BIOORGANIC CHEMISTRY 8, 339–349 (1979)

A Novel Method for the Methylation of Heterocyclic Amino Groups. Conversion of Guanosine into Its 2-*N*-Methyl- and 2-*N*,2-*N*-Dimethyl Derivatives[1]

PETER K. BRIDSON AND COLIN B. REESE[2]

Department of Chemistry, King's College, Strand, London WC2R 2LS, England

Received February 5, 1979

The heterocyclic amino-compounds **11a**, **13a**, **13b**, and **17** reacted with formaldehyde and *p*-thiocresol (**14**) in alcoholic solution to give the corresponding *N*-methylphenylthiomethyl derivatives (**16**, **15a**, **15b**, and **18a**, respectively) in satisfactory to good yields. The reactions were catalyzed by acetic acid. 2-*N*-Methylguanosine (**6a**) was obtained in good yield by treatment of **15b** with sodium borohydride followed by acidic hydrolysis, or alternatively by Raney nickel desulfurization of **15a** followed by ammonolysis of the product. Sodium borohydride reduction of **18a** gave **21** in good yield. 2-*N*,2-*N*-Dimethylguanosine (**6b**) was obtained from **19a** in three steps.

INTRODUCTION

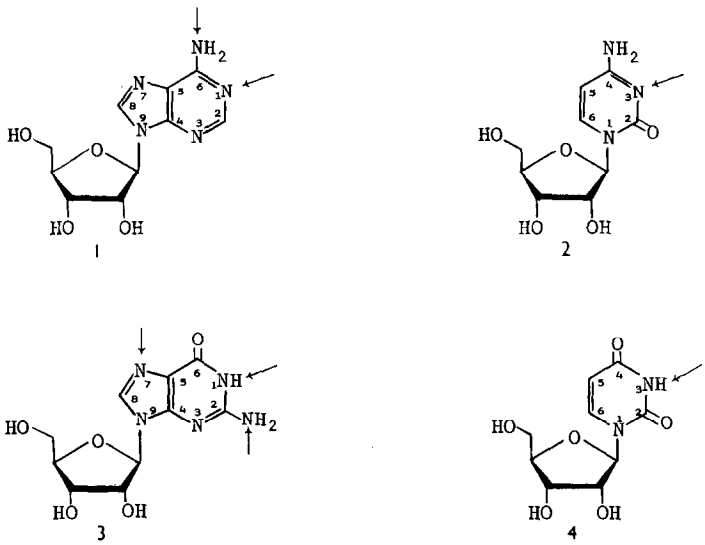
Virtually all transfer ribonucleic acid (tRNA) molecules (*1*) are composed of a number of modified ribonucleosides (*2*) in addition to adenosine (**1**), cytidine (**2**), guanosine (**3**), and uridine (**4**). Some of these modified nucleosides are simple *N*-methyl derivatives of the principal ribonucleosides (**1–4**). The known sites of *N*-methylation in tRNA molecules (*1*, *2*) are indicated by arrows in Scheme 1.

Biosynthetic studies have revealed (*2*) that *N*-methyl groups are introduced at the polynucleotide level by reaction between *S*-adenosylmethionine or methionine and the unmethylated tRNA molecules in the presence of the appropriate enzymes. Chemical studies between nucleosides (and derivatives) have led to the general conclusions that (i) adenosine (**1**) reacts (*3*) at *N*-1 with methylating agents of the type Me-X (e.g., dimethyl sulfate and methyl iodide), (ii) cytidine (**2**) reacts (*4*) at *N*-3 with methylating agents of the same type, (iii) guanosine (**3**) reacts at *N*-7 with diazomethane (*5*) and with methylating agents of the type Me-X (*3*) and (iv) uridine (**4**) reacts at *N*-3 with diazomethane (*6*). While, in the presence of potassium carbonate, guanosine (**3**) [i.e. the conjugate base of (**3**)] reacts at *N*-1 with methyl iodide (*7*), no method has yet been described for the direct and specific methylation of adenosine (**1**), cytidine (**2**), and guanosine (**3**) on their exocyclic amino groups. However, von Minden and McCloskey (*8*) have obtained mass spectrometric evidence that **1**, **2**, and **3** react on a small scale ($2–100 \times 10^{-6}$ g) with methyl iodide in the presence of methylsulfinyl ion in dimethyl sulfoxide solution to give permethylated products containing dimethylamino groups. It seems unlikely that this process will prove to be of synthetic value even when

1 This paper is dedicated to the memory of Professor George Kenner.

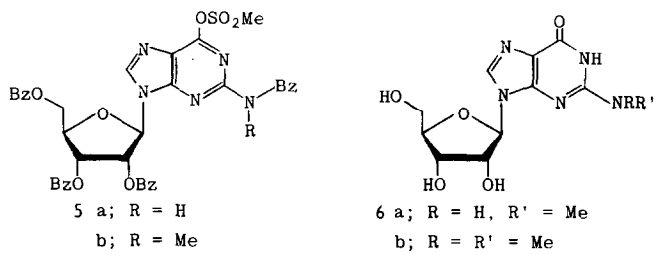
2 To whom correspondence should be addressed.

