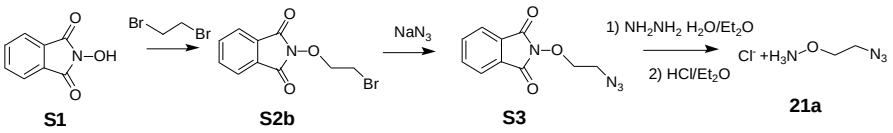
Supplementary Methods:

Synthesis of small molecule 21a-d and 13[1]

Synthesis of O-(2-azidoethyl)-hydroxylamine hydrochloride (21a)



S1 **S2b** **S3** **21a**

Synthesis of N-(2-bromoethoxy)-phthalimide (S2a) [2]

1.14 g, 7.0 mmol N-Hydroxyphthalimide **S1** (1.0 eq) was dissolved in 8.0 mL DMF and 3.0 mL 34.8 mmol 1,2-dibromoethane (~5.0 eq) was added to the solution dropwise followed by 2.0 mL, 14 mmol triethylamine. The reaction was stirred at room temperature protecting from light and monitored by TLC (EA/PE=1:1, Rf_1=0.5 and Rf_{2a}=0.85). The solid in the reaction mixture was filtered and washed with DMF twice when the TLC indicated the complete assumption of the starting material. Most of DMF was evaporated and the residue was precipitated into excess water. The solid was gathered and wash with water twice. The precipitate was removed after that the crude product was redissolved in 5.0 mL EA. The solution was washed with 1 N HCl, water and brine respectively and dried over anhydrous Na_2SO_4. Finally the solution was concentrated to give the product **S2a** (977.4 mg, yield 52.0 %). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.89 (m, 2H ArH), 7.80 (m, 2H ArH), 4.51 (t, 2H), 3.67 (t, 2H). HRMS Calcd for $[M+Na]^+$ 291.9585, found $[M+Na]^+$ 291.9569.

Synthesis of N-(2-azidoethoxy)-phthalimide (S3) [3]

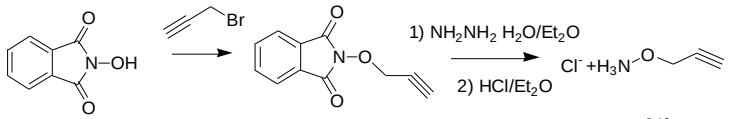
The material **S2a** (500 mg, 1.85 mmol, 1.0 eq) was dissolved in 10.0 mL acetone and to solution was added sodium azido (360 mg, 5.55 mmol, 3.0 eq) which was pre-dissolved in 2.0 mL water at an ice bath. The reaction was heated to 60 ^{o}C after stirring at the ice bath for 30 min. The reaction was monitored by TLC (EA/PE=1:2). 30.0 mL DCM was added to dilute the reaction mixture and after that the solution was washed with water and brine respectively. Then it was dried over anhydrous Na_2SO_4 and finally concentrated under reduced pressure to afford **S3** as a white solid

(184 mg, 1.46 mmol. Yield 79.0 %). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.89 (m, 2H), 7.80 (m, 2H), 4.38 (t, 2H), 3.69 (t, 2H). HRMS Calcd for $[M+Na]^{+}$ 255.0494, found $[M+Na]^{+}$ 255.0476.

Synthesis of O-(2-azidoethyl)-hydroxylamine hydrochloride (21a) [4]

To a well-stirred solution of **S3** (103 mg, 0.45 mmol, 1.0 eq) was added hydrazine hydrate (45 μL, 0.67 mmol, 1.5 eq) and the solid-hydrazine hydrate mixture was stirred at room temperature for 30 min followed by another 2 hours in 20 mL ether. The color turned from yellow solid to snow white solid in ether. The solid was gather with centrifugation and washed with ether twice. The organic part was combined and stirred with 1 N HCl in dioxane (2.0 eq) for another 2 hours. The mixture was centrifuged to get a white solid M1. ^{1}H NMR (400 MHz, DMSO-d6) δ 11.05 (s, 3H), 4.15 (q, J = 5.2, 4.4 Hz, 2H), 3.59 (t, J = 4.4 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d6) δ 73.42, 48.77. HRMS Calcd for $[M+H]^{+}$ 103.0614, found $[M+H]^{+}$ 103.0617.

Synthesis of O-(2-propynylethyl)-hydroxylamine hydrochloride (21b)



S1 **S2b** **21b**

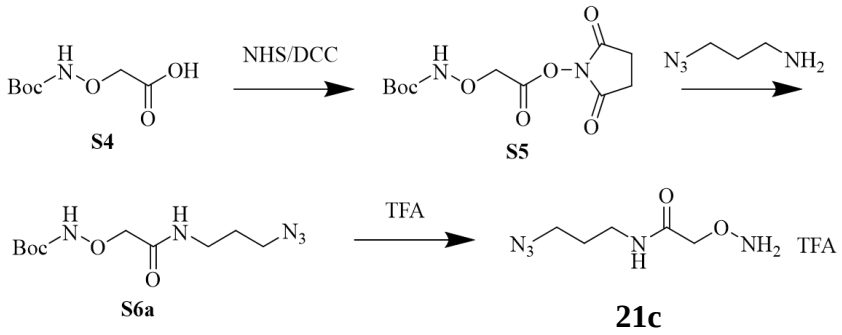
Synthesis of N-(2-propynylethoxy)-phthalimide (S2b)

The procedure was same to the synthesis of N-(2-bromoethoxy)-phthalimide (**S2a**). Characterization data matched that previously reported[5].

Synthesis of O-(2-propynylethyl)-hydroxylamine hydrochloride (21b)

The procedure was same to the synthesis of O-(2-azidoethyl)-hydroxylamine hydrochloride (**25a**). ^{1}H NMR (400 MHz, DMSO-d6) δ 11.07 (s, 3H), 4.73 (dd, J = 5.5, 2.2 Hz, 2H), 3.86 (t, J = 2.4 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d6) δ 81.51, 76.99, 62.27. HRMS Calcd for $[M+H]^{+}$ 72.0444, found $[M+H]^{+}$ 72.0451.

Synthesis of 2-Aminooxy-N-(3-azidopropyl)acetamide (21c)



S4 S5 S6a **21c**

Synthesis of 2-(Boc-aminooxy)-N-hydroxysuccinimide Ester (S5) [6]

N-Boc-aminooxyacetic acid (1.0 g 5.23c mmol 1.0 eq) was dissolved in 20 mL DCM and cooled with an ice bath. To the stirred solution was added N-hydroxysuccinimide (NHS, 662.5 mg, 5.75 mmol, 1.1 eq) followed by N, N'-dicyclohexylcarbodiimide (DCC, 1.3 g, 6.28 mmol, 1.2 eq). The solution was stirred at an ice bath for 10 min and another 2 hours at room temperature. The solution was washed with saturated $NaHCO_3$ and brine respectively after that the TLC indicated the completion of the reaction. The organic part was dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to afford **S5** as a white solid (1.42 g, 94%) and used without further purification. ^{1}H NMR (400 MHz, Methanol-d4) δ 4.78 (s, 2H), 2.88 (s, 4H), 1.50 (s, 9H).

Synthesis of 2-(Boc-aminooxy)-N-(3-azidopropyl)acetamide (S6a)

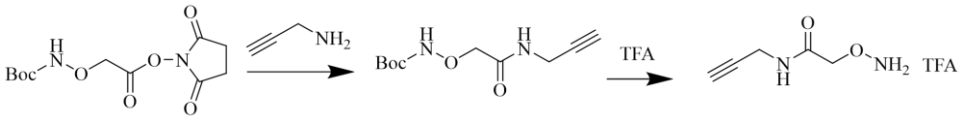
To a stirred solution of **S5** (100 mg, 0.35 mmol, 1.0 eq) in 10 mL DCM was added 3-azido-1-propanamine (40.0 mg, 0.38 mmol, 1.1 eq) followed by TEA (72 μL, 2.0 eq). The reaction was stirred at room temperature for 2 hours and monitored by TLC (EA/PE=1:1). The solution was then washed with water and brine respectively, and dried over anhydrous Na_2SO_4. The organic part was gathered and concentrated under reduced pressure to afford **S6b** as a slightly yellow oil (90 mg, 95%). 1H NMR (400 MHz, Chloroform-d) δ 8.37 (s, 1H), 7.57 (s, 1H), 4.34 (s, 2H), 3.42 (p, 4H), 1.86 (p, 2H), 1.51 (s, 9H). ^{13}C NMR (126 MHz, Chloroform-d) δ 168.47, 157.47, 82.93, 48.51, 35.79, 28.19, 27.59. HRMS Calcd for $[M+Na]^+$ 296.1335, found $[M+Na]^+$ 296.1347.

2-Aminooxy-N-(3-azidopropyl)acetamide (21c)

To a stirred solution of **S6b** (90 mg) in 8.0 mL DCM was added TFA 2.0 mL (20% TFA/DCM

solution) and stirred at room temperature for 1 hour at which time TLC indicated completion assumption of the starting material. The reaction was concentrated *in vacuo* and the crude yellow residue was taken up in an amount of water and washed with DCM twice. The water part was gathered and concentrated to give **25c** as a slight orange oil. HRMS Calcd for $[M+Na]^+$ 196.0805, found $[M+Na]^+$ 196.0814. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.24 (t, J = 5.6 Hz, 1H), 4.32 (s, 2H), 3.38 (t, J = 6.8 Hz, 2H), 3.18 (q, J = 6.7 Hz, 2H), 1.69 (p, J = 6.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 167.85, 72.37, 48.76, 36.12, 28.71. HRMS Calcd for $[M+H]^+$ 174.0991, found $[M+H]^+$ 174.0982.

Synthesis of 2-Aminooxy-N-(propargyl)acetamide (21d).



c

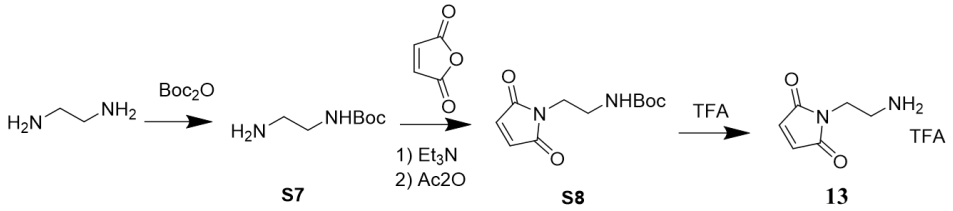
Synthesis of 2-(Boc-aminooxy)-N-propargylacetamide (S6b)

To a stirred solution of **S5** (650 mg, 2.26 mmol, 1.0 eq) in 10mL DCM was added propargylamine (186.17 mg, 3.38 mmol, 1.5 eq) followed by TEA (629 μL, 2.0 eq). The reaction was stirred at room temperature for 2 hours and monitored by TLC (EA/PE=1:1). The solution was then washed with water and brine respectively, and dried over anhydrous Na_2SO_4. The organic part was gathered and concentrated under reduced pressure to afford **S6b** as a yellow oil (462.8 mg, 90%) HRMS Calcd for $[M+Na]^+$ 251.1002, found $[M+Na]^+$ 251.1004. Other characterization data matched that previously reported[7]

2-Aminooxy-N-(propargyl)acetamide (21d)

To a stirred solution of **S6b** (462.8 mg) in 16.0 mL DCM was added TFA 4.0 mL (20% TFA/DCM solution) and stay at room temperature for 1 hour at which time TLC indicated complete assumption of the starting material. The reaction was concentrated *in vacuo* and concentrated to give **25d** as a slight brown solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.59 (t, J = 5.4 Hz, 1H), 4.36 (s, 2H), 3.93 (dd, J = 5.5, 2.5 Hz, 2H), 3.18 (t, J = 2.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d6) δ 167.90, 81.12, 73.78, 72.56, 28.11. HRMS Calcd for $[M+H]^+$ 129.0658, found $[M+H]^+$ 129.0659.

Synthesis of N-(2-Aminoethyl)maleimide (13)[8]



Synthesis of N-Boc-Ethylenediamine (S7)

Ethylenediamine (7 mL, 100 mmol) was dissolved in 100 mL chloroform and to the solution was added di-tert-butyl bicarbonate (2.185 g, 10 mmol) dropwise in an ice bath over a period of 2 hour. The reaction mixture was stirred in the ice bath for another 2 hour before heated to and stirred at room temperature for 16 hour. The precipitate was filtered and the organic phase was washed with brine and water respectively. Dried over anhydrous Na_2SO_4 and concentrated to give a colorless oil (1.4 g, 87.5 %).

Synthesis of N-(2-[(t-Boc)amino]ethyl Maleimide (S8)

Step 1: N-Boc-Ethylenediamine (320 mg, 2 mmol, 1.2 eq) and triethylamine (278 μL, 1.2 eq) was dissolved in 30 mL ethanol and to the solution was added maleic anhydride (163 mg, 1.66 mmol, 1.0 eq) in 10 mL ethanol dropwise at an ice bath. The solution was stirred at 0 ^oC for another 4 hour before the complete consumption of starting material.

Step 2: The solvent was evaporated and the medium product was redissolved in 8 mL of acetic anhydride followed by sodium acetate (193 mg, 1.1 eq). Then the reaction was heated to 65 ^oC and stirred for 1 hour before cooled to room temperature. The solution was diluted with water and extracted with ethyl acetate. The organic phase was collected and washed with saturated $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified with flash chromatography (EA/PE, 1:2) to give a white solid (312.5 mg, 78.3 %). ^1H NMR (400 MHz, Chloroform-d) δ 6.73 (s, 2H), 4.74 (s, 1H), 3.68 (t, J = 8 Hz, 2H), 3.35 (q, J = 4 Hz, 2H), 1.43 (s, 9H).

Synthesis of N-(2-Aminoethyl)maleimide Trifluoroacetate Salt (13)

N-(2-[(t-Boc)amino]ethyl maleimide (300 mg) was dissolved in 6 mL DCM with additional 2 mL

TFA. The solution was stirred at room temperature for 2 hour and finally evaporated to give a yellow oil. The crude product was redissolved in 1 mL methanol and precipitated into excess ether. The product was filtered to give a white solid compound (272.7 mg, 92 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.79 (s, 3H), 7.09 (s, 2H), 3.66 (t, J = 6.0 Hz, 2H), 2.99 (t, J = 5.8 Hz, 2H).

Expression and purification of Enzymes

The sequence and vector information of Endo-M, Endo-S WT, and Endo-S D233Q were provided in reference[1].

The sequence of alpha-L-fucosidase AlfC was obtained from GeneBank (ID 6405344).

Sequence and vector information of AlfC:

Gene sequence:

CCATGGCCCATATGATGAACGACAACGTCGCCTGGTTCAAACAGGCCAAATACGGCATGATGATCCACTGGGGTCT
GTATAGTCTGCTGGCTGGCGAATATCGCGGTGAAAGTAGTAGCGCATACGCAGAATGGATTCAGAGCAAATTCCAG
ATCCCGAACGCAGAATACGGCAATCTGGCGACCGCGTTTAATCCGCTGTACTTCGACGCGAAAAAGATTGTCGCTC
TGGCTAAACAGTGCGGTATGCAGTACCTGGTTGTTACCACCAAACATCACGACGGCTTTGCGATGTACCATAGCAA
AGTCGACGCGTATAACGTCTACGACGCAACCCCGTTTCACCGCGATATTATTGGCGAACTGGCTGAAGCTTGCCAG
AAAGCTGGTCTGAAATTTGGCCTGTACTACAGCCAGGATCTGGATTGGCACGATCCGAATGGCGGCGGTTATAAAA
GTAACGACGTCGAAACCGCAGGTACCACCTGGGATAATAGTTGGGATTTCCCGGACGAAGACCAGAAAAACTTCGA
CCTGTGCTTCGACAACAAAATCCTGCCGCAGATCAAAGAGATCATGAGCAACTACGGCGATATTGCGACCGCTTGG
TTTGATGTTCCGATGACCCTGTCTGAAGCACAGAGTCAGACCATCTACGATACCGTTCGCGAACTGCAACCGAACT
GTCTGATTAACAGCCGTCTGGGCAACGGCAAATACGATTTCGTTAGCCTGGGCGACAACGAGATCCCGAAAAACAA
AGAGGACATGAACAAAACCGACGTCGACTACAACGAAATCACCGGCTTTAAACCGAGTCCGCTGGGTCTGTACGAA
ACCGCAGGTACCATTAACGATAGCTGGGGTTTCTCCTATCACGATCAGAACTGGAAAACCCCGCGTACCCTGTATC
GTTACAAACAGCACCTGAACGACTTCGGGATCAACTACCTGCTGAACGTAGGTCTGGACCCGCTGGGTCGCGTTCC
GATGATGGCTGAAGAAAATCTGCTGGCAGCGAAAGCTCTGGAAGATGAAGCAAATCGTGCGGCCGC

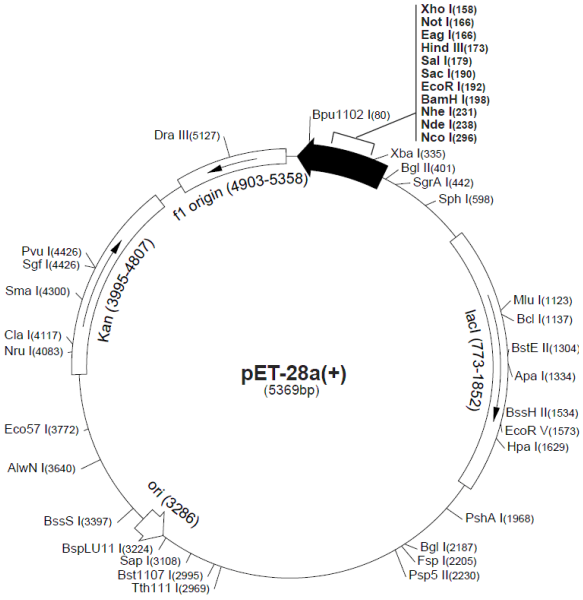
(Sequence highlighted in yellow is the restriction site of NcoI; Sequence highlighted in green is the restriction site of NdeI; Sequence highlighted in blue is the restriction site of NotI; The original sequence starts from the red residues.)

Amino acid sequence:

MNDNVAWFKQAKYGMMIHWGLYSLLAGEYRGESSSAYAEWIQSKFQIPNAEYGNLATAFNPLYFDAKKIVALAKQC
GMQYLVVTTKHHDGFAMYHSKVDAYNVYDATPFHRDIIGELAEACQKAGLKFGLYYSQDLDWHDPNGGGYKSNDVE

TAGTTWDNSWDFPDEDQKNFDLCFDNKILPQIKEIMSNYGDIATAWFDVPMTLSEAQSQTIYDTVRELQPNCLINS
RLGNGKYDFVSLGDNEIPKNKEDMNKTDVDYNEITGFKPSPLGLYETAGTINDSWGFSYHDQNWKTPRTLYRYKQH
LNDFGINYLLNVGLDPLGRVPMMAEENLLAAKALEDEANRAAALEHHHHHH

pET-28a(+):



pET-28a(+) sequence landmarks	
T7 promoter	370-386
T7 transcription start	369
His•Tag coding sequence	270-287
T7•Tag coding sequence	207-239
Multiple cloning sites (*Bam*H I - *Xho* I)	158-203
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	773-1852
pBR322 origin	3286
Kan coding sequence	3995-4807
f1 origin	4903-5358

The maps for pET-28b(+) and pET-28c(+) are the same as pET-28a(+) (shown) with the following exceptions: pET-28b(+) is a 5368bp plasmid; subtract 1bp from each site beyond *Bam*H I at 198. pET-28c(+) is a 5367bp plasmid; subtract 2bp from each site beyond *Bam*H I at 198.

Enzyme genes of Endo-M, Endo-S, and AlfC were synthesized and inserted to different vectors by contract service provided by GENEWIZ or Synbio Tech.
Vectors:
EndoS was cloned into pET30a(+) with restriction sites of NdeI and NotI.
EndoM was cloned into pET23b(+) with restriction sites of NdeI and XhoI.
Fucosidase was cloned into pET28a(+) with restriction sites of NcoI and NotI.

The four plasmids were transfected into Trans5α for amplification for later usage. Then, the harvested plasmids were transformed into *E.Coli* BL21 (DE3) for expression.

For EndoS-WT/ EndoS D233Q/ α-L-Fucosidase:
E. Coli BL21 (DE3) bacteria expressing the wild type pET-30a (+)-ndoS or D233Q pET-30a (+)-ndoS or pET28a(+)-α-L-fucosidase expression vector were first grown in 50 mL LB/Kan+ medium including 0.1 mg/mL Kanamycin at final concentration. Control the temperature at 37 ^{o}C, after a 4 hours pre-culture step (220 rpm) OD600 = 0.6-1.0 can be reached. Then add all the bacterial into 1L LB/Kan+ medium and culture for more than 12hours. When OD600=0.4-0.6 was attained, the expression bacterial was incubated by the addition of IPTG (isopropyl β-D-1 thiogalactopyranoside) with 0.1mM as the final concentration. The cultures were incubated at 16 ^{o}C overnight to get soluble protein. Cells were harvested by centrifugation at 4 ^{o}C and 10000×g for 20 min. Then the bacterial were suspended in 10 mM phosphate buffer (10mM, pH =7.4) to a total volume of 50 mL. Cells were lysed by ultrasonication and the extract was centrifuged at 4 ^{o}C and 18000×g for 20min to get supernatant.

Here we use 5mL column volume of Ni-NTA agarose (QIAGEN) to purify 1L cells lysate, and before purification procedure, the Ni-NTA affinity column should be pre-processed with several steps for equilibration. The lysate solution was loaded onto it for more than one time. After washing with 10 column volumes of phosphate buffer (10mM, pH =7.4), we can remove the non-specific binding of protein. Then wash with 10 column volumes of phosphate buffer (10mM, pH =7.4) containing 20 mM imidazole gently. This operation can remove most impurity protein and a gradient elution procedure can also be used as optimized protocol. Target protein can be eluted by 250 mM imidazole. Results of expression and purification from SDS-PAGE and western blot analysis suggested that the protein were effectively purified.

For EndoM:

E. Coli BL21(DE3)bacteria were first grown in 5 mL LB/Amp+ medium including 0.1 mg/mL ampicillin at final concentration. Control the temperature at 37 ^{o}C, after a 12 hours pre-culture step (220 rpm) OD600=0.6-1.0 can be reached. Then add all the bacterial into 1L LB/ Amp + medium and culture for more than 12hours. When OD600 = 0.4-0.6 was attained, the expression bacterial was incubated without IPTG. The cultures were incubated at 20 ^{o}C for 3 days. Cells were harvested by centrifugation at 4 ^{o}C and 10000×g for 20 min. Then the bacterial were suspended in 10 mM phosphate buffer (10mM, pH =7.4) to a total volume of 50 mL. Cells were lysed by ultrasonication and the extract was centrifuged at 4 ^{o}C and 18000×g for 20min to get supernatant.

Here we use 5mL column volume of Ni-NTA agarose (QIAGEN) to purify 1L cells lysate, and before purification procedure, the Ni-NTA affinity column should be pre-processed with several steps for equilibration. The lysate solution was loaded onto it for more than one time. After washing with 10 column volumes of phosphate buffer (10mM, pH =7.4) we can remove the non-specific binding of protein. Then wash with 10 column volumes of phosphate buffer (10mM, pH =7.4) containing 50 mM imidazole gently. Target protein can be eluted by 100 mM imidazole. Results of expression and purification from SDS-PAGE and western blot analysis suggested that the protein were effectively purified.

Reference

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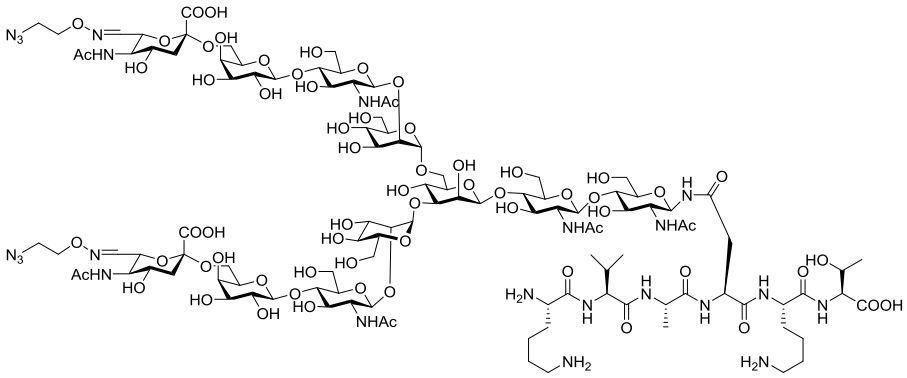
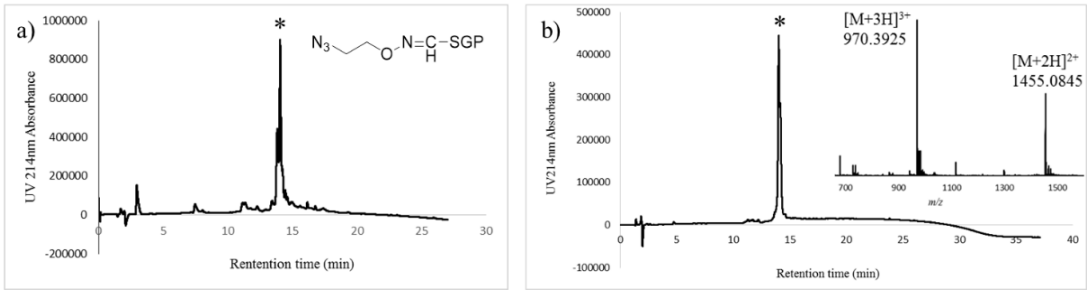
Supplementary results:

PRODUCT 4A:



HPLC and LCMS profiles



HPLC and LCMS profiles (HPLC method B) of CHO-SGP **20** and O-(2-propynyl)-hydroxylamine hydrochloride **21a** reaction. a) Aliquots removed from reaction mixture at 2 hour. The corresponding product was labeled with *. b) The HPLC profile of the product after preparative column purification. The insert is the HRMS profile of pure product.

