# An Improved Method for the Synthesis of $N^3$-Benzoylthymidine

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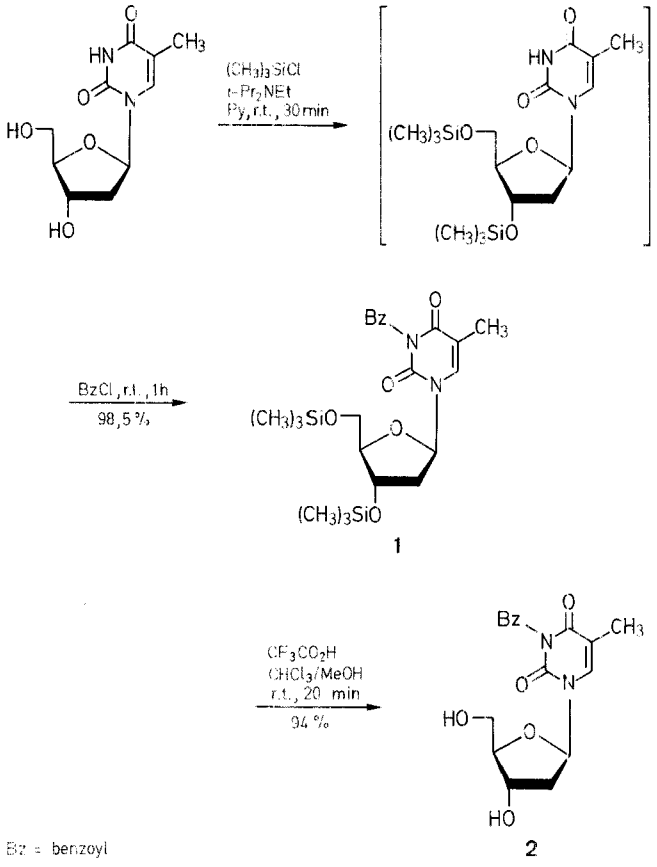
$N^3$-Benzoylthymidine **(2)**, a key intermediate for oligodeoxyribonucleotide synthesis, was synthesized in high yield *via* 3′,5′-bis-*O*-(trimethylsilyl)-$N^3$-benzoylthymidine, which could be isolated as stable crystals.

Recent studies devoted to the chemical synthesis of oligonucleotides using the phosphotriester approach revealed that the remaining reactive sites of the four common base residues should be protected as perfectly as possible to realize high yield synthesis of DNA and RNA fragments.[1-9] In the phosphoramidite method using deoxyribonucleoside methyl 3′-phosphoramidites, $N^3$-methylation of the thymidine moiety was not negligible at the deprotection stage.[10,11] Among several protecting groups[3-8] for the thymine imido function

reported until now, the benzoyl group[6] appeared the most useful, because it could be readily introduced and removed. The benzoyl and anisoyl groups were also used for protection of the imido group of uracil in oligoribonucleotide synthesis.[12,13] For practical use, key synthetic intermediates such as *N*-protected deoxyribonucleosides should be able to be prepared in a large scale, whereby bothersome isolation procedures like silica gel column chromatography can be avoided. This paper describes an improved procedure for the synthesis of $N^3$-benzoylthymidine (**2**).




Compound **2** was previously synthesized by a series of reactions involving silylation of thymidine with chlorotriisopropylsilane followed by benzoylation and then acid treatment. Our preliminary studies showed that the trimethylsilyl group was too labile to use for the reaction. However, it has now been found that this transient protecting group[14] can indeed be employed under certain conditions to obtain **2**.

Thymidine was allowed to react with 2.5 equiv of chlorotrimethylsilane in the presence of 5 equiv of ethyldiisopropylamine in pyridine for 1 h and successively with 1.5 equiv of benzoyl chloride for 1 h. When the mixture was treated with potassium dihydrogen phosphate in ice-water, a large amount of precipitate appeared as colorless needles. This compound was determined to be 3',5'-bis-*O*-(trimethylsilyl)-$N^3$-benzoylthymidine (**1**) from its NMR spectrum and elemental analysis.

