Supporting Information

Photocaging the C6 Carboxylate of β-Glucuronide Prodrugs Enables Spatiotemporally Controlled Release of Anticancer Agents via a Dual Activation Strategy

Junjie Fu,[a] Dianya Yong,[a] Jie Liu,[a] Yunying Tan,[a] Hongyu Yang,[a] Jing Hu[b,c] and Jian Yin[*a,c]

[a]Key Laboratory of Carbohydrate Chemistry and Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Lihu Avenue 1800, Wuxi, Jiangsu 214122, China.

[b]Wuxi School of Medicine, Jiangnan University, Wuxi, 214122, China.

[c]Institute of Future Food Technology, JITRI, Yixing 214200.

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1. Supplementary Figures and Tables

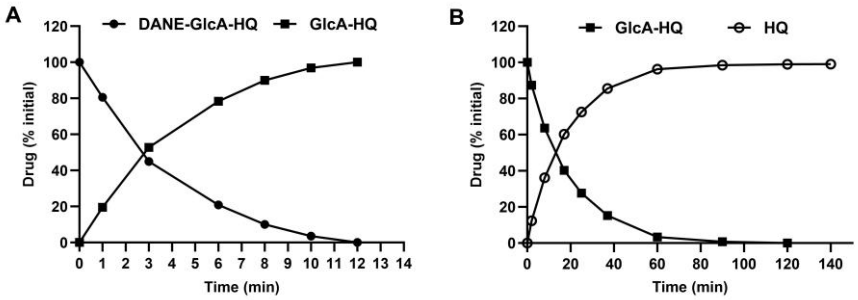


Fig. S1 Two-step release kinetics of DANE-GlcA-HQ. (A) Photoconversion of DANE-GlcA-HQ to GlcA-HQ under 365 nm irradiation. (B) Enzymatic hydrolysis of GlcA-HQ to release HQ catalyzed by β-GUS (30 U mL^{-1}).

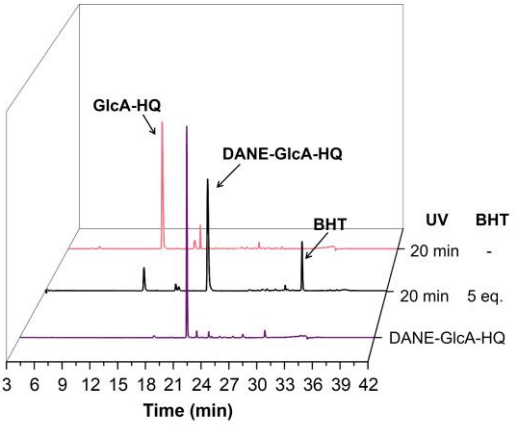


Fig. S2 Radical trapping experiment for the photolysis of DANE-GlcA-HQ. HPLC chromatograms of DANE-GlcA-HQ after 365 nm irradiation for 20 min in the absence and presence of butylated hydroxytoluene (BHT).

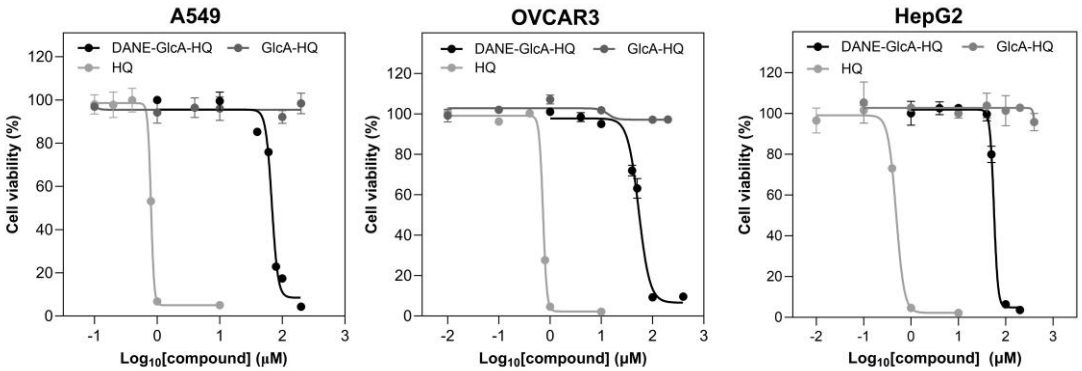


Fig. S3 Comparison of cytotoxicity of HQ, GlcA-HQ, and DANE-GlcA-HQ in cancer cell lines in the absence of UV irradiation, mean ± SD, n = 3.

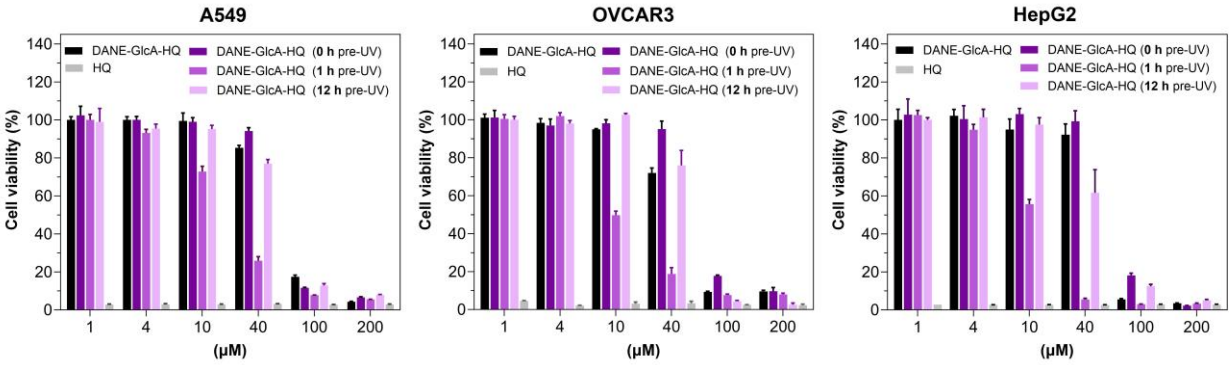


Fig. S4 Effect of pre-UV incubation time on the UV-triggered cytotoxicity of DANE-GlcA-HQ, mean ± SD, n = 3.

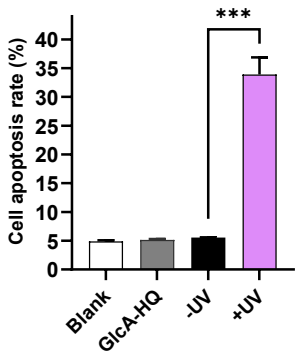


Fig. S5 Quantitative analysis of the percentage of apoptosis cells (Q2+Q3 regions) after indicated treatments, mean ± SD, n = 3, $^{***}P < 0.001$.

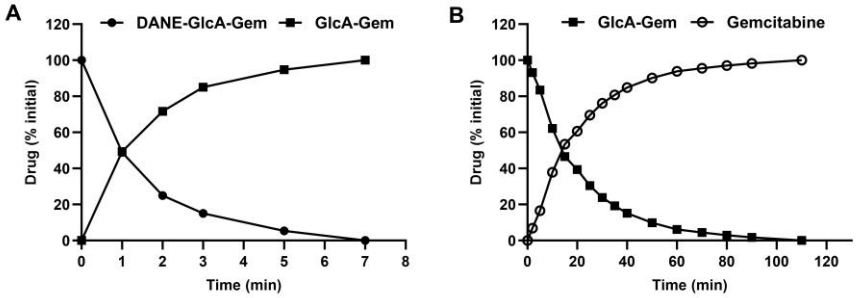


Fig. S6 Two-step release kinetics of DANE-GlcA-Gem. (A) Photoconversion of DANE-GlcA-Gem to GlcA-Gem under 365 nm irradiation. (B) Enzymatic hydrolysis of GlcA-Gem to release Gem catalyzed by β-GUS (5 U mL^{-1}).

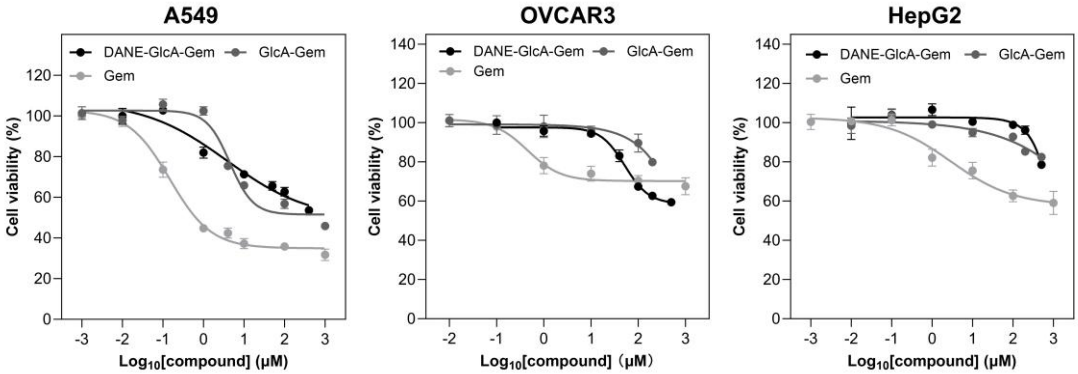


Fig. S7 Comparison of cytotoxicity of Gem, GlcA-Gem, and DANE-GlcA-Gem in cancer cell lines in the absence of UV irradiation, mean ± SD, n = 3.

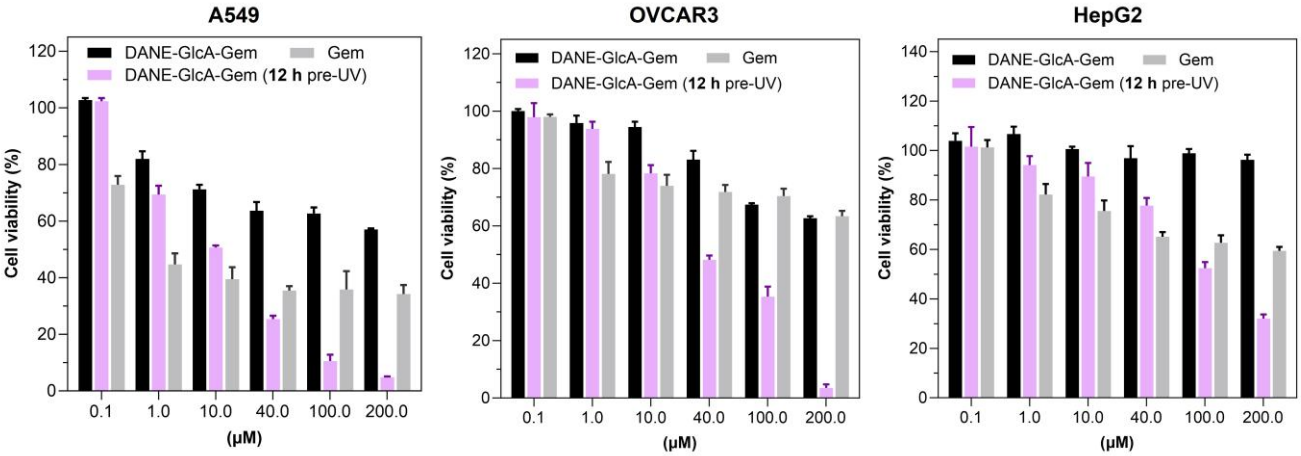


Fig. S8 Cytotoxicity of DANE-GlcA-Gem upon UV irradiation after 12 h of incubation.

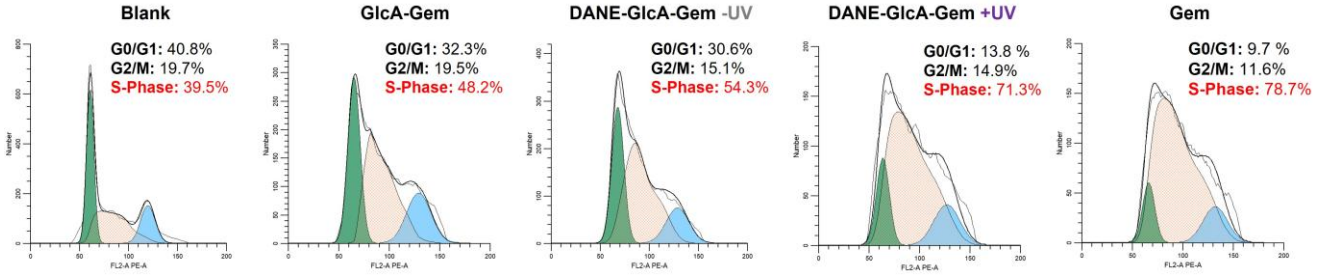


Fig. S9 The effects of DANE-GlcA-Gem on cell cycle of A549 cells with and without UV irradiation.

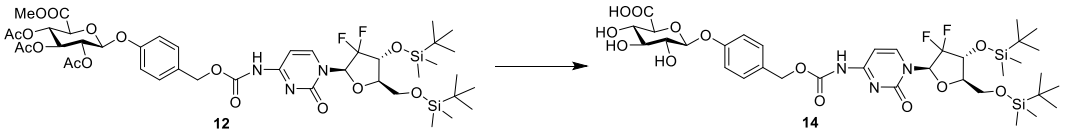
Table S1. Effect of pre-incubation time (0, 1, 12 h) on UV-triggered cytotoxicity (IC_{50}) of DANE-GlcA-HQ.

Tumor Cells	IC_{50} (μM)[a]						
	-UV	+UV (0 h pre-UV)	LDSI[b]	+UV (1 h pre-UV)	LDSI[b]	+UV (12 h pre-UV)	LDSI[b]
A549	67.93 ± 1.16	70.34 ± 0.50	0.96	20.34 ± 3.19	3.34	52.55 ± 0.02	1.29
OVCAR3	53.69 ± 2.02	69.00 ± 0.13	0.77	9.74 ± 0.66	5.51	48.09 ± 2.61	1.11
HepG2	57.01 ± 5.45	87.75 ± 0.30	0.65	10.43 ± 0.33	5.47	44.62 ± 1.61	1.28

[a]Data are presented as mean ± SD, n = 3.

[b]LDSI = IC_{50}(-UV)/IC_{50}(+UV)

Table S2. Optimization of the deacetylation/methylation conversion from compound **12** to **14**.



Entry	Reaction Conditions	Yield
1	MeONa (1 eq.), H_2O (1 eq.), MeOH, r.t., 0.5 h	0
2	MeONa (1 eq.), H_2O (1 eq.), MeOH, 0 °C, 0.5 h	0
3	MeONa (1 eq.), MeOH, 0 °C, 0.5 h	0
4	1) MeONa (1 eq.), MeOH, 0 °C, 0.5 h; 2) LiOH (2 eq.), H_2O, MeOH, 0 °C, 1 h	1) 81% 2) 49%
5	1) MeONa (1 eq.), MeOH, 0 °C, 0.5 h; 2) NaOH (3 eq.), Acetone	1) 81% 2) 74%

Table S3 LogP values and IC_{50} values against cancer cells for compounds Gem, GlcA-Gem, and DANE-GlcA-Gem.

Compounds	LogP	IC_{50} (μM)[a]								
		A549			OVCAR3			HepG2		
		−UV	+UV	LDSI[b]	−UV	+UV	LDSI[b]	−UV	+UV	LDSI[b]
Gem	-1.70	0.48 ± 0.06	0.44 ± 0.07	0.91	> 200	> 200	-	> 200	> 200	-
GlcA-Gem	-1.29	> 200	> 200	-	> 200	> 200	-	> 200	> 200	-
DANE-GlcA-Gem	1.07	> 200	11.06 ± 0.88	> 10	> 200	33.48 ± 2.36	> 10	> 200	84.46 ± 6.25	> 10

[a]Data are presented as mean ± SD, n = 3.

[b]LDSI = IC_{50}(-UV)/IC_{50}(+UV)

2. Analytical Studies

2.1 On demand release of reporter molecule from the model molecules

The in vitro response of the model molecule was detected by HPLC. The column used was Eclipse XDB-C18 (5 μm, 4.6×150 mm), the injection volume was 10 μL, mobile phase A was water + 0.1% trifluoroacetic acid, mobile phase B was acetonitrile. The method was: 10% acetonitrile (0 min ~ 2 min), 10% ~ 95% acetonitrile (2 min ~ 17 min), 95% ~ 10% acetonitrile (19 min ~ 20 min), gradient elution, flow rate was 1.0 mL·min^{-1}, UV detection wavelength was 313 nm.

2.2 On demand release of HQ from DANE-GlcA-HQ

The in vitro response of prodrug DANE-GlcA-HQ was detected by HPLC. The drug was prepared as a 1 mM PBS solution, the column used was Eclipse XDB-C18 (5 μm, 4.6×150 mm), injection volume was 10 μL, mobile phase A was water + 0.1% trifluoroacetic acid, mobile phase B was acetonitrile. The method was: 5% acetonitrile (0 min ~ 1 min), 5% ~ 15% acetonitrile (1 min ~ 15 min), 15% ~ 70% acetonitrile (15 min ~ 30 min), 70% ~ 95% acetonitrile (30 min ~ 32 min), 5% acetonitrile (33 min ~ 43 min), gradient elution, flow rate was 1.0 mL·min^{-1}. UV detection wavelength was 260 nm.

2.3 On demand release of Gem from DANE-GlcA-Gem

The in vitro response of prodrug DANE-GlcA-Gem was detected by HPLC. The drug was prepared as a 1 mM PBS solution, the column used was Eclipse XDB-C18 (5 μm, 4.6×150 mm), injection volume was 10 μL, mobile phase A was water + 0.1% trifluoroacetic acid, mobile phase B was acetonitrile. The method was 2% acetonitrile (0 min ~ 6 min), 2% ~ 10% acetonitrile (6 min ~ 8 min), 10% ~ 45% acetonitrile (8 min ~ 24 min), 45% ~ 2% acetonitrile (24 min ~ 26 min), 2% acetonitrile (26 min ~ 30 min), gradient elution, flow rate was 1.0 mL·min^{-1}, UV detection wavelength was 270 nm.

2.4 Determination of Octanol-Water Partition Coefficient

Add the sample to be tested into 4 mL of water-saturated n-octanol until insoluble precipitate appears in the solution. After centrifugation, take the supernatant, dilute it with methanol by a certain factor, and measure the absorbance (A). Calculate the initial concentration (C_0) of each drug in n-octanol based on the standard curve. Mix the aforementioned 4 mL of n-octanol solution with 4 mL of n-octanol-saturated water, place the mixture in a shaker, and shake for 1 hour. Take samples from the oil phase and the water phase separately, dilute them with methanol by a certain factor, and measure the absorbance. Calculate the concentrations in the oil phase (C_{oil}) and water phase (C_w). The octanol-water partition coefficient (P) is then calculated using the formula: $P = C_{oil} / C_w$.

The logarithm of P is then taken to obtain Log P.

2.5 Cell Uptake Experiment

Cells in the logarithmic growth phase were digested using 0.25% trypsin (containing EDTA), resuspended in complete medium, and gently pipetted to form a single-cell suspension. After cell counting, the suspension was adjusted to an appropriate concentration and seeded into 12-well plates at 1 mL/well (3,000 cells/well). Plates were gently rocked to ensure uniform cell distribution and incubated in a humidified incubator at 37 °C with 5% CO_2 for 24 h. Cell adhesion and growth status were monitored via microscopy. Following medium removal, drug-containing medium was added. The light-exposure group was incubated at 37 °C/5% CO_2 for 20 min, irradiated with a 365 nm UV lamp for 4 min, and transferred to a 37 °C incubator without CO_2 for an additional 30 min. Post-treatment, drug-containing medium was aspirated, and cells were washed twice with PBS. Cells were fixed with 4% paraformaldehyde (500 μL/well) at room temperature for 15 min. After fixation, paraformaldehyde was removed, and cells were washed twice with PBS. DAPI solution (300 μL/well) was added for nuclear staining (10 min, RT). After staining, cells were washed twice with PBS. Finally, 500 μL PBS was added per well, and images were captured using an inverted fluorescence microscope（Axio Vert A1, Germany）. Data were analyzed with ZEISS ZEN 3.8 software.

2.6 MTT Assay

Cells in the logarithmic growth phase were digested with 0.25% trypsin (containing EDTA), resuspended in complete medium, and gently pipetted to form a single-cell suspension. After cell counting, the suspension was adjusted and seeded into 96-well plates at 100 μL/well (5,000 cells/well). Plates were gently rocked for uniform distribution and incubated at 37 °C with 5% CO_2 for 24 h, with cell adhesion and growth confirmed microscopically. High-concentration drug stock solutions (DMSO-dissolved) were serially diluted with medium to ensure < 0.5% DMSO in working solutions. Original medium was aspirated and replaced with 100 μL/well drug-containing medium, followed by incubation at 37 °C/5% CO_2. Control groups were incubated for 36 h, while test groups received 365 nm UV irradiation at 0, 1, and 12 h: DANE-GlcA-HQ underwent 12 min total irradiation (3 × 4 min pulses, 30 min intervals), and DANE-GlcA-Gem received 8 min total irradiation (2 × 4 min pulses, 60 min interval). Post-treatment, drug-medium was aspirated. Under light-protected conditions, 20 μL/well MTT solution (5 mg/mL) was added, and plates were incubated at 37 °C/5% CO_2 for 4 h. After aspirating MTT solution, 150 μL/well DMSO was added, and plates were agitated at 60 rpm, 25 °C for 10 min to dissolve formazan crystals. The absorbance value of each well was detected using a BioTek Synergy H4 multidetection microplate plate reader (Agilent, USA) at the wavelength of 490 nm.

