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# SYNTHESIS OF A CLOSE ANALOG OF THE REPEATING UNIT OF THE ANTIFREEZE GLYCOPROTEINS OF POLAR FISH*†

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ABSTRACT

The protected glycopeptide *N*-(benzyloxycarbonyl)-L-alanyl-[*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)]-L-threonyl-L-alanine 2,2,2-trichloroethyl ester (**21**) was made by coupling the respective disaccharide and tripeptide blocks. The disaccharide block was generated by coupling tetra-*O*-benzoyl-α-D-galactopyranosyl bromide to allyl 2,4,6-tri-*O*-benzyl-α-D-galactopyranoside and converting the product into *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl chloride (**6**) *via* the 1-propenyl glycoside and the free (1-OH) sugar. Alternatively, the 1-propenyl intermediate was obtained directly by using 1-propenyl 2,4,6-tri-*O*-benzyl-α-D-galactopyranoside (**10**) as the acceptor in the initial coupling reaction. An efficient 3-step synthesis of **10** was accomplished by the dibutyltin oxide-assisted, selective crotylation of allyl α-D-galactopyranoside at O-3, followed by benzylation and treatment of the product with potassium *tert*-butoxide. The *N*-benzyloxycarbonyl (Z) and *N*-*tert*-butoxycarbonyl (Boc) 2,2,2-trichloroethyl esters of Thr–Ala and Ala–Thr–Ala were formed by sequential coupling. The silver triflate-promoted glycosylation of the Z-protected dipeptide and tripeptide by 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride, and of the tripeptide by **6**, proceeded with excellent α-stereoselectivity. From the disaccharide tripeptide **21**, the carboxyl-deprotected and fully deprotected derivatives were prepared.

## INTRODUCTION

Antifreeze glycoproteins (AFGP), present in the body fluids and tissues of several species of polar fish, lower the freezing point of water by a non-colligative mechanism. These proteins (peptides) have a simple, repeating-unit structure,

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which, at its most regular, may be expressed as Ala–[Ala–Thr(Gal$\beta$→3GalNAc$\alpha$)–Ala]$_n$–Ala, and they exhibit some antifreeze properties at rather short chain-lengths[1,2]. Thus, it is tempting to suppose that insights into the relationship between specific structural features of the AFGP and their activity might be gained by the chemical synthesis of analogs.

In view of our interest in $\beta$-(1→3)-linked oligosaccharides of D-galactose[3], an attractive possibility was the preparation of protected derivatives of the glycotripeptide Ala–Thr(Gal$\beta$→3Gal$\alpha$)–Ala (**25**). Polymers (oligomers) of **25**, if they could be obtained, would differ from the natural AFGP only in having D-galactose as the inner sugar of the disaccharide moiety instead of 2-acetamido-2-deoxy-D-galactose.

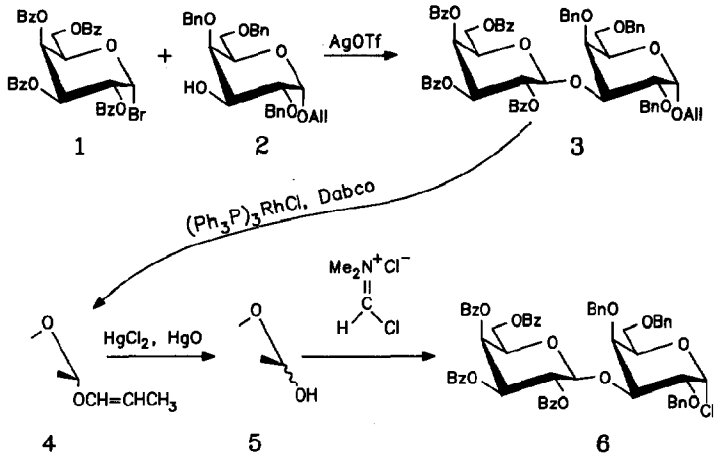
When we began this work, only a few syntheses of glycopeptides had been recorded in the literature. Thus, it was not clear which of the obvious alternative routes was to be preferred. The major possibilities were: (*a*) the coupling of the protected disaccharide to protected threonine, and use of the resulting glycosylated amino acid in the stepwise construction of the peptide chain; and (*b*) the synthesis of a protected form of Ala–Thr–Ala, and glycosylation of this tripeptide. Rather arbitrarily, the convergent approach (*b*) was selected and, *via* this approach, a substituted glycotripeptide (**22**) was prepared that was suitable for polymerization studies.

## RESULTS

*The disaccharide moiety.* — Since the protected disaccharide was to be coupled to the protected tripeptide by an $\alpha$ linkage, we desired a derivative of $\beta$-D-Gal*p*-(1→3)-D-Gal having a non-participating (in the neighboring-group sense) substituent on O-2 of the reducing unit. One of our "standardized intermediates"[4], namely, allyl 2,4,6-tri-*O*-benzyl-$\alpha$-D-galactopyranoside (**2**), was chosen as the synthon for this residue. The silver trifluoromethanesulfonate (triflate)-catalyzed glycosylation of **2** with tetra-*O*-benzoyl-$\alpha$-D-galactopyranosyl bromide (**1**, Scheme 1) then gave the fully substituted disaccharide glycoside **3** (81% yield after chromatography). The presence of a doublet at $\delta$ 5.32 ($J$ 8 Hz) in the $^1$H-n.m.r. spectrum of **3** established the $\beta$ configuration of the newly coupled residue. The $\alpha$-linked anomer was not formed at a detectable level.

