Supplementary Information

Oligomannose glycopeptide conjugates elicit antibodies targeting the glycan core rather than its extremities

Dung N. Nguyen, Bokai Xu, Robyn L. Stanfield, Jennifer K. Bailey, Satoru Horiya, J. Sebastian Temme, Deborah R. Leon, Celia C. LaBranche, David C. Montefiori, Catherine E. Costello, Ian A. Wilson, and Isaac J. Krauss*

*Corresponding author
Email: kraussi@brandeis.edu

Table of contents

Fig. S1. Structure of linkers used in conjugation ........................................................................................................... 4
Fig. S2. Group 6 (sequential) serum selectivity for binding peptides, glycopeptides, and CRM197-linker .............. 5
Fig. S3. LC-MS of purified peptide 10V1S and glycopeptide 10V1S ........................................................................ 6
Fig. S4. LC-MS of purified peptide 10F12M, 10F12M-cyclohexanol, glyco10F12M-Man1, and glyco10F12M-Manz(1-3) ........................................................................................................................................... 7
Fig. S5. LC-MS of purified glyco10F12M-Manz(1-6), glyco10F12M Man3 branched, glyco10F12M Man3 linear, glyco10F12M Man4 linear .................................................................................................................................................. 8
Fig. S6. LC-MS of purified glyco10F12M-Man5, glyco10F12M-Man9, glyco10F12M-Man9GlcNAc2 ................. 9
Fig. S7. MALDI-TOF MS analysis of CRM-glyco10V1S, BSA-10V1S, and BSA-glyco10V1S conjugates ......... 10
Fig. S8. MALDI-TOF MS analysis of BSA-peptide 10F2,10F5M,10F6,10F8 conjugates ................................................. 11
Fig. S9. MALDI-TOF MS analysis of BSA-glyco10F2, glyco10F5M, glyco10F6, glyco10F8 conjugates ............... 12
Fig. S10. MALDI-TOF MS analysis of BSA-10F12M, BSA-10F12Mcyclohexanol, BSA-10F12M Man1, and BSA-10F12M Man2 (1-3) conjugates .................................................................................................................. 13
Fig. S11. MALDI-TOF MS analysis of BSA-10F12M Man2 (1-6), BSA-10F12M Man3 branched, BSA-10F12M Man3 linear, and BSA-10F12M Man4 linear conjugates ............................................................................................................. 14
Fig. S12. MALDI-TOF MS analysis of BSA-10F12M-Man5, BSA-10F12M-Man9, and BSA-10F12M Man9GlcNAc2 conjugates ....................................................................................................................... 15

Crystal structure determination of 2G12-glycopeptide complexes ................................................................. 16
Fig. S13. Wall-eyed stereo view of glycopeptide 10V1S ................................................................. 17
Fig. S14. Superposition of 10V1S with 2G12+Man3 (6MNF) .......................................................... 18
Fig. S15. Stability of glycopeptide 10F5M in crystallization buffer .................................................. 19
Fig. S16. ELISA of all groups binding to 293F SOSIP trimer at 200 ng/well ........................................ 20
Fig. S17. Binding of 2G12 to glycopeptide 10F12M .......................................................................... 20
Fig. S18. Serum mannosidase trimming graph and chromatograms for N-terminal tryptic peptide of g10F6. 21
Fig. S19. MALDI-TOF MS analysis of CRM197-AcBr ................................................................. 21
Fig. S20. MALDI-TOF MS analysis of alkyne-CRM197-AcBr .......................................................... 22
Fig. S21. MALDI-TOF MS analysis of alkyne-CRM197-Ac-glycopeptide 10F6 ................................. 22
Table S1. 10V1 vs 10V1S sequence and Kds .................................................................................... 23
Table S2. Surface area (Å²) buried on Fab by glycopeptides 10V1S or 10F5M ...................................... 24
Table S3. Surface area (Å²) buried on glycopeptide 10V1S or 10F5M by Fab ...................................... 24
Table S4. Hydrogen bonds from Fab to glycopeptide 10V1S ............................................................ 25
Table S5a. TZMB1 Neutralization assay results of post-dose 4 sera from multi-immunogen study ...... 26
Table S5b. TZMB1 Neutralization assay results of post-dose 6 sera from multi-immunogen study ...... 27
Table S6. P-values for glycan binding comparisons when groups are considered separately ............. 28
Table S7: Crystal structure data collection and refinement statistics ............................................... 29
Table S8: P-values for serum binding to autologous vs. heterologous glycopeptides ......................... 31
Table S9. Effect of mannose on Dose 6 serum binding to BG505SOSIP.664T332N ............................ 32
Table S10. Relative quantification of serum mannosidase trimming of g10F6 conjugate ................. 33
Synthetic procedures for peptide 10F12M, glycopeptide 10F12M- Man1, Man2, Man3, Man4, Man5, Man9, Man5GlcNAc2 and BSA-maleimide ................................................................. 34

General conjugation procedures: CRM-g10V1S, BSA- peptide10V1S, g10V1S, g10F2, g10F5M, g10F6, g10F8, 10F12M- Man1, Man2(1-3), Man2(1-6) ,10F12M-Man3 branched, 10F12M-Man3 linear, 10F12M-Man4, 10F12M-Man5, 10F12M-Man9, 10F12M-Man5GlcNAc2, and cyclohexanol-azide ........................................ 34

Rabbit studies and ELISA procedure ............................................................................................. 36
Synthetic procedure for CRM197-AcBr and alkyne-CRM197-AcBr .............................................. 37
Conjugation procedure for alkyne-CRM197-Ac-glycopeptide 10F6 ............................................. 37
In vitro trimming of alkyne-CRM197-Ac-glycopeptide 10F6 using rabbit serum ......................... 37
Identification and Quantification of Intact and Trimmed Glycoforms of the Tryptic Glycopeptides from g10F6 by Mass Spectrometry (MS) Analysis .................................................................................................................. 38
LC/MS characterization data of peptides and glycopeptides ................................................................................................................................. 39
Glycan synthesis methods and data ........................................................................................................................................................................... 41
NMR spectra of glycans and synthetic intermediates ........................................................................................................................................ 58
Fig. S1 Structure of linkers used in conjugation. a CRM$_{197}$-glycopeptides used in rabbit immunization. b CRM$_{197}$ + linkers used in rabbit control group in multi-immunogen study. c BSA-glycopeptide used for coating plates in ELISA. d BSA+linkers used in ELISA assay. e Groups 2-6 post-dose 4 serum binding to BSA+linkers depicted in d.
Fig. S2 Group 6 (sequential) serum selectivity for binding peptides, glycopeptides, and CRM\textsubscript{197} + linker. Data are presented with geometric mean and geometric standard deviation. Statistical significance was determined by one-way ANOVA followed by multiple comparisons with Tukey’s post-hoc test.
Fig. S3 LC-MS of purified peptide 10V1S and glycopeptide 10V1S.
Fig. S4 LC-MS of purified peptide 10F12M, 10F12M-cyclohexanol, glyco10F12M-Man1, and glyco10F12M-Man3(1-3).
Fig. S5 LC-MS of purified glyco10F12M-Man$_3$(1-6), glyco10F12M Man$_3$ branched, glyco10F12M Man$_3$ linear, glyco10F12M Man$_4$ linear.
Fig. S6 LC-MS of purified glyco10F12M-Man₅, glyco10F12M-Man⁹, glyco10F12M-Man₅GlcNAc₂.
**Fig. S7** MALDI-TOF MS analysis of CRM-glyco10V1S, BSA-10V1S, and BSA-glyco10V1S conjugates. The red numbers indicate the number of glycopeptides per CRM protein molecule. More closely clustered peaks at lower m/z are doubly charged ions.
Fig. S8 MALDI-TOF MS analysis of BSA-peptide 10F2, 10F5M, 10F6, 10F8 conjugates. The red numbers indicate the number of glycopeptides per CRM protein molecule.
**Fig. S9** MALDI-TOF MS analysis of BSA-glyco10F2, glyco10F5M, glyco10F6, glyco10F8 conjugates. The red numbers indicate the number of glycopeptides per CRM protein molecule. More closely clustered peaks at lower m/z are doubly charged ions.
Fig. S10 MALDI-TOF MS analysis of BSA-10F12M, BSA-10F12M-cyclohexanol, BSA-10F12M Man$_1$, and BSA-10F12M Man$_2$ (1-3) conjugates. The red numbers indicate the number of glycopeptides per CRM protein molecule.
Fig. S11 MALDI-TOF MS analysis of BSA-10F12M Man$_2$ (1-6), BSA-10F12M Man$_3$ branched, BSA-10F12M Man$_3$ linear, and BSA-10F12M Man$_4$ linear conjugates. The red numbers indicate the number of glycopeptides per CRM protein molecule. More closely clustered peaks at lower m/z are doubly charged ions.
Fig. S12 MALDI-TOF MS analysis of BSA-10F12M-Man$_5$, BSA-10F12M-Man$_9$, and BSA-10F12M Man$_5$GlcNAc$_2$ conjugates. The red numbers indicate the number of glycopeptides per CRM protein molecule. More closely clustered peaks at lower m/z are doubly charged ions.
Crystal structure determination for 2G12-glycopeptide complexes

IgG 2G12 was expressed in 293 Freestyle cells and purified by protein A chromatography. IgG was cleaved to Fab with 2% papain for 3 hours before inactivation with 200 mM iodoacetamide. The cleavage mixture was applied to a protein A column and the unbound Fab was further purified on a S200 16/60 column (GE).

Fab was mixed with peptide at a 1:3 (Fab:peptide) molar ratio and the complex was purified by size exclusion chromatography with a Superdex 200 16/60 column. Each Fab-glycopeptide complex was concentrated to 10 mg/ml.

The 2G12-10V1S peptide complex was crystallized in 24-well sitting drop trays (Hampton Research) in condition 6B of Footprint 1 screen, which corresponded to 1.0 M sodium citrate tribasic dihydrate, 10mM sodium borate, pH 8.5. Crystals were cryoprotected with well solution augmented with glycerol to a final concentration of 30%.

2G12-10F5M peptide complex was crystallized in 24-well sitting drop trays with a well solution of 0.2M LiSO₄, 17.5% PEG400, 0.1M Tris, pH 8.5. Crystals were cryoprotected with the well solution plus PEG400 at a final concentration of 27.5%. All crystals were cryocooled by rapid plunging into liquid nitrogen and data were collected at SSRL beamline 12-2 using a Dectris Pilatus 6M detector. Data were processed and scaled with HKL-2000 and molecular replacement was carried out with Phaser using 2G12 Fab coordinates from PDB 4RBP as a model. Refinement was carried out with Phenix.refine and final statistics for data collection and refinement are outlined in Table S7.

For both complexes, the Fab chain identifiers are L and K (light chains) and H and M (heavy chains). The 10V1S glycopeptide has chain identifiers A and C. Glycans in 10F5M are labeled A and B. In Fab1 (LH), the V₇ domain is paired with the V₉ domain, while in Fab2 (KM), the V₇' domain is paired with the V₉ domain (see Fig. 3).

The 2G12-10V1S complex crystals have one domain-swapped Fab dimer and two glycopeptides in the asymmetric unit, with one peptide bound to each Fab within the dimer (Figs. 3 and S13). Glycopeptide A is better ordered and has lower B values than C (38 Å² for A versus 57 Å² for C), likely due to crystal contacts from a symmetry-related Fab. Thus, glycopeptide A is used for most of the analysis and discussion. Out of the 40-residue peptide, residues 18-33 and 19-33 are visible in peptides A and C. The peptide forms a hairpin, with a type VIII non-hydrogen bonded reverse turn around residues 23-26 (IPWY). Pro24 adopts a cis conformation. Residue 20 is homopropargylglycine (HPG) to which a Man₉ glycans is attached. All 9 mannose moieties are visible in the electron density and adopt a gg-gg (3-4' and 4'-B both adopt a gauche rotamer) rotameric arrangement. The primary glycan binding site in each Fab binds to the terminal Manα1-2 Man moieties from the D3 arm (mannose B and D3) (Fig. 3a). The Fab-glycopeptide interface is extensive with 854Å² and 884Å² buried on the glycopeptide and Fab, respectively (Tables S2 and S3). About 60% of the glycopeptide contribution is from peptide. The Fab contacts glycopeptide with CDR’s L3, H1’, H2’, and H3’, with some contributions from heavy-chain framework residues (Table S2); the largest contribution comes from CDR H2’. There are 10 hydrogen bonds from the glycopeptide to the Fab, with 6 from the peptide component and 4 from the carbohydrate (Table S4).
Fig. S13 Wall-eyed stereo view of glycopeptide 10V1S. Residues 18-33 of the 40-residue peptide are visible in the crystal structure.
Fig. S14. Superposition of 10V1S with 2G12+Man₈ (6MNF). The 10V1S heavy chain and carbohydrate are colored pink and yellow, while the 2G12/Man₈ heavy chain and carbohydrate are both colored white. In both the 10V1S structure and 6MNF, there are 6 hydrogen bonds involving ThrH33, ThrH52a, SerH53 and AspH100d to the D3 or 4' mannose moieties.
**Fig. S15** Stability of glycopeptide 10F5M in crystallization buffer. UPLC chromatograms of glycopeptide 10F5M are shown a) prior to and b) after incubation in crystal growth medium. The conditions for growth of 10F5M-2G12 cocrystals were room temperature for 2 days in 0.2M Li$_2$SO$_4$, 17.5% PEG400, 0.1M Tris, pH 8.5. To test stability, glycopeptide 10F5M was dissolved in the same mixture for a week at room temperature, then exchanged back to water by Amicon filtration and analyzed by UPLC/ESI/MS.
**Fig. S16** ELISA of all groups binding to 293F SOSIP trimer at 200 ng/well.

**Fig. S17** Binding of 2G12 to glycopeptide 10F12M. Binding was measured by a bead-based radioactive binding assay. Glycopeptide 10F12M was produced by *in vitro* translation of the peptide followed by click glycan attachment, and contained a C-terminal LGHis-FLAG sequence instead of the shorter GCA sequence of synthetic 10F12M shown in Fig. 8. The sequence of 10F12M, including tags for this assay, is XSYVTVIPAXNXPEARLGIYVSHXPGRGKALYGYSGSLGHHHHHRDYKDDEDK (X = homopropargylglycine).
Fig. S18. Glycan analysis showing high mannose glycoform distribution for alkyne-CRM$_{197}$-Ac-glycopeptide 10F6 collected at different time points. a) nanoUPLC/MS Total Ion Chromatogram of tryptic digest of conjugate, with colored superimposed Extracted Ion Chromatograms for two 10F6 tryptic glycopeptides. b) Closeup of EICs for all glycoforms of N-terminal glycopeptide (fXLXFIR) at t = 0 and 17 hr exposure to serum. c) Graph of glycoform relative abundance vs. time. d) Representative data (time point 17h) showing Extracted Ion Chromatograms corresponding to distinct glycoforms of the glycopeptide in b and c.

Figure S19. MALDI-TOF MS analysis of CRM$_{197}$-AcBr.
**Figure S20.** MALDI-TOF MS analysis of alkyne-CRM$_{197}$-AcBr.

**Figure S21.** MALDI-TOF MS analysis of alkyne-CRM$_{197}$-Ac-glycopeptide 10F6. The red numbers indicate the number of glycopeptides per CRM protein molecule. More closely clustered peaks at lower m/z are doubly charged ions.
Table S1. a 10V1 vs 10V1S sequences and $K_D$. $X$ = Man$_9$-Cy- “click” glycosylated homopropargylglycine residue, as depicted in Fig. 1. b BioLayer Interferometry (BLI) measurement of 2G12 interacting with surface-immobilized synthetic 10V1S glycopeptide. The $K_D$ for 10V1 was published previously $^6$. Uncertainties reported are standard errors of measurement.
**Table S2.** Surface area (Å$^2$) buried on Fab by glycopeptides 10V1S or 10F5M. Contacts made to the neighboring Fab within the domain-swapped dimer are indicated.

|       | 10V1S | 10F5M |
|-------|-------|-------|
|       | Glycopeptide | Glycopeptide | Glycan A | Glycan |
| L1    | 0     | 0     | 14       | 2       |
| L2    | 0     | 0     | 0        | 0       |
| L3    | 93    | 84    | 74       | 50      |
| H1    | 45    | 44    | 51       | 48      |
| H2    | 288   | 303   | 76       | 109     |
| H3    | 141   | 140   | 191      | 130     |
| H framework | 138 | 147 | 0 | 0 |
| H1 (from neighboring Fab) | 38 | 41 | 0 | 0 |
| H framework (from neighboring Fab) | 111 | 120 | 0 | 0 |
| **Total** | **854** | **879** | **405** | **339** |

**Table S3.** Surface area (Å$^2$) buried on glycopeptide 10V1S or 10F5M by Fab.

|       | 10V1S | 10F5M |
|-------|-------|-------|
|       | Fab1  | Fab2  | Fab1  | Fab2  |
| Peptide |        |       |       |       |
| Man3   | 28    | 38    | 14    | 1     |
| Man4   | 0     | 0     | 3     | 53    |
| ManC   | 27    | 0     | 72    | 71    |
| ManD1  | 0     | 0     | 133   | 131   |
| Man4   | 58    | 49    | 21    | 18    |
| ManA   | 28    | 26    | 8     | 1     |
| ManD2  | 0     | 0     | 49    | 15    |
| ManB   | 72    | 73    | 9     | 5     |
| ManD3  | 143   | 143   | 0     | 0     |
| Total carbohydrate | 356 | 329 | 359 | 317 |
| Total peptide+carbohydrate | 884 | 859 | N/A | N/A |
Table S4. Hydrogen bonds from Fab to glycopeptide 10V1S.

| Peptide residue | atom | Fab residue | atom | Distance (Å) |
|-----------------|------|-------------|------|--------------|
| TyrA26          | OH   | ArgH57      | O    | 2.46         |
| SerA22          | OG   | TyrH59      | OH   | 2.78         |
| SerA22          | OG   | GluH(M)75   | OE2  | 2.52         |
| TyrA28          | OH   | GluH(M)75   | OE2  | 2.70         |
| ArgA21          | NH1  | AspH(M)76   | OD2  | 3.10         |
| ArgA21          | NH2  | AspH(M)76   | OD2  | 3.15         |

| Carbohydrate    | atom | Fab residue | atom | Distance (Å) |
|-----------------|------|-------------|------|--------------|
| ManA D3         | O2   | ThrH33      | N    | 2.74         |
| ManA D3         | O5   | ThrH33      | OG1  | 2.92         |
| ManA D3         | O6   | ThrH33      | OG1  | 2.92         |
| ManA 4’         | O2   | ThrH52a     | OG1  | 2.64         |
| ManA 4’         | O2   | SerH53      | OG   | 3.02         |
| ManA D3         | O4   | AspH100d    | N    | 3.01         |
| Animal ID | Groups | ID#8075 | ID#7847 | ID#9212 | ID# |
|-----------|--------|---------|---------|---------|-----|
| 19251     | 1      | <20     | <20     | <20     | <20 |
| 19252     | 1      | <20     | <20     | <20     | <20 |
| 19253     | 1      | <20     | <20     | <20     | <20 |
| 19254     | 1      | <20     | <20     | <20     | <20 |
| 19255     | 1      | <20     | <20     | <20     | <20 |
| 19251     | 1      | <20     | <20     | <20     | <20 |
| 19263     | 3      | <20     | <20     | <20     | <20 |
| 19264     | 3      | <20     | <20     | <20     | <20 |
| 19265     | 3      | <20     | <20     | <20     | <20 |
| 19266     | 3      | <20     | 23      | <20     | <20 |
| 19267     | 3      | <20     | <20     | <20     | <20 |
| 19268     | 3      | <20     | <20     | <20     | <20 |
| 19269     | 4      | <20     | <20     | <20     | <20 |
| 19270     | 4      | <20     | 24      | <20     | <20 |
| 19271     | 4      | <20     | <20     | <20     | <20 |
| 19272     | 4      | <20     | <20     | <20     | <20 |
| 19273     | 4      | <20     | <20     | <20     | <20 |
| CH01-31   | >25    | 3.00    | <0.01   | <0.01   | <0.01 |

1Values are the serum dilution at which relative luminescence units (RLUs) were reduced 50% compared to virus control wells (no test sample).

