Conversion of *N*-acetylneuraminic acid glycosyl chloride into dibenzyl glycosyl phosphate: *O*-glycosylation in the absence of a promoter

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Readily accessible *N*-acetylneuraminic acid (Neu5Ac) glycosyl chloride, which was regarded to be a poor glycosyl donor, was shown to react with dibenzyl phosphoric acid salts in the absence of glycosylation promoters to give the corresponding β-Neu5Ac dibenzyl glycosyl phosphate in high yield.

Key words: sialic acids, *N*-acetylneuraminic acid, glycosyl chloride, glycosyl phosphates, monosaccharides, NMR spectroscopy.

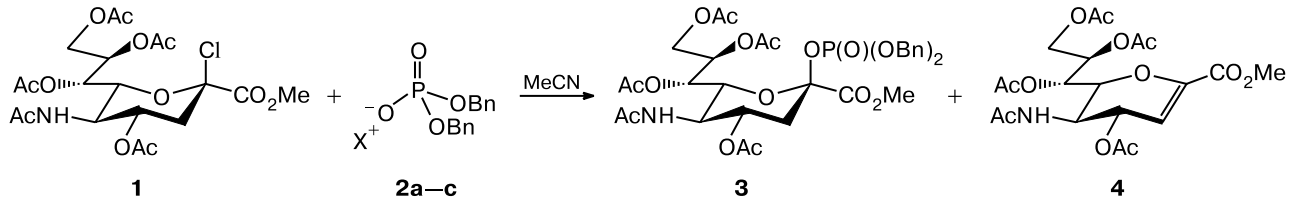
The development of new efficient procedures for the synthesis of oligosaccharides containing sialic acid residues and, in particular, *N*-acetylneuraminic acid (Neu5Ac) residue, which are responsible for various immunological, neurobiological, oncological, and other biological processes,[1] is an important problem of modern synthetic carbohydrate chemistry.

The preparation of various glycosyl donors proposed[2] for this purpose is often very laborious. Presently, readily accessible glycosyl chloride[2,3] **1** (Scheme 1) is little used because of its low reactivity. However, it has recently been found that this compound can react with MeOH in the absence of an added promoter to give the corresponding acetylated glycoside in high yield.[3a]

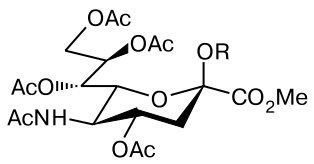
It is of interest to search for other *O*-glycosylation reactions in which chloride **1** can efficiently be used. In the present study, we examined the reactions of this compound with dibenzyl phosphate (**2a**) and its salts **2b** and **2c** (Scheme 1). The expected reaction product, *viz.*, dibenzyl glycosyl phosphate **3**, can subsequently be transformed into cytidine 5′-monophosphate *N*-acetylneuraminic acid[4,5] (CMP-Neu5Ac), which serves as a glycosyl donor in enzymatic sialylation.[2]

The synthesis of Neu5Ac β-glycosyl phosphate **3** has been described[4,5] in connection with the preparation of CMP-Neu5Ac and investigation of its glycosylating potential.[4] Compound **3** was prepared by phosphitylation of protected Neu5Ac hemiacetal (**5a**) with $(BnO)_2PNPr^i_2$ followed by oxidation of triester **6a**[4] as well as by glycosylation of dibenzyl phosphoric acid (**2a**) with Neu5Ac glycosyl phosphite **6b**.[5] An attempt to glycosylate dibenzyl phosphoric acid (**2a**) with glycosyl chloride **1** in

Scheme 1

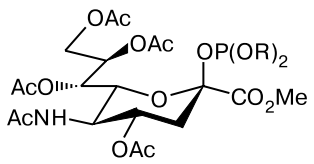


X = H (**a**), Pr^i_2EtNH (**b**), Bu_4N (**c**)



5a,b

R = H (**a**), Ac (**b**)



6a,b

R = Bn (**a**), Et (**b**)

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the presence of AgOTf as a promoter was unsuccessful. In this case, only the elimination product, glycal **4**, and hydrolysis product **5a** were isolated from the reaction mixture.[4]

Our experiments demonstrated that acid **2a** did not react (TLC data) with chloride **1** in the absence of a promoter under the conditions used (MeCN, 20 °C). However, chloride **1** was smoothly transformed into β-glycosyl dibenzyl phosphate **3** in the reaction with more nucleophilic ammonium dibenzyl phosphates (**2b,c**). In all cases, the reactions afforded glycal **4** as the only by-product. Separation of this product from phosphate **3** is a rather laborious procedure because of similar chromatographic mobilities of these compounds.

