A Second Generation Synthesis of the MBr1 (Globo-H) Breast Tumor Antigen: New Application of the *n*-Pentenyl Glycoside Method for Achieving Complex Carbohydrate Protein Linkages

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Dedicated to Dr. Bert Fraser-Reid on the occasion of his 65th birthday

Abstract: A new synthesis of the hexasaccharide MBr1 antigen (globo-H) is reported. A revised construction with improved efficiency was necessary because an anti-cancer vaccine containing this antigen is entering phase II and phase III clinical trials for prostate cancer. The key feature of this second generation synthesis is the preparation of globo-H as its *n*-pentenyl glycoside. This group serves as an anomeric protecting group and as a linker for bioconjugation to carrier protein. The resultant synthesis allows for the production of suitable quantities of globo-H for clinical trials.

Keywords: antigens · carbohydrates · glycosides · glycoconjugates · immunology

Introduction

Active specific immunotherapy via vaccination attempts to stimulate an immune response in a tumor-bearing host through the use of a tumor-associated antigen. It has been known for some time that specific types of glycolipids or glycoproteins, which may be detectable in normal tissue types by immunohistology, are significantly more expressed in tumors of that tissue.[1] Furthermore, high levels of expression on tumor cells can often provoke an antibody response. However, this initial antibody response is usually ineffective in providing immunoprotection or immunorejection and disease can result. Possibly, tumor cells are capable of sending a variety of decoys, which disguise their initial growth and allow for the tumor antigens to be seen as "self" by the immune system.[2] Accordingly, the idea of using synthetically derived, cell-free glycoconjugates as versions of tumor-associated immunostimmulatory antigens in the development of anti-tumor vaccines to break the tolerance of the immune response is attractive.[3] The use of cancer vaccines containing carbohydrate antigens of fully synthetic origin to induce an anti-cancer immune response is in fact showing considerable promise for eventual therapy. Investigations using carbohydrate-based vaccines have thus far been conducted with the hope that patients immunized in an adjuvant setting might produce antibodies reactive with cancer cells, and that the production of such antibodies might mitigate against tumor spread, thereby creating a more favorable prognosis.[4]

Cancer carbohydrate antigens such as Tn,[5] TF, GD_3,[6] GM_2,[7] KH-1,[8] Le^y,[9] fucosyl GM_1,[10] and globo-H[11] are suitable targets for both active and passive immunotherapies because they have been characterized as being over-expressed on the surface of malignant cells in a variety of cancers. Obviously, such discoveries can only follow efforts that are achieved as a result of painstaking analysis. Moreover, due to major advances in the areas of monoclonal antibody (mAb) technology and immunohistology, the antigens have been immunocharacterized by suitable mAb's and therefore have relevant serological markers available for immunological studies. However, the required delicate, yet complex, characterizations are even more impressive when it is considered that the availabilities of such carbohydrates is typically limited to sub-milligram levels following isolation and purification of specimens from human cancer tissue collections. Consequently, in the absence of an enzymatically mediated synthesis, the task of delivery of carbohydrate-based antigens in amounts required to study immunotherapy at the preclinical and clinical level falls to organic synthesis.

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Indeed our laboratory has been interested in the development of anti-cancer vaccines using vaccine constructs prepared by total chemical synthesis for some time.[12] For example, a construct containing the MBr1 antigen globo-H **1a**,[13,14] is one of our most clinically promising anti-cancer vaccines (Figure 1).[15] It has been demonstrated in phase I

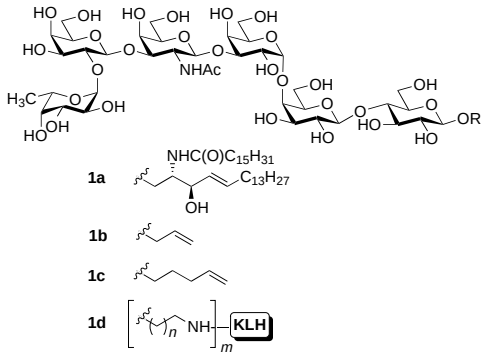

Figure 1. Structures of synthetically derived globo-H constructs.

clinical trials that the vaccine **1d** ($n=1$) is safe in humans as regards to autoimmunity problems and induces specific antibodies against tumor cells carrying on their cell surface the same antigenic structure contained in the vaccine. As part of a program to evaluate our globo-H based vaccine **1d** in phase II and phase III human clinical trials for prostate and breast cancers, we required a rapid and efficient synthesis by which suitable quantities could be produced. Herein, we describe the synthesis of globo-H pentenyl glycoside **1c** in the context of describing our most recent approach harmonizing the goals of a convergent total synthesis with the need for bioconjugation to carrier protein.

Results and Discussion

The previous synthesis of globo-H from our laboratories[16] utilized all glycal building blocks[17] for the rapid construction this complex oligosaccharide. These investigations relied on a highly convergent [3+3] coupling to generate the hexasaccharide core contained in the final target. In this approach, a flexible terminal glycal was maintained throughout the hexasaccharide construction (see structure **2**, Figure 2). The glycal was then used to install the ceramide side chain present en route to globo-H glycolipid **1a** or its allyl glycoside **1b**. The

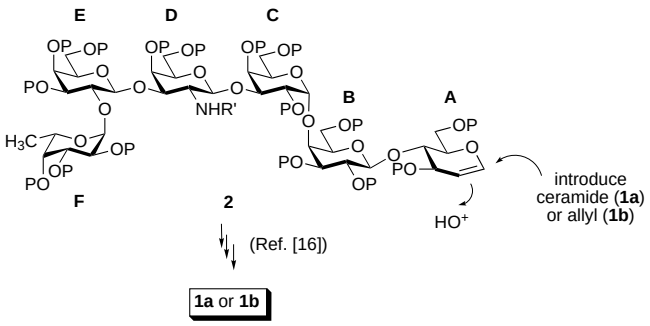

Figure 2. Previous strategy for the synthesis of **1a** and **1b**.

synthesis of **1a** served to facilitate the proof of structure and immunocharacterization of globo-H. The allyl glycoside **1b** was employed for immunoconjugation to biocarrier proteins. The previous protocols were effective in producing adequate quantities of synthetic material for proof of structure, immunocharacterization, conjugation, mouse vaccinations, and phase I human clinical trials.

The requirements for the current investigations were to meet the demand of a single target formation for advanced clinical trials. The target must be so functionalized as to anticipate the need for its conjugation to carrier protein and, that the resulting construction must be appropriately efficient as to allow for the generation of suitable material for clinical purposes. In the context of a second-generation strategy, we envisioned a synthetic route that began with the spacer required for bioconjugation in place rather early in the synthesis. This strategy would obviate the need to install the bioconjugation linker from the terminal glycal during the end-game transformations (vide infra).

It will be appreciated that the presentation of the globo-H antigenic hexasaccharide is crucial for an effective immune response. Studies have shown that a coherent vaccine can be achieved through bioconjugation to carrier protein keyhole limpet hemocyanin (KLH).[18] In designing a requisite linker toward the goal of conjugation to KLH, we took cognizance of several requirements. For the vaccine to be given a chance to be immunofunctional, the molecular recognition of the synthetic tumor antigen by the immune system must not be compromised in conjugating the carbohydrate domain to biocarrier. Among various strategies of spacer-linker combinations, we had success using allyl glycosides to link carbohydrates to proteins, although the optimum setting has not necessarily been determined. In our previous synthesis of **1b**, as was typical in our program aimed at total synthesis of tumor-associated antigens, the glycal was maintained through the global deprotection to the peracetate stage (see Figure 3, **2**→**3**).[19] Epoxidation of the glycal, followed by treatment with allyl alcohol gave the corresponding allyl glycoside **4**. However, with the resident neighboring acetates as the sole source of stereochemical control, only modest stereoselectivity was realized in this step (see formation of **4′**). We had considered and evaluated the possibility of carrying the allyl glycoside while the resident protecting groups were as originally protected. At this stage the epoxidation and allyl glycoside formation was indeed stereospecific (see **2**→**5**). However, it was then found that deprotection of the benzyl ethers and benzene sulfonamide groups in **5** by Birch reduction to yield **1b** was not possible with the allyl glycoside already in place. Though the reasons for the instability were not precisely determined, it appeared that the allyl group was cleaved and the resultant free sugar suffered further chemical damage. To avoid destruction of the allyl linking group we were forced to resort to conducting the epoxidation and allyl glycosidation sequence after deprotection and peracetylation (see **3**→**1b**). However, the trade-off was a serious erosion of stereoselectivity resulting in losses of hard won fully synthetic hexacyclic epitope. Nonetheless, with this strategy, removal of the ester protecting groups in **4** yielded fully deprotected allyl glycoside **1b** poised for bioconjugation.

