## An Efficient One-Pot Synthesis of a Fully Protected 2′-Deoxycytidine 3′-Monophosphate

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Considerable progress in the field of genetic engineering has led to an increasing demand for synthetic oligonucleotides[1,2]. In recent years the phosphotriester approach has been one of the preferred synthetic methods[3]. In this method, fully protected 2′-deoxyribonucleoside 3′-monophosphates such as **7** are key building blocks and are characterized by permanent protecting groups on both the base and phosphate moieties as well as temporary ones on the 5′-OH and the phosphate.

The synthesis of **7** usually involves at least three steps: (a) benzoylation, (b) tritylation, (c) phosphorylation, and, with some methods, the phosphorylation stage alone consists of several steps[4,5,6]. Overall yields are in the order of 30% with respect to the initial 2′-deoxyribonucleoside. Thus, there is still room for improvement in synthetic procedures in order to obtain better yields and reduce the time required for synthesis.

An improved method for selective $N^4$-benzoylation and subsequent tritylation of 2-deoxycytidine **(1)** (without isolation of **3**) has been reported[7]. The latter method uses an activated ester of the type pentafluorophenyl benzoate or cheaper pentachlorophenyl benzoate in pyridine to give **3**. Finally, the 5′-OH group in **3** is protected by reaction with 4,4′-dimethoxytrityl chloride **(4)** to give compound **5**.

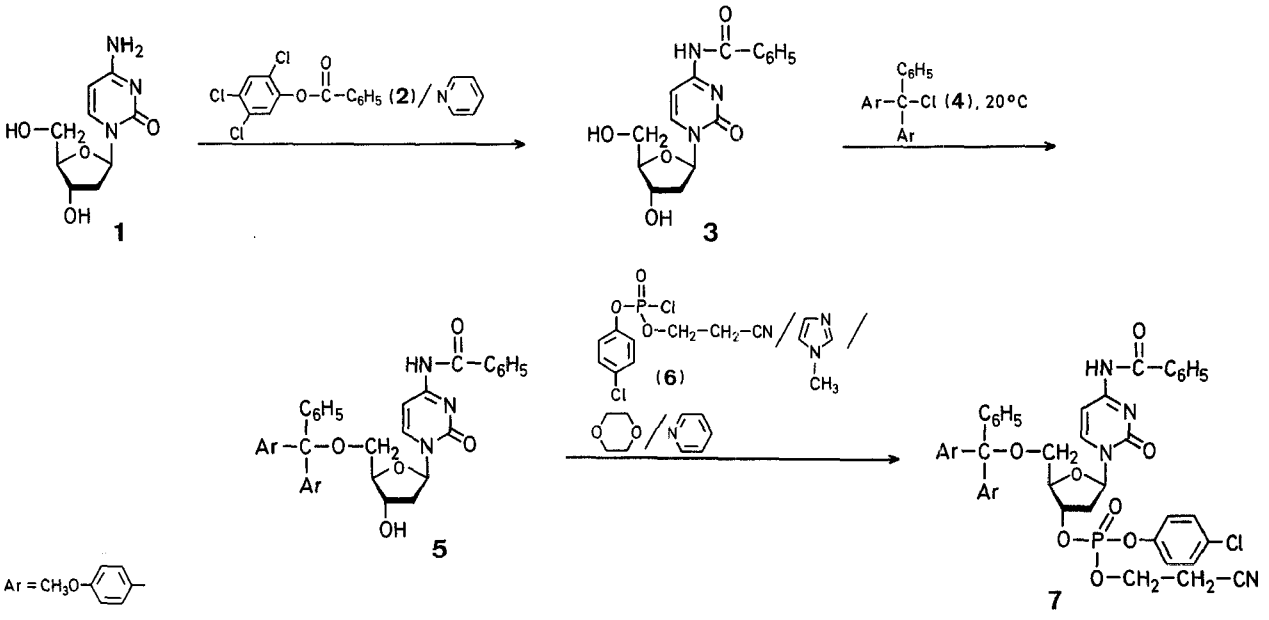
We now report a one-pot procedure for the preparation of **7** using a modification of the above method[7] followed by direct phosphorylation using the monofunctional phosphorylating reagent **6**[8]. 2,4,5-Trichlorophenyl benzoate **(2)** reacts with 2-deoxycytidine **(1)** giving the $N^4$-benzoylated derivative **3**. Addition of 4,4′-dimethoxytrityl chloride **(4)** gives **5** and subsequent addition of **6** in dioxan/pyridine in the presence of methylimidazole gives **7** in a yield of 60% with respect to the starting compound **1**. The advantage of this procedure is that **7** can be prepared without isolation of **3**[7] or **5** by column chromatography. This greatly simplifies the procedure and reduces the time required for synthesis.

2-Deoxycytidine was obtained from Pharma-Waldorf, 2,4,5-trichlorophenol and methylimidazole from Merck, and benzoyl chloride from Prolabo; the latter two compounds were distilled before use; 4,4′-dimethoxytrityl chloride was obtained from Aldrich-Europe.

