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Synthesis of 3-β-D-Ribofuranosylwye, the Most Probable Structure for Wyosine from *Torulopsis utilis* Phenylalanine Transfer Ribonucleic Acid

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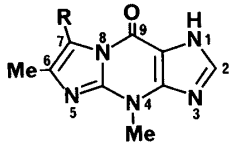
Treatment of 5-(methylamino)-1-β-D-ribofuranosylimidazole-4-carboxamide (**5a**) with CNBr in acetate buffer gave the 5-cyanomethylamino derivative **6a**, which was cyclized to 3-methylguanosine (**7**) in the presence of NaOEt. Cyclocondensation of **7** with bromoacetone in the presence of K_2CO_3 provided 3-β-D-ribofuranosylwye (**2**), the most probable structure for the fluorescent nucleoside from *Torulopsis utilis* phenylalanine transfer ribonucleic acid ($tRNA^{Phe}$). The glycosidic bonds of **2** and **7** have been shown to be unusually subject to cleavage under either acidic or basic conditions, but proved to be less labile under neutral conditions, as had been reported. The base moiety of **2** is also cleaved under basic conditions.

Keywords——wyosine; 3-methylguanosine; cyclocondensation; imidazole nucleoside; N-cyanation; base-catalyzed cyclization; glycosidic bond cleavage; ring fission

Since the finding[1] of a strongly fluorescent substance adjacent to the 3′ end of the anticodon of yeast phenylalanine transfer ribonucleic acid ($tRNA^{Phe}$), the chromophore and its congeners have been isolated from various eukaryotic $tRNAs^{Phe}$ and their structures have been elucidated as **1**.[2] Two fluorescent nucleosides, wyosine[2b,3] from *Torulopsis utilis* $tRNA^{Phe}$ and wybutosine[1b,4,5] from yeast $tRNA^{Phe}$, have been isolated and their bases have been determined to be wye (**1a**)[2a,b] and wybutine (**1b**),[2c,e] respectively. Thiebe and Zachau reported that ribose was detected in the hydrolyzate of wybutosine by thin-layer chromatography (TLC).[4] Comparison of the chemical properties and ultraviolet (UV) spectra of these nucleosides with those of model compounds has suggested that their sugar moieties are attached to the 3-position.[2b,5-7] Since all the nucleosides which have been isolated from tRNAs and fully characterized have so far proved to be β-D-ribofuranosides or 2-*O*-methyl-β-D-ribofuranosides, it is natural to assign the 3-β-D-ribofuranosides of **1a**, **b** to wyosine and wybutosine as the most probable structures. A prominent feature of these nucleosides is exceptional susceptibility of the glycosidic bonds to acidic hydrolysis.[3,4] However, Reese and Whittall[8] have reported that the glycosidic bond of 3-β-D-ribofuranosylwye (**2**), the putative structure for wyosine, is unlikely to be as labile as that of wyosine on the basis of rate studies on the hydrolysis of nucleosides structurally related to **2**. As wyosine and wybutosine were isolated from tRNAs in extremely minute quantities, rigorous identification of the position and the structure of the sugar moieties has to rest on chemical synthesis.

Nakatsuka *et al.*[9] reported the first synthesis of **2** and reported also that **2** was identical with wyosine in terms of the UV spectrum and chromatographic behavior. Such identification, however, is apparently unsatisfactory for full characterization of wyosine. Furthermore, the reported molar extinction coefficients for **2**[9] are unacceptably smaller than those for the 3-alkyl analogues (type **13**)[6,7] of **2**. We wish to present here a detailed account of our own synthesis and studies on the hydrolysis of **2**.[10]



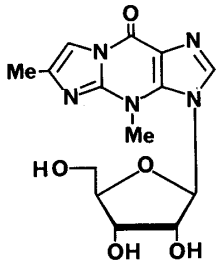
1

a: R=H

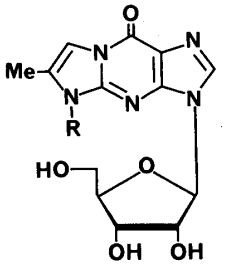
b—d: R=$CH_2CHR'CHNHCO_2MeCO_2Me$

(**b**: R'=H, **c**: R'=OH, **d**: R'=O_2H)

e: R=$CH_2CH(OH)CH(NH_2)CO_2H$



2



3: R=H
4: R=Me

Chart 1

Condensation of **1a** with tetra-*O*-acetyl-β-D-ribofuranose was reported to take place at the 1-position of **1a**.[2b,5] Reaction of 4-demethyl-**2** (**3**) with diazomethane was reported to give 5-methyl-**3** (**4**) in 89% yield.[2b] We obtained **4** by methylation of **3** with methyl iodide in the presence of K_2CO_3[11] though a complex mixture of products was formed in the absence of base.[12] We have developed the synthesis of 3,9-dialkylguanines (type **12**) and converted them into 3-alkylwyes (type **13**) as a model for the synthesis of **2**.[7] We have also established the synthesis of the requisite imidazole nucleosides **5**[13] to apply the procedures of the model synthesis at the nucleoside level.

Taking into account difficulties that purification of unprotected nucleosides might encounter, we first tried the reaction of 5-(methylamino)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**5b**)[9,13a,b] with CNBr according to the model synthesis.[7] The expected 5-(cyanomethylamino)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**6b**), mp 156—157 °C, was obtained in 39% yield. The correctness of the structure **6b** was confirmed by elemental analyses and by spectral similarity to the model compounds.[7] When **6b** was treated with NaH in Me_2NCHO,[7] a mixture of many products was obtained. Such complexity of the products is presumably due to O→N acetyl migration of the product(s) initially formed. Although separation of the desired 2′,3′,5′-tri-*O*-acetyl-3-methylguanosine[9] failed, treatment of the mixture with NH_3–MeOH afforded 3-methylguanosine (**7**) as the dihydrate, mp *ca.* 180 °C (dec.), in 21% yield. The structure of **7** was supported by spectral similarity to 3,9-dialkylguanines (type **12**)[7] and by acid hydrolysis (giving 3-methylguanine (**9**)[14] in high yield).

