Strategy in Oligosaccharide Synthesis: An Application to a Concise Total Synthesis of the KH-1(adenocarcinoma) Antigen

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Abstract: A concise and potentially practical synthesis of the title compound has been achieved. The route features a high degree of convergence and economy of synthetic operations. A key step is the concurrent introductory addition of three α-L-fucosyl residues at required hydroxyl acceptor sites (see **37** → **39**). Conjugation to carrier protein was achieved, and a route to include truncated structures for investigations for antibody specificity was accomplished.

Background

Many tumors are characterized by the appearance of large and unusual oligosaccharide subtypes.[1,2] These structures tend to emerge covalently bound to proteins in the form of cell surface glycoproteins. Alternatively, the anomalous carbohydrates can be encountered as glycolipids, adhering to cell surfaces through attractive molecular forces rather than via classical covalent bonds. The isolation, immunocharacterization, and structural identification of large carbohydrate-based tumor antigens constitutes a highly complex and challenging undertaking.

The possibility of exploiting these tumor-associated carbohydrate ensembles to provoke productive immune responses to the transformed state has occurred to glycobiologists, immunologists, and clinicians for some time.[3,4] Progress along these lines had to await breakthroughs in isolation, and purification techniques, as well as in the ability to assign connectivity and stereochemistry of carbohydrate domains through spectroscopic methods. Fortunately, major advances in these areas have provided a series of interesting structures (proposed with varying degrees of rigor) that might form the basis of strategies to achieve active immunity. However, a complicating feature, not easily overcome, is the difficulty associated with isolating small amounts of elaborate glycoconjugate structures from tissue collections of patients. Moreover, retrieval of the intact carbohydrate lattice by severing its covalent attachment to bioconjugating molecules (proteins or lipids) is only rarely possible.

These complexities pose large challenges as well as important opportunities for organic synthesis. There is, first, a great need for synthesizing the epitope structures themselves. However, superimposed on this goal is the challenge of delivering the tumor antigens in a favorable molecular framework for eliciting therapeutically useful, active immunity. The ultimate success of the chemistry end of the enterprise, then, is measured not only by the attainment of the total synthesis of the epitope sector, but also by the incorporation of this epitope into carrier protein. The total construct becomes the subject of immunological evaluation as part of a coherent vaccinology program.

The strategy and methodology which we have been employing for synthesizing the oligosaccharide epitopes are conveniently grouped under the term "glycal assembly".[5] The logic of the method has been amply reviewed. The employment of glycal assembly in the construction of epitope structures including the follow-up bioconjugation and mouse immunization studies have resulted in a vaccine (Globo-H)[6,7] which is currently undergoing advanced clinical evaluation. Two other fully synthetically derived vaccines are at the stage of advanced, preclinical processing.

In this paper, we deal with what is perhaps the most formidable carbohydrate-based tumor antigen thus far character-

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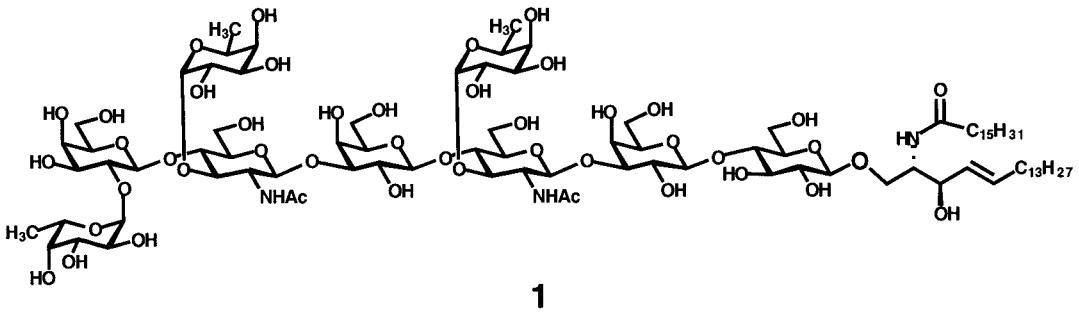

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Figure 1.

ized. The KH-1 antigen (**1**; see Figure 1)[8] was isolated from human colonic adenocarcinoma cells by using antibodies generated against the classical Le^y determinant. System **1**, in the form of glycolipid conjugates, was found to be present on the cell surface of all adenocarcinoma cells thus far studied. Furthermore, its presence has never been detected in normal colonic extracts.

Monoclonal antibodies[9a] were raised against this antigen and found to bind specifically to compound **1**. On the basis of these studies, Hakomori et al.[9b] postulated that the KH-1 antigen is a highly specific marker for malignancy and premalignancy involving colonic adenocarcinoma. Recently, an X-ray crystal structure of an antitumor antibody BR96 in complex with the nonanoate ester derivative of Le^y tetrasaccharide was reported by Jeffrey et al.[10] The view of the antibody−Le^y complex provided by this determination suggested that the BR96 antibody has unused binding capacity which might also recognize structures larger than the Le^y tetrasaccharide (such as the KH-1 antigen).

The difficulties associated with isolation and separation of complex carbohydrates from human colonic cancer tissue have been such that compound **1**, either as a glycolipid or as a protein conjugate, has not been available for evaluation. Thus, chemical synthesis could offer a viable alternative to produce workable quantities of complex systems such as the KH-1 antigen (**1**). Our interests were not limited to KH-1 (**1**), but included congeners[11] which would be bioconjugated to the appropriate protein carrier systems. Below, we describe (vide infra) the total synthesis of the KH-1 antigen **1**.[12] We also describe a protocol for reaching a suitable bioconjugatable analogue, as well as a truncated heptasaccharide congener. In addition, we also relate the upgrading of the KH-1 epitope for conjugation to carrier protein.

Synthetic Planning

In conducting this project, we also hoped to address the important issue of "strategy" in oligosaccharide synthesis. Of course, in this field, as opposed to "conventional" natural product synthesis, the basic building blocks are rather restricted and tend to bear obvious homology with the readily recognized components of the target system. Nonetheless, there are considerable opportunities for the realization of formats, which might lead to major synthetic economies. Thus, strategy in oligosaccharide synthesis tends to focus on programs for optimal conciseness in identifying specific centers for glycosylation, and for the attainment of stereocontrol in these couplings. *A cardinal strategic objective in oligosaccharide assembly is that of gaining maximum relief from blocking group manipulations*. From these perspectives, we came to favor a plan that would build a hexasaccharide (cf. structure **4**), so differentiated in terms of its protecting patterns as to allow for the simultaneous unveiling of the three free hydroxyls destined for fucosylation at a strategic point of our choosing. The three fucosylations would then be conducted concurrently.

Broadly speaking, our plan called for inclusion of three kinds of blocking groups in structure **4** (see Figure 2). The two nitrogen centers carry special R functions. The three oxygen centers to be fucosylated carry unique blocking groups (R*). The remaining hydroxyls are protected by a general blocking group (P). The three unique protecting groups would be cleaved in a single operation (**4** → **3**). Hopefully, the three immunologically defining α-L-fucose entities could be introduced in one concurrent synthetic operation (see structure **2**). The terminal glycal in **2** would be used to provide access to the native KH-1 antigen (**1**) or to bioconjugates, en route to evaluatable antiadenocarcinoma vaccines.

Considerable thought was also directed to assembling the hexasaccharide with minimal protection−deprotection maneuvers which would still be consistent with sound management of the complex network of hydroxyl groups. Toward this end, we found advantages in drawing from principles which have come forth from the logic of glycal assembly.[5] Thus, differentiated glycal types **6** and **7** (see Figure 3) were to be derived from D-glucal by exploiting reliable reactivity preferences of the C6, C3, and C4 hydroxyls (C6 > C3 > C4).[13] Moreover, the clean fashioning of an α-epoxide from appropriate galactal derivatives (cf. **5**) is well-known. Also well-known is the excellent β-galactosyl-donating capacity of properly chosen epoxides.[14]

Coupling of such a galactal-derived epoxide (**5**) to acceptors **6** and **7** would give rise to lactal-related disaccharides **8** and **9**, respectively. The C2′ hydroxyl of the lactal derivative **8** could be uniquely protected with R* in anticipation of future fucosylation at this site (see **10** bearing two unique R* blocking groups). In another arm of the effort, the same hydroxyl groups of **8** could be protected with a standard blocking (P) group to

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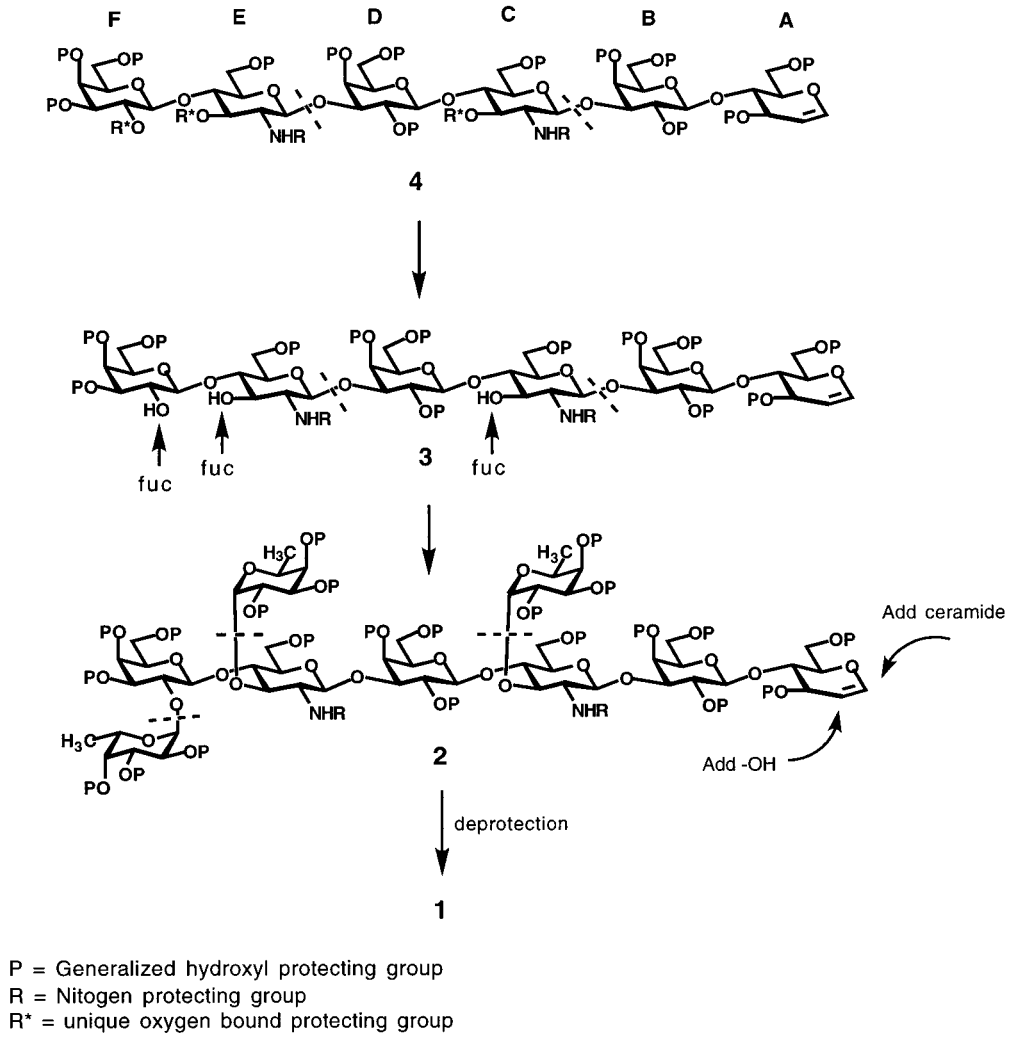

Figure 2.

afford **11**. Thus, segment **11** bears one unique blocking group (R*) anticipating fucosylation amidst more robust blocking groups (P). In some fashion (vide infra), lactal derivative **9** would be so arranged as to function as a glycosyl acceptor at C3′ (see system **12**).

Azaglycosylative coupling[6b,15] of **12** with **11** would provide tetrasaccharide **13**. In a manner to be discussed, the C3 hydroxyl of the remote galactose of **13** would be identified as an azaglycosyl acceptor site (see **14**). Coupling of **14** at this site with an azaglycosyl donor derived from **10** would afford **4**, thus feeding back in to the prospectus adumbrated in Figure 2.

Needless to say, considerable trial and error was to be necessary before translating this program to the realm of practice. An account of the manner in which implementation was accomplished in a highly concise fashion is described below.

Discussion and Results

The specific galactal derivative corresponding to formal structure **5** was the epoxide **17**. This compound was readily and stereospecifically obtained by action of dimethyldioxirane[16] on **16** (see Scheme 1). Compound **16** was synthesized through benzylation of the previously reported **15**.[17] As has been demonstrated many times,[5] the cyclic carbonate blocking group, engaging C3 and C4 of a 6-monoprotected galactal, has a very powerful α-directing effect in the epoxidation reaction, and a strong β-directing effect in the use of such an epoxide for galactosylation. These findings proved to be applicable to this synthesis (vide infra).

The specific glucals corresponding to formal structures **6** and **7** were **18** and **20**, respectively. The dibenzyl derivative **18** had been reported from our laboratory by controlled dibenzylation of D-glucal.[13b] The preparation of **20** involved a readily achieved mono-triethylsilylation of **19**. Compound **19** had been reported in the literature in very low yield by the monobenzylation of D-glucal at C6.[18] An improved but still unoptimized preparation of **19** was accomplished as part of our studies. With the required monosaccharide structures well in hand, glycal assembly could commence. Thus, coupling of **17** with **20** occurred under mediation by zinc chloride, affording **21** in 65% yield.

The lone hydroxyl group at C2′ of lactal product **21** was protected as its acetate (see compound **22**). In terms of our overall logic, this acetyl group serves as a general protecting

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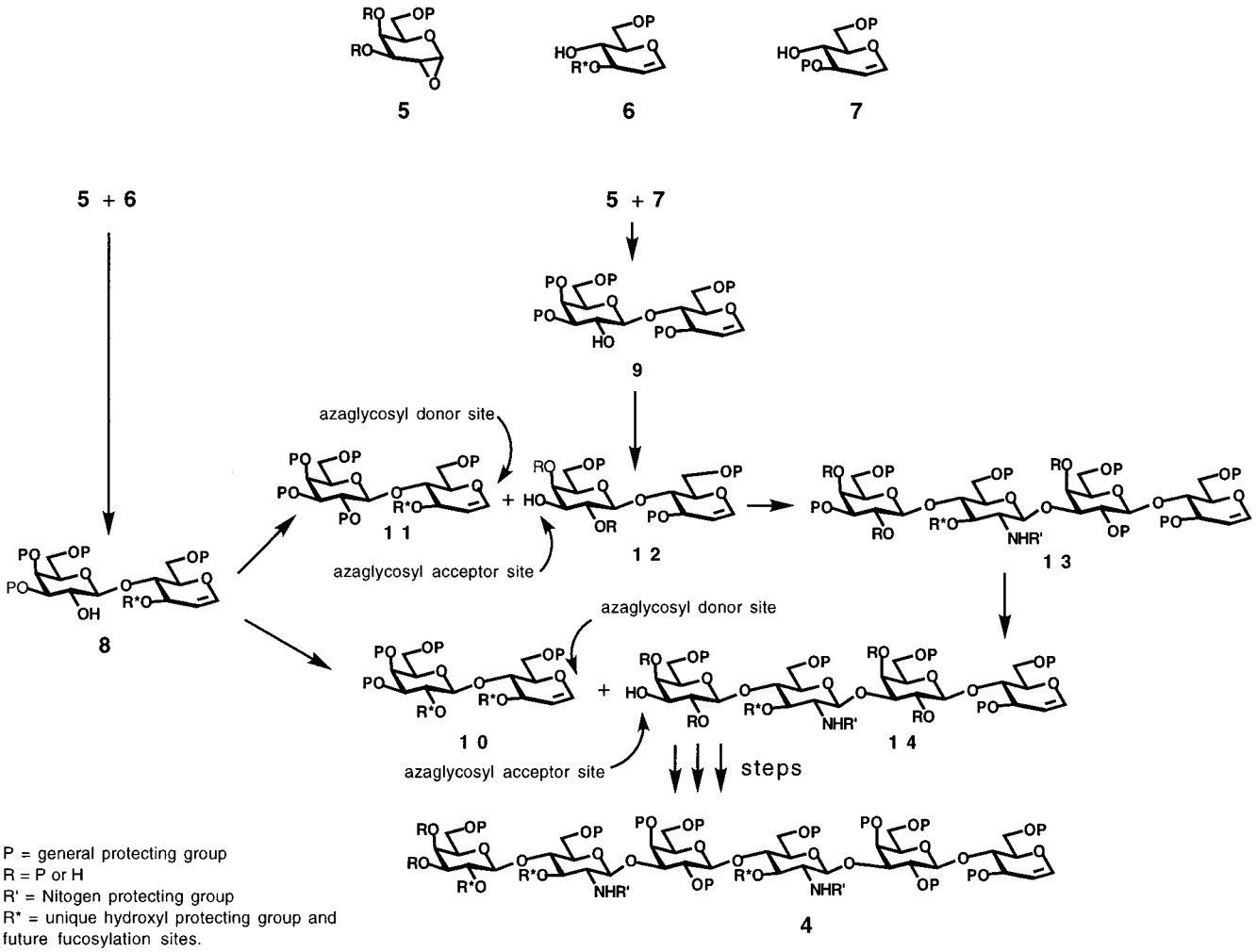

Figure 3.

group, P. In parallel fashion, and according to plan, the same C2′ hydroxyl in compound **21** could be converted as its triethylsilyl ether (see compound **23**). *It will thus be recognized that these simply derived lactal derivatives **22** and **23** already carried the three "unique" (triethylsilyl) functions at the sites anticipated for 3-fold fucosylation.*

Similarly, couplings of dibenzyl glucal acceptor **18** with galactosyl donor **17** afforded a 55% yield of **24**. With disaccharide building blocks **22**, **23**, and **24** in hand, the program for reaching a hexasaccharide system, generalized as **4**, was carried forward.

