Glycomimetic Ligands for the Human Asialoglycoprotein Receptor

Sreeman K. Mamidyala[a‡], Sanjay Dutta[a‡], Boris A. Chrunyk[b], Cathy Preville[b], Hong Wang[b], Jane M. Withka[b], Alexander McColl[b], Timothy A. Subashi[b], Steven J. Hawrylik[b], Matthew C. Griffor[b], Sung Kim[b], Jeffrey A. Pfefferkorn, David A. Price[b], Elnaz Menhaji-Klotz[b], Vincent Mascitti[b]*, and M.G. Finn[a]*

[a]Department of Chemistry, The Scripps Research Institute, 10550 N. Torrey Pines. Rd., La Jolla, CA 92037, USA

[b]Pfizer Global Research & Development, Eastern Point Road, Groton, CT 06340, USA

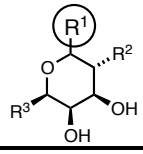
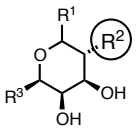
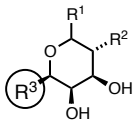
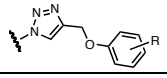
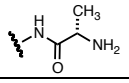
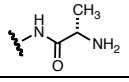
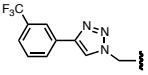
Supporting Information

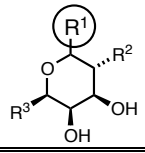
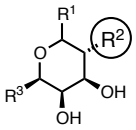
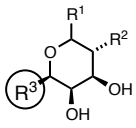
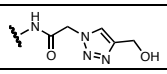
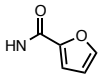
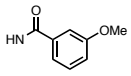
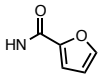
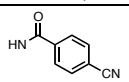
Contents of This File

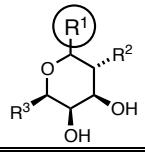
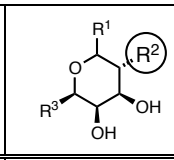
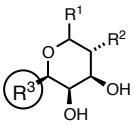
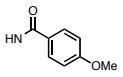
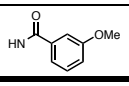
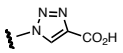
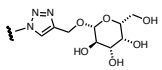
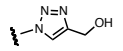
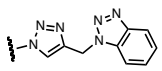
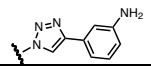
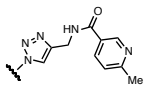
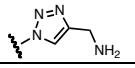
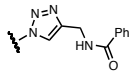
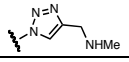
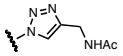
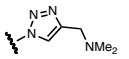
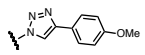
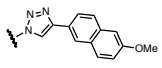
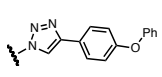
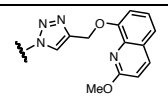
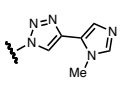
Accompanying File (Supporting Information, part b)

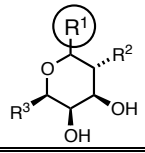
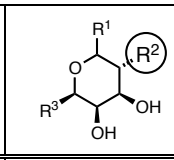
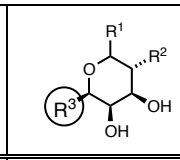
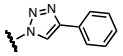
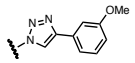
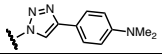
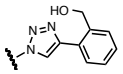
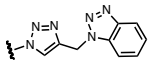
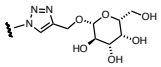
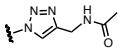
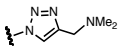
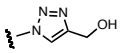
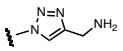
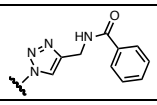
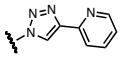
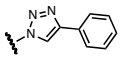
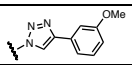
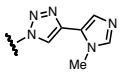
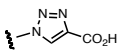
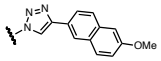
A. Complete SPR Results. Table S1 shows the structures and binding constants of all compounds contributing to this study, from surface plasmon resonance measurements as described in the text and below. Values denoted as "ca. XXX μM" and "> 1 mM" are approximate or given as a lower bound either because binding behavior was not well-resolved or because only one or two replicate measurements were performed, since these compounds were of lesser interest. In some instances, slower kinetic behavior was observed, but this could not be fit to simple kinetic models and usually exhibited Ru values above the maximum for 1:1 interaction, suggesting either nonspecific interactions or multisite binding. No K_D values were attributed to these compounds, denoted by "slow kinetics" in the "notes" column.

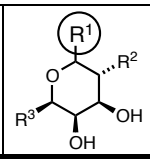
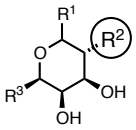
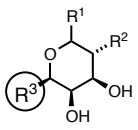
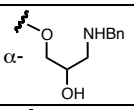
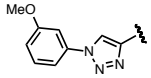
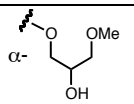
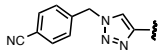
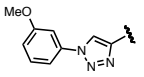
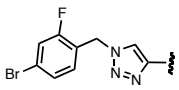
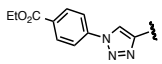
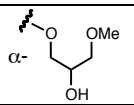
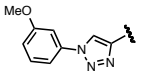
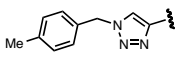
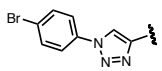
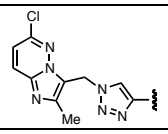
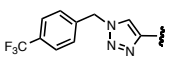
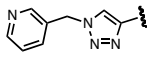
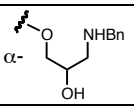
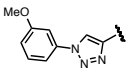
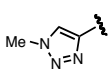
Table S1. Complete results from SPR studies, organized by compound type and in order of decreasing affinity within each type, except for reference compounds listed at the beginning of each section. The blue background marks those structures included in Figure 2 in the main paper.

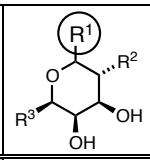
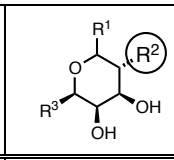
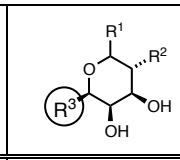
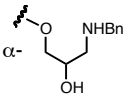
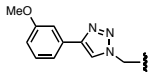
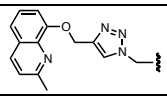
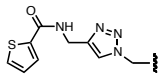
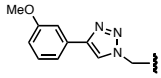
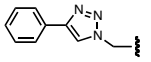
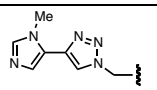
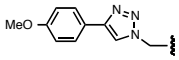
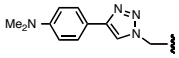
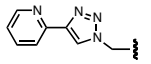
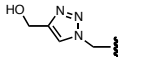
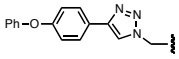
Cpd. ID				**K_d** (n = # SPR replicate measurements)	**notes**	**EIMS m/z calc; obs** [a]	**HRMS calc; obs** $(M+H)^+$ unless otherwise noted
1a	H		CH_2OH	20-40 μM [b]			
1b	β-OMe		CH_2N_3	IC_{50} = 46 μM [c]			
1c	β-OMe			IC_{50} = 48 μM [c]			
GalNAc	OH	$NHCOCH_3$	CH_2OH	40.4 ± 9.5 μM (n=45)			
	OMe	OH	CH_2OH	880 ± 200 μM (n=12)			
lactose	β-D-glucose	OH	CH_2OH	257 ± 23 μM (n=6)			
	β-Me	NH_2	CH_2OH	ca. 570 μM (n=2)		177.1; 178.1	178.1074; 178.107 200.0893; 200.0892 $(M+Na)^+$
3a1	β-Me	$NHCOCH_3$	CH_2OH	41.0 ± 7.0 μM (n=3)		219.1; 220.1	220.1179; 220.1182 242.0999; 242.0996 $(M+Na)^+$
3a6	β-Me	$NHCOCF_3$	CH_2OH	14.8 ± 2.0 μM (n=8)		273.1; 296.0 $(M+Na)^+$	274.0897; 274.0894
3a10	β-Me	$NHCOCH_2CF_3$	CH_2OH	33.1 ± 1.4 μM (n=8)		287.1; 288.1	288.1053; 288.105 310.0873; 310.0867 $(M+Na)^+$
3a2	β-Me	NHCO<u>n</u>Pr	CH_2OH	175 ± 9.5 μM (n=4)	EIMS on tris(benzyl ether)	517.3; 518.3	248.1492; 248.1493 270.1312; 270.1314 $(M+Na)^+$
3a3	β-Me	NHCO<u>i</u>Pr	CH_2OH	189 ± 22.0 μM (n=4)	EIMS on tris(benzyl ether)	517.3; 518.3	248.1492; 248.1490 270.1312; 270.1309 $(M+Na)^+$

Cpd. ID				K_d (n = # SPR replicate measurements)	notes	EIMS m/z calc; obs [a]	HRMS calc; obs $(M+H)^+$ unless otherwise noted
3a4	β-Me	NHCOtBu	CH_2OH	254 ± 4.0 µM (n=4)	EIMS on tris(benzyl ether)	531.3; 532.3	262.1649; 262.1647 284.1468; 284.1470 $(M+Na)^+$
3a8	β-Me	$NHCOCH_2CO_2H$	CH_2OH	199 ± 102 µM (n=4)		263.1; 264.1	286.0897; 286.0902 $(M+Na)^+$
3a9	β-Me	$NHCOCH_2NH_2$	CH_2OH	>500 µM (n=4)	compare to **1b** and **1c**, all with β-aminoacetamide substituents	234.1; 235.1	235.1288; 235.1298 257.1108; 257.1109 $(M+Na)^+$
3a17	β-Me		CH_2OH	490 ± 210 µM (n=4)		316.1; 317.1	317.1456; 317.1453
3a11	β-Me		CH_2OH	> 1 mM (n=2)	EIMS on tris(benzyl ether)	541.2; 542.3	276.1442; 276.1442 298.1261; 298.1261 $(M+Na)^+$
3a14	β-Me		CH_2OH	> 1 mM (n=2)	EIMS on tris(benzyl ether)	581.3; 582.3	312.1442; 312.1438 334.1261; 334.1260 $(M+Na)^+$
	β-4-OMePh	NH_2	CH_2OH	> 1 mM (n=2)		269.1; 270.1	270.1336; 270.1332 292.1155; 292.1157 $(M+Na)^+$
3b1	β-4-OMePh	$NHCOCH_3$	CH_2OH	202 ± 58.5 µM (n=3)			312.1442; 312.1445 334.1261; 334.1265 $(M+Na)^+$
3b6	β-4-OMePh	$NHCOCF_3$	CH_2OH	9.8 ± 0.7 µM (n=4)		365.1; 366.1	366.1159; 366.1157 388.0978; 388.0979 $(M+Na)^+$
3b11	β-4-OMePh		CH_2OH	181 ± 4.0 µM (n=4)		367.2; 368.1	368.1704; 368.1708 390.1523; 390.1528 $(M+Na)^+$
3b4	β-4-OMePh	NHCOtBu	CH_2OH	182 ± 27.5 µM (n=4)		353.2; 354.2	354.1911; 354.1910 376.1731; 376.1732 $(M+Na)^+$
3b7	β-4-OMePh	NHBoc	CH_2OH	ca. 250 µM (n=2)		369.2; 392.2 $(M+Na)^+$	370.1860; 370.1868 392.1680; 392.1678 $(M+Na)^+$
3b16	β-4-OMePh		CH_2OH	347 ± 113 µM (n=4)			
3b15	β-4-OMePh		CH_2OH	ca. 360 µM (n=2)			
3b12	β-4-OMePh	NHCOPh	CH_2OH	ca. 380 µM (n=2)		373.2; 374.1	374.1598; 374.1601 396.1418; 396.1421 $(M+Na)^+$
3b2	β-4-OMePh	NHCOnPr	CH_2OH	ca. 730 µM (n=2)		339.2; 340.1	340.1755; 340.1759 362.1574; 362.1578 $(M+Na)^+$
3b3	β-4-OMePh	NHCOiPr	CH_2OH	>1 mM (n=2)		339.2; 340.1	340.1755; 340.1752 362.1574; 362.1574 $(M+Na)^+$

Cpd. ID				K_d (n = # SPR replicate measurements)	notes	EIMS m/z calc; obs [a]	HRMS calc; obs $(M+H)^+$ unless otherwise noted
3b13	β-4-OMePh		CH_2OH	>1 mM (n=2)			404.1704; 404.1709 426.1523; 426.1526 $(M+Na)^+$
3b14	β-4-OMePh		CH_2OH	>1 mM (n=2)			
4a19	β-Me		CH_2OH	24.2 ± 4.8 μM (n=4)		273.1; 274.1	274.1034; 274.1033 296.0853; 296.0860 $(M+Na)^+$
4a14	β-Me		CH_2OH	113 ± 6.5 μM (n=4)		421.2; 422.1	422.1769; 422.1779 444.1589; 444.1594 $(M+Na)^+$
4a15	β-Me		CH_2OH	134 ± 26.3 μM (n=8)			260.1241; 260.1238 282.1060; 282.1058 $(M+Na)^+$
4a12	β-Me		CH_2OH	ca. 200 μM (n=2)	α-Ar analogue **4b12** binds better (40 μM)		
4a4	β-Me		CH_2OH	ca. 212 μM (n=2)		320.2; 321.1	
4a13	β-Me		CH_2OH	ca. 300 μM (n=2) (slow kinetics?)			378.1772; 378.1771 400.1591; 400.1596 $(M+Na)^+$
4a16	β-Me		CH_2OH	ca. 600 μM (n=2)			276.1666; 276.1667 $(M + NH_4)^+$
4a20	β-Me		CH_2OH	ca. 620 μM (n=2)	EIMS on tris(benzyl ether)	632.3; 633.3	363.1663; 363.1675 385.1482; 385.1490 $(M+Na)^+$
4a17	β-Me		CH_2OH	> 0.7 mM (n=2)			
4a21	β-Me		CH_2OH	ca. 840 μM (n=2)	α-Ar analogue binds better (93 μM)		323.1326; 323.1327 $(M+Na)^+$
4a18	β-Me		CH_2OH	> 1 mM (n=2)	α-Ar analogue binds much better (95 μM)	286.2; 287.1	287.1714; 287.1712 309.1533; 309.1533 $(M+Na)^+$
4a2	β-Me		CH_2OH	> 1 mM (n=2)		335.2; 336.1	336.1554; 336.1554 358.1373; 358.1374 $(M+Na)^+$
4a7	β-Me		CH_2OH	> 1 mM (n=2)		385.2; 386.1	386.1710; 386.1714 408.1530; 408.1532 $(M+Na)^+$
4a8	β-Me		CH_2OH	> 1 mM (n=2)		397.2; 398.1	398.1710; 398.1714 420.1530; 420.1532 $(M+Na)^+$
4a9	β-Me		CH_2OH	> 1 mM (n=2)			417.1769; 417.1774
4a11	β-Me		CH_2OH	> 1 mM (n=2)		309.1; 310.1	310.1510; 310.1508 332.1329; 332.1323 $(M+Na)^+$

