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Note

Preparation of *S*-(N^6,N^6-dimethyladenosyl)-L-methionine

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During recent studies to delineate the structural features of the adenine moiety of *S*-adenosyl-L-methionine necessary to produce maximal activity in the enzyme 1-aminocyclopropane-1-carboxylic acid synthase[1] (ACC-S), we required (±)*S*-(N^6,N^6-dimethyladenosyl)-L-methionine (**5**). This compound has been previously reported[2] as a possible substrate and/or inhibitor of the transmethylase enzymes catechol *O*-methyltransferase, phenylethanolamine *N*-methyltransferase, histamine *N*-methyltransferase, and hydroxyindole *O*-methyltransferase. However, no physical properties of the compound were reported and the physical properties of the precursor *S*-(N^6,N^6-dimethyladenosyl)-L-homocysteine[3] (**4**) were suspect when compared with other *S*-adenosyl-L-homocysteine derivatives in which the adenosine had been modified.

Although N^6,N^6-dimethyladenosine [6-dimethylamino-9-(β-D-ribofuranosyl)-purine, **2**] is commercially available, it is expensive ($250/g, Sigma). We therefore started the synthesis with inosine (9-β-D-ribofuranosylhypoxanthine), which was converted in two steps into 6-chloro-9-(β-D-ribofuranosyl)purine (**1**) via dimethyl chloromethyleneammonium chloride by the method of Zemlička[4]. The chloro derivative was converted into N^6,N^6-dimethyladenosine **2** in 92% yield by a modification of the procedure of Zemlička[5]. The original method reported a yield of 50%. Other routes to **2** involve methylation of adenosine either reductively with $NaBH_4$/HCHO in 65% yield[6] or directly with trimethylphosphate–tetrabutylammonium fluoride in 55% yield[7]. N^6,N^6-Dimethyladenosine was converted into 5′-chloro-5′-deoxy-N^6,N^6-dimethyladenosine (**3**) by the method of Ichino[8]. The key step in this synthesis is the displacement of chloride by the monosodium salt of L-homocysteine. This method has been used in our laboratory for the preparation of similar analogues[9]. The sulfonium salt was prepared by treatment of *S*-(N^6,N^6-dimethyladenosyl)-L-homocysteine (**4**) with methyl iodide and silver acetate in acetic acid–formic acid[10]. Purification of the sulfonium salt was accomplished by C_{18} reverse-phase low-pressure chromatography with the product eluting off with 0.1M ammonium acetate (pH 3.5) near the void volume.

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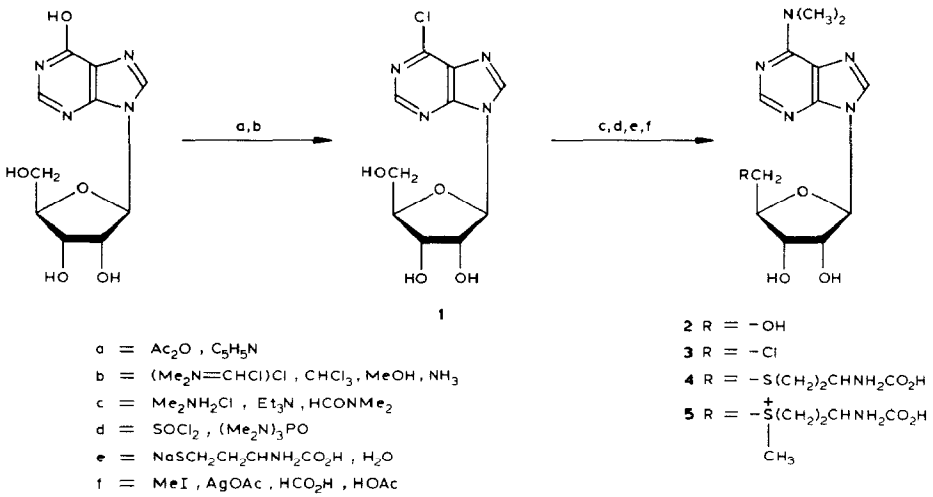

The procedure reported here allows the preparation of large amounts of the important biological compound **2**, a key intermediate in the synthesis of several biologically interesting nucleotide analogues, as well as the synthesis of *S*-(N^6,N^6-dimethyladenosyl)-L-homocysteine (**4**) and the title compound, *S*-(N^6,N^6-dimethyladenosyl)-L-methonine (**5**).

