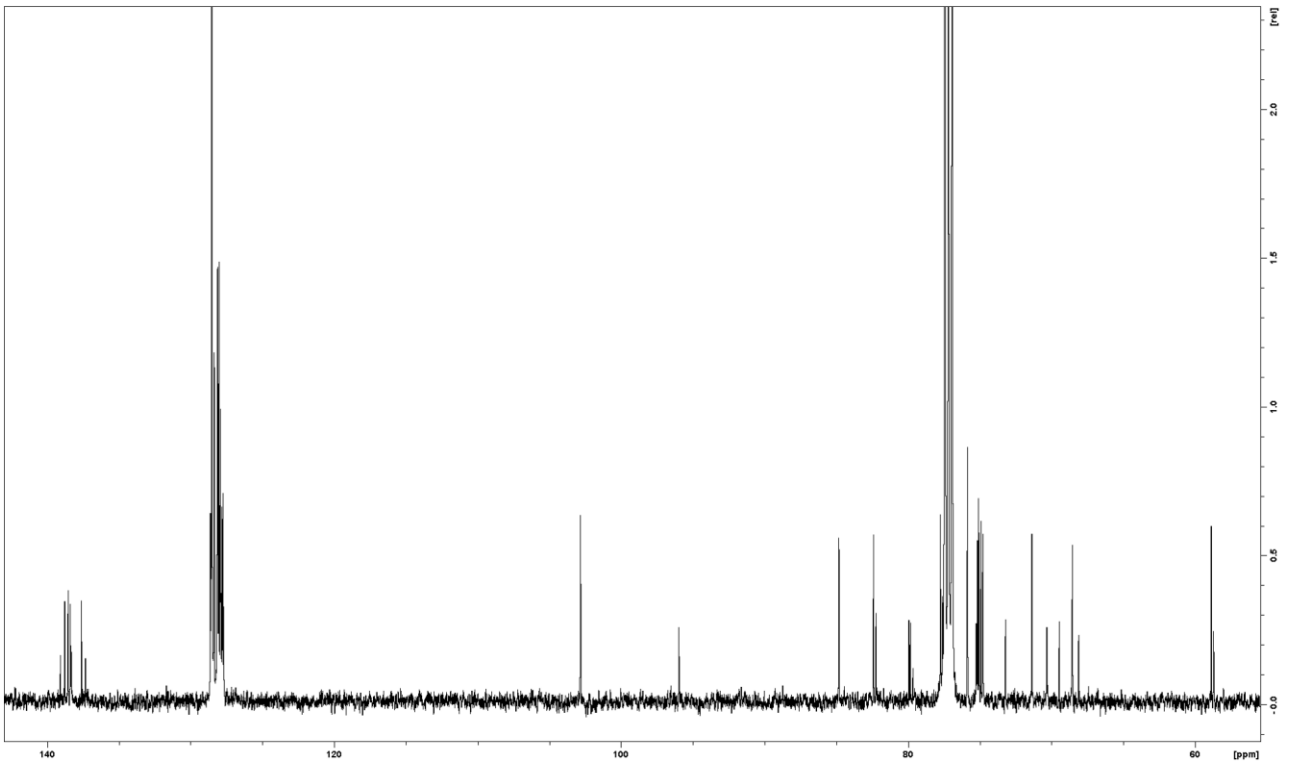
Supplementary figure 5. ^{1}H NMR spectrum of 1,2,3,4-tetra-*O*-benzyl-D-glucopyranose (500.13 MHz, 23 °C, $CDCl_3$).



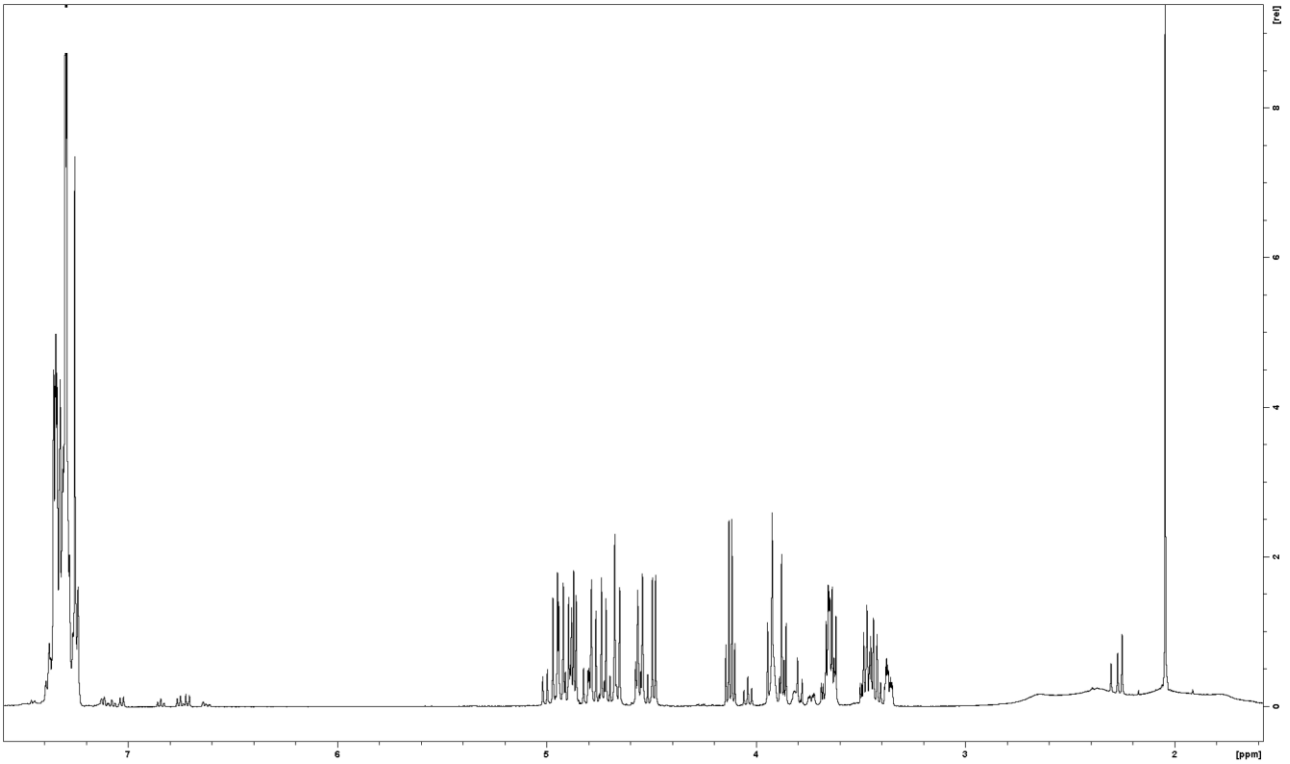
Supplementary figure 6. ^{13}C NMR spectrum of 1,2,3,4-tetra-*O*-benzyl-D-glucopyranose (125.76 MHz, 23 °C, $CDCl_3$)



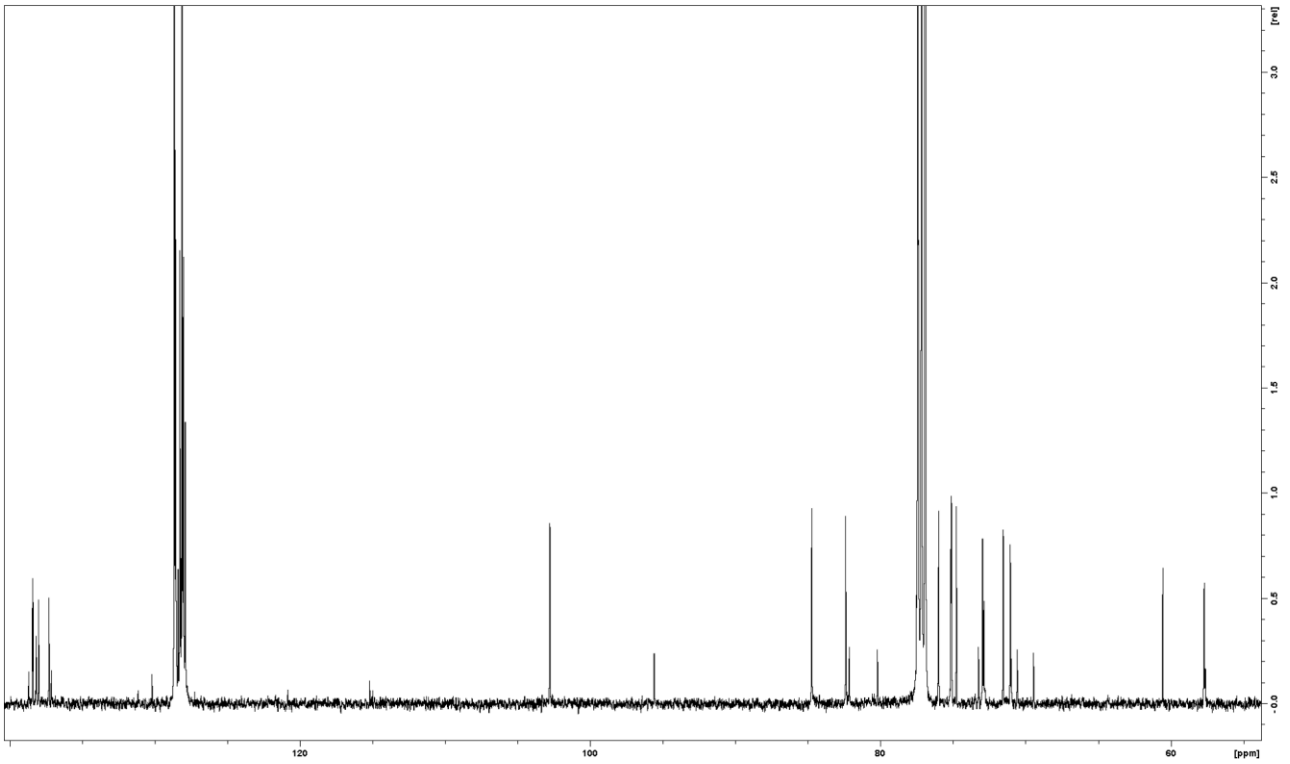
Supplementary figure 7. ^{1}H NMR spectrum of **5** (500.13 MHz, 23 °C, $CDCl_3$).



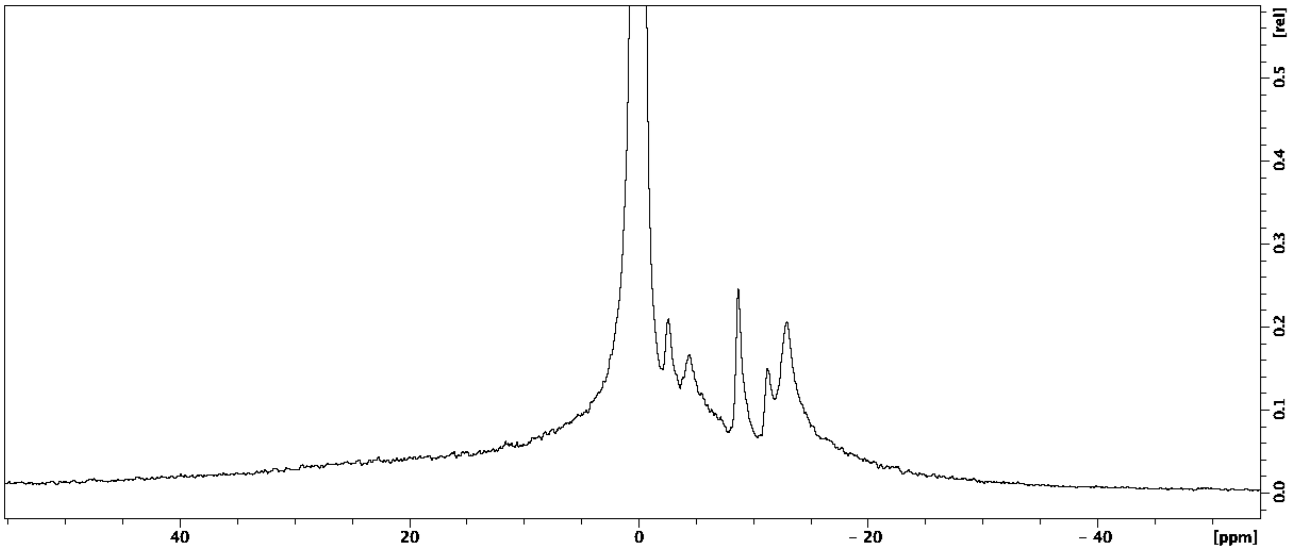
Supplementary figure 8. ^{13}C NMR spectrum of **5** (125.76 MHz, 23 °C, $CDCl_3$)



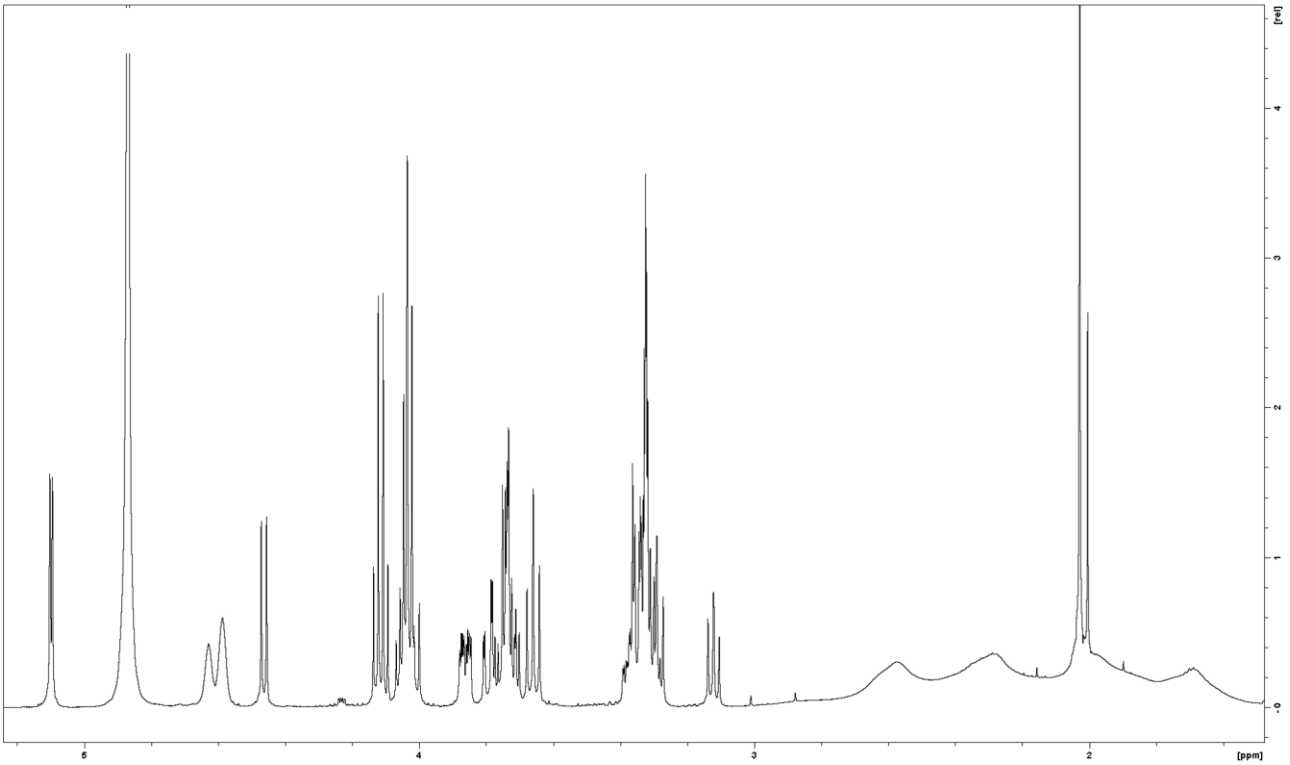
Supplementary figure 9. ^{1}H NMR spectrum of **6** (500.13 MHz, 23 °C, $CDCl_3$).



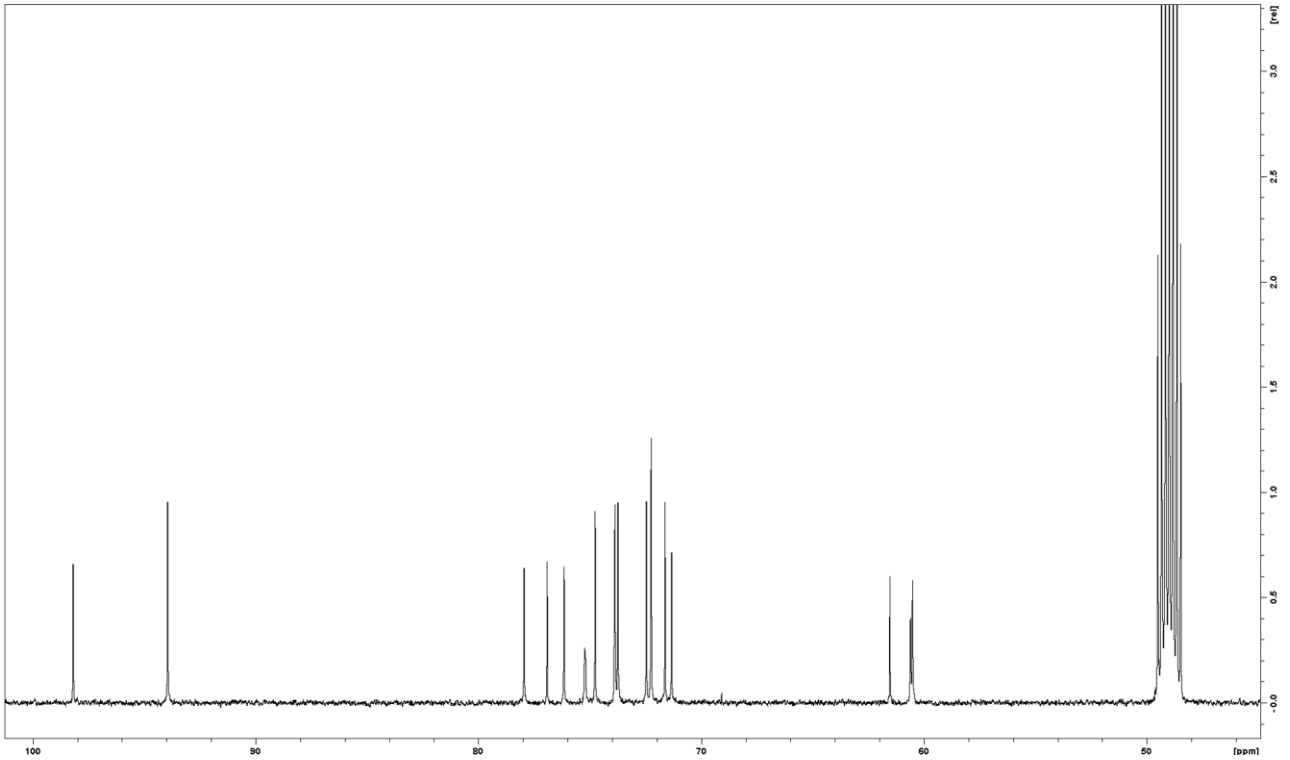
Supplementary figure 10. ^{13}C NMR spectrum of **6** (125.76 MHz, 23 °C, $CDCl_3$).



