Synthesis of 4,9-Dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine and the "Y" Base from *Saccharomyces cerevisiae* Phenylalanine Transfer RNA

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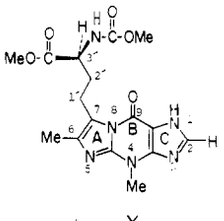
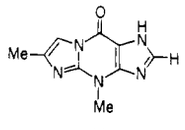
An efficient synthesis of the "Y" base compounds has been developed using 7-benzyl-3-methylguanine as the key intermediate. A study of the regioselectivity of the alkylation of this intermediate, plus the high yields in this synthesis, confirms the proposed structure for the fluorescent bases isolated from the phenylalanine transfer RNA of *Torulopsis utilis* and *Saccharomyces cerevisiae*.

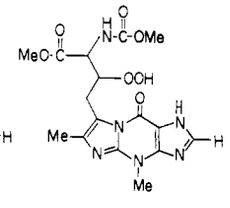
Because of their distinctive physical properties and/or because of their ability to be selectively altered, the modified nucleosides occurring in transfer RNA (tRNA) have been used to study the tertiary structure of tRNA and its interactions with other molecules.[1] Some of the most distinctive of these modified nucleosides are the "Y" type compounds,[2] which are still the only nucleic acid bases possessing a condensed tricyclic skeleton. Y bases have been isolated from the phenylalanine tRNA of several eukaryotic species, including *Saccharomyces cerevisiae*,[3] wheat germ,[4] beef liver,[5] rat liver,[6] and *Torulopsis utilis*,[7] but at least one eukaryotic species does not possess Y bases in its phenylalanine tRNA.[8] In contrast to most other modified nucleosides, the Y compounds have been of special interest because of their intense fluorescence.[9]



1 $Y_{S.C.}$



2 $Y_{T.U.}$



3 $Y_{L.}$

To date the structures of three Y bases have been determined. The planar structure of the Y base **1** isolated from $tRNA^{Phe}$ of *S. cerevisiae* was determined by comparing its spectroscopic data obtained with 300 μg of sample with various synthetic models[10] and was subsequently "confirmed" by a "synthesis" in which the yield of the last step was 2%.[11] The absolute configuration of the side chain was established as *S* by a microozonolysis of Y base which yielded dimethyl (*S*)-2-methylcarbamoylglutarate.[11] The structures of the *Torulopsis utilis* Y base **2**[7] and the Y base isolated from mammalian liver[12] and *Lupinus luteus* **3**,[13] which contains the rare hydroperoxide function, were determined by correlation (and by the synthesis of Y_{TU} **2**[7]). The possible presence of Y bases other than **1**–**3** have also been reported.[14] Interestingly, the $tRNA^{Phe}$ isolated from brain tumor has been shown to be deficient in the Y bases.[15,16]

The structure of the Y nucleosides, however, still remains to be proven unambiguously by synthesis. It has been proposed to be the 3-β-D-ribofuranoside[17] because the synthetic Y_{TU}-1-β-D-ribofuranoside resisted hydrolysis by 2 N hydrochloric acid at 37 °C, which was in contrast to the well-known lability of Y nucleosides to mild acid.[18] On the other hand, Reese and Whittall[19] synthesized a 3,4-cyclo-β-D-ribofuranoside and found it to be acid resistant; they have thus suggested the possibility that the Y nucleosides could be 2′-deoxyribonucleosides. The skeleton of the Y_{SC} nucleoside has been shown to be biosynthesized from guanine,[20,21] whereas the side chain is apparently derived from methionine.[22]

Since it is difficult to isolate more than a few hundred micrograms of these bases from natural sources, an efficient synthesis of these compounds is necessary in order to study their chemical and physical properties. This paper discusses our synthetic studies on these naturally occurring modified bases.

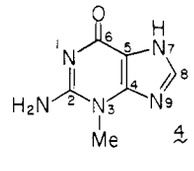
Results and Discussion

Our goal was to develop an efficient synthesis of the simplest of the naturally occurring Y bases, **2**, with the expectation that this route could be used for the syntheses of other bases such as **1** and **3**. Noting the vigorous conditions needed to form the B and C rings, it seemed that the best route involved building ring A onto the preformed 3-methylguanine nucleus. The necessary 3-methylguanine (**4**) was prepared by several modifications of the literature procedure,[23,24] which resulted in an increase of overall yield from 3 to 35%.

It seemed reasonable to form the A ring by causing an α-bromo ketone to react with 3-methylguanine (**4**). After this work had commenced, it was reported that chloroacetaldehyde reacts well with adenosines and cytosines under acidic conditions (pH 3.5–4.5) to form tricyclic and dicyclic compounds, respectively, but no detectable reaction occurred with guanines under these conditions.[25,26] More recently, Leonard and co-workers have reported that under slightly higher pHs (6.4) chloroacetaldehyde also reacts with guanosine to give a linear tricyclic compound possessing basically the Y base nucleus, i.e., 1,N^2-ethenoguanine (hydrogens instead of the two methyls in structure **2**); this compound, however, is only weakly fluorescent.[27]

Reaction of α-Bromo Ketones with 3-Methylguanine. The alkylation–condensation reaction of an appropriate α-bromo ketone with 3-methylguanine is the only published synthesis of **1** and **2**,[7,11] but this was not a satisfactory route because of the side reactions, poor solubility properties of 3-methylguanine, etc.[28]

Although 3-methylguanine is completely insoluble in most solvents, it is slightly soluble in dimethylformamide and dimethylacetamide. It is more soluble in dimethyl sulfoxide, water (pH >8), and ethanol (pH >8), but these solvents cause substantial decomposition of the α-bromo ketone. To exem-



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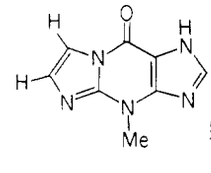

