1 H), 5.97 (2 s, 2 H), 4.16 (s, 3 H), 4.00 (s, 3 H), 3.72 (s, 3 H), 3.59 (s, 3 H); UV (EtOH) 264, 295, 309, 350 nm; fluorescence 453 nm; HRMS, m/z 424.1151 (M^+), calcd for $C_{23}H_{20}O_8$ 424.1155.

Acknowledgment. We thank Drs. N. G. Patel and E. L. Jenner for providing us natural justicidin P and its spectra. The NMR studies by Dr. G. S. Reddy are greatly appreciated. We also thank Prof. B. M. Trost for helpful discussions.

Registry No. 1, 86012-93-3; 2, 25001-57-4; 4, 86012-94-4; 5, 86012-95-5.

Chemical Synthesis of Some Mono- and Digalactosyl *O*-Glycopeptides†

J. M. Lacombe and A. A. Pavia*

Laboratoire de Chimie Bioorganique, Faculté des Sciences d'Avignon, 84000 Avignon, France

Received June 29, 1982

Chemical syntheses of several *O*-glycopeptides containing *O*-galactosylthreonine are reported. Two different approaches have been investigated to determine the strategy best adapted to the synthesis of any desired *O*-glycopeptide. Glycosylation of an adequately protected threonine-containing peptide was shown to be less successful than a stepwise strategy using an appropriate *O*-glycosyl amino acid as starting material. This route was shown to have more potential since it allows construction of complex glycopeptides containing both glycosyl amino acids and unglycosylated hydroxy amino acids. Various α- and β-*O*-glycopeptides are described in which the threonine molecule linked to galactose is either C-terminal or N-terminal or inserted in the peptide chain.

We have recently reported the one-step synthesis of *O*-glycosyl amino acids relevant to glycoproteins.[1] The procedure involved the reaction of a fully benzylated reducing sugar with the appropriate derivative of a serine, threonine, or hydroxyproline molecule, in the presence of trifluoromethanesulfonic anhydride, followed by removal of all protecting groups by hydrogenolysis in the presence of 10% palladium-on-charcoal as catalyst. We now wish to report the synthesis of several threonine-containing *O*-glycopeptides of known anomeric configuration in which the threonine molecule linked to galactose is either C- or N-terminal or inserted in the peptide core.

In the past few years, natural abundance carbon-13 nuclear magnetic resonance has been used to gain dynamic and structural information about carbohydrate residues of large glycoproteins.[2-4] Application of this technique to the structural study of glycoproteins seems promising, but there are still some temporary limitations. One of these is the lack of ^{13}C NMR data of relevant model compounds needed to make specific assignments in the spectra of glycoproteins. Knowledge of ^{13}C NMR chemical shifts of appropriate model compounds may facilitate the use of this technique to study carbohydrate–peptide linkages in intact glycoproteins.

In addition, the glycopeptides reported in this communication were required in the course of our research to provide a better understanding of the structural and conformational properties of the glycosidic bond in glycopeptides as well as the role of the carbohydrate moiety in the binding of metal cation with membrane glycoproteins.

Results and Discussion

One approach to the synthesis of glycopeptides is the synthesis of the core peptide containing the desired hydroxy amino acid followed by glycosylation. To be suitable, this approach requires the glycosylation method to be highly stereoselective. Moreover, since the glycosylation reaction depends upon the reactivity of the hydroxyl group, this reactivity should not be altered by the incorporation of serine, threonine, or hydroxyproline in the peptide chain. In fact, as seen below, this latter requirement was not achieved in all cases. An alternative approach requires the synthesis of adequately protected *O*-glycosyl amino acids, followed by elongation at either or both the C- or N-terminals. As done previously,[5-8] we followed the latter strategy. This route has more potential since it allows construction of complex glycopeptides containing both glycosyl amino acids and unglycosylated hydroxy amino acids. It should be noted that Guillemin and co-workers[9] attempted to adapt this route to the solid-phase synthesis of *N*-glycopeptides related to somatostatin.

In order to determine the strategy best adapted to the synthesis of any desired glycopeptide, both of these routes were investigated. Peptide blocking strategies are well-known. However, in the case of glycopeptides, there are additional requirements: (i) Deprotection of peptide residues to allow coupling at the C- and N-terminal must be both selective and compatible with carbohydrate hydroxyl-protecting groups. (ii) The preparation of glycopeptides with the α-anomeric configuration precludes the presence of participating neighboring groups on the carbohydrate moiety. Therefore, benzyl derivatives were preferred to acetyl or benzoyl derivatives. (iii) Amino acid protecting groups, on the other hand, must be stable in acid medium because of the acid-catalyzed nature of the glycosylation reaction.[10] Ideally, the removal of glycosyl

† This work was supported by a research grant from the Délégation Générale à la Recherche Scientifique et Technique (D.G.R.S.T.), No. 81 F 0388.

(1) J. M. Lacombe, A. A. Pavia, and J. M. Rocheville, *Can. J. Chem.*, **59**, 482-489 (1981).
(2) K. Dill and A. Allerhand, *J. Biol. Chem.*, **254**, 4524-4531 (1979).
(3) E. Berman, A. Allerhand, and A. L. DeVries, *J. Biol. Chem.*, **255**, 4407-4410 (1980).
(4) E. Berman, D. E. Walters, and A. Allerhand, *J. Biol. Chem.*, **256**, 3853-3857 (1981).
(5) J. Martinez, A. A. Pavia, and F. Winternitz, *Carbohydr. Res.*, **50**, 15-22, 148-151 (1976).
(6) H. G. Gard and R. W. Jeanloz, *Carbohydr. Res.*, **52**, 245-250 (1976).
(7) H. G. Garg, R. W. Jeanloz, *Pept. Proc. Am. Pept. Symp. 5th*, 477-479 (1977); *Chem. Abstr.*, **89**, 454489 (1978).
(8) M. G. Vafina and W. A. Derevitzkaya, *Izv. Akad. Nauk SSSR, Ser. Khim.* 1475 (1965).
(9) S. Lavielle, N. Ling, R. Saltman, and T. Guillemin, *Carbohydr. Res.*, **89**, 229-236 (1981).

0022-3263/83/1948-2557$01.50/0

Table I. Carbon-13 NMR Chemical Shifts for Glycopeptides Carrying the Galactosyl Residue at the N-Terminal Threonine[a]

compounds[b]	CO ester	CO amide	CO urethane	C1 galactose	Cα Gly	Cα[c] Thr	Cα Thr	CH_3[c] Thr	CH_3 Thr
Bzl_4-α-D-Gal→ Z-L-Thr-ONp (3)	168.5		156.7	99.2		60.14		19.30	
Bzl_4-β-D-Gal→ Z-L-Thr-ONp (4)	168.3		156.6	101.1		59.5		16.5	
Bzl_4-α-D-Gal→ Z-L-Thr-Gly-OBzl (7)	168.55	169.25	155.6	97.37	40.7	56.3		15.3	
Bzl_4-β-D-Gal→ Z-L-Thr-Gly-OBzl (8)	169.28	169.78	156.6	103.9	41.4	57.5		16.8	
Bzl_4-α-D-Gal→ Z-L-Thr-Thr-OBzl (9)	169.2	170.4	155.7	98.6		56.7	57.9	15.2	19.8
Bzl_4-β-D-Gal→ Z-L-Thr-Thr-OBzl (10)	169.3	170.3	156.5	104.8		57.2	58.3	15.6	20.0

