Supporting Information for

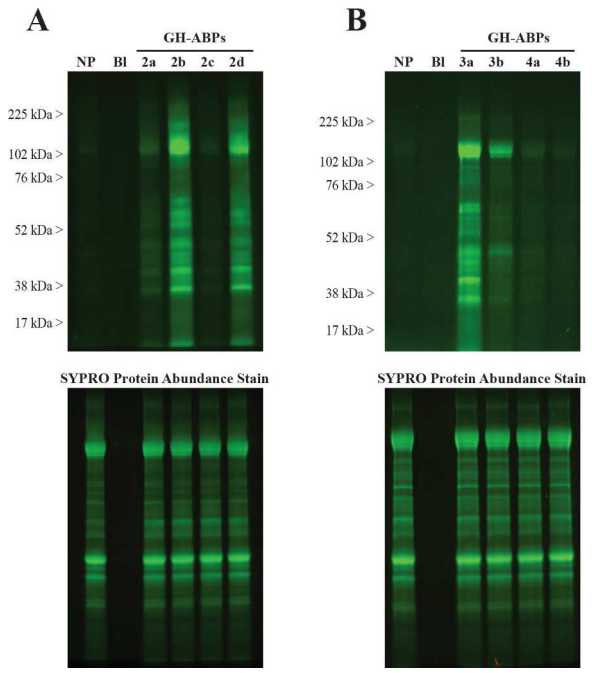
A Suite of Activity-Based Probes for Cellulose Degrading Enzymes

Lacie M. Chauvigné-Hines[‡#], Lindsey N. Anderson[‡], Holly M. Weaver, Joseph N. Brown, Phillip K. Koech, Carrie D. Nicora, Beth A. Hofstad, Richard D. Smith, Michael J. Wilkins, Stephen J. Callister, Aaron T. Wright*

Biological Sciences Division, Pacific Northwest National Laboratory, 902 Battelle Blvd, Richland, Washington, 99352

*email: aaron.wright@pnnl.gov

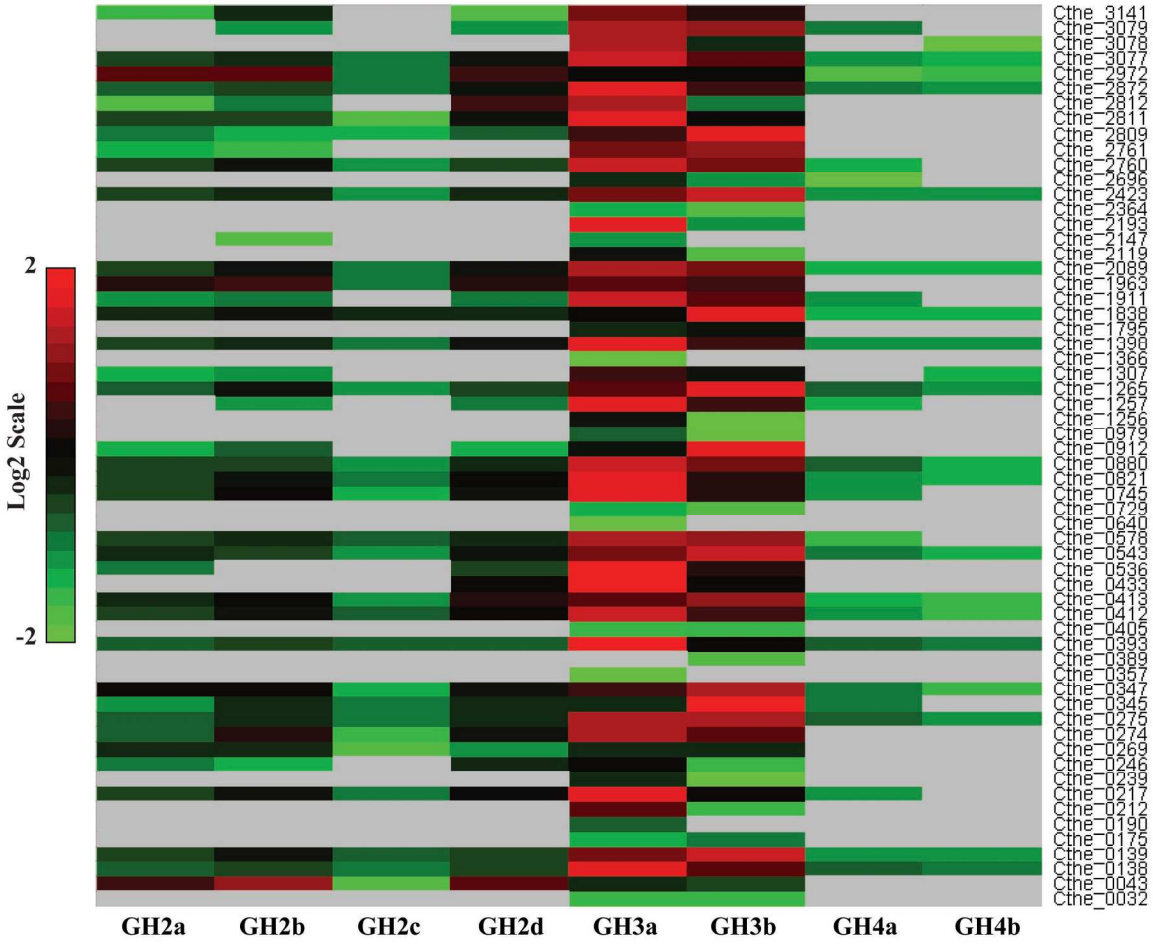
SUPPORTING FIGURE S1



Supporting Figure S1. (**A/B**) Top panel - *C. thermocellum* secretome samples, containing cellulosome proteins, were incubated at 1 mg/mL with individual **GH-ABPs** (75 μM). Click chemistry was used to append the fluorophores rhodamine-azide and -alkyne, and proteins were separated by SDS-PAGE and imaged to reveal the fluorescent ABP-labeled proteins. NP = no probe control, in which no probe was added, but all click chemistry reagents were added; BL = blank, no probe, click chemistry reagents, or sample added. (**A/B**) Bottom panel – SYPRO fluorescent protein stains

of the labeled gels shown in the top panel. This clearly shows that sample loading is identical for all samples, and that probe labeling is due to protein activity, not simply abundance.

SUPPORTING FIGURE S2



Supporting Figure S2. Heatmap showing all carbohydrate active enzymes labeled by the **GH-ABP** probe suite. The rows are scaled, such that the reactivity of each probe toward a protein can be compared; the scale is log2; gray spaces indicate no measurement. All measurements represent the average of three sample replicates per **GH-ABP**.

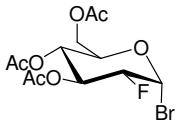
PROBE SYNTHESIS

General Procedures and Materials. NMR spectra were recorded at 25 °C on a Varian Oxford 500 MHz spectrometer at the following frequencies: 499.8 MHz (^{1}H) and 125.7 MHz (^{13}C); spectra were calibrated to

the chemical shift of tetramethylsilane (δ = 0 ppm). Spectra were assigned with appropriate ^{1}H and ^{13}C NMR experiments. Chemical shifts are in ppm, coupling constants in Hertz (Hz), and multiplicities indicated with: singlet (s), doublet (d), triplet (t), doublet of doublets (dd), doublet of doublet of doublets (ddd), doublet of triplets (dt), and multiplet (m). ESI mass spectra were obtained with a LCQ Deca spectrometer. High-resolution mass spectra were obtained with a Exactive Orbitrap mass spectrometer (Thermo Scientific). Unless otherwise noted, a Grace Davison Discovery Sciences Reveleris medium pressure liquid chromatographer fitted with commercial silica cartridges was used for purification of ABPs and intermediates. Starting mono- and disaccharide material for the syntheses of all **GH-ABPs** were purchased from Carbosynth Ltd. Other reagents were purchased from Sigma-Aldrich, Acros, or Alfa Aesar and used as received unless stated otherwise. Dry solvents were obtained via a LC Technology Solutions, Inc., SP-1 solvent drying system, or purchased, and reactions were carried out in an inert nitrogen or argon environment.

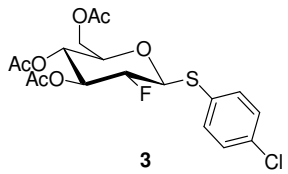
Synthesis of Glycoside Hydrolase Activity-Based Probes (GH-ABPs):

Synthesis of GH1-ABP.



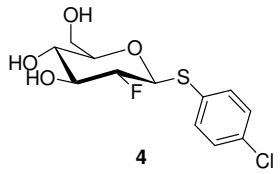
2

*1-Bromo-3,4,6-tri-O-acetyl-2-deoxyl-2-fluro-α-D-glucopyranoside (**2**).* 3,4,6-tri-*O*-acetyl-1,2-deoxy-1,2-fluoro-β-D-glucopyranoside (**1**, 499 mg, 1.6 mmol) was dissolved in 33% HBr/AcOH (2 mL) and acetic anhydride (198 μL) at rt. The resulting orange solution stirred overnight in the dark. The reaction was diluted with CH_2Cl_2 and extracted with cold DI H_2O (3x), dried over Na_2SO_4, filtered, and concentrated by rotary evaporation. Toluene (2 x 10 mL) was added to the residue and then removed by rotary evaporation. Lastly, Et_2O/Hex (1/1) was added to the residue and subsequently removed by rotary evaporation to yield **2** (564 mg, 95%) as a pale yellow solid. ^{1}H NMR (500 MHz, CD_3OD): δ 6.76 (d, *J* = 4.5 Hz, 1H), 5.57 (q, *J* = 11 Hz, 1H), 5.13 (t, *J* = 15 Hz, 1H), 4.66 (ddd, *J* = 9, 5 Hz, *J*(H,F) = 49 Hz, 1H), 4.64-4.62 (m, 2H), 4.13 (d, *J* = 11.5 Hz, 1H), 2.05 (s, 3H), 2.04 (s, 6H).



3

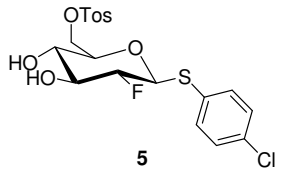
*4-Chlorophenyl-3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-1-thio-β-D-glucopyranoside (**3**).* To a solution of crude bromide **2** (564 mg, 1.5 mmol) in $CHCl_3$ (15 mL), was added a solution of tetrabutylammonium hydrogen sulfate (102 mg, 0.3 mmol) in DI H_2O (2 mL) followed by 4-chlorothiophenol (330 mg, 2.3 mmol). The mixture was cooled in an ice-water bath and a solution of KOH (170 mg, 3.03 mmol) in H_2O (20 mL) was added drop wise over a period of 15 min. After the addition was complete, the mixture was allowed to slowly warm to rt overnight. The organic phase was separated, washed with H_2O, dried over Na_2SO_4 and concentrated by rotary evaporation. The residue was purified by flash column chromatography (2:1 Hex:EtOAc) providing the thioglycoside **3** (650 mg, 98%) as a clear oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.52 (d, *J* = 8.5 Hz, 2H), 7.02 (d, *J* = 9 Hz, 2H), 5.35-5.29 (m, 1H), 4.95 (t, *J* = 10 Hz, 1H), 4.65 (d, *J* = 9.5 Hz, 1H), 4.17 (m, 2H), 3.73-3.72 (m, 1H), 2.09 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.97, 170.28, 170.03, 170.01, 134.83, 134.69, 130.03,129.05, 129.00, 88.40, 86.88, 83.66 (*J* = 90 Hz), 75.56, 73.91 (*J*(C,F) = 80 Hz), 68. 24 (*J*(C,F) = 30 Hz), 19.49, 19.40, 19.34. HRMS *m/z* (M^+) calcd for $C_{18}H_{20}ClFO_7S$: 434.06, observed: 457.049 [M+Na].



4

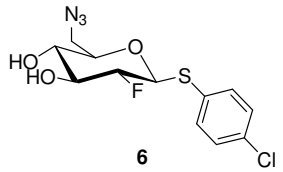
*4-Chlorophenyl-2-deoxy-2-fluoro-1-thio-β-D-glucopyranoside (**4**).* Compound **3** (620 mg, 1.4 mmol) was dissolved in dry MeOH (14 mL) and treated with a catalytic amount of 30% wt. $NaOCH_3$/MeOH at rt for 1 h. The solution was neutralized with Amberlite IR-15 H^+ resin, filtered, and the solvent removed by rotary evaporation to yield **4** (404 mg, 92%) as a brown solid. ^{1}H NMR (500 MHz, CD_3OD): δ 7.55 (d, *J* = 8.5 Hz, 2H), 7.34 (d, *J* = 8.5 Hz, 2H), 4.77 (d, *J* = 9.5 Hz, 1H), 3.94 (t, *J* = 9 Hz, 1H), 3.84 (m, 1H), 3.67-3.60

(m, 2H), 3.38-3.34 (m, 1H), 3.14 (d, *J* = 10 Hz, 1H). ^{13}C NMR (125 MHz, CD_3OD): δ 134.16, 134.03, 131.24, 128.89, 90.88, 89.40, 84.14 (*J*(C,F) = 90 Hz), 81.07, 76.34 (*J* (C,F) = 70 Hz), 73.35, 69.88, 61.39.



