SUPPLEMENTARY MATERIAL

Synthesis and cytotoxicity of the conjugates of diterpenoid isosteviol and *N*-acetyl-D-glucosamine

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Abstract

A series of conjugates of diterpenoid isosteviol (16-oxo-*ent*-beyeran-19-oic acid) and *N*-acetyl-D-glucosamine was synthesized and their cytotoxicity against some human cancer cell lines (M-Hela, MCF-7, Hep G2, Panc-1, PC-3), as well as normal human cell lines (WI-38, Chang liver) were assayed. Most of the conjugates was found to be cytotoxic against the mentioned cancer cell lines in the range of IC_{50} values 13 – 89 μM. Two lead compounds **14a** and **14b** showed selective cytotoxicity against M-Hela (IC_{50} 13 and 14 μM) that was two times as high as the cytotoxicity of the anti-cancer drug Tamoxifen in control (IC_{50} 28 μM). It was found that cytotoxic activity of the lead compounds against M-Hela cells is due to induction of apoptosis.

Keywords: Isosteviol, glucosamine, conjugates, diterpenoids, cytotoxicity

Experimental

Chemistry

General metods

The ^{1}H spectra were recorded on 400 MHz Brucker Advance. ^{13}C NMR spectra were obtained in the above instrument operating at 100.6 MHz. Melting points were obtained on Electrothermal IA 9100 (Bibby Scientific, Great Britain). The MALDI mass spectra were recorded on an Ultraflex III

TOF/TOF mass spectrometer (Bruker Daltonic GmbH, Germany) operated in the linear mode with the registration of positively charged ions or negatively charged ions. A Nd:YAG laser (λ = 355 nm, repetition rate 100 Hz) was used. The mass spectrum was obtained with an accelerating voltage of 25 kV and an ion extraction delay time of 30 ns. The resulting mass spectrum was formed due to multiple laser irradiation of the crystal (50 shots). The metal target MTP AnchorChipTM was used. Portions (0.5 μl) of a 1% matrix solution in acetonitrile and of a 0.1% sample solution in methanol were consecutively applied onto the target and evaporated. The data was obtained using the FlexControl program (Bruker Daltonik GmbH, Germany) and processed using the FlexAnalysis 3.0 program (Bruker Daltonik GmbH, Germany). The ESI MS measurements were performed using an AmazonX ion trap mass spectrometer (Bruker Daltonic GmbH, Germany) in positive (and/or negative) mode in the mass range of 70–3000. The capillary voltage was −3500 V, nitrogen drying gas – 10 $L \bullet min^{-1}$, desolvation temperature – 250 °C. A methanol/water solution (70:30) was used as a mobile phase at a flow rate of 0.2 mL/min by binary pump (Agilent 1260 chromatograph, USA). The sample was dissolved in methanol to a concentration of 10^{-6} $g \bullet L^{-1}$. Optical rotations were measured using a Perkin Elmer-341 automatic digital polarimeter (Perkin Elmer, USA) on λ = 589 нм, 20°C. The elemental analysis was carried out on a CHNS analyzer EuroEA3028-HT-OM (Eurovector SpA, Italy). The samples were weighed on Sartorius CP2P (Germany) microbalances in tin capsules. Callidus 4.1 software was used to perform quantitative measurements and evaluate the data received. Flash chromatography was performed on silica gel 60 (40-63 μm, Buchi, Sepacore). Thin-layer chromatography was carried out on plates with silica gel (Sorbfil, Russia). Spots of compounds were visualized by heating the plates (at ca. 150 °C) after immersion in a 5% H_2SO_4 and 95% H_2O. Anhydrous solvents were purified and dried (where appropriate) according to standard procedures. D-Glucosamine hydrochloride were purchased from abcr GmbH (Germany).

Isosteviol **1** were synthesized by analogous to the literature (Khaibullin et al 2009) from sweetener Sweta (Stevian Biotechnology Corp.). Derivative **3a** was prepared according to protocols described in (Garifullin et al. 2015). Compounds **3b-c** и **4a-b** were synthesized and published in early publication (Sharipova et al. 2018). 3,4,6-Tri-O-acetyl-2-deoxy-2-[2,2,2-trichloroethoxycarbonyl(amino)]-α-bromo-D-glucopyranose **9** were synthesized by analogous to the literature (Lioux et al. 2005), spectral data were in keeping with published ones (Higashi et al. 1990).

19-(5-Hydroxypentylaza)-16,19-dioxo-*ent*-beyeran 5. A solution of 2 g (6.3 mmol) of isosteviol **1** in 2 mL of $SOCl_2$ was heated for 2 h at 40°C under argon. Excess $SOCl_2$ was distilled off under reduced pressure, the residue was treated with anhydrous methylene chloride, the mixture was stirred,

the solvent was distilled off, and the residue was dried *in vacuo*. Freshly prepared 16-oxo-ent-beyeran-19-oyl chloride **2** was dissolved in 10 mL of anhydrous CH_2Cl_2, and the solution was added dropwise under argon to ice cooled solution of 2.17 g (21 mmol) of 5-amino-butane-1-ol in 5 mL of anhydrous methylene chloride. The mixture was stirred at room temperature for 7 h. Reaction mixture was washed with water (3×10 mL) and dried over $CaCl_2$. The residue was purified by chromatography using petroleum ether–ethyl acetate (3:2 → 1:4) as eluent. Yield 2.1 g (83%) as colorless needle-like crystals; m.p. 157.5-157.9 °C; $[\alpha]_D^{20}$ = -45.9 (1, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.8–2.05 (m, 24H, *ent*-beyerane, $(CH_2)_3$ - spacer), 0.77 (s, 3H, H-20), 0.96 (s, 3H, H-17), 1.16 (s, 3H, H-18), 2.64 (dd, 1H, *J* = 18.6, 3.8 Hz, H-15α), 3.18-3.26 (m, 2H, H-5''), 3.63 (t, 2H, *J* = 6.3 Hz, H-1''), 5.65 (t, 1H, *J* = 5.4 Hz, N<u>H</u>C(O)CH_3). ESI-MS *m/z* 404.3 $[M+H]^+$, 426.3 $[M+Na]^+$. Anal.,%: C, 74.27; H, 10.05; N, 3.52. $C_{25}H_{41}NO_3$. Calcd., %: C, 74.40; H, 10.24; N, 3.47. *M* 403.60.

General procedure for the synthesis of the glycosides 11, 20a-b. 3,4,6-Tri-O-acetyl-2-deoxy-2-[2,2,2-trichloroethoxycarbonyl(amino)]-α-bromo-D-glucopyranose **9** (1 mol), K_2CO_3 (1.5 mol), and TBAB (0.25 mol) were added to a stirred solution of isosteviol **1** (1 mol) or its derivatives **4a** or **4b** (1 mol) in the mixture CH_2Cl_2/H_2O 1:1 (40 mL) and the reaction mixture was refluxed until isosteviol **1** or its derivatives **4a** or **4b** had completely reacted, as indicated by TLC (30–40 h). After cooling to 25 °C, $CHCl_3$ was added and the mixture washed with water and brine. The organic layer was dried over $MgSO_4$, filtered, concentrated under reduced pressure, the residue was used without further purification in the next step. A suspension of compounds **10**, **19a** or **19b** (1 mol) and Zn powdered (10 mol) in AcOH (10 mL) was stirred for 10 min at room temperature, then Ac_2O (10 mol) was added, and the mixture was stirred for 24 h at room temperature. The suspension was filtered, washed with CH_2Cl_2 and concentrated. The residue was diluted with CH_2Cl_2 and washed with water. The organic layer was separated, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography eluted with petroleum ether/EtOAc to afford the corresponding compounds **11**, **20a** or **20b**.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl-16-oxo-*ent*-beyeran-19-oate (11). Yield 0.17 g (25 %), colorless oil, $[\alpha]_D^{20}$ = -25.5 (1.75, $CHCl_3$). Eluent: petroleum ether/EtOAc = 4:1 → 1:2. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.73–1.93 (m, 18H, *ent*-beyerane), 0.69 (s, 3H, H-20), 0.97 c (s, 3H , H-17), 1.18 c (s, 3H , H-18), 1.91 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.20 (d, 1H, H-3eq, *J* = 13.5 Hz), 2.57 (dd, 1H, *J* = 18.8, 3.6 Hz, H-15α), 3.72–3.80 (m, 1H, H-5'), 4.04–4.10 (m, 1H, H-6'a), 4.26 (dd, 1H, *J* = 12.3, 5.15 Hz, H-6'b), 4.32–4.41 (m, 1H, H-2'), 5.04–5.15 (m, 2H, H-4', H-3'), 5.46 (d, 1H, *J* = 9.8 Hz, N<u>H</u>C(O)CH_3), 5.64

(d, 1H, *J* = 8.8 Hz, H-1'). MALDI-MS: *m/z*: 671.3 [*M*+H+Na]$^+$. Anal.,%: C 63.50; H 7.59; N 2.19. $C_{34}H_{49}NO_{11}$. Calcd., %: C, 63.04; H 7.62; N 2.16. *M* 647.75.

(3,4,6-Tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyloxopentyl)-16-oxo-*ent*-beyeran-19-oate (20a). Yield 0.25 g (32 %), colorless oil, $[\alpha]_D^{20}$ = -29.7° (2.95, $CHCl_3$). Eluent: petroleum ether/EtOAc = 1:1 → EtOAc. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.79–2.19 (m, 25H, *ent*-beyerane, $(CH_2)_3$ - spacer), 0.69 (s, 3H , H-20), 0.96 (s, 3H , H-17), 1.17 (s, 3H , H-18), 1.89 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.30–2.42 (m, 2H, H-2''), 2.60 (dd, 1H, *J* = 18.6, 3.3 Hz, H-15α), 3.76–3.84 (m, 1H, H-5'), 4.00 (q, 2H, *J* = 6.5 Hz, H-6''), 4.10 (dd, 1H, *J* = 12.4, 1.7 Hz, H-2'), 4.19–4.29 (m, 2H, H-6'), 5.11 (t, 1H, *J* = 9.5 Hz, H-3'), 5.17 (t, 1H, *J* = 9.8 Hz, H-4'), 5.72 (d, 1H, *J* = 8.8 Hz, H-1'), 5.87 (d, 1H, *J* = 9.4 Hz, N<u>H</u>C(O)CH_3). MALDI-MS: *m/z*: 784.7 [*M*+Na]$^+$, 800.7 [*M*+K]$^+$. Anal.,%: C 62.98; H 7.49; N 1.87. $C_{40}H_{59}NO_{13}$ Calcd., %: 63.06; H 7.81; N 1.84. *M* 761.90.

(3,4,6-Tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyloxononyl)-16-oxo-*ent*-beyeran-19-oate (20b). Yield 0.16 g (20 %), colorless oil, $[\alpha]_D^{20}$= -20.9 (0.7; CH_3OH). Eluent: petroleum ether/EtOAc = 3:1 → 1:2. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.70 (s, 3H , H-20), 0.97 (s, 3H , H-17), 1.18 (s, 3H , H-18), 0.84–1.87 (m, 32H, *ent*-beyerane, $(CH_2)_7$ - spacer), 1.90 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.17 (d, 1H, *J* = 13.0 Hz, H-3eq), 2.36 (t, 2H, *J* = 7.5 Hz, H-2''), 2.62 (dd, 1H, *J* = 18.6, 3.7 Hz, H-15α), 3.76 – 3.83 (m, 1H, H-5'), 3.96 – 4.04 (m, 2H, H-10''), 4.05-4.12 (m, 1H, H-6'a), 4.22–4.28 (m, 2H, H-2', H-6'b), 5.08 – 5.20 (m, 2H, H-3', H-4'), 5.72 (d, 1H, *J* = 8.8 Hz, H-1'), 5.87 (d, 1H, *J* = 9.6 Hz, N<u>H</u>C(O)CH_3). MALDI-MS: *m/z*: 840.8 [*M*+Na]$^+$, 856.7 [*M*+K]$^+$. Found: C 64.54; H 8.29; N 1.75. $C_{44}H_{67}NO_{13}$. Calcd., %: C 64.61; H 8.26; N 1.71. *M* 818.00.

General procedure for the synthesis of the glycosides 14a-c, 17. 3,4,6-Tri-O-acetyl-2-deoxy-2-[2,2,2-trichloroethoxycarbonyl(amino)]-α-bromo-D-glucopyranose **9** (1 mol), and $ZnCl_2$ (1 mol) were added to a stirred solution of corresponding compounds **3a-c** or **5** (1 mol) in anhydrous CH_2Cl_2 (25 mL) and the reaction mixture was stirred at room temperature. When complete disappearance of starting compounds **3a-c** or **5** on TLC was observed (24–48 h), reaction mixture was filtered, CH_2Cl_2 was added and the organic solution washed with 5% $NaHCO_3$ solution and water. The organic layer was dried over Na_2SO_4, filtered, concentrated *in vacuo*, the residue was used without further purification in the next step. A suspension of compounds **13a-c** or **16** (1 mol) and Zn powdered (10 mol) in AcOH (10 mL) was stirred for 10 min at room temperature, then Ac_2O (10 mol) was added,

and the mixture was stirred for 24 h at room temperature. The suspension was filtered, washed with CH_2Cl_2 and concentrated. The residue was diluted with CH_2Cl_2 and washed with water. The organic layer was separated, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography eluted with petroleum ether/EtOAc to afford the corresponding compounds **14a-c** or **17**.

3',4',6'-Tri-*O*-acetyl-2'-deoxy-2'-acetamido-α-D-glucopyranosylbutyl 16-oxo-*ent*-beyeran-19-oate 14a. Yield 0.39 g (52 %), colorless oil, $[\alpha]_D^{20}$ = 16.6 (0.65, CH_2Cl_2). Eluent: petroleum ether/EtOAc = 5:1 → 1:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.80–1.96 (m, 22H, *ent*-beyerane, $(CH_2)_2$ - spacer), 0.72 (s, 3H , H-20), 0.97 с (s, 3H , H-17), 1.20 с (s, 3H , H-18), 1.96 с (s, 3H, 2 CH_3CO), 2.01 с (s, 3H, 2 CH_3CO), 2.02 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO), 2.19 д (d, 1H, *J* = 14.4 Hz, H-3eq), 2.62 (dd, 1H, *J* = 18.5, 3.6 Hz, H-15α), 3.41–3.49 (m, 1H, H-1''a), 3.70–3.78 м (m, 1H, H-1''b), 3.88–3.94 (m, 1H, H-5'), 4.01–4.14 (m, 3H, H-6'a, H-4''), 4.23 (dd, 1H, *J* = 12.3, 4.5 Hz, H-6'b), 4.30–4.37 (m, 1H, H-2'), 4.84 (d, 1H, *J* = 3.6 Hz, H-1'), 5.11 (t, 1H, H-4', *J* = 9.9 Hz), 5.19 (t, 1H, *J* = 9.9 Гц, H-3'), 5.83 (d, 1H, *J* = 9.3 Гц, N<u>H</u>C(O)CH_3). MALDI-MS: *m/z*: 742.3 $[M+Na]^+$. Anal.,%: C 63.45; H 8.04; N 1.93. $C_{38}H_{57}NO_{12}$. Calcd., %: C 63.40; H 7.98; N 1.95. *M* 719.86.

3',4',6'-Tri-*O*-acetyl-2'-deoxy-2'-acetamido-α-D-glucopyranosylhexyl 16-oxo-*ent*-beyeran-19-oate 14b. Yield 0.32 g (30 %), colorless oil, $[\alpha]_D^{20}$ = 11.3 (0.6, $CHCl_3$). Eluent: petroleum ether/EtOAc = 4:1 → 1:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.78–1.90 (m, 26H, *ent*-beyerane, $(CH_2)_4$ - spacer), 0.71 (s, 3H , H-20), 0.97 (s, 3H , H-17), 1.19 (s, 3H , H-18), 1.95 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 2.18 (d, 1H, *J* = 13.5 Hz, H-3eq), 2.62 (dd, 1H, *J* = 18.5, 3.7 Hz, H-15α), 3.40–3.47 (m, 1H, H-1''a), 3.65–3.70 (m, 1H, H-1''b), 3.90–3.95 (m, 1H, H-5'), 3.98–4.11 (m, 3H, H-6'a, H-6''), 4.23 (dd, 1H, *J* = 12.3, 4.6 Hz, H-6'b), 4.30–4.37 (m, 1H, H-2'), 4.83 (d, 1H, *J* = 3.7 Hz, H-1'), 5.11 (t, 1H, , *J* = 9.9 Hz, H-4'), 5.20 (t, 1H, *J* = 9.9 Hz, H-3'), 5.71 (d, 1H, *J* = 9.5 Hz, N<u>H</u>C(O)CH_3). MALDI-MS: *m/z*: 770.6 $[M+Na]^+$, 786.6 $[M+K]^+$. Anal.,%: C 64.30; H 8.19; N 1.85. $C_{40}H_{61}NO_{12}$. Calcd., %: C 64.24; H 8.22; N 1.87. *M* 747.91.

