Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 338 (2003) 1039–1043
CARBOHYDRATE RESEARCH
www.elsevier.com/locate/carres

# Practical synthesis of the 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucosides of Fmoc-serine and Fmoc-threonine and their benzyl esters

Ivone Carvalho,[a,b,c] Shona L. Scheuerl,[b] K.P. Ravindranathan Kartha,[b,c] Robert A. Field[b,c,*]

[a]*Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto 14040 930, Brazil*
[b]*School of Chemistry and Centre for Biomolecular Sciences, The Purdie Building, University of St Andrews, St Andrews, Fife KY16 9ST, UK*
[c]*Centre for Carbohydrate Chemistry, School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich NR4 7TJ, UK*

Received 21 October 2002; accepted 10 February 2003

**Abstract**

Mercuric bromide-promoted glycosylation of Fmoc-Ser-OBn and Fmoc-Thr-OBn with 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl chloride in refluxing 1,2-dichloroethane gave the corresponding β-glycosides in good yields (64 and 62%, respectively). Direct coupling of the commercially available Fmoc-Ser-OH and Fmoc-Thr-OH carboxylic acids under similar conditions gave the corresponding β-glycosides, possessing free carboxyl groups, in moderate yields (50 and 40%, respectively).


*Keywords:* Serine; Threonine; Benzyl ester; Free carboxylic acid; Mercuric bromide; Glycosylation

## 1. Introduction

Dynamic O-glycosylation of nuclear and cytoplasmic proteins is thought to play a complimentary and/or orthogonal role to protein phosphorylation in the regulation of signal transduction processes. Proteins ranging from the myc-oncogene product and tumour suppressor p53 to the beta-amyloid precursor protein, RNA polymerase II and a variety of chromatin- and microtubule-associated proteins are all subject to reversible addition of β-linked *N*-acetylglucosamine.[1,2] Similarly in plants, response to giberellins is thought to be at least partially regulated by the action of the spindly O-GlcNAc transferase.[3] Since the addition of O-GlcNAc often occurs at potential phosphorylation sites, the typical site-directed mutagenesis approach to investigate the role of either process is complicated.[4] Efforts have therefore been made to synthesise non-hydrolysable analogues of O-GlcNAc-Ser, by replacing the linking anomeric oxygen by sulfur[5] and carbon,[6] for incorporation into glycopeptide probes for biochemical analyses. In addition, as characterisation of the key O-GlcNAc β-*N*-acetylglucosaminidase progresses[7] there is a need to consider synthetic routes to analogues of O-linked β-GlcNAc to probe the molecular basis of its recognition and cleavage.

Although 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl chloride (**1**) is often used as a glycosyl donor for glycosylation of simple alcohols, there are some limitations due to its low reactivity and the parallel formation of a relatively stable oxazoline by-product, **2**.[8] Alternatively, 1,2-*trans*-glycosides can be prepared via an acid catalysed reaction of the same oxazoline derivative **2**.[9] For glycosyl-amino acid synthesis, the coupling of appropriately protected glycosyl halides with the protected amino acid in the presence of a promoter affords the desired products in variable yield and/or with poor stereoselectivity.[10] Even a simple change of ester protecting group can have a major impact on reaction yield.[8a] The oxazoline procedure has therefore been widely used for glycosyl-amino acid

* Corresponding author. Tel.: +44-1603-593983; fax: +44-1603-592003
*E-mail address:* r.a.field@uea.ac.uk (R.A. Field).

