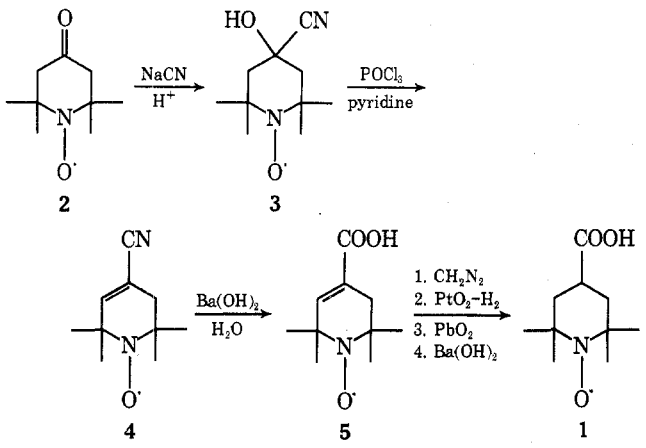
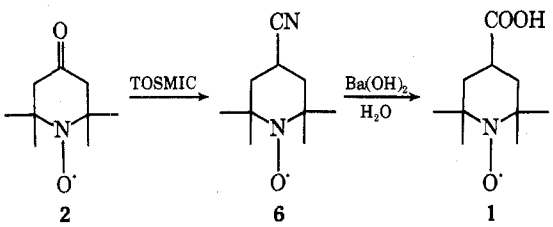
Scheme I



Recently Hsia et al.[1] reported the preparation of 1 as outlined in Scheme I. Using this reported procedure, we were unable to reduce 1-oxyl-4-carboxyl-2,2,6,6-tetramethyl-1,2,5,6-tetrahydropyridine (5) to the desired product (1). Palladium on charcoal, which we have found useful for other similar reductions, also failed to give a satisfactory yield of 1.

Toward this goal, we have devised an unequivocal synthesis of 5 using tosylmethyl isocyanide as originally reported by Van Leusen.[2] Treatment of 1-oxyl-2,2,6,6-tetramethyl-4-piperidone (2) with tosylmethyl isocyanide in the presence of base resulted in a high yield of 1-oxyl-4-cyano-2,2,6,6-tetramethylpiperidine (6). Hydrolysis of 6 to the



acid gave a product melting 20°C lower than that reported by Hsia et al.[1] It may be noted that the unsaturated acid, 5, has a reported melting point of 190–192°C, whereas the reduced (1) was reported to melt at 195–196°C. Furthermore, elemental analysis is unable to distinguish between the two compounds (1 and 5), given the generally accepted error of ±0.3%. To substantiate our findings, we performed a high-resolution mass spectral study of 1-oxyl-4-cyano-2,2,6,6-tetramethylpiperidine (6) and 1-oxyl-4-carboxyl-2,2,6,6-tetramethylpiperidine (1).

Field ionization mass spectra confirmed the molecular ions to be *m/e* 181 for 6 and *m/e* 200 for 1.

Experimental Section

Melting points were obtained on a Thomas-Hoover melting point apparatus and are corrected. The microanalysis was performed by M-H-W Laboratories, Garden City, Mich. Infrared spectra were recorded on a Perkin-Elmer Model 727 spectrophotometer. High-resolution mass spectra were recorded on a Varian CH-5 mass spectrometer.

1-Oxyl-4-cyano-2,2,6,6-tetramethylpiperidine (6). To a stirred solution of 1-oxyl-2,2,6,6-tetramethyl-4-piperidone (2, 1.0 g, 5.9 mmol) and tosylmethyl isocyanide (1.17 g, 6.0 mmol, Aldrich Chemical Co.) in 40 ml of dimethoxyethane at 0°C was added 2 equiv (1.34 g) of potassium *tert*-butoxide dissolved in 20 ml of a 1:1 mixture of dimethoxyethane and *tert*-butyl alcohol. The mixture was stirred at 0°C for 45 min, the temperature was then raised to 20°C, and the mixture was stirred for an additional 1 hr. At that time, 100 ml of water was added and the mixture was extracted three times with 25-ml portions of ether. The ether extracts were combined and dried over anhydrous magnesium sulfate. The ether was then removed in vacuo to give approximately 0.9 g of a red powder. A portion of this product was twice recrystallized from ether, giving red needles: mp 146.5–147°C; ir 2250 cm^{-1} (–C≡N); *m/e* 181.1338.

