Carbohydrate Research, 251 (1994) 69–79
Elsevier Science B.V., Amsterdam

Synthesis of allyl 3-deoxy- and 4-deoxy-β-D-galactopyranoside and simultaneous preparations of Gal(1 → 2)- and Gal(1 → 3)-linked disaccharide glycosides †

Reiko T. Lee and Yuan C. Lee *

Department of Biology and the McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Maryland 21218 (USA)

(Received March 17th, 1993; accepted June 21st, 1993)

ABSTRACT

Syntheses of galactose derivatives that are useful in probing the binding specificity of galactose-specific lectins are reported. These include allyl 3-deoxy- and 4-deoxy-β-D-*xylo*-hexopyranoside and several disaccharide glycosides having Gal(1 → 2) and Gal(1 → 3) linkages. The β-linked Gal disaccharide isomers were produced using 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide as glycosyl donor and the 4,6-*O*-benzylidene derivatives of allyl β-D-galactopyranoside, α-D-glucopyranoside, α-D-mannopyranoside, and 2-acetamido-2-deoxy-α-D-galactopyranoside as acceptors. Only the Gal(1 → 3)-linked disaccharide was obtained when the benzylidene derivatives of the mannopyranoside and 2-acetamido-2-deoxygalactopyranoside were used. Attempts at the preparation of Gal(α, 1 → 2)Gal and Gal(α, 1 → 3)Gal disaccharide glycosides were made using the same strategy, but employing the 1-trichloroacetimidate or 1-*N*-methylacetimidate of 2,3,4,6-tetra-*O*-benzyl-D-galactopyranose as the glycosyl donor. The latter imidate produced a mixture of Gal(α, 1 → 2)Gal and Gal(α, 1 → 3)Gal derivatives as major products, but the former gave the Gal(β, 1 → 2)Gal isomer as the major product.

1. INTRODUCTION

Specific interactions between carbohydrates and proteins, as exemplified by carbohydrate ligands binding to a lectin, often involve the close apposition of hydrophobic groups as well as the formation of a number of hydrogen bonds between hydroxyl and acetamido groups of carbohydrates and complementary groups in protein side chains and backbones[1,2]. Deoxy sugar derivatives are, therefore, very useful in defining the importance of a specific hydroxyl groups for such interactions [2].

† Dedicated to Professor C.E. Ballou.

* Corresponding author.

SSDI 0008-6215(93)E0330-4

Galactose/GalNAc-specific lectins are abundant both in plant and animal kingdoms. We have previously reported the preparation of 3-deoxy- and 4-deoxy-GalNAc glycosides [3], and proved them useful in probing the binding specificity of lectins that bind GalNAc strongly. However, some lectins, e.g., viscumin (toxic lectin of mistletoe)[4], bind GalNAc very poorly compared to Gal. Here, we report the preparation of allyl glycosides of 3-deoxy- and 4-deoxy-β-D-*xylo*-hexopyranose (3-deoxy- and 4-deoxy-Gal), which are needed for probing binding by this type of galactose lectins.

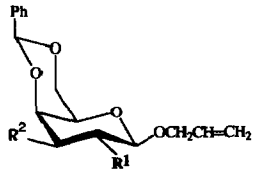
Another important factor in lectin–carbohydrate interactions is the size of the sugar-combining area. Some lectins recognize only one sugar residue[5], while others recognize two or more sugar residues in specific linkages[2]. In order to investigate whether a Gal-recognizing lectin requires certain specific disaccharide structure(s), we wanted to synthesize many Gal-containing disaccharides. Here, we describe preparations of allyl glycosides of various Gal(β, 1 → 2)- and Gal(β, 1 → 3)-linked disaccharides, as well as some glycosides of disaccharides having an α-linked D-galactosyl group.

RESULTS AND DISCUSSION

In all the syntheses described here, the allyl group was chosen as the aglycon because of its small size and its potential for derivatization[6], or removal with generation of the reducing disaccharide[7]. The key intermediate was allyl 4,6-*O*-benzylidene-β-D-galactopyranoside[8] (**1**), which served as the starting material for the two title deoxy derivatives as well as the glycosyl acceptor for the preparation of the galactosyl galactosides **18** and **19**. Similarly, allyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**22**), allyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**27**), and allyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (**30**, ref 9) served as acceptors for the preparation of the galacotsyl glucosides **25** and **26**, the galactosyl mannoside **29**, and the galactosyl 2-acetamido-2-deoxygalactosides **33** and **34**, respectively.

