Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information for

TUNING THE LEAVING GROUP IN 2-DEOXY-2-FLUOROGLUCOSIDE RESULTS IN IMPROVED ACTIVITY-BASED RETAINING β-GLUCOSIDASE PROBES

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General experimental procedures. All chemicals were used as received unless stated otherwise. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-300 (300/75 MHz), a Bruker AV-400 (400/100 MHz) and a Bruker DMX-600 (600/150 MHz) spectrometer. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard. Coupling constants are given in Hz. All given ^{13}C-APT spectra are proton decoupled. IR-spectra were recorded on a Shimadzu FTIR-8300. Flash chromatography was performed on Fluka silica gel 60 (0.04 – 0.063 mm). TLC-analysis was conducted on DC-alufolien (Merck, Kieselgel60, F254) with detection by UV-absorption (254 nm) where applicable and by spraying with 20% sulfuric acid in ethanol followed by charring at ~150 °C or by spraying with a solution of $(NH_4)_6Mo_7O_{24}\cdot H_2O$ (25 g/l) and $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ (10 g/l) in 10% sulfuric acid in water followed by charring at ~150 °C. TLC-MS analysis was performed on a Camag TLC-MS Interface combined with an API165 (SCIEX) mass spectrometer (eluted with *tert*-butylmethylether/EtOAc/MeOH, 5/4/1, v/v/v + 0.1% formic acid, flow rate 0.1 mL/min). LC-MS analysis was performed on a Jasco 980 HPLC system with API165 (SCIEX) ESI-MS and 3300 ELSD detector (Grace). Standard eluents used were A: 100% H_2O, B: 100% acetonitrile, C: 1% TFA in H_2O. Eluents used with acid-sensitive compounds were A: 100% H_2O, B: 100% acetonitrile, C: 100 mM NH_4OAc in H_2O. Column used was Phenomenix Gemini C18 column (3 µm, 4.6x50mm). All analyses were 13 min, with a flow-rate of 1 ml/min. HPLC purification was performed on a preparative LC-MS system (Agilent 1200serie) with an Agilent 6130 Quadruple MS detector and an Agilent G1968D active splitter (split ratio = 927:1; freq. = 1,429 Hz; vol. = 300 nL); the eluents used were A: 0.1% TFA in H_2O, B: 100% acetonitrile, or with acid-sensitive compounds A: 20 mM NH_4OAc in H_2O, B: 100% acetonitrile; the column used was Phenomenix Gemini C18 (5 µm, 10 x 250 mm), with a flow rate of 5 ml/min. Absorption (4MU assay) was measured on an LS55 fluorimeter (Perkin Elmer) with λ_{ex} 366 nm and λ_{em} 455 nm. Fluorescent scanning of slab gels was performed on a Typhoon Variable Mode Imager (600 PMT, medium sensitivity, pixel size 200 µm), using λ_{ex} 488 and λ_{em} 520 nm for green fluorescent BODIPY dyes, and λ_{ex} 532 and λ_{em} 610 nm for red fluorescent BODIPY dyes.

Synthesis of the probes. The stereoselectivity of the electrophilic fluorination of D-glucal with Selectfluor® has been shown to depend greatly on the protecting group pattern.[1] Whereas the per-acetylated D-glucal roughly produced a 1 : 1 epimeric *gluco* : *manno*

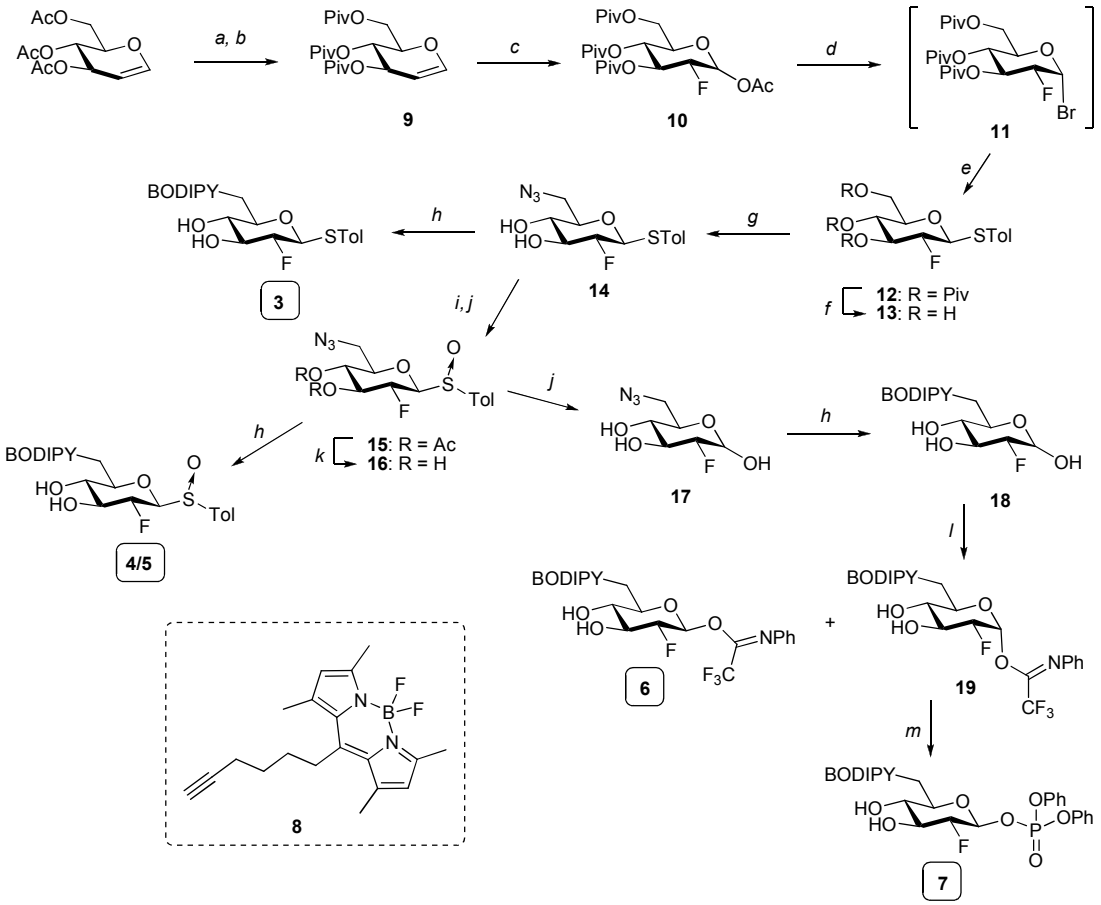
[1] Dax, K.; Albert, M.; Ortner, J.; Paul B. *Carb.Res.* **2000**, *327*, 47-86.

mixture, the per-pivaloylated D-glucal **9** revealed a high preference for the *gluco* epimer.[1] Therefore, this strategy was applied here in the synthesis of probes **3**-**7** as depicted in Scheme 1. Thus, commercially available 3,4,6-tri-*O*-acetyl-D-glucal was deacetylated using Zemplén conditions, and the triol was directly pivaloylated to give **9** in 60% over two steps. Fluorination using Selectfluor in $MeNO_2/H_2O$ yielded 66% of the *gluco* epimer **10** after ensuing acetylation and column chromatography. Subsequent anomeric bromination (HBr/AcOH) and direct substitution with *p*-thiocresol using phase-transfer conditions exclusively gave β-thioglucoside **12** in 96% over two steps. The pivaloyl esters were removed by prolonged treatment with NaOMe in MeOH (5 days) to produce triol **13**. The azido functionality was introduced by selective tosylation of 6-OH (Ts-Cl, tetramethylethylenediamine) and substitution with NaN_3 while heating at 80 ºC overnight to yield product **14** in 63% over two steps. Compound **14** was used in the copper-catalyzed click reaction with alkyne **8** to produce direct probe **3** in 44% yield. To produce probes **4**-**7**, compound **14** was first acetylated and subsequently treated with NBS in acetone/H_2O. Because it was observed before that the anomeric thio functionality was readily oxidized with aqueous NBS,[2] these conditions were applied in this synthetic scheme. In this way, sulfoxide **15** (mixture of diastereomers on sulfur) was obtained in 59% yield, next to hemiacetal byproduct (29%). Removal of the acetyls in **15** (NaOMe, MeOH) provided compound 1**6**, which was coupled to the BODIPY-moiety to produce a diastereomeric mixture of sulfoxides **4/5**. Using RP-HPLC the diastereomers were separated to give direct probes **4** and **5** in 20% and 18% yield, respectively. Sulfoxide **16** was efficiently hydrolyzed towards hemiacetal **17** (94%) by treatment with NBS for 3 h. To access the more labile anomeric imidate probe **6** and phosphate probe **7**, it was decided to install the BODIPY-moiety prior to anomeric leaving group introduction. Thus, hemiacetal **17** was connected to alkyne **8** under the standardized click conditions to produce compound **18** in 53%. Subsequently, an anomeric mixture of *N*-phenyl trifluoroacetimidates was produced under mild basic conditions, which were resolved using RP-HPLC (NH_4OAc). Subsequent lyophilization afforded the pure β-anomer **6** in 15% and α-anomer **19** in 10%. In a first attempt to obtain anomeric phosphate **7**, the anomeric mixture of imidates was treated with diphenylphosphoric acid to give immediate and quantitative conversion to an anomeric mixture of phosphates. While this mixture was separable on RP-HPLC, the β-phosphate **7** did not withstand lyophilization in the presence of

[2] Witte, M. D.; Walvoort, M. T. C.; Li, K.-Y.; Kallemeijn, W. W.; Donker-Koopman, W. E.; Boot, R. G.; Aerts, J. M. F. G.; Codée, J. D. C.; van der Marel, G. A.; Overkleeft, H. S. *Chembiochem* **2011**, *12*, 1263-1269.

aqueous NH_4OAc. To circumvent this hydrolysis, pure α-imidate **19** was substituted by diphenylphosphate in an S_N2-like reaction to yield β-phosphate **7**, which was purified using flash column chromatography and subsequently lyophilized under neutral conditions.

Scheme 1. Synthesis of 2-fluoro β-glucoside probes **3**-**7**

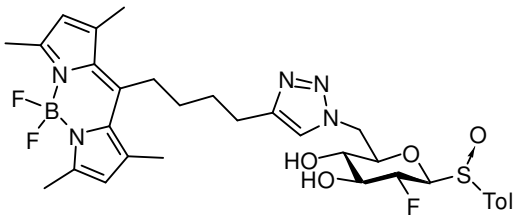


Reagents and conditions: a) NaOMe, MeOH; b) Piv-Cl, DMAP, pyridine (**9**: 60% two steps); c) *i*. Selectfluor®, $MeNO_2/H_2O$; *ii.* Ac_2O, pyridine, DCM (**10**: 66%); d) HBr/AcOH, DCM; e) TolSH, TBAB, KOH, $CHCl_3/H_2O$ (**12**: 96%, two steps); f) NaOMe, MeOH (**13**: quant.); g) *i*. Ts-Cl, TMEDA, MeCN; *ii.*NaN_3, DMF, 80 ºC (**14**: 63% over two steps); h) BODIPY-alkyne **8**, sodium ascorbate, $CuSO_4$, DMF, 80 ºC (**3**: 44%, **4**: 20%, **5**: 18%, **18**: 53%); i) Ac_2O, pyridine; j) NBS, acetone/H_2O (**15**: 39% over two steps, **17**: 94%); k) NaOMe, MeOH (**16**: quant.); l) $CF_3C(NPh)Cl$, K_2CO_3, acetone (**6**: 15%, **19**: 10%); m) $HOP(O)(OPh)_2$, DCM (**7**: 59%).

Probe 3. Compound **14** (20 mg, 67 μmol) and BODIPY-alkyne **8** (24 mg, 73 μmol) were together dissolved in DMF (1.5 mL) and treated with sodium ascorbate (10 μL, 1M solution in H_2O) and $CuSO_4$ (7 μL, 1M solution in H_2O). The resulting mixture was stirred at 80 ºC for 2

days, during which time the addition of sodium ascorbate and $CuSO_4$ was repeated twice. The mixture was allowed to cool to RT and diluted with EtOAc and H_2O. The organic phase was washed with sat. aq. NaCl, dried over Na_2SO_4 and the product was obtained using flash column chromatography (silica gel, 4% MeOH in DCM) followed by lyophilization as an orange solid (Yield: 18.8 mg, 29.3 µmol, 44%). TLC: R_f 0.32 (DCM/MeOH, 9/1, v/v); IR (neat, cm^{-1}): 894, 1065, 1508, 1551, 3394; ^1H NMR ($CDCl_3$/MeOH-d_4, 400 MHz, HH-COSY, HSQC): δ 7.26 (d, 2H, J = 8.0 Hz, CH_{arom}), 7.05 (d, 2H, J = 7.9 Hz, CH_{arom}), 6.06 (s, 2H, CH pyrrole), 4.79 (dd, 1H, J = 2.1, 14.5 Hz, H-6), 4.57 (d, 1H, J = 9.3 Hz, H-1), 4.46 (dd, 1H, J = 7.0, 14.5 Hz, H-6), 3.94 (dt, 1H, J = 9.0, 49.6 Hz, H-2), 3.68 (dt, 1H, J = 7.7, 15.4 Hz, H-3), 3.50-3.60 (m, 1H, H-5), 3.09 (t, 1H, J = 9.4 Hz, H-4), 2.99 (dd, 2H, J = 6.6, 10.1 Hz, CH_2), 2.74 (t, 2H, J = 7.5 Hz, CH_2), 2.50 (s, 6H, CH_3), 2.39 (s, 6H, CH_3), 2.31 (s, 3H, CH_3 STol), 1.85-1.94 (m, 2H, CH_2), 1.63-1.71 (m, 2H, CH_2); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 153.9, 147.3, 145.8, 140.2, 138.8 (C_q), 133.9 (CH_{arom}), 131.3 (C_q), 129.8 (CH_{arom}), 126.7 (C_q), 123.2 (CH triazole), 121.7 (CH pyrrole), 89.1 (d, J = 186 Hz, C-2), 84.4 (d, J = 24 Hz, C-1), 77.4 (C-5), 76.0 (d, J = 18 Hz, C-3), 69.9 (d, J = 8 Hz, C-4), 50.5 (C-6), 31.3, 29.6, 28.0, 25.2 (CH_2), 21.2 (CH_3STol), 16.4, 14.4 (CH_3); LC-MS: R_t 9.22 min (C18 column, linear gradient 10 → 90% B in 13.5 min); HRMS: $[M+H]^+$ calcd for $C_{32}H_{40}BF_3N_5O_3S$ 642.28915, found 642.28954.

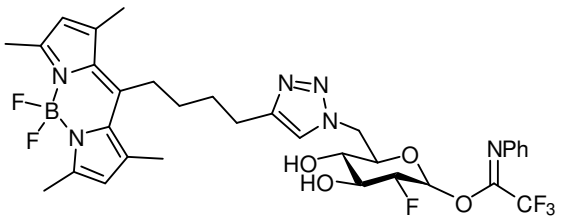
Probes **4** and **5**. Compound **16** (25 mg, 78 µmol) and BODIPY-alkyne **8** (28 mg, 85 µmol)



were together dissolved in DMF (1 mL) and treated with sodium ascorbate (12 µL, 1M solution in H_2O) and $CuSO_4$ (8 µL, 1M solution in H_2O). The resulting mixture was stirred at 80 ºC for 2 days, during which time the addition of sodium ascorbate and $CuSO_4$ was repeated twice. The mixture was allowed to cool to RT and diluted with EtOAc and H_2O. The organic phase was washed with sat. aq. NaCl, dried over Na_2SO_4 and the product was isolated using flash column chromatography (silica gel, 10% MeOH in DCM). The two diastereomers were separated using RP-HPLC followed by lyophilization to yield **4** (Yield: 10.1 mg, 15.3 µmol, 20%) and **5** (Yield: 9.5 mg, 14.4 mmol, 18%) both as orange solids. TLC: R_f 0.45 (DCM/MeOH, 8.5/1.5, v/v); IR (neat, cm^{-1}): 984, 1080, 1200, 1508, 1551, 3406. Spectroscopic data for product **4**: ^1H NMR (MeCN-d_3, 600 MHz, HH-COSY, HSQC): δ 7.32 (d, 2H, J = 8.2 Hz, CH_{arom}), 7.16 (d, 2H, J = 7.9 Hz,

CH_{arom}), 6.76 (s, 1H, CH triazole), 6.08 (bs, 2H, CH pyrrole), 4.57 (d, 1H, *J* = 14.8 Hz, H-6), 4.47 (dt, 1H, *J* = 9.3, 50.4 Hz, H-2), 4.14 (dd, 1H, *J* = 8.4, 14.8 Hz, H-6), 4.01 (dd, 1H, *J* = 2.9, 9.7 Hz, H-1), 3.67 (dt, 1H, *J* = 8.9, 15.5 Hz, H-3), 3.34 (t, 1H, *J* = 8.3 Hz, H-5), 3.14 (t, 1H, *J* = 9.4 Hz, H-4), 2.89 (dddd, 2H, *J* = 5.0, 12.9, 13.0, 25.2 Hz, CH_2), 2.41-2.59 (m, 2H, CH_2), 2.36 (s, 12H, CH_3), 2.28 (s, 3H, CH_3 STol), 1.64-1.78 (m, 2H, CH_2), 1.33-1.52 (m, 2H, CH_2); ^{13}C-APT NMR (MeCN-d_3, 150 MHz, HSQC): δ 148.3, 147.8, 142.7, 136.7 (C_q), 130.8, 126.2, 123.0, 122.6 (CH_{arom}), 90.7 (d, *J* = 24 Hz, C-1), 88.6 (d, *J* = 183 Hz, C-2), 80.5 (C-5), 76.0 (d, *J* = 17 Hz, C-3), 71.4 (d, *J* = 8 Hz, C-4), 51.6 (C-6), 31.8, 30.4, 29.0, 25.8 (CH_2), 21.7 (CH_3 STol), 16.6, 14.6 (CH_3); LC-MS: R_t 7.79 min (C18 column, linear gradient 10 → 90% B in 13.5 min); HRMS: $[M+Na]^+$ calcd for $C_{32}H_{39}BF_3N_5O_4SNa$ 680.26601, found 680.26583. Spectroscopic data for product **5**: ^{1}H NMR (MeCN-d_3, 600 MHz, HH-COSY, HSQC): δ 7.46 (d, 2H, *J* = 11.7 Hz, CH_{arom}), 7.34 (d, 2H, *J* = 8.1 Hz, CH_{arom}), 6.17 (bs, 2H, CH pyrrole), 4.73 (dd, 1H, *J* = 2.1, 14.7 Hz, H-6), 4.40-4.51 (m, 3H, H-1, H-2, H-6), 3.75 (ddd, 1H, *J* = 2.1, 7.4, 9.6 Hz, H-5), 3.66-3.69 (m, 1H, H-3), 3.10 (t, 1H, *J* = 9.2 Hz, H-4), 3.03-3.07 (m, 2H, CH_2), 2.78 (t, 2H, *J* = 7.3 Hz, CH_2), 2.46 (s, 6H, CH_3), 2.44 (s, 6H, CH_3), 2.40 (s, 3H, CH_3 STol), 1.89-1.95 (m, 2H, CH_2), 1.67-1.72 (m, 2H, CH_2); ^{13}C-APT NMR (MeCN-d_3, 150 MHz, HSQC): δ 130.6, 125.8, 123.7 (CH_{arom}), 122.7 (CH pyrrole), 93.1 (d, *J* = 24 Hz, C-1), 87.8 (d, *J* = 185 Hz, C-2), 79.6 (C-5), 76.2 (d, *J* = 18 Hz, C-3), 71.1 (d, *J* = 8 Hz, C-4), 51.4 (C-6), 31.9, 30.5, 29.0, 25.9 (CH_2), 20.3 (CH_3 STol), 16.6 (CH_3); LC-MS: R_t 8.00 min (C18 column, linear gradient 10 → 90% B in 13.5 min); HRMS: $[M+H]^+$ calcd for $C_{32}H_{40}BF_3N_5O_4S$ 658.28407, found 658.28426.

