A Broadly Applicable Method for the Efficient Synthesis of α-*O*-Linked Glycopeptides and Clustered Sialic Acid Residues

Jacob B. Schwarz, Scott D. Kuduk, Xiao-Tao Chen, Dalibor Sames, Peter W. Glunz, and Samuel J. Danishefsky*

Contribution from the Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021, and Department of Chemistry, Columbia University, New York, New York 10027

Received September 17, 1998

Abstract: The total syntheses of complex sialylated cell-surface antigens have been accomplished. The target systems include 2,3-STF, STn, 2,6-STF, and glycophorin antigens. In addition, an α-*O*-linked serine glycoside of an entire Lewis blood group (Y) antigen has been assembled.

Introduction

As part of a multidisciplinary program targeted to the enlistment of the human immune system for recognition of various tumors and for mounting a clinically helpful response, we have directed our attention to the mucin class of glycoproteins. Mucins represent a family of cell-surface glycoproteins often associated, in aberrant glycoforms, with tumors of epithelial tissues.[1] In the first phase of our investigations, reported in an earlier publication, we have demonstrated that the trimeric clusters of Tn and TF glycoepitopes, conjugated as such to the Pam_3Cys lipophilic immunostimulant[2] or to KLH (keyhole limphet hemocyanin) immuno-protein carrier,[3] are immunogenic as judged by antibody production. Furthermore, the clustered motifs provoke robust production of antibodies, with promising cell-surface reactivity for those tumors expressing the respective antigen. Seemingly, our fully synthetic vaccine constructs, with trimeric arrangement of Tn epitopes, are able to mimic the cell-surface presentation of mucin *O*-linked antigens.[3] That proof-of-principle study provided particularly strong incentives to develop generally applicable schemes for the preparation of even more complex members of this class. In particular, we focused on the development of synthetic methodology to reach sialylated members of the TF family of mucin antigens including the glycophorin family. The state of sialylation (usually accomplished intracellularly by sialyltransferases or by de-sialylation within a cell by sialidases) is a critical determinant in cell-surface recognition of glycoproteins. In stepping up the complexity level of our goals, we would be confronting a long-standing problem in oligosaccharide and glycoconjugate synthesis. The crux of the difficulty has been the problematic character of synthesizing carbohydrate domains *O*-linked to the key amino acids, serine and threonine, with strong stereochemical control in the formation of the α-glycosidic linkage. In previous publications we have shown that the conduct of the glycosylation in the context of a fully assembled complex glyco-domain (such as a glycal) is fraught with considerable uncertainty as to the eventual α/β ratios.[2,4] While success was achieved in a few cases, seemingly small structural variations in fact profoundly affect the quality of the various steps in the progression from terminal glycal to *O*-linked serine or threonine glycoside in ways that are difficultly interpretable.[2] After much study of many cases, we are unable to provide a generally reliable protocol that would deliver the required serine or threonine glycoside to mature glycodomains with high α-selectivity to *any substrate of our choosing*. Given the potentially great importance of the synthesis of complex cell-surface molecular mimics in which serine or threonine residues are omnipresent in α-linkages, we viewed this uncertain situation as a serious detriment to progress. Herein we report a remarkably general solution to reach our goal systems. The method has been tested under stringent challenges such as are involved in the stereoselective syntheses of particularly complex and potentially invaluable cell surface molecular mimics. The assembly logic we describe is that of a "cassette" modality rather than reliance on a maximally convergent approach cited above. In the traditional regime,[4] a full glycodomain is assembled. An α-linkage to serine or threonine is then fashioned at the reducing end *of the fully mature domain*. Aside from the unpredictable glycosylation ratios in any new case, another disadvantage of this strategy is that difficulties in the yield or stereoselectivity during installation of the α-*O*-linked serine or threonine are borne out by the full glycodomain. Furthermore, in the traditional approach the protecting groups throughout the domain must be compatible with installation of the glycosyl donor functionality, and with the coupling step to the serine or threonine. Most serious is the nonreliability of the stereochemistry of this type of glycosylation in any new case. *In our new approach, a terminal GalNAc residue bearing an in-place serine or threonine is used as a generalized acceptor to be appended to a suitable donor linkage*.[5] Hopefully we would be facing less problematic glycosylation challenges associated with the saccharide−saccharide coupling in our assembly process. Ideally, such couplings would be conducted with a suitably

(1) Carlstedt, I.; Davies, J. R. *Biochem. Soc. Trans.* **1997**, *25*, 214. Finn, O. J. et al. *Immunol. Rev.* **1995**, *145*, 61.

(2) Kuduk, S. D.; Schwarz, J. B.; Chen, X.-T.; Sames, D.; Glunz, P. W.; Govindaswami, R.; Livingston, P. O.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1998**, *120*, 12474.

(3) Ragupathi, G.; Park, T. K.; Zhang, S.; Kim, I. J.; Graber, L.; Adluri, S.; Lloyd, K. O.; Danishefsky, S. J.; Livingston, P. O. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 125.

(4) Sames, D.; Chen, X.-T.; Danishefsky, S. J. *Nature* **1997**, *389*, 587.

10.1021/ja9833265 CCC: $18.00
Published on Web 03/11/1999

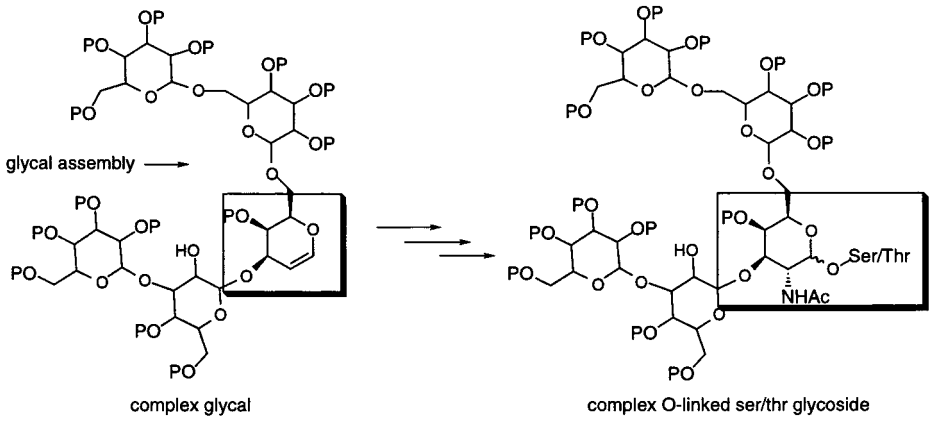


Figure 1.

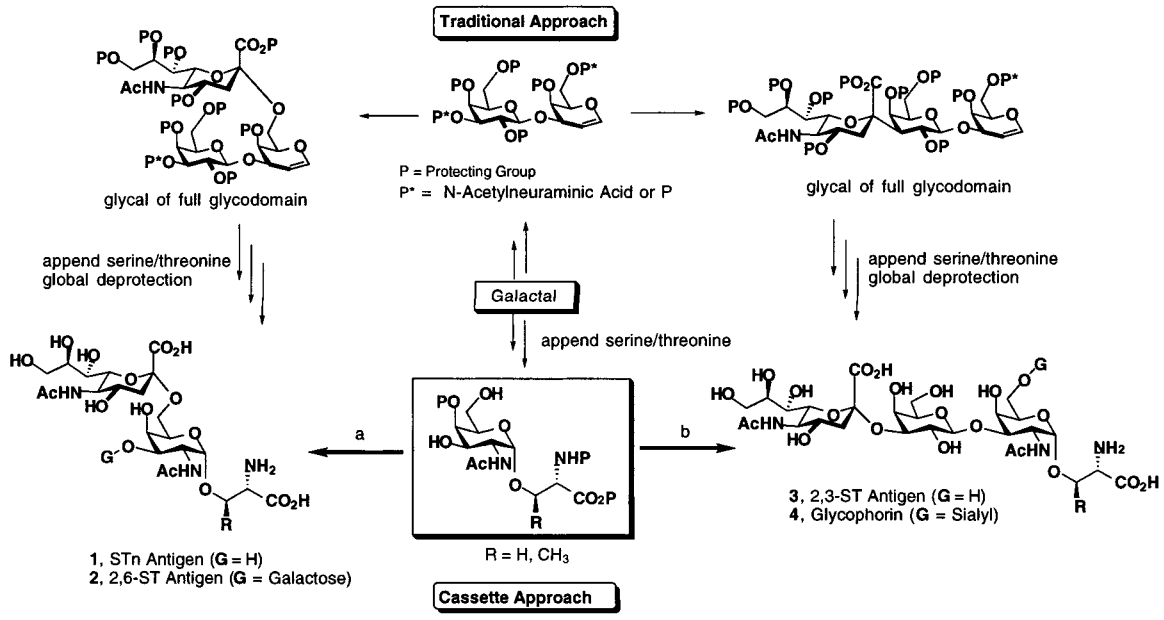


Figure 2.

protected serine or threonine residue (properly protected) *in place* at the "reducing end" of the acceptor. Figure 2 contrasts both approaches drawing particular attention (see bold arrows) to the bond being established between the cassette and the rest of the domain.[6]

Four distinct subgoals had to be achieved to implement the new program. First we had need to synthesize acceptor systems (cassettes) with α-*O*-linked serine or threonine residues, in place, in the context of GalNAc or latent GalNAc units. Moreover, these GalNAc units must bear differentiated or readily differentiable acceptor sites. Furthermore, the actual glycosylation with the acceptor cassette bearing the in-place *O*-linked serine or threonine had to proceed smoothly. Finally, global deprotection of the fully assembled construct must be achievable without undermining the stability of the potentially fragile linkage connecting the carbohydrate and amino acid domains.

In the work described herein, we set about to field test our notions regarding these challenges in the context of synthesizing sialylated antigens which are α-*O*-linked to peptides. Clearly, the inclusion of these sialic acid residues represents a significant extension in the complexity level of the undertaking. There was a clear rationale for undertaking such additional chemical challenges. Thus, the STn antigen **1**, which contains a sialic acid residue at position 6 of galactose is abundantly expressed in major epithelial tumors of the breast, ovary, colon, and stomach.[7] The presence of sialic acid moieties at either the 6-*O* position or the 3′-*O* position of the TF disaccharide is found in the 2,6-STF[8] and 2,3-STF[9] antigens **2** and **3**, respectively. The presence of sialic acid moieties on both the 6-position of the GalNAc and the 3-position of the galactose is the distinctive feature of the parent member of the family, glycophorin **4**. This motif is found in the context of a major erythrocyte membrane

(5) In practice since the assembly state of the 2,6-STF antigen (**2**) worked reasonably well by convergent glycal assembly, we did not resynthesize this goal structure through full implementation of the cassette method with two separate donors.

(6) For other cassette related approaches, see Meinjohanns, E.; Meldal, M.; Paulsen, H.; Schleyer, A.; Bock, K. *J. Chem. Soc., Perkin. Trans. 1* **1996**, 985. Mathieux, N.; Paulsen, H.; Meldal, M.; Bock, K. *J. Chem. Soc., Perkin. Trans. 1* **1997**, 2359. Nakahara, Y.; Iijima, H.; Ogawa, T. *Tetrahedron Lett.* **1994**, *35*, 3321. Liebe, B.; Kunz, H. *Tetrahedron Lett.* **1994**, *35*, 8777.

(7) Itzkowitz, S. H.; Yuan, M.; Montgomery, C. K.; Kjeldsen, T.; Takahashi, H. K.; Bigbee, W. L.; Kim, Y. S. *Cancer Res.* **1989**, *49*, 197. MacLean, G. D.; Reddish, M. A.; Koganty, R. R.; Longenecker, B. M. *J. Immunother.* **1996**, *19*, 59.

(8) (a) Fukuda, M.; Carlsson, S. R.; Klock, J. C.; Dell, A. *J. Biol. Chem.* **1986**, *261*, 12796. (b) Saitoh, O.; Gallagher, R. E.; Fukuda, M. *Cancer Res.* **1991**, *51*, 2854.

(9) Lloyd, K. O.; Burchell, J.; Kudryashov, V.; Yin, B. W. T.; Taylor-Papadimitriou, J. *J. Biol. Chem.* **1996**, *271*, 33325.

