A Convergent Total Synthesis of the Mucin Related F1α Antigen by One-Pot Sequential Stereoselective Glycosylation

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A convergent total synthesis of F1α 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 $TrB(C_6F_5)_4$ or TfOH, and following glycosylation of glycosyl amino acid **5** by further addition of *N*-iodosuccinimide(NIS) afforded protected F1α in 80 or 89% overall yield, respectively. By the subsequent deprotection, F1α 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 F1α antigens, Galβ-(1→4)GlcNAcβ-(1→6)GalNAcα-(1→3)Ser and -(1→3)Thr, represent examples of aberrant carbohydrate epitopes found on mucins associated with gastric adenocarcinomas.[1] Therefore, chemical total synthesis of F1α 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 F1α. In this communication, we would like to report a convergent total synthesis of F1α antigen (**1**, serine type) by one-pot sequential glycosylation.

The retro synthetic analysis of F1α (**1**) is shown in Figure 1. The results of our previously reported procedure indicated that the fully protected F1α 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 function[9] 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 $SOCl_2$ in DMF, followed by anomeric substitution with NaSPh to afford the corresponding thioglycoside **6** in good yield. Then, α-selective glycosylation

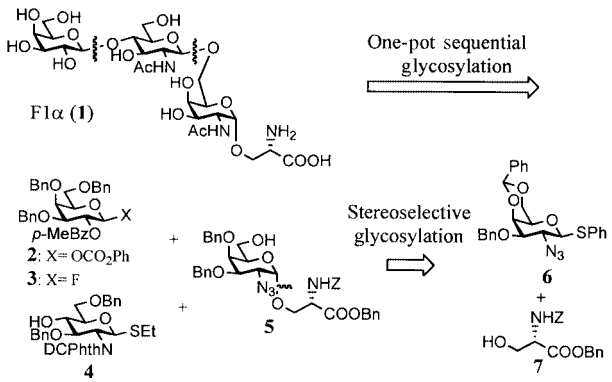
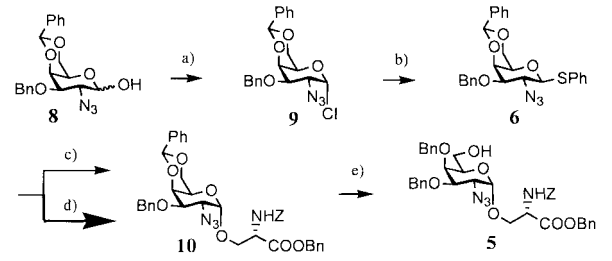


Figure 1. Retro synthesis of F1α (**1**).

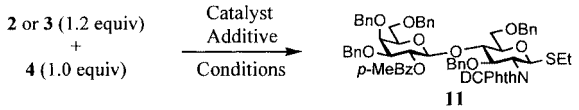
of **7** with **6** was examined by using various catalysts and solvents, and it was revealed that the glycosylation proceeded not in good yield when a combination of a catalytic amount of protic acid (such as TfOH or $HClO_4$) and NIS[11] was used because partial deprotection of benzylidene acetal took place during the glycosylation, and α-selectivity was not observed when the reaction was carried out in Et_2O. When trityl tetrakis(pentafluorophenyl)borate [$TrB(C_6F_5)_4$] was used as a Lewis acid together with NIS[4] 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 $BH_3{\cdot}Et_3N$ and $BF_3{\cdot}OEt_2$[13] 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) $SOCl_2$ / CH_2Cl_2–DMF, r.t. b) NaSPh / THF, 0 °C– r.t. 71% 2 steps. c) 20 mol% $TrB(C_6F_5)_4$, **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) $BH_3{\cdot}NEt_3$, $BF_3{\cdot}OEt_2$ / CH_2Cl_2 61%.

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



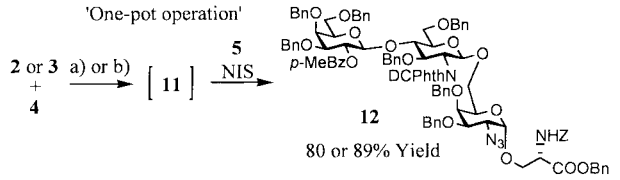
Entry	Donor	Catalyst	Additive	Conditions	Yield / %
1	**2**	$TrB(C_6F_5)_4$[b]	Drierite	CH_2Cl_2, –15 °C, 6 h	14
2	**2**		Drierite	BTF, –15 °C, 6 h	61
3	**2**		MS 5A	BTF, –15 °C, 4 h	69
4[a]	**2**		MS 5A	BTF, –15 °C, 5 h	**84**
5	**3**	TfOH[c]	MS 5A	CH_2Cl_2, –20 °C, 1 h	**94**

[a]1.8 equiv of donor **2** was used. [b]30 mol%. [c]20 mol%.

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

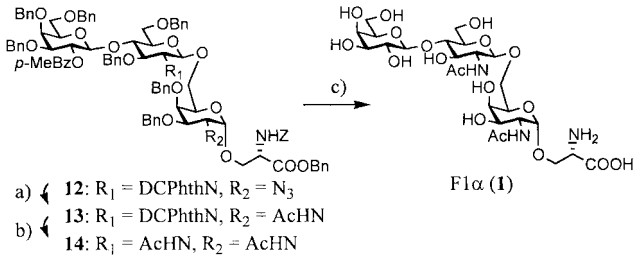
The $TrB(C_6F_5)_4$-catalyzed glycosylation of **4** with phenylcarbonate donor **2** proceeded more smoothly in trifluoromethylbenzene (BTF) compared with that in CH_2Cl_2 and the desired disaccharide **11** was obtained in good yield (Table 1, Entries 1–3).[4] After optimization, the best condition 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 $TrB(C_6F_5)_4$ 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 F1α **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% $TrB(C_6F_5)_4$, MS 5A / BTF, –15 °C, 5 h, then **5** (5.0 equiv), NIS (2.0 equiv) / BTF–CH_2Cl_2, –30 °C, 2 h, 80% (based on **4**). b) **3** (1.2 equiv), **4** (1.0 equiv), 20 mol% TfOH, MS 5A / CH_2Cl_2 –20 °C, 1 h, then **5** (1.5 equiv), NIS (2.0 equiv) / CH_2Cl_2, –20 °C, 1 h, 89% (based on **4**).

Transformation of **12** into F1α 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 acetate[15] was used in ethanol at 70 °C. On the other hand, the deprotection under standard conditions (hydrazine hydrate, ethylenediamine, and $NaBH_4$-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 F1α (**1**) in 84% yield.[16]



Scheme 3. Global deprotection of **12**. a) AcSH / Py, r.t. 85%. b) NH_2NH_2·AcOH / EtOH, 70 °C , then Ac_2O / Py, 82% (2 steps). c) H_2, 20% $Pd(OH)_2$–C / THF-MeOH-H_2O, then 0.1 M NaOH aq / MeOH, 0 °C, 84% (2 steps).

Thus, a convergent total synthesis of F1α 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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16 Selected ^{1}H NMR (500 MHz, D_2O, 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 ^{13}C NMR (125 MHz, D_2O, δ = TMS); δ 97.7 (C-1), 101.1 (C-1'), 102.5 (C-1''); HRMS (*m/z*): $[M + H]^+$ calcd for $C_{25}H_{44}N_3O_{18}$, 674.2626; found 674.2620). $[\alpha]^{20}_D$ = +54.0° (*c* 0.1, H_2O); FT-IR (KBr): 1643, 1072, 1049 cm^{-1}; Anal. Calcd for $C_{25}H_{49}N_3O_{21}\cdot 3H_2O$: C, 41.26; H, 6.79; N, 5.77%. Found: C, 41.46; H, 7.17; N, 5.97%.