

## Supporting Information

### **N-alkylated Aziridines are Easily-prepared, Potent, Specific and Cell-permeable Covalent Inhibitors of Human $\beta$ -glucocerebrosidase**

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#### **General methods for synthetic chemistry**

Analysis of all compounds were conducted at the Lakehead University Instrument Laboratory (LUIL). NMR was performed on a Varian Unity Inova 500 MHz spectrometer in a deuterated solvent with TMS as the internal standard where  $J$  (coupling constant) values are estimated in hertz (Hz). Low resolution mass spectrometry (LRMS) was performed on a Dionex UHPLC-Bruker amaZon X Ion Trap mass spectrometer while high resolution mass spectrometry (HRMS) was performed on a Waters Micromass Global Ultima Q-TOF in ESI mode. Microanalyses of aziridine **11** was measured for C, H, N using Elementar Vario EL and were within  $\pm 0.4\%$  of theoretical values. Thin layer chromatography (TLC) and silica gel column chromatography were performed using TLC Silica Gel 60 F<sub>254</sub> (EMD) and SiliaFlash®P60 (SiliCycle), respectively. All other reagents and solvents were used as purchased without further purification unless stated otherwise. ( $\pm$ )-(1R,2R,3S,4R,5R,6S)-3,4,5,6-tetrakis(benzyloxy)cyclohexane-1,2-diol was prepared following literature procedures.

## Synthesis of aziridines 9, 10 and 11

### (±)-(1R,2S,3S,4R,5S,6R)-2-azido-3,4,5,6-tetrakis(benzyloxy)cyclohexyl methanesulfonate (S1)

Methanesulfonyl chloride (1.43 mL 18.49 mmol) was added dropwise to a solution of tetrabenzyl inositol (2.00 g, 3.70 mmol) in pyridine (8 mL) at 0 °C. After addition, the reaction mixture was stirred at room temperature for overnight under argon. The solution was then concentrated under reduced pressure and the residue was dissolved in EtOAc (100 mL) and successively washed with water (100 mL), 1 M HCl aqueous (100 mL), saturated NaHCO<sub>3</sub> aqueous (100 mL), brine (100 mL), and dried (Na<sub>2</sub>SO<sub>4</sub>). After filtration, solvents were removed from the filtrate to obtain a yellow syrup that was dissolved in dry DMF (20 mL). To this solution NaN<sub>3</sub> (241 mg, 3.71 mmol) was added and the reaction mixture was stirred at room temperature under argon for 2 days. The reaction mixture was then concentrated under reduced pressure and the residue was dissolved in EtOAc (100 mL) and successively washed with water (100 mL), brine (100 mL) and dried (Na<sub>2</sub>SO<sub>4</sub>). After filtration, solvents were removed from the filtrate and the crude product was purified by silica gel flash chromatography using Hexanes-EtOAc (12:1 v/v) as an eluent to obtain the title compound **S1** as an orange oil (1.01 g, 42%).<sup>18</sup> <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.97 (s, 3H), 3.44 (t, *J* = 9.4 Hz, 1H), 3.53-3.61 (m, 4H), 4.40 (t, *J* = 9.8 Hz, 1H), 4.80-4.90 (m, 8H), 7.20-7.35 (m, 20H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 39.43, 64.76, 76.02, 76.04, 76.16, 76.25, 77.13, 77.39, 77.59, 77.64, 80.39, 80.81, 80.89, 82.19, 83.04, 137.45, 137.58, 138.04, 138.11.

### (±)-(2S,3R,4R,5S)-2,3,4,5-tetrakis(benzyloxy)-7-azabicyclo[4.1.0]heptanes (S2)

To a solution of azido mesylate **S1** (2.00 g, 3.11 mmol) in anhydrous diethyl ether (30 mL) LiAlH<sub>4</sub> (270 mg, 7.12 mmol) was slowly added at 0 °C and stirred at room temperature for overnight under argon. The reaction mixture was then quenched with EtOAc (20 mL) and stirred for 30 minutes. The mixture was successively washed with water (2 x 50 mL), brine (50 mL), and dried (Na<sub>2</sub>SO<sub>4</sub>). After filtration, solvents were removed from the filtrate and the crude product was purified by silica gel flash chromatography using hexanes-EtOAc (3:1 v/v) as an eluent to obtain the title compound **S2** as a white

solid (970 mg, 60%).<sup>18</sup> <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.33 (d, *J* = 6.0 Hz, 1H), 2.48 (dd, *J* = 6.0, 3.2 Hz, 1H), 3.43 (dd, *J* = 10.4, 7.8 Hz, 1H), 3.63 (t, *J* = 9.3 Hz, 1H), 3.84 (d, *J* = 7.8 Hz, 1H), 3.87, (d, *J* = 3.0 Hz, 1H), 4.68-4.85 (m, 8H), 7.23-7.40 (m, 20H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 33.23, 34.37, 72.84, 72.90, 75.36, 75.85, 79.66, 79.84, 81.31, 84.28, 127.49, 127.54, 127.59, 127.64, 127.75, 127.80, 127.87, 127.87, 127.91, 128.04, 128.13, 128.21, 128.31, 128.34, 128.40, 128.50, 138.17, 138.84, 138.89, 138.91. MS 522.3 (M + H)<sup>+</sup>.

**General procedure for the synthesis of (±)-(2S,3R,4R,5S)-2,3,4,5-tetrakis(benzyloxy)-7-alkyl-7-azabicyclo[4.1.0]heptanes (S3-S5)** Potassium carbonate (132 mg, 0.96 mmol) and the appropriate 1-iodoalkane (0.38 mmol) were added to a solution of aziridine **S2** (0.25 g, 0.48 mmol) in dry DMF (15 mL) and allowed to stir at 50 °C for overnight under argon. After reaction, the mixture was concentrated to dryness under reduced pressure. The residue was dissolved in EtOAc (50 mL) and successively washed with water (50 mL), brine (50 mL), and dried (Na<sub>2</sub>SO<sub>4</sub>). After filtration, solvents were removed from the filtrate and the crude product was purified by silica gel flash chromatography using hexanes-EtOAc (8:1 v/v) as an eluent to obtain the respective title compound **S3**, **S4** or **S5**. Physical and spectroscopic data for **S3** - **S4** are listed below.

**(±)-(2S,3R,4R,5S)-2,3,4,5-tetrakis(benzyloxy)-7-butyl-7-azabicyclo[4.1.0]heptanes (S3)**

Yield: 54.5%, yellow oil; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.90 (t, *J* = 7.3 Hz, 3H) 1.36 (qd, *J* = 7.5, 2.5 Hz, 2H), 1.46-1.53 (m, 2H) 1.60 (d, *J* = 6.1 Hz, 1H), 1.83 (dd, *J* = 6.1, 3.3 Hz, 1H) 2.03 (dt, *J* = 11.4, 7.4 Hz, 1H), 2.38 (dt, *J* = 11.4, 7.2 Hz, 1H), 3.37 (dd, *J* = 10.4, 8.0 Hz, 1H), 3.59 (dd, *J* = 10.4, 8.5 Hz, 1H), 3.78 (dd, *J* = 8.5, 3.3 Hz, 1H), 3.80 (d, *J* = 8.0 Hz, 1H), 4.68-4.84 (m, 8H), 7.22-7.39 (m, 20H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 14.20, 20.59, 31.82, 41.75, 42.71, 60.81, 72.63, 72.92, 75.47, 75.93, 80.12, 80.52, 81.30, 84.50, 127.47, 127.52, 127.61, 127.84, 127.87, 127.89, 128.02, 128.17, 128.32, 128.34, 128.40, 128.53, 138.27, 139.02, 139.07, 139.10. MS 578.3 (M + H)<sup>+</sup>.

**(±)-(2S,3R,4R,5S)-2,3,4,5-tetrakis(benzyloxy)-7-hexyl-7-azabicyclo[4.1.0]heptane (S4)**

Yield: 65.5%, white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.90 (t,  $J = 7.0$  Hz, 3H), 1.24-1.38 (m, 6H), 1.52 (quintet,  $J = 7.4$  Hz, 2H), 1.62 (d,  $J = 6.3$  Hz, 1H), 1.84 (dd,  $J = 6.0, 3.3$  Hz, 1H), 2.04 (dt,  $J = 11.3, 7.5$  Hz, 1H), 2.39 (dt,  $J = 11.3, 7.5$  Hz, 1H), 3.38 (dd, 10.4, 8.0 Hz, 1H), 3.58-3.62 (dd, 10.4, 8.5 Hz, 1H), 3.79 (dd,  $J = 8.5, 3.3$  Hz, 1H), 3.81 (d,  $J = 7.9$  Hz, 1H), 4.69-4.85 (m, 8H), 7.23-7.40 (m, 20H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  14.21, 22.72, 27.18, 29.68, 31.95, 41.79, 42.71, 61.19, 72.63, 72.93, 75.49, 75.93, 80.11, 80.53, 81.35, 84.50, 127.48, 127.52, 127.61, 127.84, 127.88, 128.00, 128.17, 128.33, 128.35, 128.40, 128.53, 138.28, 139.03, 139.07, 139.11. MS 606.4 (M + H) $^+$ .

**( $\pm$ )-(2S,3R,4R,5S)-2,3,4,5-tetrakis(benzyloxy)-7-octyl-7-azabicyclo[4.1.0]heptanes (S5)**

Yield: 59.3%, yellow oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.88 (t,  $J = 7.1$  Hz, 3H), 1.31 (m, 10H), 1.52 (quintet,  $J = 6.3$  Hz, 2H), 1.61 (d,  $J = 6.2$  Hz, 1H), 1.83 (dd,  $J = 6.1, 3.2$  Hz, 1H), 2.02 (dt,  $J = 11.4, 7.4$  Hz, 1H), 2.39 (dq,  $J = 11.4, 7.4$  Hz, 1H), 3.37 (dd, 10.4, 8.0 Hz, 1H), 3.59 (dd, 10.4, 8.6 Hz, 1H), 3.78 (dd,  $J = 8.6, 3.3$  Hz, 1H), 3.80 (d,  $J = 8.0$  Hz, 1H), 4.69-4.85 (m, 8H), 7.21-7.40 (m, 20H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  14.20, 22.74, 27.48, 29.35, 29.67, 29.68, 31.95, 41.77, 42.67, 61.17, 72.59, 72.90, 75.45, 75.91, 80.08, 80.50, 81.31, 84.46, 127.44, 127.49, 127.58, 127.81, 127.85, 127.96, 128.15, 128.37, 128.50, 138.25, 139.00, 139.04, 139.08. MS 634.4 (M + H) $^+$ .

