Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 709–712

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Lipase-Catalyzed Acetylation of *N*-Acetylneuraminic Acid Derivative

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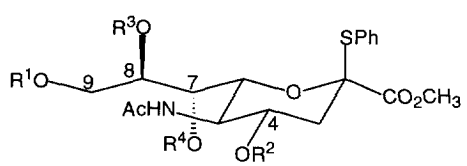
Received 20 November 1998; accepted 22 January 1999

Abstract: A facile preparation of triacetylated derivative of 2-phenylthioglycoside of *N*-acetylneuraminic acid (**4**) was achieved by treatment with lipase PS in vinyl acetate. The major product **4** has a free hydroxyl group at C-7. Results of time-course HPLC analysis indicate that the reactivity of the hydroxyl groups under this condition is in the following order; C-9 > C-4 > C-8 > C-7.

Recently glycoconjugates on cell surface have been shown to play an important role in many biological and pathological processes.[1-3] Among the various sugar residues on the glycoconjugates, sialic acids have attracted much attention because they are usually the terminal residue of the sugar chains and make direct interactions with receptors and enzymes of interest.[4-6] Up to date, more than 30 sialic acids have been characterized.[4] Many of these naturally occurring sialic acids are *O*-acetylated derivatives of *N*-acetylneuraminic acid (Neu5Ac).[7] Therefore, efforts have been focused on the preparation of sialic acid analogs bearing modification on their skeletons. These sialic acid derivatives could serve as useful research tools to probing the structure of sialic acid-recognizing proteins and as potential therapeutic agents.[8-10] Some of the syntheses, such as KDN,[11] 5-azido-5-deacetamido-Neu[12] and 7-azido-7-deoxy-Neu5Ac,[13] have been achieved by the use of Neu5Ac aldolase, while some were prepared by manipulation of Neu5Ac itself with a tedious protection-deprotection scheme.[14] In this paper we report a lipase-catalyzed transesterification reaction for the facile preparation of a sialic acid derivative **4**, which carries a free OH group at C-7 position.



1 $R^1 = R^2 = R^3 = R^4 = H$

2 $R^1 = Ac$, $R^2 = R^3 = R^4 = H$

3 $R^1 = R^2 = Ac$, $R^3 = R^4 = H$

4 $R^1 = R^2 = R^3 = Ac$, $R^4 = H$

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00060-8

Lipases have been widely used in selective acylations and deacylations of polyhydroxyl compounds, including carbohydrates, in either organic solvents or aqueous solution.[15-20] Good selectivity and yields could often be achieved with this group of biocatalysts, which has made them a reagent of choice in organic synthesis. Therefore, in this study we explore their application in preparing sialic acid derivatives. Compound **1**, methyl (phenyl 5-acetamido-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-2-nonulopyranosid)-onate,[21,22] was used as a starting material to study lipase-catalyzed acetylations in the presence of vinyl acetate. It carries four hydroxyl groups at the C-4, C-7, C-8 and C-9, respectively. The phenylthiol group not only serves as the anomeric protecting group but also enables this series of analogs prepared from compound **1** to connect to suitable glycosyl acceptors under activation conditions.[23] Since our preliminary screenings indicated that lipase PS (*Pseudomonas sp.*; Amano Pharmaceutical Co., Ltd. Japan) gave the best performance on this substrate, it was thus chosen for further studies.

Reaction condition for the transesterification is briefly described as follows. Compound **1** (150 mg) dissolved in 100 mL of vinyl acetate was incubated with 2 g of lipase PS at 45°C on a shaker (200 rpm/min). Small aliquots were removed and subjected to reversed phase HPLC analysis at different time of intervals.

