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Synthesis of Mono- and Disaccharide Amino-acid Derivatives for Use in Solid Phase Peptide Synthesis

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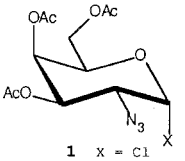
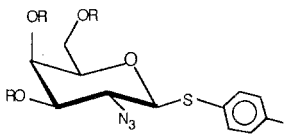
N-Fluorenylmethyloxycarbonyl-protected serine and threonine derivatives, carrying O-glycosidically α- or β-linked peracetylated β-D-Gal*p*-(1-3)-D-GalNAc*p* carbohydrate chains, were prepared. These derivatives are intended for use in solid phase glycopeptide synthesis. Suitably protected mono- and disaccharide thioglycosides were used as carbohydrate intermediates. These were activated by treatment with bromine to give the glycosyl bromides, which were then used in silver triflate-promoted glycosidations of N-fluorenylmethyloxycarbonyl amino-acid phenacyl esters. Removal of the phenacyl esters with zinc gave the target free acids.

Synthetic glycopeptides, which are useful as models for glycoproteins or as haptens in immunological studies, can be obtained, principally, in two different ways [1, 2]. 1) Suitably protected oligopeptides are first prepared and then coupled to carbohydrate derivatives. 2) Glycosylated and suitably protected amino-acids are first prepared and then used as monomers in a solution or solid phase peptide synthesis. The former approach [3-5] is often associated with peptide solubility problems and this is one of the reasons why low yields of glycopeptides have been obtained. The latter approach has been used more frequently [1, 6, 7], most often with a solution peptide synthesis protocol. Only few reports [8, 9] have appeared on the use of glycosylated amino-acids in a solid phase synthesis.

Progress in this field has until recently been hampered by the lack of a peptide synthesis protocol that does not employ repeated use of acids such as trifluoroacetic acid or hydrogen fluoride, which both can attack glycosidic linkages. However, with the advent [10-13] of the fluorenylmethyloxycarbonyl (FMOC) amino protective group, which requires only a brief treatment with a weak base [14] for deprotection, and resins such as SASRIN® (Bachem AG, Bubendorf, Switzerland) [15] or HYCRAM® [8] which require only mild conditions for cleavage, the situation has changed. A solid phase peptide synthesis protocol based on these features would not damage glycosidic bonds, and therefore solid phase glycopeptide synthesis should now be possible.



1 X = Cl
2 X = Br



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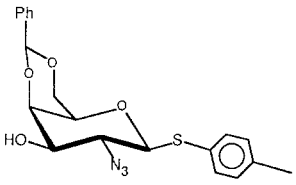
4 R = H



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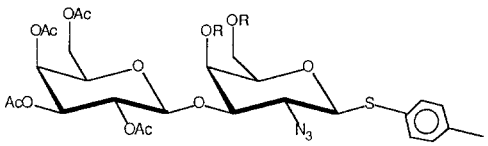
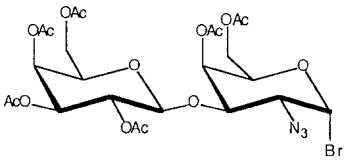


6 R = >CHPh
7 R = Ac



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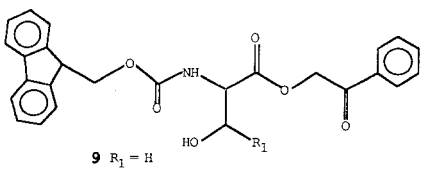
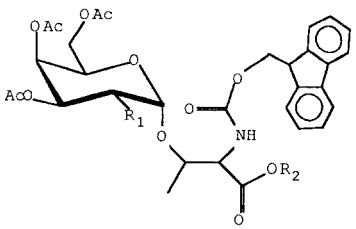
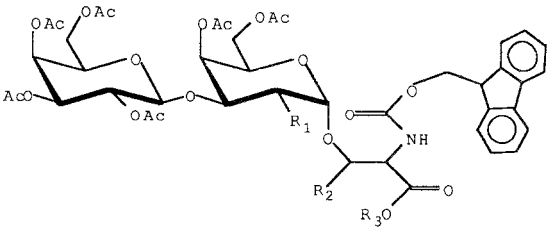


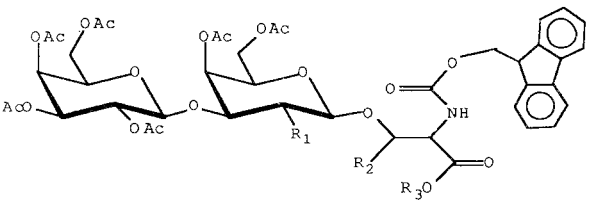
9 R_1 = H
10 R_1 = CH_3



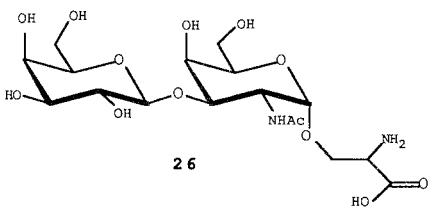
11 R_1 = N_3 , R_2 = $PhCOCH_2$
12 R_1 = NHAc , R_2 = $PhCOCH_2$
13 R_1 = NHAc , R_2 = H



14 $R_1 = N_3$ $R_2 = H$ $R_3 = CH_2COPh$ **20** $R_1 = N_3$ $R_2 = CH_3$ $R_3 = CH_2COPh$
16 $R_1 = NHAc$ $R_2 = H$ $R_3 = CH_2COPh$ **22** $R_1 = NHAc$ $R_2 = CH_3$ $R_3 = CH_2COPh$
18 $R_1 = NHAc$ $R_2 = H$ $R_3 = H$ **24** $R_1 = NHAc$ $R_2 = CH_3$ $R_3 = H$



15 $R_1 = N_3$ $R_2 = H$ $R_3 = CH_2COPh$ **21** $R_1 = N_3$ $R_2 = CH_3$ $R_3 = CH_2COPh$
17 $R_1 = NHAc$ $R_2 = H$ $R_3 = CH_2COPh$ **23** $R_1 = NHAc$ $R_2 = CH_3$ $R_3 = CH_2COPh$
19 $R_1 = NHAc$ $R_2 = H$ $R_3 = H$ **25** $R_1 = NHAc$ $R_2 = CH_3$ $R_3 = H$



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Crucial for such a synthesis is the availability of glycosylated, FMOC-protected amino-acids that carry suitable protective groups on the carbohydrate moiety. As part of a project aimed at efficient solid phase synthesis of *O*-linked and *N*-linked glycopeptides, we have now prepared the glycosylated FMOC-serine and threonine derivatives **13**, **18**, **19**, **24 a**nd **25**, the detailed syntheses of which are presented in this paper. Compounds **18** and **24** contain the common β-D-Gal*p*-(1-3)-α-D-GalNAc*p* core structure of *O*-linked carbohydrate chains in glycoproteins. Compounds **19** and **25** contain the corresponding β-linked disaccharide structure. These were prepared for comparision purposes, since they were easily available from by-products in the glycosidation reactions. The use of **13**, **18**, **19**, **24** and **25** in peptide synthesis will be reported in a coming paper.

