Synthesis of Glycoconjugate Vaccines against *Shigella dysenteriae* Type 1

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Syntheses of a hexadecasaccharide and smaller fragments corresponding to one−four repeating units of the O-specific polysaccharide of *Shigella dysenteriae* type 1 are reported in a reactive aglycon-linked from. Two tetrasaccharide donor/acceptor repeating units were assembled from monosaccharide precursors in a stepwise fashion and used in a linear, iterative manner to construct the higher-membered saccharides using Schmidt's glycosylation technique that proved superior to others tested. Single-point attachment of the saccharides to human serum albumin, using a secondary heterobifunctional spacer, afforded a range of glycoconjugates for a detailed evaluation of their immunological characteristics.

Introduction

Bacterial polysaccharides such as capsular polysaccharides (CPSs),[1] lipooligosaccharides,[2] and lipopolysaccharides (LPSs)[3] have been investigated for their use as antibacterial vaccines since the discovery that purified CPSs of pneumococci are immunogenic in humans[4] and offer specific protection against homologous serotypes.[5] These early discoveries led to the development of a four-valent vaccine in 1945 that contained type-specific CPSs.[5] The success at that time of penicillin and the sulfonamides in the treatment of bacterial infections subdued interests in prevention and research in this area which was restimulated only after the recognition that pneumococci can develop resistance to antibiotics.[6] Currently, there are two 23-valent vaccines licensed in the United States[7] targeting the adult population[8] that provide approximately 90% protection against all pneumococcal infections in immunocompetent adults for up to 8 years.[9] CPS vaccines have also been licensed against other encapsulated bacteria including four serotypes of *Neisseria meningitidis* and *Salmonella typhi*.

The human immune response to polysaccharides is developmentally regulated and in comparison to proteins that can provoke immune response in the newborn, maturation of antibody production against polysaccharides does not usually occur before two years of age.[10,11] Immunocompromised adults and the elderly also react poorly to purified polysaccharides. A solution to this problem was discovered by Goebel and Avery who showed that nonimmunogenic saccharides (haptens) can be converted to immunogenic materials by their covalent attachment to proteins.[12] Although the details of the sequence through which the conjugates elicit saccharide-specific response are not well understood, it is generally accepted that conjugation converts the T-(thymus-derived) cell independent (TI) saccharides to thymus-dependent (TD) complexes.[13] As the first step in the cascade of the immunological events, the conjugate is recognized and internalized by the B (bone-marrow derived) cells that process and present the protein−saccharide complex to T-cell receptors. Subsequently, the antigen-specific recognition process leads to the production of hapten-specific immunoglobulins. The demonstration that T-cell epitope peptides can also mediate hapten-specific antibody response supports this view and adds a further dimension to research on glycoconjugates for use as human vaccines.[14] The principle of TI → TD conversion by conjugation to proteins was exploited to develop the first carbohydrate-based vaccine against *Haemophilus influenzae* type b (Hib) for use in infants,[15] and in countries where the currently available Hib vaccines[16] are routinely used, cases of childhood menin-

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gitis caused by this bacterium are virtually eliminated.[17] The conjugate technology was extended to the CPSs of pneumococci[18] and other encapsulated bacteria.[19]

Prominent among the outermost molecular components of virulent, nonencapsulated bacteria are lipooligosaccharides[2] and LPSs.[3] Fully developed LPSs confer serotype-specificity and may be protective antigens. It has been suggested that humoral antibodies directed at the O-specific polysaccharide (O-SP) components of LPSs are "important for protection".[20] It has, indeed, been demonstrated that protein conjugates of O-SPs of several human enteropathogenic bacteria are safe and immunogenic in humans, elicit high titers of serum antibodies that can be boosted by subsequent vaccinations, and may offer protection against the homologous bacteria.[21] Improvement of the conjugate vaccine technology depends on our understanding of the effect of the structural variables on immunogenicity. These include the saccharide size, the average number of saccharide chains per conjugate molecule, the distance between the saccharide and the protein, the site of attachment on the protein, and the role of the terminal *vs* the internal epitopes.[22] Available data indicate that for heteropolysaccharides the minimum length of an oligosaccharide to elicit polysaccharide-specific antibodies should correspond to two repeating units.[13,23] In rare instances, protein conjugates of saccharides larger than two repeating units could also be evaluated and were found to elicit increased antibody response specific to the native polysaccharide as compared to the smaller congeners.[24] An increase in the saccharide chain/protein ratio within a limited range caused similar effects. However, a comprehensive evaluation of the role of saccharide size and loading over an *extended* range of variables is still missing. A major hindrance to progress in this area is that chemically well-defined higher-membered saccharides have been difficult to obtain either by degradation of the native polysaccharides or through chemical synthesis.

Shigella dysenteriae type 1 is a Gram-negative human pathogen that causes endemic and epidemic dysentery worldwide. Despite its discovery a century ago, there is still no licensed vaccine against this bacterium[25] that is resistant to available antibiotics in several countries.[26] An experimental vaccine consisting of a protein conjugate of the O-SP[27] (**A**) of the organism elicited significant anti-

$$[3)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow2)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow3)\text{-}\alpha\text{-D-Glc}p\text{NAc}\text{-}(1\rightarrow3)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow]_n$$

(**A**)

O-SP antibody levels that may confer protection.[28] While enhancement of the immunogenicity of this vaccine is of great interest, it poses major challenges due to our ignorance of structure−activity relationships for glycoconjugate vaccines. We surmized that an improved vaccine might be constructed from chemically defined extended oligosaccharide fragments corresponding to the polysaccharide **A**.[29] In this paper we first describe total syntheses of tetra- to hexadecasaccharide fragments of the O-SP. We also report a scheme for the single-point attachment of the spacer-equipped oligosaccharides[30] to human serum albumin as the model carrier to provide neoglycoproteins of diverse carbohydrate−saccharide ratios for use in immunologic studies that will assess the influence of several submolecular parameters on immunogenicity.[31]

Results and Discussion

The repeating unit of the O-SP (**A**) of *Sh. dysenteriae* type 1 contains α-linked D-galactose, *N*-acetyl-D-glucosamine, and L-rhamnose residues. Our overall synthetic design called for the assembly of a complete repeating unit for use in sequential condensation reactions to afford oligomers of this unit. While the tetrasaccharide frame along the polysaccharide chain may be shifted to define three other chemical repeating units, we selected the frame shown by **A** as the sequence of the key synthetic intermediates for the following reasons. First, the anomeric stereoselectivity of α-rhamnosylation is usually higher compared to the formation of either the α-galactosyl or α-glucosaminyl linkages; thus the use of a glycosyl donor unit corresponding to sequence **A** should ensure a high degree of anomeric selectivity for the larger targets. Second, the reactivity of the HO-3 group of the Rha residue, which is the linkage position for that unit (and the acceptor site of the selected intermediate target), usually exceeds that of the HO-2 of the Gal residue that could be the west-end terminal unit for the alternative frame.

The key stages of the construction of the oligosaccharide−protein conjugates are summarized in Figure 1.

(16) Commercial conjugate vaccines against *Haemophilus influenzae* type b: ProHiBit (Connaught), HibTITER (Lederle-Praxis), PedvaxHIB (MSD), ActHIB (Merieux/Connaught).

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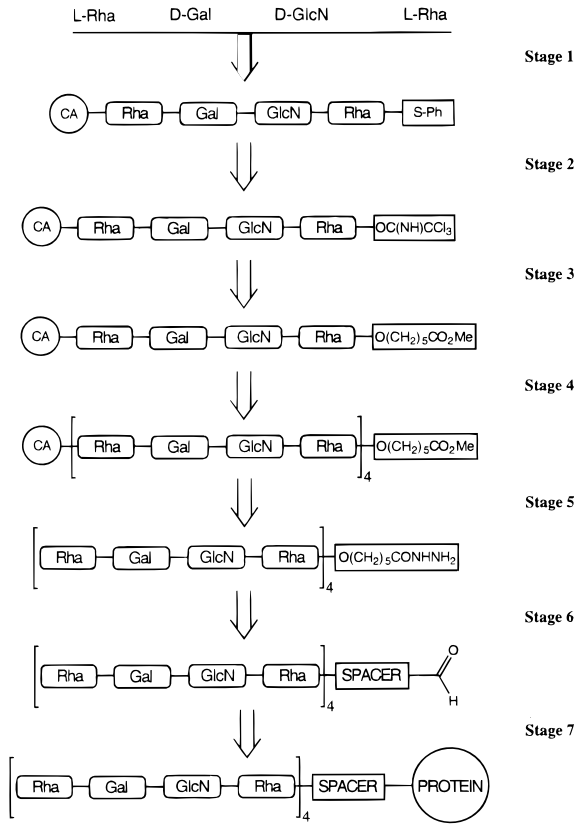

Figure 1. Key stages of the glycoconjugate synthesis.

First, a complete tetrasaccharide unit was assembled as a thioglycoside from monosaccharide precursors in a stepwise manner. Second, the phenylthio glycoside was converted to the reactive donor/acceptor intermediate. Third, this unit was condensed with the heterobifunctional aglycon. Fourth, three iterative selective deprotection and chain elongation cycles with the tetrasaccharide donor afforded the tetramer of the repeating unit. Fifth, global removal of the protecting groups by saponification and hydrogenolysis followed by hydrazinolysis afforded the tether-linked hexadecasaccharide hydrazide. Sixth, extension of the spacer moiety with a secondary spacer gave a saccharide-spacer construct featuring a terminal aldehydo group. Seventh, tethering of the aldehydo spacer-equipped saccharide to human serum albumin yielded the target neoglycoprotein.

The synthetic strategy described here follows a "classical" protecting group scenario whereby slight reactivity differences among the hydroxyl groups of *monosaccharide* precursors were exploited to prepare selectively functionalized/activated intermediates. We relied on Paulsen's principles[32] of the influence of type of protecting groups on donor/acceptor reactivities. We also made use of our experience gained in the synthesis of related oligosaccharides.[33] We focused on a blocking group scheme that (1) can be expected to promote the targeted anomeric stereoselectivity, (2) is straightforward to install, (3) is stable under glycosylation conditions, and (4) fulfills the requirements of efficient partial and complete deprotection under mild conditions. We also relied heavily on the advantages of thioglycosides as donors and acceptors.[34]

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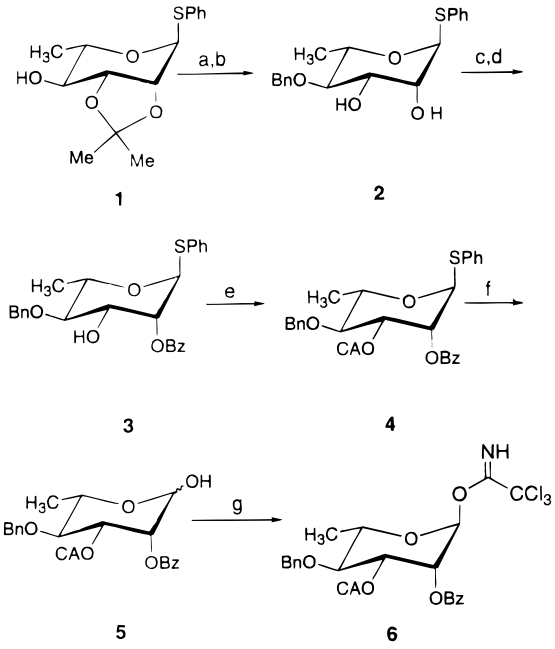
Scheme 1[a]



[a] Reagents and conditions: (a) 1.3 equiv of BnBr, NaH, DMF, 0 °C, 2 h; (b) AcOH−H_2O, 90 °C, 3 h, 89% for two steps; (c) 1.2 equiv of $(MeO)_3CPh$, 10-camphorsulfonic acid (cat.) CH_2Cl_2, 23 °C, 1 h; (d) AcOH−H_2O, 10 min, 92% for two steps; (e) 1.5 equiv of $(ClCH_2CO)_2O$, C_5H_5N, 0 °C, 15 min, 77%; (f) 1.2 equiv of $(CF_3CO_2)_2Hg$, CH_2Cl_2−H_2O, 2 h, 87%; (g) 1.1 equiv of CCl_3CN, 0.2 equiv of 1,8-diazabicyclo[5.4.0]undec-7-ene, CH_2Cl_2, 0 °C, 1 h, 98%.

Monosaccharide Synthons. Thiorhamnoside **3** was selected as the key intermediate for the Rha residues, in which the phenylthio functionality is recognized as a versatile protecting group that is stable under numerous reaction conditions. Additionally, it can be used to activate the anomeric carbon atom either directly or after conversion to other intermediates. Compound **3** features a permanent benzyl group at O-4 that is anticipated to increase the acceptor reactivity of HO-3 and a benzoyl group at O-2 that should promote the targeted anomeric selectivity by neighboring group participation without a propensity to migrate to the *cis*-oriented HO-3 group that might occur with an *O*-acetyl group in this system. The synthesis of **3** started with a two-step conversion of the isopropylidene derivative[33h] **1** involving treatment with benzyl bromide in the presence of sodium hydride followed by hydrolysis in aqueous acetic acid (→ **2**) (Scheme 1). Reaction of the diol **2** with trimethyl orthobenzoate in the presence of a catalytic amount of CSA gave an intermediate that was treated without isolation with aqueous acetic acid to afford the equatorial alcohol **3** in

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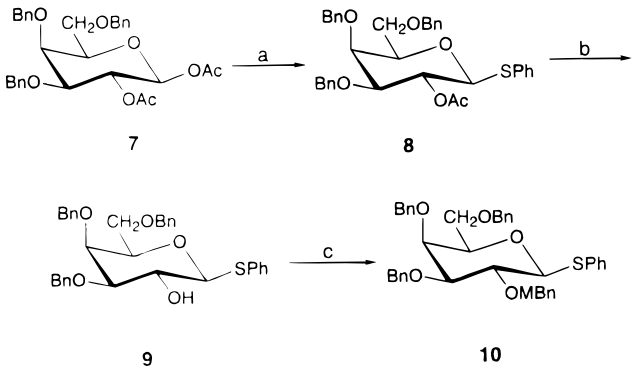
Scheme 2[a]



[a] Reagents and conditions: (a) 2.1 equiv of $PhSSiMe_3$, $BF_3{\cdot}Et_2O$ (cat.), CH_2Cl_2, 0 → 23 °C, 25 min, 87%; (b) NaOMe (cat.), MeOH, 23 °C, 24 h, 97%; (c) 1.3 equiv of MBnCl, NaH, DMF, 0 → 23 °C, 95%.

92% overall yield. Treatment of **3** with chloroacetic anhydride in pyridine readily gave **4** that was converted to the hemiacetal **5** by treatment[35] with the thiophilic reagent $(CF_3CO_2)_2Hg$. Next, compound **5** was treated with trichloroacetonitrile in the presence of DBU to afford the α-imidate **6** in 98% yield.[36]

The synthesis of the galactosyl donor **10** started from the diacetate[37] **7** that was treated[38] with $PhSSiMe_3$ to afford thiogalactoside **8** in 87% yield (Scheme 2). Acetate **8** was subjected to Zemplén deacetylation (→ **9**, 97%) followed by etherification with 4-methoxybenzyl chloride in the presence of sodium hydride to afford phenylthio galactoside **10** in 95% yield. Alternative galactosyl donor **16** was available from our earlier studies.[33d,e]

Compound **11** was prepared as described.[33g,39]

Tetrasaccharide Repeating Unit. Having readied the monosaccharide precursors, we turned to their assembly in the form of a protected tetrasaccharide repeating unit. Silver trifluoromethanesulfonate-mediated condensation of the chloride **11** and the rhamnose acceptor **3** afforded the disaccharide thioglycoside **12** in 63% yield (Scheme 3). This was converted to the acceptor **15** in the following sequence. First, chemoselective removal of the three *O*-acetyl groups by HBF_4 in MeOH provided the triol **13** (79% yield) that was sequentially treated with 4-methoxybenzaldehyde dimethyl acetal and monochloroacetic anhydride to afford the fully protected intermediate **14**. The acetal moiety was then replaced by acetyl groups in a two-step, one-pot conversion involving HBF_4-assisted methanolysis followed by acetylation with acetic anhydride and pyridine. Subsequently, the monochloroacetyl group was removed by treatment with thiourea[40] in DMF and pyridine to give the alcohol **15**. In preliminary experiments compound **15** displayed remarkable stability when treated with methyl trifluoromethanesulfonate (MeOTf) that readily activates methyl- and ethylthio glycosides.[41] This observation set the stage for the elongation of the phenylthio disaccharide **15** by MeOTf-catalyzed condensation with the methylthio galactoside **16** to afford the targeted intermediate trisaccharide that could, however, not be purified to homogeneity. At this juncture we decided to convert the azido group in this intermediate into a protected amino group instead of deferring the reduction to the final stages of the synthetic operations.[42] The azido group was reduced[43] using the Staudinger reaction to afford the free amine **17** (58% combined yield for two steps). The 2,2,2-trichloroethoxycarbonyl (TCEC) group was selected to temporarily protect the amino function, since this moiety is known to be stable under numerous reaction conditions including glycosylation.[44] Thus, **17** was reacted with 2,2,2-trichloroethoxychloroformate (TCEC-Cl), and then the intermediate so obtained was treated[45] with DDQ that removed the 4-methoxybenzyl group to afford the trisaccharide acceptor **18** in 82% yield (for two steps). Boron trifluoride etherate-mediated condensation of **18** with the rhamnose donor **6** gave the fully protected tetrasaccharide **19** in 89% yield. Preliminary experiments indicated that while **19** can be an efficient glycosyl donor with a reactive primary alcohol under activation by NIS/TfOH,[46,47] it is not suitable for the targeted chain elongation sequence. The corresponding glycosyl chloride obtained from **19** by chlorinolysis was also suboptimal for oligosaccharide building. This prompted us to convert the phenylthio glycoside **19** into the corresponding imidate **21** in a two-step sequence involving $(CF_3CO_2)_2Hg$-mediated hydrolysis of **19** to afford the hemiacetal **20** followed by reaction with CCl_3CN and DBU as reported above for compound **6**.

