Repurposing iminosugar-based glycosidase inhibitors as drug candidates for SARS-CoV-2 virus via molecular modeling and in vitro studies

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Supplementary material

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1. Computational modelling

All structures shown in **Figures S1-S19** can be downloaded as PDB files at the address https://www.chem.bg.ac.rs/~mario/SmartRep/

1.1. Positions of ligands and interactions with the binding site of α-glucosidase II

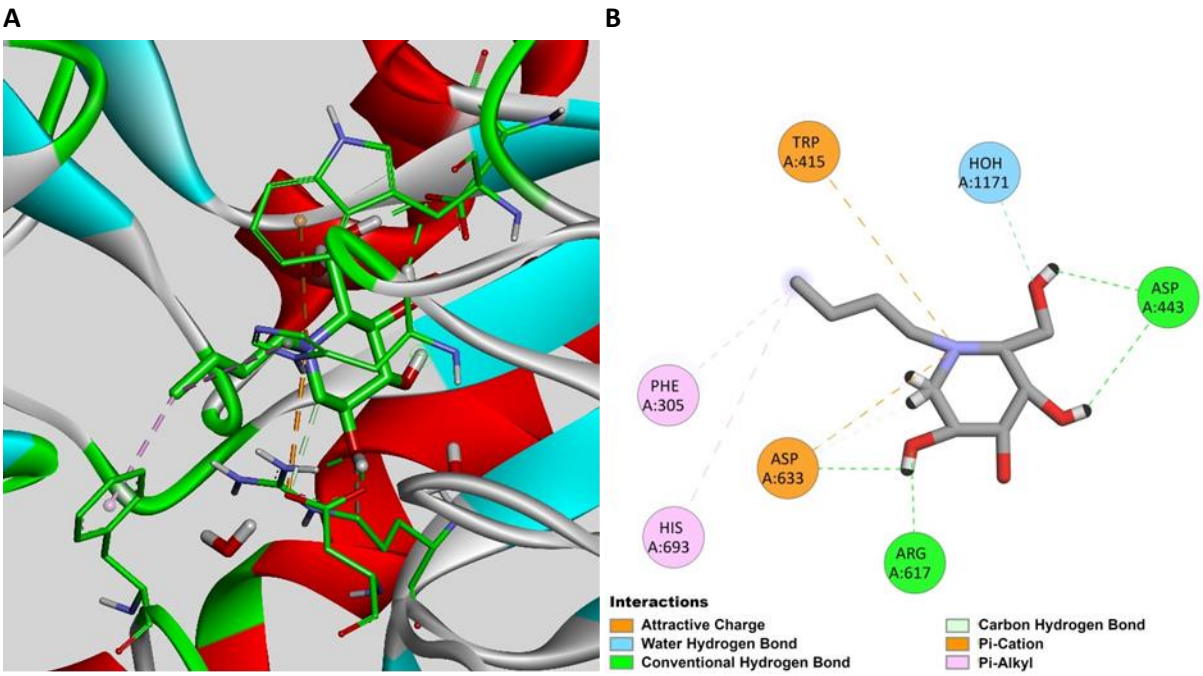


Figure S1 Position of ligand **1** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-glucosidase II.

A **B**

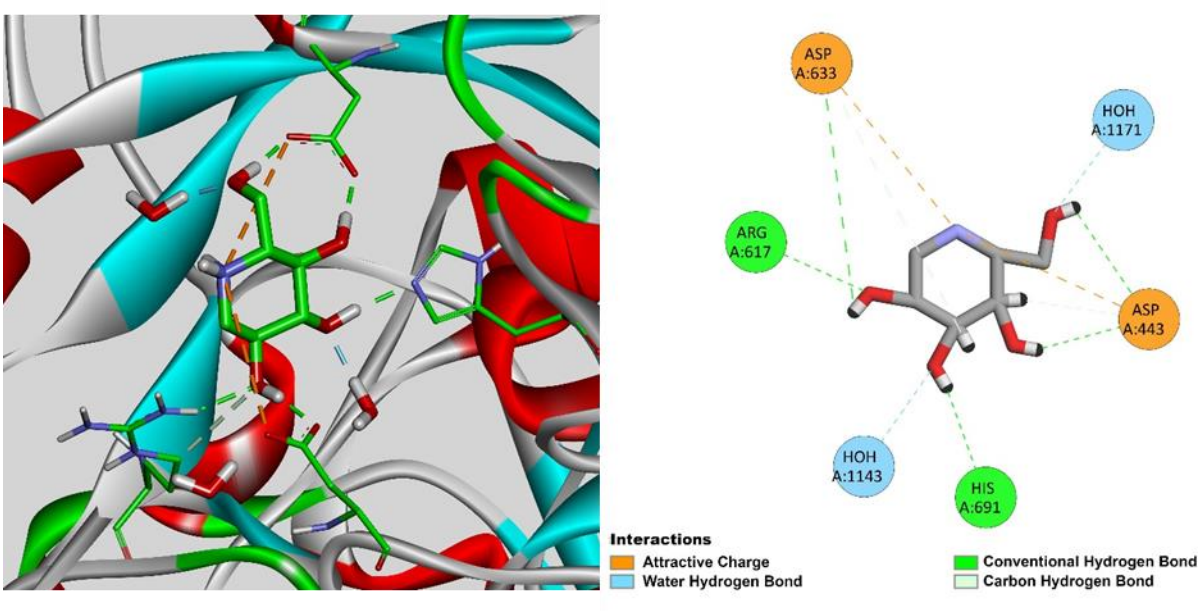


Figure S2 Position of ligand **2** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-glucosidase II.

A **B**

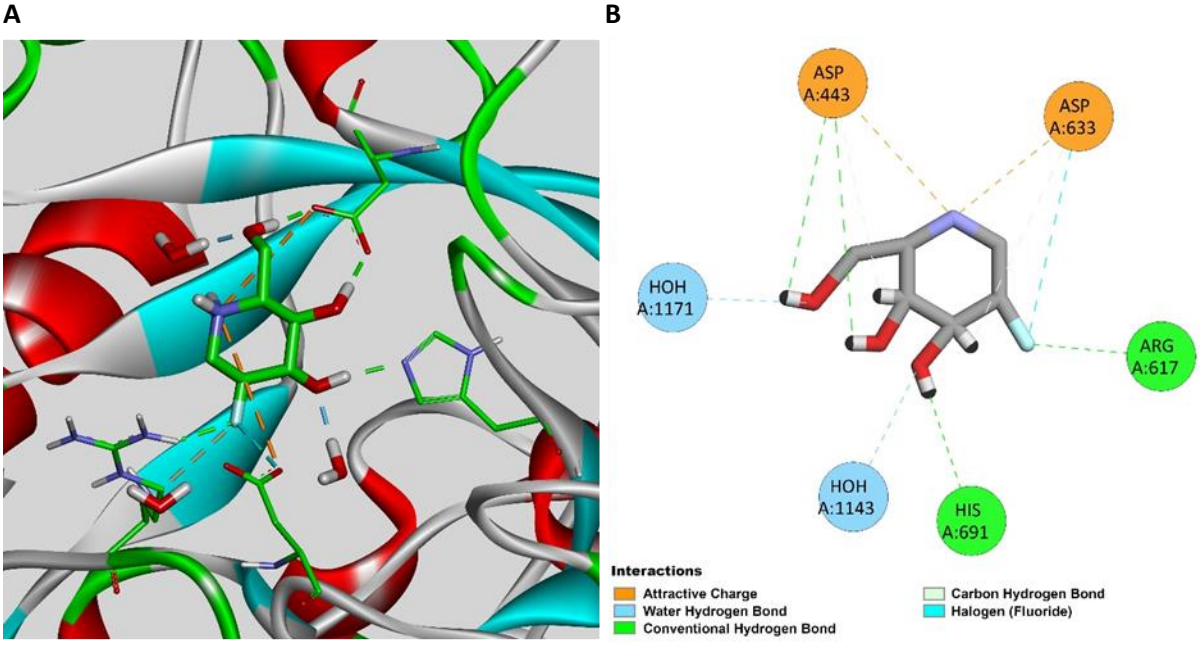


Figure S3 Position of ligand **5** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-glucosidase II.

A B

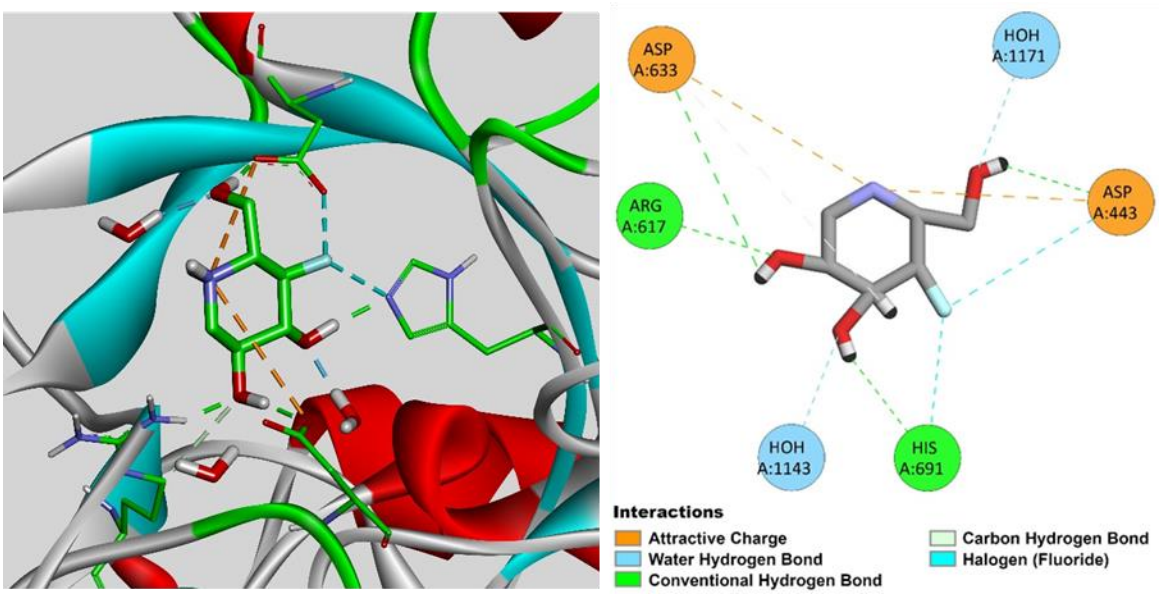


Figure S4 Position of ligand **6** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-glucosidase II.

A B

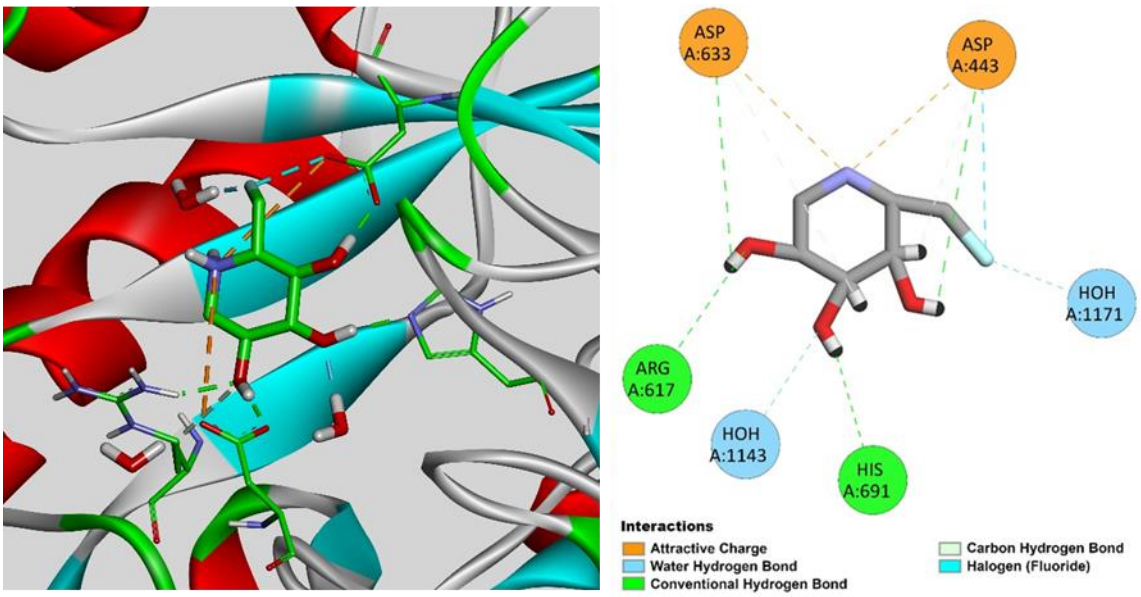


Figure S5 Position of ligand **7** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-glucosidase II.

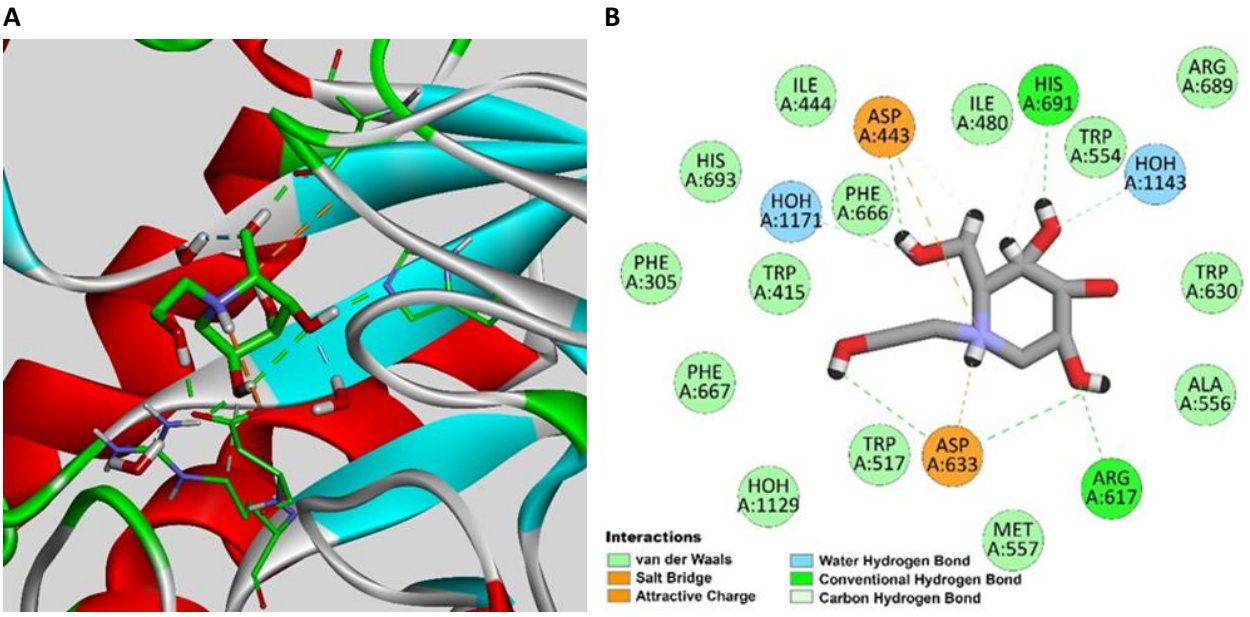


Figure S6 Position of ligand **8** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-glucosidase II.

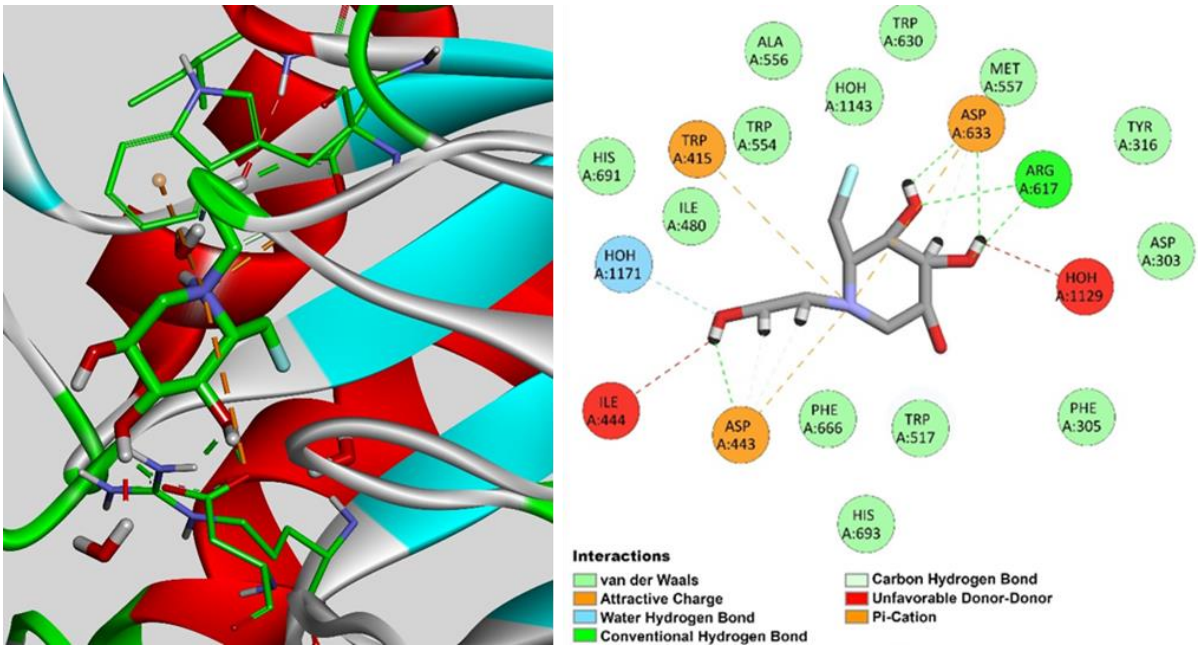
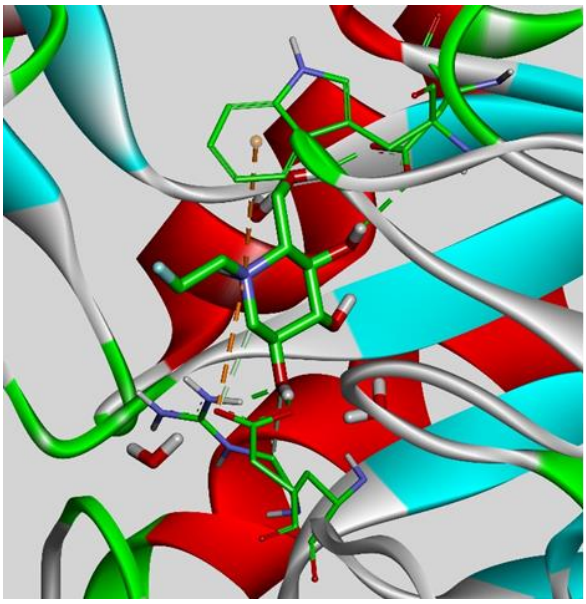


Figure S7 Position of ligand **9** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-glucosidase II.

A



B

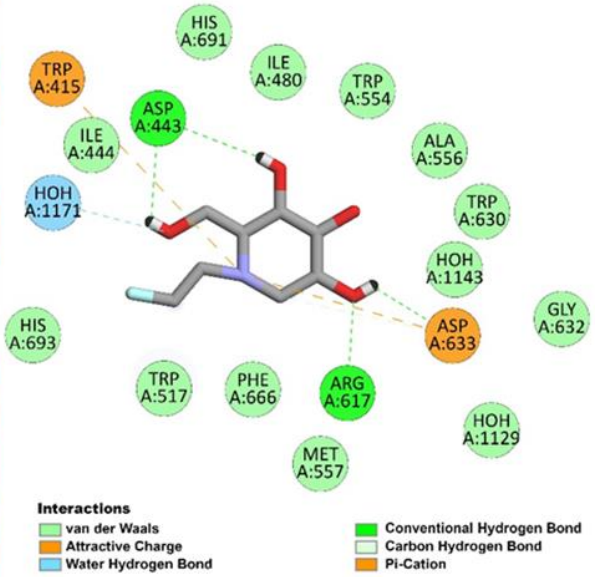
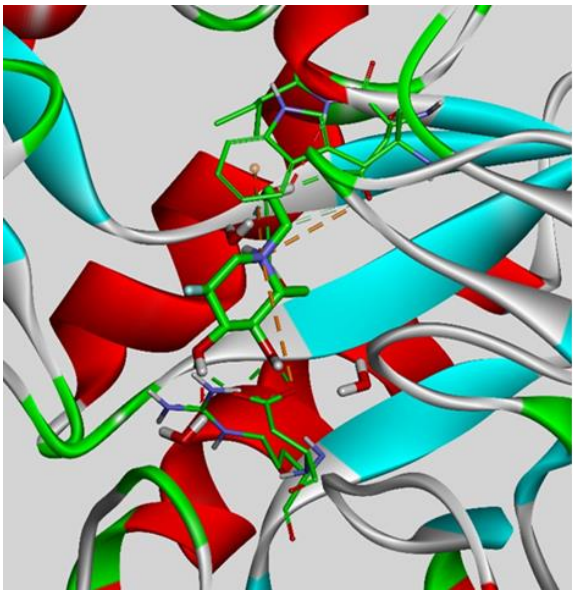


Figure S8 Position of ligand **10** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-glucosidase II.

A



B

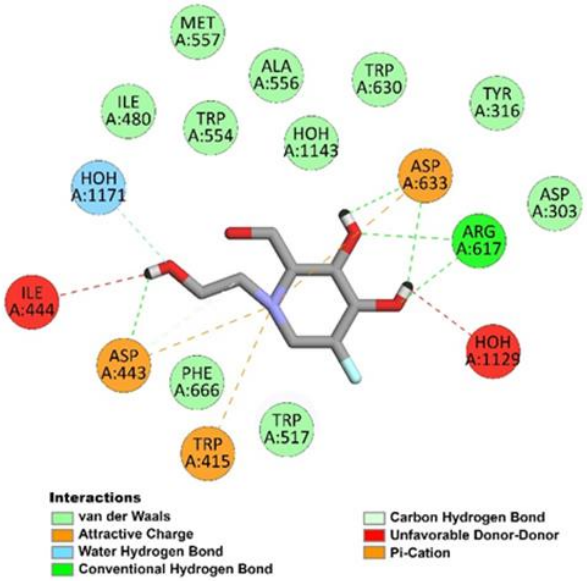


Figure S9 Position of ligand **11** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-glucosidase II.

A B

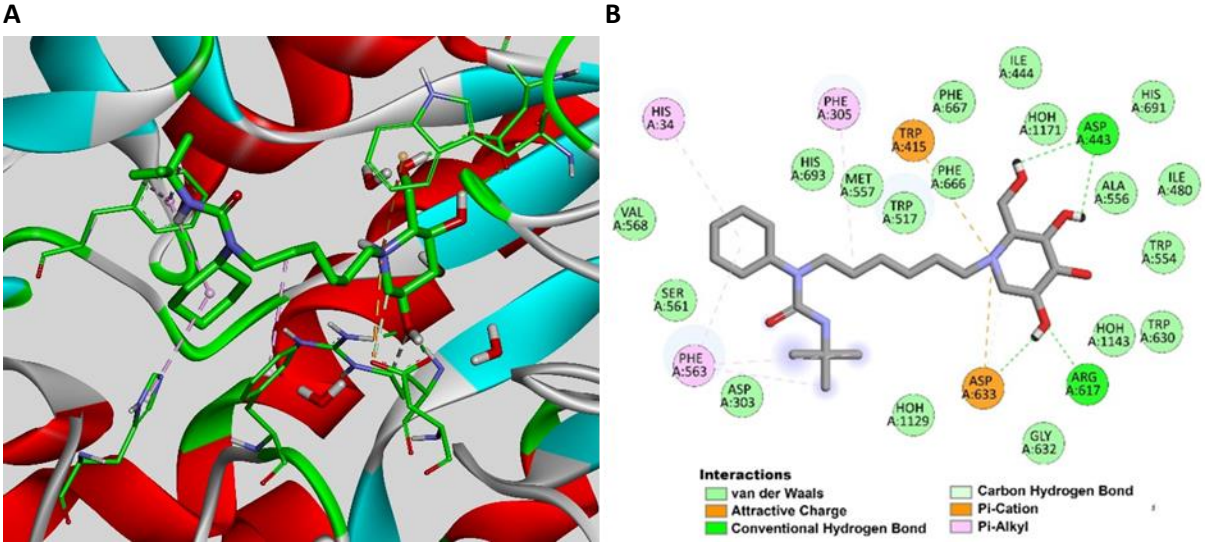


Figure S10 Position of ligand **12** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-glucosidase II.

A B

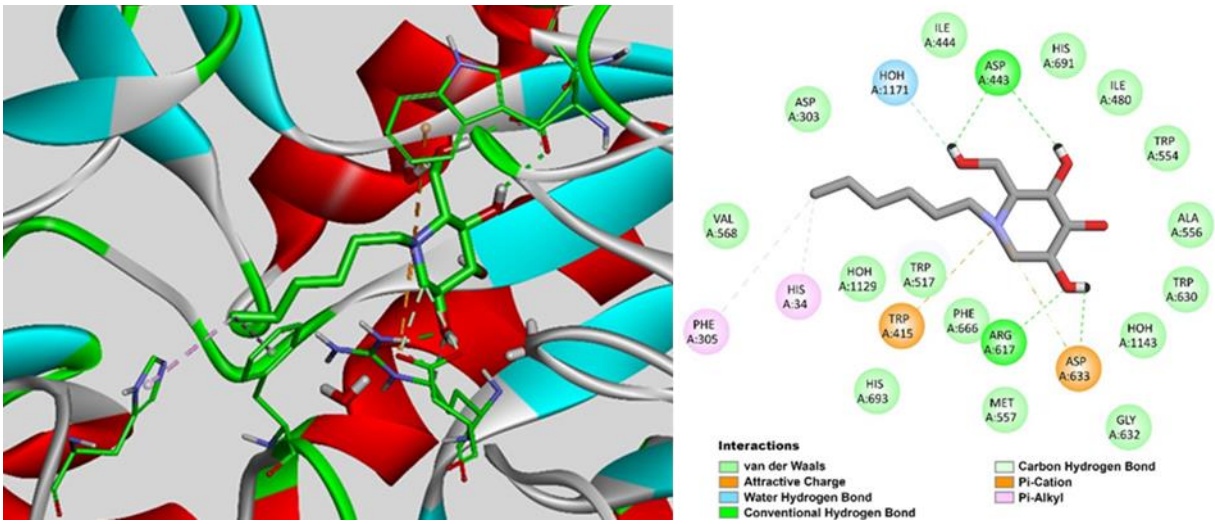


Figure S11 Position of ligand **13** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-glucosidase II.

A B

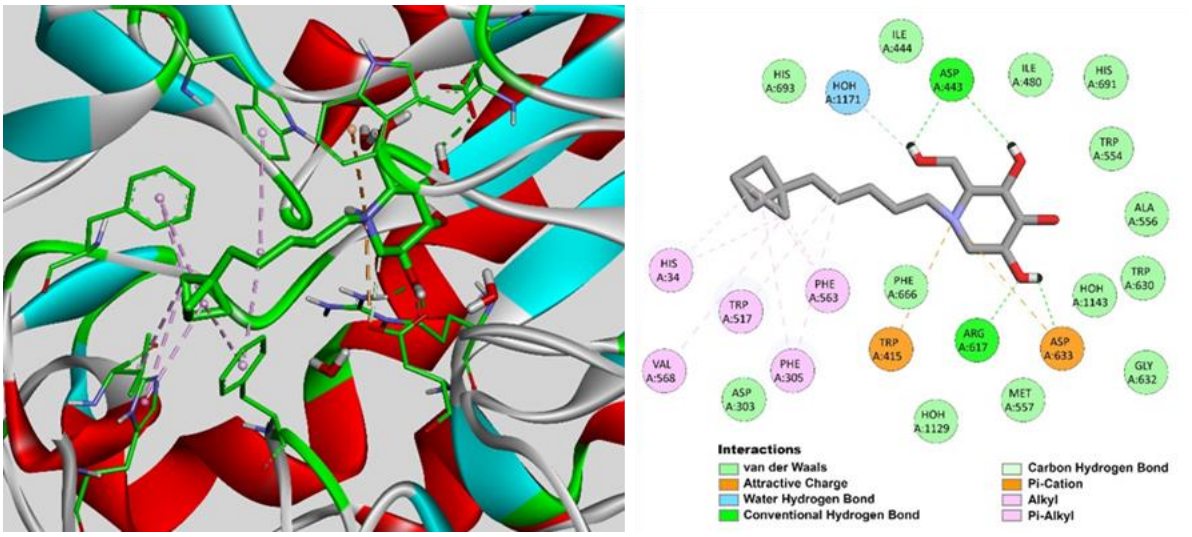


Figure S12 Position of ligand **22** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-glucosidase II.

