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Journal of Molecular Catalysis B: Enzymatic 3 (1997) 209–212

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

# Regioselective acetylation of primary hydroxyl function of sialic acid and selective hydrolysis of its derivatives with lipase from *Candida Rugosa* [1]

Armandodoriano Bianco [a,*], Cristiana Melchioni [a], Giancarlo Ortaggi [a], Paola Romagnoli [a], Mario Brufani [b]

[a] *Dipartimento di Chimica, Università 'La Sapienza', P. le Aldo Moro 5, 00185 Rome, Italy*
[b] *Dipartimento di Scienze Biochimiche, Università 'La Sapienza', P. le Aldo Moro 5, 00185 Rome, Italy*

Sialic acids are a family of nine-carbon sugars, which are derivatives of neuraminic acid. The most natural occurring sialic acid is 5-*N*-acetyl-neuraminic acid that is recognised as a receptor determinant by more viruses than any other determinant known. In particular influenza viruses have been shown to require sialic acid as a fundamental component of the cellular receptors for attachment to cell surfaces [1].

These viruses are enveloped by a host cell-derived lipid membrane which contains numerous copies of two distinct types of viral proteins: haemagglutinin (HA) and neuraminidase (NA). These proteins are responsible for influenza virus infection [2].

The HA of influenza A and B viruses is a glycoprotein that binds to terminal sialic acid residues as a first step of viral infection.

Viral attachment is followed by receptor mediated endocytosis, after which the viral and cell membranes fuse, allowing the viral nucleocapsid to enter the cytoplasm [3].

The NA, a viral receptor-destroying enzyme, is an aldolase which catalyses the cleavage of the $\alpha$-(2,3) and $\alpha$-(2,6) glycosidic linkages between terminal sialic acid and sugar residues of glycoproteins and glycolipids. The receptor-destroying activity is thought to be essential for the maintenance of virus mobility e.g. by means of the prevention of self-aggregation, to facilitate release of the virus from the cell surface [4] and to prevent virus activity from being destroyed by mucins, which are rich in sialic acids.

It has been a few years since we began to be interested in chemical modifications of sialic acid to synthesise new inhibitors as anti-influenza drugs.

Our research is aimed at the synthesis of sialic acid analogues which are able to inhibit the receptor-destroying activity of the NA and to form, with the principal binding site of the HA, a stronger bind than natural sialic acid.

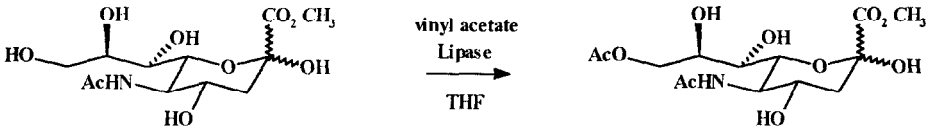
In the course of our work we performed the regioselective acetylation of methyl 5-*N*-acetyl-neuraminate **1** with lipase from *Candida Rugosa* (EC 3.1.1.3).

The chemical procedure adopted is described in Scheme 1.

* Corresponding author.

[1] Work supported by Istituto Pasteur, Fondazione Cenci Bolognetti, Università 'La Sapienza', Rome, Italy.


*PII* S1381-1177(96)00050-1




1 2

Scheme 1.

The 9-acetyl derivative **2** is a useful intermediate to prepare selectively C-9 functionalised analogues of sialic acid.

Furthermore, we have established the effects of lipase on the selective deprotection of the ester function in derivatives which we synthesise.

Use of lipase allows deprotection to be carried out in aqueous solvent; under this conditions, the sialic acid and some of its derivatives are more soluble than in other solvents.

In particular we performed the hydrolysis of methyl 5-*N*-acetyl-neuraminate **1** with lipase. The chemical procedure adopted is described in Scheme 2 (reaction a).

By treatment with lipase we obtained the hydrolysis of the ester function at C-1 in a very high yield.

Similar results have been obtained using as starting material methyl-2 $\alpha$-methoxysialoside **4** (see Scheme 2, reaction b).

## 1. Experimental

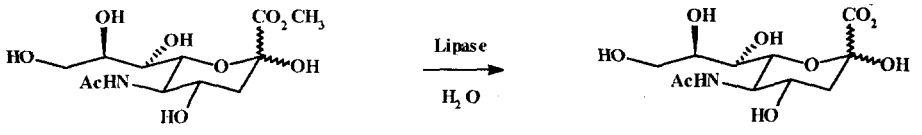
### *1.1. General*

$^1$H-NMR spectra were measured with a Gemini 200 MHz spectrometer. Products purification was obtained by solid–liquid column chromatography on Merck silica gel deactivated with 10% $H_2O$. Merck TLC 'Kiesel Gel 60 $F_{254}$' has been used to monitor the reactions, using 2 N $H_2SO_4$ as spray reagent.