We first turned to the merger of systems derived from **22** and **24**. In the latter case, it would be necessary to identify a specific glycosylation acceptor site at C3′ of a substituted galactal related to **24**. It will be recalled (see generalized structure **12** in Figure 3) that we had deliberately not specified the status of the neighboring oxygens at C2′ and C4′ in the galactose segment of the lactal (for **12**, R = protecting group or R = H). At first glance, it would seem to be necessary to develop specific protection at C2′ and C4′ of this acceptor moiety in order to pinpoint C3′ as the azaglycosyl acceptor site, and we were certainly prepared to pursue possibilities along these lines. A more exciting alternative view of the problem presented itself. *Perhaps even if C2′, C3′, and C4′ were all unprotected hydroxyl groups, the effective glycosyl acceptor site would be at C3′.* Certainly, there was encouraging precedent in the field of sialic acid acceptor sites[19] to suggest that a nondifferentiated vicinal triol of this type might be reasonably selective or even specific for reaction at C3′. To investigate this scenario for conciseness, compound **24** was subjected to basic conditions which served to cleave the cyclic carbonate, generating the potential triol acceptor **25** (see Scheme 2). Addition of *N*-iodobenzenesulfonamide to compound **22**, under the usual conditions, gave rise to the iodosulfonamide derivative **26**. Rearrangement of the sulfonamido function with concurrent thiolation was accomplished through the action of lithium hexamethyldisilazane in the presence of ethanethiol on compound **26**. This treatment was followed by acetylation (to restore any C3′ alcohol to the acetate state), thereby affording compound **27**. Thus, we had in hand the two components necessary to produce a tetrasaccharide.

In the event, glycosylation did occur at primarily C3′. Union of **25** and **27**, under the agency of methyl triflate in the presence of di-*tert*-butylpyridine, afforded compound **28** as the major product (see Scheme 3). Although this route constituted an extremely concise synthesis of tetrasaccharide **28**, the yield of this coupling event was problematic. The maximum yield obtained was 55%. More typical yields were in the range of 35−45%. Closer examination of the highly polar region of the chromatogram revealed an isomeric tetrasaccharide from a structure we tentatively assign as **29** (isolated in a ratio of 3:1−

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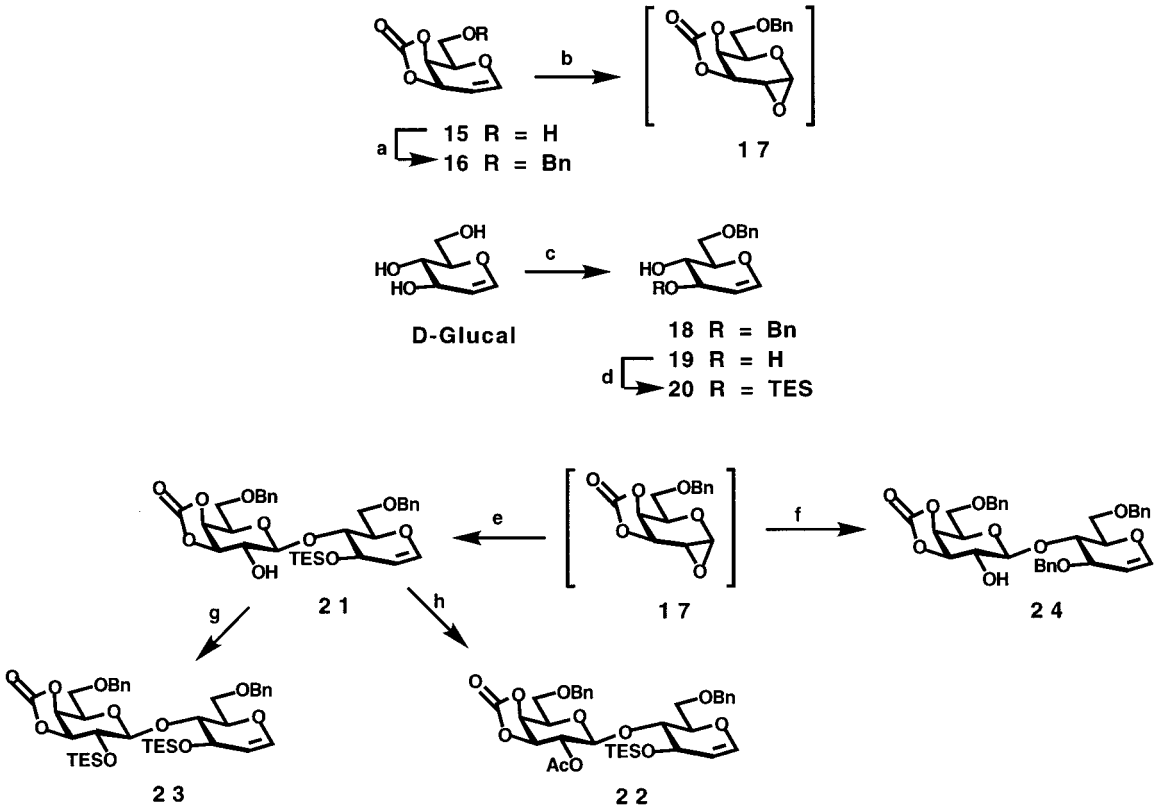
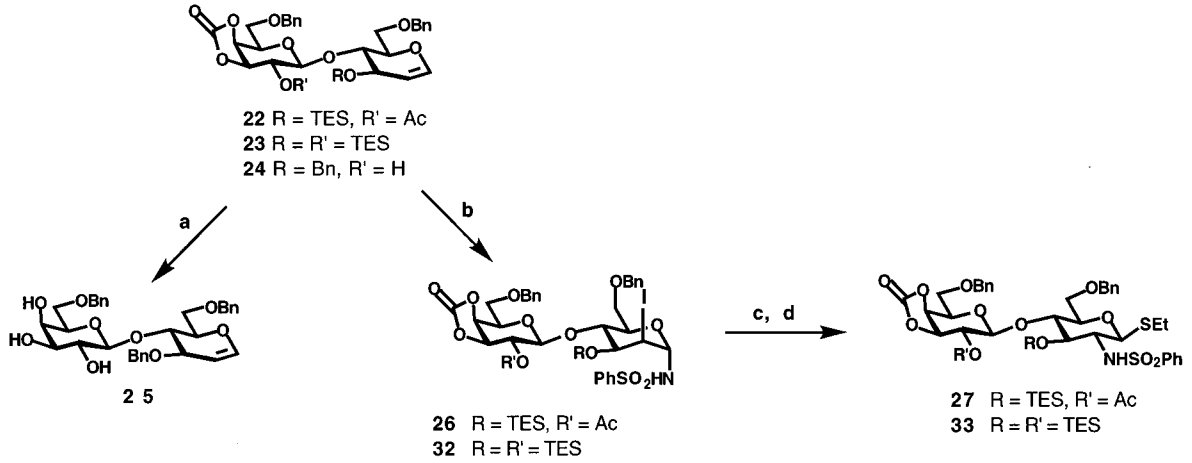
Scheme 1[a]



[a] Reagents: (a) NaH, DMF, BnBr (85%); (b) 3,3-dimethyldioxirane, CH_2Cl_2; (c) LHMDS, BnBr, DMF (30−40%); (d) TESCl, imidazole, CH_2Cl_2 (76%); (e) **20**, $ZnCl_2$, THF (65%); (f) **18**, $ZnCl_2$, THF (55%); (g) TESOTf, Et_3N, CH_2Cl_2 (92%); (h) Ac_2O, Et_3N, DMAP, CH_2Cl_2 (85%).

Scheme 2[a]



[a] Reagents: (a) K_2CO_3, MeOH (80%); (b) $I(coll)_2ClO_4$, $PhSO_2NH_2$, 4 Å MS, CH_2Cl_2 (81% for **26**, 92% for **32**); (c) LHMDS, EtSH, DMF (91% for **33**); (d) (only for **27**) Ac_2O, py, CH_2Cl_2 (95% over 2 steps).

2:1 favoring **28**). The massive polarity differences (R_f = 0.3 for **28** and 0.03 for **29** in 1:1 EtOAc−hexanes) between **28** and **29** presumably reflects different accessibilities of the two alcohol linkages to the silica surface in the two compounds. Acetylation of presumed **29** provided a triacetate assigned as **30.** Thus, the presumption that the C3′ hydroxyl in **27** would function as a fully regiospecific acceptor site turned out to be optimistic. However, synthetically useful selectivity in this reaction was achieved. While this route was certainly very concise in that it avoided the need for specific blocking functions at carbons 2′ and 4′ of the lactal derived from **24**, the formation of significant amounts of isomeric tetrasaccharide was cause for concern.

We hoped to improve upon the regiochemistry of this reaction by employing another glycosylation protocol that we had first demonstrated in a context of the synthesis of sialyl Le^x derivatives.[20] To implement this idea, we returned to the iodosulfonamide **26** with the hope that it itself might function as the eventual lactosamine donor. The glycosyl acceptor molecule would once again be compound **25**. However, here, the reaction would be mediated by bis(tri-*n*-butyltin)oxide in the presence of silver tetrafluoroborate. This reaction did, indeed, lead to an 84% yield of the previously encountered diol

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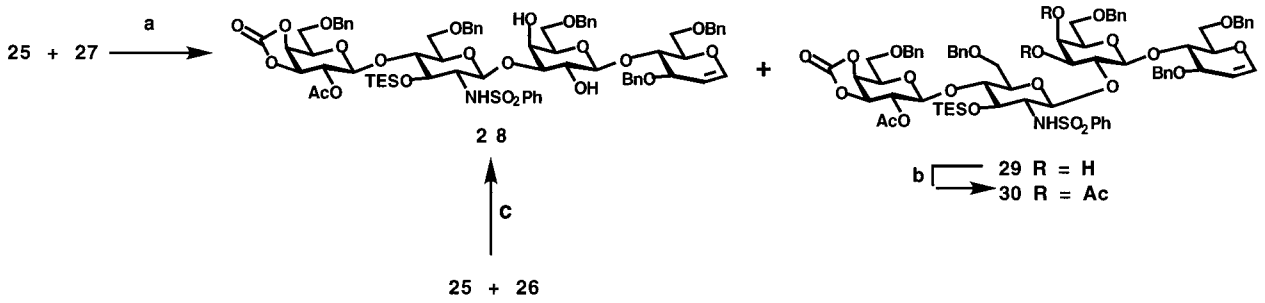
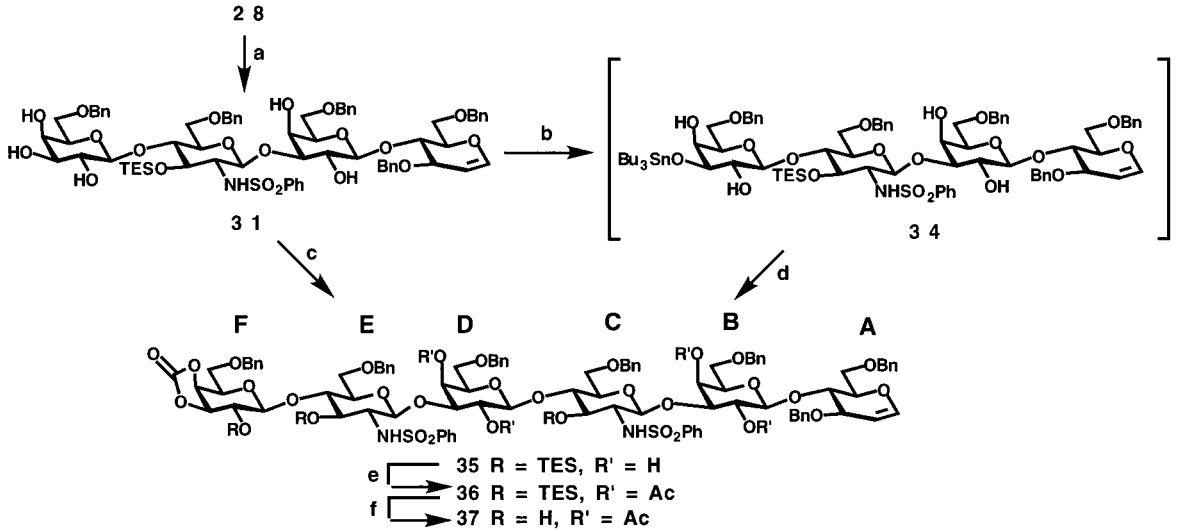
Scheme 3[a]



[a] Reagents: (a) MeOTf, di-*tert*-butylpyridine, Et_2O/CH_2Cl_2 (2:1), 4 Å MS (3:1−2:1 **28**/**29**, 35−55% for **28**); (b) Ac_2O, Py, DMAP, CH_2Cl_2 (95%); (c) (i) $(Bu_3Sn)_2O$ (2.2 equiv), PhH, **25** (4 equiv), reflux (ii) $AgBF_4$, THF, 4 Å MS (84%).

Scheme 4[a]



[a] Reagents: (a) K_2CO_3, MeOH (85%); (b) $(Bu_3Sn)_2O$, benzene, reflux; (c) **33**, MeOTf, di-*tert*-butylpyridine, Et_2O/CH_2Cl_2 (2:1), 4 Å MS (60%); (d) **32**, $AgBF_4$, THF, 4 Å MS (62%); (e) Ac_2O, Pyr, DMAP, CH_2Cl_2 (>95%); (f) TBAF/AcOH (93%).

tetrasaccharide **28**. While this direct "rollover" constituted a very pleasing and attractive solution to the assembly of **28**, this method is also not without its shortcomings in that it required recourse to excesses (4 equiv) of glycosyl acceptor **25** for high-yield coupling. In the absence of excess acceptor, it was difficult to drive the process to completion. On the other hand, the recovery of unused glycosyl acceptor, after the tin-mediated methodology, was very high (ca. 60−90%).

In summary on this point, the formation of tetrasaccharide **28** can be conducted through two methods. The reaction of donor **27** with acceptor **25**, in near stoichiometric equivalency, results in regioselective, but nonspecific, glycosylation at the desired C3′ site, giving rise to ca. 35−55% yields of tetrasaccharide. By contrast, recourse to "direct rollover" methodology, employing the tributylstannylated derivative of acceptor **25**, incurs the disadvantage of requiring significant excesses of acceptor. Since the recovery of the unreacted acceptor was high, we are currently favoring the direct method.

