A Dimroth rearrangement of pyrimidine nucleosides

David Loakes,*[a] Daniel M. Brown[a] and Stephen A. Salisbury[b]

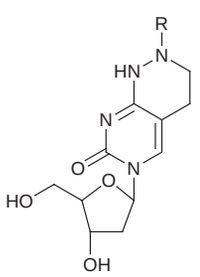
[a] *Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge, UK CB2 2QH*
[b] *Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, UK CB2 1EZ*

Received (in Cambridge) 27th January 1999, Accepted 29th March 1999

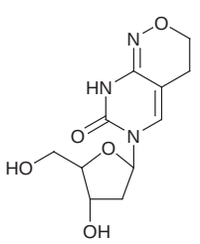
N^4-Acylamino-2′-deoxycytidine derivatives, **4a**–**c**, undergo acid-promoted cyclisation to give [1,2,4]triazolo-[4,3-*c*]pyrimidin-5(6*H*)-ones, **5a**–**c**, in the presence of pyridinium chloride. This reaction has been demonstrated for a series of analogues. Treatment of the cyclised products in basic media gives rise to a novel Dimroth-type rearrangement leading to [1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-ones, **7a**–**c**. The crystal structure of one such product, **15**, was confirmed by X-ray analysis.

Introduction

As part of our ongoing work to prepare nucleoside analogues with ambiguous base-pairing properties, we have been attempting to synthesise bicyclic N^4-amino derivatives, *e.g.* **1**. Such compounds are related to the bicyclic analogue **2**,[1] which we have previously reported and which shows ambivalent hydrogen-bonding behaviour, forming stable base-pairs with both deoxyadenosine and deoxyguanosine. However, this analogue behaves predominantly as the thymidine-like tautomer[2] and we believed that an N^4-amino derivative would be less discriminating than **2** as a result of the measured tautomeric constant of N^4-aminocytosine compared with that of N^4-hydroxycytosine.[3] Our initial studies had shown that the parent derivative (**1**, R = H) derived from **3** (R = CH_2CH_2Cl) with hydrazine was too unstable for further manipulations, and so we decided to investigate acylated derivatives. To this end we reacted the C^4-triazolopyrimidine derivative **3** (R = CH_2CH_2Cl) with hydrazine followed by acetylation. The compound we obtained did not have the expected characteristics of **1** (R = Ac), so to understand what had occurred we repeated the synthesis starting from **3** (R = Me). Recently we reported the cyclisation of N^4-acetylamino-5-methyl-2′-deoxycytidine[4] and subsequent rearrangement. In this paper we present the complete data for this reaction as well as for some other N^4-acylamino-2′-deoxycytidine derivatives.



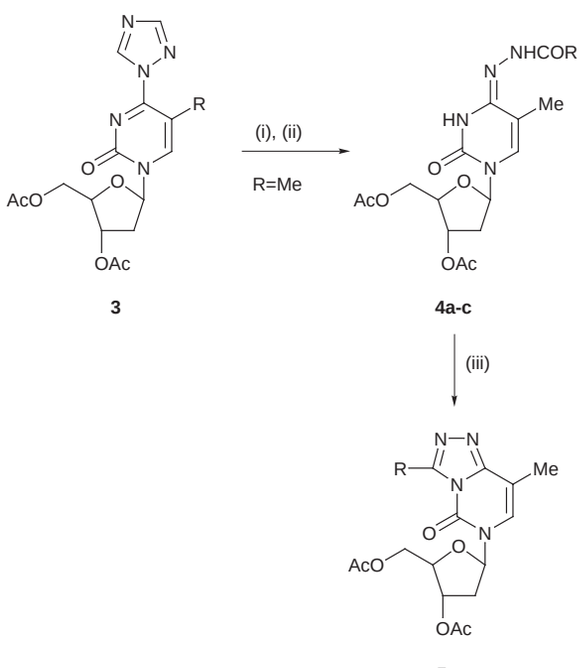
1



2

Results

Conversion of 3′,5′-di-*O*-acetylthymidine to the triazolo derivative **3** (R = Me), then treatment with hydrazine, gave the corresponding N^4-amino-2′-deoxycytidine derivative. This was reacted with various acylating agents; acetic, benzoic and phenoxyacetic (PAC) anhydrides in pyridine at room temperature, as shown in Scheme 1. The products were indeed the expected acylamino derivatives **4a**–**c**. They exhibited amide bond rotamers in their ^{1}H-NMR spectra: the acetylamino derivative showed a coalescence temperature of approximately 360 K. In the reaction with acetic anhydride a second, minor product was also formed. It was clearly a single isomer, and its mass spectrum indicated loss of water. The UV spectrum showed a λ_{max} at 263 nm with both an acid- and base-shift (λ_{max} 272 nm). It was later characterised as the cyclised [1,2,4]-triazolo[4,3-*c*]pyrimidinone product **5a** (R = CH_3) by X-ray crystallography.[4]



3 **4a-c** **5a-c**

Scheme 1 *Reagents*: (i) H_2NNH_2; (ii) $(RCO)_2O$; (iii) pyridine, pyridinium chloride. **a**, R = CH_3; **b**, R = Ph; **c**, R = CH_2OPh.