Results and Discussion

The strategy used for preparation of **13, 18, 19, 24** and **25** was first to prepare the acetylated mono- and disaccharide thioglycosides [16], **3** and **7**. These were then activated and coupled to FMOC-serine and -threonine phenacyl esters, **9** and **10**. The derivatives obtained were then converted, by a sequence of reactions, to the free acids **13, 18, 19, 24** and **25**. The choice of acetyl groups for permanent blocking on the sugar moiety requires a comment. An advantage of this type of protection is the enhanced acid stability of the glycosidic linkage that it induces. Acyl groups can also [17] be smoothly removed under conditions which do not cause β-elimination or racemization of the serine and threonine moiety.

Synthetic Route

Treatment of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl chloride **1** [18] with potassium 4-methylthiophenolate in chloroform-ethanol gave the 4-methylphenyl β-thioglycoside, **3**, in 57% yield. Initially, synthesis of the analogous methyl thioglycoside was intended, this compound having been prepared before [19] in two steps (57% total yield) from 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide **2**. However, attempts to prepare the thiomethyl glycoside with a simpler, one step procedure from the halide by displacement with the anion of methanethiol were not successful (Haraldsson M, Norberg T; unpublished results), but a change to 4-methylthiophenolate anion gave a high yield of the 4-methylphenyl thioglycoside.

Treatment of **3** with sodium methoxide in methanol gave the crystalline triol, **4** (92% yield), which was then treated with α,α-dimethoxy-toluene and *p*-toluenesulfonic acid in acetonitrile to give the glycosyl acceptor, **5** (82% yield). Silver triflate promoted glycosylation of **5** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide gave the crystalline disaccharide β-thioglycoside, **6** (54% yield). Smaller amounts of acetylated **5** (3%) and 4-methylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (8%) were isolated, in accordance with expectations on this type of reaction [20].

Disaccharide thioglycosides such as **6** have been shown [19] to be useful as glycosyl donors. However, **6** was first converted (86% yield) to the corresponding peracetylated derivative, **7**, by hydrolysis in aqueous acetic acid followed by acetylation with acetic anhydride in pyridine. This operation was performed to reduce the number of deprotection steps later, and also to enhance the acid-stability of the intended glycosylated amino-acid. Having prepared mono- or disaccharide glycosyl donors, suitably carboxyl-protected FMOC-amino-acid derivatives had to be prepared for use as glycosyl acceptors. The phenacyl ester was chosen for carboxyl protection, since it can be removed by mild treatment with activated zinc in acetic acid [21].

Treatment of L-threonine with fluorenylmethyloxycarbonyl chloride gave *N*-fluorenylmethyloxycarbonyl-L-threonine [22], which was treated *in situ* with α-bromoacetophenone and potassium fluoride [23] in *N,N*-dimethylformamide to give *N*-fluorenylmethyloxycarbonyl-L-threonine phenacylester, **10**, in 46% total yield.

Similar treatment of L-serine gave *N*-fluorenylmethyloxycarbonyl-L-serine phenacylester, **9**, in 68% total yield.

The monosaccharide bromide, **2**, prepared as described [18] or by brief treatment of **3** with bromine was reacted with **10** and silver triflate to give the α derivative, **11** (21% yield). Only small amounts of the corresponding β derivative could be isolated. The stereoselectivity was thus similar to that reported for analogous reactions [24]. Treatment of the azido derivative, **11**, with thioacetic acid [25] at room temperature for 48 h gave the corresponding 2-acetamido derivative, **12** (76% yield). Finally, the phenacyl group was removed by treatment with activated zinc in acetic acid [21] to give the target amino-acid derivative, **13** (83% yield).

The disaccharide bromide **8**, prepared from **7** by brief treatment [19] with bromine, was treated with the serine acceptor **9** and silver triflate to give a mixture of α (**14**, 52%) and β (**15**, 25%) glycosidation products. With **8** as donor and the threonine derivative **10** as acceptor, the yields under the same conditions were 39% of the α (**20**) and 31% of the β (**21**) derivative.

Two points should be noted here: firstly, the stereoselectivity with the disaccharide donor is poorer than with a monosaccharide donor, which is in accordance with results reported before [24]. Secondly, the stereoselectivity in the reaction between the disaccharide donor and threonine was poorer than in the reaction with serine, which has also been reported previously [24].

All four disaccharide derivatives were separately subjected to treatment with thioacetic acid, which gave compounds **16**, **17**, **22** and **23**. These were carboxyl deprotected as above to give compounds **18**, **19**, **24** and **25**.

To find suitable conditions for later *O*-deacetylation, the derivative **18** was treated with 0.1 M sodium methoxide in methanol at room temperature while monitoring the reaction by TLC. After 45 min, a single spot remained and the mixture was processed to give a 51% yield of **26** [26, 27]. No elimination or racemization products could be detected in the mixture.

Experimental

Melting points are corrected. Evaporations were performed at <40°C bath temperature. Optical rotations were recorded at 20°C (c= 0.4-0.7, chloroform) unless otherwise stated, using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded at 25°C for solutions in C^2HCl_3 unless otherwise stated, using a Bruker AM 500 or a JEOL JNM-GSX 270 spectrometer (TMS = δ 0.0). Only selected NMR data are reported. In the assignments of disaccharides the carbons or protons of the 3-linked galactose are followed by an apostrophe. The FAB-MS spectra were recorded with a VG ZAB-SE mass spectrometer. Silica gel 60 F-254 (Merck, Darmstadt, W. Germany) was used for TLC, detection by u.v. or by charring with 8% sulfuric acid. Column chromatography was performed on silica gel (Matrex Silica Si, 60 Å, 35-70 μm, Amicon). Elemental analyses were not obtained for some syrupy or amorphous compounds. These were purified by column chromatography, and characterized by NMR spectroscopy. Organic solvents were dried over sodium (toluene), or over molecular sieves 4 Å, (heated to 280°C for >2 weeks. Kebo, Stockholm, Sweden). Powdered molecular sieves, Union Carbide 4 Å (Fluka, Buchs, Switzerland) were used in reactions where so stated.

