nature

https://doi.org/10.1038/s41586-020-2545-9

Supplementary information

Lysosome-targeting chimaeras for degradation of extracellular proteins

In the format provided by the authors and unedited

Steven M. Banik, Kayvon Pedram, Simon Wisnovsky, Green Ahn, Nicholas M. Riley & Carolyn R. Bertozzi ✉

Supplementary Information for

Lysosome targeting chimeras for degradation of extracellular proteins

Steven M. Banik, Kayvon Pedram, Simon Wisnovsky, Green Ahn, Nicholas M. Riley, Carolyn R. Bertozzi*

*Correspondence should be addressed to C.R.B. (bertozzi@stanford.edu).

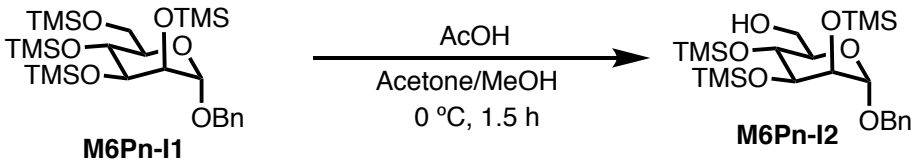
This PDF file includes:

Other Supplementary Information for this manuscript include the following:

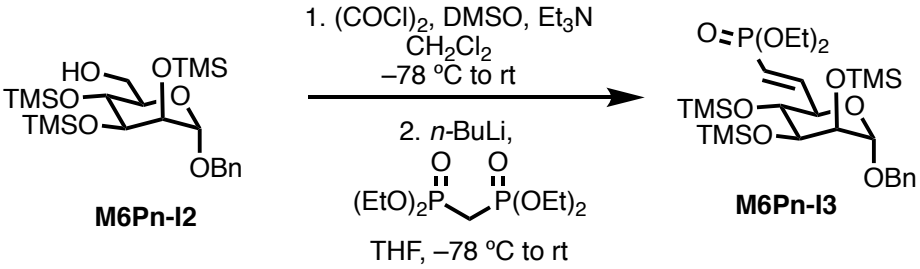
Chemical Analysis Instrumentation

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on an Inova-600 spectrometer or a Varian-400 spectrometer at 25 °C, are reported in parts per million downfield from tetramethylsilane, and are referenced to the residual protium resonances of the NMR solvent ($CDCl_3$: 7.26 [$CHCl_3$], CD_3OD: 3.31 [MeOH], D_2O: 4.79 [H_2O]). Proton-decoupled carbon-13 nuclear magnetic resonance (^{13}C {^{1}H} NMR) spectra were recorded on an Varian-400 spectrometer at 25 °C, are reported in parts per million downfield from tetramethylsilane, and are referenced to the carbon resonances of the NMR solvent ($CDCl_3$: 77.16, CD_3OD: 49.00). Chemical shifts for phosphorus-31 nuclear magnetic resonance (^{31}P NMR) were recorded on a Varian Mercury-400 spectrometer at 25 °C, are reported in parts per million downfield from H_3PO_4 (δ = 0). Data are represented as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, sept = septet, m = multiplet), coupling constants in Hertz (Hz), integration. High-resolution mass spectrometry data were obtained on a Thermo Exactive benchtop Orbitrap.

Synthesis of Mannose-6-Phosphonate *N*-Carboxyanhydride Monomers:



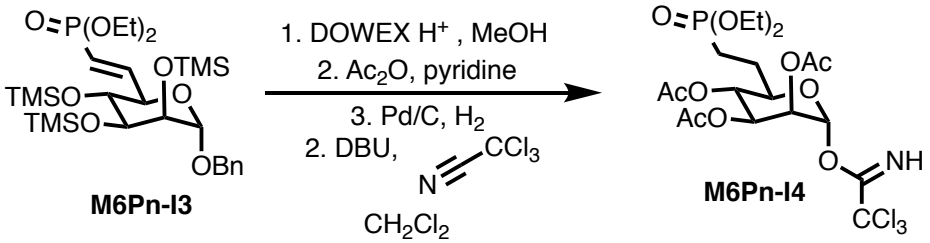
M6Pn-I2. To **M6Pn-I1**[47] (5.65 g, 10.1 mmol, 1.0 equiv.) was added acetone (22 mL) and methanol (30 mL). The mixture was cooled to 0 °C, and acetic acid (1.11 mL) was added. The reaction was stirred for 1.5 hours at 0 °C, after which solid $NaHCO_3$ (1.79 g, 21.3 mmol, 2.10 equiv.) was added. The reaction mixture was concentrated under reduced pressure and the resulting residue was purified by flash column chromatography on silica gel (0 to 15% ethyl acetate in hexanes) to give a colorless oil (2.80 g, 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.28 (m, 5H), 4.73–4.70 (d, *J* = 12.2 Hz, 1H), 4.69 (s, 1H), 4.51–4.48 (d, *J* = 12.2 Hz, 1H), 3.92–3.59 (m, 6H), 2.01 (s, 1H), 0.17 (s, 9H), 0.16 (s, 9H), 0.12 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.6, 128.4, 127.7, 127.6, 100.3, 74.1, 73.7, 72.5, 69.0, 68.1, 62.2, 0.66, 0.58, 0.35. ESI-HRMS Calc'd [M+NH_4^+]=504.2633; found 504.2627.



M6Pn-I3. To oxalyl chloride (0.649 mL, 5.87 mmol, 1.1 equiv.) was added dichloromethane (10 mL), and the mixture was cooled to -78 °C. To the reaction mixture was added DMSO (0.834 mL, 11.74 mmol, 2.2 equiv.), and the reaction was stirred for 10 minutes at -78 °C. To the reaction

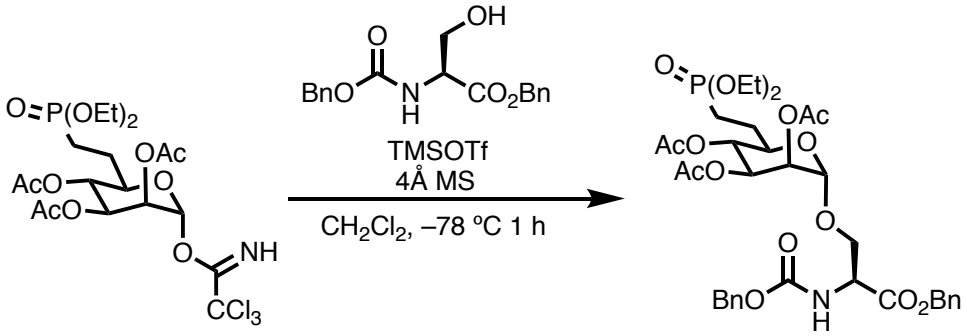
[47] Cowden, W. B. Phosphosugars and phosphosugar-containing compounds having anti-inflammatory activity. WO1990001938A1 (2001).

mixture was added a solution of **M6Pn-I2** (2.60 g, 5.34 mmol, 1.0 equiv.) in dichloromethane (10 mL) dropwise, and the reaction was stirred at -78 °C. After 45 minutes, triethylamine (3.72 mL, 26.7 mmol, 5.0 equiv) was added, and the mixture was allowed to warm to room temperature and stirred for 1 hour. The reaction mixture was diluted with dichloromethane (150 mL) and washed with water (3 X 50 mL). The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure to give an orange residue. To tetraethyl methylenediphosphonate (2.31 g, 8.01 mmol, 1.5 equiv.) was added tetrahydrofuran (30 mL) and the reaction mixture was cooled to –78 °C. To the reaction mixture was added *n*-BuLi (2.5 M in hexanes, 2.67 mL, 6.67 mmol, 1.25 equiv.). After 45 minutes, the orange residue obtained above was added slowly as a solution in tetrahydrofuran (10 mL), and the reaction mixture was stirred at -78 °C for two hours, then allowed to warm to room temperature overnight. The reaction mixture was diluted with ethyl acetate (250 mL) and washed with water (2 X 50 mL). The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel (0 to 40% ethyl acetate in hexanes) to give a colorless oil (1.93 g, 53% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.21 (m, 5H), 6.85 (ddd, *J* = 22.8, 17.2, 4.2 Hz, 1H), 6.05 (ddd, *J* = 22.0, 17.2, 1.8 Hz, 1H), 4.70 (m, 1H), 4.67–4.45 (d, *J* = 12.2 Hz, 1H), 4.48–4.45 (d, *J* = 12.2 Hz, 1H), 4.14 – 4.05 (m, 6H), 3.83 – 3.80 (m, 2H), 3.69 (app. t, *J* = 9.6 Hz, 2H), 1.33 (td, *J* = 7.1, 1.3 Hz, 4H), 0.16 (s, 9H), 0.13 (s, 9H), 0.10 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.9 (d, J = 5.9 Hz), 137.5, 128.4, 127.7, 127.6, 117.0 (d, *J* = 189.2 Hz), 100.2, 73.5, 73.3, 72.8, 71.5, 69.1, 61.6 (t, *J* = 6.1 Hz), 16.4 (d, *J* = 6.2 Hz), 0.74, 0.56, 0.32. ^{31}P NMR (162 MHz, $CDCl_3$) δ 18.5. ESI-HRMS Calc'd [M+H$^+$, -3 X TMS]=403.1522; found 403.1514.