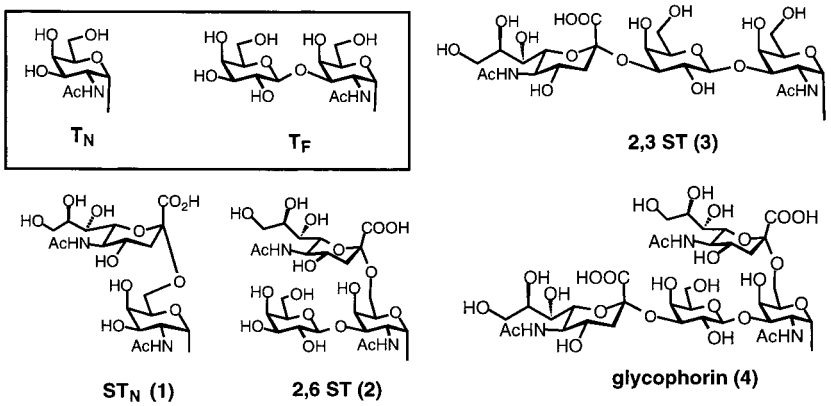
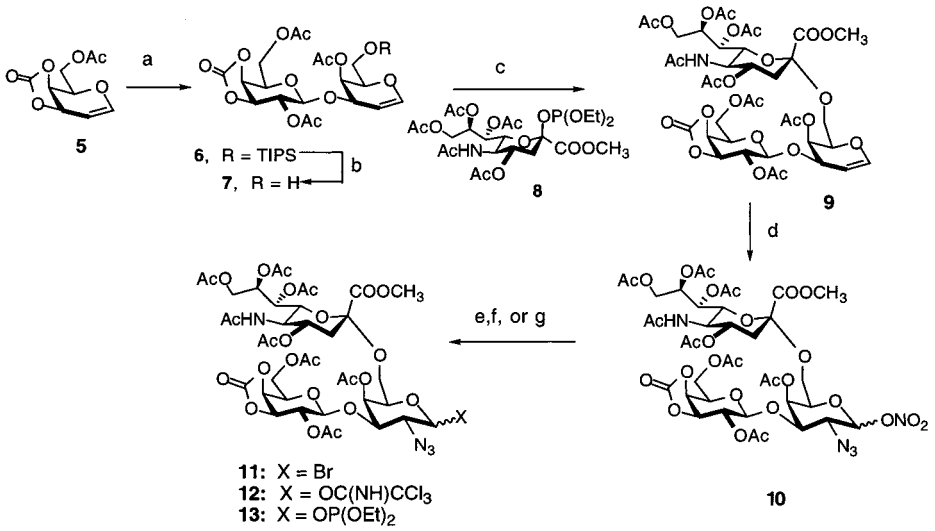


Figure 3.

Scheme 1



Reagents: (a) 1. DMDO, CH_2Cl_2; 2. 6-*O*-TIPS-galactal, $ZnCl_2$, -78 to 0 °C; 3. Ac_2O, Et_3N, DMAP, 75%.; (b) TBAF, AcOH, THF, 80%; (c) **8**, TMSOTf, THF:toluene (1:1), -60 to -45 °C, 84%, α:β 4:1; (d) NaN_3, CAN, CH_3CN, -15 °C, 60%; (e) LiBr, CH_3CN, 75%; (f) 1. PhSH, $^iPr_2NEt$, CH_3CN, 82%; 2. Cl_3CCN, $K_2C\,O_3$, CH_2Cl_2, 80%; (g) 1. PhSH, $^iPr_2NEt$; 2. $ClP(OEt)_2$, $^iPr_2NEt$, THF, 72%

glycoprotein.[10] Moreover, the presence of the 2,3-STF antigen **3** on breast tumors has been demonstrated,[9] and the expression of the 2,6-STF antigen **2** on cells of myelogenous leukemia has also been identified.[8] Our target structures are identified in Figure 3. From a chemical standpoint, the effective introduction of sialic acid residues into glycoconjugates has been a challenge for many years.[11] Careful considerations, as to which sialylation protocols would prove to be workable with either a glycal linkage in place or with α-*O*-linked glycosyl amino acid acceptors, would be a critical element of the synthetic design. In the studies described below, the two approaches discussed above were considered for accommodating the inclusion of sialic acid residues: (1) assembly of an entire glycodomain in the form of an advanced glycal, followed by the attachment of the amino acid; (2) an alternative approach wherein a "cassette", comprising a reactive acceptor having the *O*-linked amino acid attached to the GalNac residue, is united with a donor already equipped with a sialic acid. Such an approach would provide a suitably protected precursor to target structures **1**−**4** (Figure 2).

(10) Thomas, D. B.; Winzler, R. J. *J. Biol. Chem.* **1969**, *244*, 5943.

(11) Reviews: Okamoto, K.; Goto, T. *Tetrahedron* **1990**, *46*, 5835. (a) Deninno, M. P. *Synthesis* **1991**, 583. (b) Ito. Y.; Gaudino, J. J.; Paulson, J. C. *Pure Appl. Chem.* **1993**, *65*, 753.

Results and Discussion

1. Synthesis of 2,6-STF Glycosyl Serine/Threonine via the Modular Glycal Approach. To construct the glycosylated amino acid building blocks corresponding to the 2,6-STF[12] antigen for incorporation into a peptide backbone, we turned to the practice of glycal assembly (Scheme 1).[13] In the event, 6-*O*-TIPS galactal was coupled with compound **5**, to provide disaccharide **6** in 75% yield. After deblocking of the 6-*O*-TIPS moiety with buffered TBAF−HOAc, sialylation was attempted with various sialic acid donors. The diethyl phosphite donor[14] **8** proved to be most effective and afforded trisaccharide **9** in 84% yield as a separable 4:1 α:β mixture of anomers. Other donors such as a sialyl chloride[15] gave similar yields but with poorer sialic anomer selectivity.

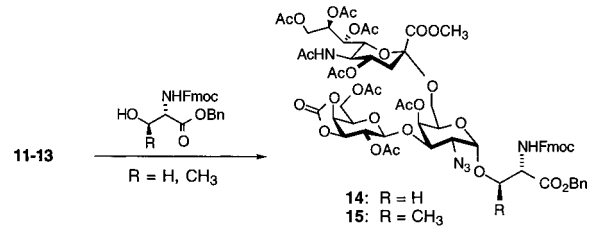
(12) For previous synthesis of 2,6-STF, see (a) Iijima, H.; Ogawa, T. *Carbohydr. Res.* **1989**, *186*, 95. (b) Qiu, D.; Koganty, R. R. *Tetrahedron Lett.* **1996**, *37*, 595. (c) Qiu, D.; Koganty, R. R. *Tetrahedron Lett.* **1997**, *38*, 961.

(13) Danishefsky, S. J.; Bilodeau, M. T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1381.

(14) (a) Martin, T. J.; Schmidt, R. R. *Tetrahedron Lett.* **1992**, *33*, 6123. (b) Martin, T. J.; Brescello, R.; Toepfer, A.; Schmidt, R. R. *Glycoconjugate J.* **1993**, *10*, 16.

(15) Ratcliffe, M. R.; Venot, A. P.; Abbas, Z. S. Eur. Pat. Appl. EP 319, 253. *Chem Abstr.* **1990**, *112*, 175281a.

Table 1.

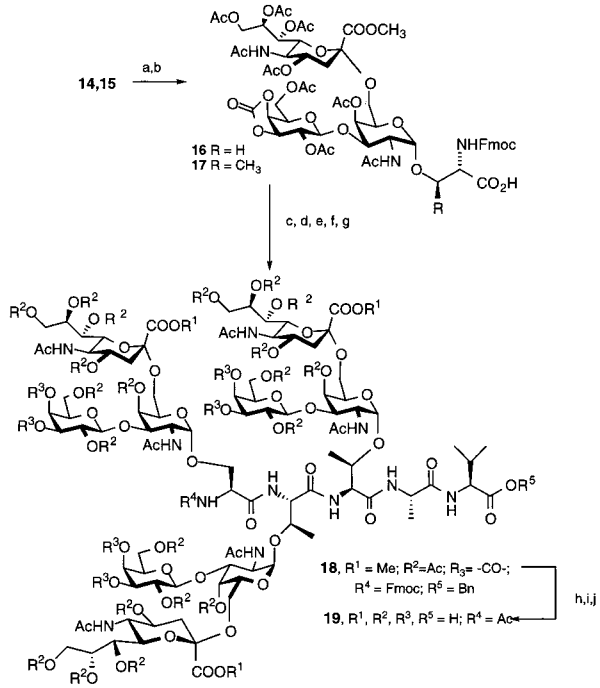


X	Catalyst / Promoter	R = H (**14**) α : β (%)	R = CH_3 (**15**) α : β (%)
- Br (**11**α)	$AgClO_4$ (1.5 eq), CH_2Cl_2, rt	2.6 : 1 (70%)	α only (74%)
- O(CNH)CCl_3 (**12**β)	BF_3•OEt_2 (0.5 eq), THF, -30° C	12 : 1 (65%)	α only (63%)
- O(CNH)CCl_3 (**12**α,β **1:1**))	BF_3•OEt_2 (0.5 eq), THF, -30 °C	4 : 1 (66%)	α only (60%)
- OP(OEt)$_2$ (**13**α,β 1:1)	BF_3•OEt_2 (0.5 eq), THF, -30 °C	30 : 1 (30%)	——

We now had to deal with the introduction of the *O*-linked amino acid to a donor ultimately derived from glycal **9**. The resultant galactal underwent smooth azidonitration[16] resulting in installation of the latent galactosamine for eventual glycosylation of serine or threonine. Conversion of compound **10** to a number of candidate donor linkages was achieved. Direct displacement of the anomeric nitrate to the α-bromide with LiBr/CH_3CN furnished anomeric bromide **11** in 75% yield. Reduction of the nitrate to the hemiacetal (PhSH, $^iPr_2NEt$) could be followed by conversion to the trichloroacetimidate **12** in 80% yield.[17] Alternatively treatment of the hemiacetal with chlorodiethyl phosphite gave the unstable phosphite donor **13**. Both **12** and **13** were obtained as mixtures of anomers in a 1:1 ratio. The results of attempts to glycosylate *N*-Fmoc-protected serine and threonine benzyl ester with donors **11**−**13** are shown in Table 1. With these donors, the coupling to protected threonine acceptors occurred with complete stereoselectivity, exclusively yielding the desired α-linked product **15**. While not proceeding with equivalent stereocontrol, the coupling of donors **12** and **13** with protected serine acceptors still provided the desired α-*O*-linked anomer as the major products in the indicated yields. The diethyl phosphite donor **13** afforded the highest selectivity. Noteworthy was the fact that a mixture of **12**α and **12***β* gave a lower selectivity than pure **12***β*. We had previously found that related donors where the 6-*O*-sialic acid in **11**−**13** is replaced by an acetate, the selectivity for both serine and threonine drops off to 2:1 α:*β* at best.[2] However, the types of difficulties as exemplified in the glycophorin synthesis (vide infra) in generalizing serine or threonine glycosylation ratios led us to favor the cassette approach. At this stage, our initial objective was achieved: a rapid, efficient glycodomain assembly via glycal logic, with resident protecting groups suitable for subsequent peptide coupling steps, and mild basic deprotection to conclude the synthesis was realized.[4] We first describe the construction of the 2,6-STF antigen motif.

2. Assembly of CD43-Derived Glycopeptides with Clustered 2,6-STF Epitopes. Having accomplished the synthesis of the 2,6-STF antigen, albeit in a nongeneral fashion, we set our sights on clustering such a substructure to produce a mimic portion of the CD43 glycopeptide.[18] The azido groups of trisaccharides **14** and **15** were reduced with thiolacetic acid[19] (78% yield) followed by quantitative hydrogenolytic removal

Scheme 2



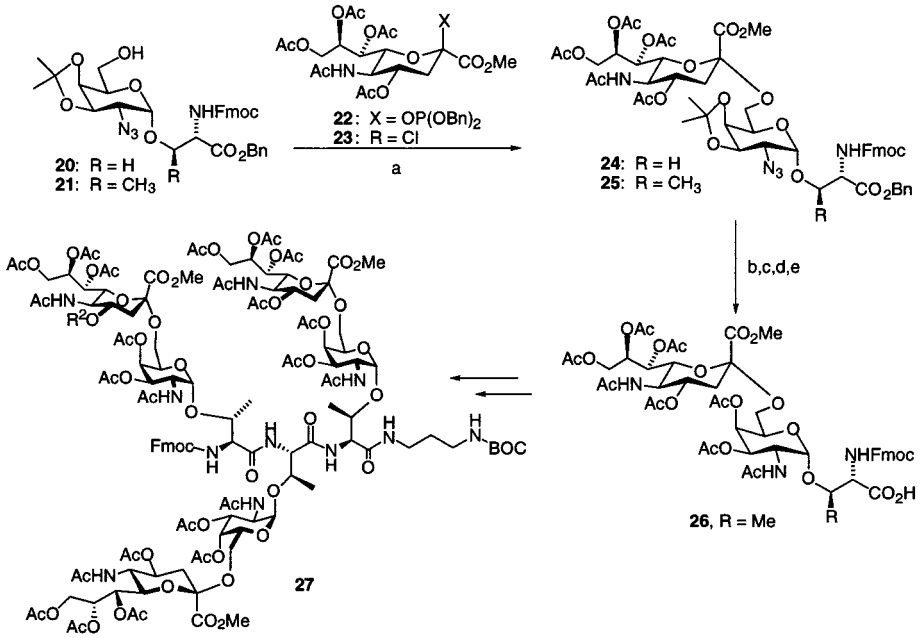
Reagents: (a) AcSH, 78%; (b) H_2, Pd/C, MeOH/H_2O, 100%; (c) H_2N-Ala-Val-OBn, IIDQ, CH_2Cl_2, 85%; (d) KF, DMF, 18-crown-6, 95%; (e) **17**, IIDQ, 87%; (f) KF, DMF 18-crown-6, 93%; (g) **15**, IIDQ, 90%; (h) 1. KF, DMF, 18-crown-6; 2. Ac_2O, CH_2Cl_2 (i) H_2, Pd/C, MeOH/H_2O; (j) NaOH, MeOH/H_2O, pH 10-10.5, 80%

of the benzyl ester, to afford **16** and **17**. These compounds served as suitable building units for the glycopeptide assembly. The glycopeptide backbone was elaborated from the carboxy to amino terminus direction (Scheme 2). Trisaccharide **17** was coupled to Ala-Val-benzyl ester (IIDQ), and iterative peptide coupling steps between the N-terminus of the peptide and the protected glycosyl amino acid, gave the desired pentapeptide **18** in high yield (average 85% for each coupling step). Finally, the carbamate linkage was deprotected and the amine capped with an acetyl group. After global deprotection (NaOH−H_2O, MeOH), the desired CD43 glycoprotein N-terminus **19** containing the clustered 2,6-STF epitopes was obtained in 80% yield and high purity as determined by reverse-phase HPLC (MeOH/H_2O, C18, 215 nm). This cluster is currently being readied for immunoconjugation and study.