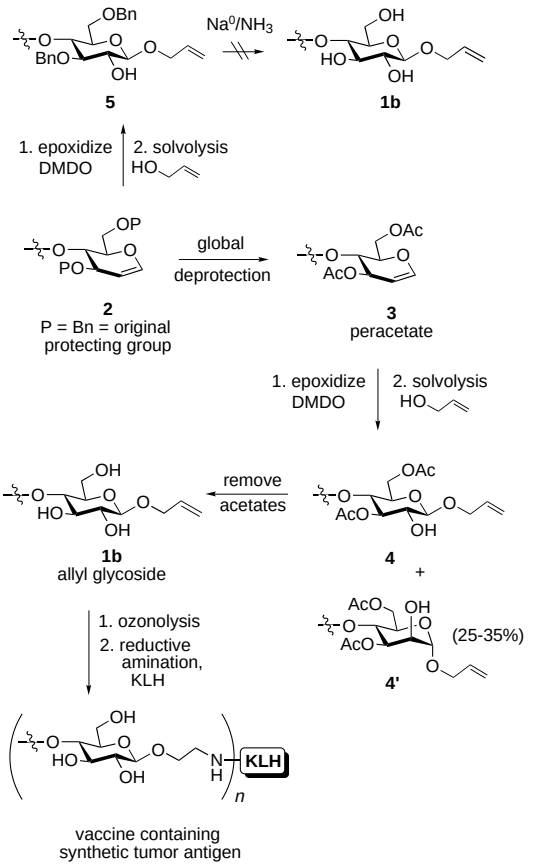

Figure 3. Summary of the previous synthesis of **1b** and **1d**.

The conundrum associated with the allyl glycoside approach detailed above invited an alternative solution which, in general terms, is formulated below (Figure 4). Thus, a hexasaccharide would be constructed containing a glycoside that would enable linkage to carrier protein, already in place. Indeed this group would already have been incorporated at the reducing end of the acceptor in the [3+3] coupling step. For successful implementation of this significant new variation of the globo-H synthesis (and by implication, other complex tumor-associated antigens), four conditions had to be met. First, the trisaccharide acceptor containing the glycoside construct had to be readily synthesizable. The glycoside construct had to be compatible with the [3+3] coupling. Clearly, the construct, in contrast to the allyl glycoside, had to survive benzyl ether and benzenesulfonamide deprotection. Finally, conjugation had to be implementable.

With these goals in mind, we chose to direct our synthesis to produce the *n*-pentenyl glycoside (NPG)[20] of the hexacyclic MBr1 antigen **1c**. We hoped that the pentenyl olefin, in contrast to the allyl function, would withstand the requisite Birch reduction step. Having survived the synthesis and deprotection phases, the terminal pentenyl function could now be oxidatively cleaved and the resulting γ-oxybutyryl aldehyde used for reductive amination.

To explore this possibility, we focused our attention on a plan involving the same DEF trisaccharide donor sector used in our previous synthesis. Our retrosynthetic analysis is shown in Figure 5. For maximum convergency, we desired the ABC acceptor containing the aforediscussed pentenyl glycosidic linker. The hexasaccharide core would then be assembled via a convergent [3+3] ABC+DEF coupling reaction using our sulfonamido glycosidation protocol.[21] Our previous results had indicated that the presence of a free hydroxyl at C4 of the reducing end galactose (Figure 5, see asterisk) in the DEF donor would be necessary to direct the formation of the

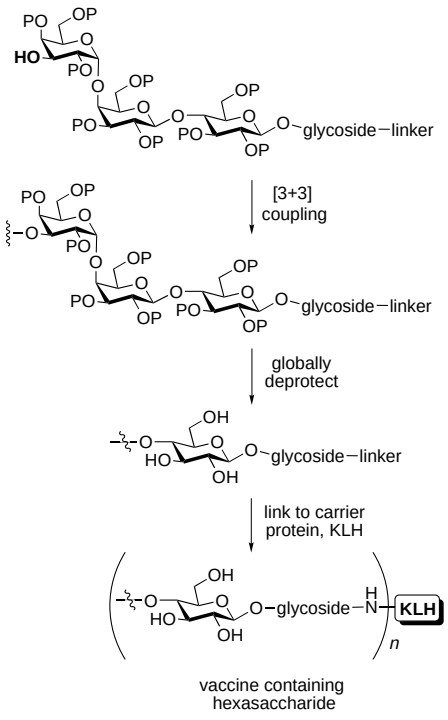

Figure 4. Synthetic requirements for our second generation procedure.

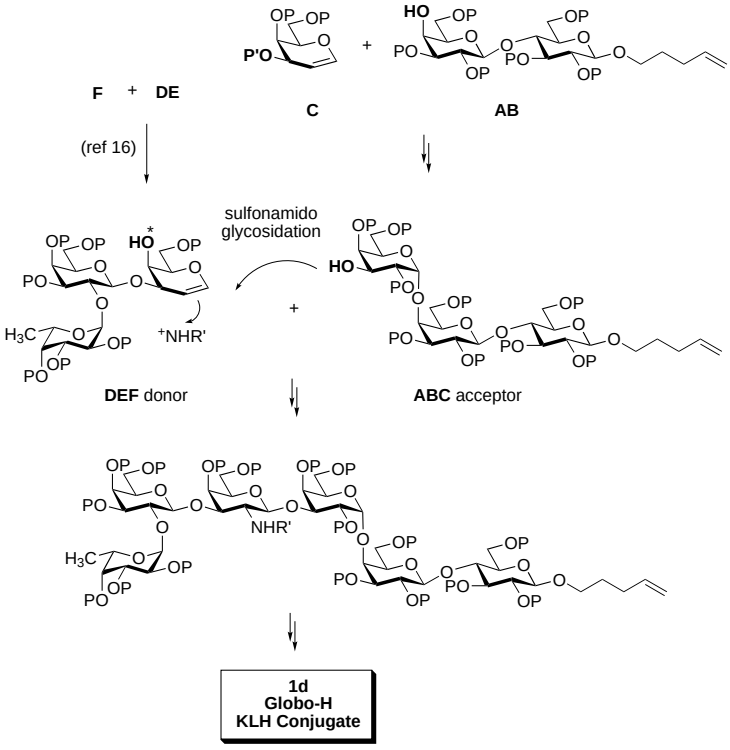

Figure 5. Retrosynthesis of **1**.

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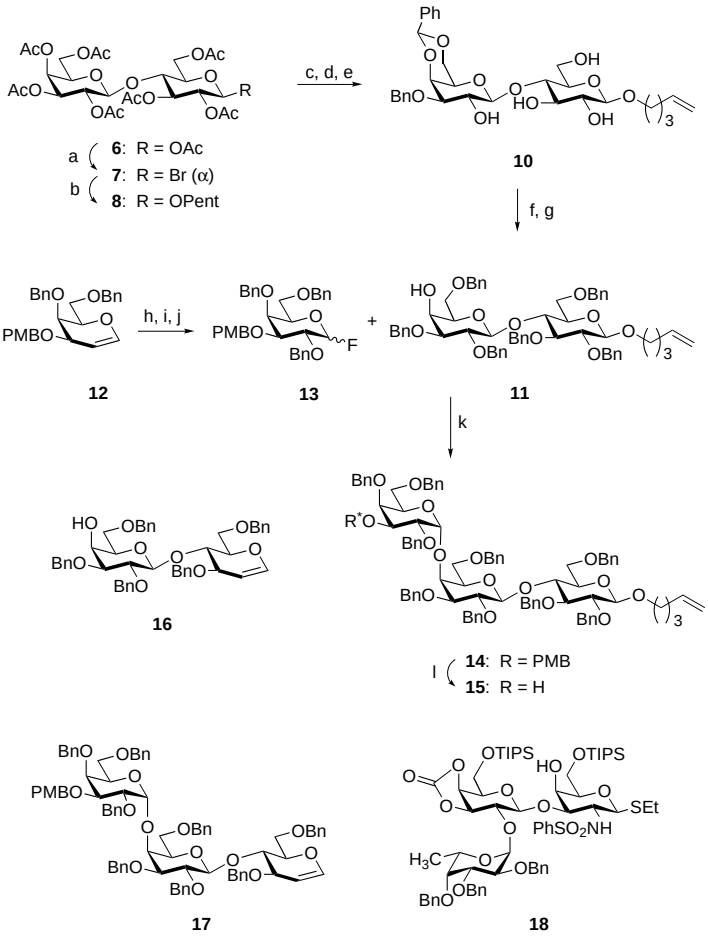
required β-linkage in the sulfonamido glycosidation.[22] The sequencing of the [3+3] coupling was expected to take place as shown, owing to the higher reactivity of the equatorial C3 hydroxyl group (see bold) in the acceptor as compared with the necessary axial C4 hydroxyl group in the donor trisaccharide. Important to the strategy described herein is that, once the hexasaccharide is assembled, only protecting group manipulations would be required to reach the pro-vaccine antigen.