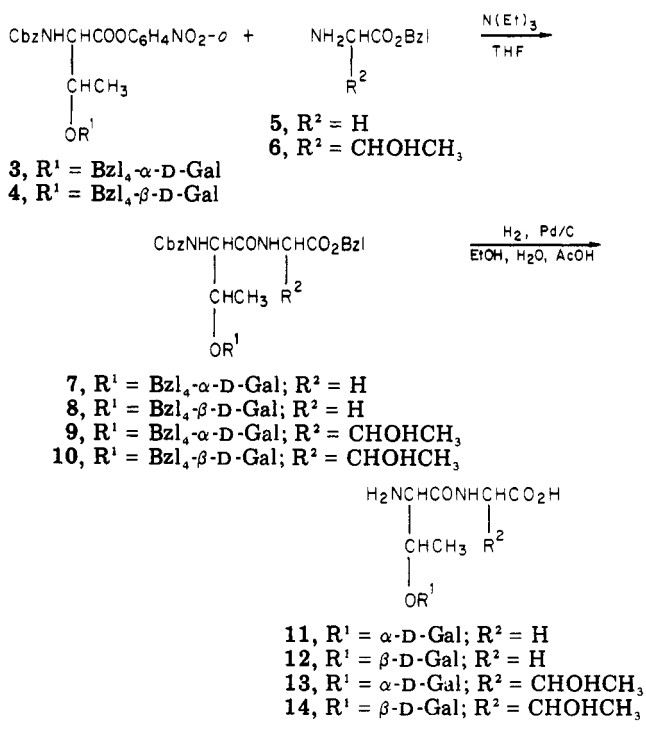
[a] Chemical shifts in ppm relative to Me_4Si as internal reference in $CDCl_3$. [b] Abbreviations: Bzl_4 = 2,3,4,6-tetra-*O*-benzyl; Z = *N*-(benzyloxycarbonyl); OBzl = benzyl ester; ONp = *o*-nitrophenyl ester; Bzl_4-α-D-Gal→Z-L-Thr-Gly-OBzl = benzyl *N*-(benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-L-threonylglycinate. [c] Indicates glycosylated threonine.

and amino acid protecting groups should require one step. (iv) Finally, since *O*-glycopeptides readily undergo β-elimination, none of the reaction conditions should be very basic.

The choice of threonine instead of serine was determined by the presence of the methyl group on the former. This methyl signal represents an extremely sensitive probe, which allowed monitoring both the stereochemistry of the anomeric linkage[11] and the racemization at either or both the asymmetric amino acid centers by ^{13}C NMR spectroscopy.[12] Scheme I shows the synthetic route used to prepare glycopeptides with galactose linked to the N-terminal threonine. The synthesis began with a trifluoromethanesulfonic anhydride condensation[1] of *N*-(benzyloxycarbonyl)-L-threonine *o*-nitrophenyl ester (1), prepared according to Bodanszky,[13] with 2,3,4,6-tetra-*O*-benzyl-D-galactopyranose (2). Pure α- and β-galactosyl derivatives (3 and 4) were obtained after silica gel column chromatography and condensed respectively with glycine and threonine benzyl esters (5 and 6) as in a previous report[5] and in the presence of a small amount of 1-hydroxybenzotriazole (HOBT). The reaction mixture was worked up as usual, and the crude residue was purified by column chromatography. Pure 7, 8, 9, and 10 were obtained in good yield. The corresponding ^{13}C NMR data, reported in Table I, are in excellent agreement with expectations. Removal of protecting groups by hydrogenation in a water–ethanol–acetic acid mixture using palladium-on-charcoal as catalyst afforded pure *O*-(α-D-galactopyranosyl)-L-threonylglycine (11), *O*-(β-D-galactopyranosyl)-L-threonylglycine (12), *O*-(α-D-galactopyranosyl)-L-threonyl-L-threonine (13), and *O*-(β-D-galactopyranosyl)-L-threonyl-L-threonine (14).

A second approach to compounds 7 and 8 involved a BOP ((benzotriazolyl-1-oxy)tris(dimethylamino)-phosphonium hexafluorophosphate) synthesis[14] employing *N*-(benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-L-threonine (15) and the corresponding β isomer (16) with glycine benzyl ester (5). This route was less satisfactory since the hydrolysis of methyl *N*-(benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-L-threoninate and the corresponding β isomer by treatment with a basic resin to produce 15 and 16 was accompanied by degradation of the starting material (the β-elimination mentioned before). Moreover, the peptide coupling reaction did not work as well as above. For instance, compound 16 was obtained in only 40% yield (see Experimental Section, synthesis of 8, Procedure B).

Scheme I



The synthesis of glycopeptides carrying the galactosyl residue at the C-terminal threonine is shown in Scheme II. In this particular case, ((9-fluorenylmethyl)oxy)-carbonyl (Fmoc) was preferred to (*tert*-butyloxy)carbonyl (Boc) for temporary protection of the amino group. Several attempts to glycosylate Boc-threonine benzyl ester in order to prepare Boc analogues of compounds 20 and 21 were unsuccessful. This behavior was ascribed to the presence of trace amounts of free amino acid resulting from partial hydrolysis of the Boc substituent. Previous work[16]

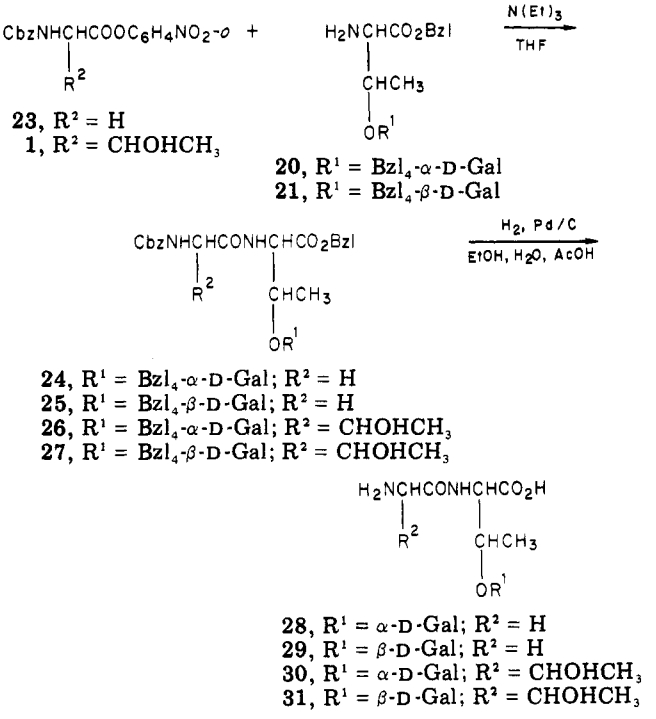
(10) A. A. Pavia and S. N. Ung-Chhun, *Can. J. Chem.*, **59**, 482–489 (1981).
(11) A. A. Pavia, S. N. Ung-Chhun, and J. M. Lacombe, *Nouv. J. Chim.*, **5**, 101–108 (1981).
(12) A. A. Pavia and J. M. Lacombe, *J. Org. Chem.*, following paper in this issue.
(13) M. Bodanszky, M. Kondo, M. L. Link, and G. F. Sigler, *J. Org. Chem.*, **39**, 444–447 (1974); M. Bodanszky, K. W. Funk, and M. L. Link, *ibid.*, **38**, 3565–3570 (1973).
(14) B. Castro, J. R. Dormoy, G. Evin, and C. Selve, *Tetrahedron Lett.*, **14**, 1219–1222 (1974); B. Castro, G. Evin, C. Selve, and R. Seyer, *Synthesis*, 413 (1977).
(15) M. Bodanszky and V. Du Vigneaud, *J. Am. Chem. Soc.*, 81, 5688–5691 (1959).

Table II. Carbon-13 NMR Chemical Shifts for Glycopeptides Carrying the Galactosyl Residue at the N-Terminal Threonine[a]

compounds[b]	CO ester	CO amide	CO urethane	C1 Gal	Cα Gly	Cα[c] Thr	Cα Thr	CH_3[c] Thr	CH_3 Thr	C9 fluorene
Bzl_4-α-D-Gal→ Fmoc-L-Thr-OBzl (18)	170.6		156.9	98.6		54.9		19.2		47.4
Bzl_4-β-D-Gal→ Fmoc-L-Thr-OBzl (19)	170.3		156.6	102.2		59.18		17.6		47.3
Z-Gly-L-Thr(Bzl_4-α-D-Gal)-OBzl (24)	170.5	169.6	156.3	97.5	44.2	56.8		19.4		
Z-Gly-L-Thr(Bzl_4-β-D-Gal)-OBzl (25)	169.9	169.6	156.6	102.3	44.4	57.3		17.8		
Z-L-Thr-L-Thr(Bzl_4-α-D-Gal)-OBzl (26)	171.6	170.2	156.6	98.5		57.4	58.8	18.1	19.1	
Z-L-Thr-L-Thr(Bzl_4-β-D-Gal)-OBzl (27)	171.1	170.1	156.4	102.1		57.3	59.1	18.0	18.0	

[a-c] See Table I.