When the unprotected imidazole nucleoside **5a**[13] was treated with CNBr, 5-(cyanomethylamino)-1-β-D-ribofuranosylimidazole-4-carboxamide (**6a**), mp 183—185 °C (dec.), was obtained in 32% yield after purification on a charcoal column. The identity of this product was established by transforming it into **6b** by acetylation in 91% yield. Cyclization of **6a** with NaH failed. Treatment of **6a** with NaOEt in EtOH afforded **7** and a postulated by-product **8**.[7b] The time-course of the reaction followed by high-performance liquid chromatography (HPLC) showed that **7** and **8** were formed rapidly and **8** was cyclized slowly to **7**, which in turn decomposed gradually to **9**. The use of $NaOCHMe_2$ instead of NaOEt had been found to be effective for suppressing the formation of this type of by-product.[10b] However, this method gave a disappointing result in this case owing to the poor solubility of **6a** in Me_2CHOH. Once **9** is contained in the product, it is difficult to remove by recrystallization. Thus, an appropriate combination of concentration of NaOEt, reaction temperature, and reaction time is crucial for large-scale preparation of pure **7**. We found that the reaction in 1 M NaOEt in EtOH at 30 °C for 23 h gave **7** in 63% yield. Although the product was found to be contaminated by a trace of **9** by HPLC, it was pure enough for use as the starting material for

the synthesis of **2**. The reaction at 4 °C for 2 weeks gave pure **7** in 49% yield. A five-step synthesis of **7** in 19% overall yield from **5b** has been reported,[9] but the molar extinction coefficients recorded are about 70% of those of the present sample.

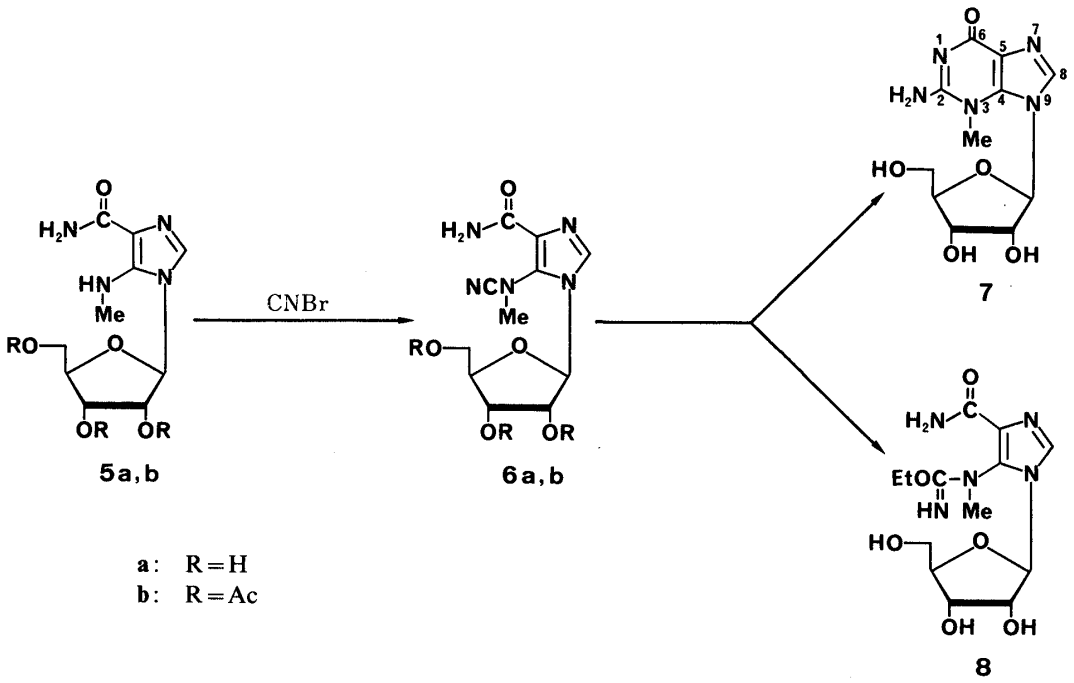


Chart 2

A prominent feature of **7** is the remarkable instability of the glycosidic bond: **7** underwent hydrolysis at a rate [pseudo-first-order rate constant (k_{obs}) 9.8×10^{-1} min^{-1}, half life ($t_{1/2}$) 42 s] 1.7×10^5 times faster than that (k_{obs} 5.7×10^{-6} min^{-1})[11] of guanosine in 0.1 N aq. HCl at 25 °C. Unlike common ribonucleosides, **7** underwent glycosidic bond cleavage easily even under basic conditions as found in the synthetic procedure described above. Ienaga and Pfleiderer[6] reported the synthesis of 3,9-dimethylguanine (**12**) as a model of the synthesis of **7**. However, we found that **7** decomposed completely under conditions (in concentrated aq. NH_3 at 100 °C for 2 h) similar to those employed in the last step in their synthesis of **12**. Thus, the direct application of their method to the synthesis of **7** seems to have little hope of success.