**General procedure for the synthesis of ( $\pm$ )-(2S,3R,4R,5S)-7-alkyl-7-azabicyclo[4.1.0]heptane-2,3,4,5-tetraols (9-11)**

$\text{NH}_3$  (~20mL) was condensed into a three neck flask at  $-78$  °C using a dry ice condenser. Metallic Na (200 mg, 8.70 mmol) was added to the stirring liq.  $\text{NH}_3$  producing a persistent deep blue color. A solution of ( $\pm$ )-(2S,3R,4R,5S)-2,3,4,5-tetrakis(benzyloxy)-7-alkyl-7-azabicyclo[4.1.0]heptanes **S3** (0.22 mmol), **S4** (0.46 mmol) or **S5** (0.40 mmol) in THF (3 mL) was cooled to  $-78$  °C and added to the blue ammonia solution via a cannula and stirred for 4 hours. After reaction, the mixture was quenched with water (15 mL) and allowed to warm to room temperature to slowly evaporate out the excess  $\text{NH}_3$ . The reaction mixture was then

concentrated under reduced pressure to and the residue was redissolved in water (50 mL), and washed with diethyl ether (50 mL). The aqueous phase was concentrated to dryness under reduced pressure. The residue was purified by silica gel flash chromatography using CHCl<sub>3</sub>-MeOH (4:1 v/v) as an eluent to obtain the respective title compound **9**, **10** or **11**. Physical and spectroscopic data for **9-11** are listed below.

**(±)- (2S,3R,4R,5S)-7-butyl-7-azabicyclo[4.1.0]heptane-2,3,4,5-tetraol (9)**

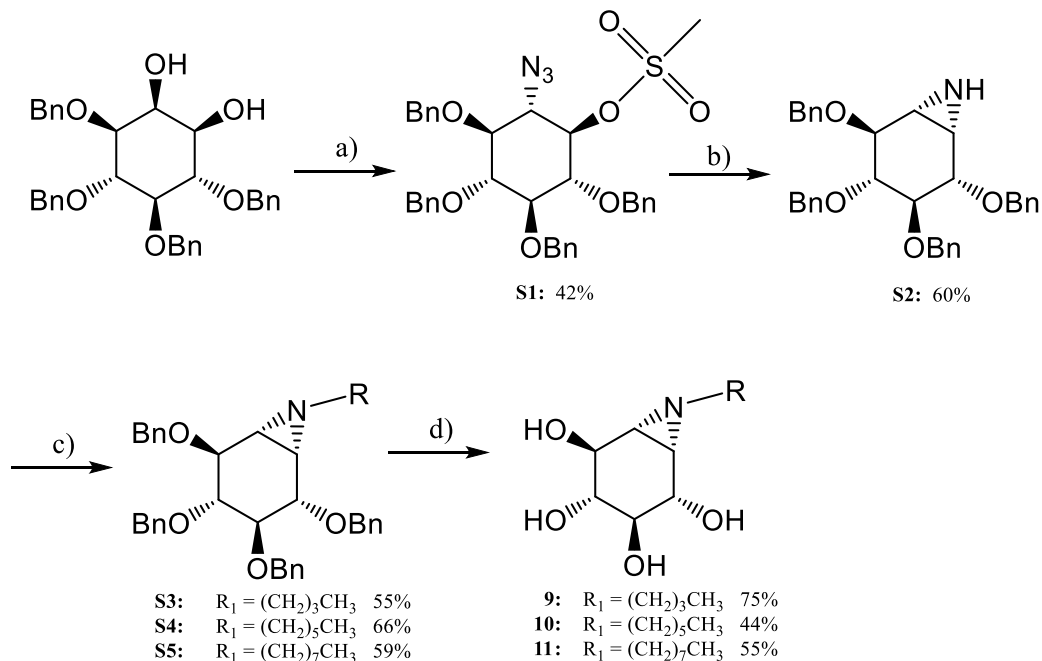
Yield: 75%, white powder; <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 0.95 (t, *J* = 7.1 Hz, 3H), 1.41 (q, *J* = 7.7 Hz, 2H), 1.58 (t, *J* = 7.2 Hz, 2H), 1.66 (d, *J* = 6.1 Hz, 1H), 1.95(m, 1H), 2.18 (dt, *J* = 9.5, 8.2 Hz, 1H), 2.40 (dt, *J* = 9.5, 8.2 Hz, 1H), 3.08 (t, *J* = 9.0 Hz, 1H), 3.25 (t, *J* = 9.2 Hz, 1H), 3.65 (d, *J* = 8.0 Hz, 1H), 3.72 (d, *J* = 8.5 Hz, 1H). <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 13.04, 20.12, 31.36, 44.05, 44.43, 60.38, 71.73, 72.04, 72.67, 76.44. HRMS (ESI): *m/z* Calcd for [C<sub>10</sub>H<sub>19</sub>NO<sub>4</sub> + H]<sup>+</sup>: 218.1392; found: 218.1399.

**(±)- (2S,3R,4R,5S)-7-hexyl-7-azabicyclo[4.1.0]heptane-2,3,4,5-tetraol (10)**

Yield: 44%, white powder; <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 0.93 (t, *J* = 6.8 Hz, 3H), 1.31-1.41 (m, 6H), 1.60 (quintet, *J* = 7.4 Hz, 2H), 1.66 (d, *J* = 6.2 Hz, 1H), 1.95 (t, *J* = 4.7 Hz, 1H), 2.17 (dt, *J* = 11.5, 7.5 Hz, 1H), 2.39 (dt, *J* = 11.5, 7.5 Hz, 1H), 3.08 (t, *J* = 9.3 Hz, 1H), 3.25 (t, *J* = 9.5 Hz, 1H), 3.65 (d, *J* = 8.1 Hz, 1H), 3.72 (dd, *J* = 8.3, 3.3 Hz). <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 13.02, 22.27, 26.70, 29.13, 31.61, 44.04, 44.45, 60.69, 71.72, 72.04, 72.68, 76.43. HRMS (ESI): *m/z* Calcd for [C<sub>12</sub>H<sub>23</sub>NO<sub>4</sub> + H]<sup>+</sup>: 246.1705; found: 246.1707.

**(±)- (2S,3R,4R,5S)-7-octyl-7-azabicyclo[4.1.0]heptane-2,3,4,5-tetraol (11)**

Yield: 55%, white powder; <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 0.92 (t, *J* = 6.6 Hz, 3H), 1.28-1.40 (m, 10H), 1.60 (quintet, *J* = 7.2 Hz, 2H), 1.65 (d, *J* = 6.3 Hz, 1H), 1.95 (t, *J* = 4.7 Hz, 1H), 2.17 (dt, *J* = 11.4, 7.6 Hz, 1H), 2.39 (dt, *J* = 11.4, 7.5 Hz, 1H), 3.08 (t, *J* = 9.3 Hz, 1H), 3.25 (t, *J* = 9.3 Hz, 1H), 3.65 (d, *J* = 8.1 Hz, 1H), 3.72 (dd, *J* = 8.5, 3.6 Hz, 1H). <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 13.05, 22.34, 27.02, 29.01, 29.16, 29.33, 31.64, 44.03, 44.45, 60.69, 71.73, 72.04, 72.68, 76.45. HRMS (ESI): *m/z* Calcd for [C<sub>14</sub>H<sub>27</sub>NO<sub>4</sub> + H]<sup>+</sup>: 274.2018; found: 274.2022. Elemental analysis calculated (%) for C<sub>14</sub>H<sub>27</sub>NO<sub>4</sub>: C: 61.51 N: 5.12 H: 9.96 Measure: 61.61 N: 5.02 H: 9.792



**Scheme S1: Synthesis of racemic conduritol aziridines 9, 10 and 11.** *Reagents and Conditions:* (a) 1) Mesyl chloride, pyridine, rt, overnight, 2) NaN<sub>3</sub>, DMF, rt, 2 days; (b) LiAlH<sub>4</sub>, ether, 0°C – rt, overnight; (c) K<sub>2</sub>CO<sub>3</sub>, C<sub>8</sub>H<sub>17</sub>I or C<sub>6</sub>H<sub>13</sub>I or C<sub>4</sub>H<sub>9</sub>I, DMF, 50°C, overnight; (d) Na<sub>(s)</sub>, NH<sub>3(l)</sub>, THF, -78°C, 4 h.

## Enzymology

All kinetic assays were performed in a Biotek Synergy4 plate reader. Human GBA3 was purchased from R & D Systems (Minnesota, USA) and used following the manufacturer's instructions. The human  $\beta$ -glucocerebrosidase (GBA1) analogue, imiglucerase, was obtained from patient leftovers of Cerezyme® (a kind gift from Dr. Lorne Clarke). The enzyme is prepared in a solution of 424 units of imiglucerase, 340 mg mannitol, 104 mg trisodium citrate, 36 mg disodium hydrogen citrate, and 1.06 mg polysorbate 80 in 10 mL water. A portion of GBA1 was diluted in the assay buffer (50 mM acetate, 0.2 % v/v Triton X-100, 0.3 % w/v sodium taurocholate and pH 5.5) and used immediately in the assay. For GBA1 both the chromogenic substrate 2,4-dinitrophenyl  $\beta$ -D-glucopyranoside (2,4-DNP-Glc,  $K_M = 1.6$  mM) and the fluorogenic substrate 4-methylumbelliferyl  $\beta$ -D-glucopyranoside (4-MUGlc, experimentally determined  $K_M = 250$   $\mu$ M) were used to measure enzymatic rates by monitoring the release of 2,4-dinitrophenolate (2,4-DNP,  $\lambda_{max}$ : 400 nm) or release of 4-methylumbelliferone ( $\lambda_{ex}$ : 340 nm,  $\lambda_{em}$ : 465 nm)

at 37°C. For GBA3, only 4-MUGlc was used as a fluorogenic substrate (experimentally determined  $K_M = 10.3 \mu\text{M}$ )

### **Time-dependent inactivation of GBA1 and GBA3**

A continuous-release assay was used to determine  $k_i$  and  $K_i$  values for compounds **9** – **11** towards GBA1 (see Scheme S1). A range of eight concentrations of the inhibitor predicted to surround the  $K_i$  value were incubated with enzyme (~ 4.14 nM) and substrate 4-MUGlc (6 mM) in buffer (50 mM acetate, 0.2 % v/v Triton X-100, 0.3 % w/v sodium taurocholate and pH 5.5). Reagents were preheated to 37 °C for 10 minutes before initiating the reaction by the addition of enzyme to the inhibitor/substrate mixture. Bleaching of the fluorophore was corrected for by subtracting substrate blanks containing buffer and substrate without added enzyme. Apparent rate constants  $K_{\text{obs}}$  were obtained by fitting the data to Equation S1 using the GraphPad Prism software. The observed rate constants were then fit to Equation S2 as a function of [I] to determine  $k_i$  and  $K_{i\text{app}}$ .  $K_i$  is then determined from Equation S3 using the experimentally determined  $K_M$  value of the substrate 4-MUGlc. An identical method was used to determine the  $k_i$  and  $K_i$  values of **11** for GBA3 except 0.1 M MES buffer and 40  $\mu\text{M}$  of 4-MUGlc was used.