A B

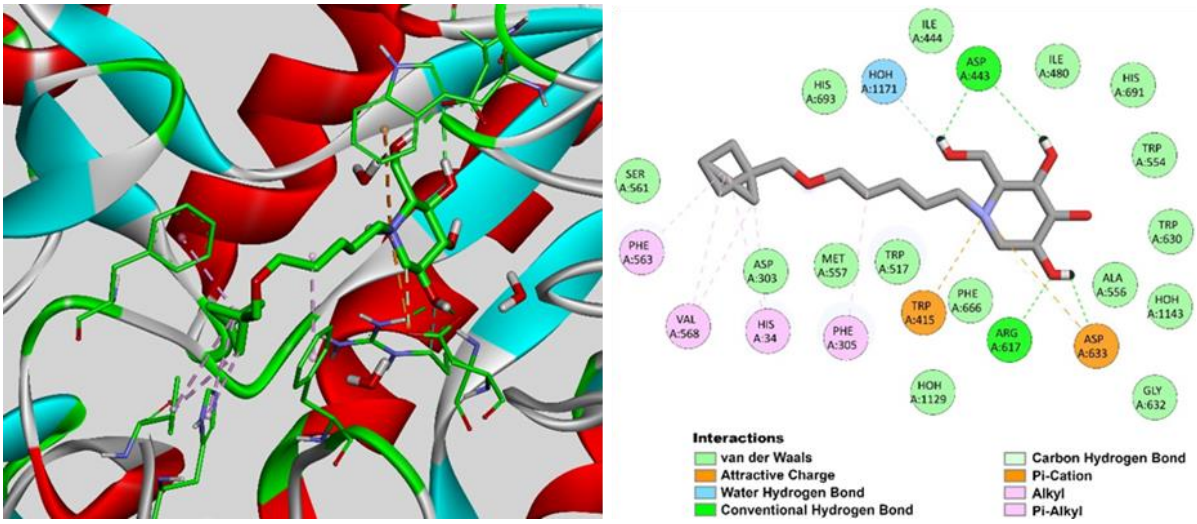


Figure S13 Position of ligand **38** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-glucosidase II.

A **B**

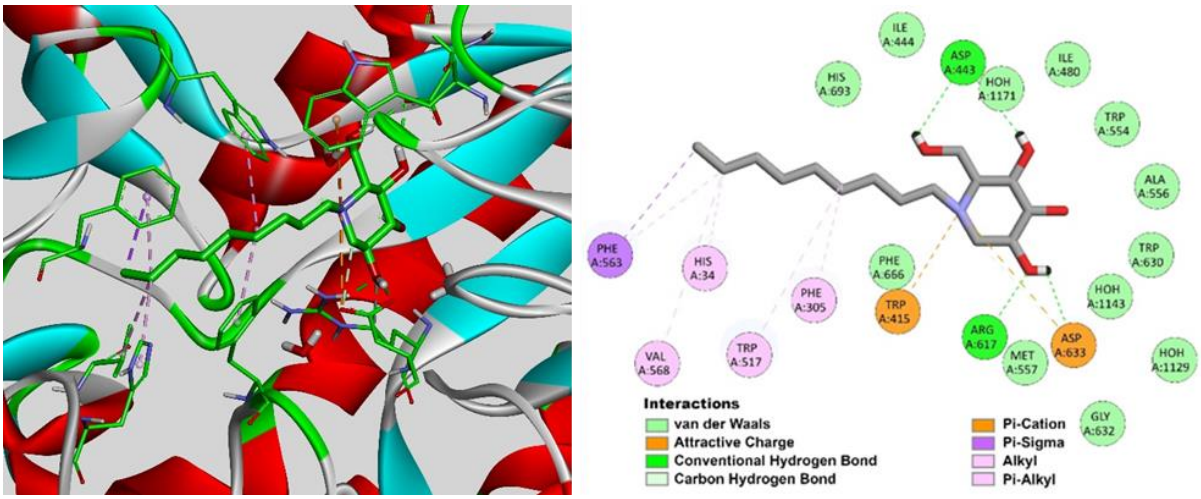


Figure S14 Position of ligand **76** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-glucosidase II.

A **B**

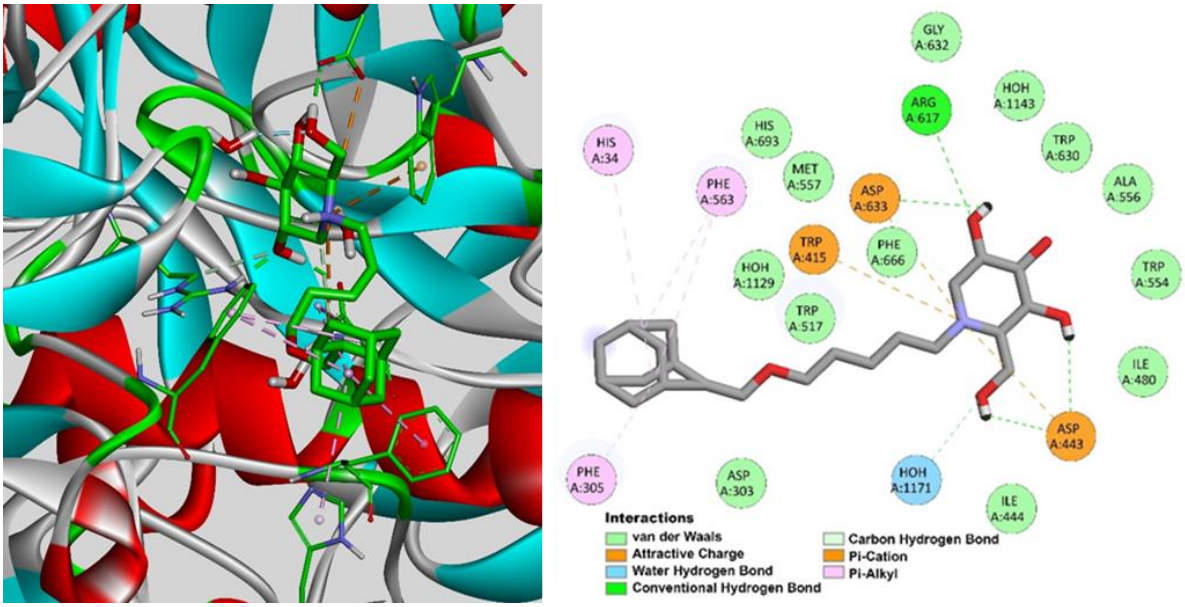


Figure S15 Position of ligand **77** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-glucosidase II.

1.2. Positions of ligands and interactions with the binding site of α-galactosidase A

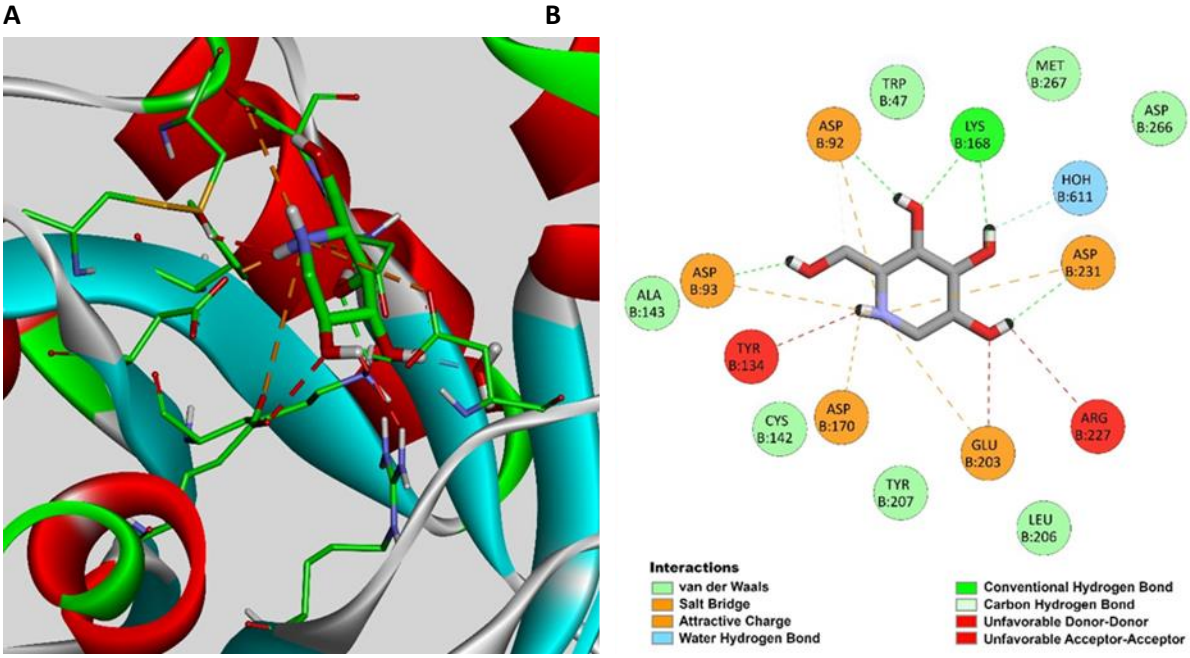


Figure S16 Position of ligand **4** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-galactosidase A.

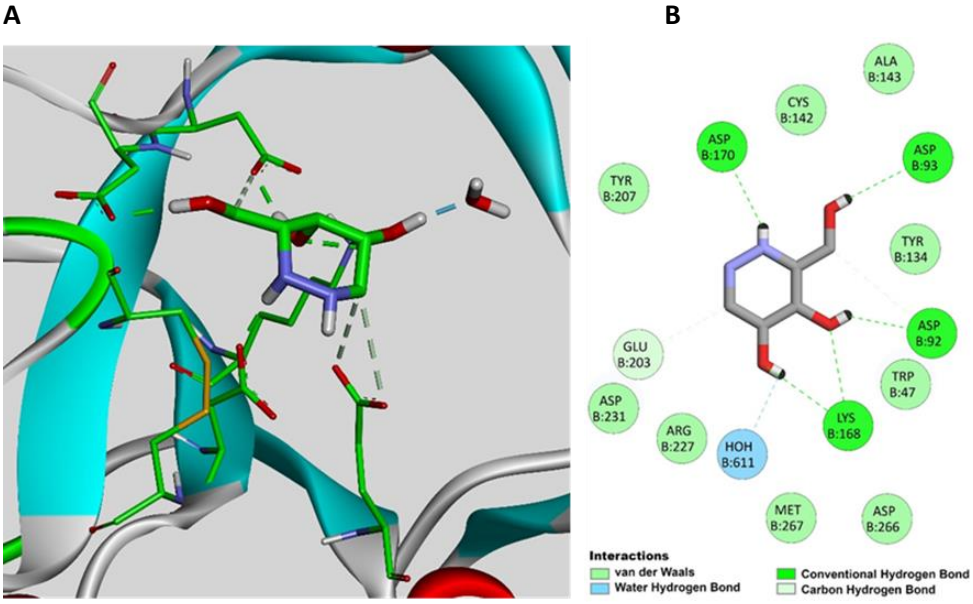


Figure S17 Position of ligand **40** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-galactosidase A.

A B

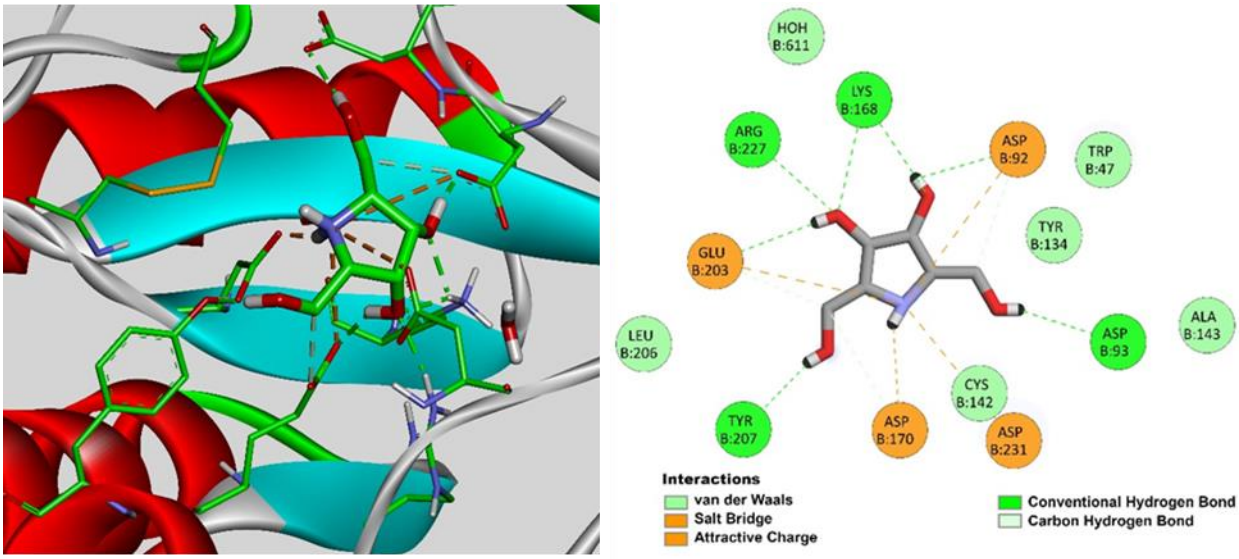


Figure S18 Position of ligand **41** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-galactosidase A.

A B

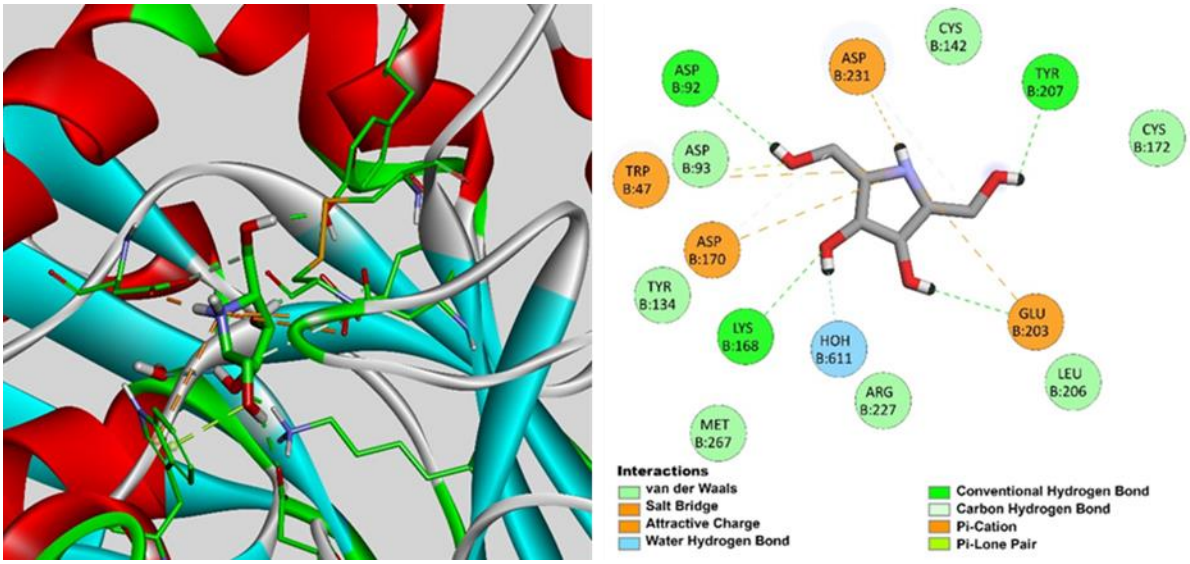


Figure S19 Position of ligand **42** (**A**) and interactions with amino acid residues (**B**) in the binding site of α-galactosidase A.

1.3. Properties of the α-glucosidase II binding site surface

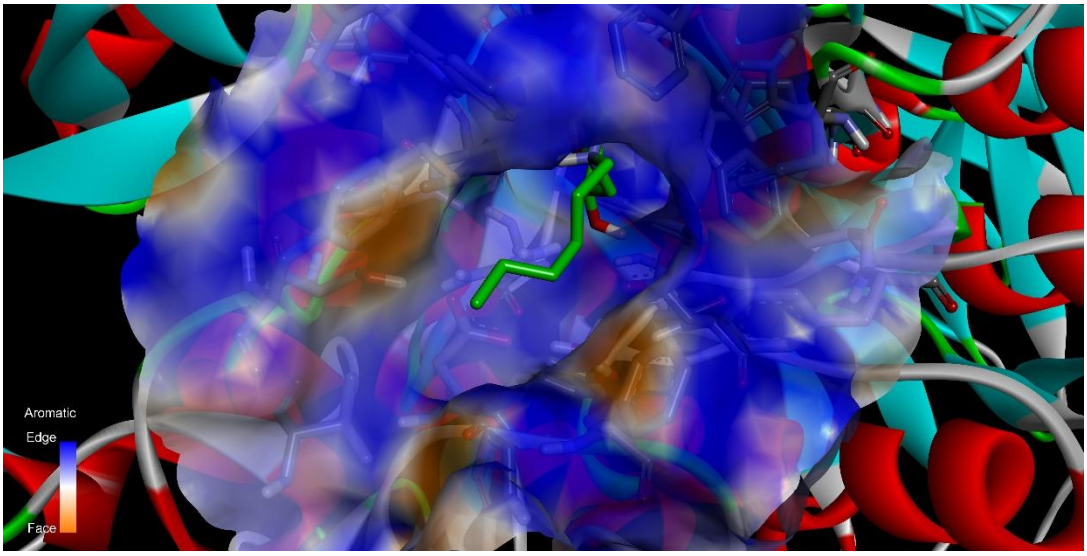


Figure S20 Aromatic properties of the α-glucosidase II binding site surface with compound **1** bound.

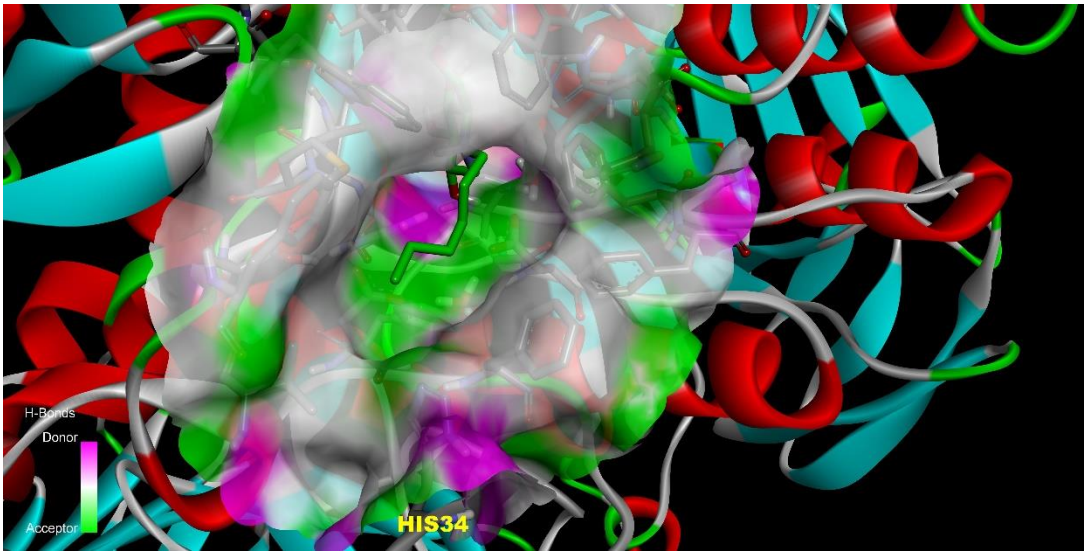


Figure S21 H-bond properties of the α-glucosidase II binding site surface with compound **1** bound.

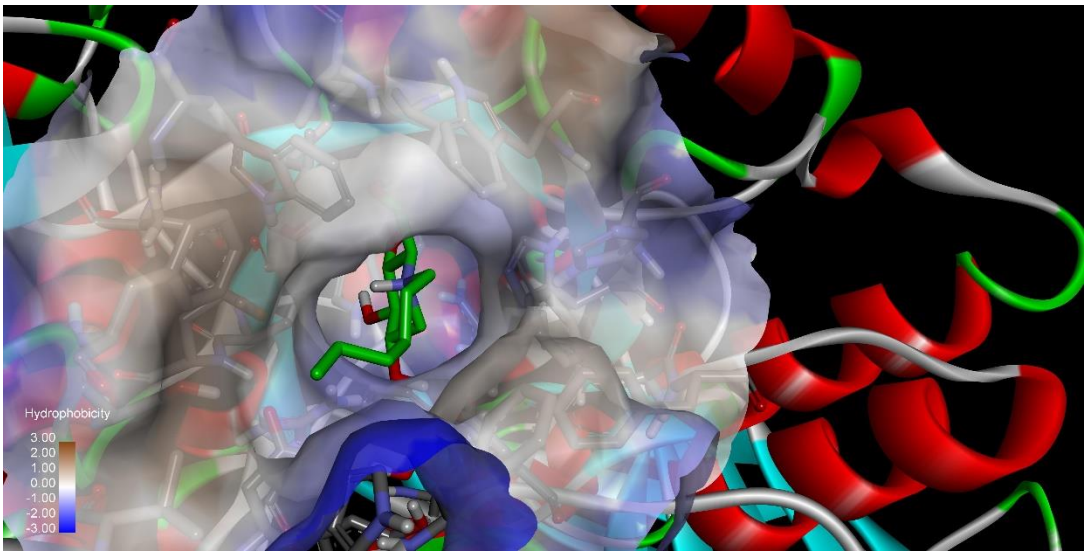


Figure S22 Hydrophobic α-glucosidase II binding site surface with compound **1** bound.

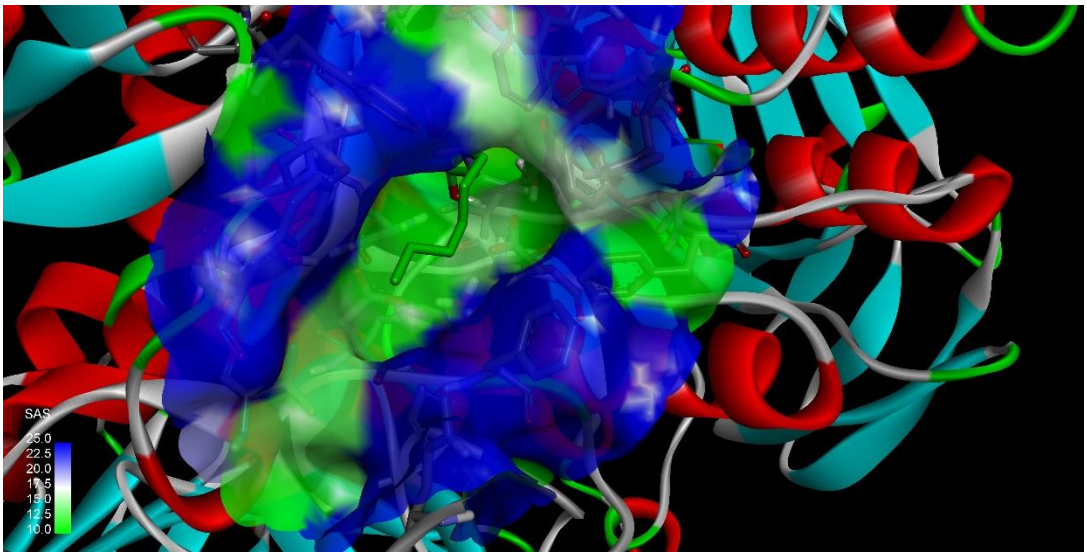


Figure S23 Solvent accesible α-glucosidase II binding site surface with compound **1** bound.

1.4. Supraposition of two molecules bound in α-galactosidase

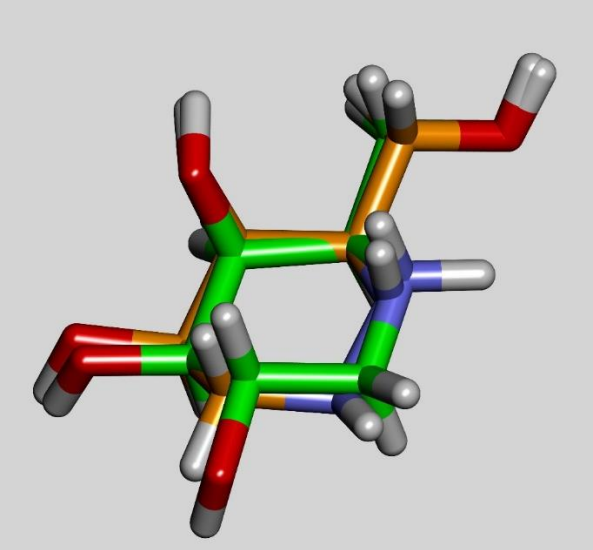


Figure S24 Best binding poses of **4** (green carbons) and **40** (orange carbons). Although they take almost the same position in the binding site of α-galactosidase A, the lack of vital interactions leads to lower binding score for **40.**

1.5. Table 1S: Tabular representation of ligand-protein interactions in the binding pocket of α-Glu II for compounds 1, 22, 76 and 77

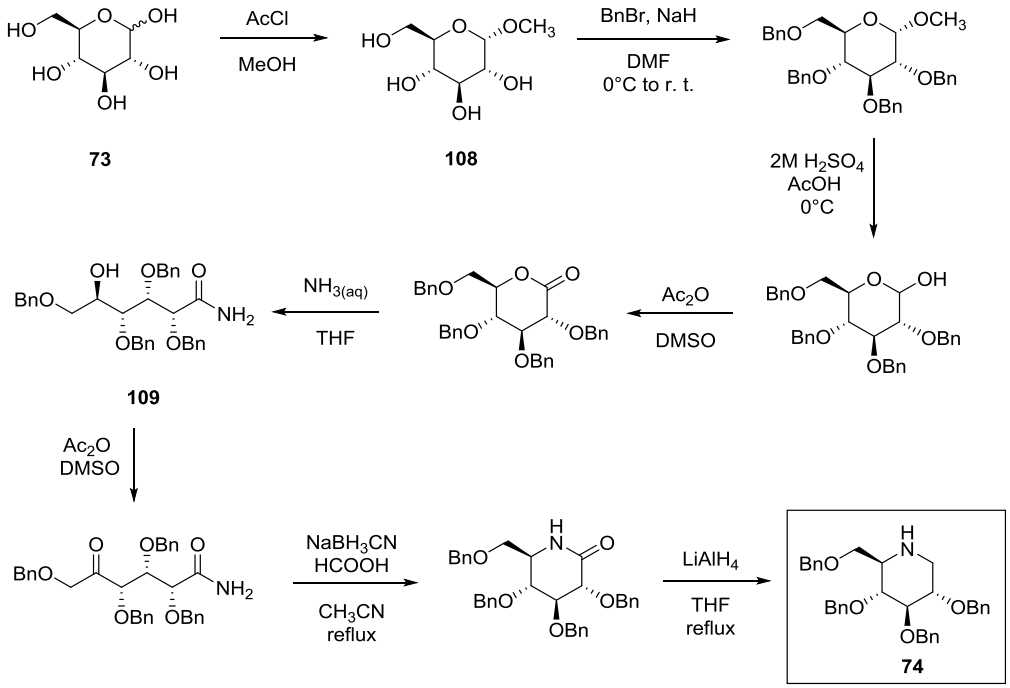
Can be dowloaded at:

https://www.chem.bg.ac.rs/~mario/SmartRep/

(Item #20)

2. Synthesis of α-glucosidase inhibitors

Compound **74** (the key intermediate in synthesis of DNJ) was prepared from *α*-glucose **73** by a modified literature procedure (Scheme 1).[1] The obtained spectral data are in accordance with the literature data.



Scheme S1 Synthesis of the key intermediate **74**.