Note: Values in bold are considered positive for neutralizing antibody activity in the post-immune sample based on the criterion of >3X the observed background against the SVA-MLV negative control pseudovirus.
Table S5b. TZMbl Neutralization assay results of post-dose 6 sera from multi-immunogen study.

| Animal ID | Group | ID#6565 | ID#7847 | ID#7012 | ID#730 | ID#7190 | ID#7204 |
|-----------|-------|---------|---------|---------|---------|---------|---------|
| 19251     | 1     | <20     | 994     | <20     | <20     | <20     | 69      |
| 19252     | 1     | <20     | 151     | <20     | <20     | <20     | 40      |
| 19253     | 1     | <20     | 1172    | 30      | <20     | <20     | 152     |
| 19254     | 1     | <20     | 1166    | <20     | <20     | <20     | 768     |
| 19255     | 1     | <20     | 1475    | <20     | <20     | <20     | 528     |
| 19256     | 1     | <20     | 45      | <20     | <20     | <20     | 239     |
| 19257     | 2     | <20     | 524     | 200     | <20     | <20     | 367     |
| 19259     | 2     | <20     | 870     | 203     | 25      | <20     | 206     |
| 19260     | 2     | <20     | 255     | 34      | <20     | <20     | 349     |
| 19261     | 2     | <20     | 976     | <20     | <20     | <20     | 905     |
| 19262     | 2     | <20     | 1089    | <20     | <20     | <20     | 662     |
| 19263     | 3     | <20     | 2004    | <20     | <20     | <20     | 48      |
| 19264     | 3     | <20     | 640     | <20     | <20     | <20     | 356     |
| 19265     | 3     | <20     | 959     | <20     | <20     | <20     | 406     |
| 19266     | 3     | <20     | 774     | <20     | <20     | <20     | 424     |
| 19268     | 3     | <20     | 619     | <20     | <20     | <20     | 265     |
| 19269     | 4     | <20     | 1239    | 49      | <20     | <20     | 162     |
| 19270     | 4     | <20     | 394     | <20     | <20     | <20     | 131     |
| 19271     | 4     | <20     | 304     | <20     | <20     | <20     | 386     |
| 19273     | 4     | <20     | 599     | <20     | <20     | <20     | 351     |
| 19274     | 4     | <20     | 306     | <20     | <20     | <20     | 60      |
| 19275     | 5     | <20     | 581     | <20     | <20     | <20     | 290     |
| 19277     | 5     | <20     | 1105    | <20     | <20     | <20     | 280     |
| 19278     | 5     | <20     | <20     | <20     | <20     | <20     | 437     |
| 19279     | 5     | <20     | 244     | <20     | <20     | <20     | 591     |
| 19280     | 5     | <20     | 53      | <20     | <20     | <20     | 22      |
| 19281     | 6     | <20     | 689     | <20     | <20     | <20     | 155     |
| 19283     | 6     | <20     | 84      | <20     | <20     | <20     | 257     |
| 19284     | 6     | <20     | 292     | <20     | <20     | <20     | 509     |
| 19285     | 6     | <20     | 44      | <20     | <20     | <20     | 700     |
| 19286     | 6     | <20     | 1097    | <20     | <20     | <20     | 286     |
| CH01-31   |       | 2.14    | 0.03    | 0.02    | <25     | <0.01   |

ID<sub>50</sub> in TZM-bl Cells<sup>1</sup>

| SVA-MLV | MW965.26 | BG505ΔCT /T332N | JR-FL | SVA-MLV/GnTI- | JR-FL/GnTI- |
|---------|----------|-----------------|-------|---------------|-------------|
| Neg Ctrl | Clade C  | Tier 1A         | Clade A | Tier 2        | Clade B     |
| Tier 2   |          | Tier 2          |        |               | Tier 2      |

1<sup>Values are the serum dilution at which relative luminescence units (RLUs) were reduced 50% compared to virus control wells (no test sample).
Note: Values in bold are considered positive for neutralizing antibody activity in the post-immune sample based on the criterion of >3X the observed background against the SVA-MLV negative control pseudovirus.\**
| Glycans compared                      | Mean diff. (log IgG titer) | Adjusted p-value |
|---------------------------------------|----------------------------|------------------|
| Groups 2-5 (aggregate)                |                            |                  |
| Cy10F12M vs. Man1Cy10F12M             | -.32                       | .011             |
| Cy10F12M vs. Man2Cy10F12M             | -1.07                      | <.0001           |
| Man1Cy10F12M vs. Man2Cy10F12M        | -.75                       | <.0001           |
| Group 2 (10F5M)                       |                            |                  |
| Cy10F12M vs. Man1Cy10F12M             | .054                       | .973             |
| Cy10F12M vs. Man2Cy10F12M             | -0.79                      | .141             |
| Man1 Cy10F12M vs. Man2Cy10F12M       | -0.85                      | .0184            |
| Group 3 (10F2)                        |                            |                  |
| Cy10F12M vs. Man1Cy10F12M             | -.26                       | .293             |
| Cy10F12M vs. Man2Cy10F12M             | -.96                       | .0014            |
| Man1 Cy10F12M vs. Man2Cy10F12M       | -.70                       | .0145            |
| Group 4 (10F6)                        |                            |                  |
| Cy10F12M vs. Man1Cy10F12M             | -.80                       | .141             |
| Cy10F12M vs. Man2Cy10F12M             | -2.1                       | .0072            |
| Man1 Cy10F12M vs. Man2Cy10F12M       | -1.3                       | .0564            |
| Group 5 (10F8)                        |                            |                  |
| Cy10F12M vs. Man1Cy10F12M             | -.26                       | .0926            |
| Cy10F12M vs. Man2Cy10F12M             | -.57                       | .0322            |
| Man1 Cy10F12M vs. Man2Cy10F12M       | -.31                       | .234             |
| Group 6 (sequential)                  |                            |                  |
| Cy10F12M vs. Man1Cy10F12M             | -.33                       | .214             |
| Cy10F12M vs. Man2Cy10F12M             | -.94                       | .235             |
| Man1Cy10F12M vs. Man2Cy10F12M        | -.61                       | .288             |

**Table S6.** P-values for comparison of serum binding to BSA-10F12M antigens bearing cyclohexyl (Cy), CyMan1 and CyMan2 (1-3 linked) when groups are considered in aggregate or separately. P-values are calculated by matched one-way ANOVA, followed by Tukey’s post-hoc test. Overall p values for the ANOVA indicated significance for all groups except group 6 (aggregated groups: p < .0001, Group 2: p = .0356; Group 3: p = .0002; Group 4: p = .0011; Group 5: p = .0162; Group 6: p = .124). ANOVA for this table was performed to compare only these three antigens, whereas ANOVA for Fig. 8b, c was performed to compare all antigens.
|                       | 2G12+10V1S          | 2G12+10F5M          |
|-----------------------|---------------------|---------------------|
| **Beamline**          | SSRL 12-2           | SSRL 12-2           |
| **Wavelength (Å)**    | 0.97950             | 0.97950             |
| **Resolution range (Å)** | 42.4 - 2.3 (2.34-2.30) | 46.8-3.6 (3.68-3.60) |
| **Space group**       | P2₁2₁2₁             | I2₂₂                |
| **Unit cell**         | 45.22, 131.18, 169.54 | 137.64, 146.31, 148.21 |
| **Total reflections** | 247,212 (11,195)    | 150,342 (10,062)    |
| **Unique reflections**| 45,804 (2195)       | 17,760 (1170)       |
| **Multiplicity**      | 5.4 (5.1)           | 8.5 (8.6)           |
| **Completeness (%)**  | 99.5 (97.6)         | 99.8 (99.9)         |
| **Mean I/σ(I)**       | 8.8 (1.9)           | 5.8 (1.5)           |
| **Wilson B-value (Å²)** | 27          | 82                 |
| R<sub>merge</sub> (%) | 17.4 (>100)         | 29.6 (>100)         |
| R<sub>free</sub> (%)  | 19.3 (>100)         | 31.6 (>100)         |
| R<sub>pim</sub> (%)   | 9.5 (50.6)          | 14.2 (48.0)         |
| CC<sub>1/2</sub> (%)  | 99.0 (51.0)         | 92.0 (72.0)         |
| **Reflections [R<sub>work</sub>(R<sub>free</sub>)]** | 43,502 (2230) | 16,789 (927) |
| **R<sub>work</sub> (%)** | 19.7 (28.4)       | 21.9 (33.4)         |
| **R<sub>free</sub> (%)** | 23.7 (31.3)       | 24.8 (37.9)         |
| **No. non-hydrogen atoms** |                       |                     |
| Fab                   | 6586                | 6573                |
| Ligands               | 477                 | 198                 |
| Solvent               | 441                 | 0                   |
| Protein residues      | 898                 | 869                 |
| RMS (bonds)           | 0.009               | 0.005               |
| RMS (angles)          | 1.35                | 1.06                |
| Ramachandran favored (%) | 96.4            | 96.4                |
| Ramachandran allowed (%) | 3.6               | 3.6                 |
| Ramachandran outliers (%) | 0.0              | 0.0                 |
| Clashscore<sup>f</sup> | 2.8               | 6.7                 |
| Average B-value (Å²)  | 33                  | 90                  |
| Fab                   | 32                  | 90                  |
Glycopeptide   47   93
Solvent       33   NA

\(^a\)Numbers in parentheses are for highest resolution shell

\(^b\)R_{merge} = \Sigma_{hkl} \Sigma_{i=1,n} |I_i(hkl)| - <I(hkl)> | / \Sigma_{hkl} \Sigma_{i=1,n} I(hkl)

\(^c\)R_{meas} = \Sigma_{hkl} \sqrt{(n/n-1)} \Sigma_{i=1,n} |I_i(hkl)| - <I(hkl)> | / \Sigma_{hkl} \Sigma_{i=1,n} I(hkl)

\(^d\)R_{pim} = \Sigma_{hkl} \sqrt{(1/n-1)} \Sigma_{i=1,n} |I_i(hkl)| - <I(hkl)> | / \Sigma_{hkl} \Sigma_{i=1,n} I(hkl)

\(^e\)CC_{1/2} = Pearson Correlation Coefficient between two random half datasets

\(^f\)Number of unfavorable all-atom steric overlaps \(\geq 0.4\)Å per 1000 atoms
Table S8. P-values for serum binding to autologous vs. heterologous glycopeptides

| Group 2 (10F5M vaccinated) | comparison | average Δ log EC₅₀ titer | adjusted p-value |
|---------------------------|------------|------------------------|-----------------|
| 10F5M vs. 10F2 response   | .254       |                       | .0025           |
| 10F5M vs. 10F6 response   | .285       |                       | .0013           |
| 10F5M vs. 10F8 response   | .291       |                       | .0016           |

| Group 3 (10F2 vaccinated)  | comparison | average Δ log EC₅₀ titer | adjusted p-value |
|----------------------------|------------|------------------------|-----------------|
| 10F2 vs. 10F5M response    | .153       |                       | .24             |
| 10F2 vs. 10F6 response     | .277       |                       | .10             |
| 10F2 vs. 10F8 response     | .276       |                       | .029            |

| Group 4 (10F6 vaccinated)  | comparison | average Δ log EC₅₀ titer | adjusted p-value |
|----------------------------|------------|------------------------|-----------------|
| 10F6 vs. 10F5M response    | .428       |                       | .034            |
| 10F6 vs. 10F2 response     | .445       |                       | .033            |
| 10F6 vs. 10F8 response     | .364       |                       | .033            |

| Group 5 (10F8 vaccinated)  | comparison | average Δ log EC₅₀ titer | adjusted p-value |
|----------------------------|------------|------------------------|-----------------|
| 10F8 vs. 10F5M response    | .527       |                       | .022            |
| 10F8 vs. 10F2 response     | .482       |                       | .020            |
| 10F8 vs. 10F6 response     | .233       |                       | .035            |

For each rabbit, post dose 2-4 titers (Fig. 5) were log transformed and averaged over the three doses. Matched one-way ANOVA was performed, comparing each rabbit’s serum selectivity for its own (autologous) glycopeptide immunogen vs. the other glycopeptides. Adjusted p-values were calculated with Dunnett’s multiple comparison test.
### Table S9. Effect of mannose on Dose 6 serum binding to BG505SOSIP.664T332N

| Group 1 | rabbit ID | A: EC<sub>50</sub> titer (0 mM mannose) | B: EC<sub>50</sub> titer (554 mM mannose) | Mannose effect (log (A/B)) | Average |
|---------|-----------|------------------------------------------|------------------------------------------|----------------------------|---------|
|         | 51-6      | 1645                                     | 1082                                     | 0.18                       |         |
|         | 52-6      | 544                                      | 418                                      | 0.11                       |         |
|         | 53-6      | 2570                                     | 1913                                     | 0.13                       |         |
|         | 54-6      | 4595                                     | 4021                                     | 0.06                       |         |
|         | 55-6      | 3295                                     | 2401                                     | 0.14                       |         |
|         | 56-6      | 1568                                     | 1511                                     | 0.02                       |         |
| Group 2 | 57-6      | 2272                                     | 1515                                     | 0.18                       | 0.11    |
|         | 59-6      | 9553                                     | 8066                                     | 0.07                       |         |
|         | 60-6      | 2164                                     | 1472                                     | 0.17                       |         |
|         | 61-6      | 1954                                     | 1536                                     | 0.10                       |         |
|         | 62-6      | 1823                                     | 1195                                     | 0.18                       |         |
| Group 3 | 63-6      | 3333                                     | 2444                                     | 0.13                       | 0.08    |
|         | 64-6      | 2327                                     | 2180                                     | 0.03                       |         |
|         | 65-6      | 2588                                     | 1936                                     | 0.13                       |         |
|         | 66-6      | 3324                                     | 3504                                     | -0.02                      |         |
|         | 68-6      | 4318                                     | 3175                                     | 0.13                       |         |
| Group 4 | 69-6      | 6111                                     | 5112                                     | 0.08                       | 0.10    |
|         | 70-6      | 2905                                     | 1931                                     | 0.18                       |         |
|         | 71-6      | 1312                                     | 943                                      | 0.14                       |         |
|         | 73-6      | 2315                                     | 1648                                     | 0.15                       |         |
|         | 74-6      | 1922                                     | 1563                                     | 0.09                       |         |
| Group 5 | 75-6      | 1402                                     | 1066                                     | 0.12                       | 0.10    |
|         | 77-6      | 3924                                     | 3605                                     | 0.04                       |         |
|         | 78-6      | 1995                                     | 1697                                     | 0.07                       |         |
|         | 79-6      | 714                                      | 556                                      | 0.11                       |         |
|         | 80-6      | 1458                                     | 1014                                     | 0.16                       |         |
| Group 6 | 81-6      | 1954                                     | 1462                                     | 0.13                       |         |
|         | 83-6      | 2316                                     | 1826                                     | 0.10                       |         |
|         | 84-6      | 3607                                     | 3517                                     | 0.01                       |         |
|         | 85-6      | 5248                                     | 4751                                     | 0.04                       |         |
|         | 86-6      | 4662                                     | 4396                                     | 0.03                       |         |

| bnAb controls | EC<sub>50</sub> (nM) 0 mM mannose | EC<sub>50</sub> (nM) 554 mM mannose | EC<sub>50</sub> (nM) 554 mM glycerol | Mannose effect | Glycerol effect |
|---------------|------------------------------------|------------------------------------|-------------------------------------|----------------|----------------|
| 2G12          | 2.8                                | 129.58                             | 2.24                                | 1.67           | -0.10          |
| b12           | 4.21                               | 18.34                              | 9.43                                | 0.64           | 0.35           |
| VRC01         | 8.53                               | 14.17                              | 10.08                               | 0.22           | 0.07           |
Table S10. Relative quantification of serum mannosidase trimming of g10F6 conjugate

| Tryptic glycopeptide 1 (formylXLXFIR)* | Full | -1 Man | -2 Man | -3 Man | -4 Man | -5 Man | -6 Man |
|----------------------------------------|------|--------|--------|--------|--------|--------|--------|
| 0                                     | 98.62| 1.13   | 0.61   | -0.20  | 0.00   | -0.16  | 0.00   |
| 4                                     | 61.06| 24.16  | 10.16  | 4.71   | 0.00   | -0.10  | 0.00   |
| 17                                    | 21.59| 26.84  | 16.75  | 20.87  | 7.73   | 1.46   | 4.76   |
| 24                                    | 15.99| 20.78  | 14.11  | 27.65  | 11.66  | 4.05   | 5.76   |
| 48                                    | 11.38| 17.28  | 12.61  | 28.65  | 14.47  | 4.24   | 11.37  |

| Tryptic glycopeptide 2 (XQYYVYHAPLLTXV)* | Full | -1 Man | -2 Man | -3 Man | -4 Man | -5 Man | -6 Man |
|------------------------------------------|------|--------|--------|--------|--------|--------|--------|
| 0                                       | 90.78| 2.47   | 6.81   | -0.05  | 0.00   | 0.00   | 0.00   |
| 4                                       | 52.17| 27.37  | 14.94  | 3.64   | 1.56   | 0.32   | 0.00   |
| 17                                      | 17.39| 20.21  | 14.86  | 8.94   | 18.66  | 7.72   | 11.78  |
| 24                                      | 9.11 | 12.67  | 14.46  | 21.67  | 16.88  | 7.71   | 16.64  |
| 48                                      | 6.35 | 10.46  | 5.88   | 22.64  | 20.46  | 8.82   | 23.99  |

*X* refers to click-glycosylated homopropargylglycine residue. Data are graphed in Figure 9d and SI Figure S18. See Methods for LC and mass spectrometric quantification procedures.
Synthesis of peptide 10F12M

10F12M (XSYTVTPAXNXPEARLGIVSHXPGRGKALLYSGSGC(StBu)A, X = homopropargylglycine) was synthesized according to previously reported methods and was prepared with two C→S mutations (bold S’s) from the original evolved sequence, 10F12. These mutations were deleterious to 2G12 binding ($K_D > 20$ nM, Fig. S16, vs. 0.77 nM for 10F12). Briefly, starting with 120 mg of trityl ChemMatrix® resin loaded with 0.28 mequiv/g serine (33.5 µmol scale), 41.8 mg of crude peptide 10F12M was obtained. HPLC purification (Waters Symmetry 300 C4, 5 µm, 10×250 mm, 4 mL/min, 10-45% MeCN in H2O with 0.1% formic acid, over 60 min, retention time: 28.4 min) of 22 mg of crude peptide yielded 3.5 mg of pure 10F12M, corresponding to 16% overall yield if all crude peptide had been purified. LR ESI-MS: observed average m/z of multiply charged ions $1091.14$ [M+4H]$^4^+$, $1454.47$ [M+3H]$^3^+$, corresponding to 4360.49 observed average mass, calculated mass for $C_{195}H_{311}N_{56}O_{53}S_{7}$: 4362.05.

