Studies Related to Synthesis of Glycophosphatidylinositol Membrane-Bound Protein Anchors. 5.[1] *n*-Pentenyl Ortho Esters for Mannan Components[2]

Carmichael Roberts,[3a] Robert Madsen,[3b] and Bert Fraser-Reid*

Contribution from Paul M. Gross Chemical Laboratory, Department of Chemistry, Duke University, Durham, North Carolina 27708

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Abstract: Procedures for rapid assembly of multigram amounts of mannan components have been examined. Although these studies are reported in the context of the mannan moiety of the glycan anchors of membrane-bound glycoproteins, the procedures should be applicable to the wider family of mannose-containing glycoproteins. Readily prepared *n*-pentenyl ortho esters of mannose are shown to be versatile substrates that can serve as glycosyl donors in their own right or be used to furnish mannosyl bromides or *n*-pentenyl α-D-mannosides. Thus three glycosyl donors of different reactivities and stabilities are obtainable from the same precursor, all three being activated under mild conditions. Two approaches are described. In the first, a portion of the starting *n*-pentenyl ortho ester is converted into an *n*-pentenyl glycoside (NPG) by acid-catalyzed rearrangement, while another portion is titrated with bromine to give a glycosyl bromide. These are coupled under Koenigs−Knorr conditions to give an *n*-pentenyl disaccharide which is then processed to become a glycosyl acceptor. A third portion of the ortho ester, after suitable protecting group adjustments, is also titrated with bromine and coupled to the disaccharide acceptor to give the desired trimannan. The instability of glycosyl bromides detracts from this route, and so a second approach which avoids their use completely was pursued in which NPG obtained from the acid-catalyzed rearrangement was converted into a vicinal dibromide. The latter is then able to serve as a glycosyl acceptor for coupling to a donor obtainable by reaction of the *n*-pentenyl ortho ester with halonium ion. The dibromopentanyl disaccharide produced then becomes an acceptor for a donor derived from *n*-pentenyl mannoside. The second approach uses no unstable reactants, is therefore experimentally less demanding, and can be operated conveniently on a large scale.

The roles that glycoproteins play in cellular interactions[4] have long been recognized, but recent years have seen an explosion of interest owing, in part, to technological advances that have made their isolation more tractable and their structure proof less formidable.[5] Although there has long been suspicion of a class of glycoproteins that were substantially different from the better known transmembrane-anchored variety,[6] it was only recently that this was firmly established. Thus, in 1985, Ferguson, Low, and Cross provided evidence for a protein covalently linked to a cell surface *via* a glycophosphatidylinositol (GPI) anchor as a result of their study of the variant surface glycoprotein (VSG) of the protozoan parasite *Trypanosoma brucei*.[7] The same year, Bouvier et al.[8] and Haldar et al.[9] reported that the surface glycoproteins of certain *Leismanica* and *Plasmodium* species, respectively, had several structural features in common with the above-mentioned VSG. The structure for the latter was finally determined to be **1a** by an elegant combination of chemical degradation and spectroscopic analyses.[10] That these pathogenic parasites should show such similarities was interesting, and this intensified in 1988 when structure **1b** was assigned to the GPI anchor of rat brain Thy-1 glycoprotein.[11,12] Since 1985, partial structural data have accumulated for over 100 GPI membrane-anchored proteins from a variety of organisms along the evolutionary pathway,[13,14] and these have led to the proposal of the generalized structure[15] indicated in Scheme 1. The highlighted pentasaccharide core appears to be conserved in nearly every structure.[16] A noteworthy feature is the presence of an extra mannose (Gly_1 = Manα1) in structures **1b,d−f**, which are derived from higher eukaryotes.

That such a high degree of structural homology should be found for compounds **1a−g** obtained from species ranging from

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(3) (a) C.R. is the recipient of a NIH Award for Underrepresented Minorities. (b) R.M. thanks the Danish Technical Research Council for a P.D. Fellowship.

(4) Sharon, N. *Complex Carbohydrates: Their Chemistry, Biosynthesis and Functions*; Addison-Wesley: Reading, MA, 1975. Lennarz, W. J., Ed. *The Biochemistry of Glycoproteins and Proteoglycans*; Plenum Press: New York, 1980. Sharon, N.; Lis, H. *Chem. Eng. News* **1981**, *59* (March 30), 21.

(5) Drickamer, K.; Carver, J. Carbohydrates and Glycoconjugates Upwardly Mobile Sugars Gain Status as Information-Bearing Molecules. In *Current Opinion in Structural Biology*, October 1992, Vol. 2, No. 5, pp 653−654. Sharon, N.; Lis, H. *Sci. Am.* **1993**, *66* (January), 82.

(6) Juliano, R. L., Rothstein, A., Eds. *Cell Surface Glycoproteins Structure Biosynthesis, and Biological Functions*; Current Topics in Membranes and Transport, Vol. 11; Bronner, F., Kleinzeller, A., Eds., Academic Press: New York, 1978.

(7) Ferguson, M. A. J.; Low, M. G.; Cross, G. A. M. *J. Biol. Chem.* **1985**, *260*, 14547.

(8) Bouvier, J.; Etges, R. J.; Bordier, C. *J. Biol. Chem.* **1985**, *260*, 15504.

(9) Haldar, K.; Ferguson, M. A. J.; Cross, G. A. M. *J. Biol. Chem.* **1985**, *260*, 4969.

(10) Ferguson, M. A. J.; Homans, S. W.; Dwek, R. A.; Rademacher, T. W. *Science* **1988**, *239*, 753.

(11) Homans, S. W.; Ferguson, M. A. J.; Dwek, R. A.; Rademacher, T. W.; Anand, R.; Williams, A. F. *Nature* **1988**, *333*, 269.

(12) For a picturesque model of the Thy-1 glycoprotein, see the following: *Chem. Eng. News*, **1993**, *71* (jan 11), 26.

(13) Ferguson, M. A. J.; Williams, A. F. *Annu. Rev. Biochem.* **1988**, *57*, 2985. Low, M. G. *Biochim. Biophys. Acta* **1989**, *988*, 427. Cross, G. A. M. *Annu. Rev. Cell. Biol.* **1990**, *6*, 1. Thomas, J. R.; Dwek, R. A.; Rademacher, T. W. *Biochemistry* **1990**, *29*, 5413.

(14) For a recent review, see: Englund, P. T. *Annu. Rev. Biochem.* **1993**, *62*, 121.

(15) Robinson, P. J. *Immunology Today* **1991**, *12*, 35.

(16) Rosse, W. F. *Semin. Hematol.* **1993**, *30*, 219.

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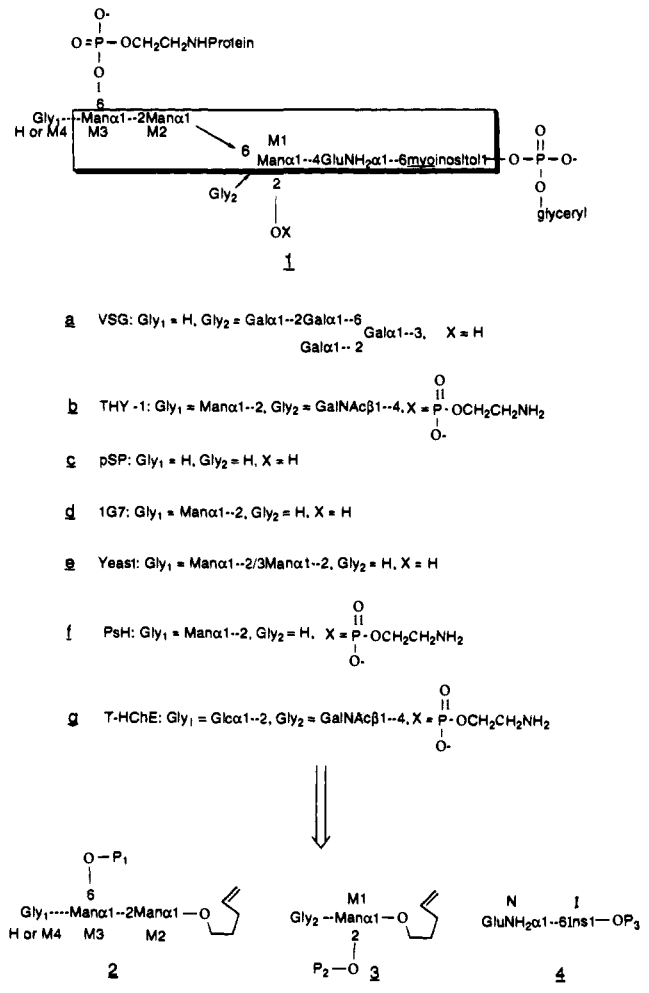
Scheme 1



single-cell eukaryotes[17] to humans is intriguing. At present, no concrete correlation between the occurrence of GPI anchors and protein function has been promulgated;[18] however, that they are implicated in transmembrane signal transduction seems to be secure.[19] Evidence has also been produced to suggest that GPI-type anchors allow for greater lateral mobility of the glycoprotein than do their transmembrane counterparts.[20]

Knowledge about their biosynthetic pathway is only slowly emerging,[14,21] but already, targets of opportunity for the development of chemotherapeutic agents designed for specific intervention have been identified.[22]

A recent discovery which further complicates the picture is the existence, on cell surfaces of certain protozoans, of GPIs that do not anchor proteins to the lipid bilayer.[23] Many of these protein-free GPIs are comprised of the same basic pentasaccharide core as found in **1**. A possible role is that this coating protects the plasma membrane of the parasite from insect or mammalian hydrolases and, hence, enhances their chances of survival.[24]

The investigation of membrane anchors gains further urgency because of evidence for a possible biosynthetic connection between GPI membrane-anchored proteins and mediators of insulin action.[25-27] Ready synthetic routes to these structures, and/or segments thereof, would help to clarify their biological roles and also assist in elucidation of the biosynthetic pathway(s)—hence our interest in this area of investigation.

