SUPPLEMENTARY INFORMATION

Synthetic Procedures

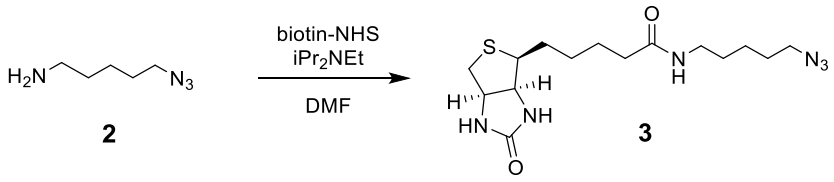
Materials

Materials obtained from commercial suppliers were used without further purification. All other chemicals and reagents were purchased from Sigma Aldrich. Deuterated NMR solvents were purchased from Cambridge Isotope Laboratories.

Instrumentation

NMR spectra were recorded on a Varian VX 500 MHz spectrometer or a Varian 400 MHz spectrometer. Mass spectra were recorded at UCSD Chemistry and Biochemistry Mass Spectrometry Facility utilizing an Agilent 6230 HR-ESI-TOF mass spectrometer. Reverse-phase HPLC purification (CLIPEUS, C18, 5 um, 10 x 240 mm, Higgins analytical) and analysis (Eclipse, XDB-C18, 5um, 4.6 x 150 mm) were carried out on an Agilent 1200 series instrument or Beckman Coulter System Gold 127P Solvent Module.

Methods



2 **3**

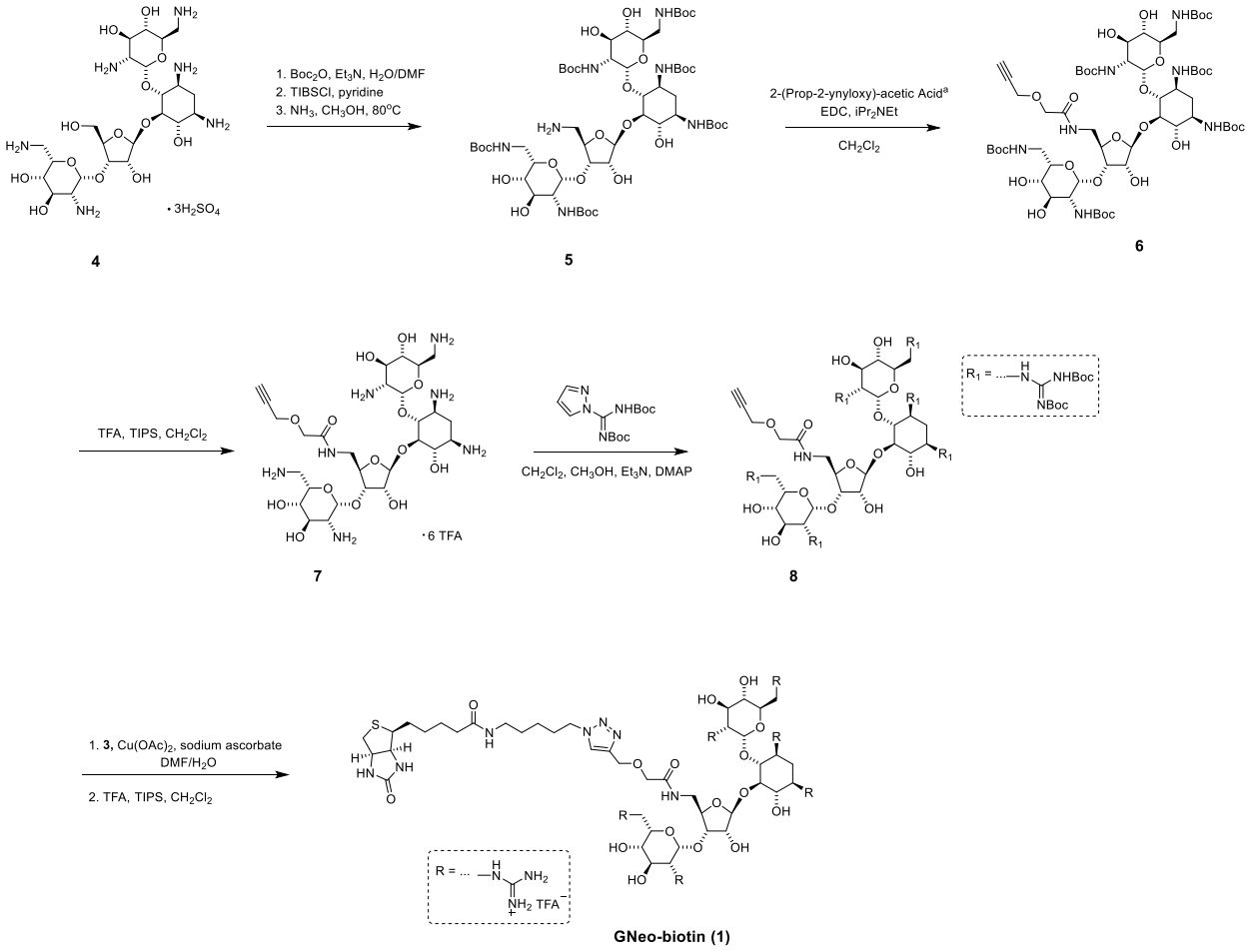
Scheme 1. Synthesis of biotin linker.

