Synthesis of Kojidextrins and Their Protein Conjugates. Incidence of Steric Mismatch in Oligosaccharide Synthesis†

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Kojidextrins are biologically important oligosaccharides that are involved in many physiological processes including protein glycosylation and bacterial growth. As part of our project to explore the role kojidextrins may play in bacterial pathogenesis, here we report synthetic routes to kojibiose (**54**), -triose (**58**), -tetraose (**64**), and -pentaose (**69**) equipped with α-linked (hydrazinocarbonyl)-pentyl aglycon, using linear and convergent strategies. In the search for a rapid convergent strategy for the construction of extended kojidextrins, four kojibiose donors (**1**−**4**) were synthesized that contain acyl- and ether-type protecting groups in various ratios. These were tested to probe the influence of diverse protecting group assemblies on their glycosyl donor ability. Attempted condensation of these donors with kojitriose and -tetraose acceptors failed to give the desired products apparently because of steric mismatch between the donor and the acceptor moieties. A one-pot procedure was developed for the covalent attachment of the synthetic saccharides through their hydrazido group to human serum albumin (HSA) using Tietze's squarate method to give neoglycoproteins containing up to 28 saccharide units per HSA.

Introduction

Kojidextrins are biologically important oligosaccharides containing α-(1→2)-linked D-glucose residues in a linear arrangement. Their simplest representative is the disaccharide kojibiose that is found among the fermentation products of rice,[1] in the core region of the O-antigenic polysaccharide of the enteric bacterium *Escherichia coli* K-12,[2] in the capsular polysaccharides of *Streptococcus pneumoniae* Types 12A[3a] and 12F,[3b] in native dextrans,[4] and in the teichoic acid[5] from *Streptococcus faecalis* strain 39. Kojibiose is the only kojidextrin found in mammals, where it is part of the lipid-linked oligosaccharide precursor for protein glycosylation.[6] Kojitriose occurs in bacterial teichoic acids[5] and in native dextrans,[4] and kojitetraose and -pentaose constitute part of the glucans secreted by *Rhizobium meliloti* J 7017.[7] Kojihexaose[8] was identified as an extracellular saccharide of a slow-growing strain of *Rhizobium japonicum* strain 561, and an antigenic polysaccharide isolated from *Mycobacterium tuberculosis* strains H37 and A33, termed polysaccharide II, is said to be poly-α-(1→2)-D-glucan.[9] We became interested in this unusual group of oligosaccharides because of their value in immunochemical studies related to pathogenic bacteria that contain kojidextrins on their surface.

The chemical synthesis of kojipentaose and smaller kojidextrins has been reported by us and by others either in free form or as glycosides bearing an alkyl group as the aglycon.[10−13] Earlier, we used a stepwise strategy and found that the overall yield rapidly declines as the chain length of the oligosaccharide increases.[10g,h] Therefore, we have chosen to systematically explore a blockwise approach that could be conducive to rapid construction of higher kojidextrins in favorable yields. The rationale for this approach is that the glycosyl residue at O-2 is a nonparticipating moiety,[14] and therefore, it would assist the formation of α-glycosidic linkage. In the present study, we prepared a series of kojibiose derivatives (**1**−**4**) in which the number of reactivity-enhancing, ether-type protecting groups in the nonreducing end residue varies between 1 and 4 and tested their reactivity as glycosyl donors. A common feature of compounds **1**−**4** is a chemoselectively removable protecting group at HO-2′, which is the site of future chain extension. It was anticipated that glycosylation experiments with these compounds would allow the assessment of the influence of the ether-type protecting groups on the reactivities of

† This paper is dedicated to Professor Hans Paulsen on occasion of his 75th birthday.

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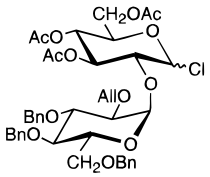
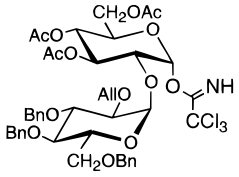
such donor moieties and would lead toward the development of an efficient kojibiosyl donor/acceptor building block. Therefore, we describe here the synthesis of kojibiose derivatives **1**−**4** and then examine their use as glycosyl donors in a blockwise approach to higher kojidextrins.



1



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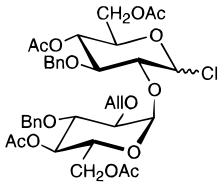

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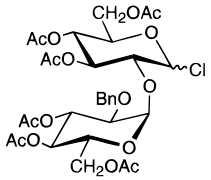


4

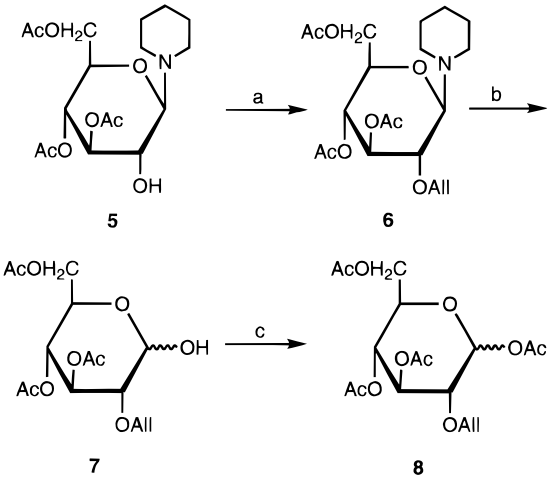
All = allyl

Bn = benzyl

Results and Discussion

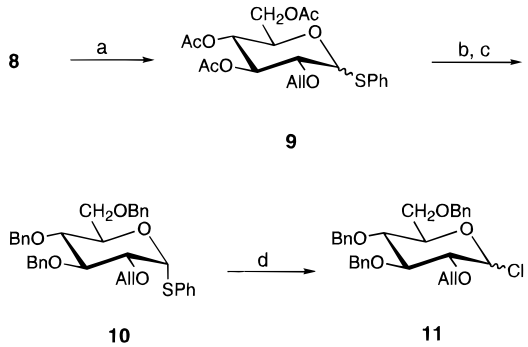
Preparation of the Disaccharide Building Blocks. (I) The Disaccharide Synthons 1 and 2. The key intermediate to disaccharides **1** and **2** was the glucosyl chloride **11** which was prepared by two independent routes.[15] In the first approach, the readily available (3,4,6-tri-*O*-acetyl-*β*-D-glucopyranosyl)piperidine[16] (**5**) was selected as the starting material and converted to the tetraacetate[12a] **8** through a three-step sequence involving allylation at O-2 (allyl iodide, Ag_2O → **6**), hydrolytic removal of the anomeric piperidino moiety (AcOH, H_2O → **7**), and acetylation in 82% overall yield (Scheme 1). Treatment of **8** with $PhSSiMe_3/Me_3SiOTf$[17] afforded thioglucoside **9** in 86% yield as a 94:6 mixture of the α and the *β* anomers (Scheme 2). Addition of PhSH to the allyl group in this reaction could be suppressed by using the reagent $PhSSiMe_3$ in a slightly less than equimolar amount. Base-catalyzed transesterification (NaOMe) followed by *O*-benzylation gave the fully protected α thioglucoside **10** (81%). Next, we investigated the conversion of the thioglucoside **10** into glucosyl chloride **11**. The standard reagent for such conversions[10g] is chlorine, which could not be used for **10** because it would abolish the double bond in the allyl group. On the other hand, reaction of **10** with α,α-dichloromethyl methyl ether[18,19] (DCMME) in the presence of Me_3SiOTf gave a *ca*. 2:1 mixture of the α and the *β* chloride[12b] **11** in a nearly quantitative reaction (^1H NMR). Unfortunately, product recovery was not efficient since chromatographic purification caused extensive losses and the α chloride **11**α could be isolated in 41% yield only. An alternative route to **11** started from the readily available tosyl ester derivative[15b] **12**, which was benzylated to give **13** (Scheme 3). The temporary tosyl group was then removed by treatment with $NaBH_4$ in Me_2SO to furnish the alcohol **14** in 71% yield.[20] Allylation at O-2 (→**15**) followed by acid-catalyzed removal[21] of the acetal gave the diol **16**. Benzylation (→**17**) followed by selective cleavage of the glycosidic linkage afforded the hemiacetal **18** from which the chloride **11** was obtained with the Vilsmeyer−Haack reagent generated[22] *in situ* from $(COCl)_2$ and DMF in 69% yield.

Scheme 1[a]



[a] Reagents and conditions: (a) 5 equiv of $CH_2{=}CHCH_2I$, Ag_2O, DMF, 23 °C, 2 h, 85%; (b) AcOH, 2:1 Me_2CO-H_2O, reflux, 15 min, quant; (c) Ac_2O/C_5H_5N, 23 °C, 1 h, 96%.

Scheme 2[a]



[a] Reagents and conditions: (a) 1.05 equiv of $PhSSiMe_3$, 0.25 equiv of $CF_3SO_2OSiMe_3$, $(CH_2Cl)_2$, 83 °C, 12 h, 86%; (b) NaOMe (cat.), MeOH, 23 °C, 3 h; (c) 1.5 equiv of NaH, 1.1 equiv of BnBr, DMF 0 → 23 °C, 3 h, 81% for two steps; (d) 5 equiv of CCl_2HOCH_3, 0.5 equiv of $CF_3SO_2OSiMe_3$, CH_2Cl_2, 0 °C, 1 h, 41%.

(15) An earlier work by Takeo[12b] used methyl 2-*O*-allyl-4,6-*O*-benzylidene-α-D-glucopyranoside as the precursor, which was prepared by several groups [(a) Küster, J. M.; Dyong, I. *Liebigs Ann. Chem.* **1975**, 2179−2189. (b) Jenkins, D. J.; Potter, B. V. L. *Carbohydr. Res.* **1994**, *265*, 145−149. (c) Dasgupta, F.; Garegg, P. J. *Synthesis* **1994**, 1121−1123] by regioselective monoallylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside. Careful reproduction of these protocols gave mixtures of the 2-*O*- and 3-*O*-allyl derivatives from which the isolation of the 2-*O*-allyl derivative proved to be impractical.

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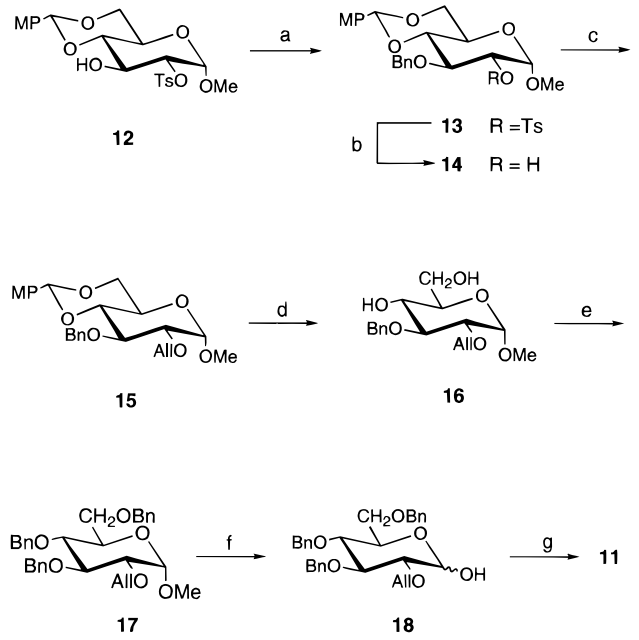
Scheme 3[a]

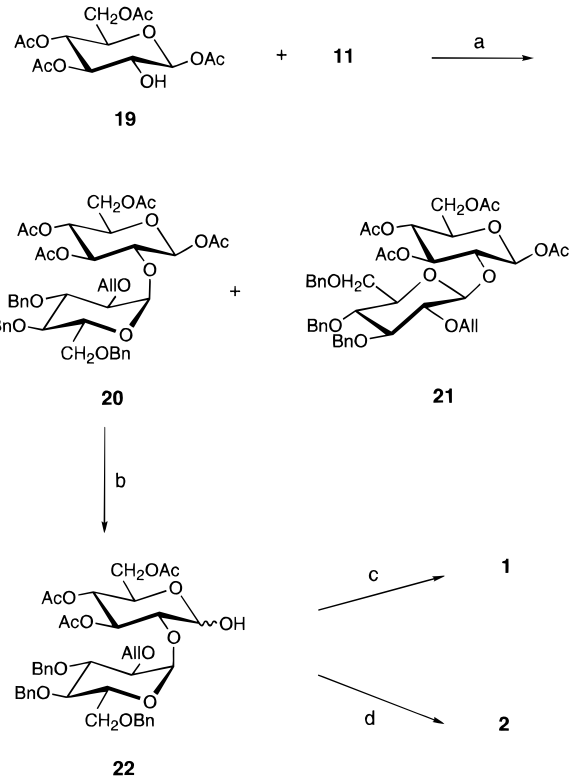


[a] Reagents and conditions: (a) 2.4 equiv of BnBr, 8 equiv of BaO, $Ba(OH)_2 \cdot 8H_2O$, DMF, 23 °C, 8 h, 88%; (b) 14 equiv of $NaBH_4$, Me_2SO, 150 °C, 24 h, 71%; (c) 1.5 equiv of NaH, 1.3 equiv of $CH_2{=}CHCH_2Br$, DMF, 0 → 23 °C, 2 h, 88%; (d) $AcOH{-}H_2O$, reflux, 15 min, 91%; (e) 1.5 equiv of NaH, 1.3 equiv of BnBr, DMF, 0 → 23 °C, 3 h, 92%; (f) HCl, $AcOH{-}H_2O$, reflux, 90 min, 68%; (g) 3 equiv of $(COCl)_2$, DMF (cat.), CH_2Cl_2, 0 °C, 3 h, 69%.

Condensation of chloride **11** with the known acceptor[23] **19** ($AgClO_4/Ag_2CO_3$) proceeded in 87% yield to give a 4:1 mixture of compounds **20** and **21** from which the α-linked disaccharide **20** was isolated in 56% yield (Scheme 4). Anomeric deacetylation[24] of **20** (NH_2NH_2/DMF) afforded hemiacetal **22** (92%) treatment of which with the Vilsmeyer−Haack reagent gave the glycosyl chloride **1** as a 1:1 mixture of the α and *β* anomers in 92% yield. The α imidate **2** was obtained by reaction[25] of **22** with trichloroacetonitrile in the presence of DBU in 85% yield.

(II) The Disaccharide Synthon 3. The Reducing-end Moiety. The (trimethylsilyl)ethyl glucoside **29** was selected as the reducing-end synthon. The precursor was the readily available 1,2,4,6-tetra-*O*-acetyl-3-*O*-benzyl-*β*-D-glucopyranose[26] (**23**), which was converted to the 2-(trimethylsilyl)ethyl glucoside **25** *via* the intermediacy of the chloride **24** in 70% overall yield (Scheme 5). Transesterification (Zemplén) was sluggish at ambient temperature[27] but was completed within 90 min in quantitative yield at 50 °C to provide the triol **26**. Selective protection of the HO-4 and -6 groups was performed as follows. First, a 4-methoxybenzylidene group was installed at both HO-4 and -6. HO-2 was then blocked with a monochloroacetyl group (→**27**, 74%). The highly acid-sensitive acetal group was removed[21] by HBF_4 in anhydrous MeOH. This was followed by *O*-acetylation (Ac_2O/Py) to give **28** in 83% overall yield. Finally, the

Scheme 4[a]



[a] Reagents and conditions: (a) 1.3 equiv of **19**, $AgClO_4$, Ag_2CO_3, 4 Å molecular sieves, CH_2Cl_2, 0 → 23 °C, 4 h, 87%; (b) 1.5 equiv of NH_2NH_2, DMF, 23 °C, 2 h, 92%; (c) 2.7 equiv of $(COCl)_2$, DMF (cat.), CH_2Cl_2, 0 → 23 °C, 4 h, 92%; (d) 30 equiv of CCl_3CN, 0.6 equiv of DBU, 0 °C, 90 min, 85%.

chloroacetyl group was selectively cleaved with hydrazine dithiocarbonate according to van Boeckel[28] to afford the acceptor moiety **29** bearing the 2-(trimethylsilyl)ethyl aglycon in 75% yield.

The Non-Reducing-End Moiety. The precursor to the non-reducing-end synthon was the tetraacetate[26] **23**, which was converted with $PhSH/BF_3 \cdot Et_2O$ to phenylthio glucoside[29] **30** in 86% yield (Scheme 6). Transesterification (Zemplén) furnished the triol **31** in a nearly quantitative yield. The introduction of the allyl group at O-2 was modeled after the sequence presented for **17**. Thus, **31** was converted to the cyclic acetal **32** (96%) with anisaldehyde dimethyl acetal followed by allylation at O-2 to give **33** in 96% yield. Replacement of the methoxybenzylidene group by acetyls [(i) HBF_4/MeOH, → **34** (78%); (ii) Ac_2O/Py] gave the key thioglycoside intermediate **35** in 96% yield. The anomeric phenylthio group in **35** was substituted by chlorine using DCMME[19] in the presence of $ZnCl_2 \cdot Et_2O$ to afford the α-chloride **36** in 94% yield. Alternatively, acetylation of the diol **16** followed by acetolysis (→**37**) and reaction with DCMME/$ZnCl_2 \cdot Et_2O$ gave the chloride **36** in 65% overall yield as shown in Scheme 7. Condensation of the glucoside acceptor **29** and the donor **36** was next investigated. Using 0.2 molar equiv of $AgClO_4$ as the promoter with Ag_2CO_3 gave the α-linked disaccharide **38** in 62% yield (Scheme 8). Trifluoroacetic acid-mediated removal[30] of the aglycon af-

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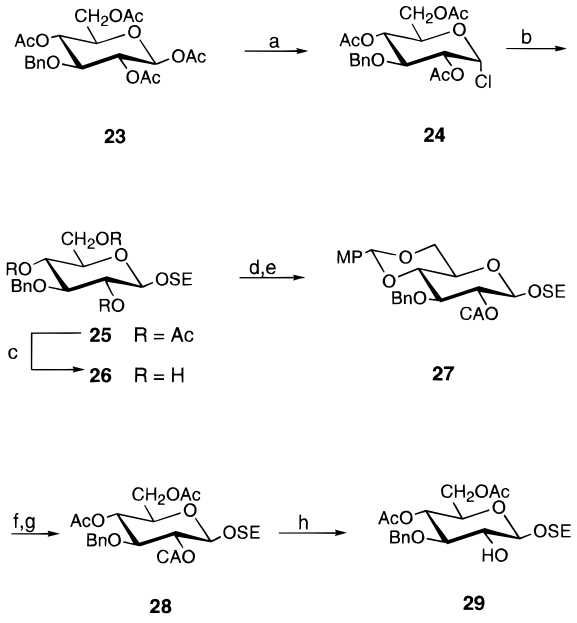
(26) Freudenberg, K.; Plankenhorn, E. *Liebigs Ann. Chem.* **1938**, *536*, 257−266.
(27) For related observations, see: (a) Szurmai, Z.; Lipták, A. *Carbohydr. Res.* **1982**, *107*, 33−41. (b) Szurmai, Z.; Lipták, A.; Schnatzke, G. *Carbohydr. Res.* **1990**, *200*, 201−208. (c) Borbás, A; Lipták, A. *Carbohydr. Res.* **1993**, *241*, 99−116.
(28) van Boeckel, C. A. A.; Beetz, T. *Tetrahedron Let.* **1983**, *24*, 3775.
(29) The synthesis of compound **30** from the same precursor in 42% yield was reported: Chénedé, A.; Perrin, E.; Rekaï, E. D.; Sinaÿ, P. *Synlett* **1994**, 420−422.
(30) For an excellent review on the use of 2-(trimethylsilyl)ethyl glycosides in oligosaccharide synthesis, see: Magnusson, G. *Trends Glycosci. Glycotechnol.* **1992**, *4*, 358−367.

