Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplemental Information for:

Beta-galactosidase activity-based probe for detection of cellular senescence by mass cytometry

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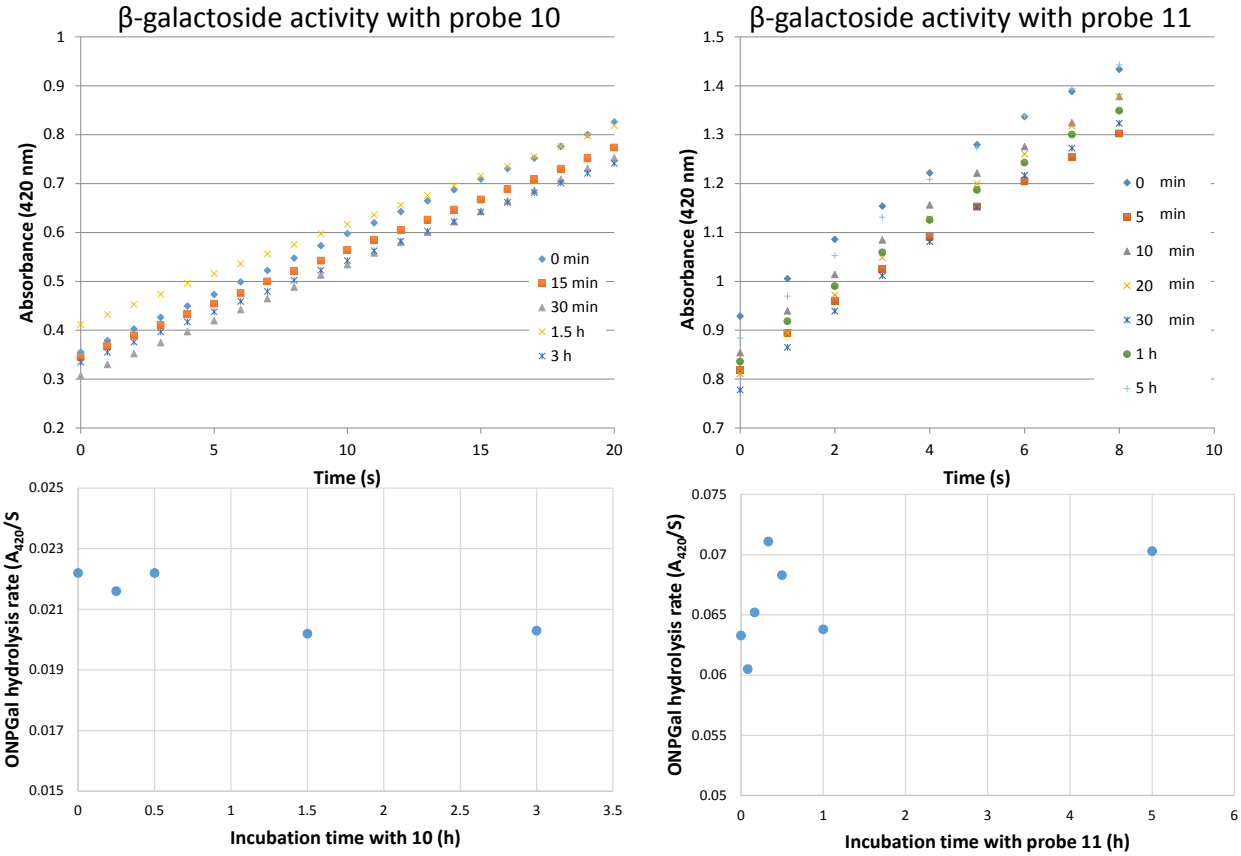


Figure S1. Inhibition of *E. coli* β-galactosidase upon incubation with probes **10** or **11**. Absorbance changes upon ONPG hydrolysis by *E. coli* β-gal. Enzyme (2 µM) was incubated with a) Probe **10** (500 µM) or b) Probe **11** (500 µM) at 37 °C in buffer (100 mM MOPS pH 7.5, $MgCl_2$ 10mM, NaCl 10 mM each and TCEP 1 mM). Aliquots were taken at the indicated time points and added to a cuvette containing ONPGal (0.6 mM) in the same buffer. Absorbance (420 nm) after addition was monitored. The rates of ONPGal release did not change significantly over the

course of probe incubation.

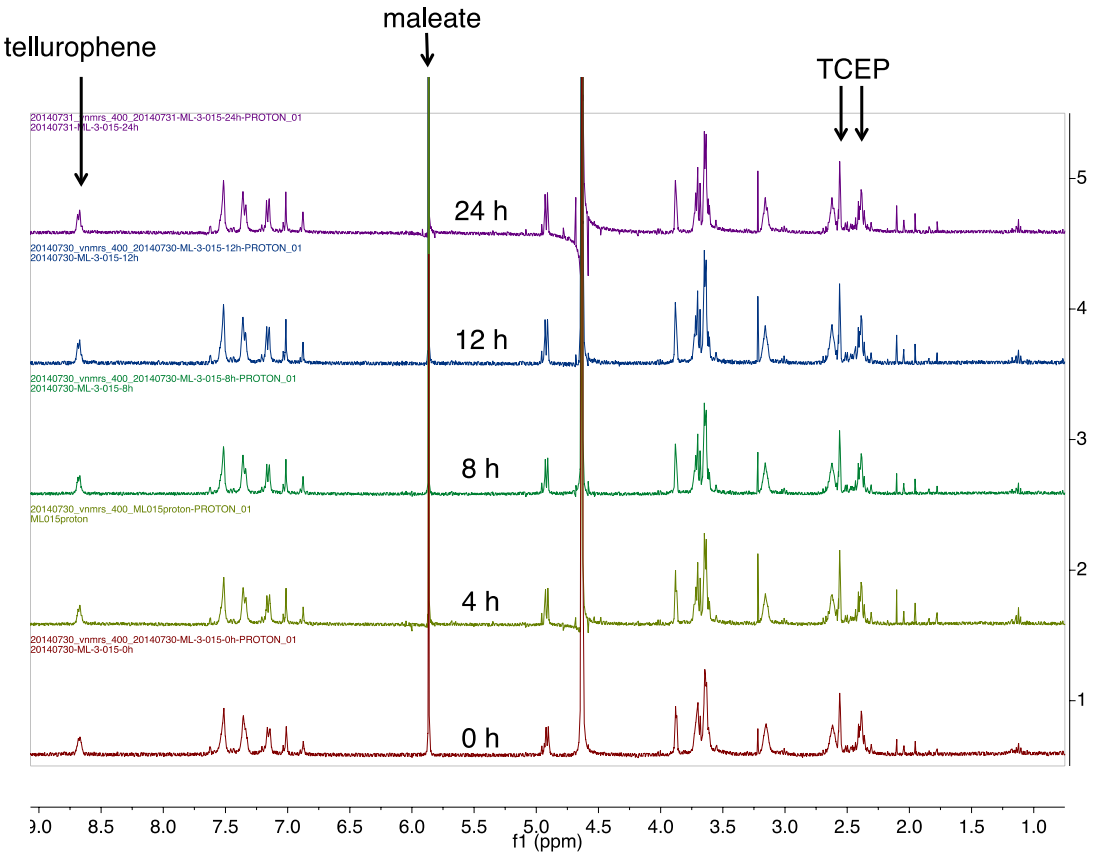


Figure S2: NMR spectra measuring the stability of **10** (10 mM) over 24 h at room temperature. The signal corresponding to a tellurophene proton was chosen as the analyte reference peak. Sodium maleate (10 mM) was used as an internal integration standard. Buffer conditions: 100 mM deuterated sodium phosphate, 10 mM $MgCl_2$, 10 mM NaCl, 1 mM TCEP, pH 7.5, ~20% d_6-DMSO.

Figure S3: NMR spectra measuring the stability of **11** (10 mM) over 24 h at room temperature. The signal corresponding to a tellurophene proton was chosen as the analyte reference peak. Sodium maleate (10 mM) was used as an internal integration standard. Buffer conditions: 100 mM deuterated sodium phosphate, 10 mM $MgCl_2$, 10 mM NaCl, 1 mM TCEP, pH 7.5, ~20% d_6-DMSO.

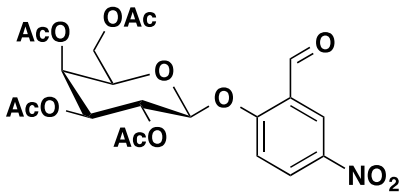
Materials

Dry solvents (Acros Organics), reagent-grade solvents (Fisher), 2,3,4,6-tetraacetyl-α-D-galactopyranosyl bromide (Carbosynth), diethylaminosulfur trifluoride (Alfa Aesar), *E. coli* β-galactosidase (Worthington) and all other compounds (Sigma-Aldrich) were used as supplied. Silica chromatography was performed with SiliCycle Silica-P Flash Silica Gel. Desalting of organic compounds was performed using Varian Bond Elute C18 cartridges. Centrifugal spin-filters were

supplied by Millipore. PD-10 size-exclusion columns were supplied by GE Life Sciences. Buffers for studies using *E. coli* β-galactosidase were supplemented with 10 mM $MgCl_2$, 10 mM NaCl and 1 mM TCEP, unless otherwise stated.

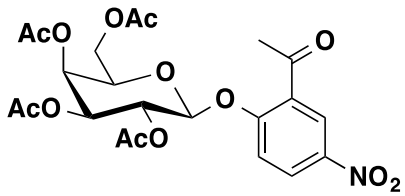
Synthesis

1-(2-formyl-4-nitrophenyl)-2-3,4,6-tetraacetyl-β-D-galactopyranose (**1**)
Cheng, T.-C.; *et al. J. Am. Chem. Soc.*, **2012**, *134*, 3103–3110.