Whereas the reaction with acetic anhydride gave rise to a small amount (~10%) of the cyclised product **5**, reaction with benzoic anhydride gave only a single product. This was characterised as the benzoylamino derivative **4b** (R = Ph). The reaction with phenoxyacetic anhydride, however, gave an approximately equal mixture of the phenoxyacetylamino derivative, **4c**, and its cyclised product, **5c**. The uncyclised product proved difficult to isolate as it cyclised even during

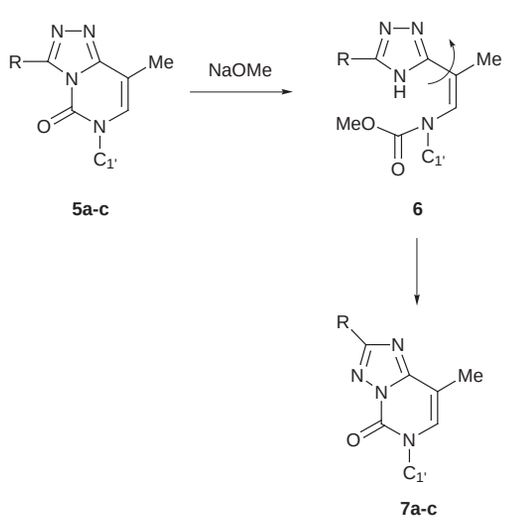
Table 1 UV λ_{max} of the products **4a**–**c**, **5a**–**c** and **7a**–**c** at pH 1, 7 and 12

			4			**5**			**7**		
	R	pH	1	7	12	1	7	12	1	7	12
a	CH_3		294	281	300	275	263	272	281	273	274
b	Ph		294	281	329	271	271	269	281	278	281
c	CH_2OPh		293	275	306	266	266	268	275	278	274

column chromatography, and was therefore isolated in only 18% yield.

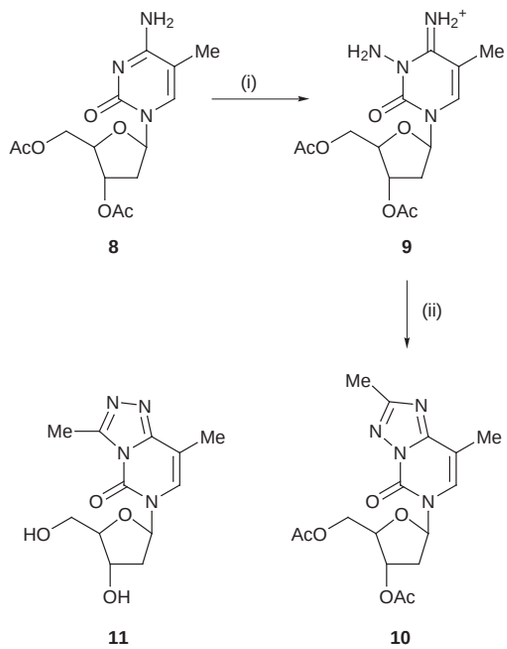
Cyclisation of the acetylamino derivative **4a** was investigated under a range of conditions, and was found to be an acid-promoted reaction. Indeed the reaction occurs in the presence of up to 1 M HCl, but under these conditions there is also considerable degradation of the product. The best reagent was found to be pyridinium chloride in pyridine. During the acylation reaction the cyclisation step must have been catalysed by pyridinium acetate (or phenoxyacetate). Under optimal conditions at 50 °C the cyclisation (**4a** ⟶ **5a**) was complete within 4 hours. Under the same cyclisation conditions the benzoylamino derivative took over 1 week, whilst formation of the PAC derivative was complete within 2 hours. In the absence of acid the rate of cyclisation in pyridine is greatly reduced, although it does occur under prolonged reaction time. Moreover, when carried out in pure 2,6-lutidine (2,6-dimethylpyridine) with lutidinium chloride the rate was of the same order as that in pyridine alone. We assume that the base catalysis must be associated with the dehydration step with consequent prevention of the back-reaction.

When the cyclised product **5a** was treated with sodium methoxide in methanol to remove the sugar-protecting groups a new product was formed which had different UV characteristics. The NMR and mass spectra were consistent with the loss of the two sugar acetyl protecting groups. The UV λ_{max} showed a bathochromic shift of approximately 10 nm, and did not have a basic shift that was observed for the parent diacetates **5a**–**c**. The UV λ_{max} of each of the products (**4**, **5** and **7**) are summarised in Table 1. The product was tentatively characterised as the rearranged [1,2,4]triazolo[1,5-*c*]pyrimidinone nucleoside **7** (Scheme 2).