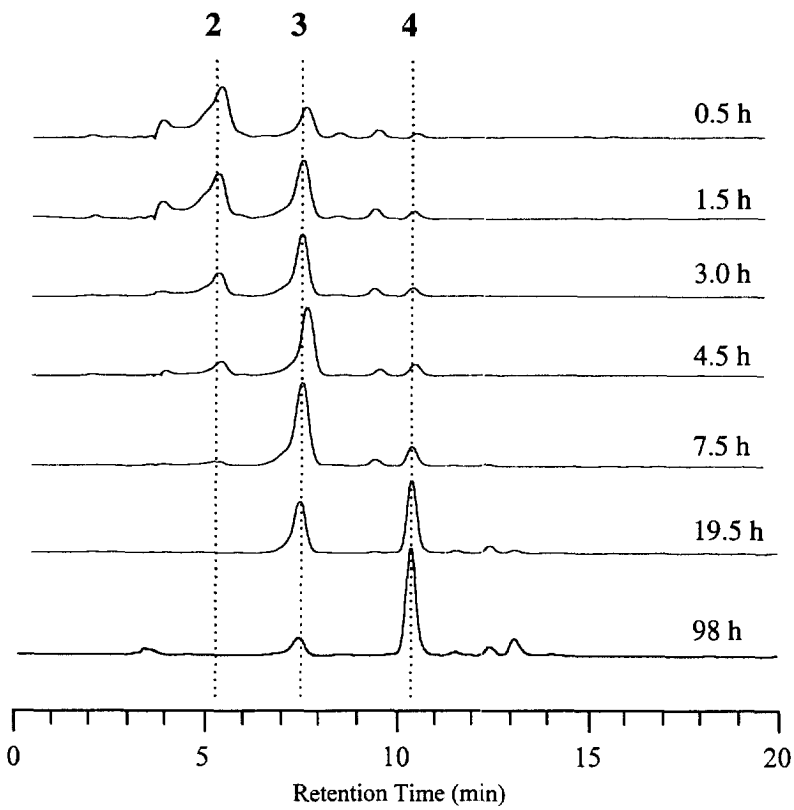


Figure 1. Time courses of acetylation of 2-thiophenyl-*N*-acetylneuraminic acid derivative (**1**) by lipase PS. HPLC was performed on a C_{18} column (0.46 x 25 cm) with a 20 min gradient from solvent A (CH_3CN:H_2O = 26:74) to solvent B (CH_3CN:H_2O = 82:18); flow rate, 1 mL/min; UV 254 nm detection. Compound numbers are shown on top of the corresponding peaks.

Results of these time-course HPLC chromatograms are shown in Figure 1. Two major products were subsequently observed during the reaction, one with retention time 7.5 min (compound **3**, di-acetylated product) and the other with retention time 10.2 min (compound **4**, tri-acetylated product). The former was dominating in the early stage of the reaction (3-7.5 h), whereas the latter was the major product left after 98 h. Another product (retention time = 5.2 min) which appeared only at the very beginning stage (< 4.5 h) and quickly decreased thereafter was assumed to be a monoacetylated product **2**. The elution behavior of these products on reversed phase HPLC fits well with the degree of acetylation, more acetylations leading to longer retention times. The *O*-acetylated positions of compounds **3** and **4** were inferred by the downfield shift of the corresponding signals for H-4, H-8 and H-9 as compared with those of compound **1**.[24]

Under our reaction conditions, the four hydroxyl groups of compound **1** undergo acetylation at different rates. The reactivity of these four hydroxyl groups can be deduced from the order of appearance of the partially acetylated products **2**, **3** and **4**. Time course HPLC profiles (Figure 1) clearly show that these products appeared in the following sequence, **1** → **2** → **3** → **4**. At first, mono-9-acetylated product **2** was formed due to the highest reactivity of the primary C-9 hydroxyl group. Although compound **2** was not isolated, this observation is consistent with a previous report that regioselective mono-*O*-acetylation of methyl *N*-acetylneuraminate at C-9 could be accomplished by lipase catalysis.[25] However, the reactions in our system didn't stop at this stage. Along with the increase of incubation time, compound **3** (4,9-diacetylated product)[24] was gaining height and became the dominant form between 3–7.5 h. It indicates that the C-4 hydroxyl group has the higher reactivity than the other secondary hydroxyl groups (C-7 and C-8). As the reaction further proceeded, the C-8 hydroxyl group was also acetylated to offer product **4** (4,8,9-triacetylated product),[24] the major product in the mixture after 98 h (yield > 75% based on HPLC analysis). Therefore, the reactivity of the hydroxyl groups of compound **1** is C-9 > C-4 > C-8 > C-7.

In conclusion, we have established a mild enzymatic acetylation reaction for *N*-acetylneuraminic acid derivative in vinyl acetate. The major product **4** carries a free hydroxyl group at C-7, which is an important intermediate for introducing other functionalities at this position. C-7 functionalized analogs could be prepared and the thioglycoside design at the anomeric position would allow this series of derivatives to become sialyl donors under appropriate conditions.[23]

Acknowledgment

This work was supported by the National Science Council (NSC 87-2113-M-002-017).

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