Oligosaccharides from "Standardized Intermediates". Synthesis of a Branched Tetrasaccharide Glycoside Related to the Blood Group B Determinant

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Abstract: A branched tetrasaccharide glycoside (**16**) isomeric with the human blood group B determinant (type 1) was synthesized from "building block" derivatives of the component monosaccharides. Initially a 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-substituted-α-D-galactopyranosyl chloride (**1a** or **1b**) was coupled to propyl 2-acetamido-4,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**2**), and the resulting protected disaccharide glycoside (**3a** or **3b**) was selectively deblocked at position 4′. Tetra-*O*-benzyl-α-D-galactopyranosyl bromide (**6**) was coupled to the deblocked product (**7**) to give a protected trisaccharide glycoside (**12**), which on being selectively deblocked at position 2′ yielded **13**. Finally, 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide (**11**) was coupled to **13**, and the product (**15**) was de-O-benzylated to **16**. The unsubstituted forms (**8**, **14**) of the intermediate disaccharide (**7**) and trisaccharide (**13**) glycosides were obtained by suitable deblocking procedures.

The chemical synthesis of oligosaccharides composed of more than one type of sugar unit and having more than one type of intersugar linkage (heterooligosaccharides) has been a major objective of research in carbohydrate chemistry during the past decade. Stimulated by evidence pointing to oligosaccharide structures as major carriers of biological information, several research groups have contributed to progress in the field. Improved methods for the stereocontrolled coupling of sugar derivatives have been worked out, and a variety of complex oligosaccharides having up to seven monosaccharide units have been synthesized.

In achieving these successes, investigators at first focused on specific oligosaccharides, notably the determinant structures of the human blood group substances, and devised synthetic schemes for producing the target compounds. In a typical approach, an activated sugar carrying transient protecting groups was first coupled to an acceptor sugar. The coupling product was then processed, perhaps in several steps, to achieve *selective* protection of its hydroxy groups. The terminal sugars were added in sequence, usually with an intervening deblocking step, and finally all protecting groups were removed. Since some of the intermediates, and the selective protection sequences, could be used in other syntheses, the approach has considerable flexibility. However, it defers many manipulations of the protecting groups until late in the synthesis and requires the creation of a new scheme for each substantially different end product.

An alternative approach, being explored in our laboratory, is to prepare derivatives of the component monosaccharides having persistent protecting groups at all appropriate positions and, on those sugars destined for interior positions, a temporary protecting group at each position where the chain is to be extended. After the coupling of two of these "standardized intermediates", the removal of a temporary protecting group is the only operation required to prepare the product for the addition of the next sugar unit. Moreover, a given intermediate of this type should be usable in any synthesis where a residue of the parent sugar is desired in the particular linkage configuration for which the intermediate is designed. This approach is routinely employed in peptide and oligonucleotide synthesis, and it can be used in both blockwise and sequential schemes. The number of examples of its application to oligosaccharides is growing rapidly.[2-4]

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(2) Srivastava, V. K.; Sondheimer, S. J.; Schuerch, C. *Carbohydr. Res.* **1980**, *86*, 203–14. Eby, R.; Schuerch, C. *Ibid.* **1981**, *92*, 149–53. Kováč, P.; Hirsch, J. *Ibid.* **1981**, *90*, C5–C7. Bhattacharjee, A. K.; Zissis, E.; Glaudemans, C. P. J. *Ibid.* **1981**, *89*, 249–54.

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In pursuit of the systematic approach, we have prepared substituted sugars for use as precursors of reducing-end and α-linked interior D-glucopyranose units,[6] reducing-end and α-linked interior D-galactopyranose units,[7] β-linked, interior 2-acetamido-2-deoxy-D-glucopyranose (*N*-acetyl-D-glucosamine) units,[8] and β-linked, interior D-galactopyranose units.[9,10] All these intermediates carry *O*-benzyl persistent protecting groups and other substituents as required in their intended roles. Most have been employed in the synthesis of di- or trisaccharides, in our laboratory or elsewhere, showing in part that they function according to design.[11,12]

In the present paper we describe the synthesis, from standard "building blocks", of the *n*-propyl β-glycoside (**16**) of the branched heterotetrasaccharide *O*-α-L-fucopyranosyl-(1→2)-[*O*-α-D-galactopyranosyl-(1→4)]-*O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranose. This tetrasaccharide is an isomer of the type 1 terminal structure of the human blood group B substances.[13] The corresponding, naturally occurring tetrasaccharide segment, which has the structure α-L-Fuc*p*-(1→2)[α-D-Gal*p*(1→3)]β-D-Gal*p*(1→3)β-D-Glc*p*NAc, has been synthesized by Paulsen and Kolář.[14] Syntheses of the group B trisaccharide α-L-Fuc*p*(1→2)[α-D-Gal*p*(1→3)]D-Gal have been described by Lemieux and Driguez in a pioneering paper,[15] by Jacquinet and Sinaÿ,[16] and by David et al.[17] Related tri-

