Synthesis of 7-Methyl-3-β-D-ribofuranosylwye, the Putative Structure for the Hypermodified Nucleoside Isolated from Archaebacterial Transfer Ribonucleic Acids[1)]

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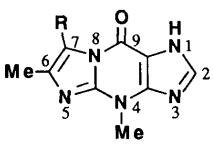
The Vilsmeier–Haack reaction of 3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)wye (7c) followed successively by reduction with sodium borohydride and catalytic hydrogenolysis afforded the 7-methyl derivative 12c, which provided the title compound 12a on deprotection. Compound 12c was more effectively produced by direct hydrogenolysis of the 7-formyl derivative 8c, especially by use of Pearlman's catalyst. Similar treatment of 1-benzyl-7-formylwye (14) led to a better synthesis of 7-methylwye (1b), the fluorescent base isolated from Archaebacterial transfer ribonucleic acids. Although hydrogenolysis of the 6-formyl compound 11 took place smoothly even over ordinary palladium on charcoal to afford 12c, this route had a bottleneck in the step of transformation of 8c into 11.

Compound 12a proved to be highly sensitive to acidic hydrolysis at the glycosyl bond and the rate determined in 0.1 N hydrochloric acid at 25 °C was virtually the same as that of 3-β-D-ribofuranosylwye (7a).

Keywords hypermodified nucleoside synthesis; fluorescent nucleoside; tricyclic nucleoside; archaebacterial tRNA; Vilsmeier–Haack formylation; organozinc reagent; hydrogenolysis; nucleoside hydrolysis; hydrolysis rate

All the known phenylalanine transfer ribonucleic acids ($tRNAs^{Phe}$) have modified components at the position next to the 3′-end of the anticodon (the 37-position). The nucleoside hitherto identified at that position of most eubacterial $tRNAs^{Phe}$ is *N*-isopentenyl-2-(methylthio)adenosine (**3**); the only known exception is 1-methylguanosine (**2**) of *Mycoplasma capricolum*. Two types of modification have been discovered at the 37-position of eukaryotic $tRNAs^{Phe}$. One is the so-called Y base family **1a, c—f** and the other is 1-methylguanosine (**2**).[2)] The latter has been demonstrated to be the first obligatory intermediate of the Y-type component in $tRNA^{Phe}$ of *Xenopus laevis* oocytes.[3)] The highly fluorescent Y family members **1a, c—f** are specific to the 37-position of eukaryotic $tRNAs^{Phe}$: none of them has ever been isolated from other natural sources. It was therefore a notable event when McCloskey *et al.* discovered a new member of **1** in unfractionated tRNAs of archaebacteria, which are phylogenetically distinguished from the other two primary kingdoms. They reported the isolation of a new fluorescent nucleoside from three extremely thermophilic archaebacterial tRNAs and elucidated the structure of its base as 7-methylwye (**1b**),[4)] by direct comparison with an authentic specimen synthesized by our group.[5)] We present herein a detailed account of the synthesis of 7-methyl-3-β-D-ribofuranosylwye (**12a**), the most probable structure for the parent nucleoside of **1b**. A preliminary report of this work has been published.[6)]



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a: R = H

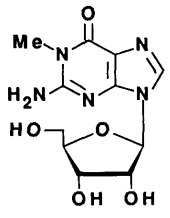
b: R = Me

c: R = $CH_2CH_2-C(CO_2Me)(H)NHCO_2Me$

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

e: R = $CH_2CH(OH)CH(NHCO_2Me)CO_2Me$

f: R = $CH_2CH(O_2H)CH(NHCO_2Me)CO_2Me$



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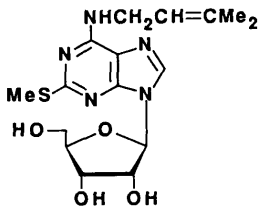

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We have already reported the synthesis of 3-β-D-ribofuranosylwye (**7a**) by cyclocondensation of 3-methylguanosine (**6a**) with bromoacetone.[7)] Consequently, it seemed that similar treatment of **6a** with 3-bromo-2-butanone instead of bromoacetone might provide the most straightforward means of access to **12a**. This method, however, did not work effectively and this was not so surprising in view of a failure in an attempt to obtain **1b** by an analogous reaction with 3-methylguanine.[5b)] Our synthesis of 7-methylwye (**1b**) had been achieved by either cyclocondensation of 7-benzyl-3-methylguanine with 3-bromo-2-butanone followed by catalytic hydrogenolysis or more effectively by Vilsmeier–Haack reaction of 1-benzyl-1,4-dihydro-4,6-dimethyl-9*H*-imidazo[1,2-*a*]purin-9-one followed successively by sodium borohydride reduction and catalytic hydrogenolysis.[5a,b)] For the latter synthesis at the nucleoside level, we expected 3-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)wye (**7b**) to be a good substrate in view of the predictable instability of the target compound **12a** under acidic as well as basic conditions by analogy with that of **7a**[7)]: the protecting groups at the sugar moiety should be removed simultaneously by the catalytic hydrogenolysis in the last step under neutral conditions. Compound **7b** should be provided from 5-(methylamino)-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**4b**)[8)] according to our reported procedure for the synthesis of **7a** from **4a** as depicted in Chart 1.[7)] The first step required for the desired transformation was *N*-cyanation of **4b** with cyanogen bromide. We had found that this type of reaction took place only in an aqueous medium and was markedly retarded by addition of an organic solvent such as methanol.[9)] Because **4b** is hardly soluble in water, the problem was what solvent to use, and how much of it should be added. The additive

solvents tested were methanol, *N*,*N*-dimethylformamide (DMF), *N*,*N*-dimethylacetamide (DMAc), dimethyl sulfoxide, dioxane, and tetrahydrofuran (THF). The best result was obtained when an equal mixture of acetate buffer (pH 5) and dioxane was used: **5b** was obtained in 46% yield. Cyclization of **5b** by treatment with sodium hydride[9] or sodium isopropoxide[10] afforded the protected guanosine **6b** in 62—73% yield. This compound was led to the fluorescent tricycle **7b** by treatment with bromoacetone in the presence of potassium carbonate in 59% yield. When the Vilsmeier–Haack reaction of **7b** was conducted at room temperature, the product isolated was 4,9-dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine-7-carboxaldehyde, which was presumably formed through cleavage of the desired **8b** at the glycosyl bond. The nucleoside **8b** was successfully obtained in the reaction at −30 °C for 5 h in 63% yield. Treatment of **8b** with sodium borohydride afforded the alcohol **9b** in 94% yield. Nevertheless, catalytic hydrogenolysis of **9b** over palladium on charcoal took place only slowly, giving a complex mixture of products in which **12a** could not be identified.