The disclosure in 1988 of structural assignments for the GPI anchors[10,11] coincided with the discovery of *n*-pentenyl glycosides (NPGs) in our laboratory.[28,29] The complexities of **1a,b** provided a challenging context in which the synthetic capabilities of NPGs could be developed and dovetailed well with our earlier interest in inositol phosphates.[30]

In view of the structural homology, a retrosynthetic plan may be envisaged, as outlined in Scheme 1, in which synthetic strategies and/or subunits are common to both targets. Indeed such a basic plan is evident in other approaches to the VSG anchor including Ogawa's heptasaccharide,[31] van Boom's disaccharides,[32] Ley's pentasaccharide,[33] and the GlcNH$_2$α1→6 myoinositol-P-diglyceride published recently by Cottaz, Brimacombe, and Ferguson.[34]

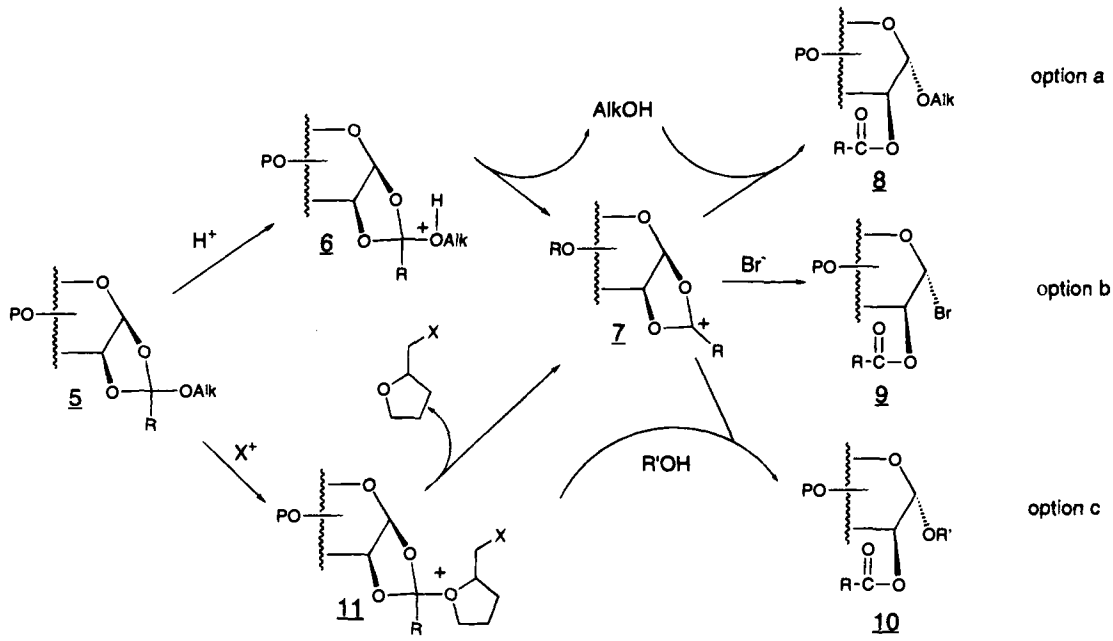
Strategy directed at the mannan components was of special interest since most developments would also be applicable to the widely distributed families of high-mannose glycoproteins.[35]

Retrosynthetic Analysis

The retrosynthetic plan in Scheme 1 indicates that the mannan moiety **2** is to be attached as the final glycosidation step. In the cases of targets such as **1b,d−f** where there is a trimannan residue, provision has to be made for future installation of the phosphodiester moiety. With respect to the phosphorylated sites of **1b,f,g**, the ideal strategy would be to utilize protecting groups that could be selectively removed in any order, once the entire anchor has been assembled. By corollary, these protecting groups would also have to be compatible with the chemical manipulations for assembly.

(17) Roberts, W. L.; Santikarn, S.; Reinhold, V. N.; Rosenberry, T. L. *J. Biol. Chem.* **1988**, *263*, 18776.
(18) Ferguson, M. A. J. *Curr. Opin. Struct. Biol.* **1991**, *1*, 522.
(19) For example, see: Presky, D. H.; Low, M. G.; Shevach, E. M. *J. Immunol.* **1990**, *144*, 860. Schmitz, B.; Klein, R. A.; Duncan, L. A.; Egge, H.; Gunawan, J.; Peter-Katalinic, J.; Dabrowski, Y.; Dabrowski, J. *Biochem. Biophys. Res. Commun.* **1987**, *146*, 1055. Cross, G. A. M. *Cell* **1987**, *48*, 179. Low, B. G. *Biochem. J.* **1987**, *244*, 1. Saltiel, A. R.; Cuatrecases, P. *Am. J. Physiol.* **1988**, *255*, C1.
(20) Phelps, B. M.; Prinmakoff, P.; Koppel, D. E.; Low, M. G.; Myles, D. G. *Science* **1988**, *249*, 1780. Edidin, M.; Stroynowski, I. *J. Cell Biol.* **1991**, *112*, 1143. McConville, M.; Ferguson, M. A. J. *Biochem. J.* **1993**, *204*, 305.
(21) Toutant, J.-P.; Richards, M. K.; Drall, J. A.; Rosenberry, T. L. *Eur. J. Biochem.* **1990**, *187*, 31. Mayor, S.; Menon, A. K.; Cross, A. M.; Ferguson, M. A. J.; Dwek, R. A.; Rademacher, T. W. *J. Biol. Chem.* **1990**, *265*, 6164. Masterson, W. J.; Raper, J.; Doehring, T. L.; Hart, G. W.; Englund, P. T. *Cell* **1990**, *62*, 73. Pvoti, A.; Conzelmann, A. *J. Biol. Chem.***1993**, *268*, 7215.
(22) Doering, T. L.; Raper, J.; Buxbaum, L. U.; Adams, S. P.; Gordon, J. I.; Hart, G. W.; Englund, P. T. *Science* **1991** *252*, 1851. Degasperi, R.; Thomas, L. J.; Sugyama, E.; Change, H. M.; Beck, P. J.; Orlean, P.; Albright, C.; Waneck, G.; Sambrook, J. F.; Warren, C. D. *Science* **1990**, *250*, 988.
(23) Turco, S. J.; Descoteaux, A. *Annu. Rev. Microbiol.* **1992**, *46*, 65.
(24) Tolsen, D. L.; Turco, S. J.; Beecroft, R. P.; Pearson, T. W. *Mol. Biochem. Parasitol.* **1989**, *35*, 109. Pimento, P. F. P.; Saraiva, E. M. B.; Sacks, D. L. *Exp. Parasitol.* **1991**, *72*, 191.
(25) Saltiel, A. R.; Siegel, M. I.; Jacobs, S.; Cuztrecasas, P. *Proc. Natl. Acad. Sci.* **1982**, *79*, 3513. Saltiel, A.; Jacobs, S.; Siegle, M.; Cuatrecasas, P. *Biochem. Biophys. Res. Commun.* **1981**, *102*, 1041.
(26) Seals, J. R.; Czech, M. P. *J. Biol. Chem.* **1980**, *235*, 6529.
(27) Malchoff, C. D.; Huang, L.; Gillespie, N.; Palasi, C. V.; Schwartz, F. W.; Ceng, K.; Hewlett, E. L.; Larner, J. *Endocrinology* **1987**, *120*, 1327.
(28) Mootoo, D. R.; Date, V.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988**, *110*, 2662.
(29) Fraser-Reid, B.; Udodong, U. E.; Wu, Z.; Ottosson, H.; Merritt, J. R.; Rao, C. S.; Roberts, C.; Madsen, R. *Synlett* **1992**, 927.
(30) Yu, K.-L.; Fraser-Reid, B. *Tetrahedron Lett.* **1988**, *29*, 979.
(31) Murakata, C.; Ogawa, T. *Carbohydr. Res.* **1992**, *235*, 95.
(32) Verduyn, R.; Beliën, J. J. A.; Dreef-Tromp, C. M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1991**, *32*, 6637. Verduyn, R.; Elie, C. J. J.; Dreef, C. E.; van der Marel, G. A.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1990**, *109*, 591.
(33) Boons, G.-J.; Grice, P.; Leslie, R.; Ley, S. V.; Yeung, L. L. *Tetrahedron Lett.* **1993**, *34*, 9525.
(34) Cottaz, S.; Brimacombe, J. S.; Ferguson, M. A. J. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2945.
(35) Lis, H.; Sharon, N. *J. Biol. Chem.* **1978**, *253*, 3468. Li, E.; Kornfield, S. *J. Biol. Chem.* **1979**, *254*, 1600. Dorland, L.; van Halbeek, H.; Vliegenthart, J. F. G.; List, H.; Sharon, N. *J. Biol. Chem.* **1981**, *256*, 7708.

