The Syntheses of Pyrimido-pyridazinone and Pyrrolidino-pyrimidinone 2′-Deoxynucleoside Derivatives

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Dedicated to Prof. *Albert Eschenmoser* on the occasion of his 75th birthday

Nucleosides that have ambivalent tautomeric properties have value in a variety of nucleic-acid hybridisation applications and as mutagenic agents. We describe here synthetic studies directed to stable derivatives based on N^4-aminocytosine. Treatment of the 5-(chloroethyl)-4-(triazol-1-yl)pyrimidine-nucleoside derivative **1** with benzylhydrazine leads to the formation of the 6,6-bicyclic pyrimido-pyridazin-7-one **6**, in addition to the 5,6-bicyclic derivative **7**. The 6,6-bicyclic benzyl derivative **6** was converted to its 5′-triphosphate for studies with DNA polymerases. Reaction of the triazole **1** with hydrazine, followed by acetylation, led to the desired acetylated 6,6-bicyclic derivative **12**. However, the latter compound undergoes acyl migration followed by ring contraction to the 5,6-bicyclic compound **13** on treatment with base.

Introduction. – In our previous paper [1], we reported on the reaction of various hydrazine analogues with the triazolo derivative **1** in an attempt to prepare the bicyclic N^4-amino-2′-deoxycytidine derivatives **2**. We observed that the parent compound, **2** (R = H), was unstable, undergoing either air oxidation or rearrangement to the five-membered analogue **3** (R = H). The rearrangement reaction to the five-membered bicyclic compounds we observed is base-catalysed. The reaction of **1** with methylhydrazine gave rise to the fixed tautomeric isomer **4**, arising from displacement of the triazolyl group with the more nucleophilic methylamino group [2] of methylhydrazine. The latter bicyclic compound was also found to undergo base-catalysed rearrangement to the five-membered bicyclic analogue **3** (R = Me). When the exocyclic N^4-amino N-atom carries a proton, it readily undergoes this base-catalysed rearrangement to the five-membered analogue **3** [1]. We believed that if we could prepare analogues that were substituted at this position, they should not undergo rearrangement. However, our previous attempts with methyl hydrazine gave **4** as the only isolated product.

Our interest in such bicyclic compounds arises because they are expected to show ambiguous base-pairing properties in DNA (RNA) duplexes. Incorporation into DNA (RNA) oligonucleotides could be either as their phosphoramidite monomers or enzymatically as their 5′-triphosphates. We [3–5] and others [6–8] have shown that 4-(aminooxy)pyrimidine derivatives are mutagenic both as 5′-triphosphate substrates [9][10] and as template bases [5]. However, such analogues have tautomerism constants (K_T) of the order of 10–100 in favour of the imino form [11]. We anticipate that N^4-amino derivatives will have different K_T values (the tautomerism constant for N^4-aminocytosine is 30, in favour of the amino form [12], *i.e.*, 0.03 in favour of the imino

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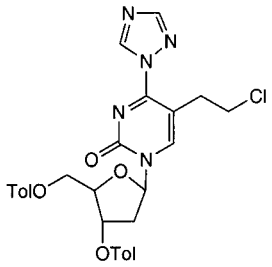

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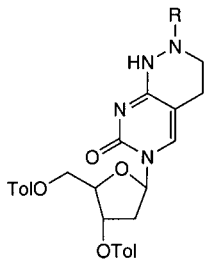

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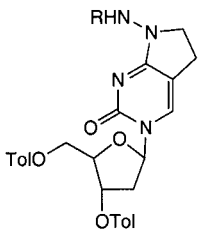

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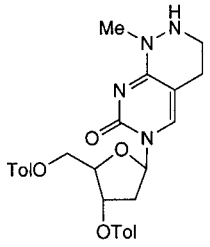