0045-2068/79/030339—11$02.00/0
Copyright © 1979 by Academic Press, Inc.
All rights of reproduction in any form reserved.
Printed in Great Britain



SCHEME 1

appropriate protecting groups are used. Recently, we reported (*9*) that the protected guanosine derivative (**5a**), which may be prepared in two steps from guanosine (**3**), reacts with diazomethane at *N*-2 and that the product (**5b**) may be unblocked to give 2-*N*-methylguanosine (**6a**).



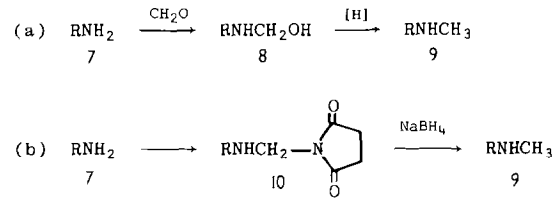
SCHEME 2

Although 6-*N*-alkyl derivatives (*10*) of adenosine (**1**) and 4-*N*-alkyl derivatives (*11*) of cytidine (**2**) (see Scheme 1) may be obtained readily and in good yields from relatively easily accessible nucleoside starting materials, this has not been the case for 2-*N*-methyl- and 2-*N*,2-*N*-dimethyl-guanosines (**6a** and **6b**). Both of the latter compounds occur widely (*1*) in tRNA. We have therefore undertaken a search for a convenient general method for the methylation of heterocyclic amino-compounds on their exocyclic nitrogen atoms with the particular view of developing satisfactory procedures for the conversion of guanosine into its 2-*N*-methyl- and 2-*N*,2-*N*-dimethyl-derivatives (**6a** and **6b**, respectively).

DISCUSSION

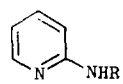
As "amidine-like" heterocyclic amino-compounds [e.g. adenosine (**1**) and cytidine (**2**)] generally react with methylating agents of the type Me-X on their endocyclic nitrogen atoms, it seemed that a reductive methylation process, based on formaldehyde,

would be more likely to be suitable for the present purpose. The general principle of such a process is indicated in Scheme 2a. Indeed, both aliphatic and aromatic primary amines have been methylated by treatment with formaldehyde and formic acid (*12*) or sodium cyanoborohydride (*13*). The reduction step (**8** → **9**, Scheme 2a) has also been effected by catalytic hydrogenolysis (*14*).



SCHEME 3

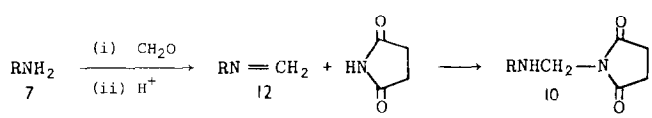
As these procedures (Scheme 2a) often lead directly to dimethyl or to mixtures of mono- and dimethyl derivatives, our attention was drawn to a related procedure (Scheme 2b). A few years' ago, Kadin (*15*) reported that the products (**10**) obtained (*16*) by treating aromatic primary amines (**7**) with formaldehyde and succinimide react with sodium borohydride in warm dimethyl sulfoxide solution to give the corresponding *N*-methyl derivatives (**9**). The latter compounds may usually be isolated in satisfactory to good yields. It is especially noteworthy in the present context that Kadin (*15*) was able to convert 2-aminopyridine (**11a**) into 2-methylaminopyridine (**11b**) in 41% yield by this procedure.



11 a; R = H
b; R = Me

SCHEME 4

Unfortunately, we were unable to prepare a 2-*N*-succinimidomethylene-guanosine derivative. A possible course for the formation of succinimidomethylene derivatives (**10**) is indicated in Scheme 3. If the amino-compound (**7**) is only weakly nucleophilic, the formation of **12** (via **8**) would not be favored, and the chances of its being trapped by reaction with the conjugate base of succinimide would thereby be decreased. Furthermore, it occurred to us that succinimide might not be a particularly suitable trapping agent in that its conjugate base is not an especially good nucleophile.

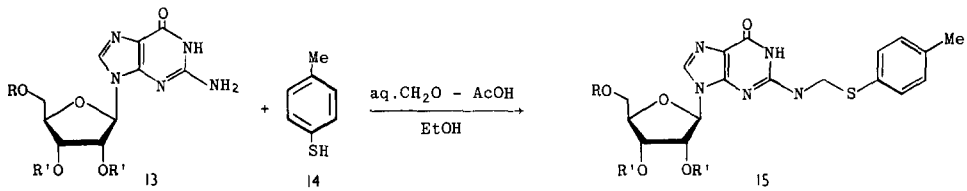


SCHEME 5

We then thought that thiophenols might have better properties than succinimide for this purpose. Thiophenols are generally stronger acids than succinimide[3] and are thus appreciably more dissociated in neutral and weakly acidic media. Furthermore, the conjugate bases of thiophenols (e.g., PhS^-) are known to be powerful nucleophiles (*18*). It further seemed likely that the adducts obtained (see below) would be convertible into

[3] The pK_a's of succinimide and thiophenol are 9.62 and 6.5, respectively (*17*).

the corresponding *N*-methyl derivatives by reduction either with sodium borohydride or Raney nickel. We now report the results of some preliminary experiments carried out with *p*-thiocresol (**14**).