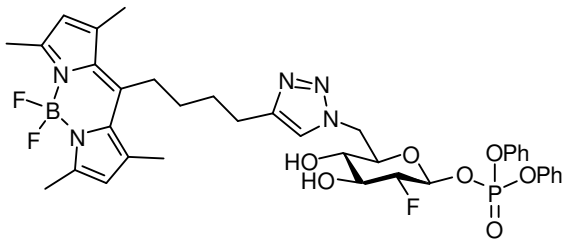
Probe **6**. A solution of compound **18** (17 mg, 32 µmol) in acetone (2 mL) was cooled to 0 ºC,



followed by the addition of *N*-phenyl trifluoroacetimidoyl chloride (10 µL, 63 µmol) and K_2CO_3 (6 mg, 43 µmol). The reaction was stirred at RT overnight, after which time the mixture was diluted with EtOAc. The organic phase was washed with sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 87% EtOAc in PE) yielded an anomeric mixture of imidates. The anomers were separated using RP-HPLC to give β-anomer **6** (Yield: 3.4 mg, 4.8 µmol, 15%) and α-anomer **19** (Yield: 2.2 mg, 3.0 µmol, 10%) both as orange solids. TLC: R_f 0.64 (DCM/MeOH, 8.5/1.5, v/v); IR

(neat, cm^{-1}): 986, 1082, 1161, 1202, 1510, 1551, 1719, 3383. Spectroscopic data for the β anomer **6**: ^{1}H NMR (MeCN-d_3, 600 MHz, HH-COSY, HSQC, T = 335 K): δ 7.57 (s, 1H, CH triazole), 7.31 (t, 2H, *J* = 7.9 Hz, CH_{arom}), 7.14 (t, 1H, *J* = 7.5 Hz, CH_{arom}), 6.76 (d, 2H, *J* = 7.5 Hz, CH_{arom}), 6.18 (s, 2H, CH pyrrole), 5.68 (bs, 1H, H-1), 4.81 (dd, 1H, *J* = 1.7, 14.6 Hz, H-6), 4.42 (dd, 1H, *J* = 8.2, 14.7 Hz, H-6), 4.33 (dt, 1H, *J* = 8.4, 51.5 Hz, H-2), 3.72-3.79 (m, 1H, H-3), 3.65-3.72 (m, 1H, H-5), 3.35 (t, 1H, *J* = 9.3 Hz, H-4), 3.01 (t, 2H, *J* = 8.8 Hz, CH_2), 2.59-2.71 (m, 2H, CH_2), 2.49 (s, 6H, CH_3), 2.41 (s, 6H, CH_3), 1.78-1.86 (m, 2H, CH_2), 1.54-1.67 (m, 2H, CH_2); ^{13}C-APT NMR (MeCN-d_3, 150 MHz, HSQC, T = 330 K): δ 154.9, 148.6, 148.5, 144.5, 142.6 132.6 (C_q), 130.2, 125.9 (CH_{arom}), 123.5 (CH triazole), 122.9 (CH pyrrole), 120.3 (CH_{arom}), 95.9 (d, *J* = 25 Hz, C-1), 92.3 (d, *J* = 187 Hz, C-2), 77.0 (C-5), 75.6 (d, *J* = 17 Hz, C-3), 72.1 (d, *J* = 8 Hz, C-4), 51.7 (C-6), 32.1, 30.7, 29.3, 26.1 (CH_2), 16.8, 14.8 (CH_3); LC-MS: R_t 9.75 min (C18 column, linear gradient 10 → 90% B in 13.5 min); HRMS: $[M+H]^+$ calcd for $C_{33}H_{38}BF_6N_6O_4$ 707.29463, found 707.29472. Spectroscopic data for the α anomer **18**: ^{1}H NMR (MeCN-d_3, 600 MHz, HH-COSY, HSQC, T = 335 K): δ 7.52 (s, 1H, CH triazole), 7.33 (t, 2H, *J* = 7.9 Hz, CH_{arom}), 7.14 (t, 1H, *J* = 7.5 Hz, CH_{arom}), 6.74 (d, 2H, *J* = 7.9 Hz, CH_{arom}), 6.29 (bs, 1H, H-1), 6.17 (s, 2H, CH pyrrole), 4.75 (dd, 1H, *J* = 2.1, 14.6 Hz, H-6), 4.41-4.52 (m, 2H, H-2, H-6), 4.03-4.08 (m, 1H, H-5), 3.97 (dt, 1H, *J* = 9.3, 12.9 Hz, H-3), 3.31 (t, 1H, *J* = 9.6 Hz, H-4), 3.05 (t, 2H, *J* = 8.6 Hz, CH_2), 2.72-2.83 (m, 2H, CH_2), 2.48 (s, 6H, CH_3), 2.43 (s, 6H, CH_3), 1.85-1.93 (m, 2H, CH_2), 1.64-1.74 (m, 2H, CH_2); ^{13}C-APT NMR (MeCN-d_3, 150 MHz, HSQC, T = 330 K): δ 154.6, 148.2, 148.2, 142.4, 132.2 (C_q), 129.8, 125.4 (CH_{arom}), 123.5 (CH triazole), 122.6 (CH pyrrole), 120.0 (CH_{arom}), 93.7 (C-1), 89.7 (d, *J* = 190 Hz, C-2), 73.6 (C-5), 72.4 (d, *J* = 14 Hz, C-3), 71.6 (d, *J* = 7 Hz, C-4), 51.4 (C-6), 31.9, 30.4, 29.0, 26.0 (CH_2), 16.6, 14.6 (CH_3); LC-MS: R_t 9.60 min (C18 column, linear gradient 10 → 90% B in 13.5 min); HRMS: $[M+H]^+$ calcd for $C_{33}H_{38}BF_6N_6O_4$ 707.29463, found 707.29459.

Probe **7.** α-Imidate **19** (2.2 mg, 3 μmol) was dissolved in dry DCM (1.5 mL) under an argon



atmosphere. The resulting solution was cooled to 0 ºC and treated with diphenyl phosphate (~ 1 mg, 3.5 μmol) for 20 min, after which time the reaction was halted by the addition of sat. aq. $NaHCO_3$ (2 mL). The mixture was diluted with EtOAc, the organic layer was washed with sat. aq. NaCl, dried over

Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 10% MeOH in EtOAc) and subsequent lyophilization afforded the title compound as an orange amorphous solid (Yield: 1.4 mg, 1.8 µmol, 59%); TLC: R_f0.22 (EtOAc); IR (neat, cm^{-1}): 974, 1080, 1161, 1202, 1510, 1551, 2292, 3337; ^{1}H NMR (MeCN-d_3, 600 MHz, HH-COSY, HSQC): δ 7.38-7.45 (m, 4H, CH_{arom}), 7.25-7.31 (m, 2H, CH_{arom}), 7.20-7.25 (m, 4H, CH_{arom}), 6.17 (s, 2H, CH pyrrole), 5.49 (ddd, 1H, *J* = 2.7, 7.3, 7.1 Hz, H-1), 4.73 (dd, 1H, *J* = 1.8, 14.7 Hz, H-6), 4.45 (dd, 1H, *J* = 7.5, 14.8 Hz, H-6), 4.20 (dt, 1H, *J* = 8.4, 51.3 Hz, H-2), 3.83-3.87 (m, 1H, H-5), 3.70-3.77 (m, 1H, H-3), 3.25 (t, 1H, *J* = 9.3 Hz, H-4), 2.95-2.99 (m, 2H, CH_2), 2.56-2.61 (m, 2H, CH_2), 2.46 (s, 6H, CH_3), 2.39 (s, 6H, CH_3), 1.74-1.80 (m, 2H, CH_2), 1.57-1.65 (m, 2H, CH_2); ^{13}C-APT NMR (MeCN-d_3, 150 MHz, HSQC): δ 154.6, 148.3, 148.2, 142.4, 132.2 (C_q), 131.1, 130.1, 126.9, 123.8, 123.6 (CH_{arom}, CH triazole), 122.6 (CH pyrrole), 121.1, 121.1 (CH_{arom}), 97.7 (dd, J = 6, 25 Hz, C-1), 92.9 (dd, J = 9, 187 Hz, C-2), 76.4 (C-5), 74.7 (dd, J = 2, 17 Hz, C-3), 71.3 (d, J = 8 Hz, C-4), 51.1 (C-6), 31.9, 30.4, 28.9, 25.8 (CH_2), 16.6, 14.6 (CH_3); ^{31}P NMR (MeCN-d_3, 162 MHz): δ -12.44; LC-MS: R_t 9.44 min (C18 column, linear gradient 10 → 90% B in 13.5 min); HRMS: $[M+H]^+$ calcd for $C_{37}H_{43}BF_3N_5O_7P$ 768.29398, found 768.29416.

3,4,6-Tri-*O*-pivaloyl-D-glucal (9). 3,4,6-Tri-*O*-acetyl-D-glucal (13.6 g, 50.0 mmol) was dissolved in MeOH (500 mL) and treated with NaOMe (0.27 g, 5 mmol) overnight at RT. The mixture was neutralized by the addition of AcOH, and the solvents were evaporated. The residue was repeatedly co-evaporated with toluene. The crude triol (~24 mmol) was dissolved in pyridine (120 mL) and DMAP (cat.) was added. The resulting mixture was cooled to 0 ºC and Piv-Cl (14.5 mL, 117.8 mmol) was added. The mixture was stirred overnight at RT, after which time the reaction was halted by the addition of MeOH. The solvents were evaporated, the residue was dissolved in EtOAc and washed with H_2O and sat. aq. NaCl. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 100% PE) yielded the title compound as a colored oil (Yield: 5.77 g, 14.5 mmol, 60% over two steps). The spectroscopic data were in full accord with those reported previously.[3] ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 6.46 (dd, 1H, *J* = 1.2, 6.2 Hz, H-1), 5.30-5.33 (m, 1H, H-3), 5.28 (dd, 1H, *J* = 5.9, 7.4 Hz, H-4), 4.82 (dd, 1H, *J* = 3.1, 6.2 Hz, H-2), 4.33 (dd, 1H, *J* = 5.5, 11.7 Hz, H-6), 4.25-4.30 (m, 1H, H-5), 4.21 (dd, 1H, *J* = 2.5, 11.7 Hz, H-6), 1.23 (s, 9H, CH_3 tBu), 1.19 (s,

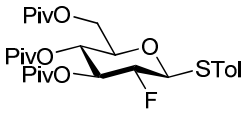
[3] Takahashi, Y.; Vasella, A. *Helv.Chim.Acta* **1992**, *75*, 1563-1571.

9H, CH_3 tBu), 1.18 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 178.1, 177.7, 176.5 (C=O Piv), 145.6 (C-1), 99.0 (C-2), 74.1 (C-5), 67.5 (C-3), 66.6 (C-4), 61.3 (C-6), 38.8, 38.7, 38.7 (C_q tBu), 27.0, 27.0, 27.0 (CH_3 tBu).

Acetyl 2-deoxy-2-fluoro-3,4,6-tri-*O*-pivaloyl-β-D-glucopyranoside (10). 3,4,6-Tri-*O*-pivaloyl-D-glucal **9** (5.77 g, 14.48 mmol) was dissolved in nitromethane/H_2O (60 mL, 5/1, v/v), and Selectfluor (6.16 g, 17.38 mmol) was portion-wise added at RT. The resulting mixture was stirred for 2 days, followed by heating at reflux (95 ºC) for 1 h. The mixture was cooled to RT and concentrated *in vacuo*. The residue was taken up in EtOAc and washed with sat. aq. $NaHCO_3$ (2x) and sat. aq. NaCl (2x). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was subsequently dissolved in DCM (50 mL) and treated with Ac_2O (1.6 mL) and pyridine (2.1 mL) overnight. The mixture was concentrated in the presence of toluene, and the product was isolated using flash column chromatography (silica gel, 9% EtOAc in PE) as a colorless oil (Yield: 4.56 g, 9.56 mmol, 66%, α : β = 1 : 2). The spectroscopic data were in full accord with those reported previously.[4] TLC: R_f 0.53 (PE/EtOAc, 5/1, v/v);^{1}H NMR ($CDCl_3$, 300 MHz, HH-COSY, HSQC): δ 6.41 (d, 0.5H, *J* = 3.8 Hz, H-1α), 5.80 (dd, 1H, *J* = 3.0, 8.1 Hz, H-1β), 5.59 (dd, 0.5H, *J* = 10.0, 21.0 Hz, H-3α), 5.44 (dt, 1H, *J* = 9.3, 14.2 Hz, H-3β), 5.10-5.18 (m, 0.5H, H-4α), 5.10 (t, 1H, *J* = 9.6 Hz, H-4β), 4.65 (ddd, 0.5H, *J* = 4.0, 9.6, 39.1 Hz, H-2α) 4.44 (dt, 1H, *J* = 8.2, 17.0 Hz, H-2β), 4.07-4.20 (m, 3.5H, H-5α, H-6α, H-6β), 3.92 (ddd, 1H, *J* = 2.5, 4.6, 10.0 Hz, H-5β), 2.20 (s, 1.5H, CH_3 Ac-α), 2.17 (s, 3H, CH_3 Ac-β), 1.21 (s, 13.5H, CH_3 tBu -α/β), 1.19 (s, 13.5H, CH_3 tBu-α/β), 1.18 (s, 4.5H, CH_3 tBu-α), 1.16 (3, 9H, CH_3 tBu-β); ^{13}C-APT NMR ($CDCl_3$, 75 MHz, HSQC): δ 177.8, 176.9, 176.4 (C=O Piv), 168.6 (C=O Ac), 91.2 (d, *J* = 24 Hz, C-1β), 88.5 (d, *J* = 191 Hz, C-2β), 88.3 (d, *J* = 22 Hz, C-1α), 86.6 (d, *J* = 194 Hz, C-2α), 73.0 (C-5β), 72.1 (d, *J* = 19 Hz, C-3β), 70.0 (C-5α), 69.9 (d, *J* = 19 Hz, C-3α), 66.9 (d, *J* = 7 Hz, C-4β), 66.5 (d, *J* = 7 Hz, C-4α), 61.3 (C-6β), 61.1 (C-6α), 38.7, 38.7 (C_q tBu), 26.9, 26.9 (CH_3 tBu), 20.7 (CH_3 Ac-α), 20.6 (CH_3 Ac-β).

[4] Bucher, C.; Gilmour, R. *Angew.Chem. Int. Ed.* **2010**, *49*, 8724-8728.