5

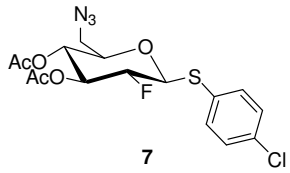
*4-Chlorophenyl-2-deoxy-2-fluoro-6-O-tosyl-1-thio-β-D-glucopyranoside (**5**).* Tosyl chloride (345 mg, 1.8 mmol) was added in portions over 15 min to a cooled 0 °C solution of **4** (373 mg, 1.2 mmol) in dry pyridine (5 mL). The resulting yellow solution was allowed to slowly reach rt overnight, and stirred for a further 24 h. The reaction was diluted with EtOAc and washed with brine (3x), dried over Na_2SO_4, filtered, and concentrated by rotary evaporation. The residue was purified by flash column chromatography (2:1 EtOAc:Hex) yielding **5** (439 mg, 79%) as a white solid. ^{1}H NMR (500 MHz, CD_3OD): δ 7.81 (d, *J* = 8 Hz, 2H), 7.42 (d, *J* = 5 Hz, 4H), 7.26 (d, *J* = 8.5 Hz, 2H), 4.71 (d, *J* = 9.5 Hz, 1H), 4.34 (d, *J* = 11 Hz, 1H), 4.15-4.12 (m, 1H), 3.85 (dt, *J*(H,F) = 50 Hz, *J* = 9 Hz, 1H), 3.78-3.53 (m, 2H), 3.22 (t, *J* = 9.5 Hz, 1H), 2.42 (s, 3H). ^{13}C NMR (125 MHz, CD_3OD): δ 134.22, 134.10, 133.04, 130.83, 129.97, 128.86, 127.99, 90.63, 89.14 (*J*(C,F) = 95 Hz), 77.46, 76.09, 75.65 (*J*(C,F) = 30 Hz), 69.45, 69.39, 69.12, 20.47. HRMS *m/z* (M^+) calcd for $C_{19}H_{20}ClFO_6S_2$: 462.94, observed: 485.026 [M+Na] and 501.019 [M+K].



6

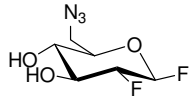
*4-Chlorophenyl-6-azido-2-deoxy-2-fluoro-1-thio-β-D-glucopyranoside (**6**).* A solution of **5** (439 mg, 0.95 mmol) and sodium azide (616 mg, 9.5 mmol) in DMF (7 mL) was heated at 70 °C overnight. The reaction was cooled rt and the solid materials were filtered through a bed of celite washing with EtOAc. The filtrate was concentrated by rotary evaporation to ~ 100 mL, extracted with brine (2x), H_2O (2x), dried over Na_2SO_4, filtered, and concentrated by rotary evaporation. The resulting crude product was purified by flash column chromatography (2:1 EtOAc:Hex) to yield **6** (279 mg, 88%) as a white solid. ^{1}H NMR (500 MHz,

CD_3OD): δ 7.55 (d, *J* = 8.5 Hz, 2H), 7.35 (d, *J* = 8.5 Hz, 2H), 4.79 (d, *J* = 9.5 Hz, 1H), 3.88 (dt, *J* = 9 Hz, *J*(H,F) = 50 Hz, 1H), 3.66-3.49 (m, 3H), 3.88-3.60 (m, 1H), 3.25 (t, *J* = 9.5 Hz, 1H). HRMS *m/z* (M^+) calcd for $C_{12}H_{13}ClFN_3O_3S$: 337.77, observed: 398.033 [M+2Na+H_2O+H].



7

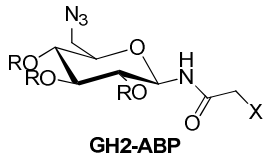
*4-Chlorophenyl 3,4-di-O-acetyl-6-azido-2-deoxy-2-fluoro-1-thio-β-D-glucopyranoside (**7**).* To a solution of **6** (279 mg, 2.6 mmol) in dry pyridine (33 mL) was added acetic anhydride (3.4 mL). The reaction mixture was stirred overnight at rt. The solvent was removed azeotropically by addition of water. The resulting residue was dissolved in EtOAc and washed successively with sat. $NaHCO_3$, 1M HCl, H_2O, sat. $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and concentrated by rotary evaporation to yield **7** (342 mg, 98%) as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.53 (d, *J* = 9.5 Hz, 2H), 7.34 (d, *J* =10 Hz, 2H), 5.32 (dt, *J* = 14 Hz, 9 Hz, 1H), 4.91 (t, *J* = 9.5 Hz, 1H), 4.66 (d, *J* = 9.5 Hz, 1H), 4.11 (dt, *J* = 9 Hz, J(H,F) = 49 Hz, 1H), 3.71-3.67 (m, 1H), 3.41-3.26 (m, 2H), 2.07 (s, 3H), 2.03 (s, 3H). HRMS *m/z* (M^+) calcd for $C_{16}H_{17}ClFN_3O_5S$: 417.06, observed: 440.045 [M+Na] and 456.039 [M+K].



GH1-ABP

*6-Azido-1,2-deoxy-1,2-fluoro-β-D-glucopyranoside (**GH1-ABP**).* To a solution of **7** (319 mg, 0.76 mmol) in a mixture of acetone (12 mL) and DI H_2O (2 mL) at rt was added N-bromosuccinimide (1.35 g, 7.6 mmol). The reaction mixture stirred for 3 h after which aqueous $NaHCO_3$ (8 mL) was added followed by CH_2Cl_2 (40 mL). The organic layer was washed successively with sat. $NaHCO_3$, H_2O, and brine. The organic phase was dried over Na_2SO_4 and concentrated by rotary evaporation to yield a pale yellow solid, which was used without further purification. The residue was dissolved in a mixture of dry THF (8 mL) and dry CH_2Cl_2 (8 mL), and cooled to -40 °C after which was added DAST (931 μL, 7.6 mmol). The

reaction mixture was allowed to slowly warm to rt overnight and was stirred a further day. The reaction mixture was cooled to 0 °C and quenched with sat. $NaHCO_3$. The resulting mixture was diluted with CH_2Cl_2, and the organic layer was washed with sat. $NaHCO_3$, H_2O, and brine. The organic layer was dried over Na_2SO_4 and concentrated by rotary evaporation. The resulting residue was purified by gradient flash column chromatography (1:2 Et_2O/Hex to 2:3 Et_2O/Hex) to give 3,4-di-*O*-acetyl-6-azido-1,2-deoxy-1,2-fluoro-β-D-glucopyranoside. To a solution of 3,4-di-*O*-acetyl-6-azido-1,2-deoxy-1,2-fluoro-β-D-glucopyranoside (140 mg, 0.48 mmol) in dry MeOH (80 mL) was added a catalytic amount of 30% wt. $NaOCH_3$/MeOH at rt for 1h. The solution was neutralized with Amberlite IR-15 H^+ resin, filtered, and the solvent removed by rotary evaporation to yield **GH1-ABP** (58 mg, 36% over 3 steps) as a yellow residue. ^{1}H NMR (500 MHz, CD_3OD): δ 5.37 (ddd, J = 10 Hz, 3.5 Hz, J(H,F) = 53.5 Hz, 1H), 3.67-3.56 (m, 3H), 3.48-3.46 (m, 2H), 3.69 (t, J = 9.5 Hz, 1H). ^{13}C NMR (125 MHz, CD_3OD): δ 106.84 (J(C,F) = 103 Hz, 92.34 (J(C,F) = 92 Hz), 7.61 (J = 19.5 Hz), 73.93 (J = 39 Hz, 68 Hz), 69.92 (J = 29 Hz), 51.38. HRMS *m/z* (M^+) calcd for $C_6H_9F_2N_3O_3$: 209.06, observed: 208.052 [M-H].

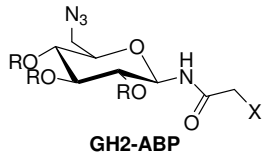


GH2-ABP

a: R = H, X = Br
c: R = H, X = I

Synthesis of GH2a/2c-ABPs; General Procedure. 6-azido-1,6-dideoxy-β-D-glucopyranosylamine (**8**, 0.32 mmol) was dissolved in anhydrous DMF (500 μL) and cooled to -10 °C, and subsequently treated with either bromoacetic anhydride (0.32 mmol) or iodoacetic anhydride (0.32 mmol). The reaction proceeded at -10 °C for 4 h until TLC (3:2 EtOAc/Hex) indicated the reaction was complete. The reaction was poured into 10 volumes of Et_2O resulting in a white precipitate, which was further cooled at -10 °C for 16 h. The Et_2O was removed and the white solid was triturated (8x) with Et_2O. The solid was further dried under high vacuum resulting in **GH2a-ABP** (92 mg, 51%) and **GH2c-ABP** (44.4 mg, 38%) as white solids. **GH2a-ABP**: ^{1}H NMR (500 MHz, CD_3OD): δ 4.91 (d, J = 10 Hz, 1H), 3.88 (s, 2H), 3.55-3.38 (m, 6H). ^{13}C NMR (125 MHz, CD_3OD): δ 170.34, 81.52, 78.86, 78.57, 74.01, 72.06, 52.57, 28.82. HRMS *m/z* (M^+) calcd for $C_8H_{13}BrN_4O_5$: 324.01, observed: 325.014 [M+H] and 327.014 [M+H] (due to bromine isotope). **GH2c-**

ABP: ^{1}H NMR (500 MHz, CD_3OD): δ 3.74 (s, 2H), 3.54-3.46 (m, 3H), 3.97-3.62 (m, 2H), 3.25 (t, *J* = 9 Hz, 1H). ^{13}C NMR (125 MHz, CD_3OD): δ 172.24, 81.47, 78.87, 78.51, 74.04, 72.04, 52.54, -2.19. HRMS *m/z* (M^+) calcd for $C_8H_{13}IN_4O_5$: 371.99, observed: 372.998 [M+H].

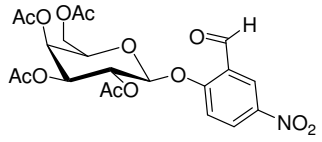


GH2-ABP

b: R = Ac, X = Br
d: R= Ac, X = I

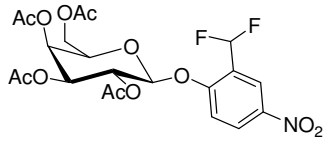
Synthesis of GH2b/2d-ABPs; General Procedure. In a round bottom flask, 2,3,4-Tri-*O*-acetyl-1-amino-6-azido-1,6-dideoxy-β-D-glucose (**8**, 0.32 mmol) was dissolved in anhydrous DMF (300 μL) and cooled to -10 °C, subsequently treated with either bromoacetic anhydride (0.32 mmol) or iodoacetic anhydride (0.32 mmol). The reaction proceeded at -10 °C for 4 h until TLC (3:2 EtOAc/Hex) indicated the reaction was complete. The reaction was diluted with CH_2Cl_2 and washed with DI H_2O (2x), dried over Na_2SO_4, and concentrated by rotary evaporation to give pure **GH2b-ABP** (59 mg, 41%) and **GH2d-ABP** (149 mg, 99%) as white solids. **GH2b-ABP**; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.21 (d, *J* = 8.5 Hz, 1H), 5.33 (t, *J* = 10 Hz, 1H), 5.22 (t, *J* = 10 Hz, 1H), 5.08 (t, *J* = 10 Hz, 1H), 5.01 (t, *J* = 10 Hz, 1H), 3.91-3.79 (m, 3H), 3.48-3.45 (m, 1H), 3.31 (dd, *J* = 20 Hz, 5 Hz, 1H), 2.08 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.57, 169.64, 169.22, 166.27, 78.33, 74.36, 72.13, 69.77, 68.90, 50.27, 27.77, 20.34. HRMS *m/z* (M^+) calcd for $C_{14}H_{19}BrN_4O_8$: 450.04, observed: 451.045 [M+Na]. **GH2d-ABP**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.28 (broad s, 1H), 5.29 (t, *J* = 10 Hz, 1H), 5.22 (t, *J* = 10 Hz, 1H), 5.06 (t, *J* = 10 Hz, 1H), 4.96 (t, *J* = 10 Hz, 1H), 3.83-3.79 (m, 1H), 3.72-3.63 (m, 2H), 3.45 (d, *J* = 15 Hz, 1H), 4.32 (dd, *J* = 13.5 Hz, 5 Hz, 1H), 2.07 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H). ^{13}C NMR (125 MHz, CD_3CN): δ 710.75, 170.61, 170.05, 169.77, 78.61, 75.16, 73.66, 71.06, 69.78, 51.47, 21.02, 20.86, 20.79, -1.22. HRMS *m/z* (M^+) calcd for $C_{14}H_{19}IN_4O_8$: 498.02, observed: 499.031 [M+H].