Scheme 2

The structure of the rearranged product **7a** (R = CH_3) was confirmed by independent synthesis.[5] Amination of the 5-methyl-2′-deoxycytidine derivative **8** with 2,4-dinitrophenoxyamine gave the N^3-amino derivative **9** (Scheme 3). This was reacted with trimethyl orthoacetate to give **10**, which was identical to that obtained by the reaction **5**→**7a** after acetylation of the latter product.



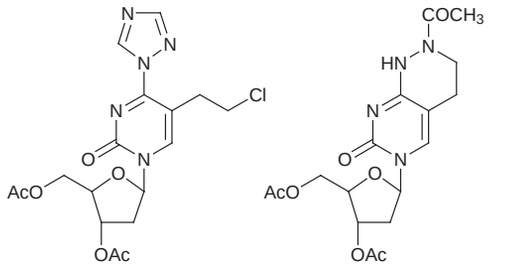
Scheme 3 *Reagents*: (i) 2,4-Dinitrophenoxyamine; (ii) trimethyl orthoacetate, acetic anhydride.

The rearrangement reaction **5a** ⟶ **7a** was quantitative (by UV and TLC analysis), and rapid, but could be monitored at room temperature by UV using 10^{-4} M sodium methoxide where it showed two isosbestic points. The half-life for the reaction was ~5 minutes in methanol, and ~10 minutes using sodium ethoxide in ethanol. Using a variety of conditions (triethylamine, pyridine and dimethylamine in methanol) we were unable to detect the accumulation of any intermediate. However, in the absence of any direct evidence we believe that the reaction proceeds *via* the intermediate ester **6**. Carrying out the reaction in aq. sodium hydroxide led to some unidentified degradation products. The major isolated product was shown by NMR and UV comparisons to be deacetylated but unrearranged [4,3-*c*] product.

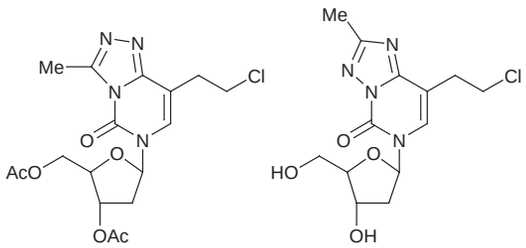
Finally, we return to the series of reactions which led us into this work. In an attempt to prepare the compound **13** we had reacted the triazolo derivative **12** (≡**3**, R = CH_2CH_2Cl) (see above) with hydrazine, followed by acetylation with acetic anhydride in pyridine (Chart 1). Though there was more than one product formed, the major product was incorrectly assumed to be **13**. Treatment with sodium methoxide to deprotect the sugar gave the product we have now characterised by X-ray crystallography as the [1,5-*c*] triazolo derivative **15** (Fig. 1). Evidently, the reactivity of the N^4-amino group of the hydrazino intermediate was insufficient to effect immediate cyclisation by nucleophilic displacement of the chloride. Acylation, however, enables the cyclisation onto N^3 to produce the bicyclic [4,3-*c*] triazolo product **14** which rearranged to the [1,5-*c*] isomer **15** under the conditions chosen for deacetylation.

Discussion

Brown and co-workers have reported the rearrangement of [1,2,4]triazolo[4,3-*c*]pyrimidines. In their work, treatment of N^4-



12 **13**



14 **15**

Chart 1

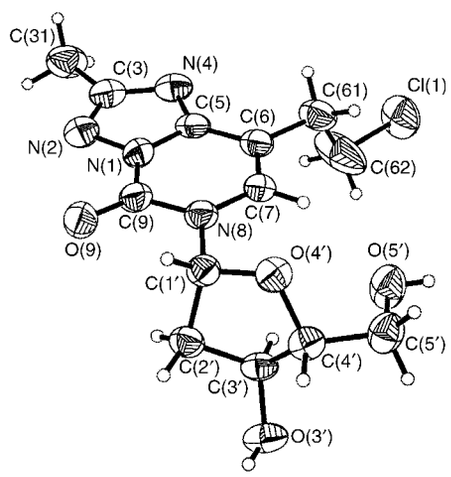
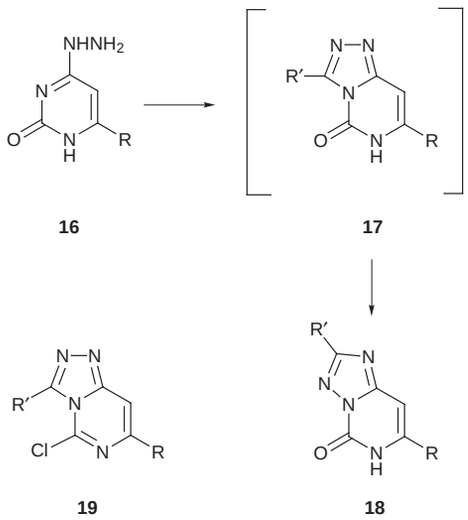


Fig. 1 Thermal ellipsoid (50% probability) plot of the Dimroth-rearranged product **15**.

aminocytosine derivatives **16** with formic acid, triethyl orthoacetate or triethyl orthobenzoate led to the intermediates **17**, which rearranged to the [1,5-*c*] isomers **18**[6] (Scheme 4) (R and R′ as defined in refs. 6 and 7). The intermediates **17** could not be isolated: conversion of the O^2 carbonyl group to chloro in **12** allowed the isolation of the [4,3-*c*] isomer **19**,[7] though they appear not to have reported the corresponding [1,5-*c*] derivative. Whereas the rearrangement **17**→**18** can be envisaged as proceeding *via* an isocyanate intermediate, this is not possible with either of the nucleosides described here, nor with the chloro compound **19**, since these species lack the N^1–H function. With the nucleosides we have postulated that the Dimroth rearrangement proceeds through an ester intermediate **6**; this is also not feasible with **19**.