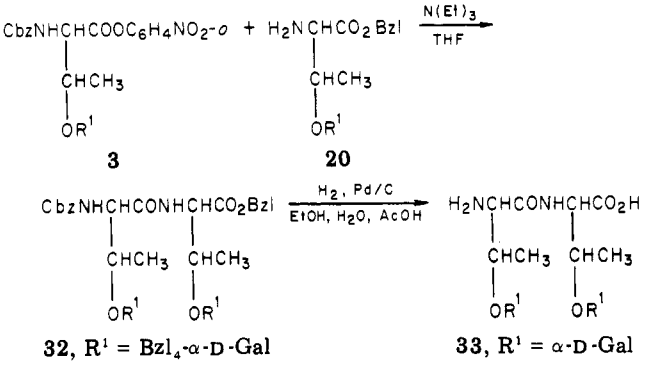
Scheme II



showed that the presence of a base such as pyridine or collidine prevents the glycosylation step. O-(2,3,4,6-Tetra-O-benzyl-α- and -β-D-galactopyranosyl)-L-threonine benzyl esters (20 and 21) were obtained by treatment of Fmoc derivatives 18 and 19 with piperidine. Condensation with N-(benzyloxycarbonyl)-glycine (23) and L-threonine (1) yielded the desired pure glycopeptides 24, 25, 26, and 27 after silica gel column chromatography. Carbon-13 NMR data reported in Table II confirm the structure and anomeric configuration of the above compounds. Catalytic reduction, as before, afforded glycyl-O-(α-D-galactopyranosyl)-L-threonine (28), glycyl-O-(β-D-galactopyranosyl)-L-threonine (29), L-threonyl-O-(α-D-galactopyranosyl)-L-threonine (30), and L-threonyl-O-(β-D-galactopyranosyl)-L-threonine (31) in almost quantitative yield.

Finally, N-(benzyloxycarbonyl)-O-(α-D-galactopyranosyl)-L-threonine o-nitrophenyl ester (3) was condensed with O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-L-threonine benzyl ester (20) to yield, after hydrogenation, O-(α-D-galactopyranosyl)-L-threonyl-O-(α-D-galactopyranosyl)-L-threonine (33) as shown in Scheme III.

Scheme III

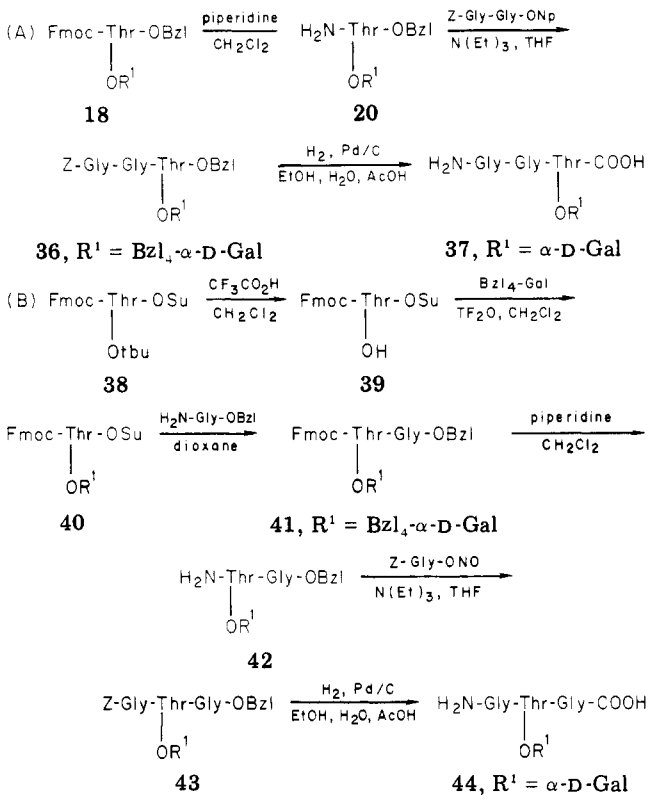


Alternatively, compounds 7 and 24 were obtained from the corresponding dipeptides 34 and 35. For reasons already mentioned, Boc protection was precluded. Dicyclohexylcarbodiimide (DCC) reaction of N-(benzyloxycarbonyl)-L-threonine with the p-toluenesulfonate salt of glycine benzyl ester[15] led to the desired peptide 34. The latter was reacted with 2,3,4,6-tetra-O-benzyl-D-galactopyranose (2) in the presence of trifluoromethanesulfonic anhydride at room temperature. Pure 7 was obtained in 85% yield after column chromatography. In this particular case, the high α-stereoselectivity of the glycosylation should be noted. On the other hand, the reactivity of the hydroxyl group seemed to be lowered, since it was necessary to perform the reaction at room temperature, whereas glycosylation of amino acid is usually carried out at approximately −15 °C. In contrast, glycosylation of peptide 35, obtained by a BOP coupling between N-(benzyloxycarbonyl)glycine and threonine benzyl ester, was far less satisfactory. Compound 24 was obtained in only 40% yield, but again, the α-stereoselectivity was reasonably good. So far, no evident reason can be put forward to account for the lower yield observed when a C-terminal peptide is glycosylated as compared to that of the corresponding N-terminal peptide.

In order to delineate the utilization of a glycosyl amino acid in peptide synthesis, two further experiments were performed. By analogy with the synthesis of compound 24, benzyl O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-L-threoninate (20) was coupled to N-(benzyloxycarbonyl)-glycylglycine o-nitrophenyl ester to form the desired galactosyl tripeptide 36 in 80% yield. Catalytic reduction of compound 36 afforded glycylglycyl-O-(α-D-galactopyranosyl)-L-threonine (37) as shown in Shcme IVA. The facile synthesis of 36 shows that the use of glycosyl amino acids in peptide synthesis is practical.

(16) A. A. Pavia, S. N. Ung-Chhun, and J. M. Rocheville, *Carbohydr. Res.*, 79, 79–90 (1980).

Scheme IV



Working along this line, we wished to demonstrate that glycopeptides could be obtained by a stepwise peptide-lenthening strategy on both sides of an appropriately protected glycosyl amino acid. The synthetic pathway to prepare compound **44** is reported in Scheme IVB. The strategy precluded the utilization of the of *N*-benzyloxycarbonyl group. In that particular case the *N*-hydroxysuccinimide ester was preferred to the *o*-nitrophenyl derivative, since the higher reactivity of the former resulted in a significant reduction of the reaction time. Consequently, the risks for the alkali-labile Fmoc protecting group to be cleaved are minimized. The major disadvantage of enhancing the reactivity of the starting material is the formation of a byproduct, which was briefly investigated as the ester resulting from the autocondensation of two molecules of **39**.

Condensation of **39** with 2,3,4,6-tetra-*O*-benzyl-D-galactose (**2**), as usual, gave a 4:1 anomeric mixture of **40α** and **40β**, which was not purified at this stage. The crude product was coupled to glycine benzyl ester and the resulting mixture purified by column chromatography to afford pure **41**. The latter was treated with a solution of piperidine in dichloromethane to give *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-L-threonylglycine benzyl ester (**42**). Compound **42** was coupled to *N*-(benzyloxycarbonyl)glycine *o*-nitrophenyl ester to give the desired tripeptide **43** in 80% yield. On catalytic hydrogenation, **43** afforded pure glycyl-*O*-(α-D-galactopyranosyl)-L-threonylglycine (**44**).

