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A New Class of Potent Guanine Antimetabolites. Synthesis of 3-Deazaguanine, 3-Deazaguanosine, and 3-Deazaguanylic Acid by a Novel Ring Closure of Imidazole Precursors

Sir:

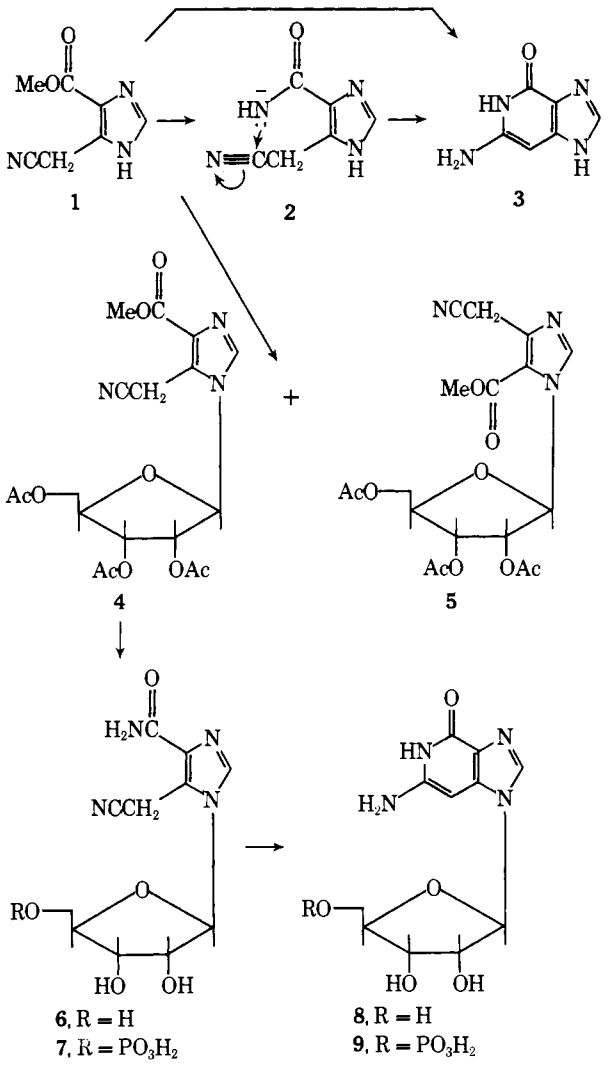
We wish to report the synthesis of 6-aminoimidazo[4,5-*c*]pyridin-4(5*H*)-one (3-deazaguanine, **3**), its nucleoside, 6-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridin-4(5*H*)-one (3-deazaguanosine, **8**), and the corresponding 5′-nucleotide, 6-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridin-4(5*H*)-one 5′-phosphate (3-deazaguanylic acid, **9**) from the requisite 5-cyanomethylimidazole-4-carboxamide which in the presence of base was found to undergo a unique ring closure to the desired 3-deazaguanine derivatives.

The increased interest in the importance of guanine nucleotide metabolism has stimulated renewed efforts to study these biochemical pathways in various microbiological and mammalian systems.[1] Antimetabolites have proved to be powerful biochemical tools employed to probe such pertinent enzymatic transformations.

de Bode and Salemink[2] recently reported a series of unsuccessful attempts to synthesize 3-deazaguanine from ring closure procedures of certain diaminopyridine derivatives. Our own approach was based on ring closure of imidazole intermediates, which had previously proved successful in the synthesis of imidazo[4,5-*c*]pyridin-4,6(5*H*,7*H*)-dione (3-deazaxanthine).[3] Preliminary success achieved in the synthesis of 6-amino-4-bromoimidazo[4,5-*c*]pyridine from 4(5)-cyano-5(4)-cyanomethylimidzole[4] did not result in the desired 3-deazaguanine (**3**) since the 4-bromo group proved to be exceptionally inert toward nucleophilic substitution.[4]

