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Chemoenzymatic preparation of 4-*O*-acetyl sialic acid derivative

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Abstract

4-*O*-Acetyl sialic acid derivative could be easily prepared from the hydrolysis of **1** by lipase OF via the migration of acetyl groups.

Keywords: sialic acid; chemoenzymatic; lipase OF.

Sialic acids are the essential components of glycoconjugates which are important in cell–cell recognition at cell surfaces.[1] Many derivatives of sialic acids have been isolated and identified. Among them, the most common modification in sialic acids is the acetylation of the hydroxyl groups to form *O*-acetyl esters. More than 25 different acetylated sialic acids have been found in nature, and some have been demonstrated to involve biological functions.[2] For example, 4-*O*-acetylated sialic acids are resistant to all known sialidase,[3] and 9-*O*-acetyl sialic acid in the non-reducing terminal glycoconjugates would generate a new epitope related to human melanoma cells.[4] Since 4-*O*-acetylsialyl glycosides exhibit a remarkable inhibition of influenza A virus,[5] the 4-*O*-acetyl sialyl derivative could be the key intermediates to develop anti-influenza drugs. In this communication, we developed a novel, simple and efficient chemoenzymatic method to prepare **2**, a 4-*O*-acetyl sialyl derivative.

Compound **1**, which serves as the starting material for the chemoenzymatic transformations, was prepared according to the procedure of Marra and Sinaÿ.[6]

A straightforward route to prepare compound **2** was obtained via direct deacetylation of **1** (a mixture of β/α diastereomers) using lipase OF (*Candida rugosa*) in a one-pot procedure (Scheme 1). After stirring a solution of **1** (119 mg, 0.204 mmol, β/α = 5/1) with lipase OF (0.3 g) in phosphate buffer solution (pH 7, 7 mL) containing 5% ethanol for 72 h at 45°C, compound **2** was isolated in 79% combined yield.[7] According to our studies, lipase OF had the ability to remove acetyl groups in primary alcohol, but not in the secondary alcohol. Therefore, the acetyl

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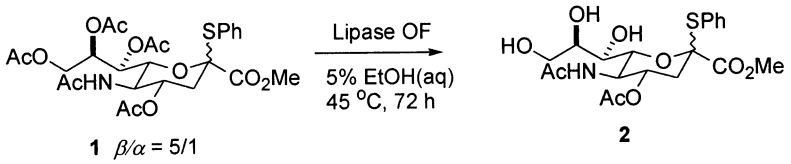
group in C-9 of **1** was the first acetyl group to be removed. Based on an earlier report of acetyl migration in sialic acid,[2,8] the acetyl groups at the C-8 and C-7 positions would then migrate to the C-9 position where they would be cleaved by the enzyme to generate **2**.[9] The C-4 acetyl group was remarkably stable in this lipase-catalyzed hydrolysis.



1 β/α = 5/1

2

Scheme 1.

In conclusion, it is noteworthy to emphasize that compared with the previous reports,[10] this chemoenzymatic method can be used to efficiently prepare **2**, which is an important building block (donor) in the synthesis of 4-*O*-acetylsialyl glycosides.

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7. The two α/β isomers could be easily separated by a flash column chromatography eluted with a gradient of eluents (CH_2Cl_2/MeOH: 20/1 to 10/1). The α isomer is less polar relative to the β isomer. The α isomer (14% yield): a white solid. LRMS (EI) *m*/*z* 457 (M^+, 55%), 348 (16%), 288 (7%). $[\alpha]_D^{26}$ −0.404 (*c* 0.16, MeOH). ^{1}H NMR (400 MHz, D_2O) δ 7.38–7.54 (m, 5H), 4.88 (ddd, J=13.1, 11.2, 5.0 Hz, 1H), 4.09 (t, J=10.5 Hz, 1H), 3.70–3.79 (m, 2H), 3.65 (d, J=10.6 Hz, 1H), 3.59 (s, 3H), 3.50–3.57 (m, 2H), 2.91 (dd, J=13.1, 5.0 Hz, 1H), 2.02–2.07 (d+s, J=12.6 Hz, 4H), 1.90 (s, 3H). ^{13}C NMR (100 MHz, D_2O) δ 174.2, 172.9, 169.8, 136.2, 130.4, 128.8, 127.2, 86.3, 74.4, 71.0, 69.8, 67.5, 62.3, 53.0, 48.6, 36.2, 21.5, 19.8. The β isomer (65% yield): a white solid. HRMS (FAB) calcd for $C_{20}H_{27}NO_9S$ ($M+Na^+$) 480.1304, found 480.1298. $[\alpha]_D^{26}$ −138.14 (*c* 0.255, MeOH). ^{1}H NMR (400 MHz, D_2O) δ 7.35–7.52 (m, 5H), 5.33 (ddd, J=14.8, 11.3, 4.7 Hz, 1H), 4.60 (d, J=10.5 Hz, 1H), 4.11 (t, J=10.3 Hz, 1H), 3.74–3.80 (m, 2H), 3.59–3.68 (m, 2H), 3.57 (s, 3H), 2.71 (dd, J=13.7, 4.7 Hz, 1H), 2.19 (dd, J=13.7, 11.3 Hz, 1H), 2.03 (s, 3H), 1.98 (s, 3H). ^{13}C NMR (100 MHz, D_2O) δ 174.2, 172.9, 169.8, 135.3, 129.8, 128.9, 128.2, 89.1, 70.9, 69.5, 67.9, 62.6, 52.9, 49.1, 36.2, 21.6, 19.9.
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