2.7 Cell Apoptosis Assay

Cells in the logarithmic growth phase were digested with 0.25% trypsin (containing EDTA), resuspended in complete medium, and gently pipetted to form a single-cell suspension. After cell counting, the suspension concentration was adjusted and seeded into 6-well plates at 2 mL/well (20,000 cells/well). Plates were gently rocked for uniform cell distribution and incubated at 37 °C with 5% CO_2 for 24 h. Cell adhesion and growth status were confirmed via microscopy. After medium removal, drug-containing medium was added and incubated for 36 h. Post-treatment, adherent cells were collected and washed three times with pre-cooled PBS (pH 7.4) (centrifugation at 2000 r/min for 5 min each). After stained using Annexin V-FITC Apoptosis Detection Kit (C1062L, Beyotime, China) according to the manufacturer's instruction, the apoptotic cells were analyzed by a flow cytometry (Accuri™ C6 Plus, BD Biosciences, USA).

2.8 Cell Cycle analysis

Cells in the logarithmic growth phase were digested using 0.25% trypsin (containing EDTA). After digestion, the cells were resuspended in complete culture medium and pipetted to form a single-cell suspension. The cell suspension concentration was adjusted based on cell counting, and cells were seeded into 6-well plates at 2 mL per well (20,000 cells per well). The plates were gently rocked to ensure even cell distribution. Subsequently, the plates were incubated in a humidified incubator at 37 °C with 5% CO_2 for 24 h. Cell attachment and growth status were monitored via microscopy during the culture period. The culture medium was then removed and replaced with drug-containing medium for a further 36 h incubation. Following drug treatment termination, the adherent cells were collected and washed three times with cold PBS (pH 7.4) (centrifugation at 2000 r/min for 5 min per wash). Cell pellets were fixed by adding 1 mL of 70% ethanol per tube and incubating overnight at 4 °C. The fixed cells were washed twice with PBS, and stained with staining solution containing RNase A and propidium iodide in the dark for 15 minutes using Cell Cycle and Apoptosis Analysis Kit (C1052, Beyotime, China). The cell cycle was analyzed by a flow cytometer (Accuri™ C6 Plus, BD Biosciences, USA).

2.9 Wound healing assay

Cells in the logarithmic growth phase were digested using 0.25% trypsin (containing EDTA). After digestion, the cells were resuspended in complete culture medium and pipetted to form a single-cell suspension. The cell suspension concentration was adjusted based on cell counting, and cells were seeded into 6-well plates at 2 mL per well (100,000 cells per well). The plates were gently rocked to ensure even cell distribution. Subsequently, the plates were incubated in a humidified incubator at 37 °C with 5% CO_2 for 24 h. Cell attachment and growth status were monitored via microscopy during the incubation. After 24 h of culture, a straight scratch wound was created across the cell monolayer in each well using a sterile pipette tip. The culture medium was then removed, and the cells were washed gently with PBS. Images of the scratch wounds were captured at 0 h under a

microscope. Following imaging, the PBS was aspirated and replaced with drug-containing medium for a further 24h incubation. After drug treatment, the drug-containing medium was removed, and the cells were washed gently with PBS. The cells were imaged with an optical microscope (CKX53, Olympus, Japan). The wound healing rates were quantified using ImageJ.

2.10 Crystal Violet Staining Assay

Cells in the logarithmic growth phase were digested with 0.25% trypsin (containing EDTA) and then resuspended in complete medium to create a cell suspension by pipetting. The cell suspension concentration was adjusted based on cell counting, and the cells were seeded into 6-well plates at 2 mL per well (800 cells per well). The plates were gently shaken to ensure even cell distribution. Subsequently, the plates were incubated in a humidified incubator at 37 °C with 5% CO_2 for 12 days. During the culture period, microscopic examination was performed to confirm cell attachment and growth status. After removing the culture medium, the cells were incubated with drug-containing medium for 36 h. Following drug treatment, the drug-containing medium was aspirated, and the cells were washed twice with PBS. Then, 1 mL per well of 4% paraformaldehyde was added to fix the cells at room temperature for 15 minutes. After fixation, the paraformaldehyde was removed, and the cells were washed twice with PBS. Next, 1 mL per well of crystal violet staining solution was added, and the cells were stained at room temperature for 15 minutes. After staining, the cells were rinsed with water to remove any residual crystal violet from the inner walls of the wells. The plates were allowed to air-dry before being photographed.

2.11 Statistical analysis

Data in this study were presented as the means with standard errors (means ± SD). Differences among groups were evaluated using one-way and two-way analysis of variance (ANOVA) with GraphPad Prism 7.0 software. The $P < 0.05$ was considered statistically significant.

3. Chemical Synthesis

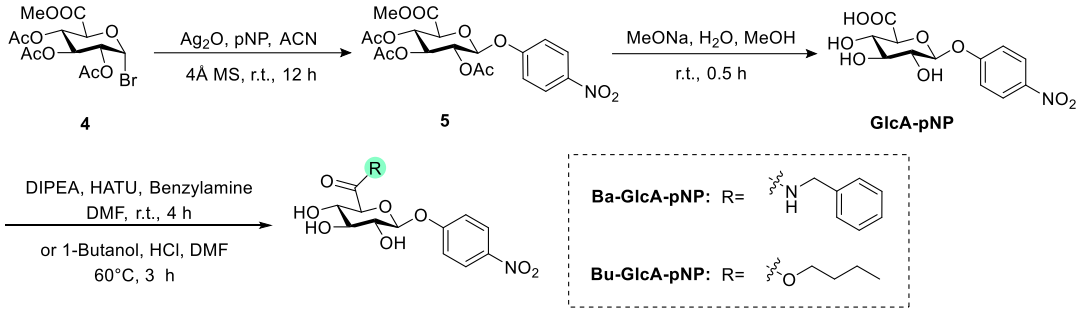
3.1 General information

Commercially available reagents were obtained from J&K scientific, Energy Chemical, Adamas-beta, and Bidepharm. All regents were used without further purification, and all reactions were carried out under argon atmosphere unless otherwise stated.

Analytical thin-layer chromatography (TLC) was carried out on pre-coated silica gel plate (0.2 mm thickness). Spots were visualized with a UV lamp (254 nm) or sugar stain (0.1% (v/v) 3-methoxyphenol, 2.5% (v/v) sulfuricacid in EtOH). Silica gel (200–300 mesh) were used for chromatography purification process.

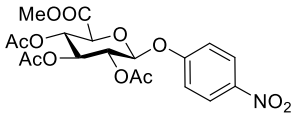
NMR spectra were recorded on a Brüker Avance III 400 MHz (Germany) or Brüker Avance NEO 600 MHz (Germany). All NMR chemical shifts (δ) were recorded in ppm and coupling constants (*J*) were reported in Hertz (Hz).

3.2 Synthesis of Bu-GlcA-pNP and Ba-GlcA-pNP



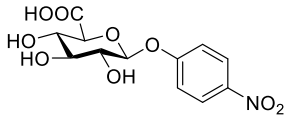
Scheme S1. The synthetic route to Bu-GlcA-pNP and Ba-GlcA-pNP

Methyl (1-*O*-(4-nitrophenyl)-2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate, 5[1,2]



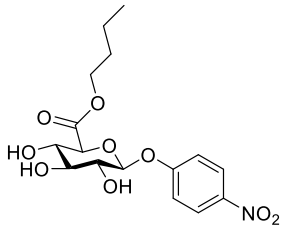
Methyl 1-bromo-2,3,4-tri-*O*-acetyl-α-D-glucopyranuronate **4** (1 g, 2.52 mmol, 1.0 eq.) was dissolved in MeCN (20 mL) and stirred. Then p-Nitrophenol (350 mg, 2.52 mmol, 1.0 eq.), Ag_2O (590 mg, 2.52 mmol, 1.0 eq.), and 4Å molecular sieves (4Å MS) were added. The mixture was stirred for another 12 h at room temperature under an argon atmosphere in the dark. When TLC showed the completion of the reaction, the mixture was filtered through celite to remove the molecular sieves and Ag_2O, washed with DCM. After dried over Na_2SO_4 and concentrated, the residue was purified with silica gel chromatography using PE and EA (10:1, v/v) to afford compound **5** as white foam (596.53 mg, 1.31 mmol, yield 52%). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.24 – 8.17 (m, 2H, Ar-H), 7.11 – 7.05 (m, 2H, Ar-H), 5.42 – 5.34 (m, 2H, 1-H, 2-H), 5.33 – 5.26 (m, 2H, 3-H, 4-H), 4.27 – 4.24 (d, *J* = 9.3 Hz, 1H, 5-H), 3.71 (s, 3H, $COOCH_3$), 2.06 (s, 3H, CH_3(Ac)), 2.06 (s, 3H, CH_3(Ac)), 2.05 (s, 3H, CH_3(Ac)).

4-Nitrophenyl β-D-glucuronide, GlcA-pNP



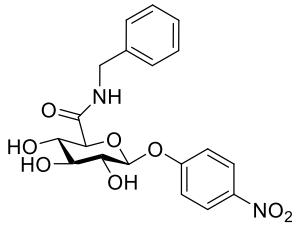
Compound **5** (1.19 mg, 2.63 mmol, 1.0 eq.) was dissolved in methanol (20 mL). With the reaction mixture cooled at 0 °C, a solution of sodium methoxide in methanol (5 M, 530 µL, 2.63 mmol, 1.0 eq.) and water (47 µL, 2.63 mmol, 1.0 eq.) were added. The mixture was then warmed to room temperature and stirred for 0.5 h under an argon atmosphere. When TLC showed the completion of the reaction, the pH was adjusted to slightly acidic by adding ion exchange process. The resin was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was extracted with EA and water. The aqueous phase was collected and concentrated under reduced pressure to afford GlcA-pNP as white foam (529.2 mg, 1.68 mmol, yield 64%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.21 (d, *J* = 9.2 Hz, 2H, Ar-H), 7.23 (d, *J* = 9.2 Hz, 2H, Ar-H), 5.16 (d, *J* = 7.6 Hz, 1H, 1-H), 4.08 (d, *J* = 9.7 Hz, 1H, 5-H), 3.68 – 3.62 (m, 1H, 3-H), 3.58 – 3.51 (m, 2H, 2-H, 4-H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 170.5, 162.2, 142.8, 125.2, 116.4, 100.2, 75.8, 75.2, 73.0, 71.4, 48.0, 47.9, 47.8, 47.6, 47.5, 47.3, 47.2.

1-*O*-(4-Nitrophenyl)-β-D-glucopyranuronic acid butyl ester, Bu-GlcA-pNP



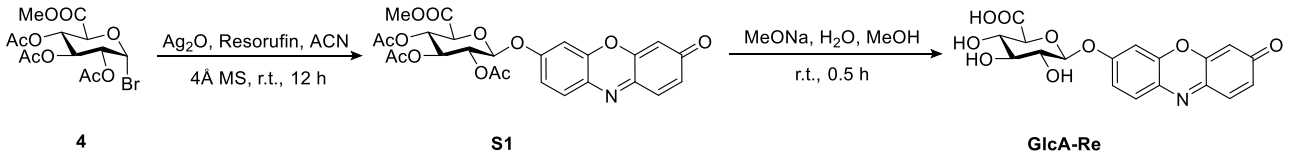
GlcA-pNP (23 mg, 0.07 mmol, 1 eq.) was dissolved in a mixture of 230 µL DMF and 920 µL anhydrous n-butanol. The solution was heated to 60°C, followed by the dropwise addition of 46 µL concentrated hydrochloric acid, and the mixture was stirred for 3 h. When TLC showed the completion of the reaction, the mixture was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using PE and EA (9:1, v/v) to afford Bu-GlcA-pNP as white foam (13.6 mg, 0.036 mmol, yield 52%). ^{1}H NMR (600 MHz, DMSO-*d*6) δ 8.24 – 8.20 (m, 2H, Ar-H), 7.26 – 7.22 (m, 2H, Ar-H), 5.60 (d, J = 4.8 Hz, 1H, OH), 5.45 (d, J = 5.8 Hz, 1H, 1-H), 5.32 (d, J = 1.6 Hz, 1H, OH), 5.31 (s, 1H, OH), 4.13 (d, J = 9.6 Hz, 1H, 5-H), 4.11 – 4.03 (m, 2H, OCH_2), 3.42 (dt, J = 9.3, 4.4 Hz, 1H, 4-H), 3.38 – 3.34 (m, 2H, 2-H, 3-H), 1.59 – 1.51 (m, 2H, CH_2), 1.35 – 1.28 (m, 2H, CH_2), 0.86 (t, J = 7.4 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-*d*6) δ 169.1, 162.4, 142.4, 126.3, 117.0, 99.8, 76.0, 75.7, 73.3, 71.7, 64.9, 40.6, 40.4, 40.2, 40.0, 39.8, 39.6, 39.4, 30.5, 19.0, 14.0. HRMS: m/z calculated $[M + H]^+$ = 372.1289, found 372.1295.

***N*-Benzyl-1-(4-nitrophenyl)-β-D-glucopyranosyluronamide, Ba-GlcA-pNP**



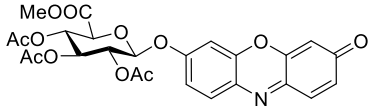
GlcA-pNP (31.5 mg, 0.10 mmol, 1 eq.) and DIPEA (53 μL, 0.3 mmol, 3 eq.) were dissolved in anhydrous N,N-dimethylformamide (DMF, 3 mL). Subsequently, HATU (44 mg, 0.11 mmol, 1 eq.) was added with the reaction mixture cooled at 0 °C, and the mixture was stirred for 2 h. Then, benzylamine (30 μL, 0.11 mmol, 1 eq.) was added, and the mixture was warmed to room temperature and stirred for an additional 4 h. When TLC showed the completion of the reaction, the mixture was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using PE and EA (1:6, v/v) to afford Ba-GlcA-pNP as white foam (25.88 mg, 0.06 mmol, yield 64%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.42 (t, *J* = 6.1 Hz, 1H, NH), 8.25 – 8.21 (m, 2H, Ar-H), 7.27 – 7.19 (m, 5H, Ar-H), 7.18 – 7.15 (m, 2H, Ar-H), 5.73 (d, *J* = 5.4 Hz, 1H, OH), 5.44 (d, *J* = 5.9 Hz, 1H, OH), 5.30 (d, *J* = 7.8 Hz, 1H, 1-H), 4.79 (dd, *J* = 10.0, 9.3 Hz, 1H, 4-H), 4.28 (dd, *J* = 15.1, 6.2 Hz, 1H, Ar-CH_2), 4.23 (d, *J* = 10.0 Hz, 1H, 5-H), 4.17 (dd, *J* = 15.2, 5.8 Hz, 1H, Ar-CH_2), 3.56 (td, *J* = 9.2, 5.9 Hz, 1H, 2-H), 3.47 (ddd, *J* = 9.1, 7.7, 5.4 Hz, 1H, 3-H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.3, 162.5, 142.3, 139.5, 128.7, 127.5, 127.2, 126.2, 117.0, 100.2, 76.5, 76.3, 73.2, 71.3, 42.3, 40.5, 40.3, 40.1, 39.9, 39.7, 39.5, 39.3. HRMS: m/z calculated $[M + H]^+$ = 405.1292, found 405.1299.

3.3 Synthesis of DANE-GlcA-pNP and DANE-GlcA-Re



Scheme S2. The synthetic route to GlcA-Re

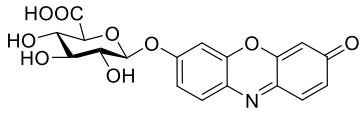
Methyl 2,3,4-tri-*O*-acetyl-1-*O*-(3-oxo-3*H*-phenoxazin-7-yl)-β-D-glucopyranosiduronate, S1[3]



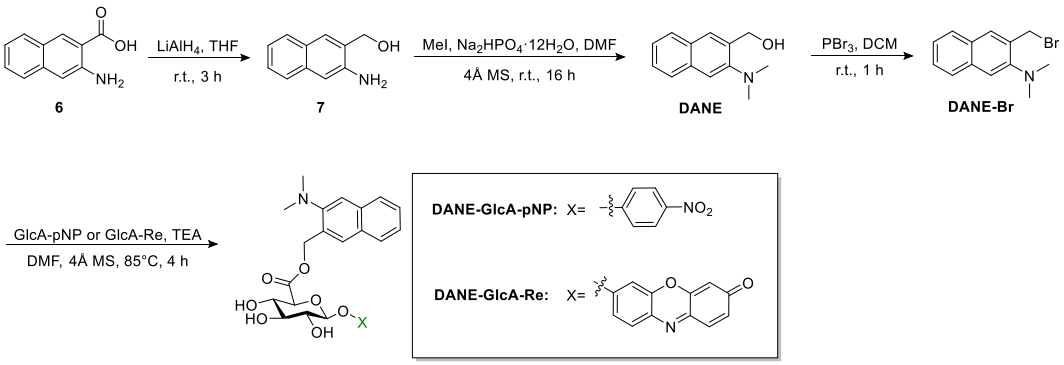
Methyl 1-bromo-2,3,4-tri-*O*-acetyl-α-D-glucopyranuronate **5** (1 g, 2.52 mmol, 1.0 eq.) was dissolved in anhydrous MeCN (20 mL). Resorufin (537.24 mg, 2.52 mmol, 1.0 eq.), Ag_2O (590 mg, 2.52 mmol, 1.0 eq.), and 4Å MS were added. The mixture was stirred for another 12 hours at room temperature under an argon atmosphere in the dark. When TLC showed the completion of the reaction, the mixture was filtered through celite to remove the

molecular sieves and Ag_2O, washed with DCM. After dried over Na_2SO_4 and concentrated, the residue was purified with silica gel chromatography using PE and EA (10:1, v/v) to afford Compound **S1** as an orange solid (693.79 mg, 1.31 mmol, yield 52%). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.72 (d, *J* = 8.9 Hz, 1H, Re-H), 7.41 (d, *J* = 9.8 Hz, 1H, Re-H), 7.00 (dd, *J* = 8.8, 2.6 Hz, 1H, Re-H), 6.94 (d, *J* = 2.6 Hz, 1H, Re-H), 6.84 (dd, *J* = 9.8, 2.0 Hz, 1H, Re-H), 6.32 (d, *J* = 2.1 Hz, 1H, Re-H), 5.40 – 5.35 (m, 2H, 2-H, 4-H), 5.31 (m, 2H, 3-H, 1-H), 4.30 (d, *J* = 9.4 Hz, 1H, 5-H), 3.73 (s, 3H, OCH_3), 2.07 (s, 3H, CH_3(Ac)), 2.06 (s, 6H, CH_3(Ac)).

1-*O*-(3-Oxo-3*H*-phenoxazin-7-yl)-β-D-glucopyranosiduronic acid, GlcA-Re[3]

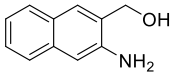


Compound **S1** (200 mg, 0.37 mmol, 1.0 eq.) was dissolved in methanol (10 mL). With the reaction mixture cooled at 0 °C, a solution of sodium methoxide in methanol (5 M, 74 µL, 0.37 mmol, 1.0 eq.) and water (6 µL, 0.37 mmol, 1.0 eq.) were added. The mixture was then warmed to room temperature and stirred for 0.5 h under an argon atmosphere. When TLC showed the completion of the reaction, the pH was adjusted to slightly acidic by adding ion exchange process. The resin was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was extracted with EA and water. The aqueous phase was collected and concentrated under reduced pressure to afford GlcA-Re as a red foam (79.41 mg, 0.20 mmol, yield 54%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.79 (d, *J* = 8.9 Hz, 1H, Re-H), 7.54 (d, *J* = 9.8 Hz, 1H, Re-H), 7.17 (s, 1H, Re-H), 7.11 (d, *J* = 8.9 Hz, 1H, Re-H), 6.79 (d, *J* = 9.8 Hz, 1H, Re-H), 6.30 (s, 1H, Re-H), 5.13 (d, *J* = 7.3 Hz, 1H, 1-H), 3.69 (d, *J* = 9.9 Hz, 1H, 5-H), 3.32 – 3.12 (m, 3H, 2-H, 3-H, 4-H).



Scheme S3. The synthetic route to DANE-GlcA-pNP and DANE-GlcA-Re

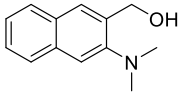
3-Amino-2-naphthalenemethanol, 7[4]



3-Amino-2-naphthoic acid **6** (133.5 mg, 0.71 mmol, 1.0 eq.) was dissolved in anhydrous THF (5 mL) under stirring.

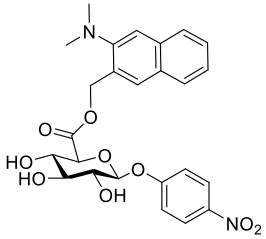
Lithium aluminum hydride (81 mg, 2.13 mmol, 3.0 eq.) was added portionwise at 0 °C. The reaction mixture was warmed to room temperature and stirred for 3 h. When TLC showed the completion of the reaction, the mixture was diluted with diethyl ether (5 mL) and quenched sequentially with water (100 µL) and aqueous NaOH (4 M, 100 µL) at 0 °C. The resulting mixture was extracted with diethyl ether and H_2O. After dried over Na_2SO_4 and concentrated, the residue was purified with silica gel chromatography using PE and EA (10:1, v/v) to afford compound **7** as a yellow solid (90 mg, 0.63 mmol, yield 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.63 (s, 1H, Ar-H), 7.52 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.31 – 7.22 (m, 1H, Ar-H), 7.15 – 7.06 (m, 1H, Ar-H), 6.92 (d, *J* = 2.1 Hz, 1H, Ar-H), 5.29 (td, *J* = 5.5, 1.9 Hz, 1H, OH), 5.22 (s, 2H, NH_2), 4.56 (dd, *J* = 5.6, 2.1 Hz, 2H, CH_2).

