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Large-Scale Synthesis of a Key Catalytic Reagent for Phosphorus Protection in Building Blocks for Isopolar Phosphonate Oligonucleotide Preparation

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Abstract:

A simple procedure for the large-scale synthesis of 4-methoxy-2-pyridinemethanol 1-oxide (5), a key compound in modern triester synthesis of oligonucleotides, employing Büchi R 152 evaporator as a reaction apparatus was elaborated. Two crucial reaction steps, both strongly exothermic, were satisfactorily handled. Our procedure provides a high-purity, light- and heat-stable product in a satisfactory yield.

Introduction

A search for nucleophilic catalysts more powerful than pyridine[1] or *N*-methylimidazole[2,3] in phosphotriester methodology of oligonucleotide synthesis[1,4] has led to the introduction of various 4-substituted derivatives of pyridine *N*-oxide **1**.[5] These *O*-nucleophiles, when added to the reaction mixture in the presence of arylsulfonyl chloride strongly accelerate the condensation reaction between the phosphodiester and the OH-component, leading to the formation of a neutral phosphotriester species. The idea of intramolecular catalysis using a phosphorus-protecting group based on a pyridine *N*-oxide derivative was verified[6] in practice by introduction of a 4-methoxy-*N*-oxido-2-picolyl group. Since that time, this group has also been successfully applied to the protection of the phosphonate moiety in various nucleoside phosphonic acids **2**−**5** from which the internucleotide linkage-modified oligonucleotides[7−15] were synthesized by phosphotriester-type condensation method. Considering further investigation in the area of isopolar phosphonate nucleotide analogues, the elaboration of a reliable synthesis of 4-methoxy-2-pyridinemethanol 1-oxide (**10**) as the superior reagent for the protection of phosphonate moiety is highly desirable. In addition, the usefulness of the compound **10** as the sugar hydroxyl-protecting group in ribonucleosides[16] as well as the starting compound for the preparation of arylsulfonylhydrazones of 2-formylpyridine derivatives[17,18] (as compounds with antineoplastic activities) is also well-known.There are two procedures described in the literature[16,19] for the synthesis of 4-methoxy-2-pyridinemethanol 1-oxide (**10**). One of them[16] utilizes a simple reduction of 4-substituted 2-formylpyridine 1-oxides obtainable by a laborious multistep synthesis, whereas the other,[19] apparently much simpler one, provided, in our hands, very variable yields of low-purity product **10**. Despite employing two chromatographical steps on silica gel and crystallization we obtained a light- and heat-sensitive gray product. Under the conditions given, the synthesis of pure **10** in 10 g amounts would thus be very problematic and laborious. Herein we describe a procedure enabling the simple, reliable, and safe large-scale synthesis of 4-methoxy-2-pyridinemethanol 1-oxide (**10**).

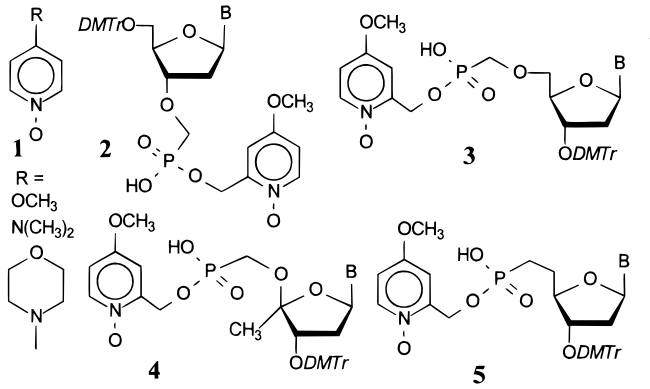

Figure 1.