Synthesis of glycopeptides 10F12M – Man$_1$, Man$_2$, Man$_3$, Man$_4$, Man$_5$, Man$_6$, Man$_7$GlcNAc:

Glycans were attached by Copper-Assisted Alkyne Azide Cycloaddition chemistry as reported previously. Briefly, starting with 0.42 mg (97 nmol, 1 equiv) of peptide 10F12M and 0.68 mg (427 nmol, 4.4 equiv) of Man$_9$cyclohexyl azide. HPLC purification (Waters Symmetry 300 C4, 5 µm, 10×250 mm, 4 mL/min, 10-45% MeCN in H2O with 0.1% formic acid, over 60 min, retention time 15.4 min) to afford 0.28 mg of pure glycopeptide 10F12M-Man$_n$ (quantified by BCA assay), corresponding to 22% yield. LR ESI-MS: observed average m/z of multiply charged ions $1345.70$ [M+8H]$^8^+$, $1537.80$ [M+7H]$^7^+$, corresponding to 10759.76 observed average mass, calculated average mass for $C_{435}H_{715}N_{71}O_{235}S_{7}$: 10763.80.

Synthesis of BSA-maleimide

BSA (Fisher BioReagents, BP9706-100) (0.7 mg, 10.5 nmol) was dissolved in 0.63 mL of PBS buffer (pH 7.5). SM-PEG$_4$-NHS (0.7 mg, 1.36 µmol, 130 equiv) was added to the solution to have the final concentration of 1 mg/mL. The reaction stood at room temperature for 30 min. Excess SM-PEG$_4$-NHS was removed by buffer exchanged through an Amicon centrifugal filter (30 kDa cutoff, Ultra-0.5, 4 rounds of dilution with PBS pH 6.5). The molecular weight of the activated BSA-maleimide was determined by MALDI-TOF MS analysis. A 6991.6-dalton average mass increase indicated an average of 17.5 linkers. A yield of 0.72 mg of activated BSA-maleimide was obtained based on BCA assay.

General conjugation procedure: CRM-g10V1S, BSA- peptide10V1S, g10V1S, g10F2, g10F5M, g10F6, g10F8, 10F12M-Man$_1$ branched, 10F12M-Man$_3$ linear, 10F12M-Man$_4$, 10F12M-Man$_5$, 10F12M-Man$_6$, 10F12M-Man$_7$GlcNAc:

Conjugations to CRM197 were performed according to previously reported methods. For 10V1S, CRM197 from Reagent Proteins (expressed in Pseudomonas fluorescens) was used. For other glycopeptides, ecoCRM197 from Fina Biosciences (expressed in E. coli) was used; this was identical to the Reagent Proteins CRM197 by MALDI-TOF mass spectrometry and SDS PAGE, and provided similar glycopeptide-specific titer. Briefly, g10V1S (2.9 mg, 310 nmol) in 150 µL of water was treated with 12.31 µL of 500 mM TCEP-HCl/ 1M Tris-HCl buffer (pH 7.8, 20 equiv). Complete deprotection of the cysteine was confirmed by UPLC-ESI-MS after the reaction stood overnight at room temperature under N$_2$. Excess TCEP was removed by buffer-exchanged through an Amicon centrifugal filter (3-kDa cutoff, Ultra-0.5, 20 min in the first round of filtration, and 30 min for the second round) with PBS buffer (pH 6.5). The deprotected glycopeptide was added to the freshly made CRM$_{197}$-maleimide (1.7 mg quantified by BCA, 27.5 nmol, ~22 average linkers/CRM) in PBS buffer (pH 6.5). The solution stood overnight under N$_2$ at room temperature. The CRM$_{197}$-glycopeptide 10V1S conjugate was purified by using an Amicon centrifugal filter (30 kDa cutoff, Ultra-0.5, 5 min for at least 4 rounds of filtration) to remove salts and unreacted glycopeptide. MALDI-TOF MS analysis indicated the distribution of conjugates with an average loading of 8. The CRM$_{197}$-glycopeptide 10V1S conjugates were capped with β-mercaptoethanol (0.29 µL neat, 32 mg, 4.1 µmol, 100 equiv, 1h) in 1 mL PBS (pH 6.5), purified and buffer-exchanged to water by Amicon centrifugal filter (30 kDa cutoff, Ultra-0.5, 5 min for at least 4 rounds of filtration). BCA quantification assay (corrected to add carbohydrate.
content) indicated 4 mg of CRM197-glycopeptide 10V1S conjugate (containing 2.2 mg of glycopeptide antigen). BSA conjugates of g10V1S, g10F2, g10F5M, g10F6, g10F8, 10F12M-Man, branched, 10F12M-Man linear, 10F12M-Man6, 10F12M-Man5, 10F12M-Man4, and 10F12M-Man2GlcNAc2 were prepared in an analogous manner with the lower-density BSA-maleimide reported above, yielding 72.6 µg BSA-g10V1S, 305 µg BSA-g10F2, 265 µg BSA-g10F5M, 282 µg BSA-g10F6, 263 µg BSA-g10F8, 54 µg BSA-10F12M-Man6 branched, 48 µg BSA-10F12M-Man linear, 62 µg BSA-10F12M-Man4, 69 µg BSA-10F12M-Man5, 64 µg BSA-10F12M-Man9, and 49 µg BSA-10F12M-Man2GlcNAc2 all with average loadings of ~4.

Conjugation procedure (6M guanidine PBS): BSA-peptide 10F2, 10F5M, 10F8

Peptides 10F2, 10F5M, 10F8 were each dissolved in 0.5% acetic acid for quantification by BCA assay, and appropriate aliquots of appropriate size for the reaction below were lyophilized and redissolved in 6M guanidine PBS buffer for the conjugation. Generally, capping was performed with slightly substoichiometric BME to avoid driving the reversible loss of glycopeptides from the conjugate.

Representative procedure for 10F5M: Peptide 10F5M (0.143 mg, 33 nmol) was dissolved in 230 µL 6M guanidine PBS buffer (pH 6.5). TCEP (0.6 µL of 500 mM aqueous solution, 9 equiv) was added and the mixture stood under N2 overnight, after which time UPLC-ESI-MS indicated complete deprotection of the cysteine. Excess TCEP was removed by Amicon centrifugal filter (3-kDa cutoff, 2 rounds of filtration: 20 min and then 30 min) with using 6M guanidine PBS buffer (pH 6.5). The deprotected peptide (50 µL volume) was added to activated BSA-maleimide (0.1 mg, 1.31 nmol, ~25 maleimide linkers, in 85 µL of the same buffer) and the solution stood overnight at room temperature under N2. The BSA-peptide 10F5M conjugate was purified by Amicon centrifugal filter (30-kDa, 4 rounds, 5 min each round) with water to remove salts and unreacted peptide. MALDI-TOF MS analysis indicated an average loading of 5. The BSA-peptide 10F5M was then diluted to 100 µL PBS/Guanidine and capped with mercaptoethanol (2.6 µL of 10mM solution in PBS pH 6.5, 20 equiv., 1h). The conjugate was then purified and buffer-exchanged with water using 30K Amicon filter (4 rounds: 5 min). BCA quantification assay indicated a yield of 72.5 µg of the BSA-peptide 10F5M conjugate. BSA conjugates of 10F2, 10F5M, and g10F8 were prepared in an analogous manner with the lower-density BSA-maleimide reported above, yielding, 154 µg 10F2-BSA, 72.5 µg 10F5M-BSA, and 34.4 µg 10F8-BSA, all with average loadings of ~4.

Conjugation procedure (0.5% acetic acid PBS): BSA-10F12M, 10F12M-cyclohexanol, 10F12M-Man, 10F12M-Man6

HPLC-purified lyophilized 10F12M peptide was dissolved in 10% acetic acid and diluted to 0.5% acetic acid solution before quantification by BCA assay (1.26 mg/mL result). This peptide 10F12M solution (260µL, 0.129 mg, 29.6 nmol) was transferred to a 0.5 mL low protein binding Eppendorf tube which was placed in a two-neck flask flushed with nitrogen. TCEP (0.6 µL of 500 mM aqueous solution, 10 equiv) was added and the solution was incubated under N2 atmosphere in a water bath at either 45°C overnight or 60°C for 7 hours, after which time UPLC-ESI-MS showed complete deprotection of the cysteine. Sometimes, another 10 equiv of TCEP was needed for the deprotection to go to completion. Excess TCEP was removed by Amicon centrifugal filter (3-kDa cutoff, 2 rounds of filtration: 20 min and then 30 min) with 0.5% acetic acid in PBS buffer (pH 5). The deprotected peptide (50 µL volume) was added to the activated BSA-maleimide (0.125 mg, 1.7 nmol, 17.4 maleimide linkers, in 101 µL of the same buffer) and the solution stood under N2 atmosphere overnight at room temperature. The BSA-peptide conjugates were then purified by Amicon centrifugal filter (30-kDa, 4 rounds, 5 min each round) with 0.5% AcOH/H2O to remove salts and unreacted peptide. The conjugates were capped with mercaptoethanol (1.4 µL of 10 mM solution, 8 equiv, 1h), then purified using 3K Amicon centrifugal filter with 0.5% AcOH/H2O (4 rounds: 5 min each round). BCA assay indicated 0.123 mg of BSA-peptide 10F12M conjugate. BSA conjugates of 10F12M-cyclohexanol, 10F12M-Man, and 10F12M-Man(1-3), and 10F12M-Man2(1-6) were prepared in an analogous manner, yielding 84 µg BSA-10F12M-cyclohexanol, 52 µg BSA-10F12M-Man, 50 µg BSA-10F12M-Man2(1-3), 17µg BSA-10F12M-Man2(1-6), all with average loadings of ~4.
Conjugation procedure (6M guanidine and 1% acetic acid): BSA-peptide 10F6

HPLC-purified lyophilized 10F6 (2.2 mg) was dissolved in 10% acetic acid and diluted to 0.5% acetic acid solution (0.5 ml) before quantification by BCA assay (1.797 mg/mL result). Solution containing 27 μg of peptide 10F6 (15 μL) was transferred to a 0.5 mL low protein binding Eppendorf tube which was lyophilized. The lyophilized peptide 10F6 was then dissolved in 50 μL 6M guanidine PBS buffer (pH 6.5) and placed in a two-neck flask flushed with nitrogen. TCEP (0.6 μL of 100 mM aqueous solution, 10 equiv) was added and the solution stood overnight at room temperature after which time UPLC-ESI-MS showed complete deprotection of the cysteine. Excess TCEP was removed by Amicon centrifugal filter (3-kDa cutoff, 2 rounds of filtration: 20 min and then 30 min) with 6M guanidine in PBS buffer (pH 6.5). The deprotected peptide was added to the activated BSA-maleimide (0.05 mg, 0.68 nmol, 17.7 maleimide linkers, in 42.4 μL of the same buffer) and the solution stood overnight at room temperature under N\textsubscript{2}. The peptide-BSA conjugates were purified by Amicon centrifugal filter (30-kDa, 4 rounds, 5 min each round) with 1% AcOH/\textsubscript{H}\textsubscript{2}O to remove salts and unreacted peptide. The conjugate was then capped with mercaptoethanol (0.68 μL of 10 mM solution, 10 equiv, 1h), and purified using 30K Amicon filter with 1% AcOH/\textsubscript{H}\textsubscript{2}O (4 rounds: 5 min each round). BCA quantification assay indicated a yield of 34.4 μg of the BSA-peptide 10F6 conjugate.

Synthesis of cyclohexanol-azide

Cyclohexanol-azide was synthesized by previously reported method \textsuperscript{8}. Briefly, imidazole-sulfonyl-azide·HCl (0.52 mmol, 109 mg) was added to a solution of 4-aminocyclohexanol (0.434 mmol, 50 mg), CuSO\textsubscript{4} (4.35 μmol, 1.1 mg), and K\textsubscript{2}CO\textsubscript{3} (0.478 mmol, 66 mg) in 2 mL MeOH. The mixture was stirred overnight, and then concentrated in vacuo. Water (5 mL) and concentrated HCl (0.25 mL) were then added to the residue and the mixture was extracted with 10 mL of EtOAc for 3 times. The organic layer was washed 3 times with brine (10 mL), dried over MgSO\textsubscript{4}, and concentrated. The crude was purified by flash column chromatography (1:1 hexane/\textsubscript{Et}\textsubscript{OA}c). Product-containing fractions were combined and dried under vacuum for 1.5 h, affording 34 mg of pure product, corresponding to 55% yield.

Pilot rabbit study

Three groups of three female New Zealand White rabbits were used to test the dose response to CRM\textsubscript{197}-glycopeptide 10V1S conjugates. Each group was immunized subcutaneously with CRM-g10V1S conjugate containing either 10μg, 50 μg or 100 μg antigen formulated in 50μL Adjuplex adjuvant, 4 times at 4-week intervals and blood was collected 2 weeks after each immunization. A prebleed was collected just before the first immunization.

Multi-immunogen rabbit study

Six groups of six female New Zealand White rabbits were used for the multi-immunogen study. Group 1 rabbits as a control group received subcutaneous immunizations of 50 μg CRM\textsubscript{197}-maleimide (with BME cap) and 50 μL Adjuplex. Group 2,3,4,5 were immunized with CRM\textsubscript{197}-glycopeptide 10F5M, CRM\textsubscript{197}-glycopeptide 10F2, CRM\textsubscript{197}-glycopeptide 10F6, CRM\textsubscript{197}-glycopeptide 10F8, respectively, each containing 50 μg of respective glycopeptides per dose. Group 6 received sequential immunizations of 10F5M, 10F2, 10F6 and 10F8 glycopeptide conjugates with adjuvant as above. Four immunizations were performed at 4-week intervals and blood was collected 2 weeks after each immunization, with a pre-bleed just before the first immunization. All rabbits received 2 booster injections of 50 μg BG505.SOSIP.664 (T332N) and 50 μL Adjuplex. Throughout the study, 5 animals (roughly one per group) died of unknown causes with no obvious relation to the immunizations.

ELISA analysis

High-protein-binding flat-bottomed Maxisorp ELISA plates (Nunc-Immuno) were coated with 120 ng/mL antigen in coating buffer (50 mM carbonate/bicarbonate buffer, pH 9.6, 100 μL/well) and incubated at 4 °C overnight. The
wells were washed twice with PBS-0.05% Tween 20 (PBS-T) and then blocked for 1 h at room temperature with 5% fat-free milk PBS-T (200 µL/well). After washing again twice with PBS-T, the wells were then incubated with either 3-fold or 4-fold serial dilutions of rabbit serum (starting at different concentrations: either 1:10 or 1:100) in 1% fat-free milk in PBS-T for 2 h at room temperature. The wells were washed 3 times before incubating with 100 µL of a horseradish peroxidase (HRP) conjugated sheep anti-rabbit antibody (Novex, part number A16172) at 1: 10,000 dilutions for 1 h at room temperature. After 3 washes, the wells were developed by adding 100 µL of 3,3′,5,5′-tetramethylbenzidine (TMB solution, Abcam Ab171522) for 3 min. The reaction was stopped by adding 100 µL of 1 M sulfuric acid and absorbance was measured at 450 nm wavelength. All measurements were performed in triplicate.

**Synthesis of CRM197-AcBr**

CRM197 (1.0 mg, 17 nmol) was dissolved in 0.9 mL of PBS buffer (pH 7.5). Bromoacetamido-PEG5-NHS ester (BroadPharm, BP-20569, BrCH₂(CO)NH(CH₂CH₂O)₅(CH₂)₂-CO-NHS) (1.2 mg, 2.5 µmol, 147 equiv) was added to the solution to have the final concentration of 1 mg/mL. The reaction stood at room temperature for 1.5 hours. Excess bromoacetamido-PEG₅-NHS ester was removed by buffer exchange through an Amicon centrifugal filter (30 kDa cutoff, Ultra-0.5, 4 rounds of dilution with PBS pH 8.5). The molecular weight of the activated BSA-bromoacetamido (BSA-AcBr) was determined by MALDI-TOF MS analysis. A 6283.8-dalton average mass increase indicated an average of 17 linkers. A yield of 1.1 mg of activated BSA-AcBr was obtained based on BCA assay.

**Synthesis of Alkyne-CRM197-AcBr**

Alkyne-PEG₅-NHS (Sigma-Aldrich, catalog # 764191, HCC-CH₂-(OCH₂CH₂)₅-CO-NHS) (0.4 mg, 0.93 µmol, 200 equiv) was added to the solution of CRM197-AcBr (0.3 mg, 4.6 nmol) in 167 µL of PBS buffer (pH 7.5) to a final concentration of 1 mg/mL. The reaction stood at room temperature for 1.5 h. Excess alkyne-PEG₅-NHS was removed by buffer exchange through an Amicon centrifugal filter (30 kDa cutoff, Ultra-0.5, 4 rounds of dilution with PBS pH 8.5). The molecular weight of alkyne-CRM197-AcBr was determined by MALDI-TOF MS analysis. A 1012.7-dalton average mass increase indicated an average of 3.5 alkyne linkers. A yield of 0.25 mg of alkyne-CRM197-AcBr was obtained based on BCA assay.