In order to obtain a derivative of allyl β-D-galactopyranoside with only the 3-OH unprotected, **1** was partially benzoylated, and the formed mixture of monobenzoylated derivatives (**2** and **3**) was resolved by chromatography. To obtain an analogous glycoside with only the 4-OH free, **1** was per-*O*-benzylated, de-*O*-benzylidenated, and then mono-*O*-benzoylated at the 6-OH group. These compounds having a single free OH group were converted to the deoxy glycosides via the corresponding imidazol-1-ylthiocarbonyl (ITC) derivatives[10].

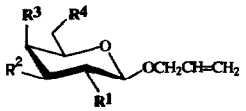
Galactosylation resulting in predominantly β-linked disaccharides was carried out in the presence of $Hg(CN)_2$, with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide[11] as the donor reagent. With **1** and **22** as glycosyl acceptors, two β-linked disaccharide glycosides were obtained, while only one disaccharide products was obtained with **27** as the glycosyl acceptor. The positional isomers were easily separable by silica gel chromatography. Identification of positional isomers de-



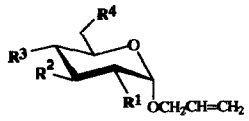
	R^1	R^2
1	OH	OH
2	OH	OBz
3	OBz	OH
4	OBz	OCS-Im
5	OBz	H
6	OBn	OBn
7	O-β-GalAc$_4$	OH
8	OH	O-β-GalAc$_4$
9	O-β-GalBn$_4$	OH
10	O-α-GalBn$_4$	OH
11	OH	O-α-GalBn$_4$
12	OH	O-β-GalBn$_4$

Im = 

pended mainly on the ^{1}H NMR, including NOE measurements and selective decoupling. The H-4 signals of the deprotected galactosyl galactosides (**18, 19, 36, 37, 38**, and **39**) were diagnostic for the linking position (2- or 3-). The H-4 and H-4′ signals showed similar chemical shifts when the linkage was via O-2, while the H-4 signal shifted considerably downfield in the case of the 3-linked disaccharides. The chemical shifts of H-1 and H-4 of these glycosides are listed in Table I. Further



	R^1	R^2	R^3	R^4
13	OH	H	OH	OH
14	OBn	OBn	OH	OH
15	OBn	OBn	OH	OBz
16	OBn	OBn	OCS-Im	OBz
17	OBn	OBn	H	OBz
18	O-β-Gal	OH	OH	OH
19	OH	O-β-Gal	OH	OH
20	O-β-GalAc$_4$	OH	OH	OH
21	OH	O-β-GalAc$_4$	OH	OH



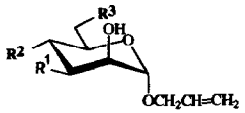
	R¹	R²	R³	R⁴
22	OH	OH	—Bzd—	
23	O-β-GalAc$_4$	OH	—Bzd—	
24	OH	O-β-GalAc$_4$	—Bzd—	
25	O-β-Gal	OH	OH	OH
26	OH	O-β-Gal	OH	OH

proofs for the linkage positions in the two isomers **18** and **19** were obtained from periodate oxidation and from an independent synthesis using **2** as the glycosyl acceptor.

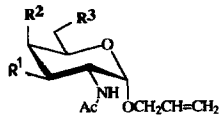
To prepare α-linked Gal disaccharides (**37** and **38**), the trichloroacetimidate method of Schmidt and co-workers[12] and the *N*-methylacetimidate method of Jacquinet and Sinaÿ[8] were applied. The galactosyl donor in these reactions was one of the imidates of 2,3,4,6-tetra-*O*-benzyl-D-galactopyranose and **1** was the glycosyl acceptor. Both methods produced four isomeric disaccharide glycosides, i.e., two anomers for each positional isomer, as shown by TLC. However, the trichloroacetimidate method gave almost exclusively a β-linked isomer (**36**), while two α-linked isomers (**37** and **38**) were major products by the *N*-methylacetimidate method (see Table II).

