Supplementary material

Design of glycosyltransferase inhibitors targeting human *O*-GlcNAc Transferase (OGT)

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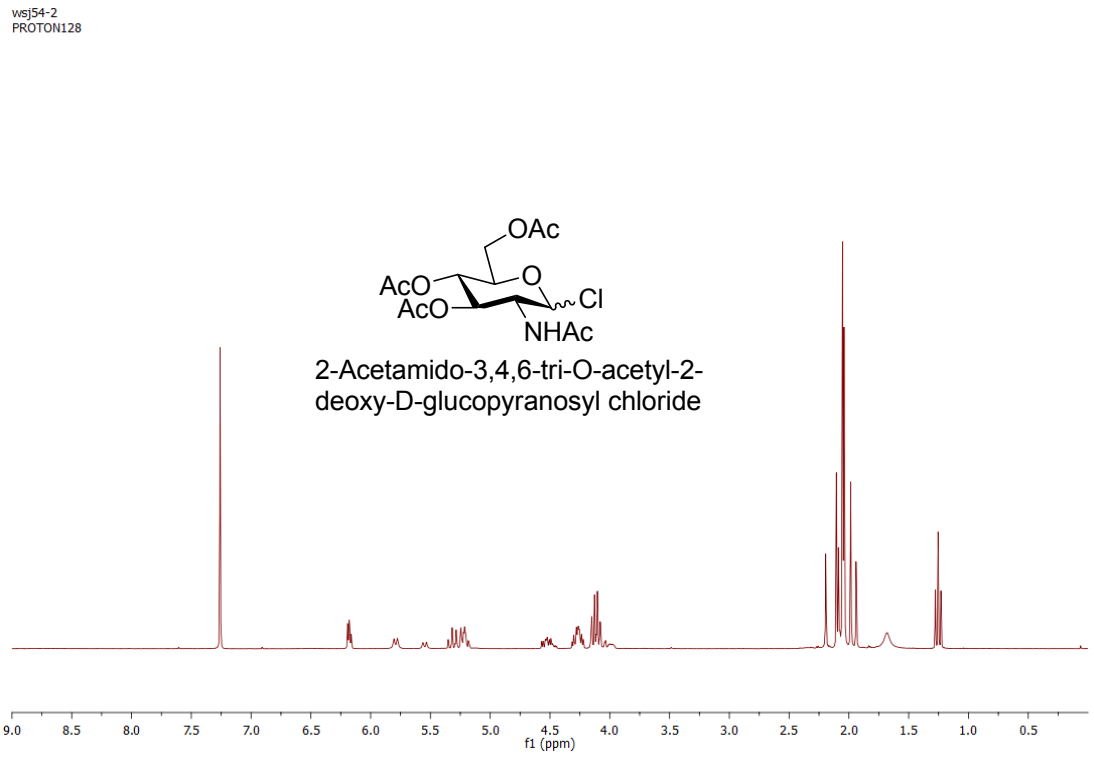
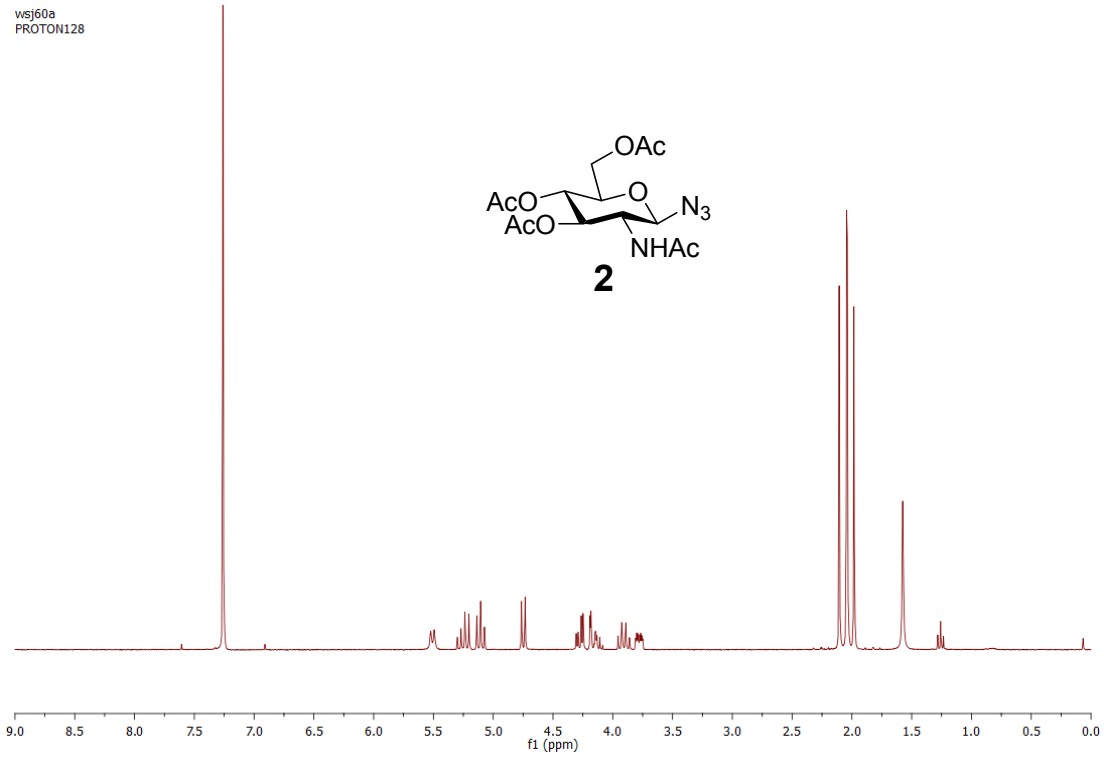
2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranosyl chloride (Ref: *Organic Letters*, **2002**, *Vol. 4*, No. 9, 1467-1470.)

2-Acetamido-2-deoxy-D-glucopyranose (5.0 g, 22.6 mmol, 1 eq.) was added to stirred acetyl chloride (10 mL) and the resulting suspension was stirred magnetically at r.t. overnight. Dichloromethane (40 mL) was added into the reaction and the resulting solution was poured into a mixture of ice (40 g) and water (10 mL) with stirring. The organic layer was separated immediately and run into a mixture of saturated aq. $NaHCO_3$ (40 mL) and ice with stirring, the neutralization being completed in a separating funnel. The organic layer was then separated and dried over $MgSO_4$. The organic layer was then filtered and the residue was washed with CH_2Cl_2 (15 mL). The resulting deep yellow solution was concentrated to about 10 mL under vacuum. Anhydrous Et_2O (50 mL) was added and the product crystallized 5 min later. The pale yellow solid was then filtered, washed with anhydrous ether (2×15 mL) and allowed to dry with vacuum for 5 min. A mixture of α and β anomers (α:β = 2:1) was afforded (4.627 g, 12.67 mmol, 56%). R_f = 0.71, 0.54 (EA, two spots for α and β anomers, respectively); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.23 – 6.13 (m, 1H), 5.79 (d, J = 8.6 Hz, 0.65H, $NH_{\alpha\ anomer}$), 5.55 (d, J = 9.1 Hz, 0.35H, $NH_{\beta\ anomer}$), 5.37 – 5.16 (m, 2H, H_3, H_4), 4.57 – 3.93 (m, 4H, H_2, H_5, H_{6a}, H_{6b}), 2.21 – 1.91 (m, 12H, 4×COCH_3).



2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (2) (Ref: *Nucleosides, Nucleotides and Nucleic Acid*, **2001**, 20(8), 1555-1569.)

A solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranosyl chloride (4.093 g, 11.2 mmol, 1.0 eq.) and NaN_3 (1.304 g, 20 mmol, 1.8 eq) in DMF (20 mL) was heated at 80°C for 16 hours. Then, heating was stopped, ice was added into the reaction and the reaction mixture was extracted with EtOAc (3×90 mL). The organic layer was washed with saturated $NaHCO_3$ (80 mL), H_2O (2×80 mL) and brine (80 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated. The residue was purified by silica gel column chromatography (PE:EtOAc = 1:2) to afford compound **2** as a white foam (1.908 g, 5.1 mmol, 46%). R_f = 0.38 (PE:EtOAc = 1:2); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) = 5.51 (d, J = 8.7 Hz, 1H, N*H*), 5.31 – 5.06 (m, 2H, H_3, H_4), 4.75 (d, J = 9.3 Hz, 1H, H_1), 4.28 (dd, J = 12.5, 4.8 Hz, 1H, H_{6a}), 4.20 – 4.13 (m, 1H, H_{6b}), 3.90 (ddd, 1H, H_2), 3.78 (ddd, J = 10.0, 4.8, 2.3 Hz, 1H, H_5), 2.11 (s, 3H), 2.04 (s, 3H, COCH_3), 2.04 (s, 3H, COCH_3), 1.98 (s, 3H, COCH_3).



6-Bromo-*N*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)picolinamide (3) 6-Bromopicolinic acid (812 mg, 4.02 mmol, 3.0 eq.) and HOBt (633 mg, 4.69 mmol, 3.5 eq.) were co-evaporated with toluene (3×15 mL) and THF (3×6 mL). The mixture was dried under vacuum for another 1 hour. Then, the mixture was dissolved in dry THF (8 mL) under argon and cooled to 0°C. DIC (720 μL, 4.69 mmol, 3.5 eq.) was added dropwise at 0°C. After addition, the ice-bath was removed and the reaction was stirred at r.t. for 30 min. At the same time, 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside azide (**2**) (500 mg, 1.34 mmol, 1.0 eq.) was dissolved in dry THF (8 mL) under argon and cooled to 0°C. PMe_3 (2.7 mL, 1 M in THF, 2.14 mmol, 2.0 eq.) was added into the flask at 0°C and the reaction was stirred at 0°C for 30 min. When there was no bubbling (~30 min), the solution was transferred into the flask containing the acid. Then, the color of the reaction mixture changed from colorless to orange. The reaction mixture was stirred with an ice-bath for 1 hour, then, the ice-bath was removed and the reaction was stirred at r.t. for 16 hours. The reaction mixture was diluted with water (150 mL), extracted with EtOAc (3×200 mL). The combined organic layers were washed with satd Na_2CO_3 (3×100 mL), H_2O (100 mL) and brine (100 mL). The organic phase was dried (Na_2SO_4), concentrated and dried with vacuum. The residue was purified by silica gel column chromatography (PE to PE:EtOAc = 3:7) to afford compound **3** as a yellow foam [678 mg, 95%, with 0.17 eq. contaminant 1,3-diisopropylurea, according to *Synlett.* **2010**, *8*, 1276-1280, data of 1,3-diisopropylurea], ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 1.14 (d, J = 6.4 Hz, 12H), 3.87 – 3.80 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) =23.5, 42.2, 157.0]. R_f = 0.19 (PE:EtOAc = 1:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 8.71 (d, J = 9.3 Hz, 1H, N*H*CO), 8.11 (dd, J = 7.5, 0.7 Hz, 1H, H_{Pyr}), 7.74 – 7.58 (m, 2H, 2 H_{Pyr}), 6.16 (d, J = 9.1 Hz, 1H, N*H*Ac), 5.36 (dd, J = 9.7, 9.3 Hz, 1H, H_1), 5.22 – 5.13 (m, 2H, H_3, H_4), 4.39 (dd, J = 9.7, 9.1 Hz, 1H, H_2), 4.28 (dd, J = 12.5, 4.3 Hz, 1H, H_{6a}), 4.10 (dd, J = 12.5, 2.0 Hz, 1H, H_{6b}), 3.91 – 3.59 (m, 1H, H_5), 2.10 – 2.01 (m, 9H, 3×COCH_3), 1.85 (s, 3H, COCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 171.7 (*C*OCH$_3$), 171.0 (*C*OCH$_3$), 170.9 (*C*OCH$_3$), 169.4 (*C*OCH$_3$), 164.0 (CONH), 149.7 (C_{Pyr}), 141.1 (C_{Pyr}), 139.6 (C_{PyrH}), 131.6 (C_{PyrH}), 121.7 (C_{PyrH}), 78.0 (C_1), 74.0 (C_5), 73.4 (C_3), 68.0 (C_4), 61.9 (C_6), 53.3 (C_2), 23.1 (CO*C*H$_3$), 20.88 (CO*C*H$_3$), 20.86 (CO*C*H$_3$), 20.7 (CO*C*H$_3$); HR-ESI-MS (positive mode) *m*/*z*: calcd. for $C_{20}H_{25}BrN_3O_9$, $[M+H]^+$ 530.0769, found 530.0776.