0008-6215/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0008-6215(03)00071-5

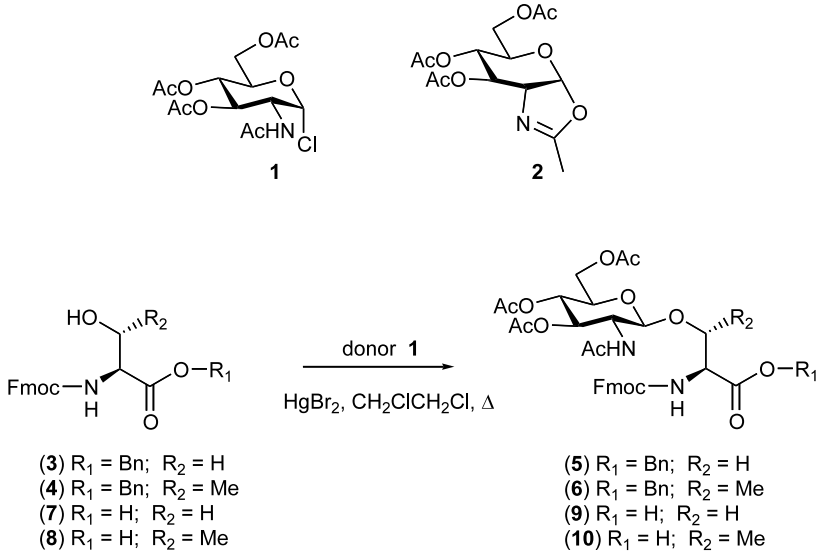
synthesis,[11] although efforts continue to be made to improve this procedure[12] and to devise practical alternatives.[13] Having considered the various different building blocks, we chose to revisit the stereoselective formation of the β-D-GlcNAc-Ser/Thr using the readily available glycosyl donor 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl chloride (**1**). Herein we report improvements to the preparation of **1**, together with its coupling to appropriately protected serine and threonine building blocks (Fig. 1).

## 2. Results and discussion

The preparation of 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl chloride (**1**) has been a literature standard[14] for many years and routinely gives robust, but often not exceptional, yields even in inexperienced hands. We have found that, as for the preparation of the corresponding sialic acid glycosyl chloride,[15] presaturating the acetyl chloride reaction medium used to effect O-acetylation and concurrent glycosyl chloride formation with anhydrous hydrogen chloride increases reaction rates and generally leads to improved yields (15–20% improvement) of glycosyl chloride **1**.

Classical Helferich glycosylation conditions[16] were initially investigated for coupling known[13c] Fmoc-Ser-OBn **3** and Fmoc-Thr-OBn **4** to give the target glycosyl serine and threonine benzyl esters, **5** and **6**, respectively. Glycosylation reactions were attempted under various reaction conditions with mercuric cyanide and mercuric bromide. Using this approach, serine β-glycoside **5** was obtained in poor yield (10%). A slight improvement in yield was obtained (23%) when the reaction was carried out in refluxing benzene in the presence of mercuric cyanide alone. This procedure is described for the corresponding *N*-benzyloxycarbonyl-protected acceptor in 70% yield, although the methyl ester gave only 34% of the desired adduct.[8a] On the other hand, glycosylation was successfully achieved using mercuric bromide as the sole promoter. On refluxing a 1,2-dichloroethane solution of glycosyl chloride **1** (2 equivalents) and protected serine **3** or threonine **4**, in the presence of mercuric bromide (1.1 equivalents) *N*-(9-fluorenylmethoxycarbonyl)-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-L-serine benzyl ester (**5**) and *N*-(9-fluorenylmethoxycarbonyl)-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-L-threonine benzyl ester (**6**) were obtained in 64 and 62% yield, respectively. The β configuration of the glycosidic bond was established in both cases from $^{1}H$ and $^{13}C$ NMR spectra (serine glycoside **5**: H-1 doublet at $\delta$ 4.67, $J_{1,2}$ 8.4 Hz and C-1 at $\delta$ 100.6 ppm; threonine glycoside **6**: H-1 doublet at $\delta$ 4.65, $J_{1,2}$ 8.1 Hz and C-1 at $\delta$ 98.50 ppm). The reaction conditions described proved effective over a range of scales (0.5–55 mmol glycosyl chloride) without significant changes in the overall yield. We suspect that the oxazoline may be an intermediate in the reaction process, but we have so far been unable to confirm this.