2-(Hydroxymethyl)-3-(dimethylamino)naphthalene, DANE[4]



Compound **7** (110.8 mg, 0.64 mmol, 1.0 eq.) and $Na_2HPO_4{\cdot}12H_2O$ (1.02 g, 3 mmol, 4.7 eq.) were dissolved in anhydrous DMF (20 mL).Then iodomethane (250 µL, 4 mmol, 6.3 eq.) and 4Å MS were added. The reaction mixture was stirred under argon atmosphere at room temperature overnight. When TLC showed the completion of the reaction, the reaction was quenched by dropwise addition of ice-water (5 mL). The mixture was extracted with diethyl ether (3 × 20 mL) and washed with saturated NaCl solution (10 mL). After dried over Na_2SO_4 and concentrated, the residue was purified with silica gel chromatography using PE and EA (20:1, v/v) to afford DANE as a yellow oil (70 mg, 0.35 mmol, yield 55%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.76 (dt, *J* = 7.7, 2.4 Hz, 2H, Ar-H), 7.65 (s, 1H, Ar-H), 7.55 (s, 1H, Ar-H), 7.42 (dddd, *J* = 14.6, 8.2, 6.9, 1.5 Hz, 2H, Ar-H), 5.26 (s, 1H, OH), 4.99 (s, 2H, CH_2), 2.84 (s, 6H, $N(CH_3)_2$).

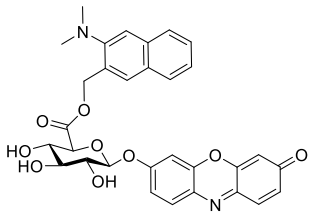
(3-(Dimethylamino)-2-(hydroxymethyl)naphthalen-1-yl)-1-(4-nitrophenyl)-β-D-glucopyranosiduronate, DANE-GlcA-pNP



DANE (191 mg, 0.95 mmol, 1.0 eq.) was dissolved in anhydrous DCM (5 mL). Phosphorus tribromide (PBr_3, 64 µL, 0.63 mmol, 0.66 eq.) was added dropwise at 0 °C. The mixture was warmed to room temperature and stirred under argon atmosphere for 1 h. When TLC showed the completion of the reaction, the reaction mixture was extracted with DCM (3 × 15 mL) and washed with saturated NaCl solution (10 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford a yellow oil (crude bromide). Then the crude bromide (0.63 mmol, 1.0 eq.), GlcA-pNP (199 mg, 0.63 mmol, 1.0 eq.), Et_3N (175 µL, 1.26 mmol, 2.0 eq.)

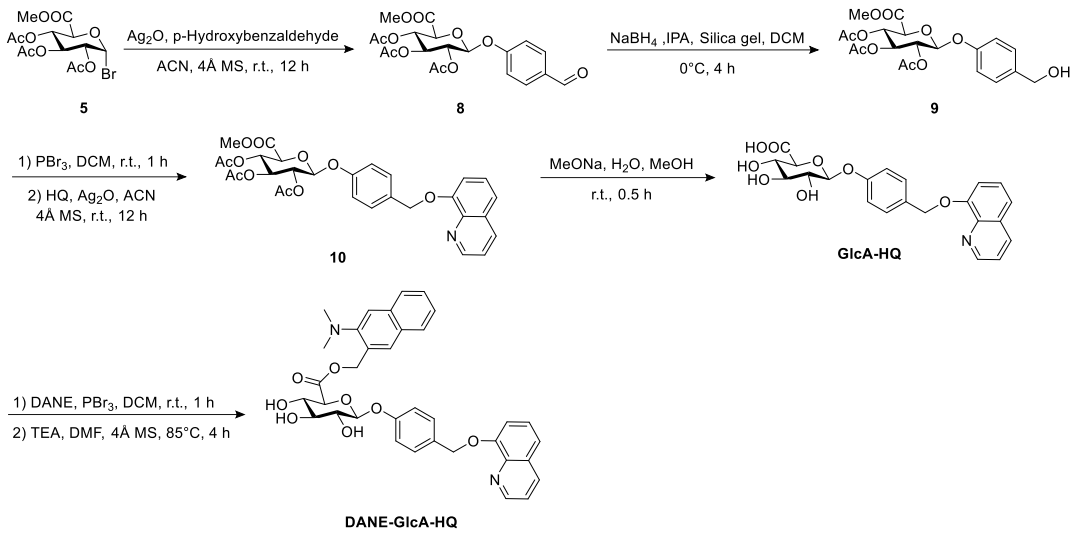
and 4Å MS were dissolved in anhydrous DMF (3 mL). The reaction mixture was stirred at 85 °C under argon for 4 h. When TLC showed the completion of the reaction, the mixture was filtered and concentrated under reduced pressure. the residue was purified with silica gel chromatography using DCM and MeOH (20:1, v/v) to afford DANE-GlcA-pNP as a yellow solid (75 mg, 0.15 mmol, yield 24%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, *J* = 9.3 Hz, 2H, Ar-H (pNP)), 7.81 (d, *J* = 7.1 Hz, 2H, Ar-H (DANE)), 7.59 – 7.50 (m, 2H, Ar-H (DANE)), 7.44 (t, *J* = 7.3 Hz, 1H, Ar-H (DANE)), 7.34 – 7.23 (m, 3H, Ar-H (pNP), Ar-H (DANE)), 5.39 (t, J = 3.3 Hz, 3H, OCH_2, 1-H), 4.32 (d, *J* = 9.7 Hz, 1H, 5-H), 3.52 (d, *J* = 9.1 Hz, 1H, 3-H), 3.43 - 3.39 (m, 2H, 2-H, 4-H), 2.71 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.0, 162.3, 150.8, 142.4, 133.8, 130.4, 129.7, 128.3, 127.7, 127.1, 126.8, 126.2, 124.9, 116.9, 116.0, 99.7, 76.0, 75.7, 73.2, 71.7, 63.1, 45.3, 40.6, 40.4, 40.2, 40.0, 39.8, 39.5, 39.3. HRMS: m/z calculated $[M + H]^+$ = 499.1711, found 499.1716.

[2-((3-(Dimethylamino)-2-naphthalenyl)methyl)]1-*O*-(3-oxo-3*H*-phenoxazin-7-yl)-β-D-glucopyranosiduronate, DANE-GlcA-Re



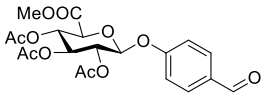
DANE (60 mg, 0.30 mmol, 2.0 eq.) was dissolved in anhydrous DCM (5 mL). PBr_3 (19 µL, 0.20 mmol, 1.33 eq.) was added dropwise at 0 °C. The mixture was warmed to room temperature and stirred under argon atmosphere for 1 h. When TLC showed the completion of the reaction, the reaction mixture was extracted with DCM (3 × 5 mL) and washed with saturated NaCl solution (10 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford a yellow oil (crude bromide). Then the crude bromide (0.3 mmol, 2.0 eq.), GlcA-Re (58 mg, 0.15 mmol, 1.0 eq.), Et_3N (41 µL, .0.3 mmol, 2.0 eq.) and 4Å MS were dissolved in anhydrous DMF (3 mL). The reaction mixture was stirred at 85 °C under argon for 4 h. When TLC showed the completion of the reaction, the mixture was filtered and concentrated under reduced pressure. the residue was purified with silica gel chromatography using DCM and MeOH (20:1, v/v) to afford DANE-GlcA-pNP as a yellow solid (6.8 mg, 0.012 mmol, yield 8%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.76 – 7.72 (m, 3H, Ar-H (DANE, Re), 7.67 – 7.63 (m, 2H, Ar-H (DANE)), 7.50 (d, *J* = 2.2 Hz, 1H, Ar-H (Re)), 7.43 (s, 1H, Ar-H (DANE)), 7.33 (d, *J* = 8.2 Hz, 1H, Ar-H (Re)), 7.26 (t, *J* = 7.6 Hz, 1H, Ar-H (DANE)), 7.20 (dd, *J* = 8.8, 2.6 Hz, 1H, Ar-H (Re)), 7.14 (d, *J* = 2.7 Hz, 1H, Ar-H (Re)), 7.09 (t, *J* = 7.7 Hz, 1H, Ar-H (DANE)), 5.56 (d, *J* = 13.0 Hz, 1H, CH_2), 5.47 (d, *J* = 12.9 Hz, 1H, CH_2), 5.32 (d, *J* = 7.8 Hz, 1H, 1-H), 4.33 (d, *J* = 9.8 Hz, 1H, 5-H), 3.82 – 3.77 (m, 1H, 3-H), 3.65 – 3.61 (m, 2H, 2-H, 4-H), 2.77 (s, 6H, $N(CH_3)_2$). HRMS: m/z calculated $[M + H]^+$ = 573.1868, found 573.1859.

3.3 Synthesis of DANE-GlcA-HQ



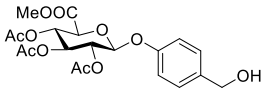
Scheme S4. The synthetic route to DANE-GlcA-HQ

Methyl 2,3,4-tri-*O*-acetyl-1-(4-formylphenyl)-β-D-glucopyranosiduronate, 8[5]



Methyl 1-bromo-2,3,4-tri-*O*-acetyl-α-D-glucopyranuronate **4** (5 g, 12.59 mmol, 1.0 eq.) was dissolved in MeCN (30 mL) and stirred. Then p-Hydroxybenzaldehyde (1.54 g, 12.59 mmol, 1.0 eq.), Ag_2O (2.92 g, 12.59 mmol, 1.0 eq.), and 4Å MS were added. The mixture was stirred for another 12 h at room temperature under an argon atmosphere in the dark. When TLC showed the completion of the reaction, the mixture was filtered through celite to remove the molecular sieves and Ag_2O, washed with DCM. After dried over Na_2SO_4 and concentrated, the residue was purified with silica gel chromatography using PE and EA (10:1, v/v) to afford compound **8** as white foam (3.50 g, 8 mmol, yield 63.6%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.92 (s, 1H, CHO), 7.85 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.11 (d, *J* = 8.7 Hz, 2H, Ar-H), 5.40 – 5.27 (m, 4H, 1-H, 2-H, 3-H, 4-H), 4.25 (d, *J* = 9.1 Hz, 1H, 5-H), 3.71 (s, 3H, $COOCH_3$), 2.08 – 2.04 (m, 9H, CH_3(Ac)).

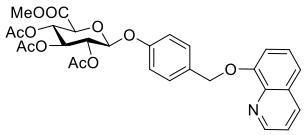
Methyl 2,3,4-tri-*O*-acetyl-1-(4-(hydroxymethyl)phenyl)-β-D-glucopyranosiduronate, 9



Compound **8** (1.34 g, 3.00 mmol, 1.0 eq.), $NaBH_4$（227 mg, 6.00 mmol, 2.0 eq.), isopropanol (5 mL), and silica gel were added to anhydrous DCM (20 mL). The reaction mixture was stirred at 0 °C for 4 h. When TLC showed the completion of the reaction, the reaction was quenched by dropwise addition of ice-water (5 mL). The mixture was extracted with DCM (3 × 25 mL) and washed sequentially with saturated $NaHCO_3$ solution (20 mL) and saturated NaCl solution (20 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced

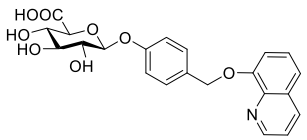
pressure to afford compound **9** as a white solid (1.41 g, 2.76 mmol, yield 92%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.34 – 7.28 (m, 2H, Ar-H), 7.02 – 6.95 (m, 2H, Ar-H), 5.37 – 5.25 (m, 3H, 2-H, 3-H, 4-H), 5.13 (d, *J* = 7.2 Hz, 1H, 1-H), 4.64 (d, *J* = 4.7 Hz, 2H, CH_2), 4.17 (d, J = 9.5 Hz, 1H, 5-H), 3.73 (s, 3H, $COOCH_3$), 2.08 – 2.01 (m, 9H, CH_3 (Ac)). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 170.1, 169.3, 169.2, 166.9, 156.3, 136.3, 128.5, 117.4, 99.4, 77.2, 77.0, 76.8, 72.8, 72.0, 71.2, 69.2, 64.8, 52.9, 20.6, 20.5.

Methyl 2,3,4-tri-*O*-acetyl-1-(4-((quinolin-8-yloxy)methyl)phenyl)-β-D-glucopyranosiduronate, 10



Compound **9** (984 mg, 2.24 mmol, 1.0 eq.) was dissolved in anhydrous DCM (20 mL). PBr_3(107 µL, 1.12 mmol, 0.5 eq.) was added dropwise at 0 °C. The mixture was warmed to room temperature and stirred for 1 h. When TLC showed the completion of the reaction, the reaction mixture was extracted with DCM (3 × 25 mL) and washed sequentially with saturated $NaHCO_3$ solution (20 mL) and saturated NaCl solution (20 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude bromide. The crude bromide (2.24 mmol, 1.0 eq.), HQ (325 mg, 2.24 mmol, 1.0 eq.), Ag_2O (520 mg, 2.24 mmol, 1.0 eq.), and 4Å MS were added to anhydrous MeCN (20 mL). The reaction mixture was stirred at room temperature under argon overnight. When TLC showed the completion of the reaction, the mixture was filtered through celite to remove molecular sieves and Ag_2O, and washed with DCM. The residue was purified by silica gel chromatography using PE and EA (4:1, v/v) to afford compound **10** as a white solid (420 mg, 1.18 mmol, yield 52.8%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.96 (dd, *J* = 4.2, 1.7 Hz, 1H, Ar-H (HQ)), 8.13 (dd, *J* = 8.3, 1.7 Hz, 1H, Ar-H (HQ)), 7.51 – 7.41 (m, 3H, Ar-H (HQ)), 7.41 – 7.36 (m, 2H, Ar-H), 7.03 – 6.94 (m, 3H, , Ar-H, Ar-H (HQ)), 5.40 – 5.28 (m, 5H, CH_2, 2-H, 3-H, 4-H), 5.12 (d, *J* = 7.2 Hz, 1H, 1-H), 4.16 (d, *J* = 9.5 Hz, 1H, 5-H), 3.71 (s, 3H, $COOCH_3$), 2.05 (s, 3H, CH_3 (Ac)), 2.04 (s, 3H, CH_3 (Ac)), 2.04 (s, 3H, CH_3 (Ac)). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 170.1, 169.3, 169.2, 166.9, 156.5, 154.4, 149.4, 140.6, 135.9, 132.2, 129.6, 128.8, 126.6, 121.6, 120.0, 117.4, 110.1, 99.4, 77.3, 77.1, 76.8, 72.8, 72.0, 71.2, 70.4, 69.2, 52.9, 20.6, 20.5. HRMS: m/z calculated $[M + H]^+$ = 568.1813, found 568.1825.

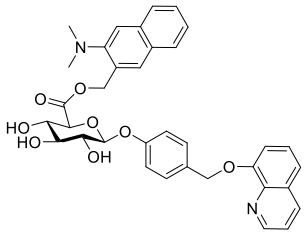
(4-((Quinolin-8-yloxy)methyl)phenyl) β-D-glucopyranosiduronic acid, GlcA-HQ



Compound **10** (420 mg, 1.18 mmol, 1.0 eq.) was dissolved in methanol (10 mL). With the reaction mixture cooled at 0 °C, a solution of sodium methoxide in methanol (5 M, 236 µL, 1.18 mmol, 1.0 eq.) and water (21 µL, 1.18 mmol, 1.0 eq.) were added. The mixture was then warmed to room temperature and stirred for 0.5 h under an argon atmosphere. When TLC showed the completion of the reaction, the pH was adjusted to slightly acidic by

adding ion exchange process. The resin was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was extracted with EA and water. The aqueous phase was collected and concentrated under reduced pressure to afford GlcA-HQ as white foam (277.2 mg, 0.65 mmol, yield 55%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.79 (dd, *J* = 4.3, 1.7 Hz, 1H, Ar-H (HQ)), 8.28 (dd, *J* = 8.3, 1.7 Hz, 1H, Ar-H (HQ)), 7.52 (dd, *J* = 8.3, 4.3 Hz, 1H, Ar-H (HQ)), 7.48 – 7.43 (m, 5H, Ar-H, Ar-H (HQ)), 7.11 (d, *J* = 8.6 Hz, 2H, Ar-H), 5.33 (s, 2H, CH_2), 4.92 (d, *J* = 7.2 Hz, 1H, 1-H), 3.79 (d, *J* = 8.9 Hz, 1H, 5-H), 3.52 (qdd, *J* = 10.4, 8.0, 3.4 Hz, 3H, 2-H, 3-H, 4-H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 168.9, 157.1, 153.6, 150.7, 148.3, 139.4, 136.8, 134.0, 131.0, 130.1, 129.9, 129.7, 128.9, 128.5, 127.2, 126.8, 126.4, 125.9, 124.3, 121.5, 119.5, 116.4, 115.7, 110.0, 100.9, 75.9, 75.4, 73.2, 71.5, 69.8, 63.5, 48.0, 47.9, 47.8, 47.6, 47.5, 47.3, 47.2, 44.3. HRMS: m/z calculated $[M - H]^-$ = 428.1351, found 428.1357.

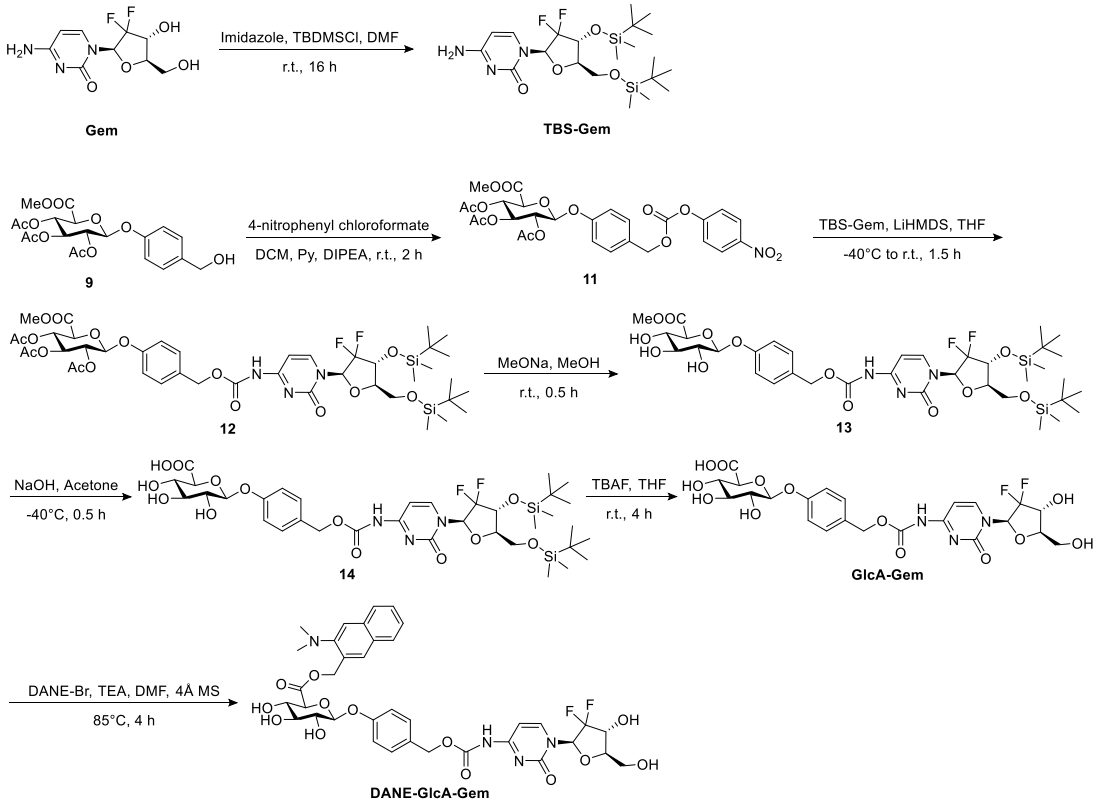
[2-(Dimethylamino)-3-naphthalenemethyl] (4-((quinolin-8-yloxy)methyl)phenyl) β-D-glucopyranosiduronate, DANE-GlcA-HQ



DANE (140.70 mg, 0.70 mmol, 1.5 eq.) was dissolved in anhydrous DCM (5 mL). Phosphorus tribromide (PBr_3, 64 µL, mmol, 0.7 eq.) was added dropwise at 0 °C. The mixture was warmed to room temperature and stirred under argon atmosphere for 1 h. When TLC showed the completion of the reaction, the reaction mixture was extracted with DCM(3 × 10 mL) and washed with saturated NaCl solution (10 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford a yellow oil (crude bromide). Then the crude bromide (0.70 mmol, 1.5 eq.), GlcA-HQ (200 mg , 0.46 mmol, 1.0 eq.), Et_3N (194 µL, 1.40 mmol, 2.0 eq.) and 4Å MS were dissolved in anhydrous DMF (5 mL). The reaction mixture was stirred at 85 °C under argon for 4 h. When TLC showed the completion of the reaction, the mixture was filtered and concentrated under reduced pressure. the residue was purified with silica gel chromatography using DCM and MeOH (20:1, v/v) to afford DANE-GlcA-pNP as a yellow solid (73 mg, 0.12 mmol, yield 26%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.76 (dd, *J* = 4.3, 1.7 Hz, 1H, Ar-H (HQ)), 8.28 (dd, *J* = 8.4, 1.7 Hz, 1H, Ar-H (HQ)), 7.84 (s, 1H, Ar-H (DANE)), 7.71 (d, *J* = 8.2 Hz, 1H, Ar-H (DANE)), 7.56 (d, *J* = 8.1 Hz, 1H, Ar-H (DANE)), 7.52 (dd, *J* = 8.3, 4.3 Hz, 1H, Ar-H (HQ)), 7.46 – 7.39 (m, 5H, Ar-H, Ar-H (HQ)), 7.34 (ddd, *J* = 8.2, 6.8, 1.3 Hz, 1H, Ar-H (DANE)), 7.24 (ddd, *J* = 8.1, 6.8, 1.3 Hz, 1H, Ar-H (DANE)), 7.17 (dd, *J* = 6.8, 2.2 Hz, 1H, Ar-H (HQ)), 7.12 – 7.07 (m, 2H, Ar-H), 5.53 – 5.43 (m, 2H, CH_2), 5.27 (s, 2H, CH_2), 5.06 (d, J = 7.6 Hz, 1H, 1-H), 4.16 (d, *J* = 9.8 Hz, 1H, 5-H), 3.80 – 3.73 (m, 1H, 3-H), 3.58 – 3.53 (m, 2H, 2-H, 4-H), 2.72 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (151 MHz, Methanol-d_4) δ 168.9, 157.1, 153.6, 150.7, 148.3, 139.4, 136.8, 134.0, 131.0, 130.1, 129.9, 129.7, 128.9, 128.5, 127.2, 126.8, 126.4, 125.9, 124.3, 121.5, 119.5, 116.4, 115.7, 110.0, 100.9, 75.9, 75.4, 73.2, 71.5, 69.8, 63.5, 48.0, 47.9, 47.8, 47.6, 47.5, 47.3, 47.2, 44.3. HRMS: m/z calculated

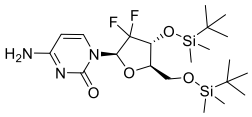
$[M + H]^+$ = 611.2388, found 611.2382.

