Facile Nucleophilic Displacement on a 4-Triazolylpyrimidine Deoxynucleoside: Single-step Synthesis of *N*-Acylated 5-Methyldeoxycytidines

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A 4-triazolyl substituted pyrimidine nucleoside undergoes nucleophilic displacement with the sodium salts of amides and thiols at room temperature in 1,4-dioxane exclusively at the 4-position to provide a simple and efficient preparation of 4-*N*-acylated and 4-thiolated analogues, respectively.

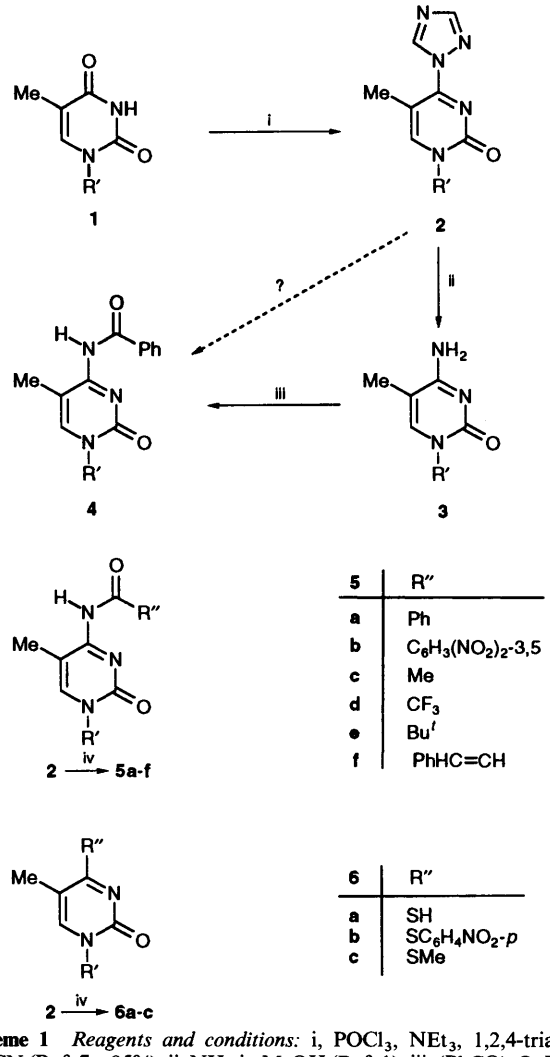
In recent years, the *N*-acyl protection of amino groups of nucleosides has received much attention because of their usefulness in phosphodiester and phosphotriester approaches to oligonucleotide synthesis.[1] Largely, the research on *N*-acyl protected nucleosidic bases to date has been concentrated on: (1) the chemoselective introduction of protecting groups;[2] (2) selecting more stable *N*-acyl groups to withstand the stepwise synthesis of oligonucleotides;[3] (3) use of lipophilic acyl groups that could serve as a purification handle;[4] and (4) studying the rate of complete deprotection under mild conditions.[5]

In our oligonucleotide research programme, large quantities of *N*-acyl protected 5-methyl-2′-deoxycytidine derivative **4** were required for the synthesis of new oligonucleotides with improved antisense properties.[6] The conventional route for the synthesis of the protected nucleoside **4** involves the conversion of the thymine residue **1** into a 4-triazolylthymine derivative **2**, which on ammonolysis provides the 5-methylcytosine derivative **3**. The latter compound can then be selectively *N*-acylated to furnish **4** by established procedures,[2] thus, requiring a three-step procedure to convert **1** into **4**.

We wanted to develop a more efficient procedure for the synthesis of **4** and explored the possibilities of directly converting **2** into **4**. The observations that the triazolyl derivative **2** can be easily and quantitatively converted into 4-*O*-alkylthymidines[7] by treatment with appropriate alcohol/base suggested the possibility that conversion of the triazolide **2** into *N*-acylated **4** might be carried out in one step utilizing a suitable amide/base combination.

In this communication, we describe for the first time a short and efficient method for the conversion of the triazolide **2** into *N*-acyl protected 5-methyl-2′-deoxycytidines **5a–f**, as useful building blocks for oligonucleotide synthesis. The one-pot methodology for the preparation of the protected nucleosides **5a–f** is illustrated in Scheme 1. The starting material **2** (R′ = 3-*O*-*tert*-butyldiphenylsilyl-5-*O*-*tert*-butyldimethylsilyl-β-D-*erythro*-pentofuranosyl) was readily prepared from thymidine following the literature procedure.[7a]

First, we studied the conversion of **2** into the *N*-benzoyl-5-methyl-2′-deoxycytidine derivative **5a**. This could be best achieved by treating **2** (1 equiv.) with benzamide and NaH (4:4 equiv.) in 1,4-dioxane or THF at room temperature (23 °C), followed by neutralization with acetic acid and extraction with CH_2Cl_2 (Table 1). Other conditions utilizing MeCN gave no reaction and recovery of the starting material. The yields of transformation of **2** into **5a** was unaffected by increasing the amount of NaH from 4 to 10 equiv., whereas, decreasing the amount of NaH below 4 equiv. lowers the yield of **5a**. Similarly, treatment of **2** with 3,5-dinitrobenzamide, 2,2,2-trifluoroacetamide, trimethylacetamide and cinnamide in the presence of



Scheme 1 *Reagents and conditions:* i, $POCl_3$, NEt_3, 1,2,4-triazole, MeCN (Ref. 7a, 95%); ii, NH_3 in MeOH (Ref. 1); iii, $(PhCO)_2O$, DMF (Ref. 2a, 91%); iv, amide or thiol (4 equiv.), NaH (4 equiv.), 1,4-dioxane or THF (see Table 1, 70–98%)

NaH furnished very good yields of the *N*-acyl derivatives; 3,5-dinitro-*N*-benzoyl **5b**, *N*-trifluoroacetyl **5d**, *N*-trimethylacetyl **5e**, and *N*-cinnamyl **5f**, respectively.