Classical Dimroth rearrangements usually occur under basic conditions. In the earlier work of Brown and Nagamatsu, pyrimidine and pyrimidinone systems had been shown to undergo Dimroth-type rearrangement in both acidic and basic media.[8] In our case, working with pyrimidine nucleosides we have shown that the rearrangement **5**→**7** occurs only in basic solution, and only when an ester intermediate may be formed. In aq. sodium hydroxide the Dimroth rearrangement does not occur, presumably because the intermediate would involve a carboxylate ion, which could not recyclise and should undergo



16 **17** **19** **18**

Scheme 4

decarboxylation leading to further degradation products. The formation of the unrearranged but deacetylated [4,3-*c*] product (60%) presumably also reflects a slower initial ring-cleavage than in the methoxide-catalysed reaction.

We therefore believe that we have demonstrated a novel Dimroth-type rearrangement. We have crystal structures for both the cyclised [1,2,4]triazolo[4,3-*c*]pyrimidinone[4] and the Dimroth-rearranged [1,2,4]triazolo[1,5-*c*]pyrimidinone, **15**, described in this paper. We have also shown that these reactions are general to N^4-acylamino(deoxy)cytidine derivatives, and may therefore provide a method for preparing further analogues for biological experiments.

Experimental

General methods

^{1}H-NMR spectra were obtained on a Bruker DRX-300 in d_6-DMSO. Coupling constants (*J*) are given in Hz. UV spectra were recorded on a Perkin-Elmer Lambda 2 spectrophotometer in aq. methanol (1%, v/v). TLC was carried out on pre-coated F_{254} silica plates, and column chromatography with Merck Kieselgel 60. Unless otherwise stated, reactions were worked up as follows: the solvent was removed and the product dissolved in chloroform. The resulting solution was washed with saturated aq. sodium bicarbonate, dried over sodium sulfate and evaporated to dryness. Ether refers to diethyl ether.

Synthesis

N^4-Acetylamino-1-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-5-methylcytosine 4a. To a solution of the triazolo compound **3** (R = Me) (1.5 g, 4 mmol) in acetonitrile (10 cm^3) was added anhydrous hydrazine (137 mm^3, 4.4 mmol) and the solution stirred at room temperature for 2 hours. The solution was evaporated, the product dissolved in pyridine (20 cm^3), acetic anhydride (0.56 cm^3, 6 mmol) was added and the solution stirred at room temperature overnight. The solvent was removed and the title product **4a** worked up and chromatographed ($CHCl_3$–2% MeOH) to give a white foam (1.19 g, 90%). A small amount of the cyclised product **5a** was also isolated (0.16 g). ^{1}H-NMR spectra are as previously described;[4] λ_{max}/nm 281 (ε/dm^3 mol^{-1} cm^{-1} 9100), λ_{min}/nm 255; pH 1 λ_{max}/nm 294 (11 800); pH 12 λ_{max}/nm 300 (19 300); *m*/*z* (EI) 364 $(M - H_2O)^+$. Accurate mass measurement (on M^+) gives $C_{16}H_{20}N_4O_6$ 364.1383, deviation +1.476 MMU, 4.0 ppm.

N^4-Benzoylamino-1-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-5-methylcytosine 4b. The benzoylamino derivative was prepared, as described for the acetylamino

compound, using 1.5 g (4 mmol) of the triazolo derivative **3** (R = Me) and benzoic anhydride (1.8 g, 8 mmol). The single product was worked up and chromatographed ($CHCl_3$–5% MeOH) to give an off-white solid, which was recrystallised from ethanol to give title compound **4b** as a pale yellow powder (1.42 g, 80%), mp 197.5–199 °C (Found: C, 56.49; H, 5.48; N, 12.48. $C_{21}H_{24}N_4O_7$ requires C, 56.8; H, 5.4; N, 12.6%); δ_H (a number of peaks were split into two signals due to amide-bond rotamers, in the ratio 4:3) 1.85, 1.97 (3H, 2 × s, CH_3), 2.06 (6H, s, 2 × $COCH_3$), 2.23–2.39 (2H, m, H_2-2′), 4.12 (1H, br s, H-4′), 4.23 (2H, br s, H_2-5′), 5.18 (1H, br s, H-3′), 6.18 (1H, t, *J* 6.9, H-1′), 6.99 (0.6H, s, 0.6 × H-6), 7.47–7.61, 7.87–7.95 (5.4H, 2 × m, Ph, 0.4 × H-6), 9.32, 10.30, 10.48, 10.71 (2H, 4 × br s, 2 × NH); λ_{max}/nm 281 (13 400), 219 (18 100); λ_{min}/nm 257; pH 1 λ_{max}/nm 294 (16 700), 227 (18 300); pH 12 λ_{max}/nm 329 (18 200); *m*/*z* (FAB) 445.9 $(M + H)^+$. Accurate mass measurement on M + H, 445.17153. $C_{21}H_{25}N_4O_7$ requires *m*/*z* 445.17233, deviation 1.8 ppm.