Construction of the Target Oligosaccharides. As the first step of oligosaccharide building, the tetrasaccharide donor **21** was condensed with the primary spacer[48] **22** under $BF_3{\cdot}Et_2O$ catalysis to afford **23** in 68% yield. Next, the monochloroacetyl group was removed by reaction with thiourea in DMF−C_5H_5N to give **24** in 96% yield. The acceptor so obtained was condensed with 1.5 equiv of the tetrasaccharide **21** to provide the octasaccharide **25** in 91% yield. Two more cycles involving the removal of the monochloroacetyl group and glycosylation with the donor **21** using $BF_3{\cdot}Et_2O$ as the activator yielded the dodeca- **27** and hexadecasaccharides **28**. We note, that although 2.6 equiv of the donor **21** were used for the final condensation, the isolated yield of the hexadecasaccharide **28** was only 48%, mirroring the decreased nucleophilicity of the acceptor moiety. The use

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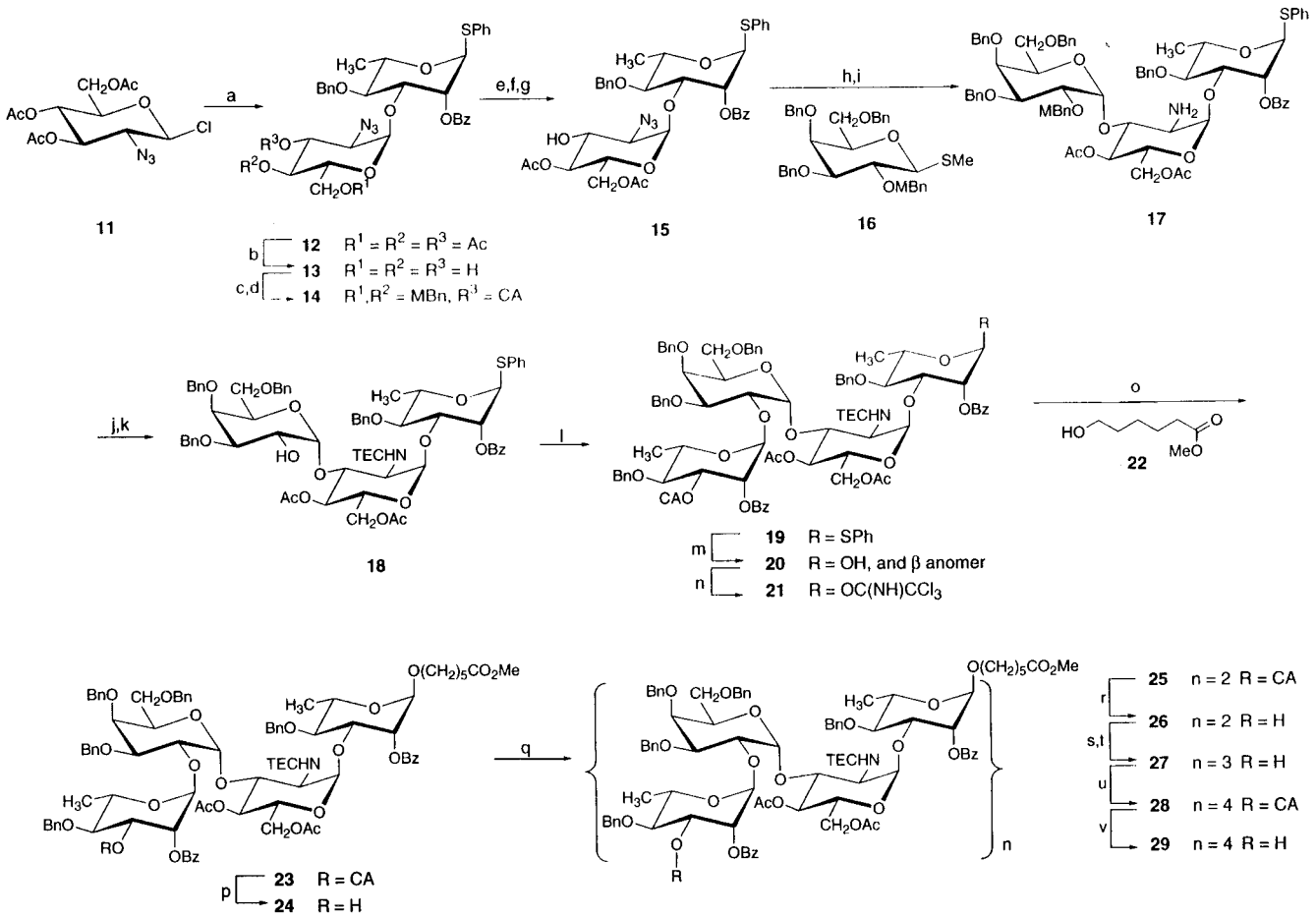
Scheme 3[a]



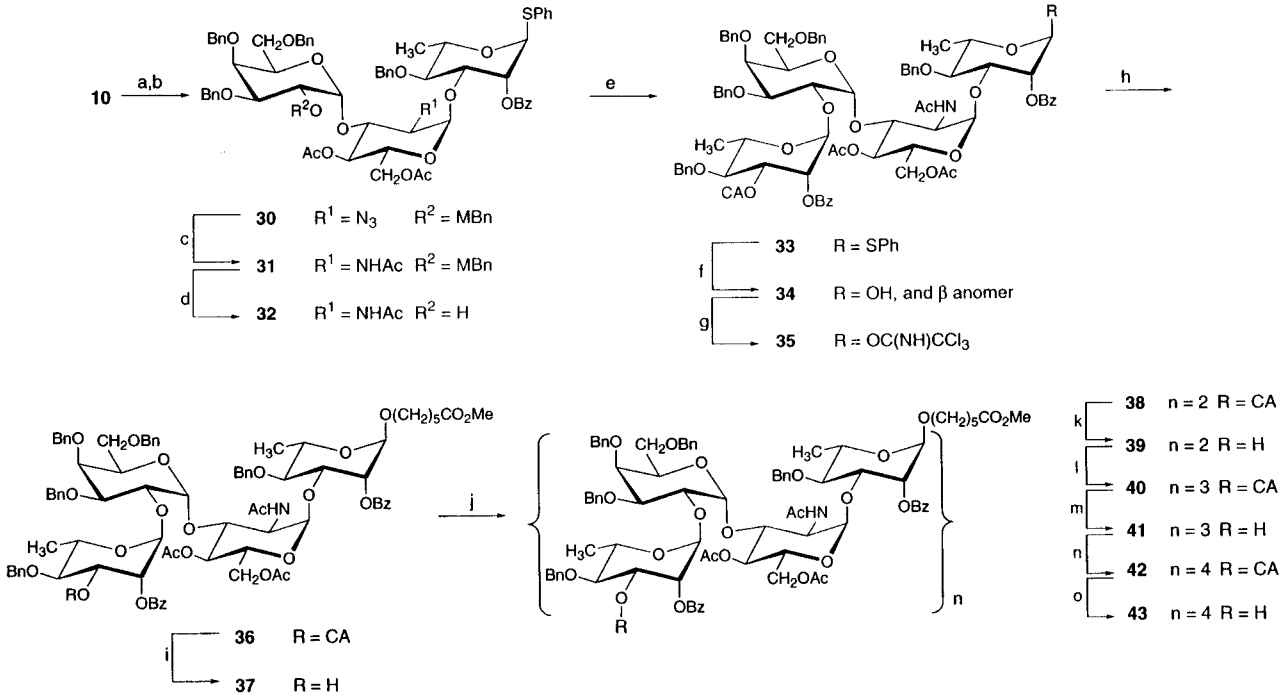
[a] Reagents and conditions: (a) 1.6 equiv of **3**, 1.5 equiv of CF_3SO_3Ag, 0.9 equiv of 2,6-di-*tert*-butyl-4-methylpyridine, CH_2Cl_2, 0 °C, 2 h, 64%; (b) $HBF_4{\cdot}Et_2O$, MeOH, 23 °C, 72 h, 79%; (c) 5.4 equiv of 4-methoxybenzaldehyde dimethyl acetal, 10-camphorsulfonic acid (cat.), CH_2Cl_2, 23 °C, 20 min; (d) 2.2 equiv of $(ClCH_2CO)_2O$, C_5H_5N, 0 °C, 10 min, 92% for two steps; (e) $HBF_4{\cdot}Et_2O$, MeOH−CH_2Cl_2, 0 → 23 °C, 30 min; (f) Ac_2O, C_5H_5N, 4-dimethylaminopyridine (cat.), 0 → 23 °C, 1 h; (g) 5.5 equiv of thiourea, DMF−C_5H_5N, 23 °C, 24 h, 71% for three steps; (h) 1.4 equiv of **16**, 1.7 equiv of CF_3SO_3Me, 2.9 equiv of 2,6-di-*tert*-butyl-4-methylpyridine, Et_2O, 23 °C, 66 h; (i) 2.2 equiv of PPh_3, CH_2Cl_2, 35−40 °C, 48 h, then H_2O, 35−40 °C, 24 h, 58% for two steps; (j) 5.8 equiv of $CCl_3CH_2OC(O)Cl$, $NaHCO_3$, acetone−H_2O, 0 °C, 3 min; (k) 2.0 equiv of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, CH_2Cl_2−H_2O, 23 °C, 3 h, 82% for two steps; (l) 3.5 equiv of **6**, 0.33 equiv of $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C, 2 h, 89%; (m) 2.0 equiv of $(CF_3CO_2)_2Hg$, CH_2Cl_2−H_2O, 30 min, 93%; (n) 9.7 equiv of CCl_3CN, 0.5 equiv of 1,8-diazabicyclo[5.4.0]undec-7-ene, CH_2Cl_2, 23 °C, 1 h, 85%; (o) 3.5 equiv of **22**, 1.4 equiv of $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C, 2 h, 68%; (p) 45 equiv of thiourea, DMF−C_5H_5N, 23 °C, 24 h, 96%; (q) 1.5 equiv of **21**, 3.1 equiv of $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C, 15 min, 91%; (r) 24 equiv of thiourea, DMF−C_5H_5N, 23 °C, 24 h, 82%; (s) 2 equiv of **21**, 4 equiv of $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C, 3 h; (t) 33 equiv of thiourea, DMF−C_5H_5N, 23 °C, 24 h, 62% for two steps; (u) 2.6 equiv of **21**, 10 equiv of $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C, 3 h, 48%; (v) 120 equiv of thiourea, DMF−C_5H_5N, 23 °C, 24 h, 82%.

of $CF_3SO_3SiMe_3$ instead of $BF_3{\cdot}Et_2O$ failed to improve the yield. The isolated yields of the pure oligosaccharide intermediates are generally lower than the actual yields of formation because of the losses experienced during the purification phase due to incomplete separation.

A Second Route to the Targets. In an attempt to improve the overall yields, we examined a slightly different approach to the target oligosacccharides using a tetrasaccharide donor that already carries the acetamido function (Scheme 4). Here, thiogalactoside **10** was treated with chlorine and the glycosyl chloride so obtained was allowed to react with the acceptor **15** under activation by silver trifluoromethanesulfonate to afford the trisaccharide **30** in 83% yield. Next, the azido group was converted to acetamido group in a one-pot, two-step reaction to afford **31** in 87% combined yield. Subsequently, the 4-methoxybenzyl group was removed by oxidation with ceric(IV) ammonium nitrate[49] to afford the trisaccharide acceptor **32** in 97% yield. As the final step of the tetrasaccharide assembly, condensation of the trisaccharide **32** with the rhamnosyl imidate **6** was carried out under $BF_3{\cdot}Et_2O$ activation to yield the α-linked tetrasaccharide **33** in 92% yield. The overall yield of the **15** → **33** sequence is 61%, which represents a nearly 50% improvement over the yield achieved in the closely related **15** → **19** sequence, which was 42%. Routine conversion of the tetrasaccharide thioglycoside to the corresponding imidate **35** was achieved via the intermediacy of the hemiacetal **34** as described above for the related compound **21**, in 67% overall yield. In reactions similar to those already presented, the spacer-linked tetra- (**36**), octa- (**38**), dodeca- (**40**), and hexadecasaccharides (**42**) were prepared by iterative condensation cycles. In summary on these glycosylations we note that the use of *catalytic* amounts of the promoter $CF_3SO_3SiMe_3$ proved to be superior over $BF_3{\cdot}Et_2O$, and

(49) Johannson, R.; Samuelsson, B. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2371.

Scheme 4[a]



[a] Reagents and conditions: (a) 1.7 equiv of **10**, 3.4 equiv of 2,6-di-*tert*-butyl-4-methylpyridine, Cl_2 (excess), CH_2Cl_2, 0 °C, 5 min, then hex-1-ene (excess); (b) **15**, 4.1 equiv of CF_3SO_3Ag, 1.3 equiv of 2,6-di-*tert*-butyl-4-methylpyridine, CH_2Cl_2, −78 °C, 2 h, 83%; (c) 4.8 equiv of PPh_3, CH_2Cl_2, 35−40 °C, 72 h, then H_2O, 35−40 °C, 48 h, then Ac_2O, 23 °C, 10 min, 87%; (d) 1.2 equiv of $(NH_4)_2Ce(NO_3)_6$, MeCN−H_2O, 23 °C, 20 min, 97%; (e) 4.0 equiv of **6**, 0.2 equiv of $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C, 2 h, 92%; (f) 1.1 equiv of $(CF_3CO_2)_2Hg$, CH_2Cl_2−H_2O, 40 min, 97%; (g) 9.0 equiv of CCl_3CN, 0.2 equiv of 1,8-diazabicyclo[5.4.0]undec-7-ene, CH_2Cl_2, 0 → 23 °C, 1 h, 69%; (h) 4.6 equiv of **22**, 0.04 equiv of $CF_3SO_3Si(CH_3)_3$, 0 °C, 2 h, 73%; (i) 15 equiv of thiourea, DMF−C_5H_5N, 23 °C, 24 h, 95%; (j) 2 equiv of **35**, 1.4 equiv of $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C, 2 h, 46%; (k) 25 equiv of thiourea, DMF−C_5H_5N, 23 °C, 24 h, 96%; (l) 4.5 equiv of **35**, 0.1 equiv of $CF_3SO_3Si(CH_3)_3$, 23 °C, 3 h, 72%; (m) 72 equiv of thiourea, DMF−C_5H_5N, 23 °C, 24 h, 89%; (n) 2.8 equiv of **35**, 0.15 equiv of $CF_3SO_3Si(CH_3)_3$, 23 °C, 5 h, 66%; (o) 130 equiv of thiourea, DMF−C_5H_5N, 23 °C, 26 h, 73%.

the overall yields in the condensations involving the acetamido group-containing donor **35** were generally higher than the corresponding reactions performed with *N*-TCEC-protected intermediates, establishing the utility of the acetamido approach that is further augmented in the deprotection phase of the syntheses.