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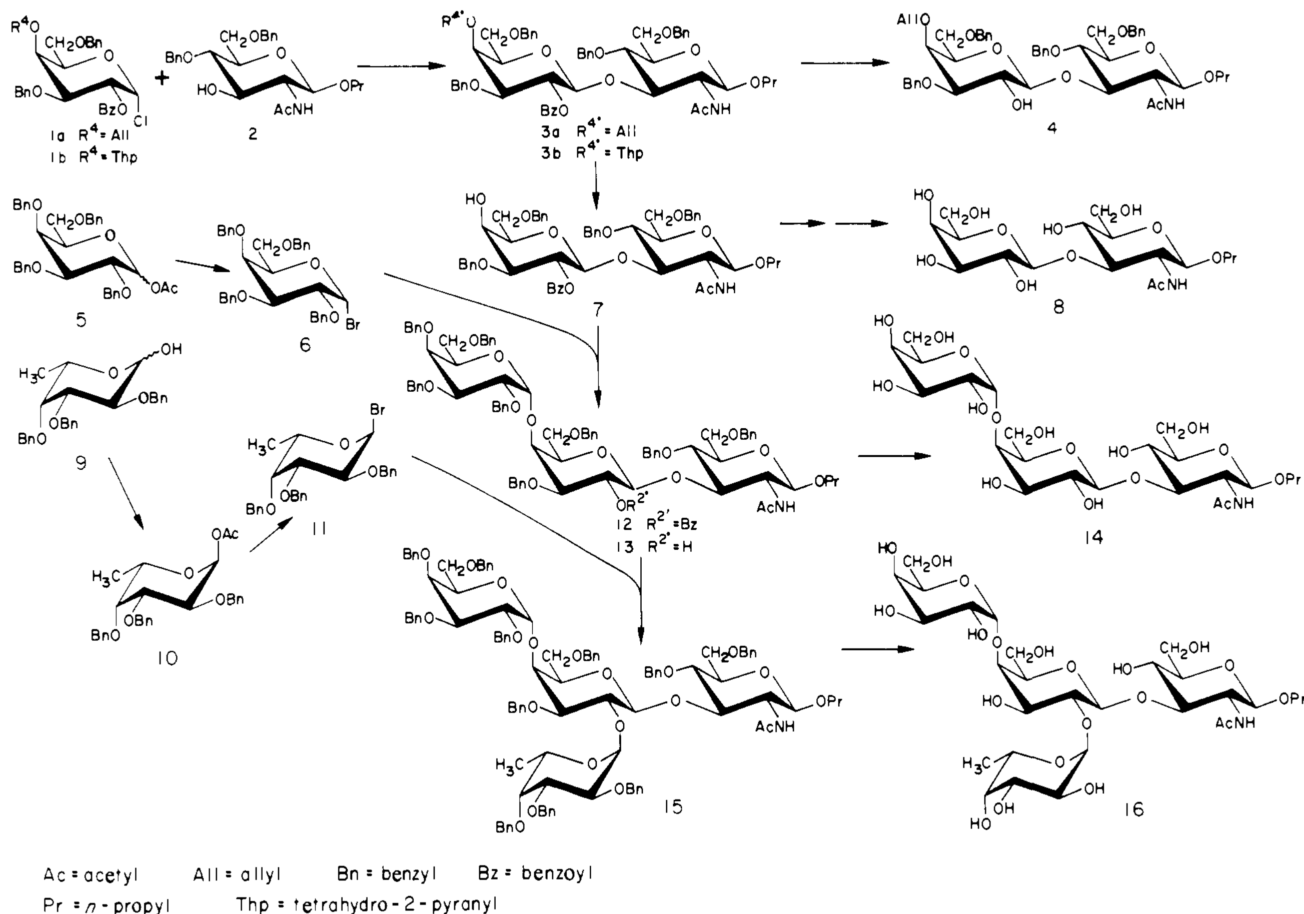

saccharides have been prepared in the laboratories of Kochetkov,[18] Lemieux,[13] and David.[17]

Synthesis of the Tetrasaccharide

The tetrasaccharide glycoside **16** was assembled from the four building blocks **1a** (or **1b**), **2**, **6**, and **11**. In all these compounds, the benzyl ether group was used as the persistent protecting group. Propyl 2-acetamido-4,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside[9] (**2**), having a hydroxy group open for chain extension at position 3, was chosen as the precursor of the reducing-end unit. This compound, a byproduct of earlier work on derivatives of *N*-acetyl-D-glucosamine, was readily prepared. A possibly disadvantageous property was the presence at the anomeric position of an *n*-propyl group, which cannot easily be removed, and hence was retained in the final product. However, the propyl group should have no adverse effects on any biochemical tests performed with **16**. 4-*O*-Allyl-2-*O*-benzoyl-3,6-di-*O*-benzyl-α-D-galactopyranosyl chloride (**1a**) and its 4-*O*-tetrahydropyranyl analogue (**1b**) were designed[9] as precursors of β-linked interior galactose residues having chain extension at position 4, with branching at position 2 if desired. A test of the suitability of **1a** and **1b** for this purpose was a primary rationale for the synthesis of **16**. The last two derivatives, 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide (**6**) and 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide (**11**), are routinely employed as precursors of α-linked nonreducing D-galactose and L-fucose residues, respectively. The entire synthesis was accomplished in six steps, comprising three cycles of coupling followed by partial (**3** → **7**, **12** → **13**) or complete (**15** → **16**) deprotection.

