Convenient Synthesis of *O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-serine and -threonine Building Blocks for Solid-phase Glycopeptide Assembly

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The suitably protected building blocks for solid-phase glycopeptide synthesis, N^{α}-Fmoc-Ser(Ac_3-β-D-GlcNAc)-OPfp, **11**, and N^{α}-Fmoc-Thr(Ac_3-β-D-GlcNAc)-OPfp, **12**, have been synthesized by stereoselective glycosidation of the 2-allyloxycarbonylamino glycosyl donor **7** with N^{α}-Fmoc-Ser-OPfp, **3**, and N^{α}-Fmoc-Thr-OPfp, **4**, followed by Pd^0-catalysed allyl transfer from the *N*-allyloxycarbonyl group in the presence of acetic anhydride.

2-Acetamido-2-deoxy-β-D-glucopyranose (GlcNAc) O-glycosidically linked to serine and threonine has been reported[1,2] as a characteristic form of nuclear protein glycosylation. Unlike most other types of protein-bound saccharides the *O*-GlcNAc moiety occurs as a monosaccharide and often attached at multiple sites on the same protein. The O-linked GlcNAc residues are found predominantly on intracellular proteins within the nucleoplasmic and cytoplasmic compartments of cells.[3] These include nuclear pore and cytoskeletal as well as numerous chromatin proteins including transcription factors. The functions of the O-linked GlcNAc are not yet fully understood but it might be important in blocking phosphorylation sites and it has been suggested that the saccharide might be involved in mediating important protein–protein associations.[2] Only few *O*-GlcNAc-bearing proteins have been characterized and some glycopeptide fragments have been isolated in order to identify the nature of the glycosylation sites. Determination of their location is essential to study the structural and functional significance of the saccharide. However, *O*-GlcNAc attachment sites have not been unequivocally determined[2] and chemical syntheses of O-linked GlcNAc glycopeptides offer, therefore, a useful tool for this purpose.

The most efficient approach to the preparation of *O*-glycopeptides involves the use of protected glycosyl amino acids as building blocks in a stepwise assembly of peptides.[4] In general, the synthesis of the glycosyl amino acid building blocks requires multistep procedures implying activation of the carboxylic group after selective removal of the protecting group and in some cases exchange of the N^{α}-protecting group.[4,5] We have described an alternative strategy which simplifies this approach. It involves the direct glycosylation of the active ester derivatives N^{α}-Fmoc-Ser-OPfp, N^{α}-Fmoc-Thr-OPfp[6–9] and N^{α}-Fmoc-Tyr-OPfp.[10]

On the other hand, glycosylation with 2-amino-2-deoxy sugar derivatives has received particular attention within the field of glycosylation reactions.[11] In fact, many of the glycosylation methods have been specifically modified for glycosylation with 2-amino-2-deoxy carbohydrate derivatives. In particular, the 1,2-*trans* glycosylations require glycosyl donors containing participating protective groups in the 2 position. Thus, first syntheses of 2-acetamidoglucopyranosyl amino acids were performed using 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[12] as well as the oxazoline[13] derivative as glycosyl donors. However, the reactions required high temperature, acidic conditions and often the yields were poor. Furthermore, these conditions are incompatible with the temperature-sensitive pentafluorophenyl (Pfp) esters.

Alternatively, *N*-allyloxycarbonyl- (Aloc) and *N*-trichloroethoxycarbonyl-(Teoc) glucosamine derivatives have been stereoselectively glycosidated[14] with Z-Ser/Thr-OBu^t, Z-Ser-Ala-OBu^t and Teoc-Ser-Ala-OBu^t. Both N-protective groups, Aloc and Teoc, have been chemoselectively removed to afford the corresponding *N*-acetyl derivatives after acetylation. The α-glycosidically linked Ser and Thr derivatives of 2-azido-2-deoxy-D-glycopyranose has been obtained through the azido-glycosylation method.[15]

Other strategies involve the use of a building block bearing an acetamido precursor, *i.e.* azide,[16] or an orthogonal N-protective group, *i.e.* the dithiasuccinimidoyl (Dts) group.[17] In both cases, the *N*-acetyl group has been generated after glycopeptide assembly in the solid phase. However, a 2-amino-2-deoxyglucopyranosyl amino acid building block containing the *N*-acetyl group prior to its incorporation onto a peptide is more convenient. In this report, we described the synthesis of such O-linked GlcNAc serine and threonine building blocks bearing fluoren-9-ylmethoxycarbonyl (Fmoc) on the aglycone α-amino group and a Pfp ester as an activator of the carboxylic group.

