Selective formation of α-glycosyl phosphate from *N*-acetylneuraminic acid glycosyl chloride

A. M. Shpirt, L. O. Kononov, V. I. Torgov, and V. N. Shibaev*

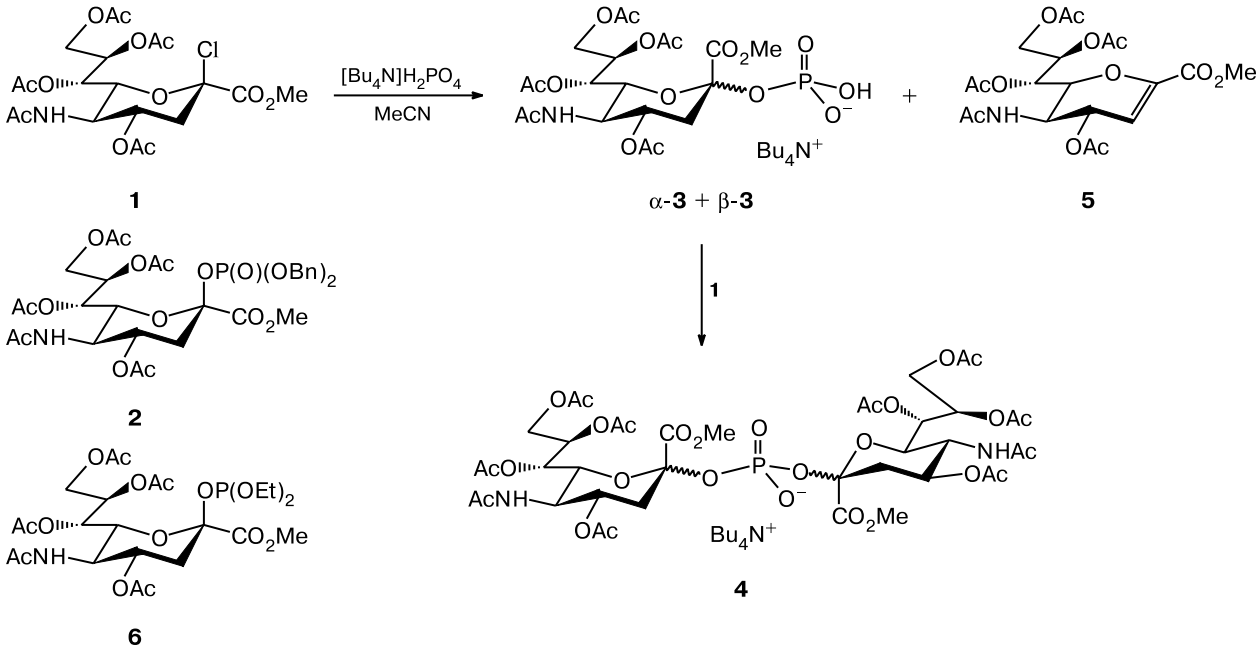
N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences,
47 Leninsky prosp., 119991 Moscow, Russian Federation.
Fax: +7 (095) 135 5328. E-mail: kononov@ioc.ac.ru

Recently, we have shown[1] that readily available β-glycosyl chloride **1** derived from *N*-acetylneuraminic acid (Neu5Ac), which has been previously regarded as a nonreactive glycosyl donor, reacts efficiently with salts of dibenzylphosphoric acid in the absence of glycosylation promoters giving glycosyl dibenzyl phosphate **2** with the β-anomeric configuration in a high yield (Scheme 1). The stereoselective formation of β-phosphate **2**, *i.e.*, the overall retention of the configuration of the anomeric center upon nucleophilic substitution of the Cl atom in glycosyl chloride **1**, may be due to anomerization of the α-phosphate formed initially, as the β-isomers of Neu5Ac are known to be thermodynamically more stable.[2] Note that in the syntheses of other glycosyl phosphates using dibenzyl phosphate salts, it is often possible to isolate the kinetically controlled reaction product with the inverted configuration of the anomeric center.[3]

We found that the reaction of tetrabutylammonium dihydrogen phosphate as the phosphate nucleophile with glycosyl chloride **1** allows the preparation of Neu5Ac α-glycosyl phosphate (α-**3**) isolated as the major reaction product (see Scheme 1). Previously,[4] the related Neu5Ac phenyl phosphate with the α-configuration was isolated as the minor product upon the reaction of glycosyl phosphite **6** with $PhOP(O)(OH)_2$ at −40 °C (α : β ≈ 1 : 6).