Preparation of the Hydrazides 45, 47, 49, and 50. Tetrasaccharide **24** was first treated with Zn in AcOH to remove the TCEC moiety, followed by *N*-acetylation with Ac_2O.[44] Next the material so obtained was exposed to NaOMe in MeOH to remove the *O*-acetyl and the *O*-benzoyl groups. The de-acylated material was stirred under H_2 in the presence of palladium-on-charcoal to afford the free tetrasaccharide as the methyl ester **44** (Chart 1). The octa- (**26**) and dodecasaccharides (**27**) were similarly deprotected to provide **46** and **48**, respectively, except that Zn was substituted by Cd[50] for compound **27**. We note, that complete de-*O*-acylation of the larger saccharides (as followed by ^1H NMR spectroscopy) needed unexpectedly long reaction times (up to 1 week) that could not be shortened even with larger amounts of NaOMe. Treatment of methyl esters **44**, **46**, and **48** with hydrazine converted them to the corresponding hydrazides **45**, **47**, and **49**, which were purified by size-exclusion chromatography using Biogel P-2 as the adsorbent and 0.02 M C_5H_5N−AcOH buffer as the eluant. The deprotection of hexadecasaccharide **29** proceeded similarly to that of the dodecasaccharide **27**, except that the final purification of the hydrazide was performed through a Biogel P-4 column to afford **50**. The oligosaccharides obtained as shown in Scheme 4 were similarly processed except that the Zn (Cd) treatment was omitted. The structural identity and purity of all intermediates and final products were verified by extensive use of NMR and mass spectroscopic measurements and elemental analyses. Figure 2 shows the partial, ^1H NMR spectrum of the hexadecasaccharide−hydrazide **50** that demonstrates a high degree of purity. A partial assignment of the anomeric resonances was possible by comparison with di- to hexasaccharide fragments for which rigorous assignments have been published (refs 33d,e). The doublet at 5.59 ppm (J = 3.4 Hz) corresponds to the anomeric protons of the four Gal units. The three-proton doublets at 5.11 ppm (J = 1.6 Hz), 5.06 ppm (J = 1.6 Hz), and 5.03 ppm (J = 3.6 Hz) represent the interchain Rha, Rha, and GlcN residues, respectively. The one-proton doublets at 5.08 ppm (J = 1.6 Hz) and 4.80 ppm (J = 1.7 Hz) correspond to the Rha residues at the "reducing" and at the "nonreducing" end, respectively, while the doublet at 4.99 ppm (3.5 Hz) belongs to the GlcN residue next to the "reducing" end Rha unit. The chemical shifts and coupling constants of the signals representing three and four protons, respectively, coincide with the corresponding signals for the O-specific polysaccharide suggesting a high degree of conformational similarity of the inner regions of **50** to the polysaccharide.

Synthesis of the Secondary Spacer. Having accomplished the synthesis of the targeted oligosaccharides,

(50) Hancock, G.; Galpin, I. J.; Morgan, B. A. *Tetrahedron Lett.* **1982**, *23*, 249.

Chart 1

44 R = O-$(CH_2)_5$-CO_2Me

45 R = O-$(CH_2)_5$-$CONHNH_2$

46 R = O-$(CH_2)_5$-CO_2Me

47 R = O-$(CH_2)_5$-$CONHNH_2$

48 R = O-$(CH_2)_5$-CO_2Me

49 R = O-$(CH_2)_5$-$CONHNH_2$

50 R = O-$(CH_2)_5$-$CONHNH_2$

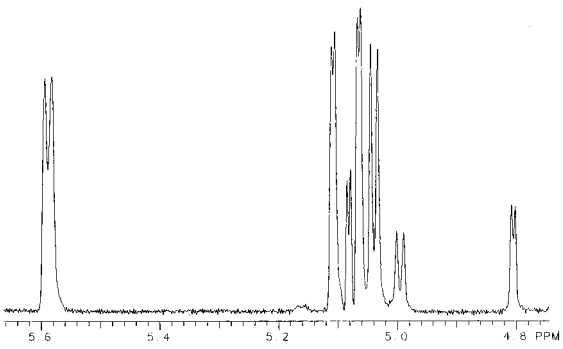


Figure 2. Partial ^1H NMR spectrum of compound **50** (300 MHz, D_2O, 320 K).

our attention turned to their attachment to proteins under mild conditions. The hydrazido spacer-equipped saccharides may be linked to proteins directly, e.g., by carbodiimide-mediated coupling to the carboxyl groups exposed on the surface of the proteins or through acyl-azide intermediates to amino groups.[51] While these approaches have often been used in many laboratories, they may cause untoward changes on the protein such as inter- and/or intramolecular condensation, *N*-acyl-urea formation, and protein nitrosylation that are difficult to avoid and characterize. The technique of reductive amination[52] (1) averts these problems and (2) was reported to be suitable for the preparation of neoglycoconjugates having very high saccharide-to-protein ratios.[53] Therefore, we designed a secondary spacer for the introduction of an aldehydo moiety in the saccharide-spacer construct.[54] Since condensation of the aldehyde with the ϵ-amino groups of the lysine residues of the protein followed by reduction converts the protein's primary amino groups into secondary ones, the protein's net charge remains virtually unchanged.[53] Simultaneously, the secondary spacer will increase the distance

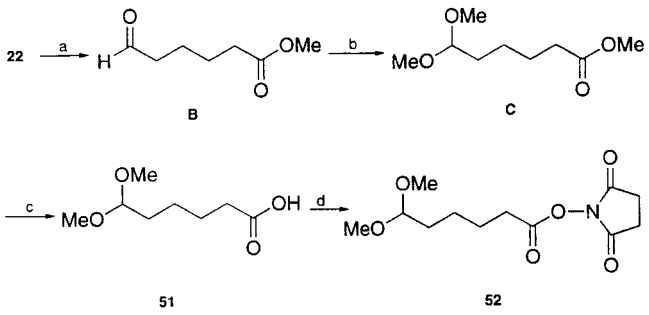
(51) For a recent review, see: Lee, R. T.; Lee, Y. C. *Glycosciences, Status and perspectives*; Gabius, H.-J., Gabius, S., Eds.; Chapman & Hall: 1997; p 56.

(52) Gray, G. R. *Arch. Biochem. Biophys.* **1974**, *163*, 426.

(53) Lee, R. T.; Wong, T.-C.; Lee, R.; Yue, L.; Lee, Y. C. *Biochemistry* **1989**, *28*, 1856.

(54) Other approaches to ω-aldehydoalkyl glycosides include (a) ozonolysis of *O*-allyl glycosides (Bernstein, M. E.; Hall, L. D. *Carbohydr. Res.* **1980**, *78*, C1). (b) Swern oxidation of ω-hydroxyalkyl glycosides (Pozsgay, V. *Glycoconj. J.* **1993**, *10*, 133). (c) use of ω-dioxolane-type aglycons (Palomimo, J. C. C.; Rensoli, M. H.; Verez-Bencomo, V. *J. Carbohydr. Chem.* **1996**, *15*, 137). (d) Use of a secondary spacer that features a protected aldehydo moiety (ref 53).

Scheme 5[a]



[a] Reagents and conditions: (a) 1.2 equiv of $(COCl)_2$, 2.3 equiv of Me_2SO, CH_2Cl_2, −50 °C, 1 h, *i*-Pr_2EtN; (b) $Me_2C(OMe)_2$, 4-toluenesulfonic acid (cat.), 23 °C, 30 min; (c) 1 N LiOH, MeOH−H_2O, 23 °C, 45 min, citric acid, 78% for 3 steps; (d) 1 equiv of *N*-hydroxysuccinimide, 1.1 equiv of 1,3-dicyclohexylcarbodiimide, EtOAc, 23 °C, 3 h, 90%.

between the saccharide and the spacer moieties, thus leaving all of the saccharide chain unshielded by the protein that may be important in the biological recognition processes.

The new heterobifunctional spacer **52** carries an activated carboxyl group for anchoring this moiety to the terminal amino group of the saccharide-spacer constructs and a masked aldehydo function for condensation with the protein's amino groups. Compound **52** was obtained from the alcohol **22** as shown in Scheme 5. Swern oxidation[55] of **22** followed by treatment of the aldehyde **B** with 2,2-dimethoxypropane in the presence of PTS gave an intermediate to which structure **C** was assigned without characterization. Treatment of this material with LiOH followed by citric acid gave the acid **51** in 78% overall yield.[56] Next, **51** was converted to the activated ester **52** by reaction with *N*-hydroxysuccinimide and *N*,*N*-dicylcohexylcarbodiimide.

Synthesis of the Glycoconjugates. The final stage of the glycoconjugate synthesis commenced with the condensation of the saccharide constructs with the secondary spacer. Thus, the hydrazido group-containing oligosaccharides were treated with an excess of the secondary spacer **52** in DMF to gave the acetal intermediates **D** that were purified by size-exclusion chromatography on a Biogel P4 column (Scheme 6). The carbohydrate-containing fractions[57] were combined and freeze-dried. Treatment of the residues so obtained in aqueous acetic acid at pH 2.65 for 6 h unmasked the aldehydo groups (→ **E**). The volatiles were removed by freeze-drying and the residues treated with a buffered solution of HSA and $NaCNBH_3$ for 4 days followed by removal of the low molecular weight components by filtration in a diafiltration apparatus through a 10 kDa molecular weight cutoff membrane. The conjugates were obtained as white amorphous substances after freeze-drying and were characterized by MALDI-TOF mass spectroscopy.[58] A typical MALDI-TOF mass spectrum of a hexadecasaccharide conjugate is shown in Figure 3. The molecular weight of the singly charged molecules having different incorporation levels is centered around 92 kDa, which is the *average* molecular weight. The well-resolved spectrum allows the observation of the individual structures also at the doubly charged ion cluster region centered around 46 kDa. From these data the average incorporation levels were calculated and are summarized in Table 1. Incorporation levels up to 23 saccharide chains per HSA molecule were achieved (out of the 58 lysine residues[60]) with incorporation yields being in the 15 to 45% range.

Conclusions

In summary, we have demonstrated the feasibility of synthetic routes to higher-membered oligosaccharides corresponding to the O-SP of *Sh. dysenteriae* type 1 in quantities that allow their detailed evaluation in animal experiments and human trials. Both approaches were based on iterative condensation of tetrasaccharide repeating units (**21** and **35**) that were equipped with a trichloroacetimidyl group at the anomeric carbon atom of the east-end residue and with a selectively removable monochloroacetyl group at the west-end moiety, the difference being that in **21** the amino group carried a TCEC protecting group throughout the synthetic sequence whereas in **35** the acetamido moiety of the final product was already accommodated. Consistently higher yields were achieved in the latter approach that thus became the method of choice for extension of this project toward the synthesis of vaccines under microbiologically controlled conditions for human experiments. We developed a novel conjugation method involving the use of the heterobifunctional spacer **52** that introduces an ω-aldehydoalkyl tail to the saccharide construct for attachment to proteins under the conditions of the reductive amination. Using the new spacer a number of neoglycoproteins were prepared whose saccharide to protein weight ratio varies from 0.05 to 0.71, with oligosaccharide chains in the range of 5−23 per molecule of the protein. The evaluation of the immunogenicity of the conjugates in mice is in progress and will be the subject of a forthcoming publication.[61]

Experimental Section

General Methods. All chemicals were commercial grade and used without purification. Solvents for chromatography were distilled prior to use. Anhydrous solvents were obtained from Aldrich. Human serum albumin (defatted) was purchased from Sigma and was purified by ultrafiltration through a YM10 Diaflow membrane in an Amicon ultrafiltration cell using five changes of water, followed by freeze-drying. Column chromatography was performed on silica gel 60 (0.040−0.063 mm). Melting points were taken on a Meltemp capillary melting point apparatus and are uncorrected. Optical rotations were measured at 23 °C. The ^1H and ^{13}C NMR spectra were recorded at 300 and 75.5 MHz, respectively. Internal references: TMS (0.000 ppm for ^1H for solutions in $CDCl_3$), acetone (2.225 ppm for ^1H and 31.00 ppm for ^{13}C for solutions

(55) Mancuso, A. J.; Huang, S. L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480.

(56) A related aldehydo-acid having one less carbon has been reported: Nakamura, Y.; Ito, A.; Shin, C. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 2151.

(57) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. *Anal. Chem.* **1956**, *28*, 350.

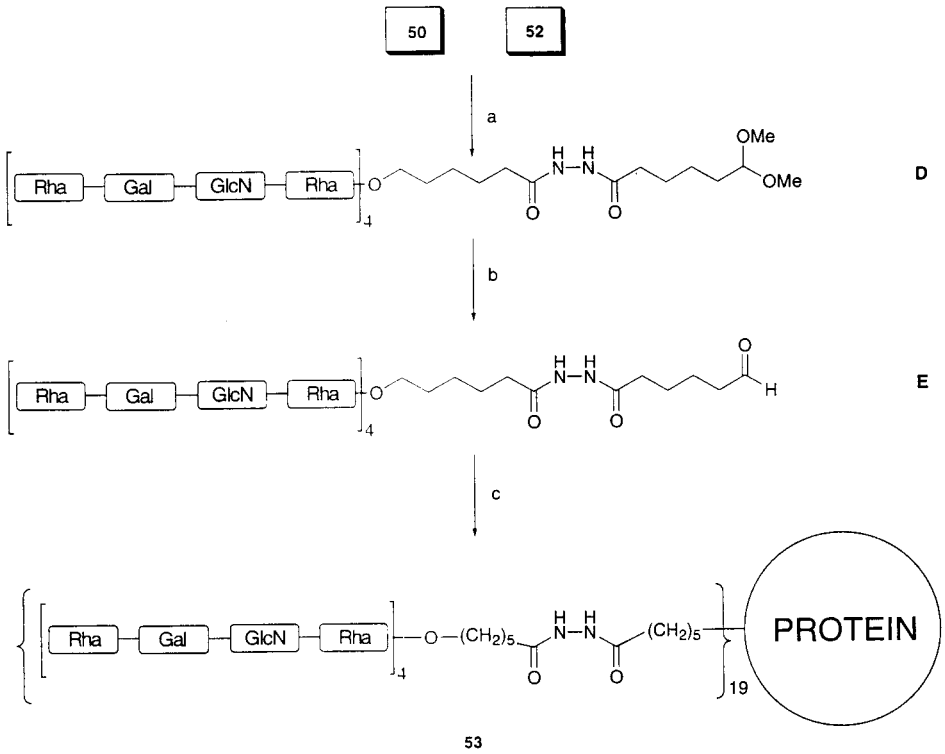
(58) Characterization of the saccharide−protein conjugates by MALDI-TOF mass spectrometry avoids the errors of the traditionally used colorimetric procedures for saccharide determination that may reach 50%: Dambra, A. J.; Baugher, J. E.; Concannon, P. E.; Pon, R. A.; Michon, F. *Anal. Biochem.* **1997**, *250*, 228.

(59) Pozsgay, V.; Dubois, E. P.; Pannell, L. *J. Org. Chem.* **1997**, *62*, 2832.

(60) (a) Carter, D. C.; Ho, J. X. *Adv. Protein Chem.* **1994**, *45*, 153. (b) Peters, D. T., Jr. *Adv. Clin. Chem.* **1970**, *13*, 37.

(61) Patent application has been filed: Pozsgay, V.; Robbins, J. B.; Schneerson, R. *Fed. Reg.* **1998**, *63*, 1117.

Scheme 6[a]



[a] Reagents and conditions: (a) 2.3 μmol of **50**, 17 equiv of **52**, DMF, 23 °C, 4 h; (b) AcOH−H_2O, 23 °C, 6 h; (c) 0.018 μmol of human serum albumin (= 1.05 μmol amino groups), pH 7.0 buffer, $NaCNBH_3$, 23 °C, 4 d.