To circumvent this obstacle, we turned to 3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)wye (**7c**),[11] readily available from 3-β-D-ribofuranosylwye (**7a**)[7] by treatment with acetic anhydride in pyridine. The same compound **7c** became more easily accessible according to the procedure recently reported by Chattopadhyaya's group.[12] The Vilsmeier–Haack reaction of **7c** at −25 °C gave **8c** in 92% overall yield based on **7a**.[13] Reduction of **8c** with sodium borohydride in anhydrous THF at room temperature[14] afforded the alcohol **9c**, although the yield was mediocre owing to partial migration of an acetyl group to the 7-hydroxy group. Catalytic hydrogenolysis of **9c** was carried out over 10% palladium on charcoal in ethanol at 60 °C. Under these conditions, **9c** was transformed into 7-ethoxy-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)wye (**10**) and then **12c** was slowly formed. Such an easy nucleophilic displacement of the hydroxy group by ethanol can be rationalized in terms of the stabilized carbocation generated from the alcohol **12c** owing to the electron-donating nature of the heterocycle.[5b] Analogous high reactivities had been observed with 1-benzyl-7-(hydroxymethyl)wye under acidic conditions[5b] and with 1-benzyl-7-bromowye.[5c] Because the overall yield (9%) of **12c** from **8c** through **9c** was intolerably low, we next attempted to convert **8c** into **12c** without isolation of **9c**: catalytic hydrogenation of **8c** under similar conditions gave a similar product pattern to that observed in the hydrogenolysis of **9c** and we obtained **12c** in 32% yield. We considered that the difficulty of the hydrogenolysis of **9c** or **10** reflected the high reactivity at the carbon center where hydrogenolysis should take place.[15] If this is the case, hydrogenolysis of **11**, a positional isomer of **8c**, would give a better result.

The requisite **11** was incidentally obtained in the course of the attempted synthesis of 3-β-D-ribofuranosylwybutine (**13**),[16] the most probable structure for wybutosine isolated

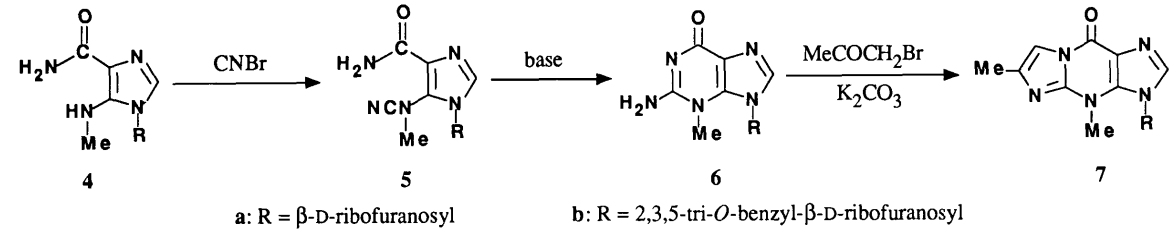


Chart 1

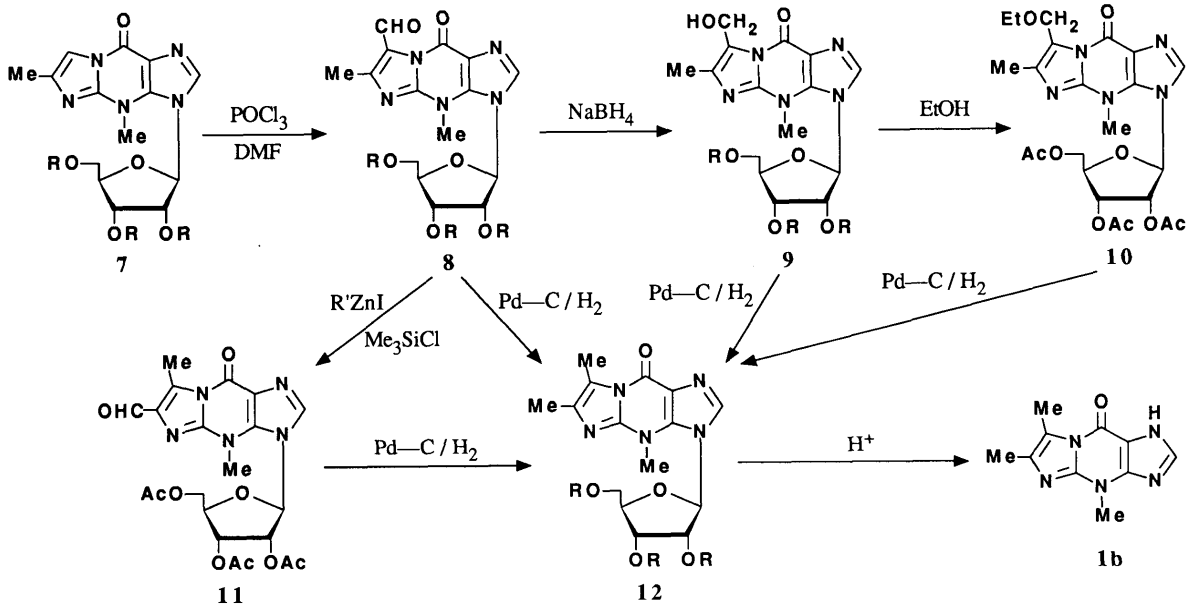
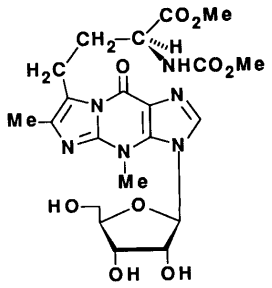