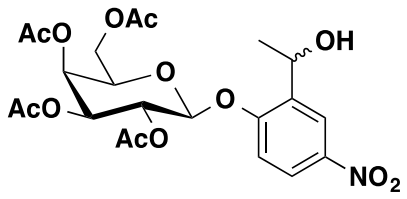
2,3,4,6-tetraacetyl-α-D-galactopyranosyl bromide (1.0 g, 2.4 mmol), 2-hydroxy-5-nitrobenzaldehyde (0.43 g, 2.7 mmol), and Ag_2O (0.84 g, 3.6 mmol) were added to an oven-dried round-bottom flask, kept under a steady flow of N_2. The flask was covered with aluminum foil, and anhydrous CH_3CN (43 mL) was added. The mixture was stirred at room temperature for 22.5 h in the dark, then filtered through a pad of celite. The filtrate was concentrated on a rotary evaporator. The crude product was purified *via* flash chromatography (stationary phase, silica gel; mobile phase, DCM, gradient of 1%–2% MeOH) to afford compound **1** (1.05 g, 87%) as a light yellow solid. (Spectra in agreement with literature). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.33 (s, 1H, C<u>H</u>O), 8.71 (d, *J* = 3.0 Hz, 1H, Ar-H), 8.42 (dd, *J* = 9.0 Hz, 3.0 Hz, 1H, Ar-H), 7.25 (d, *J* = 9.0 Hz, 1H, Ar-H), 5.61 (dd, *J* = 10.5 Hz, 8.0 Hz, 1H, H-2), 5.51 (dd, *J* = 3.5 Hz, 1.0 Hz, 1H, H-4), 5.28 (d, *J* = 7.5 Hz, H-1), 5.18 (dd, *J* = 10.5 Hz, 3.5 Hz, 1H, H-3), 4.15-4.25 (m, 3H, H-5, H-6a, H-6b), 2.20, 2.08, 2.07, 2.03 (s, 3H, 4 x COC<u>H</u>$_3$).

1-(2-acetyl-4-nitrophenyl)-2-3,4,6-tetraacetyl-β-D-galactopyranose (2)



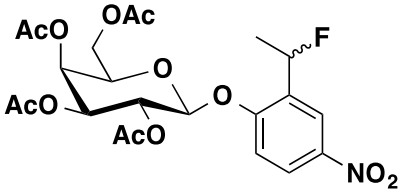
Tetrabutylammonium bromide (0.285 g, 0.883 mmol), 2-hydroxy-5-nitroacetophenone (0.400 g, 2.21 mmol) and 2,3,4,6-tetraacetyl-α-D-galactopyranosyl bromide (0.363 g, 0.883 mmol) were added to a 100 mL round-bottom flask. DCM (8.7 mL) and 1 M NaOH (8.7 mL) were added and the mixture was stirred vigorously overnight at 35 °C. The mixture was cooled to room temperature, diluted with DCM (200 mL) and washed with 1 M NaOH (2 x 200 mL), water (200 mL) and brine (200 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated to yield a pale yellow solid. The crude product was purified *via* column chromatography (stationary phase, silica gel; mobile phase, 3:2 EtOAc:pentanes, 0.1% triethylamine) to afford **6** (0.229 g, 51%) as a pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.46 (d, *J* = 3.0 Hz, 1H, Ar-H), 8.26 (dd, *J* = 4.0 Hz, 3.0 Hz, 1H, Ar-H), 7.21 (d, *J* = 9.5 Hz, 1H, Ar-H), 5.54 (dd, *J* = 10.5 Hz, 7.5 Hz, 1H, H-2), 5.46 (dd, *J* = 3.5 Hz, 0.5 Hz, 1H, H-4), 5.30 (d, *J* = 8.0 Hz, 1H, H-1), 5.14 (dd, *J* = 10.5 Hz, 3.5 Hz, 1H, H-3), 4.12-4.20 (m, 3H, H-5, H-6a, H-6b), 2.56 (s, 3H, ArCOC<u>H</u>$_3$), 2.17, 2.03, 2.02, 1.98 (s, 3H, 4 x -COOC<u>H</u>$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 197.24, 170.22, 170.03, 169.94, 169.24, 159.01, 142.99, 130.45, 127.92, 126.01, 115.44, 98.58, 77.24, 71.70, 70.58, 68.00, 66.59, 61.27, 31.35, 20.62, 20.60, 20.48. ESI-MS *m/z* calcd. for $C_{22}H_{25}NO_{13}$ 511.13, found 534.11 $[M+Na]^+$.

1-(2-(1-hydroxyethyl)-4-nitrophenyl)-2-3,4,6-tetraacetyl-β-D-galactopyranose (3)



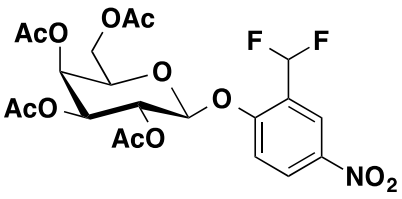
2 (0.229 g, 0.447 mmol) was dissolved in dry methanol (20 mL) in a 50 mL round-bottom flask. Sodium borohydride (0.017 g, 0.45 mmol) was added over five minutes with stirring. After 15 minutes, 1 M HCl (1 mL) was added to quench the reaction. The mixture was concentrated, diluted with DCM (100 mL), washed with 1 M HCl (100 mL), saturated sodium bicarbonate (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated to afford **3** (0.195 g, 78%) as a pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (dd, *J* = 2.5 Hz, 0.5 Hz, 1H, Ar-Ha), 8.29 (dd, *J* = 2.5 Hz, 0.5 Hz, 1H, Ar-Hb), 8.04 (dd, *J* = 5.5 Hz, 5.0 Hz, 1H, Ar-Ha), 8.02 (dd, *J* = 5.5 Hz, 5.0 Hz, 1H, Ar-Hb), 7.06 (d, *J* = 9.0 Hz, 1H, Ar-Ha), 7.04 (d, *J* = 9.0 Hz, 1H, Ar-Hb), 5.50 (dd, *J* = 10.5 Hz, 6.0 Hz, 1H, Ha-2), 5.48 (dd, *J* = 10.5 Hz, 6.0 Hz, 1H, Hb-2), 5.45 (app. d, *J* = 3.0 Hz, 2H, Ha/b-4), 5.22 (d, *J* = 8.0 Hz, 1H, Ha-1), 5.18 (d, *J* = 8.0 Hz, Hb-1), 5.16 (app. t, *J* = 4.0 Hz, 1H, Ha-3), 5.13 (app. t, *J* = 4.0 Hz, 1H, Hb-3), 5.09 (q, *J* = 5.5 Hz, 1H, ArC<u>Ha</u>(OH)-), 5.01 (q, *J* = 5.5 Hz, 1H, ArC<u>Hb</u>(OH)-), 4.10-4.20 (m, 6H, H-5a/b, Ha/b-6a, Ha/b-6b), 2.15, 2.11, 2.05, 2.03, 2.02, 2.00, 1.97, 1.97 (s, 3H, 8 x -COOC<u>H$_3$</u>), 1.44 (d, *J* = 6.5 Hz, 3H, -CH$_2$(F)-C<u>Ha$_3$</u>), 1.34 (d, *J* = 6.5 Hz, 3H, -CH$_2$(F)-C<u>Hb$_3$</u>). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.31, 170.29, 170.14, 170.11, 169.95, 169.94, 169.74, 169.40, 157.62, 157.12, 143.12, 143.07, 137.10, 136.69, 123.86, 123.79, 122.21, 122.00, 114.11, 113.82, 98.68, 97.96, 77.39, 71.39, 71.35, 70.45, 70.35, 68.33, 68.19, 66.80, 66.78, 63.97, 63.90, 61.38, 61.34, 23.56, 23.41, 20.59, 20.57, 20.50, 20.49, 20.39, 20.39. ESI-MS *m/z* calcd. for $C_{22}H_{27}NO_{13}$ 513.15, found 536.14 $[M+Na]^+$.

1-(2-(1-fluoroethyl)-4-nitrophenyl)-2-3,4,6-tetraacetyl-β-D-galactopyranose (4)



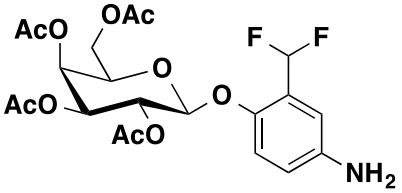
Diethylaminosulfur trifluoride (0.06 mL, 0.456 mmol) was added to a solution of **3** (0.195 g, 0.380 mmol) in dry DCM (5.4 mL). The reaction was stirred at room temperature under N_2 overnight, then quenched by the addition of ice (50 mL) and extracted into DCM (2 x 50 mL). The combined organic extracts were washed with water (100 mL) and brine (100 mL), dried over $MgSO_4$, filtered and concentrated to yield a pale yellow solid. The crude product was purified *via* flash chromatography (stationary phase, silica gel; mobile phase, DCM, 5% MeOH, 0.1% triethylamine) to afford compound **4** (0.162 g, 83%) as a pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (app. d, *J* = 3.0 Hz, 2H, Ar-Ha/b), 8.15 (m, 2H, Ar-Ha/b), 7.13 (m, 2H, Ar-Ha/b), 5.79 (app. q, *J* = 6.5 Hz, 1H, ArC<u>Ha</u>(F)-), 5.70 (app. q, *J* = 6.5 Hz, 1H, ArC<u>Hb</u>(F)-), 5.52 (dd, *J* = 10.5 Hz, 8.0 Hz, 2H, Ha/b-2), 5.48 (m, 2H, Ha/b-4), 5.12-5.15 (m, 4H, Ha/b-1, Ha/b-4), 4.14-4.24 (m, 6H, H-5a/b, Ha/b-6a, Ha/b-6b) 2.18, 2.07, 2.00 (s, 3H, 8 x -COOC<u>H$_3$</u>), 1.65 (d, *J* = 6.0 Hz, -CH$_2$(F)-C<u>Ha$_3$</u>), 1.60 (d, *J* = 6.5 Hz, -CH$_2$(F)-C<u>Hb$_3$</u>). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.19, 170.04, 169.87, 169.42, 157.36, 157.31, 143.19, 132.38, 132.22, 125.00, 124.99, 121.87, 121.79, 114.32, 99.05, 85.75, 84.40, 77.32, 71.52, 70.29, 68.04, 66.71, 61.38, 21.28, 21.09, 20.55, 20.53, 20.43. ESI-MS *m/z* calcd. for $C_{22}H_{26}FNO_{10}$ 515.14, found 533.18 $[M+NH_4]^+$.

1-(2-difluoromethyl-4-nitrophenyl)-2-3,4,6-tetraacetyl-β-D-galactopyranose (5)



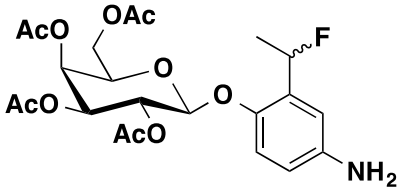
Dimethylaminosulfur trifluoride (0.146 mL, 1.50 mmol) was added to a solution of **1** (0.622 g, 1.25 mmol) in dry DCM (16 mL). The reaction was stirred at room temperature under N_2 for 6.5 h, then quenched by the addition of ice (100 mL) and extracted into DCM (2 x 100 mL). The combined organic extracts were washed with water (100 mL) and brine (100 mL), dried over $MgSO_4$, filtered and concentrated to yield a yellow oil. The crude product was purified *via* flash chromatography (stationary phase, silica gel; mobile phase, DCM, 5% MeOH, 0.1% triethylamine) to afford compound **5** (0.577 g, 89%) as a viscous yellow liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (dd, *J* = 3.0 Hz, 1.5 Hz, 1H, Ar-H), 8.33 (dd, *J* = 9.0 Hz, 2.0 Hz, 1H, Ar-H), 7.22 (d, *J* = 9.5 Hz, 1H, Ar-H), 6.85 (t, *J* = 54.5 Hz, 1H, C<u>H</u>F_2), 5.57 (dd, *J* = 10.5 Hz, 8.0 Hz, 1H, H-2), 5.50 (app d, *J* = 3.5 Hz, 1H, H-4), 5.16 (d, *J* = 8.0 Hz, H-1), 5.15 (dd, *J* = 11.0 Hz, 3.5 Hz, 1H, H-3), 4.15-4.25 (m, 3H, H-5 H-6a, H-6b), 2.20, 2.09, 2.06, 2.03 (s, 3H, 4xCOC<u>H_3</u>).