3. Synthesis of STn Glycosyl Serine/Threonine via the Cassette Approach. In our synthesis of the blood-group determinant F1α,[20] it had been shown that lactosylation of a building block containing an acceptor hydroxyl at the 6-*O*-position could be effected. This strategy provided a fully protected precursor to free F1α. It was hoped that, in a related way, we could gain access to the STn antigen with high synthetic economy using protected glycosyl amino acid derivatives (see cassettes **20** and **21**) and a sialic acid-containing donor as a counterpart of the lactosylation system.[21] A synthesis of the STn monomer and its incorporation into a peptide was recently reported by Kunz.[22] Indeed, when either of the glycosylated serine or threonine cassettes **20** or **21**, respectively, were exposed

(16) Lemieux, R. U.; Ratcliffe, R. M. *Can. J. Chem.* **1979**, *57*, 1244.
(17) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Biochem.* **1994**, *50*, 84−123.
(18) Carlstedt, I.; Davies, J. R. *Biochem. Soc. Trans.* **1997**, *25*, 214.
(19) (a) Bieldfeldt, T.; Peters, S. Meldal, M.; Bock, K.; Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 857. (b) Rosen, T.; Lico, I. M.; Chu, D. T. W. *J. Org. Chem.* **1988**, *53*, 1580.
(20) Chen, X.-T.; Sames, D.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1998**, *120*, 7700−7709.
(21) For previous synthesis of STn via glycosylation of a disaccharide see: (a) Iijima, H.; Ogawa, T. *Carbohyr. Res.* **1988**, *172*, 183. For a related example using a similar type of cassette, see (b) Liebe, B.; Kunz, H. *Tetrahedron Lett.* **1994**, *35*, 8777. (c) Elofsson, M.; Salvador, L. A.; Kihlberg, J. *Tetrahedron* **1997**, *53*, 369. (d) Yule, J. E.; Wong, T. C.; Gandhi, S. S.; Qiu, D.; Riopel, M. A.; Koganty, R. R. *Tetrahedron Lett.* **1995**, *36*, 6839.
(22) Liebe, B.; Kunz, H. *Helv. Chim. Acta* **1997**, *80*, 1473.

Scheme 3

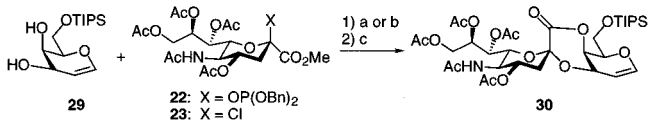


Reagents: a) TMSOTf, THF, 4Å mol sieves, -40 °C (R = Me, X = $OP(OBn)_2$, 37%; AgOTf, DTBP, $CaSO_4$, THF, -78 °C (R = H, X = Cl, 50%); b) AcSH, pyr, 87%; c) 80% AcOH; d) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 84% (two steps); e) H_2, Pd/C, MeOH/H_2O, 100%.

to a phosphite donor **22** or chloride donor **23**, the desired disaccharides **24** and **25** were obtained (Scheme 3). The stereochemistry of the addition was determined to be α from the chemical shift pattern at C3 in the ^{1}H NMR spectrum of the sialyl residue. In the case of **25**, although the overall α:*β* selectivity was 4:1, separation of the anomers proved to be tedious and the yield of pure α-adduct was only 37%. Kunz has made similar observations in both selectivity and yields, using sialyl xanthate donors.[23] Noteworthy, but not helpful, was the fact that sialylation using CH_2Cl_2 as solvent instead of THF led to the exclusive formation of the undesired *β*-anomer. In preparation for the impending peptide coupling, a short synthetic sequence was necessary. The phase commenced with the reduction of the azide linkage of **25** (AcSH, pyridine, 87%). Removal of the acetonide, reprotection as the acetates, and hydrogenolysis of the benzyl ester provided the compound **26**, necessary for clustering in 84% two-step yield. An iterative peptide coupling process, as employed above for the 2,6-STF series, afforded **27**. This tripeptide is differentially protected on each terminus to enable further elaboration. Conjugation of **27** to an immunostimulant or suitable derivative is now underway.

4. Synthesis of 2,3-STF Glycosyl Threonine via the Cassette Approach. Given the success of the synthesis and clustering of the 2,6-STF and STn antigens, we turned our attention toward the 2,3-STF antigen, which has been associated with human breast cancer.[8] The chemical synthesis of 2,3-STF by a similar approach has been recently reported[24] as has a fully enzymatic procedure.[25] Initially, as implemented in the 2,6-STF synthesis, it was our strategy that construction of the trisaccharide portion, would be followed by azidonitration and subsequent attachment of the full carbohydrate donor to a protected serine or threonine acceptor. Initial efforts at preparing trisaccharide

Scheme 4



Reagents: (a) TMSOTf, THF, -40 °C (X = $OP(OBn)_2$); (b) AgOTf, DTBP, CH_2Cl_2, -78 °C (X = Cl); (c) DBU, 0 °C, 40-50%

met with many complications. A serious problem was the difficult sialylation at the 3′-*O*-position of a suitably protected disaccharide glycal (TF core). This approach was also plagued by extremely poor yields and low selectivities.[26]

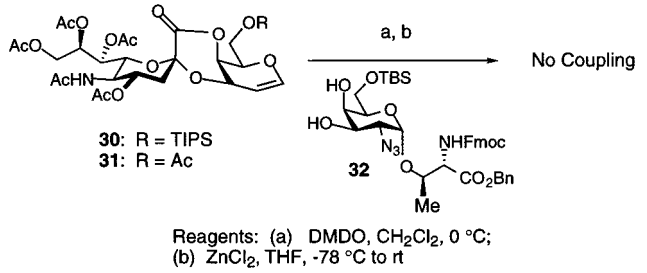
As an alternative approach, we sought to apply the cassette technology.[2,6] The goal would involve coupling of the appropriately protected cassette with a suitable donor derived from a disaccharide such as **30**. Thus, sialylation of compound **29** was carried out using phosphite donor **22** mediated by TMSOTf,[13] or alternatively with donor chloride **23** mediated by AgOTf.[27] The reaction mixture was then treated with DBU at 0 °C for 1 h to afford compound **30** in 40−50% yield for the two steps.[26,28]

The cassette coupling was attempted using the strategy previously employed by us for the TF antigen.[2] Thus, treatment of **30** with DMDO was followed by exposure to acceptor **32** in the presence of $ZnCl_2$. However, no coupling was observed. It was thought that the TIPS group in **30** was again too demanding as a steric presence. However, even with an acetate at the corresponding position (see **31**), no coupling was observed.

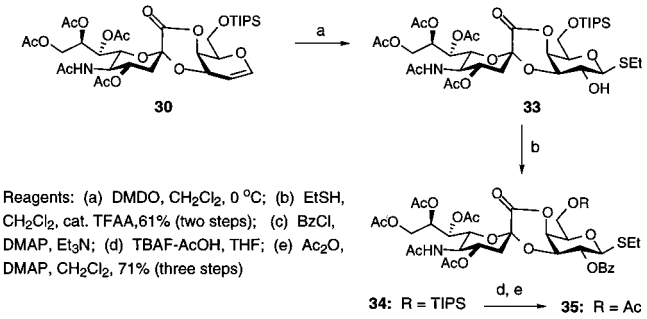
In previous studies, we and others have shown that thioethyl glycosides, suitably protected at C2 (pivalate, benzoate), can serve as excellent *β*-glycosidation donors in a variety of glycosylation reactions.[29] We were intrigued with the possibility of a convergent coupling of an inactivated thio donor such as compound **33** with acceptor **32**. In the event, treatment of **30**

(23) Liebe, B.; Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 618.
(24) Nakahara, Y.; Nakahara, Y.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1997**, *38*, 7211.
(25) Synthesis: Kren, V.; Thiem, J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 893.
(26) Woo, J. W.; Kuduk, S. D.; Danishefsky, S. J. Unpublished results.
(27) Gervay, J.; Peterson, J. M.; Oriyama, T.; Danishefsky, S. J. *J. Org. Chem.* **1993**, *58*, 5465.
(28) Ercegovic, T.; Magnusson, G. *J. Org. Chem.* **1995**, *60*, 3378.

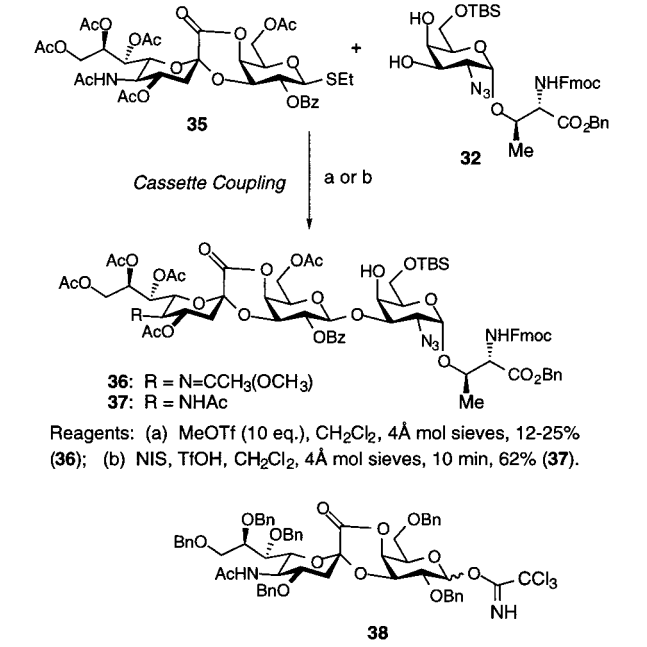
Scheme 5



Scheme 6



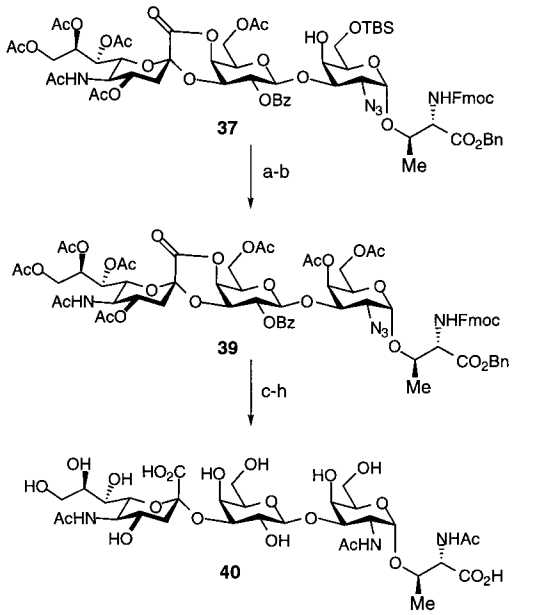
Scheme 7



with DMDO, followed by epoxide opening with ethanethiol in the presence of catalytic trifluoroacetic acid, provided **33** in 61% yield, to serve as the thiodonor. Benzoylation at C2 afforded **34**, and subsequent conversion of the 6-*O*-TIPS group to acetate furnished the projected donor **35** in 71% yield with only a single purification step. The critical coupling step was at hand.

Glycosylation of **34** with acceptor **32** was first investigated using our previously developed conditions (MeOTf, DTBP).[29a] No coupling was observed. Our first response to this setback was to replace the 6-*O*-TIPS group with an acetoxy function as in donor **35**, which would hopefully be less encumbered based upon our previous observations with TF.[2] Although some coupling products were obtained, the reaction proved quite inefficient and gave generally poor yields (ca. 25%). Another consequence of using methyl triflate as the promoter, was the formation of the compound **36** at the C-5 amide of the sialic acid. Cleavage of the methyl group required an additional hydrolytic step.

Scheme 8



After surveying a variety of alternative coupling conditions, it was found that recourse to the Fraser-Reid *N*-iodosuccinimide−triflic acid promoter system[30] afforded compound **37** in ca. 60% yield with good reproducibility. Of course, in the absence of a methylating potential in the promoter, no imidate byproduct was observed.