Although we have shown iodine in the presence of DDQ to be an effective promoter system for glycosylation of simple alcohols with glycosyl chloride donor **1**,[17] attempts to use this system with acceptors **3** and **4** proved fruitless (yields ~10%, mixed anomers), as did the use of iodine monochloride and iodine monobromide (yields ~20%, mixed anomers).[18]




(**3**) $R_1$ = Bn; $R_2$ = H
(**4**) $R_1$ = Bn; $R_2$ = Me
(**7**) $R_1$ = H; $R_2$ = H
(**8**) $R_1$ = H; $R_2$ = Me

(**5**) $R_1$ = Bn; $R_2$ = H
(**6**) $R_1$ = Bn; $R_2$ = Me
(**9**) $R_1$ = H; $R_2$ = H
(**10**) $R_1$ = H; $R_2$ = Me

Fig. 1. Glycosylation of Fmoc-Ser/Thr-OBn/OH.

For further use in solid phase glycopeptide synthesis, the α-amino acid carboxyl groups of **5** and **6** need to be released, for example by hydrogenolysis, to give carboxylic acids **9** and **10**. In order to cut out this extra chemical step and the associated chromatography, and for comparison with oxazoline chemistry,[9] the direct coupling of glycosyl chloride **1** to serine and threonine derivatives possessing free carboxyl groups (i.e. **7** and **8**, respectively) was investigated. Under the conditions described above for the corresponding benzyl esters, Fmoc-Ser-OH **7** and Fmoc-Thr-OH **8** gave the corresponding β-glycosides **9** and **10** in 50 and 40% yield, respectively. Although the yields are not high, the target glycosyl amino acid building blocks can be accessed directly from readily available protected amino acids under relatively mild conditions. In addition, formation of the glycosyl halide donor reagent is essentially stereospecific and high yielding, whereas access to the alternative oxazoline donor generally requires prior synthesis of the anomeric β-acetate, which can be low yielding.[19]

In summary, in our hands $HgBr_2$-mediated glycosylation with 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl chloride (**1**) is at least competitive with the oxazoline procedure for the synthesis of the *O*-acetylated benzyl esters and free acids of β-D-GlcNAc-Ser and β-D-GlcNAc-Thr.

## 3. Experimental

Thin-layer chromatography (TLC) was performed on Silica Gel 60 $F_{254}$ (Merck) detected by immersion in a 5% ethanolic solution of $H_2SO_4$, followed by heating ($>100$ °C). Normal-phase column chromatography was performed using Silica Gel 60 (0.063–0.200 mm). Concentration of organic extracts was typically carried out below 40 °C and at water pump pressure. Unless otherwise stated, NMR spectra were obtained in $CDCl_3$ (referenced to $\delta$ 77.0 or residual $CHCl_3$ at $\delta$ 7.27 ppm for $^{13}C$ and $^{1}H$, respectively) or $D_2O$ (referenced to added acetone at $\delta$ 31.00 or 2.25 ppm for $^{13}C$ and $^{1}H$, respectively). Dichloroethane was freshly distilled from $CaH_2$. 2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl chloride (**1**) was prepared essentially as described by Horton, except that the acetyl chloride reaction medium was presaturated with gaseous hydrogen chloride prior to use.

### 3.1. *N*-(9-Fluorenylmethoxycarbonyl)-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-L-serine benzyl ester (5)

A mixture of 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl chloride (**1**, 181 mg, 0.50 mmol) and *N*-(9-fluorenylmethoxycarbonyl)-L-serine benzyl ester (**3**) (104 mg, 0.25 mmol) in 1,2-dichloroethane (2.0 mL) was refluxed with mercuric bromide (198 mg, 0.55 mmol) for 9 h, when TLC [Hex–EtOAc (7:3)] showed the reaction to be complete. The resulting amber mixture was concentrated in vacuo and the residue was purified by a silica gel column chromatography [eluent: EtOAc–Hex (7:3)]. The desired glycoside **5** (119 mg, 64%) was obtained as an amorphous solid; $[\alpha]_D$ −4.6 (*c* 1, $CHCl_3$) (Lit.,[13c] $[\alpha]_D$ −3.7); $R_f$ 0.25 [EtOAc–Hex (7:3)]; $\delta_H$ ($CDCl_3$, 300 MHz) 7.77 (2 H, d, *J* 7.8, Fmoc Ph), 7.64 (2H, d, *J* 7.8, Fmoc Ph), 7.40–7.26 (9 H, m, Fmoc Ph, Bn), 5.81 (1 H, d, *J* 8.7, N*H*Thr), 5.35 (1 H, d, *J* 8.4, N*H*Ac), 5.25 (1 H, t, $J_{3,4}$ 9.6, H-3), 5.20 (2 H, AB, OC$H_2$Ph), 5.02 (1 H, t, $J_{3,4}$ 10, H-4), 4.67 (1 H, d, $J_{1,2}$ 8.4, H-1), 4.55–4.38 (3 H, m, C$H_2$ Fmoc, C*H* Ser), 4.28–4.19 (3 H, m, H-6, C*H* Fmoc, C$H_{2a}$ Ser), 4.08 (1 H, dd, *J* 2.2, $J_{6,6'}$ 12.3, H-6′), 3.85 (1 H, dd, *J* 2.7, *J* 10.8, C$H_{2b}$ Ser), 3.70 (1 H, apparent t, $J_{1,2}$, $J_{2,3}$ 10, H-2) 3.64–3.59 (1 H, m, H-5), 2.04, 2.03, 2.02, 1.82 (12 H, 4 × s, COC$H_3$); $\delta_C$ ($CDCl_3$, 75 MHz) 170.8, 170.7, 169.5, 169.4 (*C*OCH$_3$, *C*OCH$_2$Ph), 156.1 (*C*O Fmoc), 143.6, 143.7, 141.3 (*C*quat. Fmoc), 135.3 (*C*quat. OCH$_2$Ph) 128.5, 128.4, 128.2, 127.7, 127.1, 125.1, 119.9 (Fmoc Ph, *C*HPh), 100.6 (C-1), 71.9, 71.7 (C-5, C-3), 68.9, 68.3 (C-4, CH$_2$ Ser), 67.3 (O*C*H$_2$Ph), 66.7 (*C*H$_2$ Fmoc) 61.8 (C-6), 54.6, 54.1 (C-2, CH Ser), 47.0 (*C*H-Fmoc), 22.8, 20.7 (CO*C*H$_3$). ESIMS found *m*/*z* 764.3025 [$M + NH_4^+$]. Calcd for $C_{39}H_{42}N_2O_{13}{\cdot}NH_4$ 764.3031.

### 3.2. *N*-(9-Fluorenylmethoxycarbonyl)-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-L-threonine benzyl ester (6)

A mixture of 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl chloride (**1**, 181 mg, 0.50 mmol) and *N*-(9-fluorenylmethoxycarbonyl)-L-threonine benzyl ester **4** (108 mg, 0.25 mmol) in 1,2-dichloroethane (2.0 mL) was treated as described for the preparation of glycoside **5**. The residue was eluted from a column of silica gel with EtOAc–Hex (7:3) to give the product **6** (117 mg, 0.16 mmol, 62%) as an amorphous solid; $[\alpha]_D$ −15.6 (*c* 1, $CHCl_3$); $R_f$ 0.26 [EtOAc–Hex (7:3)]; $\delta_H$ ($CDCl_3$, 400 MHz) 7.76 (2 H, d, *J* 7.6, CH Fmoc Ph), 7.64 (2 H, d, *J* 7.8, CH Fmoc Ph), 7.41–7.26 (9 H, m, Fmoc Ph, OCH$_2$Ph), 5.83 (1 H, d, *J* 9.1, N*H*Thr), 5.54 (1 H, d, *J* 8.4, N*H*Ac), 5.25 (1 H, apparent t, $J_{2,3}$ 9.9, H-3), 5.21, 5.14 (2 H, AB, $J_{AB}$ 12.2, OC$H_2$Ph), 5.00 (1 H, t, $J_{3,4}$ 9.9, H-4), 4.65 (1 H, d, $J_{1,2}$ 8,1, H-1), 4.47–4.40 (3 H, m, C$H_2$ Fmoc, βC*H*Thr), 4.35 (1 H, dd, *J* 7.3, *J* 10.7, αC*H*Thr), 4.24 (1 H, t, *J* 7.3, C*H*Fmoc), 4.19 (1 H, dd, $J_{5,6}$ 4.4, $J_{6,6'}$ 12.3, H-6), 4.02 (1 H, dd, $J_{5,6'}$ 2.2, $J_{6,6'}$, H-6′), 3.67 (1 H, dd, $J_{1,2}$, $J_{2,3}$, H-2), 3.51–3.47 (1 H, m, H-5), 2.03, 2.02, 1.99, 1.93 (12 H, 4 × s, COC$H_3$), 1.20 (3 H, d, *J* 6.3, C$H_3$ Thr); $\delta_C$ ($CDCl_3$, 100 MHz) 170.9, 170.6, 170.4, 170.0 (*C*OCH$_3$),

169.4 (*C*OCH$_2$Ph), 156.8 (*C*O Fmoc), 144.0, 141.3 (*C*quat. Fmoc Ph), 135.5 (*C*quat. OCH$_2$Ph), 128.6, 128.5, 128.3, 127.7, 127.1, 125.5, 119.9 (*C*H Ph), 98.5 (C-1), 74.5 (β*C*HThr), 71.9, 71.7 (C-5, C-3), 68.5 (C-4), 67.3 (OCH$_2$Ph, *C*H$_2$ Fmoc), 61.9 (C-6), 58.7 (α*C*HThr), 55.3 (C-2), 47.3 (*C*H Fmoc), 23.3, 20.7, 20.5, 20.6 (CO*C*H$_3$), 17.0 (*C*H$_3$ Thr). ESIMS found *m*/*z* 778.3187 [M + NH$_4^+$]. Calcd for $C_{40}H_{44}N_2O_{13}\cdot NH_4$ 778.3187.

### 3.3. *N*-(9-Fluorenylmethoxycarbonyl)-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-L-serine (9)

A mixture of 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl chloride (**1**, 181 mg, 0.50 mmol) and commercial *N*-(9-fluorenylmethoxycarbonyl)-L-serine (**7**, 82 mg, 0.25 mmol) in 1,2-dichloroethane (1.5 mL) was treated as described for the preparation of **5**. The reaction mixture was washed with aq soln of 3% EDTA and the organic layer dried over $MgSO_4$ and concentrated. The residue was eluted from a column of silica gel with $CHCl_3$–AcOH (8:0.3 and then 8:0.6) to give the product **9** (83 mg, 50%) as an amorphous solid; $[\alpha]_D$ −14.8 (*c* 0.29, $CHCl_3$)(Lit.,[13c] $[\alpha]_D$ −14.7); $R_f$ 0.45 [$CHCl_3$–MeOH–AcOH (8:1:0.1)]; $\delta_H$ ($CDCl_3$/$CD_3OD$, 400 MHz) 7.78 (2 H, d, *J* 7.3, Fmoc Ph), 7.64 (2 H, d, *J* 7.6, Fmoc Ph), 7.43–7.31 (4 H, m, Fmoc Ph), 5.20 (1 H, t, $J_{2,3}$ 9.8, H-3), 5.02 (1 H, t, $J_{3,4}$ 9.8, H-4), 4.63 (1 H, d, $J_{1,2}$ 8.6, H-1), 4.49 (1 H, dd, *J* 10.6, *J* 6.5, C$H_{2a}$Fmoc), 4.43–4.41 (1 H, m, C*H* Ser), 4.39 (1 H, dd, *J* 10.6, *J* 6.8, C$H_{2b}$Fmoc), 4.27–4.17 (2 H, m, H-6, C*H* Fmoc), 4.19 (1 H, dd, *J* 10.9, *J* 4.0, C$H_{2a}$ Ser), 4.12 (1 H, dd, $J_{5,6'}$ 2.1, $J_{6,6'}$ 12.2, H-6′), 3.89 (1 H, dd, *J* 10.9, *J* 3.3, C$H_{2b}$ Ser), 3.84 (1 H, dd, $J_{1,2}$, $J_{2,3}$, H-2), 3.69 (1 H, ddd, $J_{4,5}$ 9.1, $J_{5,6}$ 4.5, $J_{5,6'}$, H-5), 2.08, 2.03, 2.02, 1.86 (12 H, 4 × s, COC$H_3$); $\delta_C$ ($CDCl_3$/$CD_3OD$, 100 MHz) 172.0, 171.1, 170.6, 169.8 (*C*OCH$_3$), 160.5 (*C*O Fmoc), 143.7, 141.2 (*C*quat), 127.7, 125.0, 119.9 (*C*H Ph), 100.7 (C-1), 72.5 (C-3), 71.7 (C-5), 69.0, 68.8 (C-4, *C*H$_2$ Ser), 67.0 (*C*H$_2$ Fmoc), 62.1 (C-6), 54.2, 54.0 (C-2, *C*H Ser), 47.1 (*C*H Fmoc), 22.4, 20.5, 20.4 (CO*C*H$_3$). ESIMS found *m*/*z* 674.2557 [M + NH$_4^+$]. Calcd for $C_{40}H_{44}N_2O_{13}\cdot NH_4$ 674.2561.

### 3.4. *N*-(9-Fluorenylmethoxycarbonyl)-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-L-threonine (10)

A mixture of 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl chloride (**1**, 181 mg, 0.50 mmol) and commercial *N*-(9-fluorenylmethoxycarbonyl)-L-threonine (**8**, 86 mg, 0.25 mmol) in 1,2-dichloroethane (1.5 mL) was treated as described for the preparation of **9**. The residue was eluted from a column of silica gel with $CHCl_3$–AcOH (8:0.3 and then 8:0.6) to give the product **10** (67 mg, 40%) as an amorphous solid; $[\alpha]_D$ −14.4 (*c* 1, in MeOH)(Lit.,[9] $[\alpha]_D$ + 14.7); $R_f$ 0.55 [$CHCl_3$–MeOH (30:1)]; $\delta_H$ ($CDCl_3$, 270 MHz) 7.73 (2 H, d, *J* 7.3, CH Fmoc Ph), 7.61 (2 H, d, *J* 6.6, CH Fmoc Ph), 7.38–7.24 (4 H, m, C*H* Fmoc Ph), 6.12 (1 H, d, *J* 9.2, N*H*), 6.03 (1 H, d, *J* 9.2, N*H*), 5.26 (1 H, t, $J_{2,3}$ 9.6, H-3), 5.09 (1 H, t, $J_{3,4}$ 9.6, H-4), 4.64 (1 H, d, $J_{1,2}$ 8.3, H-1), 4.50–3.60 (9 H, m, C$H_2$ Fmoc, 2 C*H* Thr, *H*-6, C*H* Fmoc, H-6′, H-2, H-5), 2.04, 1.98, 1.97, 1.93 (12 H, m, COC$H_3$), 1.18 (3 H, d, *J* 6.3, C$H_3$ Thr); $\delta_C$ ($CDCl_3$, 67.5 MHz) 171.3, 170.9, 170.8, 169.3 (*C*OCH$_3$), 156.8 (*C*O Fmoc), 143.7, 141.1 (*C*quat.), 127.6, 127.0, 125.1, 119.9 (*C*H Ph), 99.4 (C-1), 71.7, 71.5, 70.9, 68.2, 67.3 (C-3, C-4, C-5, 2 *C*H Thr, *C*H$_2$ Fmoc), 62.0 (C-6), 52.3 (C-2), 47.0 (*C*H Fmoc), 22.9, 20.8, 20.7, 20.5 (CO*C*H$_3$), 17.3 (*C*H$_3$ Thr) (NMR data in accord with the literature[20]); ESIMS found *m*/*z* 671.2453 [M + NH$_4^+$]. Calcd for $C_{40}H_{44}N_2O_{13}$ 671.2452. The optical rotation noted above for compound **10** is at odds with the literature.[9] However, the sense of the rotation we report herein is in keeping with that of the corresponding pentafluorophenyl ester.[21]

## Acknowledgements

These studies were supported by the MRC and the Weston Foundation. Support in the form of a research leave fellowship from FAPESP is gratefully acknowledged (IC). We thank Julia Bilke for performing some preliminary glycosylation experiments. We are indebted to the EPSRC Mass Spectrometry Service Centre, Swansea for invaluable support.