### **General methods for the cell experiments**

All chemical reagents were purchased from Sigma-Aldrich or Fisher Scientific and used without further purification. Protease Inhibitor Cocktail inhibitor was purchased from Sigma-Aldrich (P8340). Fluorogenic enzyme substrates including 4-methylumbelliferyl  $\beta$ -D-galactopyranoside (4-MUGal), 4-methylumbelliferyl  $\beta$ -D-mannopyranoside (4-MUM $\beta$ ), 4-methylumbelliferyl  $\alpha$ -D-mannopyranoside (4-MUM $\alpha$ ), 4-methylumbelliferyl *N*-acetyl- $\beta$ -D-glucosaminide (4-MUAGlc), 4-methylumbelliferyl  $\alpha$ -D-glucopyranoside (4-MUGlc $\alpha$ ) and 4-methylumbelliferyl  $\beta$ -D-glucopyranoside were purchased from Sigma-Aldrich. Pierce BCA Protein assay reagent A (Bicinchonic acid and tartrate in an alkaline carbonate buffer) and B (4% copper sulfate pentahydrate solution) was purchased from Fisher Scientific and the

assay performed following product instructions. HeLa cervical cancer and fibroblasts (J2/3T3) were used for all cell assays. All inhibitor solutions were prepared in Millipore water and diluted into the appropriate buffer or media as indicated.

### **Cell culture and preparation of cell lysates**

HeLa cervical cancer cells were seeded into 24 well plates (Fisher culture-treated plates) and grown to 80 % confluency in Dulbecco's Modified Eagle's Medium (DMEM, 1 mL) supplemented with 10 % FBS in a 5% CO<sub>2</sub> atmosphere. Fibroblast cells were grown under identical conditions as HeLa cells except 0.1 % gentamicin sulfate, 1 mM sodium pyruvate, and 1 % penicillin/streptomycin solution were added. Prior to treatment with inhibitor, cells were washed twice with Phosphate Buffered Saline (PBS) and then incubated in serum free DMEM (990  $\mu$ L). Aziridine **11** or CBE **1** (stock solution prepared using Millipore water) were added to various wells at the indicated concentration and incubated at 37 °C under 5% CO<sub>2</sub> for the indicated length of time. Cells were washed twice with PBS, lysed with lysis buffer (10 mM Tris•HCl, 150 mM NaCl, 1 % Triton X-100, 1 mM EDTA, pH 7.5, 1 % protease inhibitor cocktail), centrifuged at 16000 x g for 10 minutes at 4°C and the supernatant stored at -80°C until use.

### **Cell lysate assays**

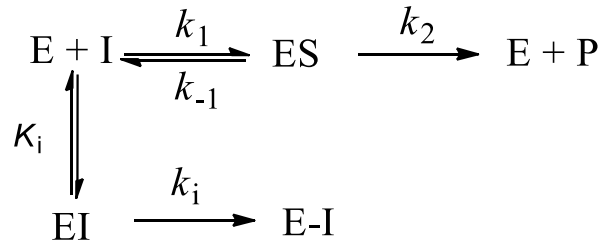
In Costar flat bottom, black, 96 well plates, cell lysate (20  $\mu$ L) was diluted in run buffer (80  $\mu$ L, 10 mM 4-MUGlc, 0.1 % BSA, 1.2 % Sodium taurocholate, 0.1 M KPO<sub>3</sub>, pH 5.5) and incubated at 37 °C for 20 minutes with shaking (set at medium built into the Synergy 4 Biotek plate reader). Aliquots (50  $\mu$ L) of the enzymatic reaction were removed and added to a stop buffer (200  $\mu$ L, 0.1 M NaOH, 0.1 M glycine, pH 10) and the total amount of 4-methylumbelliferone released was measured ( $\lambda_{ex}$ : 365 nm,  $\lambda_{em}$ : 448 nm). To correct for spontaneous hydrolysis of 4-MuGlc lysis buffer was used in the place of cell lysate. Data was represented as a relative value to control lysates which were prepared from cells treated with millipore water instead of the inhibitor solution. All lysate activity was normalized to the total protein concentration in each lysate sample determined by a BCA assay. Data was determined in replicate from a minimum of two independent trials.



## Specificity of Aziridine 11 against other enzyme classes

HeLa cells were treated with CBE **1** or aziridine **11** at the indicated concentrations in serum free DMEM and allowed to incubate at 37°C overnight. Cell lysates were prepared as described above and incubated with fluorogenic substrates under the following conditions to assay for other glycosidase enzymes. To assay for  $\beta$ -galactosidase activity, 4-MUGal (0.5 mM) was used in 0.1 M NaAc at pH 3.5. To assay for  $\beta$ -mannosidase activity, 4-MUM $\beta$  (10 mM) was used in 0.25 M NaAc at pH 4.0. To assay for  $\alpha$ -mannosidase activity, 4-MUM $\alpha$  (0.5 mM) was used in 0.25 M NaAc at pH 4.5. To assay for  $\beta$ -hexosaminidase A and B activity, 4-MUAGlc (3 mM) was used in 0.1 mM citrate, 0.2 mM phosphate at pH 5.0. To assay for  $\alpha$ -glucosidase activity, 4-MUGlc $\alpha$  (10 mM) was used in 0.1 mM NaAc at pH 4.0.

Scheme S1: **General scheme for an irreversible mechanism-based inhibitor in the continuous-release assay.**

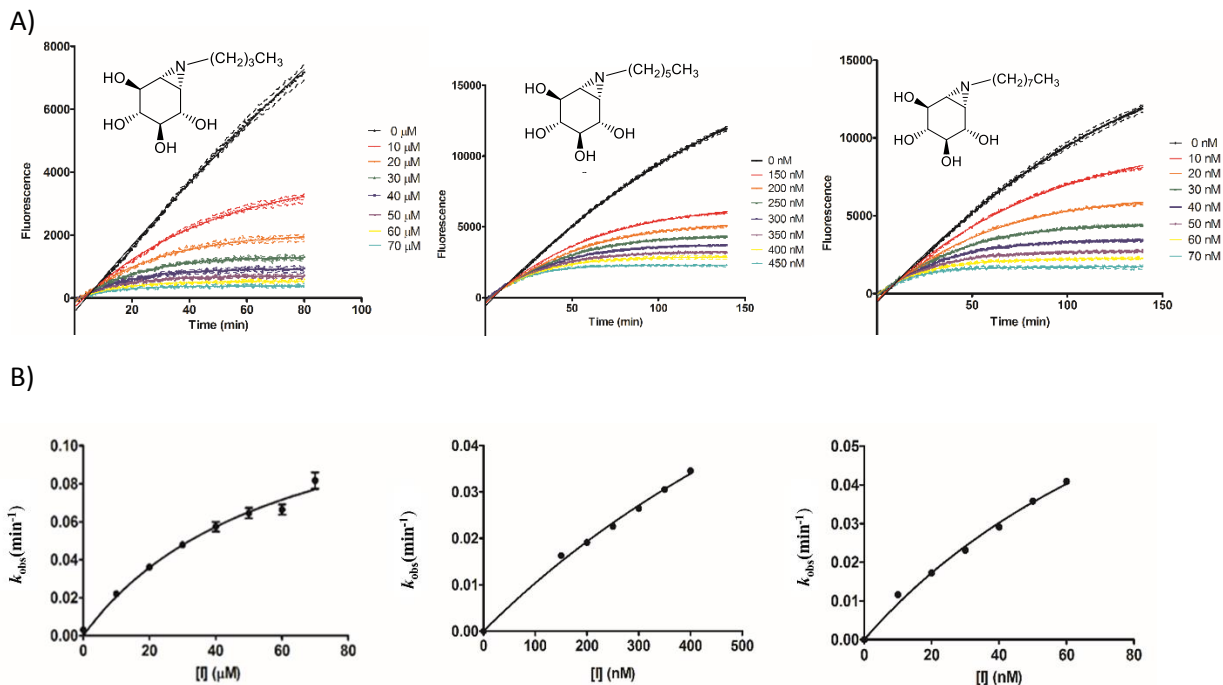


**Equation S1** 
$$y = (y_{max} - y_0)(1 - \exp^{-K_{obs}X}) + y_0$$

Where y is fluorescence in RFU and X is time in minutes.

**Equation S2** 
$$k_{obs} = \frac{k_i[I]}{K_{iapp} + [I]}$$

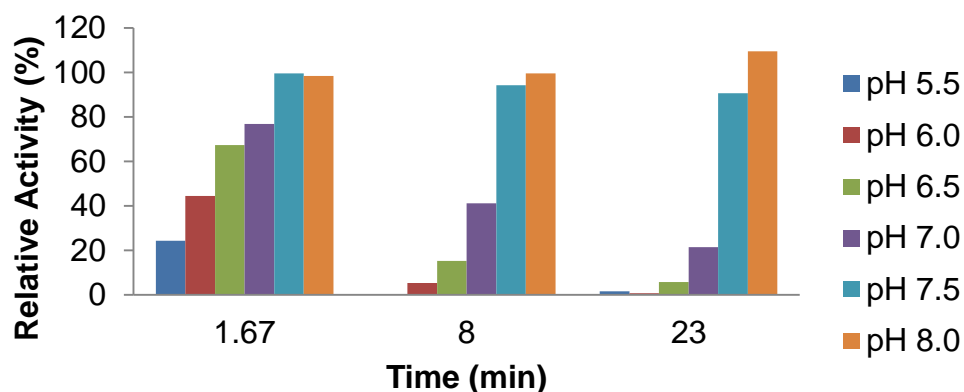
**Equation S3** 
$$K_{iapp} = K_i \left(1 + \frac{[S]}{K_m}\right)$$



**Supplementary Figure S1: Inactivation of GBA1 by aziridines 9, 10 and 11** A) Continuous-release inhibition assay of GBA1 at the indicated concentration of aziridine using an average of three individual measurements each corrected for photobleaching and spontaneous hydrolysis. Data points represent the mean values. Solid lines represent best fit according to the **Equation S1** while dashed lines represent standard deviation. B)  $K_{obs}$  versus  $[I]$  plots where data points represent the mean  $K_{obs}$  values and error bars the standard deviation. Solid line represents the best fit according to **Equation S2**.