1-(2-difluoromethyl-4-aminophenyl)-2-3,4,6-tetraacetyl-β-D-galactopyranose (6)



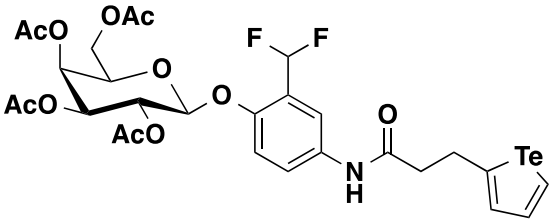
Pd/C (5% Pd, 0.220 g) was added to a stirring solution of **5** (1.08 g, 2.08 mmol) in ethyl acetate (5 mL) in a 25 mL three-necked round-bottom flask. The flask was purged with N_2, then H_2, then placed under 2.04 atm of fresh H_2 overnight at room temperature with stirring. Pd/C was filtered through celite, and the filtrate was concentrated to afford pure **6** (0.990 g, 97%) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (s, 1H, Ar-H), 6.87 (dd, *J* = 2.8 Hz, 1.6 Hz, 1H, Ar-H), 6.80 (t, *J* = 55.6 Hz, 1H, C<u>H</u>F_2), 5.47 (dd, *J* = 10.8 Hz, 8.0 Hz, 1H, H-2), 5.44 (dd, *J* = 3.2 Hz, 0.8 Hz, 1H, H-4), 5.08 (dd, *J* = 10.8 Hz, 3.6 Hz, 1H, H-3), 4.86 (d, *J* = 8.0 Hz, 1H, H-1), 4.00-4.26 (m, 3H, H-5, H-6a, H-6b), 2.19, 2.08, 2.06, 2.01 (s, 3H, 4 x COC<u>H_3</u>). ^{19}F NMR (376 MHz, $CDCl_3$) δ –108.43 (dd, *J* = 300.8 Hz, 56.4 Hz, 1F), –122.67 (dd, *J* = 300.8 Hz, 56.4 Hz, 1F).

1-(2-(1-fluoroethyl)-4-aminophenyl)-2-3,4,6-tetraacetyl-β-D-galactopyranose (7)



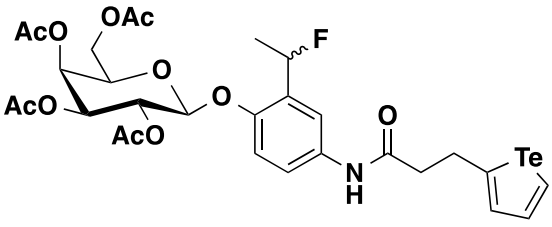
Pd/C (5% Pd, 0.049 g) was added to a stirring solution of **4** (0.162 g, 0.315 mmol) in ethyl acetate (3 mL) in a 25 mL three-necked round-bottom flask. The flask was purged with N_2, then H_2, then placed under 2.04 atm of fresh H_2 overnight at room temperature with stirring. Pd/C was filtered out with celite, and the filtrate was concentrated to yield a yellow solid. The crude product was purified *via* flash chromatography (stationary phase, silica gel; mobile phase, DCM, 6% MeOH, 0.1% triethylamine) to afford **7** (0.126 g, 52%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.89 (app. d, *J* = 8.5 Hz, 2H, Ar-Ha/b), 6.75 (s, 2H, Ar-Ha/b), 6.56 (m, 2H, Ar-Ha/b) 5.79 (app. q, *J* = 6.5 Hz, 1H, ArC<u>Ha</u>(F)), 5.69 (app. q, *J* = 6.5 Hz, 1H, ArC<u>Hb</u>(F)), 5.42 (m, 4H, Ha/b-2, Ha/b-4), 5.07 (m, 2H, Ha/b-3), 4.83 (d, *J* = 7.5 Hz, 2H, Ha/b-1), 4.00-4.21 (m, 6H, Ha/b-5, Ha/b-6a, Ha/b-6b), 3.61 (br s, 4H, -N<u>H_2</u>), 2.16, 2.07, 2.04, 1.99 (s, 3H, 8 x -COOC<u>H_3</u>), 1.55 (d, *J* = 6.5 Hz, -CH_2(F)-C<u>Ha_3</u>), 1.51 (d, *J* = 6.5 Hz, -CH_2(F)-C<u>Hb_3</u>). DART-MS *m/z* calcd. for $C_{22}H_{28}FNO_{10}$ 485.17, found 508.2 $[M+Na]^+$.

1-(2-difluoromethyl-4-(3-(tellurophen-2-yl)propanamido)phenyl)-2-3,4,6-tetraacetyl-β-D-galactopyranose (8)



3-(tellurophen-2-yl)propanoic acid (0.36 g, 1.43 mmol) was added to a solution of **6** (0.78 g, 1.6 mmol) in anhydrous ethyl acetate (8 mL) under a steady flow of N_2. After cooling the solution to 0 °C, pyridine (0.44 mL) and propylphosphonic anhydride (T3P, 1.9 mL, 2.79 mmol, 50% wt. % in ethyl acetate) were added sequentially. The mixture was stirred at room temperature for 48 h, then diluted with DCM (100 mL), and washed with saturated $NaHCO_3$ (3 x 100 mL), water (100 mL), and brine (100 mL). The organic extract was dried over $MgSO_4$, filtered, and concentrated to yield **8** (0.938 g, 81%) as a light yellow solid. **8** was sufficiently pure and used in the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (dd, *J* = 6.8 Hz, 1.2 Hz, 1H, TeAr-H), 7.96 (app. s, 1H, TeAr-H), 7.76 (dd, *J* = 9.2 Hz, 0.4 Hz, 1H, Ar-H), 7.53 (dd, *J* = 10.8 Hz, 4.0 Hz, 1H, TeAr-H), 7.45 (br s, 1H, NH), 7.33 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.04 (d, *J* = 9.2 Hz, 1H, Ar-H), 6.77 (t, *J* = 55.2 Hz, 1H, -C<u>H</u>F$_2$), 5.47 (dd, *J* = 10.8 Hz, 8.0 Hz, 1H, H-2), 5.44 (app. d, *J* = 2.8 Hz, 1H, H-4), 5.09 (dd, *J* = 10.4 Hz, 3.2 Hz, 1H, H-3), 4.95 (d, *J* = 8.0 Hz, 1H, H-1), 4.04-4.22 (m, 3H, H-5, H-6a, H-6b), 3.25 (t, *J* = 6.8 Hz, 2H, -OC-C<u>H</u>$_2$-CH$_2$), 2.64 (t, *J* = 6.8 Hz, 2H, -CH$_2$-C<u>H</u>$_2$-CTe), 2.15, 2.03, 2.02, 1.98 (s, 3H, 3 x -COC<u>H</u>$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.33, 170.15, 170.04, 169.98, 169.59, 148.53, 136.96, 135.93, 133.27, 125.23, 124.03, 118.00, 116.20, 110.76, 101.07, 100.22, 77.20, 71.23, 70.53, 68.11, 66.76, 61.36, 40.85, 32.27, 20.67, 20.64, 20.57, 20.55. DART-MS *m/z* calcd. for $C_{28}H_{31}F_2NO_{11}Te$ 725.09, found 726.1 $[M+H]^+$.

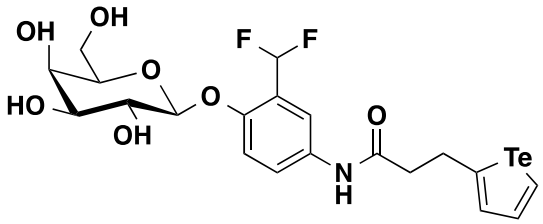
1-(2-(fluoroethyl)-4-(3-(tellurophen-2-yl)propanamido)phenyl)-2-3,4,6-tetraacetyl-β-D-galactopyranose (9)



7 (0.110 g, 0.227 mmol), 3-(tellurophen-2-yl)propanoic acid (0.052 g, 0.206 mmol), pyridine (0.06 mL) and ethyl acetate (0.13 mL) were added to a 25 mL round-bottom flask at 0 °C under N_2. T3P (50 wt. % in ethyl acetate, 0.27 mL) was added dropwise and the solution was stirred at room temperature for 20 h. The solution was diluted with DCM (40 mL) and washed with saturated sodium bicarbonate (3 x 40 mL), water (40 mL) and brine (40 mL). The organic extract was dried over $MgSO_4$, filtered and concentrated to yield a pale yellow solid. The crude product was purified *via* column chromatography (stationary phase, silica gel; mobile phase, 3:2 EtOAc:pentanes, 0.1% triethylamine) to afford **9** (0.090 g, 55%) as a pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.69 (dd, *J* = 7.0 Hz, 1.5 Hz, 2H, TeAr-Ha/b), 7.62 (dd, *J* = 8.5 Hz, 2.5 Hz, 2H, Ar-Ha/b), 7.57 (dd, *J* = 7.0 Hz, 4.0 Hz, 2H, TeAr-Ha/b), 7.44 (br s, 2H, NHa/b), 7.37 (dd, *J* = 3.5 Hz, 1.0 Hz, 2H, TeAr-Ha/b), 7.32 (app. d, *J* = 3.0 Hz, 2H Ar-Ha/b), 7.02 (dd, *J* = 9.0 Hz, 1.0 Hz, 2H, Ar-Ha/b), 5.81 (app. q, *J* = 6.0 Hz, 1H, ArC<u>H</u>a(F)-), 5.71 (app. q, *J* = 6.5 Hz, 1H, ArC<u>H</u>b(F)-), 5.49 (dd, *J* = 10.5 Hz, 8.0 Hz, 2H, Ha/b-2), 5.45 (dd, *J* = 3.5 Hz, 0.5 Hz, 2H, Ha/b-4), 5.10 (dd, *J* = 10.5 Hz, 3.0 Hz, 2H, Ha/b-3), 4.94 (d, *J* = 8.0 Hz, 2H, Ha/b-1),

