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Di-*tert*-butylsilylene-Directed α-Selective Synthesis of 4-Methylumbelliferyl T-Antigen

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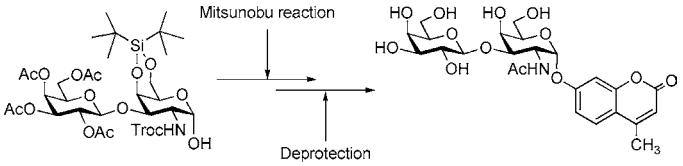
ABSTRACT



We have succeeded in the facile synthesis of 4-methylumbelliferyl T-antigen as a substrate for *endo*-α-*N*-acetylgalactosaminidase by exploiting the combination of the di-*tert*-butylsilylene effect and the Mitsunobu reaction.

endo-α-*N*-Acetylgalactosaminidase is a glycosidase of widespread occurrence in the bacteria kingdom.[1] The enzyme hydrolyzes the *O*-glycosidic α-linkage between T-antigen [β-D-Gal-(1→3)-α-D-GalNAc] and a serine or threonine residue in mucin-type glycoprotein.[2] To elucidate the substrate specificity of this enzyme, or screen the new species from other living organisms, sensitive synthetic fluorogenic T-antigen probes are intensively desired.

In this paper, we report the synthesis of 4-methylumbelliferyl (4-MU) T-antigen **1** (Figure 1) as a sensitive fluorogenic probe, featuring a di-*tert*-butylsilylene (DTBS)-directed α-selective Mitsunobu reaction.

4-MU glycosides have been popular type of fluorogenic probe for hydrolases because of the potent fluorometric property of the phenolic counterpart liberated by enzymatic hydrolysis.[3] However, the 4-MU glycoside synthesis is generally difficult.[4] In particular, the synthesis of α-glycosaminides such as the title compound is extremely arduous in order to circumvent the participatory effects of the *N*-acetyl group. In their synthesis of 4-MU-α-GalNAc, Lemieux and co-workers utilized 2-azidogalactosyl chloride as a glycosyl donor as it possesses a nonparticipatory group at C2. Unfortunately, its synthesis required many laborious manipulations. Moreover, the glycosyl donor could only be coupled to 4-methylumbelliferone (4-MU-OH) in poor yield (33%).[5] To solve this synthetic issue, we envisaged using

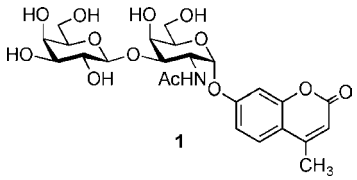


Figure 1. Structure of 4-methylumbelliferyl T-antigen.

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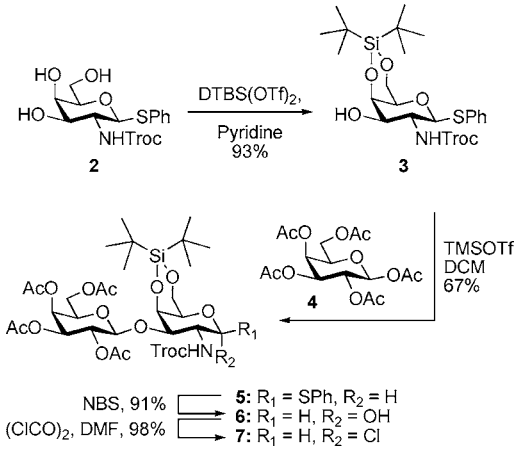
our DTBS-directing α-selective galactosylation[6] for 4-MU T-antigen synthesis.

Taking the advantage of the compatibility of our α-selective galactosylation with acyl functionality on C-2 amino groups, we designed the *N*-2,2,2-trichloroethoxycarbonyl (Troc)-protected disaccharide **7** as a DTBS glycosyl donor. Treatment of the readily accessible 2-*N*-Troc galactothioglycoside **2**[7] with $DTBS(OTf)_2$ in pyridine[8] gave 4,6-silylated **3** in 93% yield, which was then orthogonally glycosylated with the 1,2,3,4,6-penta-*O*-acetyl-*β*-D-galactopyranose **4** catalyzed by trimethylsilyl trifluoromethanesulfonate[9] to afford disaccharide **5** in 67% yield. The hemiacetalization of **5** with NBS in aqueous acetone[10] produced **6**. Finally, the hemiacetal **6** was converted into the corresponding chloride **7** by the action of Vilsmeier's reagent[11] (Scheme 1).

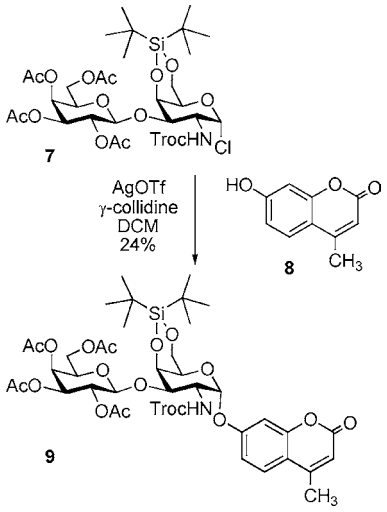
Scheme 1. Preparation of Gal *β*(1→3) GalN Disaccharide



With the glycosyl chloride **7** in hand, we then subjected it to a DTBS-directing α-glycosidation with 4-methylumbelliferone. Initially, we attempted reaction of the α-chloride **7** with 4-methylumbelliferone **8** in the presence of the silver triflate−*γ*-collidine complex.[5] This reaction provided the α-glycoside **9** exclusively in 24% yield together with the hemiacetal **6** as the main byproduct (Scheme 2). However, the yield could not be elevated any further.

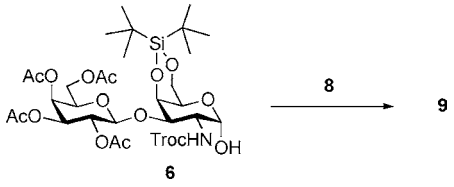
Accordingly, we next investigated the utility of the Mitsunobu reaction in this capacity.[12] Thus, the hemiacetal

Scheme 2. Condensation of **7** and 4-Methylumbelliferone **8**



6 was reacted with **8** in the presence of various combinations of trialkyl phosphines (TPP,[12] TBP,[13,15] DPPE[14]) and azo-compounds (DEAD,[12] ADDP,[13] TMAD,[15] DIAD[12,14]) as summarized in Table 1. Surprisingly, the anomeric configuration of **6** was mostly retained; the α-glycoside **9** predominating in all these reactions. Interestingly, the yield of **9** increased when the reaction was performed at higher

Table 1. 4-Methylumbelliferylation by Mitsunobu Reaction



entry	phosphine/azo compd[b]	MU-OH (equiv)	solvent	*T* (°C)	% yield[c] (α/β)
1[d]	TPP/DEAD	3.0	THF	80	47:5
2[e]	TBP/ADDP	3.0	THF	80	20:−
3[f]	TBP/TMAD	3.0	THF	80	8:−
4[g]	DPPE/DIAD	3.0	THF	80	no reaction
5	TPP/DEAD	3.0	toluene	130	74:8
6	TBP/ADDP	3.0	toluene	130	62:15
7	TPP/DEAD	8.0	toluene	130	80:9

[a] Every reaction was conducted under reflux condition. [b] TPP, triphenylphosphine, TBP, tributylphosphine, DPPE, 1,2-bis(diphenylphosphino)-ethane, DEAD, diethyl azodicarboxylate, ADDP, 1,1′-(azodicarbonyl)dipiperidine, TMAD, 1,1′-azobis(*N*,*N*′-dimethylformamide), DIAD, diisopropyl azodicarboxylate. [c] Isolated yield. [d] See ref 12. [e] See ref 13. [f] See ref 15. [g] See ref 14.

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(7) Compound **2** was derived from galactosamine hydrochloride through four-step manipulation (65% overall) according to the method of 2-*N*-Troc glucothioglycoside: Yan, F.; Mehta, S.; Eichler, E.; Wakarchuk, W. W.; Gilbert, M.; Schur, M. J.; Whitfield, D. M. *J. Org. Chem.* **2003**, *68*, 2426−2431.
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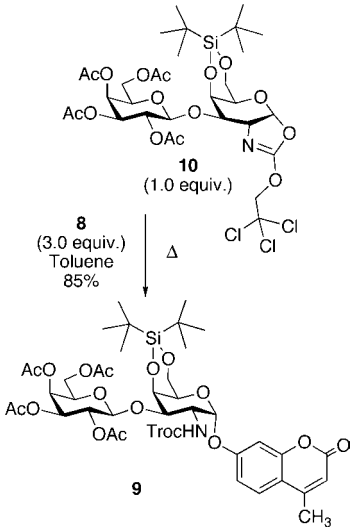
temperature (entries 5 and 6). Additionally, the use of excess 4-MU-OH (8.0 equiv) led to the optimum product yield (80%) (entry 7).

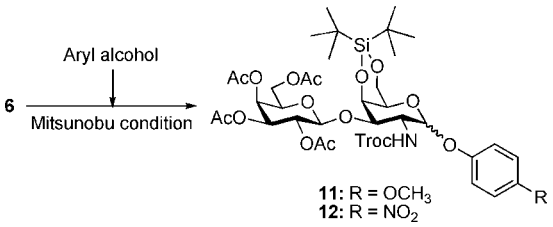
In contrast, in entries 1 and 2, the trichloroethoxyoxazole **10** was observed as a major byproduct. Furthermore, we observed the transitory formation of **10** during all our reactions according to TLC monitoring. This result implied that the oxazole **10** is a reaction intermediate; in fact, the isolated oxazole **10** reacted with 4-MU-OH in toluene under reflux to afford α-glycoside **9** in 85% yield (Scheme 3).

Scheme 3. Coupling of **10** and **8** in the Absence of Activators



Significantly, we have shown that this reaction can produce other α-aryl glycosides **11** and **12** in moderate yields (Table 2). Taking into consideration these results, we hypothesize that the coupling reactions proceed as follows: (i) initial formation of the oxazole intermediate results from triphenylphosphine oxide elimination, (ii) oxocarbenium ion formation then occurs, (iii) "through-space" electron-donation[16] from axially oriented C4 or C6 hydroxyl places the ^{t}Bu moiety closer to the anomeric carbon, and (iv) attack of 4-MU-OH is then restricted to the α-face of anomeric center due to steric hampering by the DTBS group (Scheme 4).

Table 2. Various Arylations by Mitsunobu Reaction[a]



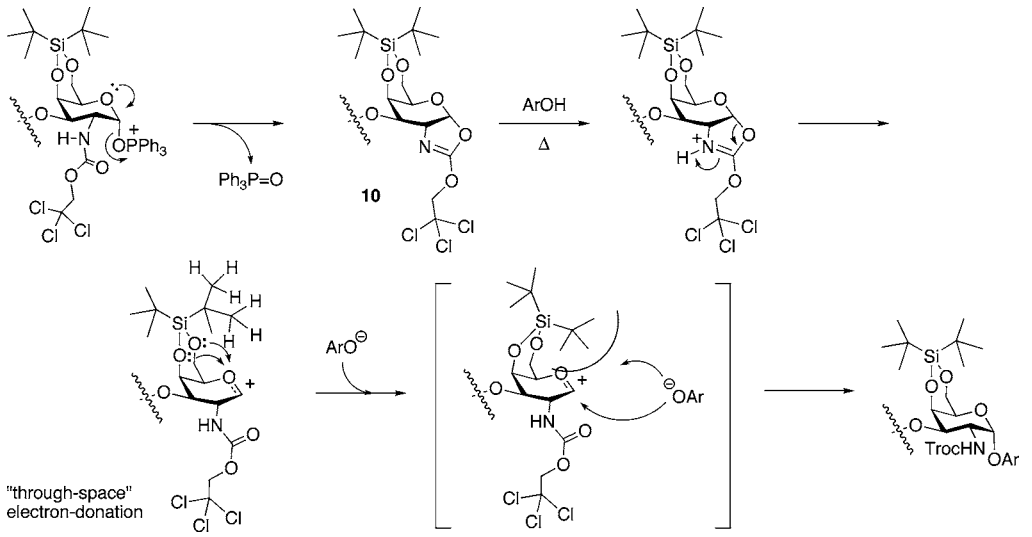
entry	aryl alcohol	product	% yield (α/β)
1	MPOH	**11**	56:–
2	PNPOH	**12**	41:17

[a] All reaction conditions are the same as entry 5 in Table 1.

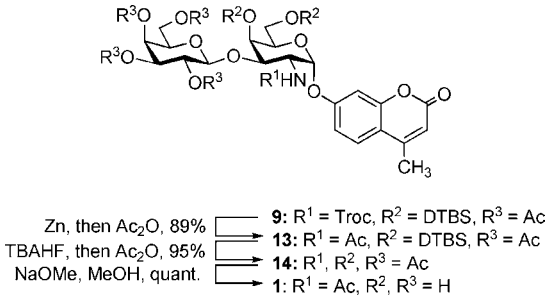
Finally, as depicted in Scheme 5, 4-MU glycoside **9** was transformed into **1**. Thus, deprotection of Troc group by the action of Zn and subsequent acetylation provided acetamide **13** in 89% yield. Removal of 4,6-*O*-DTBS group by TBAHF[17] and sequential acetylation yielded fully acetylated 4-MU T-antigen **14** in 95% yield, which was subjected to de-*O*-acetylation[18] to afford free 4-methylumbelliferyl T-antigen **1**.

In conclusion, we have found a new method for forming α-4-MU galactosaminide based upon the DTBS effect and the Mitsunobu reaction. The synthesized 4-MU T-antigen will serve as a powerful probe for enzymatic studies, e.g., seeking the unknown *endo*-α-*N*-acetylgalactosaminidase.

Scheme 4. Expected Reaction Mechanism on Aryl Glycosylation



Scheme 5. Final Deprotections



Zn, then Ac_2O, 89% → **9:** R^1 = Troc, R^2 = DTBS, R^3 = Ac
TBAHF, then Ac_2O, 95% → **13:** R^1 = Ac, R^2 = DTBS, R^3 = Ac
NaOMe, MeOH, quant. → **14:** R^1, R^2, R^3 = Ac
→ **1**: R^1 = Ac, R^2, R^3 = H

Now we are also undertaking the synthesis of other tumor-associated glycan antigen probes having 4-MU.

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Supporting Information Available: Full experimental details and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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