Scheme 2



We settled upon a chloroacetyl ester for O-6 of the M3 residue of **2**, an acetyl ester for O-2 of the M1 residue of **3**, and an allyl ether on the inositol of **4**, this also being the order for the selective deprotections. Notably all of these protecting groups are compatible with NPG coupling strategies.[29]

In this manuscript, we focus on aspects of the chemistry of *n*-pentenyl mannoside donors geared at segment **2** (Scheme 1). In the accompanying manuscript, we deal with segments **3** and **4** and give a full account of the assembly to give the cores of **1a**[36] and **1b**.[37]

Results and Discussion

Ortho esters are readily prepared derivatives[38] which may be regarded as masked esters in that they are stable to base, but which, in the presence of mild acids, undergo stereoelectronically directed rearrangement.[39,40] In the cases of 1,2-ortho esters, e.g. **5**, (Scheme 2), acid-catalyzed rearrangement in the absence of an added alcohol causes the alkoxy moiety to be transferred to the anomeric center, i.e. **5**→**8**.[41] This result can be interpreted as involving protonation of the alkoxy group as in **6**, which triggers formation of dioxolenium ion **7**, the latter then reacting with the ejected alcohol to give the *trans*-1,2-glycoside **8**.

In the case of an *n*-pentenyl ortho ester, an additional mode of reaction is possible. Thus, in the presence of an halonium ion, the ortho ester **5** (Alk = pentenyl) might be expected to proceed to the furanylium ion **11** and thence to the same dioxolenium ion **7** with ejection of 2-(halomethyl)tetrahydrofuran. Since the latter is non-nucleophilic, the subsequent fate of **7** depends on the nucleophile that is available. Thus, if the electrophile is molecular bromine, the bromide ion released upon formation of **11** (X = Br) would react with **7** to give the glycosyl bromide **9**.[42] Alternatively, if an alcohol R'OH is present (e.g., a partially protected sugar), coupling would result to give **10** (e.g., R' = sugar).

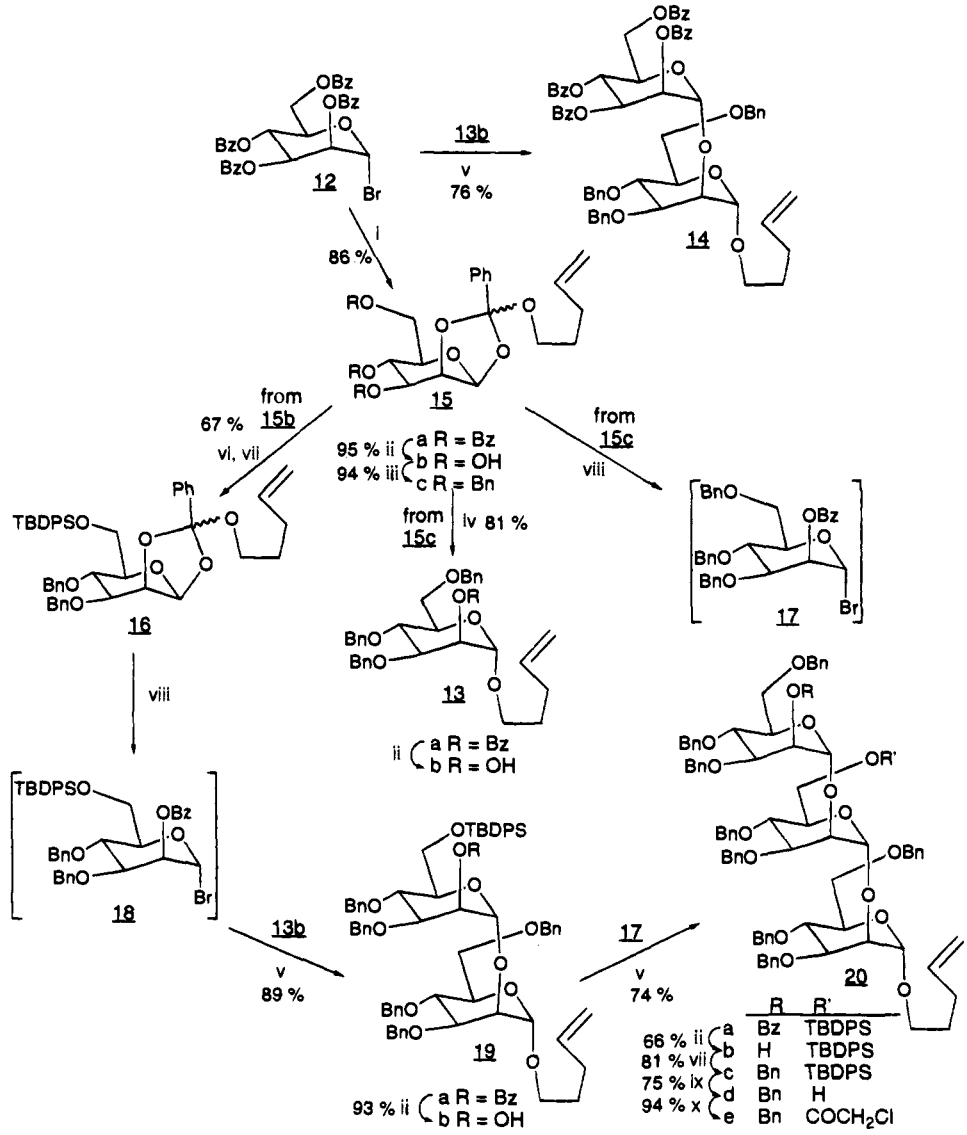
Thus there are three options for the use of a given *n*-pentenyl ortho ester **5** (Alk = pentenyl), depending on the nucleophile that is available for reaction with dioxolenium ion **7**. In option c, ortho ester **5** can serve as a glycosyl donor in its own right leading to **10**. In options a and b, **5** (Alk = pentenyl) is a progenitor of two other glycosyl donors **8** and **9**. The three donors, **5**, **8**, and **9** have vastly different chemical properties and stabilities. The bromide **9** is most conveniently prepared[42] by dropwise addition of molecular bromine to a solution of **5** until the brown color just persists. Because of the high reactivity of glycosyl bromides,[43] it is best to use the resulting material **9**, without purification, by adding the glycosyl acceptor and silver triflate directly.