Tolyl 2-deoxy-2-fluoro-3,4,6-tri-*O*-pivaloyl-1-thio-β-D-glucopyranoside (12). A solution of



compound **10** (0.93 g, 1.97 mmol) in dry DCM (3 mL) was cooled to 0 ºC, and HBr/AcOH (33 wt%, 1.8 mL, 9.85 mmol) was added. The resulting solution was stirred at RT overnight, after which time it was poured in ice-water. The organic phase was diluted with EtOAc, washed with H_2O, sat. aq. $NaHCO_3$ and sat. aq. NaCl, dried over Na_2SO_4, and concentrated in vacuo in the presence of toluene. The crude anomeric bromide **10** was used in the next reaction without further purification. TLC: R_f 0.80 (PE/EtOAc, 5/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 6.52 (d, 1H, *J* = 4.2 Hz, H-1), 5.66 (dt, 1H, *J* = 9.6, 20.4 Hz, H-3), 5.15 (t, 1H, *J* = 10.0 Hz, H-4), 4.49 (ddd, 1H, *J* = 4.3, 9.4, 49.5 Hz, H-2), 4.32 (dt, 1H, *J* = 3.2, 10.4 Hz, H-5), 4.14-4.20 (m, 2H, H-6), 1.21 (s, 9H, CH_3 tBu), 1.18 (s, 9H, CH_3 tBu), 1.17 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 177.4, 176.6, 176.1 (C=O Piv), 86.6 (d, *J* = 194 Hz, C-2), 85.5 (d, *J* = 21 Hz, C-1), 72.5 (C-5), 70.3 (d, *J* = 18 Hz, C-3), 65.6 (d, *J* = 7 Hz, C-4), 60.5 (C-6), 38.6, 38.6, 38.6 (C_q tBu), 26.8, 26.8 (CH_3 tBu). A solution of crude bromide **10** (~1.97 mmol) in $CHCl_3$ (20 mL) was cooled to 0 ºC, followed by the addition of *p*-thiocresol (0.37 g, 2.96 mmol) and TBAB (0.13 g, 0.39 mmol, dissolved in 3 mL H_2O). A solution of KOH (0.22 g, 3.94 mmol) in H_2O (3 mL) was drop-wise added, and the reaction was allowed to stir for 2 h. The mixture was diluted with EtOAc and washed with sat. aq. NaCl. The organic phase was dried over Na_2SO_4, and the title compound was obtained by flash column chromatography (silica gel, 9% EtOAc in PE) as a colorless oil (Yield: 1.02 g, 1.89 mmol, 96% over two steps).TLC: R_f 0.59 (PE/EtOAc, 5/1, v/v); $[\alpha]_D^{20}$ -2.2 (*c* 1, DCM); IR (neat, cm^{-1}): 1036, 1138, 1726, 1740; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.46 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 7.11 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 5.40 (dt, 1H, *J* = 9.3, 13.7 Hz, H-3), 4.99 (t, 1H, *J* = 9.9 Hz, H-4), 4.71 (d, 1H, *J* = 9.5 Hz, H-1), 4.04-4.25 (m, 3H, H-2, H-6, H-6), 3.78 (dd, 1H, *J* = 4.6, 10.1 Hz, H-5), 2.34 (s, 3H, CH_3 STol), 1.21 (s, 3H, CH_3 tBu), 1.15 (s, 3H, CH_3 tBu), 1.14 (s, 3H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 177.3, 176.7, 176.0 (C=O Piv), 138.4 (C_q), 133.9, 129.5 (CH_{arom}), 126.6 (C_q), 87.1 (d, *J* = 190 Hz, C-2), 84.1 (d, *J* = 23 Hz, C-1), 75.9 (C-5), 73.1 (d, *J* = 20 Hz, C-3), 66.7 (d, *J* = 7 Hz, C-4), 61.5 (C-6), 38.5, 38.4, 38.4 (C_q tBu), 26.8, 26.7 (CH_3 tBu), 20.9 (CH_3 STol); HRMS: $[M+Na]^+$ calcd for $C_{28}H_{41}FO_7SNa$ 563.24492, found 563.24459.

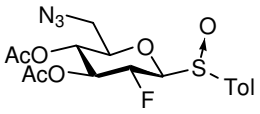
Tolyl 2-deoxy-2-fluoro-1-thio-β-D-glucopyranoside (13). A solution of compound **12** (0.84 g, 1.55 mmol) in MeOH (20 mL) was treated with NaOMe (cat.) and stirred at RT for 5 days. The mixture was quenched by the addition of Amberlite-H^+, filtered off and concentrated *in vacuo*. The product was used in the next reaction without further purification. (Yield: 0.45 g, 1.54 mmol, quant.). The spectroscopic data were in full accord with those reported previously.[5] TLC: R_f 0.46 (EtOAc); IR (neat, cm^{-1}): 766, 1009, 1047, 1364, 1614, 3277; ^{1}H NMR ($CDCl_3$/MeOH-d_4, 400 MHz, HH-COSY, HSQC): δ 7.45 (d, 2H, J = 8.0 Hz, CH_{arom}), 7.14 (d, 2H, J = 8.0 Hz, CH_{arom}), 4.64 (d, 1H, J = 9.6 Hz, H-1), 3.99 (dt, 1H, J = 9.2, 49.7 Hz, H-2), 3.87 (dd, 1H, J = 2.5, 12.2 Hz, H-6), 3.73 (dd, 1H, J = 4.7, 12.2 Hz, H-6), 3.63-3.70 (m, 1H, H-3), 3.32-3.39 (m, 2H, H-4, H-5), 2.35 (s, 3H, CH_3 STol); ^{13}C-APT NMR ($CDCl_3$/MeOH-d_4, 100 MHz, HSQC): δ 138.4 (C_q Tol-CH_3), 133.3, 129.5 (CH_{arom}), 127.2 (C_q STol), 89.5 (d, J = 186 Hz, C-2), 84.5 (d, J = 24 Hz, C-1), 79.9 (C-5), 75.9 (d, J = 18 Hz, C-3), 69.4 (d, J = 8 Hz, C-4), 61.4 (C-6), 20.7 (CH_3 STol); LC: R_t 5.53 (C18 column, linear gradient 10 → 90% B in 13.5 min); TLC-MS: m/z = 311.1 ($M+Na^+$).

Tolyl 6-azido-2,6-di-deoxy-2-fluoro-1-thio-β-D-glucopyranoside (14). Triol **13** (0.72 g, 2.50 mmol) was co-evaporated with dry acetonitrile (2x) and dissolved in acetonitrile (25 mL) under an argon atmosphere. To the mixture Ts-Cl (0.71 g, 3.75 mmol) and TMEDA (0.57 mL, 3.75 mmol) were added. The reaction was stirred for 2 h, after which time the mixture was diluted with EtOAc and 1M aq. HCl. The organic phase was washed with sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 66% EtOAc in PE) furnished the 6-*O*-tosyl intermediate as a colorless oil (Yield: 0.77 g, 1.74 mmol, 70%). A solution of the tosylate (0.77 g, 1.74 mmol) and sodium azide (0.34 g, 5.22 mmol) in DMF (17 mL) was heated at 80 ºC overnight. The mixture was diluted with EtOAc, washed with sat. aq. $NaHCO_3$ (2x) and H_2O (2x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 66% EtOAc in PE) afforded the title compound as a colorless oil (Yield: 0.49 g, 1.56 mmol, 90%). The spectroscopic data were in full accord with those reported previously.[5] TLC: R_f 0.37 (PE/EtOAc, 1/1, v/v); IR (neat, cm^{-1}): 729, 1038, 1067, 1290, 2102, 3339; ^{1}H NMR ($CDCl_3$,

[5] Witte, M. D.; Walvoort, M. T. C.; Li, K.-Y.; Kallemeijn, W. W.; Donker-Koopman, W. E.; Boot, R. G.; Aerts, J. M. F. G.; Codée, J. D. C.; van der Marel, G. A.; Overkleeft, H. S. *Chembiochem* **2011**, *12*, 1263-1269.

400 MHz, HH-COSY, HSQC): δ 7.46 (d, 2H, *J* = 8.1 Hz, CH_{arom}), 7.13 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 4.54 (dd, 1H, *J* = 0.8, 9.6 Hz, H-1), 4.40 (bs, 1H, 3-OH), 4.17 (bs, 1H, 4-OH), 3.95 (dt, 1H, *J* = 9.1, 49.6 Hz, H-2), 3.66 (dt, 1H, *J* = 7.1, 14.6 Hz, H-3), 3.54 (d, 1H, *J* = 12.1 Hz, H-6), 3.37-3.41 (m, 2H, H-4, H-5), 3.34 (d, 1H, *J* = 13.3 Hz, H-6), 2.33 (s, 3H, CH_3 STol); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 139.1 (C_q Tol-CH_3), 134.7, 129.7 (CH_{arom}), 126.0 (C_q STol), 89.2 (d, *J* = 185 Hz, C-2), 84.1 (d, *J* = 24 Hz, C-1), 78.2 (C-5), 76.2 (d, *J* = 18 Hz, C-3), 69.7 (d, *J* = 7 Hz, C-4), 51.0 (C-6), 21.1 (CH_3 STol).

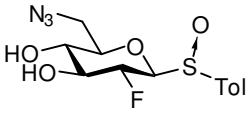
Tolyl 3,4-di-*O*-acetyl-6-azido-2,6-dideoxy-1-thio-β-D-glucopyranosyl $(S)_{R/S}$-oxide (15).



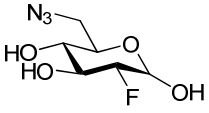
Compound **14** (1.13 g, 3.6 mmol) was treated with pyridine/Ac_2O (20 mL, 3/1, v/v) at RT overnight. The mixture was diluted with EtOAc, washed with sat. aq. NaCl (3x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 25% EtOAc in PE) yielded the 3,4-*O*-acetylated intermediate as an amorphous solid (Yield: 0.98 g, 2.47 mmol, 69%). A solution of this compound (0.60 g, 1.5 mmol) in acetone/H_2O (16 mL, 3/1, v/v) was cooled to 0 ºC and treated with NBS (0.80 g, 4.5 mmol) for 40 min, after which time the reaction was quenched by the addition of sat. aq. $Na_2S_2O_3$ (5 mL). The mixture was diluted with EtOAc, washed with H_2O and sat. aq. NaCl. The organic layer was dried over Na_2SO_4, concentrated in vacuo and purified using flash column chromatography (silica gel, 66% EtOAc in PE) to yield the title compound as a white amorphous solid (Yield: 0.35 g, 0.86 mmol, 57%, A : B = 1.7 : 1), next to the hydrolyzed product (Yield: 0.13 g, 0.44 mmol, 29%). TLC: R_f 0.22 (PE/EtOAc, 2/1, v/v); IR (neat, cm^{-1}): 727, 907, 1026, 1047, 1209, 1227, 1748, 2104; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.57 (d, 1.2H, *J* = 8.4 Hz, CH_{arom}-B), 7.55 (d, 2H, *J* = 8.5 Hz, CH_{arom}-A), 7.36 (d, 1.2H, *J* = 9.6 Hz, CH_{arom}-B), 7.34 (d, 2H, *J* = 8.3 Hz, CH_{arom}-A), 5.30-5.46 (m, 1.6H, H-3A, H-3B), 4.94-5.00 (m, 0.9H, H-2B, H-4B), 4.91 (t, 1H, *J* = 9.6 Hz, H-4A), 4.80-4.85 (m, 0.8H, H-2A, H-2B), 4.71 (t, 0.5H, *J* = 8.9 Hz, H-2A), 4.52 (dd, 1H, *J* = 3.9, 9.2 Hz, H-1A), 4.19 (dd, 0.6H, *J* = 3.1, 9.7 Hz, H-1B), 3.77 (ddd, 1H, *J* = 3.2, 5.8, 9.8 Hz, H-5A), 3.54 (ddd, 1H, *J* = 4.2, 5.3, 9.5 Hz, H-5B), 3.40 (dd, 1H, *J* = 3.3, 13.9 Hz, H-6A), 3.36 (5.9, 13.8 Hz, H-6A), 3.23-3.28 (m, 1.2H, H-6B), 2.43 (s, 1.8H, CH_3 STol-B), 2.42 (s, 3H, CH_3 STol-A), 2.10 (s, 1.8H, CH_3 Ac-B), 2.05 (s, 3H, CH_3 Ac-A), 2.02 (s, 3H, CH_3 Ac-A), 2.01 (s, 1.8H, CH_3 Ac-B); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.9 (C=O Ac-B), 169.8, 169.3 (C=O Ac-A), 169.2 (C=O Ac-B), 142.4 (C_q B), 142.4 (C_q A), 134.7 (C_qSTol-A), 134.5 (C_q STol-B), 129.8 (CH_{arom}-B), 129.8 (CH_{arom}-A), 125.2 (CH_{arom}-B),

125.0 (CH_{arom}-A), 92.1 (d, *J* = 23 Hz, C-1A), 90.1 (d, *J* = 23 Hz, C-1B), 85.0 (d, *J* = 190 Hz, C-2B), 83.9 (d, *J* = 189 Hz, C-2A), 77.6 (C-5A, C-5B), 73.2 (d, *J* = 20 Hz, C-3B), 73.1 (d, *J* = 20 Hz, C-3A), 68.5 (d, *J* = 7 Hz, C-4B), 68.2 (d, *J* = 7 Hz, C-4A), 50.9 (C-6B), 50.7 (C-6A), 21.4 (CH_3 STol-B), 21.4 (CH_3 STol-A), 20.5, 20.5, 20.4 (CH_3 Ac); HRMS: $[M+Na]^+$ calcd for $C_{17}H_{20}FN_3O_6SNa$ 436.09491, found 436.09448.

6-Azido-2,6-dideoxy-1-thio-β-D-glucopyranosyl $(S)_{R/S}$-oxide (16). Compound **15** (65 mg, 0.16 mmol) was dissolved in MeOH (2 mL) and treated with NaOMe (cat.) for 90 min. The mixture was neutralized by the addition of Amberlite-H^+, filtered and concentrated *in vacuo*. The title compound was used in the next reaction without further purification (Yield: quant., A : B = 1.7 : 1). TLC: R_f 0.18 (PE/EtOAc, 1/3, v/v); IR (neat, cm^{-1}): 1003, 1032, 1065, 1078, 2102, 3333; ^1H NMR (MeOH-d_4, 400 MHz, HH-COSY, HSQC): δ 7.56 (d, 1.2H, *J* = 8.2 Hz, CH_{arom}-B), 7.55 (d, 2H, *J* = 8.2 Hz, CH_{arom}-A), 7.36 (d, 1.2H, *J* = 8.4 Hz, CH_{arom}-B), 7.35 (d, 2H, *J* = 8.1 Hz, CH_{arom}-A), 4.66 (dd, 1H, *J* = 3.1, 9.3 Hz, H-1A), 4.53 (dt, 0.6H, *J* = 8.9, 50.1 Hz, H-2B), 4.36 (dt, 1H, *J* = 9.0, 44.7 Hz, H-2A), 4.38-4.45 (m, 0.6H, H-1B), 3.67-3.76 (m, 0.6H, H-3B), 3.65 (dt, 1H, *J* = 8.8, 16.4 Hz, H-3A), 3.52-3.57 (m, 2H, H-5A, H-6A), 3.35-3.41 (m, 1.6H, H-6A, H-6B), 3.25-3.30 (m, 1.8H, H-4B, H-5B, H-6B), 3.21 (t, 1H, *J* = 9.3 Hz, H-4A), 2.38 (s, 4.8H, CH_3 STol-A, CH_3 STol-B); ^{13}C-APT NMR (MeOH-d_4, 100 MHz, HSQC): δ 143.8 (C_q A), 143.7 (C_q B), 136.0 (C_q STol-A), 135.6 (C_q STol-B), 130.9 (CH_{arom}-B), 130.8 (CH_{arom}-A), 126.6 (CH_{arom}-B), 126.5 (CH_{arom}-A), 93.2 (d, *J* = 24 Hz, C-1A), 91.4 (d, *J* = 24 Hz, C-1B), 88.9 (d, *J* = 186 Hz, C-2B), 88.4 (d, *J* = 186 Hz, C-2A), 81.2 (C-5A), 81.1 (C-5B), 76.8 (d, *J* = 18 Hz, C-3A), 76.7 (d, *J* = 17 Hz, C-3B), 71.3 (d, *J* = 8 Hz, C-4B), 70.9 (d, *J* = 8 Hz, C-4A), 52.5 (C-6B), 52.4 (C-6A), 21.5 (CH_3 STol-B), 21.5 (CH_3 STol-A); HRMS: $[M+H]^+$ calcd for $C_{13}H_{17}FN_3O_4S$ 330.09183, found 330.09193.

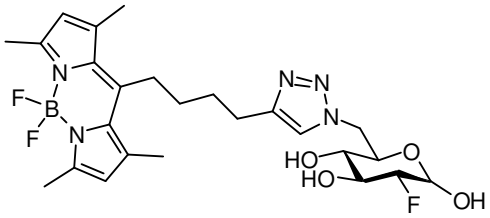


6-Azido-2,6-dideoxy-2-fluoro-α/β-D-glucopyranose (17). A solution of compound **16** (53 mg, 0.16 mmol) in acetone/H_2O (2 mL, 3/1, v/v) was treated with NBS (85 mg, 0.48 mmol) for 3 h at RT. The reaction was quenched by the addition of sat. aq. $Na_2S_2O_3$ (1 mL) and subsequently diluted with EtOAc and H_2O. The aqueous phase was extracted with EtOAc (2x), the combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Purification using flash column chromatography (silica gel, 75% EtOAc in PE) yielded the title compound as a colorless oil (Yield: 31 mg,



0.15 mmol, 94%, α : β = 1 : 1). TLC: R_f 0.35 (PE/EtOAc, 1/3, v/v); IR (neat, cm^{-1}): 816, 1001, 1051, 1177, 1290, 1694, 1771, 2104, 3329; ^{1}H NMR (MeOH-d_4, 300 MHz, HH-COSY, HSQC): δ 5.25 (d, 1H, *J* = 3.7 Hz, H-1α), 4.68 (dd, 1H, *J* = 2.5, 7.7 Hz, H-1β), 4.17 (ddd, 1H, *J* = 3.7, 9.3, 49.8 Hz, H-2α), 3.78-4.02 (m, 2H, H-2β, H-3α), 3.22-3.60 (m, 7H, H-3β, H-5α, H-5β, 2 x H-6α, 2 x H-6β); ^{13}C-APT NMR (MeOH-d_4, 100 MHz, HSQC): δ 95.8 (d, *J* = 21 Hz, C-1β), 94.7 (d, *J* = 182 Hz, C-2β), 92.0 (d, *J* = 188 Hz, C-2α), 91.5 (d, *J* = 22 Hz, C-1α), 76.5 (C-5), 76.2 (d, *J* = 18 Hz, C-3β), 72.7 (d, *J* = 17 Hz, C-3α), 72.3 (d, *J* = 8 Hz, C-4), 72.2 (d, *J* = 8 Hz, C-4), 71.8 (C-5), 52.7, 52.7 (C-6α, C-6β); TLC-MS: *m*/*z* = 230.1 ($M+Na^+$).