Anal. Calcd for $C_{10}H_{17}N_2O$: C, 66.26; H, 9.45; N, 15.46. Found: C, 66.03; H, 9.63; N, 15.32.

1-Oxyl-4-carboxyl-2,2,6,6-tetramethylpiperidine (1). To a solution of 2 g of 1-oxyl-4-cyano-2,2,6,6-tetramethylpiperidine (6) in 25 ml of methanol was added a solution of 6 g of barium hydroxide and 0.5 g of sodium hydroxide in 100 ml of water. The mixture was refluxed for 24 hr, cooled, and extracted with chloroform. The remaining aqueous solution was cooled, acidified with 10% hydrochloric acid, and extracted exhaustively with chloroform. The chloroform solution was dried over anhydrous magnesium sulfate and evaporated in vacuo, giving 1.9 g of a red powder: mp 171–172°C from hexane–benzene (1:2); ir 1680 (–C=O), 3300–3100 cm^{-1} broad (–OH); *m/e* 200.1271.

Anal. Calcd for $C_{10}H_{18}NO_3$: C, 59.98; H, 9.06; N, 6.99. Found: C, 60.04; H, 9.19; N, 6.80.

Acknowledgment. This study was supported in part by a grant from NIH (NS 10823). We wish to thank Dr. Robert Harless for the mass spectral data.

Registry No.—1, 37149-18-1; 2, 2896-70-0; 6, 38078-71-6; tosylmethyl isocyanide, 36635-61-7.

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A Convenient Preparation of *S*-Adenosylhomocysteine and Related Compounds

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Received June 30, 1975

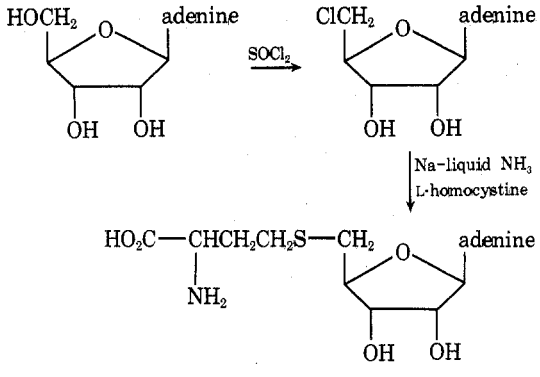
Since the discovery of *S*-adenosylmethionine,[2] a great variety of *S*-adenosylmethionine-dependent biological transmethylation reactions have been demonstrated.[3] A general feature of most *S*-adenosylmethionine-dependent methyltransferases is the inhibition produced by the demethylated product *S*-adenosyl-L-homocysteine. Because of its possible significance as a biological regulatory mechanism,[3b] this product inhibition has stimulated considerable research interest. Numerous compounds structurally related to *S*-adenosyl-L-homocysteine have been synthesized as potential inhibitors of this class of enzymes.[4]

Because of the high substrate specificity of the enzyme capable of synthesizing *S*-adenosylhomocysteine,[5] analogues of this compound have been prepared by chemical synthesis. The general route for the synthesis of *S*-adenosylhomocysteine analogues was modeled after the procedure first described by Baddiley and Jamieson.[6] This route involves (1) the synthesis of the parent nucleoside, if not commercially available; (2) protection of the 2′,3′-hydroxyl groups of the nucleoside using an isopropylidene protecting group; (3) activation of the nucleoside 5′ position by formation of the corresponding 5′-tosylate; (4) condensation of the intermediate 5′-tosylate with *S*-benzyl-L-homocysteine (or related compounds); and (5) removal of the isopropylidene protecting group using dilute acid. This standard procedure has proven quite successful for the synthesis of a broad spectrum of *S*-adenosylhomocysteine analogues.[4] A

variation of this procedure using a phenylboronate ester as a protecting group was recently reported.[4n] The general procedures outlined above suffer from several disadvantages which include (1) the necessity to protect the 2',3'-hydroxyl groups of the nucleoside; and (2) a stability problem with the intermediate 5'-tosylate.