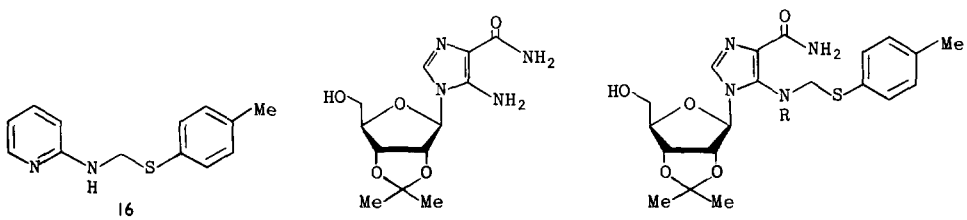


13 14 15

a; R = R' = Ac
b; R = H, R'R' = $Me_2C<$

SCHEME 6

When 2',3',5'-tri-*O*-acetylguanosine (**13a**) was heated, under reflux, for 4 hr with just under 3 molecular equivalents of aqueous formaldehyde and just over 3 molecular equivalents of *p*-thiocresol (**14**) in the presence of acetic acid in ethanol solution, the desired product (**15a**) was obtained in 84% isolated yield. Indeed, much of the product was precipitated as a homogeneous (tlc) crystalline solid when the reaction mixture was cooled. 2',3'-*O*-Isopropylidene-2-*N*-(*p*-methylphenylthiomethyl)guanosine (**15b**) was similarly prepared from 2',3'-*O*-isopropylideneguanosine (**13b**) in 76% yield. The structures assigned to **15a** and **15b** are based on spectroscopic and analytical data (see Experimental) and on their conversions to 2-*N*-methylguanosine (**6a**) (see below).



16 17

18 a; R = H
b; R = 4-$MeC_6H_4SCH_2$

SCHEME 7

In order to investigate the scope of the *p*-thiocresol-formaldehyde alkylation reaction, 2-aminopyridine (**11a**) was heated with a stoichiometric quantity of *p*-thiocresol (**14**) and a slight excess of formaldehyde, under reflux, in methanol solution for 1 hr. 2-(*p*-Methylphenylthiomethyl)aminopyridine (**16**) was thereby obtained and isolated as a crystalline solid in 65% yield. This reaction proceeded readily under mild conditions without the addition of an acid catalyst. The reaction between *p*-thiocresol (**14**), formaldehyde, and the 2',3'-*O*-isopropylidene derivative (**17**) of 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (AICA-riboside) was also investigated. This experiment was regarded as a particularly important test of the new reaction, as the 5-*N*-methyl derivative of AICA-riboside was required in the synthesis of the then unreported[4] 3-*N*-methylguanosine, the precursor of the so-called Y-ribosides. The latter hypermodified nucleosides are located adjacent to the anticodons of phenylalanine-

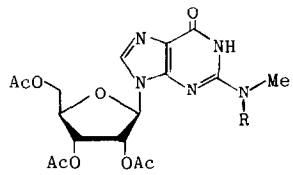
[4] Very recently, two independent syntheses of 3-*N*-methylguanosine have been described in the literature (*19*).

specific tRNAs (*20*). Preliminary experiments involving model compounds had suggested (*21*) that AICA-riboside would react with methylating agents of the type Me-X (see above) on *N*-3 and that 5-*N*-acyl derivatives of AICA-riboside would react with diazomethane also on *N*-3. Although there was some indication (*21*) that 5-amino-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide (**17**) reacted with formaldehyde and succinimide (Scheme 3), attempts to find reproducible experimental conditions for the formation of the desired product, even in modest yield, were unsuccessful.

However, when **17** was heated, under reflux, for 1 hr with *p*-thiocresol (ca. 4.5 molecular equivalents) and aqueous formaldehyde (ca. 4.1 molecular equivalents) in the presence of acetic acid in methanol solution, it was converted into a mixture of the desired product (**18a**, 54%) and what was believed to be the bis-*p*-methylphenylthiomethyl derivative (**18b**, ca. 30%). The 5-*N*-mono-*p*-methylphenylthiomethyl derivative (**18a**) was not obtained crystalline, but its structure followed from its spectroscopic properties and from the fact that it reacted with sodium borohydride to give the corresponding 5-*N*-methyl derivative (**21**, see below) which was, in turn, converted into 2′,3′-*O*-isopropylidene-3-*N*-methylguanosine (*21*). As 5-amino-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide (**17**) is a relatively valuable starting material, an attempt was made to suppress the formation of **18b**; when **17** was heated with *p*-thiocresol (ca. 2.4 molecular equivalents) and aqueous formaldehyde (ca. 2.5 molecular equivalents), under gentle reflux, in dioxan solution in the absence of acetic acid for 2 hr and the products then fractionated by chromatography, unreacted starting material (**17**; 65%), **18a** (26%) and almost no **18b** were obtained. The yield of **18a**, based on consumed starting material (**17**), was 74% in the latter experiment. Neither of these experiments has been optimized, but it seems likely that the yield of **18a** could be improved by changing the reaction conditions.