Our experience with the DEF trisaccharide sector has been that its synthesis is fairly concise, requiring six transformations starting from 6-*O*-TIPS galactal and tri-*O*-benzyl fluoro fucose.[16] The primary encumbrance to our synthetic effort had been that of producing the ABC acceptor in glycal form. For purposes of a second-generation approach, the acceptor trisaccharide component can be dissected into a lactose derivative containing the desired NPG bearing a differentiated hydroxyl at C4′ and an appropriate C-ring donor (Figure 5). The galactose donor monosaccharide also requires differential protection at C3, for eventual ABC+DEF coupling, and needs careful attention to efficiently allow for the required α-glycosidic linkage joining the AB+C domains.

We begin with the synthesis of the requisite ABC acceptor, as shown in Scheme 1. Our method takes advantage of readily available lactose octaacetate **6**. Conversion of **6** to the known α-bromo donor **7**[23] was followed by silver carbonate mediated glycosylation with pentenyl alcohol as acceptor, to give **8** (Pent = $CH_2CH_2CH_2CH{=}CH_2$) in 75% yield on a 100 g scale.[24] Similar processing of **7** with silver triflate as promoter resulted in only 17% yield of the desired product. Thus, with the formation of **8**, we had in an early stage of the synthesis successfully installed the linker to be used for late stage bioconjugation.

Subsequent steps were designed to generate a free acceptor site at C4′ of **8** for an eventual AB+C coupling give the ABC trisaccharide (Scheme 1). Removal of the ester protecting groups in **8** to give pentenyl lactoside **9** was followed by a stannane mediated monobenzylation to selectively give the C3′ benzyl ether.[25] In a second step, the C4′ and C6′ hydroxyls were engaged as a benzylidene acetal to provide compound **10** as the only observable product.[26] Finally, perbenzylation of the remaining hydroxyl groups in **10** and regioselective reductive cleavage of the benzylidene with sodium cyanoborohydride and anhydrous HCl gave the C4′ alcohol **11**.[27] Thus, using well known transformations starting from lactose octaacetate **6**, the AB pentenyl glycoside acceptor **11** was obtained in seven steps and in 20% overall yield.

With large quantities of the protected pentenyl glycoside **11** available, we turned our attention to the AB+C coupling to form the trisaccharide acceptor **14**. The previous synthesis of glycal **17** (Scheme 1) required careful preparation of the highly activated β-fluoro donor **13** from glycal **12**.[16] The C3 PMB ether contained in **12** was strategically incorporated to allow for eventual ABC+DEF coupling upon selective deprotection of this group. In the course of this work, we discovered that α-**13** could be formed conveniently in high yield and on large scale. Accordingly, α-donor **13** was prepared from differentially protected glycal **12** by epoxidation and exposure to HF/pyridine to yield the *cis* fluorohydrin derivative and subsequent conversion of the resulting C2-hydroxyl to its benzyl ether. The anomeric α:β selectivity was demonstrated to be 10:1 and the overall yield in transforming **12** into **13** was 76%.



Scheme 1. Reagents: a) HBr, Ac_2O, AcOH, 96%; b) PentOH, Ag_2CO_3, CH_2Cl_2, 4 Å molecular sieves, 75%; c) NaOMe, MeOH; then Dowex-H^+; d) BnBr, Bu_2SnO, Bu_4NI, C_6H_6, 54% two steps; e) $PhCH(OMe)_2$, CSA, CH_3CN, 72%; f) BnBr, NaH, DMF, Et_4NI, 97%; g) $NaCNBH_3$, HCl, Et_2O, THF, 79%; h) DMDO, CH_2Cl_2; i) HF/pyridine, 85% two steps; j) BnBr, NaH, DMF, 95%; k) $Cp_2Zr(OTf)_2$, toluene/THF 5:1, 80% (α), α:β 10:1; l) DDQ, CH_3CN, H_2O, 84%.

We were now in a position to compare the effectiveness of the AB+C coupling using previously prepared β-**13** and our newly prepared α-**13** with the AB acceptor **11**. We also examined the synthetic optimization of glycal trisaccharide **17** as a model case (see **13**+**16** →**17**), because of its presumed sensitivity to overly demanding coupling promoters. In these investigations it was discovered that the reduced reactivity of α-fluoro donors could be attenuated by conducting the couplings with highly fluorophilic promoters in judiciously chosen solvents, as summarized in Table 1. Our previous coupling procedure using the predominantly β-fluoro donor **13** and glycal **16** to give glycal trisaccharide **17** employed Muykiyama coupling conditions[28] and proceeded in 54% yield with modest anomeric selectivity (entry 1, Table 1). Investigations using other promoters with α-**13** are shown in entries 2 and 3, but produced little satisfaction in terms of

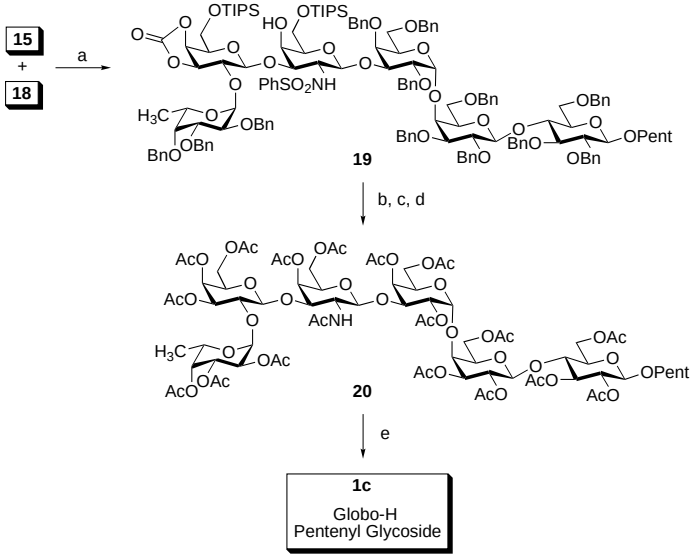
Table 1. Coupling conditions used to generate the ABC trisaccharide.

Compound	Acceptor AB	Donor C	Promoter, Solvent	α:β selectivity	Product yield [%]
1	**16**[a]	**13** (β)[a]	$SnCl_2$, $AgClO_4$ Et_2O	3:1	54% (α), 18% (β) **17**
2	**16**	**13** (10α:1β)	$Sn(OTf)_2$ toluene/THF 5:1	8:1	40% (α), 5% (β) **17**
3	**16**	**13** (10α:1β)	Cp_2ZrCl_2, $AgClO_4$ CH_2Cl_2/Et_2O	2.7:1	55% (α) **17**
4	**16**	**13** (10α:1β)	$Cp_2Zr(OTf)_2$ toluene/THF 10:1	10:1	72% (α), 8% (β) **17**
5	**11**	**13** (β)	$SnCl_2$, $AgClO_4$ Et_2O	3:1	42% (α) **14**
6	**11**	**13** (10α:1β)	$Cp_2Zr(OTf)_2$ toluene/THF 5:1	10:1	80% (α), 8% (β) **14**

[a] See reference [16].

overall efficiency. However, the preparation of glycal **17** was successfully extended to include the described α-donor **13** using strongly fluorophilic $Cp_2Zr(OTf)_2$[26] promotion (73% yield, entry 4). Gratifyingly, these optimized glycosidation conditions for formation of **17** were successfully applied to the AB+C coupling employing pentenyl glycoside **11** to provide trisaccharide **14** in yields that rivaled the parent reaction (80% yield, entry 6). Muykiyama coupling of β-**13** with **11** yielded only 42% of trisaccharide **14** (entry 5). Satisfied with the events leading to smooth formation of large quantities of **14**, we positioned ourselves to investigate the [3+3] coupling. The discharge of the lone PMB group in **14** could be effected in excellent yield (92%), thus completing the assembly of the desired ABC pentenyl acceptor **15**.

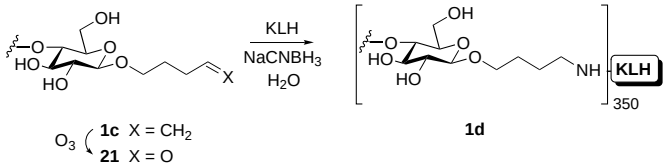
The key step and final transformations completing the synthesis of **1c** are shown in Scheme 2. Treatment of the known DEF donor **18**[16] (see Scheme 1) with MeOTf[30] in the presence of acceptor **15** smoothly provided hexasaccharide **19** in 60% yield. The configuration of the new anomeric center of **19** was confirmed to be β-configured, as was expected based on our earlier precedents. The [3+3] coupling yield using trisaccharide acceptor **15** was comparable to the [3+3] procedure using the glycal-based acceptor corresponding to **17**. The tremendous advantage of using **15**, however, is manifested in the steps which follow.