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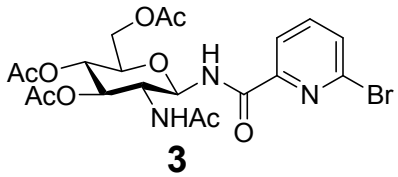

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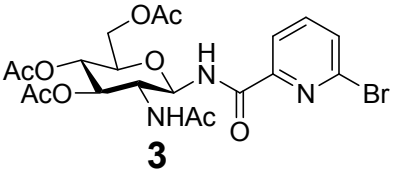
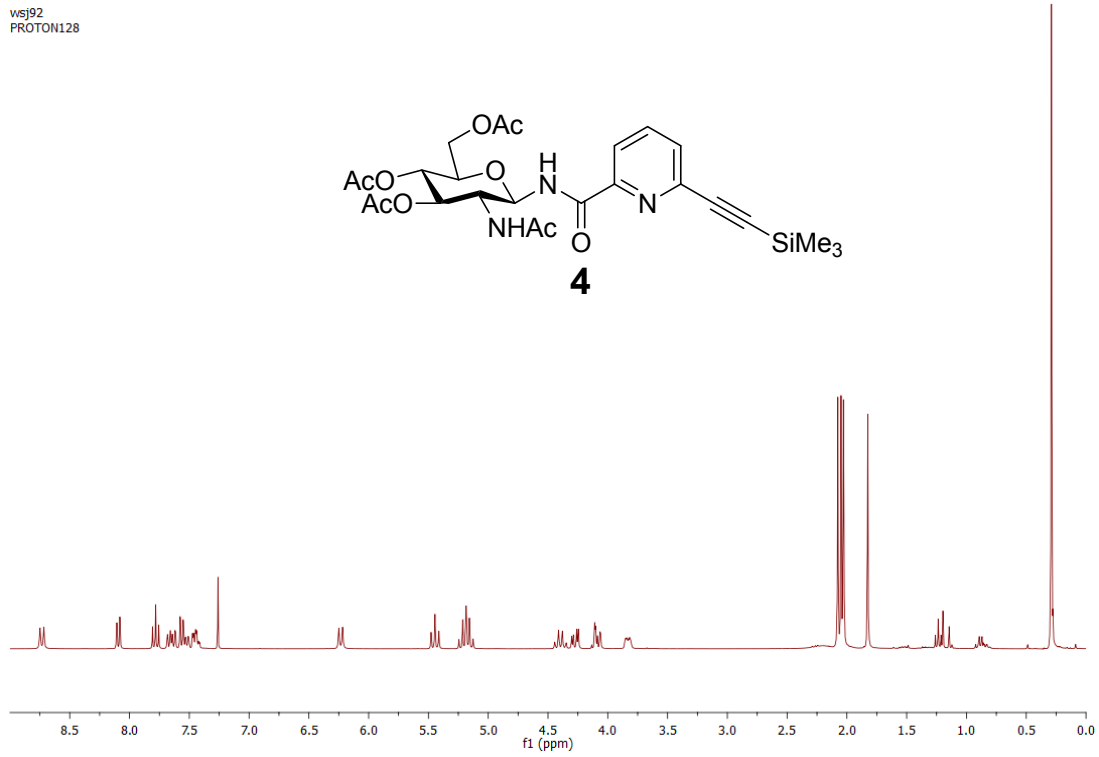


3

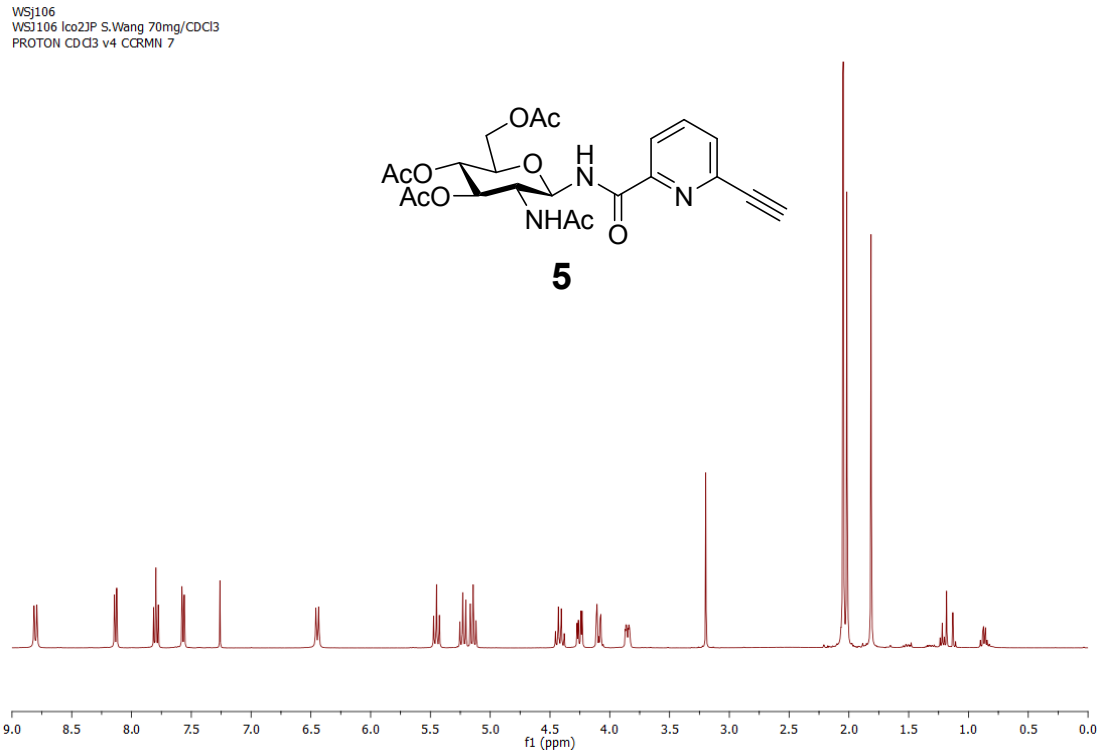
6-(2-Trimethylsilylethynyl)-*N*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)picolinamide (4)

A flask containing compound **3** (543 mg, 1.0 mmol, 1.0 eq.), $Pd(PPh_3)_4$ (115 mg, 0.1 mmol, 0.1 eq.) and CuI (19 mg, 0.1 mmol, 0.1 eq.) in toluene (25 mL) was bubbled with argon. Then, trimethylsilylacetylene (0.43 mL, 3.0 mmol, 3.0 eq.) and diisopropylamine (0.36 mL, 2.6 mmol, 2.6 eq.) were injected. The reaction was stirred at r.t. for 24 hours with protection of light. Then, the reaction mixture was poured into 100 mL satd aq. NH_4Cl solution, extracted with EtOAc (3×100 mL). The combined organic layers were washed with H_2O (100 mL) and brine (100 mL). The organic phase was dried and the solvent was evaporated. The residue was then purified by silica gel column chromatography (PE to PE:EtOAc = 4:6) to afford compound **4** (552 mg, 98%, with a little contaminant at aromatic zone ~7.5 ppm). R_f = 0.32 (PE:EtOAc = 1:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 8.73 (d, J = 9.7 Hz, 1H, N*H*CO), 8.09 (d, J = 7.8 Hz, 1H, H_{Pyr}), 7.78 (t, J = 7.8 Hz, 1H, H_{Pyr}), 7.58 (d, J = 7.8 Hz, 1H, H_{Pyr}), 6.23 (d, J = 9.4 Hz, 1H, N*H*Ac), 5.44 (t, J = 9.7, 9.4 Hz, H_1), 5.26 – 5.10 (m, 2H, H_3, H_4), 4.40 (ddd, J = 10.1, 9.7, 9.4 Hz, 1H, H_2), 4.27 (dd, J = 12.5, 4.3 Hz, 1H, H_{6a}), 4.09 (dd, J = 12.5, 4.6 Hz, 1H, H_{6b}), 3.88 – 3.78 (m, 1H, H_5), 2.08 (s, 3H, COC*H*$_3$), 2.05 (s, 3H, COC*H*$_3$), 2.03 (s, 3H, COC*H*$_3$), 1.83 (s, 3H, COC*H*$_3$), 0.29 (s, 9H, $SiMe_3$); ESI-MS (positive mode) *m*/*z*: $[M+H]^+$ 548.2, $[M+Na]^+$ 570.2.



6-Ethynyl-*N*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)picolinamide (5)

TBAF (1.4 mL, 1 M in THF, 1.4 mmol, 3.0 eq.) was added dropwise into a flask containing compound **4** (250 mg, 0.46 mmol, 1.0 eq.) in MeOH (5 mL). The reaction was stirred at r.t. for 3 hours. Then, the reaction mixture was poured into water (50 mL), extracted with CH_2Cl_2 (4×50 mL). The combined organic layers were dried over Na_2SO_4. Then, the solvent was evaporated, the residue was purified by silica gel column chromatography (PE to PE:EtOAc = 3:7) to afford compound **5** as a yellow foam (144 mg, 0.30 mmol, 66%). R_f = 0.21 (PE:EtOAc = 1:3); $[\alpha]_D$ = -56.4 (*c* = 2.2, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 8.80 (d, *J* = 9.6 Hz, 1H, N*H*CO), 8.13 (d, *J* = 7.7 Hz, 1H, H_{Pyr}), 7.80 (t, *J* = 7.7 Hz, 1H, H_{Pyr}), 7.57 (d, *J* = 7.7 Hz, 1H, H_{Pyr}), 6.45 (d, *J* = 9.3 Hz, 1H, N*H*Ac), 5.45 (dd, *J* = 9.7, 9.6 Hz, 1H, H_1), 5.28 – 5.19 (m, 1H, H_3), 5.26 – 5.09 (m, 1H, H_4), 4.42 (ddd, *J* = 9.7, 10.1, 9.3 Hz, 1H, H_2), 4.25 (dd, *J* = 12.5, 4.3 Hz, 1H, H_{6a}), 4.09 (dd, *J* = 12.5, 2.0 Hz, 1H, H_{6b}), 3.85 (ddd, *J* = 9.8, 4.3, 2.0 Hz, 1H, H_5), 3.20 (s, 1H, C≡CH), 2.05 (s, 6H, 2×COC*H*$_3$), 2.02 (s, 3H, COC*H*$_3$), 1.81 (s, 3H, COC*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 171.5 (*C*OCH$_3$), 170.9 (s, 2C, 2×*C*OCH$_3$), 169.4 (*C*OCH$_3$), 164.6 (*C*ONH), 149.0 (C_{Pyr}), 141.3 (C_{Pyr}), 137.7 (C_{PyrH}), 130.5 (C_{PyrH}), 122.4 (C_{PyrH}), 81.9 (C≡*C*H), 79.6 (C_1), 78.5 (*C*≡CH), 73.9 (C_5), 73.5 (C_3), 68.2 (C_4), 62.0 (C_6), 53.2 (C_2), 23.1 (CO*C*H$_3$), 20.8 (s, 2C, 2×CO*C*H$_3$), 23.7 (CO*C*H$_3$); HR-ESI-MS (positive mode) *m*/*z*: calcd. for $C_{22}H_{25}N_3NaO_9$ $[M+Na]^+$ 498.1483, found 498.1487.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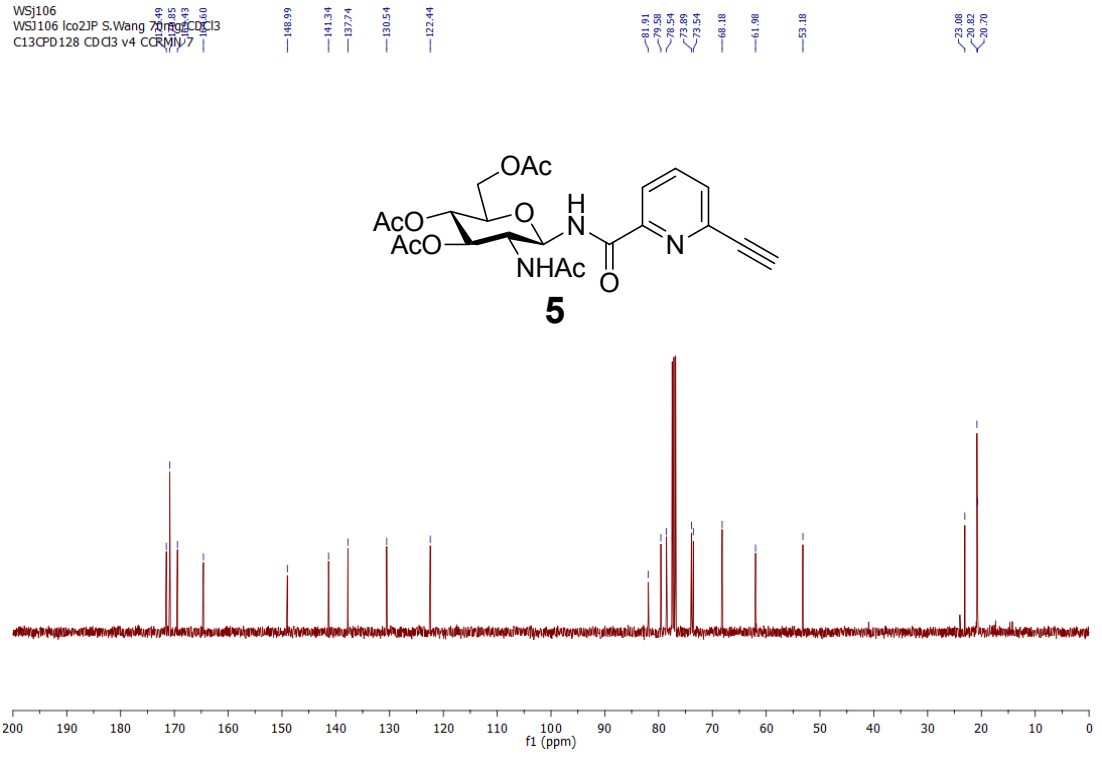
f1 (ppm)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AcO
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H
N
NHAc
O
N
5
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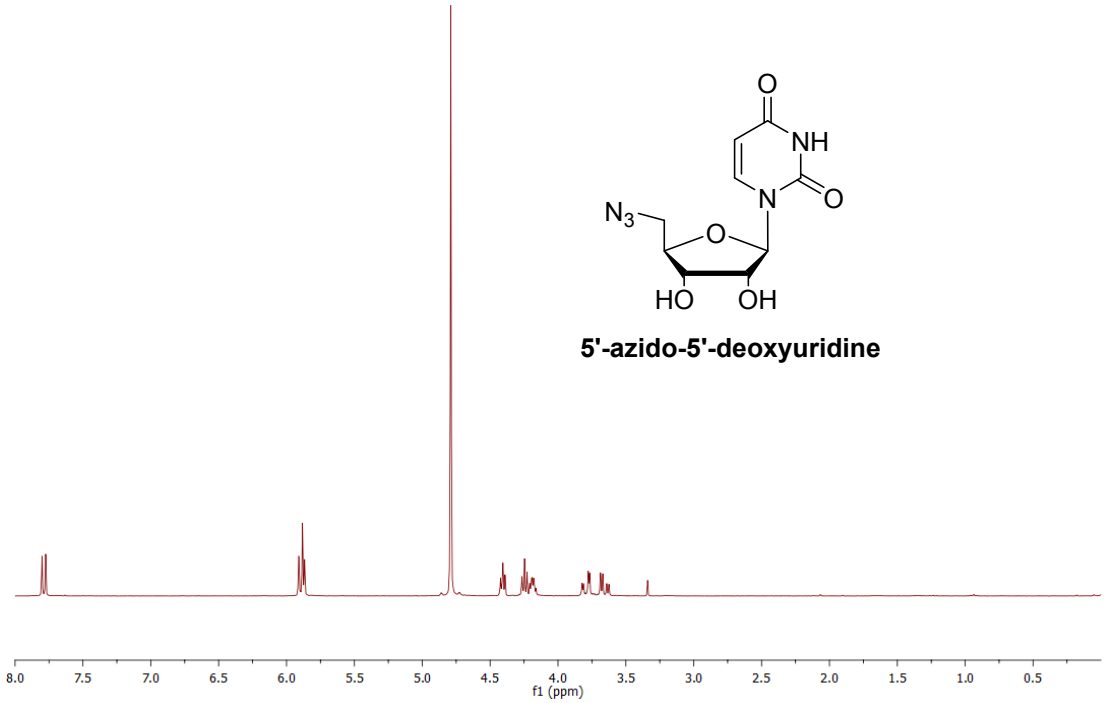
f1 (ppm)