The reaction of glycosyl chloride **1** with $[Bu_4N]H_2PO_4$ (4.5 equiv.) in MeCN (20 °C, 3.5—4 h) gave a mixture of glycosyl phosphates α-**3**, β-**3** and **4** and glycal **5**[5] (see Scheme 1). Using reversed-phase chromatography (Mega Bond Elut C18 cartridge (Varian), gradient elution with H_2O—MeCN (0→20% MeCN, deionized water)), we were able to isolate pure glycosyl phosphates α-**3** (yield 27%) and β-**3** (yield 12%), and a mixture of phosphodiester **4** anomers (yield 13%), which was apparently formed in the

Scheme 1



Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 2, pp. 470—472, February, 2005.

reaction of glycosyl chloride **1** with phosphoric monoesters **3**.

The structure of phosphates **3** and **4** follows from ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectroscopy (Bruker AC-200, $CDCl_3$) and mass spectrometry (Finnigan LCQ, electrospray ionization (ESI), MeCN, detection of negative ions). All the signals in the NMR spectra of phosphates **3** and **4** are broadened compared to the signals in the spectra of glycosyl phosphate **2** with the protected phosphoric acid residue,[1] which may be related to exchange processes or to aggregation of the solute molecules.

The assignment of anomeric configurations of glycosyl phosphates α-**3** and β-**3** is based on a lower-field position of the $H_{eq}(3)$ signal in the ^{1}H NMR spectrum of the α-anomer (δ 3.00) with respect to that for the β-anomer (δ 2.68; *cf.* Ref. 6: δ 2.73 for the product of debenzylation of compound **2**).

The signals in the ^{31}P NMR spectra of anomeric glycosyl phosphates **3** are clearly discernible: δ ≈ −5.0 for α-**3** and −3.9 for β-**3**. Note that, although each particular spectrum contains only one signal for the respective anomers, the spectra of different samples of glycosyl phosphates **3** are somewhat different (for example, for α-**3**, δ −4.9, −5.0 and −5.2). This "scatter" in the position of signals may imply the presence of different ionic forms of phosphate **3**.

The ^{1}H NMR spectrum of phosphoric diester **4** exhibits two singlets of equal intensity (δ 3.78 and 3.82 (CO_2Me)), which suggests the presence of two types of *N*-acetylneuraminic acid residues in the molecule. The ^{31}P NMR spectrum exhibits a set of signals expected of phosphoric diesters containing two Neu5Ac residues with unusual high-field chemical shifts (δ −10.1, −12.7 and −13.1).

The mass spectra of glycosyl phosphates α,β-**3** contain the expected molecular ion peak (found: *m/z* 569.9 [M − Bu_4N]; calculated for $C_{20}H_{29}NO_{16}P$: 570.1), its fragments (found: *m/z* 538.1 [M − Bu_4N − OMe − H]; calculated for $C_{19}H_{25}NO_{15}P$: 538.1), and a peak for the dimer (found: *m/z* 1382.1 [M_2 − Bu_4N]; calculated for $C_{56}H_{94}N_3O_{32}P_2$: 1382.53) containing two Neu5Ac residues per tetrabutylammonium cation. Meanwhile, the mass spectrum of phosphoric diester **4** exhibits only the molecular ion peak (found: *m/z* 1043.2 [M − Bu_4N]; calculated for $C_{40}H_{56}N_2O_{28}P$: 1043.28).

The stereoselective synthesis of *N*-acetylneuraminic acid phosphate with the α-configuration first proposed in this study is attractive as regards experimental simplicity and availability of the reactants. The moderate yield is compensated for by the small number of steps, because there is no need to remove protective groups from the phosphoric acid residue.

This work was financially supported by the Russian Foundation for Basic Research (Projects No. 02-03-32271 and No. 04-03-32854) and by the Foundation of the Russian Federation President (Program for the Support of Leading Scientific Schools of the Russian Federation, grant NSh-1557.2003.3).

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Received December 10, 2004