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Chemoenzymatic synthesis of C8-modified sialic acids and related α2–3-and α2–6-linked sialosides

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ABSTRACT

Naturally occurring 8-*O*-methylated sialic acids, including 8-*O*-methyl-*N*-acetylneuraminic acid and 8-*O*-methyl-*N*-glycolylneuraminic acid, along with 8-*O*-methyl-2-keto-3-deoxy-D-glycero-D-galacto-nonulosonic acid (Kdn8Me) and 8-deoxy-Kdn were synthesized from corresponding 5-*O*-modified six-carbon monosaccharides and pyruvate using a sialic acid aldolase cloned from *Pasteurella multocida* strain P-1059 (PmNanA). In addition, α2–3- and α2–6-linked sialyltrisaccharides containing Neu5Ac8Me and Kdn8Deoxy were also synthesized using a one-pot multienzyme approach. The strategy reported here provides an efficient approach to produce glycans containing various C8-modified sialic acids for biological evaluations.

Sialic acids have been widely found in higher animals and some microorganisms. They are commonly found at the termini of the glycan chains on glycoproteins and glycolipids.[1] Sialic acids constitute a structurally diverse family of nine-carbon acidic monosaccharides with more than 50 members having been identified. *N*-Acetylneuraminic acid (Neu5Ac), *N*-glycolylneuraminic acid (Neu5Gc), and 2-keto-3-deoxy-D-glycero-D-galacto-nonulosonic acid (Kdn) are the three basic forms of sialic acids which are distinguished from one another by different substituents at carbon-5.[2–4] Additional modifications at different hydroxyl groups of sialic acids include *O*-acetylation as the most frequently occurring modification. 8-*O*-Methylation of sialic acids is also commonly observed. For example, 8-*O*-methylated sialic acids have been reported in starfish *Asterias rubens* as the components of gangliosides[5–8] and glycoproteins.[9] Several 8-*O*-methylated sialic acid forms observed include 8-*O*-methyl-*N*-acetylneuraminic acid (Neu5Ac8Me **1**, Fig. 1), 8-*O*-methyl-*N*-glycolylneuraminic acid (Neu5Gc8Me **2**, Fig. 1), and their *O*-acetylated derivatives.[10,11] Neu5Ac8Me has also been found in the sperm and eggs of teleost fish,[12] in human red blood cell membrane,[13] and in mouse tissues.[14] As 8-*O*-methylated sialic acids are resistant to sialidases,[15] 8-*O*-methylation of sialic acid may play important biological roles. Nevertheless, the significance of naturally occurring C8-modified sialic acid derivatives is currently unclear.

Only a few methods have been reported for chemical synthesis of sialosides containing Neu5Ac8Me.[16–18] These methods, however, are inefficient, lengthy, and tedious. In comparison, enzyme-catalyzed reactions often offer great advantages and are considered attractive and practical approaches for the synthesis of sialosides including those containing uncommon sialic acid forms. Recently, Withers et al. reported the chemical synthesis of C8-modified sialic acids and their application in the CMP-sialic acid synthetase-catalyzed synthesis of CMP-sialic acid derivatives. *Campylobacter jejuni* α2–3-sialyltransferase Cst-I-catalyzed formation of α2–3-linked sialyllactose containing C8-modified sialic acids was also achieved from CMP-sialic acid derivatives.[19] Nevertheless, both chemical and enzymatic syntheses of sialosides containing C8-modified sialic acids so far have been limited to Neu5Ac-based structures and with α2–3-sialyl linkage.