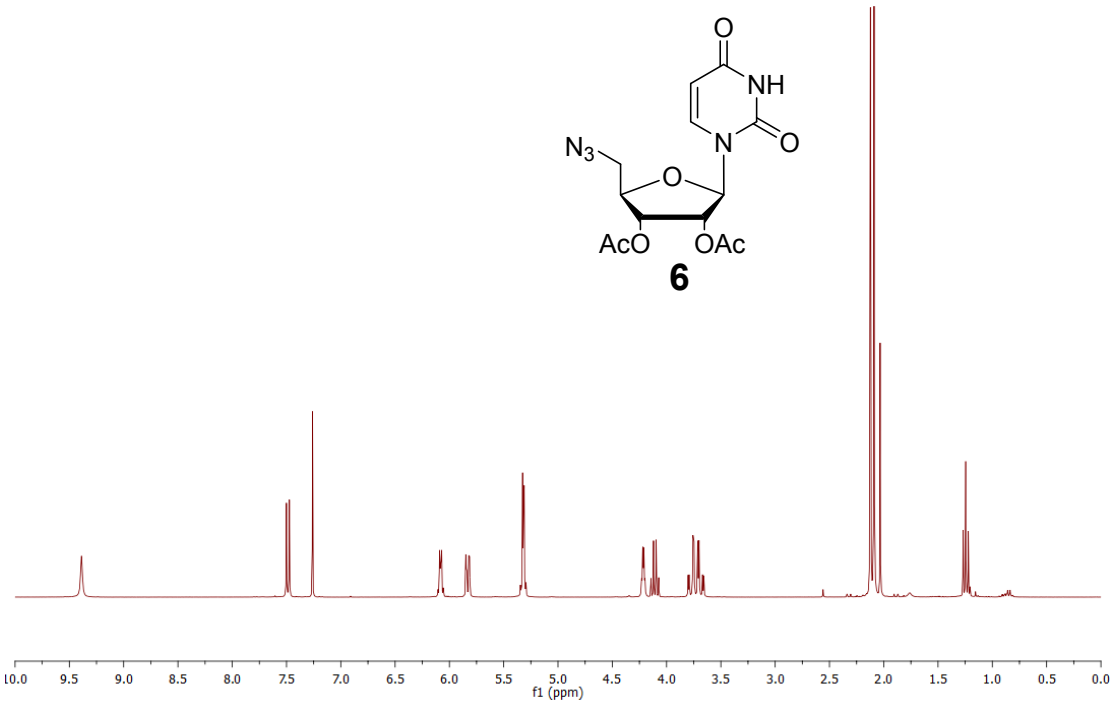
2',3'-Di-*O*-acetyl-5'-azido-5'-deoxy-uridine (6) (*J.C.S. Perkin I*, **1980**, 306-310.)

To a stirred mixture of uridine (5 g, 20.5 mmol, 1.0 eq.), triphenylphosphine (8.056 g, 30.7 mmol, 1.5 eq.), NaN_3 (61.5 mmol, 4.0 g, 3.0 eq.) and tetrabutylammonium iodide (1.515 g, 4.1 mmol, 0.2 eq) in DMF (50 mL) was added carbon tetrabromide (10.2 g, 30.75 mmol, 1.5 eq.) and the resulting mixture was stirred at r.t. overnight. Then, the reaction mixture was co-evaporated with toluene (15×40 mL) and the residue was purified by silica gel column chromatography (EtOAc to MeOH:EtOAc = 5:95) to afford 5'-azido-5'-deoxy-uridine (5.22 g, 19.4 mmol, 95%). R_f = 0.45 (MeOH:CH_2Cl_2, 1:9); ^{1}H NMR (300 MHz, D_2O) δ (ppm) = 7.81 (d, J = 8.1 Hz, 1H, H_{6Uri}), 5.93 – 5.85 (m, 2H, H_{5Uri}, H_{1Rib}), 4.43 (t, J = 4.9 Hz, 1H), 4.27 (t, J = 5.6 Hz, 1H), 4.21 (dd, J = 8.6, 5.0 Hz, 1H), 3.81 (dd, J = 13.7, 3.1 Hz, 1H, H_{5aRib}), 3.68 (dd, J = 13.7, 4.9 Hz, 1H, H_{5bRib}).

In a flask containing 5'-azido-5'-deoxy-uridine (232 mg, 0.86 mmol, 1eq.) in pyridine (7 mL) was added Ac_2O (2 mL). After addition, the reaction mixture was stirred at r.t. overnight under argon. Then, the reaction mixture was poured into satd aq. $NaHCO_3$ solution (100 mL) and extracted by EtOAc (3×100 mL). The organic phase was washed with 1N HCl (2×100 mL) and brine (2×100 mL), dried (Na_2SO_4) and evaporated. The residue was purified by silica gel column chromatography (PE:EtOAc = 1:1) to afford compound **6** as a white foam (251 mg, 0.71 mmol, 83%). R_f = 0.13 (PE:EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 9.39 (s, 1H, NH_{Uri}), 7.49 (d, J = 8.2 Hz, 1H, H_{6Uri}), 6.08 (dd, J = 3.6, 2.1 Hz, 1H, H_{1Rib}), 5.83 (dd, J = 8.1, 2.1 Hz, 1H, H_{5Uri}), 5.36 – 5.27 (m, 2H, H_{2Rib}, H_{3Rib}), 4.21 (dd, J = 3.1, 3.0 Hz, 1H, H_{4Rib}), 3.79 (dd, J = 13.7, 3.1 Hz, 1H, H_{5aRib}), 3.66 (dd, J = 13.7, 4.9 Hz, 1H, H_{5bRib}), 2.12 (s, 3H, COCH_3), 2.09 (s, 3H, COCH_3).



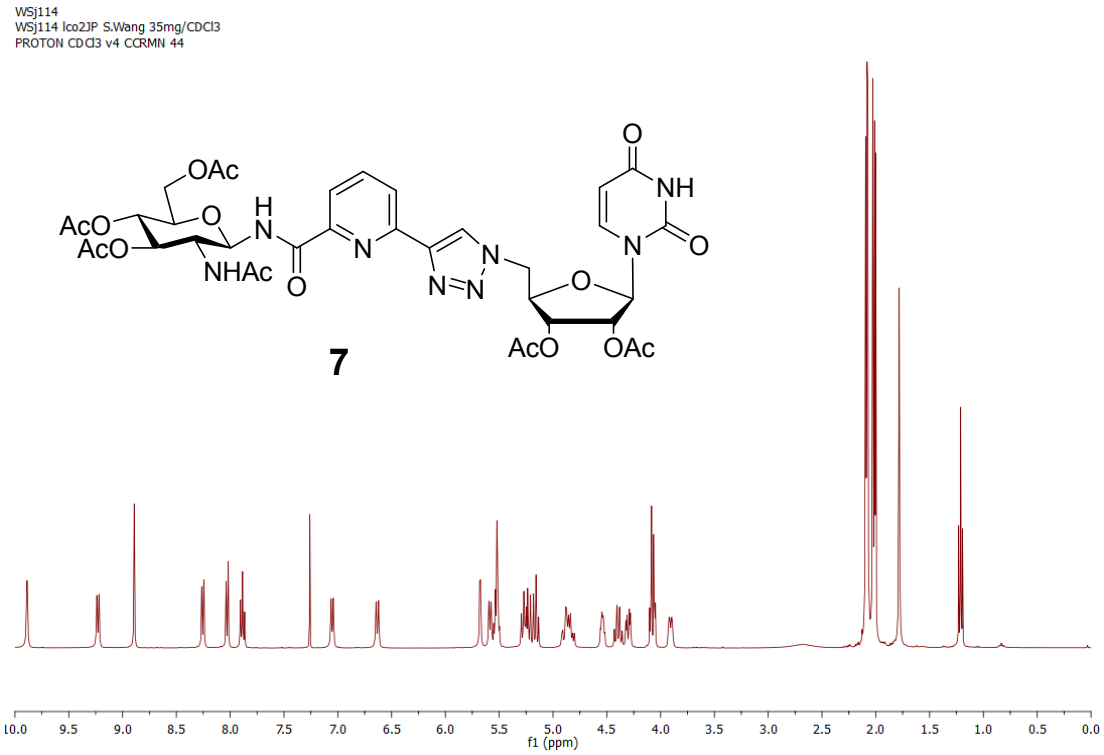
5'-azido-5'-deoxyuridine



6

6-[1-(2',3'-Di-*O*-acetyl-uridin-5'-yl)-1*H*-1,2,3-triazol-4-yl]-*N*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)picolinamide (7)

To a solution of compound **5** (89 mg, 0.19 mmol, 1.0 eq.) and compound **6** (66 mg, 0.19 mmol, 1.0 eq.) in *t*BuOH/H_2O (5.6 mL/280μL) were added $CuSO_4$ (1.2 mL, 0.1 M in H_2O, 0. 12 mmol, 0.6 eq.) and sodium ascorbate (2.3 mL, 0.1 M in H_2O, 0.23 mmol, 1.2 eq.). The reaction was stirred at 35°C for 64 hours. Then, the reaction mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (3×30 mL). The combined organic layers were dried over Na_2SO_4. Then, the solvent was evaporated. The residue was purified by silica gel column chromatography (100% PE to 100% EtOAc) to afford compound **7** as a white foam (105 mg, 0.13 mmol, 68%). R_f = 0.41 (EtOAc); $[\alpha]_D$ = +0.3 (c = 0.7, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 9.89 (s, 1H, N*H*CO$_{Uri}$), 9.23 (d, *J* = 8.3 Hz, 1H, N*H*CO), 8.89 (s, 1H, $H_{triazole}$), 8.25 (d, *J* = 7.8 Hz, 1H, H_{Pyr}), 8.03 (d, *J* = 7.8 Hz, 1H, H_{Pyr}), 7.88 (t, *J* = 7.8 Hz, 1H, H_{Pyr}), 7.05 (d, *J* = 8.1 Hz, 1H, H_{6Uri}), 6.63 (d, *J* = 8.9 Hz, 1H, N*H*Ac), 5.68 (d, *J* = 3.0 Hz, 1H, H_{1Rib}), 5.58 (d, *J* = 8.1 Hz, 1H, H_{5Uri}), 5.56 – 5.48 (m, 2H, H_{2Rib}, H_{3Rib}), 5.32 – 5.12 (m, 3H, $H_{1GlcNAc}$, $H_{3GlcNAc}$, $H_{4GlcNAc}$), 4.90 (dd, *J* = 14.3, 3.2 Hz, 1H, H_{5aRib}), 4.83 (dd, *J* = 14.3, 5.9 Hz, 1H, H_{5bRib}), 4.54 (ddd, *J* = 9.8, 5.9, 3.2 Hz, 1H, H_{4Rib}), 4.39 (ddd, *J* = 9.8, 10.1, 8.9 Hz, 1H, $H_{2GlcNAc}$), 4.30 (dd, *J* = 12.5, 4.3 Hz, 1H, $H_{6aGlcNAc}$), 4.08 – 4.03 (m, 1H, $H_{6bGlcNAc}$), 3.94 – 3.85 (m, 1H, $H_{5GlcNAc}$), 2.09 (s, 3H, COC*H*$_3$), 2.08 (s, 3H, COC*H*$_3$), 2.08 (s, 3H, COC*H*$_3$), 2.03 (s, 3H, COC*H*$_3$), 2.01 (s, 3H, COC*H*$_3$), 1.78 (s, 3H, COC*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 172.1, 171.8, 170.9, 169.8, 169.6 (*C*OCH$_3$), 165.5 (NH*C*O), 163.1 (*C*=O$_{Uri}$), 150.3 (*C*=O$_{Uri}$), 149.0 ($C_{triazole}$), 148.3, 147.8, 141.9 (C_{6Uri}), 138.5 (C_{PyrH}), 125.6 ($C_{triazoleH}$), 122.8 (C_{PyrH}), 121.5 (C_{PyrH}), 103.0 (C_{5Uri}), 92.0 (C_{1Rib}), 80.6 ($C_{1GlcNAc}$), 80.4 (C_{4Rib}), 73.5 ($C_{5GlcNAc}$), 73.0 (C_{2Rib} or C_{3Rib}), 72.8 ($C_{3GlcNAc\ or}$ $C_{4GlcNAc}$), 71.1 (C_{2Rib} or C_{3Rib}), 68.5 ($C_{3GlcNAc\ or}$ $C_{4GlcNAc}$), 62.1 ($C_{6GlcNAc}$), 52.6 ($C_{2GlcNAc}$), 51.5 (C_{5Rib}), 23.0 (CO*C*H$_3$), 20.9 (CO*C*H$_3$), 20.8 (CO*C*H$_3$), 20.7 (CO*C*H$_3$), 20.51 (CO*C*H$_3$), 20.46 (CO*C*H$_3$); HR-ESI-MS (positive mode) *m*/*z*: calcd. for $C_{35}H_{41}N_8O_{16}$ $[M+H]^+$ 829.2635, found 829.2606.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O
NH
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OAc
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6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
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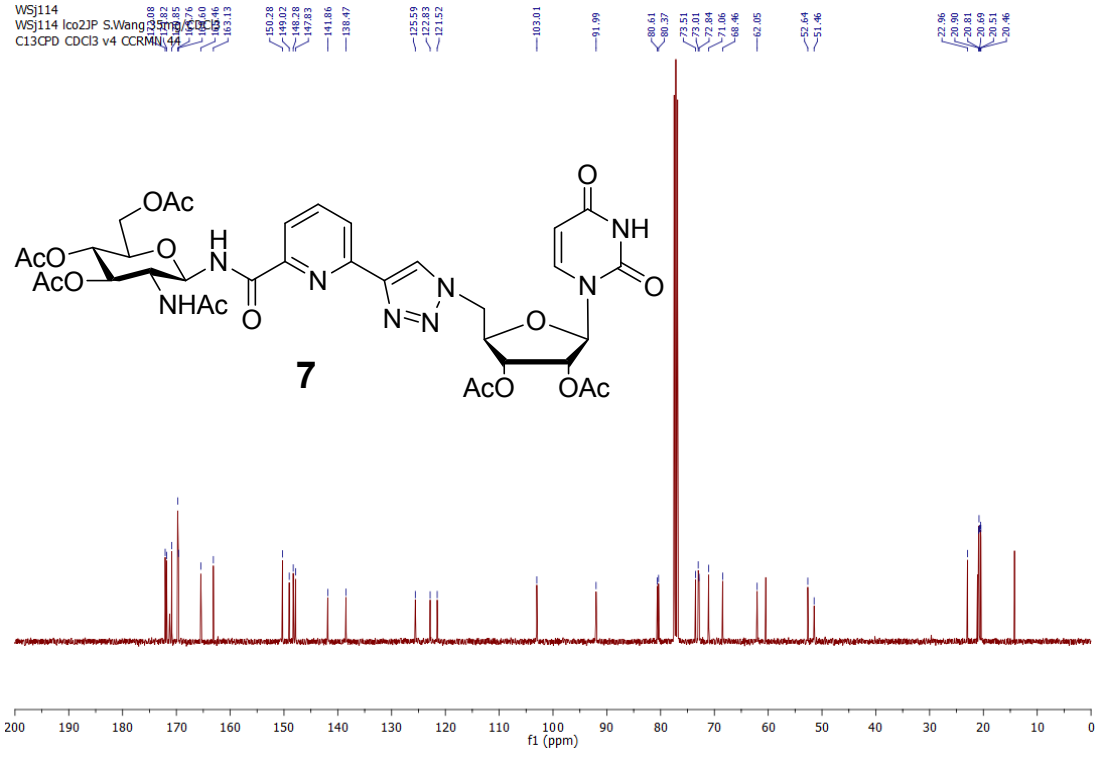
1.0
0.5
0.0
f1 (ppm)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WSj114 lco2JP S.Wang 35mg/CDCl3
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N
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NH
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N
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AcO
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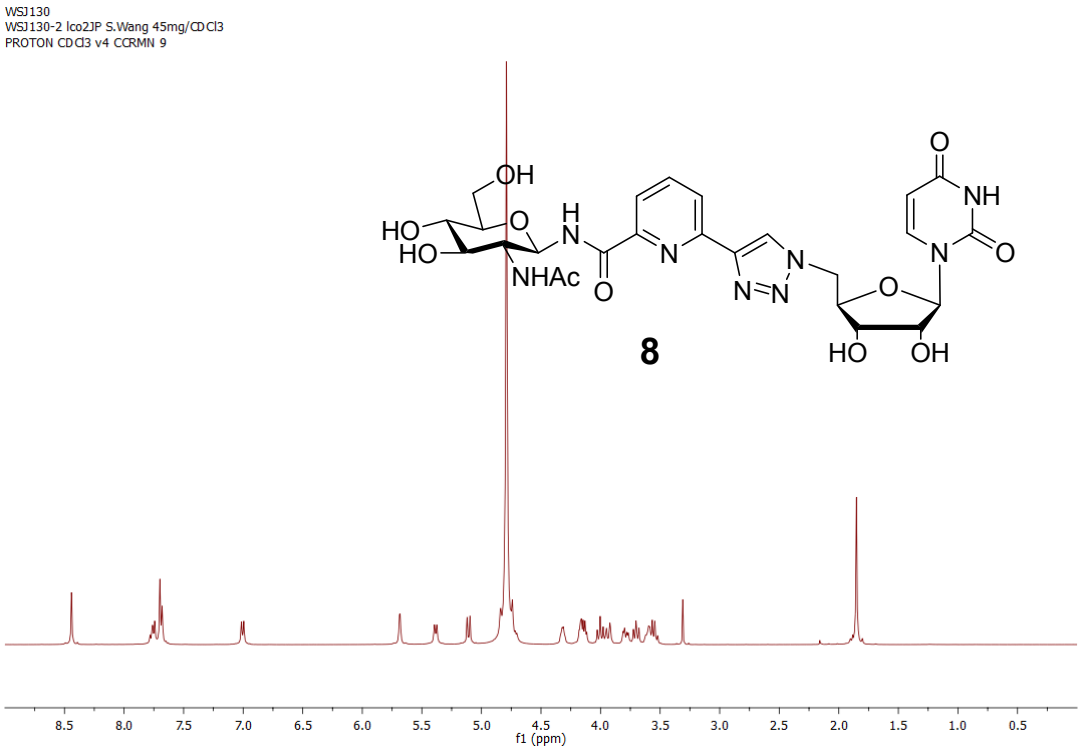
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