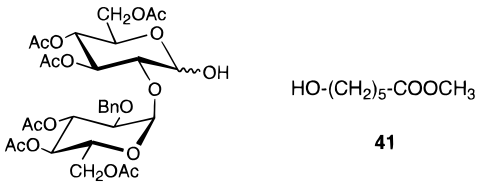
Scheme 5[a]



[a] Reagents and conditions: (a) 4.6 equiv of CCl_2HOCH_3, 0.1 equiv of $ZnCl_2$, CH_2Cl_2, 23 °C, 20 min, 91%; (b) 3 equiv of SEOH, 0.9 equiv of *s*-collidine, 4 Å molecular sieves, 1.9 equiv of CF_3SO_2OAg, 0 °C, 3 h, 79%; (c) NaOMe (cat.), MeOH, 50 °C, 90 min, 99%; (d) 1.8 equiv of 4-methoxybenzaldehyde dimethyl acetal, camphorsulfonic acid (cat.), DMF, 23 °C, 2 h; (e) 3.3 equiv of $(ClCH_2CO)_2O$, C_5H_5N, 0 °C, 1 h, 74% for two steps; (f) $HBF_4{\cdot}Et_2O$, MeOH, 0 → 23 °C, 1 h; (g) Ac_2O, C_5H_5N, 23 °C, 12 h, 83% for two steps; (h) 3 equiv of $NH_2NH_2CS_2$, dioxane, 23 °C, 1 h, 76%. Key: CA = chloroacetyl; MP = 4-(methoxy)phenyl; SE = 2-(trimethylsilyl)ethyl.

forded the hemiacetal **39** (87%), which was converted to the target glycosyl chloride **3** with $(COCl)_2$/DMF in 82% yield.

(III) The Disaccharide Synthon 4. Compound **4** was obtained as a 6:4 mixture of the α and β anomers by chlorination of the kojibiose hemiacetal[31] **40** with *in situ* generated Vilsmeyer−Haack reagent.



40

41

Assembly of the Oligosaccharides. Our synthetic plan called for a chain extension initiated at the reducing end, using 5-(methoxycarbonyl)pentanol[32] (**41**) as the aglycon. First, we examined the condensation of the disaccharide **4** with the alcohol **41** in the presence of $AgClO_4/Ag_2CO_3$. Although the condensation proceeded in a high yield, the resulting mixture of the α and the β anomers could not be separated. Similar difficulties were encountered in attempting Koenigs−Knorr-type condensation of **41** with monosaccharide donors having a nonparticipating group at O-2. Therefore, this approach (not described in the Experimental Section) was abandoned. Earlier, we found[10g,33] that reaction of either an isolated or an *in situ* generated β-glucosyl bromide with an alcohol in the presence of a base gave the corresponding α-glucoside exclusively. We next examined the reaction of the donor **43**, obtained quantitatively (^{1}H NMR) from the thioglucoside **42**, with the alcohol **41**. As expected, the α-glucoside **44** was obtained as the only product in exclusive stereoselectivity (Scheme 9). In this transformation, the intermediate formation of the α-bromide (^{1}H NMR), which was eventually converted into the product α-glucoside, evidently through the β-bromide, was also observed. The process, being catalyzed by *in situ* formed endogenous bromide ions, was termed "nonclassical halide ion catalysis."[33] Catalytic hydrogenolysis of **44** gave the acceptor **45** for the subsequent chain extension. The glycosylation of compound **45** with monosaccharide donors was next studied. The reaction of **45** with the chloride **11** afforded a *ca.* 3:1 mixture of the α- (**50**) and the β-linked (**49**) disaccharides in a combined yield of 74% (Scheme 10). An improved stereoselectivity was obtained when **45** was condensed with the known[23] 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl chloride (**51**) to give the α-linked disaccharide **52** in 78% yield. This was debenzylated in the presence of Pd/C in ethanol to afford compound **53** as a crystalline material. Reacting the disaccharide acceptor **53** with the monosaccharide donor **36** in the presence of AgOTf afforded the desired trisaccharide **55** in 79% yield (Scheme 11).

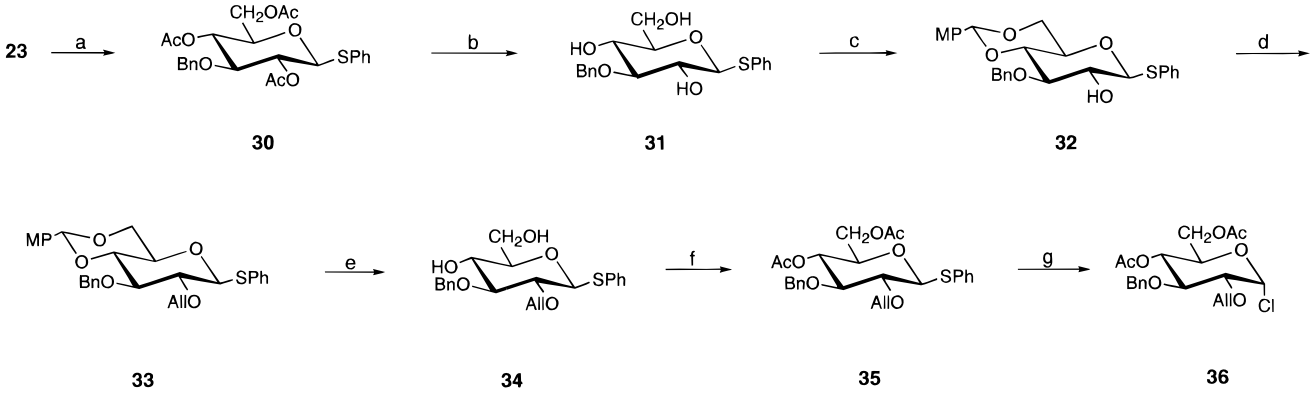
Next the glycosylation reactions with the disaccharide donors were examined. The reaction of the monosaccharide acceptor **45** with the disaccharide donor **3** proceeded equally well to provide the trisaccharide **59** in 70% yield. This was deallylated[34] to give the trisaccharide acceptor **60** in 84% yield (Scheme 12). Surprisingly, attempted condensation of the disaccharide acceptor **53** with the disaccharide chloride **3** under various Koenigs−Knorr conditions failed, and the major product in this reaction was tentatively identified as a trehalose-type tetrasaccharide formed from the donor **3**. Reaction of **53** with the chloride **1** under $AgClO_4$ catalysis was more successful, and the targeted tetrasaccharide **61** could be isolated in 54% yield (Scheme 13). A slightly improved yield (66%) was obtained in the condensation of the acceptor **53** with the chloride **4** to give the tetrasaccharide **65**. Deallylation of **61** and debenzylation of **65** afforded the tetrasaccharide acceptors **62** and **66**, respectively.

Having prepared the tri- (**60**) and tetrasaccharide acceptors (**62** and **66**) and the disaccharide donors (**1**−**4**), their condensation was next examined. Surprisingly, no condensation was obtained between any of the following acceptor/donor pairs under a variety of Koenigs−Knorr and Schmidt conditions: trisaccharide acceptor **60** and disaccharide donor **3**; tetrasaccharide acceptor **62** and disaccharide donors **1**, **2**, and **4**; and tetrasaccharide acceptor **66** and disaccharide donor **4** (Table 1). On the other hand, reaction of the tetrasaccharide alcohol **66** with the monosaccharide donor **36** gave the fully protected pentasaccharide **67** in 38% yield (Scheme 14).

The protected (methoxycarbonyl)pentyl glycosides **45**, **53**, **55**, **62**, and **67** were deprotected by standard procedures and converted to the corresponding hydrazides **47**, **54**, **58**, **64**, and **69** as shown in Schemes 9−11, 13, and 14 for covalent attachment to proteins as described below.

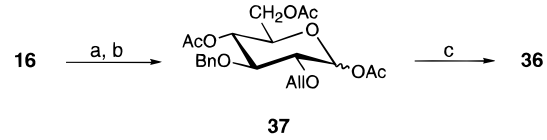
(31) Dubois, E.; Neszmélyi, A.; Lotter, H.; Pozsgay, V. *Tetrahedron Let.* **1996**, *37*, 3627−3630.
(32) Sabesan, S.; Paulson, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 2068−2080.
(33) Pozsgay, V.; Coxon, B. *Carbohydr. Res.* **1995**, *277*, 171−178.
(34) (a) Bose, R.; Scheffold, R. *Angew. Chem.* **1976**, *88*, 578−579. (b) Ogawa, T.; Nakabayashi, S. *Carbohydr. Res.* **1981**, *93*, c1-c5.

Scheme 6[a]



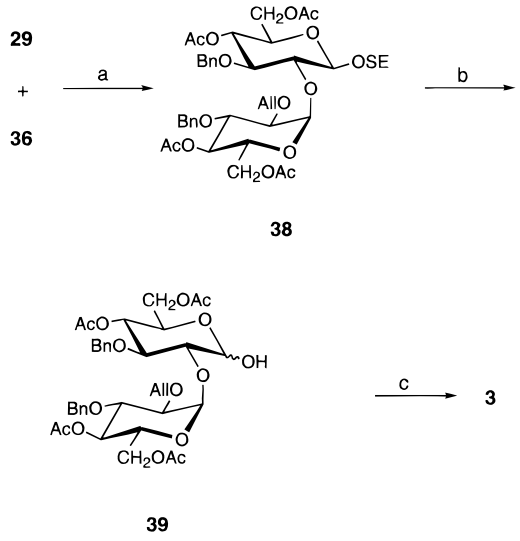
[a] Reagents and conditions: (a) 4 equiv of PhSH, 1.2 equiv of $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C, 30 min, 88%; (b) NaOMe (cat.), MeOH, 23 °C, 24 h, 99%; (c) 2.2 equiv of 4-methoxybenzaldehyde dimethyl acetal, camphorsulfonic acid (cat.), DMF, 23 °C, 2 h, 96%; (d) 1.4 equiv of NaH, 1.7 equiv of $CH_2{=}CHCH_2Br$, DMF, 0 → 23 °C, 1 h, 96%; (e) $HBF_4{\cdot}Et_2O$, MeOH−EtOAc, 0 → 23 °C, 30 min, 78%; (f) Ac_2O, C_5H_5N, 23 °C, 1 h, 96%; (g) 10 equiv of CCl_2HOCH_3, 0.15 equiv of $ZnCl_2$, CH_2Cl_2, 0 °C, 2 h, 94%. Key: Ph = phenyl.

Scheme 7[a]



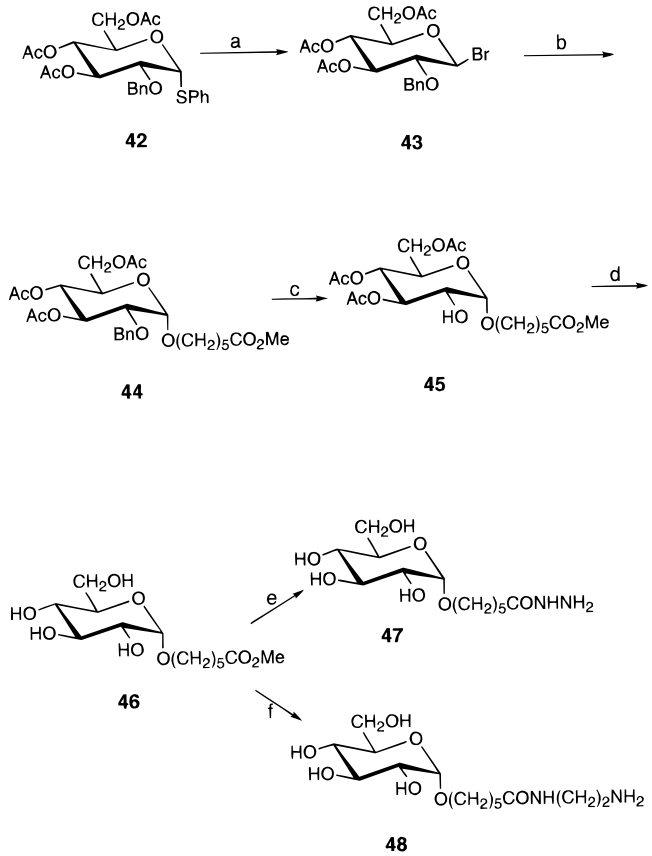
[a] Reagents and conditions: (a) Ac_2O, C_5H_5N, 23 °C, 12 h, 83%; (b) H_2SO_4 (cat.), Ac_2O, 0 °C, 5 min, 73% for two steps; (c) 1.8 equiv of CCl_2HOCH_3, 0.1 equiv of $ZnCl_2$, CH_2Cl_2, 0 → 23 °C, 30 min, 91%.

Scheme 8[a]



[a] Reagents and conditions: (a) 1.5 equiv of **36**, $AgClO_4$, Ag_2CO_3, 4 Å molecular sieves, CH_2Cl_2, 0 → 23 °C, 4 h, 52%; (b) CF_3COOH, CH_2Cl_2, 0 → 23 °C, 1 h, 87%; (c) 3.3 equiv of $(COCl)_2$, DMF (cat.), CH_2Cl_2, 0 → 23 °C, 4 h, 82%.

Scheme 9[a]

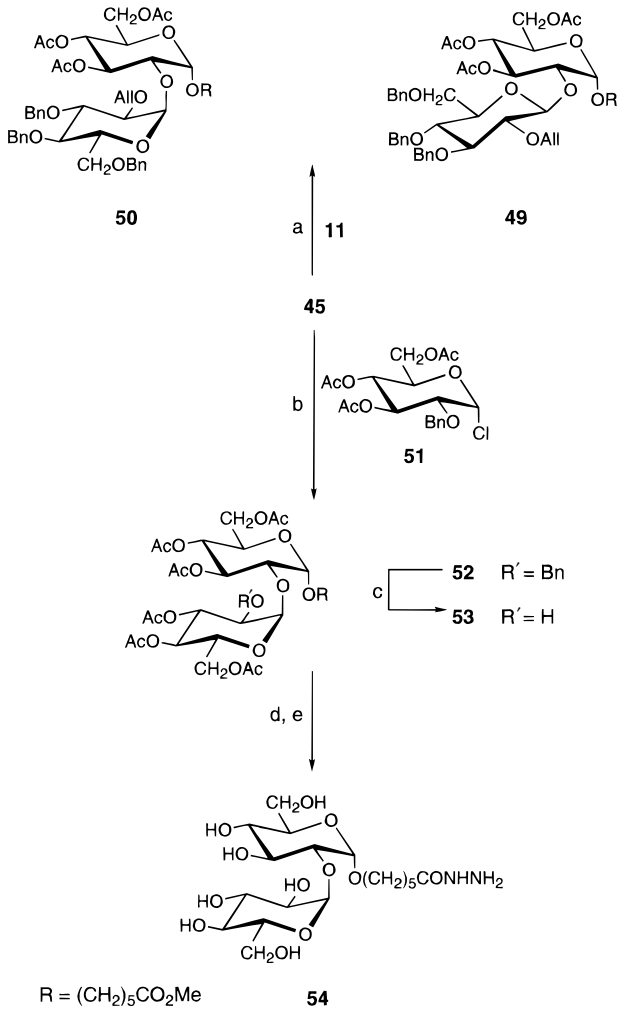


[a] Reagents and conditions: (a) 1 equiv of Br_2, CH_2Cl_2, 0 → 23 °C, 5 min; (b) $HO(CH_2)_5CO_2Me$ (excess), Et^iPr_2N, 23 °C, 36 h, 80% for two steps; (c) H_2/Pd−C, EtOH−AcOH, 23 °C, 200 psi, 24 h, 83%; (d) NaOMe (cat.), MeOH, 23 °C, 12 h, quant; (e) NH_2NH_2 (excess), EtOH, 23 °C, 36 h, 80%; (f) $NH_2(CH_2)_2NH_2$ (excess), 80 °C, 12 h, 83%.

The structures of all intermediates and unprotected saccharides were verified by ^1H and ^{13}C NMR spectroscopy, which gave the expected chemical shift and homo- and heteronuclear coupling patterns. Of particular note is the observation of unusual broadening of several of the resonances in the ^{13}C NMR spectra of the pentasaccharides **68** and **69**. These include three of the five anomeric carbon resonances and three of the four interglycosidically linked C-2 carbon signals and very likely reflect rigidity around the glycosidic linkages in the internal regions of the saccharide chain.

In summary, we have demonstrated that various kojibiose donors (**1**−**4**) bearing acetyl as well as benzyl and/or allyl protecting groups can be used for block-synthesis of kojidextrins in [2 + 1] and [2 + 2] condensation reactions. In these reactions, no significant differences were seen among the donors **1**−**4** that could be attributed to differences in the blocking group pattern. However, the disaccharide blocks **1**−**4** could not be condensed with tri- or tetrasaccharide alcohols, indicating extensive clashing between the acceptor moiety and the disaccharide donor unit.

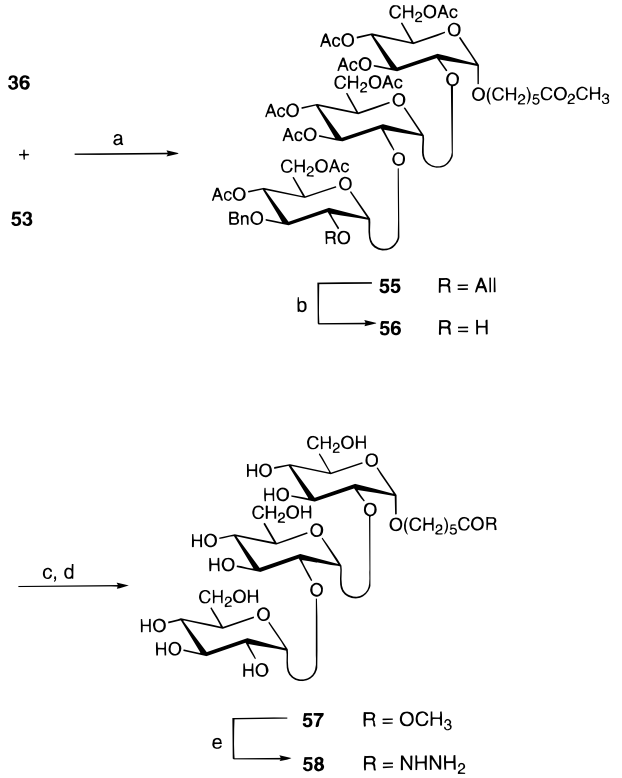
Scheme 10[a]



[a] Reagents and conditions: (a) 1.4 equiv of **11**, $AgClO_4$, Ag_2CO_3, 4 Å molecular sieves, CH_2Cl_2, 0 °C, 80 min, 17% for **49**, 57% for **50**; (b) 1.8 equiv of **51**, $AgClO_4$, Ag_2CO_3, 4 Å molecular sieves, CH_2Cl_2, 23 °C, 4 h, 78%; (c) H_2/Pd−C, EtOH−AcOH, 23 °C, 300 psi, 24 h, 86%; (d) NaOMe (cat.), MeOH, 23 °C, 16 h; (e) NH_2NH_2 (excess), EtOH, 23 °C, 12 h, 72% for two steps.