### Inactivation of GBA1 by aziridine 11 at various pH's

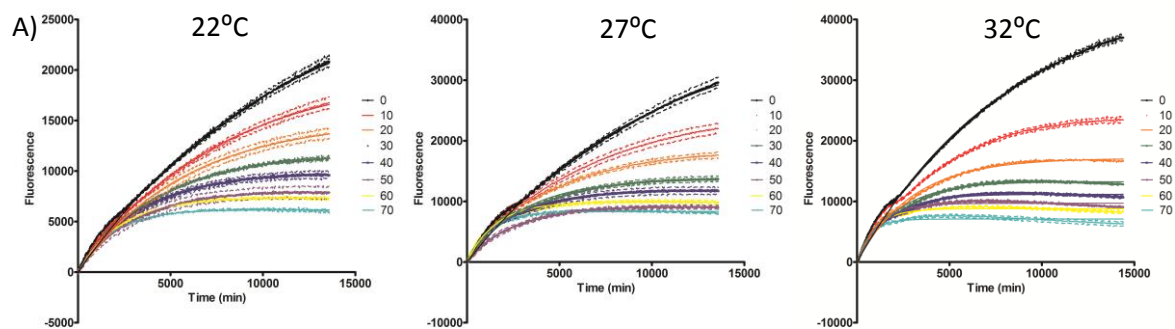
Aziridine **11** (50 nM) was incubated with GBA1 (41.4 nM) in assay buffer (50 mM acetate, 0.2 % v/v Triton X-100, 0.3 % w/v sodium taurocholate at pH 5.5 - 8) for 1.6, 8 and 23 minutes at the indicated pH. Aliquots (10  $\mu$ L) of the inactivation solution were withdrawn and diluted 20-fold in 96 well plates containing 2  $\times$   $K_M$  of the substrate 2,4-DNP- $\beta$ -D-Glc (190  $\mu$ L). Dilution of the reaction mixture effectively halts further inactivation by diluting the inhibitor and active site competition by excess substrate. The reaction rates were measured by the release of 2,4-dinitrophenolate ( $\lambda_{max}$ : 400 nm) and expressed as a percentage of the rate obtained for GBA1 incubated at the same pH for the equivalent time but without aziridine.

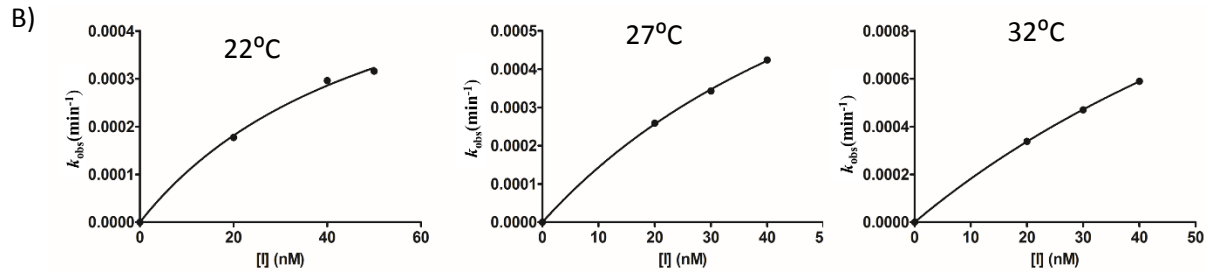


**Supplementary Figure S2:** The residual GBA1 activity after incubation with aziridine **11** (50 nM) at pH higher than the optimal activity. Data represents residual activity relative to a control assay where GBA1 was incubated under identical conditions with no inhibitor added. Inhibition of GBA1 is dramatically reduced at pH 7 and above suggesting that the active site general acid, Glu-235, must be protonated for efficient inactivation. These data suggest that the alkylated aziridine **11** is indeed mechanism-based and reacting efficiently only with the catalytically active enzyme.

### Inactivation of GBA1 activity at various temperatures

To determine the rate of enzyme inactivation at different temperatures, a range of eight concentrations of the inhibitor **11** predicted to surround the  $K_i$  value were incubated with GBA1 (4.14 nM) and substrate 4-MUGlc (6 mM) in assay buffer (50 mM acetate, 0.2 % v/v Triton X-100, 0.3 % w/v sodium taurocholate and pH 5.5). Reagents were preheated to the desired temperature for 10 minutes before initiating the reaction by the addition of enzyme to the inhibitor/substrate mixture. Bleaching of the fluorophore was corrected by subtracting blanks containing the substrate in assay buffer.

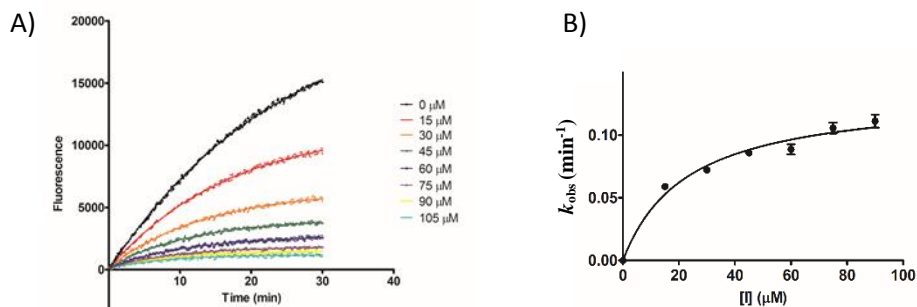




**Supplementary Figure S3: Inactivation of GBA1 by aziridine 11 at varying temperatures A)** Continuous-release inhibition assay of GBA1 at the indicated concentration of aziridine and temperature each corrected for photobleaching and spontaneous hydrolysis. Data points represent the mean values. Solid lines represent best fit according to the **Equation S1** while dashed lines represent standard deviation. B)  $K_{obs}$  versus  $[I]$  plots where data points represent the mean  $K_{obs}$  values and error bars the standard deviation. Solid line represents the best fit according to **Equation S2**.

**Supplementary Table S1 – The effects of temperature on the  $k_i$  and  $K_i$  inactivation parameters of aziridine 11 with GBA1**

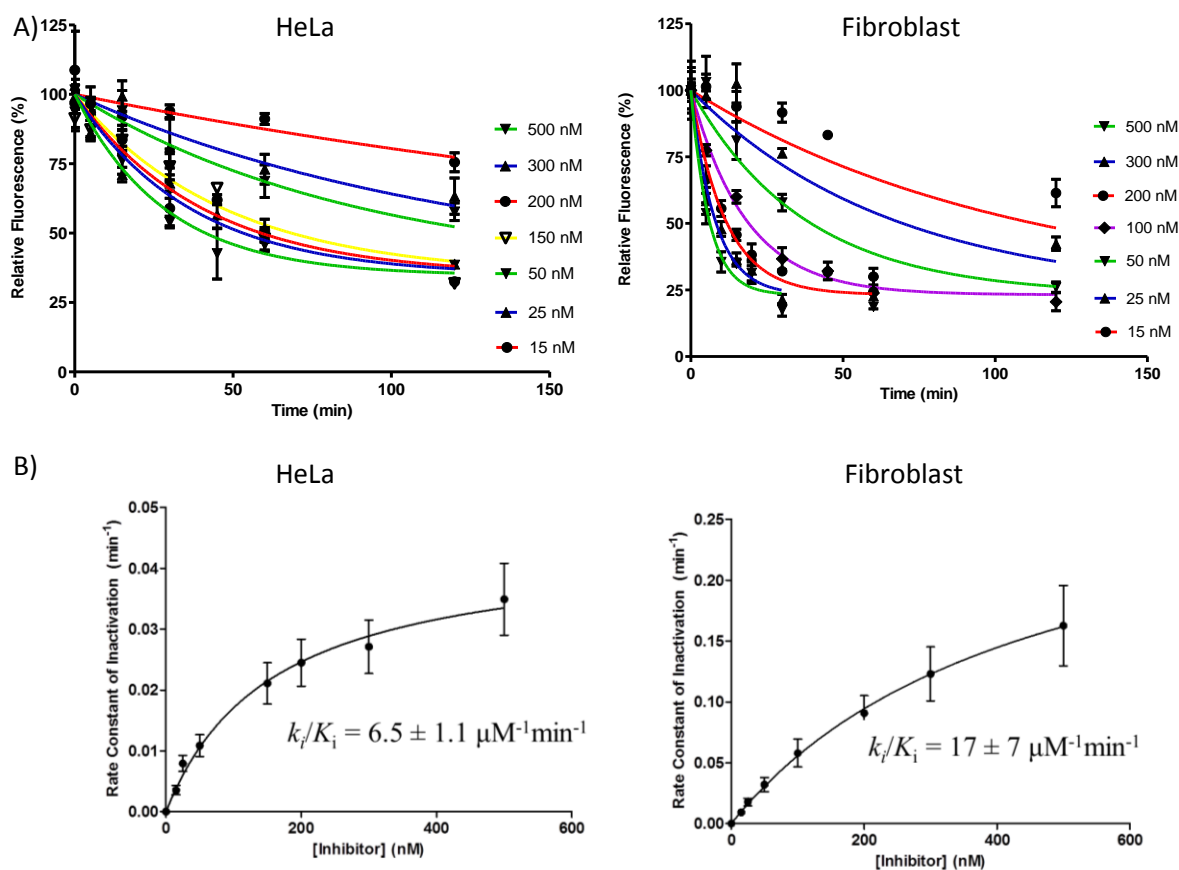
Temperature (°C)	$k_i$ (min <sup>-1</sup> )	$K_i$ (nM)	$k_i/K_i$ ( $\mu\text{M}^{-1}\text{min}^{-1}$ )
37	$0.12 \pm 0.014$	$4.8 \pm 0.075$	$25 \pm 3.4$
32	$0.0023 \pm 0.00073$	$4.8 \pm 0.19$	$0.48 \pm 0.17$
27	$0.0012 \pm 0.00055$	$2.9 \pm 0.20$	$0.41 \pm 0.22$
22	$0.00067 \pm 0.000052$	$2.2 \pm 0.29$	$0.030 \pm 0.0063$



**Supplementary Figure S4: Inactivation of GBA3 by aziridine 11** A) Continuous-release inhibition assay of GBA3 at the indicated concentration of aziridine using an average of three individual measurements each corrected for photobleaching and spontaneous hydrolysis. Data points represent the mean values. Solid lines represent best fit according to the **Equation S1** while dashed lines represent standard deviation. B)  $K_{obs}$  versus  $[I]$  plots where data points represent the mean  $K_{obs}$  values and error bars the standard deviation. Solid line represents the best fit according to **Equation S2**.