*4-Methylphenyl 3,4,6-Tri-O-acetyl-2-azido-2-deoxy-1-thio-β-D-galactopyranoside (**3**)*

Potassium hydroxide (2.97 g, 53 mmol) and 4-methylthiophenol (6.59 g, 53 mmol) were dissolved in ethanol (92 ml). A solution of the chloride, **1** (16.1 g, 46 mmol) in chloroform (92 ml) was added over 30-60 min and the mixture was then stirred for a further 3 h. The reaction mixture was diluted with dichloromethane, washed with aqueous sodium hydrogen carbonate, dried (magnesium sulphate) and concentrated to give crude **3**, which was purified by column chromatography (toluene/ethyl acetate/hexane, 1/1/1 by vol). Pure, amorphous **3** was obtained (11.4 g, 26.1 mmol, 57%), $[\alpha]_D$ -8°.

NMR data: ^{13}C, δ 20.5, 20.6, 20.7, 21.2 (CH_3COO, CH_3Ph), 59.3 (C-2), 66.6 (C-6), 61.6, 73.0, 74.4 (C-3, 4, 5), 86.7 (C-1), 129.0, 129.9, 134.0, 138.9 (Aromatic C), 169.7, 169.9, 170.4 (CH_3COO); ^1H, δ 3.63 (t, $J_{1,2} = J_{2,3}$ 10.2 Hz, H-2), 3.86 (t, $J_{5,6a} = J_{5,6b}$ 6.5 Hz, H-5), 4.16-4.22 (m, H-6a, 6b), 4.47 (d, $J_{1,2}$ 10.2 Hz, H-1), 4.85 (dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.2 Hz, H-3), 5.34 (d, $J_{3,4}$ 3.2 Hz, H-4).

*4-Methylphenyl 2-Azido-2-deoxy-1-thio-β-D-galactopyranoside (**4**)*

Methanolic sodium methoxide (0.5 M, 5 ml) was added to a solution of **3** (1.56 g, 3.57 mmol) in methanol (50 ml). After 1 h, the mixture was neutralized with Dowex 50 H^+ ion exchange resin. Filtration and concentration gave crude **4** (1.02 g, 3.30 mmol, 92%) as a crystalline solid. Recrystallization from ethyl acetate gave material with m.p. 120°C, $[\alpha]_D$ +23° (methanol).

NMR data ($C^2H_3O^2H$):^{13}C, δ, 21.1 (CH_3Ph), 62.5, 64.4, 69.6, 75.2, 80.6 (C-2, 3, 4, 5, 6), 88.2 (C-1), 130.4, 130.6, 133.7, 139.1 (Aromatic C).

Analytical data. Calculated for $C_{13}H_{17}N_3O_4S$: C, 50.2; H, 5.5. Found: C, 49.8; H, 5.5.

*4-Methylphenyl 2-Azido-4,6-O-benzylidene-2-deoxy-1-thio-β-D-galactopyranoside (**5**)*

p-Toluenesulfonic acid (100 mg, 0.58 mmol) was added to a solution of **4** (3.01 g, 9.68 mmol) and α,α-dimethoxytoluene (1.91 g, 12.6 mmol) in acetonitrile (35 ml). After 2 h the mixture was concentrated and purified by column chromatography (toluene/ethyl acetate, 6/4 by vol). Pure, syrupy **5** was obtained (3.16 g, 7.92 mmol, 82%), $[\alpha]_D$ -24°.

NMR data: ^{13}C, δ 21.1 (CH_3Ph), 61.9, 69.1, 69.7, 73.0, 74.3 (C-2, 3, 4, 5, 6), 84.9 (C-1), 101.2 (CHPh).

*4-Methylphenyl 2-Azido-4,6-O-benzylidene-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-1-thio-β-D-galactopyranoside (**6**)*

A solution of silver triflate (3.16 g, 12.3 mmol) in dry toluene (47 ml) was added, while cooling to -30°C and stirring, to a solution of **5** (3.27 g, 8.20 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (4.34 g, 10.7 mmol) in dry dichloromethane (70 ml) containing powdered molecular sieves (7.7 g). The mixture was stirred at -30°C to -20°C for 20 min. Pyridine (0.45 ml) was then added and the solid was filtered off and thoroughly washed with dichloromethane (4 x 50 ml). The filtrate was washed, successively, with

aqueous 0.5 M sodium thiosulphate, water, and aqueous sodium hydrogen carbonate, then dried (magnesium sulphate) and concentrated. Column chromatography of the residue (toluene/ethyl acetate, 7/3 by vol) gave the following fractions identified by NMR: a) acetylated **5** (0.09 g), b) 4-methylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (0.29 g), c) impure and unidentified fractions (0.23 g), and d) pure **6** (3.20 g, 4.39 mmol, 54%). Recrystallization from dichloromethane/hexane gave material with m.p. 116°C, $[\alpha]_D$ -39°.

NMR data: ^{13}C, δ 20.5, 20.6, 20.6 (CH_3CO), 21.1 (CH_3Ar), 59.9, 61.5 (C-2,6'), 66.6, 68.6, 69.2, 69.8, 70.8, 70.9, 74.7, 80.8 (C-3, 4, 5, 6, 2', 3', 4', 5'), 85.7 (C-1), 100.7 (PhCH), 102.1 (C-1'), 125-138 (Aromatic C), 169.2, 170.0, 170.1 (CH_3CO) ; ^1H, δ 1.97, 2.01, 2.04, 2.13 (4s, CH_3COO), 2.31 (s, CH_3Ph), 3.45 (m, H-5), 3.52 (dd, $J_{2,3}$ 10.0, $J_{3,4}$ 3.2 Hz, H-3), 3.70 (t, $J_{1,2}$ 9.8, $J_{2,3}$ 10.0 Hz, H-2), 3.88 (dt, $J_{5',6'a} = J_{5',6'b}$ 6.5, $J_{4'5'}$ 1.3 Hz, H-5'), 4.01 (dd, $J_{5,6a}$ 1.6, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.10 (dd, $J_{5',6'a}$ 6.4, $J_{6'a,6'b}$ 11.2 Hz, H-6'a), 4.16 (dd, $J_{5',6'b}$ 6.4, $J_{6'a,6'b}$ 11.2 Hz, H-6'b), 4.23 (d, $J_{3,4}$ 3.2 Hz, H-4), 4.38 (dd, $J_{5,6b}$ 1.7, $J_{6a,6b}$ 12.3 Hz, H-6b), 4.38 (d, $J_{1,2}$ 9.8 Hz, H-1), 4.76 (d, $J_{1',2'}$ 8.0 Hz, H-1'), 4.99 (dd, $J_{2',3'}$ 10.4, $J_{3',4'}$ 3.5 Hz, H-3'), 5.22 (dd, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.4 Hz, H-2'), 5.37 (dd, $J_{3',4'}$ 3.5, $J_{4',5'}$ 1.3 Hz, H-4'), 5.50 (s, CHPh).