Treatment of **7** with bromoacetone in Me_2NCHO in the presence of K_2CO_3 at 30 °C gave **2**, mp 235 °C (dec.), in 52% yield. The structure of **2** was assignable on the basis of spectral similarity to 3-alkylwyes (type **13**),[7] and it was confirmed by acidic hydrolysis to give **1a** in good yield. A fluorescent by-product was obtained in 15% yield. The mass spectrum (MS) and the nuclear magnetic resonance (NMR) spectrum as well as the elemental analyses suggested that it was a wye having a 2-oxopropyl group, and the structure 1-(2-oxopropyl)wye (**10**) was assigned to this compound on the basis of UV spectral similarity to 1-methyl-[2b] or 1-D-ribofuranosylwye.[2b] Nakatsuka *et al.*[9] reported the synthesis of **2** by cyclocondensation of 2′,3′,5′-tri-*O*-acetyl-3-methylguanosine with bromoacetone followed by deacetylation. They also reported that the glycosidic bonds of **2** and **7** were so weak that the nucleosides were gradually decomposed even in the solid state. Our samples of **2** and **7**, however, have been kept unchanged at room temperature for several years without special precautions. The reported molar extinction coefficients for **2** obtained in H_2O are about a half of those of the present sample. Recently, Golankiewicz and Folkman[15] reported an alternative synthesis of **2**.[12] The spectral characteristics and stability of their sample matched our results.

Compound **2** was found to be extraordinarily unstable in acidic media. The rate (k_{obs} 4.4×10^{-1} min^{-1}, $t_{1/2}$ 95 s) of hydrolysis of the glycosidic bond of **2** at 25 °C in 0.1 N aq. HCl is of the same order of magnitude as that of **7**. It is noteworthy that the glycosidic bond of **2** underwent hydrolysis at a rate (k_{obs} 1.7×10^{-2} min^{-1}, $t_{1/2}$ 41 min) similar to that reported for wybutosine ($t_{1/2}$ *ca.* 70 min)[4] at pH 2.9 and 37 °C. These results definitively refute the suggestion that wyosine and wybutosine are unlikely to be ribonucleosides.[8]

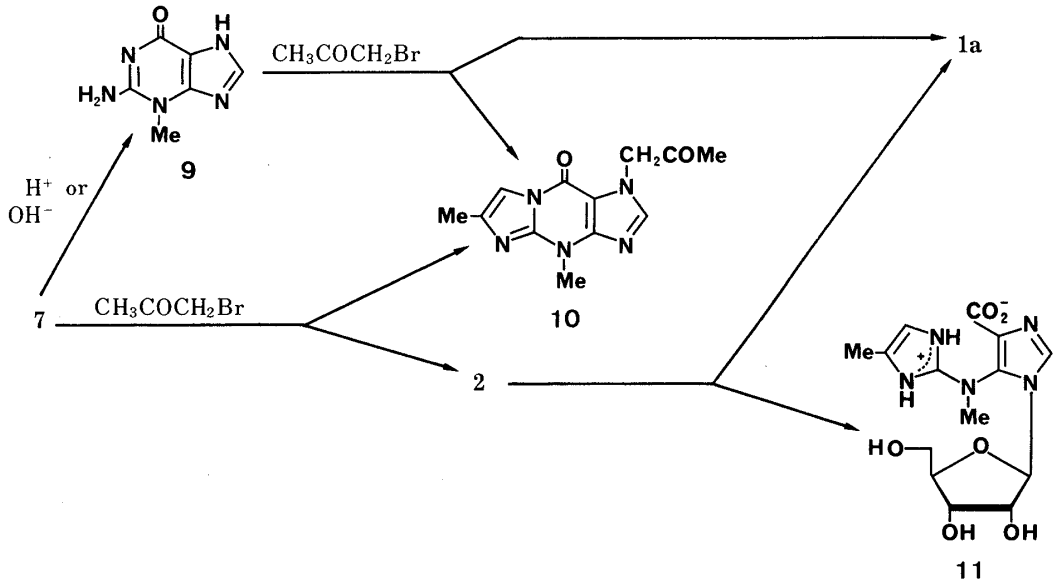


Chart 3

When **2** was dissolved in 0.1 N aq. NaOH at room temperature, cleavage of the glycosidic bond and ring fission at the base moiety took place simultaneously to provide **1a** in 12% yield and 5-[methyl(4-methylimidazol-2-yl)amino]-1-β-D-ribofuranosylimidazole-4-carboxylic acid (**11**) in 52% yield. Compound **11** was obtained as a caramel, and attempts to convert **11** into a crystalline derivative were unsuccessful. The UV spectra of **11** showed that the chromophore had been changed. The NMR spectrum suggested the structure **11**, which was further supported by the molecular ion at *m/e* 353 in the field desorption mass spectrum (FD-MS). 3-Methylwye (**13**)[7] was then treated similarly with the aim of obtaining the crystalline analogue of **11**. Although the product was a caramel again, the exact mass obtained by electron impact mass spectroscopy (EI-MS) was consistent with the expected structure. Close similarity in the UV spectra of **11** and **14** further supported the structure **11**. The reaction of **2** in 0.1 N aq. NaOH at 37 °C was analyzed by use of HPLC. It was found that **2** was competitively transformed into **1a** and **11** (k_{obs} 1.1×10^{-2} min^{-1}, $t_{1/2}$ 63 min) and **1a** was formed in *ca.* 30% yield. Compound **11** further decomposed under these conditions.