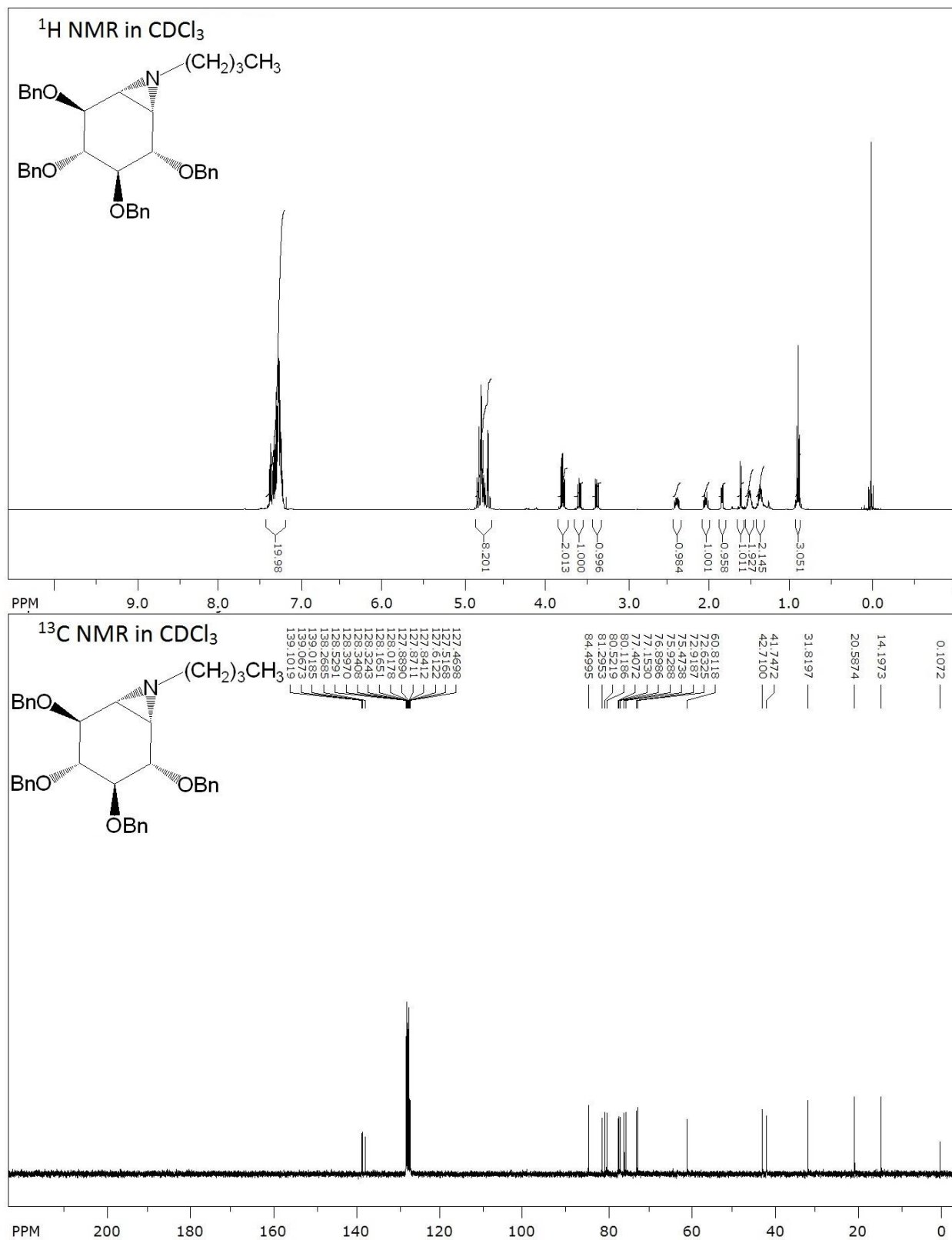
### $k_i/K_i$ in live HeLa and fibroblast cells

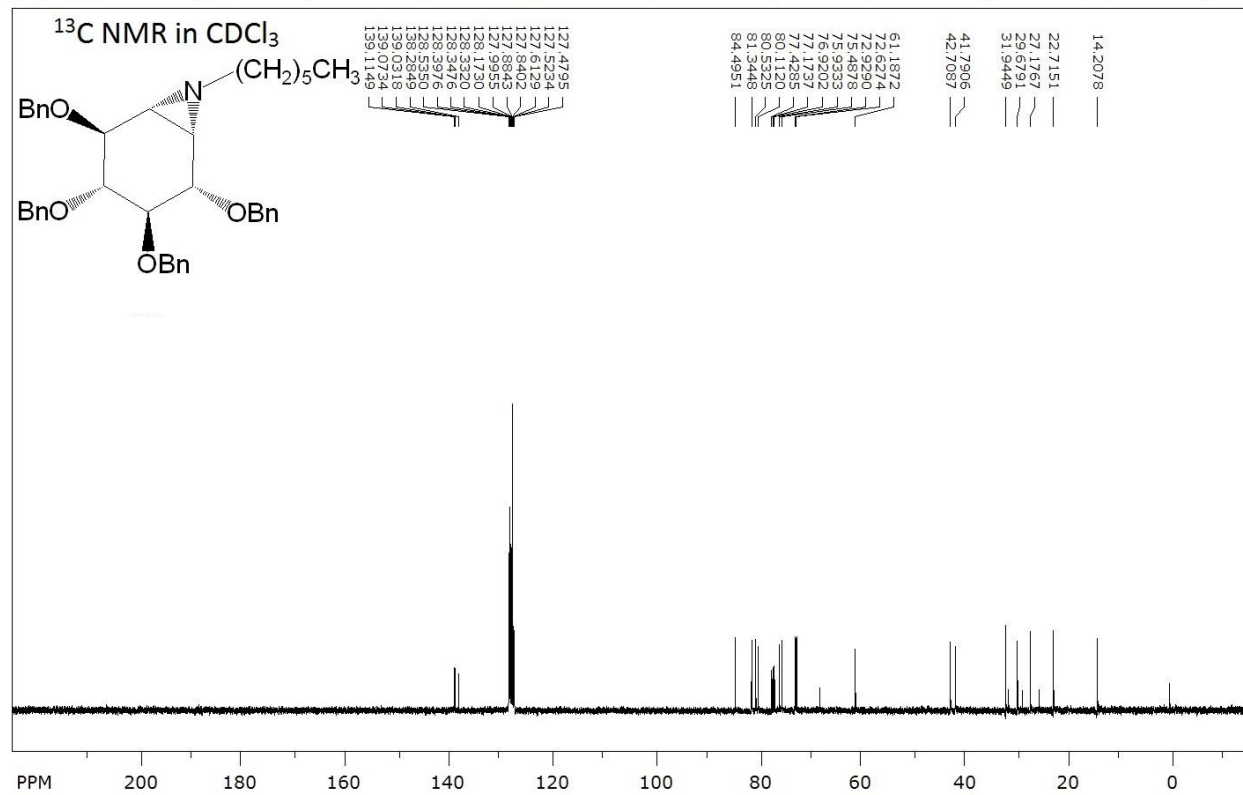
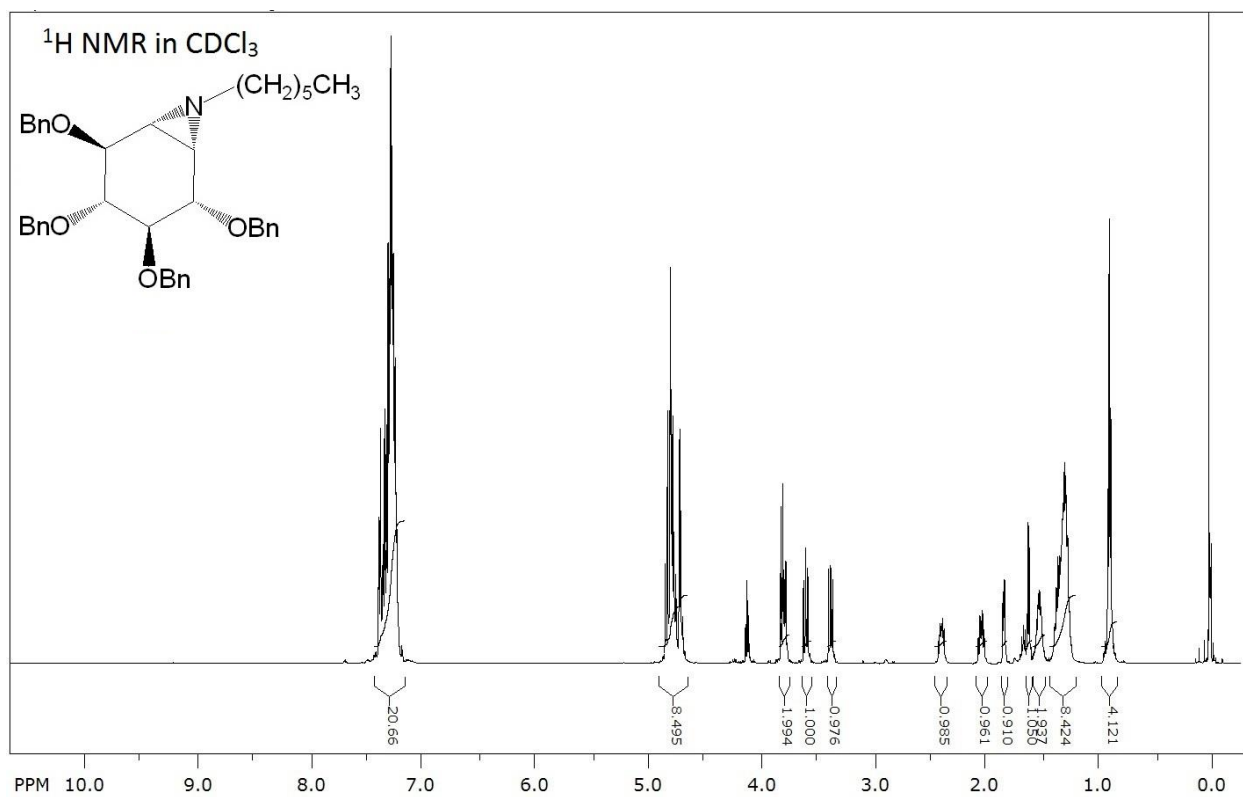
HeLa cervical cancer and fibroblasts cells were treated with aziridine **11** at a range of eight concentrations in serum free DMEM and allowed to incubate for the indicated lengths of time. The cells were washed with PBS (2x), lysed with lysis buffer (100  $\mu$ L, 10 mM Tris•HCl, 150 mM NaCl, 1 % Triton X-100, 1 mM EDTA, pH 7.5, 1% protease inhibitor cocktail) centrifuged at 16000 rpm for 10 minutes at 4°C and the supernatant stored at -80°C until the lysate assay was performed.