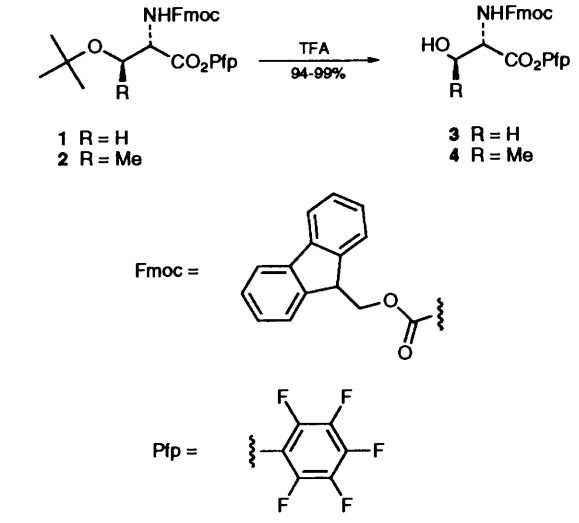
Results and Discussion

Compounds **3** and **4** were obtained by treatment of N^{α}-Fmoc-Ser(Bu^t)-OPfp **1** and N^{α}-Fmoc-Thr(Bu^t)-OPfp **2** with neat trifluoroacetic acid (TFA) (Scheme 1). The yields were almost quantitative (94 and 99%, respectively). The purity of products **3** and **4** was evaluated by NMR spectroscopy and by comparison of their physical properties with those of an authentic sample. Purification by chromatography was not required.

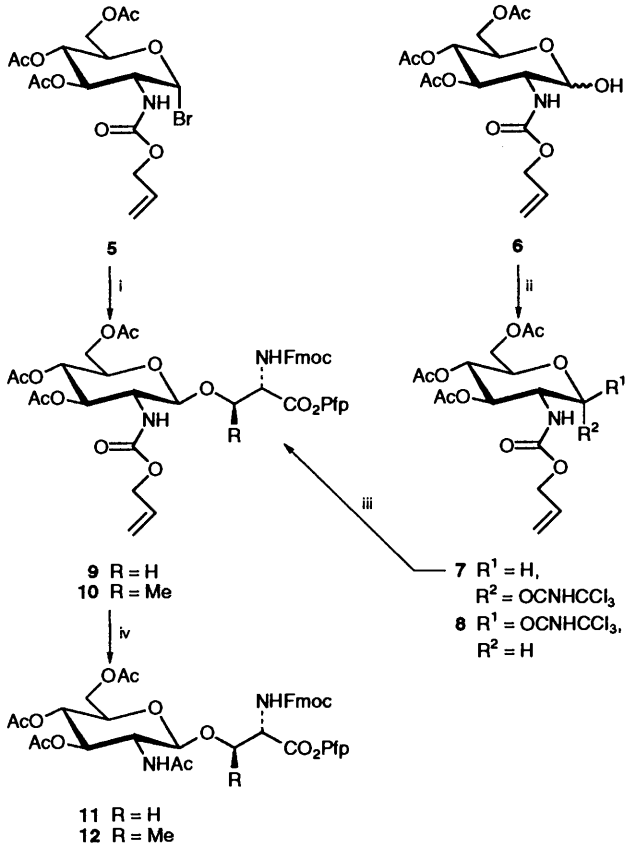
Among all the amino-protective groups reported in the literature[11] for glycosidations of 2-amino-2-deoxy sugars, the Aloc group showed some advantages for our purposes. First, it could be involved in C-2 anchimeric assistance to afford 1,2-*trans* glycosides. Secondly, it could be easily removed in a chemospecific manner. And thirdly, the Aloc group has previously been used as an orthogonal protective group in solid-phase peptide synthesis.[18]

The silver triflate-mediated glycosylation of N^{α}-Fmoc-Ser-OPfp **3** with the glycosyl bromide[19] **5** was carried out in dichloromethane at room temperature. Compound **9** was obtained in moderate yields (37%) due in part to the instability of glycosyl donor **5**.