A somewhat related approach had been previously employed by Ogawa using trichloroacetimidate **38**.[31] It should be noted here that thiodonor **35** was highly unactivated due the presence of the acetates, while donor **38** was an electron rich perbenzylated system. Thus, although we have exploited a "disarmed"[32] glycosyl donor in the case of thiodonor **35**, the use of the NIS/TfOH[30] promoter system was successful toward 2,3-STF. We have also extended this approach to other "disarmed" glycosyl donors (vide infra). Global deprotection starting with **37** to expose the free antigen was accomplished in a straightforward manner (Scheme 8). Hence, removal of the 6-*O*-TBS group on the GalNAc residue was achieved with 1 N HCl. Acetylation of the free hydroxyl groups provided trisaccharide **39** with all resident hydroxyl groups protected as esters in 85% yield. At this stage the stereochemistry of the NIS coupling product was determined to be β by COSY and HETCOR experiments. Reduction of the azide with thiolacetic acid proceeded in 56% yield. Deprotection of the amino acid portion was effected by

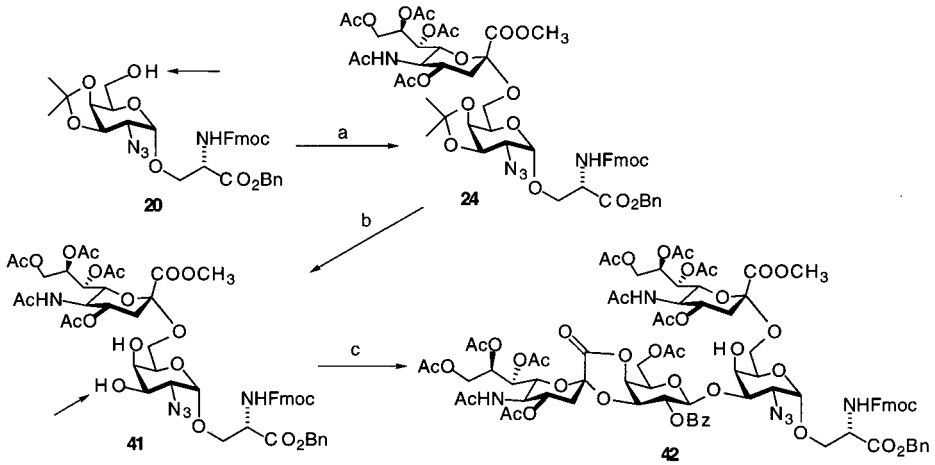
(29) (a) Seeberger, P. H.; Eckhardt, M.; Gutteridge, C. E.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 10064. (b) Garegg, P. J.; Hentrichson, C.; Norberg, T. *Carbohydr. Res.* **1983**, *116*, 162. Reviews on thioglycosides: (c) Fujed, P.; Garegg, P. J.; Lonn, H.; Norberg, T. *Glyconconjugate J.* **1987**, *4*, 97. (d) Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503.

(30) Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313.

(31) Nakahara, Y.; Nakahara, Y.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1997**, *38*, 7211.

(32) (a) Mootoo, D. R.; Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988**, *110*, 5583. (b) Halcomb, R. L.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 6661. (c) Veeneman, G. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 275. (d) Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331.

Scheme 9



Reagents: (a) **23** (X= Cl), AgOTf, $CaSO_4$, DTBP, THF, -78°C; (b) AcOH, 12 hr; (c) **35**, NIS, TfOH, CH_2Cl_2, 4Å mol sieves, 10 min, 53%

carbamate removal (morpholine), and capping of the free amine was achieved by acetylation. Subsequent hydrogenolysis of the benzyl ester, hydrolysis of the acetates with sodium hydroxide in aqueous methanol, and removal of the 2′-*O*-benzoate (NaOMe, MeOH, 65 °C) provided *N*-acetyl-2,3-STF **40**. While the yield of the global deprotection sequence was only 44% overall, some of the loss could be accounted for via β-elimination of the amino acid under the harsh basic conditions.[33] Clustering of the antigen and immunological evaluation can be realized in an analogous fashion as for the 2,6-STF and STn glycopeptides and will be reported in due course.

5. Synthesis of a Glycophorin Glycosyl Threonine Precursor via a Modified [2 + 2] Cassette Approach. Having successfully accessed the other members of the ST antigen family, we directed our attentions toward an even more complicated venture. The glycophorin antigen is encountered on a major glycoprotein found on the human erythrocyte membranes.[34] To date, only Ogawa has reported a total synthesis of an *O*-linked glycophorin. However, the Ogawa routes were plagued by poor selectivity in the amino acid glycosylations,[35] or by recourse to a series of mono-glycosylations, which required significant manipulating of protecting groups.[36] For application of our methodology, a properly *O*-linked disaccharide acceptor would be required. For this purpose we required an undifferentiated acceptor such as **41**. In this undertaking, in an overall sense (see arrows) we would be using cassette **20** with two different donors in a properly timed way. Accordingly, sialylation of **20** with sialic acid donor **23** afforded **24**, followed by acetonide cleavage using AcOH to yield compound **41** in 48% yield for the two steps. We hoped that the glycosylation (i.e. sialylgalactosylation) would occur at C3 of the galactose segment of **41** (see arrow) which is equatorial. In the event, coupling of **41**, prepared as shown, with **35** using the Fraser-Reid conditions[30,32] afforded the protected *O*-linked tetrasaccharide **42** in 53% yield.

5. Synthesis of a Le^y Hexasaccharide Glycosyl Serine Precursor via a Modified [5 + 1] Cassette Approach. As a further extension of this new methodology, we examined an even more ambitious "disarmed" glycosylation[30] process related to an ongoing Le^y project in our laboratory.[37] In this regard, we were interested in producing α-*O*-linked Le^y structures. One such construct was hexasaccharide **45**, which could be obtained in a [5 + 1] cassette coupling with a pentasaccharide donor. In the event, thio-donor **43** and cassette **44** were coupled via NIS/TfOH in an exemplary 79% yield. *It will not go unnoticed that* ***45*** *constitutes the basic substructure by which a Lewis Y blood group determinant can be presented in the context of an advanced cell-surface mimic.* We are currently exploiting this remarkable cassette coupling method.

Summary

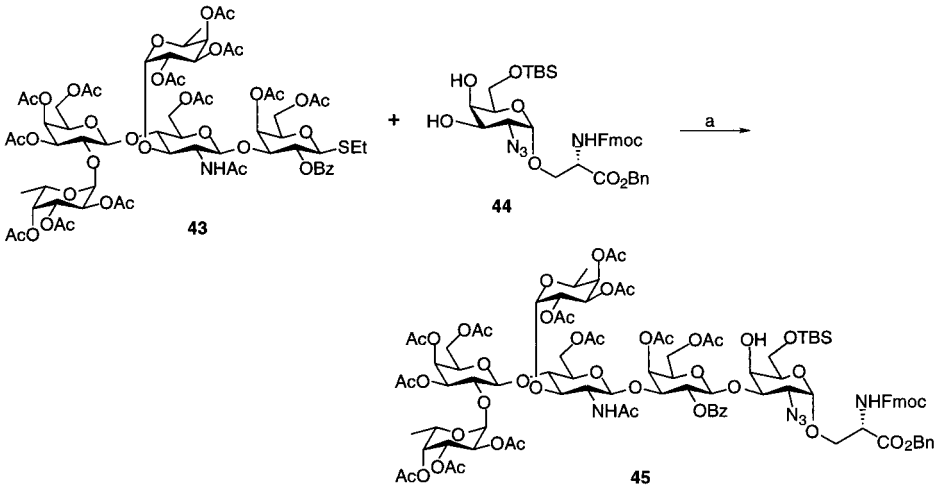
In conclusion, we have successfully prepared the 2,3-STF, STn, 2,6-STF, and glycophorin antigens utilizing both the classical and cassette approaches. Clustered forms of these antigens are now being studied for their immunological profiles. Also, the synthesis of an α-*O*-linked serine glycoside of an entire Lewis blood group (Y) antigen has been accomplished via the cassette methodology. While complexities will undoubtedly be encountered on a case-to-case basis, we believe that the results shown here constitute validation and broad demonstration that the required chemistry can be achieved in the general case. Increasingly sophisticated and, we hope, increasingly realistic cell-surface molecular mimics can be now assembled and evaluated both as regards to spectroscopy and immune recognition.

Experimental Section

Glycal 6. Galactal **5** (1.96 g, 9.89 mmol) was dissolved in 100 mL of anhydrous CH_2Cl_2 and cooled to 0 °C. DMDO (0.06 M solution in acetone, 200 mL, 12 mmol) was added via cannula to the reaction flask. After 1 h the starting material was consumed as judged by TLC. The solvent was removed with a stream of N_2, and the crude epoxide was dried in vacuo for 1 h at ambient temperature. The crude residue was dissolved up in 33 mL of THF, and 6-*O*-TIPS galactal (2.50 g, 8.24 mmol) in 20 mL THF was added. The resulting mixture was cooled to −78 °C, and $ZnCl_2$ (1.0 M solution in ether, 9.80 mL, 9.80 mmol) was added dropwise. The reaction was allowed to warm slowly to ambient temperature and stirred 12 h. The mixture was diluted with EtOAc and washed with $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography

(33) Meldal, M.; Bielfeldt, T.; Peters, S.; Jensen, K. J.; H. Paulsen,; Bock, K. *Int. J. Pept. Protein Res.* **1994**, *43*, 529.
(34) Thomas, D. B.; Winzler, R. J. *J. Biol. Chem.* **1969**, *244*, 5943.
(35) Iijima, H.; Ogawa, T. *Carbohydr. Res.* **1989**, *186*, 107.
(36) (a) Nakahara, Y.; Iijima, H.; Ogawa, T. *Tetrahedron Lett.* **1994**, *35*, 3321. (b) Nakahara, Y.; Iijima, H.; Ogawa, T. *Carbohydr. Lett.* **1994**, *1*, 99.
(37) Glunz, P. W.; Hintermann, S.; Lloyd, K. O.; Danishefsky, S. J. Unpublished results.

Scheme 10



(40 → 45 → 50 → 60% EtOAc in hexanes) to yield 3.36 g of product which was immediately acetylated. The material was dissolved in 50 mL of dry CH_2Cl_2, and triethylamine (19.2 mL) and DMAP (20 mg) were added. The solution was cooled to 0 °C at which time acetic anhydride (9.90 mL) was added dropwise. The reaction was stirred at ambient temperature 12 h. The solvent was removed in vacuo and the crude material purified by flash chromatography (50% EtOAc in hexanes) to give 3.30 g (75%) of glycal **6**: ^{1}H NMR ($CDCl_3$) δ 6.42 (d, J = 6.3 Hz, 1H, H-1, glycal), 4.35 (1/2 AB, dd, J = 11.5, 6.8 Hz, 1H, H-6′a), 4.28 (1/2 AB, dd, J = 11.5, 6.1 Hz, 1H, H-6′b). HRMS (EI) calcd for $C_{28}H_{44}O_{13}SiK$ (M + K): 655.2188; found: 655.2154.

Glycal 7. Glycal **6** (1.50 g, 2.43 mmol) was dissolved in 24 mL of THF and cooled to 0 °C. A mixture of TBAF (5.80 mL, 5.83 mmol) and acetic acid (0.34 mL, 5.83 mmol) was added to the substrate at 0 °C. The reaction was stirred at 30 °C for 5 h. The solution was partitioned between EtOAc and saturated $NaHCO_3$, and the phases separated. The organic phase was washed with saturated $NaHCO_3$, brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (80 → 85 → 90% EtOAc/ hexane) to yield 0.9 g (80%) of glycal **7**: ^{1}H NMR ($CDCl_3$) δ 6.38 (dd, J = 6.3, 1.8 Hz, 1H, H-1, glycal), 5.39 (m, 1H, H-4), 2.22 (s, 3H), 2.16 (s, 3H), 2.13 (s, 3H). HRMS (EI) calcd for $C_{19}H_{24}O_{13}K$ (M + K): 499.0854; found: 499.0885.

Glycal 9. A flame-dried flask was charged with sialyl phosphite donor **8** (69 mg, 0.11 mmol) and acceptor **7** (40 mg, 0.085 mmol) in the drybox (argon atmosphere). The mixture was dissolved in 0.6 mL of dry THF. Dry toluene (0.6 mL) was added, and the solution was slowly cooled to −60 °C to avoid precipitation. Trimethylsilyl triflate (2.4 μL) was added, and the mixture was stirred at −45 °C for 2 h. The reaction was quenched at −45 °C with 2 mL of saturated $NaHCO_3$, warmed until the aqueous phase melted, and poured into EtOAc. The organic phase was washed with sat. $NaHCO_3$, dried (Na_2SO_4), and concentrated. ^{1}H NMR analysis of the crude material revealed a 4:1 ratio of α:β isomers. The mixture was separated by flash chromatography on silica gel (2 → 2.5 → 3 → 3.5 → 4% MeOH in CH_2Cl_2) to yield 50 mg (63%) of glycal **9**: ^{1}H NMR ($CDCl_3$) δ 6.42 (d, J = 6.2 Hz, 1H), 5.37 (m, 1H), 5.32−5.29 (m, 4H), 5.26−5.24 (m, 1H), 5.12−5.10 (m, 2H), 4.98 (d, J = 3.5 Hz, 1H), 4.92−4.85 (m, 1H), 4.83−4.80 (m, 3H), 4.54 (m, 1H), 4.45 (dd, J = 3.0, 13.5 Hz, 1H), 4.33−4.20 (m, 3H), 4.22−4.02 (m, 7H), 3.96 (dd, J = 10.9, 7.6 Hz, 1H, H-2), 2.59 (dd, J = 12.9, 4.6 Hz, 1H, H-2eq NeuNAc), 2.30 (dd, J = 12.9, 7.2 Hz, 1H, H-2ax NeuNAc), 2.16 (s, 3H), 2.14 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.88 (s, 3H, C*H*$_3$CONH); FTIR (neat) 2959, 1816, 1745, 1684, 1662 cm^{-1}. HRMS (EI) calcd for $C_{39}H_{51}NO_{25}K$ (M + K): 972.2386; found: 972.2407.