Synthesis of GH3a,3b-ABPs.



11

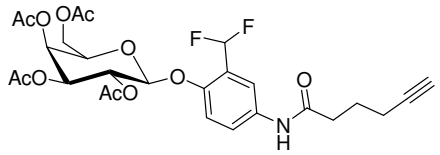
*2-Formyl-4-nitro-1-phenoxyl-2,3,4,6-tetra-O-acetyl-α-D-galactopyranose (**11**).* To a cold (0 °C) solution of commercially available 1-bromo-1-deoxy-2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose (**9**, 400 mg, 0.973 mmol) in CH_2Cl_2 (10 mL) was added a cold (0 °C) mixture of tetrabutylammonium bromide (370 mg, 1.15 mmol) and 2-hydroxy-5-nitrobenzaldehyde (**10**, 368 mg, 2.2 mmol) in 1M NaOH (5 mL). The resulting yellow solution was allowed to slowly warm to rt overnight. The reaction was diluted with CH_2Cl_2 and washed with 1M NaOH (4x). The organic layer was then dried over Na_2SO_4 and concentrated by rotary evaporation. The orange crude product was dry loaded for purification via gradient flash column chromatography (2:1 Hex/EtOAc to 1:1 EtOAc/Hex) to yield **11** (210 mg, 43%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.31 (d, *J* = 5 Hz, 1H), 8.70 (s, 1H), 8.40 (d, *J* = 5 Hz, 1H), 7.25 (d, *J* = 5 Hz, 1H), 5.59 (q, *J* = 10 Hz, 1H), 5.49 (broad s, 1H), 5.27 (t, *J* = 10 Hz, 1H), 5.17 (m, 1H), 4.23-4.13 (m, 3H), 2.18 (s, 3H), 2.07-2.00 (m, 9H). HRMS *m/z* (M^+) calcd for $C_{21}H_{23}NO_{13}$: 497.12, observed: 520.104 [M+Na].



12

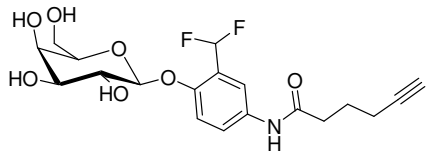
*2-Difluromethyl-4-nitro 1-phenoxyl-2,3,4,6-tetra-O-acetyl-α-D-galactopyranose (**12**).* To a cold (0 °C) solution of **11** (210 mg, 0.42 mmol) in dry CH_2Cl_2 (6 mL) was added diethylaminosulfur trifluoride (DAST) (207 μL, 1.7 mmol). The solution was allowed to slowly reach rt overnight at which point the reaction was determined complete by TLC (2:1 Hex/EtOAc). The reaction was diluted with CH_2Cl_2 and extracted with brine (3x). The organic layer was dried over Na_2SO_4 and concentrated by rotary evaporation to give pure **12** (188 mg, 85%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.50 (s, 1H), 8.34 (d, *J* = 10 Hz, 1H), 7.22 (d, *J* = 10 Hz, 1H), 6.85 (t, *J(H,F)* = 55 Hz, 1H), 5.58 (q, *J* = 10 Hz, 1H), 5.51 (d, *J* = 5 Hz,

1H), 5.16-5.13 (m, 2H), 4.26-4.15 (m, 4H), 2.21 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H). HRMS *m/z* (M^+) calcd for $C_{21}H_{23}F_2NO_{12}$: 519.12, observed: 542.06 [M+Na].



GH3b-ABP

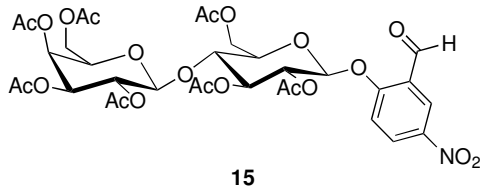
*4-(hex-5-yneamide)-2-difluromethyl 1-phenoxyl-2,3,4,6-tetra-O-acetyl-α-D-galactopyranose (**GH3b-ABP**).* Compound **12** (249 mg, 0.48 mmol) was dissolved in EtOAc (3 mL), and 10% Pd on carbon (56 mg) was added. The reaction mixture was purged with H_2 for 5 min. The reaction was then maintained under a balloon of H_2 for 48 h until TLC (2:1 Hex/EtOAc) indicated no starting material. The Pd/C was filtered through a bed of celite and the filtrate concentrated by rotary evaporation to yield **13** (190 mg, 81%), a pale yellow solid. The amine **13** was used in the subsequent step without purification. 5-Hexynoic acid (46 μL, 0.42 mmol), EDCI (87.4 mg, 0.46 mmol), HOBt (69.8 mg, 0.46 mmol) and NMM (83 μL, 0.76 mmol) were dissolved in dry DMF (3 mL), and stirred for 15 min. The amine **13** (191 mg, 0.38 mmol) dissolved in dry DMF (2 mL) was added drop wise over 5 min. The resulting solution was stirred for 48 h at rt. The reaction was diluted with EtOAc and washed with H_2O (2x), sat. $NaHCO_3$ (2x), 1M HCl (2x), and brine (2x). The organic layer was dried over Na_2SO_4, filtered and concentrated by rotary evaporation to give crude product, which was purified by flash column chromatography (2:1 EtOAc/Hex), then preparative TLC to give **GH3b-ABP** (88 mg, 40%) as a white solid. ^{1}H NMR (500 MHz, CD_3OD): δ 7.77 (s, 1H), 7.70 (d, *J* = 10 Hz, 1H), 7.20 (d, *J* = 10 Hz, 1H), 6.83 (t, *J*(H,F) = 55 Hz, 1H), 5.47 (d, *J* = 5 Hz, 1H), 5.41-5.37 (m, 1H), 5.28-5.25 (m, 2H), 4.33 (t, *J* = 10 Hz, 1H), 4.20 (d, *J* = 10 Hz, 2H), 2.50 (t, *J* = 10 Hz, 2H), 2.29-2.27 (m, 3H), 2.18 (s, 3H), 2.05 (s, 6H), 1.98 (s, 3H), 1.92-1.85 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.66, 170.38, 1470.20, 170.08, 169.64, 150.88, 133.60, 123.71, 117.75, 116.17, 110.78, 100.21, 83.34, 71.18, 70.51, 69.44, 68.09, 66.74, 61.36, 50.80, 35.67, 23.77, 20.61, 20.53 (2C), 17.72 (2C). HRMS *m/z* (M^+) calcd for $C_{27}H_{31}F_2NO_{11}$: 583.14, observed: 584.195 [M+H].



GH3a-ABP

*4-(Hex-5-yneamido)-2-difluromethyl 1-deoxy-1-phenoxyl-α-D-galactopyranoside (**GH3a-ABP**).* **GH3b-ABP** (42.4 mg, 0.073 mmol) was dissolved in dry MeOH (3 mL) and treated with a catalytic amount of 30% wt. $NaOCH_3$/MeOH at rt for 1 h. The solution was neutralized with Amberlite IR-15 H^+ resin, filtered, and the solvent removed by rotary evaporation to yield **GH3a-ABP** (29.4 mg, 97%) as a red-brown solid. ^{1}H NMR (500 MHz, CD_3OD): δ 7.79 (s, 1H), 7.63 (d, *J* = 10 Hz, 1H), 7.29-7.05 (m, 2H), 3.99 (d, *J* = 5 Hz, 1H), 3.80-3.75 (m, 4H), 3.68-3.65 (m, 1H), 3.56 (dd, *J* = 10 Hz, 5 Hz, 1H), 2.50 (t, *J* = 5 Hz, 2H), 2.29-2.27 (m, 3H), 1.88 (m, 2H). ^{13}C NMR (125 MHz, CD_3OD): δ 173.56, 155.32, 137.29, 127.06, 120.81, 120.40, 114.78, 112.92, 106.31, 86.35, 79.37, 77.10, 74.44, 72.55, 72.43, 64.61, 38.73, 27.90, 20.90. HRMS *m/z* (M^+) calcd for $C_{19}H_{23}F_2NO_7$: 415.14, observed: 416.149 [M+H].

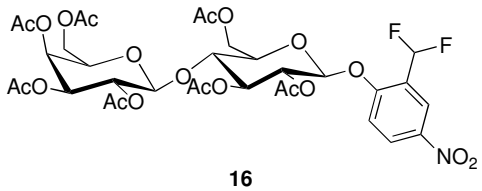
Synthesis of GH4a,4b-ABPs.



15

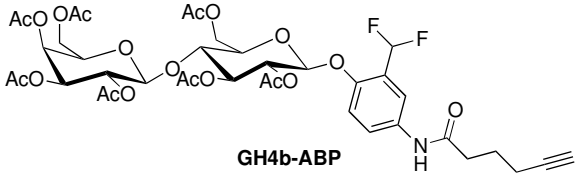
*2-Formyl-4-nitro 2,3,4,6,2′,3′,4′,6′-hepta-O-acetyl-1-phenoxyl-α-D-lactopyranose (**15**).* To a cold (0 °C) solution of commercially available 1-bromo-1-deoxy-2,3,4,2′,3′,4′,6′-hepta-*O*-acetyl-α-D-lactopyranose (**14**, 500 mg, 0.72 mmol) in CH_2Cl_2 (5.4 mL) was added a cold (0 °C) mixture of tetrabutylammonium bromide (272 mg, 1.63 mmol) and 2-hydroxy-5-nitrobenzaldehyde (**10**, 272 mg, 0.84 mmol) in 1M NaOH (4 mL). The resulting yellow solution slowly warmed to rt overnight. The reaction was diluted with CH_2Cl_2 and washed with 1M NaOH (4x). The organic layer was then dried over Na_2SO_4 and concentrated by rotary evaporation. The orange crude product was dry loaded for purification via flash column chromatography

(1:1 EtOAc/Hex) to yield **15** (322 mg, 57%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.35 (s, 1H), 8.76 (d, *J* = 5 Hz, 1H), 8.45 (dd, *J* = 10 Hz, 5 Hz, 1H), 7.25 (d, *J* = 10 Hz, 1H), 6.56 (d, *J* = 5 Hz, 1H), 5.56 (t, *J* = 10 Hz, 1H), 5.37 (ddd, *J* = 15 Hz, 10 Hz, 5 Hz, 1H), 5.16-5.12 (m, 1H), 4.98 (dd, *J* = 10 Hz, 5 Hz, 1H), 4.56-4.50 (m, 3H), 3.97-3.85 (m, 2H), 2.16-1.97 (m, 21H).



16

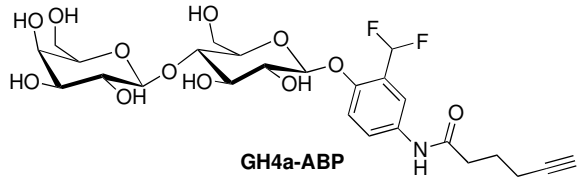
*2-Difluromethyl-4-nitro 2,3,4,6,2′,3′,4′,6′-hepta-O-acetyl-1-phenoxyl-α-D-lactopyranose (**16**).* To a cold (0 °C) solution of **15** (331 mg, 0.41 mmol) in dry CH_2Cl_2 (6 mL) was added DAST (201 μL, 1.64 mmol). The solution was slowly warmed to rt overnight. The reaction was diluted with CH_2Cl_2 and extracted with brine (3x), dried over Na_2SO_4, and concentrated by rotary evaporation to give pure **16** as a yellow solid (301 mg, 91%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.29 (s, 1H), 8.49 (s, 1H), 8.34 (d, *J* = 10 Hz, 1H), 7.18 (d, *J* = 10 Hz, 1H), 6.81 (t, *J(H,F)* = 55 Hz, 1H), 5.37 (s, 1H), 5.32 (t, *J* = 5 Hz, 2H), 5.25-5.20 (m, 3H), 5.15-5.11 (m, 1H), 4.98 (dd, *J* = 10 Hz, 5 Hz, 1H), 4.57-4.51 (m, 3H), 4.17-4.07 (m, 2H), 3.96-3.89 (m, 2H), 2.16 (s, 3H), 2.10 (s, 3H), 2.07-2.06 (s, 6H), 1.97 (s, 3H). HRMS *m/z* (M^+) calcd for $C_{33}H_{39}F_2NO_{20}$: 807.20, observed: 830.187 [M+Na].