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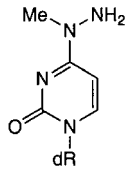
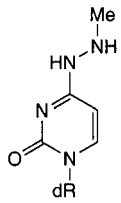
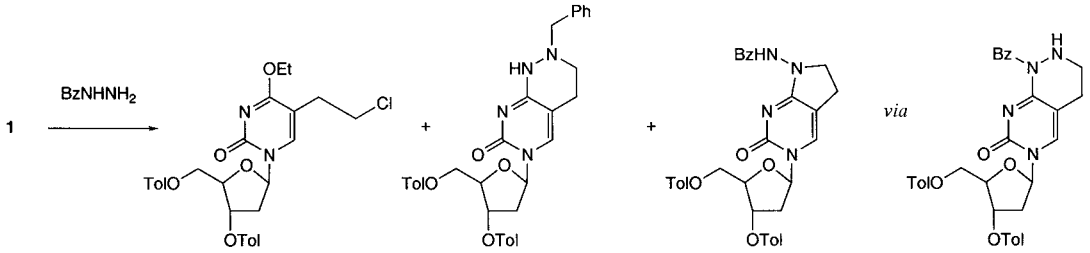
form as compared to N^4-hydroxy derivatives) so, in an attempt to reduce this bias, bicyclic analogues of **2** have been investigated. In this paper, we report on the synthesis of some such analogues. The reaction of cytidine with methylhydrazine under conditions of bisulfite addition to the C(5)=C(6) bond gives rise to both substitution products shown below [13]. However, for this work, we were unable to use bisulfite-addition chemistry, as when the C(5) position is substituted bisulfite addition does not occur. The N^4-(methylamino) compound is mutagenic in *E. coli* [13].





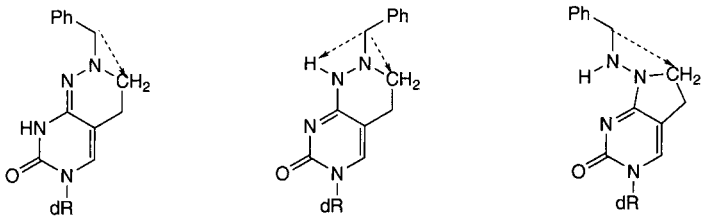
Results and Discussion. – The reaction of benzylhydrazine hydrochloride with **1** was carried out under a variety of conditions. No reaction occurred at room temperature in CH_2Cl_2, THF, MeCN, or EtOH as solvent, and Et_3N or K_2CO_3 as base. However, when the reaction temperature was raised to refluxing in EtOH with Et_3N as base, the triazole **1** disappeared, and three products were formed. One of the products was identified as the O^4-ethyl derivative **5**, the other two products each showed $PhCH_2$ (Bz) groups in their ^{1}H-NMR spectra (see *Scheme 1*).

Scheme 1



5 **6** **7** **8**

To characterise the two remaining products, 2D-COESY and NOESY-NMR spectra were obtained, and as a result they were identified as the desired product **6** and a five-membered isomer **7**. The latter product must have derived from reaction with benzylhydrazine to give **8**, where the Bz group is attached to the N^4-nitrogen, followed by rearrangement as described earlier [1] to give the five-membered analogue **7**. The product **6** can exist in two different tautomeric forms, and, from the NMR spectral data, this appears to be solvent-dependent. In DMSO, there is an NOE only between the benzyl CH_2 (3.92 ppm) and the CH_2 protons of the six-membered ring (2.50 ppm), whereas, in $CDCl_3$, there is an additional NOE to the exchangeable NH (10.13 ppm) signal (see below). In the five-membered analogue **7**, the NH proton appears to form an internal H-bond to the pyrimidine ring N-atom. Therefore, by using a more hindered hydrazine derivative we have been able to prepare the desired six-membered bicyclic derivative, and shown that a tautomeric equilibrium exists dependent on solvent polarity.