Analytical data. Calculated for $C_{34}H_{39}N_3SO_{13}$: C, 56.0; H, 5.4. Found: C, 55.7; H, 5.4.

4-Methylphenyl 4,6-Di-O-acetyl-2-azido-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-1-thio-β-D-galactopyranoside (7)

A solution of **6** (3.21 g, 4.41 mmol) in 80% aqueous acetic acid (10 ml) was heated to 80°C for 1 h. The mixture was concentrated and co-evaporated with toluene. Pyridine (8 ml) and acetic anhydride (5 ml) were added and the reaction was left overnight at room temperature. Concentration and co-evaporation with toluene gave **7** (3.08 g). NMR of the crude product showed at least 90% purity, $[\alpha]_D$ +4.

NMR data: ^{13}C, δ 21.1 (CH_3Ar), 60.9, 61.4, 62.6 (C-2, 6, 6'), 66.7, 68.0, 68.7, 70.6, 70.8, 75.1, 79.5 (C-3, 4, 5, 2', 3', 4', 5'), 86.7 (C-1), 101.3 (C-1'), 127.1, 129.6, 133.6, 138.8 (Aromatic C), 169.3, 169.6, 170.0, 170.2, 170.4, 170.5 (CH_3CO).

*N-(9-Fluorenylmethyloxycarbonyl)-L-serine Phenacylester (**9**)*

9-Fluorenylmethyloxycarbonyl chloride (1.30 g, 5.02 mmol) in dioxane (7.5 ml) was added, while cooling in ice, over one hour to L-serine (0.53 g, 5.0 mmol) in 10% aqueous sodium carbonate (10 ml) and dioxane (5 ml). The reaction was kept at 0°C for one hour and then at room temperature overnight. Ice-water (150 ml) was added and the reaction mixture was washed with diethyl ether (2 x 40 ml). The aqueous layer was cooled in ice while 2 M HCl was added to pH 2. The aqueous layer was then extracted with ethyl acetate (2 x 30 ml). The combined organic phases were washed with 0.1 M HCl (30 ml), dried with magnesium sulphate and concentrated to an oil.

Potassium fluoride (0.64 g, 11 mmol) and α-bromoacetophenone (1.0 g, 5.0 mmol) were stirred in *N,N*-dimethylformamide (5 ml) at room temperature for 1 min. The crude oil from above in *N,N*-dimethylformamide (5 ml) was added and the mixture was stirred overnight, then diluted with diethyl ether (25 ml) and washed with water (3 x 20 ml). The organic phase

Table 1: ^{1}H NMR δ and *J* values (in parenthesis) for derivatives **11**, **12**, **14**, **15**, **20** and **21** (C^2HCl_3 solution, 30°C, TMS = δ 0.00) and for derivatives **13**, **18**, **19**, **24** and **25** ($C^2H_3O^2H$ solution, 30°C, TMS = δ 0.00).

Spin system	GalNAc							Gal						
Proton	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	H-1'	H-2'	H-3'	H-4'	H-5'	H-6a'	H-6b'
J	1,2	2,3	3,4	4,5	5,6a	5,6b	6a,6b	1',2'	2',3'	3',4'	4',5'	5',6a'	5'6b'	6a',6b'
11	5.53 (3.7)	3.80 (11.2)	5.32 (3.2)	5.48	4.28 (7.4)	4.36 (7.6)	4.42 (11.5)							
12	5.41 (3.7)	4.68 (11.4)	5.18 (3.1)	5.44	4.30 (7.1)	4.11 (5.8)	4.15 (11.3)							
13	4.97 (3.8)	4.40 (11.4)	5.10 (3.0)	5.43	4.23	4.47	4.57							
14	5.84 (3.4)	3.93 (10.8)	3.66 (3.6)	5.33				4.66 (7.9)	5.17 (10.5)	4.98 (3.5)	5.11			
15	4.39 (7.9)	3.65 (10.3)	3.54 (3.5)	5.33	3.74	4.02	4.15 (11.7)	4.70 (7.8)	5.15 (10.5)	5.01 (3,5)	5.36 (0.9)	3.87	4.09	4.15 (11.3)
18	4.79 (3.7)	4.38 (11.0)	4.01 (3.5)	5.39	4.17	3.92	4.12	4.70 (7.3)	4.98 (10.4)	5.02 (3.4)	5.34 (0.8)	3.96	4.12	4.12
19	4.55 (8.4)	3.95	3.90	5.37	4.10			4.72 (7.7)	5.01 (10.5)	5.07 (3.4)	5.35 (1.2)	4.04		
20	5.51 (4.0)	4.05 (10.7)	3.78 (4.0)	5.49 (0.3)	(7.4)	4.00 (4.3)	4.19 (11.0)	4.74 (8.0)	5.18 (10.6)	5.01 (3.2)	5.34 (1.0)	3.90 (6.6)	4.09 (6.6)	4.17 (11.3)
21	4.64 (7.8)	3.66 (10.5)	3.62 (3.2)	5.35 (1.5)	3.80 (7.0)	3.98 (5.8)	4.15 (11.5)	4.74 (8.0)	5.17 (10.5)	5.04 (3.4)	5.38 (2.0)	3.90 (6.5)	4.10 (7.0)	4.18 (11.1)
24	4.87 (3.8)	4.35 (11.2)	3.90 (3.5)	5.38 (1.5)	4.22	3.97 (7.8)	(11.4)	4.64 (7.9)	4.99	4.99 (3.0)	5.35 (1.1)	3.99 (6.8)	4.11 (6.4)	4.16 (11.2)
25	4.75 (7.6)	3.96 (9.0)	4.04 (3.1)	3.99 (0.9)	3.90 (6.6)	4.00	(11.4)	4.54 (8.4)	5.02 (10.5)	5.08 (3.4)	5.36 (1.1)	4.04		

was dried with magnesium sulphate and concentrated to give crude **9** ,which was purified by column chromatography (toluene/ethyl acetate 7/3 by vol) giving pure **9** (1.52 g, 3.42 mmol, 68%) as a white crystalline solid, mp 105-108°C, $[\alpha]_D$ -23°.