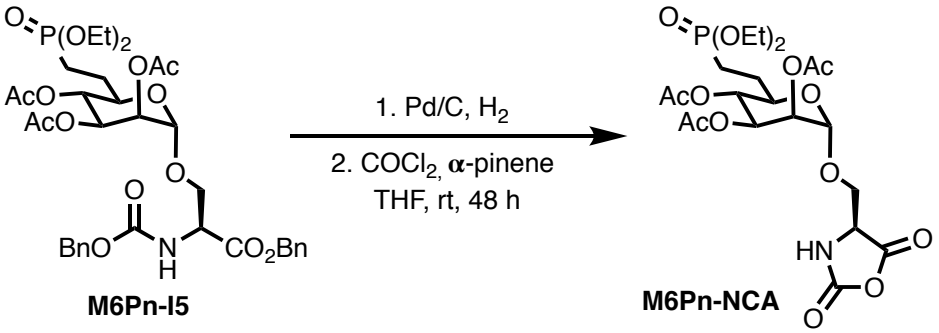
M6Pn-I4. To **M6Pn-I3** (1.93 g, 3.12 mmol, 1.0 equiv) was added methanol (40 mL) and DOWEX 50 WX8-200 (1.0 g). The mixture was stirred for 1 hour at room temperature. The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was dissolved in pyridine (20 mL), and acetic anhydride (10 mL) was added. The reaction was allowed to stir overnight, then concentrated under reduced pressure. The residue was dissolved in dichloromethane (100 mL) and washed with 1M HCl (50 mL) and water (50 mL). The organic layer was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. To the resulting residue was added 10% Pd/C (666 mg, 0.624 mmol, 0.2 equiv.), and methanol (40 mL), and the reaction mixture was degassed and placed under an atmosphere of hydrogen. After 24 hours, additional Pd/C was added (2.00 g), and the reaction was stirred under an atmosphere of hydrogen for an additional 24 hours. Upon completion, the reaction mixture was filtered through celite and concentrated. To the resulting residue was added dichloromethane (50 mL), trichloroacetonitrile (3.12 mL, 31.2 mmol, 10.0 equiv.), and DBU (0.046 mL, 0.0312 mmol, 0.1 equiv.). The reaction mixture was allowed to stir for 1 hour at room temperature, then concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel (30 to 100% ethyl acetate in hexanes) to give a pale yellow foam (1.35 g, 74% over 4 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H), 6.09 (s, 1H), 5.33–5.31 (m, 1H), 5.22 (dd, *J* = 10.0, 3.4 Hz, 1H), 5.07 (app. t, *J* = 10.0 Hz, 1H), 4.00–3.88 (m, 4H), 3.82 (app. t, *J* = 8.1 Hz, 1H), 2.06 (s, 3H), 1.95 (s, 3H), 1.87 (s, 3H), 1.82–1.43 (m, 4H), 1.18 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.6, 169.5, 169.5, 159.5, 94.2, 90.4, 72.2 (d, *J* = 18.0 Hz), 68.7, 67.9 (d, *J* =

16.8 Hz), 61.5 (d, *J* = 6.9 Hz), 61.4 (d, *J* = 7.0 Hz), 24.1 (d, *J* = 4.0 Hz), 21.6, 20.6, 20.6, 20.5, 20.1, 16.4 (d, *J* = 5.9 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 31.2. ESI-HRMS Calc'd [M+H$^+$]=584.0622; found 584.0607.



M6Pn-I4 **M6Pn-I5**

M6Pn-I5. To **M6Pn-I4** (1 g, 1.71 mmol, 1.25 equiv.) was added Z-Ser-OBn (451 mg, 1.37 mmol, 1.0 equiv.), 3 g freshly activated 4Å MS, and dichloromethane (50 mL). The reaction mixture was cooled to -78 °C and TMSOTf (0.060 mL, 0.342 mmol, 0.2 equiv.) was added and the reaction mixture was stirred at -78 °C for 1 h. Triethylamine (1 mL) was added to reaction mixture, and the resulting mixture was filtered over celite and concentrated. The resulting residue was purified by flash column chromatography on silica gel (30 to 100% ethyl acetate in hexanes) to give a white foam (1.02 g, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.28 (m, 10H), 5.84 (d, *J* = 8.0 Hz, 1H), 5.31 – 4.95 (m, 8H), 4.63 (s, 1H), 4.55 (d, *J* = 7.7 Hz, 2H), 4.09–3.95 (m, 4H), 3.92–3.90 (m, 2H), 3.72–3.68 (m, 1H), 2.10 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H), 1.84–1.66 (m, 4H), 1.27 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.7, 169.6, 169.3, 155.8, 136.0, 135.0, 128.5, 128.4, 128.4, 128.3, 128.1, 128.0, 97.7, 69.8 (d, *J* = 17.2 Hz), 68.5 (d, *J* = 40.4 Hz), 67.5, 67.1, 61.5 (d, *J* = 4.6 Hz), 61.5 (d, *J* = 5.4 Hz), 54.2, 24.1 (d, *J* = 4.1 Hz), 20.8 (d, *J* = 143.3 Hz), 20.6, 20.6, 20.5, 20.1, 16.3 (d, *J* = 6.0 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 31.3. ESI-HRMS Calc'd [M+H$^+$]=752.2683; found 752.2670.

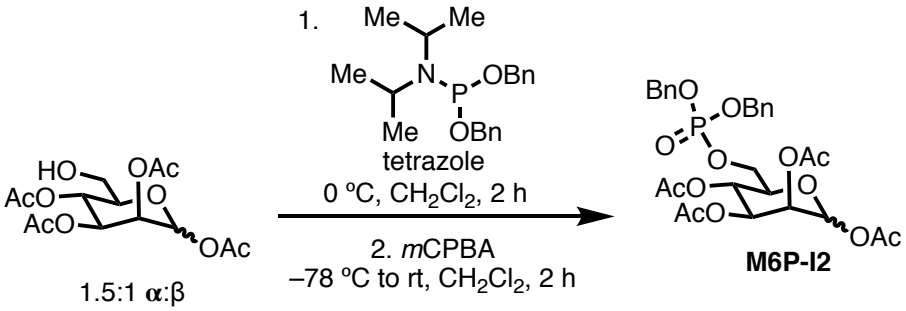


M6Pn-I5 **M6Pn-NCA**

M6Pn-NCA. To **M6Pn-I5** (500 mg, 0.665 mmol, 1.0 equiv.) was added 10% Pd/C (71 mg, 0.067 mmol, 0.1 equiv.), and methanol (15 mL), and the reaction mixture was degassed and placed under an atmosphere of hydrogen. After 12 hours, the reaction mixture was filtered over celite and concentrated to give the corresponding amino acid. The resulting residue was dissolved in tetrahydrofuran (15 mL), and α-pinene (422 µL, 2.66 mmol, 4.00 equiv), and phosgene (950 µL, 1.33 mmol, 2.00 equiv.) were added. The reaction mixture was allowed to stir for 48 hours, then concentrated under reduced pressure into a cold trap in a fumehood. The resulting tan residue was purified by flash column chromatography on anhydrous silica gel (0 to 30% anhydrous

tetrahydrofuran in anhydrous dichloromethane) to give a white foam (420 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 – 8.73 (m, 1H), 5.24 (dd, *J* = 3.4, 1.8 Hz, 1H), 5.11 (dd, *J* = 10.1, 3.4 Hz, 1H), 5.02 (app. t, *J* = 9.9 Hz, 1H), 4.79 (d, *J* = 1.8 Hz, 1H), 4.36 (app. t, *J* = 2.3 Hz, 1H), 4.25–3.96 (m, 6H), 3.85 (dd, *J* = 9.6, 2.2 Hz, 1H), 2.11 (s, 3H), 2.05 (s, 3H), 1.94 (s, 3H), 1.92–1.60 (m, 4H), 1.35 (t, *J* = 7.0 Hz, 3H), 1.31 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2, 169.9, 169.3, 168.9, 152.2, 96.8, 69.9, 69.3, 68.7, 68.5 (d, *J* = 2.6 Hz), 64.2, 62.5 (d, *J* = 7.0 Hz), 62.1 (d, *J* = 6.7 Hz), 58.1, 25.3 (d, *J* = 5.7 Hz), 20.9, 20.9, 20.7, 20.3 (d, *J* = 143.1 Hz), 16.6 (d, *J* = 5.9 Hz), 16.5 (d, *J* = 6.1 Hz) ^{31}P NMR (162 MHz, $CDCl_3$) δ 29.4. ESI-HRMS Calc'd [M+H$^+$]=554.1639; found 554.1627.

Synthesis of Mannose-6-Phosphate *N*-Carboxyanhydride Monomers:

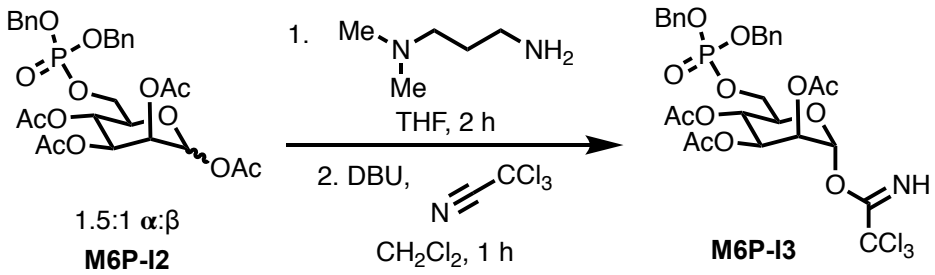


1.5:1 α:β
M6P-I1

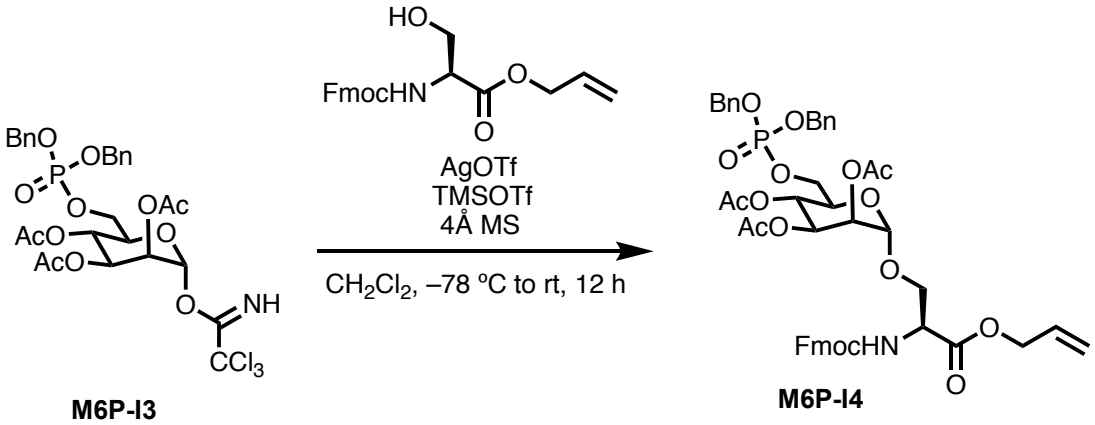
M6P-I2

M6P-I2. To **M6P-I1**[48] (5.00 g, 14.4 mmol, 1.00 equiv.) was added 1*H*-tetrazole (2.03 g, 28.8 mmol, 2.00 equiv.) and dichloromethane (288 mL). The mixture was cooled to 0 °C, and dibenzyl *N,N*-diisopropylphosphoramidite (7.26 mL, 21.6 mmol, 1.5 equiv.) was added dropwise over 5 minutes. After 2 hours, the reaction mixture was cooled to -78 °C, and *m*CPBA (12.91 g of 77% *m*CPBA, 57.6 mmol, 4 equiv.) was added as a solid. The reaction was allowed to warm to 0 °C. After two hours, saturated $Na_2S_2O_3$ (100 mL) was added, and the organic layer was removed, washed 2X with saturated $NaHCO_3$ (150 mL), dried with $MgSO_4$, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel (10 to 90% ethyl acetate in hexanes) to give a colorless, viscous oil (8.01 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$, two diastereomers) 7.31–7.23 (m, 10H), 6.25 (d, *J* = 3.7 Hz, 1H), 5.66 (d, *J* = 8.3 Hz, 1H), 5.39 (t, *J* = 9.9 Hz, 1H), 5.18 (t, *J* = 9.4 Hz, 1H), 5.09 – 4.90 (m, 8H), 4.16 – 3.93 (m, 3H), 3.73 (dd, *J* = 9.9, 1.6 Hz, 1H), 2.06 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.95 (s, 6H), 1.93 (s, 3H), 1.92 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.1, 170.0, 169.5, 169.2, 169.1, 168.7, 168.6, 135.7, 135.6, 128.5, 128.5, 127.9, 127.9, 127.8, 91.5, 88.8, 73.0 (d, *J* = 8.1 Hz), 72.7, 70.2 (d, *J* = 8.1 Hz), 70.1, 69.7, 69.4 (d, *J* = 1.6 Hz), 69.4, 69.0, 67.7, 67.6, 65.0 (d, *J* = 5.2 Hz), 64.9 (d, *J* = 5.8 Hz), 20.7, 20.6, 20.5, 20.4, 20.3. ^{31}P NMR (162 MHz, $CDCl_3$) δ -1.3, -1.5. ESI-HRMS Calc'd [M+H$^+$]=609.1737; found 609.1728.

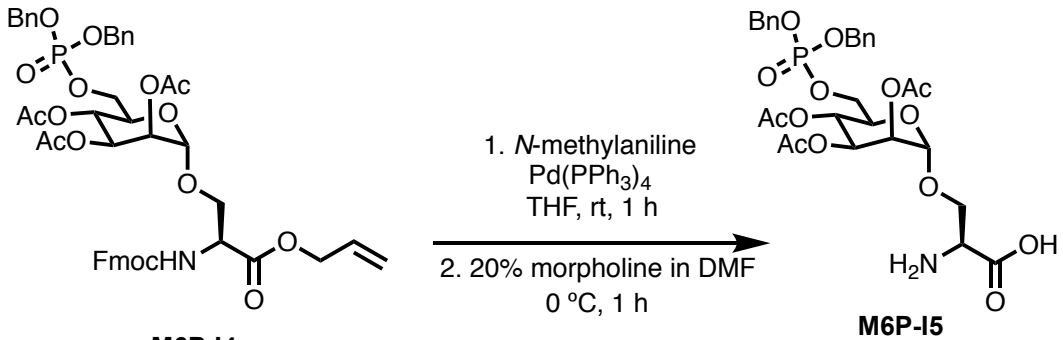
[48] Lin, K. & Kasko, A. M. Effect of Branching Density on Avidity of Hyperbranched Glycomimetics for Mannose Binding Lectin. *Biomacromolecules* **14**, 350–357 (2013).



M6P-I3. To **M6P-I2** (8.01 g, 13.2 mmol, 1.00 equiv.) was added tetrahydrofuran (66 mL), followed by 3-(dimethylamino)-1-propylamine (4.15 mL, 33.0 mmol, 2.5 equiv.). The reaction was allowed to stir 1.5 hours until complete consumption of the starting material was observed. The reaction mixture was diluted with dichloromethane (250 mL) and washed with 1M HCl (100 mL) and water (100 mL). The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. To the resulting residue was added dichloromethane (250 mL), trichloroacetonitrile (6.62 mL, 66.0 mmol, 5.0 equiv.), and DBU (0.197 mL, 1.32 mmol, 0.1 equiv.). The reaction mixture was allowed to stir for 1 hour at room temperature, then concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel (10 to 85% ethyl acetate) to give a pale yellow, viscous oil (6.10g, 65% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H), 6.20 (s, 1H), 5.38 (s, 1H), 5.36 – 5.27 (m, 2H), 4.97 (app. t, *J* = 6.8 Hz, 4H), 4.25 – 3.89 (m, 3H), 2.02 (s, 3H), 1.96 (s, 3H), 1.94 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.7, 169.6, 169.4, 159.4, 135.7 (d, *J* = 4.1 Hz), 135.6 (d, *J* = 4.1 Hz), 128.5, 128.4, 127.9, 127.8, 94.3, 71.7 (d, *J* = 7.8 Hz), 69.4 (d, *J* = 2.2 Hz), 69.3 (d, *J* = 2.1 Hz), 68.7, 67.7, 65.4 (d, *J* = 5.2 Hz), 65.1, 20.6, 20.6, 20.6. ^{31}P NMR (162 MHz, $CDCl_3$) δ -1.35. ESI-HRMS Calc'd [M+H$^+$]=710.0728; found 710.0714.

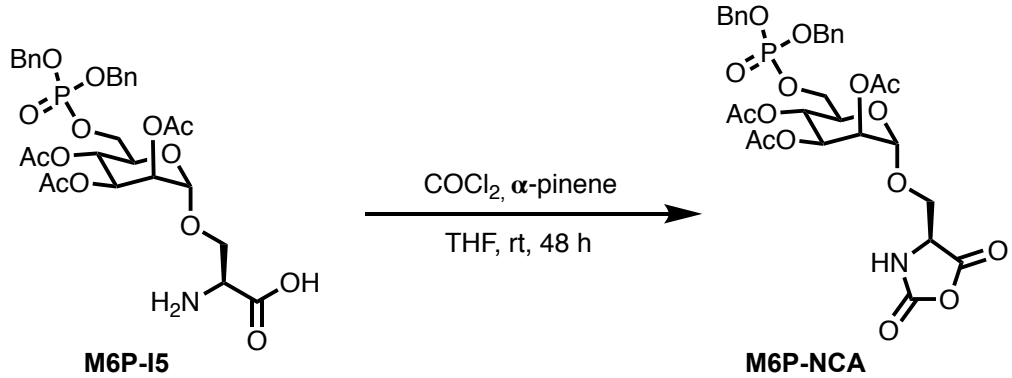


M6P-I4. To **M6P-I3** (4.00 g, 5.63 mmol, 1.0 equiv.) was added Fmoc-Ser allyl ester (2.48 g, 6.76 mmol, 1.2 equiv.), AgOTf (2.89 g, 11.26 mmol, 2.0 equiv.), freshly activated 4Å MS (12.0 g), and dichloromethane (320 mL). The reaction mixture was cooled to -78 °C and TMSOTf (0.255 mL, 1.41 mmol, 0.25 equiv.) was added. The reaction was allowed to warm to -40 °C, stirred at that temperature for 1 h, then allowed to warm to room temperature over 12 hours. Triethylamine (1.0 mL) was added to reaction mixture, and the resulting mixture was filtered over celite and concentrated. The resulting residue was purified by flash column chromatography on silica gel (10 to 100% ethyl acetate in hexanes) to give a white foam (2.33g, 45%). ESI-HRMS Calc'd [M+H$^+$]=916.2945; found 916.2925.



M6P-I4 **M6P-I5**

M6P-I5. To **M6P-I4** (2.33 g, 2.54 mmol, 1.0 equiv.) was added tetrahydrofuran (109 mL), $Pd(PPh_3)_4$ (210 mg, 0.181 mmol, 0.071 equiv.), and *N*-methylaniline (2.75 mL, 25.4 mmol, 10.0 equiv.). The reaction mixture was stirred for 1 hour at room temperature, then concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel (20 to 100% ethyl acetate in hexanes) to give a light brown foam, which was dissolved in *N*,*N*-dimethylformamide (48.0 mL) and cooled to 0 °C. Morpholine (12.0 mL) was added, and the reaction was stirred at 0 °C for 1 hour. The reaction mixture was concentrated under reduced pressure and purified by flash column chromatography on silica gel (100% ethyl acetate to 33% MeOH in ethyl acetate to 33% MeOH in ethyl acetate with 17% H_2O) to give a tan foam (743 mg, 45% over two steps). ^{1}H NMR (400 MHz, CD_3OD) δ 7.55 – 7.11 (m, 10H), 5.39–5.34 (m, 1H), 5.31 (s, 1H), 5.30–5.28 (m, 1H), 5.10–5.00 (m, 4H), 4.20–4.14 (m, 2H), 4.11–4.04 (m, 2H), 3.81–3.71 (m, 2H), 1.96 (s, 3H), 1.95 (s, 3H), 1.92 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 171.8, 171.4, 171.2, 137.2 (m), 129.8 (d, *J* = 1.2 Hz), 129.7, 129.2, 129.2, 99.5, 71.0, 70.9, 70.9, 70.8, 70.5 (d, *J* = 7.8 Hz), 70.2, 68.2, 66.9 (d, *J* = 5.3 Hz), 66.5, 55.0, 20.7, 20.6, 20.6. ^{31}P NMR (162 MHz, CD_3OD) δ -1.87. ESI-HRMS Calc'd $[M+H^+]$=654.1952; found 654.1941.



