

A Convergent Total Synthesis of the Mucin Related F1 α Antigen by One-Pot Sequential Stereoselective Glycosylation

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A convergent total synthesis of F1 α antigen, a member of the tumor-associated *O*-linked mucin glycosyl amino acids, was accomplished by one-pot sequential glycosylation. In the first step, the corresponding disaccharide was formed from galactosyl phenylcarbonate **2** or fluoride **3** and thioglycoside **4** by the promotion of TrB(C₆F₅)₄ or TfOH, and following glycosylation of glycosyl amino acid **5** by further addition of *N*-iodosuccinimide (NIS) afforded protected F1 α in 80 or 89% overall yield, respectively. By the subsequent deprotection, F1 α antigen was obtained in good yield.

During the last decade, mucins have been regarded as important substances in antitumor immunological studies due to their high expression on epithelial cell surfaces and high content of clustered α -*O*-linked carbohydrates. Mucins on epithelial tumors often contain aberrant α -*O*-linked carbohydrates, and recently found F1 α antigens, Gal β -(1 \rightarrow 4)GlcNAc β -(1 \rightarrow 6)GalNAc α -(1 \rightarrow 3)Ser and -(1 \rightarrow 3)Thr, represent examples of aberrant carbohydrate epitopes found on mucins associated with gastric adenocarcinomas.¹ Therefore, chemical total synthesis of F1 α antigens is an interesting topic because of their structures and biological properties, and their total synthesis has already been accomplished by R. R. Koganty et al. (in 1997)² and S. J. Danishefsky et al. (in 1998).³ In our previous papers,^{4,5} two types of one-pot sequential glycosylation for the synthesis of trisaccharides were reported, in which glycosyl fluoride or phenylcarbonate and thioglycoside were used in the first step, and methylglycoside was used in the second step.⁶ It was thought then that the above method would be effectively applied to rapid total synthesis of F1 α . In this communication, we would like to report a convergent total synthesis of F1 α antigen (**1**, serine type) by one-pot sequential glycosylation.

The retro synthetic analysis of F1 α (**1**) is shown in Figure 1. The results of our previously reported procedure indicated that the fully protected F1 α would be synthesized by one-pot sequential glycosylation using galactosyl donor **2** or **3**, thioglycoside **4** and glycosyl amino acid **5**. Galactosyl donor **2** or **3** having 2-*O*-*p*-methylbenzoyl (*p*-MeBz) group was prepared according to the same procedure as glucosyl donors.^{4,7,8} Thioglycoside **4** having 4,5-dichlorophthaloyl (DCPhth) amino function⁹ was chosen by considering its easiness in removing the amino protecting group and was prepared by standard protecting group manipulations. It was thought that **5** would be prepared by stereoselective glycosylation of the protected serine **7** with thioglycoside **6** using trityl salt and NIS.⁴

In the first place, stereoselective synthesis of **5** was examined (Scheme 1): that is, 1-*O*-hydroxy sugar **8**, prepared by the known procedure,¹⁰ was treated with SOCl₂ in DMF, followed by anomeric substitution with NaSPh to afford the corresponding thioglycoside **6** in good yield. Then, α -selective glycosylation

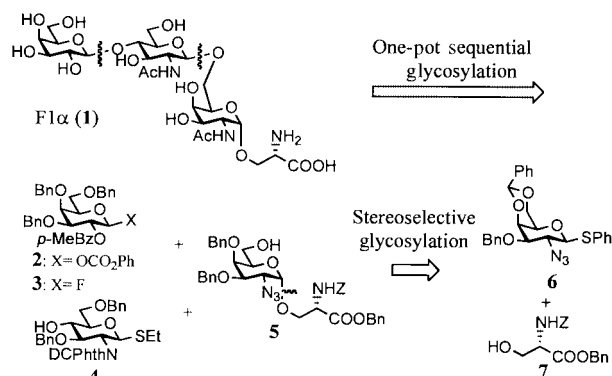
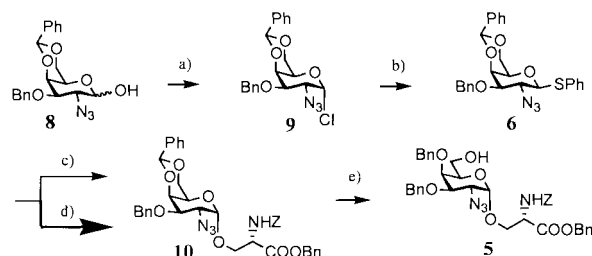


Figure 1. Retro synthesis of F1 α (**1**).

of **7** with **6** was examined by using various catalysts and solvents, and it was revealed that the glycosylation proceeded not in good yield when a combination of a catalytic amount of protic acid (such as TfOH or HClO₄) and NIS¹¹ was used because partial deprotection of benzylidene acetal took place during the glycosylation, and α -selectivity was not observed when the reaction was carried out in Et₂O. When trityl tetrakis(pentafluorophenyl)borate [TrB(C₆F₅)₄] was used as a Lewis acid together with NIS⁴ in toluene, the glycosyl amino acid **10** was obtained in high yield, however, the undesirable β -glycoside was predominantly formed (Scheme 1). On the other hand, the TrOTf (generated in situ from TrCl and AgOTf)-catalyzed glycosylation in toluene afforded **10** in high yield with good α -selectivity. It is interesting to note that the stereoselectivity was reversed only by changing the counter anion of the catalyst.¹² After separation of these isomers, regioselective benzylidene ring opening of **10 α** was carried out by using a combination of BH₃·Et₃N and BF₃·OEt₂¹³ to give alcohol **5** in moderate yield.

Next, stereoselective glycosylation of thioglycoside **4** with galactosyl donor **2** or **3** was examined in detail according to our



Scheme 1. Stereoselective synthesis of glycosyl amino acid **5**. a) SOCl₂ / CH₂Cl₂-DMF, r.t. b) NaSPh / THF, 0 °C – r.t. 71% 2 steps. c) 20 mol% TrB(C₆F₅)₄, **7** (1.5 equiv), NIS (1.5 equiv), MS 5A / toluene, –35 °C 90% (α / β = 36 / 64). d) 20 mol% TrCl-AgOTf, **7** (1.5 equiv), NIS (1.5 equiv), MS 5A / toluene, –35 °C 97% (α / β = 83 / 17). e) BH₃·NEt₃, BF₃·OEt₂ / CH₂Cl₂ 61%.

Table 1. Glycosylation of acceptor **4** with donor **2** or **3**.

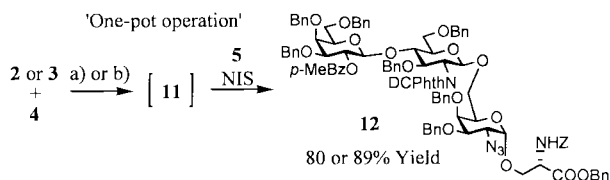
2 or 3 (1.2 equiv) + 4 (1.0 equiv)		Catalyst Additive	Conditions	Yield / %
1	2		Drierite CH ₂ Cl ₂ , -15 °C, 6 h	14
2	2	TrB(C ₆ F ₅) ₄ ^b	Drierite BTF, -15 °C, 6 h	61
3	2		MS 5A BTF, -15 °C, 4 h	69
4 ^a	2		MS 5A BTF, -15 °C, 5 h	84
5	3	TfOH ^c	MS 5A CH ₂ Cl ₂ , -20 °C, 1 h	94

^a1.8 equiv of donor **2** was used. ^b30 mol%. ^c20 mol%.

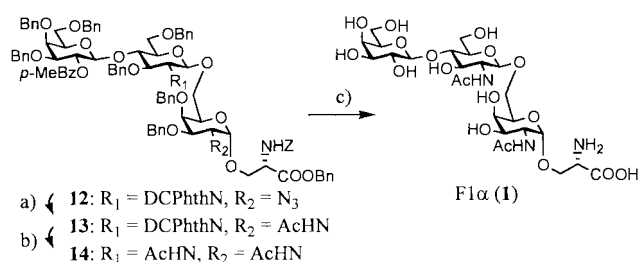
previously reported one-pot glycosylation procedure in order to optimize the reaction conditions (Table 1).

The TrB(C₆F₅)₄-catalyzed glycosylation of **4** with phenyl-carbonate donor **2** proceeded more smoothly in trifluoromethylbenzene (BTF) compared with that in CH₂Cl₂ and the desired disaccharide **11** was obtained in good yield (Table 1, Entries 1–3).⁴ After optimization, the best condition was determined as shown in Entry 4 (84%, in BTF, MS 5A, at -15 °C, 1.8 equivalent of donor). The glycosylation of **4** with glycosyl fluoride **3** was further examined by using 20 mol% of TfOH and **11** was obtained in excellent yield (94%).^{5,14}

Then, two types of one-pot sequential glycosylation were attempted as shown in Scheme 2. In the first step, glycosyl phenylcarbonate **2** or fluoride **3** was treated with thioglycoside **4** in the presence of a catalyst such as TrB(C₆F₅)₄ or TfOH, and **4** was almost completely consumed within 5 h or 1 h, respectively, which was confirmed by TLC monitoring. Next, the second glycosylation of glycosyl amino acid **5** with thus formed disaccharide was tried by successive addition of NIS in one-pot operation, and fully protected Flα **12** was stereoselectively obtained in high yield (80 or 89%, respectively).

**Scheme 2.** One-pot sequential synthesis of trisaccharide **12**. a) **2** (1.8 equiv), **4** (1.0 equiv), 30 mol% TrB(C₆F₅)₄, MS 5A / BTF, -15 °C, 5 h, then **5** (5.0 equiv), NIS (2.0 equiv) / BTF-CH₂Cl₂, -30 °C, 2 h, 80% (based on **4**). b) **3** (1.2 equiv), **4** (1.0 equiv), 20 mol% TfOH, MS 5A / CH₂Cl₂, -20 °C, 1 h, then **5** (1.5 equiv), NIS (2.0 equiv) / CH₂Cl₂, -20 °C, 1 h, 89% (based on **4**).

Transformation of **12** into Flα is demonstrated as in Scheme 3. In the first place, azido group of **12** was reduced with thioacetic acid to give **13** in 85% yield. Successive deprotection of DCPhth group of **13** smoothly proceeded to afford the desired diacetamido glycosyl amino acid **14** in high yield after acetylation (2 steps, 82%) only when hydrazine acetate¹⁵ was used in ethanol at 70 °C. On the other hand, the deprotection under standard conditions (hydrazine hydrate, ethylenediamine, and NaBH₄-reduction followed by AcOH) gave complicated mixtures. Then, removal of benzyl and benzyloxycarbonyl groups of compound **14** by hydrogenolysis, and careful saponification of *p*-MeBz group afforded the final product Flα (**1**) in 84% yield.¹⁶

**Scheme 3.** Global deprotection of **12**. a) AcSH / Py, r.t. 85%. b) NH₂NH₂·AcOH / EtOH, 70 °C, then Ac₂O / Py, 82% (2 steps). c) H₂, 20% Pd(OH)₂-C / THF-MeOH-H₂O, then 0.1 M NaOH aq / MeOH, 0 °C, 84% (2 steps).

Thus, a convergent total synthesis of Flα antigen was accomplished by one-pot sequential glycosylation and whose method proved to be applicable for the rapid assembly of various types of complex oligosaccharides. Also, it should be noted that the control of the stereoselectivity of glycosylation is influenced by the kind of the counter anion of the catalyst.

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- Selected ¹H NMR (500 MHz, D₂O, δ = TMS); δ 4.26 (1H, d, *J* = 7.9 Hz, H-1''), 4.36 (1H, d, *J* = 7.6 Hz, H-1'), 4.67 (1H, d, *J* = 3.7, H-1); Selected ¹³C NMR (125 MHz, D₂O, δ = TMS); δ 97.7 (C-1), 101.1 (C-1'), 102.5 (C-1''); HRMS (*m/z*): [M + H]⁺ calcd for C₂₅H₄₄N₃O₁₈, 674.2626; found 674.2620. [α]_D²⁰ = +54.0° (c 0.1, H₂O); FT-IR (KBr): 1643, 1072, 1049 cm⁻¹; Anal. Calcd for C₂₅H₄₀N₃O₂₁·3H₂O: C, 41.26; H, 6.79; N, 5.77%. Found: C, 41.46; H, 7.17; N, 5.97%.