Oligosaccharide–Peptide Ligation of Glycosyl Thiolates with Dehydropeptides: Synthesis of S-Linked Mucin-Related Glycopeptide Conjugates

Danica P. Galonić, Wilfred A. van der Donk,* and David Y. Gin*[a]

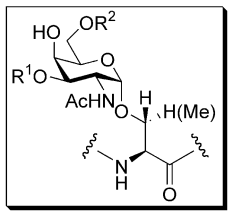
Abstract: A chemoselective strategy for oligosaccharide–peptide ligation is described in which α-thio analogues of mucin-related glycoconjugates can be readily accessed through site-selective conjugate addition of complex oligosaccharide thiolates to dehydroalanine-containing peptides. The efficiency of the ligation is highlighted by the rapid convergent assembly of thio-isosteres of the four tumor-associated carbohydrate antigens, T_N, T, ST_N, and 2,6-ST, as a pair of diastereoisomers at the newly formed cysteine stereocenter. The process proceeds in high yield and with complete retention of the α-anomeric configuration.

Keywords: carbohydrates · chemoselective ligation · dehydropeptide · glycopeptides · mucin

Introduction

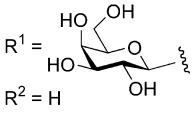
Post-translational glycosylation introduces enormous structural diversity into glycoproteins. Inflammation, immune response, as well as protein expression and folding are among the many biological events in which the carbohydrate segments play essential roles. Given the high degree of heterogeneity in natural glycoproteins and the difficulties associated with the isolation of well-defined glycopeptides from natural sources, the development of efficient chemical methods for the synthesis of carbohydrate–peptide conjugates remains a key challenge.[1] Protein glycosylation, with few exceptions, can be divided into two general classes—those in which a GlcNAc residue is β-N-linked to the side chain of Asn, and those in which a GalNAc residue is α-O-linked to the side chain of Ser or Thr. The latter is characteristic of mucins, which are O-glycosylated proteins comprising an important class of tumor-associated antigens that have received considerable attention in cancer vaccine therapies.[2] Representative mucin-associated carbohydrates are illustrated by: T_N (**1**), T (**2**), ST_N (**3**), and 2,6-ST (**4**) tumor-associated antigens.

Traditional strategies for the preparation of oligosaccharide–peptide conjugates have involved the synthesis of fully protected, pre-formed oligosaccharide/amino acid intermediates, followed by multistep elaboration of the amino acid aglycone into the requisite peptide chain.[1,3] An alternate approach involves chemoselective ligation in which site-specific attachment of the carbohydrate component occurs directly to a pre-formed peptide chain. Recent elegant advances concerning the construction of β-N-linked glycopeptides have been realized in this regard;[1,4] however, ligation strategies as applied to mucin-like glycopeptides are scarce given the inherent difficulties associated with construction of the α-*O*-GalNAc glycosidic bond,[5] especially with polypeptide glycosyl acceptors. In this context, notable accomplishments have been made in the ligation of α-glycosyl aminoxy GalNAc carbohydrates with ketone-functionalized peptides[6] to afford mucin-like conjugates incorporating an oxime functionality[7] as the linker.

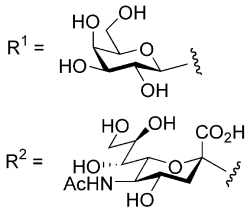


T_N Antigen (1)
R^1 = H
R^2 = H

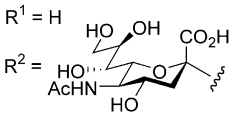
T Antigen (2)



2,6-ST Antigen (4)



ST_N Antigen (3)



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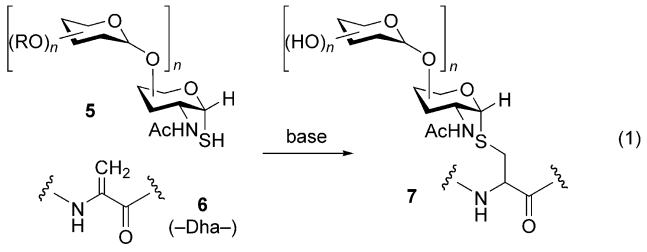
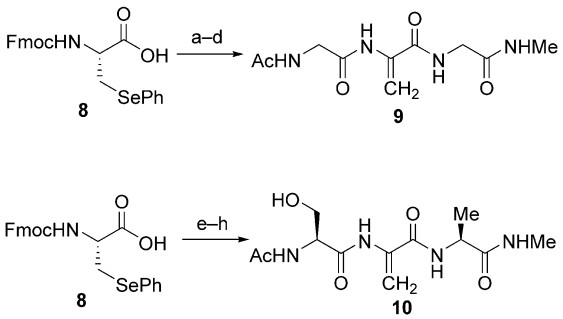
We now report a new strategy for the ligation of α-GalNAc-thiolate oligosaccharides (**5**) [Eq. (1)] with dehydroalanine (Dha)-containing peptides **6** by 1,4-conjugate addition, resulting in the efficient formation of the S-linked analogues of mucin-related α-GalNAc glycoconjugates **7**. This one-pot oligosaccharide–peptide ligation is site-selective for dehydroalanine, maintains the α-anomeric stereochemical integrity of the carbohydrate donor, and proceeds under mild conditions, providing fully deprotected oligosaccharide isosteres of the naturally occurring tumor-associated antigens. Moreover, S-linked glycopeptides have been reported to have enhanced chemical stability as well as increased resistance to enzymatic hydrolysis relative to their O-linked counterparts.[8]



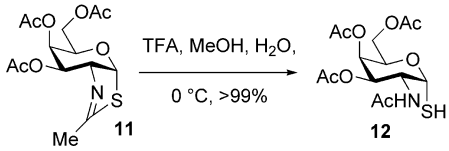
Results and Discussion

Dehydroalanine residues within peptides serve as versatile functionalities for chemoselective ligation given the paucity of electrophilic reactive sites on native amino acid residues.[9] Two tripeptides incorporating a central Dha fragment were prepared as Michael acceptors to establish the feasibility of this ligation approach (Scheme 1). *N*-Fmoc-Protected phenylselenocysteine **8** is coupled with *N*-methyl glycine by using the benzotriazol-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) coupling agent to generate the corresponding selectively protected dipeptide in 61% yield. Subsequent Fmoc deprotection and amino acylation with *N*-acetyl glycine provides the corresponding selectively protected tripeptide with a central phenylselenocysteine residue, an ideal precursor to Dha residues in complex peptide synthesis.[10] Thus, chemoselective selenide oxidation followed by elimination proceeds in near quantitative yield to afford the Dha-containing peptide **9**. A similar short synthetic sequence was employed to prepare the tripeptide Ac-Ser-Dha-Ala-NHMe (**8**→**10**).[11] Notably, this synthetic approach to Dha peptides is compatible with all naturally occurring amino acids, including those that are prone to oxidation.[10]

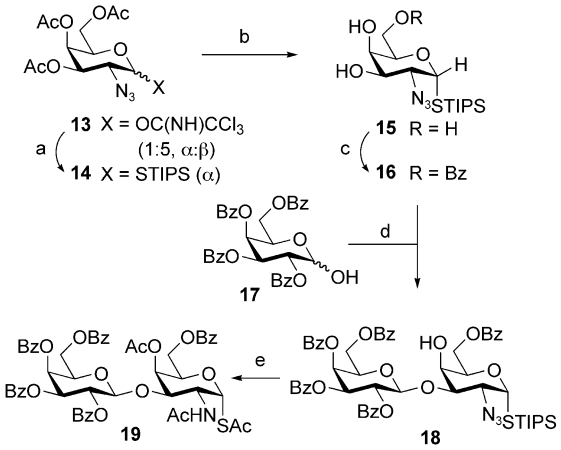


Scheme 1. a) H-Gly-NHMe, BOP, DIPEA, $CHCl_3$, 23°C, 61%; b) $(CH_2)_5NH$, CH_2Cl_2, 23°C, 91%; c) Ac-Gly-OH, BOP, DIPEA, $CHCl_3$, 23°C, 81%; d) H_2O_2, MeCN, H_2O, 23°C, >99%; e) H-Ala-NHMe, BOP, DIPEA, $CHCl_3$, 23°C, 72%; f) $(CH_2)_5NH$, CH_2Cl_2, 23°C, 75%; g) Ac-Ser(*t*Bu)-OH, BOP, DIPEA, $CHCl_3$, 23°C, 97%; h) TFA, 0°C; H_2O_2, MeOH, CH_2Cl_2, 23°C, 98%.

However, a critical challenge in establishing this ligation approach is the preparation and conjugate addition of stereo-defined GalNAc-derived α-oligosaccharyl thiols, which have not been synthesized heretofore. Thus, anomeric sulfur analogues of *all four* of the tumor-associated carbohydrate antigen segments (**1**–**4**) were synthesized to probe the feasibility, scope, and efficiency of this carbohydrate–peptide ligation. The C1-thio-analogue of the T_N-antigen **1** is prepared from 3,4,6-triacetoxy galactosyl thiazoline **11** [Eq. (2)].[12] Acid-mediated hydrolysis of the thiazoline **11** under carefully controlled conditions yields the C1-galactosyl thiopyranose **12** with complete retention of α-stereochemistry.



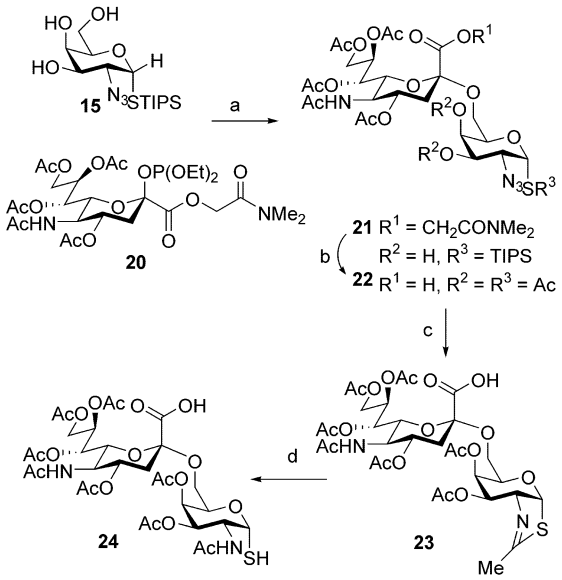
Preparation of the thio analogue of the disaccharide T-antigen **2** commences with Schmidt glycosylation[13] (Scheme 2) of triisopropylsilylthiol with 2-azido glycosyl trichloroacetimidate **13**,[14] providing the α-*S*-silyl thioglycoside **14** in good yield (85%). Saponification of the acetate esters followed by selective benzoylation of the primary C6-hydroxyl affords the thiogalactopyranoside **16**. Glycosylation of this diol acceptor proceeds chemoselectively at the equatorial C3 hydroxyl by using 2,3,4,6-tetra-*O*-benzoylgalacto-



Scheme 2. a) TIPS-SH, TMSOTf, CH_2Cl_2, Et_2O, −20→23°C, 85%; b) NaOMe, MeOH, 23°C, 85%; c) BzCl, Et_3N, CH_2Cl_2, −20°C, 75%; d) Ph_2SO, Tf_2O, CH_2Cl_2, −78→−20°C, 68%; e) PhSeH, Et_3N, pyr, 40°C; Ac_2O, 23°C, 76%.

pyranose (**17**) as the donor with our dehydrative glycosylation protocol (Ph_2SO, Tf_2O)[15] to afford the β-1,3-disaccharide **18** (68%). It is worth noting that the dehydrative glycosylation of **16** with **17** is performed in the absence of a triflic acid scavenger, allowing for in situ productive rearrangement of undesired orthoester adducts, byproducts which often plague glycosylation with C2-acyl donors.[16] Exposure of disaccharide **18** to benzeneselenol effects reduction of the C2-azide accompanied by silyl deprotection of the anomeric thiol. Subsequent treatment of the reaction mixture with excess acetic anhydride provides the fully acylated glycosyl thioacetate disaccharide **19** (76%), the T-antigen precursor for carbohydrate–peptide ligation.

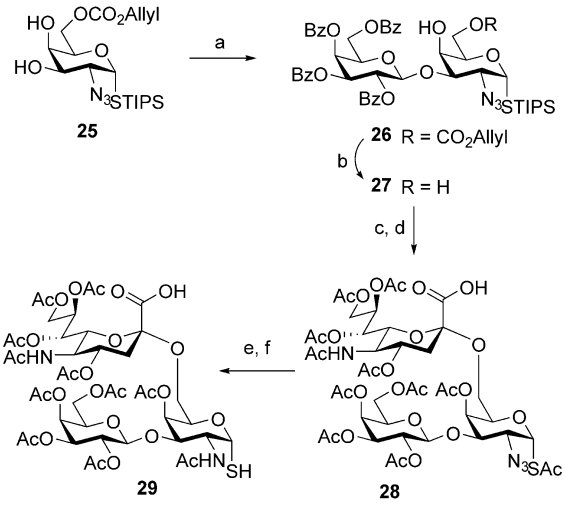
Synthesis of the thio analogue of the ST_N-antigen oligosaccharide **3** features sialylation of *S*-triisopropylsilyl-2-azido-2-deoxygalacto-1-thiopyranoside (**15**, Scheme 3) with



Scheme 3. a) TMSOTf, CH_2Cl_2, −45 °C, 82%, 6.5:1 α:β; b) NaOH, MeOH, H_2O, 23 °C; Ac_2O, DMAP, pyr, 23 °C, 66%; c) PPh_3, THF, 23 °C, >99%; d) TFA, MeOH, H_2O, 0 °C, >99%.

sialyl phosphite donor **20**, incorporating the C1-*N,N*-dimethyl glycolamide auxiliary to enhance α-selectivity.[17] The coupling proceeds efficiently to form the sialylconjugate **21** (82%; 6.5:1, α:β), which is then subjected to global deprotection followed by per-acetylation to afford the glycosyl thioacetate disaccharide **22** (66%, 2 steps). An anomeric *S*-to-*N* reductive acyl-transfer sequence is then performed involving the treatment of **22** with PPh_3 to promote 1-*S*-2-*N*-thiazoline formation (**23**) by means of an aza-Wittig-like condensation.[18] Subsequent TFA-mediated (TFA = trifluoroacetic acid) thiazoline ring opening affords the α-glycosyl thiol **24**, incorporating the naturally occurring C2-acetamido group, in near quantitative yield over both steps.

