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Mannich-Type C-Nucleosidations in the 5,8-Diaza-7,9-dicarba-purine Family[1]

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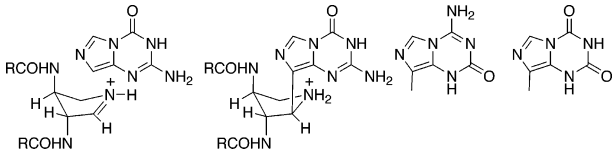
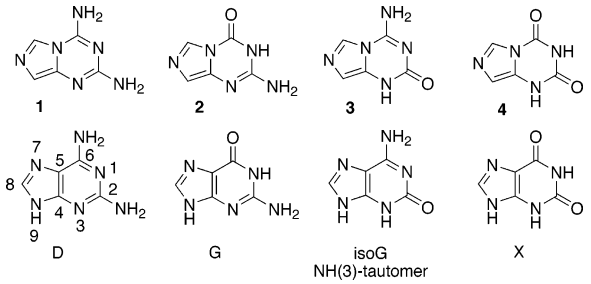
ABSTRACT



C-Nucleosidation with cyclic iminium salts occurring under mild reaction conditions and affording C-nucleosides that are isosteric with N-nucleosides of natural purines is shown to be a consistent property of the entire family of 2,6-(oxo or amino)-disubstituted 5,8-diaza-7,9-dicarba-purines.

In the context of ongoing studies directed toward a chemical etiology of nucleic acid structure, we recently reported on the synthesis[2] and some chemical properties[3] of the nucleobase-analogue 2,6-diamino-5,8-diaza-7,9-dicarba-purine **1**. The base is the 2,6-diamino derivative of a family of purinoid heterocycles **1**−**4** that are isomeric to the family of the natural purine nucleobases of which adenine and guanine are the canonical members (Scheme 1). The heterocycles of both families can be (formally) derived via isomeric generational pathways from the same ensembles of (potentially prebiotic) building blocks HCN, NH_2CN, and glycine nitrile (see Scheme 2 in ref 2). Constitutionally corresponding members of the two families possess functionally equivalent recognition elements and are expected to show similar reactivity and selectivity in Watson−Crick pairing. However, they differ in their function as nucleophilic reaction partners in nucleosidation reactions: 5,8-diaza-7,9-dicarba-purine derivatives may undergo Mannich-type nucleosidation reactions at position C-9 (purine numbering) to afford C-nucleosides that are isosteric to conventional (N-9)-nucleosides of the natural purine nucleobases. In a previous communication, we have reported such C-nucleosidations of the 2,6-diamino derivative **1**.[3] Those studies have now been extended to all other members of the 5,8-diaza-7,9-dicarba-purine family (including the adenine analogue), whereby it is found that the propensity to undergo Mannich-type C-nucleosidations is indeed a consistent property within the family and not restricted to the (most reactive) 2,6-diamino derivative.

Scheme 1. Members of the 5,8-Diaza-7,9-dicarba-purine Family Juxtaposed to Corresponding Members of the Purine Family



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(1) Chemistry of α-Aminonitriles. Part 42. For Part 41, see: Ferencic, M.; Reddy, G.; Wu, X.; Guntha, S.; Nandy, J.; Krishnamurthy, R.; Eschenmoser, A. *Chem. Biodiv.* **2004**, *1*, 939.
(2) Wang, Z.; Huynh, H. K.; Han, Bo; Krishnamurthy, R.; Eschenmoser, A. *Org. Lett.* **2003**, *5*, 2067.
(3) Han, Bo; Wang, Z.; Jaun, B.; Krishnamurthy, R.; Eschenmoser, A. *Org. Lett.* **2003**, *5*, 2071.