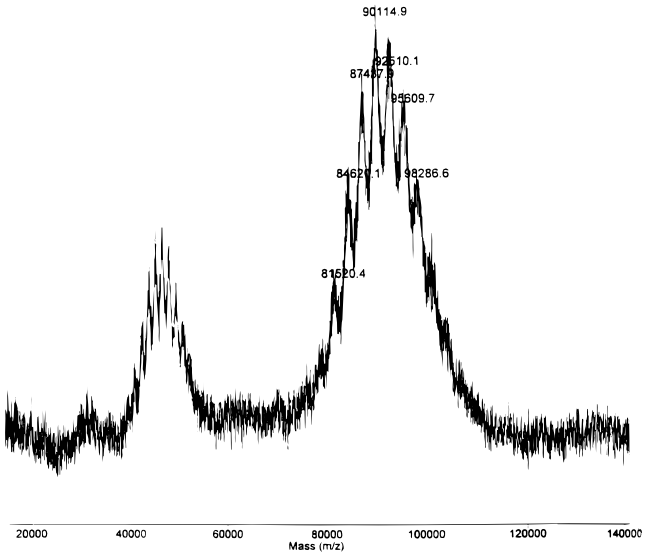


Figure 3. MALDI−TOF mass spectrum of a hexadecasaccharide−human serum albumin conjugate.

in D_2O), methanol (3.358 ppm for ^1H and 49.68 ppm for ^{13}C for solutions in D_2O), and $CDCl_3$ (77.00 ppm for ^{13}C for solutions in $CDCl_3$). Coupling constants are given in hertz. The mass spectra were recorded at the Laboratory of Analytical Chemistry, NIDDK, NIH, Bethesda, MD. Ammonia was used as the ionizing gas for the chemical ionization (CI) mass spectra. The fast atom bombardment (FAB) mass spectra were obtained using 6 keV Xe atoms to ionize samples from dithiothreitol/dithioerythritol, 3-nitrobenzyl alcohol, or glycerol as the matrix. For the MALDI-TOF mass spectra the sample was dissolved in 0.1% TFA in 50% aqueous acetonitrile and applied to the target in a sinapinic acid matrix. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

Table 1. Molecular Weight and Composition of Some of the Neoglycoproteins

		saccharide−protein ratio[b]	
conjugate	mol weight,[a] Da	g/g	mol/mol
tetraHSA-1[c,d]	70 700	0.05	5
tetraHSA-2	77 970	0.13	13
octaHSA-1	83 160	0.21	11
octaHSA-2	98 000	0.40	20
dodecaHSA-1	83 340	0.24	8
dodecaHSA-2	89 200	0.29	10
dodecaHSA-3	118 000	0.71	23
hexadecaHSA-1	78 000	0.16	4
hexadecaHSA-2	92 400	0.36	9
hexadecaHSA-3	120 000	0.71	19

[a] Determined by MALDI−TOF mass spectrometry as described in the Experimental Section. [b] The calculation is based on the molecular weight of HSA measured as 66 350 Da. [c] TetraHSA stands for the HSA conjugate of the tetrasaccharide, etc. [d] Prepared by the squaric acid method according to ref 59.

Abbreviations: Ac = acetyl, Bz = benzoyl, Bn = benzyl, CA = chloroacetyl, CI = chemical ionization, CSA = 10-camphorsulfonic acid, DBU = diazabicyclo[5.4.0]undec-7-ene, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DMF = *N*,*N*-dimethylformamide, FAB = fast atom bombardment, Gal = D-galactose, GlcN = 2-amino(azido)-2-deoxy-D-glucose, MALDI-TOF = matrix-assisted laser desorption ionization time-of-flight, MBn = 4-methoxybenzyl, MP = 4-methoxyphenyl, MS = mass spectra/spectroscopy, NIS = *N*-iodosuccinimide, Ph = phenyl, PTS = 4-toluenesulfonic acid, Rha = L-rhamnose, TCEC = 2,2,2-trichloroethoxycarbonyl, TfOH = trifluoromethanesulfonic acid.

Phenyl 4-*O*-Benzyl-1-thio-α-L-rhamnopyranoside (2). To a stirred solution of **1** (61 g, 206 mmol) in dry DMF (200 mL) was added at 0 °C NaH (12 g of a 60% suspension in oil) in portions. After 1 h, the mixture was treated with benzyl bromide (33 mL, 277 mmol). The mixture was stirred for 2 h and then treated sequentially with MeOH and H_2O. The crystalline precipitate was isolated by filtration and then

treated with 90% aqueous AcOH (300 mL) at 90 °C for 3 h. The solution was concentrated. Toluene was added to and evaporated from the residue thrice. Filtration of the resulting mixture followed by washing of the solids with hexane afforded **2** (63.5 g, 89% for two steps): mp 111−113 °C; $[\alpha]_D$ −201° (*c* 0.9, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.45 (d, 1 H, J = 1.5), 4.75, 4.70 (2 d, 1 H each, $J \sim 11$), 4.21 (dq, 1 H), 4.14 (ddd, 1 H), 3.93 (ddd, 1 H), 3.42 (t, 1 H, J = 9.3), 3.14 (d, 1 H, J = 4.0), 2.80 (d, 1 H, J = 5.5), 1.34 (d, 3 H, J = 6.2); ^{13}C δ 87.4, 81.7, 75.0, 72.5, 71.8, 68.6, 17.9; CI-MS *m/z* 364 [$(M + NH_4)^+$]. Anal. Calcd for $C_{19}H_{22}O_4S$: C, 65.87; H, 6.40. Found: C, 66.00; H, 6.42.

Phenyl 2-*O*-Benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (3). To a stirred solution of **2** (51.0 g, 147 mmol) in CH_2Cl_2 (100 mL) was added trimethyl orthobenzoate (30 mL, 175 mmol). The mixture was treated with a catalytic amount of CSA at 23 °C for 1 h, and then the solution was concentrated. To the residue was added 90% aqueous AcOH. After 10 min, the mixture was concentrated. Column chromatography of the residue (6:1 hexanes−EtOAc) afforded **3** (61.2 g, 92%) as a syrup: $[\alpha]_D$ +95° (*c* 0.8, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.60 (dd, 1 H, J = 3.4), 5.54 (d, 1 H, J = 1.7), 4.88, 4.75 (2 d, 2 H each, $J \sim 11$), 4.29 (dq, 1 H), 4.20 (ddd, 1 H), 3.56 (t, 1 H, J = 9.4), 1.40 (d, 3 H, J = 6.2); ^{13}C δ 85.8, 81.6, 75.1, 74.8, 70.9, 68.7, 18.0; CI-MS *m/z* 468 [$(M + NH_4)^+$]. Anal. Calcd for $C_{26}H_{26}O_5S$: C, 69.31; H, 5.82. Found: C, 69.05; H, 5.89.

Phenyl 2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-1-thio-α-L-rhamnopyranoside (4). To a solution of **3** (9.0 g, 20 mmol) in C_5H_5N (25 mL) at 0 °C was added chloroacetic anhydride (5.1 g, 29 mmol) under stirring. After 15 min, the solution was treated with MeOH (5 mL) and the solution was concentrated. Extractive workup (5% aqueous HCl/$CHCl_3$) followed by drying (Na_2SO_4) and crystallization from hexane−isopropyl ether afforded **4** (9.0 g, 77%): mp 67−68 °C; $[\alpha]_D$ −201° (*c* 0.9, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.78 (dd, 1 H, J = 3.2), 5.51 (d, 1 H, J = 1.8), 5.45 (dd, 1 H), 4.74, 4.69 (2 d, 1 H each, $J \sim 11$), 4.40 (dq, 1 H), 3.92, 3.84 (2 d, 1 H each, $J \sim 15$), 3.72 (t, 1 H, J = 9.6), 1.41 (d, 3 H, J 6.2); ^{13}C δ 166.5, 166.3, 85.6, 78.6, 75.3, 74.1, 71.9, 69.1, 17.9; CI-MS *m/z* 436 [$(M - C_7H_7 + H)^+$]. Anal. Calcd for $C_{28}H_{27}ClO_6S$: C, 63.81; H, 5.16. Found: C, 63.73; H, 5.14.

2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-1-thio-α,β-L-rhamnopyranose (5). To a stirred mixture of **4** (6.5 g, 12.4 mmol) in CH_2Cl_2 (80 mL) and H_2O (2 mL) at 0 °C was added $(CF_3CO_2)_2Hg$ (6.5 g, 15.2 mmol). After 2 h, the mixture was treated with aqueous 5% KI. Concentration of the organic phase gave a semisolid that was triturated with ether and hexane. The mixture was filtered, and the solids were discarded. Concentration of the mother liquor gave a syrupy residue which was purified by column chromatography (6:1 hexanes−EtOAc) to afford **5** (7.7 g, 87%) as a solid: $[\alpha]_D$ +77° (*c* 1.0, $CHCl_3$); NMR (major anomer, $CDCl_3$) ^{1}H δ 5.47−5.23 (m, 2 H), 5.08 (br s, 1 H), 4.70, 4.64 (2 d, 1 H each), 4.15 (dq, 1 H), 3.88, 3.81 (2 d, 1 H each, $J \sim 15$), 3.62 (t, 1 H), 1.39 (d, 3 H); ^{13}C δ 92.2, 78.5, 75.1, 73.6, 70.8, 67.8, 40.6, 18.1; CI-MS *m/z* 452 [$(M + NH_4)^+$]. Anal. Calcd for $C_{22}H_{23}ClO_7$: C, 60.76; H, 5.33. Found: C, 60.88; H, 5.38.

2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl Trichloroacetimidate (6). To a stirred solution of **5** (7.7 g, 17.7 mmol) and CCl_3CN (1.9 mL, 18.9 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added DBU (500 μL, 3.3 mmol). After 1 h, the solution was concentrated. Column chromatography (8:1 hexanes−EtOAc) of the residue afforded **6** (10.0 g, 98%) as a syrup: $[\alpha]_D$ +9° (*c* 1.2, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 6.32 (d, 1 H, J = 1.4), 5.72 (dd, 1 H), 5.52 (dd, 1 H, J = 3.1, J = 9.7), 4.74, 4.68 (2 d, 1 H each, $J \sim 11$ Hz), 4.13 (dq, 1 H), 3.94, 3.85 (2 d, 1 H each, $J \sim 15$), 3.73 (t, 1 H), 1.45 (d, 3 H); ^{13}C δ 94.8, 77.8, 75.5, 73.6, 70.7, 68.7, 40.6, 18.0. Anal. Calcd for $C_{24}H_{23}Cl_4NO_7$: C, 49.76; H, 4.00. Found: C, 49.64; H, 3.99.

Phenyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (8). To a stirred solution of 1,2-di-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranose (**7**) (7.5 g, 14 mmol) and (phenylthio)trimethylsilane (1.6 mL, 15.4 mmol) in dry CH_2Cl_2 at 0 °C was added $BF_3{\cdot}Et_2O$ (200 μL). The solution was allowed to reach 23 °C. After 25 min, the solution was recooled to 0 °C and then was treated with Et_3N (excess). Concentration followed by crystallization from MeOH afforded **8** (7.1 g, 87%): mp 110−111 °C; $[\alpha]_D$ +12° (*c* 0.5, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.43 (t, 1 H, J = 9.8), 4.94, 4.67, 4.57, 4.52, 4.47, 4.40 (6 d, 1 H each), 4.62 (d, 1 H, J = 10.0), 3.95 (br d, 1 H, J = 2.8), 3.68−3.61 (m, 3 H), 3.56 (dd, 1 H); ^{13}C δ 86.7, 81.4, 77.6, 74.3, 73.6, 72.8, 72.0, 69.7, 68.8; CI-MS *m/z* 602 [$(M + NH_4)^+$]. Anal. Calcd for $C_{35}H_{36}O_6S$: C, 71.89; H, 6.21. Found: C, 72.00; H, 6.26.

Phenyl 3,4,6-Tri-*O*-benzyl-1-thio-β-D-galactopyranoside (9). A solution of **8** (6.8 g) in CH_2Cl_2 (50 mL) was treated with a catalytic amount of NaOMe in MeOH at 23 °C for 24 h. The solution was treated with Dowex 50X2 (H^+) resin, filtered, and concentrated to afford **9** (6.1 g, 97%) as a crystalline material: mp 91−92 °C; $[\alpha]_D$ −10° (*c* 0.6, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 4.90, 4.66, 4.57, 4.50, 4.44, 4.42 (6 d, 1 H each), 4.54 (d, 1 H, J = 9.8), 4.00 (t, 1 H), 3.98 (dd, 1 H), 3.62−3.69 (m, 3 H), 3.48 (dd, 1 H, J = 9.7, J = 2.6 Hz); ^{13}C δ 88.5, 83.2, 77.5, 74.4, 73.6, 73.1, 72.4, 69.0, 68.7; CI-MS *m/z* 560 [$(M + NH_4)^+$]. Anal. Calcd for $C_{33}H_{34}O_5S$: C, 73.01; H, 6.31. Found: C, 72.97; H, 6.26.

Phenyl 3,4,6-Tri-*O*-benzyl-2-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside (10). To a stirred solution of **9** (6.0 g, 11.1 mmol) in DMF (25 mL) was added NaH (1.0 g of a 60% suspension in oil, ∼ 25 mmol) at 0 °C. After 30 min, the mixture was treated with 4-methoxybenzyl chloride (2 mL, 14.7 mmol). The stirred mixture was allowed to reach 23 °C. Extractive workup afforded **10** (7.0 g, 95%): mp 94−95 °C; $[\alpha]_D$ +9° (*c* 0.9, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 4.97, 4.72, 4.70, 4.66, 4.60, 4.41 (6 d, 1 H each), 4.73 (br s, 2 H), 4.62 (d, 1 H, J = 9.6 Hz), 3.97 (dd, 1 H), 3.92 (t, 1 H), 3.79 (s, 3 H), 3.65−3.56 (m, 4 H); ^{13}C δ 87.7, 84.2, 77.3, 75.3, 74.4, 73.6 (2 C), 73.4, 72.7, 68.8, 55.3; CI-MS *m/z* 680 [$(M + NH_4)^+$]. Anal. Calcd for $C_{41}H_{42}O_6S$: C, 74.29; H, 6.39. Found: C, 74.26; H, 6.45.

Phenyl (3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (12). A stirred solution of **3** (61.0 g, 135 mmol), **11** (29.0 g, 83 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (16.0 g, 77 mmol), and 4 Å molecular sieves (15 g) in CH_2Cl_2 (150 mL) was treated under argon with CF_3SO_3Ag (32.0 g, 125 mmol) at −20 °C. The mixture was allowed to reach 0 °C in 2 h before treatment with a saturated aqueous $NaHCO_3$ solution. The mixture was filtered, and the solids were washed thrice with $CHCl_3$. The organic phase was extracted with H_2O and concentrated. Column chromatography of the residue (4:1 hexanes−EtOAc) afforded **12** (40.2 g, 63.5%) as an amorphous solid: $[\alpha]_D$ +59° (*c* 1.2, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.88 (dd, 1 H), 5.57 (d, 1 H, J = 1.6), 5.44 (dd, 1 H, J = 10.5, J = 9.3), 5.36 (d, 1 H, J = 3.6), 5.00 (dd, 1 H, J = 9.6), 4.93, 4.81 (2 d, 1 H each), 4.37 (dq, 1 H), 3.72 (t, 1 H, J = 9.6), 3.31 (dd, 1 H), 2.10, 2.02, 1.89 (3 s, 3 H each), 1.45 (d, 3 H); ^{13}C δ 93.1, 86.1, 79.6, 75.9, 70.1, 69.3, 68.9, 68.0, 67.5, 61.6, 60.5, 20.7, 20.6, 20.5, 17.9; CI-MS *m/z* 781 [$(M + NH_4)^+$]. Anal. Calcd for $C_{38}H_{41}N_3O_{12}S$: C, 59.76; H, 5.41. Found: C, 59.64; H, 5.37.

Phenyl (2-Azido-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (13). A solution of **12** (50.0 g) in MeOH (200 mL) was treated with HBF_4 (∼54% in Et_2O, 15 mL) at 23 °C. After 3 days, the solution was concentrated to ∼50 mL under vacuum. The residue was treated with Et_3N at 0 °C; then most of the volatiles were removed under vacuum. Column chromatography of the residue (3:2 hexanes−EtOAc) afforded **13** (33 g, 79%) as a syrup: $[\alpha]_D$ −11° (*c* 0.6, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.84 (dd, 1 H, J = 3.1), 5.53 (d, 1 H, J = 1.6), 5.20 (d, 1 H, J = 3.8), 4.81, 4.62 (2 d, 1 H each, $J \sim 11$), 4.30 (dq, 1 H), 4.20 (dd, 1 H), 3.12 (dd, 1 H, J = 10.3), 1.32 (d, 3 H, J = 6.2); ^{13}C δ 93.7, 85.8, 79.5, 75.8, 73.2, 71.3 (2C), 69.9, 69.3, 69.0, 62.2, 61.0, 17.8; CI-MS *m/z* 721 [$(M + H)^+$], 632 [$(M + H - N_2)^+$], 542 [$(M + H - Me_3Si(CH_2)_2OH)^+$]. Anal. Calcd for $C_{32}H_{35}N_3O_9S$: C, 60.27; H, 5.53. Found: C, 59.61; H, 5.60.