Scheme 2. Reagents: a) MeOTf, CH_2Cl_2/Et_2O 1:2, 60–70%, 0°C; b) TBAF, THF; then NaOMe, MeOH; c) Na/NH_3, THF, −78°C; then MeOH; d) Ac_2O, pyridine, DMAP, 42%; e) NaOMe, MeOH, 99%.

Global deprotection began with subjection of **19** to TBAF in order to remove the silyl ethers and the cyclic carbonate. The benzyl and sulfonamido protecting groups on the resulting penta-ol were then cleaved under the action of dissolving metal reduction. This protocol was followed by peracetylation to give the isolable hexasaccharide peracetate **20**. As in earlier steps, the pentenyl linkage proved highly reliable under the listed deprotection conditions. It is again notable by contrast that the corresponding allyl glycoside (to ultimately yield **1b**) is not stable to the reducing metal conditions required for global deprotection and therefore must be installed subsequent to deprotection at the level of **2** (see Figure 2, P = Ac). Deacetylation of **20** with methoxide yielded the fully deprotected pentenyl glycoside of globo-H **1c** notably poised for bioconjugation. *Importantly*, in the second generation variation, progress toward **1d** from hexasaccharide construct **19** was greatly simplified because the need for additional functionalization to allow for conjugation had been eliminated.

Toward our goal of facilitating clinical evaluation of synthetic globo-H, we have conjugated **1c** to carrier protein KLH for purposes of creating a functional vaccine. The first step of this procedure involved ozonolysis of the pendant olefin, followed by reductive work-up, to give the uncharacterized aldehyde intermediate **21**, as shown in Scheme 3.



Scheme 3. Covalent attachment to **1c**.

Although the use of NPG's for bioconjugation has not previously been reported, it seemed reasonable to assume that this four carbon aldehyde would serve well for this purpose. Accordingly, we were pleased to find that reductive amination with KLH and sodium cyanoborohydride in phosphate buffer yielded vaccine glycoconjugate **1d** (n = 3).[31] Covalent attachment of the carbohydrate to the proteins presumably occurs through the ε-amino groups on exposed lysine residues in KLH. Hydrolytic carbohydrate analysis[32] of **1d** revealed approximately 350 carbohydrate residues per molecule of carrier protein.[33]

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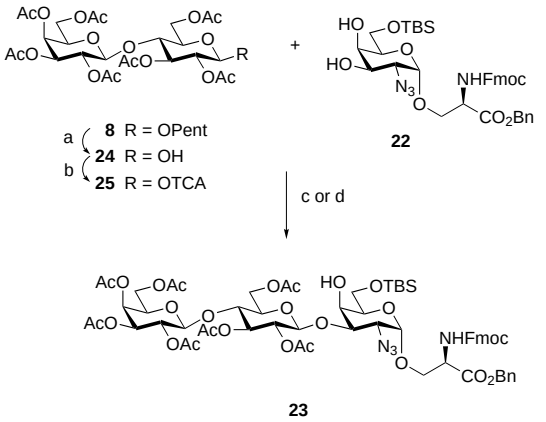
It did not escape our attention that with our current strategy which includes a reducing end NPG, the possibility to perform glycosidations to construct larger congeners or alternative linkages exists. In fact it was in this context that the NPG's were discovered and developed. Fraser-Reid and associates have very thoroughly demonstrated that NPG's can be effective donors in glycosidation reactions.[34] As part of our program to synthesize glycopeptides containing this and related tumor-associated antigens,[12, 35] we have begun to investigate the possibility of such couplings.

In the event, disaccharide **8** was used as the glycosidic donor with the glycosylated amino acid cassette acceptor **22**.[36] As shown in Scheme 4, conditions of NIS/TfOH[37] were used to



Scheme 4. Reagents: a) NBS, 1 % H_2O/CH_3CN, 53 %; b) Cl_3CCN, K_2CO_3, CH_2Cl_2, 83 %; c) **8**, NIS, TfOH, CH_2Cl_2, 4 Å molecular sieves, rt, 54 %; d) **25**, TMSOTf, CH_2Cl_2, 4 Å molecular sieves, 0 °C, 88 %.

promote the coupling and trisaccharide **23** was formed in 54 % yield. We recognized that NPG donors are not orthogonal to glycal donors. Thus, activating a NPG by standard treatment with halonium ion in the presence of a glycal acceptor could also result in glycal activation. In an effort to convert the pentenyl glycoside to alternative donors to be used with non-orthogonal acceptors, we also investigated oxidative hydrolysis reactions of **8**. Reaction of **8** with *N*-bromosuccinamide in 1 % H_2O/CH_3CN gave the free reducing alcohol **24**, as desired.[38, 39] Conversion of **24** to the trichloroacetimidate donor **25**[40] also proceeded smoothly (83 % yield). Coupling of trichloroacetamidate **25** with acceptor **22** under the action of TMSOTf yielded the same trisaccharide **23**, in an improved 88 % yield. Thus, with a simple system we have demonstrated that the pentenyl linkage contained in our generalized strategy does indeed offer alternative and broader possibilities for assembling complex glycopeptide and glycoprotein ensembles.

Conclusion

In summary, the synthesis of **1c** reported here allows facile production of globo-H. We have sharply re-worked our previous synthesis to incorporate an *n*-pentenyl glycoside, which was ultimately used for bioconjugation, in a trisaccharide precursor. Happily, the glycosidic *n*-pentenyl group was stable to the coupling promoters used throughout the construction and also the conditions required for global deprotection. The improved synthesis required fewer synthetic steps and occurs in a significantly more processible yield than the synthesis originally used to prove the globo-H structure and initiate phase I clinical trials. We are also exploring the generality of using *n*-pentenyl glycosides for immunoconjugation.[41] Not only does incorporation of the *n*-pentenyl protecting group offer improved chemical yields over fewer synthetic steps, its donor properties can also be used to efficiently generate other derivatives. Conversions analogous to those shown in Scheme 4 using **20** in the globo-H series, as well as biologically significant oligosaccharides in other series, are currently underway. Clinical results using synthetic vaccine **1d** ($n = 3$) prepared by the route described herein will be reported in due course.

Experimental Section

Peracetyl pentenyl-β-D-lactoside (8): 30 % HBr in AcOH (100 mL) was added dropwise over a period of 60 min to a cooled (ice bath) suspension of lactose octaacetate (100.0 g, 147.7 mmol), glacial acetic acid (30 mL) and acetic anhydride (30 mL). The reaction mixture stirred for 1 h and the ice bath was removed. Upon stirring for an additional 2 h at room temperature, the mixture became a homogeneous yellow solution. The solution was diluted with H_2O (1 L) and extracted with $CHCl_3$ (3×400 mL). The organic extracts were washed with H_2O (2×1 L), saturated $NaHCO_3$ (3×500 mL), dried over $MgSO_4$, and concentrated. The product was azeotroped with anhydrous benzene and dried under high vacuum to yield the lactosyl bromide (98.8 g, 96 %) which was used without further purification.

n-Pentenyl alcohol (5.0 equiv, 73.4 mL) was added to a suspension of Ag_2CO_3 (100 g, 362.6 mmol), freshly activated 4 Å molecular sieves (15 g) and a crystal of I_2 in CH_2Cl_2 (400 mL), and then the lactosyl bromide (98.8 g, 141.4 mmol) in CH_2Cl_2 (400 mL). After the solution was stirred in the dark at room temperature for 16 h, the reaction was filtered through a plug of Celite with additional CH_2Cl_2 and concentrated to a yellow oil which was purified by flash column chromatography (10 % →50 % EtOAc/hexanes) to yield the pentenyl lactoside as a white foam (74.7 g, 75 %). $[\alpha]_D^{22} = -48.9°$ ($c = 7.5$, $CHCl_3$); IR ($CHCl_3$, film): $\tilde{\nu} = 2941$, 1751, 1369, 1224, 1054 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 5.60$ (m, 1H), 5.17 (d, 1H, $J = 2.7$ Hz), 5.02 (m, 1H), 4.93 (dd, 1H, $J = 7.9$, 10.3 Hz), 4.85 (d, 1H, $J = 1.6$ Hz), 4.78 (m, 2H), 4.71 (dd, 1H, $J = 9.6$, 7.9 Hz), 4.30 (m, 3H), 3.93 (m, 3H), 3.66 (m, 3H), 3.45 (m, 1H), 3.30 (m, 1H), 1.98 (s, 3H), 1.94 (s, 3H), 1.91 (m, 2H), 1.89 (s, 3H), 1.88 (s, 6H, $2 \times CH_3$), 1.87 (s, 3H), 1.79 (s, 3H), 1.49 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 170.33$, 170.28, 170.09, 170.00, 169.74, 169.54, 169.01, 137.72, 115.00, 101.01, 100.51, 76.27, 72.76, 72.48, 71.64, 70.94, 70.58, 69.23, 69.01, 66.52, 61.97, 60.73, 29.75, 28.49, 20.80, 20.75, 20.64, 20.57, 20.45; FAB-HRMS calcd for $C_{31}H_{44}O_{18}Na^+$: 727.2425; found: 727.2418.