The trichloroacetimidate glycosylation[11,20] has been frequently used for the practical, selective syntheses of complex oligosaccarides and glycoconjugates. In particular, 2-deoxy-2-phthalimidoglycopyranosyl imidates have been used as glycosyl donors in reactions promoted either by Lewis acids,[21] such as



Scheme 1



Scheme 2 *Reagents:* i, **3**, AgOTf, 3 Å MS, CH_2Cl_2 (37%); ii, CCl_3CN, K_2CO_3, CH_2Cl_2 (**7**, 76%; **8**; 15%); iii, **3** or **4**, AgOTf, CH_2Cl_2 (**9**, 63%; **10**, 71%); iv, $Pd(PPh_3)_4$, Bu_3SnH, Ac_2O, CH_2Cl_2 (**11**, 59%; **12**, 71%)

boron trifluoride–diethyl ether and trimethylsilyl trifluoromethanesulfonate, or by AgOTf.[22]

The imidate compound was therefore investigated as an alternative glycosyl donor. The reaction of the hemiacetal **6**[23] with trichloroacetonitrile in the presence of anhydrous potassium carbonate led to a mixture of anomers (α:β 5:1) which were easily separated by silica gel chromatography. The α-anomer **7** was selected as glycosyl donor for practical reasons, even though the mixture of anomers can be used for preparative glycosylations. Condensation of compound **7** with the Pfp esters **3** and **4** in the presence of AgOTf,[22] in the absence of molecular sieves and without further neutralization gave stereoselectively the β-glycosides **9** and **10** in 63 and 71% yield, respectively, after silica gel purification (Scheme 2). Long reaction times (16–17 h) were needed for completion of the glycosylations. This is due to the combination of several factors such as the electronegative influence of the Pfp esters,[6] an unfavourable hydrogen-bonding from the amide hydrogen to the oxygen lone pair which decreases the nucleophility of the hydroxy-group acceptor,[24] as well as the reduced activation of the imidate leaving group by AgOTf.[22]

It is well known that the Aloc group can be removed under practically neutral conditions by using palladium(0) catalyst in the presence of a nucleophile.[25] Palladium(0) reacts with the Aloc moiety to form a π-allylpalladium complex which is attacked by morpholine, dimedone, *N,N'*-dimethylbarbituric acid, or Bu_3SnH[18,26] to give carbon dioxide and the free amine. However, sometimes the liberated amino group competes with those nucleophiles and N-allylation may arise as a side reaction.[27] N-Allylation may be prevented by using Bu_3SnH[28] which reacts with the π-allylpalladium complex, leading to a tributyltin carbamate and propene. The tin carbamate is cleaved *in situ* with a proton donor or an activated electrophilic carbonyl group,[27,29] including Pfp esters.

Assuming that acetic anhydride is a better acylating reagent than a Pfp ester compound, we tested the conversion of the *N*-Aloc-protected glycosyl amino acid compounds **9** and **10** into N^{α}-Fmoc-Ser(Ac_3-β-D-GlcNAc)-OPfp, **11**, and N^{α}-Fmoc-Thr(Ac_3-β-D-GlcNAc)-OPfp, **12**, by treatment with $Pd(Ph_3P)_4$, Bu_3SnH and acetic anhydride. The reactions were followed by TLC and were complete within 10 min. The products were purified by chromatography on silica gel to afford compounds **11** and **12** in 59 and 71% yield, respectively.

The application of the building blocks **11** and **12** for glycopeptide synthesis is currently under investigation.

Experimental

General Procedures.—TLC was performed on Merck Silica Gel 60 F_{254} aluminium sheets with detection by charring with sulfuric acid, and by UV light when applicable. Vacuum liquid chromatography (VLC)[30,31] was performed on Merck Silica Gel 60 H. Solvents were purchased from Labscan Ltd. Dichloromethane was distilled from P_4O_{10} and was stored over molecular sieves 3 Å under argon. Light petroleum was the 60–80 °C fraction. Concentrations were performed under reduced pressure at temperatures < 40 °C. *O-tert*-Butyl-N^{α}-(fluoren-9-ylmethoxycarbonyl)-L-serine pentafluorophenyl ester **1** and *O-tert*-butyl-N^{α}-(fluoren-9-ylmethoxycarbonyl)-L-threonine pentafluorophenyl ester **2** were purchased from Bachem. Flasks for glycosylation reactions were stored at 120 °C for at least 15 h.