We have recently developed a shorter, more convenient procedure for the preparation of *S*-adenosyl-L-homocysteine and related compounds, which we feel has several advantages over the procedures outlined above. This new procedure involves the initial formation of the 5'-chloro-5'-deoxynucleoside from the corresponding nucleoside, followed by condensation with L-homocystine (Scheme I).

Scheme I



We have used the procedure outlined in Scheme I to synthesize *S*-adenosyl-L-homocysteine, *S*-adenosyl-D-homocysteine, and numerous base- or sugar-modified derivatives, some of which are listed in Table I. The appropriate 5'-chloro-5'-deoxynucleosides were prepared in 75–100% yields from the corresponding nucleosides using thionyl chloride and hexamethylphosphoramide according to a general procedure described by Kikugawa and Ichino.[7] The 5'-chloro-5'-deoxynucleosides were condensed with L-homocystine (or D-homocystine) in sodium and liquid ammonia to yield directly the desired *S*-adenosylhomocysteine derivatives. Use of L-homocystine, rather than the *S*-benzyl-L-homocysteine, was found to afford cleaner reaction products and higher yields (30–60%). In addition, since L-homocystine, D-homocystine, or DL-homocystine are commercially available, this makes the D isomer, L isomer, or DL mixture of *S*-adenosylhomocysteine or related compounds readily accessible.

We believe that this method has several advantages over previously described procedures. The route outlined in Scheme I is shorter, requiring only two steps to the desired product from the appropriate nucleoside. In addition, the overall yields from the appropriate nucleosides to the desired products are generally 30–60%, as compared to yields of 5–25% for the four-step conversion using the isopropylidene protecting group. We have also found that the 5'-chloro-5'-deoxynucleosides are relatively stable compounds, unlike the corresponding 5'-tosylates, which are unstable and unless properly stored (0°C) decompose. Most important, however, is the fact that by using this procedure we have been able to prepare in good yield several compounds, including the N^6-methyl-3-deazaadenine derivative **3**, which was prepared in very poor yield using the earlier procedures.

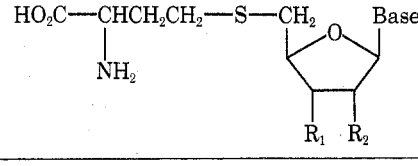
Experimental Section

Melting points were obtained on a calibrated Thomas-Hoover Uni-Melt and were corrected. Microanalyses were conducted on an F & M Model 185 C, H, N analyzer, The University of Kansas, Lawrence, Kans. The ir, NMR, and uv data were consistent with the assigned structures. Ir data were recorded on a Beckman IR-33 spectrophotometer, NMR data on a Varian Associates Model T-60 spectrophotometer (Me_4Si), and uv data on a Cary Model 14 spectrophotometer.

The following compounds were commercially available from the indicated sources: adenosine, N^6-methyladenosine (Aldrich); 2'-deoxyadenosine, D-homocystine, L-homocystine, and tubercidin (7-deazaadenosine) (Sigma). N^6-Methyl-3-deazaadenosine was prepared by a modification[4b] of the procedure of Mizuno et al.[8]

General Reaction Procedure for the Preparation of the 5'-Chloro-5'-deoxynucleosides. The conversion of the appropriate nucleoside (e.g., adenosine) to the corresponding 5'-chloro-5'-deoxynucleoside (e.g., 5'-chloro-5'-deoxyadenosine) was modeled after a procedure previously described by Kikugawa and Ichino.[7] A solution of 0.75 ml of thionyl chloride and 5.0 ml of hexamethylphosphoramide was stirred under nitrogen while 0.50 g of the nucleo-

Table I
***S*-Adenosyl-L-homocysteine and Related Compounds Prepared by the Condensation of L-Homocystine and 5'-Chloro-5'-deoxynucleosides[a,b]**