Cpd. ID				K_d (n = # SPR replicate measurements)	notes	EIMS m/z calc; obs [a]	HRMS calc; obs $(M+H)^+$ unless otherwise noted
4a1	β-Me		CH_2OH		slow kinetics	305.1; 306.1	306.1448; 306.1450 328.1268; 328.1268 $(M+Na)^+$
4a3	β-Me		CH_2OH		slow kinetics	335.2; 336.1	336.1554; 336.1542 358.1373; 358.1376 $(M+Na)^+$
4a5	β-Me		CH_2OH		slow kinetics	348.2; 349.1	
4a10	β-Me		CH_2OH		slow kinetics		
4b12	β-4-OMePh		CH_2OH	39.7 ± 5.4 (n=8)		452.2; 453.1	453.1881; 453.1886 475.1700; 475.1702 $(M+Na)^+$
4b14	β-4-OMePh		CH_2OH	42.3 ± 5.2 (n=6)		513.2; 514.2	514.2031; 514.2033 536.1851; 536.1855 $(M+Na)^+$
4b21	β-4-OMePh		CH_2OH	93.3 ± 4.4 (n=6)		392.2; 393.1	393.1769; 393.1769 415.1588; 415.1588 $(M+Na)^+$
4b18	β-4-OMePh		CH_2OH	94.5 ± 24.5 (n=4)		378.2; 379.2	379.1976; 379.1979 401.1795; 401.1799 $(M+Na)^+$
4b15	β-4-OMePh		CH_2OH	ca. 300 µM (n=4)	α-Ar analogue binds better (134 µM)	351.2; 352.1	352.1503; 352.1506 374.1323; 374.1320 $(M+Na)^+$
4b16	β-4-OMePh		CH_2OH	ca. 600 µM (n=2)	EIMS on tris(benzyl ether)	620.3; 621.3	
4b20	β-4-OMePh		CH_2OH	ca. 640 µM (n=2)		454.2; 455.1	455.1925; 455.1930 477.1745; 477.1748 $(M+Na)^+$
4b6	β-4-OMePh		CH_2OH	> 1 mM (n=2)		398.2; 399.1	399.1663; 399.1666 421.1482; 421.1485 $(M+Na)^+$
4b1	β-4-OMePh		CH_2OH	> 1 mM (n=2)	poor solubility	397.2; 398.2	398.1710; 398.1721 420.1530; 420.1530 $(M+Na)^+$
4b3	β-4-OMePh		CH_2OH	> 1 mM (n=2)	poor solubility		
4b11	β-4-OMePh		CH_2OH	> 1 mM (n=2)		401.2; 402.1	402.1772; 402.1777 424.1591; 424.1593 $(M+Na)^+$
4b19	β-4-OMePh		CH_2OH	>1 mM	α-Me analogue binds much better (24 µM); EIMS on tris(benzyl ether)	635.3; 636.2	366.1296; 366.1303
4b7	β-4-OMePh		CH_2OH	slow kinetics	poor solubility	477.2; 478.2	478.1973; 478.1975

Cpd. ID				K_d (n = # SPR replicate measurements)	notes	EIMS m/z calc; obs [a]	HRMS calc; obs $(M+H)^+$ unless otherwise noted
9	α-O-allyl	$NHCOCH_3$	CH_2OH	10.7 ± 1.9 μM (n=6)	EIMS on C3,C4-acetonide	301.2; 302.2	262.1285; 262.1285 284.1105; 284.1109 $(M+Na)^+$
11	α-O-allyl	$NHCOCH_3$	CH_2N_3	1.6 ± 0.60 μM (n=6)		286.1; 287.2	287.1350 287.1354
10	α-O-allyl	$NHCOCH_3$	CCH	44.0 ± 4.3 μM (n=4)	EIMS on C3,C4-acetonide	295.1; 296.4	256.1179; 256.1182 278.0999; 278.1000 $(M+Na)^+$
17		$NHCOCF_3$		1.2 ± 0.6 μM (n=6)	k_{on} = 4.3 x 10^4 $M^{-1}s^{-1}$ k_{off} = 0.05 s^{-1}	581.2; 582.2	582.2170; 582.2179
16		$NHCOCH_3$	CH_2N_3	6.0 ± 0.45 μM (n=4)		334.2; 335.2	335.1561; 335.1569
6e	α-O-allyl	$NHCOCH_3$		15.8 ± 0.6 μM (n=4)	EIMS on C3,C4-acetonide	453.2; 454.2	414.1772; 414.1768 436.1591; 436.1590 $(M+Na)^+$
6d	α-O-allyl	$NHCOCH_3$		22.0 ± 9.9 μM (n=8)		404.2; 405.2	405.1769; 405.1771 427.1588; 427.1592 $(M+Na)^+$
6f	α-O-allyl	$NHCOCH_3$		25.0 ± 1.0 μM (n=4)	EIMS on C3,C4-acetonide	524.1; 525.1/527.1	485.0830; 485.0835 507.0650; 507.0654 $(M+Na)^+$
6c	α-O-allyl	$NHCOCH_3$		25.9 ± 2.3 μM (n=4)	EIMS on C3,C4-acetonide	486.2; 487.2	
13		$NHCOCH_3$		29.3 ± 12.1 μM (n=4)		452.2; 453.3	451.1834; 451.1822
6g	α-O-allyl	$NHCOCH_3$		34.2 ± 6.3 μM (n=8)		402.2; 403.2	403.1976; 403.1982 425.1795; 425.1802 $(M+Na)^+$
6b	α-O-allyl	$NHCOCH_3$		34.4 ± 1.6 μM (n=4)	EIMS on C3,C4-acetonide	492.1; 493.1/495.1	453.0768; 453.0767 475.0588; 475.0586 $(M+Na)^+$
6j	α-O-allyl	$NHCOCH_3$		41.5 ± 3.0 μM (n=4)		477.2; 478.1	478.1600; 478.1593
6h	α-O-allyl	$NHCOCH_3$		41.3 ± 0.8 μM (n=4)	EIMS on C3,C4-acetonide	496.2; 497.2	457.1693; 457.1693 479.1513; 479.1515 $(M+Na)^+$
6i	α-O-allyl	$NHCOCH_3$		61.0 ± 14.0 μM (n=4)		389.2; 390.1	390.1772; 390.1778 412.1591; 412.1597 $(M+Na)^+$
14		$NHCOCH_3$		66.2 ± 29.9 μM (n=4)		527.2; 528.3	528.2453; 528.2451 550.2272; 550.2267 $(M+Na)^+$
6a	α-O-allyl	$NHCOCH_3$		104.7 ± 12.4 μM (n=6)	EIMS on C3,C4-acetonide	352.2; 353.1	313.1506; 313.1519

Cpd. ID				K_d (n = # SPR replicate measurements)	notes	EIMS m/z calc; obs [a]	HRMS calc; obs $(M+H)^+$ unless otherwise noted
15		$NHCOCH_3$		0.69 ± 0.15 µM (n=5)	data suggests additional weak binding mode at high concentration	541.3; 542.3	542.2609; 542.2611 564.2429; 564.2431 $(M+Na)^+$
8i	α-O-allyl	$NHCOCH_3$		3.2 ± 2.0 µM (n=14)		483.2; 484.2	484.2191; 484.2202
8j	α-O-allyl	$NHCOCH_3$		6.6 ± 2.5 µM (n=12)		451.2; 452.2	452.1598; 452.1606
8e	α-O-allyl	$NHCOCH_3$		8.2 ± 0.7 µM (n=12)		418.2; 419.2	419.1925; 419.1935
8b	α-O-allyl	$NHCOCH_3$		8.5 ± 1.4 µM (n=6)		388.2; 389.2	389.1819; 389.1829
8h	α-O-allyl	$NHCOCH_3$		9.0 ± 4.1 µM (n=14)		392.2; 393.2	393.1881; 393.1890
8c	α-O-allyl	$NHCOCH_3$		11.2 ± 1.0 µM (n=6)		418.2; 419.2	419.1925; 419.1928 441.1745; 441.1747 $(M+Na)^+$
8d	α-O-allyl	$NHCOCH_3$		approx. 12 µM	evidence of nonspecific binding at high concentration	431.2; 432.2	
8g	α-O-allyl	$NHCOCH_3$		12.3 ± 3.5 µM (n=10)		389.2; 390.1	390.1772; 390.1779 412.1591; 412.1595 $(M+Na)^+$
8a	α-O-allyl	$NHCOCH_3$		14.9 ± 6.9 µM (n=10)		342.2; 343.1	343.1612; 343.1616 365.1432; 365.1431 $(M+Na)^+$
8f	α-O-allyl	$NHCOCH_3$		18.6 ± 7.1 µM (n=10)		480.2; 481.2	481.2082; 481.2686 503.1901; 503.1903 $(M+Na)^+$

(a) Electrospray ionization mass spectrometry; calculated values for the uncharged compound; parent observed ions were $[M+H]^+$ unless otherwise indicated. (b) Stokmaier, D.; Khorev, O.; Cutting, B.; Born, R.; Ricklin, D.; Ernst, T. O. G.; Boni, F.; Schwingruber, K.; Gentner, M.; Wittwer, M.; Spreafico, M.; Vedani, A.; Rabbani, S.; Schwardt, O.; Ernst, B. *Bioorg. Med. Chem.* **2009**, *17*, 7254-7264. (c) Riva, C., Targeting the liver via the asialoglycopro-tein-receptor: synthesis of directed small molecule libraries for the H1-CRD. Ph.D. Thesis, University of Basel, 2006.

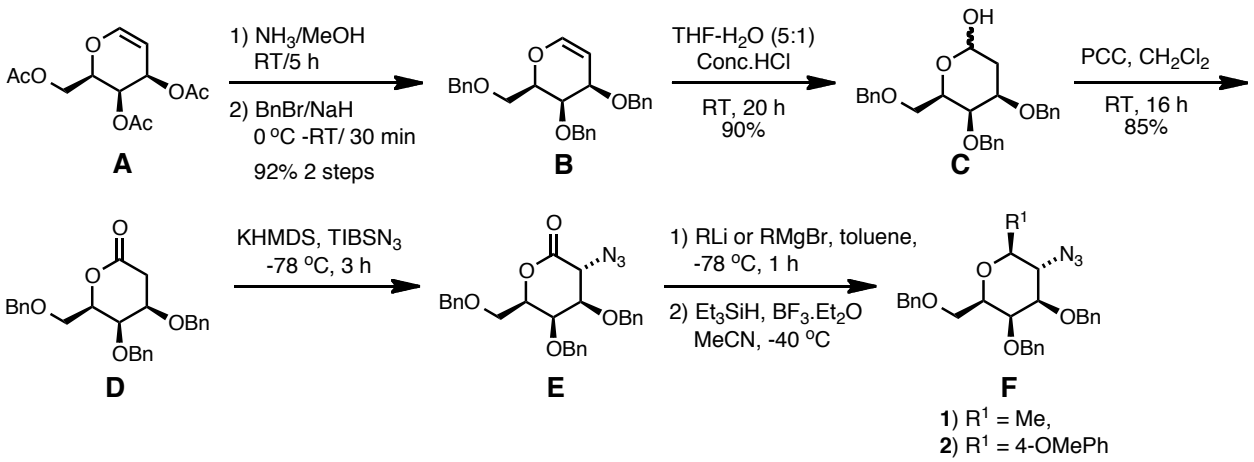
B. General. Solvents THF, acetonitrile, diethyl ether, dichloromethane, and toluene were dried by passage through activated alumina columns.[1] Chromatography was performed using SINGLE StEP pre-packed MPLC columns. Reaction progress was monitored by TLC using silica gel 60 F-254 with UV detection. Silica gel 60 (40–63 μm) was used for column chromatography. Solutions of phosphomolybdic acid or anisaldehyde/EtOH were used in addition to UV light with fluorescent TLC plates. Reactions requiring anhydrous conditions were performed under nitrogen. ^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX-600 equipped with a 5mm DCH cryoprobe, Bruker DRX-500, Varian Mercury-300 or Varian Mercury-200 MHz spectrometers in $CDCl_3$, CD_3OD, or DMSO-d_6 solvent, as indicated. NMR data are presented as follows: chemical shift (ppm), multiplicity (*s* = singlet, *d* = doublet, *t*= triplet, *q* = quartet, *quin* = quintet, *sep* = septet, *m* = multiplet, *b* = broad), coupling constant (Hz) and integration. Routine mass spectra (LCMS) were obtained using an Agilent 1100 (ESI MSD) with mobile phase composed of 9:1 CH_3CN:H_2O containing 0.1% CF_3CO_2H.