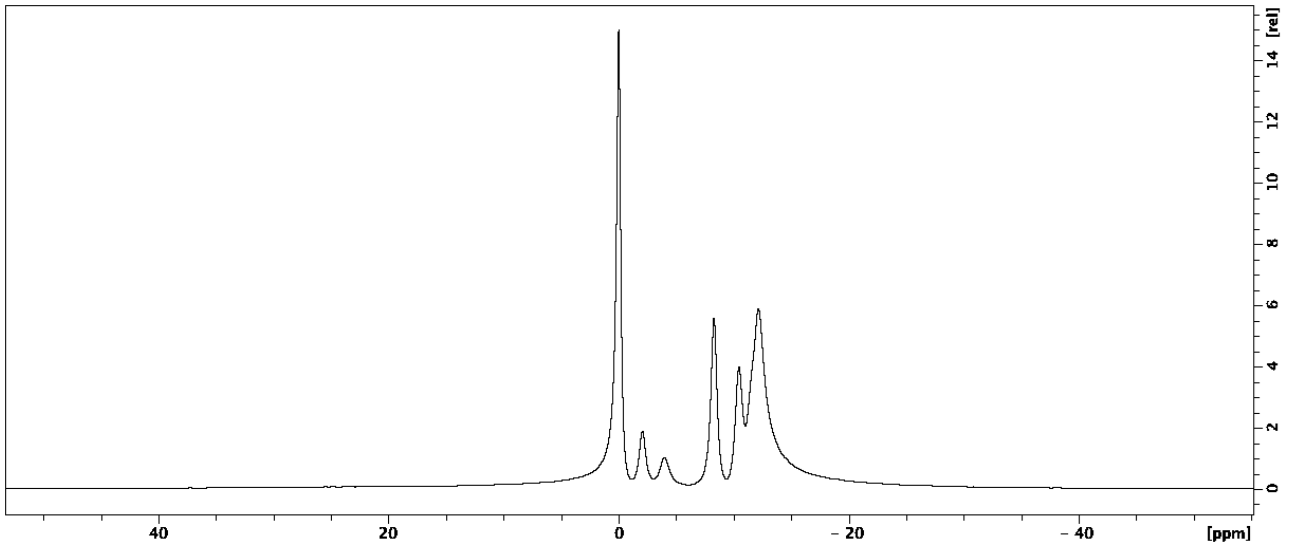
Supplementary figure 11. ^{11}B NMR spectrum of **6** (160.46 MHz, 23 °C, $CDCl_3$).



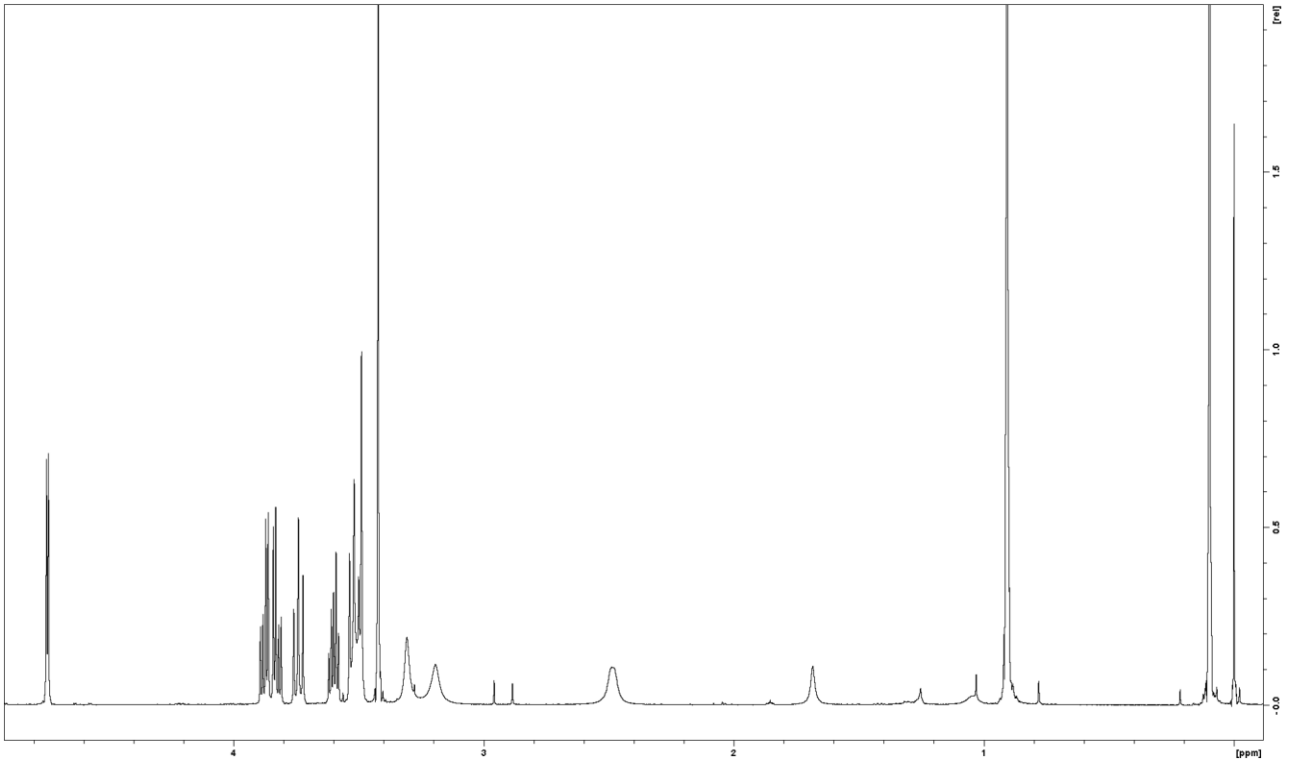
Supplementary figure 12. ^{1}H NMR spectrum of **1** (500.13 MHz, 23 °C, CD_3OD).



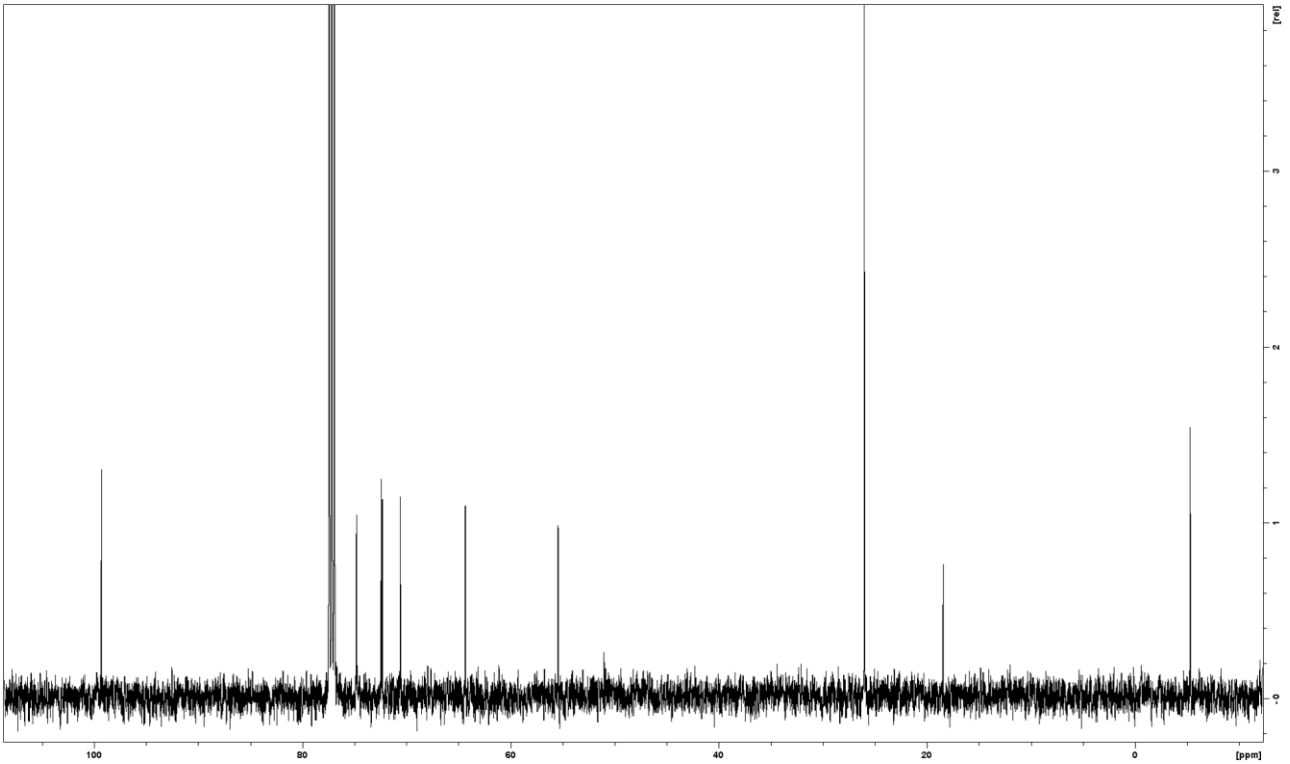
Supplementary figure 13. ^{13}C NMR spectrum of **1** (125.76 MHz, 23 °C, CD_3OD).



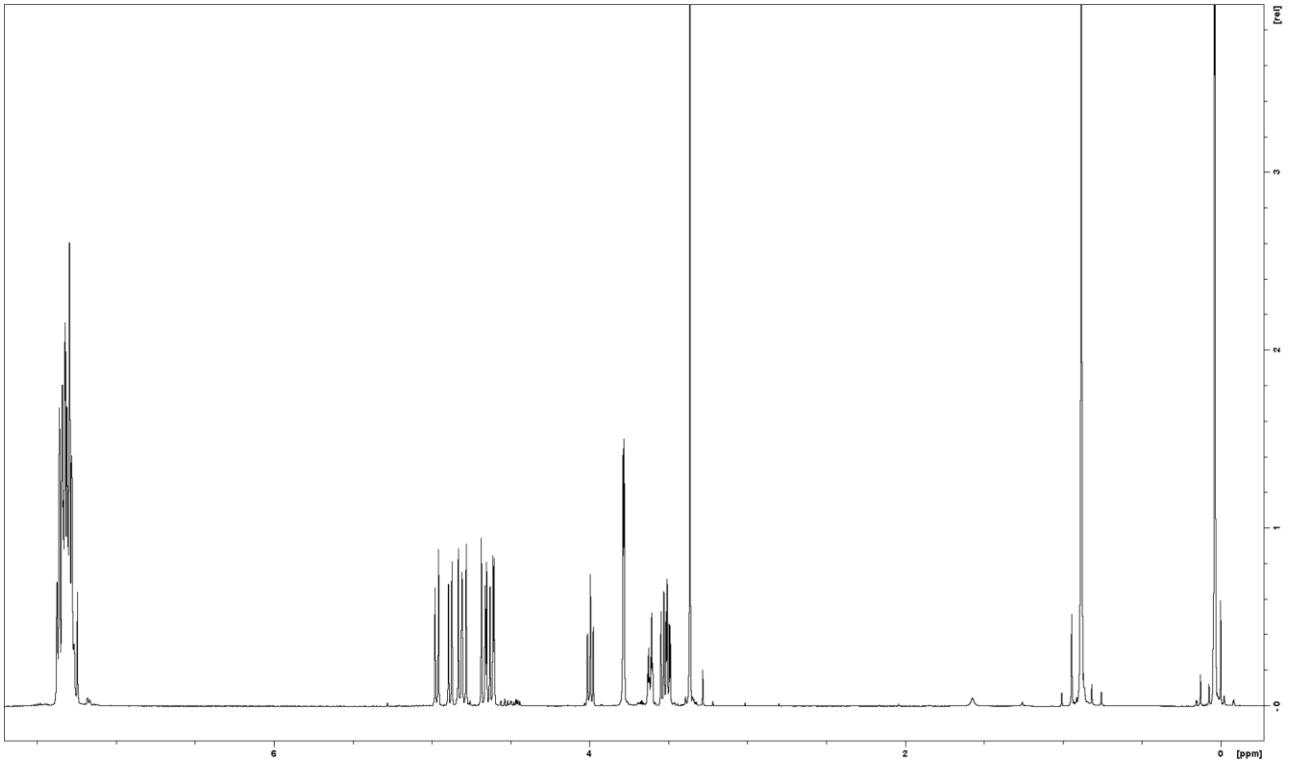
Supplementary figure 14. ^{11}B NMR spectrum of **1** (160.46 MHz, 23 °C, CD_3OD).



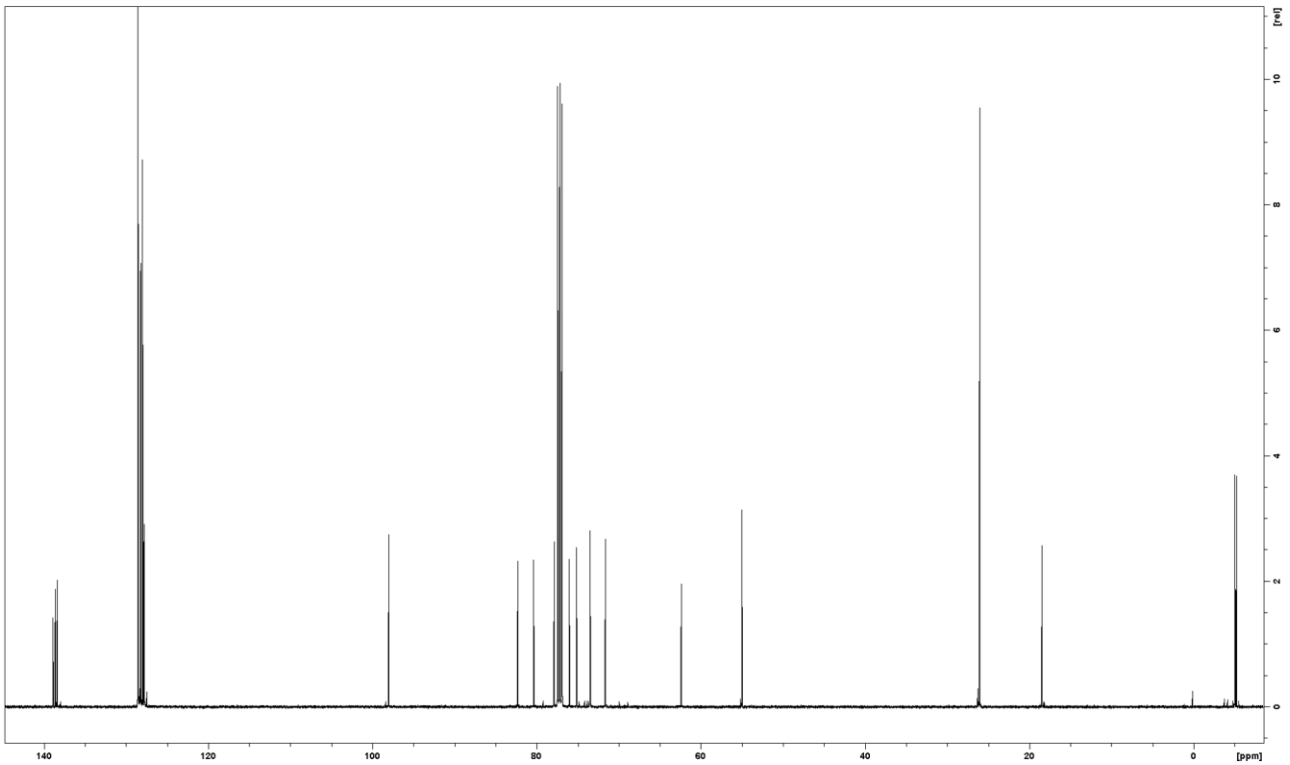
Supplementary figure 15. ^{1}H NMR spectrum of Methyl 6-*O*-tertbutyldimethylsilyl-α-D-glucopyranoside (500.13 MHz, 23 °C, $CDCl_3$).