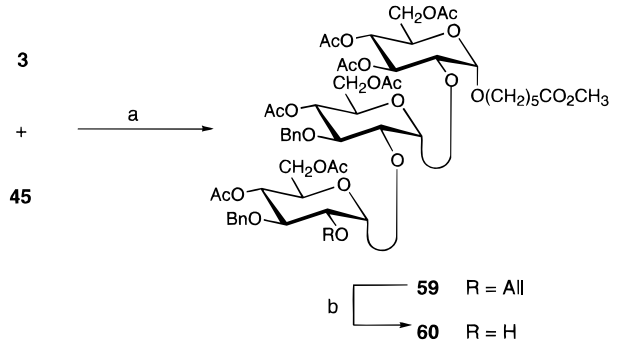
Covalent Attachment to Proteins. The primary objective of this project is to synthesize neoglycoproteins containing kojidextrins of defined structure for use as multivalent immunogens and antigens. After the multistep synthetic sequences, an efficient method for the coupling of the glycosides **47**, **54**, **58**, **64**, and **69** to proteins is essential. In this regard, the two-step squarate method proposed by Tietze[35a,b] using a 4-aminophenyl glycoside and reproposed by Hindsgaul[35c] using oligosaccharide amines appears to be an attractive approach. The first stage of the method involves coupling a terminal amino group-containing saccharide with a squaric acid diester. At pH 7, the reaction was reported[35a] to stop at the monoester−monoamide level. Subsequent exposure of the monoester and the protein to pH 9 activates the second alkoxy group and anchors the saccharide−squaric acid construct to the ϵ-amino group of the lysine residues of the protein. The possible use of (hydrazinocarbonyl)-alkyl glycosides was also advanced but not tested.[35c] Tietze reported[35a] an incorporation level of 15 disaccharide residues per molecule of bovine serum albumin (BSA), and the published data indicate that 18% of a disaccharide-squaric acid construct was incorporated in the neoglycoconjugate. Hindsgaul's data[35c] show an incorporation range of 3−10 saccharide chains per molecule of BSA, and the corresponding incorporation yield at the targeted, low incorporation levels is 74% for a monosaccharide and 36−66% for di- to tetrasaccharides. In light of the operational simplicity and the mild conditions of the coupling combined with the reported above-average yields, we decided to examine this method for the attachment of (hydrazinocarbonyl)pentyl glycosides to proteins. We sought to avoid the isolation step previously reported[35] for the monoester−monoamide intermediates and replace the published, two-step pro-

Scheme 11[a]



[a] Reagents and conditions: (a) 2.4 equiv of **36**, 2.7 equiv of 2,6-di-*tert*-butyl-4-methylpyridine, 4 Å molecular sieves, 3.2 equiv of CF_3SO_2OAg, CH_2Cl_2, 23 °C, 2 h, 76%; (b) 1.5 equiv of $PdCl_2$, 8 equiv of NaOAc, AcOH−H_2O, 23 °C, 24 h, 79%; (c) H_2/Pd−C, EtOH−AcOH, 23 °C, 200 psi, 24 h, 86%; (d) NaOMe (cat.), MeOH, 23 °C, 24 h, 73% for two steps; (e) NH_2NH_2 (excess), MeOH, 23 °C, 12 h, 72%.

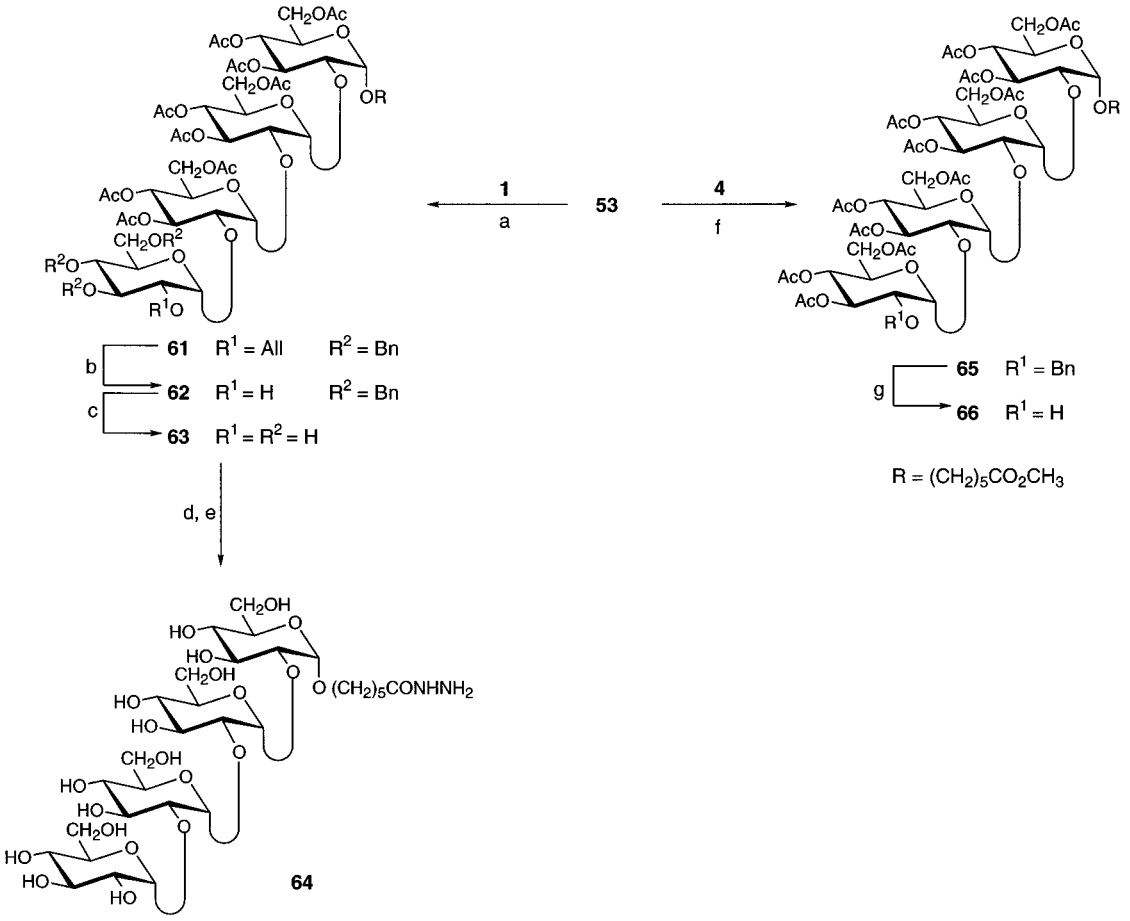
Scheme 12[a]



[a] Reagents and conditions: (a) 1.5 equiv of **45**, $AgClO_4$, Ag_2CO_3, 4 Å molecular sieves, CH_2Cl_2, 23 °C, 12 h, 70%; (b) 1.5 equiv of $PdCl_2$, 8 equiv of NaOAc, AcOH−H_2O, 23 °C, 24 h, 84%.

(35) (a) Tietze, L. F.; Schröter, C.; Gabius, S.; Brinck, U.; Goerlach-Graw, A.; Gabius, H.-J. *Bioconjugate Chem.* **1991**, *2*, 148−153. (b) Tietze, L. F.; Arlt, M.; Beller, M.; Glüsenkamp, K.-H.; Jähde, E.; Rajewsky, M. F. *Chem. Ber.* **1991**, *124*, 1215−12217. (c) Kamath, V. P.; Diedrich, P.; Hindsgaul, O. *Glycoconjugate J.* **1996**, *13*, 315−319.

Scheme 13[a]



[a] Reagents and conditions: (a) 1.25 equiv of **53**, $AgClO_4$, Ag_2CO_3, 4 Å molecular sieves, CH_2Cl_2, 23 °C, 48 h, 54%; (b) 1.5 equiv of $PdCl_2$, 8 equiv of NaOAc, AcOH−H_2O, 23 °C, 24 h, 82%; (c) H_2/Pd−C, EtOH−AcOH, 23 °C, 200 psi, 12 h, 79%; (d) NaOMe (cat.), MeOH, 23 °C, 24 h; (e) NH_2NH_2 (excess), MeOH, 23 °C, 48 h, 73% for two steps; (f) 2 equiv of **4**, $AgClO_4$, Ag_2CO_3, 4 Å molecular sieves, CH_2Cl_2, 23 °C, 48 h, 66%; (g) H_2/Pd−C, EtOH−AcOH, 23 °C, 300 psi, 12 h, 87%.

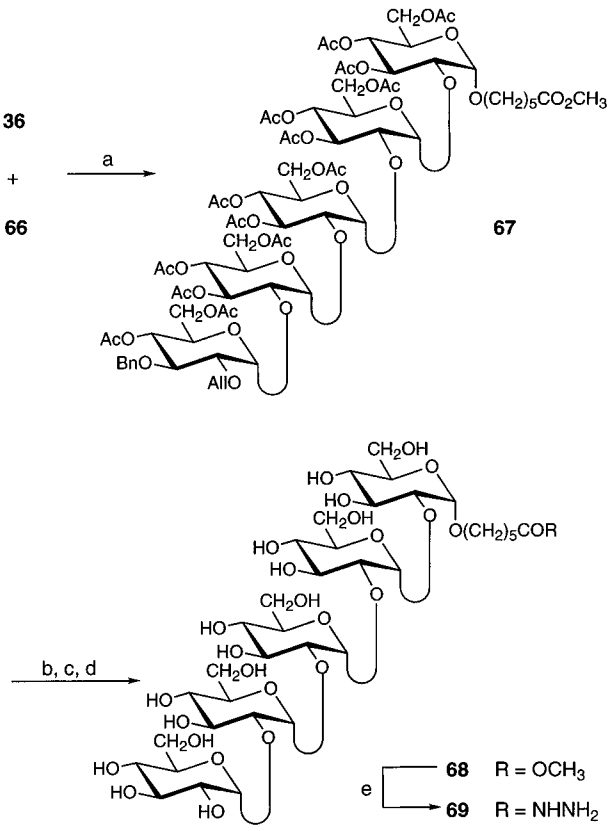
Table 1. Incidence of Mismatch between Oligosaccharide Donor/Acceptor Building Blocks

entry	donor	acceptor	entry	donor	acceptor
1	**3**	**53**	4	**2**	**62**
2	**3**	**60**	5	**4**	**62**
3	**1**	**62**	6	**4**	**66**

tocols with a one-pot procedure. For comparative purposes, the aminoethyl derivative **48** was also prepared by treatment[35c] of the ester **46** with ethylenediamine (Scheme 9) and tested for coupling efficiency. In the exploratory phase the relative carbohydrate content of the conjugates was determined by carbohydrate analysis, while the average incorporation levels of the products were calculated from average molecular masses of the glycoconjugates determined by matrix-assisted laser desorption ionization mass spectroscopy (MALDI).

Treatment of the monosaccharide hydrazide **47** with 1 molar equiv of dimethyl squarate (**70**) in pH 7 phosphate-borate buffer or in methanol containing trisisopropanolamine at 23 °C converted **47** to a faster-moving compound on thin-layer chromatography. At the later stages, the reaction was accompanied by the development of a light-violet color. The reaction mixture was treated with human serum albumin (HSA) at pH 9. In a parallel experiment, the coupling of the saccharide−squaric acid adduct to the protein was tested at pH 10. Similar treatments of the aminoethyl derivative **48** proceeded without color formation. Samples were withdrawn at day 3, and the coupling reaction was terminated after 7 days. Carbohydrate analysis of the neoglycoproteins obtained after exhaustive dialysis followed by freeze-drying indicated that pH 9 promoted slightly higher incorporations into HSA than pH 10, and at pH 9 longer reaction times increased the incorporation level. The neoglycoprotein obtained from the hydrazide **47** also had a slight violet color. Because squarate **70** did not develop color in pH 7 or pH 9 buffers in the absence of the hydrazide **47**, the color must be associated with a construct of **47** with dimethyl squarate, which retains reactivity toward the protein. Treatment of the violet saccharide−HSA conjugates with $NaBH_4$ changed the color to light-yellow without loss of the saccharide content as determined by MALDI. Gel chromatography of the crude saccharide−squarate adduct on Biogel P-2 indicated the presence of several minor compounds in addition to the major one, which, when isolated and reacted with HSA at pH 9, gave a colorless conjugate (procedure A). These observations indicate that the reaction of the hydrazide **47** with **70** is more complex than originally proposed[35] for ω-aminoalkyl/arylglycosides and some of the starting hydrazide and/or squarate may be lost during the first step, which may be inferred from the lower yields (entries 4 and 5 in Table 2) obtained with **47** relative to those found with the aminoethyl derivative **48**. When high incorporation levels were targeted, the saccharide-based yield of incorporation was in the range of 10−20% (Table 2, entries 2, 5, and 6). Even a 3.4:1 molar ratio of the saccharide to protein amino groups allowed the incorporation of 32

Scheme 14[a]



[a] Reagents and conditions: (a) 5.3 equiv of **36**, $AgClO_4$, Ag_2CO_3, 4 Å molecular sieves, CH_2Cl_2, 23 °C, 48 h, 54%; (b) 1.5 equiv of $PdCl_2$, 8 equiv of NaOAc, AcOH−H_2O, 23 °C, 48 h; (c) H_2/Pd−C, MeOH−AcOH, 23 °C, 100 psi, 12 h; (d) NaOMe (cat.), MeOH, 23 °C, 12 h, 69% for three steps; (e) NH_2NH_2 (excess), MeOH, 23 °C, 48 h, 85%.

saccharide moieties only (Table 2, entry 6). Since this appears to be the highest level of incorporation attained so far with the squarate method, the remaining 16 lysine residues of the 58 present[36] in HSA must be buried below the accessible surface of the protein. As expected, lower incorporation targets gave higher yields (Table 2, entries 4, 9, 11−13). Yields similar to those with **47** were achieved for the di- to pentasaccharides (Table 2, entries 9−13); thus, the saccharide portion in this group of compounds does not appear to interfere significantly with the anchoring process on the protein. We conclude that the isolation of the intermediate monoester-monoamide derivative is redundant since the one-pot procedure presented here (procedure B) gave incorporation levels and yields comparable to the previously reported protocols.[35]

Conclusion

While the blockwise strategy appears to be an elegant approach to larger oligosaccharides, classical protecting groups may introduce unfavorable steric demands leading to steric mismatch between the reacting partners. Such "failures" are rarely documented, possibly because of the authors' reluctance to report abortive approaches. Notable exceptions are the reports of Garegg,[37] van Boeckel,[38] Sinaÿ,[39] Hasegawa,[40] and Danishefsky,[41] who described examples of mismatch between donor and acceptor pairs. In the present study several kojibiose donors (**1**−**4**) were synthesized and used for the preparation of kojidextrins in an attempt to evaluate the combined effect of the acyl- and ether-type substituents on the glycosyl donor ability. While we found no significant differences among donors **1**−**4** due to the nature of the protecting groups in cases when condensation with the acceptor moiety did occur, we found several examples of steric mismatch in attempted [2 + *n*, *n* > 2] block condensations between α-(1→2) linked gluco-oligosaccharide moieties. Although the steric mismatch between oligosaccharide synthons is still unpredictable, it is likely that the availability of more examples of this occurrence will improve existing guidelines for designing pathways to oligosaccharides. For example, the reactivity-enhancing properties of the popular benzyl group need to be balanced against its substantial steric requirements, and the success of future oligosaccharide syntheses is likely to depend on the availability of sterically less demanding and/or flexible protecting groups.

Experimental Section

General Methods.[10g] All chemicals were commercial grade and were used without purification. 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. Solvents for chromatography were distilled prior to use. Anhydrous solvents were obtained from Aldrich. All glycosylation reactions were carried out under argon in oven-dried glassware. Column chromatography was performed on silica gel 60 (0.040−0.063 mm). The melting points 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. Coupling constants (*J*) are given in Hz. 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. Abbreviations: Ac = acetyl, All = allyl, Bz = benzoyl, Bn = benzyl, CA = chloroacetyl, MP = 4-methoxyphenyl, SE = 2-(trimethylsilyl)ethyl. Subscripts A−E refer to individual sugar residues with A standing for the reducing end unit.

***N*-(3,4,6-Tri-*O*-acetyl-2-*O*-allyl-*β*-D-glucopyranosyl)piperidine (6).** A mixture of compound[16] **5** (6.6 g, 17.7 mmol), allyl iodide (8.2 mL, 90.0 mmol), and Ag_2O (4.5 g) in DMF (20 mL) was stirred for 2 h at 23 °C. The reaction mixture was filtered and then concentrated. The residue was equilibrated between $CHCl_3$ and H_2O, and the organic layer was dried (Na_2SO_4) and concentrated. Column chromatography of the residue (3:1 hexanes−EtOAc) gave **6** (6.2 g, 85%) as an amorphous white solid: $[\alpha]_D$ +18° (*c* 1.3); NMR ($CDCl_3$) ^1H, δ 5.83 (m, 1 H), 5.26−5.17 (m, 2 H), 5.10 and 4.90 (2 dd, 2 H), 4.40−4.31 and 4.10−4.00 (2 m, 3 H), 4.22 (dd, 1 H, *J* = 4.9, *J* = 12.1), 3.95 (d, 1 H, *J* = 9.2), 3.60−3.50 (m, 2 H), 2.96−2.82 and 2.70−2.58 (2 m, 4 H), 2.07, 2.05, and 2.01 (3 s, 9 H), 1.62−1.40 (m, 6 H); ^{13}C, δ 170.7, 170.2, 169.9, 135.1, 116.8, 95.8, 75.3, 73.7, 72.7, 72.4, 69.0, 62.5, 49.1, 26.1, 24.6, 20.7; CI-MS *m*/*z* 414 [$(M + H)^+$]. Anal. Calcd for $C_{20}H_{31}NO_8$: C, 58.10; H, 7.56. Found: C, 58.26; H, 7.63.