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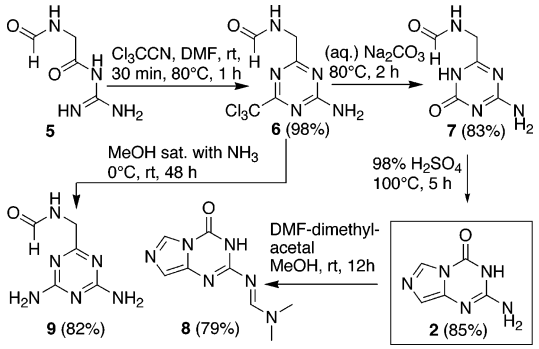
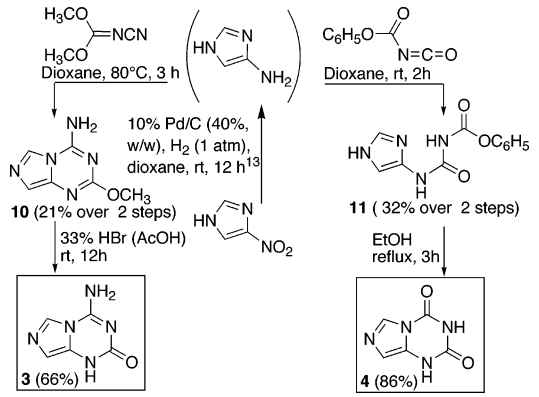
Scheme 2. Preparation of Guanine Analogue **2**



The methods used for preparing purinoids **2** (Scheme 2), **3** (Scheme 3), and **4** (Scheme 3) were chosen without regard to the potentially etiological aspects that we associate with these nucleobase analogues. *N*-Formyl-glycyl-guanidine **5**, easily obtained from *N*-formyl-glycine ethylester[4] and guanidine,[5] reacts with trichloroacetonitrile to give the trichloromethyl-triazine derivative **6**, which, following the methodology described by Kelarev et al.,[6] is hydrolytically converted to **7**. Remarkably, as well as satisfyingly, when this *N*-formyl-aminomethyl-triazine is treated with concentrated H_2SO_4 at 100 °C, a procedure reported in the literature for the cyclization of other keto-amino-triazine derivatives,[7] dehydrative cyclization proceeds regioselectively and gives the guanine analogue **2** in high yield; the isomeric cyclization product is not observed.[8] ^{1}H and ^{13}C NMR spectra of **2** are compatible with the assigned constitution;[9] the proof, hard to come by spectroscopically, is based on X-ray structure analysis[10] of the derivative **8**, formed in high yield by mild treatment of the product of cyclization with Meerwein's DMF−dimethylacetal.[11,12]

Scheme 3. Preparation of **3** and **4**



The trichloromethyl group of **6** can also be converted to an amino group of **9** by treatment with methanolic NH_3; this provides a convenient alternative to the previously reported[2] synthesis of purinoid **1**.

The preparations of **3** (the isoguanine analogue) and **4** (the xanthine analogue) start from the preformed imidazole moiety of the bicyclic heterocycles.[13,2] Treatment of 5-amino-imidazole in dioxane (prepared in situ by catalytic hydrogenation of 5-nitro-imidazole[13,14]) with *N*-cyano-isocyanate-dimethylacetal[15,16] gives the bicyclic 2-methoxy-6-amino derivative **10**, the methoxy group of which is cleaved, presumably via N-protonation(s) followed by S_N2 substitution at the methyl carbon, to the carbonyl group of **3** by HBr in acetic acid. Alternatively, reaction of 5-amino-imidazole in dioxane with phenyloxycarbonyl-isocyanate[17] affords the intermediate **11**, which smoothly cyclizes to **4** by heating in ethanolic solution.