Pent-4-enyl 3′-*O*-benzyl-4′,6′-*O*-benzylidenyl-β-D-lactoside (10): Peracetylated pentenyl lactoside **8** (18.2 g, 25.8 mmol) was dissolved in anhydrous MeOH (300 mL) and NaOMe (2.0 mL, 25 % in MeOH) was added. The reaction stirred at rt for 16 h and was neutralized with Dowex-H^+ (pH 5–6). The reaction was filtered with additional MeOH and concentrated to a white solid **9** (10.6 g, quantitative) which was used without further purification: ^1H NMR (D_2O, 400 MHz): $\delta = 5.81$ (m, 1H), 5.00 (dd, 1H, $J = 17.3$, 1.9 Hz), 4.92 (dd, 1H, $J = 8.9$ Hz), 4.74 (m, 1H), 4.39 (d, 1H, $J = 8.0$ Hz), 4.35 (d, 1H, $J = 7.8$ Hz), 3.72–3.42 (m, 12H), 3.21 (m, 1H), 2.06 (m, 2H), 1.63 (m, 2H); ^{13}C NMR (D_2O, 100 MHz): $\delta = 141.27$, 117.31, 105.42, 104.54, 80.85, 77.84, 77.24, 76.92, 75.33, 75.00, 73.44, 72.47, 71.03, 63.52, 62.56, 31.83, 30.48.

The hepta-ol **9** (1.14 g, 2.8 mmol) and dibutyltin oxide (0.76 g, 3.1 mmol) were heated at reflux in benzene (70 mL) with azeotropic water removal for 15 h. The mixture was doubled in concentration, cooled to room temperature, and benzyl bromide (0.69 mL, 5.8 mmol) and Bu_4NI (1.03 g, 2.8 mmol) were added. The mixture was heated at reflux 3.5 h, cooled, silica

gel was added to the flask, and the solvent was evaporated. The residue was applied to a column of silica gel, tin by-products were removed by flushing with hexanes, and elution (5% MeOH in CH_2Cl_2) gave the pure 3′-*O*-benzyl ether (0.76 g, 54%) as a white foam: $[\alpha]_D^{22} = +36.7^\circ$ ($c = 2.73$, $CHCl_3$); IR ($CHCl_3$, film): $\tilde{\nu} = 3371$, 2924, 2880, 1372, 1157, 1074 cm^{-1}; ^1H NMR ([D$_4$]MeOH, 400 MHz): $\delta = 7.46-7.40$ (m, 2H), 7.35–7.20 (m, 3H), 5.92–5.72 (m, 1H), 5.08–4.93 (m, 2H), 4.76 (d, 1H, $J = 11.8$ Hz), 4.65 (d, 1H, $J = 11.8$ Hz), 4.38 (d, 1H, $J = 7.8$ Hz), 4.28 (d, 1H, $J = 7.8$ Hz), 4.02 (d, 1H, $J = 2.9$ Hz), 3.95–3.63 (m, 6H), 3.61–3.48 (m, 4H), 3.43–3.20 (m, 3H), 2.20–2.10 (m, 2H), 1.78–1.65 (m, 2H); ^{13}C NMR ([D$_4$]MeOH, 100 MHz): $\delta = 139.77$, 139.47, 129.29, 129.08, 128.64, 115.19, 105.02, 104.23, 82.17, 80.74, 76.88, 76.40, 76.35, 74.71, 72.55, 71.81, 70.23, 67.02, 62.44, 61.91, 31.22, 30.07; FAB-HRMS calcd for $C_{24}H_{36}O_{11}Na^+$: 523.2155; found: 523.2137.

The 3′-*O*-benzyl ether (0.6 g, 1.20 mmol) was dissolved in acetonitrile and DMF (5:2, 7 mL), and benzaldehyde dimethylacetal (0.47 mL, 3.1 mmol) and camphorsulfonic acid (CSA) (14 mg, 60 μmol) were added. After stirring 16 h at room temperature, the mixture was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$. The organic extracts were dried ($MgSO_4$), evaporated, and following addition of ether (100 mL) to the resulting residue, pure **10**, was recovered by filtration (0.51 g, 72%): $[\alpha]_D^{22} = +111^\circ$ ($c = 2.21$, MeOH); IR ($CHCl_3$, film): $\tilde{\nu} = 3440$, 2872, 1368, 1163, 1109, 1048, 1005 cm^{-1}; ^1H NMR ([D$_4$]MeOH, 400 MHz): $\delta = 7.55-7.11$ (m, 10H), 5.82–5.69 (m, 1H), 5.45 (s, 1H), 4.98–4.83 (m, 2H), 4.64 (d, 2H, $J =$ 3.0 Hz), 4.40 (d, 1H, $J = 7.9$ Hz), 4.23 (d, 1H, $J = 3.4$ Hz), 4.18 (d, 1H, $J =$ 7.8 Hz), 4.15–3.98 (m, 2H), 3.87–3.66 (m, 4H), 3.55–3.10 (m, 7H), 2.20–2.10 (m, 2H), 1.65–1.53 (m, 2H); ^{13}C NMR ([D$_4$]MeOH, 100 MHz): $\delta =$ 139.76, 139.49, 139.47, 129.86, 129.30, 129.07, 129.03, 128.72, 127.35, 115.19, 104.69, 104.28, 102.03, 80.63, 80.17, 76.37, 76.28, 74.77, 74.73, 72.84, 70.86, 70.25, 68.17, 61.70, 31.22, 30.07. FAB-HRMS calcd for $C_{31}H_{40}O_{11}Na^+$: 611.2468; found: 611.2465.

Pent-4-enyl 2,2′,3,3′,6,6′-hexa-*O*-benzyl-β-D-lactoside (11): The tetraol **10** (0.51 g, 0.87 mmol) and Et_4NI (0.12 g, 0.43 mmol) were dried (azeotropic distillation with benzene), dissolved in DMF (5 mL) and cooled to 0°C. Benzyl bromide (0.83 mL, 7.0 mmol) was added followed by NaH (0.22 g, 60%, 5.6 mmol) and the mixture was allowed to warm to room temperature over 14 h. The mixture was diluted with ethyl acetate, washed with water, the organic layer was dried ($MgSO_4$) and evaporated. Purification of the residue by chromatography on silica gel (4:1 →2:1 hexanes/EtOAc) gave pure pentabenzyl lactoside as a white foam (0.80 g, 97%): $[\alpha]_D^{22} =$ $+129^\circ$ ($c = 1.63$, $CHCl_3$); IR ($CHCl_3$, film): $\tilde{\nu} = 3030$, 2866, 1453, 1365, 1096, 1063, 1028, 911 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 7.50-7.05$ (m, 30H), 5.80–5.65 (m, 1H), 5.38 (s, 1H), 5.10 (d, 1H, $J = 10.6$ Hz), 4.99–4.60 (m, 9H), 4.47 (d, 1H, $J = 12.1$ Hz), 4.38 (d, 1H, $J = 7.8$ Hz), 4.30 (d, 1H, $J =$ 7.8 Hz), 4.25 (d, 1H, $J = 12.1$ Hz), 4.12 (d, 1H, $J = 13$ Hz), 3.94 (d, 1H, $J =$ 3.4 Hz), 3.92–3.60 (m, 6H), 3.54 (dd, 1H, $J = 8.8$ Hz, 9.2 Hz), 3.46 (dd, 1H, $J = 2.6$ Hz, 7.0 Hz), 3.40–3.23 (m, 3H), 2.85 (s, 1H), 2.22–2.00 (m, 2H), 1.75–1.60 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 138.92$, 138.63, 138.51, 138.04, 128.80, 128.52, 128.31, 128.24, 128.17, 128.13, 128.06, 128.03, 128.00, 127.69, 127.65, 127.54, 127.49, 127.38, 127.30, 126.52, 114.84, 103.59, 102.83, 101.30, 83.01, 81.81, 79.60, 78.76, 77.65, 75.73, 75.22, 75.05, 74.97, 73.61, 72.91, 71.56, 69.27, 68.90, 68.27, 66.28, 30.18, 28.89; FAB-HRMS calcd for $C_{59}H_{64}O_{11}Na^+$: 971.4346; found: 971.4375.