BODIPY compound **18**. Compound **17** (34 mg, 164 μmol) and BODIPY-alkyne **8** (59 mg, 180 μmol) were together dissolved in DMF (1.5 mL) and treated with sodium ascorbate (12 μL, 1M solution in H_2O) and $CuSO_4$ (8 μL, 1M solution in H_2O). The resulting mixture was stirred at 80 ºC for 2 days, during which time the addition of sodium ascorbate and $CuSO_4$ was repeated twice. The mixture was allowed to cool to RT and diluted with EtOAc and H_2O. The organic phase was washed with sat. aq. NaCl, dried over Na_2SO_4 and the product was obtained using flash column chromatography (silica gel, 15% MeOH in DCM) as an orange solid (Yield: 46 mg, 86 μmol, 53%, α : β = 1.1 : 1). TLC: R_f0.59 (DCM/MeOH, 8.5/1.5, v/v); IR (neat, cm^{-1}): 984, 1061, 1200, 1508, 1551, 3429; ^{1}H NMR (MeOH-d_4, 400 MHz, HH-COSY, HSQC): δ 6.09 (s, 2H, CH pyrrole), 5.21 (d, 1H, *J* = 3.7 Hz, H-1α), 4.78 (dd, 0.9H, *J* = 2.2, 14.4 Hz, H-6β), 4.71 (dd, 1H, *J* = 2.4, 14.3 Hz, H-6α), 4.64 (dd, 0.9H, *J* = 2.5, 7.8 Hz, H-1β), 4.50 (dd, 1H, *J* = 7.4, 14.0 Hz, H-6α), 4.47 (dd, 0.9H, *J* = 7.6, 14.1 Hz, H-6β), 4.10 (ddd, 1H, *J* = 3.7, 9.4, 49.8 Hz, H-2α), 4.10 (ddd, 1H, *J* = 2.4, 7.3, 9.8 Hz, H-5α), 3.79-3.96 (m, 1.9H, H-2β, H-3α), 3.56-3.67 (m, 1.8H, H-3β, H-5β), 3.15 (t, 0.9H, *J* = 9.4 Hz, H-4β), 3.09 (t, 1H, *J* = 9.4 Hz, H-4α), 2.86-2.94 (m, 3.8H, CH_2), 2.72 (t, 3.8H, *J* = 7.5 Hz, CH_2), 2.43 (s, 11.4H, CH_3), 2.33 (s, 11.4H, CH_3), 1.79-1.90 (m, 3.8H, CH_2), 1.55-1.66 (m, 3.8H, CH_2); ^{13}C-APT NMR (MeOH-d_4, 100 MHz, HSQC): δ 154.9, 148.4, 148.3, 147.9, 142.2, 132.6 (C_q), 124.6 (CH triazole) 122.6 (CH pyrrole), 95.7 (d, *J* = 23 Hz, C-1β), 94.5 (d, *J* = 184 Hz, C-2β), 91.7 (d, *J* = 187 Hz, C-2α), 91.5 (d, *J* = 22 Hz, C-1α), 76.0 (d, *J* = 18 Hz, C-3β), 75.8 (C-5β), 72.6 (d, *J* = 17 Hz, C-3α), 72.6 (d, *J* = 7 Hz, C-4), 72.4 (d, *J* = 8 Hz, C-4), 71.0 (C-5α), 52.2, 52.1 (C-6α, C-6β), 32.2, 30.8, 28.9, 25.9 (CH_2), 16.4, 14.5



(CH_3);); LC-MS: R_t 6.86 min (C18 column, linear gradient 10 → 90% B in 13.5 min); HRMS: $[M+H]^+$ calcd for $C_{25}H_{34}BF_3N_5O_4$ 536.26505, found 536.26523.

Biological Experiments

Determination of the IC_{50} (Figure S1) Imiglucerase (12.5 µL, 20 nM) was prepared in 150 mM McIlvaine buffer (pH 5.2) containing 0.2% (w/v) taurocholate and 0.1% (v/v) Triton X-100. The enzyme was incubated with a range of probe concentrations (12.5 µL, 1 mM to 10 nM final concentration, DMSO) for 30 at 37 ºC. Then 4MUGlc (100 µL, 3.75 mM) substrate in McIlvaine buffer (pH 5.2) containing 0.2% (w/v) taurocholate, 0.1% (v/v) Triton X-100, and 0.1% (w/v) BSA was added, and the resulting mixture was incubated for 15 min at 37 ºC. The mixture was inactivated with 2.5 mL NaOH-Glycine (300 mM, pH 10.6), followed by measuring of the fluorescence of liberated 4MU (λ_{ex} 366 nm, λ_{em} 445 nm). IC_{50} values were obtained by plotting of the residual fluorescence versus the concentration (GraphPad Prism 5).

Detection limit. Imiglucerase (10 µL, 100 nM) was prepared in 150 mM McIlvaine buffer (pH 5.2) containing 0.2% (w/v) taurocholate and 0.1% (v/v) Triton X-100. The enzyme was incubated with a range of probe concentrations (10 µL, 50 µM to 10 nM final concentration, DMSO) for 60 min at 37 ºC. The sample was denatured with 5 µL Laemmli buffer (50% (v/v) 1M Tris-HCl, pH 6.8, 50% (v/v) 100% glycerol, 10% (w/v) DTT, 10% (w/v) SDS, 0.01% (w/v) bromophenol blue), boiled for 4 min at 100 ºC, and separated by electrophoresis on 7.5% (w/v) SDS-PAGE gel running continuously at 90 V, followed by fluorescent scanning.

Competition for the active site (Figure S2). Imiglucerase (10 µL, 100 nM) was prepared in 150 mM McIlvaine buffer (pH 5.2) containing 0.2% (w/v) taurocholate and 0.1% (v/v) Triton X-100. The enzyme was pre-incubated with CBE (10 µL, 20 mM in H_2O), cyclophellitol (10 µL, 2 mM in H_2O), MDW941 (10 µL, 2 µM in H_2O), or AMP-DNM (10 µL, 20 mM in H_2O) for 30 min at 37 ºC, or with 10 µL 2% (w/v) SDS and boiled for 4 min at 100 ºC. The pre-incubated mixtures were labeled with MDW933 (10 µL, 30 nM in H_2O), probe **1** (10 µL, 150 µM in H_2O), probe **6** (10 µL, 1.5 µM in H_2O), or probe **7** (10 µL, 15 µM in H_2O) for 30 min at 37 ºC. The sample was denatured with 10 µL Laemmli buffer (50% (v/v) 1M Tris-HCl, pH 6.8, 50% (v/v) 100% glycerol, 10% (w/v) DTT, 10% (w/v) SDS, 0.01% (w/v) bromophenol

blue), boiled for 4 min at 100 ºC, and separated by electrophoresis on 7.5% (w/v) SDS-PAGE gel running continuously at 90 V, followed by fluorescent scanning.

pH-dependent labeling (Figure S3). Imiglucerase (10 µL, 10 nM) was prepared in 1.5 mM McIlvaine buffer (pH 5.2) containing 0.2% (w/v) taurocholate and 0.1% (v/v) Triton X-100, and incubated with 150 mM McIlvaine buffer of pH 2-9 (25 µL), containing 0.2% (w/v) taurocholate and 0.1% (v/v) Triton X-100, for 30 min at 37 ºC. Pre-incubated enzyme was labeled with MDW941 (5 µL, 8 nM in H_2O), probe **1** (5 µL, 400 µM), probe **6** (5 µL, 4 µM), or probe **7** (5 µL, 40 µM) for 30 min at 37 ºC. The sample was denatured with 10 µL Laemmli buffer (50% (v/v) 1M Tris-HCl, pH 6.8, 50% (v/v) 100% glycerol, 10% (w/v) DTT, 10% (w/v) SDS, 0.01% (w/v) bromophenol blue), boiled for 4 min at 100 ºC, and separated by electrophoresis on 7.5% (w/v) SDS-PAGE gel running continuously at 90 V, followed by fluorescent scanning.

Labeling of mutant GBA (Figure S4). All probe solutions were prepared in 150 mM McIlvaine buffer (pH 5.2) containing 0.2% (w/v) taurocholate, 0.1% (v/v) Triton X-100, and protease inhibitor cocktail (Roche). Homogenate (20 µL) of *cos-7* cells overexpressing wild-type and acid/base mutant (E235G and E235Q) GBA was incubated with MDW1044 (20 µL, 2 µM), MDW933 (20 µL, 2 µM), probe **1** (20 µL, 200 µM), probe **6** (20 µL, 2 µM), or probe **7** (20 µL, 20 µM) for either 2 h or 24 h at 37 ºC. The samples were split in two, and one half (20 µL) was directly denatured etcetera (*vide infra*). The labeled homogenate (20 µL) was incubated with Ni-agarose beads (5 µL) and native lysis buffer (100 µL, pH 8.0) containing NaCl (300 mM) and imidazole (10 mM) while rotating for 1 h at 4 ºC. The samples were centrifuged for 3 min at 800 rpm, cleaned with wash buffer (200 µL, pH 8.0) containing NaCl (300 mM) and imidazole (20 mM) for 10 min at 4 ºC (repeated 3x). Then the nickel beads were pelleted by centrifugation for 10 min at 800 rpm and resuspended in McIlvaine buffer (20 µL, pH 5.2) containing 0.2% (w/v) taurocholate, 0.1% (v/v) Triton X-100. The sample was denatured with 10 µL Laemmli buffer (50% (v/v) 1M Tris-HCl, pH 6.8, 50% (v/v) 100% glycerol, 10% (w/v) DTT, 10% (w/v) SDS, 0.01% (w/v) bromophenol blue), boiled for 4 min at 100 ºC, and separated by electrophoresis on 7.5% (w/v) SDS-PAGE gel running continuously at 90 V, followed by fluorescent scanning. Western blotting was accomplished by transfer of the protein for 1 h at 12 V, followed by blocking of the membrane with 2% (w/v) BSA in TBST buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% (v/v) Tween-20),

overnight treatment with 1:2000 diluted primary α-myc mAb (b118, 2% (w/v) BSA in TBST), washing with TBST for 20 min (repeated 6 times), followed by 1:10,000 diluted secondary mAb IRD680 (291, 2% (w/v) BSA in TBST), subsequent washing with TBST for 20 min (repeated 6 times), and read-out on Odyssey infrared scanner.

Labeling in fibroblasts (Figure S5-S7). Wild-type human skin fibroblasts were grown to confluency (RPMI medium) for 3 days and cultured in the presence of MDW933 (0/1/10 nM), MDW1044 (0/1/10 nM), probe **1** (0/1/10 μM), probe **6** (0/1/10 μM), or probe **7** (0/1/10 μM) (probe solutions in PBS buffer) for 2 or 24 h at 37 ºC. The cells were lysed by scraping in KPi buffer (100 μL, 25 mM, pH 6.5) containing 0.1% (v/v) Triton X-100 and protease inhibitor cocktail. The protein concentration was determined using a BCA kit (Pierce), and 21 μg (2 h) or 27 μg (24 h) was loaded per lane. The homogenates (35 μL) were incubated with MDW941 (5 μL, 800 nM in McIlvaine buffer, pH 5.2, containing taurocholate, 0.1% (v/v) Triton X-10, and protease inhibitor cocktail) for 30 min at 37 ºC. The samples were denatured with 10 μL Laemmli buffer (50% (v/v) 1M Tris-HCl, pH 6.8, 50% (v/v) 100% glycerol, 10% (w/v) DTT, 10% (w/v) SDS, 0.01% (w/v) bromophenol blue), boiled for 4 min at 100 ºC, and separated by electrophoresis on 7.5% (w/v) SDS-PAGE gel running continuously at 90 V, followed by fluorescent scanning.

Figure S1. Inactivation curves of the probes

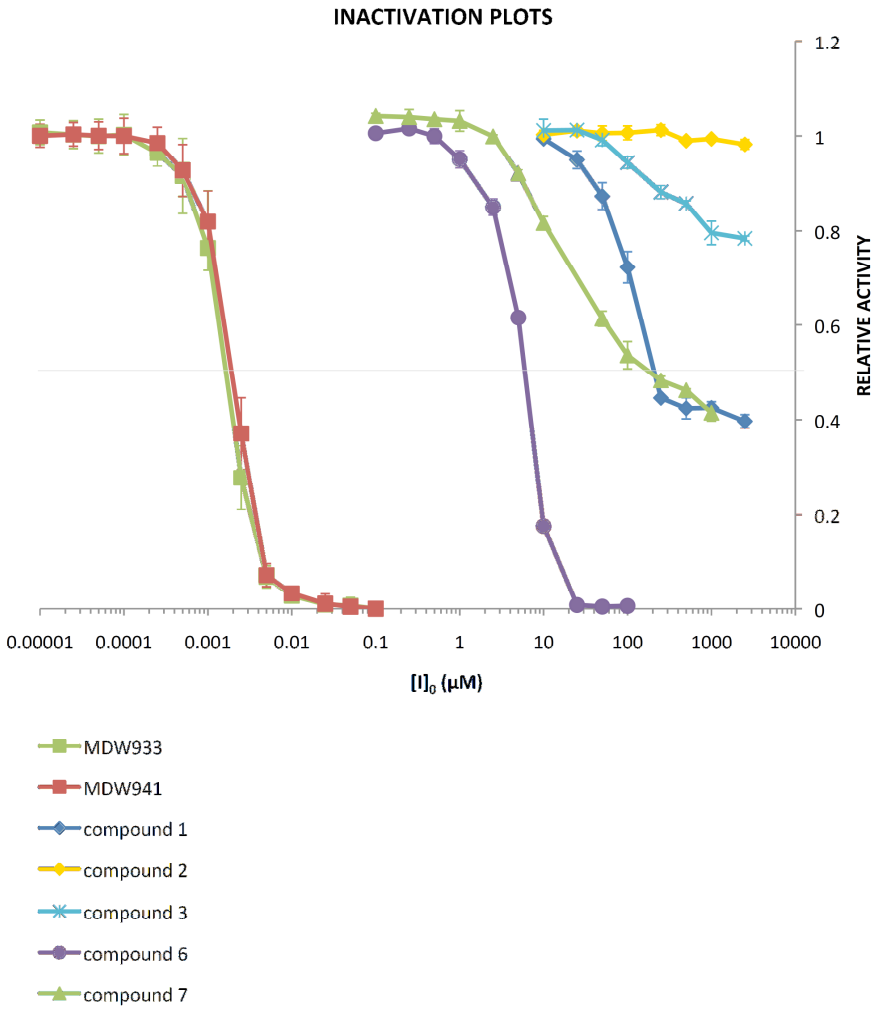
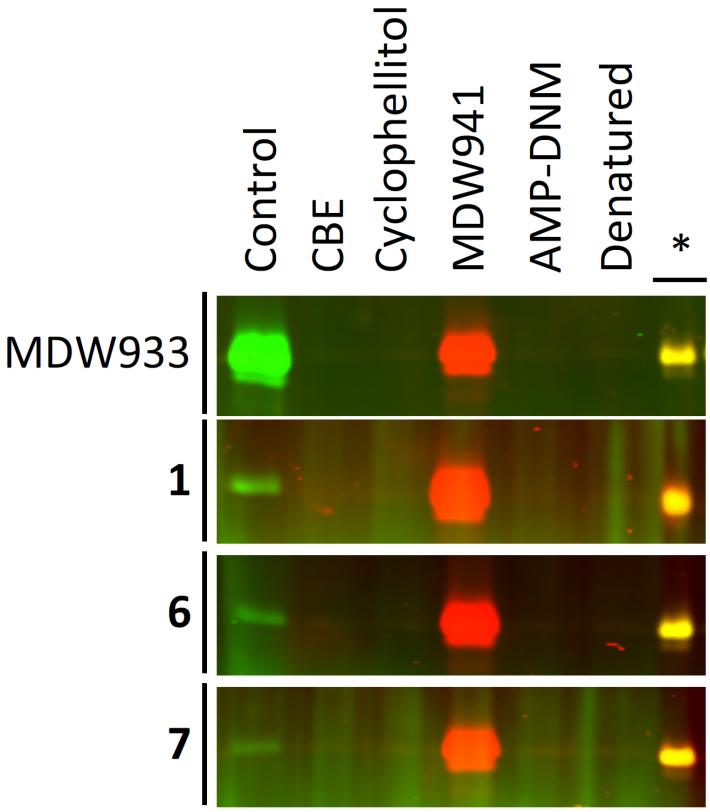
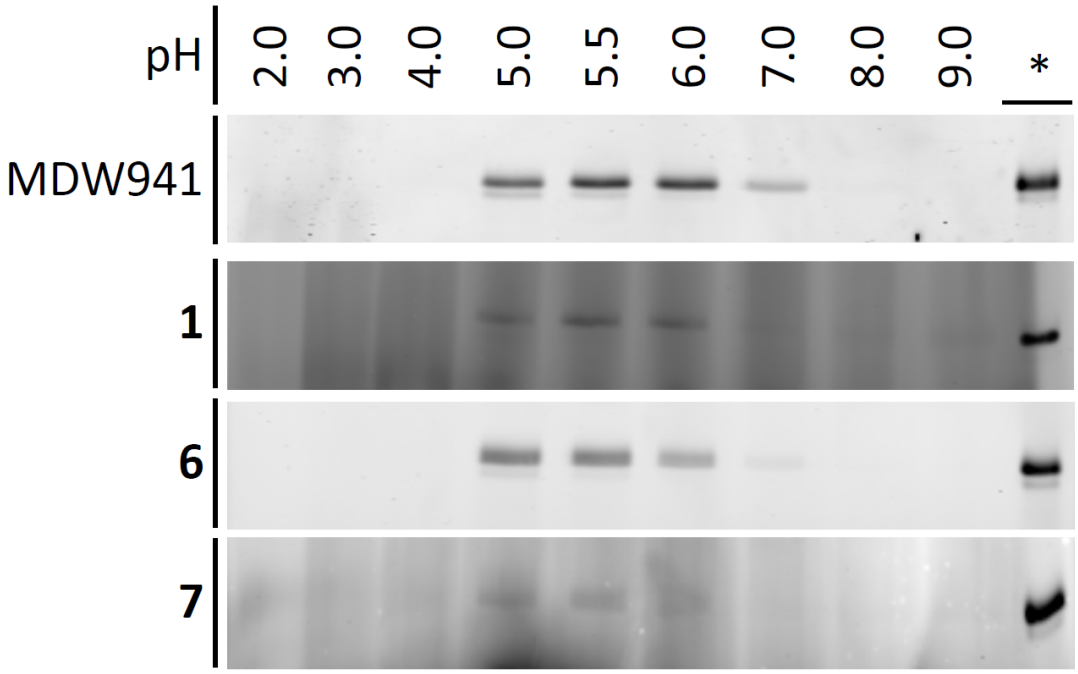