Azidonitration Product 10. Glycal **9** (0.37 g, 0.40 mmol) was dissolved in 2.2 mL of dry acetonitrile, and the solution was cooled to −20 °C. Sodium azide (38.6 mg, 0.59 mmol) and ammonium cerium nitrate (0.65 g, 1.20 mmol) were added, and the mixture was vigorously stirred at −15 °C for 12 h. The heterogeneous mixture was diluted with ethyl acetate, washed twice with ice cold water, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography to provide 0.25 g (60%) of α:β−anomeric mixture **10**: ^{1}H NMR ($CDCl_3$) δ 6.35 (d, J = 4.2 Hz, 1H, H-1, α-nitrate), 3.79 (s, 3H, methyl ester), 3.41 (dd, J = 11.0, 4.7 Hz, 1H, H-2), 2.54 (dd, J = 4.6, 12.8, H-2eq NeuNAc); ^{13}C NMR ($CDCl_3$, selected characteristic peaks) 170.99, 170.82, 170.30, 170.22, 169.81, 168.58 (acetates), 100.36, 98.34, 97.33, 94.76 (anomeric centers), 72.23, 71.77, 69.29, 68.91, 68.46, 67.70, 67.22, 62.53, 62.50, 57.53, 52.95, 21.06, 20.93, 20.83, 20.78; FTIR (neat): 2117, 1734 cm^{-1}. MS (EI) calcd: 1037.8; found 1038.4 (M + H). The azidonitrate mixture was of limited stability which precluded HRMS analysis.

α-Bromide 11. To a solution of the azidonitrates **10** (0.15 g, 0.15 mmol) in 0.6 mL of dry acetonitrile was added lithium bromide (62.7 mg, 0.73 mmol) and stirred at ambient temperature for 3 h in the dark. The heterogeneous mixture was diluted with dichloromethane, and the solution was washed twice with water, dried ($MgSO_4$), and concentrated (without heating). The residue was purified by flash chromatography (5% MeOH in CH_2Cl_2) to afford 0.12 g (75%) of α-bromide **12** which was stored under an argon atmosphere at −80 °C: ^{1}H NMR ($CDCl_3$) δ 6.54 (d, J = 3.7 Hz, 1H, H-1), 3.40 (dd, J = 10.8, 4.5 Hz, 1H, H-2), 2.57 (dd, J = 12.9, 4.5 Hz, 1H, H-2eq NeuNAc), 2.20 (s, 3H), 2.15 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.87 (s, 3H, C*H*$_3$CONH); MS (EI) calcd for $C_{39}H_{51}N_4O_{25}Br$: 1055.7; found: 1057.4 (M + H).

Trichloroacetimidates 12. Azidonitrate **10** (0.60 g, 0.58 mmol) was dissolved in 3.6 mL of acetonitrile, and the resultant solution was treated with thiophenol (0.18 mL) and diisopropylethylamine (0.10 mL). After 10 min, the solvent was removed with a stream of nitrogen. The crude material was purified by flash chromatography (2 → 2.5 → 3 → 3.5% MeOH in CH_2Cl_2) to provide 0.47 g (82%) of intermediate hemiacetal as a 1:1 mixture of α:β-anomers: ^{1}H NMR ($CDCl_3$, selected peaks) δ 5.57 (d, J = 2.9 Hz, 1H, 1α), 5.30−5.35 (m, 4H), 5.21 (d, J = 8.6 Hz, 1H, 1β), 5.11 (m, 4H), 5.04 (dd, J = 3.7, 0.5 Hz, 1H), 4.82−4.91 (m, 4H), 4.79 (dd, J = 8.4, 3.2 Hz, 2H), 4.56 (dd, J = 7.6, 5.9 Hz, 1H), 3.79 (s, 3H), 3.47 (dd, J = 10.4, 4.2 Hz, 1H), 3.34 (dd, J = 10.7, 7.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, selected peaks) δ 100.45, 100.31, 98.98, 98.30, 96.62, 92.19, 23.17, 21.00, 20.85, 20.71; FTIR (neat): 2112, 1747 cm^{-1}. HRMS (EI) calcd for $C_{39}H_{52}N_4O_{26}$ (M + K): 1031.2507; found: 1031.2539. To this hemiacetal (60 mg, 0.06 mmol) in 2.0 mL of CH_2Cl_2 were added trichloroacetonitrile (60 μL) and potassium carbonate (60 mg). After 6 h the mixture was diluted with CH_2Cl_2, filtered of excess K_2CO_3, and concentrated. The residue was purified by flash chromatography (5% MeOH in CH_2Cl_2) to provide 53.2 mg (64%, two steps) of **12** as a 1:1 mixture of α:β-anomers: ^{1}H NMR ($CDCl_3$) δ 8.76 (d, J = 5.1 Hz, 1H), 6.50 (d, J = 3.1 Hz, 1H, α-anomer), 5.65 (d, J = 8.4 Hz, 1H, β-anomer). LRMS (ES) calcd for $C_{41}H_{52}N_5O_{26}Cl_3Na$ (M + Na): 1158.1; found: 1158.2. The low stability

of **12** precluded further characterization. It is recommended to use **12** immediately in the following step.

Compound 14. A flame-dried flask was charged with donor **11** (50 mg, 0.04 mmol), 80 mg of 4 Å mol sieves, and *N*-Fmoc-L-serine benzyl ester (27.5 mg, 0.07 mmol) in the drybox. THF (0.6 mL) was added to the flask, and the mixture was cooled to −30 °C. Boron trifluoride diethyl etherate (2.8 μL, 0.022 mmol) was added, and the reaction was allowed to warm to −10 °C over 3 h. The mixture was diluted with EtOAc and washed with sat. $NaHCO_3$ while still cold. The organic phase was dried (Na_2SO_4) and concentrated, and the residue was purified by flash chromatography (2 → 2.5 → 3% MeOH in CH_2Cl_2) to provide 40 mg (66%) of **14** as a 4:1 mixture of α: β-isomers. The pure α-anomer was separated by flash chromatography (80 → 85 → 90 → 100% EtOAc:hexanes). **14**α: $[\alpha]^{23}_D$ +34.3 (*c* 0.44, $CDCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 2H), 7.62 (br d, 2H), 7.30−7.48 (m, 9H), 6.09 (br d, J = 7.9 Hz, 1H), 5.70 (d, J = 8.8 Hz, 1H), 5.34−5.39 (m, 2H), 5.30 (m, 1H), 5.20−5.27 (m, 1H), 5.17 (br t, J = 9.9 Hz, 2H), 4.79−4.95 (m, 5H), 4.80 (dd, J = 3.1 Hz, 1H), 4.60 (br s, 1H), 4.38−4.48 (m, 4H), 4.22−4.31 (m, 3H), 3.99−4.11 (m, 5H), 3.82−3.95 (m, 3H), 3.75 (s, 3H), 3.33 (dd, J = 10.2, 4.4 Hz, 1H), 2.55 (dd, J = 12.8, 4.5 Hz, 1H, H_{2eq} of NeuNAc), 2.11 (s, 3H), 2.10 (s, 3H), 2.08 (s, 6H), 2.07 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.99 (s, 3H), 1.93 (app t, J = 12.5 Hz, 1H, H_{2ax} of NeuNAc); ^{13}C NMR ($CDCl_3$) δ 170.92, 170.73, 170.60, 170.22, 170.19, 170.11, 169.81, 168.96, 167.81, 152.68, 143.79, 141.34, 134.90, 128.82, 128.70, 128.41, 127.85, 127.15, 125.12, 120.06, 100.43, 99.08, 98.66, 75.80, 73.04, 72.69, 72.25, 69.23, 69.09, 68.73, 68.49, 67.59, 67.40, 67.24, 63.78, 62.48, 62.24, 54.71, 52.91, 49.35, 48.87, 47.18, 37.64, 23.30, 23.22, 21.00, 20.84, 20.79, 20.76; IR (neat): 1746, 1673 cm^{-1}. HRMS: Calcd for $C_{66}H_{77}N_3O_{31}K$: 1446.4178; found: 1446.4140.

Compound 15. A flame-dried flask was charged with silver perchlorate (27.3 mg, 0.14 mmol), 0.12 g of 4 Å mol sieves, and *N*-Fmoc-L-threonine benzyl ester (37.3 mg, 0.086 mmol) in the drybox. Dry CH_2Cl_2 (0.72 mL) was added to the flask, and the mixture was stirred at ambient temperature for 10 min. Bromide **11** (76 mg, 0.072 mmol) in 0.46 mL of CH_2Cl_2 was added slowly over 40 min. The reaction was stirred under argon atmosphere at ambient temperature for 2 h. The mixture was then diluted with CH_2Cl_2 and filtered through Celite. The precipitate was thoroughly washed with CH_2Cl_2, the filtrate was evaporated, and the crude material was purified by flash chromatography (1 → 1.5 → 2 → 2.5% MeOH in CH_2Cl_2) to provide 74 mg (74%) of **15**. The undesired β-anomer was not detected by ^{1}H NMR and HPLC analysis of the crude material. **15**: $[\alpha]^{23}_D$ +26.8 (*c* 0.45, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 2H), 7.63 (d, J = 7.2 Hz, 2H), 7.40−7.25 (m, 8H), 5.72 (d, J = 9.2 Hz, 1H), 5.46 (s, 1H), 5.33 (m, 1H), 5.29 (d, J = 8.2 Hz, 1H), 5.23 (s, 2H), 5.11−5.04 (m, 3H), 4.87−4.71 (m, 4H), 4.4−4.39 (m, 3H), 4.33−4.25 (m, 4H), 4.09−3.97 (m, 6H), 3.79 (s, 3H), 3.66 (dd, J = 10.6, 3.7 Hz, 1H, H-3), 3.38 (dd, J = 10.7, 3.0 Hz, 1H, H-2), 2.52 (dd, J = 12.7, 4.3 Hz, 1H, H-2eq NeuNAc), 2.20 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.87 (s, 3H, C*H*3CONH), 1.35 (d, J = 6.2 Hz, Thr-CH_3); FTIR (neat) 2110, 1749; HRMS (EI) calcd for $C_{65}H_{75}N_5O_{30}K$ (M + K): 1444.4130; found: 1444.4155.

Protected 2,6-STF Threonine Acid 17. Azide **15** (47 mg, 33.4 μmol) was treated with thiolacetic acid (3 mL, distilled three times) for 27 h at ambient temperature. Excess thiolacetic acid was removed with a stream of nitrogen, followed by toluene azeotrope (4 × 5 mL). The crude product was purified by flash chromatography (1.5 → 2 → 2.5 → 3 → 3.5% MeOH in CH_2Cl_2) to yield 37 mg (78%) of reduced product which was immediately dissolved in 7.6 mL of methanol and 0.5 mL of water. After purging the system with argon, 6.5 mg of palladium catalyst (10% Pd−C) was added and the system placed under 1 atm of H_2. After 8 h the H_2 was removed by an argon flow, the catalyst was removed by filtration, and the mixture was concentrated. Flash chromatography of the residue (10% MeOH in CH_2Cl_2) provided 36 mg (78%) of acid 17: $[\alpha]^{23}_D$ +34.7 (*c* 1.75, CDCl3); ^{1}H NMR ($CDCl_3$) mixture of rotamers, selected characteristic peaks: δ 3.80 (s, 3H, methyl ester), 3.41 (m, 1H, H-2), 2.53 (m, 1H, H-2e NeuNAc)), 1.45 (d, J = 5.1 Hz, Thr-CH_3), 1.35 (d, J = 5.8 Hz, Thr-CH_3); FTIR (neat) 1818, 1747; HRMS (EI) calcd for $C_{60}H_{73}N_3O_{31}K$ (M + K): 1370.3870; found: 1370.3911.

Protected 2,6-STF Serine Acid 16. The compound **16** was prepared in 80% yield from **14** following the same procedure for the preparation of **17**: $[\alpha]^{23}_D$ +40.0 (*c* 1.75, $CDCl_3$); ^{1}H NMR ($CDCl_3$) mixture of rotamers, selected characteristic peaks: δ 3.36 (br s), 3.28 (br s), 2.52 (br d), 2.48 (br d); ^{13}C NMR ($CDCl_3$) selected peaks: δ 98.90, 98.60, 77.04, 76.79, 72.61, 69.10, 68.56, 68.43, 67.56, 62.68, 62.42, 53.09, 49.33, 47.16, 23.18, 21.02, 20.84, 20.75, 20.66, 20.22; FTIR (KBr pellet): 3362, 1750 cm^{-1}. HRMS (EI) calcd for $C_{59}H_{71}N_3O_{31}K$ (M + K): 1356.3708; found: 1356.3820.