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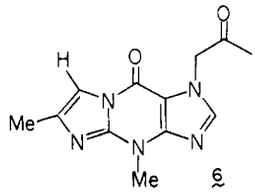

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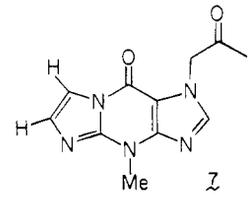

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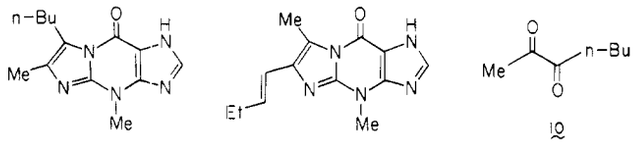
plify the side reactions encountered, the condensation of α-bromo ketone with **4** at 100 °C in DMF with diethylaniline as the base yielded not only the desired **2**, but also the desmethyl derivative **5** and the dialkylation compounds **6** and **7**. It is conceivable that compound **5** results from the reaction of 3-methylguanine with α,α′dibromoacetone formed from the disproportionation of α-bromoacetone followed by loss of formaldehyde. As would be expected, position 7 of the guanine competes with position 1 as the site of alkylation as shown by the formations of **6**, **7**, and 3-methyl-7-(2-oxopropyl)guanine. The side products **5** and **7** can be eliminated and the yield of the desired **2** raised to 25% by using water (pH 10) as the solvent, but these conditions can not be used for synthesis of the Y_{SC}, since the ester and carbamate groups are unstable in base.

When a secondary bromide is used in place of a primary bromide, other side products are formed; e.g., **9** is formed in

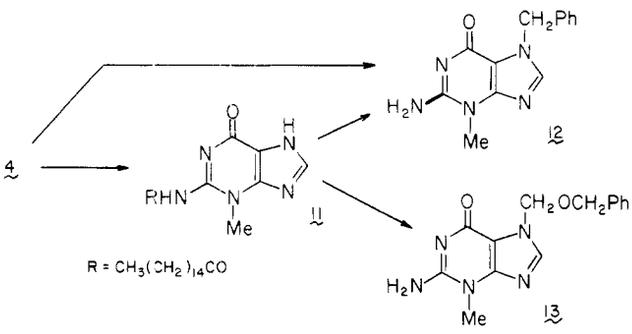


addition to **8** and **5** when 3-methylguanine is caused to react with 3-bromo-2-heptanone. Product **9** is most likely formed by oxidation of the α-bromo ketone to the diketone **10** (especially in dimethyl sulfoxide) followed by its cyclization with 3-methylguanine (**4**).[29]

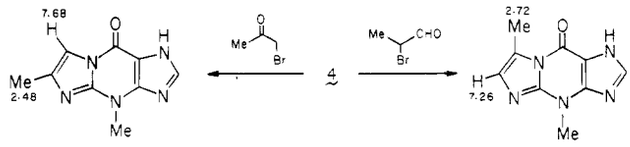
Because of the poor yields of the desired tricyclics and the multitude of products, protection of the 7 position of the 3-methylguanine is desirable to prevent alkylation of this position by the α-bromo ketone and also to make the guanine more soluble. A benzyl group was considered to be most suitable for protection in view of its regioselective attachment at the 7 position rather than the N^2 or 1 positions, its stability during the cyclization reaction, and its ease of removal even in the presence of the carbamate and ester groups of **1**.[30]

Protection of 3-Methylguanine. The desired alkylation of the 7 position of **4** was not accomplished as readily as expected. When the preformed anion of **4** (NaH in DMF) is stirred with benzyl chloride, only unreacted 3-methylguanine is recovered (>85%) after heating the mixture at 60 °C for 24 h. The same results are obtained even when the more reactive benzyl chloromethyl ether is used as the alkylating reagent. This unreactivity is in contrast to the alkylation of the anion of guanine with alkyl chloride at room temperature in DMF.[31]

On the other hand, prior protection of the 2-amino group by reacting 3-methylguanine with palmitoyl chloride in pyridine[32] to yield 3-methyl-N^2-palmitoylguanine (**11**) allowed the alkylation at N-7 to take place. In this case, reaction of **11** with potassium carbonate and benzyl chloride or benzyl chloromethyl ether followed by treatment with ammonium



Scheme I

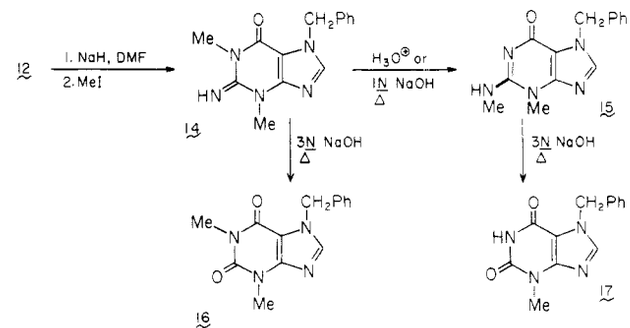


hydroxide yielded **12** and **13**, respectively. Since **11** is alkylated readily compared to **4**, the difficulty of alkylating **4** must be due to electronic rather than steric factors. Thus usage of a more reactive alkylating agent such as benzyl bromide results in direct alkylation of **4** to give **12** in good yield.

Alkylation of 7-Benzyl-3-methylguanine. The next step is the formation of the second imidazole ring by cyclization between the 1 and N^2 positions of the guanine. As with the unprotected 3-methylguanine, this can be carried out by using an α-bromo ketone. Since the orientation of the two reactive carbons of the α-bromo ketone with respect to the guanine is dependent on whether the 1 or N^2 position of the guanine is alkylated in the initial step, the site of alkylation (1 or N^2) must be determined. For the 7- or 9-substituted guanines, it is well known that alkylation under basic conditions occurs predominantly at the 1 position,[23] but it is necessary to verify that **12** is alkylated with the same regioselectivity for that position.