Melting points were determined on a Büchi apparatus. $^1$H-N.M.R. spectra were recorded in deuteriochloroform on a Varian EM-390 90 MHz spectrometer using TMS as a reference. $^{31}$P-N.M.R. spectra were measured in pyridine-$d_5$ on a Bruker WP-60, (24.3 MHz) spectrometer. Chemical shifts were measured using $H_3PO_4$ as an internal standard. For T.L.C. analysis, Merck F60 U.V. sensitive (254 nm) silica gel plates were used. Chloroform and chloroform/methanol/water (90/10/1) were used as eluents.

**2,4,5-Trichlorophenyl Benzoate (2):**

2,4,5-Trichlorophenol (6 g, 30 mmol) is dissolved in 10% aqueous sodium hydroxide solution (60 ml), benzoyl chloride (13.8 g, 11.5 ml, 100 mmol) is added and the solution is stirred in an ice bath for 15 min. The desired 2,4,5-trichlorophenyl benzoate **(2)** is obtained as a white precipitate, which is filtered off, thoroughly washed with water, and



then dried in a vacuum dessicator. After recrystallisation from ethanol, pure 2,4,5-trichlorophenyl benzoate (**2**) is obtained; yield: 6.1 g (77%); m.p. 91 °C (Ref.[9], m.p. 93 °C).

$^1$H-N.M.R. (acetone-$d_6$/TMS$_{int}$): $\delta$ = 7.3–7.4 (m, 4 H$_{arom}$); 8.1–8.2 ppm (m, 3 H$_{arom}$).

**$N^4$-Benzoyl-5′-*O*-4,4′-dimethoxyltrityl-2′-deoxycytidine 3′-*O*-(4-Chlorophenyl-2-cyanoethyl)-phosphate (7):**

2′-Deoxycytidine (**1**; 5 g, 22 mmol) is suspended in dry pyridine (50 ml) to which 2,4,5-trichlorophenyl benzoate (**2**; 10 g, 33 mmol) is added. The suspension is then placed in an oil bath and heated to 85 °C for 7.5 h until T.L.C. analysis indicates complete conversion of **1** to **3**. After cooling to room temperature (if required, **3** can be isolated by column chromatography) 4,4′-dimethoxytrityl chloride (**4**; 8.2 g, 24 mmol) is added. The reaction mixture is stirred at room temperature for 1 h until T.L.C. analysis shows complete conversion of **3** to **5**. Excess **4** is destroyed by addition of methanol (2 ml). Pyridine is removed by evaporation and the residue thoroughly dried under high vacuum. The dried material is then taken up in dioxan (95 ml) and pyridine[7] (5 ml), methylimidazole (7.2 g, 88 mmol), and reagent **6** (18.4 g, 66 mmol) are added. The reaction is carried out in a glove box under an anhydrous nitrogen atmosphere. On addition of **6**, the formation of a white precipitate is observed. The reaction mixture is stirred for 30 min (T.L.C. shows complete conversion of **1** to **7**). Excess **6** is hydrolysed by addition of 1:1 pyridine/water (20 ml) during 30 min. Dioxan and pyridine are removed by evaporation. The residue is dissolved in chloroform (500 ml) and the solution washed with aqueous sodium hydrogen carbonate (3 × 500 ml), followed by water (500 ml). The chloroform phase is concentrated to 5–10 ml and added dropwise to ether (1000 ml) to remove methylimidazole[7]. The product precipitates in the ether. After filtration through a glass fibre filter, the precipitate is redissolved in chloroform (15 ml) and then chromatographed on a silica gel column (Bio-rad, Bio-Sil A 100–200 mesh; 5 × 30 cm). Elution of non-polar impurities is carried out with 1:1 chloroform/hexane (500 ml) followed by chloroform (500 ml). The desired product is eluted with 1.5% methanol in chloroform (1000 ml). The fractions containing the desired product are concentrated to ~50 ml for purification by preparative H.P.L.C. The following instrumental and experimental parameters are used for the preparative H.P.L.C. purification:

Column: 10 $\mu$ partisil silica, 2.5 cm × 40 cm; two columns connected in series;
Pump: M.S. 17/7, ORLITA GmbH & Co. KG, Giessen/Lahn;
Flow rate: 12–15 ml/min at 200 bar;
Injection charge: 12 g in chloroform (50 ml);
Detector: Cecil U.V. 212 A variable wavelength monitor; product **7** is detected at 350 nm;
Elution: Gradient 0–60% ethyl acetate in chloroform; retention time of product **7**: 100–110 min.

The fractions containing the product are evaporated to dryness; yield: 10.9 g (55%); white powder

$^1$H-N.M.R. (CDCl$_3$)/TMS$_{int}$), diastereoisomers: $\delta$ = 2.30 (m, 2″-H); 2.70 (t, CH$_2$CN); 2.95 (m, 2′-H); 3.45 (m, 5′-H, 5″-H); 3.76 (s, OCH$_3$); 4.30 (m, 4′-H, POCH$_2$); 5.20 (m, 3′-H); 6.30 (t, 1′-H); 6.80 (d, 6-H); 6.9–7.4 (m, H$_{arom}$); 7.5 (d, 5-H); 7.7–8.1 ppm (m, H$_{arom}$).

$^{31}$P-N.M.R. (pyridine-$d_5$/H$_3$PO$_{4int}$): $\delta$ = −7.3 and −7.5 ppm, corresponding to the two diastereoisomers of **7**.

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