M.p.s were measured on a Büchi melting point apparatus and are uncorrected. Microanalysis was carried out at Leo Pharmaceutical Products (Ballerup, Denmark). Optical rotations were recorded on a Perkin-Elmer 241 polarimeter, and are given in units of 10^{-1} deg cm^2 g^{-1}. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM500 MHz spectrometer. Unless otherwise indicated the NMR experiments were performed at 300 K in $CDCl_3$. Chemical shifts are given in ppm and referenced to internal $SiMe_4$ (δ_H, δ_C 0.00). *J* Values are given in Hz. For all compounds the assignment of ^1H NMR spectra was based on 2D homonuclear chemical-shift correlation (COSY) spectra. The assignments of ^{13}C NMR spectra were based on carbon–proton shift correlation spectra.

N^{α}-(*Fluoren-9-ylmethoxycarbonyl*)-L-*serine Pentafluorophenyl Ester* **3**.—Compound **1** (11.35 g, 20.60 mmol) was treated with TFA (200 cm^3). After 30 min at room temperature the

Table 1 ^{1}H NMR data for compounds **9–12**, δ-values in ppm (J-values in Hz)

Proton	Chemical shifts and coupling constants for compound			
	9	**10**	**11**	**12**
1-H	4.71 (br s)	4.71 (8.3)	4.89 (8.3)	4.78 (8.2)
2-H	3.66 (br s)	3.64 (10.0)	3.80 (9.9)	3.83 (10.0)
3-H	5.28 (9.6)	5.21 (10.0)	5.33 (9.9)	5.31 (10.0)
4-H	5.11 (9.6)	5.11 (9.6)	5.11 (9.4)	5.11 (10.0)
5-H	3.73 (1.8, 4.7)	3.71 (4.7)	3.75 (4.5)	3.72 (2.7, 4.7)
6-H	4.18 (12.2)	4.13 (12.1)	4.17 (12.3)	4.10 (12.3)
6-H′	4.26	4.25	4.26	4.24
OAc	2.08, 2.10	2.04, 2.07, 2.10	2.08, 2.09	2.07, 2.08, 2.10
NAc			1.93	1.99
2-NH	4.95	4.95 (9.0)	5.64 (7.7)	5.65 (8.3)
C*H*=CH_2	5.84 (br s)	5.89		
CH=C*H*$_2$	5.20–5.28	5.27 (16.6)		
C*H*$_2$CH=CH_2	4.57	4.61		
α-H	4.95 (3.0)	4.76 (2.2)	4.91 (2.7, 3.6)	4.74 (2.7)
β-H	4.47 (10.4)	4.65 (6.2)	4.46 (10.6)	4.61 (6.3)
β-H′	4.03 (br s)		4.03	
γ-H		1.37		1.34
NH	6.11 (br s)	6.09 (br s)	6.12 (8.6)	6.07 (8.9)
Fmoc-CH	4.28 (7.0, 7.4)	4.29 (7.3, 7.3)	4.29 (7.1, 7.1)	4.30 (7.2, 7.2)
Fmoc-CH_2	4.44 (10.5), 4.57	4.42 (10.5), 4.53	4.48 (10.6), 4.55	4.46 (10.5), 4.52
ArH	7.82–7.30	7.80–7.30	7.80–7.30	7.80–7.30

solution was concentrated to dryness, toluene was added, and the mixture was concentrated twice to yield compound **3** (9.56 g, 94%), m.p. 139–140 °C (from diethyl ether) (lit.,[6] 144–145 °C). NMR data and $[\alpha]_D$ are in agreement with those previously reported.[6,9,32]