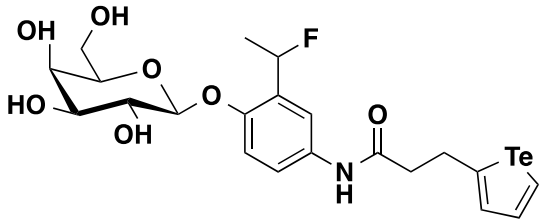
4.03-4.23 (m, 6H, Ha/b-5, Ha/b-6a, Ha/b-6b), 3.29 (t, *J* = 7.0 Hz, 4H, -OC-C<u>H</u>$_2$-CH$_2$), 2.66 (t, *J* = 7.0 Hz, -CH$_2$-C<u>H</u>$_2$-CTe), 2.18, 2.07, 2.05, 2.00 (s, 3H, 8 x -COOC<u>H</u>$_3$), 1.57 (d, *J* = 6.5 Hz, -CH$_2$(F)-C<u>Ha</u>$_3$), 1.52 (d, *J* = 6.0 Hz, -CH$_2$(F)-C<u>Hb</u>$_3$). ^{13}C NMR (126 MHz, CDCl$_3$) δ 170.35, 170.20, 170.05, 170.01, 169.52, 149.83, 148.78, 136.91, 135.77, 133.47, 131.91, 131.75, 125.14, 121.36, 117.66, 115.92, 100.38, 86.10, 84.77, 71.13, 70.68, 68.41, 66.86, 61.41, 40.76, 32.29, 21.72, 21.52, 21.05. ESI-MS *m/z* calcd. for $C_{29}H_{34}FNO_{11}{}^{130}Te$ 721.12, found 722.13 $[M+H]^+$.

1-(2-difluoromethyl-4-(3-(tellurophen-2-yl)propanamido)phenyl)-β-D-galactopyranose (10)



8 (0.076 g, 0.106 mmol) was dissolved in dry methanol (2 mL) and a 0.5 M solution of NaOMe in methanol (0.20 mL) was added dropwise to the stirring solution. After 3 h, the reaction was quenched by the addition of Dowex® 50WX2 hydrogen form resin (50-100 mesh) until neutral pH, and the solution was concentrated to yield a pale yellow solid. The crude product was desalted using a reverse-phase cartridge (stationary phase, C18; mobile phase, H$_2$O, 50%-100% MeOH) to yield **10** (0.044 g, 75%) as a pale yellow solid. ^{1}H NMR (500 MHz, CD$_3$OD) δ 8.71 (dd, *J* = 6.8 Hz, 1.2 Hz, 1H, TeAr-H), 7.77 (d, *J* = 2.5 Hz, 1H, TeAr-H), 7.63 (dd, *J* = 9.0 Hz, 2.0 Hz, 1H, Ar-H), 7.54 (dd, *J* = 7.0 Hz, 4.0 Hz, 1H, TeAr-H), 7.39 (dd, *J* = 4.0 Hz, 1.5 Hz, 1H, Ar-H), 7.27 (s, 1H, Ar-H), 7.16 (t, *J* = 55.5 Hz, 1H, -C<u>H</u>F$_2$) 4.83 (d, *J* = 8.0 Hz, 1H, H-1), 3.56-3.90 (m, 6H, H-2, H-3, H-4, H-5, H-6a, H-6b), 3.27 (t, *J* = 8.0 Hz, 2H, -OC-C<u>H</u>$_2$-CH$_2$), 2.69 (t, *J* = 7.0 Hz, 2H, -CH$_2$-C<u>H</u>$_2$-CTe). ^{13}C NMR (126 MHz, CD$_3$OD) δ 171.58, 148.43, 136.07, 135.13, 133.49, 124.14, 123.54, 117.29, 116.75, 112.97, 111.11, 109.24, 102.64, 75.70, 73.43, 70.77, 68.76, 60.94, 39.81, 31.91. DART-MS *m/z* calcd. for $C_{20}H_{23}F_2NO_7{}^{130}Te$ 557.05, found 575.09 $[M+NH_4]^+$.

1-(2-(fluoroethyl)-4-(3-(tellurophen-2-yl)propanamido)phenyl)-β-D-galactopyranose (11)



9 (0.062 g, 0.062 mmol) was dissolved in dry methanol (1.0 mL) and a 0.5 M solution of NaOMe in methanol (0.13 mL) was added dropwise to the stirring solution. After 1 h, the reaction was quenched by the addition of Dowex® 50WX2 hydrogen form resin (50-100 mesh) until neutral pH, and the solution was concentrated to yield a pale yellow solid. The crude product was desalted using a reverse-phase cartridge (stationary phase, C18; mobile phase, H$_2$O, 50%-100% MeOH) to yield **11** (0.033 g, 96%) as a pale yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD) δ 8.68 (dd, *J* = 9.0 Hz, 1.5 Hz, 2H, TeAr-Ha/b), 7.54 (app. d, *J* = 2.4 Hz, 2H, TeAr-Ha/b), 7.53 (dd, *J* = 7.2 Hz, 4.0 Hz, 2H, TeAr-Ha/b), 7.46 (dd, *J* = 9.2 Hz, 2.8 Hz, 2H, Ar-Ha/b), 7.36 (dd, *J* = 3.6 Hz, 1.2 Hz, 2H, Ar-Ha/b), 7.15 (dd, *J* = 8.8 Hz, 1.2 Hz), 6.15 (q, *J* = 6.4 Hz, 1H, ArC<u>Ha</u>(F)-), 6.04 (q, *J* = 6.4 Hz, 1H, ArC<u>Hb</u>(F)-), 4.78 (d, *J* = 8.0 Hz, 2H, Ha/b-1), 3.87 (dd, *J* = 3.2 Hz, 0.4 Hz, 2H, Ha/b-4), 3.73-3.78 (m, 6H, Ha/b-2, Ha/b-3, Ha/b-6a), 3.63 (ddd, *J* = 6.4 Hz, 5.2 Hz, 0.8 Hz, 2H, Ha/b-5), 3.54 (dd, *J* = 9.6 Hz, 3.6 Hz, 2H, Ha/b-6b), 3.23 (t, *J* = 7.2 Hz, 4H, -OC-C<u>H</u>$_2$-CH$_2$), 2.66 (t, *J* = 6.8 Hz, -CH$_2$-C<u>H</u>$_2$-CTe), 1.57 (d, *J* = 6.0 Hz, -CH$_2$(F)-C<u>Ha</u>$_3$), 1.51 (d, *J* = 6.4 Hz, -CH$_2$(F)-C<u>Hb</u>$_3$). ^{13}C NMR (126 MHz, CD$_3$OD) δ 171.47, 150.10, 150.05, 148.53, 136.07, 135.10, 133.25, 132.30, 132.14, 124.11,

120.69, 117.14, 117.06, 115.72, 102.53, 86.42, 85.09, 75.59, 73.55, 70.87, 68.78, 60.97, 39.83, 31.99, 21.52, 21.31. ^{19}F NMR (376 MHz, CD_3OD) δ -177.58 (app. sextet, *J* = 23.3 Hz, 1F, -CH_2(F)). ESI-MS *m/z* calcd. for $C_{21}H_{26}FNO_7{}^{130}Te$ 553.08, found 576.06 $[M+Na]^+$.

Stability studies

Deuterated phosphate buffer was prepared by the lyophilization of phosphate buffer (pH 7.5), followed by three additional cycles of reconstitution in D_2O and lyophilization. GalTe (~10 mM) was incubated at room temperature or at 37 °C in a 5 mm NMR tube in deuterated phosphate buffer supplemented with ~20% d_6-DMSO and 10 mM sodium maleate, as an internal standard. The tube was opened every hour for exchange of air. ^{1}H NMR spectra were obtained every 4 h.

Nucleophile tagging studies

GalTe (1 mM) was incubated with L-lysine or L-cysteine (500 mM) for four hours in buffer (100 mM MOPs pH 7.5, $MgCl_2$ 10mM, NaCl 10 mM each and TCEP 1 mM) in the presence or absence of β-galactosidase (2 μM). Reaction mixtures were loaded onto an HPLC equipped with a reverse-phase C18 column. Reaction products were eluted on a gradient of 100% to 0% water in acetonitrile, with a flow rate of 0.5 mL/min and detection by UV absorbance at 280 nm. Fractions containing compound were lyophilized and analyzed by ESI-MS.

Protein tagging studies

Thiolated maltose-binding protein (MalE) was accessed through the treatment of MalE with Traut's reagent (111 eq.; 3 eq. for every lysine residue in MalE) in PBS (pH 8.0) supplemented with EGTA (2 mM) for one hour. Unreacted Traut's reagent was separated from protein using a PD-10 size-exclusion column, and protein was eluted with MOPS buffer (pH 7.5) supplemented with EGTA (2 mM).