Fully deprotected compounds **11**, **12**, **13**, **14**, **28**, **29**, **30**, **31**, **33**, **37**, and **44** have been submitted to ^{13}C NMR spectroscopy in order to determine how the peptide bond may affect the chemical shift of the attached carbohydrate carbon atoms. Due to the fact that glycoprotein conformation may depend on the attached oligosaccharide chain, as reported occasionally in the literature, it would be of interest to obtain information about the effect of glycosylation on conformation and to ascertain whether neighboring glycosylation may produce steric interaction which also may affect protein conformation. The conclusion of this work will be reported elsewhere.[17]

Moreover, ^{13}C NMR studies of diastereoisomeric α- and β-D-galactopyranosylthreonine derivatives have shown that racemization at any of the threonine asymmetric centers could easily be detected, since all the above compounds showed distinct and different C1 and CH_3 (C′γ) resonance signals.[12] For instance, chemical shift differences of up to 8.4 and 6.9 ppm for C1 and CH_3, respectively, were noted in diastereoisomeric α- and β-D-galactopyranosyl-L-threonine, -D-threonine, -L-allothreonine, and -D-allothreonine.

These observations allowed us the conclude that, in all the compounds described herein, the extent of racemization was less than 3% (see the following paper in this issue).

Experimental Section

Carbon-13 NMR spectra were recorded on a Bruker WP80 (20.115 MHz) spectrometer and are expressed in parts per million from Me_4Si as internal standard. In deuterium oxide solutions, chemical shifts were measured with respect to 1,4-dioxane, whose chemical shift was 67.86 ppm. Optical rotations were recorded on a Perkin-Elmer M241 polarimeter. Capillary melting points were determined on a Bucchi apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed on silica gel G plates (Merk F254), and the components were visualized either by UV light or by spraying with a 10% sulfuric acid–ethanol solution followed by heating at 100 °C. Column chromatography was performed on silica gel Merck 60, 70–230 mesh, or Merck 60, 230–400 mesh, in the case of flash chromatography. Solvents used in TLC or column chromatography were as follows: A, chloroform–diethyl ether, 95:5 (v/v); B, chloroform–diethyl ether, 50:50 (v/v); C, hexane–ethyl acetate, 80:20 (v/v); D, chloroform–diethyl ether, 97:3 (v/v); E, dichloromethane–diethyl ether, 90:10 (v/v); F, dichloromethane–diethyl ether, 94:6 (v/v); G, dichloromethane–acetone, 96:4 (v/v); H, dichloromethane–acetone, 95:5 (v/v); I, chloroform–ether, 93:7 (v/v).

Abbreviations used are as follows: Boc, (*tert*-butyloxy)carbonyl; BOP, (benzotriazolyl-1-oxy)tris(dimethylamino)phosphonium hexafluorophosphate; Cbz, benzyloxycarbonyl; DCC, dicyclohexylcarbodiimide; Fmoc, ((9-fluorenyl)methyloxy)carbonyl; TF_2O, trifluoromethanesulfonic anhydride; THF, tetrahydrofuran; TEA, triethylamine; amino acid abbreviations follow IUPAC–IUB conventions.[19]

***o*-Nitrophenyl *N*-(Benzyloxycarbonyl)-L-threoninate (1).** This compound was prepared according to Bodanszky:[13] mp 73–74 °C (lit. mp 74–76 °C); $[\alpha]^{20}_D$ −66.5° (*c* 1.0, $CHCl_3$) [lit.[13] $[\alpha]^{20}_D$ −68.3° (*c* 0.6, $CHCl_3$)]; ^{13}C NMR δ 169.2 (CO ester), 156.6 (CO urethane), 67.53 and 67.3 (CH_2 benzyl and Cβ-Thr), 19.93 (CH_3-Thr).

2,3,4,6-Tetra-*O*-benzyl-D-galactopyranose (2). This compound was prepared according to Koto:[20] mp 80–82 °C (lit.[21] mp 81–82 °C); $[\alpha]^{20}_D$ −7° (*c* 1.0, $CHCl_3$) (lit.[20] $[\alpha]^{20}_D$ +18° (*c* 3.6, dioxane)].

***o*-Nitrophenyl *N*-(Benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzyl-α- and -β-D-galactopyranosyl)-L-threoninate (3 and 4).** *o*-Nitrophenyl *N*-(benzyloxycarbonyl)-L-threoninate (**1**) (1.2 g, 3 mmol) and trifluoromethanesulfonic anhydride (0.25 mL, 1.5 mmol) were mixed in cold (−15 °C) acetonitrile (30 mL) followed by the dropwise addition of 2,3,4,6-tetra-*O*-benzyl-D-galactopyranose (**2**) (0.54 g, 1 mmol) dissolved in cold (−15 °C) dichloromethane (5 mL). The reaction was monitored by TLC (solvent A). After 15 min, water (30 mL) was added and the reaction mixture extracted with diethyl ether (3 × 30 mL). The

(17) K. Dill, R. E. Hardy, M. E. Daman, M. J. Lacombe, and A. A. Pavia, *Carbohydr. Res.*, **108**, 31–40 (1982).
(18) C. Chang, M. Wagi, M. Ahmad, J. Meienhoffer, E. D. Lundell, and J. D. Heug, *Int. J. Pept. Protein Res.*, **15**, 59–66 (1980).
(19) *Pure Appl. Chem.*, **40**, 291 (1974).
(20) S. Koto, N. Morishima, Y. Myata, and S. Zen, *Bull. Chem. Soc. Jpn.*, **49**, 2639–2640 (1976).
(21) P. W. Austin, F. E. Hardy, J. G. Buchman, and J. Baddeley, *J. Chem. Soc.*, 1419 (1965).

organic phase was successively washed with water (50 mL), saturated aqueous $NaHCO_3$ (25 mL), and water (50 mL) and dried over Na_2SO_4. Filtration and concentration in vacuo gave a crude product. Flash chromatography (solvent C) of the reaction mixture to remove compound 1 (0.5 g, 70% of the excess) followed by column chromatography on silica gel (solvent D) afforded pure 3 (0.33 g, 37%) and 4 (0.29 g, 33%) as clear yellow syrups.

Compound 3: $[\alpha]^{20}_D$ +22° (c 1.1, $CHCl_3$); ^{13}C NMR, see Table I. Anal. Calcd for $C_{52}H_{52}O_{12}N_2$: C, 69.64; H, 5.80; N, 3.12. Found: C, 69.52; H, 5.85; N, 2.92.

Compound 4: $[\alpha]^{20}_D$ +6.6° (c 0.7, $CHCl_3$); ^{13}C NMR, see Table I. Anal. Calcd for $C_{52}H_{52}O_{12}N_2$: C, 69.64; H, 5.80; N, 3.12. Found: C, 69.72; H, 5.85; N, 3.18.

Benzyl N-(Benzyloxycarbonyl)-O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-L-threonylglycinate (7). (a) To a chilled (0 °C) solution of 3 (0.3 g, 0.33 mmol) in THF (15 mL) was added at once a mixture of the p-toluenesulfonate salt of benzyl glycinate (0.1 g, 0.33 mmol) and TEA (0.1 mL, 0.7 mmol) in THF (5 mL). The mixture was stirred at 0 °C for 30 min and allowed to warm to room temperature. After 18 h, HOBT (0.1 g) and additional TEA (0.5 mL) were added, and the mixture was kept at room temperature for another 6 h (TLC monitoring, solvent A). The reaction mixture was concentrated in vacuo, and the syrupy residue was dissolved in ethyl acetate (50 mL). The organic phase was successively washed with 5% aqueous citric acid (2 × 50 mL), water (50 mL), 0.05 N aqueous NaOH (5 × 25 mL), and water (50 mL). The oily residue obtained after concentration in vacuo was chromatographed on silica gel (solvent D) to afford the title compound (0.3 g, 97%) as a colorless oil: $[\alpha]^{20}_D$ +53° (c 1, $CHCl_3$); ^{13}C NMR, see Table I. Anal. Calcd for $C_{55}H_{58}O_{11}N_2$: C, 71.58; H, 6.29; N, 3.03. Found: C, 71.40; H, 6.50; N, 3.10.