NMR data

^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.41 (1.6H, dd, J = 7.2, 3.3 Hz, H1 of oxime), 6.85 (0.4H, dd, J = 6.7, 2.6 Hz, H1 of oxime), 5.07 (1H, s, H1c), 4.96 (1H, d, J = 9.6 Hz, H1a'), 4.96 (0.4 H, H6f, H6f'), 4.88 (1H, s, H1c'), 4.76 (1H, s, H1b), 4.59 (1H, t, Asn Hα), 4.53 (3H, d, J = 6.7 Hz, H1a, H1d.H1d'), 4.39 (1H, d, J = 3.5 Hz, Thr Hα), 4.37 (1H, d, J = 2.5 Hz, H1e), 4.35 (1H, d, J = 3 Hz, H1e'), 4.34-4.28 (2H, m, Lys1 Hα, Thr Hβ), 4.24 (1H, q, J = 7 Hz, Ala Hα), 4.17 (5H, m, H2b, H2 of oxime), 4.17 (1.6H, m, H6f, H6f'), 4.12 (1H, d, J = 2 Hz, H2c), 4.08 (1H, d, J= 8 Hz, Val Hα), 4.04 (1H, d, J = 2 Hz, H2c'), 3.99 (1H, t, J = 6.5 Hz, Lys2 Hα), 2.92 (4H, q, J = 7.5 Hz, Lys Hε), 2.81-2.61 (2H, m, Asn Hβ), 2.61-2.51 (2H, dd, J = 12.5 Hz, 2.5Hz, $H3f_{eq}$, $H3f'_{eq}$), 2.03-1.88 (19H, m, 6 x 3 Ac, Val Hβ), 1.87-1.66 (6H, m, Lys Hβ, $H3f_{ax}$, $H3f'_{ax}$), 1.66 (4H, m, J = 7.5 Hz, Lys Hδ), 1.43-1.32 (4H, m, Lys Hγ), 1.3 (3H, d, J = 7.5 Hz, Ala Hβ), 1.13 (3H, J = 6.5 Hz, Thr Hγ), 0.89 (6H, d, 7.0 Hz, Val Hγ). HSQC ((1H, 500 MHz)/(13C, 126 MHz), Deuterium Oxide) 7.41/149.38 (H1g/C1g), 6.85/149.11 (H1g/C1g), 5.06/99.43 (H1c/C1c), 4.96/78.27 (H1a'/C1a'), 4.95/67.43 (H6f/C6f, H6f'/C6f'), 4.87/96.94 (H1c'/C1c'), 4.69/100.39 (H1b/C1b), 4.59/49.87 (Hα Asn/Cα Asn), 4.52/99.60 (H1a/C1a, H1d/C1d, H1d'/C1d'), 4.39/57.76 (Hα Thr/Cα Thr), 4.36/103.72 (H1e/C1e, H1e'/C1e'), 4.33/53.57 (Hα Lys/Cα Lys), 4.32/67.08 (Hβ Thr/Cβ Thr), 4.23/49.46 (Hα Ala/Cα Ala), 4.17/72.10 (H2b/C2b, H2g/C2g, H6f/C6f, H6f'/C6f'), 4.12/76.41 (H2c/C2c), 4.07/59.53 (Hα Val/Cα Val), 4.04/76.23 (H2c'/C2c'), 3.99/52.63 (Hα Lys/Cα Lys), 3.46/49.76 (H3g/C3g), 2.92/39.12 (Hε Lys/Cε Lys), 2.64/36.66 (Hβ Asn/Cβ Asn), 2.58/39.18

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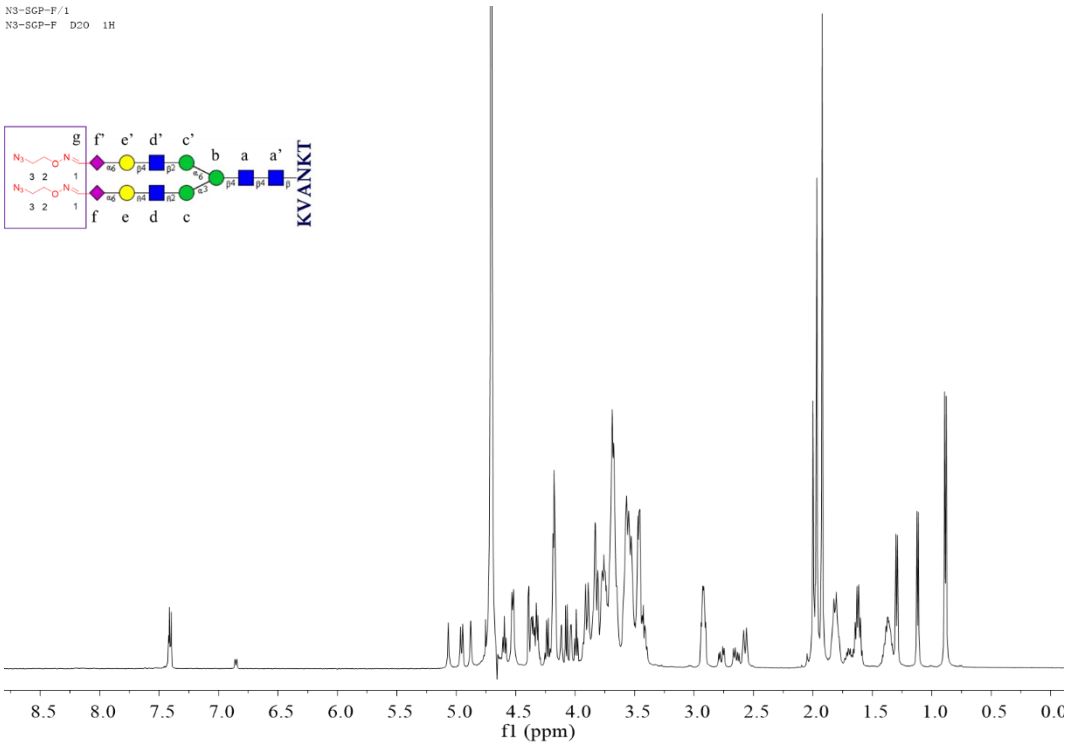
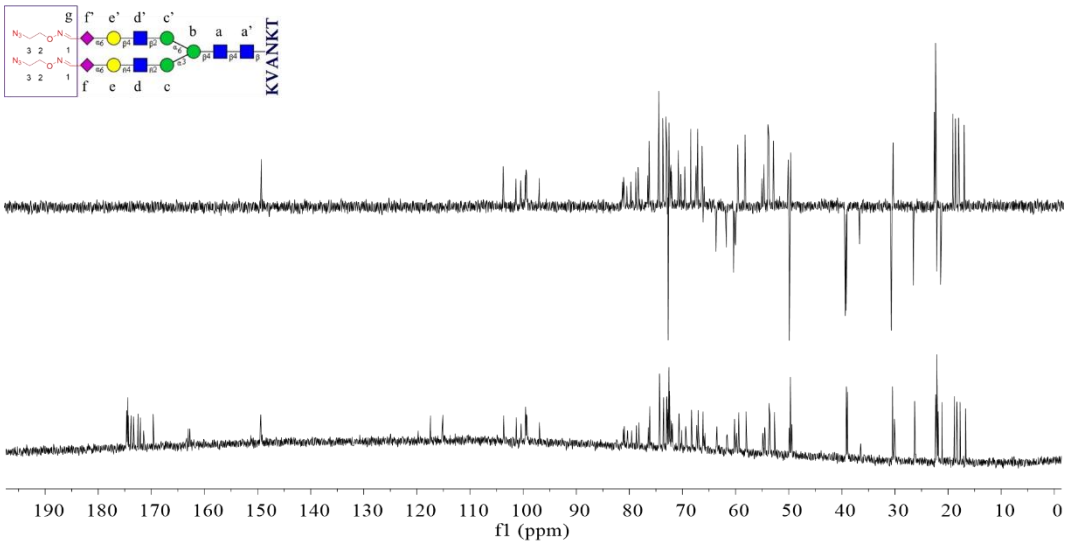
(H3f/C3f, H3f'/C3f'), 1.97/30.60 (Hβ Val/Cβ Val), 1.81/39.05 (H3f/C3f, H3f'/C3f'), 1.77/30.39 (Hβ Lys/Cβ Lys), 1.62/26.13 (Hδ Lys/Cδ Lys), 1.37/21.38 (Hγ Lys/Cγ Lys), 1.34/16.54 (Hβ Ala/Cβ Ala), 1.16/18.82 (Hγ Thr/Cγ Thr), 0.85/17.73 (Hγ Val/Cγ Val).

NMR Spectra

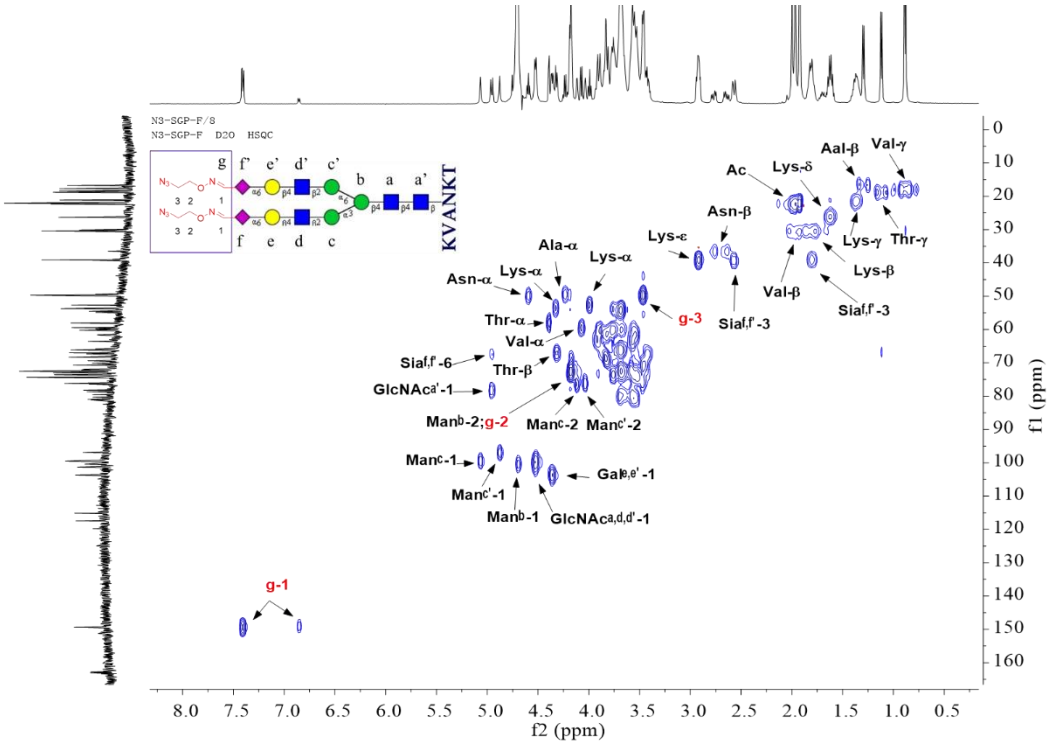


^{1}H NMR spectrum of **4a**

Nature Protocols: doi:10.1038/nprot.2017.058



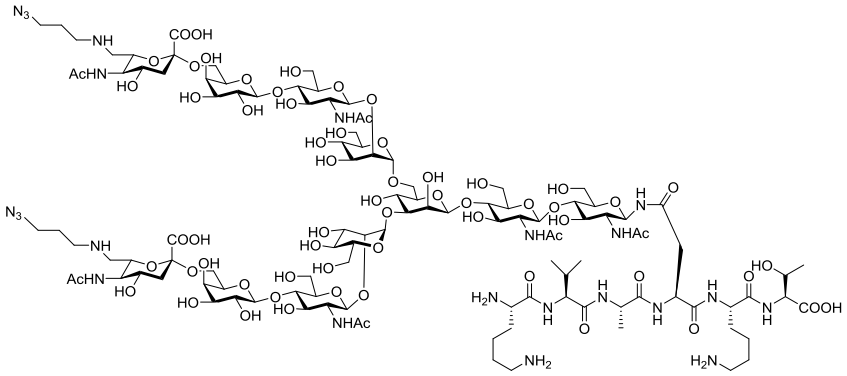
^{13}C NMR spectrum of **4a**



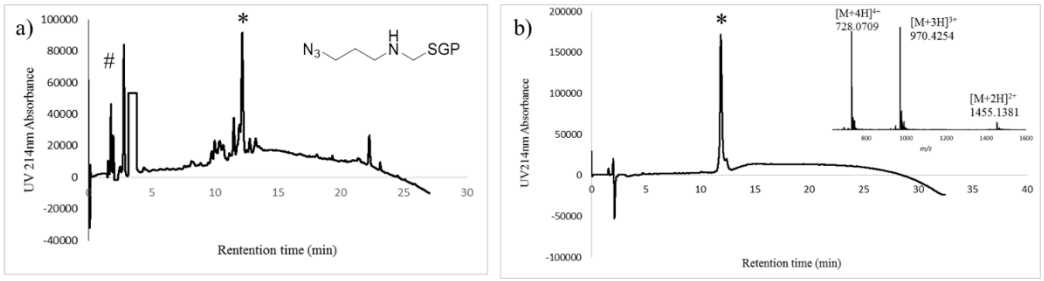
^{1}H-^{13}C HSQC NMR spectrum of **4a**

Nature Protocols: doi:10.1038/nprot.2017.058

PRODUCT 4B:



HPLC and LCMS profiles



HPLC and LCMS profiles (HPLC method B) of reductive amination reaction with 3-azido-1-propanamine. a) Aliquots removed from reaction mixture at 3 hour. The corresponding product was labeled with *. b) The HPLC profile of the product after preparative column purification. The insert is the HRMS profile of pure product.

NMR data

^{1}H NMR (500 MHz, Deuterium Oxide) δ 5.03 (1H, s, H1c), 4.93 (1H, d, J = 9.6 Hz, H1a'), 4.83 (1H, s, H1c'), 4.70 (1H, s, H1b), 4.56 (1H, t, J = 6.7 Hz, Asn Hα), 4.50 (3H, dd, J = 6.4, 5.1 Hz, H1a, H1d, H1d'), 4.37 (1H, d, J = 3.5 Hz, Thr Hα), 4.35 (1H, d, J = 2.5 Hz, H1e), 4.34 (1H, d, J = 3 Hz, H1e'), 4.30 (2H, m, Lys1 Hα, Thr Hβ), 4.21 (1H, q, J = 7.2 Hz, Ala Hα), 4.14 (1H, s, H2b), 4.09 (1H, d, J = 2.7 Hz, H2c), 4.05 (1H, d, J = 7.6 Hz, Val Hα), 4.01 (1H, d, J = 3.5 Hz, H2c'), 4.00-3.90 (3H, m, Lys Hα, H6f, H6f'), 3.24-3.04 (8H, m, -2 x -CH_2-NH-CH_2-), 2.92 (4H, q, J = 7.5 Hz, Lys Hε), 2.81-2.61 (2H, m, Asn Hβ), 2.61 - 2.51 (2H, dd, J = 12.5 Hz, 2.5Hz, $H3f_{eq}$, $H3f'_{eq}$), 2.03-1.90 (19H, m, 6 x 3 Ac, Val Hβ), 1.90-1.84 (4H, m, H3g), 1.84-1.65 (6H, m, Lys Hβ, $H3f_{ax}$, $H3f'_{ax}$), 1.59 (4H, m, J = 7.5 Hz, Lya Hδ), 1.40-1.29 (4H, m, Lys Hγ), 1.27 (3H, d, J = 7.5 Hz, Ala Hβ), 1.10 (3H, J = 6.5 Hz, Thr Hγ), 0.86 (6H, d, 7.0 Hz, Val Hγ). HSQC ((1H, 500 MHz)/(13C, 126 MHz), Deuterium Oxide) 5.05/99.53 (H1c/C1c), 4.96/78.22 (H1a'/C1a'), 4.85/96.87 (H1c'/C1c'), 4.69/100.38 (H1b/C1b), 4.59/49.84 (Hα Asn/Cα Asn), 4.53/99.58 (H1a/C1a, H1d/C1d, H1d'/C1d'), 4.38/58.04 (Hα Thr/Cα Thr), 4.37/103.51 (H1e/C1e, H1e'/C1e'), 4.32/53.51 (Hα Lys/Cα Lys), 4.31/67.02 (Hβ Thr/Cβ Thr), 4.23/49.46 (Hα Ala/Cα Ala), 4.17/70.16 (H2b/C2b), 4.11/76.42 (H2c/C2c), 4.07/59.39 (Hα Val/Cα Val), 4.03/76.11 (H2c'/C2c'), 3.99/52.73 (Hα Lys/Cα Lys), 3.43/48.19 (H4g/C4g), 3.21/48.59 (H2g/C2g), 3.16/46.14 (H1g/C1g), 2.91/39.01 (Hε Lys/Cε Lys), 2.76/36.86 (Hβ Asn/Cβ Asn), 2.59/39.43 (H3f/C3f, H3f'/C3f'), 1.98/30.12 (Hβ Val/Cβ Val), 1.80/30.30 (Hβ Lys/Cβ Lys), 1.75/39.63 (H3f/C3f, H3f'/C3f'), 1.59/26.28 (Hδ Lys/Cδ Lys), 1.36/21.42 (Hγ Lys/Cγ Lys), 1.29/16.67 (Hβ

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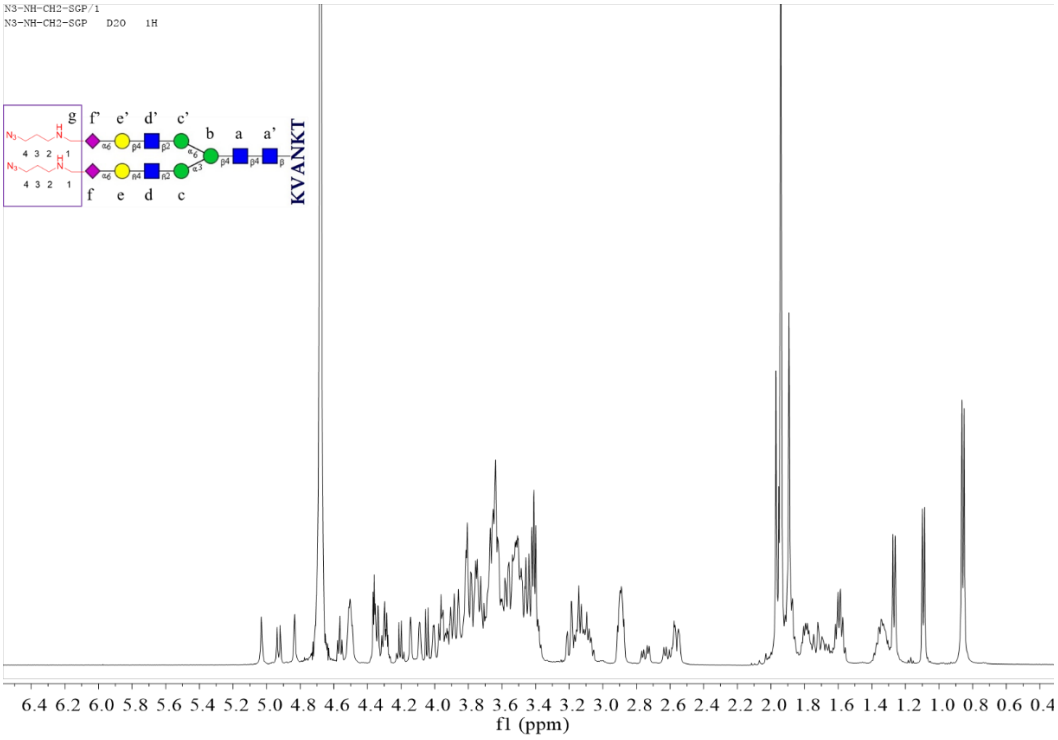
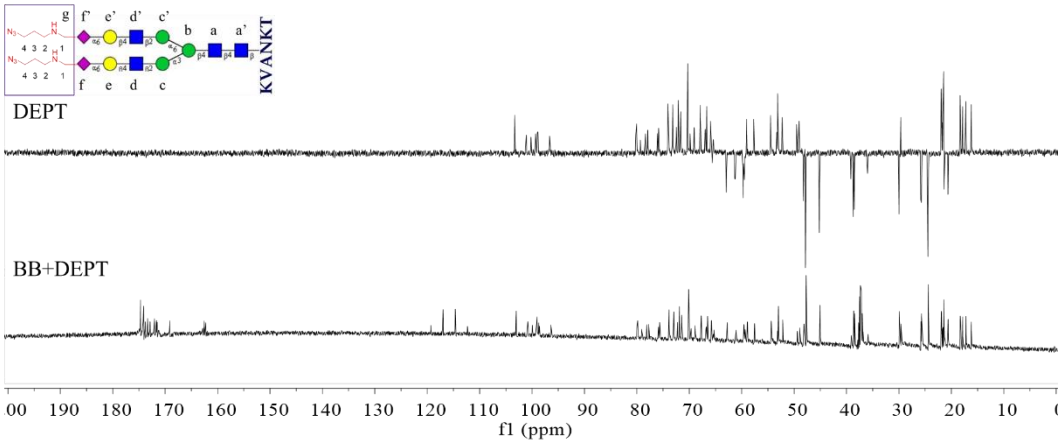
Ala/Cβ Ala), 1.12/19.03 (Hγ Thr/Cγ Thr), 0.88/18.21 (Hγ Val/Cγ Val).

NMR spectra

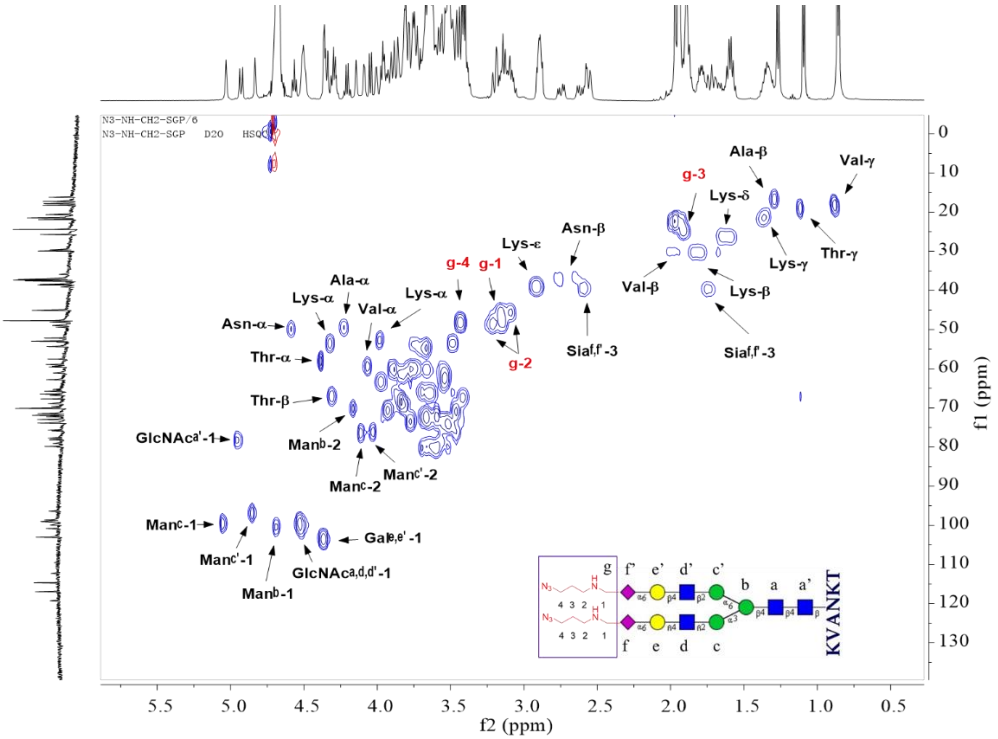


^{1}H NMR spectrum of **4b**



^{13}C NMR spectrum of **4b**

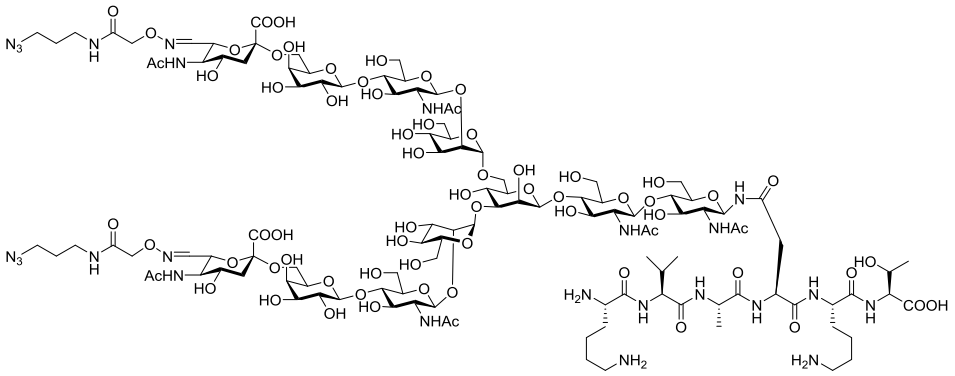
Nature Protocols: doi:10.1038/nprot.2017.058



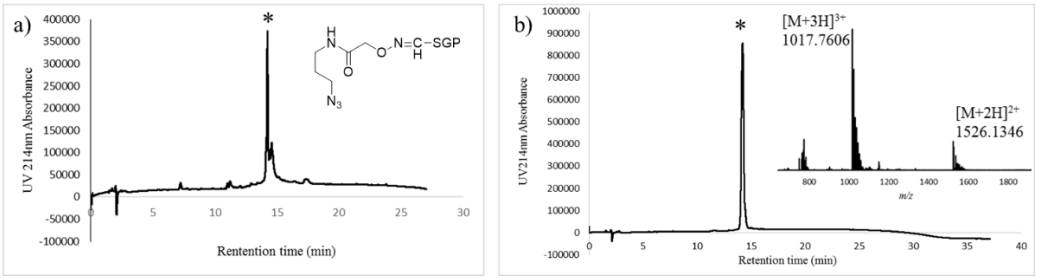
^{1}H-^{13}C HSQC NMR spectrum of **4b**

Nature Protocols: doi:10.1038/nprot.2017.058

PRODUCT 4C:



HPLC and LCMS profiles



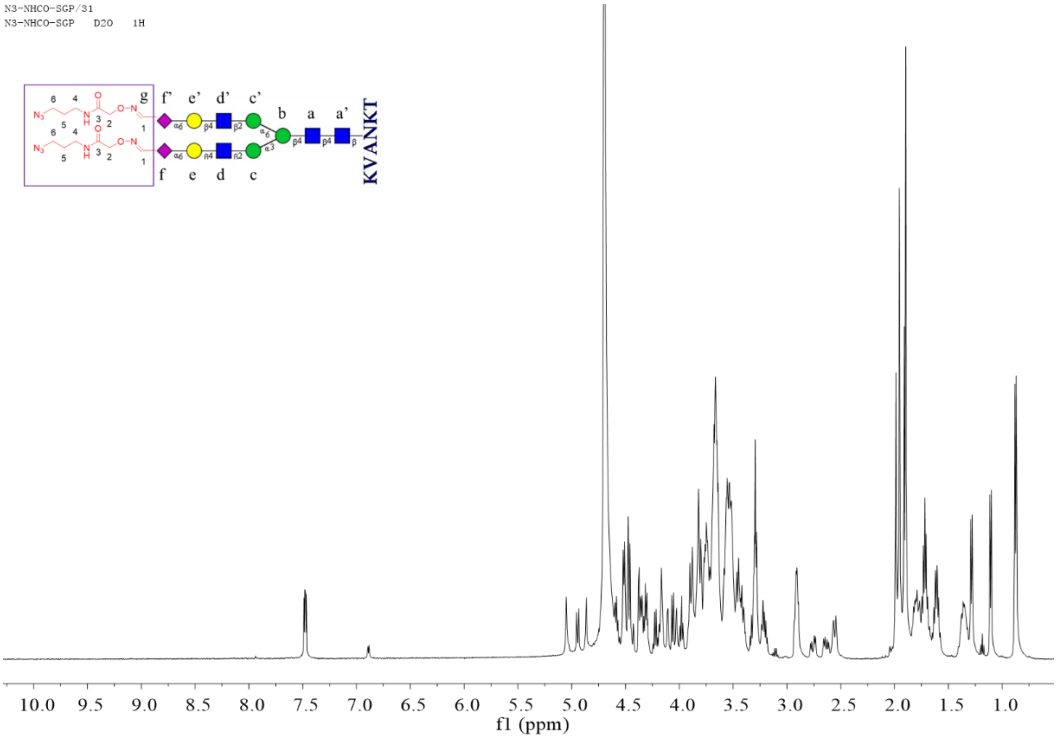
HPLC and LCMS profiles (HPLC method B) of CHO-SGP and O-(2-propynyl)-hydroxylamine hydrochloride **21c** reaction. a) The samples removed from reaction mixture at 2 hour. The corresponding product was labeled with *. b) The HPLC profile of the product after preparative column purification. The insert is the HRMS profile of pure product.

