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Cyclisation and Rearrangement of N^4-Acylaminodeoxycytidines.

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Abstract: N^4-Acetylamino-2'-deoxycytidine undergoes an acid promoted cyclisation to give a 1,2,4-triazolo[4,3-c]pyrimidinone which, under basic conditions, isomerises *via* a Dimroth-type rearrangement to the 1,2,4-triazolo[1,5-c]pyrimidinone.

As part of our work to prepare nucleosides with ambiguous base-pairing properties we wanted to study analogues of N^4-amino-2'-deoxycytidine, which is a known mutagen.[1] The un-derivatised analogue is relatively unstable, being susceptible to aerial oxidation. We therefore chose to investigate N-acylated derivatives. Treatment of the triazolo-compound (**1**) with hydrazine led rapidly to the parent N^4-amino-derivative (Scheme 1), which was then treated with acetic anhydride in pyridine, without purification of the intermediate. After overnight acetylation it was observed that two products were formed, the minor product increasing with time (or temperature). The major product was identified as being the desired N^4-acetylamino-derivative (**2**) from its ^{1}H-nmr spectrum.[2] This compound also demonstrated the expected amide bond rotamers, the nmr signals coalescing at temperatures above 80°C. The second product was not readily characterised: the ^{1}H-nmr spectrum lacked the exchangeable protons present in (**2**), its uv spectrum was different and the mass spectrum showed a mass difference corresponding to loss of water.[3] X-ray crystallography identified the product as being the cyclised 1,2,4-triazolo[4,3-c]pyrimidinone (**3**) Figure 1.[4] A related compound has been previously reported,[5] prepared by reaction of N^4-aminocytidine with ethyl acetimidate followed by ring closure at pH 4 to give the analogous triazolopyrimidinone. We have demonstrated that cyclisation of **2** occurs with acid catalysis, the best catalyst we found to be pyridinium hydrochloride in pyridine, though the reaction does occur with other acid catalysts which, however, also give rise to hydrolysis products.

When the cyclised product was deacylated with sodium methoxide, the product obtained had a different uv spectrum from its starting material, though the mass spectrum and ^{1}H-nmr spectrum were consistent with only loss of the two acetyl groups.[6] We suspected therefore that **3** had undergone a rearrangement during the deprotection, possibly to the isomeric **4** (R=H). Amination[7] of 3',5'-diacetyl-5-methyl-2'-deoxycytidine (**5**) using 2,4-dinitrophenoxyamine gave the 3,4-diaminopyrimidinone (**6**) whence trimethylorthoacetate/acetic anhydride led to the triazolopyrimidinone (**4**, R=Ac), identified with the acetylated rearrangement product[8] derived from **4**, R=H, (Scheme 2). Interestingly, treatment of the acetylamido derivative **2** with methoxide did not lead to a cyclisation or to the rearranged product, showing that in this case the open chain amido system **2**