Our preliminary report[36] relating to the synthesis of **1a** was carried out before all three options in Scheme 2 had been explored. Thus, for the synthesis of the **M2-M3** segment (Scheme 1), only option a was utilized. The mannosyl bromide **12** (Scheme 3) was converted into ortho ester **15a** by treatment with 4-penten-1-ol and lutidine.[44,45] The ester groups were replaced by benzyl ethers, **15a** → **15b** → **15c**, and then the acid-catalyzed rearrangement (option a) was employed to obtain **13a**, debenzoylation of which afforded glycosyl acceptor **13b**. Coupling of the latter to the parent bromide **12** then gave the disaccharide **14**, which was used in the synthesis of **1a**.[36]

Options a and b were explored in the context of a synthesis of the M2-M3-M4 component of the Thy-1 anchor **1b**.[37] The central unit, M3, carries a phosphodiester at the C-6 OH, and hence, this site must be equipped with a compatible temporary

(36) Mootoo, D. R.; Konradsson, P.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1989**, *111*, 8540.
(37) Udodong, U. E.; Madsen, R.; Roberts, C.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1993**, *115*, 7886.
(38) Kochetkov, K.; Klimov, E. M.; Malysheva, N. N.; Demchenko, A. V. *Carbohydr. Res.* **1991**, *212*, 77. Kochetkov, K.; Zhuin, V. M.; Klimov, E. M.; Malysheva, N. N.; Makarova, Z. G.; Ott, A. Y. *Carbohydr. Res.* **1987**, *164*, 241.
(39) King, J. F.; Allbutt, A. D. *Tetrahedron Lett.* **1967**, 49. King, J. F.; Allbutt, A. D. *Can. J. Chem.* **1970**, *48*, 1754.
(40) Deslongchamps, P. *Stereoelectronic Effects in Organic Chemistry*; Pergamon Press: New York, 1983; Chapter 3.
(41) For a historical perspective, see: Bochkov, A. F.; Zaikov, G. E. *Chemistry of the O-Glycosidic Bond*; Pergamon Press: Oxford, U.K., 1979; Chapter 2.
(42) Konradsson, P.; Fraser-Reid, B. *J. Chem. Soc., Chem. Commun.* **1989**, 1124.
(43) Igarashi, K. *Adv. Carbohydr. Chem. Biochem.* **1977**, *34*, 155. Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 155.
(44) Lemieux, R.; Takeda, T.; Chung, B. Y. *ACS Symp. Ser.* **1976**, *39*, 90.
(45) Franks, N. E.; Montgomery, R. *Carbohydr. Res.* **1964**, *6*, 286. Ogawa, T.; Nukada, T. *Carbohydr. Res.* **1988**, *136*, 135.

Scheme 3[a]



[a] (i) 4-Penten-1-ol, 2,6-lutidine, CH_2Cl_2; (ii) NaOMe; (iii) BnBr, NaH, DMF; (iv) CSA, CH_2Cl_2, 50 °C, 6 h; (v) AgOTf, 4 Å, CH_2Cl_2; (vi) $tBuPh_2SiCl$, imidazole, THF; (vii) BnBr, NaH, Bu_4NI, THF; (viii) Br_2, CH_2Cl_2, 0 °C; (ix) TBAF, THF; (x) $(ClAc)_2O$, Et_3N, CH_2Cl_2.

protecting group. This requirement was fulfilled by making use of the already prepared ortho esters **15a,b** and NPG **13b**. A portion of triol **15b** was selectively silylated prior to benzylation to give ortho ester **16**. The latter, upon titration with bromine in methylene chloride solution, gave glycosyl bromide **18**, which was used directly for coupling to acceptor **13b** under standard Koenigs–Knorr conditions to give disaccharide **19a**. Debenzoylation then readied the product, **19b**, for service as a glycosyl acceptor.

Another portion of ortho ester **15c** now served as a precursor for the bromide **17**, which was coupled to acceptor **19b** to furnish the trisaccharide **20a**. A series of manipulations were now required to install the chloroacetyl group, and after four routine operations, the trisaccharide **20e** was ready as a glycosyl donor.

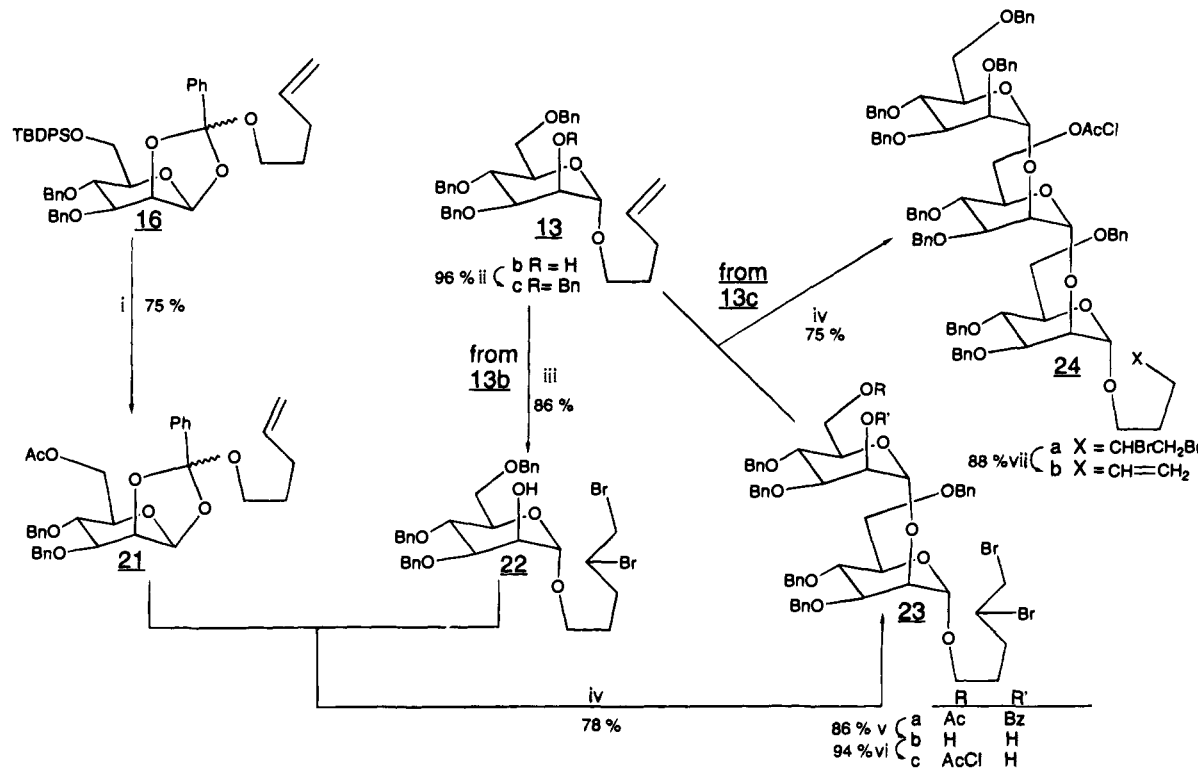
Although these four protecting group adjustments were standard and high yielding, they were nonetheless time consuming and inelegant in that they had to be carried out late in the synthesis. As long as Koenigs–Knorr methodology was to be employed, there was little alternative since glycosyl bromides **16** and **17** would not be able to tolerate protecting group manipulations.

An alternative plan was therefore designed entirely around *n*-pentenyl glycoside chemistry, and it is depicted in Scheme 4. Our objective was to maximize manipulations early in this synthetic sequence. Thus, instead of converting the differentially protected ortho ester **16** into a glycosyl bromide, as had been done in Scheme 3, we decided to make use of option c of Scheme 2 in which the ortho ester would serve as a glycosyl donor in its own right. Accordingly, the *tert*-butyldiphenylsilyl group of **16** was immediately replaced by acetyl in **21** (Scheme 4).

The other two mannopyranosyl components were now obtained from the pentenyl glycoside **13b** described in Scheme 3, one portion of which was perbenzylated to the tetrabenzyl derivative **13c** for later use. The other portion of **13b** was dibrominated, thereby postponing the ability of the NPG to function as a glycosyl donor and allowing it to serve immediately as a glycosyl acceptor.[29,46] Thus, the dibromide **22** was coupled with ortho ester **21** in keeping with option c in Scheme 2 to give disaccharide **23a**.

(46) Fraser-Reid, B.; Wu, Z.; Udodong, U. E.; Ottosson, H. *J. Org. Chem.* **1990**, *55*, 6068.

Scheme 4[a]



[a] (i) TBAF, THF then Ac_2O, DMAP, CH_2Cl_2; (ii) BnBr, NaH, Bu_4NI, DMF; (iii) Br_2, Et_4NBr, CH_2Cl_2; (iv) NIS, Et_3SiOTf, CH_2Cl_2; (v) NH_3, MeOH; (vi) $(ClAc)_2O$, Et_3N, CH_2Cl_2; (vii) Zn, Bu_4NI, EtOH then $(ClAc)_2O$.

The desired chloroacetyl group could now be installed by deesterification to diol **23b**, followed by selective acylation at the primary position. The product, **23c**, was now ready to serve as a glycosyl acceptor for the already described donor **13c**. Trisaccharide **24** was thereby obtained in excellent yield, and reductive debromination proceeded smoothly, although the chloroacetyl group was removed simultaneously. Rechloroacetylation then completed the preparation of trisaccharide **20e** as a glycosyl donor.