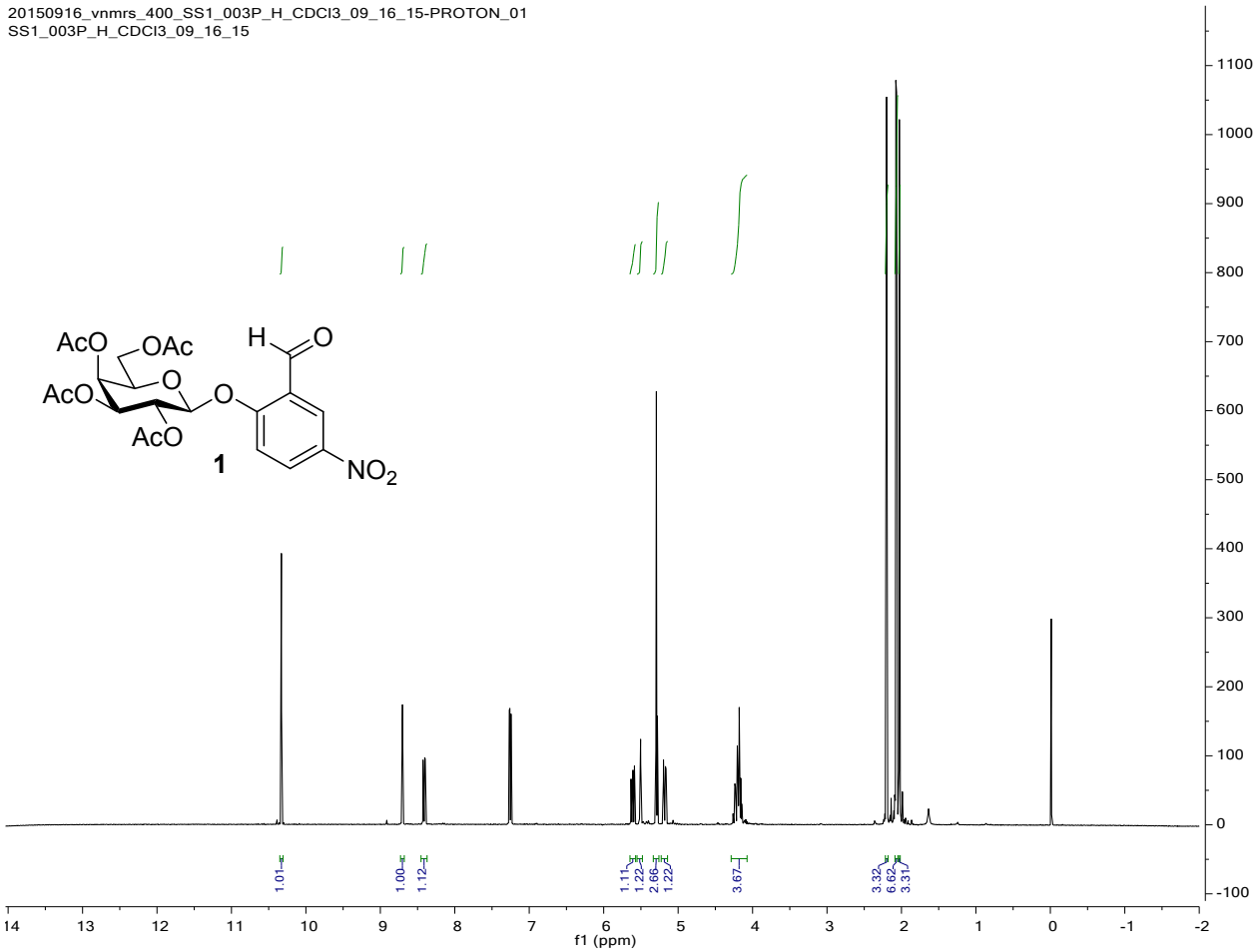
GalTe (50 μM) was incubated with unfunctionalized or thiolated maltose-binding protein (MalE, 100 μM) for four hours in buffer (100 mM MOPs pH 7.5, $MgCl_2$ 10mM, NaCl 10 mM each and TCEP 1 mM) in the presence or absence of β-galactosidase (2 μM). The reaction mixture was loaded onto amylose resin (200 μL), the resin was rinsed with MOPS buffer (10 mL), and protein was eluted with a solution of maltose (10 mM, 10 mL). Collected eluates were lyophilized, then treated with 35% HNO_3 (500 μL) and tellurium counts were quantified by ICP-MS on a Perkin-Elmer SCIEX Elan 9000 ICP-MS equipped with autosampler.

Cell studies

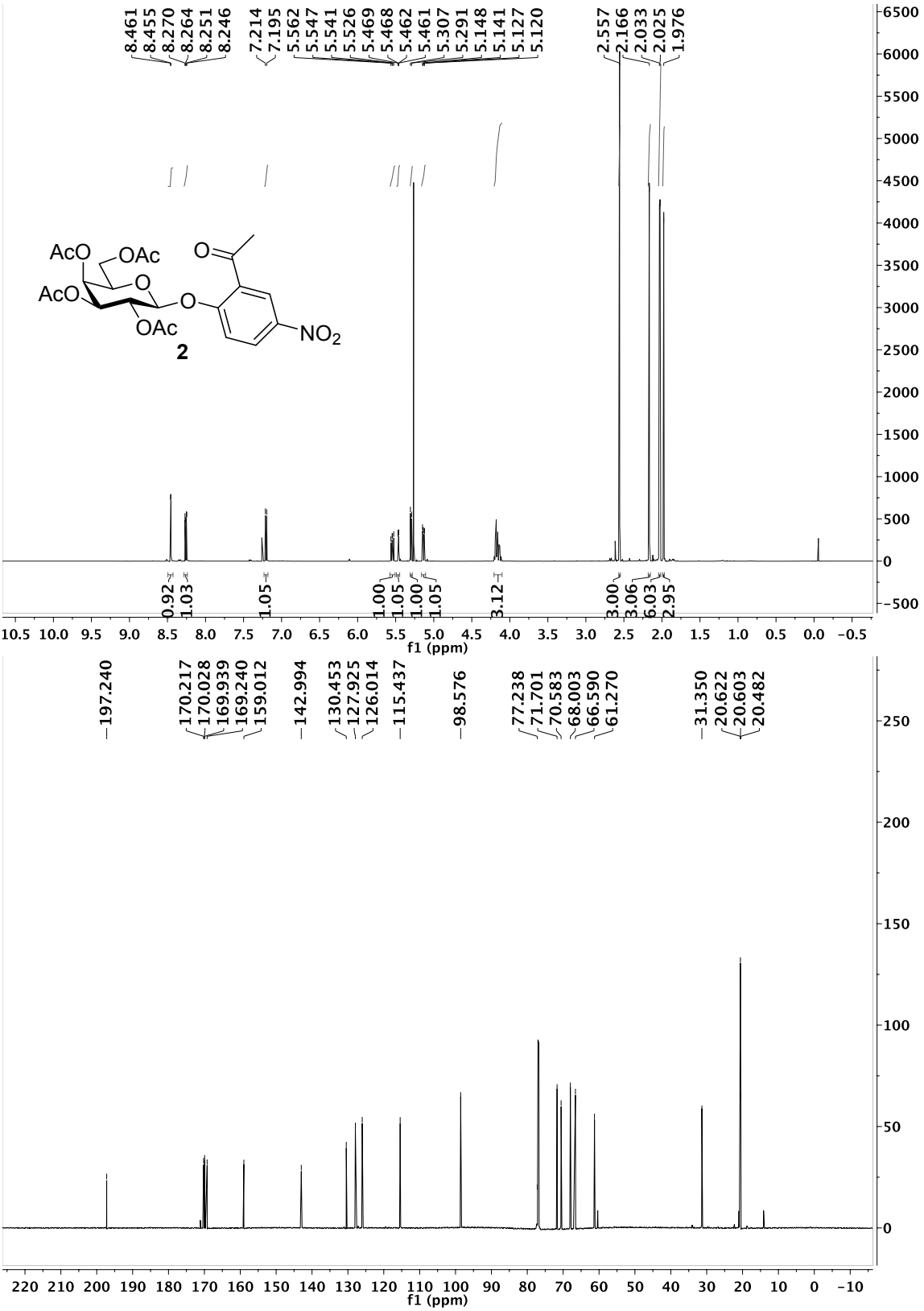
Retinal pigment epithelium (RPE) cells as well as primary human skin fibroblasts (GM05757, Coriell Institute, NJ) were cultured under standard conditions (37 °C, 5% CO_2, balance air) Cells were incubated in D-MEM (4.5 g/L) supplemented with 10 % FBS.

Senescence was induced by treating cells with 150 µM H_2O_2 at 37°C for 2 h then incubating cells in serum free D-MEM for 72 h, as previously described (Frippiat, *et al.* J. Biol. Chem 2001 276:2531-2537). Senescent and control cells were treated with 50 µM probe in serum-free media for 8 or 24 h, washed three times with PBS, and lifted with trypsin-EDTA. Cells were fixed in 10% formalin at room temperature for 20 min, then washed three times with cold PBS and stained with an iridium DNA intercalator (0.25 nM) at room temperature for 10 min. Cells were washed three times, and analyzed by mass cytometry (Fluidigm Inc., Markham, ON). Data were collected in FCS format and processed using FlowJo LLC software (Ashland, OR).