Synthesis of biotin linker (3):

The synthesis for compound **2** has been previously reported [1,2]. Biotin-NHS (218 mg, 0.64 mmol) and *N,N*-diisopropylethylamine (111 µL, 0.64 mmol) were added to a solution of **2** (0.58 mmol) in

DMF (800 µL). The reaction was stirred overnight at room temperature. The solvent was concentrated in vacuo then dissolved in CH_2Cl_2 and washed with water and brine. The compound was purified on silica gel using methanol and CH_2Cl_2 (60% yield).

^{1}H NMR (500 MHz, CD_3OD): δ 4.51 (m, 1H), 4.32 (m, 1H), 3.23 (m, 2H), 3.18 (m, 3H), 2.92 (dd, *J*=5.3 Hz, 13.2 Hz, 1H), 2.74 (d, *J*=13.2 Hz, 1H), 2.22 (t, *J*=7.4 Hz, 2H), 1.69-1.39 (m, 12H). ^{13}C NMR (126 MHz, CD_3OD) δ: 174.62, 164.57, 62.09, 60.32, 55.64, 51.03, 39.83, 38.91, 35.50, 28.61, 28.42, 28.29, 28.09, 25.56, 23.83. HRMS (m/z): $[M+Na]^+$ calcd. for C15H26N6O2S, 377.1730; found, 377.1730.



Scheme 2. Synthesis of GNeo-biotin.

Compound **5** was synthesized as previously reported [2]. Alkyne-acid linker [2-(prop-2-ynloxy)-acetic acid] was prepared as previously reported [2].

Synthesis of BocNeo-alkyne derivative (6):

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (3.2 eq) was added to a solution of 2-(prop-2-ynloxy)-acetic acid (3.2 eq) in dichloromethane (CH_2Cl_2, 0.6 ml/mmol). The mixture was stirred for 30 min. A solution of **5** (1 eq) in CH_2Cl_2 (0.2 mol/L) and *N*,*N*-diisopropylethylamine (3 eq) was then added dropwise. The mixture was then stirred for 48 h. The mixture was partitioned between CH_2Cl_2 and 5% citric acid. The organic layer was separated and washed with sodium bicarbonate and brine. The organic layer was collected, dried over sodium sulfate, filtered off, and the filtrate was evaporated. Silica gel column chromatography (0-8% MeOH in CH_2Cl_2) afforded the desired product as a colorless amorphous (46% yield).