High resolution mass spectra data were gathered on an Agilent 1100 LC with MSD TOF (Agilent model G1969A) mass spec detector running with an electrospray ionization source. The instrument acquisition and data handling was done with Agilent MassHunter TOF/Q-TOF B.02 (B11285) Patches 1.2.3 software. Samples were analyzed using direct flow injection (no chromatography column), with the following parameters: injection volume = 1.0 μL; flow rate = 0.5 mL/min, run time = 1.0 min, solvent = methanol containing 0.1% formic acid and 0.05% ammonium formate. The time-of-flight analyzer used electrospray ionization in positive mode, gas temperature = 325 °C, drying gas = 6 L/min, nebulizer = 50 psg, VCap = 3500 V, mass range was set to a width of 100 units centered on the expected m/z value, acquisition rate = 0.99 spectra/s, acquisition time = 1012.8 ms/spectrum.

C. Synthesis of 2-Azido-D-galactose Derivatives

To a solution of tri-*O*-acetyl-D-galactal (**A**, 5.0 g, 18.3 mmol) in MeOH (50 mL) was added NH_3/MeOH (8N, 3 mL). The reaction mixture was stirred at room temperature for 5 h. After completion of the reaction, the solvent was removed under reduced pressure to give D-galactal[2] as a solid (2.6 g, 97%). This material (2.5 g, 17.1 mmol) was taken up in dry DMF (20 mL), cooled to 0 °C, and solid NaH was added (4 equiv). BnBr (6.75 mL, 56.5 mmol) was then added slowly to the cooled solution. The reaction mixture was allowed to warm to room temperature with stirring over 30 min. After reaching completion (assayed by TLC), cold water was added and the mixture was extracted with diethyl ether. The organic layer was separated, dried (Na_2SO_4), and the solvent was removed by rotary evaporation. Purification of the crude product by column chromatography gave the tri-*O*-benzyl-D-galactal[2] **B** as a colorless syrup (6.2-7.0 g, 87-98%).

1. (a) Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518-1520. (b) Alaimo, P. J.; Peters, D. W.; Arnold, J.; Bergman, R. G. *J. Chem. Ed.* **2001**, *78*, 64.
2. Bovin, N. V.; Zurabyan, S. E.; Khorlin, A. Y. *Carbohydr. Res*. **1981**, *98*, 25-35



A solution of **B** (6.0 g, 14.4 mmol) in THF-H_2O (2:1, 60 mL) was treated with 2N aq. HCl (3.0 mL) and stirred at room temperature for 16 h. After completion of the reaction, CH_2Cl_2 (50 mL) was added and the mixture was washed with saturated aqueous solution of $NaHCO_3$ and water. The organic layer was separated, dried (Na_2SO_4), and evaporated to give tri-*O*-benzyl-D-galactopyranoside[3] **(C**) as a solid (5.7g, 92%).

To a solution of **C** (5.0 g, 11.5 mmol) in CH_2Cl_2 was added pyridinium chlorochromate (4.9 g, 23.0 mmol) in two portions. The reaction mixture was stirred at room temperature for 12 h, diluted with CH_2Cl_2 (50 mL), and filtered through a pad of celite. The solvent was removed by rotary evaporation and the residue was purified by column chromatography (EtOAc/hexanes) to give tri-*O*-benzyl-2-deoxy-D-galactono-1,5-lactone[3] (**D**) as a colorless syrup (4.2 g, 85%).

A solution of **D** (1.0 g, 2.3 mmol) in THF (15 mL) was cooled to -78 °C and a 15% w/w solution of potassium hexamethyldisilazane (KHMDS) in toluene (6.0 mL, 4.6 mmol) was added dropwise with vigorous stirring. After 15 min, a precooled solution of trisyl azide (1.4 g, 4.6 mmol) in THF (5.0 mL) was added dropwise. After another 5 min, AcOH (0.27 mL, 4.6 mmol) was added. The cooling bath was then removed and the mixture was stirred at room temperature overnight. After addition of H_2O (30 mL), the mixture was extracted with CH_2Cl_2 (3 x 30 mL). The combined CH_2Cl_2 layers were dried and concentrated to give a crude product that was purified by flash column chromatography (silica gel, 6:1 hexanes:EtOAc) to isolate Tri-*O*-benzyl-2-azido-2-deoxy-D-galactono-1,5-lactone[4] (**E**, 0.65 g, 60%) as a colorless oil.

A solution of **E** (0.6 g, 1.6 mmol) in toluene (15 mL) was cooled to -78 °C and 1.5 M MeLi in ether (1.6 mL, 2.2 mmol) was added dropwise with vigorous stirring. The reaction mixture was stirred for 1 h, and quenched by addition of saturated NH_4Cl solution. The mixture was extracted with ether (30 mL), and the organic layer was dried (Na_2SO_4) and evaporated to give the crude lactol. This material was dissolved in dry MeCN (15 mL) and cooled to -40 °C. Triethylsilane (1.0 mL, 8.0 mmol) was added slowly, the resulting mixture was stirred for 5 min at -40 °C, and then $BF_3 \bullet Et_2O$ (1.0 mL, 9.6 mmol) was added slowly. The mixture was stirred while warming to room temperature. Saturated $NaHCO_3$ was added and the mixture was

3. Dupradeau, F.-Y.; Hakornoriar, S.-I.; Toyokuni, T. *J. Chem. Soc. Chem. Commun*. **1995**, 221-222.

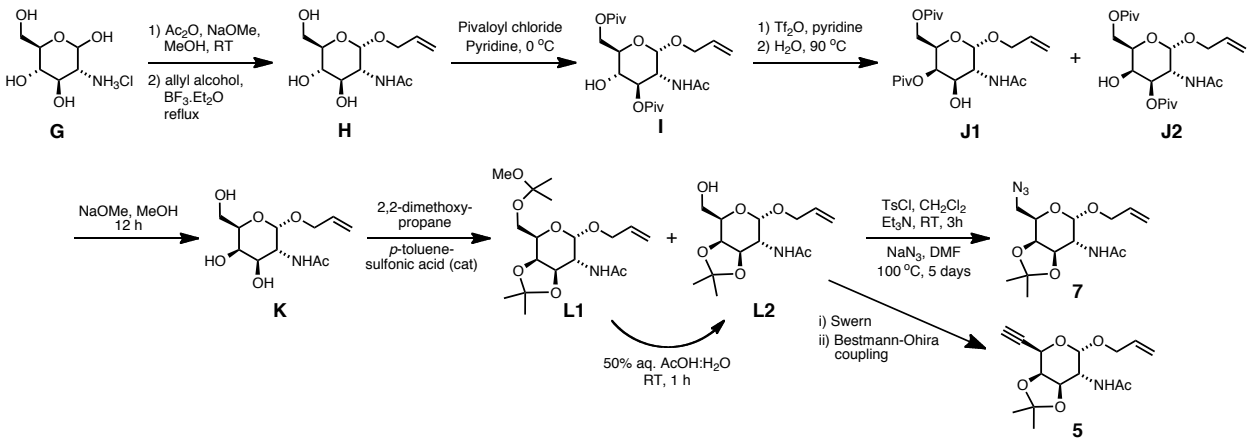
4. Dileep Kumar, J. S.; Dupradeau, F. -Y.; Strouse, M. J.; Phelps, M. E.; Tatsushi Toyokuni, T. *J. Org. Chem.* **2001**, *66*, 3220-3223.

extracted into CH_2Cl_2 (30 mL). The organic layer was separated, washed with water, dried (Na_2SO_4), and evaporated. Purification by flash column chromatography afforded methyl-tri-*O*-benzyl-2-azido-2-deoxy-D-galactopyranoside[5] (**F1**, 0.39 g, 65%) as a syrup.

The analogous procedure was performed with **E** (0.6 g, 1.6 mmol) in THF (20 mL) and 1.0 M *p*-OMePhMgBr in THF (2.5 mL, 2.2 mmol), followed by triethylsilane reduction as above. The product *p*-methoxyphenyl-tri-*O*-benzyl-2-azido-2-deoxy-*D*-galactopyranoside (**F2**, 0.5 g, 72%) was obtained as a syrup.

Following CuAAC reactions as described below to give precursors to triazoles **4a** and **4b**, *O*-benzyl deprotection was accomplished by the following general procedure. To a solution of tri-O-benzyl-D-galactose derivative (0.1 mmol) in MeOH (10 mL) was added 20% $Pd(OH)_2$ on carbon (350 mg/mmol, 0.1 g) and ammonium formate (100 mg/0.2 mmol, 0.15 g). The reaction mixture was stirred at reflux temperature for 3 h. After completion of the reaction, the mixture was filtered through a pad of Celite, and the fitrate was concentrated under vacuum to isolate the desired product.

D. Synthesis of Allyl-D-Galactosamine Derivatives



[Adapted from *Carbohydr. Res.* **1999**, *321*, 176]

To a slurry of D-glucosamine hydrochloride (10 g, 46.3 mmol) in MeOH (100 mL) was added 1.2 equiv. of NaOMe (solid). The mixture was filtered to remove solid NaCl and acetic anhydride (1.1 equiv.) was added. The reaction was stirred at room temperature for 2 h, monitored by TLC (15% MeOH/CH_2Cl_2). After completion, the solvent was removed under reduced pressure to give *N*-acetyl-D-glucosamine[6] as an off-white solid (8.5 g, 85%)

A suspension of this compound (10.0 g, 45.2 mmol) in 100 mL allyl alcohol was treated with $BF_3 \bullet Et_2O$ (2.0 mL, 15.9 mmol) and the mixture was heated at reflux under a drying tube. After 2 h a clear solution was obtained, and the mixture was cooled and the solvent removed under vacuum. The residue was recrystallized in EtOH/ether to give allyl-*N*-acetyl-α-D-glucosamine[7] (**H**) as a solid (9.0-10.0 g, 76-85%).

5. Ayadi, E.; Stanislas Czernecki, S.; Xie, J. Chem. Commun. **1996**, 347-348.
6. Liang, H.; Grindley, T. B. *J. Carbohydr. Chem*. **2004**, *23*, 71-82
7. Wong, T. C.; Townsend, R. R.; Lee, Y. C. *Carbohydr. Res*. **1987**, *170*, 27-46

A solution of compound **H** (10.0 g, 38.3 mmol) in pyridine (100 mL) was cooled to -15 °C and treated with pivaloyl chloride (11.2 mL, 91.9 mmol). The reaction mixture was stirred at room temperature for 12h. After completion of the reaction (monitored by TLC), solvent was removed and the residue was dissolved in CH_2Cl_2 (200 mL). The solution was washed with 1N HCl (50 mL), H_2O (100 mL), saturated $NaHCO_3$ (100 mL), and H_2O (100 mL). The organic layer was separated, dried (Na_2SO_4) and concentrated to give a crude product which was purified by flash column chromatography (EtOAc/hexanes) to give allyl-*N*-acetyl-3,6-*O*-pivaloyl-α-D-glucosamine[8,9] (**I**) as a white solid (12 g, 73%).

A solution of **I** (10.0 g, 23.3 mmol) in CH_2Cl_2/pyridine (1:1) was cooled to -15 °C and treated with triflic anhydride (4.7 mL, 27.9 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction (TLC), H_2O (4.2 mL) was added and the mixture was refluxed for 2 h. The mixture was then cooled, diluted with CH_2Cl_2, and washed with 1N HCl, sat. $NaHCO_3$, and H_2O in succession. The organic layer was separated, dried (Na_2SO_4) and removed by rotary evaporation. The crude product was purified by flash column chromatography to give an uncharacterized mixture of **J1** and **J2** as an off-white solid (6.0-7.0 g, 60-70%).

A solution of **J** (6.0 g, 13.9 mmol) in MeOH (30 mL) was treated with NaOMe (1.8 g, 34.7 mmol) and was stirred at room temperature for 12 h. After completion, the reaction was neutralized with IR-120 (H^+) resin, filtered, and the resin washed with MeOH (30 mL). The solvent was removed under reduced pressure to give allyl-*N*-acetyl-α-D-galactosamine[9] (**K**) as a white solid, which was further purified by silica gel chromatography (elution with MeOH/CH_2Cl_2) (3.6 g, 99%).

To a mixture of **K** (3.0 g, 11.5 mmol) and 2,2-dimethoxypropane (20 mL) was added *p*-toluenesulfonic acid (PTSA) (0.2 g). The reaction mixture was stirred at room temperature for 5 h. After the starting material was consumed, the reaction was neutralized with triethylamine and the solvent was removed. The crude mixture was purified by flash column chromatography (EtOAc/hexanes/CH_2Cl_2) to give a mixture of compounds **L1** and **L2**. To this mixture in MeOH (20 mL) was added 50% aq. AcOH (10 mL) and the mixture was stirred at room temperature for 1 h. After disappearance of the top spot on TLC, the reaction was neutralized with Na_2CO_3 and the solvent was removed by rotary evaporation. The residue was dissolved in EtOAc, washed with water, and the organic layer was separated, dried (Na_2SO_4) and concentrated. The crude product was purified by flash column chromatography (EtOAc/hexanes/CH_2Cl_2) to give allyl-*N*-acetyl-3,6-isopropylidene-α-D-galactosamine[9] (**L2**) as an off-white solid (2.5, 73%).

In an alternative procedure, compound **K** (1.63 mmol) was suspended in 7.5 mL of anhydrous DMF and treated with 2,2-dimethoxypropane (5 mL, 40.6 mmol) and camphorsulfonic acid (0.38 mmol, 0.23 equiv). The mixture was stirred for 3 h at room temperature. After completion of reaction as monitored by TLC, the reaction mixture was neutralized with triethylamine, filtered and concentrated under vacuum at 60°C. The product was purified by column chromatography as described above to obtain **L2** (43 % yield).