Chart 2

from yeast $tRNA^{Phe}$.[17] We have already reported the synthesis of wybutine (**1c**), the base excised from wybutosine, by means of the Wittig reaction between 1-benzyl-7-formylwye (**14**) and (*R*)-[2-carboxy-2-[(methoxycarbonyl)amino]ethyl]triphenylphosphonium chloride as a key step.[5a] The same strategy would provide access to wybutosine from **8b**, **c**. The Wittig reaction of **8b** or **8c**, however, failed to afford the desired olefin. For comparison, we performed a similar reaction with 3-benzyl-7-formylwye (**15**) as a model experiment, once again obtaining a discouraging result. Of other methods of C–C bond formation we tried to apply for the synthesis of **13**, a notable one was the "remote Reformatsky reaction" proposed by Tamaru *et al.*[18]: the reaction of **8c** with the organozinc reagent[19] prepared from (*R*)-3-iodo-*N*-(methoxycarbonyl)alanine methyl ester,[5a] in the presence of chlorotrimethylsilane did not afford the desired product but gave the rearranged aldehyde **11** in 31% yield. Although the mechanism of this reaction remains to be solved, analogous transformations in this ring system under basic conditions have been reported.[5a,c] We failed to improve the yield of **11** through several variations of this procedure. Hydrogenolysis of **11** over 10% palladium on charcoal indeed took place smoothly to afford **12c**. Accordingly, if the yield of the transformation of **8c** into **11** could have been improved, the procedure through **11** would have led to a better synthesis of **12c**.

Undaunted, we again focused on the direct hydrogenolysis of **8c** and found that the replacement of the catalyst alone led to a satisfactory result: when **8c** was hydrogenated over Pearlman's catalyst,[20] **12c** was produced in 62% yield.[21] Similar treatment of **14** afforded **1b**, which we had previously obtained in 50% yield from **14** through reduction with sodium borohydride followed by hydrogenolysis over ordinary palladium on charcoal,[5a,b] in 81% yield. Chattopadhyaya's group recently reported the synthesis of **12c** from 3,5-dihydro-6,7-dimethyl-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-imidazo[1,2-*a*]purin-9-one (**16**) in 65% yield using their own method for selective *N*-methylation at the 4-position.[12] Although they obtained **16** by cyclocondensation of guanosine with 2-bromobutanone followed by acetylation, the yield of the cyclocondensation might be poor by analogy with that observed in the reaction with 3-methylguanosine (**6a**) (*vide supra*). They gave no information in this respect.



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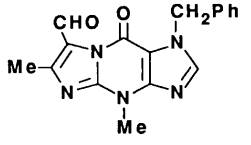

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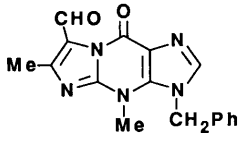

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Deprotection of **12c** was performed by treatment with saturated methanolic ammonia at 0 °C to afford the target compound **12a** as a hemihydrate in 93% yield. The structure of **12a** thus obtained was supported by the self-consistent synthetic routes, the correct elemental analyses, the reasonable ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum, and its mild acidic hydrolysis to **1b**[5b] in 86% yield. The *N*-glycosidic bond of **12a** proved to be extremely susceptible to acidic hydrolysis: the pseudo-first-order rate constant (4.7×10^{-1} min^{-1}) determined in 0.1 N hydrochloric acid at 25 °C was practically equal to that for **7a**.[7,11b,22] Glemarec *et al.* also reported the identical rate constant for the hydrolysis of **12a** under the same conditions.[22] Although the ultimate identification of the nucleoside from natural sources[4] was difficult because of the extremely minute amount available, it was identical with the present sample of synthetic **12a** as judged by fast atom bombardment mass spectrometry and high-performance liquid chromatography.[6] These results further support the proposal that the structure of the new fluorescent nucleoside from the archaebacterial tRNAs is **12a**.[4] In addition, the present synthesis offers the potential for synthesizing some analogs of **12a** oxidized at the 6- or 7-methyl group; such compounds may occur in unidentified tRNAs.

Experimental

General Notes All melting points were determined by using a Yamato MP-1 capillary melting point apparatus and are corrected. Spectra reported herein were recorded on a Hitachi 320 UV spectrophotometer using solutions in 95% aqueous EtOH, 0.01 N hydrochloric acid (pH 2), 0.005 M phosphate buffer (pH 7), and 0.1 N aqueous sodium hydroxide (pH 13), a Hitachi M-80 mass spectrometer, a JASCO J-500C spectropolarimeter equipped with a JASCO DP-500N data processor, or a JEOL JNM-FX-100 NMR spectrometer at 25 °C with tetramethylsilane as an internal standard. Optical rotations were measured with a JASCO DIP-181 polarimeter using a 1-dm sample tube. Elemental analyses were performed by Mr. Y. Itatani and his associates at Kanazawa University. Flash chromatography was performed on silica gel according to the reported procedure.[23] The following abbreviations are used: br = broad, d = doublet, dd = doublet-of-doublets, dt = doublet-of-triplets, m = multiplet, q = quartet, s = singlet, sh = shoulder, t = triplet.

5-(Cyanomethylamino)-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)-1*H*-imidazole-4-carboxamide (5b) Cyanogen bromide (18.5 g, 175 mmol) was added to a solution of **4b**[8] (9.15 g, 16.9 mmol) in a mixture of dioxane (230 ml) and 1 M acetate buffer (pH 5) (230 ml), and the whole was gently stirred at 26—28 °C, plugged with a cork stopper. Further cyanogen bromide (18.5 and 9.25 g) was added 3 and 5 d after the start of the reaction, respectively. Stirring was continued for a total of 8 d. The resulting solution was concentrated *in vacuo* to one-third of the inital volume, neutralized with saturated aqueous sodium bicarbonate, and extracted with dichloromethane (4 × 110 ml). The combined organic phases were dried over magnesium sulfate and concentrated *in vacuo* to leave a brown oil (10.15 g). This was purified in three portions by flash chromatography [column diameter, 60 mm; ethyl acetate–hexane (5 : 1, v/v)] to afford **5b** (4.41 g, 46%) as a colorless solid, mp 108—120 °C (dec.). Recrystallization from MeOH gave an analytical sample as colorless needles, mp 120—123 °C (dec.); $[\alpha]_D^{27}$ −39.8° (c = 1.05, MeOH); UV λ_{max} (95% EtOH) 233 nm (sh) (ε 7900); ^{1}H-NMR ($CDCl_3$) δ: 3.19 (3H, s, NMe), 3.52 and 3.70 [1H each, dd, J = 11, 2.5 Hz, C(5′)-H_2], 4.04—4.42 [3H, m, C(4′)-, C(3′)-, and C(2′)-H], 4.45—4.75 (6H, m, three PhCH_2's), 5.47 (1H, br, NH), 5.81 [1H, d, J = 5.5 Hz, C(1′)-H], 6.88 (1H, br, NH), 7.09—7.40 (15H, m, three Ph's), 7.55 [1H, s, C(2)-H]. *Anal.* Calcd for $C_{32}H_{33}N_5O_5$: C, 67.71; H, 5.86; N, 12.34. Found: C, 67.63; H, 5.87; N, 12.07.