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Table 2. Covalent Attachment of Oligosaccharides 47, 48, 54, 58, 64, and 69 to Human Serum Albumin Using Dimethyl Squarate 70 as the Linker Moiety

entry	saccharide	general procedure[a]	ratio of saccharide to protein amino groups[b]	incorporation level[c] (mol^{-1})	incorporation yield[d] (%)
1	**47**	A	1:1	23	34
2	**47**	B	4:1	28	12
3	**47**	B	1:1	23	40
4	**47**	B	1:1.7	21	61
5	**47**	C	3.6:1	21	10
6	**48**	C	3.4:1	32	17
7	**48**	B	1:1	24	41
8	**48**	C	1:1	22	38
9	**54**	B	1:2	16	55
10	**58**	A	1:2.1	9	33
11	**64**	B	1:2	15	51
12	**64**	C	1:2	11	38
13	**69**	B	1:2	12	42

[a] For general procedure, see the Experimental Section. [b] The molar ratio of the saccharide to protein amino groups is based on the initial amount of the saccharide. [c] Determined by MALDI-TOF mass spectrometry. [d] Based on the initial amount of the saccharide.

3,4,6-Tri-*O*-acetyl-2-*O*-allyl-α,*β*-D-glucopyranose (7). A solution of **6** (6.2 g, 15.0 mmol) and AcOH (5 mL) in 2:1 acetone−H_2O (150 mL) was boiled under reflux for 15 min. The solution was diluted with toluene and concentrated to give hemiacetal[12b] **7** (quantitative, α/*β* ratio ∼4:1) as a colorless syrup, which was used without purification in the next step: NMR ($CDCl_3$) ^{1}H (selected data), *δ* 5.83 (m, 1 H), 5.38 (d, 0.8 H, *J* = 3.7), 4.76 (d, 0.2 H, *J* = 7.8), 3.53 (dd, 0.8 H, *J* = 9.8), 3.32 (dd, 0.2 H, *J* = 8.3), 2.09−2.02 (9 H); ^{13}C, *δ* 170.8, 169.8, 133.8, 118.2, 116.9, 97.3, 90.4, 79.6, 77.7, 74.0, 73.2, 72.2, 71.8, 71.5, 68.7, 68.4, 66.9, 62.3, 62.1, 20.8−20.6.

1,3,4,6-Tetra-*O*-acetyl-2-*O*-allyl-α,*β*-D-glucopyranose (8). A solution of **7** in pyridine (30 mL) was treated with Ac_2O (30 mL) at 23 °C for 1 h. Column chromatographic purification (3:1 hexanes−EtOAc) gave **8** (ref 12b) (5.6 g, 96%, α/*β* ratio ∼1:1) as a colorless syrup: NMR ($CDCl_3$) ^{1}H (selected data), *δ* 6.35 (d, 0.5 H, *J* = 3.6), 5.79 (m, 1 H), 5.61 (d, 0.5 H, *J* = 8.1), 3.65 (dd, 0.5 H, *J* = 9.9), 3.52 (dd, 0.5 H, *J* = 9.2), 2.17−2.02 (12 H); CI-MS *m*/*z* 406 [$(M + NH_4)^+$].

Phenyl 3,4,6-Tri-*O*-acetyl-2-*O*-allyl-1-thio-α,*β*-D-glucopyranoside (9). A solution of **8** (10.0 g, 25.8 mmol), $PhSSiMe_3$ (5.2 mL, 27.1 mmol), and Me_3SiOTf (1.2 mL, 6.5 mmol) in dry $(CH_2Cl)_2$ (250 mL) was stirred under reflux for 12 h. The mixture was cooled to 23 °C and extracted with aqueous $NaHCO_3$ and H_2O. The organic layer was dried (Na_2SO_4) and concentrated. Column chromatography of the residue (3:1 hexanes−EtOAc) gave **9** (9.7 g, 86%; α/*β* ratio 94:6) as an amorphous white solid: NMR ($CDCl_3$) for **9**α ^{1}H, *δ* 5.88 (m, 1 H), 5.76 (d, 1 H, *J* = 5.5), 5.35 (dd, 1 H, *J* = 9.6), 5.32−5.26 and 5.23−5.19 (2 m, 2 H), 5.02 (dd, 1 H, *J* = 10.0), 4.56 (ddd, 1 H, *J* = 2.2, *J* = 5.2), 4.30 (dd, 1 H, *J* = 12.3), 4.28−4.04 (m, 2 H), 3.99 (dd, 1 H), 3.88 (dd, 1 H, *J* = 9.9), 2.06, 2.05, and 2.02 (3 s, 9 H); ^{13}C, *δ* 170.5, 169.8, 133.8, 133.4−127.4, 118.0, 86.3, 71.5, 62.1, 20.8, 20.6; selected data for **9***β* ^{1}H, *δ* 4.64 (d, 1 H, *J* = 9.9); CI-MS *m*/*z* 456 [$(M + NH_4)^+$]. Anal. Calcd for $C_{21}H_{26}O_8S$: C, 57.52; H, 5.98; S, 7.31. Found: C, 57.45; H, 5.94; S, 7.23.

Phenyl 2-*O*-Allyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-glucopyranoside (10). A solution of **9** (9.0 g, 20.5 mmol) in dry MeOH (150 mL) was treated with a catalytic amount of NaOMe at 23 °C for 3 h. The mixture was neutralized (Dowex 50 × 8−100, H^+), filtered, and concentrated. A solution of the residue in dry DMF (80 mL) was treated at 0 °C with NaH (60% in oil, 3.7 g, approx. 92 mmol) and benzyl bromide (8.1 mL, 67.8 mmol) and then was stirred at 23 °C for 3 h. The usual workup afforded a solid from which pure **10** (9.7, 81%) was obtained by crystallization (ether−hexanes): mp 76−78 °C; $[\alpha]_D$ +184° (*c* 1.1); NMR ($CDCl_3$) ^{1}H, *δ* 7.60−7.10 (m, 20 H), 5.95 (m, 1 H), 5.75 (d, 1 H, *J* = 4.2), 5.32 and 5.20 (2 m, 2 H), 4.99, 4.85, 4.79, 4.60, 4.50, and 4.42 (6 d, 12 H, *J* = 10.8−12.0), 4.33 (ddd, 1 H, *J* = 9.9, 1.9), 4.28−4.12 (m, 2 H), 3.87−3.82 (m, 2 H), 3.78 (dd, 1 H, *J* = 3.8, 10.7), 3.75−3.66 (m, 1 H), 3.62 (dd, 1 H); ^{13}C, *δ* 138.2, 137.9, 134.5, 131.4−127.0, 117.9, 86.9, 82.5, 79.7, 77.4, 75.8, 75.1, 73.4, 71.6, 71.1, 68.5; CI-MS *m*/*z* 600 [$(M + NH_4)^+$]. Anal. Calcd for $C_{36}H_{38}O_5S$: C, 74.20; H, 6.57; S, 5.50. Found: C, 74.28; H, 6.61; S, 5.58.

2-*O*-Allyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl Chloride (11). (a) A solution of **10** (1.0 g, 1.7 mmol) in dry CH_2Cl_2 (6 mL) was treated at 0 °C with α,α-dichloromethyl methyl ether (0.78 mL, 8.59 mmol) and Me_3SiOTf (0.16 mL, 0.9 mmol) for 1 h. Diisopropylethylamine (excess) was added, and the solution was concentrated. Column chromatography of the residue (20:1 → 4:1, hexanes-EtOAc containing 0.1% of diisopropylethylamine) gave **11**α (362 mg, 41%) as a colorless syrup: $[\alpha]_D$ +89° (*c* 1.4) [lit.[12b] $[\alpha]_D$ +86° (*c* 2.2, CH_2Cl_2)]; NMR ($CDCl_3$) ^{1}H, *δ* 7.50−7.10 (m, 15 H), 6.20 (d, 1 H, *J* = 3.8), 5.93 (m, 1 H), 5.35−5.30 and 5.24−5.20 (2 m, 2 H), 4.96, 4.84, 4.80, 4.60, 4.51, and 4.48 (6 d, 12 H, *J* = 10.7−12.0), 4.20−4.17 (m, 2 H), 4.12−4.06 (m, 1 H), 4.00 (t, 1 H, *J* = 9.2), 3.81−3.65 (m, 4 H); ^{13}C, *δ* 138.4, 137.9, 137.6, 134.1, 128.4−127.7, 118.2, 93.5, 81.3, 79.8, 76.3, 75.8, 75.2, 73.4, 73.5, 72.1, 67.7.

(b) To a solution of hemiacetal **18** (2.5 g, 5.1 mmol) and DMF (0.1 mL) in CH_2Cl_2 (25 mL) was added at 0 °C oxalyl chloride (1.3 mL, 14.9 mmol). After 4 h the reaction mixture was concentrated. Short column chromatography (1:1 hexanes−EtOAc) of the residue gave **11** (2.4 g, 69%, α/*β* ratio 7:3) as a colorless syrup: NMR ($CDCl_3$) selected data for **11***β* ^{1}H, *δ* 5.16 (d, 0.3 H, *J* = 8.5); ^{13}C, *δ* 134.3, 117.6, 90.4, 72.1, 68.3.

Methyl 3-*O*-Benzyl-4,6-*O*-benzylidene-2-*O*-(4-toluenesulfonyl)-α-D-glucopyranoside (13). A mixture of tosylate[15b] (51.0 g, 97 mmol), benzyl bromide (28 mL, 235 mmol), BaO (117.0 g, 0.8 mol), and $Ba(OH)_2{\cdot}8H_2O$ (31.0 g, 98 mmol) in dry DMF (250 mL) was stirred at 23 °C for 8 h. The mixture was filtered, and the filtrate was diluted with $CHCl_3$. The organic layer was washed with H_2O, dried (Na_2SO_4), and concentrated. Recrystallization of the residue (EtOH) gave **13** (54.3 g, 88%) as a white solid: mp 119−121 °C (lit.[20] mp 121.5−123.5 °C), $[\alpha]_{578}$ +4° (*c* 1.6) [lit.[20] $[\alpha]_{578}$ +2° (*c* 1.1)].

Methyl 3-*O*-Benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (14). A mixture of **13** (30.0 g, 57.0 mmol) and $NaBH_4$ (30.0 g, 0.79 mol) in Me_2SO (80 mL) was stirred at 150 °C for 24 h. After being cooled to 50 °C, the reaction mixture was diluted with H_2O and stirred at 23 °C for 1 h. Filtration afforded a solid that was recrystallized from EtOH to give **14** (15.1 g, 71%): mp 183−184 °C (lit.[20] mp 183−185 °C); $[\alpha]_{578}$ +86° (*c* 1.8) [lit.[20] $[\alpha]_{578}$ +87° (*c* 0.9)].

Methyl 2-*O*-Allyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (15). To a solution of **14** (30.0 g, 80.6 mmol) in dry DMF (100 mL) were added at 0 °C NaH (60% in oil, 4.9 g, approx. 121 mmol) and allyl bromide (8.9 mL, 105.2 mmol). The mixture was stirred at 23 °C for 2 h. The usual workup followed by crystallization (EtOH) gave **15** (29.1 g, 88%) as a white solid: mp 92−94 °C (lit.[15a] mp 93−94 °C); $[\alpha]_D$ +10° (*c* 1.2) [lit.[15a] $[\alpha]_D$ +10.6° (*c* 0.88)]; NMR ($CDCl_3$) ^{1}H, *δ* 7.60−7.20 (m, 10 H), 5.93 (m, 1 H), 5.56 (s, 1 H), 5.29 and 5.20 (2 m, 2 H), 4.88 and 4.83 (2 d, 2 H, *J* = 11.4), 4.80 (d, 1 H, *J* = 3.7), 4.38−4.14 (m, 3 H), 4.00 (dd, 1 H, *J* = 9.2), 3.65−3.49 (m, 2 H), 3.61 (dd, 1 H, *J* = 9.3), 3.52 (dd, 1 H, *J* = 9.3), 3.44 (s, 3 H); ^{13}C, *δ* 138.7, 137.4, 134.8, 128.9−126.0, 117.8, 101.2, 99.2, 82.1, 79.2, 78.5, 75.3, 73.1, 69.1, 62.3, 55.3.

Methyl 2-*O*-Allyl-3-*O*-benzyl-α-D-glucopyranoside (16). A solution of **15** (20.0 g, 48.5 mmol) in AcOH (50 mL) and

H_2O (30 mL) was stirred under reflux for 15 min and then was concentrated under vacuum. Toluene was added and evaporated several times. Column chromatographic purification of the residue (1:1 → 1:0 EtOAc−hexanes) gave **16** (14.3 g, 91%) as a colorless syrup: $[\alpha]_D$ +50° (*c* 1.3) [lit.[15a] $[\alpha]_D$ +48.8° (*c* 1.48)].

Methyl 2-*O*-Allyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (17). A solution of **16** (14.3 g, 44.1 mmol) in dry DMF (60 mL) was treated at 0 °C with NaH (60% in oil, 5.3 g, approximately 132 mmol) and benzyl bromide (13.6 mL, 114.6 mmol). The mixture was stirred for 3 h, during which time it was allowed to reach 23 °C. The solution was cooled to 0 °C and was treated with MeOH and H_2O. Extractive work-up ($CHCl_3/H_2O$) followed by column chromatographic purification (4:1 hexanes−EtOAc) of the residue gave **17** (20.5 g, 92%) as a colorless syrup: $[\alpha]_D$ +44° (*c* 1.3) [lit.[12b] $[\alpha]_D$ +44.5° (*c* 1.2)]; NMR ($CDCl_3$) ^{1}H, δ 7.50−7.10 (m, 15 H), 5.98 (m, 1 H), 5.35−5.15 (m, 2 H), 4.94, 4.83, 4.78, 4.63, 4.49, and 4.47 (6 d, 12 H, J = 10.9−12.2), 4.83 (d, 1 H, J = 3.5), 4.30−4.10 (m, 2 H), 3.94 (t, 1 H, J = 9.3), 3.80−3.60 (m, 4 H), 3.51 (dd, 1 H, J = 9.3), 3.42 (s, 3 H); ^{13}C, δ 138.8, 138.3, 138.0, 134.8, 128.3−127.6, 117.8, 98.2, 82.0, 79.8, 77.6, 75.7, 75.0, 73.5, 72.6, 70.1, 68.5, 55.1.

2-*O*-Allyl-3,4,6-tri-*O*-benzyl-α,β-D-glucopyranose (18). A solution of **17** (14.4 g, 28.6 mmol) in a 4:1 mixture 1 M aqueous HCl and AcOH (250 mL) was stirred under reflux for 90 min. The usual workup afforded **18** (9.6 g, 68%, α/β ratio 7:3) as a white solid: mp 135−136 °C (lit.[12b] mp 134−136 °C); NMR ($CDCl_3$) **18**α ^{13}C, δ 138.2, 137.9, 134.5, 127.9−127.6, 117.9, 91.3, 81.6, 80.0, 77.6, 75.7, 75.0, 73.5, 72.4, 70.3, 68.6; **18**β ^{13}C, δ 138.2, 137.9, 134.4, 127.9−127.6, 117.2, 97.3, 84.6, 82.9, 77.7, 75.7, 75.0, 73.7, 72.4, 70.3, 68.9.

***O*-(2-*O*-Allyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (20) and *O*-(2-*O*-Allyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→2)-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (21).** A stirred mixture of **19** (1.9 g, 3.6 mmol), **6** (950 mg, 2.7 mmol), Ag_2CO_3 (843 mg), and 4 Å powdered molecular sieves (3.3 g) in dry CH_2Cl_2 (40 mL) was stirred at 23 °C for 1 h with exclusion of light and then cooled to 0 °C. $AgClO_4$ (310 mg) was added, and the mixture was stirred at 23 °C for 4 h and then filtered through Celite. The insoluble material was washed several times with $CHCl_3$. The combined filtrate and washings were extracted successively with saturated aqueous $NaHCO_3$ and H_2O, dried (Na_2SO_4), and concentrated. The residue in pyridine (7 mL) was treated with Ac_2O (7 mL) and a catalytic amount of 4-(dimethylamino)pyridine for 12 h at 23 °C. Concentration followed by extractive workup ($CHCl_3/H_2O$) and column chromatography (5:2, hexanes−EtOAc) afforded **21** (111 mg, 5%) as a colorless syrup: $[\alpha]_D$ +20.5° (*c* 0.9); NMR ($CDCl_3$) ^{1}H, δ 7.45−7.10 (m, 15 H), 5.89 (m, 1 H), 5.74 (d, 1 H, J = 7.9), 5.28 (t, 1 H, J = 9.5), 5.22 and 5.13 (2 m, 2 H), 5.02 (dd, 1 H, J = 9.8), 4.91−4.46 (m, 6 H), 4.40 (d, 1 H, J = 7.8), 4.31 (dd, 1 H, J = 4.5, 12.5), 4.29−4.22 and 4.15−4.02 (2 m, 3 H), 3.90−3.82 (m, 2 H,), 3.78−3.65 (m, 2 H), 3.62 (dd, 1 H, J = 9.6), 3.51 (t, 1 H, J = 8.9), 3.41−3.35 (m, 1 H), 3.21 (dd, 1 H, J = 8.9), 2.07, 2.05, 2.04, and 2.02 (4 s, 12 H); ^{13}C, δ 170.5, 169.9, 169.7, 169.0, 138.4, 138.0, 137.8, 134.9, 128.3−127.5, 116.9, 103.9, 92.1, 84.7, 81.3, 77.3, 76.7, 75.5, 74.9, 74.6, 74.5, 73.9, 73.4, 72.5, 68.8, 68.1, 61.6, 20.8, 20.7, 20.6; CI-MS m/z 838 [(M + NH_4)$^+$]. Anal. Calcd for $C_{44}H_{52}O_{15}$: C, 64.38; H, 6.39. Found: C, 64.49; H, 6.45.

Further elution gave a 1:1 mixture of **20** and **21** (585 mg, 26.4%) and then **20** (1.24 g, 56%) as a colorless syrup: $[\alpha]_D$ +62° (*c* 1.6); NMR ($CDCl_3$) ^{1}H, δ 7.40−7.05 (m, 15 H), 5.83 (2 m, 1 H), 5.77 (d, 1 H, J = 8.2), 5.30 (t, 1 H, J = 9.6), 5.26 and 5.18 (m, 2 H, 5.19 (d, 1 H, J = 3.3), 5.04 (dd, 1 H, J = 9.8), 4.84, 4.80, 4.73, 4.60, 4.45, and 4.43 (6 d, 6 H, J = 10.8−12.1), 4.31 (dd, 1 H, J = 4.4, J = 12.5), 4.22−4.02 (m, 3 H), 3.43 (dd, 1 H, J = 9.8), 2.13, 2.08, 2.02, and 1.96 (4 s, 12 H); ^{13}C, δ 170.5, 169.9, 169.8, 168.4, 138.5, 138.4, 137.7, 134.5, 128.3−127.4, 117.7, 97.4, 93.5, 81.2, 79.7, 77.0, 75.6, 74.8, 73.6, 73.5, 72.6, 72.5, 71.1, 68.2, 67.8, 61.5, 21.1, 20.8, 20.7, 20.6; CI-MS m/z 838 [(M + NH_4)$^+$]. Anal. Calcd for $C_{44}H_{52}O_{15}$: C, 64.38; H, 6.39. Found: C, 64.30; H, 6.40.

