Supporting Information for:

A hydrophilic azacyclooctyne for Cu-free click chemistry

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General Synthetic Procedures.

All chemical reagents were purchased from Sigma-Aldrich, Acros, and TCI chemicals and used without purification unless noted otherwise. Anhyd DMF and MeOH were purchased from Aldrich or Acros in sealed bottles; all other solvents were purified as described by Pangborn *et al.*[1] In all cases, magnesium sulfate was used as a drying agent and solvent was removed by reduced pressure with a Buchi Rotovapor R-114 equipped with a Welch self-cleaning dry vacuum. Products were further dried by reduced pressure with an Edwards RV5 high vacuum. Thin layer chromatography was performed with Silicycle® 60 Å silica gel plates. Unless otherwise specified, R_f values are reported in the solvent system the reaction was monitored in. Flash chromatography was performed using Merck 60 Å 230-400 mesh silica or a Biotage Flash+® system with Biotage® 10S, 10M, 40S or 40M prepacked silica gel columns.

All ^{1}H and ^{13}C NMR spectra are reported in ppm and referenced to solvent peaks. Spectra were obtain on Bruker AVB-400®, DRX-500®, or AV-500® instruments. IR spectra were obtained using a Varian 3100 FT-IR using thin films on NaCl plates. Optical rotations were measured using a Perkin Elmer 241 polarimeter. High resolution fast atom bombardment (FAB) and electrospray ionization (ESI) mass spectra were obtained from the UC Berkeley Mass Spectrometry Facility. Elemental analysis was performed at the UC Berkeley Microanalytical Facility.

Experimental Procedures.

(2*S*, 3*S*, 4*R*) *N*-allyl, *N*-(methyl succinyl)-4-hydroxy-2,3-dimethoxyhex-5-ene amine (2). Pyranoside **1**[2] (18.2 g, 63.8 mmol, 1 equiv) was dissolved in 19:1 1-propanol/H_2O (1.5 L) in an Erlenmeyer flask equipped with an overhead stirring unit. To this solution, allylamine (150 mL, 2.0 mol, 31 equiv), zinc (223.9 g, 3.423 mol, 54 equiv, acid treated), and $NaBH_3CN$ (18.97 g, 301.9 mmol, 5 equiv) were added. The reaction was heated to 90 °C and monitored by TLC (EtOAc) for the disappearance of **1** (R_f = 0.7). Upon reaction completion (approx 1 h), the mixture was cooled to rt and filtered through Celite. The filtrate was evaporated to dryness and dissolved in 6:4:1 MeOH/CH_2Cl_2/1.5M HCl (1.32 L) and stirred for 1 h (adding 3M HCl as necessary to keep the solution acidic) at which point, H_2O (300 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 600 mL). The organics were dried, decanted, and evaporated to a residue. The resulting crude amine was dissolved in MeOH (600 mL, anhyd). To this solution, *N,N*-diisopropylethylamine (12.2 mL, 70.0 mmol, 1.1 equiv) followed by methyl succinyl chloride (8.6 mL, 70 mmol, 1.1 equiv) were added and the mixture was stirred at rt under N_2 for 1 h, at which point the reaction was quenched with H_2O (100 mL) and the MeOH was removed via rotary evaporation. To the resulting aqueous solution, H_2O (500 mL) was added and extracted with CH_2Cl_2 (3 x 650 mL). The organic extracts were combined, dried, decanted, and evaporated to dryness. The crude product was purified via flash chromatography using 3 Biotage 40M columns with a gradient solvent system starting with 25:1 toluene/acetone and ending with 3:1 toluene/acetone (product begins to

[1] Pangborn, A.B.; Giardello, M.A.; Grubbs, R.H.; Rosen, R.K.; Timmers, F.J. *Organometallics* **1996**, *15*, 1518.
[2] Jones, K.; Wood, W.W. *Carb. Res.* **1986**, *155*, 217.

elute at 10:1 toluene/acetone) to result in pure **2** as a colorless oil (14.8 g, 44.9 mmol, 70%). R_f = 0.6 in 1:1 toluene/acetone. $[\alpha]_D^{28}$ -38.8° (*c* 0.943, CH_2Cl_2). 1:0.5 mixture of rotamers (designated rot) ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.98-5.88 (m, 1H, 1rotH), 5.79-5.71 (m, 1H, 1rotH), 5.36 (d, *J* = 17.2 Hz, 1rotH), 5.35 (d, *J* = 17.2 Hz, 1H), 5.24-5.11 (m, 3H, 3rotH), 4.32-4.27 (m, 1H, 1rotH), 4.16-4.13 (m, 1H, 1rotH), 4.07-4.03 (m, 1H), 3.98-3.94 (m, 1rotH), 3.78 (dd, *J* = 13.9, 3.6 Hz, 1H), 3.72-3.67 (m, 4H, 3rotH), 3.63-3.59 (m, 2rotH), 3.52 (s, 3H, 3 rotH), 3.45-3.38 (m, 3H, 4rotH), 3.25-3.21 (m, 1H, 1rotH), 3.15 (t, *J* = 4.2 Hz, 1H), 2.85-2.80 (m, 1rotH), 2.73-2.60 (m, 5H, 3rotH), 2.33 (d, *J* = 6.5 Hz, 1rotH). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.9, 173.7, 172.2, 172.1, 138.4, 133.6, 132.8, 117.2, 116.7, 116.31, 116.25, 83.4, 82.7, 80.5, 80.4, 72.4, 71.5, 60.7, 60.6, 59.8, 59.7, 52.02, 51.95, 51.9, 48.9, 48.3, 48.2, 29.5, 29.2, 28.2, 28.1. IR: 3441 (b), 3082, 2981, 2933, 2832, 1737, 1641 cm^{-1}. HRMS (FAB) calcd for $C_{16}H_{28}NO_6$ $[M + H]^+$: 330.191663; found: 330.192190. Anal. calcd for $C_{16}H_{27}NO_6$: C, 58.34; H, 8.26, N, 4.25; found: C, 58.41; H, 8.22, N, 4.38.

(5*R*, 6*S*, 7*S*, Z) *N*-(methyl succinyl)-5-hydroxy-6,7-dimethoxyazacyclooct-3-ene (3). Compound **2** (790 mg, 2.40 mmol, 1 equiv) was dissolved in CH_2Cl_2 (200 mL, anhyd) and heated to reflux while stirring under N_2. Once at reflux, Grubbs second generation catalyst (163.3 mg, 0.1927 mmol, 0.08 equiv) was added and the reaction was carefully monitored by TLC in 1:1 toluene/acetone for the disappearance of **2** (R_f = 0.6) adding more catalyst if necessary (at 2.25 h 61 mg catalyst added). Upon completion (approx 5 h), the mixture was cooled to rt, evaporated to dryness, and *immediately* purified via flash chromatography using a Biotage 40M column with a gradient of 8:1 toluene/acetone, 6:1 toluene/acetone, 4:1 toluene/acetone. This procedure resulted in pure **3** as a brown oil (500 mg, 1.66 mmol, 69%, R_f = 0.4). $[\alpha]_D^{28}$ -82.6° (*c* 1.12, CH_2Cl_2). 1:0.18 mixture of rotamers. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.63 (ddd, *J* = 11.9, 6.4, 2.0 Hz, 1H), 5.56-5.54 (m, 2rotH), 5.48-5.45 (m, 1H), 4.41-4.30 (m, 2rotH), 4.35 (t, *J* = 8.0 Hz, 1H), 4.30 (apparent d, *J* = 17.5 Hz, 1H), 4.07 (dd, *J* = 13.8, 3.1 Hz, 1H), 3.73 (dd, *J* = 17.2, 5.0 Hz, 1H, 2rotH), 3.66 (s, 3H, 3rotH), 3.62-3.56 (m, 4H, 3rotH), 3.50 (s, 3H), 3.45 (s, 3rotH), 3.42-3.39 (m, 2rotH), 3.27 (s, 1H), 3.08 (apparent dd, *J* = 9.0 Hz, 5.6 Hz, 1rotH), 2.93 (dd, *J* = 9.4, 7.7 Hz, 1H, 1rotH), 2.81 (dd, *J* = 13.8, 9.6 Hz, 1H), 2.68-2.52 (m, 4H, 4rotH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.3, 173.1, 171.5, 134.5, 132.4, 125.3, 123.7, 85.5, 85.2, 81.4, 80.5, 68.1, 66.8, 60.8, 60.1, 58.0, 57.8, 51.6, 47.8, 46.0, 45.3, 45.2, 29.0, 28.7, 28.3. IR: 3472 (b), 2933, 2828, 1735, 1636 cm^{-1}. HRMS (FAB) calcd for $C_{14}H_{24}NO_6$ $[M + H]^+$: 302.160363; found: 302.159780.

(6*R*, 7*S*, Z) *N*-(methyl succinyl)-6,7-dimethoxy-5-oxoazacylclooct-3-ene. To a solution of **3** (436 mg, 1.51 mmol, 1 equiv) in CH_2Cl_2 (100 mL, anhyd), pyridinium chlorochromate (494 mg, 2.29 mmol, 1.5 equiv) was added. The mixture was heated to 40 °C and stirred under N_2 overnight. The following day, the reaction was cooled to rt, H_2O (75 mL) was added, and extracted with CH_2Cl_2 (3 x 100 mL). The organic extracts were combined, dried, decanted, and evaporated to dryness to result in crude product, which was purified via flash chromatography using a Biotage 40S columnwith a gradient solvent system of 8:1 toluene/acetone, 6:1 toluene/acetone, 4.5:1 toluene/acetone to yield pure (6*R*, 7*S*, Z) *N*-(methyl succinyl)-6,7-dimethoxy-5-oxoazacylclooct-3-ene as a clear oil (360 mg, 1.21 mmol, 80%). R_f = 0.6 in 1:1 toluene/acetone. $[\alpha]_D^{28}$ +35.5° (*c* 4.46,

CH_2Cl_2). 1:1 mixutre of rotamers. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.17 (apparent d, J = 11.8 Hz, 1H), 5.95-5.88 (m, 2H), 5.69 (d, J = 11.9 Hz, 1H), 4.26 (dd, J = 17.4, 4.3 Hz, 1H), 4.11 (d, J = 19.7 Hz, 1H), 3.96 (d, J = 19.7 Hz, 1H), 3.81-3.71 (m, 6H), 3.61-3.59 (m, 8H), 3.48-3.30 (m, 13H), 2.62-2.40 (m, 7H), 2.36-2.31 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 203.2, 202.2, 173.8, 173.7, 172.5, 171.9, 137.5, 132.5, 129.1, 126.3, 89.1, 87.3, 81.1, 79.3, 59.6, 59.09, 59.07, 58.3, 51.9, 51.8, 50.4, 49.7, 48.5, 46.8, 29.1, 29.0, 28.02, 27.95. IR: 3590, 3516, 2944, 2830, 1743, 1691, 1655 cm^{-1}. HRMS (FAB) calcd for $C_{14}H_{22}NO_6$ $[M + H]^+$: 300.144713; found: 300.144130. Anal. calcd for $C_{14}H_{21}NO_6$: C, 56.18; H, 7.07, N, 4.68; found: C, 56.17; H, 7.09, N, 4.57.

(3*S*, 4*R*) *N*-(methyl succinyl)-3,4-dimethoxy-5-oxoazacyclooctane (4). (6*R*, 7*S*, *Z*) *N*-(methyl succinyl)-6,7-dimethoxy-5-oxoazacylclooct-3-ene (331 mg, 1.11 mmol, 1 equiv) was dissolved in EtOH (60 mL) and 10 % Pd/C (27.8 mg) was added. The mixture was stirred overnight at rt under H_2 (1 atm). The following day, the mixture was filtered through Celite and the filtrate was evaporated to dryness to yield **4** (295 mg, 0.980 mmol, 89%). R_f = 0.5 in 1:1 toluene/acetone. $[\alpha]_D^{28}$ +29.7° (*c* 6.24, CH_2Cl_2). 1:0.66 mixture of rotamers. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.00 (dd, J = 14.0, 4.5 Hz, 1H), 3.87 (d, J = 8.0 Hz, 1H), 3.75-3.69 (m, 3rotH), 3.65-3.39 (m, 8H, 6rotH), 3.34 (s, 3rotH), 3.29-3.20 (m, 3H, 2rotH), 3.08 (dt, J = 14.5, 5.0 Hz, 1H), 2.69-2.25 (m, 6H, 8rotH), 2.12-2.07 (m, 3H), 1.96 (s, 1rotH). ^{13}C NMR (125 MHz, $CDCl_3$): δ 209.7, 209.0, 173.8, 173.5, 172.8, 172.4, 88.4, 86.1, 82.3, 80.4, 59.8, 59.1, 58.8, 57.9, 51.9, 51.8, 49.4, 48.3, 47.8, 47.5, 41.3, 37.7, 29.2, 28.9, 28.5, 28.3, 26.0, 25.3. IR: 3587, 3518, 2940, 2831, 1743, 1711, 1655 cm^{-1}. HRMS (ESI) calcd for $C_{14}H_{23}NO_6Na$ $[M + Na]^+$: 324.1418; found: 324.1420. Anal. calcd for $C_{14}H_{23}NO_6$: C, 55.80; H, 7.69, N, 4.65; found: C, 55.91; H, 7.77, N, 4.63.

Compound 5. Ketone **4** (1.528 g, 5.075 mmol, 1 equiv) was dissolved in 1:1 H_2O/EtOH containing 100 mM aniline[3] (40 mL). To this solution, semicarbazide hydrochloride (5.898 g, 52.66 mmol, 10 equiv) and AcOH (4 mL) was added. The reaction was stirred at rt and monitored by TLC (1:1 toluene/acetone) for the disappearance of **4** (R_f = 0.5). After the reacton was complete (approx 8 h), the reaction was neutralized with 10% NaOH and the EtOH was removed via evaporation under reduced pressure. The aqueous layer was extracted with EtOAc (4 x 75 mL). The EtOAc was combined, dried, decanted, and evaporated to dryness to result in crude semicarbazone that was directly converted to selenadiazole **5**. The crude semicarbazone was dissolved in dioxane (10 mL). A solution of SeO_2 (2.587 g, 23.31 mmol, 4.6 equiv) in 1:1 dioxane/H_2O (8 mL) was added dropwise over 4 h to the semicarbazone solution. The mixture was stirred at rt and analyzed by ESI-MS for the presence of **5** ($[M+H]^+$ = 392) and absence of semicarbazone ($[M+H]^+$ = 359, $[M+Na]^+$ = 381). Upon reaction completion (approx 3 h after addition of all SeO_2), the dioxane was removed under reduced pressure and H_2O (10 mL) was added. The aqueous solution was extracted with EtOAc (3 x 40 mL) and the organic extracts were combined, dried, decanted, and evaporated to dryness. The crude product was purified by flash chromatographed using a Biotage 40M column with CH_2Cl_2, 80:1 CH_2Cl_2/MeOH, 60:1 CH_2Cl_2/MeOH to result in pure **5** as a yellow oil (1.183 mg, 3.031 mmol, 60%). R_f = 0.3 in 60:1 CH_2Cl_2/MeOH. $[\alpha]_D^{28}$ +17.6° (*c* 1.27,

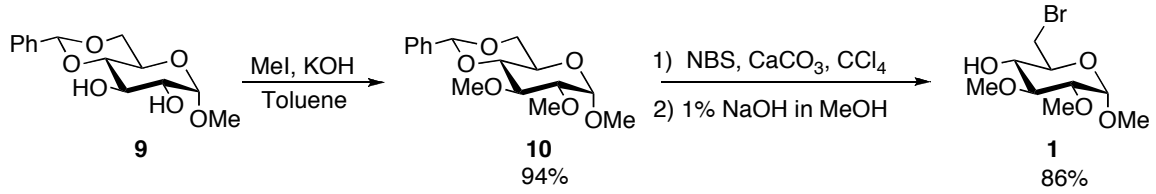
[3] Dirksen, A.; Hackeng, T.M.; Dawson, P.E. *Angew. Chem. Int. Ed.* **2006**, *45*, 7581.