NMR data: ^{13}C, δ 47.0 ($CHAr_2$), 58.4 (α-CH), 63.8 (β-CH_2), 66.6, 67.2 ($NHCOOCH_2$, OCH_2CO), 119-143 (Ar C), 156.1 (NHCOO), 170.3 ($COOCH_2$), 192.9 (OCH_2CO); ^{1}H, δ 3.92 (d, *J* 11.5 Hz, β-CH_2), 4.22 (t, *J* 7.2 Hz, $CHAr_2$), 4.32 (m, β-CH_2), 4.34 (dd, *J* 7.3, 10.5 Hz, $NHCOOCH_2$), 4.41 (dd, *J* 7.3, 10.5 Hz, $NHCOOCH_2$), 4.64 (m, α-CH), 5.28 (d, *J* 16.5 Hz, OCH_2CO), 5.68 (d, *J* 16.5 Hz, OCH_2CO), 5.95 (d, *J* 8.5 Hz, NH).

Analytical data. Calculated for $C_{26}H_{23}NO_6$: C, 70.1; H, 5.2. Found: C, 70.1; H, 5.3.

*N-(9-Fluorenylmethyloxycarbonyl)-L-threonine Phenacylester (**10**)*

L-Threonine (5.96 g, 50 mmol) was treated as described for serine, giving **10** (10.7 g, 23 mmol, 46%) as white crystals, mp 130-131°C, $[\alpha]_D$ -46°.

NMR data: ^{13}C, δ 18.8 (γ-CH_3), 47.1 ($CHAr_2$), 59.7 (α-CH), 66.6 (OCH_2CO), 67.2 ($NHCOOCH_2$), 68.2 (β-CH), 119-143 (Ar C), 156.7 (NHCOO), 170.8 ($COOCH_2$), 193.0

Table 2: ^{1}H NMR δ and *J* values (in parenthesis) for derivatives **11**, **12**, **14**, **15**, **20** and **21** (C^2HCl_3 solution, 30°C, TMS = δ 0.00) and for derivatives **18**, **19**, **24** and **25** ($C^2H_3O^2H$ solution, 30°C, TMS = δ 0.00).

Spin system	FMOC			Amino-acid				Phenacyl CH_2	
Proton	CH	CH_2a	CH_2b	NH	H-α	H-β[a]	H-γ	Ha	Hb
J	H,Ha	H,Hb	Ha,Hb	NH,α	α,β	β,γ		Ha,Hb	
11	4.33 (6.5)	4.12		5.95 (9.4)	4.57 (2.1)	4.64 (6.5)	1.44	5.32 (16.5)	5.60
12		4.47 (7.1)		5.79 (9.5)	4.56	4.53 (6.5)	1.44	5.28 (16.4)	5.60
14				5.74				5.42 (16.3)	5.52
15	4.26 (7.3)	4.44 (7.3)	4.46	5.92 (7.8)				5.46	5.46
18	4.25 (6.5)	4.44 (6.5)	4.45		4.41	3.93			
19	4.24 (6.8)	4.33 (6.8)	4.35 (10.5)						
20	4.41 (7.5)	4.34 (7.5)	4.52 (10.5)	5.95 (9.3)	4.55 (2.2)	(6.4)	1.43	5.36 (16.3)	5.58
21	4.27 (7.5)	4.42 (7.5)		5.93 (9.0)	4.61 (2.5)	4.67 (6.5)	1.47	5.34 (16.5)	5.35
24	4.28 (6.4)	4.51 (6.4)	4.58 (10.8)		4.22	4.36	1.25		
25	4.24 (7.2)	4.36 (7.2)	4.37		4.22 (2.9)	4.40 (6.7)	1.21		

[a] For serine derivatives, only one value is given.

(OCH_2CO); ^{1}H, δ 1.33 (d, *J* 6.4 Hz, γ-CH3), 4.25 (t, *J* 7.3 Hz, $CHAr_2$), 4.40 (d, *J* 7.3 Hz, $NHCOOCH_2$), 4.53 (dd, *J* 9.6, 1.9 Hz, α-CH), 4.64 (ddd, *J* 1.9, 6.4 Hz, β-CH), 5.36 (d, *J* 16.5 Hz, OCH_2CO), 5.66 (d, *J* 16.5 Hz, OCH_2CO), 5.73 (d, *J* 9.6 Hz, NH).

Analytical data. Calculated for $C_{27}H_{25}NO_6$: C, 70.6; H, 5.5. Found: C, 70.3; H, 5.5.

*N-(9-Fluorenylmethyloxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-L-threonine Phenacylester (**11**)*

A solution of silver triflate (2.83 g, 11.1 mmol) in dry toluene (70 ml) was added to a cooled (-50°C) solution of **1** [18] (2.91 g, 7.38 mmol) and **10** (5.07 g, 11.1 mmol) in dry

dichloromethane (90 ml) containing molecular sieves (2.76 g). After 15 min, pyridine (3 ml) was added and the reaction was allowed to attain room temperature. The solids were filtered off and thoroughly washed with dichloromethane. The filtrate was washed with, successively, 0.5 M sodium thiosulfate, water and aqueous sodium hydrogen carbonate, dried (magnesium sulfate) and concentrated. Column chromatography (toluene/ethyl acetate, 7/3 by vol) of the residue gave first pure **11** (1.21 g ,1.56 mmol, 21%), $[\alpha]_D$ +21°.

NMR data: ^{13}C, δ 19.1 (γ-CH_3), 20.6 (CH_3COO), 47.0 ($CHAr_2$), 58.2, 58.4, 61.8 (C-2, 6, α-CH), 66.7, 66.9, 67.47, 67.53, 68.4 (C-3, 4, 6, $NHCOOCH_2$, OCH_2CO), 76.1 (β-CH), 98.8 (C-1), 119-144 (Ar C), 156.8 (NHCOO), 169.8, 169.9, 170.0, 170.3 (CH_3COO, $COOCH_2$), 190.7 (OCH_2CO); For the ^1H data, see Tables 1 and 2. FAB mass spectroscopy showed (M^+ +1) = 773, (M^+ + Na) = 795. Further elution gave a mixture of **10** and **11** (3.63 g) in the ratio 1:3.