The benzylidene (0.63 g, 0.66 mmol) was dissolved in THF (6.6 mL) and stirred with freshly activated 4 ÅMS (0.25 g) 10 min at room temperature. In one portion $NaCNBH_3$ (0.21 g, 3.3 mmol) was added followed by anhydrous HCl (2.0 M Et_2O), dropwise until the mixture no longer bubbled (approx. 2 mL). After stirring and additional 10 min, the mixture was passed through a plug of Celite washing with ethyl acetate, the filtrate was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and the organic layers evaporated. Purification by column chromatography (9:1 hexanes/EtOAc) gave pure **11** as white solid (0.49 g, 79%): $[\alpha]_D^{22} = +200^\circ$ ($c = 1.05$, $CHCl_3$); IR ($CHCl_3$, film): $\tilde{\nu} = 3474$, 3062, 3029, 2869, 1453, 1364, 1094, 1028 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 7.40-7.06$ (m, 30H), 5.80–5.66 (m, 1H), 5.02–4.85 (m, 3H), 4.81 (d, 1H, $J = 11.0$ Hz), 4.75–4.54 (m, 6H), 4.67 (d, 1H, $J = 12.2$ Hz), 4.42–4.26 (m, 5H), 3.94 (s, 1H), 3.92–3.81 (m, 2H), 3.71 (dd, 1H, $J = 10.7$ Hz, 4.1 Hz), 3.64 (d, 1H, $J = 10.6$ Hz), 3.57 (dd, 1H, $J = 9.4$ Hz, 5.5 Hz), 3.55–3.42 (m, 3H), 3.38 (dd, 1H, $J = 5.2$ Hz, 9.6 Hz), 3.36–3.21 (m, 4H), 2.32 (s, 1H), 2.15–2.02 (m, 2H), 1.74–1.60 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 139.04$, 138.54, 138.52, 138.23, 138.09, 137.96, 137.81, 128.33, 128.15, 127.93, 127.66, 127.50, 114.80, 103.50, 102.43, 82.79, 81.68, 80.99, 79.27, 76.52, 75.22, 75.10, 74.99, 74.83, 73.37, 72.99, 72.67, 71.86, 69.10, 68.32, 68.16, 66.00, 30.11, 28.83; FAB-HRMS calcd for $C_{59}H_{66}O_{11}Na^+$: 973.4503; found: 973.4513.

α-Fluoro donor 13: A solution of 3-*O*-PMB-4,6-di-*O*-benzyl-galactal (2.24 g, 5.02 mmol) in dry CH_2Cl_2 (5 mL) under N_2 at 0°C was treated with dimethyldioxirane DMDO (0.11M, 47 mL), and the mixture was stirred until all of the glycal was consumed (~1 h, TLC 30% EtOAc in hexane) **Note**: Elevated temperature and/or excess of DMDO will prompt oxidation of the PMB group and lower reaction yield. The solvents were evaporated under vacuum at 0°C and the residue was kept under high vacuum. The flask containing galactal epoxide was charged with freshly prepared 4 Å molecular sieves (2 g), dry THF (50 mL) and cooled to 0°C. HF/pyr complex (0.79 mL, ~5 equiv) was added dropwise via syringe. The reaction mixture was left overnight to slowly reach room temperature and quenched with Et_3N (1.27 g, ~2.5 equiv) to reach pH ~7. The mixture was filtered through a pad of anhydrous $MgSO_4$ and rinsed three times with EtOAc (50 mL). The filtrate was washed with water (50 mL) and saturated $NaHCO_3$ (50 mL), dried over $MgS0_4$ and concentrated to dryness. Flash column chromatography (2:1 EtOAc/hexanes) gave fluorohydrin (2.06 g, 85% yield) as a mixture of anomers $\alpha:\beta = 10:1$. ^{19}F NMR ($CDCl_3$, 376 MHz, C_6F_6 as external standard): $\delta = 9.7$ (dd, α, $J = 54.4$, 25.0 Hz) 20.0 (dd, β, $J = 53.9$, 13.1 Hz); ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 7.38-7.24$ (m, 12H), 6.90 (d, 2H, $J = 8.7$ Hz), 5.70 (dd, 1H, $J = 54.4$, 2.8 Hz), 4.89 and 4.57 (2d, 2H, $J = 11.3$ Hz), 4.70 and 4.54 (AB q, 2H, $J = 11.2$ Hz), 4.54 and 4.46 (AB q, 2H, $J = 11.8$ Hz), 4.17 (AMX octet, 1H, $J = 2.8$, 10.1, 25.0 Hz), 4.13 (br t, 1H, $J = 6.8$ Hz), 4.06 (d, 1H, $J = 1.5$ Hz), 3.81 (s, 3H), 3.74 (dd, 1H, $J = 2.6$, 10.1 Hz), 3.60 (m, 2H).

The above mixture (8.29 g, 17.2 mmol) was dissolved in dry DMF (100 mL) containing freshly prepared 4 Å molecular sieves (3 g) under N_2 at 0°C, treated with benzyl bromide (4.41 g, 25.8 mmol, 1.5 equiv) and finally with NaH (1.24 g, 60% dispersion in oil, 30.86 mmol, 1.8 equiv), and stirred overnight at room temperature. The reaction was quenched with glacial acetic acid (0.93 g, 0.9 equiv) and the mixture filtered through a pad of anhydrous $MgSO_4$ with EtOAc (4 × 50 mL). The organic solution was washed with water (4 × 50 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash column chromatography of the residue (4:1 hexane/EtOAc) gave the title compound (9.36 g, 95%) as colorless liquid with the same ratio of anomers $\alpha:\beta = 10:1$ as the starting fluorohydrin. ^{19}F NMR ($CDCl_3$, 376 MHz, C_6F_6 as external standard): $\delta = 11.5$ (dd, α, $J = 53.7$, 25.2 Hz), 22.8 (dd, β, $J = 53.4$, 13.0 Hz). For analytical purpose pure α anomer (50 mg) was obtained using preparative HPLC. $[\alpha]_D^{22} = -54.5^\circ$ ($c = 0.55$, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz), δ 7.38–7.24 (m, 17H), 6.88 (d, 2H, $J = 8.6$ Hz), 5.58 (dd, 1H, $J = 53.7$, 2.7 Hz), 4.93 (d, 2H, $J = 11.34$ Hz), 4.56 (d, 2H, $J = 11.34$ Hz), 4.85 (AB q, 2H, $J = 11.78$ Hz), 4.72 (AB q, 2H, $J =$ 11.78 Hz), 4.73 (AB q, 2H, $J = 11.3$ Hz), 4.68 (AB q, 2H, $J = 11.3$ Hz), 4.47 (AB q, 2H, $J = 11.84$ Hz), 4.41 (AB q, 2H, $J = 11.84$ Hz), 4.09 (br t, 1H, $J =$ 6.5 Hz), 4.02 (AMX m, 1H, $J = 2.7$, 10.05, 25.2 Hz), 3.98 (br s, 1H), 3.92 (dd, 1H, $J = 2.64$, 10.05 Hz), 3.81 (s, 3H), 3.54 and 3.52 (ABX m, 2H, $J = 9.3$, 6.05, 7.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 159.20$, 138.35, 138.08, 137.71, 130.43, 129.18, 128.39, 128.25, 128.14, 127.92, 127.8, 127.78, 127.66, 113.81, 106.25 (d, $J = 229.0$ Hz), 78.09, 75.65 (d, $J = 23.5$ Hz), 74.79 ($ArCH_2$), 74.29, 73.70 ($ArCH_2$), 73.45 ($ArCH_2$), 72.71 ($ArCH_2$), 71.70 (d, $J = 2.7$ Hz) 68.26, 55.24 (CH_3O); LRMS (FAB) 586 $[M+NH_4]^+$.