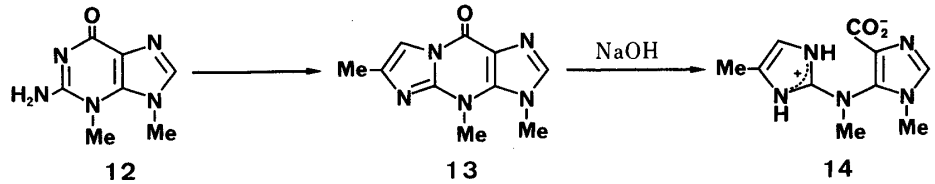


Chart 4

Wyosine[2b,3] and wybutosine[1b,4,5] have been isolated from tRNAs^{Phe} by enzymatic digestion at pH 5—8.5 and 37 °C. We found that the glycosidic bond of **2** underwent hydrolysis only slowly (k_{obs} 9.3×10^{-5} min^{-1}, $t_{1/2}$ 124 h) at pH 5.0 and 37 °C and that **2**

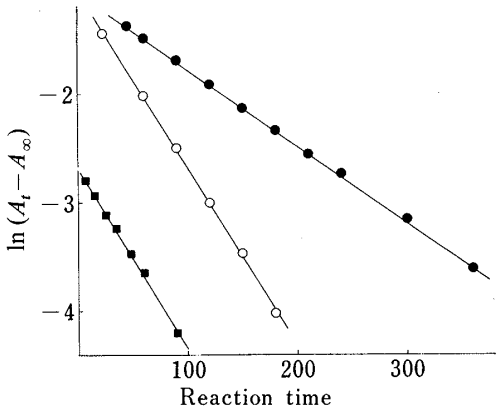


Fig. 1. First-Order Plots for the Glycosidic Bond Cleavages of **7** (○) and **2** (●) in 0.1 N aq. HCl at 25 °C and of **2** at pH 2.90, Ionic Strength 0.1, and 37 °C (■)

Reaction time, s for ○ and ●; min for ■.

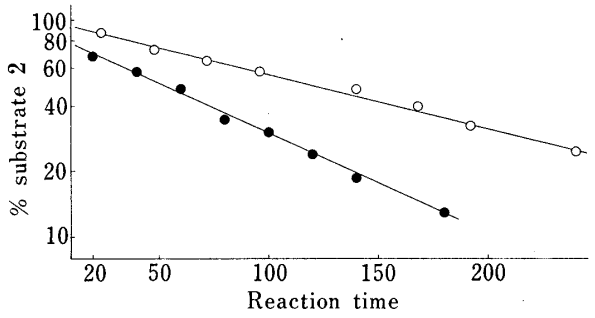


Fig. 2. First-Order Plots for the Glycosidic Bond Cleavage of **2** at pH 5.0, Ionic Strength 0.1, and 37 °C (○) and for the Disappearance of **2** in 0.1 N aq. NaOH at 37 °C (●)

Reaction time, h for ○; min for ●.

remained practically unchanged at pH 7.0—8.5 and 37 °C. Assuming that wyosine is actually **2**, these results show that the acid-catalyzed hydrolysis of the glycosidic bond alone cannot be a serious barrier to the isolation of wyosine.

In conclusion, the present work has established a facile three-step synthesis of **2** from **5a**. The rate studies on the hydrolysis of **2** further support the structural assignment of wyosine as **2**, and should help towards the isolation of wyosine and its congeners in sufficient quantities to permit unambiguous identification of the structure.

Experimental

General Notes——All melting points are corrected. MS and infared (IR) spectra were recorded on Hitachi M-80 and JASCO IRA-2 instruments, respectively. UV spectra were measured with a Hitachi 323 or 320 spectrometer using solutions in 95% aq. EtOH, 0.1 N aq. HCl (pH 1), 0.01 N aq. HCl (pH 2), 0.005 M phosphate buffer (pH 7), and 0.1 N aq. NaOH (pH 13). NMR spectra were recorded on a JEOL JNM-PS-100 or JNM-FX-100 spectrometer at 25 °C using Me_4Si as an internal standard. Optical rotations were measured with a JASCO DIP-181 polarimeter. Spectrophotometric determinations were carried out with a Hitachi 320 or 181 spectrometer. The liquid chromatographic system was a Waters Model 204 ALC which included a 6000A pump, a U6K injector, and a Model 440 absorbance detector operating at 254 nm. pH values were measured on a Hitachi-Horiba F-5 or a Toa HM 18-ET pH meter. Elemental analyses were performed by Mr. Y. Itatani and his associates at Kanazawa University. The following abbreviations are used: br = broad, d = doublet, m = multiplet, q = quartet, s = singlet, sh = shoulder, t = triplet.

5-(Cyanomethylamino)-1-β-D-ribofuranosylimidazole-4-carboxamide (6a)——CNBr (8.10 g, 76.5 mmol) was added to a solution of **5a**[13] (4.09 g, 15 mmol) in 1 M AcOH–AcONa (pH 5.0, 72 ml) and the mixture was gently stirred until the reagent went into solution. The solution was allowed to stand at room temperature in a stoppered vessel for 24 h and was then concentrated *in vacuo*. The residue was dissolved in H_2O (50 ml) and the solution was passed through a column packed with activated charcoal (20 g). The column was eluted with H_2O (1.4 l) and then with MeOH (0.8 l). The methanolic eluate was concentrated *in vacuo* and the resulting solid residue was washed with a little MeOH to give **6a** (1.41 g, 32% yield), mp 183—185 °C (dec.). Recrystallization from MeOH gave an analytical sample as colorless needles of unchanged mp. IR ν_{max}^{Nujol} cm^{-1}: 2240 (C≡N), 1680 (C=O). UV $\lambda_{max}^{95\%\ EtOH}$ 233 nm (sh) (ε 8000); $\lambda_{max}^{H_2O}$ (pH 1) 233 (sh), (7500); $\lambda_{max}^{H_2O}$ (pH 7) 235 (sh) (7600); $\lambda_{max}^{H_2O}$ (pH 13) unstable. ^{1}H-NMR (Me_2SO-d_6) δ: 3.22 (3H, s, NMe), 3.58 (2H, br, $C_{(5')}$-H_2), 3.87—4.15 (2H, m, $C_{(3')}$- and $C_{(4')}$-H), 4.29 (1H, m, $C_{(2')}$-H), 5.51 (1H, d, J=6 Hz, $C_{(1')}$-H), 7.34 and 7.46 (1H each, br, NH_2), 8.03 (1H, s, $C_{(2)}$-H). $[\alpha]_D^{26}$ −42.7±0.1° (c=0.652, H_2O). *Anal.* Calcd for $C_{11}H_{15}N_5O_5$: C, 44.44; H, 5.09; N, 23.56. Found: C, 44.26; H, 5.10; N, 23.73.