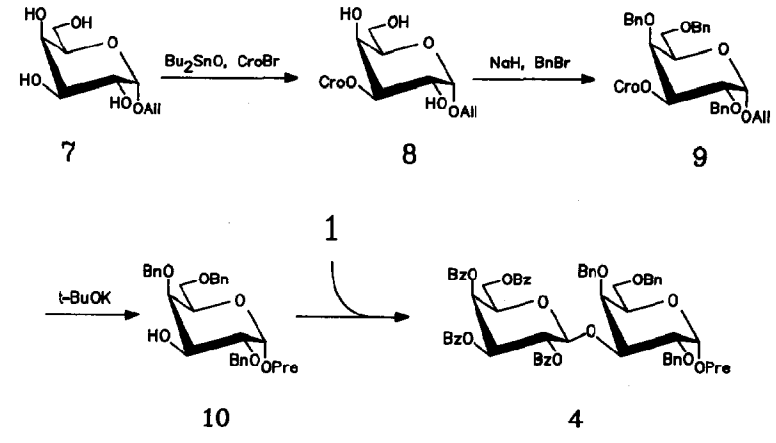
As shown in Scheme 1, the allyl glycoside **3** was successively converted into the 1-propenyl glycoside **4** and the carbinol-protected reducing disaccharide **5**. The necessary activated form of the disaccharide, namely, the glycosyl chloride **6**, was then obtained by treating **5** with the Vilsmeier reagent. To probe the configurational purity of the inter-sugar linkage in **3**–**6**, the deprotected disaccharide-alditol from **5** was examined by an n.m.r. procedure[5] sensitive to 1–2% of the $\alpha$ anomer; none was detected.

The synthesis of **6** by the foregoing method is tedious, because six steps are involved in the preparation of the glycosyl acceptor **2** from allyl $\alpha$-D-galacto-




All = $CH_2$=$CHCH_2$–; Tf = $CF_3SO_2$–
Scheme 1

pyranoside[4]. However, the finding by David *et al.*[6] that unprotected galactosides can be selectively alkylated at position 3 in the presence of dibutyltin oxide led us to test the shorter route shown in Scheme 2. In this route, the key step is the direct conversion of allyl α-D-galactopyranoside (**7**) into its 3-*O*-crotyl derivative.**8**, and the first product having HO-3 unmasked is the propenyl glycoside **10**, rather than the allyl isomer **2**. The projected glycosylation of **10** with **1** therefore leads directly to the "rearranged" disaccharide glycoside **4**.




Cro = $CH_3CH$=$CHCH_2$–; Pre = $CH_3CH$=CH–
Scheme 2

The alkylation of **7** with crotyl bromide–dibutyltin oxide proceeded with very good regioselectivity and, after the two subsequent steps, the glycosyl acceptor **10** could be isolated by chromatography in 61% overall yield. The compound, previously known as a syrup[7], was obtained crystalline. The 3-position of the free hydroxyl group was confirmed by the finding that acetylation caused a downfield shift of the n.m.r. signal for H-3. For the coupling of **1** with **10** as the glycosyl acceptor, careful attention was given to the proportions of bases (tetramethylurea, 2,6-dimethylpyridine) in the reaction mixture; when these were properly adjusted (see Experimental), the disaccharide product **4** could be obtained in yields of 65–70%. Thus, the sequence of Scheme 2, plus the hydrolysis and activation steps **4**→**5**→**6**, constitutes the preferred route to the disaccharide portion of the glycotripeptide.

*Protected tripeptides.* — The choice of protecting groups for the peptide moiety was conditioned by the requirement that these groups be selectively removable, after the coupling of disaccharide and tripeptide, without cleavage of the *O*-substituents from the sugar. On this basis, 2,2,2-trichloroethyl (Tce) was selected as the carboxyl-protecting group[8], and *tert*-butoxycarbonyl (Boc) as the amino-protecting group[9,10]. From Boc-L-alanine, the known Tce ester (**11**) was prepared and elaborated into Boc-Ala–Thr–Ala-OTce (**15B**) by standard peptide-synthesis procedures, as shown in Fig. 1.

Efforts to couple **15B** with glycosyl chlorides (see below), in either the absence or the presence of acid acceptors, yielded mixtures of degradation products. On the hypothesis that this result was due to the marked lability of the Boc group[11], *N*-(benzyloxycarbonyl)-L-alanine (Z-Ala) was substituted for Boc-Ala in the second coupling step of the peptide synthesis, and the alternative, Z-protected tripeptide **15Z** was obtained. By using Z-Thr in the first peptide coupling step, a simpler glycosyl acceptor, Z-Thr–Ala-OTce (**13Z**) was also obtained. Peptides **13Z** and **15Z** had the requisite stability, but it could not be predicted with certainty that,

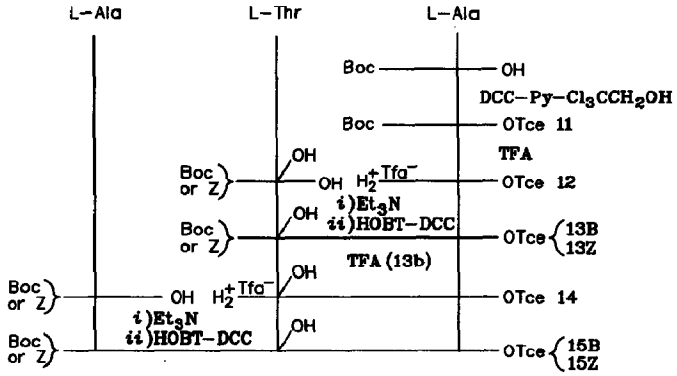



Fig. 1. Synthesis of the peptide reactants. Abbreviations: Boc, *tert*-butoxycarbonyl; DCC, dicyclohexylcarbodiimide; HOBT, 1-hydroxybenzotriazole; Py, pyridine; Tce, 2,2,2-trichloroethyl; TFA, trifluoroacetic acid; $Tfa^-$, trifluoroacetate ion; Z, benzyloxycarbonyl.

after glycosylation, the Z group could be cleaved from their *N*-termini without some *O*-debenzylation of the inner sugar residue. In the event (data not presented), the *N*-deprotection of glycosylated, *C*-deprotected **15Z** (**21**) was accomplished with satisfactory selectivity, and the product was converted into mixtures of low-molecular-weight polymers.