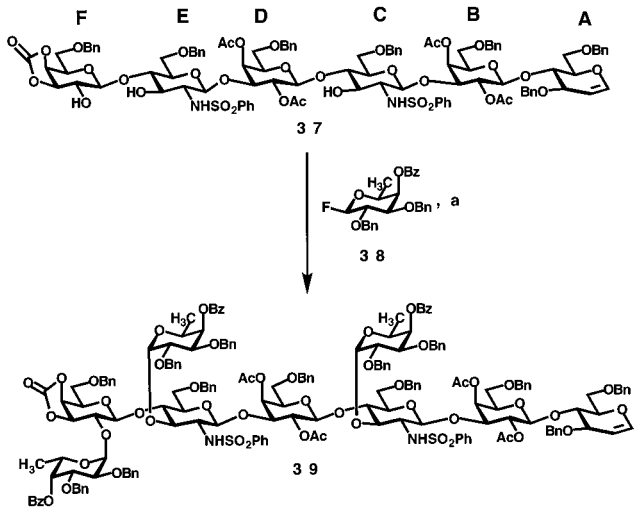
The next plateau to be reached was seen to be a hexasaccharide corresponding to a type **4** compound (see Figure 2). To further maximize the opportunities for conciseness, we were prepared to assume another significant risk in the use of undifferentiated hydroxyl groups in the acceptor site. The thought was to convert cyclic carbonate acetate **28** to pentaol **31**. This transformation was readily accomplished by treatment of **28** with potassium carbonate in methanol as shown in Scheme 4.

It was hoped that the glycosyl acceptor site of this *pentaol* would occur at the C3 hydroxyl group of the terminal galactose residue. The rationale was that the cluster of three neighboring and unprotected hydroxyl groups in the D ring in **31** would provide a greater locus of activity than the C2′ and C4′ hydroxyls of the B ring, each of which is flanked by two extensively substituted oxygens. Thus, the C2′ hydroxyl of the B ring is surrounded by the glycosidic bond to the A ring glucal as well as the glycosidic bond to the C ring glucosamine analogue. Similarly, the C4′ hydroxyl of the B ring abuts a benzyl ether protecting group at C6′ and is vicinal to a disaccharide group projecting from the oxygen from C3′.

Once again, we elected to explore the use of a thioethyl donor type to be derived from compound **23**. Toward this end, iodosulfonamidation of **23** afforded **32** which, under thiolate-mediated rearrangement of the sulfonamido group, gave rise to **33** (see Scheme 2). The coupling reaction between **28** and **33** was performed by the mediation of methyl triflate in the presence of di-*tert*-butylpyridine. Fortunately, in this case, the coupling could well be executed in acceptable yield (40−60%) without detectable formation of side product arising from glycosylation at alternative acceptor sites. In that sense, the situation was far better than in the 2 + 2 coupling of **25** + **27**, conducted through the same experimental protocol. However, we still experienced difficulties in obtaining reproducible yields.

For this reason, we elected again to examine the direct rollover method, this time using the tributylstannylated acceptor

Scheme 5[a]



[a] Reagents: (a) $Sn(OTf)_2$, di-*tert*-butylpyridine, Tol/THF (10:1), 4 Å MS (60%).

34 derived from pentaol **31** reacting with donor **32**. Using a 4-fold excess of **31** (once again unused acceptor was recovered in very high yield), a 62% yield of **35** was obtained. As before, the direct method lent itself to much more reproducible chemistry which compensated for the need to recover unused acceptor. This is the method we currently follow.

We were now ready to address the culminating stage of the plan, namely, the construction of the nonasaccharide. The preparation for this purpose simply involved cleavage of the unique (silyl) protecting groups (see system **3**, Figure 2). We had carefully prepared for the possibility of such a step by carrying relatively dischargable triethylsilyl protecting groups at the oxygens destined for fucosylation. Indeed, treatment of compound **36** with buffered tetrabutylammonium fluoride provided triol **37**. As our L-fucosyl donor, we employed compound **38**[21] which had been used several times before[5] in our program to synthesize blood group determinants and tumor antigens.

The critical step of the synthesis, *3-fold fucosylation*, was indeed accomplished through the reaction of **37** and **38**. A 60% yield of nonasaccharide **39** was obtained (see Scheme 5). Needless to say, we did not have available to us any substance for direct comparison, or any downstream product which might be used in a strategy to confirm the structure of **39**. The structure assignment would not be fully clarified until the synthesis was concluded and even then without benefit of a reference sample. Therefore, it was important to marshal convincing evidence that we had indeed generated the compound we were claiming, i.e., structure **39**. Our strategy in this regard was to return to compound **37**. If its structure could be established, we would be confident in concluding that the three fucosyl monosaccharides had been introduced at the proper positions. NMR analysis at the eight anomeric centers in the nonasaccharide, in conjunction with the information we had already accumulated at the hexa stage, would provide acceptable support of the structure of the trifucosylated product **39**.

The examination of the NMR and mass spectra of compound **37** indicated the presence of the expected four acetate groups. Fortunately NMR experiments could be used to assign the positions of these acetates. The sites of the free hydroxyl groups would thus have been established by difference.

The locations of the four acetate protecting groups were determined unequivocally through the use of several NMR experiments. The four multiplets between 5.0 and 5.45 ppm fall in the distinctive region for the protons on *O*-acetylated sugar carbons. The multiplets at 5.06 and 5.16 ppm were assigned to the H-2 protons of either sugar ring B or ring D, on the basis of their coupling patterns, and their cross peaks in the double quantum COSY experiment (DQCOSY) to protons assumed to be at anomeric carbons on the basis of chemical shifts.

The confirmation that these signals indeed arise from anomeric protons followed from the $^1H-^{13}C$ correlation experiments, where these resonances show cross peaks to carbons with the distinct anomeric carbon shift.[22] These H-2 protons, which had now been identified, show DQCOSY cross peaks to one other vicinal partner each, which must be the respective H-3's for the two pyranose rings. The peaks at 5.36 and 5.40 ppm arise from the two other sites of *O*-acetylation, i.e., at the axial hydroxyls at C4 of the rings B and D. That these centers carry the remaining acetoxy groups can be surmised from the correlation of these protons to carbonyl resonances in long-range (HMBC) $^1H-^{13}C$ correlation experiments.

Analysis of the DQCOSY experiment showed that three additional sites have cross peaks to respective H-3 protons identified from connection to the H-2 resonances. These data in the aggregate prove that the acetylation sites on sugar rings B and D are on carbons 2 and 4. The data confirm that the glycosylation linkages formed during the reaction between components **25** and **27** (or **25** and **26**) as well as between **31** and **33** (or **32** and **34**) have occurred through acceptor hydroxyl sites at C-3 of the respective residues. Using the markers for rings B and D that come from the acetates, along with the characteristic ^1H and ^{13}C shifts of the glycal, and data from the long-range $^1H-^{13}C$ correlation (HMBC) experiment, it was possible to trace the through bond connectivities around the respective sugar rings and across the glycosidic linkages to confirm the desired assembly sequence of the compound **37**. Also, all of the glycosidic linkages in compound **37** were confirmed as β-linkages on the basis of the splitting in the proton dimension of the individually resolved anomeric cross peaks in the HMQC experiment. These exhibited a coupling constant for the anomeric H-1 protons of 6.00 Hz or higher. It is on these bases that we were confident of the assignments for compounds **37** and **39**. The latter would now be advanced to our goal structures.

At this point, our remaining objectives were (i) the total synthesis of the KH-1 antigen itself, (ii) the synthesis of a form of the epitope which would be suitable for conjugation to carrier program, and (iii) the synthesis of a truncated construct which would help in ascertaining antibody specificities. In terms of reaching goals (i) and (ii) we were now in a position starting with the nonasaccharide glycal **39** to take advantage of previous experiences in the syntheses of glycolipids, particularly those in the Globo-H (breast tumor antigen) construct.[6]

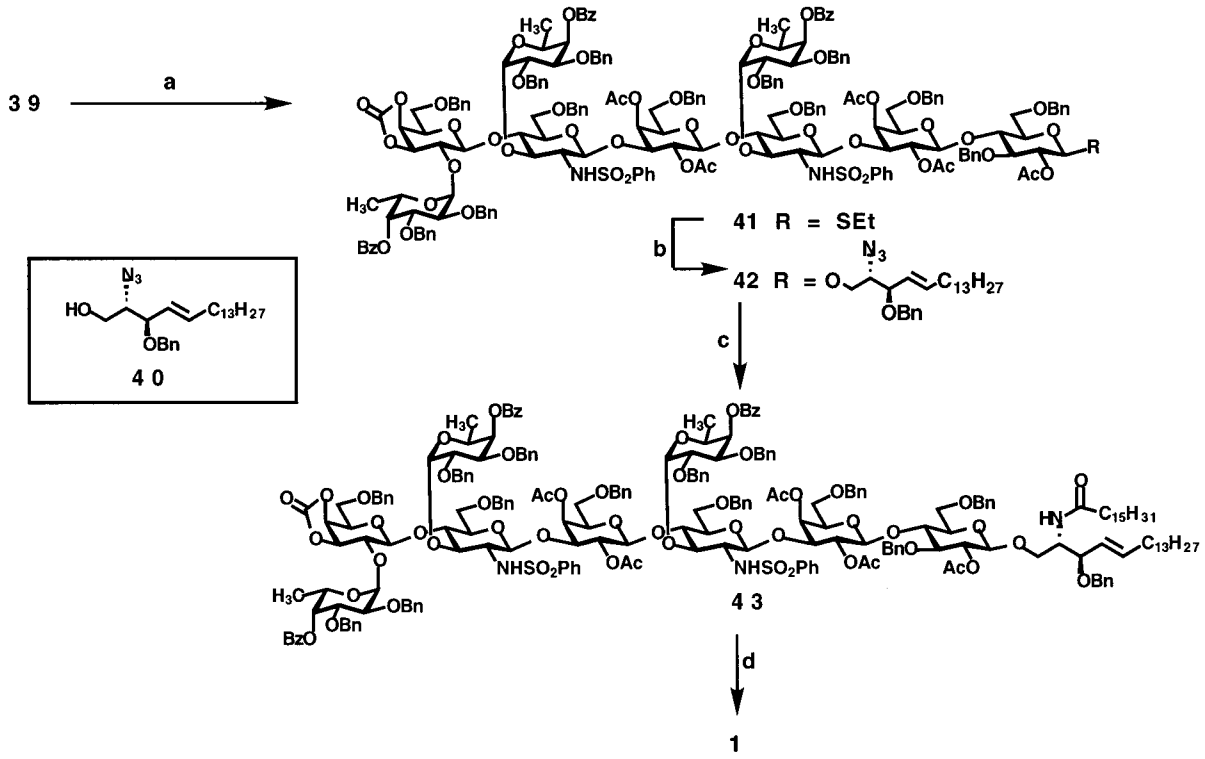
Our first step in the proposed introduction of the ceramide side chain to reach the natural KH-1 antigen (**1**) was the epoxidation of glycal **39**. While this reaction seemed to occur smoothly, attempts to use the epoxide directly as a glycosyl donor with respect to the well-known preceramide acceptor **40**[23] led to low yields of coupling. These difficulties perhaps arise from dominant conformers of a nonasaccharide epoxide in which required access to the anomeric oxido carbon by glycosyl

(21) Danishefsky, S. J.; Gervay, J.; Peterson, J. M.; McDonald, F. E.; Koseki, K.; Oriyama, T.; Griffith, D. A.; Wong, C.-H.; Dumas, D. P. *J. Am. Chem. Soc*. **1992**, *114*, 8329.

(22) Lerner, L.; Bax, A. *Carbohydr. Res*. **1987**, *166*, 35.

(23) Schmidt, R. R.; Zimmermann, P. *Tetrahedron Lett*. **1986**, *27*, 481.

Scheme 6[a]



[a] Reagents: (a) (i) 3,3-dimethyldioxirane, CH_2Cl_2; (ii) EtSH, CH_2Cl_2, H^+(cat); (iii) Ac_2O, Py, CH_2Cl_2 (60% 3 steps); (b) **40**, MeOTf, Et_2O/CH_2Cl_2 (2:1), 4 Å MS (55%); (c) Lindlar's catalyst, H_2, palmitic anhydride, EtOAc (85%); (d) (i) Na^0, NH_3, THF; MeOH quench; (ii) Ac_2O, Et_2N, DMAP, DMF, THF; (iii) MeONa, MeOH (70% 3 steps).

acceptor **40** was difficult. Given the chemical instability of glycal epoxides to Lewis acids, relatively slow glycosylation would result in low yields of glycoside, through loss of competence of the donor moiety.

Accordingly, we turned to the application of a recently developed variation of the glycal epoxy donor method.[24] This protocol started with epoxidation, followed by thiolation of the resulting epoxide of **39** and further acetylation in the usual way, leading to acetate **41** (see Scheme 6). With expectation of effective neighboring group participation of the C2 acetoxyl function available to guide a β-glycosidation, compound **41** was treated with **40** under the agency of methyl triflate. This process indeed led to the formation of glycoside **42**, in 55% yield.

From this point, the required methodology was quite familiar to us.[6] Reduction of the azide **42** was coordinated with palmitoylation of the resultant amine generated, in situ (see compound **43**). Cleavage of all aromatic groups was apparently accomplished by treatment of **43** with sodium in liquid ammonia. The resultant product was quenched with methanol. Under the basic conditions thus employed, all of the esters (including the cyclic carbonate) were cleaved. To regularize the blocking groups (vide infra), the resultant material was peracetylated. Finally, cleavage of all acetates was accomplished with sodium methoxide in methanol to give rise to the pure KH-1 antigen (**1**).

The structure assignment of the fully synthetic material is supported by a mass spectral measurement which confirmed the molecular formula. More telling was the very close correspondence of the NMR data measured on fully synthetic **1**, with the fragmentary data previously reported for the naturally occurring material. Most compelling was the extensive and self-consistent NMR analysis which had been conducted on intermediates en route to, and including, **39** (particularly compound **37**). Also, a fully rigorous NMR-based verification was possible at the stage of allyl glycoside **44**, whose synthesis was accomplished from the same **39** (vide infra).

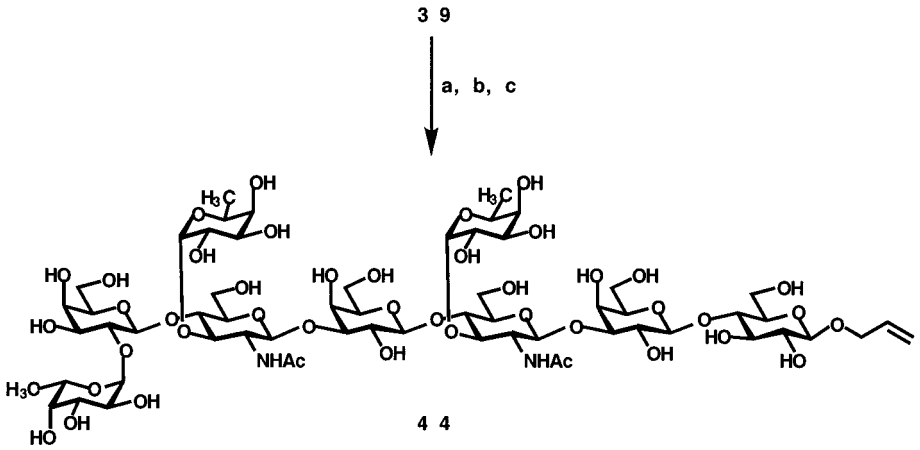
For our bioconjugation studies, we identified allyl glycoside **44** as our goal system. For this purpose we returned to the glycal **39**. Again, discharge of all the aromatic groups was accomplished by treatment of compound **39** with sodium in liquid ammonia (see Scheme 7). The remaining esters and the cyclic carbonate were cleaved after the reaction was quenched with methanol. The crude product was subjected to exhaustive acetylation, followed by epoxidation in the usual way with dimethyldioxirane. The resultant epoxide was opened by solvolysis with allyl alcohol. The crude product was fully deacylated through the action of sodium methoxide in methanol to afford compound **44** in 60% overall yield.