In connection with the verification of the substitution at the 6 and 7 positions of the tricyclic, the original structural assignment for these positions should be mentioned.[10] From the ^1H NMR spectra of a number of model compounds, it was found that the methyl groups at the 6 and 7 positions were at ~2.3 and 2.6 ppm; namely, there was a chemical shift difference of 0.3 ppm. It was rationalized that the methyl protons at the 7 position should be further downfield due to the anisotropic effect of the 9-carbonyl. A similar argument was used in the structural studies of the Y_{Tu} base **2**.[7] This rationalization could be questioned since there is a lack of proper model compounds for the prediction of the chemical shift at this position, but the synthesis of a number of model compounds supported the assigned structure.[34] The ^1H NMR data obtained from the synthesis of **2**[7] and its structural isomer as shown in Scheme I supports this assignment as long as alkylation takes place at the 1 position of 3-methylguanine.

To prove this point, we studied the alkylation of the anion of **12**. Employment of conditions similar to those for the synthesis of the tricyclics, but replacement of methyl iodide for the α-bromo ketone, produces a single benzyldimethylguanine (as determined by thin-layer chromatography and ^1H NMR) plus a small amount of unreacted 3-methylguanine. It was originally planned to identify the structure of the dimethyl derivative (either **14** or **15**) by hydrolysis to the xanthine derivative (**16** or **17**, respectively). Upon acid hydrolysis

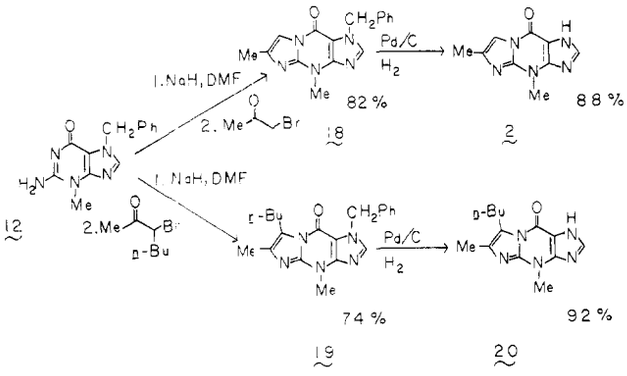


conditions (1 N HCl, 100 °C, 24 h; concentrated HCl, 100 °C, 24 h; 5 N H_2SO_4, 100 °C 24 h; 1 N HNO_3, 100 °C, 24 h) the compound was converted into another isomer, but neither isomer would hydrolyze to the xanthine derivative.[35] Unlike

the original dimethylguanine, which had two singlets at 3.42 and 3.26 ppm for the methyl groups, the rearranged compound **15** had one singlet at 3.42 ppm and one doublet at 2.74 ppm (J = 4 Hz, coupled to a single amino proton). These ^{1}H NMR data indicate that the expected guanine **14** is formed originally, but that it rearranges to the isomer **15**.[36]

Compound **14** is also converted to **15** by aqueous base,[36] but if the conditions are vigorous enough (3 N NaOH, 100 °C) **14** can be hydrolyzed to **16** before an appreciable amount of rearrangement to **15** takes place. In a similar manner **15** can be hydrolyzed to **17**. Since both **16** and **17** have been synthesized in a different manner previously,[37-40] we have verified the assigned structures for **14** and **15** and the regioselectivity of alkylation at the 1 position.

When α-bromoacetone is caused to react with the anion of **12** under the same conditions as those used with methyl iodide, a single fluorescent compound **18** was produced in 82% yield. The reaction proceeded almost as well with a secondary bromide (3-bromo-2-heptanone) to give **19** in 74% yield. The



isolated tricyclics were judged to be a single isomer in each case on the basis of the ^{1}H NMR spectra and thin-layer chromatography on silica gel in several solvent systems. Since both the α-bromo ketone and methyl iodide are completely regioselective and **14** does not rearrange to **15** under the reaction conditions, the tricyclic structures should be **18** and **19** and not any structural isomers. The ^{1}H NMR signals of the C–Me groups in **18** (2.22 ppm) and **19** (2.10 ppm), which are at chemical shifts for methyls not "peri" to the carbonyl, also corroborate these structures.

Debenzylation of Imidazo[1,2-*a*]purines. In determining hydrogenation conditions for the removal of the benzyl protecting group from **18** to yield the desired tricyclic **2**, two possible complications have to be considered, namely, the hydrogenation of the 6,7 double bond and alteration of the ester or carbamate portion of **1** under some acidic conditions.[30] Since the hydrogenation reaction proved to be much more difficult than expected, our results are described somewhat in detail.

The benzyl tricyclic compound **19** remained unchanged when its methanolic solution was stirred with 10% Pd/C under 1 atm of H_2 for 24 h. With 10% Pd/C in methanol, some debenzylation was possible when acetic acid (1–20% by volume) was added to the mixture, but this method yielded three other fluorescent compounds in about equal amounts as compared to the desired **20**. Addition of 1 N aqueous HCl along with 10% Pd/C to a methanolic solution of **19** caused clean reduction to **20**, but the rate was very slow. The above results were not improved by using the normally more readily reducible benzyloxymethyl derivative in place of the benzyl derivative.

In contrast to the other conditions, usage of 5% acetic acid and a few drops of 1 N HCl in methanol or 2-propanol with 10% Pd/C and 1 atm of H_2 led to the rapid reduction of **19** to the desired **20** in 92% yield. This method was equally successful in transforming **18** into **2**. It should be noted that under these conditions the fluorescent compound was completely destroyed if too much oxygen was present either during the reduction or in the workup until all the acetic acid was removed.

