Conversion of 'AICA-Riboside' into [^{15}N]Guanosines

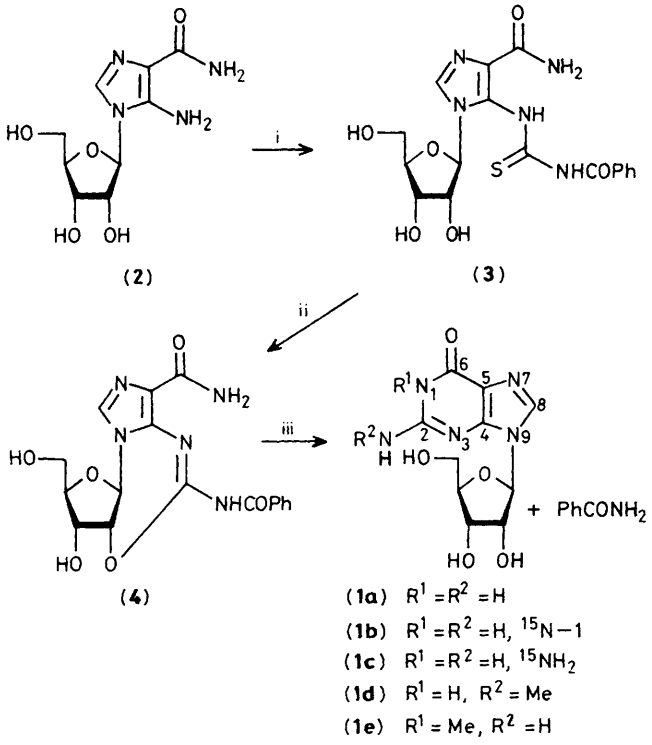
Bernard T. Golding,*[a] Pritpal K. Slaich,[a] and William P. Watson[b]

[a] *Department of Organic Chemistry, Bedson Building, The University, Newcastle upon Tyne NE1 7RU, U.K.*
[b] *Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, Kent ME9 8AG, U.K.*

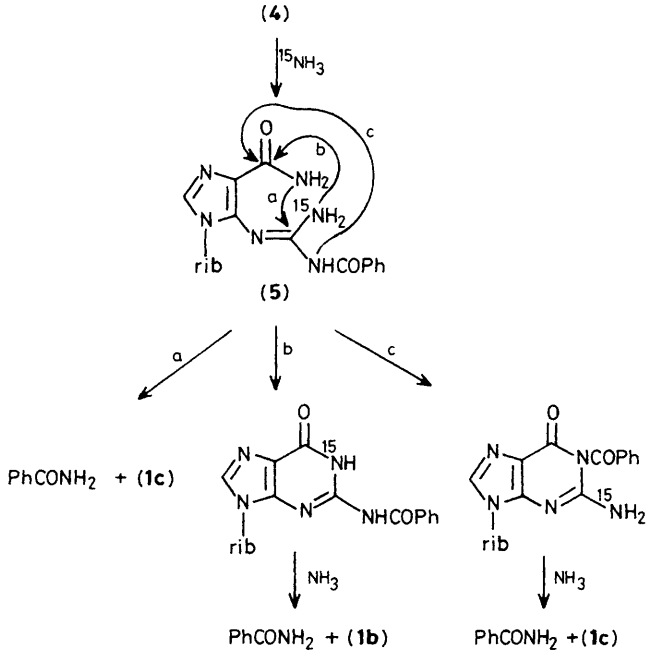
5-Amino-1-β-D-ribofuranosylimidazole-4-carboxamide ('AICA-riboside', **2**) has been converted into [^{15}N]guanosine [label either at N-1 (**1b**) or NH_2 (**1c**)]; the mechanism of formation of the pyrimidine ring in the nucleoside has been elucidated.

For studies of the reactions of guanosine (**1a**) and oligonucleotides with reactive chemical agents (*e.g.* glycidaldehyde[1,2]) we required N^2-alkyl-guanosines and ^{15}N-labelled guanosines. Okutsu and Yamazaki[3,4] have reported methods for converting 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (**2**, 'AICA-riboside'), produced by a mutant of *Bacillus subtilis*, into guanosine and other purine nucleosides. In one of their methods, riboside (**2**) was treated with benzoyl isothiocyanate to give compound (**3**), which was converted into the cyclo-imidazole nucleoside (**4**) by methylation followed by base treatment (see Scheme 1). The reaction of compound (**4**) with ammonia gave guanosine (**1a**), whilst subjecting it to primary or secondary amines was reported[3] to give N^2-alkyl- or N^2-dialkyl-guanosines, respectively. We therefore studied the reaction of compound (**4**) with [^{15}N]ammonia as a potentially convenient route to [$^{15}NH_2$]guanosine (**1c**).

We have found that reaction of compound (**4**) with 1.1 mol. equiv. of [^{15}N]ammonia {generated *in situ* from



Scheme 1. Synthesis of guanosine (**1a**), [^{15}N]guanosines (**1b**) and (**1c**), and methylguanosines (**1d**) and (**1e**). *Reagents:* i, PhCONCS; ii, MeI, NaOH; iii, NH_3, Me_2SO, 100 °C, 10 days or $MeNH_2$, 100 °C, 2 h.