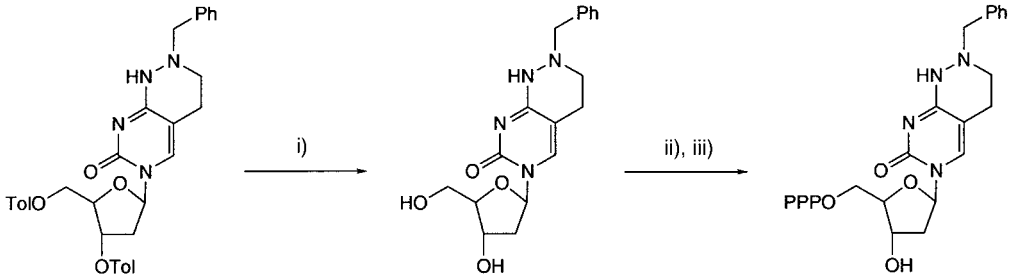


in DMSO in $CDCl_3$

To examine its DNA polymerase incorporation properties, the benzylhydrazino derivative **6** was deprotected with MeONa in MeOH to give the free nucleoside **9** (*Scheme 2*), which was then converted to its 5′-triphosphate derivative **10** [14]. Preliminary data shows that the triphosphate **10** is a reasonably good substrate for DNA polymerases, being incorporated as both dCTP and TTP, *i.e.*, opposite template G and A, respectively (data not shown).

In addition to analogues alkylated at the exocyclic N^4-amino N-atom, we decided to attempt to prepare acylated analogues: acylation can occur, in principle, at any of three

Scheme 2

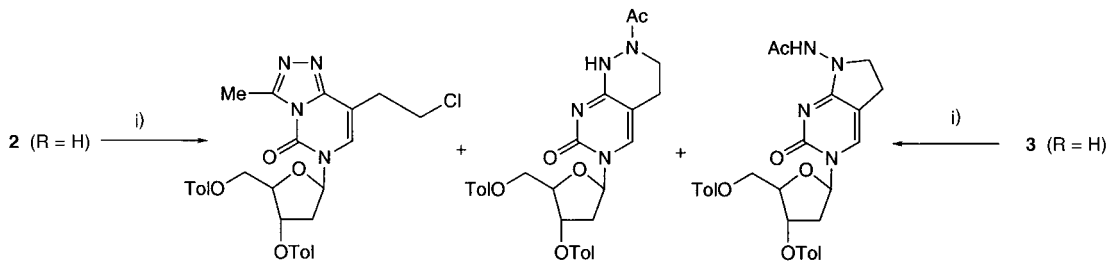


6 **9** **10**

i) MeONa/MeOH. ii) $POCl_3$. iii) Bu_3N, $P_2O_7Bu_4$

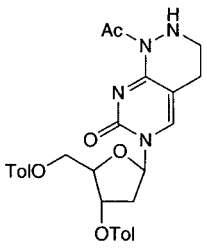
sites (including the N^3-position). To prepare an acylated derivative of the bicyclic N^4-aminocytidine derivative **2**, the triazole **1** was treated with hydrazine to give the parent bicyclic product, **2** (R = H). This was then reacted with Ac_2O in pyridine overnight without isolation of the intermediate. Three products were isolated from these reactions (*Scheme 3*). The first product was identified as the 8-(2-chloroethyl)-1,2,4-

Scheme 3



11 **12** **13**

i) Ac_2O, pyridine.



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triazolo[4,3-*c*]pyrimidin-5-one derivative **11**, analogous to that which we had previously reported [15]. This product arises from acetylation of the initially formed N^4-aminocytidine derivative, which had not cyclised with the chloroethyl side chain, followed by cyclisation of the acyl group onto the N^3-position. The second product was identified by 2D-COESY and NOESY-NMR spectra as the desired acetylated 6,6-bicyclic compound **12**, whilst the last product was characterised as the 5,6-bicyclic analogue **13**. The five-membered analogue **13** clearly arises due to acetylation at the N^4-position ($\rightarrow$ **14**), followed by rearrangement as previously described. In an attempt to detect the acetyl derivative **14**, the acetylation reaction was carried out, and aliquots were removed and quenched at various intervals. However, even after short reaction times there was no TLC evidence of any other intermediate being formed. This suggests that once the N^4-position is acetylated, giving **14**, it ring contracts spontaneously to give **13**. Comparing the reaction mixture after 1 h and overnight, there was also no difference in the ratio of the products, suggesting that the ring-contracted product **13** was not derived from **12**.

