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Monitoring glycosidase activity for clustered sugar substrates, a study on β-glucuronidase.

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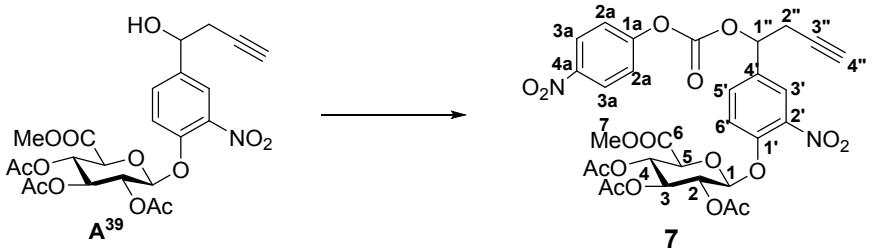
Experimental Procedures (Chemistry)

Materials

NMR spectra were recorded at room temperature with a Bruker Avance 300 Ultra Shield or eBruker Avance III 400 spectrometer and chemical shifts are reported in parts per million relative to tetramethylsilane or a residual solvent peak ($CDCl_3$: ^1H: δ=7.26, ^{13}C: δ=77.2; DMSO-d6: ^1H: δ=2.54, ^{13}C: δ=40.4; MeOD: ^1H: δ=3.31, ^{13}C: δ=49.0). Peak multiplicity is reported as: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). High resolution mass spectra HRMS where obtained by Electrospray Ionisation (ESI) on a Micromass-Waters Q-TOF Xevo G2-XS or with a Bruker Autoflex III SmartBeam spectrometer (MALDI). Low-resolution mass spectra (MS) were recorded with a Thermo electron DSQ spectrometer. All reagents were purchased from Acros Organics or Aldrich and were used without further purification. Column chromatography was conducted on silica gel Kieselgel SI60 (40-63 μm) from Merck. Reactions requiring anhydrous conditions were performed under argon. Dichloromethane was distilled from calcium hydride under nitrogen prior to use.

Compound **7**

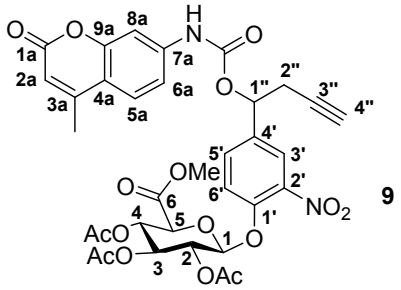


To a solution of the precursor **A**[39] (180 mg, 0.34 mmol) and 4-nitrophenyl chloroformate (140 mg, 0.68 mmol) in dry dichloromethane (3.5 mL) was added pyridine (77 μL, 0.87 mmol) at 0°C. The mixture was stirred 1 hour at room temperature, hydrolyzed with saturated aqueous $NaHCO_3$. The mixture was extracted three times with dichloromethane and the combined organic layers were dried over $MgSO_4$, filtrated and concentrated in *vacuo*. Purification by column chromatography over silica gel (petroleum ether/ethyl acetate 6/4) afforded **7** (235 mg, 0.343 mmol, quantitative yield) as a mixture of two diastereoisomers (white solid). * Compound **A** was prepared as previously described in the literature (B. Renoux, T. Legigan, S. Bensalma, C. Chadéneau, J.-M. Muller and S. Papot, *Org. Biomol. Chem.*, 2011, **9**, 8459)

^1H NMR (400 MHz, $CDCl_3$); δ 8.3 (d, 2H, J = 9.1 Hz, $2H_{2a}$), 7.9 (d, 1H, J = 1.8 Hz, $H_{3'}$), 7.65 (dd, 1H, J = 8.7 Hz and J = 1.8 Hz, $H_{5'}$), 7.4 (m, 3H, $H_{6'}$, $2H_{3a}$), 5.8 (t, 1H, J = 6.5 Hz, $H_{1''}$), 5.3 (m, 4H, H_1, H_2, H_3, H_4), 4.2 (m, 1H, H_5), 3.7 (s, 3H, H_7), 2.90 (m, 2H, $H_{2''}$), 2.10 (s, 3H, OAc), 2.09 (t, J = 2 Hz, 1H, $H_{4''}$), 2.07 (s, 3H,OAc), 2.06 (s, 3H,OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1(C=O, Ac), 169.5 (C=O, Ac), 169.3 (C=O, Ac), 166.8 (C=O, COOMe), 155.3 ($C_{carbonate}$), 149.6 (Car), 145.7 (Car), 141.1 (Car), 133.4 (Car), 132.4 (Car), 132.3 (Car), 125.5 (Car), 123.8 (Car), 121.8 (Car), 119.9 (Car), 99.4 (C_1), 77.2 ($C_{3''}$), 72.6 (C_5), 71.0 ($C_{1''}$), 70.2 ($C_{4''}$), 68.7, (3CH, C_2, C_3, C_4), 53.2 (CH_3, COOMe), 26.1 ($C_{2''}$), 20.4 ($3CH_3$,OAc); HRESI-MS: m/z 711.1280 (calcd. for $C_{30}H_{28}O_{17}N_2Na$ 711.1286 $[M+Na]^+$)

* Compound **A** was prepared as previously described in the literature (B. Renoux, T. Legigan, S. Bensalma, C. Chadéneau, J.-M. Muller and S. Papot, *Org. Biomol. Chem.*, 2011, **9**, 8459)

Compound **9**

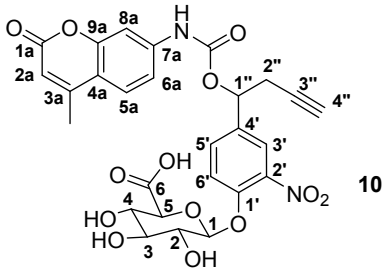


9

The activated carbonate **7** (1.28 mmol, 900 mg, 1 equiv.), the 7-amino-4-methyl-2H-chromen-2-one **8** (3.84 mmol, 619 mg, 3 equiv.) and hydroxybenzotriazole (1.28 mmol, 173 mg, 1 equiv.) were dissolved in 4.5 mL of dry dimethylformamide and diisopropylethylamine (1.54 mmol, 199 mg, 268 mL, 1.2eq) was added. The reaction mixture was heated at 50°C for 24 hours. The reaction mixture was cooled down and the DMF was evaporated under high vacuum. The crude was dissolved into water and ethyl acetate. The organic layer was separated and washed 4 times with a saturated aqueous solution of $NaHCO_3$. The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The compound **9**, obtained as a mixture of diastereoisomers, was isolated after successive purifications by automated flash-chromatography using a linear gradient of CH_2Cl_2/MeOH from 100/0 to 95/5 (349 mg, yield 38%).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm); 7.92 (t, *J* = 1.9Hz, 1H, $H_{3'}$), 7.60 (ddd, *J* = 2.2Hz, *J* = 4.1Hz, *J* = 8.6Hz, 1H, $H_{5'}$), 7.52 (dd, *J* = 0.7Hz, *J* = 8.6Hz, 1H, H_{5a}), 7.46 (d, *J* = 1.9Hz, 1H, H_{8a}), 7.38 (m, 2H, $H_{6'}$ and H_{6a}), 7.34 (br.d, *J* = 6.5Hz, 1H, NH), 6.20 (br.s, 1H, H_{2a}), 5.89 (t, *J* = 6.3Hz, 1H, $H_{1''}$), 5.33 (m, 3H, H_2, H_3 and H_4), 5.23 (d, *J* = 6.9Hz, 1H, H_1), 4.24 (d, *J* = 8.3Hz, 1H, H_5), 3.73 (s, 3H, OMe), 2.83 (m, 2H, $H_{2''}$), 2.41 (d, *J* = 0.8Hz, 3H, Me), 2.12 (d, *J* = 3.8Hz, 3H, Ac), 2.06-2.05 (m, 7H, 2Ac and $H_{1''}$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 170.2 (C=O, Ac), 169.5 (C=O, Ac), 169.4 (C=O, Ac), 166.9 (CO, COOMe), 161.2 (C=O, coumarine), 154.5 (C^{IV}_{ar}, coumarine), 152.4 (C^{IV}_{ar}, coumarine), 151.7 (C=O, NHCOO), 149.3 (C^{IV}_{ar}, nitrophenyl), 141.2 and 141.1 and 141.0 ($2C^{IV}_{ar}$, coumarine and nitrophenyl), 135.0 and 134.9 (C^{IV}_{ar}, nitrophenyl), 132.5 and 132.3 (CH_{ar}, $C_{5'}$), 125.6 (CH_{ar}, C_{5a}), 123.6 and 123.3 (CH_{ar}, $C_{3'}$), 119.9 (CH_{ar}, $C_{6'}$), 116.0 (C^{IV}_{ar}, coumarine), 114.6 (CH_{ar}, C_{6a}), 113.5 (CH_{ar}, C_{2a}), 106.3 (CH_{ar}, C_{8a}), 99.7 (CH, C_1), 78.3 (C^{IV}, $C_{3''}$), 73.3 (CH, $C_{1''}$), 72.7 (CH, C_5), 72.3 (CH, $C_{4''}$), 71.2 and 71.1 and 70.3 and 68.8 and 68.8 (3CH, C_2, C_3 and C_4), 53.2 and 53.2 (CH_3, COOMe), 26.5 and 26.5 (CH_2, $C_{2''}$), 20.7 and 20.7 and 20.6 ($3CH_3$, OAc), 18.7 (CH_3, coumarine); HRMS (ESI) *m/z*: $C_{34}H_{32}N_2NaO_{16}$ $[M+Na]^+_{calculated}$ = 747.1644, $[M+Na]^+_{measured}$ = 747.1642

Compound **10**

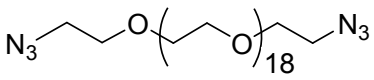


10

Compound **9** (315 mg, 0.435 mmol, 1 equiv.) was dissolved in MeOH (40 mL). The mixture was cooled at 0°C and a solution of lithium hydroxide monohydrate (192 mg, 4.57 mmol, 10.5 equiv.) in water (10 mL) was added dropwise. The mixture was stirred for 2hrs, hydrolyzed with IRC-50 acidic resin, filtrated and concentrated in *vacuo*. High degree of purity for **10** was obtained using automated reverse phase column chromatography (gradient elution 10% to 100% MeCN in H_2O (0.05% TFA) as a mixture of two diastereoisomers (148 mg, 0.25 mmol, 58 %, purity > 95%).