For the initial coupling we utilized the procedure of Hanessian and Banoub,[19] in which the glycosyl halide and the glycosyl acceptor are allowed to react in dichloromethane solution with silver trifluoromethanesulfonate (triflate) and 1,1,3,3-tetramethylurea. The procedure is designed for use with glycosyl halides having a participating *O*-acyl group at position 2. Presumably an initially formed glycosyl triflate is the active coupling reagent, and evidence suggests that the eventual 1,2-trans product is formed via an intermediate ortho ester.[20] The conjugate acid of tetramethylurea appears to serve as the catalyst for the rearrangement of the orthoester.[20,21]

Although a considerable excess of glycosyl halide is often required in coupling reactions catalyzed by silver or mercuric salts, we found that 1.2 molar equiv of **1a** sufficed for the complete glycosylation of **2**. The coupling product was tentatively identified as the β-linked disaccharide glycoside **3a** by its ^{1}H NMR spectrum, which included signals characteristic of both the donor and the acceptor moieties. A triplet at δ 5.65, attributed to H-2′, had spacings of 7.8 Hz, indicative of the trans diaxial disposition of H-1′ and H-2′, as well as H-2′ and H-3′. This triplet disappeared (by upfield shift) when **3a** was de-O-benzoylated to **4**.

When the 4-*O*-tetrahydropyranyl galactosyl chloride **1b** was coupled to **2** by the standard procedure, the tetrahydropyranyl group suffered cleavage, and the 4′-unsubstituted product **7** was obtained directly. The isolation of the fully substituted disaccharide glycoside **3b** (85%) could be accomplished only after modification of the reaction mixture by the addition of 2,6-dimethylpyridine. From this and several similar experiences, we conclude that tetramethylurea is inadequate as the sole acid acceptor in coupling reactions where one of the reactants carries an acid-labile group. However, the use of a pyridine base as a buffer requires caution, because this may cause the reaction to be arrested at the ortho ester stage.[20,22] *O*-Benzoyl glycosyl halides

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(21) In the similar procedure described by Schuerch and his co-workers (Vernay, H. F.; Rachaman, E. S.; Eby, R.; Schuerch, C. *Carbohydr. Res.* **1980**, *78*, 267–73), the silver salts of various sulfonic acids are used to convert glycosyl halides to active glycosylating agents, and coupling proceeds in the absence of base.

Table I. NMR Parameters of the Oligosaccharide Products

product	^{1}H chemical shifts, δ, (coupling constants, Hz)[a]		^{13}C chemical shifts, δ[b]			
	anomeric protons	other protons	C-1‴	C-1″	C-1′	C-1
Galβ→3GlcNAcβ→OPr (8)	4.57 (8.1) (β), 4.43 (8.1) (β)	2.05 (s) ($COCH_3$), 1.58 (m) ($CH_2CH_2CH_3$), 0.87 (t, 7.6) ($CH_2CH_2CH_3$)			104.8 (β)	102.1 (β)
Galα→3GlcNAcβ→OPr (anomer of 8, see Discussion)	5.53 (2.7) (Galα), 4.56 (8.0) (GlcNAcβ)	2.09 (s), 1.59 (m), 0.90 (t)			100.4 (α)	102.3 (β)
Galα→4Galβ→3GlcNAcβ→OPr (14)	4.95 (4.0) (Galα), 4.56 (7.7) (β), 4.50 (7.7) (β)	2.03 (s), 1.56 (m), 0.88 (t)		101.6 (α)	105.1 (β)	102.1 (β)
Galβ→4Galβ→3GlcNAcβ→OPr (anomer of 14, see Discussion)	4.60 (7.7) (β), 4.56 (7.7) (β), 4.47 (7.7) (β)	2.03 (s), 1.56 (m), 0.87 (t)		105.5[c] (β)	104.8[c] (β)	102.2 (β)
Galα→4Galβ→3GlcNAcβ→OPr 2 Fucα↑ (16)	5.20 (3.7) (α), 4.97 (4.0) (α), 4.69 (7.7) (β), 4.41 (8.5) (β)	2.10 (s), 1.57 (m), 1.23 (d, 7.0) ($CHCH_3$), 0.86 (t)	101.9[d] (α)	101.0[d] (α)	103.1 (β)	101.9 (β)