EXPERIMENTAL

General methods.—Melting points were measured with a Fisher–Johns apparatus and are uncorrected. TLC was performed on Silica Gel 60 F_{254}-coated aluminum sheets (EM Industries, Inc., Gibbstown, NJ). Preparative chromatography



	R¹	R²	R³
27	OH	—Bzd—	
28	O-β-GalAc$_4$	—Bzd—	
29	O-β-Gal	OH	OH



	R^1	R^2	R^3
30	OH	—Bzd—	
31	O-β-GalAc$_4$	—Bzd—	
32	O-α-GalAc$_4$	—Bzd—	
33	O-β-Gal	OH	OH
34	O-α-Gal	OH	OH

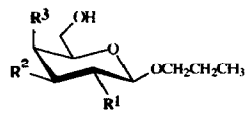
Table I

The H-1 and H-4 chemical shift values of Gal-Gal disaccharide glycosides

Compound	β-Aglycon	Inter-Gal linkage	Chemical shifts (ppm)			
			H-1	H-1′	H-4	H-4′
18	allyl	β 1-2	4.576	4.726	3.943	3.943
19	allyl	β 1-3	4.508	4.616	4.204	3.930
37	*n*-propyl	α 1-2	4.516	5.393	3.91	3.98
36	*n*-propyl	β 1-2	4.528	4.715	3.942	3.949
38	*n*-propyl	α 1-3	4.441	5.128	4.16	3.99
39	*n*-propyl	β 1-3	4.456	4.608	4.194	3.922
[a]	allyl	none	4.429		3.910	

[a] Allyl β-D-galactopyranoside.

was performed on columns of Silica Gel 60 (EM Industries). Solvents used were: *A*, 1:1 toluene–EtOAc; *B*, 1:2 toluene–EtOAc; *C*, 1:4 toluene–EtOAc; *D*, 2:1 toluene–EtOAc; *E*, 3:1 toluene–EtOAc; *F*, 3:2:1 EtOAc–2-propanol–water; *G*, 4:2:1 EtOAc–2-propanol–water; *H*, 9:1 $CHCl_3$–MeOH. Sephadex G-15 (2.5 × 136 cm) and G-10 (1.8 × 64 cm) columns were eluted with 0.1 M acetic acid, with



	R^1	R^2	R^3
35	OH	OH	H
36	O-β-Gal	OH	OH
37	O-α-Gal	OH	OH
38	OH	O-α-Gal	OH
39	OH	O-β-Gal	OH

Table II

Isomers of Gal-Gal disaccharide glycosides obtained by imidate methods

Inter-Gal linkage [a]	R_f		Relative yields [b]	
	Before [c] deprotection	After [d] deprotection	Tricholoacet-imidate method	*N*-Methylacet-imidate method
α 1-3	0.45	0.42	10	51
β 1-3	0.39	0.39	14	5
α 1-2	0.36	0.35	13	35
β 1-2	0.42	0.37	63	9

[a] The imidate-coupled products are allyl 4,6-*O*-benzylidene-2-*O*- and -3-*O*-(2,3,4,6-tetra-*O*-benzyl-α- and -β-D-galactopyranosyl)-β-D-galactopyranoside. After deprotection, they become *n*-propyl 2-*O*- and 3-*O*-α- and -β-D-galactopyranosyl-β-D-galactopyranoside. [b] Percent of the total disaccharide product. [c] R_f values obtained in TLC using solvent *D*. [d] R_f values obtained in TLC using solvent *F*.

collection of 6-mL and 3-mL fractions, respectively. Carbohydrates in the column eluates were detected by the phenol–H_2SO_4 method[13]. When a disaccharide glycoside contained two different component monosaccharides, these were determined by hydrolyzing a small amount of the product with 2 N CF_3CO_2H (100°C for 4 h), followed by evaporation and TLC using solvent *F*. The R_f values of Gal, Glc, and Man were 0.30, 0.37, and 0.42, respectively. ^{1}H NMR spectra were obtained using a Bruker Am 600 or a Bruker AMX 300 spectrometer. The standard used in the NMR measurement in $CDCl_3$ was tethermethylsilane. The signal positions in ^{1}H NMR measured in D_2O were expressed by setting the HDO peak at 27°C as 4.754 ppm.