^{1}H NMR (400 MHz, CD_3OD, δ ppm) 7.97 (d, *J* = 2Hz, $H_{3'}$), 7.72 (dd, *J* = 2Hz, *J* = 8.8Hz, $H_{5'}$), 7.67 (d, *J* = 8.7Hz, H_{5a}), 7.60 (d, *J* = 2Hz, H_{8a}), 7.44 (d, *J* = 8.8Hz, $H_{6'}$), 7.39 (dd, *J* = 2Hz, *J* = 8.8Hz, H_{6a}), 6.20 (br.s, H_{2a}), 5.90 (t, *J* = 6Hz, $H_{1''}$), 5.19 (dd, *J* = 1Hz, *J* = 7Hz, H_1), 4.04 (d, *J* = 9.7Hz, H_5), 3.63 (t, 1H, *J* = 9.2 Hz, H_4), 3.53 (m, 2H, H_2 and H_3), 2.87 (m, 2H, $H_{2''}$), 2.44 (d, *J* = 1Hz, 3H, Me), 2.38 (t, 1H, *J* = 2.5 Hz, $H_{4'}$);^{13}C NMR (100 MHz, CD_3OD, δ ppm): 173.3, 163.4, 155.6, 155.4, 154.3, 154.0, 151.0, 150.9, 144.8 (C_{IV}), 144.1(C_{IV}), 142.2 ($C_{3''}$), 135.5 (C_{IV}), 133.3, 133.2 (C_{5a}), 126.8 ($C_{5'}$), 124.9 ($C_{4''}$), 124.4 ($C_{3'}$), 118.8 ($C_{6'}$), 116.4 (C_{IV}), 116.0 (C^{6a}), 113.1 (C_{2a}), 106.5 (C_{8a}), 102.4 (C_1), 79.8, 77.3 (C_2), 76.6 (C_5), 74.4 (C_3), 72.7 (C_4), 67.9, 27.0, 18.5; HRMS (ESI) *m/z*: $C_{27}H_{23}N_2O_{13}$ $[M\text{-}H]^+_{calculated}$ = 583.1206, $[M\text{-}H]^+_{measured}$ = 583.1201.

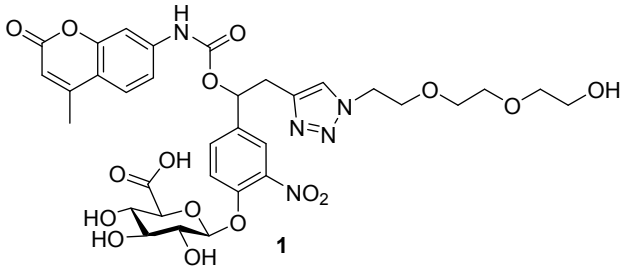
icosa-(ethylene glycol) diazide (n=18)



Mesyl chloride (103 µL, 1.3 mmol) was added dropwise at 0°C to a solution of icosaethylene glycol (500 mg, 0.55 mmol) in CH_2Cl_2 (20 mL) with triethylamine (300 µL, 2.2 mmol). After 30 minutes the reaction was heated at room temperature and stirred overnight. The mixture was concentrated under reduced pressure and used in the next step without purification. The crude product was dissolved in DMF (20 mL) and sodium azide (108 mg, 1.66 mmol) was added. The mixture was heated at 80°C overnight, and then concentrated under reduced pressure. The crude product was dissolved in DCM (15 mL) and basic resin IRN 78 (excess) was added to remove trimethylamine salt. After 1 hour the mixture was filtered through a Celite pad and the filtrate was evaporated. The solid was purified by flash column chromatography (DCM/MeOH: 98/2) to afford the diazide (359 mg, 68%) as a yellowish solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.72–3.55 (m, 76H, OCH_2), 3.37 (t, 4H, *J* = 5.1 Hz, CH_2N$_3$). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 70.7 (OCH_2), 70.1 ($CH_2CH_2N_3$), 50.7 (CH_2N_3). HRMS (ESI$^+$): Found 971.5379 $C_{40}H_{80}O_{19}N_6Na$ requires 971.5370.

Compound **1**

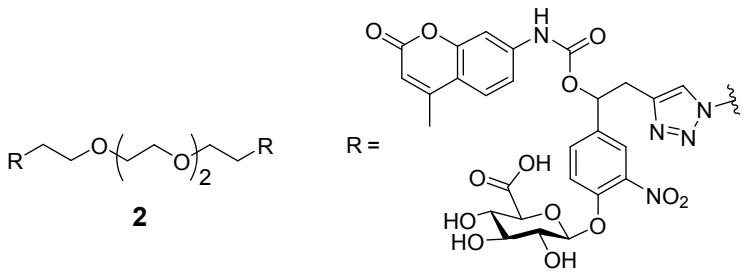


1

To a solution of mono-azide (7.2 mg, 41 µmol) and **10** (20 mg, 34.2 µmol) in dioxane-water (3 mL, 4-1) was added copper sulfate (2.5 mg, 10 µmol) and sodium ascorbate (4.1 mg, 20.5 µmol). The mixture was stirred overnight at room temperature and Quadrasil® MTU (25 mg, 25 µmol) was added to trap the residual copper. After 30 minutes the reaction was filtered through a pad of celite and the filtrate was evaporated under reduced pressure. The residue was purified by reverse phase column chromatography (H_20/ACN: 100 => 60/40) to afford **1** (22 mg, 94 %) as a yellowish solid.

^{1}H NMR (400MHz, MeOD) δ: 7.85-7.80 (m, 2H, H-3', H-4''), 7.62-7.56 (m, 2H, H-5a, H-5'), 7.49 (d, 1H, *J* = 1.93 Hz, H-8a), 7.40 (dd, 1H, *J* = 1.8 Hz, *J* = 8.8 Hz, H-6'), 7.30 (dd, 1H, *J* = 2.1 Hz, *J* = 8.7 Hz, H-6a), 6.14 (d, 1H, *J* = 1.1 Hz, H-2a), 6.05 (t, 1H, *J* = 6.5 Hz, H-1''), 5.18 (dd, 1H, *J* = 3.5 Hz, *J* = 7.5 Hz, H-1), 4.51 (t, 2H, *J* = 4.9 Hz, H-5''), 4.04 (d, 1H, *J* = 9.7 Hz, H-5), 3.83-3.77 (m, 2H, H-6''), 3.67-3.70 (m, 3H, H-4, H-10''), 3.57-3.46 (m, 10H, H-2, H-3, H-7'', H-8'', H-9''), 3.41-3.32 (m, 2H, H-2''), 2.39 (d, 3H, *J* = 1.0 Hz, Me); ^{13}C NMR (400MHz, MeOD) δ: 171.8, 163.3, 155.5, 155.3, 154.0, 150.7, 143.9 (C_{IV}), 143.7 (C_{IV}), 141.9 (C-3''), 135.9 (C_{IV}), 133.2 (C-5a), 126.7 (C-5'), 125.7 (C-4''), 124.2 (C-3'), 118.9 (C-6'), 116.3 (C_{IV}), 115.9 (C-6a), 113.1 (C-2a), 106.3 (C-8a), 102.3 (C-1), 77.3 (C-2), 76.6 (C-5), 75.5 (C-1''), 74.4 (C-3), 73.6 (C-9''), 72.6 (C-4), 70.4 (C-7'', C-8''), 62.2 (C-10''), 51.5 (C-5''), 33.4 (C-2''), 18.5 (CH_3); HRMS (ES+) m/z calcd for $C_{33}H_{38}N_5O_{16}$: 760.2314 found 760.2316.

Compound **2**



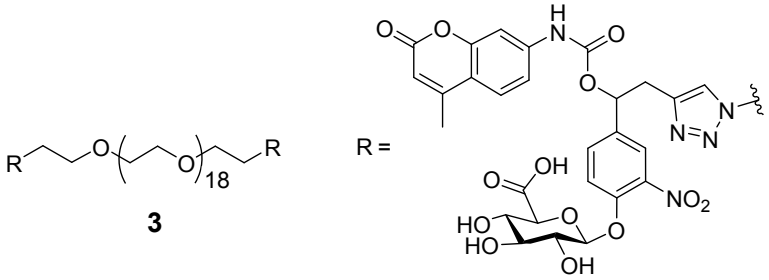
2

To a solution of diazide (4 mg, 16.4 µmol) and **10** (21.1 mg, 36.1 µmol) in dioxane-water (3 mL, 4-1) was added copper sulfate (2.46 mg, 9.84 µmol) and sodium ascorbate (3.90 mg, 19.7 µmol). The mixture was stirred overnight at room temperature and Quadrasil® MTU (25 mg, 25 µmol) was added to trap the residual copper. After 30 minutes the reaction was filtered through a Celite pad and the filtrate was evaporated under reduced pressure. The residue was purified by reverse phase column chromatography (H_20/ACN: 100 => 60/40) to afford **2** (18 mg, 78 %) as a yellowish solid.