Conclusion

In conclusion, the *n*-pentenyl ortho ester **15b** has been shown to be a very versatile precursor which can be readily prepared on a large scale, can be stored safely, and is refractory to extensive manipulation under nonacidic conditions, but is activated under neutral conditions by an halonium ion. After carrying out an appropriate protecting strategy for the hydroxyl groups, the resulting derivatives can serve as glycosyl donors in their own right or be transformed under mild conditions into glycosyl bromides or *n*-pentenyl glycosides. The former must be used *in situ*, but the latter offers a wide range of choices, notable among which is the option of postponing NPG activity by temporary dibromination of the double bond. By taking advantage of this versatility, the *n*-pentenyl ortho ester **15b** is made to serve as the sole precursor for the mannan components of the GPI anchors described in this manuscript. The key *n*-pentenyl intermediates, like the parent ortho ester itself, are stable indefinitely, may be processed on a large scale as required, and are activated by nontoxic electrophilic reagents.

Experimental Section

General Procedures. Optical rotations were determined with a Perkin-Elmer 241 polarimeter. NMR spectra were recorded on a Varian XL-300 spectrometer. Chemical shifts were measured in ppm. All NMR spectra were recorded in $CDCl_3$ using $CHCl_3$ ($\delta = 7.26$) as an internal reference for ^1H NMR spectra and $CDCl_3$ ($\delta = 77.1$) for ^{13}C NMR spectra. Mass spectral data were recorded on a JEOL JMS-SX102A mass spectrometer using the FAB technique in a *m*-nitrobenzyl alcohol matrix. Glycosyl donors, glycosyl acceptors, and silver salts were azeotroped with toluene prior to coupling reactions. All reactions were performed under an atmosphere of argon. The progress of all reactions was monitored by thin layer chromatography which was performed on aluminum plates precoated with silica gel (Merck 5554). Compounds were visualized by charring after dipping in a solution of ammonium molybdate (6.25 g) and cerium(IV) sulfate (25 g) in 10% aqueous sulfuric acid (250 mL). Anhydrous Na_2SO_4 was used to dry organic solutions during workup. Flash column chromatography was performed using Kiselgel 60 (230–400 mesh, Merck). Microanalyses were performed by Atlantic Microlab, Inc., P.O. Box 2288, Norcross, GA 30091. DMF was kept over CaH_2. Toluene was distilled from sodium, and CH_2Cl_2 was distilled from P_2O_5. THF and Et_2O were distilled from sodium/benzophenone. Absolute methanol was used as purchased. *N*-Iodosuccinimide (NIS) was recrystallized from *p*-dioxane/CCl_4.

3,4,6-Tri-*O*-benzoyl-β-D-mannopyranose 1,2-(Pent-4-enyl orthobenzoate) (15a). Following the procedure from Fletcher *et al.*,[47] D-mannose (20.5 g, 0.114 mol) in pyridine (350 mL) was cooled to 0 °C and treated with benzoyl chloride (110 mL, 0.948 mol). After stirring for 24 h, the reaction mixture was diluted with CH_2Cl_2 (700 mL), washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, respectively, dried, concentrated, and flash chromatographed (7:3 petroleum ether/EtOAc) to afford 73 g (91%) of the perbenzoylated mannose (R_f = 0.5). The pentabenzoate in CH_2Cl_2 (300 mL) was cooled to O °C and treated with a 30 wt % solution of HBr in AcOH (200 mL). After 12 h, the reaction mixture was diluted with CH_2Cl_2 (500 mL) and stirred with cold, saturated aqueous $NaHCO_3$ for 30 min. The organic phase was washed with brine, dried, concentrated, and flash chromatographed (4:1 petroleum ether/EtOAc) affording 60.3 g (88%) of the glycosyl bromide **12** (R_f = 0.5). The bromosugar **12** in CH_2Cl_2 (200 mL) was treated with 2,6-lutidine (28 mL, 0.284 mol) and 4-penten-1-ol (14.11 mL, 0.137 mol). After 5 days, the reaction mixture was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ and brine, respec-

(47) Ness, R. K.; Fletcher, H. G., Jr., Hudson, C. S. *J. Am. Chem. Soc.* **1950**, *72*, 2200.

tively, dried, concentrated, and flash chromatographed (4:1 petroleum ether/EtOAc) to afford 52 g (86%) of the desired ortho ester **15a** R_f = 0.3; ^{1}H NMR δ 8.10−7.23 (m, 20H, Ph), 5.97 (t, 1H, H-4), 5.81 (d, 1H, H-1), 5.78−5.63 (m, 2H, H-3, C*H*=CH$_2$), 5.12 (t, 1H, H-2) 4.99−4.85 (m, 2H, CH=C*H*$_2$), 4.57 (dd, 1H, CH$_2$), 4.39 (dd, 1H, CH$_2$), 4.13 (m, 1H, H-5), 3.42 (t, 2H, CH$_2$), 2.15−2.03 (m, 2H, CH$_2$), 1.73−1.61 (m, 2H, CH$_2$); ^{13}C δ NMR 166.1, 166.0, 165.2 (3Bz), 122.9 (PhC-(OR)$_3$), 114.9 (=CH$_2$), 97.9 (C-1), 30.1, 28.7 (2CH$_2$). Anal. Calcd for $C_{39}H_{36}O_{10}$: C, 70.47; H, 5.46. Found: C, 70.30; H, 5.39.

3,4,6-Tri-*O*-benzyl-β-D-mannopyranose 1,2-(Pent-4-enyl orthobenzoate) (15c). The ortho ester **15a** (27.8 g, 0.042 mol) in 230 mL of MeOH/CH$_2$Cl$_2$ (8:1) was treated with NaOMe (1 g, 0.021 mol) for 28 h. Upon completion (TLC), the solvent was removed *in vacuo* and the crude material was flash chromatographed (9:1 CH$_2$Cl$_2$/MeOH), affording 14 g (95%) of the triol **15b** (R_f = 0.4). A portion of the triol (7 g, 0.020 mol) in DMF (300 mL) was then treated with NaH (4.8 g of 60% oil dispersion, 0.115 mol) and BnBr (11.82 mL, 0.099 mL), respectively. After 19 h, the reaction mixture was cooled to 0 °C, quenched with MeOH, diluted with Et$_2$O (800 mL), washed with H$_2$O and saturated aqueous NH$_4$Cl, respectively, dried, concentrated, and flash chromatographed (4:1 petroleum ether/EtOAc) to afford 11.5 g (94%) of **15c**: R_f = 0.4; ^{1}H NMR δ 7.78−7.21 (m, 20H, Ph), 5.87 (m, 1H, C*H*=CH$_2$), 5.52 (d, 1H, H-1), 5.13−4.85 (m, H), 4.51−4.41 (m, H), 4.09−3.41 (m, H), 2.27−2.18 (m, 2H, CH$_2$), 1.82−1.70 (m, 2H, CH$_2$); ^{13}C NMR δ 122.2 (PhC(OR)$_3$) 114.9 (=CH$_2$), 97.8 (C-1), 30.3, 28.8 (2CH$_2$). Anal. Calcd for $C_{39}H_{42}O_7$: C, 75.22; H, 6.80. Found: C, 74.96; H, 6.79.

Pent-4-enyl 3,4,6-Tri-*O*-benzyl-α-D-mannopyranoside (13b). Ortho ester **15c** (8.94 g, 14.35 mmol) was treated with camphorsulfonic acid (63 mg) in CH$_2$Cl$_2$ (2 mL) at 50 °C for 6 h. Et$_3$N (0.10 mL) was then added to the reaction mixture and the solution concentrated *in vacuo*. The crude residue was dissolved in 35 mL of MeOH/CH$_2$Cl$_2$ (7:1) and treated with NaOMe (600 mg, 11.4 mmol) at room temperature for 2 h. The solution was then cooled to 10 °C, neutralized by the addition of 1% HCl in CH$_3$OH, and evaporated *in vacuo*. Flash chromatography (3:1 petroleum ether/EtOAc) of the residue gave the alcohol **13b** (6.01 g, 81%): R_f = 0.25; $[\alpha]^{20}_D$ +47.6° (*c* 1.4, CHCl$_3$); ^{1}H NMR δ 7.40−7.12 (m, 15H, Ph), 5.79 (m, 1H, C*H*=CH$_2$), 5.02 (m, 2H, =CH$_2$), 4.92 (d, 1H, *J* = 1.5 Hz, H1), 4.85 (d, *J* = 10.8 Hz, 1H), 4.64 (d, *J* = 12 Hz, 1H), 4.57 (d, *J* = 10.8 Hz, 1H), 4.48 (d, *J* = 12 Hz, 1H), 4.02 (bs, 1H, H2), 3.90, 3.62 (m, 6H, H1′b, 3, 4, 5, CH$_2$-6), 3.45 (m, 1H, H1′a), 2.51 (bs, 1H, OH), 2.11 (m, CH$_2$), 1.69 (m, CH$_2$). Anal. Calcd for $C_{32}H_{38}O_6$: C, 74.11; H, 7.39. Found: C, 74.21; H, 7.40.