The preparation of the C1-thio derivative of the 2,6-ST-trisaccharide antigen **4** initially involves chemoselective dehydrative glycosylation of the C3-hydroxyl within *S*-triisopropylsilyl 6-*O*-allyloxycarbonyl-2-azido-2-deoxygalacto-1-thiopyranoside (**25**, Scheme 4) with 2,3,4,6-tetra-*O*-benzoyl-

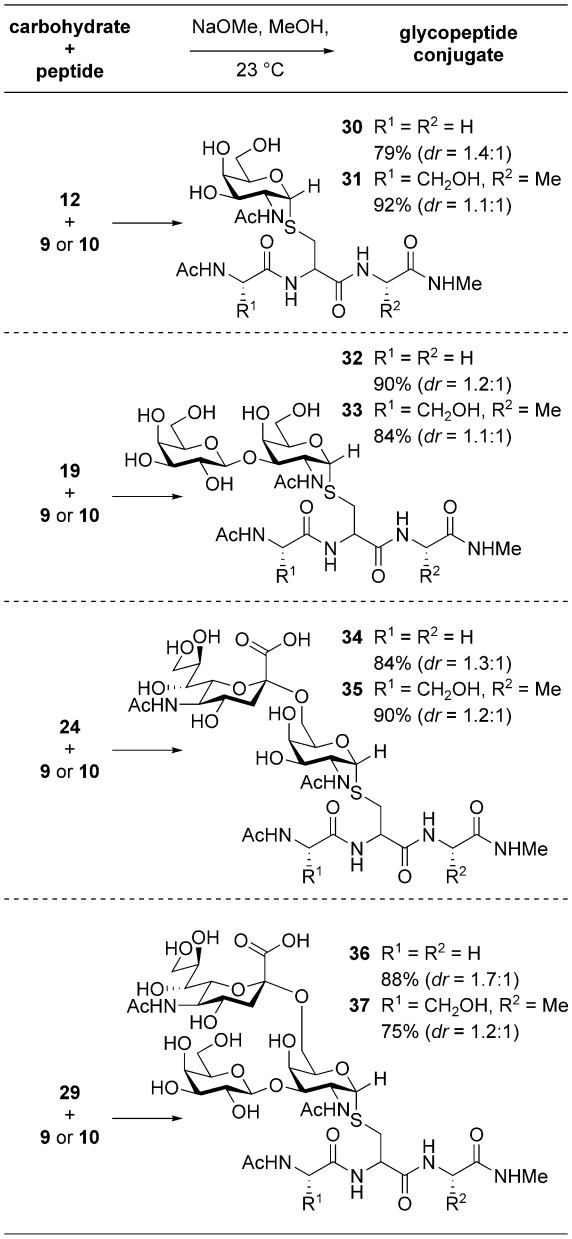


Scheme 4. a) (4-NO_2-C_6H_4)(Ph)SO, Tf_2O, **17**, CH_2Cl_2, −78→−45 °C, 70%; b) [$PdCl_2(PPh_3)_2$] (cat.), Bu_3SnH, CH_2Cl_2, H_2O, 23 °C, >99%; c) **20**, TMSOTf (cat.), CH_2Cl_2, −78 °C, 66% (4.4:1, α:β); d) NaOH, MeOH, H_2O, 23 °C; Ac_2O, pyr, DMAP, 23 °C, 51%; e) PPh_3, THF, 23 °C, 91%; f) TFA, MeOH, H_2O, 0 °C, >99%.

galactopyranose (**17**) to afford the disaccharide **26** (70%). Subsequent removal of the C6-*O*-allyloxycarbonyl group reveals the appropriate disaccharide nucleophile **27** (>99%) for sialylation. Coupling of **27** with the sialyl phosphite donor **20** in the presence of catalytic TMSOTf proceeds with good α-selectivity (66%; 4.4:1, α:β) and is followed by global deprotection and per-acetylation to afford the disaccharide sialyl conjugate **28** (51%, 2 steps). A 1-*S*-to-2-*N* reductive acyl-transfer protocol, similar to that employed in the final steps to prepare **24** (vide supra), proceeds in 91% yield (2 steps) to afford the acetylated 2,6-ST-antigen C1-thiol **29**, which can be used directly for carbohydrate–peptide ligation.

Site-selective ligations of the C1-*S*-carbohydrate donors with the tripeptides **9** and **10** proceed efficiently through 1,4-addition into the dehydroalanine residue (Table 1). For example, the C1-thio analogue of the T_N-antigen monosaccharide **12** serves as an effective nucleophile with both **9** and **10**, providing excellent yields of the α-linked T_N-antigen isosteres **30** and **31** (79%, 1.4:1 *dr*; 92%, 1.1:1 *dr*; respectively), thereby establishing the feasibility of stereoselective α-GalNAc Michael ligation.[19] Importantly, the ligation procedure is equally compatible with oligosaccharides. Disaccharides **19** and **24** undergo efficient coupling with **9** and **10** to provide the T-antigen thio-mucin linkages **32** (90%) and **33** (84%), as well as the ST_N-antigen counterparts **34** (84%) and **35** (90%). More complex oligosaccharides are also amenable to this ligation, exemplified by the coupling of the mature 2,6-ST-*S*-trisaccharide **29** with peptides **9** and **10** to afford the 2,6-ST-antigen isosteres **36** (88%) and **37** (75%). It is worth noting that both *O*-acyl-protected glycosyl thiols (**12**, **24**, and **29**) as well as glycosyl thioacetates (**19**) can be employed as carbohydrate donors, since the mild ligation conditions (MeOH, NaOMe, 23 °C) also promote concomitant O- and S-deacylation to afford fully deprotected glycopeptide isosteres in a one-pot procedure. To our knowledge,

Table 1. Site-selective ligations of the C1-*S*-carbohydrate donors with the tripeptides **9** and **10**.



this is the first report of *oligo*saccharide–peptide ligation for the preparation of thio analogues of tumor-associated carbohydrate antigens,[20,21] a process that occurs not only in high yields, but also with complete retention of the α-anomeric configuration. Moreover, the establishment of this ligation method suggests the prospect of its application with conformationally constrained dehydropeptides to stereoselectively access either D- or L-cysteine-linked mucin glycopeptide mimics.

Conclusion

In summary, a novel strategy for oligosaccharide–peptide ligation is described in which α-thio analogues of mucin-related structures can be readily accessed through site-selective 1,4-conjugate addition of complex oligosaccharide thiolates to dehydropeptides. The rapid convergent preparation of thio-isosteres of the four tumor-associated antigens, T_N, T, ST_N, and 2,6-ST, underscores the efficiencies of not only the syntheses of GalNAc-derived α-oligosaccharyl thiols, but also of the ligation event, a process that should facilitate the construction of a host of glycopeptide conjugates for biological evaluation.

Experimental Section

General: All reactions were performed in dry modified Schlenk (Kjeldahl shape) flasks fitted with a glass stopper under a positive pressure of argon, unless otherwise noted. Air- and moisture-sensitive liquids were transferred by means of a syringe. Organic solutions were concentrated by rotary evaporation below 30 °C at approximately 25 Torr. Flash column chromatography was performed employing 230–400 mesh silica gel. Thin-layer chromatography was performed by using glass plates precoated to a depth of 0.25 mm with 230–400 mesh silica gel impregnated with a fluorescent indicator (254 nm). When necessary, solvents were degassed by the freeze-pump thaw method (>3 cycles).

Dichloromethane, toluene, acetonitrile, and tetrahydrofuran were purified by passage through two packed columns of neutral alumina under an argon atmosphere. Chloroform, pyridine, diisopropylethylamine, and triethylamine were distilled from calcium hydride at 760 Torr. Methanol was distilled from magnesium oxide at 760 Torr. Millipore water was used for reactions in an aqueous solvent or co-solvent.

Infrared (IR) spectra were obtained by using a Perkin–Elmer Spectrum BX spectrophotometer referenced to polystyrene standard. Data are presented as frequency of absorption (cm^{-1}). ^1H and ^{13}C NMR spectra were recorded on Varian 400, Varian 500, Varian 750, and Varian Inova 500 NMR spectrometers; chemical shifts are expressed in ppm (δ scale) downfield from tetramethylsilane and are referenced to residual protium in the NMR solvent ($CHCl_3$: $\delta = 7.27$ ppm). Data are presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet and/or multiple resonances), integration, and coupling constants in Hertz (Hz).

Fmoc-phenylselenocysteinylglycine-*N*-methyl amide: *N,N*-diisopropylethylamine (2.1 mL, 11.9 mmol, 2.5 equiv) was added dropwise to a suspension of amino acid **8** (2.4 g, 5.3 mmol, 1.1 equiv), glycine-*N*-methyl amide hydrochloride (0.63 g, 5.0 mmol, 1.0 equiv), and BOP (2.33 g, 5.3 mmol, 1.1 equiv) in chloroform (66 mL) at 23 °C. The resulting solution was stirred at this temperature for 10 h, diluted with dichloromethane (1.4 L), and washed subsequently with 1 M aqueous hydrochloric acid (150 mL), saturated aqueous sodium bicarbonate (150 mL), and water (150 mL). The organic layer was dried (sodium sulfate), filtered, and concentrated. The residue was purified by silica-gel flash chromatography (5% methanol in dichloromethane) to afford the dipeptide (1.68 g, 61%) as a white solid. M.p. 170 °C; $R_f = 0.28$ (6% methanol in dichloromethane); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.77$ (d, $J = 7.6$ Hz, 2H), 7.56–7.27 (m, 11H), 6.61 (br s, 1H), 6.31 (br s, 1H), 5.55 (br d, $J = 5.2$ Hz, 1H), 4.42 (d, $J = 6.8$ Hz, 2H), 4.29 (m, 1H), 4.19 (t, $J = 6.6$ Hz, 1H), 3.83 (br s, 2H), 3.29 (m, 2H), 2.78 ppm (d, $J = 4.8$ Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 170.7$, 164.7, 143.8, 143.7, 141.5, 133.4, 129.7, 128.1, 127.3, 125.2, 120.3, 67.5, 47.3, 43.4, 37.1, 37.0, 26.4 ppm; FTIR (neat film): $\tilde{\nu} = 3286$, 2928, 2371, 1655, 1532, 1452, 1296, 1258, 1194, 984, 842, 741 cm^{-1}; HRMS (FAB)$^+$: *m/z* calcd for $C_{27}H_{27}N_3O_4Se$ $[M+H]^+$: 538.1245; found: 538.1246.

Phenylselenocysteinylglycine-*N*-methyl amide: A solution of piperidine in dichloromethane (5%, 200 μL, 2.00 mmol, 40 equiv) was added to a solution of Fmoc-phenylselenocystylglycine-*N*-methyl amide (100 mg, 0.19 mmol, 1 equiv) in dichloromethane (3.8 mL) at 23 °C. The resulting mixture was stirred at this temperature for 1 h, and then concentrated. The residue was purified by silica-gel flash chromatography (5% to 10% methanol in dichloromethane) to afford the amine (53 mg, 91%) as colorless oil. $R_f = 0.22$ (7% methanol in dichloromethane); ^1H NMR (500 MHz, CD_3OD): $\delta = 7.54$–7.52 (m, 2H), 7.28–7.22 (m, 3H), 3.74 (A of

AB, $J=16.8$ Hz, 1H), 3.68 (B of AB, $J=17.0$ Hz, 1H), 3.50 (X of ABX, $J_{BX}=7.1$, $J_{AX}=5.7$ Hz, 1H), 3.27 (A of ABX, $J_{AB}=12.6$, $J_{AX}=5.7$ Hz, 1H), 3.09 (B of ABX, $J_{AB}=12.6$, $J_{BX}=7.1$ Hz, 1H), 2.69 ppm (s, 3H); ^{13}C NMR (126 MHz, CD_3OD): $\delta=176.4$, 172.1, 134.2, 130.8, 130.5, 128.5, 56.2, 43.5, 33.8, 26.4 ppm; FTIR (neat film): $\tilde{\nu}=3304$, 1656, 1530, 1478, 1411, 738, 691 cm^{-1}; HRMS (FAB)$^+$: m/z calcd for $C_{12}H_{18}N_3O_2Se$ $[M+H]^+$: 314.0578; found: 314.0572.