3',4',6'-Tri-*O*-acetyl-2'-deoxy-2'-acetamido-α-D-glucopyranosyldecyl 16-oxo-*ent*-beyeran-19-oate 14c. Yield 0.15 g (18 %), colorless oil, $[\alpha]_D^{20}$ = 11.2 (1, $CHCl_3$). Eluent: petroleum ether/EtOAc = 5:1 → 1:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.80–1.91 м (m, 34H, *ent*-beyerane, $(CH_2)_8$ - spacer), 0.70 (s, 3H , H-20), 0.96 (s, 3H , H-17), 1.18 (s, 3H , H-18), 1.94 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 2.17 (d, 1H, *J* = 13.4 Hz, H-3eq), 2.61 (dd, 1H, *J* = 18.5, 3.7 Hz, H-15α), 3.37–3.46 (m, 1H, H-1''a), 3.62–3.71 (m, 1H, H-1''b), 3.89–3.96 (m, 1H, H-5'), 3.96–

4.12 (m 3H, H-6'a, H-10''), 4.22 (dd, 1H, *J* = 12.3, 4.8 Hz, H-6'b), 4.28–4.36 (m, 1H, H-2'), 4.81 (d, 1H, *J* = 3.7 Hz, H-1'), 5.09 (t, 1H, *J* = 9.8 Hz, H-4'), 5.19 (t, 1H, *J* = 10.0 Hz, H-3'), 5.67 (d, 1H, *J* = 9.6 Hz, N<u>H</u>C(O)CH_3). MALDI-MS: *m/z*: 826.7 $[M+Na]^+$, 842.7 $[M+K]^+$. Anal.,%: C 65.68; H 8.71; N 1.72. $C_{44}H_{69}NO_{12}$. Calcd., %: C 65.73; H 8.65; N 1.74. *M* 804.02.

19-(3',4',6'-Tri-O-acetyl-2'-deoxy-2'-acetamido-α,β-D-glucopyranosylpentylaza)-16,19-dioxo-ent-beyeran 17. Yield 0.34 g (30 %), colorless oil, $[\alpha]_D^{20}$ = -34.9 (0.68, $CHCl_3$). Eluent: petroleum ether/EtOAc = 3:1 → 1:2. ^{1}H NMR (400 MHz, CD_3OD): δ 0.90–2.15 (m, 25H, *ent*-beyerane, $(CH_2)_3$ - spacer), 0.79 (s, 3H , H-20), 0.94 (s, 3H , H-17), 1.16 (s, 3H , H-18), 1.90 (s, 3H, CH_3CO), 1.98 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 2.60 (dd, 1H, *J* = 18.5, 3.5 Hz, H-15α), 3.05–3.23 (m, 2H, H-4''), 3.48–3.54 (m, 1H, H-1''a), 3.75-3.80 (m, 1H, H-2'), 3.81–3.87 (m, 2H, H-5', H-1''b), 4.12 (dd, 1H, *J* = 12.3, 2.2 Hz, H-6'a), 4.27 (dd, 1H, *J* = 12.2, 4.6 Hz, H-6'b), 4.62 (d, 1H, *J* = 8.4 Hz, β-anomer (84%), H-1'), 4.81 (d, 1H, , *J* = 3.9 Hz, α-anomer (16%), H-1'), 4.97 (t, 1H, *J* = 9.7 Hz, H-4'), 5.20 (t, 1H, *J* = 9.7 Hz, H-3'), 7.17–7.23 (m, 2H, C(O)N<u>H</u>, N<u>H</u>C(O)CH_3). MALDI-MS: *m/z*: 755.5 $[M+Na]^+$, 771.4 $[M+K]^+$. Anal.,%: C 63.86; H 8.31; N 3.79. $C_{39}H_{60}N_2O_{11}$. Calcd., %: C 63.91; H 8.25; N 3.82. *M* 732.90.

General procedure for the preparation of compounds 12, 15a-c, 18. The corresponding compounds **11**, **14a-c** or **17** were dissolved in anhydrous MeOH at room temperature and the pH was adjusted to 9.0 using 0.25 N MeONa/MeOH. The deacetylation procedure was monitored by TLC and upon its completion the pH adjusted to 7.0 with acidic ion-exchange resin Amberlyst 15. After filtration, the filtrate was concentrated *in vacuo* to yield the corresponding glycosides **12**, **15a-c** or **18.**

2-deoxy-2-acetamido-β-D-glucopyranosyl-16-oxo-*ent*-beyeran-19-oate 12. Yield 0.07 g (78 %), White powder, m.p. 154.1-155.1 ^{o}C; $[\alpha]_D^{20}$= -44.5 (0.45; CH_3OH). ^{1}H NMR (400 MHz, CD_3OD): δ 0.76 (s, 3H , H-20), 0.94 (s, 3H , H-17), 1.20 (s, 3H , H-18), 0.80–2.07 (m, 18H, *ent*-beyerane), 1.98 (s, 3H , CH_3CO), 2.17 (d, 1H, *J* = 13.2 Hz, H-3eq), 2.58 (dd, 1H, *J* = 18.8, 3.6 Hz, H-15α), 3.33 – 3.51 (m, 3H, H-3', H-4', H-5'), 3.70 (dd, 1H, *J* = 12.1, 4.9 Hz, H-6'a), 3.83 (dd, 1H, *J* = 12.0, 2.2 Hz, H-6'b), 3.89 (t, 1H, *J* = 9.1 Hz, H-2'), 5.60 (d, 1H, *J* = 8.9 Hz, H-1'). ESI-MS: *m/z*: 544.4 $[M+Na]^+$. Anal.,%: C 64.29; H 8.40; N 2.65. $C_{28}H_{43}NO_8$. Calcd., %: C 64.47; H 8.31; N 2.69. *M* 521.64.

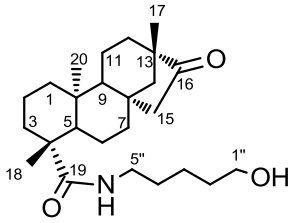
2'-Deoxy-2'-acetamido-α-D-glucopyranosylbutyl 16-oxo-*ent*-beyeran-19-oate 15a. Yield 0.13 g (68 %), colorless oil, $[\alpha]_D^{20}$ = 3.5 (1.36, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87–2.00 (m, 22H, *ent*-beyerane, $(CH_2)_2$ - spacer), 0.71 (s, 3H , H-20), 0.97 (s, 3H , H-17), 1.19 (s, 3H , H-18), 2.05 (s, 3H, CH_3CO), 2.17 (d, 1H, *J* = 13.4 Hz, H-3eq), 2.63 (dd, 1H, *J* = 18.6, 3.3 Hz, H-15α), 3.37–3.44 (m, 1H,

H-1’’a), 3.52–3.58 (m, 1H, H-1’’b), 3.61–3.66 (m, 1H, H-4’), 3.67–3.74 (m, 2H, H-6’a, H-3’), 3.75–3.80 (m, 1H, H-6’b), 3.86–3.94 (m, 1H, H-5’), 4.02–4.13 (m, 3H, H-2’, H-4’’), 4.78 (d, 1H, *J* = 3.4 Hz, H-1’), 6.32–6.41 (m, 1H, N<u>H</u>C(O)CH_3). MALDI-MS: *m/z*: 616.5 $[M+Na]^+$. Anal.,%: C 64.78; H 8.59; N 2.33. $C_{32}H_{51}NO_9$. Calcd., %: C 64.73; H 8.66; N 2.36. *M* 593.75.

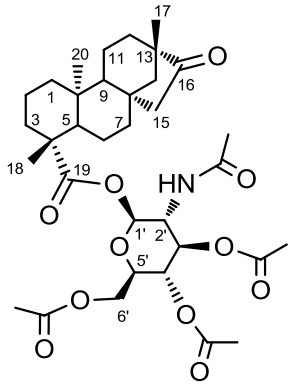
2’-Deoxy-2’-acetamido-α-D-glucopyranosylhexyl 16-oxo-*ent*-beyeran-19-oate 15b. Yield 0.21 g (94 %), colorless oil, $[\alpha]_D^{20}$ = 25.0 (0.69, CH_3OH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.80–1.93 (m, 26H, *ent*-beyerane, $(CH_2)_4$ - spacer), 0.70 (s, 3H , H-20), 0.97 (s, 3H , H-17), 1.18 (s, 3H , H-18), 2.04 (s, 3H, CH_3CO), 2.17 д (d, 1H, *J* = 13.6 Hz, H-3eq), 2.62 (dd, 1H, *J* = 18.5, 3.7 Hz, H-15α), 3.32–3.41 (m, 1H, H-1’’a), 3.52–3.59 м (m, 1H, H-1’’b), 3.60–3.79 (m, 4H, H-3’, H-4’, H-6’), 3.86–3.94 (m, 1H, H-5’), 3.97–4.10 (m, 3H, H-2’, H-6’’), 4.76 (d, 1H, *J* = 3.6 Hz, H-1’), 5.28 (br s, 3H, 3 OH) 6.45 (d, 1H, *J* = 9.1 Hz, N<u>H</u>C(O)CH_3), 6.85–7.12 (br s, 3H, 3 OH). MALDI-MS: *m/z*: 644.6 $[M+Na]^+$. Anal.,%: C 65.71; H 8.88; N 2.27. $C_{34}H_{55}NO_9$. Calcd., %: C 65.67; H 8.92; N 2.25. *M* 621.80.

2’-Deoxy-2’-acetamido-α-D-glucopyranosyldecyl 16-oxo-*ent*-beyeran-19-oate 15c. Yield 0.07 g (75%) colorless oil, $[\alpha]_D^{20}$ = 23.0 (0.71, CH_3OH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84–1.93 (m, 34H, *ent*-beyerane, $(CH_2)_8$ - spacer), 0.71 (s, 3H , H-20), 0.97 (s, 3H , H-17), 1.19 (s, 3H , H-18), 2.05 (s, 3H, CH_3CO), 2.18 (d, 1H, *J* = 13.5 Hz, H-3eq), 2.63 (dd, 1H, *J* = 18.6, 3.6 Hz, H-15α), 3.32–3.41 (m, 1H, H-1’’a), 3.54–3.74 (m, 4H, H-1’’b, H-3’, H-4’, H-6’a), 3.75–3.82 (m, 1H, H-6’b), 3.86–3.94 (m, 1H, H-5’), 3.97–4.11 (m, 3H, H-2’, H-10’’), 4.76 (d, 1H, H-1’, *J* = 3.6 Hz), 6.22 (d, 1H, *J* = 8.5 Hz, N<u>H</u>C(O)CH_3). MALDI-MS: *m/z*: 700.6 $[M+Na]^+$. Anal.,%: 67.40; H 9.31; N 2.10. $C_{38}H_{63}NO_9$. Calcd., %: C 67.33; H 9.37; N 2.07. *M* 677.91.

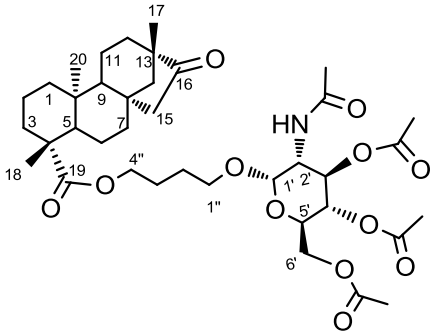
19-(2’-Deoxy-2’-acetamido-α,β-D-glucopyranosylpentylaza)-16,19-dioxo-*ent*-beyeran 18. Yield 0.25 g (89 %), colorless oil, $[\alpha]_D^{20}$ = 39.2 (0.52, CH_3OH). ^{1}H NMR (400 MHz, CD_3OD): δ 0.86–2.08 (m, 25H, *ent*-beyerane, $(CH_2)_3$ - spacer), 0.76 (s, 3H , H-20), 0.96 (s, 3H , H-17), 1.16 (s, 3H , H-18), 1.99 (s, 3H, CH_3CO), 2.62 (dd, 1H, *J* = 18.4, 3.0 Hz, H-15α), 3.07–3.25 (m, 2H, H-1’’), 3.26–3.34 (m, 1H, H-3’), 3.37–3.47 (m, 1H, H-4’), 3.52–3.92 (m, 6H, H-2’, H-5’, H-6’, H-5’’), 4.39 (d, 1H, *J* = 8.2 Hz, β-anomer (83%), H-1’), 4.76 (d, 1H, , *J* = 3.2 Hz, α-anomer (17%), H-1’), 5.09-5.39 (br s, 3H, 3OH), 5.94–6.02 (m, 1H, C(O)N<u>H</u>), 7.56-7.68 (m, 1H, N<u>H</u>C(O)CH_3). MALDI-MS: *m/z*: 629.9 $[M+Na]^+$. Anal.,%: C 65.26; H 9.03; N 4.65. $C_{33}H_{54}N_2O_8$. Calcd., %: C 65.32; H 8.97; N 4.62. *M* 606.79.



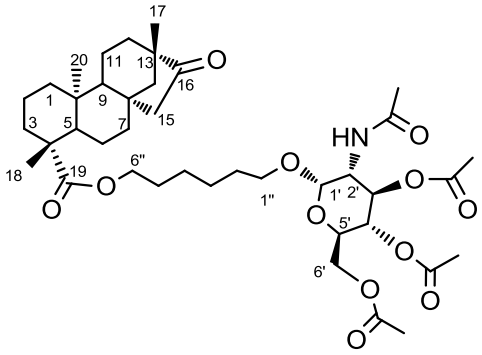
5



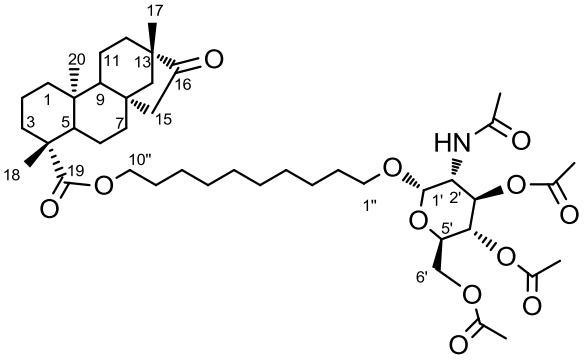
11



14a



14b



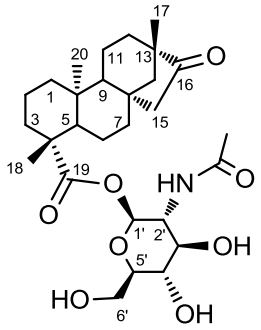
14c

Table 1S. ^{13}C NMR (100.6 MHz, $CDCl_3$) shifts for compounds **5, 11, 14a-c**

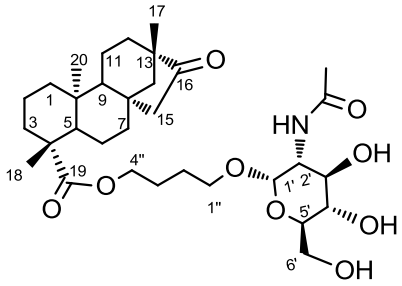
Position	^{13}C, δ				
	5	**11**	**14a**	**14b**	**14c**
1	39.65	39.76	39.96	40.01	40.03
2	19.38	19.03	19.12	19.14	19.12
3	37.45	37.41	37.46	37.49	37.47
4	43.81	44.24	44.07	44.02	43.98
5	57.72	57.28	57.21	57.25	57.25
6	22.41	21.56	21.90	21.91	21.89
7	41.89	41.59	41.64	41.69	41.71
8	48.55	48.69	48.60	48.62	48.59
9	54.92	54.87	54.82	54.87	54.86