Phenyl [2-Azido-3-*O*-chloroacetyl-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranosyl](1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (14). To a solution of the triol **13** (27.0 g, 42.4 mmol) and 4-methoxybenzaldehyde dimethylacetal (40 mL, 230 mmol) in CH_2Cl_2 (80 mL)

was added a catalytic amount of CSA at 23 °C. After 20 min, the reaction was quenched with Et_3N. The reaction mixture was stirred with hexane for 10 min (4 × 200 mL). The hexane layer was decanted, and the residue was dissolved in CH_2Cl_2 (200 mL). To this solution were added at 0 °C C_5H_5N (10 mL) and chloroacetic anhydride (16 g, 94 mmol). After 10 min, the solution was sequentially treated with MeOH and Et_3N (excess). Concentration followed by column chromatographic purification (3:1 hexanes−EtOAc) of the residue gave **14** (32 g, 92%) as an amorphous solid: $[\alpha]_D$ +29° (*c* 1.0, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.90 (dd, 1 H, J = 3.2, J = 1.6), 5.63 (t, 1 H, J = 10.0), 5.56 (d, 1 H, J = 1.6 Hz), 5.43 (s, 1 H), *5.38* (d, 1 H, J = 3.6), 4.97, 4.70 (2 d, 1 H each, $J \sim 11$), 4.37 (dq, 1 H), 3.22 (dd, 1 H, J = 10.3), 1.30 (d, 3 H, J = 6.2); ^{13}C δ 101.6, 93.9, 85.9, 79.8, 78.7, 76.6, 73.0, 70.6, 69.3, 68.8, 68.4, 62.9, 61.0, 55.3, 40.5, 17.8; FAB-MS *m*/*z* 660 [(M + H)$^+$]. Anal. Calcd for $C_{42}H_{42}ClN_3O_{11}S$: C, 60.61; H, 5.09. Found: C, 60.09; H, 5.26.

Phenyl (4,6-Di-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (15). To a solution of **14** (32.0 g, 38.5 mmol) in a mixture of MeOH (200 mL) and CH_2Cl_2 (50 mL) was added at 0 °C HBF_4 (∼54% in Et_2O, 2 mL). The solution was allowed to reach 23 °C in 30 min. The mixture was recooled to 0 °C and treated with solid $NaHCO_3$ and ice until the pH of the mixture was ∼5 as indicated by pH paper. Approximately 100 mL of the volatiles was removed by distillation. The residue was diluted with CH_2Cl_2 (400 mL). The solution was extracted with H_2O (twice), dried, (Na_2SO_4), and concentrated. To the residue were added at 0 °C C_5H_5N (10 mL), Ac_2O (20 mL), and a catalytic amount of 4-(dimethylamino)pyridine. The solution was allowed to reach 23 °C and then was treated with MeOH (∼10 mL) followed by concentration. A solution of the residue in DMF (50 mL) and C_5H_5N (3 mL) was treated with thiourea (16 g, 210 mmol) at 23 °C. After 30 min, the mixture was concentrated at <30 °C. The residue was stirred with $CHCl_3$ (100 mL) and the mixture filtered. Concentration of the mother liquor followed by column chromatographic purification of the residue (4:1 hexanes−EtOAc) gave **15** (19.7 g, 71%) as an amorphous solid: $[\alpha]_D$ +29° (*c* 1.0, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.87 (dd, 1 H), 5.56 (d, 1 H, J = 1.6), 5.29 (d, 1 H, J = 3.9), 4.84 (dd, 1 H, J = 9.3), 4.83, 4.75 (2 d, 1 H each), 4.35 (dq, 1 H), 4.26 (dd, 1 H, J = 3.2, J = 9.5), 4.18−3.93 (m, 4 H), 3.69 (t, 1 H), 3.24 (dd, 1 H J = 10.2), 2.11, 1.99 (2s, 3 H each), 1.41 (d, 3 H, J = 6.2 Hz); ^{13}C δ 93.0, 86.0, 79.7, 75.5, 73.0, 70.8, 70.2, 69.2, 69.0, 67.5, 63.0, 61.7, 20.8, 20.7, 17.9; FAB-MS *m*/*z* 739 [(M + NH_4)$^+$]. Anal. Calcd for $C_{36}H_{39}N_3O_{11}S$: C, 59.91; H, 5.45. Found: C, 60.58; H, 5.51.

Phenyl [3,4,6-Tri-*O*-benzyl-2-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl]-(1→3)-(4,6-di-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (17). A mixture of **15** (19.2 g, 26.6 mmol), **16** (22.0 g, 36.6 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (16.0 g, 78 mmol), and 4 Å molecular sieves (∼20 g) in dry ether (200 mL) was stirred for 2 h at 23 °C followed by treatment with CF_3SO_3Me (2 mL). The mixture was stirred for 66 h during which additional amounts of CF_3SO_3Me (3 mL) were added in portions. The reaction was quenched with an aqueous $NaHCO_3$ solution. Extractive workup ($CHCl_3/H_2O$) followed by chromatographic purification (6:1 hexanes−EtOAc) afforded a fraction (∼30 g) that contained the expected trisaccharide; 12.5 g of this fraction was dissolved in CH_2Cl_2 (200 mL). To this solution was added PPh_3 (16.0 g). The solution was kept at 35−40 °C for 48 h and then treated with H_2O (3 mL). The mixture was stirred at 35−40 °C for 24 h followed by concentration and column chromatographic purification of residue (2:1 hexanes−EtOAc) to give **17** (7.9 g, ∼58% from **15**) as an amorphous solid: $[\alpha]_D$ +16° (*c* 0.3, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.83 (dd, 1 H), 5.55 (d, 1 H, J = 1.4), 5.13 (d, 1 H, J = 3.7), 5.06 (dd, 1 H, J = 9.1, J = 9.4), 4.88 (2H), 4.75, 4.70, 4.65, 4.58, 4.53, 4.48 (7 d, 8 H), 3.72 (s, 3 H), 2.93 (dd, 1 H, J = 9.5), 2.10, 1.74 (2 s, 3 H each), 1.44 (d, 3 H, J = 6.2); ^{13}C δ 99.2, 95.5, 86.1, 83.0, 79.8, 78.7, 76.3, 75.7, 75.3, 74.6, 73.3 (3 C), 72.8, 70.3, 69.8, 69.2, 68.9, 68.8, 67.7, 62.0, 55.5, 55.2, 20.8, 18.0; FAB-MS *m*/*z* 1248 [(M + H)$^+$]. Anal. Calcd for $C_{71}H_{77}NO_{17}S$: C, 68.31; H, 6.22. Found: C, 68.25; H, 6.20.

Phenyl (3,4,6-Tri-*O*-benzyl-α-D-galactopyranosyl)(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (18). A stirred solution of **17** (2.8 g, 6.25 mmol) in acetone (200 mL) was treated at 0 °C sequentially with a mixture of $NaHCO_3$ (5 g) in H_2O (30 mL) and 2,2,2-trichloroethyl chloroformate (5 mL, 36.3 mmol). After 3 min, the solution was concentrated and the residue was equilibrated between $CHCl_3$ and H_2O. The organic phase was treated with DDQ (2.8 g, 12.3 mmol). After 3 h, the solution was extracted with aqueous $NaHCO_3$ and processed as usual to afford, after column chromatographic purification (3:1 hexanes−EtOAc), **18** (6.66 g, 82%) as an amorphous solid: $[\alpha]_D$ +44° (*c* 0.8, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 6.05 (m, 1 H), 5.80 (dd, 1 H, J = 3.2), 5.46 (d, 1 H, J = 1.6), 5.30 (d, 1 H, J = 3.2), 5.11 (dd, 1 H, $J \sim 9.2$), 4.34 (dq, 1 H), 2.92 (dd, 1 H, J = 9.8 Hz), 2.11, 1.92 (2 s, 3 H each), 1.43 (d, 3 H, J = 6.1); ^{13}C δ 99.4, 95.8, 92.9, 86.2, 79.8, 79.5, 75.4, 74.2, 73.9, 73.5, 73.2, 73.1, 72.4, 70.6, 69.8, 69.4, 69.3, 68.9, 68.1, 61.6, 54.2, 20.8, 20.7, 17.9. Anal. Calcd for $C_{66}H_{70}Cl_3NO_{18}S$: C, 60.81; H, 5.41. Found: C, 60.23; H, 5.46.

Phenyl (2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (19). To a stirred mixture of **18** (6.4 g, 4.92 mmol), **6** (10.0 g, 17.3 mmol), 4 Å molecular sieves (5 g), and CH_2Cl_2 (80 mL) was added at 0 °C $BF_3{\cdot}Et_2O$ (200 μL). After 2 h, the reaction mixture was filtered and concentrated. Column chromatographic purification of the residue (5:1 hexanes−EtOAc) afforded **19** (7.6 g, 89%) as an amorphous solid: $[\alpha]_D$ +40° (*c* 0.5, $CHCl_3$); NMR ($CDCl_3$) ^{13}C δ 170.8, 168.9, 165.8, 165.2, 165.0, 153.6, 98.1, 97.4, 95.9, 92.2, 86.1, 79.8, 79.6, 78.5, 75.5, 74.9, 74.1, 73.7, 73.5, 73.3, 72.9, 72.5, 72.3, 71.6, 71.3, 71.0, 70.3, 70.2, 69.4, 69.3, 68.0, 67.9, 62.0, 53.8, 40.6, 20.8, 17.9; FAB-MS *m*/*z* 1742 [($^{12}C_{88}H_{91}{}^{35}Cl_3{}^{37}ClNO_{24}S$ + Na)$^+$]. Anal. Calcd for $C_{88}H_{91}Cl_4NO_{24}S{\cdot}H_2O$: C, 60.80; H, 5.39. Found: C, 60.29; H, 5.26.

(2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranose (20). To a stirred mixture of **19** (400 mg, 0.23 mmol) in CH_2Cl_2 (10 mL) and H_2O (0.5 mL) at 0 °C was added $(CF_3CO_2)_2Hg$ (200 mg, 0.47 mmol). After 30 min, the mixture was processed as described for **5**. Column chromatographic purification (3:1 hexanes−EtOAc) of the crude product afforded **20** (353 mg, 93%) as a solid: $[\alpha]_D$ +85° (*c* 0.5, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.94 (d, 1 H, $J \sim 10$ H), 5.62, 5.58 (2 dd, 1 H each), 5.47 (dd, 1 H, J = 3.2, J = 9.6), 5.42 (br s, 1 H), 5.28, 5.13 (2 br d, 1 H each), 5.24 (t, 1 H), 2.06, 1.82 (2 s, 3 H each), 1.41, 1.27 (2 d, 3 H each, $J \sim 6.3$); FAB-MS *m*/*z* 1650 [($^{12}C_{82}H_{87}{}^{35}Cl_3{}^{37}ClNO_{25}$ + Na)$^+$]. Anal. Calcd for $C_{82}H_{87}Cl_4NO_{25}{\cdot}H_2O$: C, 59.82; H, 5.45. Found: C, 59.44; H, 5.34.

(2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl Trichloroacetimidate (21). To a stirred solution of **20** (10.6 g, 6.41 mmol) in CH_2Cl_2 (100 mL) and CCl_3CN (6.0 mL, 62 mmol) at 0 °C was added DBU (500 μL, 3.3 mmol) at 23 °C. After 1 h, the solution was processed as described for **6**. Column chromatographic purification (4:1 hexanes−EtOAc) of the residue afforded **21** (9.6 g, 85%) as an amorphous substance: $[\alpha]_D$ +58° (*c* 0.5, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 6.26 (d, 1 H, J = 2.0), 5.99 (d, 1 H, $J \sim 9.9$ Hz), 5.74, 5.63 (2 dd, 1 H each), 5.50 (dd, 1 H, J = 3.3, $J_{3,4}$ = 9.7), 5.43 (d, 1 H, J = 1.5), 5.32 (t, 1 H, $J \sim 9$), 5.29, 5.14 (2 d, 1 H each, $J \sim 3.5$ Hz), 2.06, 1.82 (2 s, 3 H each), 1.41, 1.27 (2 d, 3 H each, $J \sim 6.3$). Anal. Calcd for $C_{84}H_{87}Cl_7NO_{25}$: C, 57.37; H, 4.99. Found: C, 57.12; H, 4.99.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (23). To a stirred mixture of the imidate **21** (2.0 g, 1.13 mmol), alcohol **22** (530 mg, 3.95 mmol), 4 Å molecular sieves (3 g), and CH_2Cl_2 (20 mL) was added at 0 °C $BF_3{\cdot}Et_2O$ (200 μL). After 2 h, the mixture was treated with Et_3N (excess) and filtered. The filtrate was extracted with H_2O, dried, and concentrated. Column chromatographic purification (3:1 hexanes−EtOAc) of the residue afforded **23** (1.52 g, 68%) as a crystalline solid: mp 109−111 °C $[\alpha]_D$ +69° (*c* 0.3, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.90 (d, 1 H, *J* ∼ 9), 5.62 (dd, 1 H, *J* = 1.8, *J* = 3.3), 5.52 (dd, 1 H), 5.48 (dd, 1 H, *J* = 9.6), 5.41 (d, 1 H), 5.29 (t, 1 H, *J* ∼ 9), 5.23, 5.12 (2 d, 1 H each, *J* ∼ 3.5 Hz), 4.99, 4.75, 4.70, 4.62, 4.55, 4.49 (6 d, 1 H each), 4.77 (br s, 1 H), 2.32 (t, 2 H), 2.06, 1.81 (2 s, 3 H each), 1.36, 1.26 (2 d, 3 H each); ^{13}C, δ 174.0, 170.7, 168.7, 165.7, 165.2 (2 C), 153.6, 98.1, 97.5, 97.4, 95.8, 92.5, 79.8, 79.6, 78.5, 75.4, 74.8, 74.0, 73.7, 73.5, 73.2, 72.9, 72.4, 72.2, 71.9, 71.4, 70.7, 70.3, 69.9, 69.3, 68.0, 67.9, 67.8, 67.6, 61.6, 54.0, 51.5, 40.5, 33.8, 28.9, 25.5, 24.5, 20.7, 20.6, 17.9, 17.8; FAB-MS *m*/*z* 1702 $[(^{12}C_{87}H_{98}{}^{35}Cl_2{}^{37}ClNO_{26} + Na)^+]$. Anal. Calcd for $C_{89}H_{99}Cl_4NO_{27}$: C, 60.86; H, 5.68. Found: C, 60.76; H, 5.65.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (24). A solution of **23** (1.52 g) in a mixture of DMF (10 mL) and C_5H_5N (1 mL) was treated with thiourea (3 g) at 23 °C. After 24 h, the mixture was processed as descibed for **15**. Column chromatographic purification of the residue (3:1 hexanes−EtOAc) gave **24** (1.39 g, 96%) as an amorphous solid: $[\alpha]_D$ +55° (*c* 0.3, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.89 (d, 1 H, *J* ∼ 9 Hz), 3.66 (s, 3 H), 2.31 (t, 2 H), 2.05, 1.82 (2 s, 3 H each), 1.36, 1.29 (2 d, 3 H each); ^{13}C δ 97.94, 97.86, 97.6, 95.8, 92.5, 81.2, 79.8 (2 C), 75.5, 75.1, 74.1, 73.7, 73.3, 73.0, 72.9, 72.6, 72.1, 71.8, 70.9, 70.3, 70.1, 70.0, 69.4, 68.1, 67.9 (2C), 67.7, 61.6, 54.0, 51.5, 33.9, 29.0, 25.6, 24.6, 20.9, 20.8, 18.1, 18.0. Anal. Calcd for $C_{87}H_{98}Cl_3NO_{26}$: C, 62.20; H, 5.88. Found: C, 62.33; H, 5.96.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (25). To a stirred mixture of the imidate **21** (1.58 g, 0.90 mmol), alcohol **24** (1.04 mg, 0.62 mmol), 4 Å molecular sieves (3 g), and CH_2Cl_2 (15 mL) was added at 0 °C $BF_3{\cdot}Et_2O$ (250 μL). After 30 min, the mixture was processed as described for **23**. Column chromatographic purification (3:1 hexanes−EtOAc) of the residue afforded **25** (1.87 g, 91%) as an amorphous solid: $[\alpha]_D$ +79° (*c* 0.2, $CHCl_3$); NMR ($CDCl_3$) ^{13}C δ 174.0, 170.8, 170.5, 168.8, 168.7, 165.6, 165.4, 165.2 (2 C), 164.6, 153.7, 153.6, 99.4, 98.3, 97.6, 97.5 (3 C), 92.7, 92.2, 96.0, 95.9, 75.5, 75.4, 74.8, 74.1, 74.0, 73.9, 73.7, 73.3, 72.9, 72.5, 69.6, 67.9, 61.3, 61.6, 53.9, 53.0, 51.5, 40.6, 33.9, 29.0, 25.6, 24.6, 20.8, 20.7 (2 C), 20.4, 18.0 (3 C), 17.8; FAB-MS *m*/*z* 3313 $[(C_{169}H_{183}Cl_7N_2O_{50} + Na)^+]$. Anal. Calcd for $C_{169}H_{183}Cl_7N_2O_{50}$: C, 61.69; H, 5.61. Found: C, 61.30; H, 5.63.