[a] Determined in D_2O, with sodium 3-(trimethylsilyl)propanoate (TSP) as internal standard. [b] From Me_4Si. Determined in D_2O, with *p*-dioxane (δ 67.85) as internal standard. [c,d] These assignments may be transposed.

appear less prone to this difficulty than their *O*-acetyl congeners.[22]

No evidence for α-linked disaccharides was seen in thin-layer chromatograms of any of the products resulting from the reaction of **1a** and **1b** with **2**. Thus the coupling of these building blocks proceeds essentially stereospecifically under the conditions chosen. On the other hand, when the 2,4-di-*O*-benzoyl analogue of **1a**,**b** was used as the glycosyl donor, nearly half of the product was the α-linked disaccharide. And indeed the α product became the major product when the reaction solvent was changed to toluene, ether, or acetonitrile. The mechanism of this effect by a substitutent remote from the anomeric center is completely unknown, but the possibility of such effects must be taken into account in choosing protecting groups.

For the 4′-hydroxyl function in **3a** to be unmasked, the allyl group was isomerized to a 1-propenyl group with tris(triphenylphosphine)rhodium(I) chloride[23] and the propenyl group was removed by treatment with mercuric chloride–mercuric oxide in aqueous acetone.[24] The product was the selectively deprotected disaccharide glycoside **7**, which was also obtained from **3b** by hydrolysis of the 4′-*O*-tetrahydropyranyl group. This cleavage required only a brief treatment with acetic acid–methanol, illustrating the convenience of acid-labile protecting groups in oligosaccharide synthesis. The use of such groups in glycosyl donors was made possible by the development of methods for preparing glycosyl halides under neutral conditions.[25]

The β-linked structure of intermediates **3a**, **3b**, and **7** was confirmed by subjecting **7** to de-O-benzylation by hydrogenolysis. In the ^{1}H NMR spectrum of the resulting crystalline disaccharide glycoside **8**, there were two doublets for β-anomeric protons, and the ^{13}C NMR spectrum included lines for two β-anomeric carbons (Table I).

Proceeding to the addition of the third surgar unit, we employed bromotrimethylsilane[26] for the conversion of 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl-D-galactopyranose (**5**) into the bromide **6**, with excellent results. We then investigated various procedures for the α coupling of **6** to **7**. With the "common ion" method (tetraethylammonium bromide as the catalyst),[27] α-linked trisaccharide (**12**) was obtained, but the reaction was sluggish, and the yield was poor.[28] The use of the *N*-methylacetimidate derived from **6** as the active glycosylating agent[16] led to improved overall yields, but the product was a 4:1 mixture of **12** and its β-1″→4′ isomer. This outcome was unexpected in view of the generally good results obtained by Sinaÿ with the same imidate, but it has some precedent.[29] A good yield (72%) of **12** was finally secured by employing **6** as the glycosyl donor under modified Koenigs–Knorr conditions, with silver triflate and silver carbonate as the catalysts.[30] Evidently the hindered nature of the 4′-hydroxyl in **7** prevented the rapid glycosylation of the compound under less vigorous conditions. Our results are in accord with the suggestion of Paulsen[5,30] that the reactivity of the glycosyl donor, acceptor, and catalyst must be properly matched for successful α coupling.

The de-O-benzoylation of **12** gave an intermediate (**13**), which could be further deprotected by hydrogenolysis. Examination of the NMR spectra of the de-O-benzylated product (**14**) revealed new signals (Table I) characteristic of α-anomeric hydrogen and carbon, in addition to the β-anomeric proton and carbon signals already seen in the spectra of the disaccharide glycoside **8**. Compound **14** is therefore propyl *O*-α-D-galactopyranosyl-(1→4)-*O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside.

To prepare the glycosylating agent for the final coupling step, we undertook the conversion of 2,3,4-tri-*O*-benzyl-L-fucopyranose (**9**) into the bromide **11**. On treating **9** with acetic anhydride in pyridine at 5 °C, we obtained the crystalline α-acetate **10**, not previously isolated. Compound **11** was then efficiently generated by the reaction of **10** with bromotrimethylsilane. As anticipated from the experience of other workers, the α-fucosylation of **13** by **11** was smoothly accomplished by the common ion method. The branched tetrasaccharide product **15** (68% yield) carried only benzyl ether protecting groups and hence could be deblocked in a single operation. Hydrogenolysis gave the free propyl glycoside, and the ^{1}H and ^{13}C NMR spectra of this product confirmed the presence of an α-linked fucopyranosyl unit (Table I). The compound is therefore propyl *O*-α-L-fucopyranosyl-(1→2)-[*O*-α-D-galactopyranosyl-(1→4)]-*O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (**16**).