*Protected glycopeptides.* — For the last phase of the synthesis, namely, the α-coupling of protected sugar and protected peptide, the reaction of a modest excess (30%) of glycosyl chloride with silver triflate and acceptor peptide in dichloromethane[12] was investigated. In initial trials, the simple benzylated galactopyranosyl chloride **16** was used as the glycosyl donor (Scheme 3). With the dipeptide **13Z** and tripeptide **15Z**, this donor gave products that could be chromatographically purified as such (from **13Z**) or after carboxyl deprotection (from **15Z**). The purified glycopeptides, obtained in 60–65% yields, were assigned the α-linked structures **17** and **19** on the basis of $^1$H-n.m.r. data.

The reaction of the disaccharide chloride **6** with acceptor tripeptide **15Z** proceeded equally smoothly to furnish a readily purified coupling product (**21**) in 60% yield. This product and its carboxyl-deprotected derivative (**22**) were crystalline. The $^1$H-n.m.r. spectra of **21** and **22** were characterized by prominent, well resolved doublets in the range for α-anomeric protons (δ 5.29 and 5.14, *J* 3.8 and 3.7 Hz, respectively) and in the usual range (δ 0.90–1.35) for the side-chain $CH_3$ groups of the threonine and alanine residues. In addition, **22** showed $^{13}$C signals at δ values typical for α- (98.0) and β-anomeric (101.8) carbon atoms. Both the $^1$H and the $^{13}$C data indicate an α sugar-to-peptide linkage.

To provide a further basis for the characterization of the sugar–peptide linkage in **21** and **22**, the preparation of a reference sample of a β-linked congener was undertaken. The disaccharide glycosyl chloride **20**, which differs from **6** in having a benzoyl group instead of benzyl at O-2 of the reducing unit, was prepared from the 2-*O*-benzoyl analog of **10**. Coupling to **15Z** followed by carboxyl deprotection gave a crystalline glycopeptide (**23**) having a $^1$H-n.m.r. spectrum similar to that of **22**, but lacking a low-field anomeric proton signal. The δ values for the *C*-methyl protons of the amino acid side-chains differed from those of **22**, and the $^{13}$C-n.m.r. spectrum of **23** included lines at δ 100.5 and 102.3, as expected for two β-anomeric carbon atoms.

In the side-chain methyl region of the $^1$H-n.m.r. spectra of **21** and **22**, there was no evidence of low-intensity signals that might arise from impurities containing a β sugar-to-peptide linkage, or a D-amino acid residue resulting from partial racemization at some step of the synthesis.

The complete deprotection of **22** was accomplished by treatment with dilute methanolic sodium methoxide at 0° followed by hydrogenolysis. In the $^1$H-n.m.r. spectrum of the free glycopeptide (**25**), the previously masked signal for H-1′ was discernible. There were no minor peaks attributable to unwanted isomers.

## DISCUSSION

Subsequent to our work on compounds **21** and **22**, the synthesis of a variety of glycopeptides has been described in the literature[13]. Some of these products were prepared in the same way[14] as **21**, but in other cases disappointing results were obtained in attempts to glycosylate preformed peptides[15,16], including a protected derivative of Ala–Thr–Ala[17]. In general, better results have been obtained by first glycosylating a protected amino acid, and then using the product in the same way as other amino acid derivatives in building up the glycopeptide[11,18,19]. This approach can be made compatible with standard peptide-synthesis chemistry, and it dissociates the glycosylation step from the main sequence of reactions[11], thereby simplifying the resolution of the mixtures that often result from imperfect stereocontrol at this step.

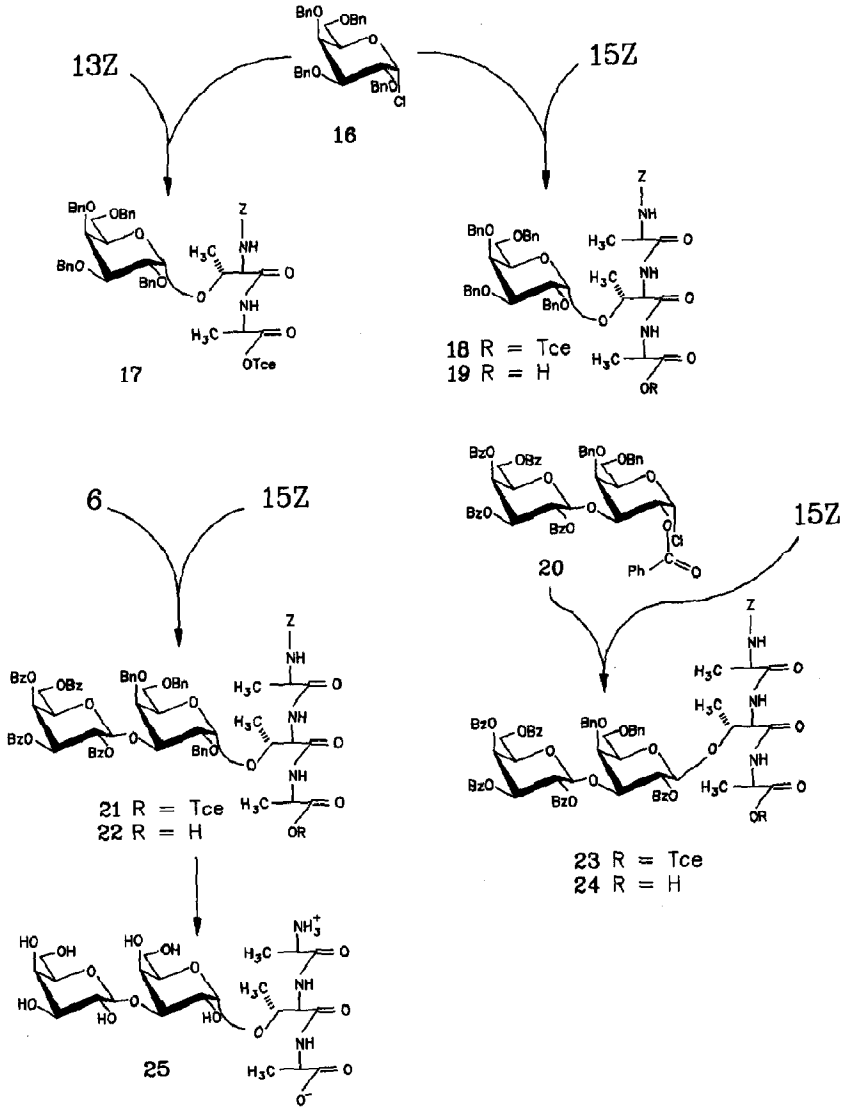
In spite of these considerations, our results show that, in some special cases, the convergent approach is very satisfactory for the synthesis of glycopeptides[18].