(b) Methyl N-(benzyloxycarbonyl)-O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-L-threoninate[1] (0.44 g, 0.5 mmol) disolved in acetone (30 mL) was stirred in the presence of resin Dowex 1 (OH^-), and the hydrolysis was monitored by TLC (solvent A). The reaction mixture was filtered, and the resin was washed with acetone and water and eluted with a 6 N hydrochloric acid–acetone (1:5, v/v) solution. Fractions containing the desired material were combined, neutralized with saturated aqueous sodium bicarbonate, and evaporated in vacuo. The remaining aqueous phase was extracted with ethyl acetate (3 × 25 mL) and the extract dried over Na_2SO_4. After evaporation, N-(benzyloxycarbonyl)-O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-L-threonine (15) was obtained as an oil (0.35 g, 87%), which was used without purification in the next step. To a solution of the above compound (0.35 g, 0.45 mmol) in dichloromethane (40 mL) was successively added BOP reagent (0.18 g, 0.45 mmol), benzyl glycinate, p-toluenesulfonate (0.16 g, 0.45 mmol), and TEA (1.4 mL, 1 mmol). After 15 min (TLC monitoring, solvent A) at room temperature, saturated NaCl aqueous solution (30 mL) was added, followed by extraction with ethyl acetate (3 × 10 mL). The organic phase was washed with 2 N hydrochloric acid (2 × 10 mL), saturated aqueous sodium bicarbonate (3 × 10 mL), and saturated aqueous NaCl (10 mL) and, finally, dried over calcium chloride. Evaporation in vacuo afforded 7 as a colorless oil, identical with that obtained in procedure a (0.32 g, 77%).

(c) Benzyl N-(benzyloxycarbonyl)-L-threonylglycinate (34) (0.4 g, 1 mmol) was dissolved in dichloromethane (10 mL) at room temperature followed by the addition of triflic anhydride (TF_2O) (0.1 mL) and 2,3,4,6-tetra-O-benzyl-D-galactopyranose (2) (0.14 g, 0.25 mmol). The mixture was allowed to react for 40 min (TLC monitoring, solvent A), and water was added. The reaction mixture was extracted with diethyl ether (3 × 25 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ (2 × 25 mL) and water (50 mL) and dried over Na_2SO_4. Concentration in vacuo gave a crude product, which was passed through a silica gel column (solvent A). Pure 7 (0.19 g, 85%) was obtained. Trace amounts of compound 8 (0.015 g) could also be isolated.

Benzyl N-(Benzyloxycarbonyl)-O-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)-L-threonylglycinate (8). (a) This compound was prepared by using the procedure described for compound 7. Column chromatography afforded 8 in 95% yield as a clear syrup which crystallized. Recrystallization from diethyl ether–petroleum ether gave 8: mp 109–110 °C; $[\alpha]^{20}_D$ +28° (c 1.0, $CHCl_3$); ^{13}C NMR, see Table I. Anal. Calcd for $C_{55}H_{58}O_{11}N_2$: C, 71.58; H, 6.29; N, 3.03. Found: C, 71.38; H, 6.65; N, 3.12.

(b) The hydrolysis of methyl N-(benzyloxycarbonyl)-O-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)-L-threoninate[1] by treatment with Dowex 1 (OH^-) as previously described required 24 h. TLC showed that methyl hydrolysis was accompanied by significant removal of 2,3,4,6-tetra-O-benzyl-galactose. Only a 40% yield of N-(benzyloxycarbonyl)-O-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)-L-threonine (16) was obtained. The latter was condensed with benzyl glycinate in the presence of BOP reagent to afford the title compound in 84% yield after column chromatography.

Benzyl N-(Benzyloxycarbonyl)-O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-L-threoninate (9). Compound 3 (0.3 g, 0.33 mmol) and benzyl threoninate (0.09 g, 0.35 mmol) were dissolved in cold (0 °C) THF containing TEA (1 mL). The reaction mixture was stirred at 0 °C for 30 min and allowed to react at room temperature (TLC monitoring, solvent E). HOBT (0.1 g) and additional TEA (0.5 mL) were added after 18 h and the reaction mixture allowed to react an additional 6 h. The crude residue obtained after concentration was dissolved in ethyl acetate (50 mL). The organic phase was successively washed with 5% aqueous citric acid (2 × 50 mL), water (50 mL), 0.05 N sodium hydroxide (5 × 25 mL), and water (50 mL) and dried over Na_2SO_4. Concentration in vacuo afforded an oily residue, which was purified by column chromatography on silica (solvent F). Compound 9 was obtained (0.3 g, 90%) as an oil: $[\alpha]^{20}_D$ +60° (c 0.5, $CHCl_3$); ^{13}C NMR, see Table I. Anal. Calcd for $C_{57}H_{62}O_{12}N_2$: C, 70.80; H, 6.41; N, 2.89. Found: C, 71.1; H, 6.72; N, 2.80.

Benzyl N-(Benzyloxycarbonyl)-O-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)-L-threonyl-L-threoninate (10). This compound was prepared by using the procedure described for 9 The title compound was obtained after column chromatography (solvent F) (0.29 g, 80%) as an oil: $[\alpha]^{20}_D$ +15.5° (c 0.9, $CHCl_3$); ^{13}C NMR, see Table I. Anal. Calcd for $C_{57}H_{62}O_{12}N_2$: C, 70.80; H, 6.41; N, 2.89. Found: C, 71.03; H, 6.63; N, 2.80.

Benzyl N-(((9-Fluorenylmethyl)oxy)carbonyl)-L-threoninate (17). The hemioxalate salt of benzyl L-threoninate (5 g, 20 mmol) was suspended in a mixture of 10% aqueous sodium bicarbonate (40 mL) and dioxane (20 mL). ((9-Fluorenylmethyl)oxy)carbonyl chloroformate (5 g) dissolved in dioxane (40 mL) was added, and the mixture was stirred vigorously for 24 h. The reaction mixture was acidified to pH 2 by the addition of 3 N HCl and extracted with ethyl acetate (3 × 100 mL). The combined extracts were washed with saturated sodium bicarbonate solution and water and then dried over Na_2SO_4. The dried solution was evaporated to a white solid, which was washed with cold diethyl ether. Recrystallization from dichloromethane–diethyl ether gave 17 (7.5 g, 87%): mp 114–115 °C [lit.[18] mp 112–113 °C]; $[\alpha]^{20}_D$ −4° (c 1.0, $CHCl_3$) [lit.[18] $[\alpha]^{20}_D$ −6.2° (ethyl acetate)]; ^{13}C NMR δ 171.15 (CO ester), 156.9 (CO urethane), 143.1, 142.9, 135.5, 125.25, 120.1 (aromatic carbons), 68.1 (Cβ-Thr), 67.44 (CH_2Fmoc and benzyl ester), 59.55 (Cα-Thr), 47.36 (C9-fluorene), 19.94 (CH_3-Thr). Anal. Calcd for $C_{26}H_{25}O_5N$: C, 72.39; H, 5.80; N, 3.24. Found: C, 72.70; H, 5.85; N, 3.20.

Benzyl N-(((9-Fluorenylmethyl)oxy)carbonyl)-O-(2,3,4,6-tetra-O-benzyl-α- and -β-D-galactopyranosyl-L-threoninate (18 and 19). Trifluoromethanesulfonic anhydride (0.25 mL, 1.5 mmol) and compound 17 (1.3 g, 3 mmol) were dissolved in a cold (−15 °C) mixture of 1:1 dichloromethane–acetonitrile (v/v). Compound 2 (0.54 g, 1 mmol) in dichloromethane (10 mL) was added over 15 min, and the foregoing mixture was allowed to warm to room temperature for an additional 10 min. When TLC (solvent A) revealed no remaining compound 2, water was added and the solution was extracted with dichloromethane (3 × 30 mL). The organic phase was then washed with saturated aqueous $NaHCO_3$ (2 × 20 mL) and water (30 mL), dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography (solvent C) of the crude product allowed removal of the excess of 17 (0.6 g, 72% of the excess) from the mixture of 18 and 19. Column chromatography on silica (solvent D) afforded 18 as a crystalline material (0.45 g, 48%) and 19 (0.31 g, 37%) as an oil.

