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Efficient Synthesis of 4-Amido-N^5-acetyl-4-deoxyneuraminic Acid and Its Application to the C-4 Modification of Sialic Acids

Zheng-Xi Gao,[†] Meng Wang,[‡] Shaozhong Wang,[‡] and Zhu-Jun Yao*[,†,‡]

State Key Laboratory of Bioorganic & Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 345 Lingling Road, Shanghai 200032, China, and Nanjing National Laboratory of Microstructures, School of Chemistry and Chemical Engineering, Nanjing University, 22 Hankou Road, Nanjing, Jiangsu 210093, China

yaoz@mail.sioc.ac.cn; yaoz@nju.edu.cn

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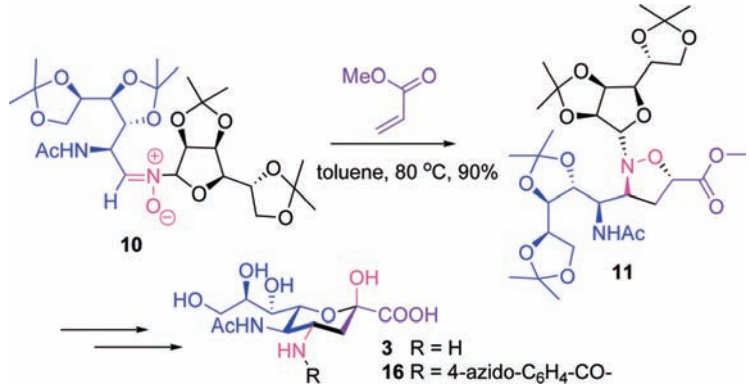
ABSTRACT



A straightforward synthesis of 4-amido-N^5-acetyl-4-deoxyneuraminic acid, a key precursor to various 4-amidoneuraminic acid analogues, has been achieved using a highly regioselective and diastereoselective [3 + 2]-cycloaddition of D-mannose-derived nitrone with methyl acrylate. Advantages of this newly developed synthesis include the use of economically available materials and reagents, the ease of operations and the excellent control of stereochemistry, as well as the convenience in application to the C-4 modifications of sialic acids.

Sialic acids are a unique family of monosaccharides in which more than 50 natural sialic acid derivatives have been identified.[1] Among these natural nine-carbon sugars, *N*-acetylneuraminic acid (Neu5Ac, Figure 1, **1**) is one of the most well-known members. In biological systems, they represent one of the most important constituents of glycoconjugates.[2] More importantly, some sialic acid-derived analogues have even achieved commercial successes in the drug market. For example, Relenza (**2**), a Neu5Ac analogue with variation at the C-4 position by substituting the 4-OH group with a guanidinyl group, has been developed as a useful drug for the treatment of type A influenza.[3] For obtaining more diverse C-4 modified Relenza-like 4-ami-

[†] Chinese Academy of Sciences.
[‡] Nanjing University.

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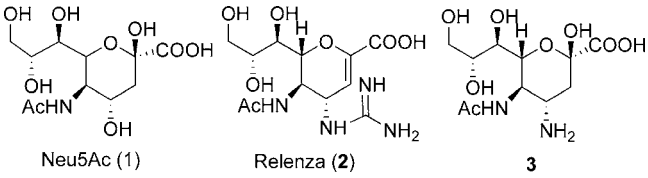

Figure 1. Neu5Ac (**1**), Relenza (**2**), and 4-amido-N^5-acetyl-4-deoxyneuraminic acid (**3**).

dosialic acid analogues, 4-amido-*N*-acetyl-4-deoxyneuraminic acid (**3**) would be an excellent common intermediate perfectly suiting the principles of diverse organic synthesis. However, few success has been achieved since the first synthesis of **3** using nonselective nitrile oxide cycloaddition with silylenopyruvate and catalytic hydrogenation of 4-oxime intermediate (1.3:1 diastereoselectivity) as key steps in 1998.[4] For today's global demand for new antiflu drugs, further studies of this useful 4-amidosialic acid derivative are thus of extreme importance for medicinal chemistry and related biomedical investigations with various sialic acid-processing enzymes.

Neu5Ac has been used as the common starting material in most commercial syntheses of sialic acid analogues, including the industrial production of Relenza.[5] Considering the relatively high cost of Neu5Ac,[6] it would be of considerable value to establish alternative routes to those useful sialic acid analogues using more economic and readily available starting materials. With such considerations, we once developed a formal synthesis of Relenza from the inexpensive sugar material D-glucono-δ-lactone in 2004.[7] In this paper, we want to report a new efficient and straightforward synthesis of 4-amido-N^5-acetyl-4-deoxyneuraminic acid (**3**) from D-glucono-δ-lactone. An azide-free protocol was adopted in this work for introducing the C-4 amido functionality by using a highly regioselective and diastereoselective 1,3-dipolar cycloaddition reaction of the corresponding nitrone and methyl acrylate.

The 1,3-dipolar cycloaddition reaction is useful in the syntheses of various natural and unnatural compounds.[8,9] Isoxazolidines, the products of cycloaddition of nitrone and olefin, are frequently employed as building blocks in synthesizing various bioactive alkaloids, amino sugars, amino acids, as well as some useful 1,3-amino alcohols after cleavage of the N−O bond (Figure 2). With proper optimi-

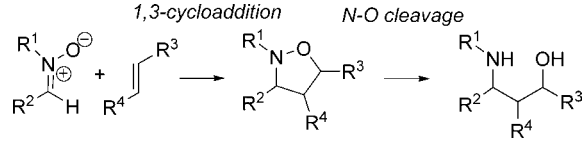

Figure 2. Substituted 1,3-amino alcohols via 1,3-dipolar cycloaddition of nitrones.

zations of conditions and substrates, satisfactory regioselectivity, stereoselectivity, and efficiency can be achieved through the concerted mechanisms.[10] For instance, 1,3-dipolar cycloaddition reaction of nitrone and olefin can introduce a new amino functionality and create as many as three new contiguous stereogenic centers in a single step.

Retrosynthetically, the α-keto ester of the open chain precursor **4** for 4-amido-N^5-acetyl-4-deoxyneuraminic acid (**3**) can be prepared from the corresponding α-hydroxy carboxylate **5** (Figure 3). Linking C-2 oxygen and C-4

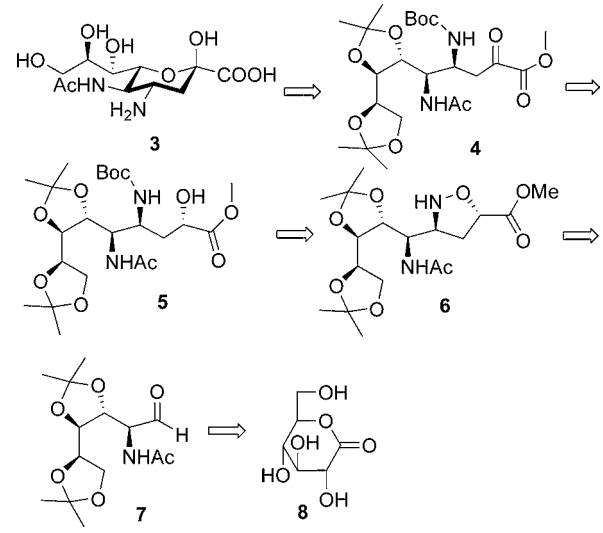

Figure 3. Retrosynthesis of 4-amido-N^5-acetyl-4-deoxyneuraminic acid (**3**).

nitrogen functionalities of compound **5** gives the isoxazolidine **6**. This isoxazolidine **6** can be easily prepared by the 1,3-dipolar cycloaddition of a proper nitrone derived from the known aldehyde **7**[11] and commercially available methyl acrylate. Aldehyde **7** can be conveniently prepared from the cheap material D-glucono-δ-lactone **8** in large scales.[7,11]

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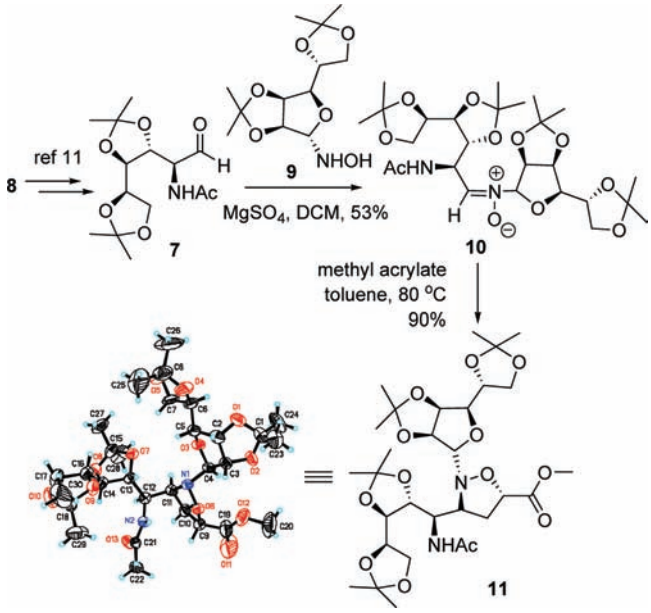
Sugar−aldehyde **7** was prepared from D-glucono-δ-lactone **8** using the procedures of our previous work[11] (Scheme 1).

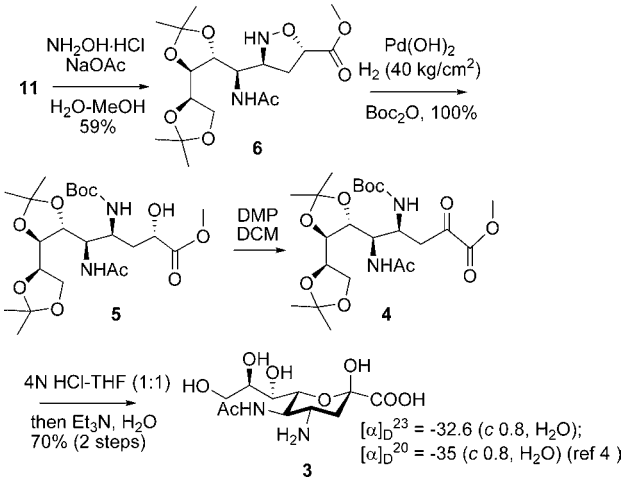
Scheme 1. 1,3-Dipolar Cycloaddition of Nitrone **10** and Methyl Acrylate



Treatment of aldehyde **7** with the sugar-derived hydroxylamine **9**[12] in the presence of $MgSO_4$ afforded the nitrone **10** as an air-stable solid. To our delight, 1,3-dipolar cycloaddition of nitrone **10** (bearing Vasella's sugar auxiliary[13]) and methyl acrylate provided the excellent diastereoselectivity.[14,15] Simply heating the mixture of the nitrone **10** and methyl acrylate in toluene afforded a major adduct isoxazolidine **11** in 90% isolated yield. However, our further attempts to expand the reaction scope of nitrone **10** with other unsaturated substrates failed to achieve satisfactory regioselectivity and diastereoselectivity, including the reaction with methyl propiolate. These results indicate that the influencing factors of the above 1,3-dipolar cycloaddition are considerably complicated and sensitive. Cycloaddition between nitrone **10** and methyl acrylate is one successful case in which all the structural elements are perfectly matched to give the desired adduct **11** with correct stereochemistries. Characterizations of isoxazolidine **11** were accomplished by 1D and 2D NMR studies and finally confirmed by an X-ray single-crystallographic analysis (see the Supporting Information).

Isoxazolidine **11** was then converted to the corresponding 1,3-amino alcohol by reductive cleavage of the N−O bond. At first, sugar auxiliary was removed from isoxazolidine **11** with hydroxylamine hydrochloride and NaOAc in a mixed solvent of MeOH−H_2O (3:1) at 70 °C (Scheme 2). Optically

Scheme 2. Completion of the Synthesis of **3**



active isoxazolidine **6** can be isolated in 59% yield after purification using routine silica gel column chromatography. However, the following reductive cleavage of the N−O bond of isoxazolidine **6** was troublesome in our initial attempts. Reactions using the commonly used conditions[16] such as Zn−acetic acid, H_2−Raney Ni, or H_2−Pd/C all failed to give the desired amino alcohol. Finally, in the presence of Boc_2O, hydrogenation of isoxazolidine **6** with catalytic amount of 20% $Pd(OH)_2$ proceeded well (40 kg/cm^3) and completed in 24 h, affording the N^4-Boc α-hydroxyl carboxylate **5** in quantitative yield. Dess−Martin oxidation of the newly born hydroxyl group of **5** provided the α-keto carboxylic acid methyl ester **4**. Without further purification, crude material **4** was directly treated with 4 N HCl in THF and then hydrolyzed with 20% Et_3N−H_2O to give the target 4-amido-N^5-acetyl-4-deoxyneuraminic acid (**3**, 70% yield for two steps). All physical data of **3** are in good agreement with those reported.[4]

As mentioned above, this newly established synthesis of 4-amido-N^5-acetyl-4-deoxyneuraminic acid (**3**) opens a new entrance to various C-4 modifications of Neu5Ac with biological interests. With considerations of future applications to the cell-surface engineering by ligation reactions,[17] a C-4 azido-functionalized Neu5Ac derivative **16** was therefore designed and synthesized using isoxazolidine **6** as the starting intermediate (Scheme 3). *N*-Acylation of **6** with 4-azido

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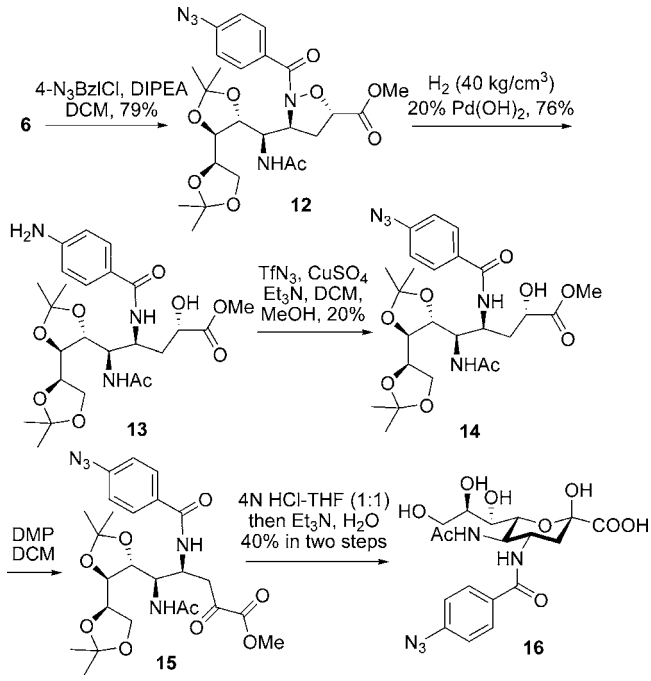
Scheme 3. Synthesis of the C-4-Modified Sialic Acid Derivative **16**



benzoic acid chloride followed by catalytic hydrogenation (40 kg/cm^3) afforded the amide **13** (60% in two steps). Treatment of aniline **13** with triflyl azide[18] in the presence of anhydrous $CuSO_4$ and Et_3N in DCM and MeOH provided the corresponding azido compound **14** in 20% yield. Dess−Martin oxidation of the alcohol **14** afforded the corresponding α-keto ester **15**. Final treatment of **15** with 4 N HCl in THF provided the C-4 azido functionalized Neu5Ac derivative **16** (40% yield for two steps).

In summary, a new straightforward efficient synthesis of 4-amido-N^5-acetyl-4-deoxyneuraminic acid starting from the cheap material D-glucono-δ-lactone has been reported. The highly regioselective and diastereoselective 1,3-dipolar cycloaddition of D-mannose-derived nitrone with methyl acrylate was developed and successfully applied in the synthesis. This newly established azide-free route is advantageous in using economically available materials, excellent control of stereochemistry, as well as the convenience in application to the C-4 modifications of sialic acids. Utilizing the isoxazolidine intermediate, a new C-4 azido-functionalized Neu5Ac analogue was synthesized with the established methodologies. Further application of our protocol to additional C-4 amido neuraminic acid derivatives of biological interests is currently under investigation in this laboratory.

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Supporting Information Available: Experimental details and characterizations of new compounds, copies of ^{1}H NMR and ^{13}C NMR spectra of new compounds and X-ray single-crystal data of compound **11** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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