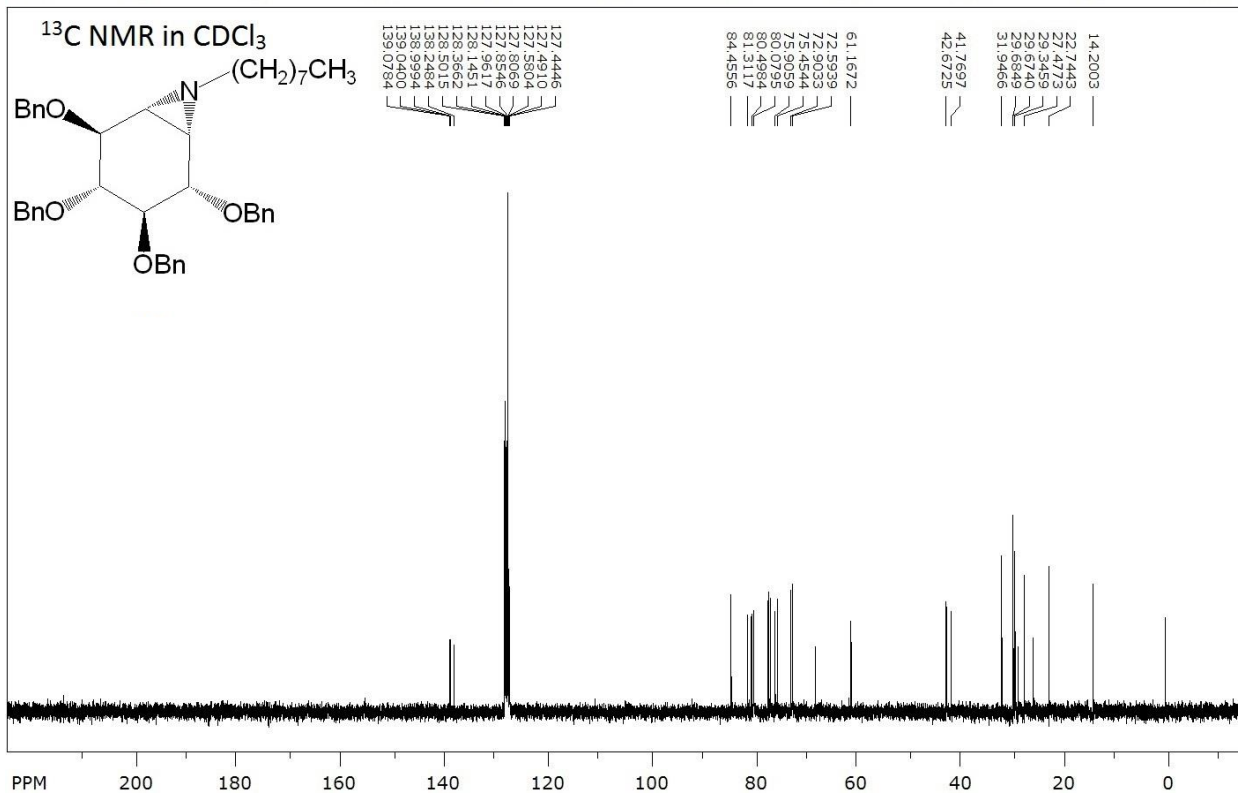
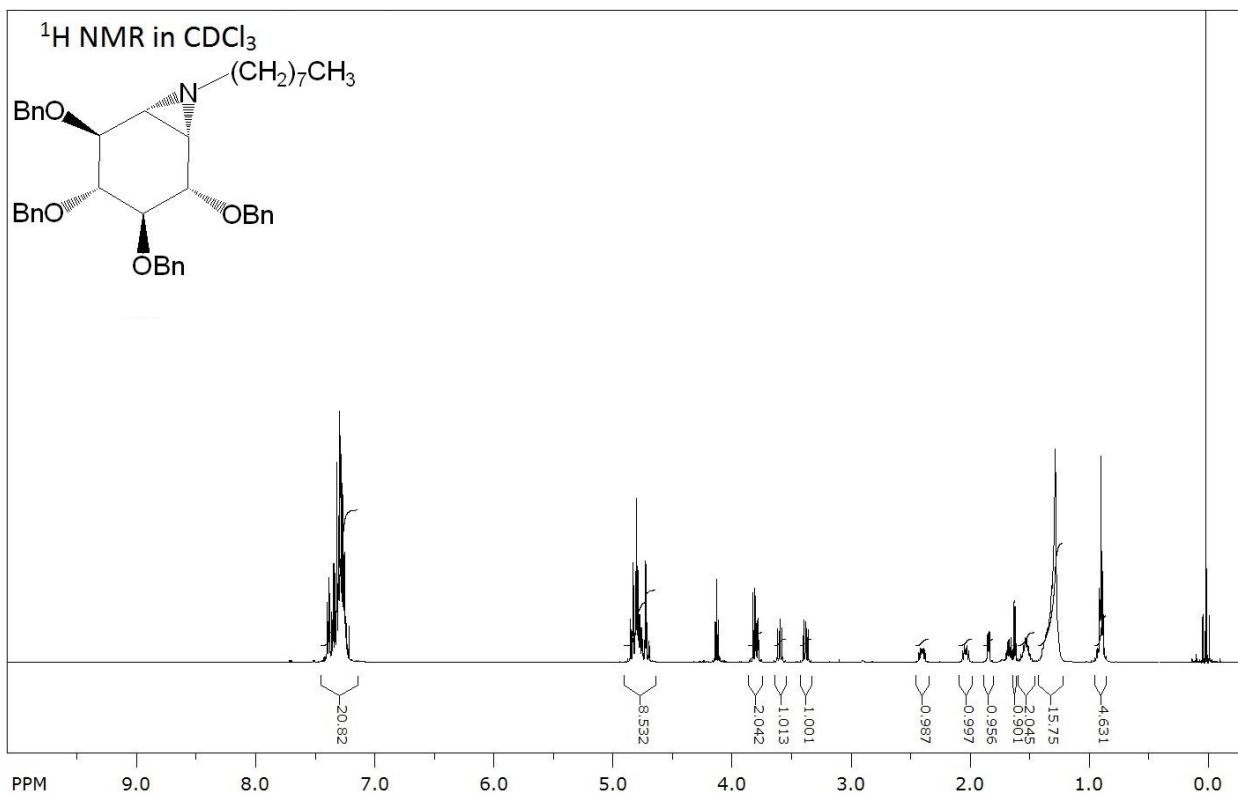


**Supplementary Figure S5: A)** One phase decay plots of the irreversible inhibition of *in vivo*  $\beta$ -glucosidase activity in lysates prepared from HeLa and fibroblasts cells treated with aziridine **11**. **B)**  $K_{obs}$  versus  $[I]$  plots. Solid lines represent best fit and data points represent the mean values  $\pm$  s.d.