3.3 Synthesis of DANE-GlcA-Gem



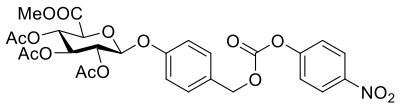
Scheme S5. The synthetic route to DANE-GlcA-Gem

5'-*O*-(tert-Butyldimethylsilyl)-2',2'-difluoro-3'-*O*-[(tert-butyldimethylsilyl)oxy]cytidine, TBS-Gem



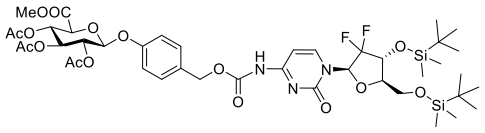
Gemcitabine (10 g, 38 mmol, 1.0 eq.), imidazole (7.75 g, 114 mmol, 3.0 eq.), and tert-butyldimethylchlorosilane (TBDMSCl, 28.64 g, 190 mmol, 5.0 eq.) were added to anhydrous DMF (50 mL). The mixture was stirred at room temperature for 30 h. When TLC showed the completion of the reaction, the solvent DMF was removed under reduced pressure. The residue was extracted with EA (3 × 35 mL). The combined organic extracts were washed with saturated $NaHCO_3$ (30 mL) and saturated NaCl (30 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford TBS-Gem as a white solid (17.75 g, 36.1 mmol, yield 95%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.66 (d, *J* = 7.5 Hz, 1H), 6.31 (dd, *J* = 10.7, 4.3 Hz, 1H), 5.74 (d, *J* = 7.4 Hz, 1H), 4.29 (td, *J* = 11.6, 7.9 Hz, 1H), 3.98 (dt, *J* = 11.9, 2.3 Hz, 1H), 3.88 (d, *J* = 7.7 Hz, 1H), 3.78 (dd, *J* = 11.8, 2.1 Hz, 1H), 0.91 (d, *J* = 13.1 Hz, 18H, TBDMS-H), 0.11 (t, *J* = 4.2 Hz, 12H, TBDMS-H).

Methyl 2,3,4-tri-*O*-acetyl-1-(4-(((((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)-β-D-glucopyranosiduronate, 11



Compound **9** (1.19 g, 2.7 mmol, 1.0 eq.), p-nitrophenyl chloroformate (2 g, 10 mmol, 3.7 eq.), *N,N*-diisopropylethylamine (DIPEA, 1 mL), and pyridine (50 μL) were added to anhydrous DCM (15 mL). The mixture was stirred at room temperature for 2 h. When TLC showed the completion of the reaction, the mixture was extracted with DCM (3 × 25 mL). The combined organic extracts were washed with saturated $NaHCO_3$ solution (20 mL) and saturated NaCl solution (20 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using PE and EA (10:1, v/v) to afford compound **11** as a white solid (1.21 g, 2 mmol, yield 75%), Then the compound **11** is immediately used in the next step of the reaction. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.29 – 8.24 (m, 2H, Ar-H), 7.41 – 7.34 (m, 4H, Ar-H), 7.04 – 7.00 (m, 2H, Ar-H), 5.38 – 5.31 (m, 2H, 2-H, 4-H), 5.28 (t, *J* = 7.8 Hz, 1H, 3-H), 5.23 (s, 2H, CH_2), 5.17 (d, *J* = 7.4 Hz, 1H, 1-H), 4.19 (d, *J* = 8.5 Hz, 1H, 5-H), 3.73 (d, *J* = 1.4 Hz, 3H, $COOCH_3$), 2.07 – 2.04 (m, 9H, CH_3 (Ac)). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 170.1, 169.4, 169.2, 166.8, 157.1, 155.5, 152.4, 145.4, 130.6, 129.3, 125.3, 121.8, 117.2, 98.8, 77.2, 77.0, 76.8, 72.6, 71.7, 71.0, 70.4, 69.0, 53.1, 20.6, 20.5. MS-ESI (*m/z*): 606.1 $[M + H]^+$.

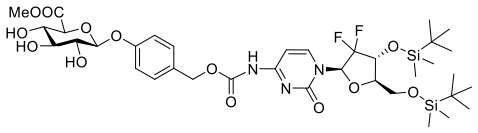
Methyl 2,3,4-tri-*O*-acetyl-1-(4-([[[(5'-*O*-(tert-butyldimethylsilyl)-2',2'-difluoro-3'-*O*-[(tert-butyldimethylsilyl)oxy]cytidin-4-yl)amino]carbonyl]oxy]methyl)phenyl)-β-D-glucopyranosiduronate, 12



Compound **11** (812 mg, 1.65 mmol, 1.0 eq.) was dissolved in anhydrous THF (10 mL). A solution of lithium bis(trimethylsilyl)amide (LiHMDS, 1.0 M in THF, 3.3 mL, 3.3 mmol, 2.0 eq.) was added dropwise at -40 °C. The mixture was stirred at this temperature for 0.5 h. Separately, TBS-Gem (1 g, 1.65 mmol, 1.0 eq.) was dissolved in anhydrous THF (10 mL). This solution was added to the reaction mixture at -40 °C. After stirring for 10 min, the reaction was warmed to room temperature and stirred for 2 h. When TLC showed the completion of the reaction, the mixture was extracted with DCM (3 × 25 mL). The combined organic extracts were washed with saturated $NaHCO_3$ solution (20 mL) and saturated NaCl solution (20 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using PE/EA (10:1, v/v) to afford compound **12** as a white solid (758 mg, 0.79 mmol, yield 48%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.05 (d, *J* = 7.6 Hz, 1H, Gem-H), 7.33 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.22 (d, *J* = 7.6 Hz, 1H, Gem-H), 7.00 (d, *J* = 8.5 Hz, 2H, Ar-H), 6.33 (dd, *J* = 10.1, 3.9 Hz, 1H, Gem-H), 5.38 – 5.23 (m, 3H, 2-H, 3-H, 4-H), 5.16 (d, *J* = 2.2 Hz, 3H, CH_2, 1-H), 4.33 (td, *J* = 11.6, 8.0 Hz, 1H, Gem-H), 4.19 (d, *J* = 9.54 Hz, 1H, 5-H), 4.01 (d,

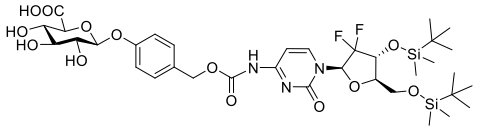
J = 12.0 Hz, 1H, Gem-H), 3.95 (d, *J* = 8.2 Hz, 1H, Gem-H), 3.80 (dd, *J* = 11.9, 2.0 Hz, 1H, Gem-H), 3.73 (s, 3H, $COOCH_3$), 2.08 – 2.02 (m, 9H, CH_3 (Ac)), 0.95 (s, 9H, TBDMS-H), 0.90 (s, 9H, TBDMS-H), 0.15 – 0.07 (m, 12H, TBDMS-H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 170.2, 169.4, 169.3, 166.8, 162.5, 156.9, 154.6, 152.1, 144.0, 144.0, 130.2, 130.0, 121.9, 117.2, 98.9, 95.0, 84.6, 81.5, 81.4, 77.3, 77.1, 76.8, 72.6, 71.8, 71.0, 69.1, 67.6, 60.0, 60.0, 53.1, 29.7, 25.9, 25.5, 20.7, 20.7, 20.6, 18.3, 18.0, -4.7, -5.3, -5.4, -5.4. HRMS: m/z calculated $[M + H]^+$ = 958.3631, found 958.3642.

Methyl 1-(4-((((5'-*O*-(tert-butyldimethylsilyl)-2',2'-difluoro-3'-*O*-[(tert-butyldimethylsilyl)oxy]cytidin-4-yl)amino)carbonyl)oxy)methyl)phenyl)-β-D-glucopyranosiduronate, 13



Compound **12** (356 mg, 0.37 mmol, 1.0 eq.) was dissolved in anhydrous MeOH (10 mL). A solution of sodium methoxide (5 M in MeOH, 75 µL, 0.37 mmol, 1.0 eq.) was added dropwise at 0 °C under an argon atmosphere. The mixture was stirred at this temperature for 0.5 h. Upon completion of the reaction, the pH was adjusted to slightly acidic using a cation-exchange resin. The crude mixture was purified by silica gel column chromatography (PE/EA = 1:1, v/v) to afford compound **13** as a white solid (249 mg, 0.30 mmol, yield 81%). ^1H NMR (400 MHz, Methanol-d_4) δ 8.18 (d, *J* = 7.7 Hz, 1H, Gem-H), 7.37 (dd, *J* = 16.6, 8.1 Hz, 3H, Gem-H, Ar-H), 7.13 – 7.06 (m, 2H, Ar-H), 6.32 – 6.24 (m, 1H, Gem-H), 5.19 (s, 2H, CH_2), 5.03 (d, *J* = 7.6 Hz, 1H, 1-H), 4.44 (td, *J* = 12.0, 8.3 Hz, 1H, Gem-H), 4.15 – 4.01 (m, 3H, Gem-H, 5-H), 3.90 (dd, *J* = 12.1, 2.2 Hz, 1H, Gem-H), 3.79 (s, 3H, $COOCH_3$), 3.64 (tt, *J* = 10.0, 6.7 Hz, 1H, 3-H), 3.57 – 3.47 (m, 2H, 2-H, 4-H), 0.98 (d, *J* = 19.0 Hz, 18H, TBDMS-H), 0.18 (dd, *J* = 8.7, 1.8 Hz, 12H, TBDMS-H).

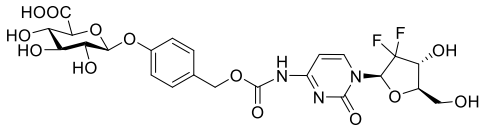
1-(4-((((5'-*O*-(tert-Butyldimethylsilyl)-2',2'-difluoro-3'-*O*-[(tert-butyldimethylsilyl)oxy]cytidin-4-yl)amino)carbonyl)oxy)methyl)phenyl) β-D-glucopyranosiduronic acid, 14



Compound **13** (200 mg, 0.24 mmol, 1.0 eq.) was dissolved in acetone (8 mL). A 1 M aqueous sodium hydroxide solution (700 µL, 0.7 mmol, 3.0 eq) was added dropwise at -40 °C, and the reaction was stirred for 0.5 h. Upon completion of the reaction, a hydrogen ion exchange resin was added to adjust the pH to weakly acidic. The mixture was then filtered, and the filtrate was concentrated under reduced pressure to afford the crude product compound **14** as a white solid (139 mg, 0.17 mmol, yield 74%). ^1H NMR (400 MHz, Methanol-d_4) δ 8.16 (d, *J* = 7.7 Hz, 1H, Gem-H), 7.34 (dd, *J* = 14.0, 7.9 Hz, 3H, Gem-H, Ar-H), 7.12 (d, *J* = 8.1 Hz, 2H, Ar-H), 6.33 – 6.22 (m, 1H, Gem-H), 5.17 (s, 2H, CH_2), 4.94 (m, 1H, 1-H), 4.44 (td, *J* = 11.9, 8.3 Hz, 1H, Gem-H), 4.11 (d, *J* = 12.1 Hz, 1H,

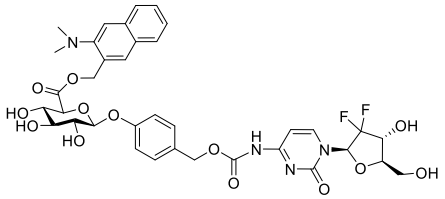
Gem-H), 4.03 (d, J = 8.3 Hz, 1H, 5-H), 3.90 (dd, *J* = 12.2, 2.3 Hz, 1H, Gem-H), 3.82 (m, 1H, Gem-H), 3.54 (m, 3H, 2-H, 3-H, 4-H), 1.00 (s, 9H, TBDMS-H), 0.95 (s, 9H, TBDMS-H), 0.17 (dd, *J* = 9.3, 2.1 Hz, 12H, TBDMS-H). HRMS: m/z calculated $[M - H]^-$ = 818.3169, found 818.3157.

1-(4-((((2',2'-Difluorocytidin-4-yl)amino)carbonyl)oxy)methyl)phenyl) β-D-glucopyranosiduronic acid, GlcA-Gem



Compound **14** (114 mg, 0.14 mmol, 1.0 eq.) was dissolved in anhydrous THF (5 mL). A solution of tetrabutylammonium fluoride (TBAF, 1 M in THF, 280 μL, 0.28 mmol, 2.0 eq.) was added dropwise to the reaction mixture at room temperature under an argon atmosphere. After stirring for 4 h, the reaction was quenched with saturated NH_4Cl solution (5 mL) and extracted with EA (3 × 10 mL). The aqueous phase was collected, concentrated under reduced pressure, and the residue was dissolved in MeOH. The resulting suspension was filtered, and the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (EA/MeOH = 5:1, v/v) to afford GlcA-Gem as a white solid (44 mg, 0.075 mmol, yield 53.5%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.33 (d, *J* = 7.6 Hz, 1H, Gem-H), 7.37 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.31 (d, *J* = 7.7 Hz, 1H, Gem-H), 7.15 – 7.09 (m, 2H, Ar-H), 6.30 – 6.22 (m, 1H, Gem-H), 5.18 (s, 2H, CH_2), 4.96 (m, 1H, 1-H), 4.37 – 4.26 (m, 1H, Gem-H), 4.03 – 3.95 (m, 2H, 5-H, Gem-H), 3.86 – 3.82 (m, 1H, Gem-H), 3.82 – 3.75 (m, 1H, Gem-H), 3.57 – 3.48 (m, 3H, 2-H, 3-H, 4-H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 174.4, 164.0, 158.0, 157.2, 156.0, 144.2, 129.7, 128.0, 122.6, 116.5, 101.2, 100.9, 95.7, 81.4, 76.3, 75.2, 73.3, 72.1, 67.1, 63.4, 58.1, 58.0, 58.0. HRMS: m/z calculated $[M - H]^-$ = 590.1439, found 590.1448.

[2-((3-(Dimethylamino)-2-naphthalenyl)methyl)] 1-(4-((((2',2'-difluorocytidin-4-yl)amino)carbonyl)oxy)methyl) phenyl)-β-D-glucopyranosiduronate, DANE-GlcA-Gem

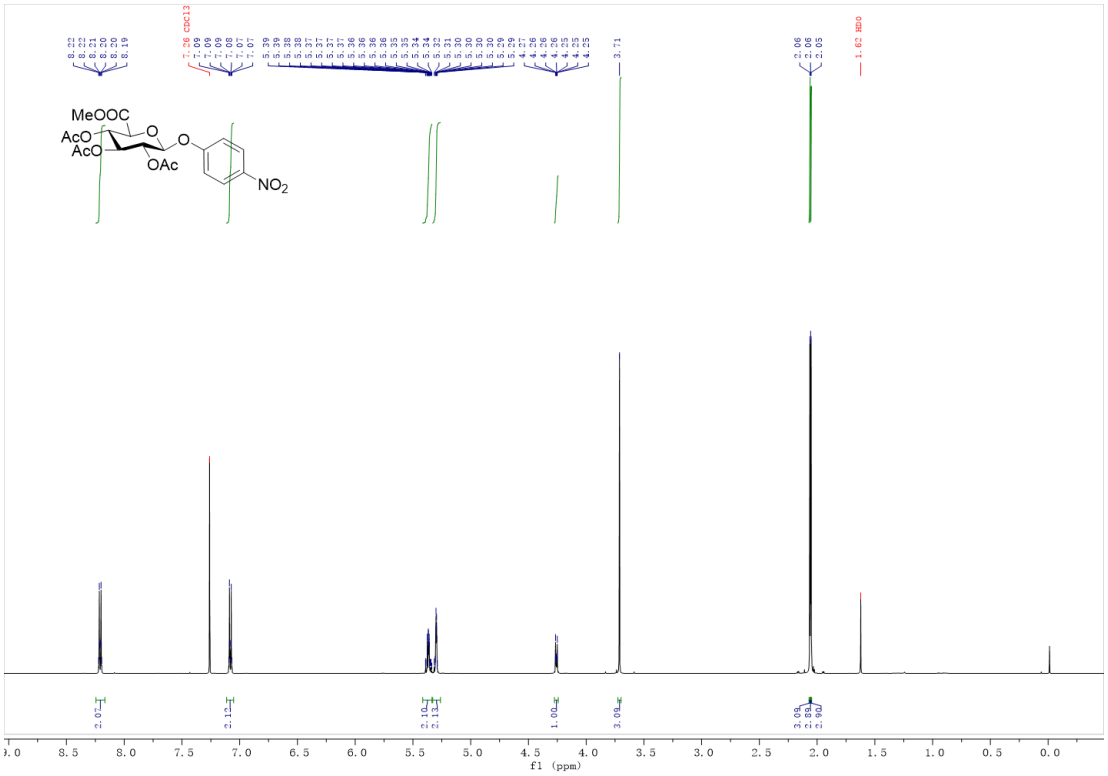


DANE (40 mg, 0.2 mmol, 1.66 eq.) was dissolved in anhydrous DCM (3 mL). Phosphorus tribromide (PBr_3, 10 μL, 0.1 mmol, 1 eq.) was added dropwise at 0 °C. The mixture was warmed to room temperature and stirred under argon atmosphere for 1 h. When TLC showed the completion of the reaction, the reaction mixture was extracted with DCM (3 × 10 mL) and washed with saturated NaCl solution (5 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford a yellow oil (crude bromide). Then the crude bromide (0.2 mmol, 1.66 eq.), GlcA-Gem (75 mg, 0.12 mmol, 1.0 eq.), Et_3N (33 μL, 0.24 mmol, 2.0 eq.) and

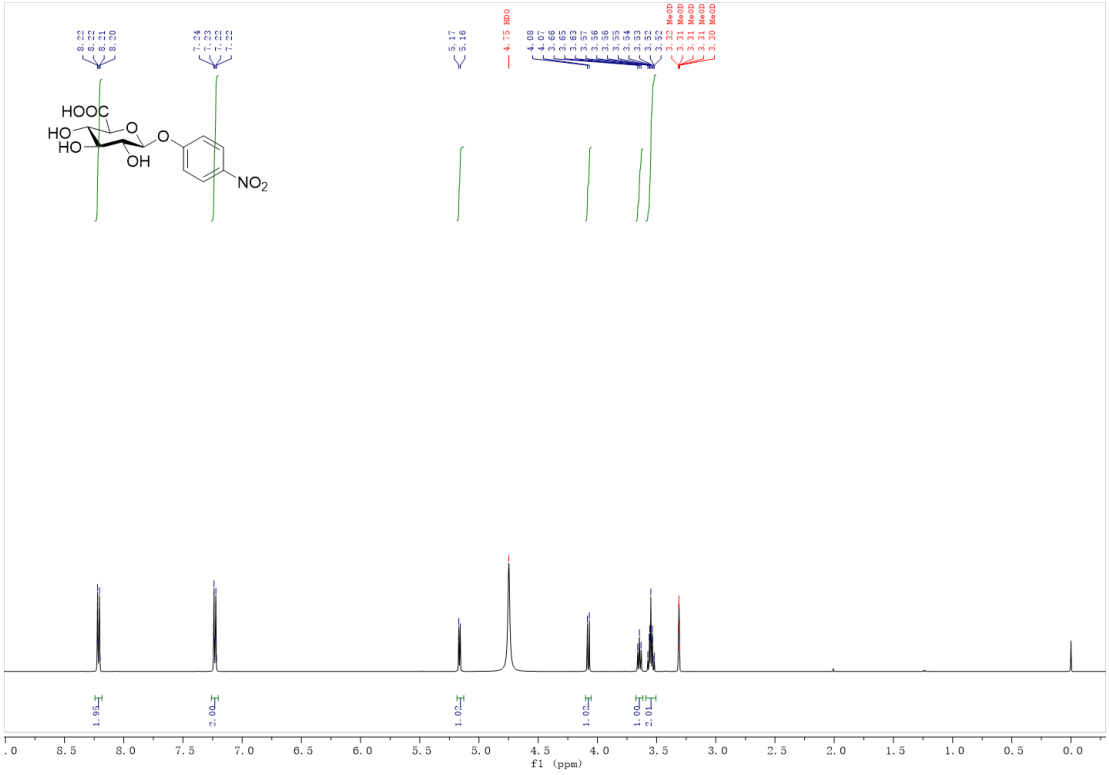
4Å MS were dissolved in anhydrous DMF (5 mL). The reaction mixture was stirred at 85 °C under argon for 4 h. When TLC showed the completion of the reaction, the mixture was filtered and concentrated under reduced pressure. the residue was purified with silica gel chromatography using DCM and MeOH (20:1, v/v) to afford DANE-GlcA-Gem as a yellow solid (19 mg, 0.025 mmol, yield 21%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.31 (d, *J* = 7.7 Hz, 1H, Gem-H), 7.84 (s, 1H, DANE-H), 7.74 (d, *J* = 7.8 Hz, 1H, DANE-H), 7.52 (d, *J* = 8.1 Hz, 1H, DANE-H), 7.49 (s, 1H, DANE-H), 7.42 – 7.38 (m, 1H, DANE-H), 7.37 – 7.33 (m, 2H, Ar-H), 7.30 (dd, *J* = 8.0, 1.6 Hz, 2H, DANE-H, Gem-H), 7.15 – 7.09 (m, 2H, Ar-H), 6.32 – 6.21 (m, 1H, Gem-H), 5.56 – 5.45 (m, 2H, $COOCH_2$), 5.16 (d, *J* = 2.2 Hz, 2H, Ph-CH_2), 5.10 (d, J = 7.6 Hz, 1H, 1-H), 4.32 (td, *J* = 12.2, 8.4 Hz, 1H, Gem-H), 4.19 (d, *J* = 9.8 Hz, 1H, 5-H), 3.99 (dp, *J* = 8.1, 2.5 Hz, 2H, Gem-H), 3.87 – 3.80 (m, 1H, 3-H), 3.80 – 3.73 (m, 1H, Gem-H), 3.59 – 3.54 (m, 2H, 2-H, 4-H), 2.76 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (101 MHz, Methanol-d_4) δ 168.9, 163.9, 157.4, 156.0, 152.9, 150.7, 144.3, 134.0, 130.1, 129.9, 129.8, 129.8, 129.7, 128.3, 127.2, 126.4, 125.9, 124.3, 120.0, 116.4, 116.4, 115.8, 100.6, 95.7, 84.7, 81.5, 75.8, 75.4, 73.2, 71.4, 69.0, 68.8, 68.6, 67.0, 63.5, 58.2, 58.2, 58.1, 48.5, 48.3, 48.1, 47.9, 44.4. HRMS: m/z calculated $[M + H]^+$ = 773.2476, found 773.2483.