NMR data

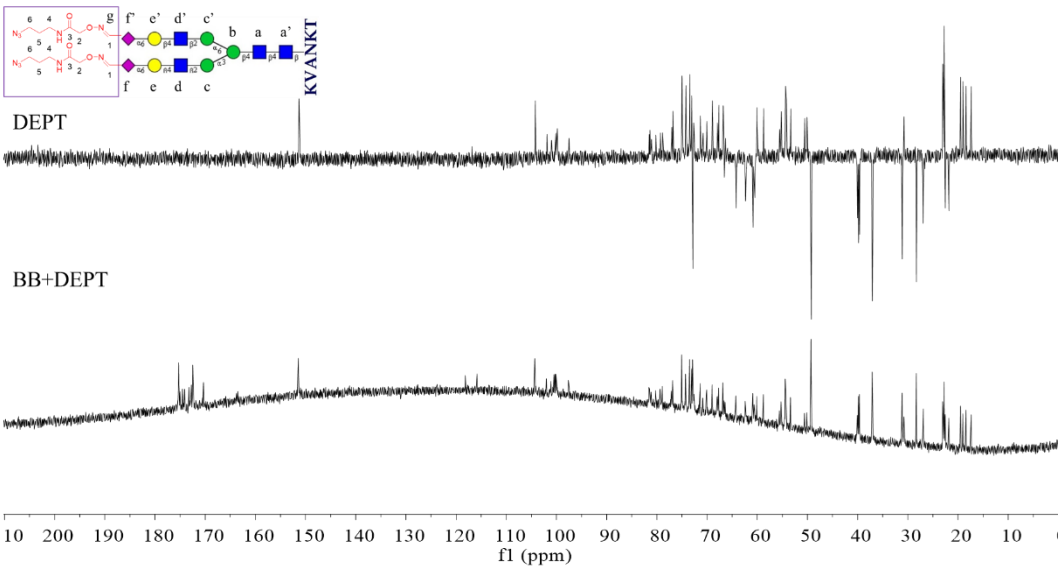
^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.48 (1.6H, dd, J = 7.0, 3.7 Hz, H1g), 6.89 (0.4H, dd, J = 7.0, 2.5 Hz, H1g), 5.05 (1.4H, s, H1c, H6f, H6f'), 4.95 (1H, d, J = 9.7 Hz, H1a'), 4.87 (1H, s, H1c'), 4.67 (1H, s, H1b), 4.60 (1H, t, Asn Hα), 4.52 (3H, d, J = 7.1 Hz, H1a, H1d, H1d'), 4.49-4.41 (4H, m, H2g), 4.38 (1H, d, Thr Hα), 4.36 (2H, dd, H1e, H1e'), 4.33-4.29 (2H, m, Lys Hα, Thr Hβ), 4.24 (1H, q, J = 7.2 Hz, Ala Hα), 4.18 (2.6H, m, H1b, H6f, H6f'), 4.11 (1H, d, J = 2.5 Hz,H2c), 4.06 (1H, d, J = 7.6 Hz, Val Hα), 4.03 (1H, d, J = 2 Hz, H2c'), 3.98 (1H, t, J = 6.7 Hz, Lys Hα), 3.37-3.15 (8H, m, H4g, H6g), 2.91 (4H, q, J = 7.6 Hz, Lys Hε), 2.70 (2H, ddd, J = 62.2, 16.4, 6.7 Hz, Asn Hβ), 2.56 (2H, dd, J = 11.0 Hz, H3f$_{eq}$, H3f'$_{eq}$), 1.80 (4H, m, Lys Hβ)1.72 (6H, m, H5g, H3f$_{ax}$, H3f'$_{ax}$), 1.43-1.31 (4H, m, Lys Hγ), 1.29 (3H, d, Ala Hβ), 1.11 (3H, d, J = 6.4 Hz, Thr Hγ), 0.88 (6H, d, J = 6.7 Hz, Val Hγ). HSQC ((1H, 500 MHz)/(13C, 126 MHz), Deuterium Oxide) 7.49/150.77 (H1g/C1g), 6.90/150.47 (H1g/C1g), 5.06/99.43 (H1c/C1c), 5.05/67.26 (H6f/C6f, H6f'/C6f'), 4.96/78.16 (H1a'/C1a'), 4.88/97.00 (H1c'/C1c'), 4.70/100.37 (H1b/C1b), 4.60/49.86 (Hα Asn/Cα Asn), 4.53/99.51 (H1a/C1a, H1d/C1d, H1d'/C1d'), 4.48/72.14 (H2g/C2g), 4.39/57.99 (Hα Thr/Cα Thr), 4.37/103.58 (H1e/C1e, H1e'/C1e'), 4.33/53.62 (Hα Lys/Cα Lys), 4.32/67.14 (Hβ Thr/Cβ Thr), 4.24/49.53 (Hα Ala/Cα Ala), 4.18/72.35 (H2b/C2b, H6f/C6f, H6f'/C6f'), 4.12/76.11 (H2c/C2c), 4.08/59.56 (Hα Val/Cα Val), 4.04/76.01 (H2c'/C2c'), 4.00/52.70 (Hα Lys/Cα Lys), 3.32/36.54 (H4g/C4g), 3.31/48.73 (H6g/C6g), 3.23/36.58 (H4g/C4g), 2.93/39.11 (Hε Lys/Cε Lys),

2.77/36.47 (Hβ Asn/Cβ Asn), 2.58/39.32 (H3f/C3f, H3f'/C3f'), 2.04/29.84 (Hβ Val/Cβ Val), 1.87/30.45 (Hβ Lys/Cβ Lys), 1.78/39.39 (H3f/C3f, H3f'/C3f'), 1.71/27.53 (H5g/C5g), 1.63/26.18 (Hδ Lys/Cδ Lys), 1.37/21.65 (Hγ Lys/Cγ Lys), 1.34/16.85 (Hβ Ala/Cβ Ala), 1.09/18.70 (Hγ Thr/Cγ Thr), 0.86/17.86 (Hγ Val/Cγ Val).

NMR spectra

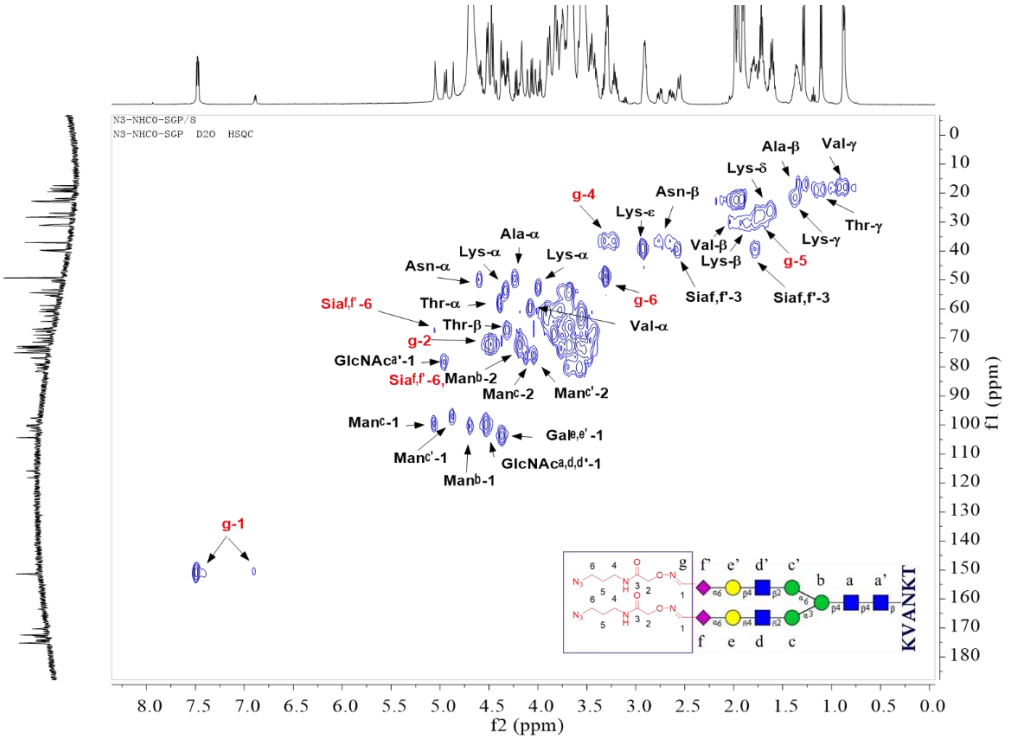


^{1}H NMR spectrum of **4c**



^{13}C NMR spectrum of **4c**

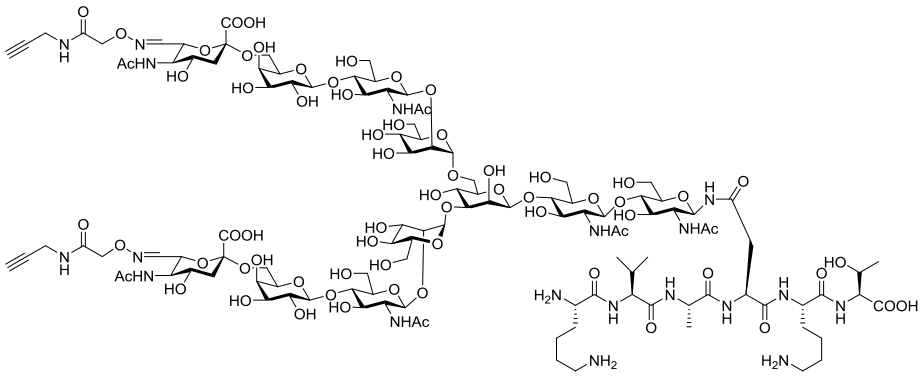
Nature Protocols: doi:10.1038/nprot.2017.058



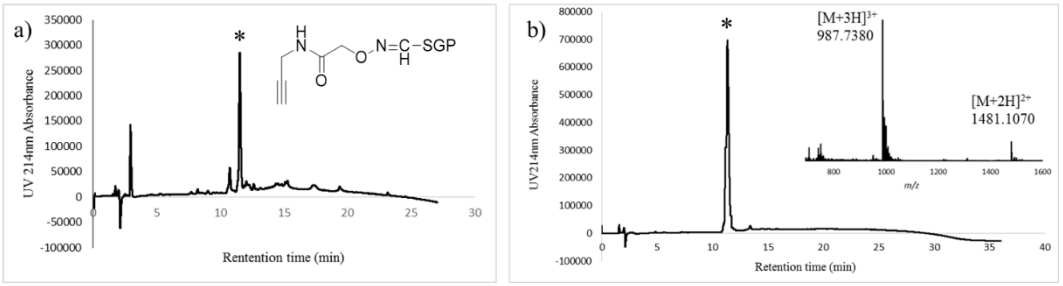
^{1}H-^{13}C HSQC NMR spectrum of **4c**

Nature Protocols: doi:10.1038/nprot.2017.058

PRODUCT 4D:



HPLC and LCMS profiles



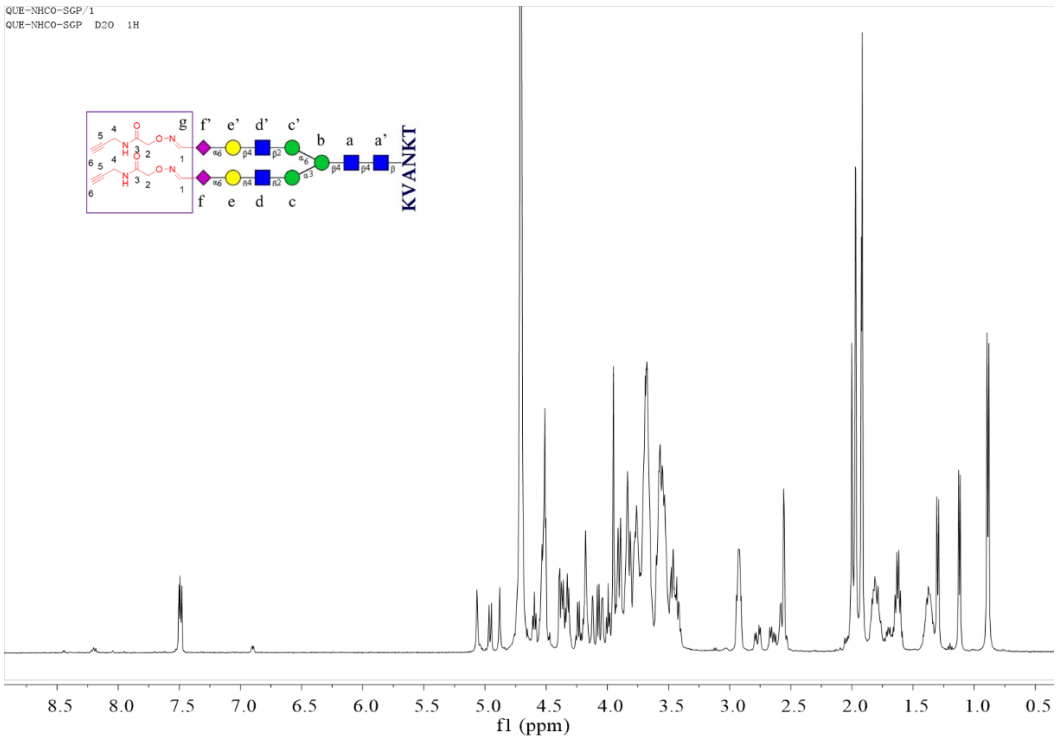
HPLC and LCMS profiles (HPLC method B) of CHO-SGP **20** and **21d** reaction. a) The samples removed from reaction mixture at 2 hour. The corresponding product was labeled with *. b) The HPLC profile of the product after preparative column purification. The insert is the HRMS profile of pure product.

NMR data

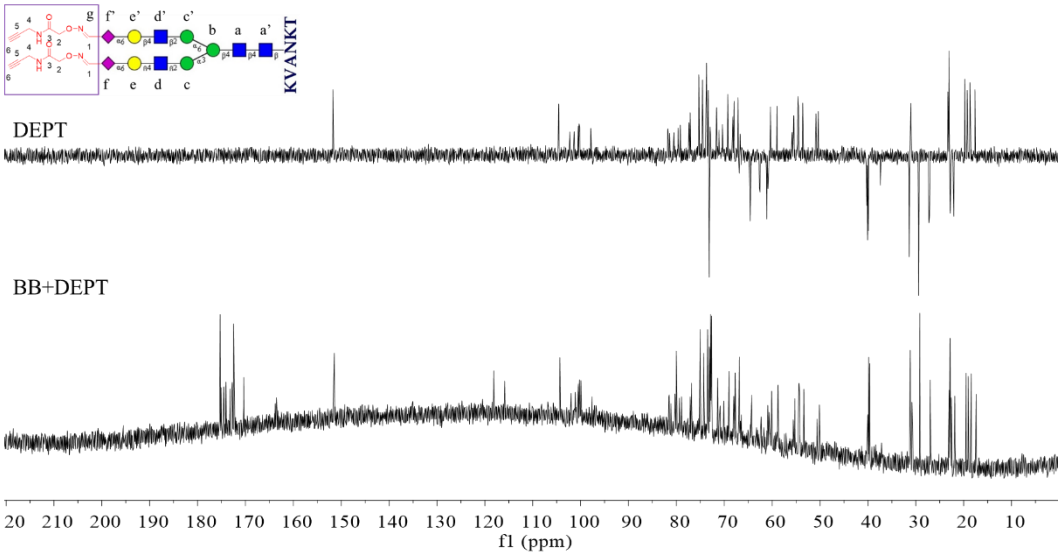
^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.39 (1.6H, dd, J = 7.2, 3.3 Hz, H1 of oxime), 6.85 (0.4H, dd, J = 6.7, 2.6 Hz, H1 of oxime), 5.07 (1H, s, H1c), 4.96 (1.4H, d, J = 9.6 Hz, H1a', H6f/f'), 4.88 (1H, s, H1c'), 4.76 (1H, s, H1b), 4.61 (5H, m, H2g, Asn Hα), 4.54 (3H, d, J = 6.7 Hz, H1a, H1d.H1d'), 4.39 (1H, d, J = 3.5 Hz, Thr Hα), 4.37 (1H, d, J = 2.5 Hz, H1e), 4.35 (1H, d, J = 3 Hz, H1e'), 4.34-4.28 (2H, m, Lys1 Hα, Thr Hβ), 4.24 I(1H, q, J = 7 Hz, Ala Hα), 4.18 (1H, s, H2b), 4.16 (1.6H, dd, J = 10.0 Hz, 7.5 Hz, H6f/f'), 4.12 (1H, d, J = 2 Hz, H2c), 4.08 (1H, d, J= 8 Hz, Val Hα), 4.04 (1H, d, J = 2 Hz, H2c') 3.99 (1H, t, J = 6.5 Hz, Lys2 Hα), 2.92 (4H, q, J = 7.5 Hz, Lys Hε), 2.86 (2H, q, H3g), 2.81-2.61 (2H, m, Asn Hβ), 2.61-2.51 (2H, dd, J = 12.5 Hz, 2.5Hz, $H3f_{eq}$, $H3f'_{eq}$), 2.03-1.88 (19H, m, 6 x 3 Ac, Val Hβ), 1.87-1.66 (6H, m, Lys Hβ, $H3f_{ax}$, $H3f'_{ax}$), 1.66 (4H, m, J = 7.5 Hz, Lya Hδ), 1.43-1.32 (4H, m, Lys Hγ), 1.3 (3H, d, J = 7.5 Hz, Ala Hβ), 1.13 (3H, J = 6.5 Hz, Thr Hγ), 0.89 (6H, d, 7.0 Hz, Val Hγ). HSQC ((1H, 500 MHz)/(13C, 126 MHz), Deuterium Oxide) 7.41/150.05 (H1g/C1g), 6.88/149.59 (H1g/C1g), 5.09/99.34 (H1c/C1c), 4.98/78.34 (H1a'/C1a'), 4.97/67.43 (H6f/C6f, H6f'/C6f'), 4.91/96.89 (H1c'/C1c'), 4.72/100.33 (H1b/C1b), 4.63/61.46 (H2g/C2g), 4.62/49.85 (Hα Asn/Cα Asn), 4.55/99.74 (H1a/C1a, H1d/C1d, H1d'/C1d'), 4.42/58.25 (Hα Thr/Cα Thr), 4.39/103.91 (H1e/C1e, H1e'/C1e'), 4.35/53.53 (Hα Lys/Cα Lys), 4.34/67.11 (Hβ Thr/Cβ Thr), 4.26/49.43 (Hα Ala/Cα Ala), 4.20/70.52 (H2b/C2b, H6f/C6f, H6f'/C6f'), 4.14/76.54 (H2c/C2c), 4.10/59.64 (Hα Val/Cα Val), 4.06/76.25 (H2c'/C2c'),

4.02/52.83 (Hα Lys/Cα Lys), 2.94/39.16 (Hε Lys/Cε Lys), 2.89/76.16 (H4g/C4g), 2.78/37.03 (Hβ Asn/Cβ Asn), 2.58/39.45 (H3f/C3f, H3f'/C3f'), 2.01/30.19 (Hβ Val/Cβ Val), 1.84/30.59 (Hβ Lys/Cβ Lys), 1.79/39.37 (H3f/C3f, H3f'/C3f'), 1.65/26.40 (Hδ Lys/Cδ Lys), 1.43/21.61 (Hγ Lys/Cγ Lys), 1.32/16.78 (Hβ Ala/Cβ Ala), 1.14/19.15 (Hγ Thr/Cγ Thr), 0.91/18.15 (Hγ Val/Cγ Val).

NMR spectra

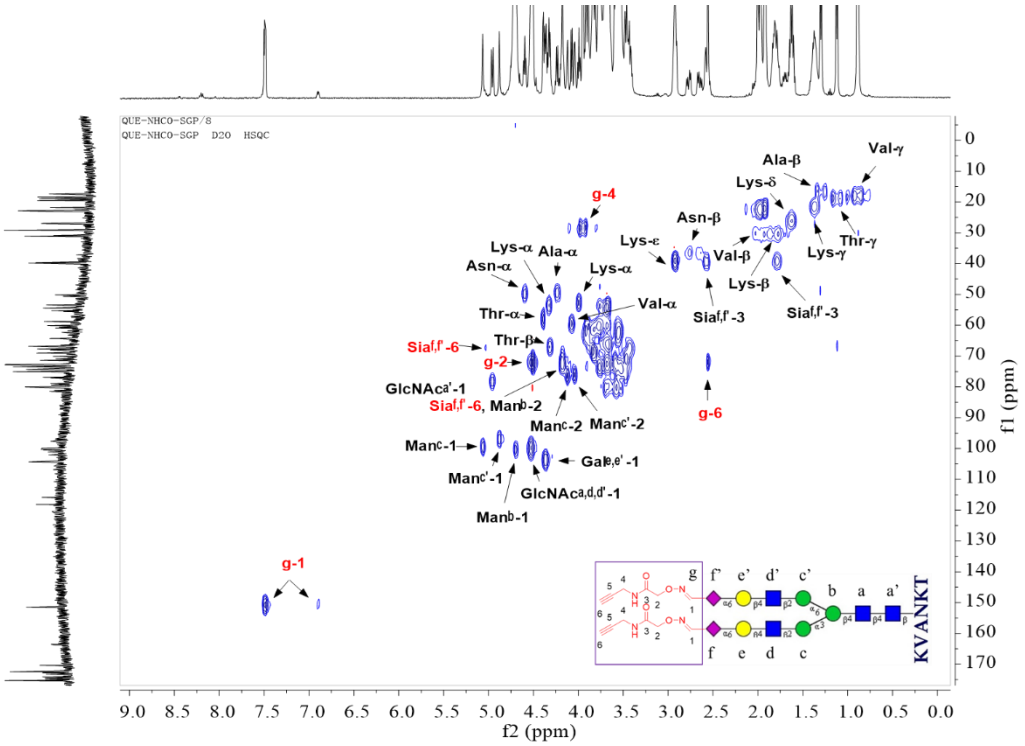


^{1}H NMR spectrum of **4d**



^{13}C NMR spectrum of **4d**

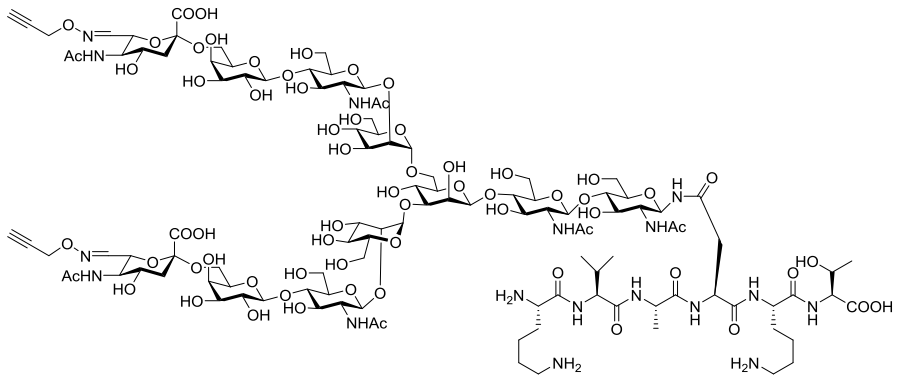
Nature Protocols: doi:10.1038/nprot.2017.058



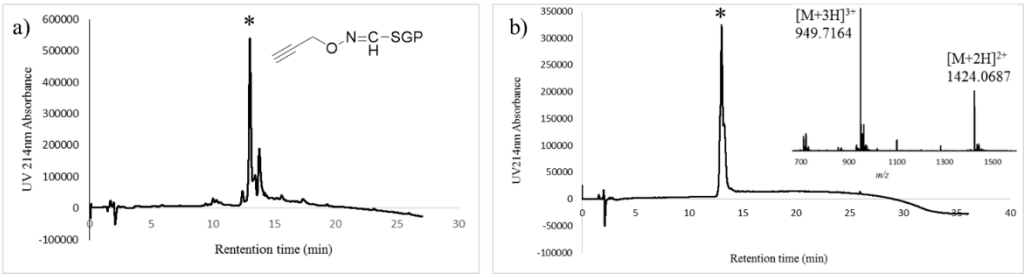
^{1}H-^{13}C HSQC NMR spectrum of **4d**

Nature Protocols: doi:10.1038/nprot.2017.058

PRODUCT 4E:



HPLC and LCMS profiles



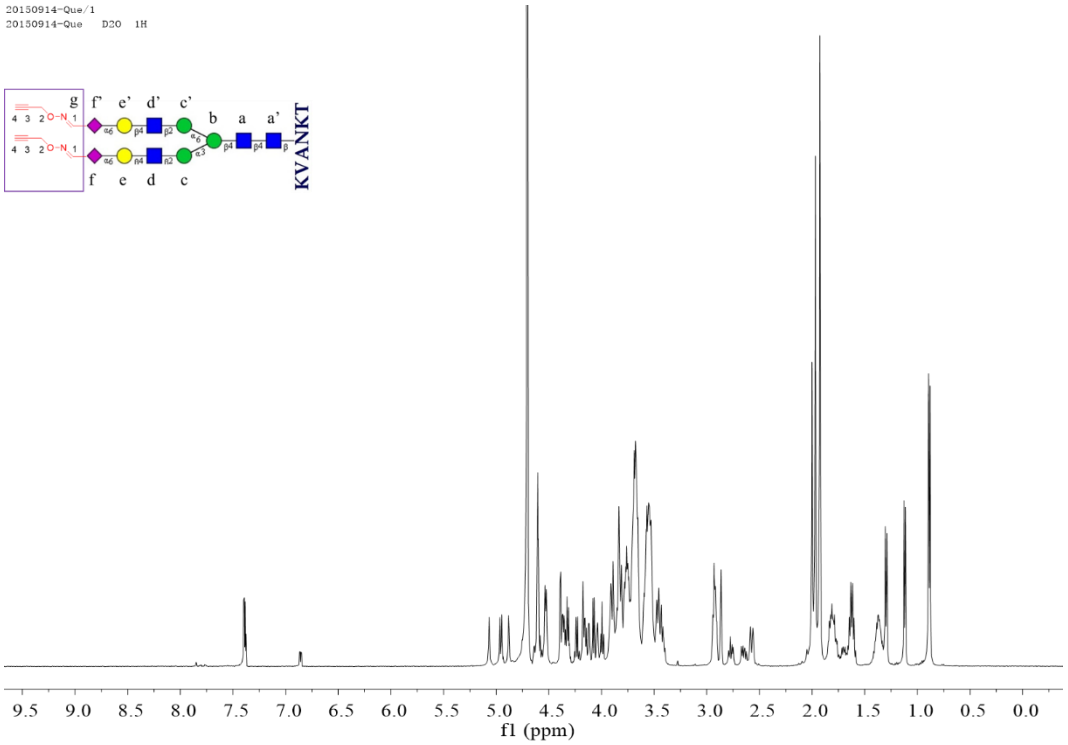
HPLC and LCMS profiles (HPLC method B) of CHO-SGP **20** and O-(2-propynyl)-hydroxylamine hydrochloride **21b** reaction. a) The samples removed from reaction mixture at 2 hour. The corresponding product was labeled with *. b) The HPLC profile of the product after preparative column purification. The insert is the HRMS profile of pure product.