^{1}H NMR (400MHz, DMSO-d6) δ: 10.41-10.35 (m, 2H, OH), 7.89-7.86 (m, 4H, H-3',H-4''), 7.69-7.61 (m, 4H, H-5_a , H-5'), 7.52-7.48 (m, 2H, H-8_a), 7.46-7.35 (m, 4H, H-6_a, H-6'), 6.21 (br s, 2H, H-2a), 6.06-5.99 (m, 2H, H-1''), 5.37-5.30 (m, 2H, OH), 5.22-5.11 (m, 4H, H-1, OH), 4.44 (t, 4H, *J* = 5.2 Hz, H-5''), 3.75-3.68 (m, 6H, H-5, H-6''), 3.43-3.22 (m, 18H, H-2, H-3, H-4, H-2'', 8*PEG, HDO), 2.37 (br s, 6H, Me); ^{13}C NMR (400MHz, DMSO-d6) δ: 173.2, 160.0, 153.8, 153.2, 152.4 (C_{IV}), 141.9 (C_{IV}), 139.7 (C-3''), 133.7 (C_{IV}), 132.1 (C-5a), 125.9 (C-5'), 123.5 (C-4''), 122.7 (C-3'), 116.9 (C-6'), 115.7 (C_{IV}), 114.4 (C-6a), 111.9 (C-2a), 104.6 (C-8a), 99.8 (C-1), 76.4 (C-2), 74.6 (C-5), 74.0 (C-1''), 72.8

(C-3), 71.7 (C-4), 69.5 (PEG), 68.8 (C-6''), 49.3 (C-5''), 31.9 (C-2''), 17.9 (CH_3); HRMS (ES-) m/z calcd for $C_{62}H_{63}N_{10}O_{29}$: 1411.3762 found 1411.3800.

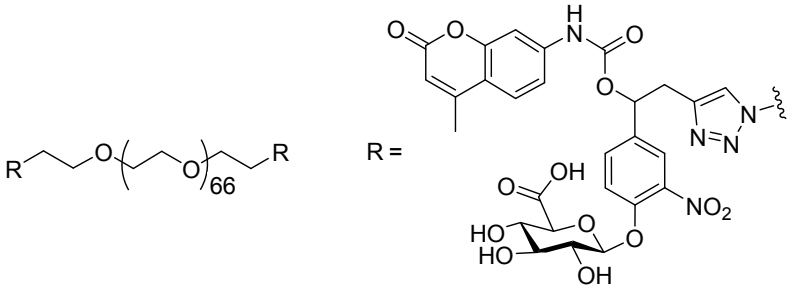
Compound **3**



Copper sulfate (0.33 mg, 1.32 µmol) and sodium ascorbate (0.52 mg, 2.63 µmol) were added to a solution of di-azide (2.5 mg, 2.63 µmol) and **10** (6.53 mg, 5.79 µmol) in dioxane-water (2 mL, 4-1). The mixture was stirred overnight at room temperature and Quadrasil® MTU (25 mg, 25 µmol) was added to trap the residual copper. After 30 minutes the reaction was filtered through a pad of celite and the filtrate was evaporated under reduced pressure. The residue was purified by reverse phase column chromatography (H_20/ACN: 100 => 60/40) to afford **3** (22 mg, 67 %) as a yellowish solid.

^{1}H NMR (400MHz, CD_3CN) δ: 7.79 (d, *J* = 1.6 Hz, 2H, H-3'), 7.62-7.52 (m, 4H, H-5_a , H-5'), 7.47-7.41 (m, 2H, H-8_a), 7.37-7.26 (m, 4H, H-6_a, H-6'), 6.13 (br s, 2H, H-2a), 5.97 (t, 2H, *J* = 6.3 Hz, H-1''), 5.18-5.11 (m, 2H, H-1), 4.45 (t, 4H, *J* = 5.1 Hz, H-5''), 4.00 (d, 2H, *J* = 9.7 Hz, H-5), 3.78-3.72 (m, 4H, H-6''), 3.62-3.46 (m, 78H, H-2, H-3, H-4, 72*PEG), 3.32-3.26 (m, 4H, H-2''), 2.38-2.32 (m, 6H, Me); ^{13}C NMR (400MHz, CD_3CN) δ: 170.4, 162.0, 154.9, 154.3, 153.2, 149.6 (C_{IV}), 142.9 (C_{IV}), 141.0 (C-3''), 135.5 (C_{IV}), 132.8 (C-5a), 126.5 (C-5'), 125.1 (C-4''), 123.6 (C-3'), 117.9 (C-6'), 115.8 (C_{IV}), 115.2 (C-6a), 112.9 (C-2a), 105.8 (C-8a), 101.2 (C-1), 76.1 (C-2), 75.6 (C-5), 74.8 (C-1''), 73.3 (C-3), 71.7 (C-4), 70.6 (PEG), 69.6 (C-6''), 50.8 (C-5''), 32.8 (C-2''), 18.4 (CH_3); HRMS (ES-) m/z calcd for $C_{94}H_{126}N_{10}O_{45}$: 2114.7879 found 2114.7898.

Compound **4**

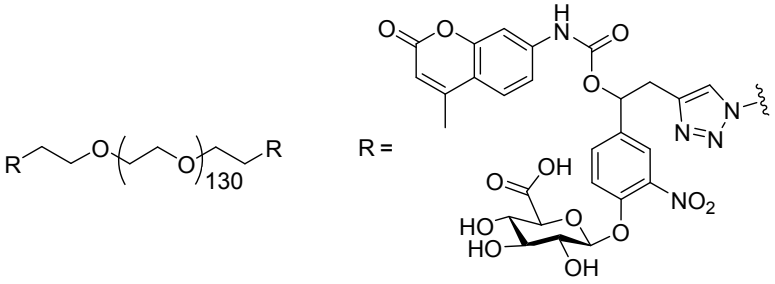


Copper sulfate (1.23 mg, 4.89 µmol) and sodium ascorbate (1.94 mg, 9.79 µmol) were added to a solution of diazide (25 mg, 8.16 µmol) and **10** (10.5 mg, 17.95 µmol) in dioxane-water (3 mL, 4-1). The mixture was stirred overnight at room temperature and Quadrasil® MTU (12.5 mg, 12.5 µmol) was added to trap the residual copper. After 30 minutes the reaction was filtered through a pad of celite and the filtrate was evaporated under reduced pressure. The residue was purified by reverse phase column chromatography (H_20/ACN: 100 => 60/40) to afford **4** (21 mg, 61%) as a yellowish solid.

^{1}H NMR (400MHz, MeOD) δ: 7.87-7.83 (m, 4H, H-3', H-4''), 7.69-7.59 (m, 4H, H-5_a , H-5'), 7.57-7.53 (m, 2H, H-8_a), 7.45-7.39 (m, 2H, H-6'), 7.38-7.31 (m, 2H, H-6_a), 6.18 (br s, 2H, H-2a), 6.08 (t, 2H, *J* = 6.4 Hz, H-1''), 5.21-5.16 (m, 2H, H-1), 4.52 (t, 4H, *J* = 4.8 Hz, H-5''), 4.03 (d, 2H, *J* = 10.3 Hz, H-5), 3.83-3.77 (m, 4H, H-6''), 3.65-3.55 (m, 270H, H-2, H-3, H-4, 264*PEG), 3.47-3.33 (m, 4H, H-2''), 2.43 (s, 6H, Me); ^{13}C NMR (400MHz, MeOD) δ: 171.6, 163.1, 155.6, 155.3, 153.9, 150.8 (C_{IV}), 144.0

(C_{IV}), 141.9 (C-3''), 135.9 (C_{IV}), 133.2 (C-5a), 126.9 (C-5'), 125.8 (C-4''), 124.2 (C-3'), 118.9 (C-6'), 116.4 (C_{IV}), 116.0 (C-6a), 113.2 (C-2a), 106.4 (C-8a), 102.4 (C-1), 77.4 (C-2), 76.7 (C-5), 75.5 (C-1''), 74.3 (C-3), 72.7 (C-4), 71.5 (PEG), 70.4 (C-6''), 51.5 (C-5''), 33.5 (C-2''), 18.6 (CH_3); HRMS (ES-) m/z calcd for $C_{190}H_{318}N_{10}O_{93}$: 4228.0462 found 4228.0454.

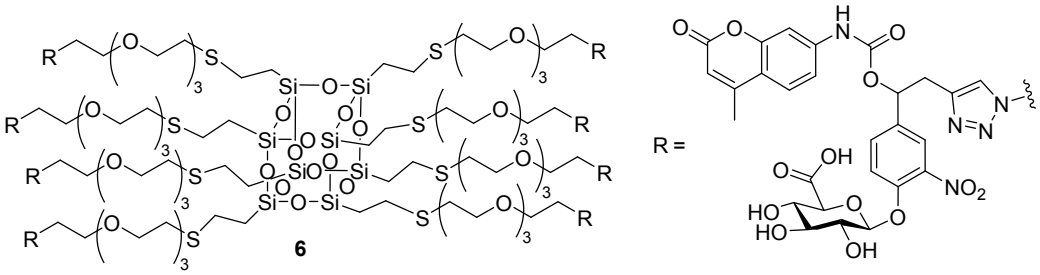
Compound **5**



To a solution of diazide (25 mg, 4.25 µmol) and **10** (5.50 mg, 9.35 µmol) in dioxane-water (3 mL, 4-1) was added copper sulfate (1.28 mg, 5.10 µmol) and sodium ascorbate (2.02 mg, 10.20 µmol). The mixture was stirred overnight at room temperature and Quadrasil® MTU (12.5 mg, 12.5 µmol) was added for trapped the residual copper. After 30 minutes the reaction was filtered through a Celite pad and the filtrate was evaporated under reduced pressure. The residue was purified by reverse phase column chromatography (H_20/ACN: 100 => 60/40) to afford **5** (19 mg, 62%) as a yellowish solid.