CH_2Cl_2). 1:0.8 mixture of rotamers. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.39 (s, 1rotH), 5.37 (d, J = 5.4 Hz, 1H), 4.36 (m, 1rotH), 4.07-4.02 (m, 2rotH), 3.91 (q, J = 5.3 Hz, 1H), 3.73 (bs, 2H), 3.67 (s, 3rotH), 3.65 (s, 3H), 3.59-3.53 (m, 4H, 3rotH), 3.45-3.39 (m, 2H, 2rotH), 3.33-3.19 (m, 3H, 5rotH), 2.91-2.83 (m, 1rotH), 2.74-2.53 (m, 5H, 1rotH), 2.48-2.36 (m, 2rotH). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.9, 173.7, 173.4, 171.7, 161.2, 159.3, 157.0, 156.3, 82.6, 80.1, 78.8, 77.9, 58.7, 58.5, 57.94, 57.88, 52.0, 51.9, 51.3, 50.5, 50.1, 49.8, 29.6, 29.1, 28.4, 28.3, 26.3, 25.4. IR: 3580, 3057, 2983, 2931, 2828, 1741, 1649 cm^{-1}. HRMS (FAB) calcd for $C_{14}H_{22}N_3O_5Se$ $[M + H]^+$: 392.072467; found: 392.071460.

(6*S*, 7*S*) *N*-(methyl succinyl)-6,7-dimethoxyazacyclooct-4-yne. Selenadiazole **5** (350 mg, 0.90 mmol, 1 equiv) was dissolved in *m*-xylene (320 mL) and heated to 115 °C. The reaction was monitored by TLC (1:1 toluene/acetone) for the disappearance of **5** (R_f = 0.60, UV active, red spot when visualized with vanillin) and appearance of azacyclooctyne methyl ester (R_f = 0.65, green spot when visualized with vanillin). Upon completion (approx 2 d), the reaction was cooled to rt and filtered, and then the filtrate was evaporated to dryness. The crude product was purified via flash chromatography using silica gel and a toluene/acetone solvent system starting at 20:1 and ending with 8:1. This procedure resulted in pure (6*S*, 7*S*) *N*-(methyl succinyl)-6,7-dimethoxyazacyclooct-4-yne as a slightly yellow oil (120 mg, 0.423 mmol, 47%, 55% brsm, R_f = 0.6). $[\alpha]_D^{28}$ +7.5° (*c* 0.64, CH_2Cl_2). 1:0.15 mixture of rotamers. ^{1}H NMR (400 MHz, D_2O): δ 4.38 (apparent d, J = 7.9 Hz, 1rotH), 4.24 (dt, J = 8.6, 2.6 Hz, 1H, 1rotH), 4.17 (dd, 14.9, 5.4 Hz, 1H), 4.06 (d, J = 14.3 Hz, 1H), 4.00 (s, 1rotH), 3.88 (t, J = 9.2 Hz, 1rotH), 3.72-3.68 (m, 4H, 3rotH), 3.55-3.44 (m, 3H, 4rotH), 3.38-3.29 (m, 4H, 3rotH), 3.05 (dd, J = 14.3, 9.1 Hz, 1H, 1rotH), 2.90-2.64 (m, 5H, 5rotH), 2.33 (dt, J = 16.9, 3.2 Hz, 1H), 2.25 (apparent d, J = 16.8 Hz, 1rotH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.6 (broad), 172.5, 171.6, 99.0, 96.0, 93.0, 91.0, 87.0, 85.2, 77.8, 77.4, 59.8, 59.3, 57.5, 57.4, 56.4, 55.7, 53.2, 52.3, 52.02, 51.98, 29.5, 29.3, 28.7, 28.3, 22.1, 21.1. IR: 3489, 2931, 2827, 2203, 1736, 1648 cm^{-1}.HRMS (FAB) calcd for $C_{14}H_{22}NO_5$ $[M + H]^+$: 284.149798; found: 284.150650.

(6*S*, 7*S*) *N*-(succinate)-6,7-dimethoxyazacyclooct-4-yne (6). (6*S*, 7*S*) *N*-(methyl succinyl)-6,7-dimethoxyazacyclooct-4-yne (27.3 mg, 0.0964 mmol, 1 equiv) was dissolved in 2:1 H_2O/dioxane (1.5 mL) and LiOH (45.7 mg, 1.91 mmol, 20 equiv, crushed) was added. The reaction was stirred overnight at rt. The following day the mixture was neutralized with 3M HCl and the dioxane was removed via reduced pressure. Additional H_2O (3 mL) was added to the resulting aqueous solution, this solution was acidified with 3M HCl and extracted with EtOAc (5 x 10 mL). The organic extracts were combined, dried, decanted, and evaporated to dryness. The crude product was purified via flash chromatography using silica gel and a gradient solvent system starting with 8:1 toluene/acetone and ending with 1:1 toluene/acetone. This procedure resulted in pure **6** as an off-white solid (17.1 mg, 0.0636 mmol, 66%). R_f = 0.3 - 0.4 in 1:1 toluene/acetone. $[\alpha]_D^{28}$ -14.6° (*c* 0.357, H_2O). 1:0.1 mixture of rotamers. ^{1}H NMR (400 MHz, D_2O): δ 4.37 (dt, J = 7.8, 2.3 Hz, 1rotH), 4.13 (dt, J= 8.7, 2.8 Hz, 1H, 1rotH), 4.18 (dd, J = 14.9, 5.4 Hz, 1H), 4.06 (d, J = 14.3 Hz, 1H), 3.91 (s, 1rotH), 3.89 (t, J = 8.4 Hz, 1rotH), 3.71 (t, J = 8.5 Hz, 1H), 3.56 (s, 3H, 3rotH), 3.56-3.46 (m, 2rotH), 3.37-3.28

(m, 4H, 2rotH), 3.04 (dd, J = 14.3, 9.0 Hz, 1H, 1rotH), 2.88-2.64 (m, 5H, 5rotH), 2.32 (dt, J = 16.6, 3.4 Hz, 1H), 2.24 (apparent d, 16.8 Hz, 1rotH). ^{13}C NMR (125 MHz, D_2O, no rotamer peaks tabulated): δ 177.0, 174.9, 99.0, 89.7, 84.1, 76.1, 58.0, 56.4, 54.1, 51.9, 29.0, 27.7, 20.6. IR: 3434, 2935, 2830, 2358, 2207, 1729, 1642 cm^{-1}. HRMS (ESI) calcd for $C_{13}H_{19}NO_5Na$ $[M + Na]^+$: 292.1155; found: 292.1157.



Scheme S1. Synthesis of methyl 6-bromo-6-deoxy-2,3-di-*O*-methyl-α,D-glucopyranoside, 11, from commercially available protected glucopyranoside 9.

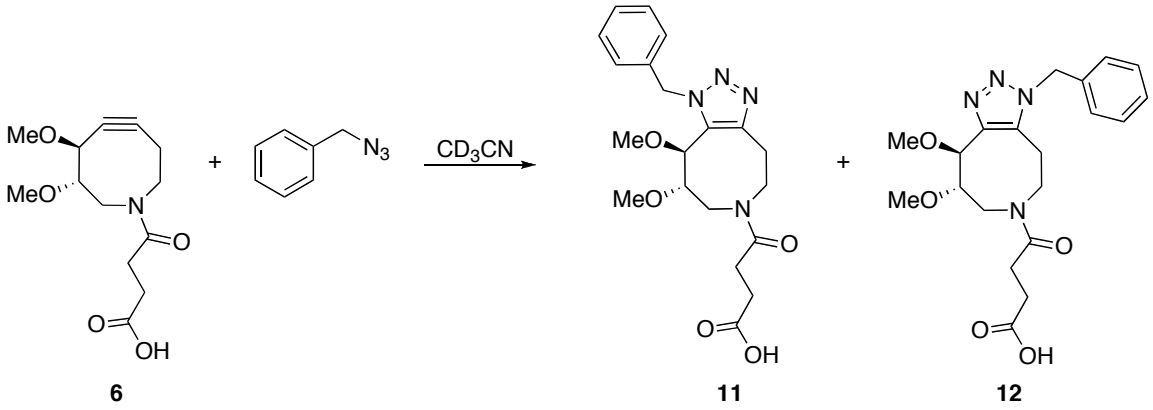
Methyl 4,6-*O*-benzylidine-2,3-di-*O*-methyl-α,D-glucopyranoside (10). Methyl 4,6-*O*-benzylidene-α,D-glucopyranoside (1.416 g, 5.176 mmol, 1 equiv, Acros) was dissolved in toluene (55 mL, anhyd). To this solution, KOH was added (1.73 g, 30.8 mmol, 6 equiv) followed by MeI (2.20 mL, 35.3 mmol, 7 equiv). The mixture was heated to reflux while stirring under N_2 and monitored by TLC (1:1 hexanes/EtOAc) for the disappearance of **9** (R_f = 0.2). Upon reaction completion (approx 4 h), the mixture was cooled to rt and toluene (50 mL) was added and the organic layer was washed with H_2O (3 x 30 mL). The organic extracts were evaporated to dryness and twice azeotroped (50 mL) with toluene to result in **10** as a white powder (1.516 g, 4.885 mmol, 94%, R_f = 0.7). Mp 123.2-124.0 °C (lit.[4] 121-123 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48-7.46 (m, 2H), 7.34-7.28 (m, 3H), 5.51 (s, 1H), 4.82 (d, J = 3.4 Hz, 1H), 4.25 (dd, J = 9.9, 4.5 Hz, 1H), 3.79 (td, J = 5.1, 4.4 Hz, 1H), 3.70 (t, J = 10.1 Hz, 1H), 3.66 (t, J = 9.2 Hz, 1H), 3.60 (s, 3H), 3.52 (s, 3H), 3.50 (t, J = 9.3 Hz, 1H), 3.41 (s, 3H), 3.26 (dd, J = 9.2, 3.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.4, 129.0, 128.3, 126.1, 101.4, 98.4, 82.2, 81.5, 79.9, 69.1, 62.3, 61.1, 59.4, 55.3. HRMS (FAB) calcd for $C_{16}H_{23}O_6$ $[M + H]^+$: 311.14946; found: 311.14930.

Methyl 6-bromo-6-deoxy-2,3-di-*O*-methyl-α,D-glucopyranoside (1). Methyl 4,6-*O*-benzylidene-α,D-glucopyranoside (37.97 g, 122.4 mmol, 1 equiv) was dissolved in CCl_4 (1.5 L, anhyd) and $CaCO_3$ (13.54 g, 135.3 mmol, 1.11 equiv) was added. This mixture was heated to reflux under N_2. *N*-bromosuccinimide (24.228 g, 136.13 mmol, 1.11 equiv, recrystallized) was then added, and the reaction was monitored by TLC (1:1 hexanes/EtOAc) for the disappearance of **10** (R_f = 0.7). Upon completion (approx 1 h), the reaction was cooled to rt and evaporated to dryness. The residue was dissolved in CH_2Cl_2 (1 L) and washed with 10% Na_2SO_3 (1 x 1 L) and sat. $NaHCO_3$ (1 x 1 L). Each aqueous wash was extracted with CH_2Cl_2 (2 x 500 mL). All organic layers were combined, dried, decanted, and evaporated to dryness. The residue was dissolved in a solution of 1% NaOH in MeOH (1.5 L). After 1 h, the solution was neutralized with 3M HCl and evaporated to dryness. The residue was dissolved in H_2O (1.5 L) and extracted with CH_2Cl_2 (8 x 500 mL). The organic layers were combined, dried, decanted, and evaporated to dryness. The crude product was purified by flash chromatography on 5

[4] Capon, B.; Overend, W.G.; Sobell, M. *Tetrahedron* **1961**, *16*, 106.

Biotage 40M columns with a gradient solvent system of 4:1 hexanes/EtOAc to 1:1 hexanes/EtOAc to result in pure **1** as a clear oil (30.10 g, 105.6 mmol, 86%, $R_f = 0.3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.73 (d, J = 3.5 Hz, 1H), 3.60-3.53 (m, 3H), 3.49 (s, 3H), 3.49-3.43 (m, 1H), 3.35-3.24 (m, 8H), 3.11 (dd, J = 9.1, 3.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 97.3, 82.7, 81.7, 71.6, 69.9, 61.2, 58.5, 55.3, 33.5. HRMS (FAB) calcd for $C_9H_{17}BrO_5Li$ $[M + Li]^+$: 291.041939; found: 291.041570.

Determination of the second-order rate constant for the reaction of DIMAC (6) and benzyl azide.



6 **11** **12**

Scheme S2: Reaction of DIMAC (6) with benzyl azide.

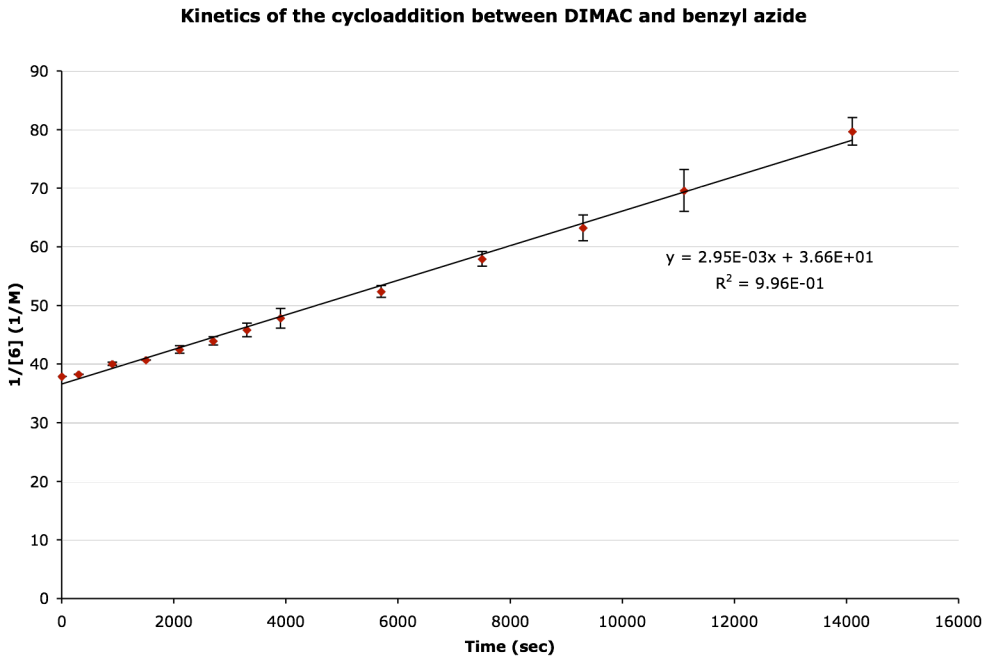
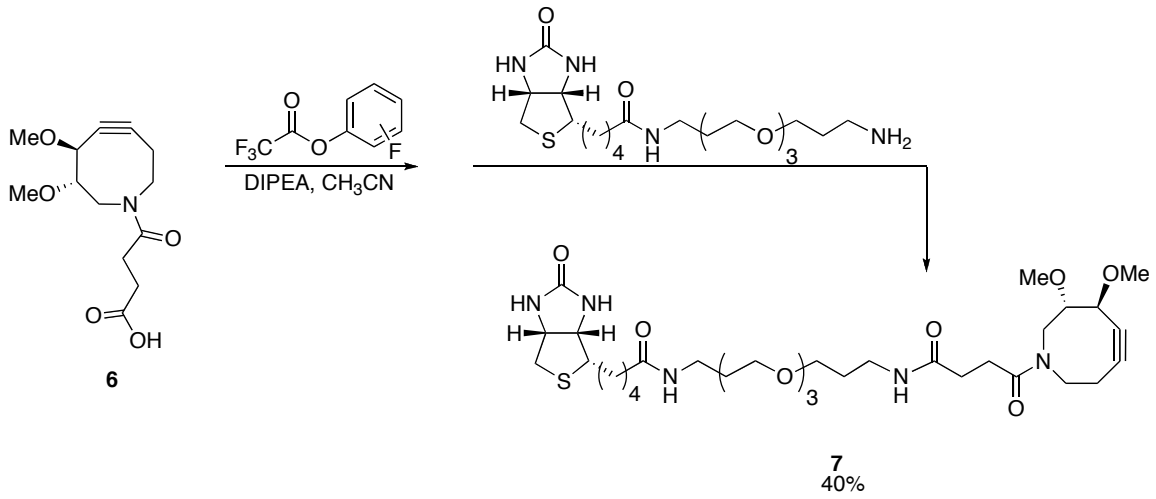


Figure S1. The reaction in Scheme S2 was monitored by ^{1}H NMR for 4 h at 25 °C. DIMAC and benzyl azide were separately dissolved in CD_3CN and mixed together in a 1:1 ratio at concentrations between 20 and 30 mM. *Tert*-butyl acetate (approx 0.3 equiv) was used as an internal standard. The percent conversion was calculated by the

disappearance of DIMAC relative to the *tert*-butyl acetate as determined by integration. No products other than **11** and **12** were apparent by ^{1}H NMR. The triazole isomers were produced in a ~ 1:1.4 ratio. The second-order rate constant was determined by plotting 1/[**6**] versus time. The plot was fit to a linear regression and the slope corresponds to the second-order rate constant. Shown are data from two replicate experiments.



