Total Syntheses of Tumor-Related Antigens N3: Probing the Feasibility Limits of the Glycal Assembly Method

Hyunjin M. Kim,[†,‡,§] In Jong Kim,[†,||] and Samuel J. Danishefsky*[,†,‡]

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

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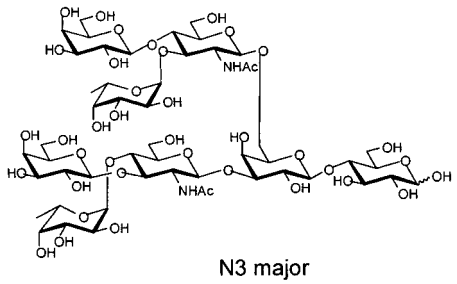
Abstract: The total syntheses of two octasaccharide antigens isolated from human milk, **1** and **2**, and their corresponding allyl glycosides, **3** and **4**, have been achieved by utilizing the glycal method. Convergent assembly of the core hexasaccharides and concurrent introduction of two α-L-fucosyl moieties at the late stage of the syntheses provided these complex carbohydrates in a concise manner. With synthetic material obtained, biological evaluations of these antigens as potential gastrointestinal cancer immunotherapeutic agents have been initiated.

Background

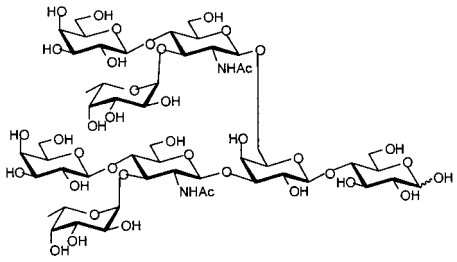
Human milk contains numerous oligosaccharides. Their compositions are largely dependent on the blood type of the lactating mother. These oligosaccharides can be classified as either "acidic" or "neutral," depending on the presence or absence of sialic acid moieties.[1] Due to the difficulties associated with the isolation of homogeneous carbohydrate entities in decent quantity from such sources, and due to the intricacies of the determination of function in the complex arena of glycobiology, the specific roles of such carbohydrates are not yet known, though progress is certainly being realized.[2] For instance, it has been found that some of these entities present in milk, which survive the digestive process of the baby, are similar to tumor-associated antigens.[3] Given the roles of carbohydrates in inter- and intracellular signaling, such oligosaccharide constellations may have a role in infant growth and development.

Herein we describe research related to two such milk-derived antigens[1,4] known as N3 major and N3 minor. These substances isolated from human milk are found in "neutral" oligosaccharide fractions. The compounds are the octasaccharides, difucosyllacto-*N*-hexaose (**1**) and difucosyllacto-*N*-neohexaose (**2**) (Figure 1). The major N3 component, which will henceforth be called "N3 major", is difucosyllacto-*N*-hexaose (**1**). Inspection of its structure reveals the presence of Le^a and Le^x moieties branching from a central lactose framework. By contrast, the minor component of N3 (compound **2**) displays two Le^x subunits projecting from the lactose core. These two octasaccharides will be collectively described as N3 antigens.



N3 major

Difucosyllacto-*N*-hexaose (**1**)



N3 minor

Difucosyllacto-*N*-neohexaose (**2**)

Figure 1.

Although the Le^a trisaccharide moiety is not known per se to be a tumor-associated antigen, Le^x has been so recognized, and it is indeed closely related to various cancers. In fact, the Le^x antigen is often referred to as an oncofetal antigen due to its transient expression during ontogeny and its reappearance in tumors.[5] Increased expression of Le^x antigen has been observed in most common human cancers including those of leukocyte, stomach, liver, lung, and colon origin. It is interesting to note that the cell surface expression of Le^x antigen occurs with a higher frequency in early gastric cancer than in advanced stage disease.[6] In addition, Le^x is present in 81% of the cases of histologically classified undifferentiated gastric cancer and 67% of differentiated gastric cancer. Since the N3 antigens are

† Sloan-Kettering Institute for Cancer Research.
‡ Columbia University.
§ Present address: Division of Chemistry and Chemical Engineering, California Institute of Technology, 1201 E. California Blvd (Mail Code: 164-30), Pasadena, CA 91125.
|| Present address: Kanazawa University, Faculty of Pharmaceutical Sciences, Takara-machi 13-1, Kanazawa, 920, Japan.

(1) Kobata, A. *Methods Enzymol.* **1972**, *28*, 262.
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(6) Dohi; et al. *Gastroenterol. Jpn.* **1989**, *24*, 238.

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composed of either Le^a and Le^x or two Le^x antigens, it would not be too surprising for them to be overexpressed in various tumors. As it turns out, the N3 antigens have been particularly associated with gastrointestinal (GI) cancer cells.

A recently filed patent claims the use of the N3 antigen as a probe for gastrointestinal cancer.[7] In the patent, antibody titers to N3 antigens in the sera of GI cancer patients were measured and it was found that the titers are higher for the GI cancer sera than for the normal sera. Using a cutoff titer of 1:80, 93% of normal sera fell below this titer, while 87% of early stage, 58% of intermediate stage, and 75% late stage carcinoma sera had titers above this dilution. It is of particular interest that the percentage of patients having anti-N3 titer over 1:80 was greater in the early stage than in the later stages. Thus, it was envisioned that measurement of anti-N3 antibody titers could be a useful detection method for early stage GI cancer. This finding and claim argued to us the case for initiating studies of N3 antigens in cancer vaccinology.

However, the supply of N3 antigens is quite limited due to the difficulties associated with isolation of complex carbohydrates. Even though the antigens are commercially available at rather high prices ($278.00/0.1 mg for N3 major and $901.00/0.1 mg for N3 minor),[8] they can be obtained only as anomeric mixtures of "free reducing end" carbohydrates. Their utility is limited for subsequent biological studies. Development of viable syntheses of N3 antigens can perhaps alleviate the above-mentioned problems.

Earlier, our laboratory has focused on the synthesis of allyl and pentenyl glycosides of various tumor-associated antigens. Such glycosides have proven to be appropriately versatile substrates for subsequent biological studies.[9] Accordingly, glycosides **3** and **4** (Figure 2) were identified as end points of this study even though they are not the naturally isolated species of N3 antigens.

A total synthesis venture aimed at the N3 antigens was of interest, not only for potential medical reasons. It was also anticipated that the synthesis would be challenging and would help to probe the perimeters of feasibility of the glycal assembly logic of oligosaccharide total synthesis. As will be chronicled in detail, our expectations of the difficulties and learning opportunities were surpassed in reality. Indeed, as will be described, a major reassessment of synthetic design was necessary to reach our goal. However, eventually, the objectives were met. In this paper, we describe (vide infra) the total syntheses of N3 allyl glycosides **3** and **4** as well as the natural products **1** and **2**.

Preliminary Synthetic Studies

The initial strategy for reaching N3 major reflected earlier realizations of glycal assembly methodology in reaching Le^x and various other carbohydrate antigens.[9] Thus, we charted a synthesis of N3 major by anticipating late stage mergers of donors derived from glycals **5** and **7** (Scheme 1) in some undefined sequence with appropriate glycal acceptors. The first acceptor type to be employed was formulated as **6**. The second glycal acceptor would be a differentiated pentasaccharide glycal

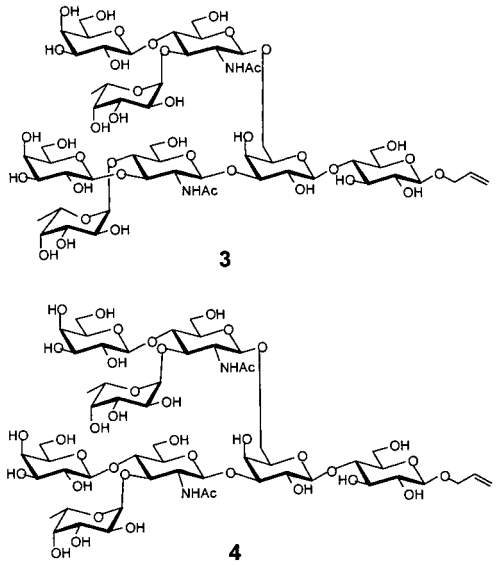

Figure 2.

derived (cf. **8**) from the initial coupling of **6** with **5** or **7**. We were aware, through earlier experiences, of the reactivity differences of hydroxyl groups on lactal **6** (C6′ > C3′ > C2′ > C4′) and planned to join the Le^a trisaccharide **5** first on C3′-hydroxyl of **6**, followed by coupling of Le^x **7** on the C6′-hydroxyl of the resultant pentasaccharide. In this way, we hoped to avoid the late stage installation of a trisaccharide on the less reactive C3′-hydroxyl group of the pentasaccharide. To pursue the approach we favored, the C6′-hydroxyl group of the original lactal **6** would have to be equipped with a suitable protecting group P* that could be differentiated from other existing masking units in the system and unmasked at a propitious time.

The synthesis of **12** (corresponding to a specific form of **6** in Scheme 1) commenced with the known lactal derivative **10**[10] which was treated with TBAF in THF (Scheme 2). Treatment of this product with sodium methoxide in methanol led to the lactal tetraol **11** in 88% yield. We came to favor protecting the 6′-hydroxyl group as a levulinate ester. The C6′-alcohol could be exposed in a variety of ways without unmasking other protective arrangements (for example triisopropyl silyl ethers). Accordingly, the C6′-OH of **11** was selectively levulinoylated to provide **12**. From previous synthetic studies directed toward KH-1,[11] we were convinced that the glycosidation would occur selectively on the C3′-OH in the presence of unmasked C2′- and C4′-hydroxyl groups. Accordingly, no further protection on the triol **12** was performed.

Turning to the synthesis of Le^a trisaccharide donor **20** (**5** in Scheme 1), we started with protection of 6-*O*-triisopropylsilylglucal (**13**). Treatment of this compound with triphenylsilyl chloride in the presence of Et_3N and DMAP afforded **14** in 72% yield. The remaining hydroxyl group of **14** was then glycosylated with fluorofucosyl donor **15**[12] according to the Mukaiyama protocol.[13] The resultant crude disaccharide was not purified. Removal of its triphenylsilyl function afforded **16** in 64% in over 2 steps. The reaction between **16** and the epoxide derivative of **17** through mediation by anhydrous zinc chloride provided

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(8) BioCarb Chemicals, 1997 catalogue.
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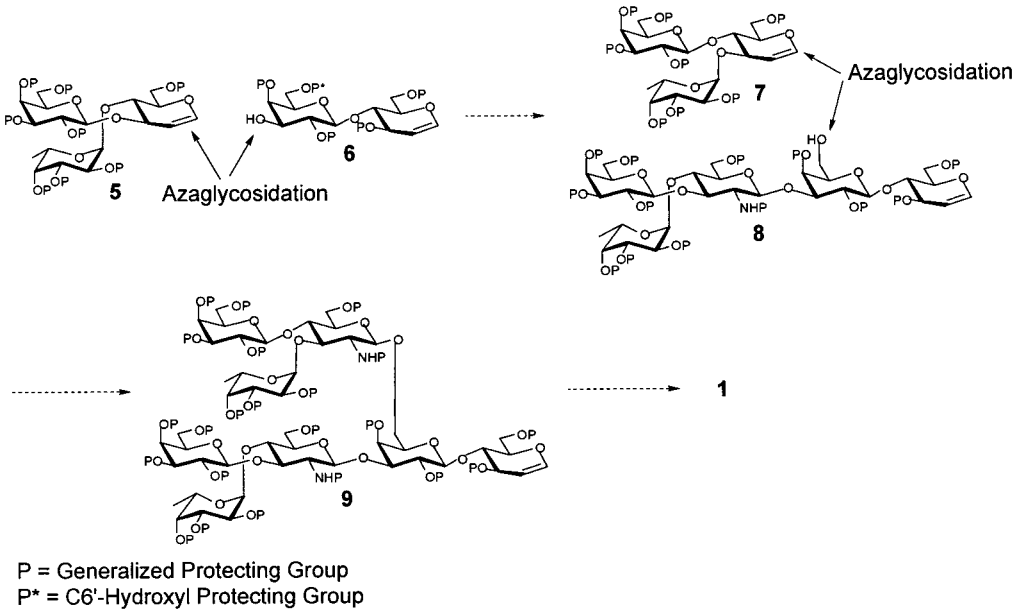
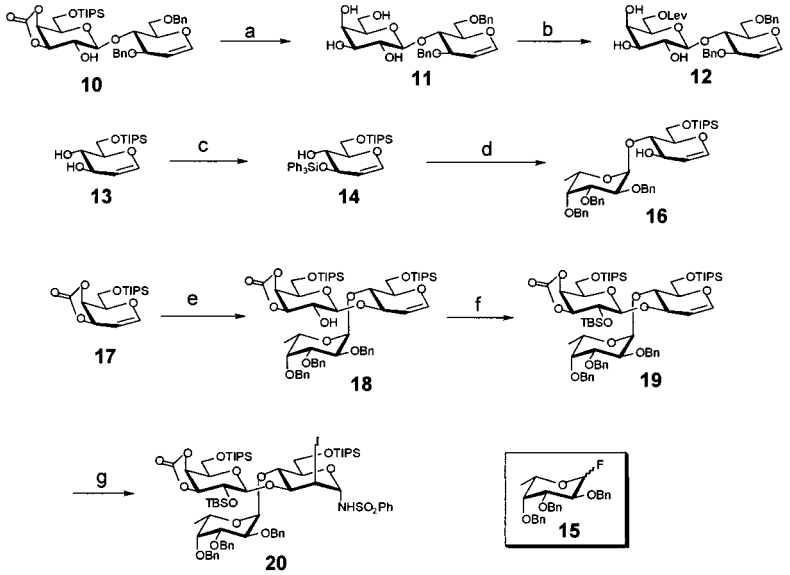
Scheme 1

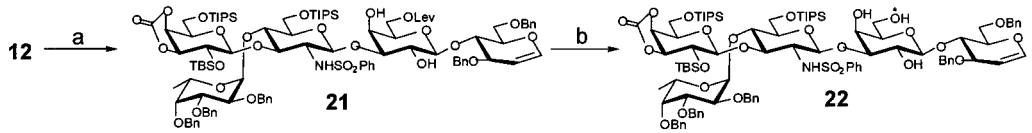


Scheme 2[a]



[a] Reagents: (a) (i) TBAF, THF; (ii) NaOMe, MeOH (88%); (b) levulinic acid, 2-chloro-1-methylpyridinium iodide, Et_3N, dioxane (64%); (c) Ph_3SiCl, Et_3N, DMAP, CH_2Cl_2 (72%); (d) (i) **15**, $AgClO_4$, $SnCl_2$, di-*tert*-butylpyridine, 4 Å MS, Et_2O; (ii) K_2CO_3, MeOH, THF (64% over two steps); (e) (i) DMDO, CH_2Cl_2; (ii) **16**, $ZnCl_2$, THF (76%); (f) TBSOTf, Et_3N, CH_2Cl_2 (94%); (g) I(*sym*-coll)$_2ClO_4$, $PhSO_2NH_2$, 4 Å MS, CH_2Cl_2 (89%).

