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New Bicyclic Nucleosides Related to 6-Azaisocytidine§

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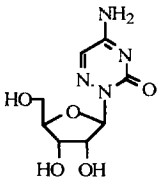
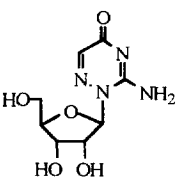
Abstract: Ribosidation of azolo-triazines related to 6-azaisocytosine gives fluorescent nucleosides (**6a/6b**) and/or novel betaine-like nucleosides (**7a/7b**), depending on the reaction conditions. The structure of **7a** (debenzoylated) has been confirmed by X-ray crystallography. By heating in the presence of Me_3SiOTf, betaines **7** rearrange to afford first N8-substituted isomers **8** and eventually **6**.

5-Azacytidine, 6-azauridine, and 6-azacytidine (**1**) are the most important azanucleosides isolated or synthesised so far, from a clinical point of view.[1] Isocytosine and isocytidine derivatives, including 6-aza-isocytosine, are also well-known compounds,[2] but 6-azaisocytidine (**2**) has been much less investigated.[3] In this context, we focussed our attention on ring-fused systems **3** and **4** that could arise from **1** and **2**, respectively (eg, by treatment with $BrCH_2CHO$ or by reaction of their N-amino derivatives with $CH(OMe)_3$). The interest of nucleosides with five- or six-membered rings fused to purines[4] and pyrimidines[2] relies on their use as fluorescent probes, their natural occurrence in tRNA from several sources, and the fact that they are readily formed when certain mutagens (vinyl chloride epoxide, chloroacetaldehyde, etc.) interact with DNA and RNA.[5] Obviously, an alternative approach to this set of "anomalous" or rare polycyclic nucleosides could be based on the direct glycosidation of the preformed polynitrogenated bicyclic systems. We report here that **4a** and **4b** derivatives can be actually obtained via ribosidation of imidazo-triazinone **5a** and triazolo-triazinone **5b**,[6] respectively, but, even more interestingly, that these products are often accompanied by betaine-like isomers[7] of unusual features.



1



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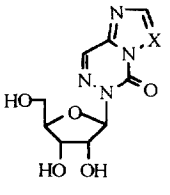
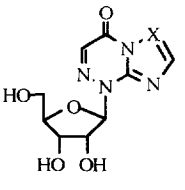
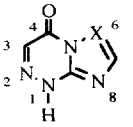


3a, X=CH
3b, X=N



4a, X=CH
4b, X=N

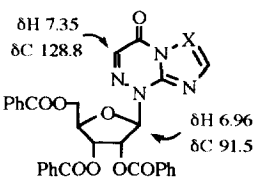


5a, X=CH
5b, X=N

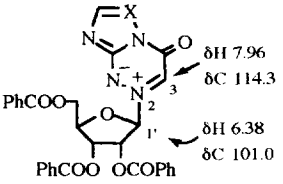
Thus, heating of **5a** (1.60 mmol) with Me_3SiCl (40 µl) and $(Me_3Si)_2NH$ (2.4 ml),[8] with removal of the reagent excess in vacuo, dissolution in CH_3CN (3 ml), addition via syringe to a solution of 2,3,5-tri-*O*-benzoyl-

§ Dedicated to Prof. Satoshi Ōmura on the occasion of his 60th birthday.

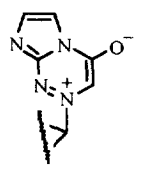
β-D-ribofuranosyl bromide (0.80 mmol) in CH_3CN (1 ml), cooling to –40 °C, addition of silver trifluoromethylsulfonate (AgOTf, 0.80 mmol) in CH_3CN (0.6 ml),[9] stirring at rt for 20 h, and separation by column chromatography afforded a small amount (ca 5% yield) of a product with an intense blue fluorescence at λ 455 nm (excitation at 370 nm), the ^1H and ^{13}C NMR chemical shifts for the nitrogenated moiety almost identical to those of **5a**, and the β-D-ribofuranose expected system ($J_{1'2'}$ = 3.3). Structure **6a**, ie 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[2,1-*c*][1,2,4]triazin-4(1*H*)-one, was attributed to this nucleoside. On the other hand, the more polar, major product (90% yield, referred to the monosaccharide amount employed, as throughout the present work) was hardly fluorescent (λ 462 nm, excitation at 430 nm), but its NMR spectra were remarkable in several aspects, since the anomeric proton and carbon appeared ca 0.6 ppm and 10 ppm at higher and lower field, respectively, in relation to the corresponding signals of **6a**, while C3 appeared 14.5 ppm at higher field than C3 of **6a**. However, the remaining NMR data suggested that we were dealing with a β-D-ribofuranosyl derivative ($J_{1'2'}$ = 2.1). Betaine-like structure **7a** was tentatively assigned to this nucleoside in view of these evidences and of a nuclear Overhauser experiment (showing a correlation between H3 and H1' that was not observed in the case of **6a**). In other words, under these reaction conditions (see entry 1 of the Table), it seemed that attack through N2, which leads to betaine nucleoside **7a**, largely predominated. On the other hand, when **5a**, without previous silylation, was treated with 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl bromide in a 2:1 molar ratio, in refluxing CH_3CN for 6 h, **6a** was exclusively isolated (see Table, entry 2); however, at shorter reaction times, TLC and ^1H NMR indicated the presence of **6a**, **7a**, and a third nucleoside (to which structure **8a** was assigned later, see below) in the reaction mixture.