Pent-4-enyl 2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranoside (13c). The pentenyl glycoside **13b** (2.0 g, 3.86 mmol) in DMF (80 mL) was treated with NaH (0.308 g of 60% of dispersion, 7.71 mmol) and BnBr (0.917 mL, 7.71 mmol), respectively. After 8 h, the reaction mixture was cooled to 0 °C, quenched with MeOH, diluted with Et$_2$O (300 mL), washed with H$_2$O and saturated aqueous NH$_4$Cl, respectively, dried, concentrated, and flash chromatographed (9:1 petroleum ether/EtOAc) to afford 2.25 g (96%) of **13c**: R_f = 0.25; ^{1}H NMR δ 7.42−7.16 (m, 20H, Ph), 5.82 (m, 1H, C*H*=CH$_2$), 5.08−4.88 (m, 3H), 4.84−4.61 (m, 6H), 4.06−3.91 (m, 3H), 3.88−3.37 (m, 5H), 3.42−3.37 (m, 2H, CH$_2$), 2.14−2.06 (m, 2H, CH$_2$), 1.70−1.62 (m, 2H, CH$_2$); ^{13}C NMR δ 138.6, 138.5, 138.4, 138.1 (4Bn), 115.0 (=CH$_2$), 98.0 (C-1″), 30.4, 28.7 (2CH$_2$). Anal. Calcd for $C_{39}H_{44}O_6$: C, 76.95; H, 7.29. Found: C, 76.73; H, 7.25.

Pent-4-enyl 2-*O*-(2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (14). A mixture of bromide **12** (240 mg, 0.36 mmol), alcohol **13b** (145 mg, 0.28 mmol), *N,N,N′,N′*-tetramethylurea (0.07 mL, 0.55 mmol), and activated, powdered 4 Å molecular sieves in CH$_2$Cl$_2$ (5 mL) was stirred at −20 °C for 20 min. At this time, AgOTf (92 mg, 0.36 mmol) was added to the reaction mixture and the reaction allowed to proceed at room temperature for an additional 4 h. The reaction mixture was then diluted with CH$_2$Cl$_2$ (25 mL) and filtered, and the filtrate was washed with 10% aqueous Na$_2$S$_2$O$_3$ and saturated aqueous NaHCO$_3$. The organic extract was dried and evaporated *in vacuo*. Flash chromatography (9:1 petroleum ether/EtOAc) of the crude product afforded the disaccharide **14** (233 mg, 76%): R_f = 0.5; $[\alpha]^{20}_D$ −28.6° (*c* 1.14, CHCl$_3$); ^{1}H NMR δ 8.14−7.05 (m, 35H, Ph), 6.16 (t, 1H, *J* = 9.9 Hz, H4′), 6.03 (m, 2H, H2′, H3′), 5.33 (bs, 1H, H1′), 5.84 (m, 1H, C*H*=CH$_2$), 5.01 (m, 4H, H1″, =CH$_2$, PhC*H*), 4.84−4.61 (m, 6H, PhC*H* × 5, H6′), 4.52 (dd, 1H, *J* = 4.2), 4.06−3.86 (m, 3H), 3.72−3.37 (m, 1H, CH$_2$), 2.05 (m, 2H, CH$_2$), 1.60 (m, 2H, CH$_2$); ^{13}C NMR δ 166.2, 165.6, 165.3, 165.1 (4Bz), 115.0 (=CH$_2$), 99.5 (C1a, $J_{C,H}$ = 173.6 Hz), 98.79 (C1b, $J_{C,H}$ = 168.7 Hz), 30.3, 28.7 (2CH$_2$). Anal. Calcd for $C_{66}H_{64}O_{15}$: C, 72.25; H, 5.88. Found: C, 72.28; H, 5.88.

3,4-Di-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-mannopyranose 1,2-(Pent-4-enyl orthobenzoate) (16). β-D-Mannopyranose 1,2-(pent-4-enyl orthobenzoate) **15b** (12.3 g, 34.8 mmol) in THF (100 mL) was cooled to 0 °C and treated sequentially with imidazole (7.14 g, 104.4 mmol) and *tert*-butyldiphenylsilyl chloride (10.89 mL, 41.7 mmol). After the mixture was stirred for 24 h, the THF was removed under reduced pressure. The crude product was then diluted with EtOAc and washed with saturated aqueous NaHCO$_3$ followed by brine. The organic layer was dried, concentrated, and flash chromatographed (4:1 EtOAc/CH$_2$Cl$_2$) to afford 16.56 g (28.62 mmol) of the 6-*O*-silylated ortho ester to which was added a mixture of THF (200 mL) and NaH (4.59 g of 60% oil dispersion, 114.48 mmol) at 0 °C. After 15 min, the reaction mixture was treated with Bu$_4$NI (1.05 g, 2.85 mmol) followed by BnBr (13.77 mL, 114.48 mmol), warmed to room temperature, and stirred for 14 h. The mixture was then cooled to 0 °C and quenched with MeOH, and the solvent was removed under reduced pressure. The crude residue was then diluted with CH$_2$Cl$_2$, washed with saturated aqueous NaHCO$_3$ and brine, respectively, dried, concentrated, and flash chromatographed (4:1 petroleum ether/EtOAc) to afford 17.49 g (67%) of **16**: R_f = 0.72; ^{1}H NMR δ 7.71−7.23 (m, 25H, Ph), 5.79 (m, 1H, OCH$_2$CH$_2$CH$_2$C*H*=CH$_2$), 5.41 (d, 1H, H-1), 5.09−4.69 (m, 7H), 3.93−3.72 (m, 6H), 3.36 (m, 1H, OC*H*$_2$CH$_2$CH$_2$-CH=CH$_2$), 2.17−2.08 (m, 2H, OCH$_2$CH$_2$C*H*$_2$CH=CH$_2$), 1.72−1.62 (m, 2H, OCH$_2$C*H*$_2$CH$_2$CH=CH$_2$), 1.18 (s, 9H, tBu); ^{13}C NMR δ 122.9 (PhC(OR)$_3$), 115.1 (=CH$_2$), 98.0 (C-1), 30.4, 28.8 (2CH$_2$).

Pent-4-enyl 2-*O*-(2-*O*-Benzoyl-3,4-di-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsllyl)-α-D-mannopyranosyl)-3,4,6-trl-*O*-benzyl-α-D-mannopyranoside (19a). To the ortho ester **16** (10.4 g, 13.5 mmol, 2.52 equiv.) was added dry CH$_2$Cl$_2$ (20 mL), and the solution cooled to 0 °C was treated dropwise with 2.5 M Br$_2$ in CH$_2$Cl$_2$ until a faint yellow color persisted. TLC (4:1 petroleum ether/EtOAc) indicated the consumption of the starting material (R_f = 0.6), and a new product, presumed to be **18** (R_f = 0.7), was formed. The orange solution was then concentrated under reduced pressure and the resulting syrup dried for 10 min *in vacuo*. In the meantime, the alcohol **13b** (4.66 g, 8.98 mmol, 1 equiv), dissolved in 10 mL of CH$_2$Cl$_2$, was transferred to a slurry of AgOTf (3.93 g, 15.29 mmol, 1.7 equiv) in 15 mL of CH$_2$Cl$_2$ containing 2.0 g of powdered, activated 4 Å molecular sieves and stirred for 5 min at −60 °C. A solution of **18** in 9 mL of CH$_2$Cl$_2$ was then added dropwise to the slurry under argon, and the temperature was raised to −40 °C. After 10 min TLC indicated complete consumption of alcohol **13b**. The reaction was quenched by vigorous stirring with saturated aqueous NaHCO$_3$, diluted with CH$_2$Cl$_2$, and then filtered through Celite. The organic solution was washed with brine, dried, and concentrated. Flash chromatography (4:1 petroleum ether/EtOAc) afforded 10.81 g (89%) of **19a**: R_f = 0.63; $[\alpha]^{20}_D$ +7.9° (*c* 1, CHCl$_3$); ^{1}H NMR δ 8.20−7.15 (m, 40H, Ph), 5.87 (d, *J* = 1.6 Hz, 1H, H-2), 5.76 (m, 1H, C*H*=CH$_2$), 5.40 (d, *J* = 1.6 Hz, 1H, H-1′), 5.02−4.49 (m, 11H), 4.82 (d, 1H, H-1″), 4.20−3.70 (m, 12H), 3.63 (m, 1H, OCH$_2$), 3.29 (m, 1H, OCH$_2$), 2.12−2.03 (m, 2H, CH$_2$), 1.75−1.61 (m, 2H, CH$_2$), 1.15 (s, 9H, 3 CH$_3$); ^{13}C NMR δ 165.6 (Bz), 114.9 (=CH$_2$), 99.2 (C-1′), 98.9 (C-1″), 30.3, 28.6 (2 CH$_2$). Anal. Calcd for $C_{75}H_{82}O_{12}Si$: C, 74.85; H, 6.87. Found: C, 74.89; H, 6.91.