A solution of oxalyl chloride (0.23 mL, 2.64 mmol) in dry CH_2Cl_2 (10 mL) was cooled to -78 °C under a nitrogen atmosphere. Dry DMSO (0.37 mL, 5.28 mmol) was slowly added, and

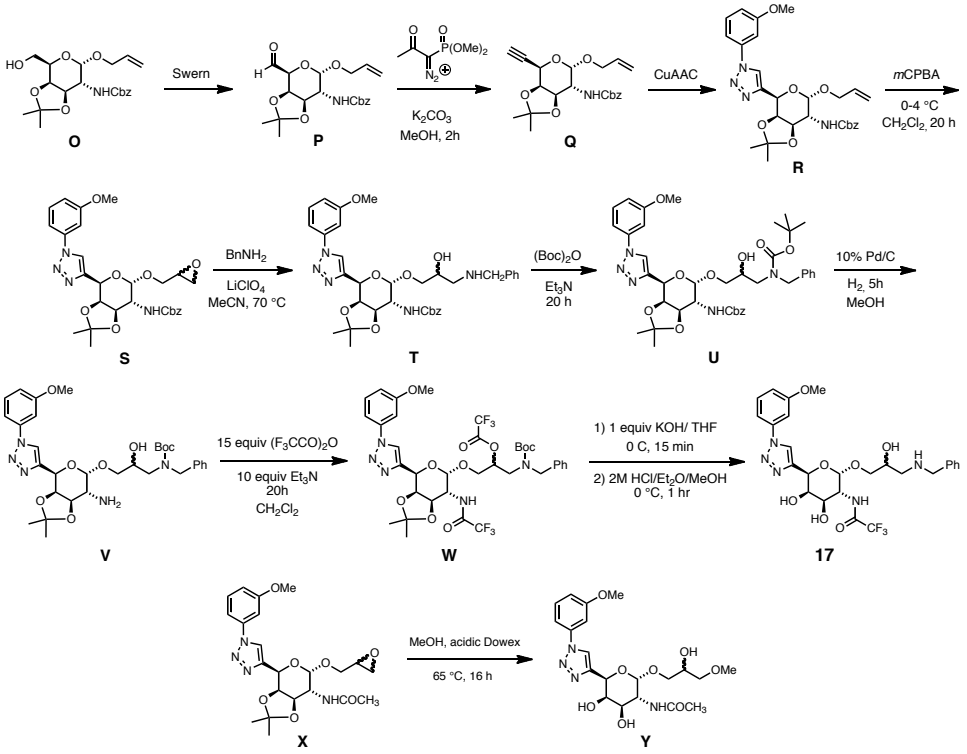
8. Ljevakovi, D.; Tomic, S.; Tomasic, J. *Carbohydr. Res*. **1988**, *182*, 197-205

9. a) Rye, C. S.; Withers, S. G. *J. Am. Chem. Soc.* **2002**, *124*, 9756-9767. b) Cipolla, L.; Ferla, B. L.; Lay, L.; Peri, F.; Nicotra, F. *Tetrahedron: Asymmetry* **2000**, *11,* 295–303

the reaction mixture was stirred for 10 min. A solution of **L2** (0.4 g, 1.32 mmol) in CH_2Cl_2 was then added by syringe to the cooled solution. After stirring for 15 min, Et_3N (0.74 mL, 5.28 mmol) was added and the reaction mixture was allowed to warm slowly to room temperature and was stirred for 30 min. The reaction was quenched by dilution with CH_2Cl_2 (20 mL) and washing with water (20 mL) . The organic layer was separated, dried (Na_2SO_4) and concentrated to give the crude intermediate aldehyde (0.38 g). This compound was dissolved in MeOH (10 mL), treated with K_2CO_3 (0.35 g, 2.5 mmol), and stirred for 10 min. at RT. The Bestmann-Ohira[10] reagent (dimethyl-1-diazo-2-oxopropylphosphonate, 0.36 g, 1.9 mmol) was then added and the reaction was stirred for 2h. After completion, the mixture was dried (Na_2SO_4), concentrated and the residue purified by flash column chromatography to give **5** as a white solid (0.27 g, 73%).

To a solution of compound **L2** (0.3 g, 1.0 mmol) in dry CH_2Cl_2 (10 mL) was added triethylamine (0.2 mL, 1.5 mmol), and the reaction mixture was cooled to 0 °C. A solution of *p*-toluenesulfonyl chloride (TsCl, 0.2 g, 1.1 mmol, in CH_2Cl_2) was added; the reaction mixture was allowed to warm to room temperature and was stirred for 3 h. After completion of the reaction, it was diluted with CH_2Cl_2 and washed with water. The organic layer was separated, dried, and concentrated to give the 6-tosylated intermediate (0.40 g, 90%). The tosylate (0.14 mmol) was dissolved in 1.3 mL of DMSO, and NaN_3 (10 equiv) dissolved in 0.2 mL of water was added. The reaction mixture was stirred at 100 °C for 4 days, and was then diluted with EtOAc (30 mL) and washed with water (20 mL). The organic layer was separated, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (EtOAc/hexanes) to give allyl-*N*-acetyl-6-azido-3,6-*O*-isopropylidene-α-D-galactosamine (81% yield).

E. Synthesis of N-Trifluoroacetamide-D-Galactosamine Derivatives



10. (a) Roth, G. J.; Liepold, B.; Stephan G. Müller, S. G.; Bestmann, H, J. *Synlett*. **1996**, 521-522. (b) Roth, G. J.; Liepold, B.; Stephan G. Müller, S. G.; Bestmann, H, J. *Synthesis* **2004**, 59-61

[Note: the procedures described here for epoxidation and ring opening are representative of those used for the preparation of **14**-**16**, as well as **17**.] N-Cbz protected alcohol **O**[11] was converted to aldehyde **P** by Swern oxidation (isolated yield of 83%). Aldehyde **P** was converted to alkyne **Q** using the Bestmann-Ohira procedure as described in the preceding section (see the preparation of **5**) in 66% yield. Alkyne **Q** was then clicked with 3-ethynylanisole by standard CuAAC reaction conditions as described below to generate the triazole **R**.

Compound **R** (130 mg, 0.24 mmol) was dissolved in dry CH_2Cl_2 and kept on ice. To this solution was added 3-chloroperoxybenzoic acid (70-75%, 2.6 equiv, 150 mg) and the mixture was stirred at 0-4 ^oC for 20 h. The reaction was judged to have gone to completion by TLC, and the mixture was washed with saturated aqueous $NaHCO_3$. The combined organic layers were dried with $MgSO_4$, and the solvent was removed by rotary evaporation. The resulting epoxide was purified by column chromatography, eluting with EtOAc/hexane/CH_2Cl_2) to give the diastereomeric mixture of epoxides **S** in 65% yield.

For epoxide opening by methanol (for the synthesis of **13** and **16**), the following procedure is representative. Epoxide **X** (25 mg, 0.042 mmol) was dissolved in 1.5 mL anhydrous methanol containing 20 mg of Dowex 50W X8 -200 mesh (ion exchange resin, acidic) and the mixture was heated at 65 °C for 16 h. Filtration, washing, and concentration provided the acetonide-deprotected intermediate **Y** in quantitative yield.

For epoxide opening by benzylamine: comound **S** (35 mg, 0.06 mmol) was dissolved in 2 mL dry acetonitrile. To this solution was added $LiClO_4$ (1 equiv) and benzylamine (10 equiv, 0.08 mL), and the mixture was heated at reflux (70 ^oC bath temperature) for 20 h. The reaction mixture was cooled, evaporated to dryness, redissolved in CH_2Cl_2, and washed with 0.1 N HCl. The organic layers were dried and purified by column chromatography (eluting with CH_2Cl_2/MeOH, to give amine **T** as a diastereomic mixture in 92% yield.

Compound **T** (0.06 mmol) was dissolved in 5 mL of dry CH_2Cl_2 and was treated with di-*tert*-butyldicarbonate (0.36 mmol) and triethylamine (0.3 mmol). The mixture was stirred for 20 h, and then was washed with water and back-extracted with CH_2Cl_2. The combined organic layers were dried and the product purified by column chromatography (gradient elution with CH_2Cl_2/MeOH to give **U** in 98% yield. (The crude material was occasionally contaminated with small amounts of what appears to be the O-*tert*-butyl ether.) Removal of the benzyloxycarbamate group in compound **U** (0.07 mmol) was accomplished by hydrogenation (1 atm) in the presence of catalytic (15% w/w) 10% Pd/C in methanol for 5 h. Compuond **V** (80% yield) was isolated by filtration of the reaction mixture through Celite and evaporation of the solvent, and was used without further purification.

Compound **V** was converted to the bis(trifluroacetamide) derivative by reacting with 15 equiv trifluoroacetic anhydride and 10 equiv triethylamine in CH_2Cl_2 for 20 h. After completion of the reaction the products were extracted from water with CH_2Cl_2, and the organic layer was dried, evaporated, and the product purified by column chromatography (elution with MeOH and CH_2Cl_2) in 95% isolated yield. Hydrolysis of the trifluoroacetamide ester to the corresponding monotrifluroacetamide was performed by treatment with 1 equiv. KOH on ice for 15 min, giving quantitative yield. The resulting intermediate was deprotected (both the *tert*-

11. See Fukuda, Y.; Sasai, H.; Suami, T. Bull. Chem. Soc. Jpn.1982, *55*, 1574-1578, for preparation of the analogous OMe glycoside. The same procedures were used to prepare compound **O**.

butylcarbamate and acetonide) by treatment with 2 M HCl in 1:1 Et_2O:MeOH to give the final trifluoroacetamide derivative **17** in 95 % yield.

F. General Procedure for Click Chemistry

Using solution-phase catalyst. To a solution of alkyne (0.58 mmol, 1 equiv) and azide (1.2 equiv) in MeOH (2.0 mL) was added THPTA[12] or TBTA[13] (5 mol%), $CuSO_4$ (1 mol %) in a small amount of water, and sodium ascorbate (10 mol%) in a small amount of water. The reaction was vigorously stirred for 5 h at room temperature, and was then diluted with CH_2Cl_2 (10 mL) and water (10 mL). The organic phase was separated and the aqueous phase was further extracted with CH_2Cl_2 (2 x 10 mL). The combined organic phases were dried over sodium sulfate, concentrated by rotary evaporation, and the residue was purified by chromatography over a short silica gel column to isolate the desired product.

Using a resin-bound catalyst. The strongly-binding $BimPy_2$ ligand reported earlier[14] was immobilized on a flexible tether to a NovaPEG resin. The preparation and properties of this ligand are described separately.[15] To the NovaPEG-$BimPy_2$ resin (5 mol% ligand with respect to limiting azide or alkyne reagent) was added MeoH, 5 mol% aq. $CuSO_4$, and alkyne or azide (0.05 mmol). The mixture was shaken for a minute, followed by addition of azide or alkyne (0.06 mmol) and 10 mol% aqueous sodium ascorbate solution. The mixture was sealed and was tumbled gently at room temperature throughout the reaction period. The reaction was monitored by TLC and after completion; the resin was filtered, washed with MeOH. The solvent was removed under reduced pressure to give the crude compound. The crude residue was purified by column chromatography to afford the triazole derivatives (90-95% yields).

G. General procedure for *O*-benzyl deprotection[10]

To a solution of tri-O-benzyl-D-galactose derivatives (1 mmol) in MeOH (10 mL) was added 20% $Pd(OH)_2$ (10% w/w) and ammonium formate (0.3 g). The reaction mixture was stirred at reflux temperature for 2 h. After completion of the reaction, the mixture was filtered through a pad of Celite, and the fitrate was concentrated under vacuum to isolate the desired product.

H. Surface Plasmon Resonance measurements

Preparation of biotinylated ASGPR. ASGPr H1 wild type was expressed as an insoluble inclusion body (IB) in *E. coli.* The cell pellet was re-suspended in TBS buffer pH 8.0 with 0.1 mg/mL lysozyme + 25 µg/mL DNase + 10 µg/mL RNase + 10 mM $MgCl_2$ and stirred on ice until well blended. The mixture was passed through a microfluidizer twice and centrifuged 30 minutes at 30,000 x g. The resultant IB pellet was washed with TBS buffer pH 8.0 + 3M urea and centrifuged and this process was repeated three additional times with water. The IB pellet was

12. Hong, V.; Presolski, S. I.; Ma, C.; Finn, M. G. *Angew. Chem. Int. Ed*, **2009**, *48*, 9879-9883.
13. Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 2853-2855.
14. Presolski, S.I.; Hong, V.; Cho, S.-H.; Finn, M.G. *J. Am. Chem. Soc.* **2010**, *132*, 14570-14576.
15. Presolski, S.I.; Manzenrieder, F.; Mamidyala, S.K.; Finn, M.G. **2011**, submitted.

solubilized in 10 mM Tris + 8M urea + 100 mM β-mercaptoethanol (pH 8.5), stirred for approximately 20 minutes, then centrifuged to discard any precipitate. For the following manipulations, "buffer T" refers to 20 mM Tris + 0.5 M NaCl + 25 mM $CaCl_2$. The supernatant was diluted to approximately 0.5 mg/mL in buffer T + 2mM β-mercaptoethanol + 8M urea (pH 8.0), then dialysed vs. 8-10x excess volume of buffer T + 2mM β-mercaptoethanol + 2 M urea (pH 8.0) overnight at 4°C. Dialysis was repeated in buffer T + 1mM β-mercaptoethanol + 1 M urea (pH 8.0) for approximately 24 h at 4°C with several volume changes with a final dialysis vs. buffer T + 5 mM GSH + 1 mM GSSG (pH 7.5) with 3 volume changes. The resulting sample was centrifuged and the supernatant was loaded onto an *N*-acetyl-D-galactosamine agarose packed Pharmacia XK 26 column, equilibrated in buffer T + 2mM TCEP (pH 8.0). The column was washed with this buffer until baseline was re-established. The bound protein was eluted with 20 mM Tris + 0.5 M NaCl + 2 mM TCEP + 2 mM EDTA (pH 8.0). The final yield was approximately 50 mg from 5 L (70 wgm.) *E.coli* cell pellet.