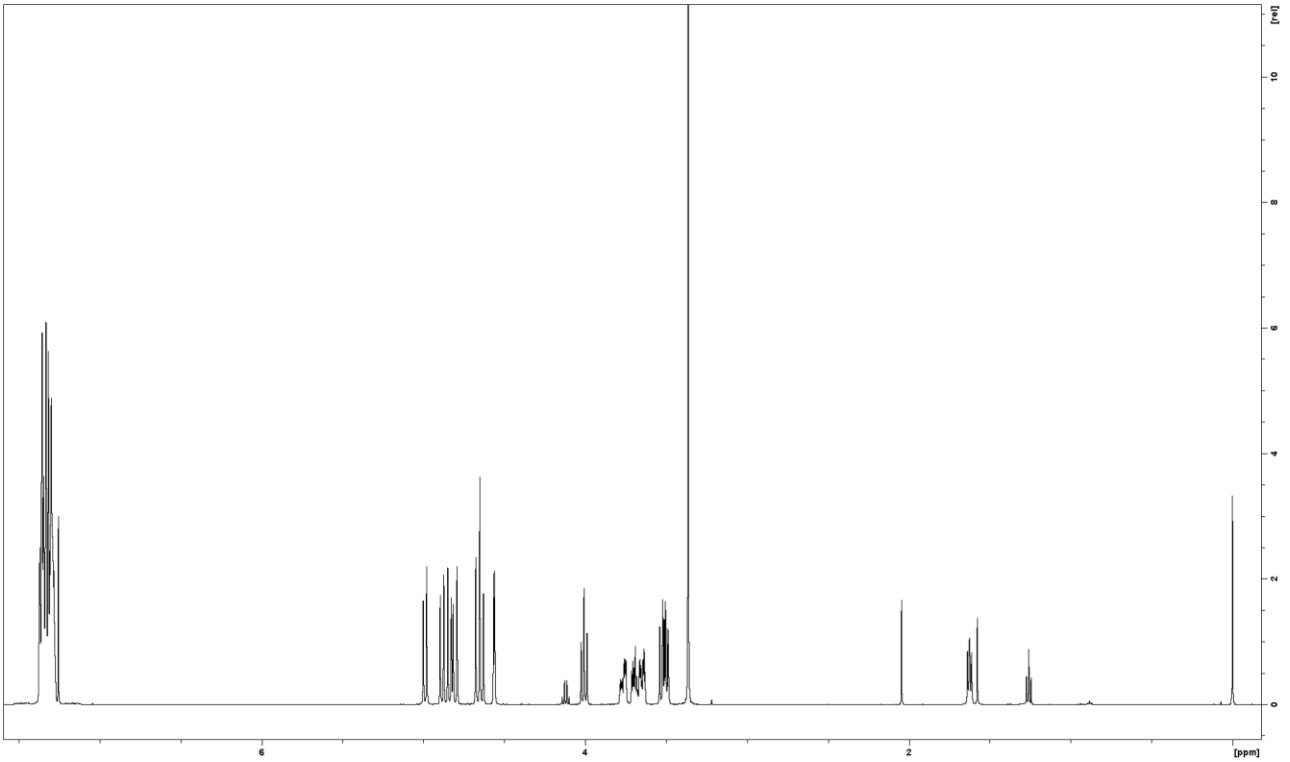
Supplementary figure 16. ^{13}C NMR spectrum of Methyl 6-*O*-tertbutyldimethylsilyl-α-D-glucopyranoside (125.76 MHz, 23 °C, $CDCl_3$).



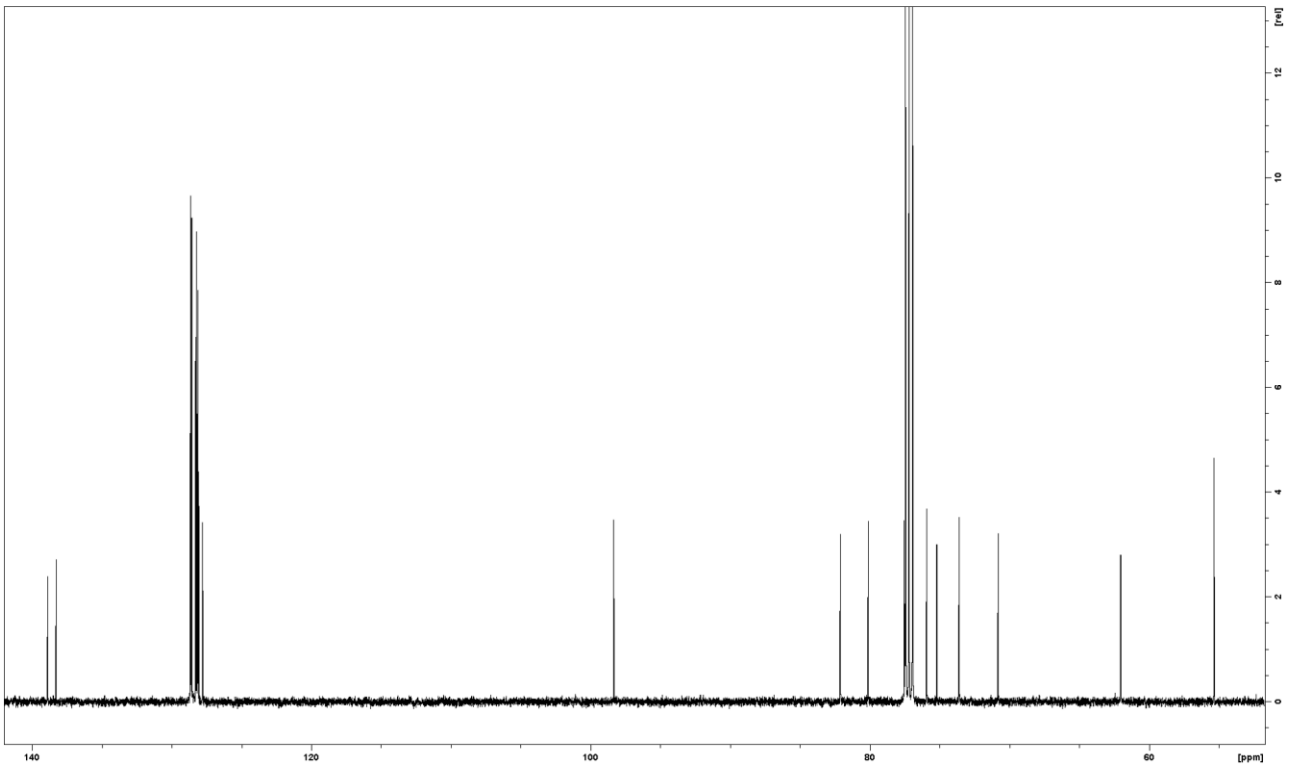
Supplementary figure 17. ^{1}H NMR spectrum of **7** at 23 °C (500.13 MHz, 23 °C, $CDCl_3$).



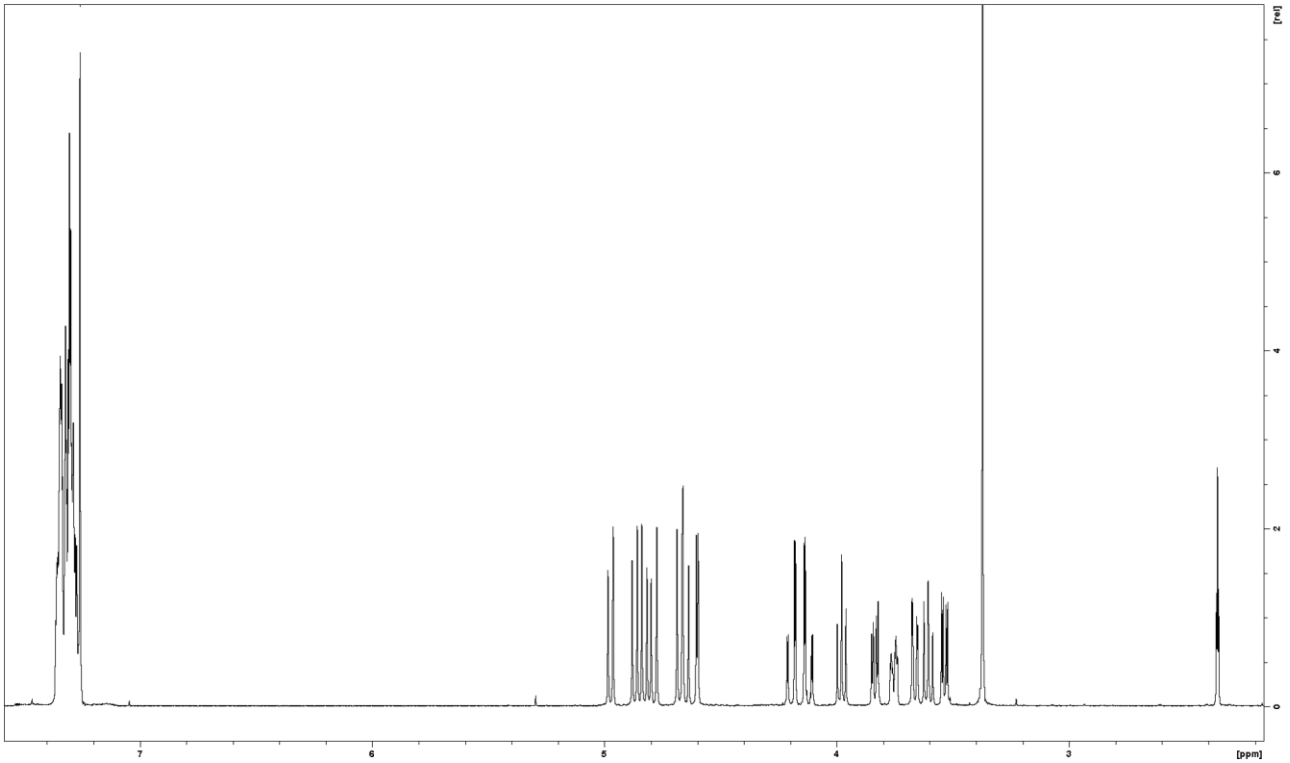
Supplementary figure 18. ^{13}C NMR spectrum of **7** (125.76 MHz, 23 °C, $CDCl_3$).



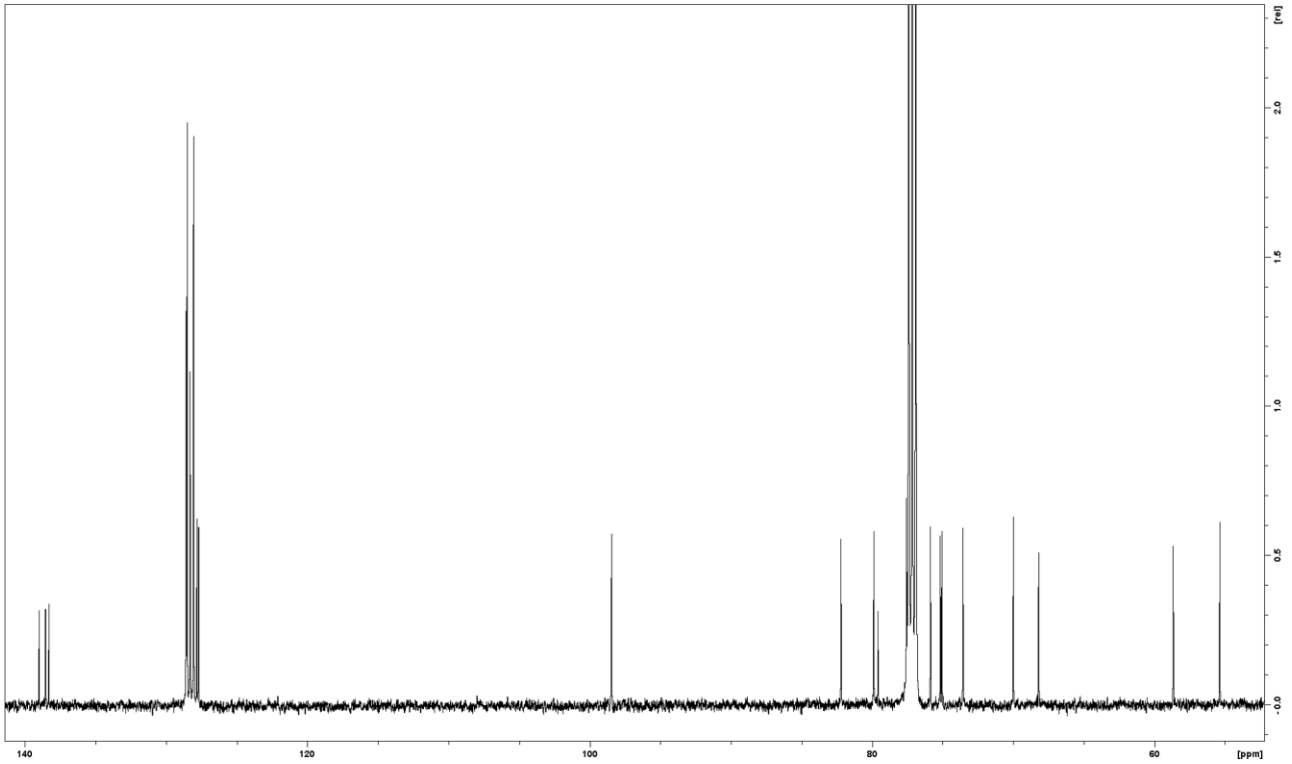
Supplementary figure 19. ^{1}H NMR spectrum of Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (500.13 MHz, 23 °C, $CDCl_3$).



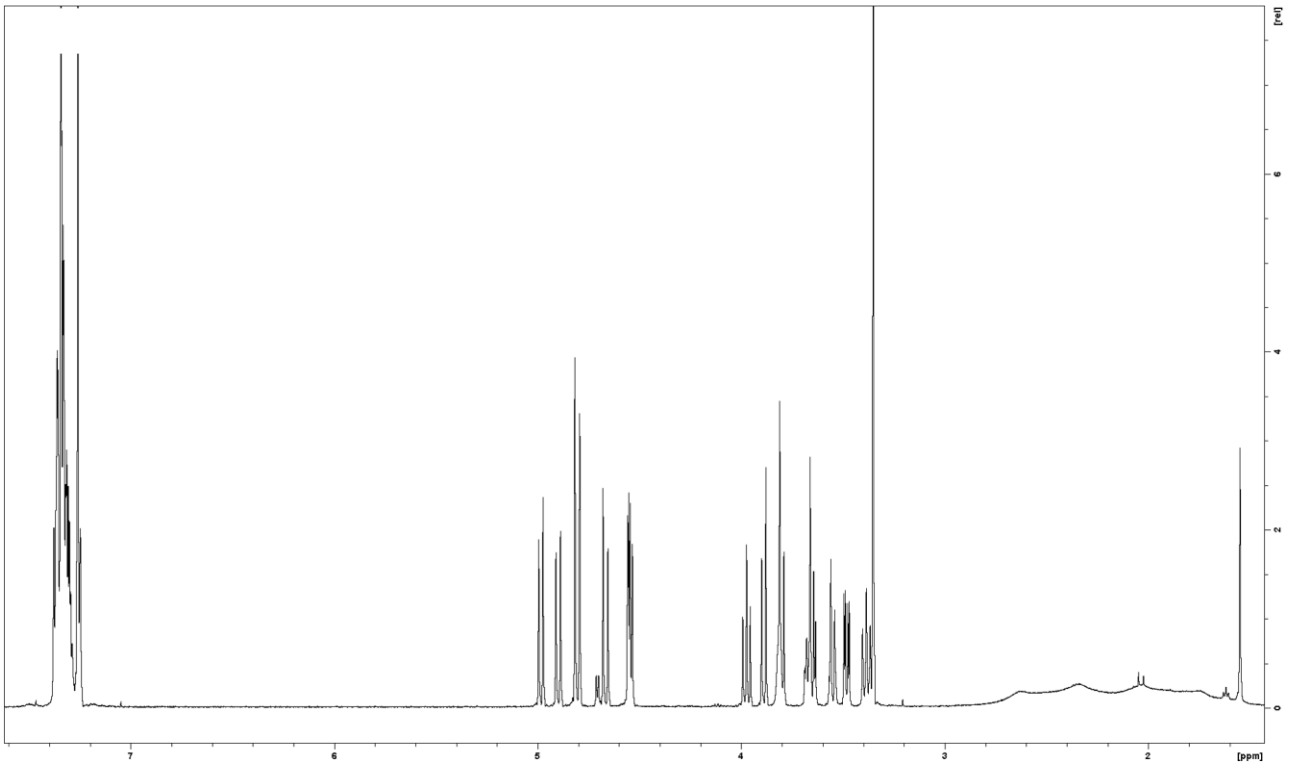
Supplementary figure 20. ^{13}C NMR spectrum of Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (125.76 MHz, 23 °C, $CDCl_3$).



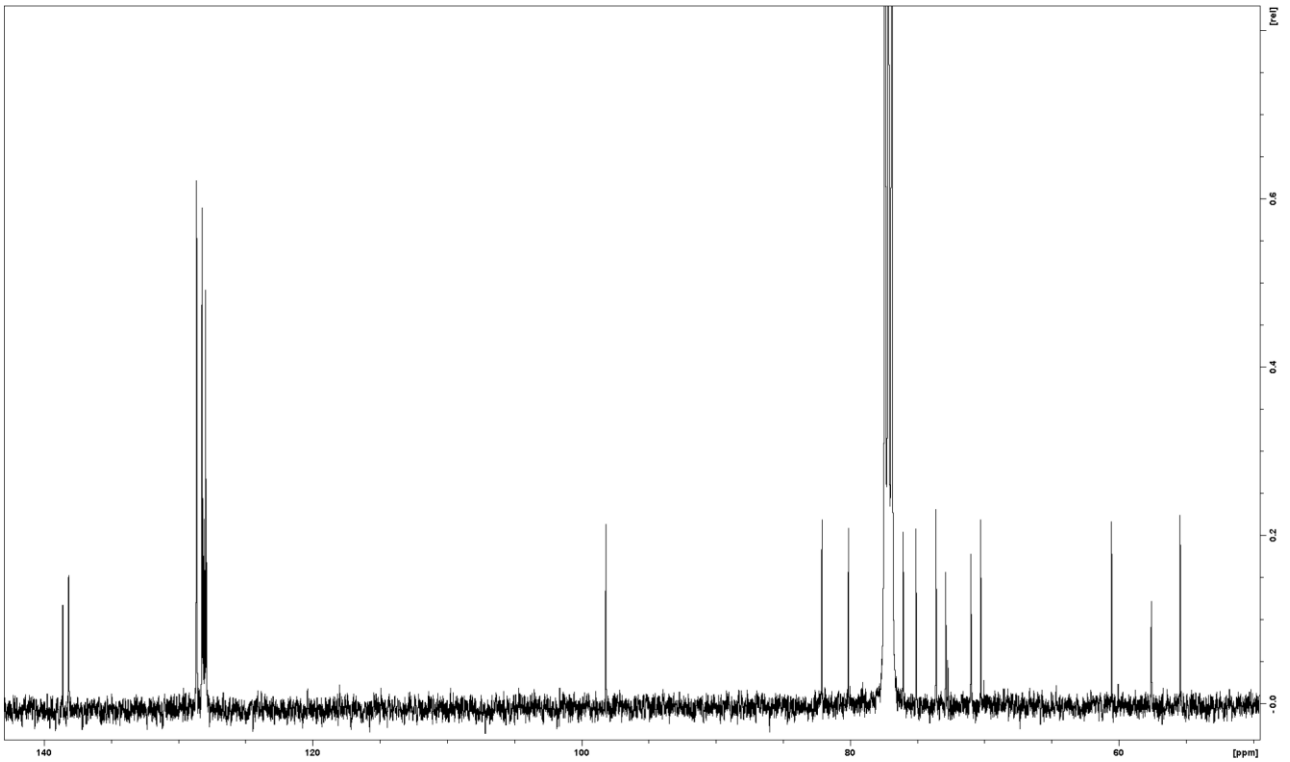
Supplementary figure 21. ^{1}H NMR spectrum of **8** (500.13 MHz, 23 °C, $CDCl_3$).