6-[(1-Uridin-5'-yl)-1*H*-1,2,3-triazol-4-yl]-*N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl) picolinamide (8)

In a flask containing compound **7** (104.7 mg, 0.126 mmol, 1.0 eq.) in MeOH (3 mL) was slowly added solid NaOMe until pH ≥ 9. Then, the reaction mixture was stirred at r.t. for 3 hours. The reaction mixture was concentrated and the residue was purified by C18 reverse phase column chromatography (100% H_2O to 30% MeOH/H_2O) to afford compound **8** as a white foam (66 mg, 0.11 mmol, 85%). R_f = 0.50 (EA:MeOH = 2:3); $[\alpha]_D$ = +19.5 (c = 0.8, MeOH); ^{1}H NMR (400 MHz, D_2O) δ (ppm) = 8.44 (s, 1H, $H_{triazole}$), 7.83 – 7.69 (m, 3H, H_{Pyr}), 7.00 (d, J = 7.9 Hz, 1H, H_{6Uri}), 5.69 (d, J = 2.2 Hz, 1H, H_{1Rib}), 5.38 (d, J = 7.9 Hz, 1H, H_{5Uri}), 5.11 (d, J = 9.6 Hz, 1H, $H_{1GlcNAc}$), 4.87 – 4.68 (m, 2H, H_{5Rib}), 4.34 – 4.28 (m, 1H, H_{4Rib}), 4.19 – 4.09 (m, 2H, H_{2Rib}, H_{3Rib}), 4.04 – 3.89 (m, 2H, $H_{2GlcNAc}$, $H_{6aGlcNAc}$), 3.79 (dd, J = 12.4, 4.8 Hz, 1H, $H_{6bGlcNAc}$), 3.75 – 3.65 (m, 1H, $H_{3GlcNAc}$), 3.65 – 3.50 (m, 2H, $H_{4GlcNAc}$, $H_{5GlcNAc}$), 1.85 (s, 3H, NHCOCH_3); ^{13}C NMR (100 MHz, D_2O) δ (ppm) = 175.1, 168.5, 166.9, 153.4, 147.9, 147.6, 146.8, 141.8 (C_{6Uri}), 139.4 (C_{PyrH}), 126.1 ($C_{triazoleH}$), 123.3 (C_{PyrH}), 122.0 (C_{PyrH}), 102.5 (C_{5Uri}), 91.4 (C_{1Rib}), 80.7 (C_{4Rib}), 79.8 ($C_{1GlcNAc}$), 78.0 ($C_{5GlcNAc}$), 74.2 ($C_{3GlcNAc}$), 72.9 (C_{2Rib} or C_{3Rib}), 70.4 (C_{3Rib} or C_{2Rib}), 70.2 ($C_{4GlcNAc}$), 61.1 ($C_{6GlcNAc}$), 54.6 ($C_{2GlcNAc}$), 51.2 (C_{5Rib}), 22.4 (NHCOCH_3); HR-ESI-MS (positive mode) m/z: calcd. for $C_{25}H_{30}N_8NaO_{11}$ $[M+Na]^+$ 641.1926, found 641.1911.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NH
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6.5
6.0
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3.0
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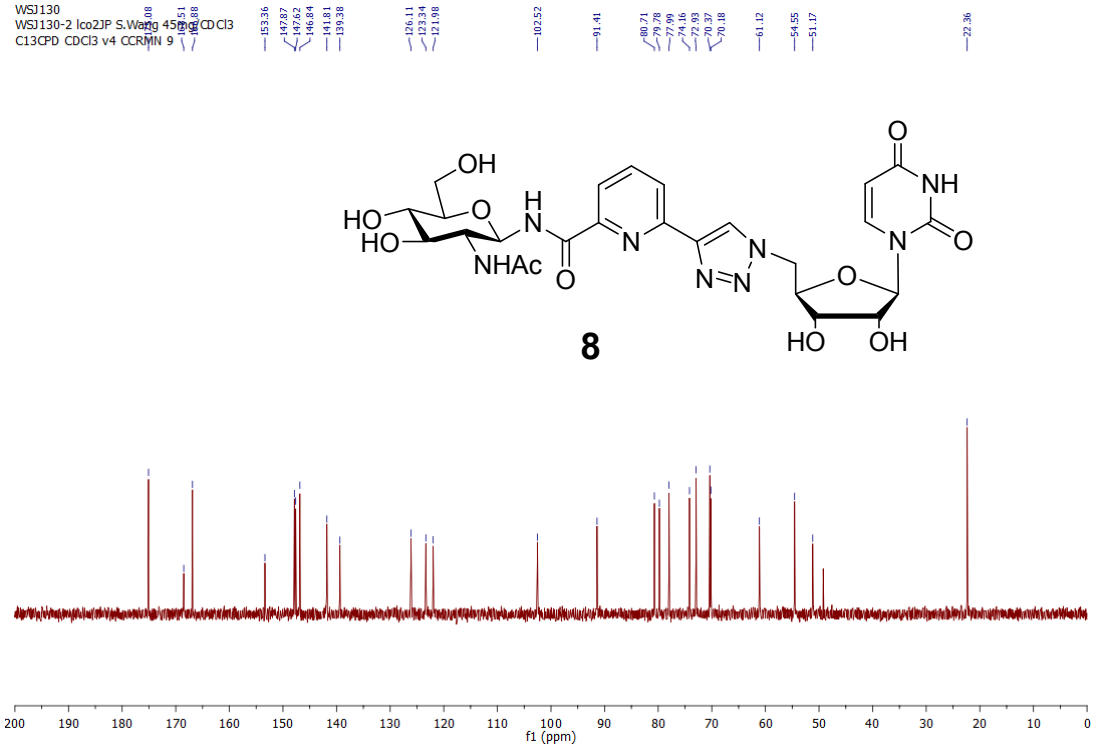
0.5
f1 (ppm)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WSJ130-2 lco2JP S.Wang 45mg/CDCl3
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77.99
74.16
72.93
70.37
70.18
61.12
54.55
51.17
22.36
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H
N
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N
N
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N
O
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N
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OH
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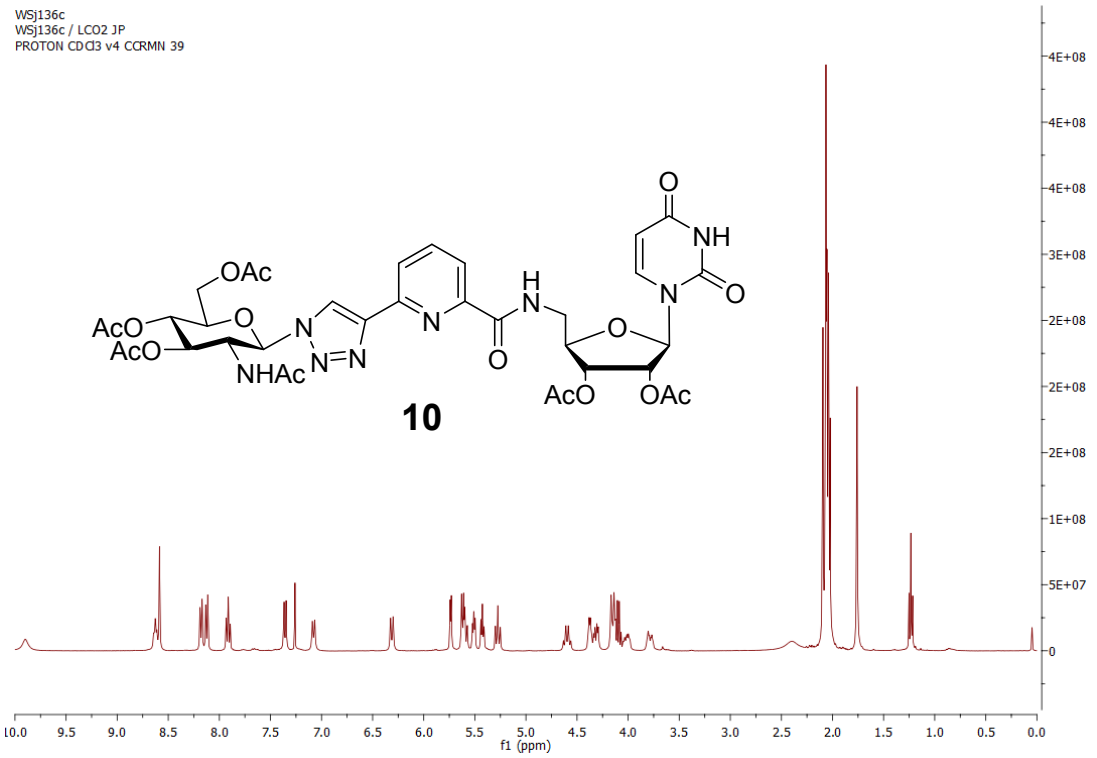
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

6-[(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl]-*N*-(2',3'-di-*O*-acetyl-uridin-5'-yl)picolinamide (10)