In summary, the use of the benzyl protecting group at the 7 position of 3-methylguanine allows it to be converted to the "Y" bases **2** and **20** in overall yields of approximately 60%. This synthesis establishes the structure of the "Y_T" base and hence the skeleton of the other related "Y" bases.

Synthesis of the Y Base from *Saccharomyces cerevisiae*. Although the synthesis of the Y compounds via the route described above gave good yields for simple Y compounds, e.g., **2** and **20**, a number of problems arose in using this synthesis for making the more complicated molecule Y_{SC} **1**. One of the problems was that the reported synthesis of **23** was lengthy;[11] in addition, this synthesis did not allow a ready route to the optically active compound.

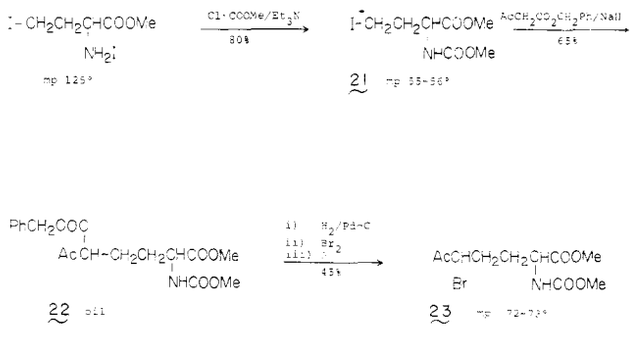
We therefore considered a number of alternative routes where the tricyclic system would be constructed first, and then the amino acid side chain would be added. The advantage of this route was that a derivatized L-homoserine could be used to yield the optically active naturally occurring **1**.[42] Unfortunately, the three attempted routes failed because of the instability or reactivity of the tricyclic heterocyclic system.[42,43]

It has been reported that **23** reacts with 3-methylguanine directly to give **1**; however, the yield was only 2% based on purine and using 3 equiv of **23**.[11] In addition, a number of other fluorescent compounds were formed and had to be separated by silica gel chromatography. The synthesis of **23** is given in Scheme II and the Experimental Section.

It was expected that the use of 7-benzyl-3-methylguanine should give a much cleaner reaction and higher yields of the desired Y base. Using 1 equiv of **23** in place of bromoacetone gave a 6% yield of 1-benzyl-Y_{SC} and no other fluorescent products were produced. Increasing the reaction time, bromo ketone addition time, and reaction temperature did not increase the product yield. The major problem was a decomposition of the bromo ketone by an internal cyclization process. It had been previously noted that the yield of tricyclic bases could be increased at higher temperatures in a dimethylformamide–tetrahydrofuran (1:3, v/v) mixture when the bromo ketone was exceptionally unstable.[43] These conditions did not give an increased yield in the case of cyclization with **23**. In all cases, most of the 7-benzyl-3-methylguanine did not react and was recovered.

The low tricyclic yield with **23** cannot be attributed to steric hindrance since other secondary bromo ketones, such as 2-bromo-2-heptanone, reacted readily. The internal cyclization of **23** is apparently more facile than the alkylation of the purine system. The reaction of potassium *tert*-butoxide, methylmagnesium bromide, or triethylamine instead of sodium hydride with 7-benzyl-3-methylguanine (**12**) prior to the addition of **23** did not improve the yield of 1-benzyl-Y_{SC}. However, it was finally found that reaction of **12** with 1 equiv of **23**

Scheme II



in DMF at 50 °C with 5 equiv of *N,N*-diethylaniline gave a 20% yield of 1-benzyl-Y_{SC}.

This product could be converted to Y_{SC} under the same conditions that converted 1-benzyl-Y_{TU} to Y_{TU}. Therefore, the synthesis of Y_{SC} described here has been considerably improved from that previously reported.[11] These synthetic studies also chemically corroborate the structures forwarded for the Y bases.

Experimental Section

All melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Jasco (Model 13A-1) or a Perkin-Elmer (Model 621) grating infrared spectrophotometer. The peaks are given in reciprocal centimeters with polystyrene film as the standard. Ultraviolet spectra were recorded on a Cary 14 recording spectrophotometer. Fluorescence measurements were conducted on a Perkin-Elmer MPF-2A spectrophotofluorimeter fitted with a xenon lamp and are uncorrected using 310 nm as excitation wavelength for the emission spectra. Proton magnetic resonance spectra (^{1}H NMR) were recorded on Varian A-60A, T-60, or HA-100 spectrometers. Chemical shifts are in δ units with respect to tetramethylsilane as the internal standard and $(CD_3)_2SO$ as the solvent unless otherwise specified. Low-resolution mass spectra were recorded on a Jeol JMA-07 spectrometer and high-resolution spectra on a DuPont CEC 21-110B with PFK as the standard for peak matching. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn.

Analytical and preparative thin-layer chromatography plates were made using Merck silica gel G. The preparative plates were prewashed with methanol and the desired bands were eluted from the plates using the same solvent as for developing the plates. Column chromatography was done on Merck silica gel 60 and at room temperature. The dimethylformamide was stirred with potassium hydroxide pellets and then distilled from CaH prior to use. All other organic solvents were distilled and stored over molecular sieves.

2,6-Diamino-1,4-dihydro-1-methyl-4-pyrimidone. The procedure of Roth et al.[24] was followed until the purification of the crude pyrimidone. Instead of purification as the sulfate salt, the free base was purified in the following manner. To a 75 °C solution (120 mL of water/10 g of pyrimidone) was added 40% aqueous sodium hydroxide until the solution had cleared. Addition of charcoal, followed by filtration through Celite and acidification to pH 6, yielded a white precipitate, which when filtered and dried weighed 200 g (65% yield): mp 284 °C.