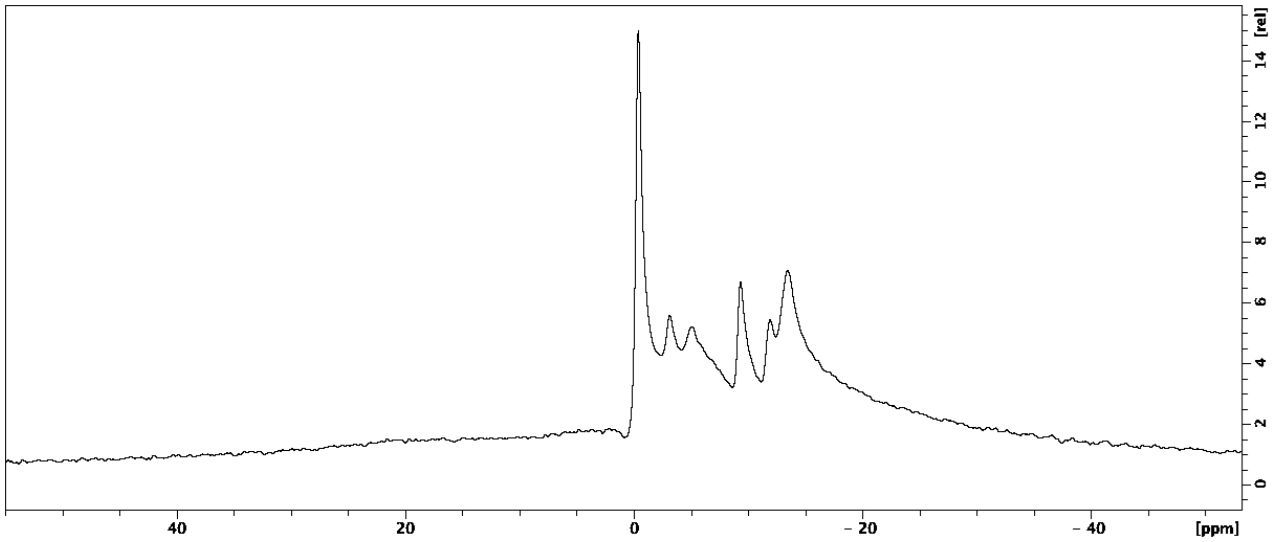
Supplementary figure 22. ^{13}C NMR spectrum of **8** (125.76 MHz, 23 °C, $CDCl_3$).



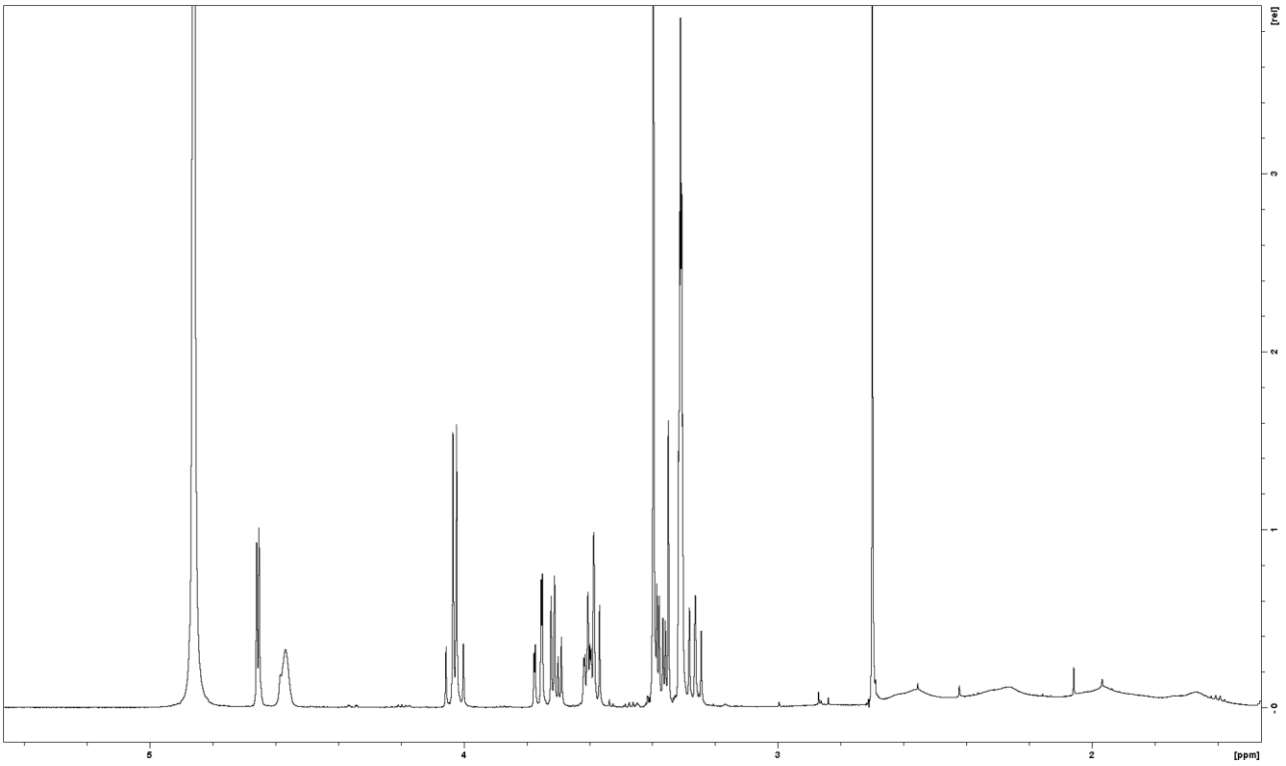
Supplementary figure 23. ^{1}H NMR spectrum **9** (500.13 MHz, 23 °C, $CDCl_3$).



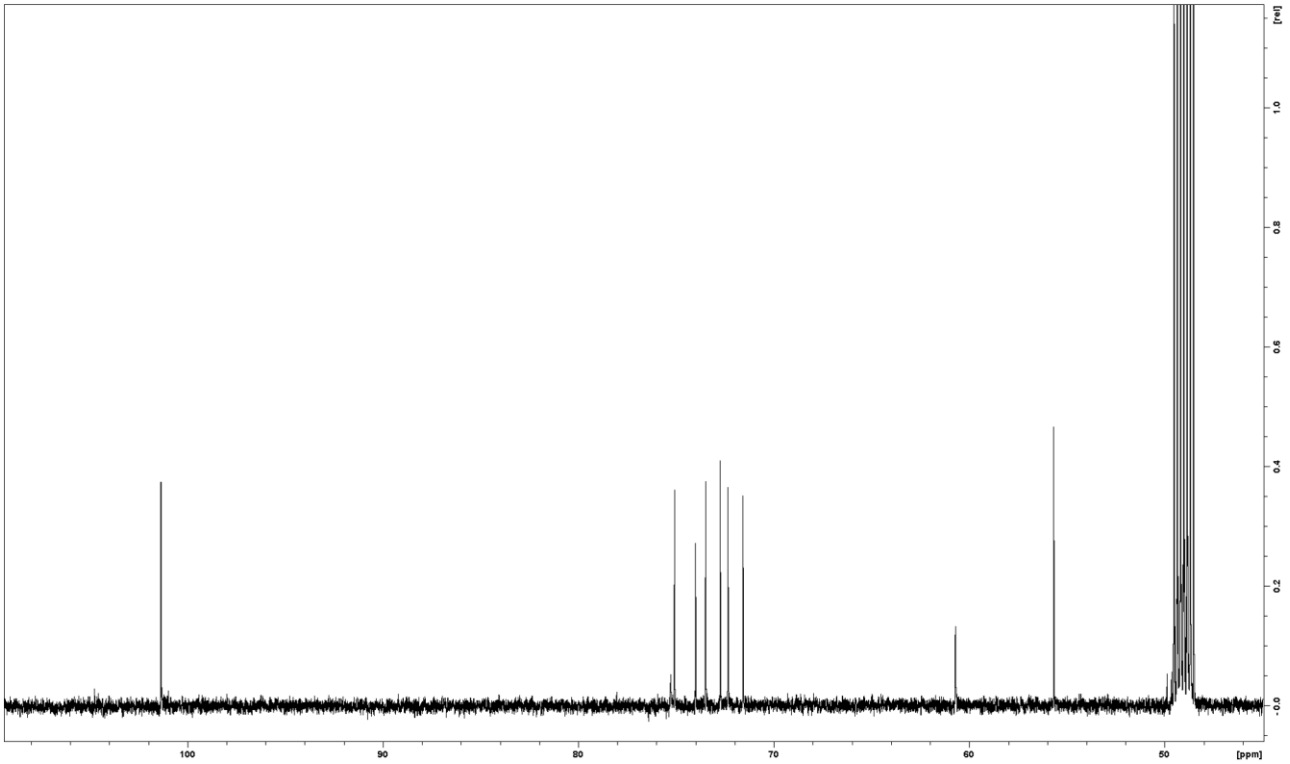
Supplementary figure 24. ^{13}C NMR spectrum of **9** (125.76 MHz, 23 °C, $CDCl_3$).



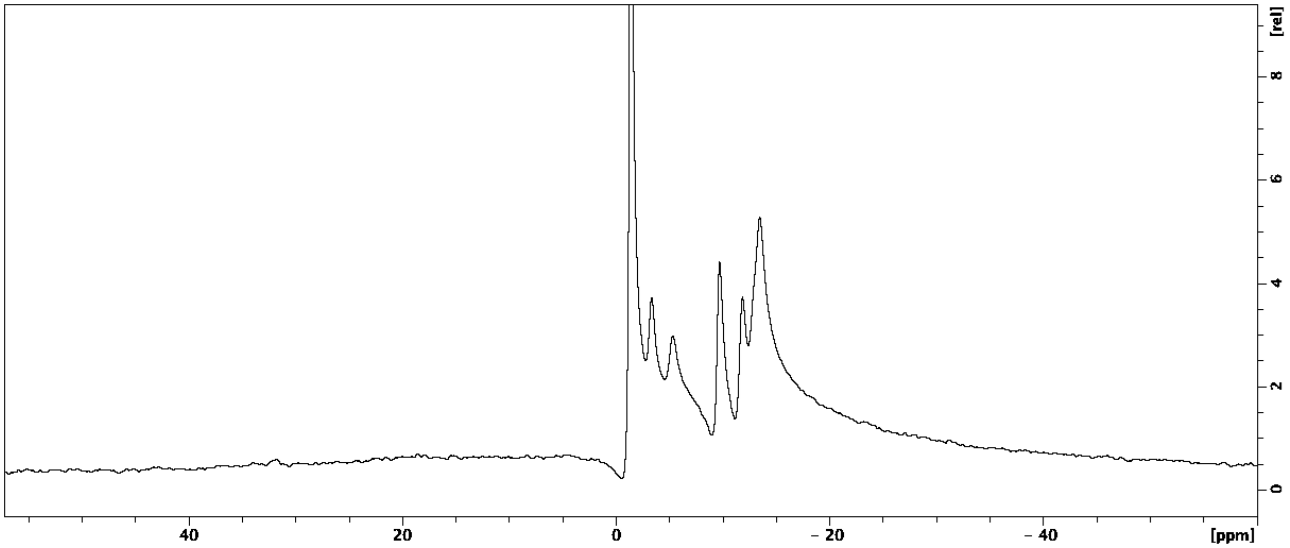
Supplementary figure 25. ^{11}B NMR spectrum of **9** (160.46 MHz, 23 °C, $CDCl_3$).



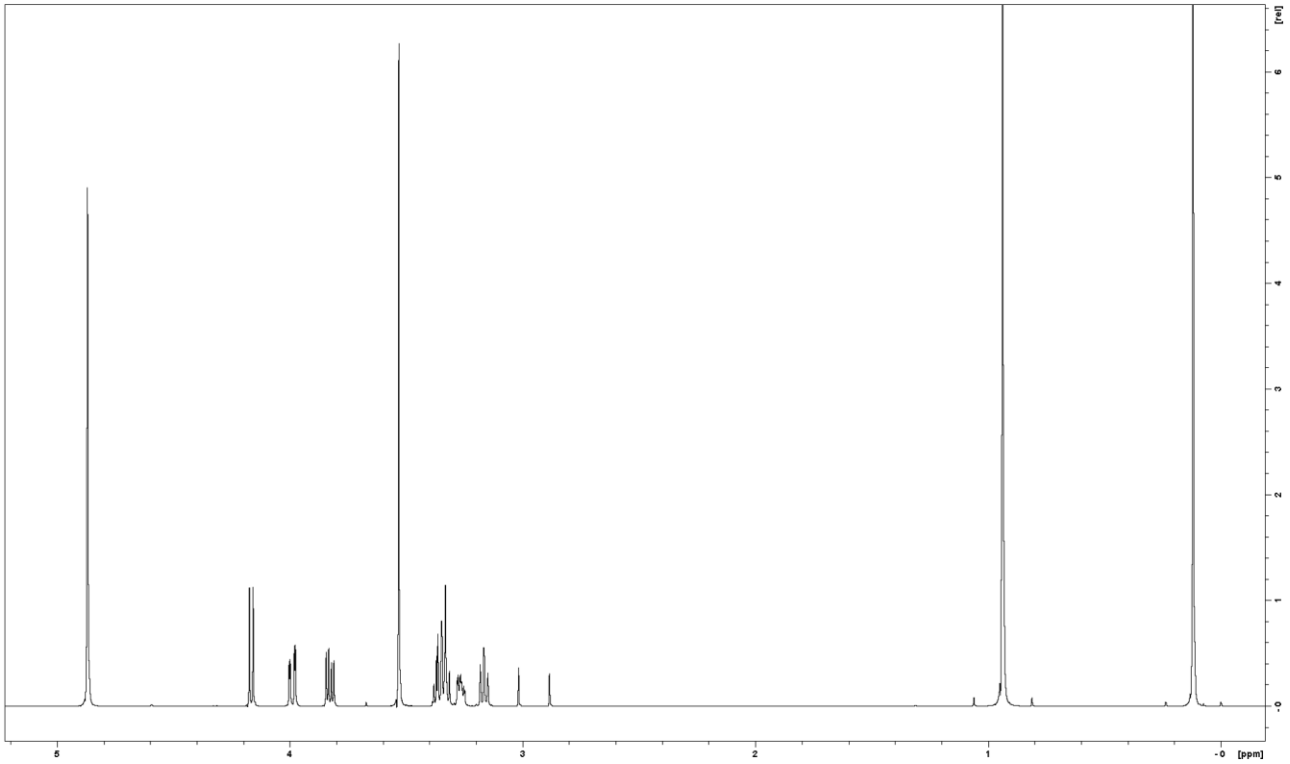
Supplementary figure 26. ^{1}H NMR spectrum of **2** (500.13 MHz, 23 °C, CD_3OD).



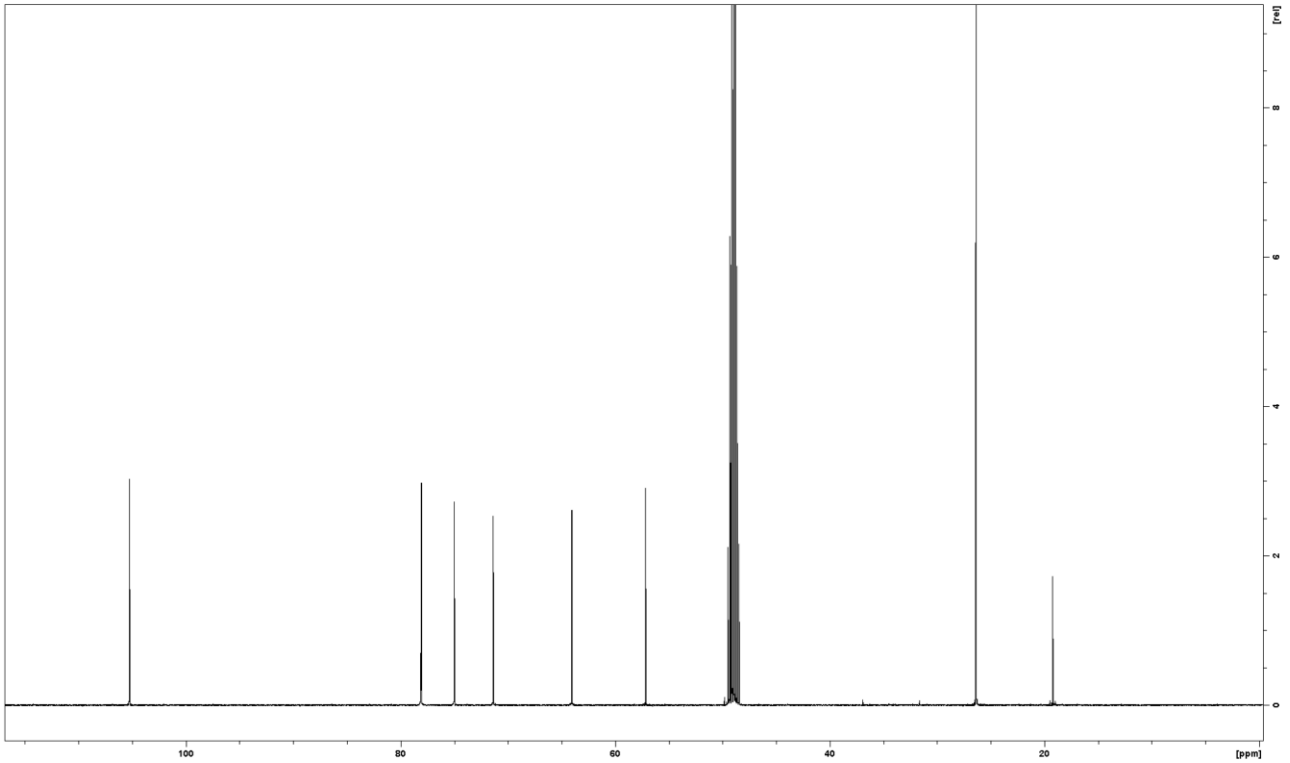
Supplementary figure 27. ^{13}C NMR spectrum **2** (125.76 MHz, 23 °C, CD_3OD).