*N-(9-Fluorenylmethyloxycarbonyl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonine Phenacylester (**12**)*

Compound **11** (1.34 g, 1.73 mmol) was dissolved in thioacetic acid (2 ml) and left at room temperature for two days. The reaction mixture was then purified by column chromatography (toluene/ethyl acetate, 7/23 by vol) to give **12** (1.04 g, 1.31 mmol, 76%), $[\alpha]_D$ +17°

NMR data: ^{13}C, δ 18.5 (γ-CH_3), 20.6, 20.7, 20.8 (CH_3COO), 22.8 (CH_3CONH), 47.1, 47.4 (C-2, $CHAr_2$), 58.4, 62.2 (α-CH, C-6), 66.5, 67.2, 67.4, 67.5, 68.6 (C-3, 4, 5, $NHCOOCH_2$, OCH_2CO), 76.7 (β-CH_2), 99.9 (C-1), 120-141 (Ar C), 157.0 (OCONH), 170.3 (CH_3COO), 170.9 (CH_3CONH), 191.5 (OCH_2CO). For the ^1H data, see Tables 1 and 2.

*N-(9-Fluorenylmethyloxycarbonyl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonine (**13**)*

Activated zinc (2.08 g, 32 mmol) and **12** (0.69 g, 0.87 mmol) were vigorously stirred for 4 h in 80% aqueous acetic acid (3 ml). The solids were filtered off and the filtrate concentrated and purified by column chromatography (ethyl acetate/acetic acid, 9/1 by vol) to give **13** (0.48 g, 0.72 mmol, 83%), $[\alpha]_D$ +90°.

NMR data ($C^2H_3O^2H$): ^{13}C, δ 20.2 (γ-CH_3), 21.5, 21.6 (CH_3COO), 23.9 (CH_3CONH), 49.6 ($CHAr_2$) 60.8, 60.9, 64.3, 68.6, 69.1, 69.7, 70.6 (C-2, 3, 4, 5, 6, $NHCOOCH_2$, α-CH), 78.6 (β-CH), 101.7 (C-1), 121-146 (Ar C), 160.1 (NHCOO), 172.9, 173.0 (CH_3COO), 174.3, 174.6 (CH_3CONH, COOH). For the ^1H data, see Table 1.

*N-(9-Fluorenylmethyloxycarbonyl)-O-[4,6-di-O-acetyl-2-azido-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-serine Phenacylester (**14**) and N-(9-Fluorenylmethyloxycarbonyl)-O-[4,6-di-O-acetyl-2-azido-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-galactopyranosyl]-L-serine Phenacylester (**15**)*

Bromine (0.52 g, 3.27 mmol) was added to a crude solution of the thioglycoside **7** (1.58 g, 2.19 mmol) in dry dichloromethane (35 ml) containing molecular sieves (9.0 g). After 10 min, cyclooctadiene (0.2 ml) was added. The alcohol **9** (1.46 g, 3.28 mmol) was added and the mixture was cooled to -40°C. A solution of silver triflate (1.12 g, 4.36 mmol) in dry

toluene (12 ml) was added. After 10 min, pyridine (3.5 ml) was added and the reaction mixture was allowed to attain room temperature. The solids were filtered off and thoroughly washed with dichloromethane (4 x 50 ml). The filtrate was washed, successively, with 0.5 M sodium thiosulfate, water, and sodium hydrogen carbonate, dried with magnesium sulfate and concentrated. The residue was purified by column chromatography (toluene/ethyl acetate, 6/4 by vol) to give first **14** as an oil (1.19 g ,0.40 mmol, 52%), $[\alpha]_D$ +67°. NMR data: ^{13}C, δ 20.5, 20.6 (CH_3COO), 47.0 ($CHAr_2$), 54.3 (α-CH), 59.5, 61.0, 62.7 (C-2, 6, 6'), 63.9, 66.6, 66.7, 66.8, 67.2, 68.0, 68.7, 69.3, 69.4, 70.7, 74.6 (C-3, 4, 5, 2', 3', 4', 5', $NHCOOCH_2$, β-CH_2, OCH_2CO, $NHCOOCH_2$), 99.1 (C-1), 101.5 (C-1'), 119-144 (Ar C), 156.2 (NHCOO), 169.5, 169.6, 170.0, 170.2, 170.3, 170.5 (CH_3COO, CHCOO), 191.3 (OCH_2CO). For the ^1H data, see Tables 1 and 2. The next fraction was **15** (0.56 g, 0.54 mmol, 25%) $[\alpha]_D$ +15°. NMR data: ^{13}C, δ 20.4, 20.5 (CH_3COO), 46.9 ($CHAr_2$), 54.1 (α-CH), 60.9, 61.9, 63.0, 66.6, 66.8, 67.2, 67.9, 68.6, 69.5, 70.4, 70.7, 71.4 (C-2, 3, 4, 5, 6, 2', 3', 4', 5', 6', $NHCOOCH_2$, β-CH_2, OCH_2CO), 101.3, 102.2 (C-1, 1'), 119-144 (Ar C), 155.8 (OCONH), 169.0, 169.2, 169.5, 169.9, 170.1, 170.2, 170.4 (CH_3COO, CHCOO), 191.1 (OCH_2CO). For the ^1H data, see Tables 1 and 2.

*N-(9-Fluorenylmethyloxycarbonyl)-O-[2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-serine Phenacylester (**16**)*

Compound **14** (1.00 g, 0.96 mmol) was dissolved in thioacetic acid (3 ml). After two days the reaction mixture was purified by column chromatography (toluene/ethyl acetate, 1/2 by vol) to give **16** (0.73 g, 0.69 mmol, 72%), $[\alpha]_D$ +49°.

NMR data: ^{13}C, δ 20.6-21.3 (CH_3COO), 23.2 (CH_3CONH), 47.1 ($CHAr_2$), 48.7 (C-2), 54.4 (α-CH), 61.1, 62.8 (C-6, 6'), 66.9, 67.0, 67.4, 68.2, 68.6, 68.9, 69.3, 70.7, 70.9, 73.0 (C-3, 4, 5, 2', 3', 4', 5', β-CH_2, $NHCOOCH_2$, OCH_2CO), 98.7 (C-1), 101.3 (C-1'), 120-144 (Ar C), 156.0 (NHCOO), 169.6-170.7 (CH_3COO, CHCOO, CH_3CONH, $CHCOOCH_2$), 193 (OCH_2CO).