Acetylglycylphenylselenocysteinylglycine-*N*-methyl amide: *N,N*-diisopropylethylamine (435 μL, 2.5 mmol, 2.5 equiv) was added dropwise to a solution of phenylselenocysteinylglycine-*N*-methyl amide (314 mg, 1.0 mmol, 1.0 equiv), acetyl glycine (131 mg, 1.1 mmol, 1.1 equiv), and BOP (499 mg, 1.1 mmol, 1.1 equiv) in chloroform (26 mL) at 23 °C. The solution was stirred at this temperature for 4 h, diluted with 5% methanol in dichloromethane (500 mL) and washed sequentially with 1 M aqueous hydrochloric acid (40 mL), saturated aqueous sodium bicarbonate solution (40 mL), and saturated aqueous sodium chloride solution (40 mL). The combined aqueous washes were saturated with sodium sulfate and extracted with 5% methanol in dichloromethane (6×100 mL). The combined organic layers were dried (sodium sulfate), filtered, and concentrated. The residue was purified by silica-gel flash chromatography (7% methanol in dichloromethane), and re-purified by the same method (dry-loaded, 12% methanol in dichloromethane), to afford tripeptide (334 mg, 81%) as a white solid. M.p. 214 °C; $R_f=0.17$ (10% methanol in dichloromethane); ^{1}H NMR (500 MHz, CD_3OD): $\delta=7.57$–7.55 (m, 2H), 7.31–7.27 (m, 3H), 4.46 (X of ABX, $J=8.6$, 5.3 Hz, 1H), 3.85 (A of AB, $J=16.8$ Hz, 1H), 3.79 (A of AB, $J=16.8$ Hz, 1H), 3.77 (B of AB, $J=16.4$ Hz, 1H), 3.69 (B of AB, $J=16.6$ Hz, 1H), 3.40 (A of ABX, $J_{AB}=13.0$, $J_{AX}=5.3$ Hz, 1H), 3.18 (B of ABX, $J_{AB}=13.0$, $J_{AX}=8.6$ Hz, 1H), 2.72 (s, 3H), 2.01 ppm (s, 3H); ^{13}C NMR (126 MHz, CD_3OD): $\delta=174.4$, 173.2, 172.4, 172.1, 134.4, 130.6, 130.5, 128.7, 55.5, 43.9, 43.7, 29.3, 26.4, 22.5; FTIR (neat film): $\tilde{\nu}=3278$, 2426, 1625, 1528, 1437 ppm; HRMS (FAB)$^+$: m/z calcd for $C_{16}H_{23}N_4O_4Se$ $[M+H]^+$: 415.0885; found: 415.0884.

Tripeptide 9: Hydrogen peroxide (30%, 64 μL, 0.52 mmol, 2 equiv) was added to a solution of acetylglycylphenylselenocysteinylglycine-*N*-methyl amide (107 mg, 0.26 mmol, 1 equiv) in a mixture of water (3.1 mL) and acetonitrile (3.1 mL) at 23 °C. The solution was stirred at this temperature for 7 h. Excess H_2O_2 was neutralized with dimethyl sulfide (100 μL). The reaction was diluted with toluene (~15 mL), then concentrated, and residue was purified by silica-gel flash chromatography (5% to 14% methanol in dichloromethane) to give the dehydroalanine-containing tripeptide **9** (66 mg, >99%) as a white solid. M.p. 152–153 °C; $R_f=0.31$ (14% methanol in dichloromethane); ^{1}H NMR (500 MHz, CD_3OD): $\delta=5.85$ (s, 1H), 5.58 (s, 1H), 3.94 (s, 2H), 3.89 (s, 2H), 2.75 (s, 3H), 2.03 ppm (s, 3H); ^{13}C NMR (126 MHz, CD_3OD): $\delta=174.3$, 172.3, 171.0, 167.1, 137.3, 108.4, 44.3, 44.0, 26.4, 22.5 ppm; FTIR (neat film): $\tilde{\nu}=3291$, 2476, 1649, 1517, 1405 cm^{-1}; HRMS (FAB)$^+$: m/z calcd for $C_{10}H_{16}N_4NaO_4$ $[M+Na]^+$: 279.1069; found: 279.1070.

Fmoc-phenylselenocysteinylalanine-*N*-methyl amide: Diisopropylethylamine (0.9 mL, 5.2 mmol, 2.5 equiv) was added dropwise to a suspension of amino acid **8** (1.30 g, 2.3 mmol, 1.1 equiv), alanine-*N*-methyl amide hydrochloride (0.29 g, 2.1 mmol, 1.0 equiv), and BOP (1.02 g, 2.3 mmol, 1.1 equiv) in chloroform (38 mL) at 23 °C. The resulting mixture was stirred at this temperature for 24 h. The reaction mixture was diluted with chloroform (1.4 L) and washed subsequently with 1 M aqueous hydrochloric acid solution (150 mL), saturated aqueous sodium bicarbonate solution (150 mL), and water (150 mL). The organic layer was dried (sodium sulfate), filtered, and concentrated. The residue was purified by silica-gel flash chromatography (5% methanol in dichloromethane) to give the dipeptide (0.82 g, 72%) as a white solid. M.p. 229 °C; $R_f=0.36$ (7% methanol in dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=7.78$ (d, $J=7.5$ Hz, 2H), 7.58–7.27 (m, 11H), 6.56 (d, $J=7.6$ Hz, 1H), 6.29 (br s, 1H), 5.58 (d, $J=4.6$ Hz, 1H), 4.45–4.33 (m, 4H), 4.20 (t, X of ABX, $J=6.8$ Hz, 1H), 3.30 (A of ABX, $J_{AB}=12.2$, $J_{AX}=6.0$ Hz, 1H), 3.24 (B of ABX, $J_{AB}=12.2$, $J_{BX}=12.2$, 6.4 Hz, 1H), 2.79 (d, $J=4.3$ Hz, 3H), 1.36 ppm (d, 3H, $J=7.0$ Hz); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta=172.1$, 170.0, 143.8, 141.5, 135.5, 133.2, 129.6, 128.1, 127.3, 125.2, 120.3, 67.5, 55.3, 49.3, 47.2, 29.5, 26.5, 18.2 ppm; FTIR (neat film): $\tilde{\nu}=3291$, 1642, 1538, 1262, 735 cm^{-1}; HRMS (FAB)$^+$: m/z calcd for $C_{28}H_{30}N_3O_4Se$ $[M+H]^+$: 552.1402; found: 552.1400.

Phenylselenocysteinylalanine-*N*-methyl amide: Piperidine (50% solution in dichloromethane, 1.7 mL, 30.5 mmol, 20 equiv) was added dropwise to a solution of Fmoc-phenylselenocysteinylalanine-*N*-methyl amide (0.84 g, 1.52 mmol, 1 equiv) in dichloromethane (31 mL) at 23 °C. The resulting solution was stirred at this temperature for 1.5 h. The reaction mixture was concentrated, and the residue was purified by silica-gel flash chromatography (7% methanol in dichloromethane) to give the amine (375 mg, 75%) as a white solid. M.p. 130 °C; $R_f=0.34$ (10% methanol in dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.76$ (d, $J=7.1$ Hz, 1H), 7.55–7.53 (m, 2H), 7.30–7.26 (m, 3H), 6.39 (br s, 1H), 4.34 (pent, $J=7.0$ Hz, 1H), 3.50–3.42 (m, 2H), 3.03 (dd, $J=12.4$, 8.3 Hz, 1H), 2.77 (d, $J=4.8$ Hz, 3H), 1.71 (s, 2H), 1.36 ppm (d, $J=7.08$ Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta=173.7$, 172.6, 133.3, 129.5, 128.9, 127.7, 54.6, 48.8, 33.8, 26.4, 17.8 ppm; FTIR (neat film): $\tilde{\nu}=3305$, 1648, 1515, 1478, 1438, 691 cm^{-1}; HRMS (FAB)$^+$: m/z calcd for $C_{13}H_{20}N_3O_2Se$ $[M+H]^+$: 330.0720; found: 330.0721.

Acetylseryl-(*O*-*tert*-butyl)-phenylselenocysteinylalanine-*N*-methyl amide: Diisopropylethylamine (269 μL, 1.55 mmol, 1.5 equiv) was added dropwise to a suspension of acetylserine-*tert*-butyl ether (236 mg, 1.1 mmol, 1.1 equiv), phenylselenocysteinylalanine-*N*-methyl amide (339 mg, 1.0 mmol, 1.0 equiv), and BOP (507 mg, 1.14 mmol, 1.1 equiv) in chloroform (15 mL) at 23 °C. The resulting solution was stirred at this temperature for 6 h. The reaction mixture was diluted with chloroform (800 mL) and washed subsequently with 10% aqueous citric acid solution (80 mL), saturated aqueous sodium bicarbonate solution (80 mL), and saturated aqueous sodium chloride solution (80 mL). The organic layer was dried (sodium sulfate), filtered, and concentrated. The residue was purified by silica-gel flash chromatography (dry loaded, 5% to 7% methanol in dichloromethane), to give the tripeptide (513 mg, 97%) as a white solid. M.p. 262 °C; $R_f=0.55$ (14% methanol in dichloromethane); ^{1}H NMR (400 MHz, CD_3OD): $\delta=7.55$–7.53 (m, 2H), 7.30–7.26 (m, 3H), 4.53 (X of ABX, $J_{BX}=5.7$, $J_{AX}=5.2$ Hz, 1H), 4.33 (t, X of ABX, $J=5.3$ Hz, 1H), 4.21 (q, $J=7.4$ Hz, 1H), 3.65 (A of ABX, $J_{AB}=9.3$, $J_{AX}=5.0$ Hz, 1H), 3.59 (B of ABX, $J_{AB}=9.3$, $J_{BX}=5.7$ Hz, 1H), 3.36 (A of ABX, $J_{AB}=13.7$, $J_{AX}=5.2$ Hz, 1H), 3.19 (B of ABX, $J_{AB}=13.7$, $J_{BX}=5.7$ Hz, 1H), 2.70 (s, 3H), 2.02 (s, 3H), 1.30 (d, $J=7.27$ Hz, 3H), 1.20 ppm (s, 9H); ^{13}C NMR (126 MHz, CD_3OD): $\delta=175.4$, 174.2, 173.3, 172.3, 134.2, 130.9, 130.6, 128.7, 75.2, 62.7, 55.1, 51.0, 50.9, 28.1, 27.9, 27.9, 26.6, 22.8, 16.7 ppm; FTIR (neat film): $\tilde{\nu}=3287$, 2433, 1626, 1451 cm^{-1}; HRMS (FAB)$^+$: m/z calcd for $C_{22}H_{35}N_4O_5Se$ $[M+H]^+$: 515.1773; found: 515.1773.

Tripeptide 10: Trifluoroacetic acid (1.7 mL) was added to acetylseryl-(*O*-*tert*-butyl)phenylselenocysteinylalanine-*N*-methyl amide (89 mg, 0.17 mmol) at 0 °C, and the resulting solution was stirred at this temperature for 11.5 h. The reaction mixture was concentrated to give acetylserylphenylselenocysteinylalanine-*N*-methyl amide. Hydrogen peroxide (30%, 47 μL, 0.34 mmol, 2 equiv) was added to a solution of the unpurified acetylserylphenylselenocysteinylalanine-*N*-methyl amide (79 mg, 0.17 mmol, 1 equiv) in a mixture of methanol (4.7 mL) and dichloromethane (4.7 mL) at 23 °C, and the resulting solution was stirred at this temperature for 1 h. The reaction mixture was neutralized by the addition of dimethylsulfide (100 μL), and concentrated. The residue was purified by silica-gel flash chromatography to give tripeptide **10** (51 mg, 98%) as a white solid. M.p. 238 °C; $R_f=0.40$ (20% methanol in dichloromethane); ^{1}H NMR (500 MHz, CD_3OD): $\delta=5.85$ (d, $J=0.8$ Hz, 1H), 5.69 (d, $J=0.5$ Hz, 1H), 4.44 (t, X of ABX, $J=5.5$ Hz, 1H), 4.36 (q, $J=7.1$ Hz, 1H), 3.87 (A of ABX, $J_{AB}=11.0$, $J_{AX}=5.4$ Hz, 1H), 3.79 (B of ABX, $J_{AB}=11.0$, $J_{BX}=5.6$ Hz, 1H), 2.74 (s, 3H), 2.04 (s, 3H), 1.38 ppm (d, $J=7.4$ Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD): $\delta=175.7$, 174.0, 172.3, 166.3, 137.1, 109.9, 62.9, 57.6, 51.2, 26.5, 22.6, 17.9 ppm; FTIR (neat film): $\tilde{\nu}=3294$, 1654, 1508 cm^{-1}; HRMS (FAB)$^+$: m/z calcd for $C_{12}H_{21}N_4O_5$ $[M+H]^+$: 301.1512; found: 301.1513.