In a new approach, designed to yield 3-deazaguanine (**3**) directly by ring closure, the required key imidazole intermediate, methyl 5(4)-cyanomethylimidazole-4(5)-carboxylate[5] (**1**, Scheme I), mp 170–171° dec, was obtained in 77% yield from methyl 5(4)-carbamoylmethylimidazole-4(5)-carboxylate[3] and refluxing phosphorus oxychloride. Treatment of **1** with liquid ammonia (8 days, 100°) provided 3-deazaguanine (**3**) as light-sensitive yellow needles [75%, mp >350° (H_2O)]: $\lambda_{max}^{pH\ 1}$ 273 (ε 11,320), 311 (ε 6380); $\lambda_{max}^{pH\ 11}$ 262 (ε 9630), 298 (ε 7780). The intermediate to **3**, 5(4)-cyanomethylimidazole-4(5)-carboxamide (**2**) (mp 231–232° dec), was obtained in 77% yield by interrupting the reaction of **1** and ammonia after 48 hr. Compound **2** was smoothly cyclized to **3** with aqueous sodium carbonate. This ring closure has now been shown in our laboratory to be of general application in the synthesis of various condensed aminopyridone systems. The mechanism is visualized as occurring by base abstraction of an amide proton followed by attack of the generated anion on the nitrile carbon.

Scheme I



The requisite imidazole nucleoside, methyl 5-cyanomethyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylate (**4**) was obtained in quantitative yield from the condensation of 1 equiv of methyl 5(4)-cyanomethyl-1-trimethylsilylimidazole-4(5)-carboxylate (silylated **1**) with 1 equiv of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose in the presence of 1.44 molar equiv of stannic chloride. The yield and ratio of positional isomers in this ribosylation procedure markedly depends on the ratio of stannic chloride to silylated **1** and the blocked ribofuranose since the same condensation carried out with 0.72 molar equiv of stannic chloride afforded, after silica gel chromatography, a 29.5% yield of **4** and a 34.5% yield of the other positional isomer, methyl 4-cyanomethyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-5-carboxylate (**5**) (white needles, mp 92–93° (EtOH)).

Treatment of **4** with liquid ammonia (3 hr, 100°) provided the versatile intermediate, 5-cyanomethyl-1-β-D-ribofuranosylimidazole-4-carboxamide (**6**) (81%, mp 90–91° dec (MeOH)). When **6** was refluxed (0.5 hr) with aqueous sodium carbonate in ethanol, 3-deazaguanosine (**8**) was formed and crystallized from the reaction solution (85%, white microcrystals, mp 255–257° dec): $\lambda_{max}^{pH\ 1}$ 284 (ε 13,100), 308 sh (ε 7050); $\lambda_{max}^{pH\ 11}$ 272 (ε 11,900), 295 sh

Table I. Some Pertinent ^{13}C Chemical Shifts of 4- and 5-Substituted Imidazole Anions and Their Ribofuranosyl Derivatives

Compound	Chemical shift, ppm[a]		
	C_2	C_4	C_5
Anion of 10[b]	145.7	130.1	133.7
Anion of 1[b]	143.5	124.8	136.4
4	136.8	128.0	130.7
	(136.5)[c]	(126.8)	(129.4)
5	139.9	138.4	118.4
	(136.5)	(138.4)	(117.8)

[a] ^{13}C NMR spectra of 20% DMSO-d_6 solutions were obtained on a Bruker HX-90 NMR spectrometer operating at 22.62 MHz in the Fourier transform mode at a probe temperature of 35°. Chemical shifts are measured from DMSO-d_6, converted to TMS scale using the relationship δTMS = δDMSO-d_6 + 39.5 ppm. [b] The anions of various heterocycles were formed by neutralization with LiOH in DMSO-d_6. [c] Values in parentheses are predicted chemical shifts using α- and β-substitution shifts of +7 and −2 ppm, respectively.

(ϵ 8950). Alternatively, when **4** was treated with liquid ammonia (22 hr, 110°) deblocking, ammonolysis, and ring closure occurred simultaneously to provide **8** in 20–30% yield.

