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Note

New methods for the synthesis of *N*-benzoylated uridine and thymidine derivatives; a convenient method for *N*-debenzoylation

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Abstract

An improved procedure for the synthesis of *N*-benzoyl-2′,3′-*O*-isopropylidene uridine via one-step selective *N*-benzoylation of 2′,3′-*O*-isopropylidene uridine has been developed. An efficient synthetic route to *N*-benzoyl thymidine via initial tribenzoylation, followed by selective hydrolysis of the benzoates is also described. De-*N*-benzoylation of *N*-benzoylated thymidine and uridine derivatives can be conveniently effected under neutral conditions, by heating with benzyl alcohol.

Keywords: Selective protection and deprotection; *N*-Benzoyl uridine and thymidine

Since the introduction of the chemical synthesis of oligonucleosides, a myriad of protecting groups for nucleosides has been developed.[1] Among the protecting groups which have been explored for the bases in uridine and thymidine, the benzoyl group has emerged as the most important and commonly employed.[2,3] While this group provides a number of significant advantages as a means of N^3 protection, such as ease of introduction and stability towards many commonly encountered reaction conditions, deprotection is usually performed under basic conditions and the concentrated NH_4OH–methanol procedure[3] is perhaps the most useful. Notably, Köster and co-workers[4] have reported interesting data concerning the stability of *N*-acyl groups towards a potent deacylating system (0.2 M NaOH–MeOH).

During a recent study, we required *N*-benzoylated pyrimidine derivatives as precursors, but due to the base sensitivity of our final products, we needed to conduct the subsequent deprotection under neutral conditions. For practical purposes, efficient synthetic routes to the *N*-benzoylated key intermediates were sought. This paper describes an improved procedure for the synthesis of *N*-benzoyl-2′,3′-*O*-isopropylidene uridine (**2**) via one-step selective *N*-benzoylation of 2′,3′-*O*-isopropylidene uridine (**1**), two new synthetic methods for *N*-benzoyl thymidine **5** and a convenient method for *N*-debenzoylation of the *N*-benzoylated nucleoside derivatives under neutral conditions.

Despite the relatively simple structure of *N*-benzoyl-2′,3′-*O*-isopropylidene uridine (**2**), which can be envisaged as a useful synthetic intermediate, we were unable to find any reference to its synthesis. Synthesis of *N*-benzoyl uridine from uridine by trimethylsilylation, treatment with benzoyl chloride and hydrolysis has been described.[2b] Direct reaction of 2′,3′-*O*-isopropylidene uridine (**1**) with benzoyl chloride and pyridine was reported to produce the 5′-*O*-benzoyl derivative.[5] *N*-Benzoylation of uridine under phase transfer conditions was also conducted on the precursor protected at the 3′- and 5′-OH groups by silylation.[6] Thus direct *N*-benzoylation of uridine in the presence of the unprotected primary 5′-OH group poses a challenge.

We undertook an extensive investigation of the influence of reaction conditions, and especially the nature of

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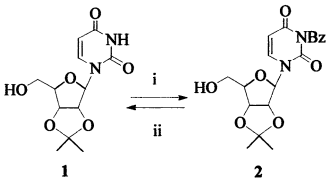
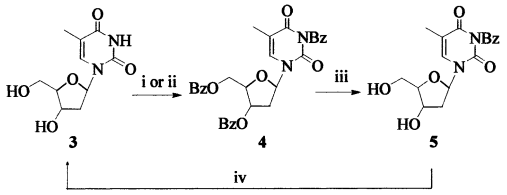


Scheme 1. (i) BzCl (1.1 equiv), Et_3N (1.1 equiv), CH_2Cl_2, rt, 6 h, 56%; (ii) BnOH, 90 °C, 30 h, 85%.