GH4b-ABP

*4-(Hex-5-yneamide)-2-difluromethyl-1-phenoxyl-2,3,4,6,2′,3′,4′,6′-hepta-O-acetyl-1-phenoxyl-α-D-lactopyranose (**GH4b-ABP**).* The nitro **16** (211 mg, 0.26 mmol) was dissolved in EtOAc (7 mL), and to this was added 10% Pd on carbon (30.4 mg). The reaction mixture was purged with H_2 for 5 min. The reaction was then maintained under a balloon of H_2 for 2 h until TLC (2:1 Hex/EtOAc) indicated no starting material. The Pd/C was filtered through a bed of celite, and the filtrate concentrated by rotary evaporation

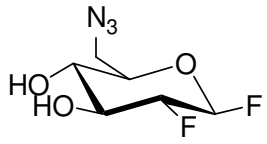
to give amine **17** (187 mg, 93%) as a pale yellow oil. The amine **17** was used in the subsequent step with out purification. 5-hexynoic acid (29 μL, 0.26 mmol), EDCI (56 mg, 0.29 mmol), HOBt (44.4 mg, 0.29 mmol) and NMM (53 μL, 0.48 mmol) were dissolved in dry DMF (3 mL) and stirred for 15 min. The amine **17** (187 mg, 0.24 mmol), dissolved in dry DMF (3 mL), was added drop wise over 5 min. The resulting solution stirred for 48 h at rt. The reaction was diluted with EtOAc and washed with H_2O (2x), sat. $NaHCO_3$ (2x), 1M HCl (2x), brine (2x), dried over Na_2SO_4 and concentrated by rotary evaporation. The crude product was purified by flash column chromatography (2:1 EtOAc/Hex), then preparative TLC, to give **GH4b-ABP** (52 mg, 25%) as a pale yellow oil. ^{1}H NMR (500 MHz, CD_3OD): δ 7.75 (s, 1H), 7.68 (d, *J* = 10 Hz, 1H), 7.18 (d, *J* = 10 Hz, 1H), 6.80 (t, *J(H,F)* = 55 Hz, 1H), 5.36 (d, *J* = 5 Hz, 1H), 5.32 (t, *J* = 10 Hz, 1H), 5.24 (d, *J* = 5 Hz, 1H), 5.16-5.11 (m, 2H), 5.04-5.01 (m, 1H), 4.72 (d, *J* = 5 Hz, 1H), 4.55 (d, *J* = 15 Hz, 1H), 4.21-4.13 (m, 4H), 3.97 (t, *J* = 10 Hz, 2H), 2.50 (t, *J* = 10 Hz, 2H), 2.29-2.24 (m, 3H), 2.13 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.93 (s, 3H), 1.89-1.85 (m, 2H). ^{13}C NMR (125 MHz, CD_3OD): δ 173.73, 172.24, 172.02, 171.93, 171.63, 171.42, 171.35, 171.15, 152.05, 135.53, 124.69, 124.54, 118.71, 117.41, 112.57, 102.04, 100.16, 84.09, 77.54, 74.13, 74.00, 72.58, 72.45, 71.79, 70.66, 70.32, 68.59, 63.45, 62.31, 36.46, 25.59, 21.08, 20.70 (5C), 20.59, 18.63. HRMS *m/z* (M^+) calcd for $C_{39}H_{47}F_2NO_{19}$: 871.27, observed: 872.278 [M+H].



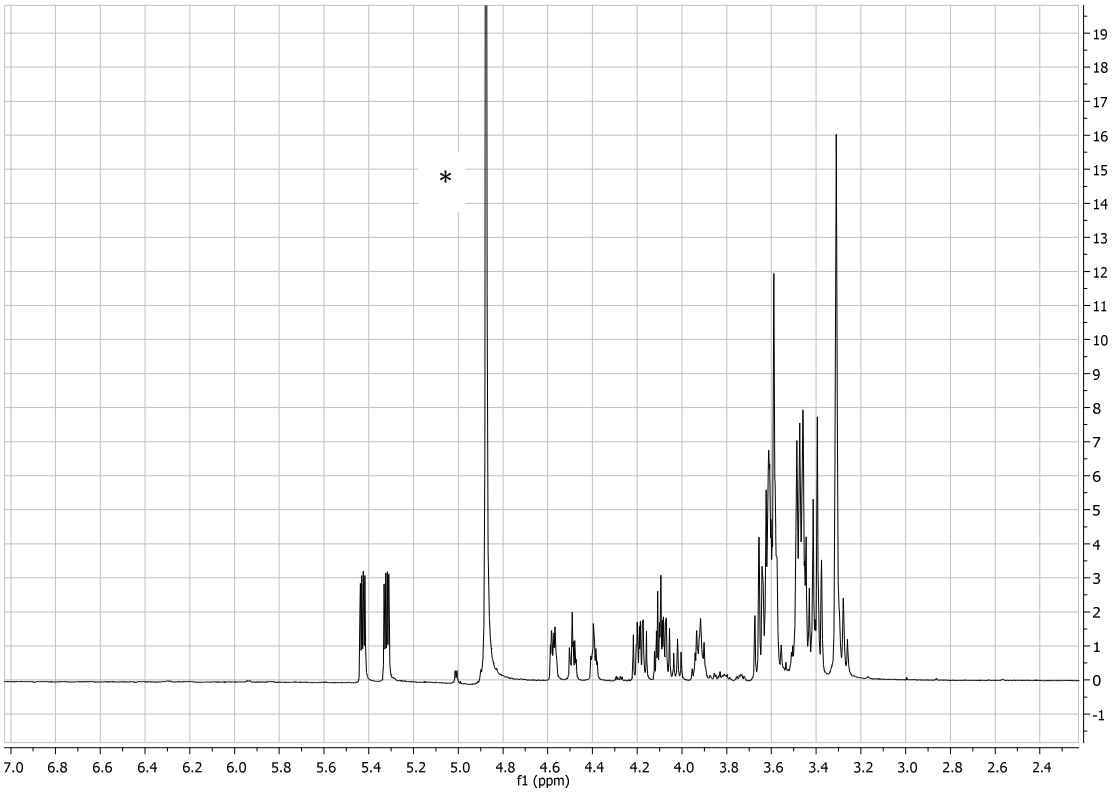
GH4a-ABP

*4-(Hex-5-yneamide)-2-difluromethyl 1-deoxy-1-phenoxyl-α-D-lactopyranose (**GH4a-ABP**).* **GH4b-ABP** (38 mg, 0.043 mmol) was dissolved in dry MeOH (3 mL) and treated with a catalytic amount of 30% wt. $NaOCH_3$/MeOH solution at rt for 1 h. The solution was neutralized with Amberlite IR-15 H^+ resin, filtered, and the solvent removed by rotary evaporation to yield **GH4a-ABP** (25 mg, 99%) as a yellow solid. ^{1}H-NMR (500 MHz, CD_3OD): δ 7.78 (d, *J* = 5Hz, 1H), 7.65 (d, *J* = 5 Hz, 1H), 7.26 (s, 1H), 7.15 (t, *J*(H,F) = 55 Hz, 1H), 4.39 (d, *J* = 10 Hz, 1H), 3.93-3.47 (m, 13H), 2.50 (t, *J* = 10 Hz, 2H), 2.29-2.26 (m, 3H), 1.91-1.85 (m, 2H). ^{13}CNMR (125 MHz, CD_3OD): δ 173.72, 152.77, 135.18, 125.91, 124.82, 118.59,

118.13, 112.50, 105.04, 103.23, 84.09, 79.98, 77.10, 76.73, 74.81, 74.47, 72.55, 70.30 (2C), 62.51, 61.59, 36.48, 25.64, 18.65. HRMS m/z (M^+) calcd for $C_{25}H_{33}F_2NO_{12}$: 577.20, observed: 578.204 [M+H].