^{1}H NMR (500MHz, MeOD) δ: 7.88-7.82 (m, 4H, H-3', H-4''), 7.67 (d, 2H, *J* = 8.7 Hz, H-5a), 7.64-7.60 (m, 2H, H-5'), 7.58 (br s, 2H, H-8_a), 7.43 (dd, 2H, *J* = 2.5 Hz, *J* = 8.8 Hz, H-6'), 7.37 (d, 2H, *J* = 8.7 Hz, H-6_a), 6.20 (br s, 2H, H-2a), 6.08 (t, 2H, *J* = 6.5 Hz, H-1''), 5.20-5.16 (m, 2H, H-1), 4.53 (t, 4H, *J* = 4.9 Hz, H-5''), 4.04 (d, 2H, *J* = 9.9 Hz, H-5), 3.79-3.75 (m, 4H, H-6''), 3.70-3.50 (m, 526H, H-2, H-3, H-4, 520*PEG), 3.45-3.33 (m, 4H, H-2''), 2.44 (s, 6H, Me); ^{13}C NMR (500MHz, MeOD) δ: 171.7, 163.1, 155.6, 155.3, 154.0, 150.8 (C_{IV}), 144.1 (C_{IV}), 141.9 (C-3''), 135.9 (C_{IV}), 133.2 (C-5a), 126.9 (C-5'), 125.7 (C-4''), 124.2 (C-3'), 118.9 (C-6'), 116.5 (C_{IV}), 116.1 (C-6a), 113.2 (C-2a), 106.4 (C-8a), 102.4 (C-1), 77.4 (C-2), 76.7 (C-5), 75.6 (C-1''), 73.7 (C-3), 72.7 (C-4), 71.6 (PEG), 70.4 (C-6''), 51.5 (C-5''), 33.6 (C-2''), 18.6 (CH_3); HRMS (ES-) m/z calcd for $C_{318}H_{574}N_{10}O_{157}$: 7045.7239 found 7045.7138.

Compound **6**



To a solution of COSS-N_3 (10 mg, 3.97 µmol) and **10** (20.5 mg, 34.99 µmol) in dioxane-water (4 mL, 4-1) was added copper sulfate (2.40 mg, 9.54 µmol) and sodium ascorbate (3.78 mg, 10.08 µmol). The mixture was stirred overnight at room temperature and Quadrasil® MTU (25 mg, 25 µmol) was added for trapping the residual copper. After 30 minutes the reaction was filtered through a Celite pad and the filtrate was evaporated under reduced pressure. The residue was purified by Sephadex LH-20 (H_20/THF: 1/1) to afford **6** (16.6 mg, 58%) as a yellowish solid.

^{1}H NMR (500MHz, D_2O/THF-d8) δ: 7.86-7.75 (m, 16H, H-3', H-4''), 7.75-7.60 (m, 16H, H-5a, H-5'), 7.60-7.46 (m, 16H, H-8_a, H-6'), 7.40-7.30 (m, 8H, H-6_a), 6.18-6.10 (m, 8H, H-2a), 6.03-5.95 (m, 8H, H-1''), 5.22-5.10 (m, 8H, H-1), 4.54-4.48 (m, 16H, H-5''), 3.92-3.79 (m, 40H, H-5, H-6'', H-7''), 3.59-3.46 (m, 88H, H-2, H-3, H-4, 64*PEG), 3.42-3.33 (m, 8H, H-2_a''), 3.33-3.26 (m, 8H, H-2_b''), 3.14-2.86 (m, 32H, H-8'', H-9''), 2.41 (br s, 24H, Me), 1.13-0.96 (m, 16H, H-10''); ^{13}C NMR (500MHz, D_2O/THF-d8) δ: 173.9, 162.3, 155.3, 154.8, 153.8, 150.9 (C_{IV}), 143.5 (C_{IV}), 140.8 (C-3''), 135.3 (C_{IV}), 133.7 (C-5a), 126.8 (C-5'), 125.5 (C-4''), 124.1 (C-3'), 119.0 (C-6'), 115.9 (C_{IV}), 115.7 (C-6a), 113.0 (C-2a), 105.9 (C-8a), 101.9 (C-1), 76.8 (C-2), 75.5 (C-1''), 74.0 (C-3), 72.6 (C-4), 71.0, 70.9 (PEG), 70.2 (C-5), 68.2 (C-6''), 64.7 (C-7''), 51.7 (C-8''), 51.1 (C-5''), 47.3 (C-9''), 33.2 (C-2''), 18.7 (CH_3), 14.7 (C-10'').

Experimental procedures (molecular dynamic simulations)

Elongated structures of compounds 2 to 5 were generated with the "Ligand Maker and Modeler" tool of the CHARMM-GUI webpage [1,2]. A set of folded structures were generated, minimized and ranked according to their energy with the ConfGen application of the SCHROEDINGER suite [3,4] using standard parameters. For compound 2 and 3 the three highest ranking folded structures and for compound 4 and 5 the five highest ranking structures were used to perform 20-ns long molecular dynamics simulations.

All molecular dynamics trajectories were generated in the isothermal-isobaric ensemble at 300K with the program NAMD2.12 [5] using the CHARMM36 force field [6,7]. Long-range electrostatic interactions were calculated using the particle-mesh Ewald method [8]. A smoothing function was applied to truncate short-range electrostatic interactions. The Verlet-I/r-RESPA multiple time-step propagator [9] was used to integrate the equation of motions using a time step of 2 and 4 fs for short- and long-range forces, respectively. All bonds involving hydrogen atoms were constrained using the Rattle algorithm [10]. Missing force-field parameters were generated using the "Ligand Maker and Modeler" tool of the CHARMM-GUI webpage [1,2]. After solvation of the system, first a 2.5 ns long equilibration of the solvent (water and ions) and second an unrestrained 2.5 ns long equilibration was performed. Afterwards a 20-ns long production run was carried out for each of the systems. From all simulations the average distance *d* (as in Graph 1 in the main text) between the two glucuronide ligands was computed as the average over the distances between the centre of mass of the heavy atoms of the sugar ring of the glucuronide ligand determined for 10.000 snapshots extracted each 0.002 ns from the generated trajectories using VMD [11].

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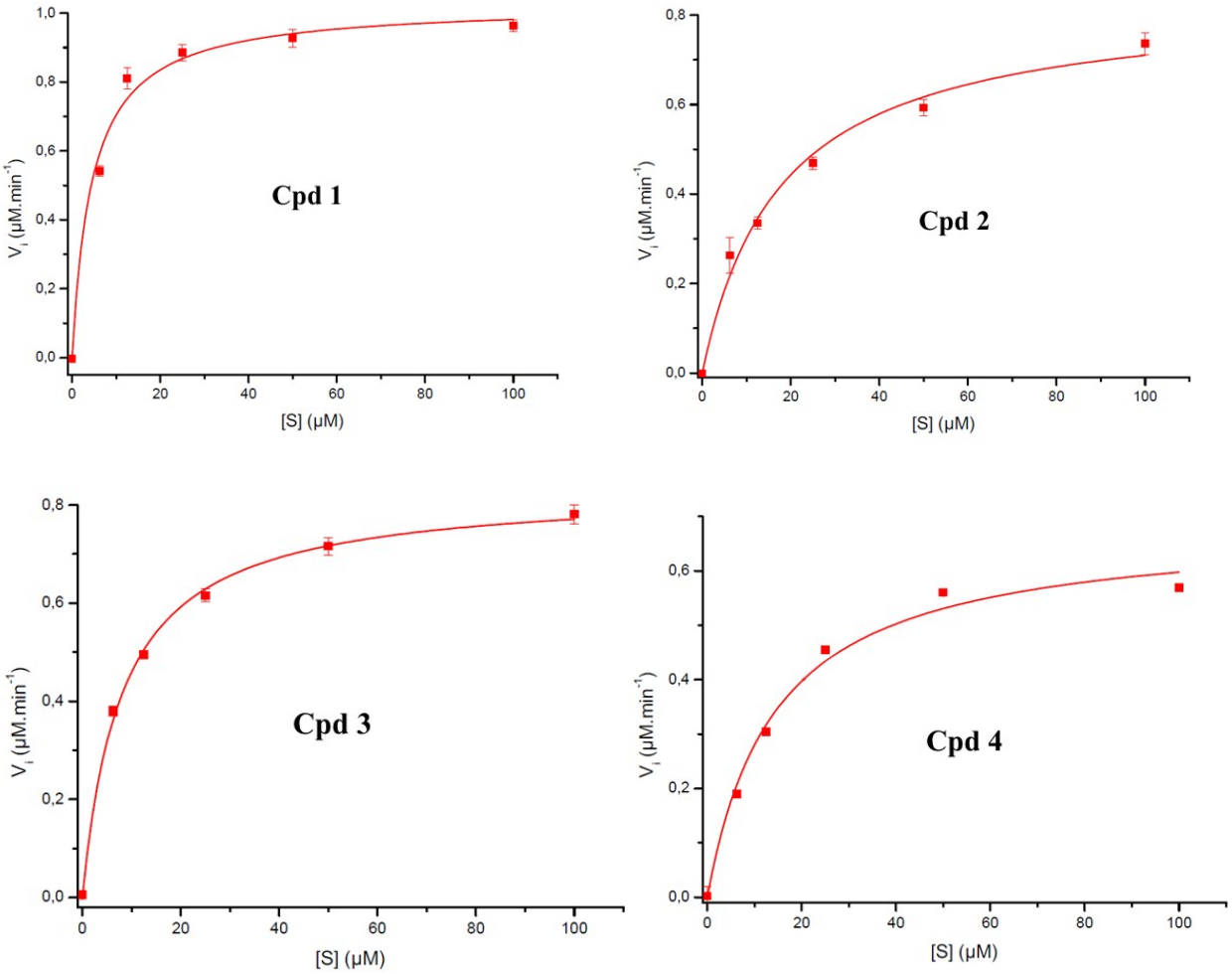
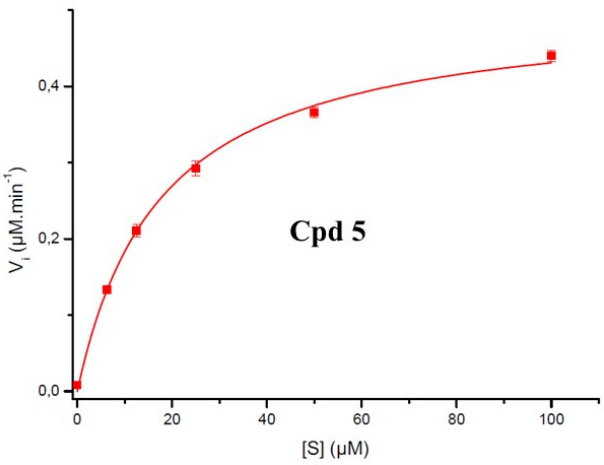
Enzyme kinetics study using a fluorescence assay.