Scheme 3[a]

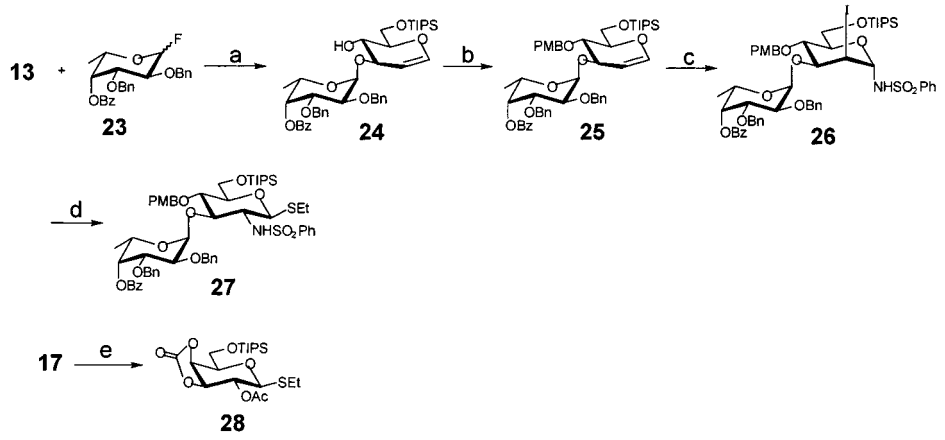


[a] Reagents: (a) (i) $(Bu_3Sn)_2O$, benzene; (ii) **20**, $AgBF_4$, 4 Å MS, THF (45%); (b) H_2NNH_2, AcOH, pyridine (93%).

the trisaccharide glycal **18**. The newly generated hydroxyl group of glycal **18** was protected as its TBS ether, and the resulting glycal **19** was, in turn, transformed to iodosulfonamide donor **20** by treatment with I(*sym*-coll)$_2ClO_4$ and benzenesulfonamide.

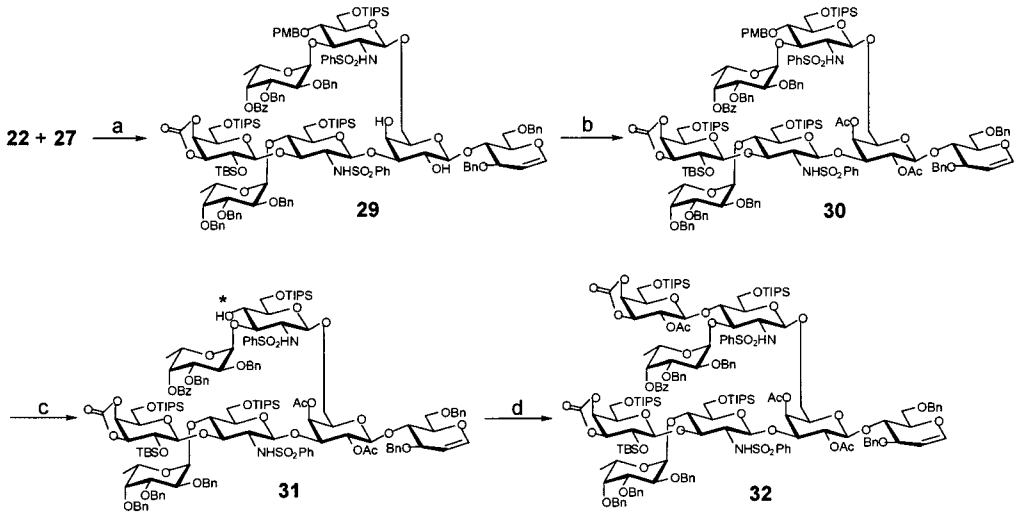
The acceptor lactal **12** was converted to its tributylstannyl ether derivative and combined with **20** in the presence of $AgBF_4$ (Scheme 3). As shown in our synthesis of the KH-1 antigen, the formation of stannyl ether resulted in selective activation of C3′-OH so that the subsequent glycosidation would occur only at the activated position.[11] The well-documented predilection of the iodosulfonamide glycosylative rearrangement reaction to afford β-glycosidic linkages was another consideration favoring this route.[14] Indeed, the desired pentasaccharide **21** was obtained in 45% yield as the sole recognized product in the azaglycosylation. However, the low reactivity of the Le[a] donor **20** necessitated elevated temperature (45 °C), long reaction times (100 h), and a large excess (10 equiv) of a stannyl ether

Scheme 4[a]



[a] Reagents: (a) $AgClO_4$, $SnCl_2$, di-*tert*-butylpyridine, 4 Å MS, Et_2O (55%); (b) *p*-methoxybenzyl chloride, NaH, DMF (83%); (c) I(*sym*-coll)$_2ClO_4$, $PhSO_2NH_2$, 4 Å MS, CH_2Cl_2 (88%); (d) EtSH, LHMDS, DMF (95%); (e) (i) EtSH, $ZnCl_2$, THF; (ii) Ac_2O, DMAP, pyridine, CH_2Cl_2 (23%).

Scheme 5[a]



[a] Reagents: (a) MeOTf, di-*tert*-butylpyridine, 4 Å MS, Et_2O, CH_2Cl_2 (52% + 18% α isomer); (b) Ac_2O, DMAP, pyridine, CH_2Cl_2 (58%); (c) DDQ, di-*tert*-butylpyridine, CH_2Cl_2, H_2O (57%); (d) **28**, MeOTf, di-*tert*-butylpyridine, 4 Å MS, CH_2Cl_2, Et_2O (96%).

derivative of **12** to achieve a moderate coupling yield. For the moment, we accepted this limitation as the synthesis was explored further. The levulinate ester of **21** was cleanly cleaved through hydrazinolysis to provide pentasaccharide C6′-OH acceptor **22**.[15]

The stage was now set for the anticipated merger of the Le^x moiety with the pentasaccharide **22** at its C6′ hydroxyl group (see asterisk). Unfortunately, attempted coupling protocols with various trisaccharide Le^x donors were not successful. Inadequate reactivities of these bulky Le^x trisaccharide donors were perceived to be an obstacle to glycosidation. Thus, it was deemed necessary to devise more activated and less bulky donors that could be converted to Le^x at later stage. The concept of using a fully developed trisaccharide donor was set aside. Instead, the disaccharide thioglycoside donor **27** was synthesized as shown in Scheme 4. Thus, following published procedures, 6-*O*-TIPS glucal (**13**) was selectively fucosylated at the C3 alcohol to give disaccharide **24** in 55% yield.[16] The remaining C4-OH of **24** was protected as its PMB ether to yield the glycal **25**. The treatment of **25** with I(*sym*-coll)$_2ClO_4$ and benzenesulfonamide gave iodosulfonamide **26** in 88% yield. Subsequently, **26** was cleanly converted to donor **27** by the action of basic ethanethiolate. Similarly, the required terminal galactal donor **28** was obtained in a two-step sequence from 6-*O*-TIPS galactal 3,4-cyclic carbonate (**17**) (see Scheme 4).

In the event, coupling between pentasaccharide **22** and thiodonor **27** provided the desired heptasaccharide **29** in 52% yield along with its α-linked isomer (as a 3:1 ratio in favor of the desired *β*-anomer, Scheme 5). The remaining task for the completion of the N3 major synthesis was to install the last remaining galactose moiety. Before proceeding to this goal, we sought to mask the two remaining hydroxyl groups to prevent the glycosidation on the undesired hydroxyl group. After many experiments, effective conditions to acetylate both hydroxyl groups were found, and the diacetylated heptasaccharide **30** was obtained in 58% yield. The PMB ether of **30** was cleaved by the action of DDQ. The reaction was buffered with di-*tert*-butylpyridine to minimize the decomposition of glycal double bond. The hydroxyl group (see asterisk) of heptasaccharide **31** was coupled with thioglycoside **28** in the presence of MeOTf

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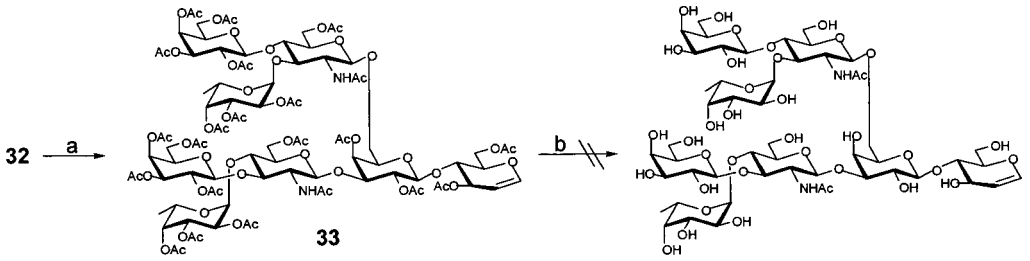
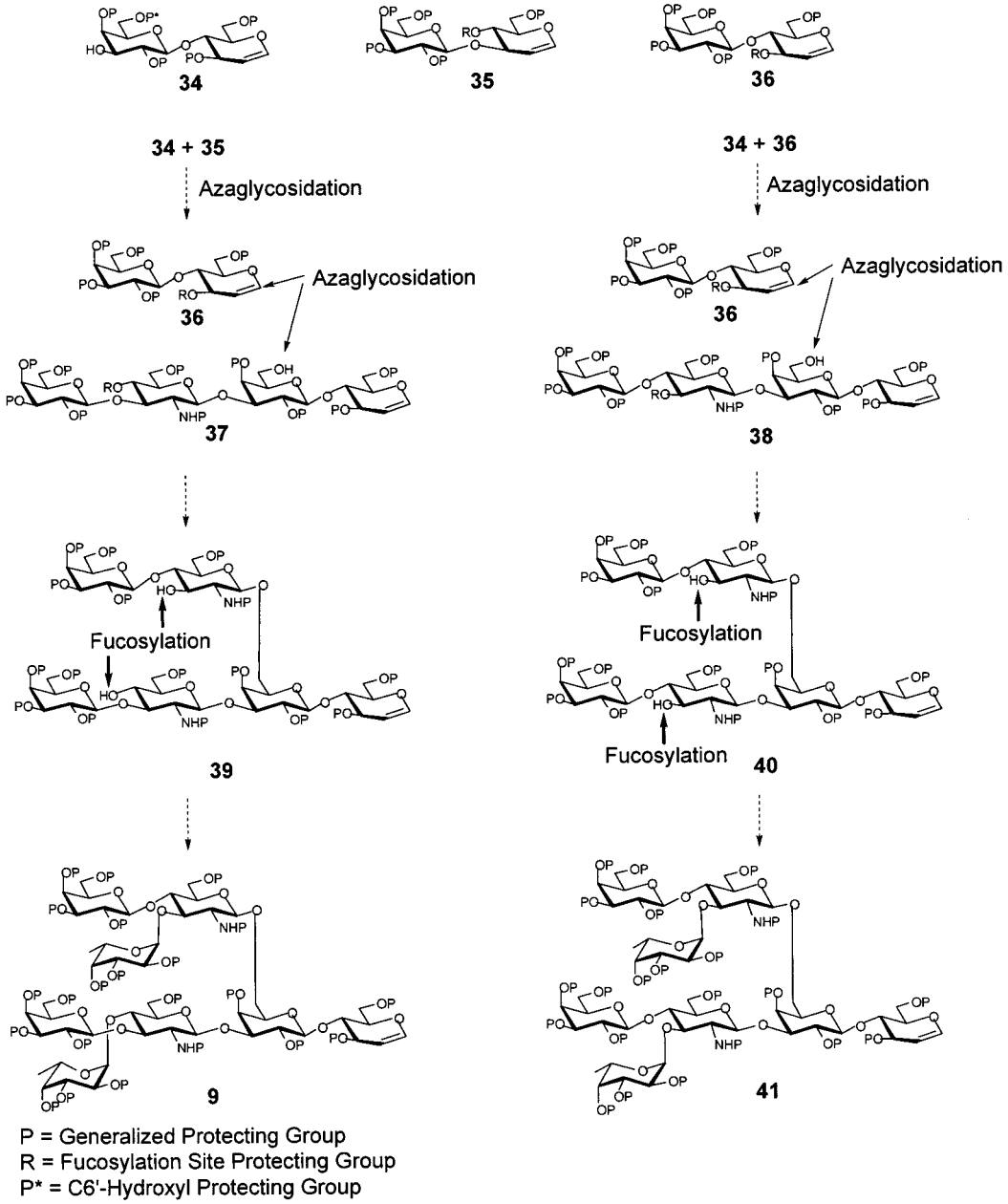
(16) Danishefsky, S. J.; Gervay, J.; Peterson, J. M.; McDonald, F. E.; Koseki, K.; Griffith, D. A.; Oriyama, T.; Marsden, S. P. *J. Am. Chem. Soc.* **1995**, *117*, 1940.

Scheme 6[a]



[a] Reagents: (a) (i) TBAF, THF; (ii) Ac_2O, DMAP, Et_3N, CH_2Cl_2; (iii) NaOMe, MeOH; (iv) Na, NH_3; (v) Ac_2O, DMAP, pyridine (43%); (b) NaOMe, MeOH.

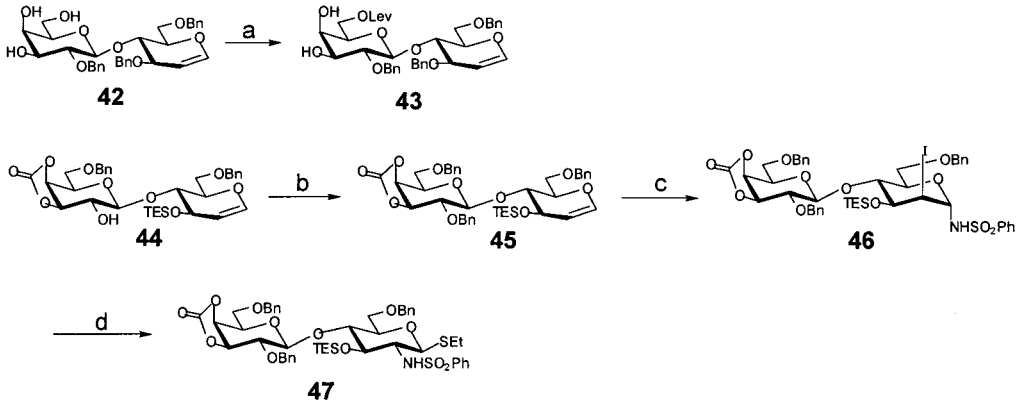
Scheme 7



to afford an octasaccharide. The structure of the oligosaccharide was tentatively assigned as **32**.