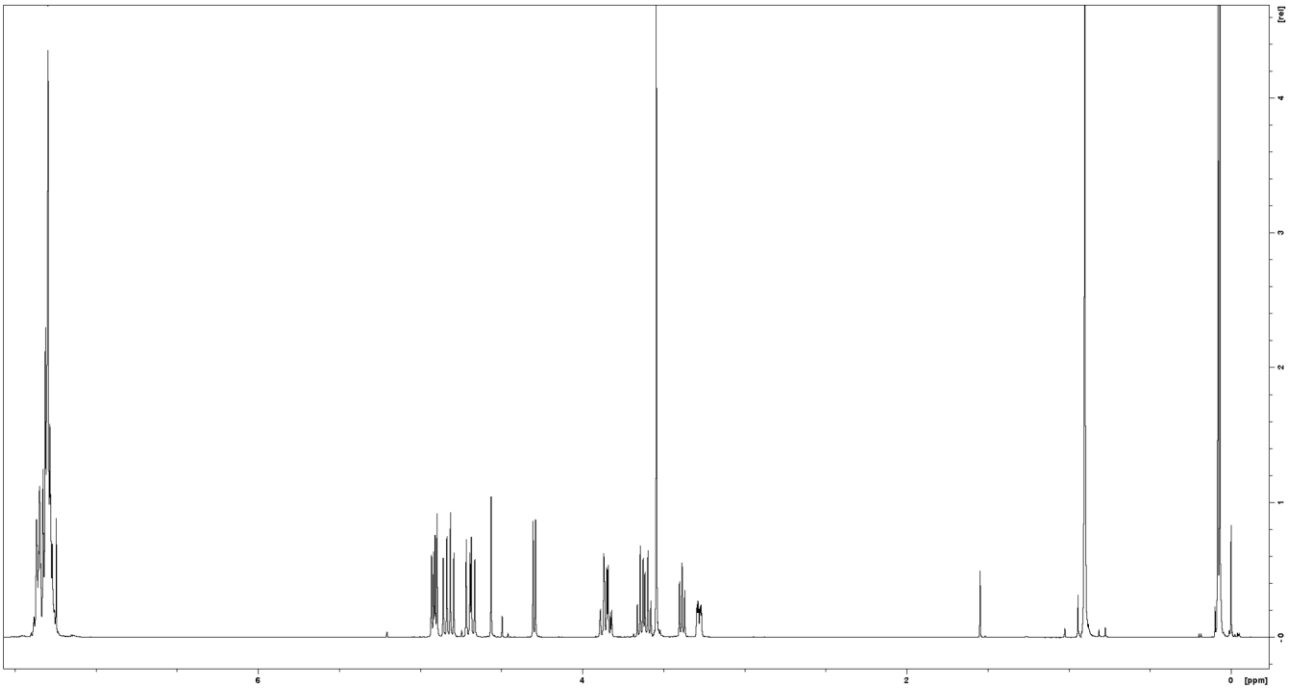
Supplementary figure 28. ^{11}B NMR spectrum of **2** (160.46 MHz, 23 °C, CD_3OD).



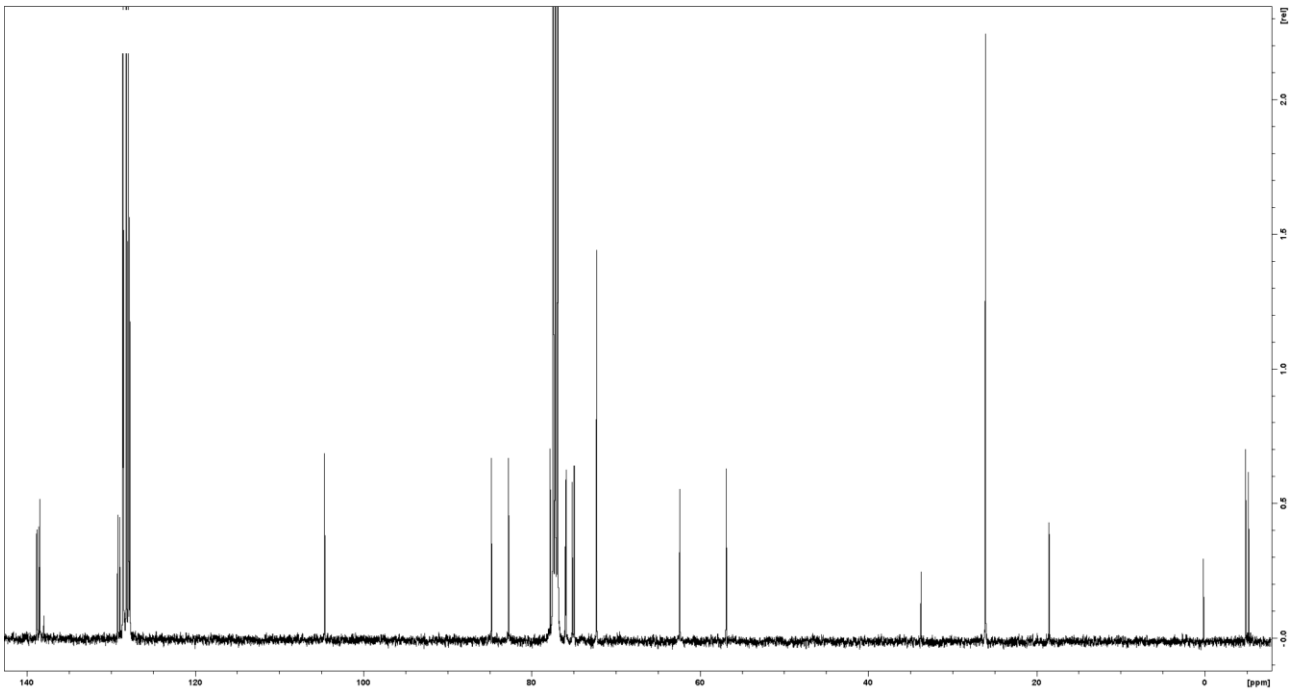
Supplementary figure 29. ^{1}H NMR spectrum of Methyl 6-*O*-tertbutyldimethylsilyl-β-D-glucopyranoside (500.13 MHz, 23 °C, CD_3OD).



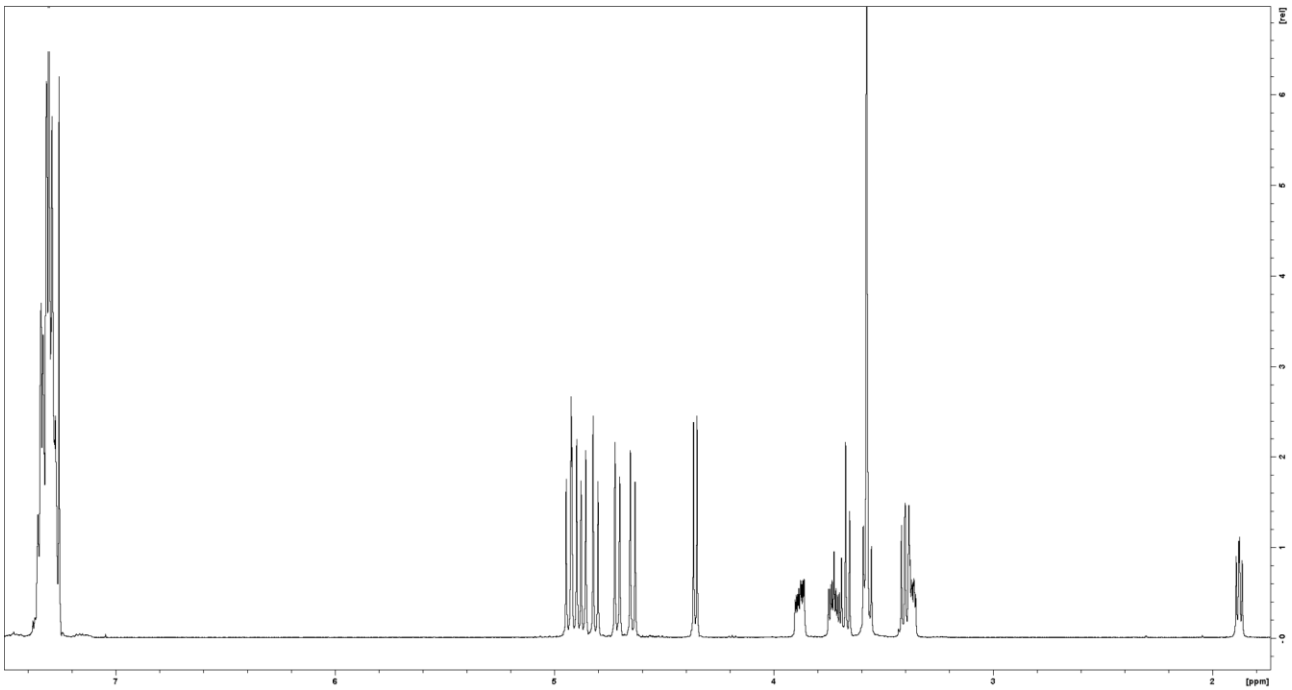
Supplementary figure 30. ^{13}C NMR spectrum of Methyl 6-*O*-tertbutyldimethylsilyl-β-D-glucopyranoside (125.76 MHz, 23 °C, CD_3OD).



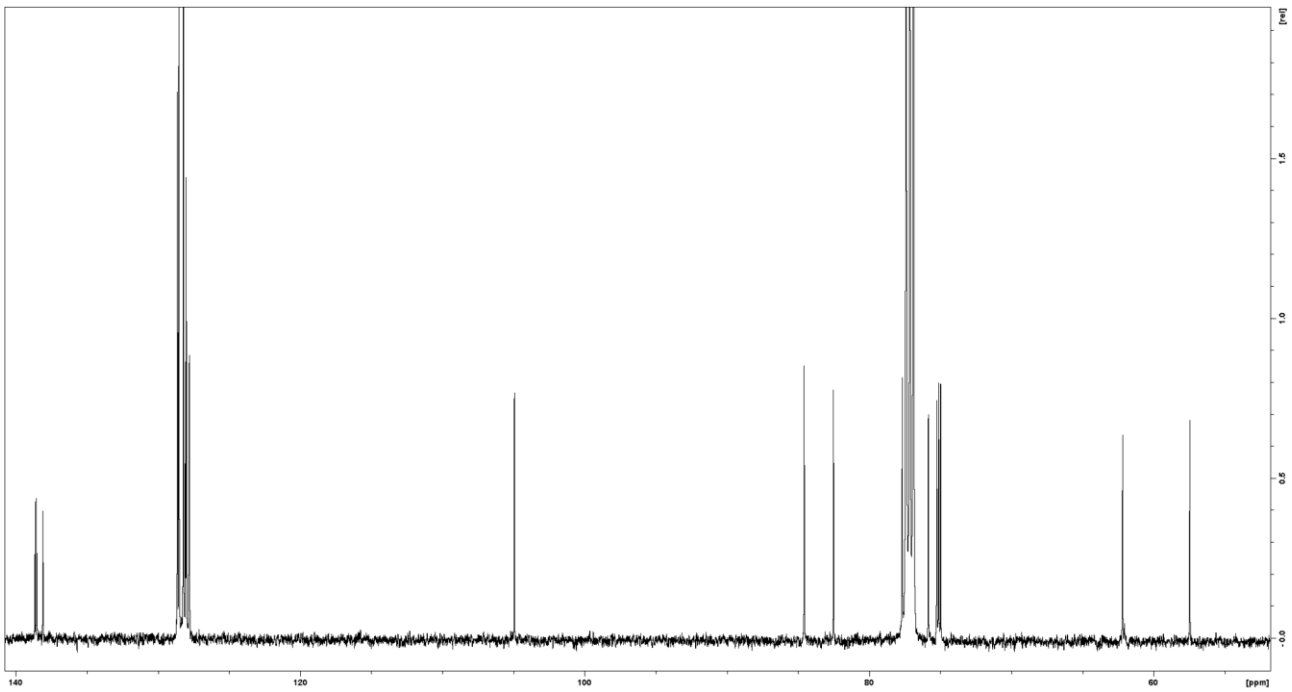
Supplementary figure 31. ^{1}H NMR spectrum of **10** (500.13 MHz, 23 °C, $CDCl_3$).



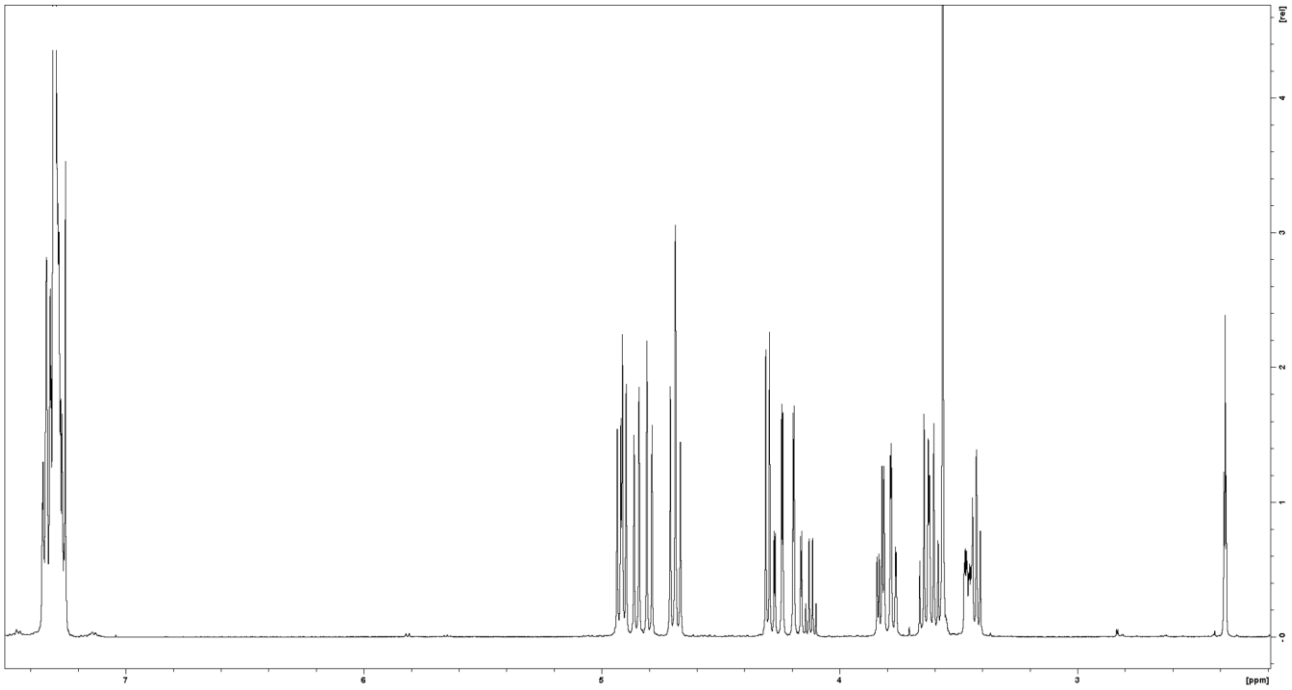
Supplementary figure 32. ^{13}C NMR spectrum of **10** (125.76 MHz, 23 °C, $CDCl_3$).



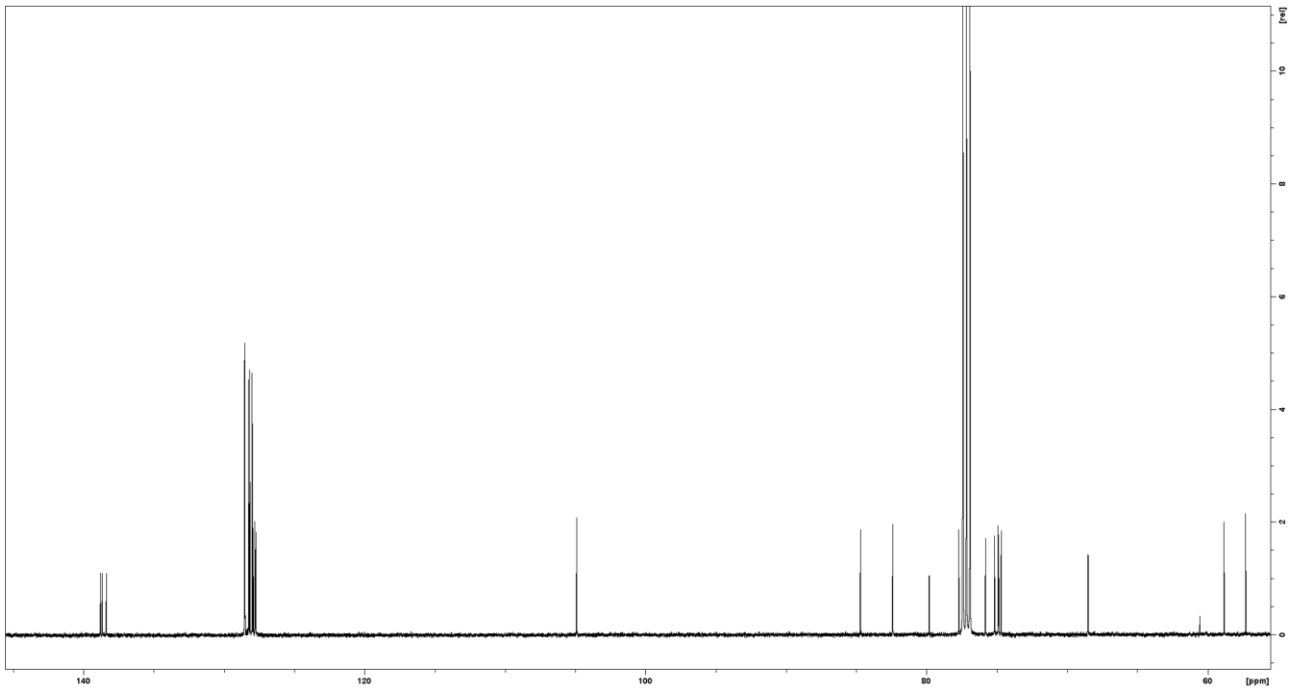
Supplementary figure 33. ^{1}H NMR spectrum of Methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (500.13 MHz, 23 °C, $CDCl_3$).