M6P-I5 **M6P-NCA**

M6P-NCA. To **M6P-I5** (350 mg, 0.536 mmol, 1.0 equiv.) was added 12 mL tetrahydrofuran, α-pinene (340 µL, 2.14 mmol, 4.00 equiv.), and phosgene (765 µL, 1.07 mmol, 2.00 equiv.). The reaction mixture was allowed to stir for 48 hours, then concentrated under reduced pressure into a cold trap in a fumehood. The resulting tan residue was purified by flash column chromatography on anhydrous silica gel (0 to 30% anhydrous tetrahydrofuran in anhydrous dichloromethane) to give a white foam (290 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.40–7.25 (m, 10H), 5.25 (dd, *J* = 3.5, 1.8 Hz, 1H), 5.17–4.95 (m, 6H), 4.80 (s, 1H), 4.33–4.30 (m, 1H), 4.25–4.18 (m, 1H), 4.11–3.95 (m, 3H), 3.82 (dd, *J* = 10.1, 2.8 Hz, 1H), 2.09 (s, 3H), 1.95 (s, 3H), 1.88 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) 170.3, 169.8, 169.4, 168.3, 152.1, 135.4 (d, *J* = 6.7 Hz), 135.3 (d, *J* = 6.9 Hz), 129.0, 129.0, 128.9, 128.8, 128.3, 128.0, 97.1, 70.2 (d, *J* = 1.0 Hz), 70.12 (d, *J* = 1.9

Hz), 69.5 (d, *J* = 1.8 Hz), 69.0, 68.5, 67.70 (d, *J* = 6.3 Hz), 66.6, 64.7, 58.0, 20.8, 20.7, 20.6. δ ^{31}P NMR (162 MHz, $CDCl_3$) δ -3.18. ESI-HRMS Calc'd [M+H^+]=680.1744; found 680.1735.

General Procedure for M6Pn/M6P-containing Glycopolypeptide Synthesis
Polymerization is performed in a glovebox under a N_2 atmosphere. The polymerization catalyst was prepared fresh following the procedure of Kramer *et al*.[21], using $Ni(COD)_2$ and 2,2'-bipyridyl. To an oven dried 1-mL vial was added 20 mg M6Pn-NCA, 4.2 mg Alanine NCA, and 161 µL freshly prepared catalyst mixture (stoichiometry relative to initiator = 5:5:1). The reaction mixture turned from deep purple to red, and was incubated for 48 hours at ambient temperature in the glovebox. After 48 hours, the vial was removed from the glovebox and the reaction mixture quenched with acidic methanol and evaporated under reduced pressure. For both M6Pn and M6P containing polymerization reactions, reactions proceeded slowly without alanine-NCA used as a comonomer, while reaction completion of 1:1 mixtures was obtained within 48 hours.

General Procedure for M6Pn-Containing Polymer Deprotection
To the resulting residue obtained above was added 1 mL anhydrous DCM, 50 µL TMSBr (10 equiv. per phosphonate group), and 30 µL pyridine (10 equiv. per phosphonate). The reaction mixture was stirred at room temperature overnight sealed under N_2. After concentrating under vacuum, the reaction mixture was suspended in a 1:1:1 mixture of 2 mL MeOH with 2 mL THF and 2 mL saturated aqueous K_2CO_3. After 48 hours, 2 mL H_2O was added, in which some of the precipitate dissolved to yield a cloudy solution that was further stirred for 24 hours. The reaction mixture was neutralized to pH = 7 using concentrated HCl, and evaporated to dryness under reduced pressure. The resulting residues and salts were dissolved in H_2O and dialyzed in a 3.5 KDa MWCO dialysis cassette against H_2O for 3 days, with H_2O changes every 8 hours for the first 24 hours, followed by every 12 hours subsequently. The dialysate was removed from the cassette and any precipitate was removed by filtration through a 0.2 µM filter. The resulting solution was lyophilized to yield white solids.

General Procedure for M6P-Containing Polymer Deprotection
To the residue obtained above was added Pd/C (100 mol% Pd per phosphate group) and a dichloromethane/methanol mixture (1:1, 2 mL total/20 mg NCA used in polymerization), and the reaction was stirred under a balloon of H_2 for 48 hours. After filtration through a 0.2 µM filter, the resulting residue was concentrated under vacuum and resuspended in a 1:1:1 mixture of 2 mL MeOH with 2 mL THF and 2 mL saturated aqueous K_2CO_3. After 48 hours, 2 mL H_2O was added, in which some of the precipitate dissolved to yield a cloudy solution that was further stirred for 24 hours. The reaction mixture was neutralized to pH = 7 using concentrated HCl, and evaporated to dryness under reduced pressure. The resulting residues and salts were dissolved in H_2O and dialyzed in a 3.5 KDa MWCO dialysis cassette against H_2O for 3 days, with H_2O changes every 8 hours for the first 24 hours, followed by every 12 hours subsequently. The contents of the cassette were removed and filtered through a 0.2 µM filter. The resulting solution was lyophilized to yield a white solid.

Synthesis of Poly(GalNAc-co-Ala) and poly(Mannose-co-Ala): As previously described[21,49].

[49] Zhou, M. N. *et al.* N-Carboxyanhydride Polymerization of Glycopolypeptides That Activate Antigen-Presenting Cells through Dectin-1 and Dectin-2. *Angew. Chem. Int. Ed Engl.* **57**, 3137–3142 (2018).

Glycopolypeptide Molecular Weight Analysis with Aqueous GPC
Gel permeation chromatography (GPC) was carried out in aqueous 0.1M $NaNO_3$ + 0.01M NaN_3 or PBS using an OHpak SB-803 HQ Shodex column with the dimensions 8.0 x 300 mm a particle size of 6 µm connected in series with a DAWN multiangle laser light scattering (MALLS) detector and an Optilab T-rEX differential refractometer (both from Wyatt Technology), dn/dc values were obtained for each injection by assuming 100% mass elution from the columns.

Glycopolypeptide GPC Molecular Weights, Dispersities, and ^{1}H NMR Glycosylated Serine:Alanine Ratios:

poly(Mannose-co-Ala): M_n=18.9 KDa, Đ = 1.51, 2.4:1
poly(GalNAc-co-Ala): M_n=27.1 KDa, Đ = 1.24, 1.2:1
poly(M6Pn-co-Ala)$_{short}$: M_n=9.75 KDa, Đ = 1.32, 1:1.6
poly(M6Pn-co-Ala)$_{long}$: M_n=37.1 KDa, Đ = 1.42, 1:0.9
poly(M6P-co-Ala)$_{short}$: M_n=8.20 KDa, Đ = 1.58, 1:1.2
poly(M6P-co-Ala)$_{long}$: M_n=24.7 KDa, Đ = 1.75, 1:1.2

Representative Procedure for Polymer N-terminal Biotinylation
To 1.0 mg of poly(M6Pn)$_{short}$ (M_n = 9.7 KDa, 103 nmoles) was added PBS (0.5 mL), and 40 equiv. Biotin-Sulfo-NHS (1.8 mg). The reaction was allowed to incubate for 12 hours at room temperature, then purified with a NAP-25 column, eluting with water. The eluent was then dialyzed against H_2O for 48 hours, with 6 water changes. Yield: 896 µg. The resulting material was dissolved in 231 µL PBS to make a 400 µM stock solution.

Representative Procedure for Polymer N-terminal Azide-Functionalization
To 1.0 mg of poly(M6Pn)$_{short}$ (M_n = 9.7 KDa, 103 nmoles) was added PBS (0.5 mL), and 50 equiv. NHS-PEG_4-N_3 (100 µL of a 20 mg/mL stock) was added. The reaction was allowed to incubate for 12 hours at room temperature, then purified with a NAP-25 column, eluting with water. The eluent was then dialyzed against water for 48 hours, with 6 water changes.

Representative Procedure for Polymer N-terminal BCN-Functionalization
To 500 µg of poly(M6Pn)$_{short}$ (M_n = 9.7 KDa, 51.5 nmoles) was added PBS (1.0 mL) and 25 equiv. BCN-NHS (360 µL of a 1 mg/mL stock in DMSO). The reaction was allowed to incubate for 12 hours at room temperature, then purified with a NAP-25 column, eluting with water. The eluent was then dialyzed against H_2O for 48 hours, with 6 water changes. Yield: 424 µg.

Representative Procedure for Antibody Labeling with BCN-NHS (Cetuximab)
1 mL of a 2 mg/mL solution of cetuximab was concentrated with a 30K spin filter and subsequently diluted to 1000 µL with PBS. 100 µg (25 equiv.) of NHS-BCN in DMSO (1 mg/mL solution, 100 uL) was added, and the reaction was allowed to incubate at rt overnight. After incubation, the reaction mixture was filtered and diluted eight times using a 500 µL, 30 KDa Amicon Centrifugal Filter.

Representative Procedure for Antibody Labeling with NHS-PEG_4-N_3 (Cetuximab)
1 mL of a 2 mg/mL solution of cetuximab was concentrated with a 30K spin filter and subsequently

diluted with 1000 µL PBS. 137 µg (25 equiv., 14 µL) of NHS-PEG4-N3 in DMSO (10 mg/mL, 137 uL) was added, and the reaction was allowed to incubate at rt overnight. After incubation, the reaction mixture was filtered and diluted eight times using a 500 µL, 30 KDa Amicon Centrifugal Filter.

Representative Procedure for Antibody-Glycopolypeptide Conjugation

To poly(M6Pn)$_{short}$-N_3 (136 µg, 20 equiv.) was added ctx-BCN (100 µg, 50 µL in PBS) and the reaction was allowed to incubate for 3 days at room temperature. The reaction mixture was filtered 9X with a 50 KDa Amicon Centrifugal Filter, then resuspended in 100 µL 1X PBS to give a 5.94 µM solution in PBS (89% recovery). Protein concentration was determined by A_{280} absorbance.