The utility of *N*-*p*-methylphenylthiomethyl derivatives as intermediates in the preparation of *N*-methyl derivatives of heterocyclic amino-compounds was readily demonstrated with guanosine. When 2′,3′-*O*-isopropylidene-2-*N*-(*p*-methylphenylthiomethyl)guanosine (**15b**) was treated with an excess of sodium borohydride in dimethyl sulfoxide solution for 1 hr at 100°C and the products subjected to acidic hydrolysis, 2-*N*-methylguanosine (**6a**) was obtained in 64% yield. The desired reduction could also be effected with Raney nickel. Treatment of 2′,3′,5′-tri-*O*-acetyl-2-*N*-(*p*-methylphenylthiomethyl)guanosine (**15a**) with Raney nickel in boiling aqueous methanol solution, followed by ammonolysis of the resulting 2′,3′,5′-tri-*O*-acetyl-2-*N*-methylguanosine (**19a**), gave 2-*N*-methylguanosine (**6a**) in 37% yield. Apparently, no product other than **19a** was detected in the Raney nickel reduction reaction, and it is not clear why the yield was so low.

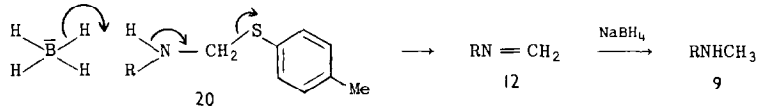
The synthesis of 2-*N*,2-*N*-dimethylguanosine (**6b**) was then investigated. While the formation of a bis-*p*-methylphenylthiomethyl derivative (**18b**) was observed in the reaction between *p*-thiocresol (**14**), formaldehyde, and 5-amino-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide (**17**), such bis-alkylation was not observed in the case of the guanosine derivatives (**13a** and **13b**) examined. This result may have been due partly to the limited solubility of the mono-alkylated products (**15a** and **15b**, respectively) in the reaction medium. However, when 2′,3′,5′-tri-*O*-acetyl-2-*N*-methylguanosine (**19a**) was heated, under reflux, with ca. 3 molecular



19 a; R = H
b; R = 4-MeC$_6$H$_4$SCH$_2$
c; R = Me

SCHEME 8

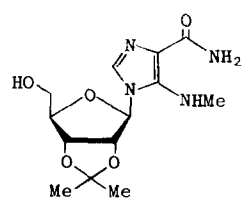
equivalents of *p*-thiocresol (**14**) and ca. 6 molecular equivalents of formaldehyde in the presence of acetic acid in ethanol solution, **19b** was obtained in virtually quantitative yield. When the latter compound (**19b**) was heated, under reflux, with Raney nickel in aqueous methanol solution, both 2',3',5'-tri-*O*-acetyl-2-*N*,2-*N*-dimethylguanosine (**19c**, 30%) and **19a** (32%) were obtained. Treatment of **19c** with methanolic ammonia gave 2-*N*,2-*N*-dimethylguanosine (**6b**). Raney nickel desulfurization of **19b** was rather unsatisfactory in that it led to a slightly higher yield of **19a** than of the desired product (**19c**). However, no attempt was made to reduce **19c** with sodium borohydride, as it was assumed that the reaction of *p*-methylphenylthiomethyl derivatives (**20**) of primary amino-compounds with sodium borohydride (Scheme 5), like the reaction (see above) of the corresponding succinimide derivatives (**10**), proceeded via an initial elimination process (*15*).



20 12 9

SCHEME 9

Finally, the reaction between 5-(*p*-methylphenylthiomethyl)amino-1-(2',3'-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide (**18a**) and sodium borohydride was investigated. When **18a** was heated, under reflux, with an excess of sodium borohydride in 1,2-dimethoxyethane solution for 20 min, 5-methylamino-1-(2',3'-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide (**21**) was obtained as the sole nucleoside product and isolated as a crystalline solid in 76% yield.



21

SCHEME 10

In conclusion, we believe that the reaction of amino-compounds with formaldehyde and *p*-thiocresol (or some other thiol), followed by reduction of the resulting derivatives, is likely to prove to be a generally effective method of *N*-methylation. It seems probable that the procedure could be improved by a number of modifications. Thus, in the case

of the alkylation of the AICA-riboside derivative (**17**), it is possible that bis-alkylation would be suppressed by using a bulkier thiol than *p*-thiocresol (**14**). In the case of derivatives of secondary amino-compounds (e.g., **19b**), it might possibly prove to be beneficial if the oxidation level of the sulfur atom were increased before the reduction step was attempted. It is further conceivable that the alkylation reaction could be made more general by using aldehydes other than formaldehyde. Modifications such as these are now under investigation in our laboratory.