***N*^{4}-Phenoxyacetylamino-1-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-5-methylcytosine 4c.** Prepared, as described for the acetyl derivative, using 1.5 g (4 mmol) of the triazolo derivative **3** (R = Me) and phenoxyacetic anhydride (2.3 g, 8 mmol). The reaction was worked up and chromatographed ($CHCl_3$–1% MeOH) to give the cyclised product **5b** (1.07 g, 59%) and with $CHCl_3$–2% MeOH to give the title compound **4c** as a white foam (0.34 g, 18%); δ_H(d_6-DMSO) (a number of peaks were split into two signals due to amide-bond rotamers, in the ratio 3:2) 1.80, 1.86 (3H, 2 × s, CH_3), 2.06 (6H, s, 2 × $COCH_3$), 2.17–2.42 (2H, m, H_2-2′), 4.12 (1H, br s, H-4′), 4.23 (2H, br s, H_2-5′), 4.57, 4.97 (2H, 2 × br s, OCH_2), 5.17 (1H, br s, H-3′), 6.18 (1H, t, *J* 7, H-1′), 6.84–7.05 (4H, m, H-6, 0.67 × Ph), 7.23–7.33 (2.25H, m, 0.33 × Ph), 9.81, 10.01, 10.13, 10.31 (2H, 4 br s, 2 × NH); λ_{max}/nm 275 (10 000), 212sh (18 700); λ_{min}/nm 253; pH 1 λ_{max}/nm 293 (12 800), 208sh (24 700); λ_{min}/nm 249; pH 12 λ_{max}/nm 306 (20 400); λ_{min}/nm 251. *m*/*z* (FAB) 475.9 $(M + H)^+$. Accurate mass measurement on M + H, 475.18289. $C_{22}H_{27}N_4O_8$ requires *m*/*z*, 475.18289, deviation −0.2 ppm.

3,8-Dimethyl-6-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)[1,2,4]triazolo[4,3-*c*]pyrimidin-5(6*H*)-one 5a. A solution of the acetylamino derivative **4a** (1 g, 2.6 mmol) in pyridine (10 cm^3) containing pyridine hydrochloride (100 mg) was heated at 50 °C for 4 hours. The solvent was removed and the title product **5a** chromatographed ($CHCl_3$–2% MeOH) to give a white foam (0.84 g, 84%); ^{1}H-NMR spectral data are as previously described;[4] λ_{max}/nm 263 (14 700); λ_{min}/nm 220; pH 1 λ_{max}/nm 275 (11 400); pH 12 λ_{max}/nm 272 (10 800); *m*/*z* (EI) 364 M^+. Accurate mass measurement gives $C_{16}H_{20}N_4O_6$ (M^+) 364.1383, deviation +0.06MMU, deviation 0.2 ppm.

3-Phenyl-6-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-8-methyl[1,2,4]triazolo[4,3-*c*]pyrimidin-5(6*H*)-one 5b. This was prepared similarly to the acetyl derivative. Compound **4a** (0.42 g, 1 mmol) in pyridine (10 cm^3) containing pyridine hydrochloride (100 mg) was heated at 50 °C for 1 week. The product was chromatographed ($CHCl_3$–2% MeOH) to give an off-white solid, which was recrystallised from ethanol to give compound **5b** as white needles (0.34 g, 84%) (remainder unreacted starting material), mp 143–144.5 °C. (Found: C, 58.86; H, 5.25; N, 12.99. $C_{21}H_{22}N_4O_6$ requires C, 59.1; H, 5.2; N, 13.1%); δ_H(d_6-DMSO) 2.05 (3H, s, $COCH_3$), 2.07 (3H, s, $COCH_3$), 2.28 (3H, s, CH_3), 2.31–2.43 (2H, m, H_2-2′), 4.22–4.25 (1H, m, H-4′), 4.28–4.30 (2H, m, H_2-5′), 5.19–5.22 (1H, m, H-3′), 6.28 (1H, t, *J* 6.8, H-1′), 7.32 (1H, s, H-7), 7.44–7.53, 7.70–7.72 (5H, m, Ph); λ_{max}/nm 271 (14 300); λ_{min}/nm 228; pH 1 λ_{max}/nm 271 (16 300); pH 12 λ_{max}/nm 269sh (13 300), 248 (30 100) (irreversible); *m*/*z* (FAB) 427.8 $(M + H)^+$. Accurate mass measurement on M + H, 427.16065. $C_{21}H_{23}N_4O_6$ requires *m*/*z*, 427.16177, deviation 2.6 ppm.