**Synthesis of alkyne-CRM197-Ac-glycopeptide 10F6-Man₅-cyclohexyl**

Glycopeptide 10F6 (0.202 mg, 16.2 nmol) in 96 µL of water was treated with 1.62 µL of 100 mM TCEP-HCl/1M Tris-HCl buffer (pH 7.8, 10 equiv). Complete deprotection of the cysteine was confirmed by UPLC-ESI-MS after the reaction stood overnight at room temperature under N₂. Excess TCEP was removed by buffer-exchange through an Amicon centrifugal filter (3-kDa cutoff, Ultra-0.5, 20 min in the first round of filtration, and 45 min for the second round) with PBS buffer (pH 8.5). The deprotected glycopeptide was added to the freshly-made alkyne-CRM197-AcBr (0.125 mg quantified by BCA, 1.9 nmol, ~17 average AcBr linkers/CRM197) in PBS buffer (pH 8.5). The solution stood overnight in the dark under N₂ at room temperature. The alkyne-CRM197-glycopeptide 10F6 conjugate was purified using an Amicon centrifugal filter (30 kDa cutoff, Ultra-0.5, 5 min for at least 4 rounds of filtration) to remove salts and unreacted glycopeptide. MALDI-TOF MS analysis indicated the distribution of conjugates with an average loading of 3. The alkyne-CRM197-glycopeptide 10F6 conjugates were capped with β-mercaptoethanol (2.28 µL from 10 mM stock, 22.8 nmol, 12 equiv, 1h) in 125 µL PBS (pH 8.5), purified and buffer-exchanged to water by Amicon centrifugal filter (30 kDa cutoff, Ultra-0.5, 5 min for at least 4 rounds of filtration). BCA quantification assay (corrected to add carbohydrate content) indicated 0.162 µg of alkyne-CRM197-glycopeptide 10F6 conjugate.

**In vitro trimming of CRM197-glycopeptide 10F6 conjugates using rabbit serum**

Alkyne-CRM-Ac-g10F6 conjugate (30 µg) was added to five 1.5 mL Eppendorf tubes, each containing 0.3 mL of rabbit serum. The serum mixtures were incubated at 37°C and the reaction was stopped at different time points (4, 17, 24, 48 h) by adding two mannosidase inhibitors (kifunensine and swainsonine, Santa Cruz Biotechnology, catalog # sc-201364 and sc-201362, respectively) directly to the serum mixture to obtain a 5 µg/mL concentration.
A 0 hr timepoint was generated by adding conjugate to serum already containing inhibitors. The quenched reactions were immediately frozen and stored at -80°C until the last time point sample was obtained.

Next, Dde-biotin-picolyl-azide (Click Chemistry Tools, catalog # 1186-5) (25 µL from 10 mM stock in DMSO) and THPTA (50 µL from 100 mM stock in PBS buffer pH 7.5) were added to the serum mixtures. The mixtures were then vortexed briefly to mix. Next, CuSO$_4$ 5H$_2$O (50 µL from 20 mM stock in PBS buffer) was added and mixed briefly. Lastly, sodium ascorbate (50 µL from 300 mM stock in PBS buffer) was added and also vortexed to mix. The click reactions were covered with aluminum foil to protect from light and they were stood for 2h at room temperature. Excess Dde-biotin-picolyl-azide linker was removed by buffer exchange through a 30K Amicon filter (6 rounds of filter, 10 min each round). The clicked CRM-Ac-g10F6 conjugates were incubated with 100 µL of NeutrAvidin resin (ThermoFisher, catalog # 29200) in 0.8 mL centrifuge columns for 1.5 h at room temperature. The NeutrAvidin resin was then washed 4 times with 200 µL water. Then, the washed resin was incubated in elution buffer (2% v/v hydrazine solution) for 1h at room temperature and washed 4 times with 200 µL water. The eluant and all the washes were combined and buffer exchanged by 30K Amicon filter with water. The recovered CRM-Ac-g10F6 conjugates were concentrated and lyophilized.

**Identification and quantification of intact and trimmed glycoforms of the tryptic glycopeptides from g10F6 by mass spectrometry (MS) analysis:**

To determine the presence of G10F6 trimmed glycoforms resulting from serum mannosidase activity on the G10F6 glycopeptide, a pure G10F6-CRM (standard) sample and the five time-point serum-incubated samples (0, 4, 17, 24, and 48 hr) described above were analyzed the same way, as described below. Prior to mass spectrometry analysis, the samples were reduced, alkylated, trypsin digested, SP-C18 desalted and concentrated. Tryptic digestion would be expected to generate two glycopeptides (formylXLFIR and XQYVYHAPLLTXVR), each containing two homopropargylglycine residues with triazole cyclohexyl linker and glycan attached (X).

Three types of MS experiments were performed on each sample: a glycopeptide identification focus nanoUPLC-MS/MS [HCD (Higher Energy Collisional Dissociation (HCD) NCE (Normalized Collision Energy) experiment at 45 v, a glycan composition focus nanoUPLC-MS/MS (HCD NCE) experiment at 15 v, and a relative quantification focus nanoUPLC-MS-only experiment. Each dried peptide/glycopeptide mixture was resuspended in 40 µL of Mobile Phase A (1% ACN/0.1% FA/Water). A one-µL aliquot was analyzed in each MS experiment. The aliquot was injected into a nanoAcquity UPLC M-Class (Waters) equipped with reversed phase columns: nanoEase™ M/Z Symmetry C18, 100 Å, 5 µm, 1/PK 180 µm x 20 mm, trap column and nanoEase™ M/Z HSS C18 T3 Col 100 Å, 1.8 µm, 1/PK 75 µm x 100 mm, analytical column (Waters). The nano-UPLC was connected online to an Orbitrap Fusion Lumos Tribid Mass Spectrometer (Thermo Scientific) equipped with a Triversa NanoMate (Advion) electrospray ionization (ESI) source operated at 1.7 kV, in order to generate a constant nanoESI plume. The sample was loaded onto the precolumn, washed for 4 min at a flow of 4 µL/min with 100% Mobile Phase A (% (1% ACN/0.1% FA/Water). After the trapping event, the peptides were eluted to the analytical column and resolved by a gradient of 3-40% mobile phase B (1% Water/0.1% FA/ACN) delivered over 40 min at a flow rate of 500 nL/min.
The data for all MS acquisition experiments were acquired as follows: the mass spectrometer was operated in positive ion mode and the Orbitrap analyzer was used as the detector for all scan events; all data were acquired in Profile mode. The sample ions were introduced into the mass spectrometer (MS) through an Ion Transfer Tube operated at 300 °C. To minimize in-source fragmentation, the source RF Lens was operated at 5%. Data from the MS tandem experiments were acquired with the following scan event parameters: The MS\(^1\) scan was set at a resolution of 120,000 @ \(m/z\) 200, over the full scan range \(m/z\) 350-1500, 1 \(\mu\)scan/spectrum, maximum injection time (ion accumulation time) of 50 ms with a target automatic gain control (AGC) of \(4 \times 10^5\) ion population. The following filters were applied to the data dependent acquisition scan events: Monoisotopic Peak Determination was set to Peptide; Charge States included 2-7, the Dynamic Exclusion was set to exclude after 1 time, for a duration of 10 s with a \(\pm 10\) ppm window, Excluding Isotopes was set to True; the Intensity Threshold was set to \(4 \times 10^4\); the Data Dependent Mode was set to Cylce Time (Top Speed Methodology) with a Master Scan every 3 s. The MS\(^2\) scan event used the Quadrupole for Isolation Mode, with an Isolation Window of \(m/z\) 1.6; the activation used was HCD at 45 % for the glycopeptide identification focus experiment or 15% for the glycan composition focus experiment. The MS\(^2\) scan range was set to Auto with \(m/z\) range set to High; the first mass was fixed to \(m/z\) 100; the AGC target was set to \(5.5 \times 10^5\) ion population and maximum injection time of 150 ms; 2 \(\mu\)scan/spectrum.

The relative quantification focus MS-only experiment MS\(^1\) scan followed the same parameters of the tandem MS\(^1\) scan events in the tandem methods, the only difference being that the MS scan range was set as \(m/z\) 350-2000. The glycopeptides were identified by manually assigning peptide backbone and glycan loss peaks in the HCD 45% tandem data; the glycan compositions of such glycopeptides were confirmed in the HCD 15% tandem data. To quantify the relative abundances of the g10F6 glycopeptides’ glycoform distributions (the full [Man\(^n\)] and trimmed glycoform versions) for the standard sample (pure glycopeptide-CRM conjugate) and all time point samples (serum-treated samples) the area under the chromatographic peak corresponding to each glycopeptide precursor ion was calculated by the Thermo Scientific Xcalibur’s Qual Browser Software, using the extracted ion chromatogram for each observed charge state. The extracted areas were normalized by charge (\(\text{Total Area} = \sum z \times (\text{Area}/z)\)). The percentage for each chromatographically resolved Man\(^n\) glycoform in the standard sample were calculated and then subtracted from the total signal observed for the corresponding Man\(^n\) glycoform at each time point. The values were also corrected to take into account a small amount of in-source fragmentation (~4-5%) observed for the standard. This corrected amount was then calculated as a percentage distribution and plotted with Excel. The results are shown in Table S10.

**LC/MS characterization data of peptides and glycopeptides:**

**Peptide 10V1S**

Peptide 10V1S was synthesized according to previously reported methods \(^7\). Briefly, starting with 85 mg of trityl ChemMatrix® resin loaded with 0.3 mequiv/g alanine (26 \(\mu\)mol scale), 50 mg of crude peptide 10V1S was obtained. HPLC purification (Waters Symmetry 300 C4, 5 \(\mu\)m, 10×250 mm, 4 mL/min, 10-45% MeCN in H\(_2\)O with 0.1% formic acid, over 60 min, retention time, \(t_R = 30.8\) min) of 14 mg of crude peptide yielded 2 mg of pure 10V1S, corresponding to 6 % overall yield if all crude peptide had been purified. LR ESI-MS: observed average \(m/z\) of multiply charged ions 1160.67 [M+4H]\(^4+\), 1547.78 [M+3H]\(^3+\), corresponding to 4639.51 observed average mass, calculated average mass for C\(_{206}H_{313}N_{59}O_{60}S_2\): 4640.18.

Glycans were “clicked” to peptides according to the procedure in Ref. \(^7\).
**Glycopeptide 10V1S**

Starting with 4.7 mg (1.1 µmol, 1 equiv) of peptide 10V1S and 6.2 mg (3.9 µmol, 3.6 equiv) of Man\(_5\)cyclohexyl azide. LR ESI-MS: observed average m/z of multiply charged ions 1181.26 [M+8H]\(^+\), 1349.52 [M+7H]\(^+\), 1574.38 [M+6H]\(^+\), 1888.93 [M+5H]\(^+\), corresponding to 9440.41 observed average mass, calculated average mass for C\(_{388}H_{1615}N_{680}O_{398}S_2\): 9441.49. RP-HPLC purification (Waters Symmetry 300 C4, 5 µm, 10×250 mm, 4 mL/min, 2-42% MeCN in H\(_2\)O with 0.1% formic acid, 60 min, retention time, \(t_R = 30.7\) min) afforded 0.13 mg of pure glycopeptide 10V1S, corresponding to 41 % yield.

**Peptide 10F12M-cyclohexanol**

Starting with 0.5 mg (115 nmol, 1 equiv) of peptide 10F12M and 0.89 mg (6.3 µmol, 55 equiv) of cyclohexanol azide. LR ESI-MS: observed average m/z of multiply charged ions 985.95 [M+5H]\(^+\), 1232.1 [M+4H]\(^+\), 1642.82 [M+3H]\(^+\), corresponding to 4924.87 observed average mass, calculated average mass for C\(_{216}H_{335}N_7O_{35}S_2\): 4926.74. RP-HPLC purification (Waters Symmetry 300 C4, 5 µm, 10×250 mm, 4 mL/min, 10-45% MeCN in H\(_2\)O with 0.1% formic acid, 60 min, retention time, \(t_R = 26.6\) min) afforded 0.13 mg of pure 10F12M-cyclohexanol, corresponding to 23 % yield.

Most glycopeptides 10F12M were quantified by BCA assay without correction factor accounting for the glycan weight, which resulted in lower yields than anticipated:

**Glycopeptide 10F12M-Man\(_5\)**

Starting with 0.5 mg (115 nmol, 1 equiv) of peptide 10F12M and 0.15 mg (504 nmol, 4.4 equiv) of Man\(_5\)cyclohexyl azide. LR ESI-MS: observed average m/z of multiply charged ions 930.06 [M+6H]\(^+\), 1115.97 [M+5H]\(^+\), 1394.34 [M+4H]\(^+\), corresponding to 5574.19 observed average mass, calculated average mass for C\(_{263}H_{395}N_7O_{35}S_2\): 5575.30. RP-HPLC purification (Waters Symmetry 300 C4, 5 µm, 10×250 mm, 4 mL/min, 10-45% MeCN in H\(_2\)O with 0.1% formic acid, 60 min, retention time, \(t_R = 21.6\) min) afforded 0.2 mg of pure glycopeptide 10F12M-Man\(_5\), corresponding to 31 % yield. Most 10F12M-derived glycopeptides were quantified by BCA assay without a correction factor to account for the glycan weight, which resulted in lower yields than anticipated.

**Glycopeptide 10F12M-Man\(_5\): (1-3)**

Starting with 0.22 mg (51 nmol, 1 equiv) of peptide 10F12M and 0.10 mg (224 nmol, 4.4 equiv) of Man\(_5\) (1-3) cyclohexyl azide. LR ESI-MS: observed average m/z of multiply charged ions 1038.16 [M+6H]\(^+\), 1245.41 [M+5H]\(^+\), 1556.71 [M+4H]\(^+\), corresponding to 6222.62 observed average mass, calculated average mass for C\(_{263}H_{395}N_7O_{35}S_2\): 6223.86. RP-HPLC purification (Waters Symmetry 300 C4, 5 µm, 10×250 mm, 4 mL/min, 10-45% MeCN in H\(_2\)O with 0.1% formic acid, 60 min, retention time, \(t_R = 20.4\) min) afforded 0.16 mg of pure glycopeptide 10F12M-Man\(_5\): (1-3), corresponding to 52 % yield.

**Glycopeptide 10F12M-Man\(_5\): (1-6)**

Starting with 0.5 mg (115 nmol, 1 equiv) of peptide 10F12M and 0.23 mg (504 nmol, 4.4 equiv) of Man\(_5\) (1-6) cyclohexyl azide. LR ESI-MS: observed average m/z of multiply charged ions 1038.47 [M+6H]\(^+\), 1245.79 [M+5H]\(^+\), 1556.64 [M+4H]\(^+\), corresponding to 6223.78 observed average mass, calculated average mass for C\(_{263}H_{395}N_7O_{35}S_2\): 6223.86. RP-HPLC purification (Waters Symmetry 300 C4, 5 µm, 10×250 mm, 4 mL/min, 10-45% MeCN in H\(_2\)O with 0.1% formic acid, 60 min, retention time, \(t_R = 20.3\) min) afforded 0.13 mg of pure glycopeptide 10F12M-Man\(_5\): (1-6), corresponding to 18 % yield.

**Glycopeptide 10F12M-Man\(_5\): (linear)**

Starting with 0.61 mg (140 nmol, 1 equiv) of peptide 10F12M and 0.39 mg (616 nmol, 4.4 equiv) of Man\(_5\)cyclohexyl azide. LR ESI-MS: observed average m/z of multiply charged ions 1146.25 [M+6H]\(^+\), 1375.50 [M+5H]\(^+\), 1718.75 [M+4H]\(^+\), corresponding to 6871.67 observed average mass, calculated average mass for C\(_{290}H_{475}N_{71}O_{115}S_{2}\):
6872.43. RP-HPLC purification (Waters Symmetry 300 C4, 5 μm, 10×250 mm, 4 mL/min, 10-45% MeCN in H2O with 0.1% formic acid, 60 min, retention time, \( t_r = 18.9 \) min) afforded 0.8 mg of pure glycopeptide 10F12M-Man\( _3 \) linear, corresponding to 83 % yield.

**Glycopeptide 10F12M-Man\( _3 \) (branched)**

Starting with 0.5 mg (115 nmol, 1 equiv) of peptide 10F12M and 0.32 mg (504 nmol, 4.4 equiv) of Man\( _\text{cyclohexyl} \) azide. LR ESI-MS: observed average m/z of multiply charged ions 1146.37 [M+6H]\(^6+\), 1375.69 [M+5H]\(^5+\), 1718.61 [M+4H]\(^4+\), corresponding to 6872.04 observed average mass, calculated average mass for \( \text{C}_{297}\text{H}_{475}\text{N}_{71}\text{O}_{115}\text{S}_{22} \): 6872.43. RP-HPLC purification (Waters Symmetry 300 C4, 5 μm, 10×250 mm, 4 mL/min, 10-45% MeCN in H2O with 0.1% formic acid, 60 min, retention time, \( t_r = 18.6 \) min) afforded 0.12 mg of pure glycopeptide 10F12M-Man\( _3 \) (branched), corresponding to 15 % yield.

**Glycopeptide 10F12M-Man\( _4 \)**

Starting with 0.5 mg (115 nmol, 1 equiv) of peptide 10F12M and 0.45 mg (573 nmol, 5 equiv) of Man\( _\text{cyclohexyl} \) azide. LR ESI-MS: observed average m/z of multiply charged ions 1254.41 [M+6H]\(^6+\), 1505.07 [M+5H]\(^5+\), 1881.04 [M+4H]\(^4+\), corresponding to 7520.32 observed average mass, calculated average mass for \( \text{C}_{315}\text{H}_{515}\text{N}_{71}\text{O}_{133}\text{S}_{22} \): 7520.99. RP-HPLC purification (Waters Symmetry 300 C4, 5 μm, 10×250 mm, 4 mL/min, 10-45% MeCN in H2O with 0.1% formic acid, 60 min, retention time, \( t_r = 18.4 \) min) afforded 0.32 mg of pure glycopeptide 10F12M-Man\( _4 \), corresponding to 37 % yield.

**Glycopeptide 10F12M-Man\( _5 \)GlcNAc:**

Starting with 0.5 mg (115 nmol, 1 equiv) of peptide 10F12M and 0.48 mg (504 nmol, 4.4 equiv) of Man\( _\text{cyclohexyl} \) azide. LR ESI-MS: observed average m/z of multiply charged ions 1167.92 [M+7H]\(^7+\), 1362.37 [M+6H]\(^6+\), 1634.63 [M+5H]\(^5+\), corresponding to 8168.27 observed average mass, calculated average mass for \( \text{C}_{339}\text{H}_{555}\text{N}_{71}\text{O}_{155}\text{S}_{22} \): 8169.55. RP-HPLC purification (Waters Symmetry 300 C4, 5 μm, 10×250 mm, 4 mL/min, 10-45% MeCN in H2O with 0.1% formic acid, 60 min, retention time, \( t_r = 16.7 \) min) afforded 0.35 mg of pure glycopeptide 10F12M-Man\( _5 \)GlcNAc, corresponding to 37 % yield.