Compound 18: mp 78–79 °C (recrystallized from diethyl ether–hexane); $[\alpha]^{20}_D$ +33° (c 1.5, $CHCl_3$); ^{13}C NMR, see Table II. Anal. Calcd for $C_{60}H_{59}O_{10}N$: C, 75.55; H, 6.19; N, 1.46. Found: C, 75.30; H, 6.23; N, 1.52.

Compound **19**: $[\alpha]^{20}_D$ +8.6° (*c* 1.4, $CHCl_3$); ^{13}C NMR, see Table II. Anal. Calcd for $C_{60}H_{59}O_{10}N$: C, 75.55; H, 6.19; N, 1.46. Found: C, 75.40; H, 6.12; N, 1.53.

***o*-Nitrophenyl *N*-(Benzyloxycarbonyl)-L-glycinate (23).** This compound was prepared from *N*-(benzyloxycarbonyl)glycine according to the procedure described by Bodanszky.[13] Its physical data were consistent with the previously reported.

Benzyl *N*-(Benzyloxycarbonyl)glycyl-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-L-threoninate (24). A mixture of **18** (0.3 g, 0.31 mmol) and piperidine (3 mL) in dichloromethane (30 mL) was stirred at room temperature for approximately 1 h. TLC monitoring (solvent A) showed the presence of 3 compounds with R_f 0.9 (dibenzofulvene), R_f 0.25 (dibenzofulvene–piperidine adduct), and R_f 0.17 (compound **20**), which gave a positive test with ninhydrin. The reaction mixture was washed with 1 N hydrochloric acid (3 × 50 mL) in order to remove both piperidine and the above adduct. The organic phase was dried over Na_2SO_4 and concentrated in vacuo to give **20**, which was used in the next step without purification. Compound **20** was dissolved in THF (15 mL) at 0 °C. To the above stirred solution were added compound **23** (0.16 g, 0.5 mmol) dissolved in THF (5 mL) and triethylamine (0.2 mL). After 30 min (TLC monitoring, solvent G), the reaction mixture was allowed to stand at room temperature for 18 h and HOBT (0.1 g) was added. After an additional 5 h, the solution was concentrated to give an oily residue. The latter was dissolved in ethyl acetate (50 mL) and the organic phase washed with 5% aqueous citric acid (2 × 50 mL), water (50 mL), 0.5 N sodium hydroxide (2 × 20 mL), and finally water (50 mL). The dried organic solution was concentrated in vacuo to afford a product that was purified by column chromatography on silica (solvent G). Compound **24** was obtained as an oil (0.25 g, 87%): $[\alpha]^{20}_D$ +50° (*c* 1.4, $CHCl_3$); ^{13}C NMR, see Table II. Anal. Calcd for $C_{55}H_{58}O_{11}N_2$: C, 71.58; H, 6.19; N, 3.03. Found: C, 71.72; H, 6.29; N, 3.33.

Benzyl *N*-(Benzyloxycarbonyl)glycyl-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-L-threoninate (25). This compound was prepared by using the same procedure described for **24** except that compound **21** was used instead of **20**. The title compound was obtained as a crystalline material after column chromatography (solvent G), (0.23 g, 80% yield): mp 111 °C (from diethyl ether–petroleum ether); $[\alpha]^{20}_D$ +6° (*c* 1.0, $CHCl_3$); ^{13}C NMR, see Table II. Anal. Calcd for $C_{55}H_{58}O_{11}N_2$: C, 71.58; H, 6.29; N, 3.03. Found: C, 71.80; H, 6.44, N, 3.33.

Benzyl *N*-(Benzyloxycarbonyl)-L-threonyl-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-L-threoninate (26). Essentially the same procedure as that described above for the preparation of **24** was followed. Compound **18** (0.2 g, 0.21 mmol) gave, after the usual workup, compound **20**, which was reacted with compound **1** (0.16, 0.4 mmol) in the presence of triethylamine (2 mL). The title compound was obtained after column chromatography (solvent G) as a colorless oil (0.19 g, 95%): $[\alpha]^{20}_D$ +26° (*c* 1.0, $CHCl_3$); ^{13}C NMR, see Table II. Anal. Calcd for $C_{57}H_{62}O_{12}N_2$: C, 70.80; H, 6.41; N, 2.89. Found: C, 71.02; H, 6.47; N, 2.64.

Benzyl *N*-(Benzyloxycarbonyl)-L-threonyl-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-L-threoninate (27). This compound was prepared by using the same procedure described for **24**, **25**, and **26**. Compound **27** was obtained as a crystalline material (0.18 g, 89%). Recrystallization from diethyl ether–petroleum ether gave a pure compound: mp 105–106 °C; $[\alpha]^{20}_D$ +15.5 (*c* 0.9, $CHCl_3$); ^{13}C NMR, see Table II. Anal. Calcd for $C_{57}H_{62}O_{12}N_2$: C, 70.80; H, 6.41; N, 2.89. Found: C, 70.99; H, 6.62; N, 2.69.

Benzyl *N*-(Benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-L-threonyl-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-L-threoninate (32). Compound **18** (0.2 g, 0.21 mmol) in dichloromethane (30 mL) was carefully treated with piperidine (TLC monitoring, solvent I) for about 1 h. The mixture was then washed with 1 N hydrochloric acid (3 × 50 mL), and the organic phase containing **20** was dried over Na_2SO_4. The oily crude residue left after evaporation was dissolved in cold (0 °C) THF (15 mL) followed by the addition of a mixture of **3** (0.16 g, 0.21 mmol) and triethylamine (1 mL) in cold (0 °C) THF (5 mL). The reaction mixture was then allowed to stand at room temperature for 24 h, after which HOBT (0.1 g) was added. The reaction was complete after 36 h (TLC, solvent F). The usual workup, as described for compound **24**, gave, after column chromatography on silica (solvent F), the title compound (0.2 g, 64%) as a colorless oil: $[\alpha]^{20}_D$ +35° (*c* 1.1, $CHCl_3$); ^{13}C NMR δ 170.0 (CO amide), 169.4 (CO ester), 98.2 and 98.9 (Cl galactopyranose), 56.6 and 58.4 (Cα-threonine), 16.5 and 17.6 (CH_3-threonine). Anal. Calcd for $C_{91}H_{96}O_{17}N_2$: C, 75.38; H, 6.45; N, 1.88. Found: C, 75.62; H, 6.80; N, 1.77.

Benzyl *N*-(Benzyloxycarbonyl)-L-threonylglycinate (34). Dicyclohexylcarbodiimide (DCC) (5.5 g) was added to a solution of *N*-(benzyloxycarbonyl)-L-threonine (6.3 g, 25 mmol), benzyl glycinate as its *p*-toluenesulfonate salt (8.4 g, 25 mmol), and triethylamine (3.5 mL) in dichloromethane (100 mL). The mixture was stirred for 4 h at room temperature. After filtration of the precipitated dicyclohexylurea, the solvent was removed, and the oily residue was redissolved in ether and filtered again. The title compound precipitated from the dried ether after the addition of hexane. Recrystallization gave pure **34** as a white crystalline material (7 g, 70%): mp 96–98 °C; $[\alpha]^{20}_D$ −18.3° (*c* 1.2, $CHCl_3$); ^{13}C NMR δ 171.61 and 169.87 (CO ester and amide), 156.96 (CO urethane), 67.4 and 67.25 (CH_2-Cbz and benzyl ester), 59.15 (Cα-Thr), 41.43 (Cα-Gly), 18.43 (CH_3-Thr). Anal. Calcd for $C_{21}H_{24}O_6N_2$: C, 63.0; H, 6.0; N, 7.0. Found: C, 63.10, H, 6.09, N, 6.90.