10	38.32	37.77	38.05	38.20	38.12
11	20.49	20.46	20.48	20.47	20.48
12	38.25	38.26	38.20	38.21	38.18
13	39.47	39.54	39.64	39.64	39.62
14	54.43	54.44	54.42	54.47	54.46
15	48.84	48.81	48.88	48.86	48.85
16	222.46	222.37	222.74	222.47	222.74
17	19.98	19.97	19.97	20.00	19.99
18	29.36	29.05	29.13	29.11	29.10
19	176.73	176.17	177.58	177.49	177.50
20	13.72	13.66	13.62	13.58	13.55
$\underline{C}H_3CO$	-	20.71	20.47	20.50	21.89
$\underline{C}H_3CO$	-	20.75	20.73	20.73	20.73
$\underline{C}H_3CO$	-	20.83	20.87	20.86	20.86
$\underline{C}H_3CONH$	-	23.42	23.29	23.33	23.32
$CH_3\underline{C}O$	-	169.39	169.45	169.44	169.42
$CH_3\underline{C}O$	-	169.86	170.76	170.01	169.94
$CH_3\underline{C}O$	-	170.70	171.65	170.78	170.78
$CH_3\underline{C}ONH$	-	171.37	171.41	171.51	171.51
1’	-	92.16	97.19	97.31	97.27
2’	-	52.79	52.29	52.11	52.07
3’	-	72.86	71.25	71.59	71.63
4’	-	68.25	68.10	67.97	67.86
5’	-	73.15	68.36	68.42	68.69
6’	-	62.00	62.18	62.20	62.18
1’’	62.75	-	63.78	64.15	64.41
2’’	40.37	-	25.70	29.44	29.48
3’’	32.35	-	25.94	28.60	29.31
4’’	30.36	-	67.96	25.96	28.62
5’’	23.37	-	-	29.83	26.29
6’’	-	-	-	67.84	26.29

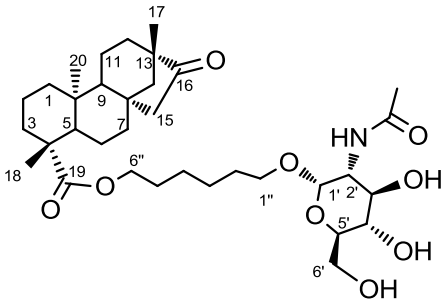
7’’	-	-	-	-	28.62
8’’	-	-	-	-	29.31
9’’	-	-	-	-	29.64
10’’	-	-	-	-	68.37



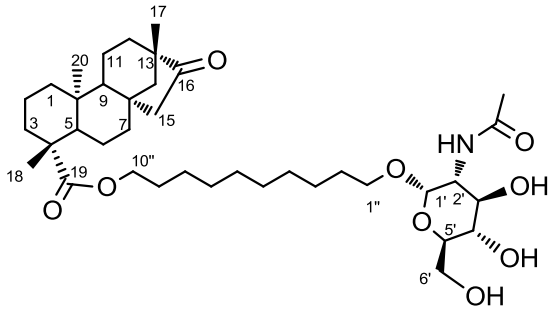
12



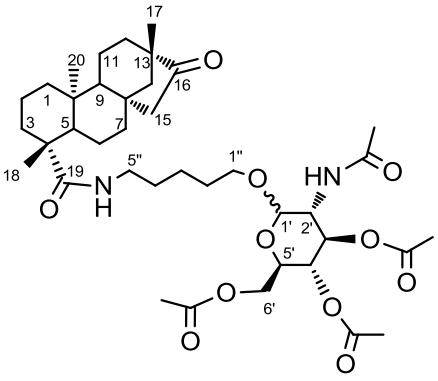
15a



15b



15c



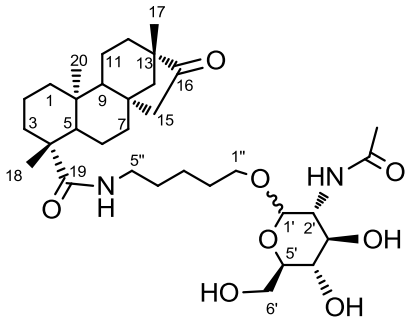
17

Table 2S. ^{13}C NMR (100.6 MHz, CD_3OD, $CDCl_3$) shifts for compounds **12, 15a-c, 17**

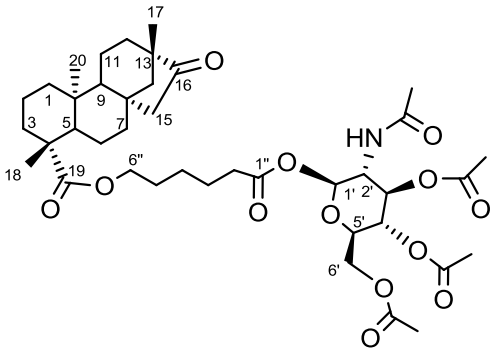
Position	**^{13}C, δ**				
	12 (CD_3OD)	**15a** ($CDCl_3$:CD_3OD =5:1)	**15b** ($CDCl_3$)	**15c** ($CDCl_3$)	**17** (CD_3OD)
1	40.82	39.66	37.01	37.04	40.76
2	20.00	18.82	16.13	16.14	20.18
3	39.00	37.77	34.47	34.48	38.81
4	45.15	43.84	38.67	38.71	44.91
5	58.25	56.91	58.26	58.35	59.04
6	22.76	21.61	18.89	18.89	22.82
7	42.40	41.28	41.00	40.99	42.73
8	49.78	solv.	45.60	45.61	49.85
9	55.92	54.46	50.88	50.91	55.54

10	38.43	37.92	35.19	35.19	38.48
11	21.43	20.20	17.49	17.49	21.49
12	39.23	37.21	35.11	35.13	39.28
13	40.65	39.43	36.64	36.63	40.61
14	55.86	54.07	51.43	51.46	55.90
15	49.82	solv.	45.88	45.87	solv.
16	225.00	223.72	219.79	219.66	224.81
17	20.15	19.56	16.98	17.00	20.41
18	29.27	28.77	22.86	23.31	29.81
19	177.75	177.77	174.53	174.53	179.35
20	14.25	13.30	10.58	10.56	14.41
$\underline{C}H_3CO$	-	-	-	-	20.54
$\underline{C}H_3CO$	-	-	-	-	20.57
$\underline{C}H_3CO$	-	-	-	-	20.60
$\underline{C}H_3CONH$	23.37	22.57	23.11	23.27	23.35
$CH_3\underline{C}O$	-	-	-	-	171.28
$CH_3\underline{C}O$	-	-	-	-	171.86
$CH_3\underline{C}O$	-	-	-	-	172.31
$CH_3\underline{C}ONH$	173.31	172.11	169.50	169.33	173.32
1’	93.84	97.29	94.63	94.60	102.17
2’	55.15	53.66	54.23	54.26	55.15
3’	76.35	71.89	69.69	65.23	74.27
4’	71.57	71.77	67.11	67.25	70.72
5’	78.75	70.44	68.98	68.90	72.91
6’	62.45	61.26	51.83	51.86	70.33
1’’	-	63.92	61.23	61.45	59.04
2’’	-	25.37	26.82	26.83	30.19
3’’	-	25.84	26.13	26.33	24.55
4’’	-	67.26	25.58	26.13	30.45
5’’	-	-	26.45	25.63	63.35
6’’	-	-	65.02	25.63	-

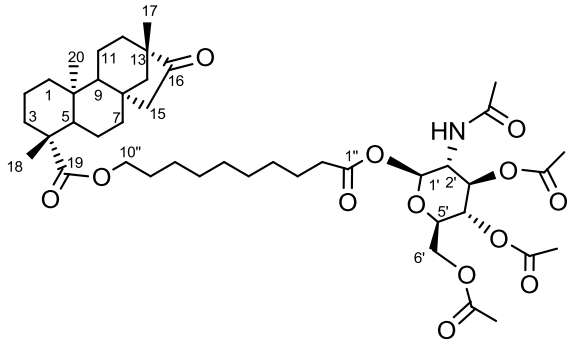
7’’	-	-	-	26.13	-
8’’	-	-	-	26.56	-
9’’	-	-	-	26.68	-
10’’	-	-	-	50.54	-



18



20a



20b

Table 3S. ^{13}C NMR (100.6 MHz, $CDCl_3$) shifts for compounds **18, 20a-b**

Position	^{13}C, δ		
	18	**20a**	**20b**
1	40.36	39.98	39.65
2	19.40	19.08	19.15
3	37.47	37.43	37.52
4	43.80	43.97	44.01
5	57.72	57.19	57.28
6	22.39	21.87	21.91
7	41.87	41.64	41.73
8	48.60	48.55	48.62
9	54.86	54.81	54.89
10	38.26	38.10	38.15
11	20.50	20.47	20.47

12	38.26	38.15	38.21
13	39.68	39.60	40.06
14	54.39	54.41	54.48
15	48.87	48.86	48.90
16	222.76	222.74	222.71
17	19.99	19.96	20.01
18	29.09	29.07	29.01
19	177.09	177.49	177.54
20	13.79	13.54	13.57
$\underline{C}H_3CO$	-	20.68	20.71
$\underline{C}H_3CO$	-	20.74	20.76
$\underline{C}H_3CO$	-	20.82	20.84
$\underline{C}H_3CONH$	23.33	23.24	23.31
$CH_3\underline{C}O$	-	169.38	169.35
$CH_3\underline{C}O$	-	170.18	170.04
$CH_3\underline{C}O$	-	170.75	170.77
$CH_3\underline{C}ONH$	172.88	172.04	172.45
1’	101.60	92.61	92.64
2’	56.25	53.25	53.21
3’	74.39	72.73	72.86
4’	70.28	68.02	67.98
5’	75.88	72.99	73.13
6’	61.40	61.81	61.84
1’’	177.02	171.20	171.24
2’’	30.35	24.13	29.13
3’’	22.46	25.69	29.18
4’’	23.47	28.25	29.22
5’’	69.79	33.97	29.39
6’’	-	63.94	24.66
7’’	-	-	26.26
8’’	-	-	28.58

9''	-	-	34.19
10''	-	-	64.41

Biology

Cytotoxicity of test compounds on cancer and normal human cell lines

Cytotoxic effects of the test compounds on human cancer and normal cells were estimated by means of the multifunctional Cytell Cell Imaging system (GE Health Care Life Science, Sweden) using the Cell Viability Bio App which precisely counts the number of cells and evaluates their viability from fluorescence intensity data. Two fluorescent dyes that selectively penetrate the cell membranes and fluoresce at different wavelengths were used in the experiments. A low-molecular-weight 4′,6-diamidin-2-phenylindol dye (DAPI) is able to penetrate intact membranes of living cells and color nuclei in blue. High-molecular propidium iodide dye penetrates only dead cells with damaged membranes, staining them in yellow. As a result, living cells are painted in blue and dead cells are painted in yellow. DAPI and propidium iodide were purchased from Sigma. The M-Hela clone 11 human, epithelioid cervical carcinoma, strain of Hela, clone of M–Hela; human breast adenocarcinoma cells (MCF-7); PANC-1 is a human pancreatic cancer cell line from the Type Culture Collection of the Institute of Cytology (Russian Academy of Sciences) and Chang liver cell line (Human liver cells) from N. F. Gamaleya Research Center of Epidemiology and Microbiology were used in the experiments. The cells were cultured in a standard Eagle's nutrient medium manufactured at the Chumakov Institute of Poliomyelitis and Virus Encephalitis (PanEco company) and supplemented with 10% fetal calf serum and 1% nonessential amino acids. The cells were plated into a 96-well plate (Eppendorf) at a concentration of 100000 cells/mL, 150 μL of medium per well, and cultured in a CO_2 incubator at 37°C. Twenty four hours after seeding the cells into wells, the compound under study was added at a preset dilution, 150 μL to each well. The dilutions of the compounds were prepared immediately in nutrient media; 5% DMSO that does not induce the inhibition of cells at this concentration was added for better solubility. The experiments were repeated three times. Intact cells cultured in parallel with experimental cells were used as a control.

Flow Cytometry Assay.

Cell Culture. M-Hela cells at 1×10^6 cells/ well in a final volume of 2 mL were seeded into six-well plates. After 24 h of incubation, various concentrations of the lead compounds **14a,b** were added to wells.

Cell Apoptosis Analysis. The cells were harvested at 2000 rpm for 5 min and, then, washed twice with ice-cold PBS, followed by resuspension in binding buffer. Next, the samples were incubated with 5 μL of annexin V-FITC and 5 μL of propidium iodide for 15 min at room temperature in the dark. Finally, the cells were analyzed by flow cytometry (Guava easy Cyte, MERCK, USA).

The experiments were repeated three times.

Cell cycle analysis. The DNA content and cell-cycle distribution after genistein treatment were estimated by flow cytometry. Cell seeding, drug treatment and ethanol fixation were similar to cell proliferation assay. After washing with PBS, genistein and daidzein-treated and -fixed Caco-2 cells were suspended in 250 μl of PBS, then 1.0 ml phosphate-citrate buffer (0.05 M, pH 4.0) was added and the suspension was incubated at room temperature for 5min to facilitate the extraction of low molecular weight DNA. Following centrifugation the cells were resuspended in 500 μl DNA staining solution (20 μg/ml propidium iodide, 200μg/ml DNase (RNase-free), and 0.1% Triton X-100) and incubated in the dark at room temperature for 30 min. Cell cycle distribution was determined by fluorescence-activated cell sorting analysis of propidium iodide-stained ethanol-fixed cells using a Guava EasyCyte (Guava easy Cyte, MERCK, USA) (Han et al., 2013).

Hemolytic activity of the lead compounds **14a,b** was estimated by comparing the optical density of a solution containing the test compound with the optical density of blood at 100% hemolysis. The experiments were repeated for three times. A 10% suspension of human erythrocytes was used as an object of investigation. An erythrocytic mass with heparin was washed three times with physiological saline (0.9% NaCl) solution, centrifuged for 10 min at 800 rpm, and resuspended in physiological saline (0.9% NaCl) solution to a concentration of 10%. The concentrations of the compounds that corresponded to the MIC values for the bacterial test strains were prepared in physiological saline (0.9% NaCl) solution (supplemented with 5% DMSO), and 450 μL of the compound at the corresponding dilution was added to 50 μL of a 10% suspension of erythrocytes. The samples were incubated for 1 h at 37°C and centrifuged for 10 min at 2000 rpm. Release of hemoglobin was controlled by measuring the optical density of the supernatant on Microplate reader Iinvitrologic (Russia) at λ 450 nm. The control sample corresponding to zero hemolysis (blank experiment) was prepared by adding of 50 μL of 10% red blood cell suspension to 450 μL of physiological saline solution (0.9% NaCl). The control sample corresponding to 100% hemolysis was prepared by adding of 50 μL of 10% red blood cell suspension to 450 μL of distilled water.

Statistical analysis

The cytometric results were analyzed by the Cytell Cell Imaging multifunctional system using the Cell Viability BioApp and Apoptosis BioApp application. The data in the tables and graphs are given as the mean ± standard error.