The enzymatic activity was measured using the fluorescence generated after the rupture of glycosidic bond of each substrate. Each assay were performed at 37 °C in PBS buffer (75 mM), pH 6,5. To determine the kinetics parameters of β-glucuronidase from *E. coli.,* purchased from Sigma Aldrich, 10 µL of enzyme at 96 nM ($[GUS]_{final}$ = 9,6 nM) were diluted in 40 µL of PBS buffer in a black 96-well microtitre plate and were incubated for 15 minutes at 37 ºC. After addition of 50 µL of adequate concentration of the different substrates in PBS buffer, the fluorescence was directly monitored throughout the reaction each minutes during 30 min at 37°C. FL signals were detected at an excitation wavelength of 355 nm and emission at 460 nm. Relative fluorescence units (RFU) were converted to the concentration of the product 4-methylumbelliferone (4-MU) according to the 4-MU standard curve. K_m and V_{max} values for each substrate were determined using Origin software to curve-fit the Michaelis-Menten plot using a single site (n = 1) nonlinear Hill fit.

$$Vo = \frac{V_{max} \cdot [S]^n}{K_m^n + [S]^n}$$

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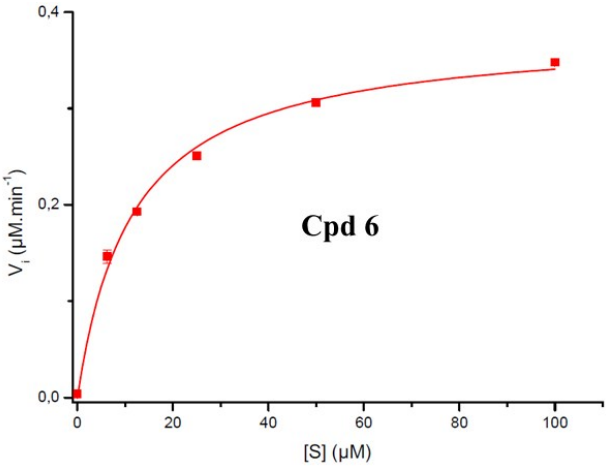
[S] (µM)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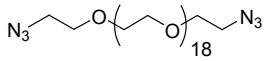
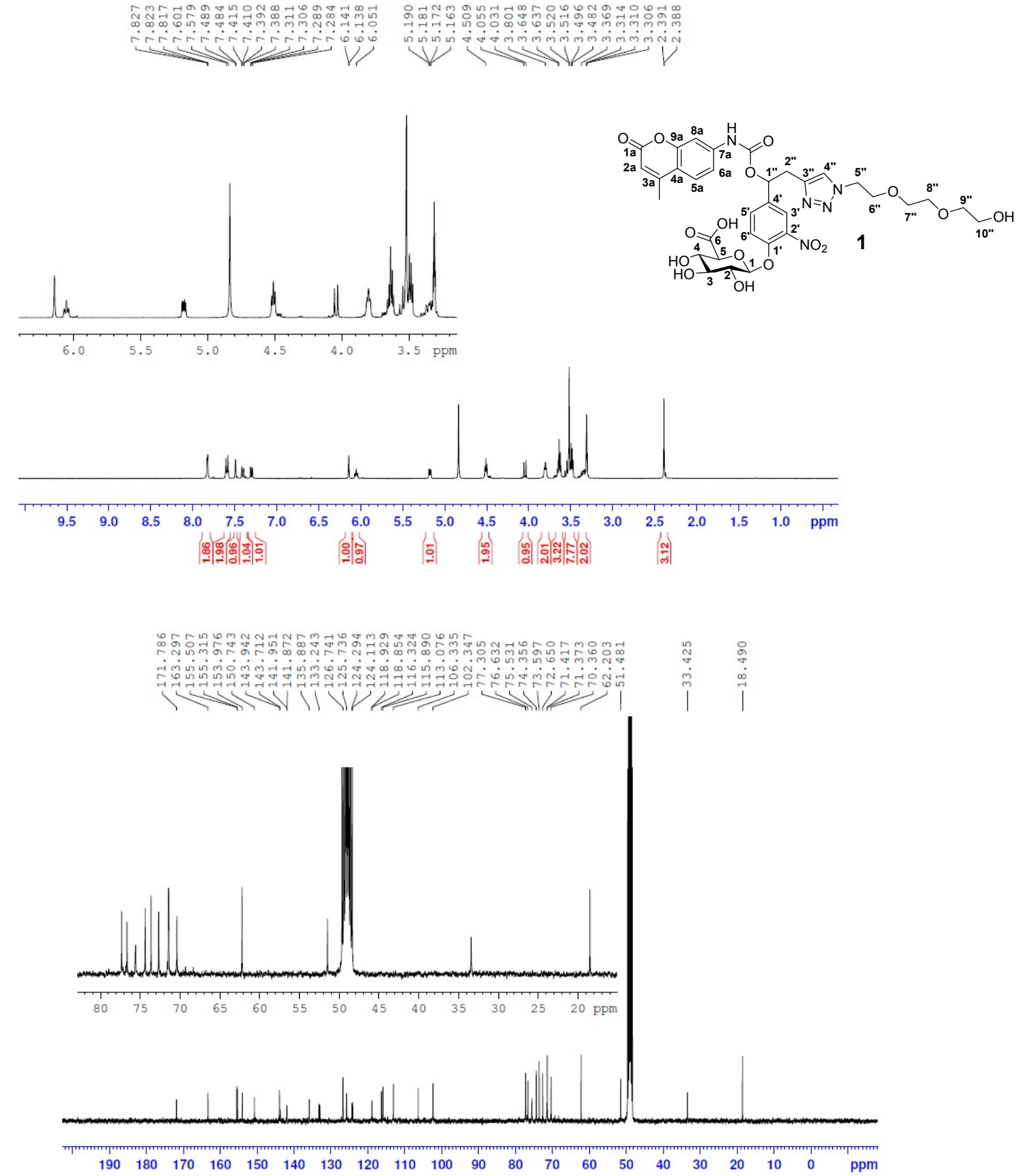
[S] (µM)