The isoguanine analogue **3** was obtained as a white solid which, in contrast to **2**, is only sparingly soluble in DMSO and insoluble in H_2O or CH_3OH. The compound is thermally stable to heating (in a closed tube) to 190 °C for 10 h. The xanthine analogue **4** crystallized in colorless cubes from ethanol; it is soluble in DMSO, H_2O, and CH_3OH, and its constitution was confirmed by X-ray analysis.[10] The UV spectra of **1**−**4**, in which all absorption maxima are hypsochromic relative to those of the corresponding purines, are reproduced in Figure 1.[9]

Scheme 4 summarizes our experiments on the C-nucleosidation of the guanine analogue **2** with the previously described pyrroline derivative **12**.[3] Treatment of 1.0 mol equiv of **2** in DMF (c = 0.2 M) with 1.1 mol equiv of **12** in the presence of 1.1 proton equivalents (p-TsOH·H_2O) at room temperature for 2 h afforded the corresponding C-nucleosides essentially quantitatively, as revealed by direct (NH)-protection of the reaction product in the reaction mixture with $(BOC)_2O/Et_3N$ followed by chromatography

(4) Martin, P. K.; Matthews, H. R.; Rapoport, H.; Thyagarajan, G. *J. Org. Chem.* **1968**, *33*, 3758.

(5) Procedure for the preparation of *N*-formyl-L-valyl-guanidine by: Hoffmann, E.; Diller, D. *Can. J. Chem.* **1965**, *43*, 3103.

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(8) Considering the harsh reaction conditions (concentrated H_2SO_4, 100° C) and the multitude of protonation sites in the substrate, we refrain from attempting to rationalize the observed regioselectivity of the cyclization.

(9) NMR and mass spectral properties, see Supporting Information.

(10) X-ray structure analysis of **4** and **8** was carried out by Dr. Bernd Schweizer, ETH-Z. Crystallographic data for the structure has been deposited with the Cambridge Crystallographic Data Center as deposition no. CCDC 242452 for **8** and no. CCDC 242453 for **4**. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Rd., Cambridge CB12 1EZ, UK (fax: +44 (1233) 336 0333; e-mail: deposit@ccdc.cam.ac.uk). Data of **4** and **8** can be found in Supporting Information.

(11) Reaction of **2** with DMF−dimethylacetal in methanol at room temperature produces, besides **8**, small amounts of a side product containing an additional dimethylamino-methylidene group at carbon C-9.

(12) Koch, G. (Novartis AG, Basel) Private communication: Underivatized **2** could be obtained in crystalline form and as such subjected to X-ray structure analysis (work by G. Koch and B. Wagner (Novartis)).

(13) Al-Shaar, A. H. M.; Chambers, R. K.; Gilmour, D. W.; Lythgoe, D. J.; McClenaghan, I.; Ramsden, C. A. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2789.

(14) Rabinowitz, J. C. *J. Biol. Chem.* **1956**, *218*, 175.

(15) Prepared by heating cyanamide in tetramethyl orthocarbonate at 95 °C in 76% yield (mp 56−58° C) according to Kantlehner et al.[16] but using tetramethyl- instead of tetraethyl-orthocarbonate in order to avoid the formation of a mixture of O- and N-ethylated isomers (see procedure in Supporting Information).

(16) Kantlehner, W.; Maier, T.; Löffler, W.; Kapassakalidis, J. J. *Liebigs Ann. Chem.* **1982**, 507.

(17) Rao, P.; Benner, S. A. *J. Org. Chem.* **2001**, *66*, 5012.