The desired 6,6-bicyclic compound **12** showed a doubling of most signals in a ratio of 3 : 1 in its ^{1}H-NMR spectrum in (D_6)DMSO. This was more obvious in $CDCl_3$, where almost every peak was duplicated, in a ratio of 4 : 1, and arises from amide rotamers. The other significant difference between the spectra in DMSO and $CDCl_3$ is the number of NH signals. In $CDCl_3$, there are two NH signals (9.80 and 10.57 ppm), whereas in DMSO there are three (9.89, 9.99 and 10.33 ppm). The presence of the third NH signal in DMSO we attribute to the fact that the tautomerism constant may be close to unity, thus we are observing the two tautomeric forms in the polar solvent, as we had previously observed in the NMR spectrum of 2,6-diamino-N^6-methoxypurine [16]. The structure of the five-membered analogue **13** was further confirmed by acetylation of **3** (R = H) [1], the two products having identical spectral data.

When the 6,6-bicyclic product **12** was treated with a catalytic amount of MeONa in MeOH, the product isolated was found to be the 5,6-bicyclic product **13**. To form this product, we believe that there must first be an Ac migration onto the N^4-amino N-atom to give **14**, followed by ring contraction. With an equivalent of methoxide, the sugar-deprotected derivative of **13** was formed (see *Exper. Part*). We can find no literature precedence for the acyl-migration reaction; it presumably proceeds *via* a three-membered ring intermediate, giving rise to the kinetically favoured acyl intermediate **14**. Once **14** has formed, under the basic conditions of the reaction, it undergoes ring contraction as previously described to give the known **13**. It would probably be possible to prepare the free nucleoside derivative of **12** by an alternative protecting-group strategy for the sugar, *e.g.*, the use of (*t*-Bu)Me_2Si protection. However, the aim of this work is to prepare analogues suitable for incorporation into DNA as either their 5′-triphosphates or as the phosphoramidite: under the conditions of oligonucleotide synthesis or during the purification of the triphosphate, basic conditions are employed. Under these conditions, the analogue would undergo acyl migration, so further work on acyl derivatives has not been pursued.

Much effort has gone into the synthesis of these bicyclic hydrazino derivatives [1][15][17][18]. An alternative strategy involved reaction of the side chain at C(5) with methylhydrazine with the intention of subsequently cyclising to C(4). This route was tested but was unsuccessful due to the required protection/deprotection

of the new alkylhydrazine side chain. However, one analogue has been prepared here, namely the benzylhydrazino derivative **9**, and this will form the subject of further work.

Experimental Part

General. Unless otherwise stated, reactions were worked up as follows: after removal of the solvent, the product was dissolved in $CHCl_3$ and washed with aq. $NaHCO_3$ solution. The combined org. fractions were dried (Na_2SO_4) and evaporated. 5-(Chloroethyl)-2′-deoxyuridine was prepared (as the 3′,5′-di-*O*-toluoyl derivative) according to the procedure of *Griengl et al*. [19]. TLC: pre-coated F_{254} silica plates. CC: *Merck* silica gel *60*. M.p.: *Gallenkamp* melting-point apparatus, uncorrected. UV Spectra: *Perkin-Elmer Lambda 2* spectrophotometer in MeOH/H_2O 10:90, unless otherwise stated. ^{1}H-NMR Spectra: *Bruker DRX 300*, in (D_6)DMSO, unless otherwise stated. NOE Experiments: *Bruker AMX-500* or *DRX 300* spectrometer. MS: *Bruker FTICR Bioapex II*.

2-Benzyl-6-(3,5-di-O-p-*toluoyl-β*-D-*2-deoxyribofuranosyl)-3,4-dihydro-8*H-*pyrimido[4,5*-c*]pyridazin-7-one* (**6**). To a soln. of the triazole **1** (6.35 g, 11 mmol) in dry EtOH (200 cm^3) was added benzylhydrazine hydrochloride (2.35 g, 12.1 mmol) followed by Et_3N (6 cm^3) under N_2, and the soln. was heated at reflux overnight. TLC (AcOEt/hexane 2:1) showed there was no starting material left. The solvent was removed and the residue was chromatographed (AcOEt/hexane 1:1–2:1, then 2–5% MeOH in $CHCl_3$). Three fractions were collected: the first fraction was further chromatographed ($CHCl_3$/2% MeOH) to give *5-(2-chloroethyl)-4*-O-*ethyl-3′,5′-di*-O-*p-toluoyl-β*-D-*2′-deoxyuridine* (**5**). Yield 0.76 g (12%). UV: λ_{max} 271 (sh, 11000), 245 (30600), λ_{min} 229; at pH 1: λ_{max} 281 (sh, 14400), 251 (27600), λ_{min} 230. ^{1}H-NMR: 1.27 (*t*, *J* = 7, Me); 2.36 (*s*, Me of toluoyl); 2.39 (*s*, Me of toluoyl); 2.46–2.62 (*m*, 1 H–C(2′), CH_2N); 2.66–2.72 (*m*, 1 H–C(2′)); 3.56 (*t*, *J* = 7, CH_2Cl); 4.30 (*q*, *J* = 7, CH_2); 4.53–4.68 (*m*, H–C(4′), 2 H–C(5′)); 5.59–5.62 (*m*, H–C(3′)); 6.28 (*t*, *J* = 7, H–C(1′)); 7.28–7.35 (*m*, 4 CH of toluoyl); 7.82–7.93 (*m*, 4 CH of toluoyl); 7.84 (*s*, H–C(6)).