5-(Cyanomethylamino)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (6b)——i) A mixture of 5-(methylamino)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**5b**)[13a,b] (3.14 g, 7.9 mmol), CNBr (4.5 g, 42 mmol), and 1 M AcOH–AcONa (pH 5, 100 ml) was gently stirred at room temperature until **5b** went

into solution. The solution was allowed to stand at room temperature for 1 d and further CNBr (2.25 g) was added. The solution was allowed to stand at room temperature for another 1 d and the mixture was brought to pH 6—7 with 10% aq. NaOH. The mixture was concentrated *in vacuo* to half the initial volume and then extracted with $CHCl_3$ (200 ml). The $CHCl_3$ extracts were dried over $MgSO_4$ and concentrated *in vacuo* to leave an oily residue, which crystallized on treatment with EtOH (0.1 ml). The resulting solid was filtered off and washed with EtOH (10 ml) to give **6b** (1.02 g), mp 150—151 °C. The combined filtrate and washings were concentrated *in vacuo* and the residue was purified by column chromatography on silica gel. Elution with $CHCl_3$–MeOH (10 : 1, v/v) gave a second crop of **6b** (0.27 g, total yield 39%), mp 149—151 °C. Recrystallization from EtOH gave an analytical sample as colorless prisms, mp 156—157 °C. IR ν_{max}^{Nujol} cm^{-1}: 2230 (C≡N), 1740 (ester C=O), 1680 (amide C=O). UV $\lambda_{max}^{95\%\ EtOH}$ 233 nm (sh) (ε 7900); $\lambda_{max}^{H_2O}$ (pH 1) 233 (sh) (7900); $\lambda_{max}^{H_2O}$ (pH 7) 235 (sh), (7800); $\lambda_{max}^{H_2O}$ (pH 13) unstable. ^{1}H-NMR ($CDCl_3$) δ: 2.15, 2.17, and 2.19 (3H each, s, three MeCO's), 3.39 (3H, s, NMe), 4.32—4.48 (3H, m, $C_{(5')}$-H_2 and $C_{(4')}$-H), 5.32—5.56 (2H, m, $C_{(3')}$-H and $C_{(2')}$-H), 5.80 (1H, br, NH), 5.86 (1H, d, J=6 Hz, $C_{(1')}$-H), 7.08 (1H, br, NH), 7.74 (1H, s, $C_{(2)}$-H). $[\alpha]_D^{26}$ −18.3±0.4° (c=0.814, H_2O). *Anal.* Calcd for $C_{17}H_{21}N_5O_8$: C, 48.22; H, 5.00; N, 16.54. Found: C, 48.15; H, 4.97; N, 16.78.

ii) A mixture of **6a** (1.55 g, 5.21 mmol), Ac_2O (16 ml), and pyridine (100 ml) was stirred at 18 °C for 2 h. EtOH (100 ml) was slowly added to the resulting solution under cooling with ice-water. The solution was allowed to stand at room temperature overnight and then concentrated *in vacuo* to leave a colorless heavy oil. This was purified by column chromatography on silica gel (100 g). Elution with $CHCl_3$–EtOH (15 : 1, v/v) gave a colorless caramel. Treatment with a little EtOH gave **6b** (2.01 g, 91% yield) as colorless prisms, mp 156—157 °C, identical with the analytical sample described above.

3-Methylguanosine (7)——i) A mixture of **6b** (1.78 g, 4.2 mmol), 50% NaH (202 mg, 4.2 mmol), and Me_2NCHO (65 ml) was stirred at 18 °C for 1 h. After addition of AcOH (252 mg, 4.2 mmol), the mixture was concentrated *in vacuo*. The residue was dissolved in cold saturated NH_3–MeOH (80 ml) and the solution was kept at 0 °C for 19 h. The mixture was concentrated *in vacuo* and the residue was washed with EtOH (60 ml). Rapid recrystallization of the insoluble solid from H_2O (2 ml) gave **7** (294 mg, 21% yield) as the dihydrate. Further recrystallization from H_2O gave colorless needles. These were dried over P_2O_5 at 2 mmHg and room temperature and then exposed to air until a constant weight was reached to give an analytical sample, mp *ca.* 180 °C (dec.). UV $\lambda_{max}^{95\%\ EtOH}$ 252 nm (sh) (ε 11200), 260 (11800); $\lambda_{max}^{H_2O}$ (pH 2) unstable; $\lambda_{max}^{H_2O}$ (pH 7) 217 (27500), 250 (10100), 265 (11700); $\lambda_{max}^{H_2O}$ (pH 13) 250 (10100), 265 (11700). ^{1}H-NMR (Me_2SO-d_6) δ: 3.38 (br, H_2O), 3.62 (2H, br, $C_{(5')}$-H_2), 3.70 (3H, s, NMe), 3.98 (1H, m, $C_{(4')}$-H), 4.10 (1H, m, $C_{(3')}$-H), 4.43 (1H, m, $C_{(2')}$-H), 4.8—5.8 (3H, br, three OH's), 5.93 (1H, d, J=5.5 Hz, $C_{(1')}$-H), 6.96 (2H, br, NH_2), 8.03 (1H, s, $C_{(8)}$-H). $[\alpha]_D^{26}$ −47.7±0.3° (c=0.340, H_2O). *Anal.* Calcd for $C_{11}H_{15}N_5O_5 \cdot 2H_2O$: C, 39.64; H, 5.75; N, 21.01. Found: C, 39.76; H, 5.45; N, 20.86.