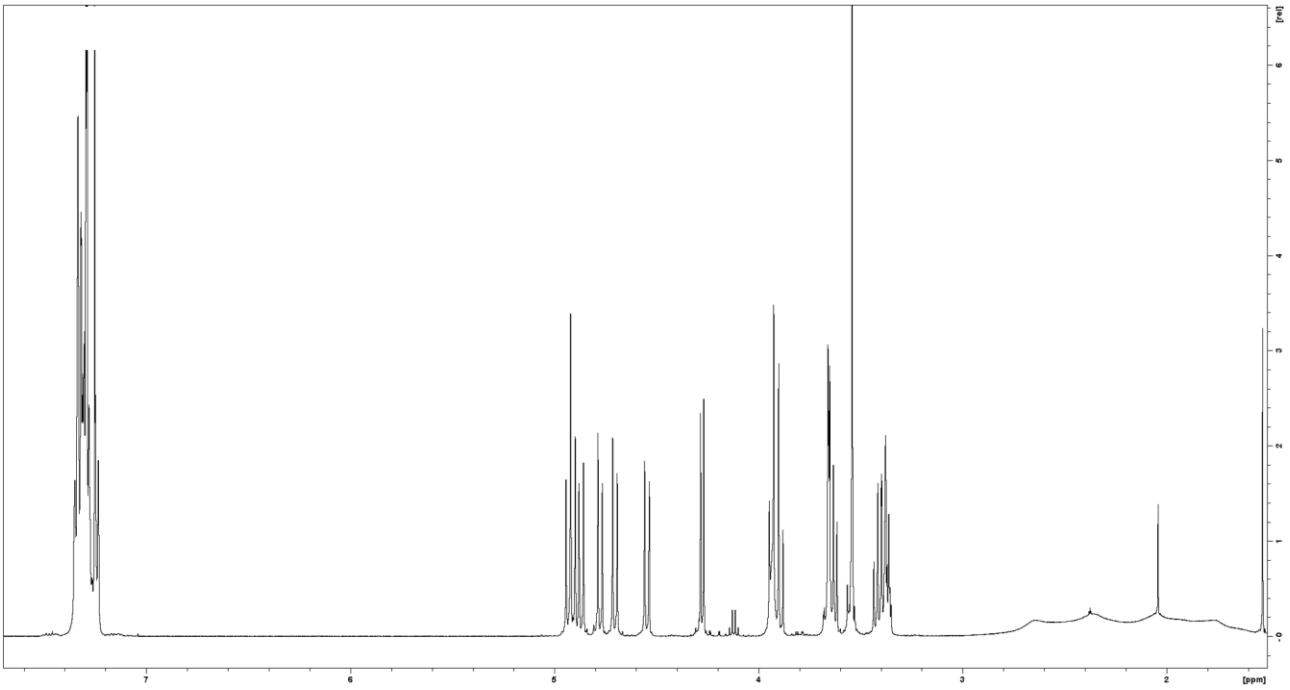
Supplementary figure 34. ^{13}C NMR spectrum of Methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (125.76 MHz, 23 °C, $CDCl_3$).



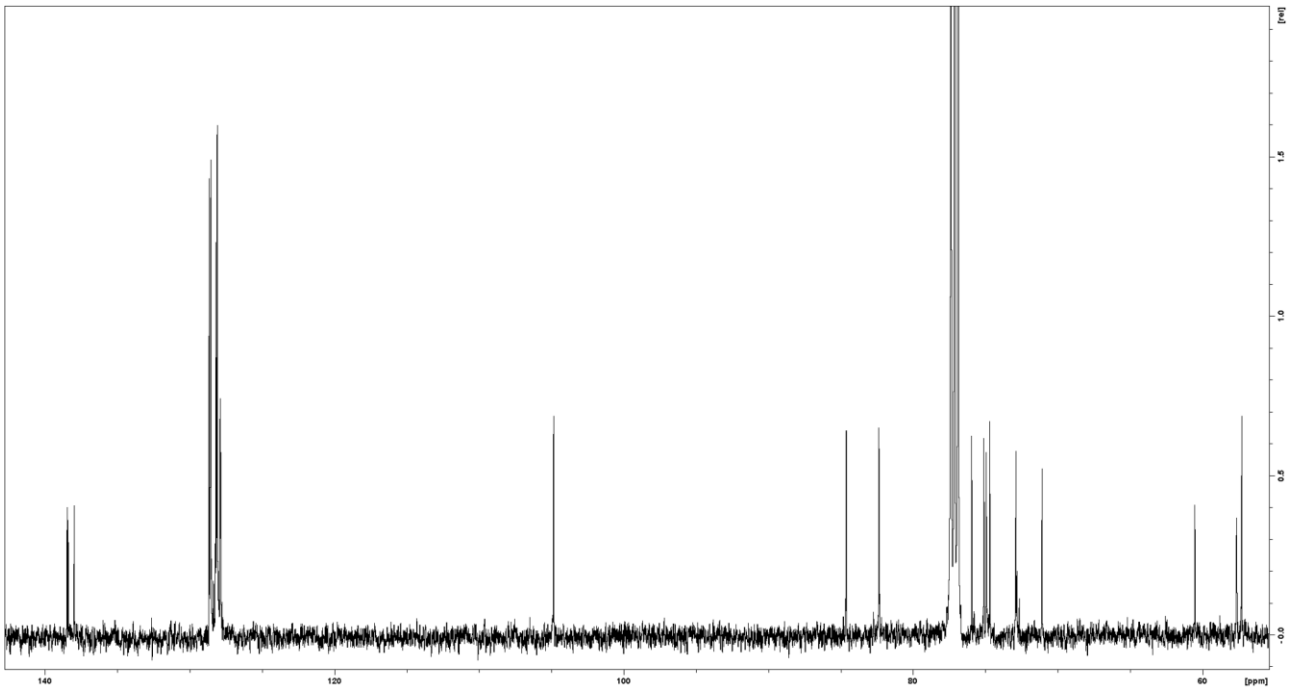
Supplementary figure 35. ^{1}H NMR spectrum of **11** (500.13 MHz, 23 °C, $CDCl_3$).



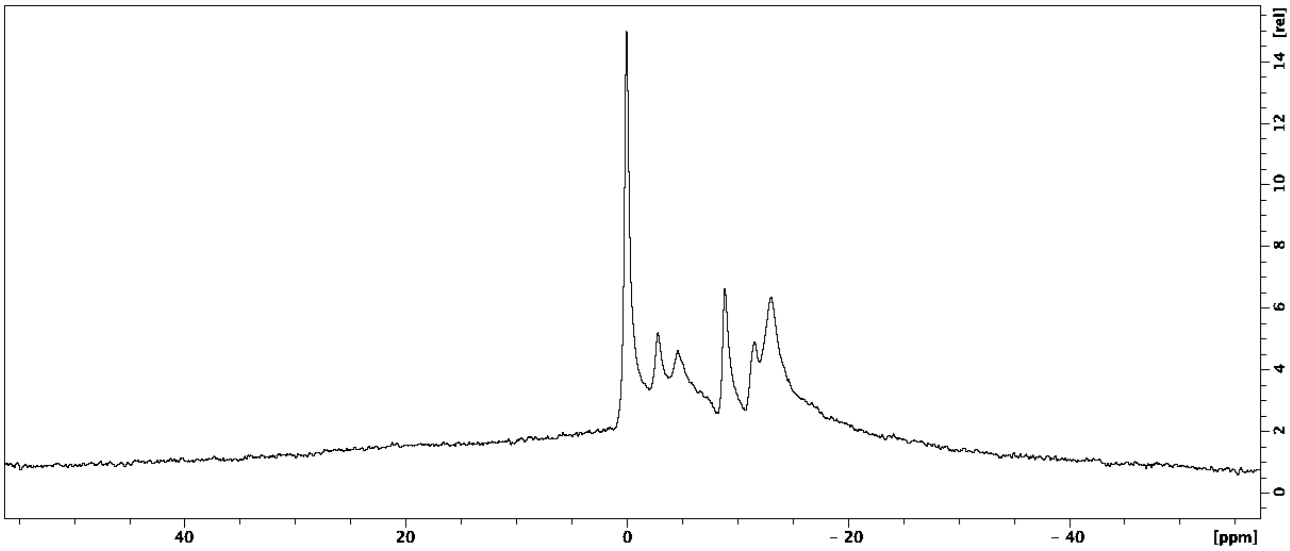
Supplementary figure 36. ^{13}C NMR spectrum of **11** (125.76 MHz, 23 °C, $CDCl_3$).



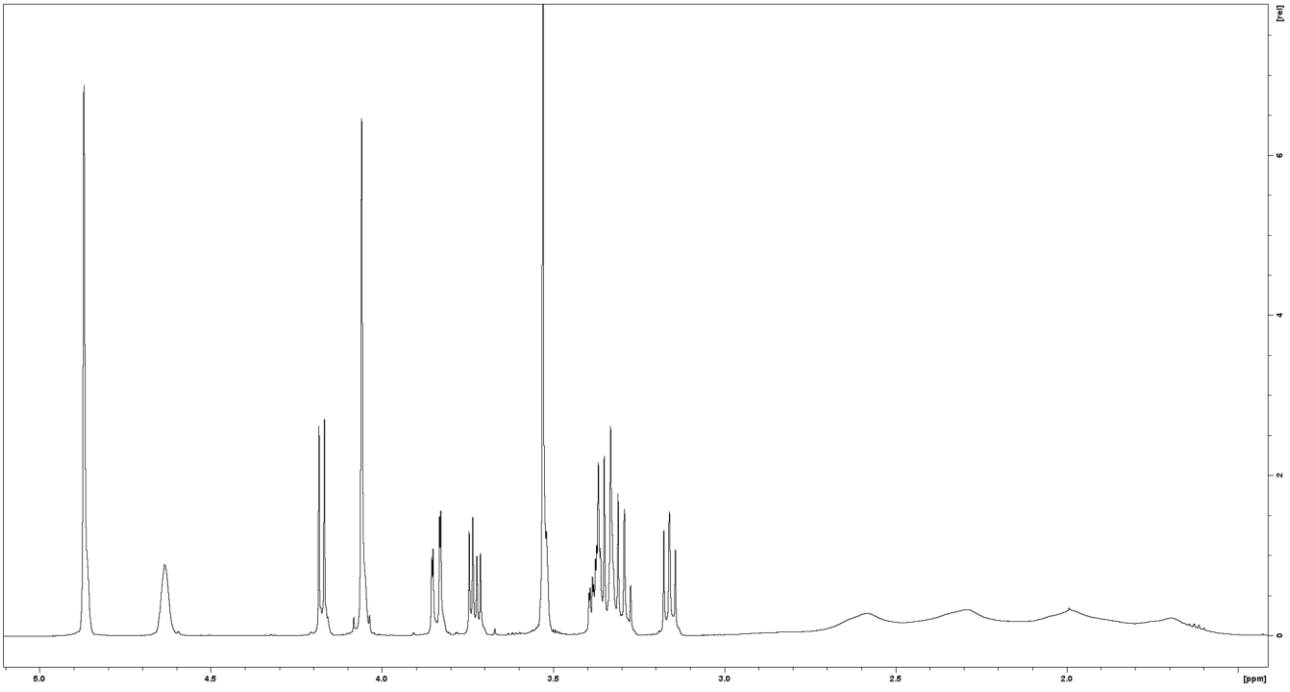
Supplementary figure 37. ^{1}H NMR spectrum of **12** (500.13 MHz, 23 °C, $CDCl_3$).



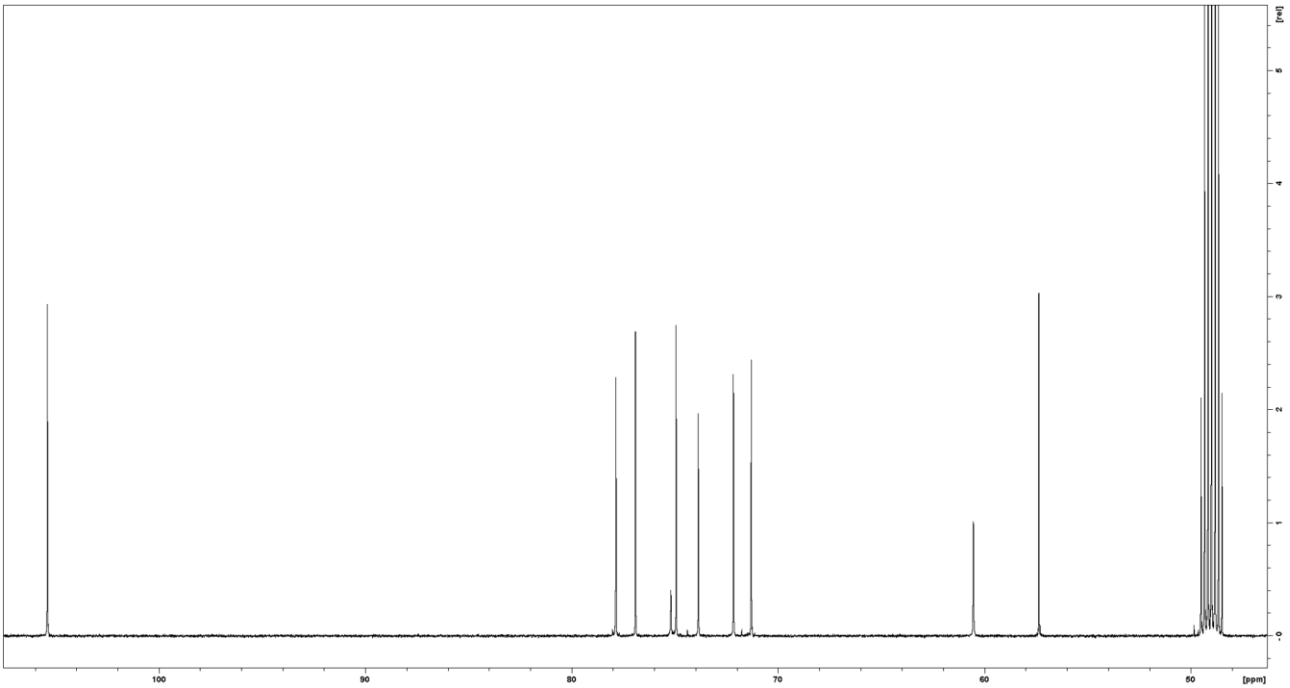
Supplementary figure 38. ^{13}C NMR spectrum of **12** (125.76 MHz, 23 °C, $CDCl_3$).



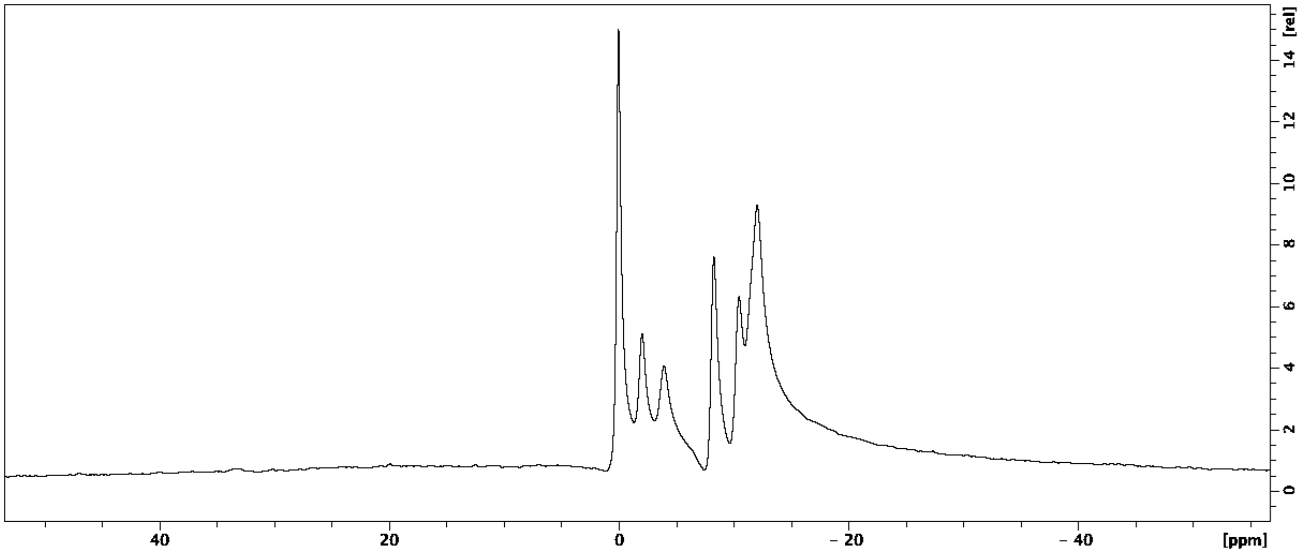
Supplementary figure 39. ^{11}B NMR spectrum of **12** (160.46 MHz, 23 °C, $CDCl_3$).



Supplementary figure 40. ^{1}H NMR spectrum of **3** (500.13 MHz, 23 °C, CD_3OD).



Supplementary figure 41. ^{13}C NMR spectrum **3** (125.76 MHz, 23 °C, $CDCl_3$).



Supplementary figure 42. ^{11}B NMR spectrum of **3** (160.46 MHz, 23 °C, CD_3OD).

3. Docking results and Ligand PDBQT files

Supplementary table 1 shows the ligand binding energies for representative binding poses to the glucose transporter in both the outward and inward-open conformations, as computed using the Autodock protocol described above. For the outward-open conformation, based on PDB ID 6N3I, "pose A" represents the estimated natural binding site of β-glucose, while "pose B" represents the other major binding site. For the inward-open conformation, based on PDB ID 4QIQ, "pose 1" represents the major binding mode, while "pose 2" represents the second-best binding mode, based on mean binding energy.