4. NMR Spectra

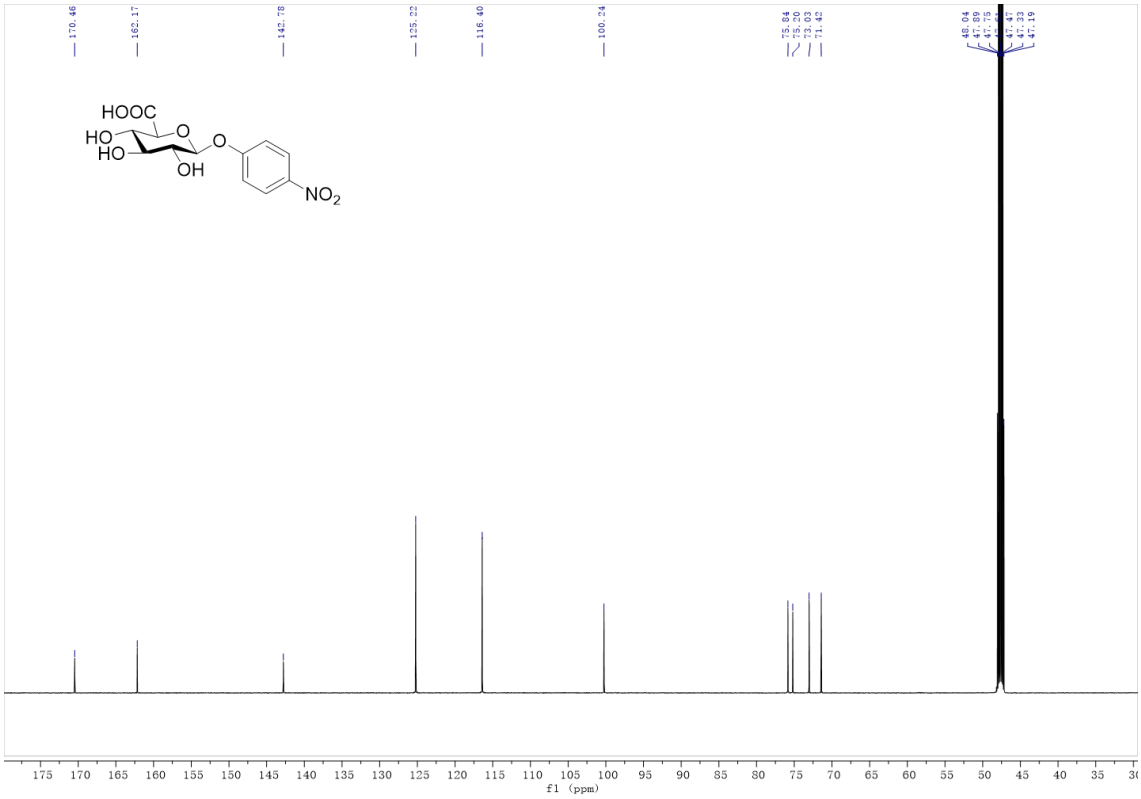
^{1}H NMR of compound **5**



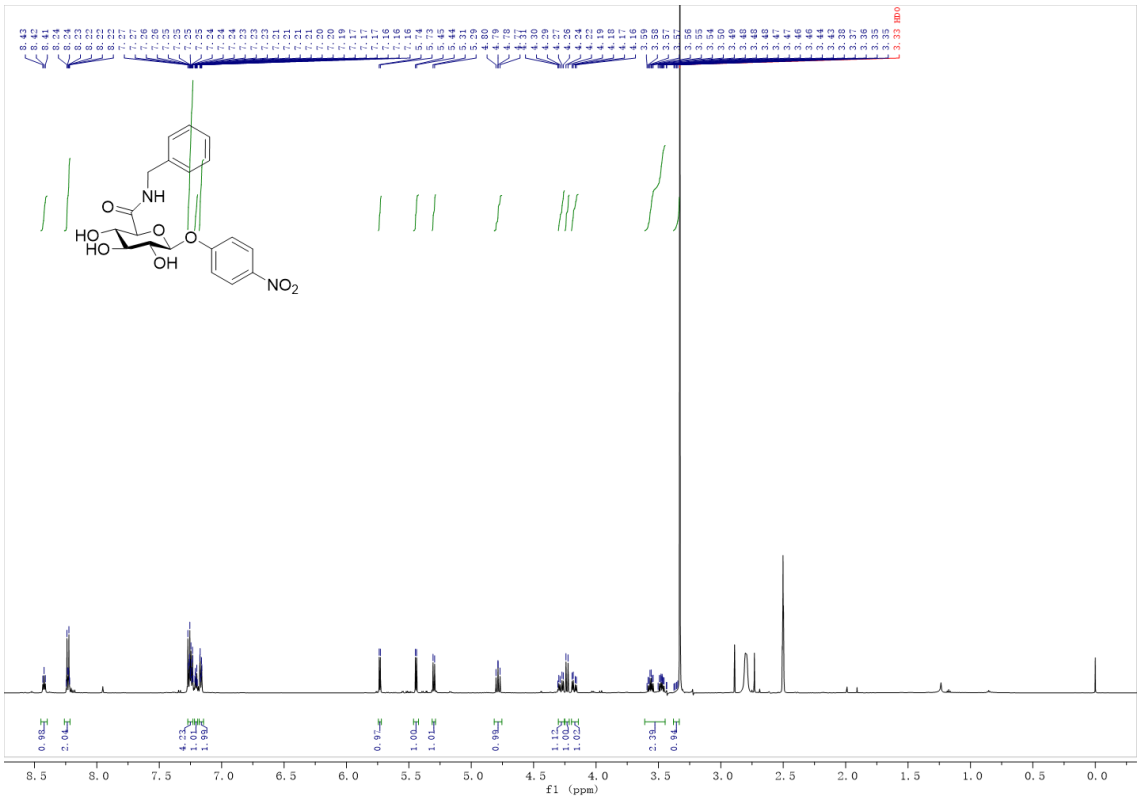
^{1}H NMR of compound GlcA-pNP



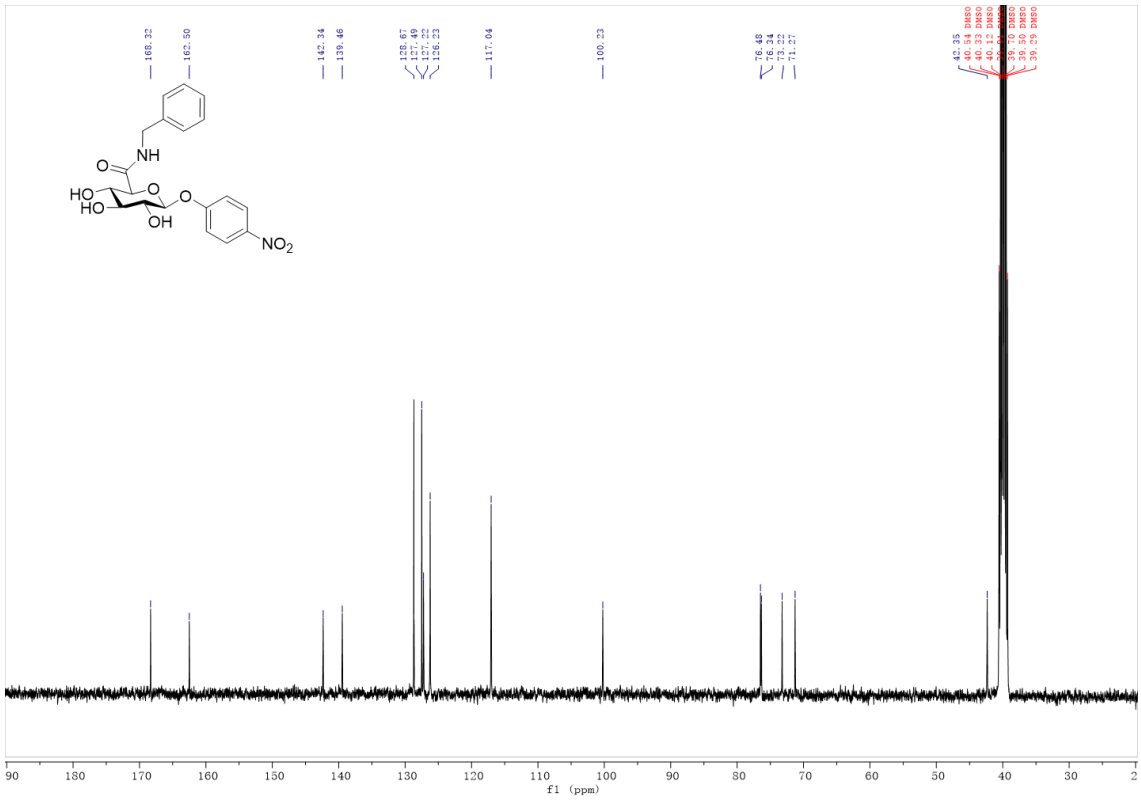
^{13}C NMR of compound GlcA-pNP



^{1}H NMR of compound Ba-GlcA-pNP



^{13}C NMR of compound Ba-GlcA-pNP



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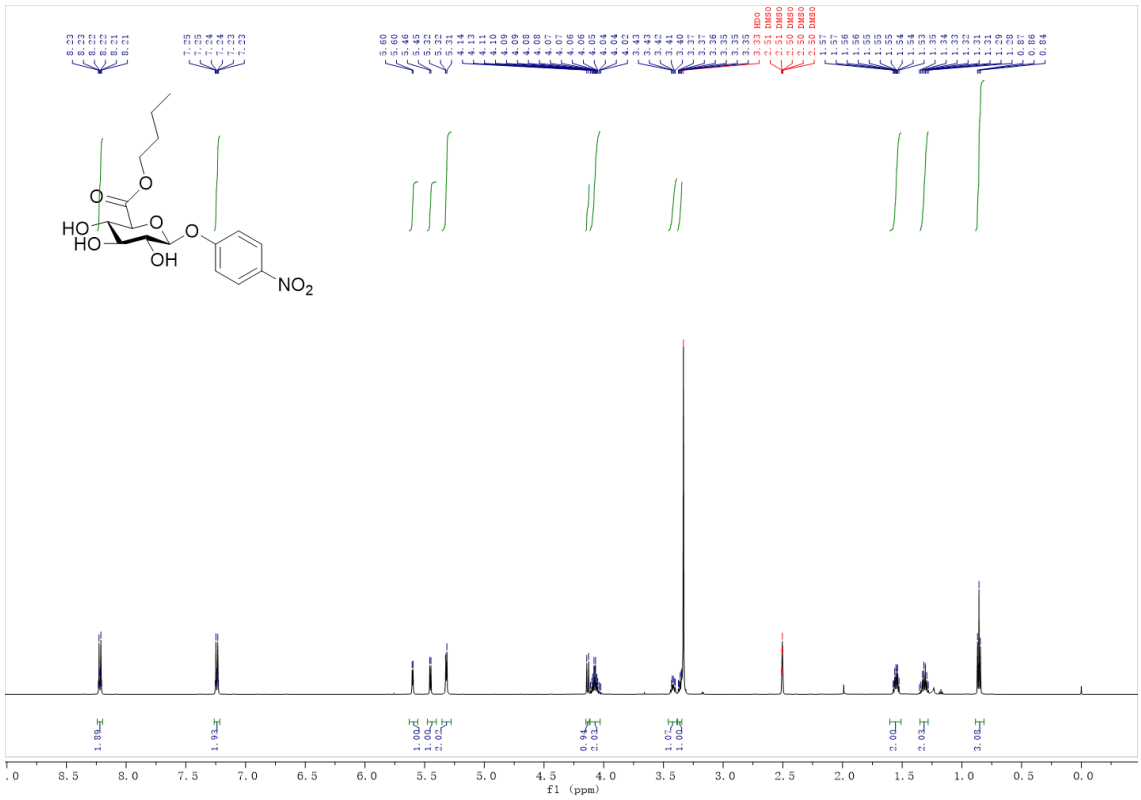
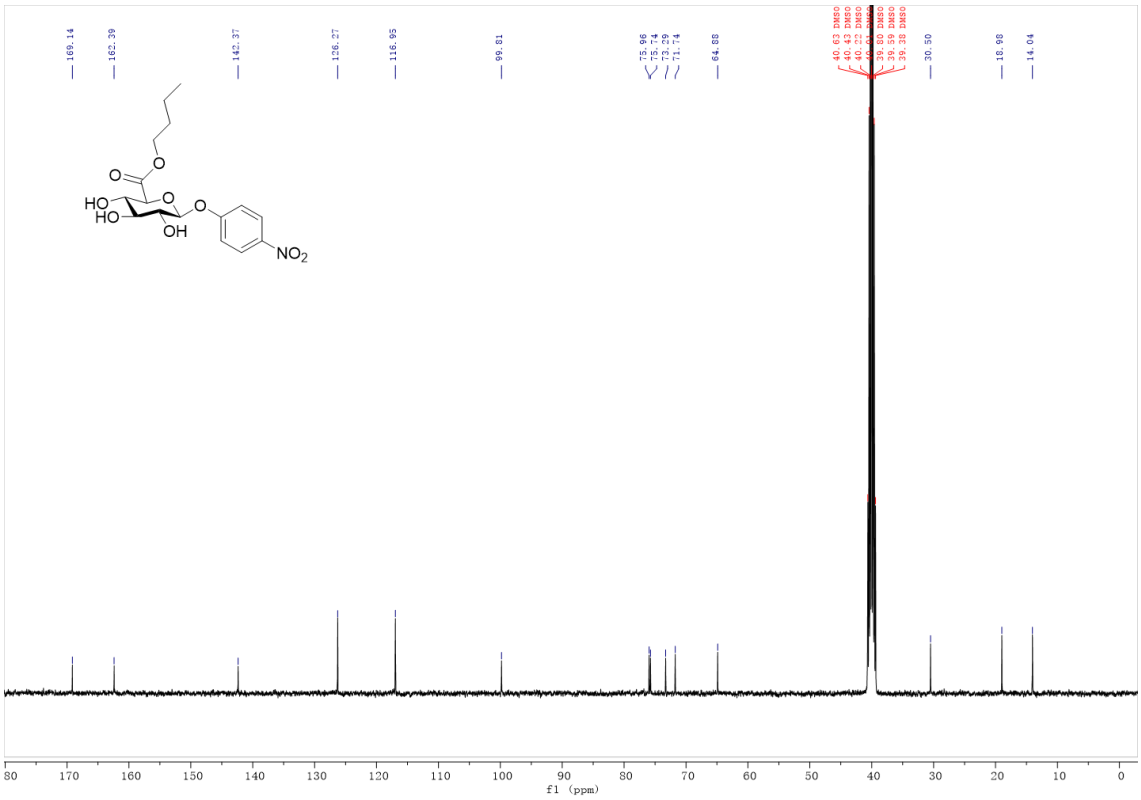
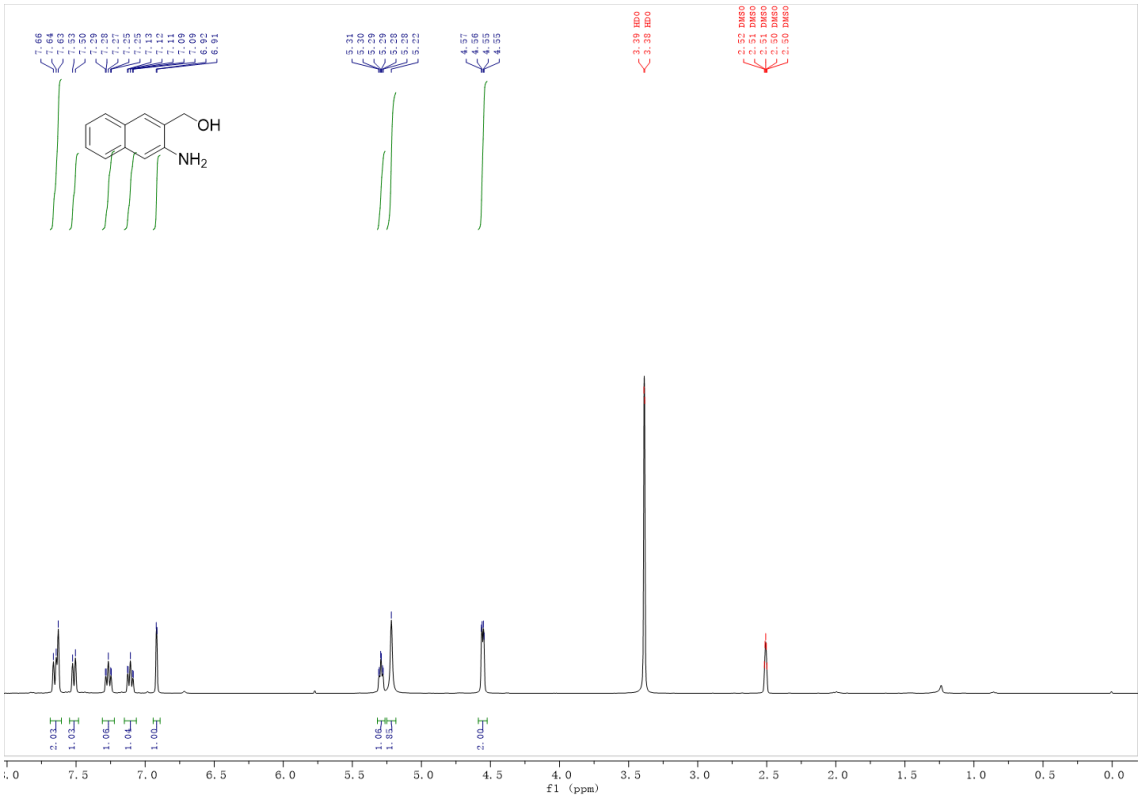