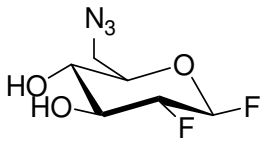


GH1-ABP

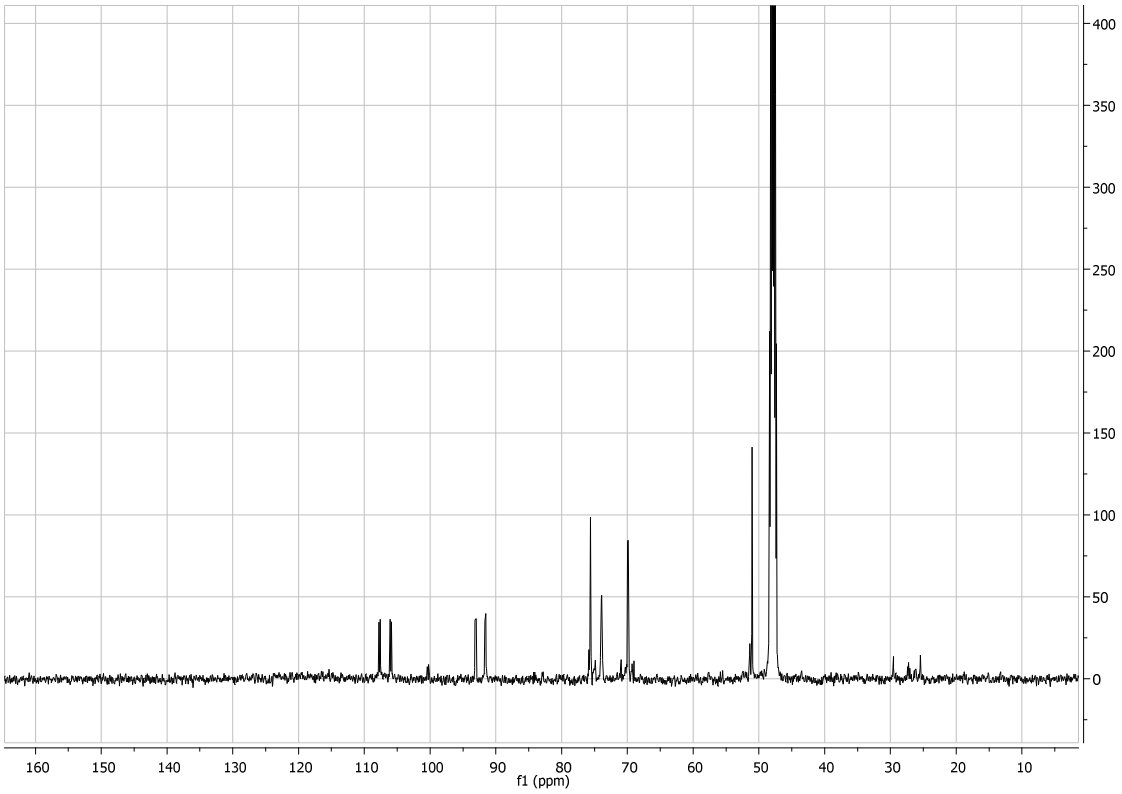


^{1}H NMR (CD_3OD) GH1-ABP

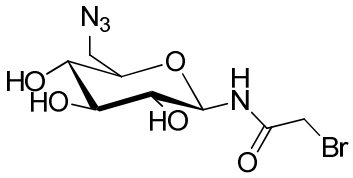
*H_2O in in CD_3OD



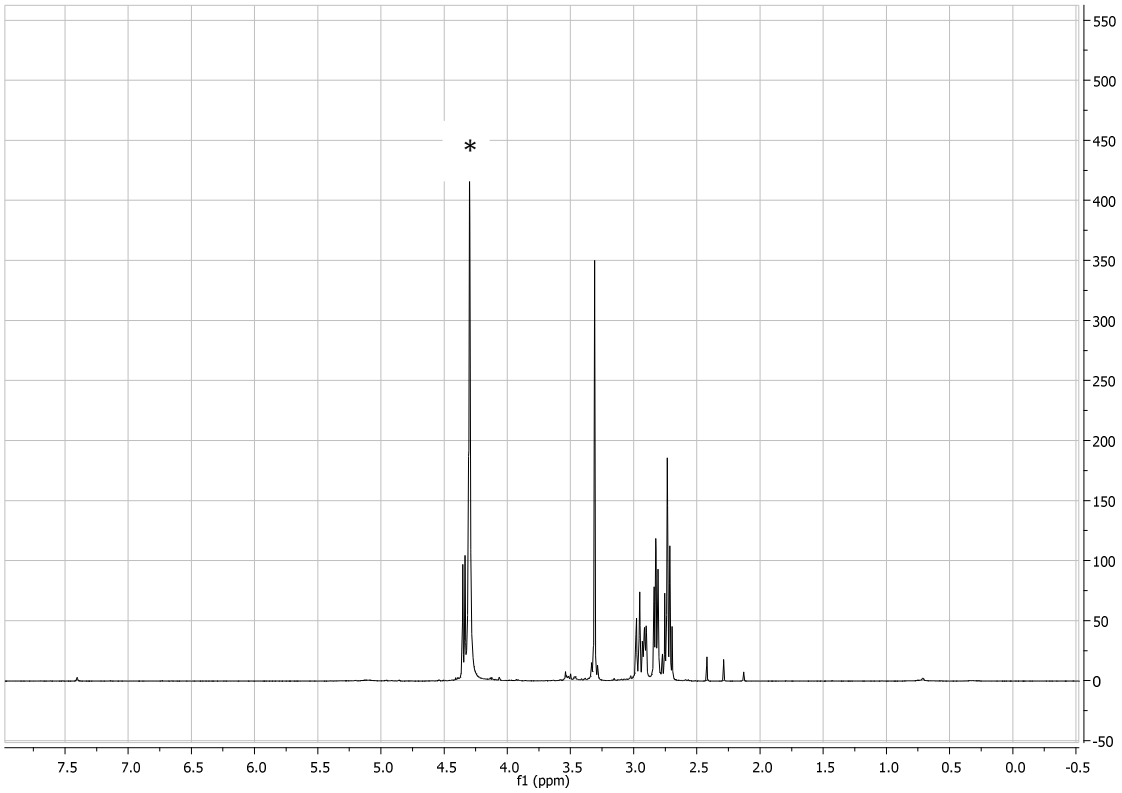
GH1-ABP



^{13}C NMR (CD_3OD) GH1-ABP

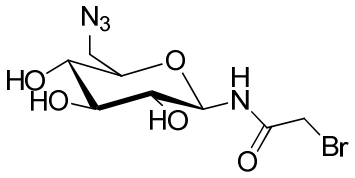


GH2a-ABP

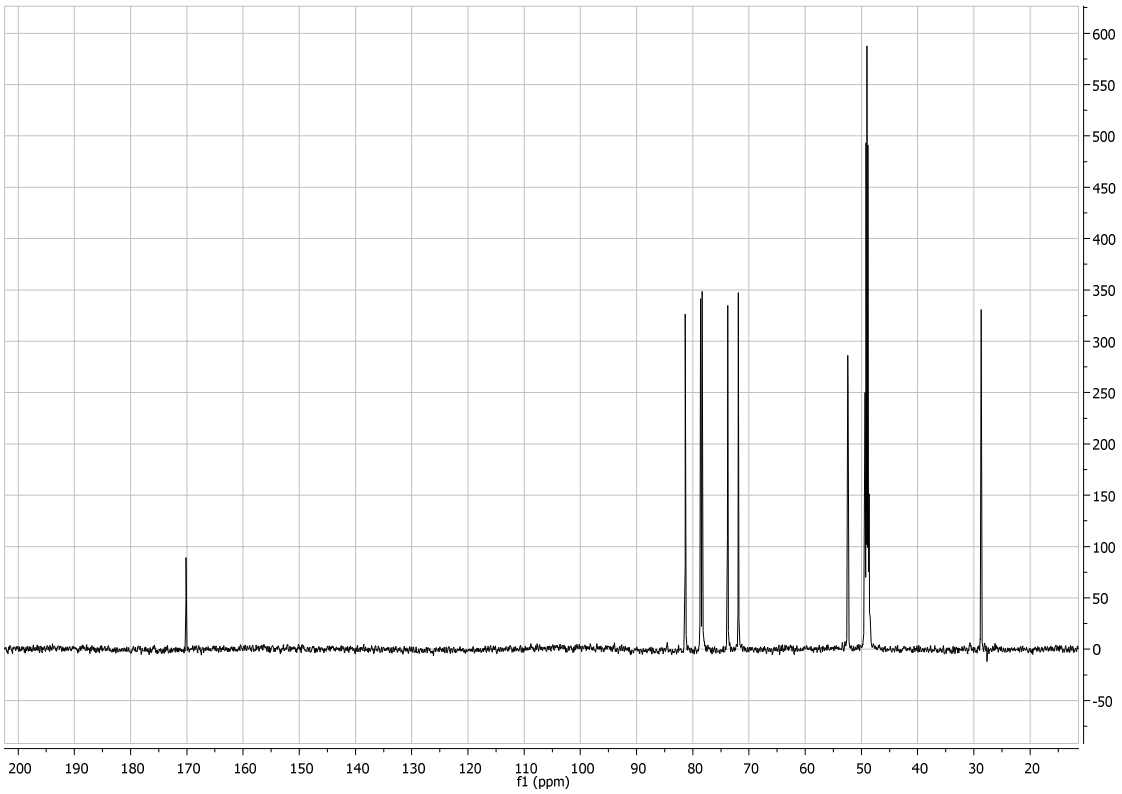


^{1}H NMR (CD_3OD) GH2a-ABP

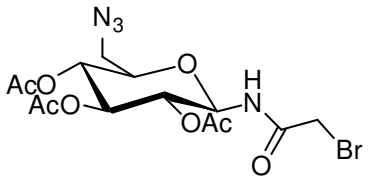
*H_2O in CD_3OD



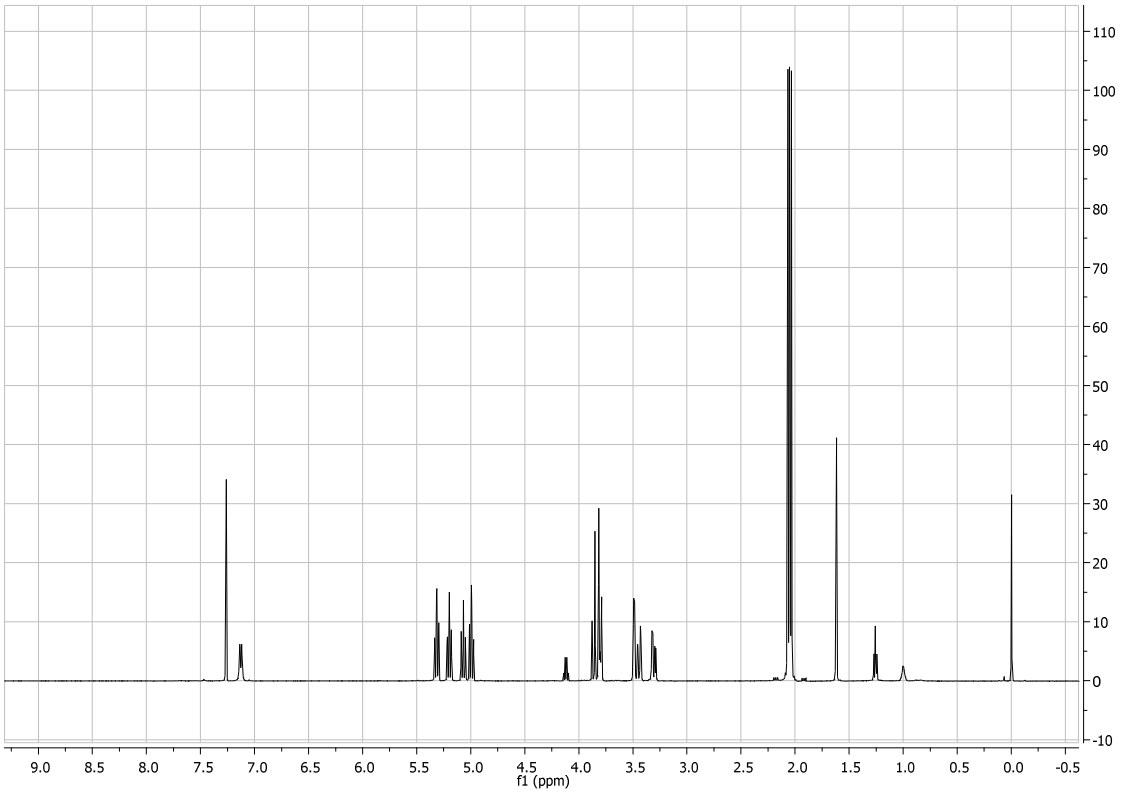
GH2a-ABP



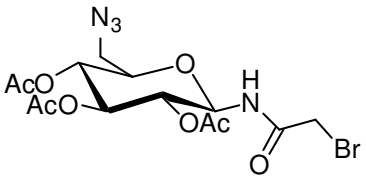
^{13}C NMR (CD_3OD) GH2a-ABP