Salt **2b** was synthesized *in situ* by the reaction of Pr^i_2NEt with dibenzyl phosphoric acid (**2a**). Salt **2c**, which is prepared by the reaction of an aqueous solution of Bu_4NOH with acid **2a** in MeCN, was isolated and dried before the reaction with chloride **1**. In all cases, the reactions were carried out with samples of salt **2b** or **2c** containing a small excess (10%) of dibenzyl phosphoric acid (**2a**) to prevent basic conditions (which can happen in the case of the inaccurate measurement of the amount of a base in the step of preparation of the salt), which favor the formation of glycal **4** from chloride **1**.[6]

The difference in the reaction rate in going from **2b** to **2c** (Table 1) can be attributable to the fact that the salt of a quaternary ammonium base $[Bu_4N]^+[OP(O)(OBn)_2]^-$ (**2c**) is more ionic than the salt of tertiary amine $[Pr^i_2NHEt]^+[OP(O)(OBn)_2]^-$ (**2b**). Apparently, in MeCN salt **2c** forms a less tight ion pair than salt **2b** and the nucleophilicity of the phosphate anion in **2c** can be expected to be higher.

Phosphate **3** was characterized by ^1H, ^{13}C, and ^{31}P NMR spectroscopy. The ^1H NMR spectrum of β-glycosyl phosphate **3** synthesized in the present study is identical to that described in the literature,[4,5] which is evidence that the configuration of compound **3** is β. The presence of the phosphoric acid residue in reaction product **3** was confirmed by the ^{31}P NMR spectrum, which has one signal (δ_P −6.0). Generally, signals in the ^{31}P NMR spectra of dibenzyl hexosyl phosphates are observed at δ_P from −2.0 to −3.0.[7] The unusually high-field position of the signal for the phosphorus atom in the spectrum of phosphate **3** is apparently associated with the electron-withdrawing effect of the methoxycarbonyl group. The ^{13}C NMR spectrum of phosphate **3** shows doublets corresponding to the C(2) (δ 100.0, $^2J_{C,P}$ = 7.5 Hz) and C(3) (δ 37.2, $^3J_{C,P}$ = 5.3 Hz) atoms with the characteristic spin-spin coupling constants between the carbon atoms and the phosphorus atom.

The β configuration of the anomeric C(2) center of phosphate **3** was additionally confirmed by the small spin-spin coupling constant between the C(1) atom (the methoxycarbonyl group) and the axial proton at the C(3) atom ($^3J_{C(1),H_{ax}(3)} < 1$ Hz). In the case of the α configuration of the anomeric center in Neu5Ac, $^3J_{C(1),H_{ax}(3)}$ would be equal to *ca.* 5—6 Hz.[8]

Stereoselectivity of the formation of β-phosphate **3**, *i.e.*, the overall retention of the configuration of the anomeric center upon the nucleophilic substitution of the Cl atom in glycosyl chloride **1**, is apparently associated with anomerization of the initially formed α-phosphate due to the known fact that β isomers of Neu5Ac are thermodynamically favorable.[2] It should be noted that it is generally possible to isolate the kinetically controlled reaction product in the synthesis of other glycosyl phosphates with the use of dibenzyl phosphoric acid salts.[9]

To summarize, we demonstrated that the reactions of glycosyl chloride **1** with dibenzyl phosphoric acid salts **2b,c** afford Neu5Ac β-phosphate **3** in high yield (72%; 80% in the reaction mixture according to NMR analysis). Thus, this method is much more efficient than the procedure for the synthesis of compound **3** based on glycosylation of dibenzyl phosphoric acid with glycosyl phosphite **6b** (53% yield).[5] The approach to the synthesis of Neu5Ac β-phosphate **3** developed in the present study is experimentally simple, and the starting reagents are readily accessible.