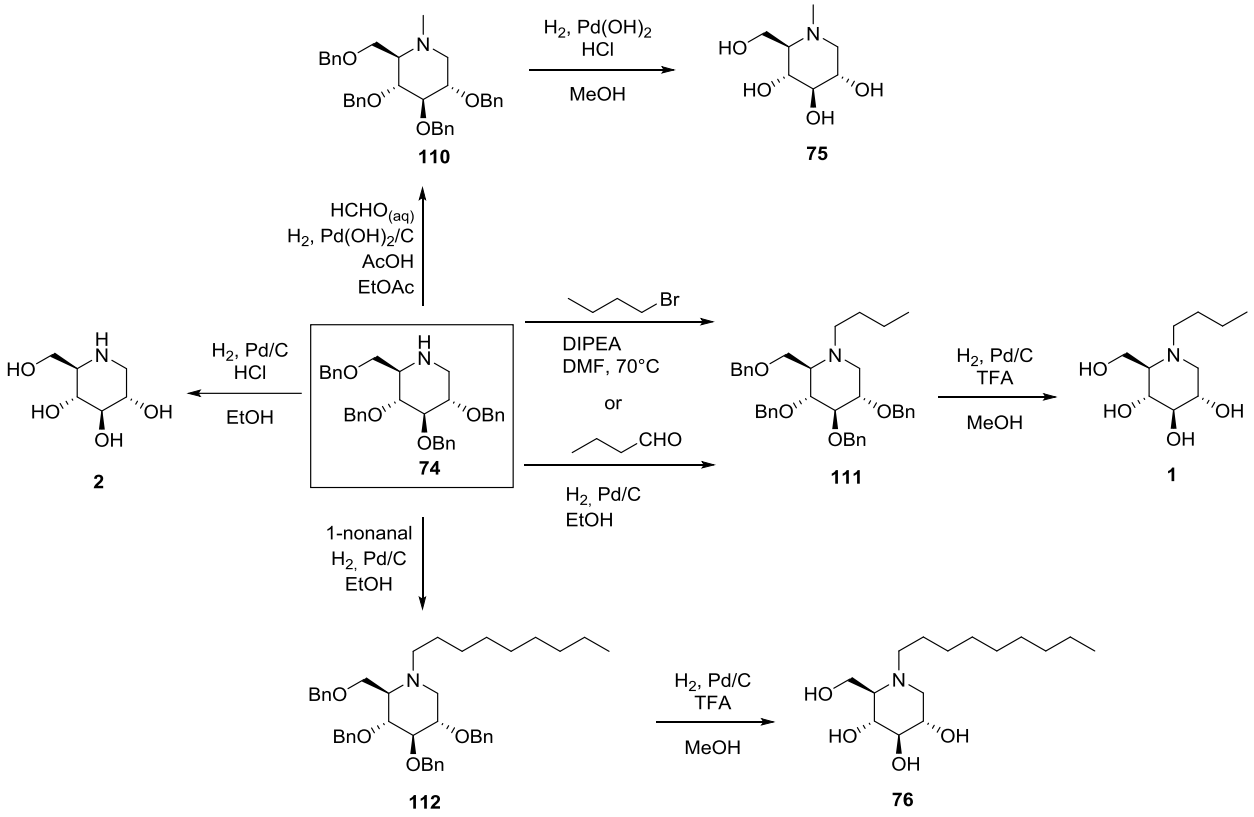
2.1. (2*R*,3*S*,4*S*,5*R*,6*S*)-2-(hydroxymethyl)-6-methoxytetrahydro-2H-pyran-3,4,5-triol (108)

To a suspension of *α*-glucose (50.0 g, 0.278 mol) in methanol (250 mL) was added acetyl chloride (2 mL, 28 mmol) dropwise and the reaction mixture was refluxed for 72 h (a clear solution was formed after 15 minutes). After the disapperance of the starting material (monitored by TLC, petroleum ether/ethyl acetate = 4:6), the reaction mixture was concentrated to 1/4 of the volume. A crystal of methyl α-D-glucopyranose was added to the residue, whereupon crystallization occured, affording 40.0 g, (74%) of product **108**, as white cristals, used in the next step without additional purification.

2.2. (2*R*,3*S*,4*R*,5*R*)-2,3,4,6-tetrakis(benzyloxy)-5-hydroxyhexanamide (109)

To a solution of 2,3,4,6-tetra-*O*-benzyl-D-gluconolactone (5.0 g; 9.3 mmol) in THF (21 mL) was added 25% $NH_{3(aq)}$ (99 mL) and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with diethyl ether (60 mL) and the aqueous layer was extraced with diethyl ether (3 x 80 mL). The organic layer was dried over anhydrous $MgSO_4$, concentrated under reduced pressure and purified by dry-flash chromatography (eluent: petroleum ether/ethyl acetate = 4:6), to afford 4.8 g (92%) of the

product **109**, as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20-7.35 (m, 20H), 6.59 (s, 1H), 5.58 (s, 1H), 4.73-4.46 (m, 8H), 4.24 (d, J = 3.3 Hz, 1H), 4.07 (dd, J = 5.5, 3.3 Hz, 1H), 3.96-3.83 (m, 2H), 3.64 (dd, J = 9.8, 3.0 Hz, 1H), 3.57 (dd, J = 9.8, 5.3 Hz, 1H), 2.83 (d, J = 4.1 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.1, 138.3, 138.2, 137.9, 136.9, 128.8, 128.5 (2C), 128.4, 128.2, 128.00 (2C), 127.9, 127.8, 80.7, 79.8, 77.8, 75.4, 74.3, 73.9, 73.5, 71.5, 71.2.



Scheme S2 Synthesis of DNJ-derived α-glucosidase inhibitors.

2.3. (2*R*,3*R*,4*R*,5*S*)-2-(hydroxymethyl)piperidine-3,4,5-triol (DNJ, 2)

To a solution of **74** (60.0 mg; 0.115 mmol) in ethanol (4 mL) were added $HCl_{(aq)}$ (1.5 M, to obtain pH=3) and 10% Pd/C (37.0 mg; 0.045 mmol) and the reaction mixture was stirred for 57 h under a hydrogen atmosphere (5 atm). The reaction mixture was then diluted with methanol, filtered, concentrated under reduced pressure and purified by column chromatography (eluent: ethyl acetate/methanol/25% $NH_{3\,(aq)}$= 1:1:0.05), to afford 15.1 mg (81%) of the product **2**, as a viscous oil. ^{1}H (400 MHz, D_2O) δ 3.86 (dd, J = 11.7, 3.0 Hz, 1H), 3.66 (dd, J = 11.7 Hz, 1H), 3.52 (ddd, J = 10.7, 9.0, 5.1 Hz, 1H), 3.35 (t, J = 9.1 Hz, 1H), 3.27 (t, J = 9.4 Hz, 1H), 3.15 (dd, J = 12.3, 5.2 Hz, 1H), 2.61-2.52 (m, 1H), 2.49 (dd, J = 12.1, 11.0 Hz, 1H). ^{13}C (100 MHz, D_2O) δ 78.2, 71.3, 70.7, 61.2, 60.4, 48.5. IR (ATR): $\tilde{\nu}$= 3317, 2892, 2462, 1964, 1377, 1097, 1039, 1017, 747, 596 cm^{-1}. HRMS (m/z) $[M+H]^+$ calcd. for $C_6H_{14}NO_4$: 164.0917, found: 164.0920.

2.4. (2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-1-methylpiperidine (110)

To a solution of amine **74** (10.7 mg; 0.02 mmol) in EtOAc (0.2 mL) were added 30% $HCHO_{(aq)}$ (9 µL), AcOH (3 µL) and $Pd(OH)_2$ (7.0 mg) and the reaction mixture was stirred 6.5 h under a hydrogen atmosphere (1 atm). The mixture was filtered, concentrated under reduced pressure and purified by column chromatography (eluent: petroleum ether/ethyl acetate = 3:2), to afford 9.8 mg (91%) of the product **110**, as a viscous oil. $[\alpha]_D^{20}$ −6.6 (*c* 0.01 in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36-7.21 (m, 18H), 7.14-7.09 (m, 2H), 4.95 (d, *J* = 11.0 Hz, 1H), 4.86 (d, *J* = 10.8 Hz, 1H), 4.80 (d, *J* = 11.0 Hz, 1H), 4.66 (dd, *J* = 15.5, 11.6 Hz, 2H), 4.48 (dd, *J* = 19.7, 12.2 Hz, 2H), 4.38 (d, *J* = 10.8 Hz, 1H), 3.75-3.53 (m, 4H), 3.47 (t, *J* = 9.1 Hz, 1H), 3.07 (dd, *J* = 11.1, 4.8 Hz, 1H), 2.31 (s, 3H), 2.10 (t, *J* = 10.8 Hz, 1H), 1.95 (d, *J* = 9.6 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.1, 138.7, 138.6, 138.0, 128.6, 128.5 (2C), 128.4 (2C), 128.0 (3C), 127.8, 127.7, 127.6, 87.4, 78.3 (2C), 75.5, 75.3, 73.7, 72.9, 67.3, 65.4, 59.1, 42.1. IR (ATR): $\tilde{\nu}$= 3088, 3063, 3030, 2863, 1605, 1496, 1454, 1362, 1318, 1252 cm^{-1}. HRMS (m/z) $[M+H]^+$ calcd. for $C_{35}H_{40}NO_4$: 538.2952, found: 538.2971.

2.5. (2*R*,3*R*,4*R*,5*S*)-2-(hydroxymethyl)-1-methylpiperidine-3,4,5-triol (75)[1,2]

Compound **75** was prepared according to the literature procedure.[1,2]
^{1}H NMR (400 MHz, CD_3OD) δ 3.89 (qd, *J* = 12.1, 2.4 Hz, 1H), 3.60-3.50 (m, 1H), 3.43 (t, *J* = 9.5 Hz, 1H), 3.20 (t, *J* = 9.1 Hz, 1H), 3.05 (dd, *J* = 11.4, 4.9 Hz, 1H), 2.53 (s, 1H), 2.36 (t, *J* = 11.1 Hz, 1H), 2.13 (d, *J* = 9.9 Hz, 1H). ^{13}C NMR (100 MHz, D_2O) δ 79.8, 70.8, 70.0, 69.7, 61.1, 58.1, 42.1.

2.6. (2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-1-butylpiperidine (111)

2.6.1. Method 1: Alkylation

To a solution of amine **74** (99.5 mg; 0.19 mmol) and DIPEA (149.0 mg; 1.15 mmol) in DMF (1 mL) was added 1-bromobutane (118.0 mg; 1.15 mmol) and the reaction mixture was stirred at 70 °C for 24 h under an argon atmosphere. The reaction mixture was then diluted with diethyl ether (70 mL), washed with water (2x15 mL) and brine (15 mL), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by dry-flash chromatography (eluent: petroleum ether/ethyl acetate = 85:15) to give compound **111** (76.0 mg, 69%) as a colorless oil.

2.6.2. Method 2: Reductive amination

A mixture of amine **74** (70.0 mg; 0.13 mmol), butanal (49.0 mg; 0.67 mmol) and 10% Pd/C (31.0 mg; 0.03 mmol) in ethanol (3.8 mL) was stirred for 24 h under a hydrogen atmosphere (4.2 atm). The reaction mixture was then filtered, concentrated under reduced pressure and purified by column chromatography (eluent: petroleum ether/ethyl acetate = 85:15) to afford compound **111** (55.9 mg, 72%) as a colorless oil. ^{1}H NMR(400 MHz, $CDCl_3$) δ 7.39-7.21 (m, 18H), 7.16-7.10 (m, 2H), 4.95 (d, *J* = 11.1 Hz, 1H), 4.87 (d, *J* = 10.9 Hz, 2H), 4.81 (d, *J* = 11.1 Hz, 1H), 4.72-4.62 (m, 2H), 4.52-4.39 (m, 3H), 3.70-3.50 (m, 4H), 3.45 (t, *J* = 9.1 Hz, 1H), 3.09 (dd, *J* = 11.2, 4.8 Hz, 1H), 2.73-2.50 (m, 2H), 2.33-2.15 (m, 2H), 1.46-1.10 (m, 4H), 0.86 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.2, 138.8 (2C), 138.0, 128.6, 128.5 (2C), 128.4 (4C), 128.0,

127.9, 127.7, 127.6, 127.5, 87.6, 78.8 (2C), 75.4, 73.3, 73.6, 72.9, 65.6, 63.9, 54.6, 52.3, 25.9, 20.8, 14.1. IR (ATR): $\tilde{\nu}$= 3088, 3061, 3030, 2958, 2910, 2867, 1497, 1453, 1360, 1118, 1089, 1063, 998, 745, 695, 675 cm^{-1}. HRMS (m/z) $[M+H]^+$ calcd. for $C_{38}H_{46}NO_4$: 580.3421, found: 580.3439.

2.7. (2*R*,3*R*,4*R*,5*S*)-1-butyl-2-(hydroxymethyl)piperidine-3,4,5-triol (miglustat, 1)

A mixture of amine **111** (127.0 mg; 0.217 mmol), trifluoroacetic acid (44 µL; 0.576 mmol) and 10% Pd/C (150.0 mg; 0.141 mmol) in methanol (3.3 mL) was stirred for 26 h under a hydrogen atmosphere (1 atm). The reaction mixture was then filtered, concentrated under reduced pressure and purified by dry-flash chromatography (eluent: ethyl acetate/methanol/25% $NH_{3\,(aq)}$= 7:3:0.05) to afford compound **1** (39.2 mg, 82%) as a colorless oil. ^{1}H (400 MHz, D_2O) δ 3.91 (qd, *J* = 12.9, 2.6 Hz, 2H), 3.60 (ddd, *J* = 10.8, 9.3, 4.9 Hz, 1H), 3.44 (t, *J* = 9.5 Hz, 1H), 3.31 (t, *J* = 9.2 Hz, 1H), 3.12 (dd, *J* = 11.6, 5.0 Hz, 1H), 2.89-2.80 (m, 1H), 2.75 - 2.65 (m, 1H), 2.48-2.35 (m, 2H), 1.57-1.46 (m, 2H), 1.37-1.27 (m, 2H), 0.93 (t, *J* = 7.4 Hz, 3H). ^{13}C (100 MHz, D_2O) δ 78.0, 69.7, 68.4, 65.0, 57.0, 54.9, 51.8, 24.9, 20.0, 13,1. IR (ATR): $\tilde{\nu}$= 3352, 2958, 2932, 2873, 1665, 1460, 1378, 1086, 1014, 644cm^{-1}. HRMS (m/z) $[M+H]^+$ calcd. for $C_{10}H_{22}NO_4$: 220.1543, found: 220.1547.

2.8. (2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-1-nonylpiperidine (112)

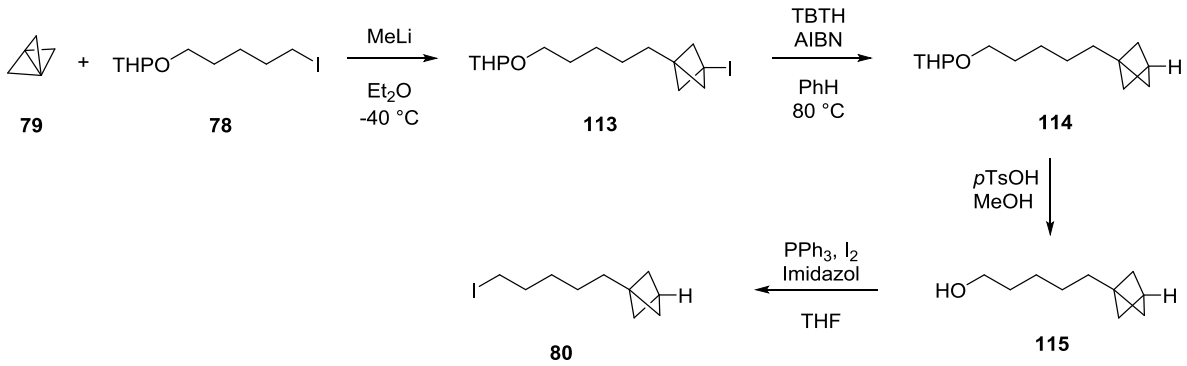
A mixture of amine **74** (140.0 mg; 0.26 mmol), nonanal (218.0 mg; 0.138 mmol) and 10% Pd/C (62.0 mg; 0.06 mmol) in ethanol (7.6 mL) was stirred for 23 h under a hydrogen atmosphere (4.2 atm). The reaction mixture was then filtered, concentrated under reduced pressure and purified by column chromatography (eluent: petroleum ether/ethyl acetate = 85:15) to afford compound **112** (121.0 mg, 70%) as a colorless oil. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.37-7.22 (m, 18H), 7.16-7.11 (m, 2H), 4.95 (d, *J* = 11.1 Hz, 1H), 4.87 (d, *J* = 10.8 Hz, 2H), 4.81 (d, *J* = 11.1 Hz, 1H), 4.71-4.61 (m, 2H), 4.51-4.39 (m, 3H), 3.71-3.51 (m, 4H), 3.45 (t, *J* = 9.1 Hz, 1H), 3.10 (dd, *J* = 11.1, 4.9 Hz, 1H), 2.71-2.52 (m, 2H), 2.36-2.28 (m, 1H), 2.23 (t, *J* = 10.8 Hz, 1H), 1.47-1.06 (m, 14H), 0.9 (t, *J* = 6.7 Hz 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.0, 138.6 (2),137.8, 128.4, 128.3 (4C), 127.8 (2C), 127.6, 127.5, 127.4, 87.4, 78.6, 75.3, 73.4, 72.7, 65.3, 63.7, 54.4, 52.4, 31.9, 29.6, 27.5, 23.5, 22.7, 14.10. IR (ATR): $\tilde{\nu}$= 3091, 3031, 2955, 2920, 2849, 1498, 1454, 1362, 1148, 1177, 1092, 1066, 1053, 734, 696 cm^{-1}. HRMS (m/z) $[M+H]^+$ calcd. for $C_{43}H_{56}NO_4$: 650.4204, found: 650.4224.

2.9. (2*R*,3*R*,4*R*,5*S*)-2-(hydroxymethyl)-1-nonylpiperidine-3,4,5-triol (76)

A mixture of amine **112** (87.0 mg; 0.134 mmol), trifluoroacetic acid (27 µL; 0.35 mmol) and 10% Pd/C (93.0 mg; 0.084 mmol) in methanol (1.9 mL) was stirred for 12 h under a hydrogen atmosphere (1 atm). The reaction mixture was then filtered, concentrated under reduced pressure and purified by dry-flash chromatography (eluent: ethyl acetate/methanol/25% $NH_{3\,(aq)}$= 7:3:0.05) to afford compound **76** (27.3 mg, 70%) as a colorless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 3.90 (qd, *J* = 12.2, 2.6 Hz, 2H), 3.60-3.51 (m, 1H), 3.44 (t, *J* = 9.4 Hz, 1H), 3.22 (t, *J* = 9.1 Hz, 4H), 3.16 (dd, *J* = 11.5, 4.9 Hz, 1H), 3.07-2.99 (m, 1H), 2.87-2.78 (m, 1H), 2.53-2.44 (m, 1H), 1.66-1.56 (m, 2H), 1.43-1.21 (m, 12H), 0.90 (t. *J* = 6.3 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 79.7, 70.9, 69.7, 67.4, 57.9, 56.6, 53.9, 33.0, 30.6, 30.5, 30.4, 28.3, 24.9, 23.7, 14.4. IR (ATR): $\tilde{\nu}$=

3348, 2956, 2925, 2855,1668,1465,1378, 1089,1031cm^{-1}. HRMS (m/z) $[M+H]^+$ calcd. for $C_{15}H_{32}NO_4$: 290.2325, found: 290.2331.

3. Synthesis of BCP fragment



Scheme S3 Synthesis of BCP fragment.

3.1. 2-((5-(Bicyclo[1.1.1]pentan-1-yl)pentyl)oxy)tetrahydro-2H-pyran (114)

To a solution of iodoalkane **78** (1.87 g; 6.29 mmol) and bicyclo[1.1.1]pentane **79**[3] (0.9 M in diethyl ether; 7.50 mL; 6.75 mmol) in anhydrous Et_2O (10 mL), a solution of MeLi in diethoxymethane (3.1 M, 1.98 mL, 6.04 mmol) was added dropwise at –40 °C, under an argon atmosphere. The reaction mixture was allowed to warm to room temperature, stirred for 24 h, and then cooled again to –40 °C, when MeOH (0.1 mL) was added. The resulting solution was poured into an ice-cold mixture of H_2O and pentane. After separation of the layers, the organic phase was washed with water, dried, and concentrated under reduced pressure. The crude iodide **113** (2.29 g) was used in the next step without purification.
A deaerated solution of iodide **113** (2.29 g, 6.29 mmol), tributyltin hydride (2.56 g, 8.80 mmol) and AIBN (50.0 mg, 0.31 mmol) in benzene (20 mL) was stirred at 80 °C under an argon atmosphere. After 1 h, a second batch of AIBN (50 mg, 0.31 mmol) was added and the reaction mixture was stirred for 1 h. After removal of the solvent under reduced pressure, the residue was purified by dry-flash chromatography (eluent: petroleum ether/ethyl acetate = 95:5) to give compound **114** (1.32 g, 88%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.59-4.55 (m, 1H), 3.90-3.84 (m, 1H), 3.76-3.69 (m, 1H), 3.53-3.47 (m, 1H), 3.40-3.35 (m, 1H), 2.43 (s, 1H), 1.88-1.78 (m, 1H), 1.74-1.68 (m, 1H), 1.67-1.47 (m, 12H), 1.40-1.24 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 98.9, 67.7, 62.2, 50.4, 45.9, 32.6, 30.9, 29.9, 27.4, 26.5 (2C), 25.6, 19.8. IR (ATR): $\tilde{\nu}$= 2961, 2927, 1460, 1281, 1194, 1048 cm^{-1}.

3.2. 5-(Bicyclo[1.1.1]pentan-1-yl)pentan-1-ol (115)

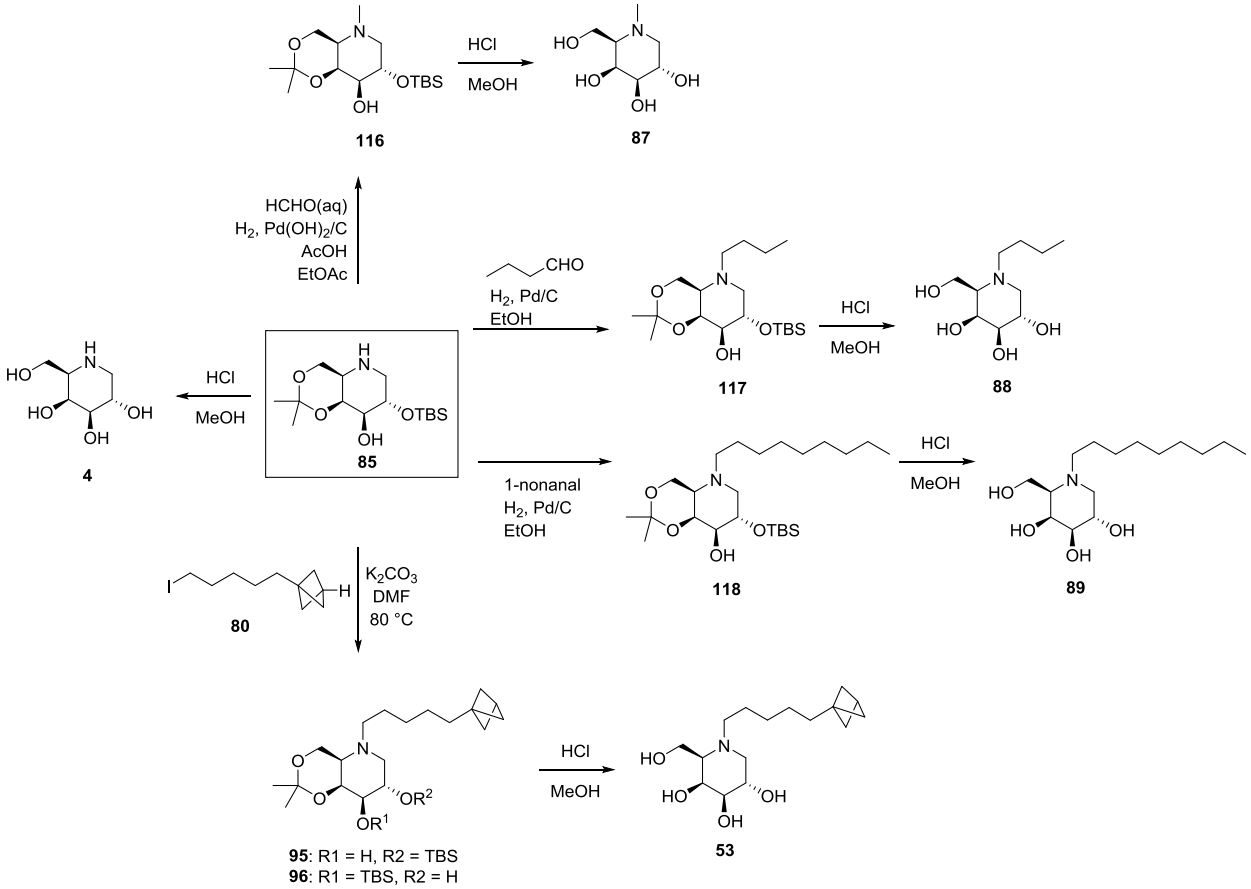
To a solution of compound **114** (1.32 g; 5.53 mmol) in methanol (20 mL) was added *p*-T_SOH (47.0 mg; 0.276 mmol) and the reaction mixture was stirred at rt. After 45 minutes triethyl amine (3 drops) was added, the mixture was concentrated under reduced pressure and the residue was purified by dry-flash

chromatography (eluent: hexane/ethyl acetate = 8:2) to give compound **115** (809 mg, 95%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62 (t, *J*=6.6, 2H), 2.44 (s, 1H), 1.63 (s, 6H), 1.57-1.52 (m, 2H), 1.40-1.24 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 63.0, 50.4, 45.8, 32.9, 32.7, 27.5, 26.5, 26.0. IR (ATR): $\tilde{\nu}$= 3331, 2960, 2929, 2867, 1461, 1280, 1194, 1054 cm^{-1}.

3.3. 1-(5-Iodopentyl)bicyclo[1.1.1]pentane (80)

To a solution of alcohol **115** (50.0 mg; 0.324 mmol), PPh_3 (127.5 mg; 0.486 mmol) and imidazole (66.1 mg; 0.972 mmol) in anhydrous THF (1.0 mL), iodine (123.8 mg; 0.486 mmol) was added portionwise at –10 °C, under an argon atmosphere. The cooling bath was removed and the reaction mixture was stirred for 15 min. The reaction mixture was diluted with diethyl ether, washed with saturated $Na_2S_2O_{3\,(aq)}$ and brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in pentane, filtered through a short pad of celite and evaporated under reduced pressure to yield 75 mg (88%) of the volatile, unstable iodide **80**, which was used in the next step without further purification.

4. Synthesis of α-galactosidase A inhibitors



Scheme S4 Synthesis of DGJ and the analogues thereof.

4.1. (2*R*,3*S*,4*R*,5*S*)-2-(hydroxymethyl)piperidine-3,4,5-triol (DGJ, migalastat, 4)[4]

The compound **4** was prepared from compound **85**[4] according to the literature procedure.[4]
^{1}H NMR (500 MHz, CD_3OD) δ 3.93-3.90 (m, 1H), 3.72 (td, *J* = 10.1, 5.3 Hz, 1H), 3.67-3.57 (m, 2H), 3.33-3.26 (m, 1H), 3.10 (dd, *J* = 12.5, 5.3 Hz, 1H), 2.63 (t, *J* = 6.5 Hz, 1H), 2.40-2.30 (m, 1H). ^{13}C NMR (125 MHz, CD_3OD) δ 77.4, 70.8, 69.9, 63.2, 61.3, 51.5.

4.2. (4a*R*,7*S*,8*S*,8a*S*)-7-((tert-butyldimethylsilyl)oxy)-2,2,5-trimethylhexahydro-4H-[1,3]dioxino[5,4-b]pyridin-8-ol (116)

To a solution of amine **85**[4] (15.5 mg; 0.047 mmol) in EtOAc (0.5 mL) were added 30% $HCHO_{(aq)}$ (28 µL), acetic acid (5 µL) and $Pd(OH)_2$ (15.0 mg) and the reaction mixture was stirred overnight under a hydrogen atmosphere (1 atm). The mixture was filtered, concentrated under reduced pressure and purified by column chromatography (ethyl acetate/methanol/25% $NH_{3\ (aq)}$= 19:1:0.05), to afford 14.8 mg (94%) of product **116**, as a colorless oil. $[\alpha]_D^{20}$ +42.5 (*c* 0.01 in MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.23-4.19 (m, 1H), 4.01-3.86 (m, 3H), 3.28 (td, *J* = 8.4, 4.1 Hz, 1H), 2.93 (dd, *J* = 11.2, 4.6 Hz, 1H), 2.35-2.37 (m, 1H), 2.30 (s, 3H), 1.96 (t, *J* = 10.7 Hz, 1H), 1.18 (s, 1H), 1.46 (s, 6H), 0.88 (s, 9H), 0.11 (s, 3H), 0.00 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 99.7, 75.4, 70.1, 69.6, 61.5, 60.9, 60.2, 42.7, 28.9, 26.0, 19.4, 18.2, -4.3, -4.4. IR (ATR): $\tilde{\nu}$= 3570, 2990, 2953, 2929, 2885, 2856, 1462, 1381, 1349, 1280, 1250, 1199 cm^{-1}. HRMS (m/z) $[M+H]^+$ calcd. for $C_{16}H_{34}NO_4Si$: 332.2252, found: 332.2259.