Benzyl *N*-(Benzyloxycarbonyl)glycyl-L-threoninate (35). *N*-(Benzyloxycarbonyl)glycine (2 g, 1 mmol), benzyl L-threoninate hemioxalate (2.6 g, 1 mmol), and BOP reagent (4.4 g, 1 mmol) were dissolved in acetonitrile (20 mL), and the mixture was stirred at room temperature. After 4 h, the solvent was evaporated and the residue redissolved in ethyl acetate (30 mL) and washed with 1 N hydrochloric acid (50 mL), saturated sodium bicarbonate solution (50 mL), and water (50 mL). Evaporation of the dried (Na_2SO_4) organic phase furnished a white solid, which was recrystallized from diethyl ether–hexane, (3 g, 70%): mp 124–125 °C; $[\alpha]^{20}_D$ +6.7° (*c* 1.5, $CHCl_3$); ^{13}C NMR δ 171.0 and 170.20 (CO ester and amide), 156.96 (CO urethane), 68.08, 67.51, 67.34 (CH_2-Cbz, CH_2-benzyl ester, and Cβ-Thr), 57.9 (Cα-Thr), 44.62 (Cα-Gly), 20.07 (CH_3-Thr). Anal. Calcd for $C_{21}H_{24}O_6N_2$: C, 63.0; H, 6.0; N, 7.0. Found: C, 62.83; H, 5.80; N, 7.2.

Benzyl *N*-(Benzyloxycarbonyl)glycylglycyl-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-L-threoninate (36). Compound **18** (0.6 g, 0.63 mmol) was treated, as previously reported for **24**, with piperidine (7.5 mL) in dichloromethane. After 1 h (TLC monitoring, solvent A), the mixture was washed with 1 N hydrochloric solution (2 × 75 mL), saturated sodium bicarbonate (50 mL), and water (50 mL). The dried organic phase was evaporated in vacuo to give an oily residue (**20**) which was redissolved in THF (40 mL) at 0 °C. To the above solution was successively added Cbz-Gly-Gly-ONO (Bachem) (0.4 g, 1 mmol) and triethylamine (0.6 mL), and the mixture was stirred for 30 min at 0 °C. After 18 h, TLC (methanol–CH_2Cl_2, 5:95 v/v) showed the presence of **20**. HOBT (0.05 g) was added and the reaction was continued for an additional 6 h. The reaction mixture was then concentrated and the residue redissolved in dichloromethane (50 mL). The organic phase was washed with 1 N hydrochloric acid (20 mL) and saturated sodium bicarbonate (25 mL), dried over Na_2SO_4, and concentrated. The oily residue was purified by column chromatography (ethyl acetate–hexane, 6:4 (v/v)) to afford pure **36** (0.56 g, 80%): $[\alpha]^{20}_D$ +34° (*c* 2.9, $CHCl_3$); ^{13}C NMR δ 170.4, 169.65, 169.4 (CO ester and amides), 156.65 (CO urethane), 97.4 (Cl-galactose), 56.8 (Cα-Thr), 44.35, 42.7 (Cα-Gly), 19.0 (CH_3-Thr). Anal. Calcd for $C_{57}H_{61}O_{12}N_3$: C, 69.86; H, 6.23; N, 4.29. Found: C, 69.60; H, 6.29; N, 4.30.

***N*-Hydroxysuccinimyl *N*-(((9-Fluorenylmethyl)oxy)carbonyl)-*O*-(*tert*-butyl)-L-threoninate (38).** This compound was prepared according to Chang and collaborators[18] from commercial (Bachem, Switzerland) *N*-(((9-fluorenylmethyl)oxy)carbonyl)-*O*-(*tert*-butyl)threonine: mp 144–145 °C [lit.[18] mp 148.5–155 °C]; $[\alpha]^{20}_D$ +16° (*c* 1.0, ethyl acetate) [lit.[18] $[\alpha]^{20}_D$ +14.5° (*c* 1.0, ethyl acetate)]; ^{13}C NMR δ 168.5 (CO imide), 167.0 (CO ester), 74.9 (Cβ-Thr), 67.6 (C-*t*-Bu), 67.3 (CH_2-Fmoc), 58.9 (Cα-Thr), 47.3 (C9-fluorene), 28.7 (CH_3-*t*-Bu), 25.65 (CH_2-imide), 20.85 (CH_3-Thr).

***N*-Hydroxysuccinimyl *N*-(((Fluorenylmethyl)oxy)carbonyl)-L-threoninate (39).** Compound **38** (3.8 g) was dissolved in a mixture of dichloromethane (25 mL) and trifluoroacetic acid (TFA, 2 mL). After 2 h, TLC (dichloromethane–acetone,

95:5 (v/v)) showed no remaining starting material and revealed the presence of a polar compound. The reaction mixture was evaporated in vacuo, and the crude residue was redissolved in dichloromethane (50 mL). The organic phase was washed with 1 N hydrochloric acid (2 × 25 mL), saturated sodium bicarbonate (2 × 25 mL), and water (25 mL). Evaporation of the dried organic phase left a white foamy material (3 g, 75%), homogeneous on TLC, which could not be crystallized: $[\alpha]^{20}_D$ −26.5° (c 1.0, $CHCl_3$); ^{13}C NMR δ 168.2 (CO imide), 167.1 (CO ester), 68.1 (Cβ-Thr), 67.7 (CH_2-Fmoc), 58.9 (Cα-Thr), 47.2 (C9-fluorene), 26.7 (CH_2-imide), 19.8 (CH_3-Thr).

***N*-Hydroxysuccinimyl *N*-(((9-Fluorenylmethyl)oxy)carbonyl)-*O*-(2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl)-L-threoninate (40α and 40β).** Compound **39** (1.29 g, 3 mmol) and trifluoromethanesulfonic anhydride (0.25 mL, 1.5 mmol) were dissolved in cold (−18 °C) acetonitrile (10 mL) followed by the addition in aliquots over a period of 5 min of a solution of compound **2** (0.54 g, 1 mmol) in cold dichloromethane (5 mL). TLC monitoring (chloroform–acetone, 96:4) showed the reaction was complete within 20 min. Water (20 mL) was then added and the mixture extracted with dichloromethane (3 × 25 mL). The organic phase was washed with saturated sodium bicarbonate (25 mL) and dried over Na_2SO_4. Flash chromatography (ethyl acetate–hexane, 17:23 (v/v)) gave 0.92 g of the mixture **40α** and **40β**. On the other hand, the excess of amino acid (0.5 g) could be recovered. The ^{13}C NMR spectrum of the above mixture showed the α:β ratio to be 80:20 (C1-α at 98.8 ppm, C1-β at 103.2 ppm). The mixture was used without purification in the next step.

Benzyl *N*-(((9-Fluorenylmethyl)oxy)carbonyl)-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-L-threonylglycinate (41). The mixture of **40α** and **40β** (0.48 g, 0.5 mmol) was dissolved in dioxane (25 mL). To the above stirred solution kept at 0 °C were successively added benzyl glycinate (0.21 g) as its *p*-toluenesulfonate salt and 25 mL of a 10% sodium bicarbonate solution. The reaction mixture was allowed to react at 0 °C for 30 min and at room temperature for an additional 1 h (TLC monitoring, hexane–acetone, 23:17). The solution was then poured into water (300 mL) and acidified to pH 2 by the addition of 2 N hydrochloric acid. Extraction of the aqueous phase with dichloromethane and concentration in vacuo of the dried, combined extract left a crude oily material, which was purified by passage through a silica gel column (ethyl acetate–hexane, 3:7). Pure compound **41** (0.37 g, 78%) and **41β** (0.093 g, 19%) were obtained.

Compound **41**: mp 126–127 °C (from dichloromethane–hexane); $[\alpha]^{20}_D$ +62.6° (c 0.5, $CHCl_3$); ^{13}C NMR δ 169.2 and 168.55 (CO amide and ester), 155.6 (CO urethane), 97.3 (C1-galactose), 55.85 (Cα-Thr), 47.4 (C9-fluorene), 40.7 (Cα-Gly), 15.2 (CH_3-Thr).