Conclusion

The construction of the branched tetrasaccharide glycoside **16** by a series of alternate coupling and selective deprotection reactions, as outlined above, demonstrates the feasibility of synthesizing a complex oligosaccharide from fully fashioned standardized building units. In particular, galactosyl chlorides **1b** and

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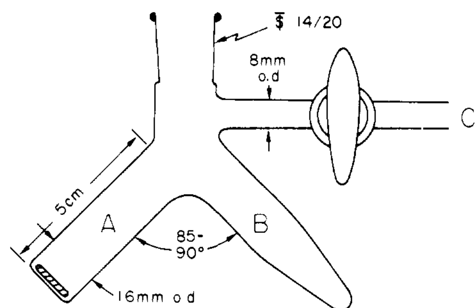

Figure 1.

1a were shown to serve effectively in their proposed role, namely, as synthons for β-linked interior D-galactopyranose units having chain extension at position 4 and also at position 2. These extensions were accomplished sequentially by the addition of a terminal α-galactose unit at position 4′ of the initial coupling product and then a terminal α-fucose unit at position 2′.

In a separate experiment, the 2′-hydroxy compound **4** was made by the de-O-benzoylation of **3a**. Given the alkali stability of the tetrahydropyranyl group, it is clear that **3b** could be converted into an analogous 2′-hydroxy derivative. Thus, each of the temporary protecting groups in **3a** and **3b** (4′-substituent, 2′-*O*-benzoyl group) can be removed independently of the other, which should in principle make it possible to add the two exterior sugars in either order. Surprisingly, in work on the synthesis of an isomer of **16**, we found that the sequence used here (coupling first at 4′, then 2′) was the only successful one. These results will be presented in a subsequent paper.

The structure **16** did not lend itself to blockwise assembly, but when a blockwise approach is indicated, it can be accomplished with minor modification of the intermediates used in the present work. Thus, instead of the chloride **1a**, one could start with its precursor,[9] the corresponding 1-propenyl glycoside having R^4 = H, and couple a protected sugar to O-4 of that compound. The resulting disaccharide could then be activated for further coupling by replacement of the anomeric propenyloxy group with chlorine.[31] Syntheses employing such disaccharide blocks will be described in forthcoming papers.

Experimental Section

The instrumental and chromatographic procedures used were described in a previous paper.[8] ^{1}H NMR spectra were recorded at 270 MHz, with decoupling as required for the identification of signals that could not be assigned unambiguously by inspection. ^{13}C NMR spectra were determined on a Bruker HX 90E instrument operating at 22.63 MHz, with *p*-dioxane as an internal standard. Chromatography on silica gel was accomplished with mixtures of ethyl acetate and *n*-hexane, acetone and chloroform, or methanol and chloroform. Elemental analyses were done at the Galbraith Laboratories, Inc., Knoxville, TN.

General Procedure for Coupling. Coupling reactions were performed in two-armed reaction vessels of the type illustrated in Figure 1. The procedure followed is now in routine use in our laboratory.

For β-glycosidic couplings by the method of Hanessian and Banoub,[19] glycosyl acceptor (e.g., **2**) is placed in the flat-bottomed arm A, along with a magnetic stirring bar, and glycosyl donor (glycosyl halide, e.g., **1a**) is placed in conical arm B. Silver triflate (trifluoromethanesulfonate) is added to the acceptor in A, and the reactor is wrapped in aluminum foil to exclude light. The assembly is then attached to a liquid nitrogen filled Dewar vessel equipped with ports in its outer wall to provide short-path access to the refrigerated inner surface. The reactants are dried by keeping the system under vacuum (~0.01 torr) for 3–4 h.

Next, dry nitrogen gas is admitted through sidearm C and allowed to flow while the reactor is detached from the Dewar, its neck is degreased, and acid acceptors (1,1,3,3-tetramethylurea, 2,6-dimethylpyridine) and solvent (dichloromethane) are added. These liquids are previously dried over 4-Å molecular molecular sieves. In the present work, the proportions of reagents, with respect to glycosyl halide, were silver triflate, 1.67 mol; tetramethylurea (arm A), 2.4–2.5 mol; dimethylpyridine, 1.3 mol; and dichloromethane (divided between chambers A and B), 10 mL/g. Dimethylpyridine is included only when one of the reactants contains an acid-labile substituent, e.g., the tetrahydropyranyl group in **1b**. After these additions, the reactor is closed at the neck (rubber stopper) and sidearm, and the glycosyl halide solution in chamber B is tipped into A with swirling. The vessel, still wrapped with aluminum foil, is mounted over a magnetic stirrer, and the reaction mixture is stirred at room temperature, usually overnight.

When the reaction is deemed complete, several drops of methanol are added to the mixture to quench any unreacted glycosyl donor and stirring is continued for ~1 h. The mixture is filtered over Celite, and the filter cake is washed with chloroform or dichloromethane. The filtrate is extracted with 5% aqueous sodium hydrogencarbonate, the organic phase is evaporated to dryness, and the coupling product is isolated by chromatography on a column of silica gel.

