Synthesis of a chromogenic proline-containing glycopeptide

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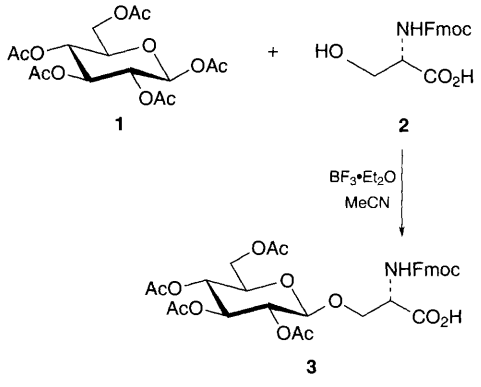
A convenient synthesis of a chromogenic glycopeptide, *O*-(β-D-glucopyranosyl)-Ser-Pro-4-nitroanilide, involving the direct glycosylation of Fmoc-Ser-OH, coupling of L-proline-4-nitroanilide and sequential deprotection without diketopiperazine formation is reported.

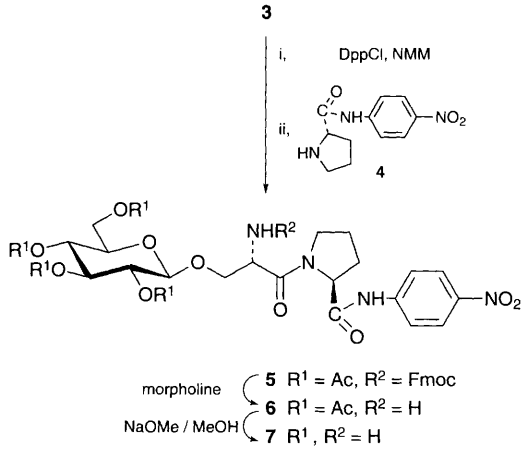
Chromogenic peptides *e.g.* substituted coumarins and β-naphthylamides and 4-nitroanilides (NH-Np) are frequently used to study the properties of hydrolytic enzymes and their effectors and particularly to determine steady state kinetic parameters. Furthermore, glycosylated peptide-4-nitroanilides are suitable for the study of the influence of glycosylation on the elementary structural units responsible for proteins folding. Important biological functions of the carbohydrate units of glycoproteins have been recently discovered[1] and initiated intensive research leading to advances in glycopeptide synthesis.[2] To the best of our knowledge, the synthesis of glycosylated peptide-4-arylamides, in particular 4-nitroanilides, has not been described. Here we report the preparation of a glycopeptide-4-nitroanilide that will be useful to study the influence of *O*-glycosylation on proline-dependent properties of peptides and proteins,[3] especially their susceptibility to protease activity and the conformation of the peptide backbone.

The first step in the preparation of the target compound **7** is the synthesis of the glycosylated acid **3**. Compound **3** represents the carbohydrate-amino acid linkage that has been found to occur in the epidermal growth factor-like domain of the bovine blood clotting factor IX.[4] It is a suitable building block for solution-phase and solid-phase Fmoc-based peptide synthesis.[5] An earlier synthesis of derivative **3**[6] and of the deacetylated analogue[7] required multi-step preparations including the temporary protection of the carboxyl function.

Recently, a one-step glycosylation has been developed by Elofsson *et al.*,[8] involving the Lewis acid-catalysed glycosylation of the carboxyl-unprotected amino acid derivatives such as Fmoc-Ser-OH **2** forming a 1,2-*trans*-*O*-glycosidic bond. This approach has been applied to a disaccharide[9] and to peracetylated GlcNAc.[10] The latter has also been coupled to a resin-bound serine derivative[11] using TMS-triflate[12] as the catalyst. We obtained the glycosylated serine derivative **3** using a gram-scale reaction. Glucose acetate **1** (5 g) and the serine derivative **2** (1 equiv.) were treated with $BF_3{\cdot}Et_3O$ (3 equiv.) in MeCN for 2 h at room temperature (Scheme 1). Workup[8] gave compound **3** (3 g) in a 35% yield. The 1,2-*trans* configuration of the glycosidic linkage was deduced from the ^1H NMR spectrum by the high coupling constant for H-1 ($J_{1,2}$ = 7.9 Hz). TMS-triflate (1.1 equiv.) worked just as well as $BF_3{\cdot}Et_2O$ in the synthesis of acid **3**. Coupling of **3** with L-proline-4-nitro-anilide **4** was performed *via* the mixed anhydride generated with diphenylphosphinic chloride (DppCl) in the presence of *N*-methyl morpholine (NMM) to give the glycosylated peptide **5** in 60% yield. The deprotection of the serine amino group and the deacetylation of the glucose moiety were delicate for two reasons. Firstly, the glycosidic bond is susceptible to strong acids and bases. Secondly, there was the possibility of side reactions at the activated arylamide function in the form of diketopiperazine formation upon removal of the urethane protecting group, especially favoured in the motif H-Xaa-Pro-OR, and methanolysis during the base-catalysed transesterification of the sugar acetates. Initial experiments to remove the amino protecting group were performed with the proline methyl ester analogue of compound **5** by treatment with morpholine.[13] The product, however, underwent intramolecular condensation to form the glycosylated diketopiperazine. In contrast, the removal of the urethane group of derivative **5** with morpholine was not accompanied by a diketopiperazine formation. It should be noted that this deprotection step emphasises the possibile use of Fmoc-based synthetic strategies with peptides bearing the 4-nitroanilide function. As expected,[14] β-elimination of the glycon did not occur. The crude product **6** was deacetylated by Zemplén-transesterification using a 1% sodium methoxide solution in methanol at pH 8.5–9[15] affording the target peptide **7** in 43% yield with respect to **5**. Compound **7** showed a UV absorbance maximum at 320 nm. A comparison of the circular dichroism of the glycosylated peptide **7** and the non-glycosylated peptide, H-Ser-Pro-NH-Np **8**, is shown in Fig. 1. The glycosylated peptide showed a maximum at 220 nm, in contrast to the non-glycosylated peptide, which did not. The structure of the target compound **7**‡ was unambiguously ascertained by ^1H and ^{13}C NMR spectroscopy. The signal due to the anomeric proton (H-1) was observed at δ 4.32. The high value of the coupling constant for H-1 ($J_{1,2}$ = 7.9 Hz) confirmed the



Scheme 1



Scheme 2

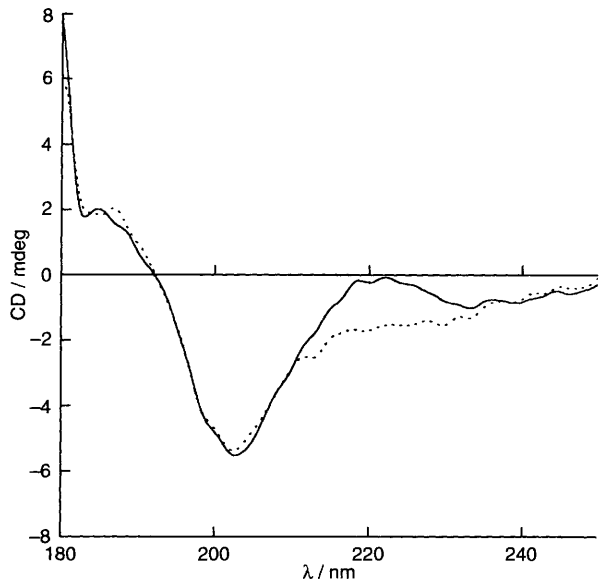


Fig. 1 CD spectrum of H-Ser-Pro-NH-Np **8** (·····) and of the corresponding glycosylated compound **7** (—), recorded using a JASCO J-710 spectropolarimeter at a sample concentration of 150 μmol in 2,2,2-trifluoroethanol at 20 °C; each measurement was the average of 8 repeated scans

1,2-*trans*-glycosidic bond. A singlet for the *N*-bound anilide proton appeared at δ 10.77.

We thank Dr G. Scherer for performing NMR spectroscopy, Dr A. Schierhorn for recording electrospray mass spectra, B. Janowski for CD measurements and A. Kaspari for providing Pro-pNA. This work was supported by a grant of the Deutsche Forschungsgemeinschaft (Fi 455/1-3).

Footnotes

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‡ All compounds obtained gave satisfactory analytical data. *Selected data* for **7**: calcd monoisotopic mass 484.2, ESMS: *m/z* 484.4 $[M + H]^+$, ^1H NMR (COSY; 500 MHz): δ ($[^2H_6]Me_2SO$ = 2.5 at 295 K): 1.92 (m, 2 H, Pro-γ), 2.04 (m, 1 H, Pro-βHa), 2.28 (m, 1 H, Pro-βHb), 3.05 (m, 2 H, H-2, H-6a, $J_{6a,b}$ = 11.4 Hz), 3.19 (m, 2 H, H-3, H-6b), 3.45 (dd, 1 H, H-5), 3.62 (m, 1 H, Pro-δHa), 3.72 (dd, 1 H, H-4), 3.82 (m, 1 H, Pro-δHb), 3.90 (dd, 1 H, Ser-βHa), 3.97 (dd, 1 H, Ser-βHb), 4.32 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1), 4.50 (m, 1 H, Ser-αH), 4.52 (m, 1 H, Pro-αH), 7.83 (m, 2 H, arom), 8.22 (m, 2 H, arom) and 10.77 (s, NH-arom). ^{13}C NMR (125 MHz): δ ($[^2H_6]Me_2SO$ = 39.5 at 295 K): 24.9 (Pro-γ), 29.3 (Ser-α), 47.2 (Pro-δ), 51.8 (Pro-β), 61.0 (2C, Pro-α, C-6), 65.9 (Ser-β), 70.0 (C-4), 73.3 (C-2), 76.2 (C-3), 77.1 (C-5), 102.65 (C-1), 119.0, 125.0 (4C, arom), 142.4, 145.0 (2C, arom), 164.9 and 170.7 (2C, C=O).

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Received, 5th October 1995; Com. 5/06527E