While BSA has one cysteine (Cys34) not engaged in disulfide bonding, this thiol group is often blocked as a mixed disulfide with small thiols such as cysteine, or as the sulfenic acid by aerobic oxidation.[16] Disulfide reduction and labeling were found to be the cleanest method for derivatization of the ASGPR binding domain among those that we tested. Mild disulfide reduction conditions were chosen so as not to disrupt buried intra-strand (solvent inaccessible) crosslinks. Thus, immediately prior to biotinylation, the ASGPR sample was first incubated with 1 mM TCEP to ensure that the sample contained a single free thiol. The protein was reacted with a 19-fold molar excess of Pierce Maleimide-PEG2-biotin reagent in PBS overnight at 4°C. Excess biotin was removed from the sample using either PD-10 columns (GE Healthcare) or Zeba Spin Desalting columns (Thermo/Pierce) using manufacturer protocols. LC-MS analysis of the product verified the presence of a single mass with addition of 525 amu consistent with the molecular weight of the maleimide adduct. Since TCEP was present throughout the maleimide reaction, if the protein had been fully reduced (making other cysteine thiols available), the result would have been a heterogeneous product containing multiple adducts, and probably misfolding or precipitation upon removal of key structural disulfide linkages. No such outcomes were observed. Furthermore, the observation of standard binding specificity (GalNAc *vs.* methyl galactoside *vs.* lactose) strongly suggests that enough of the protein on the SPR chip was properly folded and displayed.

SPR binding measurements. All SPR measurements were performed using a Biacore 3000 instrument (GE Healthcare) at 25°C. Biotinylated ASGPR was immobilized typically at 2000-3500 resonance units (Ru) using either SA sensor chips (GE Healthcare) or custom sensor chips with Neutravidin (Pierce Biochemical) immobilized by standard amine coupling to CM5 sensor chips (GE Healthcare). The running buffer was HBS (10 mM HEPES, 150 mM NaCl), 50 mM $CaCl_2$, 0.01% p20, 3% DMSO pH 7.5 or 50 mM Tris, 150 mM NaCl, 50 mM $CaCl_2$, 0.01% p20, 3% DMSO pH 7.5. [Some of the initial data sets were collected in HEPES but Tris buffer appeared to produce data with better signal-to-noise ratios. The affinity values were unchanged by this difference in buffer.] Compounds were serially diluted 3 fold from 900 μM to 3.7 μM for initial evaluation. Compound solutions were injected at 50 μL/min for 1 min followed by a 1 min dissociation in duplicate for each concentration. Data was processed using Scrubber2 (Biologic

16. Kettenhofen, N. J.; Wood, M. J. *Chem. Res. Toxicol.* **2010**, *23*, 1633-1646.

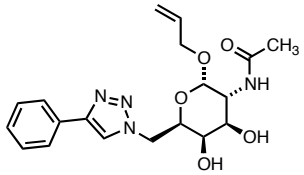
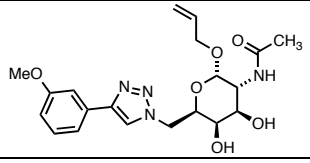
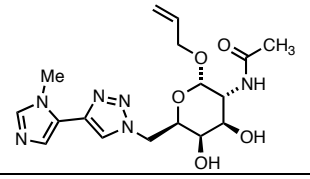
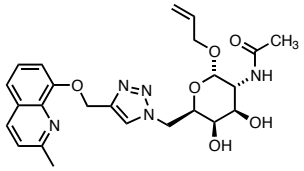
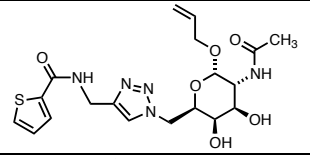
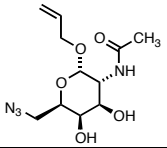
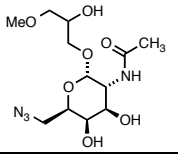
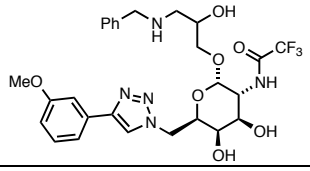
Software, Inc.) to zero, align, double reference and correct the data for excluded volume effects. Values of $1/K_{ads}$ (the immobilized-phase analogue to solution-phase K_d) for were determined by fitting the steady state binding responses for the six concentrations to a single site binding model in Scrubber2. Typical signal levels (R_{max}) achieved for GalNAc were on the order of 15 Ru consistent with approximately 50% of the surface bound protein showing activity.

In the course of these experiments, there were observed shifts in the baselines after some compound injections at the highest concentrations used (900 μM) and were often excluded from the fits of the data. Also many of the compounds failed to approach saturation and a $1/K_{ads}$ value could not be obtained. Decreasing signals can often be observed at high concentrations of analyte as the sample plug may not be uniform. Note that minor "negative" signals of the type previously reported for carbohydrates binding to maltose binding protein[17] were observed in the initial development of the ASGPR assay, but were largely eliminated upon optimization of the assay, save for occasional aberrant injections.

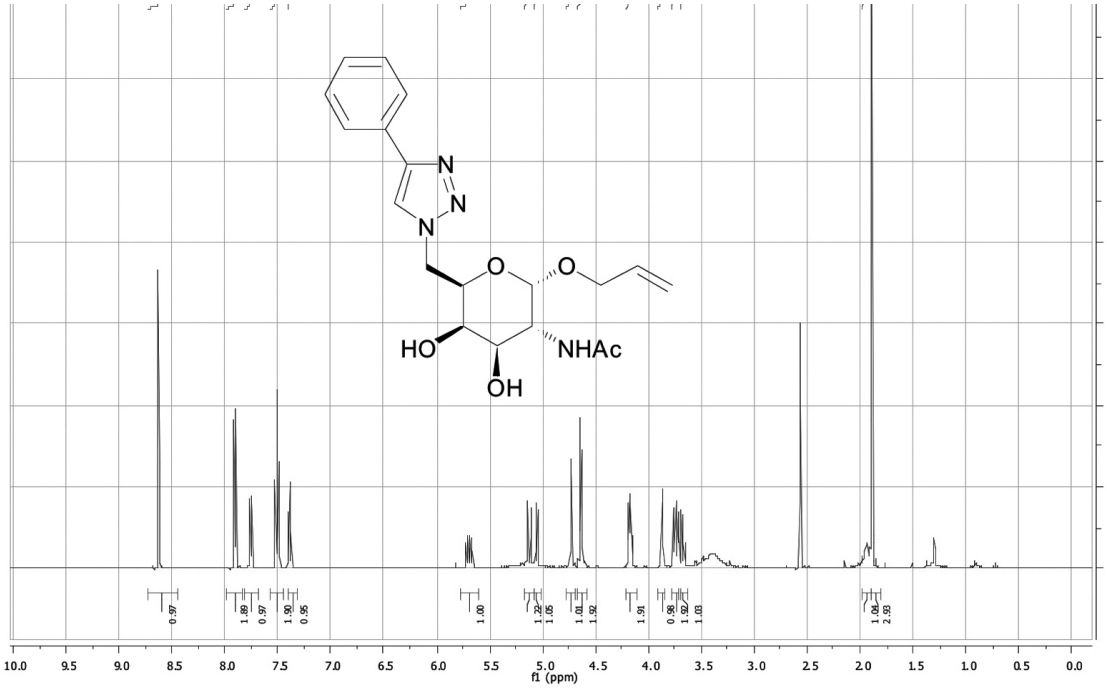
17. Gestwicki, J.E.; Hsieh, H.V.; Pitner, J.B. *Anal. Chem.* **2001**, *23*, 5732-5737.

I. Characterization data for selected high-affinity ligands

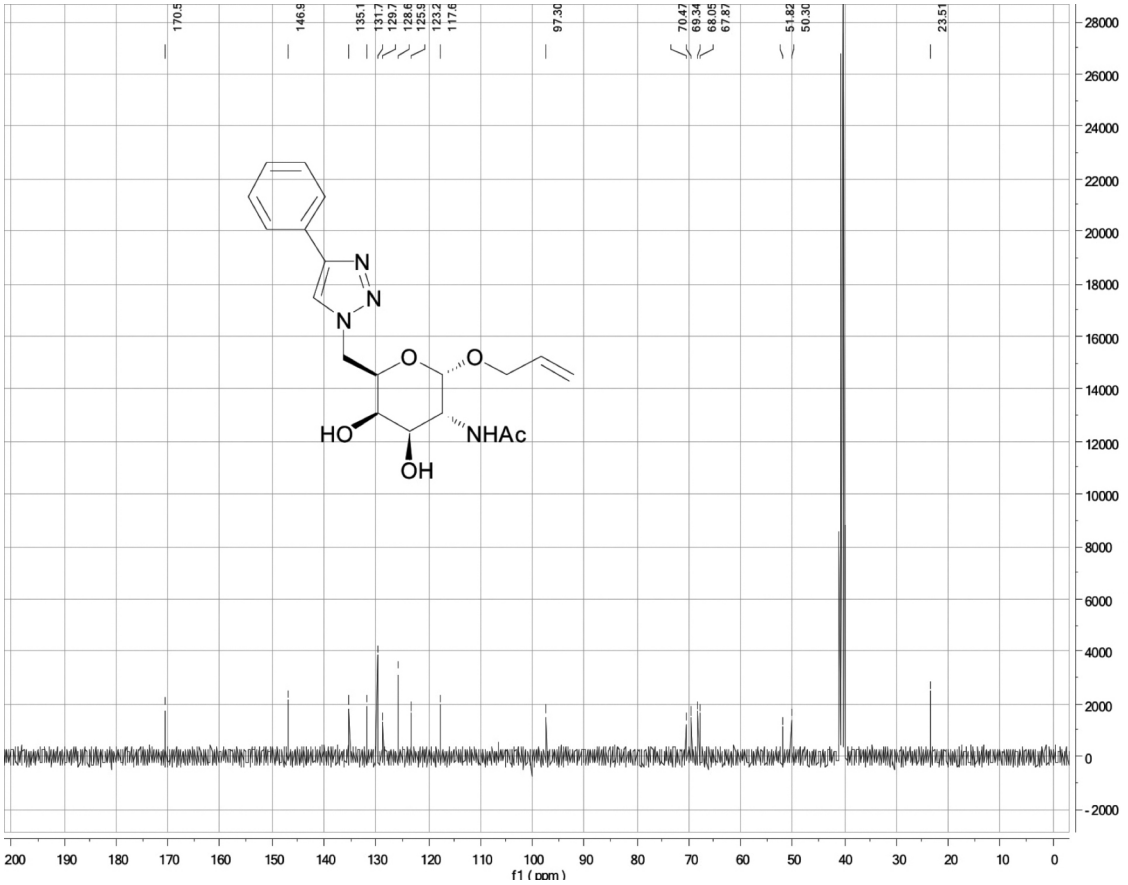
Table S2. High-resolution electrospray ionization mass spectrometry and optical rotation data.

Cpd. ID	Structure	K_d	calcd mass $(M+H)^+$	obsvd mass $(M+H)^+$	$[\alpha]_D^{23}$
8b		8.5 ± 1.4 μM	389.1819	389.1829	+174.4°
8e		8.2 ± 0.7 μM	419.1925	419.1935	+86.5°
8h		9.0 ± 4.1 μM	393.1881	393.1890	+126.7°
8i		3.2 ± 2.0 μM	484.2191	484.2202	+58.0°
8j		6.6 ± 2.5 μM	452.1598	452.1606	+94.2°
11		1.6 ± 0.60 μM	287.1350	287.1354	+82.3°
16		6.0 ± 0.45 μM	335.1561	335.1569	n/a
17		1.2 ± 0.6 μM	582.2170	582.2179	n/a

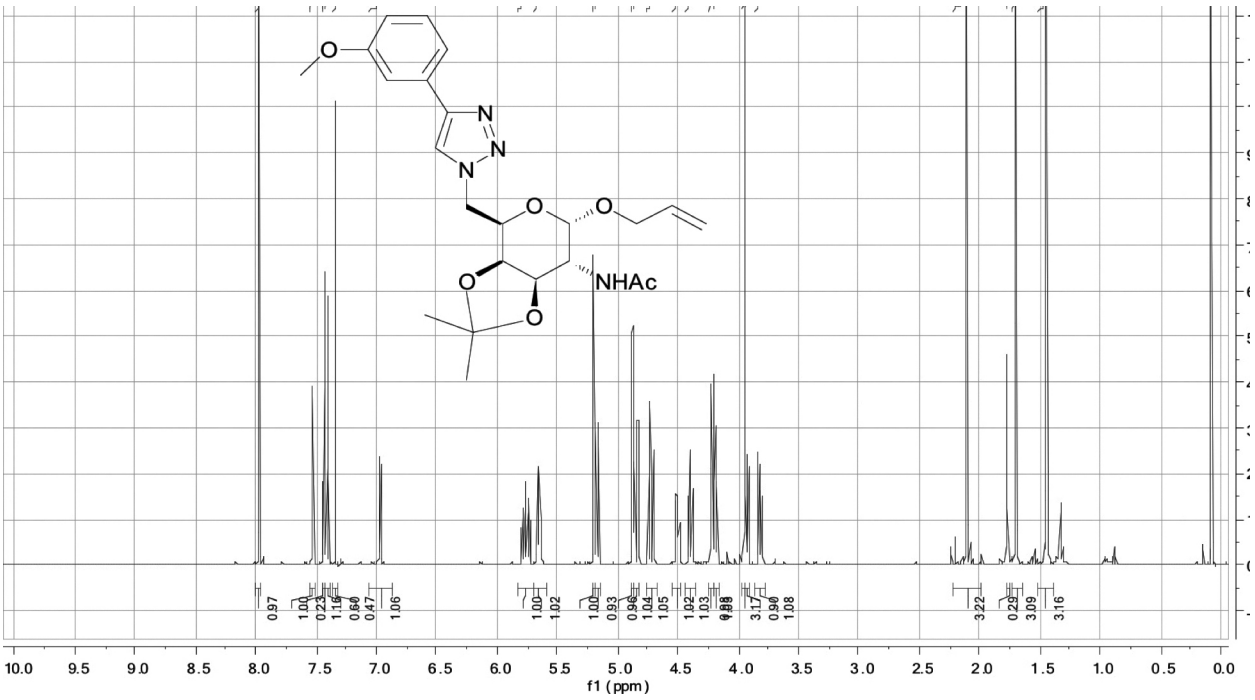
^{1}H NMR, 500MHz, DMSO-d_6, (Compound **8b**)