***O*-(2-*O*-Allyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-acetyl)-α,β-D-glucopyranose (22).** A mixture of **20** (1.3 g, 1.6 mmol) and hydrazine acetate (220 mg, 2.4 mmol) in DMF (7 mL) was kept at 23 °C for 2 h. The solution was diluted with EtOAc and then washed with H_2O. The organic layer was dried (Na_2SO_4) and concentrated. Column chromatography (3:2 hexanes−EtOAc) of the residue gave **22** (1.1 g, 92%, α/β ratio ∼ 1:1), as a colorless syrup: NMR ($CDCl_3$) ^{13}C, δ 170.7, 170.1, 169.8, 169.3, 138.2, 137.7, 137.6, 133.9 133.4, 128.4−127.6, 119.5, 118.8, 100.8, 96.8, 96.4, 90.4, 75.7, 74.6, 73.54, 73.49, 73.36, 68.1, 67.8, 62.1, 62.0, 20.8, 20.7, 20.6; CI-MS m/z 796 [(M + NH_4)$^+$]. Anal. Calcd for $C_{42}H_{50}O_{14}$: C, 64.77; H, 6.47. Found: C, 64.72; H, 6.48.

***O*-(2-*O*-Allyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α,β-D-glucopyranosyl Chloride (1).** To a stirred solution of **22** (3.5 g, 5.1 mmol) and DMF (100 μL) in CH_2Cl_2 (30 mL) was added oxalyl chloride (1.20 mL, 13.8 mmol) at 0 °C, dropwise. The solution was allowed to reach 23 °C. After 4 h, the solution was concentrated. The residue was filtered through a layer of silica gel (4.5 g) using 1:1 hexanes−EtOAc as the eluant. The solution was concentrated to give **1** (3.3 g, 92%, α/β ratio ∼ 1:1) as a colorless syrup: NMR ($CDCl_3$) ^{1}H, selected data δ 6.23 (d, 0.5 H, J = 3.9), 5.37 (d, 0.5 H, J = 8.1); ^{13}C, selected data δ 170.6, 170.5, 169.9, 169.8, 169.7, 169.5, 138.6-137.7, 134.51, 134.47, 128.4−127.6, 117.8, 117.6, 97.1, 96.5, 91.0, 89.6, 61.7, 61.3, 20.8−20.5; CI-MS m/z 814 [(M + NH_4)$^+$].

***O*-(2-*O*-Allyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl Trichloroacetimidate (2).** A solution of **22** (200 mg, 257 μmol) in CH_2Cl_2 (7 mL) was treated at 0 °C with trichloroacetonitrile (780 μL, 7.7 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (23 μL, 154 μmol) for 90 min. Removal of the volatiles followed by purification by column chromatography (5:2 hexanes−EtOAc) gave **2** (201 mg, 85%) as a colorless syrup: $[\alpha]_D$ +92° (*c* 1.1); NMR ($CDCl_3$) ^{1}H, δ 8.68 (s, 1 H), 7.45−7.05 (m, 15 H), 6.62 (d, 1 H, J = 3.6), 5.85 (m, 1 H), 5.52 (dd, 1 H, J = 9.7), 5.25 and 5.16 (2 m, 2 H), 5.12 (dd, 1 H, J = 9.9), 5.02 (d, 1 H, J = 3.2), 4.80, 4.67, 4.57, 4.45, 4.43, and 4.29 (6 d, 6 H, J = 10.9−12.1), 4.28 (dd, 1 H, J = 4.2, J = 12.2), 4.24−4.07 (m, 4 H), 4.01 (dd, 1 H, J = 9.8), 3.41 (dd, 1 H, J = 9.8), 2.06, 2.04, and 1.98 (3 s, 9 H); ^{13}C, δ 170.6, 170.0, 169.7, 160.9, 138.7−127.3, 117.4, 97.9, 93.2, 81.2, 79.4, 77.1, 75.4, 74.9, 74.6, 73.5, 72.3, 71.3, 69.9, 68.1, 67.9, 61.6, 20.8, 20.7; CI-MS m/z 941 [(M + NH_4)$^+$]. Anal. Calcd for $C_{44}H_{50}Cl_3NO_{14}$: C, 57.24; H, 5.46. Found: C, 57.15; H, 5.50.

2,4,6-Tri-*O*-acetyl-3-*O*-benzyl-α-D-glucopyranosyl Chloride (24). A solution of **23** (ref 26) (8.0 g, 18.7 mmol), α,α-dichloromethyl methyl ether (8.0 mL, 88.4 mmol), and $ZnCl_2$-Et_2O (2.2 M in CH_2Cl_2, 0.83 mL, 1.8 mmol) in dry CH_2Cl_2 (20 mL) was stirred at 23 °C for 20 min. The reaction mixture was poured in a cold saturated aqueous $NaHCO_3$, diluted with $CHCl_3$, and then washed several times with saturated aqueous $NaHCO_3$ and H_2O until neutralization. The organic layer was dried (Na_2SO_4) and concentrated. Purification of the residue by column chromatography (3:1 hexanes−EtOAc) gave **24** (6.9 g, 91%) as a colorless syrup: $[\alpha]_D$ +100° (*c* 1.0); NMR ($CDCl_3$) ^{1}H, δ 7.40−7.21 (m, 5 H), 6.31 (d, 1 H, J = 3.9), 5.15 (dd, 1 H, J = 9.7, J = 9.9), 4.97 (dd, 1 H, J = 9.8), 4.75 and 4.63 (2 d, 2 H, J = 11.7), 4.25−4.01 (m, 4 H), 2.09, 2.08, and 1.96 (3 s, 9 H); ^{13}C, δ 170.6-169.3, 137.8−127.6, 90.9, 76.6, 75.2, 73.2, 70.9, 68.5, 61.4, 20.7; CI-MS m/z 432 [(M + NH_4)$^+$]. Anal. Calcd for $C_{19}H_{23}ClO_8$: C, 55.01; H, 5.59. Found: C, 55.08; H, 5.56.

2-(Trimethylsilyl)ethyl 2,4,6-Tri-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranoside (25). A mixture of the chloride **24** (10.0 mg, 24.1 mmol), 2-(trimethylsilyl)ethanol (10.4 mL, 72.7 mmol), *sym*-collidine (2.8 mL, 21.1 mmol), and 4 Å powdered molecular sieves (7 g) in dry CH_2Cl_2 (100 mL) was stirred at 23 °C for 3 h and then cooled to 0 °C. AgOTf (11.9 g, 46.3 mmol) was added, and the mixture was stirred at 0 °C for 3 h with exclusion of light. The reaction mixture was neutralized by addition of a saturated aqueous $NaHCO_3$ and then filtered through celite. The insoluble material was washed several times with $CHCl_3$. The combined organic layers were washed successively with saturated aqueous $NaHCO_3$ and H_2O, dried (Na_2SO_4), and then concentrated. The residue was treated with pyridine (25 mL) and Ac_2O (25 mL) for 12 h at 23 °C followed by concentration. Purification of the residue by column chromatography (3:1 hexanes−EtOAc) gave **25** (9.4 g,

79%) as a colorless syrup: $[\alpha]_D$ −21° (*c* 1.9); NMR ($CDCl_3$) ^{1}H, δ 7.50−7.20 (m, 5 H), 5.12 (t, 1 H, J = 9.6), 5.04 (dd, 1 H, J = 9.6), 4.62 and 4.58 (2 d, 2 H, J = 12.2), 4.43 (d, 1 H, J = 7.9), 4.22 (dd, 1 H, J = 5.1, J = 12.2), 4.12 (dd, 1 H, J = 2.7), 4.00−3.90 and 3.62−3.49 (2 m, 3 H), 3.70 (t, 1 H, J = 9.6), 2.08, 2.01, and 1.98 (3 s, 9 H), 1.11−0.82 (m, 2 H), 0.01 (s, 9 H); ^{13}C, δ 169.3, 169.2, 137.8−127.8, 100.7, 80.2, 72.5, 72.0, 69.7, 73.6, 67.2, 62.4, 20.9, 20.8, 17.9, -1.5; CI-MS *m*/*z* 514 [(M + NH_4)$^+$]. Anal. Calcd for $C_{24}H_{36}O_9Si$: C, 58.04; H, 7.31. Found: C, 58.04; H, 7.28.

2-(Trimethylsilyl)ethyl 3-*O*-Benzyl-*β*-D-glucopyranoside (26). A solution of **25** (16.0 g, 32.3 mmol) in dry MeOH (100 mL) was treated with a catalytic amount of NaOMe at 50 °C for 1.5 h. After neutralization (Dowex 50 × 8−100, H^+) the reaction mixture was filtered then concentrated to give **26** (11.9 g, 99%) as an amorphous white solid: $[\alpha]_D$ −20° (*c* 0.5, MeOH); NMR ($CDCl_3$) ^{1}H, δ 7.45−7.27 (m, 5 H), 5.01 and 4.75 (2 d, 2 H, J = 11.6), 4.32 (d, 1 H, J = 7.6), 3.89 (dd, 1 H, J = 3.4, J = 11.8), 3.77 (dd, 1 H, J = 4.8), 1.10−0.90 (m, 2 H), 0.03 (s, 9 H); ^{13}C, δ 138.4, 128.6−128.0, 102.4, 83.6, 75.1, 74.6, 70.2, 74.6, 67.8, 62.6, 18.3, −1.4; CI-MS *m*/*z* 388 [(M + NH_4)$^+$]. Anal. Calcd for $C_{18}H_{30}O_6Si$: C, 58.35; H, 8.16. Found: C, 58.22; H, 8.05.

2-(Trimethylsilyl)ethyl 3-*O*-Benzyl-2-*O*-(chloroacetyl)-4,6-*O*-(4-methoxybenzylidene)-*β*-D-glucopyranoside (27). A solution of **26** (11.8 g, 31.9 mmol), 4-methoxybenzaldehyde dimethyl acetal (10.0 mL, 58.7 mmol), and camphorsulfonic acid (850 mg, 3.7 mmol) in dry DMF (30 mL) was stirred under vacuum at 23 °C for 2 h. The solution was diluted with $CHCl_3$ and washed several times with H_2O. The organic layer was dried (Na_2SO_4) and concentrated. A solution of the residue in pyridine (40 mL) was treated at 0 °C with chloroacetic anhydride (18.0 g, 105.3 mmol), then was allowed to reach 23 °C. After 1 h, the solution was treated at 0 °C with saturated aqueous $NaHCO_3$ and then was diluted with $CHCl_3$. The organic layer was extracted with H_2O, dried (Na_2SO_4), and concentrated. Crystallization of the residue from MeOH gave **27** (13.4 g, 74%) as a solid: mp 58−59 °C; $[\alpha]_D$ −12° (*c* 1.2); NMR ($CDCl_3$) ^{1}H, δ 7.55−7.25 and 7.05−6.90 (2 m, 9 H, aromatic), 5.57 (s, 1 H), 5.07−5.02 (m, 1 H), 4.90 and 4.66 (2 d, 2 H, J = 12.1), 4.52 (d, 1 H, J = 8.1), 4.38 (dd, 1 H, J = 5.0, 10.5), 4.02−3.40 (m, 8 H), 3.85 (s, 3 H), 1.10−0.90 (m, 2 H), 0.03 (s, 9 H); ^{13}C, δ 165.8, 137.2−127.3, 113.7, 101.3, 100.8, 81.6, 78.3, 74.5, 74.2, 68.6, 67.7, 66.3, 55.3, 40.7, 18.1, −1.5; CI-MS *m*/*z* 582 [(M + NH_4)$^+$], 565 [(M + H)$^+$]. Anal. Calcd for $C_{28}H_{37}ClO_8Si$: C, 59.51; H, 6.60. Found: C, 59.30; H, 6.62.

2-(Trimethylsilyl)ethyl 4,6-Di-*O*-acetyl-3-*O*-benzyl-2-*O*-chloroacetyl-*β*-D-glucopyranoside (28). A solution of **27** (13.0 g, 23.0 mmol) in a mixture 1:6 MeOH−EtOAc (140 mL) was treated at 0 °C with tetrafluoroboric acid (54% in diethyl ether, 1.6 mL). The solution was allowed to reach 23 °C. After 1 h, NaOAc was added to the reaction mixture until pH ∼6 was reached, and then the mixture was concentrated. A solution of the residue in $CHCl_3$ was washed with H_2O, dried (Na_2SO_4), and concentrated. Acetylation of the residue [pyridine (40 mL), Ac_2O (40 mL), 12 h, 23 °C] gave after column chromatography (8:1 → 3:1 hexanes−EtOAc) **28** (10.1 g, 83%) as a colorless syrup: $[\alpha]_D$ −16° (*c* 1.1); NMR ($CDCl_3$) ^{1}H, δ 7.40−7.20 (m, 5 H), 5.15 (dd, 1 H, J = 9.6), 5.06 (dd, 1 H, J = 9.5), 4.64 and 4.58 (2 d, 2 H, J = 11.7), 4.46 (d, 1 H, J = 8.0), 4.24 (dd, 1 H, J = 5.1, J = 12.2), 4.13 (dd, 1 H, J = 2.7), 3.99−3.93 and 3.63−3.50 (2 m, 3 H), 3.92 and 3.84 (2 d, 2 H, J = 14.8), 3.74 (t, 1 H, J = 9.5), 2.09 and 2.00 (2 s, 6 H), 1.20−0.85 (m, 2 H), 0.01 (s, 9 H); ^{13}C, δ 170.8, 169.3, 165.6, 137.7−127.9, 100.1, 80.0, 74.2, 74.0, 72.0, 69.8, 67.4, 66.2, 40.5, 20.7, 17.9, −1.5; CI-MS *m*/*z* 548 [(M + NH_4)$^+$]. Anal. Calcd for $C_{24}H_{35}ClO_9Si$: C, 54.28; H, 6.64. Found: C, 54.28; H, 6.59.

2-(Trimethylsilyl)ethyl 4,6-Di-*O*-acetyl-3-*O*-benzyl-*β*-D-glucopyranoside (29). A solution of **28** (5.6 g, 10.5 mmol) in dry dioxane (40 mL) was treated with hydrazine dithiocarbonate (approximately 30 mmol prepared as described in ref 28) at 23 °C for 1 h. The solution was concentrated, and the residue was equilibrated between $CHCl_3$ and H_2O. The organic layer was dried (Na_2SO_4) and concentrated. Column chromatography (3:1 hexanes−EtOAc) of the residue gave **29** (3.7 g, 76%) as a white solid: mp 71−72 °C (diisopropyl ether−hexanes); $[\alpha]_D$ −48° (*c* 1.3); NMR ($CDCl_3$): ^{1}H, δ 7.40−7.25 (m, 5 H), 5.05 (dd, 1 H, J = 9.6, 9.0), 4.86 and 4.70 (2 d, 2 H, J = 11.8), 4.30 (d, 1 H, J = 7.4), 4.24 (dd, 1 H, J = 5.1), 4.01 (dd, 1 H, J = 2.5, 12.2), 4.05−3.96 and 3.66−3.52 (2 m, 5 H), 2.07 and 1.97 (2 s, 6 H), 1.08−0.95 (m, 2 H), 0.04 (s, 9 H); ^{13}C, δ 170.8-169.5, 138.2, 128.4, 127.8, 102.3, 81.3, 74.5, 74.4, 72.0, 69.6, 67.7, 62.5, 20.8, 18.2, −1.4; CI-MS *m*/*z* 472 [(M + NH_4)$^+$]. Anal. Calcd for $C_{22}H_{34}O_8Si$: C, 58.13; H, 7.54. Found: C, 58.14; H, 7.51.

Phenyl 2,4,6-Tri-*O*-acetyl-3-*O*-benzyl-1-thio-*β*-D-glucopyranoside (30). A solution of **23** (ref 26) (28.5 g, 65.1 mmol) in dry CH_2Cl_2 (50 mL) was treated at 0 °C with PhSH (27 mL, 263 mmol) and $BF_3{\cdot}Et_2O$ (9.5 mL, 77 mmol). After 30 min, the mixture was extracted with cold saturated aqueous $NaHCO_3$ and H_2O. The organic layer was dried (Na_2SO_4) and concentrated. The residue was crystallized from diisopropyl ether−hexanes to give **30** (27.8 g, 88%): mp 118−122 °C; $[\alpha]_D$ −15° (*c* 1.3) [lit.[29] mp 108−109 °C (MeOH); $[\alpha]_D$ −16°]; NMR ($CDCl_3$) ^{1}H, δ 7.56−7.10 (m, 10 H), 5.08 and 5.06 (2 t, 2 H), 4.66−4.56 (m, 3 H), 4.23−4.14 (m, 2 H), 3.70 (dd, 1 H, J = 9.0, 9.6), 3.67−3.61 (m, 1 H), 2.08, 2.05, and 1.97 (3 s, 9 H); ^{13}C, δ 170.7, 169.3, 169.2, 132.5, 128.9−127.8, 86.2, 81.5, 76.1, 74.2, 71.3, 69.6, 62.5, 21.0−20.8; CI-MS *m*/*z* 506 [(M + NH_4)$^+$].

Phenyl 3-*O*-Benzyl-1-thio-*β*-D-glucopyranoside (31). A solution of **30** (55.0 g, 113 mmol) in dry MeOH (500 mL) was treated with a catalytic amount of NaOMe at 23 °C for 24 h. The usual workup followed by crystallization (diethyl ether−hexanes) gave **31** (39.0 g, 95%) as a white solid: mp 104−106 °C; $[\alpha]_D$ −64° (*c* 1.3); NMR ($CDCl_3$) ^{1}H, δ 7.57−7.19 (m, 10 H), 5.00 and 4.76 (2 d, 2 H, J = 11.5), 4.57 (d, 1 H, J = 9.0), 3.94−3.30 (2 m, 2 H), 3.40−3.38 (m, 4 H); ^{13}C, δ 132.7, 129.1−128.0, 88.6, 85.0, 79.4, 74.9, 72.6, 69.9, 62.7; CI-MS *m*/*z* 380 [(M + NH_4)$^+$]. Anal. Calcd for $C_{19}H_{22}O_5S$: C, 62.96; H, 6.12. Found: C, 62.85; H, 6.14.

Phenyl 3-*O*-Benzyl-4,6-*O*-(4-methoxybenzylidene)-1-thio-*β*-D-glucopyranoside (32). A solution of **31** (39.0 g, 108 mmol), 4-methoxybenzaldehyde dimethyl acetal (40.0 mL, 235 mmol), and camphorsulfonic acid (2.6 g, 11.2 mmol) in dry DMF (50 mL) was stirred under vacuum at 23 °C for 2 h. To the reaction mixture was added Et_3N (excess), followed by H_2O and hexanes. Filtration of the mixture gave **32** (49.6 g, 96%): mp 148−152 °C; $[\alpha]_D$ −38° (*c* 1.4); NMR ($CDCl_3$) ^{1}H, δ 7.58−7.24 and 6.95−6.87 (2 m, 14 H), 5.54 (s, 1 H), 4.95 and 4.78 (2 d, 2 H, J = 11.5), 4.63 (d, 1 H, J = 9.8), 3.82 (s, 3 H); ^{13}C, δ 133.2, 129.0−127.3, 113.6, 101.2, 88.4, 81.6, 81.0, 74.8, 72.2, 70.8, 68.6, 55.3; CI-MS: *m*/*z* 481 [(M + H)$^+$], 498 [(M + NH_4)$^+$]. Anal. Calcd for $C_{27}H_{28}O_6S$: C, 67.48; H, 5.87. Found: C, 67.22; H, 5.97.