In addition to **5**, **6** was obtained as a brown solid, which recrystallised from MeOH to give a white powder. Yield 1.29 g (20%). M.p. 205–207°. UV: λ_{max} 245 (44650), λ_{min} 225; at pH 1: λ_{max} 282 (12700), 243 (36700), λ_{min} 270, 221. ^{1}H-NMR (DMSO): 2.15 (*m*, CH_2(4), 1 H–C(2′)); 2.34 (*s*, Me of toluoyl); 2.38 (*s*, Me of toluoyl); 2.5 (*m*, CH_2N, 1 H–C(2′)); 3.92 (*s*, PhC*H*$_2$); 4.36 (*m*, H–C(4′)); 4.38–4.58 (*m*, 2 H–C(5′)); 5.53 (br. *s*, H–C(3′)); 6.26 (*m*, H–C(1′)); 6.58 (*s*, H–C(5)), 7.25–7.90 (*m*, 8 H of toluoyl, Ph); 10.13 (*s*, NH): ^{1}H-NMR ($CDCl_3$) 2.23–2.56 (*m*, CH_2N, CH_2(4), 2 H–C(2′)); 2.35 (*s*, Me of toluoyl); 2.37 (*s*, Me of toluoyl); 3.92 (*s*, PhC*H*$_2$); 4.36–4.38 (*m*, H–C(4′)); 4.46–4.61 (*m*, 2 H–C(5′), 5.52–5.54 (*m*, H–C(3′)); 6.26 (*t*, *J* = 6.5, H–C(1′)); 6.58 (*s*, H–C(5)), 7.21–7.35 (*m*, 4 CH of toluoyl, Ph); 7.86–7.91 (*m*, 4 CH of toluoyl); 10.13 (*s*, NH). FAB-MS: 595.26 ([*M* + H]$^+$). Accurate mass measurement on [*M* + H] ($C_{34}H_{35}N_4O_6$): 595.25710, deviation 2.42 ppm. Anal. calc. for $C_{34}H_{34}N_4O_6$: C 68.67, H 5.76, N 9.42; found: C 68.46, H 5.71, N 9.43.

The second fraction was identified as the five-membered analogue *1-(benzylamino)-5-(3,5-di*-O-p-*toluoyl-β*-D-*2-deoxyribofuranosyl)-2,3-dihydro-5*H-*pyrrolidino[2,3*-d*]pyrimidin-6-one* (**7**). Yield 2.9 g (44%). UV: λ_{max} 282 (sh, 15500), 246 (36850), λ_{min} 227; at pH 1: λ_{max} 298 (9600), 244 (29000), λ_{min} 269, 220. ^{1}H-NMR ($CDCl_3$): 2.18 (*m*, CH_2); 2.42–2.76 (*m*, 2 H–C(2′)); 2.35 (*s*, Me of toluoyl); 2.37 (*s*, Me of toluoyl); 2.85 (*m*, CH_2); 3.27 (*m*, CH_2); 4.03 (*s*, PhC*H*$_2$), 4.44 (*m*, H–C(4′)); 4.54 (*dd*, *J* = 3, 12, 1 H–C(5′)); 4.70 (*dd*, *J* = 3, 12, 1 H–C(5′)); 5.10 (br. *s*, NH); 5.52 (*d*, *J* = 7, H–C(3′)); 6.50 (*dd*, *J* = 6, 9, H–C(1′)); 7.20 (*m*, 11 arom. H); 7.85 (*m*, 4 arom. H). FAB-MS: 595.26 ([*M* + H]$^+$). Accurate mass measurement on [*M* + H] ($C_{34}H_{35}N_4O_6$): 595.25650, deviation 1.41 ppm.