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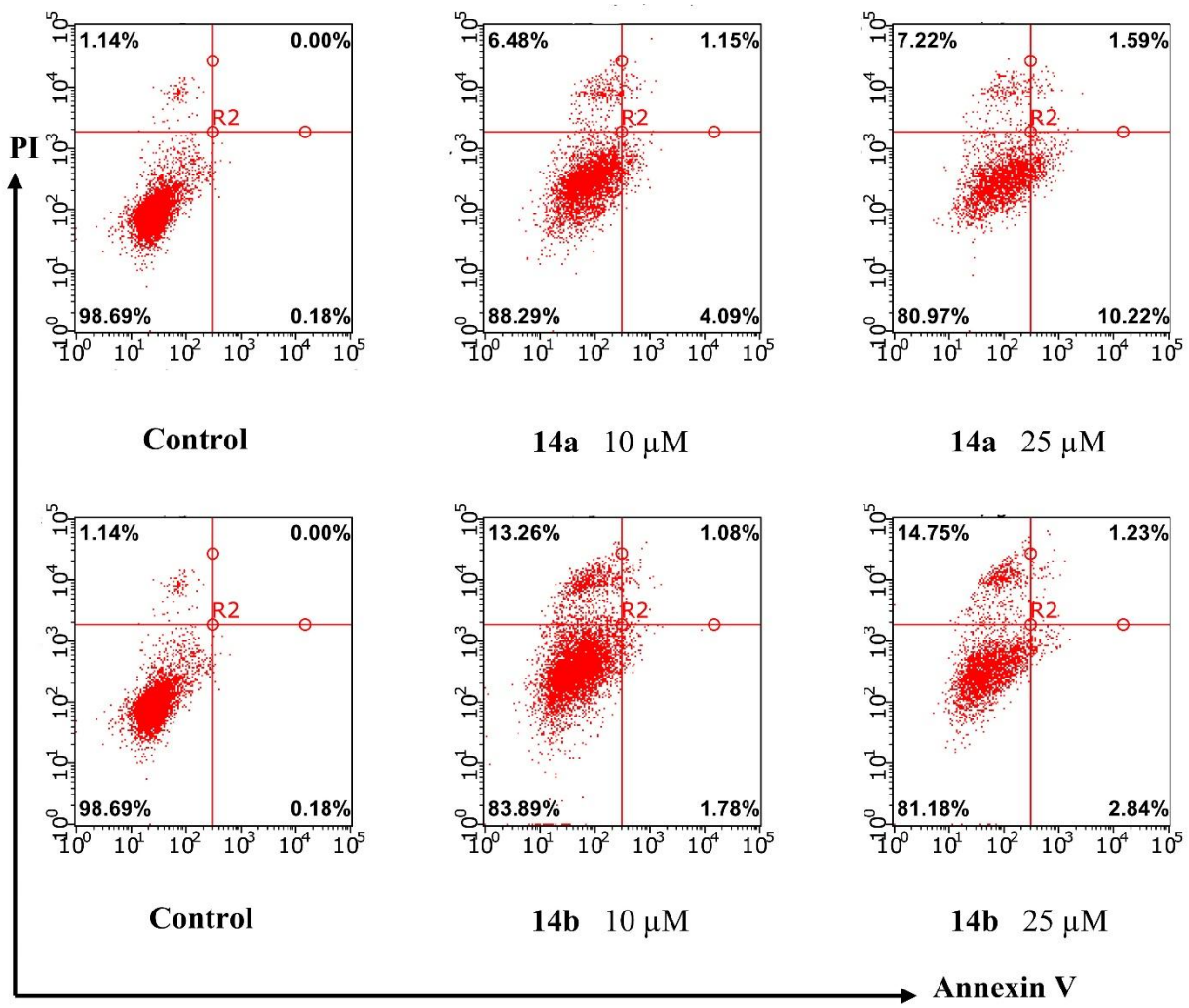

Figure S1. Apoptotic ettects of compounds **14a** and **14b** on M-Hela cells. M-Hela cells were treated with the mentioned compounds at indicated concentrations for 24 h. Apoptotic effects were measured by flow cytometry using annexin V- FITC staining protocol. The values are presented as the mean ± SD (n = 3).

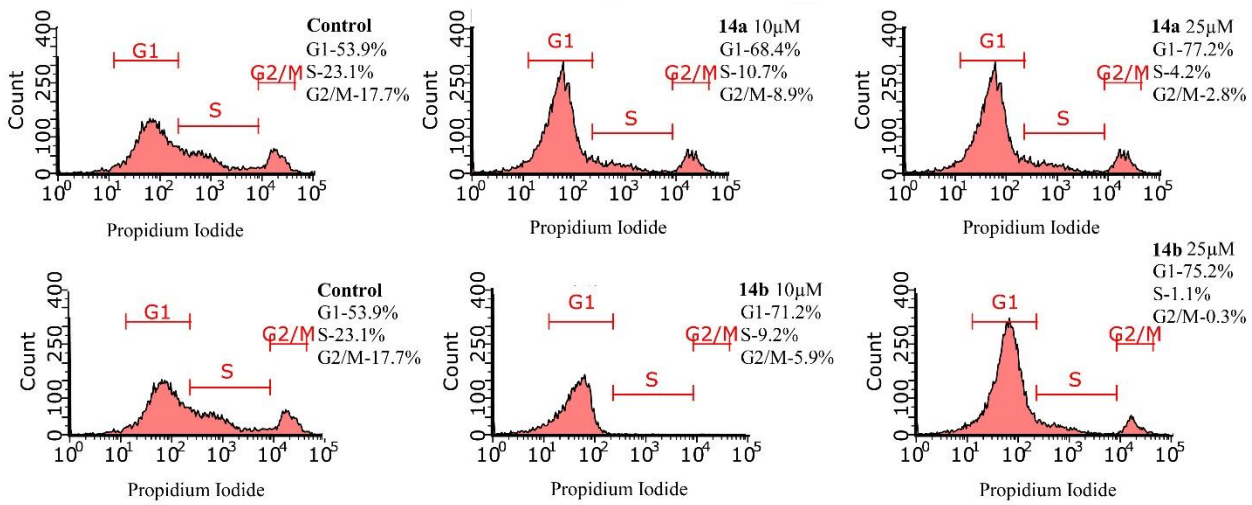

Figure S2. Effect of conjugates **14a** and **14b** on cell cycle progression of M-Hela by flow cytometry.