6a, X=CH

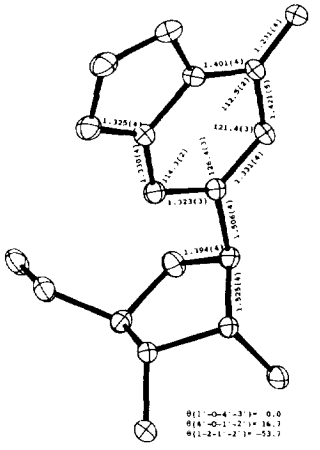


7a, X=CH



7a'

Both **7a** and its deprotected,[10] parent compound 2-β-D-ribofuranosyl-imidazo[2,1-*c*][1,2,4]triazinium-4-olate showed a strong IR band at 1680 cm^{-1} which indicated that either their structures were erroneous or that the contribution of Lewis formulae such as **7a'** to the resonance hybrids should be unusually low (to explain the relatively high degree of double-bond character of the C4–O bond). Crystals of the debenzoylated compound were submitted to X-ray analysis,[11] which confirmed the suspected structure (see the Figure) and showed a C4–O bond length of 1.231 Å (123.1 pm), only slightly longer than that of a standard carbonyl bond.



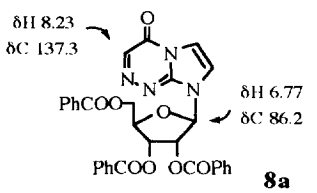
7a (debenzoylated)

Table. Reaction of **5a** and **5b** with ribofuranosyl bromide (tri-*O*-Bz), in CH_3CN

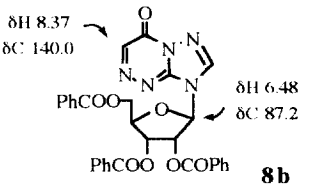
entry	substrate	reaction conditions	nucleoside, isolated yield	betaine nucl., isolated yield	other nucl., isolated yield
1	**5a** (silylated)	AgOTf, 20 h at rt	**6a**, 5%	**7a**, 90%	—
2	**5a**	reflux, 6 h	**6a**, 95%	—	—
3	**5b** (silylated)	AgOTf, 20 h at rt	**6b**, 10%	**7b**, 15%	**8b**, 10%
4	**5b** (silylated)	AgOTf, 20 h at –20 °C	—	**7b**, 45%	—
5	**5b**	reflux, 6h	**6b**, 85%	—	—

In the case of triazole derivative **5b** we isolated, after silylation and ribosidation (see Table, entry 3) as in the case of **5a**, three different nucleosides by column chromatography, in small amounts: the expected fluorescent nucleoside (**6b**),[12] the betaine nucleoside (**7b**),[12] and a third β-ribonucleoside of intermediate polarity (**8b**, the structural elucidation of which will be commented below). More interesting was the fact that, when the reaction was performed at –20 °C, the betaine nucleoside (**7b**) turned out to be the major product in the final mixture (NMR) and could be isolated in acceptable yields (see entry 4). Therefore, it seems that **7b** is kinetically favoured but it is very sensitive —more sensitive than **7a**— to the temperature under the reaction conditions. On the other hand, the direct reaction of triazole derivative **5b**, without previous silylation, with the same ribofuranosyl bromide in refluxing CH_3CN for 6 h, gave only the expected nucleoside **6b** (entry 5).

Although some mesoionic and betaine nucleosides have been reported from time to time,[13] we have demonstrated now that *in azolo-triazinone systems it is also possible to isolate betaine-like species as the major products*. Nevertheless, even the most stable of our two betaines isomerised in hot in the presence of acids. For instance, after heating **7a** with 0.1 equiv. of Me_3SiOTf in CH_3CN for 1 h, a third nucleoside was obtained as the major compound, to which structure **8a** was assigned on the basis of 2D NMR experiments (^1H-^{13}C COSY and HMBC); the spectral data of **8a** agree with those of the other 'transient' nucleoside that, as indicated in the Table, we isolated in the triazole case (see entry 3), viz **8b**. By heating for further 8 h, **8a** was completely