N$^{\alpha}$-(*Fluoren-9-ylmethoxycarbonyl*)-L-*threonine Pentafluorophenyl Ester* **4**.—Compound **2** (12.09 g, 21.00 mmol) was treated with TFA (100 cm^3). After 45 min at room temperature the solution was concentrated to dryness, toluene was added, and the mixture was concentrated twice to yield compound **4** (10.80 g, 99%), m.p. 122 °C (from diethyl ether) (lit.,[32] 126–128 °C). NMR data and $[\alpha]_D$ are in agreement with those previously reported.[8,9,32]

3,4,6-*Tri*-O-*acetyl*-2-*allyloxycarbonylamino*-2-*deoxy*-α-D-*glucopyranosyl Trichloroacetimidate* **7**.—To a mixture of compound **6**[23] (3.70 g, 9.51 mmol) and trichloroacetonitrile (10 cm^3) in dry dichloromethane containing molecular sieves (3 Å) was added anhydrous K_2CO_3 (3.83 g). The mixture was stirred overnight at room temperature and was directly subjected to VLC [ethyl acetate–light petroleum (1:2)] to give, first, *compound* **7** (3.87 g, 76%) (Found: C, 40.4; H, 4.5; N, 5.2. $C_{18}H_{23}Cl_3N_2O_{10}$ requires C, 40.5; H, 4.3; N, 5.3%; $[\alpha]_D^{25}$ +82.0 (*c* 1, $CHCl_3$); δ_H 2.08, 2.09 and 2.11 (9 H, 3 s, 3 × Me), 4.13–4.18 (2 H, m, 5- and 6-H), 4.28–4.34 (2 H, m, 2-H and 6-H′), 4.56 (1 H, dd, J 13.3, C*H*CH=CH_2), 4.62 (1 H, dd, J 13.3, C*H*′CH=CH_2), 4.95 (1 H, d, J 9.4, NH), 5.25 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 10.0, 4-H), 5.30 (2 H, m, C*H*$_2$=CH), 5.35 (1 H, t, $J_{2,3}$ = $J_{3,4}$ = 10.0, 3-H), 5.91 (1 H, m, C*H*=CH_2), 6.42 (1 H, d, $J_{1,2}$ 3.4, 1-H) and 8.84 (1 H, s, C=NH); δ_C 20.6 and 20.7 (3 × Me), 53.5 (C-2), 61.4 (C-6), 66.0 (*C*H$_2$CH=CH_2), 67.5 (C-4), 70.2 (C-5), 70.6 (C-3), 94.9 (C-1), 117.95 (CH=*C*H$_2$), 132.3 (*C*H=CH_2), 155.5 (NHCO), 160.3 (C=N) and 169.2 and 171.1 (3 × CO).

3,4,6-*Tri*-O-*acetyl*-2-*allyloxycarbonylamino*-2-*deoxy*-β-D-*glucopyranosyl trichloroacetimidate* **8** (0.79 g, 15%) was isolated as the second fraction, as a syrup, from the preparation of its epimer **7** (Found: C, 40.3; H, 4.3; N, 5.0%); $[\alpha]_D^{25}$ +20.6 (*c* 1, $CHCl_3$); δ_H 2.08, 2.09 and 2.11 (9 H, 3 s, 3 × Me), 3.93 (1 H, m, 5-H), 4.10 (1 H, dt, $J_{1,2}$ = $J_{2,NH}$ = 9.0 and $J_{2,3}$ 9.6, 2-H), 4.21 (1 H, br d, J 12.4, 6-H), 4.36 (1 H, dd, J 4.5 and 12.4, 6-H′), 4.55 (2 H, br s, C*H*$_2$CH=CH_2), 5.06 (1 H, d, NH), 5.20 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.2, 4-H), 5.29–5.34 (3 H, m, C*H*$_2$=CH and 3-H), 5.88 (1 H, m, C*H*=CH_2), 5.96 (1 H, d, 1-H) and 8.73 (1 H, s, C=NH); δ_C 20.6 and 20.7 (3 × Me), 54.8 (C-2), 61.7 (C-6), 65.8 (*C*H$_2$CH=CH_2), 68.0 (C-4), 71.9 and 72.8 (C-3 and -5), 96.2 (C-1), 117.9 (CH=*C*H$_2$), 132.4 (*C*H=CH_2), 155.3 (NHCO), 161.3 (C=N) and 169.3 and 170.6 (3 × CO).