Conjugation of **44** to carrier keyhole lympet hemocyanin (KLH) protein was accomplished through ozonolysis of the double bond of the allyl group. This treatment was followed by reductive amination of the resultant aldehyde to KLH. The procedure for accomplishing conjugation to KLH is detailed in the Experimental Section.

In preparing for immunological investigations, it would be helpful to determine the specificities of various antibodies to the structural features of the KH-1 antigen. Toward that end, it was of interest to generate truncated structures in which segments of the molecule would be deleted. In our first such effort, we directed our attention to a construct in which the three fucose residues as well as the *N*-acetyl function on the E ring would be retained (see compound **50**). However, the terminal *N*-acetylactosamine substructure would be deleted. For this purpose, a modified synthesis was initiated. We returned to

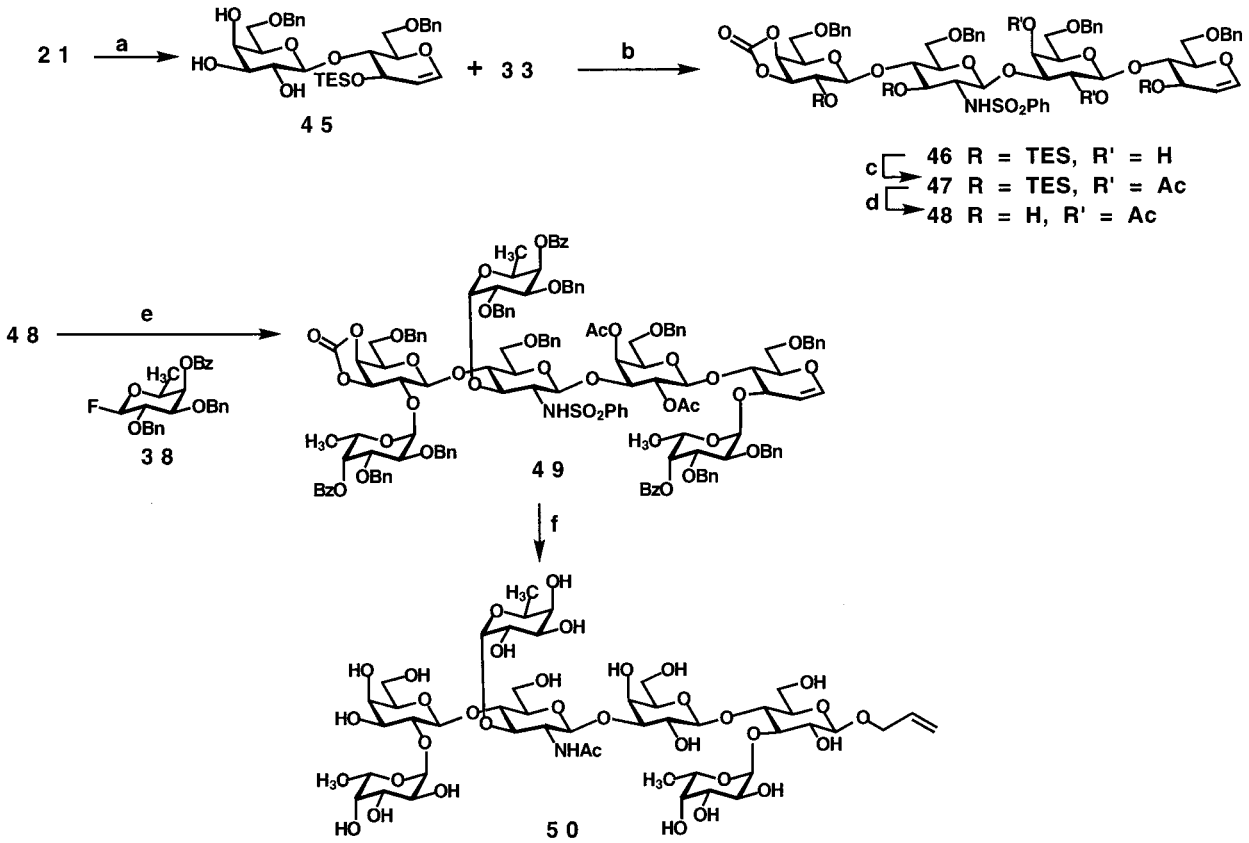
(24) Seeberger, P. H.; Eckhardt, M.; Gutteridge, C. E.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 10064.

Scheme 7[a]



[a] Reagents: (a) (i) Na^0, NH_3, THF; (MeOH quench); (ii) Ac_2O, Et_2N, DMAP, DMF, THF; (b) (i) 3,3-dimethyldioxirane, CH_2Cl_2; (ii) allyl alcohol; (c) MeONa, MeOH (60% 3 steps).

Scheme 8[a]



[a] Reagents: (a) K_2CO_3, MeOH (quant); (b) MeOTf, di-*tert*-butylpyridine, Et_2O/CH_2Cl_2 (2:1), 4 Å MS (61%); (c) Ac_2O, Py, DMAP, CH_2Cl_2 (93%); (d) 1:1 TBAF/AcOH (72%); (e) $Sn(OTf)_2$, Tol/THF (10:1), 4 Å MS (39%); (f) (i) Na^0, NH_3, THF; MeOH quench; (ii) Ac_2O, Et_2N, DMAP, THF, DMF; (iii) 3,3-dimethyldioxirane, CH_2Cl_2; (iv) allyl alcohol; (v) MeONa, MeOH (60% 3 steps).

compound **21** which would first serve as an acceptor substructure. Cleavage of the cyclic carbonate linkage gave rise to compound **45** (see Scheme 8).

The donor, to be coupled to compound **45**, was the C1 thioethyl C2 phenylsulfonamide system **33**, containing the two unique TES protecting groups in the E and F rings. Coupling of **45** with **33** occurred under the usual conditions to give an acceptable 60% yield of the tetrasaccharide **46**. Acetylation of **46** afforded a diacetate, **47**. This system now contained the three uniquely identified triethylsilyl ether functions which were cleaved through the action of TBAF (see compound **48**). Once again, it was possible to conduct 3-fold fucosylation. This chemistry was not optimized, and we accepted a 39% yield of compound **49**. The remaining steps for conversion of **49** to allyl glycoside **50** ran parallel to those used with related structures in the synthesis of compound **44**.

With serviceable routes to the various tetrasaccharide, disaccharide, and monosaccharide moieties contained in the KH-1 construct, it seems likely that other permuted structures can be readily assembled. These can be used to ascertain epitope specificities for monoclonal antibodies which are being elicited as part of this vaccine-oriented program.

Summary

All of the chemical goals identified at the outset of this project have been realized. The KH-1 antigen (**1**) has been synthesized. It has also been synthesized in conjugatable form for delivery to carrier protein (see compound **44**). Conjugation to KLH has, in fact, been achieved (see the Experimental Section). A format for synthesizing truncated versions of the KH-1 antigen in order to establish epitope specificities for various monoclonal antibodies being harvested has also been achieved (see compound **50**).

From a strategic standpoint, the strategy of 3-fold fucosylation to achieve conciseness in the synthesis was certainly vindicated. Also, the synthesis speaks forcefully to the advantages of glycal assembly and to the minimization of protecting group manipulations for purposes of differentiating functional groups. Thus, in three instances (see compounds **25**, **31**, and **45**) we took recourse to glycosyl acceptors with more than one potential glycosylation site. In the latter two cases, coupling was accomplished under nearly stoichiometric conditions utilizing 1-β-thioethyl-2α-phenylsulfonamido donors under the agency of methyl triflate. In the case of coupling of acceptors **25** and **31**, these delicate reaction conditions led to some difficulties in reproducing the best case yield. However, excellent yields in a highly reproducible fashion were obtained by treatment of the 1α-sulfonamido-2β-iodo donor type (see compounds **26** and **32**), utilizing a stannylated version of acceptor (**25** and **31**). These reactions, however, require a significant excess of glycosyl acceptor. Since the recovery yields are very high, our current technology favors this practice.

The primary focus of the KH-1 antigen problem has now shifted to issues of immunology and vaccinology. Data on these matters will soon be forthcoming.

Experimental Section

General Methods. All commercial materials were used without further purifications unless otherwise noted. The following solvents were distilled under positive pressure of dry nitrogen immediately before use: THF and ether from sodium−benzophenone ketyl, and CH_2Cl_2, toluene, and benzene from CaH_2. All the reactions were performed under N_2 atmosphere. NMR (^{1}H, ^{13}C) spectra were recorded on a Bruker AMX-400 MHz spectrometer, a Bruker Avance DRX-500 MHz spectrometer, and a Varian 600 Unity plus 600 MHz spectrometer, referenced to TMS (^{1}H NMR, δ 0.00) or $CDCl_3$ (^{13}C NMR, δ 77.0) and CD_3OD(^{13}C NMR, δ 49.05) peaks unless otherwise stated. LB = 1.0 Hz was used before Fourier transformation for all of the ^{13}C NMR. IR spectra were recorded with a Perkin-Elmer 1600 series FTIR spectrometer, and optical rotations were measured with a JASCO DIP-370 digital polarimeter using a 10 cm path length cell. Low- and high-resolution mass spectral analyses were performed with a JEOL JMS-DX-303 HF mass spectrometer. Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F_{254} plates (0.25 mm). Compounds were visualized by dipping the plates in a cerium sulfate−ammonium molybdate solution followed by heating. Flash column chromatography was performed using the indicated solvent on E. Merck silica gel 60 (40−63 μm) or Sigma H-Type silica gel (10−40 μm) for normal phase and EM Science Lichroprep RP-18 (15−25 μm) for reverse phase. Melting points are obtained with an Electrothermal melting point apparatus (series no. 9100) and are uncorrected.

6-*O*-Benzyl 3,4-*O*-Carbonate 16. To a solution of the galactal carbonate derivative **15** (5.36 g, 34.4 mmol) in dry DMF (50 mL) at 0 °C was added benzyl bromide (12.3 mL, 103 mmol), followed by NaH (60% oil dispersion, 1.50 g, 1.1 equiv). The reaction mixture was stirred for 1 h, diluted with $CHCl_3$ (50 mL), treated with brine solution (20 mL), and again stirred for 5 min. The organic layer was separated, dried ($MgSO_4$), and filtered. The filtrate was concentrated to afford a syrup. Chromatography with 1:1 EtOAc/hexanes afforded 7.66 g of compound **16** (85%) as a syrup: $[\alpha]^{23}_D = -92.0°$ (*c* 1.0, $CHCl_3$); FTIR (film) 3030, 2875, 1797, 1647, 1496, 1453, 1371, 1244, 1164, 1110, 1010, 837, 699 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.7−3.9 (m, 2H, H-6), 4.08 (br t, 1H, J = 7.4 Hz, H-5), 4.58 (s, 2H, $-CH_2Ar$), 4.90 (d, 1H, J = 7.8 Hz, H-4), 4.93 (br m, 1H, H-3), 5.14 (dd, 1H, J = 3.2, 7.7 Hz, H-2), 6.66 (d, 1H, J = 6.2 Hz, H-1), 7.28−7.45 (m, 5H, Ar−H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 67.97, 68.74, 72.41, 73.14, 73.66, 97.97, 127.77, 127.93, 128.44, 137.18, 149.06, 153.98; HRMS (FAB) calcd for $C_{14}H_{14}O_5$·Na (M + Na)$^+$ 285.084, found 285.072.

6-*O*-Benzylglucal (19). To a solution of D-glucal (10.0 g, 68.4 mmol) in dry DMF (200 mL) was added LHMDS (1.0 M solution in THF, 75.3 mL, 1.1 equiv) dropwise at −40 °C, followed by BnBr (8.18 mL, 68.4 mmol). The solution was stirred mechanically for 6 h, allowing the temperature to rise to 0 °C. Saturated solution of NH_4Cl (50 mL) was added to the reaction mixture, followed by EtOAc (200 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (3 × 50 mL). Combined organic layers were washed with brine (50 mL) and water (50 mL), dried with $MgSO_4$, filtered, concentrated, and submitted for column chromatography (1:1 EtOAc/hexanes) to obtain compound **19** as a syrup (4.80 g, 30%): $[\alpha]^{23}_D = +11.0°$ (*c* 1.0, $CHCl_3$); FTIR (film) 3342, 2871, 1642, 1656, 1231, 1101, 1027, 851, 738 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.6−3.85 (m, 5H,), 4.06 (d, 1H, J = 4.0 Hz, −OH), 4.11 (br t, 1H, H-3), 4.46 (d, 1H, J = 12.0 Hz, $-CH_2Ar$), 4.52 (d, 1H, J = 12.0 Hz, $-CH_2$-Ar), 4.57 (dd, 1H, J = 1.8, 6.0 Hz, H-2), 6.21 (d, 1H, J = 6.0 Hz, H-1), 7.15−7.35 (m, 5H, Ar−H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 69.06, 69.63, 70.28, 73.427, 76.95, 102.72, 127.29, 127.55, 128.21, 128.24, 137.60, 137.75, 143.86.

6-*O*-Benzyl-3-*O*-(triethylsilyl)glucal (20). To a solution of compound **19** (4.00 g, 16.8 mmol) in dry CH_2Cl_2 (70 mL) were added imidazole (1.26 g, 18.5 mmol) and DMAP (0.01 g), and the solution was cooled to −78 °C. At −78 °C, to the reaction mixture was added TESCl (3.10 mL, 18.5 mmol) dropwise, and the resulting solution was allowed to warm to −40 °C. The reaction mixture was stirred for 4 h, diluted with EtOAc (200 mL), and washed with water (2 × 100 mL), a saturated solution of $NaHCO_3$ (3 × 100 mL), and brine (10 mL). The organic layer was dried ($NaSO_4$), filtered, concentrated, and submitted for column chromatography (1:9 EtOAc/hexanes) to provide **20** (4.46 g,76%) as a syrup: $[\alpha]^{22}_D = +44.0°$ (*c* 1.0, $CHCl_3$); FTIR (film) 3468, 3030, 2953, 2875, 1644, 1453, 1237, 1086, 871, 737 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.55 (q, 6H, J = 7.9 Hz, $-SiCH_2CH_3$), 0.88 (t, 9H, J = 7.9 Hz, $-SiCH_2CH_3$), 2.47 (d, 1H, J = 4.1 Hz, −OH), 3.6−3.75 (m, 3H, 2H-6, H-4), 4.13 (br d, 1H, J = 6.4 Hz, H-3), 4.47 and 4.52 (2d, 2H, J = 12.0 Hz, $-CH_2Ar$), 4.55 (dd, 1H, J = 2.2, 6.2 Hz, H-2), 6.21 (d, 1H, J = 6.0 Hz, H-1), 7.10−7.40 (m, 5H, Ar−H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 4.84, 6.66, 69.05, 69.64, 70.56, 73.47, 76.97, 103.44, 127.59, 127.64, 128.27, 137.78, 143.33; HRMS (FAB) calcd for $C_{19}H_{30}O_4Si$·Na (M + Na)$^+$ 373.180, found 373.181.