3-Phenoxymethyl-6-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-8-methyl[1,2,4]triazolo[4,3-*c*]pyrimidin-5(6*H*)-one 5c. This was prepared similarly to the acetyl derivative. A solution of the phenoxyacetylamino derivative **4c** (0.34 g, 0.7 mmol) in pyridine (10 cm^3) containing pyridine hydrochloride (100 mg) was heated at 50 °C for 2 hours. The title product **5c** was chromatographed ($CHCl_3$–2% MeOH) to give a white foam (0.27 g, 83%); δ_H(d_6-DMSO) 2.06 (6H, s, 2 × $COCH_3$), 2.24 (3H, s, CH_3), 2.34–2.54 (2H, m, H_2-2′), 4.22–4.24 (1H, m, H-4′), 4.28–4.30 (2H, m, H_2-5′), 5.21–5.24 (1H, m, H-3′), 5.56 (2H, dd, *J* 12.5, OCH_2), 6.32 (1H, t, *J* 7.3, H-1′), 6.94–7.06 (3H, m, H-7, 2 × ArH), 7.27–7.32 (3H, m, 3 × ArH); λ_{max}/nm 266 (13 400); λ_{min}/nm 229; pH 1 λ_{max}/nm 226 (13 200); pH 12 λ_{max}/nm 268 (10 400); *m*/*z* (FAB) 457.9 $(M + H)^+$. Accurate mass measurement on M + H, 457.17215. $C_{22}H_{25}N_4O_7$ requires *m*/*z*, 457.17233, deviation 0.4 ppm.

2,8-Dimethyl-6-(2-deoxy-β-D-*erythro*-pentofuranosyl)[1,2,4]-triazolo[1,5-*c*]pyrimidin-5(6*H*)-one 7a. A solution of the [4,3-*c*]pyrimidinone **5a** (0.5 g, 1.4 mmol) was dissolved in methanol (10 cm^3), sodium methoxide (185 mg, 3.4 mmol) added and the solution stirred at room temperature for 2 hours. The solution was neutralised with acetic acid and then evaporated to dryness and chromatographed ($CHCl_3$–10% MeOH) to give compound **7a** as a white foam (0.29 g, 75%); ^{1}H-NMR spectral data are as previously described.[4] λ_{max}/nm 273 (8200); pH 1 λ_{max}/nm 281 (7800), 244 (4600); pH 12 λ_{max}/nm 274 (8600); *m*/*z* (EI) 281 $(M + H)^+$. Accurate mass measurement gives $C_{12}H_{17}N_4O_4$ *m*/*z*, 281.1249, deviation −0.3 ppm.

2-Phenyl-8-methyl-6-(2-deoxy-β-D-*erythro*-pentofuranosyl)-[1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-one 7b. To a solution of the nucleoside **5b** (0.6 g, 1.4 mmol) in methanol (20 cm^3) was added sodium methoxide (180 mg, 3.3 mmol) and the solution stirred at room temperature for 2 hours. The solid was filtered and recrystallised (ethanol) to give compound **7b** as white needles (0.35 g, 73%), mp 199–201 °C (Found: C, 59.11; H, 5.25; N, 16.10. $C_{17}H_{18}N_4O_4$ requires C, 59.6; H, 5.3; N, 16.3%); δ_H(d_6-DMSO) 2.22–2.27 (2H, m, H_2-2′), 2.26 (3H, s, CH_3), 3.58–3.71 (2H, m, H_2-5′), 3.85–3.89 (1H, m, H-4′), 4.29–4.32 (1H, m, H-3′), 5.15 (1H, t, *J* 4.8, 5′-OH), 5.31 (1H, d, *J* 4.1, 3′-OH), 6.40 (1H, t, *J* 6.6, H-1′), 7.52–7.54 (3H, m, Ph), 7.91 (1H, s, H-7), 8.15–8.18 (2H, m, Ph); λ_{max}/nm 278sh (11 650), 247 (38 400); λ_{min}/nm 219; *m*/*z* (FAB) 343.7 $(M + H)^+$. Accurate mass measurement on M + H, 343.13831. $C_{17}H_{19}N_4O_4$ *m*/*z*, requires 343.14063, deviation 6.7 ppm.