Here, we report a facile chemoenzymatic approach for preparative synthesis of Neu5Ac8Me (**1**), Neu5Gc8Me (**2**), Kdn8Me (**3**), and Kdn8Deoxy (**4**) from chemically synthesized C5-modified *N*-acetylmannosamine (ManNAc), *N*-glycolylmannosamine (ManNGc), and mannose derivatives using a sialic acid aldolase-catalyzed reaction. The use of 5-*O*-methyl-ManNAc and 5-deoxy-mannose as donor substrates in a one-pot three-enzyme system for preparing both α2–3- and α2–6-linked sialosides containing Neu5Ac8Me and Kdn8Deoxy are also described. These compounds are important probes for studying the importance of C8-hydroxy group at the sialic acid residue in the interaction of sialylated carbohydrates and sialic acid binding proteins.

Sialic acid aldolases are enzymes involved in the metabolism of sialic acids. They catalyze the aldol cleavage reaction in nature but the reaction is reversible and the enzymes can be used synthetically in the aldol addition direction for the formation of Neu5Ac from pyruvate and ManNAc. The sialic acid aldolase from

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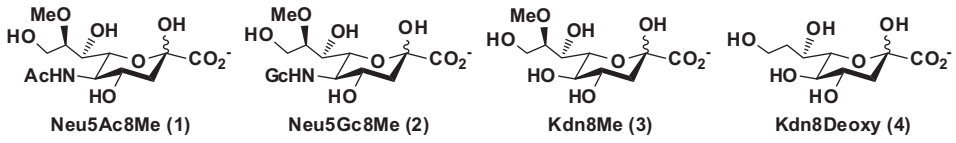


Figure 1. Structures of C8-modified sialic acid forms.

Pasteurella multocida P-1059 (PmNanA) recently cloned in our lab has shown flexible substrate specificity.[20] It is a more efficient enzyme than the *E. coli* sialic acid aldolase reported previously[21] for using 5-*O*-methyl ManNAc as a substrate.[20] Based on the extremely flexible substrate specificity of PmNanA, we hypothesize that the 5-*O*-methyl ManNGc, 5-*O*-methyl mannose, and 5-deoxy-mannose are also potential substrates for this enzyme to produce the corresponding C8-modified sialic acids **2**–**4**.

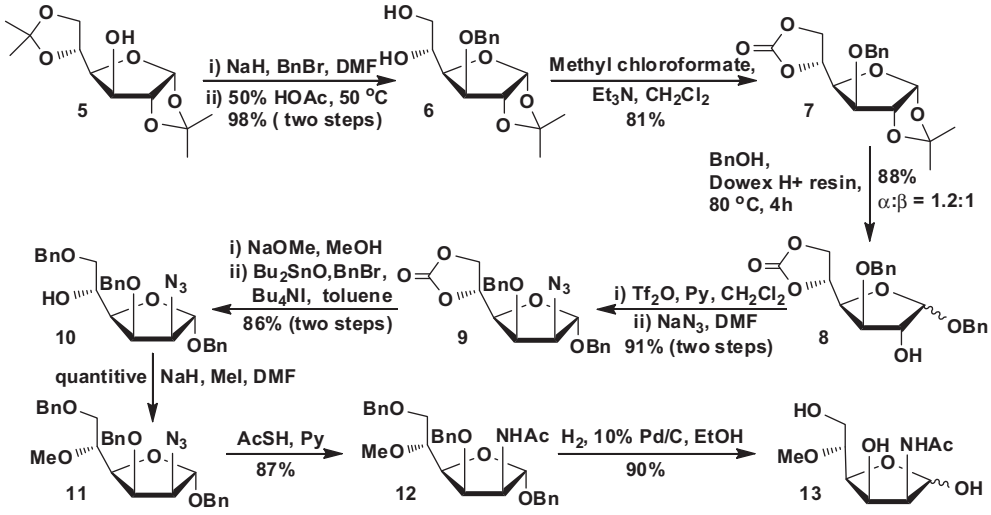
5-*O*-Methyl ManNAc **13** was synthesized from readily accessible and inexpensive 1,2:5,6-di-*O*-isopropylidene-α-D-glucopyranose (**5**).[22] As shown in Scheme 1, after benzylation of C3-OH, the 5,6-isopropylidene protecting group was selectively removed by mild acid hydrolysis and the resulting intermediate diol **6** was treated with methyl chloroformate to produce carbonate **7**.[23] Treatment of **7** with benzyl alcohol in the presence of acidic ion exchange resin[24] produced benzyl α- and β-anomers of furanoside **8** in 88% yield with a ratio of approximately 1.2:1[25], which can be separated by flash chromatography. The C2-OH of the α-anomer **8** was converted to triflate ester by treating with Tf_2O. It was then reacted with NaN_3 in DMF to produce 2-azido-2-deoxy-manopyranoside **9**. The carbonate protecting group was removed by treating **9** with sodium methoxide in methanol. Selective 6-*O*-benzylation of the azido diol was then achieved by formation of a dibutylstannylene derivative followed by alkylation with benzyl bromide.[26] The product **10** was treated with iodomethane and sodium hydride to produce methylation product **11** as the required key intermediate. The 2-azido group of **11** was converted to acetamido group by treating with AcSH in pyridine to produce **12**.[27,28] After hydrogenation in the presence of H_2 and Pd/C, 5-*O*-methyl-ManNAc **13** was produced in 90% yield.