Scheme S3. Synthesis of DIMAC- biotin conjugate 7.

DIMAC-biotin conjugate (7). DIMAC (**6**) (4.8 mg, 0.018 mmol, 1 equiv) was dissolved in CH_3CN (1 mL, anhyd) and cooled to 0 °C. *N,N*-Diisopropylethylamine (10 μL, 0.06, 3 equiv) was added and this solution was stirred under N_2 for 10. Pentafluorophenyl trifluoroacetate (10 μL, 0.058 mmol, 3 equiv) was then added dropwise and the reaction was allowed to warm to rt. The reaction was monitored by TLC (1:1 toluene/acetone) for the disappearance of **6** (R_f = 0.3-0.4). Upon reaction completion (approx 1 h), the mixture was filtered, and the filtrate was evaporated to dryness. Pentafluorophenyl activated DIMAC was purified via flash chromatography using silica gel and anhyd toluene and anhyd ether in a gradient solvent system of 10:1 toluene/ether to 4:1 toluene/ether. This product was dried under reduced pressure and immediately used for the coupling to biotin. *N*-(13-amino-4,7,10-trioxatridecanyl)biotinamide[5] (7.8 mg, 0.018 mmol, 1 equiv) was dissolved in DMF (0.5 mL, anhyd) and cooled to 0 °C. *N,N*-Diisopropylethylamine (2 drops) was added. The pentafluorophenyl activated DIMAC was dissolved in DMF (0.5 mL, anhyd) and this solution was added dropwise to the biotin solution at 0 °C. Upon addition of all activated DIMAC, the reaction was warmed to rt and monitored by ESI-MS for the formation of **7** ($[M+H]^+$ = 698, $[M+Na]^+$ = 720). Upon reaction completion (approx 6 h), the mixture was evaporated to dryness and purified by flash chromatography on silica gel. A gradient solvent system was used beginning at 50:3:1 EtOAc/MeOH/H_2O and ending with 8:3:1 EtOAc/MeOH/H_2O. This procedure resulted in pure **7** (5.0 mg, 0.0072 mmol, 40%). R_f = 0.4 in 5:3:1 EtOAc/MeOH/H_2O. ^{1}H NMR (500 MHz, D_2O): 4.58 (dd, *J* = 7.9, 4.9 Hz, 1H), 4.40 (dd, *J* = 7.9, 4.5 Hz, 1H), 4.36 (dt, *J* = 7.8, 2.0 Hz, 0.1H), 4.22 (dt, *J* = 8.6, 2.5 Hz, 1H), 4.12

[5] Wilbur, D.S.; Hamlin, D.K.; Vessella, R.L.; Stray, J.E.; Buhler, K.R.; Stayton, P.S.; Klumb, L.A.; Pathare, P.M.; Weerawarna, S.A. *Bioconjug. Chem.* **1996**, *7*, 689.

(dd, 14.9, 5.4 Hz, 0.9H), 4.05 (d, J = 14.2 Hz, 0.9H), 4.00 (d, J = 16.2 Hz, 0.1H), 3.82 (t, J = 8.4 Hz, 0.1H), 3.71-3.65 (m, 8.9H), 3.57-3.45 (m, 7.1H), 3.37-3.19 (m, 8.9H), 3.03 (dd, J = 14.3, 9.0 Hz, 1H), 2.97 (dd, J = 13.1, 5.0 Hz, 1H), 2.92-2.88 (m, 0.1H), 2.79-2.75 (m, 2.9H), 2.65-2.62 (m, 1H), 2.58-2.49 (m, 2H), 2.32 (dt, J = 16.8, 3.0 Hz, 0.9H), 2.24 (t, J = 7.2 Hz, 2.1H), 1.79-1.53 (m, 8H), 1.44-1.33 (m, 2H). ^{13}C NMR (125 MHz, D_2O): 176.7, 174.7, 174.5, 165.2, 98.9, 89.9, 84.3, 76.1, 69.5, 69.3, 68.4, 68.3, 62.0, 60.2, 58.0, 56.5, 55.3, 54.2, 52.0, 39.6, 36.3, 36.2, 35.4, 30.7, 28.24, 28.18, 27.8, 27.6, 25.1, 20.8. HRMS (ESI) calcd for $C_{33}H_{55}N_5O_9SNa$ $[M + Na]^+$: 720.3618; found: 720.3593.

Cell culture procedures.

Jurkat cells (human T-cell lymphoma) were maintained in RPMI-1640 media (Invitrogen Life Technologies) supplemented with 10% fetal calf serum (FCS), penicillin (100 units/mL), and streptomycin (0.1 mg/mL) in a 5% CO_2 water-saturated atmosphere. The cells were maintained at densities between 1×10^5 and 1.6×10^6 cells/mL.

Western blot analysis of azide-labeled cell lystates.

Jurkat cells were grown in media supplemented with or without 25 μM Ac_4ManNAz (+Az or –Az respectively) for 3 d. The cells were concentrated (500 × *g*, 3 min) and washed three times by sequential resuspension with 10 mL chilled PBS and concentration. The cell pellet was resuspended in lysis buffer (150 mM NaCl, 20 mM Tris, 1% NP-40 substitute (Sigma-Aldrich), pH 7.4; 500 μL buffer per 2.8×10^7 cells) containing protease inhibitors (Roche) and disrupted by sonication with one 30-s 6 W pulse. Following sonication, insoluble debris were removed by centrifugation (20000 × *g*, 10 min) and the supernatant was kept. The protein concentration of each lysate was determined using the Bio-RAD® D_c protein assay.

Thirty-five micrograms of total protein from each lysate was reacted with 250 μM **7**, **8**, or no reagent overnight at 25 °C. SDS-PAGE loading buffer (4X, BioRAD) was added to each sample and the proteins were separated via electrophoresis and transferred to a nitrocellulose membrane. Equal protein loading and successful transfer were confirmed by visualizing the proteins with Ponceau S. The membrane was blocked with 5-10% BSA in PBST (PBS pH 7.4 containing 0.1% Tween 20) for 2 h at rt. The membrane was incubated with horseradish peroxidase-conjugated α-biotin (1:100,000 dilution, Jackson Laboratories) in PBST for 1 h. The membrane was washed with PBST (3 x 15 min). Detection was performed by chemiluminescence using Pierce SuperSignal West Pico Chemiluminescent Substrate.

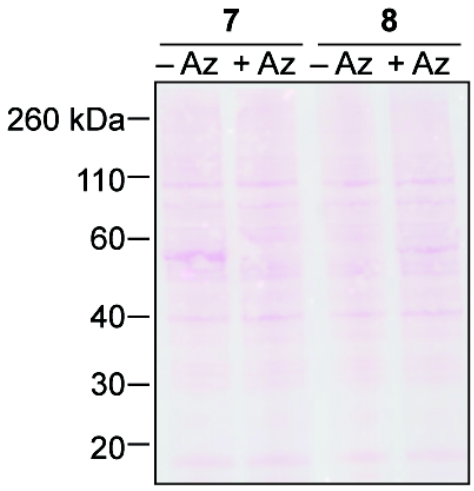


Figure S2: Ponceau S staining of Western blot in Figure 2B.

Cell surface azide labeling and detection.

Jurkat cells were incubated in untreated media or media containing 25 μM Ac_4ManNAz. After 3 d, the cells were twice concentrated (500 x *g*, 3 min, 4 °C) and resuspended in 10 mL FACS buffer (PBS containing 1% FCS, 2 x 10 mL) and cells (approx 500,000 per a well) were placed in a 96 well V-bottom plate. The cells were concentrated by centrifugation (2500 x g, 3 min, 4 °C), resuspended in 200 μL cold FACS buffer, and again concentrated by centrifugation (2500 x *g*, 3 min, 4 °C). The cells were then reacted for 1 h (unless otherwise noted) at rt with the desired reagent (no reagent, **7**, **8**, difluorinated cyclooctyne or triarylphosphine). After 1 h, the cells were thrice concentrated by centrifugation (2500 x *g*, 3 min, 4 °C) and resuspended in 200 μL cold FACS buffer. Following and additional concentration by centrifugation (2500 x *g*, 3 min, 4 °C), cells were resuspended in FACS buffer (100 μL) containing FITC-avidin (1:200 dilution of 1 mg/mL stock, Sigma-Aldrich) and incubated in the dark at 4 °C for 15 min. Following the incubation, cells were concentrated by centrifugation, resuspended in 200 μL cold FACS buffer, concentrated by centrifugation, and another FITC-avidin incubation was performed. After the second FITC-avidin labeling, the cells were thrice concentrated by centrifugation (2500 x *g*, 3 min, 4 °C) and resuspended in 200 μL cold FACS buffer. The cells were then diluted to 400 μL for flow cytometry analysis. Flow cytometry was performed on a BD Biosciences FACSCalibur flow cytometer equipped with a 488-nm argon laser. All flow cytomery experiments were performed with three replicate samples.

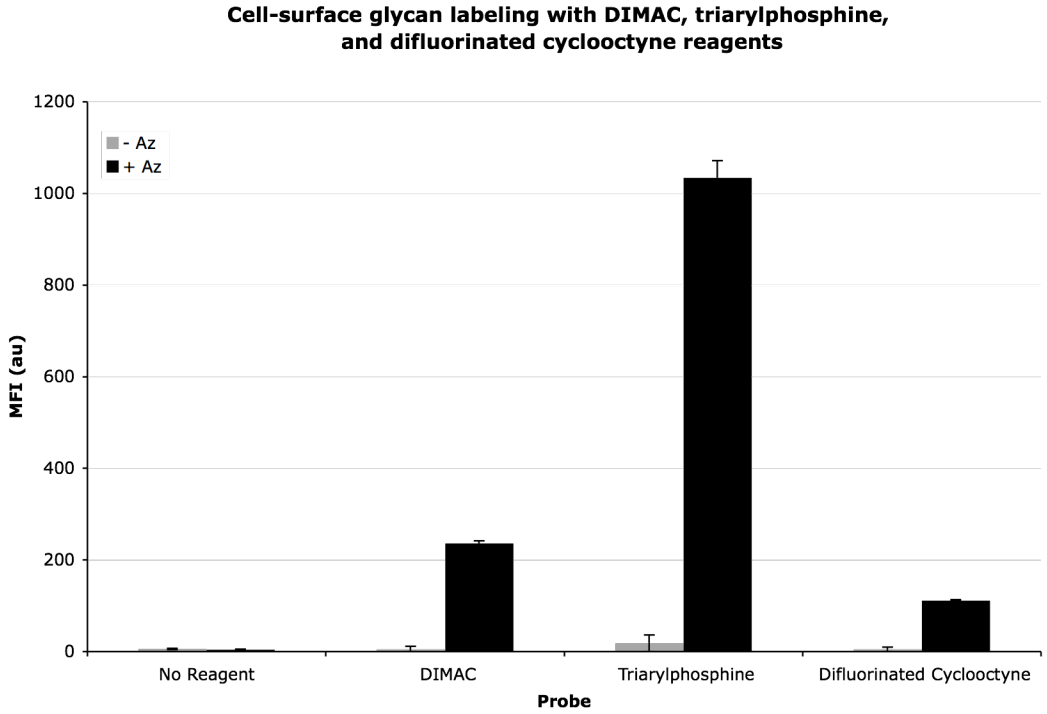


Figure S4. (A) Cell-surface glycan labeling with DIMAC-biotin conjugate **7**, a difluorinated cyclooctyne-biotin conjugate,[6] and a triarylphosphine-biotin conjugate.[7] Jurkat cells were incubated in the presence (+ Az, black bars) or absence (– Az, gray bars) of 25 μM $Ac_4ManNAz$ for 3 d. Cells were reacted with no reagent (FACS buffer), DIMAC- biotin conjugate **7** (200 μM **7** in FACS buffer), triarylphosphine-biotin conjugate (200 μM reagent in FACS buffer with 3% DMF), or difluorinated cyclooctyne-biotin conjugate (2 μM reagent in FACS buffer with 0.03% DMF) for 1 h at 25 °C, incubated with FITC-avidin, and analyzed by flow cytometry. The error bars represent standard deviations from three replicate samples. MFI = mean fluorescence intensity and has arbitrary units (au).

[6] Baskin, J.M.; Prescher, J.A.; Laughlin, S.T.; Agard, N.J.; Chang, P.V.; Miller, I.A.; Lo, A.; Codelli, J.A.; Bertozzi, C.R. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 16793-16797.
[7] Saxon, E.; Bertozzi, C.R. *Science* **2000**, *287*, 2007-2010.

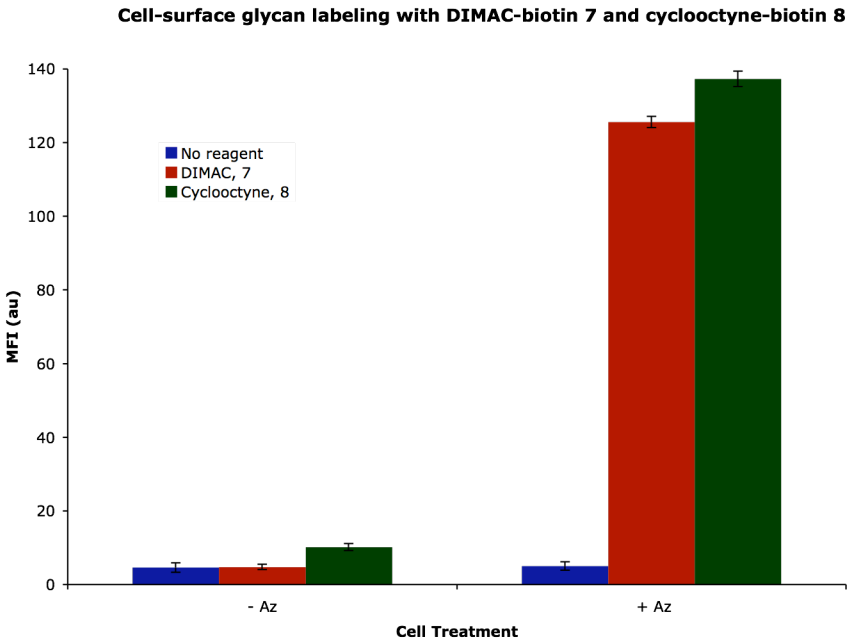


Figure S4. Cell-surface glycan labeling with DIMAC-biotin conjugate **7** and parent cyclooctyne-biotin conjugate **8.** Jurkat cells were incubated in the presence (+ Az) or absence (– Az) of $Ac_4ManNAz$ for 3 d. Cells were reacted with no reagent (FACS buffer containing 3% DMF, blue bars), DIMAC- biotin conjugate **7** (250 μM **7** in FACS buffer containing 3% DMF, red bars) or cyclooctyne-biotin conjugate **8** (250 μM **8** in FACS buffer containing 3% DMF, green bars) for 1 h at 25 °C, incubated with FITC-avidin, and analyzed by flow cytometry. The error bars represent standard deviations from three replicate samples.

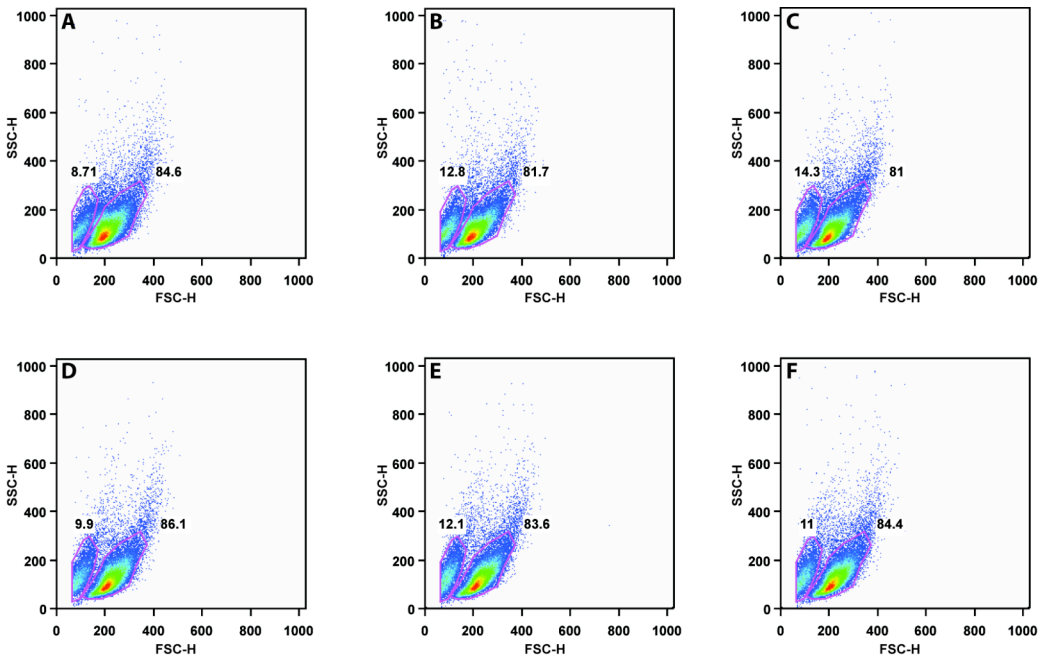


Figure S5. Representative forward-scatter (x-axis, FSC-H) and side-scatter (y-axis, SSC-H) plots for the experiment described in Figure S4. Jurkat cells were treated with (A, B, C) or without (D, E, F) 25 μM Ac_4ManNAz for 3 d and then treated with no reagent (A, D), 250 μM DIMAC-biotin conjugate **7** (B, E), or 250 μM cyclooctyne-biotin conjugate **8** (C, F) followed by FITC-avidin.

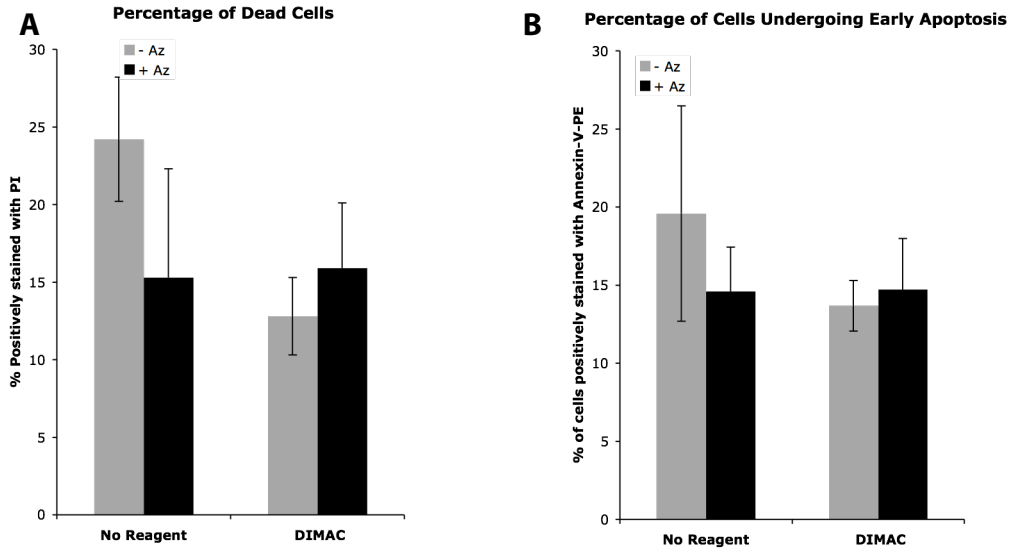


Figure S6. Cytotoxicity analysis of DIMAC-biotin **7**. (A,B) Jurkat cells were incubated in the presence (+ Az) or absence (– Az) of Ac_4ManNAz for 3 d. Cells were reacted with no reagent (FACS buffer) or DIMAC- biotin conjugate **7** (250 μM **7** in FACS buffer) for 1 h at 25 °C, incubated with FITC-avidin, and washed. Prior to flow cytometry analysis, half the cells were treated with (A) propdium iodide and the other half were treated with (B) Annexin-V-PE using the BD Pharmingen™ Annexin-V Apoptosis Detection Kit following the provided procedure.[8] The samples were diluted and analyzed by flow cytometry. The error bars represent standard deviations from three replicate samples.

[8] http://www.bdbiosciences.com/external_files/pm/doc/tds/cell_bio/live/web_enabled/6710KK_556570.pdf

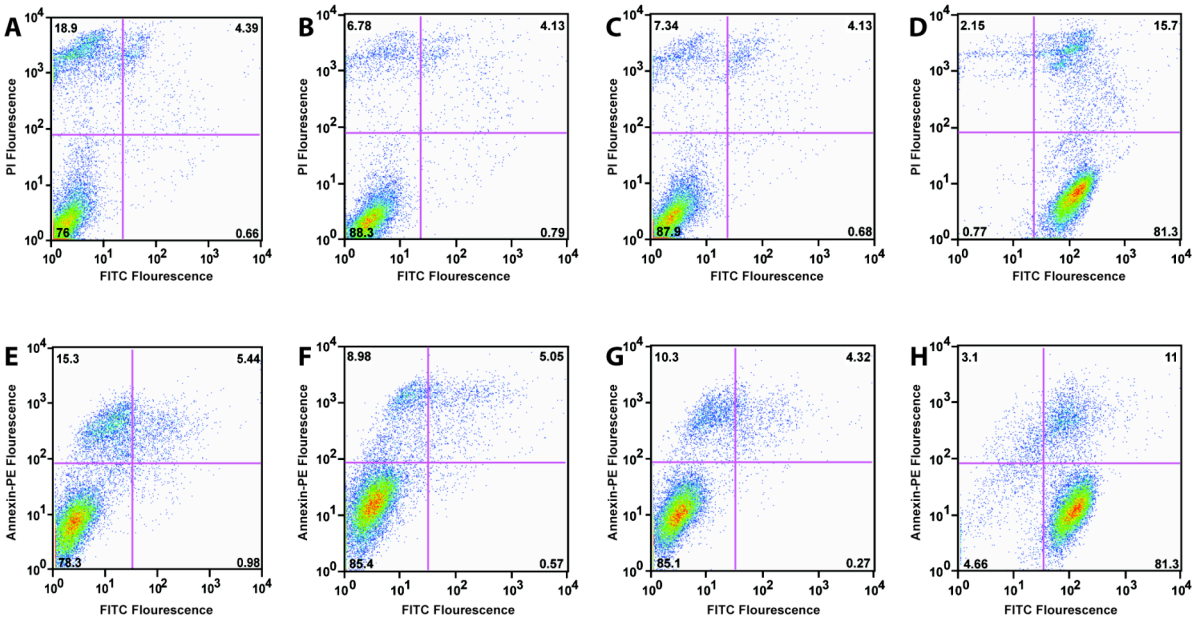


Figure S7. Representative FL2 vs. FL1 scatter plots for the flow cytometry experiments described in Figure S6. In all plots, the x-axis indicates the degree of cell-surface glycan labeling as measured by FITC fluorescence (FL1). For plots A-D, the y-axis represents the degree of propidium iodide staining (FL2, cell death marker), while the y-axis in plots E-H represents the degree of Annexin-V staining (FL2, early apoptosis marker). Jurkat cells were treated with (C, D, G, H) or without (A, B, E, F) 25 μM Ac_4ManNAz for 3 d. The cells were then treated with no reagent (A, C, E, G) or 250 μM DIMAC-biotin **7** (B, D, F, H) for 1 h at 25 °C followed by FITC-avidin. Cells were then subjected to propidium iodide (A-D) or Annexin-V-PE (E-H) and analyzed by flow cytometry.

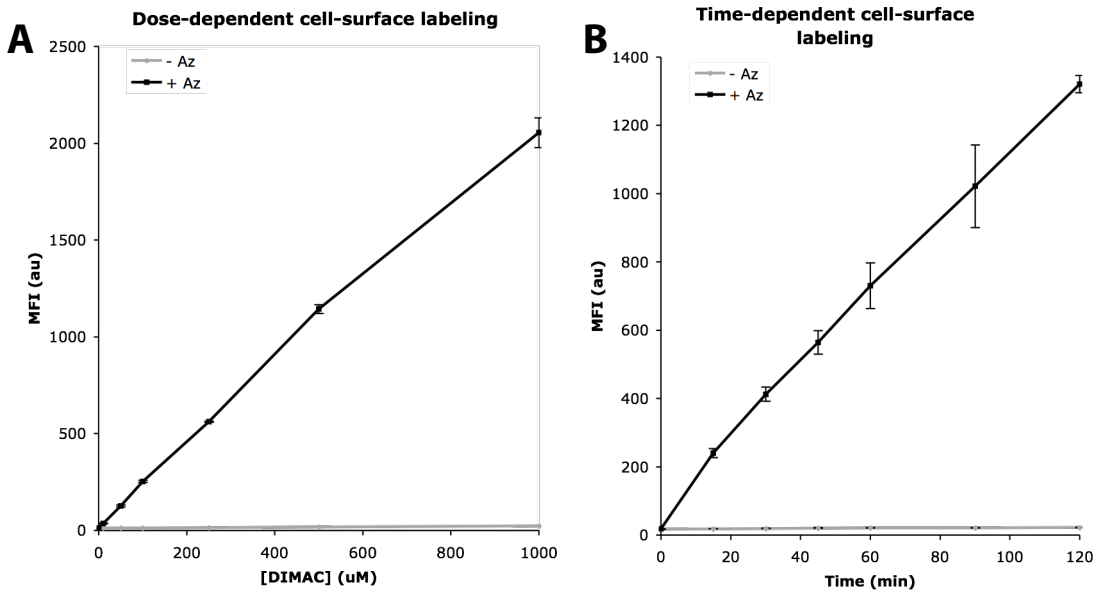
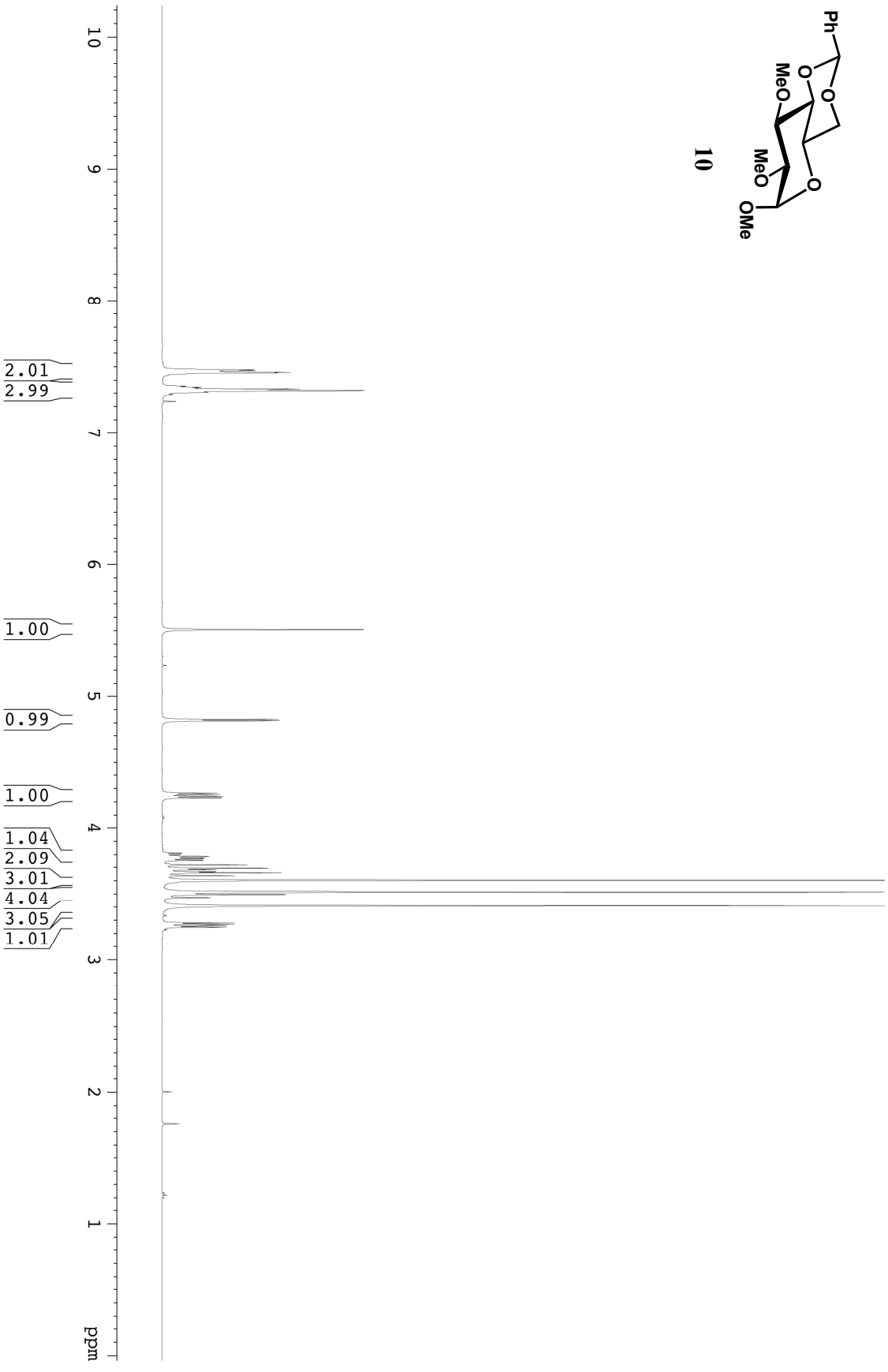


Figure S8. Dose- and time-dependent cell-surface glycan labeling with DIMAC-biotin **7**. Jurkat cells were grown in the presence (+ Az, black line) or absence (– Az, gray line) of

25 μM Ac_4ManNAz for 3 d. The cells were treated with DIMAC-biotin **7** in (A) varying concentrations for 1 h or (B) varying times at a concentration of 250 μM. The cells were then treated with FITC-avidin and analyzed by flow cytometry. The error bars represent standard deviations from three replicate samples.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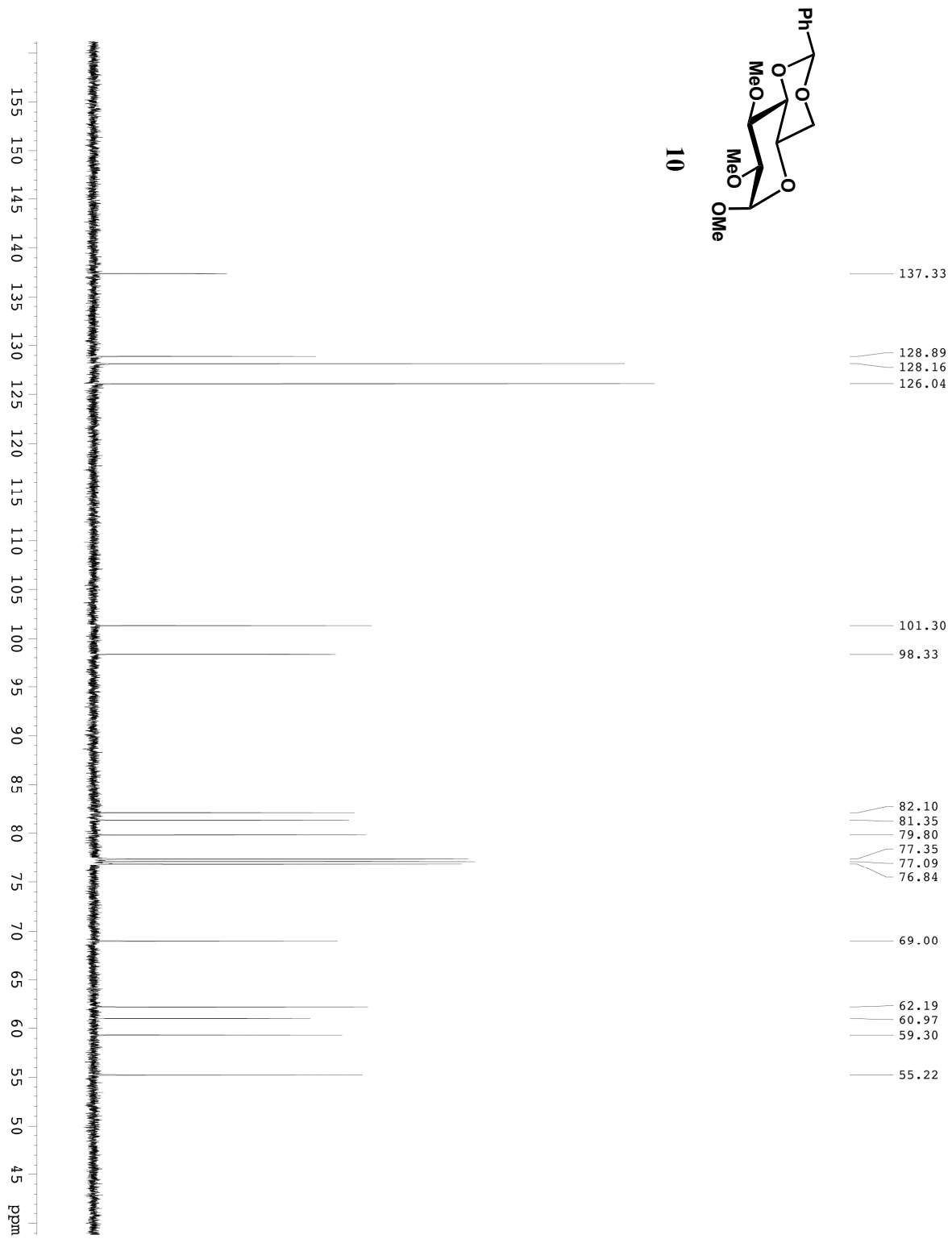
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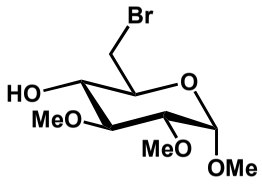
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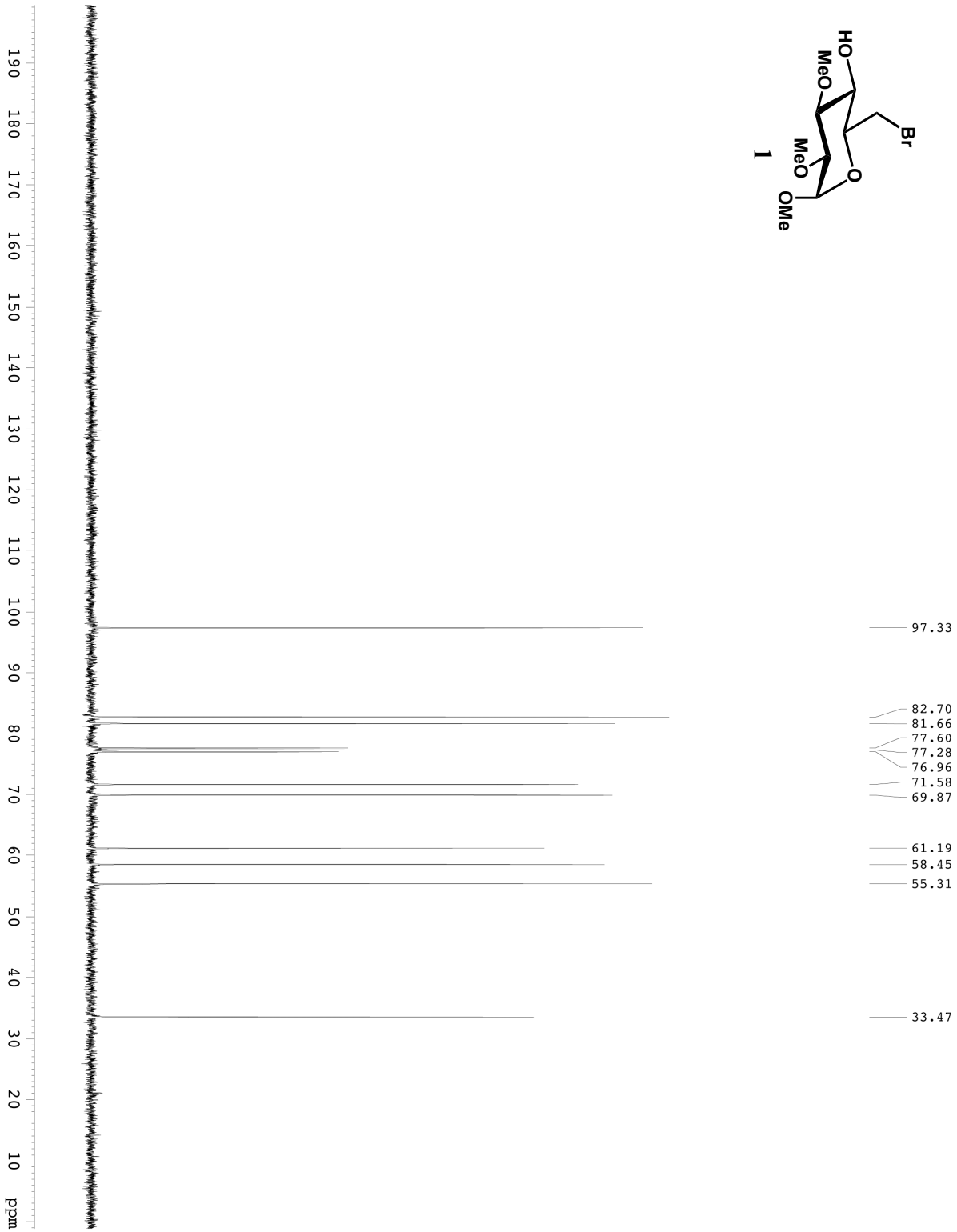
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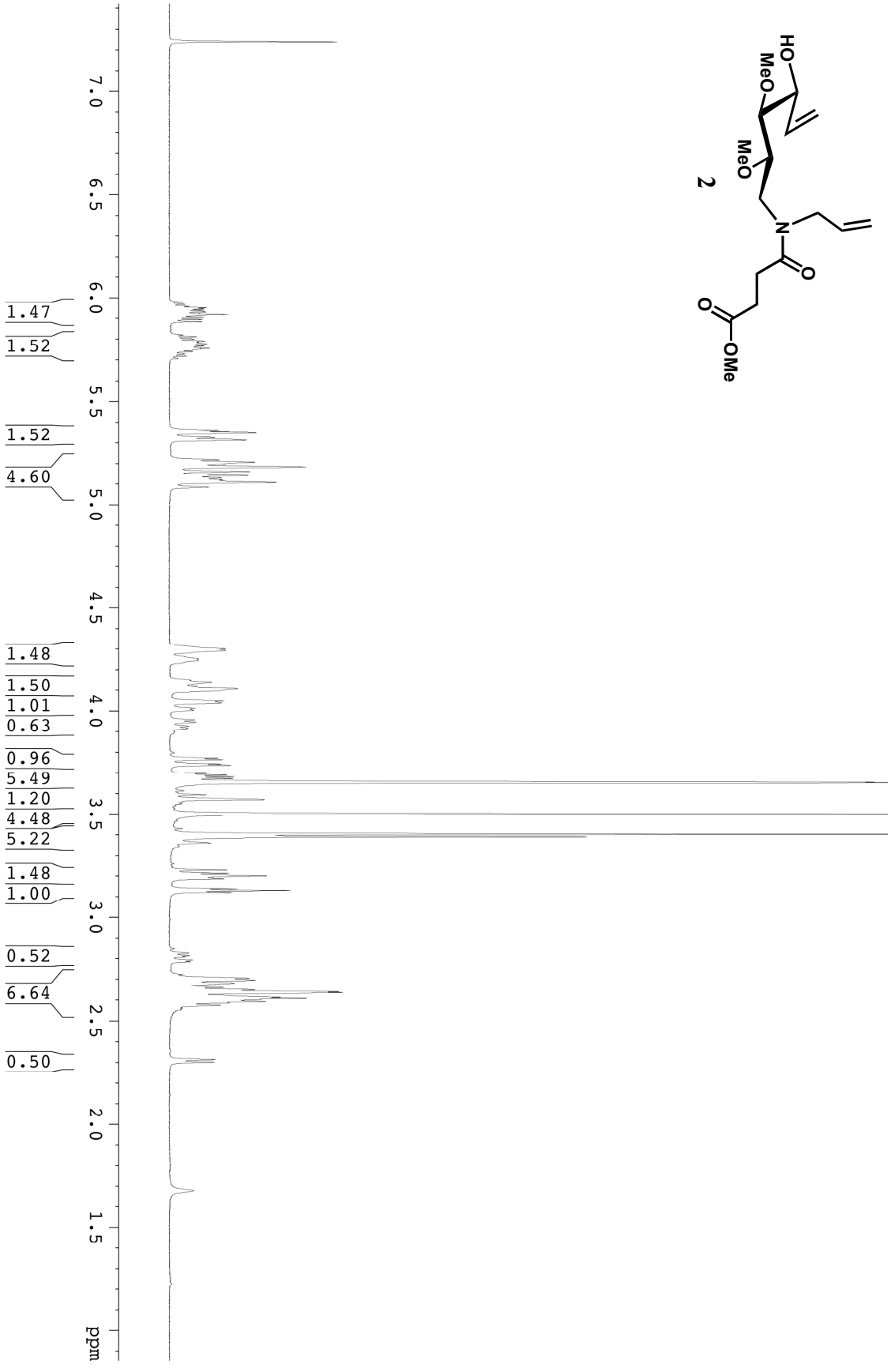
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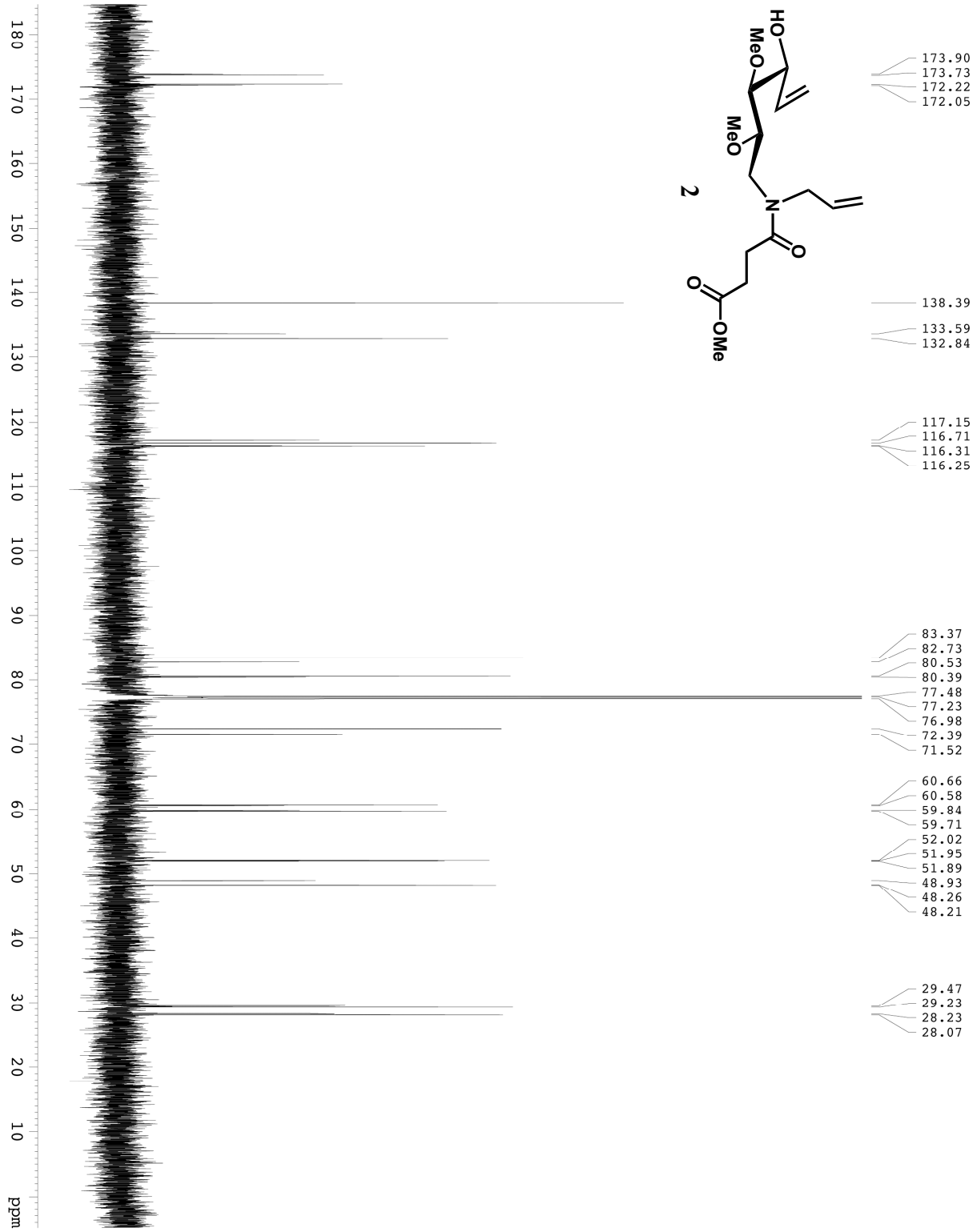
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59.71
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51.95
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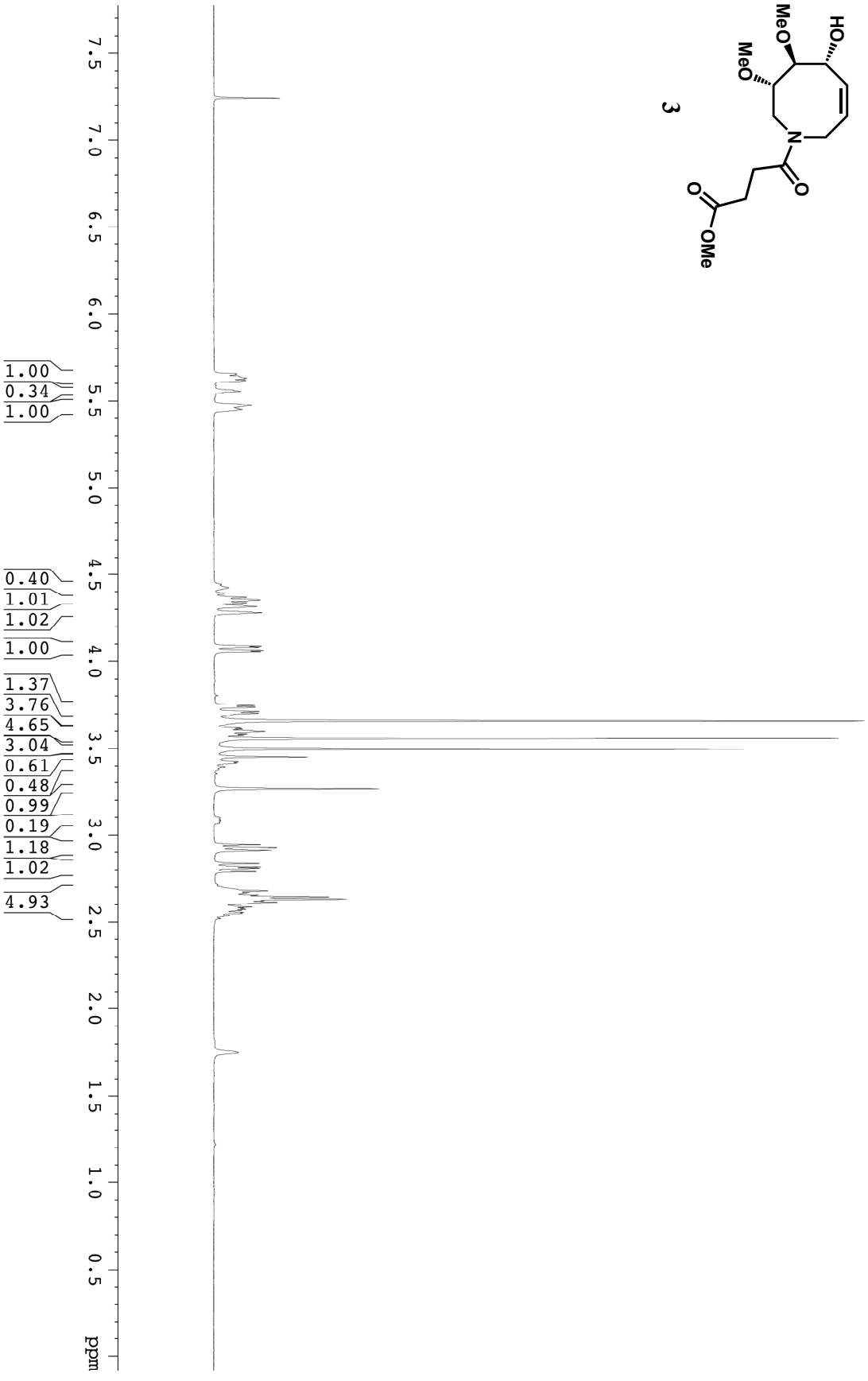
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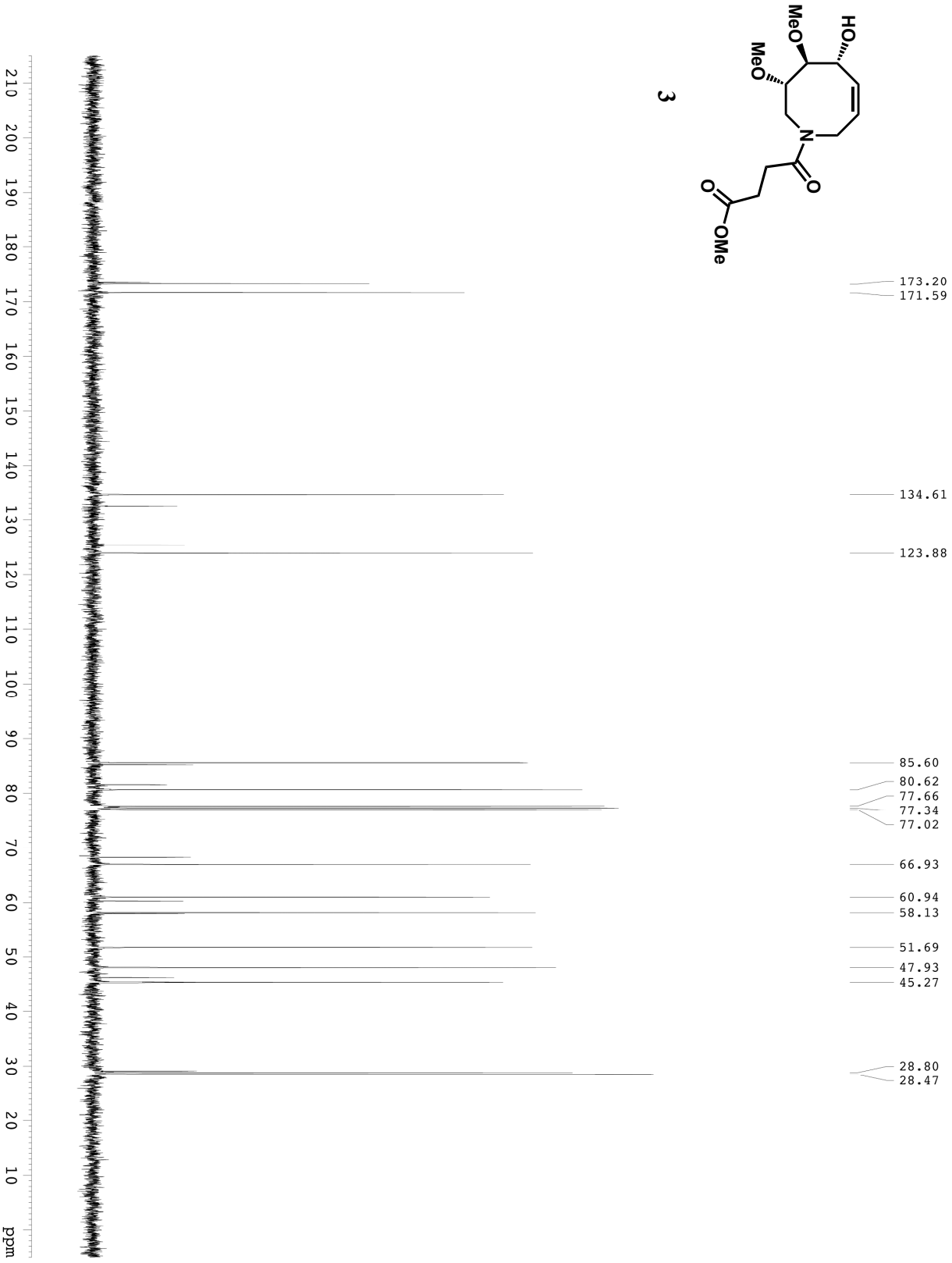
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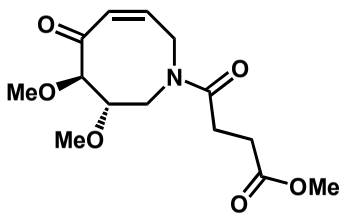

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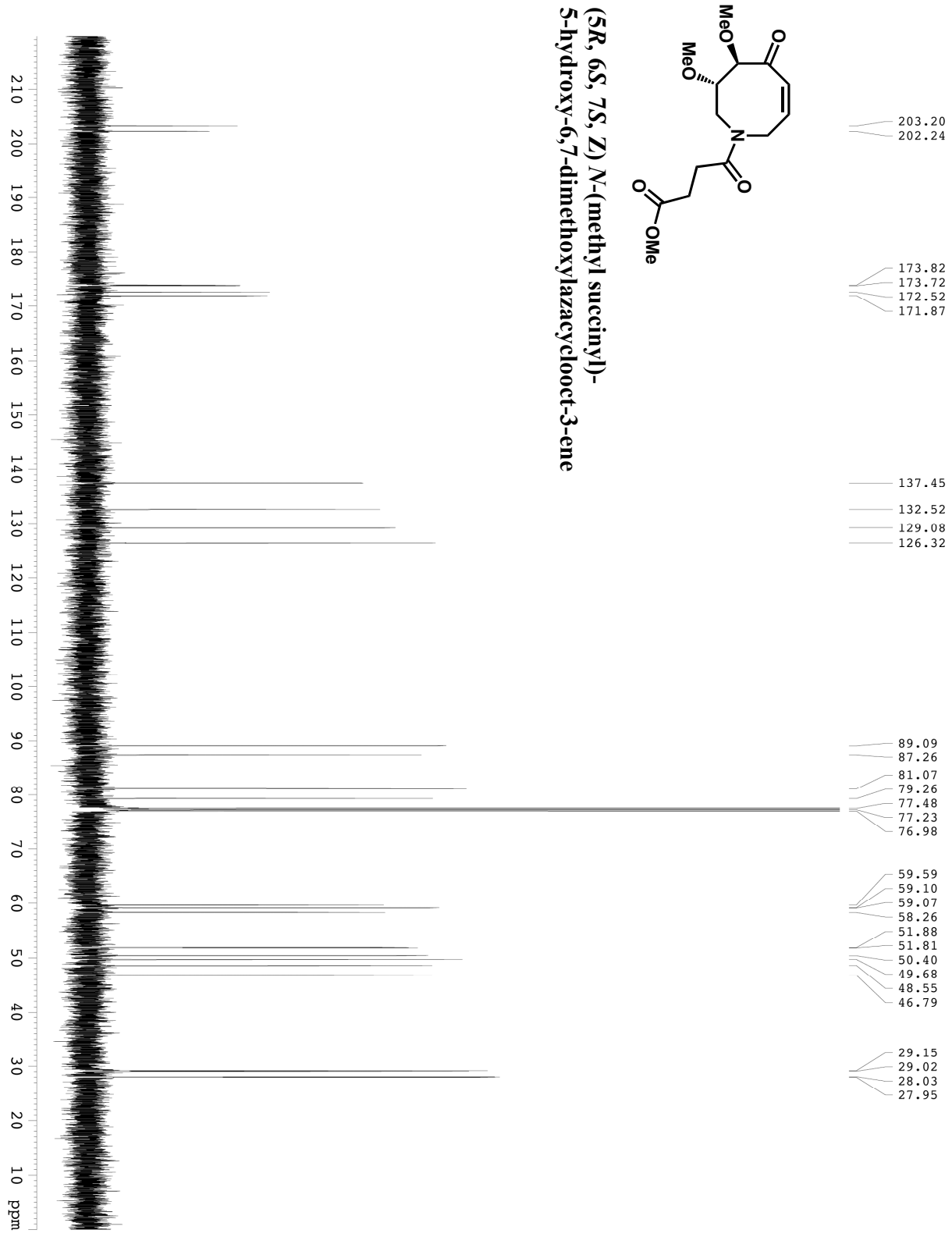
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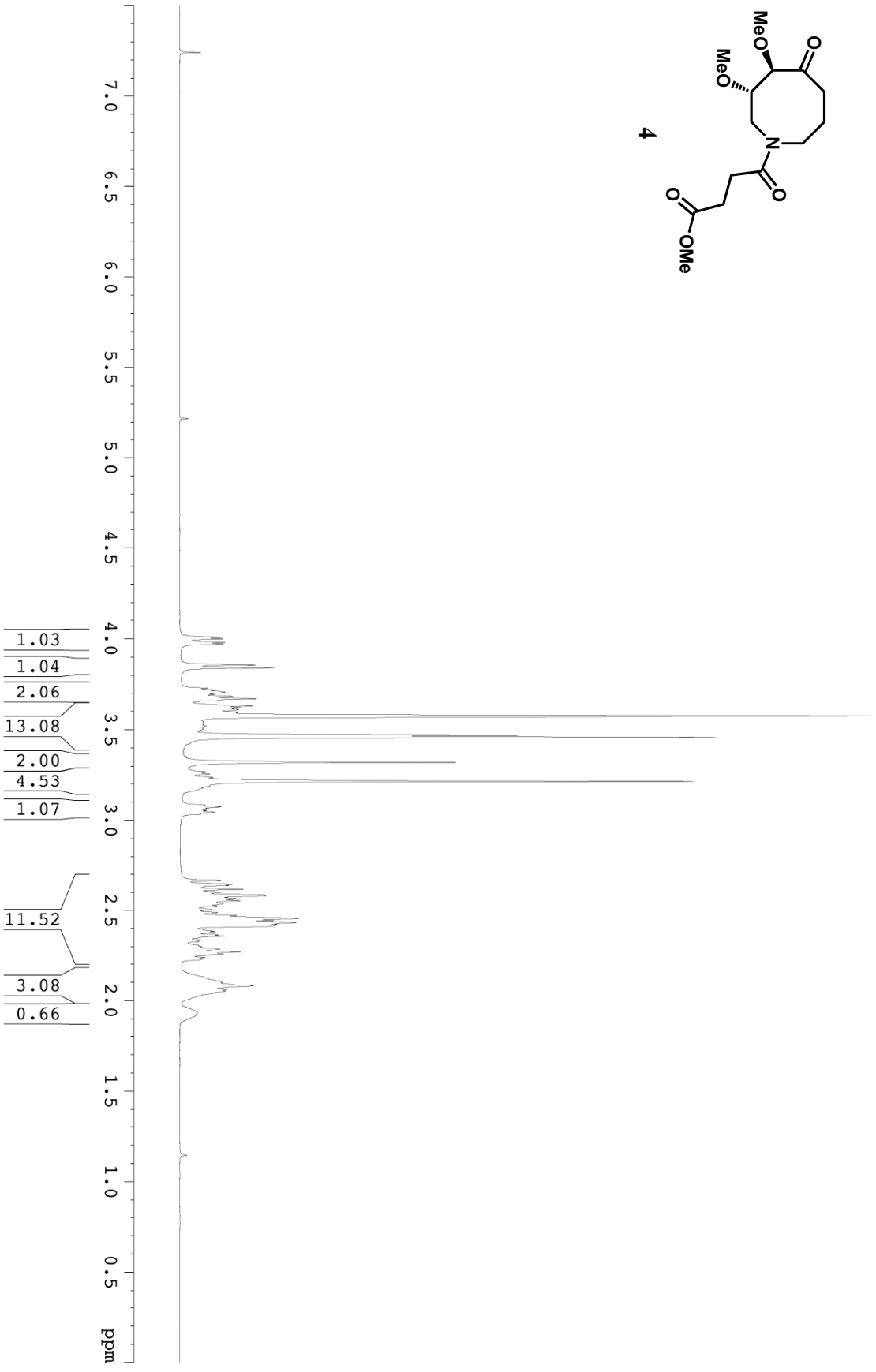
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3.0
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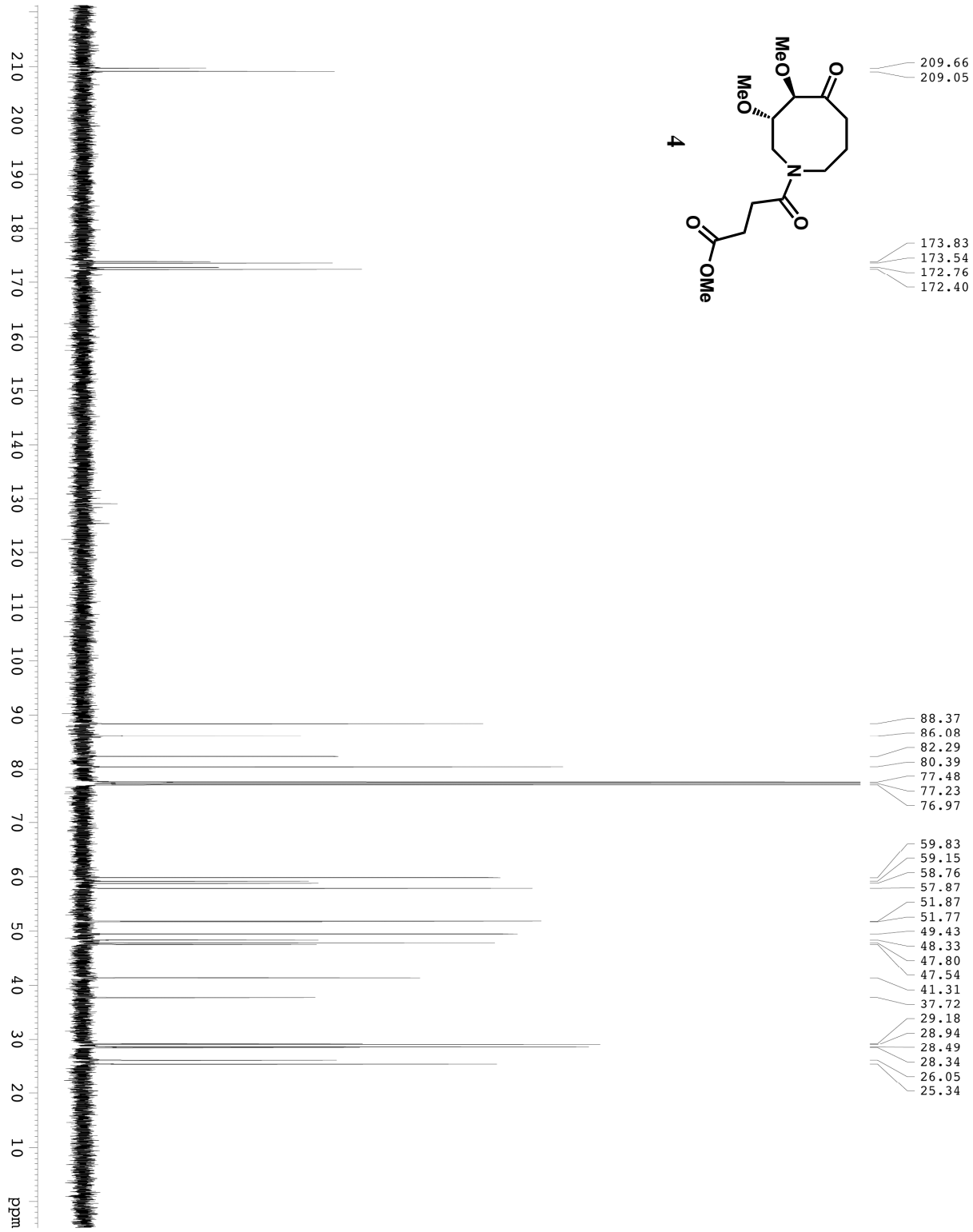
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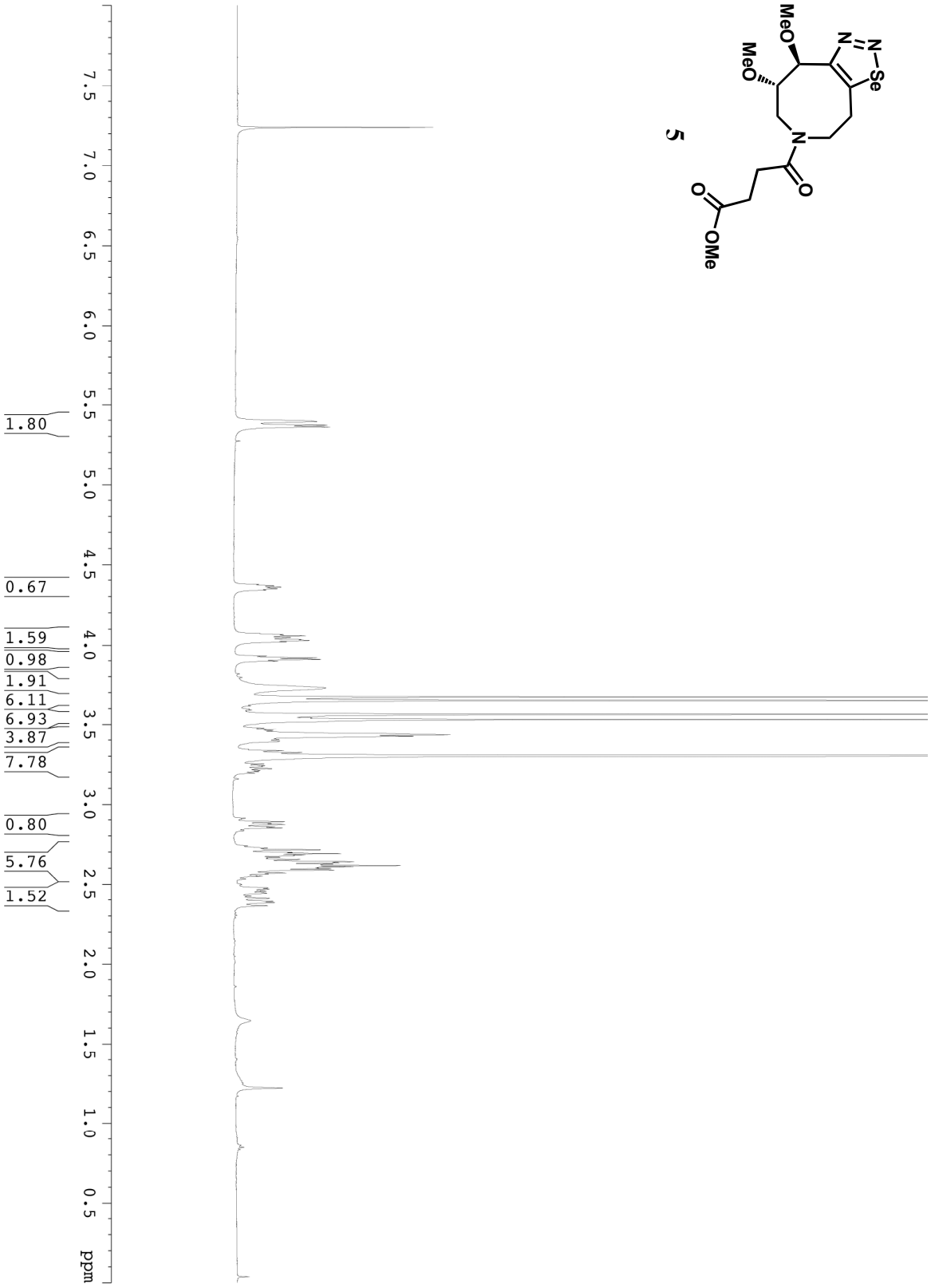
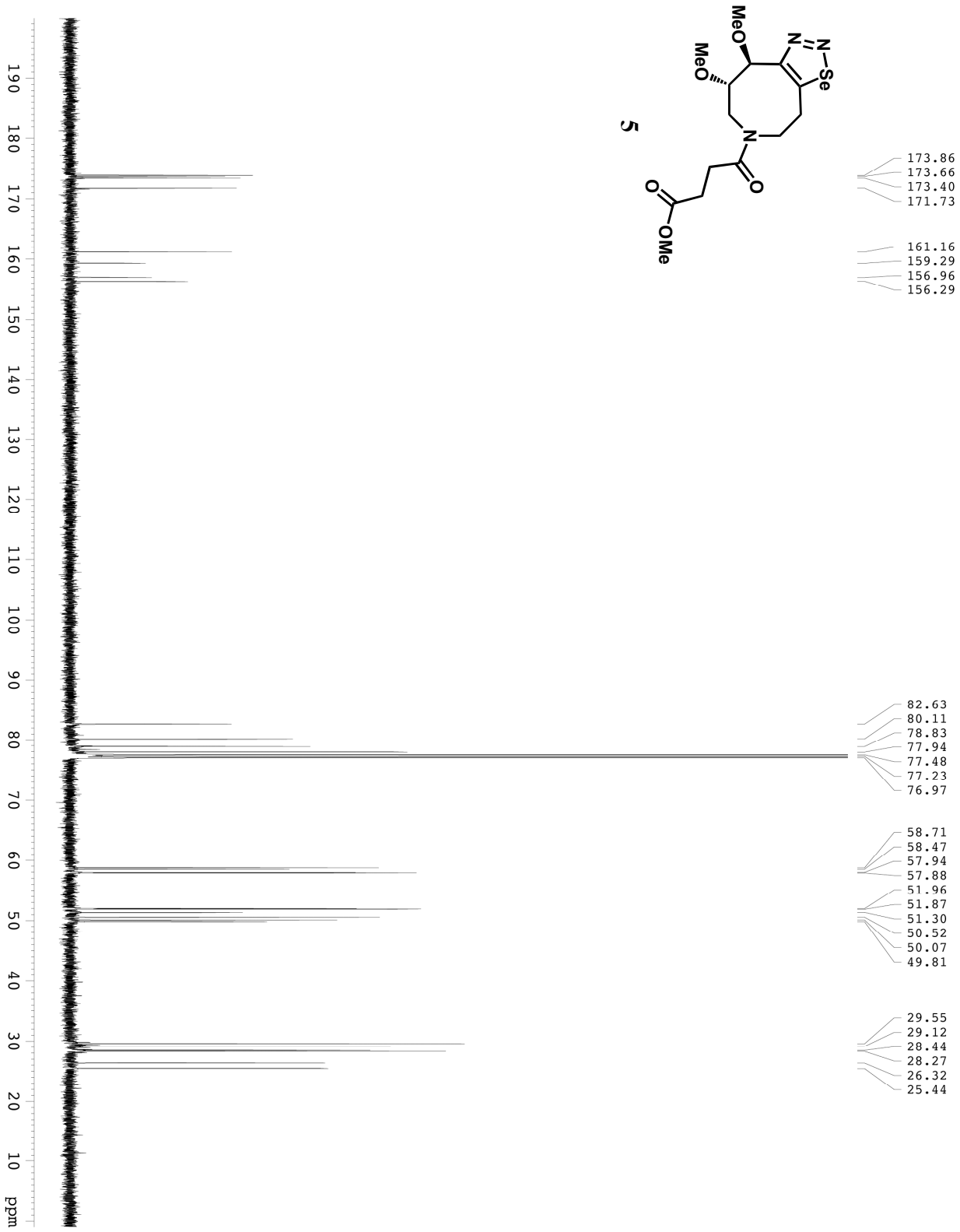
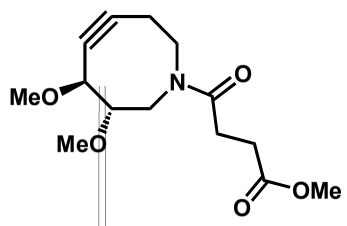
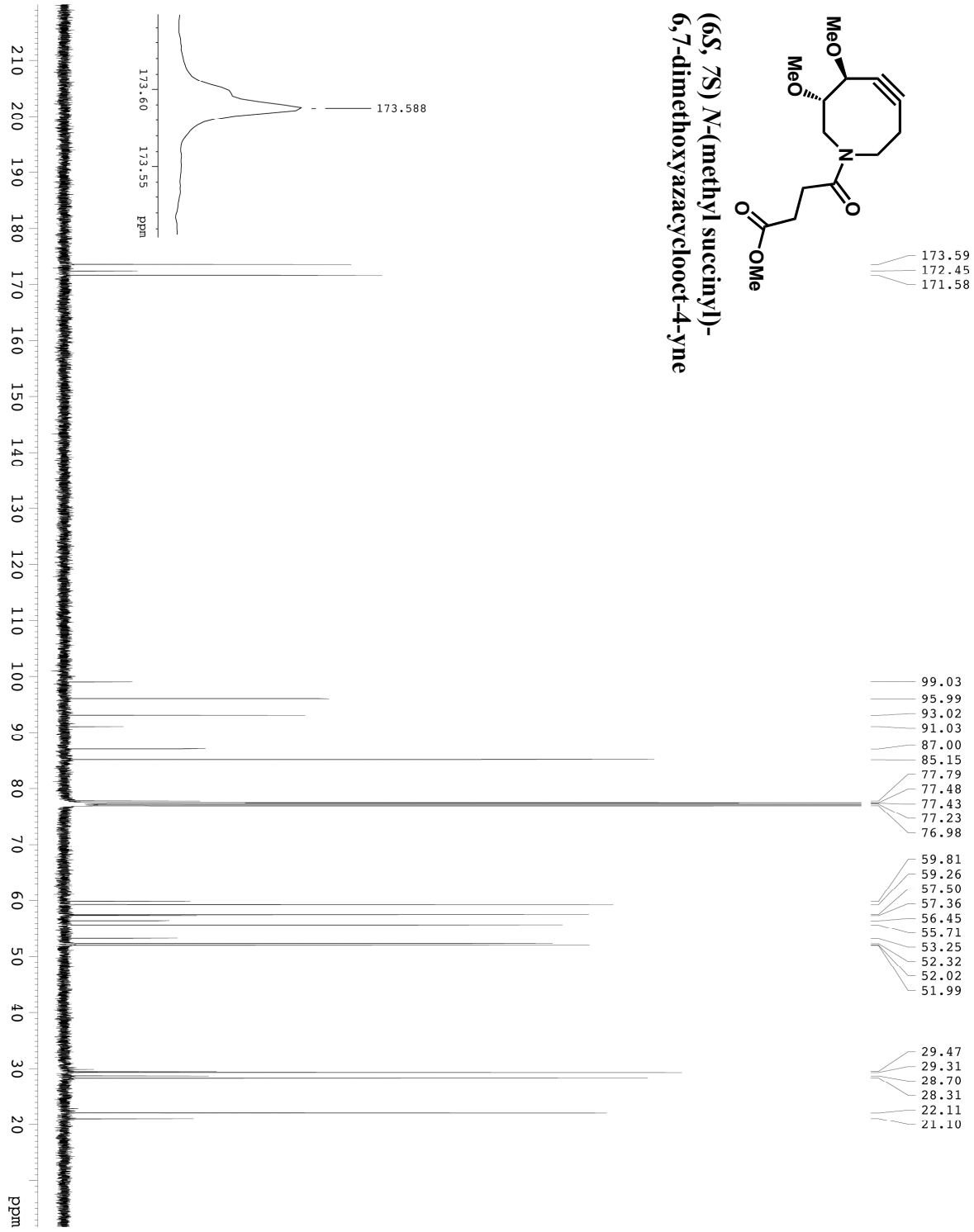
88.37
86.08
82.29
80.39
77.48
77.23
76.97
59.83
59.15
58.76
57.87
51.87
51.77
49.43
48.33
47.80
47.54
41.31
37.72
29.18
28.94
28.49
28.34
26.05
25.34
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