Scheme 2. (i) BzCl (3.6 equiv), $DMAP_{cat}$, pyridine, rt, 9 h, 89%; (ii) BzCl (3.3 equiv), KOH (3.3 equiv), $TEBAC_{cat}$, CH_2Cl_2, rt, 8 h, 83%; (iii) NaOH 1 M (2.2 equiv), pyridine, rt, overnight, 92%; (iv) BnOH, 90 °C, 30 h, 82%.

the base, on the regioselectivity of the benzoylation. When **1**, prepared following a literature procedure,[7] was treated with benzoyl chloride (1.1 equiv) in the presence of triethylamine (1.1 equiv) in dichloromethane at room temperature for 6 h, selective *N*-benzoylation of 2′,3′-*O*-isopropylidene uridine (**1**) was observed producing *N*-benzoyl-2′,3′-*O*-isopropylidene uridine **2** in 56% yield (Scheme 1). The product was fully characterised spectroscopically and by elemental analysis. Use of other bases such as DMAP (4-*N*,*N*-dimethylaminopyridine) under the same conditions gave 5′-*O*-benzoyl-2′,3′-*O*-isopropylidene uridine, consistent with the literature precedent for preferential reaction at the 5′-OH group,[5] highlighting the significance of the selective *N*-benzoylation achieved using triethylamine as base. The reason for the difference in behaviour depending on the base employed is not clear.

Synthesis of *N*-benzoyl thymidine **5** was previously reported by Sekine and co-workers.[8] Protection of the 3′- and 5′-OH groups by silylation prior to *N*-benzoylation and subsequent *O*-deprotection, was conducted,[3] reminiscent of the literature approaches developed for *N*-benzoyl uridine outlined above. However, a synthetic route to *N*-benzoyl deoxycytidine by Khorana and co-workers[9] caught our attention—they had reported initial conversion of deoxy cytidine to the *N*, 3′,5′-tribenzoyl derivative followed by selective ester hydrolysis. This strategy appeared attractive as an efficient route to *N*-benzoyl thymidine **5**, obviating the necessity for intermediate silyl protection.

Treatment of thymidine **3** with 3.6 equiv of benzoyl chloride in the presence of a catalytic amount of DMAP in pyridine for 9 h gave the *N*, 3′,5′-tribenzoyl derivative **4** in 89% yield. Alternatively, benzoylation of thymidine was also effected in 83% yield under phase transfer conditions, using KOH, benzyltriethylammonium chloride (TEBAC) and benzoyl chloride in dichloromethane for 8 h. This intermediate was subjected to selective de-*O*-acylation, which could be performed quantitatively under alkaline treatment using 1 M NaOH (2.2 equiv) in pyridine overnight. After neutralisation with Amberlite resin IR-120(H), the desired product **5** was obtained in 92% yield (Scheme 2).

Interestingly, when thymidine **3** was reacted under the conditions employed for the direct *N*-benzoylation of 2′,3′-*O*-isopropylidene uridine (**1**), i.e., benzoyl chloride, triethylamine in dichloromethane, selective *N*-protection was not achieved; instead 3′,5′-dibenzoyl thymidine **6**[10,11] was obtained in 24% yield and the tribenzoyl derivative **4** was obtained in 3% yield.

Having achieved efficient synthetic routes to the *N*-benzoylated pyrimidine derivatives **2** and **5**, our attention focused on developing a method for *N*-debenzoylation under neutral conditions. While this deprotection is usually conducted under basic conditions as outlined above, Ishido and co-workers developed specific *N*-debenzoylation of a series of perbenzoylated adenosine and cytidine derivatives by treatment with a series of phenols and aliphatic alcohols to give the corresponding benzoates with free amino groups in high yields.[12]

These results prompted us to investigate deprotection of the *N*-benzoylated thymidine and uridine derivatives **2** and **5** on treatment with alcohols; use of benzyl alcohol proved successful, although it was not among the alcohols described in the earlier report.[12] The *N*-benzoyl uridine derivative **2** was dissolved in benzyl alcohol and after stirring at 90 °C for 30 h, the expected product **1** was obtained in good yield (85%, Scheme 1). This method has been also applied to the compound **5**, and under the same conditions described above, the *N*-deprotection to thymidine **3** was effected in 82% yield (Scheme 2).

In conclusion, the present paper describes efficient methods for the synthesis of *N*-benzoyl uridine and thymidine derivatives, and a practical solution for the *N*-debenzoylation of these protected nucleosides under very mild, neutral conditions and in good yields. The selective *N*-benzoylation of 2′,3′-*O*-isopropylidene uridine (**1**) is notable. The use of these intermediates in the synthesis of multifunctionalised nucleosides is under investigation in our laboratory and will be reported in due course.