As shown in Scheme 2, to synthesize 5-*O*-methyl ManNGc **16**, the azido group in compound **11** was reduced to an amino group in the presence of 1,3-dithiopropanol and Et_3N in pyridine/H_2O.[28] The resulting amino group in **14** was readily converted to *N*-glycolyl by coupling with *N*-hydroxysuccinamide-activated glycolyl ester (glycolyl-NHS ester),[21] leading to the formation of compound **15**. Debenzylation by hydrogenolysis with H_2 and Pd/C in methanol produced the desired 5-*O*-methyl-ManNGc **16** in 76% yield.

The preparation of 5-*O*-methyl mannose **22** is outlined in Scheme 3. Commercially available 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose **17** was treated with sodium hydride and benzyl bromide to produce the corresponding benzyl α- and β-glycosides **18** in 57% and 42% yields, respectively. Partial regioselective hydrolysis of **18** was achieved at 30 °C for 20 h using 70% aqueous acetic acid to produce the desired diol **19**. Selective 6-*O*-benzylation was achieved by formation of a dibutylstannylene derivative followed by alkylation with benzyl bromide. Product **20** was treated with iodomethane and sodium hydride to produce the methylation product **21**, which was then treated with 75% TFA followed by hydrogenolytic removal of the benzyl groups to produce desired 5-*O*-methyl mannose **22** in 90% yield.

For the preparation of 5-deoxy-mannose **27** (Scheme 4), diol **23** was obtained from D-mannose in three steps.[29] Regioselective benzoylation of **23** produced partially benzoated compound **24** in 90% yield. Treatment of benzoate **24** with phenyl chlorothionoformate and pyridine produced the corresponding thiocarbonyl derivative **25** in good yield (95%). Reaction of **25** with tri-*n*-butylstannane and AIBN provided the deoxy intermediate **26** in 71% yield. Subsequent removal of the isopropylidene group using TFA/H_2O followed by debenzoylation produced the target compound 5-deoxy mannose **27** in 91% yield.

With these C-5 modified monosaccharides (**13**, **16**, **22**, **27**) on hands, the substrate specificity of PmNanA was examined. Despite of several reported unsuccessful aldolase-catalyzed enzymatic reaction of 5-*O*-methyl ManNAc with pyruvate[25] or the observation of trace amount of product,[30] our results showed that all of the C5-modified monosaccharides tested can be tolerated by the recombinant PmNanA as the substrates. The PmNanA-catalyzed aldol addition of **13**, **16**, **22**, and **27** with five equivalents of sodium pyruvate in Tris-HCl buffer (100 mM, pH 7.5) at 37 °C for 24 h



Scheme 1. Synthesis of 5-*O*-methyl ManNAc **13**.