2,3,4,6-Tetra-*O*-benzyl-D-galactopyranose was obtained from Toronto Research Chemicals, Inc., Toronto, Canada. The preparation of allyl β-D-galactopyranoside, allyl α-D-glucopyranoside, and allyl α-D-mannopyranoside has been reported[14]. Allyl 2-acetamido-2-deoxy-α-D-galactopyranoside (mp 205°C) was prepared using a BF_3-assisted glycosylation method[9]. Allyl glycosides were benzylidenated using α,α-dimethoxytoluene as described[3]. The products were crystallized from hot 95% EtOH, and amounts remaining in the mother liquors were recovered by silica gel chromatography using solvent *B*. To de-*O*-benzylidenate, a solution of the benzylidene derivative in 80% acetic acid was heated at 80°C for 1.5 h. *O*-Deacylation was carried out at room temperature by treatment with 10 mM NaOMe in dry MeOH for 3–5 h. *O*-Benzylation was carried out as described[8]. *O*-Debenzylation was carried out in either 80% acetic acid or 95% EtOH by hydrogenolysis for a few hours at atmospheric pressure in a micro-Brown hydrogenator[15]. Limited *O*-benzoylation[8] was carried out by treatment with benzoylimidazole in a $CHCl_3$ solution under reflux for several hours. The solution of benzoylimidazole was freshly prepared prior to use by reacting benzoyl chloride with a 2-fold excess of anhyd imidazole in $CHCl_3$ at 4°C and filtering off the precipitated imidazole HCl.

*Allyl 3-*O*-benzoyl-4,6,-*O*-benzylidene-β-D-galactopyranoside* (**2**) *and allyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside* (**3**).—A limited benzoylation of **1** was

carried out as described in the general methods, using a 2-fold excess of benzoylimidazole over **1**. The main products (**2** and **3**) were isolated by silica gel chromatography using solvent *E*. Crystallization from EtOAc–hexanes gave **2**; mp 164–165°C (lit.[8]: 172–173°C)*; and **3**, mp 137–138°C (lit.[8]: 144–145°C).

*Allyl 3-deoxy-β-*D*-xylo-hexopyranoside* (**13**).—Compound **3** (170 mg, 0.37 mmol) in 1,2-dichloroethane was refluxed for 7 h with a 2-fold excess of thiocarbonyldiimidazole. After evaporation of the solvent, the imidazol-1-ylthiocarbonyl (ITC) derivative (**4**) was isolated by silica gel chromatography with solvent *D*. Compound **4** was reduced by dripping it into a refluxing solution of tributyltin hydride (2.5 molar excess) in toluene. After 4 h of reflux, allyl 2-*O*-benzoyl-4,6,-*O*-benzylidene-3-deoxy-β-D-galactopyranoside (**5**) was isolated by chromatography in solvent *D* in 80% yield and crystallized from ether–hexanes; mp 86–87°C. Anal. Calcd for $C_{23}H_{24}O_6$ (396.42): C, 69.68; H, 6.10. Found: C, 68.51; H, 6.06. *O*-Debenzoylation of **5** followed by *O*-debenzylidenation and gel filtration (Sephadex G-10) gave, after freeze-drying, the desired compound **13** (57 mg, 0.28 mmol, 75%). ^{1}H NMR (D_2O): δ 1.714 (m, $J_{3ax,4}$ 3.1, $J_{3ax,2}$ 12, $J_{3ax,3eq}$ 13.8 Hz, H-3*ax*), 2.200 (m, $J_{3eq,4}$ 3.1, $J_{3eq,2}$ 5.1, $J_{3eq,3ax}$ 13.8 Hz, H-3*eq*), 3.693–3.735 (m, H-2,5,6), 3.976 (pseudo t, *J* 3.0 Hz; H-4), 4.218 (m, *J* 12.7 and 6.4 Hz, allylic H), 4.391 (m, *J* 12.7 and 5.5 Hz, allylic H), 4.431 (d, *J* 8.0 Hz, H-1), 5.272 (dd, *J* 10.4 and 1.4 Hz, olefinic H), 5.375 (dd, *J* 17.5 and 1.5 Hz, olefinic H, and 5.98 (8 lines, olefinic H).