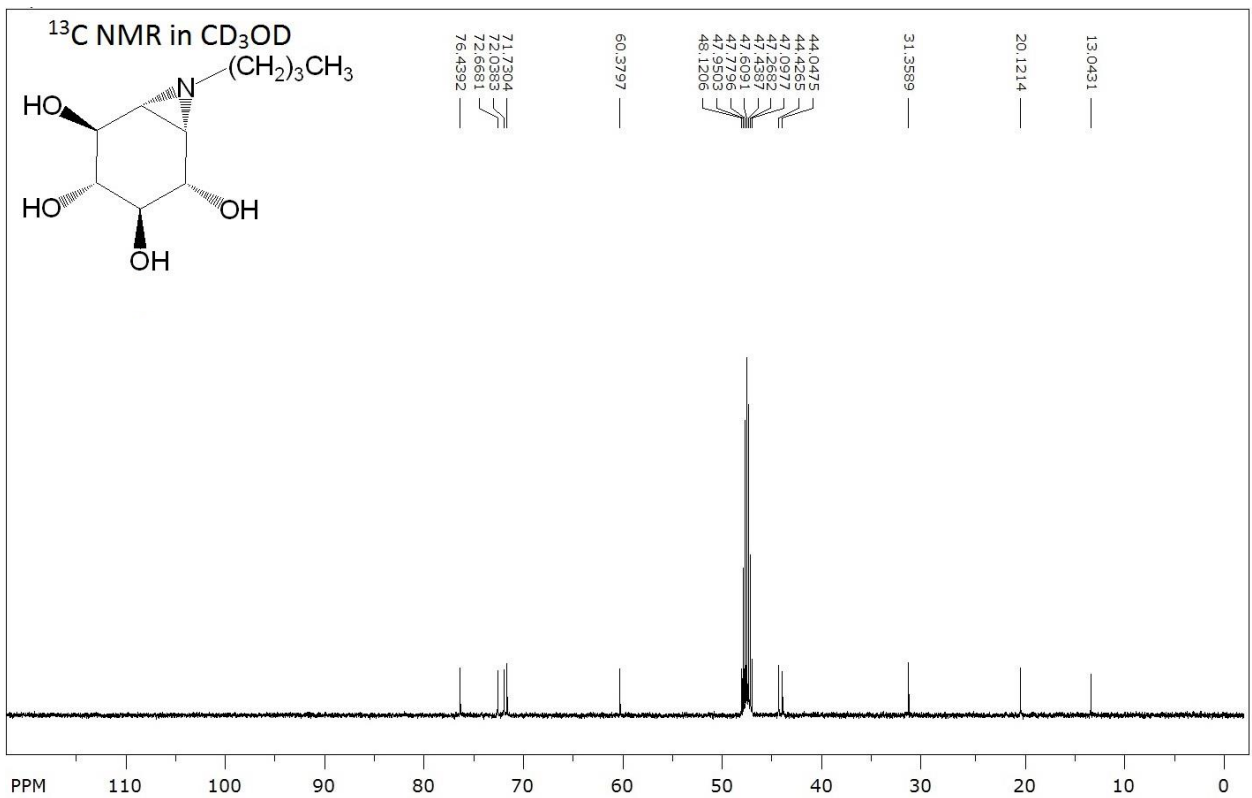
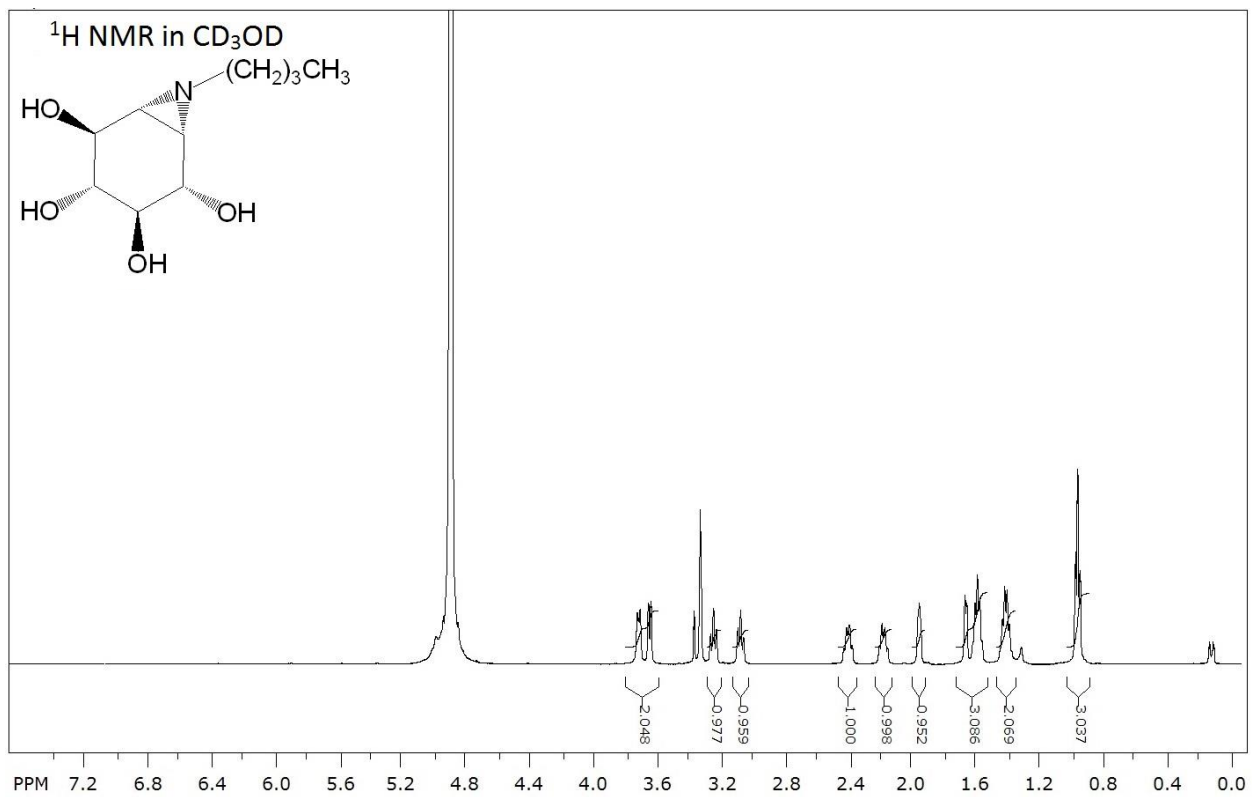
**<sup>1</sup>H and <sup>13</sup>C NMR Spectra of compounds S3, S5, S5, 9, 10, and 11**

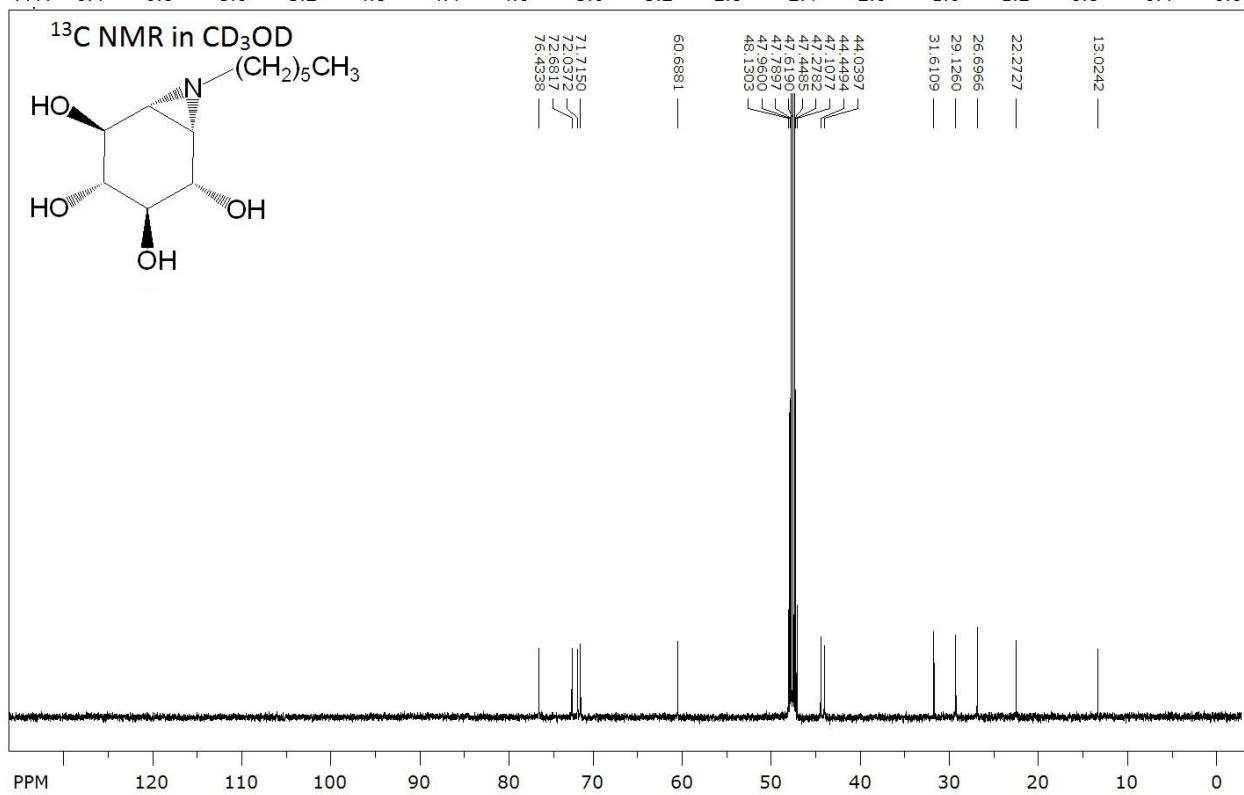
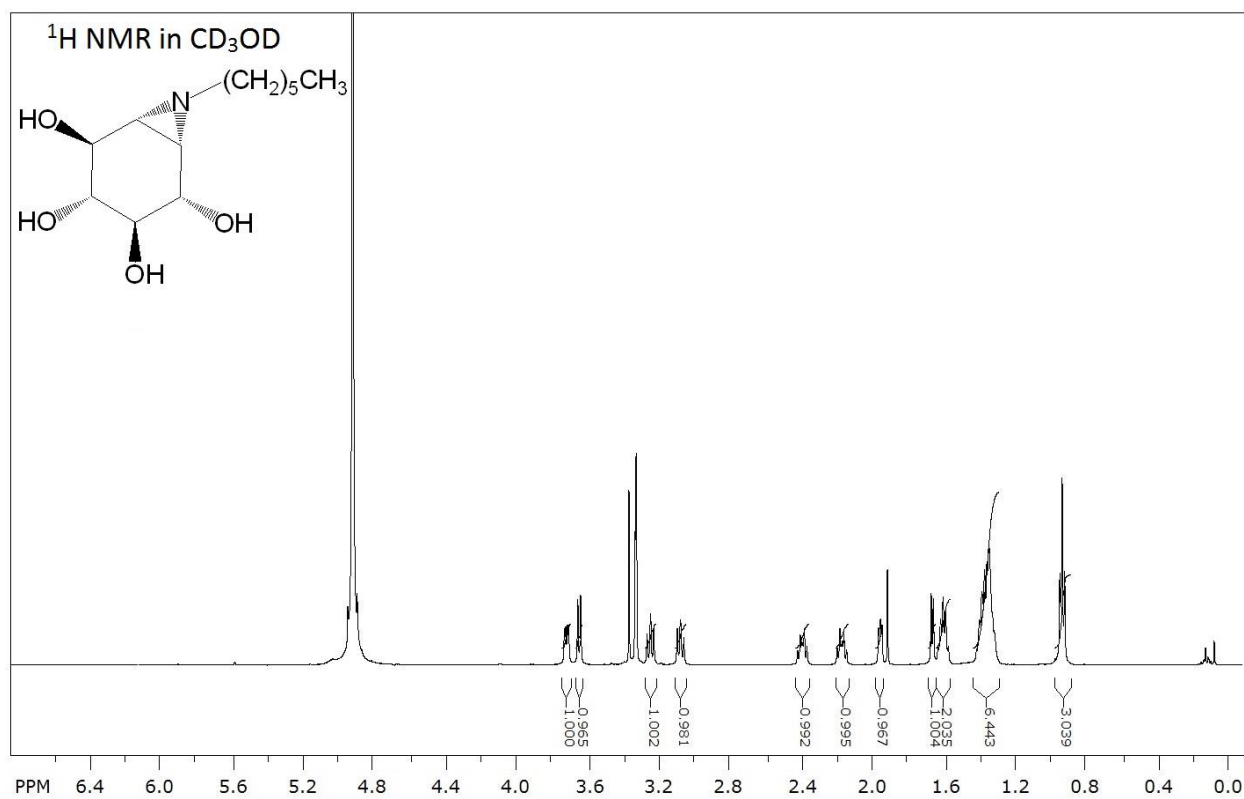