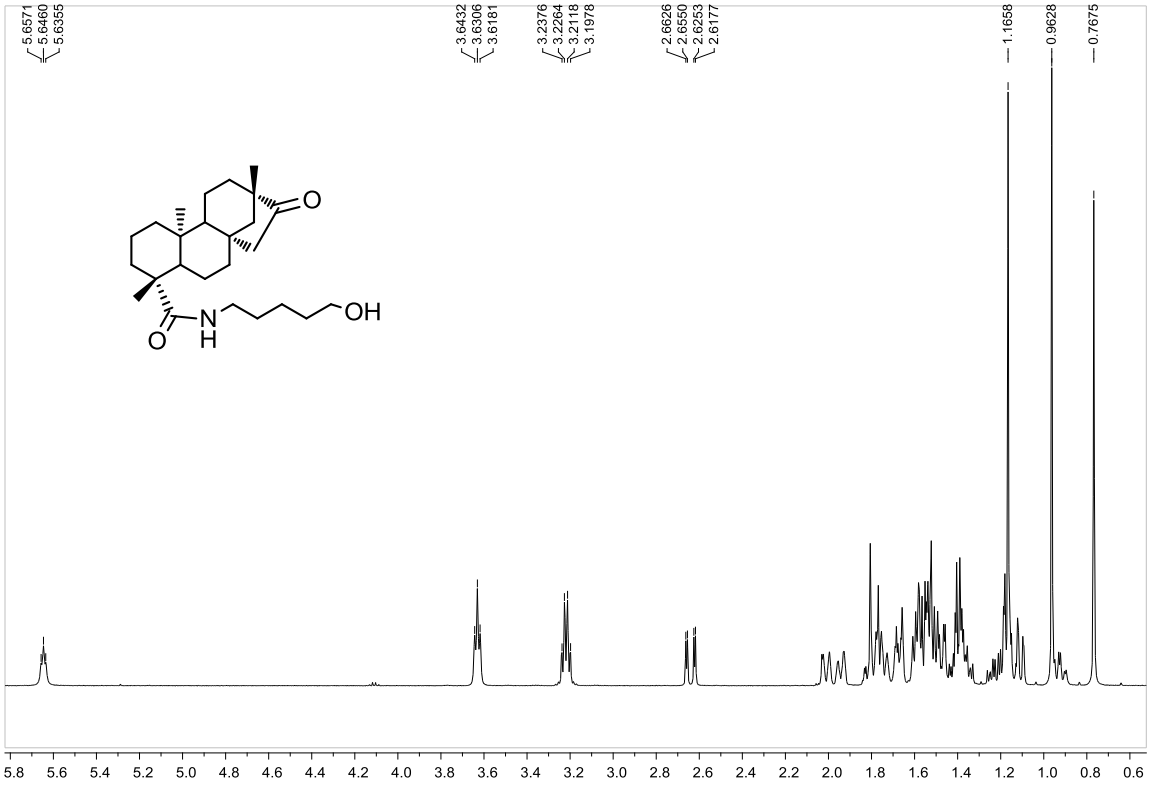

Figure S3. ^{1}H NMR shifts for compound **5** ($CDCl_3$) ppm

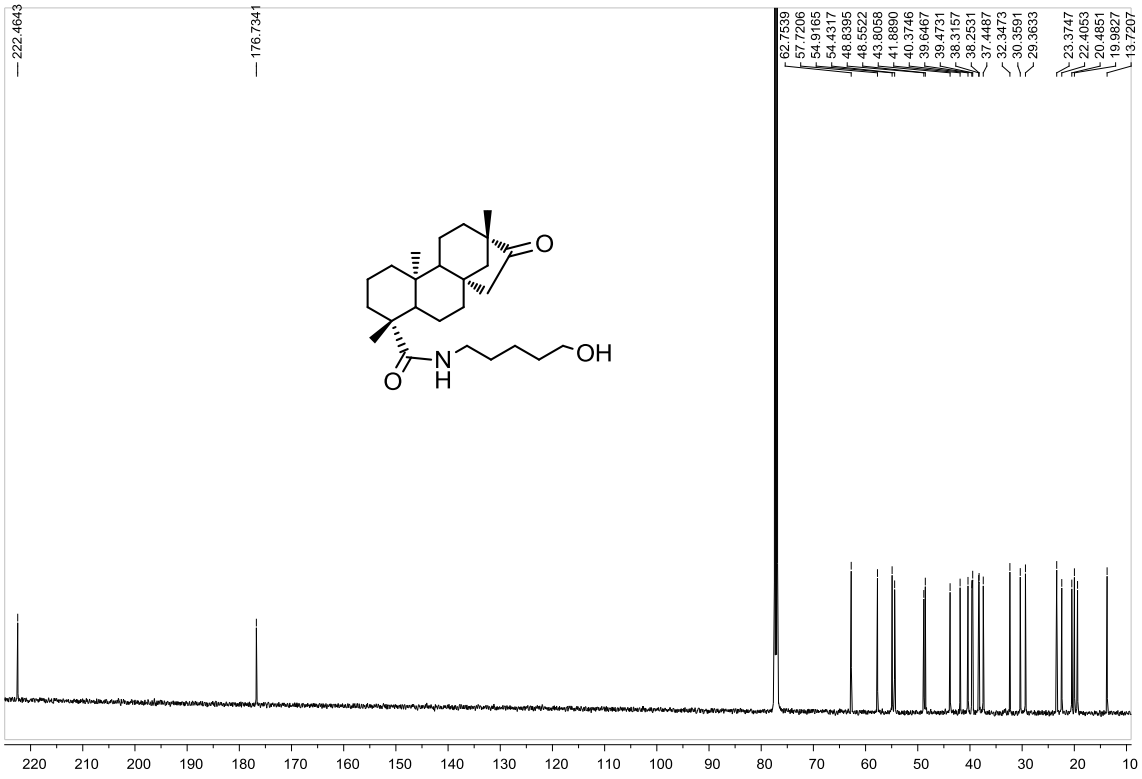

Figure S4. ^{13}C NMR shifts for compound **5** ($CDCl_3$) ppm

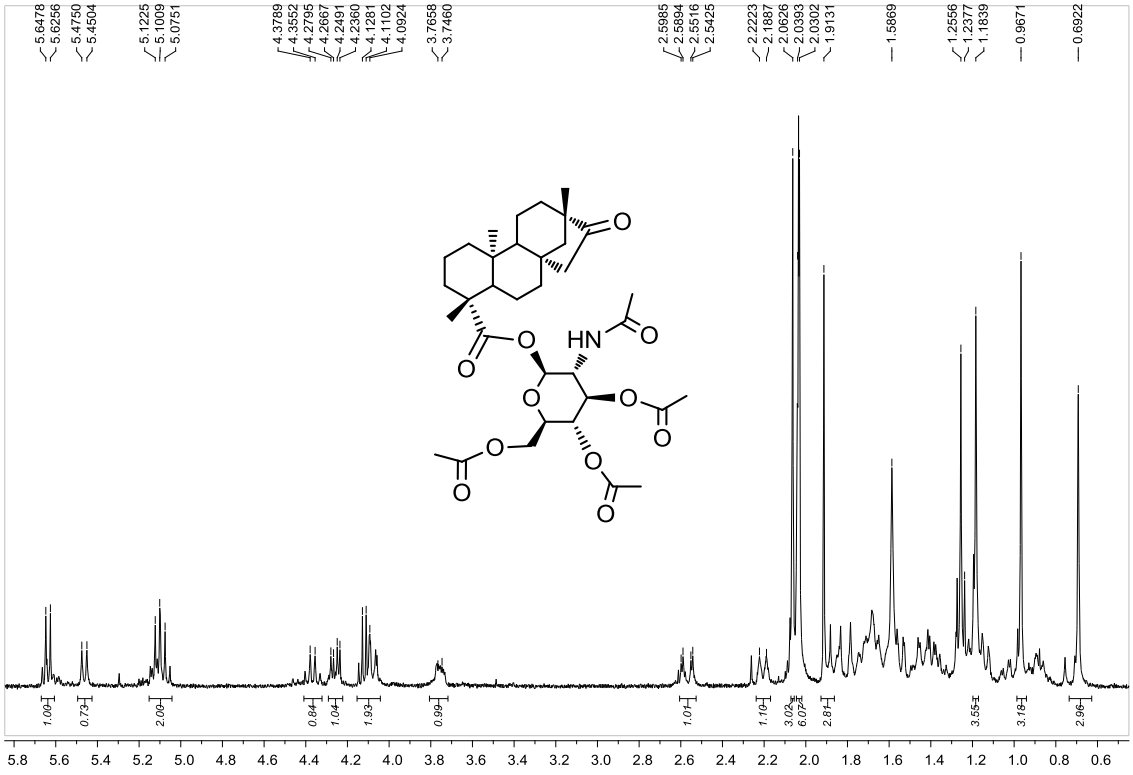

Figure S5. ^{1}H NMR shifts for compound **11** ($CDCl_3$) ppm

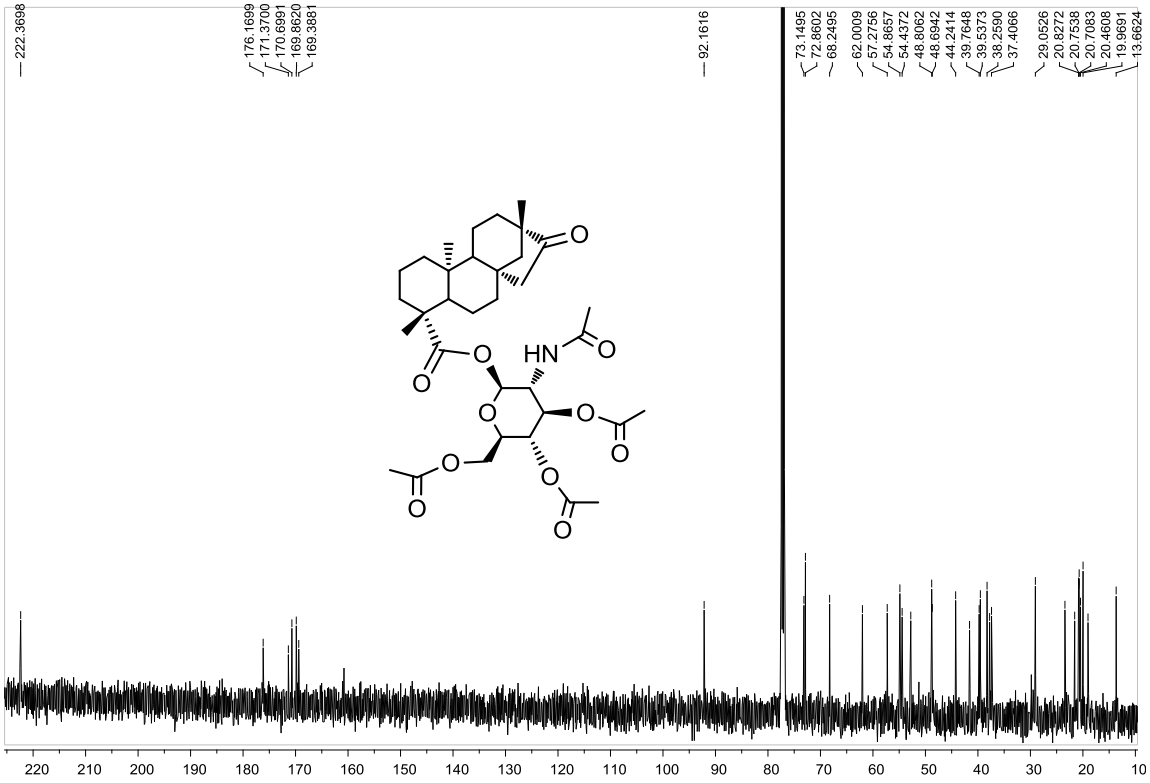

Figure S6. ^{13}C NMR shifts for compound **11** ($CDCl_3$) ppm

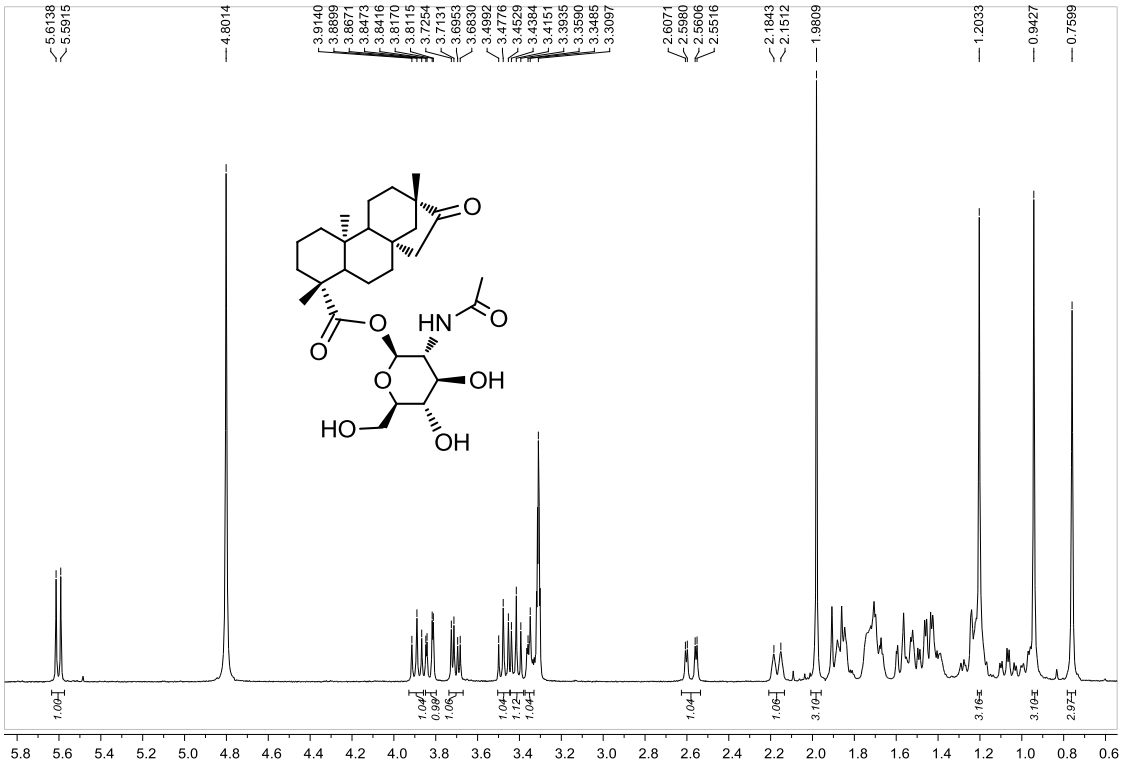

Figure S7. ^{1}H NMR shifts for compound **12** (CD_3OD) ppm

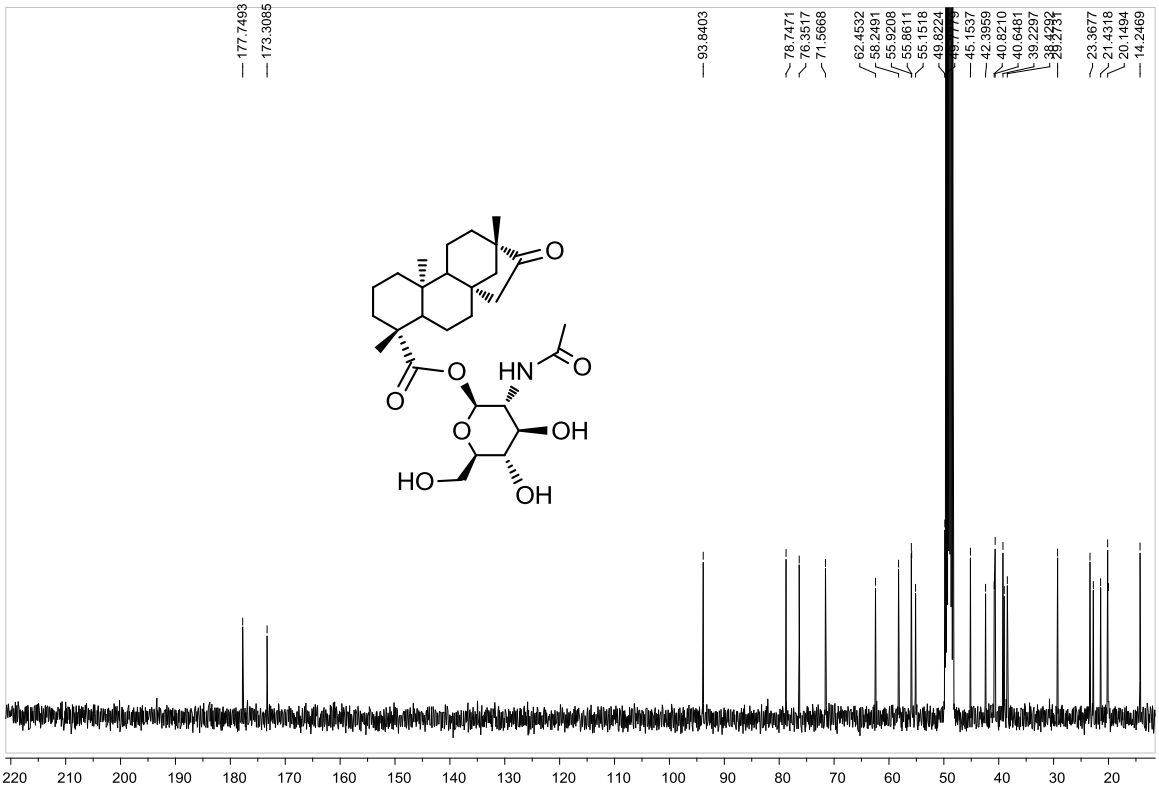

Figure S8. ^{13}C NMR shifts for compound **12** (CD_3OD) ppm

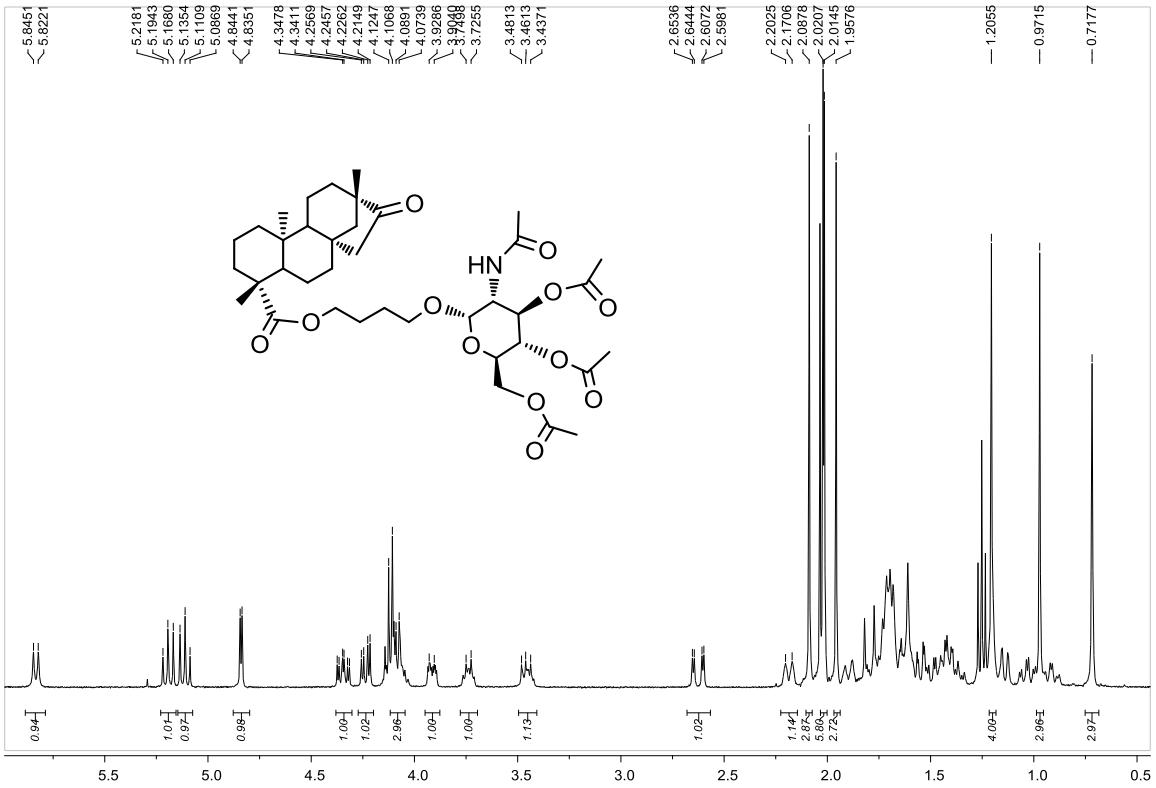