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(1) Reese, C. B. *Tetrahedron* **1978**, *34*, 3143.
(2) Efimov, V. A.; Reverdatto, S. V.; Chakhmakhcheva, O. G. *Nucleic Acids Res.* **1982**, *10*, 6674.
(3) Efimov V. A.; Buryakova A. A.; Reverdatto S. V.; Chakhmakhcheva, O. G.; Ovchinnikov, Yu. A. *Nucleic Acids Res.* **1983**, *11*, 8369.
(4) Zarytova, V. F.; Knorre, D. G. *Nucleic Acids Res.* **1984**, *12*, 2091.
(5) Efimov, V. A.; Chakhmakhcheva, O. G.; Ovchinnikov, Yu. A. *Nucleic Acids Res.* **1985**, *13*, 3651.
(6) Efimov, V. A.; Buryakova, A. A.; Dubey, I. Y.; Polushin, N. N.; Chakhmakhcheva, O. G.; Ovchinnikov, Yu. A. *Nucleic Acids Res.* **1986**, *14*, 6526.
(7) Szabo, T.; Kers, A.; Stawinski, J. *Nucleic Acids Res.* **1995**, *23*, 893.
(8) Efimov, V. A.; Choob, M. V.; Kalinkina, A. L.; Chakhmakhcheva, O. G. *Nucleosides* Nucleotides **1997**, *16*, 1475.
(9) Efimov, V. A.; Buryakova, A. A.; Choob, M. V.; Chakhmakhcheva, O. G. *Nucleosides Nucleotides* **1999**, *18*, 1393.
(10) Efimov, V. A.; Choob, M. V.; Buryakova, A. A.; Chakhmakhcheva, O. G. *Nucleosides Nucleotides* **1999**, *18*, 1425.
(11) Chakhmakhcheva, O. G.; Andrianov, M.; Buryakova, A. A.; Choob, M. V.; Efimov, V. A. *Nucleosides Nucleotides* **1999**, *18*, 1427.
(12) Točík, Z.; Kavenová, I.; Rosenberg, I. *Collect. Czech. Chem. Commun. (Symposium Series)* **1999**, *2*,109.
(13) Rinnová, M.; Rosenberg, I. *Collect. Czech. Chem. Commun. (Symposium Series)* **1999**, *2*, 60.
(14) Rejman, D.; Liboska, R.; Endová, M.; Kavenová, I.; Točík Z.; Rosenberg, I. *Collect. Czech. Chem. Commun. (Symposium Series)* **1999**, *2*, 92.
(15) Rejman, D.; Rosenberg, I. *Collect. Czech. Chem. Commun. (Symposium Series)* **1999**, *2*, 96.
(16) Mizuno, Y.; Endo, T.; Takahashi, A.; Inaki, A. *Chem. Pharm. Bull.* **1980**, *28*, 3041.
(17) Agraval, K. C.; Sartorelli, A. C. *J. Med. Chem.* **1978**, *21*, 218.
(18) Mizuno, Y.; Endo T.; Inoue, Y.; Tampo, H.; Takahashi, A.; Iigo, M.; Hoshi, A.; Kuretani, K. *Chem. Pharm. Bull.* **1980**, *28*, 1584.
(19) Mizuno, Y.; Endo, T. *J. Org. Chem.* **1978**, *43*, 684.

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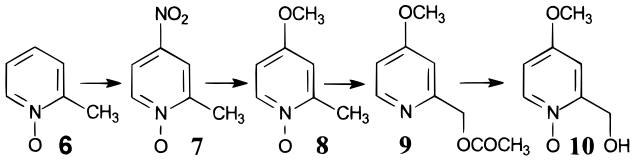
Scheme 1



Results and Discussion

The nitration of the starting, commercially available 2-picoline-*N*-oxide (**6**) (see Scheme 1) with fuming nitric acid at elevated temperature performed according to the modified procedure[20] afforded pure 4-nitro-2-picoline-*N*-oxide (**7**) in a good yield. Treatment of this compound with excess of sodium methoxide in methanol gave the expected 4-methoxy-2-picoline-*N*-oxide (**8**)[21] in nearly quantitative yield. Subsequent acetylation of the methoxy derivative **8** by acetic anhydride in chloroform under the formation of derivative **9** has been described[19] to involve a silica gel chromatography. We performed a large scale acetylation of **8** by stepwise addition of a solution of methoxy derivative **8** in glacial acetic acid to preheated acetic anhydride. This reaction arrangement was safe enough to maintain a strongly exothermic acetylation fully under control. The isolation of a key acetoxymethyl derivative **9** was accomplished by fractional distillation in vacuo and no chromatographic step was needed. We experienced that a noncontrolled, exothermic reaction having almost explosive character occurred when addition of reagents is carried out in the reverse order.