6,6′-Di-*O*-benzyl 3′,4′-Carbonate 3-*O*-Triethylsilyl Lactal Derivative 21. To a solution of compound **16** (2.99 g, 11.4 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C was added 3,3-dimethyldioxirane (300 mL, 0.0800 M solution in acetone). The reaction mixture was stirred at 0 °C for 1 h. The solvent was evaporated by N_2, and further dried in vacuo for 10 min. The resulting 1,2-anhydro sugar **17** was dissolved in a solution of compound **20** (6.00 g, 17.1 mmol) in dry THF (30 mL), and at 0 °C to the resulting solution was added a 1.0 M solution of $ZnCl_2$ in ether (5.70 mL, 0.5 equiv). The reaction mixture was stirred at room temperature for 24 h, diluted with EtOAc (50 mL), and washed with a saturated solution of $NaHCO_3$ (2 × 10 mL). The organic layer was separated, dried ($MgSO_4$), filtered, concentrated, and submitted for chromatography (2:3 EtOAc/hexanes) to provide compound **21** (4.76 g, 66%) as a syrup: $[\alpha]^{22}_D = -25.0°$ (*c* 1.0, $CHCl_3$); FTIR (film) 3439, 3030, 2910, 1804, 1725, 1647, 1453, 1371, 1243, 1074, 847, 741 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.58 (q, 6H, J = 8.0 Hz, $-SiCH_2CH_3$), 0.92 (t, 9H, J = 8.0 Hz, $-SiCH_2CH_3$), 3.51 (d, 1H, J = 2.8 Hz, −OH), 3.62 (ddd, 1H, J = 2.8, 7.2, 7.2 Hz, H-2′), 3.65−3.75 (m, 3H), 3.85 (m, 1H), 3.93 (dd, 1H, J = 4.9, 11.2 Hz), 3.99 (br t, 1H, J = 5.3, 6.48 Hz), 4.09 (br m, 1H), 4.27 (br t, 1H, J = 4.2 Hz), 4.48−4.68 (m, 6H, $-CH_2Ar$), 4.70 (dd, 1H, J = 3.4, 6.2 Hz, H-2), 4.74 (dd, 1H, J = 1.8, 7.2 Hz, H-4), 6.32 (d, 1H, J = 6.0 Hz, H-1), 7.2−7.4 (m, 10H, Ar−H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 4.75, 6.67, 65.65, 67.79, 67.93, 70.42, 71.49, 73.43, 73.58, 74.46, 75.27, 75.42, 78.05, 99.94, 102.61,

127.79, 127.85, 128.14, 128.33, 137.36, 137.53, 143.00, 153.96; HRMS calcd for $C_{33}H_{44}O_{10}Si{\cdot}Na$ $(M + Na)^+$ 651.170, found 651.368.

2′-*O*-Acetyl-6,6′-di-*O*-benzyl 3′,4′-Carbonate 3-*O*-Triethylsilyl Lactal (22). To a solution of compound **21** (3.50 g, 5.50 mmol) in CH_2Cl_2 (30 mL) were added Et_3N (3 mL), Ac_2O (3 mL), and a catalytic amount of DMAP. The reaction mixture was stirred overnight, diluted with EtOAc (50 mL), and washed with saturated solutions of $CuSO_4$ (3 × 10 mL), water (1 × 10 mL), $NaHCO_3$ (2 × 10 mL), and brine (1 × 10 mL) sequentially. The organic layer was separated, dried ($MgSO_4$), filtered, concentrated, and submitted to chromatography (1:1 EtOAc/hexanes) to provide **22** in quantitative yield: $[\alpha]^{23}_D = -42.0°$ (*c* 1.0, $CHCl_3$); FTIR (film) 2954, 2875, 1809, 1755, 1646, 1454, 1222, 1060, 743 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.58 (q, 6H, $J = 7.9$ Hz, $-SiCH_2$-CH_3), 0.92 (t, 9H, $J = 7.9$ Hz, $-SiCH_2CH_3$), 2.06 (s, 3H, $-COCH_3$), 3.63 (dd, 1H, $J = 2.9$, 10.9 Hz, H-5), 3.70 (br d, 2H, $J = 10.5$ Hz, 2H-6), 3.85 (dd, 1H, $J = 6.1$ Hz, $J = 10.92$, H-5′), 3.9−4.0 (m, 2H, 2H-6′), 4.10−4.2 (m, 2H), 4.5−4.6 (m, 4H, 2-CH_2Ar), 4.64 (dd, 1H, $J = 4.0$, 8.0 Hz, H-3′), 4.71 (dd, 1H, $J = 4.2$, 5.8 Hz, H-2), 4.84 (dd, 1H, $J = 1.0$, 8.1 Hz, H-4′), 4.90 (d, 1H, $J = 4.6$ Hz, H-1′), 4.99 (t, 1H, $J = 4.2$ Hz, H-4′), 6.30 (d, 1H, $J = 6.2$ Hz, H-1), 7.15−7.40 (m, 10H, Ar−H); ^{13}C NMR ($CDCl_3$) δ 4.78, 6.73, 20.58, 64.78, 67.94, 67.99, 69.38, 69.50, 73.19, 73.35, 73.79, 73.92, 74.56, 74.86, 96.82, 102.27, 127.60, 127.75, 127.78, 127.93, 128.29, 128.44, 137.35, 138.01, 142.99, 153.27, 168.54; HRMS calcd for $C_{35}H_{46}O_{11}Si{\cdot}Na$ $(M + Na)^+$ 693.270, found 693.273.

6,6′-Di-*O*-benzyl 3′,4′-Carbonate 2′,3-Bis(*O*-triethylsilyl) Lactal (23). To a solution of the lactal **21** (3.00 g, 4.77 mmol) in dry CH_2Cl_2 (50 mL) was added Et_3N (3.34 mL) followed by dropwise addition of TESOTf (1.61 mL, 7.15 mmol) at 0 °C. The reaction mixture was stirred for 3 h, and washed with a saturated solution of $NaHCO_3$ (2 × 15 mL). The organic layer was separated, dried ($MgSO_4$), filtered, concentrated, and submitted for chromatography (1:4 EtOAc/hexanes) to provide **23** (3.27 g, 92%) as a syrup: $[\alpha]^{23}_D = -38.0°$ (*c* 1.0, $CHCl_3$); FTIR (film) 3087, 2953, 2875, 1819, 1647, 1647, 1454, 1365, 1240, 1101, 854, 739 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.57 and 0.617 (2q, 12H, $J = 8.0$ Hz, $-SiCH_2CH_3$), 0.92 and 0.94 (2t, 18H, $J = 8.0$ Hz, $-SiCH_2CH_3$), 3.5−3.75 (m, 4H), 3.8−4.0 (m, 3H), 4.05−4.20 (m, 2H), 4.49 (dd, 1H, $J = 4.4$, 7.2 Hz), 4.50−4.62 (m, 4H, $-CH_2$Ar), 4.64 (d, 1H, $J = 5.2$ Hz, H-1′), 4.70 (dd, 1H, $J = 4.0$, 5.6 Hz, H-4′), 4.76 (br d, 1H, $J = 7.5$ Hz, H-2), 6.32 (d, 1H, $J = 6.0$ Hz, H-1); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 4.56, 4.79, 6.58, 6.76, 65.24, 67.99, 68.02, 69.48, 71.06, 73.37, 73.76, 74.24, 74.37, 75.10, 78.21, 99.21, 99.34, 102.56, 127.63, 127.77, 127.79, 127.88, 128.32, 128.43, 137.53, 138.09, 143.08, 153.87; HRMS calcd for $C_{39}H_{58}O_{10}Si_2{\cdot}Na$ 765.360 $(M + Na)^+$, found 765.347.

3,6,6′-Tri-*O*-benzyl 3′,4′-Carbonate Lactal (24). To a solution of compound **16** (2.99 g, 11.4 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C was added 3,3-dimethyldioxirane (300 mL, 0.0800 M solution in acetone). The reaction mixture was stirred at 0 °C for 1 h. The organic solvent was evaporated by N_2 stream, and further dried in vacuo for 10 min. The resulting 1,2-anhydro sugar **17** was dissolved in a solution of known 3,6-di-*O*-benzylglucal (**18**) (5.29 g, 17.2 mmol) in dry THF (30 mL). To the solution at 0 °C was added a 1.0 M solution of $ZnCl_2$ in ether (5.71 mL, 0.5 equiv). The reaction mixture was stirred at room temperature for 24 h, diluted with EtOAc (50 mL), and washed with a saturated solution of $NaHCO_3$ (2 × 10 mL). The organic layer was separated, dried ($MgSO_4$), filtered, concentrated, and submitted for chromatography (1:1 EtOAc/hexanes) to provide compound **24** (3.31 g, 48%) as a syrup: $[\alpha]^{22}_D = -38.0°$ (*c* 1.0, $CHCl_3$); FTIR (film) 3437, 3029, 2871, 1804, 1648, 1453, 1367, 1166, 1097, 1027, 739, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.55−3.62 (m, 2H), 3.62−3.70 (m, 2H), 3.70−3.78 (m, 2H), 3.95−4.11 (m, 2H), 3.95−4.11 (m, 2H), 4.17 (dd, 1H, $J = 5.4$, 7.0 Hz), 4.27 (ddd, 1H, $J = 1.1$, 1.7, 5.3 Hz), 4.44 (s, 2H, $-CH_2$Ar), 4.77 (dd, 1H, $J = 2.5$, 6.1 Hz, H-2), 6.28 (d, 1H, $J = 6.0$ Hz, H-1), 7.10−7.40 (m, 15H, Ar−H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 68.00, 68.09, 70.55, 70.63, 72.20, 73.58, 73.81, 74.58, 74.82, 75.26, 76.18, 78.47, 100.17, 101.32, 127.43 (2C), 127.56, 127.72 (2C), 127.83, 127.90, 128.00 (2C), 128.31 (2C), 128.37 (2C), 128.44 (2C), 137.28, 137.43, 138.29, 144.59, 153.97; HRMS calcd for $C_{34}H_{36}O_{10}{\cdot}Na$ $(M + Na)^+$ 627.220, found 627.220.

3,6,6′-Tri-*O*-benzyllactal (25). To a solution of compound **24** (3.00 g, 4.96 mmol) in MeOH (100 mL) was added a solution of sodium methoxide (1.00 mL, 25% by weight in MeOH) dropwise. The reaction mixture was stirred for 1 h. The volatiles were removed in vacuo. The residual syrup obtained was quickly purified by column chromatography (2.5% MeOH in EtOAc) to provide 2.68 g (91%) of **25** as a syrup: $[\alpha]^{22}_D = -14.0°$ (*c* 1.0, $CHCl_3$); FTIR (film) 3415, 3029, 2867, 1647, 1453, 1246, 1068, 735 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.48−3.56 (m, 2H), 3.62 (dd, 1H, $J = 4.8$, 8.0 Hz), 3.66−3.78 (m, 3H), 3.91 (d, 1H, $J = 4.4$ Hz), 3.97 (dd, 1H, $J = 4.0$, 8.8 Hz), 4.18−4.28 (m, 4H), 4.47 (s, 2H, $-CH_2$Ar), 4.52 (d, 1H, $J = 8.0$ Hz), 5.59 (s, 1H, $-CH_2$Ar), 4.57−4.65 (m, 2H, $-CH_2$Ar), 4.85 (dd, 1H, $J = 2.4$, 4.8 Hz, H-2), 6.41 (d, 1H, $J = 4.8$ Hz, H-1), 7.20−7.45 (m, 15H, Ar−H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 67.92, 68.86, 69.19, 69.82, 71.53, 73.35 (2C), 73.39, 73.42, 73.87, 76.26, 100.01, 103.30, 127.35, 127.42, 127.59, 127.74, 128.19, 128.29, 137.72, 137.81, 138.52, 144.57; HRMS (FAB) calcd for $C_{33}H_{38}O_9Na$ $(M + Na)^+$ 601.240, found 601.242.

Iodo Sulfonamide Disaccharide 26. To a solution of compound **22** (2.50 g, 3.72 mmol) with 4 Å molecular sieves (3.00 g) and benzenesulfonamide (2.92 g, 18.6 mmol) in CH_2Cl_2 at 0 °C was added a solution of I(*sym*-coll)$_2ClO_4$ [freshly prepared from Ag(coll)$_2ClO_4$ (8.36 g, 18.6 mmol) and I_2 (4.53 g, 18.5 mmol)] in CH_2Cl_2 (40 mL) via cannula. The ice bath was removed, and the reaction mixture was stirred at room temperature for 1 h. The resulting suspension was filtered through a pad of silica gel, and the filtrate was washed with a saturated solution of $Na_2S_2O_3$ (3 × 25 mL), followed by a saturated solution of $CuSO_4$ (5 × 25 mL) and H_2O (2 × 10 mL). The separated organic layer was dried ($MgSO_4$), filtered, concentrated, and submitted for column chromatography (5% EtOAc in CH_2Cl_2, start gradient) to obtain **26** (2.87 g, 81%) as a syrup: $[\alpha]^{23}_D = -30.0°$ (*c* 1.0, $CHCl_3$); FTIR (film) 3267, 2954, 1806, 1755, 1495, 1458, 1370, 1342, 1090, 813, 750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.66 (m, 6H, $J = 7.8$ Hz, $-SiCH_2$-CH_3) 0.95 (t, 9H, $J = 7.8$ Hz, $-SiCH_2CH_3$), 2.04 (s, 3H, $-COCH_3$), 3.44 (dd, 1H, $J = 5.6$, 10.2 Hz, H-5), 3.55−3.72 (m, 4H), 3.86 (br s, 1H), 4.11 (t, 1H, $J = 7.0$ Hz), 4.23 (br s, 1H), 4.35 (dd, 1H, $J = 2.3$, 10.0 Hz), 4.44 and 4.50 (2d, 2H, $J = 11.9$ Hz, $-CH_2$Ar), 4.57 (s, 2H, $-CH_2$Ar), 4.70 (br d, 1H, $J = 8.3$ Hz), 4.89 (br s, 1H), 4.95−5.0 (m, 2H), 5.25 (t, 1H, $J = 9.6$ Hz), 5.60 (d, 1H, $J = 9.9$ Hz), 7.2−7.5 (m, 13H, Ar−H), 7.88 (d, 2H, $J = 7.7$ Hz, Ar−H); ^{13}C NMR ($CDCl_3$) δ 4.93, 6.95, 20.64, 67.49, 67.86, 68.40, 68.46, 71.91, 72.57, 73.33, 73.94, 75.20, 79.30, 126.39, 127.35, 127.67, 127.85, 127.98, 128.09, 128.36, 128.54, 128.58, 129.10, 132.35, 132.68, 137.14, 137.91, 141.36, 153.60, 168.69; HRMS calcd for $C_{41}H_{52}INO_{13}SSiNa$ 976.190, found 976.187.

Ethylthio Sulfonamide Disaccharide 27. To a solution of iodo sulfonamide **26** (2.80 g, 2.93 mmol) in dry DMF (40 mL) at −40 °C was added EtSH (1.08 mL, 14.7 mmol), followed by dropwise addition of a solution of LHMDS (1.0 M solution in THF, 8.80 mL). The reaction mixture was stirred for 1 h, while it was allowed to warm to room temperature, quenched with a saturated solution of NH_4Cl (10 mL), and extracted with EtOAc (5 × 20 mL). The organic layer was washed with brine (15 mL), separated, dried ($MgSO_4$), filtered, and concentrated. The resulting crude material was redissolved in CH_2Cl_2 (50 mL) and treated with pyridine (1.0 mL), Ac_2O (1.0 mL), and the reaction mixture was stirred overnight. The organic layer was washed with a saturated solution of $CuSO_4$ (3 × 15 mL) and water (1 × 10 mL), and finally with a saturated solution of $NaHCO_3$ (2 × 15 mL). The organic layer was separated, dried ($MgSO_4$), filtered, concentrated, and submitted for chromatography (1:1 EtOAc/hexanes) to provide **27** (2.38 g, 91%) as a syrup: $[\alpha]^{23}_D = -4.0°$ (*c* 1.0, $CHCl_3$); FTIR (film) 3316, 2955, 2875, 1815, 1745, 1448, 1371, 1330, 1227, 1092, 897, 740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.51 (q, 6H, $J = 8.0$ Hz, $-SiCH_2CH_3$), 0.88 (t, 9H, $J = 7.9$ Hz, $-SiCH_2CH_3$), 1.09 (t, 3H, $J = 7.2$ Hz, $-SCH_2CH_3$), 2.09 (s, 3H, $-COCH_3$), 2.44 (m, 2H, $-SCH_2CH_3$), 3.48 (br m, 1H, H-2), 3.83−3.70 (m, 7H), 3.89 (br t, 1H), 3.95 (br s, 1H), 4.43 (d, 1H, $J = 5.4$ Hz, H-1), 4.48 (br d, 2H, $-CH_2$Ar), 4.53 (d, 1H, $J = 6.3$ Hz, H-1′), 4.57 (s, 2H, $-CH_2$Ar), 4.75 (br t, 1H, $J = 5.7$ Hz, H-2′), 4.84 (br d, 1H, 9.9 Hz, $-NHSO_2Ph$), 7.20−7.40 and 7.40−7.60 (m, 13H, Ar−H), 7.97 (d, 2H, $J = 7.2$ Hz, Ar−H); ^{13}C NMR ($CDCl_3$) δ 4.28, 6.65, 14.56, 20.64, 20.89, 56.96, 67.64, 70.49, 70.52, 70.57, 71.14, 73.26, 73.72, 73.96, 74.99, 75.02, 76.88, 82.48, 97.83, 126.21, 127.30, 127.61, 127.78, 127.87, 128.29, 128.38, 128.62, 128.94, 132.17,

137.32, 137.94, 141.38, 153.27, 170.99; HRMS calcd for $C_{43}H_{57}NO_{13}S_2Si{\cdot}Na$ $(M + Na)^+$ 910.290, found 910.294.