## References

1. Wells, L.; Vosseller, K.; Hart, G. W. *Science* **2001**, *291*, 2376–2378.
2. Zachara, N. E.; Hart, G. W. *Chem. Rev.* **2002**, *102*, 431–438.
3. Thornton, T. M.; Swain, S. M.; Olszewski, N. E. *Trends Plant Sci.* **1999**, *4*, 424–428.
4. Comer, F. I.; Hart, G. W. *Biochemistry* **2001**, *40*, 7845–7852.
5. Ohnishi, Y.; Ichikawa, M.; Ichikawa, Y. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1289–1291.
6. Ohnishi, Y.; Ichikawa, Y. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 997–999.
7. Wells, L.; Gao, Y.; Mahoney, J. A.; Vosseller, K.; Chen, C.; Rosen, A.; Hart, G. W. *J. Biol. Chem.* **2002**, *277*, 1755–1761.
8. For example: (a) Garg, H.G.; Jeanloz, R.W. *Carbohydr. Res.*, **1976**, *49*, 482–488; (b)Vafina, M.G.; Kim., A.; Molodstov, N.V. *Carbohydr. Res.*, **1978**, *64*, 334–338.
9. Arsequell, G.; Krippner, L.; Dwek, R.; Wong, S. Y. C. *Chem. Commun.* **1994**, 2383–2384.
10. Reviewed in Taylor, C. M. *Tetrahedron* **1998**, *54*, 11317–11362.

11. For example Pohl, T.; Waldmann, H. *J. Am. Chem. Soc.* **1997**, *119*, 6702–6710.
12. Wittmann, V.; Lennartz, D. *Eur. J. Org. Chem.* **2002**, 1363–1367.
13. For example: (a) Meinjohanns, E.; Meldal, M.; Bock, K. *Tetrahedron Lett.*, **1995**, *36*, 9205–9208; (b) Saha, U.K.; Schmidt, R.R. *J. Chem. Soc., Perkin Trans. 1*, **1997**, 1855–1860; (c) Mitchell, S.A.; Pratt, M.A.; Hruby, V.J.; Polt, R. *J. Org. Chem.*, **2001**, *66*, 2327–2342.
14. Horton, D. *Methods Carbohydr. Chem.* **1972**, *6*, 282–285.
15. Kuhn, R.; Lutz, P.; MacDonald, D. L. *Chem. Ber.* **1966**, *99*, 611–617.
16. Helferich, B.; Wedemeyer, K.-F. *Justus Liebigs Ann. Chem.* **1949**, *563*, 139–145.
17. Kartha, K. P. R.; Aloui, M.; Field, R. A. *Tetrahedron Lett.* **1996**, *37*, 8807–8810.
18. Kartha, K. P. R.; Field, R. A. *Tetrahedron Lett.* **1997**, *38*, 8233–8237.
19. Conchie, J.; Levvy, G. A. *Methods Carbohydr. Chem.* **1963**, *2*, 332–335.
20. (a) Seitz, O.; Wong, C.-H. *J. Am. Chem. Soc.* **1997**, *119*, 8766–8776;
(b) Simanek, E. C.; Huang, D.-H.; Pasternack, L.; Machajewski, T. D.; Seitz, O.; Millar, D. S.; Dyson, H. J.; Wong, C.-H. *J. Am. Chem. Soc.* **1998**, *120*, 11567–11575.
21. (a) Vargas-Berengud, A.; Meldal, M.; Paulsen, H.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2615–2619;
(b) Jensen, K. J.; Hansen, P. R.; Venugopal, D.; Berany, G. *J. Am. Chem. Soc.* **1996**, *118*, 3148–3155.