Compound **41β**: oil; $[\alpha]^{20}_D$ +11.8 (c 1.9, $CHCl_3$); ^{13}C NMR δ 104.0 (C1-Gal), 57.4 (Cα-Thr), 41.4 (Cα-Gly), 16.65 (CH_3-Thr).

Benzyl *N*-(Benzyloxycarbonyl)glycyl-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-L-threonylglycinate (43). Compound **41** (0.24 g, 0.25 mmol) was treated, as already described, with piperidine (2.5 mL) in dichloromethane (25 mL). The reaction was monitored by TLC (ethyl acetate–hexane, 3:7) and was shown to be complete in 1 h. The usual workup afforded a crude residue which was dissolved in cold (0 °C) THF (25 mL). *o*-Nitrophenyl *N*-(benzyloxycarbonyl)glycinate (Bachem, 0.16 g) in THF (10 mL) and triethylamine (0.2 mL) were added. The mixture was allowed to react at 0 °C for 30 min and at room temperature for 16 h. After that, HOBT (0.05 g) was added and the reaction mixtured worked up after 6 additional h. The residue obtained after evaporation of the dichloromethane was purified by flash chromatography (hexane–ethyl acetate, 1:1) and afforded the title compound (0.2 g, 84%) as an oil: $[\alpha]^{20}_D$ +57° (c 1.0, $CHCl_3$); ^{13}C NMR δ 169.2 and 168.6 (CO ester and amide), 156.5 (CO urethane), 97.3 (C1-galactose), 54.6 (Cα-Thr), 44.5 and 40.8 (Cα-Gly), 15.5 (CH_3-Thr). Anal. Calcd for $C_{57}H_{61}O_{12}N_3$: C, 69.86; H, 6.23; N, 4.29. Found: C, 69.92; H, 6.07; N, 4.46.

General Procedure for the Deprotection of Glycopeptides. Glycopeptides (0.5 mmol) were hydrogenated overnight in a mixture of ethanol (20 mL), acetic acid (5 mL), and water (5 mL) in the presence of 10% palladium on charcoal (1.5 g) as catalyst at room temperature. Hydrogen pressure was 4 bar. After filtration through a bed of Celite, the catalyst was thoroughly washed with hot water (2 × 15 mL), and the filtrate was concentrated in vacuo at 50 °C. The addition of water (10 mL), filtration, and concentration were repeated twice. The last washing was made with deionized (IRC or Chelex 100 resin) water (100 mL). The aqueous phase was concentrated in vacuo, and the residue was dissolved in acetone from which glycopeptides precipitated.

O-(α-D-galactopyranosyl)-L-threonylglycine (**11**):[2] yield, 90%; mp 240 °C; $[\alpha]^{20}_D$ +28° (c 1.2, H_2O).

O-(β-D-galactopyranosyl)-L-threonylglycine (**12**): yield, 92%; mp 225–230 °C dec; $[\alpha]^{20}_D$ −6.4° (c 1.1, H_2O).

O-(α-D-galactopyranosyl)-L-threonyl-L-threonine (**13**): yield, 95%; mp 240 °C; $[\alpha]^{20}_D$ +18° (c 1, H_2O).

O-(β-D-galactopyranosyl)-L-threonyl-L-threonine (**14**): yield, 90%; mp 220–225 °C dec; $[\alpha]^{20}_D$ −6° (c 1, H_2O).

Glycyl-*O*-(α-D-galactopyranosyl)-L-threonine (**28**): yield, 97%; mp 240 °C; $[\alpha]^{20}_D$ +26° (c 1, H_2O).

Glycyl-*O*-(β-D-galactopyranosyl)-L-threonine (**29**): yield, 95%; hygroscopic; $[\alpha]^{20}_D$ −1° (c 1, H_2O).

Threonyl-*O*-(α-D-galactopyranosyl)-L-threonine (**30**): yield, 92%; hygroscopic; $[\alpha]^{20}_D$ +44° (c 1, H_2O).

Threonyl-*O*-(β-D-galactopyranosyl)-L-threonine (**31**): yield, 90%; mp 230–235 °C dec; $[\alpha]^{20}_D$ +4° (c 1, H_2O).

O-(α-D-galactopyranosyl)-L-threonyl-*O*-(α-D-galactopyranosyl)-L-threonine (**33**): yield, 90%; mp 235–240 °C dec; $[\alpha]^{20}_D$ +58° (c 1.0, H_2O).

Carbon-13 NMR data for above compounds are reported elsewhere.[17]

Glycylglycyl-*O*-(α-D-galactopyranosyl)-L-threonine (**37**): mp > 230 °C; $[\alpha]^{20}_D$ +97° (c 0.9, H_2O); ^{13}C NMR δ 177.3, 172.1 and 169.2 (CO acid and amides), 100.4 (C1-Gal), 76.9 (Cβ-Thr), 72.5 (C5-Gal), 70.8, 70.7 (C3- and C4-Gal), 69.9 (C2-Gal), 62.5 (C6-Gal), 60.5 (Cα-Thr), 43.8 and 41.9 (Cα-Gly), 19.6 (CH_3-Thr).

Glycyl-*O*-(α-D-galactopyranosyl)-L-threonylglycine (**44**): mp 230 °C dec; $[\alpha]^{20}_D$ +49° (c 1.0, H_2O); ^{13}C NMR δ 176.8, 172.4, 169.2 (CO acid and amides), 100.85 (C1-Gal), 76.0 (Cβ-Thr), 72.6 (C5-Gal), 70.6 (C3- and C4-Gal), 67.9 (C2-Gal), 62.5 (C6-Gal), 59.1 (Cα-Thr), 44.8 and 42.0 (Cα-Gly), 19.1 (CH_3-Thr).

Registry No. **1**, 62087-89-2; **2**, 53081-25-7; **3**, 86161-21-9; **4**, 86117-93-3; **5** tosylate, 1738-76-7; **6**, 33640-67-4; **7**, 86088-43-9; **8**, 86117-94-4; **9**, 86088-44-0; **10**, 86117-95-5; **11**, 84653-99-6; **12**, 84710-39-4; **13**, 84654-00-2; **14**, 84710-40-7; **15**, 86088-45-1; **16**, 86117-96-6; **17**, 73724-48-8; **18**, 86088-46-2; **19**, 86117-97-7; **20**, 86088-47-3; **21**, 86117-98-8; **22**, 1138-80-3; **23**, 6154-41-2; **24**, 86088-48-4; **25**, 86117-99-9; **26**, 86088-49-5; **27**, 86118-00-5; **28**, 84654-01-3; **29**, 84710-41-8; **30**, 84710-42-9; **31**, 84654-02-4; **32**, 86088-50-8; **33**, 84654-03-5; **34**, 16305-79-6; **35**, 86088-51-9; **36**, 86088-52-0; **37**, 85774-46-5; **38**, 75530-95-9; **39**, 86088-53-1; **40α**, 86088-54-2; **40β**, 86118-01-6; **41**, 86088-55-3; **41β**, 86118-02-7; **42α**, 86088-56-4; **43**, 86088-57-5; **44**, 85774-48-7; methyl *N*-(benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-L-threoninate, 77942-97-3; methyl *N*-(benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-L-threoninate, 77942-98-4; ((9-fluorenylmethyl)oxy)carbonyl chloroformate, 86088-58-6; benzyl L-threoninate hemioxalate, 86088-59-7; dibenzofulvene, 4425-82-5; *N*-(benzyloxycarbonyl)-L-threonine, 19728-63-3; *o*-nitrophenyl *N*-(benzyloxycarbonyl)glycinate, 6154-41-2; Cbz-Gly-Gly-ONO, 86088-60-0; *N*-(((9-fluorenylmethyl)oxy)carbonyl)-*O*-*tert*-butylthreonine, 71989-35-0.