*N-(9-Fluorenylmethyloxycarbonyl)-O-[2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-galactopyranosyl]-L-serine Phenacylester (**17**)*

Compound **15** (0.40 g, 0.38 mmol) was dissolved in thioacetic acid (1 ml). After two days the reaction mixture was purified by column chromatography (toluene/ethyl acetate, 1/2 by vol) to give **17** (0.27 g, 0.25 mmol, 68%), $[\alpha]_D$ +16°.

NMR data: ^{13}C, δ 20.6-21.2 (CH_3COO), 23.6 (CH_3CONH), 47.2 ($CHAr_2$), 54.7 (α-CH), 61.1, 62.5 (C-6, 6'), 66.8, 66.9, 67.3, 68.3, 68.9, 69.2, 70.9, 71.0, 74.8 (C-3, 4, 5, 2', 3', 4', 5', β-CH_2, $NHCOOCH_2$, OCH_2CO), 99.5, 100.5 (C-1,1'), 120-144 (Ar C), 156.2 (NHCOO), 169.5-171.9 (CH_3COO, CH_3CONH, $CHCOOCH_2$, CHCOO), 191.9 (OCH_2CO).

*N-(9-Fluorenylmethyloxycarbonyl)-O-[2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-serine (**18**)*

Activated zinc (1.42 g, 22 mmol) and **16** (122 mg, 0.115 mmol) were vigorously stirred for 4 h in 80% aqueous acetic acid (1.5 ml). The solids were filtered off and the filtrate

concentrated and purified by column chromatography (ethyl acetate/acetic acid, 9/1 by vol) to give **18** (92 mg, 0.098 mmol, 85%), $[\alpha]_D$ +89°.

NMR data ($C^2H_3O^2H$): ^{13}C, δ 20.1-20.5 (CH_3COO), 22.7 (CH_3CONH), 48.1 ($CHAr_2$), 49.4, 55.7 (C-2, α-CH), 62.1, 63.6 (C-6, 6'), 67.6, 68.3, 68.6, 69.5, 69.7, 70.9, 71.5, 71.8, 74.3 (C-3, 4, 5, 2', 3', 4', 5', $NHCOOCH_2$, β-CH_2), 99.8 (C-1), 102.1 (C-1'),120-145 (Ar C), 158.1 (NHCOO), 170-174 (CH_3COO, CH_3CONH, COOH). For the ^1H data, see Tables 1 and 2. FAB mass spectroscopy showed (M^+ + 1) = 945, (M^+ + Na) = 967.

*N-(9-Fluorenylmethyloxycarbonyl)-O-[2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-galactopyranosyl]-L-serine (**19**)*

Activated zinc (1.21 g, 18.5 mmol) and **17** (297 mg, 0.28 mmol) were vigorously stirred for 4 h in 80% aqueous acetic acid (4 ml). The mixture was purified by column chromatography (ethyl acetate/acetic acid, 9/1 by vol) to give **19** (196 mg, 0.21 mmol, 75%), $[\alpha]_D$ +27°. NMR data ($C^2H_3O^2H$): ^{13}C, δ 20.4-20.8 (CH_3COO), 23.3 (CH_3CONH), 48.3 ($CHAr_2$), 53.0, 55.6 (C-2, α-CH), 62.3, 63.5 (C-6, 6'), 68.0, 68.6, 69.8, 70.2, 70.4, 71.8, 72.1, 72.5, 77.4 (C-3, 4, 5, 2', 3', 4', 5', $NHCOOCH_2$, β-CH_2), 102.2, 102.4 (C-1, 1'),120-145 (Ar C), 158.1 (NHCOO), 171-174 (CH_3COO, CH_3CONH, COOH). For the ^1H data, see Tables 1 and 2. FAB mass spectroscopy showed (M^+ + 1) = 945, (M^+ + Na) = 967.

*N-(9-Fluorenylmethyloxycarbonyl)-O-[4,6-di-O-acetyl-2-azido-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-threonine Phenacylester (**20**) and N-(9-Fluorenylmethyloxycarbonyl)-[4,6-di-O-acetyl-2-azido-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-galactopyranosyl]-L-threonine Phenacylester (**21**)*

Bromine (0.24 g, 1.53 mmol) was added to a crude solution of the thioglycoside **7** (0.74 g, 1.02 mmol) in dry dichloromethane (17 ml), containing molecular sieves (3.47 g). The bromine was allowed to react for 10 min. Cyclooctadiene (0.1 ml) was added and the bromine-red colour disappeared. The alcohol **10** (0.700 g, 1.528 mmol) was added and the mixture was cooled to -40°C. A solution of silver triflate (0.52 g, 2.04 mmol) in dry toluene (8 ml) was added . After 10 min, pyridine (2 ml) was added and the reaction mixture was allowed to attain room temperature. The solids were filtered off and thoroughly washed with dichloromethane. The filtrate was washed, successively, with 0.5 M sodium thiosulfate, water, and sodium hydrogen carbonate, dried with magnesium sulfate, and concentrated. The residue was purified by column chromatography (toluene/ethyl acetate, 6/4 by vol) to give **20** as the first fraction (0.43 g, 0.40 mmol, 39%) $[\alpha]_D$ +33°. NMR data: ^{13}C, δ 19.1 (γ-CH_3), 20.5-20.7 (CH_3COO), 47.1 ($CHAr_2$), 58.6 (α-CH), 60.3, 61.0, 63.0 (C-6, 6', 2), 66.7, 66.8, 68.8, 69.4, 70.7, 70.8, 74.8, 76.1 (C-3, 4, 5, 2', 3', 4', 5', $NHCOOCH_2$, β-CH, OCH_2CO), 98.9 (C-1), 101.5 (C-1'), 120-144 (Ar C), 156.8 (NHCOO), 169.7-170.4 (CH_3COO, $COOCH_2$, CHCOO), 192 (OCH_2CO). For the ^1H data, see Tables 1 and 2. The next fraction was **21** (0.34 g, 0.32 mmol, 31%), $[\alpha]_D$ +13°. NMR data: ^{13}C, δ 18.5 (γ-CH_3), 20.7-21.6 (CH_3COO), 47.2 ($CHAr_2$), 58.3 (α-CH), 61.1, 62.0, 63.4 (C-6, 6', 2), 66.9, 67.0, 67.6, 68.1, 69.0, 70.8, 70.9, 71.3, 76.3, 77.0 (C-3, 4, 5, 2', 3', 4', 5', $NHCOOCH_2$, β-CH, OCH_2CO), 100.6 (C-1), 101.6 (C-1'), 120-144 (Ar C), 156.9 (NHCOO), 169.5-170.7 (CH_3COO, CHCOO, CH_3CONH), 192 (OCH_2CO). For the ^1H data, see Tables 1 and 2.