Table 1. Influence of the nature of the phosphate reagent $(BnO)_2P(O)^-X^+$ (**2a—c**) on the results of the reaction with glycosyl chloride **1**

Reagent	Counter-ion	Amount of **2** (equiv.)	τ[a]/h	Yield of **3** (%)	Ratio **3** : **4**[b]
2a	H^+	1.5	12	0[c]	—
2b	$Pr^i_2EtNH^+$	4	24	72[d] (80[e])	4 : 1
2c	Bu_4N^+	4	12	63[d] (75[e])	3 : 1

[a] The time of complete conversion of chloride **1** (TLC).
[b] ^1H NMR spectroscopic data.
[c] No reaction.
[d] After column chromatography on silica gel.
[e] ^1H NMR spectroscopic data for the reaction mixture after workup.

Experimental

The reactions were performed with the use of commercial reagents $(BnO)_2P(O)OH$ (Aldrich), a 20% aqueous Bu_4NOH solution (Merck), 85% H_3PO_4 (Fluka), and AcCl (Fluka). Diisopropylethylamine (Aldrich) was distilled over CaH_2 under Ar. The solvents were distilled and purified before use according to standard procedures. Acetonitrile used for the synthesis of compound **3** was freshly distilled over CaH_2 under Ar. Thin-layer chromatography was carried out on silica gel plates Kieselgel 60 F_{254} (Merck) using AcOEt as the eluent; spots were visualized by heating plates after immersion in a 1 : 10 mixture of 85% aqueous H_3PO_4 and 95% EtOH or under UV light. Column chromatography was performed on silica gel L 100—250 μm (Chemapol, Czech Republic) using AcOEt as

the eluent. The NMR spectra were recorded on a Bruker AC-200 instrument. The ^{1}H chemical shifts are given relative to the residual signal of $CHCl_3$ (δ 7.27), the ^{13}C chemical shifts were measured relative to the signal of $CDCl_3$ (δ 77.0), and the ^{31}P chemical shifts are given relative to 75% H_3PO_4 in D_2O as the external standard (δ 0.0). The assignment of the signals in the ^{13}C NMR spectra was made based on the DEPT135 experiment. Phosphates were synthesized at room temperature (20—25 °C) in anhydrous solvents under dry argon. All reactions were carried out with the use of glycosyl chloride **1** freshly prepared from peracetate **5b** according to a modified procedure.[3a]

The ratio between phosphate **3** and glycal **4** in a mixture of the reaction products was determined by integration of the signals of H(3) and C(1)O_2C$\underline{H}_3$ in the ^{1}H NMR spectra (**3**: δ 2.62 (H_{eq}(3)), δ 3.65 (OCH_3); **4**: δ 5.95 (H(3)), δ 3.78. (OCH_3)). The yield of phosphate **3** was calculated taking into account the molar ratios between phosphate **3** and glycal **4**.

Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-non-2-ulopyranosyl chloride)onate (1). Anhydrous MeOH (1.8 mL, 0.04 mol) was slowly added dropwise to AcCl (4.5 mL, 0.06 mol) with cooling in an ice water bath. The reaction mixture was added to a cold solution of Neu5Ac acetate **5b** (102 mg, 0.19 mmol) in a mixture of anhydrous CH_2Cl_2 (4.5 mL) and AcCl (4.5 mL, 0.06 mol) and the reaction mixture was kept at +4 °C for 12 h (TLC control, R_f 0.50 (**1**), R_f 0.27 (**5b**)). Volatile components were evaporated, CCl_4 was added, and volatile components were again evaporated (5×5 mL). The residue was dried *in vacuo* (oil pump) to give glycosyl chloride **1** (114 mg), which was used without additional purification. The ^{1}H and ^{13}C NMR spectra of the product are identical to those described in the literature.[3]

Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-*O*-dibenzyloxyphosphoryl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-non-2-ulopyranosonate (3). ***A.*** Diisopropylethylamine (65 μL, 0.37 mmol) was added to a solution of dibenzyl phosphoric acid (**2a**) (115 mg, 0.41 mmol) in MeCN (1 mL). The solution of salt **2b** thus prepared was added dropwise to a solution of glycosyl chloride **1**, which was prepared from acetate **5b** (50 mg, 0.09 mmol), in MeCN (2 mL). The vessel in which salt **2b** was prepared was additionally rinsed with MeCN (2×1 mL). The course of the reaction was monitored by TLC (R_f 0.50 (**1**), R_f 0.48 (**3**)). After completion of the reaction (24 h), the reaction mixture was cooled in an ice water bath and then a cold saturated $NaHCO_3$ solution (10 mL) and $CHCl_3$ (10 mL) were added. The aqueous phase was extracted with $CHCl_3$ (3×5 mL). The organic phase was concentrated *in vacuo* (without heating). The residue was dissolved in CH_2Cl_2 and applied onto a silica gel column packed in light petroleum. The products were eluted with AcOEt and a mixture of **3** and **4** was obtained in a yield of 57 mg (4 : 1, ^{1}H NMR data, R_f 0.48 (**3**), R_f 0.49 (**4**)), *i.e.*, the yield of phosphate **3** was 72% (with respect to acetate **5b**).

B. A suspension of acid **2a** (120 mg, 0.43 mmol) in MeCN (3 mL) was stirred until complete dissolution (*ca.* 3 min). Then a 20% aqueous solution of Bu_4NOH (172 μL, 0.39 mmol) was added, the reaction mixture was stirred for 15 min, volatile components were evaporated, MeCN was added, and volatile components were again evaporated (4×2 mL). The residue was dried *in vacuo* (oil pump) for 2 h to give crude salt **2c** in a yield of 219 mg (^{31}P NMR ($CDCl_3$): δ −0.5). Then MeCN (1 mL) was added and the resulting suspension was added dropwise to a solution of chloride **1**, which was prepared from acetate **5b** (56 mg, 0.1 mmol), in MeCN (2 mL). The flask in which salt **2c** was prepared was additionally rinsed with MeCN (3×0.5 mL). The reaction mixture was stirred at 22 °C. After completion of the reaction (12 h), the products were isolated analogously to the method ***A***. After chromatography, a mixture of **3** and **4** was obtained in a yield of 50 mg (3 : 1, ^{1}H NMR data), *i.e.*, the yield of phosphate **3** was 63%.

Compound **3**. ^{1}H NMR ($CDCl_3$), δ: 1.87 (s, 3 H, AcN); 1.99, 2.00, 2.05, and 2.10 (all s, 3 H each, AcO); 1.99—2.10 (m, 1 H, H_{ax}(3)); 2.60 (dd, 1 H, H_{eq}(3), $J_{H_{eq}(3),H(4)}$ = 5.0 Hz, $J_{H_{eg}(3),H_{ax}(3)}$ = 13.6 Hz); 3.65 (s, 3 H, OMe); 4.20—4.30 (m, 3 H, H(5), H(6), H_a(9)); 4.60 (dd, 1 H, H_b(9), $J_{H_b(9),H_a(9)}$ = 10.1 Hz, $J_{H_b(9),H(8)}$ = 2.2 Hz); 4.98—5.09 (m, H(4)); 5.03 (d, OC$\underline{H}_2$Ph, $^3J_{H,P}$ = 2.2 Hz); 5.08 (d, OC$\underline{H}_2$Ph, $^3J_{H,P}$ = 3.1 Hz) (a total of 5 H); 5.35—5.40 (m, 2 H, H(7), H(8)); 5.63 (d, 1 H, C(5)NH, $J_{H(5),NH}$ = 9.5 Hz); 7.30—7.41 (m, 10 H, Ph).

^{13}C NMR ($CDCl_3$), δ: 20.7 (AcO); 22.9 (NAc); 37.2 (d, C(3), $^3J_{C,P}$ = 6.6 Hz); 48.2 (C(5)); 55.5 (MeO); 62.5 (C(9)); 68.0 (C(8)); 69.5 (C(7)); 70.1 (d, O$\underline{C}H_2$Ar, $^2J_{C,P}$ = 6.0 Hz); 71.9 (C(6)); 73.4 (C(4)); 100.0 (d, C(2), $^2J_{C,P}$ = 6.6 Hz); 128.3, 128.4, 128.6, 128.7 (Ph); 135.2 ($OCH_2\underline{C}$, $^3J_{C,P}$ = 6.6 Hz); 165.8 (C(1)); 170.0 ($CH_3\underline{C}ONH$); 170.3, 170.6, 171.0 (CO).

^{31}P NMR ($CDCl_3$), δ: −6.4.

The ^{1}H and ^{13}C NMR spectra of glycal **4** are identical to those published in the literature.[6b]

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