*Allyl 6-O-benzoyl-2,3-di-O-benzyl-β-*D*-galactopyranoside* (**15**).—Benzylation of **1** produced allyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**6**) in quantitative yield (mp 121–122°C; lit.[8]: 126–127°C). Compound **6** was *O*-debenzylidenated to form allyl 2,3-di-*O*-benzyl-β-D-galactopyranoside (**14**, mp 68–70°C from toluene–hexanes). A partial benzoylation of **14** using 1.5-fold molar excess of benzoyl imidazole gave the title compound (**15**) in 50% yield after silica gel chromatography with solvent *E*; mp 112–113°C from toluene–hexanes. Compound **15**, has the expected structure, because after imidazolthiocarbonyl formation, reduction and deprotection, the correct product, *n*-propyl 4-deoxy-β-D-xylohexopyranoside, was obtained, and its identity was confirmed by NMR (see below).

n-*Propyl 4-deoxy-β-*D*-xylo-hexopyranoside* (**35**).—Compound **15** (140 mg, 0.28 mmol) was converted to its ITC derivative (**16**) as described for **4**; however, an overnight reflux was needed to accomplish complete reaction. The reduction of **16** as described for **5** produced **17** in nearly quantitative yield (TLC). *O*-Debenzoylation and hydrogenolysis of **17** followed by gel filtration (Sephadex G-10) and freeze-drying gave **35** (47%). ^{1}H NMR (D_2O):δ 0.903 (t, *J* 7.42 Hz, CH_3), 1.385 (q, $J_{5,4ax} = J_{4ax,4eq} = J_{4ax,3}$ 12.0 Hz, H-4*ax*), 1.614 (m, *J* 7.26, 7.11, and 7.25 Hz, CH_2 of propyl), 1.964 (m, $J_{4eq,5}$ 1.5, $J_{4,eq,3}$ 5.2, $J_{4eq,4ax}$ 12.0 Hz, H-4*eq*), 3.147 (m, *J* 8.1 and 9.1 Hz, H-2), 3.58–3.85 (m, H-3,5,6), and 4.368 (d, *J* 7.90 Hz, H-1).

* The melting points of allyl 4,6-*O*-benzylidene-β-D-galactopyranoside and all of its *O*-benzoylated and *O*-benzylated derivatives reported by us are without exception 5–7°C lower than the literature values[8].

Allyl 2-O-β-D-galactopyranosyl-β-D-galactopyranoside (**18**) *and allyl 3-O-β-D-galactopyranosyl-β-D-galactopyranoside* (**19**).—The Koenigs–Knorr reaction (Helferich modification) of **1** (616 mg, 2 mmol) and a 1.2 molar excess of 2,3,4,6-tetra-*O*-acetyl-α-D-galactosyl bromide was carried out overnight at room temperature in 1:1 nitromethane–toluene (30 mL) in the presence of $Hg(CN)_2$ (600 mg, 2.4 mmol). The mixture was filtered, solvents were evaporated, and the residue was dissolved in $CHCl_3$ and extracted with 1 M NaCl and 1 M KBr. After drying with anhyd Na_2SO_4 the $CHCl_3$ solution was filtered, and the solvent was evaporated off. The residue was dissolved in 95% EtOH (10 mL), and fractionated on a Sephadex LH-20 column (5 × 190 cm), using 95% EtOH as eluant and collecting 20-mL fractions. The column resolved disaccharide derivatives from monosaccharide derivatives. Fractions containing disaccharides were combined and evaporated to dryness. The residue was further purified on a silica gel column with solvent *A*, which gave **7** and **8** in 14 and 41% yield, respectively. *O*-Deacetylation and *O*-debenzylidenation of **8** gave **19** in 26% yield (mp 215–216°C, from aq EtOH–ether). A similar process applied to **7** produced 80 mg (11%) of **18** (mp 112–115°C, from aq EtOH–ether). ^1H NMR (D_2O) for **18**: δ 3.575 (dd, *J* 10 and 7.9 Hz, H-2), 3.865 (dd, *J* 9.7 and 3.55 Hz, H-3), 3.936 and 3.950 (d, *J* 3.5 and 3.6 Hz, H-4 and H-4′), 4.244 and 4.442 (m, allylic H), 4.576 and 4.726 (d, *J* 7.6 and 7.7 Hz, H-1 and H-1′), 5.291 (dd, *J* 10.2 and 1.16 Hz, vinylic H), 5.414 (dd, *J* 16.8 and 1.6 Hz, vinylic H), and 6.017 (m, vinylic H); for **19**: δ 3.623 (dd, *J* 9.6 and 7.7 Hz, H-2), 3.930 (d, *J* 3.4 Hz, H-4′), 4.204 (d, *J* 3.4 Hz, H-4), 4.238 and 4.415 (m, allylic H), 4.508 and 4.616 (d, *J* 7.9 Hz, H-1 and H-1′), 5.300 (dd, *J* 10.3 and 1.5 Hz, vinylic H), 5.400 (dd, *J* 17.4 and 1.4 Hz, vinylic H), 5.996 (m, vinylic H). Anal. for **19**: Calcd for $C_{15}H_{26}O_{11}$ (382.36): C, 47.12; H, 6.85. Found: C, 46.99; H, 6.97.