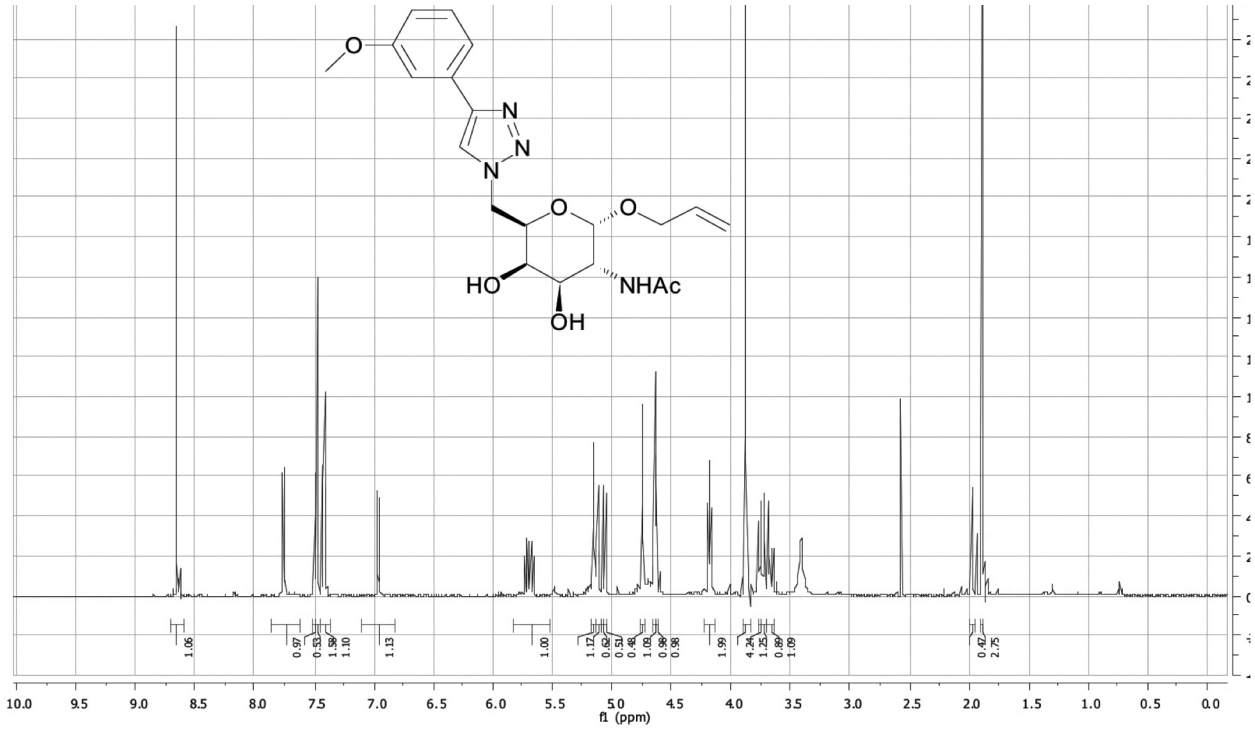
^{13}C NMR, 125 MHz, DMSO-d_6, (Compound **8b**)



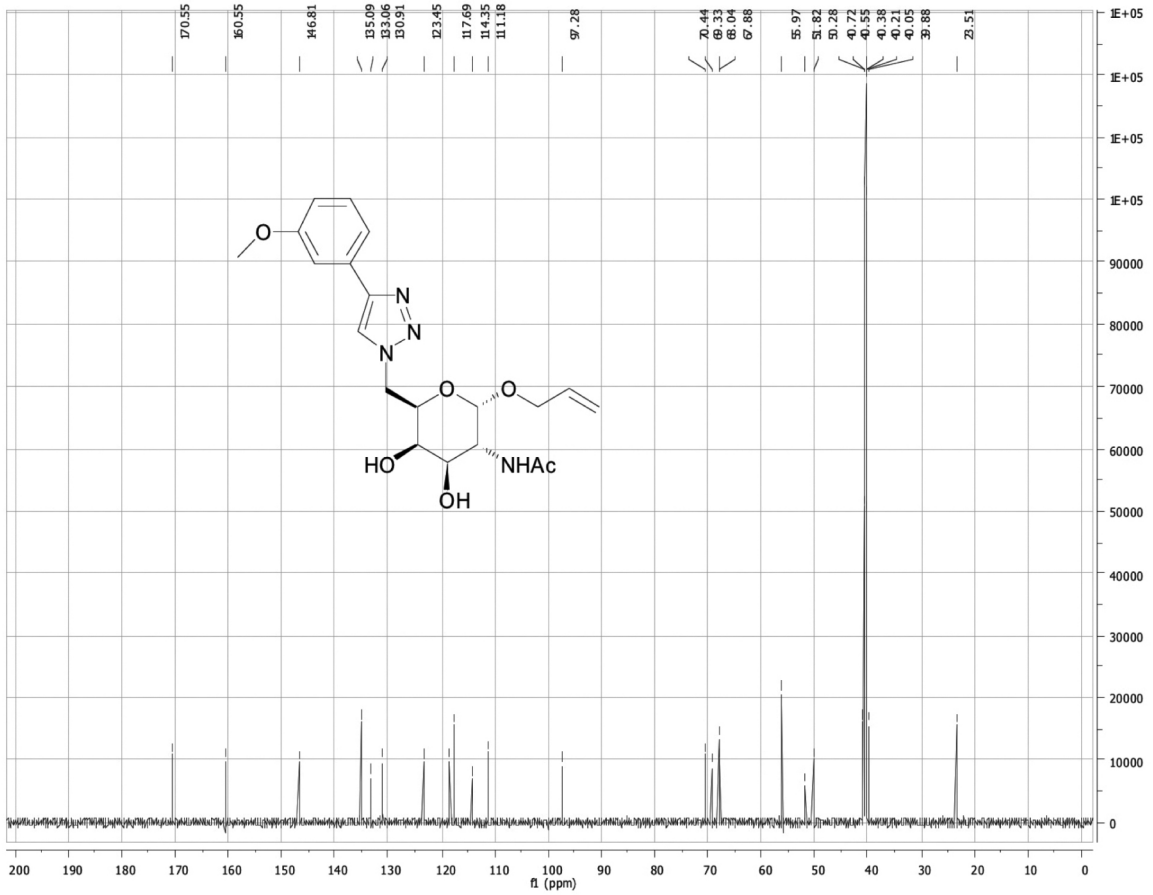
^{1}H NMR, 500MHz, $CDCl_3$, (Compound **8e**, acetonide protected)



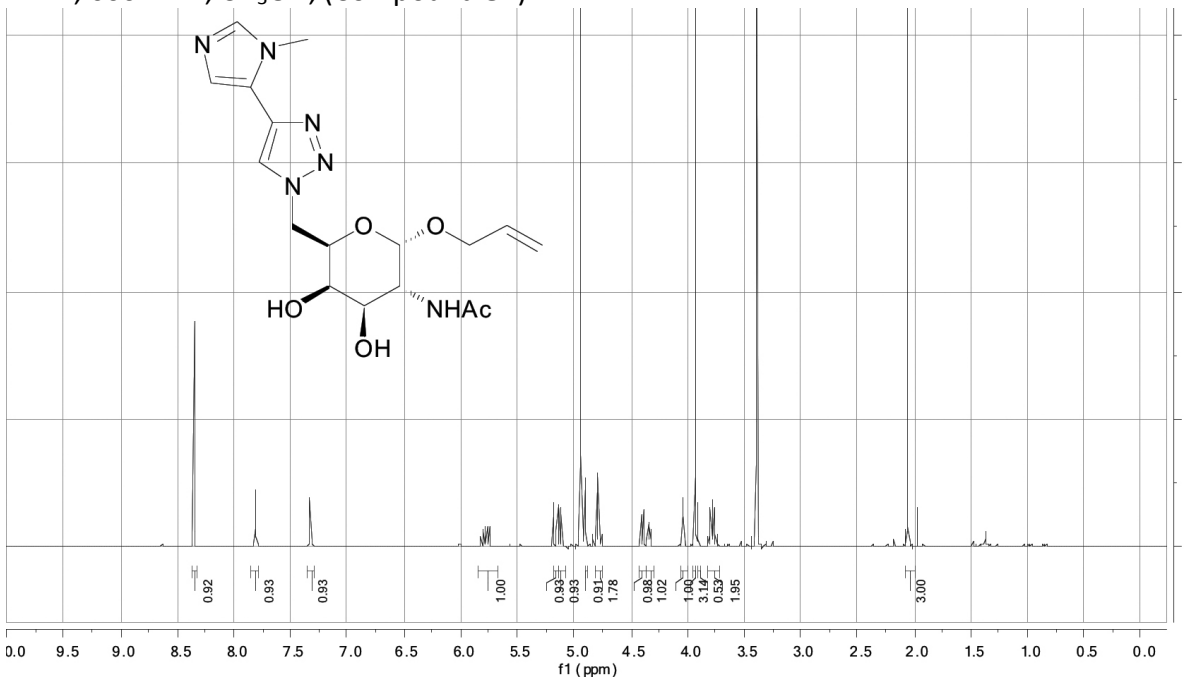
^{1}H NMR, 500MHz, DMSO-d_6, (Compound **8e**)



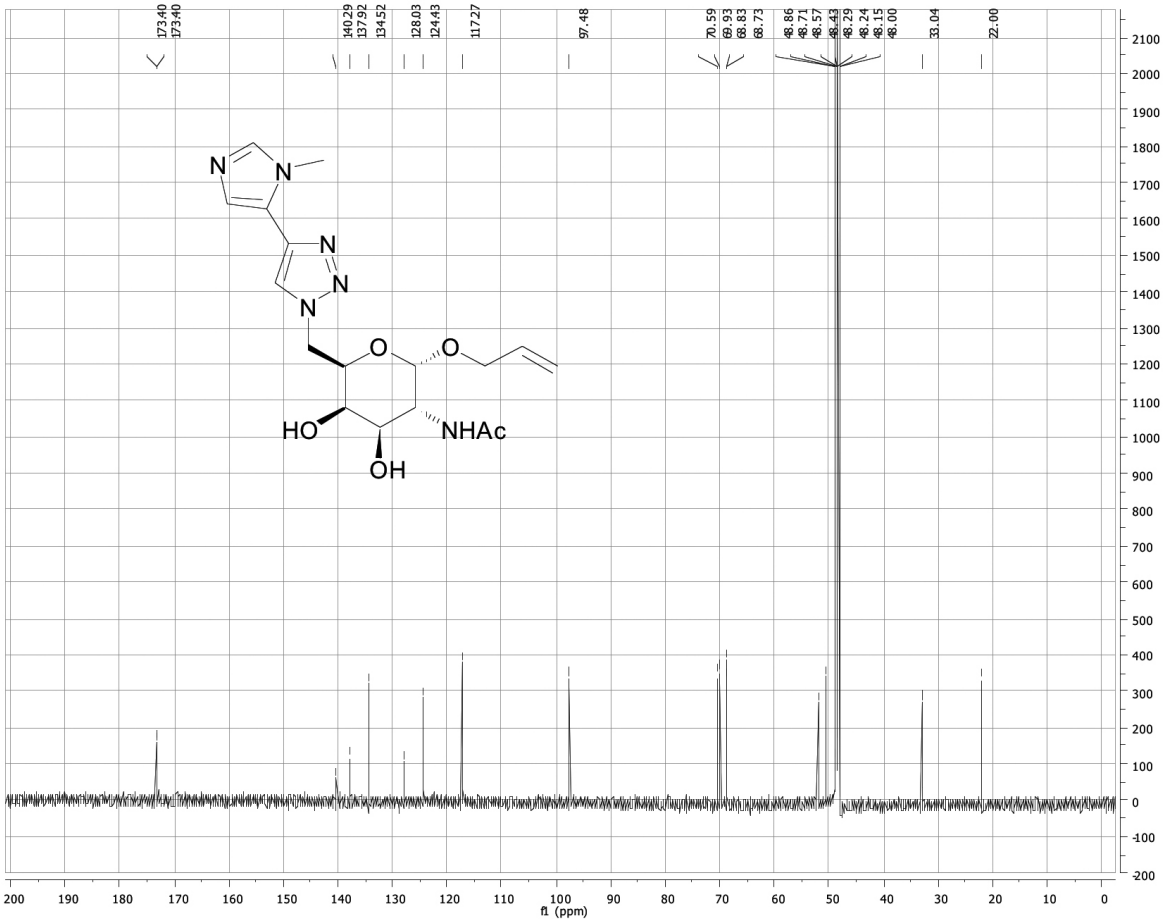
^{13}C NMR, 125 MHz, DMSO-d_6, (Compound **8e**)