EXPERIMENTAL

Ultraviolet absorption spectra were measured, usually in 95% ethanol solution, on a Perkin–Elmer Model 402 or a Cary Model 17 spectrophotometer. ^{1}H nmr spectra were obtained in $CDCl_3$ or $(CD_3)_2SO$ solution (with CD_3OD or D_2O added to exchange relatively acidic protons) on a Perkin–Elmer R12B or a Bruker HFX 90 FT spectrometer; ^{13}C nmr spectra were obtained in $CDCl_3$ or $(CD_3)_2SO$ solution on the latter spectrometer. Tlc was carried out on Merck 60 F_{254} plates which were developed in chloroform–methanol mixtures of varying proportions. Short column chromatography was carried out on Reeve Angel SO.TLC silica gel with chloroform containing added ethanol as the eluting solvent.

2′,3′,5′-Tri-O-acetyl-2-N-(p-methylphenylthiomethyl)guanosine (**15a**). 2′,3′,5′-Tri-*O*-acetylguanosine (*22*) (2.0 g, 4.9 mmol), *p*-thiocresol (2.0 g, 16 mmol), 40% aqueous formaldehyde (1.0 ml, 14 mmol), and acetic acid (1 ml) were heated together, under reflux, in ethanol (15 ml) solution for 4 hr. The products were cooled and the colorless precipitate (1.9 g) was collected by filtration. A second crop (0.35 g) was obtained from the mother liquors. Total yield, 2.25 g (84%); mp, after recrystallization from water containing a small quantity of dimethyl sulfoxide, 228°C; uv (95% ethanol): λ_{max} 258 (ε 20 000), λ_{infl} 274 nm (ε 14 500); ^{1}H nmr [$(CD_3)_2SO$, 90 MHz] δ 1.99 (3H, s), 2.10 (6H, s), 2.27 (3H, s), 4.0–4.5 (3H, m), 4.91 (2H, m), 5.68 (1H, m), 5.85–6.1 (2H, m), 7.15 (3H, m), 7.36 (2H, d, J = 8 Hz), 7.93 (1H, s), 10.80 (1H, br.s); ^{13}C nmr [$(CD_3)_2SO$, 22.63 MHz] δ 45.65 (–NHCH$_2$S–).

Anal. Calcd for $C_{24}H_{27}N_5O_8S$: C, 52.8; H, 5.0; N, 12.8. Found: C, 52.4; H, 5.0; N, 12.9%.

2′,3′-O-Isopropylidene-2-N-(p-methylphenylthiomethyl)guanosine (**15b**). 2′,3′-*O*-Isopropylideneguanosine (*23*) (2.5 g, 7.7 mmol), *p*-thiocresol (2.5 g, 20 mmol), 40% aqueous formaldehyde (1.65 ml, 23 mmol), acetic acid (4 ml), and ethanol (50 ml) were heated, under reflux, for 16 hr. The products were cooled and the colorless precipitate (2.4 g) was collected by filtration. A second crop (0.3 g) was obtained from the mother liquors. Total yield, 2.7 g (76%); mp 204–206°C; uv (95% ethanol): λ_{max} 256 (ε 19 700), λ_{infl} 272 (ε 15 200), λ_{min} 226 nm (ε 10 500); ^{1}H nmr [$(CD_3)_2SO$, 90 MHz] δ 1.35 (3H, s), 1.54 (3H, s), 2.27 (3H, s), 3.53 (2H, m), 4.13 (1H, m), 4.8–5.1 (3H, m), 5.36 (1H, dd, J = 2.3 and 5.9 Hz), 6.01 (1H, d, J = 2.3 Hz), 7.15 (3H, m), 7.37 (2H, d J = 8.2 Hz), 7.95 (1H, s), 10.78 (1H, br.s); ^{13}C nmr [$(CD_3)_2SO$, 22.63 MHz] δ 46.21 (–NHCH$_2$S–).

Anal. Calcd for $C_{21}H_{25}N_5O_5S$: C, 54.9; H, 5.5; N, 15.2. Found: C, 54.7; H, 5.5; N, 15.0%.

2-(p-Methylphenylthiomethyl)aminopyridine (**16**). A solution of *p*-thiocresol (0.25 g, 2.0 mmol) and 40% aqueous formaldehyde (0.16 ml, 2.2 mmol) in methanol (10 ml) was heated, under reflux, for 10 min. 2-Aminopyridine (0.19 g, 2.0 mmol) was then added and the reactants were heated, under reflux, for a further period of 1 hr. The products were then concentrated under reduced pressure and the residue crystallized from cyclohexane; yield, 0.304 g (65%); mp 68°C; uv (95% ethanol): λ_{max} 249, 299 (ε 19 000, 5 500) λ_{min} 282 nm (ε 4500); ^{1}H nmr ($CDCl_3$, 60 MHz) δ 2.28 (3H, s), 4.78 (2H, d, $J \sim 7$ Hz), 5.1–5.6 (1H, m), 6.3–6.8 (2H, m), 6.9–7.6 (5H, m), 8.09 (1H, m).