Tetrasaccharide Diol 28. To a solution of a disaccharide **25** (0.100 g, 0.174 mmol) and thio donor **27** (0.308 g, 0.347 mmol) with 4 Å molecular sieves (1.0 g) in dry CH_2Cl_2 (8 mL) was added di-*tert*-butylpyridine (0.311 mL, 0.694 mmol). The suspension was cooled to −10 °C, treated with MeOTf (0.156 mL, 0.694 mmol), and stirred for 2 h. The reaction mixture was warmed to 0 °C, and stirred for 24 h. The reaction mixture was quenched with Et_3N (0.1 mL), stirred for an additional 3−5 min, diluted with EtOAc (25 mL), and filtered through a pad of silica gel. The filtrate was washed with a saturated solution of $NaHCO_3$ (2 × 10 mL), and the organic layer was separated, dried ($MgSO_4$), filtered, concentrated, and submitted for chromatography (35% EtOAc in hexanes) to provide 0.134 g (55%) of **28** tetrasaccharide as a syrup. Further elution (60% EtOAc in hexane) provided the regioisomer **29** in 3:1−2:1 ratio favoring the desired product: $[\alpha]^{23}_D = -28.0°$ (*c* 1.0, $CHCl_3$); FTIR (film) 3491, 3029, 3874, 1815, 1753, 1647, 1453, 1370, 1221, 1160, 1064, 738 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.38 (q, 6H, $J = 8.0$ Hz, $-SiCH_2CH_3$), 0.76 (t, 9H, $J = 8.0$ Hz, $-SiCH_2CH_3$), 1.97 (s, 3H, $-COCH_3$), 3.2−3.32 (m, 2H), 3.35−3.55 (m, 5H), 3.55−3.7 (m, 7H), 3.7−3.8 (m, 4H), 3.95 (dd, 1H, $J = 4.6$, 11.3 Hz), 4.0−4.12 (m, 2H), 4.18 (br s, 1H), 4.3−4.65 (m, 15H), 4.7−4.8 (m, 2H), 4.89 (t, 1H, $J = 5.2$ Hz), 5.31 (d, 1H, $J = 8.4$ Hz), 6.32 (d, 1H, $J = 6.0$ Hz, H-1), 7.1−7.5 (m, 28H, Ar−H), 7.85 (d, 2H, $J = 7.4$ Hz, Ar−H); ^{13}C NMR ($CDCl_3$) δ 4.47, 6.74, 20.62, 58.45, 67.89, 68.16, 68.91, 69.63, 70.01, 70.23, 70.70, 70.74, 72.95, 73.26, 73.32, 73.40, 73.43, 73.79, 74.38, 74.75, 74.81, 75.34, 76.60, 77.19, 82.21, 97.41, 100.41, 102.53, 102.84, 127.30, 127.44, 127.50, 127.56, 127.59, 127.62, 127.76, 127.82, 127.84, 127.96, 128.18, 128.24, 128.33, 128.38, 128.46, 128.83, 132.49, 137.32, 137.89, 138.70, 140.76, 144.51, 153.43, 168.94; HRMS calcd for $C_{74}H_{89}O_{22}SSiNa$ 1426.530, found 1426.526.

Tetrasaccharide 3′,4′-Diol 29: $[\alpha]^{23}_D = -40.0°$ (*c* 1.0, $CHCl_3$); FTIR (film) 3469, 3030, 2873, 1814, 1254, 1451, 1369, 1222, 1063, 737 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.33 (q, 6H, $J = 7.7$ Hz, $-SiCH_2CH_3$), 0.73 (t, 9H, $J = 7.5$ Hz, $-SiCH_2CH_3$), 2.05 (s, 3H, $-COCH_3$), 3.11 (s, 1H, −OH), 3.30−3.50 (m, 8H), 3.60 (br m, 6H), 3.68−3.81 (m, 5H), 3.80 (dd, 1H, $J = 4.9$, 11.3 Hz), 4.02−4.12 (m, 2H), 4.14 (br s, 1H), 4.38−4.25 (m, 4H), 4.38−4.56 (m, 10H), 4.61 (dd, 2H, $J = 6.0$, 10.4 Hz), 4.61 (m, 1H), 4.70−4.77 (m, 2H), 4.89 (br t, 1H, $J = 5.2$ Hz), 5.49 (d, 1H, $J = 8.5$ Hz, $-NHSO_2Ph$), 6.31 (d, 1H, $J = 6.4$ Hz,m H-1), 7.10−7.30 (m, 25H, Ar−H), 7.32−7.45 (m, 3H, Ar−H), 7.87 (d, 2H, $J = 6.8$ Hz, Ar−H); ^{13}C NMR ($CDCl_3$) δ 4.43, 6.74, 20.74, 57.53, 67.68, 68.11, 69.54, 70.26, 70.30, 70.51, 70.70, 72.07, 72.72, 73.18, 73.34, 73.50, 73.72, 73.81, 73.85, 74.04, 74.16, 74.26, 74.38, 74.89, 70.05, 76.11, 76.59, 77.24, 96.95, 100.38, 103.09, 103.52, 127.37, 127.41, 127.46, 127.68, 127.82, 127.85, 127.99, 128.24, 128.29, 128.39, 128.41, 128.49, 128.97, 132.55, 137.37, 137.81, 137.89, 138.37, 138.65, 139.47, 144.53, 153.47, 169.69; LRMS calcd for $C_{74}H_{89}NO_{22}SSi{\cdot}Na$ 1426.5 $(M + Na)^+$, found 1426.6.

Alternative Synthesis of Tetrasaccharide Diol 28. To a solution of compound **25** (0.0983 g, 0.170 mmol) in dry benzene (90 mL) was added bis(tributyltin) oxide (0.0500 mL, 0.0935 mmol), and the resulting solution was distilled overnight with removal of water with a Dean−Stark trap. Thus formed tin ether was concentrated with a stream of dry N_2 and then further dried in vacuo. To a mixture of azeotropically dried (3 × 5 mL of benzene) compound **26** (0.0405 g, 0.0425 mmol) and freshly flame dried 4 Å molecular sieves (0.8 g) was added a solution of the tin ether in 1.8 mL of THF via cannula. Then the resulting suspension was cooled to −60 °C, and was treated with a solution of $AgBF_4$ (0.0337 g, 0.170 mmol) in 0.6 mL of THF via cannula. The reaction mixture was stirred for 2 days with exclusion of light while slowly being allowed to warm to room temperature. The reaction mixture was diluted with EtOAc (100 mL) and filtered through a pad of silica gel. The filtrate was washed with a saturated solution of $NaHCO_3$ (3 × 60 mL), and brine (1 × 60 mL). The organic layer was separated, dried (Na_2SO_4), filtered, concentrated, and submitted for chromatography (45% EtOAc in hexanes) to afford 0.0498 g (84%) of tetrasaccharide **28** as the only product. Further chromatographic separation (80% EtOAc in hexanes) afforded 0.0480 g of unused acceptor **25**.

Tetrasaccharide Pentaol 31. To a solution of tetrasaccharide **28** (0.37 g, 0.277 mmol) in MeOH (5 mL) was added K_2CO_3 (0.37 g), and the reaction mixture was stirred for 15 min. Then the solution was diluted with CH_2Cl_2 (100 mL), and filtered through a pad of silica gel, followed by a washing of the pad with EtOAc (100 mL). The filtrate was concentrated, and dried in vacuo without further purification to afford **31** (0.295 g, 85%) as a syrup: $[\alpha]^{23}_D = -18.0°$ (*c* 1.0, $CHCl_3$); FTIR (film) 3469, 3030, 2873, 1648, 1496, 1452, 1328, 1092, 909, 737 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.31 (q, 6H, $J = 6.4$ Hz, $-SiCH_2CH_3$), 0.70 (t, 9H, $J = 6.4$ Hz, $-SiCH_2CH_3$), 2.49 (br s, 1H, −OH), 2.82 (br s, 1H, −OH), 3.16 (m, 1H, $-CHNHSO_2Ph$), 3.3−3.6 (m, 12H), 3.6−3.78 (m, 6H), 3.79 (br s, 2H), 3.8−3.85 (m, 3H), 3.92 (br d, 1H, $J = 4.2$ Hz), 4.0 (br t, 1H), 4.05−4.10 (m, 2H), 4.10−4.25 (m, 3H), 4.30−4.40 (m, 6H), 4.4−4.55 (m, 7H), 4.75 (dd, 1H, $J = 2.7$, 4.9 Hz), 4.9 (d, 1H, $J = 4.2$ Hz), 6.17 (d, 1H, 6.6 Hz, $-HNSO_2Ph$), 6.31 (d, 1H, $J = 4.9$ Hz, H-1), 7.0−7.4 (m, 23H, Ar−H), 7.80 (d, 2H, $J = 6.0$ Hz, Ar−H); ^{13}C NMR ($CDCl_3$) δ 4.30, 6.72, 57.88, 68.00, 68.78, 68.84, 69.20, 70.46, 70.86, 71.39, 71.99, 73.04, 73.14, 73.31, 73.40, 73.54, 73.79, 75.72, 76.01, 76.16, 81.44, 100.15, 101.85, 102.32, 102.60, 127.30, 127.45, 127.58, 127.62, 127.65, 127.73, 128.17, 128.21, 128.30, 128.33, 128.90, 132.52, 137.71, 137.87, 137.91, 138.10, 138.62, 140.18, 144.27; HRMS calcd for $C_{71}H_{89}NO_{20}SSiNa$ 1358.536 $(M + Na)^+$, found 1358.536.

Di-triethylsilylated Iodo Sulfonamide Disaccharide 32. To a solution of lactal **23** (2.50 g, 3.36 mmol) with 4 Å molecular sieves (3.0 g) and benzenesulfonamide (2.64 g, 3.36 mmol) was added at 0 °C a freshly prepared solution of $I(sym\text{-coll})_2ClO_4$ (5 equiv) in CH_2Cl_2. The reaction mixture was stirred at room temperature for 1 h, filtered through a pad of silica gel, and washed with a saturated solution of $Na_2S_2O_3$ (3 × 25 mL), $CuSO_4$ (5 × 25 mL), and water (2 × 10 mL). The organic layer was separated, dried ($MgSO_4$), filtered, concentrated, and submitted for chromatography (5% EtOAc in CH_2Cl_2) to provide **32** (3.20 g, 92%) as a syrup: $[\alpha]^{23}_D = -19.0°$ (*c* 1.0, $CHCl_3$); FTIR (film) 3258, 2953, 2875, 1806, 1788, 1453, 1331, 1105, 849, 745 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.57 and 0.64 (2q, 12H, $J = 8.0$ Hz, $-SiCH_2CH_3$), 0.90 and 0.95 (2t, 18H, $J = 8.0$ Hz, $-SiCH_2CH_3$), 3.39 (br m, 1H, H-2), 3.60−3.70 (m, 4H), 3.78−3.83 (br m, 2H), 4.05−4.17 (m, 3H), 4.34 (dd, 1H, $J = 2.4$, 8.7 Hz), 4.45 and 4.52 (2d, 2H, $J = 12.0$ Hz, $-CH_2Ar$), 4.55 (s, 2H, $-CH_2Ar$), 4.68 (d, 1H, $J = 3.0$ Hz), 4.89 (d, 1H, $J = 8.6$ Hz), 5.29 (t, 1H, $J = 8.4$ Hz), 5.47 (d, 1H, $J = 9.6$ Hz, $-NHSO_2Ph$), 7.2−7.5 (m, 13H, Ar−H), 7.89 (d, 2H, $J = 7.6$ Hz, Ar−H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 4.54, 4.94, 6.59, 6.95, 67.94, 68.12, 68.39, 68.63, 73.12, 73.31, 73.36, 73.90, 75.26, 75.33, 76.86, 79.66, 100.04, 127.40, 127.67, 127.76, 127.93, 128.01, 128.36, 128.51, 128.60, 132.39, 137.34, 138.02, 141.31, 154.01; HRMS calcd for $C_{45}H_{64}INO_{12}SSi_2{\cdot}Na$ 1848.260 $(M + Na)^+$, found 1848.263.

Di-triethysilylated Ethylthio Sulfonamide Disaccharide 33. To a solution of iodo sulfonamide **32** (2.70 g, 2.63 mmol) in dry DMF (40 mL) was added at −40 °C EtSH (0.584 mL, 7.89 mmol), followed by dropwise addition of a solution of LHMDS (1.0 M solution in THF, 7.89 mL). The reaction mixture was stirred for 1 h while being allowed to warm to room temperature. Then the solution was neutralized with a saturated solution of NH_4Cl (10 mL), and diluted with EtOAc (50 mL). The organic layer was washed with brine (5 mL), separated, dried ($MgSO_4$), filtered, concentrated, and submitted for chromatography (3:7 EtOAc/hexanes) to provide **33** (2.30 g, 91%) as a syrup: $[\alpha]^{23}_D = -64.0°$ (*c* 1.0, $CHCl_3$); FTIR (film) 3314, 2954, 2875, 1807, 1453, 1330, 1181, 1104, 739 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.50 (q, 6H, $J = 7.9$ Hz, $-SiCH_2CH_3$), 0.624 (q, 6H, $J = 7.6$ Hz, $-SiCH_2CH_3$), 0.87 (t, 9H, $J = 7.9$ Hz, $-SiCH_2CH_3$), 0.94 (t, 9H, J= 8.0 Hz, $-SiCH_2CH_3$), 1.11 (t, 3H, $J = 7.4$ Hz, $-SCH_2CH_3$), 2.48 (m, 2H,-SCH_2CH_3), 3.35 (m, 1H, H-2), 3.85−3.68 (m, 6H), 3.86 (br m, 1H), 3.97 (br t, 1H), 4.06 (br t, 1H, $J = 6.6$ Hz), 4.49 (s, 2H, $-CH_2Ar$), 4.57 (s, 2H, $-CH_2Ar$), 4.55 (m, 1H), 4.61 (d, 1H, $J = 6.3$ Hz, H-1), 4.67 (d, 1H, $J = 4.0$ Hz, H′-4), 4.87 (d, 1H, $J = 8.8$ Hz, H-1), 5.50 (d, 1H, $J = 8.8$ Hz, $-NHSO_2Ph$), 7.2−7.4 (m, 10H, Ar−H), 7.45−7.55 (m, 3H, Ar−H), 7.94 (d, 2H, $J = 7.2$ Hz, Ar−H); ^{13}C NMR ($CDCl_3$) δ 4.28, 4.45, 6.56, 6.71, 14.58, 25.64, 57.42, 67.82, 69.05, 69.68, 70.37, 71.80, 73.13, 73.66, 73.75, 76.45, 76.64, 77.05, 82.68, 100.94, 127.52,

127.57, 127.79, 127.83, 128.22, 128.35, 128.84, 132.25, 137.49, 138.01, 140.70; HRMS (FAB) calcd for $C_{47}H_{69}NO_{12}S_2Si_2Na$ 982.380, found 982.370.