Two other methods, periodate oxidation and independent synthesis, were used to confirm the linkage positions of the two isomeric disaccharides glycosides. Before periodate oxidation, **7** and **8** (~ 40 mg each) were separately *O*-debenzylidenated and purified by silica gel chromatography (solvent H) to give **20** and **21**, respectively. These compounds were treated with sodium periodate and the periodate consumption was measured spectrophotometrically[16]. The periodate did not react with **21** during a 6-h period, whereas with **20** reaction was complete during the first half hour, and the amount of the periodate disappearing was equivalent to that consumed by the same molar quantity of allyl 2-acetamido-2-deoxy-α-D-galactopyranoside (i.e., 1 mol of vicinal OH oxidized per mol). The results indicate that **20** (and **18**) is the 2-linked isomer and **21** (and **19**) is the 3-linked isomer.

A Koenigs–Knorr reaction was carried out as described above, but using **2**, which has only one unsubstituted OH group, instead of **1** as glycosyl acceptor. Purification and deprotection as described above gave a single disaccharide product, which matched **18** by TLC, thus confirming that **18** is the 2-linked isomer.

Allyl-2-O-β-D-galactopyranosyl-α-D-glucopyranoside (**25**) *and allyl 3-O-β-D-galalctopyranosyl-α-D-glucpyranoside* (**26**).—Reaction conditions and purification

methods were similar to those described for **18** and **19**. From 2 g (6.6 mmol) of **22** and 3.4 g (8.25 mmol) of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide, **23** was obtained in 20% yield (0.84 g, 1.32 mmol, mp 162–163°C, from 95% EtOH), and the isomeric product **24** was obtained in 12% yield (0.5 g, 0.78 mmol). Upon component sugar analysis, both compounds yielded Gal and Glc in similar amounts. Deprotection, as for **7** and **8**, produced the title compounds **25** (80%, mp 199–200°C, from water–EtOH–ether) and **26** (71%, mp. 107–109°C, from water–EtOH–ether). ^{1}H NMR (D_2O) for **25**: δ 3.649 (dd, *J* 13.2 and 3.4 Hz, H-3′), 3.667 (dd, *J* 9.8 and 3.9 Hz, H-2), 3.844 (t, *J* 9.1 and 9 Hz, H-3), 3.915 (d, *J* 3.1 Hz, H-4′), 4.088 (dd, *J* 12.7 and 6.3 Hz, allylic H), 4.213 (dd, *J* 12.7 and 5.6 Hz, allylic H), 4.538 (d, *J* 7.8 Hz, H-1′), 5.194 (d, *J* 3.6 Hz, H-1), 5.259 (d, *J* 10.3 Hz, vinylic H), 5.366 (d, *J* 16.6 Hz, vinylic H), and 5.987 (8 lines, vinylic H). For **26**: δ 3.675 (dd, *J* 9.9 and 3.2 Hz, H-3′), 3.757 (dd, *J* 9.1 and 4.4 Hz, H-2), 3.912 (t, *J* 9.5 and 9.6 Hz, H-3), 4.081 (dd, *J* 12.8 and 6.1 Hz, allylic H), 4.238 (dd, *J* 12.6 and 5.3 Hz, allylic H), 4.620 (d, *J* 7.8 Hz, H-1′), 4.976 (d, *J* 3.5 Hz, H-1), 5.267 (d, *J* 10.4 Hz, vinylic H), 5.373 (d, *J* 17.2 Hz, vinylic H), and 5.983 (8 lines, vinylic H).

