Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 45 (2004) 355–358
Tetrahedron Letters

Convenient high yield and stereoselective synthesis of *O*-glycopeptides using *N*-α-Fmoc-Tyr/Ser[β-D-Glc(OAc)$_4$]OPfp generated in solution ☆

Beechanahalli P. Gangadhar,[a] Seetharama D. S. Jois[b] and Ambikaipakan Balasubramaniam[a,*]

[a]*Division of GI Hormones, Department of Surgery, University of Cincinnati Medical Center, Cincinnati, OH 45267, USA*
[b]*Department of Pharmacy, National University of Singapore, Singapore 117543*

Received 23 September 2003; revised 23 October 2003; accepted 24 October 2003

Abstract—Fmoc-AA-OPfp (AA = Tyr or Ser) (1 equiv) was reacted with β-D-Glc(OAc)$_5$ (6 equiv) in the presence of $BF_3{\cdot}Et_2O$ (6 equiv) in CH_2Cl_2 at room temperature for 2 h, and the glycosylation reaction mixture was used directly to couple to the amino group of the peptide resin without isolation and purification of the Fmoc-AA[β-D-Glc(OAc)$_4$]-OPfp. Moreover, the -OAc protecting groups of glucose was removed just prior to releasing the peptide from the resin using 6 mM NaOMe in 85% DMF-MeOH. The crude product obtained by TFA cleavage contained >90% of the target *O*-glycopeptide, and the 500 MHz ^{1}H NMR analysis revealed that the glycosylation reaction was nearly stereoselective (>97% β-anomer). This method is rapid and stereoselective, and can now be exploited for the routine synthesis of *O*-glycopeptides.

Carbohydrate moieties in glycoproteins play a crucial role in a number of biological process including cell recognition, cell adhesion, infection, and tumor metastasis.[1] Glycosylation of peptides has also been shown to increase proteolytic stability, and promote blood brain barrier (BBB) permeability.[2,3] Moreover, glycosylation enhances solubility, and may also contribute to the stabilization of peptide structures. The latter may impart differential receptor selectivity. Thus glycopeptides have attracted much attention in recent years. It is therefore desirable that a convenient and high yield method be available for the routine synthesis of glyocopeptides.

Although a significant advancement has been made in the solid and solution phase glycopeptide synthesis during the last decade,[1–3] limited availability of protected glycosylated amino acid derivatives has been impeding *O*-glycopeptide research. This could, at least in part, be attributed to the difficulties such as multiple steps, lower degree of stereoselectivity, cumbersome purification steps, and/or low yields associated with the currently used *O*-glycosylation strategies including: (1) β-D-Gal(OAc)$_5$ and $BF_3{\cdot}Et_2O$,[4] (2) α-D-Ac$_4$Glc-Br and AgOTfl;[5–7] (3) β-D-2-N$_3$-2-deoxy-Gal-Br and Ag_2CO_3/$AgClO_4$,[8] and (4) α-imino esters, glycosyl bromide, and AgOTfl.[9]

Our interest in *O*-glycopeptides emanates from the demonstration that *O*-glycosylation could promote BBB entry of peptides.[3] This is a useful strategy for the delivery of various agonists and antagonists of neuropeptide Y (NPY) that have been developed by us and other investigators.[10,11]

This is clinically important because central NPY has been implicated in the pathophysiology of obesity, seizures and mental disorders.[10–12] With this in mind, we wanted to develop a convenient glycosylation strategy that would be rapid, economical and amenable under normal conditions.

Fmoc-AA-OPfp (AA = Tyr or Ser) has been successfully glycosylated in good yields using α-D-Ac$_4$Glc-Br and

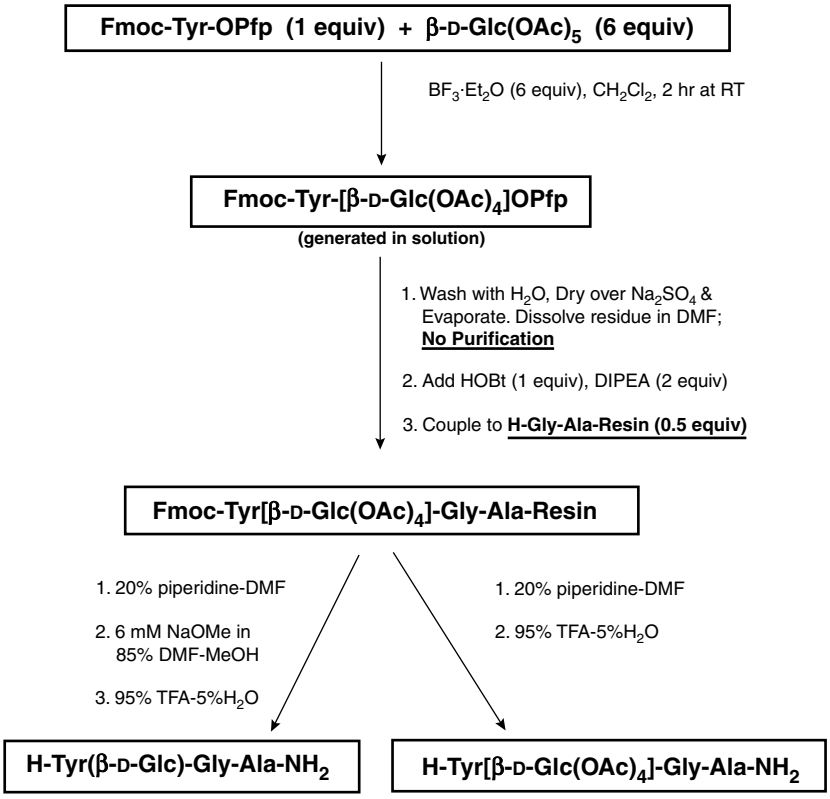
Keywords: *O*-Glycopeptides; Solid phase synthesis; Stereoselective; NMR.
☆ Supported in part by a NIH grant GM47122.
* Corresponding author. Tel.: +1-513-558-3819; fax: +1-513-558-0750; e-mail: ambi.bala@uc.edu

0040-4039/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2003.10.178

Table 1. Mass and ^{1}H 500 MHz NMR data of the *O*-glycopeptides

Compounds	M+H-found (calcd)	^{1}H NMR data for 1-H anomeric proton[a]		
		β-Anomer δ, ppm ($J_{H,H'}$, Hz)	α-Anomer δ, ppm ($J_{H,H'}$, Hz)	β:α (%)
H-Tyr(β-D-Glc)-Gly-Ala-NH_2	471.2 (470.4)	5.13 (7.52)	5.66 (3.67)	99.27:0.73
H-Tyr(β-D-Lac)-Gly-Ala-NH_2[b]	633.4 (633.3)	5.17 (7.89)	5.66 (3.49)	98.07:1.93
H-Tyr[β-D-Glc(OAc)$_4$]-Gly-Ala-NH_2	638.3 (639.4)			
H-Ser(β-D-Glc)-Gly-Ala-NH_2	563.2 (563.1)	4.53 (7.89)	4.98 (3.67)	97.36:2.63
H-Ser(β-D-Glc[(OAc)$_4$]-Gly-Ala-NH_2	471.2 (472.2)			
Ac-Trp-Arg-Tyr-(β-D-Glc)-NH_2[c]	727.4 (727.3)	4.78 (7.71)	5.54 (3.50)	98.45:1.55
Ac-Trp-Arg-Tyr-[β-D-Glc(OAc)$_4$]-NH_2	895.5 (895.4)			

[a] 500 MHz Brucker NMR instrument with crayo-probe; solvent: 100% D_2O; reference: DSS; temperature: 25 °C, (°10 °C).
[b] Additional peak was observed due to 1′-H anomeric proton.



Scheme 1.

AgOTfl.[5–7] However, this reaction is performed at temperatures from −10 to −40 °C. On the other hand, *O*-glycosylation reactions of Fmoc-AA-OH using $BF_3{\cdot}Et_2O$ and glycosylpentaacetate are performed at room temperature, but yields are somewhat lower.[4] Therefore, we reasoned that combining these two methodologies through using NH_2– and –COOH protected hydroxyl amino acids such as Fmoc-AA-OPfp and a large excess of glycosylating agents, $BF_3{\cdot}Et_2O$ and glycosylpentaacetate, one could achieve quantitative *O*-glycosylation at room temperature. Towards this, we investigated the degree of glycosylation at various time points using 1–10 equiv each of $BF_3{\cdot}Et_2O$ and glycosylpentaacetate. These studies, as monitored by TLC, revealed quantitative glycosylation could be achieved in 2 h using 6 equiv of the reagents.

Several *O*-glycosylated model tripeptides were synthesized to study the general feasibility of this strategy (Table 1). In a typical reaction, 1 equiv of Fmoc-AA-OPfp was stirred with 6 equiv each of $BF_3{\cdot}Et_2O$ and glycosylpentaacetate in CH_2Cl_2 (10 mL) under a nitrogen atmosphere at room temperature (Scheme 1).[13] Reaction was continued (generally 2 h) until the TLC[14] analysis showed the absence of free Fmoc-AA-OPfp. At this time, the reaction mixture was diluted with CH_2Cl_2 (×5), washed with water (2×20 mL), dried over anhydrous Na_2SO_4, and evaporated. The residue was dis-

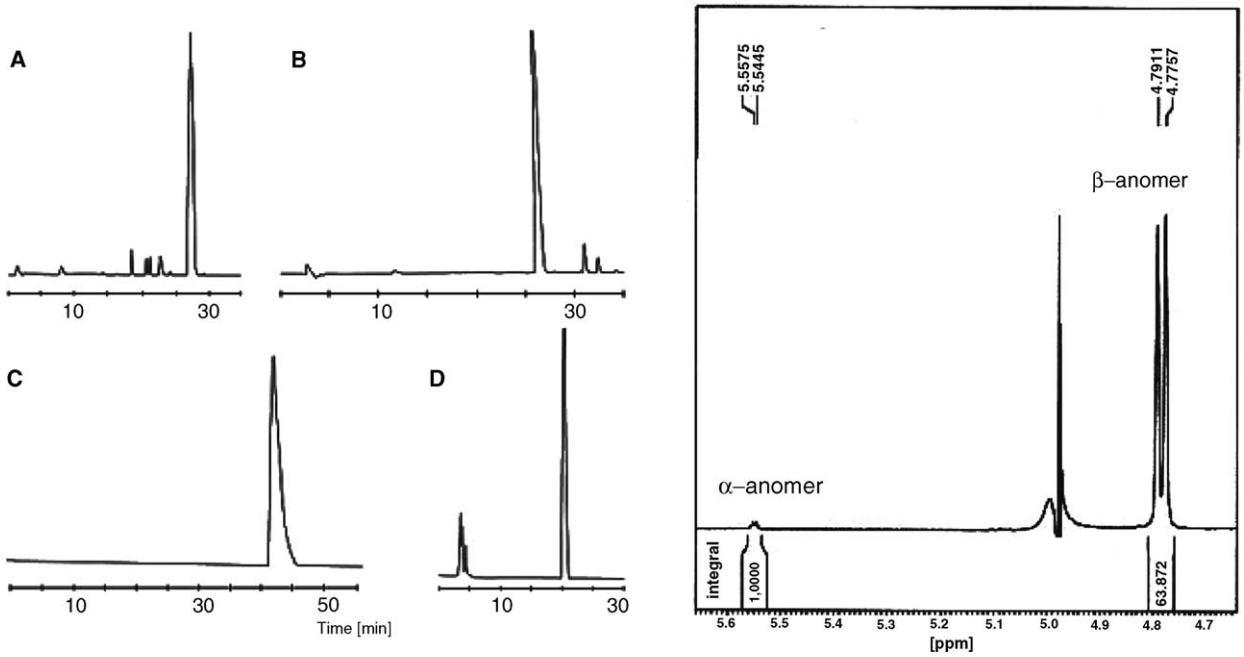


Figure 1. Left: Representative analytical reversed phase chromatograms of the crude *O*-glycopeptides obtained by TFA cleavage: (A) H-Tyr[β-D-Glc(OAc)$_4$]-Gly-Ala-NH$_2$ (R_t, 27 min); (B) H-Tyr(β-D-Glc)-Gly-Ala-NH$_2$ (R_t, 26 min); (C) H-Tyr[β-Lac(OAc)$_7$]-Gly-Ala-NH$_2$ (R_t, 41 min); (D) H-Ser[β-D-Glc(OAc)$_4$]-Gly-Ala-NH$_2$ (R_t, 23 min). *Note*: The major peaks contained the target peptides (>90% of the crude peptides). See Ref. 15 for HPLC conditions. Right: Representative 500 MHz NMR spectrum showing the anomeric proton region of *N*-α-Ac-Trp-Arg-Tyr(β-D-Glc)-NH$_2$ in D_2O at 10 °C. Data suggests that glycosylation reaction under these conditions is highly stereoselective (β anomer >98%). The peaks between 4.96 and 5.00 ppm is due to H_2O.

solved in DMF (10 mL) and used directly for acylation of the α-amnio group of the peptide resin without further purification or isolation steps (Scheme 1). At the end of stepwise solid phase synthesis, OAc-protection of the glucose was removed with 6 mM NaOMe in 85% DMF–MeOH. This solvent composition was found optimal for resin swelling and complete deprotection. Finally, the free peptide was obtained by standard TFA cleavage. Alternatively, direct treatment of the fully protected peptide resin with TFA gave the tetraacetylated glycopeptide. We have also successfully extended this strategy for the synthesis of peptides containing disaccharides (Table 1) and for the synthesis of dodecapeptides containing Thr(β-D-Glc) (not shown). Applicability of this method for the synthesis of peptides containing either Fmoc AA(α-D-Glc/Gal) or Fmoc AAα/β-D-GlcNAc/GalNAc) remains to be investigated.

Reversed phase analytical HPLC indicated that the crude products contained greater than 90% of the target glycosylated peptide (Fig. 1 left). Purified peptides also had the expected mass (Table 1). As shown in the representative 500 MHz proton NMR spectrum, all glycopeptides thus obtained contained >97% of the β-anomer suggesting that glycosylation under these conditions is stereoselective (Fig. 1 right and Table 1).

In summary, we have avoided the cumbersome purification steps, and devised a rapid, economical and convenient method for the high yield and stereoselective synthesis of *O*-glycopeptides. This method can now be exploited for the synthesis of various glycopeptides required for SAR studies as well as other applications,[1,2] and is expected to provide an added impetus for *O*-glycopeptide research.

References and Notes

1. Hojo, H.; Nakahara, Y. *Curr. Prot. Pept. Sci.* **2000**, *1*, 23–48.
2. Polt, R.; Palian, M. M. *Drugs of the Future* **2001**, *26*, 561–576.
3. Polt, R.; Porreca, F.; Szabo, L. Z.; Bilsky, E. J.; Davis, P.; Abbruscato, T. J.; Davis, T. P.; Horvath, R.; Yamamura, H. I.; Hruby, V. *J. Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7114–7118.
4. Salvador, L. A.; Elofsson, M.; Kihlberg, J. *Tetrahedron* **1995**, 5643–5656.
5. Jensen, K. J.; Meldal, M.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2119–2129.
6. Meldal, M.; Jensen, K. *J. Chem. Soc., Chem. Commun.* **1990**, 483–485.
7. Jansson, A. M.; Meldal, M.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1992**, *23*, 1699–1707.
8. Bielfeldt, T.; Peters, S.; Meldal, M.; Bock, K.; Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 857–859.
9. Polt, R.; Szabo, L.; Treiberg, J.; Li, Y.; Hruby, V. J. *J. Am. Chem. Soc.* **1992**, *114*, 10249–10258.
10. Balasubramaniam, A. *Curr. Pharm. Des.* **2003**, *9*, 1165–1175.
11. Parker, E.; Heek, M. V.; Stamford, A. *Eur. J. Pharmacol.* **2002**, *440*, 173–187.
12. Balasubramaniam, A. *Am. J. Surg.* **2002**, *183*, 430–434.
13. General method for the glycosylation of *N*-α-Fmoc-Tyr/Ser-OPfp: Fmoc-Tyr/Ser-OPfp (0.5 mmol, 1 equiv), β-D-Glc(OAc)$_5$ (1.17 g, 3 mmol, 6 equiv) and CH_2Cl_2 (10 mL) were placed in a round bottom flask and N_2 gas was bubbled for 5 min. $BF_3{\cdot}Et_2O$ (390 μL, 3 mmol, 6 equiv)

was then added and N_2 gas bubbling continued for another 5 min. The flask was stoppered and stirred for 2 h at room temperature. Completion of the reaction was checked by TLC.[14] The reaction mixture was then diluted with CH_2Cl_2 (40 mL), washed with water (2×20 mL), dried over anhydrous Na_2SO_4, evaporated and the residues was dissolved in DMF (10 mL) and used in the next step without further purification.

14. TLC plates were developed with ethylacetate–petroleum ether (1:2) and the amino acid spots were visualized using UV light. R_f, 0.64 (Fmoc-Tyr-OPfp), R_f 0.35 {Fmoc-Tyr[Glc(OAc)$_4$]-OPfp}.
15. HPLC Conditions: TARGA C_{18} Column (250×4.6 mm, 5 μm particle size); Solvent systems: Buffer X–0.1% TFA in 5% CH_3CN–H_2O, Buffer Y–0.1% TFA in 90% CH_3CN–H_2O, and Buffer Z–0.1% TFA in H_2O. Gradients: Figure 1A: 0–70% Buffer Y in Buffer X in 60 min; Figure 1B: 0–15% Buffer Y in Buffer Z in 40 min; Figure 1C: 0–60% Buffer Y in Buffer X in 60 min; Figure 1D: 0–60% Buffer Y in Buffer X in 60 min.