NMR data

^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.39 (1.6H, dd, J = 7.2, 3.3 Hz, H1 of oxime), 6.85 (0.4H, dd, J = 6.7, 2.6 Hz, H1 of oxime), 5.07 (1H, s, H1c), 4.96 (1.4H, d, J = 9.6 Hz, H1a', H6f/f'), 4.88 (1H, s, H1c'), 4.76 (1H, s, H1b), 4.61 (5H, m, H2g, Asn Hα), 4.54 (3H, d, J = 6.7 Hz, H1a, H1d.H1d'), 4.39 (1H, d, J = 3.5 Hz, Thr Hα), 4.37 (1H, d, J = 2.5 Hz, H1e), 4.35 (1H, d, J = 3 Hz, H1e'), 4.34-4.28 (2H, m, Lys1 Hα, Thr Hβ), 4.24 I(1H, q, J = 7 Hz, Ala Hα), 4.18 (1H, s, H2b), 4.16 (1.6H, dd, J = 10.0 Hz, 7.5 Hz, H6f/f'), 4.12 (1H, d, J = 2 Hz, H2c), 4.08 (1H, d, J= 8 Hz, Val Hα), 4.04 (1H, d, J = 2 Hz, H2c') 3.99 (1H, t, J = 6.5 Hz, Lys2 Hα), 2.92 (4H, q, J = 7.5 Hz, Lys Hε), 2.86 (2H, q, H3g), 2.81-2.61 (2H, m, Asn Hβ), 2.61-2.51 (2H, dd, J = 12.5 Hz, 2.5Hz, $H3f_{eq}$, $H3f'_{eq}$), 2.03-1.88 (19H, m, 6 x 3 Ac, Val Hβ), 1.87-1.66 (6H, m, Lys Hβ, $H3f_{ax}$, $H3f'_{ax}$), 1.66 (4H, m, J = 7.5 Hz, Lya Hδ), 1.43-1.32 (4H, m, Lys Hγ), 1.3 (3H, d, J = 7.5 Hz, Ala Hβ), 1.13 (3H, J = 6.5 Hz, Thr Hγ), 0.89 (6H, d, 7.0 Hz, Val Hγ). HSQC ((1H, 500 MHz)/(13C, 126 MHz), Deuterium Oxide) 7.41/150.05 (H1g/C1g), 6.88/149.59 (H1g/C1g), 5.09/99.34 (H1c/C1c), 4.98/78.34 (H1a'/C1a'), 4.97/67.43 (H6f/C6f, H6f'/C6f'), 4.91/96.89 (H1c'/C1c'), 4.72/100.33 (H1b/C1b), 4.63/61.46 (H2g/C2g), 4.62/49.85 (Hα Asn/Cα Asn), 4.55/99.74 (H1a/C1a, H1d/C1d, H1d'/C1d'), 4.42/58.25 (Hα Thr/Cα Thr), 4.39/103.91 (H1e/C1e, H1e'/C1e'), 4.35/53.53 (Hα Lys/Cα Lys), 4.34/67.11 (Hβ Thr/Cβ Thr), 4.26/49.43 (Hα Ala/Cα Ala), 4.20/70.52 (H2b/C2b, H6f/C6f, H6f'/C6f'), 4.14/76.54 (H2c/C2c), 4.10/59.64 (Hα Val/Cα Val), 4.06/76.25 (H2c'/C2c'), 4.02/52.83 (Hα Lys/Cα Lys), 2.94/39.16 (Hε Lys/Cε Lys), 2.89/76.16 (H4g/C4g), 2.78/37.03 (Hβ

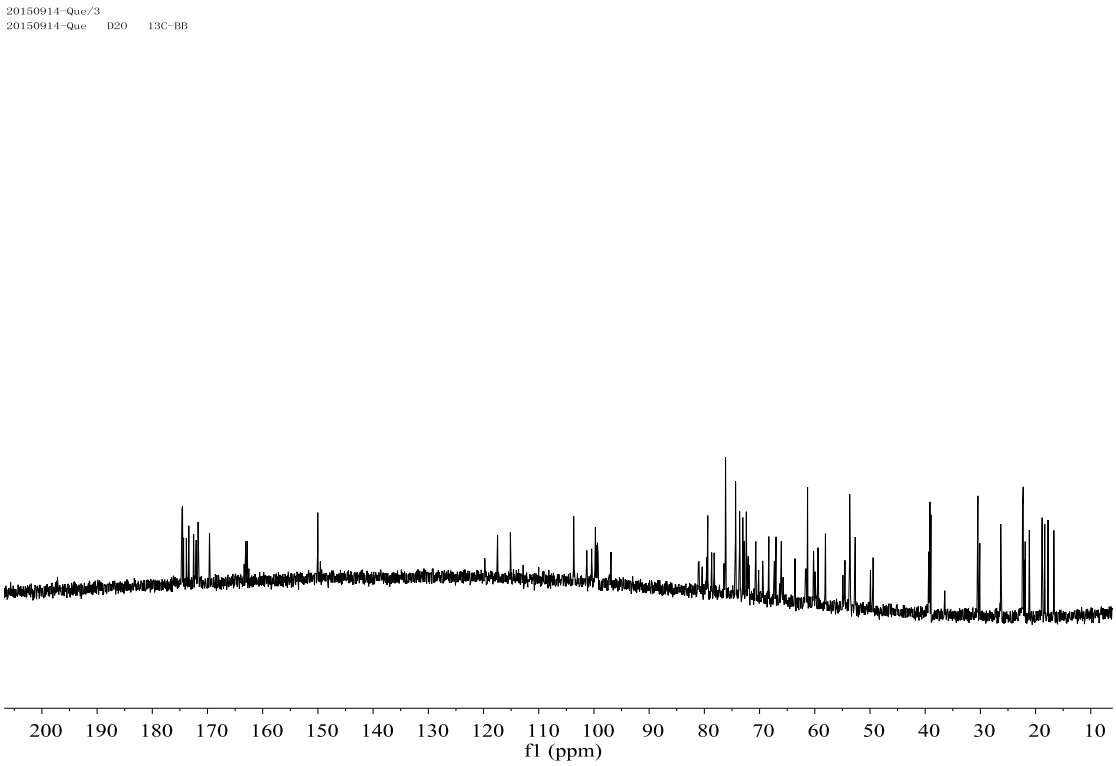
Asn/Cβ Asn), 2.58/39.45 (H3f/C3f, H3f'/C3f'), 2.01/30.19 (Hβ Val/Cβ Val), 1.84/30.59 (Hβ Lys/Cβ Lys), 1.79/39.37 (H3f/C3f, H3f'/C3f'), 1.65/26.40 (Hδ Lys/Cδ Lys), 1.43/21.61 (Hγ Lys/Cγ Lys), 1.32/16.78 (Hβ Ala/Cβ Ala), 1.14/19.15 (Hγ Thr/Cγ Thr), 0.91/18.15 (Hγ Val/Cγ Val).

NMR spectra

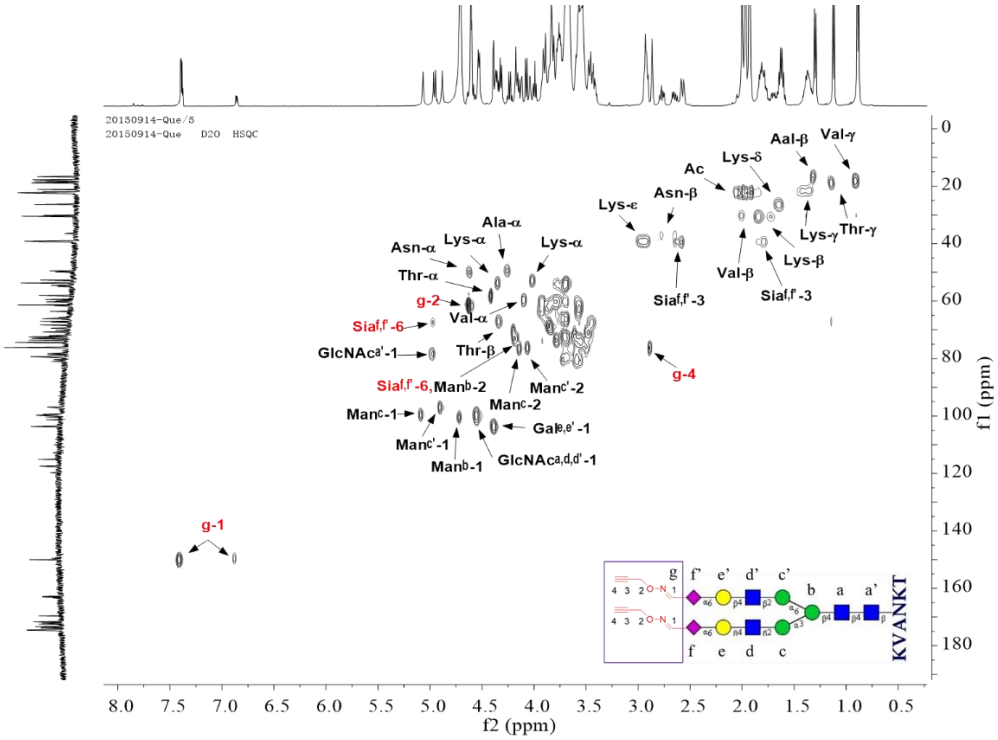


^{1}H NMR spectrum of **4e**

Nature Protocols: doi:10.1038/nprot.2017.058



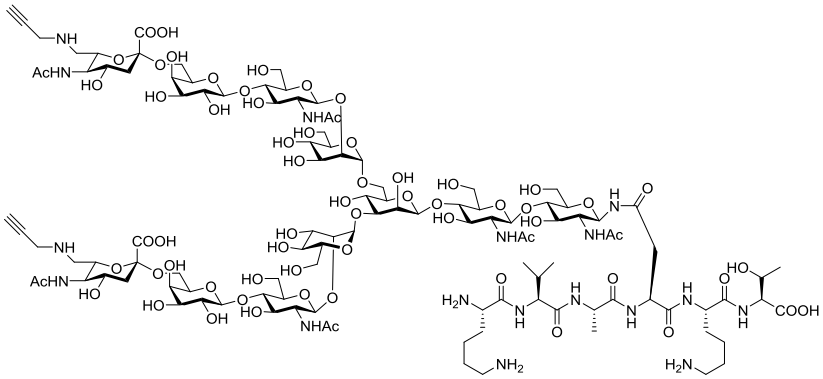
^{13}C NMR spectrum of **4e**



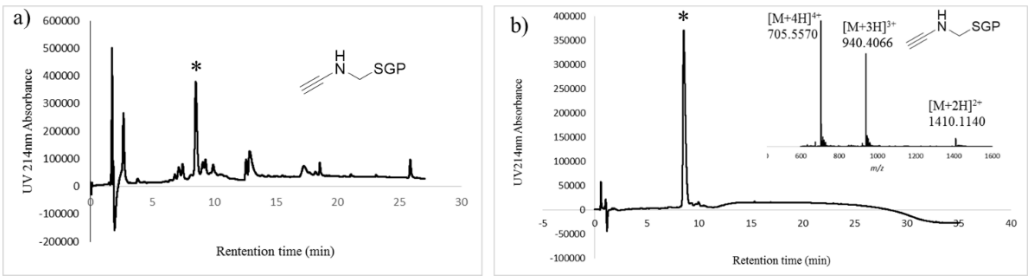
^{1}H-^{13}C HSQC NMR spectrum of **4e**

Nature Protocols: doi:10.1038/nprot.2017.058

PRODUCT 4F:



HPLC and LCMS profiles



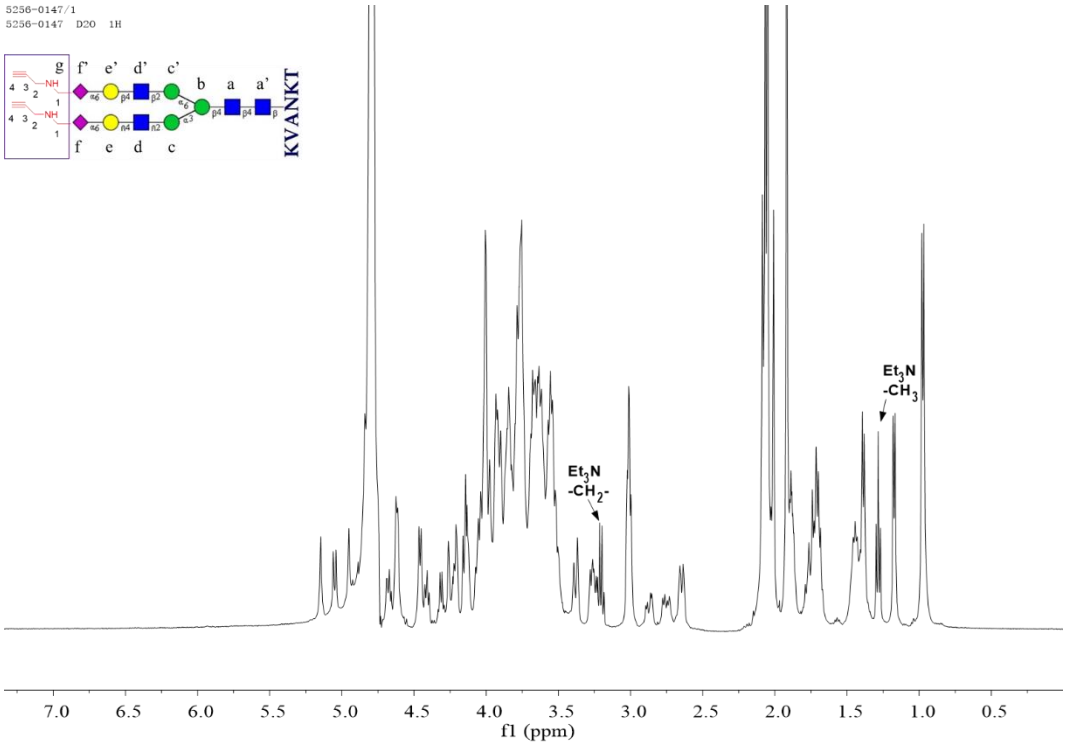
HPLC and LCMS profiles (HPLC method B) of reductive amination reaction with propargylamine. a) The samples removed from reaction mixture at 3 hour. The corresponding product was labeled with *. b) The HPLC profile of the product after preparative column purification. The insert is the HRMS profile of pure product.

NMR data

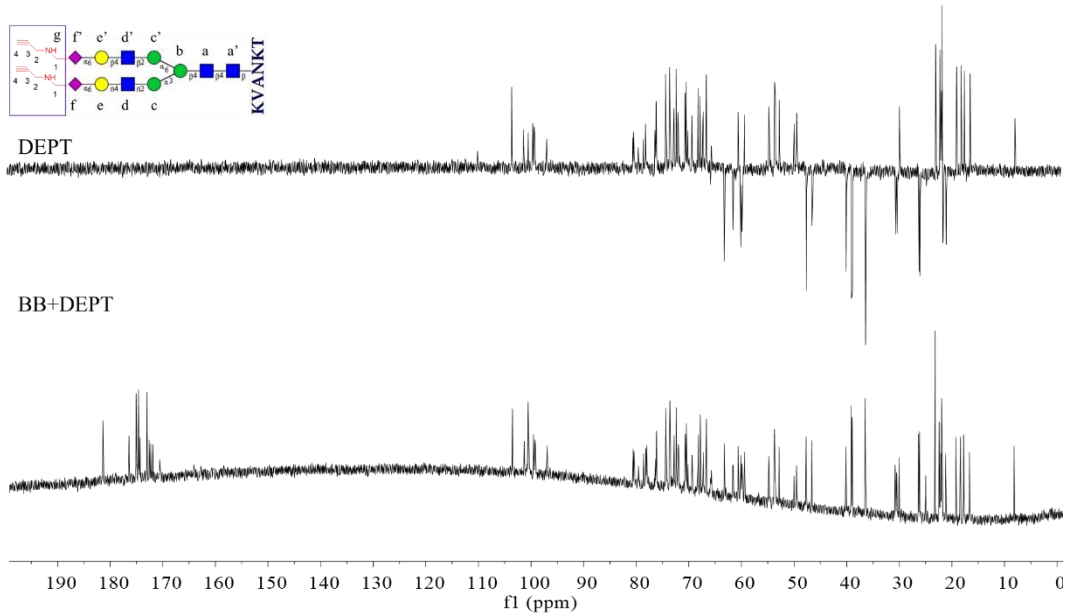
^{1}H NMR (500 MHz, Deuterium Oxide) δ 5.15 (1H, s, H1c), 5.05 (1H, d, J = 9.6 Hz, H1a'), 4.95 (1H, s, H1c'), 4.84 (1H, s, H1b), 4.67 (1H, t, J = 6.7 Hz, Asn Hα), 4.62 (3H, dd, J = 6.4, 5.1 Hz, H1a, H1d, H1d'), 4.46 (2H, dd, J = 7 Hz, H1e, H1e'), 4.41 (1H, t, Lys1 Hα), 4.31 (1H, q, J = 7.2 Hz, Ala Hα), 4.26 (1H, s, H2b), 4.22 (1H, m, Thr Hβ) 4.21 (1H, m, Thr Hα), 4.17-4.10 (3H, m, H2c, Val Hα, H2c'), 4.01 (4H, H2g), 3.43-3.16 (4H, m, H1g), 3.01 (6H, m, Lys Hε, H4g), 2.93-2.69 (2H, m, Asn Hβ), 2.69 - 2.59 (2H, dd, J = 12.5 Hz, 2.5Hz, H3f$_{eq}$, H3f'$_{eq}$), 2.15ii-1.90 (16iH, m, 5 x 3 Ac, Val Hβ), 1.97-1.81 (7H, m, Lys Hβ, Ac) 1.80-1.64 (6H, m, Lys Hβ, H3f$_{ax}$, H3f'$_{ax}$), 1.66 (4H, m, J = 7.5 Hz, Lya Hδ), 1.43 – 1.32 (4H, m, Lys Hγ), 1.3 (3H, d, J = 7.5 Hz, Ala Hβ), 1.13 (3H, J = 6.5 Hz, Thr Hγ), 0.89 (6H, d, 7.0 Hz, Val Hγ). HSQC ((1H, 500 MHz)/(13C, 126 MHz), Deuterium Oxide) 5.06/100.47 (H1c/C1c), 4.96/79.04 (H1a'/C1a'), 4.86/97.82 (H1c'/C1c'), 4.70/101.42 (H1b/C1b), 4.59/50.87 (Hα Asn/Cα Asn), 4.53/100.62 (H1a/C1a, H1d/C1d, H1d'/C1d'), 4.37/103.51 (H1e/C1e, H1e'/C1e'), 4.32/54.42 (Hα Lys/Cα Lys), 4.31/67.02 (Hβ Thr/Cβ Thr), 4.22/50.36 (Hα Ala/Cα Ala), 4.17/71.01 (H2b/C2b), 4.12/77.38 (H2c/C2c), 4.06/60.90 (Hα Val/Cα Val), 4.04/77.09 (H2c'/C2c'), 3.92/53.74 (Hα Lys/Cα Lys), 3.91/37.48 (H2g/C2g), 3.28/48.52 (H1g/C1g), 2.90/39.89 (Hε Lys/Cε Lys), 2.79/39.26 (Hβ Asn/Cβ Asn), 2.60/40.92 (H3f/C3f, H3f'/C3f'), 1.97/30.09 (Hβ Val/Cβ Val), 1.79/31.46 (Hβ Lys/Cβ Lys), 1.69/41.18 (H3f/C3f, H3f'/C3f'), 1.65/27.10 (Hδ Lys/Cδ Lys), 1.35/22.40 (Hγ Lys/Cγ Lys), 1.30/17.64 (Hβ Ala/Cβ Ala), 1.09/20.15 (Hγ Thr/Cγ Thr), 0.89/19.16 (Hγ Val/Cγ Val).

Nature Protocols: doi:10.1038/nprot.2017.058

NMR spectra

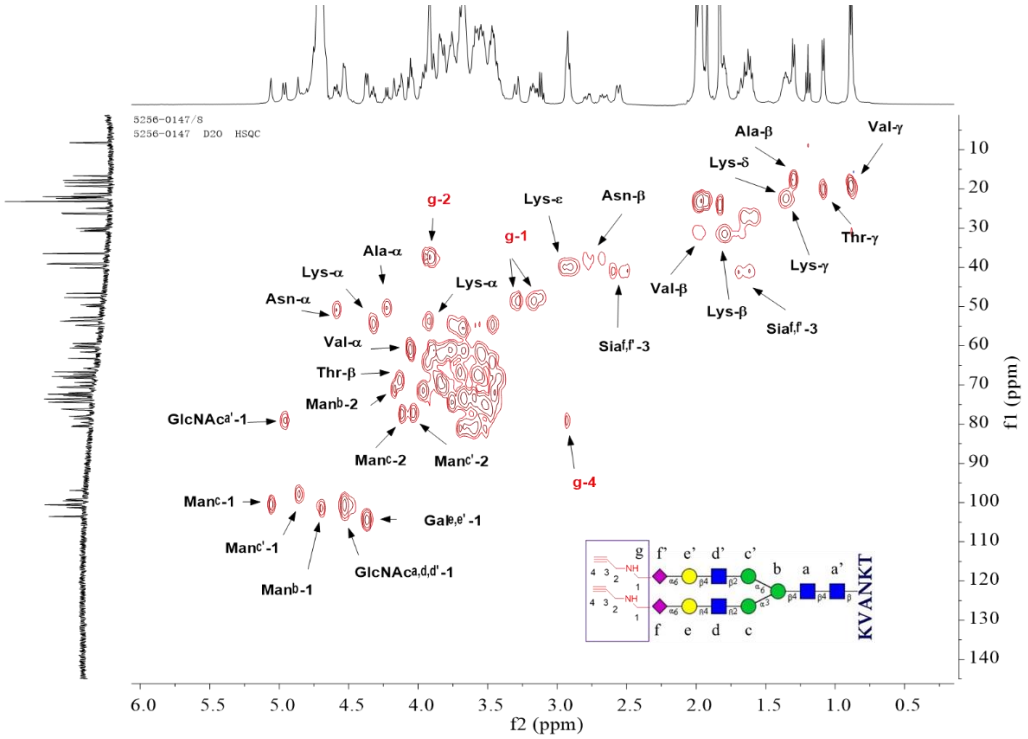


^{1}H NMR spectrum of **4f**



^{13}C NMR spectrum of **4f**

Nature Protocols: doi:10.1038/nprot.2017.058

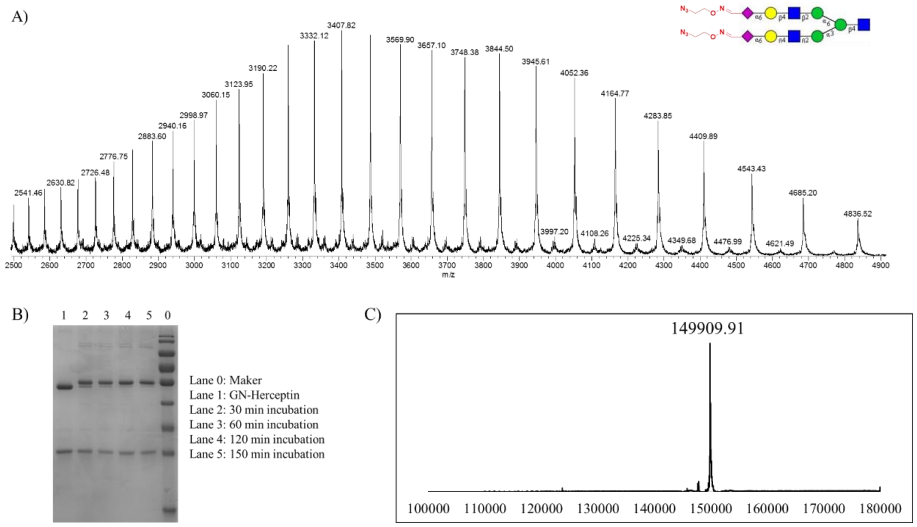


^{1}H-^{13}C HSQC NMR spectrum of **4f**

Nature Protocols: doi:10.1038/nprot.2017.058

PRODUCT 5A:

LCMS and SDS-PAGE profiles

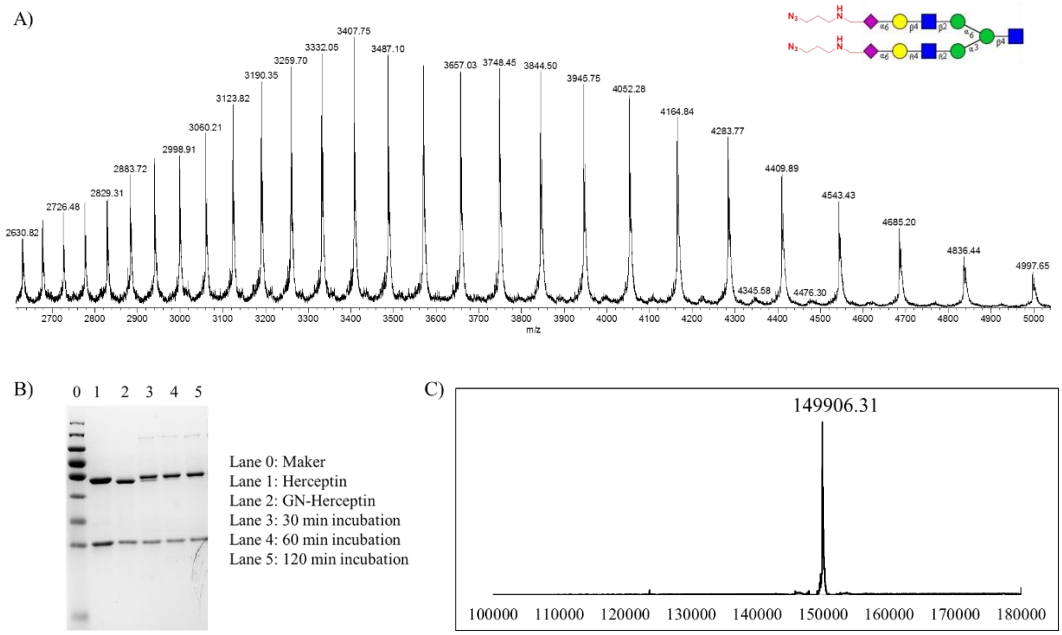


LCMS and SDS-PAGE profile of glycoengineered Herceptin **5a**. A) The charged *m/z* spectrum of **5a**, B) SDS-PAGE monitoring, C) The deconvoluted spectrum of **5a**.

Nature Protocols: doi:10.1038/nprot.2017.058

PRODUCT 5B:

LCMS and SDS-PAGE profiles

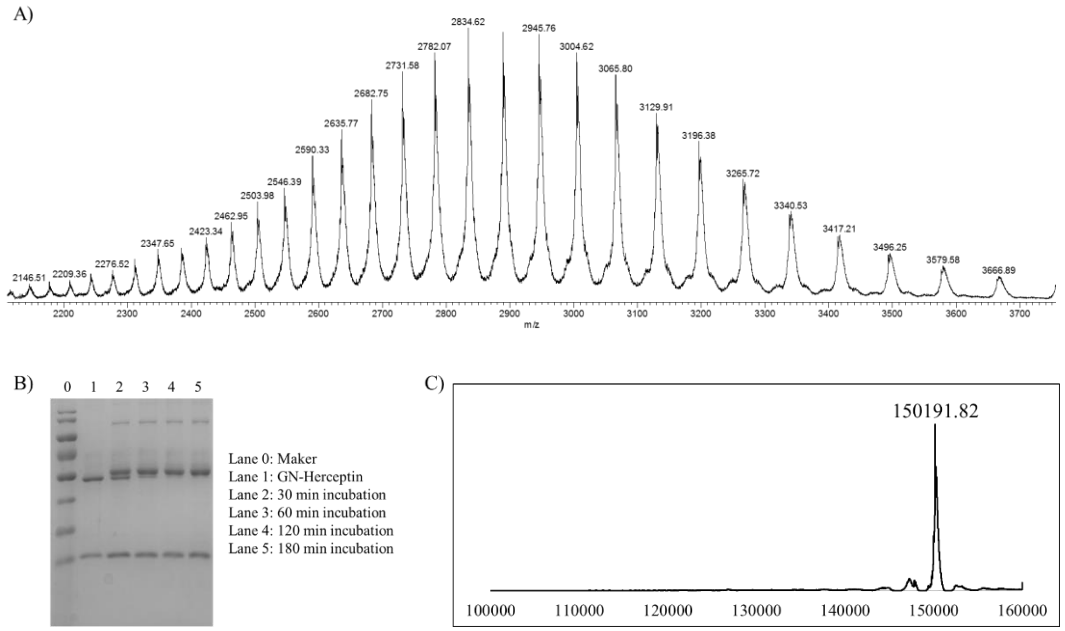


LCMS and SDS-PAGE profile of glycoengineered Herceptin **5b**. A) The charged *m/z* spectrum of **5b**, B) SDS-PAGE monitoring, C) The deconvoluted spectrum of **5b**.