Allyl 3-O-β-D-galactopyranosyl-α-D-mannopyranoside (**29**)—The Koenigs–Knorr reaction was carried out as described for **18** and **19** starting from 1 g (3.24 mmol) of **27** and **5** mmol of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide. Fractionation on the Sephadex LH-20 column revealed one major product (**28**) in the disaccharide region. Acid hydrolysis followed by TLC showed that the material contained about equal amounts of Gal and Man. The disaccharide fractions were combined and the solvent was evaporated off. The deprotected disaccharide product was chromatographed on the Sephadex G-15 column. Fractions containing only **29** were combined and concentrated, and the residue was dried in a desiccator over NaOH pellets to yield 0.48 g (1.3 mmol) of amorphous **29**. Unresolved material was chromatographed on a silica gel column (solvent *G*) to give a further crop of **29** (100 mg, 0.26 mmol); the combined yield was 47%. ^{1}H NMR of per-*O*-acetylated **29** in $CDCl_3$ indicated the intersugar linkage to be β, 1 → 3. ^{1}H NMR of **29** (D_2O) at 50°C: δ 4.106 (m, allylic H); 4.253 (m, allylic H); 4.521 (d, *J* 7.44 Hz, H-1′); 4.966 (d, *J* 1.74 Hz, H-1); 5.28–5.41 (m, 2 vinylic H); 5.95–6.08 (8 lines, vinylic H).

Allyl 2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-α-D-galactopyranoside (**33**) *and allyl 2-acetamido-2-deoxy-O-α-D-galactopyranosyl-α-D-galactopyranoside* (**34**).—The Koenigs–Knorr reaction of **30** (349 mg, 1 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide, followed by purification as described above by the Sephadex LH-20 and silica gel chromatography (solvent *C*), yielded disaccharides **31** (380 mg, 0.56 mmol; R_f 0.24) and **32** (50 mg, 0.07 mmol; R_f 0.25). *O*-Deacetylation and *O*-debenzylidenation of **31** and **32** produced **33** (222 mg, 0.52 mmol, 52% overall yield; mp 234–236°C, from aq EtOH) and **34** (22 mg, 0.06 mmol, 6% overall yield). ^{1}H NMR (D_2O) for **33**: δ 4.46 (d, *J* 8.0 Hz, H-1′), 4.94 (d, *J* 3.7 Hz, H-1); for **34**: δ 4.96 (d, *J* 4.0 Hz, H-1), 5.13 (d, *J* 4.3 Hz, H-1′), Anal. of 33: Calcd for

$C_{17}H_{29}N_1O_{11} \cdot 3H_2O$ (477.46): C, 42.76; H, 7.39; N, 2.93. Found: C, 43.10; H, 7.52; N, 2.92.

n-*Propyl 2-O-β-D-galactopyranosyl-β-D-galactopyranoside* (**36**).—2,3,4,6,-Tetra-*O*-benzyl galactopyranose (0.54 g, 1 mmol) was converted to its trichloroacetimidate derivative according to Grundler and Schmidt[12]. The imidate (~ 0.8 mmol) formed was immediately treated with **1** (0.6 mmol) in dry CH_2Cl_2 (30 mL) at room temperature for two days in the presence of BF_3-etherate (0.6 mmol). After processing[12], the product was purified on the Sephadex LH-20 column. TLC (solvent *D*) showed that the disaccharide region contained a cluster of three carbohydrate bands (R_f 0.39 to 0.49). A portion of the component with R_f 0.46 crystallized out from the ethanolic column effluent, and was shown to be allyl 4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-β-D-galactopyranoside (**9**), (65 mg, 13%; mp 172–173°C). The material that remained in the mother liquor was fractionated on a silica gel column (solvent *D*) to give a second crop of **9** (20 mg). The yield of other isomers is shown in Table II. Anal. of **9**: Clacd for $C_{50}H_{54}O_{11}$: C, 72.27; H, 6.55. Found: C, 72.34; H, 6.63. Compound 9 (60 mg, 72 μmol) was deprotected by hydrogenolysis to yield **36** in 90% yield; mp 162–164°C, from water–EtOH–ether. ^{1}H NMR data are listed in the next section.

n-*Propyl 2-O-α-D-galactopyranosyl-β-D-galactopyranoside* (**37**) *and* n-*propyl 3-O-α-D-galactopyranosyl-β-D-galactopyranoside* (**38**).—The glycosylation reaction was carried out with approximately equimolar amounts (2.4 mmol each) of 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl *N*-methylacetimidate[8] and **1** in the presence of camphorsulfonic acid. The reaction products were isolated as described for **7** and **8**. The two major isomeric disaccharide glycosides (**10** and **11**, both α-linked) and two minor products (**9** and **12**, β-linked) were isolated by silica gel chromatography (solvent *D*). **10**, 8%, mp 105–108°C, **11**, 11%, mp 144–145°C. The combined yield of the four isomers was ~ 22%.