2-Benzyl-6-(2-deoxy-β-D-*ribofuranosyl)-3,4-dihydro-8*H-*pyrimido[4,5*-c*]pyridazin-7-one* (**9**). The bicyclic compound **8** (1.4 g, 2.35 mmol) was suspended in MeOH (50 cm^3), and to this was added MeONa (140 mg, 2.6 mmol), and the soln. was stirred at r.t. for 1 h. The solvent was removed and the product chromatographed ($CHCl_3$/10% MeOH) to give a pale yellow powder. Yield 0.61 g (72%). UV: λ_{max} 295 (7800); ε(260) [μM] = 5.7. ^{1}H-NMR: 1.88–2.03 (*m*, 2 H–C(2′)); 2.45–2.50 (*m*, CH_2N); 2.59–2.63 (*m*, 2 H–C(4)), 3.41–3.54 (*m*, 2 H–C(5′)); 3.65–3.66 (*m*, H–C(4′)); 3.92 (*s*, PhC*H*$_2$); 4.16 (*m*, H–C(3′)); 4.89 (*t*, C(5′)–OH); 5.15 (*d*, C(5′)–OH); 6.13 (*t*, *J* = 6.1, H–C(1′)); 6.74 (*s*, H–C(5)); 7.21–7.34 (*m*, Ph), 9.98 (*s*, NH).

2-Benzyl-6-(2-deoxy-β-D-*ribofuranosyl)-3,4-dihydro-8*H-*pyrimido[4,5*-c*]pyridazin-7-one 5′-Triphosphate* (**10**). To a stirred soln. of **9** (100 mg, 0.28 mmol) in trimethyl phosphate (2 cm^3), was added phosphoryl chloride (39 μl, 0.42 mmol) dropwise at 0–5°. After 45 min, the reaction was simultaneously treated with bis[tributylammonium] pyrophosphate (2.55 cm^3 of 0.5M soln. in DMF, 1.39 mmol) and Bu_3N (331 μl, 1.39 mmol), and stirred at r.t. for 20 min. The soln. was then neutralised with 1.0M triethylammonium

bicarbonate (TEAB), stirred for 4 h, and then evaporated. The crude triphosphate was purified on a (diethylamino)ethyl (DEAE)-*Sephadex A-25* column using a linear gradient from 0 to 1.0M TEAB (pH 7.5). The triphosphate fractions collected (0.7–0.9M) were concentrated and finally purified by reverse-phase HPLC (*Waters semi-prep. Delta Pak* 15 µ *C-18* column) with a gradient of 0–100% 0.1M TEAB and buffer B (25% MeCN in 0.1M TEAB) at 12 ml/min. The desired triphosphate eluted in 30 min. Yield 73 mg. UV (H_2O): λ_{max} 296. ^{31}P-NMR (D_2O) ethylenediaminetetraacetic acid (EDTA) −9.56 (*d*, γ-P); −10.15 (*d*, α-P), −22.61 (*t*, β-P).