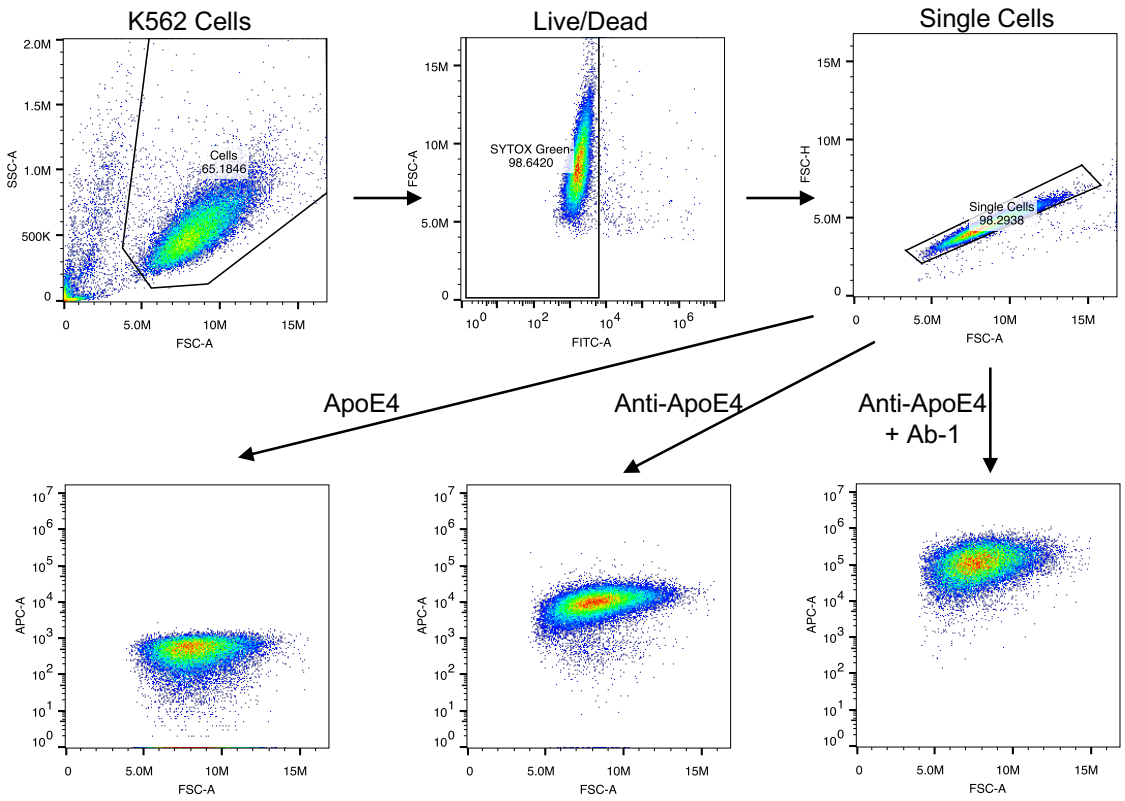
Procedure for HepG2 Proliferation Study

HepG2 cells were plated at 5,000 cells/well in a 96-well plate two days before treatment. On day 0, cells were treated with EGF (200 ng/ml) and 50 nM cetuximab or LYTAC. After 48 hours, cells were washed and fresh media was added, followed by CellTiter Reagent (Promega) and incubated for 3 hours at 37 °C. Absorbance was read by plate reader at 490 nm, according to the manufacturer's specifications.

Antibodies and Usage

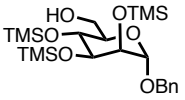
Antibody	Source (#)	Usage and dilution
Rabbit anti-EGFR	CST (D38B1)	WB, 1:1000
Goat anti-EGFR	R&D Systems (AF231)	WB, 1:1000
Mouse anti-CD71	Bio-Rad (VMA00037)	WB, 1:1000
Rabbit anti-PD-L1	CST (E1L3N)	WB, 1:500
Mouse anti-Vinculin	Bio-Rad (V284)	WB, 1:1000
Mouse anti-α-Tubulin	Millipore Sigma (T5168)	WB, 1:5000
Rabbit anti-EXOC1	Abcam (ab118798)	WB, 1:2500
Rabbit anti-CIM6PR	CST (D3V8C)	WB, 1:1000
Rabbit anti-pAKT (S473)	CST (9271)	WB, 1:1000
Rabbit anti-pEGFR (Y1068)	CST (2234)	WB, 1:1000
Rabbit anti-p44/42 MAPK	CST (20G11)	WB, 1:1000
Mouse anti-CD71	BioXCell (BE0023)	Functional
Mouse anti-PD-L1	BioXCell (BE0285)	Functional
Mouse anti-mCherry	Biorbyt (orb66657)	Functional
Mouse anti-ApoE4	Novus Biologicals (4E4)	Functional
Goat anti-Mouse IgG	Jackson ImmunoResearch (115-005-062)	Functional
Mouse anti-biotin-Alexa Fluor 488	Jackson ImmunoResearch (200-542-211)	Functional
Atezolizumab	Biovision (A1305)	Functional
Cetuximab	Eli Lilly	Flow cytometry, Functional
Mouse anti-CIM6PR	Abcam (2G11)	Flow cytometry, 1:500
Goat anti-mouse IgG-Alexa Fluor 488	Jackson ImmunoResearch (115-545-008)	Flow cytometry, 1:375
Goat anti-mouse IgG-Alexa Fluor 647	Jackson ImmunoResearch (115-605-071)	Flow cytometry, 1:375
Goat anti-human IgG-Alexa Fluor 647	Jackson ImmunoResearch (109-605-003)	Flow cytometry, 1:375