## EXPERIMENTAL

*General methods.* — Reactions were monitored by t.l.c. on glass plates coated with Silica Gel G (Merck). Spots were detected by charring with sulfuric acid. Chromatographic purifications were accomplished on columns of Silica Gel 60 (Merck), particle size 0.063–0.200 mm. $^1$H-N.m.r. spectra were recorded with a Bruker WH-270 or a Nicolet NT-200 spectrometer; $^{13}$C spectra were recorded with the NT-200 instrument. Unless otherwise specified, samples were dissolved in $CDCl_3$ containing tetramethylsilane as internal standard. When $D_2O$ was used as the solvent, chemical shifts were referenced to D*H*O at $\delta$ 4.67 (27°). Decoupling was done as required to identify signals that could not be assigned unambiguously by inspection. All reported compounds had spectra fully consistent with their assigned structures, but in most cases only the most characteristic lines are listed. Optical rotations were determined with a Perkin–Elmer model 141 polarimeter, and elemental analyses were done by the Galbraith Laboratories, Knoxville, TN.

*Allyl* O-*(2,3,4,6-tetra-*O-*benzoyl-β-*D-*galactopyranosyl)-(1→3)-2,4,6-tri-*O-*benzyl-α-*D-*galactopyranoside* (**3**). — A solution of **2** (ref. 4; 390 mg, 0.8 mmol) and silver triflate (257 mg, 1 mmol) in dichloromethane (25 mL) was stirred in a stoppered flask with Drierite (3 g) at 0°. The glycosyl donor **1** (ref. 20; 790 mg, 1.2 mmol), dissolved in a little $CH_2Cl_2$, was added, and stirring was continued for 2 h at 0°, in the absence of base[21]. Tetramethylurea (0.1 mL) and 2,6-dimethylpyridine (0.2 mL) were then added and the mixture was stirred overnight*. The solids were filtered off, the filtrate was evaporated to dryness, and the residue was chromatographed (1:5 EtOAc–hexane). Evaporation of the appropriate fractions gave **3** (690

*Probably unnecessary. The results of Garegg and Norberg[21] suggest that the reaction was complete before the bases were added.




Scheme 3

mg, 81%) as a foam, $[\alpha]_D$ +56° (*c* 1, chloroform); $^1$H-n.m.r.: δ 5.8 (m, 2 H, $=CH_2$), 5.32 (d, 1 H, *J* 8 Hz, H-1'), and 4.75 (d, 1 H, *J* 3.5 Hz, H-1).

*Anal.* Calc. for $C_{64}H_{60}O_{15}$ (1069.17): C, 71.9; H, 5.7. Found: C, 72.2; H, 5.9.

*1-Propenyl O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-(1→3)-2,4,6-tri-O-benzyl-α-D-galactopyranoside* (**4**). — (*a*) *From the allyl glycoside.* A mixture

of **3** (1 g), 1,4-diazabicyclo[2.2.2]octane ("Dabco"; 50 mg), and tris(triphenylphosphine)rhodium(I) chloride (300 mg) in 7:3:1 ethanol–toluene–water (25 mL) was boiled for 3 h under reflux. The mixture was then evaporated and the residue was extracted with ether. Evaporation of the extract gave a foamy residue which was purified by chromatography (1:5 EtOAc–hexane), to give **4** (900 mg, 90%) as a foam, $[\alpha]_D$ +35° (*c* 1, chloroform); $^1$H-n.m.r.: $\delta$ 5.32 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1'), 1.97, and 1.52 (2 d, total 3 H, *trans* and *cis* CH=CHC$H_3$).

*Anal.* Calc. for $C_{64}H_{60}O_{15}$ (1069.17): C, 71.9; H, 5.7. Found: C, 71.8; H, 5.8.