The subsequent oxidation step[19] of derivative **9** in aqueous peroxoacetic acid was considerably modified. We used an anhydrous solution of peroxoacetic acid in acetic acid prepared from acetic acid, 30% aqueous hydrogen peroxide, and acetic anhydride. In our procedure, the acetoxymethyl derivative **9** was slowly added to a solution of peroxoacetic acid at elevated temperature under strict temperature control. If necessary, the temperature of the oxidation reaction mixture can be efficiently and quickly decreased by direct addition of dry ice. Evaporation of the reaction mixture followed by hydrolysis of the acetoxy group in concentrated aqueous ammonia afforded the crude *N*-oxide **10** which was deionized on Dowex 50 (H^+ form) and purified by a two-step crystallization. The first one performed from hot ethyl acetate allowed removal of an essential amount of a gummy residue that prevent crystallization. The obtained brown crystalline compound was recrystallized from a chloroform−dichloromethane mixture in which both *N*-oxide **10** and the brownish by-products were well soluble. The crystals were washed with dichloromethane in which the coloured compounds were much better soluble than *N*-oxide **10**. Additional recrystallization from the same mixture afforded a highly pure 4-methoxy-2-pyridinemethanol 1-oxide (**10**).

Additional ~10% of the product **10** can be obtained by the following process: Mother liquors containing *N*-oxide **10** and concentrated coloured by-products were subjected to a filtration through a small column of C18 silica gel (recommended particle size >40 μm) in water to remove an essential amount of crystallization-preventing coloured compounds with high affinity towards reverse phase (a partial regeneration of the reverse phase column can be achieved by its successive washing with methanol, acetic acid, acetic acid−chloroform mixture (1−1) and finaly with methanol). The *N*-oxide **10** obtained in this purification step was crystalized from chloroform−dichloromethane mixture as described above.

The described simplified procedure afforded light- and heat-stable product **10** in an excellent purity and a good yield. Considering a shelf life of the compound **10**, we stored it in an amber bottle almost one year at room temperature without any changes in its chemical reactivity and composition.

Experimental Section

All chemicals were obtained from commercial suppliers, solvents were distilled prior to use. Chemical ractions were carried out in a Büchi R 152 evaporator. Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were measured on a Varian Unity 500 instrument (^{1}H at 500 MHz, ^{13}C at 125.7 MHz) in hexadeuteriodimethyl sulfoxide and were referenced to the solvent signal ($\delta_H = 2.50$, $\delta_C = 39.7$). UV spectra were recorded on Cary 100 (Varian). Analytical TLC was performed on silica gel plates, Silufol (Kavalier, Czech Republic) in ethyl acetate−acetone−ethanol−water (6:1:1:0.5) (system H).