2,6-Diamino-1,4-dihydro-1-methyl-5-nitroso-4-pyrimidone. To a solution at 40 °C of 25 g of 2,6-diamino-1,4-dihydro-1-methyl-4-pyrimidone, 28 g of sodium nitrite, and 8 g of sodium hydroxide in 3 L of water, glacial acetic acid was slowly added until pH 6 was reached. If precipitation occurred before the solution turned a deep magenta, aqueous sodium hydroxide was added until the precipitate dissolved and then the solution was reacidified. After a red precipitate started to form, the pH was adjusted to 5 with acetic acid. The solution stayed at room temperature for 4 h and was then filtered. After dissolving the red solid in water by adding 40% aqueous sodium hydroxide, the solution was filtered, washed with water and acetone, and dried to yield 22 g (70%): mp >300 °C.

1,4-Dihydro-1-methyl-2,5,6-triamino-4-pyrimidone Sulfate. The triaminopyrimidone sulfate was prepared according to the procedure of Roth et al.[24] except for the following simplification. After the addition of the sodium dithionite, the hot yellow solution was filtered and 6 N sulfuric acid was added until pH 1 was reached. After cooling, the solid was removed by filtration and dried to yield 12.9 g (86%): mp >300 °C.

3-Methylguanine (4). The triaminopyrimidone sulfate was converted to 3-methylguanine according to the procedure of Townsend and Robins,[23] except that the product was recrystallized from hot water with a charcoal treatment and then recrystallyzed twice more from hot water to yield white crystals in 89% yield: mp >300 °C; ^{1}H NMR δ 4.50 (s, 3), 8.08 (br, 2), 8.63 (s, 1).

7-Benzyl-3-methylguanine (12). 3-Methylguanine (0.32 g, 2 mmol) was dissolved in 100 mL of DMF through which nitrogen was bubbled for 1 h. After adding sodium hydride (96 mg of a 50% oil dispersion, 2 mmol), the solution was stirred for 3 h before adding the benzyl bromide (0.34 g, 2 mmol). After stirring for 18 h, silica gel (0.3 g) was added and the DMF was removed in vacuo. The solid was placed on top of a 100-g silica gel column, which was then washed with 300 mL of chloroform to remove unreacted benzyl bromide. Finally, 800 mL of chloroform–methanol (9:1, v/v) was used to elute the product. Recrystallization twice from methanol yielded 0.43 g (85%) of white needles: mp 269–270 °C; ^{1}H NMR δ 3.50 (s, 3), 5.50 (s, 2), 6.89 (br s, 2), 7.28 (s, 5), 8.18 (s, 1); UV λ_{max} (H_2O, pH 1) 245 (ϵ 9300), 267 (11 900); (pH 7) 248 (8100), 268 (10 400); (pH 12) 242 (9 100), 265 (11 500).

Anal. Calcd for $C_{13}H_{13}N_5O$: C, 61.17; H, 5.13; N, 27.43. Found: C, 60.94; H, 5.17; N, 27.14.

7-Benzyl-1,3-dimethylguanine (14). To a solution of **12** (128 mg, 0.5 mmol) in DMF (50 mL), which was deoxygenated under a flow of nitrogen, was added sodium hydride (24 mg of a 50% oil dispersion, 0.5 mmol). After stirring for 3 h, methyl iodide (70 mg, 0.5 mmol) was added and the mixture was stirred for 15 h. After removing the DMF in vacuo, the solid was stirred in benzene and then removed by filtration. Rapid recrystallization from water yielded 120 mg (89%) of a white solid: mp 175 °C; ^{1}H NMR δ 3.26 (s, 3), 3.42 (s, 3), 4.4 (br, 1), 5.45 (s, 2), 7.35 (s, 5), and 8.10 (s, 1). The facile rearrangement of this compound to **15** precluded obtaining an elemental analysis of this material.

7-Benzyl-N^2,3-dimethylguanine (15). A solution of **14** (114 mg, 0.4 mmol) was stirred in 1 N NaOH (5 mL) for 4 h. After neutralization with 1 N HCl and cooling, the mixture was filtered to yield 88 mg (85%) of a white solid: mp 235 °C; ^{1}H NMR δ 2.74 (d, 3, J = 4 Hz), 3.43 (s, 3), 5.43, (s, 2), 6.84 (br, 1), 7.20 (s, 5), 7.90 (s, 1); UV λ_{max} (H_2O, pH 1) 238 (ϵ 9200), 262 (11 400); (pH 7) 240 (9400), 261 (11 800); (pH 12) 236 (9200), 265 (11 700).

Anal. Calcd for $C_{14}H_{15}N_5O$: C, 62.44; H, 5.61; N, 26.01. Found: C, 62.27; H, 5.53; N, 25.95.

7-Benzyl-1,3-dimethylxanthine (16). To a refluxing solution of 3 N NaOH (5 mL) was added 7-benzyl-1,3-dimethylguanine (**14**; 114 mg, 0.4 mmol). After refluxing for 1 h, the solution was neutralized with glacial acetic acid. After cooling in a refrigerator, the product was removed by filtration. Purification by silica gel chromatography yielded 80 mg (74%) of the desired xanthine plus 9 mg (9%) of 7-benzyl-3-methylxanthine (**17**), which is derived by rearrangement prior to hydrolysis. The 7-benzyl-1,3-dimethylxanthine had: mp 157 °C (lit. mp 158 °C);[38-40] ^{1}H NMR (Me_2SO–TFA) δ 3.15 (s, 3), 3.36 (s, 3), 5.43 (s, 2), 7.25 (s, 5), 8.03 (s, 1).