A carefully optimized five-step deprotection sequence on the octasaccharide **32** was conducted. It commenced with treatment of **32** with TBAF followed by acetylation of the crude product, for purposes of purification. Subsequent, sequential deacylation of the resulting product, Birch reduction, and peracetylation provided two products in a nonconstant ratio (Scheme 6). One, as indicated by mass spectrometric analysis, was a heptasaccharide glycal lacking one of the terminal galactose units. The precise structure of this heptamer could not be fully determined by spectroscopic methods. On other occasions, an octasaccharide glycal, which was tentatively assigned as peracetyl N3 major glycal **33**, was obtained in modest yield of 43%. However,

Scheme 8[a]



[a] Reagents: (a) levulinic acid, 2-chloro-1-methylpyridinium iodide, Et_3N, dioxane (91%); (b) BnBr, NaH, DMF (90%); (c) I(*sym*-coll)$_2ClO_4$, $PhSO_2NH_2$, 4 Å MS, CH_2Cl_2 (94%); (d) EtSH, LHMDS, CH_2Cl_2 (85%).

attempted "per-deacetylation" with NaOMe in MeOH failed to produce the desired product. At best, we could identify a compound with an extra mass of 42 (corresponding to an extra acetate group). Unfortunately, several attempts to remove this "extra" acetyl group were not successful. Since per-deacylation of **33**, were it present, should have been accomplished under these conditions, failure to achieve this expected end point engendered concerns about the actual structure of the synthetic octasaccharide. Thus, while the gross molecular features of these molecules were verifiable by mass spectrometric analysis, we could not vouch for the integrity of all of the linkages of the presumed **33**. Given this impasse, a new and different approach was in order.

Modification of the Synthetic Routes to N3 Major and N3 Minor

The synthetic program directed toward N3 was redesigned with the difficulties described above in mind. The more symmetric N3 minor, rather than N3 major, was chosen as the target to test the new departures. The corresponding octasaccharide **41** became the primary synthetic goal since its glycal double bond would provide functionality to gain access to various end products including the desired allyl glycoside as well as the natural product itself (Scheme 7). A simultaneous 2-fold fucosylation strategy, of the type which was successful in the case of our KH-1 antigen synthesis,[11] was to be applied to the case at hand. In the retrosynthetic vein, the elimination of two fucose moieties would leave a hexasaccharide (cf. **40**) with two identical lactose moieties each linked to C3′- and C6′-hydroxyl groups on the central lactose unit. Provisions for future fucosylation sites would be anticipated through the use of special protecting groups (R) allowing for exposure of the free hydroxylic acceptor sites in an optimal setting. Initially, the installation of the two flanking lactose units **36** on the central lactose **34** was slated to be carried out stepwise. We were not unaware of the fact that glycosidations tend to be much more difficult when larger entities are to be merged. Since the C6′ position of lactose type **34** was expected to be more reactive, it seemed that once the synthesis had progressed to the tetrasaccharide stage, it would be easier to install the appropriate disaccharide at C6′ rather than at C3′. *Thus, the glycosidation at C3′ was to be carried out first on the lactal **34** with the C6′ position masked via a suitable protecting group, P*.*

The synthesis of N3 major posed a somewhat more difficult challenge in that it requires installation of both Le^a and Le^x subunits. However, the strategy of simultaneous difucosylation could still be appropriate. As in the case required for N3 minor, retrosynthetic "deletion" of two fucosyl moieties from the goal octasaccharide glycal, **9**, takes one back to hexasaccharide, **39** bearing Le^a and Le^x disaccharide precursors linked to the C3′ and C6′ positions of the central lactose unit. Consecutive stepwise glycosidation on C3′ with **35**, followed by reaction on C6′ of **37** with **36**, would provide the hexasaccharide **39**. With the three disaccharide building blocks, **34**−**36**, we could hope to obtain each of the N3 antigens.

Synthesis of N3 Minor

The synthesis of N3 minor focused first on the preparation of a central lactal acceptor, **43**, corresponding to the generalized **34** in Scheme 7. The program commenced with lactal **42**, previously employed in our Globo-H synthesis (Scheme 8).[17] A levulinate ester group was again selected for the protection of C6′-hydroxyl, and the desired central acceptor lactal **43** was obtained by conversion of **42** to its levulinate ester (91% yield). The synthesis of the des-fucosyl Le^x precursor, which was designated as **36** in Scheme 7, started from lactal **44**.[11] The remaining C2′-hydroxyl group of **44** was protected as its benzyl ether. Lactal **45** was then treated with I(*sym*-coll)$_2ClO_4$, in the presence of benzenesulfonamide to yield **46**. When this compound was treated with basic ethanethiolate, the desired disaccharide **47** featuring an anomeric thioethyl donor was obtained in 85% yield. The menu of building blocks necessary for N3 minor was now in hand.

The assembly process was launched via coupling between thioglycoside **47** and the central lactal acceptor **43** (Scheme 9). With methyl triflate (MeOTf) as the promoter, tetrasaccharide **48** was obtained in 80% yield. The levulinate group of **48** was then unmasked to free the C6′-hydroxyl group for the next coupling. Again, thioglycoside **47** was joined to tetrasaccharide acceptor **49** through the agency of MeOTf to give hexasaccharide **50** in 91% yield. Following treatment with acetic acid buffered TBAF, hexasaccharide **50** was successfully converted to hexasaccharide acceptor **51** *with two cleanly identified hydroxyl groups ready for the crucial bis-fucosylation reaction*.

Indeed, triol **51** was successfully fucosylated with 4 equiv of fluorofucosyl donor **23**[16,18] under promotion by Sn(OTf)$_2$.[19]

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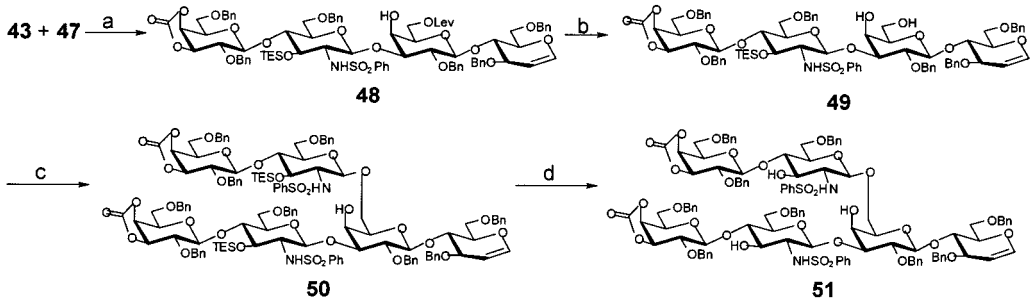
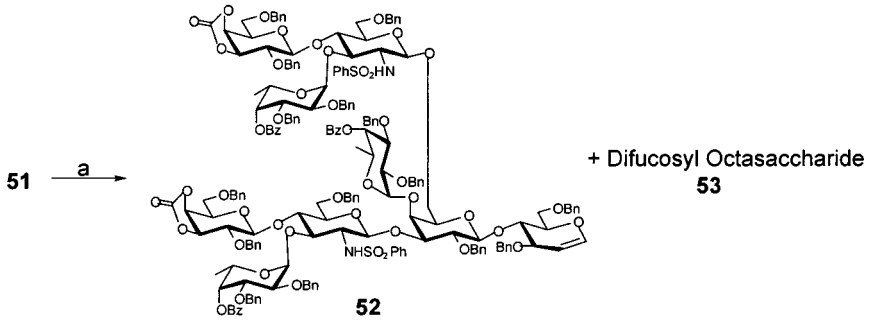
Scheme 9[a]



[a] Reagents: (a) MeOTf, di-*tert*-butylpyridine, 4 Å MS, Et_2O, CH_2Cl_2 (80%); (b) H_2NNH_2, pyridine, AcOH (87%); (c) **47**, MeOTf, di-*tert*-butylpyridine, 4 Å MS, Et_2O, CH_2Cl_2 (91%); (d) TBAF, AcOH (96%).

Scheme 10[a]



[a] Reagents: (a) 2.5 equiv. of **23**, $Sn(OTf)_2$, di-*tert*-butylpyridine, 4 Å MS, THF, toluene (30% for **52** + 55% for **53**).

This donor was shown to have a strong tendency for favoring the formation of α-linked glycosides, possibly due to the anchimeric stabilization of the oxonium transition state by the C4-benzoyl moiety. Unexpectedly, the major product turned out to be nonasaccharide **52** wherein the axial hydroxyl had also suffered fucosylation. With more careful control of equivalency of the fucosyl donor, the difucosylated octasaccharide was obtained in acceptable yield. However, reliable high-yielding methodology to accomplish bis fucosylation proved to be quite difficult.

Fucosylation on the seemingly sterically hindered axial C4′-hydroxyl group of the central lactal was unexpected and certainly unwelcome since it lowered the efficiency of the reaction. Moreover, there was a structure assignment problem in determining which two of three potential acceptor sites had been fucosylated. As is not uncommon with complex carbohydrates, structural determination of minute quantities of samples with sole reliance on NMR may not always be fully persuasive.

As a potential solution to this problem, we examined the possibility of protection of the C4′-hydroxyl group. Fortunately, acetylation was successfully carried out on tetrasaccharide **48** to give **54** in 92% yield (Scheme 11). The levulinate group of **54** was again selectively removed with buffered hydrazine solution without the removal of the newly installed acetyl group. Hexasaccharide **56** was obtained in 71% yield by MeOTf mediated coupling between the tetrasaccharide **55** and thiodonor **47**. The two triethylsilyl groups of **56** were conveniently removed by treatment with TBAF buffered with AcOH. The resulting diol **57** was then bis-fucosylated with the fucosyl fluoride **23** in the presence of $Sn(OTf)_2$ to give the octasaccharide **58**. A cardinal goal of the program had been achieved. With this material in hand, we were in a position to pursue the syntheses of a variety of N3 minor congeners, including allyl glycoside **4** as well as naturally isolated **2**.

Upon comparison of the ^{1}H NMR spectrum of the octasaccharide **58** with that of **53**, it was evident *that they were not identical*. Thus, one of two fucose moieties in **53** was on the axial C4′-hydroxyl group. It will be of interest to determine the reason for the unexpectedly enhanced reactivity of the axial C4′-hydroxyl group in **51**. For the moment this finding was not followed up.

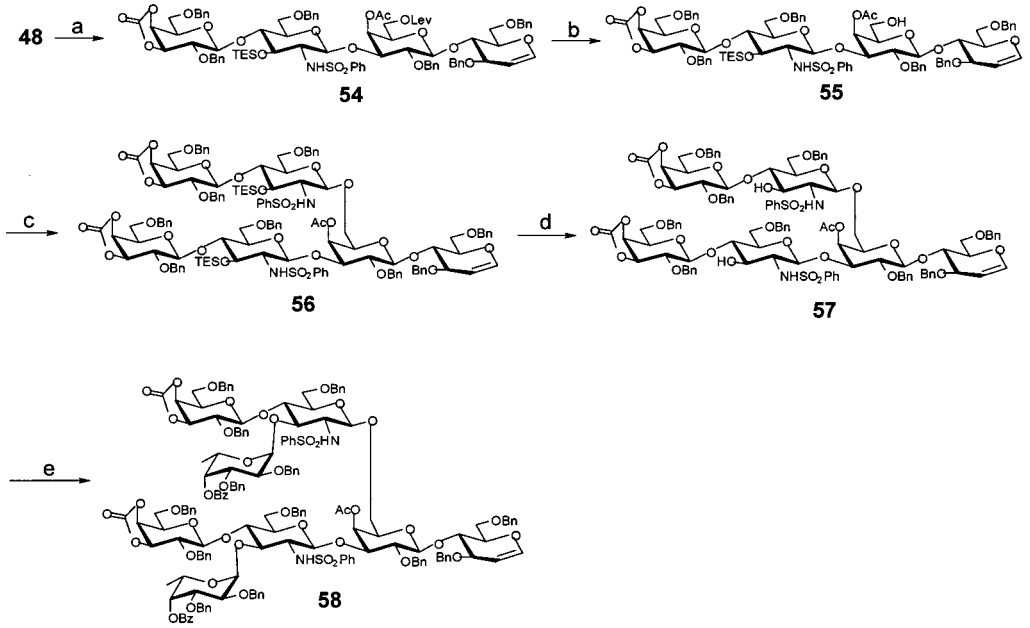
In the planning stage, a protecting group strategy had been devised to ensure that global deprotection of the fully protected N3 minor octasaccharide could be conducted concisely. Thus, all of the protecting groups of **58** could be removed by dissolving metal conditions. In the event, removal of the benzyl ether groups by Birch reduction, followed by peracetylation, provided the peracetyl octaglycal **59** in 43% overall yield (Scheme 12). Dimethyldioxirane oxidation[20a,b] of the peracetyl octacyclic glycal **59**, followed by opening of the resulting epoxide with allyl alcohol under solvolytic conditions, provided the peracetyl allyl glycoside **60** in 48% yield along with its α-mannose type isomer (an 11:9 ratio favoring **60**). In the synthesis of the final allyl glycoside, the serious stereochemical problem associated with late stage glycal epoxidation in the presence of resident acetate functions had been noted earlier. A solution to this difficulty using *n*-pentenyl glycosides has recently been described but not yet applied to the case at hand.[20c,d] Finally, removal of the acetyl esters of **60** by treatment with NaOMe in MeOH produced the allyl glycoside **4**. The structure of **4** was rigorously proven by 2 D NMR experiments including HMBC and HMQC performed by Dr. David Live.[21] Thus, the synthesis of N3 minor allyl glycoside **4** was completed. As a further proof of the structure, allyl glycoside

(19) (a) Lubineau, A.; Malleron, A. *Tetrahedron Lett.* **1985**, *26*, 1713. (b) Lubineau, A.; Le Gallic, J.; Malleron, A. *Tetrahedron Lett.* **1987**, *28*, 5041. (c) Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1987**, *28*, 6221.

(20) (a) Halcomb, R. L.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 6661. (b) Preparation of DMDO: Murray, R. W.; Jeyaraman, R. J. *J. Org. Chem.* **1985**, *50*, 2847. (c) Allen, J. R.; Ragupathi, G.; Livingston, P. O.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1999**, *10875*, 5. (d) Allen, J. R.; Allen, J. G.; Zhang, X. F.; Williams, L. J.; Zatorski, A.; Danishefsky, S. J. *Chem.*−*Eur. J.* **2000**, *6*, 1366.

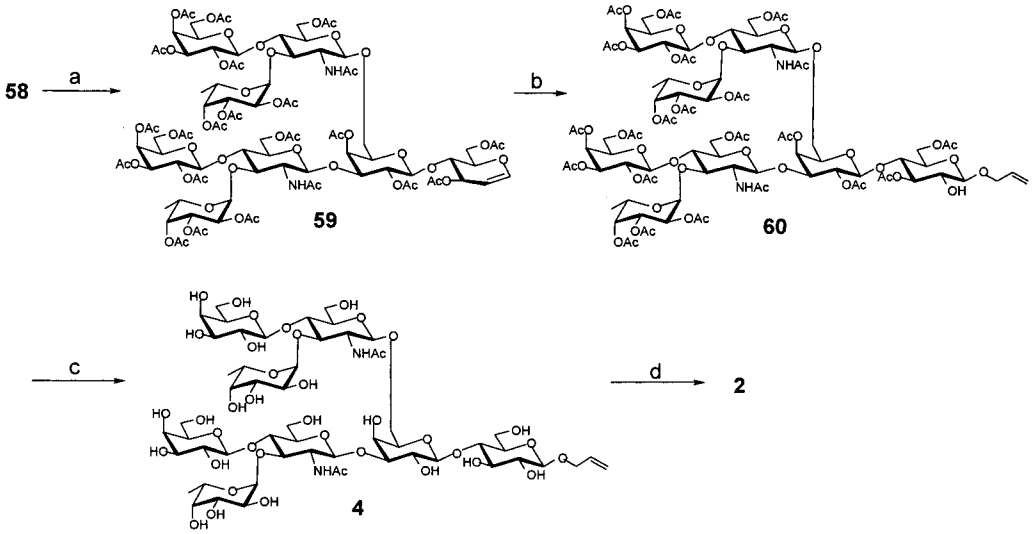
(21) Structural Biology NMR Resource, Department of Biochemistry, Medical School, University of Minnesota.