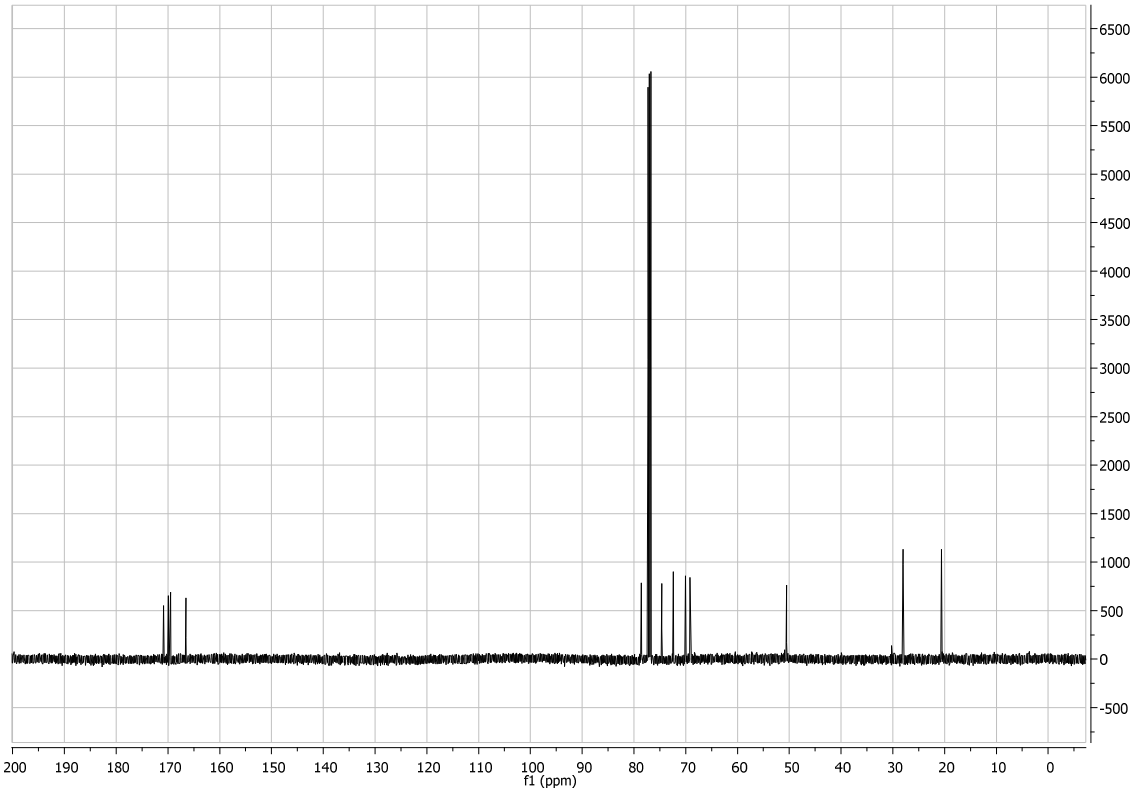
GH2b-ABP



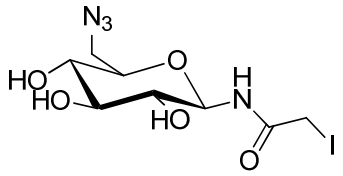
^{1}H NMR ($CDCl_3$) GH2b-ABP



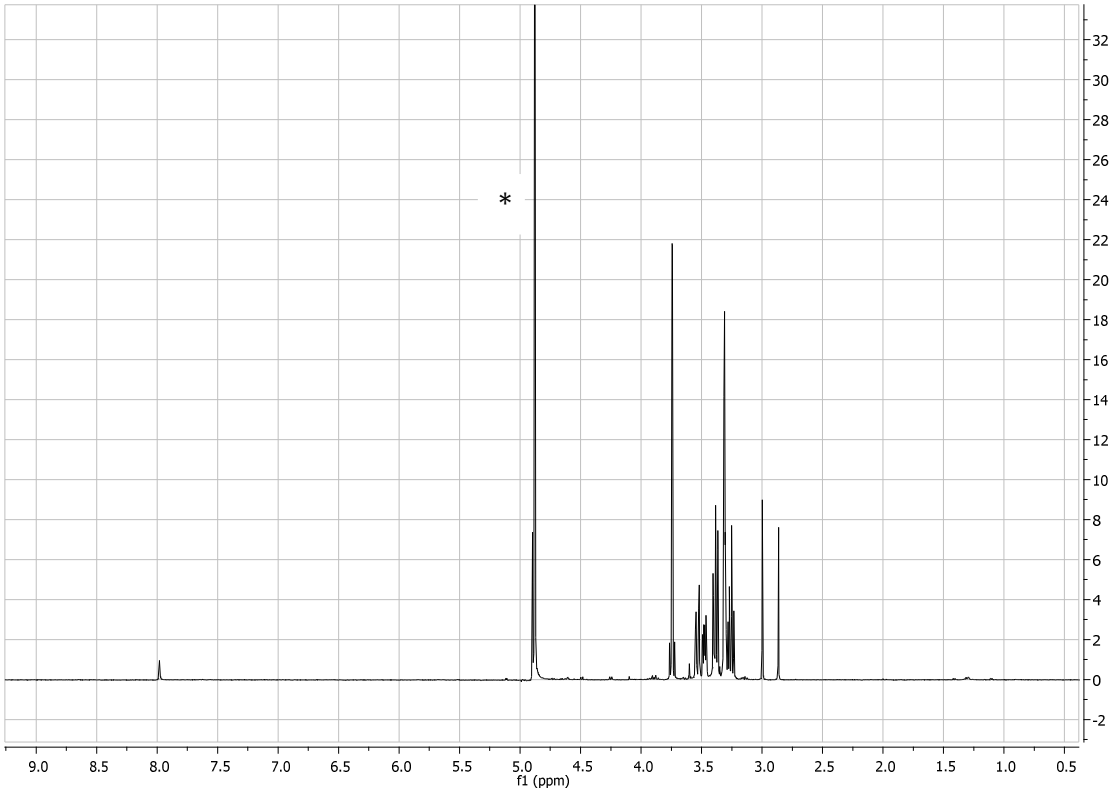
GH2b-ABP



^{13}C NMR ($CDCl_3$) GH2b-ABP

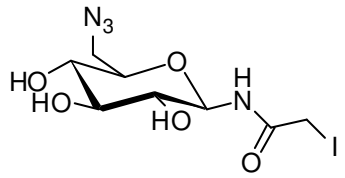


GH2c-ABP

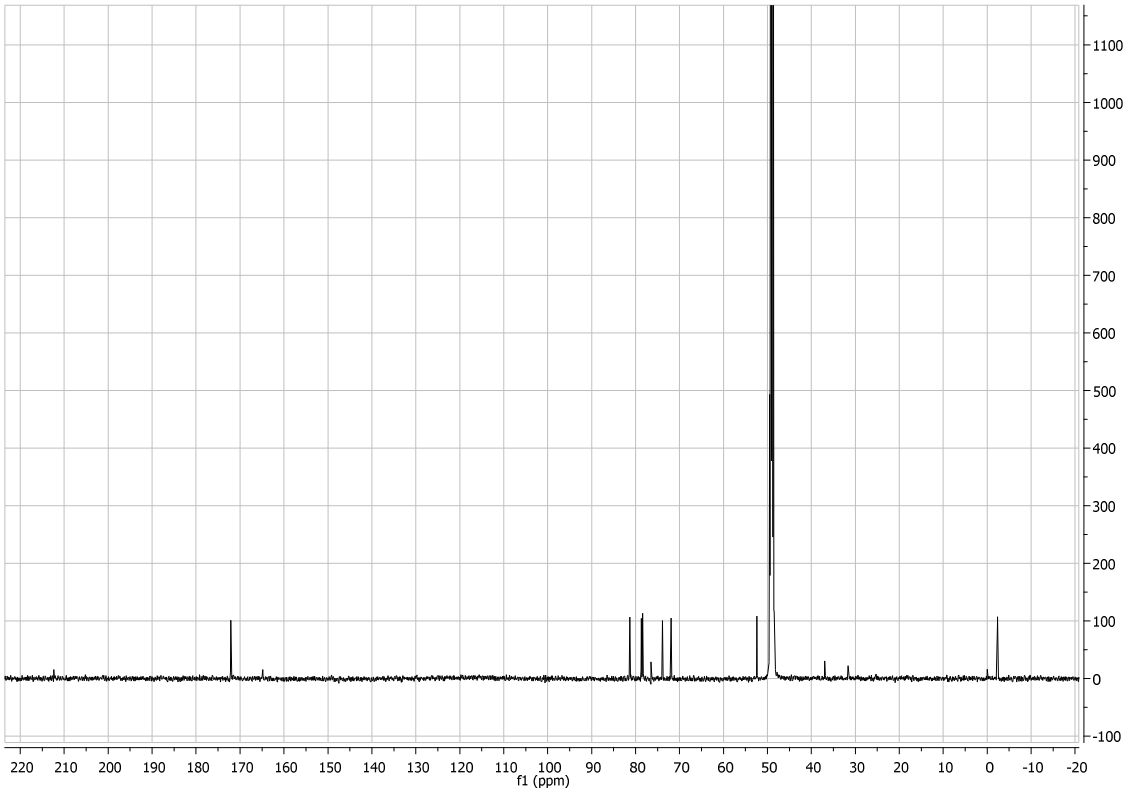


^{1}H NMR (CD_3OD) GH2c-ABP

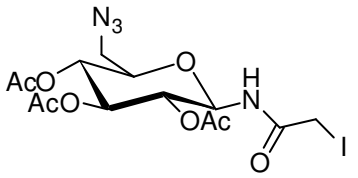
*H_2O in CD_3OD



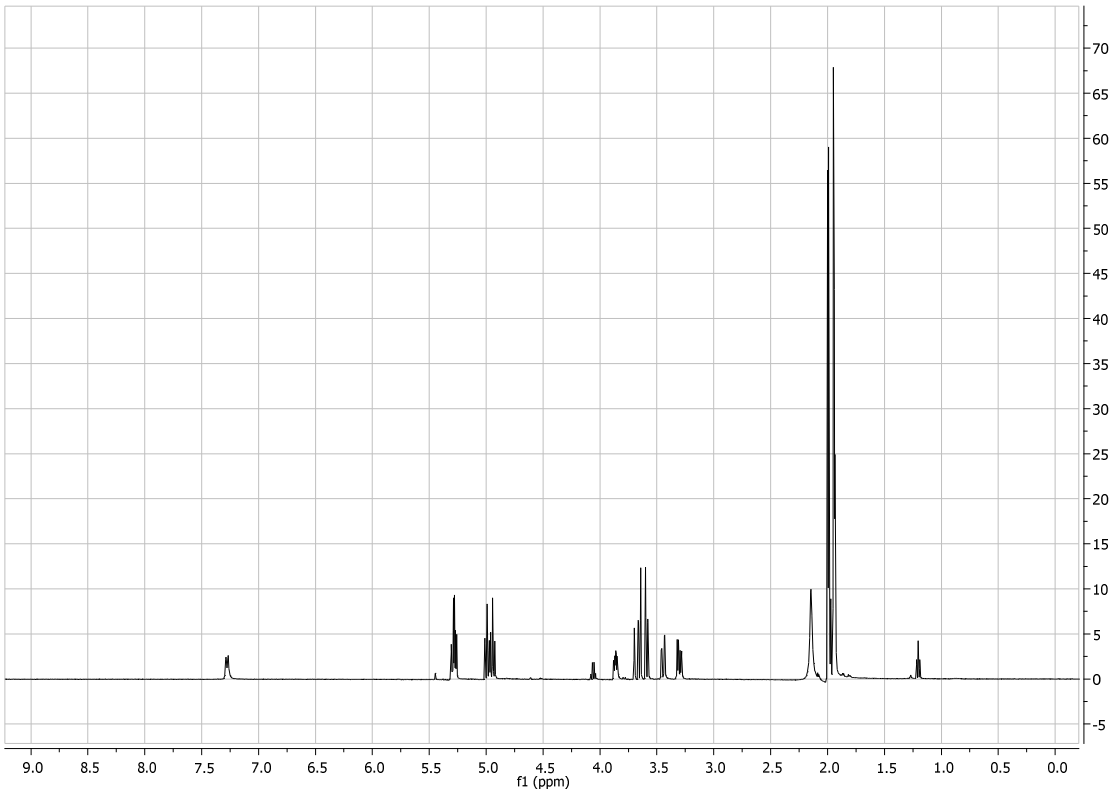
GH2c-ABP



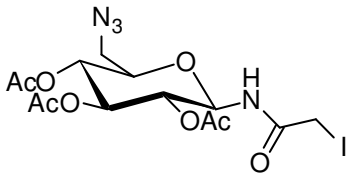
^{13}C NMR (CD_3OD) GH2c-ABP



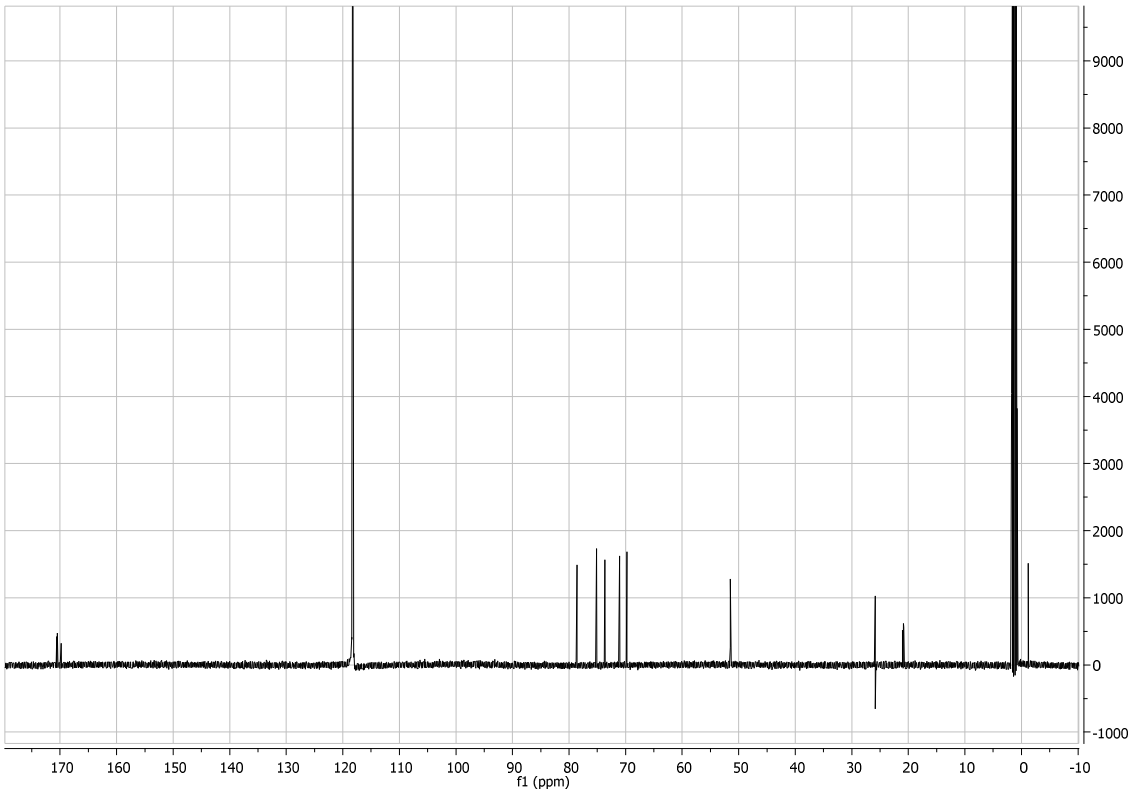
GH2d-ABP