ii) To a solution of Na (240 mg, 10.4 matom) in anhydrous EtOH (10 ml) was added **6a** (297 mg, 1.0 mmol) and the mixture was stirred at 30 °C for 23 h. The mixture was concentrated *in vacuo* and the residue was neutralized with 5 M aq. AcOH. The resulting precipitate was filtered off and recrystallized from H_2O (2 ml) to provide **7**·$2H_2O$ (210 mg, 63% yield) as colorless needles. This product was found to be contaminated by a trace of **9** by HPLC, but the IR spectrum was identical with that of the analytical sample described above.

iii) Compound **6a** (1.12 g, 3.77 mmol) was added to a solution of Na (907 mg, 39 matom) in anhydrous EtOH (40 ml) and the mixture was stirred at 4 °C for 336 h. The resulting mixture was concentrated *in vacuo* and the residue was dissolved in cold H_2O (6 ml). The solution was neutralized with 5 M aq. AcOH and cooled in a refrigerator. The resulting precipitate was filtered off, washed successively with cold H_2O (20 ml) and MeOH (10 ml), and dried to give **7**·$2H_2O$ (618 mg, 49% yield), identical with the analytical sample described above.

3,4-Dihydro-4,6-dimethyl-3-β-D-ribofuranosyl-9*H*-imidazo[1,2-*a*]purin-9-one (3-β-D-Ribofuranosylwye) (2)——A mixture of **7**·$2H_2O$ (167 mg, 0.5 mmol), K_2CO_3 (69 mg, 0.5 mmol), and Me_2NCHO (10 ml) was briefly ultrasonicated. Bromoacetone[16] (0.10 ml, 1.2 mmol) was added to the mixture and the mixture was stirred at 30 °C for 7 h. It was then concentrated *in vacuo* below 30 °C. The residue was triturated with MeOH–H_2O (1 : 4, v/v) (5 ml) and the mixture was kept in a refrigerator overnight. The resulting precipitate was filtered off, washed successively with the aq. MeOH (2 ml) and MeOH (1 ml), and dried to give **2** (72 mg), mp *ca.* 215 °C (dec.). The combined filtrate and washings were concentrated *in vacuo* and the residue was purified by preparative HPLC on a Bondapak C_{18}/Porasil B column (7 × 600 mm). Elution with MeOH–H_2O (1 : 4, v/v) gave a second crop of **2** (15 mg, total yield 52%), mp *ca.* 210 °C (dec.). Further elution of the column gave 1-(2-oxopropyl)wye (**10**) (19 mg, 15% yield) as a colorless fluorescent solid, mp 190—215 °C (dec.).

Recrystallization of the crude **2** from H_2O gave an analytical sample as colorless needles, mp 235 °C (dec., rapid heating). FD-MS *m/e*: 374 (M^++K), 358 (M^++Na), 335 (M^+), 203 [$C_9H_9N_5O^+$ (wye)], 133 [$C_5H_9O_4^+$ (ribosyl)]. UV $\lambda_{max}^{95\%\ EtOH}$ 235 nm (ε 33400), 293 (8100); $\lambda_{max}^{H_2O}$ (pH 1) unstable; $\lambda_{max}^{H_2O}$ (pH 2) 230 (35600), 277 (11800); $\lambda_{max}^{H_2O}$ (pH 7) 236 (34700), 296 (8000); $\lambda_{max}^{H_2O}$ (pH 13) 236 (35800), 295 (8200). ^{1}H-NMR (Me_2SO-d_6) δ: 2.23 (3H, d, J=1 Hz, $C_{(6)}$-Me), 3.64 (2H, br, $C_{(5')}$-H_2), 3.95—4.21 (2H, m, $C_{(3')}$-H and $C_{(4')}$-H), 4.09 (3H, s, NMe), 4.46 (1H, m, $C_{(2')}$-H), 5.12 (1H, t, J=5 Hz, $C_{(5')}$-OH), 5.31 (1H, d, J=5 Hz, $C_{(3')}$-OH), 5.70 (1H, d, J=6 Hz, $C_{(2')}$-OH), 6.13 (1H, d, J=5 Hz, $C_{(1')}$-H), 7.37 (1H, q, J=1 Hz, $C_{(7)}$-H), 8.25 (1H, s, $C_{(2)}$-H). $[\alpha]_D^{27}$ −47±3° (c=0.082, H_2O). *Anal.* Calcd for $C_{14}H_{17}N_5O_5$: C, 50.14; H, 5.11; N, 20.89. Found: C, 50.04; H, 5.13; N, 20.96.