Scheme 11[a]



[a] Reagents: (a) Ac_2O, pyridine, DMAP, CH_2Cl_2 (92%); (b) H_2NNH_2, pyridine, AcOH (96%); (c) **47**, MeOTf, di-*tert*-butylpyridine, 4 Å MS, Et_2O, CH_2Cl_2 (71%); (d) TBAF, AcOH, THF (quantitative); (e) **23**, $Sn(OTf)_2$, di-*tert*-butylpyridine, 4 Å MS, THF, toluene (70−90%).

Scheme 12[a]



[a] Reagents: (a) (i) Na, NH_3, THF; (ii) Ac_2O, pyridine, DMAP (43% over two steps); (b) (i) DMDO, CH_2Cl_2; (ii) allyl alcohol (48% over two steps + 37% α-mannose type isomer); (c) NaOMe, MeOH (88%); (d) $PdCl_2$, wet MeOH (quantitative).

4 was treated with $PdCl_2$ in wet MeOH to provide free reducing end carbohydrate **2**.[22] The ^1H NMR spectra of synthetic N3 minor **2** was identical with the available spectra of the isolated compound, thus providing additional confirmation as to completion of the N3 minor synthesis.[23]

After we accomplished the syntheses of N3 minor octasaccharide **2**, as well as its more useful congener, allyl glycoside **4**, N3 major became our next focus of research. The plan called for reaching a fully protected N3 major octasaccharide glycal intermediate, which would be convertible to the N3 major allyl glycoside. From the synthetic studies on N3 minor series, we had already confirmed the validity of a general strategy, which anticipates a bis-fucosylation reaction.

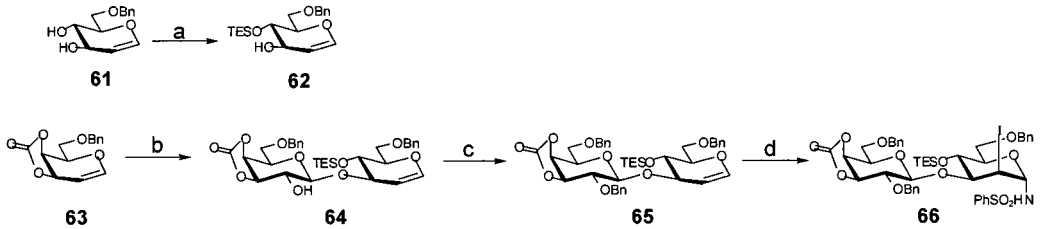
For the synthesis of N3 major, the first issue to be addressed was that of building a disaccharide precursor that would eventually become the Le[a] trisaccharide. To that end, a differently protected glucal starting material had to be assembled. Provision for several stringent criteria would be necessary. First, the glycal had to be equipped, eventually to pave the way for a free hydroxyl at C3 position to achieve the 1,3 disaccharide linkage which is present in Le[a]. Second, this glycal had to have robust and easily cleavable protecting groups at the C6 and C4 positions, respectively. The protecting group at the C4 position was to be removed at the propitious time, exposing a unique, free hydroxyl group to serve as a fucosylation site.

In a high-yielding three-step sequence, 6-*O*-benzylglucal (**61**) was converted to 6-*O*-benzyl-4-*O*-TES glucal (**62**) (Scheme 13). The previously utilized 6-*O*-benzyl-3,4-cyclocarbonate galactal (**63**) was oxidized with dimethyldioxirane, and the resulting

(22) (a) Guibé, F. *Tetrahedron*, **1999**, *53*, 13509. (b) Qian, X.; Hindsgaul, O.; Li, H.; Palcic, M. M. *J. Am. Chem. Soc*. **1998**, *120*, 2184.

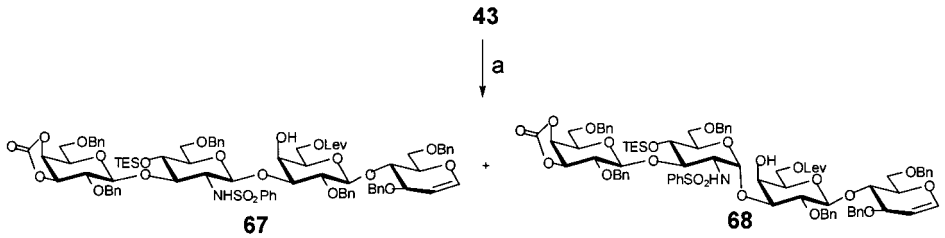
(23) Haeuw-Fievre, S.; Wieruszeski, J.-M.; Plancke, Y.; Michalski, J.-C.; Montreuil, J.; Strecker, G. *Eur. J. Biochem*. **1993**, *215*, 361.

Scheme 13[a]



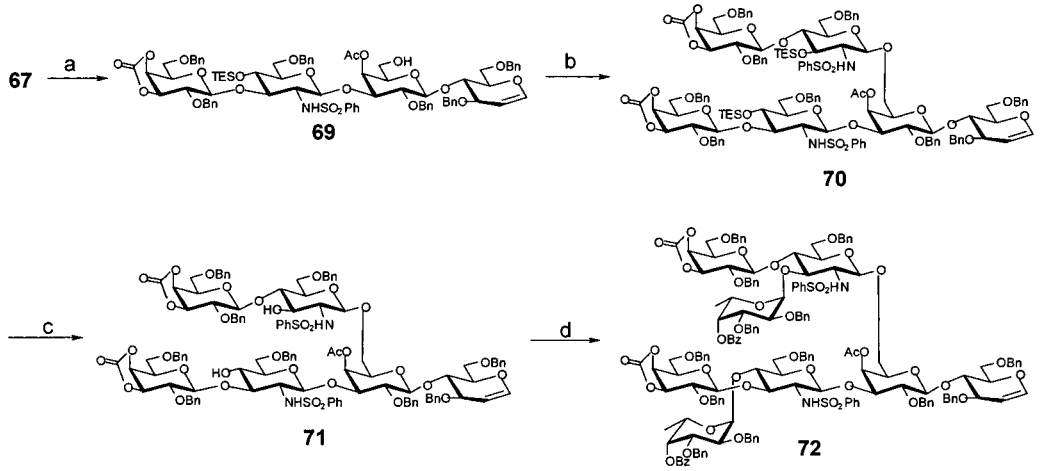
[a] Reagents: (a) (i) trimethylacetyl chloride, DMAP, pyridine, CH_2Cl_2 (88%); (ii) TESOTf, Et_3N, CH_2Cl_2 (98%); (iii) DIBAL-H, CH_2Cl_2 (97%); (b) (i) DMDO, CH_2Cl_2; (ii) **62**, $ZnCl_2$, THF (47%); (c) BnBr, NaH, DMF (82%); (d) I(*sym*-coll)$_2ClO_4$, $PhSO_2NH_2$, 4 Å MS, CH_2Cl_2 (90%).

Scheme 14[a]



[a] Reagents: (a) (i) $(Bu_3Sn)_2O$, benzene; (ii) **66**, $AgBF_4$, 4 Å MS, THF (37% **67** + 30% **68**).

Scheme 15[a]



[a] Reagents: (a) (i) Ac_2O, DMAP, pyridine, CH_2Cl_2; (ii) H_2NNH_2, pyridine, AcOH (73% over two steps); (b) **47**, MeOTf, di-*tert*-butylpyridine, 4 Å MS, Et_2O, CH_2Cl_2 (75%); (c) TBAF, AcOH (93%); (d) **23**, $Sn(OTf)_2$, di-*tert*-butylpyridine, 4 Å MS, THF, toluene (76%).

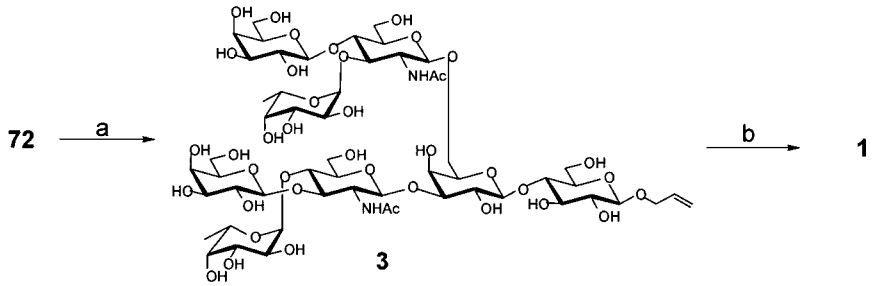
epoxide was coupled with **62**, to yield 1,3-linked disaccharide **64**. The newly generated C2′-hydroxyl group was then benzylated to give disaccharide glycal **65**. Treatment of **65** with I(*sym*-coll)$_2ClO_4$ in the presence of benzenesulfonamide provided iodosulfonamide **66** in 90% yield, thus completing the synthesis of the necessary pre-Le[a] precursor donor.

The direct use of a 1,2-iodosulfonamide carbohydrate as an azaglycosylation donor constitutes an alternative to the thioglycoside coupling.[11a] Indeed, in the synthesis of KH-1 antigen, we were able to obtain high yields of β-linked tetrasaccharides and hexasaccharide by this method. In the case at hand, the reaction between the stannyl ether derived from **43** and the iodosulfonamide **66** gave the tetrasaccharide **67** in 37% yield. *Much to our surprise,* α*-linked anomeric isomer* ***68*** *was obtained in 30% yield as well* (Scheme 14). During our study of glycal chemistry using the direct aza glycosylation reaction,[9b,c] we obtained α-linked glycosides on the rarest occasions. Such a result clearly does not fit our simple model which presupposes a 1,2-aziridine-based intermediate to account for the β-azaglycosidation phenomenon. In the case in hand, the aziridine (or aziridinium) must be short-lived, and a significant population of the donor must reflect anomeric "onium" ion character. Even though the coupling reaction to produce the tetrasaccharide **67** was far from optimal, we were able to obtain sufficient amounts, through the "direct rollover" reaction, to enable completion of our objective.

As before, the remaining hydroxyl group of **67** was acetylated, and the levulinate group was then removed by treatment with hydrazine to provide the tetrasaccharide acceptor **69** (Scheme 15). The 4 +2 glycosidic coupling between **47** and **69** afforded the hexasaccharide **70** in 75% yield. This time, no other anomeric isomer was obtained in this thioglycoside mediated glycosidation reaction. The two remaining TES groups of **70** were simultaneously removed by the treatment with TBAF buffered with AcOH to gave the diol **71**, which was in turn fucosylated with the fluorofucosyl donor **23** thus affording, unambiguously, the octasaccharide **72**. The initial goal of synthesizing fully protected octasaccharide glycal **72** had been reached.

The concluding phase of the synthesis of N3 major allyl glycoside commenced with the global deprotection of **72** under dissolving metal conditions, followed by peracetylation (Scheme

Scheme 16[a]



[a] Reagents: (a) (i) Na, NH_3, THF; (ii) Ac_2O, pyridine, DMAP (58% over two steps); (iii) DMDO, CH_2Cl_2; (iv) allyl alcohol; (v) NaOMe, MeOH (40% over 3 steps + 15% of α-mannose type isomer); (b) $PdCl_2$, wet MeOH (quantitative).

16). This deprotection sequence provided the peracetyl N3 major glycal in 58% overall yield. After a sequence consisting of DMDO oxidation,[20] solvolysis of the epoxide with allyl alcohol, and removal of the acetates with NaOMe, N3 major allylglycoside **3** was obtained along with the α-mannose type isomer. As discussed above, this result reflects a lack of stereospecificity in the epoxidation of the terminal glycal in the peracetate series. After the work described here, a solution to the problem was devised in the context of the use of *n*-pentenyl glycoside.[20c,d] In any case, the synthesis of N3 major allyl glycoside had now been achieved. Each stereochemical outcome of the glycosidation reactions along the way to **3** was confirmed by HMQC NMR analysis, and high-resolution mass spectrometry further served to confirm the molecular formula of **3**. Definite proof of the structure of synthetic N3 major was obtained at the stage of the free reducing end carbohydrate **1**. The allyl glycoside **3** was converted to free reducing end N3 major with $PdCl_2$ in wet MeOH.[22] The ^{1}H NMR spectrum of this synthetic material matched with the published spectrum of isolated N3 major fromthe natural source, thus further confirming the synthesis of N3 major.[24]

The binding of synthetic N3 to the anti-major N3 monoclonal antibodies was examined by ELISA. As expected, major N3−KLH conjugates derived from the allyl glycoside **3** were found to bind the anti-major N3 monoclonal antibodies tightly at the concentration of 25 μg/mL. Surprisingly, minor N3−KLH conjugates made from the allyl glycoside **4** bound the antibodies as well, at the same concentration. In addition, KLH conjugates of Le^x trisaccharide was synthesized and found to bind the same antibodies equally well. Apparently this particular monoclonal is focusing on Lewis^x moieties. Further experiments following up on these points are in progress.

Conclusions

The syntheses of the N3 major and minor allyl glycosides have been accomplished. Three disaccharide building blocks sufficed to assemble these systems. The syntheses successfully applied the late stage concurrent multifucosylation reaction, thus providing convergent and efficient routes to these structurally complex octasaccharides. In addition, overall protecting group strategies employed in these syntheses minimized the number of steps in the final deprotection reactions, thus providing further conciseness. The biological evaluations of these synthetic antigens are in progress, and the results will be disclosed in due course.

