Trans-glycosylation through the Reduction of C-1 Position of Sialic Acid

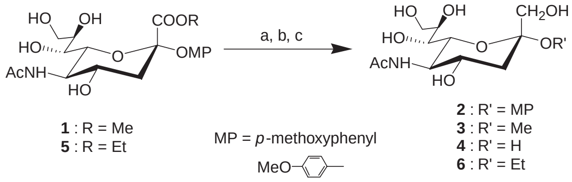
Muneyoshi Kikkawa and Teruo Yoshino*
Graduate School Division of Natural Sciences, International Christian University,
3-10-2 Osawa, Mitaka, Tokyo 181-8585

(Received August 28, 2006; CL-060973; E-mail: yoshino@icu.ac.jp)

Rapid and α-selective trans-glycosylation of C-1 sialoside alcohol with solvent alcohol was found, because 2-*p*-methoxyphenoxy group has good leaving-group properties. The kinetic studies by ^{1}H and ^{2}H NMR showed that the process was a single reaction, and proved the existence of intermediate epoxide to govern the α-selectivity.

5-Acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid (Neu5Ac) is widespread in animal tissues.[1] Usually, Neu5Ac exists at the terminus of glycoconjugate chain, and plays important roles in the nervous system and cell adhesion. In order to study the biological functions of sialoglycoconjugates, various chemical syntheses have been developed.[2,3] The difficulties of sialylation are that the anomeric position is a quaternary carbon with a high conformational barrier, 2,3-dehydrate forms easily, neighbouring group participation cannot be utilized, and the unnatural β-anomer is favoured due to greater thermodynamic stability. Hasegawa et al.[2] and Schmidt et al.[3] establish α-stereoselective sialylation using the 2-thioglycosides, 2-phosphites of sialic acids as the glycosyl donor, respectively. Since the above methods require strictly anhydrous conditions at lower temperature and use of a very strong acid, new sialylation reactions are being investigated.[4] Protection of hydroxy groups are also indispensable for all of the above attempts. An ideal synthesis, which does not require a strong acid, would be one in which the C-2 substituent is a good leaving-group, removable by a medium strength acid. On the other hand, sialoside alcohol containing oligosaccharides have not been a main theme of carbohydrate synthesis, though 1-carboxyl group of sialic acid is believed to have a big influence on *in vivo* reactions. Therefore, the purpose of this study is to establish a new mild synthetic method of such oligosaccharides, which would be useful for functional analysis of 1-carboxyl group.

We found an easy α-selective trans-glycosylation of sialic acid alcohol in the course of the $NaBH_4$-mediated reduction of the methyl ester of *p*-methoxyphenyl sialoside (Neu5Ac-OMP **1**). Precisely, reduction of the C-1 ester of sialic acid with $NaBH_4$ in MeOH was attempted (Scheme 1). Surprisingly, the product was not α-2-OMP sialoside alcohol **2** but α-2-methyl sialoside alcohol **3**. This unexpected reaction gives rise to two hypotheses; the C-1 carboxyl methyl group moved to the C-2 position, or, solvent MeOH replaced OMP group. Initially, the reduction with $NaBH_4$ in MeOH took place only 32%. Using EtOH instead of MeOH, **1** was reduced completely, giving α-2-methyl sialoside alcohol **3** in 66% yield, and hydrolyzate **4** in 11% yield. The first hypothesis would be rejected due to the fact that the reduction of ethyl ester **5** in EtOH followed by dilution with MeOH gave **3** as a major product (47%) and α-2-ethyl sialoside alcohol **6** as a minor one (11%). Conducting the reduction of **1** entirely in EtOH gave mainly **6** (53% yield) and **4** in 17% yield, supporting the second hypothesis. The spots of released HOMP and **6** were monitored by TLC after neutralization with sulfonate-type cation-exchange resin (Dowex-SO_3H), indicating that trans-glycosylation took place from intermediate alcohol **2**. Because **2** was isolated in 86% yield using AcOH for neutralization, OMP group was stable enough against AcOH (Table 1). When **2** was treated in MeOH with Dowex-SO_3H, α-selective trans-glycosylation with a methyl group was confirmed in 61% yield. Considering the above results, the reaction mechanism of trans-glycosylation is proposed as shown in Scheme 2. Dowex-SO_3H releases the OMP group from **2**, then, the C-1 alcohol forms an epoxide with the C-2 position. Finally, solvent alcohol attacks the C-2 position stereoselectively from the α-direction, producing **3** exclusively.



Scheme 1. Reduction of C-1 position of sialic acid. a) Reduction with $NaBH_4$ (3 equiv.), rt, overnight; b) Dilution; c) Neutralization.

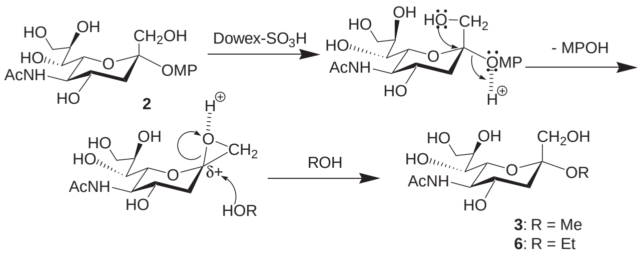
α-Selectivity of the trans-glycosylation was confirmed by comparing the product **3** with the authentic sample and its β-anomer **7**. Both α- and β-2-methyl sialic acids methyl ester[5] were reduced with $NaBH_4$. The ^{1}H NMR spectra of **3** reduced from **1** and authentic **3** are identical. The chemical shifts of H_{3e} and H_{3a} of them are lower by 0.0075 and 0.0085 ppm than those of β-anomer **7**.

A kinetic study of the trans-glycosylation of intermediate **2** to **3** was carried out by ^{1}H NMR at 23 and 0 °C. First, **2** was dissolved in CD_3OD, and then, Dowex-SO_3H was added to the sample tube. Trans-glycosylation at 23 °C completed in 5 m. At 0 °C, it took about 3.5 h, and allowed clear observation of the trans-glycosylation (Figure 1). Notably, the trans-glycosylation process was shown in the halfway of stacked spectra, and

Table 1. Reduction and trans-glycosylation

reactant	solvent for reduction	diluent	neutralization / acid catalyst	product	isolation yield/%
1	MeOH	MeOH	Dowex-SO_3H	**3**	32[a]
1	EtOH	MeOH	Dowex-SO_3H	**3**	66
1	EtOH	EtOH	Dowex-SO_3H	**6**	53
1	EtOH	MeOH	AcOH	**2**	86
2	—	MeOH	Dowex-SO_3H	**3**	61 (78*)

[a] Calculated yield from ^{1}H NMR spectra.



Scheme 2. Proposed reaction mechanism of C-1 reduction and trans-glycosylation.

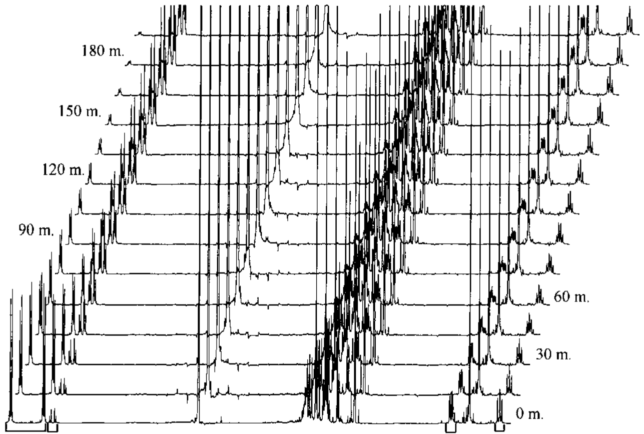


Figure 1. ^{1}H NMR spectra of kinetic experiment at 0 °C from 0 to 210 m by 15 m. H_{3a} (a) and H_{3e} (b) are slightly shifting to upfield. HOMP (c) is increasing. MP group (d) is decreasing.

the final spectrum of this experiment indicated the production of only **3** and HOMP. Thus, it is proved that the trans-glycosylation of **2** is a single reaction producing **3** exclusively.

Another kinetic study by ^{2}H NMR was performed under the same condition using C-1 deuteride of **2** (Figure 2). The signal due to ^{2}H-1of reactant has δ 3.14 (δ 7.27 for $CDCl_3$ as an internal reference), and the product has δ 3.00. Remarkably, there appeared a new signal (δ 2.72) on the way of stacked spectra and finally disappeared, indicating the formation of the intermediate epoxide.

Reduction of the per-acetate of **1** with $NaBH_4$ took place but always accompanied by deacetylation. Several other borohydrides (e.g. $NaBH_3CN$, $(CH_3)_3NBH_3$, and $Ca(BH_4)_2$) were tried with or without pH control, but they gave the same results as $NaBH_4$, or could not even induce the reduction.

The lability of the OMP group of **1** as a leaving-group was examined using Dowex-SO$_3$H. Though HOMP was released, it proceeded by heating at 40 °C for 15 h with low stereoselectivity. In the case of per-acetate of **1**, various acids other than Dowex-SO$_3$H, higher temperature, or longer time were required for releasing the OMP group, producing complex mixtures.

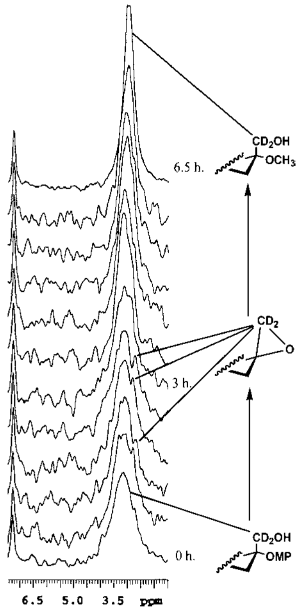


Figure 2. ^{2}H NMR spectra of kinetic experiment at 0 to 23 °C. ^{2}H-1 shifts to upper-field. The third signal attributes the epoxide, which only appears in the halfway.

In summary, α-selective trans-glycosylation assisted by the C-1 alcohol was found on all hydroxyl free sialoside alcohol **2**, due to the good leaving-group properties of the OMP group, through the intermediate epoxide. This is a single reaction, requires only 5 m at rt, and gives complete stereoselectivity and good yield. Applicability of this reaction for sialoside alcohol containing oligosaccharides and sialyl oligosaccharides synthesis is under investigation.

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