PMB trisaccharide 14: A mixture of lactoside **11** (402 mg, 0.423 mmol) and fluoro donor **13** (485 mg, 0.846 mmol, 2 equiv) was azeotroped with anhydrous benzene (3 × 10 mL) and further dried on high vacuum for 3 h. The above mixture was dissolved in toluene (3.8 mL) and transferred via cannula to a flask containing freshly prepared 4 Å molecular sieves (0.68 g) under N_2, treated with 2,6-di-*tert*-butylpyridine (143 μL) and cooled to −20°C. $(Cp)_2Zr(OTf)_2$ (225 mg, 0.381 mmol, 0.9 equiv) was suspended in THF (0.38 mL) and added via a cannula to the reaction mixture. The reaction was stirred for 72 h at 7°C in darkness. The reaction mixture was diluted with EtOAc (10 mL) and filtered through a pad of anhydrous $MgSO_4$ with EtOAc (3 × 10mL). The filtrate was washed with saturated $NaHCO_3$ (2 × 10 mL), dried over $MgSO_4$, and concentrated to dryness. Flash column chromatography (2% Et_2O/CH_2Cl_2) gave the desired α-product **14** (509 mg, 80%) and β-product (51 mg, 8%). $[\alpha]_D^{22} =$ $+24.6^\circ$ ($c = 3.90$, $CHCl_3$); IR ($CHCl_3$, film): $\tilde{\nu} = 3062$, 3029, 2919, 2868, 1612, 1513, 1496, 1364, 1303, 1248, 1028 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 7.40-6.95$ (m, 49H), 6.69 (d, 1H, $J = 8.5$ Hz), 5.73 (m, 1H), 5.00–4.93 (m, 2H), 4.92–4.84 (m, 2H), 4.82–4.73 (m, 2H), 4.72–4.63 (m, 5H), 4.61

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(d, 1H, $J = 13.0$ Hz), 4.48–4.35 (m, 5H), 4.34–4.24 (m, 4H), 4.16 (d, 2H, $J = 6.8$ Hz), 4.07 (dd, 1H, $J = 8.8$ Hz), 4.02–3.80 (m, 8H), 3.78–3.60 (m, 3H), 3.68 (s, 3H), 3.60–3.35 (m, 6H), 3.35–3.18 (m, 4H), 3.12–3.04 (m, 1H), 2.06 (m, 2H), 1.65 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 158.76$, 139.66, 139.45, 139.26, 139.16, 139.09, 138.92, 138.57, 138.52, 131.39, 129.30, 128.95, 128.70, 128.60, 129.30, 128.08, 127.95, 115.35, 114.02, 104.05, 103.35, 101.25, 83.14, 82.17, 79.91, 79.71, 77.77, 77.04, 75.69, 75.58, 75.46, 75.33, 74.17, 73.75, 73.54, 73.48, 72.65, 72.54, 69.91, 69.71, 68.80, 68.33, 68.19, 55.11, 30.14, 28.86; FAB-HRMS calcd for $C_{94}H_{102}O_{17}Na^+$: 1525.7014; found: 1525.6996.

Trisaccharide acceptor 15: A solution of PMB trisaccharide **14** (445 mg, 0.296 mmol) in CH_2Cl_2 (10 mL) at 0 °C was treated with phosphate buffer (1.5 mL, pH 7.4) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (89 mg, 1.3 equiv) and stirred at 0 °C for 5 h. The reaction mixture was diluted with EtOAc (50 mL), washed with saturated $NaHCO_3$ (2×20 mL) and water (20 mL), dried over $MgSO_4$, and concentrated to dryness. The crude material was purified by flash column chromatography (4% ether in CH_2Cl_2) to give deprotected trisaccharide **15** (344 mg, 84%) as a colorless oil. $[\alpha]_D^{22} = +28.2^\circ$ ($c = 5.70$, $CHCl_3$); IR ($CHCl_3$, film): $\tilde{\nu} = 3570$, 3062, 3029, 2913, 2868, 1496, 1453, 1364, 1208, 1095 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.77–7.06$ (m, 45H), 5.73 (m, 1H), 5.01 (dd, 1H, $J = 5.5$, 3.3 Hz), 4.95 (dd, 1H, $J = 5.8$, 2.6 Hz), 4.90 (m, 1H), 4.78 (d, 1H, $J = 10.9$ Hz), 4.75 (d, 1H, $J = 11.4$ Hz), 4.70–4.59 (6d, 6H), 4.47–4.37 (m, 5H), 4.28 (m, 3H), 4.19 (s, 2H), 4.08–3.91 (m, 6H), 3.85 (m, 2H), 3.69 (m, 5H), 3.66 (1H, d, $J = 11.0$ Hz), 3.50–3.19 (m, 9H), 3.10 (dd, 1H), 2.07 (m, 2H), 1.79 (d, 1H, OH), 1.65 (d, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta =$ 139.36, 138.72, 138.63, 138.52, 138.41, 138.29, 138.19, 138.07, 137.98, 128.35, 128.20, 128.06, 127.97, 127.66, 127.54, 127.08, 114.82, 103.55, 102.67, 99.58, 82.93, 81.67, 81.55, 79.32, 77.61, 76.90, 75.13, 75.02, 74.96, 74.80, 73.08, 72.99, 72.91, 72.01, 69.95, 69.22, 69.15, 68.34, 67.73, 67.57, 60.35, 30.19, 28.92; FAB-HRMS calcd for $C_{86}H_{94}O_{16}Na^+$: 1405.6439; found: 1405.6385.

Hexasaccharide 19: The thioethyl donor **18** (543 mg, 0.425 mmol) and acceptor **15** (587 mg, 0.425 mmol) were combined, azeotroped with anhydrous benzene (5×5 mL) and placed under high vacuum for 5 h. The mixture was then dissolved in CH_2Cl_2 (3.5 mL) and Et_2O (7.0 mL), treated with freshly prepared 4 Å molecular sieves and cooled to 0 °C. Methyl triflate (3.0 equiv, 144 µL) was added in one portion and the reaction stirred at 0 °C for 3 h. Another 144 µL of MeOTf was added and the reaction was allowed to stir for an additional 2 h at 5 °C. The reaction was quenched by the addition of solid $NaHCO_3$, filtered through Celite with EtOAc, concentrated and purified by HPLC (17% EtOAc/hexanes) to give hexasaccharide (663 mg, 60%) as a white foam. $[\alpha]_D^{22} = -9.7^\circ$ ($c = 1.00$, $CHCl_3$); IR ($CHCl_3$, film): $\tilde{\nu} = 3533$, 3343, 3087, 3030, 2940, 2865, 1790, 1496, 1453, 133, 1095 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.76$ (d, 2H, $J = 7.5$ Hz), 7.45–7.00 (m, 63H), 5.84 (m, 1H), 5.20 (s, 1H), 5.11 (d, 1H, $J = 3.2$ Hz), 5.09 (d, 1H, $J = 3.6$ Hz), 5.05 (d, 1H, $J = 3.3$ Hz), 5.03 (m, 1H), 4.92 (m, 2H), 4.86 (d, 1H, $J = 6.0$ Hz), 4.82 (m, 2H), 4.78 (1H, d, $J = 2.2$ Hz), 4.74–4.61 (m, 8H), 4.53–4.44 (4d, 4H), 4.38–4.30 (m, 4H), 4.18–3.82 (m, 20H), 3.76–3.66 (m, 5H), 3.66–3.60 (m, 2H), 3.58–3.52 (m, 2H), 3.48–3.40 (m, 2H), 3.38–3.32 (m, 2H), 3.29–3.25 (m, 3H), 3.06 (dd, 1H, $J = 10.2$ Hz), 2.86 (s, 1H), 2.74 (m, 1H), 2.16 (m, 2H), 1.74 (m, 2H), 1.23 (s, 3H, $J = 6.5$ Hz), 1.16–1.07 (m, 42H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 155.49$, 140.71, 139.37, 138.96, 138.72, 137.70, 138.66, 138.55, 138.42, 138.37, 138.10, 138.07, 138.04, 137.88, 132.07, 128.89, 128.64, 128.50, 128.27, 128.16, 128.04, 127.86, 127.68, 127.53, 127.34, 127.20, 114.79, 103.49, 103.14, 102.61, 99.63, 99.12, 97.79, 82.26, 81.61, 81.34, 80.45, 79.36, 78.95, 78.26, 77.82, 77.64, 77.45, 77.24, 77.16, 76.83, 76.45, 75.39, 75.28, 75.12, 74.98, 74.89, 74.78, 73.94, 73.13, 72.94, 72.92, 72.52, 71.91, 71.81, 71.25, 71.11, 69.35, 69.23, 69.18, 68.18, 68.11, 68.01, 67.77, 67.54, 61.98, 61.72, 56.03, 30.16, 28.88, 18.01, 18.00, 17.95, 17.92, 11.85, 11.82; LRMS (FAB) calcd for $C_{150}H_{185}NO_{32}SSi_2Na^+$: 2624; found: 2624.