N$^{\alpha}$-(*Fluoren-9-ylmethoxycarbonyl*)-O-(3,4,6-*tri*-O-*acetyl*-2-*allyloxycarbonylamino*-2-*deoxy*-β-D-*glucopyranosyl*)-L-*serine Pentafluorophenyl Ester* **9**.—*Method A*. Compound **3** (85 mg, 0.17 mmol), the bromide **5**[19] (146 mg, 0.32 mmol), AgOTf (96 mg, 0.37 mmol), and molecular sieves (3 Å) were placed in a pre-dried flask. After evacuation with an oil-pump the flask was filled with argon, and dry dichloromethane was injected (3 cm^3). The mixture was stirred for 17 h at room temperature in the dark and was then neutralized with diisopropylethylamine (0.064 cm^3). The mixture was filtered through Celite and the filtrate was concentrated. VLC [ethyl acetate–light petroleum (1:2)] yielded the *title compound* **9** (55 mg, 37%), which was crystallized from ethyl acetate–light petroleum, m.p. 160–161 °C; $[\alpha]_D^{25}$ −5.3 (*c* 1, $CHCl_3$) (Found: C, 55.3; H, 4.4; N, 3.3. $C_{40}H_{37}F_5N_2O_{14}$ requires C, 55.6; H, 4.3; N, 3.2%). ^{1}H and ^{13}C NMR data are presented in Tables 1 and 2.

Method B. Compound **3** (0.619 g, 1.25 mmol), the imidate **7** (1.00 g, 1.87 mmol), and AgOTf (0.490 g, 1.91 mmol) were placed in a pre-dried flask in the dark. After evacuation with an oil-pump the flask was filled with argon, and dry dichloromethane was injected (15 cm^3). The solution was stirred for 16 h at room temperature and then was directly subjected to VLC [ethyl acetate–light petroleum (1:2)] to yield compound **9** (0.690 g, 63%).

N$^{\alpha}$-(*Fluoren-9-ylmethoxycarbonyl*)-O-3,4,6-*tri*-O-*acetyl*-2-*allyloxycarbonylamino*)-2-*deoxy*-β-D-*glucopyranosyl*)-L-*threonine Pentafluorophenyl Ester* **10**.—Compound **4** (0.676 g, 1.33 mmol), the imidate **7** (1.063 g, 1.99 mmol), and AgOTf (0.521 g, 2.03 mmol) were placed in a pre-dried flask in the dark. After evacuation with an oil-pump the flask was filled with argon, and dry dichloromethane was injected (15 cm^3). The solution was

Table 2 ^{13}C NMR data for compounds **9–12**

Carbon	Chemical shifts (δ) for compound			
	9	**10**	**11**	**12**
C-1	100.9	98.4	100.6	98.2
C-2	56.0	56.2	54.9	55.1
C-3	71.9	71.7	71.9	71.9
C-4	68.4	68.4	68.4	68.4
C-5	72.0	71.8	72.1	71.7
C-6	61.9	61.8	61.9	61.9
*C*OMe	169.5, 170.9	169.3, 170.6, 170.9	169.4, 170.6, 170.9, 171.0	169.3, 170.5, 170.6, 171.1
OCO*Me*	20.6	20.4, 20.6, 20.7	20.6, 20.7	20.4, 20.6
NCO*Me*			23.2	23.3
CH=CH_2	118.1	117.9		
CH_2CH=CH_2	66.2	65.9		
OCONH	156.1	155.7, 156.7	156.1	156.6
CO_2Pfp	166.1	166.5	166.3	166.5
C-α	54.2	58.7	54.3	58.7
C-β	69.4	73.0	68.3	73.2
C-γ		16.4		16.5
Fmoc-CH	47.1	47.1	47.1	47.1
Fmoc-CH_2	67.5	73.0	67.2	67.3
*C*H=CH_2[a] and	120.0, 125.1,	120.0, 124.8,	120.0, 125.2,	119.9, 125.2,
Fmoc arom.-C	127.1, 127.8,	125.2, 127.1,	127.1, 127.8,	127.0, 127.1,
	132.1, 141.3,	127.4, 127.7,	128.5, 128.6,	127.7, 128.5,
	143.5, 143.6	132.4, 141.3,	132.0, 132.1,	128.6, 132.0,
		143.6, 143.7	141.3, 143.6,	132.1, 141.3,
			143.7	143.6, 143.8
Pfp-C ($J_{F,C}$)[b]	137.9 (237)	137.8 (262),	137.9 (250),	137.8 (250),
		141.0 (237)	141.0 (250)	140.9 (250)