^{1}H NMR (500 MHz, CD_3OD): δ 6.59 (d, *J*=8.1 Hz, 1H), 5.37 (s, 1H), 5.11 (s, 1H), 4.35-4.30 (m, 4H), 4.18-4.11 (m, 3H), 3.96-3.89 (m, 3H), 3.75-3.36 (m, 15H), 3.19 (m, 3H), 2.97 (t, *J*=2.8 Hz, 1H), 1.96 (d, *J*=13.0, 1H), 1.47-1.43 (m, 54 H); ^{13}C NMR (126 MHz, CD_3OD) δ: 170.62, 157.66, 157.45, 157.13, 156.94, 156.60, 156.49, 109.94, 98.89, 97.58, 85.67, 79.29, 79.05, 78.95, 78.91, 78.77, 78.52, 75.95, 74.14, 73.83, 73.22, 71.83, 71.43, 71.25, 70.27, 67.88, 67.68, 57.94, 55.45, 52.10, 51.10, 49.96, 41.16, 40.69, 34.47, 27.55, 27.45, 27.43, 27.39, 27.34. HRMS (m/z): $[M+Na]^+$ calcd. for C58H99N7O26, 1332.6532; found, 1332.6529.

Synthesis of Neo-alkyne (7):

To a solution of **6** (1 eq) and triisopropylsilane (6.0 eq) in CH_2Cl_2 (6.1 ml/mmol) was added trifluoroacetic acid (TFA, 6.1 ml/mmol) at room temperature. The mixture was stirred for 2 hours. The reaction was azeotroped in toluene 3 times and dissolved in water. The solution was washed with CH_2Cl_2 (3 times) and lyophilized to give the desired product as a colorless amorphous (84% yield).

^{1}H NMR (500 MHz, D_2O): δ 5.79 (d, *J*=3.85 Hz, 1H), 5.19 (d, *J*=3.34 Hz, 1H), 5.08 (d, *J*=1.62 Hz, 1H); ^{13}C NMR (126 MHz, D_2O) δ: 172.41, 163.26, 162.98, 162.70, 162.42, 119.65, 117.33, 115.01, 112.69, 109.86, 95.19, 94.58, 84.94, 79.56, 78.40, 77.21, 76.63, 74.87, 73.06, 72.16, 70.11, 69.65, 68.23, 67.90, 67.37, 67.01, 58.41, 52.94, 50.67, 49.42, 48.30, 40.68, 40.19, 39.79, 27.72.

Synthesis of BocGNeo-alkyne (8):

A solution of **7** (1 eq) in MeOH (0.12 mol/L) was added to CH_2Cl_2 (0.036 mol/L), triethylamine (15 eq) and *N*,*N*′-Di-Boc-1*H*-pyrazole-1-carboxamidine (15 eq), and 4-dimethylaminopyridine (1 eq) at ambient temperature. The mixture was stirred for 120 h. The mixture was partitioned between CH_2Cl_2 and 5% citric acid. The organic layer was collected, dried over sodium sulfate, filtered off, and the filtrate was evaporated. Silica gel column chromatography (0-3% MeOH in CH_2Cl_2) afforded the desired product as a colorless amorphous (63% yield).