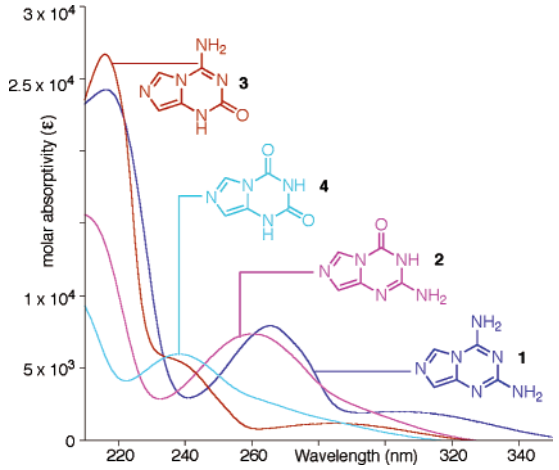


Figure 1. UV absorption spectra of purinoids **1**−**4** (c = 2.33−2.52 × 10^{-4} M in 0.1 M, pH 7, aq phosphate buffer, rt). **1**: ϵ (220 nm) = 23 300; λ_{max} = 265 nm (ϵ = 7900); ϵ (ca 310 nm) = 1900. **2**: ϵ (220 nm) = 10 200; λ_{max} = 261 nm (ϵ = 7300). **3**: ϵ (220 nm) = 24 400; λ_{sh} = 240 nm (ϵ = 5200); ϵ (ca 290 nm) = 1000. **4**: ϵ (220 nm) = 4300; λ_{max} = 237 nm (ϵ = 5900).

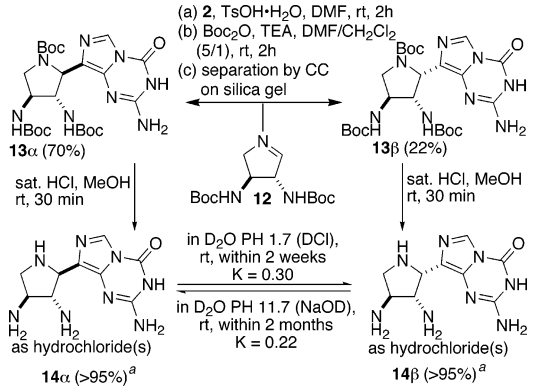
of the epimeric nucleosides to give pure α-epimer **13**α in 70% and pure epimer **13**β in 22% yield. The assignment of constitution rests on mass and ^{1}H/^{13}C NMR spectroscopic data and the assignment of configuration on NMR data observed for the corresponding deprotected nucleosides **14**α and **14**β (see below).

Keeping samples of **13**α or **13**β in methanolic HCl (saturated at room temperature) for 30 min and subsequently removing volatiles by evaporation under reduced pressure afforded the anomeric nucleosides **14**α and **14**β, respectively, as hydrochlorides[18] in the form of water-soluble white solids. The assignment of their configuration at the anomeric centers is based on NMR (J values and NOE) data.[19]

The two anomers **14**α and **14**β are configurationally stable under the conditions of deprotection (NMR). They slowly epimerize in D_2O solution (^{1}H NMR, pH 1.7) at room temperature reaching an equilibrium composition of **14**α:**14**β ∼3:1 within about 2 weeks. In alkaline D_2O solution (NaOH, pH = 11.8), at room temperature they reach equilibrium after about 2 months. The protected nucleosides **13**α and **13**β are configurationally stable in DMSO at room temperature; yet when heated in DMSO-d_6 to 100 °C, they equilibrate to a composition of **13**α:**13**β ∼1.4:1 within 1 day (^{1}H NMR).

C-Nucleosidations of the purinoids **3** and **4** with (trimeric) pyrroline **15**[3] are summarized in Scheme 5, which also gives

Scheme 4. C-Nucleosidation of Guanine Analogue **2** with BOC-Protected (3*R*)-*trans*-3,4-Diamino-1-pyrroline **12**



[a] Yields for **14** calculated as tetra-HCl-salts.