^{1}H and ^{13}C NMR

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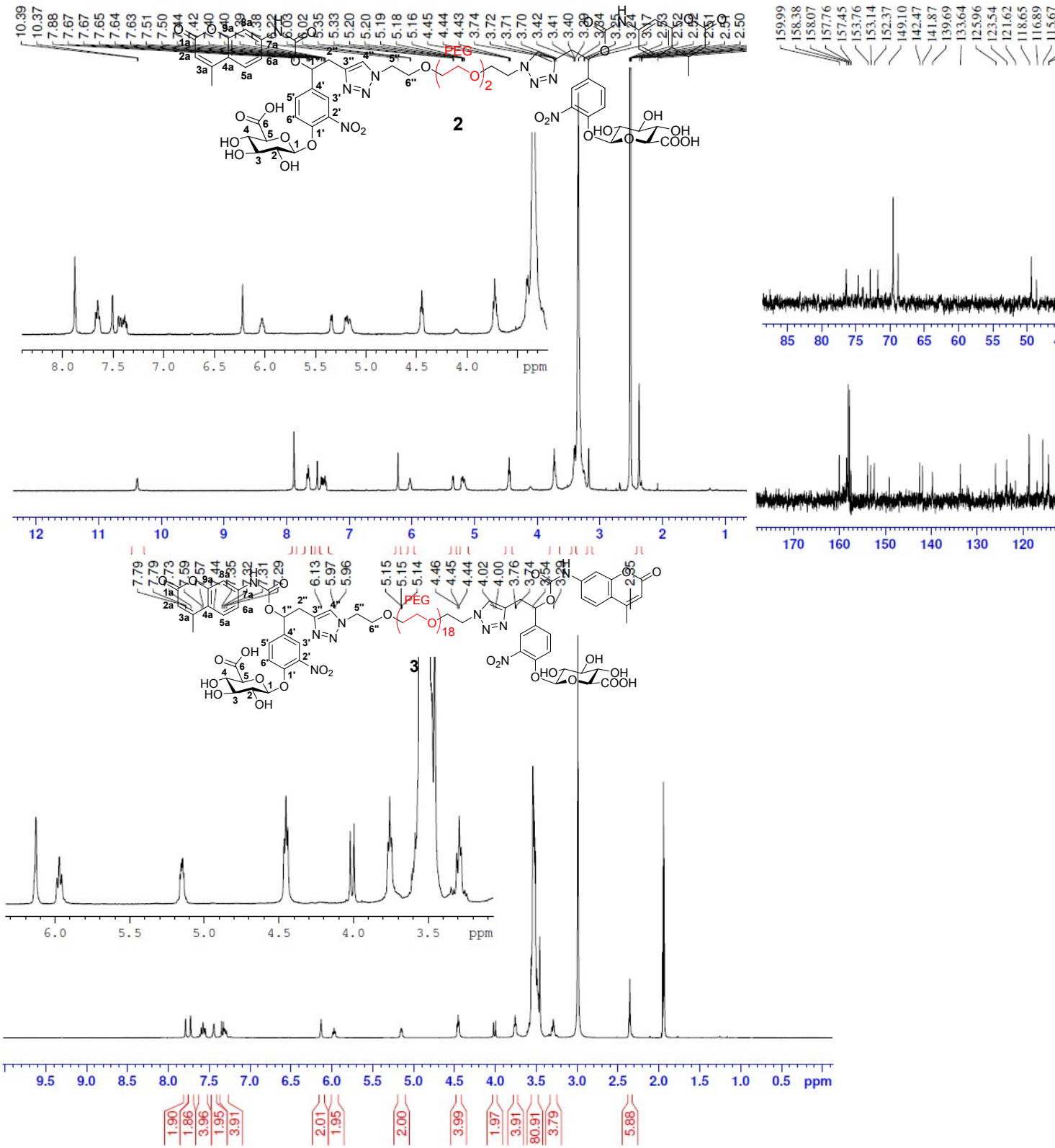
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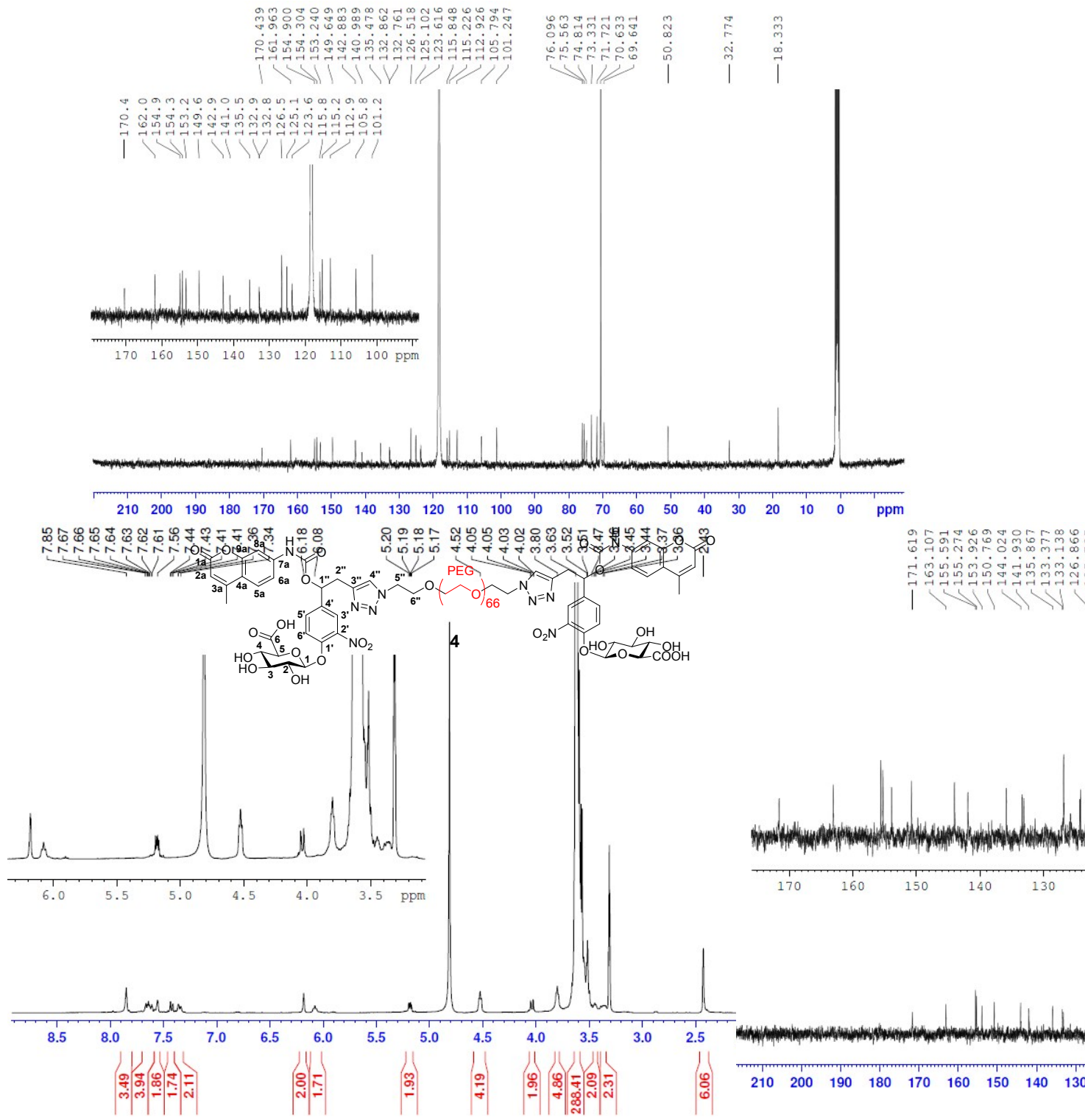
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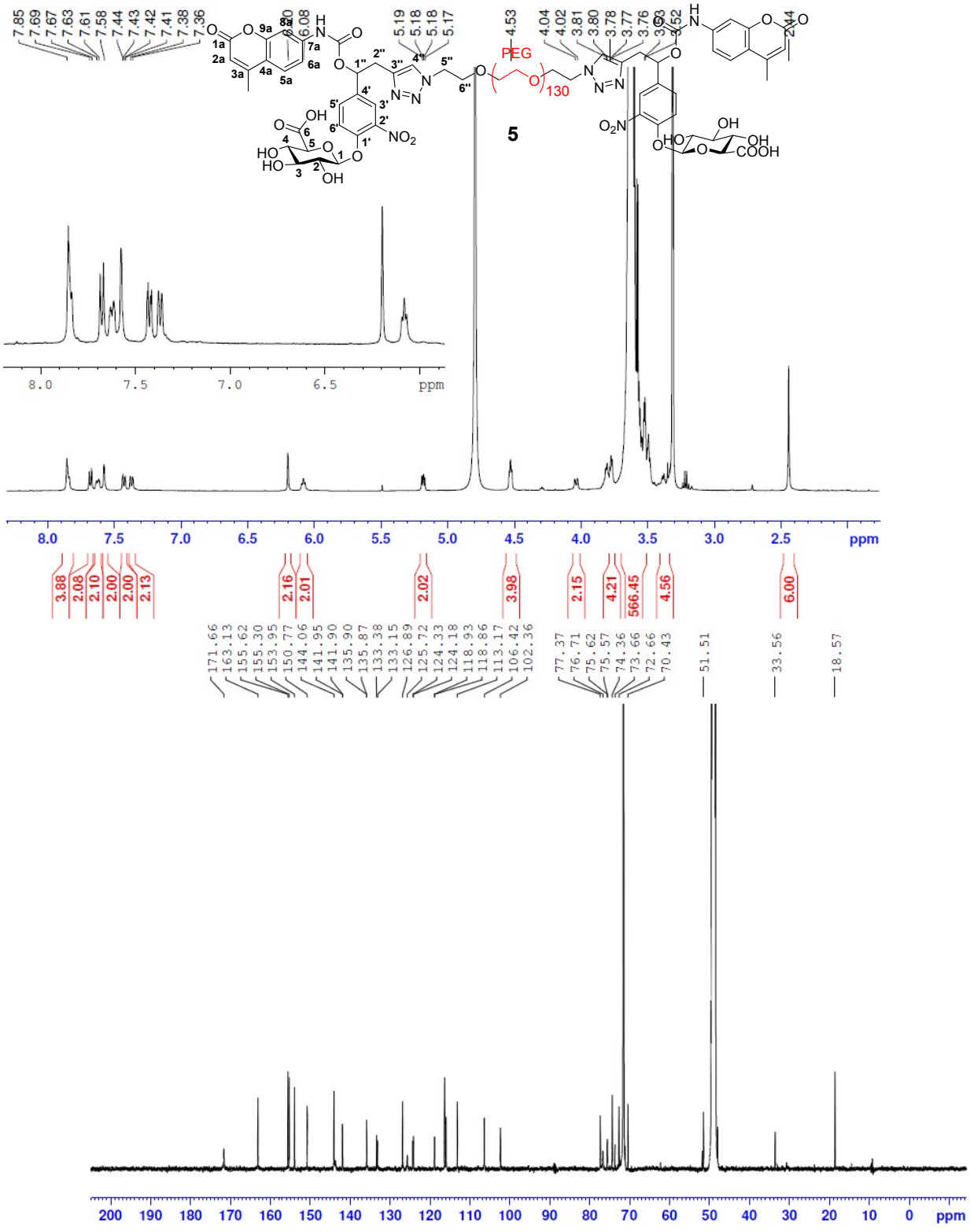
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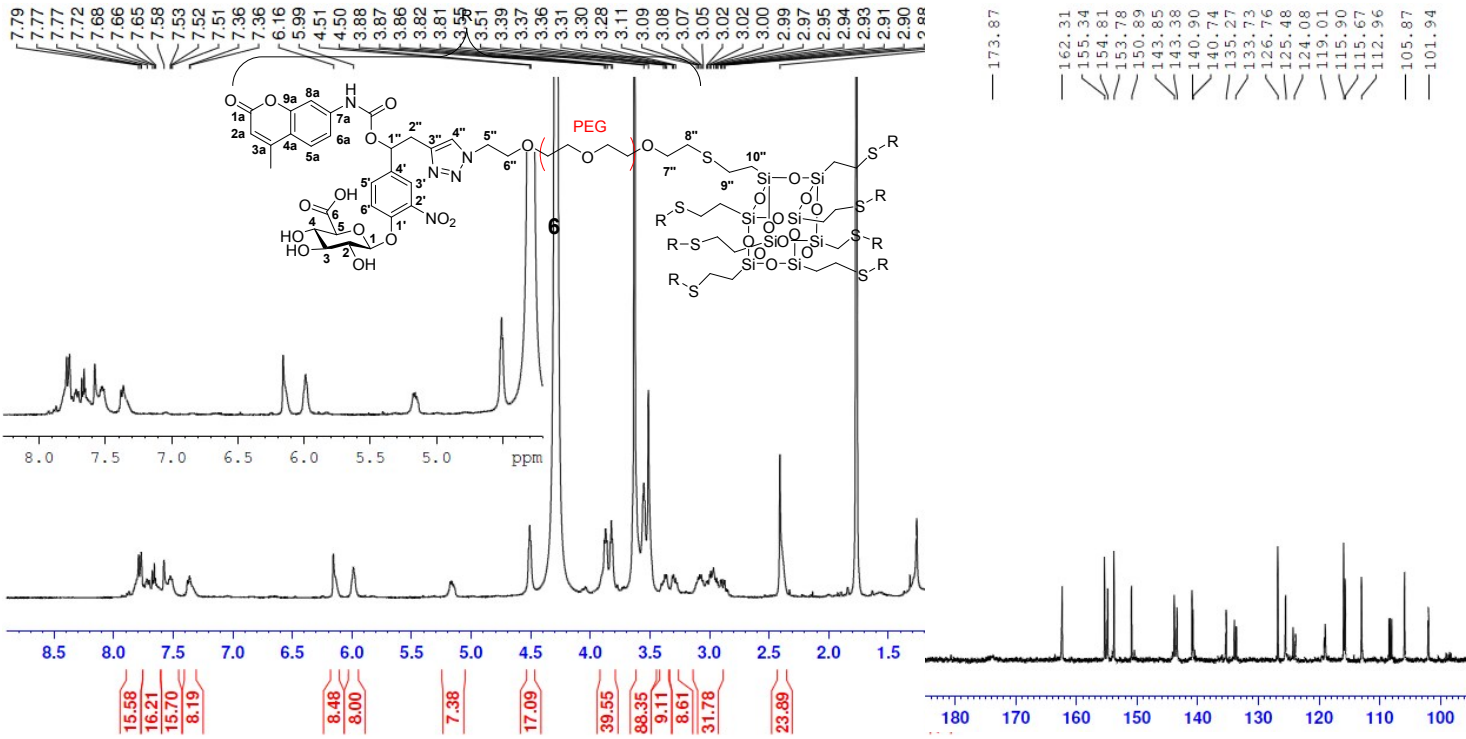
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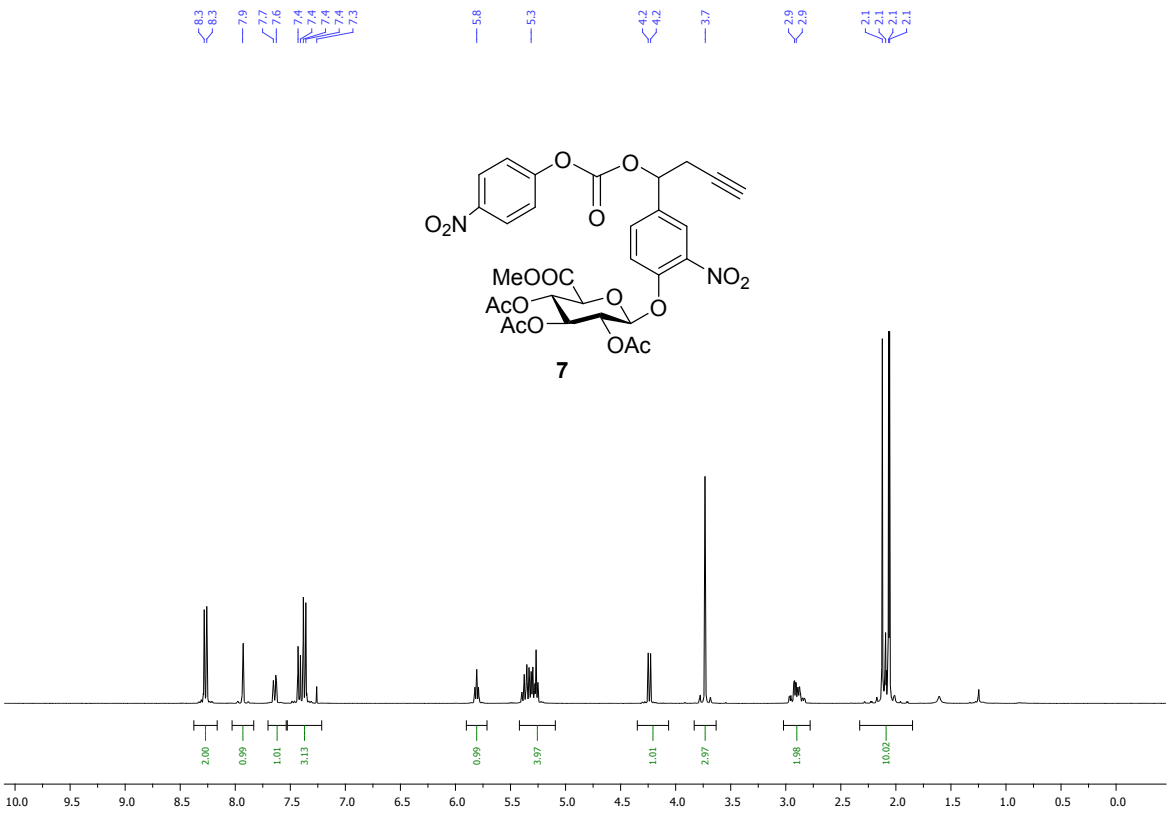
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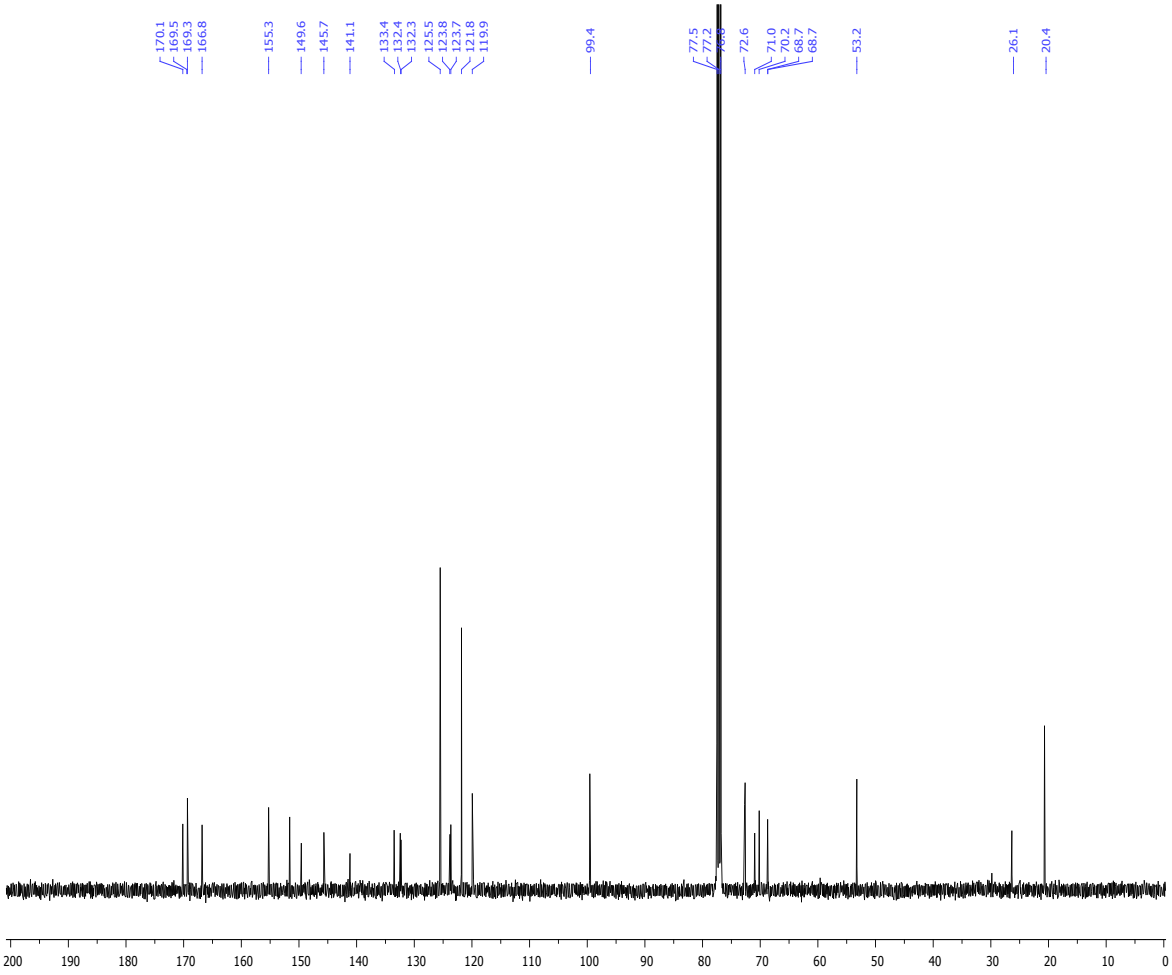
10.02