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Scheme 1

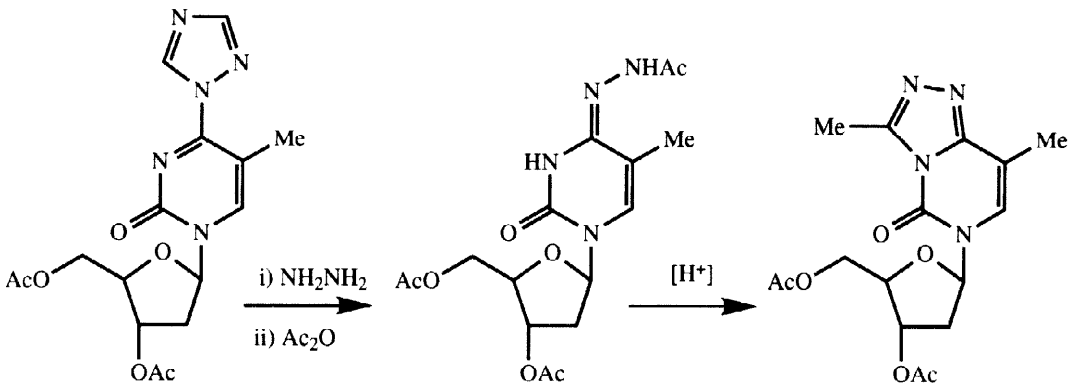
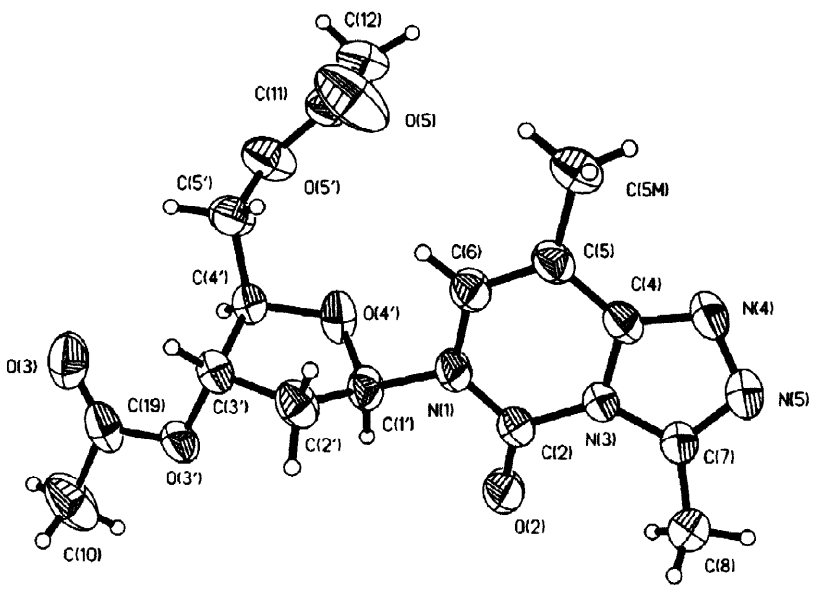
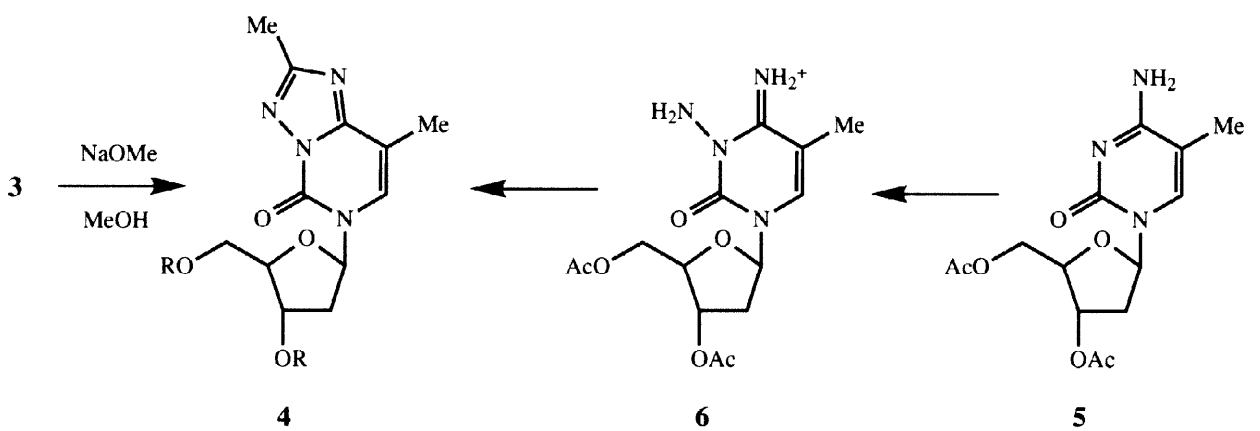


Figure 1



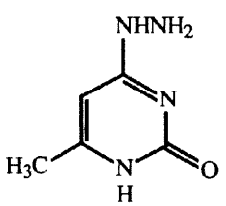
ORTEP plot of the cyclised product **3.**

Scheme 2



cannot rearrange under basic conditions. This is in distinction to the many amidine intermediates observed in other Dimroth type rearrangements.