Nature Protocols: doi:10.1038/nprot.2017.058

PRODUCT 5C:

LCMS and SDS-PAGE profiles

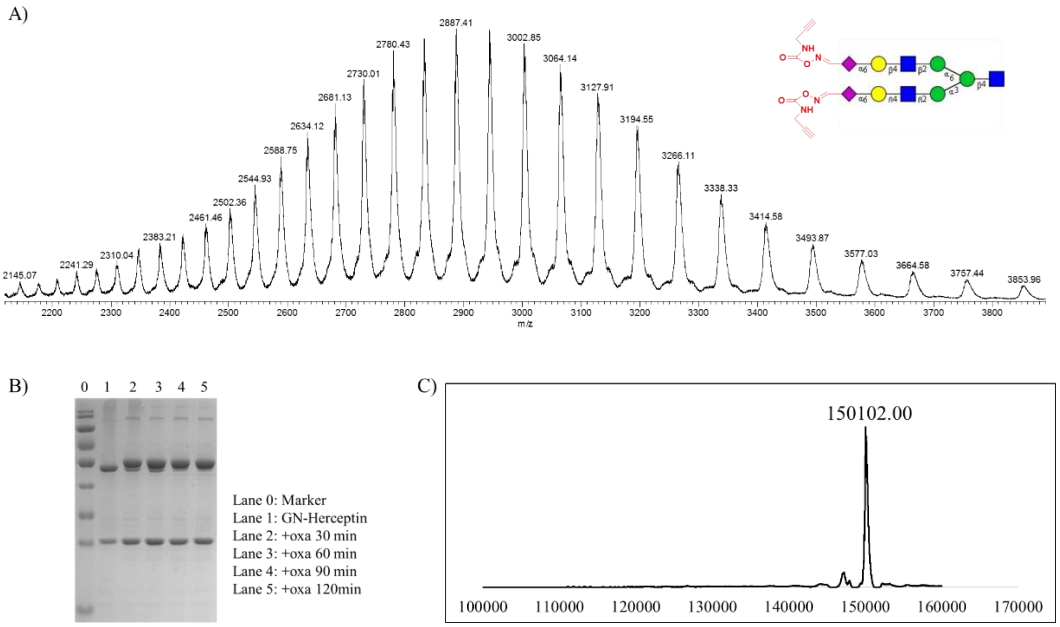


LCMS and SDS-PAGE profile of glycoengineered Herceptin **5c**. A) The charged *m/z* spectrum of **5c**, B) SDS-PAGE monitoring, C) The deconvoluted spectrum of **5c**.

Nature Protocols: doi:10.1038/nprot.2017.058

PRODUCT 5D:

LCMS and SDS-PAGE profiles

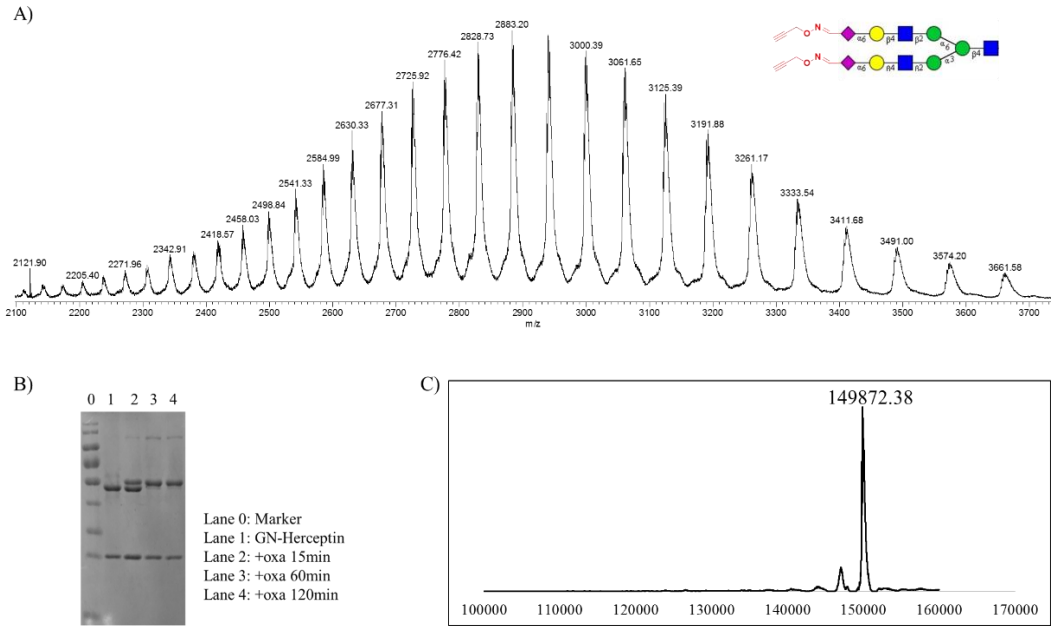


LCMS and SDS-PAGE profile of glycoengineered Herceptin **5d**. A) The charged *m/z* spectrum of **5d**, B) SDS-PAGE monitoring, C) The deconvoluted spectrum of **5d**.

Nature Protocols: doi:10.1038/nprot.2017.058

PRODUCT 5E:

LCMS and SDS-PAGE profiles

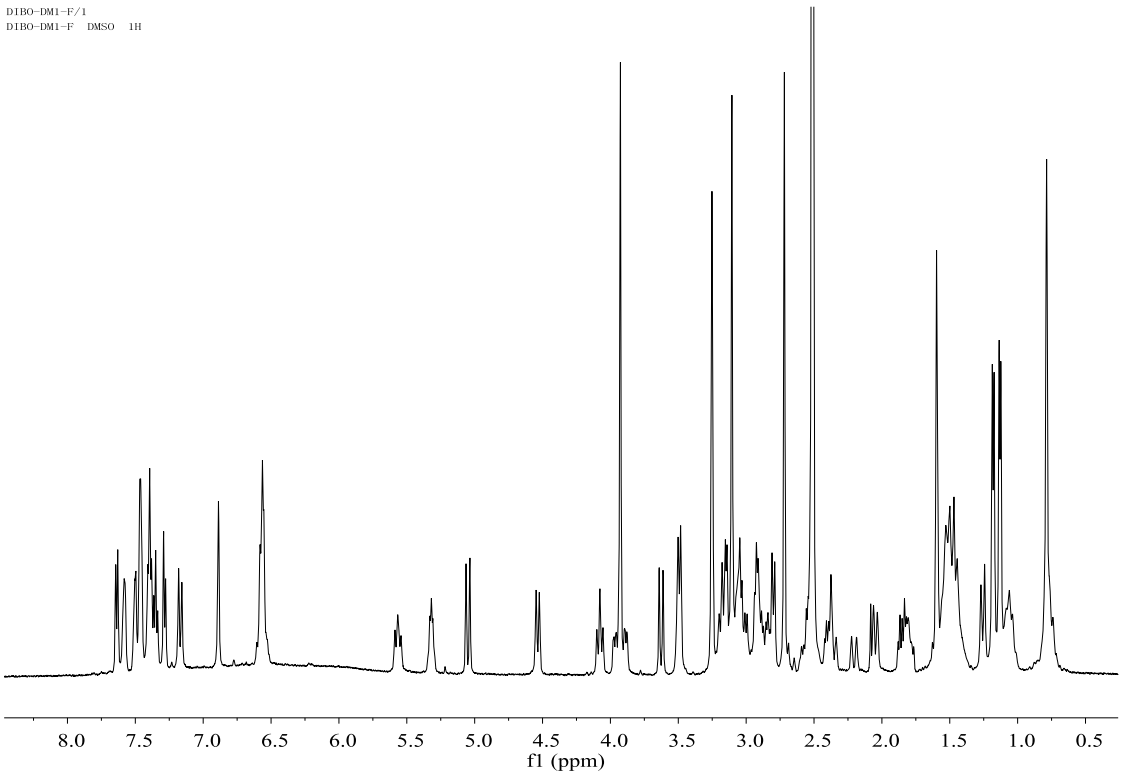


LCMS and SDS-PAGE profile of glycoengineered Herceptin **5e**. A) The charged *m/z* spectrum of **5e**, B) SDS-PAGE monitoring, C) The deconvoluted spectrum of **5e**.

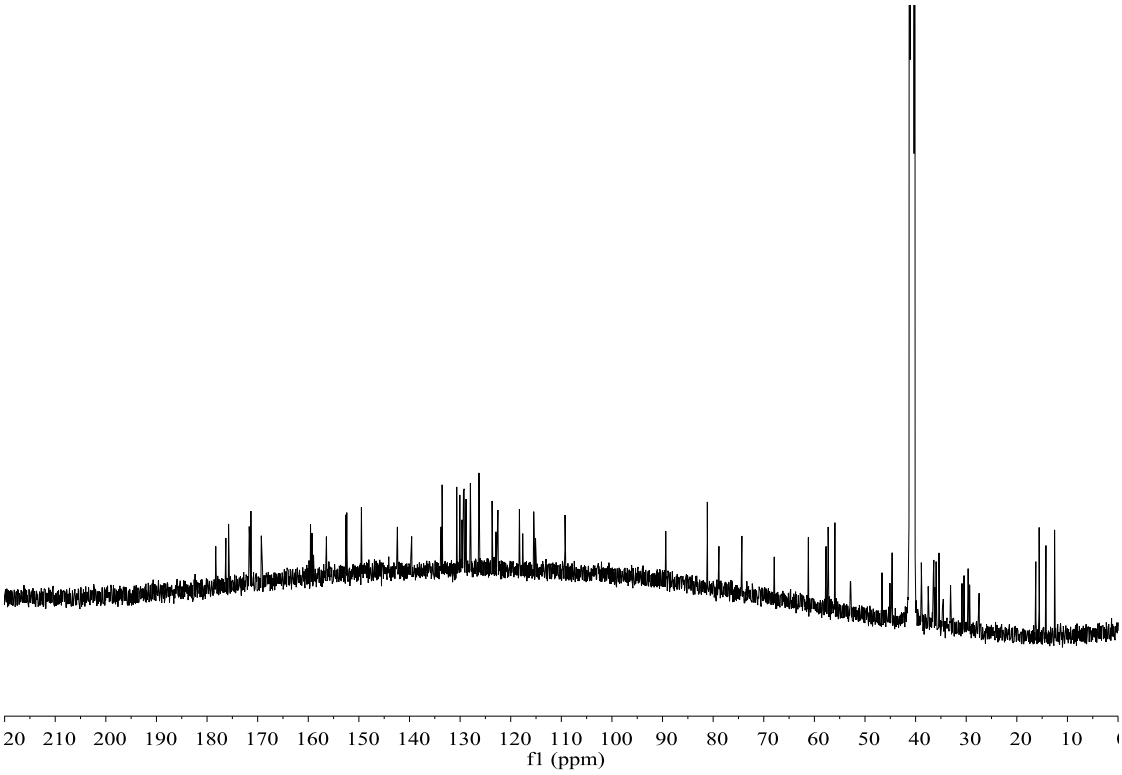
Nature Protocols: doi:10.1038/nprot.2017.058

PRODUCT 6A:

NMR spectra



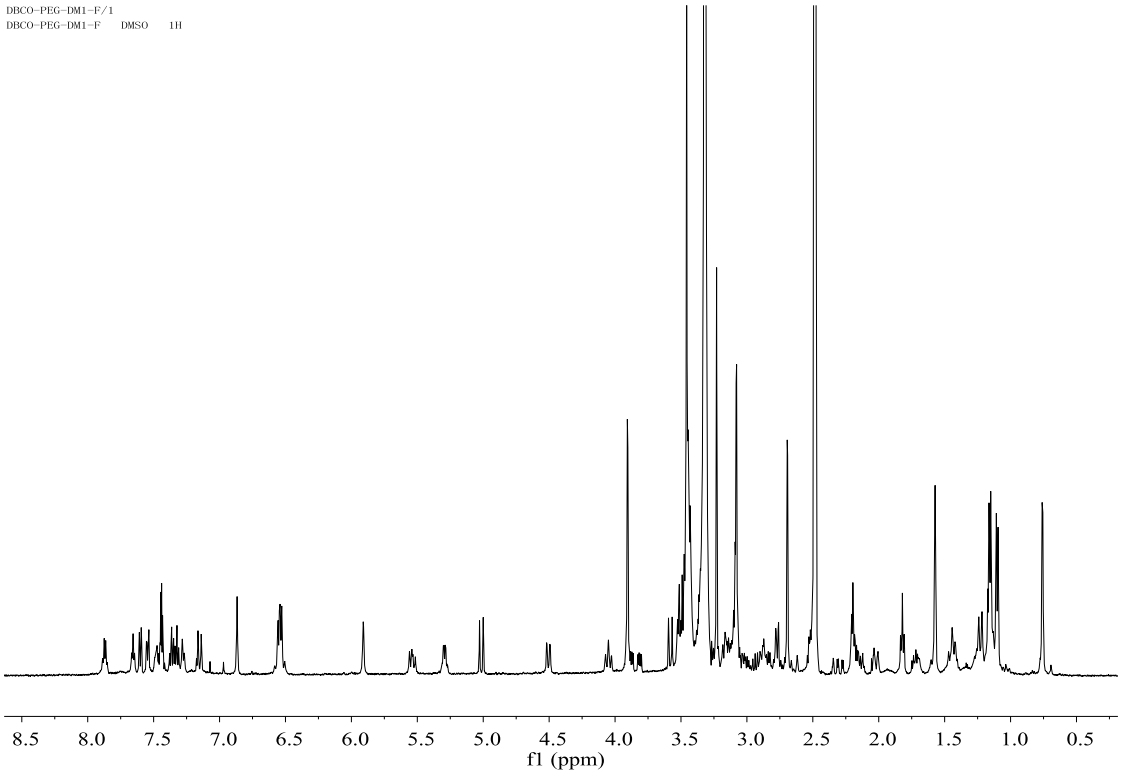
^{1}H NMR spectrum of **6a**



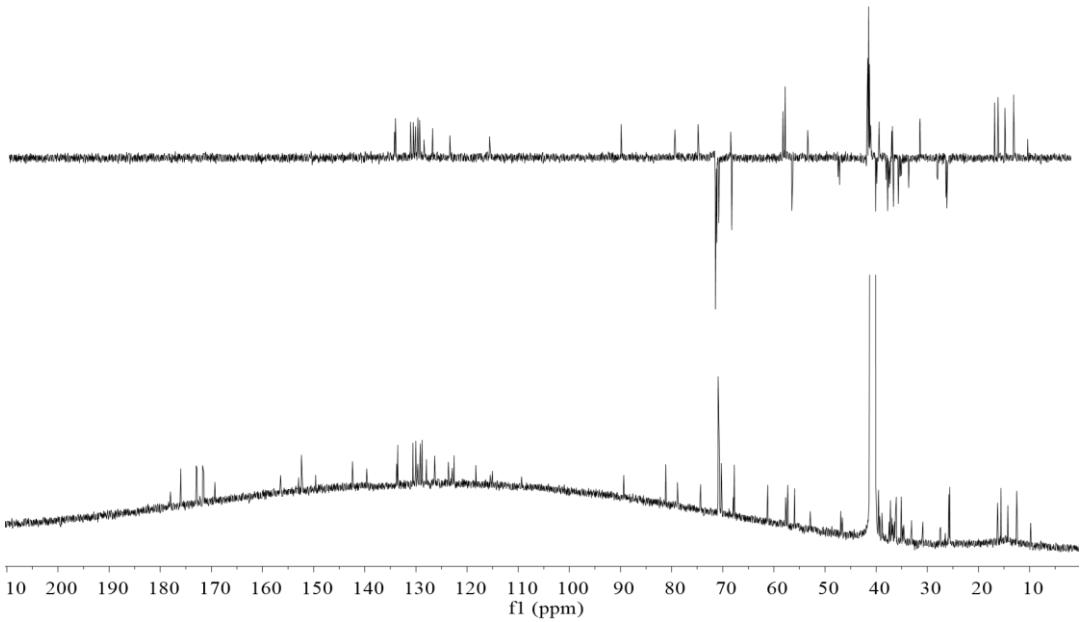
^{13}C NMR spectrum of **6a**

PRODUCT 6B:

NMR spectra



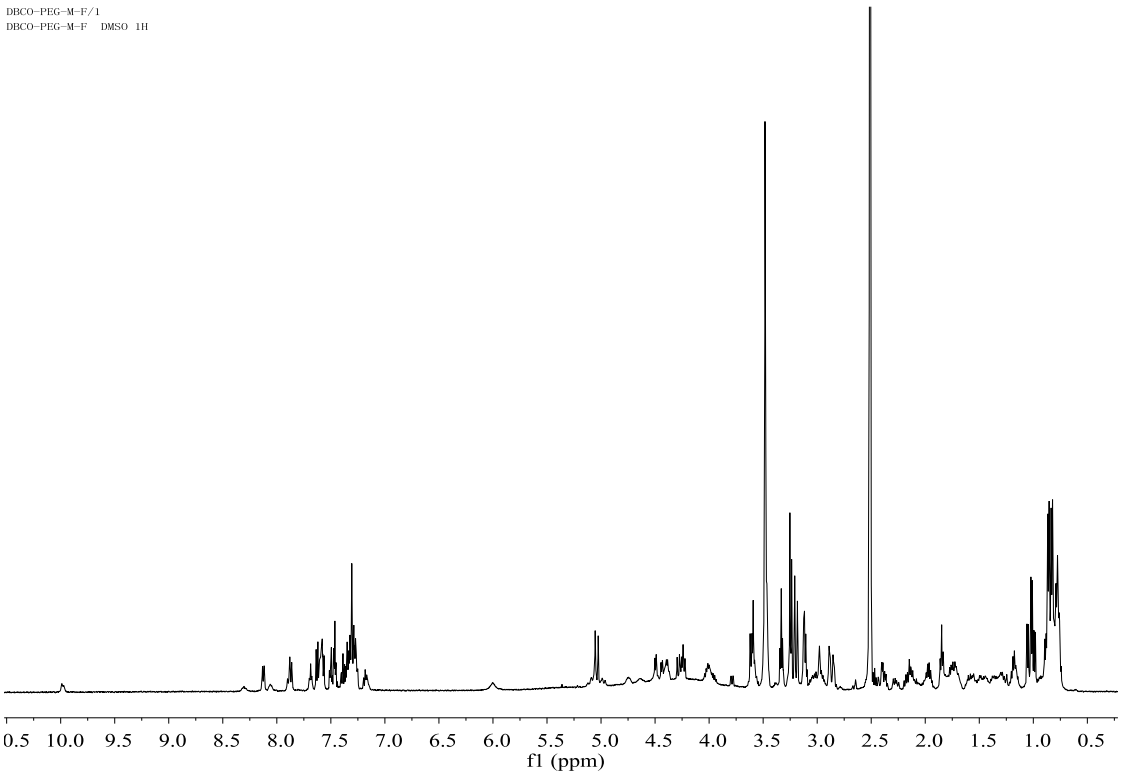
^{1}H NMR spectrum of **6b**



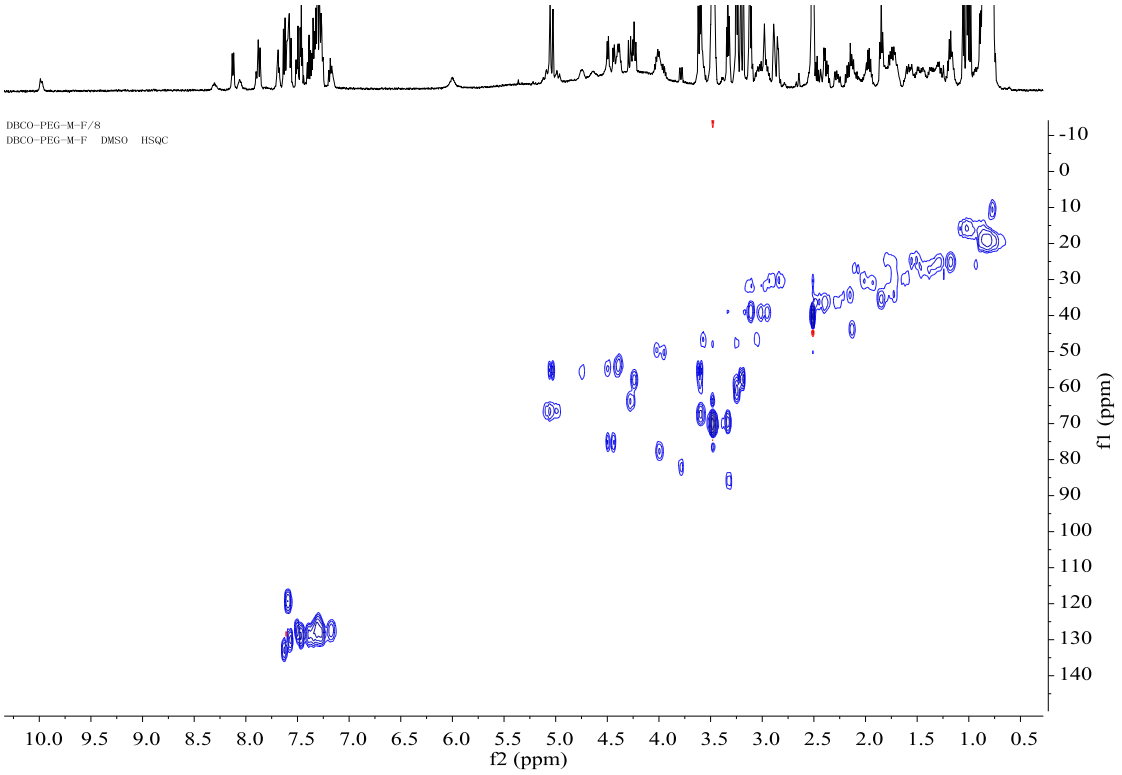
^{13}C NMR spectrum of **6b**

PRODUCT 6C:

NMR spectra



^{1}H NMR spectrum of **6c**

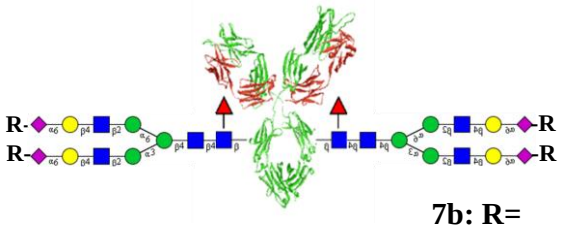


HSQC spectrum of **6c**

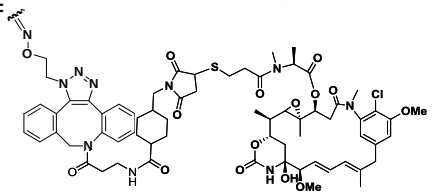
PRODUCT 7A-F:

Structural information of gsADCs **7a-f.**

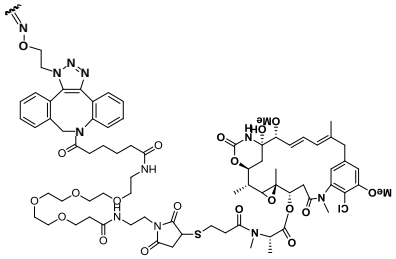
7a-f:



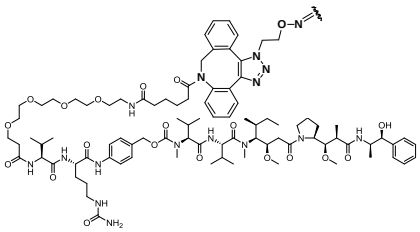
7a: R=



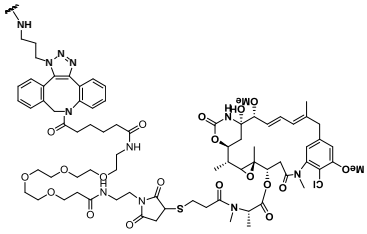
7b: R=



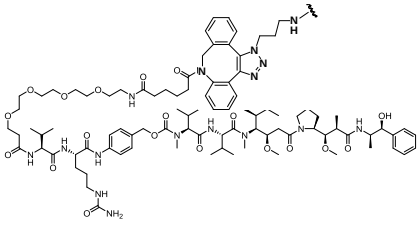
7c: R=



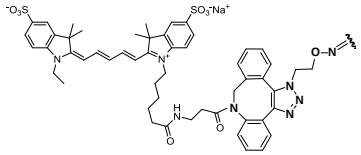
7d: R=



7e: R=

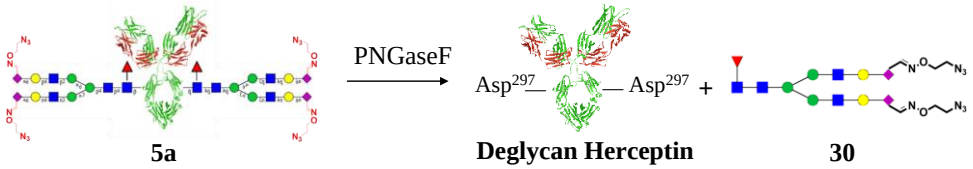


7f: R=



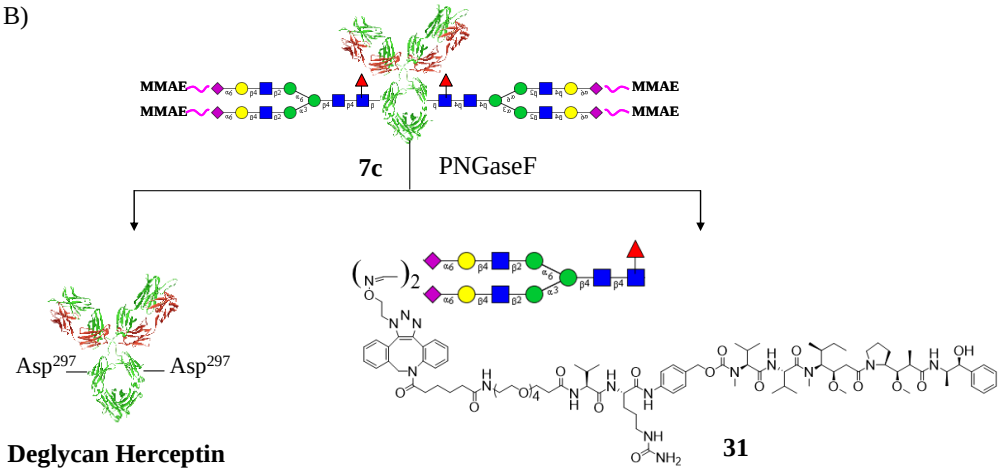
Reaction scheme of PNGaseF digestion of **5a** and **7c.**