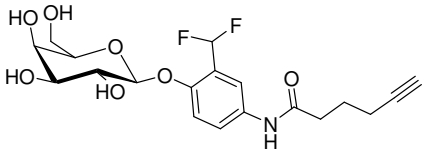
^{1}H NMR (CD_3CN) GH2d-ABP



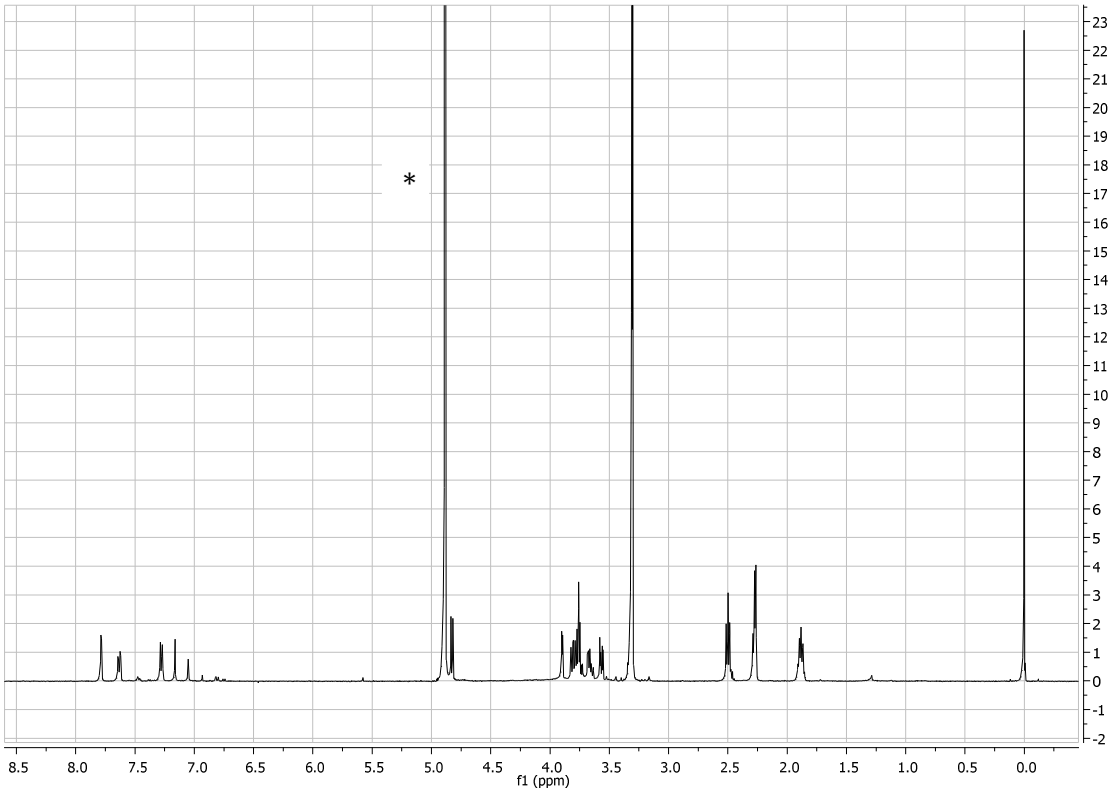
GH2d-ABP



^{13}C NMR (CD_3CN) GH2d-ABP

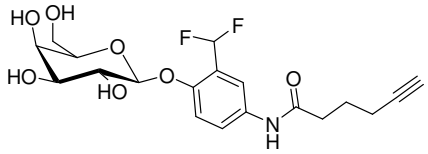


GH3a-ABP

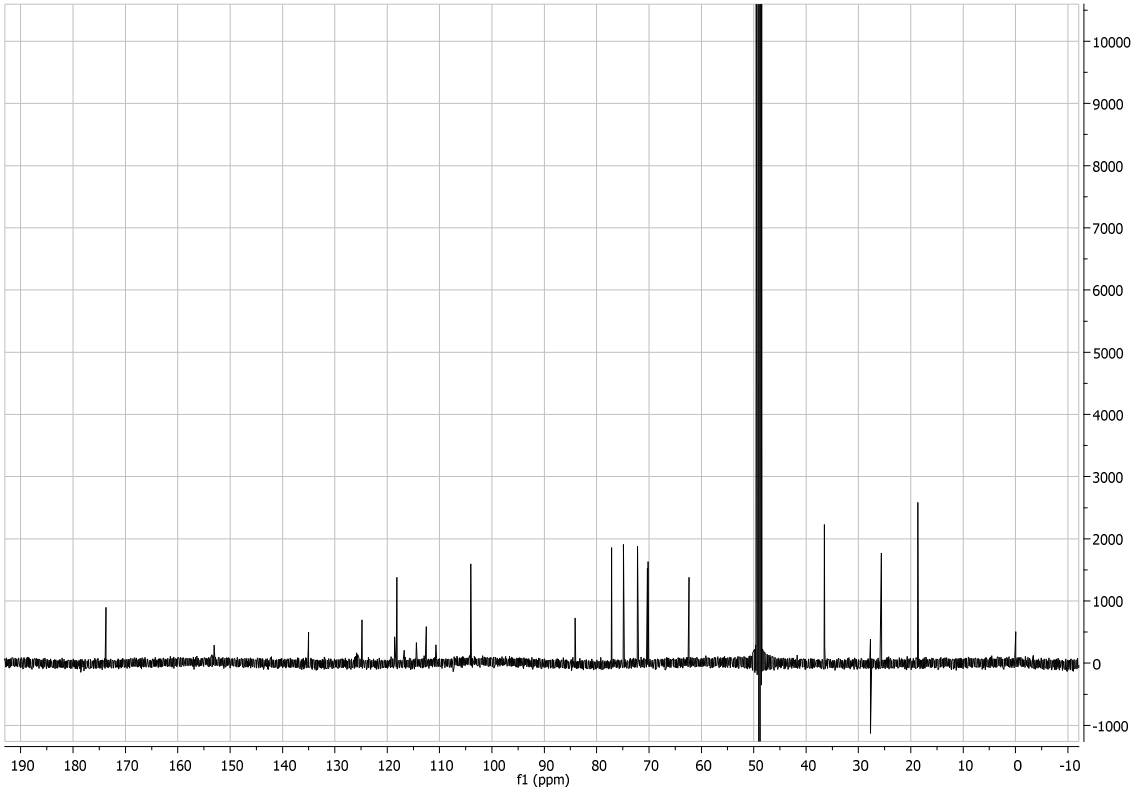


^{1}H NMR (CD_3OD) GH3a-ABP

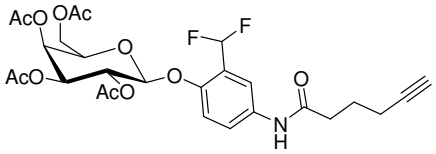
*H_2O in CD_3OD



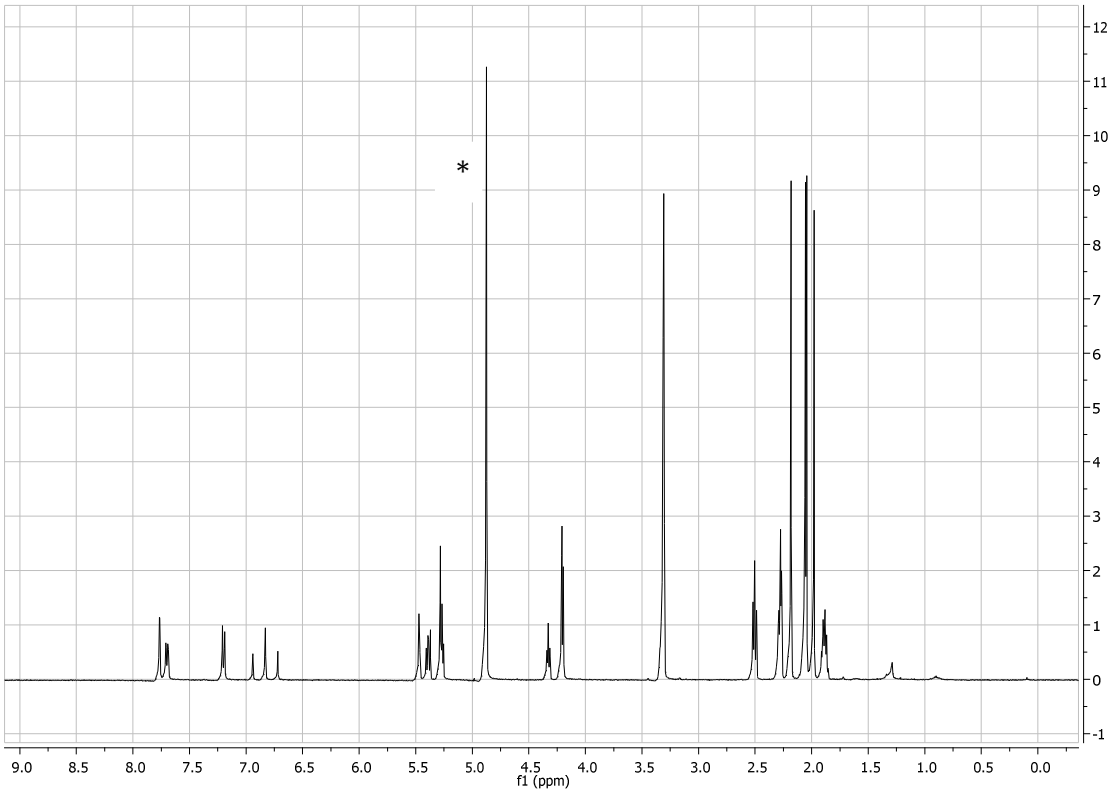
GH3a-ABP



^{13}C NMR (CD_3OD) GH3a-ABP

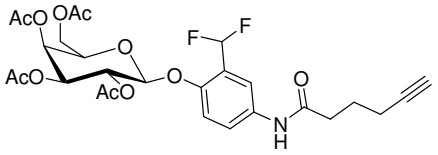


GH3b-ABP

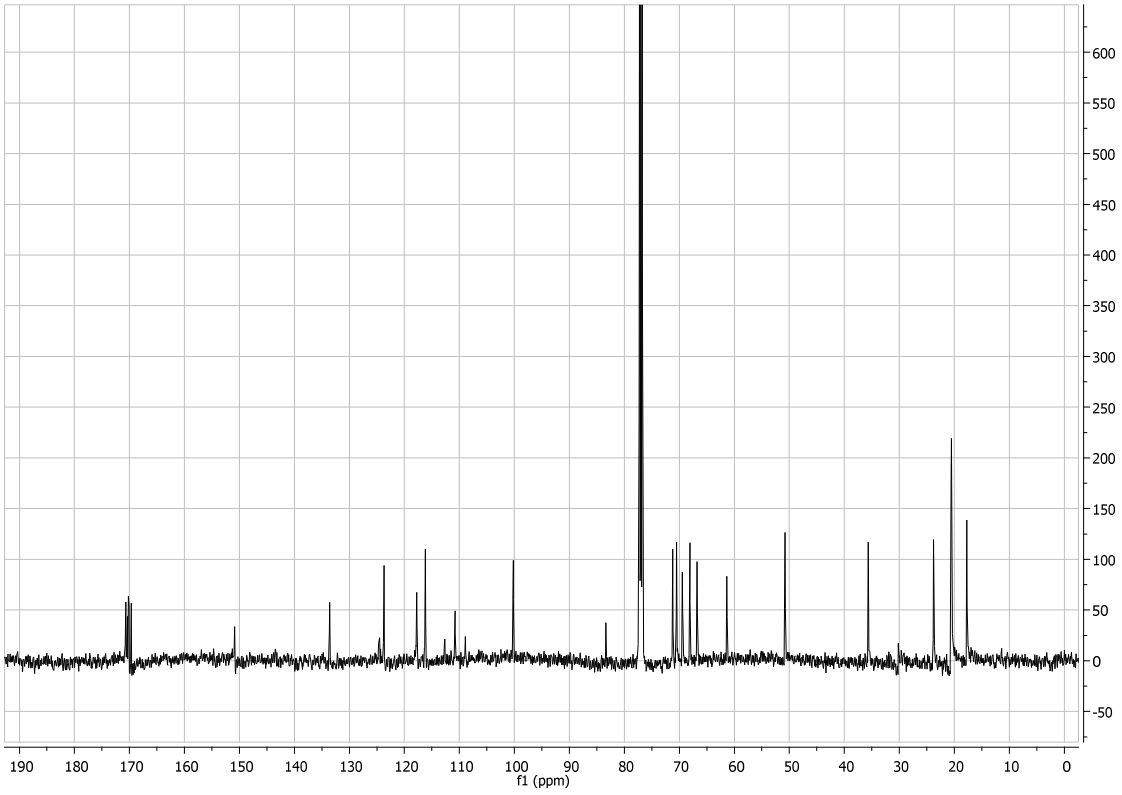


^{1}H NMR (CD_3OD) GH3b-ABP

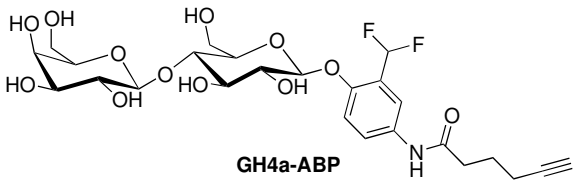