To a solution of 6-ethynyl-*N*-(2',3'-di-*O*-acetyl-uridin-5'-yl)picolinamide (**9**) (44 mg, 0.10 mmol, 1.0 eq.) and 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (**2**) (53 mg, 0.14 mmol, 1.4 eq.) in *t*BuOH/H_2O (4 mL/200μL) were added $CuSO_4$ (100 μL, 0.1 M in H_2O, 0. 01 mmol, 0.1 eq.), sodium ascorbate (200 μL, 0.1 M in H_2O, 0.02 mmol, 0.2 eq.) and DIPEA (0.1 mL, to adjust pH value around 8). Then, the reaction mixture was stirred at 38°C. After 3 h, the reaction mixture was concentrated and the residue was purified by silica gel column chromatography (60% EtOAc/PE to 10% MeOH/EtOAc) to afford compound **10** as a white foam (41 mg, 0.05 mmol, 50%). R_f = 0.10 (EtOAc); $[\alpha]_D$ = -19.3 (c = 0.7, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 9.90 (s, 1H, H_{3Uri}), 8.63 (t, *J* = 6.0 Hz, 1H, N*H*CO), 8.59 (s, 1H, $H_{triazole}$), 8.18 (d, *J* = 7.8 Hz, 1H, H_{Pyr}), 8.12 (d, *J* = 7.8 Hz, 1H, H_{Pyr}), 7.91 (t, *J* = 7.8 Hz, 1H, H_{Pyr}), 7.36 (d, *J* = 8.1 Hz, 1H, H_{6Uri}), 7.08 (d, *J* = 8.9 Hz, 1H, N*H*Ac), 6.31 (d, *J* = 9.8 Hz, 1H, $H_{1GlcNAc}$), 5.74 (d, *J* = 4.7 Hz, 1H, H_{1Rib}), 5.65 – 5.55 (m, 2H, H_{5Uri}, $H_{3GlcNAc}$), 5.51 (dd, *J* = 5.7, 4.7 Hz, 1H, H_{2Rib}), 5.43 (t, *J* = 5.7, 5.7 Hz, 1H, H_{3Rib}), 5.27 (t, *J* = 9.6, 9.6 Hz, 1H, $H_{4GlcNAc}$), 4.60 (ddd, *J* = 10.6, 9.8, 8.9Hz, 1H, $H_{2GlcNAc}$), 4.41 – 4.27 (m, 2H, $H_{4Rib,}$ $H_{6aGlcNAc}$), 4.19 – 4.11 (m, 2H, $H_{5GlcNAc,}$ $H_{6bGlcNAc}$), 4.02 (dd, *J* = 14.3, 5.6 Hz, 1H, H_{5aRib}), 3.79 (dd, *J* = 14.3, 4.4 Hz, 1H, H_{5bRib}), 2.13 – 1.99 (m, 15H, 5×COCH_3), 1.76 (s, 3H, N*H*Ac); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 171.6 (*C*OCH_3), 170.9 (*C*OCH_3), 170.8 (*C*OCH_3), 170.0 (*C*OCH_3), 169.8 (*C*OCH_3), 169.7 (*C*OCH_3), 165.1, 163.4, 150.4, 149.4, 148.6, 147.7, 141.5 (C_{6Uri}), 138.6 (C_{PyrH}), 123.3 (C_{PyrH}), 122.0 (s, 2C, C_{PyrH}, $C_{triazole}$), 103.2 (C_{5Uri}), 90.5 (C_{1Rib}), 86.0 ($C_{1GlcNAc}$), 80.4 (C_{4Rib}), 75.0 ($C_{5GlcNAc}$), 72.7 (C_{2Rib}), 72.3 ($C_{3GlcNAc}$), 70.6 (C_{3Rib}), 68.3 ($C_{4GlcNAc}$), 62.1 ($C_{6GlcNAc}$), 54.1 ($C_{2GlcNAc}$), 40.2 (C_{5Rib}), 22.85 (CO*C*H_3), 20.82 (CO*C*H_3), 20.75 (CO*C*H_3), 20.74 (CO*C*H_3), 20.60 (CO*C*H_3), 20.55 (CO*C*H_3); HR-ESI-MS (positive mode) *m*/*z*: calcd. for $C_{35}H_{41}N_8O_{16}$ $[M+H]^+$ 829.2635, found 829.2610.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AcO
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NHAc
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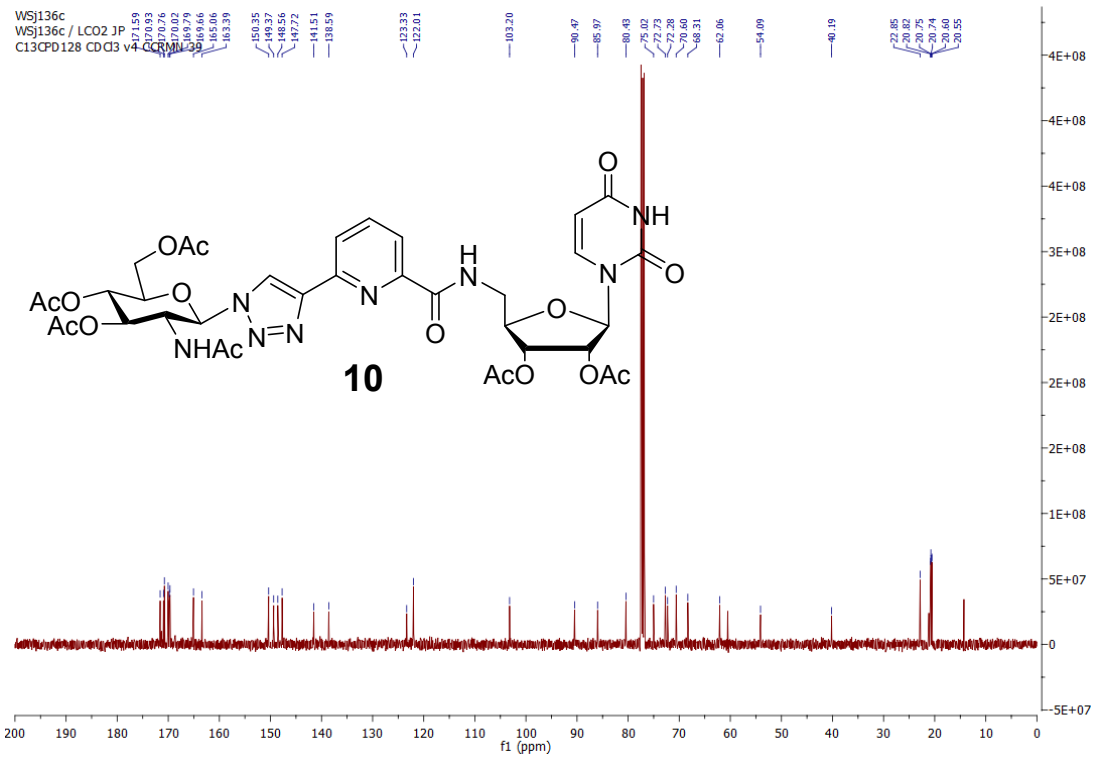
NH
N
O
O
AcO
OAc
10
f1 (ppm)


WSj136c
WSj136c / LCO2 JP
C13CPD128 CDCl3 v4 CCRMN 39
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AcO
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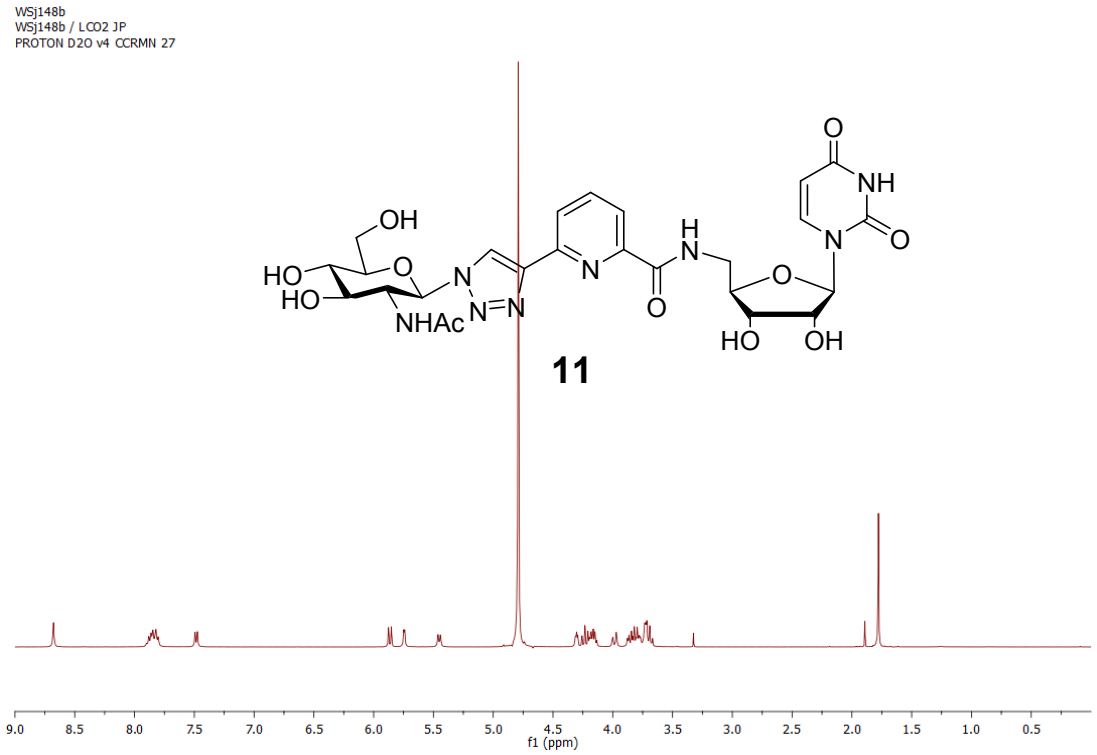
NHAc
N
N=N
N
H
N
O
O
NH
N
O
O
AcO
OAc
10
f1 (ppm)

6-[(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl]-*N*-(uridin-5'-yl) picolinamide (11)

In a 10 mL round-bottom flask containing compound **10** (41 mg, 0.05 mmol, 1.0 eq.) in CH_3OH (3 mL) was slowly added solid NaOMe until pH~9. Then, the reaction mixture was stirred at r.t. for 4 hours. DOWEX 50W×2 Resin (Fluka, 50-100 mesh, H^+ Form) was added to neutralize the reaction until pH~7. Then, the reaction mixture was filtrated and concentrated. The residue was purified by C18 reverse phase column chromatography (100% H_2O to 70% MeOH/H_2O) to afford compound **11** as a white foam (35 mg, 0.05 mmol, quant.). $[\alpha]_D$ = +19.1 (c = 0.4, H_2O); ^{1}H NMR (400 MHz, D_2O) δ (ppm) = 8.68 (s, 1H, $H_{triazole}$), 7.90 – 7.78 (m, 3H, H_{Pyr}), 7.48 (d, J = 7.9 Hz, 1H, H_{6Uri}), 5.86 (d, J = 9.6 Hz, 1H, $H_{1GlcNAc}$), 5.74 (d, J = 3.0 Hz, 1H, H_{1Rib}), 5.45 (d, J = 7.9 Hz, 1H, H_{5Uri}), 4.33 – 4.28 (m, 1H, H_{2Rib}), 4.27 – 4.11 (m, 3H), 4.02 – 3.95 (m, 1H, $H_{6aGlcNAc}$), 3.90 – 3.64 (m, 6H), 1.78 (s, 3H, N*H*Ac); ^{13}C NMR (100 MHz, D_2O) δ (ppm) = 174.5, 168.4, 166.6, 153.3, 148.5, 147.7, 146.9, 141.9 (C_{6Uri}), 139.6 (C_{PyrH}), 123.8 (C_{PyrH}), 123.4 ($C_{triazole}$), 122.0 (C_{PyrH}), 102.4 (C_{5Uri}), 91.0 (C_{1Rib}), 86.9 ($C_{1GlcNAc}$), 82.2, 79.4, 73.8, 73.5 (C_{2Rib}), 71.0, 69.7, 61.0 ($C_{6GlcNAc}$), 56.0 ($C_{2GlcNAc}$), 40.5 (C_{5Rib}), 22.0 (NHCO*C*H_3); HR-ESI-MS (positive mode) *m*/*z*: calcd. for $C_{25}H_{30}N_8NaO_{11}$ $[M+Na]^+$ 641.1926, found 641.1920.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WSj148b / LCO2 JP
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N
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HO
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11
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6.5
6.0
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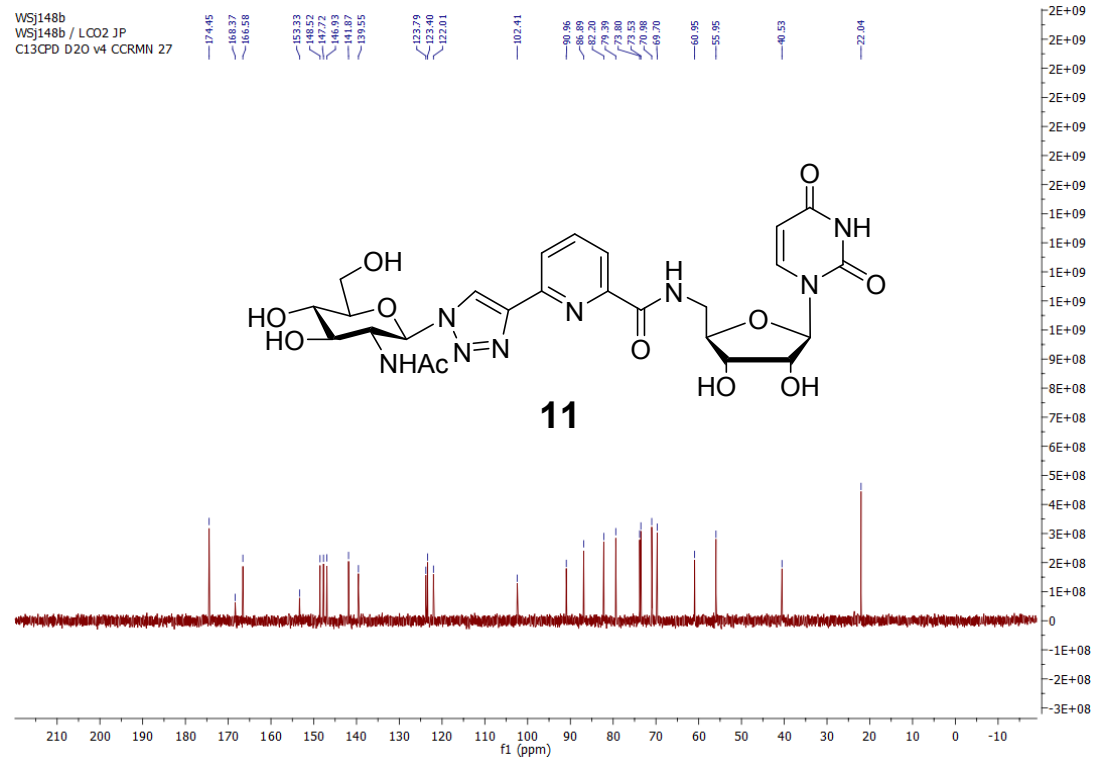
0.5
f1 (ppm)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WSj148b / LCO2 JP
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139.55
123.79
123.40
122.01
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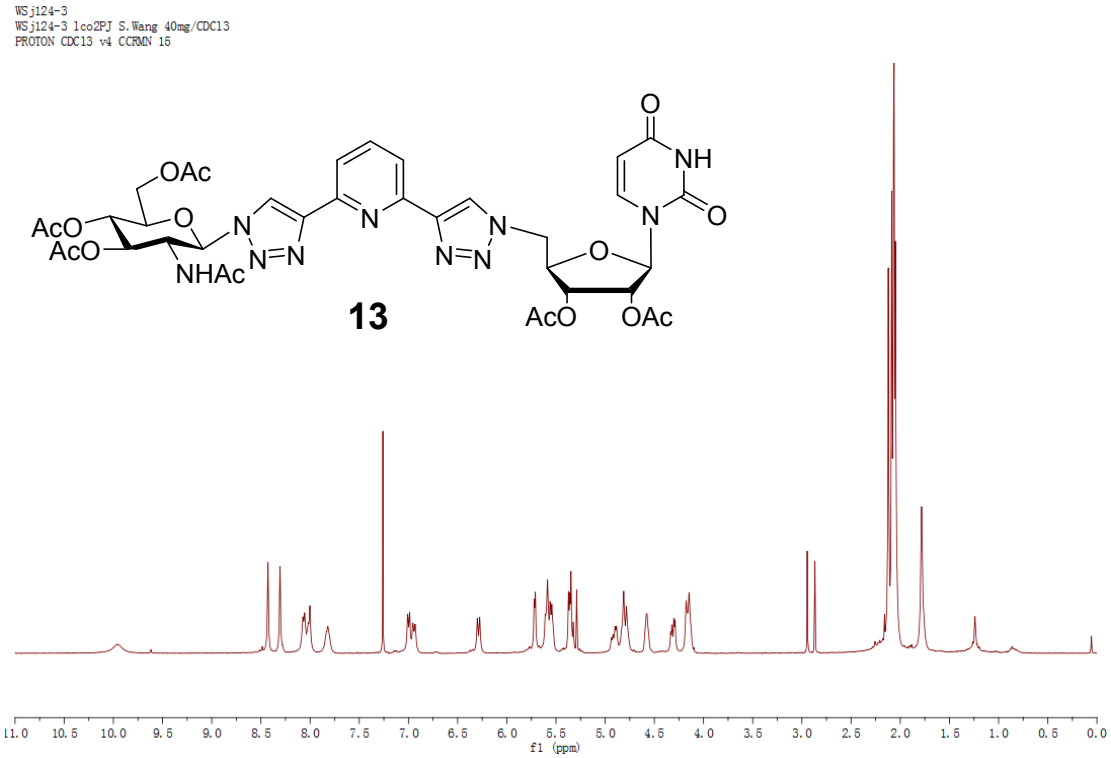
82.20
79.39
73.80
73.53
70.98
69.70
60.95
55.95
40.53
22.04
OH
HO
HO
O
NHAc
N
N=N
N
H
N
O
O
NH
N
O
HO
OH
11
2E+09
1E+09
9E+08
8E+08
7E+08
6E+08
5E+08
4E+08
3E+08
2E+08
1E+08
0
-1E+08
-2E+08
-3E+08
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