General Procedure for Peptide Coupling. Glycosyl amino acid **16** or **17** (1 equiv) and the peptide with a free amino group (1.2 equiv) were dissolved in CH_2Cl_2 (22 mL/1 mmol). The solution was cooled to 0 °C, and IIDQ (1.15−1.3 equiv) was added (1 mg in ca. 0.02 mL of CH_2Cl_2). The reaction was then stirred at ambient temperature for 8 h. The mixture was directly loaded onto a silica gel column for purification by flash chromatography.

General Procedure for *N*-Fmoc Deprotection. A substrate (1 mmol in 36 mL of DMF) was dissolved in anhydrous DMF followed by addition of KF (10 equiv) and 18-crown-6 ether (catalytic amount). The mixture was then stirred for 48 h at ambient temperature. Evaporation of DMF in vacuo was followed by purification by flash chromatography on silica gel.

Protected Glycopeptide 18. ^{1}H NMR ($CDCl_3$) δ 3.45−3.30 (m, 3 × 1H, H-2), 3.74 (s, 3H, methyl ester), 2.58−2.49 (m, 3 × 1H, H-2eq NeuNAc); FTIR (neat) 2962, 1819, 1747, 1664; MS (EI) calcd: 3760; found: 1903.8/doubly charged = 3806 (M + 2Na).

Deprotected Glycopeptide 19. The benzyl ester was hydrogenolyzed according to the procedure set forth for compound **17**. The acid was dissolved in methanol to yield a 5 μM solution. 1 M NaOH (aq) was added dropwise until the pH reached 10−10.5, and the mixture was stirred for 12 h at ambient temperature. Amberlyst-18 was added to bring the pH to ca. 4. The resin was filtered off and the mixture concentrated. The crude product was purified by reverse phase flash chromatography (LiChroprep RP-18, H_2O eluant) to provide fully deprotected **19** in 80% yield (two steps): ^{1}H NMR (D_2O) δ 4.73 (m, 2H, 2 × H-1), 4.70 (d, 1H, H-1), 4.64 (m, 3H, 3 × H-1′), 4.26−4.20 (m, 5H), 4.12−4.00 (m, 7H), 3.95−3.82 (m, 7H), 3.77−3.27 (m, 51H), 2.55−2.51 (m, 3H, 3 × H-2eq NeuNAc), 1.84−1.82 (m, 21H, CH_3-CONH), 1.52−1.45 (m, 3H, H-2ax NeuNAc), 1.20 (d, J = 7.2 Hz, 3H), 1.18 (d, J = 6.6 Hz, 3H), 1.12 (d, J = 6.2 Hz, 3H), 0.71 (d, J = 6.6 Hz, 6H, Val); ^{13}C NMR (D_2O) anomeric carbons: δ 105.06, 105.01, 100.60, 100.57, 100.53, 100.11, 99.52, 98.70; MS (FAB) $C_{96}H_{157}N_{11}O_{64}$ 2489 (M + H); MS(MALDI) 2497.

Compound 25. To a suspension of 6-acceptor **21** (R = Me, 0.41 g, 0.62 mmol), sialyl phosphite **22** (X = $OP(OBn)_2$, 0.42 g, 0.57 mmol), and 0.2 g 4 Å mol sieves in 15 mL of dry THF at −45 °C was added trimethylsilyl trifluoromethanesulfonate (23 μL, 0.11 mmol) dropwise over 2 min. The mixture was stirred at −45 °C for 12 h at which time an additional 23 μL of TMSOTf was added dropwise. The mixture was stirred 6 h at −45 °C, and then filtered through a pad of Celite and concentrated. Flash chromatography of the residue (1:1 hexanes: EtOAc → EtOAc) yielded a mixture of α- and β-anomers, which was subjected to further chromatography (4:1 EtOAc:hexanes) to afford 0.26 g (37%) of pure α anomer **25** (R = Me) as a colorless foam. $[\alpha]^{23}_D$ +32.6 (*c* 1.18, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.73 (d, J = 7.5 Hz, 2H), 7.58 (d, J = 7.5 Hz, 2H), 7.20−7.37 (m, 10H), 5.65 (d, J = 9.5 Hz, 1H), 5.41 (d, J = 9.3 Hz, 1H), 5.36 (m, 1H), 5.30 (d, J = 7.5 Hz, 1H), 5.18 (dd, J = 12.1, 5.8 Hz, 2H), 4.85 (m, 1H), 4.76 (d, J = 3.6 Hz, 1H), 4.41 (m, 3H), 4.32 (dd, J = 12.4, 2.6 Hz, 1H), 4.27 (m, 2H), 4.20 (app t, J = 7.3 Hz, 1H), 4.04−4.12 (m, 5H), 3.87 (m, 1H), 3.74 (s, 3H), 3.63 (m, 1H), 3.29 (dd, J = 7.6, 3.6 Hz, 1H), 2.56 (dd, J = 12.9, 4.7 Hz, 1H), 2.09 (s, 3H), 2.08 (s, 3H), 2.02 (m, 1H), 1.98 (s, 6H), 1.84 (s, 3H), 1.45 (s, 3H), 1.32 (d, J = 5.5 Hz, 3H), 1.31 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.81, 170.44, 170.14, 170.00, 169.94, 169.90, 156.63, 143.84, 143.57, 141.18, 141.15, 134.93, 128.56, 128.51, 128.47, 127.62, 127.59, 127.01, 126.97, 125.14, 125.06, 119.85, 109.82, 98.84, 98.59, 77.20, 72.97, 72.70, 72.37, 69.07, 68.98, 67.55, 67.48, 67.24, 67.17, 63.50, 62.26, 60.75, 58.86, 52.65, 49.22, 47.03, 37.35, 27.87, 25.97, 23.04, 20.95, 20.72, 20.68, 20.63, 18.56; IR (neat): 2986, 2109, 1745, 1668, 1666 cm^{-1}.

Protected STn Acid 26. $[\alpha]^{23}_D$ +36.7 (*c* 1.04, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.83 (d, $J = 7.5$ Hz, 2H), 7.70 (app t, $J = 6.7$ Hz, 2H), 7.41 (m, 2H), 7.32 (m, 2H), 5.41 (m, 2H), 5.33 (dd, $J = 9.2$, 2.0 Hz, 1H), 5.03 (dd, $J = 11.7$, 3.2 Hz, 1H), 4.93 (d, $J = 3.8$ Hz, 1H), 4.81 (m, 1H), 4.64 (dd, $J = 10.8$, 6.3 Hz, 1H), 4.46 (dd, $J = 10.7$, 5.9 Hz, 1H), 4.37 (m, 2H), 4.27 (m, 3H), 4.14 (m, 2H), 4.08 (dd, $J = 12.4$, 5.1 Hz, 1H), 3.97 (app t, $J = 10.5$ Hz, 1H), 3.87 (m, 1H), 3.82 (s, 3H), 3.32 (m, 1H), 2.60 (dd, $J = 12.7$, 4.6 Hz, 1H), 2.16 (s, 3H), 2.11 (s, 3H), 2.09 (s, 3H), 2.03 (m, 1H), 2.00 (s, 3H), 1.98 (s, 3H), 1.94 (s, 3H), 1.93 (s, 3H), 1.83 (s, 3H), 1.23 (d, $J = 6.4$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 175.61, 175.47, 175.34, 174.19, 174.08, 173.83, 173.63, 173.44, 173.30, 171.07, 160.91, 147.30, 147.00, 144.61, 144.58, 130.70, 130.65, 130.11, 130.05, 128.04, 127.87, 122.90, 122.83, 102.71, 101.75, 79.98, 75.16, 72.49, 71.90, 71.12, 70.89, 70.38, 69.38, 66.45, 65.33, 61.94, 55.29, 51.89, 40.74, 24.75, 24.56, 23.08, 22.78, 22.62, 22.57, 21.20; IR (neat): 3361, 2956, 1746, 1659 cm^{-1}. HRMS: Calcd for $C_{51}H_{63}N_3O_{24}$-Na: 1124.3699; found: 1124.3748.

Protected STn Cluster 27. $[\alpha]^{23}_D$ +42.0 (*c* 1.67, $CHCl_3$); ^1H NMR (CD_3OD) δ 7.83 (d, $J = 7.5$ Hz, 2H), 7.72 (app t, $J = 6.5$ Hz, 2H), 7.41 (app t, $J = 7.5$ Hz, 2H), 7.34 (app t, $J = 7.4$ Hz, 2H), 5.34 (m, 8H), 5.17 (m, 2H), 5.08 (m, 2H), 4.98 (d, $J = 3.6$ Hz, 1H), 4.90 (d, $J = 2.7$ Hz, 1H), 4.78 (m, 2H), 4.69 (br s, 1H), 4.58 (dd, $J = 10.7$, 6.6 Hz, 1H), 4.49 (dd, $J = 10.6$, 6.2 Hz, 1H), 4.42 (m, 3H), 4.27−4.35 (m, 8H), 4.18 (app t, $J = 5.9$ Hz, 1H), 4.09 (m, 6H), 3.96 (m, 2H), 3.88 (m, 2H), 3.81 (s, 3H), 3.71 (s, 3H), 3.08 (m, 3H), 2.59 (dd, $J = 12.7$, 4.6 Hz, 2H), 2.54 (dd, $J = 12.8$, 4.6 Hz, 1H), 2.17 (s, 3H), 2.16 (s, 3H), 2.11 (s, 9H), 2.09 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.00 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.98 (s, 6H), 1.97 (s, 3H), 1.97 (s, 3H), 1.94 (s, 3H), 1.93 (s, 3H), 1.92 (s, 3H), 1.83 (s, 3H), 1.82 (s, 6H), 1.45 (s, 9H), 1.37 (m, 6H), 1.32 (d, $J = 6.2$ Hz, 3H); ^{13}C NMR (CD_3OD) δ 173.78, 173.53, 173.25, 172.70, 172.40, 172.36, 172.31, 172.23, 172.06, 171.82, 171.79, 171.77, 171.54, 171.50, 169.24, 169.19, 169.14, 159.12, 158.56, 145.49, 145.22, 142.76, 128.91, 128.29, 126.32, 126.20, 121.11, 121.05, 101.24, 100.63, 100.01, 99.98, 99.95, 80.06, 79.41, 78.35, 73.34, 71.02, 70.71, 70.40, 69.24, 68.55, 67.79, 64.76, 63.44, 60.32, 58.53, 58.24, 53.48, 50.06, 38.95, 38.08, 30.91, 28.94, 23.55, 23.44, 23.39, 22.74, 21.30, 21.04, 20.87, 20.79, 19.87, 19.62; IR (neat): 2934, 2470, 1746, 1654 cm^{-1}.

Thioglycoside Donor 33. To a solution of lactone **30** (0.19 g, 0.26 mmol) in 8.5 mL of CH_2Cl_2 at 0 °C was added freshly prepared DMDO (0.06 M solution in acetone, 8.5 mL, 0.51 mmol). The solution was stirred for 1 h, and the solvent was evaporated with an N_2 flow. To the residue was added 3.0 mL of CH_2Cl_2, and the solution was cooled to −78 °C. Ethanethiol (0.38 mL, 5.11 mmol) was added, followed by trifluoroacetic anhydride (2 μL, 0.01 mmol). The mixture was allowed to warm slowly to ambient temperature over 6 h, and concentrated with an N_2 flow. Flash chromatography of the residue (4:1, EtOAc: hexanes) yielded 0.13 g (61%) of thioglycoside **33** as colorless crystals: $[\alpha]^{23}_D$ −22.8 (*c* 4.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.50 (ddd, $J = 7.6$, 2.0 Hz, 1H), 5.41 (d, $J = 10.2$ Hz, 1H), 5.23 (dd, $J = 8.2$, 1.6 Hz, 1H), 5.18 (ddd, $J = 6.2$, 2.2 Hz, 1H), 4.83 (d, $J = 4.0$ Hz, 1H), 4.36−4.31 (m, 2H), 4.23−4.08 (m, 3H), 4.00−3.88 (m, 2H), 3.72 (d, $J = 7.1$ Hz, 1H), 3.65 (dd, $J = 10.3$, 1.4 Hz, 1H), 3.51 (t, $J = 9.4$ Hz, 1H), 2.81−2.57 (m, 3H), 2.46 (dd, $J = 13.5$, 5.4 Hz, 1H), 2.14 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.01 (m, 1H), 2.01 (s, 3H), 2.00 (s, 3H), 1.87 (s, 3H), 1.30 (t, $J = 7.3$ Hz, 3H), 1.13 (bs, 21H); ^{13}C NMR ($CDCl_3$) δ 171.0, 170.6, 170.5, 170.4, 170.0, 169.8, 164.0, 95.0, 85.1, 78.6, 77.2, 74.5, 73.2, 69.7, 69.5, 68.7, 66.9, 62.7, 62.2, 49.1, 38.5, 24.3, 23.0, 20.8, 20.6, 20.5, 17.8, 15.1, 11.8; IR (film) 3364, 2942, 1749, 1667 cm^{-1}; HRMS: Calcd for $C_{36}H_{59}NO_{16}SSiNa$: 844.3222; found: 844.3227.

