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Building Blocks for Glycopeptide Synthesis: Preparation of α-*O*-Fucosylated Fmoc Serine and Threonine in One Step from L-Fucose Tetraacetate

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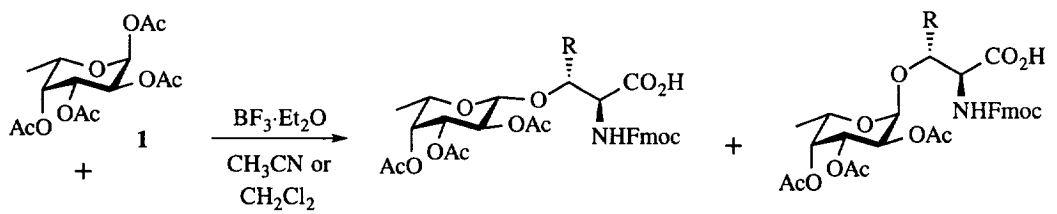
Abstract: Building blocks with *O*-acetylated fucose α-glycosidically linked to serine and threonine have been prepared (in 44 and 35% yields, respectively) by glycosylation of Fmoc-Ser/Thr-OH with L-fucose tetraacetate under boron trifluoride etherate promotion. Initially, the corresponding β-glycosides were formed as products but these rearranged to the more stable α-glycosides under the influence of the promoter. Copyright © 1996 Elsevier Science Ltd

The majority of the proteins found in nature carry structurally diverse *O*- and *N*-linked oligosaccharide moieties.[1] In order to understand the physiochemical and biological functions conferred by the carbohydrate moieties upon glycoproteins substantial efforts have been directed towards the synthesis of glycopeptides.[2,3] At present glycosylation of protected oligopeptides by chemical means does not constitute a feasible route to *O*-linked glycopeptides. Instead, the most general synthetic route to both *O*- and *N*-linked glycopeptides employs glycosylated amino acids for stepwise synthesis, preferably on solid phase. Synthetic routes to glycosylated amino acid building blocks therefore constitute the foundation for synthesis of glycopeptides. In most studies the fluoren-9-ylmethoxycarbonyl (Fmoc) group[4] has been used for α-amino protection of the glycosylated building blocks since it allows use of protective groups for amino acid side chains, and linkers to the solid phase, which are cleaved by trifluoroacetic acid. In general, acetyl protective groups have been employed for the carbohydrate moiety in order to stabilize the *O*-glycosidic linkages during cleavage with

trifluoroacetic acid, even though this may not be an absolute requirement for common monosaccharides such as Glc, Gal, Man, GlcNAc and GalNAc.[5,6]

We have recently described a method for preparation of *O*- and *S*-linked glycosylated amino acids from the readily available carbohydrate peracetates and N^{α}-Fmoc amino acids under Lewis acid promotion.[7,8] The resulting building blocks have a 1,2-*trans* β-anomeric configuration (except for mannosides), carry suitable protective groups, *i.e.* *O*-acetyl and N^{α}-Fmoc groups, and can be used directly in solid phase synthesis without protective group manipulations. However, glycoproteins that occur in nature often have their carbohydrate moieties linked through 1,2-*cis* α-*O*-glycosidic bonds.[1] For example, L-fucose α-glycosidically linked to serine or threonine, has been found in the epidermal growth factor domains of several coagulation and fibrinolytic proteins,[9] and in an insect neuropeptide.[10] Recently, the fucosylated threonine and serine derivatives **4**[11] and **7**[12] were prepared by syntheses in which formation of the desired α-glycosidic linkage was ensured by the use of non-participating benzyl ethers for the hydroxyl groups of the fucosyl donor. Since the fucose moieties of glycopeptides are acid labile, reprotection with *O*-acetyl groups was necessary[12,13] in order to allow use in solid phase synthesis, thereby adding several steps to the synthetic sequences and reducing the overall yields.



2 Fmoc-Thr-OH
5 Fmoc-Ser-OH

3 R = Me (~20%)
6 R = H (14%)

4 R = Me (35% in CH_2Cl_2)
7 R = H (44% in CH_3CN)

We reasoned that glycosylation of Fmoc threonine and serine (**2** and **5**) with L-fucose tetraacetate (**1**) should initially give the β-linked 1,2-*trans* glycosides **3** and **6** under the influence of the participating 2-*O*-acetyl group. However, if sufficient amounts of a Lewis acid was used as promoter, **3** and **6** would then rearrange to the termodynamically more stable α-glycosides **4** and **7**. Support for this hypothesis was provided by a recent report describing the use of 2,3,4-tri-O-benzoyl-α-L-fucopyranosyl bromide in stereoselective, mercury salt promoted α-glycosylations of alcohols having low reactivity.[14] We found that glycosylation of Fmoc threonine (**2**) with L-fucose tetraacetate (**1**)[15] rapidly gave a mixture of the β- and α-glycosides **3** and **4** when boron trifluoride etherate (2.8 equiv. as compared to **1**) was used as promoter in dichloromethane. Normal phase HPLC revealed that the ratio of **3** to **4** changed from ≈3:1 after 4.5 h reaction, to 1:1 after 22 h and 1:2 after 46 h. HPLC also revealed formation of increasing amounts of 3-*O*-acetylated Fmoc threonine, and the reaction was therefore terminated after 50 h. Purification of the crude product by normal phase HPLC then gave the target α-fucoside **4**[11,16] in 35% yield, and slightly impure β-fucoside **3**[16] (~20%). A larger amount of 3-*O*-acetylated Fmoc-threonine was formed when dichloromethane was replaced by acetonitrile as solvent, and consequently a lower yield of **4** (22%) was obtained. Since the synthesis of **4**

required 2 days, a larger excess of boron trifluoride etherate was used for preparation of the fucosylated serine **7**. Thus, use of 6 equivalents of boron trifluroide etherate as promoter in the glycosylation of Fmoc serine (**5**) with **1** in acetonitrile allowed the reaction time to be reduced to 4 h, and the target α-fucoside **7**[12] was isolated in 44% yield, together with the β-linked **6**[16] (14%). TLC revealed initial formation of the β-fucoside **6** which then rearranged to the thermodynamically more stable **7**, in analogy with the observations made in the synthesis of **4**. When dichloromethane was used as solvent rearrangement was slower and **6** and **7** were obtained in 23 and 28% yields, respectively after 4 h.

The synthesis of the α-fucosides **4** and **7** demonstrates that glycosylated amino acids having a 1,2-*cis* α-*O*-glycosidic linkage can be prepared using carbohydrate peracetates as glycosyl donors, provided that sufficient amounts of Lewis acid is used as promoter, and that the reaction is allowed to proceed towards the thermodynamically more stable α-glycoside. Further studies should be undertaken in order to clarify if the procedure is limited only to more reactive, deoxygenated saccharides such as fucose, or if it can be applied also to peracetates of the common, non-deoxygenated monosaccharides. It should be pointed out that, in comparison to the previous syntheses of compounds **4**[11] and **7**[12], the present procedure reduces number of synthetic steps from four or six to one, while the overall yield is increased by ~10% for both **4** and **7**. In addition, a fucosyl donor prepared in one instead of in three steps is used. However, the need for purification by HPLC somewhat restricts scaling-up of the procedure.

Procedures for Synthesis of Compounds 4 and 7. $BF_3{\cdot}Et_2O$ (105 μL, 0.85 mmol) was added to a solution of **1** (100 mg, 301 μmol) and N^{α}-Fmoc-Thr-OH (**2**, 123 mg, 361 μmol) in dry CH_2Cl_2 (3 mL) at room temperature. After 50 h the solution was diluted with CH_2Cl_2 (10 mL) and washed with water (10 mL). The aqueous phase was extracted with CH_2Cl_2 (2 x 10 mL) and the combined organic phases were dried with Na_2SO_4, filtered and concentrated. The crude product was purified by NP-HPLC on a Kromasil silica column (100 Å, 5 μm, 20 x 250 mm) using a linear gradient of EtOH in hexane (0→60% during 250 min, both eluants contained 2% HOAc) to give **3**[16] (~36 mg ~20%) and **4**[16] (64 mg, 35%).

$BF_3{\cdot}Et_2O$ (110 μL, 0.90 mmol) was added to a solution of **1** (50 mg, 150 μmol) and N^{α}-Fmoc-Ser-OH (**5**, 59 mg, 180 μmol) in dry CH_3CN (3 mL). After 4 h work-up was performed as described for **4**. The crude product was purified in 30-40 mg portions with RP-HPLC on a Kromasil C-8 column (100 Å, 5 μm, 20 x 250 mm) under isocratic conditions with 0.1% aqueous TFA/0.1% TFA in CH_3CN (1:1) to give **6**[16] (13 mg 14%) and **7**[12] (39 mg, 44%).

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15. 1,2,3,4-Tetra-*O*-acetyl-L-fucopyranose (**1**) was obtained as an anomeric mixture (α:β, 10:1) by acetylation of L-fucose with acetic anhydride in pyridine. The anomeric mixture of **1** was used as glycosyl donor after purification by flash column chromatography.
16. MS and selected ^{1}H NMR data (400 MHz): **3:** NMR ($CDCl_3$) δ 5.61 (d, 9.2 Hz, NH), 5.24 (bd, 3.0 Hz, H-4), 5.12 (dd, 10.4 and 7.7 Hz, H-2), 5.04 (dd, 10.4 and 3.0 Hz, H-3), 4.54 (d, 7.6 Hz, H-1), 4.26 (t, 7.2 Hz, Fmoc-CH), 3.81 (q, 5.8 Hz, H-5), 2.20, 2.07 and 2.00 (3 s, 3 Ac), 1.39 (d, 6.3 Hz, H-γ), 1.23 (d, 6.2 Hz, H-6). **4:** $[\alpha]_D^{25}$ -79.2° (*c* 1.4, $CHCl_3$). HRMS $(M+Na)^+$ 636.2057 calcd, 636.2048 found. NMR ($CDCl_3$) δ 5.77 (d, 9.4 Hz, NH), 5.20 (d, 3.9 Hz, H-1), 5.11 (dd, 10.0 and 3.7 Hz, H-2), 4.57 (dd, 9.6 and 1.7 Hz, H-α), 4.27 (t, 7.0 Hz, Fmoc-CH), 4.09 (q, 6.2 Hz, H-5), 2.17, 2.07 and 2.02 (3 s, 3 Ac), 1.18 (d, 6.2 Hz, H-γ), 1.11 (d, 6.4 Hz, H-6). **6:** MS $(M+Na)^+$ 622 calcd, 622 found. NMR (acetone-d_6) δ 6.55 (d, 8.4 Hz, NH), 5.20 (bm, H-4), 4.64 (m, H-1, virtually coupled to H-2,3,4), 4.44 (m, H-α), 4.11 (m, H-β,β'), 4.04 (q, 6.8 Hz, H-5), 2.14, 1.98 and 1.91 (3 s, 3 Ac), 1.15 (d, 6.4 Hz, H-6). **7:** $[\alpha]_D^{25}$ -45.8° (*c* 0.58, $CHCl_3$). NMR data were in agreement with those published in ref. 12.

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