Phenyl 2-*O*-Allyl-3-*O*-benzyl-4,6-*O*-(4-methoxybenzylidene)-1-thio-*β*-D-glucopyranoside (33). T*o* a solution of **32** (49.6 g, 103.3 mmol) in dry DMF (200 mL) were added at 0 °C NaH (60% in oil, 5.8 g, approximately 145 mmol) and allyl bromide (14.5 mL, 171 mmol). The mixture was stirred for 1 h at 23 °C. The usual workup afforded a solid from which pure **33** (51.5 g, 96%) was obtained by crystallization (EtOH): mp 144−147 °C; $[\alpha]_D$ −51° (*c* 1.1); NMR ($CDCl_3$) ^{1}H, δ 7.55−6.90 (m, 14 H), 5.99 (m, 1 H), 5.54 (s, 1 H), 5.36−5.18 (m, 2 H), 4.91 and 4.79 (2 d, 2 H, J = 11.0), 4.70 (d, 1 H, J = 9.8), 4.39−4.33 and 3.74−3.80 (2 m, 5 H), 3.82 (m, 3 H), 3.65 (t, 1 H, J = 9.2), 3.43−3.50 (m, 1 H), 3.65 (dd, 1 H, J = 9.2); ^{13}C, δ 134.6, 132.2, 129.0−127.2, 117.3, 113.6, 101.1, 88.2, 82.8, 81.2, 80.2, 75.3, 74.7, 70.3, 68.6, 55.3; CI-MS *m*/*z* 521 [(M + H)$^+$] and 538 [(M + NH_4)$^+$]. Anal. Calcd for $C_{30}H_{32}O_6S$: C, 69.21; H, 6.20. Found: C, 69.12; H, 6.22.

Phenyl 2-*O*-Allyl-3-*O*-benzyl-1-thio-*β*-D-glucopyranoside (34). A solution of **33** (51.0 g, 98.1 mmol) in a 1:1 mixture MeOH−EtOAc (260 mL) was treated at 0 °C with tetrafluoroboric acid (54% in diethyl ether, 3.0 mL) and stirred at 23 °C for 30 min. NaOAc was added to the reaction mixture until pH ∼6 was reached, and then the mixture was concentrated. A solution of the residue in $CHCl_3$ was extracted with H_2O, dried (Na_2SO_4), and concentrated. Crystallization of the residue from diisopropyl ether gave **34** (30.9 g, 78%): mp 108−110 °C; $[\alpha]_D$ −56° (*c* 1.3); NMR ($CDCl_3$) ^{1}H, δ 7.57−7.21 (m, 10 H), 6.02 (m, 1 H), 5.18−5.38 (m, 2 H), 4.98 and 4.73 (2 d, 2 H, J = 11.3), 4.66 (d, 1 H, J = 9.7), 4.45 and 4.26 (2 m, 2 H), 3.85 (dd, 1 H, J = 3.4, 11.8), 3.72 (dd, 1 H, J = 5.1), 3.54 (t, 1 H, J = 9.0), 3.46 (dd, 1 H, J = 8.5, J = 9.0), 3.37−3.29 (m, 2

H); ^{13}C, δ 134.4, 131.6, 129.0−127.6, 117.6, 87.6, 85.9, 80.5, 79.1, 75.4 74.2, 70.2, 62.7; CI-MS m/z 420 [$(M + NH_4)^+$]. Anal. Calcd for $C_{22}H_{26}O_5S$: C, 65.65; H, 6.51. Found: C, 65.47; H, 6.46.

Phenyl 4,6-Di-*O*-acetyl-2-*O*-allyl-3-*O*-benzyl-1-thio-β-D-glucopyranoside (35). A solution of **34** (33.6 g, 83.8 mmol) in pyridine (30 mL) was treated with Ac_2O (30 mL) at 23 °C for 1 h. The reaction mixture was poured into ice−water. Filtration gave **35** (38.9 g, 96%) as a white solid: mp 78−80 °C; $[\alpha]_D$ −53° (*c* 1.1); NMR ($CDCl_3$) ^{1}H, δ 7.60−7.22 (m, 10 H), 6.06−5.92 (m, 1 H), 5.54 (s, 1 H), 5.32−5.18 (m, 2 H), 5.00 (t, 1 H, J = 9.8), 4.84 and 4.64 (2 d, 2 H, J = 11.3), 4.60 (d, 1 H, J = 9.6), 4.37 (dd, 1 H, J = 5.3, 11.9), 4.26−4.16 and 3.64−3.54 (2 m, 4 H), 4.10 (dd, 1 H, J = 1.6), 3.42 (dd, 1 H, J = 9.0), 2.07 and 1.92 (2 s, 6 H); ^{13}C, δ 170.7, 169.6, 134.4, 132.2, 128.9−127.7, 117.7, 87.5, 83.7, 80.2, 75.8, 75.5, 74.3, 69.5, 62.6, 20.7; CI-MS m/z 504 [$(M + NH_4)^+$]. Anal. Calcd for $C_{26}H_{30}O_7S$: C, 64.18; H, 6.21. Found: C, 63.99; H, 6.26.

4,6-Di-*O*-acetyl-2-*O*-allyl-3-*O*-benzyl-α-D-glucopyranosyl Chloride (36). (a) A solution of the thioglucoside **35** (8.0 g, 16.5 mmol), α,α-dichloromethyl methyl ether (15.2 mL, 168 mmol), and $ZnCl_2$·Et_2O (2.2 M in CH_2Cl_2, 3 mL, 6.6 mmol) in CH_2Cl_2 (30 mL) was stirred at 0 °C for 2 h. The reaction mixture was extracted with cold saturated aqueous $NaHCO_3$ and ice−water. The organic layer was dried (Na_2SO_4) and concentrated. Purification of the residue by column chromatography (3:1 hexanes−EtOAc) gave **36** (6.4 g, 94%) as a colorless syrup: $[\alpha]_D$ +74° (*c* 2.5); NMR ($CDCl_3$): ^{1}H, δ 7.45−7.25 (m, 5 H), 6.16 (d, 1 H, J = 3.4), 6.00−5.87 (m, 1 H), 5.40−5.22 (m, 2 H), 5.07 (dd, 1 H, J = 10.0), 4.88 and 4.64 (2 d, 2 H, J = 11.6), 4.34−4.14 (m, 4 H), 4.05 (dd, 1 H, J = 1.9, 12.3), 3.93 (dd, 1 H, J = 9.4), 3.72 (dd, 1 H, J = 9.3), 2.08 and 1.93 (2 s, 6 H); ^{13}C, δ 170.6-169.5, 138.2, 133.9−127.8, 118.5, 92.8, 79.5, 78.2, 75.4, 72.3, 70.8, 68.3, 61.5 (C-6), 20.7; CI-MS m/z 430 [$(M + NH_4)^+$].

(b) A solution of **37** (6.0 g, 13.7 mmol) in dry CH_2Cl_2 (25 mL) was treated at 0 °C with α,α-dichloromethyl methyl ether (2.25 mL, 24.9 mmol) and $ZnCl_2$·Et_2O (2.2 M in CH_2Cl_2, 0.63 mL, 1.4 mmol) and then was allowed to reach 23 °C. After 30 min, the reaction mixture was processed as described above to give **36** (5.2 g, 91%).

1,4,6-Tri-*O*-acetyl-2-*O*-allyl-3-*O*-benzyl-α,β-D-glucopyranose (37). A solution of **16** (4.8 g, 14.8 mmol) in pyridine (8 mL) and Ac_2O (8 mL) was stirred at 23 °C for 2 h and then was concentrated. A solution of the residue in $CHCl_3$ was sequentially extracted with aqueous $NaHCO_3$, 2% aqueous HCl, and H_2O. The $CHCl_3$ phase was dried (Na_2SO_4) and concentrated. A solution of the residue in Ac_2O (20 mL) was treated at 0 °C with concd H_2SO_4 (0.1 mL). After 3 min, the reaction mixture was poured into cold saturated aqueous $NaHCO_3$. The solution was extracted several times with $CHCl_3$. The combined $CHCl_3$ phase was extracted with saturated aqueous $NaHCO_3$ and H_2O, dried (Na_2SO_4), and concentrated. Column chromatography of the residue (3:1 hexanes−EtOAc) gave **37** (4.71 g, 73%, α/β ratio 4:1) as a colorless syrup: NMR ($CDCl_3$): ^{1}H, δ 7.45−7.25 (m, 5 H), 6.32 (d, 0.8 H, J = 3.6), 5.88 (m, 1 H), 5.57 (d, 0.2 H, J = 8.0), 5.35−5.15 (m, 2 H), 5.10−5.02 (2 t, 1 H), 4.89−4.61 (4 d, 2 H), 3.63 (t, 0.8 H, J = 9.4), 3.52 (dd, 0.2 H, J = 9.0), 2.18, 2.16, 2.09, 2.06, 1.94, 1.93 (6 s, 9 H); ^{13}C, δ 170.3−169.1, 134.3, 134.0, 127.7−126.4, 118.2, 117.3, 93.7, 89.8, 81.6, 80.3, 78.6, 78.4, 75.3, 75.2, 73.9, 72.8, 72.4, 70.0, 68.9, 68.0, 61.94, 61.88, 21.1−20.8; CI-MS: m/z 454 [$(M + NH_4)^+$]. Anal. Calcd for $C_{22}H_{28}O_9$: C, 60.54; H, 6.47. Found: C, 60.45; H, 6.47.

2-(Trimethylsilyl)ethyl *O*-(4,6-Di-*O*-acetyl-2-*O*-allyl-3-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranoside (38). A mixture of **29** (1.7 g, 3.7 mmol), **36** (2.3 g, 5.6 mmol), Ag_2CO_3 (1.8 g), and 4 Å powdered molecular sieves (4 g) in dry CH_2Cl_2 (40 mL) was stirred at 23 °C for 1 h with exclusion of light and then was cooled to 0 °C. $AgClO_4$ (360 mg, 1.3 mmol) was added, and the mixture was stirred at 0 °C for 48 h and then was filtered through Celite. The insoluble material was washed several times with $CHCl_3$, and the combined filtrate and washings were washed successively with saturated aqueous $NaHCO_3$ and H_2O, dried (Na_2SO_4), and concentrated. Column chromatography (4:1 hexanes−EtOAc) of the residue gave an impure fraction from which pure **38** (723 mg, 23%) was obtained by successive deacetylation (NaOMe), chromatography (EtOAc), and reacetylation (pyridine/Ac_2O). Further elution gave **38** (895 mg, 29%, combined yield 52%) as a colorless syrup: $[\alpha]_D$ +20° (*c* 0.8); NMR ($CDCl_3$) ^{1}H, δ 7.45−7.25 (m, 10), 5.89 (m, 1 H), 6.67 (d, 1 H, J = 3.7), 5.40−5.19 (m, 2 H), 5.11 and 4.93 (2 dd, 2 H), 4.90−4.61 (m, 4 H), 4.56 (d, 1 H, J = 7.4), 2.08, 2.00, 1.95, 1.80 (4 s, 12 H), 1.08−0.90 (m, 2 H), 0.02 (s, 9 H); ^{13}C, δ 170.8−169.4, 138.4, 137.4, 134.1, 128.4−127.3, 117.9, 102.7, 95.3, 80.8, 79.2, 78.6, 75.6, 75.1, 74.8, 72.2, 71.8, 70.7, 69.0, 67.5, 67.3, 62.4, 61.4, 20.8, 18.5, −1.5 (CH_3Si); CI-MS m/z 848 [$(M + NH_4)^+$]. Anal. Calcd for $C_{42}H_{58}O_{15}Si$: C, 60.71; H, 7.04. Found: C, 60.49; H, 7.07

***O*-(4,6-Di-O-acetyl-2-*O*-allyl-3-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α,β-D-glucopyranose (39).** A solution of **38** (820 mg, 988 μmol) in dry CH_2Cl_2 (5 mL) was treated at 0 °C with CF_3CO_2H (4 mL). The mixture was allowed to reach 23 °C. After 12 h, the solution was diluted with toluene and concentrated. Toluene was added to and evaporated from the residue several times. Column chromatographic purification (1:1 hexanes−EtOAc) gave **39** (631 mg, 87%) as a colorless syrup: NMR ($CDCl_3$) ^{13}C, δ 170.8−169.4, 138.1, 137.4, 134.8, 133.7, 128.4−127.6, 119.0, 118.9, 97.4, 97.0, 95.6, 90.0, 62.3, 62.1, 61.8, 61.5, 20.8; CI-MS m/z 748 [$(M + NH_4)^+$]. Anal. Calcd for $C_{37}H_{46}O_{15}$: C, 60.81; H, 6.34. Found: C, 60.67; H, 6.33.

***O*-(4,6-Di-O-acetyl-2-*O*-allyl-3-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α,β-D-glucopyranosyl Chloride (3).** To a solution of **39** (1.0 g, 1.4 mmol) and DMF (150 μL) in CH_2Cl_2 (15 mL) was added at 0 °C oxalyl chloride (400 μL, 4.6 mmol) dropwise. The solution was stirred at 23 °C for 5 h and then was concentrated. Short column chromatography (3:2 hexanes-EtOAc) of the residue gave **3** (840 mg, 82%) as a colorless syrup: NMR ($CDCl_3$) ^{1}H (selected data for **3**α), δ 7.40−7.20 (m, 5 H, aromatic), 6.30 (d, 1 H, J = 2.9), 5.95 (m, 1 H), 5.38−5.20 (m, 2 H), 5.08 (d, 1 H, J = 3.6), 5.00 (dd, 1 H, J = 9.5, 10.0), 4.86 and 4.83 (2 d, 2 H, J = 11.7), 3.57 (dd, 1 H, J = 9.7), 2.09, 2.01, 1.94, 1.84 (4 s, 12 H); ^{13}C, δ 170.6−169.4, 138.3, 134.3−127.6, 118.2, 94.7, 91.0, 79.3, 78.3, 77.5, 75.7, 75.2, 72.4, 70.9, 68.9, 68.6, 68.5, 61.7, 61.4, 20.7; CI-MS m/z 766 [$(M + NH_4)^+$]. Anal. Calcd for $C_{37}H_{45}ClO_{14}$: C, 59.32; H, 6.05. Found: C, 59.15; H, 6.12.

***O*-(3,4,6-Tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α,β-D-glucopyranosyl Chloride (4).** To a solution of **40** (3.4 g, 5.0 mmol) and DMF (250 μL) in dry CH_2Cl_2 (40 mL) was added oxalyl chloride (0.50 mL, 6.6 mmol) dropwise at 0 °C. The mixture was stirred at 23 °C for 20 min. Concentration followed by a short column chromatography (1:1 hexanes−EtOAc) of the residue gave **4** (2.9 g, 83%, α/β ratio 3:2) as a colorless syrup: NMR ($CDCl_3$) ^{13}C, selected data δ 170.6−169.6, 137.4, 128.7−127.9, 97.5, 96.6, 90.8, 89.0, 61.6, 61.5, 61.3, 61.2, 20.8, 20.6, 20.5; CI-MS m/z 720 [$(M + NH_4)^+$]. Anal. Calcd for $C_{31}H_{39}ClO_{16}$: C, 52.96; H, 5.59. Found: C, 52.82; H, 5.56.

5-(Methoxycarbonyl)pentyl 3,4,6-Tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranoside (44). Bromine (320 μL, 6.2 mmol) was added to a solution of thioglucoside **42** (3.0 g, 6.1 mmol) in CH_2Cl_2 (15 mL) at 0 °C. The solution was allowed to reach 23 °C in 5 min. Hex-1-ene (1 mL), ethyldiisopropylamine (1.5 mL, 8.6 mmol), and 5-(methoxycarbonyl)pentanol[32] (8 mL) were added, and then the reaction mixture was concentrated in a rotary evaporator to remove most of the CH_2Cl_2. The residue was kept at 23 °C for 36 h. Column chromatography (3:1 hexanes−EtOAc) of the reaction mixture gave **44** (2.54 g, 80%) as a colorless syrup: $[\alpha]_D$ +75° (*c* 1.5); NMR ($CDCl_3$) ^{1}H, δ 7.40−7.25 (m, 5 H), 5.43 (dd, 1 H, J = 9.6), 4.96 (dd, 1 H, J = 9.8), 4.76 (d, 1 H, J = 3.5), 4.65 and 4.57 (2 d, 2 H, J = 12.3), 4.26 (dd, 1 H, J = 4.5, 12.2), 4.00 (dd, 1 H, J = 2.2), 3.95 (ddd, 1 H), 3.67−3.60 and 3.44−3.35 (2 m, 4 H), 3.67 (s, 3 H), 3.56 (dd, 1 H, J = 10.0), 2.32 (m, 2 H, J = 7.5), 2.07−2.00 (3 s, 9 H), 1.72−1.60 (m, 4 H), 1.46−1.35 (m, 2 H); ^{13}C, δ 170.6, 170.1, 169.9, 137.8−127.8, 96.8, 76.8, 72.9, 71.9, 68.8, 68.4, 67.1, 62.1, 51.5, 33.9, 29.0, 25.6, 24.6, 20.8, 20.7; CI-MS m/z 542 [$(M + NH_4)^+$]. Anal. Calcd for $C_{26}H_{36}O_{11}$: C, 59.53; H, 6.92. Found: C, 59.57; H, 6.89.

5-(Methoxycarbonyl)pentyl 3,4,6-Tri-*O*-acetyl-α-D-glucopyranoside (45). A mixture of **44** (2.5 g, 4.77 mmol), 10% Pd−C (200 mg), EtOH (50 mL), and AcOH (1 mL) was stirred under hydrogen at 23 °C at 200 psi for 24 h. Removal of the catalyst by filtration and the volatiles under vacuum followed by column chromatographic purification (2:1 hexanes−EtOAc) gave **45** (1.7 g, 83%) as a colorless syrup: $[\alpha]_D$ +111° (*c* 1.3); NMR ($CDCl_3$) ^{1}H, δ 5.22 (dd, 1 H, J = 9.8, 9.6), 5.01 (dd, 1 H, J = 10.0), 4.91 (d, 1 H, J = 3.9), 4.27 (dd, 1 H, J = 4.6, 12.3), 4.07 (dd, 1 H, J = 2.3), 3.95 (ddd, 1 H), 3.82−3.62 and 3.56−3.46 (2 m, 3 H), 3.69 (s, 3 H), 2.35 (m, 2 H, J = 7.3), 2.09, 2.08, 2.04 (3 s, 9 H), 1.76−1.62 (m, 4 H), 1.48−1.34 (m, 2 H); ^{13}C, δ 173.9, 171.0, 170.6, 169.5, 98.2, 73.5, 70.8, 68.4, 68.0, 67.6, 62.0, 51.7, 33.8, 28.9, 25.6, 24.5, 20.9, 20.7, 20.6; CI-MS *m*/*z* 452 [(M + NH_4)$^+$]. Anal. Calcd for $C_{19}H_{30}O_{11}$: C, 52.53; H, 6.96. Found: C, 52.36; H, 6.90.

