

# Efficient synthesis of *O*-(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-serine and -threonine building blocks for glycopeptide formation †

PERKIN

Uttam K. Saha and Richard R. Schmidt \*

Fakultät Chemie, Universität Konstanz, Postfach 5560 M 725, D-78434 Konstanz, Germany

Glucosamine donors **1–3** having *N*-TCP, *N,N*-diacetyl and *N*-Teoc protection, respectively, give with *N*<sup>o</sup>-Boc-protected serine and threonine benzyl esters **4a,b** as acceptors exclusively the  $\beta$ -glycosides; they can be transformed into *O*-GlcNAc serine and threonine derivatives **8a,b**. The high yielding *O*-glycosylation of compounds **4a,b** with trichloroacetimidate **3** and the ease of replacement of the *N*-Teoc group by the *N*-acetyl group prompted the use of *N*<sup>o</sup>-Fmoc-protected serine and threonine allyl (**9a,b**) and Pfp active esters (**12a,b**) as acceptors, thus very efficiently yielding the corresponding *O*-(*N*,*O*-acetylglucosamino) serine and threonine derivatives **11a,b** and **14a,b** as active esters.

## Introduction

Intracellular posttranslational protein glycosylation, through which *N*-acetylglucosamine (GlcNAc) residues are  $\beta$ -glycosidically linked to the hydroxy group of serine and/or threonine, has been observed for some time.<sup>1</sup> Recently, it has been shown that the  $\beta$ -amyloid precursor protein (APP), which is associated with Alzheimer's disease, is also posttranslationally modified by *O*-GlcNAc residues.<sup>2</sup> The specific functions of *O*-GlcNAc attachment to proteins have not yet been fully elucidated. Indirect evidence led to the hypothesis that the *O*-GlcNAc linkage may often have a reciprocal relationship to the regulatory effect of protein phosphorylation.<sup>3</sup> Therefore, access to glycopeptides carrying  $\beta$ -linked *O*-GlcNAc residues became of great interest.<sup>4–6</sup>

Still the most efficient approach to glycopeptide synthesis is based on *O*-acyl-protected *O*- or *N*-glycosyl amino acid active esters, for instance *N*<sup>o</sup>-fluorenylmethoxycarbonyl amino acid pentafluorophenyl esters (*N*<sup>o</sup>-Fmoc-AA-OPfp),<sup>7–10</sup> which are used as building blocks for the stepwise construction of glycopeptides at a solid phase. Therefore, efficient access to *N*-protected serine and threonine active esters already possessing *O*-linked GlcNAc residues, as described in this report, eases glycopeptide synthesis. Based on the *N*-allyloxycarbonyl (Aloc) group and the *N*-dithiasuccinoyl (Dts) group a similar approach has been recently reported.<sup>4,5</sup> However, not only the ease of formation of the glycosyl donor but also the stability of the *N*-protecting group, required for activation in the glycosylation step, and a direct and high yielding transformation of *O*-glycosyl amino acid active esters into the corresponding *O*-GlcNAc-containing compounds are important aspects in this endeavour. Methods for the synthesis of glucosamine-containing serine and threonine derivatives, in which the *N*-acetyl group is generated only after glycopeptide synthesis, have also been reported.<sup>5,8,11,12</sup>

## Results and discussion

Glucosamine can be readily transformed into glycosyl donors **1–3**;<sup>13–15</sup> they exhibit high glycosyl-donor properties because formation of stable oxazolinium intermediates is prevented by the presence of strongly electron-withdrawing groups; yet high  $\beta$ -selectivity can still be expected through neighbouring-group participation. Thus, reaction of *N*-tetrachlorophthaloyl (TCP)-protected glycosyl donor **1**<sup>13</sup> with *N*-*tert*-butoxycarbonyl (Boc)-

protected serine and threonine benzyl esters **4a,b**<sup>16</sup> furnished, in the presence of catalytic amounts of trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.01 mol equiv.), as expected the  $\beta$ -anomers **5a,b** in 75% yield (1-H: **5a**:  $\delta_{\text{H}}$  5.34,  $J_{1,2}$  8.4 Hz; **5b**:  $\delta_{\text{H}}$  5.3,  $J_{1,2}$  8.3). However, the removal of the *N*-TCP group with ethylenediamine at 60 °C<sup>17</sup> and then per-acetylation with acetic anhydride in pyridine, to afford target molecules **8a,b**, was not satisfactory; various by-products were formed (Scheme 1).

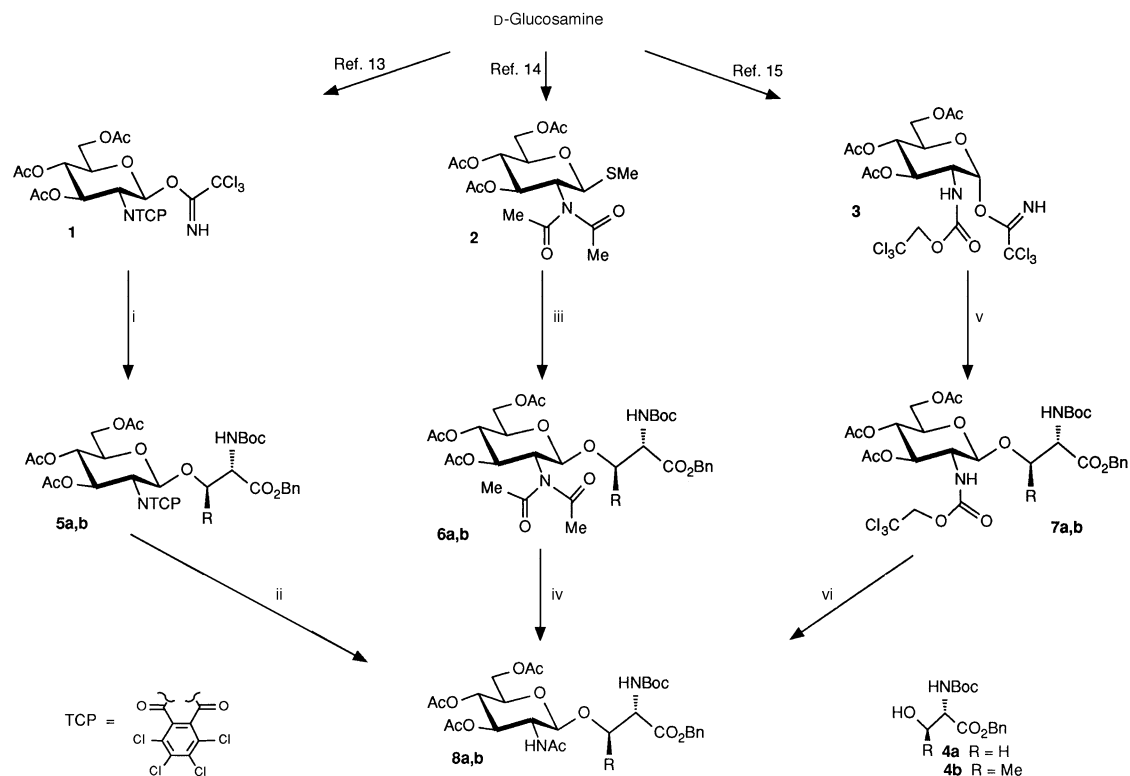
The *N,N*-diacetyl thioglycoside **2**<sup>14</sup> was activated by *N*-iodosuccinimide (NIS, 1.5 mol equiv.) and TfOH (0.01 mol equiv.), giving, by reaction with acceptors **4a,b**, the desired  $\beta$ -glycosides **6a,b** in only 50% yield (1-H: **6a**:  $\delta_{\text{H}}$  5.32,  $J_{1,2}$  7.8; **6b**:  $\delta_{\text{H}}$  5.3,  $J_{1,2}$  7.7). One of the observed side-reactions is acetyl transfer to the acceptor, as previously observed.<sup>14</sup> However, selective removal of one *N*-acetyl group in compounds **6a,b** could be readily performed with sodium methanolate in methanol; ensuing *O*-acetylation afforded compounds **8a,b** in very high yield.

The best results in terms of overall yield and convenience of the experimental procedures were obtained with *N*-trichloroethoxycarbonyl (Teoc)<sup>18</sup>-protected trichloroacetimidate **3**<sup>15,19</sup> as glycosyl donor; addition of catalytic amounts of TMSOTf (0.01 mol equiv.) afforded, with acceptors **4a,b**, the  $\beta$ -glycosides **7a,b** in 80% yield (1-H: **7a**:  $\delta_{\text{H}}$  4.61,  $J_{1,2}$  8.3; **7b**:  $\delta_{\text{H}}$  4.53,  $J_{1,2}$  8.1). Direct replacement of the *N*-Teoc group by the *N*-acetyl group, by treatment with zinc in acetic anhydride,<sup>15b</sup> gave compounds **8a,b** in 85% yield; therefore, this method seemed to be suitable for the direct generation of *O*-GlcNAc-containing active esters of serine and threonine.

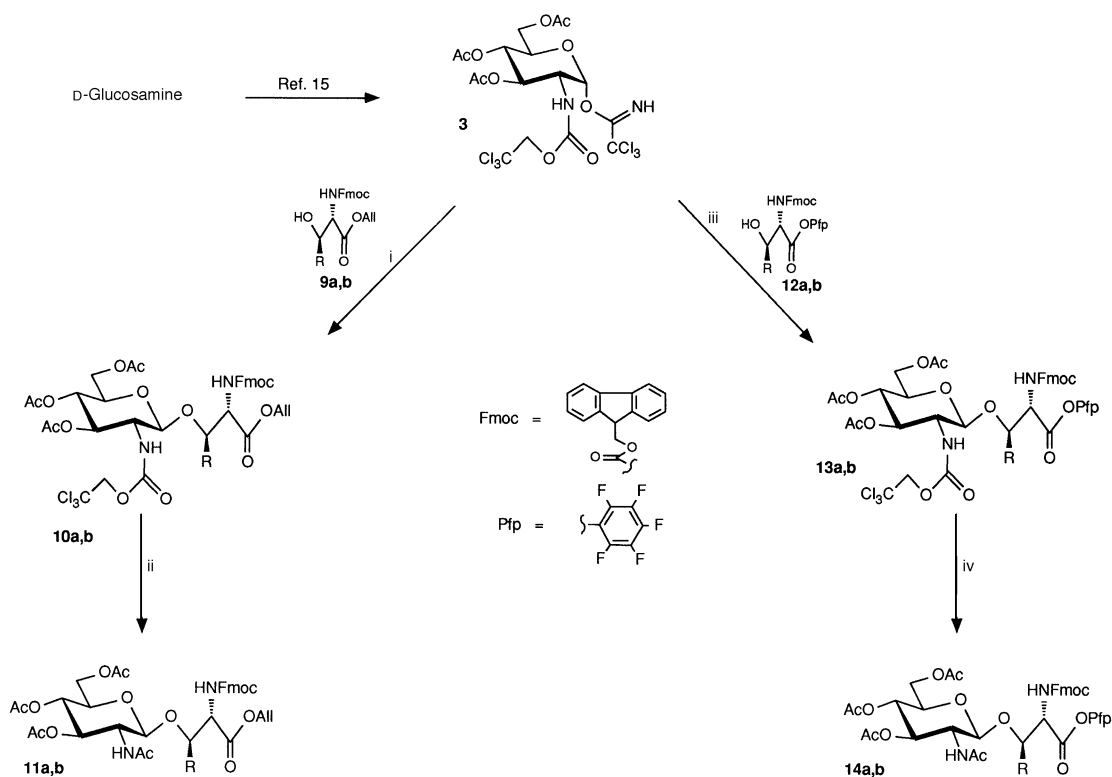
To this aim, glycosyl donor **3** was treated under the same reaction conditions with *N*<sup>o</sup>-Fmoc-protected serine and threonine allyl esters **9a,b**<sup>20</sup> (Scheme 2), thus permitting manipulations<sup>7,8</sup> of the product's orthogonal protective group in the presence of an *O*-acetyl-protected GlcNAc residue. The desired  $\beta$ -glycosides **10a,b** were obtained in this reaction in very high yield (1-H: **10a**:  $\delta_{\text{H}}$  4.7,  $J_{1,2}$  8.4; **10b**:  $\delta_{\text{H}}$  4.7,  $J_{1,2}$  8.1); again, transformation into target molecules, compounds **11a,b**, could be readily performed with zinc in acetic anhydride. This result encouraged us to use directly *N*<sup>o</sup>-Fmoc-protected serine and threonine Pfp esters **12a,b**,<sup>21</sup> typical active esters, as glycosyl acceptors; again, with Teoc derivative **3** in the presence of catalytic amounts of TMSOTf (0.01 mol equiv.) the serine  $\beta$ -glycoside **13a** was obtained in 87% yield and the threonine  $\beta$ -glycoside **13b** in 80% yield, respectively (1-H: **13a**:  $\delta_{\text{H}}$  4.67,  $J_{1,2}$  8.2; **13b**:  $\delta_{\text{H}}$  4.72,  $J_{1,2}$  8.1). Their treatment with zinc in acetic anhydride furnished cleanly the target molecules **14a,b** in 83 and 80% yield, respectively.

In conclusion, based on *N*-Teoc-protected trichloroacetimidate **3** as glycosyl donor, which is very readily available

† This paper is deemed to be Part 77 of our series 'Glycosylimidates'. For Part 76, see ref. 13b.



**Scheme 1** Reagents, conditions and yields: i, **4a,b**, TMSOTf, CH<sub>2</sub>Cl<sub>2</sub>, room temp. (R = H, Me: 75%); ii, H<sub>2</sub>N[CH<sub>2</sub>]<sub>2</sub>NH<sub>2</sub>, THF–MeCN–EtOH, 60 °C, 6 h; then Ac<sub>2</sub>O, pyridine (65%); iii, **4a,b**, NIS, TfOH, CH<sub>2</sub>Cl<sub>2</sub> (R = H, Me: 50%); iv, NaOMe, MeOH, Ac<sub>2</sub>O, pyridine (89%); v, **4a,b**, TMSOTf, Et<sub>2</sub>O, room temp. (R = H, Me: 80%); vi, Zn, Ac<sub>2</sub>O (R = H, Me: 85%)



**Scheme 2** Reagents, conditions and yields: i, **9a,b**, TMSOTf, CH<sub>2</sub>Cl<sub>2</sub>, room temp. (R = H, Me: 90%); ii, Zn, Ac<sub>2</sub>O, room temp., 6 h (R = H, Me: 85%); iii, **12a,b**, TMSOTf, CH<sub>2</sub>Cl<sub>2</sub>, room temp. (R = H: 87%; R = Me: 80%); iv, as for ii (R = H: 83%; R = Me: 80%)

from glucosamine,<sup>15</sup> β-linked *O*-GlcNAc serine and threonine derivatives can be efficiently obtained. Most importantly, the corresponding *N*<sup>F</sup>-Fmoc-protected Pfp active esters, which can be successfully employed in solid-phase glycopeptide synthesis,<sup>4,5,22</sup> are also directly and efficiently accessible *via* this approach.

## Experimental

### General procedures

<sup>1</sup>H NMR spectra were measured with a Bruker AC 250 MHz or a Bruker Avance DRX 600 MHz spectrometer, with tetramethylsilane as internal standard for solutions in deuteriochloro-

form, unless otherwise stated. *J*-Values are given in Hz. <sup>13</sup>C NMR spectra were taken on a Bruker AC (62.68 MHz) spectrometer with <sup>13</sup>CDCl<sub>3</sub> as internal standard ( $\delta_c$  77.0) for solutions in deuteriochloroform. For all compounds the assignment of <sup>1</sup>H NMR spectra was based on chemical-shift correlation (COSY) spectra. The assignment of <sup>13</sup>C NMR spectra were based on carbon–proton shift-correlation spectra (HMQC). Mass spectra were measured with a Kratos Compact MALDI 1 V5.2.0. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter, and [ $\alpha$ ]<sub>D</sub>-values are given in 10<sup>-1</sup> deg cm<sup>2</sup> g<sup>-1</sup>. TLC was performed on Merck silica gel 60 F<sub>254</sub> with detection by charring in sulfuric acid and by UV light when applicable. The silica gel used in column chromatography was Merck 60A, 230–400 mesh. The silica gel used for purification of Pfp esters was dried for at least 6 h at 200 °C prior to use, and ethyl acetate for the same purpose was dried over 3 Å molecular sieves for 24 h prior to use. Dichloromethane was distilled from CaH<sub>2</sub> and was stored over molecular sieves 3 Å under argon. Concentration were performed under reduced pressure at temperatures <40 °C. Mps were measured on a Gallenkamp melting point apparatus and uncorrected. Light petroleum refers to the fraction with distillation range 35–65 °C.

***N*<sup>n</sup>-(*tert*-Butoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido- $\beta$ -D-glucopyranosyl)-L-serine benzyl ester 5a**

To a mixture of compounds **1** (0.71 g, 1.0 mmol) and **4a** (0.29 g, 1.0 mmol) in dry dichloromethane (10 ml) was added dropwise a catalytic amount of TMSOTf (1.66  $\mu$ l, 0.01 mol equiv., diluted with 1 ml of dichloromethane). The mixture was left at room temperature for 1 h under N<sub>2</sub>. It was then neutralized with triethylamine and concentrated under reduced pressure. The thick syrupy residue was purified by flash chromatography [ethyl acetate–light petroleum (3:7)] to afford the title compound **5a** (0.63 g, 75%); [ $\alpha$ ]<sub>D</sub> +22.5 (*c* 0.84, CHCl<sub>3</sub>);  $\delta_H$ (250 MHz; CDCl<sub>3</sub>) 1.35 (9 H, s, CMe<sub>3</sub> of Boc), 1.87, 2.0 and 2.07 (total 9 H, 3 s, 3  $\times$  OAc), 3.69 (1 H, ddd, *J*<sub>5,6</sub> 2.4, *J*<sub>5,6'</sub> 4.7, *J*<sub>5,4</sub> 9.6, 5-H), 3.89 (1 H, dd, *J* <sub>$\beta,\alpha$</sub>  2.8, *J* <sub>$\beta,\beta'$</sub>  10.2,  $\beta$ -H), 4.08–4.13 (2 H, m,  $\beta$ -H' and 6-H), 4.22 (1 H, dd, *J*<sub>2,1</sub> 8.4, *J*<sub>2,3</sub> 10.1, 2-H), 4.26 (1 H, dd, *J*<sub>6,5'</sub> 4.7, *J*<sub>6,6</sub> 12.4, 6-H'), 4.3 (1 H, m,  $\alpha$ -H), 5.0 (1 H, ABd, *J* 12.3, PhCH), 5.04 (1 H, ABd, *J* 12.6, PhCH), 5.1 (1 H, d, *J* 7.5, NH), 5.12 (1 H, dd, *J*<sub>4,5</sub> 9.6, *J*<sub>4,3</sub> 9.9, 4-H), 5.34 (1 H, d, *J*<sub>1,2</sub> 8.4, 1-H), 5.66 (1 H, dd, *J*<sub>3,2</sub> 10.1, *J*<sub>3,4</sub> 9.9, 3-H) and 7.25–7.34 (5 H, m, ArH); MALDI-MS of C<sub>35</sub>H<sub>36</sub>Cl<sub>4</sub>N<sub>2</sub>O<sub>14</sub> (M, 850) *m/z* 889 (M + K)<sup>+</sup> and 873 (M + Na)<sup>+</sup>.

***N*<sup>n</sup>-(*tert*-Butoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido- $\beta$ -D-glucopyranosyl)-L-threonine benzyl ester 5b**

To a clear solution of compounds **1** (0.71 g, 1.0 mmol) and **4b** (0.31 g, 1.0 mmol) in dry dichloromethane (10 ml) was added dropwise TMSOTf (1.66  $\mu$ l, 0.01 mol equiv., diluted with 1 ml of dichloromethane). After 1 h of stirring at room temperature the mixture was neutralized with triethylamine and evaporated under reduced pressure. The residue was purified by flash chromatography [ethyl acetate–light petroleum (3:7)] to afford the title compound **5b** (0.62 g, 72%); [ $\alpha$ ]<sub>D</sub> +7.2 (*c* 0.66, CHCl<sub>3</sub>);  $\delta_H$ (250 MHz; CDCl<sub>3</sub>) 1.04 (3 H, d, *J* 6.4,  $\gamma$ -H<sub>3</sub>), 1.38 (9 H, s, CMe<sub>3</sub> of Boc), 1.87, 1.99 and 2.02 (total 9 H, 3 s, 3  $\times$  OAc), 3.58 (1 H, ddd, *J*<sub>5,6</sub> 2.4, *J*<sub>5,6'</sub> 4.7, *J*<sub>4,5</sub> 9.6, 5-H), 4.0 (1 H, dd, *J*<sub>6,5</sub> 2.4, *J*<sub>6,6'</sub> 12.4, 6-H), 4.15–4.3 (3 H, m, 2-,  $\alpha$ - and 6-H'), 4.36 (1 H, m,  $\beta$ -H), 4.98 (1 H, ABd, *J* 12.3, PhCH), 5.01 (1 H, ABd, *J* 12.6, PhCH), 5.09 (1 H, dd, *J*<sub>4,5</sub> 9.6, *J*<sub>4,3</sub> 9.9, 4-H), 5.2 (1 H, d, *J* 7.6, NH), 5.3 (1 H, d, *J*<sub>1,2</sub> 8.3, 1-H), 5.56 (1 H, dd, *J*<sub>3,2</sub> 10.1, *J*<sub>3,4</sub> 9.9, 3-H) and 7.28–7.35 (5 H, m, ArH); MALDI-MS of C<sub>36</sub>H<sub>38</sub>Cl<sub>4</sub>N<sub>2</sub>O<sub>14</sub> (M, 864) *m/z* 887 (M + Na)<sup>+</sup>.

***N*<sup>n</sup>-(*tert*-Butoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-diacetamido- $\beta$ -D-glucopyranosyl)-L-serine benzyl ester 6a**

To a mixture of compounds **2** (0.58 g, 1.5 mmol), **4a** (0.44 g, 1.5

mmol) and NIS (0.5 g, 1.8 mmol) in dry dichloromethane (15 ml) was added dropwise TfOH (1.5  $\mu$ l, diluted with 1 ml of dichloromethane) at 0 °C. The stirred mixture was left at room temperature for 30 min. It was then diluted with dichloromethane (20 ml) and washed with saturated aq. sodium hydrogen carbonate, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure. The crude product was purified by flash chromatography [ethyl acetate–light petroleum (1:3)] to give compound **6a** (0.5 g, 50%); [ $\alpha$ ]<sub>D</sub> -1.8 (*c* 0.5, CHCl<sub>3</sub>);  $\delta_H$ (250 MHz; CDCl<sub>3</sub>) 1.42 (9 H, s, Boc), 1.95, 2.0, 2.06, 2.08 and 2.35 (total 15 H, 5 s, 2  $\times$  NAc, 3  $\times$  OAc), 3.53 (1 H, dd, *J*<sub>2,3</sub> 9.6, *J*<sub>2,1</sub> 7.8, 2-H), 3.72 (1 H, ddd, *J*<sub>5,4</sub> 9.5, *J*<sub>5,6</sub> 4.7, *J*<sub>5,6'</sub> 2.4, 5-H), 3.75 (1 H, dd, *J*<sub>1</sub> 3.1, *J*<sub>2</sub> 10.4,  $\beta$ -H), 4.06 (1 H, dd, *J*<sub>6,6</sub> 12.2, *J*<sub>6,5'</sub> 2.4, 6-H'), 4.28–4.31 (2 H, m,  $\beta$ -H' and 6-H), 4.4 (1 H, m,  $\alpha$ -H), 5.04 (1 H, dd, *J*<sub>1</sub> = *J*<sub>2</sub> = 9.5, 4-H), 5.12 (1 H, ABd, *J* 12.3, PhCH), 5.19 (1 H, ABd, *J* 12.5, PhCH), 5.2 (1 H, d, *J* 7.6, NH), 5.32 (1 H, d, *J* 7.8, 1-H), 5.78 (1 H, dd, *J*<sub>3,2</sub> 9.6, *J*<sub>3,4</sub> 9.5, 3-H) and 7.34 (5 H, m, ArH); MALDI-MS of C<sub>31</sub>H<sub>42</sub>N<sub>2</sub>O<sub>14</sub> (M, 666) *m/z* 706 (M + K)<sup>+</sup> and 691 (M + Na)<sup>+</sup>.

***N*<sup>n</sup>-(*tert*-Butoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-diacetamido- $\beta$ -D-glucopyranosyl)-L-threonine benzyl ester 6b**

To a mixture of compounds **2** (0.58 g, 1.5 mmol), **4b** (0.46 g, 1.5 mmol) and NIS (0.5 g, 1.8 mmol) in dry dichloromethane (15 ml) was added TfOH (1.5  $\mu$ l, diluted with 1 ml of dichloromethane) at 0 °C. After being stirred at room temperature for 30 min, the mixture was subjected to the usual work-up and purification [ethyl acetate–light petroleum (1:3)] to give title compound **6b** (0.5 g, 47%); [ $\alpha$ ]<sub>D</sub> -6.7 (*c* 0.5, CHCl<sub>3</sub>);  $\delta_H$ (250 MHz; CDCl<sub>3</sub>) 1.05 (3 H, d, *J* 6.4,  $\gamma$ -H<sub>3</sub>), 1.4 (9 H, s, Boc), 1.94, 2.0, 2.07, 2.08 and 2.34 (total 15 H, 5 s, 2  $\times$  NAc, 3  $\times$  OAc), 3.5 (1 H, dd, *J*<sub>2,3</sub> 9.6, *J*<sub>2,1</sub> 7.7, 2-H), 3.7 (1 H, ddd, *J*<sub>5,4</sub> 9.5, *J*<sub>5,6</sub> 2.4, *J*<sub>5,6'</sub> 4.7, 5-H), 3.97 (1 H, dd, *J*<sub>6,5</sub> 2.4, *J*<sub>6,6'</sub> 12.2, 6-H), 4.16 (1 H, dd, *J*<sub>6,5'</sub> 4.7, *J*<sub>6,6</sub> 12.2, 6-H'), 4.29 (1 H, m,  $\alpha$ -H), 4.36 (1 H, m,  $\beta$ -H), 5.04 (1 H, dd, *J*<sub>1</sub> = *J*<sub>2</sub> = 9.5, 4-H), 5.12 (1 H, ABd, *J* 12.3, PhCH), 5.18 (1 H, ABd, *J* 12.6, PhCH), 5.2 (1 H, d, *J* 7.6, NH), 5.3 (1 H, d, *J*<sub>1,2</sub> 7.7, 1-H), 5.75 (1 H, dd, *J*<sub>3,4</sub> 9.5, *J*<sub>3,2</sub> 9.6, 3-H) and 7.3 (5 H, m, aromatic); MALDI-MS of C<sub>32</sub>H<sub>44</sub>N<sub>2</sub>O<sub>14</sub> (M, 680) *m/z* 701 (M + Na)<sup>+</sup>.

***N*<sup>n</sup>-(*tert*-Butoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)- $\beta$ -D-glucopyranosyl)-L-serine benzyl ester 7a**

To a mixture of compounds **3** (1.25 g, 2.0 mmol) and **4a** (0.59 g, 2.0 mmol) in dry dichloromethane (15 ml) was added dropwise TMSOTf (3.3  $\mu$ l, diluted with 1 ml of dichloromethane). After 1 h of stirring at room temperature the mixture was neutralized with triethylamine and evaporated to dryness. The residue was purified by flash chromatography [ethyl acetate–light petroleum (1:3)] to afford title compound **7a** (1.21 g, 80%); [ $\alpha$ ]<sub>D</sub> -5.6 (*c* 0.95, CHCl<sub>3</sub>);  $\delta_H$ (250 MHz; CDCl<sub>3</sub>) 1.42 (9 H, s, Boc), 1.97 (6 H, s, 2  $\times$  OAc), 2.0 (3 H, s, OAc), 3.5 (1 H, m, 2-H), 3.6 (1 H, m, 5-H), 3.79 (1 H, dd, *J* <sub>$\beta,\alpha$</sub>  2.8, *J* <sub>$\beta,\beta'$</sub>  10.2,  $\beta$ -H), 4.06 (1 H, dd, *J*<sub>6,5</sub> 2.4, *J*<sub>6,6'</sub> 12.4, 6-H), 4.22 (1 H, dd, *J*<sub>6,5'</sub> 4.7, *J*<sub>6,6</sub> 12.4, 6-H'), 4.28 (1 H, dd, *J* <sub>$\beta,\alpha$</sub>  3.5, *J* <sub>$\beta,\beta'$</sub>  10.2,  $\beta$ -H'), 4.45 (1 H, m,  $\alpha$ -H), 4.61 (1 H, d, *J*<sub>1,2</sub> 8.3, 1-H), 4.64 and 4.77 (2 H, ABd, *J*<sub>1</sub> = *J*<sub>2</sub> = 12.0, CH<sub>2</sub> of Teoc), 5.0 (1 H, dd, *J*<sub>4,5</sub> 9.6, *J*<sub>4,3</sub> 9.9, 4-H), 5.12 (1 H, d, *J* 7.5, NH), 5.12 (1 H, ABd, *J* 12.3, PhCH), 5.18 (1 H, ABd, *J* 12.6, PhCH), 5.22 (1 H, dd, *J*<sub>3,4</sub> 9.9, *J*<sub>3,2</sub> 10.2, 3-H), 5.4 (1 H, d, *J* 6.45, NH) and 7.3–7.35 (5 H, m, ArH); MALDI-MS of C<sub>30</sub>Cl<sub>3</sub>H<sub>39</sub>N<sub>2</sub>O<sub>14</sub> (M, 757) *m/z* 779 (M + Na)<sup>+</sup>.

***N*<sup>n</sup>-(*tert*-Butoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)- $\beta$ -D-glucopyranosyl)-L-threonine benzyl ester 7b**

To a clear solution of compounds **3** (1.25 g, 2.0 mmol) and **4b** (0.62 g, 2.0 mmol) in dry dichloromethane (15 ml) was added dropwise TMSOTf (3.3  $\mu$ l, 0.01 mol equiv., diluted with 1 ml of dichloromethane) at room temperature. The mixture was stirred at the same temperature for 1 h, neutralized with triethylamine,

and concentrated under reduced pressure. The crude product was purified by flash chromatography [ethyl acetate–light petroleum (1 : 3)] to give title compound **7b** (1.17 g, 76%),  $[a]_D -10.0$  (*c* 0.86, CHCl<sub>3</sub>);  $\delta_H$ (250 MHz; CDCl<sub>3</sub>) 1.17 (3 H, d,  $J_{6,3}$ ,  $\gamma$ -H<sub>3</sub>), 1.42 (9 H, s, Boc), 1.99 (3 H, s, OAc), 2.01 (6 H, s, 2 × OAc), 3.42–3.45 (2 H, m, 2- and 5-H), 3.99 (1 H, dd,  $J_{6,5}$  2.4,  $J_{6,6}$  12.4, 6-H), 4.17 (1 H, dd,  $J_{6,5}$  4.7,  $J_{6,6}$  12.4, 6-H'), 4.32 (1 H, dd,  $J_{\alpha,\beta}$  2.2,  $J_{\alpha,\text{NH}}$  9.2,  $\alpha$ -H), 4.43 (1 H, m,  $\beta$ -H), 4.53 (1 H, d,  $J_{1,2}$  8.2, 1-H), 4.7 (2 H, ABd, CH<sub>2</sub> of Teoc), 4.97 (1 H, dd,  $J_{4,5}$  9.6,  $J_{4,3}$  9.7, 4-H), 5.03 (1 H, d,  $J_{7,6}$ , NH), 5.11–5.2 (3 H, m, 3-H, CH<sub>2</sub>Ph), 5.34 (1 H, d,  $J_{9,2}$ , NH) and 7.31–7.35 (5 H, m, ArH); MALDI-MS of C<sub>31</sub>Cl<sub>3</sub>H<sub>41</sub>N<sub>2</sub>O<sub>14</sub> (M, 771) *m/z* 793 (M + Na)<sup>+</sup>.

**N<sup>w</sup>-(tert-Butoxycarbonyl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl)-L-serine benzyl ester **8a****

Compound **5a** (0.42 g, 0.5 mmol) was dissolved in 10 ml of the solvent mixture CH<sub>3</sub>CN–tetrahydrofuran (THF)–EtOH (2 : 1 : 1). To this mixture was added ethylenediamine (60.7  $\mu$ l, 0.9 mmol) and the mixture was heated at 60 °C for 6 h. The excess of reagent and solvent were removed under reduced pressure and the residue was treated with pyridine (5 ml) and acetic anhydride (2 ml) for 6 h at room temperature. The mixture was dissolved in ethyl acetate (20 ml) and washed successively with water and saturated aq. sodium hydrogen carbonate. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by flash chromatography [ethyl acetate–light petroleum (3 : 7)] to afford title compound **8a** (0.2 g, 65%). Compound **8a** was also prepared from substrates **6a** and **7a** as follows: compound **6a** (0.33 g, 0.5 mmol) was dissolved in dry methanol (5 ml). To this solution was added NaOMe (1 M solution in methanol) until the pH reached 8.5. The mixture was stirred for 1 h at room temperature, deionized with IR 120 (H<sup>+</sup>) resin, filtered, and concentrated under reduced pressure. The residue was dissolved in pyridine (5 ml) and acetic anhydride (2 ml) was added dropwise. The mixture was left overnight at room temperature. After the usual work-up and purification it gave title compound **8a** (0.27 g, 89%).

Compound **7a** (0.3 g, 0.4 mmol) was dissolved in acetic anhydride (5 ml). To this solution was added activated zinc (0.15 g). After 6 h of stirring at room temperature, the mixture was filtered through a Celite bed and concentrated under reduced pressure. The crude product was purified by flash chromatography [ethyl acetate–light petroleum (7 : 3)] to afford title compound **8a** (0.21 g, 85%);  $[a]_D -12.0$  (*c* 0.6, CHCl<sub>3</sub>) (Found: C, 55.59; H, 6.32; N, 4.52. C<sub>29</sub>H<sub>40</sub>N<sub>2</sub>O<sub>13</sub> requires C, 55.76; H, 6.45; N, 4.48%);  $\delta_H$ (250 MHz; CDCl<sub>3</sub>) 1.41 (9 H, s, Boc), 1.91 (3 H, s, NAc), 1.99, 2.0 and 2.03 (total 9 H, 3 s, 3 × OAc), 3.6 (1 H, m, 5-H), 3.72 (1 H, m, 2-H), 3.79 (1 H, dd,  $J_{\alpha,\beta}$  3.5,  $J_{\beta,\beta}$  10.4,  $\beta$ -H), 4.06 (1 H, d,  $J_{5,6}$  2.4,  $J_{6,6}$  12.3, 6-H), 4.18–4.25 (2 H, m, 6- and  $\beta$ -H'), 4.45 (1 H, m,  $\alpha$ -H), 4.68 (1 H, d,  $J_{1,2}$  8.3, 1-H), 5.0 (1 H, dd,  $J_{4,3}$  9.5,  $J_{4,5}$  9.8, 4-H), 5.12 (1 H, ABd,  $J_{12,3}$ , PhCH), 5.18 (1 H, ABd,  $J_{12,6}$ , PhCH), 5.24 (1 H, dd,  $J_{3,4}$  9.5,  $J_{3,2}$  10.4, 3-H), 5.43 (1 H, d,  $J_{7,8}$ , NH), 5.53 (1 H, d,  $J_{8,4}$ , NH) and 7.28–7.36 (5 H, m, ArH);  $\delta_C$ (62.86 MHz; CDCl<sub>3</sub>) 20.58, 20.68 and 21.23 (CH<sub>3</sub>), 28.3 (CH<sub>3</sub> of Boc), 54.01 ( $\alpha$ -C), 54.71 (2-C), 62.01 (6-C), 67.31, 68.44 (4-C), 69.33, 71.91 (3-C), 72.04 (5-C), 80.16 (CMe<sub>3</sub>), 100.8 (1-C), 128.2, 128.37, 128.52, 135.39, 155.4 (NAc), 169.36, 170.4, 170.63 and 170.8 (OAc); MALDI-MS of C<sub>29</sub>H<sub>40</sub>N<sub>2</sub>O<sub>13</sub> (M, 624) *m/z* 647 (M + Na)<sup>+</sup> and 590 (M + Na – Bu)<sup>+</sup>.

**N<sup>w</sup>-(tert-Butoxycarbonyl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl)-L-threonine benzyl ester **8b****

Following the procedure for compound **8a**, the threonine homologue **8b** was prepared from starting materials **5b**, **6b** and **7b** in 62, 86 and 82% yield, respectively;  $[a]_D -13$  (*c* 0.8, CHCl<sub>3</sub>) (Found: C, 56.28; H, 6.7; N, 4.51. C<sub>30</sub>H<sub>42</sub>N<sub>2</sub>O<sub>13</sub> requires C, 56.42; H, 6.63; N, 4.38%);  $\delta_H$ (250 MHz; CDCl<sub>3</sub>) 1.16 (3 H, d,  $J$  6.4,  $\gamma$ -H<sub>3</sub>), 1.40 (9 H, s, Boc), 1.91 (3 H, s, NAc), 1.9, 1.98 and 1.99 (total 9 H, 3 s, 3 × OAc), 3.45 (1 H, m, 5-H), 3.60 (1 H,

ddd,  $J_{1,2} = J_{2,\text{NH}} = 8.4$ ,  $J_{2,3}$  10.6, 2-H), 3.98 (1 H, dd,  $J_{6,5}$  2.4,  $J_{6,6}$  12.2, 6-H), 4.17 (1 H, dd,  $J_{6,5}$  4.7,  $J_{6,6}$  12.2, 6-H'), 4.29 (1 H, dd,  $J_{\alpha,\beta}$  2.6,  $J_{\alpha,\text{NH}}$  9.1,  $\alpha$ -H), 4.36 (1 H, m,  $\beta$ -H), 4.65 (1 H, d,  $J_{1,2}$  8.4, 1-H), 4.96 (1 H, dd,  $J_{4,3}$  9.6,  $J_{4,5}$  9.7, 4-H), 5.31 (2 H, s, CH<sub>2</sub>Ph), 5.24 (1 H, dd,  $J_{3,2}$  10.6,  $J_{3,4}$  9.6, 3-H), 5.38 (1 H,  $J_{\alpha,\text{NH}}$  9.1, NH), 5.5 (1 H, d,  $J_{2,\text{NH}}$  8.4, 2-NH) and 7.33 (5 H, m, ArH);  $\delta_C$ (62.68 MHz; CDCl<sub>3</sub>) 17.16 ( $\gamma$ -C), 20.6, 23.28 and 28.27 (CH<sub>3</sub> of Boc), 55.18 (2-C), 58.24 ( $\alpha$ -C), 61.84 (6-C), 67.04 (CH<sub>2</sub>Ph), 68.37 (4-C), 71.49 (5-C), 71.83 (3-C), 75.04 ( $\beta$ -C), 79.87 (CMe<sub>3</sub>), 98.56 (1-C), 128.2, 128.37 and 128.53 (arom C), 153.54 (NAc) and 169.37, 170.34 and 170.63 (OAc); MALDI-MS of C<sub>30</sub>H<sub>42</sub>N<sub>2</sub>O<sub>13</sub> (M, 638) *m/z* 661 (M + Na)<sup>+</sup> and 638 (M<sup>+</sup>).

**N<sup>w</sup>-(Fluoren-9-ylmethoxycarbonyl)-O-[3,4,6-tri-O-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)- $\beta$ -D-glucopyranosyl]-L-serine allyl ester **10a****

To a mixture of compounds **3** (0.62 g, 1.0 mmol) and **9a** (0.36 g, 1.0 mmol) in dry dichloromethane (10 ml) was added dropwise TMSOTf (1.6  $\mu$ l, diluted with 1 ml of dichloromethane) at room temperature. After 1 h of stirring at the same temperature the solution was neutralized with triethylamine and concentrated to dryness. The residue was purified by flash chromatography [ethyl acetate–light petroleum (3 : 7)] to give title compound **10a** (0.7 g, 90%);  $[a]_D -2.46$  (*c* 1.0, CHCl<sub>3</sub>) (Found: C, 51.93; H, 4.67; N, 3.16. C<sub>36</sub>H<sub>39</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>14</sub> requires C, 52.09; H, 4.73; N, 3.37%);  $\delta_H$ (250 MHz; CDCl<sub>3</sub>) 2.0, 2.01 and 2.05 (total 9 H, 3 s, 3 × OAc), 3.55–3.7 (2 H, m, 2- and 5-H), 3.91 (1 H, dd,  $J_{\beta,\alpha}$  3.0,  $J_{\beta,\beta}$  10.4,  $\beta$ -H), 4.1 (1 H, dd,  $J_{6,5}$  2.4,  $J_{6,6}$  12.4, 6-H), 4.2–4.3 (3 H, m, CH of Fmoc, 6-H',  $\alpha$ -H), 4.4–4.68 (7 H, m, CH<sub>2</sub>O of allyl, CH<sub>2</sub> of Teoc, CH<sub>2</sub> of Fmoc,  $\beta$ -H'), 4.7 (1 H, d,  $J_{8,4}$ , 1-H), 5.02 (1 H, dd,  $J_{4,5}$  9.4,  $J_{4,3}$  9.6, 4-H), 5.2 (1 H, dd,  $J_{3,4}$  9.6,  $J_{3,2}$  10.6, 3-H), 5.3 (3 H, m, CH<sub>2</sub>=CH of allyl, NH), 5.65 (1 H, d,  $J_{8,8}$ , NH), 5.9 (1 H, m, CH of allyl), 7.27–7.41 (4 H, m), 7.61 (2 H, dd,  $J_1 = J_2 = 6.4$ ) and 7.75 (2 H, d,  $J_{7,4}$ ) (together ArH); FAB-MS of C<sub>36</sub>H<sub>39</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>14</sub> (M, 830) *m/z* 1003/1005 (M + NaI + Na)<sup>+</sup> and 853/855 (M + Na)<sup>+</sup>.

**N<sup>w</sup>-(Fluoren-9-ylmethoxycarbonyl)-O-[3,4,6-tri-O-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)- $\beta$ -D-glucopyranosyl]-L-threonine allyl ester **10b****

To a mixture of compounds **3** (0.4 g, 0.75 mmol) and **9b** (0.29 g, 0.75 mmol) in dry dichloromethane (10 ml) was added dropwise TMSOTf (1.2  $\mu$ l, diluted with 1 ml of dichloromethane). The continuously stirred mixture was left for 1 h under N<sub>2</sub> at room temperature. It was then neutralized with triethylamine and evaporated. The residue was purified by flash chromatography [ethyl acetate–light petroleum (3 : 7)] to give title compound **10b** (0.55 g, 87%);  $[a]_D -11.35$  (*c* 0.74, CHCl<sub>3</sub>) (Found: C, 52.48; H, 4.79; N, 3.09. C<sub>37</sub>H<sub>41</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>14</sub> requires C, 52.65; H, 4.89; N, 3.31%);  $\delta_H$ (250 MHz; CDCl<sub>3</sub>) 1.22 (3 H, d,  $J$  6.3,  $\gamma$ -H<sub>3</sub>), 2.01, 2.03 and 2.05 (total 9 H, 3 s, 3 × OAc), 3.55 (1 H, m, 2-H), 3.63 (1 H, m, 5-H), 4.08 (1 H, dd,  $J_{6,5}$  2.4,  $J_{6,6}$  12.4, 6-H), 4.2–4.32 (3 H, m,  $\alpha$ -H, 6-H', CH of Fmoc), 4.35–4.48 (3 H, m,  $\beta$ -H, CH<sub>2</sub> of Fmoc), 4.60–4.72 (4 H, m, CH<sub>2</sub> of Teoc, CH<sub>2</sub>O of allyl), 4.7 (1 H, d,  $J_{1,2}$  8.1, 1-H), 5.04 (1 H, dd,  $J_{4,5}$  9.4,  $J_{4,3}$  9.6, 4-H), 5.15 (1 H, br d, NH), 5.2–5.3 (3 H, m, 3-H, CH<sub>2</sub>=CH of allyl), 5.7 (1 H, d,  $J_{8,8}$ , NH), 5.9 (1 H, m, CH=CH<sub>2</sub> of allyl), 7.27–7.41 (4 H, m), 7.63 (2 H, d,  $J$  6.2) and 7.75 (2 H, d,  $J_{7,2}$ ) (together ArH); FAB-MS of C<sub>37</sub>H<sub>41</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>14</sub> (M, 844) *m/z* 867/869 (M + Na)<sup>+</sup>.

**N<sup>w</sup>-(Fluoren-9-ylmethoxycarbonyl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl)-L-serine allyl ester **11a****

Compound **10a** (0.2 g, 0.25 mmol) was treated with zinc (0.1 g) and acetic anhydride (5 ml) for 6 h at room temperature. After the usual work-up as mentioned earlier, and purification by flash chromatography [ethyl acetate–light petroleum (7 : 3)], title compound **11a** (0.16 g, 85%) was obtained;  $[a]_D -7.1$  (*c* 0.5, CHCl<sub>3</sub>) (Found: C, 60.50; H, 5.67; N, 4.18. C<sub>35</sub>H<sub>40</sub>N<sub>2</sub>O<sub>13</sub> requires C, 60.33; H, 5.78; N, 4.02%);  $\delta_H$ (250 MHz; CDCl<sub>3</sub>) 1.83 (3 H, s, NAc), 2.0, 2.01 and 2.05 (total 9 H, 3 s, 3 × OAc), 3.65

(1 H, m, 2-H), 3.8 (1 H, m, 5-H), 3.85 (1 H, dd,  $J_{\alpha,\beta}$  3.0,  $J_{\beta,\beta'}$  10.4,  $\beta$ -H), 4.1 (1 H, dd,  $J_{6,5}$  2.4,  $J_{6,6'}$  12.4, 6-H), 4.15–4.27 (3 H, m,  $\alpha$ -H, 6-H', CH of Fmoc), 4.35–4.55 (3 H, m,  $\beta$ -H', CH<sub>2</sub> of Fmoc), 4.6–4.7 (3 H, br d, 1-H, CH<sub>2</sub>O of allyl), 5.01 (1 H, dd,  $J_{4,3}$  9.6,  $J_{4,5}$  9.5, 4-H), 5.15–5.4 (3 H, m, 3-H, CH=CH<sub>2</sub> of allyl), 5.5 (1 H, d,  $J$  8.3, NH), 5.79 (1 H, d,  $J_{\text{NH},2}$  9.0, 2-NH), 5.9 (1 H, m, CH=CH<sub>2</sub> of allyl), 7.27–7.41 (4 H, m), 7.36 (2 H, d,  $J$  6.2) and 7.76 (2 H, d,  $J$  7.2) (together ArH);  $\delta_{\text{C}}$  (62.68 MHz; CDCl<sub>3</sub>) 20.59, 20.65, 20.68, 23.15 (CH<sub>3</sub> of NAc), 47.22 (CH of Fmoc), 54.24 ( $\alpha$ -C), 54.55 (2-C), 62.01 (6-C), 66.27 (CH<sub>2</sub> of Fmoc), 66.84, 68.47 (4-C), 71.99 (3-C), 72.14 (5-C), 100.82 (1-C), 118.66, 119.98, 125.09, 127.14, 127.76, 131.42, 141.32, 143.69, 143.83 (arom C of Fmoc), 169.26 (NCO), 169.34, 170.51, 170.61 and 170.84 (OCO); FAB-MS of C<sub>35</sub>H<sub>40</sub>N<sub>2</sub>O<sub>13</sub> (M, 696)  $m/z$  735 (M + K)<sup>+</sup> and 719 (M + Na)<sup>+</sup>.

**N<sup>α</sup>-(Fluoren-9-ylmethoxycarbonyl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-L-threonine allyl ester 11b**

Compound **10b** (0.2 g, 0.23 mmol) was treated with zinc (0.1 g) and acetic anhydride (5 ml) for 6 h at room temperature. After the usual work-up and purification *title compound 11b* (0.13 g, 81%) was obtained;  $[\alpha]_{\text{D}} -12.86$  ( $c$  0.51, CHCl<sub>3</sub>) (Found: C, 60.57; H, 6.08; N, 3.71. C<sub>36</sub>H<sub>42</sub>N<sub>2</sub>O<sub>13</sub> requires C, 60.8; H, 5.97; N, 3.94%);  $\delta_{\text{H}}$  (250 MHz; CDCl<sub>3</sub>) 1.22 (3 H, d,  $J$  6.3,  $\gamma$ -H<sub>3</sub>), 1.92 (3 H, s, NAc), 2.00, 2.02 and 2.04 (total 9 H, 3 s, 3 × OAc), 3.62–3.73 (2 H, m, 2- and 5-H), 4.22 (1 H, dd,  $J_{6,5}$  2.4,  $J_{6,6'}$  12.4, 6-H), 4.25–4.28 (2 H, m, 6-H', CH of Fmoc), 4.3–4.45 (4 H, m, CH<sub>2</sub> of Fmoc,  $\beta$ - and  $\alpha$ -H), 4.62 (2 H, br s, CH<sub>2</sub>O of allyl), 4.7 (1 H, d,  $J_{1,2}$  8.2, 1-H), 5.02 (1 H, dd,  $J_{4,5}$  9.5,  $J_{4,3}$  9.6, 4-H), 5.23 (1 H, dd,  $J_{3,2}$  10.4,  $J_{3,4}$  9.6, 3-H), 5.29 (2 H, m, CH<sub>2</sub>=CH of allyl), 5.54 (1 H, d,  $J$  8.3, NH), 5.75 (1 H, d,  $J_{2,\text{NH}}$  9.0, 2-NH), 5.9 (1 H, m, CH=CH<sub>2</sub> of allyl), 7.26–7.41 (4 H, m), 7.61–7.65 (2 H, m) and 7.74 (2 H, d,  $J$  7.2) (together ArH);  $\delta_{\text{C}}$  (62.68 MHz; CDCl<sub>3</sub>) 16.84 ( $\gamma$ -C), 20.6, 20.67, 23.2 (CH<sub>3</sub> of NAc), 47.2 (CH of Fmoc), 55.26 (2-C), 58.24 ( $\alpha$ -C), 61.96 (6-C), 66.06 (CH<sub>2</sub> of Fmoc), 67.2, 68.51 (4-C), 71.72 (3-C), 71.9 (5-C), 74.27 ( $\beta$ -C), 98.49 (1-C), 118.49, 119.43, 125.24, 127.07, 127.09, 127.69, 131.71 (arom C of Fmoc), 141.29, 143.78, 143.99, 156.81, 169.37, 169.81, 170.27 and 170.62; FAB-MS of C<sub>36</sub>H<sub>42</sub>N<sub>2</sub>O<sub>13</sub> (M, 710)  $m/z$  883 (M + NaI + Na)<sup>+</sup>, 749 (M + K)<sup>+</sup> and 733 (M + Na)<sup>+</sup>.

**N<sup>α</sup>-(Fluoren-9-ylmethoxycarbonyl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-L-serine pentafluorophenyl ester 14a**

To a stirred solution of compounds **3** (1.25 g, 2.0 mmol) and **12a** (1.0 g, 2.0 mmol) in dry dichloromethane (20 ml) at room temperature was added TMSOTf (3.2  $\mu$ l in 1 ml of dichloromethane) dropwise. The mixture was stirred for 1 h at room temperature and then concentrated under reduced pressure to give a thick syrup, which was purified by flash chromatography (anhydrous silica gel) with ethyl acetate–light petroleum (3:7) to afford *compound 13a* (1.66 g, 87%);  $[\alpha]_{\text{D}} -9.06$  ( $c$  1.17, CHCl<sub>3</sub>) (Found: C, 48.78; H, 3.49; N, 3.08. C<sub>39</sub>H<sub>34</sub>Cl<sub>3</sub>F<sub>5</sub>N<sub>2</sub>O<sub>14</sub> requires C, 48.99; H, 3.58; N, 2.93%);  $\delta_{\text{H}}$  (600 MHz; CDCl<sub>3</sub>) 2.02 (9 H, s, 3 × OAc), 3.6 (1 H, ddd,  $J_{2,\text{NH}}$  6.54,  $J_{2,1}$  8.2,  $J_{2,3}$  10.2, 2-H), 3.66 (1 H, ddd,  $J_{6,5}$  2.4,  $J_{5,4}$  9.6, 5-H), 3.97 (1 H, dd,  $J_{\beta,\alpha}$  2.8,  $J_{\beta,\beta'}$  10.2,  $\beta$ -H), 4.12 (1 H, dd,  $J_{6,5}$  2.4,  $J_{6,6'}$  12.3, 6-H), 4.2 (1 H, dd,  $J_{6,5}$  4.7,  $J_{6,6'}$  12.3, 6-H'), 4.23 (1 H, dd,  $J_1$  7.1,  $J_2$  7.6, CH of Fmoc), 4.35 (1 H, dd,  $J_1$  7.1,  $J_2$  10.1, CHH of Fmoc), 4.42 (1 H, dd,  $J_{\beta,\alpha}$  3.5,  $J_{\beta,\beta'}$   $\beta$ -H'), 4.52 (1 H, dd,  $J_1$  7.6,  $J_2$  10.1, CHH of Fmoc), 4.57 (1 H, br s, CHH of Teoc), 4.67 (2 H, br d,  $J_{2,1}$  8.2, 1-H and CHH of Teoc), 4.88 (1 H, m,  $\alpha$ -H), 5.06 (1 H, dd,  $J_{4,5}$  9.6,  $J_{4,3}$  9.9, 4-H), 5.2 (1 H, br s, NH), 5.23 (1 H, dd,  $J_{3,4}$  9.9,  $J_{3,2}$  10.2, 3-H), 5.93 (1 H, br s, NH), 7.28–7.31 (2 H, m), 7.36–7.39 (2 H, m), 7.59–7.61 (2 H, m) and 7.74 (2 H, d,  $J$  7.2) (together ArH);  $\delta_{\text{C}}$  (62.68 MHz; CDCl<sub>3</sub>) 20.52, 47.05 (CH of Fmoc), 54.11 ( $\alpha$ -C), 56.12 (2-C), 61.83 (6-C), 67.23 (CH<sub>2</sub> of Fmoc), 68.43 (4-C), 71.63 (3-C), 71.98 (5-C), 100.51 (1-C), 120.0, 127.12, 127.78, 143.52, 143.65 (arom C of Fmoc), 141.26 (Pfp), 154.25, 155.95

(NCO), 166.17, 169.34, 170.56 and 170.67 (OCO); FAB-MS of C<sub>39</sub>H<sub>34</sub>Cl<sub>3</sub>F<sub>5</sub>N<sub>2</sub>O<sub>14</sub> (M, 956)  $m/z$  993/995 (M + K)<sup>+</sup>, 977/979 (M + Na)<sup>+</sup> and 955/957 (M + H)<sup>+</sup>.

Compound **13a** (1.5 g, 1.57 mmol) was treated with zinc (0.75 g) and acetic anhydride (10 ml) at room temperature for 6 h. The mixture was filtered through a Celite bed and washed with dry dichloromethane. The filtrate was evaporated to dryness and the residue was purified by flash chromatography (anhydrous silica gel) using ethyl acetate–light petroleum (7:3) to afford *title compound 14a* (1.07 g, 83%),  $[\alpha]_{\text{D}} -11.07$  ( $c$  1.21, CHCl<sub>3</sub>) (lit.,<sup>4</sup> –10.0); mp 186–187 °C (lit.,<sup>5</sup> 184 °C);  $\delta_{\text{H}}$  (600 MHz; CDCl<sub>3</sub>) 1.87 (3 H, s, NAc), 2.01 and 2.03 (9 H, 2 s, 3 × OAc), 3.68 (1 H, m, 5-H), 3.74 (1 H, ddd,  $J_{2,\text{NH}}$  6.45,  $J_{2,1}$  8.2,  $J_{2,3}$  10.02, 2-H), 3.97 (1 H, dd,  $J_{\beta,\alpha}$  2.78,  $J_{\beta,\beta'}$  10.57,  $\beta$ -H), 4.12 (1 H, dd,  $J_{6,5}$  2.4,  $J_{6,6'}$  12.4, 6-H), 4.2 (1 H, dd,  $J_{6,5}$  4.8,  $J_{6,6'}$  12.4, 6-H'), 4.23 (1 H, dd,  $J_1$  6.97,  $J_2$  7.34, CH of Fmoc), 4.39 (1 H, dd,  $J_{\alpha,\beta}$  4.0,  $J_{\beta,\beta'}$  10.57,  $\beta$ -H'), 4.40 (1 H, dd,  $J_1$  7.6,  $J_2$  10.63, CHH of Fmoc), 4.48 (1 H, dd,  $J_1$  7.13,  $J_2$  10.63, CHH of Fmoc), 4.83 (1 H, d,  $J$  8.2, 1-H), 4.86 (1 H, ddd,  $J_{\alpha,\beta}$  2.8,  $J_{\alpha,\beta'}$  4.0,  $J_{\alpha,\text{NH}}$  7.51,  $\alpha$ -H), 5.05 (1 H, dd,  $J_{4,3}$  =  $J_{4,5}$  = 9.6, 4-H), 5.27 (1 H, dd,  $J_{3,4}$  9.6,  $J_{3,2}$  10.02, 3-H), 5.6 (1 H, d,  $J$  6.45, 2-NH), 6.1 (1 H, d,  $J$  7.27, NH), 7.3 (2 H, m), 7.38 (2 H, m), 7.64 (2 H, dd,  $J_1$  5.3,  $J_2$  7.41) and 7.75 (2 H, d,  $J$  7.27) (together ArH);  $\delta_{\text{C}}$  (62.68; CDCl<sub>3</sub>) 20.58, 20.65, 23.21 (CH<sub>3</sub> of NAc), 47.11 (CH of Fmoc), 54.3 ( $\alpha$ -C), 54.9 (2-C), 61.9 (6-C), 67.22 (CH<sub>2</sub> of Fmoc), 68.25 ( $\beta$ -C), 68.37 (4-C), 71.91 (3-C), 72.12 (5-C), 100.58 (1-C), 119.98, 125.15, 127.77, 141.3, 143.66, 143.72, 156.04, 166.28, 169.35, 170.62, 170.89 and 170.95; FAB-MS of C<sub>38</sub>F<sub>5</sub>H<sub>35</sub>N<sub>2</sub>O<sub>13</sub> (M, 822)  $m/z$  995 (M + NaI + Na)<sup>+</sup>, 861 (M + K)<sup>+</sup>, 845 (M + Na)<sup>+</sup> and 823 (M + H)<sup>+</sup>.

**N<sup>α</sup>-(Fluoren-9-ylmethoxycarbonyl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-L-threonine pentafluorophenyl ester 14b**

To a stirred solution of compounds **3** (0.6 g, 1.0 mmol) and **12b** (0.5 g, 1.0 mmol) in dry dichloromethane (10 ml) was added dropwise TMSOTf (1.6  $\mu$ l in 1 ml of dichloromethane) at room temperature. After 1 h of stirring at room temperature the mixture was concentrated under reduced pressure and the residue was purified by flash chromatography (anhydrous silica gel) using ethyl acetate–light petroleum (3:7) as eluent to afford *compound 13b* (0.77 g, 80%) (Found: C, 49.36; H, 3.69; N, 3.01. C<sub>40</sub>H<sub>36</sub>Cl<sub>3</sub>F<sub>5</sub>N<sub>2</sub>O<sub>14</sub> requires C, 49.52; H, 3.74; N, 2.88%);  $[\alpha]_{\text{D}} -22.2$  ( $c$  1.0, CHCl<sub>3</sub>);  $\delta_{\text{H}}$  (250 MHz; CDCl<sub>3</sub>) 1.30 (3 H, d,  $J$  6.2,  $\gamma$ -H), 1.99, 2.01 and 2.04 (total 9 H, 3 s, 3 × OAc), 3.65 (2 H, m, 2- and 5-H), 4.12 (1 H, dd,  $J_{6,5}$  2.4,  $J_{6,6'}$  12.3, 6-H), 4.16–4.38 (3 H, m, 6-H', CH of Fmoc,  $\beta$ -H), 4.49 (1 H, dd,  $J_1$  7.6,  $J_2$  10.1, CHH of Fmoc), 4.6–4.8 (4 H, m,  $\alpha$ -H, CH<sub>2</sub> of Teoc, CHH of Fmoc), 4.72 (1 H, d,  $J_{1,2}$  8.1, 1-H), 5.06 (1 H, dd,  $J_{4,5}$  9.6,  $J_{4,3}$  9.9, 4-H), 5.23 (1 H, dd,  $J_{3,4}$  9.9,  $J_{3,2}$  10.2, 3-H), 5.37 (1 H, d,  $J$  8.3, NH), 5.96 (1 H, d,  $J$  9.0, NH), 7.26–7.4 (4 H, m), 7.65 (2 H, m) and 7.75 (2 H, d,  $J$  7.2) (together ArH);  $\delta_{\text{C}}$  (62.68; CDCl<sub>3</sub>) 16.28 ( $\gamma$ -C), 20.36, 20.51, 20.58, 47.04 (CH of Fmoc), 56.28 (2-C), 58.60 ( $\alpha$ -C), 61.75 (6-C), 67.56 (CH<sub>2</sub> of Fmoc), 68.37 (4-C), 71.49 (3-C), 71.72 (5-C), 72.77, 74.51 ( $\beta$ -C), 98.02 (1-C), 119.93, 125.13, 127.07, 127.72, 141.20, 141.23 (Pfp), 143.60, 154.27, 156.56 (NCO), 166.40, 169.29, 170.57 and 170.93; FAB MS of C<sub>40</sub>H<sub>36</sub>Cl<sub>3</sub>F<sub>5</sub>N<sub>2</sub>O<sub>14</sub> (M, 970)  $m/z$  991/993 (M + Na)<sup>+</sup>.

Compound **13b** (0.51 g, 0.51 mmol) was treated with activated zinc (0.25 g) and acetic anhydride (2 ml) at room temperature for 6 h. After the usual work-up and purification using anhydrous silica gel column chromatography [ethyl acetate–light petroleum (7:3)] *compound 14b* (0.34 g, 80%) was obtained,  $\delta_{\text{H}}$  (250 MHz; CDCl<sub>3</sub>) 1.28 (3 H, d,  $J$  6.3,  $\gamma$ -H<sub>3</sub>), 1.93 (3 H, s, NAc), 1.98, 2.01 and 2.03 (total 9 H, 3 s, 3 × OAc), 3.65 (1 H, ddd,  $J_{5,6}$  2.3,  $J_{5,6'}$  4.7,  $J_{5,4}$  9.7, 5-H), 3.75 (1 H, ddd,  $J_{2,\text{NH}}$  8.04,  $J_{2,1}$  8.2,  $J_{2,3}$  10.2, 2-H), 4.05 (1 H, dd,  $J_{5,6}$  2.3,  $J_{6,6'}$  12.2, 6-H), 4.2 (1 H, dd,  $J_{6,5}$  4.7,  $J_{6,6'}$  12.2, 6-H'), 4.24 (1 H, dd,  $J_1$  7.1,  $J_2$  7.4, CH of Fmoc), 4.35–4.5 (2 H, m, CH<sub>2</sub> of Fmoc), 4.56 (1

H, m,  $\beta$ -H), 4.67 (1 H, dd,  $J_{\alpha,\beta}$  2.8,  $J_{\alpha,\text{NH}}$  9.0,  $\alpha$ -H), 4.72 (1 H, d,  $J$  8.2, 1-H), 5.05 (1 H, dd,  $J_{4,3}$  9.5,  $J_{4,5}$  9.7, 4-H), 5.25 (1 H, dd,  $J_{3,4}$  9.5,  $J_{3,2}$  10.2, 3-H), 5.6 (1 H, d,  $J$  8.04, 2-NH), 6.03 (1 H, d,  $J$  9.0, NH), 7.25–7.4 (4 H, m), 7.63 (2 H, dd,  $J_1$  5.13,  $J_2$  7.41) and 7.75 (2 H, d,  $J$  7.27) (together ArH);  $\delta_{\text{C}}$  (62.68 MHz; CDCl<sub>3</sub>) 16.51 ( $\gamma$ -C), 20.43, 20.59, 23.39 (CH<sub>3</sub> of NAc), 47.17 (CH of Fmoc), 55.22 (2-C), 58.71 ( $\alpha$ -C), 61.89 (6-C), 67.38 (CH<sub>2</sub> of Fmoc), 68.42 (4-C), 71.82 (5-C), 73.19 (3-C), 98.2 (1-C), 119.95, 125.19, 127.06, 127.11, 127.73, 128.21, 141.31 (Pfp), 143.64, 156.64 (NCO), 166.48, 169.26, 170.39, 170.6 and 171.15; FAB MS  $m/z$  875 (M + K)<sup>+</sup>, 859 (M + Na)<sup>+</sup> and 837 (M + H)<sup>+</sup>. All other physical data of products **14a** and **14b** were identical with literature<sup>5,6</sup> values.

### Acknowledgements

This work was supported by the Alexander von Humboldt-Stiftung, the Deutsche Forschungsgemeinschaft, and the Fonds der Chemischen Industrie. Helpful discussions with J. C. Castro-Palomino, W. Dullenkopf and J. Rademann are gratefully acknowledged. U. K. S. is grateful for an AvH-Fellowship.

### References

- C. R. Torres and G. W. Hart, *J. Biol. Chem.*, 1984, **259**, 3308; G. W. Hart, R. S. Haltiwanger, G. D. Holz and W. G. Kelly, *Annu. Rev. Biochem.*, 1989, **58**, 841.
- L. S. Griffith, M. Mathes and B. Schmitz, *J. Neurosci. Res.*, 1995, **41**, 270, and references therein.
- R. S. Haltiwanger, W. G. Kelly, E. P. Roquemore, M. A. Blomberg, L.-Y. Dong, L. Kreppel, T.-Y. Chou and G. W. Hart, *Biochem. Soc. Trans.*, 1992, **20**, 264.
- A. Vargas-Berenguel, M. Meldal, H. Paulsen and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1994, 2615; E. Meinjohanns, M. Meldal, H. Paulsen and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1995, 405; E. Meinjohanns, A. Vargas-Berenguel, M. Meldal, H. Paulsen and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1995, 2165.
- K. J. Jensen, P. R. Hansen, D. Venugopal and G. Barany, *J. Am. Chem. Soc.*, 1996, **118**, 3148.
- U. Ellervik and G. Magnusson, *Carbohydr. Res.*, 1996, **280**, 251; L. Szabo, J. Ramza, C. Langdon and R. Polt, *Carbohydr. Res.*, 1995, **274**, 11.
- H. Paulsen, *Angew. Chem.*, 1990, **102**, 851; *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 823.
- H. G. Garg, K. von dem Bruch and H. Kunz, *Adv. Carbohydr. Chem. Biochem.*, 1994, **50**, 277; H. Kunz, *Angew. Chem.*, 1987, **99**, 297; *Angew. Chem., Int. Ed. Engl.*, 1987, **26**, 294.
- M. Meldal and K. J. Jensen, *J. Chem. Soc., Chem. Commun.*, 1990, 483; M. Meldal and K. Bock, *Tetrahedron Lett.*, 1990, **31**, 6987; A. M. Jansson, M. Meldal and K. Bock, *Tetrahedron Lett.*, 1990, **31**, 6991; S. Peters, T. Bielfeldt, M. Meldal, K. Bock and H. Paulsen, *Tetrahedron Lett.*, 1992, **33**, 6445; K. J. Jensen, M. Meldal and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1993, 2119.
- J. Banoub, P. Boullanger and D. Lafont, *Chem. Soc. Rev.*, 1992, **92**, 1167.
- M. Schultz and H. Kunz, *Tetrahedron Lett.*, 1992, **33**, 5319; *Tetrahedron: Asymmetry*, 1993, **4**, 1205.
- H. Paulsen and J.-P. Hölck, *Liebigs Ann. Chem.*, 1982, 1121; H. Paulsen, M. Paal and M. Schultz, *Tetrahedron Lett.*, 1983, **24**, 1759.
- For the synthesis of compound **1**, see J. C. Castro-Palomino and R. R. Schmidt, (a) *Tetrahedron Lett.*, 1995, **36**, 5343; (b) *Liebigs Ann. Chem.*, 1996, 1623; (c) the N-TCP group was independently investigated for glucosamine with the pent-4-en-1-yl group at the anomeric oxygen as leaving group; see ref. 17.
- For the synthesis of compound **2**, see J. C. Castro-Palomino and R. R. Schmidt, *Tetrahedron Lett.*, 1995, **36**, 6871.
- For the synthesis of compound **3**, see (a) H. Paulsen and B. Helpap, *Carbohydr. Res.*, 1991, **216**, 289; (b) W. Dullenkopf, J. C. Castro-Palomino, L. Manzoni and R. R. Schmidt, *Carbohydr. Res.*, 1996, **296**, 135.
- Compounds **4a,b** are commercially available.
- J. S. Debenham, R. Madsen, C. Roberts and B. Fraser-Reid, *J. Am. Chem. Soc.*, 1995, **117**, 3302.
- Instead of Teoc the abbreviation Troc has also been used; see: T. B. Windholz and D. B. R. Johnston, *Tetrahedron Lett.*, 1967, 2555, and references therein.
- For the use of *N*-Teoc in a glycosyl donor, see: S. Kusumoto, H. Yoshimura, M. Imoto, T. Shimamoto and T. Shiba, *Tetrahedron Lett.*, 1985, **26**, 909.
- M. Ciommer and H. Kunz, *Synlett*, 1991, 593.
- I. Schön and L. Kisfaludy, *Synthesis*, 1986, 303.
- U. K. Saha and R. R. Schmidt, unpublished work.

Paper 7/00210F

Received 8th January 1997

Accepted 20th February 1997