Figure S2. Competition for the active site



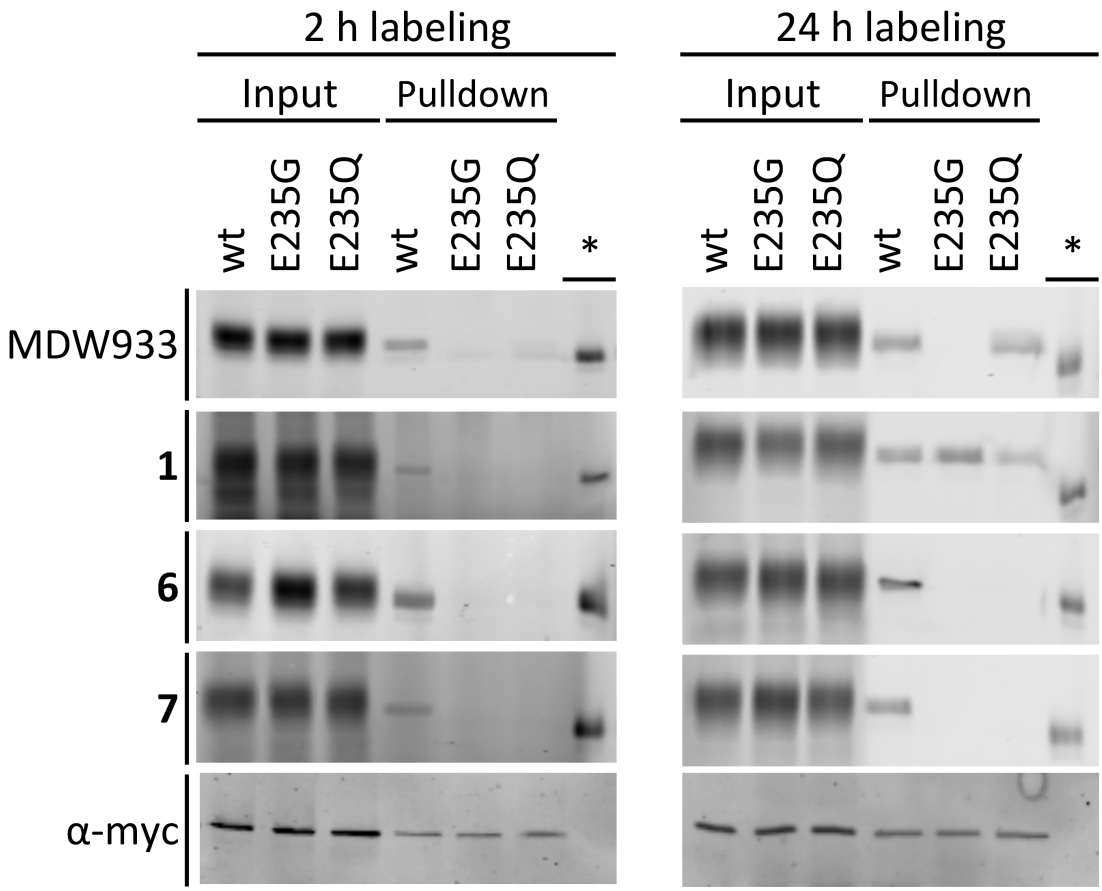
Recombinant GBA was pre-incubated with either CBE, cyclophellitol, red-fluorescent MDW941 or AMP-DNM for 30 min at 37 ºC, or denatured by SDS and boiling at 100 ºC, followed by incubation with the probe (MDW933: 10 nM, **1**: 50 µM, **6**: 500nM, **7**: 5 µM) for 30 min at 37 ºC, denatured, resolved by SDS-PAGE and visualized by scanning (✹ = imiglucerase labeled with 1nM of MDW933 and MDW941).

Figure S3. pH-dependent labeling



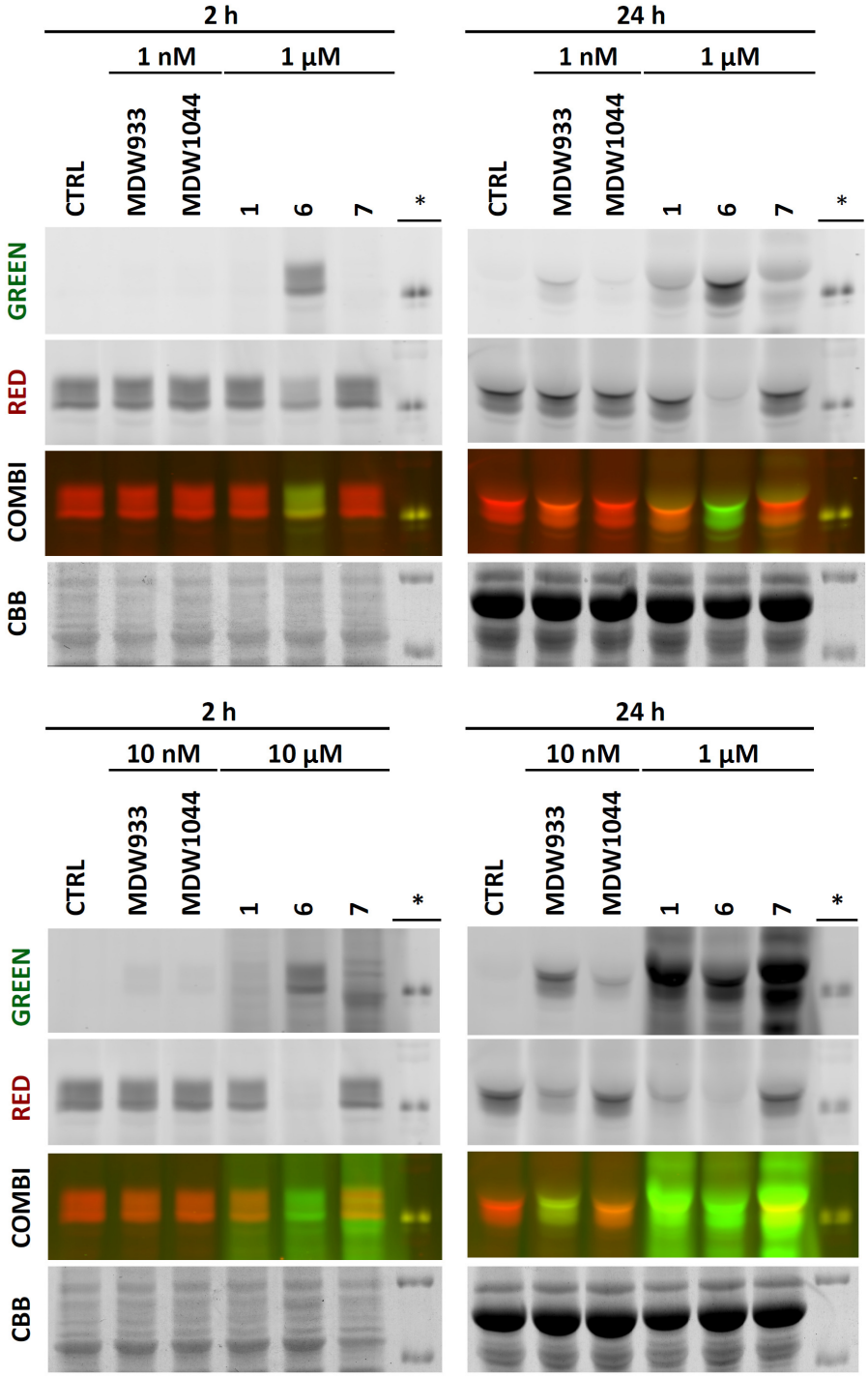
Recombinant GBA was incubated at the indicated pH for 30 min at 37 ºC, followed by incubation with the probe (MDW941: 1 nM, **1**: 50 μM, **6**: 500 nM, **7**: 5 μM) for 30 min at 37 ºC, denatured, resolved by SDS-PAGE and visualized by scanning (✹ = imiglucerase labeled with 1nM of MDW933 and MDW941).

Figure S4. Labeling of mutant GBA



Homogenates over-expressing wild-type or mutant GBA were incubated with the probe (MDW1044, MDW933: 1 µM, **1**: 100 µM, **6**: 1 µM, **7**: 10 µM) for 2 h or 24 h at 37 ºC, denatured, either directly resolved by SDS-PAGE ("input") or subjected to Ni-beads pull-down prior to SDS-PAGE ("pulldown"), and visualized by scanning (✹ = imiglucerase labeled with 1nM of MDW933 and MDW941).

Figure S5. Labeling in fibroblasts (*top*: low concentrations, *bottom*: high concentrations)[6]



Confluent fibroblasts were incubated with the probe for 2h or 24 h at 37 ºC and lysed, followed by incubation with MDW941 for 30 min. Proteins were denatured, resolved by SDS-PAGE and visualized by scanning (✷ = imiglucerase labeled with 1nM of MDW933 and MDW941).

[6] MDW1044 is an aziridine-based probe (unpublished result)

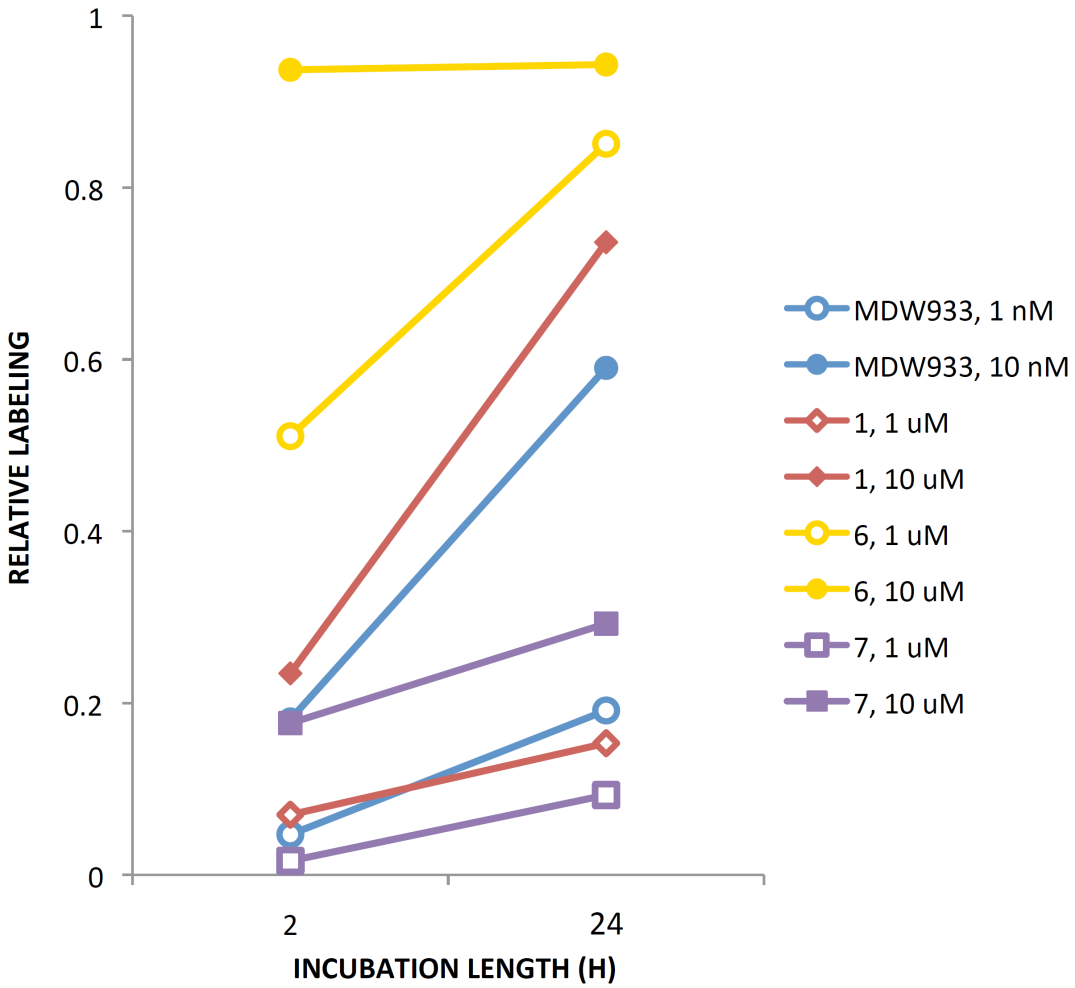
Figure S6. Quantification of residual labeling in fibroblasts

		I	II	III	IV	χ	σ	λ%
IMI CONTROL	*	3060.64	2863.74	3044.518	3212.811	3045.43	142.90	4.69

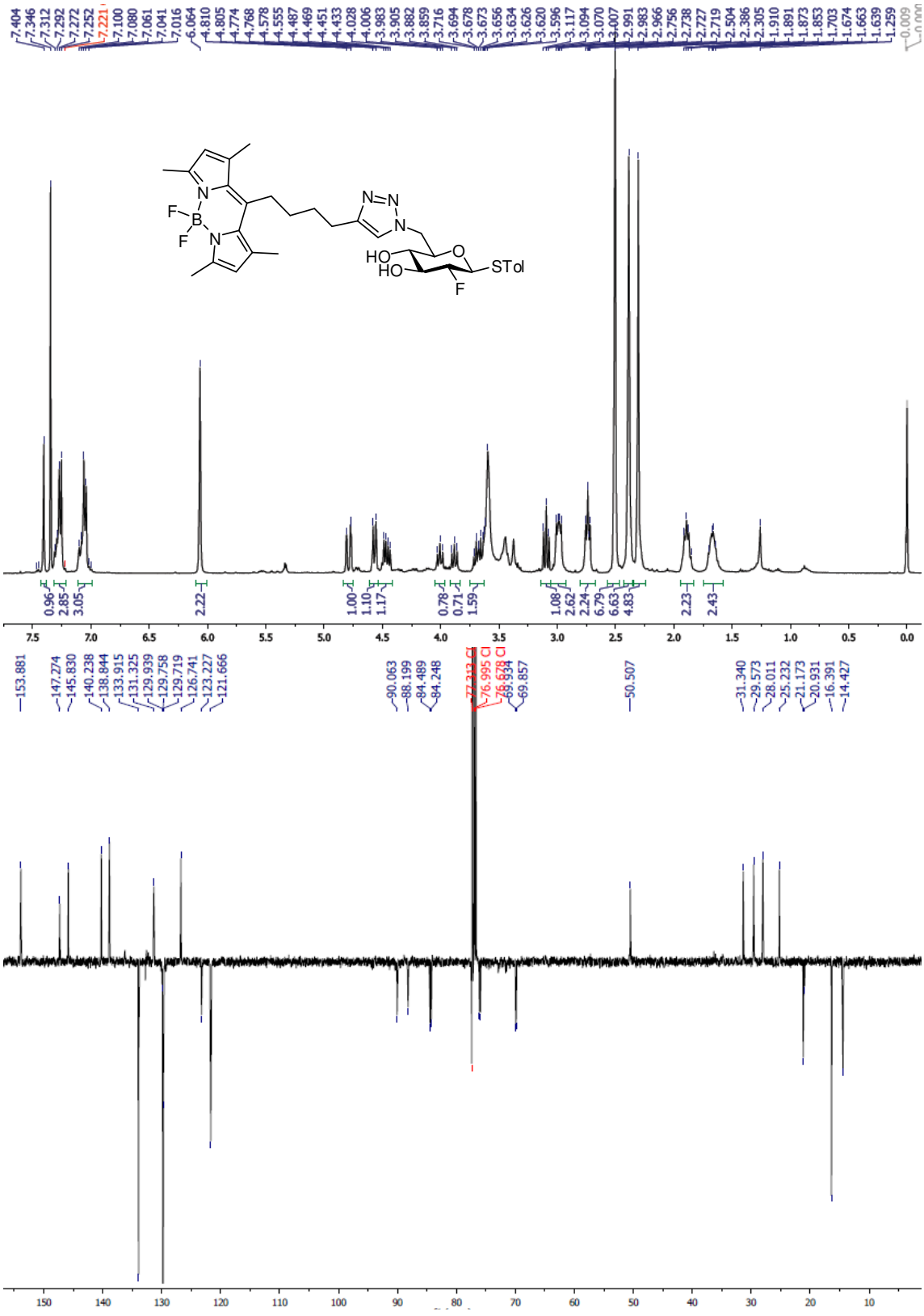
RAW QUANT		2h, l	24h, l	2h, h	24h, h
	CTRL	14584.1	13513.7	15901.9	14283.3
	MDW933	13897.4	11792.5	13024.0	5854.2
	1	13562.2	11442.2	12171.5	3765.7
	6	7136.6	2013.7	1000.7	811.3
	7	14345.3	12257.3	13092.8	10106.2