2′,3′,5′-Tri-*O*-benzyl-3-methylguanosine (6b) i) Cyclization with Sodium Hydride: Sodium hydride (60%) (140 mg, 3.5 mmol) was added to a solution of **5b** (1.98 g, 3.49 mmol) in anhydrous DMF (10 ml) and the

mixture was stirred at room temperature for 2.5 h. It was concentrated *in vacuo* and the residue was washed with hexane (5 ml), neutralized with 1 M acetate buffer (pH 5), and extracted with chloroform (3 × 10 ml). The organic phases were combined, dried over magnesium sulfate, and concentrated *in vacuo* to leave a brown oil (2.91 g). Flash chromatography [column diameter, 50 mm; benzene–MeOH (4 : 1, v/v)] afforded **6b**·H_2O (1.27 g, 62%), mp 115—145 °C. This was recrystallized from EtOH, dried over phosphorus pentoxide at 2 mmHg and 50 °C for 11 h and then exposed to air until constant weight was reached to give an analytical sample as colorless minute crystals, mp 148 °C (softened at *ca.* 100 °C); $[\alpha]_D^{23}$ −35.6° (c = 0.495, MeOH); UV λ_{max} (95% EtOH) 254 nm (sh) (ε 12000), 258 (12500); MS *m/z*: 567 (M^+); ^{1}H-NMR [$(CD_3)_2SO$] δ: 3.63 [3H, s overlapped with a two-proton broad signal due to C(5′)-H_2, NMe], 4.34 [2H, m, C(3′)- and C(4′)-H], 4.48 (2H, s, $PhC\underline{H}_2$), 4.66 (4H, s, two $PhC\underline{H}_2$'s), 4.72 [1H, m, C(2′)-H], 6.10 [1H, d, J = 5.5 Hz, C(1′)-H], 6.96 (2H, br, NH_2), 7.28 (10H, s, two Ph's), 7.34 (5H, s, Ph), 7.80 [1H, s, C(2)-H]. *Anal.* Calcd for $C_{32}H_{33}N_5O_5 \cdot H_2O$: C, 65.63; H, 6.02; N, 11.96. Found: C, 65.46; H, 5.79; N, 11.85.

ii) Cyclization with Sodium Isopropoxide: Compound **5b** (527 mg, 0.928 mmol) was dissolved in anhydrous isopropanol (65 ml), followed by addition of 0.2 M sodium isopropoxide in isopropanol (65 ml). The solution was allowed to stand at room temperature for 15 min, neutralized by addition of 5 M aqueous acetic acid (2.6 ml), and concentrated *in vacuo*. The residue was partitioned between H_2O (25 ml) and chloroform (15 ml). The aqueous layer was extracted with chloroform (3 × 15 ml). The combined organic layers were dried over magnesium sulfate and concentrated *in vacuo* to leave a slightly yellow foam. This was crystallized by treating it with a small volume of EtOH to give **6b**·H_2O (395 mg, 73%), mp *ca.* 100 °C; its infrared (IR) spectrum (Nujol) was identical with that of the analytical sample described under item (i).

3,4-Dihydro-4,6-dimethyl-3-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)-9*H*-imidazo[1,2-*a*]purin-9-one (7b) A mixture of **6**·H_2O (1.55 g, 2.65 mmol) and potassium carbonate (1.12 g, 8.1 mmol) in anhydrous DMF (36 ml) was stirred at room temperature for 1 h. Bromoacetone (2.22 g, 16.2 mmol) was then added to the mixture and stirring was continued for another 2 h. The resulting mixture was concentrated *in vacuo* and H_2O (90 ml) was added to the residue. The mixture was neutralized with 10% aqueous phosphoric acid and extracted with dichloromethane (90 ml). The aqueous layer was extracted with dichloromethane (90 ml and 2 × 40 ml). The organic phases were combined, dried over magnesium sulfate, and concentrated *in vacuo*. Flash chromatography [column diameter, 40 mm; ethyl acetate–hexane (8 : 1, v/v)] of the residue afforded **7b** (0.950 g, 59%) as a slightly yellow foam. This was crystallized from carbon tetrachloride and dried over phosphorus pentoxide at 2 mmHg and room temperature for 8 h to give an analytical sample of **7b**·1/5CCl_4 as colorless needles, mp 36—50 °C (did not show a distinct melting point); $[\alpha]_D^{27}$ −28.5° (c = 0.744, MeOH); UV λ_{max} (95% EtOH) 235 nm (ε 30200), 292 (7700); MS *m/z* 605 (M^+); ^{1}H-NMR ($CDCl_3$) δ: 2.33 (3H, d, J = 1 Hz, CMe), 3.50 (1H, dd, J = 2.2, 11 Hz) and 3.66 (1H, dd, J = 2.7, 11 Hz) [C(5′)-H_2], 4.07 (3H, s, NMe), 4.12 [1H, m, C(4′)-H)], 4.31 and 4.52 (1H each, d, J = 11.5 Hz, $PhC\underline{H}_2$), 4.39 [1H, m, C(3′)-H], 4.50 and 4.61 (1H each, d, J = 4 Hz, $PhC\underline{H}_2$), 4.68 (2H, s, $PhC\underline{H}_2$), 4.70 [1H, m, C(2′)-H], 6.17 [1H, d, J = 7 Hz, C(1′)-H], 6.96—7.41 (15H, m, three Ph's), 7.43 [1H, q, J = 1 Hz, C(7)-H], 7.64 [1H, s, C(2)-H]. *Anal.* Calcd for $C_{35}H_{35}N_5O_5 \cdot 1/5CCl_4$: C, 66.43; H, 5.54; N, 11.00. Found: C, 66.59; H, 5.54; N, 11.03.