4-Nitro-2-picoline-*N*-oxide (7). A round-bottom flask (20 L) with 2-picoline-1-oxide (Fluka) (**6**) (1350 g, 12.37 mol) and fuming nitric acid (8 L) (added at room temperature) connected to a Büchi R 152 evaporator in "reflux regime" was heated at 80 °C under gentle rotation in a tetraethylene glycol bath. The course of nitration was checked by TLC on silica gel in system H. After 8 h of reaction (complete conversion of starting material), nitric acid was distilled off at reduced pressure (approximately 6 L), the residue was cooled and diluted with ice-cold water (6 L), and the mixture was again concentrated at reduced pressure (5 mbar). This co-distillation procedure was repeated several times until pH of the distillate exceeded the value of 4. The semisolid residue was cooled in an ice bath, cold water (2 L) was added, and crystals were filtered off and washed by ice-cold water (4 × 2 L) until a neutral pH value of the filtrate was achieved. The filtrates were combined, slowly neutralized by solid sodium hydrogencarbonate to pH 7 (***Warning:*** rapid evolution of CO_2), and the solution was concentrated in vacuo (to the volume of 1 L). Cooling of this solution to 0 °C afforded, after filtration, a second crop of the crude product. Both crystalline parts were combined and dissolved under reflux in water (6 L), and the solution was filtered through Celite and concentrated at reduced pressure (to approximately 800 mL). During the concentration process, a spontaneous crystalization took place. Crystals were filtered off, washed quickly with ice-cold water (3 × 200 mL), and dried in vacuo. Yield: 1603 g (10.40 mol, 84%) of compound **7**; the compound does not melt; sublimation

(20) Kohl, B.; Sturm, E.; Senn-Bilfinger, J.; Simon, W. A.; Krueger, U. et al. *J. Med. Chem.* **1992** *3*, 51049.
(21) Katada, Y. Z. *Chem. Abstr.* **1951**, 9537.

occurred upon heating (ref[22] mp 154−156 °C).

Elemental anal.: for calcd $C_6H_6N_2O_3$ (154.12) C 46.76, H 3.92, N 18.18; found C 46.52, H 3.93, N 18.08.

HR FAB$^+$ (PEG 100, methanol) calcd for $C_6H_6N_2O_3$ 154.037842, found 155.045667 $(M + H)^+$.

^{1}H NMR: 2.42 s, 3H (CH_3); 8.08 dd, 1H, *J*(H5,H3) = 3.3, *J*(H5,H6) = 7.1 (H-5); 8.42 dd, 1H, *J*(H3,H5) = 3.3, *J*(H3,H6) = 0.5 (H-3); 8.44 dd, 1H, *J*(H6,H5) = 7.1, *J*(H6H3) = 0.5 (H-6).

^{13}C NMR: 17.29 (CH_3); 118.72 (C-5); 121.03 (C-3); 140.12 (C-6); 141.44 (C-4); 150.04 (C-2).

4-Methoxy-2-picoline-*N*-oxide (8). An ice-cold 2 M solution of sodium methoxide in methanol [prepared from metalic sodium (718 g, 31.2 mol) and anhydrous methanol (15.6 L)] was added to 4-nitro-2-picoline-*N*-oxide (**7**) (1603 g, 10.40 mol), and the resulting suspension was stirred by rotating on Büchi R 152 evaporator at room temperature. Nucleophilic displacement of the nitro group for methoxyl was checked by TLC in system H. After 8 h, the suspension was neutralized to pH 7 by adding of dry ice. The resulting thick suspension was then evaporated to dryness, the solid residue suspended in chloroform (6 L), and the suspension transferred onto the chromatography column (150 × 1500 mm) which was eluted by chloroform. Ethyl acetate can be used instead of chloroform for this disolving-washing procedure. In this case, elution of the product is much slower because of lower solubility of methoxy compound in ethyl acetate. The effluent was concentrated in vacuo and the solid residue dried at 2 mbar. Yield, 1430 g (10.28 mol, 99%) of a TLC-pure product **8**. For analytical purpose, a small sample of this compound was crystallized from water-saturated ethyl acetate); mp 56−60 °C (ref[23] 79.5−81 °C).

Elemental anal.: for $C_7H_9NO_2 \cdot H_2O$ (157.16) calcd C 53.49, H 7.05, N 8.91; found C 53.42, H 7.10, N 9.08.

HR FAB$^+$ (glycerol, methanol) calcd for $C_7H_9NO_2$ 139.063328, found 140.071154 $(M + H)^+$.