**Glycan synthesis methods**

**General synthetic methods for Man\( _1\text{s}--\text{Cy}--\text{N}_3 \) preparation**

All synthesis reagents were purchased from Sigma-Aldrich, Acros Organics, Fluka, Alfa Aesar or Strem and used without further purification unless otherwise noted. Toluene, THF, DCM, Ethyl Ether and Pentane were deoxygenated by argon purging and dried by passage through activated alumina columns, then stored under argon gas only briefly before use. DriSolv Acetonitrile, DMSO and Methanol were purchased from EMD. Amines (Et\( _3 \)N, iPr\( _2 \)NEt, Pyridine, 2,6-Lutidine and 2,6-di-t-butylpyridine) were refluxed over CaH\( _2 \) and freshly distilled before use. Glassware was flame dried or dried in a 150 °C oven. For glycosylations, carbohydrate donors and acceptors were azeotropically dried by the following procedure: the intermediate was dissolved in dry toluene, the solution was
cooled to –78 °C, vacuum was applied, and the cooling bath was removed to allow the toluene to evaporate while the mixture warmed to room temperature. The flask was then backfilled with dry nitrogen and this procedure was repeated a total of three times. SiliCycle Siliaflash P60 silica was used for flash column chromatography. Analytical thin layer chromatography (TLC) was performed using SiliCycle glass backed plates (Cat# TLG-R10011B323). TLC plates were analyzed by short wave UV illumination, or by staining with dipping in cerium-ammonium-molybdate (CAM) stain (40 g of ammonium pentamolybdate, 1.6 g of cerium (IV) sulfate, 800 mL of diluted sulfuric acid (1:9, with water, v/v)) and heating on a hot plate. All 1H and 13C NMR spectra were obtained on a Varian iNova 400 instrument in CDCl3, internally referenced to TMS, or D2O externally referenced to sodium 3–(trimethylsilyl)propanesulfonate. Chemical shifts are reported in parts per million (ppm), and coupling constants are reported in Hz. Coupling is referred to with the following abbreviations (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, app d = apparent doublet, app t = apparent triplet). For NMR spectra in which large numbers of resonances are unresolved, only the clearly–resolved “selected signals” are listed in text–format listing of data. LC/MS analysis was performed on a Waters Acquity UPLC equipped with photodiode array and Waters Micromass ZQ4000 mass detector (Column: Waters ACQUITY UPLC BEH® C18, 1.7 μm, 130Å, 2.1 x 50 mm. Waters ACQUITY UPLC BEH® HILIC, 1.7 μm, 130Å, 2.1 x 150 mm.). Optical rotation was measured using a Jasco digital polarimeter. Infrared spectra were obtained using a Nicolet IR200 spectrometer with a diamond ATR. DCM stands for dichloromethane, DDQ stands for 2,3–dichloro–5,6–dicyanobenzoquinone, DTBP stands for 2,6–di–tert–butylpyridine, EA stands for ethyl acetate, TES-H stands for triethylsilane, THF stands for tetrahydrofuran, NIS stands for N–Iodosuccinimide.

Synthesis of Man1–Cy–N3 (5)

PMB deprotected monosaccharide (3)

To a flask containing 120 mg (0.168 mmol, 1 equiv) of 19–12 was added 1.8 mL of DCM and 0.1 mL of 1M phosphate buffer pH 7, then cooled to 0 °C, and 91 mg (0.40 mmol, 2.4 equiv) DDQ was added. This mixture was allowed to stir for 6 hours, and the reaction was quenched with aqueous NaHCO3 solution. The mixture was diluted with DCM, and the organic phase was washed with water. The aqueous phase was extracted with DCM three times, and the combined organic layers were dried over MgSO4, filtered, and concentrated. Purification by flash chromatography (1:1 ethyl acetate/hexanes) afforded 83 mg (0.14 mmol, 83%) of 3 as an off–white foam. 1H NMR (400MHz, CDCl3): δ 7.89 (app d, J = 7.2 Hz, 2H), 7.59 (app t, J = 7.6 Hz, 1H), 7.53 (app t, J = 8.0, 2H), 7.49 – 7.43 (m, 2H), 7.41 – 7.29 (m, 8H), 5.52 (s, 1H), 5.03 (app d, J = 11.8 Hz, 1H), 4.63 (app d, J = 11.8 Hz, 1H), 4.74 (s, 1H), 4.32 – 4.24 (m, 2H), 3.90 – 3.81 (m, 2H), 3.81 – 3.70 (m, 2H), 3.70 – 3.60 (m, 1H), 3.35 – 3.26 (m, 1H), 3.25 – 3.15 (m, 1H), 2.33 (app d, J = 8.6 Hz, 1H), 1.81 – 2.08 (m, 4H), 1.52 – 1.16 (m, 4H). MS (ESI+): calcd. for C32H38NO8S+[M + H+] 596.23, found 596.17.

Fully deprotected cyclohexyl linked monosaccharide amine (SI-1)
Along with a stream of N\textsubscript{2}, ammonia gas was condensed against a -78 °C cold finger into a -78 °C-cooled 50 mL 3-necked flask equipped with glass-coated stir bar until 25 mL had accumulated. 70 mg (2.7 mmol, 40 equiv) Na metal was then added, and the resulting blue solution was left to stir for 1 hour. 40 mg (0.067 mmol, 1 equiv) of 3 in 4 mL of THF was then added. The reaction progress was monitored by LC–MS. After 6 hours, 165 mg (3.09 mmol, 46 equiv) NH\textsubscript{4}Cl was added to quench the reaction. At this time, the cooling bath was removed and the ammonia was blown off under a stream of N\textsubscript{2}. The resulting white solids were dissolved in water and desalted by passage through a Biorad P-2 Biogel (Fine) size exclusion column to give 16 mg of partially purified SI-1. \textsuperscript{1}H NMR (400MHz, D\textsubscript{2}O): \(\delta 4.78 (s, 1H), 3.98 – 3.86 (m, 2H), 3.86 – 3.76 (m, 1H), 3.71 (dd, J = 12.5, 6.4 Hz, 1H), 3.63 (app d, J = 9.7 Hz, 1H), 3.56 (app t, J = 9.6 Hz, 1H), 3.35 (app t, J = 6.8 Hz, 1H), 3.24 – 3.10 (m, 1H), 2.29 – 1.99 (m, 4H), 1.63 – 1.26 (m, 4H). \textsuperscript{13}C NMR (100 MHz, D\textsubscript{2}O): \(\delta 100.78, 79.06, 78.77, 75.88, 73.86, 69.62, 63.84, 51.94, 33.16, 31.86, 31.05, 30.90. \) IR (cm\textsuperscript{-1}): 3178, 2943, 1645, 1062. MS (ESI+): calcd. for C\textsubscript{12}H\textsubscript{24}NO\textsubscript{6}\textsuperscript{+}[M + H\textsuperscript{+}] 278.16, found 277.88. \([\alpha]_D\) (c 1.0, H\textsubscript{2}O, -8.5).

**Cyclohexyl linked monosaccharide azide (5, Man–Cy–N\textsubscript{3})**

Into a 20 mL vial containing 10 mg (0.036 mmol, 1.0 equiv) of crude amine SI-1 was added 0.16 mL water, 150 \(\mu\)L (0.030 mmol, 0.8 equiv) of 0.2M aqueous CuSO\textsubscript{4} and 15 mg (0.11 mmol, 3.0 equiv) of K\textsubscript{2}CO\textsubscript{3}. 1 mL of MeOH was added, followed by the addition of 1.4 mL (0.36 mmol, 10.0 equiv) of freshly prepared 0.25M TfN\textsubscript{3}\textsuperscript{13} in DCM. The resulting homogeneous reaction was left to stir at room temperature for 3 hours until complete conversion was observed by LC–MS. The reaction was quenched with 30 mg (0.36 mmol, 10.0 equiv) solid NaHCO\textsubscript{3} and concentrated in vacuo. The residue was desalted on a Biorad P-2 Biogel (Fine) size exclusion column. Reversed phase HPLC purification (Column: Waters Xbridge Prep, C\textsubscript{18}, 5\(\mu\)m, 130Å, 10x250mm. Method: 4mL/min flow rate. A= H2O/0.1%FA, B= ACN/0.1%FA. 98.2% A for 1min, then 98.2% A to 60% A over 24 minutes, then 60% to 5% A over 5 minutes. Desired product eluted at 17.9 minutes.) providing 3.5 mg (0.012 mmol, 18%, 2 steps) of Man–Cy–N\textsubscript{3} (5) as a glassy solid. \textsuperscript{1}H NMR (400MHz, D\textsubscript{2}O): \(\delta 4.80 (s, 1H), 3.98 – 3.89 (m, 2H), 3.87 – 3.78 (m, 1H), 3.73 (dd, J = 12.3, 6.2 Hz, 1H), 3.64 (dd, J = 9.6, 3.1 Hz, 1H), 3.57 (app t, J = 10.4 Hz, 1H), 3.54 – 3.44 (m, 1H), 3.40 – 3.32 (m, 1H), 2.13 – 1.95 (m, 4H), 1.56 – 1.31 (m, 4H). \textsuperscript{13}C NMR (100 MHz, D\textsubscript{2}O): \(\delta 100.71, 79.03, 78.93, 75.90, 73.90, 69.63, 63.84, 61.72, 32.99, 31.58, 31.31, 31.15. \) IR (cm\textsuperscript{-1}): 3338, 2935, 2863, 2091, 973. MS (ESI+): calcd. for C\textsubscript{12}H\textsubscript{21}N\textsubscript{3}NaO\textsubscript{6}\textsuperscript{+}[M + Na\textsuperscript{+}] 326.13, found 325.87. \([\alpha]_D\) (c 0.1, H\textsubscript{2}O, -21.0).
Synthesis of Man$_2$–Cy–N$_3$ (7)

Fully protected cyclohexyl linked disaccharide (6)

To a 25 mL flask containing 54 mg (0.083 mmol, 1 equiv) of 4 and a 10 mL flask containing 64 mg (0.12 mmol, 1.5 equiv) of SI-2 was added toluene and freeze pumped for three times. 37 μL (0.16 mmol, 2 equiv) of DTBP and 100 mg freshly dried 4Å molecular sieves were added to the 25 mL flask. 4 was redissolved in 1 mL toluene and cooled in a –20 °C salt ice bath. SI-2 was dissolved in 1 mL toluene and transferred via cannula to the 25 mL flask. The 25 mL flask was then wrapped in foil and 38 mg (0.15 mmol, 1.8 equiv) AgOTf was added. This was allowed to react for 3 hours, and the reaction was quenched with aqueous NaHCO$_3$ solution. The mixture was extracted with EA three times. The combined organic layers were dried over MgSO$_4$, filtered, and concentrated. Purification by flash chromatography (1:3 ethyl acetate/hexanes) afforded 55 mg (0.049 mmol, 59%) of 6 as an off-white foam. $^1$H NMR (400MHz, CDCl$_3$): δ 7.92 (app d, J = 7.4 Hz, 2H), 7.61 (app t, J = 7.4 Hz, 1H), 7.52 (app t, J = 7.5 Hz, 2H), 7.48 – 7.41 (m, 2H), 7.36 – 7.09 (m, 21H + CHCl$_3$), 5.67 – 5.57 (m, 2H), 4.95 – 4.84 (m, 2H), 4.79 (app d, J = 12.3 Hz, 1H), 4.70 (app d, J = 11.2 Hz, 1H), 4.63 – 4.37 (m, 6H), 4.32 (dd, J = 10.6, 5.0 Hz, 1H), 4.19 (app t, J = 9.7 Hz, 1H), 4.00 – 3.86 (m, 3H), 3.85 – 3.55 (m, 10H), 3.37 – 3.25 (m, 1H), 2.36 – 2.15 (m, 3H), 2.01 (s, 3H), 2.00 – 1.91 (m, 1H), 1.90 – 1.78 (m, 2H), 1.64 – 1.46 (m + H$_2$O, 1H), 1.42 – 1.28 (m, 1H). $^{13}$C NMR (100MHz, CDCl$_3$, selected signals): δ 169.95, 152.50, 140.28, 138.57, 138.27, 138.09, 137.82, 137.23, 133.34, 128.75, 128.72, 128.36, 128.30, 128.29, 128.21, 128.18, 128.11, 128.07, 127.87, 127.72, 127.64, 127.63, 127.56, 127.50, 125.91, 101.08, 100.05, 98.75, 78.63, 77.87, 77.67, 76.34, 75.14, 74.95, 74.88, 74.24, 73.32, 71.99, 71.59, 69.10, 68.47, 68.09, 67.31, 58.42, 53.42, 33.18, 31.48, 28.39, 20.98. IR (cm$^{-1}$): 2941, 2866, 1734, 1044, 1026, 735, 696. MS (ESI+): calcd. for C$_{63}$H$_{73}$N$_2$O$_{16}$S$^+$ [M + NH$_4^+$] 1145.47, found 1145.22. [α]$_D$(c 0.4, DCM, –24.2)

Partially deprotected cyclohexyl linked disaccharide (SI-3).

100 mg (0.0887 mmol, 1 equiv) of 6 was dissolved in 27 mL MeOH in a 50 mL flask. 1.25 mL (3.55 mmol, 40 equiv) of a 25% NaOMe solution in MeOH was added. The reaction was left stirring at room temperature under N$_2$ for 13 hours until reaction was complete based on LC–MS and TLC monitoring. Upon completion, the volume of the mixture was reduced to a half by rotary evaporation, then saturated NH$_4$Cl solution was used to quench the
reaction. The mixture was extracted with DCM three times. The combined organic layers were dried over MgSO₄, filtered and concentrated. Purification by flash chromatography (1:1 ethyl acetate/hexanes) afforded 70 mg (0.068 mmol, 77%) of SI-3 as an off–white foam. ¹H NMR (400MHz, CDCl₃): δ 7.91 (app d, J = 7.4 Hz, 2H), 7.58 (app t, J = 7.2 Hz, 1H), 7.52 (app t, J = 8.0 Hz, 2H), 7.45 – 7.12 (m, 23H + CHCl₃), 5.55 (s, 1H), 5.33 (s, 1H), 5.02 (app d, J = 7.4 Hz, 1H), 4.87 (app d, J = 11.5 Hz, 2H), 4.76 (app d, J = 12.3 Hz, 1H), 4.67 (app d, J = 11.6 Hz, 1H), 4.60 (app d, J = 11.6 Hz), 4.56 – 4.43 (m, 4H), 4.32 – 4.23 (m, 1H), 4.21 – 4.16 (m, 1H), 4.13 (app t, J = 9.6 Hz, 1H), 3.98 (dd, J = 10.1, 3.0 Hz, 1H), 3.94 – 3.81 (m, 4H), 3.81 – 3.69 (m, 5H), 3.69 – 3.59 (m, 2H), 3.44 – 3.35 (m, 1H), 3.10 (s, 1H), 2.29 – 1.81 (m, 4H), 1.58 – 1.04 (m, 4H). ¹³C NMR (100MHz, CDCl₃, selected signals): δ 141.12, 138.46, 138.30, 138.26, 137.75, 132.59, 129.13, 128.95, 128.51, 128.30, 128.29, 128.27, 128.21, 128.16, 127.92, 127.86, 127.70, 127.68, 127.58, 127.53, 126.87, 125.98, 101.46, 100.34, 100.01, 79.82, 78.63, 78.12, 75.66, 75.29, 74.89, 74.31, 73.35, 71.83, 69.29, 68.58, 67.96, 67.31, 51.58, 31.05, 30.77, 30.70, 29.27. IR (cm⁻¹): 3400, 3268, 2862, 1452, 1066, 734, 694. MS (ESI+): calcd. for C₅₉H₆₉N₂O₁₃S¹⁺[M + NH₄⁺] 1045.45, found 1045.34. [α]D (c 0.4, DCM, –22.0).

Fully deprotected cyclohexyl linked disaccharide amine (SI-4)

Along with a stream of N₂, ammonia gas was condensed against a -78 °C cold finger into a -78 °C-cooled 50 mL 3-necked flask equipped with a glass stir bar, until 25 mL had accumulated. 94 mg (4.1 mmol, 60 equiv) Na metal was then added, and the resulting blue solution was left to stir for 1 hour. 70 mg (0.068 mmol, 1 equiv) of SI-3 in 4 mL of THF was then added. The reaction progress was monitored by LC–MS. After 5 hours, 251 mg (4.70 mmol, 69 equiv) NH₄Cl was added to quench the reaction. At this time, the cooling bath was removed and the ammonia was blown off under a stream of N₂. The crude white solids were dissolved in water and desalted by passage through a Biorad P-2 Biogel (Fine) size exclusion column to give 28 mg of partially purified SI-4. ¹H NMR (400MHz, D₂O): δ 5.11 (s, 1H), 4.81 (s, 1H), 4.13 – 4.02 (m, 2H), 3.96 – 3.84 (m, 4H), 3.84 – 3.71 (m, 5H), 3.71 – 3.59 (m, 2H), 3.44 – 3.35 (m, 1H), 3.10 (s, 1H), 2.29 – 1.81 (m, 4H), 1.58 – 1.04 (m, 4H). ¹³C NMR (100 MHz, D₂O): δ 105.14, 100.63, 83.34, 79.07, 78.87, 76.14, 73.63, 73.19, 72.87, 69.63, 68.99, 63.80, 51.82, 33.28, 32.05, 31.77, 31.66. IR (cm⁻¹): 3228, 2935, 2092, 1632, 1052. MS (ESI+): calcd. for C₁₈H₃₄NO₁₁⁺[M + H⁺] 440.21, found 440.16. [α]D (c 1.0, H₂O, 6.1).

Cyclohexyl linked disaccharide azide (7, Man₂–Cy–N₃)
To a 20 mL vial containing 24 mg (0.054 mmol, 1.0 equiv) of crude amine SI-4 was added 0.27 mL water, 1.4 mg (0.0054 mmol, 0.1 equiv) of CuSO₄·5H₂O and 22 mg (0.16 mmol, 3.0 equiv) of K₂CO₃. 1 mL of MeOH was added, followed by the addition of 2.2 mL (0.54 mmol, 10.0 equiv) of freshly prepared 0.25M TfN₃ in DCM. The resulting homogeneous reaction was left to stir at room temperature for 4 hours until complete conversion was observed by LC–MS. The reaction was quenched with 45 mg (0.54 mmol, 10.0 equiv) solid NaHCO₃ and concentrated in vacuo. The residue was desalted on a Biorad P-2 Biogel (Fine) size exclusion column. Reversed phase HPLC purification (Column: Waters Xbridge Prep, C18, 5μm, 130Å, 10x250mm. Method: 4mL/min flow rate. A= H₂O/0.1%FA, B= ACN/0.1%FA. 98.2% A for 1min, then 98.2% A to 60% A over 24 minutes, then 60% to 5% A over 5 minutes. Desired product eluted at 16.4 minutes.) providing 12 mg (0.026 mmol, 38%, 2 steps) of Man₂–Cy–N₃ (7) as a glassy solid.

1H NMR (400MHz, D₂O): δ 5.11 (s, 1H), 4.81 (s, 1H), 4.13 – 4.04 (m, 2H), 3.95 – 3.86 (m, 3H), 3.87 – 3.79 (m, 2H), 3.79 – 3.72 (m, 4H), 3.71 – 3.61 (m, 2H), 3.58 – 3.46 (m, 1H), 3.45 – 3.33 (m, 1H), 2.20 – 1.80 (m, 4H), 1.54 – 1.32 (m, 4H).