Compd	Base	R_1	R_2	Mp, °C	Lit. mp, °C	% yield from 5'-chloro-5'-deoxynucleoside[f]	% yield from nucleoside
1	Adenine[c]	OH	OH	212	204[6]	75	59
2	N^6-Methyladenine	OH	OH	208–210	*d*	45	39
3	N^6-Methyl-3-deazaadenine	OH	OH	222–224	214–215	67	56
4	7-Deazaadenine	OH	OH	178–180	*e*	45	33
5	Adenine	OH	H	190–191	191[4c]	45	31
6	Adenine	H	OH	213	211[4c]	54	39

[a] The 5'-chloro-5'-deoxynucleosides were prepared from the corresponding nucleosides by the general procedure outlined in the Experimental Section. [b] The *S*-adenosylhomocysteine analogues were characterized by their NMR and uv spectra and their chromatographic properties against standard compounds. [c] *S*-Adenosyl-D-homocysteine was prepared in comparable yield by condensation of 5'-chloro-5'-deoxyadenosine and D-homocystine. [d] Previously prepared by Hildesheim et al.,[4k] but no melting point was reported. [e] Previously prepared by Coward et al.,[4g] but no melting point was reported. [f] Registry no. are, respectively, 892-48-8, 19254-36-5, 57274-13-2, 53458-85-8, 57274-14-3, 57274-15-4.

side was added. The mixture was allowed to stir for 15–20 hr at ambient temperature, then quenched with ca. 20 ml of water and concentrated in vacuo to 10 ml. For those less soluble 5′-chloronucleoside products, the aqueous solution was neutralized to pH 7–8 with 2 *N* aqueous ammonia. The resulting solution was cooled and crystals collected by filtration and washed thoroughly with ice water. The combined filtrates were applied to ca. 10 ml of ion exchange resin (Dowex 50W-X4, 50–100 mesh, H^+ form). The resin was washed well with water, then with 1 or 2 *N* NH_4OH to remove the product. The ammonia solutions were concentrated to produce a second crop of crystals. For those samples which did not crystallize on neutralization, the entire original acidic solution was passed through a large column (100–150 ml) of Dowex 50W-X4. The product was again removed by elution with NH_4OH, after first washing the column thoroughly with water. Yields averaged in the 75–100% range. The products were identified by their ir and NMR spectra and their chromatographic properties.

General Reaction Procedure for the Preparation of *S*-Adenosylhomocysteine Analogues from the 5′-Chloro-5′-deoxynucleoside and L-Homocystine. A 0.75-mmol sample (1.4 equiv) of L-homocystine was dissolved in 25 ml of liquid ammonia and treated with sodium until the solution remained blue for at least 15 min. A stirring bar was introduced and enough NH_4Cl was added to discharge the color. Then 1.05 mmol (1.0 equiv) of the appropriate 5′-chloro-5′-deoxynucleoside was added and stirring was continued for approximately 12 hr. After the ammonia had evaporated and the last traces were removed in vacuo, 5 ml of H_2O was added and the solution neutralized to pH 6 with 5% HCl. The crude mixture was then applied to an ion exchange column (30 ml of Dowex 50W-X4, 50–100 mesh, NH_4^+ form) and eluted slowly with water. The first fractions contained the inorganic ions and homocystine. The desired product along with some starting 5′-chloro-5′-deoxynucleoside was removed either by further elution with water or by elution of the column with 1 *N* and/or 2 *N* NH_4OH. The fractions containing product were concentrated in vacuo and lyophilized. Where appropriate, impure samples were further purified by using preparative TLC [Avicel, 3:2 EtOH–H_2O, or silica gel, 9 (20EtOH:2H_2O:2HOAc) + 1 (0.5 *M* phosphate buffer pH 7.0)]. Yields averaged from 40 to 75% for this condensation step, giving overall yields of 30–60% of the products from the corresponding nucleoside. The *S*-adenosylhomocysteine analogues were characterized by their ir, NMR, and uv spectra, their chromatographic properties against standard samples, and chemical analyses.