7-Benzyl-3-methylxanthine (17). To a solution of 3 N NaOH (5 mL) was added 114 mg of 7-benzyl-N^2,3-dimethylguanine (0.4 mmol). After refluxing for 2 h, the solution was neutralized with glacial acetic acid. After cooling in a refrigerator, the product was removed by filtration and recrystallized from methanol to yield 84 mg (82%): mp 271 °C (lit. mp 273 °C);[38] ^{1}H NMR (Me_2SO–TFA) δ 3.38, (s, 3), 5.43 (s, 2), 7.25 (s, 5), 8.08 (s, 1).

1-Benzyl-4,9-dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine (18). After bubbling nitrogen through a solution of 7-benzyl-3-methylguanine (255 mg, 1 mmol) in DMF (50 mL), sodium hydride (48 mg of a 50% oil dispersion, 1 mmol) was added and the mixture was stirred for 2 h. Then bromoacetone (150 mg, 1.1 mmol) in DMF (10 mL) was added and the mixture was stirred an additional 18 h under nitrogen. (Cooling the solution below room temperature when adding the bromoacetone results in a much lower yield of the tricyclic.) After adding 1 g of silica gel, the DMF was removed in vacuo and the solid was placed on top of a 30-g silica gel column. The only fluorescent band to long wavelength UV light that eluted with 2-propanol–ethyl acetate (2:8, v/v) was recrystallized from methanol to yield 121 mg (82%) of white crystals: mp 205–206 °C; ^{1}H NMR δ 2.22 (d, 3, J = 1 Hz), 3.78 (s, 3), 5.57 (s, 2), 7.34 (s, 5), 7.3 (1), 8.40 (s, 1); UV λ_{max} (90% MeOH, pH 1) 229 (ϵ 39 200), 232 (39 000), 282 (10 100); (pH 7) 229 (3500), 233 (38 000), 266 (7000), 304 (6600); (pH 12) 227 (33 000), 230 (36 100), 265 (6800), 306 (7600).

Anal. Calcd for $C_{16}H_{15}N_5O$: C, 64.04; H, 5.37; N, 24.90. Found: C, 64.15; H, 5.17; N, 24.80.

4,9-Dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine (2). The benzyl tricyclic **19** (29 mg, 0.1 mmol) and 10% Pd/C (30 mg) were added to distilled propanol (50 mL) containing acetic acid (3 mL) and 0.1 N aqueous HCl (3 drops). After flushing the flask at least four times (evacuation, followed by hydrogen) to remove almost all the oxygen present, the mixture was stirred under 1 atm of hydrogen for 6 h. The mixture was rapidly filtered and taken to dryness in vacuo. The residue was purified by preparative silica gel TLC using 2-propanol–ethyl acetate (2:8, v/v). Since the R_f of the product varied depending on impurities that were present, the product band was the main fluorescent one under long wavelength UV light. Recrystallization from methanol yielded 18 mg (88%) of white solid: mp 280 °C; ^{1}H NMR δ 2.23 (d, 3, J = 1 Hz), 3.81 (s, 3), 7.36 (q, 1, J = 1 Hz), 7.75 (s, 1); UV λ_{max} (H_2O, pH 6.8) 230 (ϵ 34 000), 265 (6400), 304 (6900); (H_2O, pH 1.3) 227 (38 160), 231 (38 000), 282 (10 400); (H_2O, pH 11.6) 230 (39 200), 273 (10 400), 300 (10 680); MS 203 (M^+).

Anal. Calcd for $C_9H_9N_5O$: C, 53.20; H, 4.47; N, 34.46. Found: C, 52.96; H, 4.62; N, 34.70.

1-Benzyl-7-butyl-4,9-dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine (19). After bubbling nitrogen through a solution of 7-benzyl-3-methylguanine (255 mg, 1 mmol) in DMF (50 mL), sodium hydride (48 mg of a 50% oil dispersion, 1 mmol) was added and the mixture was stirred for 2 h. Then 3-bromo-2-heptanone (212 mg, 1.1 mmol) in DMF (10 mL) was added and the mixture was stirred an additional 18 h under nitrogen. After adding 1 g of silica gel, the DMF was removed in vacuo and the solid was placed on top of a 30-g silica gel column. The fluorescent band to long wavelength UV lamp that eluted with 2-propanol–ethyl acetate (1:9, v/v) was recrystallized from methanol to yield 237 mg (74%): mp 164–166 °C; ^{1}H NMR δ 0.84 (m, 3), 1.33 (m, 4), 2.10 (s, 3), 2.92 (m, 2), 3.70 (s, 3), 5.58 (s, 2), 7.34 (s, 5), 8.35 (s, 1); UV λ_{max} (10% aqueous MeOH, pH 1.0) 230 (ϵ 36 100), 284 (7600); (10% aqueous MeOH, pH 6.5) 229 (29 500), 265 (6100), 315 (5200); (10% aqueous MeOH, pH 11) 230 (28 000), 266 (6200), 313 (4900).

Anal. Calcd for $C_{20}H_{23}N_5O$: C, 68.75; H, 6.63; N, 20.04. Found: C, 68.66; H, 6.57; N, 20.16.

7-Butyl-4,9-dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine (20). The benzyl tricyclic **19** (66 mg, 0.2 mmol) and 10% Pd/C (60 mg) were added to distilled 2-propanol (50 mL) containing acetic acid (3 mL) and 0.1 N aqueous HCl (3 drops). After flushing the reaction mixture at least four times (evacuation, followed by hydrogen) to remove almost all the oxygen present, the mixture was stirred under 1 atm of hydrogen for 6 h. The mixture was then rapidly filtered and taken to dryness in vacuo. The residue was purified by preparative silica gel TLC using 2-propanol–ethyl acetate (2:8, v/v). Since the R_f of the product varied depending on impurities that were present, the product band was the main fluorescent one under long wavelength UV light. Recrystallization from methanol yielded 45 mg (92%) of a white solid: mp 276–278 °C; ^{1}H NMR δ 0.97 (t, 3), 1.58 (m, 4), 2.28 (s, 3), 3.16 (t, 2), 4.00 (s, 3), 7.89 (s, 1); UV λ_{max} (10% aqueous MeOH, pH 2.1) 232 (ϵ 31 100), 283 (7600); (10% aqueous MeOH, pH 6.2) 231 (27 000), 265 (6200), 310 (5700); (10% aqueous MeOH, pH 9.4) 233 (29 000), 273 (6300), 303 (7050).