3,4-Dihydro-4,6-dimethyl-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-imidazo[1,2-*a*]purin-9-one (7c) A mixture of **7a**[7b] (106 mg, 0.316 mmol), acetic anhydride (0.3 ml), and dry pyridine (1 ml) was stirred at room temperature for 3 h. The resulting solution was concentrated *in vacuo* and the residue was dissolved in dichloromethane (10 ml). It was washed successively with 10% aqueous citric acid (2 × 5 ml) and saturated aqueous sodium bicarbonate (5 ml), dried over magnesium sulfate, and concentrated *in vacuo* to leave a colorless glass (136 mg, 93%), MS *m/z*: 461 (M^+); ^{1}H-NMR ($CDCl_3$) δ: 2.10, 2.15, and 2.18 (3H each, s, three Ac's), 2.32 [3H, d, J = 1 Hz, C(6)-Me], 4.19 (3H, s, NMe), 4.32 [2H, d, J = 3 Hz, C(5′)-H_2], 4.51 [1H, dt, J = 3, 3.5 Hz, C(4′)-H], 5.49 [1H, dd, J = 3.5, 5 Hz, C(3′)-H], 5.86 [1H, dd, J = 5, 6 Hz, C(2′)-H], 6.25 [1H, d, J = 6 Hz, C(1′)-H], 7.41 [1H, q, J = 1 Hz, C(7)-H], 7.74 [1H, s, C(2)-H].

3-Benzyl-4,9-dihydro-4,6-dimethyl-9-oxo-3*H*-imidazo[1,2-*a*]purine-7-carboxaldehyde (15) Phosphorus oxychloride (0.2 ml) was added to ice-cooled dry DMF (1.0 ml) and the mixture was stirred at room temperature for 15 min. A portion (0.6 ml) of this solution was added dropwise to a suspension of 3-benzyl-3,4-dihydro-4,6-dimethyl-9*H*-imidazo[1,2-*a*]purin-9-one[9] (147 mg, 0.50 mmol) in dry DMF (3 ml) at 0 °C. Stirring was continued for 10 h at 0 °C and the resulting mixture was poured into ice-cooled saturated aqueous sodium bicarbonate (6 ml). The precipitate that separated was collected by filtration, washed with H_2O (7 ml), and dried to give crude **15** (133 mg). This was dissolved in EtOH (400 ml) and the solution was concentrated under atmospheric pressure to 70 ml to afford **15** (105 mg, 65%), mp 269—274 °C (dec.). Further recrystallization from EtOH gave an analytical sample as colorless needles, mp 272—277 °C (dec.); UV λ_{max} (95% EtOH) 230 nm (ε 24400), 242 (sh) (18800), 320.5 (19300); MS *m/z*: 321 (M^+); ^{1}H-NMR [$(CD_3)_2SO$] δ: 2.51 (3H, s, CMe), 3.88 (3H, s, NMe), 5.79 (2H, br s, $PhC\underline{H}_2$), 7.00—7.45 (5H, m, Ph), 8.08 [1H, s, C(2)-H], 10.75 (1H, s, CHO). *Anal.* Calcd for $C_{17}H_{15}N_5O_2$: C, 63.54; H, 4.71; N, 21.79. Found: C, 63.31; H, 4.53; N, 21.56.

4,9-Dihydro-4,6-dimethyl-9-oxo-3-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)-3*H*-imidazo[1,2-*a*]purine-7-carboxaldehyde (8b) Phosphorus oxychloride (0.8 ml) was added to dry DMF (4 ml) at 0 °C under nitrogen and the mixture was stirred at room temperature for 15 min. A portion (2.28 ml) of this solution was added dropwise under nitrogen to a solution of **7b**·1/5CCl_4 (307 mg, 0.482 mmol) in dry DMF (2 ml), which was cooled at −30 °C. The mixture was stirred at −30 °C for 5 h and poured into ice-cooled saturated aqueous sodium bicarbonate (25 ml). The whole was extracted with chloroform (2 × 65 ml). The organic layers were combined, dried over magnesium sulfate, and concentrated *in vacuo*. The oily residue was purified by flash chromatography [column diameter, 20 mm; ethyl acetate–hexane (6 : 1, v/v)] to afford **8b** (194 mg, 63%) as a pale yellow foam, MS *m/z*: 633 (M^+); ^{1}H-NMR ($CDCl_3$) δ: 2.68 (3H, s, CMe), 3.51 and 3.68 [1H each, dd, J = 10.5, 2.5 Hz, C(5′)-H_2], 4.13 (3H, s, NMe), 4.20—4.73 (9H, m, C(2′)-, C(3′)-, and C(4′)-H and three $PhC\underline{H}_2$'s], 6.17 [1H, d, J = 7 Hz, C(1′)-H], 6.90—7.45 (15H, three Ph's), 7.73 [1H, s, C(2)-H], 10.96 (1H, s, CHO).

When the reaction was carried out at a temperature above 0 °C, the product isolated was 4,9-dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine-7-carboxaldehyde (mp > 300 °C) on the basis of its ^{1}H-NMR spectrum: ^{1}H-NMR [$(CD_3)_2SO$] δ: 2.53 (3H, s, CMe), 3.87 (3H, s, NMe), 8.30 [1H, s, C(2)-H], 10.70 (1H, s, CHO), 13.95 (1H, br, NH).