For α-glycosidic couplings, the manipulations are as just described. The catalysts, acid acceptors, and solvents used are those prescribed in the specific procedure being followed.

Propyl *O*-(4-*O*-Allyl-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (3a). The galactosyl chloride[9] **1a** (285 mg, 0.54 mmol) and the propyl glucosaminide[9] **2** (200 mg, 0.45 mmol) were subjected to the procedure for β-glycosidic coupling just described. Purification of the crude product on a column of silica gel gave 412 mg (98%) of the title compound as a syrup: $[\alpha]^{25}{}_D$ +16.7°, $[\alpha]^{25}{}_{436}$ +38.0° (*c* 2.07, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.33–7.08 (m, 25 H, Ph H) 6.43 (d, 1 H, $J_{NH,2}$ = 7.6 Hz, D_2O exchangeable, NH), 6.13–5.87 (m, 1 H, —C*H*=), 5.65 (t, 1 H, *J* = 7.8 Hz, H-2′), 5.52–5.19 (m, 2 H, CH=CH_2), 4.75 (d, 1 H, $J_{1',2'}$ = 7.7 Hz, H-1′), 5.15–3.10 (m, 26 H, PhCH_2, H-1, sugar CH and CH_2, OCH_2CH=, and O$CH_2$$CH_2$), 1.83 (s, 3 H, CH_3CO), 1.50–1.33 (m, 2 H, CH_2CH_2), and 0.85 (t, 3 H, CH_2CH_3).

Anal. Calcd for $C_{55}H_{63}NO_{12}$ (930.10): C, 71.02; H, 6.83; N, 1.51. Found: C, 70.80; H, 6.82; N, 1.50.

Protected Disaccharide 3b. The coupling of galactosyl chloride[9] **1b** (1.38 g, 2.43 mmol) to the glucosaminide **2** (900 mg, 2.03 mmol) was accomplished by the general procecure, with the inclusion of 2,6-dimethylpyridine in the reaction mixture. The resulting crude syrupy **propyl *O*-(2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-(tetrahydro-2-pyranyl)-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (3b)** was converted directly to **7**.

Propyl *O*-(4-*O*-Allyl-3,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (4). To a solution of compound **3a** (100 mg, 0.11 mmol) in methanol (5 mL) was added a small piece of sodium, and the mixture was boiled under reflux. After 15 min, debenzoylation was complete as judged by TLC. The solution was treated with Rexyn 101 (H^+) ion-exchange resin and then concentrated on a rotary evaporator. Crystallization of the syrupy residue from methanol–ether yielded 82 mg (92%) of pure 4: mp 84–85 °C; $[\alpha]_D{}^{25}$ −1.0°, $[\alpha]^{25}{}_{436}$ −3.6° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) similar to that of **3a** except for loss of the low-field Ph H signals, disappearance (upfield shift) of the signal for H-2′, and appearance of δ 3.13 (br s, 1 H, D_2O exchangeable, OH).

Anal. Calcd for $C_{48}H_{59}NO_{11}$ (826.00): C, 69.80; H, 7.20; N, 1.70. Found: C, 69.94; H, 7.25; N, 1.76.

2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl Bromide (6). 1-*O*-Acetyl-2,3,4,6-tetra-*O*-benzyl-α,β-D-galactopyranose[32] (200 mg, 0.34 mmol) was dissolved in dry dichloromethane (5 mL), the solution was cooled to −10 °C, and then bromotrimethylsilane (0.4 mL, 3.0 mmol) was added dropwise with stirring. The mixture was allowed to warm to room temperature and stand for 5 h. Evaporation of the solvent gave the syrupy bromide[33] 6: ^{1}H NMR ($CDCl_3$) δ 6.52 (d, 1 H, $J_{1,2}$ = 3 Hz, H-1α). According to TLC, a trace of β-anomeric bromide was present in the product, which was used without further purification.

Propyl *O*-(2-*O*-Benzoyl-3,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (7). (a) From 3a. A solution of **3a** (270 mg, 0.29 mmol), diazabicyclo[2.2.2]-octane (10 mg, 0.09 mmol), and tris(triphenylphosphine)rhodium(I) chloride (20 mg, 0.02 mmol) in ethanol (10 mL) was boiled under reflux. The conversion of the starting material to a product that was hydrolyzed by aqueous $HgCl_2$ (isomerization of the allyl group to propenyl), as demonstrated by TLC, required ~5 h. Water was added to the cooled solution, and it was extracted with chloroform. The chloroform layer was washed successively with saturated KCl, dilute HCl, saturated KCl, water, $NaHCO_3$, and water, then dried (Na_2SO_4), and evaporated. The residue was taken up in 5:1 (v/v) acetone–water (7.5 mL) and stirred with mercuric oxide (0.3 g) and a solution of mercuric chloride (0.35 g in 2.5 mL of acetone–water, added dropwise)[24]. After 5 min, the suspension was filtered, and the filtrate was evaporated to dryness. A