Several groups have reported the synthesis of 1,2,4-triazolo[4,3-c]pyrimidines,[9,10] though generally these methods did not allow the isolation of the acylated intermediate. Brown and co-workers[10,11] have examined in some detail the rearrangement of a number of 1,2,4-triazolopyrimidines, though this work has been only carried out on the free bases and not on N^1-substituted derivatives such as nucleosides. They observed that the pyrimidinone (**7**) with trimethylorthoacetate under reflux would undergo cyclisation followed by "spontaneous" rearrangement to the triazolopyrimidinone (**8**). We have demonstrated that the rearrangement (**3** → **4**) occurs only under basic conditions. Moreover, when the reaction between trimethylorthoacetate and the N^4-amino-2'-deoxycytidine derivative (**9**) was carried out as described by Brown[12] for the reaction of **7** to **8**, the product obtained was not the Dimroth rearranged 1,2,4-triazolo[1,5-c]pyrimidinone (**4**, R=Ac) analogous to that obtained by him, but the cyclised product **3**. This strongly suggests that when the reaction is carried out on the free base that the N^1-proton is required for the Dimroth rearrangement to occur, and that it may go *via* an intermediate such as the isocyanate derivative **10**. When the N^1-proton is not present as is the case with nucleoside derivatives then the Dimroth reaction cannot occur under these conditions.



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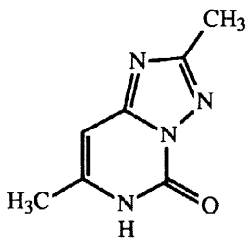

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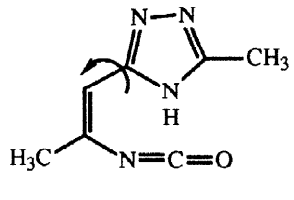

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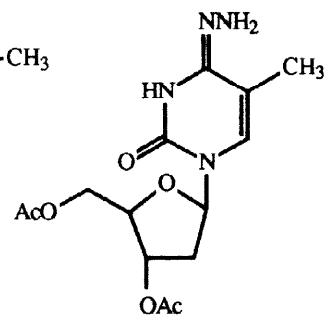

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The rearrangement of triazolopyrimidines (as opposed to pyrimidinones) as described by Brown cannot apply in the case of the rearrangements observed for the nucleoside **3** because in the former case it involves cleavage of the C^3-C^4 bond resulting, in some cases, in stable open chain ketone intermediates. In our case cleavage must occur between N^3-C^2 and we assume *via* an ester intermediate. The methoxide rearrangement (**3**→**4**) is almost instantaneous in 0.1M sodium methoxide solution, but when carried out in 10^{-4}M methoxide ($t_{1/2}$ ~ 5 mins. at rt..) the reaction can be monitored by uv, where it clearly shows two isosbestic points and therefore that no stable intermediate accumulates. In ethanol with sodium ethoxide the rate of rearrangement is only reduced by a factor of 2, and there is no reaction in pyridine. When the reaction was carried out using sodium hydroxide (0.1M) in water then the rearrangement does not occur and the product formed is the deacetylated derivative of **3**, together with degradation products, and this is consistent with our mechanism as the intermediate would have to involve a carboxylate anion which is unlikely to recyclise. The rearrangement, ought in principle to be reversible, but we have been unable to demonstrate this.

We believe therefore that we have demonstrated a novel series of reactions for N^4-acylamino-2'-deoxycytidines, and that the rearrangement reaction must proceed by a different route to that described previously for N^4-aminocytosines.

Acknowledgements: We thank Amersham International plc for financial assistance (to DL).