7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77.2
76.9
72.6
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68.7
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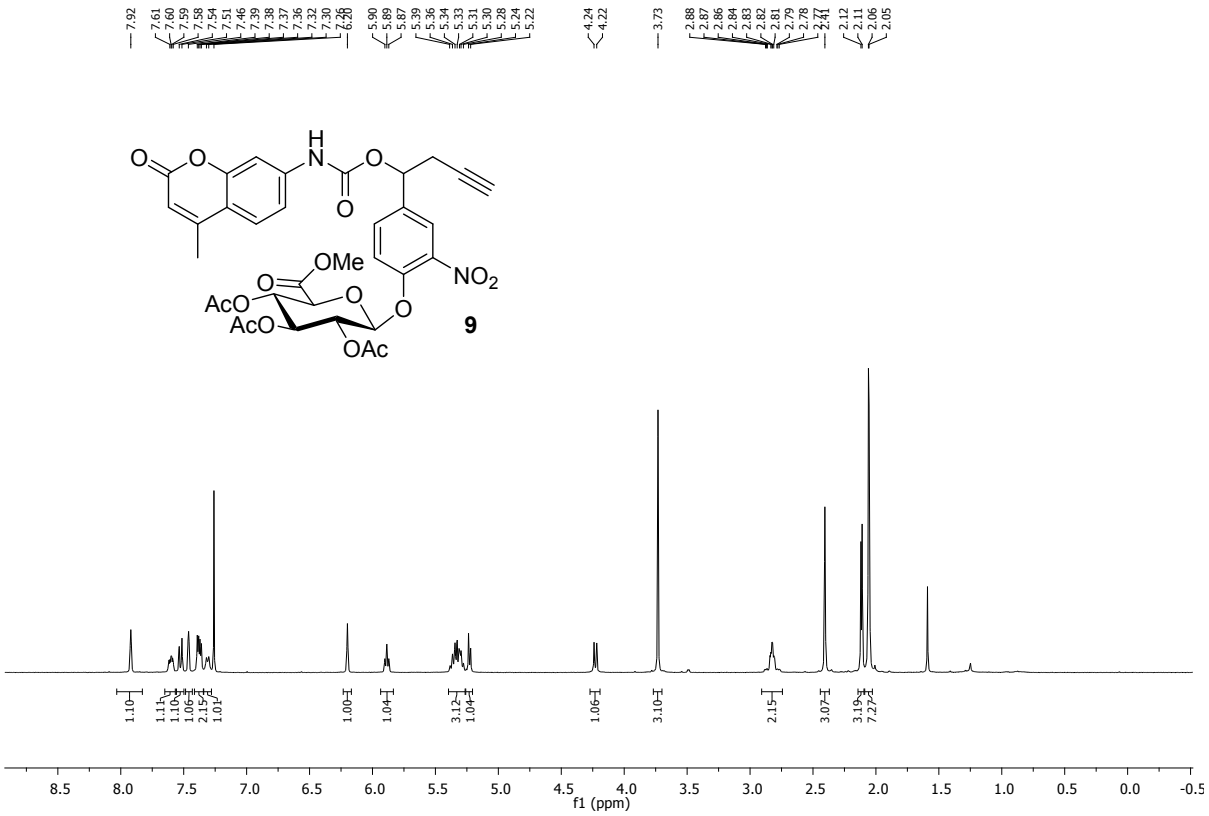
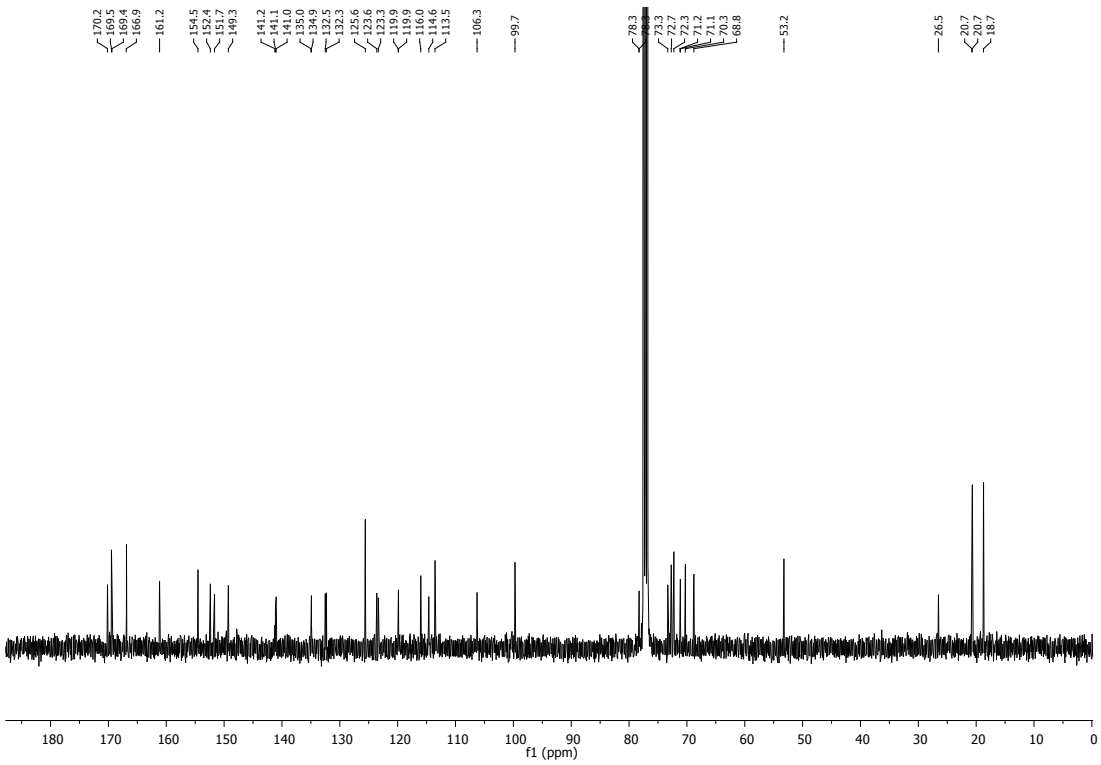
26.1
20.4