Experimental Section

Acceptor 43. To a solution of lactal **42** (2.79 g, 4.92 mmol) and 2-chloro-1-methylpyridinium iodide (2.54 g, 9.94 mmol) in 80 mL of 1,4-dioxane were added levulinic acid (0.604 mL, 5.78 mmol) and Et_3N (4.03 mL, 28.9 mmol). The yellow reaction mixture was stirred at RT (room temperature) overnight with exclusion of light. Then, the reaction mixture was transferred into a separatory funnel containing 200 mL of saturated $NaHCO_3$(aq). The aqueous layer was extracted with EtOAc (3 × 100 mL), and the combined organic layers were washed with saturated $NaHCO_3$(aq) (3 × 100 mL) and brine (1 × 100 mL), dried over Na_2SO_4, and concentrated with rotary evaporator. Upon purification by column chromatography with 55% EtOAc in hexanes, levulinate **43** (2.90 g, 89%) was obtained as colorless oil: $[\alpha]^{23}_D = +14.5°$ (*c* 0.49, $CHCl_3$); FTIR ($CHCl_3$ film) 3455, 3088, 3064, 3031, 2922, 2870, 1736, 1718, 1649, 1496, 1454, 1404, 1365, 1312, 1247, 1211, 1157, 1133, 1074, 912, 826, 738, 700 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36−7.15 (m, 15H), 6.45 (d, 1H, *J* = 6.3 Hz), 4.91−4.87 (m, 2H), 4.63−4.60 (m, 3H), 4.56 (d, 1H, *J* = 7.6 Hz), 4.53 (s, 2H), 4.38−4.31 (m, 2H), 4.24−4.19 (m, 2H), 4.12 (t, 1H, *J* = 4.0 Hz), 3.90−3.86 (m, 2H), 3.71 (dd, 1H, *J* = 10.7, 3.8 Hz), 3.58−3.52 (m, 2H), 3.47−3.44 (m, 1H), 2.72−2.60 (m, 2H), 2.53−2.42 (m, 4H), 2.22 (s, 3H); ^{13}C NMR (125 MHz,$CDCl_3$) δ 206.5, 172.7, 144.5, 138.5, 138.2, 137.7, 128.5, 128.4, 128.3, 127.9, 127.7, 127.6, 127.6, 127.5, 102.3, 99.5, 78.6, 75.7, 74.6, 73.3, 73.1, 72.8, 72.0, 71.5, 70.1, 68.1, 67.8, 62.8, 37.8, 29.7, 27.8; LRMS (EI) (*m*/*e*) calcd for $C_{38}H_{44}O_{11}Na$ $[M + Na]^+$ 699.3, found 699.4.

Lactal 45. To a solution of azeotropically dried lactal **44** (0.875 g, 1.39 mmol) in 10 mL of DMF was added benzyl bromide (0.253 mL, 2.09 mmol). The solution was then cooled to 0 °C and treated with NaH of (0.0835 g, 2.09 mmol, 60% dispersion in oil) at 0 °C. The reaction mixture was stirred at 0 °C for 5 min and at RT thereafter. After 4 h, the reaction mixture was diluted with EtOAc (100 mL) and poured into a separatory funnel containing cold saturated NH_4Cl(aq). The organic layer was separated, washed with saturated NH_4Cl(aq) (3 × 60 mL), H_2O (3 × 60 mL), and brine (1 × 60 mL), and dried over Na_2SO_4. Purification of the resulting oil by column chromatography with 15% EtOAc in hexanes yielded benzyl protected lactal **45** (0.932 g, 93%) as colorless oil: $[\alpha]^{23}_D = -12.5°$ (*c* 0.17, $CHCl_3$); FTIR ($CHCl_3$ film) 3064, 3031, 2953, 2875, 1811, 1647, 1497, 1454, 1410, 1368, 1299, 1247, 1208, 1169, 1097, 1038, 961, 938, 873, 848, 813, 742, 698 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35−7.23 (m, 15H), 6.35 (d, 1H, *J* = 6.3 Hz), 4.75−4.70 (m, 4H), 4.62−4.78 (m, 6H), 4.21 (m, 1H), 4.15 (t, 1H, *J* = 3.9 Hz), 3.94−3.89 (m, 2H), 3.85 (m, 1H), 3.69 (s, 1H), 3.67 (d, 1H, *J* = 3.2 Hz), 3.63 (dd, 1H, *J* = 11.0, 3.0 Hz), 3.55 (t, 1H, *J* = 5.5 Hz), 0.92 (t, 9H, *J* = 8.0 Hz), 0.58 (q, 6H, *J* = 7.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.8, 143.1, 138.0, 137.4, 137.0, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.6, 102.5, 99.1, 77.2, 77.1, 75.2, 74.9, 74.2, 73.73, 73.70, 73.3, 69.9, 67.9, 67.8, 65.1, 6.8, 4.8; HRMS (FAB) (*m*/*e*) calcd for $C_{40}H_{50}O_{10}SiNa$ $[M + Na]^+$ 741.3071, found 741.3042.

Iodosulfonamide 46. Azeotropically dried lactal **45** (0.640 g, 0.891 mmol), benzene sulfonamide (0.357 g, 2.23 mmol), and 4 Å molecular sieves (1 g) were suspended in 17 mL of CH_2Cl_2, and the resulting suspension was stirred at RT for 10 min. Then, the suspension was cooled to 0 °C and treated with I(*sym*-coll)$_2$$ClO_4$ (0.700 g, 1.49 mmol). The reaction mixture was stirred with exclusion of light at 0 °C for 1 h. Then, the reaction mixture was diluted with EtOAc (30 mL) and filtered through a pad of silica gel. The clear yellow filtrate was washed

(24) Dua, V. K.; Goso, K.; Dube, V. E., Bush, C. A. *J. Chromatogr.* **1985**, *328*, 259.

with saturated $Na_2S_2O_3$(aq) (3 × 100 mL), saturated $CuSO_4$(aq) (3 × 100 mL), and brine (3 × 100 mL) and dried over Na_2SO_4. Resulted crude product was purified by column chromatography with 20% EtOAc in hexanes to yield the iodosulfonamide **46** (0.883 g, 99%) as a white foam: $[\alpha]^{23}_D = -16.0°$ (*c* 0.3, $CHCl_3$); FTIR ($CHCl_3$ film) 3349, 3254, 3062, 3030, 2952, 2874, 1804, 1552, 1448, 1337, 1245, 1159, 10919, 1035, 907, 848, 813, 754, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.02−7.88 (m, 3H), 7.56−7.22 (m, 17H), 5.83 (br s, 1H), 5.32 (t, 1H, J = 8.8 Hz), 5.06 (s, 1H), 4.86 (d, 1H, J = 8.3 Hz), 4.82 (s, 1H), 4.66 (d, 1H, J = 5.6 Hz), 4.61 (d, 1H, J = 11.4 Hz), 4.57−4.51 (m, 4H), 4.41 (d, 1H, J = 11.6 Hz), 4.34 (dd, 1H, J = 8.2, 2.5 Hz), 4.06 (br s, 2H), 3.81 (br s, 1H), 3.68−3.61 (m, 5H), 3.16 (br s, 1H), 0.94 (t, 9H, J = 7.9 Hz), 0.64 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.0, 141.2, 138.0, 137.3, 136.6, 132.4, 128.63, 128.59, 128.5, 128.3, 128.0, 127.9, 127.7, 127.4, 98.5, 75.07, 74.7, 73.8, 73.4, 73.33, 73.30, 68.6, 68.3, 67.9, 7.0, 4.9; HRMS (FAB) (*m*/*e*) calcd for $C_{46}H_{56}NO_{12}SISiNa$ $[M + Na]^+$ 1024.2235, found 1024.2262.

Thioglycoside 47. A solution of azeotropically dried iodosulfonamide **46** (1.29 g, 1.29 mmol) in 43 mL of DMF was cooled to −40 °C. Then, the solution was treated with ethanethiol (0.147 mL, 1.92 mmol) and followed by dropwise addition of LHMDS (2.57 mL, 1 M in THF) at −40 °C. After being stirred at −40 °C for 3 min, the cooling bath was removed, and the reaction mixture was kept stirred at RT for 1 h. Then, the reaction mixture was transferred directly to a separatory funnel containing cold saturated NH_4Cl(aq) (ca. 100 mL) and was diluted with 100 mL of EtOAc. The organic layer was separated, washed in sequence with saturated NH_4Cl(aq) (3 × 100 mL), H_2O (3 × 100 mL), and brine (1 × 100 mL), and dried over Na_2SO_4. The oil obtained upon concentration with rotary evaporator was purified by column chromatography with 20% EtOAc in hexanes to yield thioglycoside **47** (1.09 g, 90%) as colorless oil: $[\alpha]^{23}_D = -41.7°$ (*c* 0.92, $CHCl_3$); FTIR ($CHCl_3$ film) 3310, 3063, 3031, 2953, 2927, 2874, 1809, 1454, 13701, 1330, 1162, 1093, 1036, 739, 698, 590, 553 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.90 (m, 2H), 7.48−7.25 (m, 18H), 5.75 (d, 1H, J = 9.7 Hz), 4.83 (dd, 1H, J = 7.9, 1.6 Hz), 4.74−4.61 (m, 5H), 4.56 (s, 2H), 4.49 (s, 2H), 3.96 (m, 2H), 3.85 (m, 2H), 3.81 (m, 2H), 3.72 (d, 2H, J = 6.9 Hz), 3.64 (t, 1H, J = 4.7 Hz), 3.36 (m, 1H), 2.47 (m, 2H), 1.12 (t, 3H, J = 7.5 Hz), 0.87 (t, 9H, J = 7.9 Hz), 0.50 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.9, 140.7, 138.1, 137.5, 136.6, 132.4, 128.8, 128.6, 128.5, 128.34, 128.30, 128.0, 127.9, 127.7, 127.6, 99.4, 82.8, 76.9, 76.5, 76.2, 76.0, 74.0, 73.8, 73.7, 73.3, 71.6, 70.5, 69.9, 67.8, 57.4, 25.8, 14.7, 6.8, 4.4; HRMS (FAB) (*m*/*e*) calcd for $C_{48}H_{61}NO_{12}S_2SiNa$ $[M + Na]^+$ 958.3302, found 958.3292.

N3 minor Allyl Glycoside 4. To a deep blue solution of sodium (0.0560 g, 2.43 mmol) in liquid ammonia (ca. 7 mL) under argon at −78 °C was added fully protected octasaccharide **58** (0.0460 g, 0.0150 mmol) in 2 mL of anhydrous THF. After being stirred for 45 min, the reaction mixture was quenched by addition of 3 mL of anhydrous MeOH at −78 °C. Most of ammonia was removed by a stream of argon, and the solution was diluted with 10 mL of MeOH and stirred for overnight at RT. Then, the reaction mixture was treated with Dowex 50-X8 ion-exchange resin (0.525 g), stirred for 15 min, filtered, washed with MeOH (30 mL) and ammoniacal MeOH (20 mL), and concentrated to dryness. The crude material and catalytic amount of DMAP was suspended in DMF, THF, and Et_3N (1 mL of each). Then, the reaction mixture was treated with 0.3 mL of acetic anhydride, stirred for 18 h at RT, and concentrated to dryness with rotary evaporator. Flash column chromatography of the crude material with 3% MeOH in CH_2Cl_2 provided peracetylated octasaccharide **59** (0.0145 g, 43%). A solution of the peracetylated octasaccharide **59** (0.0100 g, 0.00500 mmol) in CH_2Cl_2 (1 mL) was treated with dimethyldioxirane (0.15 mL, 0.075 M solution in acetone) at 0 °C and stirred for 45 min. Then, most of the volatiles were removed by a stream of argon until ca. 0.3 mL of the solution was left in the flask. It was treated with 3 mL of ally alcohol. After further removal of the volatiles by a stream of argon to ca. 2 mL of volume, the solution was stirred at RT for overnight and concentrated to dryness. Flash column chromatography of the crude material with 2% MeOH in CH_2Cl_2 gave two compounds [mannose type isomer, higher R_f value, 0.0038 g (37%); **60,** 0.0049 g (48%)]. These compounds were deacetylated according to the procedure described in reference (NaOMe, MeOH). Upon deacetylation, N3 minor allyl glycoside **4** (0.0027 g, 88%) was obtained from **60** and so was the mannose type isomer (0.0015 g, 64%) from the isomer of **60**.

4: mp 197 °C (decomp); $[\alpha]^{23}_D = -115.2°$ (*c* 0.07, MeOH); FTIR (KBr) 3332, 1650, 1378, 1145, 1071 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 6.04−5.97 (m, 1H), 5.41 (br d, 1H, J = 17.1 Hz), 5.31 (br d, 1H, J = 10.3 Hz), 5.15 (d, 1H, J = 3.6 Hz), 5.13 (d, 1H, J = 3.7 hz), 4.73 (d, 1H, J = 8.2 Hz), 4.66 (d, 1H, J = 7.0 Hz), 4.56 (d, 1H, J = 8.0 Hz), 4.50−4.40 (m, 4H), 4.26 (dd, 1H, J = 12.4, 6.3 Hz), 4.17 (br s, 1H), 4.04−3.87 (m, 15H), 3.86−3.79 (m, 4H), 3.77−3.60 (m, 16H), 3.52 (t, 2H, J = 8.4 Hz), 3.37 (t, 1H, J = 8.4 Hz), 2.08 (s, 3H), 2.05 (s, 3H), 1.20 (d, 6H, J = 6.3 Hz); ^{13}C NMR (125 MHz, D_2O) δ 175.1, 174.7, 133.6, 119.1, 103.4, 102.9, 102.2, 102.1, 101.3, 101.2, 99.00, 98.97, 82.2, 79.4, 75.7, 75.5, 75.3, 75.24, 75.20, 75.1, 75.0, 74.8, 73.7, 73.4, 73.2, 72.8, 72.3, 71.4, 71.0, 70.2, 69.6, 68.7, 68.1, 61.9, 60.4, 60.1, 60.0, 56.3, 56.0, 49.2, 22.9, 22.6, 15.7 (2C); HRMS (FAB) (*m*/*e*) calcd for $C_{55}H_{92}N_2O_{39}Na$ $[M + Na]^+$ 1427.5174, found 1427.5250.

Mannose type isomer: mp 235−240 °C (decomp.); $[\alpha]^{23}_D = -326.7°$ (*c* 0.03, MeOH); FTIR (KBr) 3306, 1644, 1556, 1376, 1068 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 6.05−5.97 (m, 1H), 5.40 (br d, 1H, J = 17.3 Hz), 5.31 (br d, 1H, J = 10.4 Hz), 5.16 (d, 1H, J = 3.8 Hz), 4.97 (br s, 1H), 4.74 (d, 1H, J = 8.1 Hz), 4.73 (d, 1H, J = 8.1 Hz), 4.50 (d, 1H, J = 7.6 Hz), 4.49 (d, 1H, J = 7.6 Hz), 4.43 (d, 1H, J = 7.9 Hz), 4.28 (dd, 1H, J = 12.8, 5.4 Hz), 4.16−4.10 (m, 3H), 4.05−3.87 (m, 18H), 3.85−3.80 (m, 4H), 3.77−3.69 (m, 8H), 3.67 (d, 1H, J = 3.2 Hz), 3.65−3.58 (m, 5H), 3.55−3.52 (m, 2H), 2.10 (s, 3H), 2.06 (s, 3H), 1.21 (d, 6H, J = 6.5 Hz); ^{13}C NMR (125 MHz, D_2O) δ 174.4, 174.1, 132.9, 118.1, 103.0, 102.2, 101.4 (2C), 100.7, 98.5, 98.3, 98.2, 76.5, 74.9, 74.8, 74.7, 74.6, 74.4, 73.6, 73.0, 72.7, 72.12, 72.11, 71.6, 71.1, 70.71, 70.67, 69.5, 69.3, 68.89, 68.86, 68.04, 68.00, 67.95, 67.4, 66.38, 66.35, 61.2, 59.7, 59.5, 59.3, 55.6, 55.3, 22.0, 21.9, 14.99, 14.96; LRMS (EI) *(m/e)* calcd for $C_{55}H_{92}N_2O_{39}Na$ $[M + Na]^+$ 1427.5, found 1427.8.