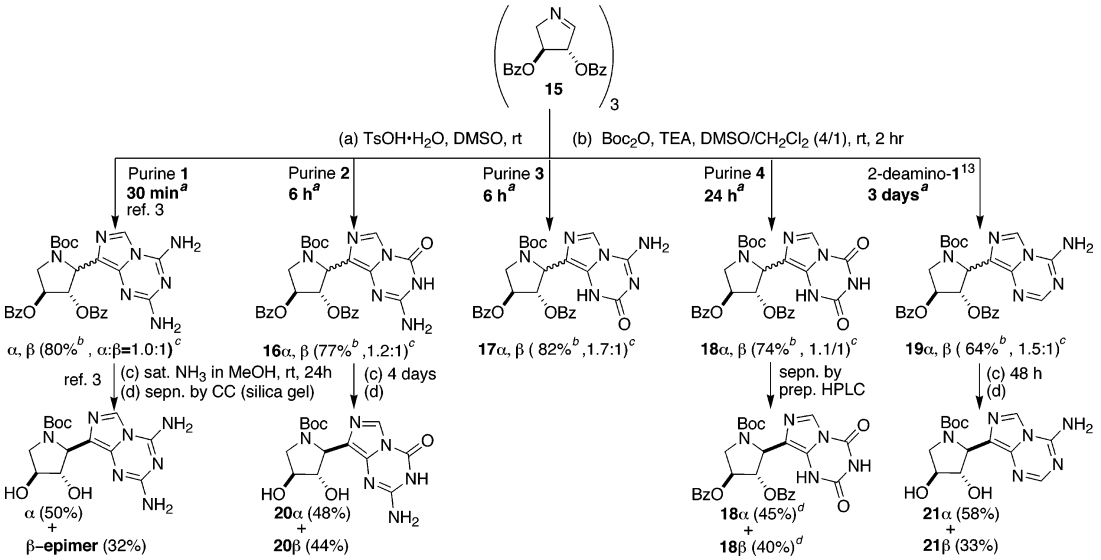
a qualitative comparison of the relative reactivity of all purinoids investigated, including the previously described[3] diamino derivative **1**, as well as its 2-deamino- (= adenine) analogue.[13] In four of the five cases, (protected) epimeric C-nucleosides have been separated by column chromatography.[9] The purinoids **1**−**4** and their adenine analogue 2-deamino-**1**[13] all undergo the C-nucleosidation with **15** in comparable yields under reaction conditions that are identical with the exception of reaction times. These vary within a factor of roughly 100 in the order **1** > **2** ∼ **3** > **4** > (2-deamino)-**1**, with the 2,6-diamino-purinoid **1** as the fastest and its 2-deamino derivative as the slowest member of the family. What may appear to be surprising in this sequence, namely, that the two oxo-amino-members **2** and **3** and even the dioxo-member **4** undergo electrophilic substitution at position C-9 faster than the adenine analogue, is a reminder of the more pronounced 10-π-electron aromaticity of members that bear only amino groups as substituents as against purinoids containing lactam groups. Mechanistic reasoning predicts that there should be an inverse relationship between the propensity of purinoids to undergo electrophilic C-nucleosidation and the stability of corresponding C-nucleosides. Experimental data seem to confirm this expectation: the nucleosides **14**α and **14**β (Scheme 4) equilibrate at room temperature within weeks at pH 1.7 and within months at pH 11.7; the corresponding nucleosides derived from **1**[3] do so within hours (rt) at pH 1.9 and within weeks at pH 11.3.

Of the four purinoids **1**−**4**, the isoguanine analogue **3** deserves a special comment with regard to its constitution. Isoguanine of the natural purine family exists, in contrast to guanine, as a mixture of tautomers in which the NH(3)-lactam tautomer (Figure 2) is not reported to be among those observed.[20] The coexistence (or accessibility) of this specific tautomer, however, has been documented in base-pairing studies in the homo-DNA[21] as well the pyranosyl-RNA series[22] by the observation of robust duplexes, the formation of which must be interpreted to rely on guanine−isoguanine base-pairs of type A (Figure 2). The type of isoguanine tautomer required in such purine−purine base-pairs becomes the only possible lactam-type tautomer in the case of the isoguanine analogue **3**. We therefore can foresee the existence of informational oligomers that may contain only puri-

(18) Estimated (by weight, after drying at room temperature/HV/overnight) to be tris- or tetrahydrochlorides; pH of solutions in H_2O ∼ 1.7 (0.072 M).