### *1.2. Methyl 5-N-acetyl-neuraminate (1)*

Sialic acid (50 mg) was dissolved in methanol (5 ml). The reaction mixture was stirred at 0°C.

a)

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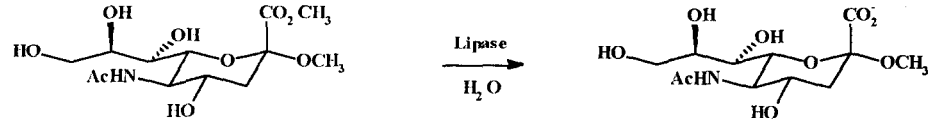
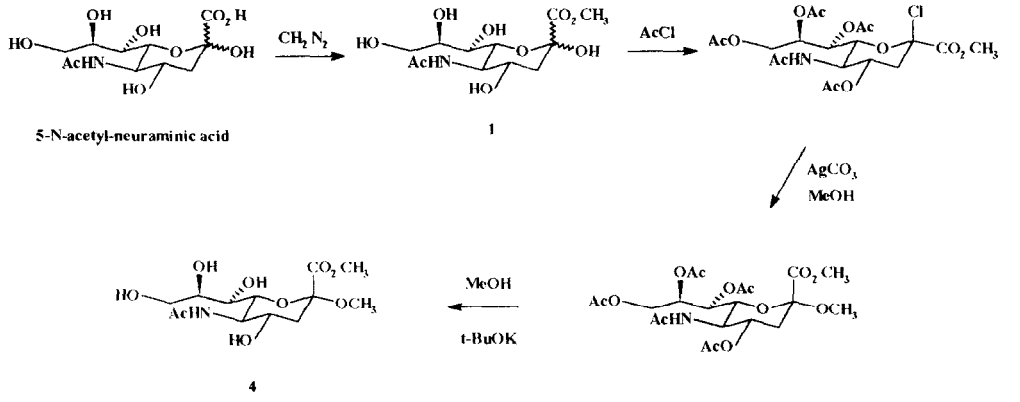
b)




4 5

Scheme 2.




Scheme 3.

Diazomethane was added until the solution became persistent yellow coloured. The volatile materials were removed in vacuo. Compound **1** was obtained as a colourless solid in quantitative yield.

$^1$H-NMR ($D_2O$): $\delta$ 1.87 (1 H, dd, $J = 11.0$ Hz, 12.0 Hz, H-3ax), 2.00 (3 H, s, NHCO$CH_3$) 2.20 (1 H, dd, $J = 5.0$ Hz, 12.0 Hz, H-3eq), 3.76 (3 H, s, $CO_2CH_3$).

### *1.3. Methyl 5-N-acetyl-9-O-acetyl-neuraminate (2)*

Compound **1** (50 mg) was dissolved in THF anhydrous (5 ml), lipase (50 mg) and vinyl acetate (1 ml) was added. The reaction mixture was stirred at room temperature. After 12 h, the lipase was denatured with methanol; the precipitate was filtered on gooch and the volatile materials were removed in vacuo. Compound **2** was obtained as a sole reaction product in a 60% yield, after chromatographic purification in $CHCl_3/CH_3OH$ 9:1.

$^1$H-NMR ($D_2O$): $\delta$ 1.87 (1 H, dd, $J = 11.0$ Hz, 12.0 Hz, H-3ax), 2.00 (3 H, s, NHCO$CH_3$) 2.05 (3 H, s, AcO), 2.22 (1 H, dd, $J = 5.0$ Hz, 12.0 Hz, H-3eq), 3.72 (3H, s, $CO_2CH_3$).

### *1.4. 5-N-acetyl-neuraminic acid (3)*

Compound **1** (50 mg) was dissolved in water (2.5 ml) and lipase was added. The reaction mixture was allowed to stand at room temperature for 1 h. The volatile materials were removed in vacuo; after chromatographic purification in AcOEt/*i*-PrOH/$H_2O$ 2:2:1 we obtained compound **3** in a 75% yield.

$^1$H-NMR ($D_2O$): $\delta$ 1.87 (1 H, dd, $J = 11.0$ Hz, 12.0 Hz, H-3ax), 2.00 (3 H, s, NHCO$CH_3$) 2.36 (1 H, dd, $J = 5.0$ Hz, 12.0 Hz, H-3eq).

### *1.5. Methyl 5-N-acetyl-2α-methoxy-neuraminate (4)*

Compound **4** was prepared according to Scheme 3 [3,5,6].

$^1$H-NMR ($D_2O$): $\delta$ 1.68 (1 H, t, $J = 12.0$ Hz, H-3ax), 1.93 (3 H, s, NHCO$CH_3$) 2.52 (1 H, dd, $J = 3.7$ Hz, 12.0 Hz, H-3eq), 3.25 (3 H, s, $OCH_3$), 3.74 (3 H, s, $COOCH_3$).

### *1.6. 5-N-acetyl-2α-methoxy-neuraminic acid (5)*

Compound **4** (50 mg) was dissolved in water (2.5 ml) and lipase was added. The reaction mixture was allowed to stand at room temperature for 2 h. The volatile materials were removed in vacuo; after chromatographic purification in AcOEt/*i*-PrOH/$H_2O$ 2:2:1 we obtained compound **5** in a 85% yield.

$^1$H-NMR ($D_2O$): $\delta$ 1.68 (1 H, t, $J = 12.0$ Hz, H-3ax), 1.93 (3 H, s, NHCO$CH_3$) 2.52 (1 H, dd, $J = 3.7$ Hz, 12.0 Hz, H-3eq), 3.25 (3 H, s, $OCH_3$).

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