Anal. Calcd for $C_{13}H_{14}N_2S$: C, 67.8; H, 6.1; N, 12.2. Found: C, 67.7; H, 6.2; N, 12.1%.

5-(p-Methylphenylthiomethyl)amino-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-imidazole-4-carboxamide (**18a**). (a) A solution of 5-amino-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide (*24*) (1.014 g, 3.4 mmol), *p*-thiocresol (1.9 g, 15.3 mmol), 40% aqueous formaldehyde (1.0 ml, 14 mmol), and acetic acid (0.5 ml) in methanol (30 ml) was heated, under reflux, for 1 hr. Methanolic ammonia (half-saturated at 0°C; 1 ml) was added to the cooled products which were then concentrated under reduced pressure and fractionated by short column chromatography (*25*).

A product with R_f 0.65 [$CHCl_3$–MeOH (4:1 v/v)], believed to be 5,5-di(*p*-methylphenylthiomethyl)amino-1-(2′,3′,-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide (**18b**), was eluted first from the column and isolated as a glass (0.58 g, ca. 30%); ^{1}H nmr ($CDCl_3$, 60 MHz) δ 1.29 (3H, s), 1.44 (3H, s), 8.26 (6H, s), 3.76 (2H, m), 4.03 (1H, m), 4.5–5.1 (6H, m), 5.39 (1H, d, $J \sim 4$ Hz), 7.01 (4H, d, $J \sim 7$ Hz), 7.29 (4H, d, $J \sim 8$ Hz), 7.70 (1H, s). The desired product (**18a**; R_f 0.53) was eluted next and isolated as a colorless glass (0.80 g, 54%); uv (95% ethanol): λ_{max} 253, λ_{infl} 265 nm; ^{1}H nmr ($CDCl_3$, 60 MHz) δ 1.35 (3H, s), 1.56 (3H, s), 2.31 (3H, s), 3.77 (2H, m), 4.31 (1H, m), 4.6–5.2 (4H, m), 5.66 (1H, br.s), 6.0–6.5 (2H, m), 7.09 (2H, d, $J \sim 8$ Hz), 7.36 (2H, d, $J \sim 8$ Hz), 7.42 (1H, s). (b) 40% Aqueous formaldehyde (1.2 ml, 16.7 mmol) was added to a solution of 5-amino-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide (2.0 g, 6.7 mmol) and *p*-thiocresol (2.0 g, 16.1 mmol) in anhydrous dioxan (100 ml). The reactants were heated, under gentle reflux, for 2 hr and the products concentrated under reduced pressure and then separated by short column chromatography. Two main fractions [(i) and (ii)] were obtained: fraction (i) consisted of the desired product (**18a**; 0.75 g, 26%) and fraction (ii) consisted of unchanged starting material (1.30 g). Yield of **18a**, based on consumed starting material (**17**), 74%.

2-N-Methylguanosine (**6a**). (a) Sodium borohydride (0.15 g, 4.0 mmol) was added to a solution of 2′,3′-*O*-isopropylidene-2-*N*-(*p*-methylphenylthiomethyl))guanosine (1.0 g, 2.2 mmol) in dimethyl sulfoxide (5 ml). The reactants were heated at 100°C for 1 hr and then cooled to room temperature. After they had been diluted with dimethyl sulfoxide (5 ml), the products were poured into rapidly stirred *M*-potassium phosphate buffer (pH 6.0, 100 ml). The colorless precipitate obtained was collected by filtration, washed thoroughly with water, and then allowed to dry. After this material had been further washed with ether, it was dissolved in 95% formic acid (20 ml) and the solution was allowed to stand at room temperature for 24 hr. The products were evaporated under reduced pressure and water was added. After further evaporation the residue was crystallized from water to give 2-*N*-methylguanosine (0.44 g, 64%), mp 235°C dec.; the

uv and ^{1}H nmr spectroscopic and tlc properties of this material were identical to those of authentic 2-*N*-methylguanosine (*26*); ^{13}C nmr [$(CD_3)_2SO$, 22.63 MHz] δ 27.6, 61.5, 70.5, 73.3, 85.2, 86.7, 116.6, 136.2, 150.9, 153.1, 156.9.

Anal. Calcd for $C_{11}H_{15}N_5O_5 \cdot H_2O$: C, 41.95; H, 5.4; N, 21.2. Found: C, 41.8; H, 5.4; N, 22.1%.

(b) 2′,3′,5′-Tri-*O*-acetyl-2-*N*-(*p*-methylphenylthiomethyl))guanosine (0.50 g, 0.92 mmol) was suspended in water–methanol (1 : 4 v/v; 25 ml) and a suspension of Raney nickel [prepared from 5 g of Ni–Al (1 : 1 w/w) alloy] in methanol (25 ml) was added. The mixture was heated, under reflux, for 1 hr and filtered. The residue was washed with hot chloroform–methanol and the combined filtrate and washings were concentrated under reduced pressure. The glass (0.21 g) so obtained was found by tlc [$CHCl_3$–MeOH (7 : 1 v/v)] to consist of a single component (R_f 0.40). This material was dissolved in methanolic ammonia (half-saturated at 0°C) and the solution allowed to stand at 20°C for 16 hr. The products were then concentrated under reduced pressure and crystallized from water to give 2-*N*-methylguanosine (0.105 g, 37%), identical to the product obtained by procedure (a) described above.