Deprotection of **10** and **11**, as described for **9**, yielded the title compounds **37** (mp 153–155°C, from aq EtOH–ether) and **38** (mp 181–183°C, from eq EtOH–ether). To obtain pure isomer **39**, the corresponding allyl glycoside **19** was hydrogenated and crystallized; mp 208–210°C, from water–EtOH–ether. ^{1}H NMR (D_2O) for **36** [Gal(β, 1 → 2)Gal(β, 1 →)OPr]: δ 0.927 (t, CH_3), 1.652 (6 lines, CH_2C of propyl), 3.58 (dd, *J* 9.9 and 7.8 Hz, H-2), 3.942 (d, *J* 4.6 Hz, H-4), 3.949 (d, *J* 4.13 Hz, H-4′), 4.528 (d, *J* 7.6 Hz, H-1), and 4.715 (d, *J* 7.6 Hz, H-1′). For **37** [Gal(α, 1 → 2)Gal(β, 1 →)OPr]: δ 0.896 (t, CH_3), 1.619 (6 lines, CH_2C of propyl), 3.91 (d, *J* 3.3 Hz, H-4), 3.98 (d, *J* 3.42 Hz, H-4′), 4.516 (d, *J* 7.68 Hz, H-1), and 5.393 (d, *J* 3.87 Hz, H-1′). For **38** [Gal(α, 1 → 3)Gal(β,1 →)OPr]: δ 0.895 (t, CH_3), 1.614 (6 lines, CH_2C of propyl), 3.99 (d, *J* 3.27 Hz, H-4′), 4.16 (d, *J* 3.29 Hz, H-4), 4.44 (d, *J* 7.89 Hz, H-1), and 5.13 (d, *J* 3.84 Hz, H-1′). For **39** [Gal(β, 1 → 3)Gal(β, 1 →)OPr], δ 0.917 (t, CH_3), 1.637 (6 lines, CH_2C of propyl), 3.922 (d, *J* 2.7 Hz, H-4′), 4.194 (d, *J* 3.18 Hz, H-4), 4.456 (d, *J* 7.92 Hz, H-1), and 4.608 (d, *J* 7.26 Hz, H-1′).

ACKNOWLEDGMENT

The authors are indebted to Dr. C. Abeygunawardana, Mr. E. Casillas, and Mr. K. Matsuoka for ^{1}H NMR measurements.

REFERENCES

1 F.A. Quiocho, *Annu. Rev. Biochem.*, 55 (1986) 287–315.
2 R.U. Lemieux, *Chem. Soc. Rev.*, 18 (1989) 347–374.
3 Y. Ichikawa, R.T. Lee, and Y.C. Lee, *J. Carbohydr. Chem.*, 9 (1990) 707–719.
4 R.T. Lee, H.-H. Gabius, and Y.C. Lee, *J. Biol. Chem.*, 267 (1992) 23722–23727.
5 R.T. Lee, Y. Ichikawa, M. Fay, K. Drickamer, M.-C. Shao, and Y.C. Lee, *J. Biol. Chem.*, 266 (1991) 4810–4815.
6 E. Eichler, J. Kihlberg, and D.R. Bundle, *Glycoconjugate J.*, 8 (1991) 69–74.
7 R. Gigg and C.D. Warren, *J. Chem. Soc.*, (1965) 2205–2210.
8 J.C. Jaquinet and P. Sinaÿ, *Tetrahedron*, 35 (1979) 365–371.
9 R.T. Lee, T.C. Wong, and Y.C. Lee, *J. Carbohydr. Chem.*, 5 (1986) 343–357.
10 J.R. Rasmussen, *J. Org. Chem.*, 45 (1980) 2725–2727.
11 K.P.R. Kartha and H.J. Jennings, *J. Carbohydr. Chem.*, 9 (1990) 777–781.
12 G. Grundler and R.R. Schmidt, *Liebigs Ann. Chem.*, (1984) 1826–1847.
13 J.F. KcKelvy and Y.C. Lee, *Arch. Biochem. Biophys.*, 132 (1969) 99–110.
14 R.T. Lee and Y.C. Lee, *Carbohydr. Res.*, 37 (1974) 193–201.
15 C.A. Brown and H.C. Brown, *J. Org. Chem.*, 31 (1966) 3989–3995.
16 E.A. Kabat and M.M. Mayer, *Experimental Immunochemistry*, 2nd ed., Thomas, Springfield, IL, 1961, pp. 542–550.