2-Phenoxymethyl-8-methyl-6-(2-deoxy-β-D-*erythro*-pentofuranosyl)[1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-one 7c. This was prepared similarly to the acetyl derivative. 0.5 g (1.1 mmol) of the pyrimidinone **5c** in methanol (10 cm^3) was treated with sodium methoxide (145 mg, 2.7 mmol) for 2 hours. The solution was evaporated after neutralisation, and the title product **7c** recrystallised from water (0.33 g, 81%), mp 157–159 °C (Found: C, 55.19; H, 5.57; N, 14.39. $C_{18}H_{20}N_4O_5 \cdot H_2O$ requires C, 55.4; H, 5.7; N, 14.4%); δ_H(d_6-DMSO) 2.21 (5H, br s, CH_3, H_2-2′), 3.57–3.71 (2H, m, H_2-5′), 3.85–3.86, (1H, m, H-4′), 4.27–4.29 (1H, m, H-3′), 5.14 (1H, t, *J* 4.9, 5′-OH), 5.25 (2H, s, CH_2), 5.30 (1H, d, *J* 4.2, 3′-OH), 6.36 (1H, t, *J* 6.5, H-1′), 6.93–7.32 (5H, m, Ph), 7.91 (1H, s, H-7); λ_{max}/nm 274 (12 700), 254sh (9000), 205sh (28 600); λ_{min}/nm 230; *m*/*z* (FAB) 373.7 $(M + H)^+$. Accurate mass measurement on M + H, 373.15122. $C_{18}H_{21}N_4O_5$ requires *m*/*z*, 373.15118, deviation −0.1 ppm.

2,8-Dimethyl-6-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)[1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-one 10. Method A. To a solution of 5-methyl-1-(3,5-di-*O*-acetyl-2-deoxy-β-D-

erythro-pentofuranosyl)cytidine **8** (0.489 g, 15 mmol) in DMF (60 cm^3)–methanol (4 cm^3) was added 2,4-dinitrophenoxyamine[9] (0.478 g, 16 mmol) and the solution stirred at room temperature for 4 days. The solution was evaporated, the residue was dissolved in water (10 cm^3) and the solution neutralised by the addition of conc. hydrochloric acid before being washed with ether, evaporated and the product recrystallised from ethanol to give 3-amino-5-methyl-1-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)cytosine hydrochloride **9** as a brown solid (5.1 g, 87%); *m/z* 341.8 (M^+).

A portion of this (746 mg, 5 mmol) was dissolved in a mixture of trimethyl orthoacetate (20 cm^3) and acetic anhydride (15 cm^3) and the solution heated at reflux for 10 h before being evaporated and the title product **10** crystallised from ethanol–ether (600 mg, 86%); δ_H(d_6-DMSO) 2.05, 2.07 (6H, 2 × s, 2 × $COCH_3$), 2.02–2.07 (2H, m, H_2-2′), 2.20 (3H, s, 8-CH_3), 2.41 (3H, s, 2-CH_3), 4.25–4.29 (3H, m, H_2-5′, H-4′), 5.23–5.25 (1H, m, H-3′), 6.38 (1H, t, *J* 6.8, H-1′), 7.56 (1H, s, H-7); λ_{max}/nm 273 (8300); pH 1 λ_{max}/nm 280 (8000); pH 12 λ_{max}/nm 273 (7950); *m/z* (EI) 364.1 (M^+).

Method B. To a solution of the nucleoside **7a** (100 mg, 0.36 mmol) in pyridine (5 cm^3) was added acetic anhydride (85 mm^3, 9 mmol) and the mixture stirred at room temperature overnight. The reaction mixture was then worked up and chromatographed ($CHCl_3$–5% MeOH) to give a white foam (0.12 g, 92%). Spectra were as described above.

3,8-Dimethyl-6-(2-deoxy-β-D-*erythro*-pentofuranosyl)[1,2,4]-triazolo[4,3-*c*]pyrimidin-5(6*H*)-one 11. To a solution of the nucleoside **5a** (0.5 g, 1.1 mmol) in acetonitrile (10 cm^3) was added 0.1 M aq. sodium hydroxide (33 cm^3, 3.3 mmol) and the solution stirred at room temperature for 2 h before being neutralised with acetic acid, evaporated and the title product **11** chromatographed ($CHCl_3$–10% MeOH) to give a white solid (0.23 g, 60%); δ_H(d_6-DMSO) 2.02–2.23 (2H, m, H_2-2′), 2.14 (3H, s, 8-CH_3), 2.72 (3H, s, 2-CH_3), 3.56–3.60 (2H, m, H_2-5′), 3.81–3.82 (1H, m, H-4′), 4.27 (1H, br s, H-3′), 5.12, 5.30 (2H, 2 × s, 3′-OH, 5′-OH), 6.28 (1H, t, *J* 6.6, H-1′), 7.46 (1H, s, H-7); λ_{max}/nm 264 (11 400); λ_{min}/nm 225; pH 1 λ_{max}/nm 278 (8700); pH 12 λ_{max}/nm 276 (10 000).