Peracetate of globo-H pentenyl glycoside 20: TBAF (1.0 M THF, 10 equiv, 2.24 mL) was added to a solution of the hexasaccharide (585 mg, 0.224 mmol) in THF (10 mL). The reaction stirred at rt for 3 d, poured into ice water and extracted with EtOAc (3×50 mL). The organic extracts were washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$ and concentrated to an oil which was purified through a short plug of silica gel with EtOAc. The resulting triol was dissolved in anhydrous MeOH (8 mL) and sodium methoxide was added (0.25 mL of a 25% solution in MeOH). The reaction stirred at rt for 18 h, neutralized with Dowex-H$^+$, filtered with MeOH washings and concentrated. THF (2.0 mL) and condensed liquid NH_3 (~25 mL) were added at −78 °C to the resulting white solid. Sodium (~500 mg) was added and the resulting blue solution stirred at −78 °C for 2 h. The reaction was quenched with anhydrous MeOH (~10 mL), brought to rt and concentrated under a stream of dry N_2 to a volume of ~5 mL. The reaction was neutralized with Dowex-H$^+$, filtered with MeOH washing and concentrated to a white solid. The white solid was dissolved in pyridine (5.0 mL) and CH_2Cl_2 (5.0 mL) and cooled to 0 °C. A crystal of DMAP was added followed by acetic anhydride (5.0 mL). The ice bath was removed and the reaction stirred at rt overnight. Concentration followed by purification by flash column chromatography (gradient elution 75% EtOAc/hexanes →100% EtOAc →5% MeOH/EtOAc) gave **20** as a white solid (168 mg, 42%): $[\alpha]_D^{22} = 4.37^\circ$ ($c = 1.85$, $CHCl_3$); IR ($CHCl_3$, film): $\tilde{\nu} = 2939$, 1747, 1370, 1229, 1066 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.66$ (d, 1H, $J = 6.5$ Hz), 5.77 (m, 1H), 5.58 (d, 1H, $J = 3.2$ Hz), 5.47 (d, 1H, $J = 3.5$ Hz), 5.39 (d, 1H, $J = 3.2$ Hz), 5.29 (dd, 1H, $J = 10.9$, 3.0 Hz), 5.24–5.06 (m, 5H), 5.04–5.02 (m, 1H), 4.99–4.85 (m, 4H), 4.74 (dd, 1H, $J = 10.9$, 2.9 Hz), 4.53–4.40 (m, 5H), 4.36 (m, 1H), 4.26 (dd, 1H, $J = 10.6$, 3.4 Hz), 4.18–4.03 (m, 6H), 3.99–3.96 (m, 2H), 3.87–3.81 (m, 3H), 3.77–3.74 (m, 1H), 3.51–3.45 (m, 1H), 3.03 (m, 1H), 2.16 (s, 3H), 2.15 (s, 3×3H), 2.13–2.11 (m, 2H), 2.10 (s, 3H), 2.09 (s, 3H), 2.08 (s, 2×3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.04 (s, 2×3H), 2.00 (s, 3H), 1.97 (s, 2×3H), 1.89 (s, 3H), 1.65 (m, 2H), 1.62 (s, 3H), 1.14 (d, 3H, $J = 6.5$ Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 172.31$, 171.55, 170.78, 170.61, 170.57, 170.48, 170.41, 170.30, 170.08, 169.75, 169.61, 169.57, 169.44, 168.96, 137.76, 115.07, 102.05, 101.29, 100.45, 99.23, 98.74, 94.29, 77.24, 77.16, 76.07, 73.68, 73.40, 73.17, 72.63, 72.34, 71.85, 71.77, 71.56, 71.34, 70.83, 70.71, 70.19, 70.08, 69.32, 69.03, 68.88, 68.09, 68.01, 67.59, 67.32, 64.48, 29.80, 28.54, 23.12, 20.90, 20.88, 20.82, 20.74, 20.73, 20.72, 20.71, 20.64, 20.62, 20.55, 20.54, 20.49, 15.91; FAB-HRMS calcd for $C_{77}H_{107}NO_{47}Na^+$: 1820.5911; found: 1820.5994.

Globo-H pentenyl glycoside 1c: The peracetate (20 mg, 0.011 mmol) was dissolved in anhydrous MeOH (2.0 mL) and sodium methoxide was added (100 µL, 25% solution in MeOH). The reaction stirred at rt for 18 h, was neutralized with Dowex-H$^+$ (~pH 6–7), filtered with MeOH washings, concentrated and purificated using RP silica gel (H_2O →1% MeOH/H_2O) then P-2 Gel (H_2O eluent) to yield a white solid (12 mg, 99%). $[\alpha]_D^{22} = 3.00^\circ$ ($c = 1.00$, MeOH); IR ($CHCl_3$, film): $\tilde{\nu} = 3374$, 2930, 1641, 1372, 1070 cm^{-1}; ^{1}H NMR (MeOH, 400 MHz): $\delta = 5.79$ (m, 1H), 5.18 (d, 1H, $J = 3.9$ Hz), 4.98 (m, 1H, $J = 7.2$ Hz), 4.91 (m, 1H), 4.87 (m, 1H), 4.51 (s, 1H), 4.49 (d, 1H, $J = 1.4$ Hz), 4.41–4.36 (m, 2H), 4.24–4.20 (m, 4H), 4.10 (d, 1H, $J = 2.5$ Hz), 4.06–4.00 (m, 3H), 3.94 (s, 1H), 3.87–3.45 (m, 22H), 3.35–3.31 (m, 2H), 3.19 (t, 1H, $J = 8.8$ Hz), 2.10 (m, 2H), 1.96 (s, 3H), 1.66 (m, 2H), 1.19 (d, 3H, $J = 6.5$ Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 174.53$, 139.53, 115.27, 105.50, 105.44, 104.30, 103.96, 102.81, 101.07, 81.29, 80.59, 80.04, 79.16, 78.00, 76.81, 76.57, 76.49, 76.45, 76.39, 75.57, 74.89, 74.69, 73.58, 72.64, 72.49, 71.56, 70.65, 70.63, 70.38, 70.31, 69.70, 68.13, 62.63, 62.59, 61.94, 61.62, 53.11, 49.90, 31.29, 30.14, 23.55, 16.76. FAB-HRMS calcd for $C_{43}H_{73}NO_{30}Na^+$: 1106.4115; found: 1106.4105.

Cassette coupling to yield trisaccharide 23: The pentenyl donor **8** (0.012 g, 0.017 mmol) and the acceptor **22** (0.024 g, 0.033 mmol) were azeotroped with anhydrous benzene and dried under high vacuum for 6 h. The mixture was dissolved in CH_2Cl_2 (0.5 mL) and a minimal amount of freshly prepared 4 Å molecular sieves (25 mg) were added with stirring. Solid *N*-iodosuccinimide (0.010 g, 0.044 mmol) was added in one portion followed by TfOH (100 µL of a solution prepared by the addition of 7.5 µL of TfOH to 1.0 mL of CH_2Cl_2, 1.0 equiv). The reaction mixture was stirred at rt overnight and quenched with solid $NaHCO_3$. Filtration through a plug of celite with additional CH_2Cl_2, followed by evaporation gave a red syrup which was purified by flash column chromatography (30% →40% EtOAc/hexanes) to give the trisaccharide product **23** (12 mg, 54%): $R_f =$ 0.29 (10% acetone/$CHCl_3$); $[\alpha]_D^{22} = +42.5^\circ$ ($c = 0.80$, $CHCl_3$); IR ($CHCl_3$, film): $\tilde{\nu} = 3029$, 2940, 2869, 1751, 1362, 1220, 1051 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.65$ (d, 2H, $J = 7.5$ Hz), 7.48 (d, 2H, $J = 7.4$ Hz), 7.29 (t, 2H), 7.23–7.21 (m, 5H), 7.19 (t, 2H), 5.71 (d, 1H, $J = 8.4$ Hz), 5.22 (d, 1H, $J = 2.6$), 5.14–5.09 (m, 2H), 5.06 (d, 1H, $J = 3.2$ Hz), 5.00 (dd, 1H, $J = 10.4$, 8.0 Hz), 4.86–4.82 (m, 2H), 4.65 (d, 1H, $J = 3.5$ Hz), 4.55 (d, 1H, $J = 10.5$ Hz), 4.39 (s, 1H, $J = 7.9$ Hz), 4.33–4.30 (m, 1H), 4.22–4.18 (m, 1H), 4.12–4.11 (m, 1H), 4.03–3.88 (m, 5H), 3.79–3.74 (m, 2H), 3.72–3.58 (m, 3H), 3.46 (m, 1H), 3.42 (dd, 1H, $J = 10.4$, 3.4 Hz), 2.77 (d, 1H, $J = 1.6$ Hz), 2.03 (s, 3H), 1.97 (s, 3H), 1.94 (s, 6H), 1.93 (s, 3H), 1.92 (s, 3H), 1.85 (s, 3H), 0.73 (s, 9H), 0.07 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 170.35$, 170.12, 170.06, 169.73, 169.64, 169.01, 155.88, 143.70, 141.28, 135.00, 128.66, 128.45,

128.32, 127.79, 127.09, 125.08, 120.04, 101.11, 100.74, 99.43, 78.20, 77.17, 75.76, 72.93, 72.71, 71.21, 70.90, 70.69, 70.52, 69.12, 68.93, 67.69, 67.59, 67.29, 66.52, 62.22, 61.18, 60.71, 58.23, 54.48, 47.08, 29.68, 25.80, 20.77, 20.62, 20.61, 20.51, 18.22, −5.50; HRMS calcd for $C_{43}H_{73}NO_{30}Na^+$: 1359.4727; found: 1359.4727.

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