The progress of the above reactions was conveniently followed by TLC. The starting material **2** exhibited a characteristic fluorescence on TLC due to the triazolyl group. The starting material **2** disappeared quickly to furnish a less polar and non-fluorescent spot for the *N*-acylated products. Quick and quantitative formation of *N*-acylated products suggest the direct displacement of the triazolyl group[9] by

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Table 1 Treatment of various amides or thiols and bases with the triazolide **2**

Product[a]	R″	Amide or thiol (equiv.)	Base (equiv.)	Solvent	Reaction time (h)	Yield[b] (%)
5a	Ph	$PhCONH_2$ (4)	NaH (4)	1,4-Dioxane	1	95
5a	Ph	$PhCONH_2$ (4)	KH (4)	THF	0.5	94
5b	$C_6H_3(NO_2)_2$-3,5	$(O_2N)_2C_6H_3CONH_2$ (4)	KH (4)	THF	0.5	79
5c	Me	$MeCONH_2$ (4)	NaH(4)	1,4-Dioxane	24	NP
5d	CF_3	CF_3CONH_2 (4)	NaH (4)	1,4-Dioxane	0.5	94
5e	Bu^t	Bu^tCONH_2 (4)	NaH (4)	1,4-Dioxane	1	70
5f	PhHC=CH	PhHC=CHCONH$_2$ (4)	NaH (4)	1,4-Dioxane	1	98
6a	SH	p-$O_2NC_6H_4SH$ (4)	NaH (4)	1,4-Dioxane	2	73
6c	SMe	MeSNa (4)	NaH (4)	1,4-Dioxane	18	94

[a] All products were identified on the basis of their ^1H NMR and mass spectral data. [b] Yields are of isolated and purified (silica gel column chromatography) products; NP = no product.[15]

amides in presence of a strong base as the mechanism for the observed reaction.

In principle, this nucleophilic displacement by amides could be extended to provide a general synthesis of various 4-*S*-alkyl or aryl substituted thymidines from a single precursor **2** *via* treatment with sulfur nucleophiles under similar conditions. This would be particularly attractive if 4-sulfanylthymidine **6a** could be prepared in a similar manner. To examine this possibility, we treated **2** with sodium methanethiolate (4 equiv.) at room temperature. The desired product[10] **6c** was obtained after 48 h in excellent yields. We next explored the reaction of **2** with *p*-nitrobenzenethiolate and NaH (4:4 equiv.) under identical conditions. Interestingly, the isolated product from this reaction was not the expected[11] **6b** but characterized as 4-sulfanylthymidine[12] **6a** (73%). Although the mechanism for the formation of **6a** is not clear, it provided an alternative route for the synthesis of a biologically important nucleoside.[13] It is worth noting that a similar transformation of the C-4-triazolyl compound to a thiol was reported by Xu *et al.* utilizing thioacetic acid as a nucleophile.[14]

In summary, to the best of our knowledge this is the first time that a 4-triazolyl group of a pyrimidine nucleoside has been displaced by a variety of amides and thiols in the presence of a base. The availability of large quantities of **5a** and other *N*-acyl protected bases by this procedure will allow efficient synthesis of oligonucleotides and their evaluation of antisense properties in biological assays. The described methodology opens the possibilities to prepare various 4-substituted nucleoside analogues of biological importance.[13]

Experimental

Typical Amidation Procedure (e.g. **2** ⟶ **5a**).—To a stirred solution of compound **2** (0.65 g, 1 mmol) in 1,4-dioxane (10 cm^3) was added a mixture of NaH (60%; 0.16 g, 4 mmol) and benzamide (0.48 g, 4 mmol) in 1,4-dioxane (10 cm^3) in one portion at room temperature under an argon atmosphere. After 1 h, TLC indicated complete consumption of the fluorescent starting material and formation of a new non-polar spot (CH_2Cl_2–MeOH, 99:1, v/v; E. Merck silica gel 60F-254 TLC plate visualized under a UV lamp). The reaction mixture was cooled (ice bath) and the solution neutralized to pH ~6 with dropwise addition (**CAUTION**: effervescence) of glacial acetic acid. The reaction mixture was then diluted with CH_2Cl_2 (200 cm^3) and washed with water (2 × 100 cm^3). The CH_2Cl_2 layer was dried ($MgSO_4$) and evaporated to provide a foamy residue. Silica gel column chromatography of the residue with CH_2Cl_2 provided compound **5a** (0.66 g, 95%).

Thiolation Procedure (e.g. **2** ⟶ **6a**, **6c**).—The thiolations were carried out in a similar manner to that described above by replacing the amide with an appropriate thiol (see Table 1 for details).

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