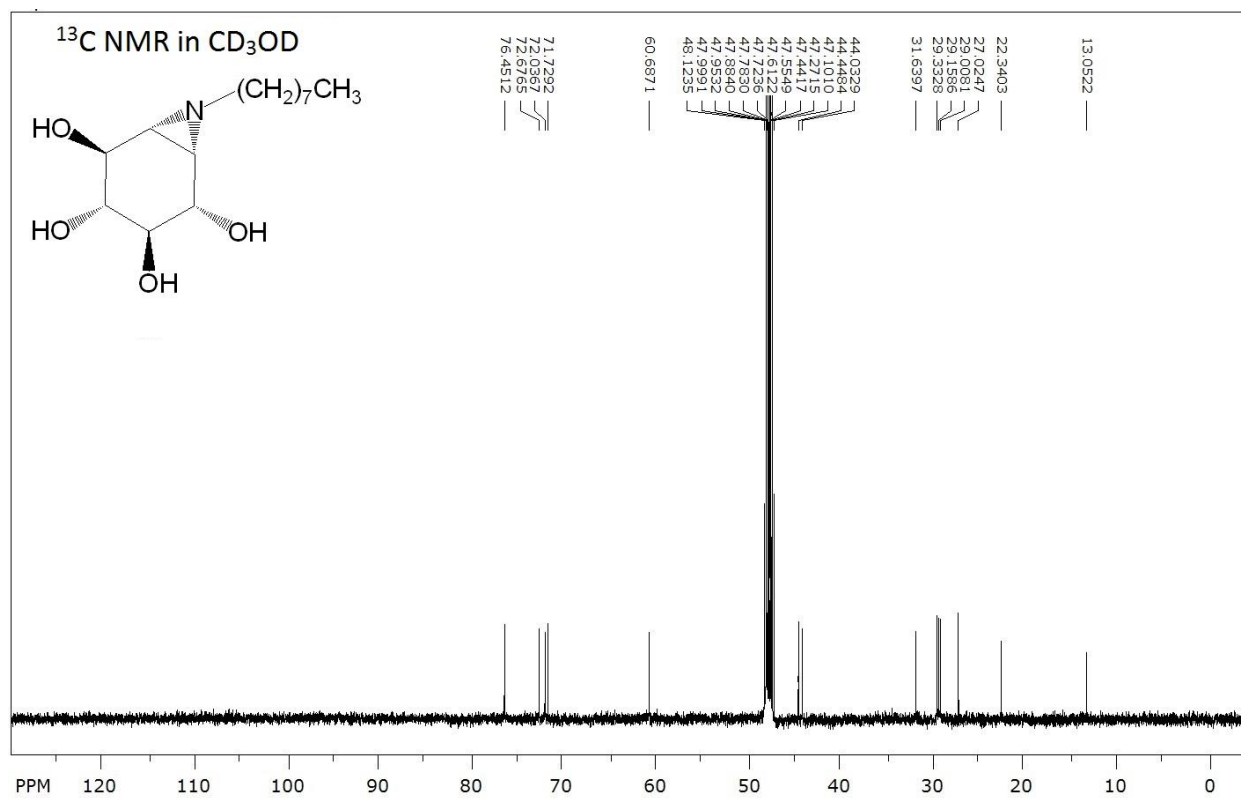
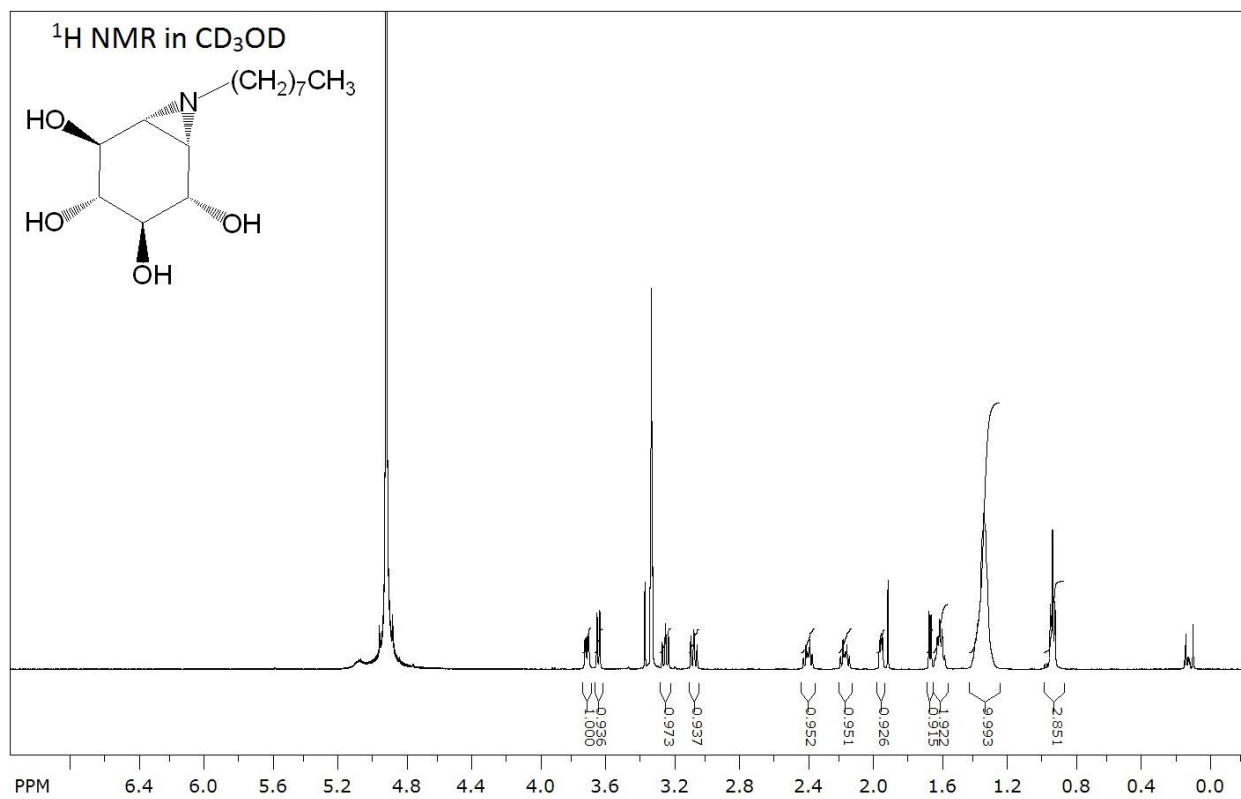




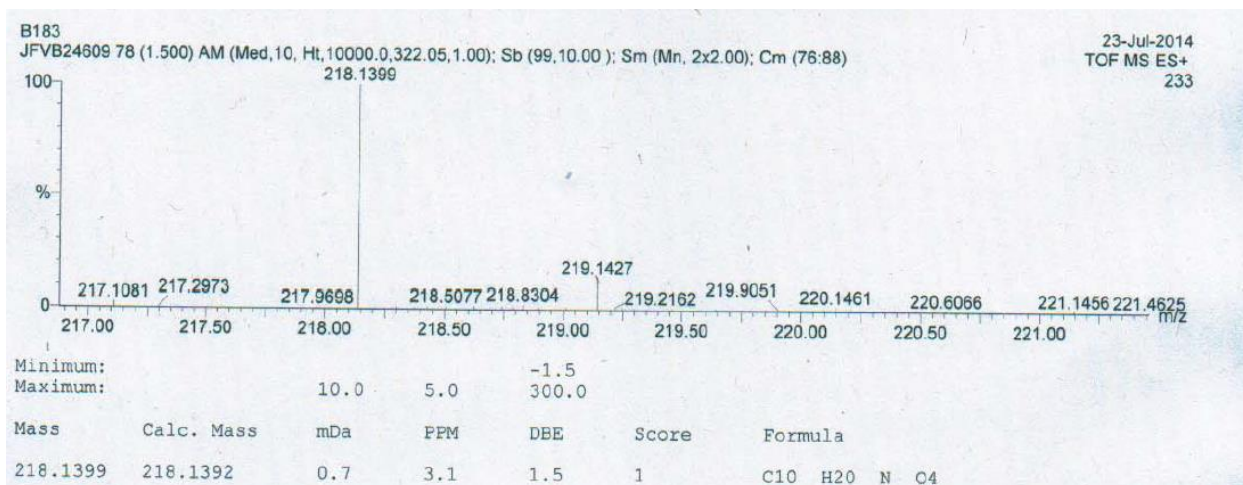




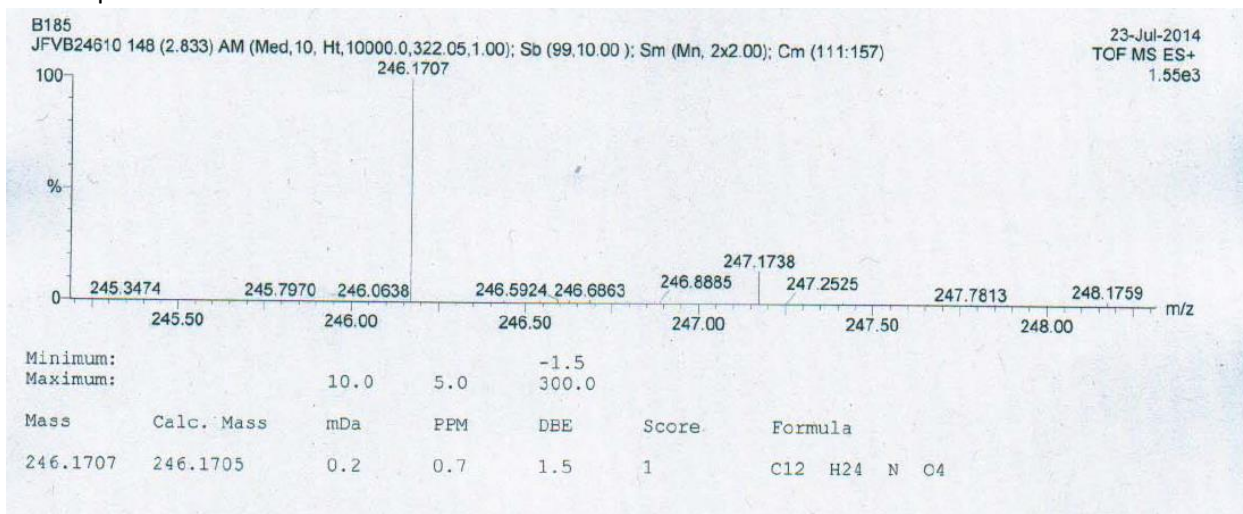




HRMS spectra of 9:



HRMS spectra of 10:



HRMS spectra of 11:

