Supporting Information: Site-selective glycosylation of hemoglobin on Cys β93

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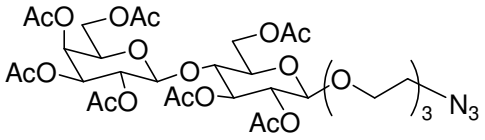
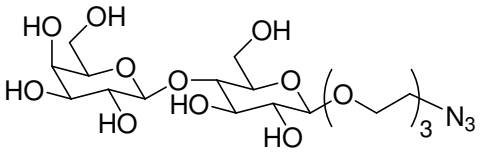
U.S.A.

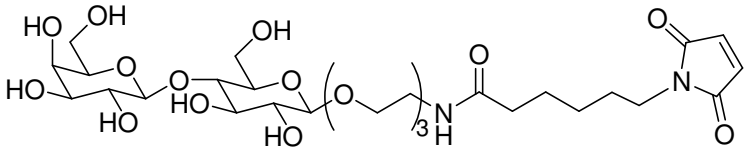


1-azido-3,6-dioxa-9-octyl-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranolsyl)-(1→4)-2,3,4,6-tetra-*O*-acetyl -β-D-galactoside (2). To a solution of peracetyl lactoside **1** (10.0 g, 14.7 mmol) in acetonitrile (150 mL) was added 2-[2-(2-chloroethoxy)ethoxy]ethanol (4.6 mL, 44.2 mmol) and 4 Å MS (Molecular Sieve) (2 g). The mixture was stirred at r.t. for 0.5 h and then cooled to 0 ºC. After that, boron trifluoride ether solution (3.9 mL, 29.4 mmol) was injected into the solution. The solution was slowly warmed to room temperature and stirred overnight. The solution was neutralized with saturated $NaHCO_3$ aqueous solution and extracted with ethyl acetate. The organic phase was dried with Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography with Hexanes/ EtOAc (1:1, v/v). The product (5.0 g, 6.35 mmol) in DMF (50 mL) was reacted with sodium azide (1.24 g, 19.1 mmol) and stirred at 80 ^oC overnight. The DMF was removed under reduced pressure and the crude product was purified by flash column chromatography with Hexanes/ EtOAc (1:1, v/v) to yield **2** (3.2 g) in an overall yield of 30% (two steps) as a colorless oil. ^1H NMR (500 MHz, $CDCl_3$) δ 1.97 (s, 3H), 2.05-2.06 (m, 9H), 2.07 (s, 3H), 2.13 (s, 3H), 2.16 (s, 3H), 3.41 (dd, *J* = 9.5, 4.3 Hz, 2H), 3.63-3.71 (m, 9H), 3.71-3.75 (m, 1H), 3.80 (t, *J* = 9.6 Hz, 1H), 3.86-3.93 (m, 2H), 4.07-4.16 (m, 3H), 4.48-4.51 (m, 2H), 4.58 (d, *J* = 8.0 Hz, 1H), 4.90 (dd, *J* = 9.5, 8.0 Hz, 1H), 4.96 (dd, *J* = 10.4, 3.5 Hz, 1H), 5.11 (dd, *J* = 10.5, 8.0 Hz, 1H), 5.20 (t, *J* = 9.4 Hz, 1H), 5.35-5.36

(m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.49, 20.61, 20.69, 20.80, 20.85, 50.69, 60.81, 62.02, 66.63, 69.05, 69.14, 70.05, 70.40, 71.00, 71.69, 72.65, 72.84, 76.29, 100.64, 101.08, 169.07, 169.65, 169.76, 170.05, 170.14, 170.34, 170.36.



1-azido-3,6-dioxa-9-octyl-*O*-(β-D-glucopyranolsyl)-(1→4)-β-D-galactoside (3). To a solution of compound **2** (3.3 g, 4.16 mmol) in MeOH (20 ml) was added sodium methoxide (22.5 mg, 0.416 mmol). The mixture was stirred at r.t. for 5 h, and neutralized with Dowex 50WX2-100 (H^+) resin. The resultant solution was then concentrated under reduced pressure to yield **3** (1.7 g, 82%) as a white solid. ^{1}H NMR (500 MHz, D_2O) δ 3.23-3.26 (m, 1H), 3.41 (t, *J* = 5.2 Hz, 2H), 3.44-3.46 (m, 1H), 3.48-3.51 (m, 1H), 3.55-3.57 (m, 3H), 3.61-3.63 (m, 7H), 3.65-3.67 (m, 4H), 3.69-3.77 (m, 2H), 3.82 (d, *J* = 3.3 Hz, 1H), 3.87-3.89 (m, 1H), 3.94-3.99 (m, 1H), 4.34 (d, *J* = 7.9 Hz, 1H), 4.42 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 50.09, 60.03, 60.98, 68.71, 69.17, 69.45, 69.55, 69.65, 70.91, 72.48, 72.78, 74.26, 74.73, 75.31, 78.31, 102.04, 102.90.



***N*-3',6'-dioxa-9'-octyl-*O*-(β-D-glucopyranolsyl)-(1→4)-β-D-galactoside-6-maleimido-hexanamide (maleimide-lactoside) (4).** To a solution of compound **3** (0.20 g, 0.40 mmol) in MeOH (10 mL) was added a catalytic amount of Pd/C. The mixture was stirred under hydrogen atmosphere at r.t. for 24 h. Consequently, the Pd/C was removed by filtration and the solution was concentrated under reduced pressure to yield the amine compound. The amine compound (50 mg, 0.106 mmol) in DMF (5.0 mL) was reacted with 6-maleimidohexanoic acid *N*-hydroxysuccinimide ester (34.0 mg, 0.111 mmol) and Et_3N (21 μL, 0.148 mmol). The solution was stirred at 40 ^{o}C overnight. The solvent was removed under reduced pressure and the residue was purified by flash chromatography with EtOAc:IPA:H_2O (6:3:1) to yield the

product as a colorless oil (26.1 mg) with an overall total yield of 37% (two steps). ^{1}H NMR (500 MHz, MeOD) δ 1.30-1.34 (m, 2H), 1.59-1.66 (m, 4H), 2.21 (t, *J* = 7.4 Hz, 2H), 3.29 (t, *J* = 8.3 Hz, 1H), 3.32-3.34 (m, 4H), 3.36-3.38 (m, 2H), 3.43-3.46 (m, 1H), 3.50-3.53 (m, 3H), 3.54-3.57 (m, 4H), 3.59-3.64 (m, 4H), 3.68-3.74 (m, 5H), 3.75-3.82 (m, 2H), 3.84-3.87 (m, 2H), 3.92 (dd, *J* = 12.2, 2.6 Hz, 1H), 4.01-4.05 (m, 1H), 4.38 (d, *J* = 7.6, 5.6 Hz, 2H), 6.83 (s, 2H). ^{13}C NMR (125 MHz, MeOD) δ 25.05, 25.92, 27.88, 35.41, 37.04, 38.92, 60.58, 61.09, 68.30, 68.90, 69.24, 69.80, 70.08, 70.14, 71.16, 73.34, 73.44, 74.90, 75.12, 75.69, 79.31, 102.90, 103.73, 133.98, 171.23, 174.73. HRMS: Calculated for $C_{28}H_{46}N_2O_{16}Na$ (M+Na^+) 689.2745; found 689.2744.

The proteolytic MS-MS data of the βChain of the glycosylated bovine hemoglobin

Trypsin

Measured m/z	Calculated M+H	Theoretical M+H	Protein Sequence	MASCOT Score	# Being detected
821.36^{1+}/411.24^{2+}	821.48	821.4073	^{1}MLTAEEK^{7}	490	19
837.53^{1+}/419.31^{2+}	837.62	837.4022	^{1}M$_{(Ox)}$LTAEEK^{7}	82	3
950.38^{1+}/475.80^{2+}	950.60	950.5094	^{8}AAVTAFWGK^{16}	1415	30
584.98^{3+}	1752.94	1752.8989	^{1}MLTAEEKAAVTAFWGK^{16}	481	12
1328.82^{1+}/664.87^{2+}	1328.74	1328.7169	^{17}VKVDEVGGEALGR^{29}	2542	47
551.30^{2+}	1101.60	1101.5535	^{19}VDEVGGEALGR^{29}	229	6
425.56^{3+}/637.83^{2+}	1274.66	1274.7256	^{30}LLVVYPWTQR^{39}	955	24
697.19^{3+}	2089.57	2089.9535	^{40}FFESFGDLSTADAVMNNPK^{58}	3403	45
702.71^{3+}/1053.70^{2+}	2106.13	2104.9484	^{40}FFESFGDLSTADAVM$_{(Ox)}$NNPK^{58}	881	14
613.32^{2+}	1225.64	1225.6245	^{65}KVLDSFSNGMK^{75}	272	9
549.17^{2+}	1097.34	1097.5296	^{66}VLDSFSNGMK^{75}	602	18
557.34^{2+}	1113.68	1113.5245	^{66}VLDSFSNGM$_{(Ox)}$K^{75}	35	1
724.14^{3+}/1085.65^{2+}	2170.30	2170.0597	^{76}HLDDLKGTFAALSELHC$_{(Cam)}$DK^{94}	287	4
843.66^{3+}	2528.98	2528.2238	^{82}GTFAALSELHC$_{(Cam)}$DKLHVDPENFK^{103}	75	2
782.76^{3+}	2346.28	2345.3704	^{95}LHVDPENFKLLGNVLVVVLAR^{115}	89	2
422.55^{3+}/633.70^{2+}	1265.40	1265.8304	^{104}LLGNVLVVVLAR^{115}	1039	22
935.10^{3+}/623.63^{3+}	1868.89	1868.9541	^{116}NFGKEFTPVLQADFQK^{131}	1416	36
474.80^{3+}/711.88^{2+}	1422.76	1422.7264	^{120}EFTPVLQADFQK^{131}	1029	21
589.36^{2+}	1177.72	1177.6800	^{132}VVAGVANALAHR^{143}	1396	18

Chymotrypsin

Measured m/z	Calculated M+H	Theoretical M+H	Protein Sequence	MASCOT Score	# Being detected
691.42^{2+}	1381.39	1381.7032	^{1}MLTAEEKAAVTAF^{13}	143	4
784.42^{2+}	1567.84	1567.7825	^{1}MLTAEEKAAVTAFW^{14}	110	3
570.02^{2+}	1139.08	1137.5786	^{3}TAEEKAAVTAF^{13}	27	1
663.13^{2+}	1325.26	1323.6579	^{3}TAEEKAAVTAFW^{14}	21	1
642.66^{3+}	1925.98	1926.0807	^{14}WGKVKVDEVGGEALGRLL^{31}	113	2
651.11^{2+}	1301.22	1300.7101	^{15}GKVKVDEVGGEAL^{27}	73	2
580.73^{3+}/870.77^{2+}	1740.54	1740.0014	^{15}GKVKVDEVGGEALGRLL^{31}	344	6
663.37^{1+}	663.37	663.3501	^{32}VVYPW^{36}	195	8
531.29^{2+}	1061.58	1061.5051	^{37}TQRFFESF^{44}	22	1
674.00^{2+}	1347.00	1346.6375	^{37}TQRFFESFGDL^{47}	40	1
782.32^{3+}/521.48^{3+}	1562.44	1562.7632	^{71}SNGMKHLDDLKGTF^{84}	243	9
527.05^{3+}	1579.15	1578.7581	^{71}SNGM$_{(ox)}$KHLDDLKGTF^{84}	25	1
603.28^{1+}	603.28	603.3348	^{85}AALSEL^{90}	42	2
418.98^{3+}/628.81^{2+}	1256.62	1256.6303	^{85}AALSELHC$_{(Cam)}$DKL^{95}	64	2
699.52^{3+}/1048.20^{2+}	2095.40	2094.9913	^{85}AALSELHC$_{(Cam)}$DKLHVDPENF^{102}	82	2
756.600^{2+}	1512.20	1510.6743	^{91}HC$_{(Cam)}$DKLHVDPENF^{102}	48	1
614.30^{3+}	1840.90	1839.9388	^{114}ARNFGKEFTPVLQADF^{129}	25	1
676.36^{2+}	1351.72	1351.6892	^{118}GKEFTPVLQADF^{129}	376	9
801.50^{3+}/1202.18^{2+}	2402.50	2402.3078	^{118}GKEFTPVLQADFQKVVAGVANAL^{140}	87	2
971.04^{2+}	1941.08	1941.0804	^{122}TPVLQADFQKVVAGVANAL^{140}	180	4
766.06^{2+}	1531.12	1530.8275	^{126}QADFQKVVAGVANAL^{140}	50	1
535.40^{2+}	1069.80	1069.6364	^{130}QKVVAGVANAL^{140}	95	3

Table 1. Data-collection statistics.

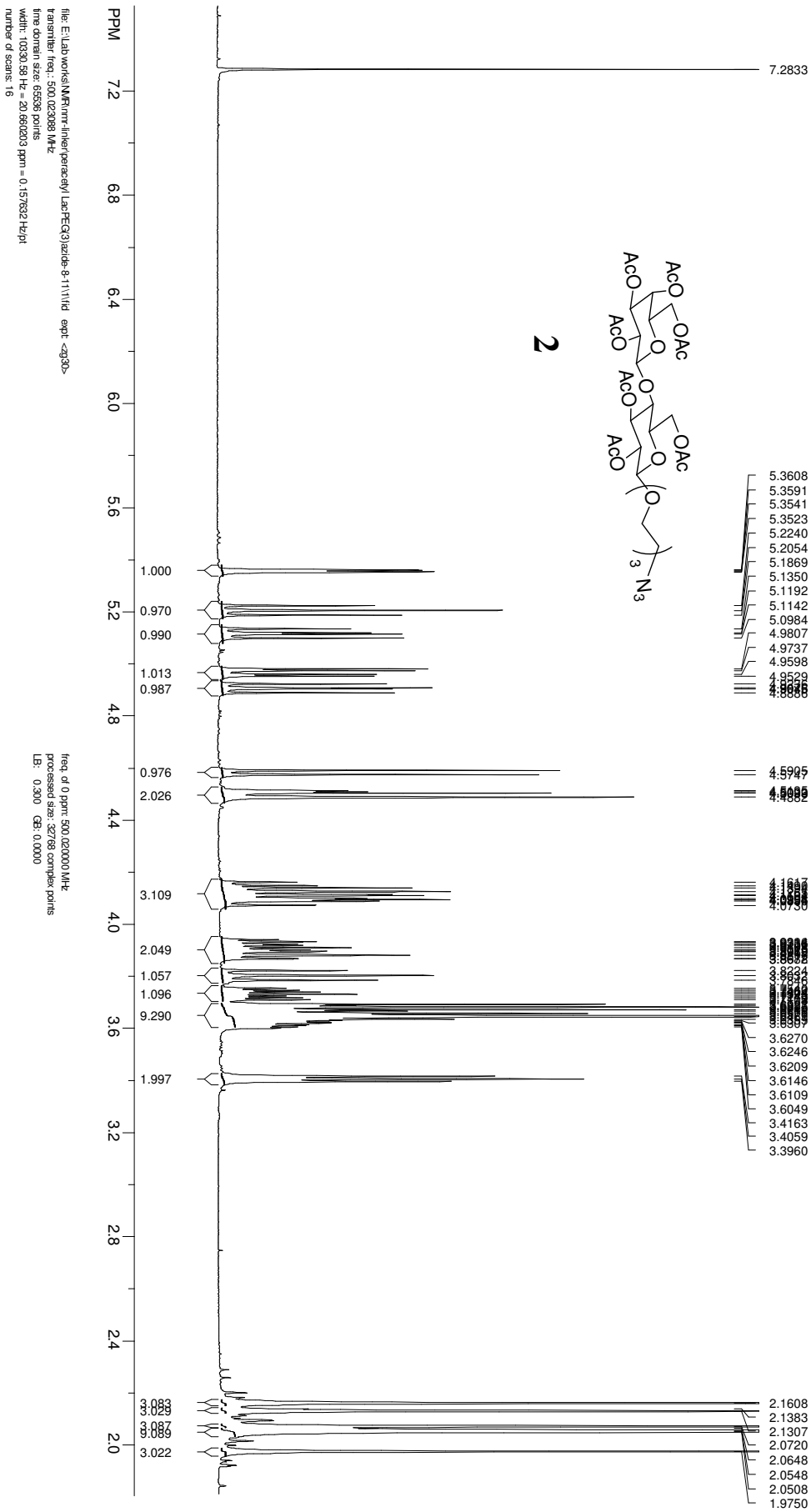
Values in parentheses are for the last shell.

Wavelength (Å)	1.54
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 62.6, b = 74.0, c = 129.8
V_M (Å^3 Da^{-1})	2.35
Solvent content (%)	47.7
Resolution range (Å)	24.5 - 3.5 (3.6 - 3.5)
No. of observed reflections	23286
No. of unique reflections	7999
Completeness (%)	96.4 (92.5)
R_{sym} (%)	13.1 (33.2)
I/I_σ	5.3 (2.4)

Table 2. Summary of crystallographic refinement.

PDB code	
Resolution limits (Å)	24.5 - 3.5
R_{cryst} (%)	26.8
R_{free} (%)	33.2
Total number of non-hydrogen atoms	4582
Total number of atoms in succinimide linked lactose	1(44)
Water molecules	8
R.m.s. deviations†(Å)	
Bond lengths	0.006
Bond angles	0.98
Dihedral angles	0.05
Overall G factor§	0.26
Average B factors $(Å)^2$	91.2
Residues in most favored regions (%)	91.1
Residues in additionally allowed regions (%)	8.9

†Target stereochemistry from literature[62] § As reported by PROCHECK[63]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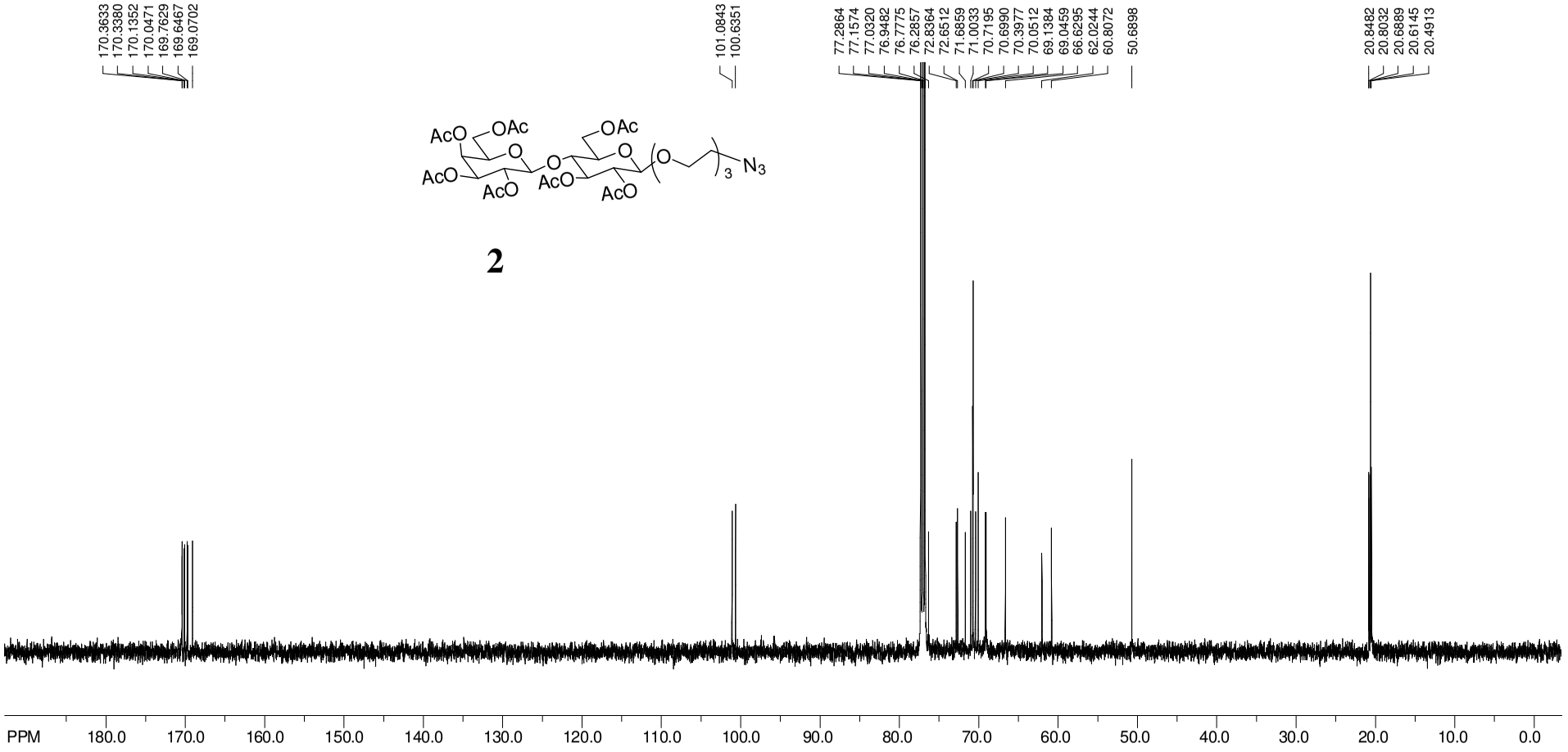

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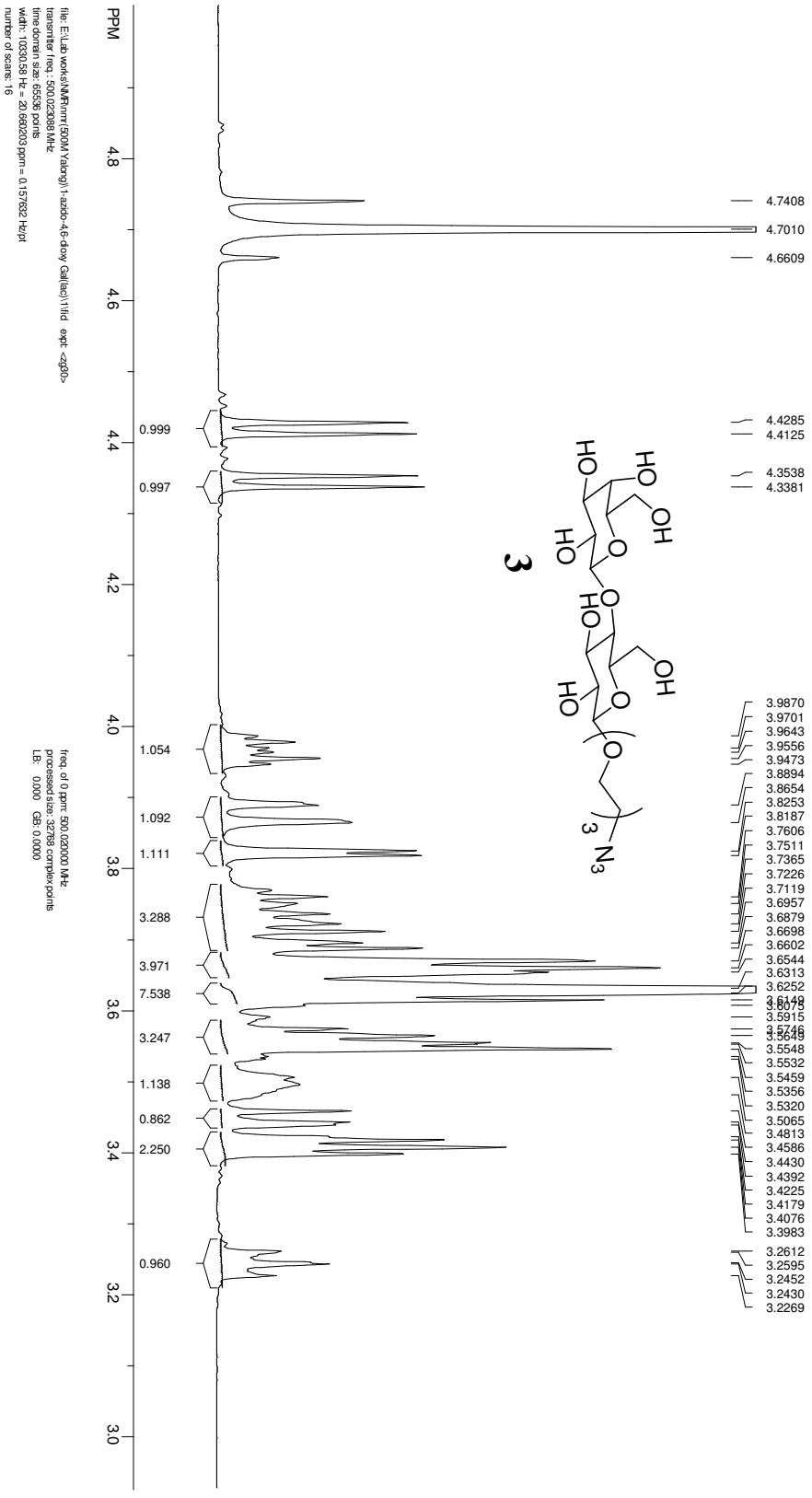
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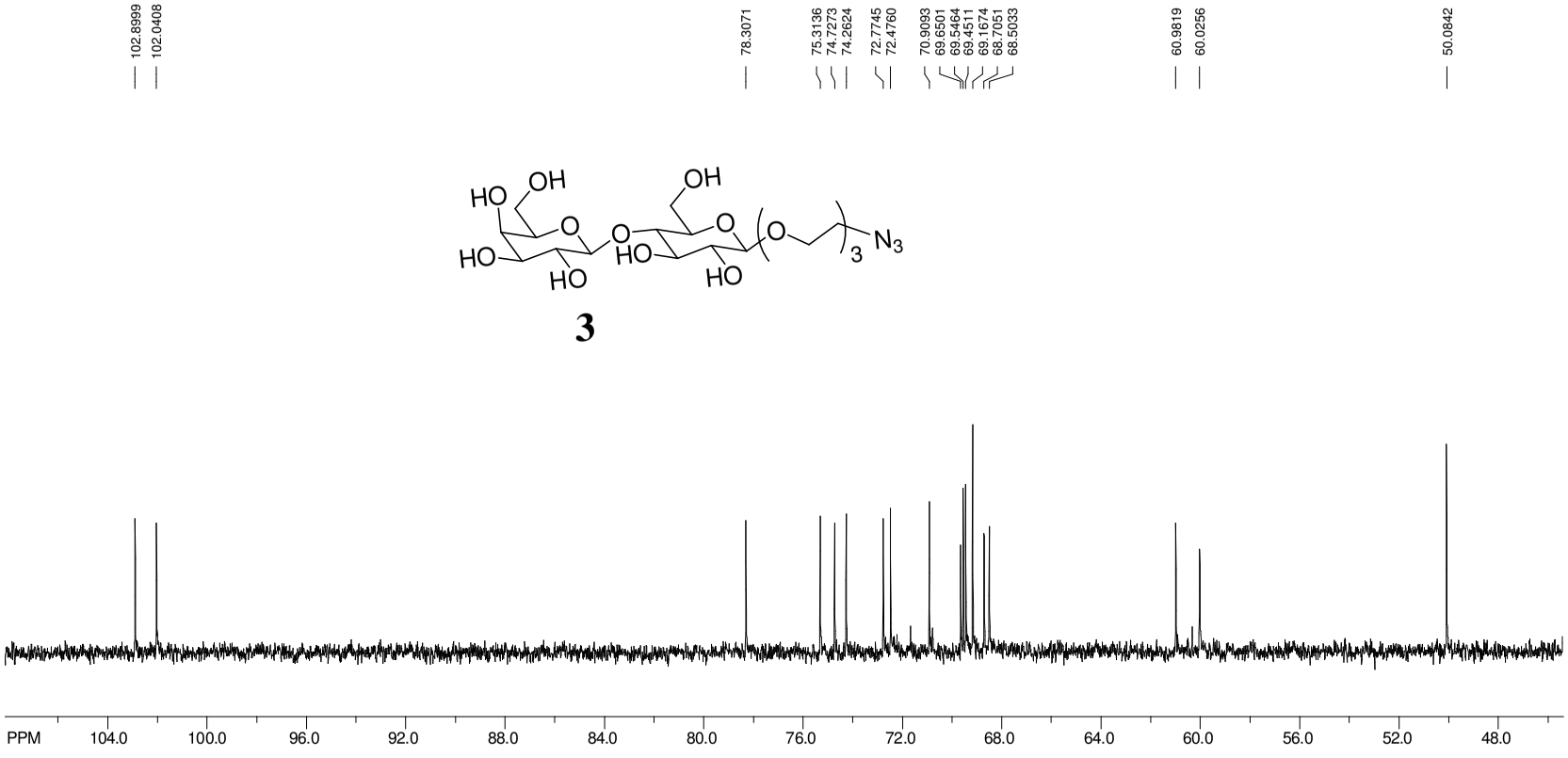

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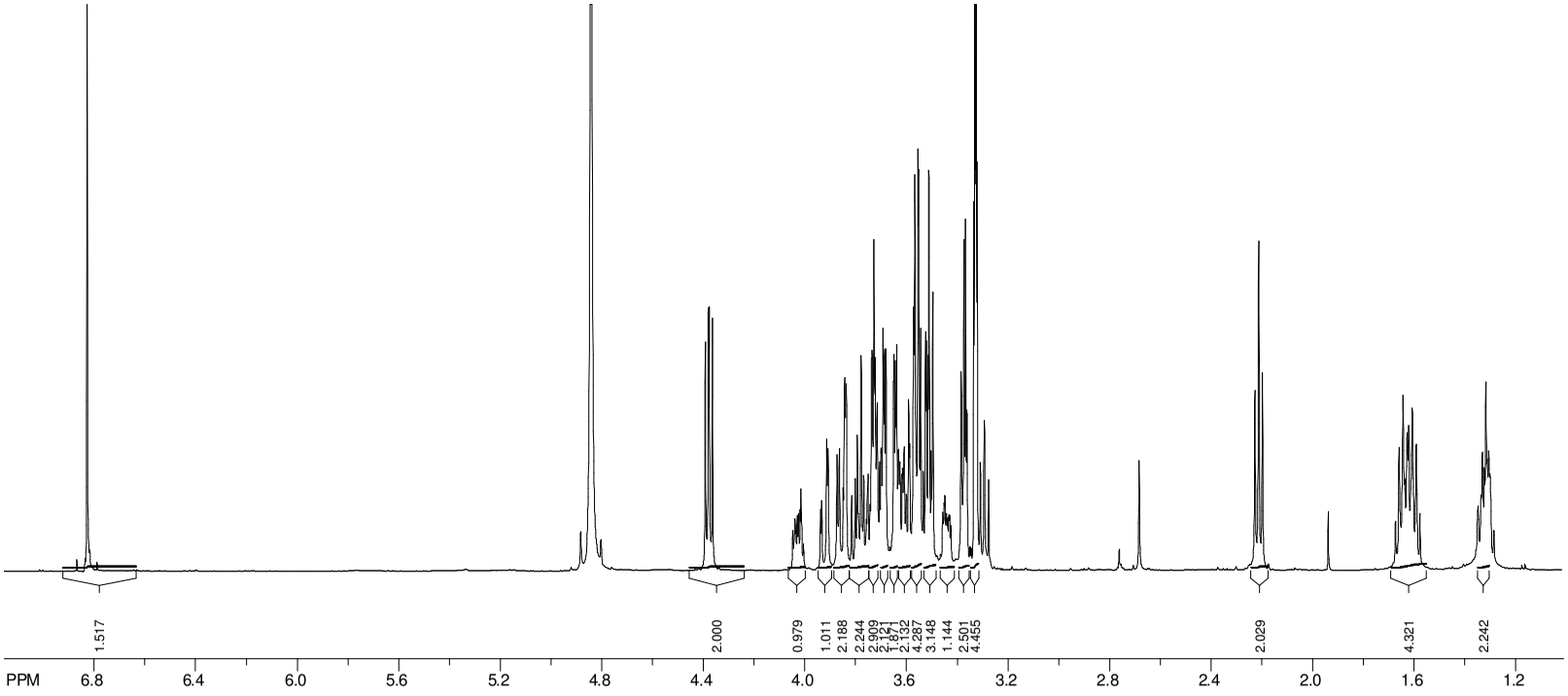

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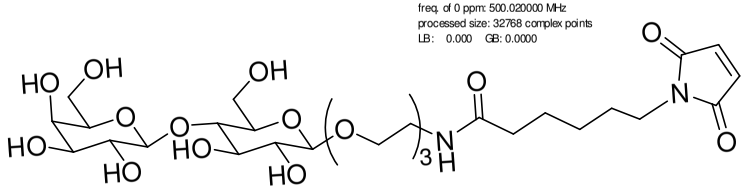

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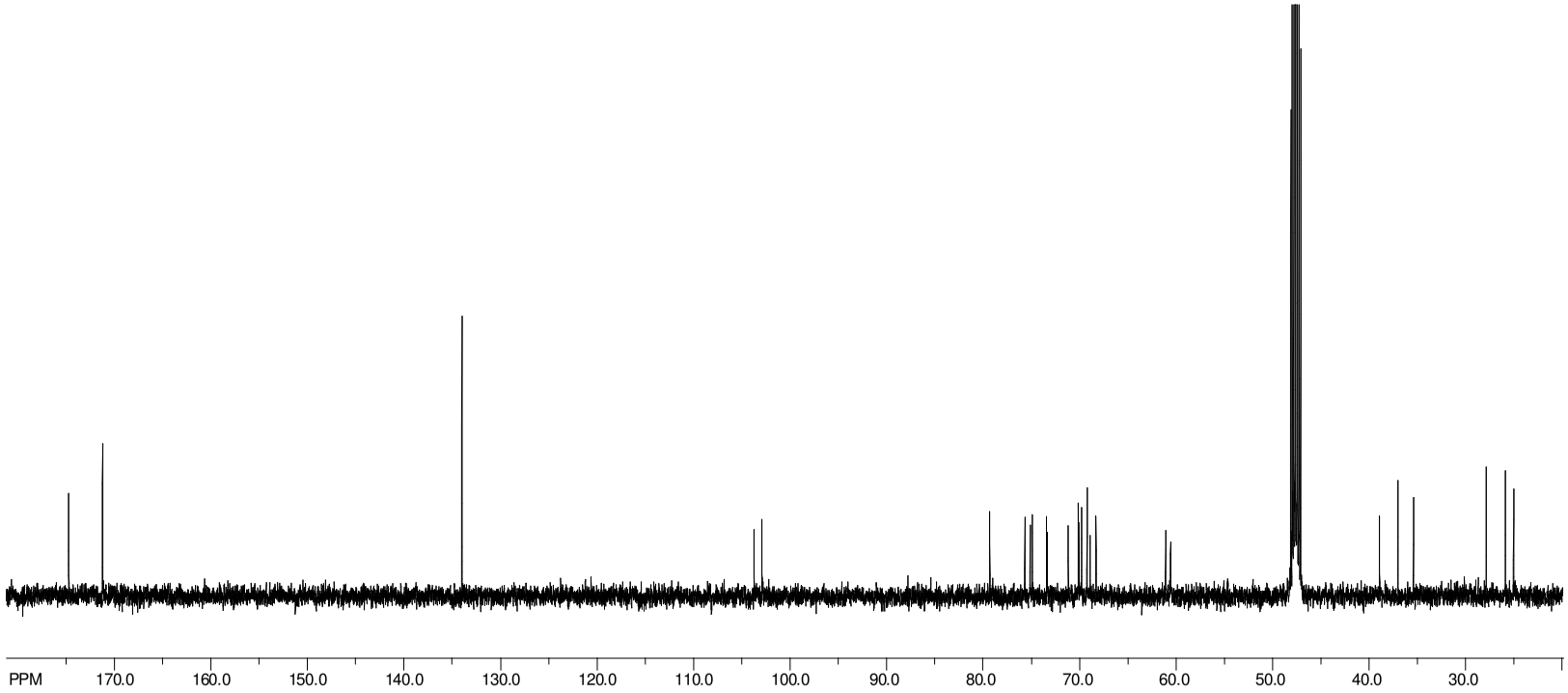

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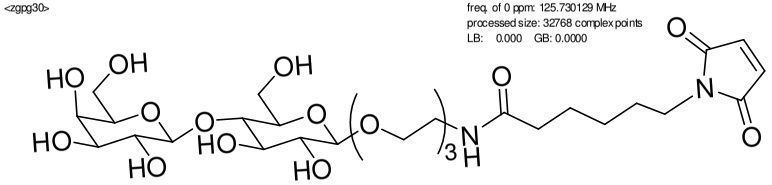

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