Hexasaccharide Tetraol 35. To a solution of disaccharide **33** (0.200 g, 0.208 mmol) and tetrasaccharide **31** (0.278 g, 0.208 mmol) in CH_2Cl_2/Et_2O (1:2, 15 mL), with 4 Å molecular sieves (1.20 g) and di-*tert*-butylpyridine (0.180 mL, 0.778 mmol) at −10 °C, was added MeOTf (0.0880 mL, 0.778 mmol). The reaction mixture was stirred for 2 h, allowed to warm to 0 °C, and stirred for 24 h. Then the suspension was diluted with EtOAc (15 mL) and filtered through a pad of silica gel, and the filtrate was washed with a saturated solution of $NaHCO_3$ (2 × 10 mL). The organic layer was separated, dried ($MgSO_4$), filtered, concentrated, and submitted for chromatography (1:1 EtOAc/hexanes) to provide **35** (0.276 g, 60%) as a syrup: $[\alpha]^{23}_D = -23.0°$ (c, 1.0, $CHCl_3$); FTIR (film) 3490, 3030, 2875, 1807, 1649, 1453, 1330, 1093, 909, 743 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.25 (m, 6H, $-SiCH_2CH_3$), 0.37 (q, 6H, J = 7.9 Hz, $-SiCH_2CH_3$), 0.71 (t, 9H, J = 7.9 Hz, $-SiCH_2CH_3$), 0.76 (t, 9H, J = 7.9 Hz, $-SiCH_2CH_3$), 0.86 (t, 9H, J = 7.9 Hz, $-SiCH_2CH_3$), 2.46 (s, 1H, −OH), 2.52 (s, 1H, −OH), 3.15 (m, 1H, $-CHNHSO_2Ph$), 3.21 (m, 1H, $-CHNHSO_2Ph$), 3.28 (dd, 1H, J = 3.0, 9.2 Hz), 3.37−3.55 (m, 7H), 3.55−3.79 (m, 14H), 3.82 (br s, 2H), 3.89 (br s, 1H), 3.94−4.11 (m, 4H), 4.18 (br s, 1H), 4.28 (m, 1H), 4.33−4.40 (m, 3H), 4.41 (s, 2H, $-CH_2Ar$), 4.44−4.47 (m, 3H), 4.49 (s, 2H, $-CH_2Ar$), 4.52 (m, 1H), 4.54 (s, 2H, $-CH_2Ar$), 4.55−4.63 (m, 2H), 4.66 (dd, 2H, J = 3.9, 6.0 Hz), 4.74 (dd, 1H, J = 2.8, 6.1 Hz), 5.28 (d, 1H, J = 7.5 Hz, $-NHSO_2Ph$), 5.51 (d, 1H, J = 8.3 Hz, $-NHSO_2Ph$), 6.32 (d, 1H, J = 6.0 Hz, H-1), 7.10−7.55 (m, 41H, Ar−H), 7.83 (d, 2H, J = 7.4 Hz, Ar−H), 7.89 (d, 2H, J = 7.5 Hz, Ar−H); ^{13}C NMR ($CDCl_3$) δ 4.36, 4.44 (2C), 6.55, 6.67, 6.87, 58.52, 58.82, 67.61, 67.76, 67.82, 68.11, 68.71, 68.94, 69.07, 69.49, 69.75, 69.78, 69.92, 70.47, 70.73, 72.51, 72.92, 73.26, 73.31, 73.34, 73.37, 73.68, 73.85, 74.26, 74.61, 75.21, 75.27, 75.75, 75.90, 76.40, 77.10, 82.97, 83.60, 99.93, 100.49, 101.64, 102.74, 102.82, 103.08, 127.20, 127.38, 127.42, 127.49, 127.54, 127.62, 127.72, 127.76, 127.88, 128.10, 128.17, 128.26, 128.30, 128.40, 128.84, 128.97, 132.38, 132.71, 137.37, 137.57, 137.87, 137.90, 138.12, 138.18, 138.76, 139.9, 140.62, 144.45, 154.16; HRMS (FAB) calcd $C_{116}H_{152}N_2O_{32}S_2Si_3Na$ 2255.900 $(M + Na)^+$, found 2255.898.

Alternative Synthesis of Hexasaccharide Tetraol 35. To a solution of compound **31** (0.125 g, 0.0932 mmol) in dry benzene (90 mL) was added bis(tributyltin) oxide (0.0272 mL, 0.0513 mmol). The resulting solution was distilled overnight with removal of water with a Dean−Stark trap. Thus formed tin ether **34** was concentrated with a stream of dry N_2 and then further dried in vacuo. To a mixture of azeotropically dried (4 × 5 mL of benzene) compound **32** (0.0224 g, 0.0233 mmol) and freshly flame dried 4 Å molecular sieves (0.76 g) was added a solution of the tin ether **34** in 2.0 mL of THF via cannula. The resulting suspension was cooled to −60 °C, and was treated with a solution of $AgBF_4$ (0.0185 g, 0.0932 mmol) in 1.0 mL of THF via cannula. The reaction mixture was stirred for 4 days with exclusion of light while slowly being allowed to warm to room temperature. The reaction mixture was diluted with EtOAc (100 mL) and filtered through a pad of silica gel. The filtrate was washed with a saturated solution of $NaHCO_3$ (3 × 60 mL) and brine (1 × 60 mL), and the organic layer was separated, dried (Na_2SO_4), filtered, concentrated, and submitted for chromatography (40% EtOAc in hexanes) to provide 0.0321 g (62%) of hexasaccharide **35** as the only product. Further elution (80% EtOAc in hexanes) provided 0.0801 g of unused acceptor **31**.

Tetraacetylated Hexasaccharide 36. To a solution of hexasaccharide **35** (0.175 g, 0.0784 mmol) in dry CH_2Cl_2 (20 mL) were added pyridine (2 mL), Ac_2O (2 mL) and DMAP (catalytic). The reaction mixture was stirred for 24 h and washed with a saturated solution of $CuSO_4$ (3 × 10 mL) and $NaHCO_3$ (3 × 10 mL), and the organic layer was separated, dried ($MgSO_4$), filtered, concentrated, and submitted for chromatography (25% EtOAc in hexanes) to give rise to **36** (0.179 g, 95%) as a syrup: $[\alpha]^{23}_D = -30.0°$ (*c* 1.0, $CHCl_3$); FTIR (film) 3030, 2953, 1809, 1748, 1453, 1369, 1221, 1094, 738 (cm^{-1}); ^1H NMR ($CDCl_3$) δ 0.24 (m, 12H, $-SiCH_2CH_3$), 0.54 (q, 6H, J = 8.1 Hz, $-SiCH_2CH_3$), 0.68 (br t, 9H, J = 7.7 Hz, $-SiCH_2CH_3$), 0.70 (br t, 9H, J = 7.8 Hz, $-SiCH_2CH_3$), 0.87 (t, 9H, J = 7.9 Hz, $-SiCH_2CH_3$), 1.86 (s, 3H, $-COCH_3$), 1.90 (s, 3H, $-COCH_3$), 2.08 (s, 2H, $-COCH_3$), 2.15 (s, 3H, $-COCH_3$), 3.03 (br d, 1H, J = 7.7 Hz, $-CHNHSO_2Ph$), 3.2−3.4 (m, 8H), 3.4−3.85 (m, 30H), 3.85−4.2 (m, 8H), 4.20−4.6 (m, 29H), 4.75 (q, 1H, J = 3.1, 6.0 Hz), 4.8 (br d, 1H, J = 8.2 Hz), 4.88 (d, 1H, J = 3.5 Hz), 5.10 (m, 2H, J = 8.8 Hz), 5.26 (d, 1H, J = 2.5 Hz), 5.33 (d, 1H, J = 8.7 Hz, $-NHSO_2Ph$), 5.42 (d, 1H, J = 2.6 Hz), 5.90 (d, 1H, J = 10.8 Hz, $-NHSO_2Ph$), 6.31 (d, 1H, J = 6.0 Hz, H-1), 7.1−7.5 (m, 41H, Ar−H), 7.82 and 7.89 (2br m, 4H, Ar−H); ^{13}C NMR ($CDCl_3$) δ 4.10, 4.14, 4.49, 6.52, 6.60, 6.64, 20.75, 20.81, 20.09, 21.46, 55.97, 56.73, 67.83, 68.41, 68.63, 68.80, 69.35, 69.82, 69.88, 70.12, 70.49, 71.09, 71.20, 71.71, 72.84, 72.95, 73.11, 73.38, 73.53, 73.60, 73.67, 73.74, 73.79, 74.10, 74.33, 74.40, 75.32, 75.78, 75.89, 76.18, 76.77, 77.20, 99.75, 100.15, 100.38, 100.53, 101.55, 102.17, 127.26, 127.34, 127.42, 127.47, 127.52, 127.58, 127.61, 127.62, 127.66, 127.73, 127.73, 127.80, 127.85, 128.14, 128.21, 128.26, 128.39, 128.41, 128.66, 128.99, 131.93, 132.60, 137.47, 137.66, 137.77, 137.92, 138.31, 138.43, 138.77, 139.96, 141.74, 144.48, 154.07, 169.44, 169.60, 169.64, 171.34; LRMS (ES) calcd for $C_{124}H_{160}N_2O_{36}S_2Si_3Na$ 2424.1 $(M + Na)^+$, found 2424.1.

Hexasaccharide Triol 37. To a solution of hexasaccharide **36** (0.175 g, 0.0725 mmol) in dry THF (5 mL) was added a solution of TBAF (1.0 M THF) and AcOH (1:1, 0.725 mL, 10 equiv). The solution was stirred at 35 °C for 24 h, diluted with EtOAc (10 mL), and washed with a saturated solution of $NaHCO_3$ (2 × 5 mL), and the organic layer was separated, dried ($MgSO_4$), filtered, concentrated, and submitted for chromatography (4:1 EtOAc/hexanes) to provide **37** (0.143 g, 93%) as a white glassy substance: $[\alpha]^{23}_D = -6.0°$ (*c* 1.0, $CHCl_3$); FTIR (film) 3472, 3028, 2870, 1805, 1745, 1369, 1225, 1161, 1069, 752 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.88, 1.92, 2.01, 2.02 (4s, 3H each, $-COCH_3$), 2.85 (br t, 1H, J = 8.2 Hz, $-CHNHSO_2Ph$), 3.02 (br q, 1H, J = 7.0 Hz, $-CHNHSO_2Ph$), 3.20 (dd, 1H, J= 7.6, 8.0 Hz), 3.27 (dd, 2H, J = 4.7, 10.0 Hz), 3.3−3.8 (m, 36H), 3.87 (br s, 2H), 4.03 (br d, 3H), 4.10 (br s, 1H), 4.2−4.65 (m, 33H), 4.66 (d, 1H, 5.1 Hz), 4.77 (q, 1H, J = 3.2 Hz), 5.01 (dd, 1H, J= 8.3, 9.7 Hz), 5.12 (dd, 1H, J = 8.2, 9.8 Hz), 5.25 (d, 1H, J = 3.2 Hz), 5.39 (d, 1H, J = 3.1 Hz), 6.32 (d, 1H, J = 6.1 Hz, H-1), 7.10−7.45 (m, 41H, Ar−H), 7.78 (m, 4H, Ar−H); ^{13}C NMR ($CDCl_3$) δ 20.69, 20.78, 21.18 (2C), 59.66 (2C), 67.79, 67.88, 68.12, 68.47, 69.42, 69.64, 70.13, 70.48, 71.14, 72.25, 72.70, 72.86, 73.11, 73.22, 73.38, 73.44, 73.50, 73.56, 73.71, 73.93, 74.29, 75.75, 77.20, 79.97, 80.25, 99.66, 100.99, 101.07, 101.18, 101.45, 101.55, 127.22, 127.29, 127.34, 127.63, 127.67, 127.73, 127.75, 127.80, 127.84, 128.00, 128.15, 128.26, 128.36, 128.41, 128.49, 128.61, 132.07, 132.20, 137.13, 137.34, 137.70, 137.71, 137.92, 138.21, 138.66, 140.96, 141.23, 144.43, 145.90, 170.04, 170.09, 170.15, 170.16; HRMS calcd for $C_{106}H_{118}N_2O_{36}S_2Na$ 2081.860, found 2081.676.

Nonasaccharide 39. To a solution of hexasaccharide **37** (0.140 g, 0.0680 mmol) and the known β-fluorofucose **38** (0.239 g, 0.530 mmol) in dry toluene (10 mL) with 4 Å molecular sieves at 0 °C was added 2,6-di-*tert*-butylpyridine (0.157 mL, 0.680 mmol) followed by a solution of $Sn(OTf)_2$ (0.228 g, 0.53 mmol) in THF (1 mL). The suspension was stirred for 36 h at room temperature and quenched with Et_3N (0.5 mL). The reaction mixture was stirred for an additional 3 min, diluted with EtOAc (25 mL), and filtered through a pad of silica gel. The organic layer was washed with a saturated solution of $NaHCO_3$ (2 × 10 mL), and separated, dried ($MgSO_4$), filtered, concentrated, and submitted for chromatography (1:1 EtOAc/hexanes) to provide **39** (0.135 g, 60%) as a syrup: $[\alpha]^{23}_D = -88.0°$ (*c* 1.0, $CHCl_3$); FTIR (film) 3029, 2869, 1817, 1745, 1722, 1452, 1270, 1096, 697 (cm^{-1}); ^1H NMR ($CDCl_3$) δ 0.87 (d, 3H, J = 6.2, $-CH_3$), 0.93 (d, 3H, J = 6.3 Hz, $-CH_3$), 1.07 (d, 3H, J = 6.4 Hz, $-CH_3$), 1.61 (s, 3H, $-COCH_3$), 1.80 (s, 3H, $-COCH_3$), 1.93 (s, 3H, $-COCH_3$), 1.98 (s, 3H, $-COCH_3$), 3.2−3.4 (m, 6H), 3.4−3.9 (m, 27H), 3.9−4.0 (m, 2H), 4.0−4.2 (m, 8H), 4.2−4.65 (m, 34H), 4.65−4.8 (m, 7H), 4.84 (d, 1H, J = 5.3 Hz), 4.89 (br t, 1H, J = 8.4 Hz), 5.05−5.15 (m, 2H), 5.28 (br s, 1H), 5.35 (d, 1H, J = 2.8 Hz), 5.40 (br s, 1H), 5.53 (br s, 3H), 5.65 (d, 1H, J= 5.6 Hz), 6.33 (d, 1H, J = 6.0 Hz, H-1), 7.0−7.3 (m, 68H, Ar−H), 7.3−7.45 (m, 9H, Ar−H), 7.45−7.57 (m, 3H), 7.65 (d, 2H, J = 7.6 Hz, Ar−H), 7.75 (d, 2H, J = 7.5 Hz, Ar−H), 7.99, 7.96, 7.94 (3d, 6H, J = 7.5 Hz, Ar−H); ^{13}C NMR ($CDCl_3$) δ 16.09, 16.13, 16.17, 20.38, 20.60, 21.16, 21.23, 56.42, 58.41, 64.97, 65.24, 65.59, 67.39, 67.40, 67.45, 67.86, 68.53, 68.71, 69.16, 69.35, 70.17, 70.25, 70.83, 70.99,

71.23, 71.25, 71.47, 71.50, 71.90, 72.55, 74.60, 73.14, 73.22, 73.39, 73.44, 73.49, 73.58, 73.66, 73.79, 73.81, 73.86, 74.72, 74.98, 75.37, 75.53, 75.71, 75.85, 76.10, 76.64, 76.94, 77.18, 96.01, 78.77, 79.40, 96.01, 97.38, 97.72, 98.67, 99.74, 100.00, 100.16, 100.24, 100.66, 127.11, 127.19, 127.25, 127.30, 127.37, 127.50, 127.60, 127.69, 127.79, 127.82, 127.88, 127.92, 128.02, 128.10, 128.12, 128.15, 128.29, 128.31, 128.39, 128.43, 128.99, 129.76, 129.84, 130.07, 132.27, 132.65, 132.90, 132.91, 133.18, 137.45, 137.48, 137.70, 137.73, 137.86, 137.90, 137.95, 138.03, 138.14, 138.17, 138.20, 138.26; LRMS calcd for $C_{187}H_{196}N_2O_{51}S_2$·2Na 3395.2 $(M + 2Na)^{2+}$, found 3395.2.