^{1}H NMR (500 MHz, CD_3OD): δ 5.85 (d, *J*=3.89 Hz, 1H), 5.08 (s, 1H), 5.02 (s, 1H), 4.58 (m, 1H), 4.39-4.32 (m, 3H), 4.28 (m, 1H), 4.16-4.08 (m, 3H), 3.92-3.85 (m, 4H), 3.77-3.69 (m, 3H), 3.54-3.48 (m, 2H), 3.22 (m, 1H), 2.25 (m, 1H), 1.59-1.4 (m, 108H); ^{13}C NMR (126 MHz, CD_3OD) δ: 170.55, 163.20, 162.92, 162.82, 162.76, 157.35, 156.57, 156.43, 156.13, 156.10, 153.29, 153.01, 152.78, 152.74, 152.60, 151.88, 151.79, 111.54, 97.92, 95.85, 87.30, 83.39, 83.31, 83.25, 83.22, 82.94, 82.66, 82.54, 81.58, 79.18, 79.13, 79.10, 79.08, 78.90, 78.84, 78.58, 78.39, 75.83, 75.65, 75.30, 74.19, 72.85, 72.19, 71.87, 70.58, 69.74, 68.13, 66.71, 58.24, 53.85, 51.59, 50.45, 48.76, 42.93, 41.34, 40.28, 33.85, 29.36, 27.44, 27.27, 27.16, 27.02, 26.97, 26.84, 26.81. HRMS (m/z): $[M+2H]^{2+}$ calcd. for C94H159N19O38, 1082.0619; found, 1082.0631.

Synthesis of GNeo-biotin (1):

8 (0.046 mmol) and **3** (0.069 mmol) were dissolved in dimethylformamide (1 mL). Cu(II) sulfate hydrate (0.01 mmol) and sodium ascorbate (0.01 mmol) were added as a solution in H_2O (150 µL). The reaction was stirred overnight at room temperature under argon. The reaction was

diluted into CH_2Cl_2 and washed with H_2O and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The protected product was dissolved in CH_2Cl_2 (1 mL) and treated with trifluoroacetic acid (1 mL) and triisopropylsilane (10 µL) for 2 hours at room temperature. The reaction was evaporated and azeotroped with toluene (3x) and purified on a C-18 reverse phase HPLC column [5-60% ACN (0.1% TFA) in H_2O (0.1% TFA) over 18 min] (46% yield).

^{1}H NMR (500 MHz, D_2O): δ 8.01 (s, 1H), 5.69 (s, 1H), 5.05 (s, 1H), 4.99 (s, 1H), 4.54 (m, 1H), 4.39 (t, *J*=6.6 Hz, 2H), 4.33 (m, 2H), 4.29 (m, 1H), 4.06 (m, 4H), 3.99 (m, 1H), 3.73 (m, 3H), 3.64 (s, 1H), 3.56-3.35 (m, 13H), 3.25 (m, 1H), 3.09 (m, 2H), 2.93 (d, 1H, *J*=12.5 Hz), 2.71 (d, 1H, *J*=12.5 Hz), 2.15 (t, *J*=6.9 Hz, 3H), 1.85 (m, 2H), 1.71-1.50 (m, 8H), 1.30 (m, 2H), 1.19 (m, 3H); ^{13}C NMR: (126 MHz, D_2O) δ 176.51, 172.22, 165.26, 163.43, 162.99, 162.71, 162.43, 157.63, 157.36, 157.26, 157.10, 157.02, 156.43, 143.05, 125.07, 119.76, 117.45, 115.13, 112.81, 110.68, 98.17, 95.98, 85.03, 79.35, 78.41, 77.12, 74.48, 73.40, 72.57, 71.89, 70.85, 69.37, 69.04, 68.63, 66.66, 63.67, 62.04, 60.20, 55.43, 55.36, 53.44, 51.93, 50.51, 50.30, 41.68, 41.60, 40.34, 39.65, 38.77, 35.42, 31.97, 28.92, 27.79, 27.67, 27.63, 25.13, 22.84. HRMS (m/z): $[M+2H]^{2+}$ calcd. for C49H89N25O16S, 658.8393; found, 658.8390.