N3 minor antigen 2. To a solution of **4** (0.0017 g, 0.0012 mmol) in wet MeOH (1 mL) was added $PdCl_2$ (0.001 g, 0.006 mmol) at RT. The mixture was stirred for 4 h. After removal of $PdCl_2$ by passing the reaction mixture through Lichroprep RP-18 pad, it was subjected to gel filtration to provide **2** (0.0017 g, quantitative) as a 1:1 (α:β) mixture of anomers: mp 180 °C (decomp); $[\alpha]^{23}_D = -40.6°$ (*c* 0.12, MeOH); FTIR (KBr) 3264, 1643, 1205, 1149, 1070 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 5.24 (d, 1/2H, J = 3.7 Hz), 5.15 (d, 1H, J = 4.0 Hz), 5.13 (d, 1H, J = 4.0 Hz), 4.73 (d, 1H, J = 8.2 Hz), 4.69 (d, 1H, J = 8.0 Hz), 4.66 (d, 1/2H, J = 7.2 Hz), 4.49 (d, 1H, J = 7.3 Hz), 4.48 (d, 1H, J = 7.4 Hz), 4.45 (d, 1H, J = 7.9 Hz), 4.16 (d, 1H, J = 3.2 Hz), 4.04−3.81 (m, 22H), 3.79−3.58 (m, 18 and 1/2H), 3.53 (d, 1H, J = 7.9 Hz), 3.51 (d, 1H, J = 8.0 Hz), 3.31 (t, 1/2H, J = 8.5 Hz), 2.08 (s, 3H), 2.05 (s, 3H), 1.20 (d, 6H, J = 6.6 Hz); LRMS (EI) *(m/e)* calcd for $C_{52}H_{88}N_2O_{39}Na$ $[M + Na]^+$ 1387.5, found 1387.8.

6-*O*-Benzyl-4-*O*-TES Glucal (62). To a solution of 6-benzylglucal (**60**) (2.62 g, 11.1 mmol) and catalytic amount of DMAP in CH_2Cl_2 (200 mL) was added pyridine (2.69 mL, 33.3 mmol). The reaction mixture was cooled to 0 °C and was treated with trimethylacetyl chloride (2.05 mL, 16.6 mmol). The reaction mixture was stirred for 24 h at RT. The reaction mixture was concentrated with rotary evaporator and redissolved in EtOAc (100 mL). The resulting solution was washed with saturated $NaHCO_3$(aq) (4 × 100 mL), saturated $CuSO_4$(aq) (2 × 100 mL), and brine (1 × 100 mL) and dried over Na_2SO_4. Chromatographic purification of crude product with 10% EtOAc in hexanes yielded pivaloate (2.57 g, 72%) as a colorless oil: $[\alpha]^{23}_D = +1.58°$ (*c* 2.79, $CHCl_3$); FTIR ($CHCl_3$ film) 3471, 3067, 3031, 2971, 2873, 1724, 1649.1, 1480, 1456, 1396, 1366, 1283, 1236, 1162, 1101, 1058, 953, 850, 827, 743, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.35−7.27 (m, 5H), 6.46 (dd, 1H, J = 6.1, 1.2 Hz), 5.22 (dt, 1H, J = 6.3, 1.8 Hz), 4.68 (dd, 1H, J = 6.1, 2.6 Hz), 4.61 (dd, 2H, J = 16.0, 12.1 Hz), 4.00 (m, 1H), 3.94 (dd, 1H, J = 9.5, 6.4 Hz), 3.81 (d, 2H, J = 4.1 Hz), 3.41 (br s, 1H), 1.21 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.1, 146.2, 137.8, 128.4, 127.7, 98.9, 77.3, 73.6, 73.1, 68.7, 68.2, 38.8, 27.0; LRMS (EI) *(m/e)* calcd for $C_{18}H_{24}O_5Na$ $[M + Na]^+$ 343.4, found 343.1.

To a solution of pivaloate (2.44 g, 7.62 mmol) in CH_2Cl_2 (120 mL) was added Et_3N (4.25 mL, 30.5 mmol). The reaction mixture was cooled to 0 °C and was treated with TESOTf (2.44 mL, 10.7 mmol). The

reaction mixture was stirred at 0 °C for 2 h. Then, the reaction mixture was concentrated with rotary evaporator and redissolved in EtOAc (100 mL). The organic solution was washed with saturated $NaHCO_3$(aq) (3 × 100 mL) and brine (1 × 100 mL) and dried over Na_2SO_4. Chromatographic purification with 3% EtOAc in hexanes afforded TES protected glucal (3.18 g, 96%) a colorless oil: $[\alpha]^{23}_D = -45.5°$ (*c* 2.73, $CHCl_3$); FTIR ($CHCl_3$ film) 3068, 3031, 2957, 2912, 2877, 1728, 1651, 1480, 1457, 1414, 1395, 1366, 1313, 1279, 1240, 1206, 1151, 1125, 1098, 1011, 959, 875, 828, 776, 742, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.34−7.26 (m, 5H), 6.43 (d, 1H, $J = 6.1$ Hz), 5.12 (t, 1H, $J = 3.8$ Hz), 4.69 (dd, 1H, $J = 6.1$, 3.3 Hz), 4.59 (dd, 2H, $J = 31.5$, 12.1 Hz), 4.05 (m, 2H), 3.76 (dd, 1H, $J = 10.5$, 5.5 Hz), 3.68 (dd, 1H, $J = 10.7$, 2.8 Hz), 1.18 (s, 9H), 0.92 (t, 9H, $J = 8.0$ Hz), 0.60 (dd, 6H, $J = 16.0$, 7.8 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.9, 145.7, 137.8, 128.3, 127.7, 127.6, 98.6, 78.2, 73.5, 71.6, 68.2, 67.3, 38.7, 27.1, 6.7, 4.8; LRMS (EI) *(m/e)* calcd for $C_{24}H_{38}O_5SiNH_4$ $[M + NH_4]^+$ 452.3, found 452.2.

To the solution of azeotropically dried TES glucal (3.18 g, 7.32 mmol) in 150 mL of CH_2Cl_2 was added DIBAl-H (14.6 mL, 1 M solution in toluene) at −78 °C. After being stirred at −78 °C for 1 h, the reaction mixture was quenched by dropwise addition of H_2O (10 mL). The reaction mixture was then warmed to RT and was treated with saturated potassium sodium tartarate aqueous solution (300 mL). The resulting mixture was stirred vigorously at RT overnight. Then, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 300 mL). All of the organic layers were combined and dried over Na_2SO_4. The crude product was purified by column chromatography with the gradient of 8% EtOAc in hexanes−10% EtOAc in hexanes to afford of 3-hydroxyglucal **62** (1.81 g,70%) as colorless oil: $[\alpha]^{23}_D$ +21.5° (*c* 1.59, $CHCl_3$); FTIR ($CHCl_3$ film) 3455, 3065, 3031, 2953, 2876, 1950, 1877, 1809, 1649, 1496, 1455, 1414, 1365, 1322, 1236, 1116, 1043, 1017, 973, 874, 777, 741, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.33−7.24 (m, 5H), 6.36 (dd, 1H, $J =$ 6.0, 1.0 Hz), 4.68 (dd, 1H, $J = 6.0$, 2.6 Hz), 4.57 (dd, 2H, $J = 27.9$, 12.1 Hz), 4.09 (d, 1H, $J = 5.2$ Hz), 3.89 (m, 1H), 3.78−3.68 (m, 3H), 2.12 (br s, 1H), 0.93 (t, 9H, $J = 8.0$ Hz), 0.63 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.3, 137.7, 128.2, 127.6, 127.5, 102.7, 78.0, 73.4, 71.5, 70.0, 69.0, 6.7, 4.9; LRMS (EI) *(m/e)* calcd for $C_{19}H_{30}O_4SiNa$ $[M + Na]^+$ 373.2, found 373.2.

Disaccharide 64. A solution of **63** (2.204 g, 8.404 mmol) in CH_2Cl_2 (20 mL) was treated with dimethyldioxirane (130 mL, 0.07 M solution in acetone) at 0 °C and stirred for 1 h. Most of the volatiles were removed by a stream of N_2. Then, the residue was treated with 20 mL of benzene, concentrated with a stream of N_2, and treated with **62** (1.477 g, 4.214 mmol) in THF (20 mL). Most of the solvent was removed by a stream of N_2. The mixture as a form of thick oil was cooled to 0 °C, treated with $ZnCl_2$ (4.4 mL, 1.0 M solution in Et_2O), and kept stirred at RT for 4 h. The reaction mixture was diluted with EtOAc (300 mL) and poured into H_2O (100 mL). The organic layer was separated, washed with H_2O (2 × 100 mL), saturated $NaHCO_3$(aq) (2 × 100 mL), and brine (1 × 100 mL), dried over Na_2SO_4, and concentrated to dryness. Flash column chromatography of the crude material with 33% EtOAc in hexanes afforded **64** (1.24 g, 47%) as a colorless oil: $[\alpha]^{23}_D = -24.4°$ (*c* 0.98, $CHCl_3$); FTIR ($CHCl_3$ film) 3454, 3064, 3031, 2953, 2912, 2878, 1951, 1806, 1648, 1496, 1455, 1413, 1369, 1245, 1074, 912, 869, 833, 740, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40−7.27 (m, 10H), 6.44 (d, 1H, $J = 6.2$ Hz), 4.79−4.75 (m, 2H), 4.65−4.52 (m, 5H), 4.47 (d, 1H, $J = 7.1$ Hz), 4.20−4.18 (m, 1H), 4.08−4.05 (m, 1H), 3.98−3.93 (m, 2H), 3.72−3.66 (m, 4H), 3.63−3.59 (m, 1H), 2.73 (d, 1H, $J = 2.7$ Hz), 0.91 (t, 9H, $J =$ 7.9 Hz), 0.57 (q, 6H, $J = 7.9$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.0, 145.5, 137.81, 137.3, 128.4, 128.3, 127.9, 127.8, 127.74, 127.65, 97.6, 96.6, 78.1, 78.0, 74.6, 73.7, 73.3, 72.7, 71.5, 70.6, 68.3, 68.0, 67.9, 6.7, 4.7; HRMS (FAB) (*m/e*) calcd for $C_{33}H_{44}O_{10}SiNa$ $[M + Na]^+$ 651.2601, found 651.2597.

Benzyl-Protected Disaccharide 65. To a solution of **64** (0.392 g, 0.623 mmol) and BnBr (0.0890 mL, 0.748 mmol) in anhydrous DMF (8 mL) was added NaH (0.0370 g, 0.935 mmol, 60% dispersion in oil) at 0 °C. The mixture was stirred at 0 °C for 1.5 h, poured into cold water (100 mL), and extracted with EtOAc (3 × 50 mL). The combined organic layer was washed with H_2O (3 × 70 mL) and brine (1 × 70 mL), dried over Na_2SO_4, and concentrated to dryness. Flash column chromatography of crude material afforded **65** (0.370 g, 82%) as colorless oil: $[\alpha]^{23}_D = -26.3°$ (*c* 1.35, $CHCl_3$); FTIR ($CHCl_3$ film) 2952, 2874, 1811, 1647, 1454, 1367, 1244, 1098, 739, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.37−7.25 (m, 15H), 6.46 (d, 1H, $J = 6.3$ Hz), 4.82−4.79 (m, 2H), 4.75−4.70 (m, 3H), 4.61 (d, 1H, $J = 11.3$ Hz), 4.58−4.53 (m, 4H), 4.14−4.10 (m, 2H), 3.99−3.95 (m, 2H), 3.72−3.68 (m, 3H), 3.62 (dd, 1H, $J = 11.0$, 2.5 Hz), 3.49 (t, 1H, $J = 4.9$ Hz), 0.93−0.90 (m, 9H), 0.63−0.58 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.9, 145.3, 138.0, 137.4, 136.9, 128.48, 128.45, 128.3, 128.1, 127.92, 127.89, 127.8 (2C), 127.5, 97.1, 96.5, 78.5, 77.2, 76.78, 74.0, 73.8, 73.7, 73.3, 72.1, 69.9, 68.5, 68.0, 67.9, 6.8, 4.7; LRMS (EI) *(m/e)* calcd for $C_{40}H_{50}O_{10}SiNa$ $[M + Na]^+$ 741.3, found 741.5.

Iodosulfonamide 66. A mixture of **65** (0.357 g, 0.496 mmol), benzenesulfonamide (0.156 g, 0.992 mmol), and freshly activated 4 Å molecular sieves (0.45 g) in CH_2Cl_2 (10 mL) was stirred for 15 min at RT, cooled to 0 °C, treated with I(*sym*-coll)$_2ClO_4$ (0.465 g), and stirred for 1 h at 0 °C. The reaction was diluted with EtOAc (150 mL), filtered through a pad of silica gel, and washed with saturated $Na_2S_2O_3$(aq) (2 × 50 mL), saturated $CuSO_4$(aq) (2 × 50 mL), H_2O (1 × 50 mL), and brine (1 × 50 mL). The organic layer was dried over Na_2SO_4 and concentrated to dryness. Flash column chromatography of crude material with 25% EtOAc in hexanes gave **66** (0.445 g, 90%) as a white foam: $[\alpha]^{23}_D$ −24.0° (*c* 1.47, $CHCl_3$); FTIR (neat) 3261, 2952, 2876, 1809, 1454, 1330, 1165, 1095, 739, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.89 (d, 2H, $J = 8.3$ Hz), 7.39−7.23 (m, 18H), 5.83 (br s, 1H), 5.47 (t, 1H, $J = 8.0$ Hz), 4.94 (d, 1H, $J = 11.4$ Hz), 4.88 (br s, 1H), 4.82−4.77 (m, 2H), 4.65 (d, 1H, $J = 11.4$ Hz), 4.56−4.48 (m, 5H), 4.02(dt, 1H, $J = 6.6$, 1.2 Hz), 3.84−3.74 (m, 5H), 3.62 (d, 2H, $J = 6.7$ Hz), 3.28 (d, 1H, $J = 9.8$ Hz), 0.86 (t, 9H, $J = 8.0$ Hz), 0.53 (q, 9H, $J =$ 7.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.7, 140.9, 138.3, 137.2, 136.7, 132.6, 128.7, 128.6, 128.5, 128.3, 128.2 (2C), 128.0, 127.9, 127.8, 127.5, 127.4, 74.0, 73.73, 73.66, 73.3, 70.1, 69.4, 67.4, 67.3, 30.1, 6.7, 4.6; LRMS (ESI) (*m/e*) calcd for $C_{46}H_{57}NO_{12}SSiNa$ $[M + Na]^+$ 1024.9, found 1024.3.