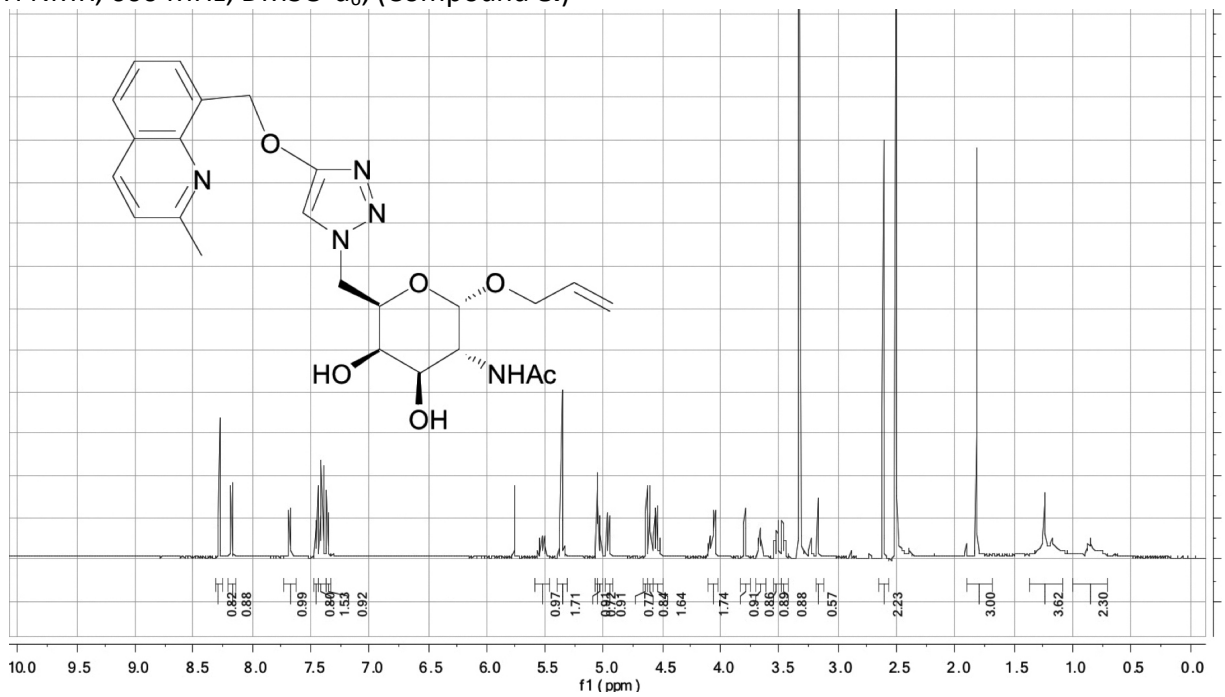
^{1}H NMR, 600 MHz, CD_3OD, (Compound **8h**)



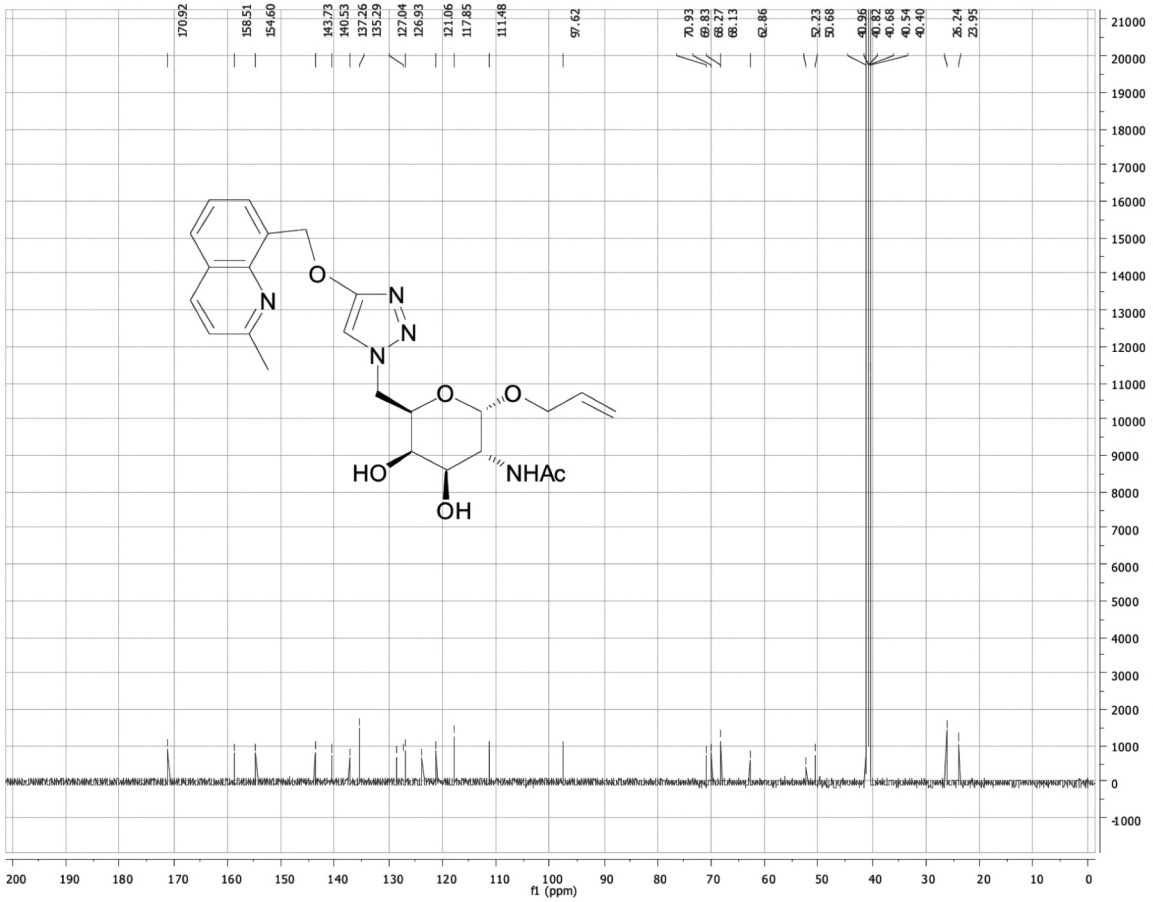
^{13}C NMR, 167 MHz, CD_3OD, (Compound **8h**)



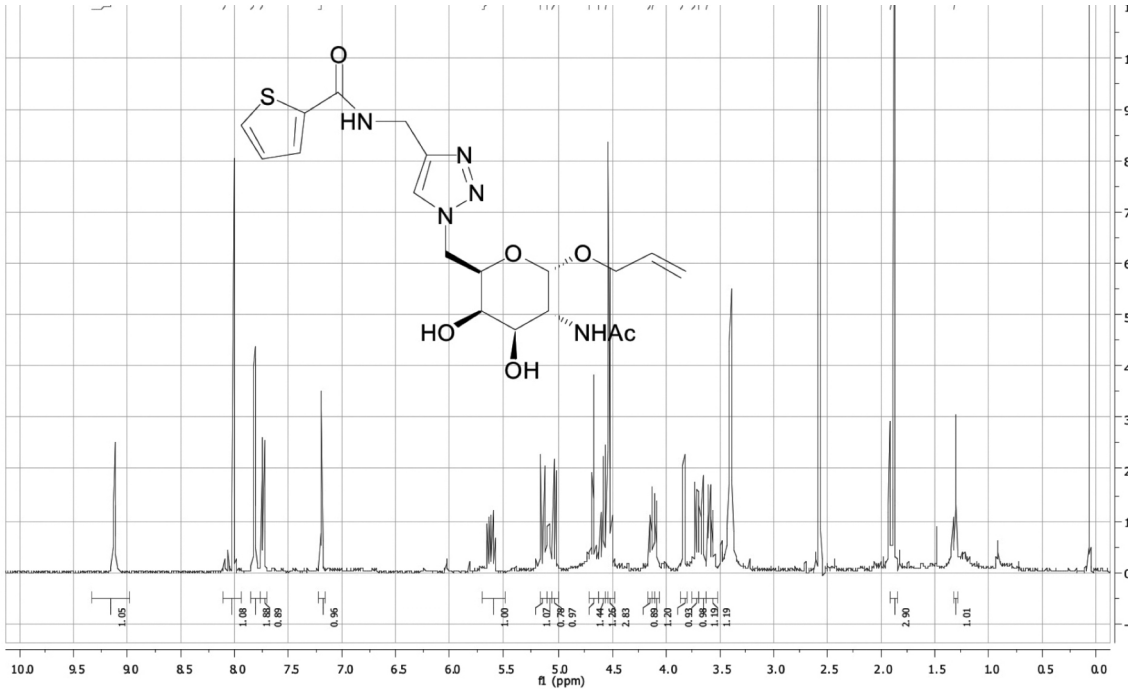
^{1}H NMR, 600 MHz, DMSO-d_6, (Compound **8i**)



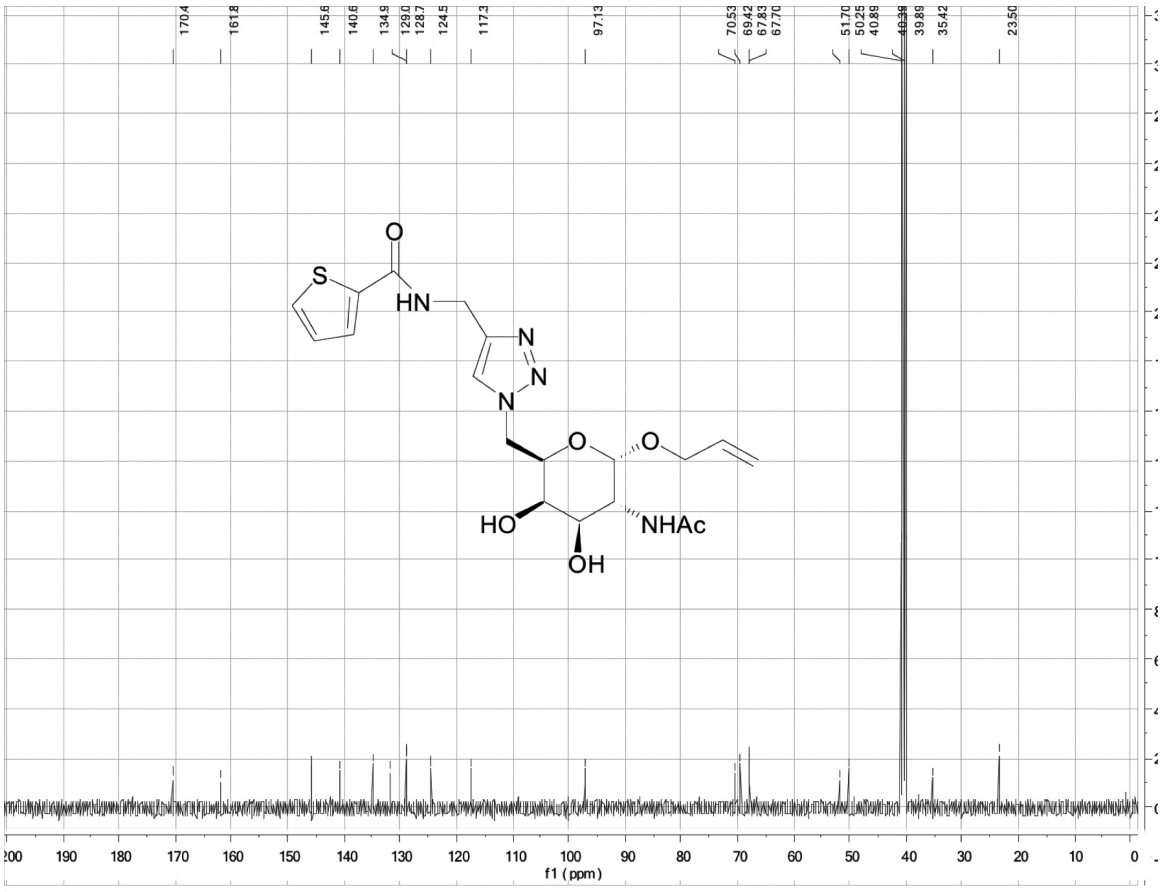
^{13}C NMR, 167 MHz, DMSO-d_6, (Compound **8i**)



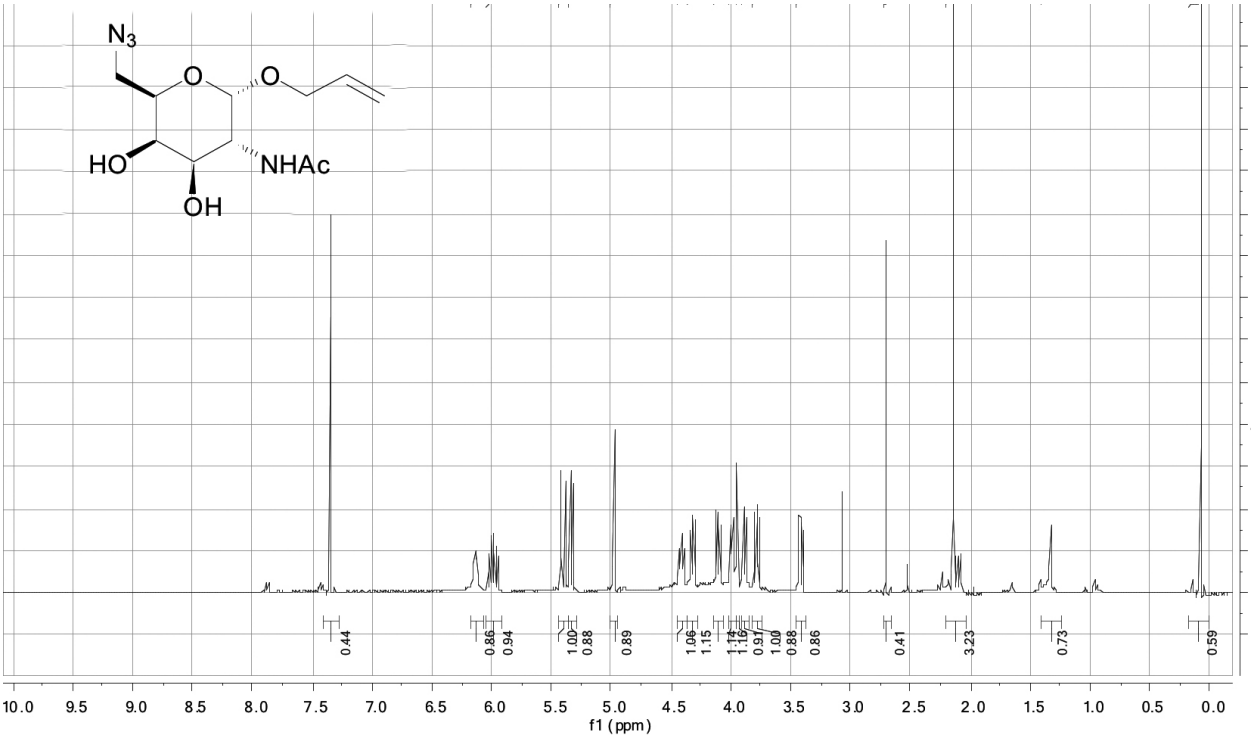
^{1}H NMR, 500 MHz, DMSO-d_6, (Compound **8j**)