A)



5a **Deglycan Herceptin** **30**

B)

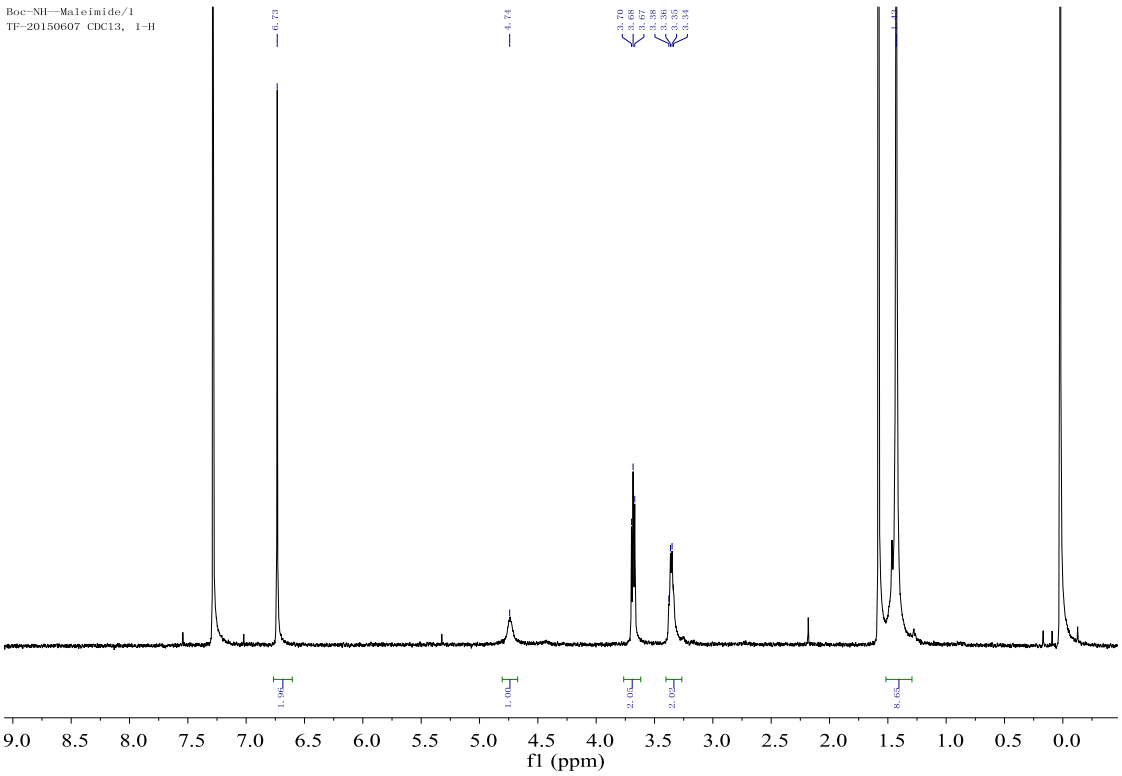


Deglycan Herceptin **31**

Nature Protocols: doi:10.1038/nprot.2017.058

PRODUCT S8:

NMR spectrum

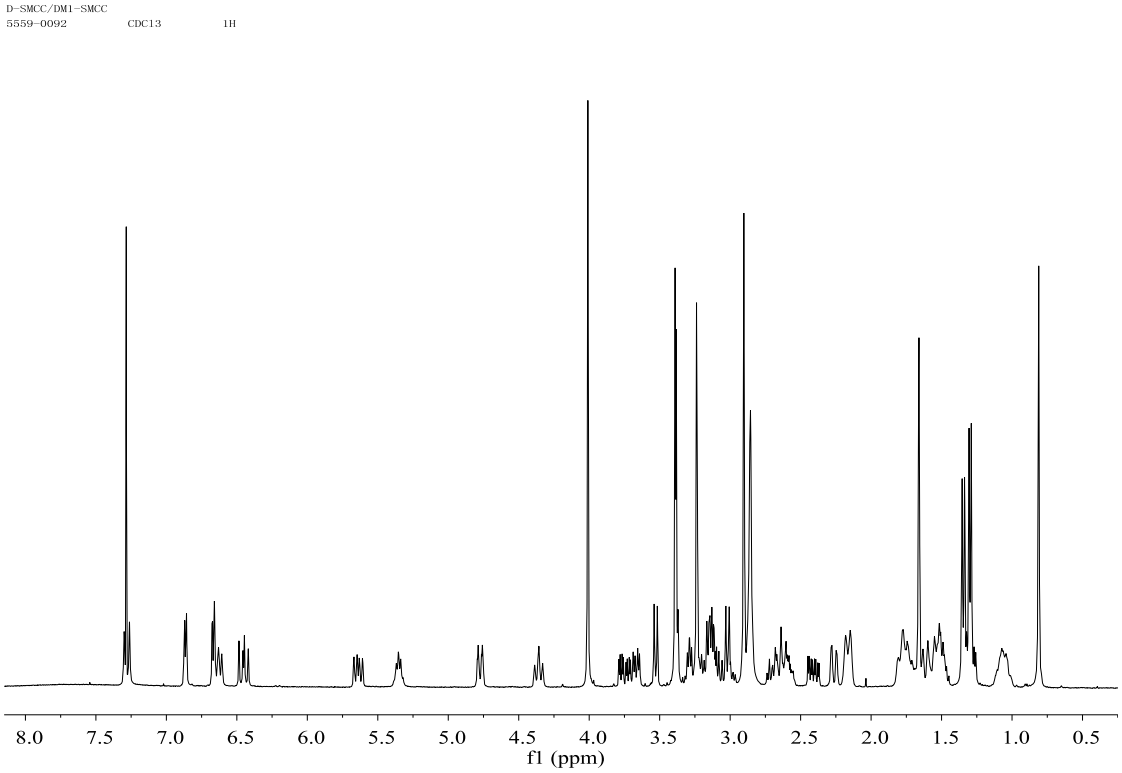


^{1}H NMR spectrum of **S8**

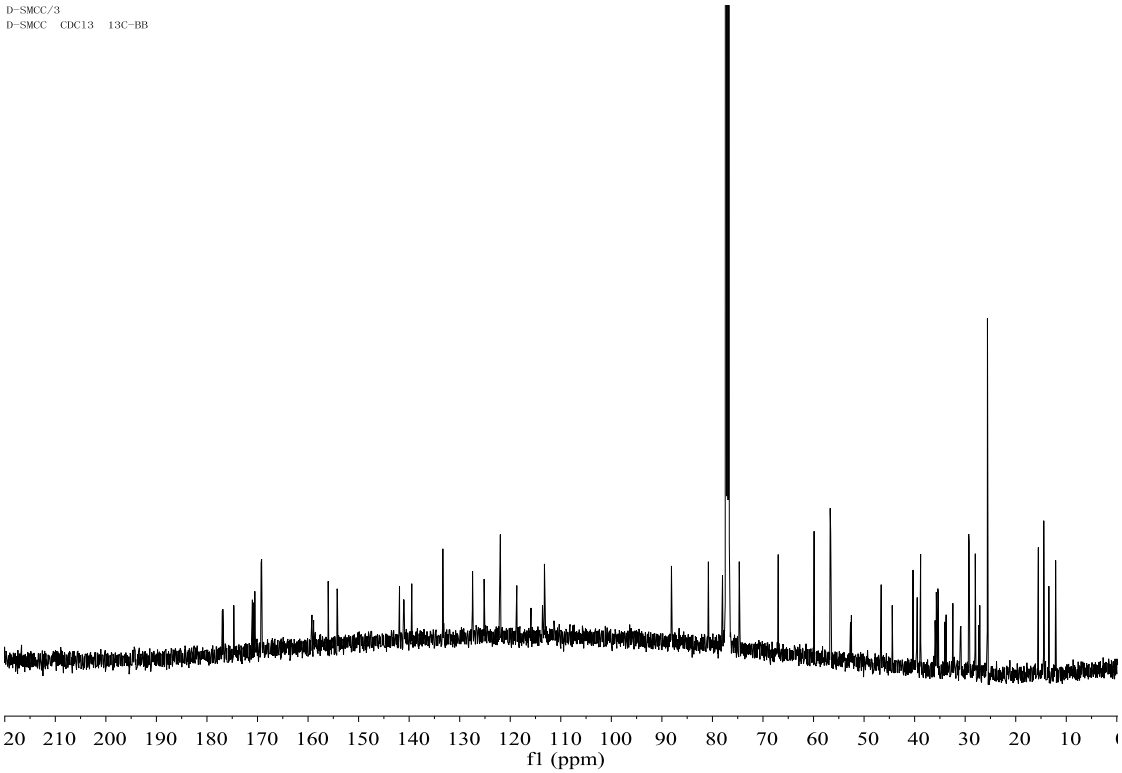
Nature Protocols: doi:10.1038/nprot.2017.058

PRODUCT 10:

NMR spectra



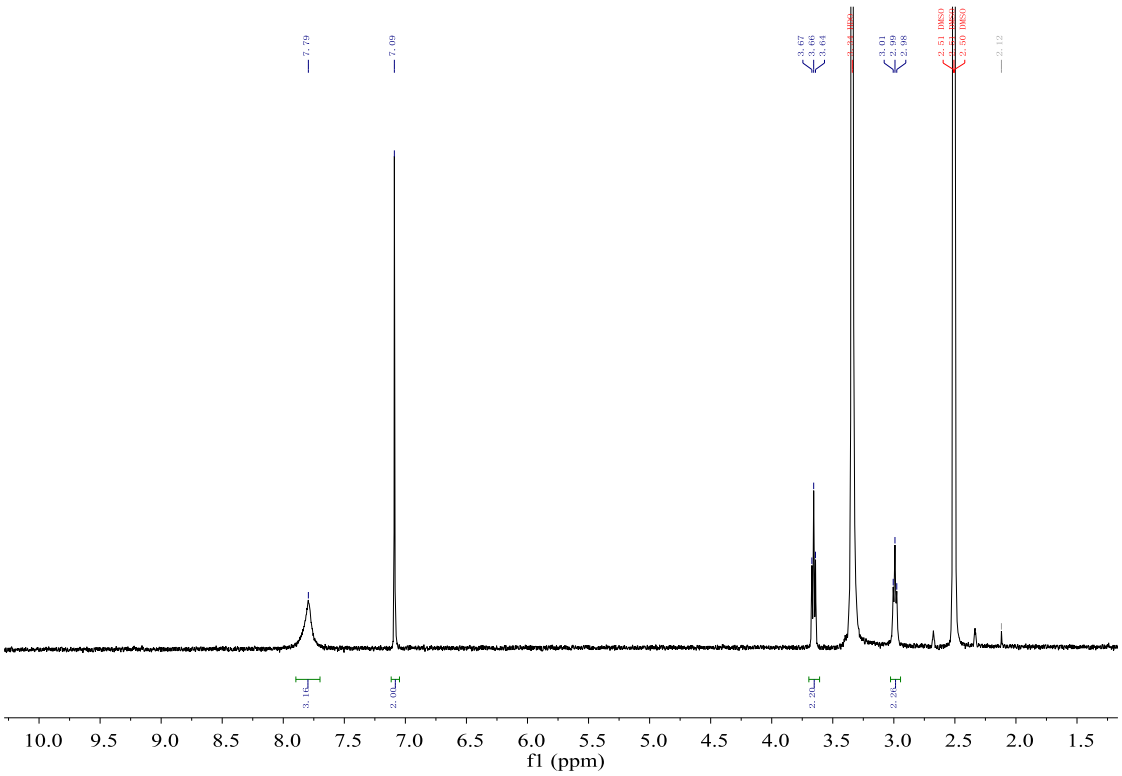
^{1}H NMR spectrum of **10**



^{13}C NMR spectrum of **10**

PRODUCT 13:

NMR spectrum

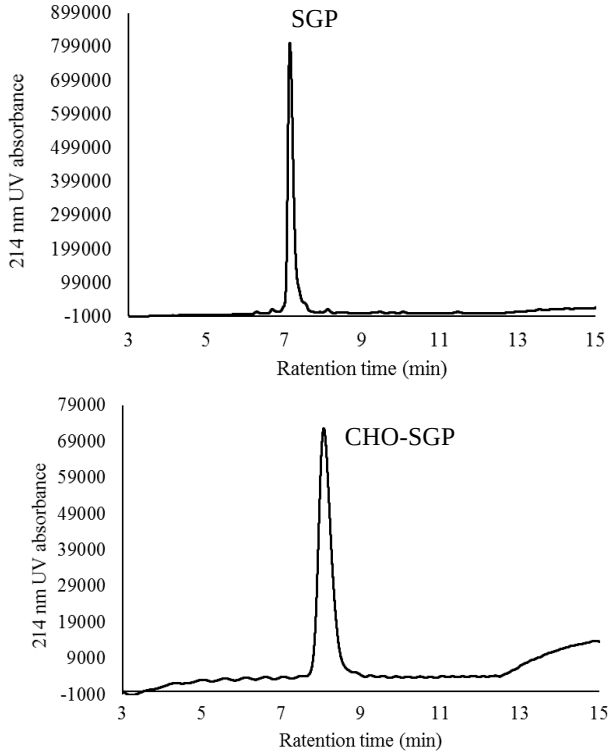


^{1}H NMR spectrum of **13**

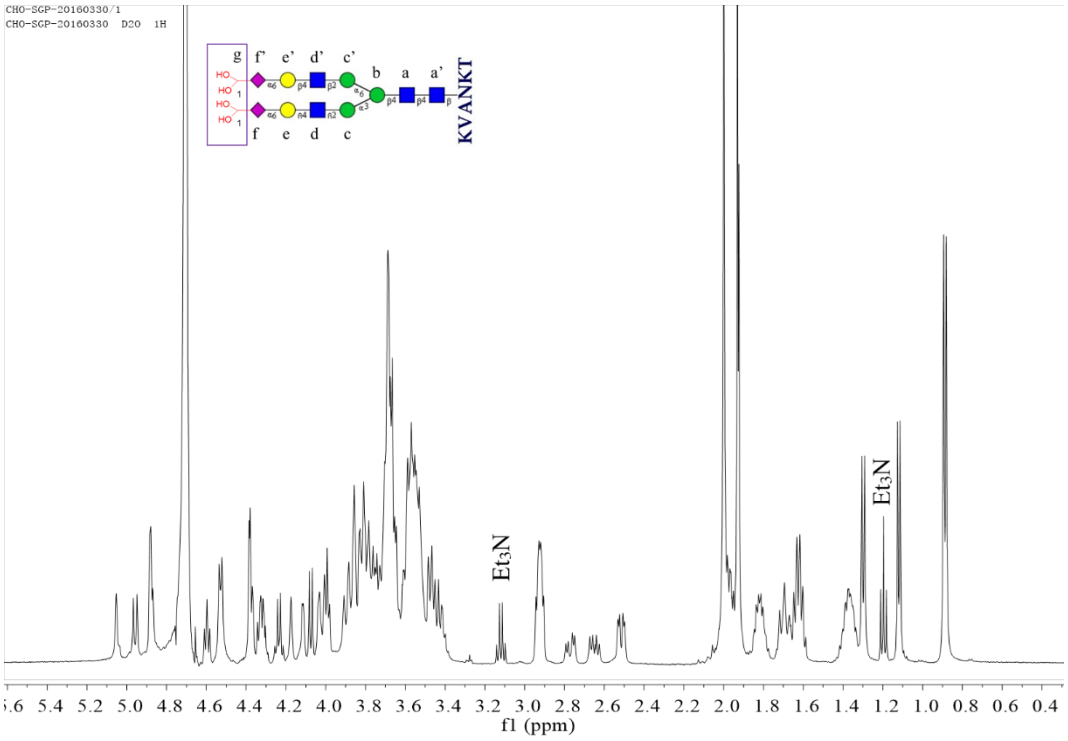
Nature Protocols: doi:10.1038/nprot.2017.058

PRODUCT 20:

HPLC profile

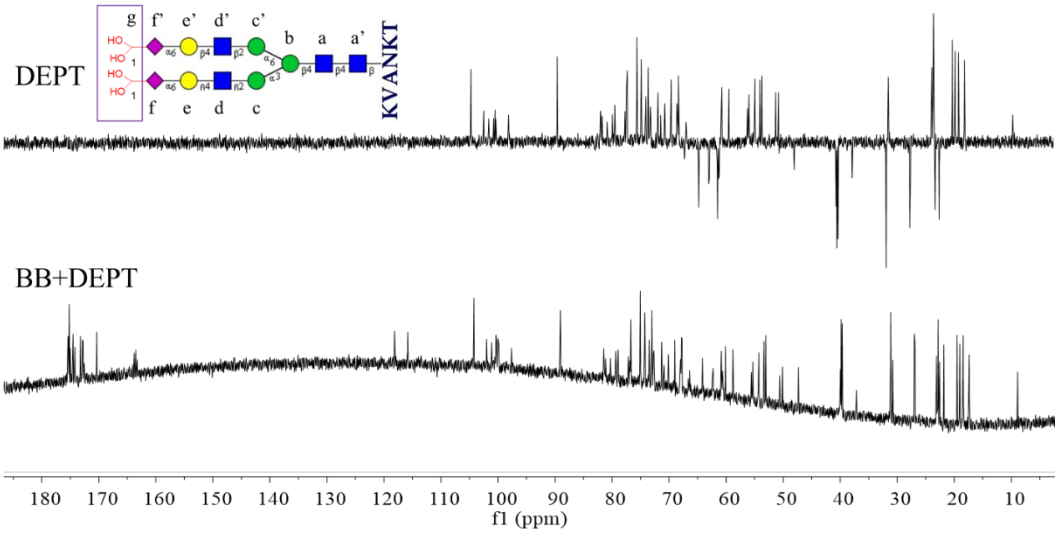


NMR spectra

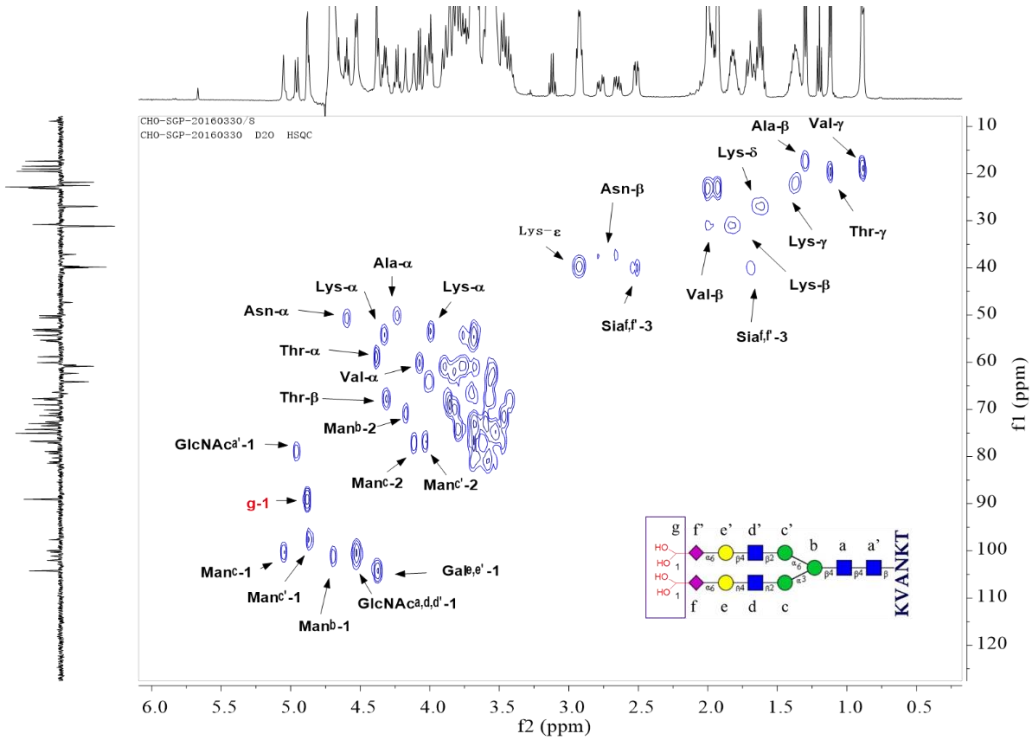


^{1}H NMR spectrum of CHO-SGP (**20**)

Nature Protocols: doi:10.1038/nprot.2017.058



^{13}C NMR spectrum of CHO-SGP (**20**)

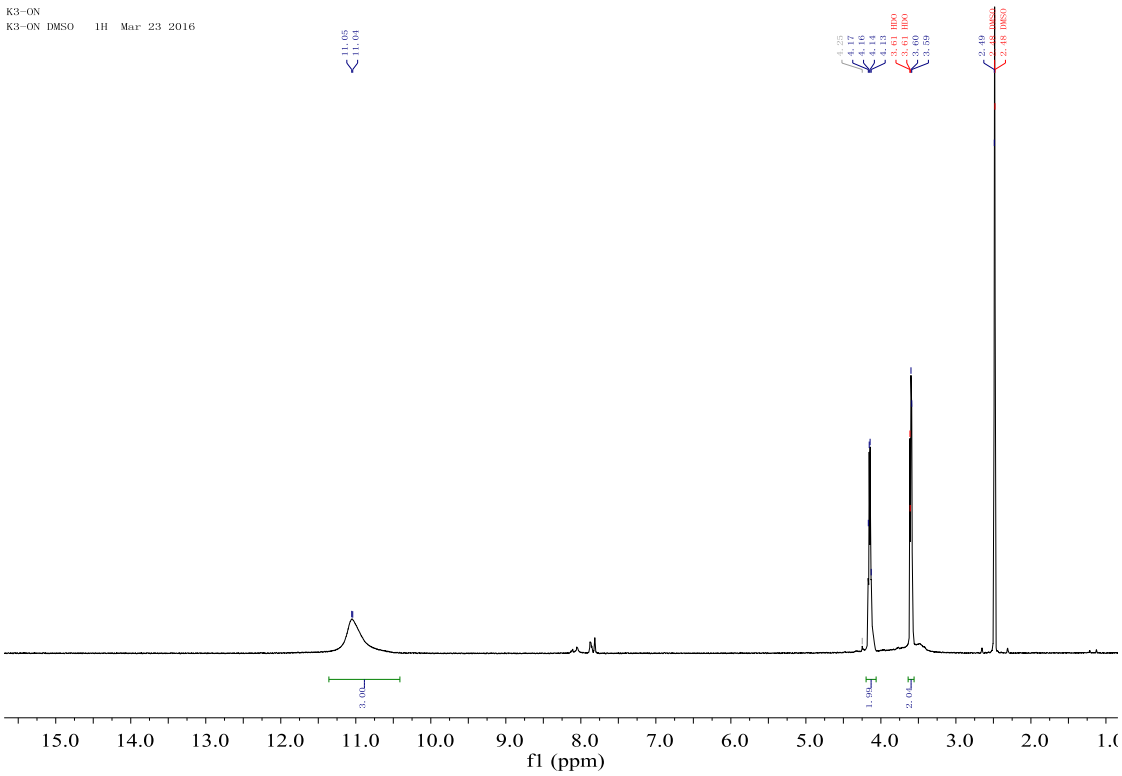


HSQC spectrum of CHO-SGP (**20**)

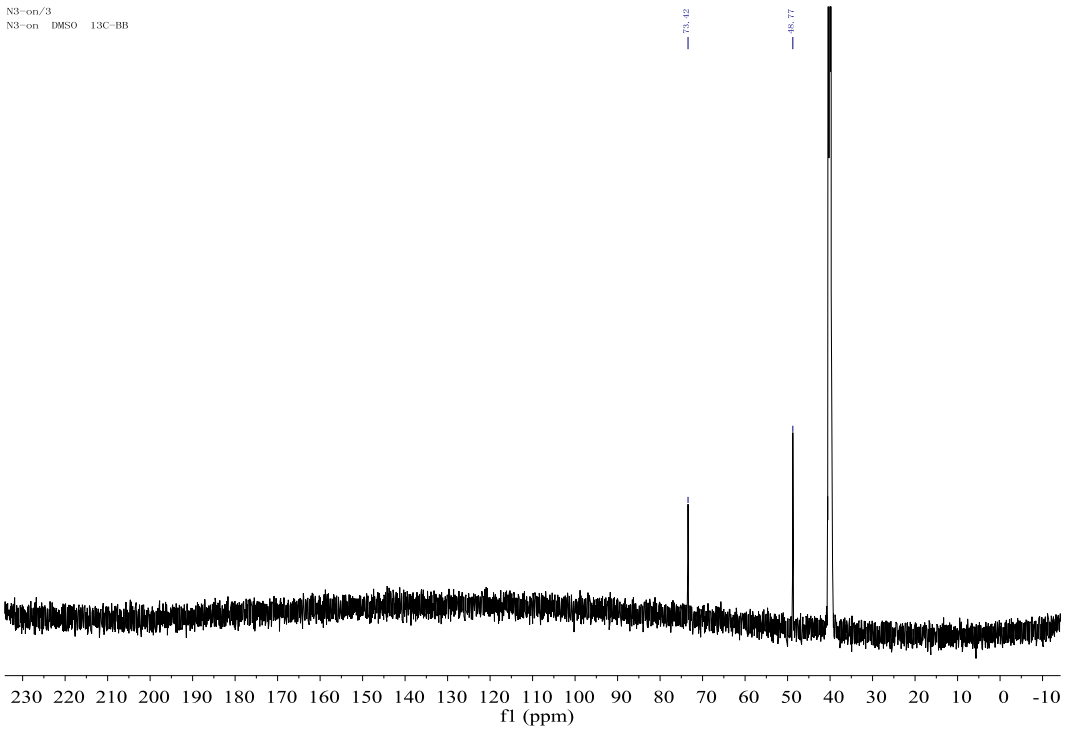
Nature Protocols: doi:10.1038/nprot.2017.058

PRODUCT 21A:

NMR spectra



^{1}H NMR spectrum of **21a**

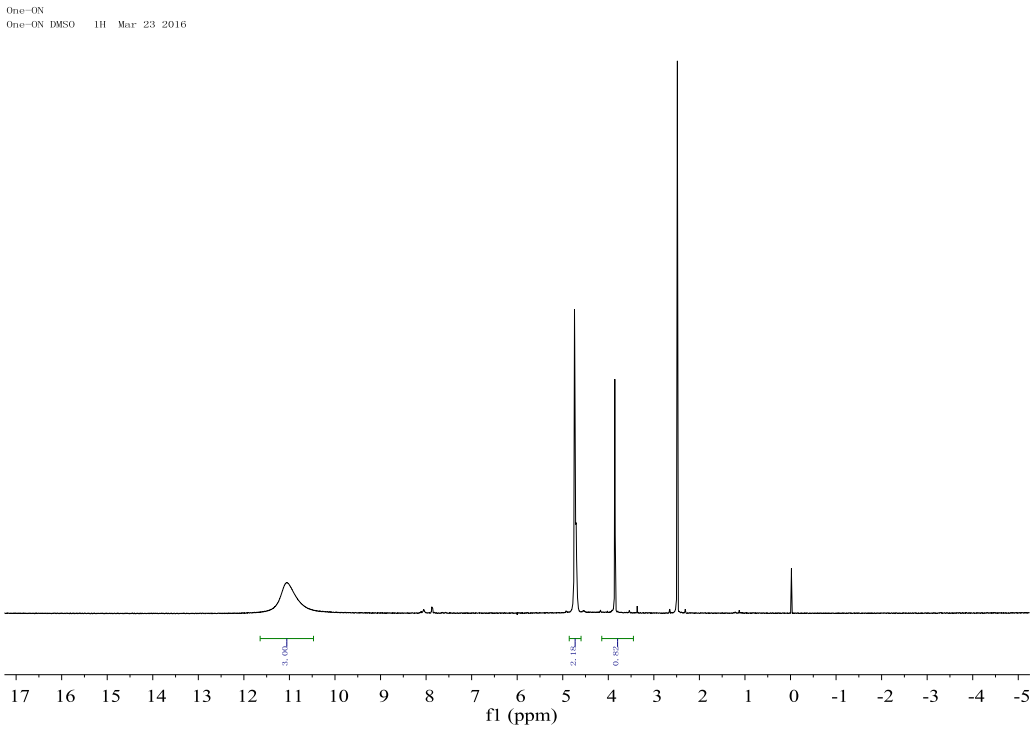


^{13}C NMR spectrum of **21a**

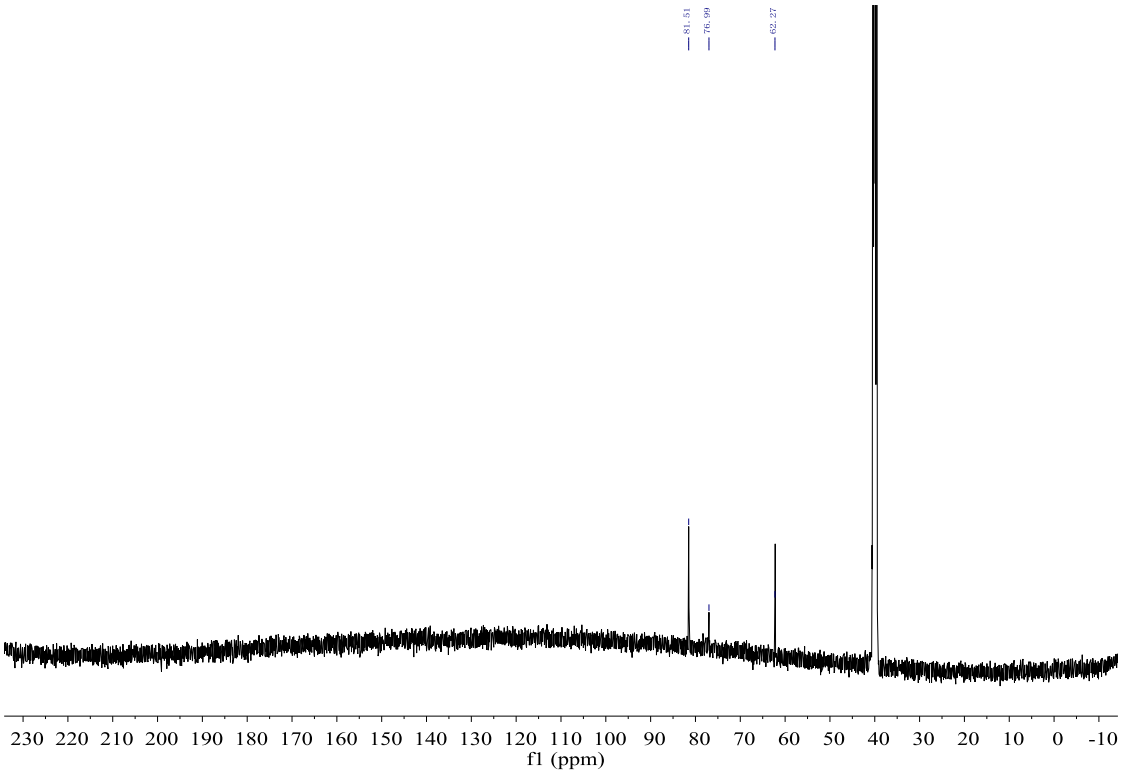
Nature Protocols: doi:10.1038/nprot.2017.058

PRODUCT 21B:

NMR spectra



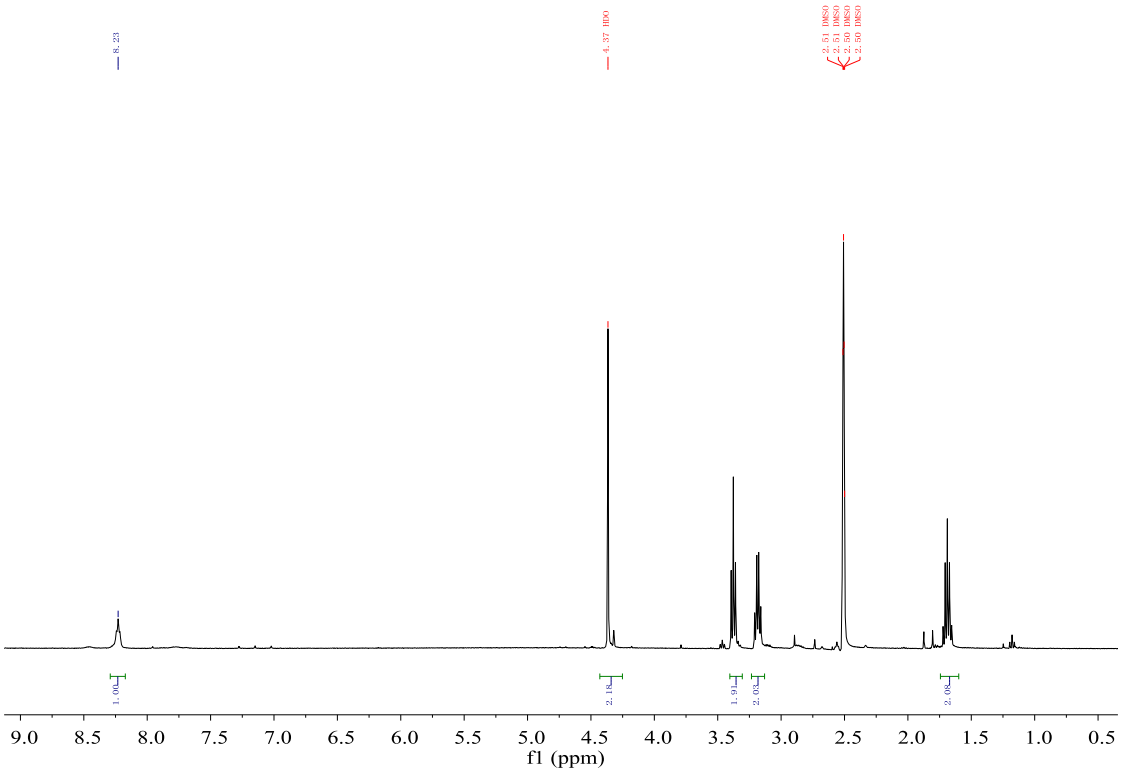
^{1}H NMR spectrum of **21b**



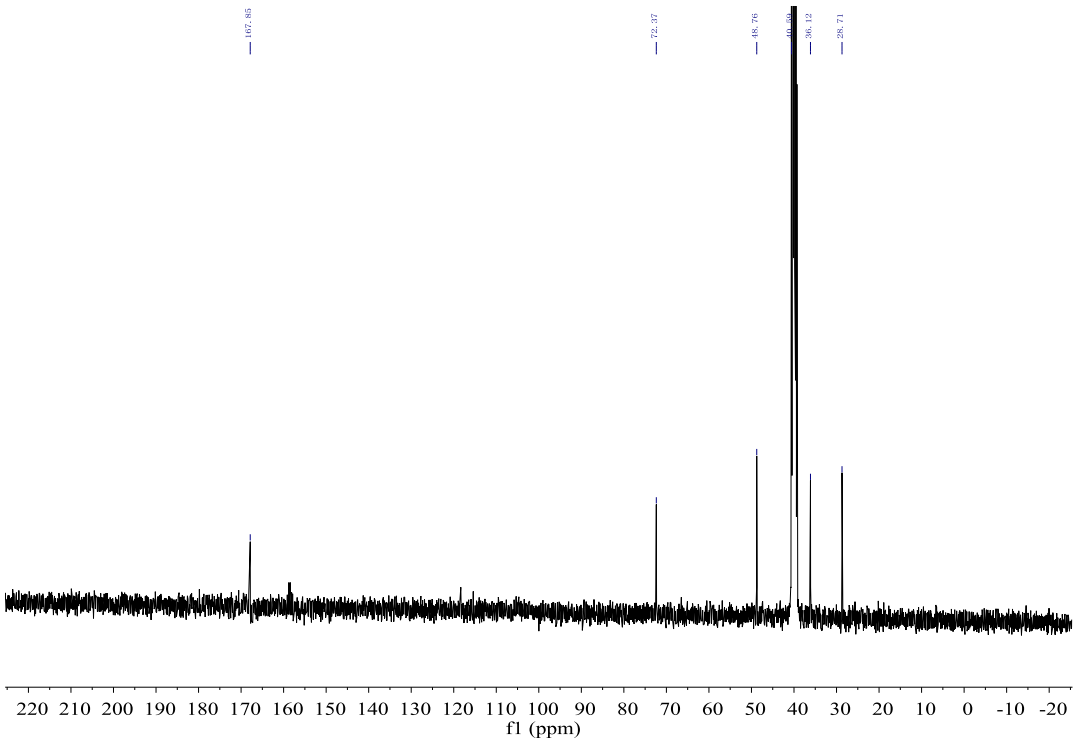
^{13}C NMR spectrum of **21b**

PRODUCT 21C:

NMR spectra



^{1}H NMR spectrum of **21c**

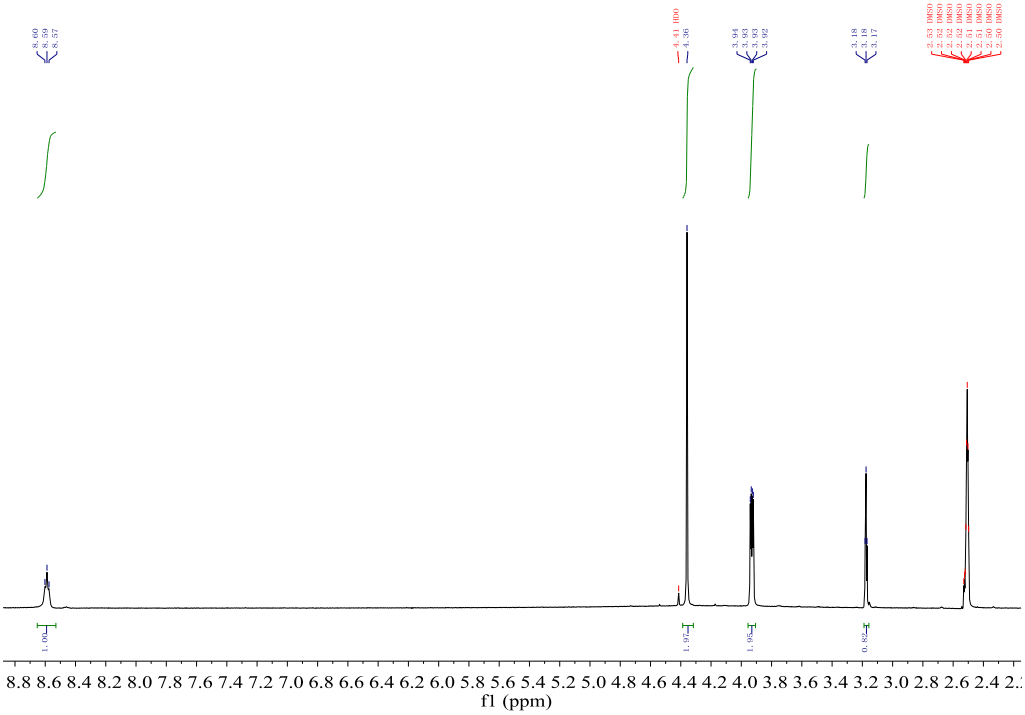


^{13}C NMR spectrum **21c**

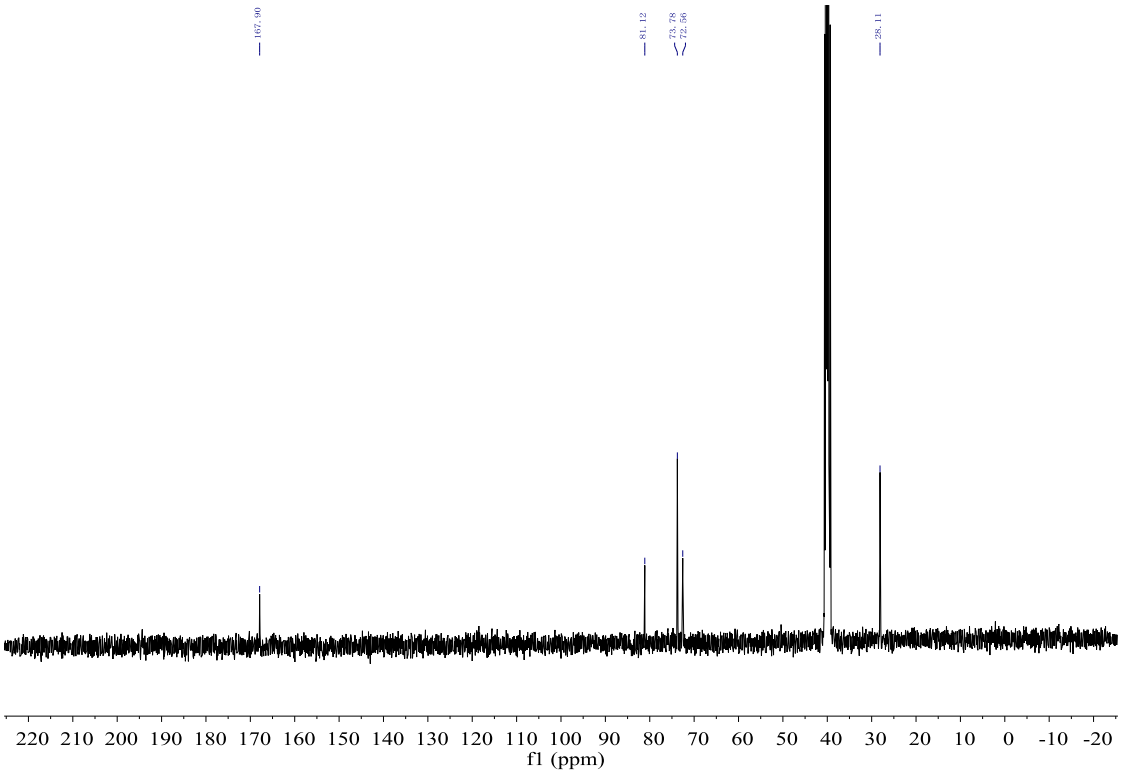
Nature Protocols: doi:10.1038/nprot.2017.058

PRODUCT 21D:

NMR spectra



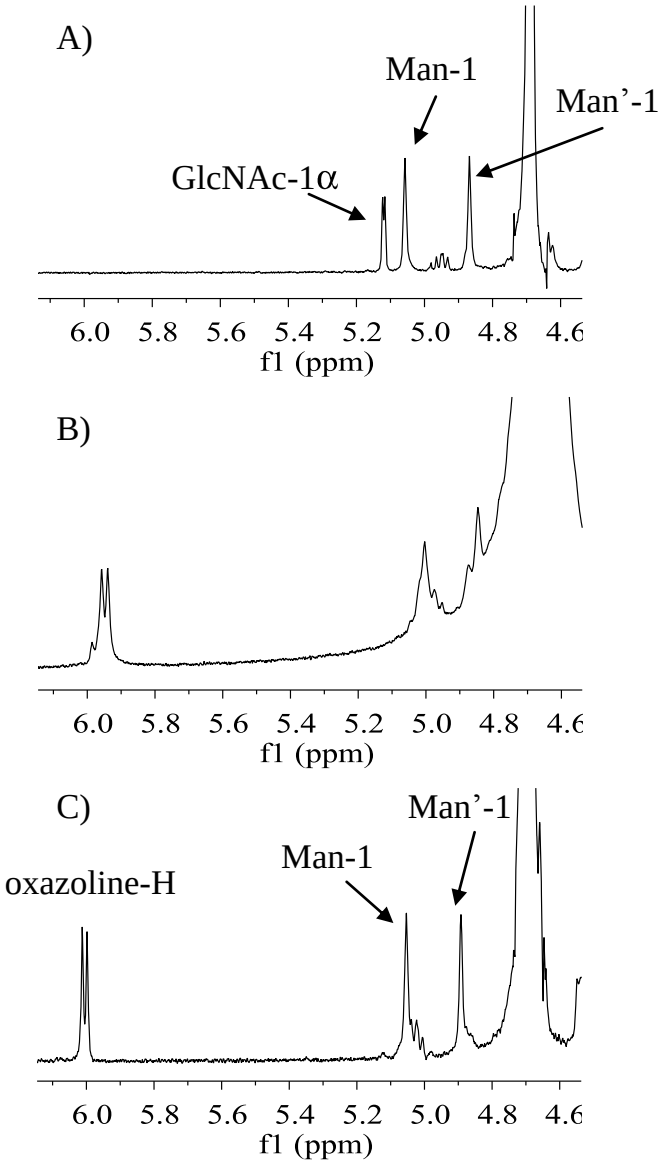
^{1}H NMR spectrum of **21d**



^{13}C NMR spectrum of **21d**

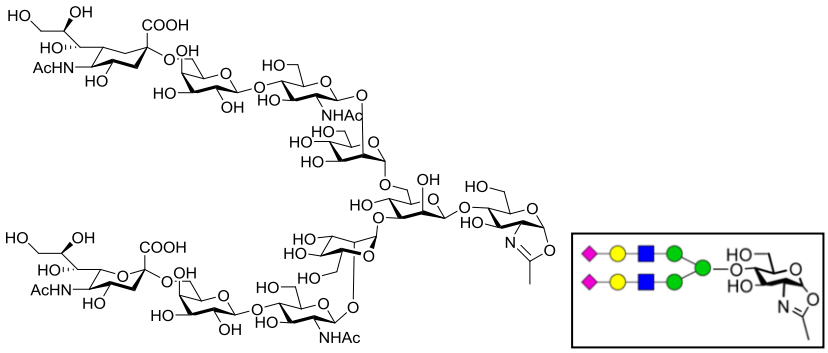
PRODUCT 22:

NMR spectra



Proton NMR monitoring on N-glycan oxazoline formation. A) NMR spectrum of SGP analogue **4a**; B) NMR spectrum of *in situ* oxazoline **22** derived from **4a**; C) NMR spectrum of purified oxazoline **22**.

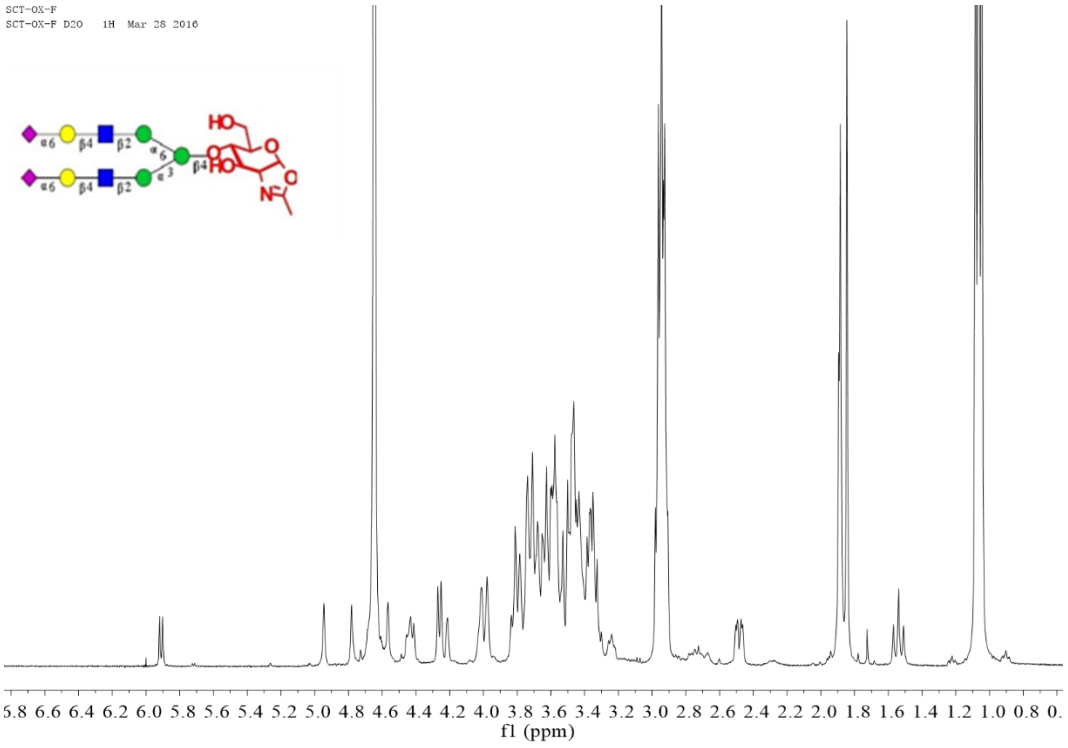
PRODUCT 23:



NMR data

^{1}H NMR (400 MHz, Deuterium Oxide) δ 5.91 (d, J = 7.3 Hz, 1H, H1 of oxa), 4.95 (s, 1H, H1c), 4.78 (s, 1H, H1c'), 4.57 (s, 1H, H1b), 4.49-4.38 (m, 2H, H1d, H1d'), 4.26 (d, J = 8.0 Hz, 2H, H1e, H1e'), 4.21 (s, 1H, H3a), 3.99 (d, J = 13.0 Hz, 4H, H2a, H2b, H2c, H2c'), 2.48 (dd, J = 12.1, 4.0 Hz, 2H, H3f$_{eq}$, H3f'$_{eq}$), 1.98-1.77 (m, 15H), 1.54 (t, J = 12.1 Hz, 2H, H3f$_{ax}$, H3f'$_{ax}$).

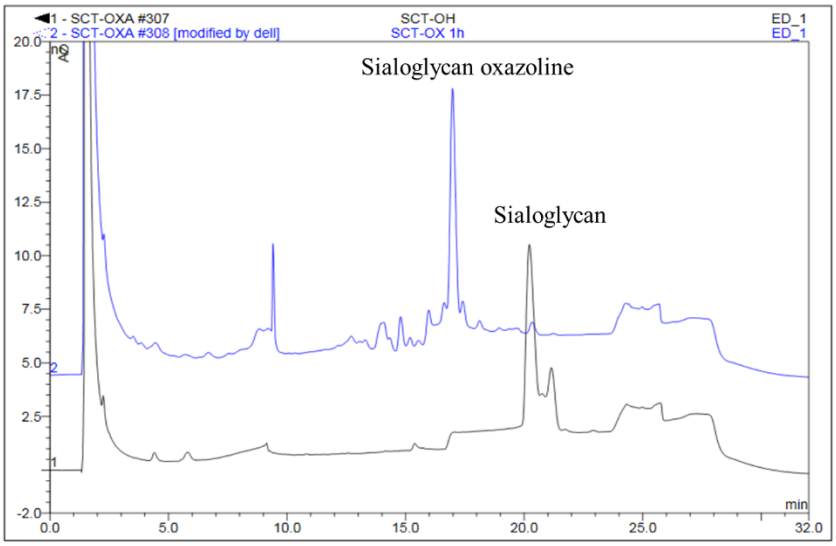
NMR spectrum



^{1}H NMR spectrum of **23**

Nature Protocols: doi:10.1038/nprot.2017.058

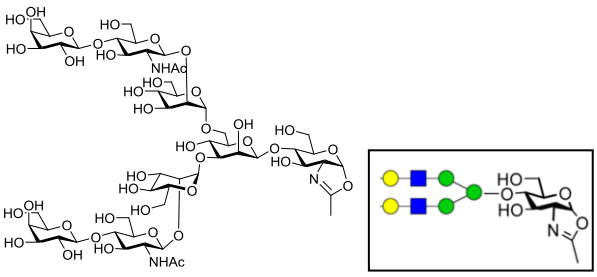
HPAEC profile



HPAEC chromatography of synthesis process of **23**. The black line (down) is the sialylated oligosaccharides released from SGP. The blue line (up) is the reaction buffer after reaction with DMC/Et_3N

Nature Protocols: doi:10.1038/nprot.2017.058

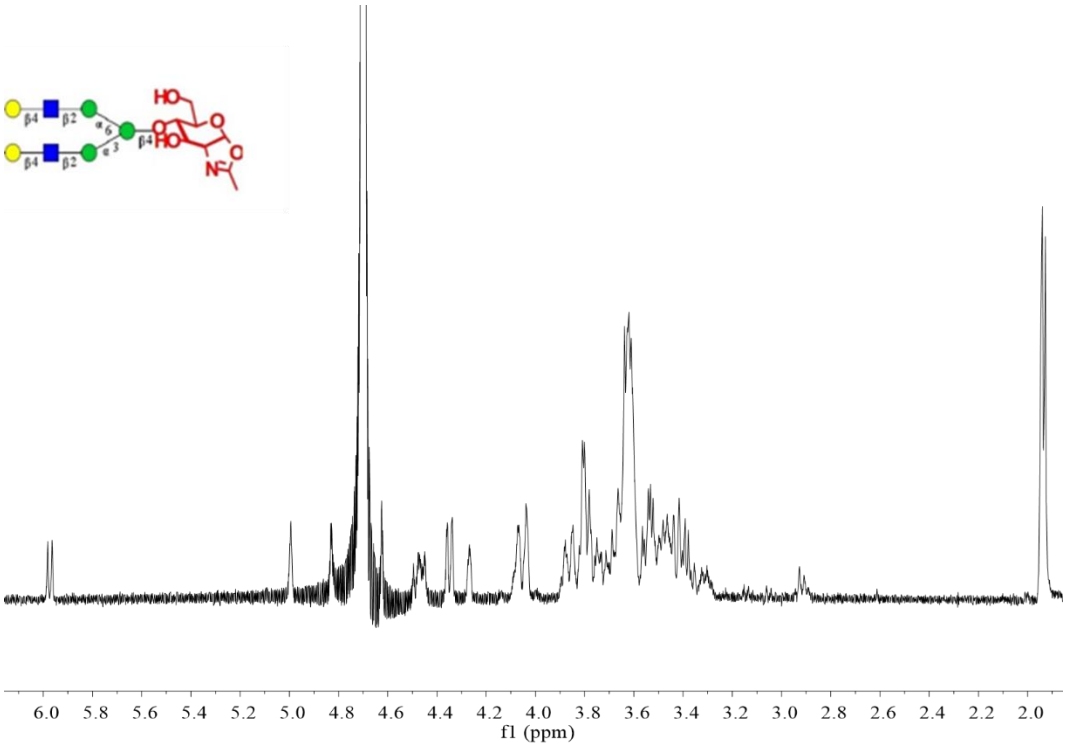
PRODUCT 24:



NMR data

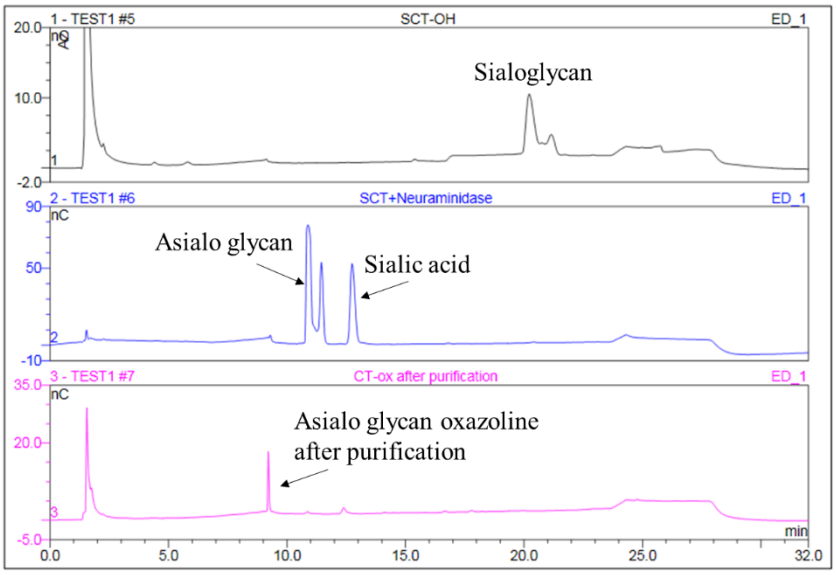
^{1}H NMR (400 MHz, Deuterium Oxide) δ 5.97 (d, J = 7.1 Hz, 1H, H1 of oxa), 4.99 (s, 1H, H1c), 4.83 (s, 1H, H1c'), 4.62 (s, 1H, H1b), 4.47 (m, 2H, H1d, H1d'), 4.35 (d, J = 8.3 Hz, 2H, H1e, H1e'), 4.27 (s, 1H, H3a), 4.05 (m, 4H, H2a, H2b, H2c, H2c'), 1.93 (d, J = 4.9 Hz, 9H, Ac).