*H_2O in CD_3OD



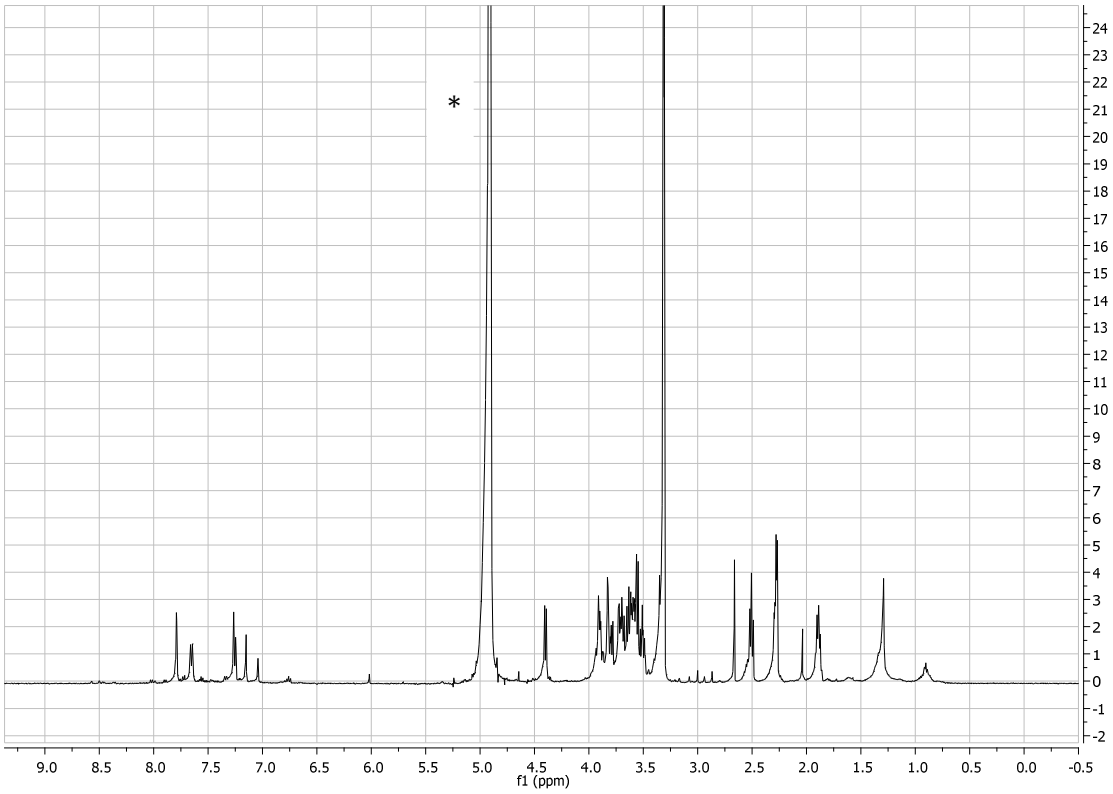
GH3b-ABP



^{13}C NMR ($CDCl_3$) GH3b-ABP

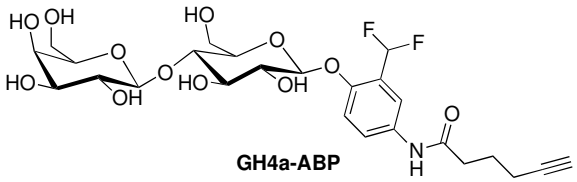


GH4a-ABP

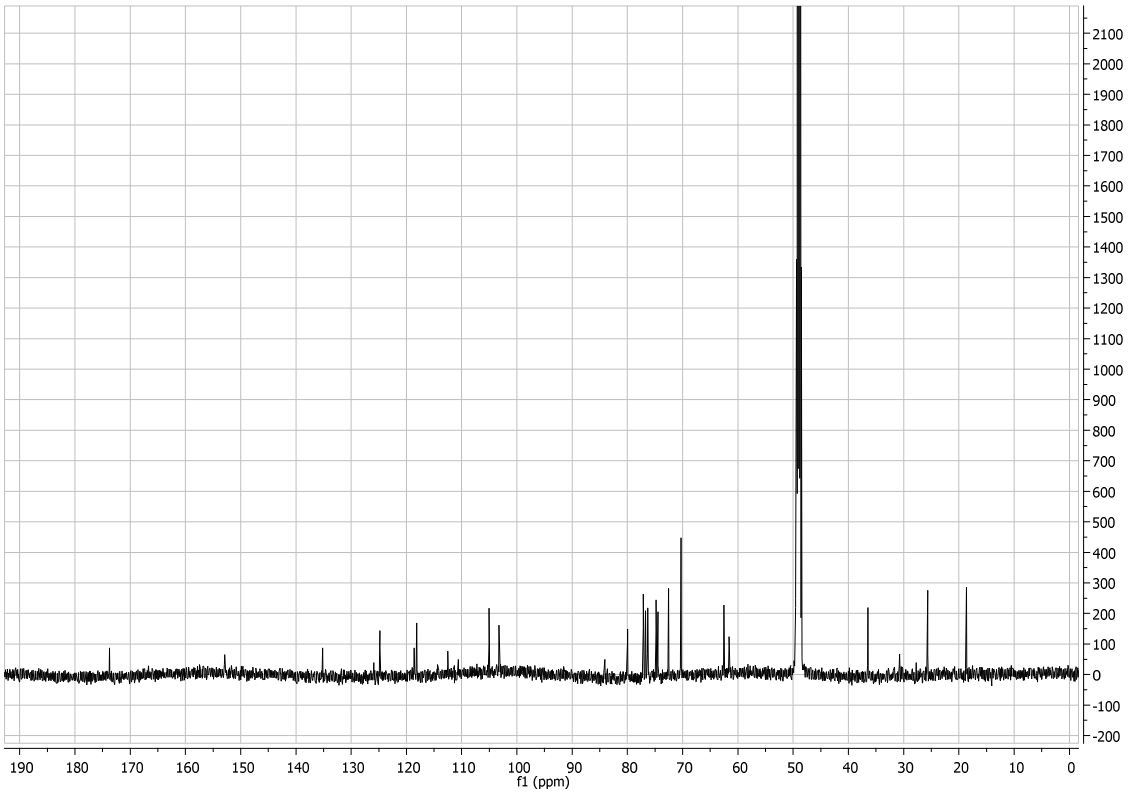


^{1}H NMR (CD_3OD) GH4a-ABP

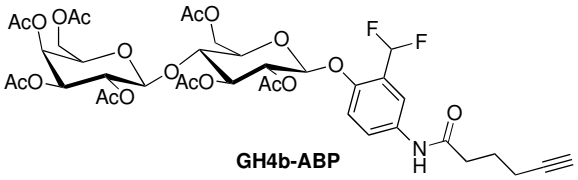
*H_2O in CD_3OD



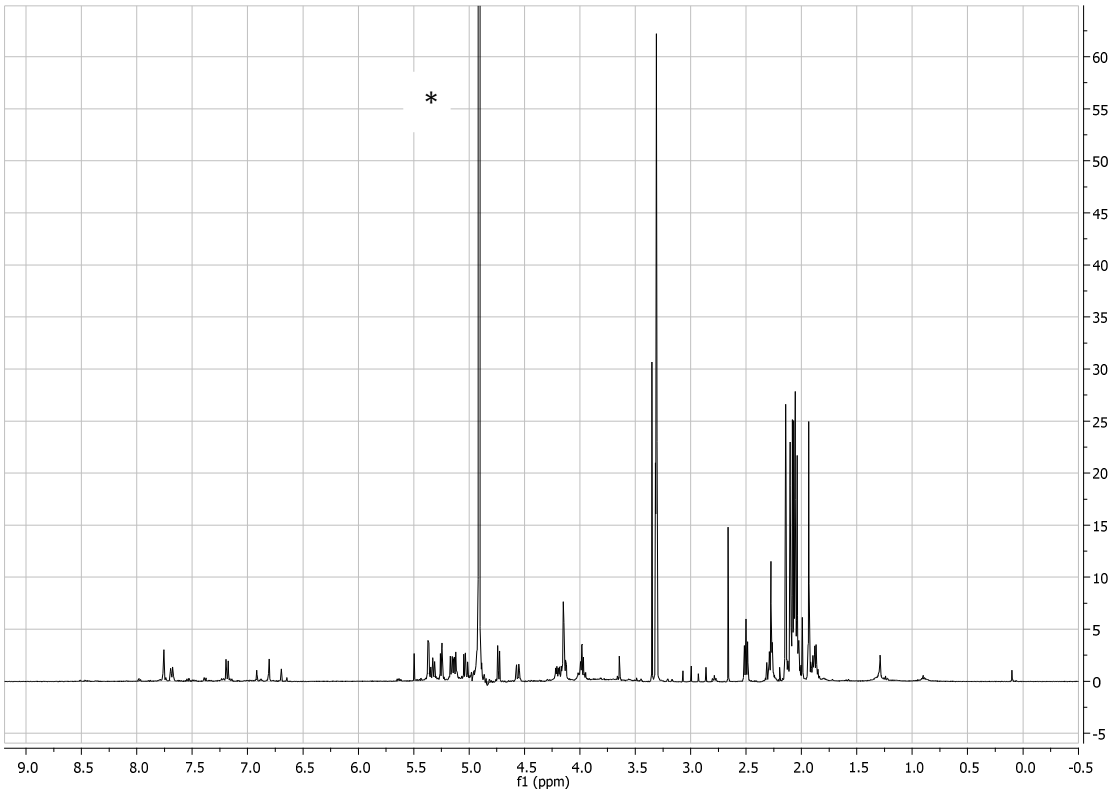
GH4a-ABP



^{13}C NMR (CD_3OD) GH4a-ABP

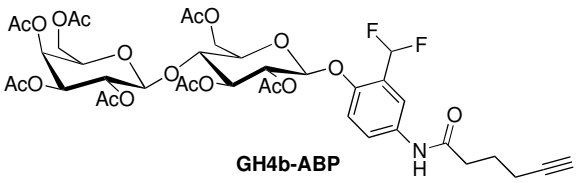


GH4b-ABP

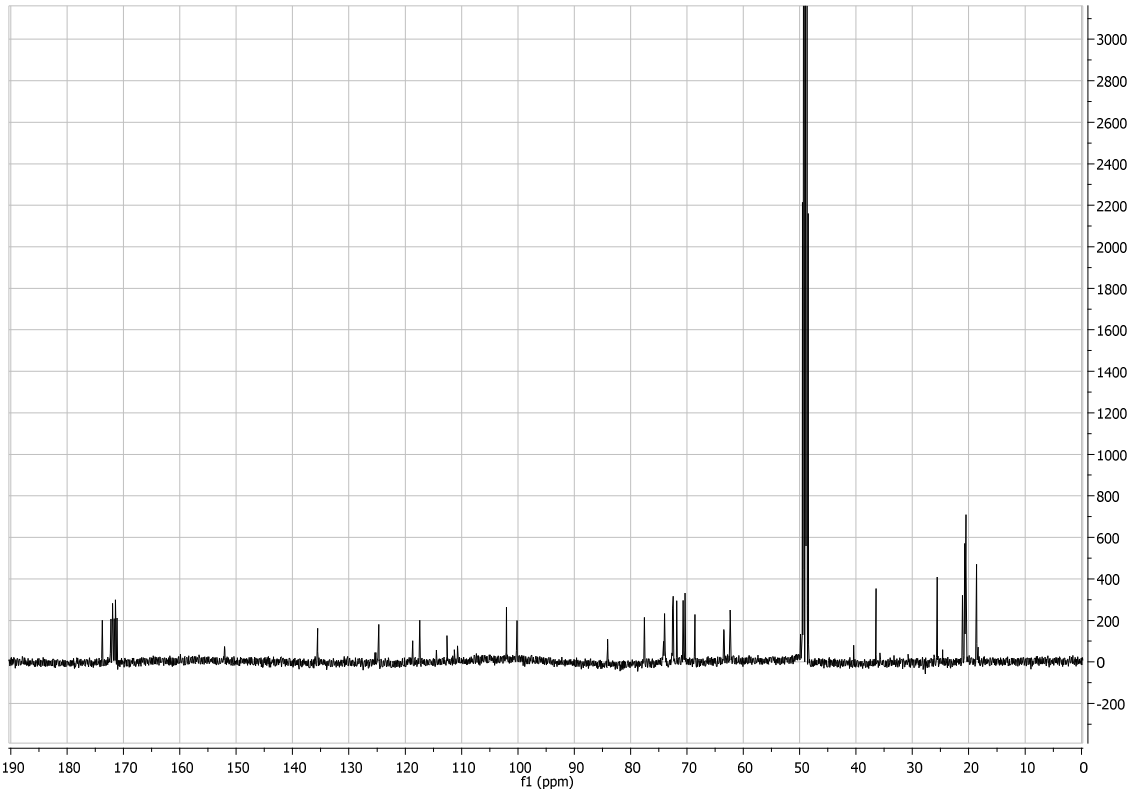


^{1}H NMR (CD_3OD) GH4b-ABP

*H_2O in CD_3OD



GH4b-ABP



^{13}C NMR (CD_3OD) GH4b-ABP