1. Experimental

^1H and ^{13}C NMR spectra were recorded on a JEOL GSX FT or on a Bruker Avance DPX 300 spectrometer. Chemical shifts (δ) are expressed in parts per million (ppm) and J values are reported in Hz. TMS

was used as an internal standard for ^1H and ^{13}C NMR. IR spectra were recorded on a Perkin–Elmer Paragon 1000 FT-IR spectrometer as liquid films or KBr discs. Thin-layer chromatography was performed on DC-Alufoilen Kieselgel $60F_{254}$ 0.2 mm plates (E. Merck) and visualised under UV light and stained with phosphomolybdic acid–aq H_2SO_4 solution.

Column chromatography was performed on Kieselgel 60 (E. Merck) 60–200 mesh. All solvents were distilled before use: hexane from calcium chloride, CH_2Cl_2 and EtOAc from phosphorous pentoxide. Dry solvents were prepared by refluxing over a drying agent under an inert atmosphere: THF was dried over sodium–benzophenone, ether, benzene and toluene were dried over $LiAlH_4$. DMF was distilled from CaH_2 under reduced pressure (ca. 15 mmHg) and stored over activated molecular sieves. Magnesium sulphate was used as drying agent.

N^3-*Benzoyl-2′,3′*-O-*isopropylidene uridine* (**2**).—Benzoyl chloride (0.55 mL, 4.8 mmol, 1.1 equiv) and triethylamine (0.67 mL, 4.8 mmol, 1.1 equiv) were added to a solution of 2′,3′-*O*-isopropylidene uridine (**1**, 1.14 g, 4.3 mmol) in CH_2Cl_2 (30 mL). The mixture was stirred at rt under nitrogen for 9 h. The solvent was evaporated. Toluene (20 mL) was introduced and evaporated. This operation was repeated twice. Flash chromatography on silica gel (1:1 EtOAc–hexane) afforded the main product **2** (0.95 g, 56%) (Found: C, 56.32; H, 5.32; N, 6.93. $C_{19}H_{20}N_2O_7{\cdot}H_2O$ requires C, 56.16; H, 5.46; N, 6.89); ν_{max} (KBr)/cm^{-1} 3440 (OH), 1748 (C=O_{Bz}), 1705, 1670 (C=C, C=O_{uracil}); δ_H (300 MHz, $CDCl_3$) 7.81–7.78, 7.60–7.20 (6 H, m, H-6, Ph), 5.74 (1 H, $J_{5,6}$ 8.1, d, H-5), 5.67–5.65 (1 H, m, H-1′), 4.88–4.86 (1 H, m, H-2′), 4.80–4.76 (1 H, m, H-3′), 4.25–4.23 (1 H, m, H-4′), 3.80–3.63 (2 H, m, CH_2-5′), 1.47, 1.25 (6 H, 2s, 2 CH_3); δ_C (75.4 MHz, $CDCl_3$) 167.4 (CO), 161.2 (C-4), 148.3 (C-2), 141.2 (C-6), 134.4, 130.2, 129.5, 128.3 (C-Ar), 113.2 ($C(CH_3)_2$), 101.1 (C-5), 94.1 (C-1′), 85.9 (C-2′), 83.2 (C-3′), 79.5 (C-4′), 61.5 (C-5′), 26.2, 24.2 ($2CH_3$); *m*/*z* (EI): 43 (98), 59 (74), 77 (80, Ph^+), 105 (100, $PhCO^+$), 173 (27), 216 (14), 277 (46), 388 (1%, M^+).

This sample is obtained as a cream solid after drying. All the attempts to crystallise it from a variety of solvents failed.

N^3,*3′,5′*-O-*Tribenzoyl thymidine* (**4**)[10]