Pent-4-enyl 2-*O*-(3,4-Dl-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (19b). The disaccharide **19a** (1.4 g, 1.16 mmol) was dissolved in 8 mL of MeOH/CH$_2$Cl$_2$ (7:1) and treated with NaOMe (50 mg, 0.92 mmol) at room temperature for 8 h. Upon completion (TLC), the solvent was removed *in vacuo* and the crude material was flash chromatographed (4:1 petroleum ether/EtOAc) affording 1.2 g (93%) of **19b**: R_f = 0.3; ^{1}H NMR δ 7.82−7.16 (m, 35H, Ph), 5.77 (m, 1H, C*H*=CH$_2$), 5.36 (d, *J* = 0.73 Hz, 1H, H-1), 5.02−4.55 (m, 13H), 4.21−3.71 (m, 13H), 3.63 (m, 1H, OCH$_2$), 3.27 (m, 1H, OCH$_2$), 2.10−2.02 (m, 2H, CH$_2$), 1.68−1.58 (m, 2H, CH$_2$), 1.14 (s, 9H, tBu); ^{13}C NMR δ 114.9 (=CH$_2$), 100.4 (C-1′), 99.1 (C-1″), 30.3, 28.7 (2CH$_2$). Anal. Calcd for $C_{68}H_{78}O_{11}Si$: C, 74.29; H, 7.15. Found: C, 74.20; H, 7.11.

Pent-4-enyl O-(2-O-Benzoyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→2)-O-(3,4-di-O-benzyl-6-O-(*tert*-butyldiphenylsilyl)-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (20a). A solution of ortho ester **15c** (2.81 g, 3.64 mmol, 1.5 equiv) in dry CH_2Cl_2 (5 mL) at 0 °C under argon was treated dropwise with 2.5 M Br_2 in CH_2Cl_2 until a faint yellow color persisted at which TLC (4:1 petroleum ether/EtOAc) indicated the consumption of the starting material **15c** (R_f = 0.7) and a new product presumed to be **17** (R_f = 0.8). The orange solution was then concentrated under reduced pressure and the resulting syrup dried for 10 min *in vacuo*. In the meantime, the alcohol **19b** (1.26 g, 1.14 mmol, 1 equiv) was dissolved in 6 mL of CH_2Cl_2, transferred to a slurry of AgOTf (1.06 g, 4.13 mmol, 1.7 equiv) in 8 mL of CH_2Cl_2 containing 2.0 g of powdered, activated 4 Å molecular sieves, and stirred for 5 min at −60 °C. A solution of **17** in 5 mL of CH_2Cl_2 was then added dropwise to the slurry, and the temperature was raised to −40 °C. After 10 min, TLC indicated complete consumption of alcohol **19b**. The reaction mixture was quenched by vigorous stirring with saturated aqueous $NaHCO_3$, diluted with CH_2Cl_2, and then filtered through Celite. The organic solution was washed with brine, dried, and concentrated. Flash chromatography (4:1 petroleum ether/EtOAc) afforded 3.25 g (74%) of **20a**: R_f = 0.75; $[\alpha]^{20}_D$ +3.28° (*c* 1, $CHCl_3$); ^{1}H NMR δ 8.13−7.05 (m, 55H, Ph), 5.69 (m, 1H, C*H*=CH_2), 5.42 (d, *J* = 1.4 Hz, 1H, H-2‴), 5.16 (d, 1H, *J* = 1.7 Hz, H-1′), 5.00−4.30 (m, 15H), 4.25−3.56 (m, 15H), 3.51 (m, 1H, OCH_2), 3.26 (m, 1H, OCH_2), 2.05−1.98 (m, 2H, CH_2), 1.65−1.56 (m, 2H, CH_2), 1.15 (s, 9H, 3 CH_3); ^{13}C NMR δ 165.4 (Bz), 114.8 (=CH_2), 100.0, 99.9, 99.0 (3C-1), 30.3, 28.6 ($2CH_2$). Anal. Calcd for $C_{102}H_{110}O_{17}Si$: C, 74.88; H, 6.78. Found: C, 74.59; H, 6.75.

Pent-4-enyl O-(2,3,4,6-Tetra-O-benzyl-α-D-mannopyranosyl)-(1→2)-O-(3,4-di-O-benzyl-6-O-(chloroacetyl)-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (20e). The trisaccharide **20a** (5.02 g, 2.94 mmol) was dissolved in 30 mL of MeOH/CH_2Cl_2 (7:1) and treated with NaOMe (300 mg, 5.50 mmol) at room temperature for 3 h. Upon completion (TLC), the solvent was removed *in vacuo* and the crude material was flash chromatographed (4:1 petroleum ether/EtOAc), affording 3.10 g (66%) of the alcohol **20b** (R_f = 0.2). THF (30 mL) was added to a cooled (ice bath) mixture of NaH (230 mg of 60% oil dispersion, 5.52 mmol) and alcohol **20b** (2.83 g, 1.84 mmol). After 15 min, the reaction mixture was treated with Bu_4NI (70 mg, 0.180 mmol) followed by BnBr (4.5 mL, 37.8 mmol) and was allowed to warm to room temperature. After 8 h, the mixture was cooled to 0 °C and quenched with MeOH and THF was removed under reduced pressure. The crude mixture was then diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ and brine, dried, concentrated, and flash chromatographed (4:1 petroleum ether/EtOAc) to afford 2.78 g of **20c** (81%) as an oil (R_f = 0.6). A solution of **20c** (2.75 g, 1.63 mmol) in dry THF (25 mL) was treated with Bu_4NF (3 mL of 1 M THF solution, 3 mmol) at room temperature. After 22 h, the reaction mixture was concentrated under reduced pressure and flash chromatography (7:3 petroleum ether/EtOAc) of the residual oil gave 1.80 g (75%) of alcohol **20d** (R_f = 0.21). A portion of **20d** (1.20 g, 0.83 mmol) was dissolved in CH_2Cl_2 (10 mL) at 0 °C and treated with chloroacetic anhydride (220 mg, 1.21 mmol) and Et_3N (0.25 mL, 1.65 mmol), respectively. After ~25 min all of the starting material was consumed (TLC 4:1 petroleum ether/EtOAc). The reaction mixture was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ and brine, dried, concentrated, and flash chromatographed (4:1 petroleum ether/EtOAc) to afford 1.18 g (94%) of **20e**: R_f = 0.42; $[\alpha]^{20}_D$ +27.2° (*c* 1, $CHCl_3$); ^{1}H NMR δ 7.40−7.15 (m, 45H, Ph), 5.81 (m, 1H, C*H*=CH_2), 5.15 (d, *J* = 1.76 Hz, 1H, H-1″), 5.05−4.40 (m, 21H), 4.19−3.60 (m, 22H), 3.39 (m, 1H, OCH_2), 2.13−2.04 (m, 2H, CH_2), 1.71−1.62 (m, 2H, CH_2); ^{13}C NMR δ 167.0 (AcCl), 115.0 (=CH_2), 99.5 (*J* = 175 Hz), 99.1 (*J* = 169 Hz), 98.8 (*J* = 171 Hz), 40.6 (AcCl), 30.3, 28.6 ($2CH_2$). Anal. Calcd for $C_{88}H_{95}ClO_{17}$: C, 72.38; H, 6.57. Found: C, 72.36; H, 6.64.

6-O-Acetyl-3,4-di-O-benzyl-α-D-mannopyranose 1,2-(Pent-4-enyl orthobenzoate) (21). The ortho ester **16** (2.98 g 3.864 mmol) was treated with Bu_4NF (25 mL of 1 M THF solution, 25.2 mmol) at room temperature. After 12 h, the reaction mixture was concentrated and flash chromatographed (7:3 petroleum ether/EtOAc) to afford 1.834 g of the corresponding alcohol (R_f = 0.31). The alcohol (1.839 g, 3.443 mmol) dissolved in CH_2Cl_2 (20 mL) at 0 °C was treated with Ac_2O (5 mL, 5.164 mmol) and DMAP (0.84 g, 6.886 mmol), respectively. After ~40 min, all of the starting material was consumed (TLC 3:1 petroleum ether/EtOAc). The reaction mixture was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ and brine, dried, concentrated, and flash chromatographed (3:1 petroleum ether/EtOAc) to afford 1.66 g (75%) of **21**: R_f = 0.32; ^{1}H NMR δ 7.71−7.23 (m, 15H, Ph), 5.81 (m, 1H, C*H*=CH_2), 5.50 (bs, 1H, H-1), 5.09−4.60 (m, 10H), 4.31−4.09 (m, 2H), 3.86 (m, 1H) 3.48 (m, 1H, OCH_2), 2.20−2.11(m, 2H, CH_2-CH=CH_2), 1.92 (s, 3H, Ac), 1.75−1.65 (m, 2H, OCH_2CH_2); ^{13}C NMR δ 170.7 (Ac), 122.9 ($PhC(OR)_3$), 115.0 (=CH_2), 97.7 (C-1), 30.3, 28.8 ($2CH_2$). Anal. Calcd for $C_{34}H_{38}O_8$: C, 71.06; H, 6.67. Found: C,-71.19, H, 6.59.