2-[(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl]-6-[1-(2',3'-di-*O*-acetyl-uridin-5'-yl)-1*H*-1,2,3-triazol-4-yl]pyridine (13)

DIPEA (0.78 mL, 4.38 mmol, 30.0 eq.) was added into a microwave tube containing 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (**2**) (54 mg, 0.15 mmol, 1.0 eq.), 2-ethynyl-6-[1-(uridin-5'-yl)-1*H*-1,2,3-triazol-4-yl]pyridine (**12**) (70 mg, 0.15 mmol, 1.0 eq.) and CuI (13 mg, 0.07 mmol, 0.5 eq.) in DMF (2 mL). The reaction mixture was heated for 15 min by microwave irradiation at 100°C. Then, the reaction mixture was diluted with EtOAc (40 mL), washed with satd aq. Na_2CO_3 solution (2×25 mL) and H_2O (30 mL). The combined aqueous phase was extracted with EtOAc (30 mL). The combined organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (100% PE to 100% EtOAc) to afford compound **13** (50 mg, 0.06 mmol, 40%). R_f = 0.16 (EtOAc); $[\alpha]_D$ = -44.0 (c = 0.1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 9.96 (s, 1H, N*H*$_{Uri}$), 8.43 (s, 1H, $H_{triazole}$), 8.31 (s, 1H, $H_{triazole}$), 8.14 – 7.95 (m, 2H, H_{Pyr}), 7.82 (s, 1H, H_{Pyr}), 7.04 – 6.88 (m, 2H, H_{6Uri}, N*H*Ac), 6.29 (d, *J* = 9.7 Hz, 1H, $H_{1GlcNAc}$), 5.72 (d, *J* = 4.2 Hz, 1H, H_{1Rib}), 5.64 – 5.51 (m, 3H, H_{5Uri}, H_{2Rib}, $H_{3GlcNAc}$), 5.43 – 5.27 (m, 2H, $H_{4GlcNAc}$, H_{3Rib}), 5.00 – 4.72 (m, 3H, H_{5aRib}, H_{5bRib}, $H_{2GlcNAc}$), 4.61 – 4.53 (m, 1H, H_{4Rib}), 4.31 (dd, *J* = 12.5, 5.1 Hz, 1H, $H_{6aGlcNAc}$), 4.20 – 4.09 (m, 2H, $H_{6bGlcNAc,}$ $H_{5GlcNAc}$), 2.15 – 2.01 (m, 15H, 5×COC*H*$_3$), 1.78 (s, 3H, NHCO*CH*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 171.5, 171.0, 170.8, 169.8, 169.7, 169.7 (*C*OCH$_3$), 163.0, 150.5, 149.9, 149.6, 148.7, 148.5, 141.6 (C_{6Uri}), 137.9 (C_{PyrH}), 124.6 ($C_{triazoleH}$), 121.2 ($C_{triazoleH}$), 119.7 (C_{PyrH}), 119.5 (C_{PyrH}), 103.6 (C_{5Uri}), 91.4 (C_{1Rib}), 85.9 ($C_{1GlcNAc}$), 80.1 (C_{4Rib}), 75.0 ($C_{5GlcNAc}$), 72.6 (C_{2Rib} or $C_{3GlcNAc}$), 72.0 (C_{2Rib} or $C_{3GlcNAc}$), 70.8 (C_{3Rib} or $C_{4GlcNAc}$), 68.4 (C_{3Rib} or $C_{4GlcNAc}$), 62.2 ($C_{6GlcNAc}$), 53.6 ($C_{2GlcNAc}$), 50.8 (C_{5Rib}), 23.0 (NHCO*C*H$_3$), 20.85 (CO*C*H$_3$), 20.83 (CO*C*H$_3$), 20.78 (CO*C*H$_3$), 20.56 (CO*C*H$_3$), 20.55 (CO*C*H$_3$); HR-ESI-MS (positive mode) *m*/*z*: calcd. for $C_{36}H_{41}N_{10}O_{15}$ $[M+H]^+$ 853.2747, found 853.2724.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AcO
AcO
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O
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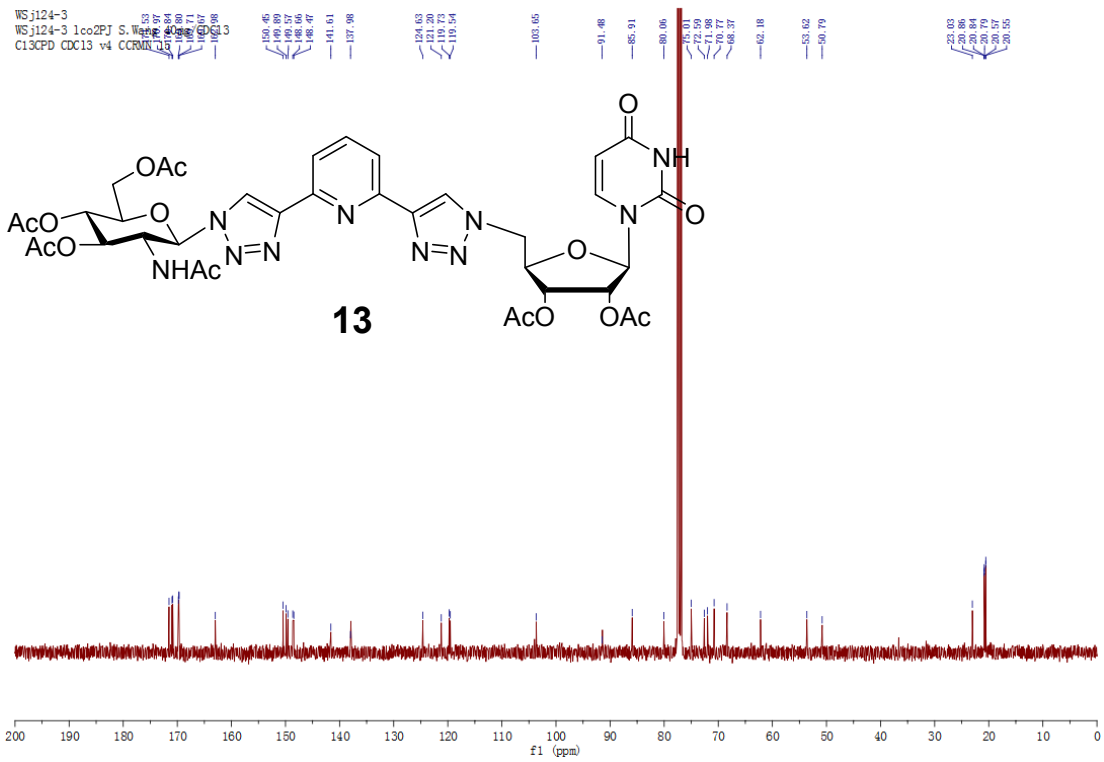
AcO
OAc
13
f1 (ppm)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AcO
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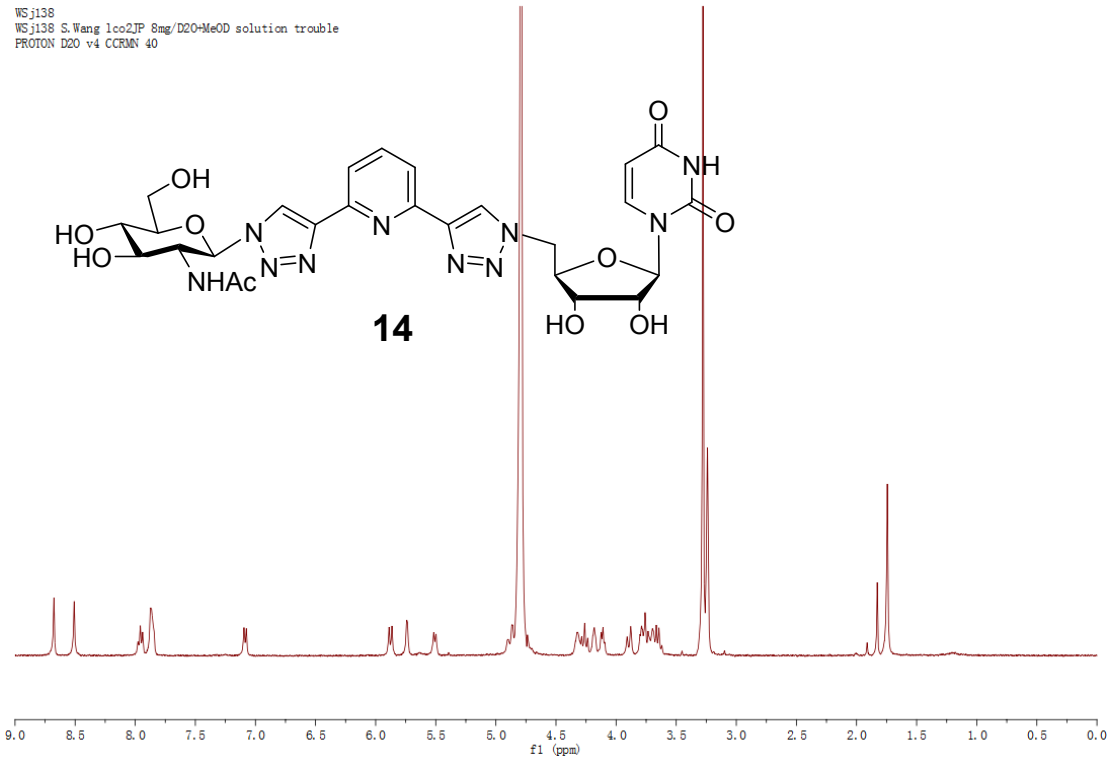
N
N=N
N
N=N
O
NH
N
O
AcO
OAc
13
f1 (ppm)

2-[(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl]-6-[1-(uridin-5'-yl)-1*H*-1,2,3-triazol-4-yl]pyridine (14)

