3-DEAZAADENOSINE-8-^{14}C

John A. Montgomery and H. Jeanette Thomas
Kettering-Meyer Laboratory
Southern Research Institute
Birmingham, Alabama 35205
Received December 12, 1977

SUMMARY

3-Deazaadenosine-8-^{14}C was prepared from 2,6-dichloro-3-deazapurine-8-^{14}C by a recently published procedure. Formylation of 3,4-diamino-2,6-dichloropyridine with ^{14}C-formic acid followed by ring closure gave the requisite 2,6-dichloro-3-deazapurine-8-^{14}C.

Key Words: 3-Deazaadenosine-8-^{14}C, 2,6-Dichloro-3-deazapurine-8-^{14}C, 4,6-Dichloroimidazo[4,5-c]pyridine-2-^{14}C, Labeled Nucleoside.

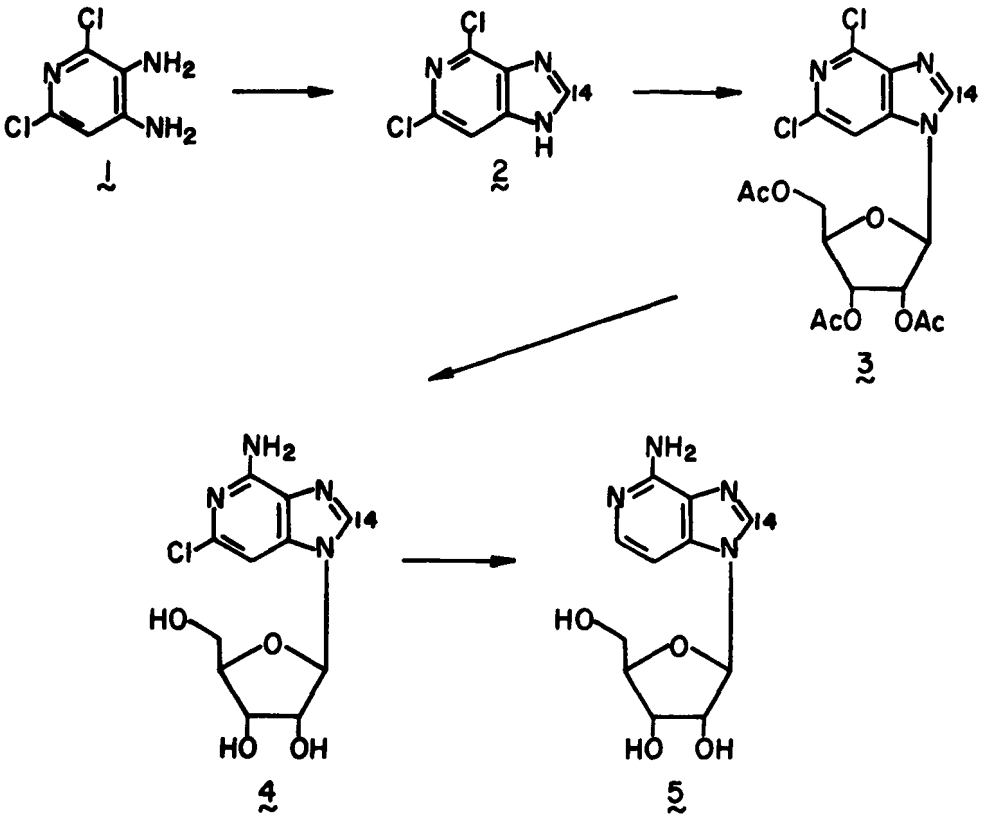
INTRODUCTION

S-3-Deazaadenosyl-L-homocysteine is a substrate of S-adenosyl-L-homocysteine hydrolase (E.C. 3.3.1.1). 3-Deazaadenosine (4-amino-1-β-D-ribofuranosylimidazo[4,5-c]pyridine) (1, 2) causes a drastic increase in the levels of S-adenosyl-L-homocysteine and S-adenosyl-L-methionine in isolated rat hepatocytes, with the formation of S-3-deazaadenosyl-L-homocysteine (3). Because of these results, radiolabeled 3-deazaadenosine was desired for a more detailed study of its metabolism.

Recent work on the synthesis of 3-deazaadenosine (4) showed that this nucleoside can best be prepared by the fusion of tetra-O-acetyl-β-D-ribofuranose with 2,6-dichloro-3-deazapurine (4,6-dichloroimidazo[4,5-c]pyridine) followed by amination and removal of the 2-chlorine by catalytic hydrogenolysis. 2,6-Dichloro-3-deazapurine is normally prepared (5) by ring closure of 3,4-diamino-2,6-dichloropyridine (1) with the ethyl orthoformate-acetic anhydride reagent (6), but such a procedure is not suitable for the introduction of ^{14}C because a large excess of ethyl orthoformate must be used for ring annulation. A trial reaction showed, however, that the closure could be effected with formic

0362-4803/78/0015-0539$01.00

acid in ethylene glycol (7); and in this case only 1.25 equivalent of formic acid had to be employed. Fusion of the 2,6-dichloro-3-deazapurine-8-^{14}C (2), prepared in this way from ^{14}C-formic acid, with tetra-O-acetyl-β-D-ribofuranose gave 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2,6-dichloro-3-deazapurine-8-^{14}C (3), which reacted with ammonia to provide 2-chloro-3-deazaadenosine-8-^{14}C (4). Reductive dechlorination with palladium-on-charcoal catalyst gave the desired 3-deazaadenosine-8-^{14}C (5).



