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SYNTHESIS OF 3,9-DIALKYLGUANINES AND 3-METHYLGUANOSINE, A KEY INTERMEDIATE FOR THE SYNTHESIS OF Y NUCLEOSIDES

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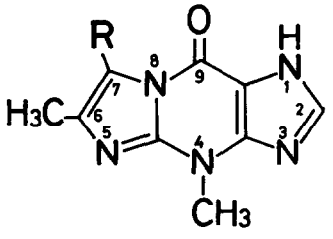
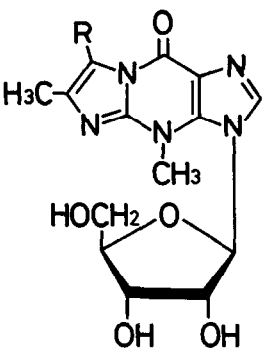
The findings[1] and structural elucidation[2] of highly fluorescent Y bases (wye (Ia); wybutine (Ib); wybutoxine (Ic))[2g] in $tRNA^{Phe}_{s}$ from various sources have focused our attention on 3-methylguanosine (Vg) as a probable biogenetic and/or synthetic precursor[2g,3] of Y nucleosides (wyosine (IIa); wybutosine (IIb); wybutoxosine (IIc))[2g] Nakatsuka and Goto[4] recently announced that they succeeded in the synthesis of 2',3',5'-tri-O-acetyl-3-methylguanosine (Vf), which was converted into Vg and IIa. Quite recently, Ienaga and Pfleiderer[5] reported the synthesis of so far unknown 3,9-dimethylguanine (Va) according to the conventional method.[6] These reports prompt us to record here our own new synthesis of 3,9-dialkylguanines (Va-e) and 3-methylguanosine (Vg).