(31) A different system for protecting and then activating the anomeric center is used by Paulsen.[4]

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chloroform solution of the residue was washed with saturated KI and then water, dried, and once again evaporated. Purification of the final residue on a column of silica gel gave 230 mg (89%) of the title compound **7**: $[\alpha]^{25}_D$ +12.9°, $[\alpha]^{25}_{436}$ +29.8° (*c* 1.6, $CHCl_3$); ^1H NMR ($CDCl_3$) similar to that of **3a** except for the loss of signals due to the allyl group and the appearance of δ 2.80 (br s, 1 H, D_2O exchangeable, OH).

Anal. Calcd for $C_{52}H_{59}NO_{12}$ (890.04): C, 70.17; H, 6.68; N, 1.57. Found: C, 70.15; H, 6.82; N, 1.53.

(b) From 3b. The crude **3b** described above was dissolved in a 7:3 (v/v) mixture of 80% aqueous acetic acid and methanol, and the solution was boiled for 1 h under reflux. The solvents were removed by evaporation, followed by the addition and evaporation of toluene as required, and the residue was chromatographed. This gave 1.53 g (85%) of **7**, identical by TLC and NMR with the product obtained in (a).

Propyl *O*-β-D-Galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (8). A solution of **7** (0.40 g) in 0.05 M methanolic sodium methoxide (11 mL) was boiled under reflux for 30 min, whereupon TLC showed that de-O-benzoylation (position 2′) was complete. The mixture was neutralized and deionized with Rexyn 101(H^+), and the resin was removed by filtration. After the addition of palladium/charcoal catalyst (10% Pd), the solution was stirred overnight under hydrogen at 1 atm of pressure, then filtered, and evaporated to dryness. Crystallization of the residue from methanol-ether afforded 0.17 g (89%) of **8** as needles: mp 216–217 °C; $[\alpha]^{25}_D$ −25.8°, $[\alpha]^{25}_{436}$ −55.2° (*c* 0.6, MeOH). The NMR data are given in Table I.

Anal. Calcd for $C_{17}H_{31}NO_{11}$ (425.43): C, 48.00; H, 7.35; N, 3.29. Found: C, 47.96; H, 7.33; N, 3.32.

1-*O*-Acetyl-2,3,4-tri-*O*-benzyl-α-L-fucopyranose (10). A solution of 2,3,4-tri-*O*-benzyl-L-fucopyranose[34] (**9**) (1.5 g, 3.45 mmol) and acetic anhydride (1 mL) in dry pyridine was kept for 4 h at 5 °C, then methanol was added, and the mixture was stirred for 15 min without cooling. Volatile components were removed by evaporation, followed by the addition and evaporation of toluene as required. Crystallization of the residue from methanol gave 1.4 g (85%) of **10** as long needles: mp 90–92 °C; $[\alpha]^{25}_D$ −71.5°, $[\alpha]^{25}_{436}$ −141° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.49–7.12 (m, 15 H, Ph H), 6.38 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 5.00–4.61 (m, 6 H, $PhCH_2$), 4.23–3.67 (m, 4 H, sugar CH), and 1.14 (d, 3 H, *J* = 6.6 Hz, CHH_3).

Anal. Calcd for $C_{29}H_{32}O_6$ (476.57): C, 73.09, H, 6.77. Found: C, 73.32; H, 7.00.

2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl Bromide (11). This was prepared as described for the corresponding galactosyl bromide 6, and it similarly contained traces of the β-anomeric bromide. The fucosyl bromide was a syrup;[34] ^1H NMR ($CDCl_3$) δ 6.54 (d, 1 H, $J_{1,2}$ ~ 2 Hz, H-1α).

Propyl *O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (12). (a) Common Ion Method. Protected disaccharide **7** (0.3 g, 0.34 mmol), tetraethylammonium bromide (0.14 g, 0.67 mmol), and powdered 4-Å molecular sieves[15] (1.3 g) were added to 6 mL of dry dichloromethane in a round-bottomed flask, and the suspension was stirred for 2 h. Tetra-*O*-benzyl-α-D-galactopyranosyl bromide (6) freshly prepared from 0.4 g (0.69 mmol) of compound **5** was dissolved in 12 mL of 1:1 (v/v) dichloromethane–*N*,*N*-dimethylformamide (dry), the solution was transferred to the reaction flask, and the contents were stirred under nitrogen for 4 days at room temperature. The reaction was then quenched and the mixture worked up as described above under General Procedure for Coupling. The yield of title compound was 160 mg (34%) of amorphous solid: $[\alpha]_D$ +50.9°, $[\alpha]^{25}_{436}$ +104° (*c* 0.75, $CHCl_3$); ^1H NMR similar to that of **7**, with additional signal intensity at δ 8.33–7.08 (now 45 H, Ph H) and 5.15–3.10 (now 39 H, $PhCH_2$, sugar CH and CH_2, and propyl OCH_2) and disappearance of the OH signal.