4.3. (2*R*,3*S*,4*R*,5*S*)-2-(hydroxymethyl)-1-methylpiperidine-3,4,5-triol (87)[5]

A solution of amine **116** (14.1 mg; 0.043 mmol) in methanol/3M $HCl_{(aq)}$ solvent mixture (0.93 mL, v/v = 3:1) was stirred at room temperature for 5 h. After the volatiles were removed under reduced pressure, the residue was purified by column chromatography (gradient ethyl acetate/methanol/25% $NH_{3\ (aq)}$= 9:1:0.05 to 1:1:0.05) to afford 5.9 mg (78%) of product **87**, as a viscous oil. $[\alpha]_D^{20}$ +0.15 (*c* 0.0067 in MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ 4.04-4.01 (m, 1H), 3.90 (td, *J* = 10.0, 5.0 Hz, 1H), 3.84 (d, *J* = 5.1 Hz, 2H), 3.28 (dd, *J* = 9.4, 3.2 Hz, 1H), 3.01 (dd, *J* = 11.3, 5.0 Hz, 1H), 2.42 (s, 3H), 2.27 (t, *J* = 4.7 Hz, 1H), 2.17 (t, *J* = 11.0 Hz, 1H). ^{13}C NMR (125 MHz, CD_3OD) δ 76.8, 72.0, 68.2 (2C), 62.3, 62.0, 42.6. IR (ATR): $\tilde{\nu}$= 3352, 2924, 2803, 1660, 1569, 1463, 1417, 1161 cm^{-1}.

4.4. (2*R*,3*S*,4*R*,5*S*)-1-Butyl-2-(hydroxymethyl)piperidine-3,4,5-triol (88)[6]

A mixture of amine **85**[4] (33.5 mg; 0.105 mmol), butanal (38.0 mg; 0.528 mmol) and 10% Pd/C (21.0 mg; 0.018 mmol) in ethanol (3.0 mL) was stirred overnight under a hydrogen atmosphere (4 atm). The mixture was filtered and concentrated under reduced pressure to afford crude amine **117**, which was used in the next step without further purification.
A solution of amine **117** (39.0 mg, 0.105 mmol) in methanol/3M $HCl_{(aq)}$ solvent mixture (1.6 mL, v/v = 3:1) was stirred at room temperature for 4 h. After the volatiles were removed under reduced pressure, the residue was purified by column chromatography (ethyl acetate/methanol/25% $NH_{3\ (aq)}$= 3:2:0.05), to

afford 11.4 mg (49%) of the product **88**, as a viscous oil. $[\alpha]_D^{20}$ −21.6 (*c* 0.47 in MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ 4.01-3.99 (m, 1H), 3.85-3.81 (m, 3H), 3.25 (dd, J_1=9.1, J_2=3.2, 1H), 3.03 (dd, J_1=11.4, J_2=5.0, 1H), 2.81-2.75 (m, 1H), 2.61-2.55 (m, 1H), 2.49 (bs, 1H), 2.21 (t, *J*=10.8, 1H), 1.54-1.47 (m, 2H) 1.35-1.27 (m, 2H), 0.95 (t, *J*=7.3, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 77.0, 72.0, 68.7, 65.3, 62.1, 57.7, 53.9, 27.1, 21.7, 14.3.

4.5. (4a*R*,7*S*,8*S*,8a*S*)-7-((tert-butyldimethylsilyl)oxy)-2,2-dimethyl-5-nonylhexahydro-4H-[1,3]dioxino[5,4-b]pyridin-8-ol (118)

A mixture of amine **85**[4] (30.5 mg; 0.096 mmol), nonanal (67.0 mg; 0.66 mmol) and 10% Pd/C (20.0 mg; 0.026 mmol) in ethanol (2.7 mL) was stirred for 2.5 h under a hydrogen atmosphere (4 atm). The mixture was filtered, concentrated under reduced pressure and purified by column chromatography (benzene/ethyl acetate = 7:3), to afford 27.3 mg (64%) of the product **118**, as a colorless oil. $[\alpha]_D^{20}$ −1.35 (*c* 0.01 in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.18-4.12 (m, 1H), 3.96-3.78 (m, 3H), 3.24 (td, *J* = 8.6, 4.2 Hz, 1H), 2.90 (dd, *J* = 11.2, 4.7 Hz, 1H), 2.64-2.43 (m, 2H), 2.29 (d, *J* = 8.1 Hz, 1H), 2,17 (s, 1H), 2.05 (t, *J* = 10.6 Hz, 1H), 1.41 (s, 6H), 1.32-1.18 (m, 14H), 0.89-0.81 (m, 12H), 0.09 (s, 3H), 0.07 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 99.7, 75.5, 70.6, 69.8, 61.1, 57.4, 56.6, 52.9, 32.0, 29.7 (2C), 29.4, 28.5, 27.6, 26.0, 24.1, 22.8, 20.0, 18.3, 14.2, -4.3 (2C). IR (ATR): $\tilde{\nu}$= 3571, 2990, 2954, 2856, 2797, 1463, 1381, 1252 cm^{-1}. HRMS (m/z) $[M+H]^+$ calcd. for $C_{24}H_{50}NO_4Si$: 444.3504, found: 444.3514.

4.6. (2*R*,3*S*,4*R*,5*S*)-2-(hydroxymethyl)-1-nonylpiperidine-3,4,5-triol (89)[7]

A solution of amine **118** (16.0 mg, 0.036 mmol) in methanol/3M $HCl_{(aq)}$ solvent mixture (0.76 mL, v/v = 3:1) was stirred at room temperature for 4.5 h. After the volatiles were removed under reduced pressure, the residue was purified by column chromatography (gradient ethyl acetate/methanol = 19:1 to 1:1), to afford 8.3 mg (80%) of the product **89**, as a viscous oil. ^{1}H NMR (500 MHz, CD_3OD) δ 4.11-4.06 (m, 1H), 3.99-3.84 (m, 3H), 3.41 (dd, *J* = 8.9, 2.8 Hz, 1H), 3.31-3.22 (m, 3H), 3.16-2.95 (m, 3H), 2.68 (t, *J* = 11.1 Hz, 1H), 1.76-1.56 (m, 2H), 1.40-1.20 (m, 10H), 0.88 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 75.2,71.3, 67.0, 66.1, 61.0, 55.4 (2C), 33.0, 30.6, 30.3, 28.0, 24.3, 23.7, 14.4.

4.7. (4a*R*,7*S*,8*S*,8a*S*)-5-(5-(bicyclo[1.1.1]pentan-1-yl)pentyl)-7-((tert-butyldimethylsilyl)oxy)-2,2-dimethylhexahydro-4H-[1,3]dioxino[5,4-b]pyridin-8-ol (95) and (4a*R*,7*S*,8*R*,8a*S*)-5-(5-(bicyclo[1.1.1]pentan-1-yl)pentyl)-8-((tert-butyldimethylsilyl)oxy)-2,2-dimethylhexahydro-4H-[1,3]dioxino[5,4-b]pyridin-7-ol (96)

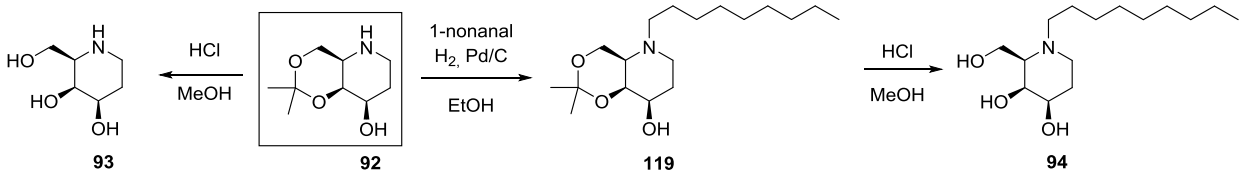
A solution of amine **85**[4] (30.0 mg; 0.095 mmol), iodide **80** (37.0 mg; 0.14 mmol) and K_2CO_3 (46.0 mg; 0.33 mmol) in DMF (0.3 mL) was stirred at 80 °C under an argon atmosphere. After 6 h, the mixture was diluted with diethyl ether, washed with saturated $NaHCO_{3(aq)}$ and H_2O, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by column chromatography (petroleum ether/ethyl acetate = 7:3), to afford 27.7 mg (63%) of the product **95** and 13.7 mg (31%) of the product **96**, both as viscous oils.

(4a*R*,7*S*,8*S*,8a*S*)-5-(5-(bicyclo[1.1.1]pentan-1-yl)pentyl)-7-((tert-butyldimethylsilyl)oxy)-2,2-dimethylhexahydro-4H-[1,3]dioxino[5,4-b]pyridin-8-ol (**95**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.19-4.14 (m, 1H), 3.97-3.79 (m, 3H), 3.26 (td, *J* = 8.5, 4.1 Hz, 1H), 2.92 (dd, *J* = 11.2, 4.7 Hz, 1H), 2.65-2.45 (m, 2H), 2.42 (s, 1H), 2.31 (d, *J* = 8.2 Hz, 1H), 2.19 (br s, 1H), 2.07 (t, *J* = 10.6 Hz, 1H), 1.61 (s, 6H), 1.43 (s, 6H), 1.40-1.17 (m, 8H), 0.89 (s, 9H), 0.10 (d, *J* = 9.3 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 99.7, 77.5, 70.6, 69.7, 61.1, 57.4, 56.6, 52.8, 50.4, 45.9, 32.7, 28.4, 27.7, 27.5, 26.6, 26.0, 24.2, 20.0, 18.2, -4.3, -4.4. IR (ATR): $\tilde{\nu}$= 3572, 3494, 2958, 2928, 2905, 2867, 1462, 1381, 1278, 1252, 1220 cm^{-1}. HRMS (m/z) $[M+H]^+$ calcd. for $C_{25}H_{48}NO_4Si$: 454.3347, found: 454.3359.

(4a*R*,7*S*,8*R*,8a*S*)-5-(5-(bicyclo[1.1.1]pentan-1-yl)pentyl)-8-((tert-butyldimethylsilyl)oxy)-2,2-dimethylhexahydro-4H-[1,3]dioxino[5,4-b]pyridin-7-ol (**96**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.06-4.02 (m, 1H), 4.02-3.92 (m, 2H), 3.85 (dd, J = 12.7, 2.9 Hz, 1H), 3.35 (dd, J = 9.3, 3.7 Hz, 1H), 3.14 (dd, J = 10.9, 4.5 Hz, 1H), 2.67-2.56 (m, 1H), 2.48-2.38 (m, 1H), 2.42 (s, 1H), 2.15 (br s, 1H), 2.11-2.02 (m, 2H), 1.61 (s, 6H), 1.48-1.16 (m, 8H), 1.42 (s, 3H), 1.40 (s, 3H), 0.92 (s, 9H), 0.12 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 99.3, 77.7, 70.4, 68.2, 61.5, 57.1, 55.9, 53.0, 50.5, 45.9, 32.7, 28.5, 27.7, 27.5, 26.6, 26.0, 24.9, 20.1, 18.4, -4.1, -4.2. IR (ATR): $\tilde{\nu}$= 3566, 2958, 2929, 2904, 2800, 1463, 1380, 1251, 1195 cm^{-1}. HRMS (m/z) $[M+H]^+$ calcd. for $C_{25}H_{48}NO_4Si$: 454.3347, found: 454.3363.

4.8. (2*R*,3*S*,4*R*,5*S*)-1-(5-(bicyclo[1.1.1]pentan-1-yl)pentyl)-2-(hydroxymethyl)piperidine-3,4,5-triol (53)

Described here is the preparation of **53** from **95**; deprotection of **96** also afforded product **53**. A solution of amine **95** (22.1 mg; 0.047 mmol) in methanol/3M $HCl_{(aq)}$ solvent mixture (1.05 mL, v/v = 3.2:1) was stirred at room temperature for 48 h. After the volatiles were removed under reduced pressure, the residue was purified by three consecutive chromatographies: column chromatography (gradient ethyl acetate/methanol/25% $NH_{3\,(aq)}$= 9:1:0.05 to 3:2:0.05), ion exchange chromatography (H_2O then 1M $NH_{3\,(aq)}$) and column chromatography (ethyl acetate/methanol/25% $NH_{3\,(aq)}$= 7:3:0.05) to afford 7.1 mg (50%) of the product **53**, as a viscous oil. $[\alpha]_D^{20}$ −12.9 (*c* 0.0059 in MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ 4.00 (dd, *J* = 3.2, 1.8 Hz, 1H), 3.86-3.79 (m, 3H), 3.25 (dd, *J* = 9.2, 3.3 Hz, 1H), 3.03 (dd, *J* = 11.3, 4.9 Hz, 1H), 2.82-2.74 (m, 1H), 2.64-2.55 (m, 1H), 2.54-2.49 (m, 1H), 2.43 (s, 1H), 2.23 (t, *J* = 10.8 Hz, 1H), 1.67 (s, 5H), 1.58-1.48 (m, 2H), 1.45-1.39 (m, 2H), 1.35-1.24 (m, 5H). ^{13}C NMR (125 MHz, CD_3OD) δ 76.9, 72.0, 68.6, 65.4, 62.1, 57.5, 54.1, 51.2, 46.8, 33.6, 28.7, 28.2, 27.5, 24.9. IR (ATR): $\tilde{\nu}$= 3366, 2960, 2867, 2241, 2078, 1622, 1423, 1354, 1194 cm^{-1}. HRMS (m/z) $[M+H]^+$ calcd. for $C_{16}H_{30}NO_4$: 300.2169, found: 300.2177.



Scheme S5 Synthesis of 4-*epi*-fagomine and the *N*-alkylated analogue.

4.9. (2*R*,3*S*,4*R*)-2-(hydroxymethyl)piperidine-3,4-diol (93)[8]

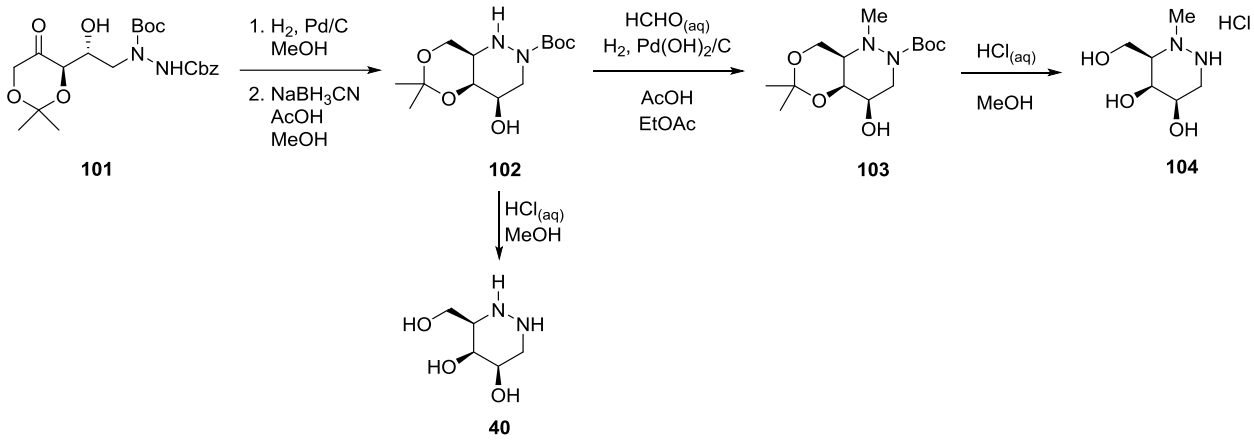
The compound **93** was prepared from **92**[8] (made using D-proline as the catalyst) according to the literature procedure.[8]
^{1}H NMR (500 MHz, CD_3OD) δ 3.97-3.94 (m, 1H), 3.86-3.73 (m, 3H), 3.37-3.29 (m, 1H), 3.27-3.20 (m, 1H), 3.01 (td, *J* = 13.4, 3.4 Hz, 1H), 2.14-2.00 (m, 1H), 1.90-1.81 (m, 1H). ^{13}C NMR (125 MHz, CD_3OD) δ 69.2, 67.8, 62.1, 61.2, 43.7, 26.2.

4.10. (4a*R*,8*R*,8a*S*)-2,2-dimethyl-5-nonylhexahydro-4H-[1,3]dioxino[5,4-b]pyridin-8-ol (119)

A mixture of amine **92**[8] (made using D-proline as the catalyst) (25.5 mg; 0.136 mmol), nonanal (95.0 mg; 0.66 mmol) and 10% Pd/C (28.0 mg; 0.026 mmol) in ethanol (3.8 mL) was stirred for 3 h under a hydrogen atmosphere (4 atm). The mixture was filtered, concentrated under reduced pressure and purified by column chromatography (gradient methylene chloride/methanol = 49:1 to 7:3), to afford 23.2 mg (54%) of the product **119**, as a colorless oil. $[\alpha]_D^{20}$ −24.8 (*c* 0.01 in MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ 4.14-4.07 (m, 1H), 4.01-3.88 (m, 2H), 3.49 (dt, *J* = 11.9, 4.1 Hz, 1H), 2.93 (dt, *J* = 11.6, 3.0 Hz, 1H), 2.72-2.62 (m, 1H) 2.52-2.42 (m, 1H), 2.26 (t, *J* = 11.8 Hz, 1H), 2.10 (s, 1H), 1.95 (qd, *J* = 12.3, 3.8 Hz, 1H), 1.66-1.56 (m, 1H), 1.53-1.36 (m, 8H), 1,36-1.18 (m, 12H), 0.87 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 101.4, 71.8, 71.3, 62.8, 58.6, 55.3, 52.2, 33.9, 31.6, 31.5, 31.3, 30.6, 29.8, 28.7, 25.6, 24.6, 20.5, 15.3. IR (ATR): $\tilde{\nu}$= 3580, 3442, 2989, 2926, 2855, 2792, 1465, 1380, 1346, 1270, 1228 cm^{-1}. HRMS (m/z) $[M+H]^+$ calcd. for $C_{18}H_{36}NO_3$: 314.2690, found: 314.2699.

4.11. (2*R*,3*S*,4*R*)-2-(hydroxymethyl)-1-nonylpiperidine-3,4-diol (94)

A solution of amine **119** (18.4 mg, 0.059 mmol) in methanol/3M $HCl_{(aq)}$ solvent mixture (1.2 mL, v/v = 3:1) was stirred at room temperature for overnight. After the volatiles were removed under reduced pressure, the residue was purified by column chromatography (gradient methylene chloride/methanol = 49:1 to 1:1), to afford 11.3 mg (70%) of the product **94**, as a viscous oil. $[\alpha]_D^{20}$ −5.8 (*c* 0.0093 in MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ 4.08-4.03 (m, 1H), 4.01-3.89 (m, 2H), 3.80-3.71 (m, 1H), 3.40-3.35 (m, 1H), 3.25-2.95 (m, 4H), 2.11 (qd, *J* = 13.1, 4.3 Hz, 1H), 1.91-1.81 (m, 1H), 1.80-1.61 (m, 2H), 1.45-1.25 (m, 12H), 0.92 (t, *J*=6.6, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 69.3, 65.9, 33.0, 30.5, 30.3, 27.9, 23.7, 14.4. IR (ATR): $\tilde{\nu}$= 3342, 2956, 2925, 2855, 1575, 1467 cm^{-1}. HRMS (m/z) $[M+H]^+$ calcd. for $C_{15}H_{32}NO_3$: 274.2377, found: 274.2384.



Scheme S6 Synthesis of AGF and the *N*-alkylated analogue.

4.12. (3*R*,4*S*,5*R*)-3-(hydroxymethyl)hexahydropyridazine-4,5-diol (AGF, 40)[8]

The compound **40** was prepared according to the literature procedure.[8]
^{1}H NMR (500 MHz, D_2O) δ 4.02-3.99 (m, 1H), 3.79 (ddd, *J* = 11.0, 5.2, 3.0 Hz, 1H), 3.64 (d, *J* = 6.3 Hz, 2H), 2.96-2.87 (m, 2H), 2.81 (dd, *J* = 12.6, 11.1 Hz, 1H). ^{13}C NMR (125 MHz, D_2O) δ 64.9, 63.7, 58.1, 57.6, 43.5. IR (ATR) $\tilde{\nu}$= 3274, 2932, 1570, 1413, 1102, 1029, 817, 657 cm^{-1}. HRMS (m/z) $[M+H]^+$ calcd. for $C_5H_{13}N_2O_3$: 149.0921; found: 149.0924.

4.13. *tert*-Butyl (4*R*,4a*S*,8a*R*)-4-hydroxy-6,6-dimethyltetrahydro-1H-[1,3]dioxino[5,4-c]pyridazine-2(3H)-carboxylate (102)[8]

A mixture of aldol **101**[8] (500.0 mg; 1.140 mmol) and 10% Pd/C (100.0 mg; 0.094 mmol) in methanol (30.0 mL) was stirred under a hydrogen atmosphere (1 atm) for 1 hour. The mixture was filtered through a short pad of celite and evaporated under reduced pressure to afford crude product, which was used in the next step without further purification.
Crude product from the previous step was dissolved in methanol (2.0 mL) and acetic acid (8.0 mL) was added to the solution. After 2 min, sodium cyanoborohydride (215.0 mg; 3.421 mmol) was added and the mixture was stirred for 30 min at room temperature. After reaction completion, the mixture was diluted with dichloromethane and washed with H_2O, saturated $NaHCO_{3(aq)}$ and brine. Organic layer was dried over anhydrous $MgSO_4$ and volatiles were removed under reduced pressure. The residue was purified by dry-flash chromatography (ethyl acetate/petroleum ether/methanol = 76:20:4), to afford 212.7 mg (65%) of the product **102**, as a white solid. mp 180-181 °C. $[\alpha]_D^{20}$ +50.9 (c 1.20, MeOH). ^{1}H NMR (500 MHz, DMSO-d6, 65 °C) δ 4.59 (d, *J* = 5.7 Hz, 1H), 4.23 (d, *J* = 11.4 Hz, 1H), 4.07-4.00 (m, 2H), 3.81 (dd, *J* = 12.2, 5.2 Hz, 1H), 3.60 (dd, *J* = 12.3, 1.6 Hz, 1H), 3.49-3.42 (m, 1H), 2.90 (t, *J* = 11.7 Hz, 1H), 2.54-2.50 (m, 1H), 1.41 (s, 3H), 1.40 (s, 9H), 1.35 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d6, 65 °C) δ 154.4, 97.9, 78.5, 66.5, 65.8, 61.3,

51.3, 45.0, 28.9, 27.8, 18.5. IR (ATR) $\tilde{\nu}$= 3417, 3250, 2983, 2973, 1695, 1502, 1385, 1177, 1066, 993, 853 cm^{-1}. HRMS (m/z) $[M+Na]^+$ calcd. for $C_{13}H_{24}N_2NaO_5$: 311.1577; found: 311.1584

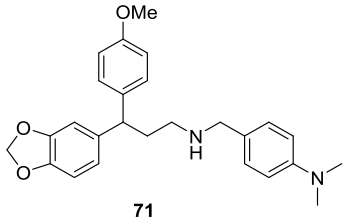
4.14. *tert*-Butyl (4*R*,4a*S*,8a*R*)-4-hydroxy-1,6,6-trimethyltetrahydro-1H-[1,3]dioxino[5,4-c]pyridazine-2(3H)-carboxylate (103)

A mixture of **102**[8] (10.0 mg; 0.035 mmol), 30% formaldehyde solution in water (19 µL; 0.208 mmol), 10% $Pd(OH)_2/C$ (10.0 mg; 0.009 mmol), ethyl acetate (300 µL) and catalytic amount of acetic acid was stirred under a hydrogen atmosphere (1 atm) for 6 hours. The mixture was diluted with ethyl acetate and filtered through a short pad of celite and volatiles were evaporated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 1:2), to afford 8.8 mg (84%) of the product **103**. ^{1}H NMR (400 MHz, $CDCl_3$) δ4.09 (m, 1H), 4.05 (d, *J* = 3.4 Hz, 1H), 4.03-3.95 (m, 1H), 3.93-3.80 (m, 1H), 3.65 (m, 1H), 3.15 (bt, *J* = 11.8 Hz, 1H), 2.84 (bs, 4H), 2.51 (d, *J* = 10.6 Hz, OH), 1.48 (s, 3H), 1.45 (s, 9H), 1.42 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ155.4, 99.4, 80.4, 67.1, 66.4, 61.7, 53.9, 39.6, 29.5, 28.4, 18.9.

4.15. (4*R*,5*S*,6*R*)-6-(Hydroxymethyl)-1-methylhexahydropyridazine-4,5-diol hydrochloride (104)

To a solution of **103** (8.3 mg; 0.027 mmol) in methanol (300 µL) a 3 M $HCl_{(aq)}$ (300 µL) was added dropwise and the resulting mixture was stirred for 24 hours at room temperature. Volatiles were removed under reduced pressure resulting in 5.4 mg (100%) of pure product **104**. ^{1}H NMR (400 MHz, D_2O) δ4.28 (m, 1H), 3.98 (d, *J* = 4.9 Hz, 2H), 3.98-3.93 (m, 1H), 3.39-3.28 (m, 2H), 3.21 (dd, *J* = 13.2, 5.2 Hz, 1H), 3.04 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.1, 67.1, 65.0, 59.6, 44.5, 41.6. HRMS (m/z) $[M+H]^+$ calcd. for $C_6H_{14}N_2O_3$: 163.1077; found: 163.1171.

5. Synthesis of non-iminosugar-type mannosidase inhibitors



71

The compound **AR 524, 71** was prepared according to the literature procedure.[9]
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17-7.09 (m, 4H), 6.80 (d, *J* = 8.3 Hz, 2H), 6.72-6.64 (m,5H), 5.88 (s, 2H), 3.88 (t, *J* = 7.8 Hz, 1H), 3.76 (s, 3H), 3.63 (s, 2H), 2.93-2.88 (s, 7H), 2.59 (t, *J* = 7.3 Hz, 2H), 2.16 (dt, *J* = 4.6 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.0, 150.0, 147.8, 145.9, 139.3, 137.1, 129.4, 128.7, 120.7, 114.0, 112.8, 108.3, 108.2, 100.9, 55.3, 53.2, 47.9, 47.3, 40.8, 35.7. IR (ATR) $\tilde{\nu}$= 2992, 2834, 2804, 1613, 1511, 1486, 1440, 1247, 1179, 1038, 936, 807, 807. HRMS (ESI) m/z calcd. for $C_{26}H_{30}N_2O_3$ 419.2329 $[M+H]^+$; found 419.2319.

6. Biochemical tests

6.1. Inhibition assay for α-glucosidase

6.1.1. Yeast α-glucosidase expression and purification

Expression of recombinant α-glucosidase in *Escherichia coli* and purification of the enzyme were performed according to modified literature procedures.[10,11]

Expression of recombinant enzyme in *Escherichia coli*

A synthetic gene encoding α-glucosidase from *Saccharomyces cerevisiae* was inserted into pET-22b(+) vector to produce recombinant protein in cytosol of *Escherichia coli*. Competent *E. coli* cells, BL21 (DE3) strain, were transformed with α-glucosidase-pET-22b(+) construct and vector pET-22b(+) without insert using standard heat-shock protocol. The vector without insert was used as a control of protein expression. The *E. coli* transformed cells were picked from sterile Luria broth (LB) solid plates supplemented with ampicillin (final 100 mg/L) and inoculated into sterile LB liquid medium with ampicillin (final 100 mg/L). Cells were incubated at 37 °C, 200 rpm overnight in the Orbital Shaker-Incubator ES-20. β-D-1-Thiogalactopyranoside (IPTG) in final concentration 0.4 mM was added when cell suspension OD600 reached 0.6 and the culture was further incubated for 18 h at 27 °C.