In a 10 mL round-bottom flask containing compound **13** (35 mg, 0.04 mmol, 1.0 eq.) in CH_3OH (3 mL) was slowly added solid NaOMe until pH~9. Then, the reaction mixture was stirred at r.t. for 3 hours. DOWEX 50W×2 Resin (Fluka, 50-100 mesh, H^+ Form) was added to neutralize the reaction until pH~7. Then, the reaction mixture was filtrated and concentrated. The residue was purified by C18 reverse phase column chromatography (100% H_2O to 80% MeOH/H_2O) to afford compound **14** (24 mg, 0.036 mmol, 91%). $[\alpha]_D$ = -28.8 (c = 0.08, DMSO); ^{1}H NMR (400 MHz, D_2O:CD_3OD = 2:1) δ (ppm) = 8.72 (s, 1H, $H_{triazole}$), 8.55 (s, 1H, $H_{triazole}$), 8.06 – 7.98 (m, 1H, H_{Pyr}), 7.91 – 7.82 (m, 2H, 2xH_{Pyr}), 7.21 (d, J = 8.0 Hz, 1H, H_{6Uri}), 5.94 (d, J = 9.7 Hz, 1H, $H_{1GlcNAc}$), 5.78 (d, J = 3.3 Hz, 1H, H_{1Rib}), 5.60 (d, J = 8.0 Hz, 1H, H_{5Uri}), 4.94 (dd, J = 14.8, 3.5 Hz, 1H, H_{5aRib}), 4.86 (dd, J = 14.8, 5.7 Hz, 1H, H_{5bRib}), 4.40 (ddd, J = 9.9, 5.7 3.5 Hz, 1H, H_{4Rib}), 4.34 (dd appears as t, J = 9.7 Hz, 1H, $H_{2GlcNAc}$), 4.28 (dd, J = 5.6, 3.3 Hz, 1H, H_{2Rib}), 4.22 – 4.15 (m, 1H, H_{3Rib}), 3.96 (dd, J = 12.4, 1.8 Hz, 1H, $H_{6aGlcNAc}$), 3.89 – 3.68 (m, 4H, $H_{3GlcNAc}$, $H_{4GlcNAc}$, $H_{5GlcNAc}$, $H_{6bGlcNAc}$), 1.82 (s, 3H, NHCOCH_3); ^{13}C NMR (100 MHz, D_2O: CD_3OD = 2:1) δ (ppm) = 174.7, 150.0, 149.8, 148.3, 141.8 (C_{6Uri}), 140.1 (C_{PyrH}), 126.4 ($C_{triazoleH}$), 123.9 ($C_{triazoleH}$), 121.2 (s, 2C, 2xC_{PyrH}), 103.6 (C_{5Uri}), 92.5 (C_{1Rib}), 87.6 ($C_{1GlcNAc}$), 81.6 (C_{4Rib}), 80.2 ($C_{3GlcNAc}$ or $C_{4GlcNAc}$ or $C_{5GlcNAc}$), 74.7 ($C_{3GlcNAc}$ or $C_{4GlcNAc}$ or $C_{5GlcNAc}$), 73.9 ($C_{3GlcNAc}$ or $C_{4GlcNAc}$ or $C_{5GlcNAc}$), 71.4 (C_{2Rib}), 70.5 (C_{3Rib}), 61.6 ($C_{6GlcNAc}$), 56.6 ($C_{2GlcNAc}$), 52.2 (C_{5Rib}), 22.6 (NHCOCH$_3$); HR-ESI-MS (positive mode) m/z: calcd. for $C_{26}H_{30}N_{10}NaO_{10}$ $[M+Na]^+$ 665.2039, found 665.2023.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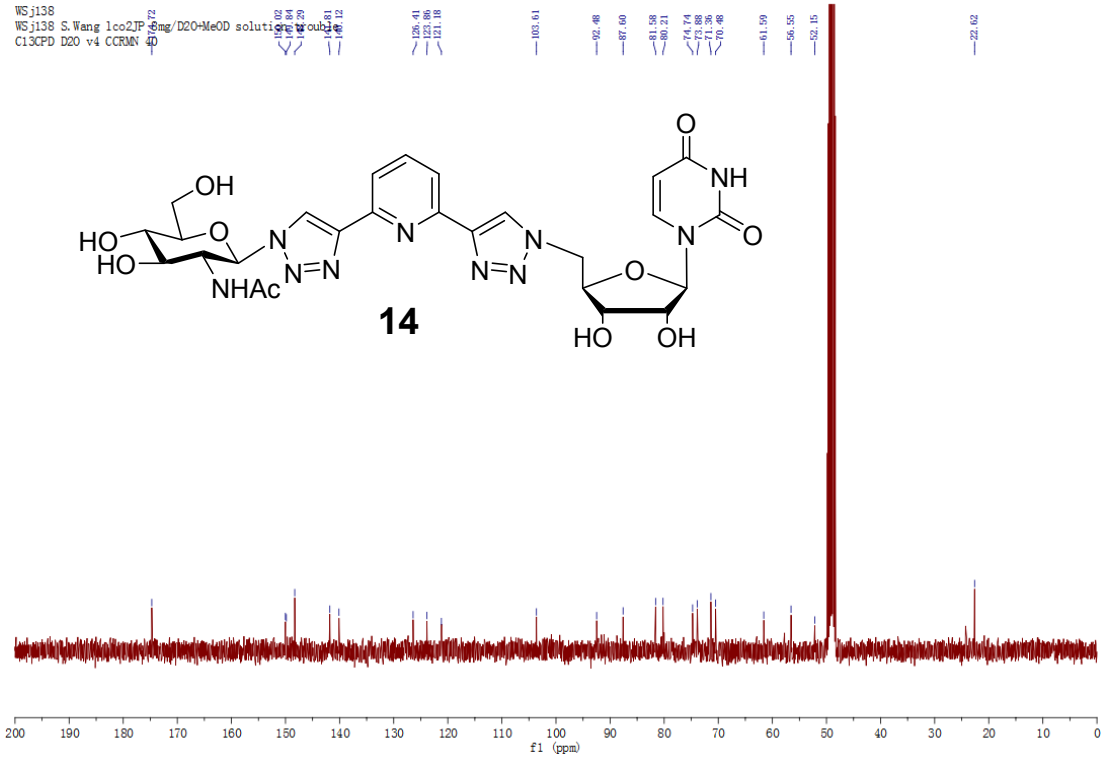
f1 (ppm)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C13CPD D2O v4 CCRMN 40
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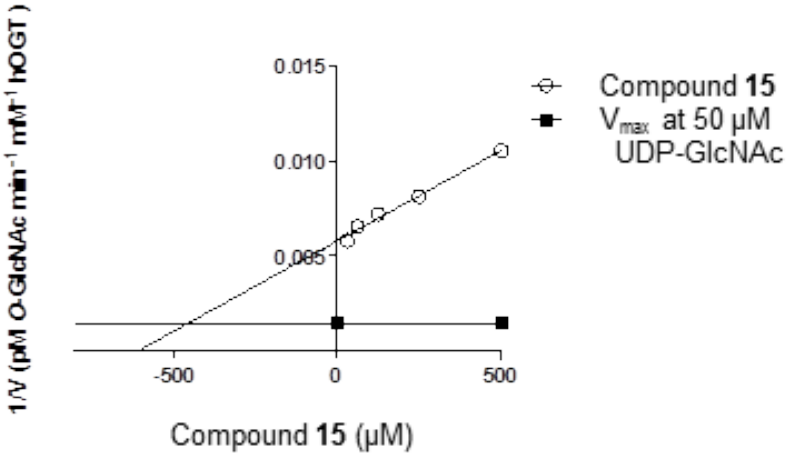
f1 (ppm)

Biological evaluations

Dixon analysis for compound 15:



Cell culture. Human breast cancer epithelial MCF7 cells and human cervix adenocarcinoma HeLa cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) (Lonza) supplemented with 10% (v/v) fetal calf serum (Lonza), 200 mM L-glutamine, 10 U/mL penicillin and 10 µg/mL streptomycin (Life Technologies, Invitrogen). Human mammary epithelial MCF10A cells were grown in the Mammary Epithelial cell Growth Medium (MEGM) composed of Mammary Epithelial Basal Medium (MEBM) supplemented with 13 mg/ml bovine pituitary extract (BPE), 10 µg/mL human epidermal growth factor (hEGF), 0.5 mg/mL hydrocortisone, and 5 mg/ml insulin (Lonza). Cells were grown at 37 °C in a humidified atmosphere enriched with 5% CO_2. To test the various compounds, cells were grown in a 24-wells plate in complete medium. When cells reached 70% confluence, medium was changed and the compound was added at the indicated concentrations (from 50 to 500 µM) for 24 or 48 hours. The cell active OGT inhibitor Ac-5SGlcNAc (2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-5-thio-α-D-glycopyranose) was used as a positive control (50 and 100 µM).[1]

Cell lysis and Western blotting. After treatment, cells were lysed on ice for 10 min in 100 µL lysis buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 0.1% (w/v) SDS, 1% (v/v) Triton-X100) containing the complete protease inhibitors cocktail (Roche Diagnostics, Meylan, France), 50 mM sodium fluoride (Sigma-Aldrich) and 100 µM orthovanadate (Sigma-Aldrich). Cell lysate was centrifuged at 20,000×g for 10 min and supernatant was collected. Protein concentration was measured using the microBCA protein assay kit (Pierce, Fisher Scientific, Illkirch, France). Proteins (20 µg) were separated by SDS-PAGE and transferred onto nitrocellulose membrane (Hybond ™-C EXTRA, GE Healthcare). Membranes were blocked in 5% (w/v) nonfat dry milk in Tris-Buffered Saline with 0.05% (v/v) Tween 20 (TBS-T) and probed with primary antibodies directed against O-GlcNAc (RL2, 1:4000) (Ozyme), and GAPDH (1:5000) (Abcam) overnight at 4 °C. After 3 washes in TBS-T, membranes were incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (anti-rabbit IgG-HRP linked, 1:30,000, GE Healthcare) for 1 h at room temperature. After several washes, blots were developed using enhanced chemiluminescence (ECL Prime Reagent, Hyperfilm™ MP; GE Healthcare).

Permeation assays. 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) solution (stock solution, 25 mg/mL in chloroform, Avanti Polar Lipids) was dried in a round bottom flask, and dispersed with buffer (PBS buffer containing inhibitors (0.01 M) or PBS buffer). The suspension was vortexed for 5-10 min until an emulsion was formed. Then, the emulsion was submitted to 5 freeze/thaw cycles (5 min in liquid N_2 / 10 min in water bath at 37°C / 1 min vortex) to yield unilamellar structures, followed by extrusion to the desired size of ca. 200 nm. To check inhibitor penetration across lipid membrane, liposomes were incubated overnight at 37°C for 24 hours in a shaking incubator. Separation of liposomes from free inhibitors was performed using gel filtration chromatography (G-25 column - GE Healthcare). Liposomes (200 nm in diameter) are eluted in the 4 mL void volume of the column. Small size molecules penetrated inside column gel, and were eluted later with more than 6 mL. After 30 mL of elution, UV absorption at 260 nm and UV spectra of 230–400 nm regions were measured for each fraction using Tecan Infinite M200 reader.

OGT inhibition in a cellular assay

To evaluate the effect of the OGT inhibitors on *O*-GlcNAc levels, MCF7 cells were treated with increasing concentrations of inhibitors. Two incubation times of 24 and 48 hours were selected since changes in O-GlcNAcylation levels can take longer than a few hours.[2] After 24 or 48 hours of treatment, intracellular *O*-GlcNAcylated proteins were analyzed by western-blot using the anti-O-GlcNAc antibody RL2. In parallel, Ac-5SGlcNAc (5S-G) was used as a positive control of OGT inhibition since this compound can act in cells.[1] No significant decrease in *O*-GlcNAc protein level was observed when using any of the inhibitors assayed at 50 or 100 µM concentrations (**Figure S1**), whereas *O*-GlcNAcylation of proteins was strongly decreased when MCF7 cells were treated with Ac-5SGlcNAc at a concentration of 50 µM.

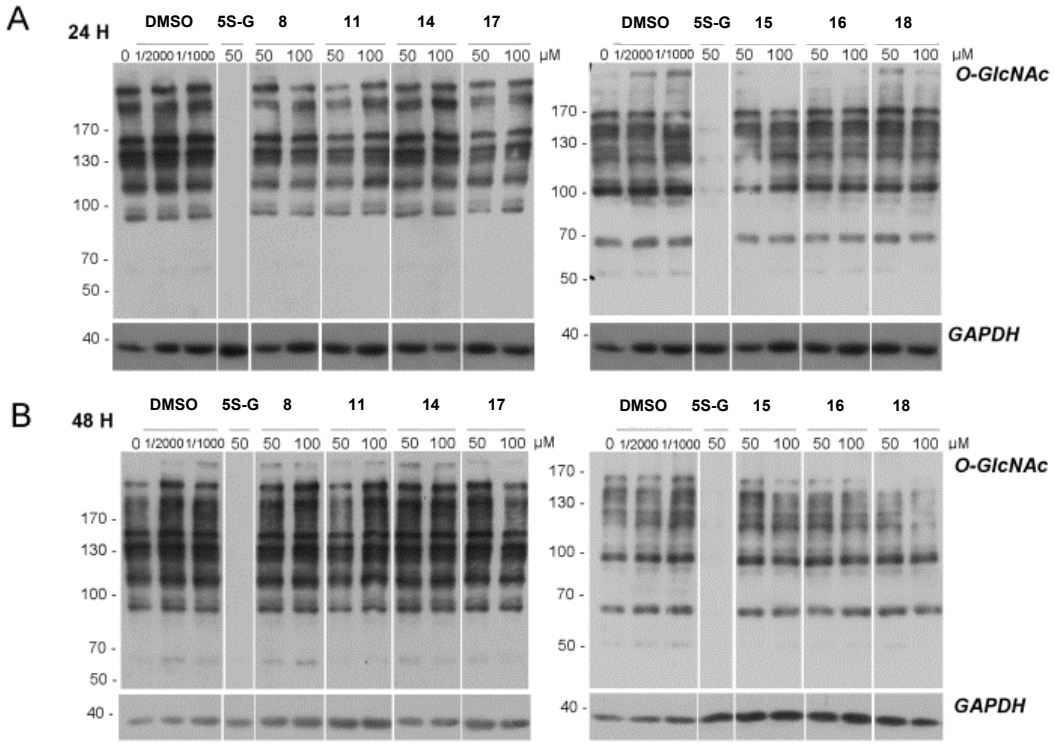


Figure S1. Inhibition of O-GlcNAcylation at inhibitor concentrations of 50 and 100 µM. MCF7 cells were treated for (**A**) 24 hours or (**B**) 48 hours with DMSO (vehicle, negative control), OGT inhibitors

8, **11**, **14-18**, or Ac-5SGlcNAc (5S-G, positive control). O-GlcNAc levels were examined by immunoblotting using the RL2 antibody. GAPDH was used as loading control. Molecular weight standards are indicated in kDa at the left. Results shown are representative of 3 independent experiments.