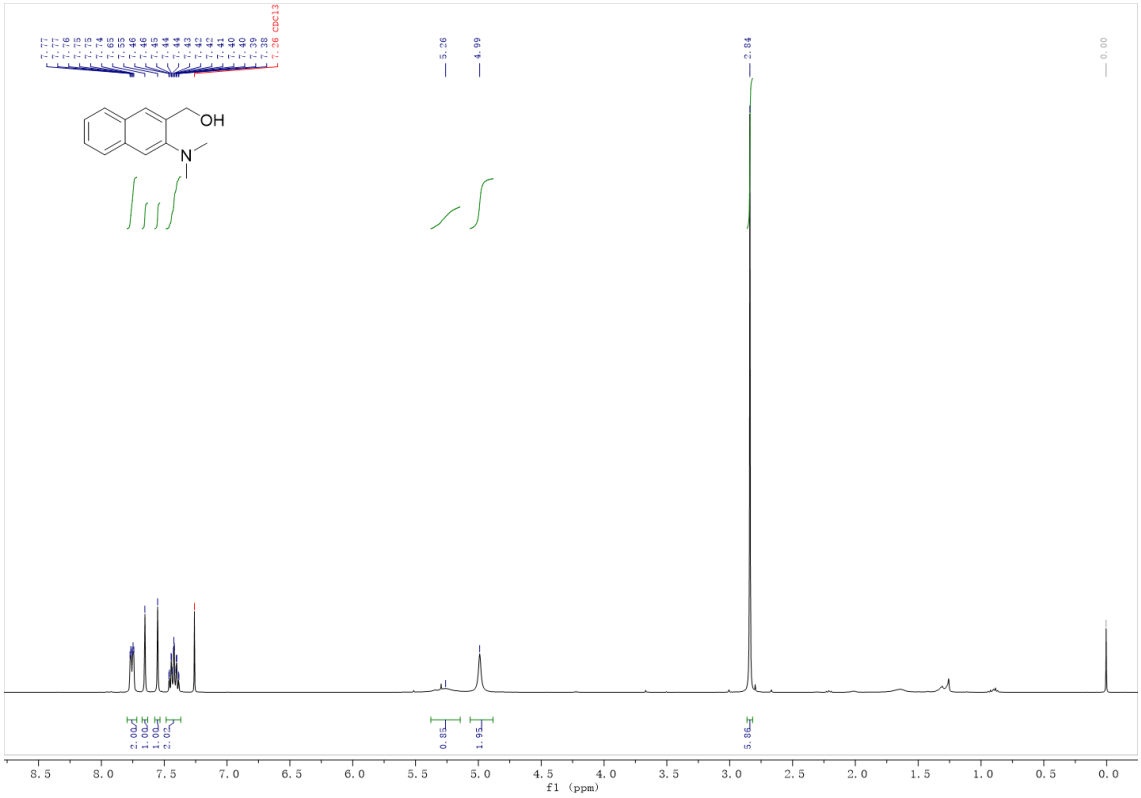
^{13}C NMR of compound Bu-GlcA-pNP



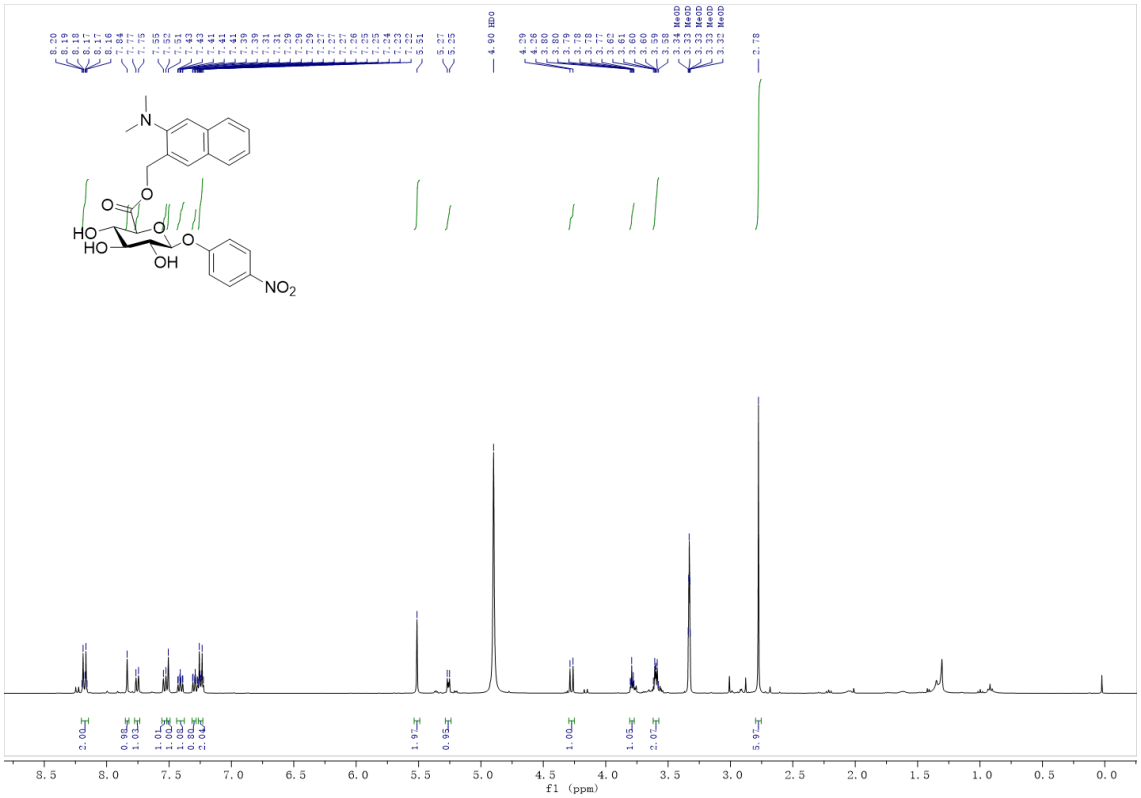
^{1}H NMR of compound **7**



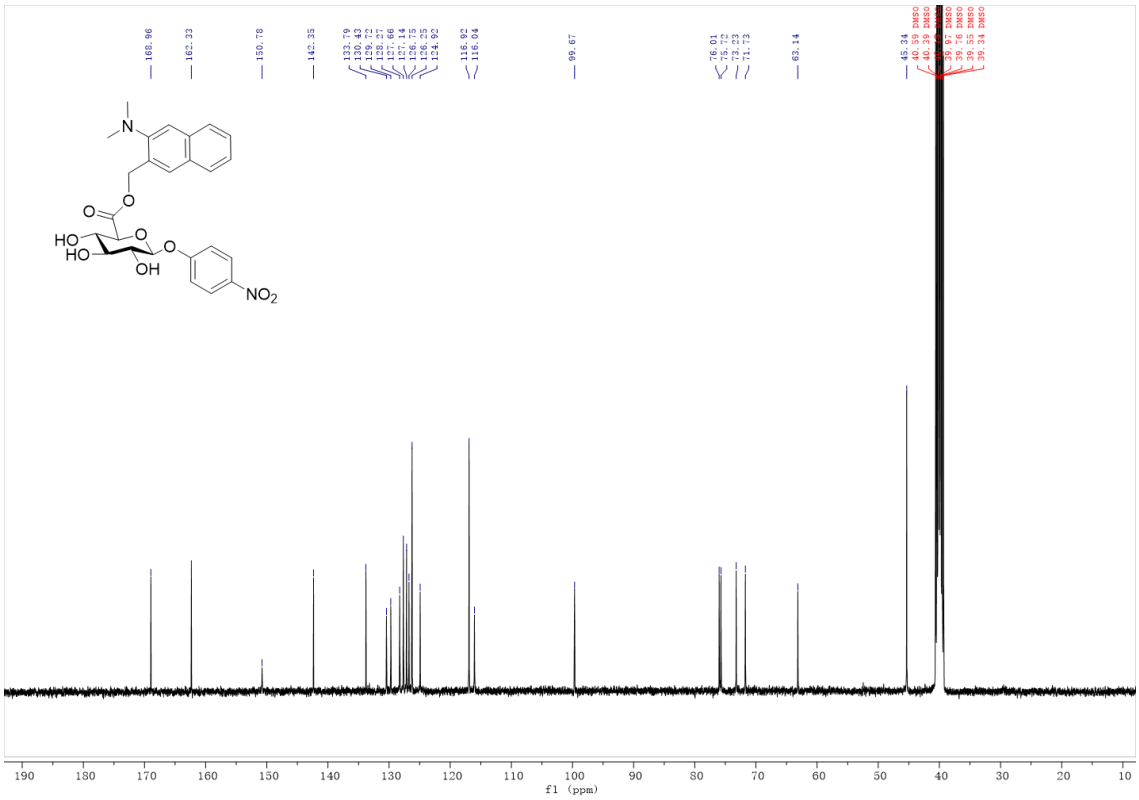
^{1}H NMR of compound DANE



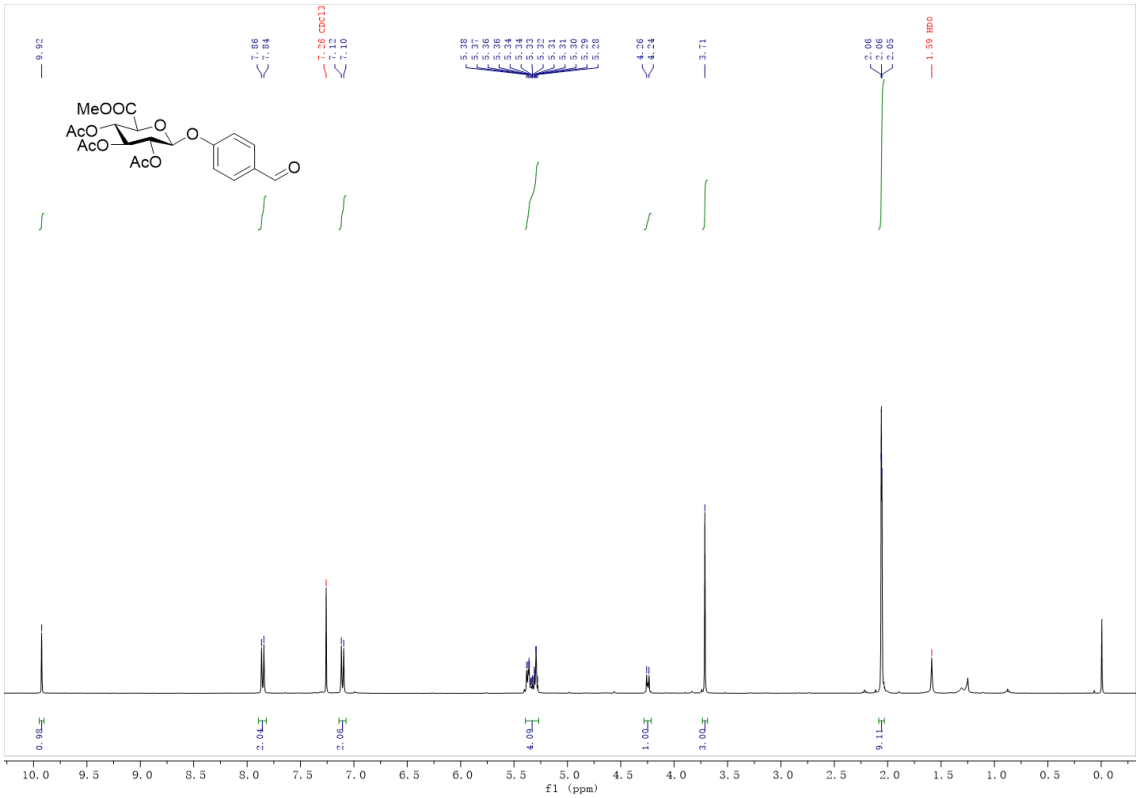
^{1}H NMR of compound DANE-GlcA-pNP



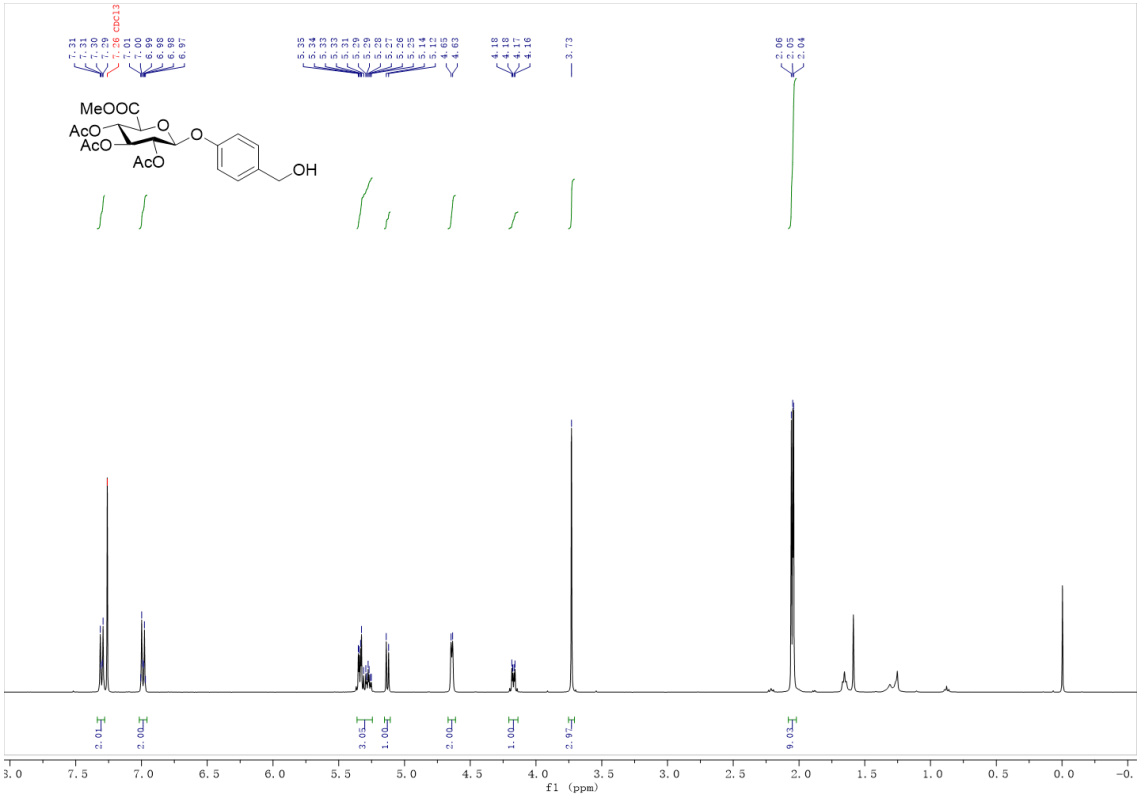
^{13}C NMR of compound DANE-GlcA-pNP



^{1}H NMR of compound **8**



^{1}H NMR of compound **9**



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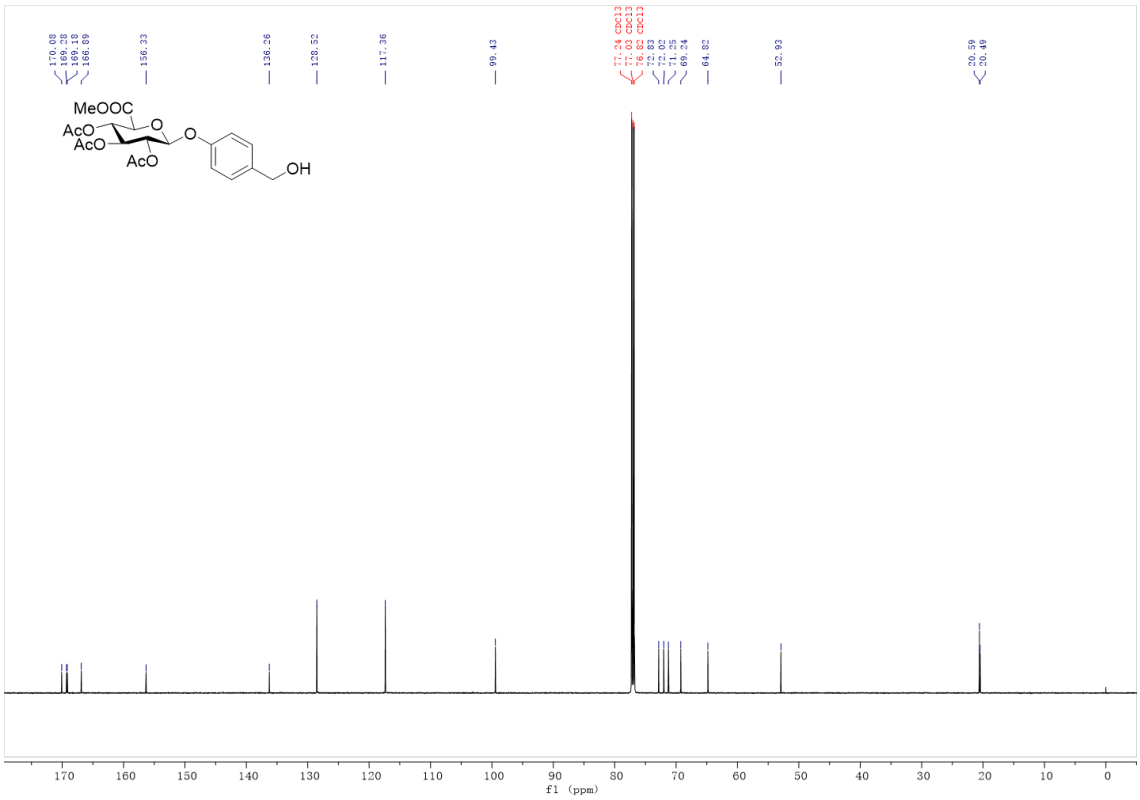
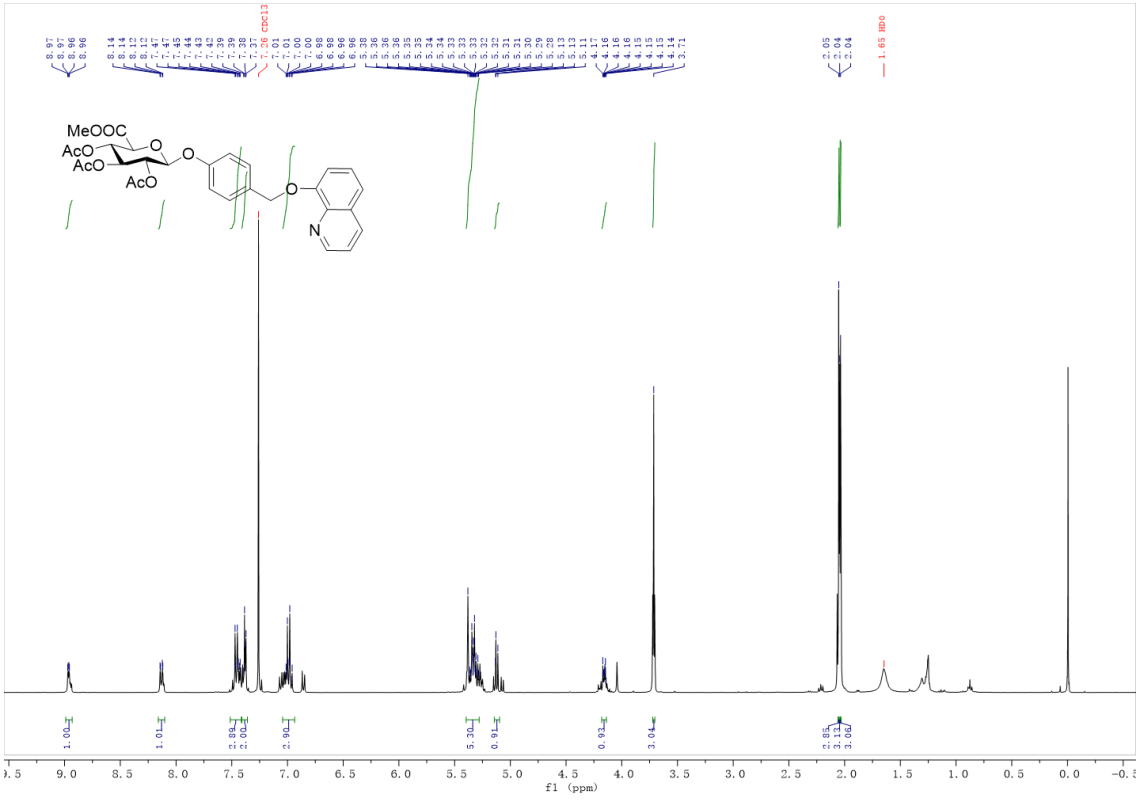
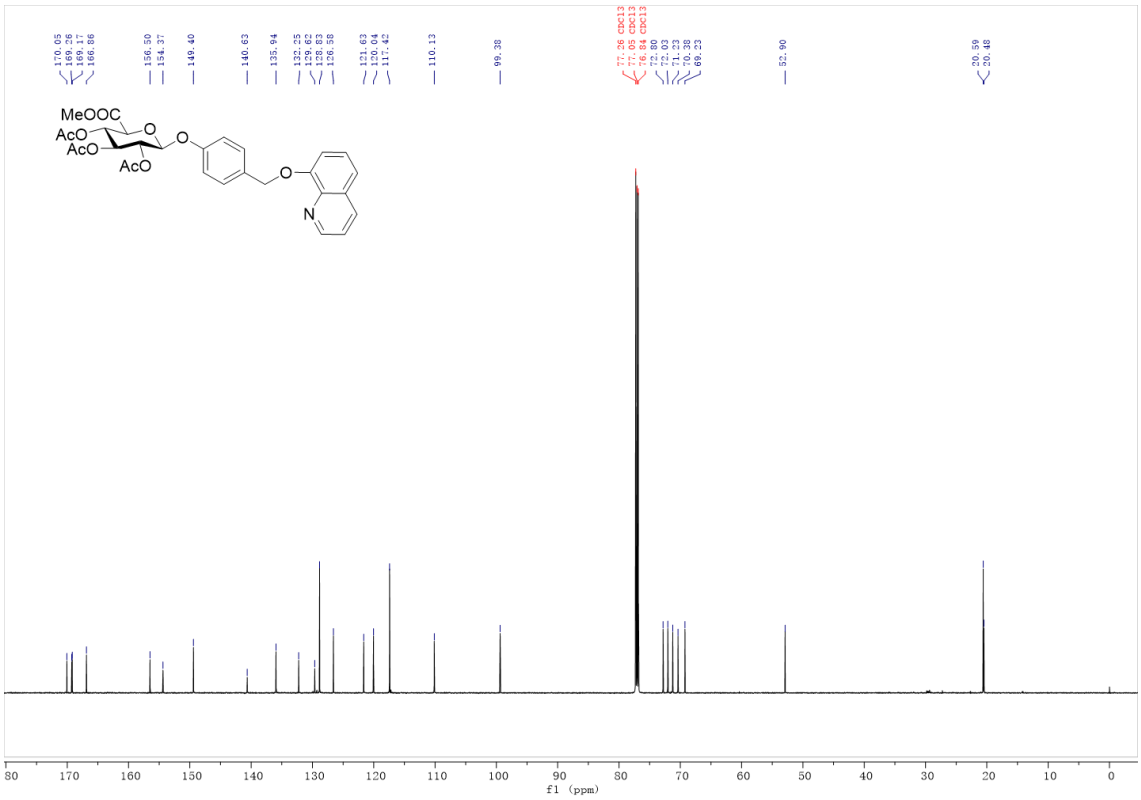