Anal. Calcd for $C_{13}H_{17}N_5O$: C, 60.21; H, 6.60; N, 27.01, Found: C, 60.21; H, 6.57; N, 26.97.

Methyl 2-Carbomethoxyamino-4-iodobutyrate (21). To a suspension of methyl 2-amino-4-iodobutyrate hydriodide[44] (50 g, 0.18 mol) in dry ether (300 mL) at 0 °C, methyl chloroformate (25.40 g, 1.5 mol) and triethylamine (36.43 g, 2.0 mol) were added while the suspension was stirred with a mechanical stirrer. After 4 h at 0 °C, the mixture was stirred overnight at room temperature. Unreacted methyl chloroformate was decomposed by the addition of 100 mL of ice water. After separating the layers, the aqueous fraction was extracted with ether and the combined ethereal fractions were washed with 0.1 N aqueous sodium thiosulfate and saturated aqueous sodium chloride. After drying the ether solution over sodium sulfate and filtration, the ether was removed in vacuo. Chromatography of the residue on 500 g of silica gel gave the desired product as the only material that eluted with 1 L of ether–hexane (2:8). The yield was 46.5 g (85%): mp 58–59 °C; ^{1}H NMR ($CDCl_3$) δ 2.34 (m, 3), 3.20 (t, 2, J = 8 Hz), 3.73 (s, 3), 3.79 (s, 3), 4.40 (m, 1), 5.75 (brd, 1, J = 8 Hz); IR (KBr) 3318, 1733, 1717, 1693, 1538, 591 cm^{-1}.

Exact mass. Calcd for $C_7H_{12}INO_4$: 420.9770. Found: 420.9765.

Methyl 5-Carbobenzyloxy-2-carbomethoxyamino-6-oxo-1-heptanoate (22). To a suspension of sodium hydride (4.2 g of a 57% oil dispersion, 0.1 mol) in dioxane (150 mL), benzyl acetoacetate (19.1 g, 0.1 mol) in 100 mL of benzene was slowly added. After stirring for 1 h, methyl 2-methylcarbamoyl-4-iodobutyrate (**21**; 25.0 g, 0.08 mol) in 50 mL of benzene was added. The solution was refluxed for 24 h while mixing with a mechanical stirrer. After cooling, the mixture was filtered and the solid was washed with dioxane. The filtrate was reduced to an oil in vacuo and then suspended in water. After adjusting the pH to 5 with 1 N HCl, the aqueous mixture was extracted with chloroform. The chloroform solution was dried with magnesium sulfate, filtered, and concentrated to an oil in vacuo. The oil was chromatographed on silica gel (200-fold ratio of silica gel to compound) and eluted with chloroform until all the benzyl acetoacetate had come off. The solvent was then changed to a 50:1 mixture of chloroform–acetone to separate the product **22** from the iodobutyrate **21**. Combining the pure fractions and rechromatography of the impure fractions yielded 19 g (55–65%) of the desired triester **22** as an oil. If the oil was colored, it was treated with 10% Pd/C (activated charcoal does not work) until the oil was colorless: ^{1}H NMR ($CDCl_3$) δ 1.84 (m, 4), 2.16 (s, 3), 3.47 (m, 1), 3.69 (s, 3), 3.72 (s, 3), 4.36 (m, 1), 4.46 (brd, 1, J = 8 Hz), 5.20 (s, 2), 7.40 (s, 5); IR (liquid film) 3360, 2955, 1716, 1523 cm^{-1}.

Exact mass. Calcd for $C_{18}H_{23}NO_7$: 365.1474. Found: 365.1480.

Methyl 2-Carbomethoxyamino-6-oxoheptanoate. To a solution of **22** (10 g, 27 mmol) in methanol, 10% Pd/C (2 g) was added and the mixture was reduced under 1 atm of hydrogen. If the **22** was colored, the catalyst became poisoned and no reduction would take place. After the uptake of 600 mL of hydrogen, the mixture was filtered through Celite and the methanol was removed in vacuo while keeping the solution cold. The residue was partitioned between ether and 1 N aqueous sodium bicarbonate. After separating the layers, the aqueous fraction was extracted with chloroform. After drying and filtering, the chloroform solution was concentrated in vacuo and decarboxylated at 0.5 mm and room temperature for 24 h to yield 5.2 g of the debenzylated acid (83%), a viscous oil: ^{1}H NMR ($CDCl_3$) δ 1.72 (m, 4), 2.18 (s, 3), 2.50 (t, 2), 3.77 (s, 3), and 3.83 (s, 3); IR (liquid film) 3330, 1742, 1710 cm^{-1}.

Exact mass. Calcd for $C_{10}H_{17}NO_5$: 231.1106. Found: 231.1112.

The acid could be isolated by recrystallizing in the cold the residue from the chloroform extraction, but it underwent decarboxylation at room temperature to the title heptanoate: ^{1}H NMR ($CDCl_3$) δ 1.86 (m, 4), 2.18 (s, 3), 3.45 (m, 1), 3.69 (s, 3), 4.38 (m, 1), 4.49 (brd, 1, J = 8 Hz).