13C NMR (100 MHz, D₂O): δ 105.15, 100.54, 83.39, 78.83, 76.12, 73.64, 73.17, 72.86, 69.62, 68.97, 63.81, 61.68, 32.82, 31.46, 31.13, 30.98. IR (cm⁻¹): 3340, 2937, 2094, 1057. MS (ESI+): calcd. for C₁₈H₃₁KN₃O₁₀⁺ [M + K⁺] 488.16, found 488.12. [α]D (c 0.5, H₂O, 3.0).

Synthesis of Man₃–Cy–N₃ (9)

Fully protected cyclohexyl linked trisaccharide (8)

50 mg (0.077 mmol, 1 equiv) of 4²⁻¹² and 111 mg (0.115 mmol, 1.5 equiv) of SI-5 ⁸⁻¹² were dissolved in toluene in a 25 mL flask and freeze pumped for three times. The dry residue was dissolved in 3 mL of acetonitrile, freshly flame-dried 4Å molecular sieves were added, and this was allowed to stir for 1 hour. The flask was then wrapped in foil, cooled to 0 °C, and 150 mg (0.184 mmol, 2.4 equiv) Sinaŷ reagent, [(p-BrC₆H₄)₃N⁺ SbCl₆⁻] was added. This was allowed to react at 0 °C for 30 minutes and then at room temperature for 30 minutes. After this time, triethylamine was added, and the reaction was filtered through celite and concentrated in vacuo. The crude residue was purified by flash chromatography with 1:3:5:1 ethyl acetate / hexanes / DCM to give 60 mg (0.038 mmol, 50%) 8, as an off-white foam. ¹H NMR (400MHz, CDCl₃): δ 7.92 (app d, J = 7.6 Hz, 2H), 7.60 (app t, J = 7.4 Hz, 1H), 7.52 (app t, J = 7.7 Hz, 2H), 7.46 – 7.36 (m, 4H), 7.36 – 7.09 (m, 35H + CHCl₃), 7.06 (app t, J = 7.4 Hz, 1H), 5.47 (s, 1H), 5.43 (s, 1H), 5.10 (s, 1H), 4.92 – 4.75 (m, 5H), 4.75 – 4.16 (m, 15H), 4.07 (app t, J = 9.5 Hz, 1H), 4.01 – 3.60 (m, 15H), 3.59 – 3.46 (m, 1H), 3.40 (app d, J = 10.9 Hz, 1H), 3.28 – 3.13 (m, 2H), 2.33 – 2.20 (m, 2H), 2.20 – 2.11 (m, 1H + Acetone), 2.08 (s, 3H), 1.59 – 1.41 (m, 1H), 1.35 – 1.13 (m, 2H + Ethyl acetate). ¹³C NMR (100MHz, CDCl₃, selected signals): δ 170.15, 152.51, 140.30, 138.66, 138.60, 138.44, 138.37, 138.29, 138.11, 138.08, 137.45, 137.45,
Partially deprotected cyclohexyl linked trisaccharide (SI-6)

55 mg (0.035 mmol, 1 equiv) of 8 was dissolved in 15 mL MeOH in a 25 mL flask. 0.4 mL (1.4 mmol, 40 equiv) of a 25% NaOMe solution in MeOH was added. The reaction was left stirring at room temperature under N₂ for 13 hours until reaction was completed based on LC–MS and TLC monitoring. Upon completion, the volume of the mixture was reduced to a half by rotary evaporation, and saturated NH₄Cl solution was added to quench the reaction. The mixture was extracted with DCM three times. The combined organic layers were dried over MgSO₄, filtered and concentrated. Purification by flash chromatography (2:3 ethyl acetate/hexanes) afforded 40 mg of SI-6 as an off–white foam. Analysis by LC–MS shows tiny amount of impurity. Reversed phase HPLC purification (Column: Waters Xbridge Prep, C18, 5μm, 130Å, 10x250mm. Method: 4mL/min flow rate. A= H₂O/0.1%FA, B= ACN/0.1%FA. 21% A to 1% A over 25 minutes, then keep for 15 minutes. Desired product eluted at 20.9 minutes.) providing 37 mg (0.025 mmol, 71%) of SI-6 as an off–white foam. ¹H NMR (400MHz, CDCl₃): δ 7.94 – 7.84 (m, 2H), 7.58 (app t, J = 7.3 Hz, 1H), 7.52 (app t, J = 7.5 Hz, 2H), 7.37 (app t, J = 8.0 Hz, 4H), 7.34 – 7.14 (m, 35H + CHCl₃), 7.08 (app t, J = 7.3Hz, 1H), 5.40 (s, 1H), 5.31 (s, 1H), 5.17 (s, 1H), 4.87 – 4.68 (m, 4H), 4.66 – 4.57 (m, 1H), 4.56 – 4.32 (m, 9H), 4.23 (dd, J = 12 Hz, 2.4 Hz, 2H), 4.19 – 4.12 (m, 1H), 4.07 – 3.98 (m, 2H), 3.95 – 3.82 (m, 2H), 3.83 – 3.68 (m, 5H), 3.65 (s, 2H), 3.52 – 3.41 (m, 1H), 3.41 – 3.33 (m, 1H), 3.27 (app d, J = 10.8 Hz, 1H), 3.21 – 3.07 (m, 2H), 1.96 – 1.87 (m, 1H), 1.86 – 1.75 (m, 2H), 1.73 – 1.63 (m, 1H), 1.44 – 1.29 (m, 1H), 1.28 – 1.04 (m, 3H). ¹³C NMR (100MHz, CDC₁₃, selected signals): δ 141.07, 138.57, 138.45, 138.38, 138.21, 138.06, 138.01, 137.37, 132.58, 129.11, 128.84, 128.42, 128.38, 128.33, 128.25, 128.23, 128.07, 127.97, 127.78, 127.75, 127.65, 127.60, 127.46, 127.42, 127.38, 127.30, 126.85, 125.94, 101.28, 99.82, 79.94, 78.37, 78.11, 75.60, 75.07, 74.93, 74.53, 74.12, 73.35, 73.19, 73.00, 72.42, 72.05, 71.94, 71.46, 69.69, 68.52, 68.45, 67.31, 51.59, 40.68, 31.02, 30.80, 29.26. IR (cm⁻¹): 3502, 3301, 3029, 2860, 1452, 1046, 734, 694. MS (ESI+): calcd. for C₉₆H₆₇N₂O₁₈S⁺ [M + NH₄⁺] 1477.66, found 1477.73. [α]D (c 1.0, DCM, −5.5).

Fully deprotected cyclohexyl linked trisaccharide amine (SI-7)
Along with a stream of N\textsubscript{2}, ammonia gas was condensed against a -78 °C cold finger into a -78 °C-cooled 50 mL 3-necked flask, equipped with glass-coated stir bar, until 25 mL had accumulated. 52 mg (2.3 mmol, 1 equiv) Na metal was then added, and the resulting blue solution was left to stir for 1 hour. 33 mg (0.023 mmol, 1 equiv) of SI-6 in 4 mL of THF was then added. The reaction progress was monitored by LC–MS. After 3 hours, 139 mg (2.60 mmol, 115 equiv) NH\textsubscript{4}Cl was added to quench the reaction. At this time, the cooling bath was removed and the ammonia was blown off under a stream of N\textsubscript{2}. The crude white solids were dissolved in water and desalted by passage through a Biorad P-2 Biogel (Fine) size exclusion column to give 50 mg crude. The mixture was desalted again and 18 mg of partially purified SI-7 was obtained. This material was used in the next step without further purification. \textsuperscript{1}H NMR (400MHz, D\textsubscript{2}O): \(\delta\) 5.36 (s, 1H), 5.05 (s, 1H), 4.80 (s, 1H), 4.13 – 4.09 (m, 1H), 4.09 – 4.05 (m, 2H), 4.00 (dd, \(J = 9.5, 3.3\) Hz, 1H), 3.96 – 3.82 (m, 4H), 3.82 – 3.61 (m, 10H), 3.39 (app t, \(J = 8.6\) Hz, 1H), 3.04 – 2.77 (m, 1H), 2.17 – 2.02 (m, 2H), 2.01 – 1.87 (m, 2H), 1.56 – 1.09 (m, 4H). \textsuperscript{13}C NMR (100 MHz, D\textsubscript{2}O): \(\delta\) 112.94, 105.12, 103.49, 100.61, 83.43, 81.27, 79.62, 78.88, 76.18, 76.08, 73.17, 73.08, 69.57, 69.05, 63.82, 51.72, 33.58, 33.24, 33.08, 32.34. IR (cm\textsuperscript{-1}): 3223, 2934, 1686, 1025. MS (ESI+): calcd. for C\textsubscript{24}H\textsubscript{44}NO\textsubscript{16}\([M + H]^{+}\) 602.27, found 602.15. [\(\alpha\)]\textsubscript{D} (c 0.5, H\textsubscript{2}O, 8.5).

Cyclohexyl linked trisaccharide azide (9, Man\textsubscript{3}–Cy–N\textsubscript{3})

Into a 20 mL vial containing 13.5 mg (0.0230 mmol, 1.0 equiv) of crude amine SI-7 was added 113 μL water, 9.2 μL (0.0023 mmol, 0.1 equiv) of 0.25 M aqueous CuSO\textsubscript{4} and 9.4 mg (0.068 mmol, 3.0 equiv) of K\textsubscript{2}CO\textsubscript{3}. 0.5 mL of MeOH was added, followed by the addition of 0.9 mL (0.226 mmol, 10.0 equiv) of freshly prepared 0.25M TfN\textsubscript{3} in DCM. The resulting homogeneous reaction was left to stir at room temperature for 3 hours until complete conversion was observed by LC–MS. The reaction was quenched with 19 mg (0.23 mmol, 10.0 equiv) solid NaHCO\textsubscript{3} and concentrated in vacuo. The residue was desalted on a Biorad P-2 Biogel (Fine) size exclusion column. Reversed phase HPLC purification (Column: Waters Xbridge Prep, C18, 5μm, 130Å, 10x250mm. Method: 4mL/min flow rate. A= H2O/0.1%FA, B= ACN/0.1%FA. 98.2% A for 1min, then 98.2% A to 60% A over 24 minutes, then 60% to 5% A over 5 minutes. Desired product eluted at 15.7 minutes.) providing 5 mg (0.008 mmol, 35%, 2 steps) of Man\textsubscript{3}–Cy–N\textsubscript{3} (9) as a glassy solid. \textsuperscript{1}H NMR (400MHz, D\textsubscript{2}O): \(\delta\) 5.34 (s, 1H), 5.03 (s, 1H), 4.77 (s, 1H), 4.11 – 4.07 (m, 1H), 4.07 – 4.03 (m, 2H), 3.98 (dd, \(J = 9.5, 3.3\) Hz, 1H), 3.93 – 3.78 (m, 5H), 3.78 – 3.56 (m, 9H), 3.53 – 3.43 (m, 1H), 3.37 (app t, \(J = 7.2\) Hz, 1H), 2.06 – 1.92 (m, 4H), 1.56 – 1.32 (m, 4H). \textsuperscript{13}C NMR (100
MHz, D$_2$O, selected signals): δ 112.96, 105.15, 103.55, 100.61, 83.51, 81.30, 78.99, 78.90, 76.21, 76.10, 73.69, 73.20, 72.96, 72.84, 69.86, 69.61, 69.08, 63.85, 61.76, 32.92, 31.56, 31.07. IR (cm$^{-1}$): 3365, 2933, 2095, 1059. MS (ESI+): calcd. for C$_{24}$H$_{42}$N$_3$O$_{16}$ [M + H$^+$] 628.26, found 628.08. [α]$_D$(c 0.18, H$_2$O, 11.8).

Synthesis of Man$_3$–Cy–N$_3$ (12)

Reduction of fully protected cyclohexyl linked disaccharide (10)

To a 10 mL flask containing 102 mg (0.0904 mmol, 1 equiv) of 6 toluene was added and freeze pumped three times. 2 mL of DCM and 300 mg freshly dried 4Å molecular sieves were added to the flask. The flask was cooled to –78 °C, and 43 μL (0.27 mmol, 3 equiv) of TES–H was added to the flask, followed by addition of 38 μL (0.31 mmol, 3.4 equiv) PhBCl$_2$. This mixture was allowed to react for 1 hour, and the reaction was quenched with triethylamine and methanol. The mixture was filtered through celite, washed with sat. NaHCO$_3$, dried over MgSO$_4$, filtered, and concentrated. Purification by flash chromatography (2:3 ethyl acetate/hexanes to 1:1 ethyl acetate/hexanes) afforded 71 mg (0.062 mmol, 68%) of 10 as an off–white foam.

$^1$H NMR (400MHz, CDCl$_3$): δ 7.91 (app d, J = 7.6 Hz, 2H), 7.59 (app t, J = 7.4 Hz, 1H), 7.50 (app t, J = 7.7 Hz, 2H), 7.43 – 7.09 (m, 25H + CHCl$_3$), 5.50 (s, 1H), 5.21 (s, 1H), 4.90 (dd, J = 26.9, 11.8 Hz, 2H), 4.78 (app t, J = 10.8 Hz, 2H), 4.65 (dd, J = 26.0, 11.1 Hz, 2H), 4.56 – 4.35 (m, 6H), 4.01 – 3.91 (m, 2H), 3.90 – 3.60 (m, 10H), 3.60 – 3.42 (m, 1H), 3.34 – 3.21 (m, 1H), 2.34 – 2.11 (m, 4H), 2.08 (s, 3H), 1.98 – 1.74 (m, 4H), 1.61 – 1.45 (m, 1H), 1.41 – 1.28 (m, 1H). $^{13}$C NMR (100MHz, CDCl$_3$, selected signals): δ 171.20, 153.70, 141.45, 139.73, 139.64, 139.38, 138.97, 134.55, 129.97, 129.63, 129.58, 129.50, 129.39, 129.38, 129.33, 129.32, 129.07, 128.98, 128.83, 128.79, 128.68, 128.60, 100.76, 81.02, 79.08, 79.06, 77.52, 77.07, 76.39, 76.34, 76.03, 75.53, 75.45, 74.55, 73.28, 73.06, 70.43, 69.90, 63.28, 61.56, 59.60, 54.63, 34.45, 32.70, 29.62, 22.17, 15.40. IR (cm$^{-1}$): 3025, 2931, 2870, 1733, 1043, 734, 696. MS (ESI+): calcd. for C$_{63}$H$_{75}$N$_2$O$_{16}$S$^+$ [M + NH$_4^+$] 1147.48, found 1147.62. [α]$_D$(c 1.0, DCM, –14.8).

Fully protected cyclohexyl linked trisaccharide (11)

To a 25 mL flask containing 70 mg (0.062 mmol, 1 equiv) of 10 and 79 mg (0.12 mmol, 2 equiv) of donor SI-8 was added toluene and freeze pumped for three times. 200 mg freshly dried 4Å molecular sieves were added and the mixture was redissolved in 2 mL DCM and cooled in a –20 °C salt ice bath for 30 minutes. The 25 mL flask
was then wrapped in foil and 8 mg (0.03 mmol, 0.5 equiv) AgOTf was added, followed by 29 mg (0.13 mmol, 2.1 equiv) of NIS. This mixture was allowed to react for 2.5 hours, and the reaction was quenched with triethylamine. The mixture was filtered through celite and extracted with EA three times. The combined organic layers were dried over MgSO₄, filtered, and concentrated. Purification by flash chromatography (1:3:1 ethyl acetate/hexanes/DCM) afforded 70 mg (0.041 mmol, 66%) of 11 as an off-white foam. 

**1H NMR (400MHz, CDCl₃):** δ 7.87 (app d, J = 7.8 Hz, 2H), 7.74 – 7.61 (m, 1H), 7.56 (app t, J = 7.5 Hz, 1H), 7.50 – 7.40 (m, 4H), 7.37 – 7.01 (m, 40H + CHCl₃), 5.71 (s, 1H), 5.52 (s, 1H), 5.20 (s, 1H), 5.11 – 4.98 (m, 2H), 4.86 (dd, J = 15.6, 11.0 Hz, 2H), 4.82 – 4.61 (m, 5H), 4.36 – 3.31 (m, 1H), 2.32 – 2.14 (m, 3H), 2.07 (s, 3H), 2.02 – 1.91 (m, 1H), 1.87 – 1.78 (m, 1H), 1.75 – 1.58 (m, 2H), 1.56 – 1.43 (m, 1H), 1.40 – 1.25 (m, 1H). 

**13C NMR (100MHz, CDCl₃, selected signals):** δ 169.95, 152.36, 140.25, 138.70, 138.51, 138.42, 138.41, 138.30, 138.23, 138.19, 138.18, 138.16, 138.09, 138.07, 138.02, 127.90, 127.79, 127.67, 127.61, 127.60, 127.55, 127.50, 127.38, 127.33, 127.21, 118.44, 118.18, 99.91, 99.63, 97.90, 80.30, 77.99, 77.68, 77.59, 76.39, 75.19, 74.96, 74.77, 74.49, 74.25, 74.10, 73.94, 73.31, 73.14, 71.97, 71.86, 71.49, 71.36, 69.46, 69.07, 68.67, 66.79, 58.43, 53.22, 33.26, 31.57, 28.51, 28.37, 20.90. IR (cm⁻¹): 2890, 2800, 1734, 1046, 733, 695. MS (ESI+): calcd. for C₉₇H₁₀₅F₂N₂O₂₂S⁺ [M + NH₄⁺] 1720.69, found 1720.78. 

[α]D (c 1.0, DCM, –3.4).

Partially deprotected cyclohexyl linked trisaccharide (SI-9)

60 mg (0.035 mmol, 1 equiv) of 11 was dissolved in 15 mL MeOH in a 25 mL flask. 0.4 mL (1.4 mmol, 40 equiv) of a 25% NaOMe solution in MeOH was added. The reaction was left stirring at room temperature under N₂ for 13 hours until reaction was completed based on LC–MS and TLC monitoring. Upon completion, the volume of the mixture was reduced to a half by rotary evaporation, and saturated NH₄Cl solution was added to quench the reaction. The mixture was extracted with DCM three times. The combined organic layers were dried over MgSO₄, filtered and concentrated. Purification by flash chromatography (1:1 ethyl acetate/hexanes to 3:2 ethyl acetate/hexanes) afforded 38 mg (0.026 mmol, 74%) of SI-9 as an off-white foam. 

**1H NMR (400MHz, CDCl₃):** δ 7.82 (app d, J = 7.4 Hz, 2H), 7.54 (app t, J = 7.3 Hz, 1H), 7.47 (app t, J = 8.4 Hz, 2H), 7.41 – 7.02 (m, 40H + CHCl₃), 5.21 (s, 1H), 4.99 (s, 1H), 4.91 (app d, J = 12.2 Hz, 1H), 4.80 (app t, J = 10.3 Hz, 2H), 4.73 – 4.55 (m, 6H), 4.56 – 4.37 (m, 8H), 4.20 – 4.02 (m, 2H), 4.02 – 3.94 (m, 1H), 3.90 – 3.55 (m, 15H), 3.52 – 3.37 (m, 1H), 3.36 – 3.24 (m, 1H), 3.14 – 2.91 (m, 1H), 2.36 (s, 2H), 1.89 – 1.66 (m, 4H), 1.37 – 1.18 (m, 1H), 1.16 – 0.90 (m, 3H). 