Representative Flow Cytometry Gating Strategy: ApoE-647 Uptake Experiment



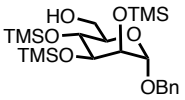
NMR Spectra

^{1}H NMR
400 MHz
$CDCl_3$

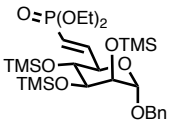


M6Pn-I2

^{13}C NMR
101 MHz
$CDCl_3$

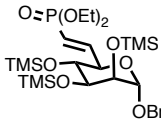


^{1}H NMR
400 MHz
$CDCl_3$

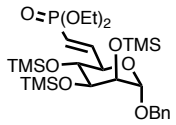


M6Pn-I3

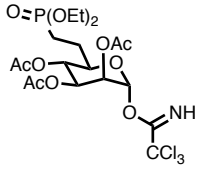
^{13}C NMR
101 MHz
$CDCl_3$



^{31}P NMR
162 MHz
$CDCl_3$

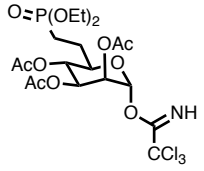


^{1}H NMR
400 MHz
$CDCl_3$

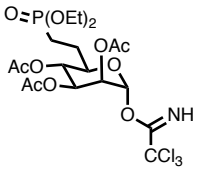


M6Pn-I4

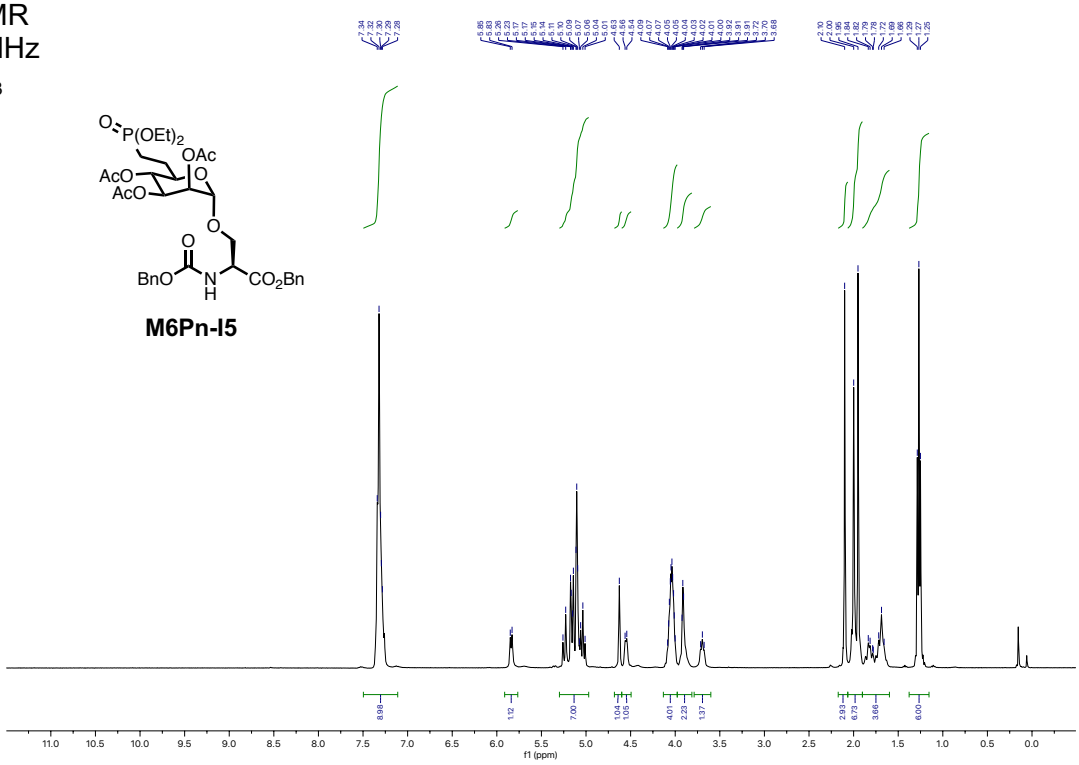
^{13}C NMR
101 MHz
$CDCl_3$



^{31}P NMR
162 MHz
$CDCl_3$

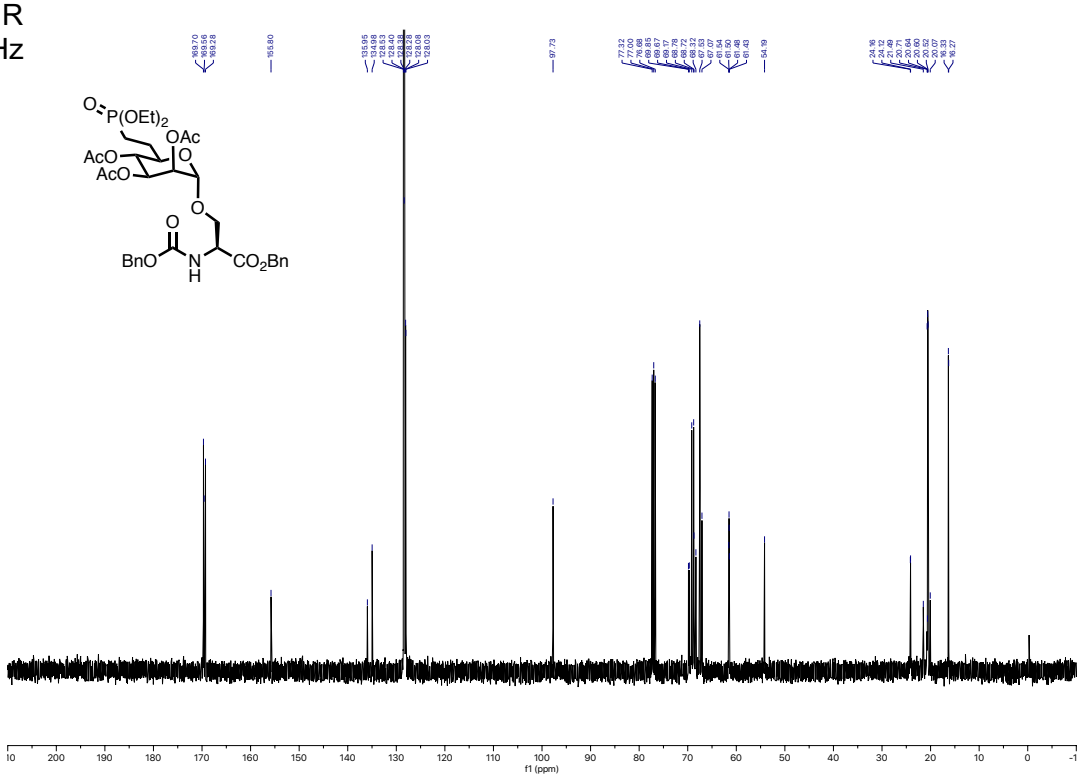


^{1}H NMR
400 MHz
$CDCl_3$

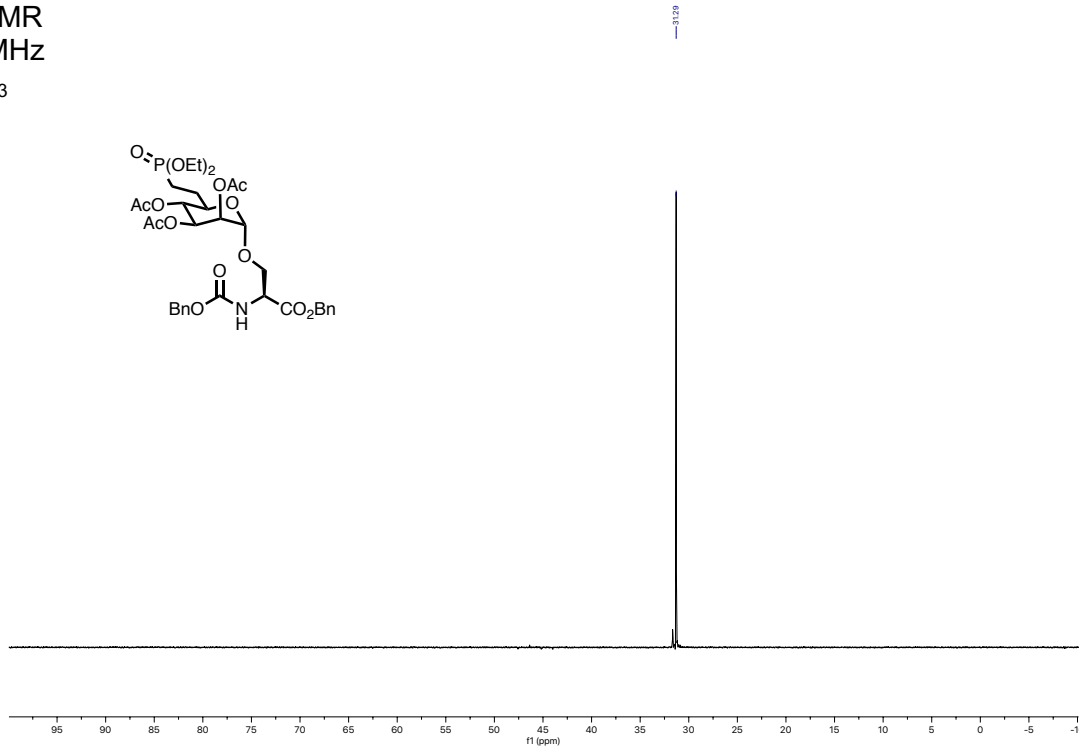


M6Pn-I5

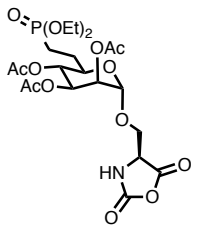
^{13}C NMR
101 MHz
$CDCl_3$



^{31}P NMR
162 MHz
$CDCl_3$

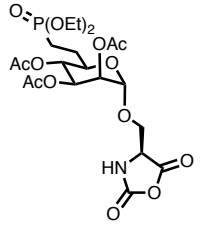


^{1}H NMR
400 MHz
$CDCl_3$