Methyl 5-Bromo-2-carbomethoxyamino-6-oxoheptanoate (23). Method A. To a solution of the above acid (5 g, 18 mmol) in methanol (25 mL) and chloroform (25 mL), bromine (3.2 g, 20 mmol) was added over a 4-h period at 0 °C. After the bromine had reacted, water (10 mL) was added and the mixture was heated to 55 °C to cause the decarboxylation. Alternatively, nitrogen was bubbled through the solution to remove the HBr; the solvent was removed in vacuo and the oil was decarboxylated at 0.5 mm for 18 h. In either case, after decarboxylation the bromo ketone was extracted into chloroform from the neutralized aqueous mixture. After drying with magnesium sulfate and filtering, the chloroform was removed in vacuo to yield 4 g of an oil. Repeated column chromatography on silica gel using chloroform–acetone (90:10, v/v) as eluting solvent was needed to separate the desired 5-bromo compound from the undesired 7-bromo and the unbrominated compounds. The purified oil could then be crystallized from ether–hexane to yield a white solid: mp 73 °C; ^{1}H NMR ($CDCl_3$) δ 2.02 (m, 2), 2.38 (s, 3), 3.78 (s, 3), 4.39 (m, 1), 5.40 (brd, 1); IR (KBr pellet) 3330, 1745, 1710, 1690, 1540, 639 cm^{-1}.

Anal. Calcd for $C_{10}H_{16}BrNO_5$: C, 38.72; H, 5.19; N, 4.51. Found: C, 38.82; H, 5.32; N, 4.45.

Method B. To a solution of methyl 2-carbomethoxyamino-6-oxoheptanoate (4.6 g, 18 mmol) in methanol (25 mL) and chloroform (25 mL), bromine (3.2 g, 20 mmol) was added over a 4-h period at 0 °C. After the bromine had reacted the solution was extracted with 10% aqueous NaCl. The chloroform fraction was worked up as above and yielded similar amounts of unbrominated, monobrominated (5- and 7-bromo), and dibrominated compounds.

Dimethyl α-(Carboxyamino)-1-benzyl-4,9-dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine-7-butyrate (or (or 1-benzyl-Y_{SC} base). After bubbling nitrogen through a solution of 7-benzyl-3-methylguanine (255 mg, 1 mmol) and purified *N,N*-diethylaniline (745 mg, 5 mmol) in dry DMF, the solution was stirred at 50 °C while methyl 5-bromo-2-carbomethoxyamino-6-oxoheptanoate (310 mg, 1 mmol) in dry DMF (5 mL) was added over 8 h. The reaction was stirred at 50 °C an additional 10 h. After adding silica gel (2 g), the solvent was removed in vacuo. The solid was placed atop a 50-g silica gel column which was first eluted with 500 mL of benzene. Then elution with 2-propanol–ethyl acetate (2:8, v/v) gave a single fluorescent band (to long wavelength UV lamp). This material was then rechromatographed on silica gel TLC plates using 2-propanol–ethyl acetate (2:8, v/v). The major fluorescent band (R_f varied) was isolated and recrystalyzed from methanol to yield 93 mg of crystals (20%): ^{1}NMR ($CDCl_3$) δ 2.12 (m, 2), 2.26 (s, 3), 3.20 (m, 2), 3.67 (s, 3), 3.70 (s, 3), 3.90 (s, 3), 4.40 (m, 1), 5.58 (s, 2), 7.36 (s, 5), 8.35 (s, 1); UV (MeOH) 237 (ϵ 24 100), 261 (4900), 313 (3200) nm.

Exact mass Calcd for $C_{23}H_{26}N_6O_5$: 466.1963. Found: 466.1958.

Dimethyl (±)-α-(Carboxyamino)-4,9-dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2*a*]purine-7-butyrate (or Y_{SC} base, 1). The 1-benzyl-Y_{SC} base (47 mg, 0.1 mmol) and 10% Pd/C were added to distilled 2-propanol (50 mL) containing acetic acid (3 mL) and 0.1 N aqueous HCl (3 drops). After flushing the reaction mixture at least four times (evacuation, followed by hydrogen) to remove almost all the oxygen present, the mixture was stirred under 1 atm of hydrogen for 6 h. The mixture was then rapidly filtered and taken to dryness in vacuo. The residue was purified by preparative silica gel TLC (20 × 40 cm plate) using 2-propanol–ethyl acetate (2:8, v/v) and then recrystallized from methanol to yield 32 mg (85%) of a white solid. The spectral properties of this racemic compound were identical with those reported in the literature.

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Registry No.—**1,** 35693-53-9; 1 benzyl derivative, 65120-77-6; **2,** 33359-03-4; **4,** 2958-98-7; **12,** 65120-78-7; **14,** 65120-79-8; **15,** 65120-80-1; **16,** 1807-85-8; **17,** 56025-86-6; **18,** 65120-81-2; **19,** 65138-60-5; **20,** 35693-54-0; **21,** 65120-82-3; **22,** 65120-83-4; **22** dibenzyl derivative, 65120-84-5; **23,** 65120-85-6; **23,** 7-bromo derivative, 65120-86-7; **23,** dibromo derivative, 65120-87-8; 2,6-diamino-1,4-dihydro-1-methyl-4-pyrimidone, 51093-34-6; 2,6-diamino-1,4-dihydro-1-methyl-5-nitroso-4-pyrimidone, 58160-46-6; 1,4-dihydro-1-methyl-2,5,6-triamino-4-pyrimidone sulfate, 65120-88-9; benzyl bromide, 100-39-0; methyl iodide, 74-88-4; bromoacetone, 598-31-2; 3-bromo-2-heptanone, 51134-59-9; methyl 2-amino-4-iodobutyrate hydriodide, 65166-01-0; methyl chloroformate, 79-22-1; benzyl acetoacetate, 5396-89-4.

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