2-Acetyl-6-(3,5-di-O-p-*toluoyl-β*-D-*2-deoxyribofuranosyl)-3,4-dihydro-8*H-*pyrimido[4,5-*c*]pyridazin-7-one* (**12**). *5-(Chloroethyl)-1-(3,5-di*-O-p-*toluoyl-2-deoxyribofuranosyl)-4-(1,2,4-triazol-1-yl)pyrimidin-2-one* (**1**; 0.5 g, 0.87 mmol) was dissolved in dry MeCN (25 cm^3), and anh. hydrazine (40 µl, 1.27 mmol) was added, and the soln. was stirred at r.t. for 2 h. The soln. was then evaporated and redissolved in pyridine (25 cm^3), and to this was added Ac_2O (0.27 cm^3, 1.38 mmol), and the soln. was stirred at r.t. overnight. TLC showed three products. The solvent was removed, and the product was worked up as usual and chromatographed (CH_2Cl_2/2% MeOH). The first product was isolated as a white foam, which crystallised from EtOH, yield 0.11 g (23%), and was characterised as *8-(2-chloroethyl)-6-(3,5-di*-O-p-*toluoyl-2-deoxy-β*-D-*ribofuranosyl)-3-methyl-1,2,4-triazolo[4,3-*c*]pyrimidin-5-one* (**11**). M.p. 150–151°. UV: λ_{max} 271 (sh, 16000), 247 (30900), λ_{min} 225; at pH 1: λ_{max} 273 (sh, 17000), 248 (30200), λ_{min} 225; at pH 12: λ_{max} 271 (sh, 14900), 244 (31600), λ_{min} 230. ^{1}H-NMR: 2.37 (*s*, Me of toluoyl), 2.39 (*s*, Me of toluoyl), 2.57–2.67 (*m*, 2 H–C(2′)); 2.72 (*s*, Me); 2.85 (*t*, *J* = 7.3, C(8)–CH_2); 3.82 (*t*, *J* = 7.1, CH_2); 4.56–4.70 (*m*, H–C(4′), 2 H–C(5′)); 5.65 (br. *s*, H–C(3′)); 6.43 (*t*, *J* = 6.8, H–C(1′)); 7.30–7.37 (*m*, 4 CH of toluoyl, H–C(7)); 7.87–7.93 (*m*, 4 CH of toluoyl). FAB-MS: 565.2 ($[M+H]^+$). Accurate mass measurement on [*M* + H]: 565.18674 (calc. for $C_{29}H_{29}N_4O_6{}^{35}Cl$ 565.18536, deviation −2.5 ppm). Anal. calc. for $C_{29}H_{29}ClN_4O_6 \cdot EtOH$: C 60.93, H 5.77, N 9.16; found: C 60.89, H 5.67, N 9.14.

The second product, a white foam, was characterised as **12**. Yield 0.13 g (27%). UV: λ_{max} 283 (11600), 244 (30800), λ_{min} 269, 221; at pH 1: λ_{max} 292 (11500), 244 (30100), λ_{min} 269, 220; at pH 12: λ_{max} 300 (17050), 242 (32400), λ_{min} 266, 228. ^{1}H-NMR ((D_6)DMSO; some signals were observed as two peaks in a ratio of 3:1): 1.87, 2.02 (2*s*, Ac); 2.37 (*s*, Me of toluoyl); 2.38 (*s*, Me of toluoyl); 2.45–2.59 (*m*, 2 H–C(2′), CH_2); 3.60–3.67 (*m*, CH_2N); 4.44–4.64 (*m*, H–C(4′), 2 H–C(5′)); 5.58–5.60 (*m*, H–C(3′)); 6.25–6.32 (*m*, H–C(1′)); 6.92, 7.13 (2*s*, H–C(5)); 7.31–7.36 (*m*, 4 CH of toluoyl); 7.87–7.92 (*m*, 4 CH of toluoyl); 9.89, 9.99, 10.33 (3*s*, NH). ^{1}H-NMR ($CDCl_3$; most signals were observed as two peaks in a ratio of 4:1: 2.23, 2.11 (2*s*, Ac); 2.26–2.59 (*m*, 2 H–C(2′), CH_2); 2.29, 2.39 (2*s*, Me of toluoyl); 2.42–2.44 (2*s*, Me of toluoyl); 3.48–3.60 (*m*, CH_2N); 4.45–4.59 (*m*, H–C(4′)); 4.61–4.81 (*m*, 2 H–C(5′)); 5.61–5.64 (*m*, H–C(3′)); 6.45–6.61(2*dd*, *J* = 5.4, H–C(1′)); 6.75, 6.96 (2*s*, H–C(5)); 7.19–7.32 (*m*, 4 CH of toluoyl); 7.88–8.04 (*m*, 4 CH of toluoyl); 9.80, 10.57 (2*s*, NH). FAB-MS: 547.3 ($[M+H]^+$), 569.2 ($[M+Na]^+$). Accurate mass measurement on [*M* + H]: 547.21750 ($C_{29}H_{31}O_7N_4$ requires 547.21930; deviation 3.3 ppm). Accurate mass measurement on [*M* + Na]: 569.19970 ($C_{29}H_{30}O_7N_4Na$ requires 569.20133, deviation 2.7 ppm).