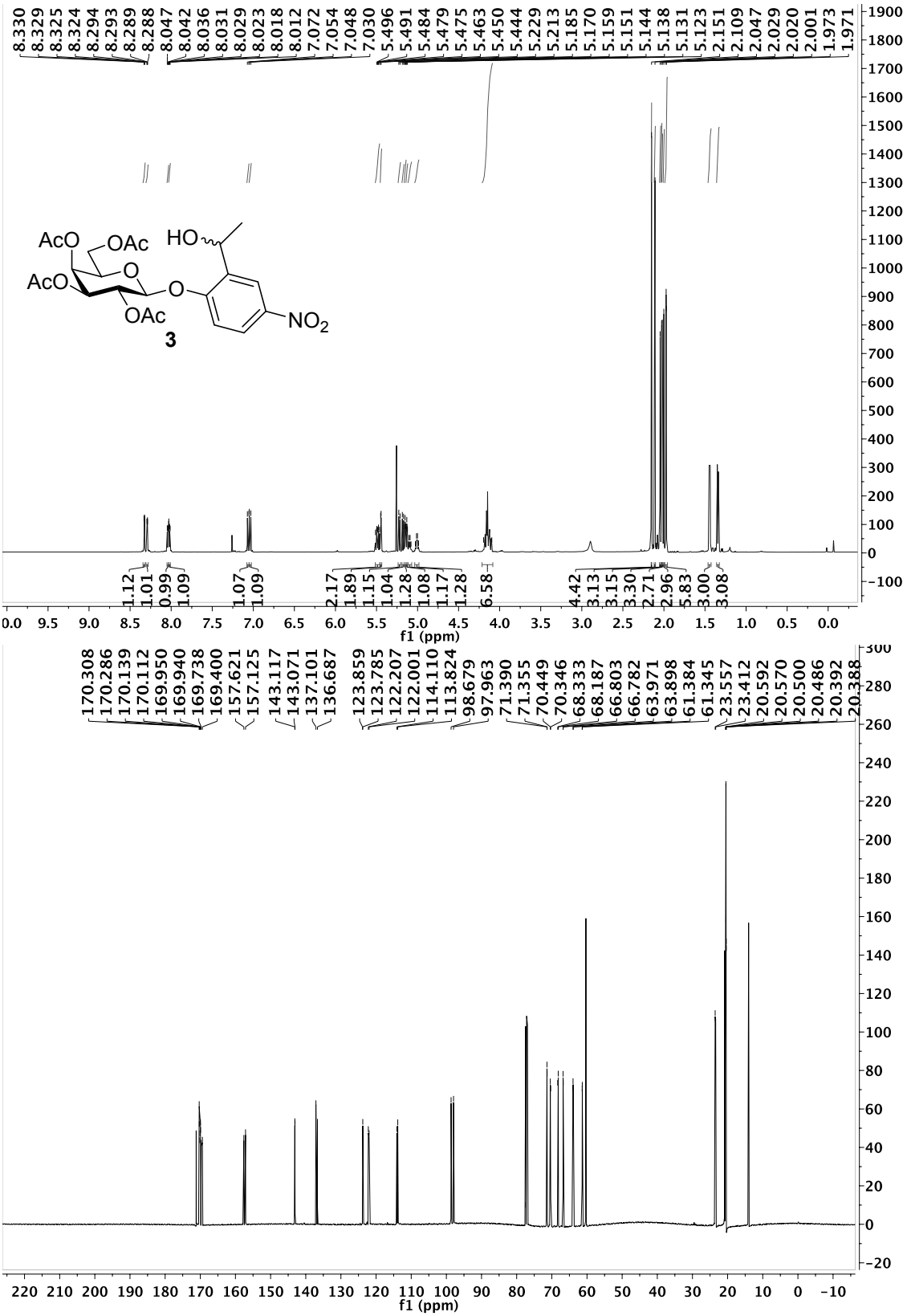
NMR spectra



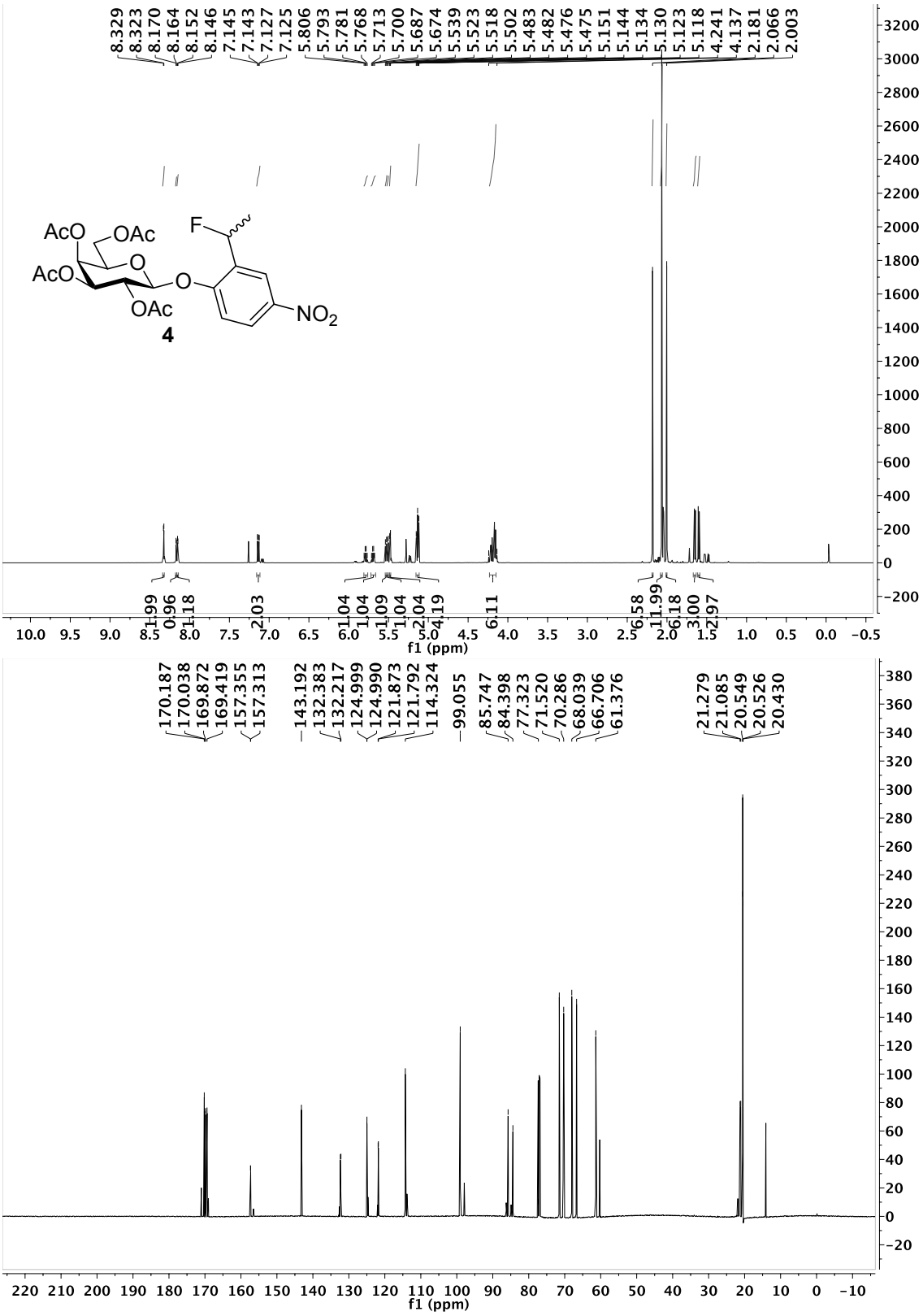
^{1}H-NMR Compound 1, (500 MHz, $CDCl_3$)



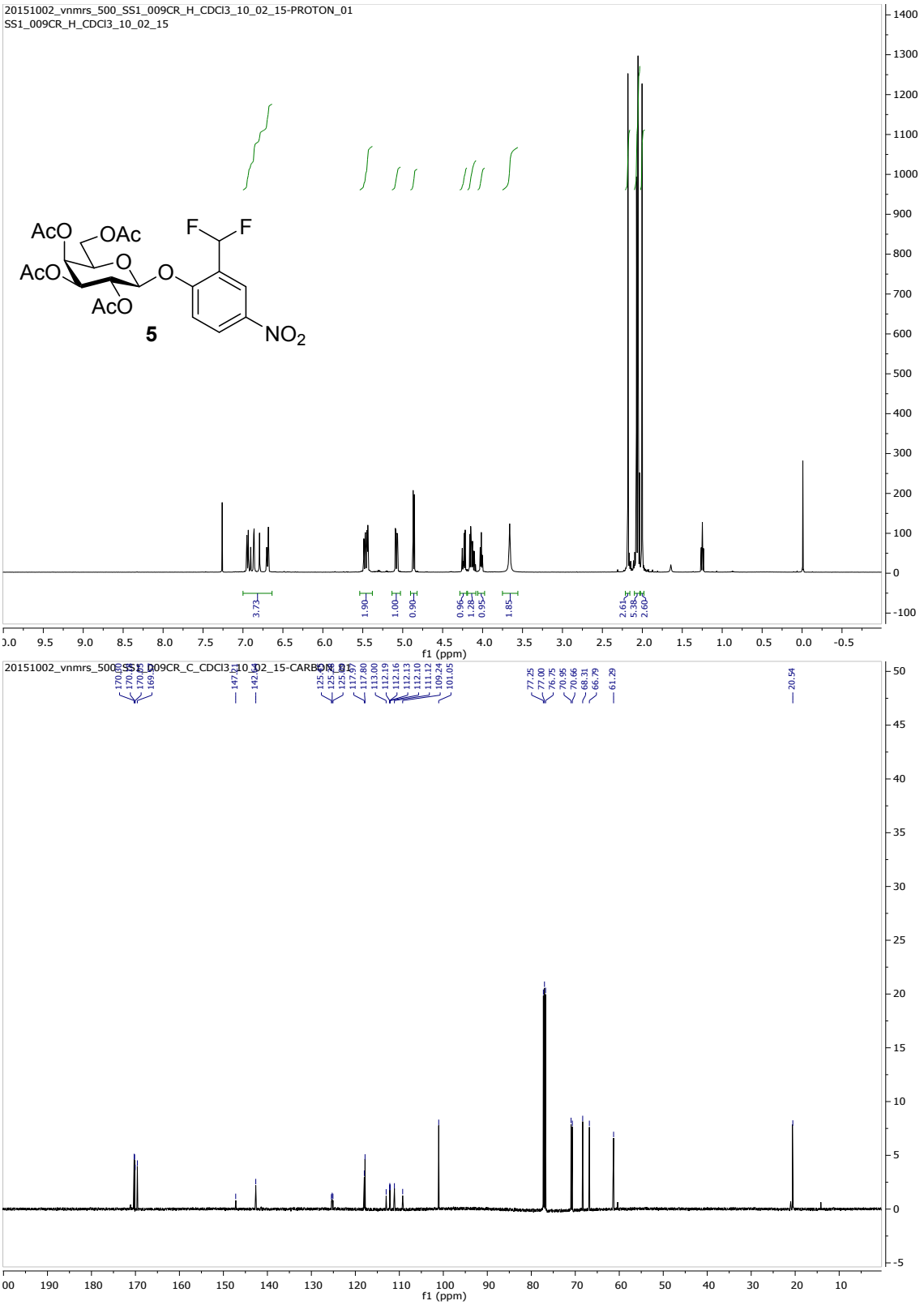
Compound **2** ^{1}H NMR (500 MHz, $CDCl_3$) (Top) and ^{13}C NMR (126 MHz, $CDCl_3$)(Bottom)



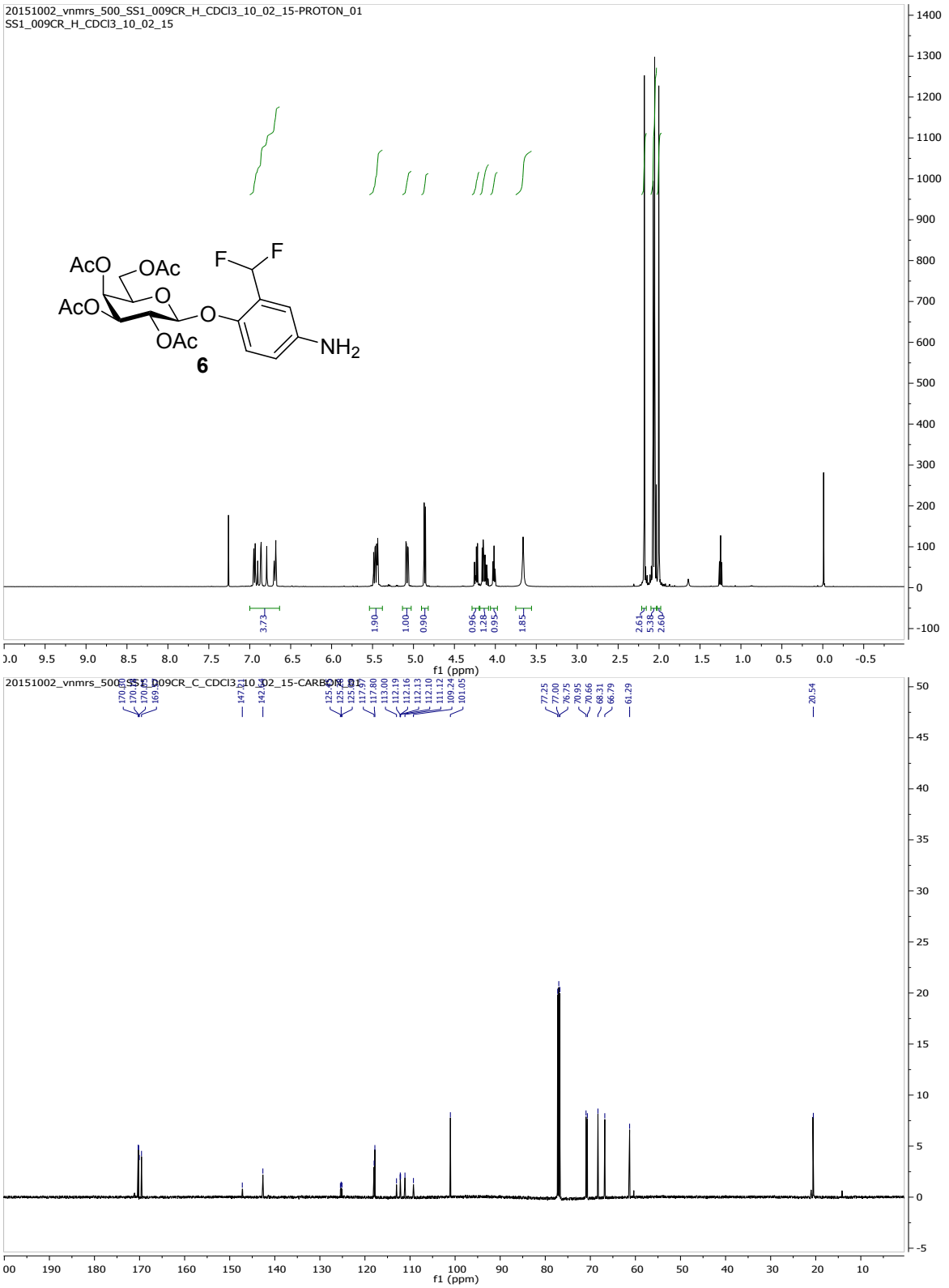
Compound **3** ^{1}H NMR (500 MHz, $CDCl_3$) (Top) and ^{13}C NMR (126 MHz, $CDCl_3$)(Bottom)