Enzyme purification

Recombinant enzyme produced by *E. coli* was harvested 18 h after induction. The culture was centrifuged in Beckman Centrifuge J-6M (30 min, 3000 rpm, 4 °C) and the collected cells were resuspended in lysis buffer (50 mM sodium phosphate, pH 7.0, 300 mM sodium chloride, 10 mM imidazole). Cells containing enzyme of interest were lysed by sonication (10 times for 10 s with 30 s breaks) on ice. The cell lysate was centrifuged for 30 min at 13400 rpm and the supernatant was filtered through 0.22 μm sterile filter.

HisTrap fast flow Ni-NTA 5 mL column was equilibrated on a HPLC system with 25 mL of 50 mM sodium phosphate, pH 7.0 buffer (supplemented with 300 mM sodium chloride and 10 mM imidazole). Afterwards, concentrated supernatant containing α-glucosidase was loaded to the column. The column was washed with 25 mL of the same buffer. Proteins were eluted using linear gradient 10–500 mM imidazole in the same buffer. Fractions with α-glucosidase activity were analysed by SDS gel electrophoresis. Pooled fractions were dialyzed against 20 mM sodium phosphate buffer, pH 7.0 with 10% glycerol and 1 mM DTT. The protein was of satisfactory purity. Electrophoregram showed the presence of a band of the correct molecular mass, about 65 kDa (Figure 25).

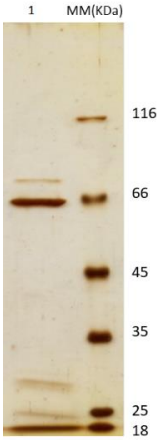
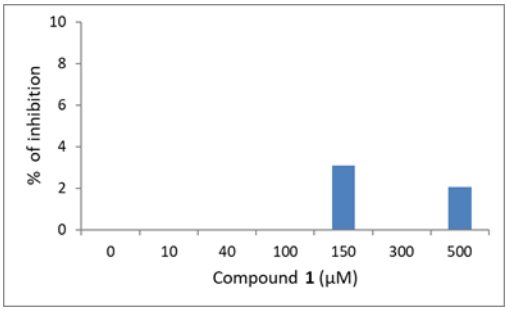


Figure S25 Silver-stained SDS electrophoregram: Sample 1 is α-glucosidase; MM stands for molecular markers

6.1.2.**Inhibition assay for α-glucosidase**

Inhibition of α-glucosidase by iminosugars was assayed by measuring hydrolysis rate of *p*-nitrophenyl glucoside, using modified literature procedure.[12] The purified enzyme was used for inhibition assays. The enzyme assay was conducted in 96-well microtiter plate by measuring the quantity of *p*-nitrophenol released from the substrate *p*-nitrophenyl α-D-glucopyranoside (pNPG) spectrophotometerically on a LKB 5060-006 Microplate Reader. Purified α-glucosidase (2.5 µg/mL) was incubated with tested inhibitors at various concentrations (0-0.5 mM) in 100 mM sodium phosphate buffer pH 7.0 for 30 min at 37 °C (Biosan PST-60H, Plate Shaker-Thermostat), and then pNPG (1.2 mM) was added (ε_{pNP} = 8.3 $mM^{-1}cm^{-1}$ at 405 nm) and the absorbance change was followed at 405 nm for 25 min. All measurements were done in triplicates. Percentage of inhibition was calculated from the residual activity in comparison to the the control sample. The linear regression analysis was performed using GraphPad linear regression calculator.[13] The results are shown in Figure 8 and Figures S26-S31.



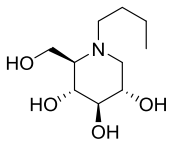


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Figure S26 Dependence of percentage of inhibition of α-glucosidase on concentration of compound **1.**

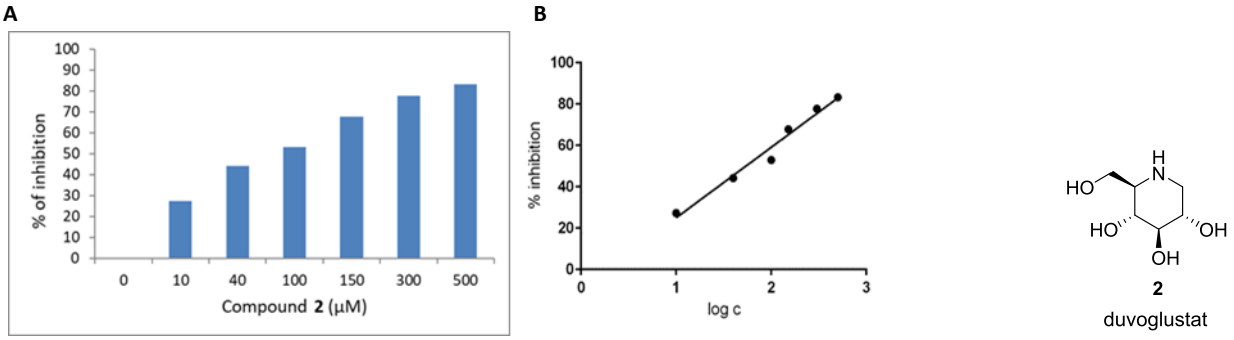


Figure S27 Dependence of percentage of inhibition of α-glucosidase on concentration (**A**) and logc (**B**) of compound **2**.

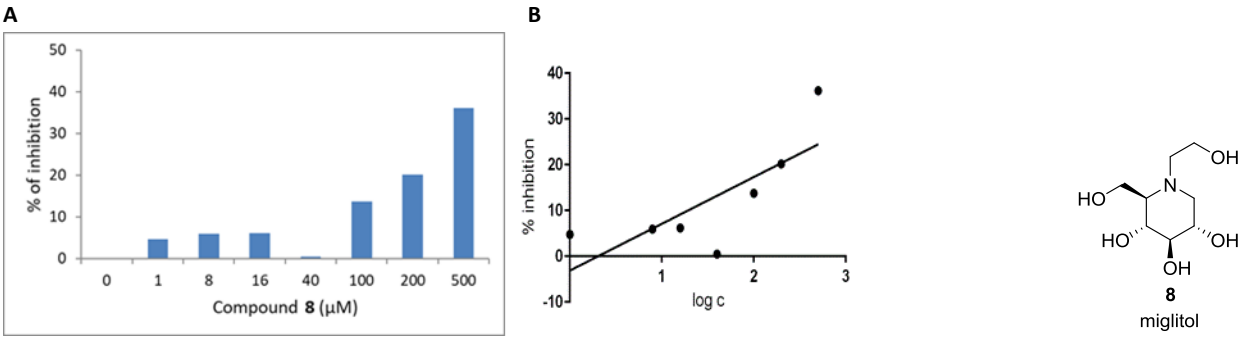


Figure S28 Dependence of percentage of inhibition of α-glucosidase on concentration (**A**) and logc (**B**) of compound **8**.

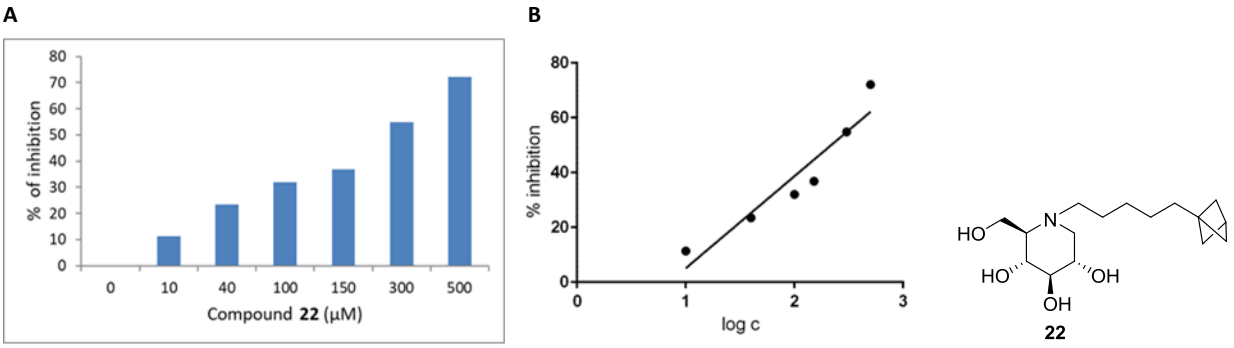


Figure S29 Dependence of percentage of inhibition of α-glucosidase on concentration (**A**) and logc (**B**) of compound **22**.

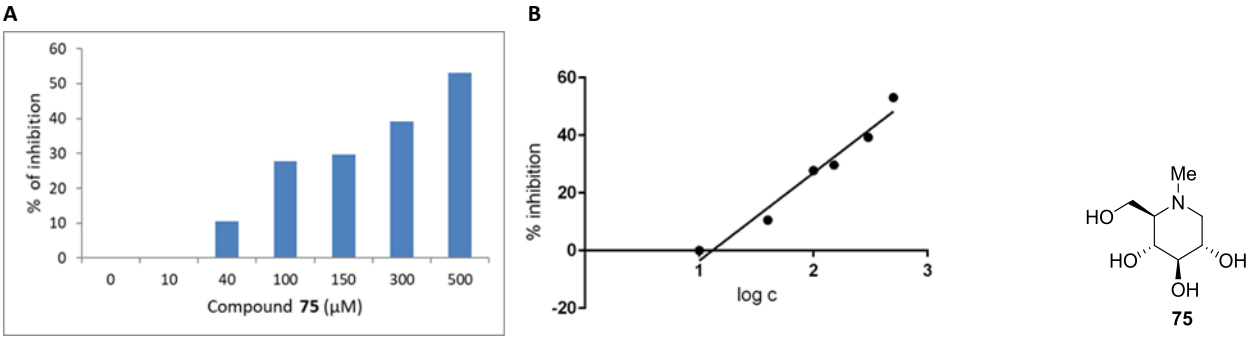


Figure S30 Dependence of percentage of inhibition of α-glucosidase on concentration (**A**) and logc (**B**) of compound **75**.

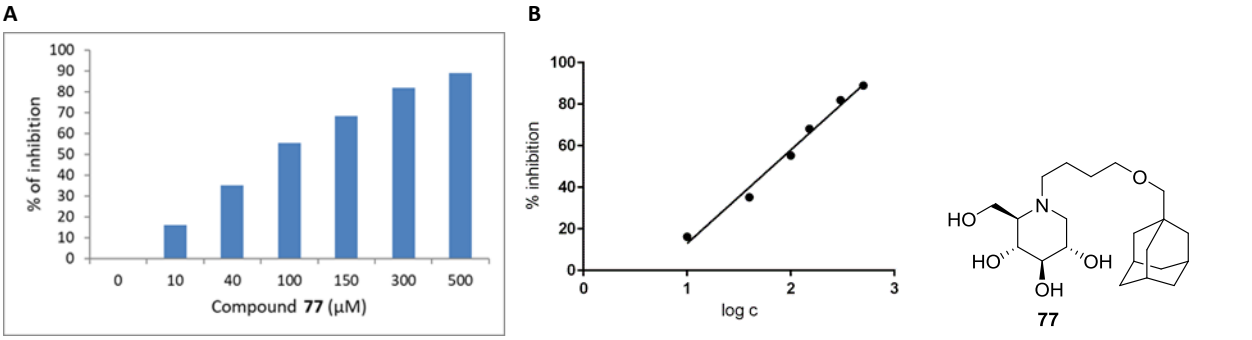


Figure S31 Dependence of percentage of inhibition of α-glucosidase on concentration (**A**) and logc (**B**) of compound **77**.

6.2. Inhibition assay for α-galactosidase

Inhibition of α-galactosidase A by iminosugars was assayed by measuring hydrolysis rate of *p*-nitrophenyl galactoside, using modified literature procedure.[14] Recombinant human α-galactosidase A was purchased from R&D Systems (Minneapolis, USA). The enzyme assay was conducted in 96-well microtiter plate by measuring the quantity of *p*-nitrophenol released from the substrate *p*-nitrophenyl α-D-galactopyranoside by the commercial α-galactosidase A. Increase in the absorbance at 405 nm was monitored for 90 min spectrophotometrically on a LKB 5060-006 Microplate Reader. The enzyme (0.1 μg/μL) was incubated with tested inhibitors at different concentrations (0.1–100 μM) in 100 mM citrate-phosphate buffer pH 4.5 for 30 min at 37 °C (Biosan PST-60H, Plate Shaker-Thermostat). Substrate *p*-nitrophenyl α-D-galactopyranoside (4 mM) was added at the end of enzyme–inhibitor incubation. Reaction aliquotes were taken during 90 min, mixed with 0.4 M sodium carbonate solution and the absorbance was measured at 405 nm. All measurements were done in triplicates. Percentage of inhibition was calculated from the residual activity in comparison to the control sample. The linear regression

analysis was performed using GraphPad linear regression calculator.[4] The results are shown in Figure 9 and Figures S32-S41.

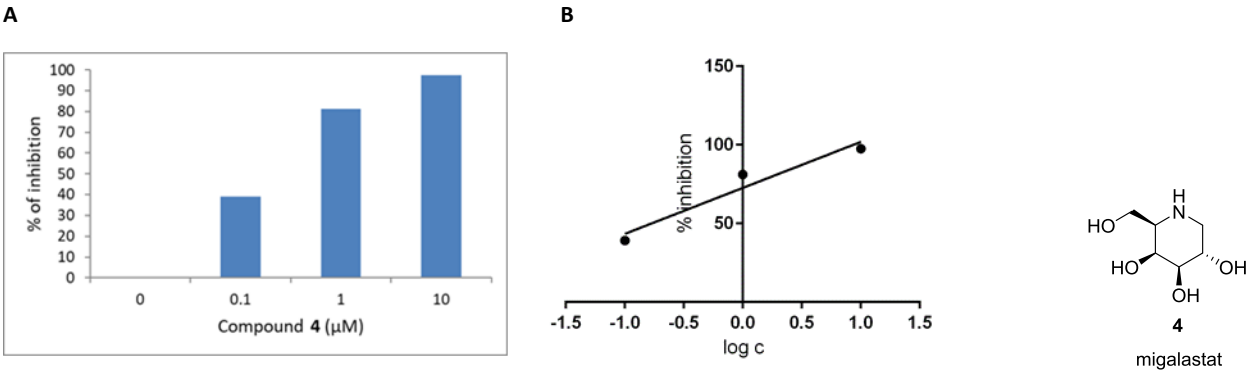


Figure S32 Dependence of percentage of inhibition of α-galactosidase A on concentration (**A**) and logc (**B**) of compound **4**.

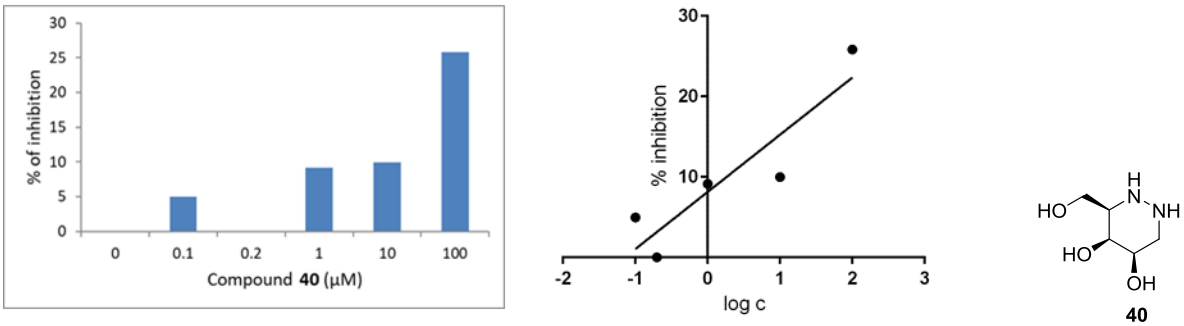


Figure S33 Dependence of percentage of inhibition of α-galactosidase A on concentration (**A**) and logc (**B**) of compound **40**.

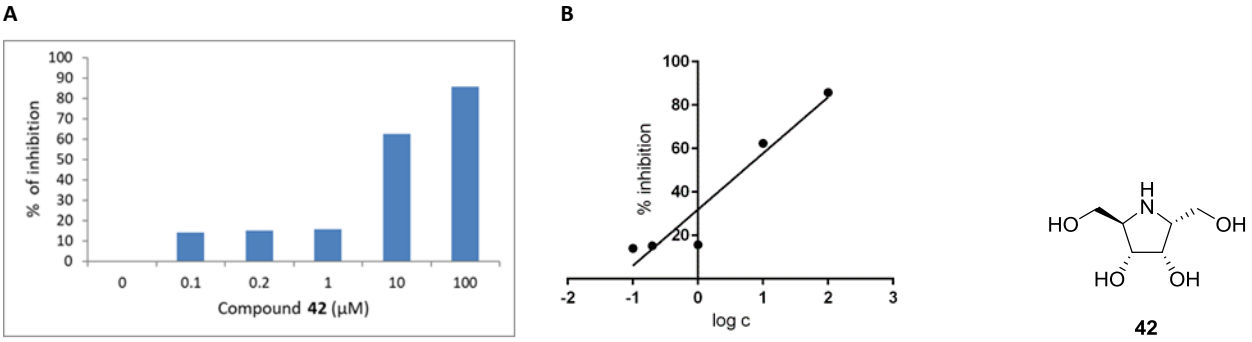


Figure S34 Dependence of percentage of inhibition of α-galactosidase A on concentration (**A**) and logc (**B**) of compound **42**.

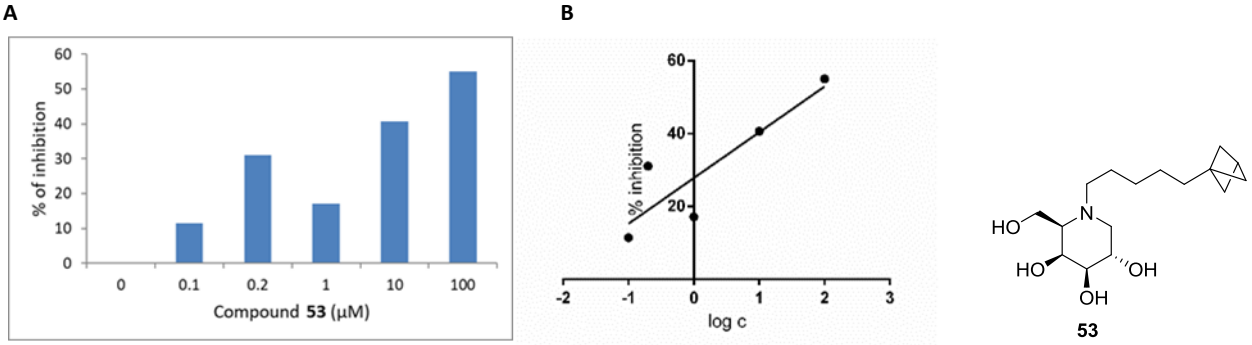


Figure S35 Dependence of percentage of inhibition of α-galactosidase A on concentration (**A**) and logc (**B**) of compound **53**.

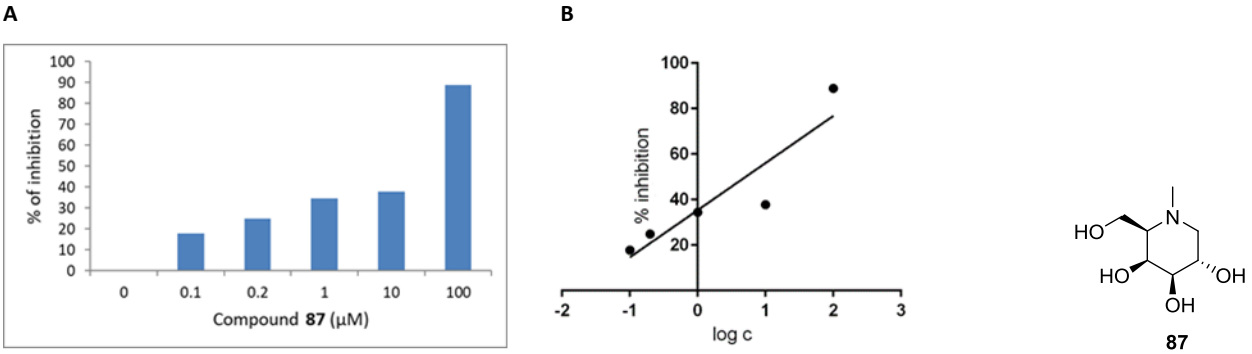
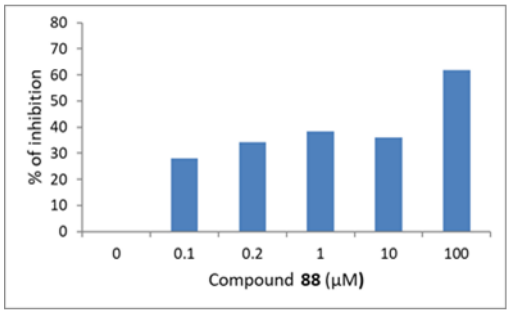
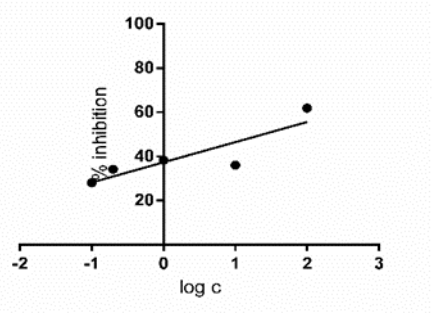


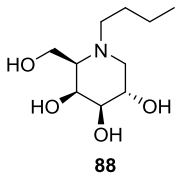
Figure S36 Dependence of percentage of inhibition of α-galactosidase A on concentration (**A**) and logc (**B**) of compound **87**.

A



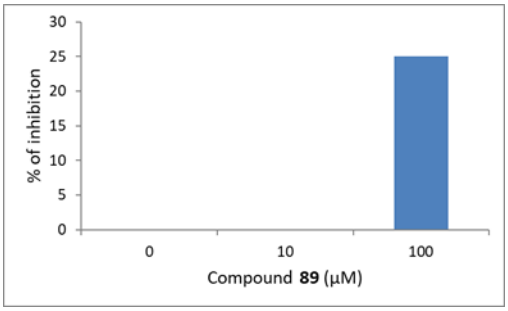
B

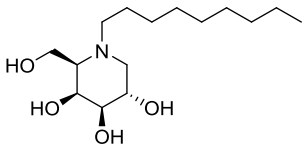




88

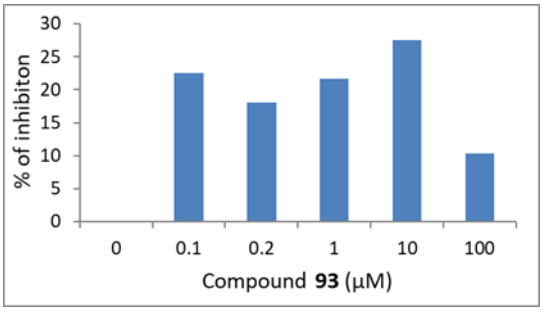
Figure S37 Dependence of percentage of inhibition of α-galactosidase A on concentration (**A**) and logc (**B**) of compound **88**.

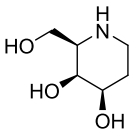




89

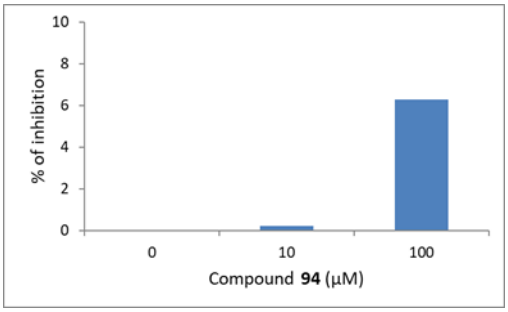
Figure S38 Dependence of percentage of inhibition of α-galactosidase A on concentration of compound **89**.

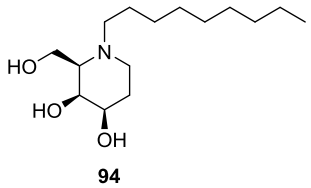




93

Figure S39 Dependence of percentage of inhibition of α-galactosidase A on concentration of compound **93**.

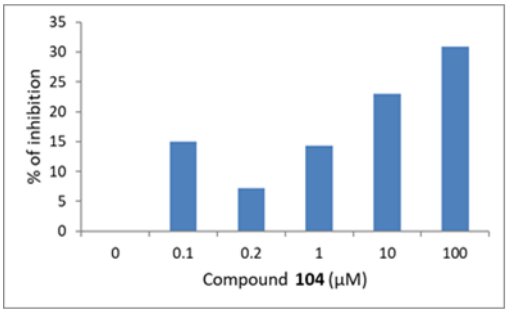




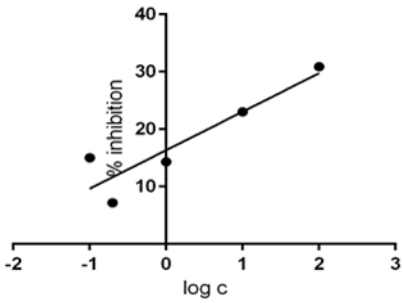
94

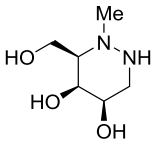
Figure S40 Dependence of percentage of inhibition of α-galactosidase A on concentration of compound **94.**

A



B





104

Figure S41 Dependence of percentage of inhibition of α-galactosidase A on concentration (**A**) and logc (**B**) of compound **104**.

7. Virology

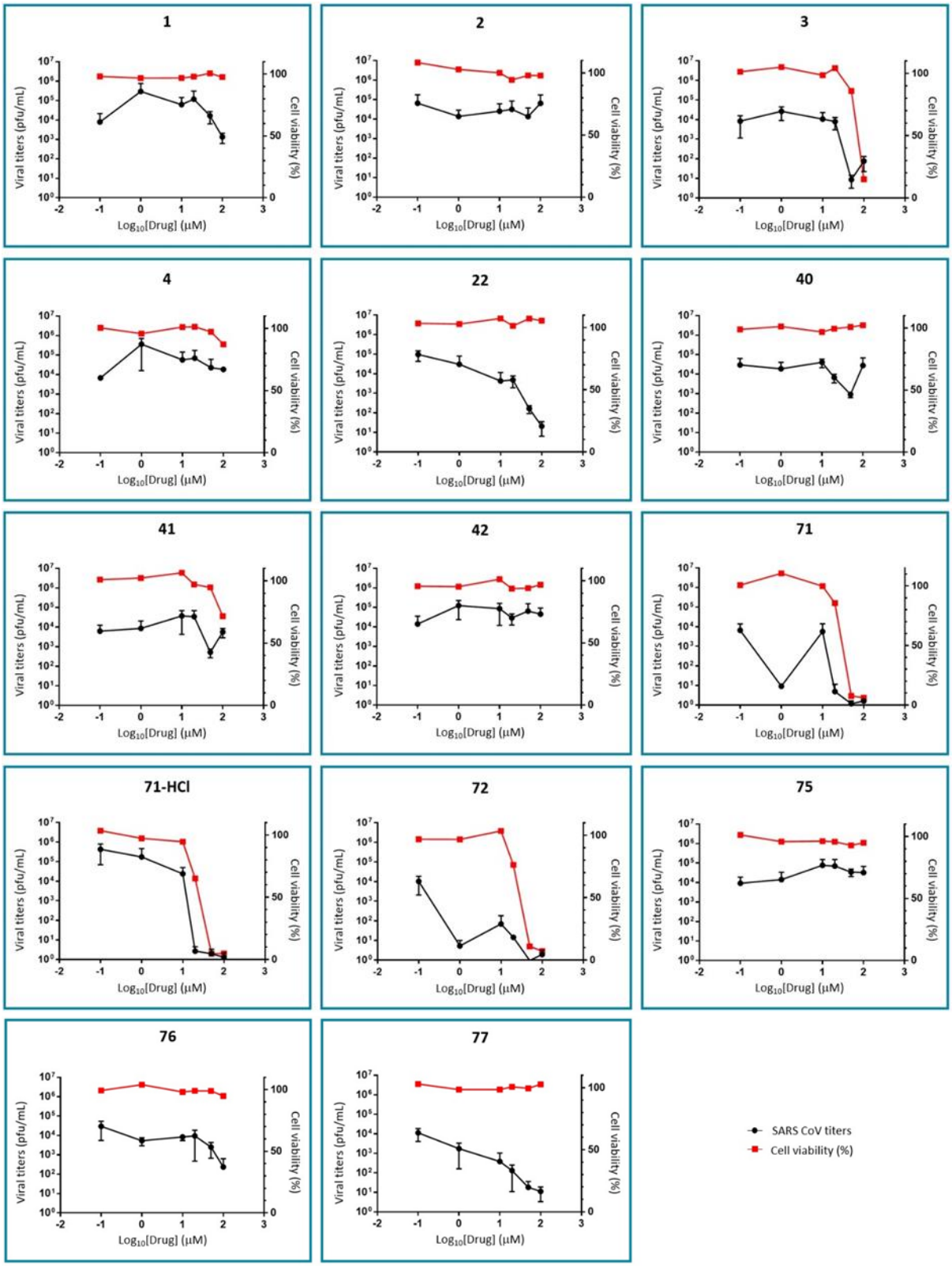


Figure S42 Antiviral activities and cell viabilities for all samples.