This cellular result was further investigated only for the inhibitor **15** which shows the largest inhibition percent *in vitro* at higher concentrations (100, 200 and 500 µM) and using three different cell lines (MCF7, MCF10A and HeLa) (**Figure S2**). After both 24 and 48 hours of treatment the OGT inhibitor **15** did not result in any decrease in levels of *O*-GlcNAcylation in any of these cell lines.

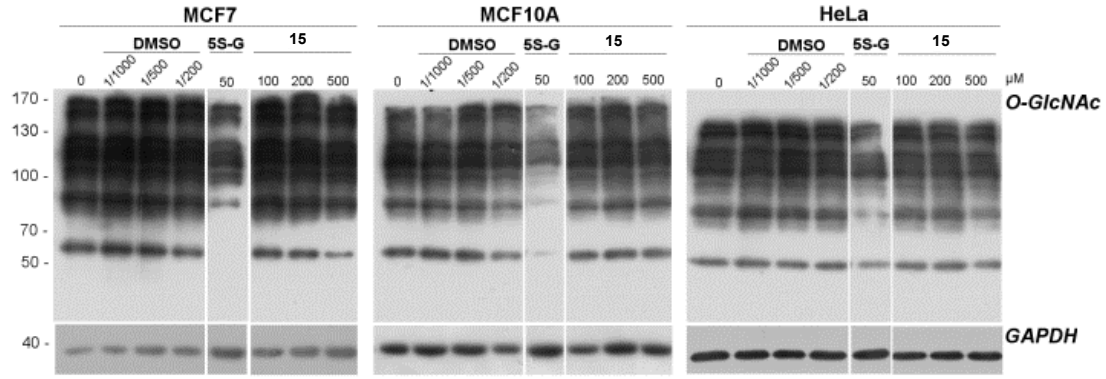


Figure S2. Inhibition of *O*-GlcNAcylation at 100, 200 and 500 µM. MCF7, MCF10A and HeLa cells were treated for 24 hours with DMSO (vehicle, negative control), inhibitor **15**, or Ac-5SGlcNAc (5S-G, positive control). *O*-GlcNAc level was examined by immunoblotting using RL2 antibody. GAPDH was used as loading control. Molecular weight standards are indicated in kDa at the left. Representative results from two independent experiments are shown.

Permeation assays

The permeation of organic molecules through a cellular membrane can be studied using various model systems. Among these systems, we have designed a straightforward assay in which the cell membrane is mimicked by the lipid bilayer of liposomes. The penetration of organic molecules into the liposomes was detected through their intrinsic UV. Two candidates were tested here: UDP-Gal analogue **20**, synthesized in a parallel study on galactosyltransferases[3] and used in this work as a model compound for permeation assays, and UDP-GlcNAc analogue **15** designed herein. Two approaches have been designed (**Figure S3**) for investigating the permeation of the studied compounds: the "out-in" method, molecule passage from outside to inside the liposomes or the "in-out" method, from the inside to outside.

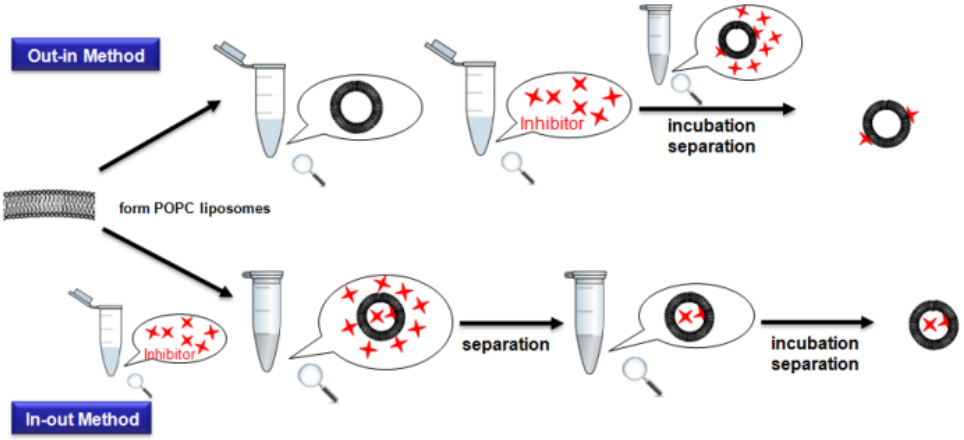


Figure S3. Schematic representation of the liposome-based permeation assays.

In the "out-in" method, liposomes were incubated with the model compound UDP-Gal analogue **20** (**Figure S4**). The liposomes were separated from the excess of organic molecules by gel filtration chromatography (**Figure S4A**). The liposome-inhibitor complex was recovered in the void volume of the column (fraction 4) whereas the soluble inhibitor was recovered in fractions 7-11. In the case of analogue **20**, a shoulder was observed at λ = 265 nm characteristic for the analogue **20**, attesting its presence in the liposome-containing fraction. Of note, UV-vis absorption was measured for the empty liposomes (**Figure S4B**) displaying no specific absorption in the range of wavelengths used. The loaded liposome fraction 4 was further incubated in buffer to test whether the molecule can travel through the liposome membrane in reverse direction. The analogue **20** was not detected in the solution (**Figure S4C**).

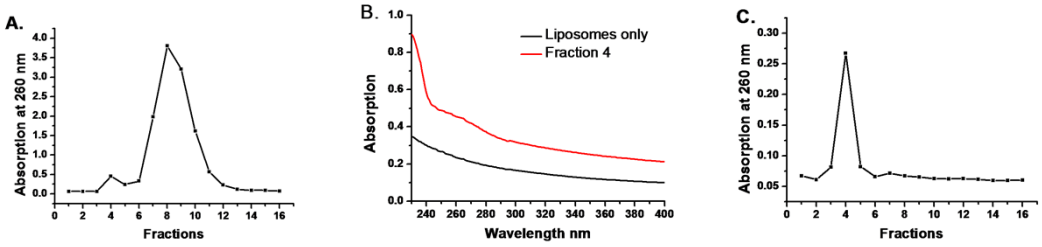


Figure S4. "Out-in" permeation tests for compound **20**. **A.** Incubation of liposomes with analogue **20** : UV absorption profile of gel filtration chromatography at 260 nm. **B.** UV spectra of liposome-**20** fraction (Fraction **4**, top), compared with liposomes only (bottom). **C.** Release of analogue **20** from liposome-**20**: UV absorption profile at 260 nm after gel filtration separation of liposomes and soluble molecules.

Alternatively, liposomes were prepared in the presence of analogue **20** ("in-out" method), and then the excess inhibitor was removed by gel filtration. The inhibitor-loaded liposomes were further incubated overnight but did not release the inhibitor into the buffer solution (**Figure S5**). Based on both methods used for the assessment of permeation, analogue **20** can be incorporated physically into liposomes as evidenced by the "out-in" method (**Figure S4**), but its passive permeation through the lipid bilayer due to osmotic pressure equilibration could not be characterized since no quantity of analogue could be detected when loaded liposomes were incubated in a buffer solution. The compound is therefore most

probably sequestered at the surface of the lipid bilayer but cannot travel through the membrane in either direction.

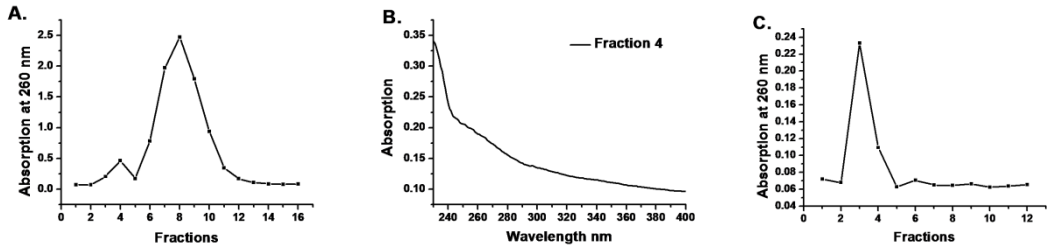


Figure S5. "In-out" permeation test for compound **20**. A. Formation of liposomes with analogue **20**: UV absorption profile of gel filtration chromatography at 260 nm. B. UV spectra of liposome-**20** fraction (**Fraction 4**). C. Release of analogue **20** from liposome-**20**: UV absorption profile at 260 nm after gel filtration separation of liposomes and soluble molecules.

The same study was performed with the best inhibitor of OGT identified here, namely compound **15**, through the "out-in" and "in-out" methods. Permeation of compound **15** could not be observed through the "out-in" method (**Figure S6**). When liposomes were prepared in the presence of compound **15** ("in-out" method), the incorporation of the organic molecule into the liposome could be readily achieved, but again release in the buffer solution could not be detected (**Figure S7**). The same conclusion can therefore be invoked: the UDP-GlcNAc analogue **15** can be incorporated into liposomes, but does not cross the membrane.

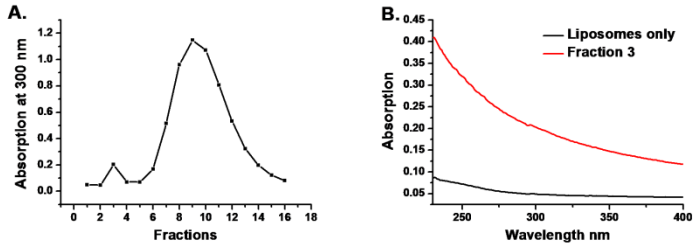


Figure S6. "Out-in" permeation tests for compound **15**. **A.** Incubation of liposomes with analogue **15**: UV absorption profile of gel filtration chromatography at 300 nm. **B.** UV spectra of Fraction 3 (top), compared with liposome only (bottom).

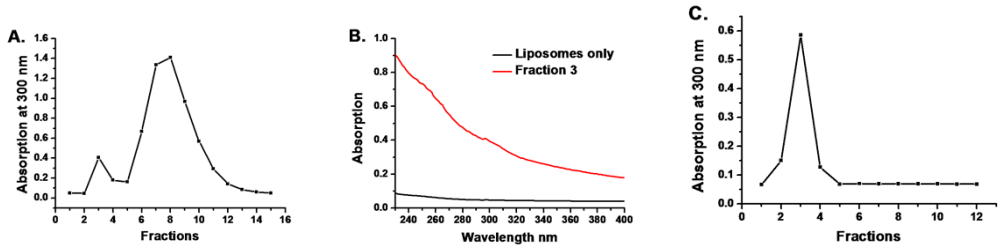


Figure S7. "In-out" permeation test for compound **15**. **A.** Formation of liposomes with analogue **5**: UV absorption profile of gel filtration chromatography at 300 nm. **B.** UV spectra of liposome-**15** fraction (**Fraction 3**, top), compared with liposomes only (bottom). **C.** Release of analogue **15** from liposome-**15**: UV absorption profile at 300 nm after gel filtration separation of liposomes and soluble molecules.

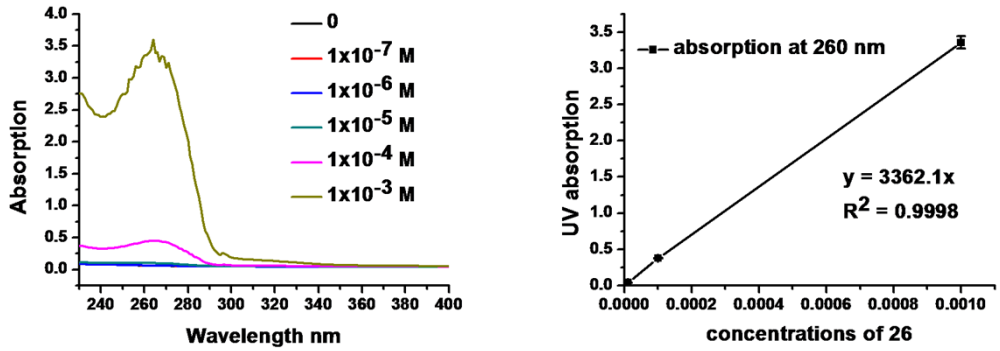


Figure S8. UV-titration of compound **20**. For UV-titration, UV absorption of **20** at concentrations of 0, 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M, 10^{-3} M in 1×PBS buffer were measured in duplicates. Linear regression fit of **20** was taken at 260 nm (λ_{max} = 263 nm).

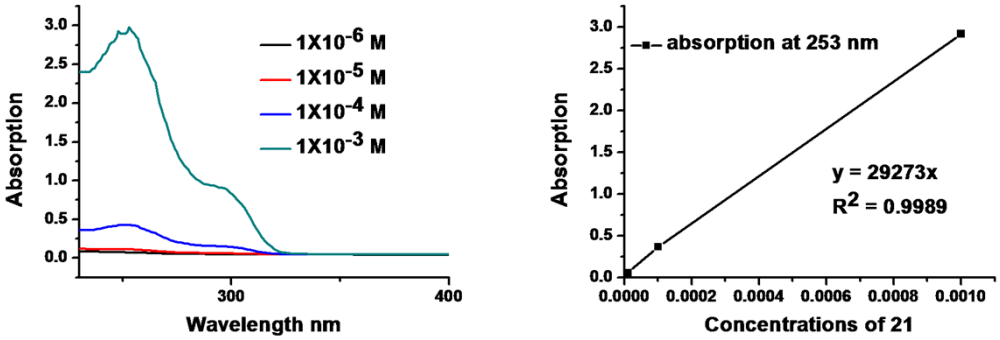


Figure S9. UV-titration of compound **15**. For UV-titration, UV absorption of **15** at concentrations of 10^{-6} M, 10^{-5} M, 10^{-4} M, 10^{-3} M in 1×PBS buffer were measured in duplicates. Linear regression fit of **15** was taken at 253 nm (λ_{max} = 253 nm and a shoulder peak at 300 nm).

References

1. T. M. Gloster, W. F. Zandberg, J. E. Heinonen, D. L. Shen, L. Deng and D. J. Vocadlo, *Nat. Chem. Biol.* 2011, **7**, 174-181.

2. L. Drougat, S. Olivier-Van Stichelen, M. Mortuaire, F. Foulquier, A.-S. Lacoste, J.-C. Michalski, T. Lefebvre and A.-S. Vercoutter-Edouart, *Biochim. Biophys. Acta* 2012, **1820**, 1839-1848.

3. S. Wang, J. A. Cuesta-Seijo, D. Lafont, M. M. Palcic and S. Vidal, *Chem. Eur. J.* 2013, **19**, 15346-15357.