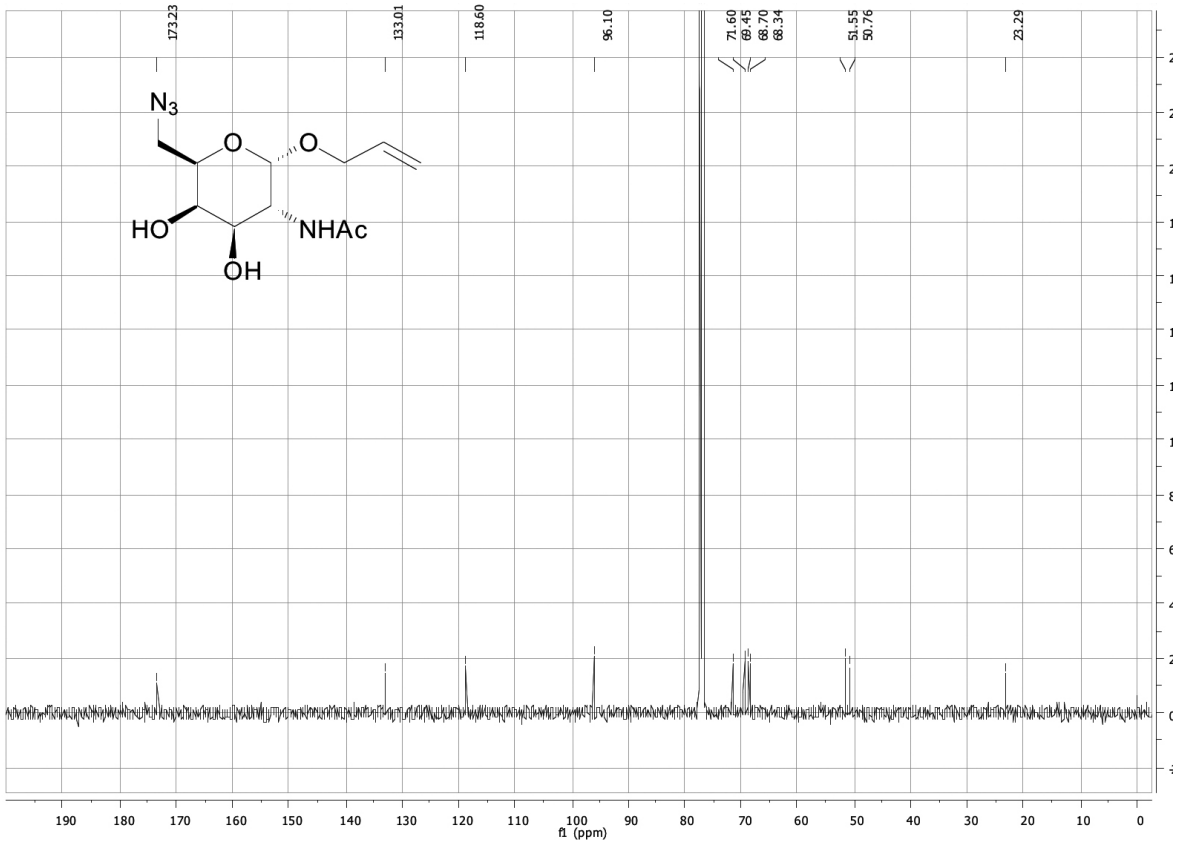
^{13}C NMR, 125 MHz, DMSO-d_6, (Compound **8j**)



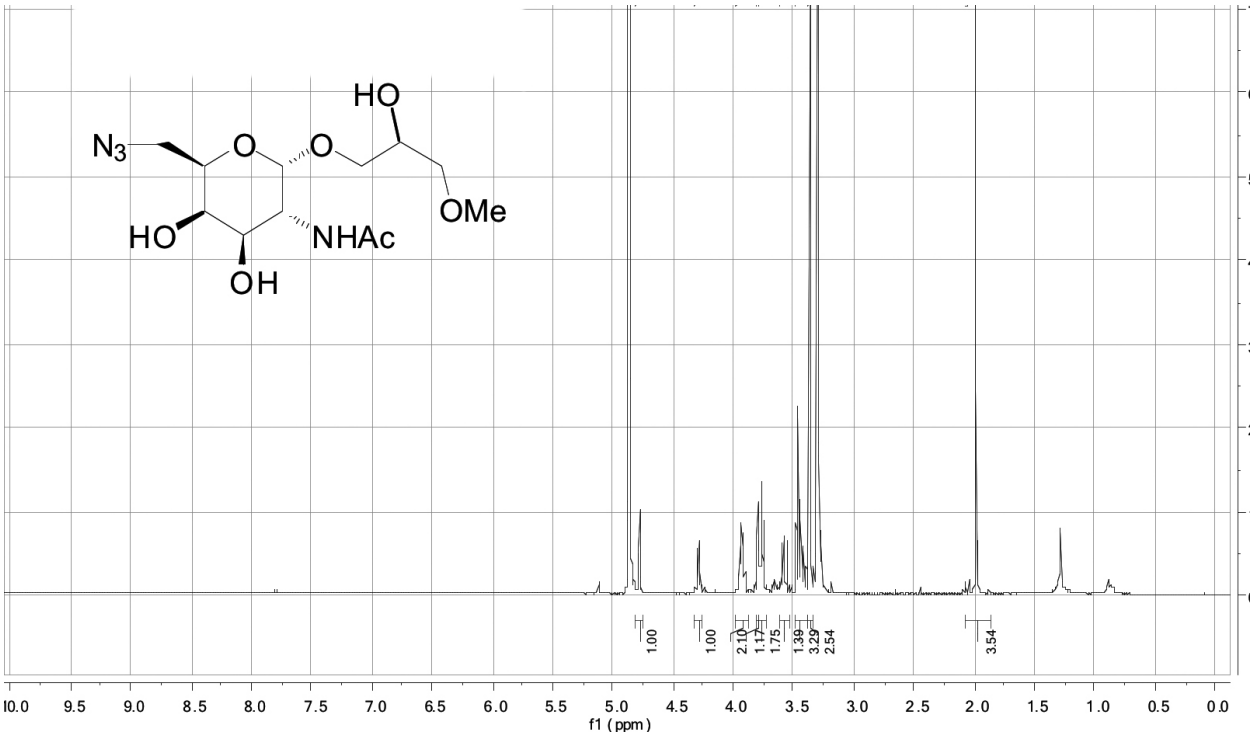
^{1}H NMR, 500 MHz, $CDCl_3$, (Compound **11**)



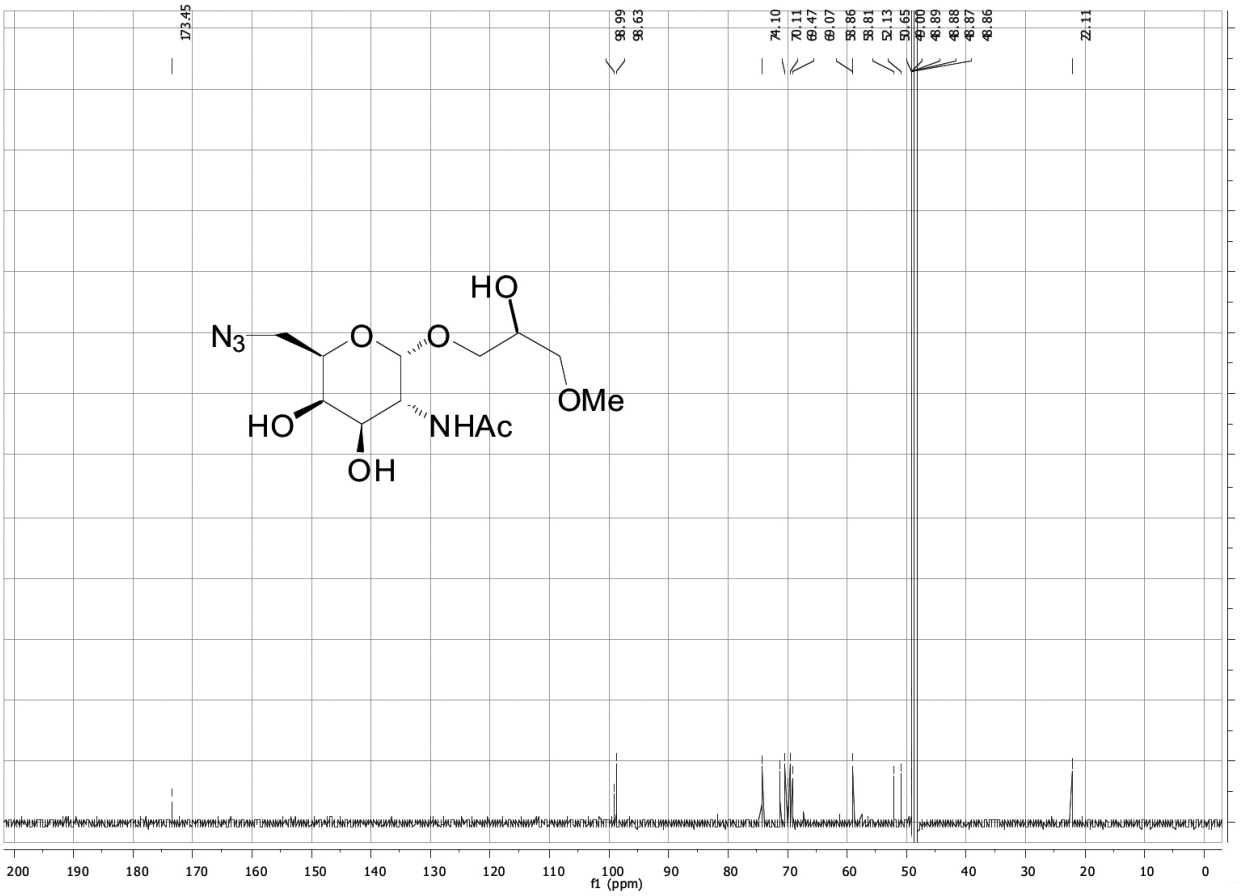
^{13}C NMR, 125 MHz, $CDCl_3$, (Compound **11**)



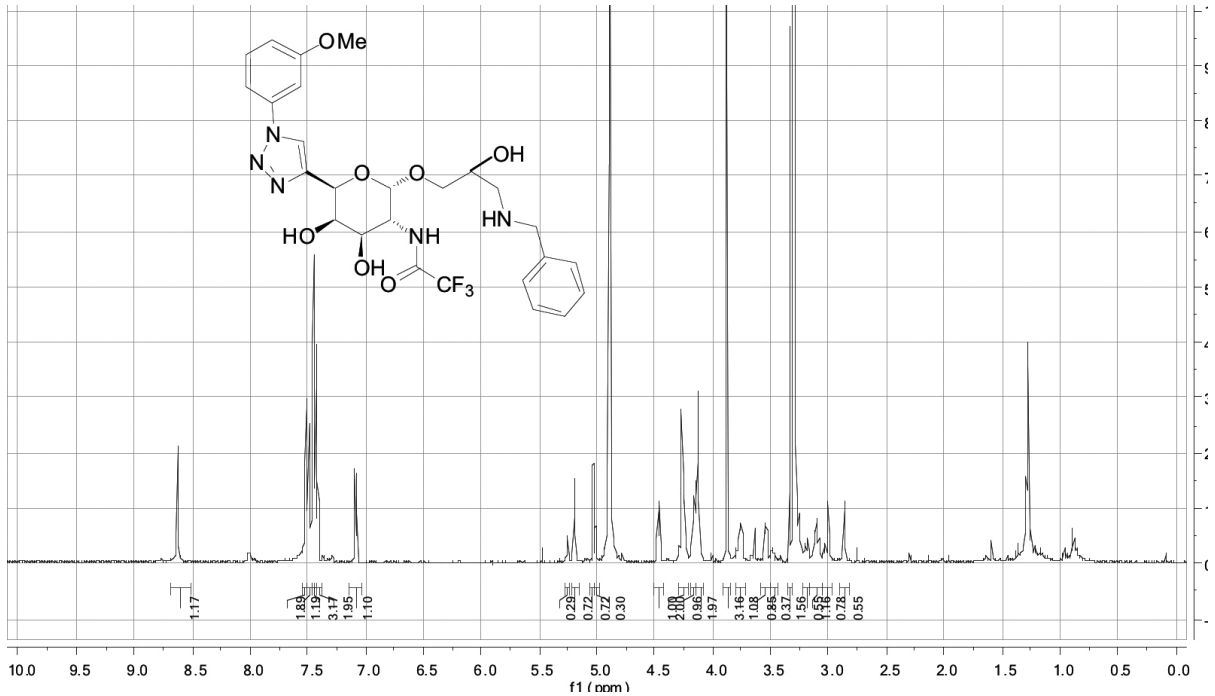
^{1}H NMR, 600 MHz, CD_3OD, (Compound **16**)



^{13}C NMR, 167 MHz, CD_3OD, (Compound **16**)



^{1}H NMR, 600 MHz, CD_3OD, (Compound **17**)



^{13}C NMR, 167 MHz, CD_3OD, (Compound **17**)