The imidazole nucleoside, **6**, was phosphorylated at 0° with phosphorus oxychloride in the presence of triethyl phosphate[6] to provide 5-cyanomethyl-1-β-D-ribofuranosylimidazole-4-carboxamide 5′-phosphate (**7**) (80%, white microcrystals, dec >160°) as the free acid, after ion-exchange chromatography. Treatment of **7** with aqueous sodium carbonate (pH 10, 100°, 0.75 hr) provided 3-deazaguanylic acid (**9**) (50%, beige microcrystals, dec >180°) as the free acid, after ion-exchange chromatography: $\lambda_{max}^{pH\ 1}$ 287 (ϵ 8400), 306 sh (ϵ 4930); $\lambda_{max}^{pH\ 11}$ 272 (ϵ 8130), 303 (ϵ 6090). Direct phosphorylation of **8** provided a mixture of products, from which **9** was isolated in 30% yield.

The ribosylation site in the imidazole nucleoside precursor, **4**, and hence the structure of 3-deazaguanosine (**8**) was established on the basis of the α and β substitution shifts (carbon-13 NMR) observed when the neutral species is compared with the anionic form.[7] The pertinent carbon-13 chemical shifts of the anion of methyl 5-cyanomethylimidazole-4-carboxylate (**1**) and the ribofuranosyl derivatives of **1**, **4**, and **5** are listed in Table I. The spectral shifts of the anion of ethyl imidazole-4-carboxylate (**10**) are included for chemical shift comparison. Large upfield shifts for the α-carbon atoms and small downfield shifts for the β-carbon atoms are evident when the neutral species, **4** and **5**, are compared with the anion of **1**. The predicted values of the chemical shifts for the α- and β-carbon atoms using substitution values of +7 ppm upfield and −2 ppm downfield,[8] respectively, are included in parentheses for comparison. Reasonable agreement between the predicted and experimental values could be obtained only if the indicated assignments of the carbon atom resonances of the two isomers as well as the indicated structures in Scheme I were used. Additional support for this structural assignment was obtained by observation of the ^1H NMR of the anomeric proton ($H_{1'}$) of **5** which was shifted 0.32 ppm downfield from the anomeric proton of **4**. This shift, attributed to the close proximity of the anisotropic carbonyl group (methyl ester) to the anomeric proton ($H_{1'}$) of **5**, has been shown to be a reliable indicator for the site of ribosylation in other nucleosides.[9]

The β-configuration of **6**, and hence **8**, was established by examination of the difference in proton chemical shifts between the methyl groups of the isopropylidene derivative, 5-cyanomethyl-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide, of **6**. A difference of 0.19 ppm was found which is characteristic of the β-configuration.[10]

Calf spleen purine nucleoside phosphorylase (E.C.2.4.2.1) which shows a high specificity for phosphorylitic cleavage of guanosine[11] was used to assay 3-deazaguanosine (**8**) spectrophotometrically. When the two compounds were assayed at 5×10^{-5} *M*, guanosine was cleaved at a rate of 81 nmol/min and 3-deazaguanosine (**8**) was cleaved at 6.3 nmol/min to give 3-deazaguanine (**3**). When **3** and excess α-D-ribofuranose 1-phosphate were incubated with the same enzyme in Tris buffer at 25°, a nucleoside was isolated from the mixture by charcoal absorption purification which proved to be identical with 3-deazaguanosine (**8**) as judged by its uv spectrum and TLC mobility.

3-Deazaguanine (**3**), 3-deazaguanosine (**8**), and 3-deazaguanylic acid (**9**) exhibit potent broad spectrum activity as inhibitors of viral cytopathic effect in KB cells in cultures of various RNA and DNA viruses.[12] It is interesting to note that the antiviral agent 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin) has been shown to be an inhibitor of guanylic acid biosynthesis.[13] 3-Deazaguanine (**3**) and its probable metabolites, **8** and **9**, are among the few antimetabolites known which exhibit similar broad spectrum antiviral activity against both RNA and DNA viruses in vitro.

3-Deazaguanine (**3**) was highly effective without appreciable toxicity against L1210 leukemia and adenocarcinoma 755 in mice.[14]

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