**13C NMR (100MHz, CDCl₃, selected signals):** δ 141.11, 138.96, 138.55, 138.43, 138.16, 137.85, 137.77, 132.48, 129.03, 128.52, 128.49, 128.34, 128.29, 128.22, 128.08, 127.96, 127.94, 127.92, 127.88, 127.85, 127.78, 127.71, 127.69, 127.66, 127.55, 127.51, 127.26, 126.81, 101.55, 99.59, 99.16, 80.94, 79.94, 79.73, 78.13, 75.22, 75.17, 75.12, 75.07, 74.78, 74.33, 74.24, 74.10, 73.39, 73.34, 72.14, 71.76, 71.38, 70.92, 69.23, 68.79, 68.67, 67.82, 66.22, 51.62, 51.28, 30.89, 30.74, 29.15. IR (cm⁻¹): 3029, 2920, 1067, 1043. MS (ESI+): calcd. for C₉₆H₉₆N₂O₁₈S⁺ [M + NH₄⁺] 1479.66, found 1479.75. [α]D (c 1.0, DCM, 10.0).
Fully deprotected cyclohexyl linked trisaccharide amine (SI-10)

Along with a stream of N₂, ammonia gas was condensed against a -78 °C cold finger into a -78 °C-cooled 50 mL 3-necked flask equipped with a glass-coated stir bar, until 25 mL had accumulated. 43 mg (1.8 mmol, 90 equiv) Na metal was then added, and the resulting blue solution was left to stir for 1 hour. 30 mg (0.021 mmol, 1 equiv) of SI-9 in 2 mL of THF was then added. The reaction progress was monitored by LC–MS. After 4 hours, 113 mg (2.11 mmol, 103 equiv) NH₄Cl was added to quench the reaction. At this time, the cooling bath was removed and the ammonia was blown off under a stream of N₂. The crude white solids were dissolved in water and desalted by passage through a Biorad P-2 Biogel (Fine) size exclusion column to give 70 mg crude. The mixture was desalted again and 13 mg of partially purified SI-10 was obtained. This material was used in the next step without further purification. ¹H NMR (400MHz, D₂O): δ 5.10 (s, 1H), 4.91 (s, 1H), 4.81 (s, 1H), 4.17 – 4.04 (m, 2H), 4.01 – 3.85 (m, 5H), 3.84 – 3.62 (m, 11H), 3.59 – 3.50 (m, 1H), 3.07 – 2.92 (m, 1H), 2.22 – 1.80 (m, 4H), 1.54 – 1.12 (m, 4H). ¹³C NMR (100 MHz, D₂O): δ 164.06, 102.38, 99.37, 98.07, 80.75, 76.85, 74.06, 73.31, 72.69, 70.78, 70.65, 70.37, 70.06, 69.94, 66.78, 66.71, 65.99, 65.63, 60.97, 48.92, 30.73, 29.73, 29.61, 29.41. IR (cm⁻¹): 3275, 2946, 1436, 1027. MS (ESI+): calcd. for C₂₄H₄₄NO₁₆⁺ [M + H⁺] 602.27, found 602.15. [α]D (c 0.26, H₂O, 3.0).

Cyclohexyl linked trisaccharide azide (12, Man₃–Cy–N₃)

Into a 20 mL vial containing 10 mg (0.017 mmol, 1.0 equiv) of crude amine SI-10 was added 83 μL water, 6.8 μL (0.0017 mmol, 0.1 equiv) of 0.25M aqueous CuSO₄ and 7 mg (0.05 mmol, 3.0 equiv) of K₂CO₃. 166 μL of MeOH was added, followed by the addition of 0.7 mL (0.17 mmol, 10.0 equiv) of freshly prepared 0.25M TfN₃ in DCM. The resulting homogeneous reaction was left to stir at room temperature for 3 hours until complete conversion was observed by LC–MS. The reaction was quenched with 18 mg (0.22 mmol, 13.0 equiv) solid NaHCO₃ and concentrated in vacuo. The residue was desalted on a Biorad P-2 Biogel (Fine) size exclusion column. Reversed phase HPLC purification (Column: Waters Xbridge Prep, C18, 5μm, 130Å, 10x250mm. Method: 4mL/min flow
rate. A = H2O/0.1%FA, B = ACN/0.1%FA. 98.2% A for 1 min, then 98.2% A to 60% A over 24 minutes, then 60% to 5% A over 5 minutes. Desired product eluted at 15.1 minutes.)

providing 6 mg (0.01 mmol, 48%, 2 steps) of Man3−Cy−N3 (12) as a glassy solid. 1H NMR (400 MHz, D2O): δ 5.09 (s, 1H), 4.90 (s, 1H), 4.79 (s, 1H), 4.07 (app d, J = 16 Hz, 2H), 4.01 – 3.84 (m, 4H), 3.83 – 3.61 (m, 12H), 3.59 – 3.38 (m, 2H), 2.13 – 1.92 (m, 4H), 1.63 – 1.29 (m, 4H). 13C NMR (100 MHz, D2O, selected signals): δ 105.23, 102.20, 100.87, 83.64, 79.24, 76.89, 76.14, 75.51, 73.65, 73.46, 73.20, 72.90, 72.77, 69.63, 69.55, 68.78, 68.47, 63.82, 61.71, 32.99, 31.59, 31.20, 31.08. IR (cm−1): 3301, 2940, 2095, 1044. MS (ESI+): calcd. for C24H42N3O16+ [M + H+] 628.26, found 628.23. [α]D (c 0.21, H2O, −7.0).

Synthesis of Mans−Cy−N3 (14)

Fully protected cyclohexyl linked pentasaccharide (13)

101 mg (0.0894 mmol, 1 equiv) of 10 and 200 mg (0.143 mmol, 1.6 equiv) of SI-119-12 in a 25 mL flask were dissolved in toluene and freeze pumped for three times. The dry residue was dissolved in 4 mL of acetonitrile, freshly flame-dried 4Å molecular sieves were added, and was allowed to stir for 1 hour. The flask was then wrapped in foil, cooled to 0 °C, and 175 mg (0.214 mmol, 2.4 equiv) Sinaï reagent, [(p−BrC6H4)3N+ SbCl6−] was added. This mixture was allowed to react at 0 °C for 30 minutes and react at room temperature for 30 minutes. After this time, triethylamine was added, and the reaction was filtered through celite and concentrated in vacuo. The crude residue was purified by flash chromatography with 1:2.5:1 ethyl acetate / hexanes / DCM to give 138 mg (0.0570 mmol, 63.8%), as an off-white foam. 1H NMR (400 MHz, CDCl3): δ 7.89 (app d, J = 7.7 Hz, 2H), 7.56 (app t, J = 7.3 Hz, 1H), 7.48 (app t, J = 7.6 Hz, 2H), 7.40 – 6.95 (m, 65H + CHCl3), 5.58 (s, 1H), 5.55 – 5.41 (m, 2H), 5.23 (app d, J = 7.7 Hz, 2H), 5.12 (s, 1H), 4.98 (s, 1H), 4.94 – 4.79 (m, 6H), 4.76 – 4.30 (m, 24H), 4.21 – 3.41 (m, 34H), 3.30 – 3.10 (m, 1H), 2.28 – 1.63 (m, 9H + ethyl acetate), 1.52 – 1.17 (m, 2H + ethyl acetate). 13C NMR (100 MHz, CDCl3, selected signals): δ 170.29, 170.14, 170.03, 152.65, 140.48, 138.80, 138.80, 138.72, 138.60, 138.41, 138.35, 138.33, 138.27, 138.06, 137.99, 137.96, 133.39, 128.88, 128.50, 128.44, 128.37, 128.33, 128.27, 128.25, 128.17, 128.10, 128.05, 127.89, 127.77, 127.74, 127.71, 127.63, 127.58, 127.49, 127.37, 100.18, 99.82, 98.32, 97.83, 79.80, 78.43, 78.08, 76.65, 75.66, 75.33, 75.29, 75.16, 75.01, 74.90, 74.47, 74.23, 73.81, 73.61, 73.46, 72.43, 72.27, 72.12, 72.08, 71.93, 71.70, 71.30, 71.20, 69.31, 69.03, 68.92, 68.76, 68.47, 66.46, 66.19, 58.59, 53.49, 33.46, 31.87, 28.71, 28.54, 21.27, 21.10, 14.36. IR (cm−1): 3030, 2940, 1738, 1047, 733, 695. MS (ESI+): calcd. for C141H157N2O33S+ [M + NH4+] 2439.04, found 2439.09. [α]D (c 1.0, DCM, 21.0).

Partially protected cyclohexyl linked pentasaccharide (SI-12)
130 mg (0.0537 mmol, 1 equiv) of 13 was dissolved in 1.5 mL MeOH and 3 mL THF in a 25 mL flask. 77 μL (2.15 mmol, 5 equiv) of a 25% NaOMe solution in MeOH was added. The reaction was left stirring at room temperature under N₂ for 13 hours until reaction was completed based on LC–MS and TLC monitoring. Upon completion, the volume of the mixture was reduced to a half by rotary evaporation, and saturated NH₄Cl solution was added to quench the reaction. The mixture was extracted with DCM three times. The combined organic layers were dried over MgSO₄, filtered and concentrated. Purification by flash chromatography (1:1 ethyl acetate/hexanes to 3:2 ethyl acetate/hexanes) afforded 73 mg (0.033 mmol, 61%) of SI-12 as an off-white foam.

**¹H NMR (400MHz, CDCl₃):** δ 7.84 (app d, J = 7.5 Hz, 2H), 7.48 (app t, J = 7.3 Hz, 1H), 7.41 (app t, J = 7.5 Hz, 1H), 7.37 – 7.07 (m, 66H + CHCl₃), 5.43 – 5.27 (m, 1H), 5.22 (s, 1H), 5.14 (s, 1H), 4.97 – 4.77 (m, 5H), 4.77 – 4.33 (m, 22H), 4.22 (s, 1H), 4.19 – 3.54 (m, 27H), 3.53 – 3.35 (m, 2H), 3.21 – 2.95 (m, 2H), 2.88 (s, 1H), 2.44 (s, 1H), 2.35 (s, 1H), 2.00 – 1.76 (m, 2H), 1.75 – 1.63 (m, 2H), 1.60 – 1.45 (m, 1H), 1.40 – 0.60 (m, 4H). **¹³C NMR (100MHz, CDCl₃, selected signals):** δ 142.09, 138.75, 138.69, 138.61, 138.59, 138.49, 138.43, 138.33, 138.23, 138.15, 137.96, 137.89, 137.79, 137.73, 132.28, 129.05, 128.74, 128.67, 128.61, 128.56, 128.50, 128.46, 128.44, 128.38, 128.25, 128.06, 128.02, 127.91, 127.87, 127.81, 127.76, 127.67, 127.45, 126.98, 101.91, 101.62, 99.93, 98.49, 96.79, 80.56, 80.29, 80.05, 80.02, 79.86, 76.63, 75.49, 75.25, 75.16, 74.98, 74.94, 74.79, 74.74, 74.66, 74.46, 74.04, 73.68, 73.56, 73.53, 72.38, 72.32, 72.22, 72.05, 71.89, 71.75, 71.61, 71.48, 69.80, 69.34, 69.06, 68.91, 68.78, 68.14, 66.07, 65.76, 51.67, 31.94, 31.85, 31.29, 29.46. **IR (cm⁻¹):** 3020, 2927, 1068, 1026. **MS (ESI+):** calcd. for C₁₃₃H₁₄₉N₂O₂₈S⁺ [M + NH₄⁺]: m/z 2255.00, found 2255.05. [α]D (c 1.0, DCM, 22.5).

Fully deprotected cyclohexyl linked pentasaccharide amine (SI-13)

Along with a stream of N₂, ammonia gas was condensed against a -78 °C cold finger into a -78 °C-cooled 50 mL 3-necked flask equipped with glass-covered stir bar, until 25 mL had accumulated. 101 mg (4.38 mmol, 140 equiv) Na metal was then added, and the resulting blue solution was left to stir for 1 hour. 70 mg (0.031 mmol, 1 equiv) of SI-12 in 4 mL of THF was then added. The reaction progress was monitored by LC–MS. After 3 hours, 270 mg (5.04 mmol, 161 equiv) NH₄Cl was added to quench the reaction. At this time, the cooling bath was removed and the ammonia was blown off under a stream of N₂. The crude white solids were dissolved in water and desalted by passage through a Biorad P-2 Biogel (Fine) size exclusion column to give 70 mg crude. The mixture was desalted again and 29 mg of partially purified SI-13 was obtained. This material was used in the next step without further purification. **¹H NMR (400MHz, D₂O):** δ 5.15 (s, 1H), 5.11 (s, 1H), 4.92 (s, 1H), 4.89 (s, 1H), 4.82 (s, 1H), 4.22 – 4.14 (m, 1H), 4.13 – 4.07 (m, 3H), 4.04 – 3.63 (m, 26H), 3.62 – 3.50 (m, 1H), 3.20 – 3.10 (m, 1H), 2.18 – 2.03 (m,
4H), 1.57 – 1.34 (m, 4H). $^{13}$C NMR (100 MHz, D$_2$O, selected signals): $\delta$ 105.21, 105.16, 102.38, 102.17, 101.12, 83.60, 81.43, 79.51, 76.93, 76.16, 75.58, 73.76, 73.63, 73.44, 73.24, 72.96, 72.92, 72.83, 72.36, 69.65, 69.61, 69.57, 68.78, 68.58, 68.14, 63.84, 63.76, 51.94, 33.39, 32.14, 31.59, 31.46. IR (cm$^{-1}$): 3273, 2969, 1024. MS (ESI+): calcd. for C$_{36}$H$_{64}$NO$_{26}$ $[M + H^+]$ 926.37, found 926.15. $[\alpha]_D$(c 1.0, H$_2$O, 42.7).

Cyclohexyl linked pentasaccharide azide (14, Man$_5$–Cy–N$_3$)

Into a 20 mL vial containing 26 mg (0.028 mmol, 1.0 equiv) of crude amine SI-13 was added 140 μL water, 11 μL (0.0028 mmol, 0.1 equiv) of 0.25M aqueous CuSO$_4$ and 11.6 mg (0.0840 mmol, 3.0 equiv) of K$_2$CO$_3$. 280 μL of MeOH was added, followed by the addition of 1.1 mL (0.167 mmol, 10.0 equiv) of freshly prepared 0.25M TfN$_3$ in DCM. The resulting homogeneous reaction was left to stir at room temperature for 4 hours until complete conversion was observed by LC–MS. The reaction was quenched with 30.6 mg (0.365 mmol, 13.0 equiv) solid NaHCO$_3$ and concentrated in vacuo. The residue was desalted on a Biorad P-2 Biogel (Fine) size exclusion column. Reversed phase HPLC purification (Column: Waters Xbridge Prep, C18, 5μm, 130Å, 10x250mm. Method: 4mL/min flow rate. A= H$_2$O/0.1% FA, B= ACN/0.1% FA. 98.2% A for 1min, then 98.2% A to 60% A over 24 minutes, then 60% to 5% A over 5 minutes. Desired product eluted at 13.6 minutes.) providing 11 mg (0.012 mmol, 39%, 2 steps) of Man$_5$–Cy–N$_3$ (14) as a glassy solid. $^1$H NMR (400MHz, D$_2$O): $\delta$ 5.12 (s, 1H), 5.09 (s, 1H), 4.90 (s, 1H), 4.87 (s, 1H), 4.79 (s, 1H), 4.19 – 4.12 (m, 1H), 4.11 – 4.04 (m, 3H), 4.03 – 3.94 (m, 2H), 3.94 – 3.61 (m, 24H), 3.59 – 3.41 (m, 2H), 2.10 – 1.94 (m, 4H), 1.54 – 1.24 (m, 4H). $^{13}$C NMR (100 MHz, D$_2$O, selected signals): $\delta$ 105.21, 102.27, 102.14, 100.83, 83.64, 81.56, 79.20, 76.91, 76.16, 75.55, 73.73, 73.63, 73.42, 73.23, 73.20, 72.92, 72.89, 72.80, 72.33, 69.63, 69.58, 68.78, 68.56, 68.47, 68.10, 63.81, 61.67, 33.01, 31.58, 31.21, 31.09. IR (cm$^{-1}$): 3340, 2931, 2996, 1027. MS (ESI+): calcd. for C$_{36}$H$_{62}$N$_3$O$_{26}$ $[M + H^+]$ 952.36, found 952.55. $[\alpha]_D$(c 0.5, H$_2$O, 41.4).

Synthesis of Man$_2$–Cy–N$_3$ (16)

Partially deprotected cyclohexyl linked monosaccharide (SI-14)
To a 10 mL flask containing 50 mg (0.065 mmol, 1 equiv) of 2 toluene was added and freeze pumped three times. 1 mL of DCM and 100 mg freshly dried 4Å molecular sieves were added to the flask. The flask was cooled to −78 °C, and 31 μL (0.19 mmol, 3 equiv) of TES–H was added to the flask, followed by addition of 28 μL (0.22 mmol, 3.4 equiv) PhBCl₂. This mixture was allowed to react for 1 hour, and the reaction was quenched with triethylamine and methanol. The mixture was filtered through celite, washed with sat. NaHCO₃, dried over MgSO₄, filtered, and concentrated. Purification by flash chromatography (2:3 ethyl acetate/hexanes) afforded 12 mg (0.016 mmol, 25%) of SI-14 as an off–white foam.¹H NMR (400MHz, CDCl₃): δ 7.92 (app d, J = 7.6 Hz, 2H), 7.61 (app t, J = 7.6 Hz, 1H), 7.52 (app t, J = 7.8 Hz, 2H), 7.40 – 7.25 (m, 8H + CHCl₃), 7.21 (app d, J = 7.6 Hz, 2H), 6.84 (app d, J = 7.6 Hz, 2H), 5.05 – 4.82 (m, 3H), 4.73 – 4.58 (m, 1H), 4.55 – 4.37 (m, 3H), 3.97 – 3.85 (m, 2H), 3.85 – 3.73 (m, 4H), 3.72 – 3.56 (m, 4H), 3.56 – 3.40 (m, 1H), 3.40 – 3.23 (m, 1H), 2.42 – 1.21 (m, 11H).¹³C NMR (100MHz, CDCl₃, selected signals): δ 159.52, 152.87, 140.58, 138.88, 138.60, 133.70, 130.51, 129.51, 129.12, 128.78, 128.75, 128.46, 128.45, 128.23, 128.12, 127.85, 114.11, 100.04, 82.43, 76.63, 76.15, 75.55, 75.13, 74.72, 74.34, 71.63, 62.90, 58.77, 53.80, 33.67, 32.00, 28.85, 28.78. IR (cm⁻¹): 3441, 2933, 2094, 1731, 1026. MS (ESI+): calcd. for C₄₂H₅₃N₂O₁₁S¹¹⁺ [M + NH₄⁺] 793.34, found 793.25. [α]D (c 1.0, DCM, −44.1).