Tetrasaccharide 67. To a solution of disaccharide acceptor **43** (0.804 g, 1.19 mmol) in anhydrous benzene (90 mL) was added bis(tributyltin) oxide (0.347 mL, 0.653 mmol). The resulting solution was heated at reflux overnight with azeotropic elimination of H_2O via a Dean−Stark trap. Removal of volatiles by N_2 stream provided the stannyl ether of **43** as oil. To a mixture of **66** (0.470 g, 0.475 mmlo) and 1 g of 4 Å molecular sieves was added the solution of the stannyl ether in dry THF (10 mL) via cannula. The reaction mixture was stirred at RT for 15 min, cooled to 0 °C, and treated with the solution of silver tetrafluoroborate (0.243 g, 1.25 mmol) in THF (2 mL). Then, the reaction was heated at 45−50 °C for 36 h. After the completion of reaction, it was filtered through a pad of Celite and washed with EtOAc (150 mL). The organic layer was washed with saturated $NaHCO_3$(aq) (3 × 50 mL) and brine (1 × 50 mL), dried over Na_2SO_4, and concentrated to dryness. Flash column chromatography of crude material with 35−50% EtOAc in hexanes afforded **67** (higher R_f value, 0.269 g, 37%) with α-isomer **68** (0.225 g, 30%) as a white foam. Also recovered was 0.4 g of acceptor **43**.

67: $[\alpha]^{23}_D = -28.2°$ (*c* 0.64, $CHCl_3$); FTIR ($CHCl_3$ film) 3514, 2874, 1809, 1736, 1718, 1454, 1367, 1094, 740, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, 2H, $J = 7.5$ Hz), 7.43−7.25 (m, 33H), 6.42 (d, 1H, $J = 6.2$ Hz), 4.93 (d, 1H, $J = 9.4$ Hz), 4.89 (dd, 1H, $J =$ 6.0, 4.3 Hz), 4.82 (t, 2H, $J = 6.0$ Hz), 4.77 (dd, 1H, $J = 7.8$, 1.6 Hz), 4.73−4.66 (m, 3H), 4.64−4.59 (m, 2H), 4.57−4.53 (m, 3H), 4.51−4.45 (m, 4H), 4.39 (d, 1H, $J = 11.2$ Hz), 4.29−4.26 (m, 1H), 4.25−4.18 (m, 2H), 4.12−4.09 (m, 1H), 3.92−3.87 (m, 2H), 3.85−3.82 (m, 2H), 3.81−3.76 (m, 2H), 3.74−3.71 (m, 1H), 3.65−3.62 (m, 3H), 3.60−3.56 (m, 1H), 3.53−3.46 (m, 2H), 3.43−3.41 (m, 2H), 2.64 (br s, 1H), 2.60−2.53 (m, 2H), 2.40−2.34 (m, 2H), 2.08 (s, 3H), 0.89 (t, 9H, $J =$ 7.9 Hz), 0.55 (q, 6H, $J = 7.9$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.3, 172.4, 153.8, 144.4, 140.7, 138.6, 138.5, 138.0, 137.8, 137.4, 137.0, 132.7, 129.0, 128.51, 128.47, 128.4, 128.33, 128.28, 128.1, 128.0, 127.9, 127.83, 127.79, 127.73, 127.71, 127.63, 127.62, 127.5, 127.0, 102.2, 100.9, 99.5, 99.2, 79.9, 78.6, 78.3, 78.1, 75.5, 74.3, 74.2, 73.7, 73.3, 73.0, 72.8, 71.8, 71.5, 70.1, 70.0, 69.9, 69.2, 68.4, 67.8, 67.5,

63.4, 56.9, 37.8, 29.7, 27.7, 6.7, 4.5; HRMS (FAB) (*m*/*e*) calcd for $C_{84}H_{99}NO_{23}SSiNa$ [M + Na]$^+$ 1572.5953, found 1572.5996.

68: $[\alpha]^{23}_D = +33.1°$ (*c* 0.48, $CHCl_3$); FTIR ($CHCl_3$ film) 3502, 2875, 1809, 1738, 1718, 1453, 1366, 1093, 738, 699 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (d, 2H, *J* = 7.9 Hz), 7.49−7.24 (m, 31H), 7.15 (d, 2H, *J* = 6.6 Hz), 6.48 (d, 1H, *J* = 6.3 Hz), 5.97 (d, 1H, *J* = 10.1 Hz), 5.01 (d, 1H, *J* = 7.4 Hz), 4.95−4.93 (m, 2H), 4.74 (d, 1H, *J* = 3.3 Hz), 4.65−4.55 (m, 5H), 4.49−4.46 (m, 3H), 4.42 (d, 1H, *J* = 10.0 Hz), 4.37 (d, 1H, *J* = 10.7 Hz), 4.34−4.21 (m, 7H), 4.11−4.08 (m, 2H), 3.97 (d, 1H, *J* = 9.1 Hz), 3.84 (dd, 1H, *J* = 10.5, 6.7 Hz), 3.79−3.74 (m, 3H), 3.65 (d, 1H, *J* = 10.5, 3.8 Hz), 3.61 (br s, 1H), 3.55−3.52 (m, 4H), 3.46−3.39 (m, 3H), 3.34 (d, 1H, *J* = 10.2 Hz), 3.18 (d, 1H, *J* = 5.3 Hz), 2.71−2.59 (m, 2H), 2.53−2.42 (m, 2H), 2.17 (bs, 1H), 2.10 (s, 3H), 0.87 (t, 9H, *J* = 7.9 Hz), 0.55 (q, 6H, *J* = 7.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.4, 172.6, 153.9, 144.5, 140.4, 138.5, 137.9, 137.7, 137.64, 137.55, 137.2, 133.1, 129.3, 128.9, 128.4, 128.34, 128.33, 128.30, 128.22, 128.17, 127.9, 127.8, 127.71, 127.69, 127.61, 127.56, 127.5, 127.3, 102.7, 100.4, 99.3, 93.2, 80.2, 79.2, 76.6, 75.7, 75.6, 75.3, 74.9, 74.3, 73.4, 73.2, 73.1, 72.9, 71.7, 71.2, 71.0, 70.8, 70.0, 69.9, 68.3, 67.8, 67.7, 64.1, 62.9, 57.6, 37.8, 29.7, 27.8, 7.0, 5.2; LRMS (EI) *(m/e)* calcd for $C_{84}H_{99}NO_{23}SSiNa$ [M + Na]$^+$ 1572.6, found 1572.7.

Tetrasaccharide Acceptor 69. To a solution of **67** (0.281 g, 0.181 mmol), a catalytic amount of DMAP, and Et_3N (0.303 mL, 2.17 mmol) in CH_2Cl_2 (15 mL) was added acetic anhydride (0.205 mL, 2.17 mmol) at 0 °C. The reaction mixture was stirred at RT for 40 h, diluted with EtOAc (50 mL), washed with saturated $NaHCO_3$(aq) (3 × 30 mL) and brine (1 × 30 mL), dried over Na_2SO_4, and concentrated to dryness. Flash column chromatography of crude material with 33% EtOAc in hexanes afforded acetylated tetrasaccharide (0.254 g, 88%) as a white foam. It was dissolved in CH_2Cl_2 (20 mL) and sequentially treated with pyridine (0.670 mL) and 1 M NH_2NH_2 in pyridine−AcOH (3:2) (0.670 mL) at RT. The reaction mixture was stirred for 4 h, diluted with EtOAc (300 mL), washed with saturated $NaHCO_3$(aq) (3 × 100 mL), saturated $CuSO_4$(aq) (3 × 100 mL), and brine (1 × 50 mL), dried over Na_2SO_4, and concentrated to dryness. Flash column chromatography of crude material gave **69** (0.194 g, 83%) as a white foam: $[\alpha]^{23}_D = -8.9°$ (*c* 0.38, $CHCl_3$); FTIR ($CHCl_3$ film) 3351, 3030, 2875, 1809, 1746, 1649, 1454, 1369, 1240, 1094, 738 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, 2H, *J* = 8.0 Hz), 7.45 (t, 1H, *J* = 8.0 Hz), 7.37−7.22 (m, 32H), 6.42 (d, 1H, *J* = 6.2 Hz), 5.19 (d, 1H, *J* = 3.3 Hz), 4.87 (dd, 1H, *J* = 6.0, 3.3 Hz), 4.79 (d, 1H, *J* = 11.6 Hz), 4.77 (d, 1H, *J* = 7.1 Hz), 4.71 (d, 1H, *J* = 7.7 Hz), 4.69 (d, 1H, *J* = 11.6 Hz), 4.63−4.48 (m, 11H), 4.44 (d, 1H, *J* = 12.0 Hz), 4.35 (d, 1H, *J* = 11.6 Hz), 4.16−4.11 (m, 3H), 3.86 (t, 1H, *J* = 4.2 Hz), 3.79−3.67 (m, 5H), 3.65−3.55 (m, 6H), 3.52−3.49 (m, 1H), 3.44−3.40 (m, 2H), 3.35−3.31 (m, 1H), 2.75 (bs, 1H), 2.01 (s, 3H), 0.86 (t, 9H, *J* = 7.9 Hz), 0.51 (q, 6H, *J* = 7.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8, 153.7, 144.6, 141.4, 138.6, 138.4, 138.1, 137.8, 137.2, 137.1, 132.3, 128.8, 128.5, 128.4, 128.32, 128.25, 128.00, 127.95, 127.8, 127.74, 127.65, 127.6, 127.5, 127.4, 127.3, 127.0, 102.4, 101.5, 100.0, 99.6, 79.5, 79.3, 78.9, 78.0, 77.8, 75.9, 74.5, 74.4, 73.8, 73.6, 73.40, 73.36, 73.2, 73.1, 72.4, 70.7, 70.4, 70.1, 69.8, 69.6, 67.7, 67.4, 60.2, 58.3, 20.7, 6.7, 4.6; HRMS (FAB) (*m*/*e*) calcd for $C_{81}H_{95}NO_{22}SSiNa$ [M + Na]$^+$ 1516.5787, found 1516.5733.

Hexasaccharide 70. A mixture of thiodonor **47** (0.201 g, 0.215 mmol), tetrasaccharide acceptor **69** (0.168 g, 0.112 mmol), di-*tert*-butylpyridine (0.097 mL, 0.43 mmol), and freshly activated 4 Å molecular sieves (1.5 g) in $Et_2O-CH_2Cl_2$ (2:1, 8.1 mL) was stirred at RT for 15 min, cooled to −40 °C, treated with MeOTf (0.0970 mL, 0.859 mmol), and slowly allowed to warm to RT for 6 h. Then, it was quenched by addition of Et_3N (2 mL), stirred for 15 min, diluted with EtOAc (300 mL), washed with saturated $NaHCO_3$(aq) (2 × 70 mL), saturated $CuSO_4$(aq) (2 × 70 mL), and brine (1 × 70 mL), dried over Na_2SO_4, and concentrated to dryness. Flash column chromatography of crude material provided hexasaccharide **70** (0.199 g, 75%) as a white foam: $[\alpha]^{23}_D = -7.0°$ (*c* 0.37, $CHCl_3$); FTIR ($CHCl_3$ film) 2874, 1811, 1747, 1454, 1370, 1092, 738, 698 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, 2H, *J* = 7.6 Hz), 7.74 (d, 2H, *J* = 7.6 Hz), 7.45−7.13 (m, 51H), 6.36 (d, 1H, *J* = 6.0 Hz), 5.15 (d, 1H, *J* = 3.5 Hz), 4.85−4.77 (m, 4H), 4.72−4.66 (m, 4H), 4.65−4.56 (m, 7H), 4.55−4.50 (m, 5H), 4.49−4.40 (m, 4H), 4.43−4.41 (m, 4H), 4.29−4.25 (m, 2H), 4.22 (d, 1H, *J* = 7.0 Hz), 4.12 (d, 1H, *J* = 5.8 Hz), 4.05−4.03 (m, 1H), 3.88−3.82 (m, 2H), 3.74−3.71 (m, 2H), 3.70−3.63 (m, 6H), 3.62−3.53 (m, 9H), 3.43 (t, 1H, *J* = 6.0 Hz), 3.37 (d, 1H, *J* = 7.3 Hz), 3.35 (d, 1H, *J* = 7.5 Hz), 3.32−3.27 (m, 3H), 3.20 (br d, 1H, *J* = 8.0 Hz), 2.75 (d, 1H, *J* = 8.3 Hz), 2.02 (s, 3H), 0.87−0.82 (m, 18H), 0.53−0.37 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.7, 153.9, 153.7, 144.4, 142.3, 141.6, 138.7, 138.5, 138.1, 138.0, 137.5, 137.32, 137.25, 137.1, 132.3, 131.9, 128.8, 128.5, 128.43, 128.41, 128.33, 128.31, 128.27, 128.22, 128.1, 128.01, 127.96, 127.93, 127.86, 127.8, 127.74, 127.68, 127.66, 127.64, 127.60, 127.57, 127.54, 127.48, 127.4, 127.1, 127.0, 102.1, 101.9, 101.5, 100.2, 100.0, 99.8, 80.0, 79.09, 79.07, 78.8, 78.7, 77.9, 77.8, 77.1, 76.14, 76.05, 75.1, 74.8, 74.6, 74.3, 73.9, 73.8, 73.7, 73.4, 73.3, 73.23, 73.20, 73.17, 71.6, 70.61, 70.56, 70.3, 70.0, 69.8, 67.94, 67.86, 67.52, 67.46, 60.1, 58.2, 20.8, 7.0, 6.8, 4.9, 4.6; HRMS (FAB) (*m*/*e*) calcd for $C_{127}H_{150}N_2O_{34}S_2Si_2Na$ [M + Na]$^+$ 2389.8846, found 2389.8948.