NMR spectrum



^{1}H NMR spectrum of **24**

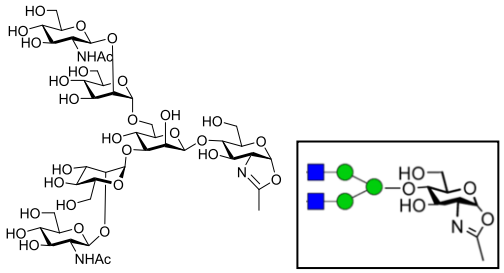
HPAEC profile



HPAEC chromatography of one-pot synthesis of **24**. The first black line is the sialylated oligosaccharides released from SGP. The second blue line is sample removed from the reaction buffer after incubation with neuraminidase. The third pink line is the final product purified with PGC after incubation with DMC/Et_3N.

Nature Protocols: doi:10.1038/nprot.2017.058

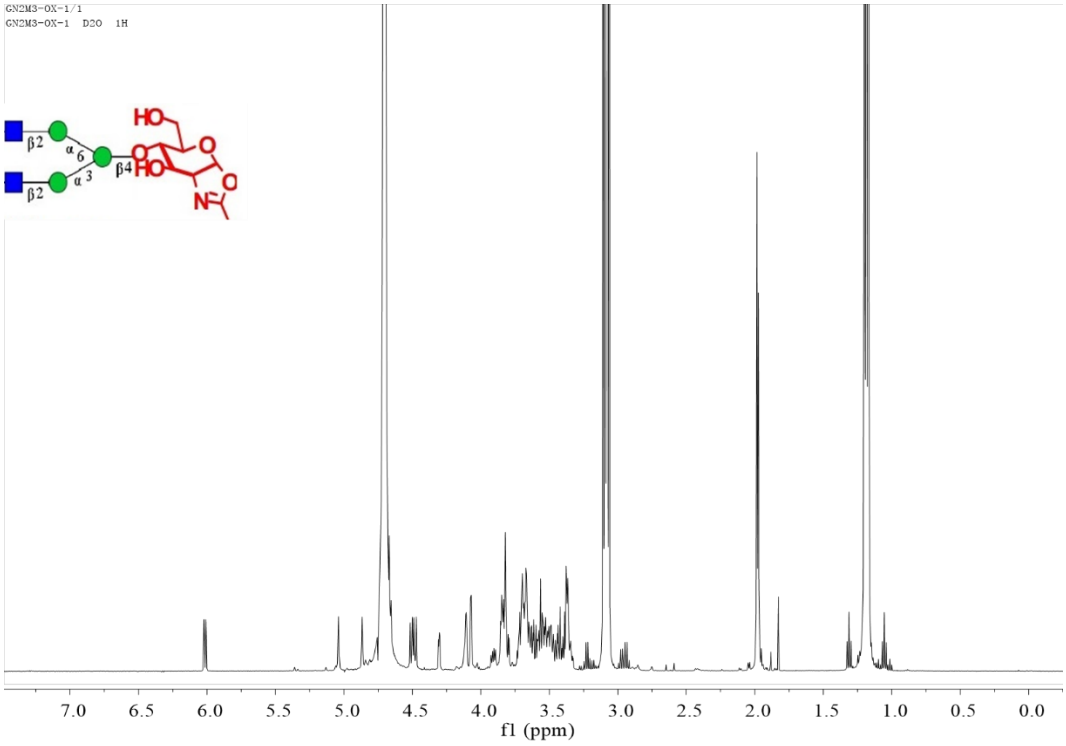
PRODUCT 25:



NMR data

^{1}H NMR (500 MHz, Deuterium Oxide) δ 6.01 (d, J = 7.3 Hz, 1H, H1 of oxa), 5.05 (s, 1H, H1c), 4.87 (s, 1H, H1c'), 4.67 (s, 1H, H1b), 4.49 (dd, J = 14.4, 8.4 Hz, 2H, H1d, H1d'), 4.32 (d, 1H, H3a), 4.16-4.03 (m, 4H, H2a, H2b, H2c, H2c'), 2.02-1.94 (m, 9H, Ac).

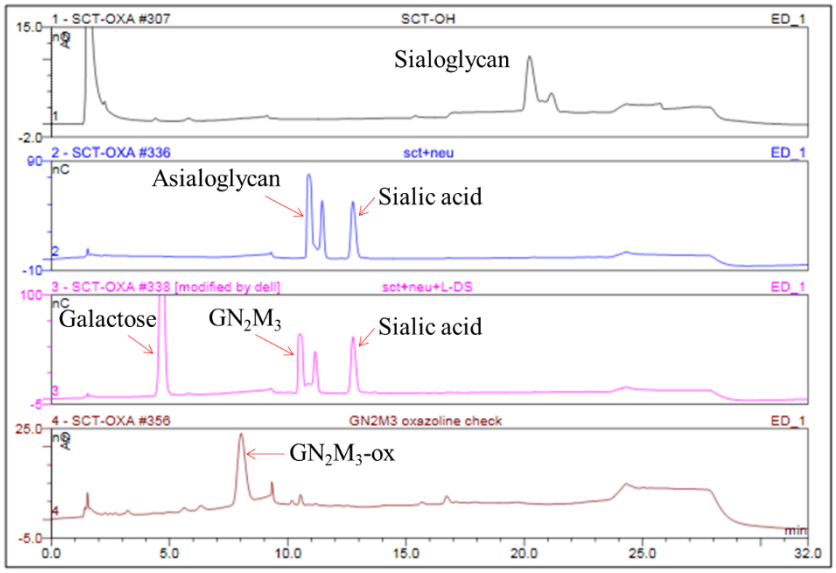
NMR spectrum



^{1}H NMR spectrum of **25**

Nature Protocols: doi:10.1038/nprot.2017.058

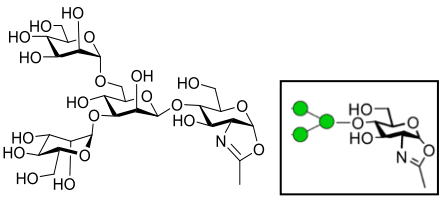
HPAEC profile



HPAEC chromatography of one-pot synthesis of GlcNAc2Man3-oxazoline **25**. The first black line is the sialylated oligosaccharides released from SGP. The second black line is sample removed from the reaction buffer after incubation with neuraminidase. The third pink line is sample removed from the reaction buffer after incubation with additional Lactase DS. The last orange one is the pure GN_2M_3-oxazoline after purification.

Nature Protocols: doi:10.1038/nprot.2017.058

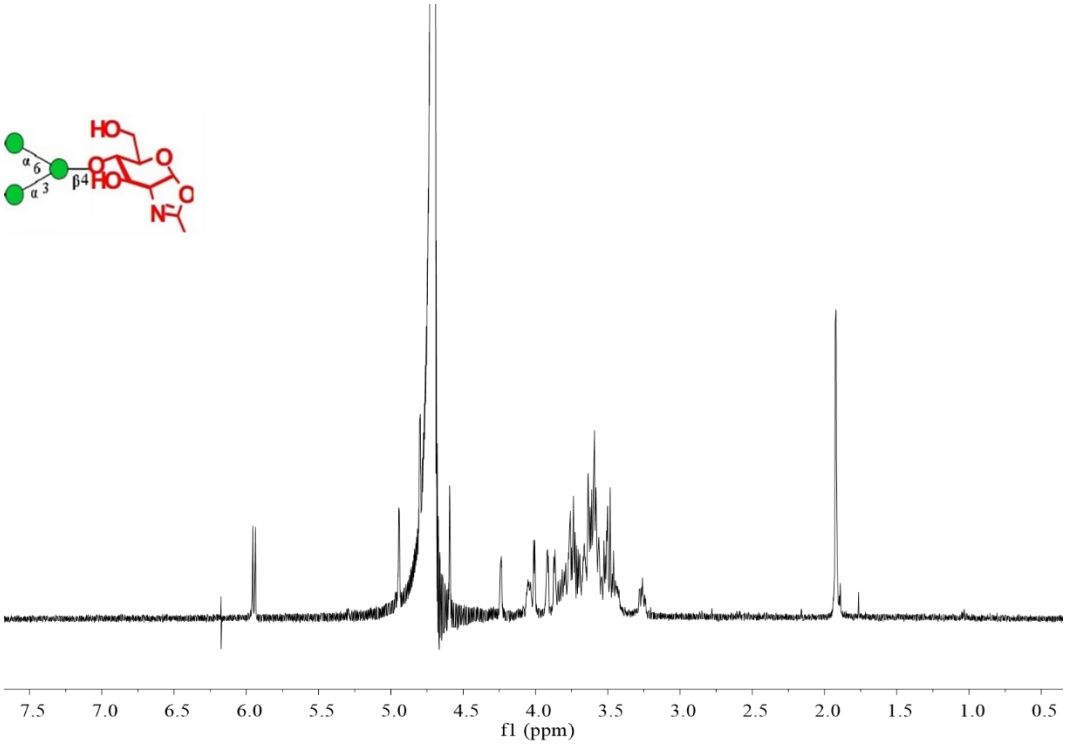
PRODUCT 26:



NMR data

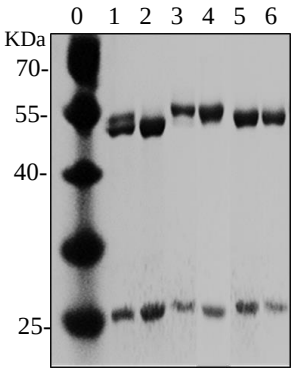
^{1}H NMR (400 MHz, Deuterium Oxide) δ 5.95 (d, J = 7.3 Hz, 1H, H1 of oxa), 4.97 – 4.93 (s, 1H, H1c), 4.80 (s, 1H, H1c'), 4.59 (s, 1H, H1b), 4.24 (s, 1H, H3a), 4.05 (m, 1H, H2a), 4.01 (d, J = 2.7 Hz, 1H, H2b), 3.92 (dd, J = 3.3, 1.7 Hz, 1H, H1c), 3.87 (d, J = 3.4 Hz, 1H, H1c'), 1.92 (d, J = 1.7 Hz, 3H, Ac of oxa).

NMR spectrum



^{1}H NMR spectrum of **26**

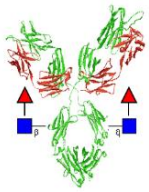
SDS-PAGE



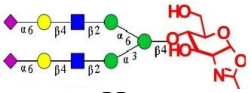
SDS-PAGE of glycoengineered Herceptins. Lane 0: Marker, Lane 1: Mixture of commercial Herceptin and Herceptin-Fucα1,6GlcNAc (**2a**), Lane 2: Herceptin-Fucα1,6GlcNAc (**2a**), Lane 3: S2G2F-Herceptin, Lane 4: G2F-Herceptin, Lane 5: G0F-Herceptin, Lane 6: M3F-Herceptin.

Nature Protocols: doi:10.1038/nprot.2017.058

S2G2F- Herceptin:

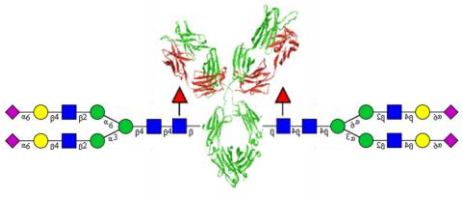


2a



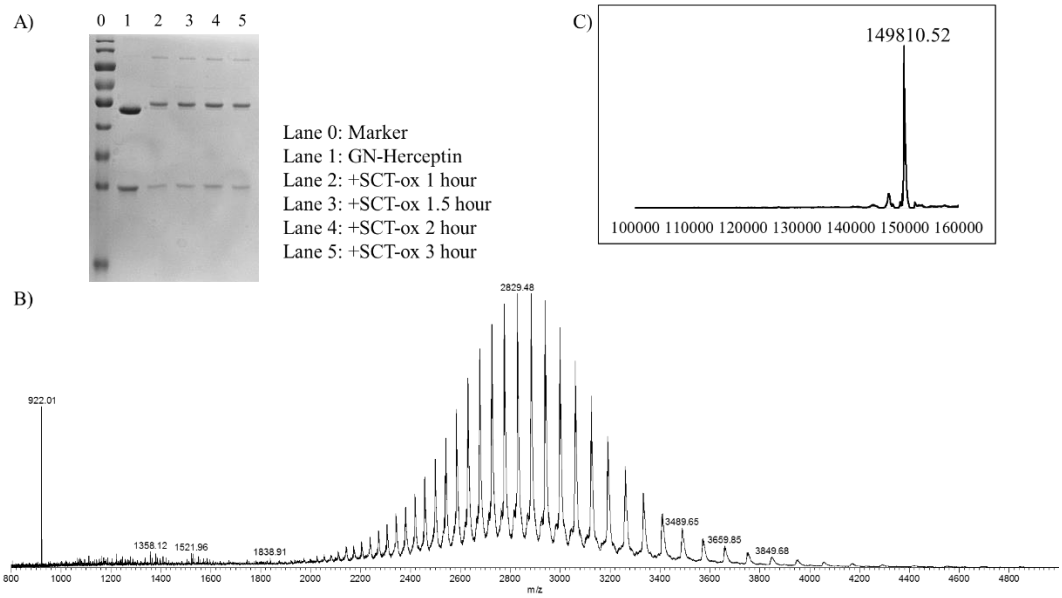
23

Endo-S D233Q



S2G2F-Herceptin

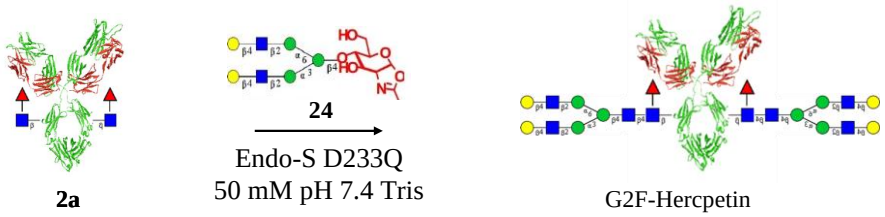
SDS-PAGE and LCMS profile



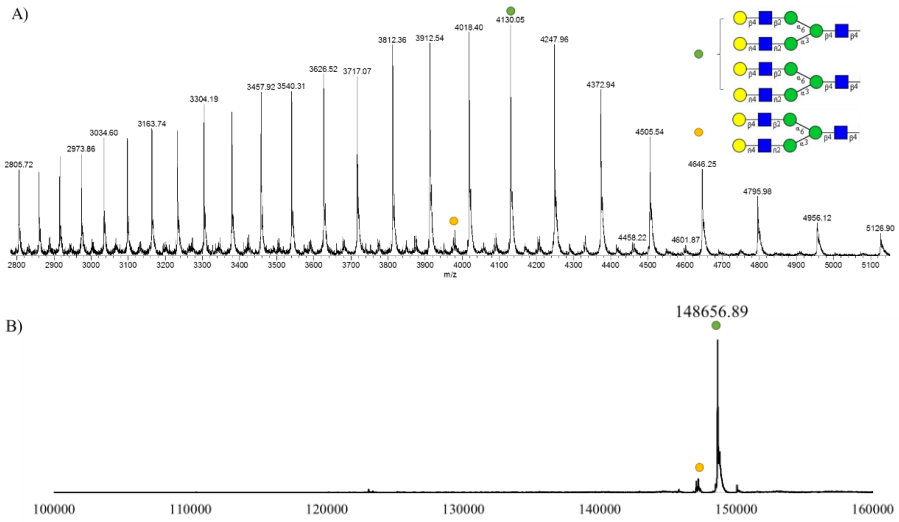
SDS-PAGE and LCMS profile of chemoenzymatic transglycosylation of (Fucα1,6)GlcNAc-Herceptin (**2a**) with oxazoline **23**. A) SDS-PAGE of transglycosylation monitoring. B) Charged mass spectrum (*m/z*) of glycoengineered S2G2F-Herceptin. C) Deconvoluted mass spectrum of S2G2F-Herceptin.

Nature Protocols: doi:10.1038/nprot.2017.058

G2F- Herceptin:



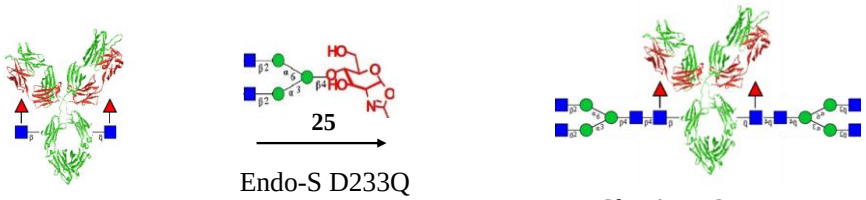
LCMS profile



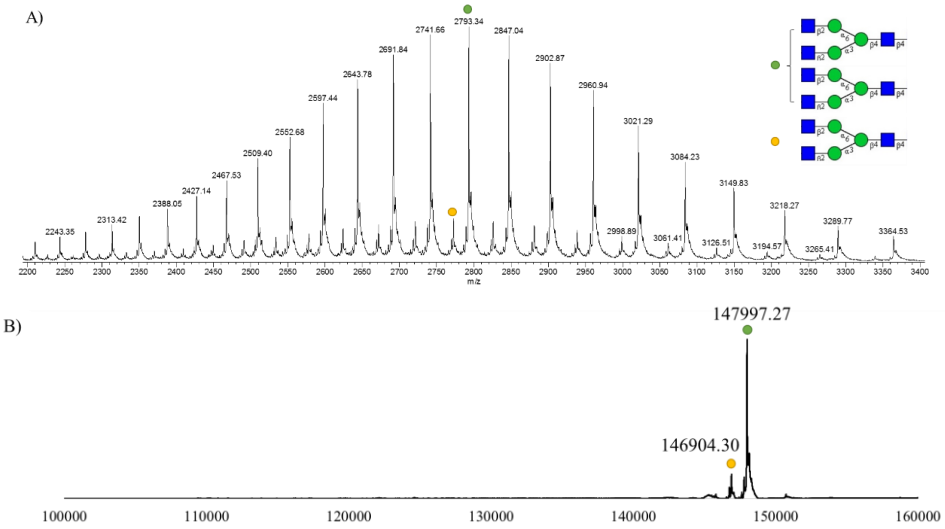
LCMS profile of glycoengineered G2F-Herceptin. A) The charged *m/z* spectrum of G2F-Herceptin, and B) The deconvoluted spectrum of G2F-Herceptin.

Nature Protocols: doi:10.1038/nprot.2017.058

GlcNAc2M3F-Herceptin:



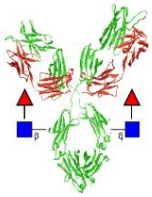
LCMS profile



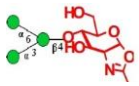
LCMS profile of glycoengineered GlcNAc2M3F-Herceptin. A) The charged *m/z* spectrum and B) deconvoluted spectrum of GlcNAc2M3F-Herceptin.

Nature Protocols: doi:10.1038/nprot.2017.058

M3F-Herceptin

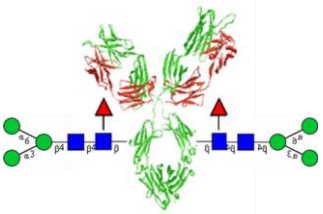


2a



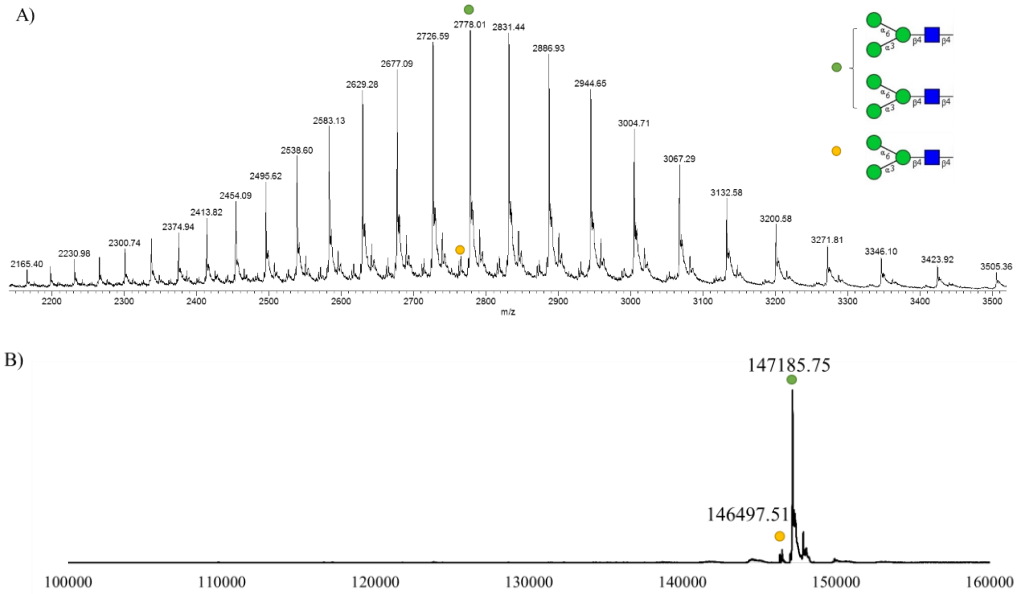
26

Endo-S D233Q
50 mM pH 7.4 Tris



M3F-Herceptin

LCMS profile

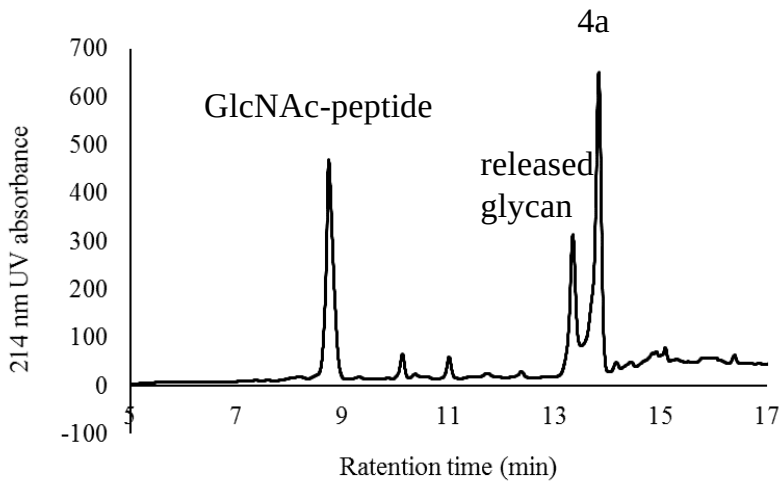


LCMS profile of glycoengineered M3F-Herceptin. A) The charged *m/z* spectrum and B) The deconvoluted spectrum of M3F-Herceptin.

Nature Protocols: doi:10.1038/nprot.2017.058

Azido-SGP hydrolysis:

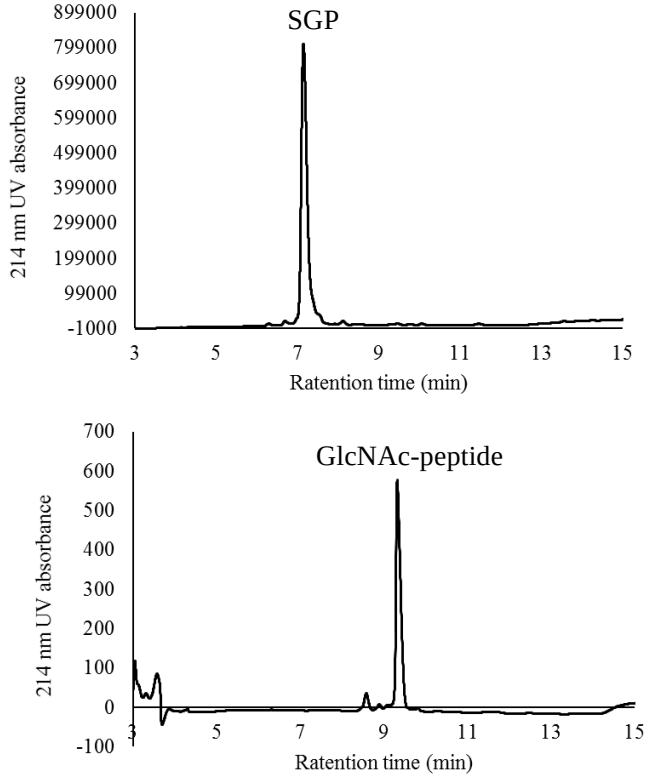
HPLC profile



HPLC monitoring on hydrolysis of azido-SGP **4a** by Endo-M.

SGP hydrolysis:

HPLC profile

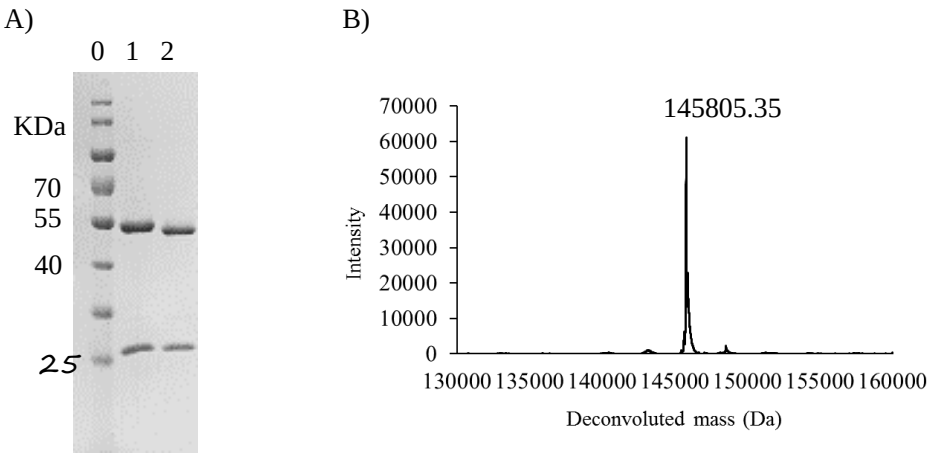


HPLC monitoring on hydrolysis of SGP by Endo-M.

Nature Protocols: doi:10.1038/nprot.2017.058

Herceptin deglycosylation:

SDS page and LCMS analysis



SDS-PAGE (panel A, lane 1: native herceptin; lane 2: deglycosylated herceptin) and LC-MS (panel B) analysis of herceptin deglycosylation by wild-type Endo-S for 2h.

Nature Protocols: doi:10.1038/nprot.2017.058