(*b*) *By direct coupling.* Propenyl glycoside **10** (400 mg, 0.82 mmol), silver triflate (251 mg, 0.98 mmol), and Drierite (4 g) were placed in a 100-mL round-bottom flask and vacuum-dried[22] for 3 h. After flushing with dry nitrogen, the flask was stoppered with a septum, then tetramethylurea (117 $\mu$L, 0.98 mmol), 2,6-dimethylpyridine (57 $\mu$L, 0.49 mmol), and dichloromethane (3 mL) were added by injection, and the mixture was cooled to 0–5°. Galactosyl bromide **1** (644 mg, 0.98 mmol), similarly dried in a separate flask, was transferred to the reaction vessel with two portions (1.5 and 1.0 mL) of dichloromethane, and the mixture was stirred at 5–10°. After 3 h, further portions (0.5 mmol each) of **1**, Ag triflate, tetramethylurea, and dimethylpyridine were added, stirring was continued for another 6 h, and then the mixture was filtered through Celite. The filtrate was washed (cold $H_2O$, cold saturated NaCl), dried ($MgSO_4$), and evaporated to dryness. Chromatography of the residue (48:1 toluene–acetone) gave **4** (594 mg, 68%), $[\alpha]_D$ +32° (*c* 1, chloroform); $^1$H-n.m.r. spectrum identical with that of the product obtained by method (*a*).

*O-(2,3,4,6-Tetra-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-*D*-galactose* (**5**). — To a solution of **4** (1 g) in 10:1 acetone–water (10 mL) were added mercuric oxide (0.5 g) and mercuric chloride (1 g). The mixture was stirred for 2 h, the solids were removed by filtration, the filtrate was evaporated, and water (20 mL) was added to the residue. The mixture was extracted with ether (2 × 25 mL), and the extract was successively washed with aqueous potassium iodide and water, dried ($Na_2SO_4$), and evaporated to a glass which was purified by column chromatography. Elution with 1:10 acetone–chloroform gave **5** (950 mg, 99%) as a foam; $[\alpha]_D$ +28° (*c* 1, chloroform; no change after 24 h).

*Anal.* Calc. for $C_{61}H_{56}O_{15}$ (1029.10): C, 71.2; H, 5.5. Found: C, 71.6; H, 5.7.

A portion (200 mg) of **5** was debenzoylated with methanolic sodium methoxide, and the solution was deionized and evaporated. The product was hydrogenolyzed over palladium–charcoal, and the resulting deprotected sugar reduced with sodium borohydride. After deionization and removal of borate by the addition and evaporation of methanol, the entire residue was taken up in $D_2O$ (0.5 mL) and examined[5] in the n.m.r. spectrometer. The doublet for H-1' appeared at $\delta$ 4.55, as expected for *O*-β-D-galactopyranosyl-(1→3)-D-galactitol, but no signal was discernible at $\delta$ 5.25, which is the chemical shift for H-1' of the α-linked anomer. This value was determined on an authentic sample, prepared by coupling **16** to **2** and converting the product into the free sugar (1-OH), which was deprotected and reduced as just described for **5**.

*Conversion of* **5** *into the glycosyl chloride.* — Vilsmeier reagent, prepared[23] from *N,N*-dimethylformamide (0.5 mL, 6.5 mmol), was washed, suspended in dry dichloromethane (10 mL), and treated with a solution of **5** (1.3 g, 1.3 mmol) in dichloromethane (5 mL). The mixture was stirred for 2 h at 25°, when t.l.c. (1:3 EtOAc–hexane) indicated complete conversion of **5**. The mixture was diluted with dichloromethane (50 mL), washed with ice-cold water (3 × 10 mL), dried ($Na_2SO_4$), and evaporated to give *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl chloride (**6**, 1.2 g); $^1$H-n.m.r.: δ 5.84 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.65 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, H-3'), 5.34 (d, 1 H, $J_{1',2'}$ 8.5 Hz, H-1'), 3.95 (dd, 1 H, $J_{2,3}$ 10 Hz, H-2). This product was used without further purification.

*1-Propenyl 2,4,6-tri-*O*-benzyl-α-*D*-galactopyranoside* (**10**). — A mixture of allyl α-D-galactopyranoside (**7**; 440 mg, 2 mmol) and dibutyltin oxide (500 mg, 2 mmol) in toluene (50 mL) was boiled under reflux for 3 h with azeotropic removal of water. The solution was then cooled to 60°, and tetrabutylammonium iodide (740 mg, 2 mmol) and crotyl bromide (2.6 mL) were added. The mixture was stirred for 20 h at 60° and then evaporated to dryness. The residue was purified by column chromatography to give allyl 3-*O*-crotyl-α-D-galactopyranoside (**8**) as a syrup.

The purified **8** (540 mg) was dissolved in *N,N*-dimethylformamide (10 mL), the solution was stirred and cooled in an ice bath, and sodium hydride (100 mg) and benzyl bromide (600 mg) were added. After 5 h at ~5–10° and 3 h at 25°, the mixture was diluted with ice-cold water (25 mL) and extracted with ether (2 × 20 mL). The extract was washed with water, dried, and concentrated to give syrupy allyl 2,4,6-tri-*O*-benzyl-3-*O*-crotyl-α-D-galactopyranoside (**9**).

Intermediate **9** was dissolved in *N,N*-dimethylformamide (10 mL) and potassium *tert*-butoxide (600 mg) was then added. The mixture was heated under nitrogen for 2 h, cooled to 25°, diluted with water (20 mL), and extracted with ether (2 × 20 mL). The extract was washed with water, dried ($Na_2SO_4$), and evaporated. The residue was purified by chromatography (1:10 acetone–chloroform) to give **10** (600 mg, 61% from **7**), m.p. 56° (from ether–hexane), $[\alpha]_D$ +42° (*c* 1, chloroform); $^1$H-n.m.r.: δ 7.45–7.15 (m, Ph–H), 6.0 (q, 1 H, $CH_3CH{=}CH_2$), 5.02 (d, $J_{1,2}$ 3 Hz, H-1), 2.40 (O*H*), 1.67, and 1.62 (2 d, total 3 H, *trans* and *cis* $CH{=}CHCH_3$).

*Anal.* Calc. for $C_{30}H_{34}O_6$ (490.60): C, 73.4; H, 7.0. Found: C, 73.7; H, 7.1.