Hexa Acceptor 71. To a solution of **70** (0.0790 g, 0.0330 mmol) in THF (5.4 mL) was dropwise added AcOH (0.011 mL, 0.20 mmol) and TBAF (0.2 mL, 1 M in THF) successively. The reaction mixture was stirred at RT for 17 h, diluted with EtOAc (150 mL), washed with saturated $NaHCO_3$(aq) (2 × 70 mL), H_2O (2 × 50 mL), and brine (1 × 50 mL), dried over Na_2SO_4, and concentrated to dryness. Flash column chromatography of crude material with 45% EtOAc in hexanes afforded **71** (0.0644 g, 93%) as a white foam: $[\alpha]^{23}_D = -14.8°$ (*c* 0.97, $CHCl_3$); FTIR ($CHCl_3$ film) 3477, 2872, 1808, 1746, 1454, 1371, 1329, 1161, 1098, 738, 699 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (bd, 2H, *J* = 7.6 Hz), 7.87 (bd, 2H, *J* = 7.1 Hz), 7.54−7.47 (m, 3H), 7.42 (bd, 2H, *J* = 7.3 Hz), 7.39−7.17 (m, 44H), 7.14 (bd, 2H, *J* = 7.5 Hz), 6.30 (d, 1H, *J* = 6.2 Hz), 5.43 (d, 1H, *J* = 7.4 Hz), 5.19 (d, 1H, *J* = 3.5 Hz), 4.93 (bs, 1H), 4.86 (dd, 1H, *J* = 6.1, 3.1 Hz), 4.81 (d, 1H, *J* = 8.7 Hz), 4.77 (d, 1H, *J* = 12.1 Hz), 4.73 (d, 1H, *J* = 8.0 Hz), 4.69 (br s, 2H), 4.66 (br d, 1H, *J* = 8.7 Hz), 4.63−4.61 (m, 5H), 4.57 (d, 1H, *J* = 5.3 Hz), 4.54−4.52 (m, 4H), 4.50 (bs, 1H), 4.47−4.44 (m, 4H), 4.40 (d, 1H, *J* = 7.9 Hz), 4.34−4.29 (m, 3H), 4.23−4.19 (m, 2H), 4.10 (bs, 1H), 3.98 (d, 1H, *J* = 12.2 Hz), 3.94 (t, 1H, *J* = 6.9 Hz), 3.85 (d, 1H, *J* = 10.4 Hz), 3.82−3.81 (m, 2H), 3.73−3.68 (m, 4H), 3.64−3.59 (m, 4H), 3.57−3.53 (m, 2H), 3.51−3.49 (m, 3H), 3.47−3.38 (m, 7H), 3.35−3.26 (m, 2H), 3.12 (dd, 1H, *J* = 10.0, 7.9 Hz), 2.85 (bd, 1H, *J* = 8.3 Hz), 2.13 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8, 153.6, 153.5, 144.5, 142.3, 142.1, 139.0, 138.5, 138.1, 137.7, 137.2, 137.1, 137.0, 136.5, 132.4, 131.8, 128.7, 128.53, 128.49, 128.47, 128.4, 128.30, 128.26, 128.2, 128.1, 128.0, 127.83, 127.78, 127.7, 127.63, 127.58, 127.55, 127.48, 127.46, 127.1, 102.0, 101.6, 101.3, 100.3, 99.9, 99.3, 76.54, 76.50, 76.3, 75.7, 75.5, 74.2, 74.0, 73.9, 73.7, 73.64, 73.61, 73.42, 73.39, 73.21, 73.18, 73.1, 72.89, 72.86, 72.7, 70.9, 70.2, 69.9, 69.4, 68.7, 68.1, 68.0, 67.9, 67.8, 67.6, 59.3, 58.5, 20.9; HRMS (FAB) (*m*/*e*) calcd for $C_{115}H_{122}N_2O_{34}S_2Na$ [M + Na]$^+$ 2161.7142, found 2161.7218.

Fully Protected N3 Major Glycal 72. A mixture of **71** (0 0352 g, 0.0160 mmol), fucosyl floride **23** (0.0370 g, 0.0820 mmol), di-*tert*-butylpyridine (0.026 mL, 0.16 mmol), and 4 Å molecular sieves (0.57 g) in toluene (2.8 mL) was stirred at RT for 15 min, cooled to −15 °C, treated with the solution of $Sn(OTf)_2$ (0.0240 g, 0.0576 mmol) in THF (0.28 mL) via cannula, and stirred at −7 °C for 12 h, 0 °C for 24 h, and RT for 5 h. Then, the reaction mixture was quenched by addition of Et_3N (2 mL) and stirred for 10 min It was poured into a separatory funnel containing EtOAc (150 mL), washed with saturated $NaHCO_3$-(aq) (2 × 50 mL) and brine (1 × 50 mL), dried over Na_2SO_4, and concentrated to dryness. Flash column chromatography of crude material with 35% EtOAc in hexanes gave **72** (0.0375 g, 76%) as white foam: $[\alpha]^{23}_D = -67.1°$ (*c* 0.83, $CHCl_3$); FTIR ($CHCl_3$ film) 2872, 1811, 1747, 1720, 1453, 1271, 1160, 1094, 911 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06−8.03 (m, 4H), 7.69 (d, 2H, *J* = 7.7 Hz), 7.56 (d, 4H, *J* = 7.4 Hz), 7.45 (t, 4H, *J* = 7.7 Hz), 7.40−7.16 (m, 71H), 6.94 (d, 1H, *J* = 3.5 Hz), 6.38 (d, 1H, *J* = 6.2 Hz), 5.58 (d, 1H, *J* = 2.6 Hz), 5.48 (d, 1H, *J* = 2.8 Hz), 5.19 (d, 1H, *J* = 3.3 Hz), 5.12 (d, 1H, *J* = 3.4 Hz), 5.00 (bs, 1H), 4.98 (d, 1H, *J* = 6.8 Hz), 4.89−4.78 (m, 6H), 4.74−4.66 (m, 6H), 4.63−4.57 (m, 7H), 4.54−4.43 (m, 12H), 4.33−4.25 (m, 5H), 4.21−4.15 (m, 3H), 4.11 (t, 1H, *J* = 4.8 Hz), 4.04 (bs, 1H), 4.01−3.91 (m, 3H), 3.84−3.76 (m, 6H), 3.73−3.60 (m, 10H), 3.53−

3.40 (m, 5H), 3.33−3.28 (m, 1H), 3.35−3.20 (m, 2H), 2.89−2.81 (m, 2H), 1.95 (s, 3H), 1.02 (t, 6H, J = 6.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.5, 166.1, 166.0, 153.6, 144.3, 142.6, 141.4, 138.8, 138.7, 138.3, 138.2, 138.10, 138.06, 137.9, 137.7, 137.6, 137.4, 137.3, 137.2, 133.0, 132.9, 132,3, 131.6, 130.02, 129.97, 129.8, 129.0, 128.8, 128.5, 128.44, 128.40, 128.36, 128.31, 128.27, 128.23, 128.15, 128.1, 127.98, 127.96, 127.90, 127.89, 127.86, 127.82, 127.75, 127.72, 127.69, 127.65, 127.62, 127.58, 127.4, 127.34, 127.29, 126.9, 101.91, 101.87, 101.0, 100.0, 99.64, 99.55, 99.5, 97.2, 79.9, 79.5, 78.5, 78.4, 77.9, 77.4, 77.2, 76.9, 76.4, 75.9, 75.6, 75.0, 74.8, 74.7, 74.5, 74.4, 74.1, 73.9, 73.73, 73.71, 73.6, 73.5, 73.4, 73.2, 72.9, 72.0, 71.8, 71.4, 71.1, 71.0, 70.5, 70.41, 70.37, 69.5, 69.2, 68.3, 67.8, 67.6, 67.3, 66.5, 66.0, 65.5, 59.0, 58.3, 20.7, 16.31, 16.28; LRMS (EI) *(m/e)* calcd for $C_{169}H_{174}N_2O_{44}S_2$-Na $[M + 2Na]^{2+}$ 1522.5 for z = 2, found 1522.6.

N3 Major Allyl Glycoside 3. To a deep blue solution of sodium (0.070 g) in liquid ammonia (ca. 7 mL) under argon at −78 °C was added fully protected octasaccharide **72** (0.058 g, 0.019 mmol) in anhydrous THF (3 mL). After being stirred for 45 min, the reaction mixture was quenched by addition of anhydrous MeOH (3 mL) at −78 °C. Most of the ammonia was removed by a stream of argon, and the solution was diluted with MeOH (6 mL) and stirred for overnight at RT. It was treated with Dowex 50-X8 ion-exchange resin (0.60 g), stirred for 15 min, filtered, washed with MeOH (20 mL) and ammoniacal MeOH (30 mL), and concentrated to dryness. A crude material and catalytic amount of DMAP was suspended in pyridine (1 mL), treated with acetic anhydride (0.5 mL), stirred at RT for 18 h, and concentrated to dryness. Flash column chromatography of crude material with 50% CH_2Cl_2 in EtOAc−100% EtOAc provided peracetylated octasaccharide (0.024 g, 58%). A solution of peracetylated octasaccharide (0.018 g, 0.0083 mmol) in CH_2Cl_2 (1 mL) was treated with 3,3′-dimethyldioxirane (0.3 mL, 0.75 M solution) at 0 °C and stirred for 45 min. Then, most of the volatiles were removed by a stream of argon until ca. 0.3 mL of mixture was left. It was treated with ally alcohol (5 mL). After further removal of volatiles being left by a stream of argon, it was stirred at RT overnight and concentrated to dryness. Flash column chromatography of the crude material with ethyl acetate gave two compounds [**A**, higher R_f value (C_6H_6–acetone), 0.0034 g (18%); **B**, 0.0083 g (45%)]. These compounds were deacetylated according to the procedure described in reference (NaOMe, MeOH). Upon deacetylation, mannose type isomer (0.0026 g, 84%) was obtained from **A** (0.005 g), and so was **3** (2.7 mg, 88%) from **B** (0.0049 g).

3 (from B): mp 225 °C (decomp); $[\alpha]^{23}_D$ = −96.0° (*c* 0.05, MeOH); FTIR (KBr) 3352, 1644, 1375, 1206, 1149, 1071 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 6.05−5.97 (m, 1H), 5.41 (dd, 1H, J = 17.3, 1.4 Hz), 5.30 (bd, 1H, J = 10.4 Hz), 5.13 (d, 1H, J = 3.9 Hz), 5.06 (d, 1H, J = 3.9 Hz), 4.72 (d, 1H, J = 8.2 Hz), 4.66 (d, 1H, J = 7.5 Hz), 4.56 (d, 1H, J = 8.0 Hz), 4.53 (d, 1H, J = 7.6 Hz), 4.48 (d, 1H, J = 7.8 Hz), 4.45 (d, 1H, J = 8.0 Hz), 4.42 (dd, 1H, J = 12.7, 5.5 Hz), 4.26 (d, 1H, J = 12.7, 6.4 Hz), 4.17 (d, 1H, J = 3.1 Hz), 4.11 (t, 1H, J = 9.8 Hz), 4.04−3.50 (m, 41H), 3.37 (t, 1H, J = 8.8 Hz), 2.08 (s, 3H), 2.06 (s, 3H), 1.21 (d, 3H, J = 6.3 Hz), 1.20 (d, 3H, J = 6.3 Hz); ^{13}C NMR (125 MHz, D_2O) δ 173.1, 172.7, 131.7, 117.1, 101.5, 101.3, 101.0, 100.2, 99.4, 97.0, 96.4, 80.2, 77.4, 74.3, 73.8, 73.6, 73.3, 73.23, 73.20, 73.1, 72.8, 71.8, 71.7, 71.2, 70.8, 70.7, 70.5, 70.33, 70.29, 69.4, 69.1, 68.9, 68.2, 67.6, 67.5, 67.0, 66.2, 66.1, 65.2, 65.1, 60.0, 59.9, 58.4, 58.1, 58.0, 54.3, 54.0, 20.9, 20.7, 13.8, 13.7; HRMS (FAB) (*m/e*) calcd for $C_{55}H_{92}N_2O_{39}Na$ $[M + Na]^+$ 1427.5128, found 1427.5175.

Mannose type isomer (from A): mp 226 °C (decomp); $[\alpha]^{23}_D$ = −21.4° (*c* 0.07, MeOH); FTIR (KBr) 3280, 1642, 1376, 1070 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 6.05−5.97 (m, 1H), 5.39 (bd, 1H, J = 17.2 Hz), 5.31 (bd, 1H, J = 10.3 Hz), 5.15 (d, 1H, J = 3.7 Hz), 5.05 (d, 1H, J = 3.4 Hz), 4.96 (bs, 1H), 4.72 (d, 1H, J = 7.8 Hz), 4.71 (d, 1H, J = 8.2 Hz), 4.53 (d, 1H, J = 7.6 Hz), 4.48 (d, 1H, J = 7.7 Hz), 4.42 (d, 1H, J = 7.9 Hz), 4.27 (dd, 1H, J = 12.8, 5.4 Hz), 4.15−4.08 (m, 4H), 4.04−3.49 (m, 41H), 2.09 (s, 3H), 2.06 (s, 3H), 1.20 (d, 6H, J = 6.4 Hz); ^{13}C NMR (125 MHz, D_2O) δ 175.1, 174.8, 133.6, 118.8, 103.7, 103.2, 103.0, 102.1, 101.4, 99.2, 98.9, 93.4, 82.3, 77.2, 76.3, 75.62, 75.58, 75.4, 75.3, 75.2, 74.3, 73.8, 72.8, 72.7, 72.5, 72.32, 72.27, 71.9, 71.4, 70.9, 70.2, 70.0, 69.61, 69.57, 69.5, 68.8, 68.7, 68.7, 68.2, 68.1, 67.2, 67.1, 62.0, 61.9, 60.4, 60.2, 60.0, 56.2, 56.0, 22.8, 22.7, 15.73, 15.71; HRMS (FAB) (*m/e*) calcd for $C_{55}H_{92}N_2O_{39}Na$ $[M + Na]^+$ 1427.5175, found 1427.5161.

N3 Major Antigen 1. To a solution of **3** (0.0015 g, 0.0011 mmol) in MeOH (1 mL) was added $PdCl_2$ (0.001 g, 0.006 mmol) at RT. The mixture was stirred for 4 h. After removal of $PdCl_2$ by passing it through Lichroprep RP-18 pad, it was subjected to gel filtration to give **1** (0.0015 g, quantitative) as a 1:1 (α:β) mixture of anomers: mp 175 °C (decomp); $[\alpha]^{23}_D$ = −46.2° (*c* 0.15, MeOH); FTIR (neat) 3318, 1644, 1207, 1149, 1072 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 5.25 (d, 1/2H, J = 3.5 Hz), 5.13 (d, 1H, J = 3.9 Hz), 5.06 (d, 1H, J = 3.7 Hz), 4.72 (d, 1H, J = 8.0 Hz), 4.69 (d, 1H, J = 8.0 Hz), 4.67 (d, 1/2H, J = 7.0 Hz), 4.53 (d, 1H, J = 7.6 Hz), 4.48 (d, 1H, J = 7.8 Hz), 4.45 (d, 1H, J = 7.9 Hz), 4.17 (d, 1H, J = 2.9 Hz), 4.11 (d, 1H, J = 9.6 Hz), 4.04−3.50 (m, 41 and 1/2H), 3.32 (t, 1/2H, J = 10.0 Hz), 2.08 (s, 3H), 2.06 (s, 3H), 1.21 (d, 3H, J = 6.3 Hz), 1.20 (d, 3H, J = 6.3 Hz); LRMS (EI) *(m/e)* calcd for $C_{52}H_{88}N2O_{34}Na$ $[M + Na]^+$ 1387.5, found 1387.6.

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Supporting Information Available: Experimental procedures for general methods for **11**, **12**, **14**, **16**, **19**−**22**, **25**−**32**, **48**, and **54**−**58** and NMR spectra (^{1}H for **1**−**4**, **43**, **45**−**48**, **54**−**58**, **62**, and **64**−**72,** ^{13}C for **3**, **4**, **43**, **45**−**48**, **54**−**58**, **62**, **64**, and **66**−**72**, and HMQC for **4**) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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