Figure S9. ^{1}H NMR shifts for compound **14a** ($CDCl_3$) ppm

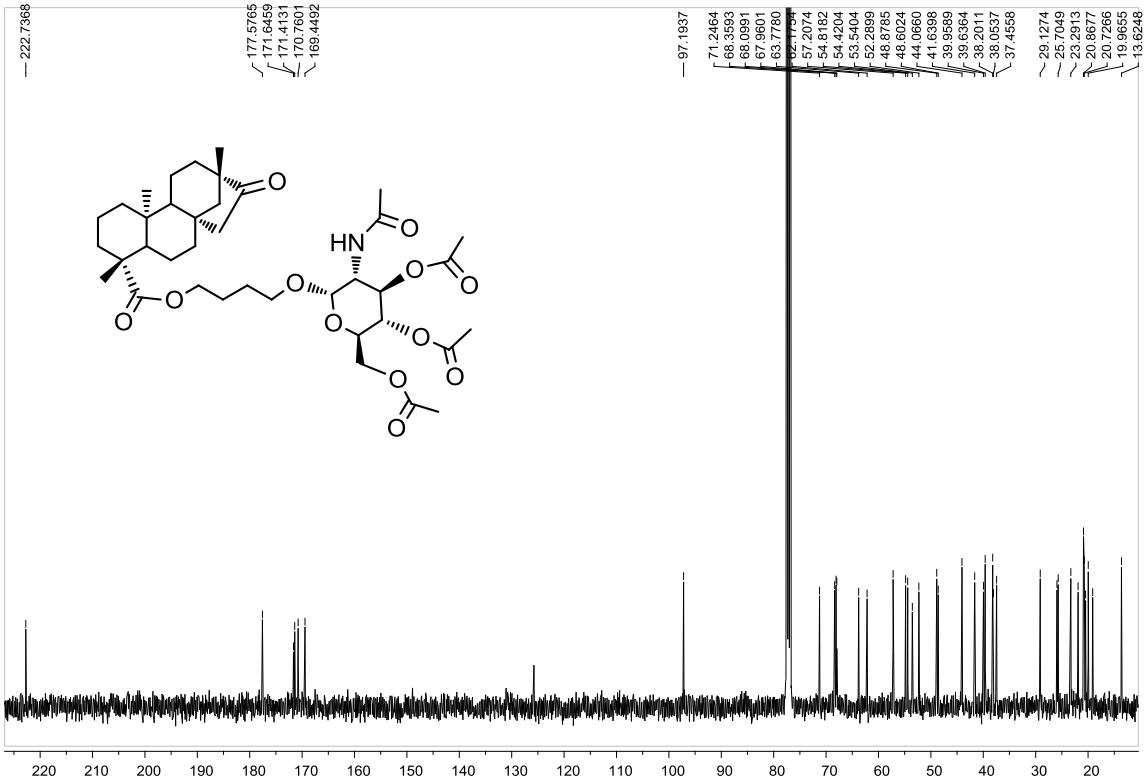

Figure S10. ^{13}C NMR shifts for compound **14a** ($CDCl_3$) ppm

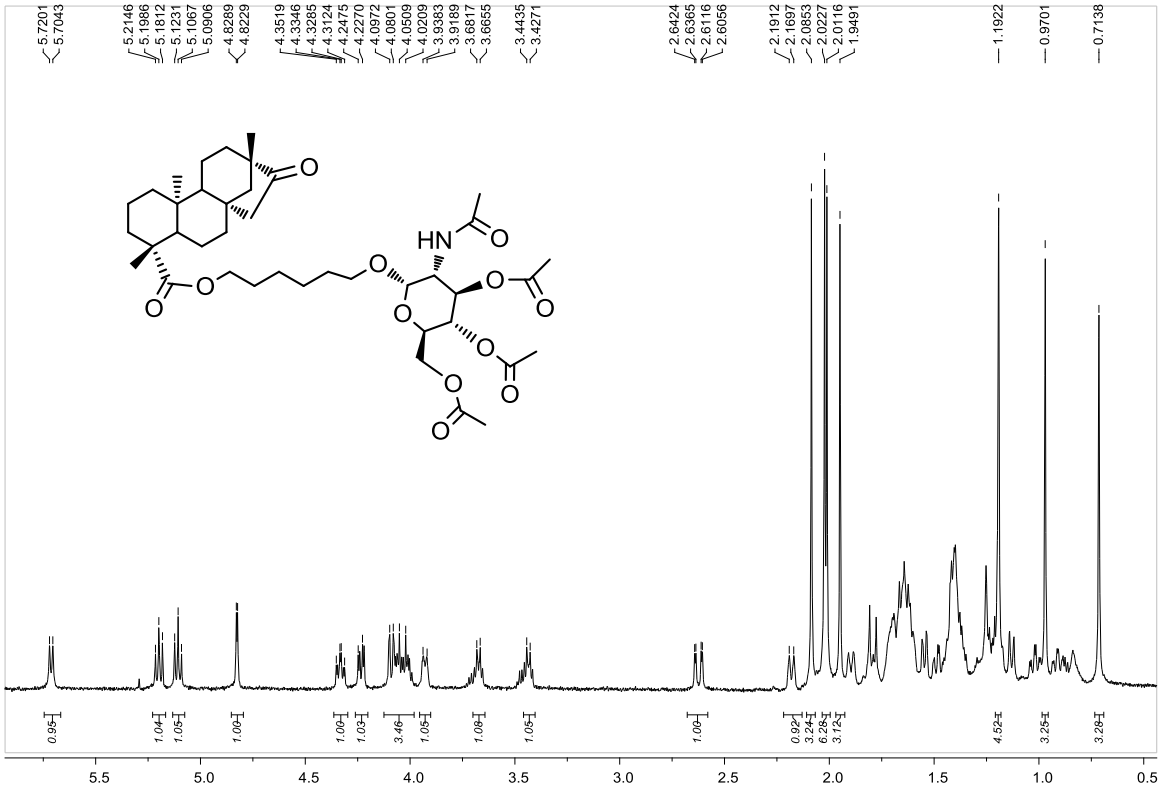

Figure S11. ^{1}H NMR shifts for compound **14b** ($CDCl_3$) ppm

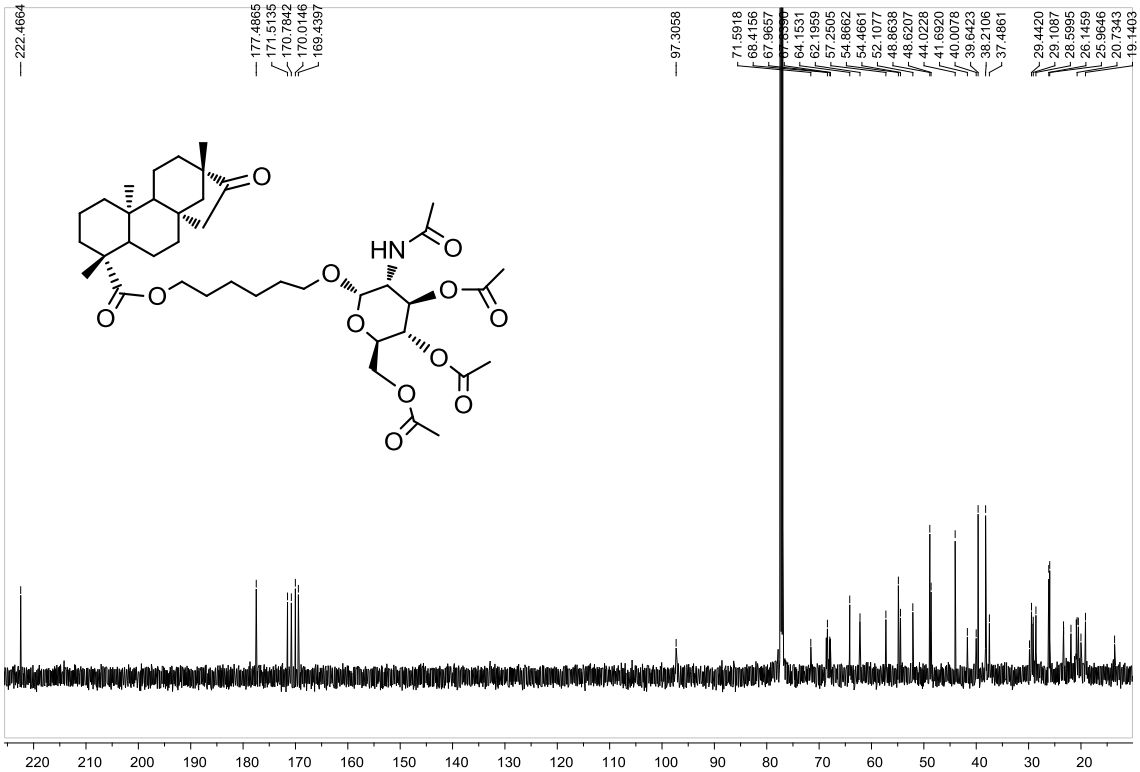

Figure S12. ^{13}C NMR shifts for compound **14b** ($CDCl_3$) ppm

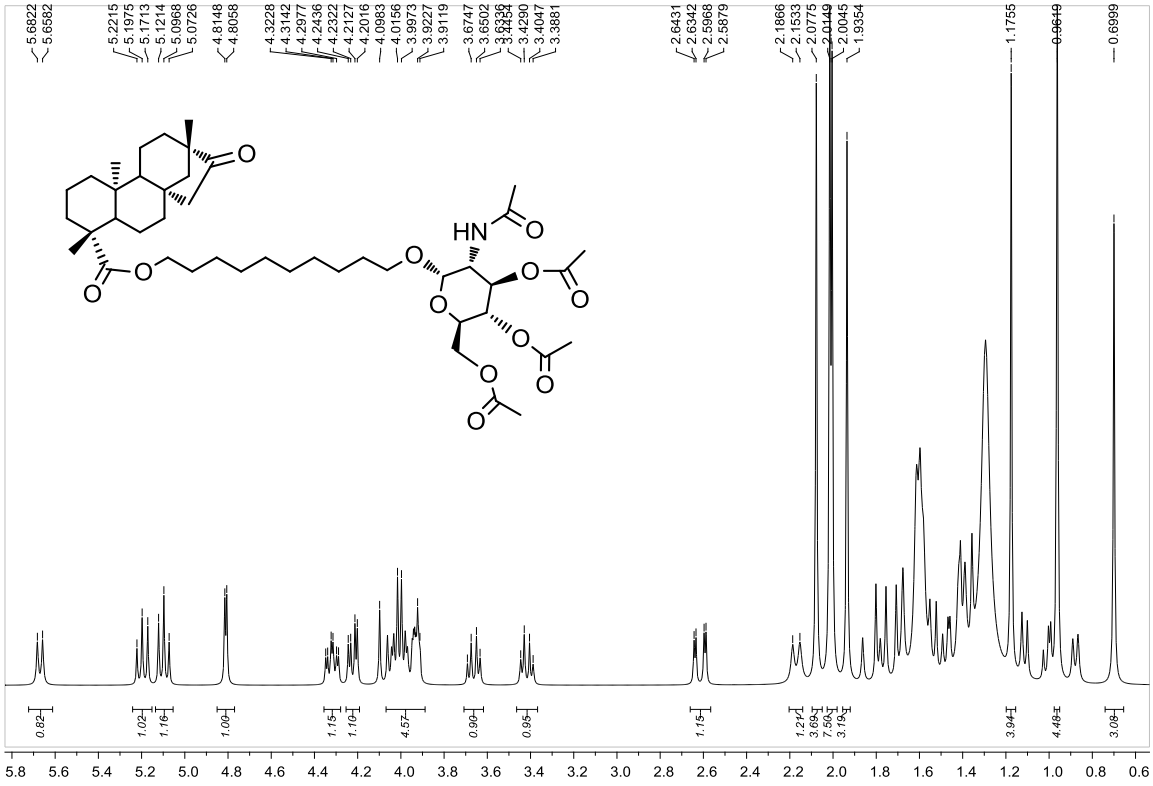

Figure S13. ^{1}H NMR shifts for compound **14c** ($CDCl_3$) ppm

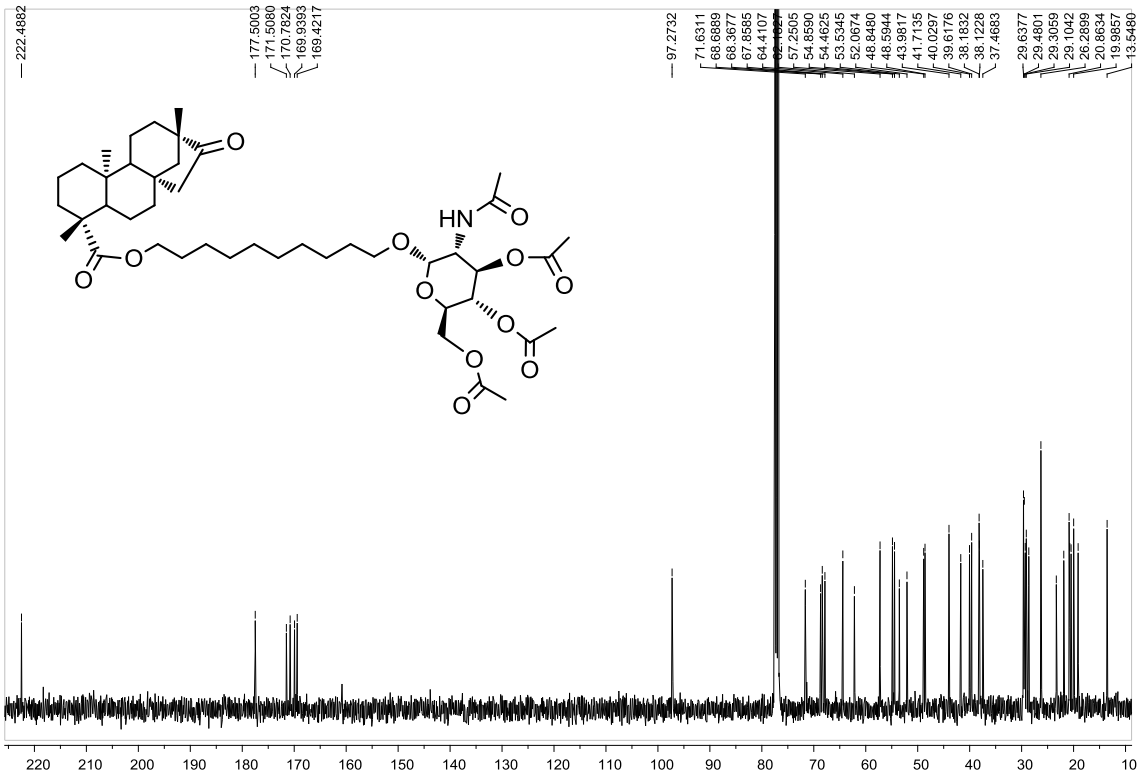

Figure S14. ^{13}C NMR shifts for compound **14c** ($CDCl_3$) ppm

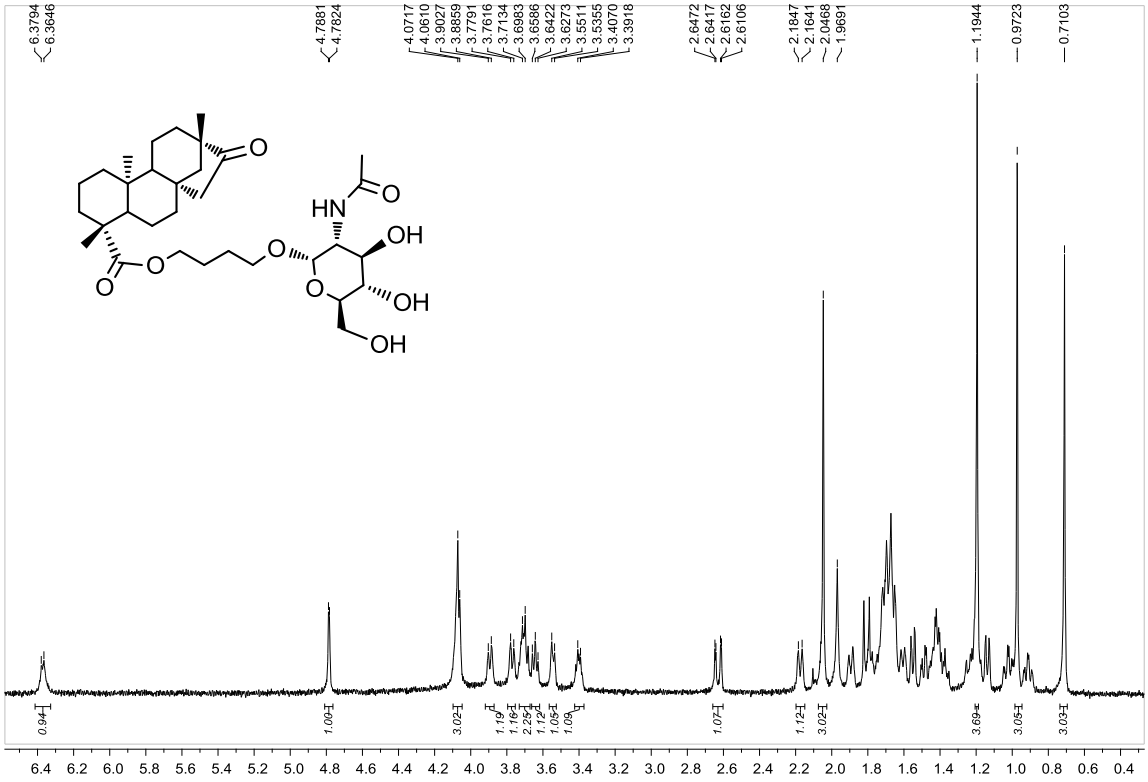

Figure S15. ^{1}H NMR shifts for compound **15a** ($CDCl_3$) ppm