The third fraction, a white solid recrystallised from EtOH, was characterised as *1-(acetylamino)-5-(3,5-di*-O-p-*toluoyl-β*-D-*2-deoxyribofuranosyl)-2,3-dihydro-5*H-*pyrrolidino[2,3-*d*]pyrimidin-6-one* (**13**). Yield 0.17 g (36%). M.p. 215–216°. UV: λ_{max} 284 (11500), 244 (35000), λ_{min} 269, 216; at pH 1: λ_{max} 297 (12700), 244 (34500), λ_{min} 269, 219; at pH 12: λ_{max} 294 (14800), 242 (35100), λ_{min} 268, 231. ^{1}H-NMR: 1.89 (*s*, Ac); 2.37 (*s*, Me of toluoyl); 2.38 (*s*, Me of toluoyl); 2.42–2.58 (*m*, H–C(2′)); 2.61–2.77 (*m*, CH_2); 6.65 (*t*, *J* = 8, CH_2N); 3.62–3.68 (*m*, H–C(4′)); 4.52–4.64 (*m*, 2 H–C(5′)); 4.46–4.48 (*m*, H–C(3′)); 6.32 (*t*, *J* = 7.7, H–C(1′)); 7.32–7.36 (*m*, 4 CH of toluoyl); 7.52 (*s*, H–C(4)); 7.86–7.92 (*m*, 4 CH of toluoyl); 10.24 (*s*, NH). FAB-MS: 547.3 ($[M+H]^+$). Accurate mass measurement on [*M* + H]: 547.22195 ($C_{29}H_{31}O_7N_4$ requires 547.21930, deviation −4.8 ppm). Anal. calc. for $C_{29}H_{30}N_4O_7 \cdot EtOH$: C 62.82, H 6.12, N 9.45; found: C 62.59, H 5.80, N 9.69.

Compound **13**: *Method B.* To a soln. of *1-amino-5-(3,5-di*-O-p-*toluoyl-β*-D-*2-deoxyribofuranosyl)-2,3-dihydro-5*H-*pyrrolidino[2,3-*d*]pyrimidin-6-one* (**3**; R = H) [15] (100 mg, 2 mmol) in pyridine (10 cm^3) was added Ac_2O (37 µl, 4 mmol), and the soln. was stirred at r.t. overnight. The reaction was worked up, and the product was chromatographed (CH_2Cl_2/2% MeOH) to give a white foam. Yield 87 mg (80%). Spectra were identical to those described above.

2-Acetyl-6-(2-deoxy-β-D-*ribofuranosyl)-3,4-dihydro-8*H-*pyrimido[4,5-*c*]pyridazin-7-one.* Compound **12** (0.45 g, 1.1 mmol) was dissolved in MeOH (20 cm^3), and to the soln. was added MeONa soln. in MeOH (2.3 cm^3, 0.5M, 1.15 mmol), and the soln. was stirred at r.t. for 20 min. The soln. was evaporated and the product chromatographed ($CHCl_3$/10% MeOH) to give a white solid. Yield 0.30 g (85%). UV: λ_{max} 272 (9400), 255 (sh, 7500), 210 (15800); λ_{min} 225; at pH 1: λ_{max} 279 (8800); at pH 12: λ_{max} 272 (8000); ε(260) [µM] = 7.5. ^{1}H-NMR: 2.14–2.30 (*m*, 2 H–C(2′)); 2.41 (*s*, Ac); 3.04 (*t*, *J* = 7, CH_2N); 3.56–3.70 (*m*, 2 H–C(5′)); 3.84–3.92

(*m*, H–C(3′)); 3.90 (*t*, *J* = 7, 2 H–C(4)); 4.26–4.30 (*m*, H–C(4)); 5.13 (*t*, C(5′)–OH); 5.30 (*d*, C(3′)–OH); 6.35 (*t*, *J* = 6.5, H–C(1′)); 7.97 (*s*, H–C(5)).

We thank *Nycomed Amersham plc* for financial assistance (to *D.L.* and *M.J.G.*).

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Received April 25, 2000