Conventional treatment of a portion of **10** with acetic anhydride–pyridine gave an acetate. $^1$H-N.m.r. data: δ 7.4–7.1 (m, Ph–H), 6.05 (m, 1 H, =C*H*O-), 5.25 (dd, 1 H, →d on irrad. at 4.2, H-3), 5.05 (d, 1 H, $J_{1,2}$ 3 Hz, H-1), 4.2 (m, including dd→d on irrad. at 5.05, H-2), and 2.0 (s, 3 H, $CH_3CO$).

N-(tert-*Butoxycarbonyl*)-L-*alanine 2,2,2-trichloroethyl ester* (**11**) *and its* N-*deprotected derivative* (**12**). — A stirred solution of *N*-(*tert*-butoxycarbonyl)-L-alanine (commercial, 2.90 g, 15 mmol) in dichloromethane (60 mL) containing pyridine (1 mL) was cooled to 0°, and dicyclohexylcarbodiimide (3.3 g, 16 mmol) was then added, followed by 2,2,2-trichloroethanol (3 g, 20 mmol). The mixture was stirred for 4 h at 0° and for 18 h at 25°. The reaction mixture was filtered and the filtrate

was evaporated. The residue was purified by chromatography (1:6 EtOAc–hexane) to give **11** (3.45 g, 70%), m.p. 67° (from ether–hexane), $[\alpha]_D$ −27° (*c* 1, chloroform); $^1$H-n.m.r.: δ 4.92, 4.62 (2 d, 2 H, $CH_2CCl_3$), 4.45 (q, 1 H, α-C*H*), 1.45 (d, 3 H, Ala $CH_3$), and 1.42 (s, 9 H, $CCH_3$ of Boc).

*Anal.* Calc. for $C_{10}H_{16}Cl_3NO_4$ (320.60): C, 37.5; H, 5.0; N, 4.4. Found: C, 37.2; H, 4.9; N, 4.4.

A solution of **11** (256 mg) in 3:7 trifluoroacetic acid–dichloromethane (3 mL) was stored for 4 h at 25°, and then concentrated to give a residue of L-alanine 2,2,2-trichloroethyl ester trifluoroacetate salt* (**12**). Trituration of the residue with ether (30 mL) furnished 254 mg (95%) of friable solid, m.p. 128–129°; $^1$H-n.m.r. ($D_2O$): δ 5.05, 4.85 (2 d, 2 H, $CH_2CCl_3$), 4.27 (q, 1 H, α-C*H*), and 1.52 (d, 3 H, β-$CH_3$).

*General procedure for protected peptides (millimole scale)*[24]. — A solution of the *C*-terminal unit (1 mmol, as the trifluoroacetate salt) in dichloromethane (15 mL) was treated with triethylamine (1 mmol) at 0°. The *N*-protected amino acid (Boc or Z, 1 mmol) was then added, followed by 1-hydroxybenzotriazole[26] (1.2 mmol) and dicyclohexylcarbodiimide (1 mmol), and the mixture was stirred for 7 h at 0° and 18 h at 25°. The precipitated dicyclohexylurea was filtered off, the filtrate was concentrated, and the residue was chromatographed (1:10 or 1:20 acetone–chloroform). The following four compounds were prepared by this procedure.

N-(tert-*Butoxycarbonyl*)-L-*threonyl*-L-*alanine 2,2,2-trichloroethyl ester* (**13B**) *and its deprotected derivative* (**14**). — Prepared from alanine ester **12** (335 mg) and *N*-(*tert*-butoxycarbonyl)-L-threonine (commercial, 219 mg), **14** (314 mg, 74%) had m.p. 105° (from ether), $[\alpha]_D$ −53°, $[\alpha]_{436}$ −111° (*c* 1, chloroform); $^1$H-n.m.r.:δ 7.1, 5.4 (2 d, 2 H, N*H*), 4.90, 4.6 (2 d, 2 H, $CH_2CCl_3$), 3.14 (s, 1 H, O*H*), 1.5 (d, 3 H, Ala $CH_3$), 1.44 (s, 9 H, $CCH_3$ of Boc), and 1.22 (d, 3 H, Thr $CH_3$).

*Anal.* Calc. for $C_{14}H_{23}Cl_3N_2O_6$ (421.71): C, 39.9; H, 5.5; N, 6.6. Found: C, 40.1; H, 5.7; N, 6.6.

The *N*-deprotection of **13B**, as described for the conversion of **11** into **12**, gave the syrupy trifluoroacetate salt of L-threonyl-L-alanine 2,2,2-trichloroethyl ester (**14**), which was used without further purification.

N-(*Benzyloxycarbonyl*)-L-*threonyl*-L-*alanine 2,2,2-trichloroethyl ester* (**13Z**). — Prepared from **12** (335 mg) and *N*-(benzyloxycarbonyl)-L-threonine (commercial, 253 mg), **13Z** (410 mg, 90%) had m.p. 93–94° (from ether), $[\alpha]_D$ −36.5° (*c* 1, chloroform); $^1$H-n.m.r.: δ 7.15 (d, 1 H, Ala N*H*), 5.85 (d, 1 H, Thr N*H*), 5.15 (s, 2 H, Ph$CH_2$), 4.93, 4.63 (2 d, 2 H, $CH_2CCl_3$), 4.33 (m, 1 H, Thr β-C*H*), 4.20 (q, 1 H, Thr α-C*H*), 3.3 (d, 1 H, O*H*), 1.47 (d, 3 H, Ala $CH_3$), and 1.2 (d, 3 H, Thr $CH_3$).

*Anal.* Calc. for $C_{17}H_{21}Cl_3N_2O_6$ (455.72): C, 44.8; H, 4.6; N, 6.2. Found: C, 45.4; H, 4.9; N, 6.0.

*The hydrobromide salt of L-alanine 2,2,2-trichloroethyl ester has been described[15].