3-Methyl-6-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-8-(2-chloroethyl)[1,2,4]triazolo[4,3-*c*]pyrimidin-5(6*H*)-one 14. To a solution of 1-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-4-(1,2,4-triazolo)-5-(2-chloroethyl)pyrimidin-2(1*H*)-one[1] **12** (0.8 g, 1.9 mmol) in dichloromethane (10 cm^3) was added anhydrous hydrazine (120 mm^3, 3.8 mmol) and the solution stirred at room temperature for 30 minutes. The solvent was evaporated, the product re-dissolved in a mixture of pyridine (10 cm^3) and acetic anhydride (0.27 cm^3, 2.9 mmol) and the solution stirred at room temperature overnight. The solvent was removed and the title product **14** worked up and chromatographed (ethyl acetate–hexane, 1 : 1) to give a white foam (0.47 g, 63%); δ_H(d_6-DMSO) 2.06 (3H, s, $COCH_3$), 2.07 (3H, s, $COCH_3$), 2.34–2.54 (2H, m, H_2-2′), 2.73 (3H, s, CH_3), 3.06 (2H, t, *J* 6.8, CH_2CH_2Cl), 3.95 (2H, t, *J* 6.9, CH_2Cl), 4.22–4.29 (3H, m, H_2-5′, H-3′), 5.21–5.25 (1H, m, H-4′), 6.30 (1H, t, *J* 6.6, H-1′), 7.31 (1H, s, H-7).

2-Methyl-8-(2-chloroethyl)-6-(2-deoxy-β-D-*erythro*-pentofuranosyl)[1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-one 15. 14 (0.45 g, 1.2 mmol) was dissolved in methanol (20 cm^3), sodium methoxide (0.5 M, 2.3 cm^3, 2.4 mmol) added and the solution stirred at room temperature for 20 minutes before being neutralised with acetic acid, evaporated to dryness and the title product **15** chromatographed ($CHCl_3$–10% MeOH) to give a white solid (0.30 g, 86%); δ_H(d_6-DMSO) 2.14–2.30 (2H, m, H_2-2′), 2.41 (3H, s, CH_3), 3.04 (2H, t, *J* 7, CH_2CH_2Cl), 3.56–3.70 (2H, m, H_2-5′), 3.84–3.92 (1H, m, H-3′), 3.90 (2H, t, *J* 7, CH_2Cl), 4.26–4.30 (1H, m, H-4′), 5.13 (1H, t, *J* 5, 5′-OH), 5.30 (1H, d, *J* 4.3, 3′-OH), 6.35 (1H, t, *J* 6.5, H-1′), 7.97 (1H, s, H-7); λ_{max}/nm 272 (9400), 255sh; λ_{min}/nm 225; pH 1 λ_{max}/nm 279 (8800); pH 12 λ_{max}/nm 272 (8000).

X-Ray structure determination of 15. Crystals (rosettes of stout needles) were obtained by slow cooling of an ethanolic solution, from which a specimen 0.7 × 0.4 × 0.1 mm was cut, and mounted on a glass fibre. Data were collected at 293 K using a Rigaku AFC7R four-circle diffractometer and MoKα radiation (λ = 0.710 69 Å) from a Rigaku RU200 rotating-anode source and graphite monochromator. Of the 1317 reflections measured in the range *h* 0–5, *k* 0–19, *l* −10 to +10, 1198 were treated as observed (>2σ). They were processed without absorption correction. Crystal data: $C_{13}H_{17}ClN_4O_4$, space group *P*2(1), *a* = 4.911(4), *b* = 16.391(5), *c* = 9.146(3) nm, β = 97.44(4)°, *Z* = 2. The structure was solved using SHELXS-86.[10] Refinement (SHELXL-93[11]) converged at *R* = 0.0370 (R_w = 0.0966) for all 1198 unique data (goodness of fit 0.934). Hydrogen atoms were added during the course of refinement according to standard methods. CCDC reference number 207/315. See http://www.rsc.org/suppdata/p1/1999/1333 for crystallographic files in .cif format.

Acknowledgements

We thank Nycomed Amersham plc for financial assistance.

References

1 P. Kong Thoo Lin and D. M. Brown, *Nucleic Acids Res.*, 1989, **17**, 10373.
2 F. Hill, D. Loakes and D. M. Brown, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 4258.
3 D. M. Brown, M. J. E. Hewlins and P. Schell, *J. Chem. Soc. C*, 1968, 1925.
4 D. Loakes, S. A. Salisbury and D. M. Brown, *Tetrahedron Lett.*, 1998, **39**, 3865.
5 M. Maeda and Y. Kawazoe, *Chem. Pharm. Bull.*, 1975, **23**, 844.
6 D. J. Brown and K. Shinozuka, *Aust. J. Chem.*, 1980, **33**, 1147.
7 D. J. Brown and T. Nagamatsu, *Aust. J. Chem.*, 1979, **32**, 1585.
8 D. J. Brown and T. Nagamatsu, *Aust. J. Chem.*, 1977, **30**, 2515.
9 T. Sheradsky, *J. Heterocycl. Chem.*, 1967, **4**, 413.
10 G. M. Sheldrick, *Acta Crystallogr.*, *Sect. A*, 1990, **46**, 467.
11 G. M. Sheldrick, in SHELXL-93 Program for the Refinement of Crystal Structures, University of Göttingen, Germany, 1993.

Paper 9/00748B