4,5-Dibromopentanyl 3,4,6-Tri-O-benzyl-α-D-mannopyranoside (22). The pentenyl glycoside **13b** (4.00 g, 7.72 mmol) and Et_4NBr (0.810 g, 3.86 mmol) were dissolved in CH_2Cl_2 (200 mL) at 0 °C. Bromine (0.400 mL, 7.72 mmol) was added dropwise, allowing the solution to decolorize between each drop. When the brown color persisted, the reaction was quenched with 10% sodium thiosulfate. The reaction mixture was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ and brine, dried, concentrated, and flash chromatographed (7:3 petroleum ether/EtOAc) to afford 4.50 g (86%) of **22**: R_f = 0.25; $[\alpha]_D^{22}$ +41.4° (*c* 1, $CHCl_3$); ^{1}H NMR δ 7.44−7.15 (m, 15H, Ph), 4.92 (d, 1H, *J* = 2Hz, H-1), 4.88−4.51 (m, 6H), 4.18 (m, 1H), 4.04 (s, 1H), 3.94−3.72 (m, 8H), 3.62 (m, 1H), 2.26 (m, 1H, OCH_2CH_2-$CH_2CHBrCH_2Br$), 1.95−1.59 (m, 3H, $OCH_2CH_2CH_2CHBrCH_2Br$); ^{13}C NMR δ 99.3 (C-1), 52.5 (CHBr), 36.3 (CH_2Br), 26.9, 26.8 ($2CH_2$). Anal. Calcd for $C_{32}H_{38}O_6Br_2$: C, 56.63; H, 5.64. Found: C, 56.50; H, 5.68.

4,5-Dibromopentanyl 2-O-(6-O-Acetyl-2-O-benzoyl-3,4-di-O-benzyl-α-D-mannopyranosyl)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (23a). The alcohol **22** (2.70 g, 4.15 mmol) was dissolved in CH_2Cl_2 (10 mL). NIS (943 mg, 5.39 mmol) and the ortho ester **21** (3.10 g, 5.39 mmol) in CH_2Cl_2 (10 mL) were added, respectively. After 5 min, a catalytic amount of Et_3SiOTf (120 μL, 0.530 mmol) was added *via syringe*. The reaction mixture was stirred for 10 min, diluted with CH_2Cl_2, washed with 10% aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$, dried, concentrated, and flash chromatographed (3:1 petroleum ether/EtOAc) to afford 3.61 g (78%) of **23a**: R_f = 0.53; $[\alpha]^{20}_D$ +11.3° (*c* 1, $CHCl_3$); ^{1}H NMR δ 8.15 −7.15 (m, 30H, Ph) 5.78 (bs, 1H, H-2′), 5.19 (bs, 1H, H-1), 4.92−4.30 (m, 13H), 4.21−3.56 (m, 13H), 3.43 (m, 1H, OCH_2), 2.29 (m, 1H, $OCH_2CH_2CH_2CHBrCH_2Br$), 2.10 (s, 3H, Ac), 1.92−1.68 (m, 3H, $OCH_2CH_2CH_2CHBrCH_2Br$), ^{13}C NMR δ 170.6 (Ac), 165.4 (Bz), 99.2 (C-1′), 98.6 (C-1″), 32.8, 26.6 (2 CH_2). Anal. Calcd for $C_{61}H_{66}Br_2O_{13}$: C, 62.78; H, 5.70. Found: C, 62.63; H, 5.69.

4,5-Dibromopentanyl 2-O-(3,4-Di-O-benzyl-6-O-(chloroacetyl)-α-D-mannopyranosyl)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (23c). The dissaccharide **23a** (3.24 g, 2.77 mmol) was dissolved in 40 mL of MeOH/acetone (20:1), and NH_3 was bubbled into the solution for 20 min at 0 °C. After 5 days the mixture was concentrated and flash chromatographed (7:3 petroleum ether/EtOAc), affording 2.41 g (86%) of diol **23b**. A portion of compound **23b** (1.71 g, 1.68 mmol) was dissolved in 24 mL of CH_2Cl_2 at 0 °C and treated with chloroacetic anhydride (316 mg, 1.85 mmol) and Et_3N (8 mL, 3.37 mmol), respectively. After 2.5 h, the reaction mixture was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ and brine, dried, concentrated, and flash chromatographed (4:1 petroleum ether/EtOAc) to obtain 1.72 g (94%) of desired alcohol **23c**: R_f = 0.25; $[\alpha]^{20}_D$ +24.2° (*c* 0.8, $CHCl_3$); ^{1}H NMR δ 7.41−7.19 (m, 25H, Ph) 5.30 (d, 1H, H-1″), 5.15 (d, 1H, H-1′), 4.91−4.28 (m, 13H), 4.21−3.56 (m, 13H), 3.43 (m, 1H, OCH_2), 2.23 (m, 1H, $CH_2CHBrCH_2Br$), 2.07 (s, 2H, AcCl), 1.91−1.68 (m, 3H, $OCH_2CH_2CH_2CHBrCH_2Br$); ^{13}C NMR δ 167.1 (AcCl), 100.7 (C-1′), 98.7 (C-1″), 32.8, 26.7 ($2CH_2$).

4,5-Dibromopentanyl O-(2,3,4,6-Tetra-O-benzyl-α-D-mannopyranosyl)-(1→2)-O-(3,4-di-O-benzyl-6-O-(chloroacetyl)-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (24). The alcohol **23c** (200 mg, 0.183 mmol) was dissolved in CH_2Cl_2 (1 mL). NIS (53.6 mg, 0.238 mmol), and the glycosyl donor **13c** (145 mg, 0.283 mmol) in CH_2Cl_2 (1 mL) were added respectively. After 5 min, a catalytic amount of Et_3SiOTf (5.3 μL, 0.023 mmol) was added *via syringe*. The reaction mixture was stirred for 30 min, diluted with CH_2Cl_2, washed with 10% aqueous $Na_2S_2O_3$ and saturated aqueous

$NaHCO_3$, dried, concentrated, and flash chromatographed (4:1 petroleum ether/EtOAc) to yield 216 mg (75%) of trisaccharide **24**: R_f = 0.51; $[\alpha]^{20}_D$ +8.24° (*c* 1, $CHCl_3$); ^1H NMR δ 7.40−7.14 (m, 45H, Ph), 5.27 (d, 1H, H1″), 5.06 (d, 1H, H-1′), 4.93−4.21 (m, 21H), 4.20−3.56 (m, 20H), 3.38 (m, 1H, OCH_2), 2.20 (m, 1H, $CH_2CHBrCH_2Br$), 2.09 (s, 2H, AcCl), 1.91−1.60 (m, 3H, $OCH_2CH_2CH_2CHBrCH_2Br$); ^{13}C NMR δ 167.3 (AcCl), 100.5 (C-1′), 98.7 (C-1″), 98.7 (C-1‴), 36.1, 26.8 (2 CH_2). Anal. Calcd for $C_{88}H_{95}Br_2ClO_{17}$: C, 65.25; H, 5.91. Found: C, 65.91; H, 6.07.

Pent-4-enyl *O*-(2,3,4,6-teTra-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4-di-*O*-benzyl-6-*O*-chloroacetyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (20e). The trisaccharide **24** (30 mg, 0.019 mmol) was dissolved in 2 mL of EtOH/acetone (3:1), and Zn (6.2 mg, 0.095 mmol) was added. After stirring for 5 min, Bu_4NI (22 mg, 0.060 mmol) was added and the reaction mixture was sonicated for 27 h. The crude suspension was then filtered through Celite, evaporated to dryness, dissolved in $CHCl_3$, washed with brine, dried, filtered, concentrated to 3 mL of $CHCl_3$, and treated with chloroacetic anhydride (4.9 mg, 0.029 mmol) and Et_3N (5.3 μL, 0.038 mmol) for 1 h. The product was then concentrated and flash chromatographed (4:1 petroleum ether/EtOAc) to obtain 24 mg (88%) of the desired trisaccharide **20e**, identical to the material prepared above.

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