(19) Minor isomer **14**β exhibits coupling constants $J_{1'2'}$ = 7.9 Hz, $J_{2'3'}$ = 6.7 Hz, $J_{3'4'si}$ = 9.3 Hz, $J_{3'4're}$ = 6.0 Hz. NOEs H1′−H2′ (m), H1′−H4′$_{re}$ (w), H3′−H4′$_{si}$ (s) indicate that H1′ is cis to both H2′ and H4′$_{re}$. For the major isomer **14**α, coupling constants $J_{1'2'}$ = 7.4 Hz, $J_{2'3'}$ = 5.8 Hz, $J_{3'4'si}$ = 8.8 Hz, $J_{3'4're}$ = 6.0 Hz together with NOEs H1′−H2′ (w), H1′−H3′ (w), H1′−H4′$_{si}$ (w), H2′−H4′$_{re}$ (m), and H3′−H4′$_{si}$ (s) are consistent with H1′ being trans to H2′ and cis to H4′$_{si}$. The similar coupling constants indicate that both epimers have the same predominant conformation of the five-membered ring with a dihedral angle H1′−C1′−C2′−H2′ near 150° for the α-isomer and near 10° for the β-isomer.

Scheme 5. C-Nucleosidation of **1**−**4** and 2-Deamino-**1** (Adenine Analogue) with (3*R*)-*trans*-3,4-Dibenzoyl-1-pyrroline **15**



[a] Reaction times of step a. [b]Yields over steps a and b. [c]Epimeric ratios determined by integration of H-1′ and H-4′$_{re}$ signals in the NMR.[9] [d]Separation yields.

noids **1**−**4** as nucleobases and where duplexation and the function of sequence recognition is exclusively based on purinoid−purinoid base-pairs of types B and C (Figure 2).

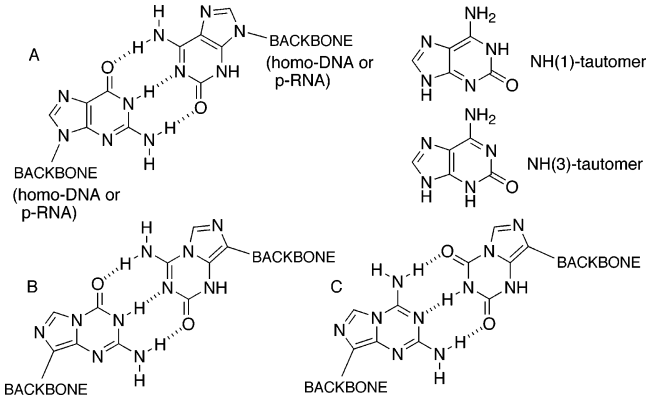


Figure 2. Purinoids **1**−**4** are expected to be able to act as nucleobases forming two Watson−Crick-type base-pairs B and C.

Whether the chemistry of the purinoid family **1**−**4** is potentially relevant in the etiological context that provided the stimulus for the present investigation in the first place will primarily depend on whether members of the family can be generated and, if so, under what conditions, from potentially prebiotic precursor molecules of the canonical nucleobases.[23] The question is to be answered experimentally. Irrespective of the outcome of such experiments, the purinoids deserve attention from a purely chemical point of view. They are special members of a growing list of artificial purinoid nucleobase variants[24] that are of interest as vehicles of research in informational chemistry,[25] medicinal chemistry,[7,26] and chemical biology.[27] What is deemed special in them is their chemical versatility for the task of attaching sets of complementary recognition elements to functional groups in any kind of substrate.

Acknowledgment. The work was supported by Novartis AG (Basel) and the Skaggs Research Foundation. We thank Dr. Guido Koch and Dr. J. Zimmermann (Novartis) for their interest in, and support of, this work.

Supporting Information Available: Procedures and data for purinoids **2**−**4**, as well as their nucleosidation reactions with **12** and **15**; CIF files for X-ray structures of compounds **4** and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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