EXPERIMENTAL

General methods. — Melting points were determined with a Mel-Temp capillary melting-point apparatus and are uncorrected. ^{1}H-N.m.r. spectra were recorded with a Bruker WH-360 (360-MHz) spectrometer. Chemical shifts are reported in p.p.m. downfield from tetramethylsilane in organic solvents and sodium 4,4-dimethyl-4-silapentanoate in D_2O. Elemental analyses were conducted by M-H-W Laboratories, Phoenix, Arizona. Specific rotations were recorded with a Perkin–Elmer model 141 polarimeter. Low-pressure chromatography was performed on a C_{18} reverse-phase column (Applied Science; Adsorbosil LC, 200–425 mesh) equilibrated and eluted with 0.2M NH_4OAc, pH 3.5, flow rate 10 mL/min; u.v. detector (260-nm wavelength monitor).

N^6,N^6-*Dimethyladenosine* (**2**). — A mixture of 6-chloro-9-(β-D-ribofuranosyl)purine[4] (**1**, 8.60 g, 0.03 mol) and dimethylamine hydrochloride (12.23 g, 0.15 mol) (dried over H_2SO_4) in *N*,*N*-dimethylformamide (50 mL) was stirred vigorously for 0.5 h at 0°. Triethylamine (20.24 g (28.0 mL), 0.2 mol) was then added and the mixture was stirred for an additional 2 h at 0° and then for 15 h at room temperature. Triethylamine hydrochloride that formed was filtered off and washed with ice-cold *N*,*N*-dimethylformamide (15 mL). *N*,*N*-Dimethylformamide was removed *in vacuo* (0.5 mm Hg at 45°) and the residue was triturated with acetone (150 mL) to give 8.2 g (92.6%) of **2**. Crystallization of this product from

methanol afforded 7.5 g (84.6%) of **2** having m.p. 183–184° (lit.[5] m.p. 182–185°); ^{1}H-n.m.r. (Me_2SO-d_6): δ 8.37 (s, 1 H, H-8), 8.21 (s, 1 H, H-2), 5.90 (d, 1 H, *J* 6.0 Hz, H-1′), 5.45 (d, 1 H, *J* 6.2 Hz, HO-2′), 5.38 (t, 1 H, *J* 4.8 Hz, HO-5′), 5.20 (d, 1 H, *J* 4.65 Hz, HO-3′), 4.55 (m, 1 H, H-2′), 4.15 (m, 1 H, H-3′), 3.96 (m, 1 H, H-4′), 3.52–3.7 (m, 2 H, H-5′), and 3.34 (d, 6 H, NMe_2).

5′-Chloro-5′-deoxy-N^6,N^6-*dimethyladenosine* (**3**). — This compound was prepared by the method of Ichino[8]. Thionyl chloride (4.0 mL) was dissolved in hexamethylphosphoric triamide (25 mL) and to the solution was added *N*^{6},*N*^{6}-dimethyladenosine (**2**, 2.5 g, 8.47 mmol). The mixture was stirred at room temperature for 15 h, and then poured into water (100 mL) and concentrated *in vacuo* to 20 mL. This solution was made neutral (pH 8.0) with 2M NH_4OH. The precipitated product was filtered off, washed with cold water, and dried to yield 2.0 g (74.5%) of **3**; m.p. 150–151° (lit.[3] m.p. 150–151°); ^{1}H-n.m.r. (Me_2SO-d_6): δ 8.26 (s, 1 H, H-8), 8.20 (s, 1 H, H-2), 6.08 (d, 1 H, *J* 5.0 Hz, H-1′), 5.40 (m, 2 H, OH), 4.70 (m, 1 H, H-4′), 4.28 (m, 2 H, H-2′ and H-3′, 3.92 (m, 2 H, H-5′), and 3.45 (s, 6 H, NMe_2).

S-(N^6,N^6-*Dimethyladenosyl*)-L-*homocysteine* (**4**). — A suspension of 5′-chloro-5′-deoxy-*N*^{6},*N*^{6}-dimethyladenosine (**3**, 0.63 g, 2.0 mmol), potassium iodide (10 mg), and the sodium salt of L-homocysteine (0.480 g, 2.5 mmol) was boiled under reflux in water (7 mL) for 2 h. The mixture became clear and t.l.c. (Avicel F, 250 μm, 9:5 1-propanol–water) showed the absence of any 5′-chloro-5′-deoxynucleoside. The mixture was allowed to cool and made acidic with 1M HCl. The solution was then applied directly to a column of Dowex 50X 4-200 (NH_4^+) resin (125 mL). The column was washed thoroughly with water (200 mL) and then eluted with M NH_4OH. The eluate was evaporated to low volume *in vacuo* and lyophilized. The product was recrystallized from ethanol–water to give 0.38 g (45%); m.p. 228–230° (lit.[3] m.p. 70°); $[\alpha]_D^{23}$ +37.9° (*c* 1, 0.2M HCl); ^{1}H-n.m.r. (D_2O): δ 8.06 (s, 1 H, H-8), 7.94 (s, 1 H, H-2), 5.87 (d, 1 H, *J* 4.9 Hz, H-1′), 4.25 (t, 1 H, *J* 5.2 Hz, H-3′), 4.19 (m, 1 H, H-4′), 3.68 (t, 1 H, *J* 5.6 Hz, αC-H), 3.19 (s, 6 H, NMe_2), 2.81 (m, 2 H, H-5′), 2.55 (t, 2 H, *J* 7.5 Hz, γC-H), and 2.02 (m, 2 H, βC-H); H-2′ overlaps with H_2O peak.