The docking studies indicate that all glucoconjugates bind significantly stronger to the transporter protein than pristine glucose. Further, upon switching from the outward-open to the inward-open conformation, the binding energy is estimated to decrease notably for the glucoconjugates; the anomers of glucose show practically no change in binding energy.

Supplementary table 1. Calculated ligand binding energies of the studied species (kcal/mol) to the outward and inward-open conformations of the glucose transporter protein.

outward-open (6N3I)	**mean binding energy (lowest binding energy) / % of docked ligands**	
	binding pose A	binding pose B
3	-6.2 (-7.1) / 31%	-6.1 (-7.3) / 47%
1β	-5.6 (-6.6) / 30%	-5.6 (-6.9) / 58%
2	-5.6 (-6.6) / 15%	-5.5 (-6.7) / 72%
1α	-5.6 (-6.5) / 36%	-5.5 (-6.7) / 54%
β-Glc	-0.9 (-1.5) / 55%	-0.7 (-1.4) / 15%
α-Glc	-0.2 (-1.0) / 10%	-0.3 (-0.7) / 80%

inward-open (4QIQ)	**mean binding energy (lowest binding energy) / % of docked ligands**	
	binding pose 1	binding pose 2
3	-4.7 (-5.6) / 33%	-4.2 (-5.3) / 11%
1β	-4.3 (-5.1) / 56%	-3.8 (-4.6) / 13%
2	-4.2 (-5.2) / 43%	-4.1 (-5.0) / 14%
1α	-3.9 (-4.7) / 69%	-3.6 (-4.6) / 9%
β-Glc	-0.9 (-1.4) / 69%	-0.8 (-1.2) / 14%
α-Glc	-0.3 (-0.9) / 55%	-0.2 (-0.7) / 4%

Ligand PDBQT files

The Autodock PDBQT ligand files, defining, *e.g.*, rotatable bonds and atomic partial charges are listed below for the studied ligands (α/β-glucose, and **1**-**3**).

α-glucose:

```
REMARK  6 active torsions:
REMARK  status: ('A' for Active; 'I' for Inactive)
REMARK    1  A    between atoms: O_12  and  C_1
REMARK    2  A    between atoms: O_11  and  C_2
REMARK    3  A    between atoms: C_3  and  O_4
REMARK    4  A    between atoms: O_10  and  C_5
REMARK    5  A    between atoms: C_6  and  C_7
REMARK    6  A    between atoms: O_8  and  C_7
ROOT
ATOM      1  C   LIG d   1       1.645   1.651   0.607  0.00  0.00     0.636 C
ATOM      2  C   LIG d   1       1.877   0.436   1.535  0.00  0.00     0.215 C
ATOM      3  C   LIG d   1       0.838  -0.665   1.233  0.00  0.00     0.471 C
ATOM      4  C   LIG d   1      -0.581  -0.071   1.371  0.00  0.00     0.175 C
ATOM      5  C   LIG d   1      -0.736   1.188   0.483  0.00  0.00     0.295 C
ATOM      6  O   LIG d   1       0.307   2.148   0.776  0.00  0.00    -0.690 OA
ENDROOT
BRANCH   1   7
```

ATOM 7 O LIG d 1 1.771 1.206 -0.728 0.00 0.00 -0.728 OA

ATOM 8 H LIG d 1 1.768 1.958 -1.358 0.00 0.00 0.449 HD

ENDBRANCH 1 7

BRANCH 2 9

ATOM 9 O LIG d 1 3.212 -0.001 1.294 0.00 0.00 -0.714 OA

ATOM 10 H LIG d 1 3.311 -0.934 1.610 0.00 0.00 0.432 HD

ENDBRANCH 2 9

BRANCH 5 11

ATOM 11 C LIG d 1 -2.034 1.953 0.785 0.00 0.00 0.340 C

BRANCH 11 12

ATOM 12 O LIG d 1 -3.147 1.098 0.485 0.00 0.00 -0.651 OA

ATOM 13 H LIG d 1 -3.297 1.056 -0.485 0.00 0.00 0.377 HD

ENDBRANCH 11 12

ENDBRANCH 5 11

BRANCH 3 14

ATOM 14 O LIG d 1 1.064 -1.649 2.249 0.00 0.00 -0.732 OA

ATOM 15 H LIG d 1 0.405 -2.377 2.153 0.00 0.00 0.425 HD

ENDBRANCH 3 14

BRANCH 4 16

ATOM 16 O LIG d 1 -1.458 -1.091 0.882 0.00 0.00 -0.735 OA

ATOM 17 H LIG d 1 -2.398 -0.756 0.913 0.00 0.00 0.435 HD

ENDBRANCH 4 16

TORSDOF 6

β-glucose:

REMARK 6 active torsions:

REMARK status: ('A' for Active; 'I' for Inactive)

REMARK 1 A between atoms: C_1 and O_12

REMARK 2 A between atoms: C_3 and C_10

REMARK 3 A between atoms: O_5 and C_4

REMARK 4 A between atoms: C_6 and O_9

REMARK 5 A between atoms: O_8 and C_7

REMARK 6 A between atoms: O_11 and C_10

```
ROOT
ATOM      1  C   LIG d   1       0.680   1.367  -0.762  0.00  0.00     0.588 C
ATOM      2  O   LIG d   1      -0.441   1.035   0.078  0.00  0.00    -0.579 OA
ATOM      3  C   LIG d   1      -0.783  -0.370   0.145  0.00  0.00     0.238 C
ATOM      4  C   LIG d   1       0.410  -1.127   0.768  0.00  0.00     0.116 C
ATOM      5  C   LIG d   1       1.699  -0.887  -0.051  0.00  0.00     0.459 C
ATOM      6  C   LIG d   1       1.956   0.612  -0.299  0.00  0.00     0.125 C
ENDROOT
BRANCH   1   7
ATOM      7  O   LIG d   1       0.834   2.752  -0.556  0.00  0.00    -0.674 OA
ATOM      8  H   LIG d   1       0.563   3.003   0.362  0.00  0.00     0.411 HD
ENDBRANCH   1   7
BRANCH   6   9
ATOM      9  O   LIG d   1       2.896   0.619  -1.381  0.00  0.00    -0.672 OA
ATOM     10  H   LIG d   1       3.212   1.537  -1.548  0.00  0.00     0.416 HD
ENDBRANCH   6   9
BRANCH   5  11
ATOM     11  O   LIG d   1       2.734  -1.416   0.790  0.00  0.00    -0.704 OA
ATOM     12  H   LIG d   1       3.605  -1.336   0.334  0.00  0.00     0.414 HD
ENDBRANCH   5  11
BRANCH   3  13
ATOM     13  C   LIG d   1      -2.035  -0.405   1.036  0.00  0.00     0.399 C
BRANCH  13  14
ATOM     14  O   LIG d   1      -3.198  -0.117   0.262  0.00  0.00    -0.704 OA
ATOM     15  H   LIG d   1      -3.158   0.805  -0.077  0.00  0.00     0.400 HD
ENDBRANCH  13  14
ENDBRANCH   3  13
BRANCH   4  16
ATOM     16  O   LIG d   1       0.040  -2.507   0.684  0.00  0.00    -0.647 OA
ATOM     17  H   LIG d   1       0.788  -3.068   1.005  0.00  0.00     0.412 HD
ENDBRANCH   4  16
TORSDOF 6
```

1α:

REMARK 8 active torsions:

REMARK status: ('A' for Active; 'I' for Inactive)

REMARK 1 A between atoms: C_1 and O_12

REMARK 2 A between atoms: O_11 and C_2

REMARK 3 A between atoms: O_10 and C_3

REMARK 4 A between atoms: C_4 and O_9

REMARK 5 A between atoms: C_5 and C_6

REMARK 6 A between atoms: C_6 and O_8

REMARK 7 A between atoms: C_17 and O_8

REMARK 8 A between atoms: C_17 and C_18

REMARK I between atoms: C_18 and C_19

REMARK I between atoms: C_19 and B_27

REMARK I between atoms: C_19 and B_21

REMARK I between atoms: B_27 and B_21

REMARK I between atoms: B_23 and B_24

REMARK I between atoms: B_24 and B_29

REMARK I between atoms: B_24 and B_25

REMARK I between atoms: B_27 and B_25

REMARK I between atoms: B_25 and B_29

ROOT

ATOM 1 C LIG d 11 -0.225 6.248 -1.847 0.00 0.00 0.496 C

ATOM 2 C LIG d 11 -0.549 7.708 -2.142 0.00 0.00 0.282 C

ATOM 3 C LIG d 11 -0.997 8.393 -0.861 0.00 0.00 0.476 C

ATOM 4 C LIG d 11 -2.187 7.663 -0.259 0.00 0.00 0.104 C

ATOM 5 C LIG d 11 -1.819 6.189 -0.054 0.00 0.00 0.306 C

ATOM 6 O LIG d 11 -1.394 5.630 -1.305 0.00 0.00 -0.499 OA

ENDROOT

BRANCH 1 7

ATOM 7 O LIG d 11 0.859 6.188 -0.969 0.00 0.00 -0.720 OA

ATOM 8 H LIG d 11 1.132 5.266 -0.885 0.00 0.00 0.483 HD

ENDBRANCH 1 7

BRANCH 2 9

ATOM 9 O LIG d 11 0.574 8.345 -2.715 0.00 0.00 -0.714 OA

ATOM 10 H LIG d 11 0.398 9.294 -2.674 0.00 0.00 0.420 HD

```
ENDBRANCH   2   9
BRANCH   3  11
ATOM     11  O   LIG d  11      -1.328   9.735  -1.210  0.00  0.00    -0.737 OA
ATOM     12  H   LIG d  11      -1.587  10.181  -0.394  0.00  0.00     0.422 HD
ENDBRANCH   3  11
BRANCH   5  13
ATOM     13  C   LIG d  11      -2.993   5.359   0.426  0.00  0.00     0.225 C
BRANCH  13  14
ATOM     14  O   LIG d  11      -2.627   4.061   0.884  0.00  0.00    -0.279 OA
BRANCH  14  15
ATOM     15  C   LIG d  11      -2.436   3.094  -0.128  0.00  0.00     0.014 C
BRANCH  15  16
ATOM     16  C   LIG d  11      -1.154   2.308   0.118  0.00  0.00     0.436 A
ATOM     17  B   LIG d  11       0.163   2.416  -0.953  0.00  0.00    -0.027 B
ATOM     18  H   LIG d  11       0.056   3.181  -1.845  0.00  0.00    -0.066 HD
ATOM     19  B   LIG d  11      -0.898   1.007  -0.965  0.00  0.00    -0.018 B
ATOM     20  B   LIG d  11       1.578   2.031   0.025  0.00  0.00     0.011 B
ATOM     21  B   LIG d  11       0.848   0.788  -1.019  0.00  0.00     0.092 B
ATOM     22  B   LIG d  11       0.269   3.009   0.710  0.00  0.00    -0.065 B
ATOM     23  H   LIG d  11      -1.741   0.814  -1.764  0.00  0.00    -0.060 HD
ATOM     24  B   LIG d  11      -0.140  -0.259   0.014  0.00  0.00     0.020 B
ATOM     25  C   LIG d  11      -1.298   0.777   0.674  0.00  0.00     0.203 A
ATOM     26  H   LIG d  11      -0.456  -1.375  -0.197  0.00  0.00    -0.066 HD
ATOM     27  B   LIG d  11       1.394   0.374   0.626  0.00  0.00    -0.005 B
ATOM     28  B   LIG d  11      -0.024   0.333   1.682  0.00  0.00     0.025 B
ATOM     29  H   LIG d  11       2.312  -0.344   0.818  0.00  0.00    -0.091 HD
ATOM     30  B   LIG d  11       1.033   1.754   1.696  0.00  0.00     0.115 B
ATOM     31  B   LIG d  11      -0.713   1.965   1.744  0.00  0.00     0.027 B
ATOM     32  H   LIG d  11       1.683   2.029   2.643  0.00  0.00    -0.108 HD
ATOM     33  H   LIG d  11      -1.432   2.346   2.590  0.00  0.00    -0.100 HD
ATOM     34  H   LIG d  11      -0.259  -0.391   2.582  0.00  0.00    -0.068 HD
ATOM     35  H   LIG d  11       2.628   2.519  -0.217  0.00  0.00    -0.098 HD
ATOM     36  H   LIG d  11       1.358   0.376  -2.002  0.00  0.00    -0.103 HD
ATOM     37  H   LIG d  11       0.260   4.164   0.936  0.00  0.00    -0.068 HD
```

ENDBRANCH 15 16

ENDBRANCH 14 15

ENDBRANCH 13 14

ENDBRANCH 5 13

BRANCH 4 38

ATOM 38 O LIG d 11 -2.482 8.320 0.979 0.00 0.00 -0.707 OA

ATOM 39 H LIG d 11 -3.423 8.241 1.163 0.00 0.00 0.443 HD

ENDBRANCH 4 38

TORSDOF 8

1β:

REMARK 8 active torsions:

REMARK status: ('A' for Active; 'I' for Inactive)

REMARK 1 A between atoms: C_1 and O_12

REMARK 2 A between atoms: C_2 and O_11

REMARK 3 A between atoms: O_10 and C_3

REMARK 4 A between atoms: O_9 and C_4

REMARK 5 A between atoms: C_6 and C_5

REMARK 6 A between atoms: O_8 and C_6

REMARK 7 A between atoms: O_8 and C_17

REMARK 8 A between atoms: C_17 and C_18

REMARK I between atoms: C_18 and B_23

REMARK I between atoms: C_18 and B_22

REMARK I between atoms: C_18 and B_21

REMARK I between atoms: B_24 and B_22

REMARK I between atoms: B_22 and B_23

REMARK I between atoms: B_23 and B_24

REMARK I between atoms: B_25 and B_24

REMARK I between atoms: B_28 and B_29

ROOT

ATOM 1 C LIG d 1 2.325 4.822 3.337 0.00 0.00 0.532 C

ATOM 2 C LIG d 1 2.737 6.288 3.452 0.00 0.00 0.111 C

ATOM 3 C LIG d 1 1.782 7.144 2.643 0.00 0.00 0.437 C

ATOM 4 C LIG d 1 1.722 6.655 1.206 0.00 0.00 0.147 C

ATOM 5 C LIG d 1 1.363 5.169 1.192 0.00 0.00 0.269 C

ATOM 6 O LIG d 1 2.328 4.444 1.970 0.00 0.00 -0.498 OA

ENDROOT

BRANCH 1 7

ATOM 7 O LIG d 1 3.198 3.979 4.031 0.00 0.00 -0.627 OA

ATOM 8 H LIG d 1 4.048 3.997 3.571 0.00 0.00 0.400 HD

ENDBRANCH 1 7

BRANCH 2 9

ATOM 9 O LIG d 1 2.679 6.742 4.796 0.00 0.00 -0.659 OA

ATOM 10 H LIG d 1 3.171 6.112 5.338 0.00 0.00 0.406 HD

ENDBRANCH 2 9

BRANCH 5 11

ATOM 11 C LIG d 1 1.361 4.601 -0.214 0.00 0.00 0.280 C

BRANCH 11 12

ATOM 12 O LIG d 1 0.749 3.320 -0.299 0.00 0.00 -0.266 OA

BRANCH 12 13

ATOM 13 C LIG d 1 1.613 2.248 0.019 0.00 0.00 -0.030 C

BRANCH 13 14

ATOM 14 C LIG d 1 0.820 0.957 0.059 0.00 0.00 0.459 A

ATOM 15 C LIG d 1 0.795 0.023 -1.279 0.00 0.00 0.194 A

ATOM 16 B LIG d 1 -0.649 -0.829 -1.443 0.00 0.00 0.064 B

ATOM 17 B LIG d 1 0.842 -1.626 -0.909 0.00 0.00 0.032 B

ATOM 18 B LIG d 1 1.785 -0.456 0.024 0.00 0.00 -0.039 B

ATOM 19 B LIG d 1 -0.638 0.840 -0.849 0.00 0.00 -0.018 B

ATOM 20 H LIG d 1 -1.058 -1.015 -2.533 0.00 0.00 -0.075 HD

ATOM 21 B LIG d 1 -0.674 -1.911 -0.043 0.00 0.00 -0.036 B

ATOM 22 B LIG d 1 -1.584 -0.378 -0.006 0.00 0.00 0.076 B

ATOM 23 H LIG d 1 -1.216 -2.960 -0.101 0.00 0.00 -0.083 HD

ATOM 24 B LIG d 1 -0.664 -0.911 1.420 0.00 0.00 0.026 B

ATOM 25 B LIG d 1 0.842 -1.679 0.862 0.00 0.00 0.081 B

ATOM 26 B LIG d 1 0.853 -0.013 1.456 0.00 0.00 0.039 B

ATOM 27 H LIG d 1 -1.199 -1.234 2.424 0.00 0.00 -0.100 HD

ATOM 28 B LIG d 1 -0.630 0.792 0.922 0.00 0.00 -0.013 B

ATOM 29 H LIG d 1 1.447 0.417 2.380 0.00 0.00 -0.090 HD

ATOM 30 H LIG d 1 -1.030 1.735 1.503 0.00 0.00 -0.075 HD

ATOM 31 H LIG d 1 1.393 -2.544 1.449 0.00 0.00 -0.103 HD

ATOM 32 H LIG d 1 -2.764 -0.324 -0.034 0.00 0.00 -0.096 HD

ATOM 33 H LIG d 1 1.427 -2.338 -1.645 0.00 0.00 -0.071 HD

ATOM 34 H LIG d 1 2.947 -0.298 -0.091 0.00 0.00 -0.056 HD

ATOM 35 H LIG d 1 -0.957 1.766 -1.495 0.00 0.00 -0.085 HD

ENDBRANCH 13 14

ENDBRANCH 12 13

ENDBRANCH 11 12

ENDBRANCH 5 11

BRANCH 3 36

ATOM 36 O LIG d 1 2.177 8.511 2.601 0.00 0.00 -0.717 OA

ATOM 37 H LIG d 1 2.243 8.823 3.512 0.00 0.00 0.433 HD

ENDBRANCH 3 36

BRANCH 4 38

ATOM 38 O LIG d 1 0.734 7.360 0.472 0.00 0.00 -0.670 OA

ATOM 39 H LIG d 1 0.857 8.298 0.673 0.00 0.00 0.424 HD

ENDBRANCH 4 38

TORSDOF 8

2:

REMARK 8 active torsions:

REMARK status: ('A' for Active; 'I' for Inactive)

REMARK 1 A between atoms: C_1 and O_12

REMARK 2 A between atoms: O_11 and C_2

REMARK 3 A between atoms: O_10 and C_3

REMARK 4 A between atoms: C_4 and O_9

REMARK 5 A between atoms: C_5 and C_6

REMARK 6 A between atoms: C_6 and O_8

REMARK 7 A between atoms: C_17 and O_8

REMARK 8 A between atoms: C_17 and C_18

REMARK I between atoms: C_18 and C_19

REMARK I between atoms: C_19 and B_21

REMARK I between atoms: B_21 and B_27

```
REMARK    I    between atoms: B_23  and  B_24
REMARK    I    between atoms: B_24  and  B_29
REMARK    I    between atoms: B_24  and  B_25
REMARK    I    between atoms: B_27  and  B_25
REMARK    I    between atoms: B_29  and  B_25
REMARK    I    between atoms: B_26  and  B_29
ROOT
ATOM      1  C   LIG d 111      -0.398   6.934  -2.146  0.00  0.00     0.539 C
ATOM      2  C   LIG d 111      -1.059   8.295  -2.322  0.00  0.00     0.172 C
ATOM      3  C   LIG d 111      -1.435   8.851  -0.960  0.00  0.00     0.479 C
ATOM      4  C   LIG d 111      -2.333   7.873  -0.221  0.00  0.00     0.115 C
ATOM      5  C   LIG d 111      -1.642   6.506  -0.146  0.00  0.00     0.324 C
ATOM      6  O   LIG d 111      -1.318   6.068  -1.474  0.00  0.00    -0.532 OA
ENDROOT
BRANCH   1   7
ATOM      7  O   LIG d 111       0.790   7.088  -1.439  0.00  0.00    -0.447 OA
ATOM      8  C   LIG d 111       1.581   5.903  -1.411  0.00  0.00     0.253 C
ENDBRANCH   1   7
BRANCH   2   9
ATOM      9  O   LIG d 111      -0.192   9.161  -3.027  0.00  0.00    -0.713 OA
ATOM     10  H   LIG d 111      -0.558  10.050  -2.927  0.00  0.00     0.436 HD
ENDBRANCH   2   9
BRANCH   3  11
ATOM     11  O   LIG d 111      -2.094  10.094  -1.194  0.00  0.00    -0.730 OA
ATOM     12  H   LIG d 111      -2.311  10.464  -0.329  0.00  0.00     0.421 HD
ENDBRANCH   3  11
BRANCH   5  13
ATOM     13  C   LIG d 111      -2.527   5.444   0.476  0.00  0.00     0.194 C
BRANCH  13  14
ATOM     14  O   LIG d 111      -1.827   4.249   0.803  0.00  0.00    -0.245 OA
BRANCH  14  15
ATOM     15  C   LIG d 111      -1.733   3.332  -0.267  0.00  0.00    -0.001 C
BRANCH  15  16
ATOM     16  C   LIG d 111      -0.771   2.222   0.109  0.00  0.00     0.469 A
```

ATOM 17 B LIG d 111 0.602 1.925 -0.850 0.00 0.00 0.067 B

ATOM 18 H LIG d 111 0.783 2.667 -1.749 0.00 0.00 -0.107 HD

ATOM 19 B LIG d 111 -0.812 0.874 -0.942 0.00 0.00 -0.051 B

ATOM 20 B LIG d 111 1.768 1.184 0.244 0.00 0.00 -0.013 B

ATOM 21 B LIG d 111 0.797 0.169 -0.850 0.00 0.00 0.075 B

ATOM 22 B LIG d 111 0.743 2.514 0.813 0.00 0.00 -0.024 B

ATOM 23 H LIG d 111 -1.611 0.904 -1.808 0.00 0.00 -0.060 HD

ATOM 24 B LIG d 111 -0.526 -0.526 0.103 0.00 0.00 0.047 B

ATOM 25 C LIG d 111 -1.390 0.815 0.660 0.00 0.00 0.184 A

ATOM 26 H LIG d 111 -1.129 -1.513 -0.127 0.00 0.00 -0.073 HD

ATOM 27 B LIG d 111 1.073 -0.335 0.836 0.00 0.00 -0.011 B

ATOM 28 B LIG d 111 -0.376 0.056 1.771 0.00 0.00 0.048 B

ATOM 29 H LIG d 111 1.731 -1.279 1.109 0.00 0.00 -0.093 HD

ATOM 30 B LIG d 111 1.038 1.121 1.865 0.00 0.00 0.114 B

ATOM 31 B LIG d 111 -0.574 1.817 1.770 0.00 0.00 -0.026 B

ATOM 32 H LIG d 111 1.663 1.226 2.862 0.00 0.00 -0.106 HD

ATOM 33 H LIG d 111 -1.216 2.404 2.557 0.00 0.00 -0.090 HD

ATOM 34 H LIG d 111 -0.877 -0.546 2.653 0.00 0.00 -0.075 HD

ATOM 35 H LIG d 111 2.929 1.344 0.086 0.00 0.00 -0.093 HD

ATOM 36 H LIG d 111 1.243 -0.399 -1.785 0.00 0.00 -0.100 HD

ATOM 37 H LIG d 111 1.040 3.634 1.024 0.00 0.00 -0.081 HD

ENDBRANCH 15 16

ENDBRANCH 14 15

ENDBRANCH 13 14

ENDBRANCH 5 13

BRANCH 4 38

ATOM 38 O LIG d 111 -2.554 8.435 1.079 0.00 0.00 -0.704 OA

ATOM 39 H LIG d 111 -3.408 8.139 1.410 0.00 0.00 0.439 HD

ENDBRANCH 4 38

TORSDOF 8

3:

REMARK 8 active torsions:

REMARK status: ('A' for Active; 'I' for Inactive)

```
REMARK    1  A    between atoms: O_12  and  C_1
REMARK    2  A    between atoms: O_11  and  C_2
REMARK    3  A    between atoms: O_10  and  C_3
REMARK    4  A    between atoms: C_4  and  O_9
REMARK    5  A    between atoms: C_5  and  C_6
REMARK    6  A    between atoms: C_6  and  O_8
REMARK    7  A    between atoms: C_17  and  O_8
REMARK    8  A    between atoms: C_17  and  C_18
REMARK       I    between atoms: C_19  and  B_20
REMARK       I    between atoms: B_21  and  B_27
REMARK       I    between atoms: B_24  and  B_23
REMARK       I    between atoms: B_24  and  B_29
REMARK       I    between atoms: B_24  and  B_25
REMARK       I    between atoms: B_25  and  B_29
REMARK       I    between atoms: B_25  and  B_27
REMARK       I    between atoms: B_26  and  B_29
ROOT
ATOM      1  C   LIG d 111       5.108   2.350  -3.011  0.00  0.00     0.291 C
ATOM      2  C   LIG d 111       6.205   3.368  -3.319  0.00  0.00     0.298 C
ATOM      3  C   LIG d 111       6.730   3.926  -2.011  0.00  0.00     0.315 C
ATOM      4  C   LIG d 111       5.591   4.517  -1.198  0.00  0.00     0.250 C
ATOM      5  C   LIG d 111       4.509   3.456  -1.000  0.00  0.00     0.164 C
ATOM      6  O   LIG d 111       4.070   2.986  -2.281  0.00  0.00    -0.355 OA
ENDROOT
BRANCH   1   7
ATOM      7  O   LIG d 111       4.575   1.755  -4.155  0.00  0.00    -0.388 OA
ATOM      8  C   LIG d 111       3.857   2.628  -5.033  0.00  0.00     0.219 C
ENDBRANCH   1   7
BRANCH   2   9
ATOM      9  O   LIG d 111       7.296   2.757  -3.997  0.00  0.00    -0.691 OA
ATOM     10  H   LIG d 111       6.927   2.190  -4.687  0.00  0.00     0.406 HD
ENDBRANCH   2   9
BRANCH   3  11
ATOM     11  O   LIG d 111       7.685   4.965  -2.204  0.00  0.00    -0.711 OA
```

ATOM 12 H LIG d 111 8.405 4.604 -2.735 0.00 0.00 0.436 HD

ENDBRANCH 3 11

BRANCH 5 13

ATOM 13 C LIG d 111 3.306 4.007 -0.259 0.00 0.00 0.294 C

BRANCH 13 14

ATOM 14 O LIG d 111 2.428 2.997 0.221 0.00 0.00 -0.272 OA

BRANCH 14 15

ATOM 15 C LIG d 111 1.526 2.510 -0.750 0.00 0.00 -0.042 C

BRANCH 15 16

ATOM 16 C LIG d 111 0.738 1.355 -0.166 0.00 0.00 0.513 A

ATOM 17 B LIG d 111 0.780 -0.168 -0.922 0.00 0.00 0.004 B

ATOM 18 H LIG d 111 1.501 -0.273 -1.850 0.00 0.00 -0.076 HD

ATOM 19 B LIG d 111 -0.619 0.894 -1.099 0.00 0.00 -0.053 B

ATOM 20 B LIG d 111 0.430 -1.322 0.364 0.00 0.00 0.020 B

ATOM 21 B LIG d 111 -0.858 -0.812 -0.754 0.00 0.00 0.089 B

ATOM 22 B LIG d 111 1.462 0.084 0.691 0.00 0.00 0.000 B

ATOM 23 H LIG d 111 -0.845 1.544 -2.056 0.00 0.00 -0.056 HD

ATOM 24 B LIG d 111 -1.839 0.403 0.085 0.00 0.00 0.049 B

ATOM 25 C LIG d 111 -0.756 1.656 0.419 0.00 0.00 0.163 A

ATOM 26 H LIG d 111 -2.961 0.689 -0.136 0.00 0.00 -0.070 HD

ATOM 27 B LIG d 111 -1.191 -0.972 0.990 0.00 0.00 -0.048 B

ATOM 28 B LIG d 111 -1.160 0.646 1.705 0.00 0.00 0.067 B

ATOM 29 H LIG d 111 -1.908 -1.807 1.417 0.00 0.00 -0.082 HD

ATOM 30 B LIG d 111 0.249 -0.414 1.883 0.00 0.00 0.089 B

ATOM 31 B LIG d 111 0.480 1.293 1.534 0.00 0.00 -0.008 B

ATOM 32 H LIG d 111 0.563 -0.845 2.937 0.00 0.00 -0.103 HD

ATOM 33 H LIG d 111 0.908 2.171 2.185 0.00 0.00 -0.086 HD

ATOM 34 H LIG d 111 -1.834 1.091 2.564 0.00 0.00 -0.079 HD

ATOM 35 H LIG d 111 0.887 -2.412 0.338 0.00 0.00 -0.096 HD

ATOM 36 H LIG d 111 -1.329 -1.518 -1.576 0.00 0.00 -0.103 HD

ATOM 37 H LIG d 111 2.630 0.119 0.839 0.00 0.00 -0.090 HD

ENDBRANCH 15 16

ENDBRANCH 14 15

ENDBRANCH 13 14

ENDBRANCH 5 13

BRANCH 4 38

ATOM 38 O LIG d 111 6.044 4.937 0.079 0.00 0.00 -0.684 OA

ATOM 39 H LIG d 111 6.865 5.426 -0.067 0.00 0.00 0.428 HD

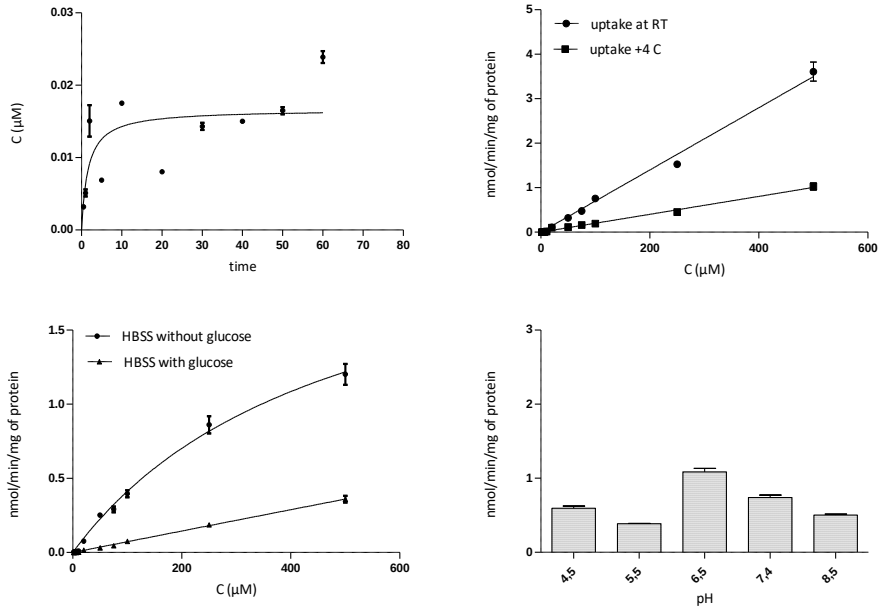
ENDBRANCH 4 38

TORSDOF 8

4. Characterization of the GLUT1 function and additional details related to affinity/uptake studies

The GLUT1 function of the CAL 27 cells were characterized with the known GLUT1 substrate [^{14}C]-D-glucose under different conditions. First, the time-dependent uptake was determined in order to find the optimal incubation time for the uptake of [^{14}C]-D-glucose. The CAL 27 cells (passages 7–20) were seeded at the density of 5 x 10^5 cells/wells onto 24-well plates. The cells were used in the characterization studies two days after seeding.

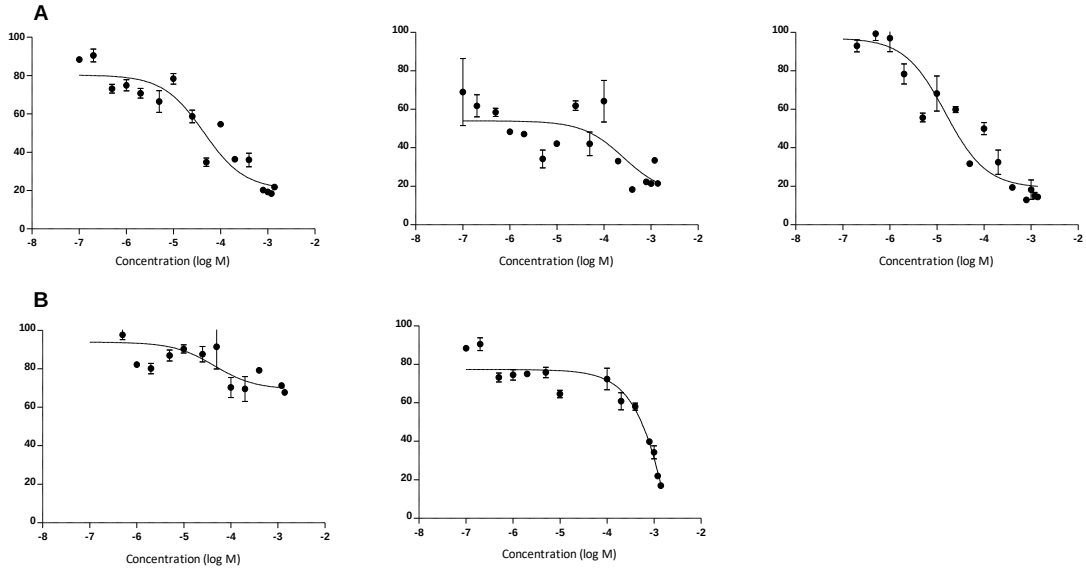
The cells were washed with pre-warmed HBSS containing NaCl (125 mM), KCl (4.8 mM), $MgSO_4$ (1.2 mM), KH_2PO_4 (1.3 mM), $CaCl_2$ (1.3 mM), glucose (5.6 mM) and HEPES (25 mM) adjusted to pH 7.4 with 1 M NaOH and the cells were pre-incubated for ten min. before the experiments. To study the time-dependent uptake of [^{14}C]-D-glucose, the cells were incubated at 10 different time points (0.5–60 min, n = 4) with 250 µl of HBSS including 1.8 µM (0.1 mCi/ml) of [^{14}C]-D-glucose. After incubation, the experiment was stopped through the addition of 500 µl of ice-cold HBSS and the cells were washed twice with ice-cold HBSS (500 µL). The cells were then lysed with 250 µl of 0.1 M NaOH and further lysed for an additional 60 min. The lysate was mixed with 1.0 ml of Emulsifier safe cocktail (PerkinElmer, Waltham, MA, USA) and the radioactivity was measured by liquid scintillation counter (MicroBeta^2 counter, PerkinElmer, Waltham, MA, USA). The optimal incubation time was determined from the linear range of the time-dependent uptake curve and was found to be 5 min. which was used in further characterization studies (*i.e.* in studies set to determine the concentration-, pH-, Na^+- and temperature-dependency).



Supplementary figure 43. Characterization of GLUT1 function in the CAL 27 cell line.

In the uptake experiments, the cell culture protocol described above was used with the exception of certain changes in incubation buffers and conditions. In order to determine the concentration-dependency, D-glucose was used at concentrations ranging from 1.8–500 µM in the HBSS buffer described above and in HBSS buffer without additional glucose. The uptake was significantly higher without additional glucose and therefore the HBSS buffer without glucose was selected for further studies. The effect of temperature was studied using an ice bath at +4 °C, with the same D-glucose concentrations (1.8–500 µM) mixed with ice-cold HBSS buffer (without additional glucose). The glucose uptake was then determined in glucose and Na^+-free buffer, by replacing NaCl with equimolar amounts of choline chloride (125 mM). Finally, the uptake was studied under different pH conditions (pH 4.5, 5.5, 6.5, 7.4 and 8.5). HEPES was replaced with MES (2-(*N*-morpholino)ethanesulfonic acid) in order to reach a more acidic pH.

Ability of compounds to bind to GLUT1



Supplementary figure 44. Inhibition of [^{14}C]-D-glucose (1.8 µM) uptake in CAL 27 cells in the presence of 0.1–1800 µM concentrations of glucoconjugates **1** (left), **2** (middle), **3** (right) (row **A**), BPA (left) and BSH (right) (row **B**).