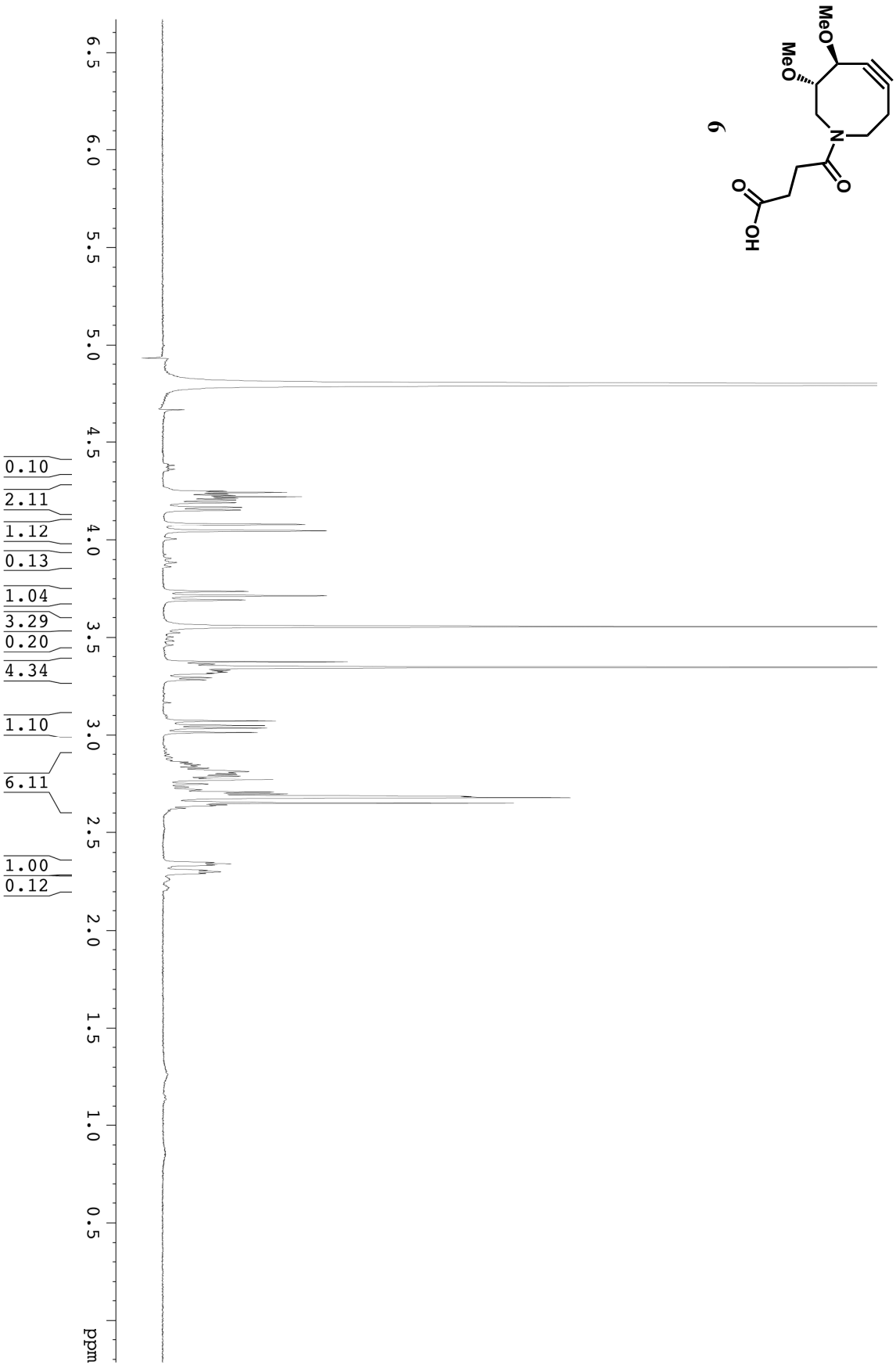

MeO
MeO
N
N
Se
N
O
O
OMe
5
1.80
0.67
1.59
0.98
1.91
6.11
6.93
3.87
7.78
0.80
5.76
1.52
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

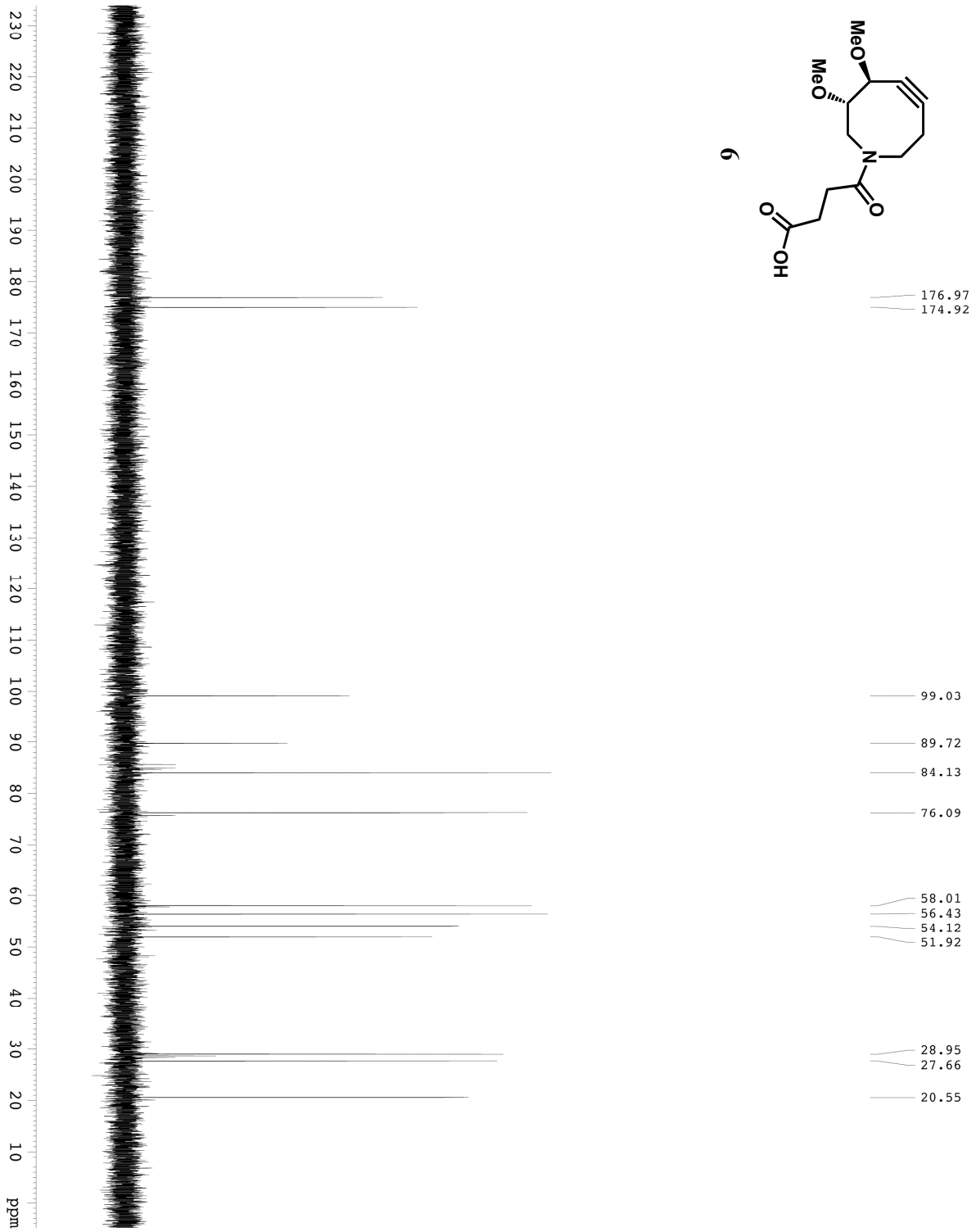

MeO
MeO
N
N
Se
O
O
O
OMe
5
173.86
173.66
173.40
171.73
161.16
159.29
156.96
156.29
82.63
80.11
78.83
77.94
77.48
77.23
76.97
58.71
58.47
57.94
57.88
51.96
51.87
51.30
50.52
50.07
49.81
29.55
29.12
28.44
28.27
26.32
25.44
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

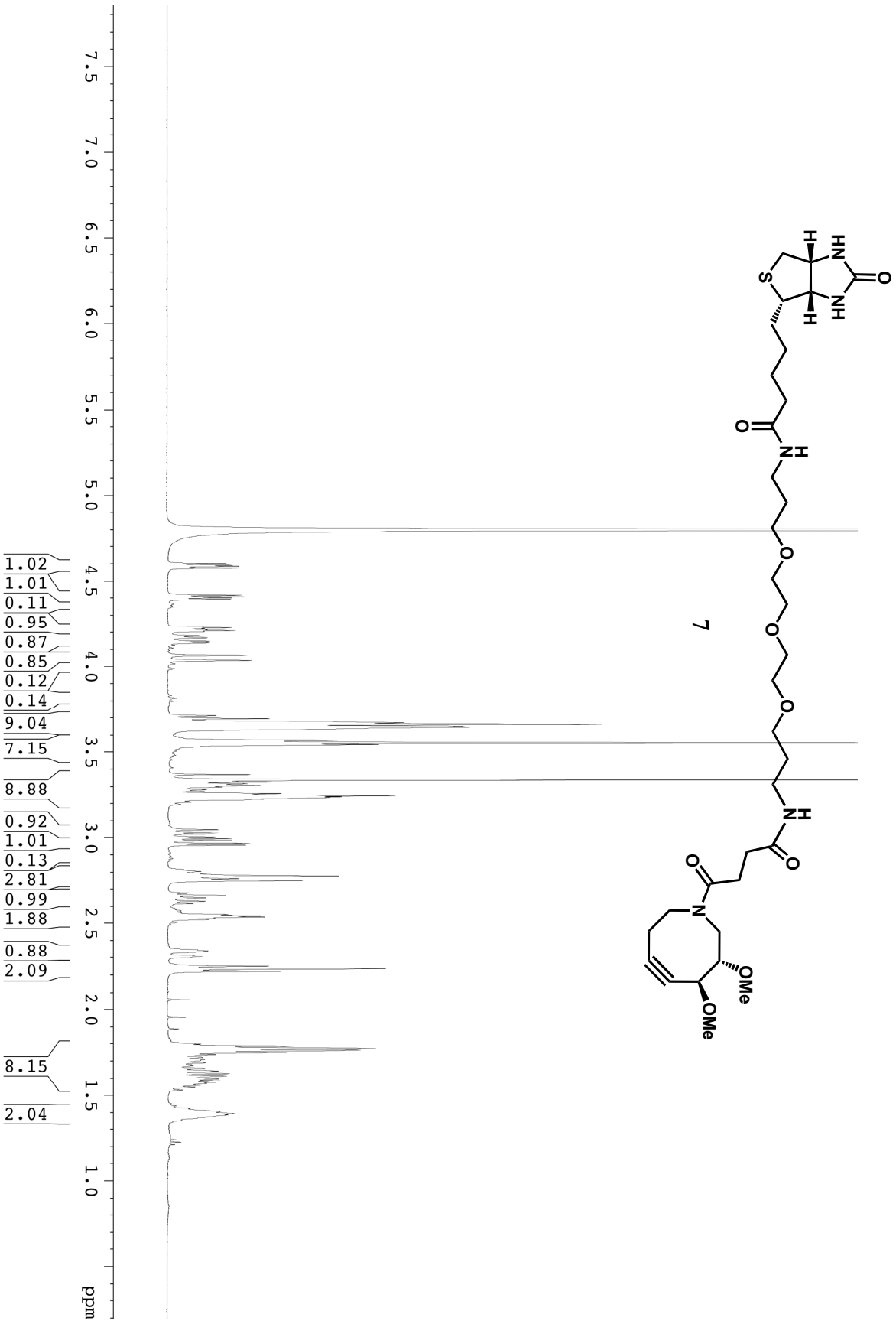


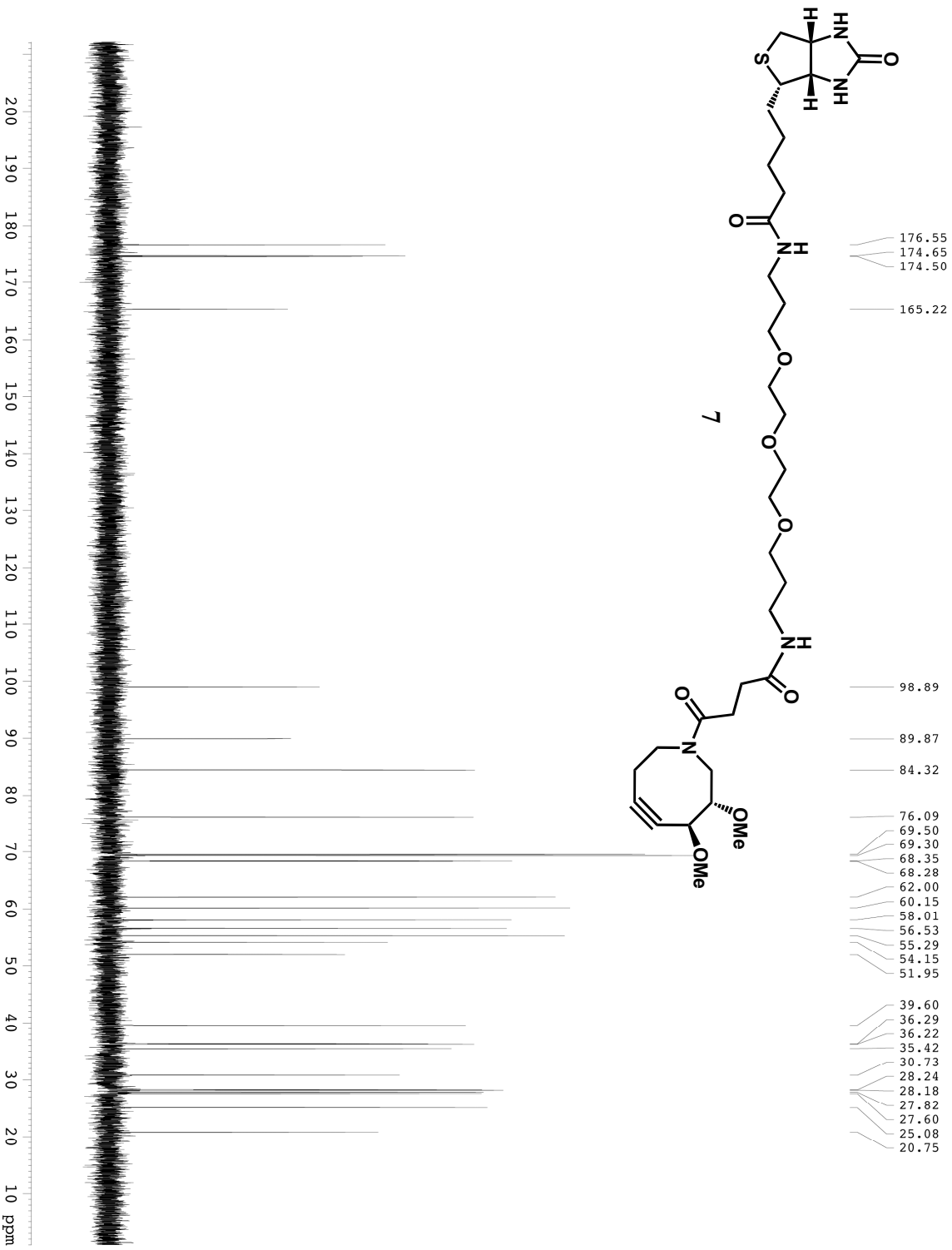
(6*S*, 7S) *N*-(methyl succinyl)-6,7-dimethoxyazacyclooct-4-yne


(6S, 7S) N-(methyl succinyl)-6,7-dimethoxyazacyclooct-4-yne
MeO
MeO
N
O
O
O
OMe
173.588
173.60
173.55
ppm
173.59
172.45
171.58
99.03
95.99
93.02
91.03
87.00
85.15
77.79
77.48
77.43
77.23
76.98
59.81
59.26
57.50
57.36
56.45
55.71
53.25
52.32
52.02
51.99
29.47
29.31
28.70
28.31
22.11
21.10
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
ppm


MeO
MeO
N
O
O
OH
9
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
0.10
2.11
1.12
0.13
1.04
3.29
0.20
4.34
1.10
6.11
1.00
0.12


MeO
MeO
N
O
O
OH
9
176.97
174.92
99.03
89.72
84.13
76.09
58.01
56.43
54.12
51.92
28.95
27.66
20.55
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.02
1.01
0.11
0.95
0.87
0.85
0.12
0.14
9.04
7.15
8.88
0.92
1.01
0.13
2.81
0.99
1.88
0.88
2.09
8.15
2.04
7
OMe
OMe


176.55
174.65
174.50
165.22
98.89
89.87
84.32
76.09
69.50
69.30
68.35
68.28
62.00
60.15
58.01
56.53
55.29
54.15
51.95
39.60
36.29
36.22
35.42
30.73
28.24
28.18
27.82
27.60
25.08
20.75
OMe
OMe
7
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm