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De novo chemoenzymatic synthesis of sialic acid†

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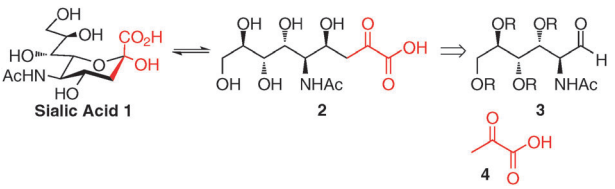
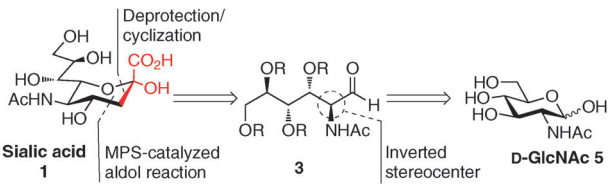
A chemoenzymatic synthesis of sialic acid from inexpensive *N*-acetyl-D-glucosamine is described. In a three-step Wittig–protection–ozonolysis strategy *manno*-configured aldehydes are obtained. Treatment with oxaloacetate in the presence of macrophomate synthase affords the signature α-keto-γ-hydroxy acid moiety with high diastereoselectivity.

Sialic acid **1** (Scheme 1) and its derivatives are ubiquitous in both mammalian and bacterial glycomes.[2] In mammals, sialic acid is typically located at the distal end of glycan chains. As a consequence, sialic acid is a key player in a variety of important biological processes, including cell–cell and cell–pathogen communication as well as tumor metastasis.[3] Surface glycoproteins of the influenza virus, for instance, recognize sialic acids on human cells in the upper respiratory tract and use them for virus entry. In a later step, budding of virus progeny from the infected cell is achieved by the action of viral sialidases that detach the viruses from the host cell.[4] As a consequence, chemically homogeneous glycans containing sialic acids and derivatives thereof have proven to be valuable as drugs and as formidable tools in biomedical research.[5]

Structurally, sialic acids differ from other common mammalian carbohydrates in several important respects: their backbones are nine carbon atoms long; they contain either a free or acylated amine at C5; and they terminate in an α-keto acid moiety (Scheme 1). These structural features render their synthesis inherently difficult. Although many elegant approaches for preparing sialic acid derivatives have been described in recent years,[6] short, flexible, and inexpensive routes to these compounds are still needed.



Scheme 1 Sialic acid **1** and its open chain form **2** with the α-keto acid motif highlighted in red. A retrosynthetic disconnection of sialic acid **1** reveals protected *manno*-configured aldehyde **3** and pyruvic acid **4** as generic precursors.



Scheme 2 Retrosynthesis of sialic acid **1** starting from *N*-acetyl-D-glucosamine **5**.

De novo syntheses that transform inexpensive and commercially available starting materials into carbohydrates or carbohydrate building blocks *via* (dia)-stereoselective C–C-bond-forming reactions have rendered many glycans chemically accessible.[7] For sialic acids, such an approach would entail introduction of the labile α-keto acid motif at a later stage by means of a stereoselective pyruvate addition to a protected aldehyde like **3** (Scheme 2). However, to date, no single chemical method has been developed that addresses this problem satisfactorily.[8]

We recently showed that the enzyme macrophomate synthase[1] (MPS) is an excellent catalyst for the stereoselective installation of the α-keto acid motif[9] and can be used for the chemoenzymatic synthesis of 3-deoxysugars such as keto-deoxy-nonulosonic acid (KDN).[10] Here, we report that this strategy also provides a convenient chemoenzymatic route to sialic acid. While there are numerous examples of enzymatic syntheses of sialic acids,[6h] the key strategic advantage of MPS is its unusual tolerance to a wide range of protecting groups. Protected building blocks are essential to allow the seamless integration of sialic acid derivatives into synthetic strategies towards large complex carbohydrates.

According to the retrosynthetic analysis shown in Scheme 2, protected aldehydes with D-*manno*-configuration are needed to access sialic acids. Since *N*-acetyl-D-mannosamine itself is prohibitively expensive (~$40 per g, Sigma-Aldrich),

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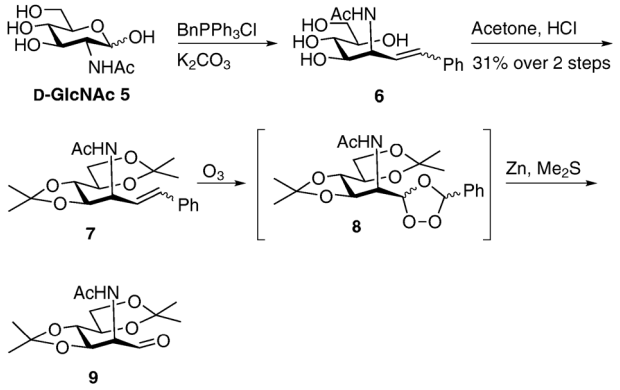
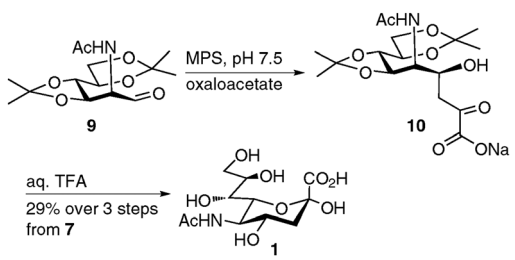


Scheme 3 Synthesis of *manno*-configured aldehyde **9** from D-GlcNAc *via* a Wittig–protection–ozonolysis sequence.

N-acetyl-D-glucosamine **5** (~$1 per g, Sigma-Aldrich) was utilized as an inexpensive alternative starting material.

Conversion of D-GlcNAc into *manno*-configured aldehydes **3** requires an inversion step and subsequent transformation to the open chain form. Subjecting **5** to the Wittig reagent benzylidene-(triphenyl)phosphorane ($BnPPh_3Cl/K_2CO_3$) accomplished both tasks (Scheme 3).[11] Due to the basic reaction conditions it is likely that equilibration of the open chain epimers D-GlcNAc and D-ManNAc occurs.[11] Reaction of the ylid with open chain D-ManNAc then leads to the formation of olefin **6**. Treatment of tetrol **6** with acetone and aqueous hydrochloric acid gave *bis*-dioxolane **7**, which was subsequently oxidized with ozone. Because α-amino aldehydes are inherently unstable,[12] they are not typically isolated or purified.[13] However, quantitative conversion without racemization was mandatory to provide a pure substrate for the subsequent aldol reaction. Unfortunately, reductive work-up of the intermediate ozonide **8** to yield aldehyde **9** proved difficult.[14] None of the standard reduction protocols involving zinc and either triphenyl phosphine or dimethylsulfide gave satisfactory yields, and epimerization of the carbon bearing the acetamido group was often observed. Nevertheless, careful optimization revealed that a mixture of finely dispersed zinc and dimethylsulfide reduced the ozonide quantitatively without any observable epimerized by-product after 24 h. The reaction proceeded cleanly and no chromatographic purification was required. Starting from D-GlcNAc, aldehyde **9** could thus be easily obtained in only three steps. This short and general route provides ready access to protected *manno*-configured aldehydes.

With aldehyde **9** in hand, the enzymatic addition of a pyruvate equivalent was investigated (Scheme 4). As α-acetamido aldehydes are particularly prone to epimerization at the α-position, neither strongly acidic nor strongly basic conditions could be used to



Scheme 4 Diastereoselective addition of pyruvate to aldehyde **9** using macrophomate synthase (MPS).

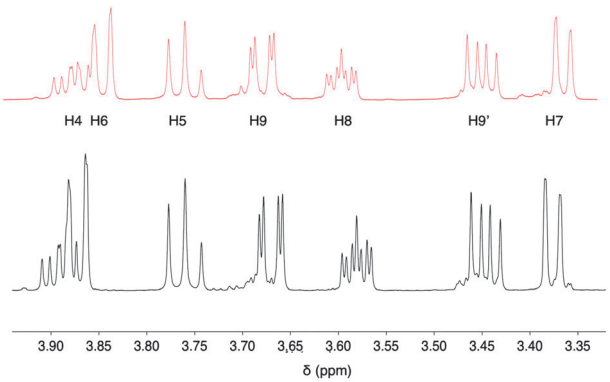


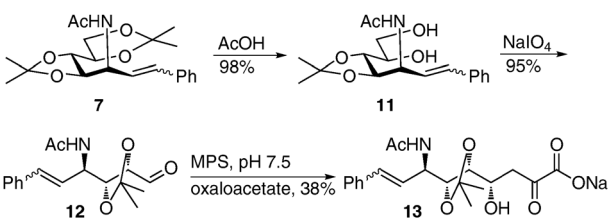
Fig. 1 ^{1}H NMR spectra of synthetic sialic acid **1** (bottom, black) and synthetic sialic acid spiked (1 : 1) with commercially available sialic acid, Sigma-Aldrich (top, red). Note: sialic acid occurs in aq. solution as a mixture of α/β-diastereomers.[15]

accomplish this task. The MPS-catalysed addition reaction takes place at near-neutral pH and thus provides an extremely mild means to add the pyruvate moiety. The reaction was performed in aqueous buffer with 5 μM MPS and magnesium(II) as a cofactor. The pyruvate enolate nucleophile is generated *in situ* by decarboxylation of oxaloacetate. Careful optimization of the reaction conditions showed that the best results were obtained at pH 7.5 and 25 °C to provide the desired 4*S* alcohol **10** as the only diastereoisomer.

^{1}H NMR analysis of the crude, deprotected cyclization product **1** was used to assess the diastereoselectivity of the enzymatic addition reaction and to provide unambiguous proof of the C4 configuration (Fig. 1). To that end, *bis*-dioxolane **10** was treated with aqueous trifluoroacetic acid to give sialic acid **1**. Fig. 1 shows the ^{1}H NMR spectrum of the crude product resulting from the deprotection of **10** (bottom). Additionally, the crude product was spiked (1 : 1 w/w) with commercially available sialic acid (top). The data show that the stereoselectivity of the MPS-catalysed pyruvate addition is excellent, with a dr > 20 : 1 in favor of the desired 4*S* diastereomer. Thus, *manno*-configured aldehydes are excellent substrates for MPS.

The outlined MPS strategy was also applicable for the synthesis of sialic acid analogs. From the same common precursor, olefin **7**, a protected γ-acetamido aldehyde, was obtained (Scheme 5). Selective hydrolysis of the terminal isopropylidene acetal of *bis*-acetal **7**, and subsequent oxidative cleavage of diol **11** gave aldehyde **12** in 93% yield over two steps. The aldehyde was an excellent substrate for MPS in the pyruvate addition reaction, with only a single C4 epimer of a sialic acid analog **13** observed.

In summary, we describe a concise route to sialic acid and its derivatives starting from inexpensive *N*-acetyl-D-glucosamine.



Scheme 5 Synthesis of a protected sialic acid analog **13** from common precursor **7**.

A three-step Wittig–protection–ozonolysis sequence provides *manno*-configured, *O*-protected aldehydes, which are then condensed with pyruvate enolate in a diastereoselective reaction catalysed by the aldolase MPS. The desired *S* selectivity for the newly formed stereogenic carbinol center proved to be excellent with a dr > 20 : 1.

The high diastereoselectivity, the mild conditions of the MPS-catalysed pyruvate addition, as well as its tolerance to a variety of protecting groups open up possibilities to access fully protected sialic acid for the assembly of sialic acid-containing oligosaccharides, novel sialidase inhibitors, and fluorinated sialic acids. The latter are the subject of ongoing studies in our laboratories.

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