Thiol 12: Water (62 μL, 5.02 mmol, 38.6 equiv) was added to a solution of thiazoline **11** (30 mg, 0.13 mmol, 1.0 equiv) in a mixture of methanol (730 μL) and trifluoroacetic acid (20 μL, 0.38 mmol, 3.0 equiv). The reaction mixture was deoxygenated and stirred at 0 °C for 5 h. The reaction mixture was concentrated to afford thiol **12** (31 mg, >99%) as a colorless oil. $R_f=0.18$ (50% diethyl ether in dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=6.01$ (br d, $J=8.2$ Hz, 1H), 5.88 (t, $J=5.9$ Hz, 1H), 5.41 (d, $J=2.3$ Hz, 1H), 5.09 (dd, $J=11.8$, 3.2 Hz, 1H), 4.75 (ddd, $J=13.4$, 8.4, 5.1 Hz, 1H), 4.54 (t, $J=6.4$ Hz, 1H), 4.17 (dd, $J=11.34$, 6.4 Hz, 1H), 4.06 (dd, $J=11.2$, 6.6 Hz, 1H), 2.17 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.99 ppm (d, $J=6.54$ Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta=171.7$, 171.5, 170.7, 170.5, 79.6, 68.0, 68.0, 67.2, 61.8, 48.8, 23.3, 21.0, 20.9,

20.9 ppm; FTIR (neat film): $\tilde{\nu}$=1748, 1661, 1372, 1233, 1086 cm^{-1}; HRMS (FAB)$^+$: *m*/*z* calcd for $C_{14}H_{22}N_1O_8S$ $[M+H]^+$: 364.1066; found: 364.1065.

Monosaccharide 14: Trimethylsilyltrifluoromethane sulfonate (210 μL, 1.13 mmol, 0.1 equiv) was added to a solution of trichloroacetimidate **13** (5.37 g, 11.3 mmol, 1.0 equiv) and tri-*iso*-propylsilane thiol (7.5 mL, 33.9 mmol, 3.0 equiv) in a mixture of dichloromethane (40 mL) and diethyl ether (40 mL) at −20 °C. The resulting mixture was stirred at this temperature for 3 h, then at 23 °C for 2 h. The reaction mixture was neutralized with *N,N*-diisopropylethylamine (0.5 mL), diluted with dichloromethane (650 mL), and washed with saturated aqueous sodium chloride solution (250 mL). The organic layer was dried (sodium sulfate), filtered, and concentrated. The residue was purified by silica-gel flash chromatography (20% ethyl acetate in hexane) to afford *S*-TIPS monosaccharide **14** (4.84 g, 85%) as a yellowish oil. R_f=0.45 (40% ethyl acetate in hexane); ^{1}H NMR (500 MHz, $CDCl_3$): δ=5.60 (d, *J*=5.1 Hz, 1H), 5.46 (d, *J*=3.2, 1.2 Hz, 1H), 5.36 (dd, *J*=11.1, 3.2 Hz, 1H), 4.73 (td, *J*=6.5, 1.2 Hz, 1H), 4.12 (dd, *J*=11.1, 5.0 Hz, 1H), 4.11 (dd, *J*=11.4, 6.4 Hz, 1H), 4.05 (dd, *J*=11.3, 6.7 Hz, 1H), 2.15 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.30 (hept, *J*=7.5 Hz, 3H), 1.14 ppm (d, *J*=7.3 Hz, 18H); ^{13}C NMR (126 MHz, $CDCl_3$): δ=170.7, 170.2, 169.9, 80.7, 69.5, 67.9, 67.6, 61.8, 59.1, 20.9, 20.8, 20.8, 18.5, 18.4, 13.0 ppm; FTIR (neat film): $\tilde{\nu}$=2947, 2868, 2110, 1753, 1370, 1229, 1082, 1048 cm^{-1}; HRMS (FAB)$^+$: *m*/*z* calcd for $C_{21}H_{38}N_3O_7SiS$ $[M+H]^+$: 504.2200; found: 504.2199.

Monosaccharide 15: Sodium methoxide (5 mg, 0.09 mmol, 0.4 equiv) was added to a solution of monosaccharide **14** (118 mg, 0.23 mmol, 1.0 equiv) in methanol (2.8 mL) at 23 °C. The solution was stirred at this temperature for 1 h, and then concentrated in vacuo. The residue was purified by silica-gel flash chromatography (8% methanol in dichloromethane) to afford triol **15** (75 mg, 85%) as a colorless oil. R_f=0.08 (7% methanol in dichloromethane); ^{1}H NMR (500 MHz, CD_3OD): δ=5.55 (d, *J*=5.0 Hz, 1H), 4.27 (ddd, *J*=6.7, 5.6, 0.9 Hz, 1H), 3.98 (dd, *J*=3.2, 1.3 Hz, 1H), 3.95 (dd, *J*=10.6, 3.0 Hz, 1H), 3.86 (dd, *J*=10.5, 5.0 Hz, 1H), 3.76 (dd, *J*=11.0, 7.1 Hz, 1H), 3.61 (dd, *J*=11.0, 5.5 Hz, 1H), 1.31 (hept, *J*=7.4 Hz, 3H), 1.16 ppm (d, *J*=7.2 Hz, 18H); ^{13}C NMR (126 MHz, CD_3OD): δ=82.7, 73.0, 70.5, 70.1, 62.8, 61.8, 19.1, 18.9, 14.2 ppm; FTIR (neat film): $\tilde{\nu}$=3338, 2865, 2105, 1056 cm^{-1}; HRMS (FAB)$^+$: *m*/*z* calcd for $C_{15}H_{31}N_3O_4NaSiS$ $[M+Na]^+$: 400.1702; found: 400.1703.

Monosaccharide 16: Benzoyl chloride (74 μL, 0.64 mmol, 1.1 equiv) was added to a solution of triol **15** (215 mg, 0.57 mmol, 1.0 equiv) and triethylamine (160 μL, 1.15 mmol, 2.0 equiv) in dichloromethane (2.5 mL) at −20 °C. The resulting solution was stirred at this temperature for 4.5 h. Additional benzoyl chloride (7 μL, 0.06 mmol, 0.11 equiv) was added, and the reaction mixture was stirred at −20 °C for a further 1.5 h. The reaction mixture was diluted with dichloromethane (150 mL) and washed with water (70 mL). The organic layer was dried (sodium sulfate), filtered, and concentrated. The residue was purified by silica-gel flash chromatography (20% to 40% ethyl acetate in hexanes) to afford diol **16** (206 mg, 75%) as a colorless oil. R_f=0.64 (50% acetone in toluene); ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.05 (m, 2H), 7.61 (m, 1H), 7.47 (m, 2H), 5.58 (d, *J*=5.1 Hz, 1H), 4.75 (dd, *J*=11.2, 7.4 Hz, 1H), 4.62 (t, *J*=6.5 Hz, 1H), 4.34 (dd, *J*=11.2, 5.8 Hz, 1H), 4.10 (ddd, *J*=10.5, 7.4, 3.3 Hz, 1H), 4.02 (br s, 1H), 3.98 (dd, *J*=10.4, 5.0 Hz, 1H), 3.09 (d, *J*=3.3 Hz, 1H), 2.65 (d, *J*=7.2 Hz, 1H), 1.27 (hept, *J*=7.2 Hz, 3H), 1.12 ppm (d, *J*=7.3 Hz, 18H); ^{13}C NMR (126 MHz, $CDCl_3$): δ=167.2, 133.7, 130.1, 129.6, 128.7, 80.6, 69.0, 68.8, 68.7, 63.0, 62.1, 18.6, 18.4, 13.0 ppm; FTIR (neat film): $\tilde{\nu}$=3368, 1945, 2867, 2108, 1722, 1272, 710 cm^{-1}; HRMS (FAB)$^+$: *m*/*z* calcd for $C_{22}H_{35}N_3O_5NaSiS$ $[M+Na]^+$: 504.1964; found: 504.1964.

Disaccharide 18: Trifluoromethanesulfonic anhydride (18 μL, 0.10 mmol, 2.1 equiv) was added to a solution of hemiacetal **17** (44 mg, 0.07 mmol, 1.5 equiv) and diphenyl sulfoxide (42 mg, 0.21 mmol, 4.2 equiv) in dichloromethane (3.2 mL) at −78 °C. The resulting mixture was stirred at this temperature for 10 min, and then at −48 °C for 1 h. A precooled (−48 °C) solution of diol **16** (24 mg, 0.05 mmol, 1 equiv) in dichloromethane (1 mL) was then added through a cannula. The resulting solution was stirred at this temperature for 1 h, followed by warming to −20 °C for 7 h. The reaction mixture was neutralized with triethylamine (135 μL), diluted with dichloromethane (110 mL), and washed sequentially with saturated aqueous sodium bicarbonate solution (2×75 mL), and saturated aqueous sodium chloride solution (75 mL). The organic layer was dried (sodium sulfate), filtered, and concentrated. The residue was purified by silica-gel flash chromatography (2.4% acetone in benzene), and repurified by the same method (1.2% ethylacetate in dichloromethane) to afford the disaccharide **18** (35 mg, 68%) as a white solid. M.p. 217 °C; R_f=0.25 (5% acetonitrile in benzene); ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.10 (m, 10H), 7.00 (m, 15H), 6.40 (dd, *J*=10.6, 8.1 Hz, 1H), 6.08 (d, *J*=3.4 Hz, 1H), 5.74 (dd, *J*=10.5, 3.6 Hz, 1H), 5.28 (d, *J*=4.5 Hz, 1H), 4.73 (m, 2H), 4.50 (dd, *J*=11.9, 8.2 Hz, 1H), 4.44 (d, *J*=8. 0 Hz, 1H), 4.43 (dd, *J*=11.3, 3.3 Hz, 1H), 4.20 (dd, *J*=11.6, 7.3 Hz, 1H), 4.09 (br s, 1H), 4.04 (dd, *J*=10.7, 2.8 Hz, 1H), 4.00 (dd, *J*=7.6, 4.4 Hz, 1H), 3.68 (dd, *J*=6.1, 4.3 Hz, 1H), 2.84 (dd, *J*=2.8, 1.6 Hz, 1H), 0.99 ppm (m, 21H); ^{13}C NMR (126 MHz, $CDCl_3$): δ=166.6, 166.2, 166.2, 166.1, 166.1, 133.9, 133.8, 133.6, 133.5, 133.4, 131.2, 130.6, 130.5, 130.5, 130.5, 130.4, 130.4, 130.4, 129.7, 129.6, 129.3, 129.2, 128.9, 128.6, 128.8, 128.7, 102.5, 81.5, 79.7, 72.7, 72.5, 70.2, 70.0, 69.2, 69.2, 65.2, 62.9, 60.6, 18.7, 18.6, 13.3 ppm; FTIR (neat film): $\tilde{\nu}$=3446, 2111, 1728, 1268, 1109, 1070, 710 cm^{-1}; HRMS (FAB)$^+$: *m*/*z* calcd for $C_{56}H_{61}N_3O_{14}NaSiS$ $[M+Na]^+$: 1082.3541; found: 1082.3541.

Disaccharide 19: Benzeneselenol (127 μL, 1.21 mmol, 14 equiv) was added to a solution of disaccharide **18** (90 mg, 0.09 mmol, 1 equiv) in a mixture of triethylamine (1.5 mL) and pyridine (1.5 mL), leading to the formation of yellow precipitate. The reaction mixture was heated to 40 °C for 1.5 h, and then cooled to 23 °C prior to the addition of acetic anhydride (470 μL, 4.31 mmol, 50 equiv). The clear solution was stirred at 23 °C for 18 h, and then concentrated in vacuo. The residue was purified by silica-gel flash chromatography (40% ethyl acetate in hexane) to afford thioacetyl disaccharide **19** (64 mg, 76%) as a white solid. M.p. 156 °C; R_f=0.28 (12% ethyl acetate in dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.14 (m, 2H), 8.02 (m, 4H), 7.95 (m, 2H), 7.77 (m, 2H), 7.65 (m, 1H), 7.53 (m, 5H), 7.43 (m, 7H), 7.26 (m, 2H), 6.31 (d, *J*=4.7 Hz, 1H), 5.98 (d, *J*=3.4 Hz, 1H), 5.77 (dd, *J*=10.2, 7.6 Hz, 1H), 5.74 (br s, 1H), 5.63 (dd, *J*=10.3, 3.4 Hz, 1H), 5.45 (d, *J*=6.9 Hz, 1H), 5.02 (d, *J*=7.6 Hz, 1H), 4.76 (m, 1H), 4.65 (dd, *J*=11.2, 6.1 Hz, 1H), 4.39 (m, 3H), 4.32 (dd, *J*=11.5, 5.6 Hz, 1H), 4.20 (t, *J*=6.4 Hz, 1H), 3.79 (dd, *J*=11.1, 3.1 Hz, 1H), 2.26 (s, 3H), 2.16 (s, 3H), 1.16 ppm (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ=170.4, 170.0, 166.4, 166.3, 165.8, 165.7, 165.7, 134.0, 134.0, 133.7, 133.6, 133.4, 130.4, 130.2, 130.1, 130.1, 130.0, 130.0, 129.6, 129.2, 129.2, 129.0, 128.9, 128.9, 128.8, 128.7, 128.6, 100.6, 75.8, 72.0, 71.6, 71.1, 70.6, 68.0, 67.7, 62.6, 62.1, 49.2, 31.7, 22.8, 20.9 ppm; FTIR (neat film): $\tilde{\nu}$=3435, 1640, 1274 cm^{-1}; HRMS (FAB)$^+$: *m*/*z* calcd for $C_{53}H_{49}NO_{17}NaS$ $[M+Na]^+$: 1026.2619; found: 1026.2623.

Disaccharide 21: Trimethylsilyltrifluoromethanesulfonate (3 μL, 0.02 mmol, 0.1 equiv) was added to a solution of sialoside **20** (108 mg, 0.19 mmol, 1.0 equiv) and triol **15** (91 mg, 0.29 mmol, 1.5 equiv) in dichloromethane (2.0 mL) at −45 °C, and the resulting solution was stirred at this temperature for 3.5 h. The reaction mixture was neutralized with triethylamine (45 μL, 0.38 mmol, 2 equiv) followed by concentration in vacuo. The residue was purified by silica-gel flash chromatography (2 to 3 to 6% methanol in dichloromethane) to afford disaccharide **21** (120 mg, 87:13 α:β, 82% total), as a colorless oil. α isomer: R_f=0.18 (6% methanol in ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ=5.62 (d, *J*=9.9 Hz, 1H), 5.55 (d, *J*=5.1 Hz, 1H), 5.35 (m, 2H), 5.18 (ddd, *J*=11.9, 10.2, 4.7 Hz, 1H), 4.92 (d, *J*=14.4 Hz, 1H), 4.78 (d, *J*=14.4 Hz, 1H), 4.42 (m, 2H), 4.21 (d, *J*=3.8 Hz, 1H), 4.15 (dd, *J*=10.8, 1.4 Hz, 1H), 4.07 (m, 4H), 3.92 (dd, *J*=10.5, 5.1 Hz, 1H), 3.85 (d, *J*=6.2 Hz, 1H), 3.00 (s, 3H), 2.99 (s, 3H), 2.82 (d, *J*=9.1 Hz, 1H), 2.69 (dd, *J*=12.7, 4.8 Hz, 1H), 2.16 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.04 (s, 3H), 1.96 (t, *J*=12.3 Hz, 1H), 1.90 (s, 3H), 1.32 (m, 3H), 1.14 ppm (dd, *J*=7.4, 2.6 Hz, 18H); ^{13}C NMR (126 MHz, $CDCl_3$): δ=171.2, 171.2, 170.6, 170.4, 170.3, 167.4, 165.6, 98.7, 80.8, 72.9, 69.9, 69.5, 69.4, 69.2, 68.4, 67.7, 62.6, 62.2, 49.3, 37.4, 36.1, 36.0, 23.4, 21.2, 21.1, 21.0, 18.7, 18.5, 13.0 ppm; FTIR (neat film): $\tilde{\nu}$=3436, 2110, 1645 cm^{-1}; HRMS (FAB)$^+$: *m*/*z* calcd for $C_{38}H_{63}N_5O_{17}NaSiS$ $[M+Na]^+$: 944.3607; found: 944.3607.

Disaccharide 22: A solution of sodium hydroxide in water (1 M, 1.5 mL, 1.5 mmol, 16 equiv) was added to a solution of disaccharide **21** (86 mg, 0.09 mmol, 1 equiv) in methanol (3.8 mL). The solution was deoxygenated, and stirred at 23 °C for 3 h. The reaction mixture was cooled to 0 °C, and neutralized by the addition of a solution of acetic acid in methanol (1 M, 1.6 mL, 1.6 mmol, 17 equiv). The reaction mixture was concentrated with toluene, and the crude product was then suspended in pyridine (1.8 mL) and acetic anhydride (950 μL, 9.7 mmol, 31 equiv) and deoxygenated. A catalytic amount of DMAP (~1 mg) was added, and the resulting

solution stirred at 23 °C for 21 h. The reaction mixture was partitioned between ethyl acetate (100 mL) and water (80 mL), and the aqueous layer was further extracted with ethyl acetate (2×100 mL). The combined organic layers were dried (sodium sulfate), filtered, and concentrated, and the residue was purified by silica-gel flash chromatography (20:160:0.1 MeOH/CH_2Cl_2/AcOH) to afford the disaccharide **22** (50 mg, 66 %) as a white solid. M.p. 188–190 °C; R_f=0.54 (4:1:1 *n*-BuOH/AcOH/H_2O); ^{1}H NMR (500 MHz, CD_3OD): δ=6.19 (d, J=5.3 Hz, 1H), 5.48 (dd, J=3.2, 1.1 Hz, 1H), 5.40 (m, 1H), 5.33 (dd, J=8.4, 1.8 Hz, 1H), 4.94 (m, 2H), 4.50 (dd, J=10.8, 1.7 Hz, 1H), 4.45 (dd, J=11.1, 5.1 Hz, 1H), 4.34 (dd, J=12.1, 2.6 Hz, 1H), 4.25 (td, J=6.6, 0.9 Hz, 1H), 4.20 (dd, J=12.4, 5.2 Hz, 1H), 3.84 (m, 2H), 3.42 (dd, J=9.4, 4.3 Hz, 1H), 2.57 (dd, J=12.3, 4.8 Hz, 1H), 2.44 (s, 3H), 2.16 (s, 3H), 2.16 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 2.02 (s, 3H), 1.96 (s, 3H), 1.84 (s, 3H), 1.62 ppm (t, J=12.0 Hz, 1H); ^{13}C NMR (126 MHz, CD_3OD): δ=193.7, 173.0, 173.7, 172.6, 172.2, 172.2, 172.1, 171.8, 171.2, 101.6, 83.7, 73.5, 73.1, 72.5, 71.7, 70.0, 69.1, 68.9, 64.1, 63.7, 59.2, 50.8, 39.7, 31.6, 22.8, 21.4, 21.1, 21.0, 20.9, 20.9, 20.8 ppm; FTIR (neat film): $\tilde{\nu}$=3369, 2113, 1744, 1618, 1370, 1230, 1040 cm^{-1}; HRMS (ESI)$^-$: m/z calcd for $C_{31}H_{41}N_4O_{19}S$ $[M-H]^-$: 805.2086; found: 805.2110.

Thiazoline 23: Triphenylphosphine (44 mg, 0.17 mmol, 1.4 equiv) was added to a solution of disaccharide **22** (95 mg, 0.12 mmol, 1 equiv) in tetrahydrofuran (2.8 mL) at 23 °C. The solution was stirred at this temperature for 16 h, and then concentrated. The residue was purified by silica-gel flash column chromatography (gradient elution: 50:300:0.1 MeOH/CH_2Cl_2/AcOH to 50:250:0.1 MeOH/CH_2Cl_2/AcOH) to afford the thiazoline **23** (90 mg, >99 %) as a white solid. M.p.=189 °C; R_f=0.20 (17 % methanol in dichloromethane); ^{1}H NMR (500 MHz, CD_3OD): δ=6.44 (d, J=6.1 Hz, 1H), 5.53 (t, J=3.3 Hz, 1H), 5.41 (ddd, J=8.7, 5.8, 2.7 Hz, 1H), 5.31 (dd, J=8.6, 2.0 Hz, 1H), 5.09 (dd, J=8.5, 3.1 Hz, 1H), 4.94 (m, 1H), 4.51 (dd, J=10.7, 1.8 Hz, 1H), 4.40 (m, 1H), 4.34 (m, 2H), 4.12 (dd, J=12.5, 5.9 Hz, 1H), 3.94 (dd, J=11.3, 7.7 Hz, 1H), 3.89 (t, J=10.6 Hz, 1H), 3.47 (dd, J=11.2, 5.2 Hz, 1H), 2.62 (dd, J=12.2, 4.7 Hz, 1H), 2.26 (d, J=1.1 Hz, 3H), 2.16 (s, 3H), 2.12 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), 1.84 (s, 3H), 1.64 ppm (t, J=12.4 Hz, 1H); ^{13}C NMR (126 MHz, CD_3OD): δ=174.8, 173.7, 172.9, 172.6, 172.2, 172.0, 171.9, 171.8, 101.8, 90.4, 75.1, 74.0, 73.0, 72.8, 72.0, 69.9, 69.2, 68.4, 63.9, 63.7, 50.8, 39.7, 22.8, 21.5, 21.4, 21.1, 21.0, 21.0, 20.9, 20.9 ppm; FTIR (neat film): $\tilde{\nu}$=3588, 1735, 1654, 1372, 1230 cm^{-1}; HRMS (ESI)$^-$: m/z calcd for $C_{31}H_{41}N_2O_{18}S$ $[M-H]^-$: 761.2085; found: 761.2086.

Thiol 24: Water (110 μL) was added to a solution of thiazoline **23** (26 mg, 0.03 mmol, 1 equiv) in methanol (1.3 mL) containing trifluoroacetic acid (50 μL, 0.62 mmol, 19 equiv). The resulting solution was deoxygenated, and stirred at 0 °C for 8 h. The reaction mixture was diluted with toluene (15 mL) and then concentrated. Residual TFA was removed from the product by azeotropic coevaporation (2×15 mL methanol; 1×15 mL tolene), followed by lyophylization (30 mL water) to afford thiol **24** (27 mg, >99 %), as a white solid. M.p. 195 °C (decomp); R_f=0.18 (17 % methanol in dichloromethane); ^{1}H NMR (500 MHz, CD_3OD): δ=5.85 (d, J=5.2 Hz, 1H), 5.49 (dd, J=3.0, 0.8 Hz, 1H), 5.43 (ddd, J=9.4, 5.3, 2.6 Hz, 1H), 5.32 (dd, J=9.4, 2.0 Hz, 1H), 5.12 (dd, J=12.0, 3.2 Hz, 1H), 4.90 (m, 1H), 4.58 (t, J=7.0 Hz, 1H), 4.55 (dd, J=11.8, 5.2 Hz, 1H), 4.27 (dd, J=9.4, 1.8 Hz, 1H), 4.24 (dd, J=12.5, 2.7 Hz, 1H), 4.09 (dd, J=12.5, 5.3 H, 1Hz), 3.96 (t, J=10.5 Hz, 1H), 3.84 (dd, J=10.0, 6.2 Hz, 1H), 3.40 (dd, J=10.0, 7.5 Hz, 1H), 2.58 (dd, J=12.6, 4.7 Hz, 1H), 2.14 (s, 3H), 2.13 (s, 3H), 2.09 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.96 (2 overlapping s, 6H), 1.84 (s, 3H), 1.75 ppm (t, J=12.4 H, 1Hz); ^{13}C NMR (126 MHz, CD_3OD): δ=180.8, 173.9, 173.7, 172.7, 172.4, 172.2, 172.0, 171.9, 171.8, 80.5, 73.2, 71.5, 70.0, 69.8, 69.4, 68.9, 68.9, 64.1, 63.8, 50.6, 49.8, 39.5, 22.8, 22.5, 21.4, 21.1, 20.9, 20.9, 20.8 ppm; FTIR (neat film): $\tilde{\nu}$=3448, 1736, 1654, 1375, 12236, 1045 cm^{-1}; HRMS (ESI)$^-$: m/z calcd for $C_{31}H_{43}N_2O_{19}S$ $[M-H]^-$: 779.2181; found: 779.2175.

Monosaccharide 25: Allylchloroformate (91 μL, 0.86 mmol, 1.3 equiv) was added portionwise to a solution of monosaccharide **15** (252 mg, 0.66 mmol, 1.0 equiv) and triethylamine (185 μL, 1.32 mmol, 2 equiv) in dichloromethane (9 mL) at −20 °C, and the solution was stirred at this temperature for 2 h. Water (5 mL) was added, and the reaction mixture was partitioned between dichloromethane (150 mL) and water (60 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by silica-gel flash column chromatography (20 % ethyl acetate in toluene) to afford diol **25** (141 mg, 46 %) as a colorless oil. R_f=0.33 (50 % ethyl acetate in hexanes); ^{1}H NMR (500 MHz, $CDCl_3$): δ=5.92 (m, 1H), 5.57 (d, J=5.0 Hz, 1H), 5.40–5.36 (m, 1H), 5.31–5.28 (m, 1H), 4.65–4.63 (m, 2H), 4.55 (t, J=6.6 Hz, 1H), 4.45 (dd, J=11.3, 6.7 Hz, 1H), 4.24 (dd, J=11.3, 6.1 Hz, 1H), 4.08 (ddd, J=10.4, 7.0, 3.4 Hz, 1H), 4.00 (br m, 1H), 3.95 (dd, J=10.4, 5.0 Hz, 1H), 2.85 (d, J=3.4 Hz, 1H), 2.62 (d, J=7.1 Hz, 1H), 1.30 (m, 3H), 1.14 ppm (d, 7.2 Hz, 18H); ^{13}C NMR (126 MHz, $CDCl_3$): δ=155.4, 131.4, 119.4, 80.5, 77.6, 77.2, 76.9, 69.1, 66.0, 61.9, 18.6, 18.4, 13.0 ppm; FTIR (neat film): $\tilde{\nu}$=3369, 2946, 2109, 1749, 1257 cm^{-1}; HRMS (FAB)$^+$: m/z calcd for $C_{19}H_{35}N_3O_6NaSiS$ $[M+Na]^+$: 484.1914; found: 484.1912.

Disaccharide 26: Trifluoromethanesulfonic anhydride (47 μL, 0.28 mmol, 1.9 equiv) was added to a solution of hemiacetal **17** (125 mg, 0.21 mmol, 1.4 equiv) and 4-nitrophenylphenyl sulfoxide (139 mg, 0.56 mmol, 3.8 equiv) in dichloromethane (9 mL) at −78 °C. The resulting mixture was stirred at this temperature for 10 min, and then at −48 °C for 1 h. A precooled (−48 °C) solution of diol **25** (65 mg, 0.15 mmol, 1 equiv) in dichloromethane (2.5 mL) was then added through a cannula. The solution was stirred at this temperature for 6 h. The reaction mixture was neutralized with triethylamine (380 μL), diluted with dichloromethane (200 mL), and washed sequentially with saturated aqueous sodium bicarbonate solution (2×125 mL) and saturated aqueous sodium chloride solution (125 mL). The organic layer was dried (sodium sulfate), filtered, and concentrated. The residue was purified by silica-gel flash chromatography (2.4 % acetone in benzene), and repurified by the same method (22 % ethylacetate in petroleum ether) to afford the disaccharide **26** (102 mg, 70 %) as a white solid. M.p. 182–183 °C; R_f=0.19 (22 % ethylacetate in petroleum ether); ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.11–7.97 (m, 6H),7.78 (m, 2H), 7.65 (m, 1H), 7.56–7.23 (m, 11H), 6.01 (dd, J=3.5, 0.9 Hz, 1H), 5.96–5.87 (m, 2H), 5.61 (dd, J=10.5, 3.5 Hz, 1H), 5.51, (d, J=4.3 Hz, 1H), 5.38–5.34 (m, 1H), 5.28–5.26 (m, 1H), 5.13 (d, J=8.0 Hz, 1H), 4.65 (dd, J=11.5, 7.4 Hz, 1H), 4.61 (dt, J=7.8, 1.3 Hz, 2H), 4.48 (dd, J=11.8, 5.5 Hz, 1H), 4.45 (t, J=7.3 Hz, 1H), 4.41 (td, J=6.0, 0.8 Hz, 1H), 4.32 (dd, J=11.6, 7.3 Hz, 1H), 4.19 (br s, 1H), 4.11 (dd, J=11.5, 4.4 Hz, 1H), 4.05 (m, 2H), 2.73 (br s, 1H), 1.27 (m, 3H), 1.10 ppm (dd, J=7.4, 4.8 Hz, 18H); ^{13}C NMR (126 MHz, $CDCl_3$): δ=166.2, 165.8, 165.7, 165.7, 155.0, 133.9, 133.6, 133.6, 133.5, 131.7, 130.2, 130.0, 130.0, 129.9, 129.4, 129.3, 129.0, 128.9, 128.8, 128.8, 128.5, 119.0, 102.4, 80.6, 78.5, 72.1, 71.7, 69.5, 68.8, 68.6, 68.2, 66.8, 62.3, 60.4, 18.5, 18.3, 12.9 ppm; FTIR (neat film): $\tilde{\nu}$=3505, 2946, 2111, 1730, 1264, 1109, 1026, 710 cm^{-1}; HRMS (FAB)$^+$: m/z calcd for $C_{53}H_{61}N_3O_{15}NaSiS$ $[M+Na]^+$: 1062.3490; found: 1062.3490.

Disaccharide 27: Tri-*n*-butyltin hydride (265 μL, 0.98 mmol, 10 equiv) was added to a deoxygenated solution of disaccharide **26** (102 mg, 0.10 mmol, 1 equiv) and *trans*-dichlorobis(triphenylphosphine)palladium(II) (7 mg, 0.01 mmol, 0.09 equiv) in dichloromethane (12.5 mL) and water (245 μL). The resulting brown solution was stirred at 23 °C for 14 min, and then concentrated in vacuo. The residue was purified by silica-gel flash column chromatography (8 % ethylacetate in dichloromethane) to afford disaccharide diol **27** (94 mg, >99 %) as a yellowish solid. M.p. 112–113 °C; R_f=0.29 (50 % ethylacetate in hexanes); ^{1}H NMR (500 MHz, C_6D_6): δ=8.22–8.20 (m, 4H), 8.15–8.13 (m, 2H), 8.00–7.98 (m, 2H), 7.16–6.70 (m, 12H), 6.38 (dd, J=10.5, 8.0 Hz, 1H), 6.05 (dd, J=3.3, 0.8 Hz, 1H), 5.71 (dd, J=10.6, 3.4 Hz, 1H), 5.30 (d, J=4.1 Hz, 1H), 4.46 (d, J=8.2 Hz, 1H), 4.44 (dd, J=10.8, 7.6 Hz, 1H), 4.31 (t, J=5.4 Hz, 1H), 4.28 (dd, J=11.8, 4.1 Hz, 1H), 4.11 (br d, J=1.7 Hz, 1H), 4.01 (m, 2H), 3.83 (m, 1H), 3.76 (m, 1H), 3.63 (ddd, J=7.7, 3.6, 0.8 Hz, 1H), 2.78 (br s, 1H), 1.87 (dd, J=8.1, 4.2 Hz, 1H), 1.12–1.01 ppm (m, 21H); ^{13}C NMR (126 MHz, $CDCl_3$): δ=166.2, 165.9, 165.7, 165.7, 134.0, 133.8, 133.6, 133.5, 130.3, 130.0, 130.0, 129.9, 129.4, 129.3, 129.0, 128.9, 128.8, 128.8, 128.5, 128.5, 102.6, 80.9, 79.0, 72.3, 71.8, 70.4, 69.8, 69.5, 68.4, 63.0, 62.6, 60.3, 18.6, 18.4, 13.0 ppm; FTIR (neat film): $\tilde{\nu}$=3580, 3472, 2949, 2110, 1729, 1267, 1108, 1070 cm^{-1}; HRMS (FAB)$^+$: m/z calcd for $C_{49}H_{58}N_3O_{13}$. NaSiS (M+H+Na)$^+$: 979.3357; found: 979.3357.

Tri-*iso*-propylsilylthio-4,7,8,9-tetra-*O*-acetyl-5-*N*-acetylamino-1-*N*,*N*-dimethylglycolamido-α-D-sialosyl-(2→6)-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-azido-1,2-dideoxy-α-D-galactopyranoside): A solution of trimethylsilyltrifluoromethanesulfonate (1.8 μL, 0.01 mmol, 0.1 equiv) in dichloromethane (16 μL) was added to a solution of sialoside **20** (51 mg, 0.08 mmol, 1.0 equiv) and disaccharide **27** (99 mg, 0.10 mmol, 1.4 equiv) in dichloromethane (2.5 mL) at −78 °C, and the solution was stirred at this temperature for 6 h. The reaction mixture was neutralized

with triethylamine (50 μL), and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (33 to 50 to 67% acetonitrile in toluene) to afford the trisaccharide as separable mixture of anomers (73 mg, 81:19 α:β, 66% total). α-isomer: M.p. 120°C; R_f=0.45 (66% acetonitrile in toluene); ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.11–8.09 (m, 2H), 8.01–7.97 (m, 4H), 7.79–7.77 (m, 2H), 7.65–7.34 (m, 10H), 7.24 (m, 2H), 6.02 (d, J=3.4 Hz, 1H), 5.89 (dd, J=10.4, 8.0 Hz, 1H), 5.85 (d, J=10.2 Hz, 1H), 5.60 (dd, J=10.3, 3.4 Hz, 1H), 5.53 (d, J=4.8 Hz, 1H), 5.39 (td, J=6.5, 2.9 Hz, 1H), 5.34 (dd, J=6.4, 2.0 Hz, 1H), 5.24 (ddd, J=12.0, 10.1, 4.6 Hz, 1H), 5.16 (d, J=7.8 Hz, 1H), 4.83 (q, J=14.4 Hz, 2H), 4.64 (dd, J=11.1, 6.5 Hz, 1H), 4.43 (dd, J=11.2, 6.9 Hz, 1H), 4.37 (m, 2H), 4.23 (m, 2H), 4.14 (m, 2H), 4.06 (dd, J=10.4, 4.8 Hz, 1H), 4.01 (dd, J=10.6, 2.7 Hz, 1H), 3.91 (dd, J=9.8, 6.2 Hz, 1H), 3.78 (dd, J=9.8, 6.1 Hz, 1H), 2.99 (m, 1H), 2.96 (s, 3H), 2.95 (s, 3H), 2.70 (dd, J=12.6, 4.8 Hz, 1H), 2.14 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.90 (t, J=12.2 Hz, 1H), 1.29 (m, 3H), 1.13 ppm (t, J=7.7 Hz, 18H); ^{13}C NMR (126 MHz, C_6D_6): δ=170.7, 170.5, 170.4, 170.4, 169.9, 168.5, 166.3, 166.2, 166.2, 166.1, 165.6, 133.8, 133.7, 133.5, 133.5, 130.6, 130.6, 130.4, 130.4, 130.0, 129.8, 129.3, 129.1, 128.9, 128.8, 128.7, 128.5, 128.3, 103.3, 99.9, 81.6, 79.7, 74.2, 72.7, 72.3, 71.6, 70.8, 70.5, 70.3, 69.4, 69.2, 69.1, 64.3, 63.7, 62.7, 62.6, 61.3, 50.0, 38.9, 35.4, 34.9, 23.1, 21.3, 21.1, 20.9, 20.8, 19.0, 18.9, 13.5 ppm; FTIR (neat film): $\tilde{\nu}$=3450, 2938, 2110, 1733, 1667, 1266, 1106, 1070, 708 cm^{-1}; HRMS (FAB)$^+$: m/z calcd for $C_{72}H_{89}N_5O_{26}NaSiS$ $[M+Na]^+$: 1522.5183; found: 1522.5177.

Trisaccharide 28: A solution of sodium hydroxide in water (1 M, 560 μL, 0.56 mmol, 30 equiv) was added to a solution of tri-*iso*-propylsilylthio-4,7,8,9-tetra-*O*-acetyl-5-*N*-acetylamino-1-*N*,*N*-dimethylglycolamido-α-D-sialosyl-(2→6)-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-azido-1,2-dideoxy-α-D-galactopyranoside) (28 mg, 0.02 mmol, 1 equiv) in methanol (1.8 mL). The resulting solution was deoxygenated, and stirred at 23°C for 3 h. The residue was diluted with toluene (~50 mL) and concentrated. The residue was suspended in pyridine (1.5 mL) and acetic anhydride (750 μL, 7.9 mmol, 427 equiv) with a catalytic amount of DMAP (~0.2 mg), and reaction mixture deoxygenated and stirred at 23°C for 15 h. The reaction was partitioned between ethyl acetate (80 mL) and 1 M hydrochloric acid (45 mL). The aqueous layer was further extracted with ethyl acetate (2×40 mL), and the combined organic layers were dried (sodium sulfate), filtered, and concentrated. The residue was purified by silica-gel flash chromatography (20:160:0.1 methanol/dichloromethane/acetic acid) to afford the trisaccharide **28** (10 mg, 51%) as a white solid. M.p. 176–177°C; R_f=0.17 (11% methanol in dichloromethane); ^{1}H NMR (500 MHz, CD_3OD): δ=6.21 (d, J=5.4 Hz, 1H), 5.48 (dd, J=3.3, 1.0 Hz, 1H), 5.37 (m, 2H), 5.31 (dd, J=7.8, 2.3 Hz, 1H), 5.13 (dd, J=10.3, 3.3 Hz, 1H), 5.07 (dd, J=10.6, 7.6 Hz, 1H), 4.95 (m, 1H), 4.55 (dd, J=12.3, 2.1 Hz, 1H), 4.36 (dd, J=12.4, 2.7 Hz, 1H), 4.29 (dd, J=10.9, 5.5 Hz, 1H), 4.12 (m, 4H), 4.04 (dd, J=7.9, 3.0 Hz, 1H), 3.88 (t, J=10.3 Hz, 1H), 3.79 (dd, J=10.8, 3.5 Hz, 1H), 3.75 (dd, J=10.9, 7.6 Hz, 1H), 3.48 (dd, J=11.1, 3.5 Hz, 1H), 2.58 (dd, J=12.1, 4.8 Hz, 1H), 2.44 (s, 3H), 2.14 (s, 6H), 2.11 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H), 1.82 (s, 3H),1.64 ppm (t, J=12.1 Hz, 1H); FTIR (neat film): $\tilde{\nu}$=3394, 2113, 1752, 1597, 1369, 1228 cm^{-1}; HRMS (ESI)$^-$: m/z calcd for $C_{43}H_{57}N_4O_{27}S$ $[M-H]^-$: 1093.2931; found: 1093.2880.

4,7,8,9-Tetra-*O*-acetyl-5-*N*-acetylamino-α-D-sialosyl-(2→6)-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4-*O*-acetyl-2-amino-1,2-dideoxy-1-*S*-2-*N*-(ethan-1-yl-1-ylidene)-α-D-galactopyranoside: Triphenylphosphine (4 mg, 0.02 mmol, 1.7 equiv) was added to a solution of trisaccharide **28** (10 mg, 0.01 mmol, 1.0 equiv) in tetrahydrofuran (600 μL) at 23°C. The solution was stirred at this temperature for 26.5 h, and concentrated. The residue was purified by silica-gel flash column chromatography (50:300:0.1 methanol/dichloromethane/acetic acid) to afford the thiazoline (9 mg, 91%) as a white solid. M.p.=195–197°C; R_f=0.20 (14% methanol in dichloromethane); ^{1}H NMR (500 MHz, CD_3OD): δ=6.30 (d, J=6.0 Hz, 1H), 5.54 (t, J=3.5 Hz, 1H), 5.41 (ddd, J=8.4, 5.8, 2.6 Hz, 1H), 5.38 (dd, J=3.4, 1.2 Hz, 1H), 5.31 (dd, J=9.0, 2.3 Hz, 1H), 5.14 (dd, J=10.0, 3.4 Hz, 1H), 5.06 (m, 2H), 4.94 (m, 1H), 4.51 (dd, J=10.8, 1.6 Hz, 1H), 4.34 (dd, J=12.4, 2.7 Hz, 1H), 4.27 (m, 1H), 4.21–4.08 (m, 6H), 3.92 (m, 2H), 3.44 (dd, J=11.1, 3.4 Hz, 1H), 2.61 (dd, J=12.2, 4.8 Hz, 1H), 2.28 (d, J=1.6 Hz, 3H), 2.15 (2 overlapping s, 6H), 2.13 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.84 (s, 3H), 1.68 ppm (t, J=12.1 Hz, 1H); ^{13}C NMR (126 MHz, CD_3OD): δ=174.9, 173.7, 172.7, 172.3, 172.2, 172.2, 172.0, 171.9, 171.7, 171.6, 169.9, 101.8, 101.7, 89.6, 79.6, 77.7, 75.0, 72.9, 72.4, 72.1, 72.0, 70.7, 70.7, 70.1, 69.4, 68.9, 63.9, 63.8, 62.6, 50.9, 39.7, 22.8, 21.5, 21.2, 21.1, 21.0, 20.9, 20.9, 20.7, 20.6 ppm; FTIR (neat film): $\tilde{\nu}$=3436, 1749, 1637, 1371, 1227, 1047 cm^{-1}; HRMS (ESI)$^-$: m/z calcd for $C_{43}H_{57}N_2O_{26}S$ $[M-H]^-$: 1049.2920; found: 1049.2919.

Thiol 29: Water (52 μL) was added to a solution of trisaccharide thiazoline (17 mg, 0.02 mmol, 1 equiv) in methanol (0.6 mL) containing trifluoroacetic acid (12 μL, 0.31 mmol, 19 equiv). The resulting solution was deoxygenated, and stirred at 0°C for 8.5 h. The reaction mixture was diluted with toluene (20 mL) and then concentrated. Residual TFA was removed from the product by azeotropic coevaporation (5×10 mL of methanol and 15 mL of toluene) to afford thiol **29** (17 mg, >99%), as a white solid. M.p. 220°C (decomp); R_f=0.22 (17% methanol in dichloromethane); ^{1}H NMR (500 MHz, CD_3OD): δ=5.69 (d, J=5.2 Hz, 1H), 5.46 (d, J=3.2 Hz, 1H), 5.40 (ddd, J=8.2, 5.3, 2.3 Hz, 1H), 5.37 (dd, J=3.4, 1.2 Hz, 1H), 5.32 (dd, J=8.6, 2.2 Hz, 1H), 5.07 (dd, J=10.5, 3.4 Hz, 1H), 5.00 (dd, J=10.5, 7.7 Hz, 1H), 4.91 (m, 1H), 4.82 (d, J=7.8 Hz, 1H), 4.58 (m, 1H), 4.45 (t, J=5.5 Hz, 1H), 4.31 (m, 2H), 4.18 (d, J=6.7 Hz, 1H), 4.11 (dd, J=12.2, 5.4 Hz, 1H), 4.05 (td, J=6.4, 0.8 Hz, 1H), 4.02 (dd, J=11.1, 3.4 Hz, 1H), 3.95 (m, 1H), 3.82 (dd, J=10.4, 7.2 Hz, 1H), 3.42 (dd, J=10.3, 4.8 Hz, 1H), 2.61 (dd, J=13.0, 4.8 Hz, 1H), 2.14 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.94 (s, 3H), 1.83 (s, 3H), 1.75 ppm (t, J=12.4 Hz, 1H); ^{13}C NMR (126 MHz, CD_3OD): δ=173.7, 173.4, 172.7, 172.3, 172.2, 172.1, 171.9, 171.7, 171.7, 171.6, 171.4, 102.3, 80.6, 73.7, 73.2, 72.2, 72.0, 71.4, 69.9, 69.1, 68.7, 64.8, 63.7, 62.5, 51.2, 50.5, 23.0, 22.8, 21.3, 21.2, 21.1, 20.9, 20.9, 20.9, 20.6, 20.6 ppm; FTIR (neat film): $\tilde{\nu}$=3451, 1744, 1645, 1374, 1228, 1052 cm^{-1}; HRMS (ESI)$^-$: m/z calcd for $C_{43}H_{59}N_2O_{27}S$ $[M-H]^-$: 1067.3026; found: 1067.2975.

Monosaccharide–tripeptide conjugate 30: A deoxygenated solution of sodium methoxide (4 mg, 0.08 mmol, 1 equiv) in methanol (760 μL) was added to a mixture of thiol **12** (28 mg, 0.08 mmol, 1.0 equiv) and tripeptide **9** (21 mg, 0.083 mmol, 1.1 equiv) at 23°C. A white precipitate was observed after 2 h. The reaction mixture was stirred at this temperature for a total of 4.5 h, and then neutralized with a solution of acetic acid in methanol (1 M, 91 μL, 0.09 mmol, 1.2 equiv) followed by concentration in vacuo. The residue was purified by silica-gel flash chromatography (25% to 50% methanol in dichloromethane), followed by LH 20 Sephadex size-exclusion chromatography (methanol) to afford glycoconjugate **30** (30 mg, 79%, dr=1.4:1) as a white solid. R_f=0.42 (50% methanol in dichloromethane); ^{1}H NMR (500 MHz, D_2O): δ=5.55 (d, J=5.6 Hz, 1.4H), 5.53 (d, J=5.6 Hz, 1H), 4.63 (dd, J=7.6, 5.1 Hz, 1.4H), 4.56 (dd, J=8.5, 6.0 Hz, 1H), 4.36–3.74 (m, 28.8H), 3.16–3.05 (m, 3.8H), 2.89 (dd, J=14.0, 8.5 Hz, 1H), 2.73 (s, 7.2H), 2.07 (s, 4.2H), 2.06 (s, 3H), 2.03 (s, 4.2H), 2.02 ppm (s, 3H); ^{13}C NMR (126 MHz, D_2O): δ=175.1, 175.0, 174.7, 174.7, 172.9, 172.8, 172.2, 172.0, 171.7, 171.6, 85.2, 83.9, 72.0, 71.9, 68.6, 68.5, 67.6, 67.5, 61.4, 61.3, 54.2, 53.4, 50.2, 50.1, 49.0, 42.7, 42.7, 42.5, 32.1, 31.1, 25.9, 22.0, 22.0, 21.8, 21.8 ppm; FTIR (neat film): $\tilde{\nu}$=3436, 2092, 1645 cm^{-1}; HRMS (FAB)$^+$: m/z calcd for $C_{18}H_{32}N_5O_9S$ $[M+H]^+$: 494.1921; found: 494.1921.

Monosaccharide–tripeptide conjugate 31: A deoxygenated solution of sodium methoxide (2 mg, 0.04 mmol, 1 equiv) in methanol (415 μL) was added to the mixture of thiol **12** (15 mg, 0.05 mmol, 1.0 equiv) and tripeptide **10** (15 mg, 0.05 mmol, 1.2 equiv) at 23°C. After 2 h, a white precipitate was observed. The reaction mixture was stirred at this temperature for a total of 4 h, and then neutralized with a solution of acetic acid in methanol (1 M, 50 μL, 0.05 mmol, 1.2 equiv) followed by concentration in vacuo. The residue was purified by silica-gel flash chromatography (25% to 50% methanol in dichloromethane), followed by LH 20 Sephadex size-exclusion chromatography (methanol) to afford glycoconjugate **31** (20 mg, 92%, dr=1.1:1) as a white solid. R_f=0.07 (25% methanol in dichloromethane); ^{1}H NMR (500 MHz, CD_3OD): δ=5.62 (d, J=5.4 Hz, 1H), 5.59 (d, J=5.4 Hz, 1H), 4.61 (dd, J=7.9, 4.8 Hz, 1H), 4.53 (t, J=7.4 Hz, 1.1H), 4.42 (m, 4.3H), 4.29 (m, 2.1H), 4.19 (m, 2.2H), 3.88–3.64 (m, 15.2H), 3.09 (m, 3.2H), 2.85 (dd, J=13.9, 7.4 Hz, 1.1H), 2.73 (s, 3H), 2.72 (s, 3.3H), 2.04 (s, 3H), 2.03 (s, 3.3H), 1.99 (s, 3H), 1.98 (s, 3.3H), 1.37 (d, J=7.4 Hz, 3.3H), 1.34 ppm (d, J=7.1 Hz, 3H); ^{13}C NMR (126 MHz, D_2O): δ=175.2, 175.2, 174.7, 174.6, 172.4, 172.3, 172.1, 171.8, 84.9, 83.6, 71.9, 71.8, 68.6, 68.5, 67.6, 67.6, 61.4, 61.2, 61.1, 55.9, 55.7, 53.8, 53.1, 50.2, 50.0, 49.0, 31.9, 31.0, 26.0, 25.9, 22.0, 22.0, 21.8, 16.6 ppm; FTIR

(neat film): $\tilde{\nu}$ = 3294, 1654, 1420, 1067 cm^{-1}; HRMS (FAB)$^+$: *m*/*z* calcd for $C_{20}H_{35}N_5O_{10}NaS$ $[M+Na]^+$: 560.2002; found: 560.2001.

Disaccharide–tripeptide conjugate 32: A deoxygenated solution of sodium methoxide (1.0 mg, 0.02 mmol, 1 equiv) in methanol (180 μL) was added to a mixture of disaccharide **19** (20 mg, 0.02 mmol, 1.0 equiv) and peptide **9** (6 mg, 0.02 mmol, 1.1 equiv) at 23 °C. After 3 h, a white precipitate was observed. The reaction mixture was stirred at 23 °C for a total of 6.5 h and then neutralized by the addition of a solution of acetic acid in methanol (1 M, 23 μL, 0.02 mmol, 1.2 equiv), followed by concentration in vacuo. The residue was purified by silica-gel flash chromatography (50% to 67% methanol in dichloromethane), followed by LH 20 Sephadex size-exclusion chromatography (methanol) to afford glycoconjugate **32** (12 mg, 90%, *dr* = 1.2:1.0) as a white solid. R_f = 0.33 (33% dichloromethane in methanol); ^{1}H NMR (500 MHz, D_2O): δ = 5.50 (d, *J* = 5.6 Hz, 1.2H), 5.48 (d, *J* = 5.6 Hz, 1H), 4.62 (dd, *J* = 7.8, 5.1 Hz, 1.2H), 4.56–4.51 (m, 3.3H), 4.48 (d, *J* = 7.8 Hz, 2H), 4.30–4.24 (m, 5H), 3.94–3.69 (m, 25.2H), 4.64–3.59 (m, 5.3H), 3.49 (dd, *J* = 9.8, 7.7 Hz, 2H), 3.13 (dd, *J* = 14.1, 6.0 Hz, 1H), 3.11 (dd, *J* = 14.5, 7.9 Hz, 1.2H), 3.06 (dd, *J* = 14.5, 5.1 Hz, 1.2H), 2.88 (dd, *J* = 14.1, 8.5 Hz, 1H), 2.71 (s, 6.6H), 2.04 (s, 3.5H), 2.04 (s, 3H), 2.00 (s, 3.6H), 1.99 ppm (s, 3H); ^{13}C NMR (126 MHz, CD_3OD): δ = 174.4, 174.2, 174.2, 174.2, 173.4, 173.2, 172.6, 173.3, 172.2, 172.2, 106.2, 87.4, 86.7, 79.0, 78.9, 77.0, 75.0, 73.4, 72.7, 70.4, 70.4, 70.0, 70.0, 63.4, 63.2, 62.8, 55.8, 55.4, 50.8, 50.8, 50.0, 44.1, 43.7, 43.7, 43.7, 34.2, 33.3, 26.5, 26.5, 22.9, 22.9, 22.6, 22.6 ppm; FTIR (neat film): $\tilde{\nu}$ = 3307, 1654, 1560, 1412, 1077 cm^{-1}; HRMS (FAB)$^+$: *m*/*z* calcd for $C_{24}H_{41}N_5O_{14}NaS$ $[M+Na]^+$: 678.2268; found: 678.2265.

Disaccharide–tripeptide conjugate 33: A deoxygenated solution of sodium methoxide (1.8 mg, 0.03 mmol, 1 equiv) in methanol (530 μL) was added to a mixture of disaccharide **19** (33 mg, 0.03 mmol, 1.0 equiv) and tripeptide **10** (12 mg, 0.04 mmol, 1.2 equiv) at 23 °C. After 2 h, white precipitate was observed. The reaction mixture was stirred at 23 °C for a total of 4 h and then neutralized by the addition of a solution of acetic acid in methanol (1 M, 40 μL, 0.04 mmol, 1.2 equiv), followed by concentration in vacuo. The residue was purified by silica-gel flash chromatography (33% to 67% methanol in dichloromethane), followed by LH 20 Sephadex size-exclusion chromatography (methanol) to afford glycoconjugate **33** (19 mg, 84%, *dr* = 1.1:1.0) as a white solid. R_f = 0.22 (50% dichloromethane in methanol); ^{1}H NMR (500 MHz, CD_3OD): δ = 5.65 (d, *J* = 5.5 Hz, 1.1H), 5.62 (d, *J* = 5.6 Hz, 1H), 4.63–4.52 (m, 3.5H), 4.43 (m, 3.6H), 4.32–4.17 (m, 5.2H), 3.90–3.68 (m, 15.2H), 3.54 (m, 3.5H), 3.46 (dd, *J* = 9.9, 3.4 Hz, 2.2H), 3.15–3.04 (m, 3.2H), 2.87 (dd, *J* = 13.8, 7.5 Hz, 1H), 2.73 (s, 3.3H), 2.72 (s, 3H), 2.04 (s, 3.3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3.3H), 1.38 (d, *J* = 7.1 Hz, 3H), 1.34 ppm (d, *J* = 7.4 Hz, 3.3H); ^{13}C NMR (126 MHz, D_2O): δ = 179.4, 179.0, 175.2, 175.2, 174.7, 172.4, 172.3, 172.1, 171.7, 104.6, 104.5, 85.1, 83.7, 77.0, 76.9, 75.1, 72.6, 71.6, 71.6, 70.7, 68.7, 68.6, 61.4, 61.3, 61.2, 61.1, 55.9, 55.7, 53.9, 53.1, 50.2, 50.1, 48.9, 48.8, 32.0, 30.9, 26.0, 25.9, 22.1, 21.8, 16.6, 16.6 ppm; FTIR (neat film): $\tilde{\nu}$ = 3307, 1654, 1560, 1375, 1076 cm^{-1}; HRMS (FAB)$^+$: *m*/*z* calcd for $C_{26}H_{45}N_5O_{15}NaS$ $[M+Na]^+$: 722.2531; found: 722.2531.