NORMALIZED	CTRL	1	1	1	1
	MDW933	0.953	0.809	0.819	0.410
	1	0.930	0.847	0.765	0.264
	6	0.489	0.149	0.063	0.057
	7	0.984	0.907	0.823	0.708

Figure S7. Labeling of GBA in fibroblasts



Probe 3.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4.810
4.805
4.774
4.768
4.578
4.555
4.487
4.469
4.451
4.433
4.028
4.006
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3.905
3.882
3.859
3.716
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3.656
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3.620
3.596
3.117
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2.305
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1.853
1.703
1.674
1.663
1.639
1.259
0.009
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F
B
F
N
N=N
N
HO
HO
O
STol
F
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3.05
2.22
1.00
1.10
1.17
0.78
0.71
1.59
1.08
2.62
2.24
6.79
6.63
4.83
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76.995 CI
76.678 CI
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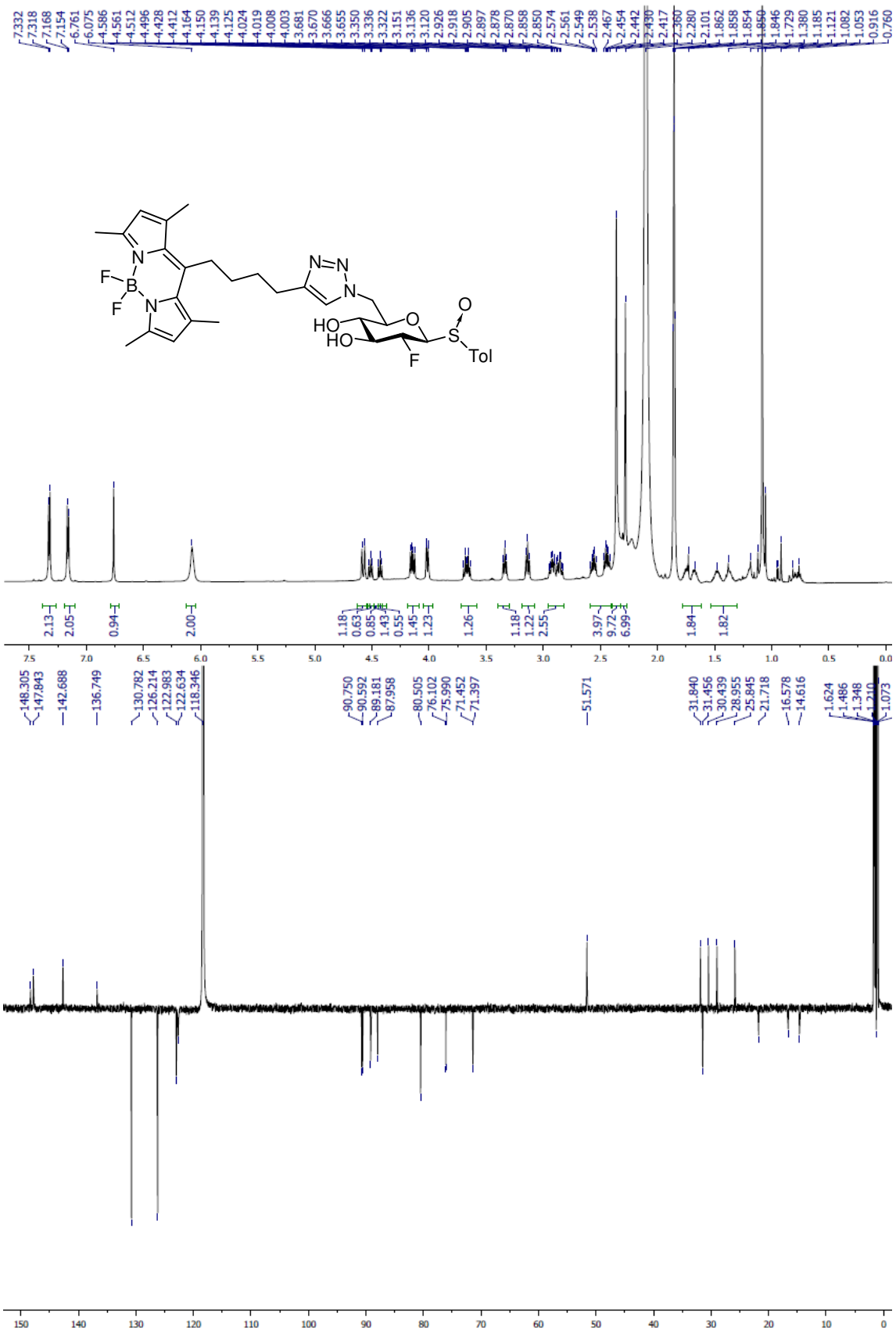
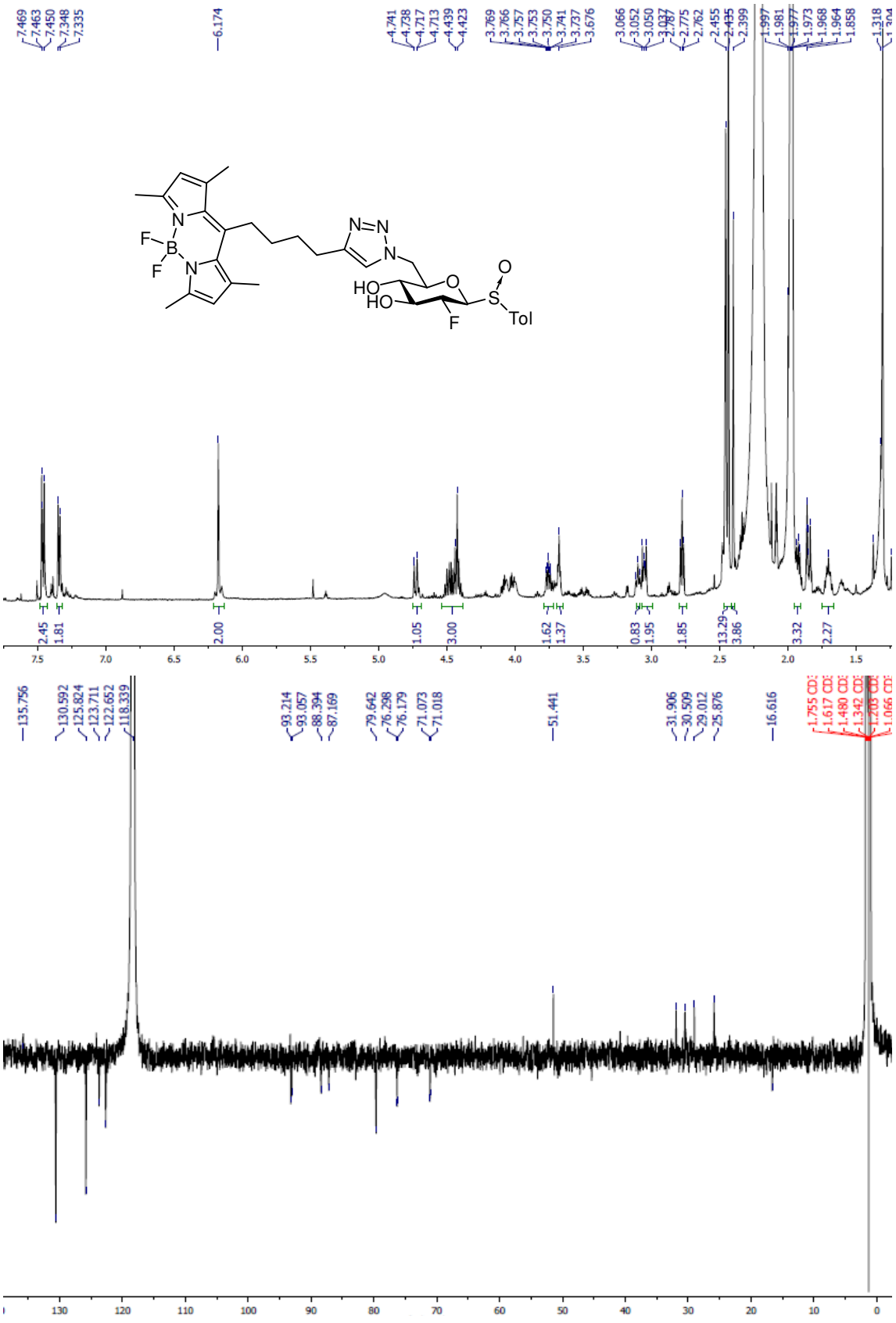
Probe 4.



Probe 5.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4.738
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4.423
3.769
3.766
3.757
3.753
3.750
3.741
3.737
3.676
3.066
3.052
3.050
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2.787
2.775
2.762
2.455
2.435
2.399
1.997
1.981
1.977
1.973
1.968
1.964
1.858
1.318
F
B
F
N
N
N=N
N
HO
HO
O
O
S
Tol
F
2.45
1.81
2.00
1.05
3.00
1.62
1.37
0.83
1.95
1.85
13.29
3.86
3.32
2.27
135.756
130.592
125.824
123.711
122.652
118.339
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93.057
88.394
87.169
79.642
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71.018
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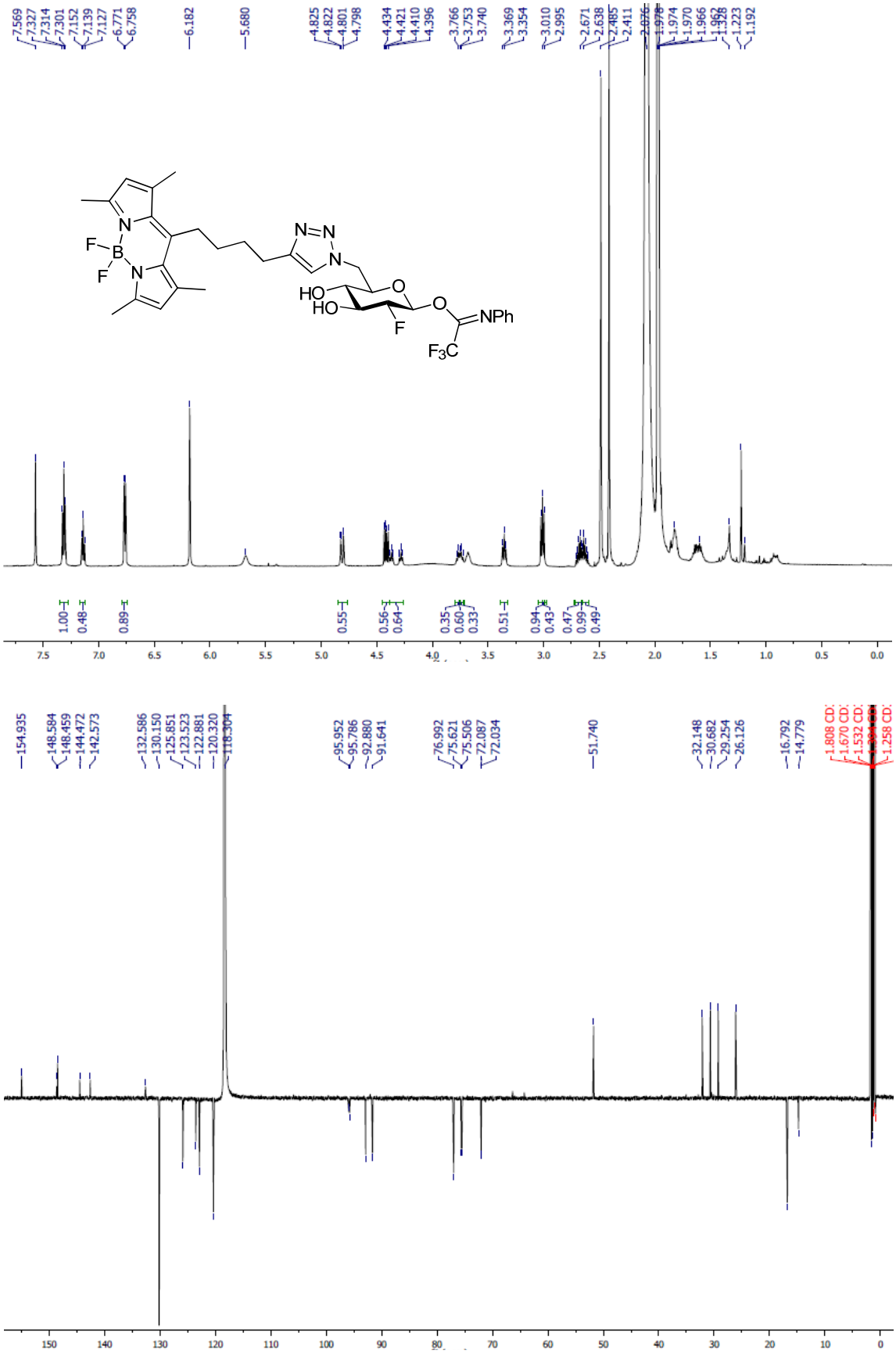
30.509
29.012
25.876
16.616

Probe 6.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4.801
4.798
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3.354
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1.328
1.223
1.192
N=N
N
O
HO
HO
F
O
NPh
F3C
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0.89
0.55
0.56
0.64
0.35
0.60
0.33
0.51
0.94
0.43
0.47
0.99
0.49
154.935
148.584
148.459
144.472
142.573
132.586
130.150
125.851
123.523
122.881
120.320
118.304
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95.786
92.880
91.641
76.992
75.621
75.506
72.087
72.034
51.740
32.148
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16.792
14.779
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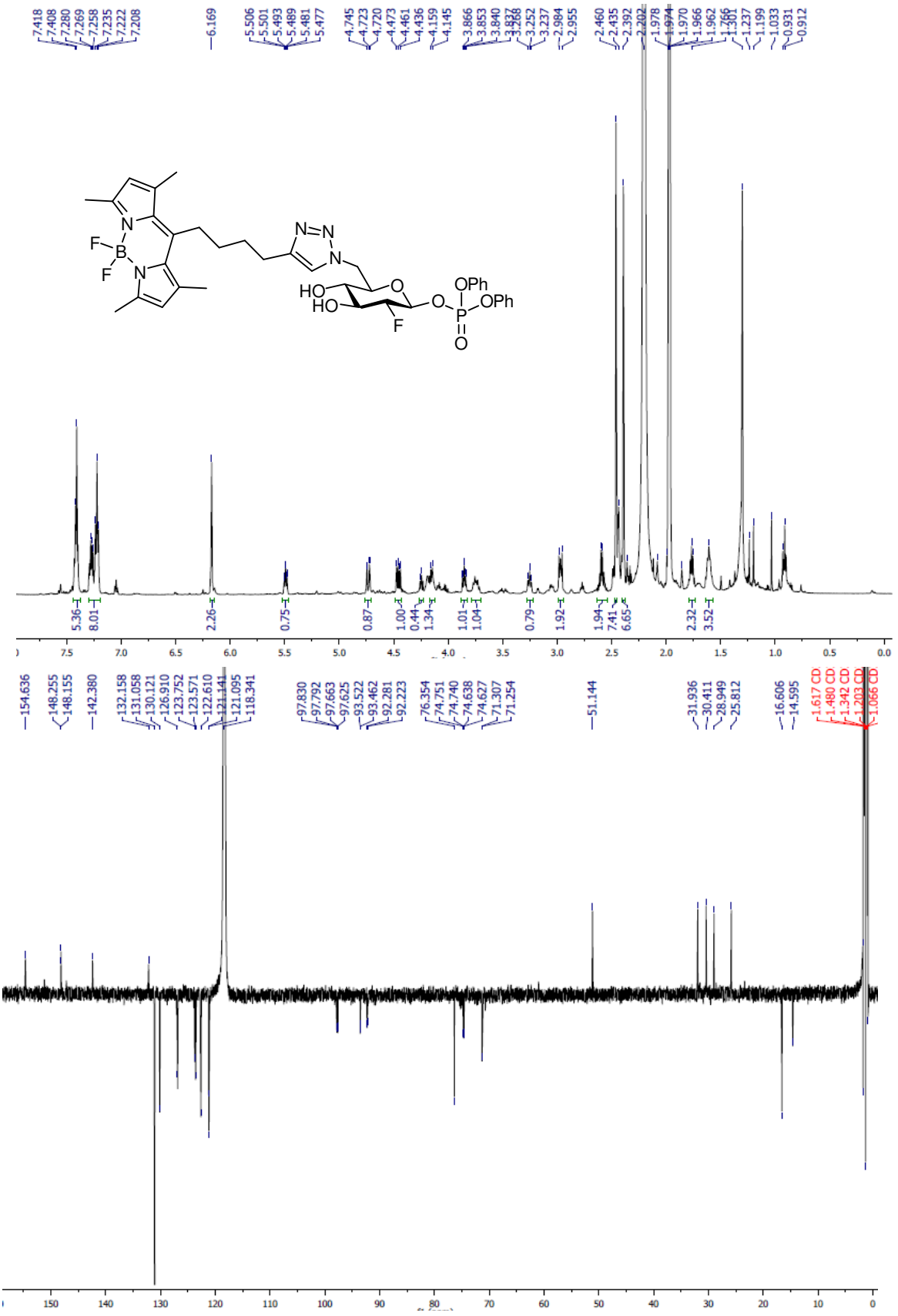
1.670 CD3
1.532 CD3
1.258 CD3