^{1}H NMR: 2.32 s, 3H (CH_3); 3.79 s, 3H (O CH_3); 6.89 dd, 1H, *J*(H5,H3) = 3.5, *J*(H5,H6) = 7.2 (H-5); 7.10 d, 1H, *J*(H3,H5) = 3.5 (H-3); 8.11 d, 1H, *J*(H6,H5) = 7.2 (H-6).

^{13}C NMR: 17.82 (CH_3); 56.23 (OCH_3); 110.61 (C-5); 112.01 (C-3); 139.54 (C-6); 148.88 (C-2); 156.31 (C-4).

2-Acetoxymethyl-4-methoxypyridine (9). A solution of 4-methoxy-2-picoline-*N*-oxide (**8**) (1430 g, 20.28 mol) in glacial acetic acid (3 L) was ***slowly added*** to acetic anhydride (4.92 L) ***preheated to 90−100 °C***. The reaction was carried out in Büchi R 152 evaporator in "reflux" regime.

Caution: This acetylation is a strongly exothermic reaction, and therefore, the addition of compound **8** ***must be strictly under control***, if the large-scale experiment is realised. We also tried to perform this reaction in reverse order, that is, by step-by-step addition of acetic anhydride to picolyl derivative **8**. After addition of ∼10% of amount of acetic anhydride, a noncontrolled *exothermic*, almost *explosive* reaction started up and a substantial amount of material was lost.

After the addition, the reaction mixture was heated at the same temperature for further 8 h. TLC in system H revealed complete disappearance of the starting material and the presence of a faster product. The solution was then subjected to fractional distillation. Acetic acid and acetic anhydride were distilled off at 30−56 °C/25 mbar, and the product at 138 °C/4−5 mbar. Yield, 1000 g (6.61 mol, 27%) of **9**.

A small sample of the product **9** (100 mg, 0.55 mmol) was deacetylated in 30% aqueous ammonia (10 mL) at room-temperature overnight. The solution was evaporated in vacuo and a semisolid residue dried at 50 °C (0.1 mbar) for 20 h. The obtained crystalline sample of 2-hydroxymethyl-4-methoxypyridine was subjected, instead of compound **9,** to all analyses; mp 67−70 °C (ref[24] 74−75 °C).

Elemental anal.: for $C_7H_9NO_2$ (139.15) calcd C 60.42, H 6.52, N 10.07; found C 60.39, H 6.60, N 10.11.

HR FAB$^+$ (glycerol, methanol) calcd for $C_7H_9NO_2$ 139.063328, found 140.071154 $(M + H)^+$.

^{1}H NMR: 3.82 s, 3H (OCH_3); 4.51 d, 2H, *J*(CH_2,OH) = 4.5 (OCH_2); 5.51 t, 1H, *J*(OH,CH_2) = 4.5 (OH); 6.90 dd, 1H, *J*(H5,H3) = 2.6, *J*(H5,H6) = 5.8 (H-5); 7.01 d, 1H, *J*(H3,H5) = 2.6 (H-3); 8.27 d, 1H, *J*(H6,H5) = 5.8 (H-6).

^{13}C NMR: 55.30 (OCH_3); 64.23 (OCH_2); 105.92 (C-5); 108.35 (C-3); 150.07 (C-6); 164.07 (C-2); 166.05 (C-4).

4-Methoxy-2-pyridinemethanol 1-oxide (10). Ice-cold 30% aqueous hydrogen peroxide (272 mL, 2.66 mol) was slowly added to acetic anhydride (1.322 L, 14.00 mol) at 0 °C under stirring and then, within 2 h, the temperature was gradually risen to 45 °C. Then, 2-acetoxymethyl-4-methoxypyridine (**9**) (1000 g, 5.5 mol) was added dropwise under stirring to the prepared solution of peroxoacetic acid, and the reaction temperature was maintained at 45 °C.

Caution: The above-mentioned oxidation step is strongly ***exothermic***. Since during addition of the pyridine derivative **9** the temperature started to rise rapidly, pieces of ***dry ice*** were added ***directly*** into the mixture to maintain the temperature near 45 °C.