The numeric data for the antiviral assays can be downloaded as .xlsx file at the address:

https://www.chem.bg.ac.rs/~mario/SmartRepPVP/

The numeric data for the cytotoxicity assays can be downloaded as .xlsx file at the address:

https://www.chem.bg.ac.rs/~mario/SmartRepCyt/

8. References

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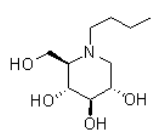
9. Copies of NMR spectra for selected compounds

(ordered by increasing compound numbers)



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7	Pulse Width	0.0000
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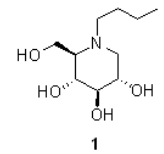
9	Spectrometer Frequency	399.73
10	Nucleus	1H



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5	Receiver Gain	30
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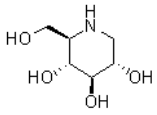
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C



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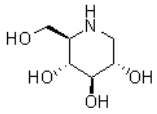
7	Pulse Width	0.0000
8	Acquisition Time	2.2807
9	Spectrometer Frequency	399.73
10	Nucleus	1H



2

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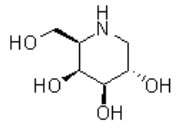
5	Receiver Gain	30
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C



4

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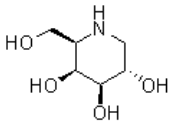
7	Pulse Width	0.0000
8	Acquisition Time	2.2807
9	Spectrometer Frequency	399.73
10	Nucleus	1H



4

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3	Temperature	25.0
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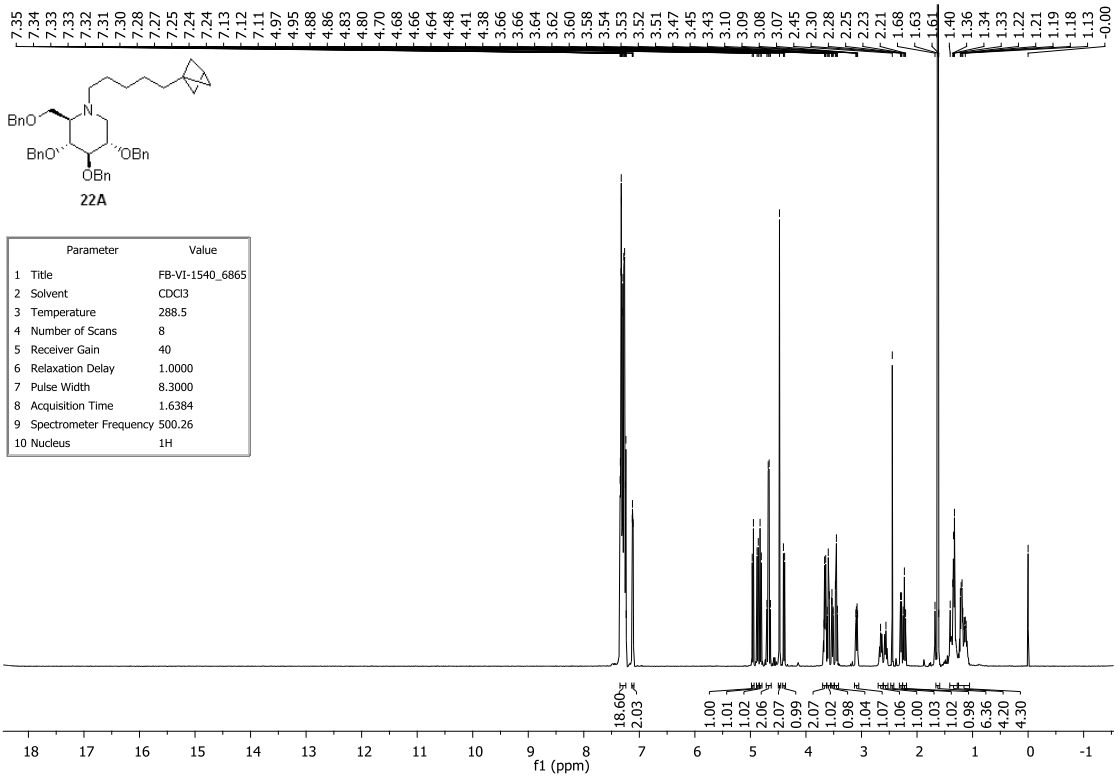
5	Receiver Gain	30
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C


BnO
BnO
OBn
OBn
22A
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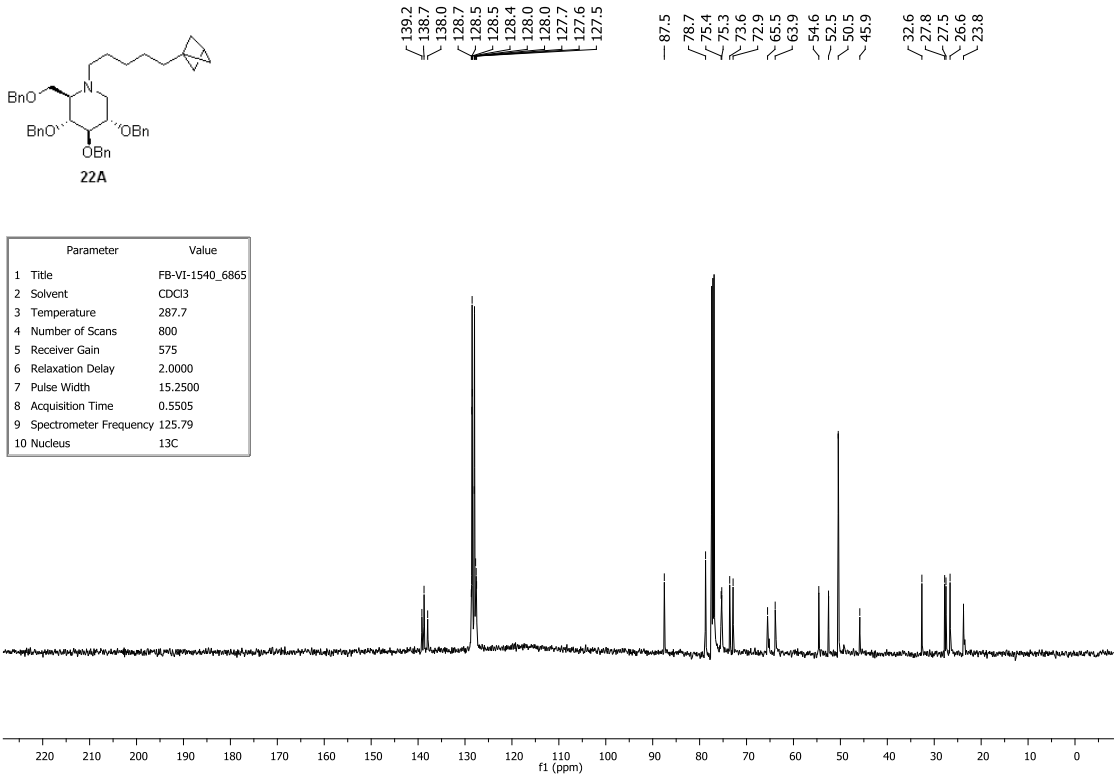
8 Acquisition Time 1.6384
9 Spectrometer Frequency 500.26
10 Nucleus 1H
f1 (ppm)


BnO
BnO
OBn
OBn
22A
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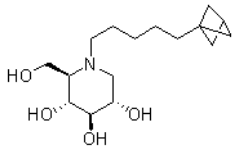
9 Spectrometer Frequency 125.79
10 Nucleus 13C
f1 (ppm)



22

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5	Receiver Gain	32
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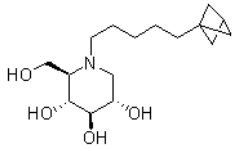
8	Acquisition Time	2.2807
9	Spectrometer Frequency	399.73
10	Nucleus	1H



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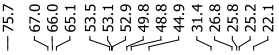

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5	Receiver Gain	30
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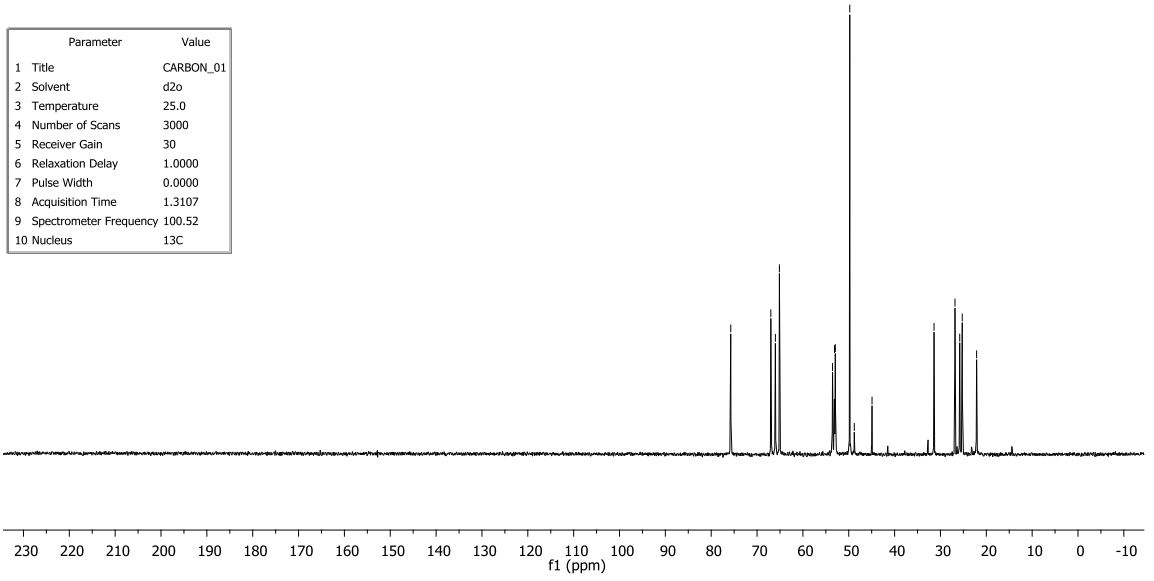
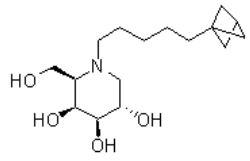
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C





53

	Parameter	Value
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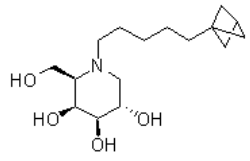

53

	Parameter	Value
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2	Solvent	MeOD
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5	Receiver Gain	2050
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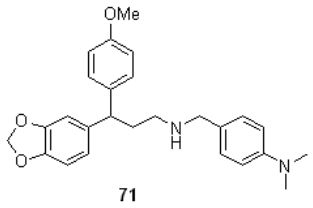
8	Acquisition Time	0.5505
9	Spectrometer Frequency	125.79
10	Nucleus	13C



71

	Parameter	Value
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5	Receiver Gain	40
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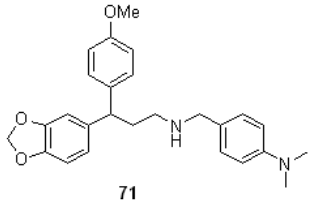
9	Spectrometer Frequency	399.73
10	Nucleus	1H



71

	Parameter	Value
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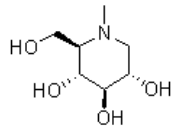
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C



75

	Parameter	Value
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5	Receiver Gain	40
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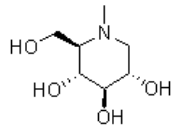
8	Acquisition Time	2.5559
9	Spectrometer Frequency	399.73
10	Nucleus	1H



75

	Parameter	Value
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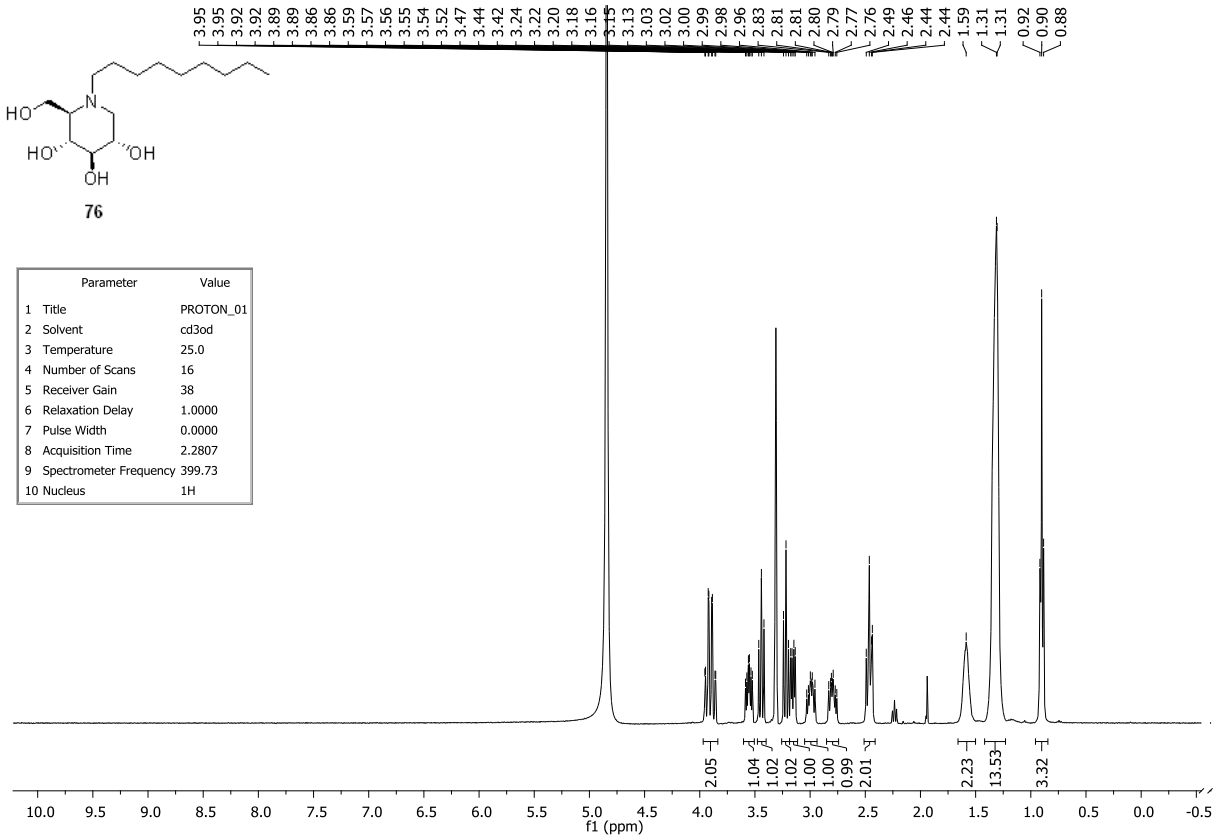
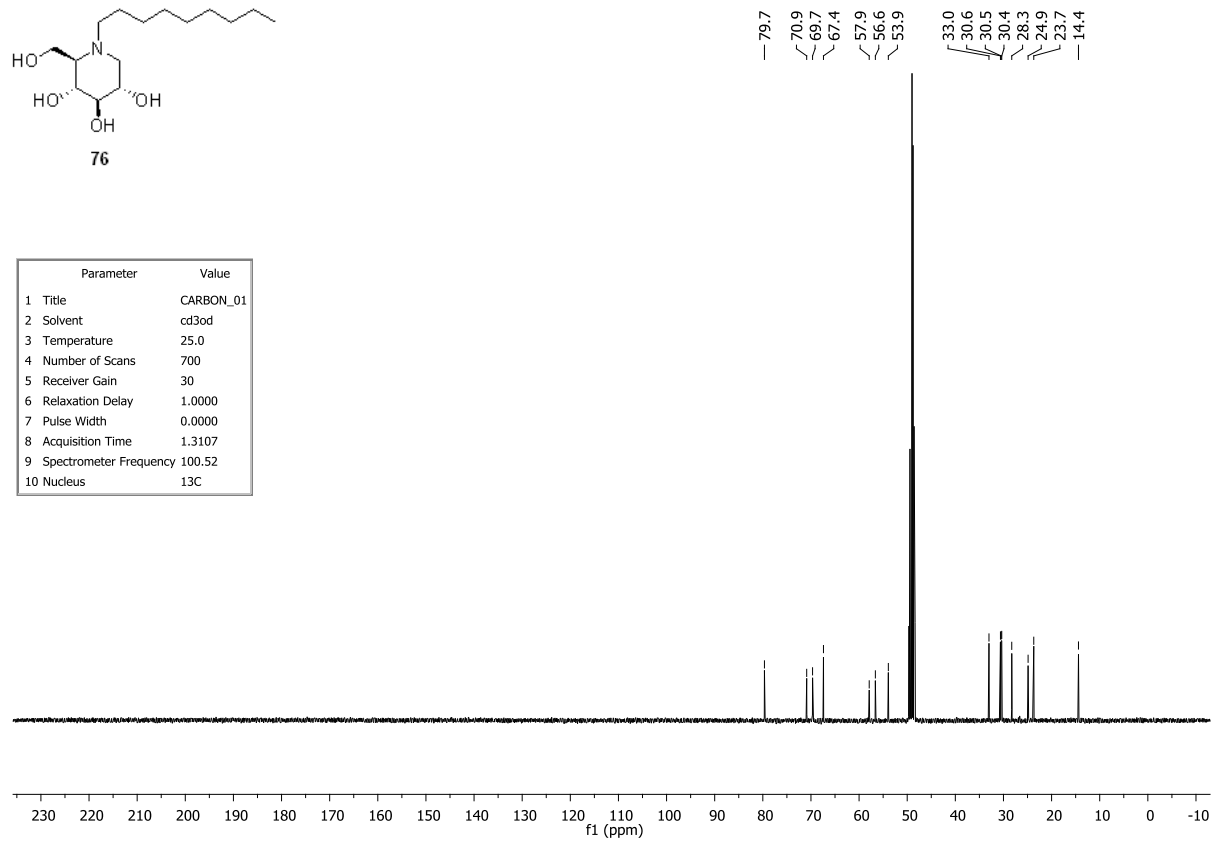
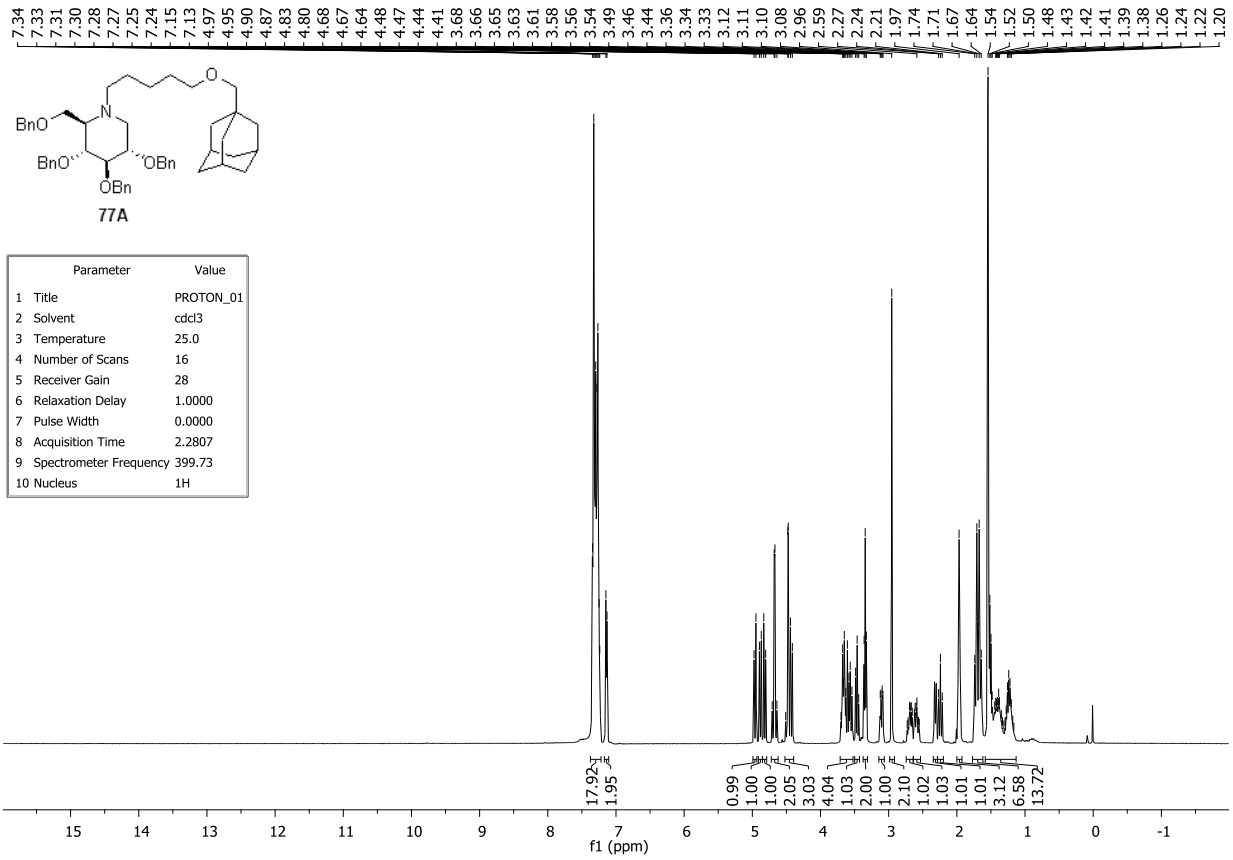
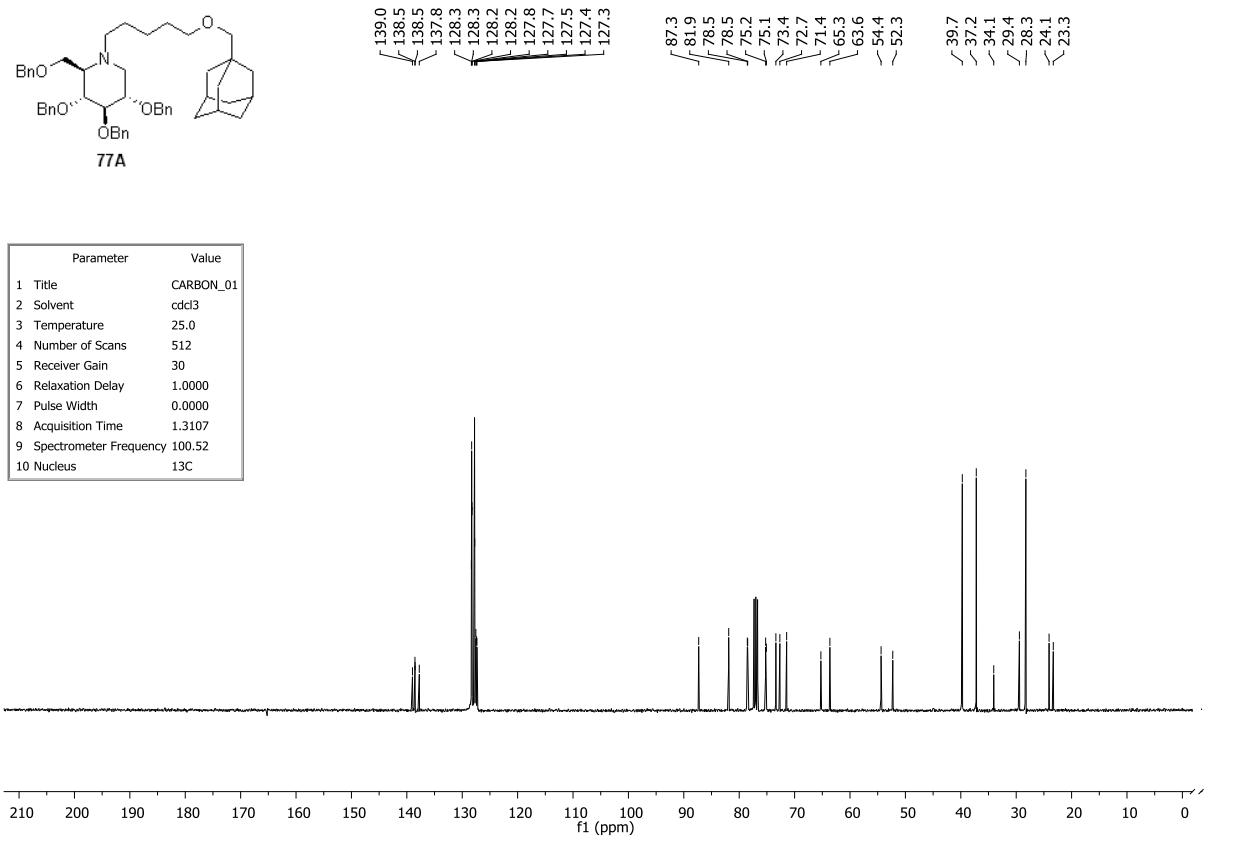
4	Number of Scans	700
5	Receiver Gain	30
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C


3.95 3.95 3.92 3.92 3.89 3.89 3.86 3.86 3.59 3.57 3.56 3.55 3.54 3.52 3.47 3.44 3.42 3.24 3.22 3.20 3.18 3.16 3.15 3.13 3.03 3.02 3.00 2.99 2.98 2.96 2.83 2.81 2.81 2.80 2.79 2.77 2.76 2.49 2.46 2.44 2.44 1.59 1.31 1.31 0.92 0.90 0.88
HO
HO
OH
OH
N
76
Parameter Value
1 Title PROTON_01
2 Solvent cd3od
3 Temperature 25.0
4 Number of Scans 16
5 Receiver Gain 38
6 Relaxation Delay 1.0000
7 Pulse Width 0.0000
8 Acquisition Time 2.2807
9 Spectrometer Frequency 399.73
10 Nucleus 1H
2.05 1.04 1.02 1.02 1.00 1.00 0.99 2.01 2.23 13.53 3.32
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