5-(Methoxycarbonyl)pentyl α-D-Glucopyranoside (46). A solution of **45** (1.6 g, 3.7 mmol) in dry MeOH (10 mL) was treated with NaOMe (cat.) at 23 °C for 12 h. The reaction mixture was treated with Dowex (50 × 8−100, H^+), filtered, and then concentrated to give **46** (1.1 g, quantitative) as an amorphous white solid: $[\alpha]_D$ +101° (*c* 0.4, H_2O); NMR (D_2O) ^{1}H, δ 4.89 (d, 1 H, J = 3.9), 3.69 (s, 3 H), 2.41 (m, 2 H, J = 7.4), 1.72−1.58 (m, 4 H), 1.47−1.34 (m, 2 H); ^{13}C, δ 178.4, 98.8, 73.9, 72.5, 72.1, 70.3, 68.7, 61.3, 52.9, 34.4, 29.0, 25.7, 24.8; CI-MS *m*/*z* 326 [(M + NH_4)$^+$]. Anal. Calcd for $C_{13}H_{24}O_8$: C, 50.64; H, 7.85. Found: C, 50.66; H, 7.90.

5-(Hydrazinocarbonyl)pentyl α-D-Glucopyranoside (47). A solution of **46** (1.0 g, 3.1 mmol) in dry EtOH (5 mL) was treated with hydrazine hydrate (2.5 mL) at 23 °C for 3 days. The usual workup followed by column chromatographic purification of the residue (1:1 EtOAc−MeOH) gave **47** (800 mg, 80%) as an amorphous white solid: $[\alpha]_D$ +99° (*c* 0.2, H_2O); NMR (D_2O) ^{1}H, δ 4.90 (d, 1 H, J = 3.5), 2.23 (m, 2 H, J = 7.4), 1.73−1.55 (m, 4 H), 1.45−1.28 (m, 2 H); ^{13}C, δ 176.5, 98.8, 73.9, 72.5, 72.1, 70.3, 68.7, 61.3, 34.4, 29.0, 25.3 (2C); CI-MS *m*/*z* 309 [(M + H)$^+$], 326 [(M + NH_4)$^+$]. Anal. Calcd for $C_{12}H_{24}N_2O_7$: N, 9.09. Found: N, 9.25.

5-[[(2-Aminoethyl)amino]carbonyl]pentyl α-D-Glucopyranoside (48). A solution of the ester **46** (250 mg, 0.8 mmol) in ethylenediamine (2 mL) was stirred at 80 °C for 12 h. The solution was concentrated under vacuum. Water was added to and evaporated from the residue several times. Column chromatographic purification of the residue (MeOH−EtOAc 1:1 then MeOH) afforded **48** (225 mg, 83%): $[\alpha]_D$ +51° (*c* 0.4); NMR (D_2O) ^{1}H, δ 4.91 (d, 1 H, J = 3.8), 3.26 (t, 2 H), 2.74 (t, 2 H), 2.29 (m, 2 H, J = 7.4), 1.71−1.58 (m, 4 H), 1.47−1.32 (m, 2 H); ^{13}C, δ 98.8, 73.9, 72.6, 72.1, 70.4, 68.7, 61.3, 42.4, 40.6, 36.4, 29.1, 25.9, 25.7; CI-MS *m*/*z* 337 [(M + H)$^+$] and 350 [(M + NH_4)$^+$]. Anal. Calcd for $C_{14}H_{28}N_2O_7$: N, 8.33. Found: N, 8.24.

5-(Methoxycarbonyl)pentyl *O*-(2-*O*-Allyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (49) and 5-(Methoxycarbonyl)pentyl *O*-(2-*O*-Allyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (50). A mixture of **45** (200 mg, 4.6 mmol), **11** (330 mg, 6.5 mmol), Ag_2CO_3 (145 mg, 526 μmol), and 4 Å powdered molecular sieves (160 mg) in dry CH_2Cl_2 (4 mL) was stirred at 23 °C for 1 h with exclusion of light and then cooled to 0 °C. $AgClO_4$ (50 mg, 241 μmol) was added, and the mixture was stirred at 0 °C for 80 min and then was filtered through celite. The insoluble material was washed several times with $CHCl_3$, and the combined filtrate and washings were washed successively with saturated aqueous $NaHCO_3$ and H_2O, dried (Na_2SO_4), and then concentrated. Column chromatography (2:1 hexanes−EtOAc) of the residue gave **49** (73.2 g, 17.3%) as a colorless syrup: $[\alpha]_D$ +51° (*c* 0.4); NMR ($CDCl_3$); ^{1}H, δ 7.40−7.10 (m, 15 H), 5.88 (m, 1 H), 5.47 (dd, 1 H, J = 9.6), 5.25 and 5.12 (2m, 2 H), 5.07 (d, 1 H, J = 3.6), 4.99 (dd, 1 H, J = 9.9), 4.92, 4.81, 4.76, 4.56, 4.48, 4.47 (6 d, 12 H, J = 10.7−12.1), 4.40 (d, 1 H, J = 7.7), 4.29 (dd, 1 H, J = 4.4, 12.3), 4.28−4.18 and 4.14−3.98 (2 m, 4 H), 3.76 (dd, 1 H, J = 9.8), 3.73−3.36 (m, 7 H), 3.63 (s, 3 H), 3.28 (dd, 1 H, J = 8.5), 2.30 (m, 2 H, J ∼ 7.5), 2.09, 2.02 (2 s, 9 H), 1.70−1.54 (m, 4 H), 1.44−1.32 (m, 2 H); ^{13}C, δ 170.7, 170.3, 169.8, 138.5, 138.0, 137.9, 135.0, 128.4−127.6, 116.7, 104.6, 98.5, 84.6, 81.3, 77.4, 77.1, 75.7, 75.0, 74.6, 73.5, 73.4, 71.8, 69.0, 68.9, 68.6, 67.0, 62.1, 51.5, 33.9, 29.0, 25.6, 24.6, 21.0, 20.8, 20.7; CI-MS *m*/*z* 924 [((M + NH_4)$^+$]. Anal. Calcd for $C_{49}H_{62}O_{15}$: C, 66.05; H, 7.01. Found: C, 66.12; H, 7.05. Further elution gave **50** (238 mg, 57%) as a colorless syrup: $[\alpha]_D$ +100° (*c* 1.1); NMR ($CDCl_3$) ^{1}H, δ 7.40−7.05 (m, 15 H), 5.89 (m, 1 H), 5.43 (dd, 1 H, J = 9.5, 9.7), 5.27 and 5.18 (2 m, 2 H), 5.02 (d, 1 H, J = 3.6), 4.99 (t, 1 H, J = 9.7), 4.95 (d, 1 H, J = 3.3), 4.88, 4.81, 4.74, 4.58, 4.46, 4.45 (6 d, 12 H, J = 10.9−12.1), 4.27 (dd, 1 H, J = 4.4, 12.2), 3.63 (s, 3 H), 3.45 (dd, 1 H, J = 9.5), 2.31 (m, 2 H, J = 7.5), 2.09, 2.03, 1.96 (3 s, 9 H), 1.74−1.60 (m, 4 H), 1.50−1.34 (m, 2 H); ^{13}C, δ 170.6, 170.0, 169.8, 138.7, 138.5, 137.8, 134.7, 128.3−127.4, 117.3, 97.0, 96.4, 81.4, 79.6, 77.2, 75.5, 75.4, 74.8, 73.5, 72.1, 71.6, 71.0, 68.8, 68.7, 68.1, 67.2, 62.1, 51.4, 33.9, 29.1, 25.7, 24.7, 20.9, 20.8, 20.7; CI-MS *m*/*z* 924 [((M + NH_4)$^+$]. Anal. Calcd for $C_{49}H_{62}O_{15}$: C, 66.05; H, 7.01. Found: C, 66.16; H, 6.99.

5-(Methoxycarbonyl)pentyl *O*-(3,4,6-Tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (52). Treatment of **45** (3.6 g, 8.2 mmol) with the chloride **51** (6.0 g, 14.5 mmol) in the presence of Ag_2CO_3 (8.0 g, 29.0 mmol), 4 Å powdered molecular sieves (3 g), and $AgClO_4$ (600 mg, 2.9 mmol) in dry CH_2Cl_2 (60 mL) at 23 °C for 24 h under the conditions described for the preparation of **49** gave, after column chromatography (2:1 hexanes−EtOAc), **52** (5.2 g, 78%) as a colorless syrup: $[\alpha]_D$ +119° (*c* 0.9); NMR ($CDCl_3$) ^{1}H, δ 7.40−7.25 (m, 5 H), 5.44 (2 t, 2 H), 4.98 (d, 1 H, J = 3.7), 4.96 (2 t, 2 H), 4.88 (d, 1 H, J = 3.3), 4.62 and 4.57 (2 d, 2 H, J = 12.0), 4.31−4.00 (m, 6 H), 3.54 (dd, 1 H, J = 9.4), 3.69−3.47 (m, 3 H), 3.66 (s, 3 H), 2.33 (m, 2 H), 2.09, 2.08, 2.04, 2.03, 2.01, and 1.98 (6 s, 18 H), 1.72−1.59 (m, 4 H), 1.47−1.38 (m, 2 H); ^{13}C, δ 174.0, 170.5−169.7, 137.4, 128.5−127.5, 98.1, 96.8, 77.6, 76.8, 73.1, 71.7 (2C), 68.9, 68.6, 68.4, 68.1, 67.2, 62.0, 61.4, 51.4, 33.8, 29.0, 25.6, 24.6, 20.8, 20.7, 20.6; CI-MS *m*/*z* 830 [((M + NH_4)$^+$]. Anal. Calcd for $C_{38}H_{52}O_{19}$: C, 56.15; H, 6.45. Found: C, 56.01; H, 6.48.

5-(Methoxycarbonyl)pentyl *O*-(3,4,6-Tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (53). Treatment of **52** (5.2 g, 6.4 mmol) with 10% Pd−C (300 mg) in EtOH (50 mL) and AcOH (1 mL) under H_2 at 23 °C at 300 psi for 24 h under the conditions described for the preparation of **45** gave **53** (4.0 g, 86%) as a white crystalline material (^{i}Pr_2O): mp 97−98 °C; $[\alpha]_D$ +153° (c 1.3); NMR ($CDCl_3$) ^{1}H, δ 5.43 (dd, 1 H, J = 9.9, 9.6), 5.12 (dd, 1 H, J = 9.6), 5.02 (dd, 1 H, J = 9.8), 5.01 (d, 1 H, J = 3.6), 4.99 (t, 1 H, J = 9.6), 4.96 (d, 1 H, J = 3.7), 4.29 and 4.25 (2 dd, 2 H, J = 4.6, 12.2), 4.08 (2 dd, 2 H), 4.02−3.93 (m, 2 H), 3.89 (dd, 1 H, J = 9.9), 3.78−3.60 and 3.50−3.40 (2 m, 3 H), 3.68 (s, 3 H), 2.47 (d, 1 H, J = 11.4), 2.34 (m, 2 H, J ∼ 7.5), 2.11, 2.09, 2.07, 2.06, 2.05, and 2.02 (6 s, 18 H), 1.72−1.61 (m, 4 H), 1.47−1.36 (m, 2 H); ^{13}C, δ 170.7−169.5, 96.7, 95.4, 74.6, 73.1, 71.0, 70.5, 68.5, 68.4, 68.3, 67.6, 67.4, 61.9, 61.6, 51.5, 33.8, 29.0, 25.6, 24.5, 20.9, 20.71, 20.67, 20.6; CI-MS *m*/*z* 740 [((M + NH_4)$^+$]. Anal. Calcd for $C_{31}H_{46}O_{19}$: C, 51.52; H, 6.42. Found: C, 51.42; H, 6.37.

5-(Hydrazinocarbonyl)pentyl *O*-α-D-Glucopyranosyl-(1→2)-α-D-glucopyranoside (54). A solution of **53** (1.0 g, 1.4 mmol) in dry MeOH (20 mL) was treated with a catalytic amount of NaOMe at 23 °C for 16 h. The mixture was neutralized (Dowex 50 × 8−100, H^+), filtered, and concentrated. A solution of the crude residue in EtOH (5 mL) was treated with hydrazine hydrate (1.5 mL) at 23 °C for 12 h. The usual workup followed by column chromatographic purification of the residue (MeOH) gave **54** (475 mg, 72%), which was obtained as an amorphous white solid after freeze-drying: $[\alpha]_D$ + 131° (*c* 0.9, H_2O); NMR (D_2O) ^{1}H, δ 5.15 and 5.08 (2 d, 2 H, J = 3.7), 2.23 (m, 2 H, J ∼ 7.2), 1.74−1.58 (m, 4 H), 1.44−1.32 (m, 2 H); ^{13}C, δ 176.6, 96.7, 95.9, 75.7, 73.5, 72.6, 72.4, 72.2, 72.1, 70.3, 70.2, 68.7, 61.3, 61.1, 34.4, 28.9, 25.67, 25.64; FAB-MS *m*/*z* 471 [((M + 1)$^+$], 493 [((M + Na)$^+$]. Anal. Calcd for $C_{18}H_{34}N_2O_{12}$: N, 5.95. Found: N, 5.53.

5-(Methoxycarbonyl)pentyl *O*-(4,6-Di-O-acetyl-2-*O*-allyl-3-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (55). A mixture of **53** (7.7 g, 10.7 mmol), **36** (10.6 g, 25.7 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (6.0 g, 29.2 mmol), and 4 Å powdered molecular sieves (5 g) in dry CH_2Cl_2 (110 mL) was stirred at 23 °C for 1 h and then was

treated with AgOTf (9.0 g, 35 mmol). After 20 min, the reaction mixture was processed as described for **49** to give, after column chromatography (3:2 hexanes−EtOAc), **55** (8.9 g, 76%) as a colorless syrup: $[\alpha]_D$ +95° (*c* 1.4); NMR ($CDCl_3$) ^{1}H, δ 7.39−7.22 (m, 5), 6.01−5.86 (m, 2 H), 5.47 and 5.43 (2 t, 2 H), 5.35−5.28 (m, 2 H), 5.18, 5.03, and 4.88 (3 d, 3 H, J = 3.3−3.6), 4.79 and 4.58 (2 d, 2 H, J = 11.5), 3.67 (s, 3 H), 2.37 (m, 2 H, $J \sim$ 7.4), 2.09 (6 H), 2.08, 2.07, 2.06 (6 H), 2.00, 1.98, and 1.90 (6 s, 24 H), 1.80−1.66 (m, 4 H), 1.58−1.44 (m, 2 H); ^{13}C, δ 170.6−169.5, 138.0, 134.1, 128.5−127.9, 119.3, 98.9, 98.2, 97.5, 79.4, 78.9, 78.6, 75.4, 73.3, 72.4, 70.8, 69.1, 69.0, 68.9, 68.8 (2C), 68.6, 68.0, 67.3, 62.2, 61.7, 61.3, 51.5, 34.0, 29.1, 25.8, 24.7, 21.0−20.7; CI-MS *m*/*z* 1116 [((M + NH_4)$^+$]. Anal. Calcd for $C_{51}H_{70}O_{26}$: C, 55.73; H, 6.42. Found: C, 55.65; H, 6.45.

5-(Methoxycarbonyl)pentyl *O*-(4,6-Di-O-acetyl-3-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (56). A mixture of **55** (3.7 g, 3.4 mmol), NaOAc (2.2 g, 27.3 mmol), and $PdCl_2$ (910 mg, 5.1 mmol) in a 20:1 mixture of AcOH−H_2O (30 mL) was stirred at 23 °C for 24 h and then was filtered through Celite. The insoluble material was washed several times with $CHCl_3$. The combined filtrate and washings were extracted successively with H_2O, aqueous $NaHCO_3$, and H_2O. The organic layer was dried (Na_2SO_4) and then concentrated. Column chromatography (1:1 hexanes−EtOAc) of the residue gave **56** (2.87 g, 79%) as a colorless syrup: $[\alpha]_D$ +131° (c 2.1); NMR ($CDCl_3$); ^{13}C δ 170.5−169.3, 138.2, 128.3−127.6, 96.4, 95.4, 93.0, 79.0, 74.6, 74.1, 72.9, 72.3, 71.2, 70.3, 68.9, 68.7, 68.6, 68.3, 68.1, 67.9, 67.5, 61.9(2C), 61.3, 51.4, 33.7, 29.0, 25.6, 24.5, 20.6; CI-MS *m*/*z* 1076 [((M + NH_4)$^+$]. Anal. Calcd for $C_{48}H_{66}O_{26}$: C, 54.44; H, 6.28. Found: C, 54.33; H, 6.32.

5-(Methoxycarbonyl)pentyl *O*-α-D-Glucopyranosyl-(1→2)-*O*-α-D-glucopyranosyl-(1→2)-*O*-α-D-glucopyranoside (57). Compound **55** (1.0 g, 945 μmol) was sequentially debenzylated [H_2, 10% Pd−C (600 mg), in EtOH (40 mL), AcOH (0.5 mL), 23 °C, 200 psi, 24 h] and deacetylated [MeOH (15 mL), NaOMe (cat.), 23 °C, 24 h] under conditions described for **52** to give **57** (436 mg, 73%) as a colorless syrup: $[\alpha]_D$ +157° (*c* 1.2, MeOH); NMR (D_2O) ^{1}H, δ 5.33, 5.19, and 5.13 (3 d, 3 H, J = 3.4−3.7), 2.42 (m, 2 H, $J \sim$ 7.4), 1.72−1.58 (m, 4 H), 1.46−1.34 (m, 2 H); ^{13}C, δ 178.4, 96.6, 96.2, 94.4, 68.6, 61.3, 61.2, 61.1, 52.9, 34.4, 29.1, 25.8, 24.9; FAB-MS *m*/*z* 633 [(M + 1)$^+$]. Anal. Calcd for $C_{25}H_{44}O_{18}$·1.5 H_2O: C, 45.52; H, 7.18. Found: C, 45.76; H, 7.00.

5-(Hydrazinocarbonyl)pentyl *O*-α-D-Glucopyranosyl-(1→2)-*O*-α-D-glucopyranosyl-(1→2)-α-D-glucopyranoside (58). Treatment of **57** (350 mg, 554 μmol) in MeOH (5 mL) with hydrazine hydrate (0.5 mL) at 23 °C for 48 h under the conditions described for **54** gave, after column chromatography (MeOH) and freeze-drying, **58** (252 mg, 72%) as an amorphous white solid: $[\alpha]_D$ +154° (*c* 0.9, H_2O); NMR (D_2O) ^{1}H, δ 5.33, 5.19, and 5.13 (3 d, 3 H, J = 3.4−3.7), 2.28−2.20, 1.78−1.58 (m, 4 H), 1.42−1.36 (m, 2 H); ^{13}C, δ 176.6, 96.7, 96.1, 94.5, 68.5, 61.3, 61.2, 61.1, 34.4, 29.1, 25.73, 25.66; FAB-MS *m*/*z* 633 [(M + 1)$^+$], 655 [((M + Na)$^+$]. Anal. Calcd for $C_{24}H_{44}N_2O_{17}$: N, 4.43. Found: N, 4.39.