4,9-Dihydro-4,6-dimethyl-9-oxo-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3*H*-imidazo[1,2-*a*]purine-7-carboxaldehyde (8c) A solution of **7c** [prepared from **7a** (758 mg, 2.26 mmol) as described above] in dry DMF (10 ml) was treated with the chloromethylenedimethylammonium chloride solution in DMF (24 ml) as described above for the preparation of **8b** and the resulting mixture was stirred at −25 °C for 4 h. It was poured into ice-cooled saturated aqueous sodium bicarbonate (200 ml) and extracted with dichloromethane (2 × 200 ml). The combined organic phases were dried over magnesium sulfate, and concentrated *in vacuo* to leave an orange oil. Flash chromatography [column diameter, 40 mm; ethyl acetate–EtOH (15 : 1, v/v)] afforded **8c** (1.022 g, 92% based on **7a**) as a slightly yellow foam, MS *m/z*: 489 (M^+); ^{1}H-NMR ($CDCl_3$) δ: 2.11, 2.188, and 2.193 (3H each, s, three Ac's), 2.62 [3H, s, C(6)-Me], 4.25 (3H, s, NMe), 4.32 [2H, d, J = 3 Hz, C(5′)-H_2], 4.53 [1H, dt, J = 3, 3.5 Hz, C(4′)-H], 5.50 [1H, dd, J = 3.5, 5.5 Hz, C(3′)-H], 5.92 [1H, dd, J = 5.5, 6 Hz, C(2′)-H], 6.28 [1H, d, J = 6 Hz, C(1′)-H], 7.79 [1H, s, C(2)-H], 10.83 (1H, s, CHO).

4,9-Dihydro-4,7-dimethyl-9-oxo-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3*H*-imidazo[1,2-*a*]purine-6-carboxaldehyde (11) The organozinc reagent was prepared in the light of literature procedures.[18,19a] A suspension of zinc–copper couple[24] (30 mg, 0.46 matom) and (*R*)-3-iodo-*N*-(methoxycarbonyl)alanine methyl ester[5a] (129 mg, 0.45 mmol) in a mixture of dry DMAc (0.055 ml) and dry toluene (0.9 ml) was sonicated under nitrogen at room temperature for 30 min, during which time the temperature of the bath rose to 30 °C, followed by addition of **8c** (147 mg, 0.3 mmol) and sonication for another 1.5 h (30—37 °C). Chlorotrimethylsilane (0.045 ml, 0.36 mmol) was added to the mixture and the whole was sonicated for 1 h (37—40 °C). Another addition of chlorotrimethylsilane (0.03 ml, 0.24 mmol) and sonication for 50 min (38—40 °C) were required for complete consumption of **8c**. The resulting mixture was diluted with dichloromethane, cooled with ice, and poured into saturated aqueous sodium bicarbonate (15 ml). The whole was filtered through Celite-545 and brought to pH 7 with 10% aqueous phosphoric acid. The organic phase was dried over magnesium sulfate and concentrated *in vacuo*. Flash chromatography [column diameter, 10 mm; hexane–ethyl acetate (1 : 20, v/v)] of the residue afforded **11** (46 mg, 31%) as a colorless glass. Crystallization from EtOH afforded colorless needles, which were dried over phosphorus pentoxide at 80 °C and 2 mmHg for 4 h followed by exposure to air until constant weight was reached to give an analytical sample of **11**·2/3H_2O, mp 159—160 °C; $[\alpha]_D^{18}$ +94.5° (c = 0.138, MeOH); UV λ_{max} (95% EtOH) 256 nm (ε 22000), 322 (16800); MS *m/z*: 489 (M^+); ^{1}H-NMR ($CDCl_3$) δ: 1.77 (br, 2/3H_2O), 2.13 (6H, s, two Ac's), 2.16 (3H,

s, Ac), 2.70 [3H, s, C(7)-Me], 4.04 (3H, s, NMe), 4.37—4.59 [a total of 3H, m, C(5′)-H_2 and C(4′)-H], 5.45 [1H, dd, J=6 Hz each, C(3′)-H], 5.71 (1H, dd, J=6, 4 Hz, C(2′)-H], 6.49 [1H, d, J=4 Hz, C(1′)-H], 8.14 [1H, s, C(2)-H], 10.85 (1H, s, CHO). *Anal.* Calcd for $C_{21}H_{23}N_5O_9 \cdot 2/3H_2O$: C, 50.30; H, 4.89; N, 13.97. Found: C, 50.19; H, 4.71; N, 13.98.

A minor product, 4,9-dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine-7-carboxaldehyde, was obtained in a separate run in 8.6% yield by flash chromatography as the most polar substance. The ^{1}H-NMR spectrum [$(CD_3)_2SO$] was identical with that of the same compound described above for the preparation of **8b**.

3,4-Dihydro-7-(hydroxymethyl)-4,6-dimethyl-3-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)-9*H*-imidazo[1,2-*a*]purin-9-one (9b) Sodium borohydride (15 mg, 0.40 mmol) was added to a solution of **8b** (150 mg, 0.237 mmol) in MeOH (8 ml). The mixture was stirred at room temperature for 30 min, neutralized with 10% aqueous phosphoric acid, and concentrated *in vacuo*. The residue was partitioned between dichloromethane (10 ml) and H_2O (10 ml). The aqueous phase was extracted with dichloromethane (5 ml). The organic phases were combined, dried over magnesium sulfate, and concentrated *in vacuo* to leave **9b** (141 mg, 94%) as a colorless glass, MS m/z: 635 (M^+); ^{1}H-NMR ($CDCl_3$) δ: 2.31 (3H, s, CMe), 3.50 (1H, dd, J=10.6, 2.3 Hz) and 3.67 (1H, dd, J=10.6, 2.6 Hz) [C(5′)-H_2], 3.95—4.75 [8H, m, C(4′)-, C(3′)-, and C(2′)-H, two PhCH_2's, and OH], 4.05 (3H, s, NMe), 4.68 (2H, s, PhCH_2), 4.83 (2H, d, J=7 Hz, CH_2OH), 6.15 [1H, d, J=7 Hz, C(1′)-H], 6.95—7.42 (10H, m, two Ph's), 7.36 (5H, s, Ph), 7.66 [1H, s, C(2)-H].

