Sialic Acid Synthesis

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Three-Step Synthesis of Sialic Acids and Derivatives**

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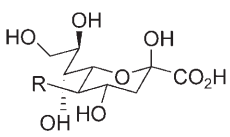
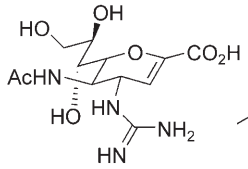
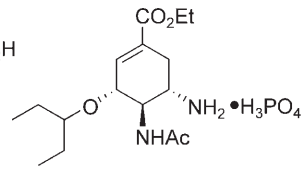
Sialic acids are a family of monosaccharides of which more than 50 natural derivatives have been identified.[1] The most well-known member of the family is *N*-acetylneuraminic acid (Neu5Ac, **1**). Other important members include the 5-glycolylamido derivative Neu5Gc (**2**) and the non-aminated derivative KDN (**3**). These compounds represent one of the most important constituents of glycoconjugates in biological systems. They are involved in cell–cell recognition, blood coagulation, fertilization, and other biological events.[2] The peripheral positioning of sialic acids also instigates their participation in the pathogenesis of a variety of diseases including inflammatory disease, cancer metastasis, and virus infection. As a result, increasing attention has been paid recently to the utilization of sialic acids and analogues as probes or inhibitors in biological and medical research.[3] Some of the sialic acid analogues have even achieved commercial success, such as Relenza (**4**) and Tamiflu (**5**) for the treatment of influenza.[4] Further studies on the roles of sialic acids in biology as well as medicine are highly warranted. However, a challenge remaining in this area is the development of an efficient method for the synthesis of sialic acids and analogues with a high degree of flexibility in structural and stereochemical alteration.[5] In this context several de novo syntheses of sialic acids have been reported.[6–8] Most of these, however, are not cost effective because the synthetic routes are lengthy and the overall yields are low. Herein we describe our own advance in this direction, in which a novel and interesting route was successfully developed to synthesize sialic acids and derivatives from inexpensive starting materials with more satisfactory proficiency.



1 Neu5Ac, R = NHAc
2 Neu5Gc, R = NHGc
3 KDN, R = OH



4 Relenza



5 Tamiflu

We began our study with the synthesis of L-*N*-acetylneuraminic acid (**6**, L-Neu5Ac), which is the enantiomer of natural D-Neu5Ac (**1**). A potential application of the synthesis was to provide a sufficient quantity of L-Neu5Ac (**6**) for screening L peptides (by phage display) or D nucleic acids (by aptamer selection) that can bind L sugars.[9] To maximize the synthetic efficiency, we designed the retrosynthetic route shown in Scheme 1. The key steps in the synthesis include: 1) the simultaneous introduction of an amino group and a vinyl group to an aldose by means of the newly developed Petasis coupling reaction[10] and 2) the concise conversion of the vinyl group to a γ-hydroxy-α-keto acid moiety by means of a diastereoselective 1,3-dipolar cycloaddition reaction and subsequent ring opening. The scope of the proposed method was expected to be highly flexible, because by utilizing various types of aldoses (or polyhydroxy aldehydes) we would be able to obtain an unlimited number of sialic acid derivatives. At the same time, the efficiency of the method would be maximized if any protection and deprotection of the hydroxy groups could be strategically avoided.

The standard Petasis reaction is a three-component condensation of an α-hydroxy aldehyde, a primary or secondary amine, and a substituted vinyl boronic acid (or aryl boronic acid). However, our initial implementation of this reaction with an aldose was not successful until we realized that the unsubstituted vinyl boronic acid is an unstable compound that deteriorates rapidly.[11] In searching for a solution to this problem, we discovered that the commercially available dibutyl vinyl boronic acid ester could also be used in the Petasis coupling. An interesting

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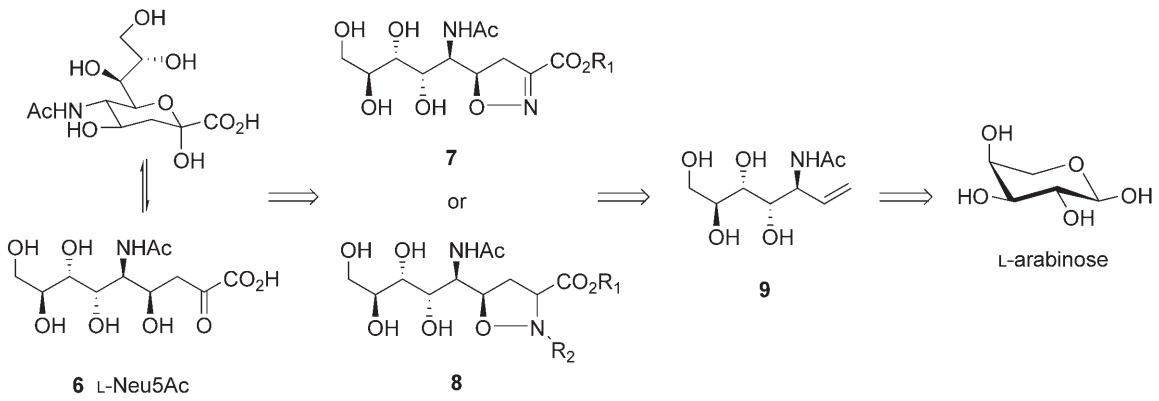
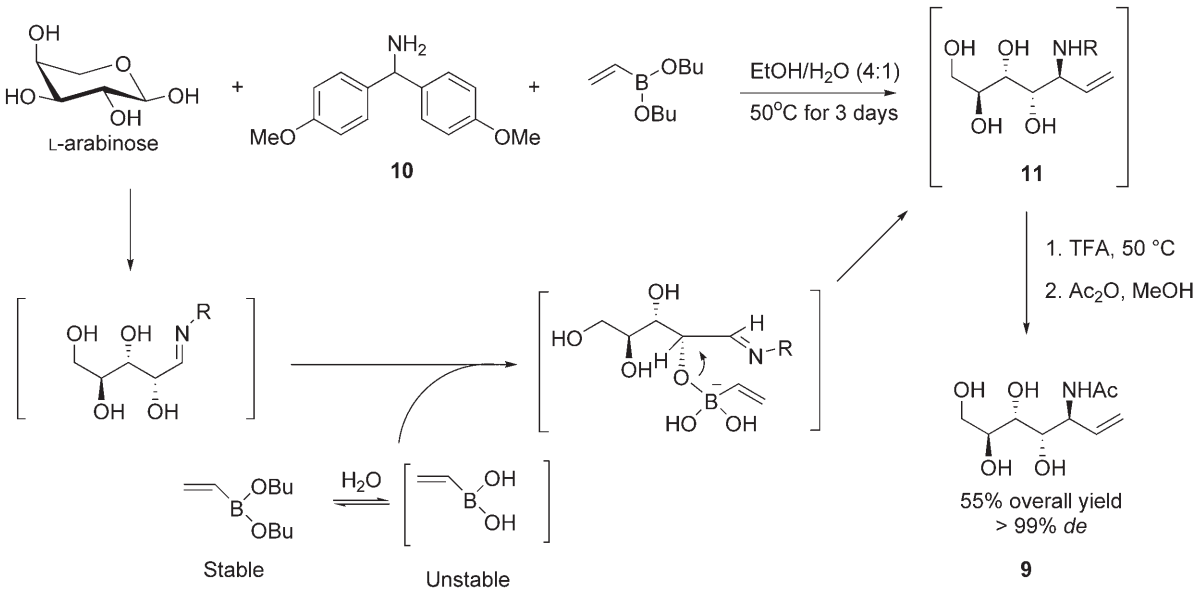


Scheme 1. Retrosynthesis of L-Neu5Ac (**6**).

aspect of this new finding was that water is necessary in the coupling of the boronic acid ester. This indicates that an in situ equilibrium between the boronic acid ester and boronic acid might occur under the reaction conditions (see Scheme 2). Owing to this exchange process, the coupling of dibutyl vinyl boronic acid ester had to be performed at a slightly higher reaction temperature (50 °C) and for a longer reaction time (72 h) in EtOH/H_2O (4:1) as compared to the standard conditions for the Petasis reaction (25 °C, 24 h, in pure EtOH).

Having solved the problem of incorporation of an unsubstituted vinyl group, we next successfully accomplished a one-pot synthesis of the key intermediate **9** (Scheme 2). In designing such a one-pot reaction, we confirmed through the NMR analysis of the crude reaction mixture that intermediate **11** was obtained as a nearly single diastereomer (> 99 % *de*). Rather than isolating compound **11**, we treated the reaction mixture with a catalytic amount of trifluoroacetic acid (TFA) and stirred the reaction overnight. After the bis(4-methoxyphenyl)methyl group had been successfully removed from the amino group, selective acetylation of the amino group was accomplished by treatment with Ac_2O/MeOH. At this stage we performed flash column chromatography to isolate compound **9**, which was obtained as a single diastereomer (> 99 % *de* as determined by ^1H and ^{13}C NMR analysis) in about 55 % yield as calculated from the starting material L-arabinose.[12]

With intermediate **9** in hand, we turned our attention to the next challenge—the conversion of the vinyl group in **9** into a γ-hydroxy-α-keto acid moiety. Our proposal was to accomplish this conversion by means of a tandem 1,3-dipolar cycloaddition and ring-opening reaction without any protection of the hydroxy groups. In our first attempt, we utilized a nitrile oxide ((*E*)-*N*-(2-ethoxy-2-oxoethylidene)methanamine oxide) as the dipole.[13] Unfortunately, although the 1,3-dipolar cycloaddition between this nitrile oxide and compound **9** proceeded smoothly to provide compound **7**, we could not selectively cleave the N–O bond in the dihydroisoxazole ring because of the presence of the C=N bond. Having failed with the nitrile oxide, we next tried to use



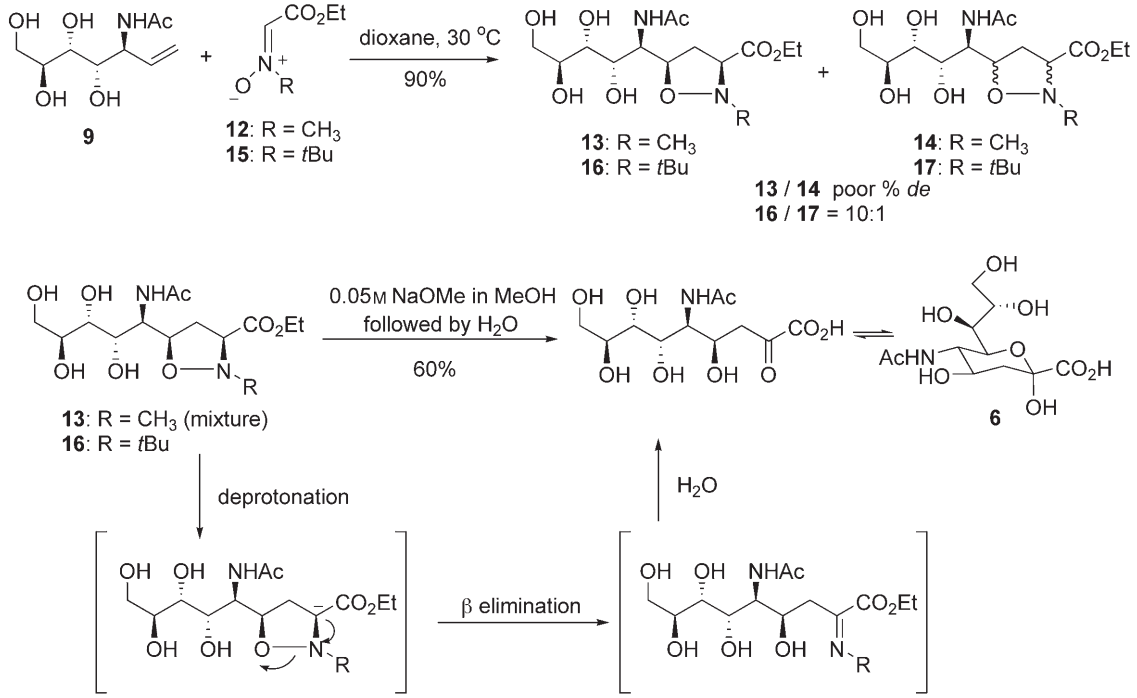
Scheme 2. One-pot synthesis of intermediate **9** and the possible mechanism of the boronic ester version of Petasis reaction. R = bis(4-methoxyphenyl)methyl.

a nitrone in the 1,3-dipolar cycloaddition.[14] It is noteworthy that in the proposed product derived from a nitrone, the carbon atom at the α position relative to the acid group would not have the desired oxidation state (see Scheme 3). Without much literature precedence, we hypothesized that a base could be used to deprotonate the α-carbon and this might prompt the β elimination of oxygen from the nitrogen atom, affording the desired α-keto group. If this hypothesis were valid, we would obtain the γ-hydroxy-α-keto acid moiety from a vinyl group in an unprecedented way. To test our hypothesis, we carried out the 1,3-dipolar cycloaddition between compound **9** and the *N*-methyl nitrone **12** (Scheme 3). Without much difficulty, the desired product was successfully obtained in fairly good yields (>80%). Nevertheless, we were disappointed to observe a complex mixture of diastereomers in the product. Considerable attempts were carried out to improve the diastereoselectivity by changing the solvent (dioxane, MeOH, and EtOH), reaction temperature (from room temperature to 80°C), and the reactant concentration (from neat to 0.05 M). Unfortunately none of the conditions were satisfactory.

At this stage we went on to test the deprotonation/β-elimination hypothesis, because at least one of the chiral centers (i.e. the chiral center at the α position relative to the ester group) would disappear anyhow. Therefore, we treated the product mixture with NaOMe (0.05 M in MeOH) followed by an aqueous workup. The hypothesized reaction proceeded smoothly, affording L-Neu5Ac (and its 4-epimer) as the final products in 60% yield. (Note: the ester group was also hydrolyzed under the same reaction conditions.) ^{1}H and ^{13}C NMR analyses showed that the ratio between L-Neu5Ac and its 4-epimer was about 3:1, which resulted from the relatively poor diastereoselectivity in the 1,3-dipolar cycloaddition. To improve the diastereoselectivity, we tested different nitrones in the 1,3-dipolar addition. After some literature searching,[15] it came to our attention that the *N*-methyl nitrone **12** (Scheme 3) should exist as a mixture of *cis* and *trans* isomers. In contrast, when a bulky *tert*-butyl group is attached to the nitrogen atom, the resulting nitrone **15** should stay predominantly in the *cis* form. Taking these facts into consideration, we expected that improved diastereoselectivity could be attained by using the *N-tert*-butyl nitrone **15** in the 1,3-dipolar addition reaction.

To our satisfaction this hypothesis turned out to be correct. Thus, when the *N-tert*-butyl nitrone **15** was utilized, only two of the four possible isomers were produced in about 90% yield from the 1,3-dipolar cycloaddition. More importantly, these two isomers were found to have markedly different R_f values by TLC analysis (R_f(major) = 0.56 vs. R_f(minor) = 0.61 in 1:4 MeOH/CH_2Cl_2). Consequently it was fairly easy to separate the two isomers by standard flash column chromatography. NOE analyses of the purified products indicate that the major isomer is compound **16.**[16] Solvent, temperature, and reactant concentration were found to exert profound influences on the product ratio **16**/**17**. Under the optimal conditions (dioxane, 30°C, reactant concentration 0.05 M), the product ration was eventually optimized to be 10:1. Next, the purified compound **16** was subjected to the base-catalyzed β elimination, which successfully afforded L-Neu5Ac (**6**) in 60% yield. ^{1}H and ^{13}C NMR spectra of the synthetic L-Neu5Ac (**6**) were identical to the previously reported data for D-Neu5Ac.[17]

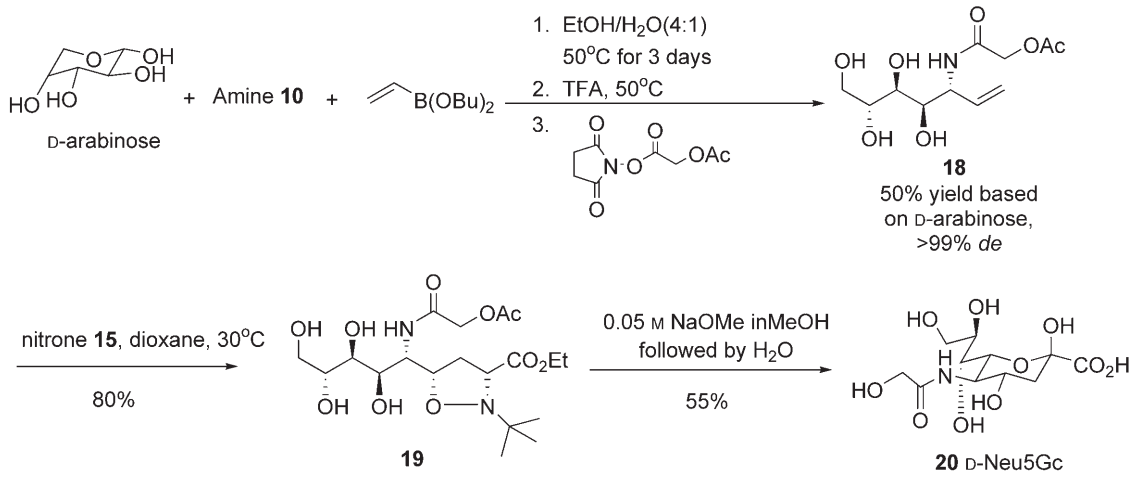
Thus, at this point we have successfully synthesized L-Neu5Ac from readily available L-arabinose. Starting from D-arabinose we also successfully synthesized D-Neu5Ac by the same three steps with an overall yield of 28%. It is important



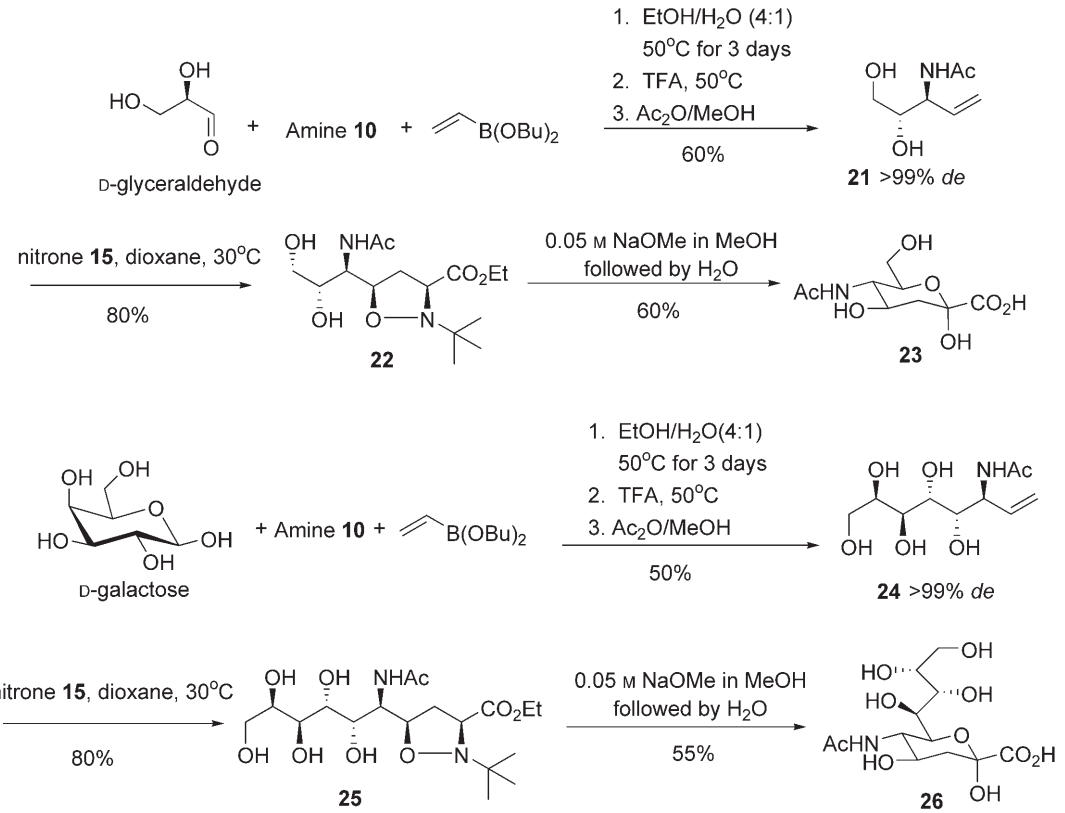
Scheme 3. Synthesis of L-Neu5Ac (**6**).

to emphasize that the newly developed synthetic method is highly flexible for the synthesis of sialic acid analogues, because various aldose sugars (or polyhydroxy aldehydes) can be employed as the starting material. To demonstrate this, we describe further syntheses of some interesting sialic acid derivatives.

D-*N*-Glycolylneuraminic acid (**20**, D-Neu5Gc; Scheme 4): D-Neu5Gc is the hydroxylated derivative of D-Neu5Ac. It is produced in many non-human mammals, yet it typically is not detectable in humans.[18] It has been proposed that the presence of D-Neu5Gc on the cell surface in non-human primates may provide immunity to HIV infection in such species. Our synthesis of D-Neu5Gc began from commercially available D-arabinose. Using the aforementioned one-pot Petasis coupling we obtained compound **18** in 50% yield (>99% *de*), which was *N*-acylated by the activated ester of 2-acetoxyacetic acid (Scheme 4). Next, compound **18** was coupled with the *N*-*tert*-butyl nitrone **15** to afford compound **19** in 80% yield after separation from the undesired isomer (d.r. 12:1) by column chromatography. Finally, base-catalyzed β elimination and ester hydrolysis of **20** gave the target product in 55% yield. Thus, D-Neu5Gc was successfully



Scheme 4. Synthesis of D-Neu5Gc (**20**).



Scheme 5. Synthesis of truncated and elongated sialic acids.

synthesized from readily available D-arabinose in three steps with an overall yield of 22%.

Truncated and elongated sialic acids: Some synthetic methods have been developed in the past for the preparation of 7-carbon analogues of sialic acids.[19] Herein, our own synthesis of the seven-carbon sialic acid analogue **23** was started from D-glyceraldehyde. Through the aforementioned "one-pot" Petasis coupling (60% yield, >99% *de*), 1,3-dipolar cycloaddition (80% yield, d.r. 8:1), and base-catalyzed β elimination and ester hydrolysis (60% yield), the target compound (**27**) was successfully obtained with an overall yield of 29% (Scheme 5). Similarly, we also synthesized the first ten-carbon analogue of sialic acid (**26**) in three steps from D-galactose with an overall yield of 22%.

In summary, in the present study we have developed a novel, efficient method for the synthesis of sialic acids and derivatives in only three steps from readily available starting materials. A boronic ester version of the Petasis coupling was developed, which facilitated the coupling of an unsubstituted vinyl group to an unprotected aldose. Next a highly diastereoselective 1,3-dipolar cycloaddition reaction was established that enabled the facile conversion of a vinyl group to an isoxazolidine ring carrying an ester group. Subsequently we discovered an unprecedented base-catalyzed ring-opening reaction for the direct conversion of the isoxazolidine to a γ-hydroxy-α-keto acid. All three reactions were completely compatible with the unprotected hydroxyl groups in carbohydrates. Armed with this novel approach, we successfully prepared several important, yet very expensive, sialic acid derivatives, including L-*N*-acetylneuraminic acid, D-*N*-glycolylneuraminic acid, a seven-carbon truncated analoguie, and a ten-carbon elongated analogue of sialic acid, in an economically competitive fashion.

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[16] In the ROESY NMR spectrum of compound **16**, there are strong H3α–H2 and H3α–H4 NOE correlations. A relatively weaker NOE correlation is also observed between H2 and H4. However, there is no NOE correlation between H3β and H2 (or between H3β and H4). These data suggest that compound **16** should be the 2*S*,4*R* isomer.

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