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Note

A simple, one-step synthesis of *N*-acetyl-9-*O*-acetylneuraminic acid by enzymic transesterification mediated by porcine pancreas lipase in pyridine

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O-Acetyl esters of sialic acid (*N*-acetylneuraminic acid, Neu5Ac) have a considerable influence on the biological function and metabolism of complex glycoconjugates. For instance, 9-*O*-acetylNeu5Ac is an essential antigenic component of disialoganglioside GD_3 in human melanoma cells[1]. Moreover, the enzymic conversion of free or glycosidically bound Neu5Ac by sialidases and *N*-acetylneuraminate lyase is retarded if OAc groups are present in the pyranose ring or the side chain of Neu5Ac. Special interest has been paid to the detection of a sialate-*O*-acetylesterase that prefers 9-*O*-acetylNeu5Ac as a substrate[2],

This substrate can be isolated on a preparative scale from bovine colostrum and human or animal sera. However, most protocols require various steps, including acid hydrolysis of glycoside bonds and chromatography on basic ion-exchange resins, which lead to variable losses of OAc groups[3]. A chemical synthesis of 9-*O*-acetylNeu5Ac has not been described and we now report a simple, one-step, high-yielding synthesis based on the finding[4] that lipase from porcine pancreas (EC 3.1.1.3) tends to mediate esterification of primary hydroxyl groups in dry pyridine. Thus, with trichloroethyl acetate and Neu5Ac, the lipase gave 64% of 9-*O*-acetyl-Neu5Ac.

When lipases (Fluka) from microbial sources (*Rhizopus arrhizus, Penicillium roqueforti, Aspergillus niger, Candida lipolytica,* and *Mucor javanicus*) and other esters (trichloroethyl butanoate, hexanoate, and octanoate) were used under the same conditions, other products, presumably di- and tri-*O*-acetyl derivatives of Neu5Ac, were obtained which are being studied further.

EXPERIMENTAL

Lipase-mediated synthesis of 9-O-acetylNeu5Ac. — To a solution of Neu5Ac (0.5 g, 1.67 mmol) in dry pyridine (10 mL) were added trichloroethyl acetate (1 g, 5 mmol) and increasing amounts of porcine pancreas lipase (50–2000 U; Fluka).

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The suspension was stirred for 1–2 days at 37°. The reaction was monitored (*a*) by t.l.c. on cellulose (Merck), prewashed with 0.1M HCl, using 1-butanol–1-propanol–0.1M HCl (1:2:1) and detection by spraying with orcinol–HCl; and (*b*) by analytical h.p.l.c.[5,6]

When the reaction was complete, the lipase was removed and the filtrate was concentrated under reduced pressure. A solution of the resulting slurry in the above solvent system was subjected to flash chromatography on cellulose (Merck) equilibrated with the same solvent. Fractions were analysed by h.p.l.c. The appropriate fractions were combined and concentrated, and the residue was crystallized from ethyl acetate to give the title compound (100% pure as shown by h.p.l.c.), $[\alpha]_D^{20}$ +34.1° (*c* 0.9, methanol). ^{1}H-N.m.r. data (360 MHz, D_2O, pD 7.0, internal Me_4Si): δ 1.83 (H-3*a*), 2.22 (H-3*e*), 4.01 (H-4), 3.92 (H-5), 4.01 (H-6), 3.60 (H-7), 3.99 (H-8), 4.40 (H-9), 4.19 (H-9′), 2.06 (Ac-5), 2.10 (Ac-9). The best yields (0.56 g, 64%) were obtained by using 1000 U of lipase and an incubation time of 2 days. Larger amounts of the enzyme or longer reaction times did not improve the yields. The ^{1}H-n.m.r. data proved[7] the identity of the product. A melting point could not be determined because the product decomposed on heating.

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