Building Blocks for Solid-phase Glycopeptide Synthesis: 2-Acetamido-2-deoxy-β-D-glycosides of FmocSerOH and FmocThrOH

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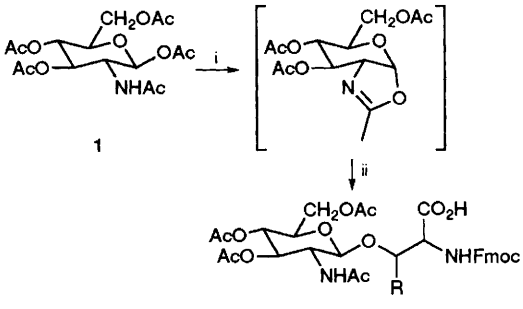
A convenient and optimized synthesis of 2-acetamido-2-deoxy-β-D-glycosides of FmocSerOH and FmocThrOH, building blocks for solid-phase synthesis of glycopeptides containing GlcNAc β-linked to Ser or Thr, is for the first time established.

2-Acetamido-2-deoxy-β-D-glycosides widely exist as important fragments of peptidoglycans, glycoproteins, mucopolysaccharides, and blood group determinants. Recently, a major new form[1] of protein glycosylation (termed *O*-GlcNAc) that is found in all eukaryotes has been described. *O*-GlcNAc is a simple, unmodified monosaccharide moiety β-glycosidically linked to the side-chain hydroxyls of serine or threonine. The natural occurrence of such *N*-acetylglucosamine glycosides has stimulated efforts towards the development of glycosylation reactions using D-glucosamine derivatives.[2]

Few routes have been found for the synthesis of 2-acetamido-2-deoxy-β-D-glycosides of Ser or Thr having a β conformation. Traditional Koenigs–Knorr[3] type reaction is one of the desired processes for the synthesis of glucosamine glycosides. In these cases, 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl halides (Cl, F) are always used as glycosyl donors[4] and both amino and carboxy protected Ser and Thr as glycosyl acceptors. However, problems associated with Koenigs–Knorr reactions are instability of the glycosyl halides, poor yields, synthesis of mixtures of α and β anomers, difficult deprotection of the amino function, toxic character of the promoters, and the necessity of several steps to obtain the desired glycosides.

Another major process for the synthesis of glucosamine glycosides is the acid catalysed reaction of oxazoline derivatives.[5] The oxazoline procedure, an extension of Koenigs–Knorr glycosylation method, and its various improvements, allowed the successful synthesis of 1,2-*trans*-2-acetamido-2-deoxy glycosides and oligosaccharides. The most commonly used sugar oxazolines[6] are the methyl oxazolines. These are reactive intermediates upon activation by an acid[7] or Lewis acids[8] will allow nucleophilic attack by a glycosyl acceptor to afford anomerically pure β-glycosides possessing the natural *N*-acetyl function, which is an extremely useful feature.

With the aim to establish a new reliable procedure for the synthesis of suitable 2-acetamido-2-deoxy-glycosides of amino acids for solid-phase peptide synthesis (SPPS), the latter approach was optimized for the 'one pot' synthesis of the two building blocks **2** and **3**, respectively (Scheme 1). The present pathway makes use of three better and convenient features. First the use of N^{α}-Fmoc[9] for the protection of the amino function eliminates the requirement of acidic conditions, to which the *O*-glycosidic linkage is sensitive, for deprotection. N^{α}-Fmoc, unlike Boc or Z groups, is labile to mild organic bases[10] such as morpholine or piperidine without β-elimination. Second the *O*-acetyl groups for the hydroxy groups of the carbohydrate moiety can be easily removed with sodium methoxide in methanol.[11] Third the α-carboxyl group of the amino acid remains free as in Eloffson's approach.[12]



2 FmocSer(β-D-pGlcNAc)OH
3 FmocThr(β-D-pGlcNAc)OH

Scheme 1 *Reagents and conditions*: i, $BF_3{\cdot}Et_2O$ (3 equiv.), CH_2Cl_2, 0 °C, then Et_3N (1 equiv.); ii, FmocSerOH (R = H) or FmocThrOH (R = Me), (1 equiv.), CH_2Cl_2–MeCN

The oxazoline of the readily available 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose **1** is prepared using an excess (3 equiv.) of boron trifluoride etherate as Lewis acid promoter. This is the glycosyl donor for the *in situ* glycosylation of the hydroxy groups of the commercially available, FmocSerOH and FmocThrOH.† The β-D-configuration of the glycosidic bond was established from the NMR spectrum‡ by the high value of the coupling constant for the anomeric proton (H-1) of $J_{1,2}$ = 8.4 Hz. No α-linked products were detected. The building block **2** has been successfully employed in the solid-phase peptide synthesis of an *O*-glycosylated nonapeptide fragment analogue of the Sendai virus nucleoprotein$_{324-332}$ wildtype: K3-*O*-GlcNAc[13] [FAPS(*O*-β-GlcNAc)NYPAL].

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Footnotes

† FmocSer (β-D-pGlcNAc)OH **2** was prepared as follows: 240 mg (0.616 mmol) of peracetylated GlcNAc **1** was placed under an argon atmosphere in a flask containing 4 Å molecular sieves and CH_2Cl_2 (4 ml) was added. After cooling to 0 °C, 200 μl (1.91 mmol, 3.1 equiv.) of freshly distilled $BF_3{\cdot}Et_2O$ are added dropwise to the suspension. After overnight stirring at room temperature the formation of the oxazoline was complete as monitored by TLC ($CHCl_3$–MeOH 10:1). Then 80 μl (0.574 mmol) of Et_3N are added dropwise at 0 °C. The reacton mixture was left stirring for 10 min and a solution of 208 mg (0.635 mmol) of FmocSerOH, in a mixture of CH_2Cl_2–acetonitrile (2:1) was added. The reaction mixture was left to stand at room temperature and periodically monitored by TLC ($CHCl_3$–MeOH–AcOH 80:10:1) and RP-HPLC (Merck 50983, Lichrospher 100 RP-18, 5 μm, 70–20% of MeCN–H_2O with 0.1% TFA in 30 min). To further enhance the yield a second batch of oxazoline (240 mg, 0.616 mmol) prepared in the same way as described above may be added after 24 h. When the reaction was complete, (from 48–150 h), the crude was neutralized at 0 °C with Et_3N, diluted with CH_2Cl_2 and filtered through Celite. The filtrate was concentrated and the crude purified by column chromatography (silica gel, $CHCl_3$–MeOH from 30:1 to 10:1). Following purification, FmocSer(β-D-pGlcNAc)OH **2** was obtained in a 55% yield. This reaction has been scaled up to a 1 g (2.5 mmol) of peracetylated GlcNAc. A similar procedure was followed for the synthesis of FmocThr(β-D-pGlcNAc)OH **3** which was obtained in a 53% yield.

‡ *Selected data* FmocSer (β-pGlcNAc)OH **2**: α^{22}_D = +27.8 (*c* = 1, MeOH); 500 MHz ^1H NMR; δ ([$^2H_6$]DMSO = 2.49 at 300 K) 1.74 (s, 3H, NHCOCH_3); 1.92, 1.98, 2.02 (s, 9H, COCH_3); 4.73, 8.4 (d, 1H, H-1). 125.7 MHz ^{13}C NMR; δ ([$^2H_6$]DMSO = 39.5 at 300 K): 20.32, 20.36, 20.45 (*C*H_3–CO); 22.53 (NH–CO*C*H_3); 100.36 (C-1); 155.63 (NH*C*O_2). ESMS: *m/z* 701.3 (M + 2 Na). FmocThr (β-pGlcNAc)OH **3**: α^{22}_D = +14.7 (*c* = 1, MeOH); 500 MHz ^1H NMR; δ ([$^2H_6$]DMSO = 2.49 at 300 K): 1.13, 7.0 (d, 3H, Thr-CH_3); 1.79 (s,

3H, $NHCOCH_3$); 1.94, 1.98, 1.99 (s, 9H, $COCH_3$); 4.78, 8.5 (d, 1H, H-1). 125.7 MHz ^{13}C NMR; δ ($[^2H_6]DMSO$ = 39.5 at 300 K): 17.44 (Thr-CH_3); 98.45 (C-1); 156.01 ($NHCO_2$). ESMS: *m/z* = 693.4 (*M* + 23).

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