5-(Methoxycarbonyl)pentyl(2-*O*-Benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (26). A solution of **25** (1.80 g, 0.55 mmol) in a mixture of DMF (10 mL) and C_5H_5N (1 mL) was treated with thiourea (4 g) at 23 °C. After 24 h the mixture was processed as descibed for **15**. Column chromatographic purification of the residue (3:1 hexanes−EtOAc) gave **26** (1.45 g, 82%) as an amorphous solid: $[\alpha]_D$ +67° (*c* 0.6, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.99, 5.90 (2 d, 2 H, *J* ∼ 9), 3.65 (s, 3 H), 2.29 (t, 2 H), 1.94, 1.78, 1.73, 1.70 (4 s, 3 H each), 1.36, 1.26 (2 d, 6 H each); ^{13}C δ 174.0, 170.6, 170.4, 169.0, 168.8, 165.9, 165.3, 165.2, 164.6, 153.7, 153.6, 99.4, 97.9, 97.7, 97.6, 97.3 (2C), 96.0, 95.8, 92.5, 92.2, 75.5, 75.4, 75.0, 74.6, 74.0, 73.9, 73.8, 73.7, 73.2, 73.1, 72.8, 72.6, 70.4, 68.8, 61.3, 61.1, 53.86, 53.78, 51.4, 33.8, 28.9, 25.5, 24.6, 20.81 (2 C), 20.8, 20.4, 17.9 (4 C); FAB-MS *m*/*z* 3236 $[(C_{167}H_{182}Cl_6N_2O_{49} + Na)^+]$. Anal. Calcd for $C_{167}H_{182}Cl_6N_2O_{49}$: C, 62.41; H, 5.71. Found: C, 62.52; H, 5.75.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (27). To a stirred mixture of the imidate **21** (1.8 g, 1.02 mmol), compound **26** (1.57 g, 0.49 mmol), 4 Å molecular sieves (2 g), and CH_2Cl_2 (15 mL) was added at 0 °C $BF_3{\cdot}Et_2O$ (250 μL). After 3 h, the mixture was treated with Et_3N (excess) and filtered. The filtrate was extracted with H_2O, dried, and concentrated. Column chromatographic purification (2:1 hexanes−EtOAc) of the residue afforded an amorphous substance (∼2.1 g) that contained the expected product. A solution of this substance in DMF (10 mL) and C_5H_5N (1 mL) was treated with thiourea (1.2 g) as described for **15**. Extractive and chromatographic workup (3:2 hexanes−EtOAc) afforded **27** (1.47 g, 62% for two steps) as an amorphous solid: $[\alpha]_D$ +76° (*c* 0.4, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.98, 5.88, 5.87 (3 d, 1 H each, *J* ∼ 9), 3.65 (s, 3 H), 2.30 (t, 2 H), 1.94, 1.76, 1.72, 1.69, 1.64, 1.60 (6 s, 3 H each) 1.35, 1.27−1.22; ^{13}C δ 174.0, 170.6, 170.4 (2 C), 169.0, 168.8 (2 C), 165.9, 165.3 (2 C), 165.2, 164.6 (2 C), 153.7, 153.6 (2 C), 99.4, 99.3, 97.9, 97.8, 97.6, 97.3 (3 C), 97.2, 96.0, 95.9, 95.8, 92.6, 92.3, 92.2, 61.3, 61.1, 60.9, 53.8 (3 C), 51.4, 33.8, 28.9, 25.5, 24.6, 20.8−20.4, 18.0 (6 C); FAB-MS *m*/*z* 4770 $[(C_{247}H_{266}Cl_9N_3O_{72} + Na)^+]$. Anal. Calcd for $C_{247}H_{266}Cl_9N_3O_{73}$: C, 62.28; H, 5.63. Found: C, 62.36; H, 5.66.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (28). To a stirred mixture of the imidate **21** (900 mg, 0.51 mmol), compound **27** (645 mg, 0.135 mmol), 4 Å molecular sieves (2 g), and CH_2Cl_2 (10 mL) was added at 0 °C $BF_3{\cdot}Et_2O$ (170 μL). After 2 h, the mixture was treated with Et_3N (excess) and filtered. The filtrate was concentrated. Column chromatography (2:1 hexanes−EtOAc) of the residue afforded an

inhomogeneous material (825 mg) which was rechromatographed to afford **28** (410 mg, 48%): $[\alpha]_D$ +78° (*c* 0.8, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.89, 5.78 (2 H), 5.69 (3 d, 4 H, $J \sim 9$), 3.67 (s, 3 H), 2.28 (t, 2 H, $J \sim 7.5$), 1.90, 1.82, 1.70 (6 H), 1.63, 1.60, 1.55, 1.50 (7 s, 24 H). Anal. Calcd for $C_{329}H_{351}Cl_{13}N_4O_{96}$: C, 62.15; H, 5.56. Found: C, 61.92; H, 5.60. Subsequent elution afforded a mixture (270 mg) that contained **28** and an unidentified compound in a ~1:1 ratio.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (29). A solution of **28** (360 mg, 56.6 mmol) in DMF (5 mL) and C_5H_5N (500 μL) was treated with thiourea (500 mg) as described for **15**. Extractive and chromatographic workup (1:1 hexanes−EtOAc) afforded **29** (290 mg, 82%) as an amorphous solid: $[\alpha]_D$ +77° (*c* 0.4, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.98, 5.88, 5.86 (2H) (3 d, 4 H, $J \sim 9$), 3.65 (s, 3 H), 2.30 (t, 2 H, $J \sim 7.4$), 1.95, 1.76, 1.72, 1.70, 1.64, 1.62, 1.59, 1.58 (8 s, 3 H each); FAB-MS *m*/*z* 6414 [(M + Cs)$^+$]. Anal. Calcd for $C_{327}H_{350}Cl_{12}N_4O_{95}$: C, 62.52; H, 5.62. Found: C, 62.25; H, 5.64.

Phenyl [3,4,6-Tri-*O*-benzyl-2-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl]-(1→3)-(4,6-di-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (30). To a solution of **10** (3.31 g, 5.0 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (2.2 g, 10.7 mmol) in CH_2Cl_2 (20 mL) was added at 0 °C an excess of Cl_2 in CCl_4. After 5 min, hex-1-ene was added until the yellow color of Cl_2 disappeared. This solution was added by a syringe to a stirred mixture of **15** (2.20 g, 3.05 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (1.0 g, 4.0 mmol), 4 Å molecular sieves (~5 g), and CH_2Cl_2 (20 mL). The mixture was cooled to −78 °C and was treated with CF_3SO_3Ag (3.2 g, 12.4 mmol). After 2 h, the mixture was processed as described for **12** to afford **30** (3.22 g, 83%) as an amorphous solid: $[\alpha]_D$ +22° (*c* 0.4, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.92 (dd, 1 H, $J = 3.1$, $J = 1.7$), 5.57 (d, 1 H, $J = 1.6$), 5.38, 5.01 (2 d, 1 H each, $J \sim 3.7$), 5.14 (dd, 1 H, $J = 9.1$, $J = 9.4$), 3.74 (s, 3 H), 3.69 (t, 1 H, $J \sim 9.5$), 3.57 (t, 1 H, $J \sim 8.6$), 2.12, 1.74 (2 s, 3 H each), 1.43 (d, 3 H, $J = 6.2$); ^{13}C δ 113.6, 106.3, 98.8, 93.0, 86.1, 79.5, 79.0, 75.6, 74.9, 74.8, 74.7, 74.5, 73.3, 73.2, 72.8, 72.7, 69.9 (2 C), 69.2, 68.9, 68.4, 67.5, 62.1, 61.9, 55.2, 20.8, 20.7, 17.9; FAB-MS *m*/*z* 1406 [(M + Cs)$^+$]. Anal. Calcd for $C_{71}H_{75}N_3O_{17}S$: C, 66.91; H, 5.93. Found: C, 66.76; H, 5.94.

Phenyl [3,4,6-Tri-*O*-benzyl-2-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl]-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (31). To a solution of **30** (33.0 g, 25.9 mmol) in CH_2Cl_2 (270 mL) was added PPh_3 (33.0 g, 126 mmol). The solution was kept at 35−40 °C for 72 h and then treated with H_2O (10 mL). The mixture was stirred at 35−40 °C for 48 h. The organic layer was extracted with H_2O, dried (Na_2SO_4), and treated with Ac_2O (8 mL) at 23 °C for 10 min. The solution was concentrated. Column chromatographic purification of the residue (2:1 hexanes−EtOAc) gave **31** (29.0 g, 87%) as a crystalline solid: mp 92−94 °C; $[\alpha]_D$ +22° (*c* 0.4, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.81 (d, 1 H, $J \sim 9.5$), 5.76 (dd, 1 H), 5.48 (d, 1 H, $J = 1.7$), 5.17 (dd, 1 H, $J = 10.2$, $J = 9.1$), 5.14, 4.99 (2 d, 1 H each, $J \sim 3.5$ Hz), 4.34 (dq, 1 H), 4.24 (dd, 1 H), 3.75 (s, 3 H), 3.60 (t, 1 H, $J = 9.8$ Hz), 2.10, 1.71, 1.48 (3 s, 3 H each), 1.44 (d, 3 H, $J = 6.3$); ^{13}C δ 170.7, 169.8, 168.9, 165.1, 99.0, 93.8, 86.1, 79.9, 79.0, 75.4, 75.3, 75.2, 75.0, 74.5, 73.3, 73.2, 73.1, 70.4, 70.1, 69.8, 69.4, 69.2, 68.0, 61.9, 55.2, 51.7, 22.6, 20.8, 20.7, 18.0; FAB-MS *m*/*z* 1289 [(M + H − H_2)$^+$]. Anal. Calcd for $C_{73}H_{79}NO_{18}S$: C, 67.94; H, 6.17. Found: C, 68.01; H, 6.20.

Phenyl [3,4,6-Tri-*O*-benzyl-α-D-galactopyranosyl]-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (32). To a solution of **31** (22 g, 17.1 mmol) in MeCN (150 mL) and H_2O (10 mL) was added $(NH_4)_2Ce(NO_3)_6$ (11.0 g, 20 mmol) at 23 °C. After 20 min, the mixture was treated with aqueous $NaHSO_3$. Extractive workup followed by column chromatographic purification (1:1 hexanes−EtOAc) of the residue afforded **32** (19.4 g, 97%) as a syrup: $[\alpha]_D$ +45° (*c* 0.2, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.82 (d, 1 H, $J \sim 9$ Hz), 5.75 (dd, 1 H, $J = 3.1$), 5.48 (d, 1 H, $J = 1.5$), 5.15, 4.98 (2 d, 1 H each, $J \sim 3.4$), 5.09 (dd, 1 H, $J = 9.4$, $J = 10.3$), 2.09, 1.91, 1.57 (3 s, 3 H each), 1.47 (d, 3 H, $J \sim 6.3$); ^{13}C δ 170.7, 170.4, 170.0, 165.1, 100.6, 93.6, 86.1, 79.8, 79.5, 75.7, 75.3, 74.5, 74.1, 73.3, 73.2, 73.1, 70.6, 70.2, 70.1, 69.5, 69.3, 68.7, 68.0, 61.5, 51.6, 22.5, 20.8 (2 C), 18.1; FAB-MS *m*/*z* 1171 [($C_{65}H_{71}NO_{17}S$ + H)$^+$]. Anal. Calcd for $C_{65}H_{71}NO_{17}S$·EtOAc: C, 65.86; H, 6.33. Found: C, 65.59; H, 6.15.

Phenyl (2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (33). To a stirred mixture of **32** (18.9 g, 16.2 mmol), **6** (32.3 g, 56 mmol), 4 Å molecular sieves (10 g), and CH_2Cl_2 (360 mL) was added at 0 °C BF_3·Et_2O (400 μL, 3.25 mmol). After 2 h, the reaction mixture was filtered and the filtrate concentrated. Column chromatographic purification of the residue (1:1 hexanes−EtOAc) afforded **33** (23.6 g, 92%) as an amorphous solid: $[\alpha]_D$ +40° (*c* 0.5, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.73 (d, 1 H, $J \sim 9$), 5.71, 5.63 (2 dd, 1 H each), 5.45, 5.31 (2 d, 1 H each), 5.43 (dd, 1 H, $J = 3.3$, $J = 9.4$), 5.21 (dd, 1 H, $J = 9$, $J = 10$), 5.03, 4.92 (2 d, 1 H each, $J \sim 3.5$ Hz), 2.08, 1.99, 1.57 (3 s, 3 H each), 1.39, 1.20 (2 d, 3 H each, $J \sim 6.3$ Hz); ^{13}C δ 170.7, 170.0, 169.3, 165.8, 165.2, 98.8, 98.6, 94.8, 86.0, 79.6, 78.8, 78.5, 75.6, 75.3, 75.1, 74.6, 74.5, 74.0, 73.7, 73.4, 72.7, 70.8, 70.2, 69.7, 69.6, 69.5, 68.5, 68.0, 67.9, 61.3, 51.8, 40.4, 22.7, 20.8, 20.7, 18.0, 17.7; FAB-MS *m*/*z* 1586 [($C_{87}H_{92}ClNO_{23}S$ + H)$^+$]. Anal. Calcd for $C_{87}H_{92}ClNO_{23}S$: C, 65.84; H, 5.84. Found: C, 65.59; H, 5.79.

(2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-L-rhamnopyranose (34). To a stirred mixture of **33** (23.4 mg, 14.8 mmol) in CH_2Cl_2 (200 mL) and H_2O (10 mL) at 0 °C was added $(CF_3CO_2)_2Hg$ (11.0 g, 24.6 mmol). After 40 min, the mixture was processed as described for **5**. Column chromatographic purification (first hexane, then 1:1 hexanes−EtOAc) of the crude product afforded **34** (21.0 g, 97%) as an amorphous solid: NMR ($CDCl_3$) ^{1}H δ 5.79 (d, 1 H, $J \sim 9.5$), 5.62, 5.48 (2 dd, 1 H each), 5.41 (dd, 1 H, $J = 3.5$, $J = 9.4$), 5.29, 5.25 (2 d, 1 H each), 5.17 (dd, 1 H, $J \sim 10$ Hz, $J \sim 9$ Hz), 5.04, 4.91 (2 d, 1 H each), 2.04, 1.97, 1.60 (3 s, 3 H each), 1.35, 1.17 (2 d, 3 H each, $J \sim 6.3$); ^{13}C δ 170.9, 170.3, 169.3, 165.9, 165.5, 165.3, 98.9, 98.7, 94.6, 92.2, 79.6, 78.8, 78.6, 75.7, 75.1, (2 C), 74.7, 74.6, 73.7 (2 C), 73.5, 73.4, 72.8, 70.2, 69.9, 69.8, 69.5, 68.4, 68.3, 68.2, 68.0, 61.3, 51.7, 40.5, 22.9, 20.9, 20.7, 18.2, 17.7; FAB-MS *m*/*z* 1494 [($C_{81}H_{88}ClNO_{24}$ + H)$^+$]. Anal. Calcd for $C_{81}H_{88}ClNO_{24}$: C, 65.07; H, 5.93. Found: C, 64.21; H, 5.90.