Recrystallization of the crude **10** from H_2O gave an analytical sample as colorless needles, mp 231—232 °C. EI-MS *m/e*: 259 (M^+), 216 ($M^+ - CH_3CO$), 202 ($M^+ - CH_3COCH_2$). IR ν_{max}^{Nujol} cm^{-1}: 1735 (ketone C=O). UV $\lambda_{max}^{95\%\ EtOH}$ 231 nm (ε 32500), 237 (32400), 260 (6300), 311 (7200); $\lambda_{max}^{H_2O}$ (pH 1) 229 (37800), 234 (39300), 254 (4900), 289 (9300); $\lambda_{max}^{H_2O}$ (pH 7 and 13) 232 (33300), 264 (6300), 313 (7100). ^{1}H-NMR ($CDCl_3$) δ: 2.35 (3H, d, *J*=1 Hz, $C_{(6)}$-Me), 2.37 (3H, s, MeCO), 3.97 (3H, s, NMe), 5.26 (2H, s, CH_2), 7.32 (1H, q, *J*=1 Hz, $C_{(7)}$-H), 7.66 (1H, s, $C_{(2)}$-H). *Anal.* Calcd for $C_{12}H_{13}N_5O_2$: C, 55.59; H, 5.05; N, 27.02. Found: C, 55.35; H, 5.08; N, 27.23.

5-[Methyl(4-methylimidazol-2-yl)amino]-1-β-D-ribofuranosylimidazole-4-carboxylic Acid (11)——A solution of **2** (67 mg, 0.2 mmol) in 0.1 N aq. NaOH (20 ml) was allowed to stand at room temperature for 19 h. It was brought to pH 7 with 10% aq. HCl, and concentrated *in vacuo* to *ca.* 1 ml. The resulting precipitate was collected by filtration and dried to give **1a** (5 mg, 12% yield) as colorless prisms, mp >300 °C, identical with an analytical sample described below. The filtrate was purified by reversed-phase chromatography on a Lobar column (Merck Art. 11804). Elution with H_2O–MeOH (95:5, v/v) gave **11** (37 mg, 52% yield) as a caramel. FD-MS *m/e*: 353 (M^+), 309 ($M^+ - CO_2$). UV $\lambda_{max}^{H_2O}$ (pH 1) 218 nm (ε *ca.* 14000), 266 (sh) (*ca.* 1700); $\lambda_{max}^{H_2O}$ (pH 7) 217 (*ca.* 14000); $\lambda_{max}^{H_2O}$ (pH 13) 269 (sh) (*ca.* 1600). ^{1}H-NMR (Me_2SO-d_6) δ: 1.97 (3H, br, CMe), 3.19 (3H, s, NMe), 3.58 (2H, m, $C_{(5')}$-H_2), 3.82 (1H, m, $C_{(4')}$-H), 4.06 (1H, m, $C_{(3')}$-H), 4.22 (1H, m, $C_{(2')}$-H), 4.3—5.0 (5H, m, three OH's and two NH's), 5.35 (1H, d, *J*=5 Hz, $C_{(1')}$-H), 6.23 (1H, br, $C_{(5'')}$-H), 7.87 (1H, s, $C_{(2)}$-H).

1-Methyl-5-[methyl(4-methylimidazol-2-yl)amino]imidazole-4-carboxylic Acid (14)——A solution of **13**[7] (217 mg, 1 mmol) in 0.1 N aq. NaOH (200 ml) was allowed to stand at room temperature for 22 h. It was neutralized with 10% aq. HCl and then concentrated to a small volume. The resulting precipitate was collected by filtration to recover the starting material (20 mg, 9% yield). The filtrate was applied to a Lobar column (Merck Art. 11804) and the column was eluted with H_2O–MeOH (90:10, v/v) to give **14** (110 mg, 47% yield) as a caramel. EI-MS *m/e*: 235.1073 (M^+) (Calcd for $C_{10}H_{13}N_5O_2$: 235.1069), 191.1162 ($M^+ - CO_2$) (Calcd for $C_9H_{13}N_5$: 191.1171). UV $\lambda_{max}^{H_2O}$ (pH 1) 216 nm (ε *ca.* 12000), 263 (sh) (*ca.* 1600); $\lambda_{max}^{H_2O}$ (pH 7) 218 (*ca.* 12000); $\lambda_{max}^{H_2O}$ (pH 13) 266 (sh) (*ca.* 1900). ^{1}H-NMR (Me_2SO-d_6) δ: 1.98 (3H, d, *J*=1 Hz, CMe), 3.21 (3H, s, N^5Me), 3.37 (3H, s, $N_{(1)}$-Me), 4.2—5.5 (2H, br, two NH's), 6.25 (1H, br, $C_{(5')}$-H), 7.61 (1H, s, $C_{(2)}$-H).

1,4-Dihydro-4,6-dimethyl-9*H*-imidazo[1,2-*a*]purin-9-one[17] (Wye) (1a)——i) A solution of **2** (80 mg, 0.24 mmol) in 0.1 N aq. HCl (10 ml) was allowed to stand at room temperature overnight. The solution was neutralized with saturated aq. $NaHCO_3$ and then cooled. The resulting precipitate was filtered off, washed with a little H_2O, and dried to give **1a** (42 mg), mp >300 °C, identical with a specimen prepared according to the reported procedure[2b] (see below). The combined filtrate and washings were extracted with $CHCl_3$ using a continuous extractor. The $CHCl_3$ extracts were dried over $MgSO_4$ and concentrated *in vacuo* to give a second crop of **1a** (6 mg, total yield 100%). Recrystallization from H_2O gave an analytical sample as colorless prisms, mp >300 °C. UV $\lambda_{max}^{95\%\ EtOH}$ 230 nm (ε 35400), 258 (6300), 305 (7500); $\lambda_{max}^{H_2O}$ (pH 1) 227 (40400), 231 (40400), 257 (sh) (5200), 284 (10200); $\lambda_{max}^{H_2O}$ (pH 7) 230 (36100), 266 (6400), 307 (7100); $\lambda_{max}^{H_2O}$ (pH 13) 230 (38000), 275 (7800), 301 (9400). ^{1}H-NMR (Me_2SO-d_6) δ: 2.24 (3H, d, *J*=1 Hz, CMe), 3.81 (3H, s, NMe), 7.38 (1H, q, *J*=1 Hz, $C_{(7)}$-H), 8.20 (1H, s, $C_{(2)}$-H), 13.37 (1H, br, NH). *Anal.* Calcd for $C_9H_9N_5O$: C, 53.19; H, 4.46; N, 34.47. Found: C, 53.36; H, 4.39; N, 34.55.