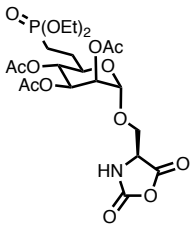


M6Pn-NCA

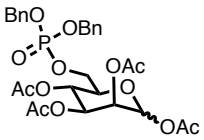
^{13}C NMR
101 MHz
$CDCl_3$



^{31}P NMR
162 MHz
$CDCl_3$

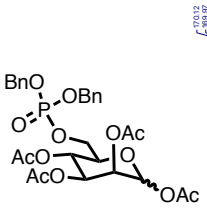


^{1}H NMR
400 MHz
$CDCl_3$

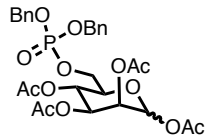


M6P-I2

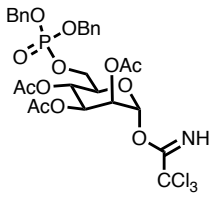
^{13}C NMR
101 MHz
$CDCl_3$



^{31}P NMR
162 MHz
$CDCl_3$

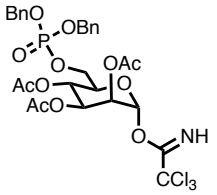


^{1}H NMR
400 MHz
$CDCl_3$

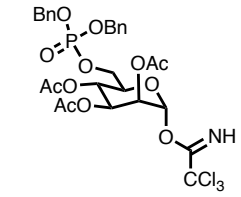


M6P-I3

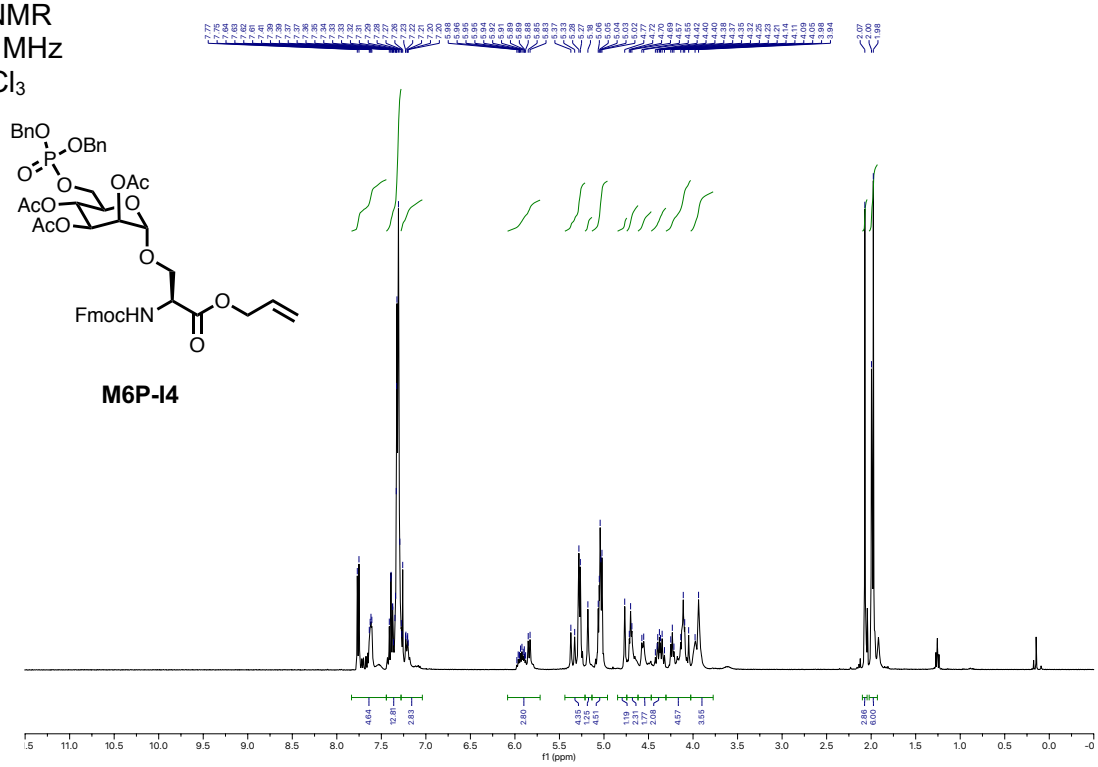
^{13}C NMR
101 MHz
$CDCl_3$



^{31}P NMR
162 MHz
$CDCl_3$

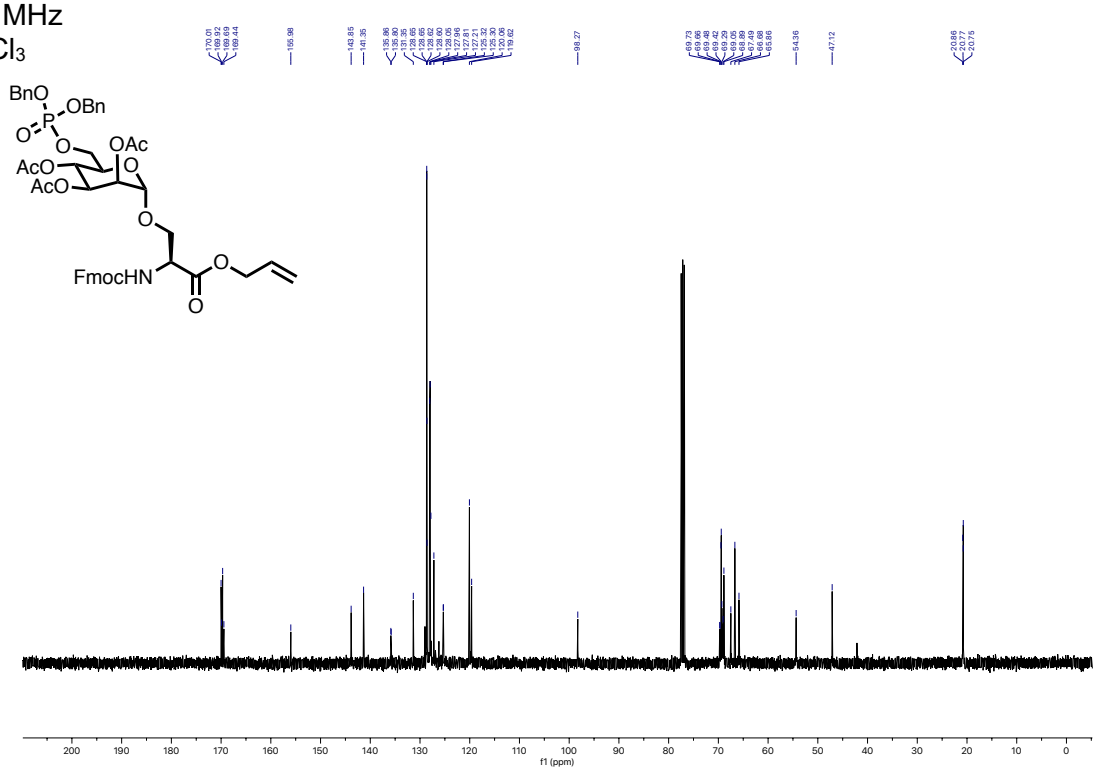


^{1}H NMR
400 MHz
$CDCl_3$

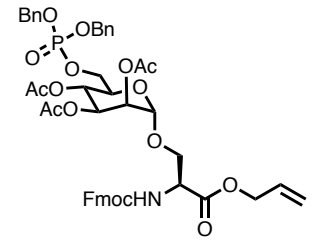


M6P-I4

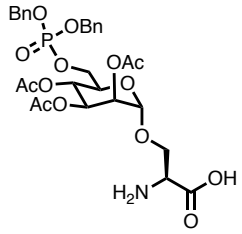
^{13}C NMR
101 MHz
$CDCl_3$



^{31}P NMR
162 MHz
$CDCl_3$

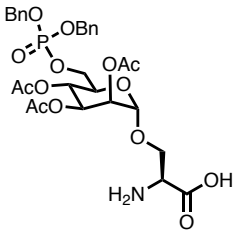


^{1}H NMR
400 MHz
CD_3OD

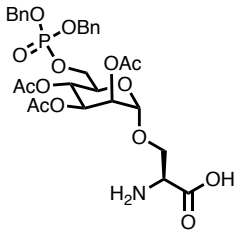


M6P-I5

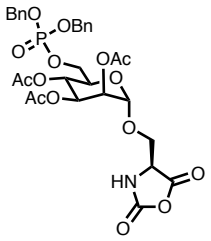