(2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzyl-α-L-rhamnopyranosyl Trichloroacetimidate (35). A stirred solution of **34** (20.5 g, 13.7 mmol) in CH_2Cl_2 (200 mL) and CCl_3CN (12.0 mL, 124 mmol) at 0 °C was added DBU (500 μL, 3.3 mmol). After 1 h, the solution was processed as described for **6**. Column chromatographic purification (4:1 hexanes−EtOAc) of the residue afforded **35** (21.5 g, 69%) as an amorphous

substance: $[\alpha]_D$ +61° (*c* 0.5, $CHCl_3$); NMR ($CDCl_3$) ^1H δ 6.29 (d, 1 H, $J = 2.2$), 5.7 (d, 1 H, $J \sim 10$), 5.64, 5.62 (2 dd, 1 H each), 5.42 (dd, 1 H, $J = 3.4$, $J = 9.4$), 5.30 (d, 1 H), 5.20 (dd, 1 H, $J = 10.3$, $J = 9.0$), 5.01, 4.90 (2 d, 1 H each), 2.03, 2.00, 1.63 (3 s, 3 H each), 1.39, 1.17 (2 d, 3 H each, $J \sim 6.3$); ^{13}C δ 170.4, 170.1, 169.3, 165.9, 165.3, 165.2, 160.2, 98.9, 98.8, 95.6, 94.1, 79.0, 78.9, 78.6, 75.9, 75.5, 75.2, 74.7, 74.6, 73.82, 73.78, 73.6, 73.4, 72.8, 71.3, 70.2, 69.8, 69.5, 68.4, 68.1, 68.0 (2 C), 61.3, 51.8, 40.5, 23.0, 20.9, 20.8, 18.2, 17.7. Anal. Calcd for $C_{83}H_{88}Cl_4N_2O_{24}$: C, 60.81; H, 5.41. Found: C, 60.10; H, 5.34.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (36). To a stirred mixture of the imidate **35** (1.12 g, 0.68 mmol), alcohol **22** (450 mg, 3.1 mmol), and CH_2Cl_2 (5 mL) was added at 0 °C $CF_3SO_3SiMe_3$ (5 μL, 26 μmol). After 2 h, the mixture was treated with Et_3N (excess), extracted with H_2O, dried, and concentrated. Column chromatographic purification (3:1 hexanes−EtOAc) of the residue afforded **36** (805 mg, 73%) as an amorphous solid: $[\alpha]_D$ +71° (*c* 0.2, $CHCl_3$); NMR ($CDCl_3$) ^1H δ 5.72 (d, 1 H, $J \sim 10$), 5.63 (dd, 1 H, $J = 1.7$, $J = 3.4$), 5.42 (dd, 1 H, $J = 3.4$, $J \sim 8$), 4.80 (br d, 1 H), 5.28 (d, 1 H), 5.20 (dd, 1 H, $J = 10.5$, $J = 9.2$), 4.97, 4.89 (2 d, 1 H each, $J \sim 3.5$), 2.34 (t, 2 H, $J \sim 7.4$), 2.04, 2.01, 1.61 (3 s, 3 H each), 1.36, 1.66 (2 d, 3 H each); ^{13}C δ 174.0, 170.7, 170.1, 169.3, 165.8, 165.5, 165.2, 99.0, 98.9, 97.3, 95.3, 79.5, 78.7, 78.6, 76.3, 75.3, 75.2, 74.7, 74.5, 74.2, 74.0, 73.8, 73.3, 72.8, 70.2, 69.7 (2 C), 69.3, 68.3, 68.2, 67.9, 67.8, 61.0, 51.8, 51.4, 40.4, 33.8, 28.9, 25.6, 24.5, 22.9, 21.0, 20.9, 18.1, 17.6; FAB-MS *m/z* 1622 $[(C_{88}H_{100}ClNO_{26} + H)^+]$. Anal. Calcd for $C_{88}H_{100}ClNO_{26}$: C, 65.12; H, 6.21. Found: C, 64.52; H, 6.19.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (37). A solution of **36** (740 mg, 0.45 mmol) in a mixture of DMF (4 mL) and C_5H_5N (0.5 mL) was treated with thiourea (0.5 g, 6.7 mmol) at 23 °C. After 24 h, the mixture was processed as descibed for **15**. Column chromatographic purification of the residue (3:1 hexanes−EtOAc) gave **37** (670 mg, 95%) as an amorphous solid: $[\alpha]_D$ +55° (*c* 0.3, $CHCl_3$); NMR ($CDCl_3$) ^1H δ 5.63 (d, 1 H, $J \sim 10$), 5.45, 4.95 (2 dd, 1 H each), 5.34 (d, 1 H, $J = 1.3$), 5.16 (t, 1 H, $J = 9.3$), 4.98, 4.86 (2 d, 1 H each, $J \sim 3.5$), 4.79 (d, 1 H), 2.33 (t, 2 H, $J \sim 7.5$), 2.04, 1.94, 1.54 (3 s, 3 H each), 1.33, 1.23 (2 d, 3 H each); ^{13}C δ 174.0, 170.7, 170.1, 169.0, 166.0, 165.5, 99.2, 98.2, 97.4, 94.7, 81.6, 79.6, 79.2, 76.1, 75.34, 75.28, 74.7, 74.6, 73.6, 73.4, 73.1, 72.9, 72.0, 70.3, 69.9, 69.7, 69.3, 68.5, 68.2 (2 C), 67.9, 67.8, 61.2, 51.8, 51.5, 33.9, 29.0, 25.6, 24.5, 22.8, 21.0, 20.7, 18.1, 18.0; FAB-MS *m/z* 1546 $[(C_{86}H_{99}NO_{25} + H)^+]$.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (38). To a stirred mixture of the imidate **35** (3.8 g, 2.33 mmol), alcohol **37** (1.8 g, 1.16 mmol), 4 Å molecular sieves (3 g), and CH_2Cl_2 (20 mL) was added at 0 °C $BF_3{\cdot}Et_2O$ (200 μL, 1.6 mmol). After 1 h, the mixture was processed as described for **36**. Column chromatographic purification (3:2 hexanes−EtOAc) of the residue afforded **38** (1.63 g, 46%) as an amorphous solid: $[\alpha]_D$ +78° (*c* 0.3, $CHCl_3$); NMR ($CDCl_3$) ^1H δ 2.32 (t, 2 H, J ~ 7.5), 2.00, 1.93, 1.82, 1.80, 1.76, 1.47 (6 s, 3 H each), 1.7−1.52, 1.41−1.32 (2 m, 6 H), 1.38, 1.24, 1.16, 0.96 (4 d, 3 H each); ^{13}C δ 174.0, 170.6−168.9, 165.8, 165.6, 165.26, 165.23, 165.2, 99.5, 99.3, 99.1, 98.0, 97.9, 97.5, 96.0, 93.9, 61.1, 60.9, 51.5, 51.6, 51.3, 33.9, 29.0, 25.6, 24.6, 23.1, 22.6, 21.0, 20.9, 20.7, 20.4, 18.1 (2 C), 17.9, 17.3; FAB-MS *m/z* 3024 $[(C_{167}H_{185}ClN_2O_{48} + H)^+]$. Anal. Calcd for $C_{167}H_{185}ClN_2O_{48}{\cdot}H_2O$: C, 65.94; H, 6.20. Found: C, 65.50; H, 6.18.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (39). A solution of **38** (1.57 g, 0.52 mmol) in a mixture of DMF (5 mL) and C_5H_5N (1 mL) was treated with thiourea (1 g, 13 mmol) at 23 °C. After 24 h, the mixture was processed as descibed for **15**. Column chromatographic purification of the residue (1:1 hexanes−EtOAc) gave **39** (1.47 g, 96%) as an amorphous solid: $[\alpha]_D$ +70° (*c* 0.3, $CHCl_3$); NMR ($CDCl_3$) ^1H δ 5.81, 5.76 (2 d, 1 H each, $J \sim 10$), 5.21, 5.06 (2 dd, 1 H each), 2.32 (t, 2 H, $J \sim 7.5$), 1.98, 1.84, 1.82, 1.80, 1.71, 1.48 (6 s, 3 H each), 1.38, 1.24, 1.16, 1.09 (4 d, 3 H each); ^{13}C δ 174.0, 170.6, 170.5, 170.4, 169.7, 169.0, 168.9, 166.0, 165.5, 165.3, 165.1, 99.3, 99.1, 98.3, 97.93, 97.90, 97.5, 95.2, 93.9, 81.6, 80.1, 79.6, 79.4, 79.1, 78.9, 78.0, 76.2, 75.3, 75.2, 74.7, 74.6, 74.3, 74.2, 73.8, 73.5, 73.4, 73.3, 73.2, 73.1, 72.8, 72.6, 72.4, 71.0, 70.3, 69.7, 69.6, 69.4, 69.3, 68.7, 68.2, 68.1, 67.9, 67.8, 67.6, 67.5, 74.7, 61.1, 60.0, 51.5, 51.3, 51.4, 33.8, 28.9, 25.5, 24.6), 23.0, 22.6, 20.93, 20.88, 20.7, 20.4, 18.1, 17.9, 17.8, 17.7; FAB-MS *m/z* 2948 $[(C_{165}H_{184}N_2O_{47} + H)^+]$. Anal. Calcd for $C_{165}H_{184}N_2O_{47}$: C, 67.24; H, 6.29. Found: C, 66.65; H, 6.32.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (40). To a stirred mixture of the imidate **35** (2.0 g, 1.22 mmol), compound **39** (0.80 g, 0.27 mmol), and CH_2Cl_2 (5 mL) was added at 23 °C $CF_3SO_3SiMe_3$ (5 μL, 26 μmol). After 3 h, the mixture was treated with Et_3N (excess), extracted with H_2O, dried (Na_2SO_4), and concentrated. Column chromatographic purification (5:4 hexanes−EtOAc) of the residue afforded **40** (860 mg, 72%) as an amorphous substance: $[\alpha]_D$ +82° (*c* 0.3, $CHCl_3$); NMR ($CDCl_3$) ^1H δ 5.85−5.72 (m, 4 H), 3.66 (s, 3 H), 2.32 (t, 2 H, $J \sim 7$); ^{13}C δ 174.0, 170.7, 170.6, 169.9, 169.7, 169.4, 168.92, 168.89, 165.8, 165.6, 165.4, 165.3, 165.2, 164.8, 99.5 (2 C), 99.4, 99.3, 99.2, 98.0, 97.9 (3 C), 97.5 (2 C), 96.1, 60.4, 51.5, 40.6, 33.8, 29.0, 25.6, 24.6, 23.1, 22.8, 22.6, 21.0−20.4, 18.0−17.2; FAB-MS *m/z* 4425 $[(C_{246}H_{270}ClN_3O_{70} + H)^+]$. Anal. Calcd for $C_{246}H_{270}ClN_3O_{70}$: C, 66.78; H, 6.15. Found: C, 66.52; H, 6.20.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (41). A solution of **40** (820 mg, 0.18 mmol) in a mixture of DMF (5 mL) and C_5H_5N (1 mL) was treated with thiourea (1 g, 13 mmol) at 23 °C for 24 h. The usual workup followed by column chromatographic purification of the residue (5:4 hexanes−EtOAc) gave **41** (720 mg, 89%) as an amorphous solid: $[\alpha]_D$ +19° (*c* 0.3, $CHCl_3$); NMR ($CDCl_3$)

^{13}C δ 174.0, 170.7−168.9, 166.1, 165.6, 165.5, 165.3, 165.1, 164.9, 99.3 (3C), 98.4, 98.0 (4C), 97.5, 95.4, 94.2, 94.0, 61.1, 61.0, 60.8, 51.5, 51.5, 51.3, 51.1, 33.9, 29.0, 25.6, 24.6, 23.1, 22.9, 22.7, 20.9−20.4, 18.1−17.7; FAB-MS *m/z* 4348 [($C_{244}H_{269}N_3O_{69}$ + H)$^+$]. Anal. Calcd for $C_{244}H_{269}N_3O_{69}$: C, 67.41; H, 6.24. Found: C, 67.29; H, 6.30.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (42). To a stirred solution of the imidate **35** (780 mg, 0.48 mmol) was added compound **41** (720 g, 0.17 mmol) in CH_2Cl_2 (4 mL) at 23 °C $CF_3SO_3SiMe_3$ (5 μL, 26 μmol). After 5 h, the mixture was treated with Et_3N (excess), extracted with H_2O, and concentrated. Column chromatographic purification (5:4 hexanes−EtOAc) of the residue afforded **42** (640 mg, 66%) as an amorphous substance: $[\alpha]_D$ +78° (*c* 0.6, $CHCl_3$); NMR ($CDCl_3$) ^{13}C δ 170.7, 170.5 (2 C), 170.43, 170.40, 169.83, 169.80, 169.6, 169.4, 168.87, 168.83 (2 C), 165.8, 165.5, 165.4, 165.3, 165.2, 165.1, 164.81, 164.77, 99.5, 99.3, 98.1, 98.0, 97.4, 96.1, 94.32, 94.27, 93.9, 61.0, 60.8, 60.7 (2 C), 51.6, 51.3, 51.1 (2 C), 51.4, 40.4, 33.8, 28.9, 25.5, 24.5, 23.1, 22.8 (2 C), 22.6, 20.92, 20.86 (3 C), 20.7, 20.4 (3 C); FAB-MS *m/z* 5825 [($C_{325}H_{355}ClN_4O_{92}$ + H)$^+$]. Anal. Calcd for $C_{325}H_{355}ClN_4O_{92}$: C, 67.02; H, 6.14. Found: C, 66.03; H, 6.09.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (43). A solution of **42** (600 mg, 0.1 mmol) in a mixture of DMF (3 mL) and C_5H_5N (0.5 mL) was treated with thiourea (1 g, 13 mmol) at 23 °C for 26 h. The usual workup followed by column chromatographic purification of the residue (5:4 hexanes−EtOAc) gave **43** (435 mg, 73%) as an amorphous solid: $[\alpha]_D$ +81° (*c* 0.3, $CHCl_3$); NMR ($CDCl_3$) ^{1}H δ 5.77, 5.75 (2 H), 5.74 (3 d, 4 H, *J* ∼ 9), 2.32 (t, 2 H, *J* ∼ 7.5), 1.98, 1.84, 1.81, 1.79, 1.72 (9 H), 1.71 (6 H), 1.59, 1.58, 1.47 (8 s, 36 H); ^{13}C δ 174.0, 170.7, 170.53 (2 C), 170.50, 170.3, 169.9, 169.8, 169.7, 169.0, 168.9 (3 C), 166.1, 165.5, 165.46, 165.4, 165.3, 165.1, 164.9, 164.8, 99.3 (4 C), 98.4, 98.2, 98.1 (5 C), 97.5, 95.4, 94.4, 94.3, 94.0, 61.1, 60.0 (2 C), 60.7, 51.5, 51.3, 51.2 (2 C), 51.5, 33.9, 29.7, 25.6, 24.6, 23.1, 22.9 (2 C), 22.7, 20.98, 20.94, 20.93 (2 C), 20.8, 20.4 (3 C), 18.1, 18.0 (2 C), 17.95, 17.90, 17.74 (2 C), 17.7; FAB-MS *m/z* 5748 [($C_{323}H_{354}N_4O_{91}$ + H − H_2)$^+$], 5771 [($C_{323}H_{354}N_4O_{91}$ + Na)$^+$]. Anal. Calcd for $C_{323}H_{354}N_4O_{91}$: C, 67.49; H, 6.21. Found: C, 67.35; H, 6.22. Subsequent elution afforded a fraction (85 mg) that contained **43** and a small amount of an unidentified impurity.

5-(Methoxycarbonyl)pentyl α-L-Rhamnopyranosyl-(1→2)-*O*-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (44). A mixture of **24** (480 mg), Zn powder (3 g), AcOH (10 mL), and H_2O (1 mL) was stirred at 23 °C for 3 h. The mixture was filtered. Approximately half of the volatiles were removed under vacuum below 25 °C. The residue was equilibrated between $CHCl_3$ and 2% aqueous solution of the disodium salt of ethylenediaminetetraacetic acid. The organic phase was washed with H_2O, dried, (Na_2SO_4) and concentrated. A solution of the residue in C_5H_5N (2 mL) was treated at 0 °C with Ac_2O (2 mL). After 10 min, the solution was concentrated below 25 °C. A solution of the residue in dry MeOH (25 mL) was treated at 23 °C with NaOMe (∼100 mg). After 72 h, the solution was treated with Dowex 50X8-100 (H^+), then concentrated. The residue was purified through a layer of silica gel using EtOAc and then 10:1 EtOAc∼MeOH as eluant. The intermediate so obtained was dissolved in a mixture of EtOH (10 mL) and AcOH (1 mL) and the solution stirred under H_2 in the presence of Pd−C (10%, ∼0.2 g) at 200 psi for 24 h. The mixture was filtered and the filtrate concentrated. A solution of the residue in H_2O was freeze-dried to afford **44** (215 mg, 83% for four steps) as an amorphous solid: $[\alpha]_D$ +64° (*c* 0.3, H_2O); NMR (D_2O) ^{1}H δ 5.58 (d, 1 H, *J* = 3.4), 5.08 (d, 1 H, *J* = 1.2), 5.00 (d, 1 H, *J* = 3.2), 4.81 (d, 1 H, *J* = 1.3), 3.69 (s, 3 H), 3.52, 3.48 (2 t, 1 H each, *J* ∼ 9.7), 2.41 (t, 2 H, *J* ∼ 7.4), 2.05 (s, 3 H), 1.68−1.57, 1.43−1.28 (2 m, 12 H); ^{13}C δ 102.3, 100.3, 98.4, 94.8, 68.5, 61.4, 60.6, 52.9, 52.7, 34.4, 28.9, 25.7, 24.8, 22.8, 17.4; FAB-MS *m/z* 804 [($C_{33}H_{57}NO_{21}$ + H)$^+$].