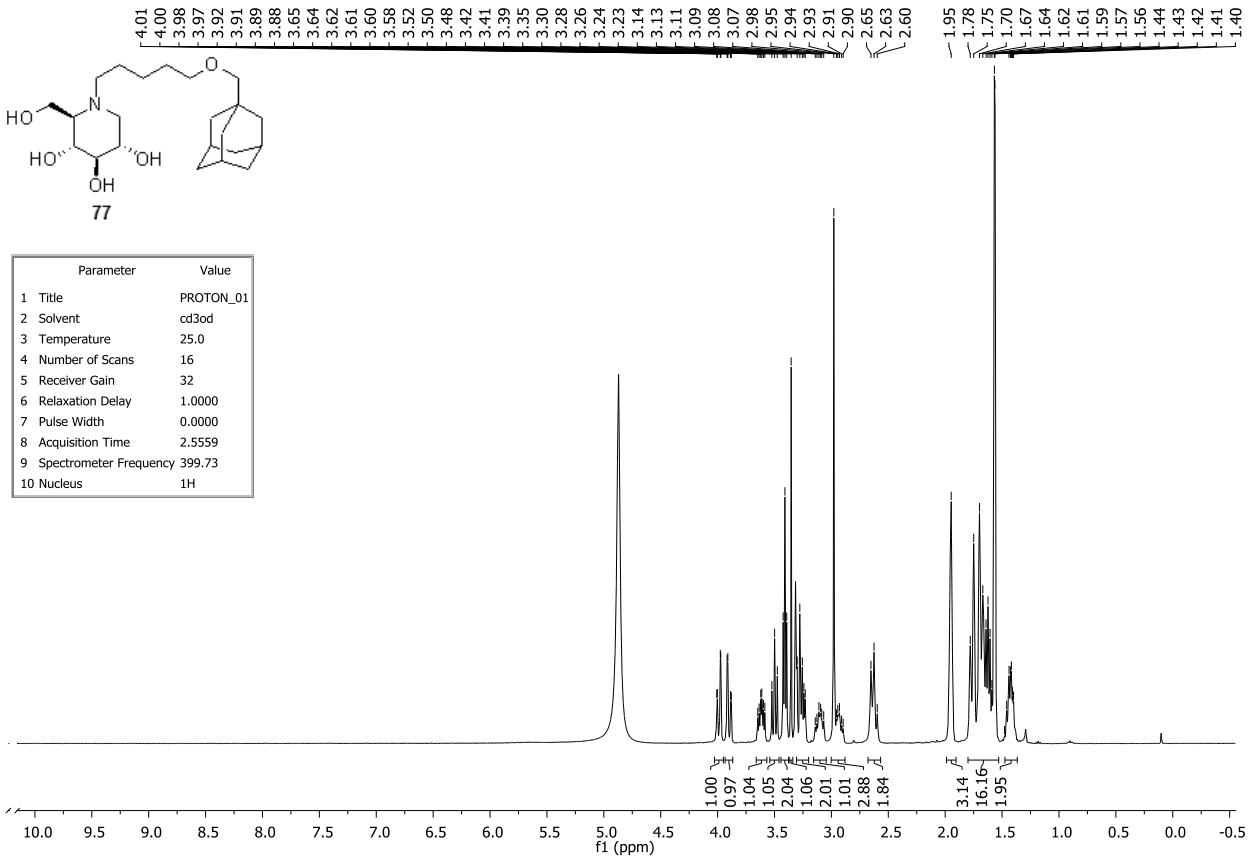

79.7 70.9 69.7 67.4 57.9 56.6 53.9 33.0 30.6 30.5 30.4 28.3 24.9 23.7 14.4
HO
HO
OH
OH
N
76
Parameter Value
1 Title CARBON_01
2 Solvent cd3od
3 Temperature 25.0
4 Number of Scans 700
5 Receiver Gain 30
6 Relaxation Delay 1.0000
7 Pulse Width 0.0000
8 Acquisition Time 1.3107
9 Spectrometer Frequency 100.52
10 Nucleus 13C
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

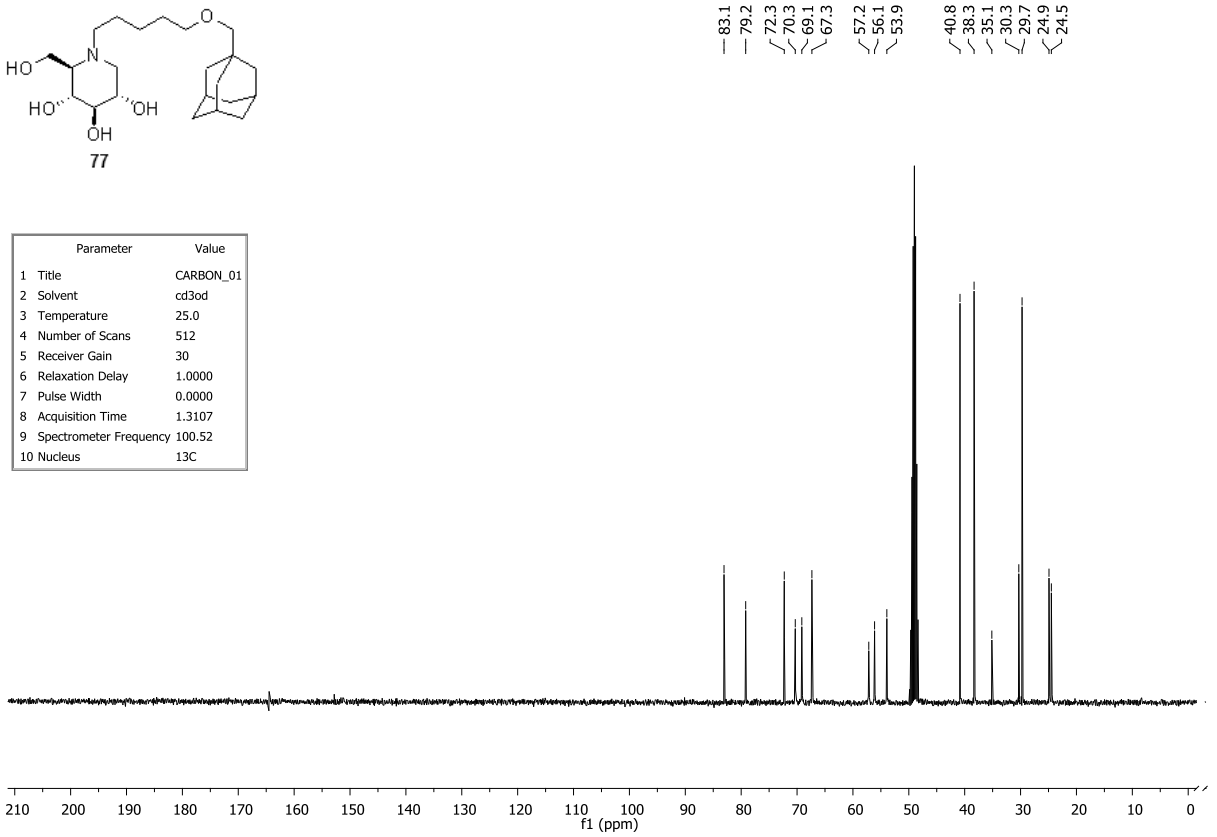


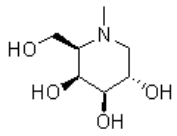
	Parameter	Value
1	Title	PROTON_01
2	Solvent	cdcl3
3	Temperature	25.0
4	Number of Scans	16
5	Receiver Gain	28
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	2.2807
9	Spectrometer Frequency	399.73
10	Nucleus	1H



	Parameter	Value
1	Title	CARBON_01
2	Solvent	cdcl3
3	Temperature	25.0
4	Number of Scans	512
5	Receiver Gain	30
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C

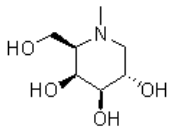

4.01 4.00 3.98 3.97 3.92 3.91 3.89 3.88 3.65 3.64 3.62 3.61 3.60 3.58 3.52 3.50 3.48 3.42 3.41 3.39 3.35 3.30 3.28 3.26 3.24 3.23 3.14 3.13 3.11 3.09 3.08 3.07 2.98 2.95 2.94 2.93 2.91 2.90 2.65 2.63 2.60 1.95 1.78 1.75 1.70 1.67 1.64 1.62 1.61 1.59 1.57 1.56 1.44 1.43 1.42 1.41 1.40
HO
HO
OH
OH
N
O
77
Parameter Value
1 Title PROTON_01
2 Solvent cd3od
3 Temperature 25.0
4 Number of Scans 16
5 Receiver Gain 32
6 Relaxation Delay 1.0000
7 Pulse Width 0.0000
8 Acquisition Time 2.5559
9 Spectrometer Frequency 399.73
10 Nucleus 1H
1.00 0.97 1.04 1.05 2.04 1.06 2.01 1.01 2.88 1.84 3.14 16.16 1.95
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


83.1 79.2 72.3 70.3 69.1 67.3 57.2 56.1 53.9 40.8 38.3 35.1 30.3 29.7 24.9 24.5
HO
HO
OH
OH
N
O
77
Parameter Value
1 Title CARBON_01
2 Solvent cd3od
3 Temperature 25.0
4 Number of Scans 512
5 Receiver Gain 30
6 Relaxation Delay 1.0000
7 Pulse Width 0.0000
8 Acquisition Time 1.3107
9 Spectrometer Frequency 100.52
10 Nucleus 13C
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



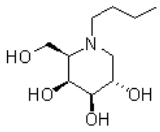
87

	Parameter	Value
1	Title	PROTON_01
2	Solvent	cd3od
3	Temperature	25.0
4	Number of Scans	16
5	Receiver Gain	40
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	2.5559
9	Spectrometer Frequency	399.73
10	Nucleus	1H



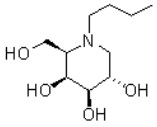
87

	Parameter	Value
1	Title	CARBON_01
2	Solvent	cd3od
3	Temperature	25.0
4	Number of Scans	700
5	Receiver Gain	30
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C



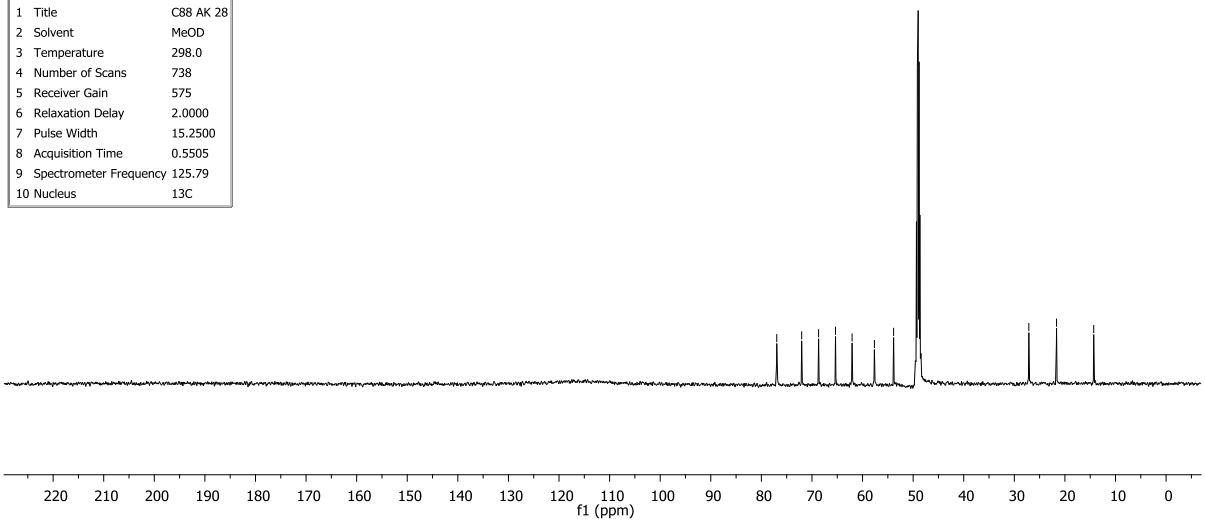
88

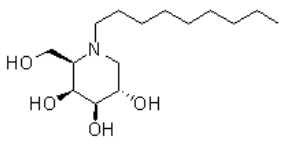
	Parameter	Value
1	Title	C88 AK 28
2	Solvent	MeOD
3	Temperature	298.0
4	Number of Scans	8
5	Receiver Gain	72
6	Relaxation Delay	1.0000
7	Pulse Width	8.3000
8	Acquisition Time	1.6384
9	Spectrometer Frequency	500.26
10	Nucleus	1H



88

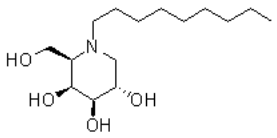
	Parameter	Value
1	Title	C88 AK 28
2	Solvent	MeOD
3	Temperature	298.0
4	Number of Scans	738
5	Receiver Gain	575
6	Relaxation Delay	2.0000
7	Pulse Width	15.2500
8	Acquisition Time	0.5505
9	Spectrometer Frequency	125.79
10	Nucleus	13C





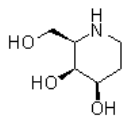
89

	Parameter	Value
1	Title	PROTON_01
2	Solvent	cd3od
3	Temperature	25.0
4	Number of Scans	16
5	Receiver Gain	38
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	2.2807
9	Spectrometer Frequency	399.73
10	Nucleus	1H

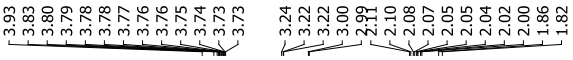


89

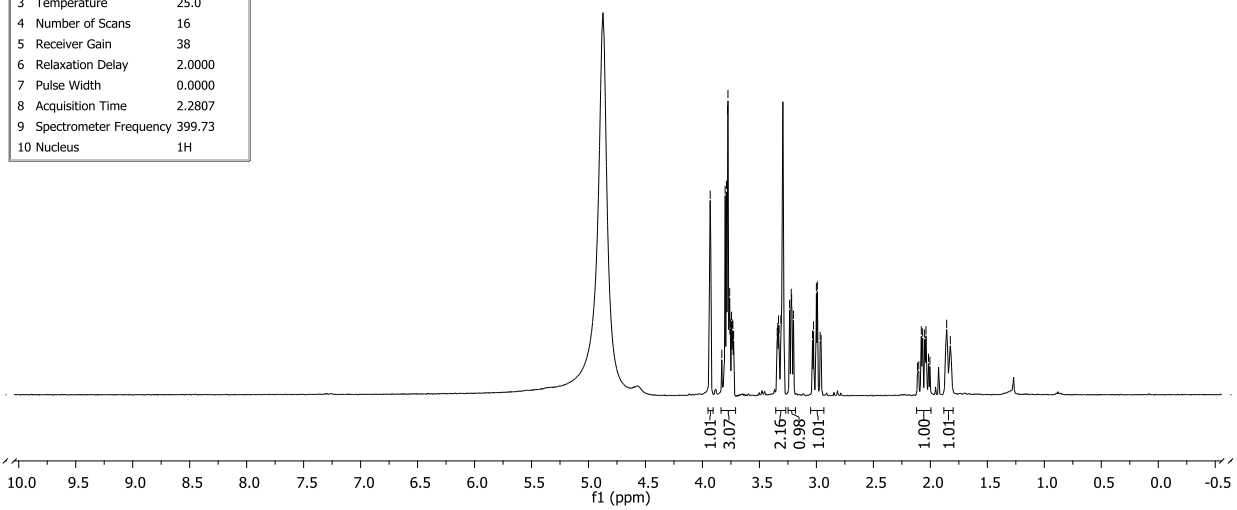
	Parameter	Value
1	Title	CARBON_01
2	Solvent	cd3od
3	Temperature	25.0
4	Number of Scans	700
5	Receiver Gain	30
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C

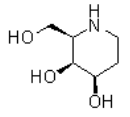


93

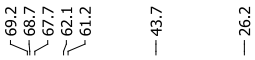


	Parameter	Value
1	Title	PROTON_01
2	Solvent	cd3od
3	Temperature	25.0
4	Number of Scans	16
5	Receiver Gain	38
6	Relaxation Delay	2.0000
7	Pulse Width	0.0000
8	Acquisition Time	2.2807
9	Spectrometer Frequency	399.73
10	Nucleus	1H

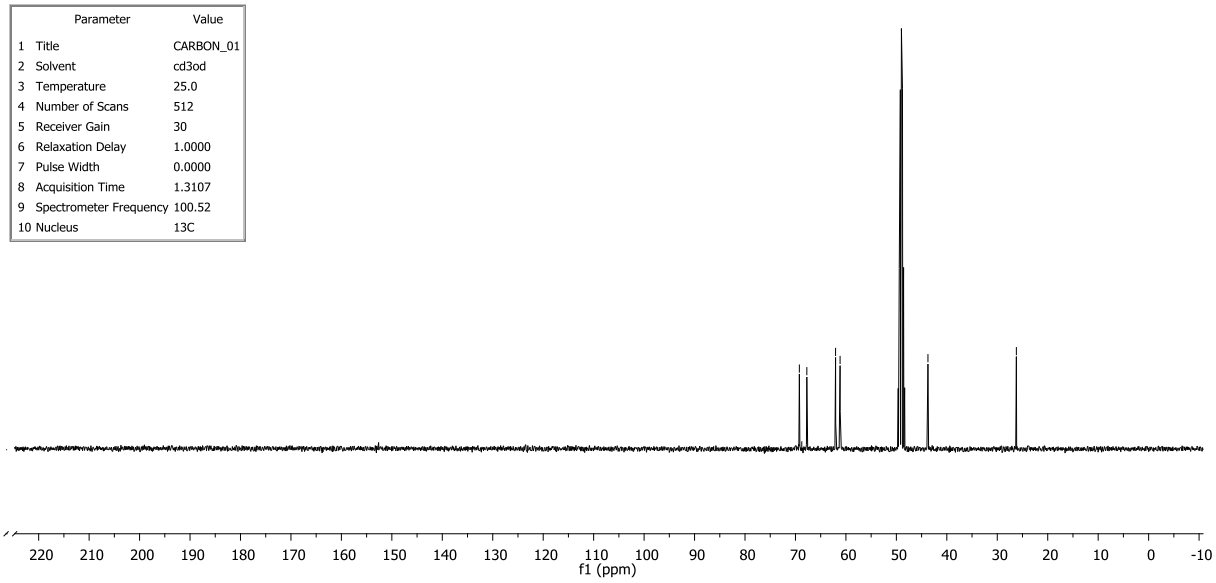


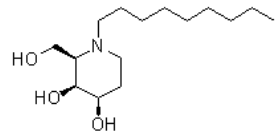


93



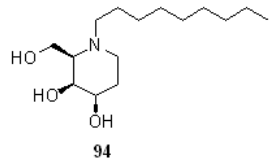
	Parameter	Value
1	Title	CARBON_01
2	Solvent	cd3od
3	Temperature	25.0
4	Number of Scans	512
5	Receiver Gain	30
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C





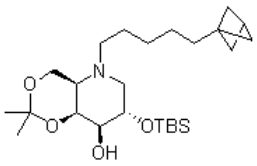
94

	Parameter	Value
1	Title	PROTON_01
2	Solvent	cd3od
3	Temperature	25.0
4	Number of Scans	16
5	Receiver Gain	38
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	2.2807
9	Spectrometer Frequency	399.73
10	Nucleus	1H



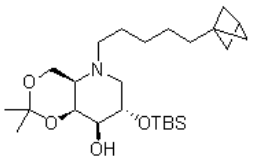
94

	Parameter	Value
1	Title	CARBON_01
2	Solvent	cd3od
3	Temperature	25.0
4	Number of Scans	700
5	Receiver Gain	30
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C



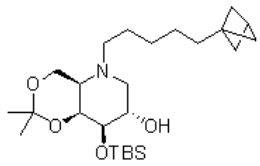
95

	Parameter	Value
1	Title	PROTON_01
2	Solvent	cdcl3
3	Temperature	25.0
4	Number of Scans	16
5	Receiver Gain	30
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	2.5559
9	Spectrometer Frequency	399.73
10	Nucleus	1H



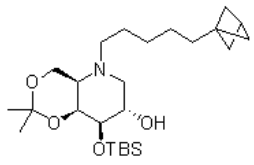
95

	Parameter	Value
1	Title	CARBON_01
2	Solvent	cdcl3
3	Temperature	25.0
4	Number of Scans	256
5	Receiver Gain	30
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C



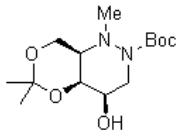
96

	Parameter	Value
1	Title	PROTON_01
2	Solvent	cdcl3
3	Temperature	25.0
4	Number of Scans	16
5	Receiver Gain	34
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	2.5559
9	Spectrometer Frequency	399.73
10	Nucleus	1H



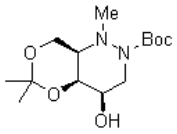
96

	Parameter	Value
1	Title	CARBON_01
2	Solvent	cdcl3
3	Temperature	25.0
4	Number of Scans	256
5	Receiver Gain	30
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C



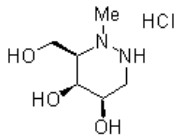
103

	Parameter	Value
1	Title	PROTON_01
2	Solvent	cdcl3
3	Temperature	27.0
4	Number of Scans	16
5	Receiver Gain	42
6	Relaxation Delay	2.0000
7	Pulse Width	0.0000
8	Acquisition Time	2.5559
9	Spectrometer Frequency	399.73
10	Nucleus	1H



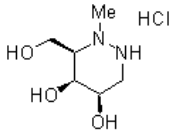
103

	Parameter	Value
1	Title	CARBON_01
2	Solvent	cdcl3
3	Temperature	27.0
4	Number of Scans	700
5	Receiver Gain	30
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C



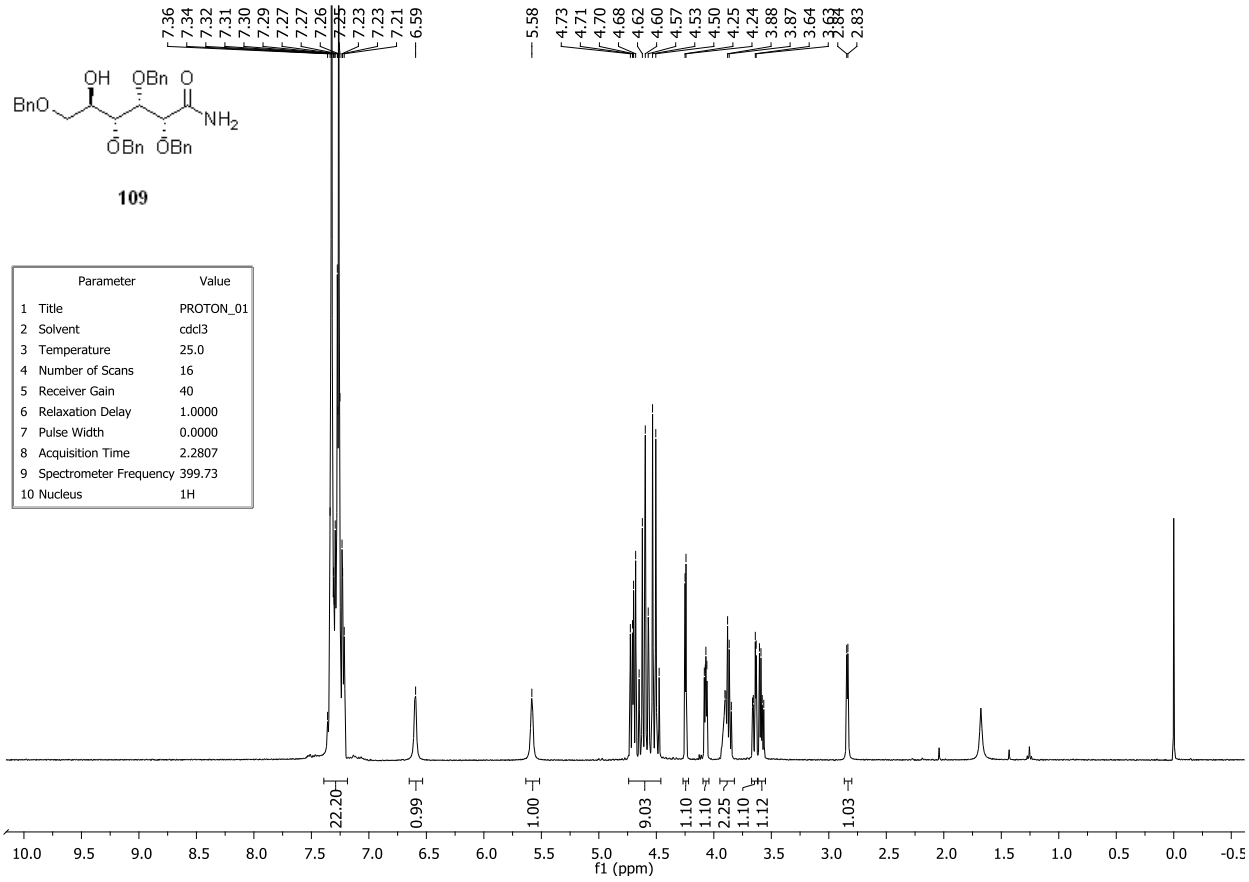
104

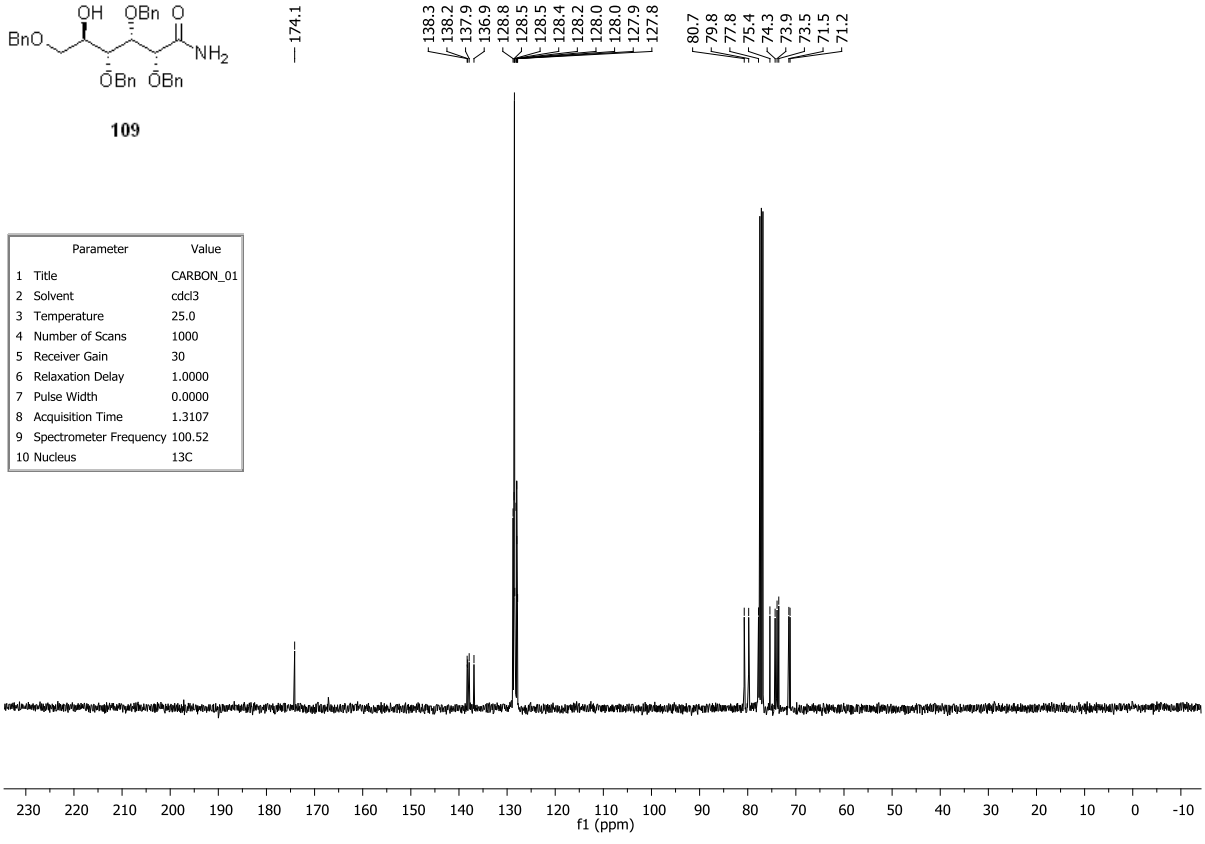
	Parameter	Value
1	Title	PROTON_01
2	Solvent	d2o
3	Temperature	27.0
4	Number of Scans	64
5	Receiver Gain	42
6	Relaxation Delay	2.0000
7	Pulse Width	0.0000
8	Acquisition Time	2.2807
9	Spectrometer Frequency	399.73
10	Nucleus	1H