5-(Methoxycarbonyl)pentyl *O*-(4,6-Di-*O*-acetyl-2-*O*-allyl-3-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-*O*-(4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (59). Treatment of **45** (522 mg, 1.2 mmol) with the chloride **3** (600 mg, 0.80 mmol) in the presence of Ag_2CO_3 (420 mg, 1.5 mmol), 4 Å powdered molecular sieves (600 mg), and $AgClO_4$ (145 mg, 700 mmol) in dry CH_2Cl_2 (20 mL) at 23 °C for 12 h under the conditions described for **49** gave, after column chromatography (2:3 hexanes−EtOAc), **59** (645 mg, 70%) as a colorless syrup: $[\alpha]_D$ +105° (*c* 1.0); NMR ($CDCl_3$) ^{13}C, δ 170.7−169.5, 138.1, 137.6, 134.3, 128.4−127.8, 118.8, 97.2, 96.8, 95.4, 78.7, 78.6, 77.7, 76.9, 76.0, 75.07, 74.99, 72.6, 71.0, 69.4, 69.2, 69.1, 68.6, 68.5, 68.2, 67.2, 62.1, 61.6 (2C), 51.5, 33.9, 29.3, 25.9, 24.7, 20.7; FAB-MS *m*/*z* 1279 [(M + Cs)$^+$]. Anal. Calcd for $C_{56}H_{75}ClO_{25}$: C, 58.63; H, 6.50. Found: C, 58.53; H, 6.48

5-(Methoxycarbonyl)pentyl *O*-(4,6-Di-*O*-acetyl-3-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-*O*-(4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (60). A mixture of **59** (350 mg, 0.30 mmol), $PdCl_2$ (81 mg, 0.47 mmol), and NaOAc·3H_2O (330 mg, 2.4 mmol) in 20:1 AcOH−H_2O (10 mL) was stirred at 23 °C for 24 h and then processed as described for compound **56** to afford the alcohol **60** (337 mg, 84%) as a colorless syrup: NMR ($CDCl_3$) ^{1}H, δ 7.43−7.09 (m, 10 H), 5.53 (t, 1 H, J = 9.8), 5.13, 5.10, and 4.95 (3 d, 3 H, J = 3.4−3.6), 5.03 (2H) and 4.96 (2 t, 3 H), 4.93, 4.76, 4.62, and 4.56 (4 d, 4 H, J = 10.9−11.5), 3.66 (s, 3 H), 2.34 (t, 2 H, J = 7.6), 2.12, 2.09 (6H), 2.07, 2.03, 1.92, and 1.90 (6 s, 21 H), 1.76−1.61 (m, 4 H), 1.50−1.35 (m, 2 H); ^{13}C, δ 170.9−169.4, 137.8, 137.4, 128.5−127.7, 95.7, 94.8, 92.0, 75.5 75.4, 61.9, 61.7, 61.5, 51.5, 33.8, 29.0, 25.6, 24.7, 20.8; FAB-MS *m*/*z* 1129 [((M + Na)$^+$].

5-(Methoxycarbonyl)pentyl *O*-(2-*O*-Allyl-3,4,6-tri-*O*-benzyl-α-D-glucopyraosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (61). Treatment of **53** (3.8 g, 4.0 mmol) with the chloride **1** (3.2 g, 5.3 mmol) in the presence of Ag_2CO_3 (3.1 g, 11.2 mmol), 4 Å powdered molecular sieves (5.0 g), and $AgClO_4$ (215 mg, 1.0 mmol) in dry CH_2Cl_2 (40 mL) at 23 °C for 48 h under the conditions described for **49** gave, after column chromatography (2:1 hexanes−EtOAc), **61** (3.2 g, 54%) as a colorless syrup: $[\alpha]_D$ +20.5° (*c* 0.9); NMR ($CDCl_3$) ^{13}C, δ 170.6-169.6, 138.3, 138.2, 137.7, 134.3, 128.4−127.6, 119.1, 99.3, 99.0, 98.5, 97.1, 81.9, 80.0, 78.8, 77.2 (2C), 75.7, 75.0, 73.6, 73.2, 72.3, 72.0, 71.4, 70.6, 69.3 (2C), 69.2, 68.8, 68.4, 68.0, 67.9 (2C), 67.2, 62.5, 61.4 (2C), 51.4, 34.0, 29.2, 25.9, 24.7, 21.0, 20.8, 20.7, 20.6, 20.5; CI-MS *m*/*z* 1482 [((M + NH_4)$^+$]. Anal. Calcd for $C_{73}H_{94}O_{32}$: C, 59.10; H, 6.39. Found: C, 59.17; H, 6.41. Further elution gave the starting compound **53** (1.5 g, 39.5%).

5-(Methoxycarbonyl)pentyl *O*-(3,4,6-Tri-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-*O*-3,4,6-tri-O-acetyl-α-D-glucopyranoside (62). A mixture of **61** (2.2 g, 1.5 mmol), NaOAc (1.6 g, 11.8 mmol), and $PdCl_2$ (400 mg, 2.3 mmol) in a 20:1 mixture of AcOH−H_2O (21 mL) was stirred at 23 °C for 24 h. Workup as described for **56** gave, after column chromatography (3:2 hexanes−EtOAc) of the residue, **62** (1.8 g, 82%) as a colorless syrup: $[\alpha]_D$ +153° (*c* 1.0); NMR ($CDCl_3$) ^{13}C, δ 170.6−169.6, 138.7−137.8, 128.4−127.5, 96.8, 96.1, 95.2, 94.6, 82.5, 76.9, 75.7, 75.2, 74.7, 73.5, 73.0, 72.2, 71.8, 71.3, 71.2, 70.5, 68.9 (2C), 68.5, 68.3, 67.9, 67.6, 67.3, 62.2, 61.47, 61.39, 51.4, 33.9, 29.1, 25.7, 24.6, 20.8, 20.7, 20.6; FAB-MS *m*/*z* 1466 [((M + Na)$^+$]. Anal. Calcd for $C_{70}H_{90}O_{32}$: C, 58.25; H, 6.28. Found: C, 58.17; H, 6.26.

5-(Methoxycarbonyl)pentyl *O*-α-D-Glucopyranosyl-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (63). Compound **62** (2.4 g, 1.66 mmol) in a mixture of EtOH (50 mL) and AcOH (1 mL) was hydrogenolyzed in the presence of 10% Pd−C (300 mg) at 23 °C at 200 psi for 12 h. Filtration, followed by concentration and column chromatography (98:2 EtOAc−MeOH) of the residue, gave **63** (1.54 g, 79%) as a colorless syrup: $[\alpha]_D$ +177° (*c* 0.7); NMR ($CDCl_3$) ^{1}H, δ 5.45−5.26 (3 t, 3 H), 5.33, 5.31, 5.15, 4.91 (4 d, 4 H, J = 3.3−3.5), 5.09−4.96 (3 t, 3 H), 3.69 (s, 3 H), 2.37 (m, 2 H, $J \sim$ 7.4), 2.11−2.02 (27 H), 1.76−1.60 (m, 4 H), 1.50−1.38 (m, 2 H), ^{13}C, δ 170.7−169.6, 97.4, 95.5, 93.9 (2C), 51.6, 33.8, 29.0, 25.5, 24.5 20.9, 20.7, 20.6, 20.5; CI-MS *m*/*z* 1190 [((M + NH_4)$^+$]. Anal. Calcd for $C_{49}H_{72}O_{32}$·0.5H_2O: C, 49.45; H, 6.18. Found: C, 49.36; H, 6.11.

5-(Hydrazinocarbonyl)pentyl *O*-α-D-Glucopyranosyl-(1→2)-*O*-α-D-glucopyranosyl-(1→2)-*O*-α-D-glucopyranosyl-(1→2)-α-D-glucopyranoside (64). Compound **62** (1.0 g, 853 μmol) was deacetylated [NaOMe (cat.), MeOH (10 mL), 23 °C, 24 h] and then treated in MeOH (5 mL) with hydrazine hydrate (0.5 mL) at 23 °C for 48 h as described for **54** to give, after column chromatography (MeOH) and freeze-drying, **64** (509 mg, 73%) as an amorphous white solid: $[\alpha]_D$ +166° (*c* 0.9, H_2O); NMR (D_2O) ^{1}H, δ 5.39 (2H), 5.26, and 5.19 (3 d, 4 H, J = 3.2−3.6), 2.28−2.20 (m, 2 H), 1.74−1.58 (m, 4 H), 1.44−1.32 (m, 2 H); ^{13}C, δ 176.5, 96.4, 96.1, 94.4, 94.2, 68.6, 61.3 (3C), 61.1, 34.4, 29.3, 25.9, 25.7; FAB-MS *m*/*z* 795 [(M + 1)$^+$], 817 [((M + Na)$^+$]. Anal. Calcd for $C_{30}H_{54}N_2O_{22}$: N, 3.52. Found: N, 3.58.

5-(Methoxycarbonyl)pentyl *O*-(3,4,6-Tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (65). Treatment of **53** (1.85 g, 2.56 mmol) with the chloride **4** (1.95 g, 5.12 mmol) in the presence of Ag_2CO_3 (1 g, 3.6 mmol), 4 Å powdered molecular sieves (4 g), and $AgClO_4$ (150 mg, 725 μmol) in dry CH_2Cl_2 (50 mL) at 23 °C for 30 h under the conditions described for **49** gave, after column chromatography (1:1 hexanes−EtOAc), **65** (2.4 g, 66%) as a colorless syrup: $[\alpha]_D$ +156° (*c* 0.8); NMR ($CDCl_3$) δ 7.50−7.35 (m, 5 H), 5.54−5.22 (4 t, 4 H), 5.21, 5.13, 4.91, and 4.80 (4 d, 4 H, *J* = 3.1−3.4), 5.06−4.90 (m, 4 H), 4.66 and 4.58 (2 d, 2 H, *J* = 12.0), 3.67 (s, 3 H), 2.40−2.30 (m, 2 H), 2.10−1.90 (m, 36 H), 1.80−1.65 (m, 4 H), 1.55−1.40 (m, 2 H); ^{13}C, δ 170.6-169.6, 137.0, 129.0−128.2, 99.2, 98.9, 97.6, 96.7, 79.7, 78.2, 77.0, 76.5, 74.2, 72.2, 72.0, 71.6, 71.1, 69.3, 68.9, 68.7 (2C), 68.5, 67.9 (3C), 67.2, 62.5, 61.7, 61.4, 61.1, 51.4, 33.9, 29.2, 25.8, 24.6, 20.6; CI-MS *m*/*z* 1406 [$((M + NH_4)^+$]. Anal. Calcd for $C_{62}H_{84}O_{35}$: C, 53.60; H, 6.09. Found: C, 53.60; H, 6.07.

5-(Methoxycarbonyl)pentyl *O*-(3,4,6-Tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (66). A mixture of compound **65** (1.4 g, 1.0 mmol), 10% Pd−C (200 mg) in EtOH (50 mL), and AcOH (1 mL) was stirred under hydrogen at 23 °C at 300 psi for 12 h. Filtration through a layer of Celite followed by concentration and column chromatography of the residue (1:2 hexanes−EtOAc) gave **66** (1.2 g, 87%) as a colorless syrup: $[\alpha]_D$ +181° (*c* 0.7); NMR ($CDCl_3$) ^{13}C, δ 170.9−169.7, 95.7, 95.5, 93.7, 93.2, 74.7, 73.6, 73.1, 72.6, 71.4, 71.2, 71.1, 70.1, 68.9 (2C), 68.5 (2C), 68.3, 68.0 (2C), 67.5 (2C), 62.1, 61.7, 61.4 (2C), 51.5, 33.8, 29.0, 25.6, 24.6, 20.6; CI-MS *m*/*z* 1316 [$((M + NH_4)^+$]. Anal. Calcd for $C_{55}H_{78}O_{35}$ H_2O: C, 50.15; H, 6.12. Found: C, 50.12; H, 6.12.

5-(Methoxycarbonyl)pentyl *O*-(4,6-Di-O-acetyl-2-*O*-allyl-3-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-3,4,6-tri-O-acetyl-α-D-glucopyranoside (67). A mixture of **66** (1.5 g, 1.15 mmol), **36** (2.5 g, 6.1 mmol), Ag_2CO_3 (600 mg), 4 Å powdered molecular sieves (5 g), and $AgClO_4$ (110 mg, 531 μmol) in dry CH_2Cl_2 (40 mL) was stirred at 23 °C for 48 h under the conditions described for **49** to give after column chromatography (1:1 hexanes−EtOAc) **67** (768 mg, 38%) as a colorless syrup: $[\alpha]_D$ +141° (*c* 1.2); NMR ($CDCl_3$) ^{13}C, δ 170.5−169.5, 137.7, 133.9, 128.5−127.8, 119.8, 100.5, 99.3, 99.1, 97.2, 96.9, 80.7, 80.3, 78.4, 78.2, 78.1, 75.2, 74.9, 73.2, 72.34, 72.29, 71.9, 70.1, 69.7, 69.1, 69.0, 68.9, 68.8, 68.7, 68.6, 68.3, 67.6, 67.5, 67.0, 62.4, 61.78, 61.72, 61.1, 51.4, 33.9, 29.2, 25.8, 24.6, 21.0−20.5; FAB-MS *m*/*z* 1698 [$((M + Na)^+$], 1808 [$(M + Cs)^+$]. Anal. Calcd for $C_{75}H_{102}O_{42}$: C, 53.73; H, 6.14. Found: C, 53.66; H, 6.10.

Subsequent elution gave **66** (854 mg, 57%) as a colorless syrup.

5-(Methoxycarbonyl)pentyl *O*-α-D-Glucopyranosyl-(1→2)-*O*-α-D-glucopyranosyl-(1→2)-*O*-α-D-glucopyranosyl-(1→2)-*O*-α-D-glucopyranosyl-(1→2)-α-D-glucopyranoside (68). Compound **67** (1.0 g, 597 μmol) was sequentially deallylated [$PdCl_2$ (160 mg, 900 μmol), $NaOAc{\cdot}H_2O$ (650 mg, 4.8 mmol) in a 20:1 mixture AcOH−H_2O (21 mL), 48 h, 23 °C], debenzylated [H_2, 10% Pd−C (500 mg), MeOH (25 mL), AcOH (0.25 mL), 23 °C, 100 psi, 24 h], and deacetylated [NaOMe (cat.), MeOH (10 mL), 23 °C, 12 h] to give **68** (395 mg, 69%) as an amorphous white solid: $[\alpha]_D$ +172° (*c* 1.5, MeOH); NMR (D_2O) ^{1}H, δ 5.51, 5.41 (2 H), 5.29 and 5.24 (4 d, 5 H, *J* = 3.3−3.4), 2.42 (m, 2 H), 1.76−1.58 (m, 4 H), 1.42−1.30 (m, 2 H); ^{13}C, δ 178.4, 96.0, 95.5, 93.3 (br, 3C), 75.5 (br, 2C), 75.3, 74.9 (br), 68.7, 61.3 (br, 2C), 61.2, 61.1, 61.0, 52.9, 34.3, 29.0, 25.6, 24.9; FAB-MS *m*/*z* 979 [$((M + Na)^+$]. Anal. Calcd for $C_{37}H_{64}O_{28}{\cdot}3H_2O$: C, 43.96; H, 6.98. Found: C, 43.93; H, 6.79.

5-(Hydrazinocarbonyl)pentyl *O*-α-D-Glucopyranosyl-(1→2)-*O*-α-D-glucopyranosyl-(1→2)-*O*-α-D-glucopyranosyl-(1→2)-*O*-α-D-glucopyranosyl-(1→2)-α-D-glucopyranoside (69). Treatment of **68** (300 mg, 314 μmol) in MeOH (5 mL) with hydrazine hydrate (0.5 mL) at 23 °C for 48 h under the conditions described for **54** gave, after purification on Biogel P-4 (0.02 M pyridine−AcOH), **69** (255 mg, 85%) as an amorphous white solid: $[\alpha]_D$ +152° (*c* 1.0, H_2O); NMR (D_2O) ^{1}H, δ 5.49, 5.40 (2H), 5.27, and 5.22 (4 d, 5 H, *J* = 3.3−3.6), 2.23 (m, 2 H, *J* = 7.3), 1.76−1.58 (m, 4 H), 1.41−1.30 (m, 2 H); ^{13}C, δ 176.5, 96.2, 95.6, 93.7 (3 C), 76.0 (br, 2C), 75.5, 75.2 (br), 68.7, 61.2 (br, 3C), 61.1, 61.0, 34.4, 29.1, 25.7, 24.6; FAB-MS *m*/*z* 957 [$(M + H))^+$]. Anal. Calcd for $C_{36}H_{64}N_2O_{27}$: N, 2.93. Found: 2.84.

Dimethyl Squarate-mediated Coupling of Oligosaccharides 47, 48, 54, 58, 64, and 69 to Human Serum Albumin. Procedure A. To a solution of hydrazide **47** (53 mg, 172 μmol) in pH 7.00 phosphate−borate buffer (2 mL) was added 3,4-dimethoxy-3-cyclobutene-1,2-dione (29 mg, 206 μmol) at 22 °C. The pH of the solution was kept at 7.0 by addition of 0.05 M sodium tetraborate. After 45 min, the solution was freeze-dried. The major fraction obtained after purification of the residue through a Biogel P-2 column (90 × 2 cm) using 0.02 M pyridine−acetic acid buffer as the eluant was freeze-dried to afford an off-white residue (61 mg). A solution of this residue (21.5 mg, 51.4 μM) and human serum albumin (58 mg, 0.089 μM) in 1 mL of pH 9 phosphate−borate buffer was kept at 22 °C for 3 days. The mixture was concentrated in an Amicon ultrafiltration cell (10 or 50 mL) equipped with a YM-10 membrane using five changes of ion-exchanged water followed by freeze-drying to afford a colorless amorphous solid in 70−85% yield.

Procedure B. A solution of the hydrazide **47** (26.1 mg, 84 μmol), 3,4-dimethoxy-3-cyclobutene-1,2-dione (12.5 mg, 88 μmol), and trisisopropanolamine (13.4 mg, 70 μmol) in MeOH (2.5 mL) was kept at 22 °C for 12 h and then was concentrated. To a solution of the residue in pH 9 buffer was added human serum albumin (24 mg, 0.362 μmol). After 7 days at 22 °C, the mixture was processed as described in Procedure A.

Procedure C. To a solution of hydrazide **47** (18.9 mg, 61.4 μmol) in pH 7.00 phosphate−borate buffer (3 mL) was added 3,4-dimethoxy-3-cyclobutene-1,2-dione (8.7 mg, 61.2 μmol) at 22 °C. (For compound **48** the pH was adjusted to 7.0 by addition of KH_2PO_4 solution before addition of compound **70**.) The pH of the solution was kept at 7.0 by addition of 0.05 M sodium tetraborate. After 4 h, the solution was treated with human serum albumin (20 mg, 0.3 μM) and its pH was adjusted to 9.0 with sodium tetraborate. After 7 days at 22 °C the mixture was processed as described in procedure A.

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