ii) The reported procedure[2b] was slightly modified. A mixture of 3-methylguanine (**9**)[14] (826 mg, 5 mmol), bromoacetone[16] (753 mg, 5.5 mmol), *N*,*N*-diethylaniline (4.5 ml), and 90% v/v aq. Me_2NCHO (45 ml) was stirred at 90 °C for 4 h. The mixture was concentrated *in vacuo* and the residue was dissolved in 10% aq. Na_2CO_3 (40 ml). The solution was extracted twice with $CHCl_3$ (50 and 30 ml). The $CHCl_3$ extracts were concentrated *in vacuo* and *N*,*N*-diethylaniline was removed by coevaporation with H_2O. The resulting residue was washed with a little C_6H_6 to give **10** (92 mg, 7% yield), identical with the analytical sample described above.

The aqueous layer was brought to pH 5 with concentrated aq. HCl and extracted with $CHCl_3$ using a continuous extractor. The $CHCl_3$ extracts were concentrated *in vacuo* to give **1a** (419 mg, 41% yield), mp >300 °C, identical with the analytical sample described above.

Glycosidic Bond Cleavage of 7——i) A solution of **7**·$2H_2O$ (30 mg, 0.09 mmol) in 0.1 N aq. HCl (3 ml) was kept at 25 °C for 10 min, neutralized with 1 N aq. NaOH, then concentrated *in vacuo*. The resulting residue was recrystallized from H_2O and dried over P_2O_5 at 2 mmHg and 110 °C for 3.5 h to give **9** (13 mg, 87% yield), mp >300 °C, identical with an authentic sample.[14d]

ii) A solution of **7**·$2H_2O$ (3.3 mg) in concentrated aq. NH_3 (0.2 ml) was heated at 100 °C for 2 h in a sealed tube. Compound **9** was formed as the sole UV-absorbing product (checked by TLC) and the yield was estimated to be 92% on the basis of the UV absorbance.

iii) Cleavage Rate in 0.1 N aq. HCl at 25 °C: A solution of **7** in approximately 0.1 N aq. HCl was prepared from a solution (4.5 ml) of **7**·$2H_2O$ (0.103 mg) and 1 N aq. HCl (0.5 ml) in the same way as reported for the hydrolysis of 3-methylinosine.[18] An appropriate amount of the mixture was transferred to a cuvette, which was placed in a cell compartment maintained at 25 °C, with as little delay as possible. Absorbance (A_t) of the mixture at selected times were determined at 246 nm. The absorbance (A_∞) on completion of the reaction agreed with that of an equimolar solution of pure **9**.[14d] A plot of $\ln(A_t - A_\infty)$ against time gave a straight line and k_{obs} 9.8×10^{-1} min^{-1} ($t_{1/2}$ 42 s) was estimated by linear regression analysis (Fig. 1).

Rate Studies on the Hydrolysis of 2——In 0.1 N aq. HCl at 25 °C: The rate of cleavage of the glycosidic bond was

measured in a manner similar to that described for **7** except that absorbances were determined at 265 nm. A good pseudo-first-order plot (k_{obs} 4.4×10^{-1} min^{-1}, $t_{1/2}$ 95 s) was obtained (Fig. 1).

At pH 2.90 and 37 °C: Compound **2** (1.504 mg) was dissolved in 0.01 M phosphate buffer (pH 2.90 at 37 °C; the ionic strength was adjusted to 0.1 with KCl) and the volume was brought to 50 ml. The solution was kept at 37 ± 0.05 °C. At intervals, aliquots were removed from the mixture and the optical densities were determined at 285 nm. The infinity reading (A_∞) agreed with the absorbance of a solution of an equimolar concentration of **1a**. A good pseudo-first-order plot (k_{obs} 1.7×10^{-2} min^{-1}, $t_{1/2}$ 41 min) was obtained (Fig. 1).

At pH 5.00 and 37 °C: A solution (50 ml) of **2** (2.106 mg) in 0.1 M acetate buffer (pH 5.00 at 37 °C and adjusted to ionic strength 0.1 with KCl) was kept at 37 ± 0.05 °C. The reaction was followed by HPLC [μBondapak C_{18}; H_2O–MeOH (7 : 3, v/v)]. Concentrations of **2** and **1a** in the reaction mixture were estimated by using calibration lines which had been constructed by plotting the product of half-width and peak height against the known concentration of pure **2** or **1a**. Compound **2** was found to be quantitatively converted into **1a** under these conditions and k_{obs} 9.3×10^{-5} min^{-1} ($t_{1/2}$ 124 h) was obtained (Fig. 2).

In 0.1 N aq. NaOH at 37 °C: Aliquots (2.0 ml) of a solution (20 ml) of **2** (2.126 mg) in 0.1 N NaOH were kept at 37 ± 0.05 °C in sealed tubes. At intervals, the solutions were mixed with 0.2 M aq. NaH_2PO_4 (2.0 ml) and the concentrations of **2** and **1a** were determined by HPLC in the same way as described above. For the decrease of **2**, k_{obs} 1.1×10^{-2} min^{-1} ($t_{1/2}$ 63 min) was obtained. The formation of **1a** was estimated to be about 30%.

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