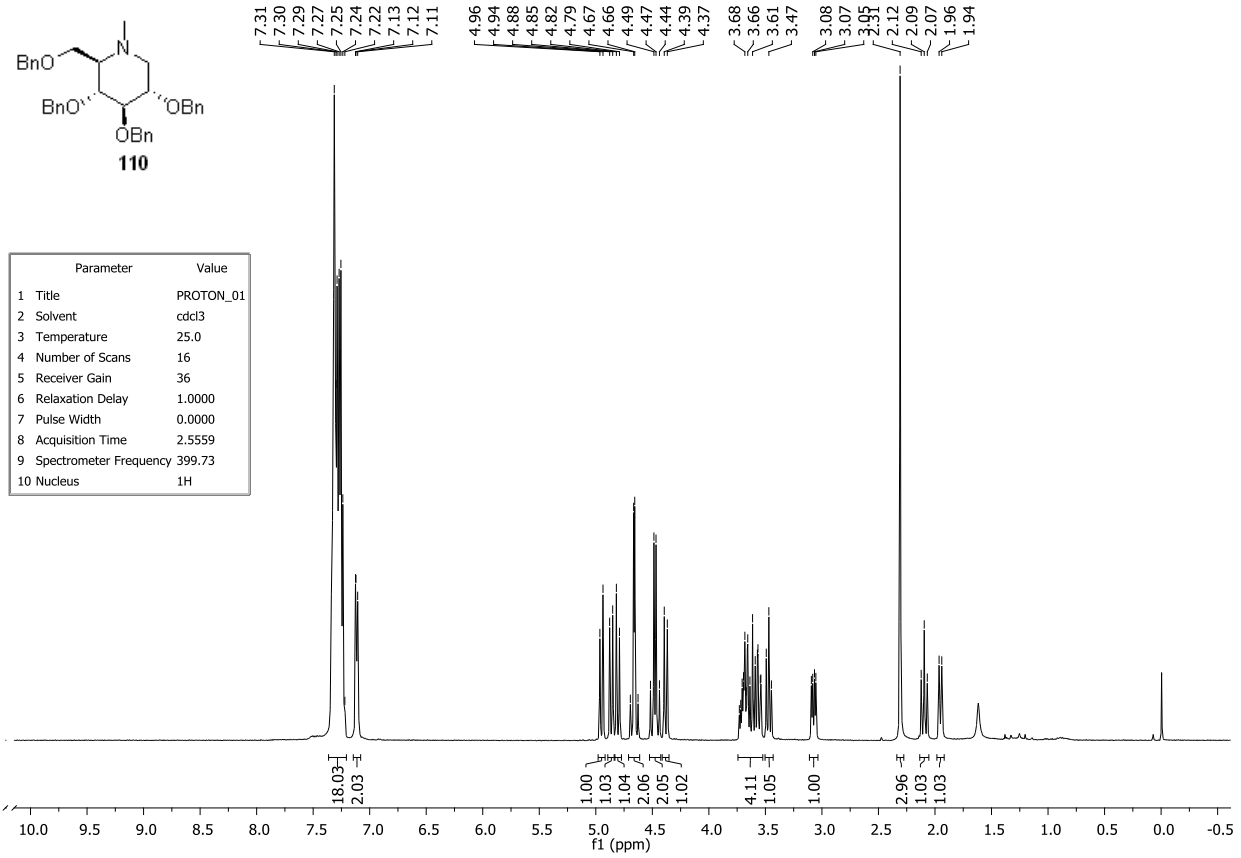


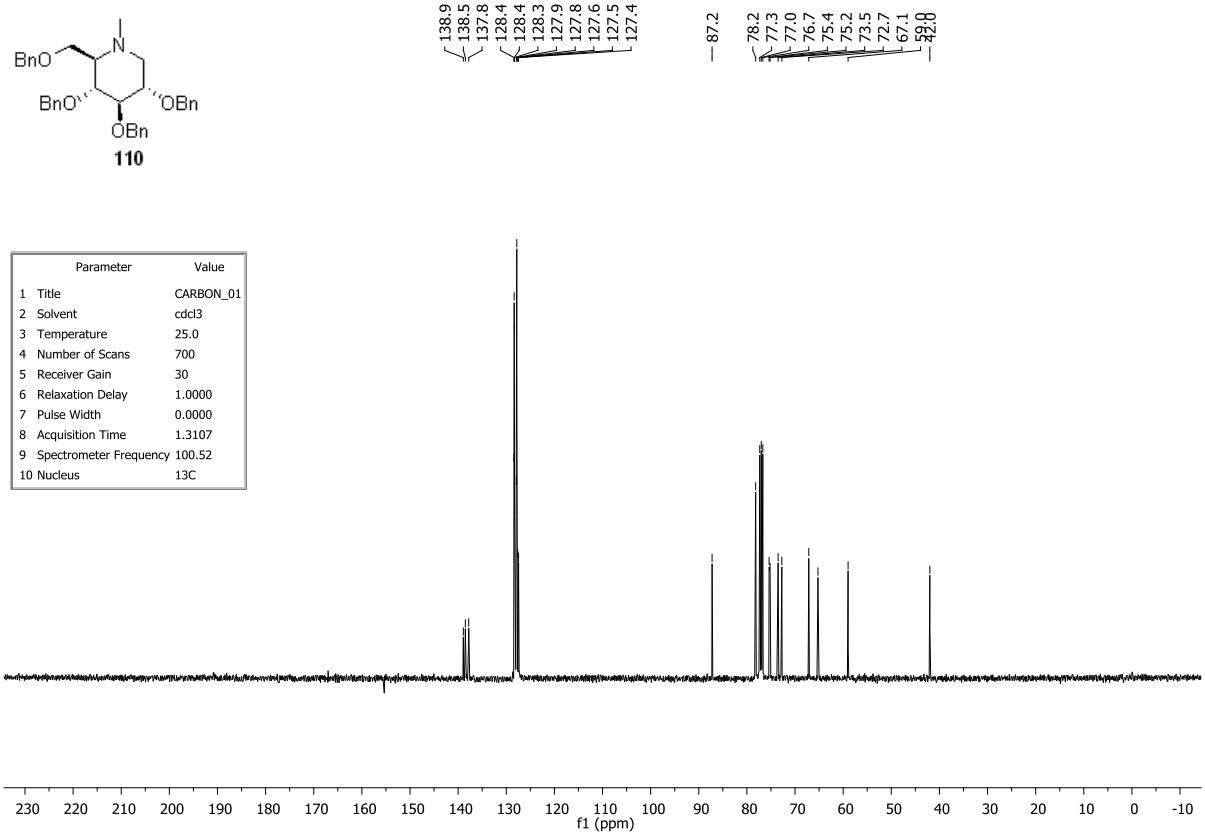
104

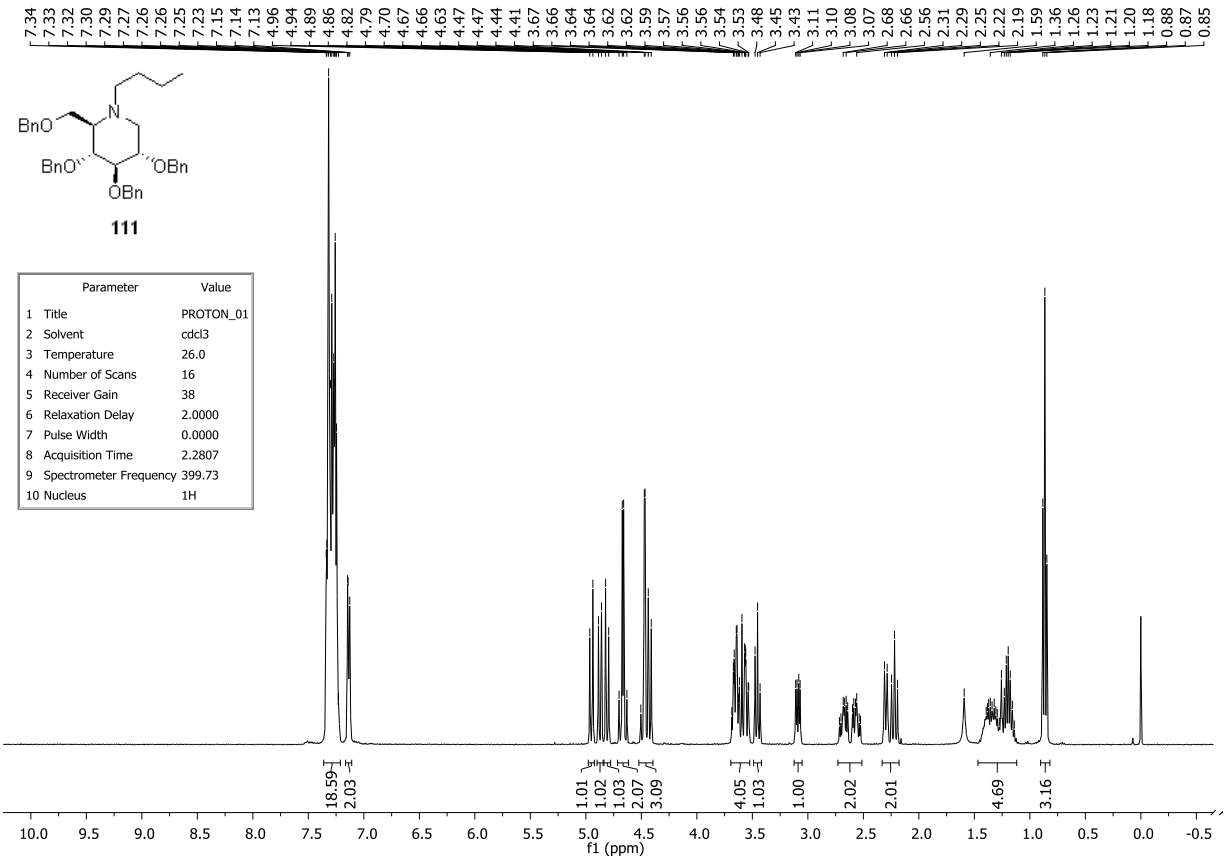
	Parameter	Value
1	Title	CARBON_01
2	Solvent	d2o
3	Temperature	27.0
4	Number of Scans	700
5	Receiver Gain	30
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C

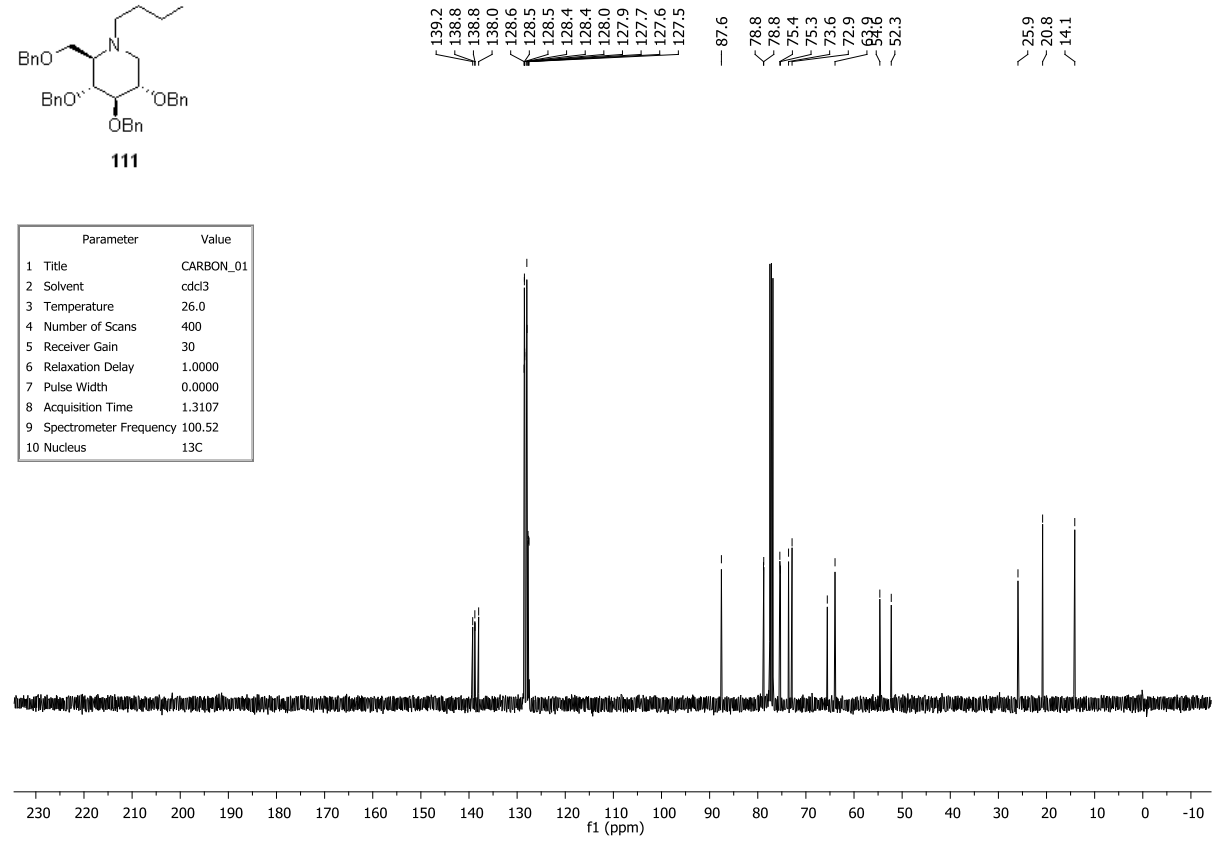

7.36
7.34
7.32
7.31
7.30
7.29
7.27
7.27
7.26
7.25
7.23
7.23
7.21
6.59
5.58
4.73
4.71
4.70
4.68
4.62
4.60
4.57
4.53
4.50
4.25
4.24
3.88
3.87
3.64
2.83
OH
OBn
O
BnO
NH2
OBn
OBn
109
Parameter
Value
1 Title
PROTON_01
2 Solvent
cdcl3
3 Temperature
25.0
4 Number of Scans
16
5 Receiver Gain
40
6 Relaxation Delay
1.0000
7 Pulse Width
0.0000
8 Acquisition Time
2.2807
9 Spectrometer Frequency
399.73
10 Nucleus
1H
22.20
0.99
1.00
9.03
1.10
1.10
2.25
1.10
1.12
1.03
f1 (ppm)

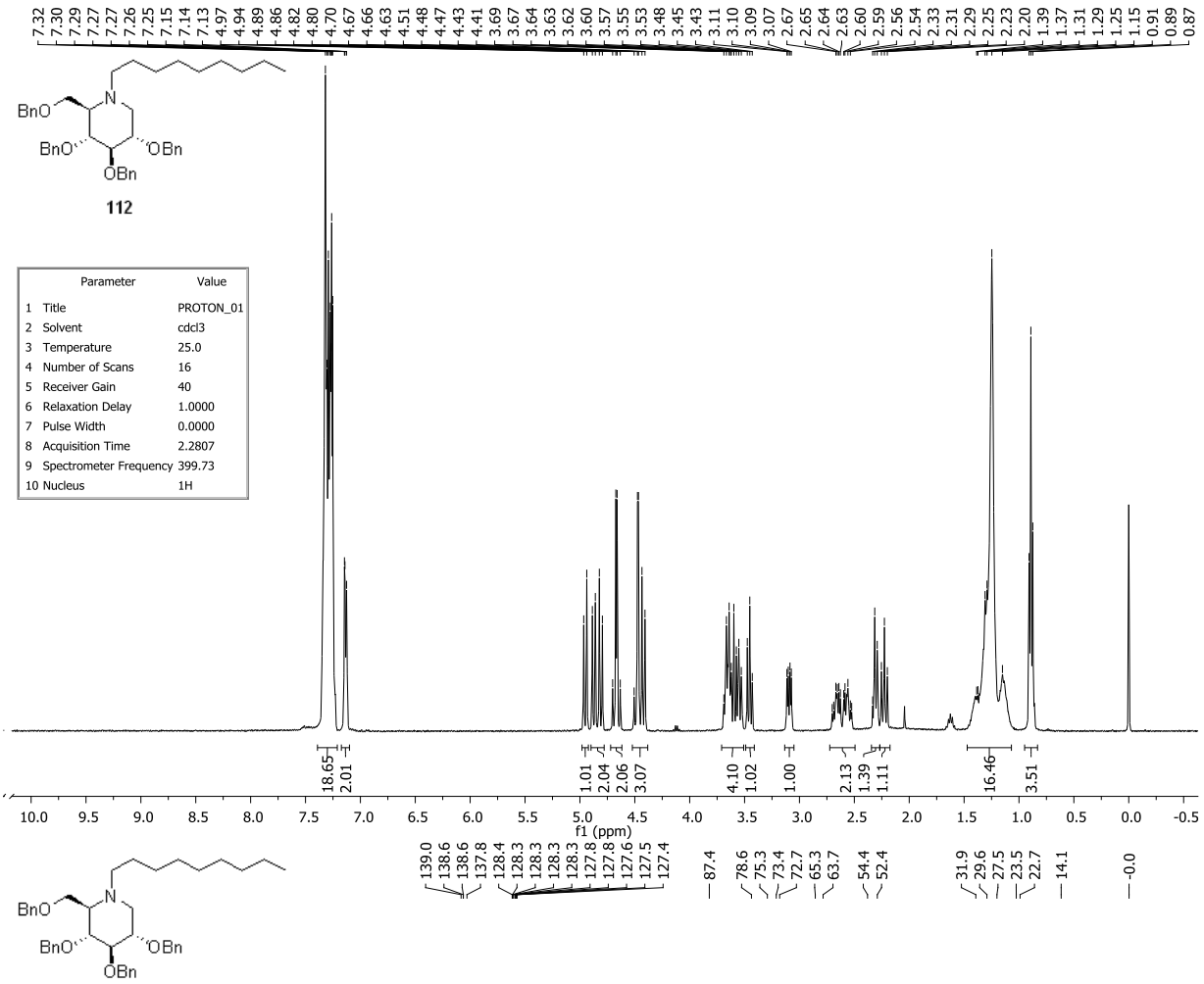

OH
OBn
O
BnO
NH2
OBn
OBn
109
174.1
138.3
138.2
137.9
136.9
128.8
128.5
128.5
128.4
128.2
128.0
128.0
128.0
127.9
127.8
80.7
79.8
77.8
75.4
74.3
73.9
73.5
71.5
71.2
Parameter
Value
1 Title
CARBON_01
2 Solvent
cdcl3
3 Temperature
25.0
4 Number of Scans
1000
5 Receiver Gain
30
6 Relaxation Delay
1.0000
7 Pulse Width
0.0000
8 Acquisition Time
1.3107
9 Spectrometer Frequency
100.52
10 Nucleus
13C
f1 (ppm)


BnO
BnO
OBn
OBn
110
Parameter Value
1 Title PROTON_01
2 Solvent cdcl3
3 Temperature 25.0
4 Number of Scans 16
5 Receiver Gain 36
6 Relaxation Delay 1.0000
7 Pulse Width 0.0000
8 Acquisition Time 2.5559
9 Spectrometer Frequency 399.73
10 Nucleus 1H
7.31 7.30 7.29 7.27 7.25 7.24 7.22 7.13 7.12 7.11
4.96 4.94 4.88 4.85 4.82 4.79 4.67 4.66 4.49 4.47 4.44 4.39 4.37
3.68 3.66 3.61 3.47
3.08 3.07 3.05 2.12 2.09 2.07 1.96 1.94
18.03 2.03 1.00 1.03 1.04 2.06 2.05 1.02 4.11 1.05 1.00 2.96 1.03 1.03
f1 (ppm)

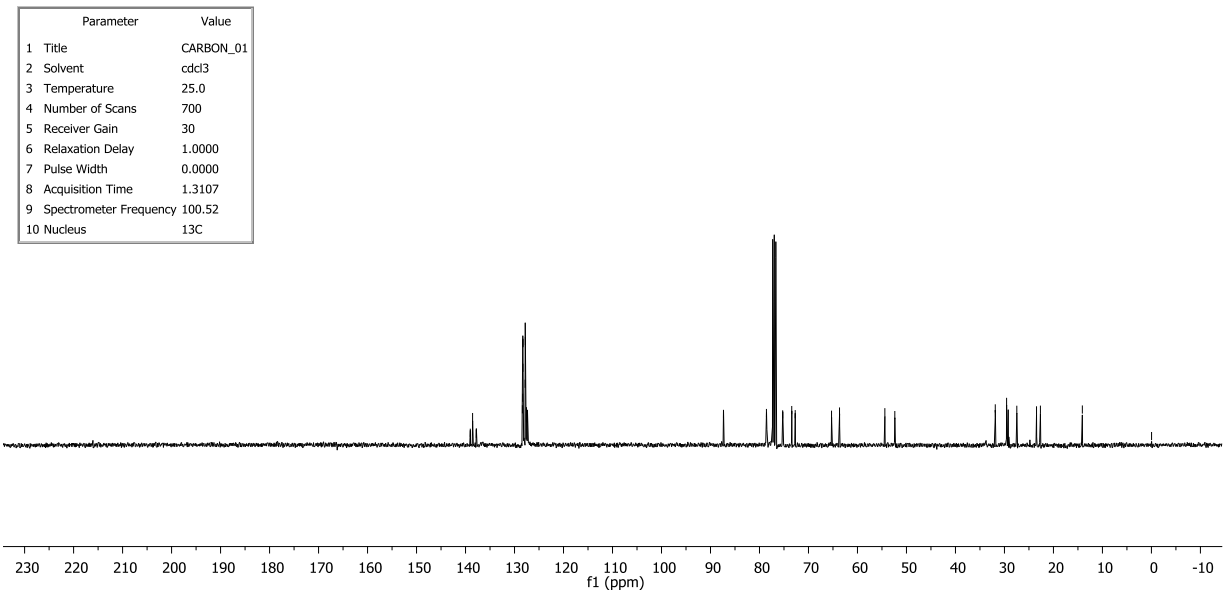

BnO
BnO
OBn
OBn
110
Parameter Value
1 Title CARBON_01
2 Solvent cdcl3
3 Temperature 25.0
4 Number of Scans 700
5 Receiver Gain 30
6 Relaxation Delay 1.0000
7 Pulse Width 0.0000
8 Acquisition Time 1.3107
9 Spectrometer Frequency 100.52
10 Nucleus 13C
138.9 138.5 137.8 128.4 128.4 128.3 127.9 127.8 127.6 127.5 127.4
87.2
78.2 77.3 77.0 76.7 75.4 75.2 73.5 72.7 67.1 59.0 42.0
f1 (ppm)

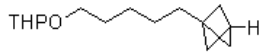

BnO
BnO
OBn
OBn
111
Parameter Value
1 Title PROTON_01
2 Solvent cdcl3
3 Temperature 26.0
4 Number of Scans 16
5 Receiver Gain 38
6 Relaxation Delay 2.0000
7 Pulse Width 0.0000
8 Acquisition Time 2.2807
9 Spectrometer Frequency 399.73
10 Nucleus 1H
f1 (ppm)


BnO
BnO
OBn
OBn
111
Parameter Value
1 Title CARBON_01
2 Solvent cdcl3
3 Temperature 26.0
4 Number of Scans 400
5 Receiver Gain 30
6 Relaxation Delay 1.0000
7 Pulse Width 0.0000
8 Acquisition Time 1.3107
9 Spectrometer Frequency 100.52
10 Nucleus 13C
f1 (ppm)


BnO
BnO
OBn
OBn
112
Parameter Value
1 Title PROTON_01
2 Solvent cdcl3
3 Temperature 25.0
4 Number of Scans 16
5 Receiver Gain 40
6 Relaxation Delay 1.0000
7 Pulse Width 0.0000
8 Acquisition Time 2.2807
9 Spectrometer Frequency 399.73
10 Nucleus 1H
f1 (ppm)

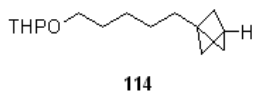
BnO
BnO
OBn
OBn
112


Parameter Value
1 Title CARBON_01
2 Solvent cdcl3
3 Temperature 25.0
4 Number of Scans 700
5 Receiver Gain 30
6 Relaxation Delay 1.0000
7 Pulse Width 0.0000
8 Acquisition Time 1.3107
9 Spectrometer Frequency 100.52
10 Nucleus 13C
f1 (ppm)



114

	Parameter	Value
1	Title	PROTON_01
2	Solvent	cdcl3
3	Temperature	25.0
4	Number of Scans	16
5	Receiver Gain	26
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	2.2807
9	Spectrometer Frequency	399.73
10	Nucleus	1H

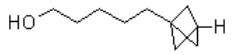


114

	Parameter	Value
1	Title	CARBON_01
2	Solvent	cdcl3
3	Temperature	25.0
4	Number of Scans	256
5	Receiver Gain	30
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C

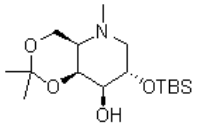
115

	Parameter	Value
1	Title	PROTON_01
2	Solvent	cdcl3
3	Temperature	25.0
4	Number of Scans	16
5	Receiver Gain	28
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	2.2807
9	Spectrometer Frequency	399.73
10	Nucleus	1H



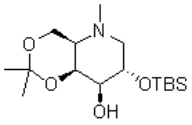
115

	Parameter	Value
1	Title	CARBON_01
2	Solvent	cdcl3
3	Temperature	25.0
4	Number of Scans	512
5	Receiver Gain	30
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C



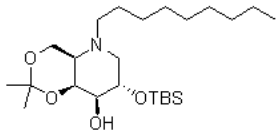
116

	Parameter	Value
1	Title	PROTON_01
2	Solvent	cdcl3
3	Temperature	25.0
4	Number of Scans	16
5	Receiver Gain	34
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	2.2807
9	Spectrometer Frequency	399.73
10	Nucleus	1H



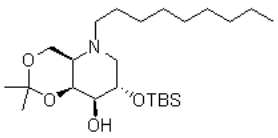
116

	Parameter	Value
1	Title	CARBON_01
2	Solvent	cdcl3
3	Temperature	25.0
4	Number of Scans	512
5	Receiver Gain	30
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C



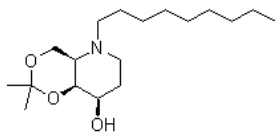
118

	Parameter	Value
1	Title	PROTON_01
2	Solvent	cdcl3
3	Temperature	25.0
4	Number of Scans	16
5	Receiver Gain	32
6	Relaxation Delay	2.0000
7	Pulse Width	0.0000
8	Acquisition Time	2.2807
9	Spectrometer Frequency	399.73
10	Nucleus	1H



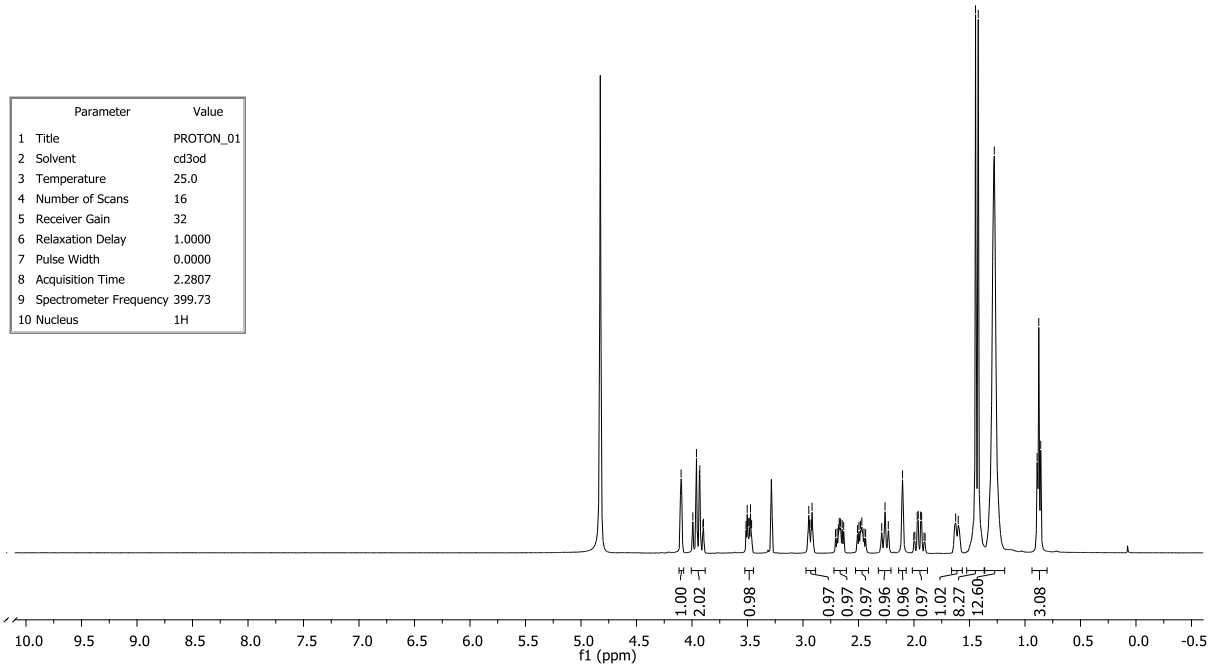
118

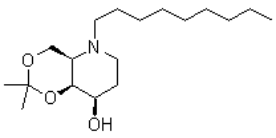
	Parameter	Value
1	Title	CARBON_01
2	Solvent	cdcl3
3	Temperature	25.0
4	Number of Scans	512
5	Receiver Gain	30
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C



119

	Parameter	Value
1	Title	PROTON_01
2	Solvent	cd3od
3	Temperature	25.0
4	Number of Scans	16
5	Receiver Gain	32
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	2.2807
9	Spectrometer Frequency	399.73
10	Nucleus	1H





119

	Parameter	Value
1	Title	CARBON_01
2	Solvent	cd3od
3	Temperature	25.0
4	Number of Scans	512
5	Receiver Gain	30
6	Relaxation Delay	1.0000
7	Pulse Width	0.0000
8	Acquisition Time	1.3107
9	Spectrometer Frequency	100.52
10	Nucleus	13C