Ia, b, c



IIa, b, c

a: R = H

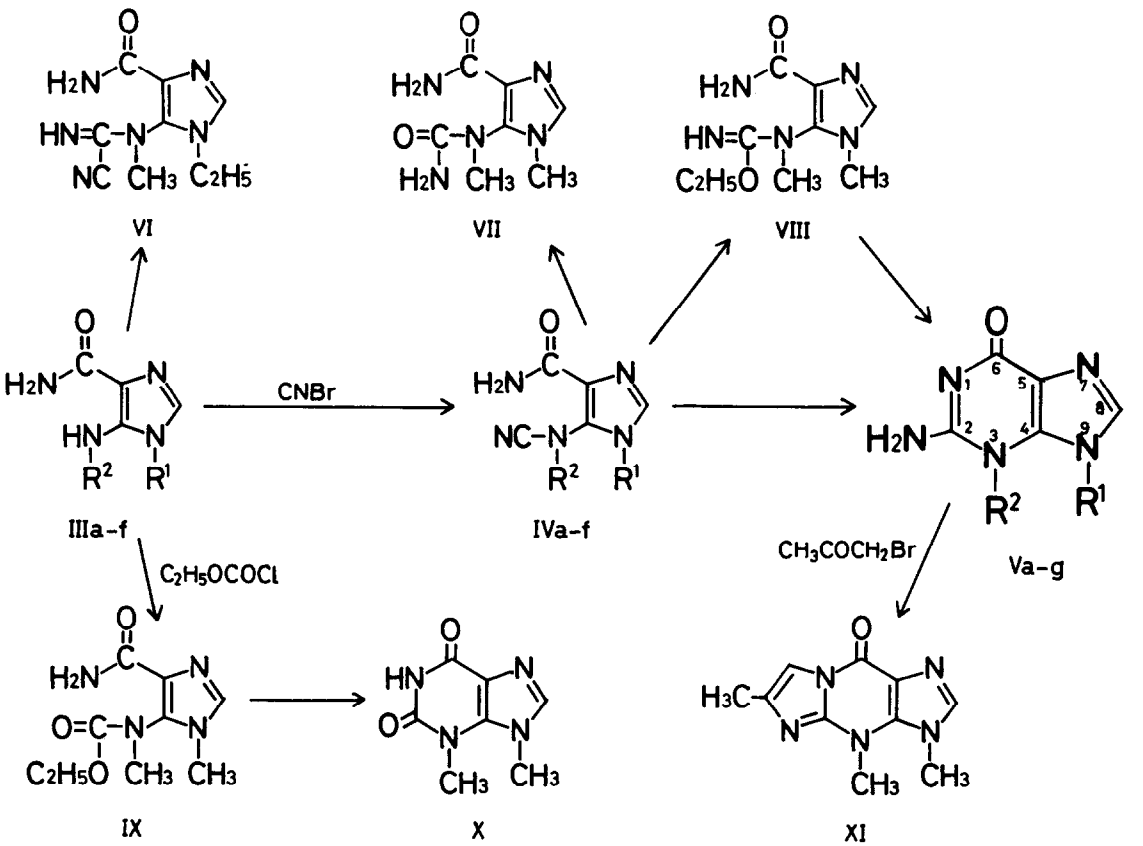
b; R = $CH_2CH_2C(COOCH_3)(H)NHCOOCH_3$

c: R = $CH_2CH(OOH)C(COOCH_3)(H)NHCOOCH_3$

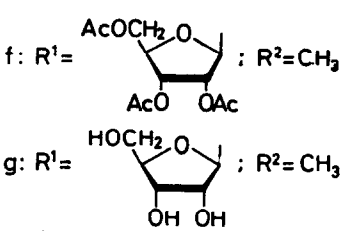
Probably the most promising means of obtaining 3,9-dialkylguanine (V) is the cyclization of appropriately substituted imidazole or pyrimidine derivatives. Expecting 1-alkyl-5-(alkylamino)-imidazole-4-carboxamides (III) to be versatile intermediates for the synthesis of various 3,9-dialkylpurines, we have reported the general synthesis of III.[7] We then tried the cyanation of III with CNBr, although Yamazaki et al.[6,8] described that neither 5-aminoimidazole-4-carboxamide (AICA) nor its 1-β-D-ribofuranoside gave the corresponding cyanamide in the reactions with CNBr under various reaction conditions. When a solution of IIId[7] and an excess of CNBr in acetate buffer at pH 5 was allowed to stand at room temperature for a few hours, 5-(cyanomethylamino)-1-ethylimidazole-4-carboxamide (IVd) (33% yield; mp 190–191°; IR ν_{max}^{Nujol} 2225 cm^{-1}; Mass m/e: 193 (M^+), 176 (M^+-NH_3), 150 (M^+-NH_3-CN); UV $\lambda_{max}^{H_2O}$ (pH 1) 235 nm (inflection) (ε 6200); $\lambda_{max}^{H_2O}$ (pH 7) 241 (7500); $\lambda_{max}^{H_2O}$ (pH 13) unstable; PMR (Me_2SO-d_6) δ:[9] 1.37 (3H, t, J=7 Hz), 3.24 (3H, s), 3.97 (2H,

q, J=7 Hz), 7.22 and 7.34 (1H each, exchangeable with D_2O), 7.71 (1H, s)][10] was obtained after extraction with $CHCl_3$ and purification of the extracts by silica gel column chromatography. We have found that the reaction is retarded with decrease in pH and that the yield of IVd is decreased in the reaction at pH 6 or above, owing to the formation of 5-[(cyanoiminomethyl)methylamino]-1-ethylimidazole-4-carboxamide (VI) [mp 214–215° (dec.)]. In a similar manner, IVa [31% yield; mp 233–235° (dec.)[11]], IVb (43% yield; mp 174–175°), IVc [51% yield; mp 212–214° (dec.)], and IVe (44% yield; mp 156–158°) were obtained from IIIa,[7,12] IIIb,[7] IIIc,[7] and IIIe,[13] respectively.

Cyclization of IVa to 3,9-dimethylguanine (Va) could be achieved by various means. Being dissolved in 0.1 N NaOH at room temperature, IVa rapidly changed into Va (48% yield) and 1-methyl-5-(1-methylureido)imidazole-4-carboxamide (VII) [25% yield; mp 266–268° (dec.)]. Even in plain water at reflux, IVa cyclized to Va (65% yield). Treatment of IVa with NaOEt in EtOH at room tem-



a: $R^1=R^2=CH_3$
b: $R^1=CH_3$; $R^2=C_2H_5$
c: $R^1=CH_3$; $R^2=C_6H_5CH_2$
d: $R^1=C_2H_5$; $R^2=CH_3$
e: $R^1=R^2=C_2H_5$



perature gave Va (41% yield) together with 5-(2-ethyl-1-methyl-1-isoureido)-1-methylimidazole-4-carboxamide (VIII) (34% yield; mp 154–155°). This compound (VIII) was transformed into Va (77% yield) by heating with EtONa in EtOH. Thus, Va was prepared more effectively (86% yield) by direct heating of IVa at reflux with EtONa in EtOH for 7 hr. On treatment with NaH (0.2 equivalent mole) in DMF at room temperature, IVa cyclized to Va (87% yield) within 30 min.