References and notes

1. Brown, D. M.; Hewlins, M. J. E. and Schell, P.; (1968), *J. Chem. Soc. (C)*, 1925-1929.
2. λmax 281nm (ε=9100), λmin 255nm, pH 1 λmax 294nm (ε=11800), pH 12 λmax 300nm (ε=19300). ^{1}H-nmr (d^6-DMSO) δ (ppm) 300K 1.77 (2H, s, 0.6xNHCOCH$_3$), 1.87 (4H, s, C^5-CH$_3$, 0.3xNHCOCH$_3$), 2.05 (6H, s, 2xCOCH$_3$), 2.15-2.40 (2H, m, H2', H2"), 4.10 (1H, br. s, H4'), 4.22 (2H, br. s, H5', H5"), 5.16 (1H, br. s, H3'), 6.12-6.19 (1H, m, H1'), 6.88, 6.89 (1H, 2xs, H6), 9.10, 10.22 (0.3H, 0.7H, 2 x s, NH), 9.85, 9.99 (0.3H, 0.7H, 2xs, NH).
360K 1.83 (3H, s, C^5-CH$_3$), 1.95 (3H, br. s, NHCOCH$_3$), 2.05 (6H, s, 2xCOCH$_3$), 2.20-2.43 (2H, m, H2', H2"), 4.11-4.12 (1H, m, H4'), 4.24-4.30 (2H, m, H5', H5"), 5.18-5.22 (1H, m, H3'), 6.14 (1H, t, J=7.1 Hz, H1'), 7.35 (1H, s, H6), 9.72 (1H, br, NH), 9.92 (1H, br, NH). Coalescence temperature approximately 360K.
3. λmax 263nm (ε=14700), λmin 220nm, pH 1 λmax 275nm (ε=11400), pH 12 λmax 272nm (ε=10800). ^{1}H-nmr (d^6-DMSO) δ (ppm) 2.06 (6H, s, 2xCOCH$_3$), 2.18 (3H, s, C^5-CH$_3$), 2.72 (3H, s, CH$_3$), 2.32-2.54 (2H, m, H2', H2"), 4.20-4.24 (1H, m, H4'), 4.27-4.29 (2H, m, H5', H5"), 5.21-5.23 (1H, m, H3'), 6.30 (1H, t, J=6.7 Hz, H1'), 7.19 (1H, s, H6). m/z (EI) 364 M$^+$. Accurate mass measurement gives C$_{16}$H$_{20}$N$_4$O$_6$ 364.1383, deviation 0.2ppm.
4. X-ray data were collected on a Rigaku AFC7R 4 circle diffractometer with a Rigaku RU200 rotating anode source and a graphite monochromator. Crystal data: C$_{16}$H$_{20}$N$_4$O$_6$, space group P2$_1$, a=8.484(2), b=10.782(2), c=9.702(3) Å, ß=99.48(2)°, V=875.7(4) Å^3, Z = 2, Dc = 1.382 g.cm^{-3}, F(000) = 384, MoKα = 0.71069 Å. Structure was solved using SIR92, refinement (SHELXL93) converged at R = 0.582 for all 2110 unique data (1987 I > 2 sigma I). No absorption correction was made. Detailed X-ray crystallographic data are available from the Cambridge Crystallographic Data Centre.
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6. λmax 273nm (ε=8200), pH 1 λmax 281nm (ε=7800), 244 nm (ε=4600), pH 12 λmax 274nm (ε=8600). ^{1}H-nmr (d^6-DMSO) δ (ppm) 2.13-2.22 (2H, m, H2', H2"), 2.17 (3H, s, C^5-CH$_3$), 2.40 (3H, s, CH$_3$), 3.56-3.68 (2H, m, H5', H5"), 3.83-3.86 (1H, m, H4'), 4.25-4.30 (1H, m, H3'), 5.13 (1H, t, 5'-OH), 5.29 (1H, d, 3'-OH), 6.36 (1H, t, J=6.6 Hz, H1'), 7.83 (1H, s, H6). m/z (EI) 281 (M+H)$^+$. Accurate mass measurement gives C$_{12}$H$_{17}$N$_4$O$_4$ 281.1249, deviation -0.3ppm.
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8. λmax 273nm (ε=8300), pH 1 λmax 280nm (ε=8000), pH 12 λmax 273nm (ε=7950). ^{1}H-nmr (d^6-DMSO) δ (ppm) 2.05, 2.07 (6H, 2 x s, 2 x COCH$_3$), 2.02-2.07 (2H, m, H2', H2"), 2.20 (3H, s, C^5-CH$_3$), 2.41 (3H, s, CH$_3$), 4.25-4.29 (3H, m, H5', H5", H4'), 5.23-5.25 (1H, m, H3'), 6.38 (1H, t, J=6.8 Hz, H1'), 7.56 (1H, s, H6).
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