N-(tert-*Butoxycarbonyl*)-L-*alanyl*-L-*threonyl*-L-*alanine 2,2,2-trichloroethyl ester* (**15B**). — Prepared from **14** (436 mg) and *N*-(*tert*-butoxycarbonyl)-L-alanine (189 mg), **15B** (400 mg, 81%) had m.p. 104° (from ether), $[\alpha]_D$ −57° (*c* 1, chloroform); $^1$H-n.m.r.: δ 7.5, 7.2, 5.2 (3 d, 3 H, N*H*), 4.95, 4.63 (2 d, 2 H, $CH_2CCl_3$), 2.70 (br. s, 1 H, O*H*), 1.52 (d, 3 H, Ala $CH_3$), 1.42 (s, 9 H, $CCH_3$ of Boc), 1.4 (d, 3 H, Ala $CH_3$), and 1.17 (d, 3 H, Thr $CH_3$).

*Anal.* Calc. for $C_{17}H_{28}Cl_3N_3O_7$ (492.78): C, 41.4; H, 5.7; N, 8.5. Found: C, 41.5; H, 5.7; N, 8.7.

Examination of a hydrolyzed sample in the amino-acid analyzer gave a value of 19.0:9.9 for the ratio Ala:Thr.

N-*(Benzyloxycarbonyl)*-L-*alanyl*-L-*threonyl*-L-*alanine 2,2,2,-trichloroethyl ester* (**15Z**). — Prepared from **14** (436 mg) and *N*-(benzyloxycarbonyl)-L-alanine (commercial, 223 mg), **15Z** (450 mg, 85%) had m.p. 168° (from ether), $[\alpha]_D$ −47.5° (*c* 2, chloroform); $^1$H-n.m.r.: δ 7.45–7.25 (m, 6 H, Ph–H and N*H*), 7.17, 5.58 (2 d, 2 H, N*H*), 5.15 (s, 2 H, $PhCH_2$), 4.95, 4.65 (2 d, 2 H, $CH_2CCl_3$), 3.5 (br. s, 1 H, O*H*), 1.48, 1.42 (2 d, 6 H, Ala $CH_3$), and 1.22 (d, 3 H, Thr $CH_3$).

*Anal.* Calc. for $C_{20}H_{26}Cl_3N_3O_7$ (526.80): C, 45.6; H, 5.0; N, 8.0. Found: C, 45.8; H, 5.1; N, 8.0.

*General procedures for glycosylation*[27] *and deprotection of the terminal carboxyl group*[8,25]. — A mixture of the Z-protected peptide (1.0 mmol), silver triflate (1.0 mmol), and Drierite (6 g) in dichloromethane (30 mL) was cooled to 0°. The glycosyl chloride (1.3 mmol), dissolved in a little dichloromethane, was added and the mixture was stirred for 2 h at 0°. Tetramethylurea (0.2 mL) and 2,6-dimethylpyridine (0.4 mL) were then added and stirring was continued for 6 h at 25°. Finally, the solids were filtered off, the filtrate was concentrated, and the residual crude product was purified by chromatography.

For carboxyl deprotection, the glycopeptide trichloroethyl ester (0.1 mmol) was dissolved in 90% aqueous acetic acid (3 mL), and zinc powder (500 mg) was added to the stirred, ice-cold solution. After being stirred for 4 h at 0°, the suspension was filtered, the filter cake was washed with aqueous 90% acetic acid, and the combined filtrate and washings were evaporated to dryness. Ethyl acetate (15 mL) and M hydrochloric acid were added to the residue, the mixture was stirred, and the ethyl acetate phase was separated, washed with water, dried ($Na_2SO_4$), and concentrated. The resulting glycopeptide acid was purified by chromatography or recrystallization.

These procedures served for the preparation of the following protected glycopeptides. For use with quantities other than those illustrated, the amounts of reagents and solvents were adjusted proportionately.

*Galactosylation of the protected dipeptide* **13Z**. — Methyl β-D-galactopyranoside (commercial) was benzylated[28], and the product was hydrolyzed to give 2,3,4,6-tetra-*O*-benzyl-D-galactopyranose[29]. A portion (700 mg) of the benzylated sugar was converted into the glycosyl chloride[23] (**16**) and this, with **13Z** (456 mg), gave *N*-(benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-

(1→3)-L-threonyl-L-alanine 2,2,2-trichloroethyl ester (**17**; 630 mg, 64%), $[\alpha]_D$ +51.5° (*c* 1, chloroform); $^1$H-n.m.r.: δ 7.7 (d, 1 H, Ala N*H*), 7.4–7.2 (m, Ph–H), 5.9 (d, 1 H, Thr N*H*), 5.27 (d, 1 H, *J* 3.1 Hz, Gal H-1), 1.15 (d, 3 H, Ala C$H_3$), and 0.95 (d, 3 H, Thr C$H_3$).

N-*(Benzyloxycarbonyl)*-L-*alanyl*-[O-*(2,3,4,6-tetra*-O-*benzyl*-α-D-*galactopyranosyl-(1→3)*]-L-*threonyl*-L-*alanine* (**19**). — Galactosyl chloride **16** (from 700 mg of precursor) and protected tripeptide **15Z** (527 mg) were coupled, and one-tenth of the resulting **18** was carboxyl-deprotected. Chromatographic purification (10:1 chloroform–acetone) then gave **19** (58 mg, 63% from **15Z**), m.p. 64–65°, $[\alpha]_D$ +47° (*c* 1, chloroform); $^1$H-n.m.r.: δ 7.75, 7.57 (2 d, 2 H, N*H*), 7.37–7.2 (m, Ph–H), 5.75 (d, 1 H, N*H*), 5.2 (d, 1 H, *J* 2.2 Hz, Gal H-1), 1.33, 1.07 (2 d, 6 H, Ala C$H_3$), and 1.0 (d, 3 H, Thr C$H_3$); $^{13}$C-n.m.r. ($CDCl_3$): δ 98 (Gal C-1).