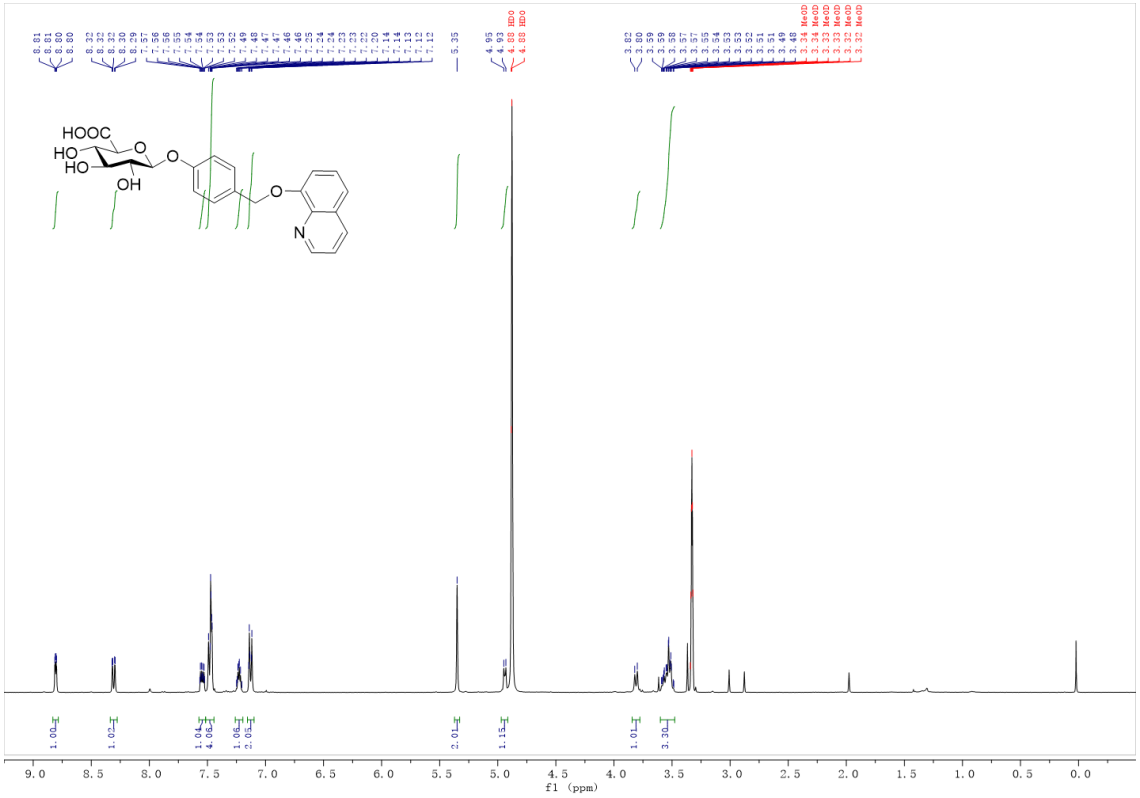
^{1}H NMR of compound **10**



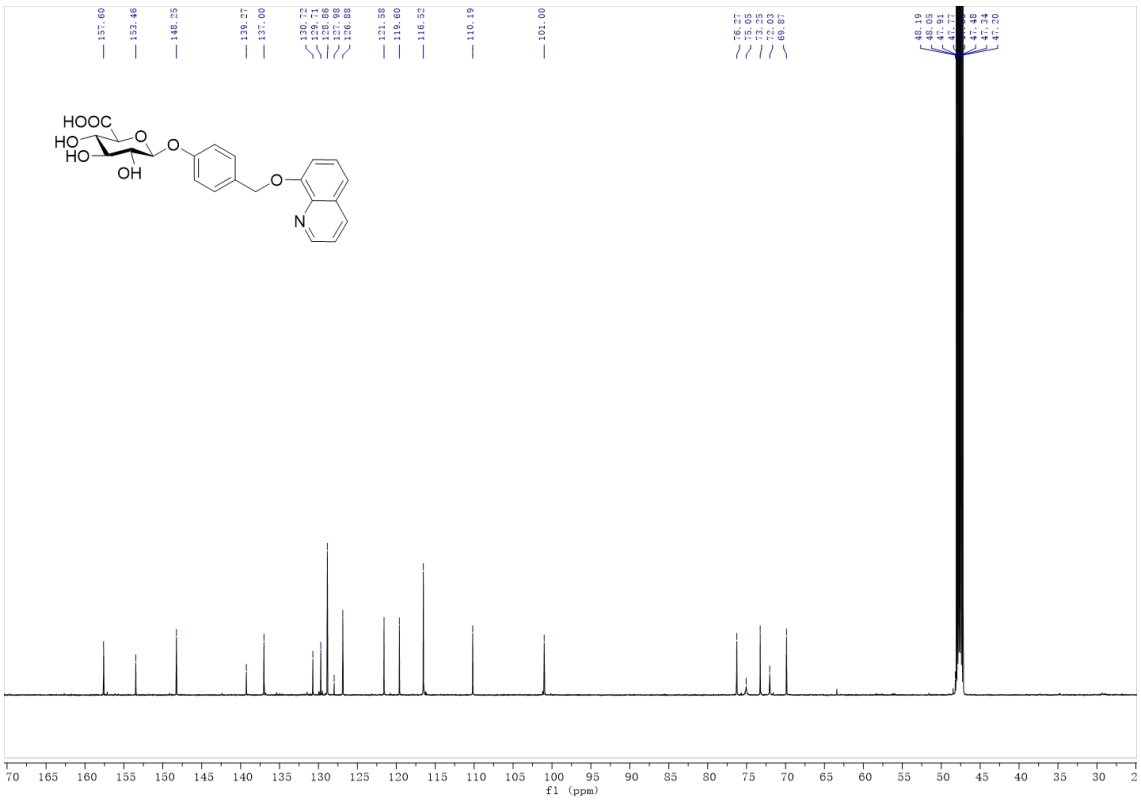
^{13}C NMR of compound **10**



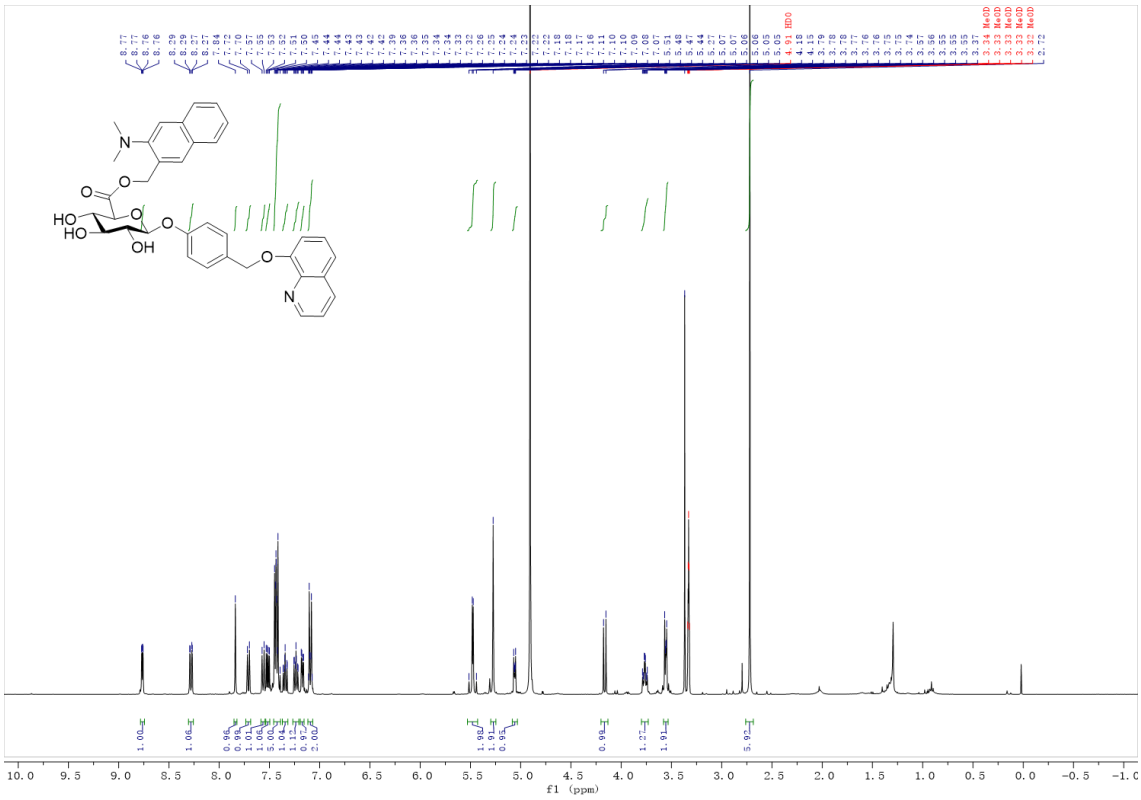
^{1}H NMR of compound GlcA-HQ



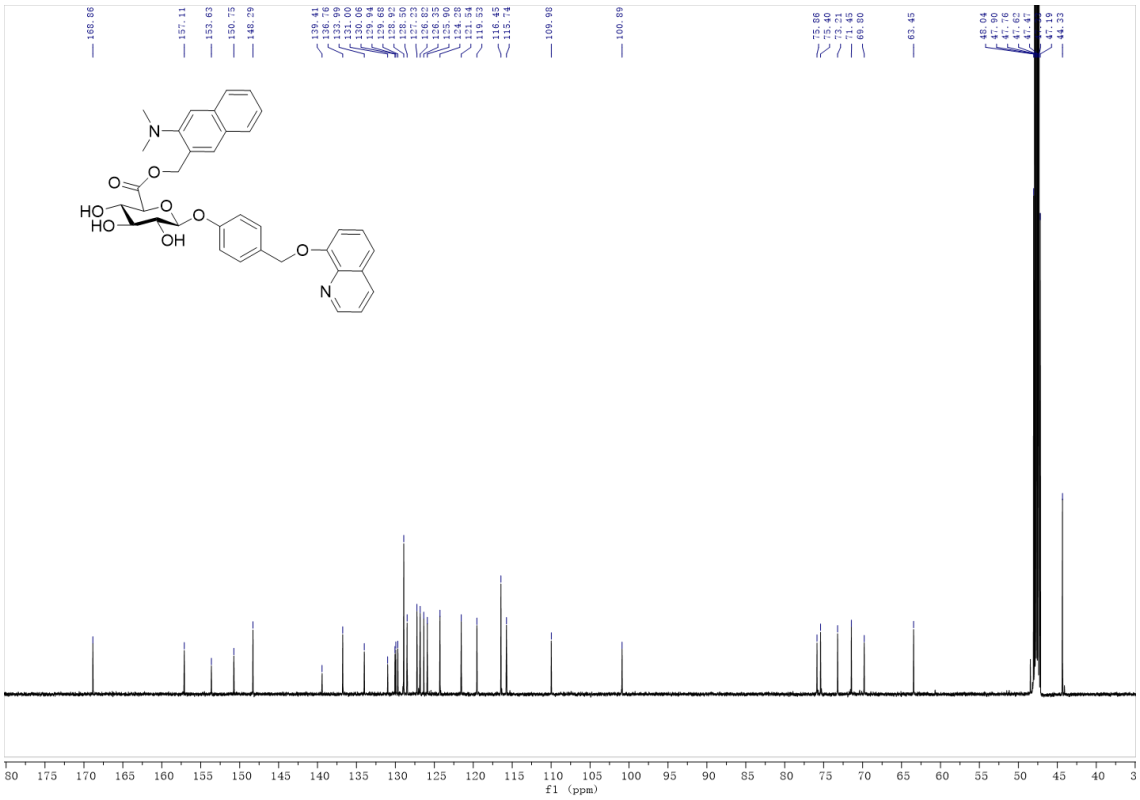
^{13}C NMR of compound GlcA-HQ



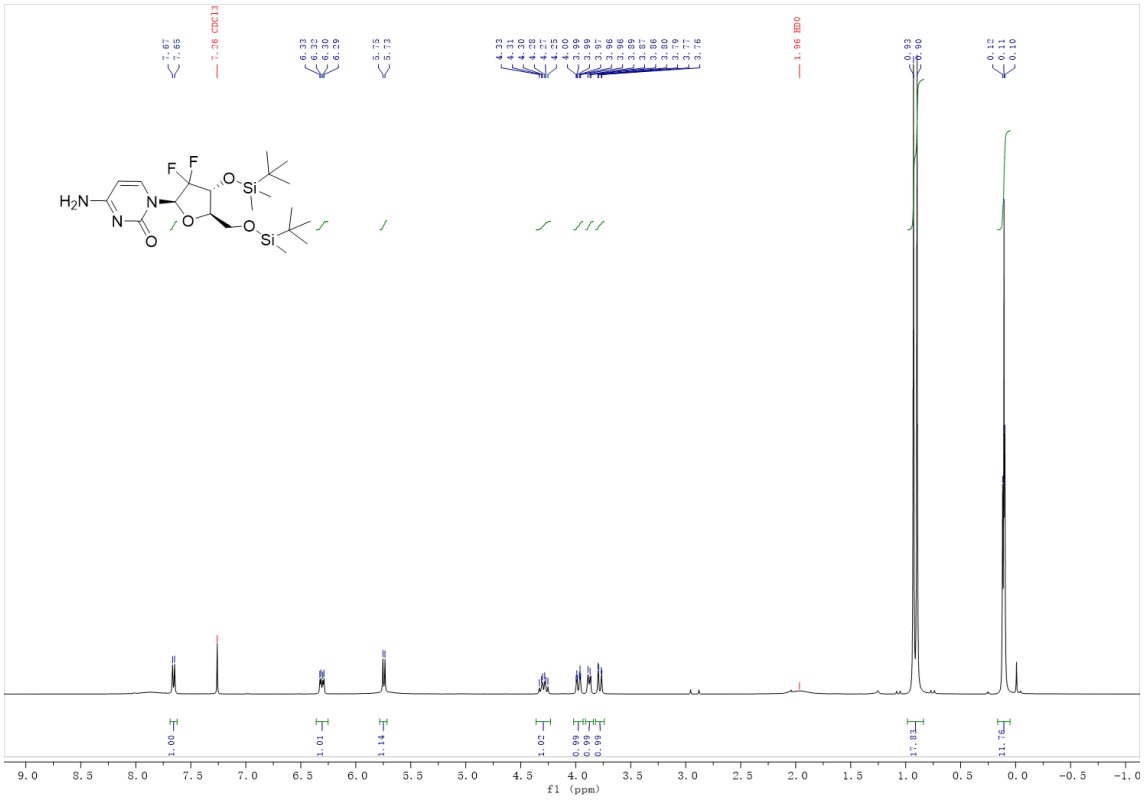
^{1}H NMR of compound DANE-GlcA-HQ



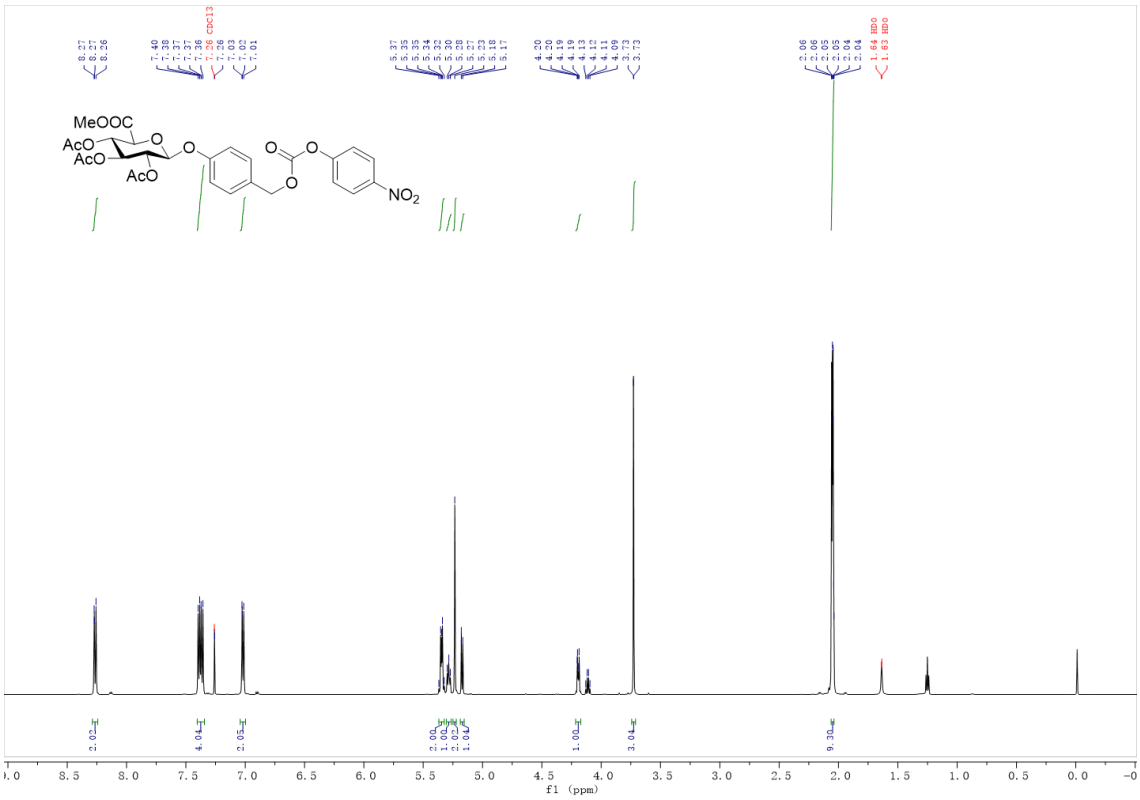
^{13}C NMR of compound DANE-GlcA-HQ



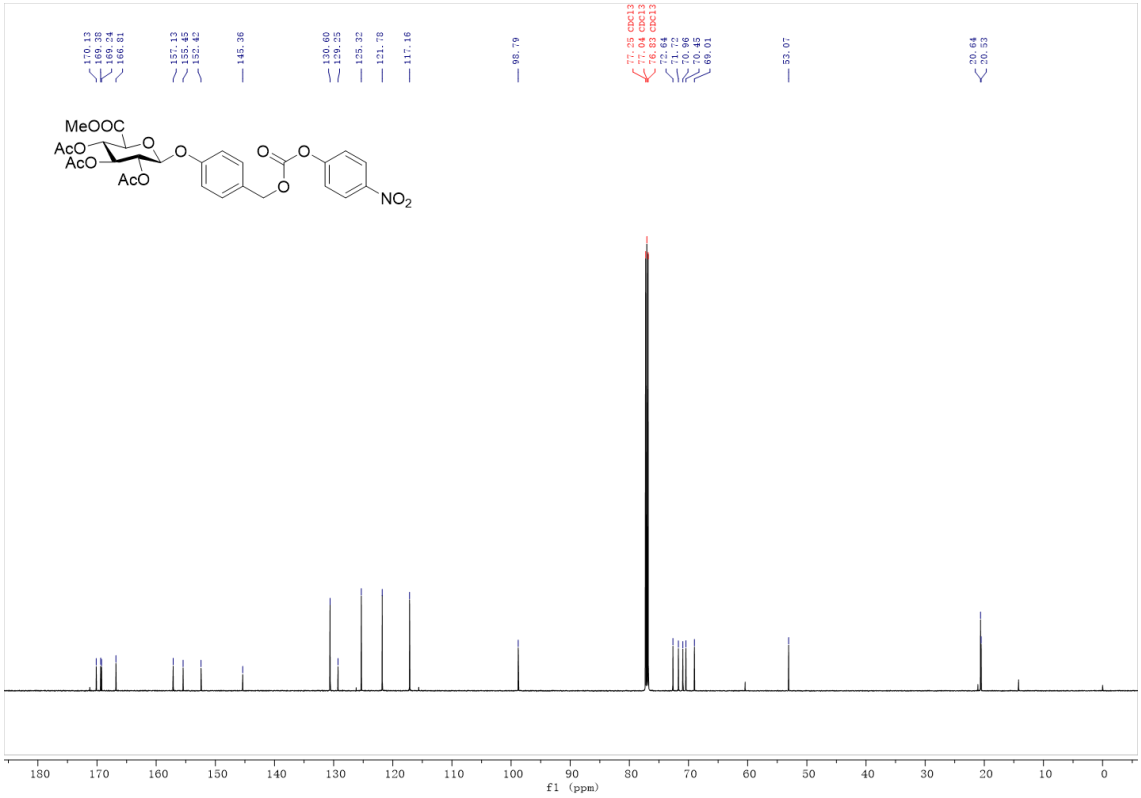
^{1}H NMR of compound TBS-Gem



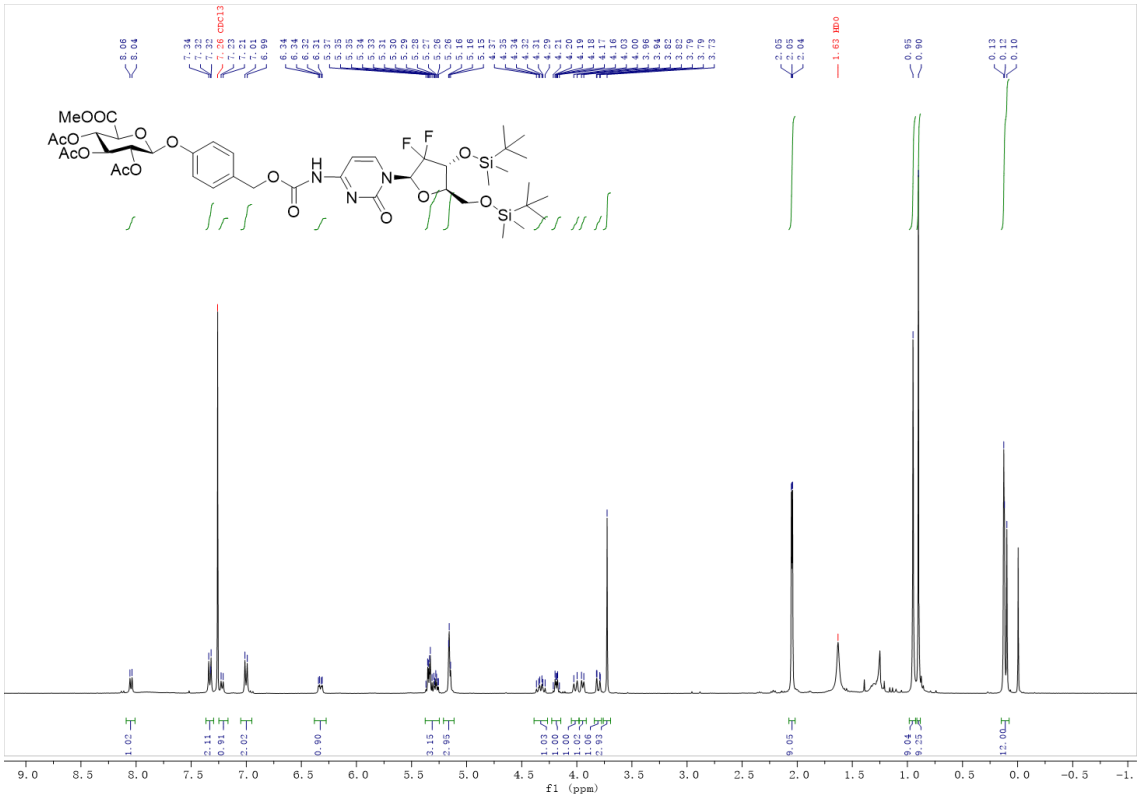
^{1}H NMR of compound **11**



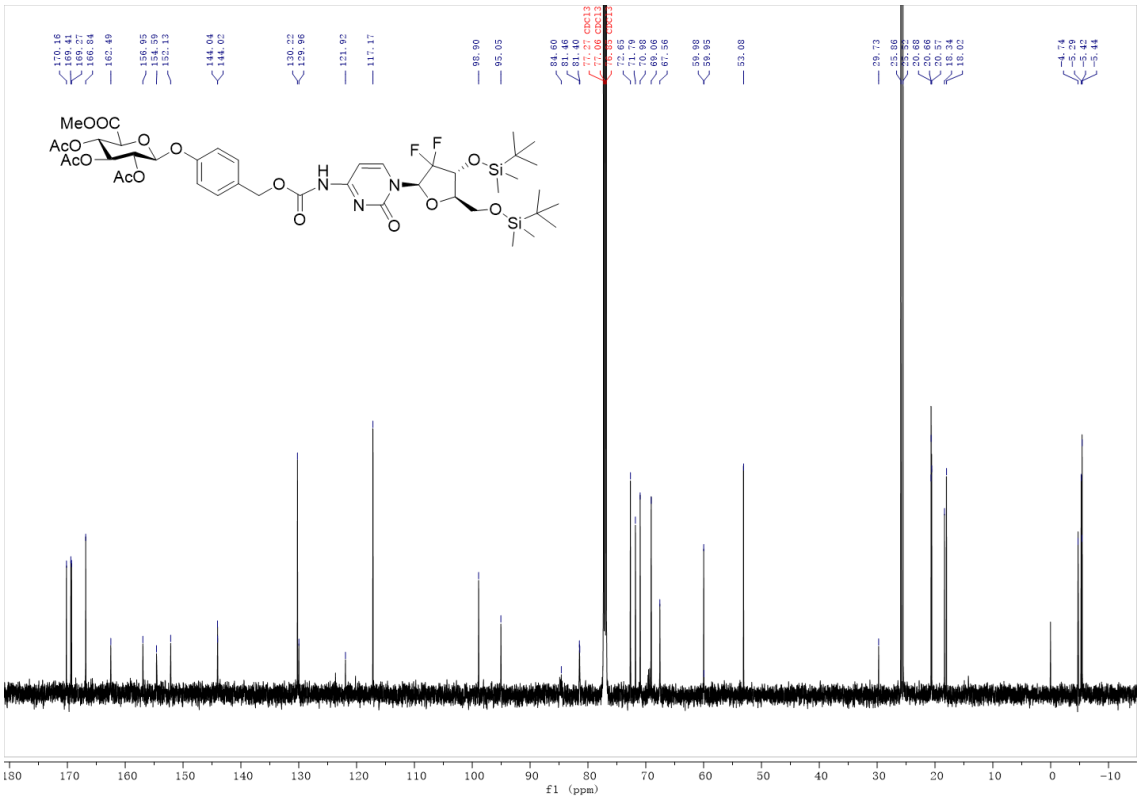
^{13}C NMR of compound **11**



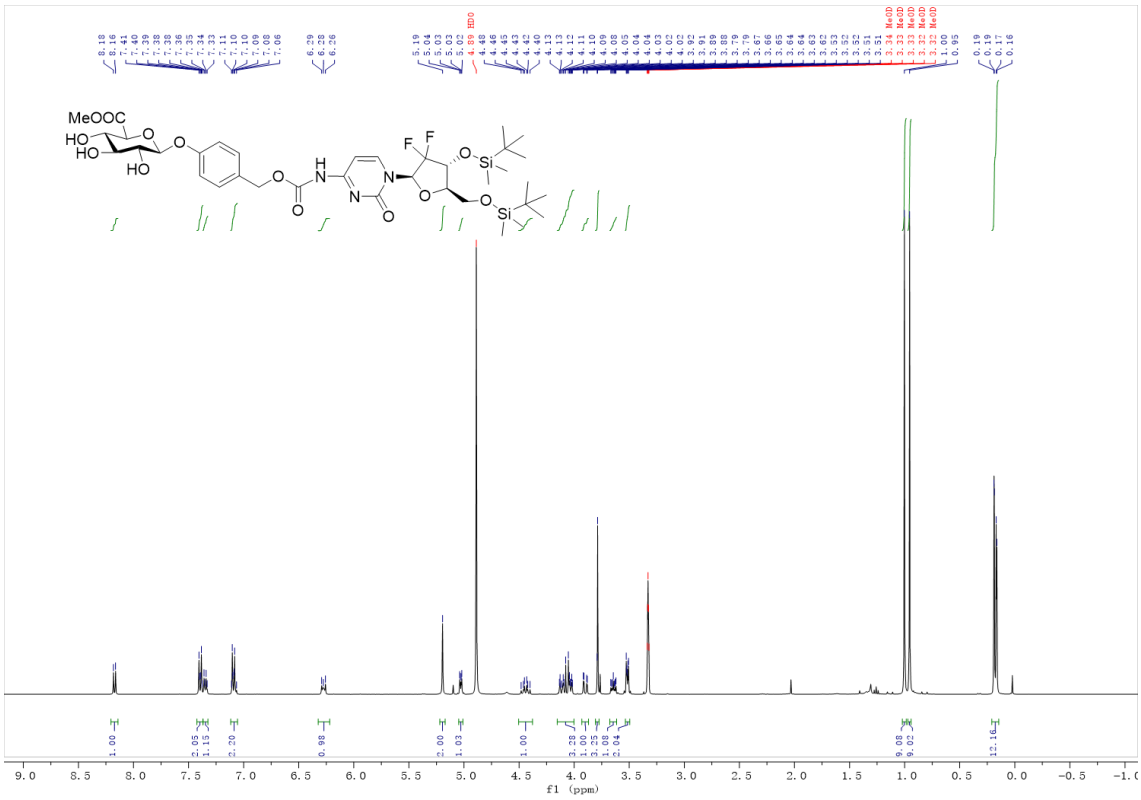
^{1}H NMR of compound **12**



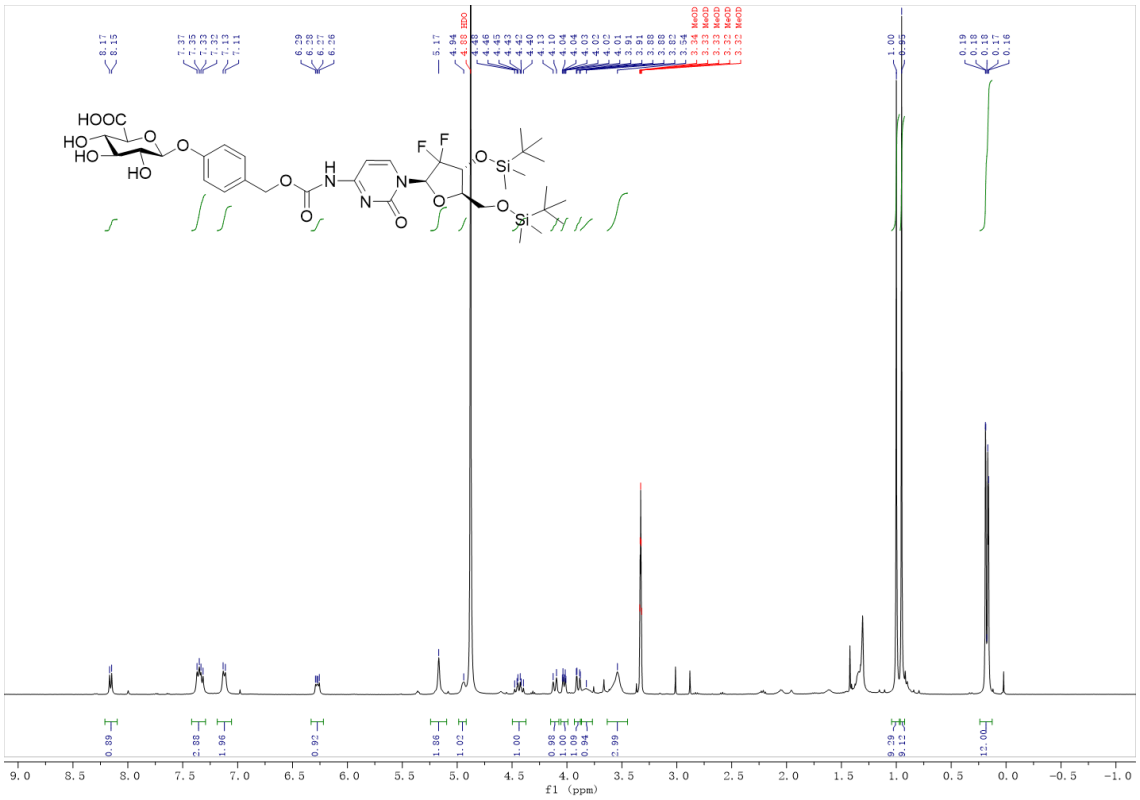
^{13}C NMR of compound **12**



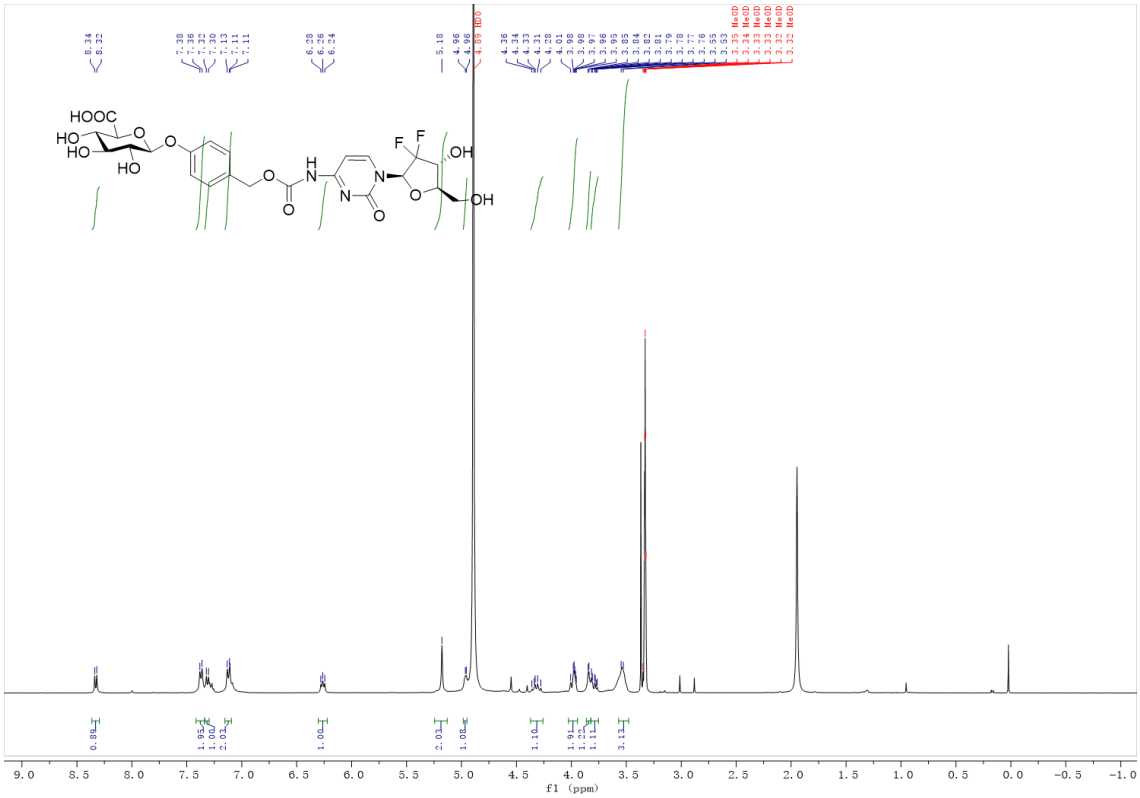