Anal. Calc. for $C_{16}H_{24}N_6O_5S$: C, 46.59; H, 5.86. Found: C, 46.41; H, 5.79.

(±)S-(N^6,N^6-*Dimethyladenosyl*)-L-*methionine* (**5**). — This compound was prepared by the following modification of the procedure of Monem[10]. To a solution of *S*-(*N*^{6},*N*^{6}-dimethyladenosyl)-L-homocysteine (**4**, 100 mg, 0.25 mmol) in 1 mL of 1:1 (v/v) acetic acid–98% formic acid was added successively 0.3 mL (5.5 mmol) of iodomethane and 1.8 mmol of silver acetate. The mixture was stirred for 4 h at room temperature. The resulting slurry was centrifuged to remove the precipitated silver iodide and the acetic acid–formic acid solution was diluted with water (2 mL) and extracted with ether (3 × 20 mL) to remove the excess of iodomethane. The aqueous solution was purged with nitrogen to remove any excess of ether and loaded onto a C_{18} reverse-phase column operating under low pressure. The product was eluted with 0.1M ammonium acetate. Fractions containing the product were

pooled and lyophilized to give **5** (93 mg, 73%); ^{1}H-n.m.r. (D_2O); δ 8.06 (s, 1 H, H-8), 8.0 (s, 1 H, H-2), 5.89 (d, 1 H, *J* 4.9 Hz, H-1'), 4.67 (bs, 1 H, H-2'), 4.36 (t, 1 H, *J* 5.0 Hz, H-3'), 3.76 (m, 1 H, H-4'), 3.52 (bs, 1 H, αC-H), 3.23 (s, 6 H, NMe_2), 3.55 (m, 2 H, H-5'), 2.77 (m, 2 H, γC-H), [2.79 (s, 1.2 H, SCH_3), 2.75 (s, 1.8 H, SCH_3)]*, and 2.16 (m, 2 H, βC-H). The residual solid was immediately dissolved in 1.0mM HCl and stored at −20°.

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REFERENCES

1 R. W. Woodard in E. Usdin, R. T. Borchardt, and C. R. Creveling (Eds.), *1-Aminocyclopropane-1-carboxylic acid synthase, A Unique SAM-Utilizing Enzyme,* in *Biochemistry of* S-*Adenosylmethionine and Related Compounds,* Macmillan, 1982, pp. 621–625.
2 R. T. Borchardt, Y. S. Wu, J. A. Huber, and A. F. Wycpalek, *J. Med. Chem.*, 19 (1976) 1104–1110.
3 R. T. Borchardt, J. A. Huber, and Y. S. Wu, *J. Med. Chem.*, 19 (1976) 1094–1099.
4 J. Zemlička and F. Šorm, *Coll. Czech. Chem. Commun.*, 30 (1965) 1880–1889.
5 J. Zemlička, S. Chládek, D. Ringer, and K. Quiggle, *Biochemistry,* 14 (1975) 5239–5249.
6 M. Morr and L. Ernst, *J. Chem. Res. Synop.*, (1981) 90–91.
7 K. K. Ogilvie, S. L. Beaucage, M. F. Gillen, and D. W. Entwistle, *Nucleic Acid Res.*, 6 (1979) 2261–2273.
8 K. Kikugawa and M. Ichino, *Tetrahedron Lett.*, 2 (1971) 87–90.
9 K. Ramalingam and R. W. Woodard, *J. Org. Chem.*, 49 (1984) 1291–1293.
10 M. Pankaskie and M. A. Monem, *J. Med. Chem.*, 23 (1980) 121–127.
11 M. L. Stolowitz and M. J. Minch, *J. Am. Chem. Soc.*, 103 (1981) 6015–6019.

*The methylation of all *S*-adenosyl-L-homocysteine derivatives studied to date[11] has given a mixture of stereoisomers, at the sulfur center, in which the unnatural *R* isomer is produced in 20% enantiomeric excess. This deviation from the expected racemic mixture (50% *R*: 50% *S* at the sulfur center) is probably due to asymmetric induction by the ribose portion of the nucleoside or another adjacent chiral center.