*Anal.* Calc. for $C_{52}H_{59}N_3O_{12}$ (918.05): C, 68.0; H, 6.5; N, 4.6. Found: C, 67.7; H, 6.5; N, 4.5.

N-*(Benzyloxycarbonyl)*-L-*alanyl*-[O-*(2,3,4,6-tetra*-O-*benzoyl*-β-D-*galactopyranosyl)-(1→3)*-O-*(2,4,6-tri*-O-*benzyl*-α-D-*galactopyranosyl)-(1→3)*]-L-*threonyl*-L-*alanine 2,2,2-trichloroethyl ester* (**21**). — Prepared from disaccharide-chloride **6** (0.65 mmol, from 670 mg of **5**) and protected tripeptide **15Z** (264 mg, 0.5 mmol), **21** (470 mg, 61%) had m.p. 155° (from ether–hexane), $[\alpha]_D$ +48° (*c* 1, chloroform); $^1$H-n.m.r.: δ 5.68 (dd, 1 H, sugar H-3′), 5.29 (d, 1 H, *J* 3.8 Hz, sugar H-1), 1.35, 1.22 (2 d, 6 H, Ala C$H_3$), and 0.98 (d, 3 H, Thr C$H_3$).

*Anal.* Calc. for $C_{81}H_{80}Cl_3N_3O_{21} \cdot 0.5\ H_2O$ (1546.90): C, 62.9; H, 5.3; N, 2.7. Found: C, 62.8; H, 5.6; N, 3.0.

N-*(Benzyloxycarbonyl)*-L-*alanyl*-[O-*(2,3,4,6-tetra*-O-*benzoyl*-β-D-*galactopyranosyl)-(1→3)*-O-*(2,4,6-tri*-O-*benzyl*-α-D-*galactopyranosyl)-(1→3)*]-L-*threonyl*-L-*alanine* (**22**). — Prepared from **21** (155 mg) by the general *C*-deprotection procedure, **22** (130 mg, 91%) had m.p. 150° (from ether), $[\alpha]_D$ +46° (*c* 1, chloroform); $^1$H-n.m.r.: δ 5.69 (dd, 1 H, sugar H-3′), 5.14 (d, 1 H, *J* 3.7 Hz, sugar H-1), 1.35, 1.15 (2 d, 6 H, Ala C$H_3$), and 0.90 (d, 3 H, Thr C$H_3$); $^{13}$C-n.m.r. ($CDCl_3$): δ 98.0 (sugar C-1) and 101.8 (sugar C-1′).

*Anal.* Calc. for $C_{79}H_{79}N_3O_{21}$ (1406.50): C, 67.5; H, 5.7; N, 3.0. Found: C, 67.4; H, 5.8; N, 2.9.

N-*(Benzyloxycarbonyl)*-L-*alanyl*-[O-*(2,3,4,6-tetra*-O-*benzoyl*-β-D-*galactopyranosyl)-(1→3)*-O-*(2*-O-*benzoyl-4,6-di*-O-*benzyl*-β-D-*galactopyranosyl)-(1→3)*]-L-*threonyl*-L-*alanine* (**24**). — Galactosyl bromide **1** was coupled to 1-propenyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-galactopyranoside[4] by a method similar to that used in preparing compound **15** of ref. 3. Work-up by chromatography (99:1 toluene–acetone) gave a disaccharide product (513 mg from two runs, procedure not optimized). This was converted, as described for the transformation **4→5→6**, into an intermediate having the properties expected of *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-D-galactopyranosyl chloride (**20**).

Compound **20** (138 mg, 0.13 mmol) and protected tripeptide **15Z** (53 mg, 0.1

mmol) on coupling and *C*-deprotection furnished **24** (100 mg, 70%), m.p. 146° (from ether), $[\alpha]_D$ +14.7° (*c* 1, chloroform); $^{13}$C-n.m.r. ($CDCl_3$): $\delta$ 102.3 (sugar C-1′) and 100.5 (sugar C-1).

*Anal.* Calc. for $C_{79}H_{77}N_3O_{22}$ (1420.48): C, 66.8; H, 5.5; N, 3.0. Found: C, 67.2; H, 5.5; N, 2.9.

*Complete deprotection of the disaccharide–tripeptide* **22**. — A solution of **22** (25 mg) in a little methanol was cooled to 0° and neutralized by the dropwise addition of methanolic 0.1M sodium methoxide. One further drop of NaOMe rendered the solution basic to pH paper (blue color), and further small increments of NaOMe were added to maintain this condition while the solution was kept in a stoppered flask at 0°. After 2 days, when debenzoylation was complete (t.l.c., 6:1 chloroform–methanol), the solution was deionized with Amberlite IR-120 ($H^+$) and filtered from the resin. Following the addition of 10% palladium-charcoal (50 mg), the solution was stirred for 40 h under hydrogen at 1 atm., then filtered, and evaporated to dryness. Trituration of the residue with ether (4 × 2 mL) left L-alanyl-[*O*-β-D-galactopyranosyl-(1→3)-*O*-α-D-galactopyranosyl-(1→3)]-L-threonyl-L-alanine (**25**) as a white powder (6 mg, 60%). $^1$H-N.m.r. data ($D_2O$): $\delta$ 5.15 (d, 1 H, *J* 3.0 Hz, sugar H-1), 4.67 (d, 1 H, *J* 9.0 Hz, sugar H-1′), 1.90, 1.85 (2 d, 6 H, Ala C$H_3$), and 1.75 (d, 3 H, Thr C$H_3$).

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