*N-(9-Fluorenylmethyloxycarbonyl)-O-[2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-threonine Phenacylester (**22**)*

Compound **20** (0.428 g, 0.404 mmol) was dissolved in thioacetic acid (1 ml). After two days the residue was purified by column chromatography (toluene/ethyl acetate, 1/2 by vol) to give **22** (0.326 g, 0.303 mmol, 75%) as an amorphous solid, $[\alpha]_D$ +22°. NMR data: ^{13}C, δ 17.5 (γ-CH_3), 20.3-20.6 (CH_3COO), 22.8 (CH_3CONH), 47.1 ($CHAr_2$), 48.9 (C-2), 58.5, 60.8, 62.9 (α-CH, C-6, 6'), 66.7, 66.8, 67.1, 67.9, 68.5, 69.3, 70.4, 70.7, 72.6, 76.8 (C-3, 4, 5, 2', 3', 4', 5', $NHCOOCH_2$, β-CH, OCH_2CO), 99.4 (C-1), 101.1 (C-1'), 120-144 (Ar C), 156.5 (NHCOO), 169-171 (CH_3COO, CH_3CONH, CHCOO), 192.7 (OCH_2CO).

*N-(9-Fluorenylmethyloxycarbonyl)-O-[2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-galactopyranosyl]-L-threonine Phenacylester (**23**)*

Compound **21** (0.40 g, 0.38 mmol) was dissolved in thioacetic acid (1 ml). After two days the residue was purified by column chromatography (toluene/ethyl acetate, 1/2 by vol) to give **23** (0.27 g, 0.25 mmol, 68%) as an amorphous solid, $[\alpha]_D$ +16°. NMR data: ^{13}C, δ 17.4 (γ-CH_3), 20.1-20.6 (CH_3COO), 23.3 (CH_3CONH), 46.9 ($CHAr_2$), 54.6 (C-2), 58.4, 60.7, 61.9 (α-CH, C-6, 6'), 66.4, 66.5, 66.9, 67.8, 68.9, 70.6, 70.9, 74.4, 74.9 (C-3, 4, 5, 2', 3', 4', 5', $NHCOOCH_2$, β-CH, OCH_2CO), 97.2 (C-1), 100.0 (C-1'), 119-144 (Ar C), 156.3 (NHCOO), 169.1-170.7 (CH_3COO, CH_3CONH, CHCOO), 191.7 (OCH_2CO).

*N-(9-Fluorenylmethyloxycarbonyl)-O-[2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-threonine (**24**)*

Compound **22** (229 mg, 0.213 mmol) and activated zinc (1 g, 15 mmol) in 80% aqueous acetic acid (2 ml) were stirred at room temperature. After 3 h the mixture was purified by column chromatography (ethyl acetate/acetic acid, 9/1 by vol) to give **24** (185 mg, 0.193 mmol, 91%) as an amorphous solid, $[\alpha]_D$ +67°. NMR data ($C^2H_3O^2H$): ^{13}C, δ 18.7 (γ-CH_3), 20.0-20.4 (CH_3COO), 21.9 (CH_3CONH), 46.2, 49.1 ($CHAr_2$, C-2), 59.9, 61.9, 63.8 (α-CH, C-6, 6'), 67.2, 68.2, 68.5, 69.7, 70.9, 71.3, 71.8, 74.9, 77.3 (C-3, 4, 5, 2', 3', 4', 5', $NHCOOCH_2$, β-CH), 100.7 (C-1), 102.1 (C-1'), 120.6-145 (Ar C), 158.8 (NHCOO), 169-173 (CH_3COO, CH_3CONH, COOH). For ^1H data, see Tables 1 and 2. FAB mass spectroscopy showed $(M^+ + 1) = 959$, $(M^+ + Na) = 981$.

*N-(9-Fluorenylmethyloxycarbonyl)-O-[2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-galactopyranosyl]-L-threonine (**25**)*

Compound **23** (240 mg, 0.223 mmol) and activated zinc (1.0 g, 15 mmol) in 80% aqueous acetic acid (2 ml) were stirred at room temperature. After 3 h the mixture was purified by column chromatography (ethyl acetate/acetic acid, 9/1 by vol) to give **25** (207 mg, 0.216 mmol, 97%) as an amorphous solid, $[\alpha]_D$ +31°.NMR data ($C^2H_3O^2H$): ^{13}C, δ 17.4 (γ-CH_3), 20.0-20.4 (CH_3COO), 23.0 (CH_3CONH), 47.8 ($CHAr_2$), 53.0 (C-2), 59.8, 61.8, 62.7 (α-CH, C-6, 6'), 67.6, 68.3, 69.8, 69.9, 71.3, 71.6, 71.7, 76.1, 77.0 (C-3, 4, 5, 2', 3', 4', 5', $NHCOOCH_2$, β-CH), 100.8 (C-1), 101.9 (C-1'),120-146 (Ar C), 158.5 (NHCOO), 170-174 (CH_3COO, CH_3CONH, COOH). For the ^1H data, see Tables 1 and 2. FAB mass spectroscopy showed $(M^+ + 1) = 959$, $(M^+ + Na) = 981$.

*O-[2-Acetamido-2-deoxy-3-O-(β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-serine (**26**)*

Sodium methoxide (0.5 M) was added dropwise to a solution of **18** (81.6 mg, 0.086 mmol) in methanol (10 ml), to pH 12. The mixture was kept at room temperature for 30 min and then neutralized with aqueous hydrochloric acid (0.1 M). The mixture was concentrated to 2-4 ml and purified on a Bio-Gel P-2 column to give pure **26** (22.4 mg, 0.044 mmol, 51%) as a white solid. NMR data (^2H_2O) : ^{13}C, d 23.1 (CH_3CONH), 49.4 (α-CH), 55.5 (C-2), 62.0, 62.2 (C-6, 6'), 67.6 (β-CH_2), 69.6, 69.7, 71.6, 72.1, 73.5, 77.0, 77.6 (C-3, 4, 5, 2', 3', 4', 5'), 99.2 (C-1), 105.6 (C-1'), 172.7, 175.7 (COOH, CH_3CONH); the ^1H values were as reported [26, 27].

Note Added in Proof

Paulson H, Merz G and Weichert U [Angew Chem Int Edn Engl (1988) 27:1365-67] have recently reported a solid phase synthesis of O-glycopeptide sequences using a similar approach to that described here.

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