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Simplified beta-glycosylation of peptides

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ABSTRACT

A simple and effective activating system for *S*-phenyl thioglycosides, namely N-iodosuccinimide and catalytic copper(I) triflate, promotes beta-O-glycosylation at the serine and threonine hydroxyls of "mono-," di-, and tripeptides. The same activator combination promotes carboxamide beta-N-glycosylation of asparagine-containing mono-, di, and tri-peptides, as well as a nucleoside carboxamide and a sulfonamide. An important feature of the copper(I) triflate method is that undesired amide *O*-glycosylation is largely circumvented. For both sets of biologically important acceptor sites (HO— and —CONH$_2$), a *beta*-GlcNAc-equivalent donor is demonstrated to provide the linkages efficiently. Streamlined deprotection sequences have been developed based on global hydrogenolysis that lead smoothly to the parent glycopeptides. The core glycopeptide region for biological protein *N*-glycosylation, represented by N^4-(β-*N*-acetyl-D-2-glucosaminyl)-Asp-Gly-Thr-OH, has been prepared in solution, purified, and characterized as the fully deprotected (mono)glycosylated tripeptide.

1. Introduction

Enzyme mediated O- and N-glycosylation of proteins, particularly at the side chain residues of serine, threonine, and asparagine, is an important biological process that determines the properties, function, and fate of the resulting glycoproteins.[1–4] To take a recent example: cryo-EM and other evidence indicates that single N-glycosylated Asn154 of the Zika virus glycoprotein shell may function to enable attachment of the virus to host cells.[5–7] The analogous but simpler glycosylated peptides and amino acids, including the blood group antigens,[8,9] have their own distinctive biological roles.[10,11] The chemical preparation of N-glycopeptides[12–14] has developed along two parallel lines, with notable successes for both: (1) N-acylation of glycosylamines with activated aspartic acid derivatives,[15,16] and (2) direct O- and N-glycosylation of protected peptides with activated glycosyl donors.[17–21] In either case, elaboration of the glycosylated amino acid or small glycopeptide into a more complex glycopeptide can often be achieved by well precedented solution or resin-supported peptide coupling methods.[22–28] The direct glycosylation approach, while more versatile in principle, and more similar to the biological process itself, is limited by the difficulty of carrying out a chemical glycosylation on a complex acceptor with multiple Lewis basic sites. These can include the various amide and carbonyl-bearing protecting groups, which can undergo wasteful carbonyl-O-glycosylations[29–31] in competition with reaction at the desired acceptor site, namely serine/threonine —OH or asparagine —CONH$_2$. Thus, the chemical yields of these glycosylation reactions can be unacceptably low. The development of a simple and efficient process for O- and N- glycosylation of peptides that need not be optimized for each new specific case would benefit multistep preparations, especially those limited by availability of material, that cannot themselves be taken through extensive optimization. Part of this optimization should include mild and efficient deprotection protocols.

In addressing an analogous situation in nucleoside glycosylation, namely, how can glycosylation be carried out at an acceptor site that is not necessarily the most reactive one, we recently found a practical solution.[32] This features identifying an activator/Lewis acid combination that promotes reversibility of the initial glycoslation at a competing acceptor site, such as an amide carbonyl, and allows a slower but more permanent glycosylation at the desired site. In this paper we apply similar reasoning to develop procedures for peptide O- or N-glycosylation, and further sharpen the process by streamlining the deprotection steps.

2. Results and discussion

2.1. Beta-glycosylation of serine hydroxyl

Thioglycoside donors have advantages for glycosylation that

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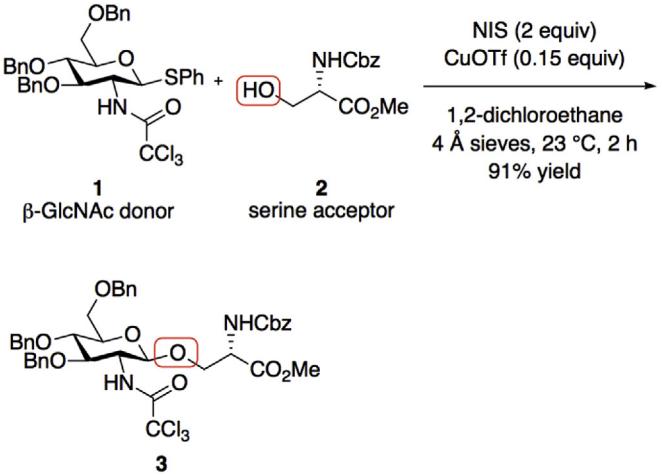
include their simplicity and ease of preparation, their chemical stability, and the versatility of the various activation methods.[33–36] The beta-GlcNAc donor **1**, with phenylthio as the activatable leaving group, and trichloroacetamido as the participating group at C-2,[37] was evaluated as a glycosylation donor with commercial N-Cbz serine methyl ester (**2**) as the protected amino acid ("monopeptide") acceptor. A qualitative screen of acids (triflic acid, TMS-triflate) and metal triflates[38–40] in combination with N-iodosuccinimide (NIS) led to the selection of copper(I) triflate, commercially available as a 2:1 toluene complex, as an effective catalytic promoter (91% yield, Scheme 1). This yield compares favorably with the best literature methods for serine beta O-GlcNAc-ylation.[41–46] The best promoter for nucleoside O-glycosylation, namely, indium(III) triflate,[32] was not as effective for the peptide example. Although we have no direct evidence, it is possible that Cu(I) is better than the other promoters because it effectively isomerizes mis-glycosylated imidate-type byproducts.

By application of the same reaction conditions, with **1** and also with the analogous *galacto* donor[47] **4**, to the glycosylation of several other protected serine and threonine acceptors, the glycopeptides in Fig. 1 were prepared in the yields shown. H-1 NMR spectroscopic analysis (*J* at the anomeric H) indicates that products have exclusively the *beta* glycosyl stereochemistry. The original acceptor atoms are highlighted with red boxes.

Similarly, a dipeptide and a tripeptide acceptor were converted to glycopeptides **11**–**14** by using donors **1** and **4** (Fig. 2). Yields are somewhat reduced compared with the monopeptides, but are nevertheless acceptable for acceptors with this number of competing Lewis basic sites. The acceptor atoms are highlighted with red boxes.

The use of hydrogenolyzable protecting and precursor groups on both the donor and acceptor moieties enables a one step deprotection protocol, as shown in Scheme 2. No fewer than eight individual hydrogenolytic steps (as indicated by the red arrows) occur during the palladium hydroxide mediated deprotection of **6**, which leads to the hydrochloride of the GlcNAc serine derivative[48] **15** in 91% overall yield. Examination of the H-1 and C-13 NMR spectra indicates that hydrogenolysis is complete. There are, for example, no signals attributable to a mono-benzyl or a chloroacetamido byproduct. Likewise, no stereoisomers, such as might have been produced had partial epimerization occurred at the serine *alpha* carbon, are observed. The same efficient procedure provides glycopeptides **16**–**19** from the respective glycosylation products (Scheme 2). Because of the presence of aqueous HCl during



Scheme 1. Prototypical serine O-glycosylation.

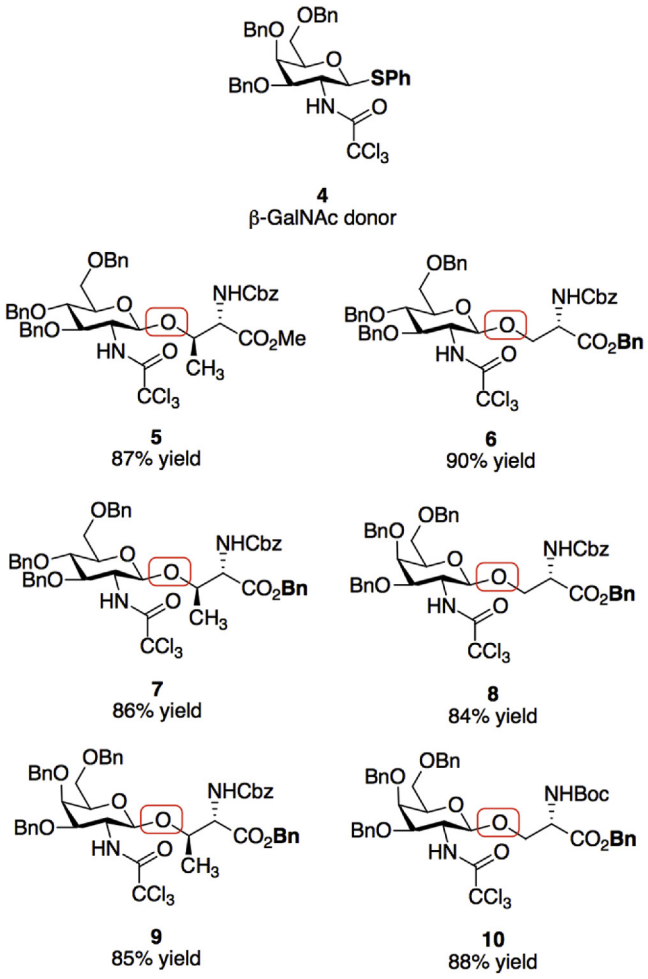


Fig. 1. O-Glycosylation of serine hydroxyls.

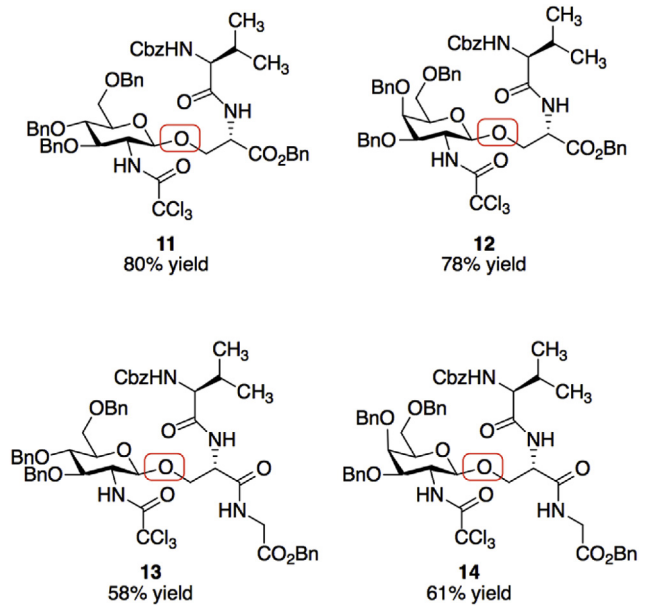
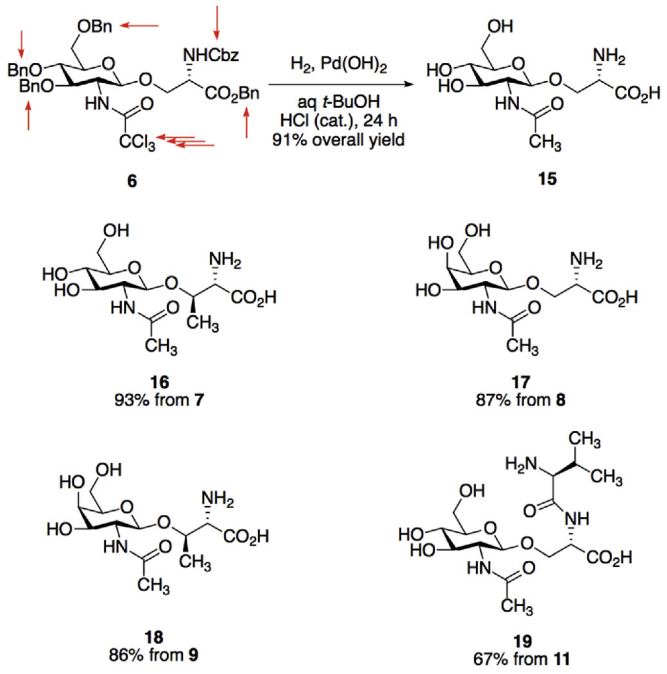


Fig. 2. Dipeptide and tripeptide serine O-glycosylations.



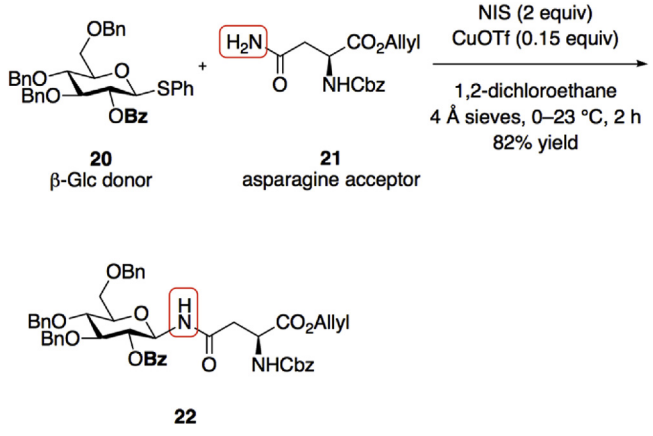
Scheme 2. Global deprotection of glycopeptides.

hydrogenolysis, the glycopeptides **15**–**19** are obtained as their hydrochloride salts.[49] The pyranoside H-1/H-2 vicinal coupling constants ($J = 6.6–8.4$ Hz) confirm the beta anomeric stereochemistry. A notable feature of the mild final hydrogenolysis step is that competing beta elimination from the threonine derivatives,[13] which can occur under acidic or basic conditions, is avoided.

2.2. Beta-N-glycosylation of asparagine carboxamide

N-Glycosylation of the carboxamide group in asparagine derivatives comes with the particular challenge of avoiding or reversing O-glycosylation at the amide carboxyl oxygen. The copper(I) triflate procedure proved effective in this regard. Thus, protected asparagine **21** reacted with the beta-glucopyranosyl donor **20** to give glycopeptide **22** in 82% yield without significant O-glycosylation interference (Scheme 3).

The Cu(I) glycosylation method proved to be successful for various carboxamide acceptors, as exemplified by the glycosylated



Scheme 3. Prototypical beta-N-glycosylation of asparagine carboxamide.

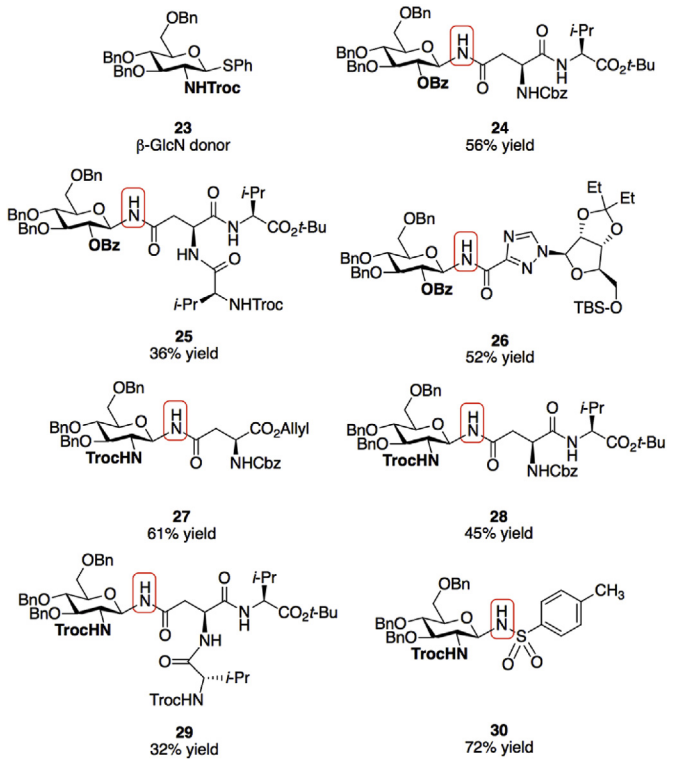


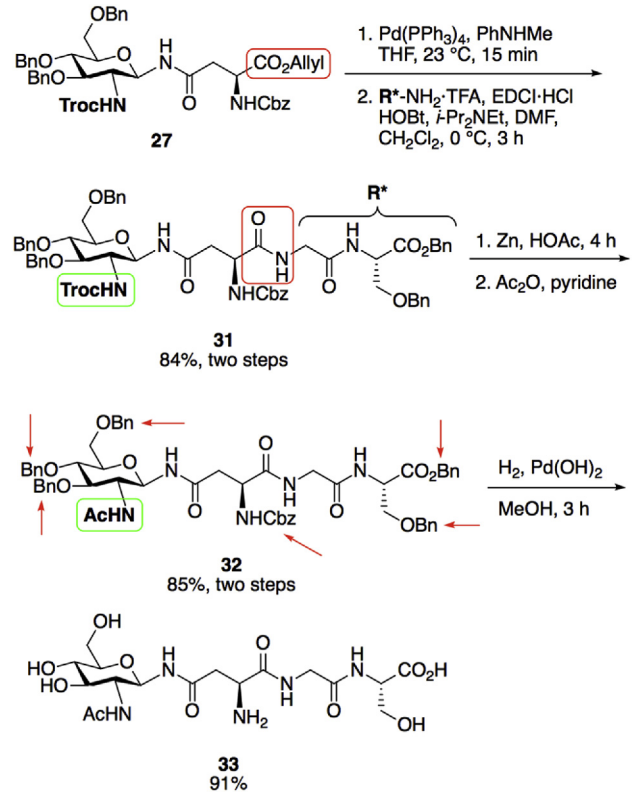
Fig. 3. Carboxamide beta-N-glycosylations.

products shown in Fig. 3. The application to more complex dipeptide and tripeptide acceptors also worked, but the yields were reduced (see **24** and **25**). N-Glucosylation of the carboxamide group of protected ribavirin was carried out to give **26**. The combination of the GlcN donor **23** (bearing N-Troc) with asparagine-containing mono-, di, and tripeptide acceptors gave the respective N-glycopeptides **27**, **28**, and **29**. Glycosylation of tripeptide acceptors in particular can be quite challenging,[19] so the reduced yields are nevertheless acceptable. The Troc protecting group, 2,2,2-trifluoroethoxycarbonyl, is effective at directing beta glucosaminidation while also maintaining reactivity of the donor.[50] A sulfonamide acceptor[51] was also N-glycosylated with this donor (see **30**).

The N-glucosaminidated asparagine peptides in Fig. 3 (**27**, **28**, and **29**) feature the important biological linkage between asparagine and the pentasaccharide of N-linked glycoproteins. In order to establish a deprotection protocol for an elaborated glycopeptide, the preparation of the core N-glucosamidated tripeptide portion of natural glycoproteins,[13] namely **33**, was undertaken (Scheme 4). Related or protected versions of **33** have been reported.[16,17,52] The asparagine derivative **27** was converted to an Asp-Gly-Thr tripeptide version (**31**) by deprotection[53] of the allyl ester and then coupling to the appropriately protected dipeptide amine. The deprotection sequence began with conversion of N-Troc to N-acetyl (green boxes), which was accomplished by zinc treatment and then acetylation[54] to provide **32**. Global hydrogenolytic deprotection of the tripeptide at six sites (red arrows) gave the free glycopeptide **33** in excellent yield. The pyranoside H-1/H-2 vicinal coupling constant ($J = 8.0$ Hz) confirms the beta anomeric stereochemistry.

2.3. Other acceptor substrates

Acceptor substrates for glycosylation (**34**–**43**) that are not explicitly shown above are displayed in Fig. 4. Their provenance is described in the Experimental Section.



Scheme 4. Preparation of the tripeptide glycoprotein core.

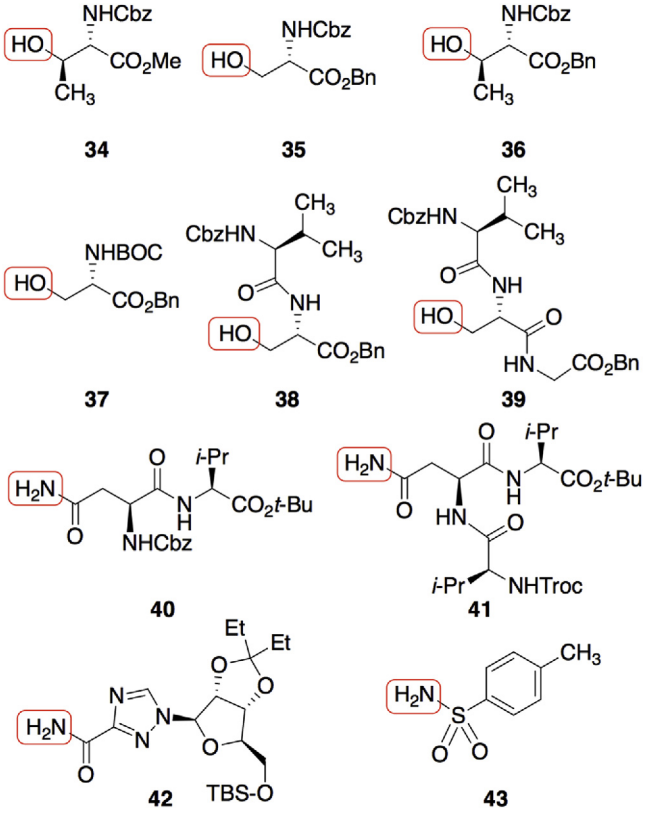


Fig. 4. Additional acceptor substrates used in this study.

3. Conclusion

The use of catalytic Cu(I) triflate as a co-activator of simple thioglycosides is featured in the beta-O-glycosylation of eleven serine-containing mono-, di-, and tripeptides, and the N-glycosylation of seven asparagine-containing mono-, di-, and tripeptides as well as two miscellaneous amides. Yields are competitive or better compared with prior methods, suggesting that the role of Cu(I) in these reactions may include facilitating the isomerization of intermediates that are wrongly carbonyl-O-glycosylated. The selection of hydrogenolyzable protecting and precursor groups (O-benzyl ethers and esters, N-Cbz, and N-trichloroacetyl) enables efficient global deprotection to provide the parent glycopeptides, including an asparagine N-GlcNAc-ylated tripeptide that represents the concensus core of natural N-glycoproteins. Limitations of this method include the decreasing yields with increasing acceptor peptide length, and the inapplicability of hydrogenolysis to sulfur containing peptides. Catalytic copper(I) promotion of glycosylation holds promise for further application to alpha selective or more elaborated glycosyl donors as well as additional complex acceptors with competing Lewis basic sites.

4. Experimental section

4.1. General glycosylation procedure

A 10 mL vial containing the peptide acceptor (0.10 mmol), copper(I) triflate (commercial toluene complex, 10.5 mg, 0.02 mmol), activated 4 Å molecular sieves (150 mg), the glycosyl donor (0.15 mmol) and 1,2-dichloroethane (1 mL) was stirred at 0 °C (external ice bath) for 15 min. N-iodosuccinimide (45 mg, 0.20 mmol) was added, and the reaction mixture was allowed to stir at 0 °C or room temperature for 2 h as needed for disappearance of donor. The reaction mixture was filtered through a 0.45 μM PTFE syringe filter, and the cake was rinsed with dichloromethane (2 × 5 mL). The organic solution was washed with 10 mL of saturated aq sodium bisulfite, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by using a pre-packed silica gel column or preparative silica thin layer chromatography plate to afford the glycosylation product.

4.2. O-glycosylations

4.2.1. N-((2S,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-2-(phenylthio)tetrahydro-2H-pyran-3-yl)-2,2,2-trichloroacetamide **1**[37]

A solution of commercial 1,3,4,6-tetra–*O*-acetyl-2-deoxyl-2-[(trichloroacetyl)amino]-*β*-D-glucopyranose (5.5 g, 11.2 mmol) in dry dichloromethane (100 mL) was treated sequentially with thiophenol (2.3 mL, 22.4 mmol) and boron trifluoride etherate (4.3 mL, 33.6 mmol). The mixture was stirred for 15 h, and then was quenched with saturated aq sodium bicarbonate (100 mL). The organic phase was dried and then concentrated, and the residue was purified by silica gel column chromatography, eluting with 4:6 ethyl acetate/hexane, to give the thioglycoside triacetate (4.1 g, 68% yield) as a white solid.

A solution of above thioglycoside triacetate (4.0 g, 7.37 mmol) in anhydrous methanol (80 mL) was treated with sodium methoxide (40 mg, 0.74 mmol). The solution was stirred for 2 h, and then was neutralized with Dowex-H $^{+}$ resin. The resin was removed by filtration, and the filtrate was concentrated to give 3.07 g of the triol as a white solid. A solution of this material in dry dimethylformamide (40 mL) was stirred at −10 °C, and then was treated sequentially with benzyl bromide (5.2 mL, 43.92 mmol) and sodium hydride (60% in oil, 1.77 g, 43.92 mmol).[55] The reaction mixture was

stirred under nitrogen below 0 °C for 2 h, and then was quenched with 2 mL of methanol. Ethyl acetate (150 mL) was added, and the organic solution was washed sequentially with water (100 mL) and brine (100 mL), dried, and then concentrated. The residue was purified by flash chromatography, eluting with 5:95 ethyl acetate/hexane, to give **1** (3.62 g, 72% yield) as a white powder: ^{1}H NMR (400 Hz, $CDCl_3$) δ 7.56 (d, 2 H, J = 1.7 Hz), 7.20–7.54 (m, 18 H), 6.89 (d, 1 H, J = 10.5 Hz), 5.13 (d, 1 H, J = 12.5 Hz), 4.78 (dd, 1 H, J = 6.6 and 13.4 Hz), 4.68 (d, 1 H, J = 13.4 Hz), 4.64 (d, 1 H, J = 11.1 Hz), 4.60 (d, 1 H, J = 9.9 Hz), 4.56 (d, 1 H, J = 14.9 Hz), 4.10 (dd, 1 H, J = 10.3 and 11.9 Hz), 3.82 (dd, 1 H, J = 2.9 and 13.5 Hz), 3.77 (dd, 1 H, J = 4.9 and 13.5 Hz), 3.57–3.70 (m, 1 H), 1.57 (water peak); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.6, 138.3, 137.9, 137.7, 133.2, 132.2, 129.2, 128.7, 128.6, 128.5, 128.4, 128.1, 128.05; 128.0, 127.9, 127.8, 127.7, 92.6, 84.7, 81.5, 79.4, 78.4, 75.5, 74.9, 73.6, 69.0, 56.9; LC-ESI-MS $[M+Na]^+$ calcd for $C_{35}H_{34}Cl_3NO_5SNa$ 709.11, found 709.28.

4.3. Methyl ((benzyloxy)carbonyl)-L-serinate **2**

This acceptor is available commercially.

4.3.1. Methyl N-((benzyloxy)carbonyl)-O-((2R,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-3-(2,2,2-trichloroacetamido) tetrahydro-2H-pyran-2-yl)-L-serinate **3**

By following the general procedure, *gluco* donor **1** (103.1 mg, 0.15 mmol) was combined with commercial methyl ((benzyloxy) carbonyl)-*L*-serinate (acceptor **2**, 25.3 mg, 0.10 mmol) at room temperature for 2 h. After work-up, purification by thin-layer chromatography with 6:4 hexane/ethyl acetate as the eluant afforded **3** (75.5 mg, 91%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27–7.35 (m 18 H), 7.18–7.20 (m, 2 H), 6.98 (d, 1 H, J = 7.6 Hz), 5.70 (d, 1 H, J = 8.2 Hz), 5.07–5.13 (m, 2 H), 4.84 (d, 1 H, J = 7.6 Hz), 4.77 (d, 1 H, J = 10.3 Hz), 4.75 (d, 1 H, J = 10.1 Hz), 4.68 (d, 1 H, J = 11.2 Hz), 4.50–4.60 (m, 4 H), 4.30 (dd, 1 H, J = 3.0 and 10.4 Hz), 4.05 (t, 1 H, J = 8.9 Hz), 3.80 (dd, 1 H, J = 2.9 and 10.0 Hz), 3.69–3.74 (m, 6 H), 3.57–3.60 (m, 2 H), 1.85 (water peak); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 161.9, 156.1, 137.9, 137.7, 136.2, 128.5, 128.44, 128.42, 128.2, 128.1, 127.9, 127.8, 127.7, 99.4, 92.4, 79.5, 77.9, 75.0, 74.8, 74.6, 73.5, 69.1, 68.6, 67.1, 57.5, 54.2, 52.7; HR-ESI-MS $[M + H]^+$ calcd for $C_{41}H_{44}Cl_3N_2O_{10}$ 829.2056, found 829.2055.

4.3.2. N-((2S,3R,4R,5R,6R)-4,5-bis(benzyloxy)-6-((benzyloxy) methyl)-2-(phenylthio)tetrahydro-2H-pyran-3-yl)-2,2,2-trichloroacetamide **4**[47]

Triethylamine (16.5 mL, 116.0 mmol) was added to a stirred suspension of D-galactosamine hydrochloride (12.5 g, 58.0 mmol) in methanol (100 mL) at 0 °C. Trichloroacetyl chloride (6.5 mL, 58.0 mmol) was then added by drops. The suspension was warmed to room temperature and stirred for 48 h. The reaction mixture was filtered through a plug of Celite, washed with MeOH, and then concentrated. The residue was dissolved in pyridine (100 mL) and cooled to 0 °C. Acetic anhydride (50 mL) was added by drops, and the solution was allowed to warm to room temperature and stirred for 16 h. The reaction mixture was filtered through a Celite plug, concentrated, and dissolved in ethyl acetate (150 mL). The organic solution was washed sequentially with 1 M hydrochloric acid (3 × 50 mL), saturated aqueous sodium bicarbonate (3 × 50 mL), and brine (100 mL), and then dried and concentrated. The residue was purified by flash chromatography eluting with 1:3 ethyl acetate/hexane to provide 15.0 g of the 2-deoxy-2-trichloroacetamidogalactopyranose tetra-acetate.

A stirred solution of the 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranose described above (15.0 g, 30.4 mmol) in dry dichloromethane (100 mL) was treated sequentially and dropwise with thiophenol (4.1 mL, 39.6 mmol) and trimethylsilyl trifluoromethanesulfonate (5.5 mL, 30.4 mmol). The resulting dark red solution was stirred for 18 h, and then was poured into a vigorously stirred solution of aq sodium bicarbonate (10.0 g in 30 mL water). After 30 min of stirring, the layers were separated, and the aqueous layer was extracted with dichloromethane (100 mL). The combined organic extract was dried and then concentrated. The residue was purified by flash chromatography, eluting with 1:4 ethyl acetate/hexane, to provide 9.0 g of the thioglycoside as a brown solid.

A solution of above thioglycoside (9.0 g, 16.58 mmol) in anhydrous methanol (100 mL) was treated with sodium methoxide (0.5 M in methanol, 830 μL, 1.66 mmol). The reaction was stirred at room temperature under nitrogen for 2 h, and then was quenched with Dowex-H^+ resin. The reaction mixture was filtered, and the filtrate was concentrated. A stirred solution of the residue in anhydrous dimethylformamide (100 mL) was cooled to −10 °C. Benzyl bromide (3.3 mL, 27.6 mmol) and sodium hydride (60% in mineral oil, 1.11 g, 27.6 mmol) were added sequentially. After 2 h the reaction was carefully quenched with 1 mL of methanol. Ethyl acetate (150 mL) was added, and the organic solution was washed sequentially with water (2 × 100 mL) and brine (100 mL), and then dried and concentrated. The residue was purified by flash chromatography, eluting with 1:9 ethyl acetate/hexane, to give **4** (4.9 g, 43% yield) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51–7.53 (m, 2 H), 7.18–7.37 (m, 18 H), 6.82 (d, 1 H, J = 7.4 Hz), 5.28 (d, 1 H, J = 10.2 Hz), 4.88 (d, 1 H, J = 11.4 Hz), 4.67 (d, 1 H, J = 11.2 Hz), 4.56 (d, 1 H, J = 11.2 Hz), 4.52 (d, 1 H, J = 11.2 Hz), 4.51 (d, 1 H, J = 11.2 Hz), 4.45 (d, 1 H, J = 11.2 Hz), 4.26 (dd, 1 H, J = 2.7 and 10.5 Hz), 4.06 (d, 1 H, J = 2.1 Hz), 3.88–3.95 (m, 1 H), 3.67–3.76 (m, 3 H), 1.64 (water peak); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.7, 138.5, 137.9, 137.4, 132.8, 132.4, 129.0, 128.8, 128.7, 128.5, 128.3, 128.2, 128.17, 128.02, 128.0, 127.9, 127.8, 127.6, 92.6, 84.5, 78.5, 74.6, 73.7, 72.7, 72.5, 68.6, 53.8; LC-ESI-MS $[M+Na]^+$ calcd for $C_{35}H_{34}Cl_3NO_5SNa$ 708.11, found 708.29.

4.3.3. Methyl N-((benzyloxy)carbonyl)-O-((2R,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-3-(2,2,2-trichloroacetamido) tetrahydro-2H-pyran-2-yl)-L-threoninate **5**

By following the general procedure, *gluco* donor **1** (103.1 mg, 0.15 mmol) was combined with threonine acceptor **34** (26.7 mg, 0.10 mmol) at room temperature for 2 h. After work-up, purification by thin-layer chromatography with 7:3 hexane/ethyl acetate as the eluant afforded **5** (73.4 mg, 87%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.35 (m 18 H), 7.19–7.21 (m, 2 H), 7.04 (d, 1 H, J = 7.6 Hz), 5.75 (d, 1 H, J = 8.7 Hz), 5.09–5.16 (m, 2 H), 4.91 (d, 1 H, J = 8.2 Hz), 4.78–4.83 (m, 2 H), 4.69 (d, 1 H, J = 10.1 Hz), 4.60 (d, 1 H, J = 10.1 Hz), 4.55 (d, 1 H, J = 12.0 Hz), 4.48 (d, 1 H, J = 12.1 Hz), 4.40–4.42 (m, 1 H), 4.35 (dd, 1 H, J = 2.6 and 8.8 Hz), 4.14 (t, 1 H, J = 9.2 Hz), 3.71–3.76 (m, 2 H), 3.68 (s, 3 H), 3.42–3.52 (m, 2 H), 2.17 (water peak), 1.22 (d, 3 H, J = 6.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 161.9, 156.8, 138.0, 138.0, 137.9, 136.4, 128.59, 128.56, 128.5, 128.48, 128.2, 128.1, 128.0, 127.9, 127.86, 127.8, 127.78, 97.3, 92.5, 79.7, 78.4, 75.0, 74.8, 74.5, 73.7, 68.7, 67.1, 57.8, 58.6, 52.5, 17.4; HR-ESI-MS $[M + H]^+$ calcd for $C_{42}H_{46}Cl_3N_2O_{10}$ 843.2213, found 843.2208.

4.3.4. Benzyl N-((benzyloxy)carbonyl)-O-((2R,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-3-(2,2,2-trichloroacetamido) tetrahydro-2H-pyran-2-yl)-L-serinate **6**

By following the general procedure, *gluco* donor **1** (103.1 mg, 0.15 mmol) was combined with serine acceptor **35** (32.9 mg, 0.10 mmol) at room temperature for 2 h. After work-up, purification by thin-layer chromatography with 7:3 hexane/ethyl acetate as the eluant afforded **6** (81.6 mg, 90%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25–7.33 (m, 23 H), 7.18–7.20 (m, 2 H), 6.93 (d,

1 H, $J = 7.6$ Hz), 5.71 (d, 1 H, $J = 8.3$ Hz), 5.16–5.18 (m, 2 H), 5.06–5.12 (m, 2 H), 4.84 (d, 1 H, $J = 7.7$ Hz), 4.78 (d, 1 H, $J = 5.0$ Hz), 4.75 (d, 1 H, $J = 5.0$ Hz), 4.67 (d, 1 H, $J = 11.0$ Hz), 4.58 (d, 1 H, $J = 11.4$ Hz), 4.54 (d, 1 H, $J = 11.4$ Hz), 4.47 (d, 1 H, $J = 12.1$ Hz), 4.35 (dd, 1 H, $J = 2.9$ and 10.1 Hz), 4.06 (t, 1 H, $J = 9.0$ Hz), 3.81 (dd, 1 H, $J = 3.0$ and 10.1 Hz), 3.68–3.72 (m, 2 H), 3.46–3.55 (m, 2 H), 1.64 (water peak); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.7, 162.0, 156.1, 138.0, 137.9, 137.8, 136.3, 135.5, 128.7, 128.6, 128.58, 128.5, 128.49, 128.4, 128.2, 128.19, 128.1, 128.0, 127.97, 127.94, 127.9, 127.88, 127.8, 99.4, 92.5, 79.6, 78.1, 77.5, 75.1, 75.0, 74.7, 73.7, 69.3, 68.7, 67.4, 67.2, 57.9, 54.4, 29.8; HR-ESI-MS $[M + H]^+$ calcd for $C_{47}H_{48}Cl_3N_2O_{10}$ 905.2369, found 905.2358.

4.3.5. Benzyl N-((benzyloxy)carbonyl)-O-((2R,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-3-(2,2,2-trichloroacetamido)tetrahydro-2H-pyran-2-yl)-L-threoninate **7**

By following the general procedure, *gluco* donor **1** (103.1 mg, 0.15 mmol) was combined with threonine acceptor **36** (34.3 mg, 0.10 mmol) at room temperature for 2 h. After work-up, purification by thin-layer chromatography with 7:3 hexane/ethyl acetate as the eluant afforded **7** (79.1 mg, 86%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24–7.35 (m, 23 H), 7.17–7.20 (m, 2 H), 6.89 (d, 1 H, $J = 7.5$ Hz), 5.73 (d, 1 H, $J = 8.9$ Hz), 5.19 (d, 1 H, $J = 12.4$ Hz), 5.11–5.14 (m, 2 H), 4.89 (d, 1 H, $J = 8.1$ Hz), 4.79 (t, 2 H, $J = 10.6$ Hz), 4.66 (d, 1 H, $J = 11.0$ Hz), 4.57 (d, 1 H, $J = 11.0$ Hz), 4.45–4.48 (m, 2 H), 4.36–4.40 (m, 1 H), 4.10 (t, 1 H, $J = 9.9$ Hz), 3.63–3.70 (m, 2 H), 3.33–3.39 (m, 2 H), 1.71 (water peak), 1.21 (app d, 2 NH, $J = 6.3$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 161.9, 156.8, 138.0, 138.0, 137.9, 136.4, 135.6, 128.6, 128.59, 128.55, 128.5, 128.4, 128.39, 128.2, 128.16, 128.1, 128.0, 127.94, 127.9, 127.8, 127.78, 127.7, 97.1, 92.5, 79.6, 78.4, 75.1, 75.0, 74.8, 74.5, 73.6, 68.6, 67.3, 67.1, 58.9, 58.7, 17.4; HR-ESI-MS $[M + H]^+$ calcd for $C_{48}H_{50}Cl_3N_2O_{10}$ 919.2526, found 919.2521.

4.3.6. Benzyl N-((benzyloxy)carbonyl)-O-((2R,3R,4R,5R,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-3-(2,2,2-trichloroacetamido)tetrahydro-2H-pyran-2-yl)-L-serinate **8**

By following the general procedure, *galacto* donor **4** (103.1 mg, 0.15 mmol) was combined with serine acceptor **37** (32.9 mg, 0.10 mmol) at room temperature for 2 h. After work-up, purification by thin-layer chromatography with 7:3 hexane/ethyl acetate as the eluant afforded **8** (76.1 mg, 84%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.37 (m, 23 H), 7.16–7.18 (m, 2 H), 6.82 (d, 1 H, $J = 7.0$ Hz), 5.66 (d, 1 H, $J = 8.3$ Hz), 5.20 (d, 1 H, $J = 12.5$ Hz), 5.14 (d, 1 H, $J = 12.6$ Hz), 5.10 (d, 1 H, $J = 12.4$ Hz), 5.05 (d, 1 H, $J = 12.3$ Hz), 4.87–4.91 (m, 2 H), 4.65 (d, 1 H, $J = 11.3$ Hz), 4.57 (d, 1 H, $J = 11.3$ Hz), 4.47–4.52 (m, 2 H), 4.43 (d, 1 H, $J = 11.5$ Hz), 4.42 (d, 1 H, $J = 11.5$ Hz), 4.33 (dd, 1 H, $J = 2.7$ and 10.1 Hz), 4.17 (dd, 1 H, $J = 2.5$ and 11.0 Hz), 4.02 (d, 1 H, $J = 2.3$ Hz), 3.73–3.78 (m, 2 H), 3.56–3.64 (m, 3 H), 1.61 (water peak); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.7, 162.1, 156.1, 138.3, 137.8, 137.5, 136.2, 135.4, 128.6, 128.57, 128.53, 128.5, 128.4, 128.3, 128.2, 128.1, 128.09, 128.0, 127.9, 127.8, 127.79, 127.6, 99.4, 92.5, 77.3, 74.9, 73.7, 73.6, 72.5, 72.4, 69.2, 68.3, 67.3, 67.1, 55.7, 54.3; HR-ESI-MS $[M + H]^+$ calcd for $C_{47}H_{48}Cl_3N_2O_{10}$ 905.2369, found 905.2352.

4.3.7. Benzyl N-((benzyloxy)carbonyl)-O-((2R,3R,4R,5R,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-3-(2,2,2-trichloroacetamido)tetrahydro-2H-pyran-2-yl)-L-threoninate **9**

By following the general procedure, *galacto* donor **4** (103.1 mg, 0.15 mmol) was combined with threonine acceptor **36** (34.3 mg, 0.10 mmol) at room temperature for 2 h. After work-up, purification by thin-layer chromatography with 7:3 hexane/ethyl acetate as the eluant afforded **9** (78.2 mg, 85%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20–7.35 (m, 25 H), 6.84 (d, 1 H, $J = 7.1$ Hz, 1 H); 5.68 (d, $J = 8.7$ Hz, 1 H); 5.06–5.15 (m, 3 H); 4.93 (d, $J = 8.2$ Hz, 1 H); 4.85 (d, $J = 11.3$ Hz), 4.63 (d, 1 H, $J = 11.3$ Hz), 4.54 (d, 1 H, $J = 11.3$ Hz), 4.49 (d, 1 H, $J = 11.3$ Hz), 4.38–4.42 (m, 1 H), 4.34 (d, 1 H, $J = 11.3$ Hz), 4.33 (d, 1 H, $J = 11.3$ Hz), 4.16 (dd, 1 H, $J = 2.6$ and 11.0 Hz), 3.99 (d, 1 H, $J = 2.2$ Hz), 3.57–3.70 (m, 2 H), 3.66–3.53 (m, 2 H), 1.61 (water peak), 1.18 (d, 3 H, $J = 6.3$ Hz); ^{13}C NMR (125 Hz, $CDCl_3$): 170.1, 161.9, 156.8, 138.4, 137.9, 137.5, 136.4, 135.5, 128.7, 128.6, 128.5, 128.3, 128.26, 128.2, 128.14, 128.1, 128.02, 128.0, 127.89, 127.8, 127.7, 97.1, 92.6, 77.0, 74.9, 74.4, 73.5, 73.5, 72.6, 72.3, 68.1, 67.2, 67.0, 58.9, 55.9, 17.2; HR-ESI-MS $[M + H]^+$ calcd for $C_{48}H_{50}Cl_3N_2O_{10}$ 919.2526, found 919.2520.

4.3.8. Benzyl O-((2R,3R,4R,5R,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-3-(2,2,2-trichloroacetamido)tetrahydro-2H-pyran-2-yl)-N-(tert-butoxycarbonyl)-L-serinate **10**

By following the general procedure, *galacto* donor **4** (103.1 mg, 0.15 mmol) was combined with serine acceptor **37** (29.5 mg, 0.10 mmol) at room temperature for 2 h. After work-up, purification by thin-layer chromatography with 7:3 hexane/ethyl acetate as the eluant afforded **10** (76.8 mg, 88%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24–7.38 (m, 24 H), 7.12–7.16 (m, 1 H), 6.87 (d, 1 H, $J = 7.0$ Hz), 5.38 (d, 1 H, $J = 8.6$ Hz), 5.23 (d, 1 H, $J = 12.6$ Hz), 5.11 (d, 1 H, $J = 12.6$ Hz), 4.91 (d, 1 H, $J = 11.2$ Hz), 4.89 (d, 1 H, $J = 11.2$ Hz), 4.66 (d, 1 H, $J = 11.3$ Hz), 4.58 (d, 1 H, $J = 11.2$ Hz), 4.52 (d, 1 H, $J = 11.3$ Hz), 4.43–4.47 (m, 2 H), 4.36 (dd, 1 H, $J = 2.5$ and 9.8 Hz), 4.22 (dd, 1 H, $J = 2.2$ and 11.0 Hz), 4.03 (d, 1 H, $J = 2.0$ Hz), 3.57–3.75 (m, 6 H), 1.67 (water peak), 1.41 (s, 9 H); ^{13}C NMR (125 Hz, $CDCl_3$) δ 170.1, 162.1, 155.5, 138.4, 137.8, 137.5, 135.6, 128.7, 128.6, 128.41, 128.37, 128.13, 128.12, 128.1, 128.0, 127.9, 127.8, 99.4, 92.6, 80.1, 77.3, 75.0, 73.7, 72.6, 72.4, 69.6, 68.3, 67.2, 55.9, 53.9, 28.4; HR-ESI-MS $[M + H]^+$ calcd for $C_{44}H_{50}Cl_3N_2O_{10}$ 871.2526, found 871.2525.

4.3.9. Benzyl N-(((benzyloxy)carbonyl)-L-valyl)-O-((2R,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-3-(2,2,2-trichloroacetamido)tetrahydro-2H-pyran-2-yl)-L-serinate **11**

By following the general procedure, *gluco* donor **1** (103.1 mg, 0.15 mmol) was combined with Val-Ser acceptor **38** (42.8 mg, 0.10 mmol) at room temperature for 2 h. After work-up, purification by thin-layer chromatography with 6:4 hexane/ethyl acetate as the eluant afforded **11** (80.4 mg, 80%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, 1 H, $J = 8.8$ Hz), 7.14–7.37 (m, 23 H), 7.14–7.16 (m, 2 H), 6.75 (d, 1 H, $J = 7.4$ Hz), 5.37 (d, 1 H, $J = 8.1$ Hz), 5.18 (s, 2 H), 5.11 (d, 1 H, $J = 12.5$ Hz), 5.05 (d, 1 H, $J = 12.5$ Hz), 4.19–4.77 (m, 2 H), 4.66 (d, 1 H, $J = 10.7$ Hz), 4.59 (d, 1 H, $J = 12.1$ Hz), 4.52 (d, 1 H, $J = 12.1$ Hz), 4.49 (d, 1 H, $J = 12.1$ Hz), 4.36 (dd, 1 H, $J = 1.4$ and 11.3 Hz), 3.98 (dd, 1 H, $J = 9.1$ and 18.0 Hz), 3.87–3.90 (m, 2 H), 3.76 (t, 1 H, $J = 8.6$ Hz), 3.66–3.71 (m, 2 H), 3.24–3.27 (d, 1 H, $J = 9.1$ Hz), 2.00–2.05 (m, 1 H), 0.98 (d, 3 H, $J = 6.7$ Hz), 0.97 (d, 3 H, $J = 6.7$ Hz); ^{13}C NMR (125 Hz, $CDCl_3$) δ 171.2, 169.0, 162.0, 156.8, 138.1, 138.0, 137.8, 136.0, 135.1, 128.8, 128.7, 128.6, 128.5, 128.44, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.3, 101.9, 93.1, 81.9, 78.0, 75.2, 75.1, 74.6, 73.5, 68.9, 68.6, 67.8, 67.2, 61.5, 57.1, 53.1, 30.5, 19.4, 18.7; HR-ESI-MS $[M + H]^+$ calcd for $C_{53}H_{57}Cl_3N_3O_{10}$ 1004.3035, found 1004.3225.

4.3.10. Benzyl N-(((benzyloxy)carbonyl)-L-valyl)-O-((2R,3R,4R,5R,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-3-(2,2,2-trichloroacetamido)tetrahydro-2H-pyran-2-yl)-L-serinate **12**

By following the general procedure, *galacto* donor 4 (103.1 mg, 0.15 mmol) was combined with Val-Ser acceptor **38** (42.8 mg, 0.10 mmol) at room temperature for 2 h. After work-up, purification by thin-layer chromatography with 6:4 hexane/ethyl acetate as the eluant afforded **12** (78.4 mg, 78%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, 1 H, $J = 8.4$ Hz), 7.26–7.37 (m, 25 H), 6.75 (d, 1 H, $J = 7.4$ Hz), 5.39 (d, 1 H, $J = 8.2$ Hz), 5.16 (s, 2 H), 4.96 (s, 1 H),

4.90 (d, 1 H, $J = 11.4$ Hz), 4.64 (d, 1 H, $J = 11.5$ Hz), 4.56–4.60 (m, 2 H), 4.48 (d, 1 H, $J = 11.6$ Hz), 4.40–4.46 (m, 2 H), 4.32 (d, 1 H, $J = 10.8$ Hz), 4.21 (dd, 1 H, $J = 8.8$ and 18.8 Hz), 3.97 (t, 1 H, $J = 7.2$ Hz), 3.93 (s, 1 H), 3.85 (dd, 1 H, $J = 2.0$ and 10.7 Hz), 3.69 (dd, 1 H, $J = 2.0$ and 10.8 Hz), 3.60 (t, 1 H, $J = 8.4$ Hz), 3.53 (t, 1 H, $J = 5.3$ Hz), 3.36 (t, 1 H, $J = 6.1$ Hz), 2.64 (broad, 1 H), 2.03–2.08 (m, 1 H), 1.27 (broad, 1 H), 0.95–0.98 (d, $J = 6.7$ Hz, 6 H); ^{13}C NMR (125 Hz, $CDCl_3$) δ 171.2, 169.1, 162.2, 156.6, 138.4, 137.9, 137.6, 136.1, 135.2, 128.7, 128.62, 128.6, 128.5, 128.4, 128.35, 128.3, 128.24, 128.2, 128.0, 127.9, 127.8, 127.5, 101.4, 93.1, 74.7, 73.6, 73.55, 72.2, 71.8, 68.6, 68.4, 67.6, 66.9, 61.1, 54.7, 53.0, 30.8, 19.4, 18.4; HR-ESI-MS $[M + H]^+$ calcd for $C_{53}H_{57}Cl_3N_3O_{11}$ 1004.3053, found 1004.3033.

4.3.11. Benzyl N-(((benzyloxy)carbonyl)-L-valyl)-O-((2R,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-3-(2,2,2-trichloroacetamido)tetrahydro-2H-pyran-2-yl)-L-serylglycinate **13**

By following the general procedure, *gluco* donor **1** (103.1 mg, 0.15 mmol) was combined with Val-Ser-Gly acceptor **39** (48.5 mg, 0.10 mmol) at room temperature for 2 h. After work-up, purification by thin-layer chromatography with 6:4 hexane/ethyl acetate as the eluant afforded **13** (61.6 mg, 58%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.34 (m, 23 H), 7.19–7.20 (m, 2 H), 7.09 (m, 1 H), 6.78 (d, 1 H, $J = 5.3$ Hz), 5.43 (d, 1 H, $J = 6.3$ Hz), 5.14 (s, 2 H), 5.08–5.11 (m, 2 H), 4.77–4.81 (m, 4 H), 4.68–4.70 (m, 2 H), 4.55–4.57 (m, 2 H), 4.46 (d, 1 H, $J = 9.4$ Hz), 3.48–4.07 (m, 5 H), 3.89 (dd, 1 H, $J = 3.5$ and 14.7 Hz), 3.70–3.81 (m, 6 H), 3.55 (d, 1 H, $J = 6.5$ Hz), 2.05–2.09 (m, 1 H), 1.86 (broad, 1 H), 0.84–0.91 (m, 6 H); ^{13}C NMR (125 Hz, $CDCl_3$) δ 171.7, 169.6, 169.5, 162.2, 156.7, 137.9, 137.8, 136.2, 135.3, 128.8, 128.7, 128.64, 128.6, 128.5, 128.4, 128.1, 128.04, 128.0, 127.97, 127,9, 100.6, 92.7, 80.4, 78.1, 75.2, 74.9, 74.8, 73.6, 68.9, 68.6, 67.3, 61.0, 57.8,52.6, 41.5, 30.8, 19.4, 18.0; HR-ESI-MS $[M + H]^+$ calcd for $C_{54}H_{60}Cl_3N_4O_{12}$ 1061.3268, found 1061.3246.

4.3.12. Benzyl N-(((benzyloxy)carbonyl)-L-valyl)-O-((2R,3R,4R,5R,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-3-(2,2,2-trichloroacetamido)tetrahydro-2H-pyran-2-yl)-L-serylglycinate **14**

By following the general procedure, *galacto* donor **4** (103.1 mg, 0.15 mmol) was reacted with Val-Ser-Gly acceptor **39** (48.5 mg, 0.10 mmol) at room temperature for 2 h. After work-up, purification by thin-layer chromatography with 6:4 hexane/ethyl acetate as the eluant afforded **14** (64.8 mg, 61%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25–7.37 (m, 25 H), 7.11 (d, 1 H, $J = 4.4$ Hz), 6.99 (t, 1 H, $J = 4.2$ Hz), 6.73 (d, 1 H, $J = 5.4$ Hz), 5.28 (d, 1 H, $J = 6.4$ Hz), 5.14 (s, 2 H), 5.09 (d, 1 H, $J = 9.7$ Hz), 5.04 (d, 1 H, $J = 9.6$ Hz), 4.89 (d, 1 H, $J = 9.1$ Hz), 4.81 (d, 1 H, $J = 5.6$ Hz), 4.66 (d, 1 H, $J = 9.2$ Hz), 4.58 (d, 1 H, $J = 9.1$ Hz), 4.51 (d, 1 H, $J = 9.2$ Hz), 4.49 (d, 1 H, $J = 9.1$ Hz), 4.44 (d, 1 H, $J = 9.4$ Hz), 4.12 (dd, 1 H, $J = 3.7$ and 8.1 Hz), 4.01–4.08 (m, 5 H), 3.96 (dd, 1 H, $J = 4.2$ and 15.9 Hz), 3.64–3.71 (m, 4 H), 2.07–2.13 (m, 1 H), 1.69 (broad, 1 H), 0.95 (d, 3 H, $J = 5.3$ Hz), 0.89 (d, 3 H, $J = 5.3$ Hz); ^{13}C NMR (125 Hz, $CDCl_3$) δ 171.5, 169.6, 169.5, 162,4, 156.6, 138.4, 137.8, 137.5, 136.3, 135.3, 128.8, 128.7, 128.68, 128.63, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 100.5, 92.8, 78.0, 77.4, 74.8, 73.7, 73.7, 72.3, 72.0, 68.3, 67.3, 67.2, 60.7, 55.3, 52.6, 41.6, 31.1, 19.4, 17.9; HR-ESI-MS $[M + H]^+$ calcd for $C_{54}H_{60}Cl_3N_4O_{12}$ 1061.3268, found 1061.3242.

4.4. Deprotection procedures

4.4.1. O-((2R,3R,4R,5S,6R)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-L-serine **15**

A solution of 81.5 mg (0.090 mmol) of glycopeptide **6** in 3.0 mL of a 5:1 *t*-butanol/water mixture was treated with 40.0 mg of palladium hydroxide (20 wt % on activated carbon) and 20 μL of 1 N aq hydrochloric acid. The reaction was purged 3 times with a balloon filled with hydrogen gas, and then stirred under positive hydrogen pressure for 12 h. The reaction was then filtered through Celite and concentrated to give the known[48] glycosylated serine product **15** (25.2 mg, 91% yield) as its hydrochloride: ^{1}H NMR (400 MHz, DMSO) δ 8.34 (trace of phthlate contaminant), 7.92 (d, 1 H, $J = 6.3$ Hz), 4.43 (d, 1 H, $J = 6.6$ Hz), 4.16 (s, 1 H), 4.05 (dd, 1 H, $J = 2.9$ and 9.1 Hz), 3.48–3.96 (broad, 4 H), 3.95 (d, 1 H, $J = 8.4$ Hz), 3.68 (d, 1 H, $J = 9.5$ Hz), 3.47 (dd, 1 H, $J = 3.6$ and 9.1 Hz), 3.39 (t, 1 H, $J = 7.4$ Hz), 3.30 (t, 1 H, $J = 6.8$ Hz), 3.07–3.11 (m, 2 H), 1.84 (s, 3 H); ^{13}C NMR (125 MHz, CD_3OD) δ 167.3102.2, 78.1, 75.6, 71.8, 66.9, 62.4, 56.7, 54.2, 23.0; HR-ESI-MS $[M + H]^+$ calcd for $C_{11}H_{21}N_2O_8$ 309.1292, found 309.1292.

4.4.2. O-((2R,3R,4R,5S,6R)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-L-threonine **16**

Hydrogenolysis of **7** (79.1 mg, 0.086 mmol) by following the same procedure as for **15** gave the known[23] glycosylated threonine product **16** (25.9 mg, 93% yield) as its hydrochloride: ^{1}H NMR (400 MHz, CD_3OD) δ 4.45 (d, 1 H, $J = 8.2$ Hz), 4.25 (t, 1 H, $J = 6.0$ Hz), 3.92 (d, 1 H, $J = 11.8$ Hz), 3.83 (d, 1 H, $J = 5.0$ Hz), 3.62–3.69 (m, 2 H), 3.44–3.47 (m, 1 H), 3.30 (overlap with CD_3OD, 2 H), 2.00 (s, 3 H), 1.35 (d, $J = 5.8$ Hz, 3 H); ^{13}C NMR (125 MHz, CD_3OD) δ 169.6, 101.0, 77.8, 75.2, 74.1, 71.8, 62.3, 58.5, 57.1, 22.9, 18.4; HR-ESI-MS $[M + H]^+$ calcd for $C_{12}H_{23}N_2O_8$ 323.1449, found 323.1447.

4.4.3. O-((2R,3R,4R,5R,6R)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-L-serine **17**

Hydrogenolysis of **8** (71.6 mg, 0.079 mmol) by following the same procedure as for **15** gave the known[9] glycosylated serine product **17** (21.3 mg, 87% yield) as its hydrochloride: ^{1}H NMR (400 MHz, CD_3OD) δ 4.41 (d, 1 H, $J = 8.4$ Hz), 4.09–4.13 (m, 2 H), 3.96–4.01 (m, 3 H), 3.71–3.84 (m, 2H), 3.58 (dd, 1 H, $J = 2.9$ and 10.6 Hz), 3.54 (dd, 1 H, $J = 4.6$ and 6.9 Hz), 2.00 (s, 3 H); ^{13}C NMR (125 MHz, CD_3OD) δ 170.1, 168.3, 102.5, 77.0, 72.8, 69.6, 66.8, 62.6, 60.7, 53.5, 23.1; HR-ESI-MS $[M + H]^+$ calcd for $C_{11}H_{21}N_2O_8$ 309.1292, found 309.1292.

4.4.4. O-((2R,3R,4R,5R,6R)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-L-threonine **18**

Hydrogenolysis of **9** (78.2 mg, 0.085 mmol) by following the same procedure as for **15** gave the known[9] glycosylated threonine product **18** (23.6 mg, 86% yield) as its hydrochloride: ^{1}H NMR (400 MHz, CD_3OD) δ 4.40 (d, 1H, $J = 8.3$ Hz), 4.20 (t, 1 H, $J = 6.3$ Hz), 3.91 (dd, 1 H, $J = 8.6$ and 10.0 Hz), 3.70–3.81 (m, 4 H), 3.57 (dd, 1 H, $J = 2.3$ and 10.8 Hz), 3.50–3.53 (m, 1 H), 1.98 (s, 3 H), 1.32 (d, 1 H, $J = 5.3$ Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 174.6, 169.5, 101.4, 76.9, 73.9, 72.4, 69.6, 62.6, 58.7, 54.0, 22.9, 18.4; HR-ESI-MS $[M + H]^+$ calcd for $C_{12}H_{23}N_2O_8$ 323.1449, found 323.1449.

4.4.5. N-(L-valyl)-O-((2R,3R,4R,5S,6R)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-L-serine **19**

Hydrogenolysis of **11** (80.4 mg, 0.080 mmol) by following the same procedure as for **15** gave **19** (21.9 mg, 67% yield) as its hydrochloride: ^{1}H NMR (400 MHz, CD_3OD) δ 5.47 (d, 1 H, $J = 0.8$ Hz), 4.65 (t, 1 H, $J = 4.6$ Hz), 4.44 (d, 1H, $J = 8.4$ Hz), 4.10 (dd, 1 H, $J = 6.8$ and 10.6 Hz), 3.90 (dd, 1 H, $J = 4.3$ and 9.2 Hz), 3.87 (d, 1 H, $J = 12.4$ Hz), 3.74 (d, 1 H, $J = 6.0$ Hz), 3.60–3.68 (m, 2 H), 3.40–3.44 (m, 1 H), 3.32 (d, 1 H, $J = 0.9$ Hz), 2.16–2.24 (m, 1 H), 1.99 (s, 3 H), 1.08 (t, 3 H, $J = 7.5$ Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 174.4, 172.2, 169.6, 102.9, 78.1, 75.6, 72.0, 69.1, 62.6, 59.8, 57.1, 54.0, 31.6, 23.1, 18.8, 18.0; HR-ESI-MS $[M + H]^+$ calcd for $C_{16}H_{30}N_3O_9$ 408.1977, found 408.1978.

4.5. N-glycosylations

4.5.1. (2S,3R,4S,5R,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-2-(phenylthio)tetrahydro-2H-pyran-3-yl benzoate **20**[56]

A solution of 33% HBr solution in acetic acid (3.18 mL, 53.1 mmol) was added dropwise to a solution of α-D-glucopyranose pentabenzoate (6.20 g, 8.85 mmol) in 50 mL of dry dichloromethane. The reaction mixture was stirred under nitrogen for 16 h, and then diluted with 100 mL of dichloromethane. The organic solution was washed sequentially with 100 mL each of water, saturated aq sodium bicarbonate, and water. The organic phase was dried and concentrated. A solution of the residue in 50 mL of nitromethane was stirred with molecular sieves (4 Å, 1.50 g) under nitrogen for 1 h. The flask was then covered with foil, and treated sequentially with γ-collidine (1.50 mL, 11.36 mmol), dry methanol (0.34 mL, 8.9 mmol), and *tert*-butylammonium bromide (5.0 mmol, 1.62 g). After 16 h, triethylamine (0.4 mL) was added, the solid was removed by filtration, and the filtrate was washed with 100 mL of saturated aq sodium bicarbonate. The aqueous layer was further extracted with dichloromethane (2 × 50 mL). The combined organic extracted was washed with water, dried, and then concentrated. The residue was debenzoylated and benzylated by following the literature procedure.[57] The resulting product was purified by column chromatography on silica gel, eluting with 3:7 ethyl acetate/hexane, to give the known orthoester (3.57 g, 71% yield) as a light yellow gel.

The orthoester described above (3.57 g, 6.28 mmol) was combined with molecular sieves (4 Å, 1.5 g) and dry acetonitrile (40 mL), and the resulting mixture was stirred under nitrogen for 30 min. Thiophenol (2.35 g, 21.3 mmol) and mercuric(II) bromide (0.076 g, 0.211 mmol) were added sequentially, and the mixture was heated at reflux for 2.5 h. The reaction mixture was cooled, filtered, and then concentrated. A solution of the residue in dichloromethane (20 mL) was washed sequentially with 1% aq sodium hydroxide (30 mL) and water (2 × 30 mL), dried, and then concentrated. The residue was purified by column chromatography on silica gel, eluting with 1:9 ethyl acetate/hexane, to give thioglycoside donor **20** (3.04 g, 75% yield) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03–8.05 (m, 2 H), 7.61–7.8 (m, 1H), 7.44–7.50 (m, 4 H), 7.19–7.36 (m, 13 H), 7.11–7.14 (m, 5 H), 5.29 (dd, 1 H, J = 7.6 and 8.0 Hz), 4.82 (d, 1 H, J = 9.2 Hz), 4.79 (d, 1 H, J = 8.4 Hz), 4.73 (d, 1 H, J = 8.8 Hz), 4.64 (d, 1 H, J = 8.4 Hz), 4.56–4.61 (m, 3 H), 3.82–3.87 (m, 2 H), 3.73–3.78 (m, 2 H), 3.61–3.64 (m, 1 H), 1.56 (broad, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.4, 138.4138.1, 137.8, 133.4, 133.1, 132.7, 130.1, 130.0, 129.0, 128.6, 128.6, 128.5, 128.4, 128.2, 128.2, 127.9, 127.9, 127.8, 86.3, 84.5, 79.7, 75.5, 75.3, 73.7, 72.6, 69.2; LC-ESI-MS $[M+Na]^+$ calcd for $C_{40}H_{38}O_6SNa$ 669.23, found 669.19.

4.5.2. Allyl ((benzyloxy)carbonyl)-L-asparaginate **21**[19]

A stirred solution of *N*-(benzyloxycarbonyl)-*L*-asparagine (2.0 g, 7.51 mmol) in 35 mL dimethylformamide was treated with allyl bromide (0.78 mL, 9.01 mmol) and sodium iodide (1.64 g, 11.0 mmol). After 24 h, the reaction mixture was partitioned between ethyl acetate (150 mL) and water (150 mL). The aqueous layer was further extracted with ethyl acetate (2 × 50 mL), and the combined organic extract was washed sequentially with 1.2 M aq hydrochloric acid (100 mL), saturated aq sodium bicarbonate (100 mL), and brine (100 mL), dried, and then concentrated. The residue was purified by chromatography on silica gel eluting with 1:3 ethyl acetate/hexane to afford **21** (1.79 g, 78%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.31 (m, 5 H), 6.03 (d, 1 H, J = 4.0 Hz), 5.94–5.86 (m, 1 H), 5.57 (broad, 1 H), 5.45 (broad, 1 H), 5.32 (d, 1 H, J = 16.0 Hz), 5.25 (d, 1 H, J = 4.0 Hz), 5.13 (s, 2 H), 4.66 (d, 2 H, J = 4.0 Hz), 4.64–4.62 (m, 1 H), 3.00 (dd, 1 H, J = 4.0 and 16.0 Hz), 2.78 (dd, 1 H, J = 4.0 and 16.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 171.1, 156.4, 136.4, 131.7, 128.7, 128.3, 128.2, 118.8, 67.2, 66.4, 50.9, 37.2; LC-ESI-MS $[M+H]^+$ calcd for $C_{15}H_{19}N_2O_5$ 307.13, found 307.10.

4.5.3. (2R,3R,4S,5R,6R)-2-((S)-4-(allyloxy)-3-(((benzyloxy)carbonyl)amino)-4-oxobutanamido)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-3-yl benzoate **22**

By following the general procedure, *gluco* donor **20** (97.0 mg, 0.15 mmol) was combined with asparagine acceptor **21** (30.6 mg, 0.10 mmol) at 0 °C for 2 h. After work-up, purification by thin-layer chromatography with 6:4 hexane/ethyl acetate as the eluant afforded **22** (70.95 mg, 82%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, 2 H, J = 7.2 Hz), 7.58 (t, 1 H, J = 7.2 Hz), 7.42–7.45 (m, 2 H), 7.27–7.34 (m, 15 H), 7.10–7.17 (m, 5 H), 6.49 (d, 1 H, J = 9.2 Hz), 5.90 (d, 1 H, J = 9.2 Hz), 5.54–5.61 (m, 1H), 5.22 (t, 1 H, J = 9.2 Hz), 5.13 (t, 1 H, J = 9.2 Hz), 5.05–5.08 (m, 3H), 5.00 (d, 1 H, J = 10.6 Hz), 4.80 (d, 1 H, J = 4.5 Hz), 4.77 (d, 1 H, J = 4.5 Hz), 4.72 (d, 1 H, J = 10.5 Hz), 4.65 (d, 1 H, J = 10.5 Hz),4.47–4.55 (m, 4 H), 4.35 (dd, 1 H, J = 5.8 and 13.1 Hz), 4.20 (dd, 1 H, J = 5.8 and 13.5 Hz), 3.90 (t, 1 H, J = 9.5 Hz), 3.86 (t, 1 H, J = 9.5 Hz), 3.74–3.79 (m, 2 H), 2.85 (dd, 1 H, J = 4.3 and 16.9 Hz), 2.69 (dd, 1 H, J = 4.3 and 16.9 Hz), 1.64 (broad, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 170.4, 167.0, 156.2, 138.0, 137.8, 137.78, 136.4, 133.8, 131.5, 130.0, 129.1, 128.7, 128.6, 128.57, 128.56, 128.5, 128.2, 128.17, 128.21, 128.1, 128.06, 128.0, 127.94, 127.9, 118.4, 83.1, 78.5, 77.7, 77.4, 76.8, 75.7, 75.3, 73.8, 73.6, 68.1, 67.1, 66.1, 50.5, 37.8; HR-ESI-MS $[M + H]^+$ calcd for $C_{49}H_{51}N_2O_{11}$ 843.3487, found 843.3464.

4.5.4. 2,2,2-Trichloroethyl ((2S,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-2-(phenylthio)tetrahydro-2H-pyran-3-yl)carbamate **23**[19]

A solution of the commercial β-D-thioglycoside triacetate, (2R,3*S*,4R,5R,6*S*)-2-(acetoxymethyl)-6-(phenylthio)-5-(((2,2,2-trichloroethoxy)carbonyl)amino)tetrahydro-2*H*-pyran-3,4-diyl diacetate, (2.5 g, 4.36 mmol) in methanol (50 mL) was treated with sodium methoxide (24 mg, 0.44 mmol). The reaction mixture was allowed to stir overnight, and then was neutralized with Dowex 50x8-100 acidic resin. The resin was removed by filtration, and the filtrate was concentrated. The residue was dried azeotropically with toluene (3 × 5 mL) and then was taken to the next step without further purification.

A solution of the above triol (approximately 4.36 mmol) in dimethylformamide (20 mL) was treated with 60% sodium hydride suspension in mineral oil (697 mg, 17.44 mmol), and the resulting mixture was stirred at 0 °C for 30 min. Benzyl bromide (2.6 mL, 21.80 mmol) was added, and the mixture was stirred at room temperature for 24 h. The reaction mixture was carefully quenched with 100 mL of saturated aqueous ammonium chloride, and the mixture was extracted with ethyl acetate (2 × 50 mL). The combined extract was dried and then concentrated to a residue, which was purified by column chromatography on silica gel, eluting with 1:9 ethyl acetate/hexane, to give the thioglycoside donor **23** (2.0 g, 64% yield) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.54 (m, 2H), 7.21–7.35 (m, 18 H), 5.09 (d, 1 H, J = 8.5 Hz), 4.95 (d, 1 H, J = 10.5 Hz), 4.70–4.84 (m, 5 H), 4.61–4.63 (m, 2 H), 4.55 (d, 1 H, J = 12.0 Hz), 3.89 (t, 1 H, J = 8.5 Hz), 3.79 (d, 1 H, J = 11.0 Hz), 3.75 (dd, 1 H, J = 4.0 and 11.5 Hz), 3.66 (t, 1 H, J = 9.0 Hz), 3.58 (broad, 1 H), 3.45–3.48 (m, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.9, 138.4, 138.1, 132.2, 129.1, 128.6, 128.6, 128.5, 128.2, 128.1, 128.0, 127.95, 127.8, 127.7, 85.9, 82.4, 79.4, 78.6, 77.4, 75.4, 75.0, 74.6, 73.6, 69.1, 56.8; LC-ESI-MS $[M+Na]^+$ calcd for $C_{36}H_{36}Cl_3NO_6SNa$ 738.12, found 738.29.

*4.5.5. (2R,3R,4S,5R,6R)-4,5-bis(benzyloxy)-2-((S)-3-(((benzyloxy) carbonyl)amino)-4-(((S)-1-(tert-butoxy)-3-methyl-1-oxobutan-2-yl)amino)-4-oxobutanamido)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-3-yl benzoate **24***

By following the general procedure, *gluco* donor **20** (97.0 mg, 0.15 mmol) was combined with Asp-Val acceptor **40** (42.1 mg, 0.10 mmol) at 0 °C for 2 h. After work-up, purification by thin-layer chromatography with 6:4 hexane/ethyl acetate as the eluant afforded 24 (53.7 mg, 56%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, 2 H, J = 7.8 Hz), 7.56 (t, 1 H, J = 7.3 Hz), 7.42 (t, 2 H, J = 7.8 Hz), 7.11–7.35 (m, 20 H), 6.99 (d, 1 H, J = 8.7 Hz), 6.78 (d, 1 H, J = 9.2 Hz), 6.26 (d, 1 H, J = 7.3 Hz), 5.23 (t, 1 H, J = 9.2 Hz), 5.16 (t, 1 H, J = 9.2 Hz), 5.07 (d, 1 H, J = 12.0 Hz), 5.02 (d, 1 H, J = 12.0 Hz), 4.80 (d, 1 H, J = 10.6 Hz), 4.79 (d, 1 H, J = 11.1 Hz), 4.74 (d, 1 H, J = 11.1 Hz), 4.65 (d, 1 H, J = 12.1 Hz), 4.53 (d, 1 H, J = 10.6 Hz), 4.48 (d, 1 H, J = 12.1 Hz), 4.43 (m, 1H), 4.05 (m, 1H), 3.92 (dd, 1 H, J = 8.6 and 9.2 Hz), 3.85 (dd, 1 H, J = 8.6 and 9.2 Hz), 3.77 (dd, 1 H, J = 2.4 and 10.6 Hz), 3.73 (d, 1 H, J = 10.6 Hz), 3.61 (d, 1 H, J = 9.6 Hz), 2.79 (dd, 1 H, J = 3.9 and 16.0 Hz), 2.60 (dd, 1 H, J = 5.8 and 16.0), 1.95–1.99 (m, 1H), 1.67 (broad, 1 H), 1.43 (s, 9H), 0.76 (d, 3 H, J = 7.3 Hz), 0.73 (d, 3 H, J = 6.8 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 170.5, 170.2, 167.0, 156.3, 138.0, 137.83, 137.8, 136.2, 133.6, 130.1, 129.3, 128.6, 128.5, 128.4, 128.2, 128.15, 128.1, 128.0, 127.99, 127.95, 127.9, 127.86, 83.3, 81.9, 78.5, 77.4, 76.7, 75.6, 75.2, 73.7, 73.6, 68.2, 67.2, 57.7, 51.4, 37.4, 31.2, 28.1, 18.8, 17.6; HR-ESI-MS $[M + H]^+$ calcd for $C_{55}H_{64}N_3O_{12}$ 958.4485, found 958.4455.

*4.5.6. tert-Butyl (6S,9S,12S)-9-(2-(((2R,3R,4S,5R,6R)-3-(benzoyloxy)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)amino)-2-oxoethyl)-1,1,1-trichloro-6,12-diisopropyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oate **25***

By following the general procedure, *gluco* donor **20** (97.0 mg, 0.15 mmol) was combined with Val-Asp-Val acceptor **41** (56.2 mg, 0.10 mmol) at 0 °C for 2 h. After work-up, purification by thin-layer chromatography with 6:4 hexane/ethyl acetate as the eluant afforded **25** (39.6 mg, 36%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, 2 H, J = 9.6 Hz), 7.56–7.63 (m, 2 H), 7.43 (t, 2 H, J = 9.5 Hz), 7.26–7.33 (m, 7 H), 6.94–7.16 (m, 7 H), 6.95 (d, 1 H, J = 10.5 Hz), 5.17–5.23 (m, 2 H), 4.72–4.80 (m, 3 H), 4.67 (d, 1 H, J = 9.9 Hz), 4.64 (d, 1 H, J = 10.0 Hz), 4.52 (d, 1 H, J = 13.8 Hz), 4.48 (d, 1 H, J = 15.2 Hz), 4.05 (dd, 1 H, J = 6.8 and 10.0 Hz), 3.90–3.97 (m, 2 H), 3.82 (t, 1 H, J = 11.7 Hz), 3.75 (broad, 1 H), 3.63 (d, 1 H, J = 12.0 Hz), 2.70 (dd, 1 H, J = 3.6 and 16.0 Hz), 2.54 (dd, 1 H, J = 7.4 and 16.0 Hz), 2.43 (broad, 2 H), 2.06–2.10 (m, 1H), 1.93–1.98 (m, 1 H), 1.43 (s, 9 H), 0.93 (d, 3 H, J = 8.4 Hz), 0.87 (d, 3 H, J = 8.4 Hz), 0.77 (d, 3 H, J = 8.6 Hz), 0.74 (d, 3 H, J = 8.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 170.9, 170.3, 169.7, 167.1, 154.8, 138.0, 137.8, 133.6, 130.2, 129.5, 128.6, 128.57, 128.5, 128.2, 128.1, 128.0, 127.96, 127.9, 95.6, 83.2, 81.9, 78.5, 77.7, 77.4, 76.7, 75.7, 75.2, 74.8, 73.8, 73.8, 73.6, 68.3, 60.5, 57.9, 49.9, 37.2, 31.4, 31.1, 28.2, 19.3, 18.8, 17.7, 17.6; HR-ESI-MS $[M + H]^+$ calcd for $C_{55}H_{67}N_4O_{13}$ 1097.3843, found 1097.3801.

*4.5.7. (2R,3R,4S,5R,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-2-(1-((3aR,4R,6R,6aR)-6-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-diethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-1H-1,2,4-triazole-3-carboxamido)tetrahydro-2H-pyran-3-yl benzoate **26***

By following the general procedure, *gluco* donor **20** (97.0 mg, 0.15 mmol 1.5 equiv) was combined with protected ribavirin acceptor **42** (42.6 mg, 0.10 mmol) at 0 °C for 2 h. After work-up, purification by thin-layer chromatography with 8:2 hexane/ethyl acetate as the eluant afforded **26** (50.1 mg, 52%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, 2 H, J = 7.8 Hz), 7.87 (d, 1 H, J = 9.3 Hz), 7.55 (t, 1 H, J = 7.3 Hz), 7.39–7.42 (m, 2 H), 7.27–7.37 (m, 10 H), 7.11–7.19 (m, 8 H), 6.09 (s, 1 H), 5.51 (t, 1 H, J = 9.8 Hz), 5.39 (d, 1 H, J = 6.4 Hz), 5.31 (d, 1 H, J = 9.3 Hz), 4.96 (d, 1 H, J = 5.7 Hz), 4.84 (d, 1 H, J = 10.7 Hz), 4.80 (d, 1 H, J = 10.7 Hz), 4.72 (d, 1 H, J = 11.2 Hz), 4.66 (d, 1 H, J = 12.2 Hz), 4.58 (d, 1 H, J = 10.7 Hz), 4.51 (d, 1 H, J = 12.2 Hz), 4.37 (t, 1 H, J = 6.8 Hz), 3.97 (t, 1 H, J = 8.8 Hz), 3.92 (t, 1 H, J = 9.3 Hz), 3.82 (dd, 1 H, J = 2.4 and 10.7 Hz), 3.77 (d, 1 H, J = 10.7 Hz), 3.71 (d, 1 H, J = 9.3 Hz), 3.57 (dd, 1 H, J = 7.3 and 10.7 Hz), 3.43 (dd, 1 H, J = 7.3 and 10.7 Hz), 1.79 (q, 2 H, J = 7.3 Hz), 1.73 (broad, 1 H), 1.65 (q, 2 H, J = 7.3 Hz), 1.01 (t, 3 H, J = 7.3 Hz), 0.90 (t, 3 H, J = 7.3 Hz), 0.81 (s, 9 H), −0.09 (s, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.2, 158.7, 158.0, 138.1, 137.9, 137.8, 133.5, 130.0, 129.3, 128.6, 128.55, 128.5, 128.4, 128.1, 128.07, 128.0, 127.96, 127.9, 127.8, 118.0, 101.9, 93.6, 89.8, 84.6, 83.3, 82.5, 78.2, 77.7, 77.4, 77.0, 75.6, 75.2, 73.8, 73.4, 68.3, 63.4, 29.5, 29.2, 25.9, 18.3, 8.5, 7.8, 0.1, −5.3; HR-ESI-MS $[M + H]^+$ calcd for $C_{53}H_{67}N_4O_{11}Si$ 963.4570, found 963.4539.

*4.5.8. Allyl N^2-((benzyloxy)carbonyl)-N^4-((2R,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-3-(((2,2,2-trichloroethoxy) carbonyl)amino)tetrahydro-2H-pyran-2-yl)-L-asparaginate **27***

By following the general procedure, *gluco* donor **23** (107.5 mg, 0.15 mmol) was combined with asparagine acceptor **21** (30.6 mg, 0.10 mmol) at 0 °C for 2 h. After work-up, purification by thin-layer chromatography with 6:4 hexane/ethyl acetate as eluant afforded **27** (55.7 mg, 61%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.17 (m, 20 H), 6.89 (d, 1 H, J = 8.3 Hz), 5.98 (d, 1 H, J = 9.2 Hz), 5.87–5.79 (m, 1 H), 5.26 (dd, 1 H, J = 1.5 and 16.9 Hz), 5.18 (dd, 1 H, J = 1.5 and 10.7 Hz), 5.13 (d, 1 H, J = 12.1 Hz), 5.08 (d, 1 H, J = 12.1 Hz), 4.84–4.89 (m, 2 H), 4.79 (d, 1 H, J = 10.6 Hz), 4.76 (d, 1 H, J = 12.1 Hz), 4.64–4.70 (m, 3 H), 4.57–4.59 (m, 2 H), 4.55 (d, 1 H, J = 10.6 Hz), 4.47 (d, 1 H, J = 7.6 Hz), 3.81 (t, 1 H, J = 9.2 Hz), 3.69–3.76 (m, 2 H), 3.59 (dd, 1 H, J = 7.2 and 9.2 Hz), 3.41–3.48 (m, 2 H), 2.86 (dd, 1 H, J = 4.3 and 16.9 Hz), 2.68 (dd, 1 H, J = 4.4 and 16.9 Hz), 1.87 (broad, 2 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 156.2, 156.0, 137.8, 137.5, 136.4, 131.7, 129.1, 128.8, 128.6, 128.55, 128.23, 128.2, 128.1, 127.9, 118.6, 95.4, 80.12, 80.1, 78.4, 77.4, 76.7, 75.1, 74.9, 74.6, 73.8, 68.1, 67.2, 66.3, 55.8, 50.6, 37.8; HR-ESI-MS $[M + H]^+$ calcd for $C_{45}H_{49}Cl_3N_3O_{11}$ 912.2427, found 912.2420.

*4.5.9. tert-Butyl N^2-((benzyloxy)carbonyl)-N^4-((2R,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-3-(((2,2,2-trichloroethoxy)carbonyl)amino)tetrahydro-2H-pyran-2-yl)-L-asparaginyl-L-valinate **28***

By following the general procedure, *gluco* donor **23** (107.5 mg, 0.15 mmol) was combined with Val-Asp acceptor **40** (42.1 mg, 0.10 mmol) at 0 °C for 2 h. After work-up, purification by thin-layer chromatography with 6:4 hexane/ethyl acetate as the eluant afforded **28** (46.3 mg, 45%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28–7.40 (m, 18 H), 7.18–7.19 (m, 2 H), 7.02 (d, 1 H, J = 8.2 Hz), 6.43 (d, 1 H, J = 7.3 Hz), 5.13 (d, 1 H, J = 12.0 Hz), 5.09 (d, 1 H, J = 12.0 Hz), 4.90 (d, 1 H, J = 12.1 Hz), 4.86 (dd, 1 H, J = 8.2 and 9.2 Hz), 4.85 (d, 1 H, J = 12.0 Hz), 4.80 (d, 1 H, J = 11.1 Hz), 4.62–4.70 (m, 4 H), 4.57 (d, 1 H, J = 11.1 Hz), 4.48 (broad, 1H), 4.47 (d, 1 H, J = 12.1 Hz), 4.28 (dd, 1 H, J = 4.9 and 8.7 Hz), 3.80 (t, 1 H, J = 9.2 Hz), 3.67–3.76 (m, 2 H), 3.60 (m, 1 H), 3.47–3.49 (m, 2 H), 2.78 (dd, 1 H, J = 3.4 and 16.4 Hz), 2.58 (dd, 1 H, J = 5.8 and 16.4 Hz), 2.05–2.10 (m, 1 H), 1.44 (s, 9 H), 0.82 (d, 6 H, J = 6.8 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 170.6, 170.4, 156.1, 137.9, 137.8, 137.5, 136.2, 129.0, 128.8, 128.7, 128.6, 128.5, 128.3, 128.2, 128.17, 128.1, 127.9, 95.5, 82.0, 80.0, 78.5, 77.4, 76.7, 75.14, 75.1, 74.6, 73.8, 68.2, 67.3, 58.0, 55.9, 51.4, 37.6, 31.3, 28.2, 19.0, 17.7; HR-ESI-MS $[M + H]^+$ calcd for $C_{51}H_{62}Cl_3N_4O_{12}$ 1027.3424, found 1024.3408.

4.5.10. tert-Butyl N^4-((2R,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-3-(((2,2,2-trichloroethoxy)carbonyl)amino) tetrahydro-2H-pyran-2-yl)-N^2-(((2,2,2-trichloroethoxy)carbonyl)-L-valyl)-L-asparaginyl-L-valinate ***29***

By following the general procedure, *gluco* donor **23** (107.5 mg, 0.15 mmol) was combined with the Val-Asp-Val acceptor **41** (42.1 mg, 0.10 mmol) at 0 °C for 2 h. After work-up, purification by thin-layer chromatography with 6:4 hexane/ethyl acetate as the eluant afforded **29** (37.4 mg, 32%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, 1 H, J = 8.7 Hz), 7.18–7.40 (m, 15 H), 5.66 (d, 1 H, J = 10.1 Hz), 4.79–4.91 (m, 4 H), 4.63–4.71 (m, 4 H), 4.56 (d, 1 H, J = 13.5 Hz), 4.48 (d, 1 H, J = 15.1 Hz), 4.24 (dd, 1 H, J = 5.8 and 10.5 Hz), 4.09 (t, 1 H, J = 7.1 Hz), 3.60–3.81 (m, 4 H), 3.46 (d, 1 H, J = 11.3 Hz), 2.72 (dd, 1 H, J = 3.8 and 20.0 Hz), 2.54 (dd, 1 H, J = 7.5 and 19.8 Hz), 2.06–2.18 (m, 2 H), 1.85 (broad, 2 H), 1.44 (s, 9 H), 0.98 (d, 3 H, J = 8.4 Hz), 0.93 (d, 3 H, J = 8.4 Hz), 0.85–0.86 (m, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 170.9, 170.4, 170.0, 156.1, 137.9, 137.8, 137.5, 129.1, 128.8, 128.6, 128.58, 128.2, 128.1, 128.0, 95.5, 82.0, 80.1, 80.0, 78.5, 77.4, 76.7, 75.3, 75.1, 74.8, 74.6, 73.8, 68.2, 60.6, 58.2, 55.8, 49.9, 37.1, 31.4, 31.2, 28.2, 19.4, 19.0, 17.9, 17.7; HR-ESI-MS $[M + H]^+$ calcd for $C_{51}H_{66}Cl_6N_5O_{13}$ 1166.2783, found 1166.2765.

4.5.11. 2,2,2-Trichloroethyl ((2R,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-2-((4-methylphenyl)sulfonamido)tetrahydro-2H-pyran-3-yl)carbamate ***30***

By following the general procedure, donor **23** (107.5 mg, 0.15 mmol) was combined with 4-methylbenzenesulfonamide (17.1 mg, 0.10 mmol) at 0 °C for 2 h. After work-up, purification by thin-layer chromatography with 4:1 hexane/ethyl acetate as the eluant afforded **30** (56.0 mg, 72%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (m 2 H), 7.17–7.39 (m, 17 H), 6.03 (d, 1 H, J = 8.9 Hz), 4.87 (d, 1 H, J = 7.8 Hz), 4.83 (d, 1 H, J = 11.6 Hz), 4.79 (d, 1 H, J = 10.9 Hz), 4.75 (d, 1 H, J = 12.0 Hz), 4.63–4.70 (m, 3 H), 4.58 (d, 1 H, J = 11.0 Hz), 4.43 (d, 1 H, J = 12.1 Hz), 4.31 (d, 1 H, J = 12.1 Hz), 3.63–3.69 (m, 2 H), 3.51–3.61 (m, 2 H), 3.38–3.43 (m, 2 H), 2.35 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.8, 143.4, 138.9, 138.0, 137.8, 137.5, 129.4, 128.9, 128.6, 128.54, 128.5, 128.1, 127.9, 127.8, 127.3, 95.3, 84.4, 80.5, 78.3, 76.5, 75.1, 74.9, 74.8, 73.8, 68.4, 56.1, 40.5, 21.6; LC-ESI-MS $[M+H]^+$ calcd for $C_{37}H_{39}Cl_3N_2O_8S$ 777.15, found 777.13.

4.6. Glycoprotein core tripeptide synthesis

4.6.1. Benzyl O-benzyl-N-N2-((benzyloxy)carbonyl)-N4-((2R,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-3-(((2,2,2-trichloroethoxy)carbonyl)amino)tetrahydro-2H-pyran-2-yl)-L-asparaginylglycyl-L-serinate ***31***

A solution of glycosylated asparagine **27** (35.0 mg, 0.038 mmol) and *N*-methylaniline (8.2 mg, 0.077 mmol) in tetrahydrofuran (1 ml) was treated with *tetrakis*(triphenylphosphine)palladium(0) (4.0 mg, 3.8 μmol). After 15 min, the reaction mixture was concentrated, the residue was dissolved in 2 mL of 10:1 dimethyl sulfoxide/water, and the resulting solution was purified by preparative reverse phase medium pressure chromatography, eluting with a gradient from 10% to 100% acetonitrile/water. The fractions containing product were combined and dried by lypholizer to afford 33 mg of the carboxylic acid containing a trace amount of triphenylphosphine.

The preparation of the dipeptide coupling partner benzyl *O*-benzyl-*N*-glycyl-L-serinate 2,2,2-trifluoroacetate salt is given following this paragraph. A stirred and cooled (ice bath) solution of the carboxylic acid described above (33 mg, 0.037 mmol) and benzyl O-benzyl-*N*-glycyl-L-serinate 2,2,2-trifluoroacetate salt (34 mg, 0.075 mmol) in dry dichloromethane and dry dimethylformamide (10:1, 1 mL) was treated sequentially with *N*,*N*-diisopropylethylamine (15 mg, 0.113 mmol), 1-hydroxybenzotriazole (10 mg, 0.075 mmol), and 1-(3-dimethyl aminopropyl)-3-ethylcarbodiimide hydrochloride (12 mg, 0.075 mmol). After 3 h, the reaction mixture was partitioned between dichloromethane (10 mL) and 1.2 M hydrochloric acid (10 mL). The aqueous layer was further extracted with dichloromethane (2 × 5 mL). The combined organic extract was washed sequentially with saturated aq sodium bicarbonate (10 mL) and brine (10 mL), dried, and concentrated. The residue was purified by preparative reverse phase medium pressure chromatography, eluting with 10%–100% acetonitrile/water. The fraction containing product were conbined and dried by lypholizer to give the glycosylated tripeptide **31** (39 mg, 0.032 mmol, 84% over 2 steps) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13–7.34 (m, 28 H), 7.05 (d, 1 H, J = 8.7 Hz), 6.97 (d, 1 H, J = 8.0 Hz), 6.32 (d, 1 H, J = 8.6 Hz), 5.49 (d, 1 H, J = 8.3 Hz), 5.20 (d, 1 H, J = 12.7 Hz), 5.07–5.15 (m, 2 H), 4.99 (t, 1 H, J = 9.2 Hz), 4.89 (d, 1 H, J = 12.2 Hz), 4.79–4.83 (m, 1 H), 4.75 (d, 1 H, J = 12.3 Hz), 4.72 (d, 1 H, J = 12.3 Hz), 4.71 (d, 1 H, J = 12.3 Hz), 4.60 (d, 1 H, J = 12.3 Hz), 4.57 (d, 1 H, J = 12.2 Hz), 4.51 (d, 1 H, J = 10.8 Hz), 4.49 (d, 1 H, J = 12.4 Hz), 4.44 (d, 1 H, J = 12.5 Hz), 4.37 (d, 1 H, J = 12.3 Hz), 4.11 (dd, 1 H, J = 6.6 and 16.8 Hz), 3.86 (dd, 1 H, J = 4.0 and 10.0 Hz), 3.60–3.76 (m, 4 H), 3.46–3.54 (m, 2 H), 2.91 (dd, 1 H, J = 3.3 and 16.1 Hz), 2.72 (broad, 2 H), 2.55 (q, 1 H, J = 7.6); ^{13}C NMR (125 Hz, $CDCl_3$) δ 171.9, 171.4, 170.3, 169.1, 156.0, 138.0, 137.8, 137.78, 137.3, 136.0, 135.2, 128.8, 128.75, 128.7, 128.6, 128.58, 128.56, 128.4, 128.3, 128.28, 128.2, 128.1, 128.0, 127.9, 127.89, 95.6, 81.2, 79.8, 78.2, 77.4, 76.7, 75.1, 74.7, 73.7, 73.3, 69.3, 68.3, 67.7, 67.6, 56.2, 52.7, 43.1; LC-ESI-MS $[M+H]^+$ calcd for $C_{61}H_{65}Cl_3N_5O_{14}$ 1196.35, found 1196.42.

4.6.2. Benzyl O-benzyl-N-(tert-butoxycarbonyl)-L-serinate[58]

A stirred solution of commercial *N*-(*tert*-butoxycarbonyl)-*O*-benzyl-*L*-threonine (2.00 g, 6.46 mmol) in dimethylformamide (15 mL) at 0 °C was treated sequentially with triethylamine (0.90 mL, 6.46 mmol) and benzyl bromide (0.76 mL, 6.46 mmol). After 12 h, the reaction mixture was diluted with ethyl acetate (100 mL), and the solution was washed successively with aq citric acid (100 mL), saturated aq sodium bicarbonate (100 mL), and brine (100 mL), dried, and then concentrated in *vacuo*. The residue was purified by chromatography on silica, eluting with 1:9 ethyl acetate/hexane, to afford the ester (1.10 g, 85% yield) as colorless syrup: ^{1}H NMR (400 MHz, $CDCl_3$) 7.21–7.32 (m, 10 H), 5.43 (d, 1 H, J = 8.7 Hz), 5.23 (d, 1 H, J = 12.4 Hz), 5.14 (d, 1 H, J = 12.4 Hz), 4.50 (d, 1 H, J = 12.0 Hz), 4.43 (d, 1 H, J = 12.1 Hz), 3.91 (dd, 1 H, J = 2.6 and 9.4 Hz), 3.69 (dd, 1 H, J = 2.9 and 9.3 Hz), 1.44 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) 170.4, 155.4, 137.4, 135.4, 128.4, 128,3, 128.2, 128.0, 127.6, 127.5, 79.7, 73.1, 70.0, 67.0, 54.1, 28.2. LC-ESI-MS $[M+H]^+$ calcd for $C_{22}H_{28}NO_5$ 386.20, found 386.18.

4.6.3. Benzyl O-benzyl-N-glycyl-L-serinate trifluoroacetate salt

A solution of benzyl *O*-benzyl-*N*-(*tert*-butoxycarbonyl)-*L*-serinate (see above; 1.10 g, 2.85 mmol) in trifluoroacetic acid (5 mL) and dichloromethane (20.0 mL) was stirred at room temperature for 3 h, and then concentrated. The residue was used for the next reaction without further purification.

A stirred and cooled (ice bath) solution of the crude trifluoroacetate salt described above (approximately 2.85 mmol) and (*tert*-butoxycarbonyl)glycine (509 mg, 2.90 mmol) in dry dichloromethane and dry dimethylformamide (10:1, 28.0 mL) was treated sequentially with 1-hydroxybenzotriazole (579 mg, 4.27 mmol), *N*,*N*-diisopropylethylamine (1.25 mL, 8.55 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (818 mg, 4.27 mmol). After 13 h, the reaction mixture was dilated with dichloromethane (50 mL), and the organic solution was washed with 1 M hydrochloric acid (50 mL). The aqueous layer was further extracted with dichloromethane (2 × 50 mL), and the

combined organic extract was washed sequentially with saturated aq sodium bicarbonate and brine, dried, and then concentrated. The residue was purified by using a prepacked C-18 reverse-phase column (80 g), eluting with 5%–100% acetonitrile/water. The fractions containing product were combined and then dried by lypholizer to afford the dipeptide (945 mg, 75% over two steps), which was used as obtained for the deprotection step.

The solution of above dipeptide in dichloromethane (10 mL) and trifluoroactetic acid (2 mL) was stirred for 3 h, and then concentrated. The residue was purified by using a prepacked C-18 reverse phase column (80 g), eluting with 5%–100% acetonitrile/water. The fractions containing product were combined and then dried by lypholizer to afford the dipeptide salt (970 mg, 74% over 3 steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.31 (m, 5 H), 6.03 (d, 1 H, J = 4.0 Hz), 5.94–5.86 (m, 1 H), 5.57 (broad, 1 H), 5.45 (broad, 1 H), 5.32 (d, 1 H, J = 16.0 Hz), 5.25 (d, 1 H, J = 4.0 Hz), 5.13 (s, 2 H), 4.66 (d, 2 H, J = 4.0 Hz), 4.64–4.62 (m, 1 H), 3.00 (dd, 1 H, J = 4.0 and 16.0 Hz), 2.78 (dd, 1 H, J = 4.0 and 16.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 167.5, 138.9, 137.0, 129.5, 129.34, 129.3, 129.2, 128.8, 128.76, 74.3, 70.4, 68.2, 54.4, 41.4; HR-ESI-MS $[M + H]^+$ calcd for $C_{19}H_{23}N_2O_4$ 343.1652, found 343.1655.

4.6.4. Benzyl N-N4-((2R,3R,4R,5S,6R)-3-acetamido-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)-N2-((benzyloxy)carbonyl)-L-asparaginylglycyl-O-benzyl-L-serinate **32**

A stirred solution of glycosylated tripeptide **31** (39 mg, 0.032 mmol) in acetic acid (1 mL) was treated with zinc dust (43.0 mg). After 4 h. The suspension was filtered, the filter cake was washed with acetic acid (1 mL), and the filtrate was concentrated. A stirred solution of the residue in pyridine (1 ml) was treated with acetic anhydride (33 mg, 0.32 mmol). After 24 h, the reaction mixture was concentrated and then dissolved in dimethylsulfoxide (1 mL). The solution was by reverse phase preparative medium pressure chromatography, eluting with a gradient of 10%–100% acetonitrile/water. The fractions containing product were conbined and dried by lypholizer to give **32** (29 mg, 0.027 mmol, 85% over 2 steps) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14–7.35 (m, 30 H), 7.02 (d, 1 H, J = 7.9 Hz), 6.27 (d, 1 H, J = 8.6 Hz), 5.39 (d, 1 H, J = 7.2 Hz), 5.17 (d, 1 H, J = 12.2 Hz), 5.12 (d, 1 H, J = 12.2 Hz), 5.11 (d, 1 H, J = 12.2 Hz), 5.07 (d, 1 H, J = 12.2 Hz), 4.75–4.82 (m, 4 H), 4.61 (d, 1 H, J = 12.0 Hz), 4.56 (d, 1 H, J = 12.0 Hz), 4.52 (d, 1 H, J = 12.2 Hz), 4.48 (d, 1 H, J = 12.1 Hz), 4.45 (d, 1 H, J = 12.0 Hz), 4.39 (d, 1 H, J = 11.9 Hz), 4.08 (d, 1 H, J = 16.9 Hz), 3.86–3.93 (m, 2 H), 3.66–3.77 (m, 5 H), 3.41–3.47 (m, 2 H), 2.91 (dd, 1 H, J = 3.0 and 16.4 Hz), 2.52 (dd, 1 H, J = 4.6 and 16.4 Hz), 2.00 (s, 3 H); ^{13}C NMR (125 Hz, $CDCl_3$) δ 171.5, 171.4, 170.1, 169.0, 168.5, 138.1, 138.0, 137.9, 137.3, 136.1, 135.3, 128.9, 128.8, 128.7, 128.65, 128.6, 128.57, 128.54, 128.5, 128.4, 128.3, 128.28, 128.2, 128.1, 128.06, 128.0, 127.97, 127.9, 81.4, 78.2, 77.4, 76.8, 75.2, 74.4, 73.7, 73.2, 69.4, 68.3, 67.7, 52.7, 43.2, 23.1; LC-ESI-MS $[M+H]^+$ calcd for $C_{60}H_{66}N_5O_{13}$ 1064.47, found 1064.52.

4.6.5. N4-((2R,3R,4R,5S,6R)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-L-asparaginylglycyl-L-serine **33**

A solution of protected tripeptide **32** (25 mg, 0.023 mmol) in methanol (2 mL) was combined with palladium(II) hydroxide (0.0046 mmol, 33 mg, 10% on carbon). A hydrogen atmosphere was supplied with balloon. After 3 h, the suspension was filtered and the filtrate concentrated to give the deprotected tripeptide **33** (10 mg, 91% yield) as a white powder: ^{1}H NMR (400 MHz, CD_3OD) δ 5.00 (d, 1 H, J = 8.0 Hz), 4.49 (t, 1 H, J = 5.8 Hz), 4.23 (t, 1 H, J = 5.8 Hz), 4.00 (m, 2 H), 3.90 (dd, 1 H, J = 4.5 and 10.9 Hz), 3.84 (d, 2 H, J = 12.2 Hz), 3.72 (t, 1 H, J = 9.9 Hz), 3.64 (dd, 1 H, J = 4.2 and 12.1 Hz), 3.48 (t, 1 H, J = 8.4 Hz), 3.32 (s, 2 H), 2.90 (dd, 1 H, J = 4.9 and 17.3 Hz), 2.76 (dd, 1 H, J = 7.5 and 17.0 Hz), 1.96 (s, 3 H); ^{13}C NMR (125 Hz, CD_3OD) δ 174.5, 173.2, 171.2, 171.0, 169.8, 80.3, 79.8, 76.2, 71.9, 62.8, 62.7, 56.3, 56.2, 51.2, 43.4, 36.9, 22.9; HR-ESI-MS $[M + H]^+$ calcd for $C_{17}H_{31}N_5O_{11}$ 480.1936, found 480.1938.

4.7. Additional acceptors

4.7.1. Methyl ((benzyloxy)carbonyl)-L-threoninate **34**

This acceptor is available commercially.

4.7.2. Benzyl ((benzyloxy)carbonyl)-L-serinate **35**[59]

Esterification of *N*-Cbz-(*L*)-serine by following the same procedure as for **36** (below) gave **35** (89% yield) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.35 (m, 10 H), 5.73 (d, 1 H, J = 6.3 Hz), 5.22 (s, 2 H), 5.12 (s, 2 H), 4.48–4.50 (m, 1 H), 3.95–4.00 (broad, 2 H), 2.17 (broad, 1 H), 1.62 (broad, 1 H); ^{13}C NMR (125 Hz, $CDCl_3$) δ 170.5, 156.4, 136.2, 135.2, 128.7, 128.6, 128.6, 128.3, 128.3, 128.2, 67.6, 67.3, 63.3, 56.3; LC-ESI-MS $[M+H]^+$ calcd for $C_{18}H_{19}NO_5$ 330.35, found 330.36.

4.7.3. Benzyl ((benzyloxy)carbonyl)-L-threoninate **36**[60]

A solution of (2*S*,3R)-2-benzyloxycarbonylamino-3-hydroxybutanoic acid (1.0 g, 4.0 mmol) in DMF (15.0 mL) and deionized water (5.0 mL) was treated with cesium carbonate (644.5 mg, 2.0 mmol), stirred for 30 min, concentrated, and then azeotropically dried with toluene (3 × 10 mL). A solution of benzyl bromide (0.71 mL, 5.9 mmol) in DMF (10.0 mL) was then added. After 24 h, water (15 mL) was added, and the mixture was extracted with ethyl acetate (2 × 30 mL). The organic extract was dried over anhydrous sodium sulfate and concentrated. The residue was purified by flash column chromatography, eluting with 1:3 ethyl acetate/hexane, to give **36** (1.149 g, 84% yield) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.35 (m, 10 H), 5.60 (d, 1 H, J = 8.7 Hz), 5.17–5.24 (m, 2 H), 5.13 (s, 2 H), 4.37–4.39 (m, 2 H), 1.97 (broad, 1 H), 1.63 (broad, 1 H), 1.23 (d, 1 H, J = 6.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2, 156.9, 136.2, 135.3, 128.6, 128.5, 128.5, 128.2, 128.0, 68.0, 67.4, 67.2, 59.5, 19.9; LC-ESI-MS $[M+H]^+$ calcd for $C_{19}H_{21}NO_5$ 344.14, found 344.03.

4.7.4. Benzyl (tert-butoxycarbonyl)-L-serinate **37**

This acceptor is available commercially.

4.7.5. Benzyl ((benzyloxy)carbonyl)-L-valyl-L-serinate **38**

A solution of *N*-(benzyloxycarbonyl)-*L*-valine (2.51 g, 10.0 mmol) and benzyl *L*-serinate (1.95 g, 10.0 mmol) in dry dichloromethane and dry dimethylformamide (10:1, 100 mL) was treated sequentially with 1-hydroxybenzotriazole (1.61 g, 12.0 mmol), *N*,*N*-diisopropylethylamine (3.80 mL, 24.0 mmol), and 1-(3-dimethyl aminopropyl)-3-ethylcarbodiimide hydrochloride (2.29 g, 12.0 mmol) at 0 °C. After stirring for 3 h, the reaction mixture was poured into a mixture of dichloromethane (100 mL) and 1.2 M aq hydrochloric acid (150 mL). The aqueous layer was further extracted with two portions of dichloromethane (50 mL). The combined organic extract was washed sequentially with saturated aq sodium bicarbonate (100 mL) and brine (100 mL), dried over magnesium sulfate, and concentrated. The residue was purified by flash chromatography on silica gel, eluting with 3:97 methanol/dichloromethane, to afford **38** (3.35 g, 78% yield) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.35 (m, 10 H), 6.87 (d, 1 H, J = 7.2 Hz), 5.44 (d, 1 H, J = 8.1 Hz), 5.18–5.24 (m, 2 H), 5.10 (d, 1 H, J = 12.0 Hz), 5.04 (d, 1 H, J = 12.0 Hz), 4.70–4.72 (m, 1 H), 3.90–4.02 (m, 3 H), 2.04–2.13 (m, 1 H), 1.69 (broad, 2 H), 0.97 (d, 3 H, J = 6.6 Hz), 0.93 (d, 3 H, J = 6.6 Hz); ^{13}C NMR (125 Hz, $CDCl_3$)

δ 171.9, 170.3, 157.0, 136.2, 135.2, 128.7, 128.6, 128.6, 128.4, 128.3, 128.2, 67.6, 67.3, 62.9, 60.7, 54.8, 31.3, 19.3, 18.1; HR-ESI-MS $[M+H]^+$ calcd for $C_{23}H_{29}N_2O_6$ 429.2020, found 429.2025.

4.7.6. Benzyl ((benzyloxy)carbonyl)-L-valyl-L-serylglycinate **39**

A solution of dipeptide ester **38** (1.2 g, 2.80 mmol) in methanol (30 mL) was treated with 2 N sodium hydroxide (2.8 mL, 5.60 mmol) at room temperature. The mixture was stirred for 5 h and then concentrated. The residue was partitioned between ethyl acetate (50) mL and 2 N aqueous HCl (50 mL). The organic layer was separated, dried over Na_2SO_4, and then concentrated to afford 1.1 g of the crude dipeptide acid as a white solid that was used directly in the next step.

A solution of the crude dipeptide acid (1.1 g, 2.80 mmol) and benzyl glycinate hydrochloride (564 mg, 2.80 mmol) in dry dichloromethane and dry dimethylformamide (10:1, 30 mL) was treated sequentially with 1-hydroxybenzotriazole (402 mg, 3.0 mmol), *N*,*N*-diisopropylethylamine (1.0 mL, 6.0 mmol) and 1-(3-dimethyl aminopropyl)-3-ethylcarbodiimide hydrochloride (763 mg, 3.0 mmol) at 0 °C. After stirring for 3 h, the reaction mixture was poured into a mixture of dichloromethane (100 mL) and 1.2 M aqueous hydrochloric acid (100 mL). The organic layer was separated and the aqueous layer was extracted with two additional portions of dichloromethane (50 mL each). The combined organic extract was washed sequentially with saturated aq sodium bicarbonate (100 mL) and brine (100 mL), dried over magnesium sulfate, and then concentrated. The residue was purified by flash chromatography on silica gel, eluting with 4:96 methanol/dichloromethane, to afford **39** (1.0 g, 74% yield) as a white powder: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (t, 1 H, J = 5.4 Hz), 7.89 (d, 1 H, J = 7.8 Hz), 7.27–7.34 (m, 10 H), 5.20 (s, 2 H), 4.98–5.05 (m, 2 H), 4.86 (t, 1 H, J = 5.6 Hz), 4.33–4.38 (m, 1 H), 3.89–3.95 (m, 3 H), 3.55 (t, 2 H, J = 5.4 Hz), 1.96–2.01 (m, 1 H), 0.84 (d, 3 H, J = 6.7 Hz), 0.79 (d, 3 H, J = 6.7 Hz); ^{13}C NMR (125 Hz, DMSO-d_6) δ 171.1, 170.4, 169.6, 156.2, 137.1, 135.9, 128.4, 128.3, 128.1, 127.9, 127.8, 127.6, 65.9, 65.5, 61.8, 60.1, 64.8, 40.8, 30.4, 19.2, 17.9; HR-ESI-MS $[M+H]^+$ calcd for $C_{25}H_{32}N_3O_7$ 486.2235, found 486.2244.

4.7.7. tert-Butyl ((benzyloxy)carbonyl)-L-asparaginyl-L-valinate **40**[19]

A stirred solution of *N*-(benzyloxycarbonyl)-*L*-asparagine (1.88 g, 7.06 mmol) and *L*-valine *tert*-butyl ester hydrochloride (1.48 g, 7.06 mmol) in dry dichloromethane and dry dimethylformamide (10:1, 70 mL) was treated sequentially with 1-hydroxybenzotriazole (1.14 g, 8.48 mmol), *N*,*N*-diisopropylethylamine (2.24 mL, 14.12 mmol) and 1-(3-dimethyl aminopropyl)-3-ethylcarbodiimide hydrochloride (1.62 g, 8.48 mmol) at 0 °C. After 3 h, the reaction mixture was partitioned between dichloromethane (50 mL) and 1.2 M aqueous hydrochloric acid (100 mL). The aqueous layer was extracted with additional dichloromethane (2 × 50 mL), and the combined organic extract was washed sequentially with saturated aq sodium bicarbonate (50 mL) and brine (50 mL), dried, and concentrated. The residue was purified by silica gel flash chromatography eluting with 4:96 methanol/dichloromethane to afford **40** (2.40 g, 82% yield) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31–7.42 (m, 5H), 6.42 (d, 1 H, J = 7.2 Hz), 5.91 (s, 1 H), 5.47 (s, 1 H), 5.14 (s, 2 H), 4.58 (m, 1 H), 4.35 (dd, 1 H, J = 4.3 and 8.7 Hz), 2.96 (dd, 1 H, J = 3.4 and 16.0 Hz), 2.62 (dd, 1 H, J = 6.8 and 16.0 Hz), 2.17 (m, 1 H), 1.46 (s, 9 H), 0.91 (d, 3 H, J = 6.8 Hz), 0.89 (d, 3 H, J = 6.8 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.7171.2, 170.5, 156.3, 136.2, 128.4, 128.1, 128.0, 81.8, 66.9, 57.9, 51.6, 37.0, 31.1, 28.0, 18.9, 17.5; LC-ESI-MS $[M+H]^+$ calcd for $C_{21}H_{32}N_3O_6$ 422.23, found 422.13.

4.7.8. tert-Butyl ((2,2,2-trichloroethoxy)carbonyl)-L-valyl-L-asparaginyl-L-valinate **41**

Palladium-on-carbon (0.30 g, 10 wt%) was added to a solution of *N*-(benzyloxycarbonyl)-*L*-asparaginyl-*L*-valine **40** (3.00 g, 7.63 mmol) in methanol (76.0 mL). The reaction mixture was stirred under a hydrogen atmosphere for 3 h, filtered through a pad of Celite, and then concentrated. The residue was used for the next reaction without further purification.

A stirred solution of the above residue (approximately 7.63 mmol) and ((2,2,2-trichloroethoxy)carbonyl)-*L*-valine (2.67 g, 9.16 mmol) in dry dichloromethane and dry dimethylformamide (10:1, 76.0 mL) was treated sequentially with 1-hydroxybenzotriazole (1.45 g, 10.7 mmol), *N*,*N*-diisopropylethylamine (2.24 mL, 15.3 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.05 g, 10.7 mmol) at 0 °C. After 13 h, the reaction mixture was partitioned between dichloromethane (100 mL) and 1 M aq hydrochloric acid (100 mL). The aqueous layer was further extracted with dichloromethane (2 × 50 mL), and the combined organic extract was washed sequentially with saturated aq sodium bicarbonate and brine, dried, and concentrated. The residue was purified on a C-18 reverse-phase column (80 g), eluting with 5%–100% acetonitrile/water. The desired fractions were conbined and concentrated by lypholizer to afford **41** (2.55 g, 59% yield over 2 steps) as a white powder: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.16 (d, 1 H, J = 9.5 Hz), 7.79 (d, 1 H, J = 10.4 Hz), 7.74 (d, 1 H, J = 11.0 Hz), 7.33 (s, 1 H), 6.93 (s, 1 H), 4.82 (d, 1 H, J = 15.5 Hz), 4.72 (d, 1 H, J = 15.5 Hz), 4.62 (m, 1 H), 3.99 (dd, 1 H, J = 7.0 and 10.3 Hz), 3.88 (dd, 1 H, J = 9.0 and 10.7 Hz), 2.49 (dd, 1 H, J = 6.7 and 19.4 Hz), 2.40 (app q, 1 H, J = 9.4), 1.99 (m, 1 H), 1.37 (s, 9 H), 0.81–0.89 (m, 12 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.4, 170.9, 170.6, 170.2, 154.6, 96.1, 80.6, 73.5, 60.5, 60.0, 57.9, 51.7, 49.3, 37.1, 30.3, 30.0, 27.6, 19.1, 18.8, 18.1, 17.8; HR-ESI-MS $[M+H]^+$ calcd for $C_{21}H_{36}Cl_3N_4O_7$ 561.1644, found 561.1650.

4.7.9. 1-((3aR,4R,6R,6aR)-6-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-diethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-1H-1,2,4-triazole-3-carboxamide **42**

A solution of Ribavirin (2.44 g, 10.00 mmol) in 3-pentanone (20 mL) was treated with trimethyl orthoformate (3.18 g, 30.00 mmol) and 4-methylbenzenesulfonic acid monohydrate (0.38 g, 2.00 mmol). The mixture was stirred at 50 °C for 2 h, and then concentrated. A solution of the residue in 10 mL of dimethyl sulfoxide was chromatographed with a pre-packed C-18 reverse phase column (120 g), eluting with 1:19 acetonitrile/water. The fractions containing product were combined and lyophilized to afford the Ribavirin ketal (2.72 g, 87% yield) as a white powder: ^{1}H NMR (400 MHz, CD_3OD) δ 8.71 (s, 1 H), 6.21 (d, 1 H, J = 1.5 Hz), 5.28 (dd, 1 H, J = 1.5 and 6.3 Hz), 4.96 (dd, 1 H, J = 2.0 and 6.3 Hz), 4.39 (m, 1 H), 3.62 (dd, 1 H, J = 5.4 and 11.7 Hz), 3.56 (dd, 1 H, J = 5.4 and 11.7 Hz), 1.78 (q, 2 H, J = 7.8 Hz), 1.65 (q, 2 H, J = 7.3 Hz), 0.98 (t, 3 H, J = 7.3 Hz), 0.89 (t, 3 H, J = 7.8 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 163.2, 158.3, 146.7, 118.9, 95.3, 90.7, 86.5, 83.6, 63.3, 30.2, 30.1, 8.7, 8.0; LC-ESI-MS $[M+H]^+$ calcd for $C_{13}H_{21}N_4O_5$ 313.15, found 313.00.

A solution of the above ketal (2.72 g, 8.71 mmol) in 10 mL of dimethylformamide was treated sequentially with imidazole (1.19 g, 17.42 mmol) and *tert*-butyldimethylsilyl chloride (1.97 g, 13.06 mmol). The mixture was stirred at room temperature for 12 h, then was diluted with ethyl acetate (100 mL). The solution was washed sequentially with water (100 mL) and brine (100 mL), dried over magnesium sulfate, and then concentrated. The residue was purified by silica gel chromatography, eluting with 1:4 ethyl acetate/hexane, to give **42** (3.53 g, 95% yield) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1 H), 7.02 (s, 1 H), 6.55 (broad, 1 H), 6.05 (s, 1 H), 5.32 (d, 1 H, J = 5.8 Hz), 4.83 (d, 1 H, J = 6.3 Hz), 4.51 (dd, 1 H, J = 4.4 and 4.9 Hz), 3.74 (dd, 1 H, J = 4.4 and 11.2 Hz), 3.64

(dd, 1 H. $J = 4.9$ and 11.2 Hz), 1.78 (q, 2 H, $J = 7.3$ Hz), 1.63 (q, 2 H, $J = 7.3$ Hz), 0.97 (t, 3 H, $J = 7.3$ Hz), 0.89 (t, 3 H, $J = 7.3$ Hz), 0.82 (s, 9 H), 0.03 (s, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.2, 157.2, 144.0, 118.2, 95.4, 89.5, 85.7, 82.0, 63.7, 29.5, 29.2, 26.0, 18.4, 8.6, 7.8, 5.4, 5.4; HR-ESI-MS $[M + H]^+$ calcd for $C_{19}H_{35}N_4O_5Si$ 427.2371, found 427.2380.

4.7.10. *p-Toluenesulfonamide* ***43***

This acceptor is available commercially.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.tet.2018.04.082.

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