Scheme 2. Routes for formation of guanosine from the intermediate (**5**).

[^{15}N]ammonium chloride (96.9 atom % ^{15}N) by treatment with 1 mol equiv. of LiOH} in dimethyl sulphoxide (10 days at 100 °C) gave [^{15}N]guanosine (55%), isolated after evaporation of the reaction mixture and column chromatography of the residue on silica gel (Merck 7754) [elution with ethanol–ammonia (*d* 0.880) (4 : 1)]. This contained >90% of molecules having one atom of ^{15}N [analysis by fast atom bombardment (f.a.b.) mass spectrometry]. According to analysis by n.m.r. spectroscopy the ^{15}N was distributed between N-1 and N^2 in a 4 : 1 ratio: δ_H [300 MHz, $(CD_3)_2SO$], 3.58 (2 H, m, 2 × H-5), 3.88 (1 H, m, H-4), 4.09 (1 H, m, H-3), 4.40 (1 H, m, H-2), 5.05 [1 H, t, *J* 5 Hz, OH (C-5)], 5.15 [1 H, d, *J* 4.5 Hz, OH (C-2)], 5.42 [1 H, d, *J* 6 Hz, OH (C-3)], 5.72 (1 H, d, *J* 6 Hz, H-1), 6.45 (0.8 H, s, $^{14}NH_2$), 6.45 [0.2 H, d, $J(^{15}NH)$ 90 Hz, $^{15}NH_2$], 7.93 (1H, s, H-8), 10.63 (0.3 H, s, ^{14}NH), and 10.63 [0.7 H, d, $J(^{15}NH)$ 88 Hz, ^{15}NH]; δ_N [30.4 MHz, $(CD_3)_2SO$, external $^{15}NO_3^-$ reference] −302.6 [0.75 ^{15}N, t, $J(^{15}NH)$ 91 Hz] and −228 p.p.m. [0.25 ^{15}N, d, $J(^{15}NH)$ 87 Hz]. A mixture of guanosines (**1a**) (5—10%), (**1b**) (~75%), and (**1c**) (15—20%) was therefore obtained.

Yamazaki and Okutsu[4] suggested that ammonia reacts with (**4**) to give the benzoylguanidino intermediate (**5**) which cyclises to guanosine by nucleophilic attack of the carbamido nitrogen at the guanidino group, followed by elimination of benzamide (pathway a, Scheme 2). However, guanosine could be formed either by pathway b or c of Scheme 2, the former giving [^{15}N-1]guanosine (**1b**) and the latter giving [$^{15}NH_2$]guanosine (**1c**). To support the contention that these are the actual routes to guanosine we have synthesised the cycloimidazole nucleoside (**4**) from potassium [^{15}N]thiocyanate (95 atom % ^{15}N) (reaction with benzoyl chloride[5]) and have converted it into [^{15}N]guanosine by reaction with ammonia (1.1 mol. equiv.) in dimethyl sulphoxide (10 days at 100 °C). This guanosine contained 90% of singly labelled ^{15}N molecules (f.a.b. analysis), being a mixture of guanosines (**1b**) (15—20%) and (**1c**) (~75%) according to n.m.r. analysis [^{1}H, 300 MHz, $(CD_3)_2SO$; ^{15}N, 30.4 MHz, $(CD_3)_2SO$].

Further evidence for the competing pathways of Scheme 2 was obtained by re-examination of the reaction of nucleoside (**4**) with methylamine. According to ref. 3, this gives *N*^{2}-methylguanosine (**1d**) (29%), but we find that 1-*N*-methylguanosine (**1e**) is also formed. Thus, heating compound (**4**) with an excess of 30% (w/v) methylamine in water (2 h; 100 °C) gave (**1e**) as well as (**1d**) (2 : 1 ratio by h.p.l.c. analysis). The methylguanosines were identified by comparison with authentic samples by n.m.r. and mass spectroscopy and h.p.l.c.

Our studies show that the cycloimidazole nucleoside (**4**) is not an ideal precursor of *N*^{2}-substituted guanosines, because of the co-production of the 1-*N*-substituted isomer. However, the ^{15}N-labelled guanosines described are suitable for studying reactions of guanosines with alkylating agents, and for incorporation into oligonucleotides.

We thank Dr. M. N. S. Hill (Newcastle) and Dr. P. Regan (Shell) for f.a.b. mass spectra, and the S.E.R.C. for a CASE studentship (to P. K. S.).

Received, 13th February 1986; Com. 199

References

1 B. T. Golding, P. K. Slaich, and W. P. Watson, Proceedings of IARC Meeting (Lyon, France, 1984) 'The Role of Cyclic Nucleic Acid Adducts in Carcinogenesis and Mutagenesis,' ed. B. Singer and H. Bartsch, IARC Scientific Publications, 1985.
2 B. T. Golding, P. K. Slaich, and W. P. Watson, *J. Chem. Soc., Chem. Commun.*, 1986, 515.
3 M. Okutsu and A. Yamazaki, *Nucleic Acids Res.*, 1976, **3**, 237.
4 A. Yamazaki and M. Okutsu, *J. Heterocycl. Chem.*, 1978, **15**, 353.
5 A. Takamizawa, K. Hirai, and K. Matsui, *Bull. Chem. Soc. Jpn.*, 1963, **36**, 1214.