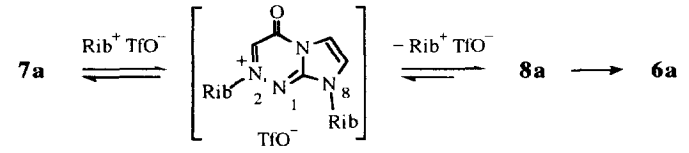


8a



8b

converted into the normal nucleoside, **6a**.[14] In the absence of Me_3SiOTf, **7a** was more stable.[15] Moreover, in other independent experiments we noted that: (i) betaine **7a** was converted into **8a** when treated with 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl bromide (Rib-Br) and AgOTf in CH_3CN; (ii) **7a**, when treated with equimolar amounts of **5a**-TfOH in refluxing CH_3CN, gave eventually **6a**; and (iii) **6a** was found to be stable under all the above-mentioned conditions. Thus, it appears that the ribofuranosyl cation (Rib^+) catalyses the isomerisation of **7a** to **8a**, likely through the intermediate arising from the attack of Rib^+ at N8 of **7a**, which is favoured by steric reasons (in relation to that at N1). When pure **7a** is heated in the presence of Me_3SiOTf, we believe that small amounts of Rib^+, arising now from the **7a** + Me_3SiOTf ⇌ **5a** (silylated) + Rib^+ TfO^- equilibrium —the reverse of the ribosidation reaction—, catalyse the first isomerisation (**7a** → **8a**), while the second one (**8a** → **6a**) is probably intramolecular. In short, these rearrangements may be summarised as in the next Scheme. Concerning the isolation of betaines **7**, we attribute the success of the experiments of entries 1 and 4 of the Table



to the relatively low temperatures and the use of a defect of Rib^+ TfO^- in relation to **5**. At higher temperatures, the presence of Me_3SiOTf (which is a product in entries 1, 3, and 4) or protic acid (HBr, ie **5**-HBr, is the co-product in the direct reaction, entries 2 and 5) diminishes the chance of obtaining betaines **7**, since their ribosidation step is reversible and they undergo the above rearrangements or a similar series of events (with Rib-Br/Δ in place of Rib^+ TfO^- in entries 2 and 5) to afford at last the thermodynamically stable isomers, **6**.

In summary, the present results show that betaine-like structures can be generated (and suggest that they may have participated as transient species more frequently than previously thought) in the glycosidation of certain nucleobases. Work is in progress in connection with the glucosidation of **5** as well as with the synthesis of **3** and related structures.

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10. NaOMe/MeOH at rt, then Amberlite IRC-50; 70% yield.
11. Enraf-Nonius CAD4 four-circle diffractometer, $Mo_{K\alpha}$ radiation, graphite monochromator, 293(2) K, 0.1 × 0.1 × 0.2 mm, orthorhombic, $P2_12_12_1$, a = 5.371(7), b = 8.945(2), c = 23.383(4) Å, V = 1123(2) Å^3, Z = 4, ρ = 1.586 g cm^{-3}, 1944 collected relections in the range $1.74 \leq \Theta \leq 29.98$, 1916 independent reflections, 1330 observed reflections with $I > 2\sigma(I)$, 221 refined parameters, R = 0.0467, ωR = 0.1378, H atoms (omitted in the Figure for the sake of clarity) located from a difference synthesis and refined with an overall isotropic temperature factor, structure solved by direct methods using SHELXS computer program (Sheldrick, G. M. *Acta Cryst.* **1990**, *A46*, 467) and refined by full-matrix least-square method with SHELXS93 computer program. Further information has been deposited at the Cambridge Crystallographic Data Centre.
12. Selected spectral data of **6b** (as throughout this work NMR spectra were recorded in $CDCl_3$ at 300 MHz for ^1H and 75.4 MHz for ^{13}C, J values are given in Hz, and IR spectra were obtained in KBr): δH1' 6.81 ($J_{1'2'}$ = 3.3), δC1' 92.5, δH3 7.52, δC3 133.5; $\tilde{\nu}$(C4-O) 1730 cm^{-1}. Of **7b**: δH1' 6.38 ($J_{1'2'}$ = 2.4), δC1' 101.6, δH3 8.33, δC3 122.1; $\tilde{\nu}$(C4-O) 1690 cm^{-1}.
13. Bambury, R. E.; Feeley, D. T.; Lawton, G. C.; Weaver, J. M.; Wemple, J. *J. Chem. Soc., Chem. Commun.* **1984**, 422; *J. Med. Chem.* **1984**, *27*, 1613, and ref. therein. The work of Wemple et al., who isolated 4-cyano-1-β-D-ribofuranosyl-pyridazinium-3-oxide from urine in mice and synthesised this metabolite and a few related products by reaction of the corresponding hydroxyazines with tetra-*O*-acetyl-β-D-ribofuranose and $SnCl_4$ in $ClCH_2CH_2Cl$, may be considered a landmark in this field. (We found, however, that these conditions were not appropriate for obtaining betaines **6a/6b**.) Following our experimental procedure ca 85% yields of the betaine-like ribofuranosides of 3-hydroxypyridine and pyridazin-3-one could be achieved; unfortunately, 5-hydroxypyrimidine, pyrazinone, and 6-azauracil did not afford betaines in acceptable yields but standard nucleosides under any of the mentioned (and other attempted) conditions.
14. Triazole-containing betaine **7b** underwent analogous isomerisations much more rapidly than imidazole-containing betaine **7a** (eg, complete conversion of **7b** into **6b** took only 1 h with 0.1 equiv. of Me_3SiOTf in refluxing CH_3CN, as compared to 9–10 h in the case of **7a** to **6a**).
15. After refluxing in CH_3CN for 6 h (without Lewis or protic acids), only a 25% of **7a** was converted into **8a** and **6a**.

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