2-N,2-N-Dimethylguanosine (**6b**). A solution of 2′,3′,5′-tri-*O*-acetyl-2-*N*-methylguanosine [0.296 g, 0.70 mmol; prepared by treating 2′,3′,5′-tri-*O*-acetyl-2-*N*-(*p*-methylphenylthiomethyl)guanosine with sodium borohydride in 1,2-dimethoxyethane-dimethyl sulfoxide solution, reacting the partially deacetylated products with acetic anhydride in pyridine solution and crystallizing the product from propan-2-ol], *p*-thiocresol (0.26 g, 2.1 mmol), 40% aqueous formaldehyde (0.3 ml, 4.2 mmol) and acetic acid (0.3 ml) in ethanol (12 ml) was heated, under reflux, for 16 hr. The cooled products were evaporated under reduced pressure and purified by short column chromatography to give 2′,3′,5′-tri-*O*-acetyl-2-*N*-methyl-2-*N*-(*p*-methylphenylthiomethyl)guanosine as a colorless glass (0.39 g); ^{1}H nmr ($CDCl_3$, 60 MHz) δ 2.05, 2.10 (9 H, ss), 2.22 (3H, s), 3.17 (3H, s), 4.41 (3H, m), 5.14 (2H, m), 5.5–6.1 (3H, m), 7.00 (2H, d, $J \sim 8$ Hz), 7.40 (2H, d, $J \sim 8$ Hz), 7.85 (1H, s).

A suspension of Raney nickel [prepared from 5 g of Ni–Al (1 : 1 w/w) alloy] in methanol–water (4 : 1 v/v; 20 ml) was added to a solution of the above product (0.90 g, 1.6 mmol) in methanol (25 ml). The reactants were heated, under reflux, for 1 hr and then filtered. The residue was washed with hot methanol and the combined filtrate and washings were evaporated under reduced pressure. The residue obtained was separated into two fractions [(a) and (b)] by short column chromatography. The material eluted first (fraction (a)) was identified by ^{1}H nmr spectroscopy as 2′,3′,5′-tri-*O*-acetyl-2-*N*,2-*N*-dimethylguanosine (yield 0.21 g, 30%); fraction (b) was identified as 2′,3′,5′-tri-*O*-acetyl-2-*N*-methylguanosine (0.216 g, 32%).

When fraction (a) was treated with ammonia in methanol solution, it was quantitatively converted into 2-*N*,2-*N*-dimethylguanosine. After recrystallization from water, the latter compound had mp 242°C dec.; its uv and nmr spectroscopic and tlc properties were identical to those of authentic 2-*N*,2-*N*-dimethylguanosine (*26*); ^{13}C nmr [$(CD_3)_2SO$, 22.63 MHz] δ 37.6, 61.3, 70.2, 73.2, 84.9, 86.6, 115.8, 136.4, 150.8, 152.8, 157.3.

Anal. Calcd for $C_{12}H_{17}N_5O_5 \cdot 0.2\ H_2O$: C, 45.8; H, 5.4; N, 22.2. Found: C, 45.9; H, 5.6; N, 22.2%.

5 - Methylamino - 1 - (2′,3′ - O - isopropylidene - β - D - ribofuranosyl)imidazole - 4 -

carboxamide (**21**). Sodium borohydride (0.16 g, 4.2 mmol) was added to a solution of 5-(*p*-methylphenylthiomethyl)amino-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)-imidazole-4-carboxamide (1.0 g, 2.3 mmol) in 1,2-dimethoxyethane (4 ml). The reactants were heated, under reflux, for 20 min and then cooled. The solid precipitate was broken up and acetone (0.25 ml) and acetic acid (0.25 ml) were added. The solution was decanted from the gummy residue which was then extracted with chloroform (3 × 5 ml). The supernatant and the extracts were combined, concentrated under reduced pressure and the residue purified by short column chromatography. Yield of pure product, 0.55 g (76%); mp, after cyrstallization from ethanol, 137°C; uv (95% ethanol): λ_{max} 265 (ε 8900), λ_{min} 222 nm (ε 4600); ^{1}H nmr [$(CD_3)_2SO$–D_2O, 90 MHz] δ 1.34 (3H, s), 1.53 (3H, s), 2.82 (3H, s), 3.54 (2H, m), 4.18 (1H, m), 4.87 (1H, dd, J = 2.6 and 6.2 Hz), 5.06 (1H, dd, J = 3.5 and 6.2 Hz), 5.73 (1H, d, J = 3.5 Hz), 7.59 (1H, s); ^{13}C nmr [$(CD_3)_2SO$, 22.63 MHz] δ 25.1, 26.9, 34.7, 61.1, 80.9, 83.6, 85.5, 89.0, 113.4, 129.8, 144.7, 166.0.