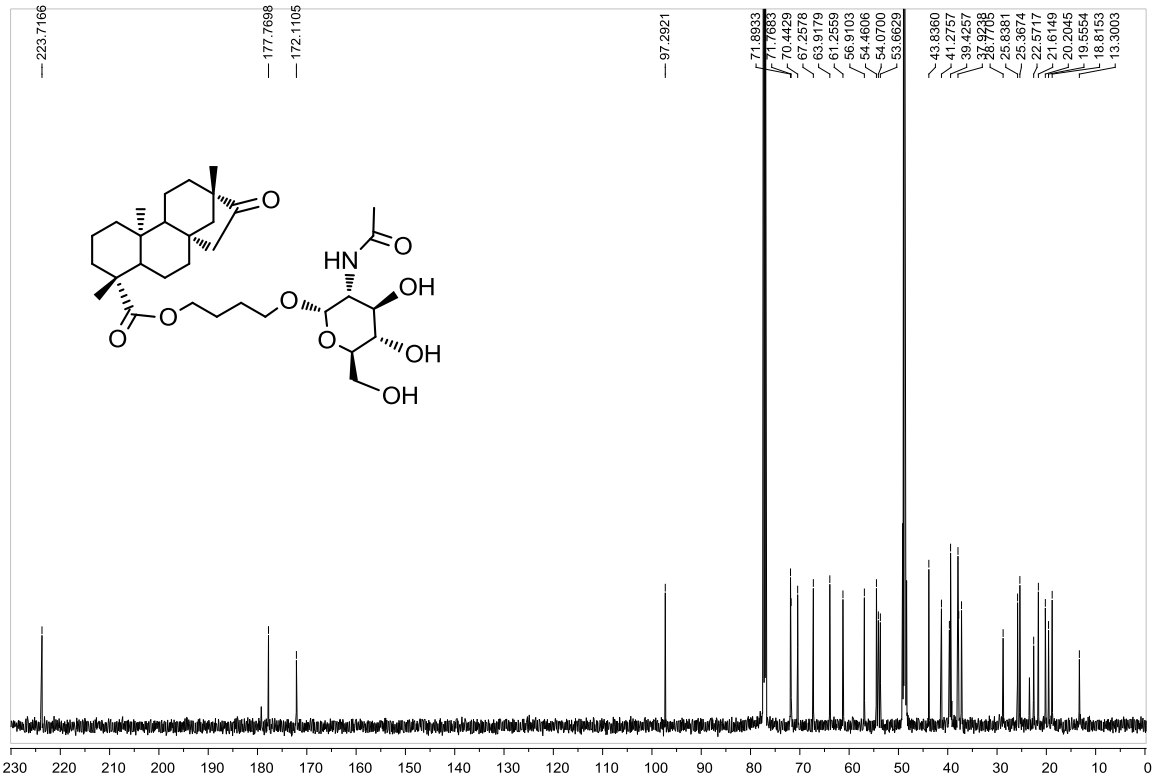

Figure S16. ^{13}C NMR shifts for compound **15a** ($CDCl_3$+ CD_3OD) ppm

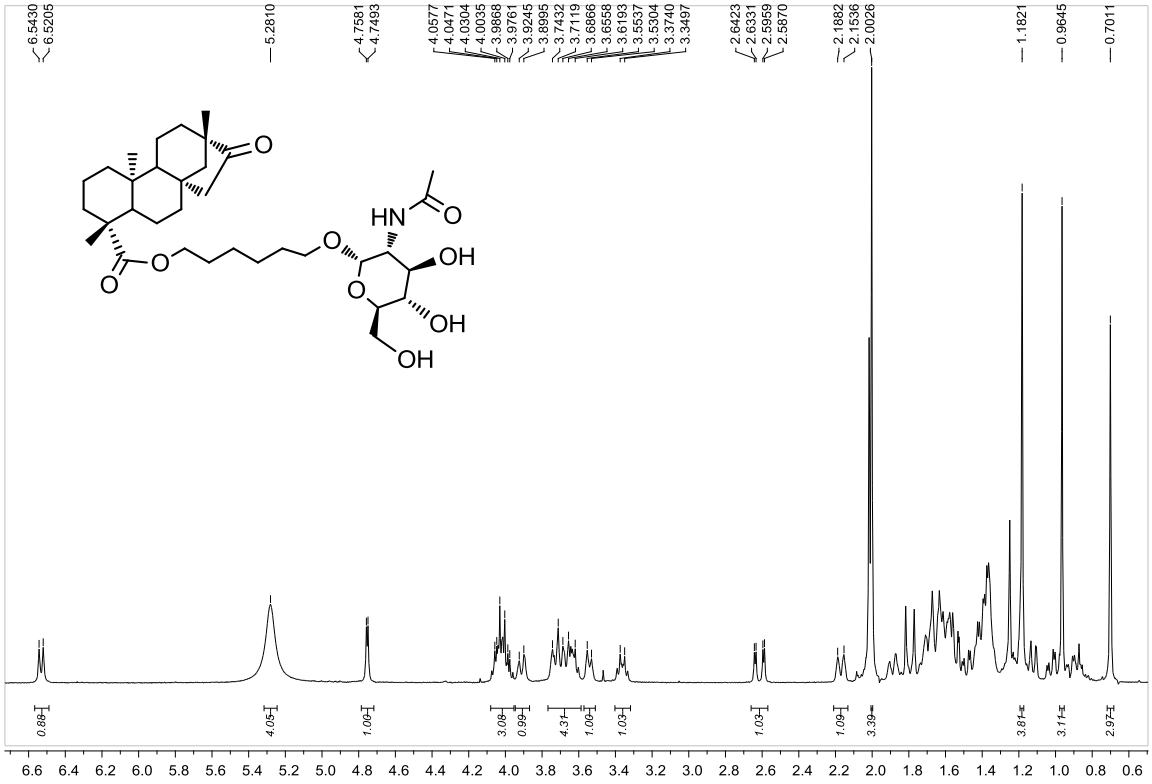

Figure S17. ^{1}H NMR shifts for compound **15b** ($CDCl_3$) ppm

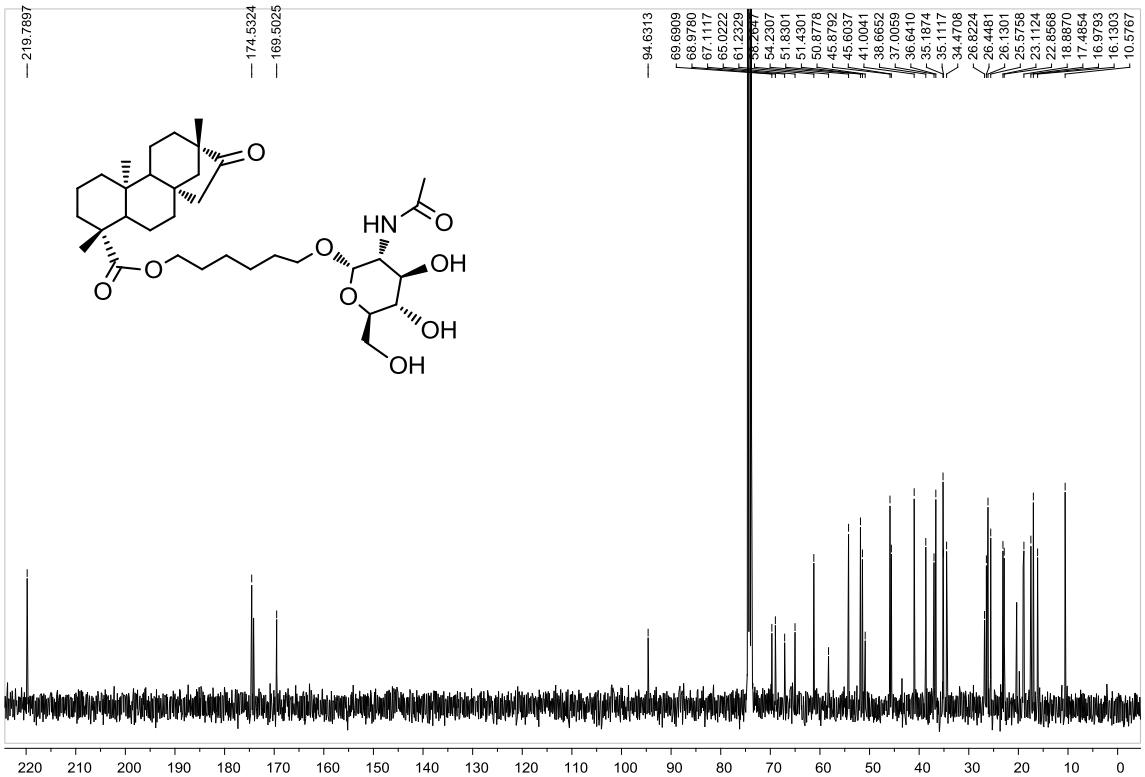

Figure S18. ^{13}C NMR shifts for compound **15b** ($CDCl_3$) ppm

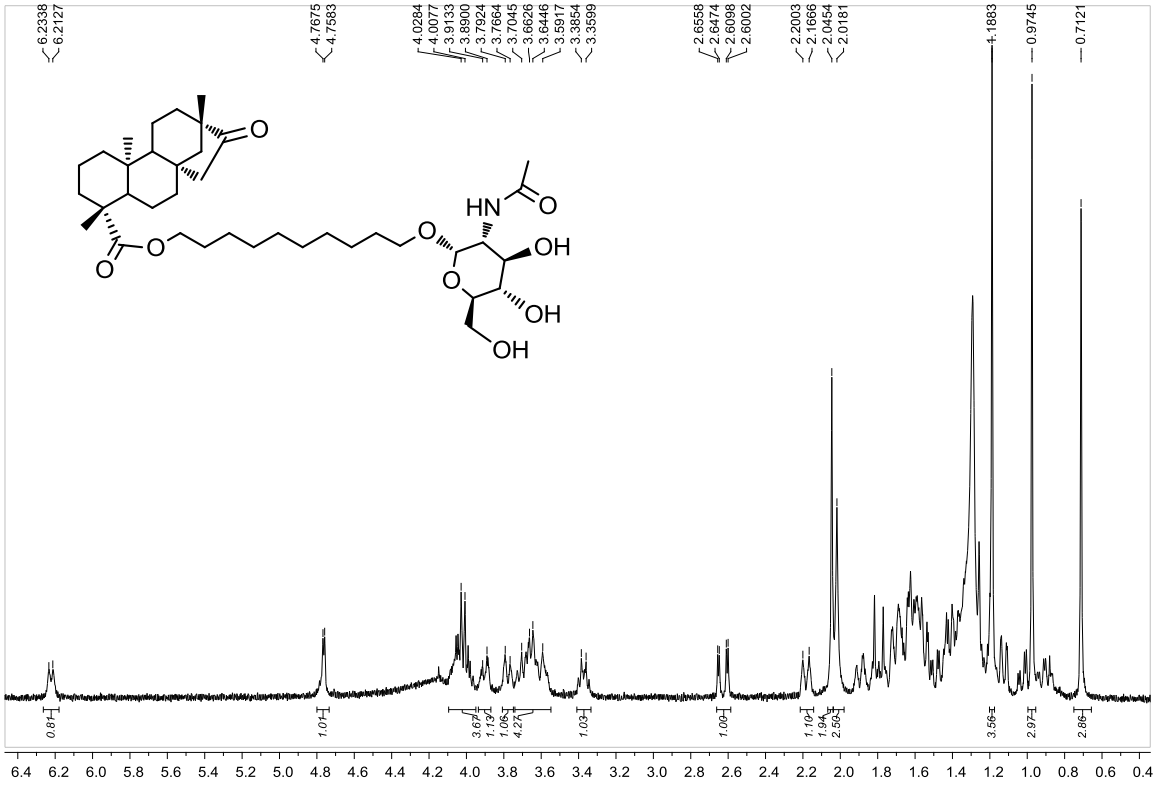

Figure S19. ^{1}H NMR shifts for compound **15c** ($CDCl_3$) ppm

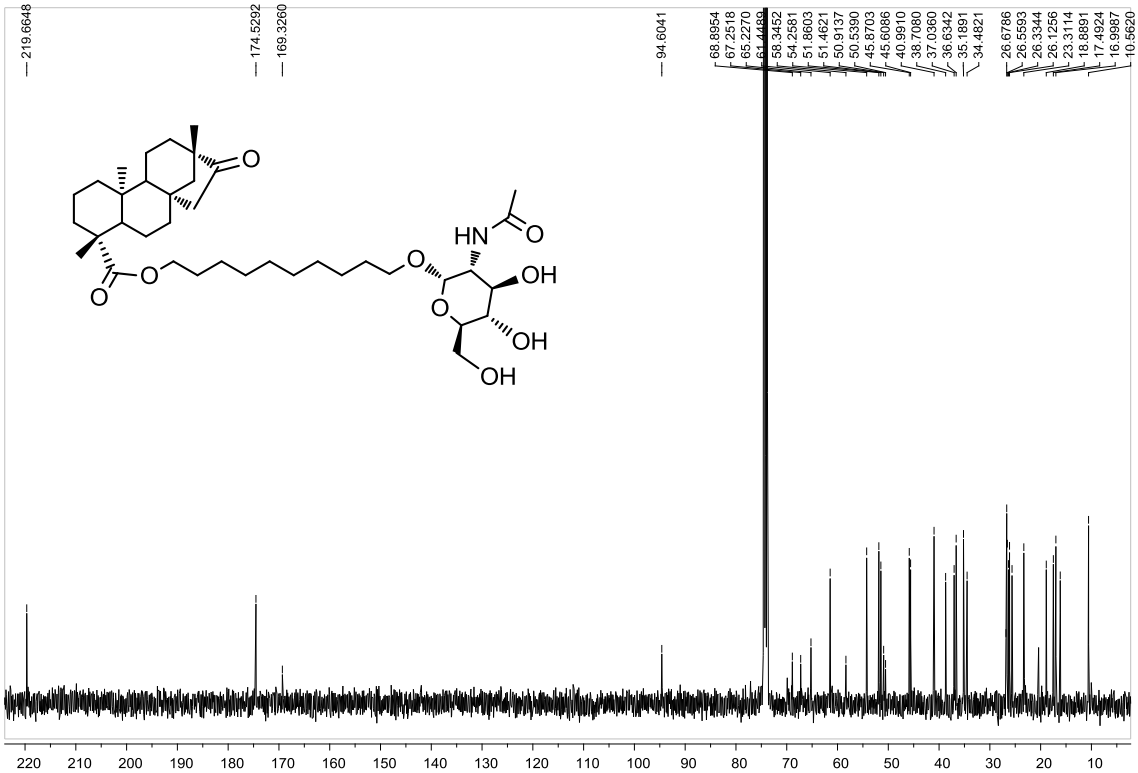

Figure S20. ^{13}C NMR shifts for compound **15c** ($CDCl_3$) ppm

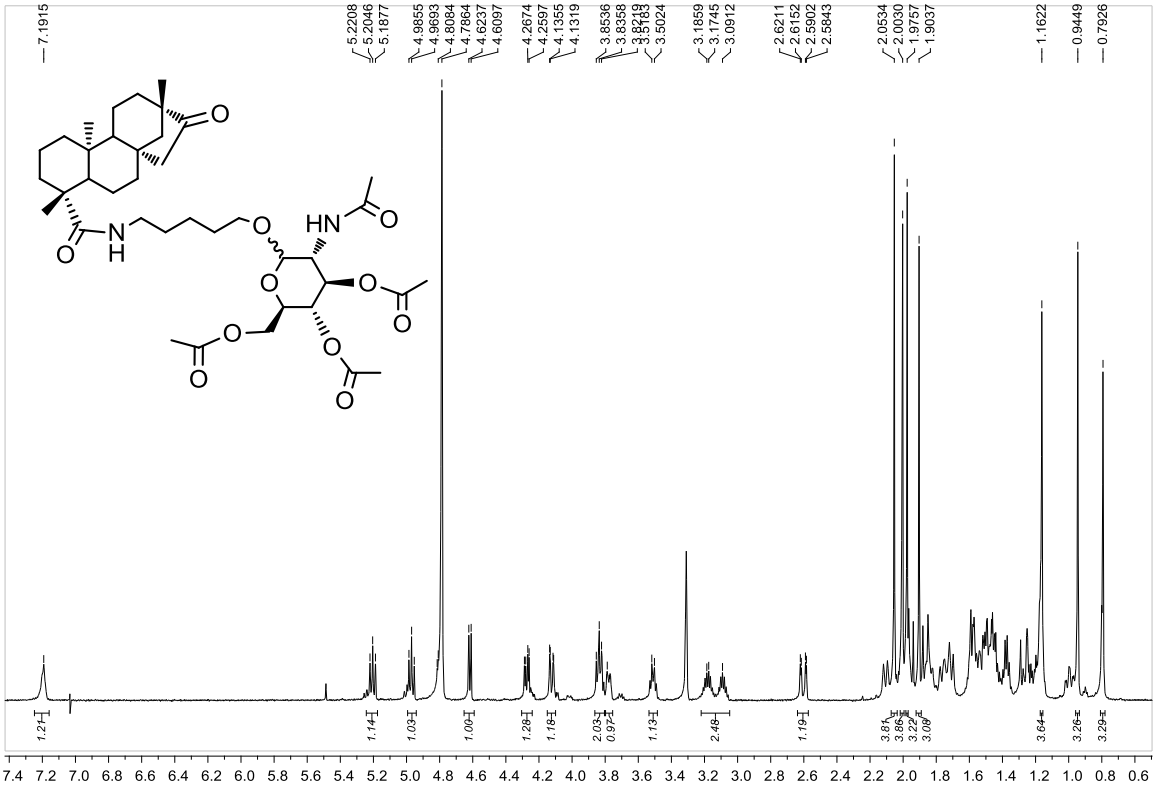

Figure S21. ^{1}H NMR shifts for compound **17** (CD_3OD) ppm

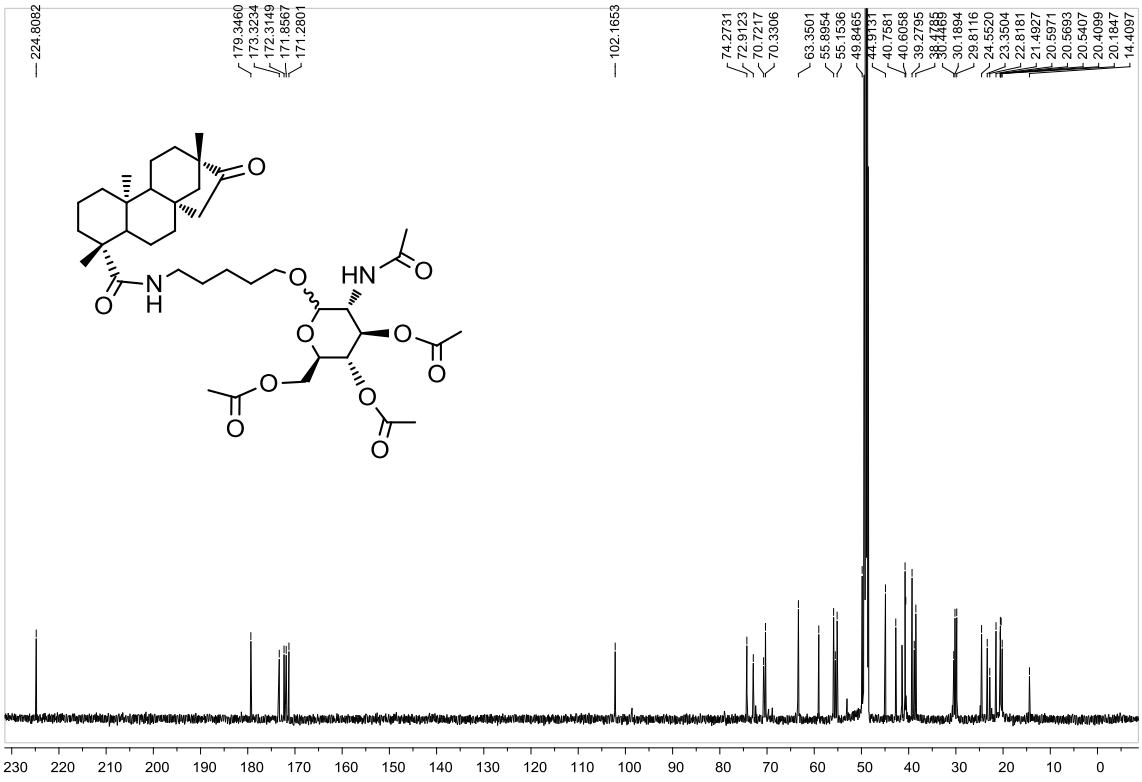