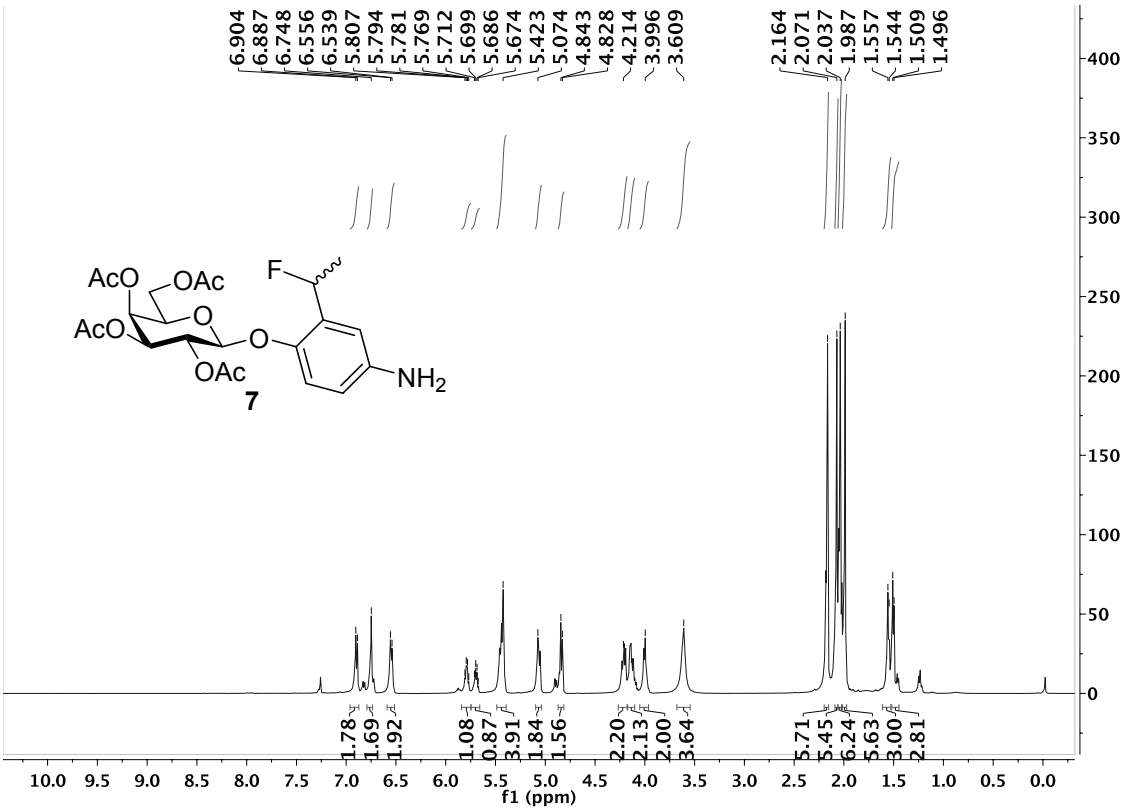
Compound **4** ^{1}H NMR (500 MHz, $CDCl_3$) (Top) and ^{13}C NMR (126 MHz, $CDCl_3$)(Bottom)



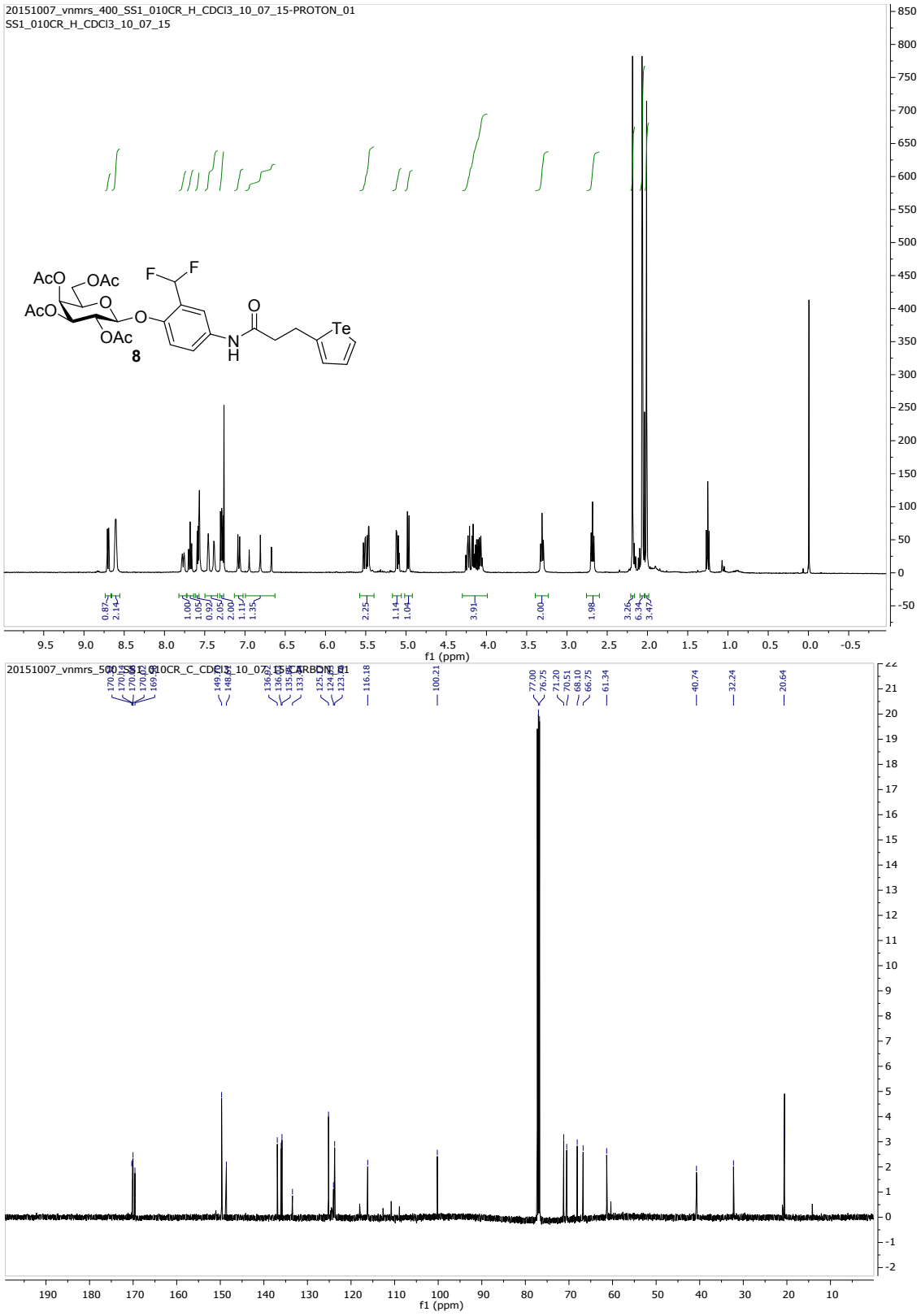
Compound **5** ^{1}H NMR (500 MHz, $CDCl_3$) (Top) and ^{13}C NMR (126 MHz, $CDCl_3$)(Bottom)