3,4-Dihydro-7-(hydroxymethyl)-4,6-dimethyl-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-imidazo[1,2-*a*]purin-9-one (9c) Sodium borohydride (50 mg, 1.3 mmol) was added to a solution of **8c** (489 mg, 1.0 mmol) in dry THF (30 ml) and the mixture was stirred at room temperature for 40 min. It was neutralized with acetic acid and concentrated *in vacuo* to a small volume. The residue was partitioned between dichloromethane (20 ml) and H_2O (20 ml). The aqueous phase was extracted with dichloromethane (10 ml). The combined organic phases were dried over magnesium sulfate and concentrated *in vacuo* to leave a colorless foam. Flash chromatography [column diameter, 30 mm; ethyl acetate–EtOH (4:1, v/v)] afforded a colorless glass (33 mg) as the rapidly eluted substance. The ^{1}H-NMR spectrum [($CDCl_3$) δ: 2.04, 2.11, 2.14, and 2.18 (3H each, s, four Ac's), 2.33 [3H, s, C(6)-Me], 4.15 (3H, s, NMe), 4.31 [2H, d, J=3 Hz, C(5′)-H_2], 4.50 [1H, dt, J=3, 3.5 Hz, C(4′)-H], 5.48 [1H, dd, J=3.5, 5 Hz, C(3′)-H], 5.56 [2H, s, C(7)-CH_2], 5.86 [1H, dd, J=5, 6 Hz, C(2′)-H], 6.22 [1H, d, J=6 Hz, C(1′)-H], 7.74 [1H, s, C(2)-H] of this compound suggested that it was 7-(acetyloxymethyl)-3,4-dihydro-4,6-dimethyl-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-imidazo[1,2-*a*]purin-9-one. It was difficult to isolate this compound in a pure state because of its instability. Compound **9c** was obtained from the more polar fractions as a colorless glass (176 mg, 36%), MS m/z: 491 (M^+); ^{1}H-NMR ($CDCl_3$) δ: 2.11, 2.15, and 2.18 (3H each, s, three Ac's), 2.29 [3H, s, C(6)-Me], 4.00 (1H, t, J=7 Hz, OH), 4.16 (3H, s, NMe), 4.32 [2H, d, J=3 Hz, C(5′)-H_2], 4.51 [1H, dt, J=3, 4 Hz, C(4′)-H], 4.81 (2H, d, J=7 Hz, CH_2OH), 5.49 [1H, dd, J=4, 5 Hz, C(3′)-H], 5.86 [1H, dd, J=5, 6 Hz, C(2′)-H], 6.24 [1H, d, J=6 Hz, C(1′)-H], 7.75 [1H, s, C(2)-H].

3,4-Dihydro-4,6,7-trimethyl-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-imidazo[1,2-*a*]purin-9-one (12c) i) From **9c**: A solution of **9c** (79 mg, 0.16 mmol) in EtOH (10 ml) was hydrogenated over 10% palladium on charcoal at *ca.* 60 °C and atmospheric pressure for 10 h. The catalyst was filtered off and extracted with dichloromethane using a Soxhlet extractor. The extracts were concentrated *in vacuo* to leave a colorless glass (11 mg). The filtrate was concentrated *in vacuo* to leave a colorless glass (65 mg), whose ^{1}H-NMR spectrum indicated that it was a mixture (molar ratio, 2:1) of 7-ethoxy-3,4-dihydro-4,6-dimethyl-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-imidazo[1,2-*a*]purin-9-one (**10**) and **12c**. This was combined with the product obtained from the extracts of the catalyst and purified by flash chromatography [column diameter, 10 mm; ethyl acetate–EtOH (10:1, v/v)] to give **12c** (4 mg), a mixture of **12c** and **10**, and **10** [colorless needles from EtOH, mp 156—157 °C; MS m/z: 519 (M^+); ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, t, J=7 Hz, MeCH_2), 2.10, 2.14, and 2.18 (3H each, s, three Ac's), 2.32 [3H, s, C(6)-Me], 3.64 (2H, q, J=7 Hz, MeCH_2), 4.13 (3H, s, NMe), 4.31 [2H, d, J=3 Hz, C(5′)-H_2], 4.50 [1H, dt, J=3, 3.5 Hz, C(4′)-H], 4.95 (2H, s, CH_2OEt), 5.48 [1H, dd, J=3.5, 5 Hz, C(3′)-H], 5.83 [1H, dd, J=5, 6 Hz, C(2′)-H], 6.22 [1H, d, J=6 Hz, C(1′)-H], 7.71 [1H, s, C(2)-H]]. The fractions containing **10** were combined and hydrogenated again in EtOH (10 ml) over 10% palladium on charcoal (50 mg) under the same conditions for 10 h. The reduction was continued for another 11 h after addition of more catalyst (50 mg). The catalyst was filtered off and continuously extracted with dichloromethane. The extracts were combined with the filtrate and concentrated *in vacuo* to leave a colorless glass (37 mg). This was purified by layer chromatography on silica gel [ethyl acetate–EtOH (10:1, v/v)] to afford a second crop of **12c** (19 mg, the total yield was 25%), ^{1}H-NMR ($CDCl_3$) δ: 2.11 and 2.15 (3H each, s, two Ac's), 2.18 [6H, s and dull q, Ac and C(6)-Me], 2.63 [3H, dull q, J=0.7 Hz, C(7)-Me], 4.10 (3H, s, NMe), 4.31 [2H, d, J=3 Hz, C(5′)-H_2], 4.49 [1H, dt, J=3, 4 Hz, C(4′)-H], 5.48 [1H, dd, J=4, 5 Hz, C(3′)-H], 5.85 [1H, dd, J=5, 6 Hz, C(2′)-H], 6.21 [1H, d, J=6 Hz, C(1′)-H], 7.66 [1H, s, C(2)-H].