Acknowledgment. The authors gratefully acknowledge support of this project by a Research Grant from the National Institute of Neurological Communicative Disorders and Stroke (NS-10918). The excellent technical assistance of Robert Miller is gratefully acknowledged.

Registry No.—**1**, 979-92-0; **2**, 53228-06-1; **3**, 53199-58-9; **4**, 57344-98-6; **5**, 57274-11-0; **6**, 57274-12-1; L-homocystine, 626-72-2; D-homocystine, 6027-15-2.

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Synthesis and Characterization of 5-Hydroperoxymethyluracil (Thy$^{\alpha}$OOH)

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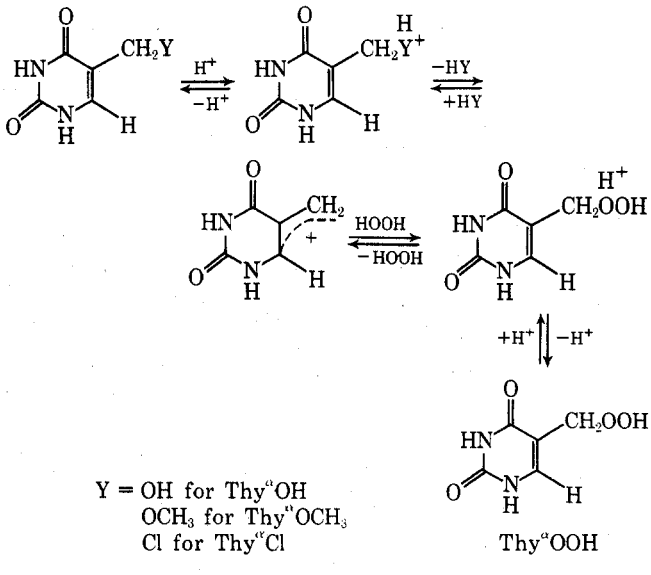
Received August 22, 1975

Cadet and Teoule[1] have shown that radiolysis of thymine (Thy)[2] and thymidine (dThd) in pH 1.7–7 aerated aqueous solutions results in the formation of Thy$^{\alpha}$OOH. Swinehart et al.[3] studied the γ-ray-induced production of [^{3}H]H_2O from [^{3}H]Thy in single- and double-stranded DNA and suggested the formation of Thy·$^{\alpha}$, a probable intermediate for the formation of Thy$^{\alpha}$OOH and other analogous products, to explain the observed [^{3}H] release. Earlier, the same suggestion was made by Wang and Alcantara[4] about the photooxidation of Thy in aqueous solutions. These findings indicate the possible importance of Thy$^{\alpha}$OOH in radiobiology and photobiology, and thus the study of the effects of Thy$^{\alpha}$OOH on biological compounds and systems seems to be warranted, especially in view of the effects of *cis*-5,6-dihydro-6-hydroperoxy-5-hydroxythymine (ho^{5}ho$_2$^{6}hThy, 6-TOOH)[5] on neighboring bases, cells, chromosomes, etc.[6]

Because access to sufficient quantities of Thy$^{\alpha}$OOH is necessary for similar studies, improved methods have been developed with analogous starting materials, two of which are novel for the preparation of Thy$^{\alpha}$OOH. These syntheses give Thy$^{\alpha}$OOH in yields of ~90%, which is considerably greater than in the previous method.[7] In addition, Thy$^{\alpha}$OOH exhibits some interesting photochemical and chemical behavior. Furthermore, purified Thy$^{\alpha}$OOH is in fact rather stable, contrary to the early belief,[1] and that is convenient for our intended studies.

Results and Discussion

These syntheses are straightforward and give excellent yields of Thy$^{\alpha}$OOH. Considering the ease of reaction and the requirement of concentrated HCl, acid-catalyzed formation of an electrophilic center is probably involved as shown in the following scheme.



Because Cl is a much better leaving group than OH and OCH_3 under the present reaction condition, acid catalysis is necessary for the reactions of Thy$^{\alpha}$OH and Thy$^{\alpha}$$OCH_3$