Correct analyses were obtained for Va as monohydrate [mp>300°; PMR (Me_2SO-d_6) δ:[9] 3.67 (3H, s, 3-Me),[14] 3.91 (3H, s, 9-Me),[14] 6.6–7.1 (2H, exchangeable with D_2O), 7.53 (1H, s); p*K*a 4.62 ± 0.08]. The 3,9-dimethylguanine structure was supported by close resemblance between UV spectra of Va [$\lambda_{max}^{H_2O}$ (pH 1) 248 nm (ε 12500); $\lambda_{max}^{H_2O}$ (pH 7) 216 (30100), 247 (8600), 266 (11400); $\lambda_{max}^{H_2O}$ (pH 13) 247 (8800), 265.5 (11000)] and those reported for 2′,3′-O-isopropylidene-3,5′-cycloguanosine[15] or 3,5′-cycloguanosine.[15b] Further support for the structure rested on alkaline hydrolysis of Va. Holmes and Robins[15b] reported that 3,5′-cycloguanosine underwent hydrolysis to give 3,5′-cycloxanthosine on heating in 1 N NaOH. Similar treatment of Va gave a mixture of 3,9-dimethylxanthine (X) (21% yield), VII (34% yield), and IIIa (3.5% yield). The structure of X was confirmed by direct comparison with a sample {mp>300° [lit.[16] mp 349° (dec.)]} derived from IIIa *via* 5-[(ethoxycarbonyl)-methylamino]-1-methylimidazole-4-carboxamide (IX) (mp 204.5–206°). An alternative isoguanine structure[17] for Va was ruled out by a marked difference between Va and 3,9-dimethylisoguanine[18] in UV spectrum.

When Va was treated with bromoacetone in Me_2SO in the presence of NaH, 3-methylwye [XI: $C_{10}H_{11}N_5O$; 22% yield; mp 292–294° (dec.) (lit.[5] 296–297°); PMR (Me_2SO-d_6) δ:[9] 2.22, 4.04, and 4.08 (3H each, s), 7.33 and 7.74 (1H each, s)], a model compound of wyosine (IIa), was obtained. UV spectra of XI [$\lambda_{max}^{H_2O}$ (pH 1) 228 nm (ε 42800), 278 (11400); $\lambda_{max}^{H_2O}$ (pH 7) 234 (40100); 264 (4000); 297 (7700); $\lambda_{max}^{H_2O}$ (pH 13) 234 (39700), 264 (4000), 297 (7500)] closely resemble those[2a] reported for IIa.

Reactions of IVb-e with NaH (0.2–1.2 equivalent mole) similar to the above cyclization of IVa furnished the corresponding 3,9-dialkylguanines [Vb: $C_8H_{11}N_5O$; 88% yield; mp>300°; Vc: $C_{13}H_{13}N_5O\cdot H_2O$; 77% yield; mp 280–282° (dec.); Vd: $C_8H_{11}N_5O\cdot H_2O$; 91% yield; mp>300°; Ve: $C_9H_{13}N_5O$; 82% yield; mp 270–278° (dec.)[11]]. The structures of Vb-e were confirmed by the similarity to Va in UV spectrum.

5-(Methylamino)-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (IIIf)[13] was treated in the same manner as described above for the synthesis of Va-e, and 3-methylguanosine [Vg: $C_{11}H_{15}N_5O_5\cdot 2H_2O$; mp ca. 180° (dec);[11] PMR (Me_2SO-d_6) δ:[9] 3.38 (H_2O), 3.62 (m) and 3.70 (s) (a total of 5H), 3.98, 4.10, and 4.43 (1H each, m), 4.8–5.8 (3H, exchangeable with D_2O), 5.93 (1H, d, *J* = 5.5 Hz), 6.96 (2H, broad, exchangeable with D_2O), 8.03 (1H, s); UV $\lambda_{max}^{H_2O}$ (pH 2) unstable; $\lambda_{max}^{H_2O}$ (pH 7) 217 nm (ε 27500), 250 (10100), 265 (11700); $\lambda_{max}^{H_2O}$ (pH 13) 250 (10100), 265 (11700)] was obtained after deacetylation of the product (Vf) with NH_3–MeOH. UV spectrum of Vg at pH 1 changed completely in 10 min at 20° into what was very similar to that reported for 3-methylguanine.[19] Such instability of Vg is interesting in connection with the unusual susceptibility of Y nucleosides to acidic hydrolysis.[1,2]

This work provides a general method of obtaining 3,9-dialkylguanine (V) and a shorter route to 3-methylguanosine (Vg).

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