References:

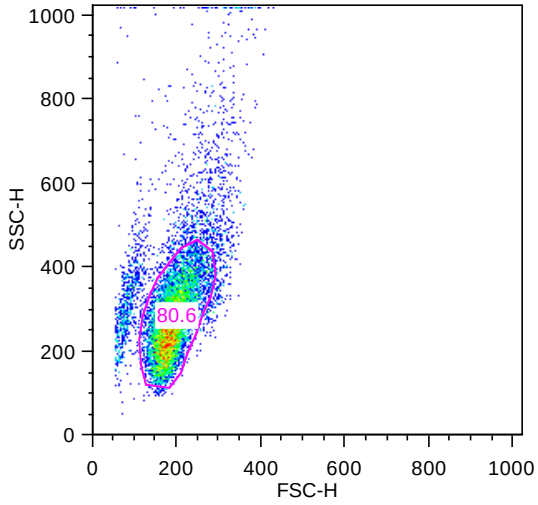
1 Chouhan, G. & James, K. CuAAC macrocyclization: high intramolecular selectivity through the use of copper-tris(triazole) ligand complexes. *Org Lett* **13**, 2754-2757, doi:10.1021/ol200861f (2011).

2 Dix, A. V. *et al.* Cooperative, heparan sulfate-dependent cellular uptake of dimeric guanidinoglycosides. *Chembiochem* **11**, 2302-2310, doi:10.1002/cbic.201000399 (2010).

Supplementary Figures

Gating Strategy

All cell analyzed by flow cytometry were gated using forward and side scattering as shown below.



Supplementary Tables

Supplementary Table 1:

Disaccharide composition of A375 HS

Disaccharide Structure		A375 Wildtype	A375 *KDM2B*$^{-/-}$
Structure Code[a]	**Unit Formula**[b]	**Abundance (% Total Disaccharide)**[c]	
D0H0	DUA-GlcNH$_2$	-	-
D0A0	DUA-GlcNAc	58.7 ± 0.5	57.9 ± 1.9
D0H6	DUA-GlcNH$_2$6S	-	-
D2H0	DUA2S-GlcNH$_2$	-	-
D0S0	DUA-GlcNS	16.3 ± 0.1	18.0 ± 0.08
D0A6	DUA-GlcNAc6S	7.8 ± 0.5	9.8 ± 0.6
D2A0	DUA2S-GlcNAc	0.71 ± 0.5	0.26 ± 0.05

D2H6	DUA2S-GlcNH$_2$6S	-	-
D0S6	DUA-GlcNS6S	5.7 ± 0.2	5.3 ± 0.3
D2S0	DUA2S-GlcNS	5.8 ± 0.6	5.2 ± 0.3
D2A6	DUA2S-GlcAc6S	-	-
D2S6	DUA2S-GlcNS6S	4.9 ± 1.1	3.5 ± 0.6

[a] The disaccharide structure code is described in (Lawrence, et al. Nat. Methods 2008)
[b] DUA = 4,5-unsaturated uronic acid
[c] –, not detected

Supplementary Table 2:

Sulfation and N-substitution of glucosamine units

Unit	**A375 Wildtype**	**A375 *KDM2B*$^{-/-}$**
	Constituents/100 disaccharides	
Unsubstituted glucosamine	-	-
N-acetylglucosamine	67 ± 1	68 ± 1
N-sulfoglucosamine	33 ± 1	32 ± 1
Uronyl-2-*O*-sulfates	11 ± 1	9 ± 1
Glucosaminyl 6-*O*-sulfates	18 ± 0.6	19 ± 1

Supplementary Table 3:

qPCR Primer Sequences

Primer (Human)	**Forward (5'-3')**	**Reverse (5'-3')**
YWHAZ	CCTGCATGAAGTCTGTAACTGAG	GACCTACGGGCTCCTACAACA
KDM2B	GGGTTCCCCTGATATTTCGAGA	GCTCCCCACTAGGAGTTTGAC
SULF1	GAAGGAGAAGAGACGGCAGA	CAGAAAGATCCCAGGTTCCA
HS6ST2	CCGTCCAGGAACTTCCACTA	GACCAGTCATCGCCAGTGTA
HS3ST3A1	CAGTGCCCTCTCCACCTC	GCCAGGCAGTAGAAGACGTAA