Fully protected cyclohexyl linked disaccharide (15)

To a 25 mL flask containing 22 mg (0.028 mmol, 1 equiv) of SI-14 and 36 mg (0.057 mmol, 2 equiv) of donor SI-8 toluene was added and freeze pumped three times. 100 mg freshly dried 4Å molecular sieves were added to the 25 mL flask. SI-14 was redissolved in 2 mL DCM and cooled in a −20 °C salt ice bath for 30 minutes. The 25 mL flask was then wrapped in foil and 3.6 mg (0.014 mmol, 0.5 equiv) AgOTf was added, followed by 14 mg (0.060 mmol, 2.1 equiv) of NIS. This mixture was allowed to react for 2.5 hours, and the reaction was quenched with triethylamine. The mixture was filtered through celite and extracted with EA three times. The combined organic layers were dried over MgSO₄, filtered, and concentrated. Purification by flash chromatography (1:3:1 ethyl acetate/hexanes/DCM) afforded 18 mg (0.013 mmol, 46%) of 15 as an off–white foam.¹H NMR (400MHz, CDCl₃): δ 7.88 (app d, 2H), 7.72 – 7.62 (m, 1H), 7.52 – 7.44 (m, 4H), 7.36 – 7.02 (m, 27H + CHCl₃), 6.83 (app d, 2H), 5.71 (s, 1H), 5.09 – 4.98 (m, 2H), 4.97 – 4.86 (m, 2H), 4.86 – 4.76 (m, 1H), 4.76 – 4.59 (m, 2H), 4.53 – 4.34 (m, 8H), 4.09 – 3.89 (m, 3H), 3.88 – 3.79 (m, 6H), 3.77 – 3.69 (m, 2H), 3.64 – 3.57 (m, 2H), 3.57 – 3.46 (m, 4H), 3.43 – 3.26 (m, 1H), 2.36 – 2.17 (m, 3H), 2.13 – 2.00 (m, 1H), 1.96 – 1.79 (m, 1H), 1.78 – 1.68 (m, 1H), 1.64 – 1.38 (m, 2H).¹³C NMR (100MHz, CDCl₃, selected signals): δ 160.66, 153.93, 141.85, 140.38, 140.09, 139.87, 139.86, 139.38, 134.72, 131.72, 130.72, 130.20, 129.98, 129.81, 129.77, 129.69, 129.65, 129.55, 129.37, 129.27, 129.20, 129.12, 129.05, 129.02, 128.87, 120.01, 119.76, 115.24, 101.72, 99.37, 83.76, 79.44, 78.05, 76.48, 76.21, 76.02, 75.72, 75.43, 75.24, 74.70, 73.01, 72.91, 72.52, 71.10, 70.33, 68.71, 60.03, 56.77, 54.78, 34.95, 33.34, 30.16, 29.96. IR (cm⁻¹): 3020, 2820, 1733, 1084. MS (ESI+): calcd. for C₇₆H₸₅F₂N₂O₁₁S⁺ [M + NH₄⁺] 1365.54, found 1365.51. [α]D (c 0.5, DCM, −37.8).
PMB deprotected disaccharide (SI-15)

To a flask containing 15 mg (0.013 mmol, 1 equiv) of 15 was added 1 mL of DCM and 0.06 mL of 1M pH 7 phosphate buffer. This mixture was cooled to 0 °C, and 6 mg (0.03 mmol, 2.4 equiv) DDQ was added, allowed to stir for 6 hours, and the reaction was quenched with aqueous NaHCO₃ solution. The mixture was diluted with DCM, and the organic phase was washed with water. The aqueous phase was extracted with DCM three times, and the combined organic layers were dried over MgSO₄, filtered, and concentrated. Purification by flash chromatography (1:2 ethyl acetate/hexanes) afforded 7 mg (0.006 mmol, 46%) of SI-15 as an off–white foam. ¹H NMR (400MHz, CDCl₃): δ 7.88 (app d, J = 8.0 Hz, 2H), 7.74 – 7.04 (m, 31H + CHCl₃), 5.70 (s, 1H), 5.23 – 5.02 (m, 2H), 4.80 – 4.97 (m, 2H), 4.80 – 4.29 (m, 9H), 4.14 – 3.32 (m, 16H), 2.49 (app d, J = 10.1 Hz, 1H), 2.39 – 2.18 (m, 2H), 2.18 – 2.06 (m, 1H), 1.97 – 1.81 (m, 1H), 1.81 – 1.66 (m, 1H), 1.52 – 1.39 (m, 2H). MS (ESI⁺): calcd. for C₆₈H₇₅F₂N₂O₁₆S⁺ [M + NH₄⁺] 1245.48, found 1245.48.

Partially deprotected cyclohexyl linked disaccharide (SI-16)

12 mg (0.011 mmol, 1 equiv) of SI-15 was dissolved in 5 mL MeOH and 2 mL THF in a 25 mL flask. 111 μL (0.40 mmol, 40 equiv) of a 25% NaOMe solution in MeOH was added. The reaction was left stirring at room temperature under N₂ for 13 hours until reaction was completed based on LC–MS and TLC monitoring. Upon completion, the volume of the mixture was reduced to a half by rotary evaporation, and saturated NH₄Cl solution was added to quench the reaction. The mixture was extracted with DCM three times. The combined organic layers were dried over MgSO₄, filtered and concentrated. Purification by flash chromatography (1:2 ethyl acetate/hexanes) afforded 6 mg (0.006 mmol, 55%) of SI-16 as an off–white foam. ¹H NMR (400MHz, CDCl₃): δ 7.81 (app d, J = 7.2 Hz, 2H), 7.55 (app t, J = 7.6 Hz, 1H), 7.48 (app t, J = 8 Hz, 2H), 7.36 – 7.23 (m, 23H + CHCl₃), 7.21 – 7.10 (m, 2H), 5.03 – 4.95 (m, 2H), 4.92 – 4.76 (m, 2H), 4.72 – 4.61 (m, 2H), 4.60 – 4.53 (m, 2H), 4.53 – 4.43 (m, 4H), 4.11 – 4.02 (m, 1H), 3.96 (app d, J = 7.4 Hz, 1H), 3.91 – 3.52 (m, 11H), 3.40 (app t, J = 9.1 Hz, 1H), 3.35 – 3.20 (m, 1H), 3.12 – 2.97 (m, 1H), 2.47 – 2.35 (m, 2H), 1.97 – 1.74 (m, 3H), 1.40 – 1.08 (m, 4H), 1.08 – 0.92 (m, 1H).

Fully deprotected cyclohexyl linked disaccharide amine (SI-17)
Along with a stream of N\textsubscript{2}, ammonia gas was condensed against a -78 °C cold finger into a -78 °C-cooled 50 mL 3-necked flask equipped with a glass-coated stir bar, until 25 mL had accumulated. 8 mg (0.4 mmol, 60 equiv) Na metal was then added, and the resulting blue solution was left to stir for 1 hour. 6 mg (0.006 mmol, 1 equiv) of \textit{SI}-16 in 2 mL of THF was then added. The reaction progress was monitored by LC–MS. After 3 hours, 21 mg (0.40 mmol, 69 equiv) NH\textsubscript{4}Cl was added to quench the reaction. At this time, the cooling bath was removed and the ammonia was blown off under a stream of N\textsubscript{2}. The crude white solids were dissolved in water and desalted by passage through a Biorad P-2 Biogel (Fine) size exclusion column to give 4 mg of partially purified \textit{SI}-17. This material was used in the next step without further purification. \textsuperscript{1}H NMR (400MHz, D\textsubscript{2}O): \textsuperscript{δ} 4.95 (s, 1H), 4.84 (s, 1H), 4.04–3.89 (m, 4H), 3.88–3.62 (m, 8H), 3.60–3.47 (m, 1H), 3.30–3.17 (m, 1H), 2.25–2.06 (m, 4H), 1.60–1.35 (m, 4H). MS (ESI\textsuperscript{+}): calcd. for C\textsubscript{18}H\textsubscript{34}NO\textsubscript{11} \([M + H\textsuperscript{+}]\) 440.21, found 440.35.

Cyclohexyl linked disaccharide azide (\textit{16}, Man\textsubscript{2}–Cy–N\textsubscript{3})

Into a 20 mL vial containing 2.6 mg (0.0060 mmol, 1.0 equiv) of crude amine \textit{SI}-17 was added 29 μL water, 2.3 μL (0.0006 mmol, 0.1 equiv) of 0.25M aqueous CuSO\textsubscript{4} and 2.4 mg (0.017 mmol, 3.0 equiv) of K\textsubscript{2}CO\textsubscript{3}. 60 μL of MeOH was added, followed by the addition of 0.23 mL (0.060 mmol, 10.0 equiv) of freshly prepared 0.25M TfN\textsubscript{3} in DCM. The resulting homogeneous reaction was left to stir at room temperature for 4 hours until complete conversion was observed by LC–MS. The reaction was quenched with 6.3 mg (0.075 mmol, 13.0 equiv) solid NaHCO\textsubscript{3} and concentrated in vacuo. The residue was desalted on a Biorad P-2 Biogel (Fine) size exclusion column. Reversed phase HPLC purification (Column: Waters Xbridge Prep, C18, 5μm, 130Å, 10x250mm. Method: 4mL/min flow rate. A= H\textsubscript{2}O/0.1% FA, B= ACN/0.1% FA. 98.2% A for 1min, then 98.2% A to 60% A over 24 minutes, then 60% to 5% A over 5 minutes. Desired product eluted at 16.2 minutes.) providing 1.4 mg (0.0030 mmol, 50%, 2 steps) of Man\textsubscript{2}–Cy–N\textsubscript{3} (\textit{16}) as a glassy solid. \textsuperscript{1}H NMR (400MHz, D\textsubscript{2}O): \textsuperscript{δ} 4.91 (s, 1H), 4.78 (s, 1H), 4.00–3.95 (m, 1H), 3.95–3.86 (m, 3H), 3.86–3.58 (m, 8H), 3.56–3.40 (m, 2H), 2.15–1.93 (m, 4H), 1.53–1.34 (m, 4H). \textsuperscript{13}C NMR (100 MHz, D\textsubscript{2}O): \textsuperscript{δ} 102.23, 101.05, 79.33, 77.06, 76.08, 75.53, 73.89, 73.46, 72.79, 69.70, 69.56, 68.80, 63.82, 61.76, 33.19, 31.72, 31.37, 31.25. IR (cm\textsuperscript{-1}): 3338, 2933, 2094, 1046, 974. MS (ESI\textsuperscript{+}): calcd. for C\textsubscript{18}H\textsubscript{34}NaO\textsubscript{11} \([M + Na\textsuperscript{+}]\) 483.23, found 483.40. [α]D (c 0.06, H\textsubscript{2}O, 32.1). \textsuperscript{1}H NMR Spectrum of \textit{3} (400MHz, CDCl\textsubscript{3})
$^1$H NMR Spectrum of SI-1 (400MHz, D$_2$O)
$^{13}$C NMR Spectrum of SI-1 (100MHz, D$_2$O)
\(^1\text{H NMR Spectrum of 5 (400MHz, D}_2\text{O)}\)
$^{13}$C NMR Spectrum of 5 (100MHz, D$_2$O)
$^1$H NMR Spectrum of 7 (400MHz, CDCl₃)

[Image of a chemical structure and a spectrum graph]
$^{13}$C NMR Spectrum of 7 (100MHz, CDCl$_3$)
$^1$H NMR Spectrum of SI-3 (400MHz, CDCl$_3$)
$^{13}$C NMR Spectrum of SI-3 (100MHz, CDCl$_3$)
$^1$H NMR Spectrum of SI-4 (400MHz, D$_2$O)
$^{13}$C NMR Spectrum of SI-4 (100MHz, D$_2$O)
$^1$H NMR Spectrum of 7 (400MHz, D$_2$O)
\(^{13}\text{C}\) NMR Spectrum of 7 (100MHz, D\(_2\)O)
\(^1\)H NMR Spectrum of 8 (400MHz, CDCl\(_3\))
$^{13}$C NMR Spectrum of 8 (100MHz, CDCl$_3$)
$^1$H NMR Spectrum of **SI-6** (400MHz, CDCl₃)
$^{13}$C NMR Spectrum of SI-6 (100MHz, CDCl$_3$)
$^1$H NMR Spectrum of SI-7 (400MHz, D$_2$O)
$^{13}$C NMR Spectrum of SI-7 (100MHz, D$_2$O)
$^1$H NMR Spectrum of 9 (400MHz, D$_2$O)
$^{13}$C NMR Spectrum of 9 (100MHz, D$_2$O)
$^1$H NMR Spectrum of 10 (400MHz, CDCl$_3$)
$^{13}$C NMR Spectrum of 10 (100MHz, CDCl$_3$)
$^1$H NMR Spectrum of 11 (400MHz, CDCl$_3$)
$^{13}$C NMR Spectrum of 11 (100MHz, CDCl$_3$)
$^1$H NMR Spectrum of SI-9 (400MHz, CDCl$_3$)
$^{13}$C NMR Spectrum of SI-9 (100MHz, CDCl$_3$)
$^1$H NMR Spectrum of SI-10 (400MHz, D$_2$O)
$^{13}$C NMR Spectrum of SI-10 (100MHz, D₂O)
$^1$H NMR Spectrum of 12 (400MHz, D$_2$O)
$^{13}$C NMR Spectrum of 12 (100MHz, D$_2$O)
$^1$H NMR Spectrum of 13 (400MHz, CDCl$_3$)
$^{13}$C NMR Spectrum of 13 (100MHz, CDCl$_3$)
$^1$H NMR Spectrum of SI-12 (400MHz, CDCl₃)
$^{13}$C NMR Spectrum of SI-12 (100MHz, CDCl$_3$)
$^1$H NMR Spectrum of SI-13 (400MHz, D$_2$O)
$^{13}$C NMR Spectrum of SI-13 (100MHz, D$_2$O)
$^1$H NMR Spectrum of 14 (400MHz, D$_2$O)
$^{13}$C NMR Spectrum of 14 (100MHz, D$_2$O)
H NMR Spectrum of SI-14 (400MHz, CDCl₃)
$^{13}$C NMR Spectrum of SI-14 (100MHz, CDCl$_3$)
$^1$H NMR Spectrum of 15 (400MHz, CDCl₃)
$^{13}$C NMR Spectrum of 15 (100MHz, CDCl$_3$)
$^1$H NMR Spectrum of SI-15 (400MHz, CDCl$_3$)
$^1$H NMR Spectrum of SI-16 (400MHz, CDCl$_3$)
$^1$H NMR Spectrum of SI-17 (400MHz, D$_2$O)
$^1$H NMR Spectrum of 16 (400MHz, D$_2$O)
$^{13}$C NMR Spectrum of 16 (100MHz, D$_2$O)
References

1. Stura, E. A.; Nemerow, G. R.; Wilson, I. A., Strategies in the crystallization of glycoproteins and protein complexes. J. Cryst. Growth 1992, 122 (1), 273-285.
2. Otwinowski, Z.; Minor, W., Processing of X-ray diffraction data collected in oscillation mode. Methods Enzymol. 1997, 276, 307-326.
3. McCoy, A. J.; Grosse-Kunstleve, R. W.; Adams, P. D.; Winn, M. D.; Storoni, L. C.; Read, R. J., Phaser crystallographic software. J. Appl. Crystallogr. 2007, 40 (4), 658-674.
4. Stanfield, R. L.; De Castro, C.; Marzaioli, A. M.; Wilson, I. A.; Pantophlet, R., Crystal structure of the HIV neutralizing antibody 2G12 in complex with a bacterial oligosaccharide analog of mammalian oligomannose. Glycobiology 2015, 25 (4), 412-419.
5. Afonine, P. V.; Grosse-Kunstleve, R. W.; Echols, N.; Headd, J. J.; Moriarty, N. W.; Mustyakimov, M.; Terwilliger, T. C.; Zwart, P. H.; Adams, P. D., Towards automated crystallographic structure refinement with phenix.refine. Acta Crystallogr. Sect. D. Biol. Crystallogr. 2012, 68 (Pt 4), 352-367.
6. Horiya, S.; Bailey, J. K.; Temme, J. S.; Guillon Schlippe, Y. V.; Krauss, I. J., Directed evolution of multivalent glycopeptides tightly recognized by HIV antibody 2G12. J. Am. Chem. Soc. 2014, 136 (14), 5407-5415.
7. Bailey, J. K.; Nguyen, D. N.; Horiya, S.; Krauss, I. J., Synthesis of multivalent glycopeptide conjugates that mimic an HIV epitope. Tetrahedron 2016, 72 (40), 6091-6098.
8. van Kalkeren, H. A.; Bruins, J. J.; Rutjes, F. P. J. T.; van Delft, F. L., Organophosphorus-Catalysed Staudinger Reduction. Adv Synth Catal 2012, 354 (8), 1417-1421.
9. MacPherson, I. S.; Temme, J. S.; Habeshian, S.; Felczak, K.; Pankiewicz, K.; Hedstrom, L.; Krauss, I. J., Multivalent glycocluster design through directed evolution. Angew. Chem. Int. Ed. 2011, 50 (47), 11238-11242.
10. Temme, J. S.; Drzyzga, M. G.; MacPherson, I. S.; Krauss, I. J., Directed evolution of 2G12-targeted nonamannose glycoclusters by SELMA. Chem. Eur. J. 2013, 19 (51), 17291-17295.
11. Crich, D.; Li, W.; Li, H., Direct chemical synthesis of the β-mannans: linear and block syntheses of the alternating β-(1→3)-β-(1→4)-mannan common to rhodotorula glutinis, rhodotorula mucilaginosa, and leptospira biflexa. J. Am. Chem. Soc. 2004, 126 (46), 15081-15086.
12. Geng, X.; Dudkin, V. Y.; Mandal, M.; Danishefsky, S. J., In pursuit of carbohydrate-based HIV vaccines, part 2: The total synthesis of high-mannose-type gp120 fragments—evaluation of strategies directed to maximal convergence. Angew. Chem. Int. Ed. 2004, 43 (19), 2562-2565.
13. Cavender, C. J.; Shiener, V. J., Trifluoromethanesulfonyl azide. Its reaction with alkyl amines to form alkyl azides. J. Org. Chem. 1972, 37 (22), 3567-3569.
14. Waleczak, M. A.; Danishefsky, S. J., Solving the convergence problem in the synthesis of triantennary N-glycan relevant to prostate-specific membrane Antigen (PSMA). J. Am. Chem. Soc. 2012, 134 (39), 16430-16433.