Benzoate Thioglycoside Donor 35. To a solution of thio-donor **33** (0.21 g, 0.26 mmol) in 4.0 mL of CH_2Cl_2 were added triethylamine (0.18 mL, 1.28 mmol), benzoyl chloride (0.15 mL, 1.28 mmol), and DMAP (0.31 g, 2.55 mmol), and the solution was stirred at ambient temperature for 16 h. The mixture was partitioned between EtOAc (50 mL) and sat. $NaHCO_3$ (50 mL), and the phases were separated. The organic phase was washed with brine (50 mL), dried (Na_2SO_4), and concentrated. To the crude benzoate **34** in 10 mL of THF at 0 °C were added acetic acid (0.89 mL, 15.6 mmol) and TBAF (1.0 M solution in THF, 3.90 mL, 3.90 mmol). The solution was allowed to warm slowly to ambient temperature and stirred for 16 h. The solution was partitioned between EtOAc (60 mL) and H_2O (60 mL), and the phases were separated. The organic phase was washed with sat. $NaHCO_3$ (60 mL) and brine (60 mL), dried (Na_2SO_4), and concentrated. To the crude alcohol was added triethylamine (0.72 mmol, 5.20 mmol), acetic anhydride (0.49 mL, 5.20 mmol), and DMAP (10 mg, 0.08 mmol), and the solution was stirred for 6 h at ambient temperature. The mixture was partitioned between EtOAc (50 mL) and sat. $NaHCO_3$ (50 mL), and the phases were separated. The organic phase was washed with brine (50 mL), dried (Na_2SO_4), and concentrated. Flash chromatography of the residue (4:1, EtOAc:hexanes) provided 0.15 g (71% from **33**) of thioethyl glycoside **35** as a pale yellow solid. $[\alpha]^{23}_D$ +1.56 (*c* 1.58, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.00 (d, $J = 7.2$ Hz, 2H), 7.58 (t, $J = 7.4$ Hz, 1H), 7.44 (t, $J = 7.6$ Hz, 2H), 5.50 (ddd, $J = 16.1$, 11.0, 5.4 Hz, 1H), 5.40 (m, 1H), 5.24 (m, 3H), 5.08 (d, $J = 3.6$ Hz, 1H), 4.63 (dd, $J = 12.0$, 3.1 Hz, 1H), 4.55 (d, $J = 10.0$ Hz, 1H), 4.34 (m, 3H), 4.10 (ABq, $J = 10.4$ Hz, 1H), 3.94 (m, 2H), 3.65 (d, $J = 10.5$ Hz, 1H), 2.68 (m, 2H), 2.48 (dd, $J = 13.8$, 5.4 Hz, 1H), 2.10 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), 1.85 (s, 3H), 1.77 (dd, $J = 13.7$, 11.7 Hz, 1H), 1.22 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 170.82, 170.65, 170.61, 170.39, 170.30, 169.90, 165.16, 163.14, 133.39, 129.77, 129.14, 128.39, 95.09, 82.87, 74.59, 72.71, 72.62, 69.23, 68.87, 68.42, 66.93, 62.79, 62.18, 48.99, 38.04, 24.07, 23.03, 20.77, 20.70, 20.66, 20.55, 14.84; IR (neat): 2966, 1746, 1676 cm^{-1}. HRMS: Calcd for $C_{36}H_{45}NO_{18}SNa$: 834.2255; found: 834.2269.

Compound 37. A mixture of thiodonor **35** (0.10 g, 0.12 mmol) and 3-acceptor **32** (0.18 g, 0.25 mmol) was azeotroped with dry benzene (4 × 5 mL), and the flask was backfilled with nitrogen and placed under high vacuum for 1 h. Molecular sieves (4 Å, 0.5 g), CH_2Cl_2 (5.0 mL), and NIS (69 mg, 0.31 mmol) were added, and the mixture was cooled to 0 °C. Triflic acid (freshly prepared 1% solution in CH_2Cl_2, 1.84 mL, 0.12 mmol) was added dropwise over 5 min. The mixture was warmed to ambient temperature immediately following addition, allowed to stir for 10 min, and then partitioned between EtOAc (50 mL) and sat. $NaHCO_3$ (50 mL). The phases were separated, and the aqueous phase was back-extracted with EtOAc (20 mL). The combined organic phases were washed with sat. $Na_2S_2O_3$ (50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated. Flash chromatography of the residue (4:1 EtOAc:hexanes) furnished 0.11 g (62%) of trisaccharide **37** as a colorless crystalline solid. $[\alpha]^{23}_D$ +29.6 (*c* 1.65, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.02 (d, $J = 7.3$ Hz, 2H), 7.77 (d, $J = 7.7$ Hz, 2H), 7.56 (m, 2H), 7.26−7.50 (m, 12H), 5.59 (d, $J = 9.5$ Hz, 1H), 5.51 (ddd, $J = 15.9$, 11.2, 5.5 Hz, 1H), 5.59 (d, $J = 9.5$ Hz, 1H), 5.21 (br s, 4H), 5.07 (m, 3H), 4.85 (d, $J = 8.0$ Hz, 1H), 4.66 (m, 2H), 4.19−4.48 (m, 10H), 4.13 (br s, 1H), 4.66 (m, 2H), 4.19−4.48 (m, 10H), 4.13 (br s, 1H), 4.09 (d, $J = 10.4$ Hz, 1H), 4.04 (m, 1H), 3.94 (m, 3H), 3.78 (m, 4H), 3.64 (d, $J = 10.4$ Hz, 1H), 3.45 (dd, $J = 10.5$, 3.9 Hz, 1H), 2.11 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.00 (s,3H), 1.99 (s, 3H), 1.86 (s, 3H), 1.78 (m, 1H), 1.29 (d, $J = 6.3$ Hz, 3H), 0.86 (s, 9H) 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 170.95, 170.66, 170.39, 169.95, 165.30, 163.02, 156.70, 143.92, 143.63, 141.24, 134.81, 133.41, 129.74, 129.11, 128.58, 128.54, 128.49, 128.36, 128.01, 127.71, 127.09, 127.02, 125.17, 125.11, 119.96, 100.80, 99.49, 95.16, 78.46, 76.17, 72.78, 72.14, 71.75, 71.54, 71.25, 70.92, 70.05, 69.18, 68.57, 68.33, 67.61, 67.33, 67.07, 63.05, 62.25, 62.21, 58.79, 58.70, 49.23, 47.11, 37.97, 25.83, 23.10, 20.82, 20.73, 20.71, 20.63, 20.55, 18.78, 18.28, 18.00, 17.88, 17.84, 11.89, −5.35, −5.50; IR (neat): 2953, 2931, 2111, 1744, 1689 cm^{-1}. HRMS: Calcd for $C_{72}H_{87}N_5O_{27}SiNa$: 1504.5255; found: 1504.5202.

Peracetylated 2,3-STF Trisaccharide 39. To trisaccharide **37** (70 mg, 0.05 mmol) in 1.0 mL of THF was added 1.0 mL of 1 N HCl. The solution was stirred for 30 min and then partitioned between EtOAc (30 mL) and sat $NaHCO_3$ (30 mL). The phases were separated, and the organic phase was washed with brine (30 mL), dried (Na_2SO_4), and concentrated. To the residue were added CH_2Cl_2 (2.0 mL), acetic anhydride (0.1 mL), triethylamine (0.1 mL), and DMAP (2 mg), and the solution was stirred at ambient temperature for 12 h. The mixture was partitioned between EtOAc (30 mL) and sat. $NaHCO_3$ (30 mL). The phases were separated, and the organic phase was washed with brine (30 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (4:1 hexanes:EtOAc) to afford 58 mg (85%) of peracetylated trisaccharide **39** as a colorless crystalline

solid. $[\alpha]^{23}_D$ +40.0 (*c* 0.90, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.02 (d, *J* = 7.4 Hz, 2H), 7.78 (d, *J* = 7.4 Hz, 2H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.37−7.53 (m, 6H), 7.29 (m, 6H), 5.61 (d, *J* = 9.4 Hz, 1H), 5.51 (m, 2H), 5.19 (m, 4H), 5.08 (d, *J* = 4.3 Hz, 1H), 5.00 (d, *J* = 3.5 Hz, 1H), 4.81 (d, *J* = 7.9 Hz, 1H), 4.75 (d, *J* = 3.8 Hz, 1H), 4.58 (dd, *J* = 11.9, 3.3 Hz, 1H), 4.47 (dd, *J* = 10.3, 7.1 Hz, 1H), 4.27−4.42 (m, 5H), 4.17−4.25 (m, 2H), 4.10 (m, 2H), 3.90−4.02 (m, 3H), 3.86 (dd, *J* = 11.8, 8.2 Hz, 1H), 3.63 (d, *J* = 10.5 Hz, 1H), 3.46 (dd, *J* = 10.6, 3.7 Hz, 1H), 2.49 (dd, *J* = 13.8, 5.5 Hz, 1H), 2.11 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 2.09 (s, 3H), 2.00 (s, 6H), 1.98 (s, 3H), 1.85 (s, 3H), 1.75 (app t, *J* = 13.7 Hz, 1H), 1.26 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 170.89, 170.65, 170.36, 169.99, 169.82, 169.50, 165.25, 162.92, 156.70, 143.83, 143.61, 141.27, 134.78, 133.40, 129.72, 129.19, 128.63, 128.59, 128.45, 128.37, 127.76, 127.09, 127.04, 125.11, 125.06, 120.01, 100.95, 99.22, 95.08, 75.29, 72.51, 72.26, 71.74, 71.19, 70.20, 69.54, 69.20, 68.21, 68.05, 67.67, 67.36, 66.78, 63.18, 62.38, 62.22, 59.34, 58.72, 49.28, 47.10, 38.03, 23.11, 20.82, 20.75, 20.71, 20.65, 20.56, 18.38; IR (neat): 3356, 2961, 2111, 1744 cm^{-1}. HRMS: Calcd for $C_{70}H_{77}N_5O_{29}$-Na: 1474.4602; found: 1474.4595.

Azide Reduction of 39. Trisaccharide **39** (77 mg, 0.053 mmol) was deprotected according to the procedure set forth for azide reduction of **17** with AcSH. Purification by flash chromatography (1:1 hexanes:EtOAc → EtOAc) afforded 44 mg (56%) of a colorless oil. $[\alpha]^{23}_D$ +43.2 (*c* 1.65, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.00 (d, *J* = 7.5 Hz, 2H), 7.79 (d, *J* = 7.5 Hz, 2H), 7.62 (m, 2H), 7.25−7.53 (m, 12 H), 5.65 (d, *J* = 9.4 Hz, 1H), 5.45 (m, 1H), 5.41 (br s, 2H), 5.27 (d, *J* = 10.3 Hz, 1H), 5.15 (m, 2H), 5.01 (d, *J* = 3.3 Hz, 1H), 4.90 (d, *J* = 11.9 Hz, 1H), 4.76 (d, *J* = 3.4 Hz, 1H), 4.72 (d, *J* = 7.7 Hz, 1H), 4.63 (m, 1H), 4.52 (m, 2H), 4.25−4.43 (m, 6H), 4.18 (m, 2H), 4.08 (m, 2H), 3.93 (m, 2H), 3.84 (dd, *J* = 11.5, 8.2 Hz, 1H), 3.64 (d, *J* = 10.5 Hz, 1H), 2.50 (dd, *J* = 13.5, 5.0 Hz, 1H), 2.11 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.01 (s, 3H), 1.99 (s, 6H), 1.95 (s, 3H), 1.81 (s, 3H), 1.73 (app t, *J* = 12.7 Hz, 1H), 1.23 (s, 3H), 1.22 (d, *J* = 5.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 171.13, 170.02, 170.66, 170.41, 170.37, 169.96, 169.77, 164.89, 162.95, 156.58, 143.72, 141.37, 141.32, 134.41, 133.62, 129.99, 129.81, 129.15, 128.83, 128.76, 128.55, 128.49, 127.80, 127.13, 127.08, 124.93, 124.87, 120.08, 99.85, 99.61, 95.11, 74.15, 72.53, 72.33, 71.57, 71.40, 70.25, 69.15, 68.74, 68.25, 67.95, 67.60, 66.94, 66.78, 63.25, 62.67, 62.21, 58.64, 49.28, 48.71, 47.20, 37.97, 29.65, 23.08, 22.64, 20.80, 20.74, 20.72, 20.65, 20.56, 18.25; IR (neat): 3333, 2927, 1745, 1670 cm^{-1}. HRMS: Calcd for $C_{72}H_{81}N_3O_{30}Na$: 1490.4802; found: 1490.4814. To this oil (29 mg, 0.020 mmol) was added morpholine (2 mL), and the mixture was stirred at ambient temperature 1 h. Excess morpholine was removed by azeotroping with toluene (3 × 5 mL). To the crude free amine in 2.0 mL of CH_2Cl_2 were added acetic anhydride (0.1 mL) and diisopropylethylamine (0.1 mL), and the solution was stirred at ambient temperature 1 h. The mixture was partitioned between EtOAc (20 mL) and saturated $NaHCO_3$ (20 mL). The phases were separated, and the organic phase was dried (Na_2SO_4) and concentrated. Flash chromatography of the residue (3 → 4 → 5 → 6 → 7 → 8% MeOH in CH_2Cl_2) provided 22.8 mg of a colorless solid. The product contained ca. 10% of a byproduct, presumably owing to some lactone opening by morpholine (supported by mass spectral analysis). The mixture was used in the next step without purification. Hydrogenolysis of the benzyl ester according to the procedure set forth for compound **17** was effected and the product used without purification. To the crude acid was added methanol (0.5 mL), 0.1 N aq NaOH (0.5 mL), and the mixture was stirred at ambient temperature 24 h. DOWEX-50H was added to lower the pH to ca. 4, the mixture was filtered through a cotton plug to remove the resin, and the solution was concentrated. To the residue was added methanol (2.0 mL), and then sodium methoxide (25 wt % in MeOH) dropwise until the pH reached 10−10.5 (ca. 5 drops). The solution was heated to reflux 16 h and cooled, and DOWEX-50H was added to lower the pH of the solution to ca. 4. The mixture was filtered through a cotton plug to remove the resin and concentrated. Purification of the residue by reverse-phase column chromatography (LiChroprep RP-18, H_2O eluant) provided 7.1 mg (44%) of *N*-acetyl-2,3-STF **40** as a colorless crystalline solid. $[\alpha]^{23}_D$ +56.1 (*c* 0.27, MeOH); ^1H NMR (D_2O) δ 4.95 (d, *J* = 3.8 Hz, 1H), 4.53 (d, *J* = 7.8 Hz, 1H), 4.36 (dd, *J* = 6.6, 2.6 Hz, 1H), 4.26 (m, 2H), 4.20 (br d, *J* = 2.6 Hz, 1H), 4.05 (m, 3H), 3.92 (d, *J* = 3.2 Hz, 1H), 3.86 (m, 4H), 3.72 (m, 4H), 3.62 (m, 4H), 3.51 (dd, *J* = 9.6, 8.0 Hz, 1H), 2.75 (dd, *J* = 12.4, 4.6 Hz, 1H), 2.12 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.78 (t, *J* = 12.2 Hz, 1H), 1.24 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (D_2O, external CS_2 reference) δ 176.14, 174.97, 174.61, 174.10, 173.98, 104.57, 99.63, 99.00, 77.23, 76.74, 75.62, 74.78, 72.78, 71.91, 70.95, 69.00, 68.80, 68.45, 68.12, 67.37, 62.53, 61.28, 61.02, 59.26, 51.66, 48.57, 39.77, 22.39, 22.05, 21.97, 18.26; HRMS: Calcd for $C_{31}H_{49}N_3O_{22}Na_3$: 884.2501; found: 884.2551.