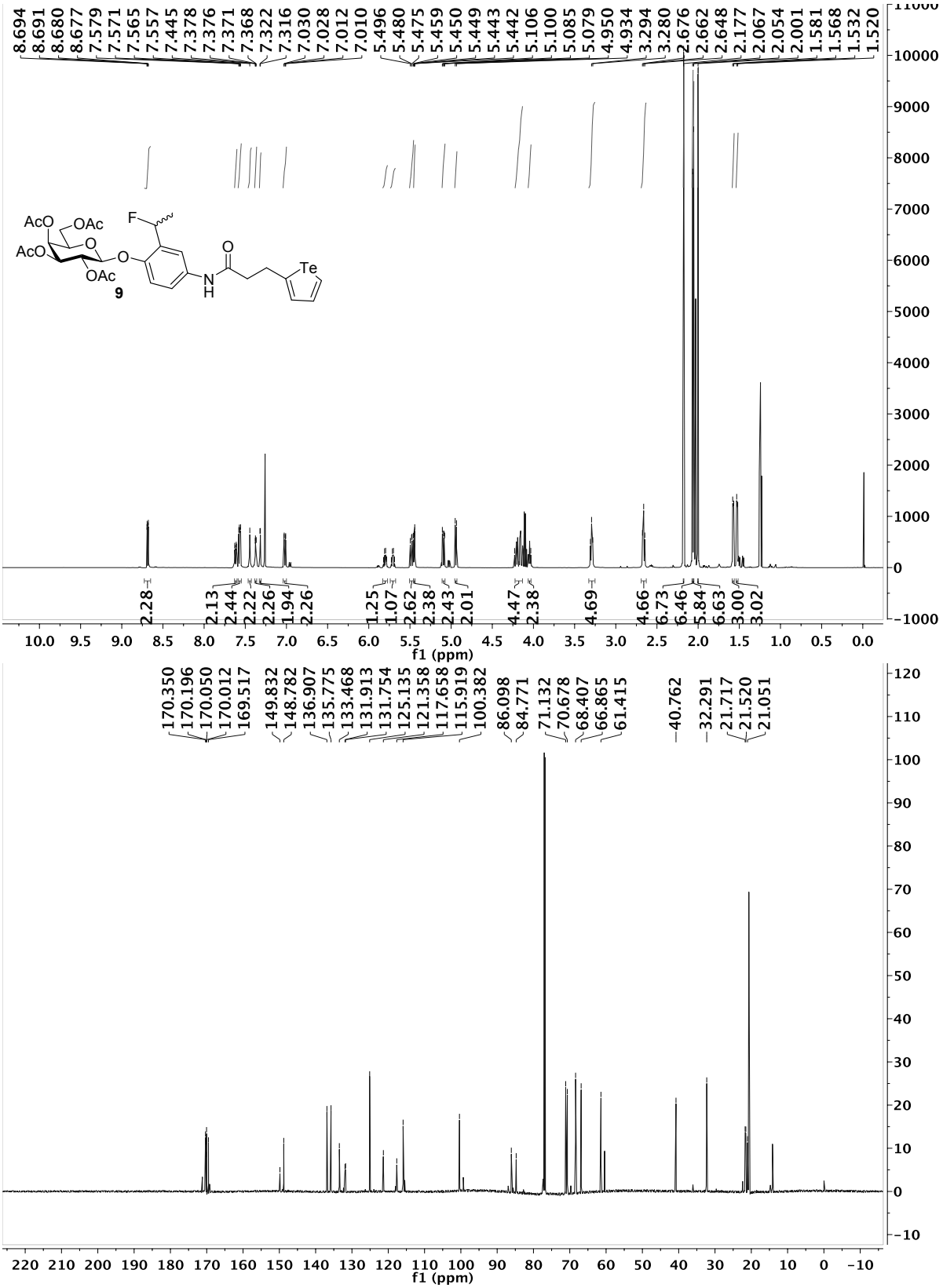
Compound **6** ^{1}H NMR (500 MHz, $CDCl_3$) (Top) and ^{13}C NMR (126 MHz, $CDCl_3$)(Bottom)



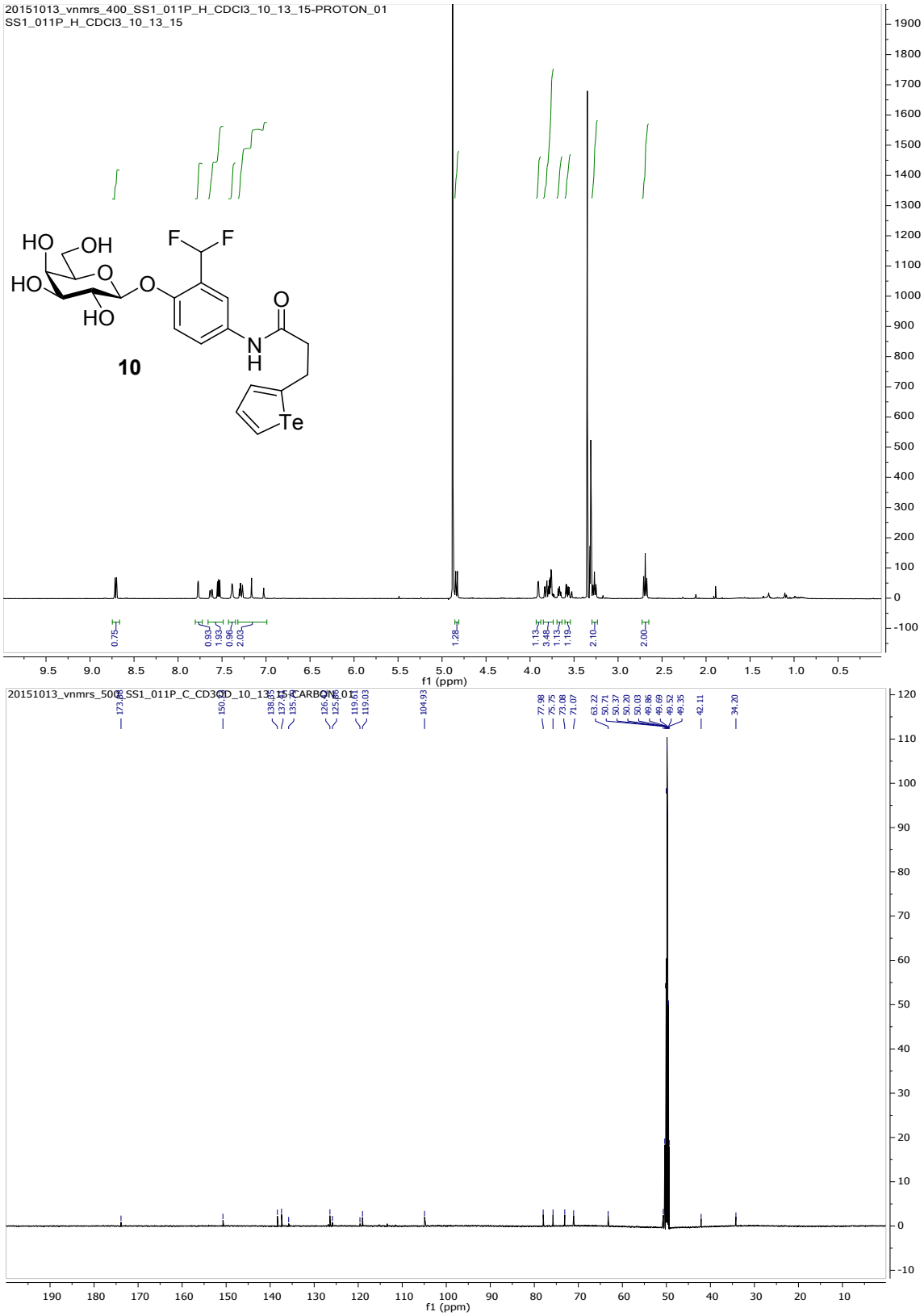
Compound **7** ^{1}H NMR (500 MHz, $CDCl_3$)



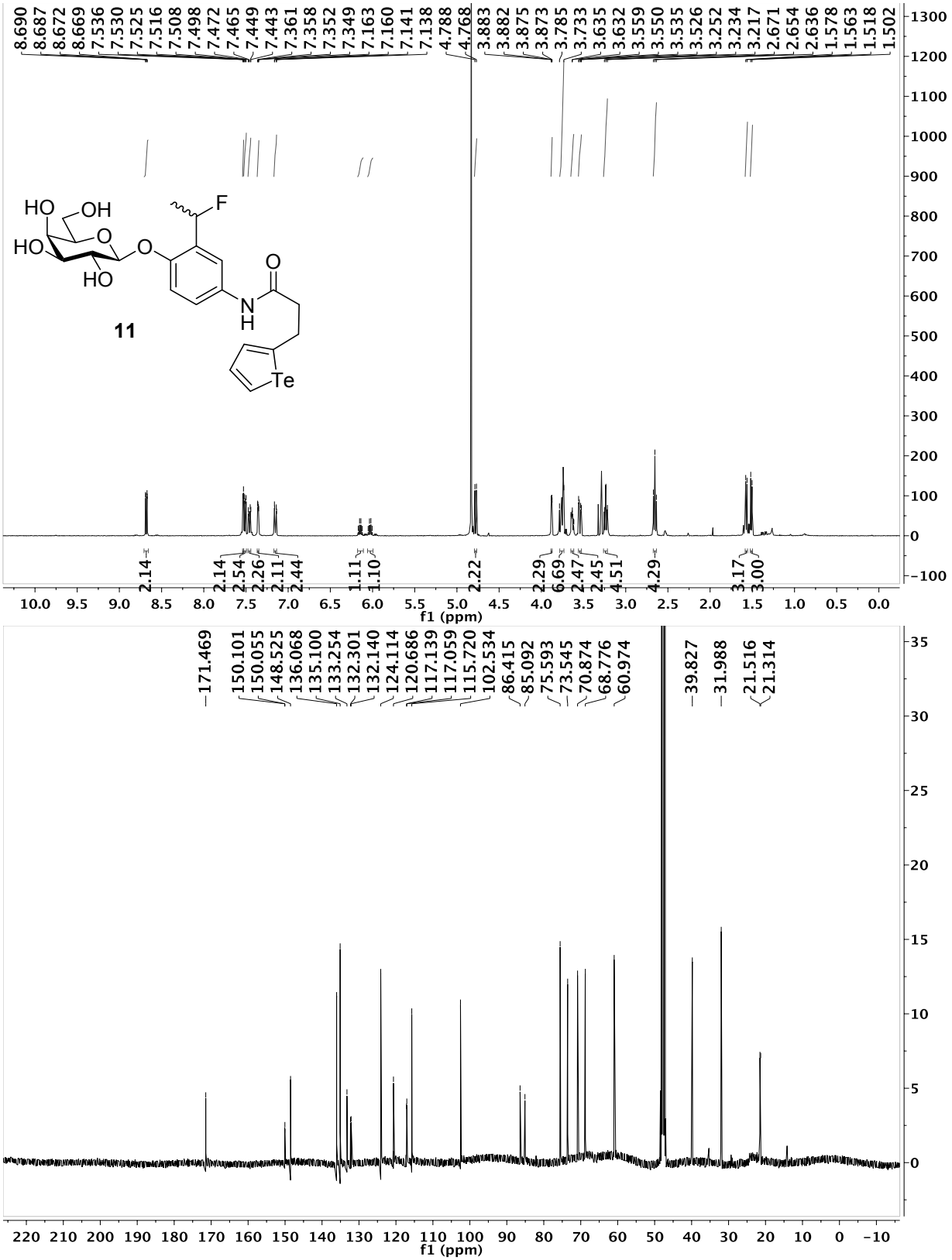
Compound **8** ^{1}H NMR (500 MHz, $CDCl_3$) (Top) and ^{13}C NMR (126 MHz, $CDCl_3$)(Bottom)



Compound **9** ^{1}H NMR (500 MHz, $CDCl_3$) (Top) and ^{13}C NMR (126 MHz, $CDCl_3$)(Bottom)



Compound **10** ^{1}H NMR (500 MHz, CD_3OD) (Top) and ^{13}C NMR (126 MHz, CD_3OD)(Bottom)



Compound **11** ^{1}H NMR (500 MHz, CD_3OD) (Top) and ^{13}C NMR (126 MHz, CD_3OD)(Bottom)