Compound **1** gave a clear spot on TLC without any decomposition. Since it has been generally held that a trimethylsilyl group attached to nucleoside hydroxyls was not stable during the usual work-up procedure,[14] these findings were somewhat surprising. However, McLaughlin et al. have very recently reported a similar, but not *N*-protected species, i.e., 2',3',5'-tris-*O*-(trimethylsilyl)guanosine, which was obtained in 54% yield from guanosine.[15]

The previous $N^3$-benzoylation[3] using ethyldiisopropylamine caused considerable coloration, so that repeated chromatography was necessary for the purification of **2**; in the present study, the successful isolation of **1** eliminated this tedious procedure. The yield of **1** was almost quantitative (98.5%). The trimethylsilyl groups were easily removed from **1** by treatment with trifluoroacetic acid in chloroform/methanol to give **2** in 94.4% yield. Compound **2** thus obtained was analytically pure and could be used for various transformations without further purification.

Several attempts to isolate 2',3',5'-tris-*O*-(trimethylsilyl)-$N^3$-benzoyluridine in a similar manner from uridine were unsuccessful.[12]

In conclusion, the present method enhances considerably the synthetic utility of **2** in this field.

**3',5'-Bis-*O*-(trimethylsilyl)-$N^3$-benzoylthymidine (1):**
To a solution of thymidine (7.26 g, 30 mmol), which is rendered anhydrous by repeated coevaporation with dry pyridine, in dry pyridine (100 mL) are added ethyldiisopropylamine (26.1 mL, 150 mmol) and chlorotrimethylsilane (9.52 mL, 75 mmol). After the mixture has stirred at room temperature for 30 min, benzoyl chloride (5.22 mL, 4.5 mmol) is added. The resulting mixture is stirred for 1 h and then $KH_2PO_4$ (25 g) and ice-water (120 g) and are added to the mixture with cooling. After the mixture has been stirred for several minutes, a crystalline precipitate appears. The crystals are collected by filtration and washed with water (500 mL). After air-drying and subsequent drying over $P_2O_5$ *in vacuo* in a desiccator, **1** is obtained; yield: 14.5 g (98.5%); m.p. 162–164°C; Rf 0.40 ($CH_2Cl_2$/MeOH, 20:1, v/v).

| | | | | |
|---|---|---|---|---|
| $C_{23}H_{34}O_6N_2Si_2$ | calc. | C 56.30 | H 6.98 | N 5.71 |
| (490.7) | found | 56.24 | 6.86 | 5.75 |

$^1$H-NMR (60 MHz, $CDCl_3$/TMS): $\delta$ = 0.12 (s, 9 H, $(CH_3)_3Si$); 0.17 (s, 9 H, $(CH_3)_3Si$); 1.95 (s, 3 H, $CH_3$); 2.18 (m, 2 H, 2'-H); 3.78 (m, 2 H, 5'-H); 3.91 (m, 1 H, 4'-H); 4.34 (m, 1 H, 3'-H); 6.27 (t, 1 H, $J$ = 7 Hz, 1'-H); 7.15–8.05 (m, 6 H, ArH and 6-H).

**$N^3$-Benzoylthymidine (2):**
To a solution of **1** (10.0 g, 20.4 mol) in $CHCl_3$/MeOH (200 mL, 1:1) is added $CF_3CO_2H$ (0.5 mL). After being kept at room temperature for 20 min, the mixture is transferred to a separatory funnel containing $CHCl_3$ (200 mL). The $CHCl_3$ solution is washed with 5% aq. $NaHCO_3$ (100 mL) and the washing is back-extracted with $CHCl_3$ (6 × 100 mL). The organic phases are collected, dried ($Na_2SO_4$), and filtered. The filtrate is evaporated to a gum. This gummy material is coevaporated several times with toluene and dissolved in 1,2-dichloroethane (10 mL). The solution is poured into a stirred solution of $Et_2O$/hexane (850 mL, 1:7.5). The resulting hygroscopic solid is collected and dried *in vacuo* in a desiccator over $P_2O_5$ overnight to give **2**; yield: 6.8 g (94.4%).

This sample is obtained as a slightly yellowish foamy solid after drying, and is homogeneous on TLC. All the attempts to crystallize it from a variety of solvents failed. The $^1$H-NMR spectrum of this sample is identical to that of the authentic material.[6]

| | | | | |
|---|---|---|---|---|
| $C_{17}H_{18}O_6N_2$ | calc. | C 58.96 | H 5.24 | N 8.09 |
| (346.3) | found | 58.75 | 5.31 | 7.90 |

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