^{13}C NMR
101 MHz
CD_3OD



^{31}P NMR
162 MHz
CD_3OD

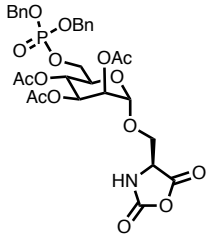


^{1}H NMR
400 MHz
$CDCl_3$

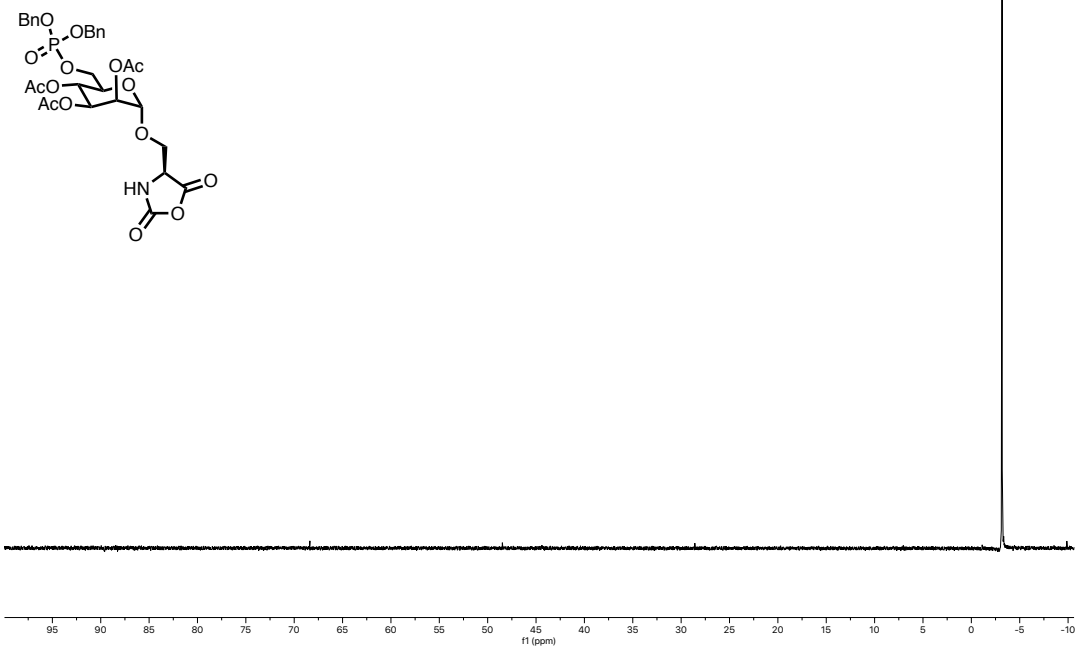


M6P-NCA

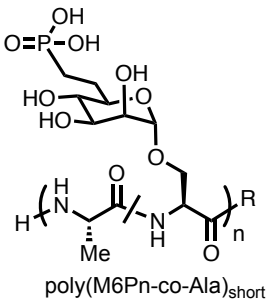
^{13}C NMR
101 MHz
$CDCl_3$



^{31}P NMR
162 MHz
$CDCl_3$

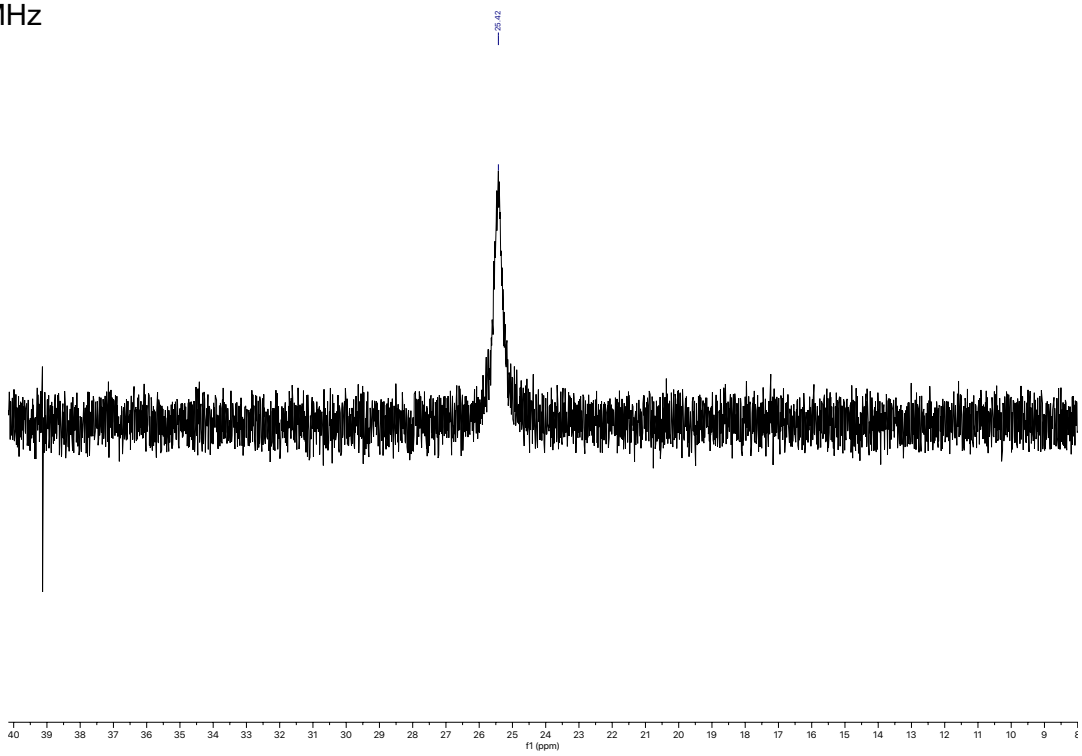


^{1}H NMR
600 MHz
D_2O

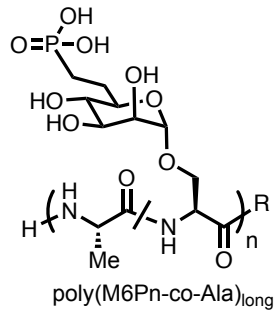


poly(M6Pn-co-Ala)$_{short}$

^{31}P NMR
162 MHz
D_2O

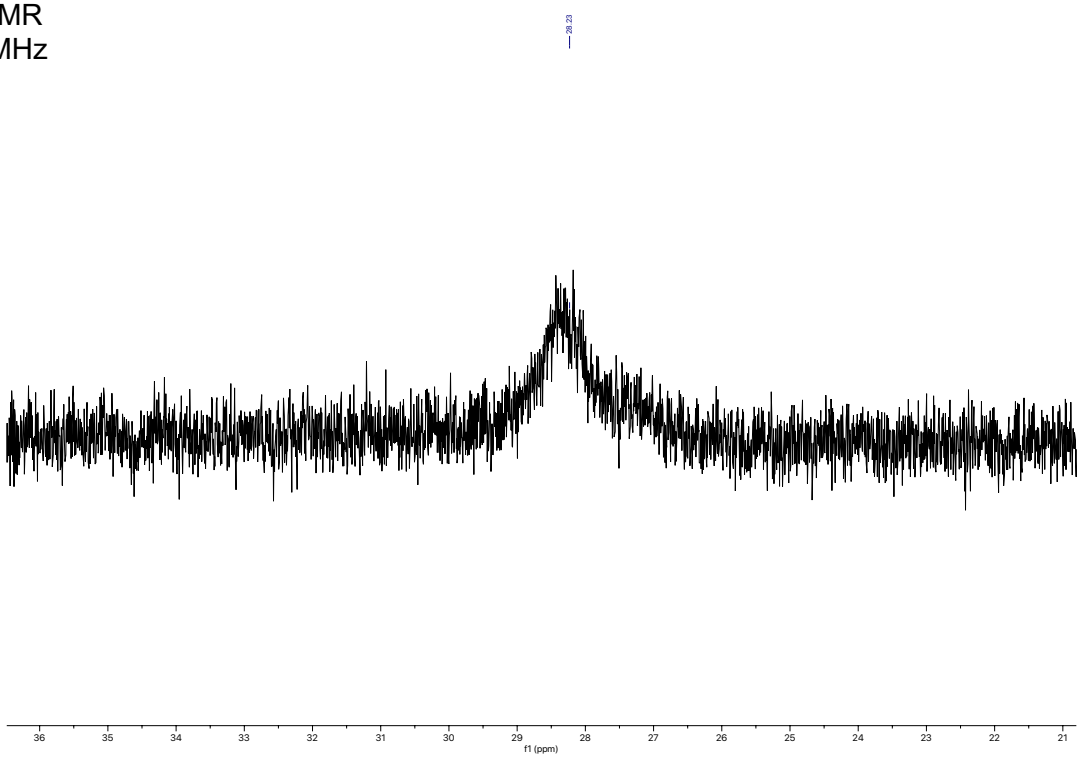


^{1}H NMR
600 MHz
D_2O

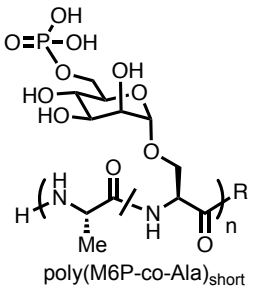


poly(M6Pn-co-Ala)$_{long}$

^{31}P NMR
162 MHz
D_2O

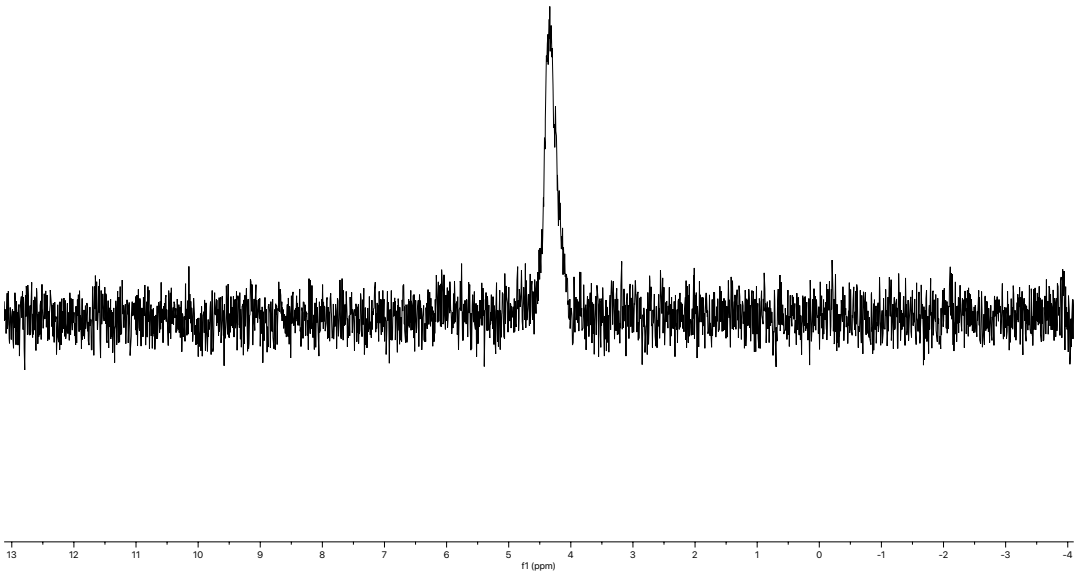


^{1}H NMR
600 MHz
D_2O

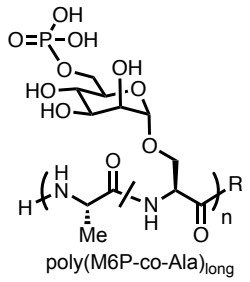


poly(M6P-co-Ala)$_{short}$

^{31}P NMR
162 MHz
D_2O

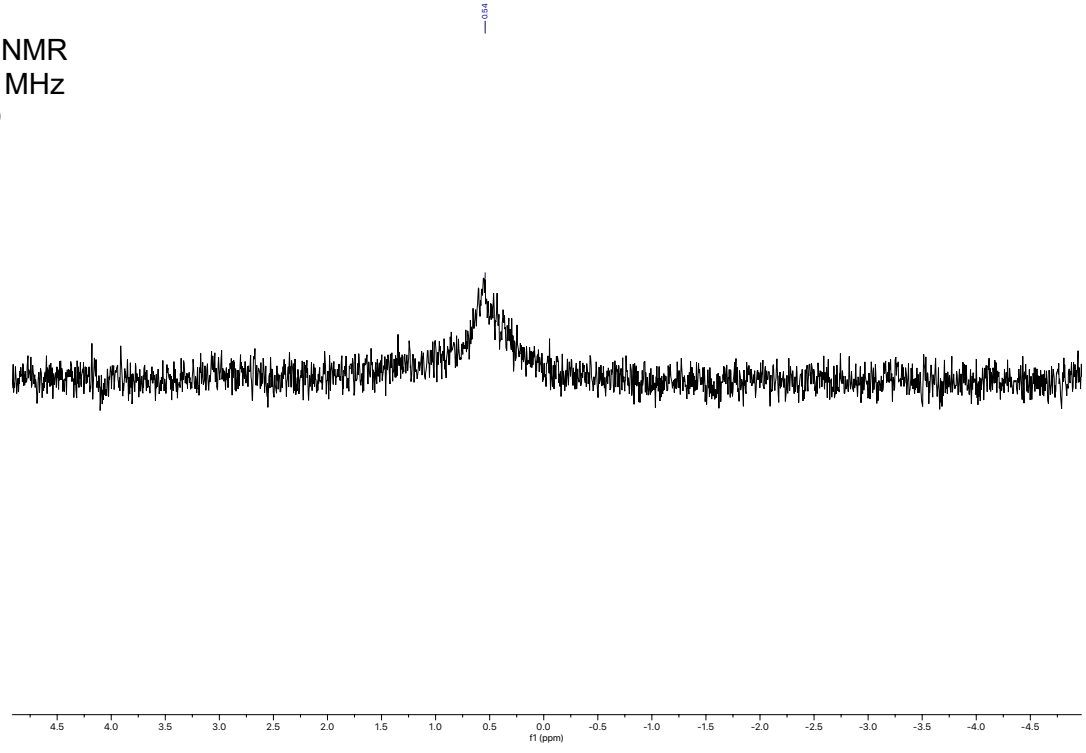


^{1}H NMR
600 MHz
D_2O



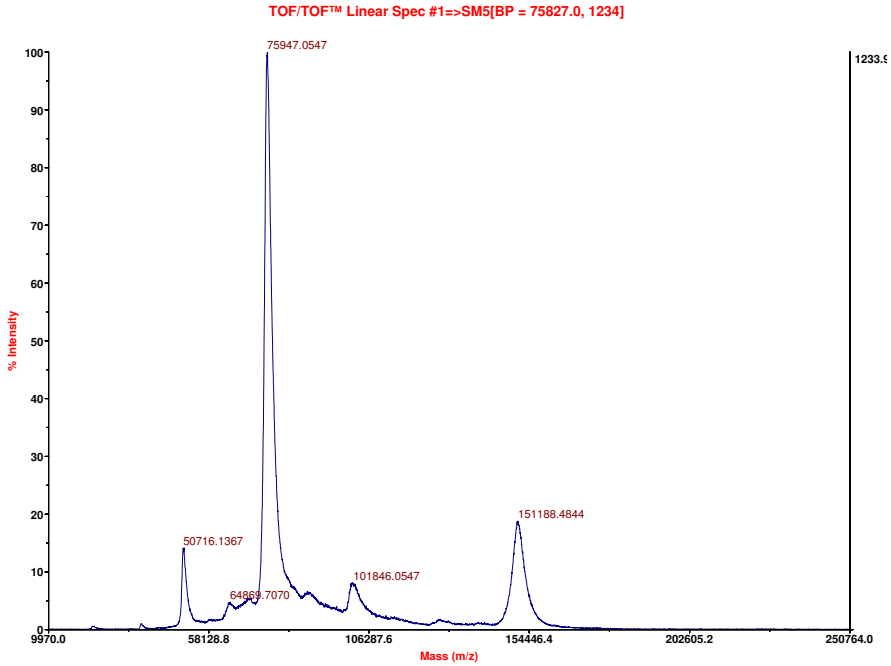
poly(M6P-co-Ala)$_{long}$

^{31}P NMR
162 MHz
D_2O



MALDI-MS Results for Ab-2 Construction:

Ctx



Ctx-N_3