ii) From **8c** Using 10% Palladium on Charcoal: A solution of **8c** (245 mg, 0.50 mmol) in EtOH (15 ml) was hydrogenated over 10% palladium on charcoal (250 mg) at *ca.* 60 °C and atmospheric pressure for 8 h. More catalyst (250 mg) was added and the reduction was continued for another 12 h. The catalyst was filtered off and the filtrate was concentrated *in vacuo* to leave a colorless glass (0.15 g). This was purified by flash chromatography [column diameter, 20 mm; ethyl acetate–EtOH (10:1, v/v)] to give **12c** (48 mg) as a colorless glass, and a mixture of **12c** and other products (0.03 g). The catalyst was continuously extracted with dichloromethane using a Soxhlet extractor to give additional crude **12c** (0.04 g). These crude fractions containing **12c** were combined and purified by flash chromatography (column diameter, 10 mm) using the same solvent to afford a second crop of **12c** (28 mg, the total yield was 32%), identical (^{1}H-NMR spectrum) with that described under method (i).

iii) From **8c** Using Palladium Hydroxide on Charcoal: A mixture of **8c** (245 mg, 0.5 mmol), palladium hydroxide on carbon[20] (490 mg), and EtOH (15 ml) was shaken under hydrogen at *ca.* 60 °C and atmospheric pressure for 15 h. The resulting mixture was filtered and the catalyst was washed with hot EtOH (50 ml). The combined filtrate and washings were concentrated *in vacuo* and the residue was purified by flash chromatography in the same way as described under item (ii) to afford **12c** (106 mg) as a colorless foam. The catalyst was extracted with dichloromethane using a Soxhlet extractor. A second crop (16 mg) of **12c** was obtained from this fraction by flash chromatography. Further purification of the combined fractions containing **12c** and **10** by flash chromatography afforded a third crop of **12c** (25 mg, the total yield was 62%) and **10** (18 mg, 7%).

iv) From **11**: A mixture of **11**·2/3H_2O (37 mg, 0.074 mmol) in EtOH (8 ml) was hydrogenated over 10% palladium on charcoal (37 mg) at *ca.* 60 °C and atmospheric pressure for 9 h, followed by the hydrogenation with more catalyst (37 mg) for another 5 h. The resulting mixture was filtered and the catalyst was washed with hot EtOH (50 ml). The filtrate and washings were combined and concentrated to afford **12c** (23 mg, 66%) as a colorless glass.

3,4-Dihydro-4,6,7-trimethyl-3-β-D-ribofuranosyl-9*H*-imidazo[1,2-*a*]purin-9-one (12a) A solution of **12c** (285 mg, 0.599 mmol) in saturated methanolic ammonia (10 ml) was kept at 0 °C for 5 h and concentrated *in vacuo*. The solid residue was washed with cold EtOH (7 ml) and dried to afford **12a**·1/2H_2O (200 mg, 93%) as a colorless solid, mp *ca.* 170 °C (dec.). Recrystallization from H_2O (brief heating in previously boiled H_2O and quick cooling with ice water to *ca.* 40 °C) gave colorless needles, which were dried over phosphorus pentoxide at 2 mmHg and room temperature for 24 h and then exposed to air until constant weight was reached to give an analytical sample of **12a**·1/2H_2O, mp *ca.* 190—200 °C (dec.); $[\alpha]_D^{17}$ −35° (c=0.166, MeOH); CD ($c=3.01\times10^{-5}$ M, H_2O) $[\theta]^{20}$ −7300 (244 nm) (neg. max.); UV λ_{max} (95% EtOH) 239 nm (ε 28600), 280 (sh) (4800), 297 (5700); λ_{max} (H_2O, pH 2) 233 (30700), 278 (10300); λ_{max} (H_2O, pH 7) 240 (29900), 301 (5400); λ_{max} (H_2O, pH 13) 240 (31200), 301 (5500); ^{1}H-NMR [$(CD_3)_2SO$] δ: 2.11 [3H, q, J=0.7 Hz, C(6)-Me], 2.56 [3H, q, J=0.7 Hz, C(7)-Me], 3.63 [2H, m, C(5′)-H_2], 3.98 [1H, m, C(4′)-H], 4.01 [3H, s, NMe], 4.12 [1H, m, C(3′)-H], 4.45 [1H, m, C(2′)-H], 5.11 (1H br, 5′-OH), 5.30 (1H, d, J=5 Hz, 3′-OH), 5.69 (1H, d, J=6 Hz, 2′-OH), 6.08 [1H, d, J=5 Hz, C(1′)-H], 8.17 [1H, s, C(2)-H]. *Anal.* Calcd for $C_{15}H_{19}N_5O_5 \cdot 1/2H_2O$: C, 50.28; H, 5.63; N, 19.54. Found: C, 50.02; H, 5.38; N, 19.66.

1,4-Dihydro-4,6,7-trimethyl-9*H*-imidazo[1,2-*a*]purin-9-one (1b) i) By Hydrolysis of **12a**: A solution of **12a**·1/2H_2O (5.7 mg, 0.016 mmol) in 0.1 N hydrochloric acid (2 ml) was allowed to stand at room temperature for 1 h, then neturalized with 1 N aqueous sodium hydroxide, and extracted wtih dichloromethane using a continuous extractor. Evaporation of the solvent from the organic phase gave a colorless solid, which was recrystallized from MeOH to afford **1b**·H_2O (3.2 mg, 86%) as colorless needles, identical (IR spectrum) with an authentic sample.[5b]

ii) By Hydrogenolysis of **14**: A suspension of **14** (161 mg, 0.5 mmol) in EtOH (65 ml) was hydrogenated over Pearlman's catalyst[20] (322 mg) at *ca.* 60 °C and atmospheric pressure for 8 h. More catalyst (322 mg) was added and the hydrogenation was continued for another 6 h. The catalyst was filtered off and extracted with MeOH using a Soxhlet extractor. The

extracts were combined with the filtrate. Removal of the solvent by evaporation afforded crude **1b**·H_2O (95 mg, 81%), which was identical in terms of IR spectrum and chromatographic behavior with an authentic specimen.[5b]

Rate of Hydrolysis of the Glycosidic Bond of 12a in 0.1 N Hydrochloric Acid at 25 °C The rate was determined according to the reported procedures for 3-methylinosine,[25] **6a**,[7b] and **7a**,[7b] by following the absorbance of the reaction mixture at 277 nm. In two separate runs, a pseudo-first-order rate constant of $(4.7 \pm 0.1) \times 10^{-1}\,\mathrm{min}^{-1}$ (half-life 88 s) was obtained.

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References and Notes

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