^{1}H NMR of compound **13**



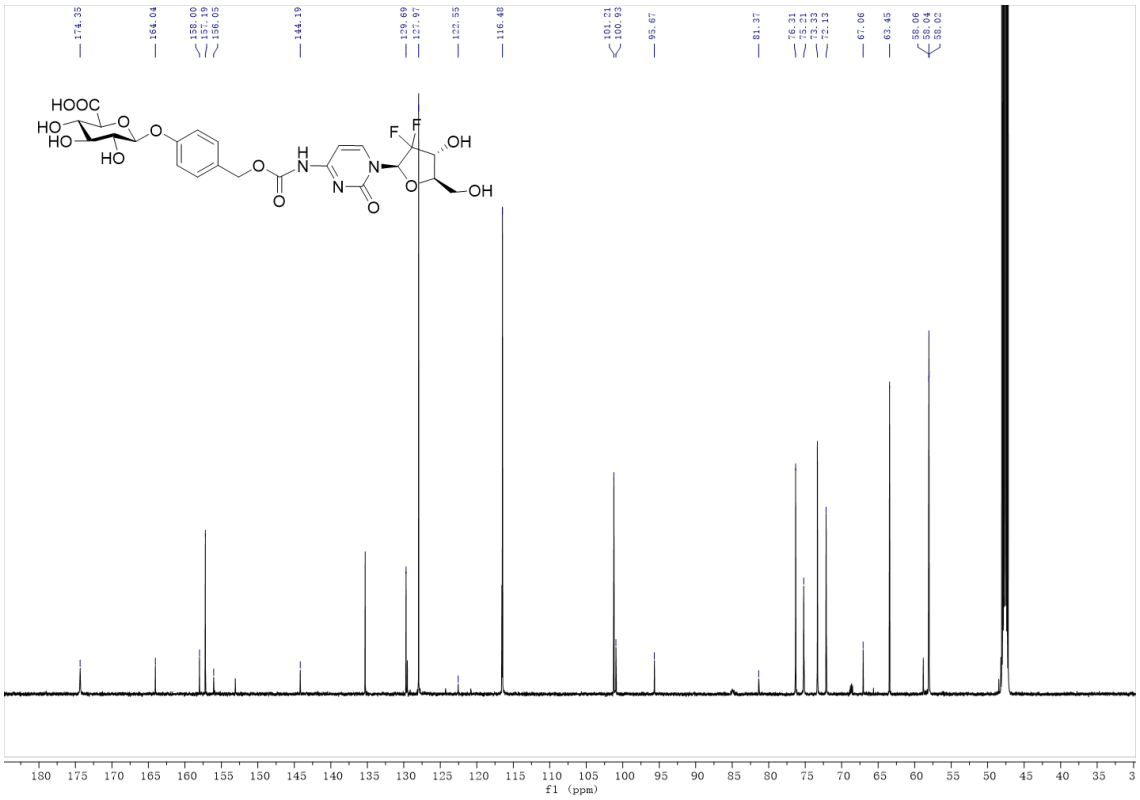
^{1}H NMR of compound **14**



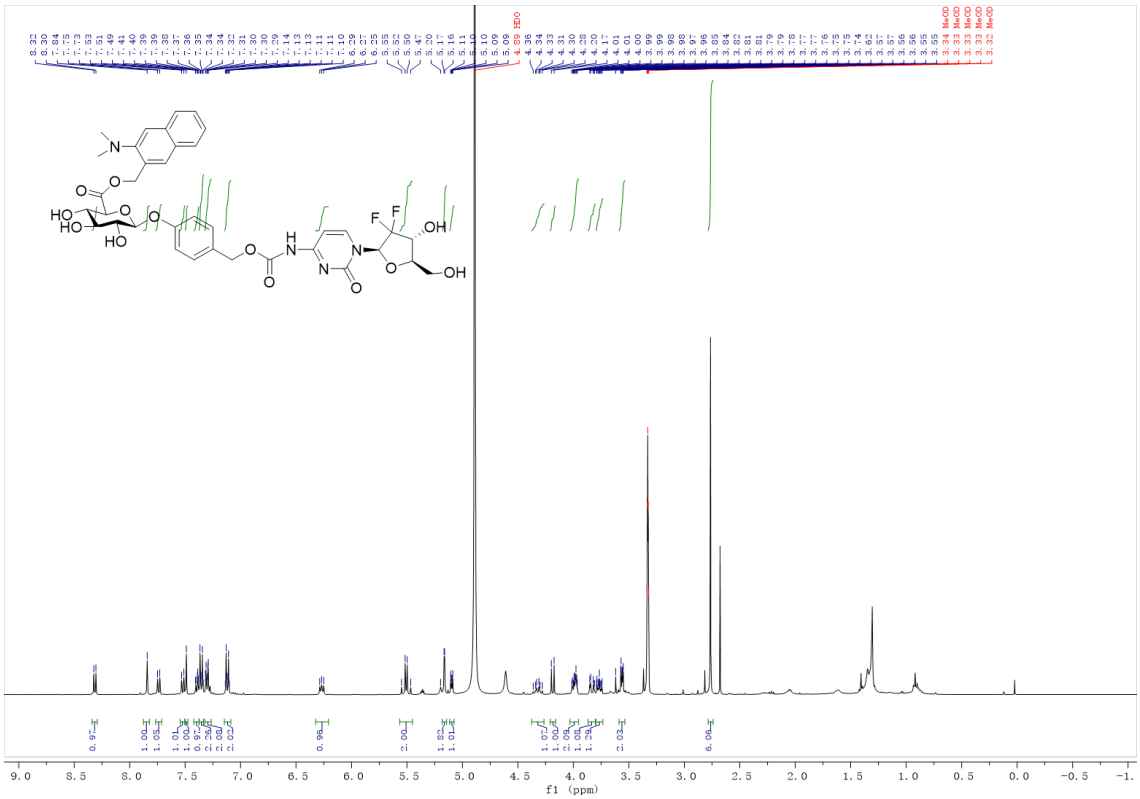
^{1}H NMR of compound GlcA-Gem



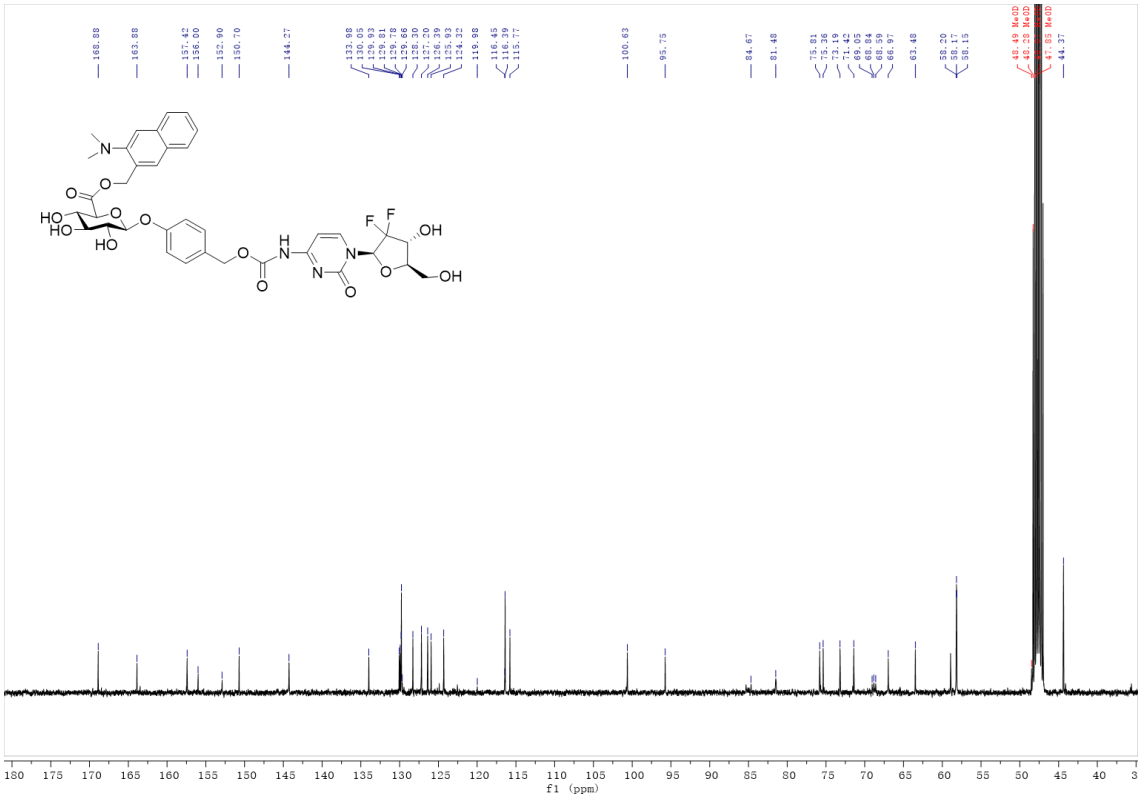
^{13}C NMR of compound GlcA-Gem



^{1}H NMR of compound DANE-GlcA-Gem



^{13}C NMR of compound DANE-GlcA-Gem



5. Reference

1. R. Walther, M. T. Jarlstad Olesen and A. N. Zelikin, *Org. Biomol. Chem.*, 2019, **17**, 6970-6974.
2. R. H. Nimmo-Smith, *Biochim. Biophys. Acta.* , 1961, **50**, 166-169.
3. G. Magro, R. E. S. Bain, C. A. Woodall, R. L. Matthews, S. W. Gundry and A. P. Davis, *Environ Sci Technol.*, 2014, **48**, 9624-9631.
4. V. Lovrinčević, Y. Guo, D. Vuk, I. Škorić, J. Ma and N. Basarić, *J. Org. Chem.*, 2023, **88**, 15176-15188.
5. Y. L. Leu, S. R. Roffler and J. W. Chern, *J. Med. Chem.*, 1999, **42**, 3623-3628.