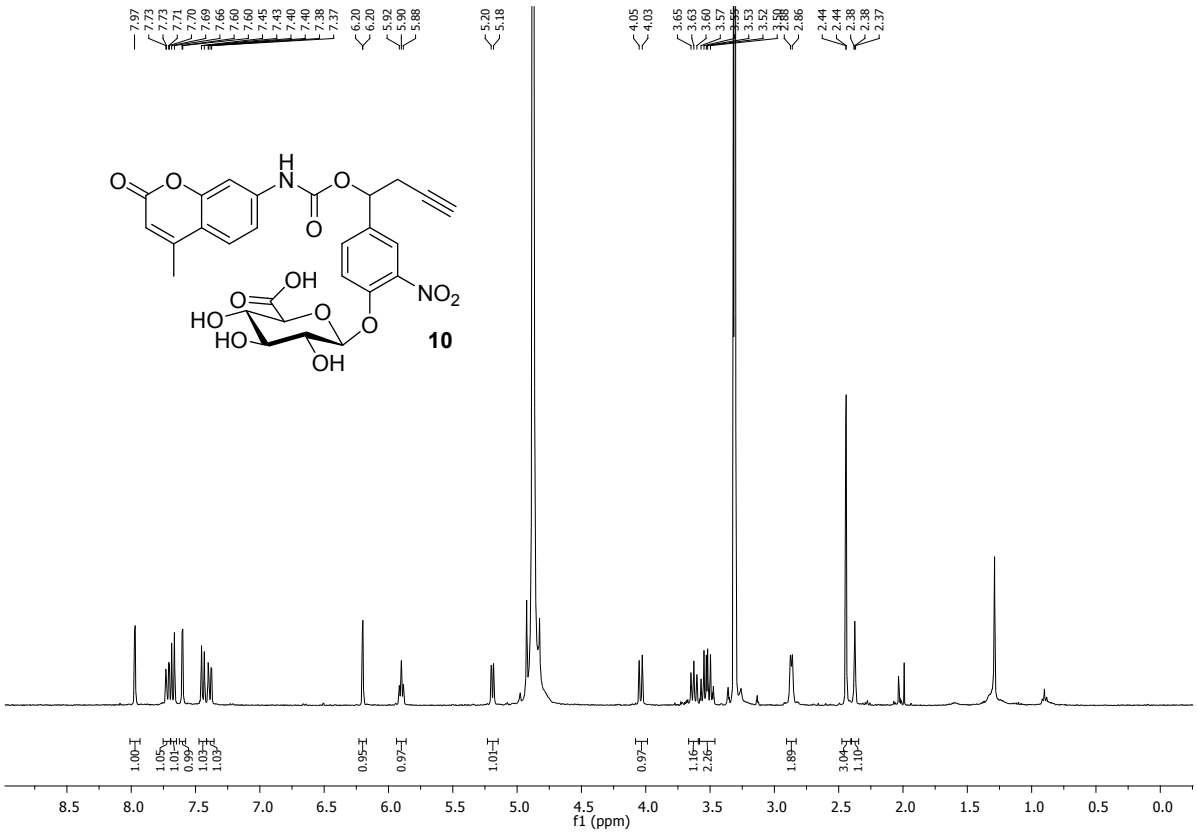
^{1}H NMR spectrum (400 MHz, 298 K, $CDCl_3$) of **9**



^{13} C NMR spectrum (100 MHz, 298 K, $CDCl_3$) of **9**



[1]H NMR spectrum (400 MHz, CD_3OD) of **10**



10

[13] C NMR spectrum (100 MHz, 298 K, CD_3OD) of ***10***