Figure S22. ^{13}C NMR shifts for compound **17** (CD_3OD) ppm

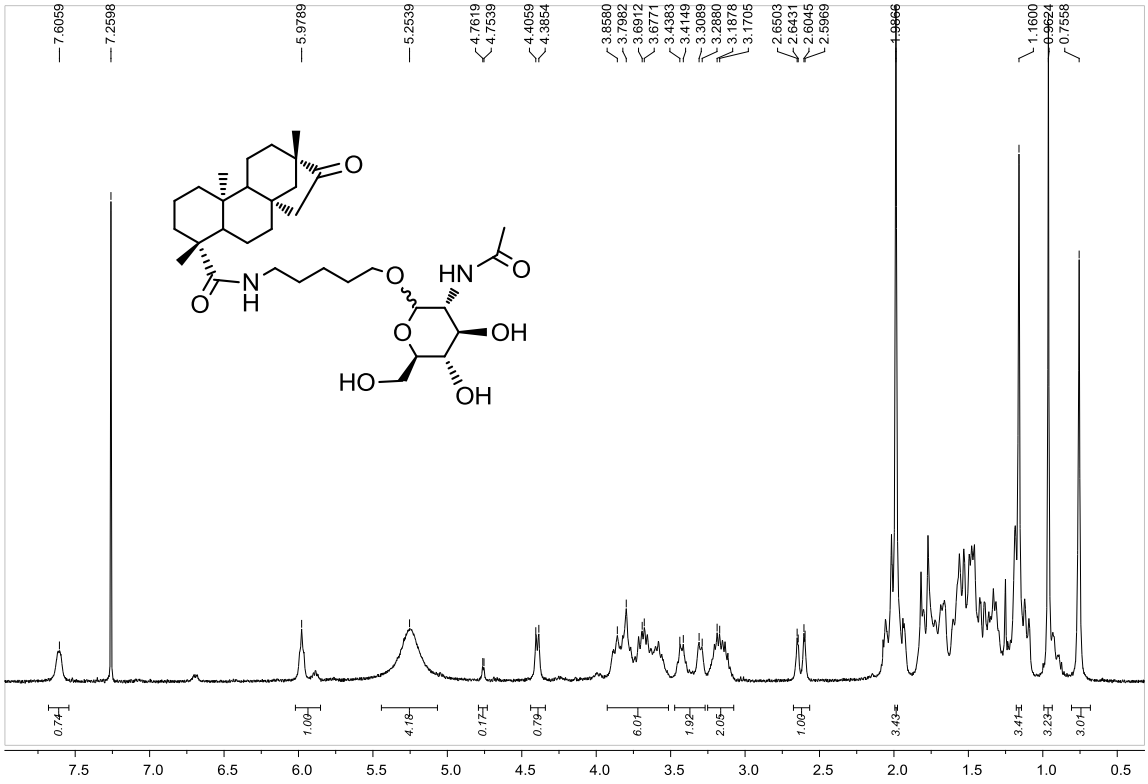

Figure S23. ^{1}H NMR shifts for compound **18** ($CDCl_3$) ppm

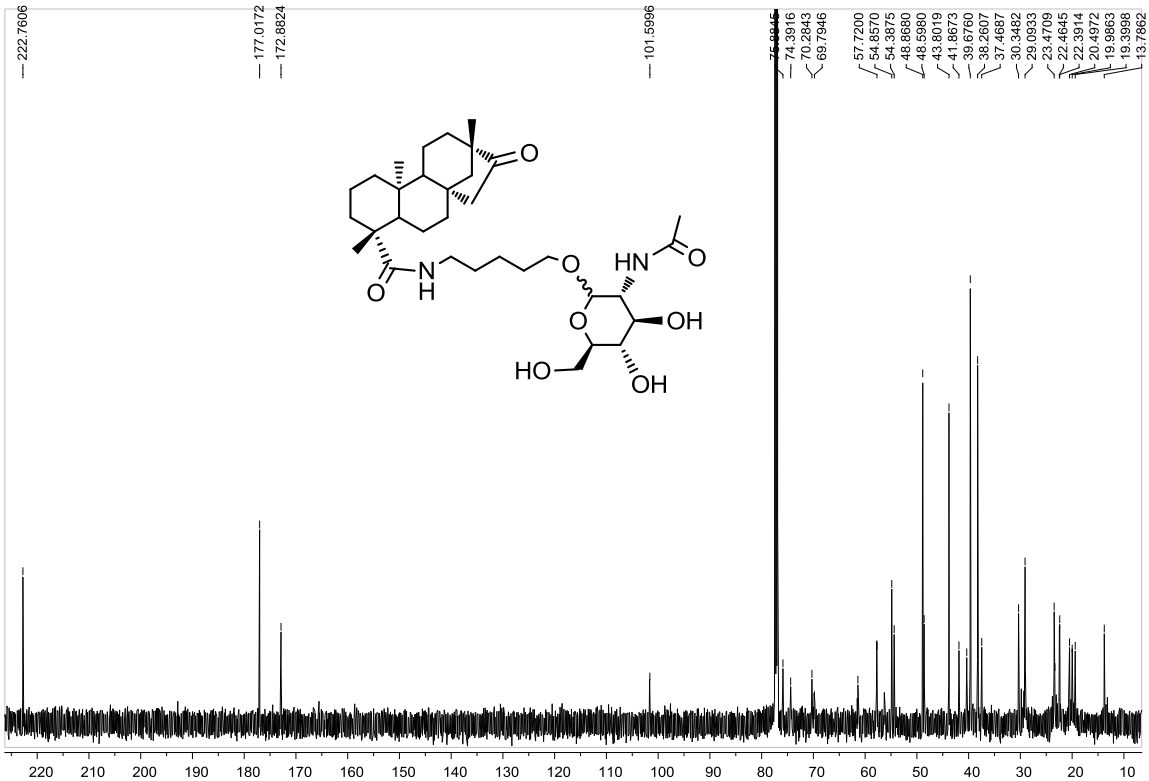

Figure S24. ^{13}C NMR shifts for compound **18** ($CDCl_3$) ppm

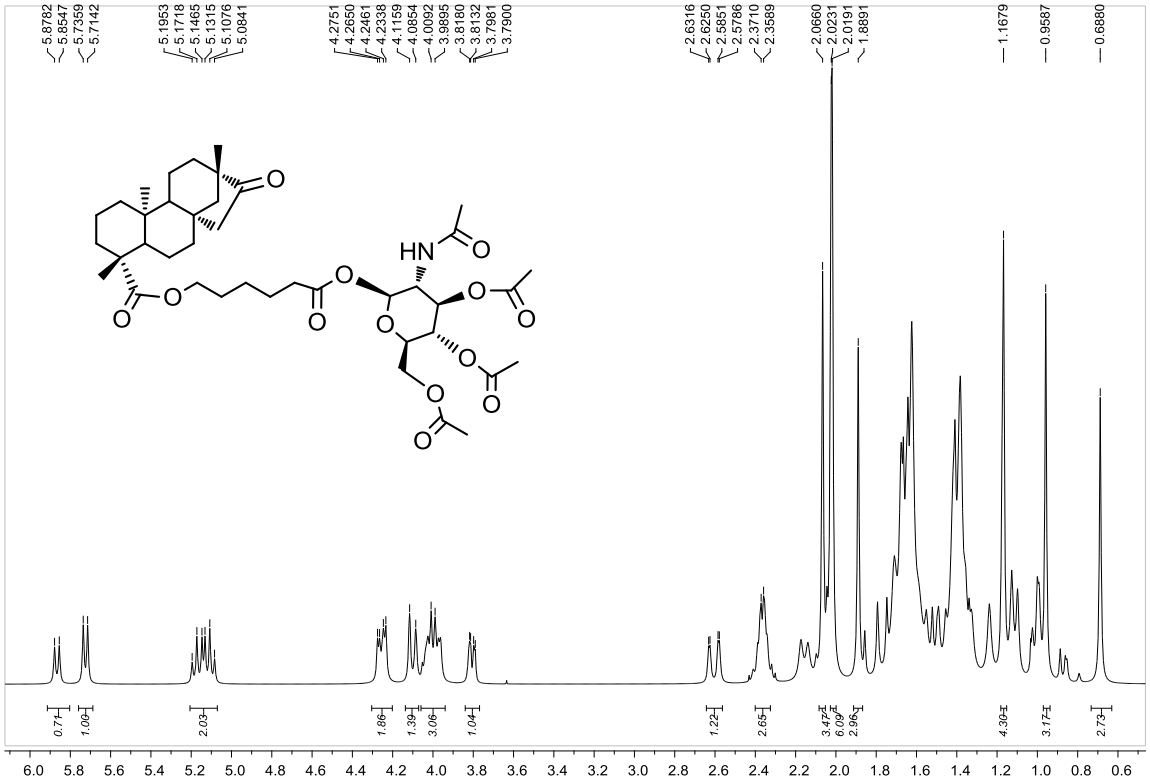

Figure S25. ^{1}H NMR shifts for compound **20a** ($CDCl_3$) ppm

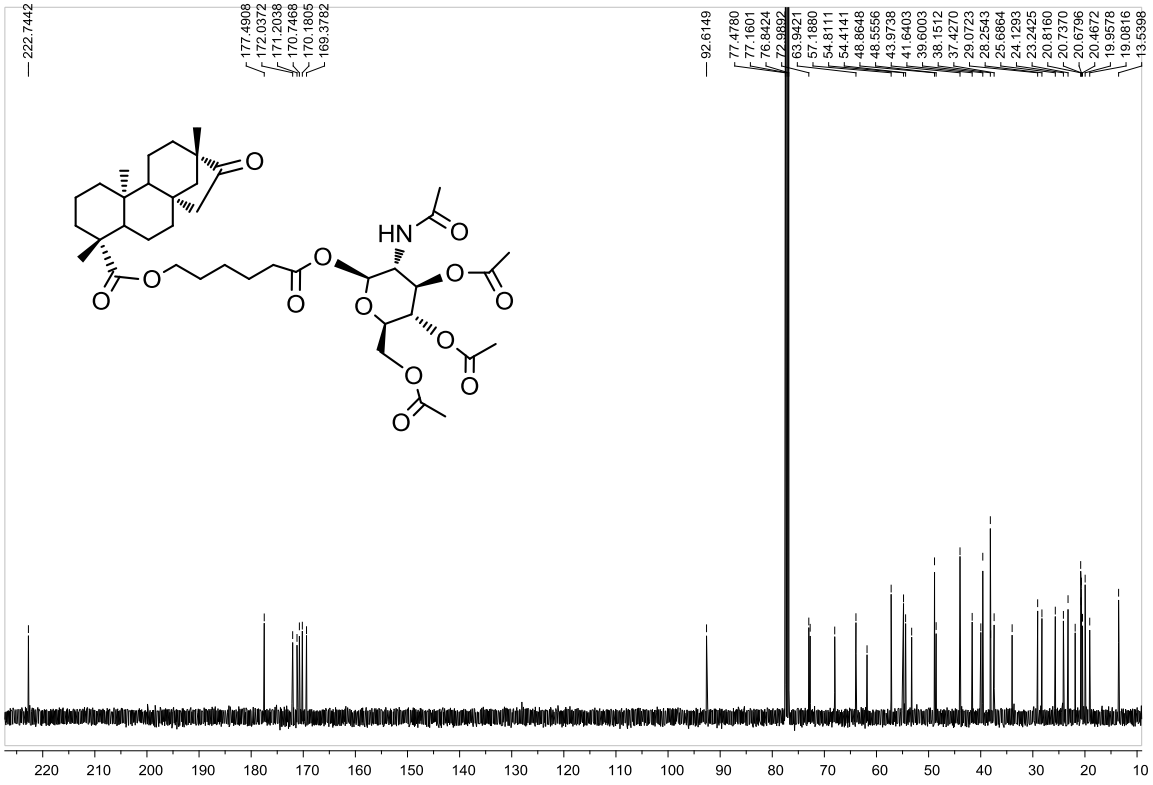

Figure S26. ^{13}C NMR shifts for compound **20a** ($CDCl_3$) ppm

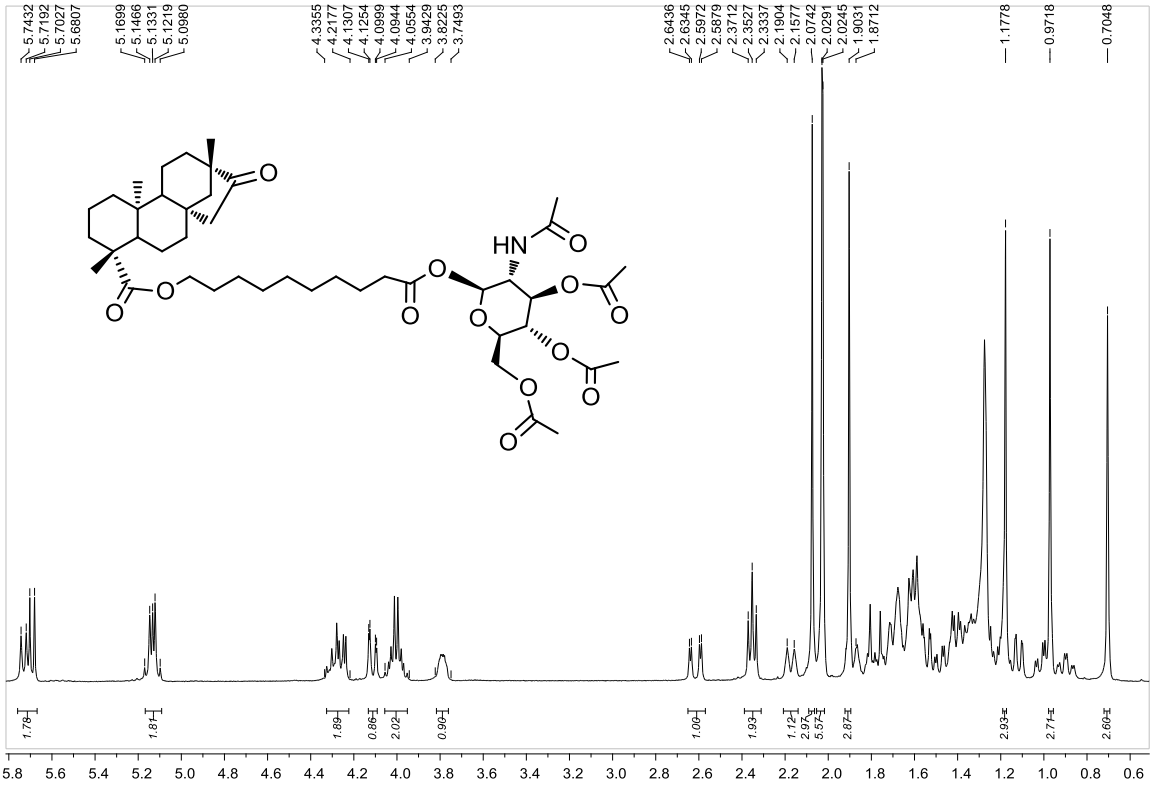

Figure S27. ^{1}H NMR shifts for compound **20b** ($CDCl_3$) ppm

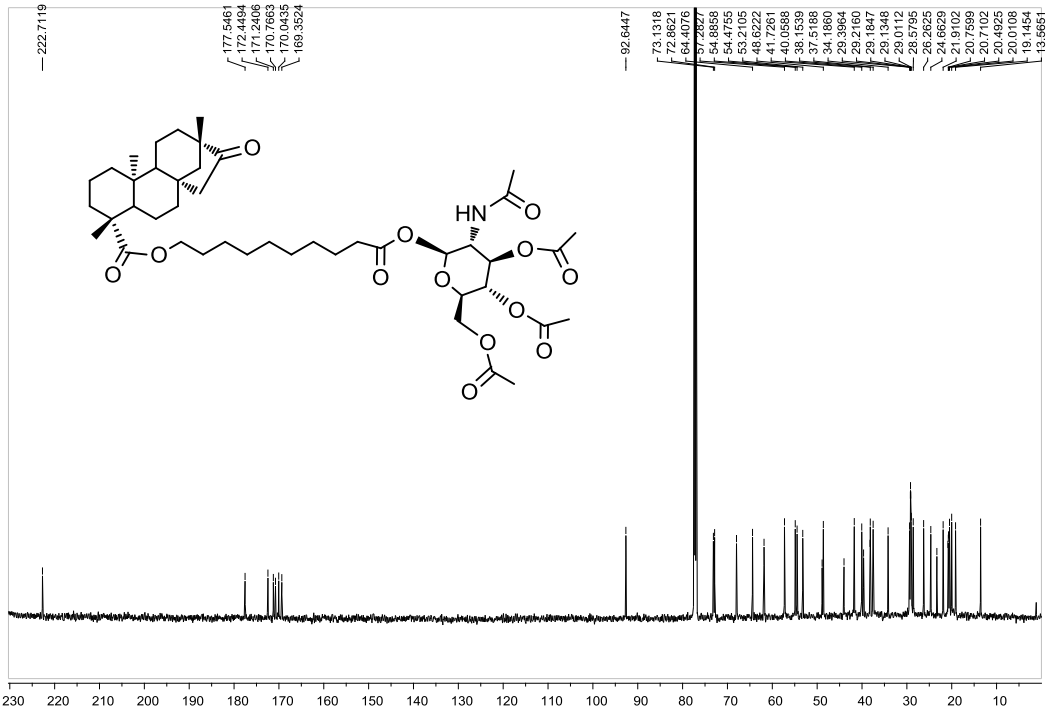

Figure S28. ^{13}C NMR shifts for compound **20b** ($CDCl_3$) ppm