[a] For compounds **9** and **10**. [b] These values are the one-bond ^{19}F–^{13}C coupling constants in Hz.

stirred for 15 h at room temperature and then was directly subjected to VLC [ethyl acetate–light petroleum (1 : 2)] to yield *title compound* **10** (0.829 g, 71%), which was crystallized from diethyl ether, m.p. 184–185 °C; $[\alpha]_D^{25}$ −19.0 (*c* 1, $CHCl_3$) (Found: C, 55.8; H, 4.5; N, 3.2. $C_{41}H_{39}F_5N_2O_{14}$ requires C, 56.0; H, 4.5; N, 3.2%; M, 878.77). ^1H and ^{13}C NMR data are presented in Tables 1 and 2.

O-(2-*Acetamido*-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-β-D-*gluco*-*pyranosyl*)-N$^{\alpha}$-(*fluoren*-9-*ylmethoxycarbonyl*)-L-*serine Penta*-*fluorophenyl Ester* **11**.—$Pd(PPh_3)_4$ (17 mg, 0.015 mmol) was added to a well stirred solution of compound **9** (354 mg, 0.409 mmol) in dry dichloromethane (20 cm^3) containing acetic anhydride (2.2 cm^3) under argon, followed by immediate addition of Bu_3SnH (0.215 cm^3). After 15 min at room temperature the solvent was evaporated off. The residue was purified by VLC [ethyl acetate–light petroleum (2 : 1)] to yield *title compound* **11** (200 mg, 59%), which was crystallized from diethyl ether, m.p. 207 °C; $[\alpha]_D^{25}$ −10.0 (*c* 1, $CHCl_3$) (Found: C, 55.3; H, 4.3; N, 3.4. $C_{38}H_{35}F_5N_2O_{13}$ requires C, 55.5; H, 4.3; N, 3.4%). ^1H and ^{13}C NMR data are presented in Tables 1 and 2.

O-(2-*Acetamido*-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-β-D-*gluco*-*pyranosyl*)-N$^{\alpha}$-(*fluoren*-9-*ylmethoxycarbonyl*)-L-*threonine Pentafluorophenyl Ester* **12**.—$Pd(Ph_3P)_4$ (14 mg, 0.012 mmol) was added to a well stirred solution of compound **10** (302 mg, 0.344 mmol) in dry dichloromethane (15 cm^3) containing acetic anhydride (1.9 cm^3) under argon, followed by immediate addition of Bu_3SnH (0.181 cm^3). After 10 min at room temperature the solvent was evaporated off. The residue was purified by VLC [ethyl acetate–light petroleum (2 : 1)] to yield *title compound* **12** (202 mg, 71%), which was crystallized from diethyl ether, m.p. 175 °C (decomp.); $[\alpha]_D^{25}$ −30.6 (*c* 1, $CHCl_3$) (Found: C, 55.9; H, 4.5; N, 3.3. $C_{39}H_{37}F_5N_2O_{13}$ requires C, 56.0; H, 4.5; N, 3.4%). ^1H and ^{13}C NMR data are presented in Tables 1 and 2.