After addition, the reaction mixture was set aside at the same temperature for 12 h. The solution was then concentrated in vacuo (45 °C in bath, 4−5 mbar). The crude product, the 2-acetoxymethyl-4-methoxypyridine-*N*-oxide was used for final deacetylation without further purification. The residue was dissolved in concentrated aqueous ammonia (2 L), and the resulting solution was set aside overnight at room temperature and then concentrated in vacuo (50 °C in bath, 8−10 mbar). The highly viscous, dark red-brown residue was dissolved in water (4 L), and the resulting solution was applied onto a column of Dowex 50 (H^+) (5 L). The column was washed with water until UV absorbing compounds were eluted, and then the column was washed with 10% aqueous ammonia. This ammonia effluent was evaporated to dryness, the solid residue extracted with hot ethyl acetate (10 × 1 L), and the dark solution containing gummy residue was filtered through Celite. The combined filtrates were concentrated to approximately 3 L, and the solution was set aside to crystallize in a refrigerator overnight. Crystals were filtered off, washed by a small volume of

(22) Hom, R. K.; Chi, D. Y.; Katzenellenbogen, J. A. *J. Org. Chem*. **1996**, *8*, 2624.

(23) Bojarska, D. *Recl. Trav. Chim. Pays-Bas* **1959**, *78*, 981, 985.

(24) Schultz; Fedders *Arch. Pharm. (Weinheim Ger.)* **1977**, *310*, 128, 135.

dichloromethane (100 mL), and dried in vacuo. Mother liquors were concentrated in vacuo, the semisolid residue was dissolved in water (2 L), and the dark solution was pumped, after previous filtration through Celite, on a chromatography column (25 × 500 mm) charged with octadecyl silica gel (40−60 μm) at the flow rate of 15 mL/min. Elution of the column with water (approximately 2 L) afforded a light-brown effluent which was evaporated, the solid residue was dried in vacuo and recrystallized from hot ethyl acetate as described above to afford a further crop of *N*-oxide **10**. All of the obtained crude crystalline parts were combined and dissolved in refluxing chloroform (5 mL/g), and the solution, diluted with 2 vols of dichloromethane, was set aside overnight in a refrigerator. The crystals were collected, washed with small amount of cold dichloromethane, and dried in vacuo. Recrystalization under the same conditions afforded pure 4-methoxy-2-pyridinemethanol 1-oxide (**10**) in the yield of 240 g (1.55 mol, 23%) as light- and heat-stable white needles; mp 143−143.5 °C (ref[19] 135−137 °C).

Elemental anal.: for $C_7H_9NO_3$ (155.15) calcd C 54.19, H 5.85, N 9.03; found C 53.93, H 5.85, N 9.09.

HR FAB$^+$ (glycerol, methanol) calcd for $C_7H_9NO_3$ 155.058243, found 156.066068 $(M + H)^+$.

UV spectra in neutral (50% aqueous methanol), λ_{max} 261 nm, λ_{min} 226 nm; in acidic (0.01 M HCl in 50% aqueous methanol) λ_{max} 261 nm, λ_{min} 224 nm; in basic (0.01 M NaOH in 50% aqueous methanol) λ_{max} 261 nm, λ_{min} 227 nm.

^{1}H NMR: 3.83 s, 3H (OCH_3); 4.54 d, 2H, $J(CH_2,OH)$ = 5.6 (OCH_2); 5.71 t, 1H, $J(OH,CH_2)$ = 5.6 (OH); 6.95 dd, 1H, J(H5,H3) = 2.5, J(H5,H6) = 8.0 (H-5); 7.06 d, 1H, J(H3,H5) = 2.5 (H-3); 8.13 d, 1H, J(H6,H5) = 8.0 (H-6).

^{13}C NMR: 56.25 (OCH_3); 58.58 (OCH_2); 108.24 (C-5); 110.50 (C-3); 139.61 (C-6); 152.50 (C-2); 156.89 (C-4).

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