Method A. Benzoyl chloride (1.7 mL, 14.8 mmol, 3.6 equiv) and dimethylaminopyridine DMAP (40 mg) were added to a solution of thymidine **3** (1 g, 4.1 mmol) in pyridine (30 mL). The mixture was stirred at rt for 9 h. The solvent was evaporated. Toluene (20 mL) was introduced and evaporated. This operation was repeated twice. Flash chromatography on silica gel (1:1 EtOAc–hexane) afforded *N*,3′,5′-*O*-tribenzoyl thymidine (**4**, 2.04 g, 89%) as a white solid, mp 121–122 °C (EtOH) (lit,[10] mp 124–125 °C) δ_H (300 MHz, $CDCl_3$) 8.18–7.38 (16 H, m, H-6, 3 Ph), 6.49–6.44 (1 H, m, H-1′), 5.68–5.66 (1 H, m, H-3′), 4.83–4.65 (2 H, m, 2 H-5′), 4.55–4.52 (1 H, m, H-4′), 2.75–2.40 (2 H, m, 2 H-2′), 1.66 (3 H, s, CH_3); δ_C (75.4 MHz, $CDCl_3$) 168.8, 165.9, 162.8 (3 CO), 162.5 (C-4), 149.2 (C-2), 135.1, 134.6, 134.5, 133.7, 131.5, 130.5, 130.4, 129.7, 129.5, 129.3, 129.0, 128.9, 128.6 (C-6, C-Ar), 111.5 (C-5), 85.1 (C-4′), 82.7 (C-1′), 74.9 (C-3′), 64.2 (C-5′), 37.8 (C-2′), 12.8 (CH_3).

Method B. Pulverised KOH (0.15 g, 2.7 mmol, 3.3 equiv), TEBAC (20 mg, 0.08 mmol, 0.1 equiv) and benzoyl chloride (0.31 mL, 2.7 mmol, 3.3 equiv) were added to a solution of thymidine **3** (0.2 g, 0.83 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred at rt for 8 h, then extracted twice with water (10 mL). After drying, the organic phase was evaporated to dryness under reduced pressure. Flash chromatography on silica gel (1:1 EtOAc–hexane) afforded *N*,3′,5′-*O*-tribenzoyl thymidine (**4**, 0.38 g, 83%).

N^3-*Benzoyl thymidine* (**5**)[8].—A solution of NaOH (8.14 mL, 1 M, 8.14 mmol, 2.2 equiv) was added to a mixture of *N*^{3},3′,5′-*O*-tribenzoyl thymidine (**4**, 2.04 g, 3.7 mmol) in freshly distilled pyridine (20 mL) and the mixture was stirred vigorously overnight. (The efficiency of this process is critically dependent on the quality of the pyridine employed—distilling just before use is recommended.) The solution was neutralised with Amberlite resin IR-120(H). The ion exchanger was washed with CH_2Cl_2 and flash chromatography on silica gel (7:3 EtOAc–hexane) afforded *N*-benzoyl thymidine **5** as a cream solid (1.17 g, 92%) δ_H (300 MHz, $CDCl_3$) 7.95–7.30 (6 H, m, H-6, H-Ar), 6.15–6.06 (1 H, m, H-1′), 4.41–4.32 (1 H, m, H-3′), 3.88–3.72 (1 H, m, H-4′), 3.72–3.50 (2 H, m, 2 H-5′), 2.28–2.20 (2 H, m, 2 H-2′), 1.85 (3 H, s, CH_3); δ_C (75.4 MHz, $CDCl_3$) 167.5 (CO), 162.5 (C-4), 149.0 (C-2), 135.6, 134.3, 130.3, 129.5, 128.2 (C-Ar, C-6), 110.0 (C-5), 86.0 (C-4′), 85.3 (C-1′), 70.3 (C-3′), 61.2 (C-5′), 39.2 (C-2′), 11.6 (CH_3).

General procedure for the debenzoylation.—The compound (1 mmol) was dissolved in 1 mL of benzyl alcohol and heated at 90 °C for 30 h. The solvent was evaporated under vacuum and flash chromatography on silica gel afforded the *N*-debenzoylated derivative.

Purification conditions and yield of the product are as follows.

2′,3′-O-*Isopropylidene uridine* (**1**).—Silica gel-column chromatography (EtOAc) of the crude product of 1 mmol synthesis (0.39 g of **2**) afforded **1** (0.24 g, 85%) as a cream solid. This product showed ^1H and ^{13}C NMR spectral data identical to those of an authentic sample of **3**.

Thymidine (**3**).—Silica gel-column chromatography (20:1 CH_2Cl_2–EtOH) of the crude product of 1 mmol synthesis (0.24 g of **5**) afforded **3** (0.28 g, 82%) as a white solid. This product is identical in all respects to the commercial material.

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