Disaccharide Acceptor 41. 6-Acceptor **20** (190 mg, 0.29 mmol), $CaSO_4$ (400 mg), and AgOTf (125 mg, 0.58 mmol) were combined in a flask under argon, at which time 5.0 mL of THF and DTBP (0.15 mL, 0.58 mmol) were added. After stirring at ambient temperature for 30 min, the reaction was cooled to −78 °C, and chloride donor **23** (X = Cl, 0.30 g, 0.58 mmol) in 5.0 mL of THF was added dropwise over 1 h. The reaction was allowed to warm to −10 °C and held at this temperature 4 h and then filtered through Celite and concentrated. The crude product **24** was treated with 80% HOAc for 16 h and concentrated. The resultant oil was purified by silica gel chromatography (2 → 3 → 4% MeOH in CH_2Cl_2) to provide 0.15 g of **41** (48% from **20**) as a white foam: $[\alpha]^{23}_D$ +61.8 (*c* = 0.25, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.87 (s, 3H), 1.90 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.11 (s, 3H), 2.15 (s, 3H), 2.27 (dt, *J* = 13.0, 1.7 Hz, 1H), 2.53 (dd, *J* = 12.9, 4.6 Hz, 1H), 2.77 (d, *J* = 8.9 Hz, 1H), 3.29 (d, *J* = 4.4 Hz, 1H), 3.69−4.57 (m, 12H), 4.84−4.87 (m, 2H), 5.16 (d, *J* = 9.2 Hz, 1H), 5.02−5.34 (m, 6H), 5.39 (dt, *J* = 11.0, 5.4 Hz, 1H), 5.50 (d, *J* = 9.6 Hz, 1H), 5.88 (d, *J* = 8.2 Hz, 1H), 7.22−7.47 (m, 9H), 7.60 (d, 2H), 7.75 (d, 2H); ^{13}C NMR ($CDCl_3$) δ 171.1, 170.8, 170.5, 170.3, 170.1, 170.0, 169.6, 169.0, 168.0, 155.8, 143.7, 143.6, 141.1, 135.0, 128.5, 128.4, 127.6, 127.0, 125.1, 125.0, 119.0, 99.2, 98.6, 94.8, 72.7, 69.2, 69.0, 68.8, 68.1, 67.5, 63.3, 62.7, 62.5, 60.1, 54.4, 53.2, 53.0, 49.1, 46.9, 36.8, 36.0, 22.9, 20.9, 20.8, 20.7, 20.6; IR (film) 3357, 3067, 2956, 2110, 1745, 1664, cm^{-1}. FAB HRMS *m/e* calcd for (M + Na) $C_{51}H_{59}N_5O_{21}Na$ 1100.3600, found 1100.3589.

Tetrasaccharide 42. Sialyated acceptor **41** (58 mg, 0.054 mmol) and thioglycoside **35** (22 mg, 0.027 mmol) were azeotroped with benzene (3 × 5 mL). NIS (15.2 mg, 0.068 mmol), 0.1 g of 4 Å mol sieves, and 2.0 mL of CH_2Cl_2 were then added. A freshly prepared solution of triflic acid (1% solution in CH_2Cl_2, 0.24 mL) was then added dropwise. After 5 min, the reaction was judged complete by TLC and quenched with triethylamine. Flash chromatography (3 → 3.5 → 4 → 4.5 → 5% MeOH in CH_2Cl_2) afforded 26 mg (53%) of **42** as a white film: $[\alpha]^{23}_D$ +20.8 (*c* = 1.25, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.02 (d, *J* = 6.7 Hz, 2H), 7.77 (d, *J* = 6.7 Hz, 2H), 7.60 (t, *J* = 6.8 Hz, 2H), 7.53 (t, *J* = 7.2 Hz, 1H), 7.04−7.44 (m, 11H), 5.84 (d, *J* = 8.3 Hz, 1H), 5.51 (dt, *J* = 10.7, 5.4 Hz, 1H), 5.16−5.38 (m, 10H), 5.06 (bs, 1H), 4.85 (bm, 1H), 4.77 (d, *J* = 7.9 Hz, 1H), 4.75 (bs, 1H), 4.61 (bd, *J* = 8.3 Hz, 2H), 3.75−4.48 (m, 22H), 3.65 (d, *J* = 10.5 Hz, 1H), 3.55 (dd, *J* = 9.7, 5.8 Hz, 1H), 3.48 (dd, *J* = 10.4, 3.4 Hz, 1H), 2.61 (bs, 1H), 2.56 (dd, *J* = 12.8, 4.6 Hz, 1H), 2.51 (dd, *J* = 13.9, 5.5 Hz, 1H), 2.12 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.87 (s, 3H), 1.86 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 171.0, 170.9, 170.7, 170.6, 170.4, 170.3, 170.2, 170.0, 169.9, 169.8, 168.0, 165.3, 163.0, 155.8, 143.8, 143.7, 141.2, 135.0, 133.4, 129.7, 129.1, 128.6, 128.5, 128.4, 128.3, 127.8, 127.1, 125.2, 120.0, 100.8, 99.0, 98.7, 95.1, 72.8, 72.7, 72.2, 71.2, 69.4, 69.2, 69.0, 68.9, 68.8, 68.0, 67.7, 67.6, 67.2, 67.0, 66.3, 62.5, 62.0, 58.3, 54.4, 53.4, 52.8, 49.3, 47.1, 38.0, 37.5, 29.7, 23.1, 23.0, 21.0, 20.8, 20.7, 20.6, 20.5; IR (film) 3366, 3065, 2959, 2111, 1744, 1687, 1533, 1369, 1225 cm^{-1}. FAB HRMS *m/e* calcd for (M + Na) $C_{85}H_{98}N_6O_{39}Na$ 1849.5767, found 1849.5766.

Hexasaccharide 45. A mixture of thioglycoside **43** (70.8 mg, 0.05 mmol) and 3-acceptor **44** (68.3 mg, 0.1 mmol) was azeotroped with toluene (3 × 5 mL). To the mixture was added powdered 4 Å mol sieves (0.2 g), and 3.0 mL of CH_2Cl_2, and the mixture was stirred 30 min at ambient temperature. The mixture was cooled to 0 °C, at which time NIS (26.7 mg, 0.12 mmol) and freshly prepared triflic acid solution (1% solution in CH_2Cl_2, 0.42 mL, 0.05 mmol) were added. The red mixture was stirred at 0 °C for 15 min and then diluted with EtOAc. The organic phase was washed with sat. $NaHCO_3$, 10% $Na_2S_2O_3$ and brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (1:1 → 2:1 EtOAc:CH_2Cl_2) to afford 80.1 mg (79%) of **45** as a colorless solid. $[\alpha]^{23}_D$ −26.4 (*c* 1.00, $CHCl_3$); ^1H

NMR ($CDCl_3$) δ 8.10 (d, J = 7.4 Hz, 2H), 7.79 (d, J = 7.5 Hz, 2H), 7.59 (d, J = 7.0 Hz, 2H), 7.54 (t, J = 7.2 Hz, 1H), 7.43−7.24 (m, 12H), 5.86 (d, J = 8.5 Hz, 1H), 5.52−5.47 (m, 2H), 5.35−5.32 (m, 4H), 5.18−5.05 (m, 5H), 5.04−4.98 (m, 3H), 4.95−4.88 (m, 3H), 4.80 (d, J = 7.9 Hz, 1H), 4.72 (d, J = 3.3 Hz, 1H), 4.59−4.56 (m, 2H), 4.51 (dd, J = 11.7, 5.7 Hz, 1H), 4.43−4.37 (m, 2H), 4.33−4.23 (m, 2H), 4.21−4.07 (m, 6H), 4.03−3.84 (m, 5H), 3.80−3.73 (m, 4H), 3.44 (d, J = 10.3 Hz, 1H), 3.43 (d, J = 10.5 Hz, 1H), 3.21−3.13 (m, 1H), 2.83 (s, 1H), 2.21 (s, 3H), 2.18 (s, 3H), 2.16 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 1.99 (s, 6H), 1.27 (s, 3H), 1.14 (d, J = 5.6 Hz, 6H), 0.86 (s, 9H), 0.04 (s, 6H); ^{13}CNMR ($CDCl_3$) δ 171.37, 171.23, 171.10, 170.96, 170.91, 170.87, 170.85, 170.74, 170.54, 170.39, 170.17, 169.96, 169.92, 165.79, 156.31, 144.18, 141.69, 135.43, 134.09, 130.24, 129.51, 129.05, 129.01, 128.92, 128.84, 128.17, 127.50, 125.58, 125.54, 120.43, 102.39, 100.83, 100.69, 99.87, 96.62, 96.09, 78.11, 77.30, 74.25, 73.76, 73.52, 73.30, 72.96, 72.04, 71.81, 71.33, 71.26, 71.10, 71.03, 69.81, 69.38, 68.71, 68.61, 68.23, 68.10, 67.99, 67.95, 67.67, 67.29, 65.45, 64.36, 62.95, 62.20, 60.95, 58.84, 58.76, 54.87, 47.51, 26.25, 22.97, 21.47, 21.30, 21.26, 21.14, 21.08, 21.05, 20.99, 18.69, 16.28, 15.99, −4.98, −5.07; IR (neat): 2935, 2110, 1746 cm^{-1}. FAB HRMS *m*/*e* calcd for (M + Na) $C_{100}H_{127}N_5O_{45}SiNa$: 2168.7470; found 2168.7545.

Acknowledgment. This research was supported by the National Institutes of Health (Grant numbers: AI 16943 (S.J.D.), Sloan-Kettering Institute Core Grant (CA-08748)). Postdoctoral Fellowship Support is gratefully acknowledged by J.B.S. (NIH-F3218804), S.D.K. (US Army Breast Cancer Research Fellowship DAMD 17-98-1-8154), P.W.G. (American Cancer Society, PF98026), and D.S. (Irvington Institute for Immunological Research). We gratefully acknowledge Dr. George Sukenick (NMR Core Facility, Sloan-Kettering Institute) for NMR and mass spectral analyses.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **9**, **15**, **16**, **17**, **18**, **19**, **25**, **26**, **27**, **33**, **35**, **37**, **39**, **39b**, **40**, **41**, **42**, **45**; ^{1}H NMR spectra for compounds **11**, **12**, **14**; full chemical names for new compounds **25**, **27**, **33**, **39**, **40**, **41**, **42**, **45** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA9833265