Anal. Calcd for $C_{13}H_{20}N_4O_5$: C, 50.0; H, 6.45; N, 17.9. Found: C, 49.8; H, 6.5; N, 17.7%.

ACKNOWLEDGMENTS

We thank the Science Research Council and Roche Products Limited (C.A.S.E. Studentship awarded to P.K.B.) for support of this work. We also thank Dr. J. Jiricny and Dr. K. J. Divakar for their help and the Ajinomoto Company for a generous gift of AICA-riboside.

REFERENCES

1. M. SPRINZL, F. GRÜTER, AND D. H. GAUSS, *Nucleic Acids Res.* **5**, r15 (1978).
2. R. H. HALL, "The Modified Nucleosides in Nucleic Acids." Columbia University Press, New York and London, 1971.
3. J. W. JONES AND R. K. ROBINS, *J. Amer. Chem. Soc.* **85**, 193 (1963).
4. P. BROOKES AND P. D. LAWLEY, *J. Chem. Soc.* 1348 (1962).
5. J. A. HAINES, C. B. REESE, AND LORD TODD, *J. Chem. Soc.* 5281 (1962).
6. J. A. HAINES, C. B. REESE, AND LORD TODD, *J. Chem. Soc.* 1406 (1964).
7. A. D. BROOM, L. B. TOWNSEND, J. W. JONES, AND R. K. ROBINS, *Biochemistry* **3**, 494 (1964).
8. D. L. VON MINDEN AND J. A. MCCLOSKEY, *J. Amer. Chem. Soc.* **95**, 7480 (1973).
9. P. K. BRIDSON, W. MARKIEWICZ, AND C. B. REESE, *J.C.S. Chem. Commun.* 447 (1977).
10. J. A. JOHNSON, H. J. THOMAS, AND H. J. SCHAIFFER, *J. Amer. Chem. Soc.* **80**, 699 (1958).
11. J. J. FOX, D. VAN PRAAG, I. WEMPEN, I. L. DOERR, L. CHEONG, J. E. KNOLL, M. L. EIDINOFF, A. BENDICH, AND G. B. BROWN, *J. Amer. Chem. Soc.* **81**, 178 (1959); I. WEMPEN, R. DUSCHINSKY, L. KAPLAN, AND J. J. FOX, *J. Amer. Chem. Soc.* **83**, 4755 (1961).
12. M. L. MOORE, *Org. React.* **5**, 301 (1949).
13. R. F. BORCH AND A. I. HASSID, *J. Org. Chem.* **37**, 1673 (1972).
14. J. A. DAVIES, C. H. HASSALL, AND I. H. ROGERS, *J. Chem. Soc.* (*C*) 1358 (1969).
15. S. B. KADIN, *J. Org. Chem.* **38**, 1348 (1973).
16. M. B. WINSTEAD, K. V. ANTHONY, L. L. THOMAS, R. G. STRACHAN, AND H. J. RICHWINE, *J. Chem. Eng. Data* **7**, 414 (1962); *Chem. Abstr.* **58**, 6722f (1963).
17. A. ALBERT AND E. P. SERJEANT, "Ionization Constants of Acids and Bases," pp. 132, 135. Methuen, London, 1962.
18. S. R. HARTSHORN, "Aliphatic Nucleophilic Substitution," p. 49. Cambridge University Press, 1973.

19. S. NAKATSUKA, T. OHGI, AND T. GOTO, *Tetrahedron Lett.* 2579 (1978); T. ITAYA AND K. OGAWA, *Tetrahedron Lett.* 2907 (1978).
20. U. L. RAJBHANDARY, R. D. FAULKNER, AND A. STUART, *J. Biol. Chem.* **243**, 575 (1968); B. S. DUDOCK, G. KATZ, E. K. TAYLOR, AND R. W. HOLLEY, *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **27**, 342 (1968); D. YOSHIKAMI, G. KATZ, E. B. KELLER, AND B. S. DUDOCK, *Biochim. Biophys. Acta* **166**, 714 (1968); L. M. FINK, L. W. LANKS, T. GOTO, AND I. B. WEINSTEIN, *Biochemistry* **10**, 1873 (1971).
21. P. K. BRIDSON, Ph.D. Thesis, London University, 1978, p. 75ff.
22. H. BREDERECK, *Chem. Ber.* **80**, 401 (1947).
23. H. P. M. FROMAGEOT, B. E. GRIFFIN, C. B. REESE, AND J. E. SULSTON, *Tetrahedron* **23**, 2315 (1967).
24. G. SHAW, D. V. WILSON, AND C. P. GREEN, *J. Chem. Soc.* 2650 (1964).
25. B. J. HUNT AND W. RIGBY, *Chem. and Ind.* 1868 (1967).
26. A. YAMAZAKI, I. KUMASHIRO, AND T. TAKENISHI, *J. Org. Chem.* **32**, 3032 (1967).