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References

1 C.-R. Torres and G. W. Hart, *J. Biol. Chem.*, 1984, **259**, 3308.
2 G. W. Hart, R. S. Haltiwanger, G. D. Holt and W. G. Kelly, *Annu. Rev. Biochem.*, 1989, **58**, 841; in *Carbohydrate Recognition in Cellular Function*, ed. G. Bock and S. Harnett, Wiley, Chichester, 1989, p. 102.
3 K. P. Kearse and G. W. Hart, *Arch. Biochem. Biophys.*, 1991, **290**, 543.
4 M. Meldal, in *Neoglycoconjugates: Preparation and Application*, ed. Y. C. Lee and R. T. Lee, Academic Press, Orlando, 1994, p. 145.
5 H. Paulsen, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 823; H. Kunz, *Angew. Chem., Int. Ed. Engl.*, 1987, **26**, 294.
6 M. Meldal and K. J. Jensen, *J. Chem. Soc., Chem. Commun.*, 1990, 483.
7 M. Meldal, S. Mouritsen and K. Bock, *ACS Symp. Ser.*, 1993, **519**, 19.
8 A. M. Jansson, M. Meldal and K. Bock, *Tetrahedron Lett.*, 1990, **31**, 6991.
9 A. M. Jansson, M. Meldal and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1992, 1699.
10 K. J. Jensen, M. Meldal and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1993, 2119.
11 J. Banoub, P. Boullanger and D. Lafont, *Chem. Rev.*, 1992, **92**, 1167.
12 H. G. Garg and R. W. Jeanloz, *Carbohydr. Res.*, 1976, **52**, 246.
13 M. G. Vafina, A. Kim and N. V. Molodtsov, *Carbohydr. Res.*, 1978, **64**, 334.
14 M. Schultz and H. Kunz, *Tetrahedron Lett.*, 1992, **33**, 5319.
15 H. Paulsen and J.-P. Hölck, *Liebigs Ann. Chem.*, 1982, 1121.
16 S. Peters, T. Bielfeldt, M. Meldal, K. Bock and H. Paulsen, *J. Chem. Soc., Perkin Trans. 1*, 1992, 1163.
17 E. Meinjohanns, M. Meldal, H. Paulsen and K. Bock, presented at the XVIIth International Carbohydrate Symposium, Ottawa, 1994.
18 A. Loffet and H. X. Zhang, *Int. J. Pept. Protein Res.*, 1993, **42**, 346; H. Kunz and C. Unverzagt, *Angew. Chem., Int. Ed. Engl.*, 1984, **23**, 436.
19 P. Boullanger, J. Banoub and G. Descotes, *Can. J. Chem.*, 1987, **65**, 1343.

20 R. R. Schmidt and J. Michel, *Angew. Chem., Int. Ed. Engl.*, 1980, **19**, 73; R. R. Schmidt, *Angew. Chem., Int. Ed. Engl.*, 1986, **25**, 212.
21 R. R. Schmidt and G. Grundler, *Angew. Chem., Int. Ed. Engl.*, 1983, **22**, 776.
22 S. P. Douglas, D. M. Whitfield and J. J. Krepinsky, *J. Carbohydr. Chem.*, 1993, **12**, 131.
23 P. Boullanger, M. Jouineau, B. Bouammali, D. Lafont and G. Descotes, *Carbohydr. Res.*, 1990, **202**, 151.
24 R. Polt, L. Szabó, J. Treiberg, Y. Li and V. J. Hruby, *J. Am. Chem. Soc.*, 1992, **114**, 10249.
25 I. Minami, Y. Ohashi, I. Shimuzu and J. Tsuji, *Tetrahedron Lett.*, 1985, **26**, 2449; Y. Hayakawa, H. Kato, M. Uchiyama, H. Kajino and R. Noyori, *J. Org. Chem.*, 1986, **51**, 2400.
26 H. Kunz, *Pure Appl. Chem.*, 1993, **65**, 1223.
27 F. P. J. T. Rutjes, M. Paz, H. Hiemstra and W. N. Speckamp, *Tetrahedron Lett.*, 1991, **32**, 6629.
28 O. Dangles, F. Gubé, G. Balavoine, S. Lavielle and A. Marquet, *J. Org. Chem.*, 1987, **52**, 4984.
29 E. C. Roos, P. Bernabé, H. Hiemstra and W. N. Speckamp, *Tetrahedron Lett.*, 1991, **32**, 6633.
30 N. M. Targett, J. P. Kilcoyne and B. Green, *J. Org. Chem.*, 1979, **44**, 4962.
31 J. C. Coll and B. F. Bowden, *J. Nat. Prod.*, 1986, **49**, 934.
32 L. Kisfaludy and I. Schön, *Synthesis*, 1983, 325.

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