Probe 7.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5.489
5.481
5.477
4.745
4.723
4.720
4.473
4.461
4.436
4.159
4.145
3.866
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3.840
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3.268
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2.955
2.460
2.435
2.392
2.202
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1.237
1.199
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0.912
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8.01
2.26
0.75
0.87
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1.34
1.01
1.04
0.79
1.92
1.94
7.41
6.65
2.32
3.52
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148.255
148.155
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132.158
131.058
130.121
126.910
123.752
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122.610
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92.281
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76.354
74.751
74.740
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71.307
71.254
51.144
31.936
30.411
28.949
25.812
16.606
14.595
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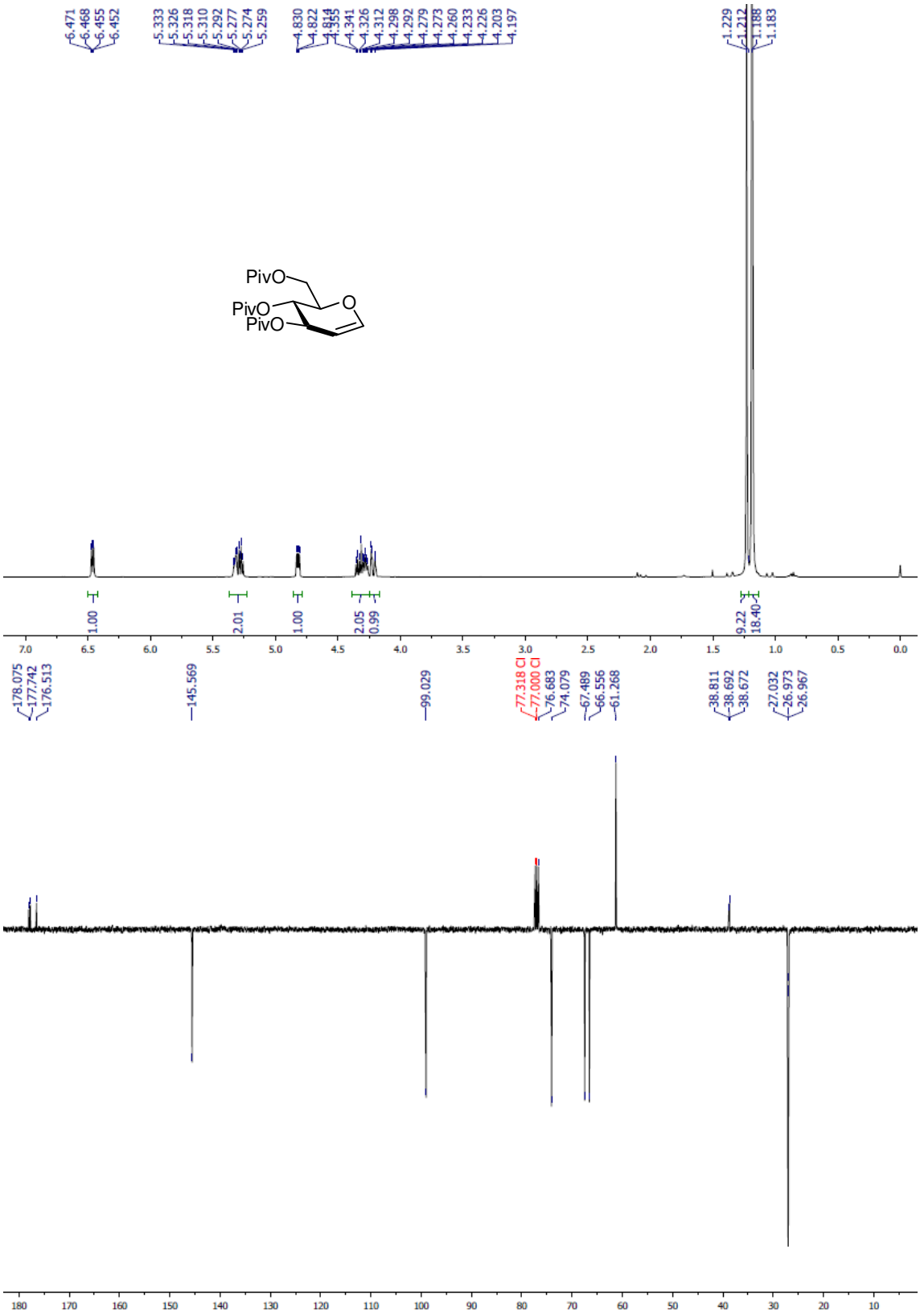
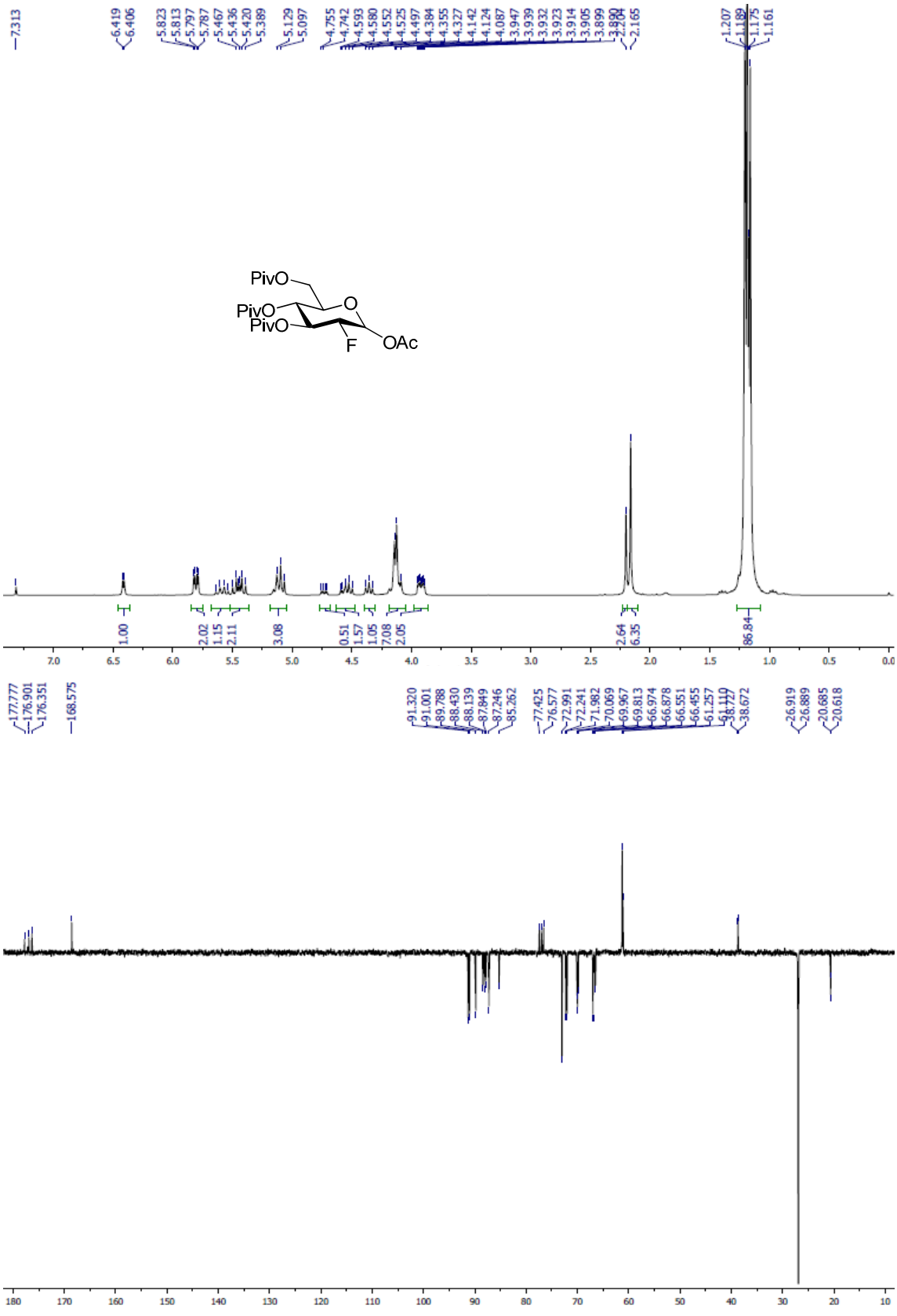
1.480 CD
1.342 CD
1.203 CD
1.066 CD

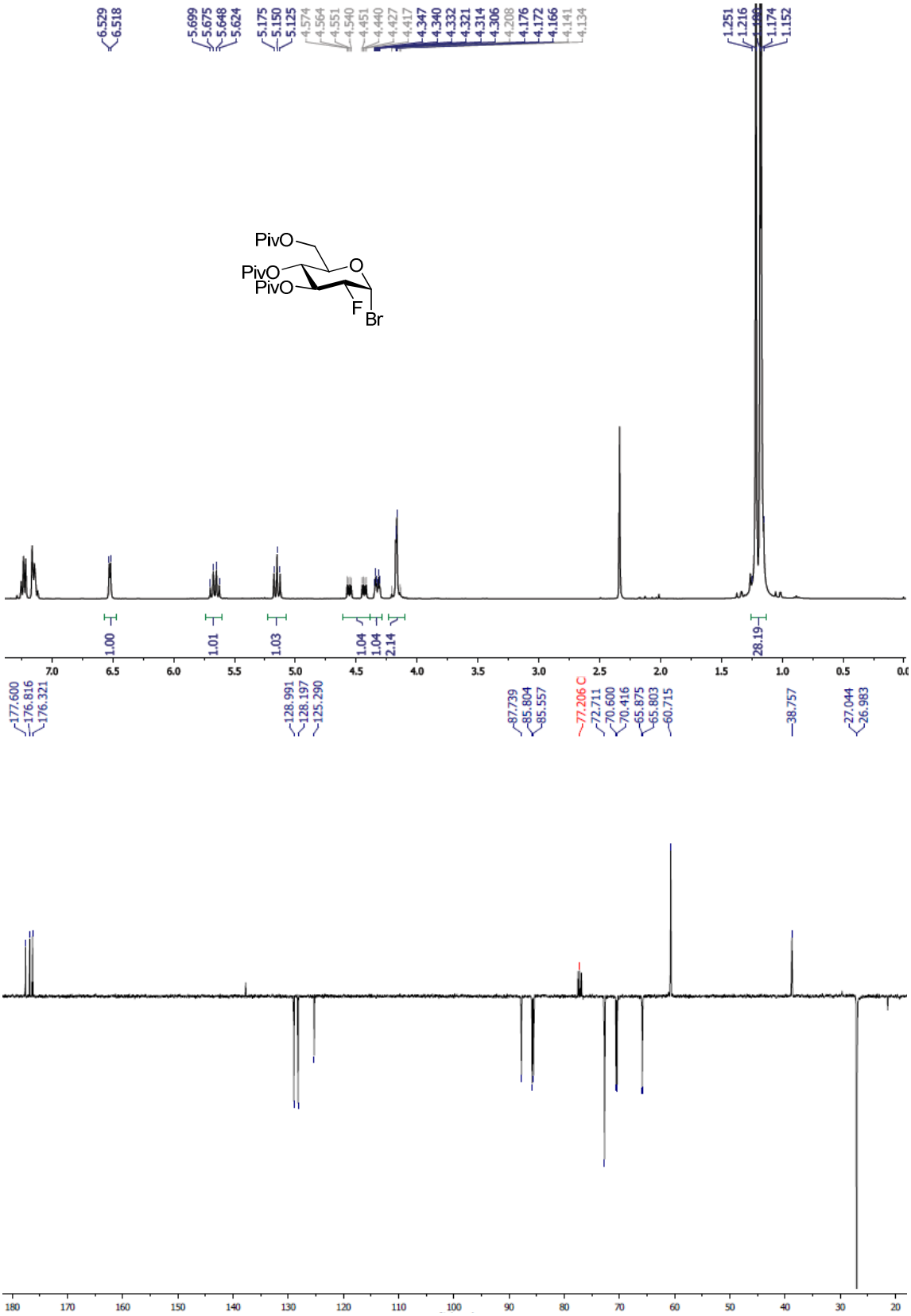
3,4,6-Tri-*O*-pivaloyl-D-glucal (9).



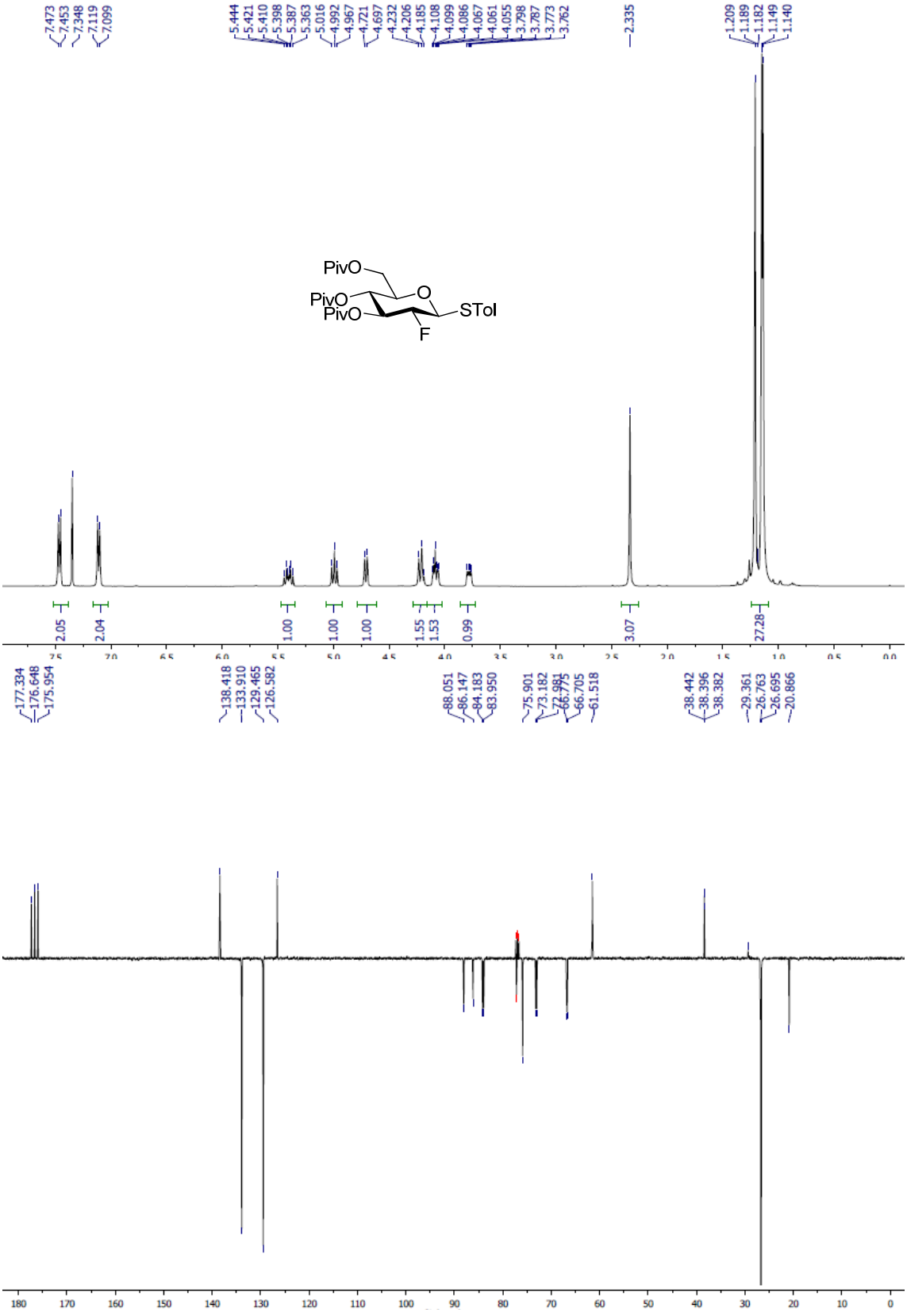
1-*O*-Acetyl-2-deoxy-2-fluoro-3,4,6-tri-*O*-pivaloyl-β-D-glucopyranoside (10).



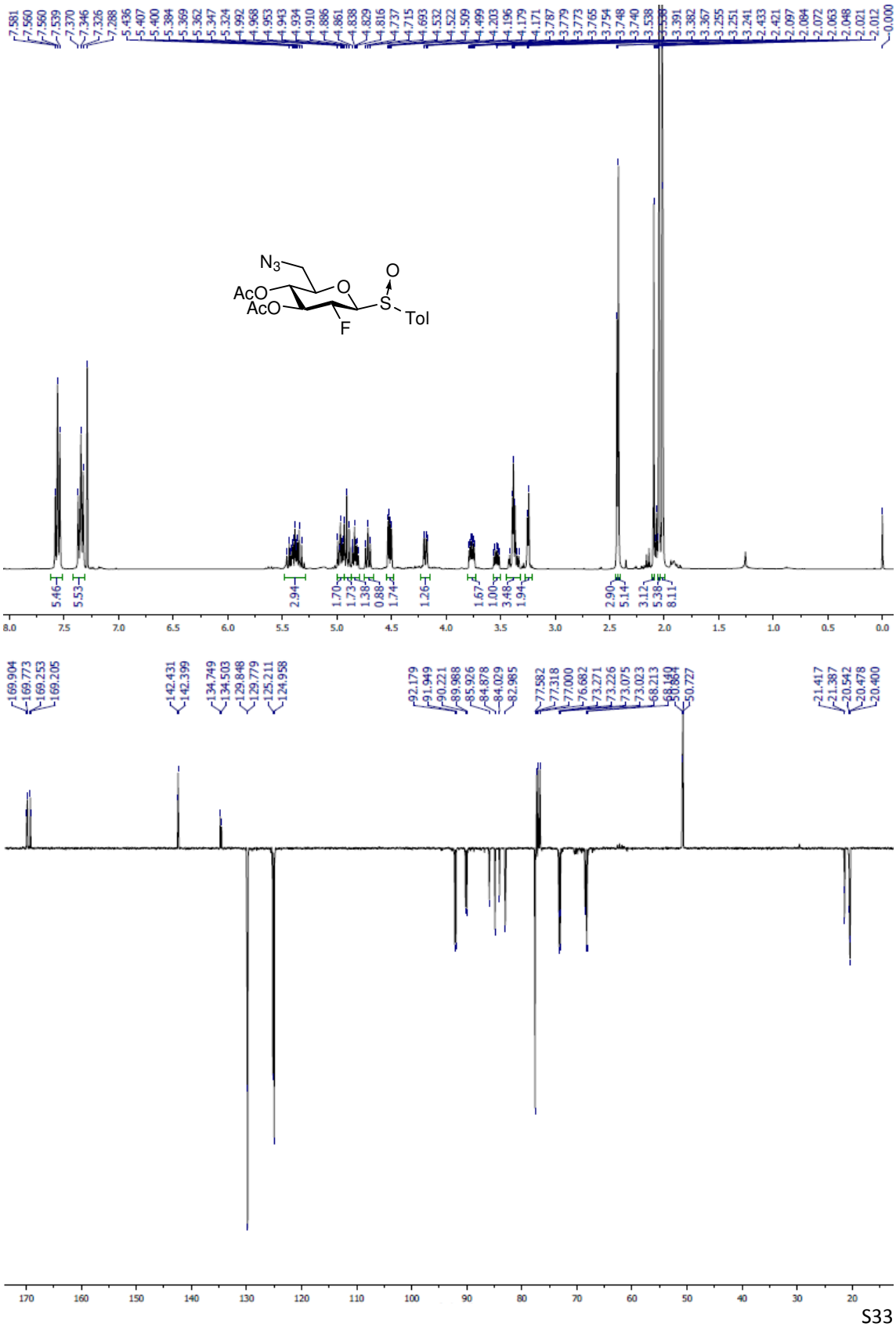
2-Deoxy-2-fluoro-3,4,6-tri-*O*-pivaloyl-α-D-glucopyranosyl bromide (11).