Disaccharide–tripeptide conjugate 34: A deoxygenated solution of sodium methoxide (2.6 mg, 0.05 mmol, 4 equiv) in methanol (570 μL) was added to a mixture of thiol **24** (10 mg, 0.01 mmol, 1 equiv) and tripeptide **9** (4 mg, 0.01 mmol, 1.2 equiv) at 23 °C. The reaction mixture was stirred at 23 °C for 14 h and then neutralized by the addition of a solution of acetic acid in methanol (1 M, 52 μL, 0.05 mmol, 4.2 equiv) followed by concentration in vacuo. The residue was purified by silica-gel flash chromatography (33% to 67% methanol in dichloromethane), followed by LH 20 Sephadex size-exclusion chromatography (methanol) to afford glycoconjugate **34** (8 mg, 84%, *dr* = 1.3:1.0) as a white solid. R_f = 0.15 (4:4:1:1 MeOH/EtOAc/AcOH/H_2O); ^{1}H NMR (500 MHz, CD_3OD): δ = 5.61 (d, *J* = 5.5 Hz, 1H), 5.53 (d, *J* = 1.3 Hz, 1.3H), 4.67 (t, *J* = 7.1 Hz, 1H), 4.57 (dd, *J* = 8.0, 3.9 Hz, 1.3H), 4.41 (m, 3.9H), 4.34 (dd, *J* = 7.4, 4.4 Hz, 1.3H), 4.06–3.51 (m, 40H), 3.22 (dd, *J* = 14.4, 8.3 Hz, 1.3H), 3.14 (dd, *J* = 13.8, 6.9 Hz, 1H), 3.00 (dd, *J* = 14.4, 4.2 Hz, 1H), 2.82 (m, 3.7H), 2.75 (s, 3H), 2.74 (s, 3.9H), 2.08 (s, 3.9H), 2.04 (s, 3H), 2.02 (s, 7H), 1.96 (s, 7H), 1.57 ppm (m, 2.3H); ^{13}C NMR (126 MHz, CD_3OD): δ = 175.8, 175.8, 175.7, 174.7, 174.7, 174.3, 174.1, 174.1, 173.8, 173.4, 173.4, 173.2, 173.2, 172.4, 172.3, 87.7, 87.1, 74.9, 74.7, 73.1, 73.0, 72.3, 72.0, 70.4, 70.3, 69.8, 69.7, 69.5, 69.4, 65.5, 65.4, 64.8, 64.7, 56.1, 56.0, 54.3, 54.1, 52.0, 50.1, 49.8, 44.4, 44.1, 44.0, 43.9, 42.5, 42.0, 34.6, 26.5, 23.1, 22.9, 22.8, 22.8, 22.8 ppm; FTIR (neat film): $\tilde{\nu}$ = 3294, 1654, 1560, 1376, 1035 cm^{-1}; HRMS (ESI)$^-$: *m*/*z* calcd for $C_{29}H_{47}N_6O_{17}S$ $[M-H]^-$: 783.2718; found: 783.2738.

Disaccharide–tripeptide conjugate 35: A deoxygenated solution of sodium methoxide (1.1 mg, 0.02 mmol, 2 equiv) in methanol (400 μL) was added to a mixture of thiol **24** (8 mg, 0.01 mmol, 1 equiv) and tripetide **10** (4 mg, 0.01 mmol, 1.2 equiv) at 23 °C. The reaction mixture was stirred at 22 °C for 14 h, when another portion of sodium methoxide (1.1 mg, 0.02 mmol, 2 equiv) in methanol (200 μL) was added, and the resulting solution was stirred for an additional 7 h. The reaction mixture was neutralized by the addition of a solution of acetic acid in methanol (1 M, 46 μL, 0.05 mmol, 4.2 equiv), followed by concentration in vacuo. The residue was purified by silica-gel flash chromatography (50% to 67% methanol in dichloromethane), followed by LH 20 Sephadex size-exclusion chromatography (methanol) to afford glycoconjugate **35** (8 mg, 90%, *dr* = 1.2:1.0) as a white solid. R_f = 0.28 (4:4:1:1 MeOH/EtOAc/AcOH/H_2O); ^{1}H NMR (500 MHz, CD_3OD): δ = 5.59 (d, *J* = 5.4 Hz, 1H), 5.58 (d, *J* = 5.5 Hz, 1.2H), 4.50 (dd, *J* = 8.1, 6.2 Hz, 1.2H), 4.41 (m, 5.7H), 4.3 (m, 4.6H), 4.00–3.51 (m, 36.5H), 3.26 (dd, *J* = 14.2, 9.8 Hz, 1.2H), 3.12 (m, 2H), 2.83 (m, 3.7 Hz), 2.73 (2 overlapped singlets, 6.6H), 2.10 (s, 3H), 2.04 (s, 3.6H), 2.02 (s, 7.2H), 1.97 (s, 3H), 1.96 (s, 3H), 1.67 (m, 2.2H), 1.39 (d, *J* = 7.2 Hz, 3H), 1.34 ppm (d, *J* = 3.6 Hz, 3.6H); ^{13}C NMR (126 MHz, CD_3OD): δ = 175.8, 175.7, 175.7, 175.5, 174.1, 174.1, 174.0, 173.9, 173.7, 172.7, 172.3, 170.5, 102.3, 102.1, 74.9, 74.7, 73.1, 73.0, 71.9, 71.8, 70.4, 70.4, 70.2, 69.7, 69.7, 69.4, 65.1, 64.7, 63.5, 63.2, 57.8, 57.7, 57.5, 56.3, 55.3, 54.2, 54.1, 52.0, 51.9, 51.2, 51.1, 50.0, 49.8, 26.6, 26.6, 23.1, 22.9, 22.8, 22.7, 22.7, 18.1 ppm; FTIR (neat film): $\tilde{\nu}$ = 3307, 1654, 1560, 1034 cm^{-1}; HRMS (ESI)$^-$: *m*/*z* calcd for $C_{31}H_{51}N_6O_{18}S$ $[M-H]^-$: 827.2981; found: 827.3016.

Trisaccharide–tripeptide conjugate 36: A deoxygenated solution of sodium methoxide (1.3 mg, 0.02 mmol, 4 equiv) in methanol (300 μL) was added to a mixture of thiol **29** (6 mg, 0.006 mmol, 1.0 equiv) and tripeptide **9** (3 mg, 0.01 mmol, 1.9 equiv) at 23 °C. After 1.5 h, a white precipitate was observed. The reaction mixture was stirred at 23 °C for a total of 16.5 h, and then neutralized by the addition of a solution of acetic acid in methanol (1 M, 25 μL, 0.02 mmol, 4.2 equiv) followed by concentration in vacuo. The residue was purified by silica-gel flash chromatography (33% to 75% methanol in dichloromethane), followed by LH 20 Sephadex size-exclusion chromatography (methanol) to afford glycoconjugate **36** (5 mg, 88%, *dr* = 1.7:1.0) as a white solid. R_f = 0.08 (4:4:1:1 MeOH/EtOAc/AcOH/H_2O); ^{1}H NMR (500 MHz, CD_3OD): δ = 5.64 (d, *J* = 5.4 Hz, 1H), 5.56 (d, *J* = 5.6 Hz, 1.7H), 4.58 (m, 6.6H), 4.44 (d, *J* = 7.6 Hz, 2.7H), 4.39 (dd, *J* = 8.4, 3.5 Hz, 1.7H), 4.23 (m, 2.7H), 4.10–3.38 (m, 82.2H), 3.27 (dd, *J* = 14.4, 8.2 Hz, 1.7H), 3.17 (dd, *J* = 14.0, 6.8 Hz, 1H), 3.01 (dd, *J* = 14.5, 4.2 Hz, 1H), 2.82 (m, 4.4H), 2.76 (s, 3H), 2.74 (s, 5.1H), 2.08 (s, 5.1 Hz), 2.05 (s, 3H), 2.02 (s, 3H), 2.01 (s, 5.1H), 1.95 (s, 8.1H), 1.58 ppm (m, 2.7H); ^{13}C NMR (188 MHz, CD_3OD): δ = 176.1, 175.5, 175.4, 174.6, 174.5, 174.1, 173.9, 173.9, 173.2, 173.0, 172.3, 172.2, 172.0, 106.0, 102.4, 87.3, 78.7, 78.6, 76.8, 74.9 (multiple resonances of pyranoside carbons), 74.7, 74.5, 72.9 (multiple resonances of pyranoside carbons), 72.5, 71.8, 70.4, 70.3, 70.3, 70.1 (multiple resonances of pyranoside carbons), 69.7, 69.6, 69.5, 65.5, 64.5 (multiple resonances of pyranoside carbons), 62.5 (multiple resonances of pyranoside carbons), 55.8, 54.1, 50.5, 44.2, 43.8, 42.2, 41.6, 34.3, 30.8 (multiple resonances of methyl carbons), 26.3 (multiple resonances of methyl carbons), 22.7 ppm (multiple resonances of methyl carbons); FTIR (neat film): $\tilde{\nu}$ = 3307, 1654, 1594, 1077, 1033 cm^{-1}; HRMS (ESI)$^-$: *m*/*z* calcd for $C_{35}H_{57}N_6O_{22}S$ $[M-H]^-$: 945.3247; found: 945.3234.

Trisaccharide–tripeptide conjugate 37: A deoxygenated solution of sodium methoxide (1.4 mg, 0.026 mmol, 4 equiv) in methanol (345 μL) was added to a mixture of thiol **29** (7 mg, 0.007 mmol, 1.0 equiv) and tripeptide **10** (3 mg, 0.01 mmol, 1.7 equiv) at 23 °C. The reaction mixture was stirred at 23 °C for a total of 17 h, during which time a white precipitate was formed. The reaction mixture was neutralized by the addition of a solution of acetic acid in methanol (1 M, 28 μL, 0.03 mmol, 4.2 equiv) followed by concentration in vacuo. The residue was purified by silica-gel flash chromatography (33% to 75% methanol in dichloromethane), followed by LH 20 Sephadex size-exclusion chromatography (methanol) to afford glycoconjugate **37** (5 mg, 75%, *dr* = 1.2:1.0) as a white solid. R_f = 0.10 (4:4:1:1 MeOH/EtOAc/AcOH/H_2O); ^{1}H NMR (500 MHz, CD_3OD): δ = 5.62 (d, *J* = 5.6 Hz, 2.2H), 4.56–3.44 (m, 53H), 3.14 (m, 2H), 2.83 (m, 4.6 Hz), 2.73 (s, 6.6H), 2.11 (s, 3H), 2.04 (s, 3.6H), 2.01 (s, 6.6H), 1.96

(m, 6.6H), 1.72 (t, $J=11.3$ Hz, 1H), 1.66 (t, $J=12.0$ Hz, 1H), 1.39 (d, $J=7.2$ Hz, 3H), 1.35 ppm (d, $J=7.3$ Hz, 3.6H); ^{13}C NMR (188 MHz, CD_3OD): $\delta=175.7$, 175.5, 175.5, 175.4, 174.5, 174.0, 173.9, 173.9, 173.7, 173.5, 173.2, 172.5, 172.2, 106.0, 102.2, 102.0, 85.8, 78.8 (multiple resonances of pyranoside carbons), 76.8, 74.9, 74.8, 74.6, 74.5, 73.0 (multiple resonances of pyranoside carbons), 72.8 (multiple resonances of pyranoside carbons), 72.5, 71.4, 71.3, 70.2 (multiple resonances of pyranoside carbons), 69.9, 69.6 (multiple resonances of pyranoside carbons), 65.1, 64.5 (multiple resonances of pyranoside carbons), 63.4, 63.1, 62.5, 62.5, 57.6, 57.3, 56.2, 54.0, 53.9, 51.1, 51.0, 50.5, 42.3, 40.9, 32.8, 30.8 (multiple resonances of methyl carbons), 26.5 (multiple resonances of methyl carbons), 22.7 (multiple resonances of methyl carbons), 22.6 (multiple resonances of methyl carbons), 17.9 ppm; FTIR (neat film): $\tilde{\nu}=3306$, 1638, 1377, 1074 cm^{-1}; HRMS (ESI)$^-$: m/z calcd for $C_{37}H_{61}N_6O_{23}S$ $[M-H]^-$: 989.3509; found: 989.3499.

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