Anal. Calcd for $C_{86}H_{93}NO_{17}$ (1412.68): C, 73.12; H, 6.64; N, 0.99. Found: C, 72.93; H, 6.70; N, 0.71.

(b) Silver-Assisted Coupling. A suspension of compound **7** (0.2 g, 0.22 mmol), silver carbonate (0.17 g, 0.62 mmol), and silver triflate (0.16 g, 0.62 mmol) in dry dichloromethane (6 mL) was stirred at −25 °C. The galactosyl bromide (6) from 0.35 g (0.60 mmol) of **5**, in dichloromethane (6 mL), was added dropwise, the mixture was stirred in the dark for 3 h at −25 °C and then for 12 h at 5 °C, quenched, and worked up according to the general procedure. The title trisaccharide **12** was obtained as a pure syrup (0.227 g, 72%) having the same chromatographic mobility, specific rotation, and ^1H NMR spectrum as the product obtained by (a).

Propyl *O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (13). Compound **12** (0.125 g) was de-O-benzoylated in the same way as its disaccharide precursor (see **7** → **8**, above). Purification on a column of silica gel gave 0.103 g (89%) of pure **13** as an amorphous solid: $[\alpha]^{25}_D$ +42.6°, $[\alpha]^{25}_{436}$ +83.7° (*c* 1.03, $CHCl_3$); ^1H NMR ($CDCl_3$) similar to that of **12** except for the loss of the lowest field Ph H signals, disappearance (upfield shift) of the signal for H-2′, and appearance of δ 5.20 (d, 1 H, *J* = 3.0 Hz, H-1″).

Anal. Calcd for $C_{79}H_{89}NO_{16}$ (1308.57): C, 72.51; H, 6.86; N, 1.07. Found: C, 72.10; H, 6.83; N, 1.00.

Propyl *O*-α-D-Galactopyranosyl-(1→4)-*O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (14). Compound **13** (60 mg) in methanol (10 mL) was treated with 10% palladium/charcoal catalyst (80 mg), and the suspension was stirred overnight under hydrogen at 1 atm. Removal of the catalyst and evaporation of the filtrate left a quantitative yield (~30 mg) of the trisaccharide glycoside as an amorphous solid: $[\alpha]^{25}_D$ +43.5° (*c* 1.07, MeOH). The NMR data are given in Table I.

Anal. Calcd for $C_{23}H_{41}NO_{16}\cdot 5H_2O$ (677.65): C, 40.77; H, 7.59; N, 2.07. Found: C, 40.53; H, 6.81; N, 2.27.

Propyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-[*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→4)]-*O*-(3,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (15). The procedure for the α-fucosylation of **13** was the same as that employed in (a) for the α-galactosylation of **7** (see under **12**, above). Along with the acceptor **13** (100 mg, 0.08 mmol) in dry dichloromethane (2 mL), tetraethylammonium bromide (43 mg, 0.2 mmol) and powdered 4-Å molecular sieves (0.4 g) were used. The fucosyl bromide **11**, freshly prepared from 100 mg (0.21 mmol) of **10**, was dissolved in dichloromethane (2 mL) and *N*,*N*-dimethylformamide (2 mL). Purification of the crude coupling product on a column of silica gel afforded 89 mg (68%) of pure **15**: glass; $[\alpha]^{25}_D$ +3.4°, $[\alpha]^{25}_{436}$ +7.7° (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$) similar to that of **13** except for additional signal intensity at δ 8.33–7.08 (now 55 H, Ph H) and 5.15–3.10 (now 50 H, $PhCH_2$, sugar CH and CH_2, propyl OCH_2) and new signals at δ 5.76 (d, 1 H, $J_{1,2}$ = 3.7 Hz, α-anomeric H) and 1.21 (d, 3 H, *J* = 6.6 Hz, CH_3 of fucose).

Anal. Calcd for $C_{106}H_{117}NO_{20}$ (1725.09): C, 73.80; H, 6.84; N, 0.81. Found: C, 73.38; H, 6.59; N, 0.67.

Propyl *O*-α-L-Fucopyranosyl-(1→2)-[*O*-α-D-galactopyranosyl-(1→4)]-*O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (16). The hydrogenolysis of **15** (65 mg), accomplished as described above (see **13** → **14**), furnished **16** as an amorphous solid in near quantitative yield: $[\alpha]^{25}_D$ −2.0° (*c* 0.61, MeOH). The NMR data are given in Table I.

Anal. Calcd for $C_{29}H_{51}NO_{20}\cdot 5H_2O$ (823.79): C, 42.28; H, 7.46; N, 1.70. Found: C, 41.88; H, 7.00; N, 1.62.

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