EXPERIMENTAL

2,6-Dichloro-3-deazapurine-8-^{14}C (2)

A solution of 1 (214 mg, 1.09 mmol), concentrated hydrochloric acid (1 mmol), formic acid [3.66 mg (0.08 mmol) of ^{14}C-formic acid (specific activity 60.7 mCi/mmol) diluted with 59.4 mg (1.29 mmol) of formic acid: total

1.37 mmol] in 2.4 ml of ethylene glycol was heated at 80–85°. The reaction, monitored by thin-layer chromatography (silica gel GF developed in 95:5 chloroform-methanol solvent), required 4 days. The solution was evaporated to dryness in vacuo at 100° to give 2 as a buff-colored solid.

9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-2,6-dichloro-3-deazapurine-8-^{14}C (3)

A mixture of 2, tetraacetyl ribose (636 mg, 2.00 mmol), and p-toluenesulfonic acid (10 mg) was fused with stirring at 160–165°/125 mm. The resulting melt solidified after 7 min. A solution of the melt in chloroform (100 ml) was washed with saturated aqueous sodium bicarbonate solution (50 ml), twice with water (50 ml), dried over magnesium sulfate, and evaporated to dryness in vacuo to give 3 as a syrup.

2-Chloro-3-deazaadenosine-8-^{14}C (4).

A solution of 3 in 70 ml of ethanol saturated at 0° with ammonia was heated in a stainless steel bomb at 140° for 3 days and then evaporated to dryness in vacuo to give 4 as a dark syrup.

3-Deazaadenosine-8-^{14}C (5)

A solution of 4 in 50 ml of water containing 66 mg of 30% palladium-on-charcoal catalyst and 1 ml of 1 N sodium hydroxide was hydrogenated at ambient temperature and 40 psi for 24 hr. Examination of the reaction by thin-layer chromatography (silica gel GF developed in 3:1 chloroform-methanol solvent) indicated incomplete reaction. Therefore, 66 mg more catalyst was added and hydrogenation resumed for 24 hr. The process was repeated, adding 12 mg more catalyst. The total reaction time was 3 days. The solution was filtered and evaporated to dryness in vacuo. An aqueous solution of the residue was streaked across one 8-in. Brinkmann silica gel plate (20 x 20 cm, 2-mm thickness). The plate was developed three times in 3:1 chloroform-methanol solvent system. The major uv-absorbing band was removed and extracted with boiling

methanol. Evaporation of the methanol solution gave 5 as a glass that crystallized from 4 ml of water; yield 65 mg. Evaporation of the filtrate gave a second crop; yield 12 mg. Further extraction of the silica gel band with methanol gave another crop; yield 5 mg. Further extraction of the catalyst with water gave still another crop; yield 3.5 mg. The crops were combined to give 85.5 mg, which was dissolved in methanol and streaked across one 8-in. Brinkmann silica gel plate. The plate was developed in methanol. The major band was removed and extracted with warm methanol. Evaporation of the methanol solution gave a residue that crystallized from 2 ml of methanol as a fluffy, white solid; yield 56 mg: specific activity determined by liquid scintillation counting 12.6 μCi/mg or 3.37 mCi/mmol. From the filtrate a second crop was obtained; yield 9 mg: specific activity 10.9 μCi/ mg or 2.90 mCi/ mmol. The chemical purity of each crop was determined by thin-layer chromatography (Analtech silica gel GF, methanol). The radiochemical purity was determined by uv light and radioactivity scans of the thin-layer chromatogram with a Packard radiochromatogram scanner, Model 7201, and both crops were found to be 99% radiopure.

ACKNOWLEDGMENTS

This work was supported by funds from the National Institutes of Health. The authors wish to thank Dr. Peter Chiang for his encouragement in this work.

REFERENCES

1. Rousseau R. J., Townsend L. B., and Robins R. K. - Biochemistry 5: 756 (1966)
2. May J. A. and Townsend L. B. - J. Chem. Soc., Perkin Trans. I: 125 (1975)
3. Chiang P. K., Richards H. H., and Cantoni G. L. - Mol. Pharmacol. 13: 939 (1977)

4. Montgomery J. A., Shortnacy A. T., and Clayton S. D. - J. Heterocycl. Chem. 14: 195 (1977)

5. Rousseau R. J. and Robins R. K. - J. Heterocycl. Chem. 2: 196 (1965)

6. Montgomery J. A. - J. Am. Chem. Soc. 78: 1928 (1956)

7. Montgomery J. A. - Organic Syntheses with Isotopes. 759, Ed. by A. Murray, III, and D. L. Williams, Interscience Publishers, Inc., New York (1958)