Nonasaccharide Thioglycoside 41. To a solution of a nonasaccharide **39** (0.0502 g, 0.0150 mmol) in dry CH_2Cl_2 (1 mL) with 4 Å molecular sieves (0.10 g) was added a solution of dimethyldioxirane in acetone (ca. 0.08 M, 3 mL). The solution was stirred for 45 min, and the solvent was removed with a stream of dry N_2, and further dried in vacuo (10 min). The resultant crude epoxide was dissolved in CH_2Cl_2 (1 mL), and was treated with EtSH (1 mL) and TFAA (0.005 mL) at −78 °C. The reaction mixture was stirred for 6 h while warming to room temperature. Volatiles were evaporated with a stream of dry N_2 and in vacuo. The crude mixture was dissolved in CH_2Cl_2 (1 mL) and then treated with acetic anhydride (0.5 mL) and pyridine (0.5 mL). After 24 h of stirring, the reaction mixture was concentrated under reduced pressure and purified by flash chromatography (2:3 EtOAc/hexanes) to afford thioglycoside **41** (0.0302 g, 60%) as a syrup: $[\alpha]^{23}_D = -0.030°$ (*c* 1.0, $CHCl_3$); FTIR (film) 3064, 2875, 1808, 1744, 1720, 1453, 1369, 1250, 1090, 730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (d, 3H, $J = 6.0$ Hz, $-CH_3$), 0.93 (d, 3H, $J = 6.1$ Hz, $-CH_3$), 1.07 (d, 3H, $J = 6.2$ Hz, $-CH_3$), 1.18 (dt, 3H, $J = 1.5$ Hz, $-SCH_2CH_3$), 1.67 (s, 3H, $-COCH_3$), 1.81 (s, 3H, $-COCH_3$), 1.92 (s, 3H, $-COCH_3$), 1.97 (s, 3H, $-COCH_3$), 2.05 (s, 3H, $-COCH_3$), 2.62 (m, 2H, $-SCH_2CH_3$), 3.10 (br m, 1H), 3.2−3.4 (m, 7H), 3.4−3.75 (m, 24H) 3.8 (m, 3H), 3.82 (dd, 2H), 3.85−4.0 (m, 4H), 4.0−4.2 (m, 4H), 4.2−4.5 (m, 26H), 4.5−4.63 (m, 1H), 4.63−4.8 (m, 8H), 4.8−5.0 (m, 5H), 5.11 (d&m, 2H), 5.21 (d, 1H), 5.35 (d, 1H), 5.40 (br s, 1H), 5.54 (br s, 2H), 5.64 (br s, 1H), 7.0−8.2 (m, 90H, Ar−H); LRMS calcd for $C_{191}H_{204}N_2O_{53}S_3$·$NH_4$ 3369.2 $(M + NH_4)^+$, found 3469.0.

Azide 42. To a solution of thioglycoside **41** (0.0290 g, 0.00860 mmol), azidohyrin **40** (0.0357 g, 10.0 equiv) at 0 °C in dry Et_2O/CH_2Cl_2 (2:1, 1.5 mL), and 4 Å molecular sieves (0.10 g) was added MeOTf (0.00389 mL, 4 equiv). The reaction mixture was stirred while warming to room temperature. After 24 h of stirring, the suspension was quenched with Et_3N (0.020 mL), diluted with EtOAc (5 mL), filtered through a pad of silica gel, and washed with a saturated solution of $NaHCO_3$ (2 × 5 mL), and the organic layer was separated, dried ($MgSO_4$), filtered, concentrated, and submitted for column chromatography (1:1 EtOAc/hexanes) to provide azide **42** (0.0181 g, 55%) as a syrup: $[\alpha]^{23}_D = -17.4°$ (*c* 0.7, $CHCl_3$); FTIR (film) 3030, 2923, 2100, 1814, 1744, 1602, 1451, 1368, 1269, 1222, 1096, 743 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80 (br t, 3H, $-CH_3$), 0.85 (d, 3H, $J = 6.3$ Hz, $-CH_3$), 0.93 (d, 3H, $J = 6.3$ Hz, $-CH_3$), 1.07 (d, 3H, $J = 6.4$ Hz, $-CH_3$), 1.18 (br s, 23H, aliphatic $-CH_2$), 1.33 (br m, 2H) 1.52 (s, 3H, $-COCH_3$), 1.81 (s, 3H, $-COCH_3$), 1.94 (s, 3H, $-COCH_3$), 2.02 (s, 6H, 2-$COCH_3$), 3.20 (m, 2H), 3.3 (m, 5H), 3.38−3.43 (m, 5H), 3.5−3.75 (m, 20H), 3.75−3.83 (br d, 2H), 3.85 (br q, 2H, $J = 5.8$, 8.8 Hz), 3.9−4.3 (m,4H), 4.3−4.54 (m, 3H), 4.54−4.63 (m, 7H), 4.63−4.82 (m,7H), 4.83 (d, 1H, $J = 5.0$ Hz), 4.89 (t, 1H, $J = 9.6$ Hz), 5.10 (d, 1H, $J = 3.5$ Hz), 5.12 (m, 1H), 5.26 (br s, 1H), 5.34 (m, 1H), 5.39 (br d, 2H, $J = 8.2$ Hz), 5.47 (d, 1H, $J = 8.2$ Hz), 5.53 (br s, 2H), 5.60 (d, 1H, $J = 5.5$ Hz), 5.69 (dt, 1H, $J = 6.6$, 14.7 Hz), 7.0−7.3 (m, 80H, Ar−H), 7.33−7.43 (m, 8H), 7.5 (m, 4H), 7.65 (d, 2H, $J = 7.6$ Hz, Ar−H), 7.78 (d, 2H, $J = 7.5$ Ar-H), 7.97 (m, 6H, Ar−H); LMRS calcd for $C_{214}H_{239}N_5O_{55}S_2$·2$NH_4$ 3858.5 $(M + 2NH_4)^{2+}$, found 3859.0.

Protected KH-1 Antigen 43. To a solution of azide **42** (0.0181 g, 0.00473 mmol) in EtOAc (3 mL) were added Lindlar's catalyst (0.050 g) and palmitic anhydride (0.0102 g, 0.0200 mmol), and the reaction mixture was stirred at room temperature under a H_2 atmosphere for 24 h. The reaction mixture was filtered through a pad of silica gel, rinsed with EtOAc (20 mL), concentrated, and submitted for chromatography (1:1 EtOAc/hexanes) to provide amide **43** (0.0165 g, 85%) as a syrup: $[\alpha]^{23}_D = -36.0°$ (*c* 0.5, $CHCl_3$); FTIR (film) 3025, 2923, 1814, 1745, 1657, 1451, 1367, 1266, 1095, 742 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80 (m, 6H, $-CH_3$), 0.85 (d, 3H, $J = 6.0$ Hz, $-CH_3$), 0.93 (d, 3H, $J = 6.4$ Hz, $-CH_3$), 1.07 (d, 3H, $J = 6.4$ Hz, $-CH_3$), 1.18 (br s, 48H, aliphatic $-CH_2$), 1.6 (m, 4H), 1.84 (s, 6H, 2-$COCH_3$), 2.0 (s, 3H, $-COCH_3$), 2.08 (s, 6H, $-COCH_3$) 3.2 (br m, 1H), 3.3 (br m, 6H), 3.3−3.75 (m, 27H), 3.78 (m, 3H), 3.85 (m, 2H), 3.95 (m, 3H), 4.2 (dd,4H), 4.15−4.5 (m, 23H), 4.5−4.75 (m, 9H), 4.75−4.8 (m, 4H), 4.8−4.95 (m, 3H), 5.1 (br s, 2H), 5.26 (m, 2H), 5.57 (m, 2H), 5.59 (m, 2H), 7.00−7.35 (m, 79H, Ar−H), 7.4 (m, 8H, Ar−H), 7.5 (m, 3H, Ar−H), 7.65 (d, 2H), 7.75 (2d, 2H), 7.96 (m, 6H); ^{13}C NMR (anomeric carbons) δ 98.06, 100.11 (2C), 100.47, 101.28, 102.74, 102.89, 102.97, 103.16; LRMS calcd for $C_{230}H_{271}N_3O_{56}S_2$·$NH_4$ 4052.8 $(M + NH_4)^+$, found 4052.0.

KH-1 Antigen (1). To a blue solution of sodium (0.018 g) in liquid ammonia (5 mL) under N_2 at −78 °C was added a solution of protected KH-1 derivative **43** (0.0202 g, 0.00500 mmol) in dry THF (1 mL). After 45 min of reflux at −78 °C, the reaction mixture was quenched with absolute MeOH (5 mL). Most of the ammonia was removed with a stream of dry nitrogen, and the solution was diluted with MeOH (5 mL) and stirred overnight. The solution was neutralized with Et_3N·HCl, stirred for an additional 15 min, and dried with a stream of dry nitrogen. The crude material obtained was then suspended in DMF (1.0 mL), THF (1.0 mL), and Et_3N (1.0 mL) and treated with Ac_2O (1 mL) and a catalytic amount of DMAP. The reaction mixture was stirred for 24 h. The solution was concentrated, passed through a pad of silica gel with the help of EtOAc, and again concentrated. The syrup obtained was dissolved in MeOH (5 mL), treated with MeONa (0.005 mL), neutralized with Dowex 50-X8 after 24 h, filtered, and concentrated to give 0.00711 g (70%) of KH-1 antigen (**1**). An analytical sample was prepared by RP column chromatography, eluting with water−5% methanolic water, followed by lyophilization to deliver **1** as a white foam: ^1H NMR (DMSO) δ 0.95 (m, 3H), 1.1−1.35 (3d, 9H, $-CH_3$), 1.38 (br m, multiple protons, aliphatic $-CH_2$), 1.5 (m, 9H), 1.85 (s, 6H, $-CHNHCOCH_3$), 1.9 (m, 2H), 2.0−2.20 (m, 6 H), 3.0−4.0 (m, multiple protons), 4.01 (q, 1H, $J = 6.5$ Hz), 4.17 (d, 1H, $J = 7.5$ Hz), 4.27 (br d, 1H), 4.34 (br m, 1H), 4.40 (d, 1H, $J = 7.0$ Hz), 4.59 (m, 1H), 4.65 (d, 1H, $J = 8.0$ Hz), 4.67 (d, 1H, $J = 4.0$ Hz), 4.72 (d, 1H, $J = 7.0$ Hz), 4.73 (d, 1H, $J = 7.5$ Hz), 4.87 (d, 1H, $J = 3.5$ Hz), 4.97 (d, 1H, $J = 3.5$ Hz), 5.37 (dd, 1H, $J = 7.0$ Hz, 15.5 Hz), 5.55 (dt, 1H, 6.5 Hz, 15.0 Hz); ^{13}C NMR (DMSO, anomeric carbons) δ 101.79, 102.40, 103.60, 103.78, 104.51 (2C), 105.18, 106.70, 106.77; HRMS (FAB) calcd for $C_{92}H_{163}N_3O_{45}$·Na 2053.046 $(M + Na)^+$, found 2053.049.

Allyl Glycoside 44. To a solution of sodium (0.060 g) in liquid ammonia (8 mL) under N_2 at −78 °C was added a solution of nonasaccharide glycal **39** (0.0469 g, 0.0140 mmol) in dry THF (2 mL). After 45 min of reflux at −78 °C, the reaction mixture was quenched with absolute MeOH (5 mL). Most of the ammonia was removed with a stream of dry nitrogen and then diluted with MeOH (5 mL) and stirred overnight. The basic solution was neutralized with Dowex 50-X8 (0.557 g), and the mixture was stirred for an additional 15 min, and filtered. The resins were washed with a saturated solution of NH_3 in MeOH (4 × 50 mL), and all filtrates were combined and dried with a stream of dry nitrogen. The crude material obtained was then suspended in DMF (1.0 mL), THF (1.0 mL), and Et_3N (1.0 mL), treated with Ac_2O (1 mL) and a catalytic amount of DMAP, and stirred for 24 h. The solution was concentrated, passed through a pad of silica gel with the help of EtOAc, and concentrated. The syrup obtained was dissolved in CH_2Cl_2 and then at 0 °C under N_2 treated with dimethyldioxirane solution in acetone (ca. 0.08 M, 6 mL) and stirred for 45 min. Solvent was removed with a stream of N_2. The resultant syrup (0.0400 g) was redissolved in allyl alcohol (5 mL). After 24 h, excess allyl alcohol was evaporated, and the crude syrup was dissolved in MeOH (5 mL) and treated with MeONa (25% in MeOH, 0.060 mL). After 24 h, the mixture was neutralized with Dowex 50-X8, filtered, and concentrated to provide allyl glycoside **44** (0.0130 g, 60%). An analytical sample was prepared by RP column chromatography, eluting with water−5% methanolic water, followed by lyophilization to obtain a white foam: $[\alpha]^{23}_D = -58.0°$ (*c* 0.6, H_2O); ^1H NMR (D_2O) δ 1.12 (d, 3H, $J = 6.5$ Hz, $-CH_3$), 1.21 (d, 3H, $J = 7.0$ Hz, $-CH_3$), 1.24 (d, 3H, $J = 6.5$ Hz, $-CH_3$), 2.00 (1.35 (3d, 9H, $-CH_3$), 2.0 (s, 6H, $-COCH_3$), 3.31 (br t, 1H, $-CHNHAc$), 3.4−4.0 (m, multiple protons), 4.06 (d, 1H, $J = 3.0$ Hz), 4.13 (d, 1H, $J = 3.0$ Hz), 4.22 (m, 2H), 4.41 (d, 1H, $J = 8.0$ Hz,

anomeric), 4.49 (d, 1H, $J = 8.0$ Hz, anomeric), 4.51 (d, 1H, $J = 8.5$ Hz, anomeric), 4.69 (d, 2H, $J = 8.5$ Hz, 2 anomeric), 4.79 (q, 1H, $J = 7.0$ Hz), 4.86 (q, 1H, $J = 7.0$ Hz), 5.09 (d, 1H, $J = 4.0$ Hz, anomeric), 5.10 (d, 1H, $J = 4.0$ Hz, anomeric), 5.25 (d, 1H, $J = 3.0$ Hz, anomeric) 5.36 (d, 1H, $J = 17$ Hz, $-CH{=}CH_2$), 5.95 (m, 1H, $-CH{=}CH_2$); ^{13}C NMR (D_2O, anomeric carbons) δ 101.31 (2C), 102.20, 102.91, 103.79, 104.50, 105.23 (2C), 105.70; HRMS (FAB) calcd for $C_{61}H_{102}N_2O_{43}$·Na 1573.575 $(M + Na)^+$, found 1573.568.

Synthesis of KH-1 Aldehyde. Ozone gas was bubbled through a solution of 0.004 g of KH-1 allyl glycoside **50** in 5 mL of methanol for 10 min while the solution was stirred vigorously at −78 °C. The excess ozone was then displaced with nitrogen over a period of 5 min. To the solution was added 0.10 mL of methyl sulfide. The reaction mixture was stirred for 2 h at room temperature.

Conjugation of KH-1 Aldehyde with KLH. To a solution of 0.002 g of KH-1 aldehyde and 0.004 g of keyhole lympet hemocyanin (KLH) in 1.0 mL of 0.1 M phosphate-buffered saline (PBS), pH 7.2, was added 0.0020 g of sodium cyanoborohydride, and the mixture was incubated under gentle agitation at 37 °C. After 16 h, an additional 0.001 g of sodium cyanoborohydride was added, and the incubation was continued for another 22 h. The unreacted KH-1 aldehyde was removed completely with multiple washes using a Amicon Centriprep with molecular weight cutoff value of 30 000, with 6−7 changes of PBS at 4 °C. The epitope ratio was determined by estimating protein content by BioRad dye binding protein assay and carbohydrate by a HPAEC-PAD assay. The epitope ratio was 141:1.

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Supporting Information Available: Experimental procedures for **30**, **45**, **46**, **47**, **48**, **49**, and **50** and NMR spectra (^1H for **1**, **16**, **19**, **20**−**33**, **35**−**37**, **39**, and **41**−**50**, ^{13}C for **16**, **19**, **20**−**33**, **35**−**37**, **39**, and **45**−**49**, and HMQC for **1**, **43**, **44**, and **50**) (67 pages). See any current masthead page for ordering information and Web access instructions.

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