5-(Hydrazinocarbonyl)pentyl α-L-Rhamnopyranosyl-(1→2)-*O*-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (45). A solution of **44** (185 mg) and NH_2NH_2 (1 mL) in dry MeOH (5 mL) was kept at 23 °C for 7 d. The solution was concentrated. H_2O was added to and distilled from the residue several times. The residue so obtained was purified by gel filtration through Biogel P-2 using 0.02 M C_5H_5N−AcOH, containing 0.01% 1,1,1-trichloro-2-methyl-2-propanol to give **45** (165 mg, 89%): $[\alpha]_D$ +73° (*c* 0.2, H_2O); NMR (D_2O) ^{1}H δ 5.60 (d, 1 H, *J* = 3.6), 5.08 (br s, 1 H), 5.00 (br d, 1 H, *J* = 3.3), 4.79 (br s, 1 H), 2.22 (t, 2 H, *J* ∼ 7.1), 2.06 (s, 3 H), 1.67−1.56, 1.42−1.33 (2 m, 6 H), 1.31, 1.30 (2 d, 3 H each); ^{13}C δ 176.6, 175, 102.4, 100.3, 98.4, 94.9, 61.5, 60.8, 52.88, 34.4, 29.0, 25.7, 25.6, 22.8, 17.5, 17.4; FAB-MS *m/z* 804 [($C_{32}H_{57}N_3O_{20}$ + H)$^+$].

5-(Methoxycarbonyl)pentyl α-L-Rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (46). Compound **26** was treated as described for the preparation of compound **44**, to afford **46** as an amorphous material: $[\alpha]_D$ +83° (*c* 0.5, H_2O); ^{1}H NMR (D_2O) δ 5.60 (d, 2 H, *J* ∼ 3.6), 5.14, 5.08, 5.05, 4.80 (4 br s, 1 H each), 5.04, 4.99 (2 d, 1 H each, *J* ∼ 3.4), 3.69 (s, 3 H), 2.41 (t, *J* ∼ 7.3), 2.06, 2.05 (2 s, 3 H each), 1.34 (3 H), 1.31 (3 H), 1.30 (6 H); FAB-MS *m/z* 1484 [($C_{59}H_{100}N_2O_{39}$ + Na)$^+$].

5-(Hydrazinocarbonyl)pentyl α-L-Rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (47). Compound **46** was treated with NH_2NH_2 as described for the preparation of **45**, to afford **47** as an amorphous solid: $[\alpha]_D$ +81° (*c* 0.5, H_2O); NMR (D_2O) ^{1}H δ 5.60 (d, 2 H, *J* ∼ 3.6), 5.11 (br d, 1 H), 5.08 (br d, 1 H), 5.05 (br d, 1 H,), 5.045 (d, 1 H, *J* ∼ 3.5), 5.00 (d, 1 H, *J* ∼ 3.4), 4.80 (br d, 1 H), 2.28−2.18, 1.66−1.55 (2 m, 6 H), 2.06, 2.05 (2 s, 3 H each), 1.34, 1.31, 1.30 (3 d, 12 H, *J* ∼ 6.2 Hz); ^{13}C δ 174.99, 174.94, 102.8, 102.4, 102.3, 100.3, 98.4 (2 C), 94.9 (2 C), 61.5 (2 C), 60.9, 60.7, 52.8, 34.4 (br), 28.9, 25.7, 25.6, 22.9, 17.6, 17.5 (2 C), 17.4; FAB-MS *m/z* 1462 [($C_{59}H_{100}N_2O_{39}$ + H)$^+$].

5-(Methoxycarbonyl)pentyl α-L-Rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-

D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (48). A mixture of compound **27** (440 mg, 92.3 μmol), Cd powder (1.5 g), AcOH (1.5 mL), and DMF (1.5 mL) was stirred at 23 °C for 8 h. The mixture was diluted with EtOAc and filtered, and the filtrate was concentrated. Toluene was added to and evaporated from the residue to afford a syrup that was treated with C_5H_5N (5 mL) and Ac_2O (5 mL) at 23 °C for 3 h. Concentration followed by column chromatographic purification (1:1 hexanes−EtOAc) afforded an intermediate (230 mg) as an amorphous substance: $[\alpha]_D$ +77° (*c* 0.3, $CHCl_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 99.9, 99.6, 99.3 (2 C), 98.2, 98.1 (2 C), 97.9, 97.5, 96.5, 94.2, 94.0, 61.0, 60.8, 60.7, 51.7, 51.3, 51.1, 51.5, 33.8, 29.0, 25.6, 24.6, 23.2, 22.9, 22.7, 20.9−20.4, 18.11 (2 C), 18.0, 17.9, 17.8, 17.2; FAB-MS *m/z* 4390 [$(C_{246}H_{271}N_3O_{70} + H)^+$]. Anal. Calcd for $C_{246}H_{271}N_3O_{70}$: C, 67.31; H, 6.22. Found: C, 67.02; H, 6.30. This material was sequentially deacylated (NaOMe in MeOH) and then hydrogenolyzed (H_2/Pd−C in EtOH−AcOH) as described for **44** to afford **48**: $[\alpha]_D$ +81° (*c* 0.3, H_2O); ^{13}C NMR (D_2O) δ 178.4, 175.0, 174.9 (2 C), 102.8 (2 C), 102.4, 102.3 (2 C), 100.3, 98.4 (3 C), 94.1 (3 C), 61.5 (3 C), 60.9 (2 C), 60.8, 52.9, 52.8 (3 C), 34.4, 28.9, 25.7, 24.8, 22.9 (3 C), 17.6 (2 C), 17.5 (4 C); FAB-MS *m/z* 2141 [$(C_{85}H_{143}N_3O_{57} + Na)^+$].

5-(Hydrazinocarbonyl)pentyl α-L-Rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside(49). Procedure a. Compound **48** was treated with NH_2NH_2 as described for the preparation of **45** to afford **49** as an amorphous solid: $[\alpha]_D$ +80° (*c* 0.2, H_2O); NMR (D_2O) ^1H δ 5.59 (d, 3 H, $J \sim 3.7$), 5.11 (d, 2 H, $J \sim 1.7$), 5.08 (d, 1 H, $J \sim 1.7$), 5.06 (d, 2 H, $J \sim 1.7$), 5.04 (d, 2 H, $J \sim 3.4$), 5.00 (d, 1 H, $J \sim 3.4$), 4.80 (d, 1 H, $J \sim 1.7$), 2.06 (s, 6 H), 2.05 (s, 3 H), 1.33 (d, 6 H, $J \sim 6.1$), 1.30 (d, 3 H, $J \sim 6.3$), 1.29 (d, 9 H, $J \sim 6.1$); ^{13}C δ 176.6, 175.0, 179.5 (2 C), 102.8 (2 C), 102.4, 102.3 (2 C), 100.3, 98.4 (3 C), 94.9 (3 C), 68.5, 61.5 (3 C), 60.9 (2 C), 60.7, 52.8, 34.4, 28.9, 25.7, 25.6, 22.9, 17.6 (2 C), 17.5 (3 C), 17.4; FAB-MS *m/z* 2118 [$(C_{84}H_{143}N_5O_{56} + H)^+$].

Procedure b. To a solution of **41** (550 mg) in MeOH (5 mL) and CH_2Cl_2 (2 mL) was added a solution of NaOMe in MeOH until the pH of the solution reached 12 as detected by a pH paper strip. After 5 d, the solution was treated with Dowex 50X8-100 (H^+). The mixture was filtered, and the filtrate was concentrated. Column chromatographic purification of the residue (first 1:1 hexanes−EtOAc and then EtOAc and finally 10:1 EtOAc−MeOH) gave an intermediate (315 mg) which was hydrogenolyzed (H_2/PdC in EtOH−AcOH) as described for **44**. The usual workup afforded an amorphous material, a solution of which in EtOH (3 mL) was treated with NH_2NH_2 (0.5 mL) at 23 °C for 4 d. The solution was concentrated. H_2O (3 × 30 mL) was added to and evaporated from the residue to afford a semisolid. This was dissolved in H_2O (30 mL), and the solution was freeze-dried. The latter cycle was repeated two more times to afford **49** (200 mg) that was further purified as described in procedure a.

5-(Hydrazinocarbonyl)pentyl α-L-Rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (50). Procedure a. A mixture of compound **29** (550 mg, 87.5 mmol), Cd (5 g), AcOH (5 mL), and DMF (5 mL) was stirred at 23 °C for 2 h. The mixture was diluted with EtOAc and filtered, and the filtrate was concentrated. Toluene was added to and evaporated from the residue to afford a syrup which was treated with C_5H_5N (5 mL) and Ac_2O (5 mL) at 23 °C for 3 h. Concentration followed by column chromatographic purification (1:1 hexanes−EtOAc) gave a homogeneous fraction {340 mg, $[\alpha]_D$ +76° (*c* 0.3, $CHCl_3$), ^{13}C NMR ($CDCl_3$) δ 99.9, 99.6, 99.3 (3 C), 98.2, 98.1, 98.0 (4 C), 97.5, 96.5, 94.4, 94.3, 94.0, 61.1, 60.8, 60.7 (2 C), 51.8, 51.3, 51.1 (2 C), 51.5, 33.8, 29.0, 25.6, 24.6, 23.2, 22.9 (2 C), 22.7, 18.11, 18.09, 17.99, 17.97, 17.9, 17.74, 17.70, 17.2; FAB-MS *m/z* 5791 [$(C_{325}H_{356}N_4O_{92} + H)^+$]}. Then 330 mg of this fraction was treated with NaOMe in MeOH at 23 °C for 7 days. Next the solution was treated with Dowex 50X2 (H^+) resin, filtered, and concentrated. The residue was purified through a short column of silica gel (EtOAc). The fractions containing the major product were pooled and concentrated. The residue was dissolved in a mixture of EtOH (10 mL) and AcOH (1 mL) and was stirred under hydrogen in the presence of Pd−C (10%, ∼0.2 g) at 200 psi for 24 h. Filtration through a layer of silica gel followed by concentration afforded an amorphous substance after freeze-drying: $[\alpha]_D$ +82° (*c* 0.2, H_2O); FAB-MS *m/z* 2777 [$(C_{111}H_{186}N_4O_{75} + H)^+$]. A solution of this material in EtOH (2 mL) was treated with hydrazine (0.5 mL) at 23 °C for 3 days. The solution was concentrated. Water was added to and evaporated from the residue several times. The residue was purified through a Biogel P-4 column which was eluted with 0.02 M pyridine−AcOH to afford **50** (62 mg, 26% for four steps) as a white amorphous substance: $[\alpha]_D$ +78° (*c* 0.1, H_2O); NMR (D_2O) ^1H, δ 5.59 (d, 4 H, $J \sim 3.4$), 5.11 (d, 3 H, $J \sim 1.6$), 5.08 (d, 1 H, $J \sim 1.7$), 5.06 (d, 3 H, $J \sim 1.6$), 5.04 (d, 3 H, $J \sim 3.6$), 5.00 (d, 1 H, $J \sim 3.5$), 4.80 (d, 1 H, $J \sim 1.7$), 2.06 (s, 9 H), 2.05 (s, 3 H), 1.34 (d, 9 H, $J \sim 6.1$), 1.31 (d, 3 H, $J \sim 6.3$), 1.30 (d, 12 H, $J \sim 6.1$); ^{13}C δ 102.6 (3 C), 102.2, 102.1 (3 C), 100.3, 98.4 (4 C), 94.9 (4 C), 61.4 (4 C), 61.0 (3 C), 60.9, 52.7 (4 C), 28.8, 26.1, 25.6, 25.5, 22.8 (4 C), 17.6 (3 C), 17.4 (4 C), 17.3; FAB-MS *m/z* 2777 [$(C_{111}H_{186}N_4O_{75} + H)^+$].

Procedure b. Compound **43** (435 mg) was treated as described in procedure b for compound **49** to afford **50** (145 mg, 72%) as an amorphous solid.

6,6-Dimethoxyhexanoic Acid (51). To a solution of $(COCl)_2$ (5.47 mL, 62.7 mmol) in CH_2Cl_2 (50 mL) was added at −60 °C a solution of DMSO (8.90 mL, 125 mmol) in CH_2Cl_2 (20 mL) under stirring, in a period of 5 min. The solution was stirred for 2 min after the addition was complete. To this solution was added dropwise a solution of methyl 6-hydroxyhexanoate (**22**, 7.9 g, 54 mmol) in CH_2Cl_2 (40 mL). The opaque reaction mixture was stirred at −50 °C for 1 h and then was allowed to reach −10 °C. After 15 min, the solution was treated with Hünig's base (30 mL) and then was allowed to reach 23 °C. The solution was extracted with H_2O (3 × 50 mL), dried (Na_2SO_4), and concentrated to afford a syrup. To a solution of this material in 2,2-dimethoxypropane (60 mL) was added a catalytic amount of 4-toluenesulfonic acid. After 30 min, approximately half of the volatiles were removed. The solution so obtained was treated with Et_3N (excess) and then concentrated. The residue was equilibrated between $CHCl_3$ and H_2O. The organic layer was dried (Na_2SO_4) and concentrated to give a syrup. To a solution of this material in MeOH (100 mL) was added 1 N aqueous LiOH (80 mL) at 23 °C. After 45 min, MeOH was removed by distillation. The solution was extracted with ether thrice. To the aqueous solution was added solid citric acid until the pH of the solution reached 3.5 as estimated by indicator paper. The clear solution was extracted with $CHCl_3$ (3 × 50 mL). The combined organic phase was washed with H_2O and concentrated. Column chromatographic purification of the residue (EtOAc) afforded **51** (7.4 g, 78%) as a clear liquid whose purity is estimated to be >95% (NMR): NMR ($CDCl_3$) ^1H δ 4.37 (t, 1 H, $J = 5.4$), 3.32 (s, 6 H), 2.37 (t, 2 H), 1.71−1.59 and 1.48−1.36 (2 m, 6 H); ^{13}C δ 179.0 (*C*=O), 104.3, 52.7, 33.8, 32.1, 24.5, 24.1; CI-MS *m/z* 194 [$(M + NH_4)^+$].

***N*-Hydroxysuccinimide Ester of 6,6-Dimethoxyhexanoic Acid (52).** To a solution of **51** (478 mg, 2.71 mmol) in EtOAc (10 mL) were added *N*-hydroxysuccinimide (312 mg, 2.71 mmol) and 1,3-dicyclohexylcarbodiimide (614 mg, 2.98 mmol). The mixture was stirred at 23 °C for 3 h and then diluted with ether (10 mL) followed by filtration. The mother

liquor was concentrated to afford **52** as a syrup that was used without further purification. The purity of this material is estimated to be >90% (^{1}H NMR). During storage at 0 °C a small amount of a crystalline material separated. NMR ($CDCl_3$) ^{1}H δ 4.37 (t, 1 H, $J \sim 5.5$), 3.31 (s, 6 H), 2.85 (br s, 4 H), 2.63 (t, 2 H), 1.83−1.45 (3 m, 6 H).

Procedure for the Covalent Attachment of the Oligosaccharides to Human Serum Albumin. To a solution of **50** (6.5 mg, 2.3 μmol) in DMF (200 μL) was added **52** (∼11 mg, 40 μmol). After 4 h, the solution was applied to a Biogel P-4 column (25 × 1 cm) that was eluted with H_2O. The carbohydrate-containing fractions were pooled and freeze-dried to give an amorphous solid [NMR (D_3O): ^{1}H δ 4.50 (t, 1 H), 3.33 (s, 6 H)]. To a solution of the residue in H_2O (1 mL) was added AcOH until the pH of the solution reached ∼2.65. After 6 h, the solution was freeze-dried. To the residue was added human serum albumin (1.2 mg) followed by a solution of $NaCNBH_3$ (0.21 mg) in pH 7.0 borax−phosphate buffer (30 μL). After 2 days, the solution was treated with an additional amount of $NaCNBH_3$ (0.21 mg in pH 7.0 borax−phosphate buffer). After an additional 2 days the solution was transferred to a 10 mL Amicon diafiltration apparatus equipped with a YM-10 Diaflo membrane. The solution was filtered using five changes of H_2O. A solution of the residue in H_2O was freeze-dried to give **53** as an amorphous substance that had an average MW of 120 kDa (MALDI-TOF MS).

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