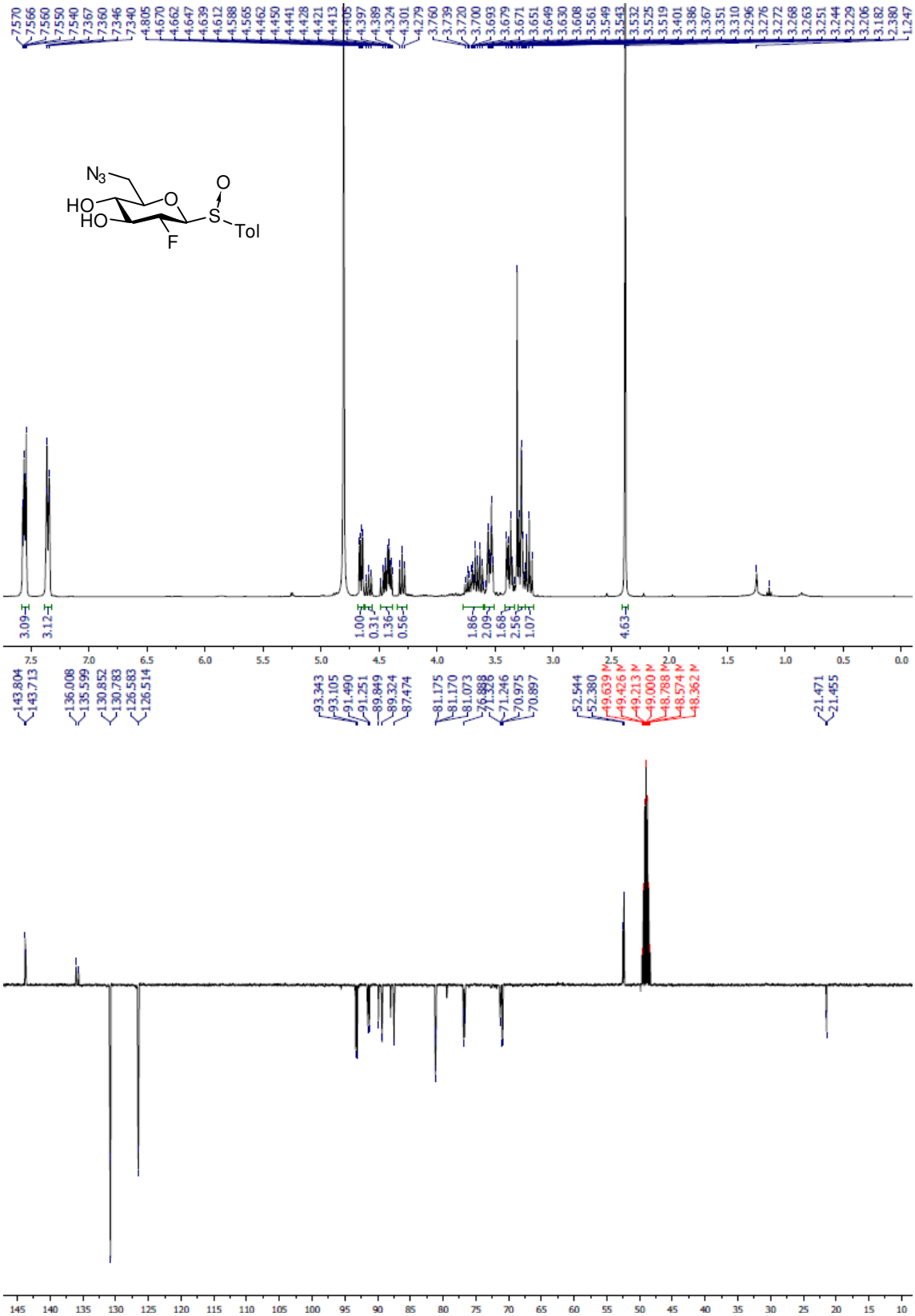
Tolyl 2-deoxy-2-fluoro-3,4,6-tri-*O*-pivaloyl-1-thio-β-D-glucopyranoside (12).



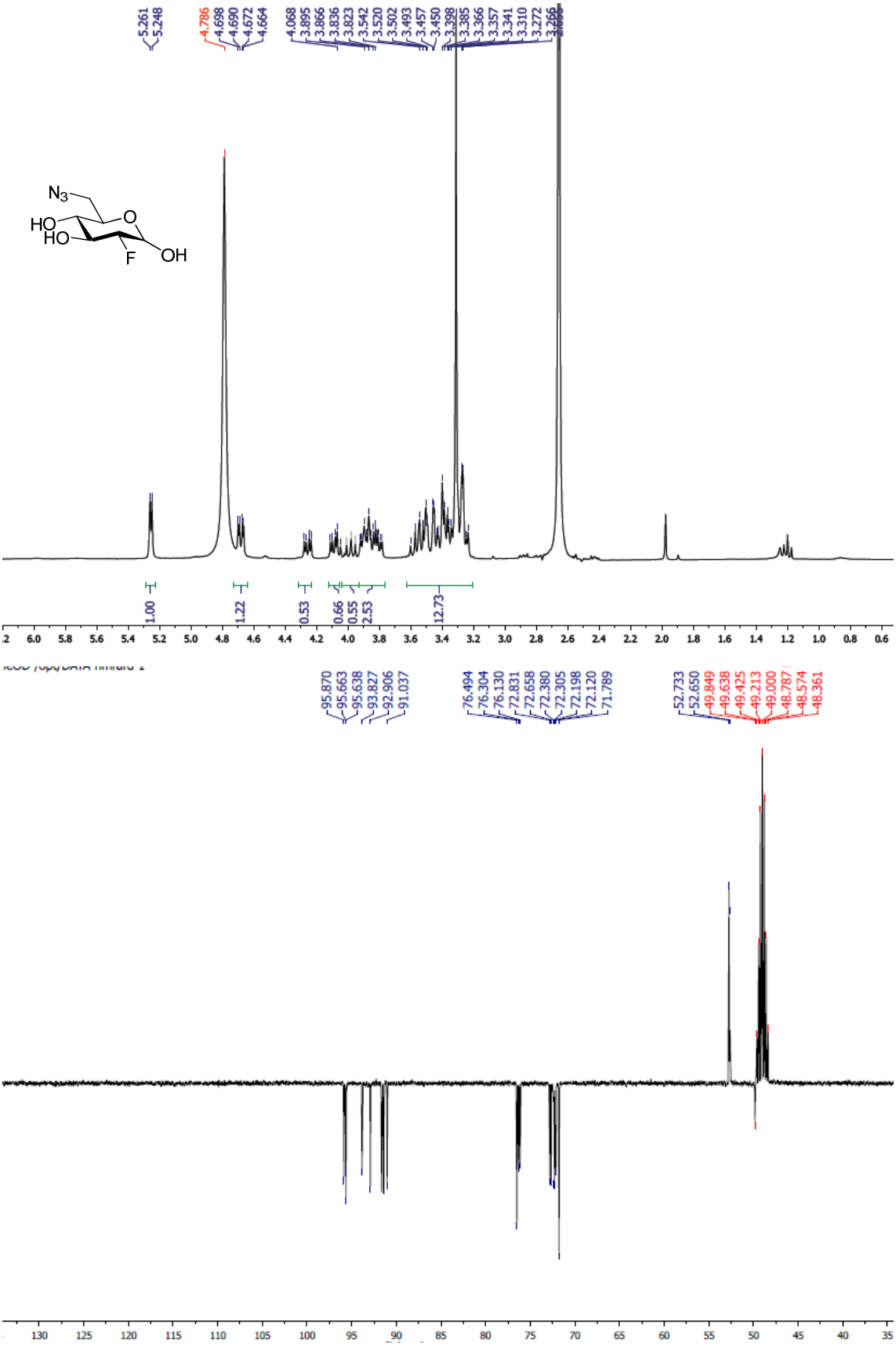
Tolyl 3,4-di-*O*-acetyl-6-azido-2,6-dideoxy-2-fluoro-1-thio-β-D-glucopyranosyl $(S)_{R/S}$-oxide (15).



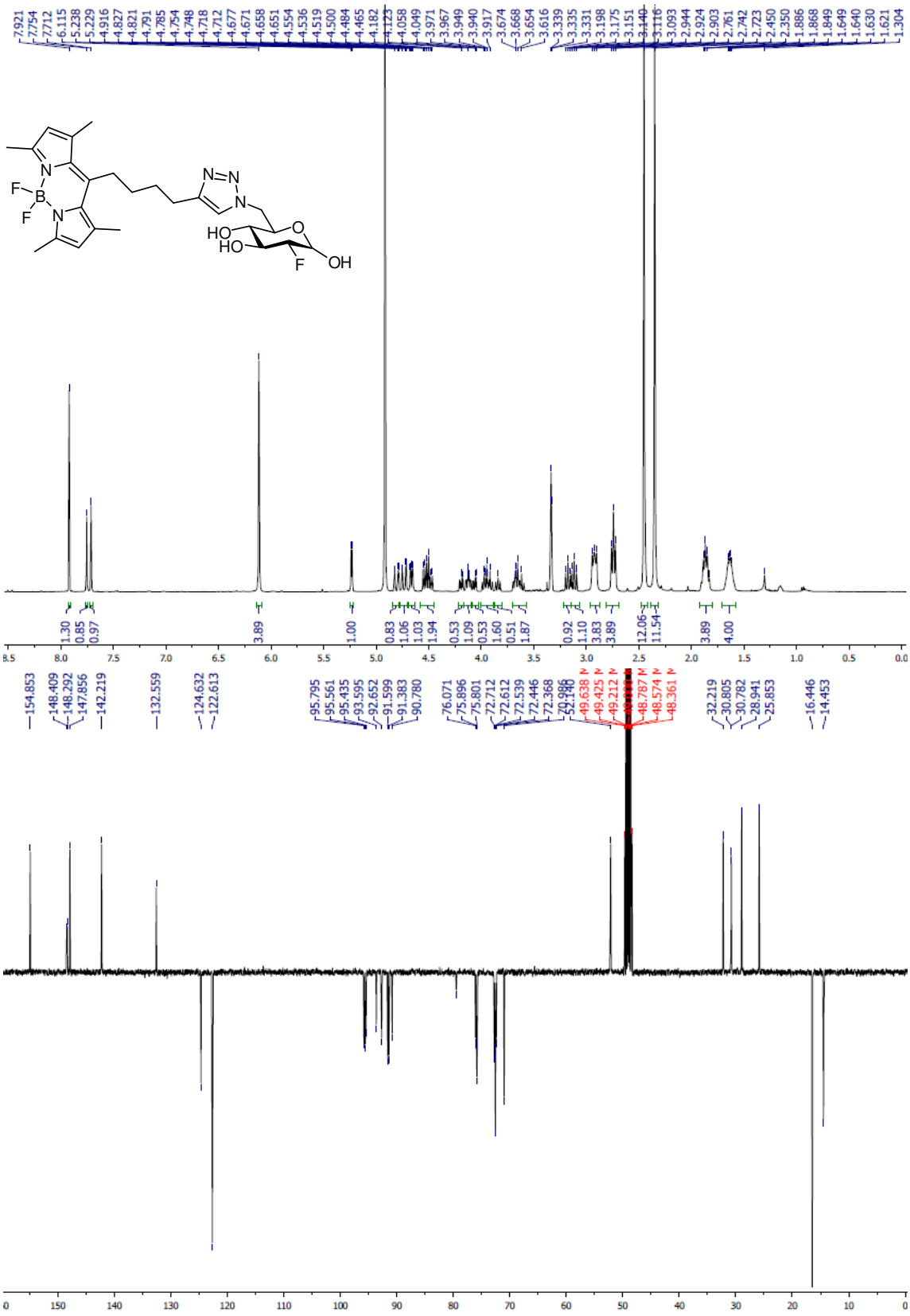
6-Azido-2,6-dideoxy-2-fluoro-1-thio-β-D-glucopyranosyl $(S)_{R/S}$-oxide (16).



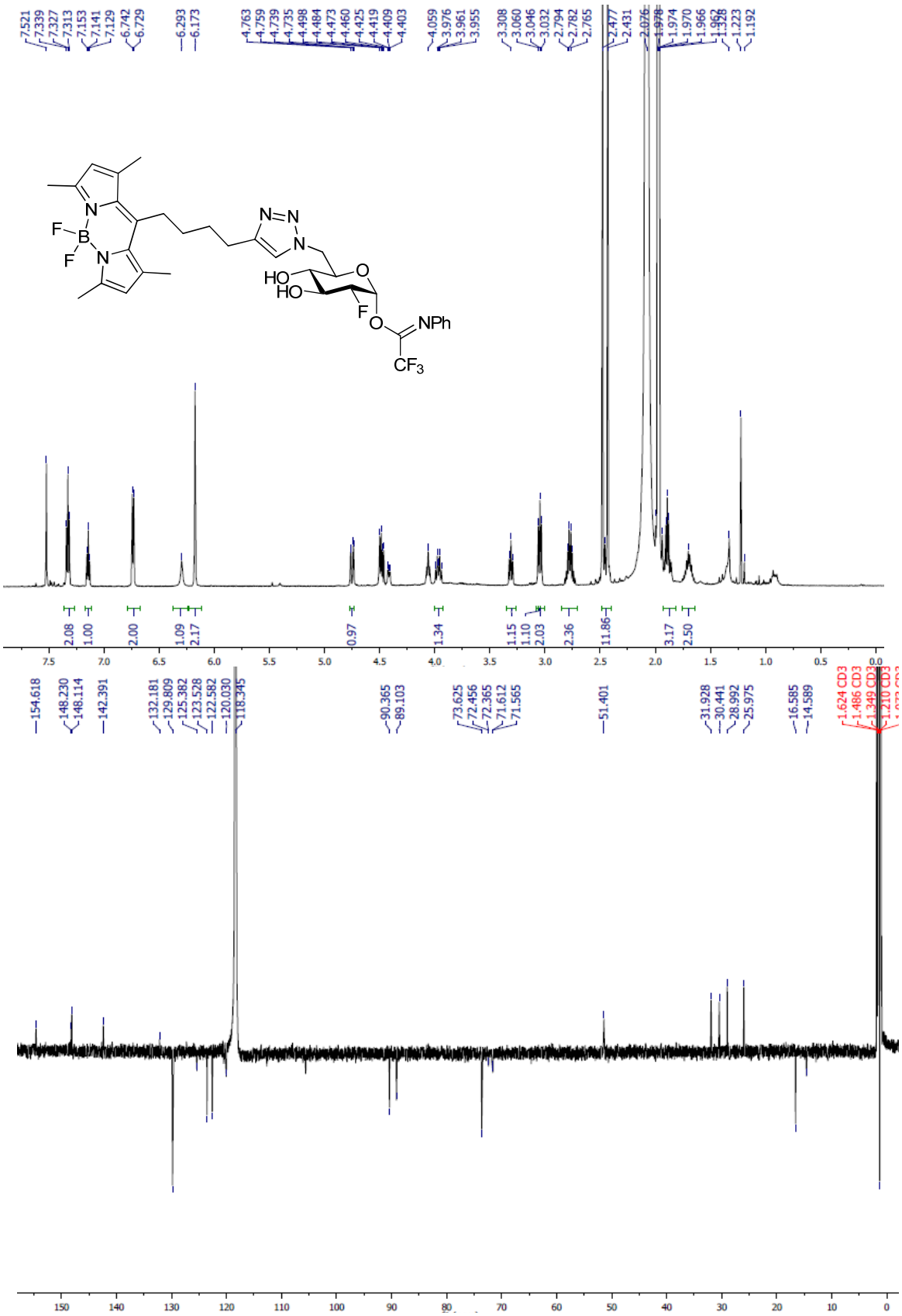
6-Azido-2,6-dideoxy-2-fluoro-α/β-D-glucopyranose (17).



Bodipy compound **18**.



a-imidate (**19**)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4.759
4.739
4.735
4.498
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3.032
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1.223
1.192
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B
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N
N
N=N
N
HO
HO
O
F
O
NPh
CF3
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2.00
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1.15
1.10
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72.365
71.612
71.565
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28.992
25.975
16.585
14.589
1.624 CD3
1.486 CD3
1.349 CD3
1.210 CD3