Notes

5′-Chloropuromycin. Inhibition of Protein Synthesis and Antitrypanosomal Activity

Robert Vince,* Heejoo Lee, A. S. Narang,

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455

and Frances N. Shirota

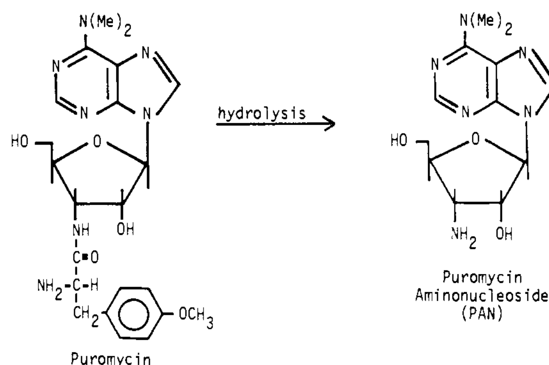
Medical Research Laboratories, Veterans Administration Medical Center, Minneapolis, Minnesota 55417.
Received March 30, 1981

A facile, two-step conversion of puromycin aminonucleoside (PAN) into 5′-deoxy-PAN (**5**) via 5′-chloro-5′-deoxy-PAN (**1**) was accomplished. Replacement of the 5′-OH group of PAN with H or Cl resulted in the elimination of kidney toxicity associated with the administration of PAN. The corresponding puromycin derivatives, 5′-chloro-5′-deoxypuromycin (**4**) and 5′-deoxypuromycin (**6**), derived from **1** and **5**, respectively, were compared in a ribosomal peptidyltransferase assay. Both compounds were excellent substrates for the transpeptidation reaction, confirming our previous observations with **6** that the 5′-OH of puromycin is not essential for activity at the ribosomal level. Thus, **4** represents a new puromycin derivative that retains puromycin-like activity at the ribosomal site but is capable of releasing only a nonnephrotoxic aminonucleoside upon enzymatic release of the *p*-methoxyphenylalanyl side chain. The chloro derivative **4** exhibited significant antitrypanosomal activity in mice infected with *Trypanosoma rhodesiense*. The 5′-deoxy derivative **6** was inactive against trypanosomes.

The antibiotic puromycin derives its antimicrobial and antitumor activities from its ability to cause a premature release of growing polypeptide chains from ribosomes.[1,2] The successful use of puromycin against trypanosomiasis and amebiasis in mice[3-5] and humans[6,7] indicated promising clinical use as a chemotherapeutic agent. However, toxicity studies soon revealed the development of nephrotoxic manifestations that have subsequently precluded the use of puromycin in the treatment of human or animal infectious diseases.[8] The nephrotoxicity of puromycin has been ascribed to the enzymatic release of the aminonucleoside 6-(dimethylamino)-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)purine (PAN) by hydrolysis of the amino acid side chain (Scheme I).[9] Metabolic studies have demonstrated that PAN can be monodemethylated by liver enzymes[10,11] and subsequently converted to the 5′-nucleotide.[12] It has been suggested that the nucleotide may be the active metabolite of PAN which induces kidney toxicity.[12,13]

In a program to design active puromycin molecules that, if hydrolyzed at the peptide bond, would release only nontoxic aminonucleosides, we have previously prepared

Scheme I. Conversion of Puromycin to Puromycin Aminonucleoside



5′-deoxypuromycin (**6**) from its aminonucleoside, 5′-deoxy-PAN (**5**).[13,14] Because of the lengthy synthetic scheme involved in the total synthesis of **6**, we have recently investigated the direct conversion of PAN to 5′-deoxy-PAN (**5**) via the 5′-chloro-5′-deoxy-PAN (**1**). In addition to its use as a synthetic intermediate, we were interested in assessing the ability of **1** to resist conversion to a nephrotoxic metabolite. Also in this paper, 5′-chloro-5′-deoxypuromycin is examined with respect to its ability to participate in the ribosomal peptidyltransferase reaction and inhibit protein biosynthesis.

Results and Discussion

Synthesis. The synthetic route for the preparation of 5′-chloro-5′-deoxypuromycin is outlined in Scheme II. Thus, the conversion of PAN to 5′-chloro-5′-deoxy-PAN (**1**) was accomplished with thionyl chloride in triethyl phosphate. The aminonucleoside, **1**, was condensed with *N*-(*tert*-butyloxycarbonyl)-L-*p*-methoxyphenylalanine (**2**) using dicyclohexylcarbodiimide and *N*-hydroxysuccinimide. Removal of the *tert*-butyloxycarbonyl blocking group from **3** with anhydrous trifluoroacetic acid gave the desired 5′-chloro-5′-deoxypuromycin (**4**) as expected.

As illustrated in Scheme III, 5′-deoxy-PAN was obtained by reduction of **1** with tributyltin hydride in the presence

(1) D. Nathans, *Proc. Natl. Acad. Sci. U.S.A.*, **51**, 585 (1964).
(2) R. R. Traut and R. E. Monro, *J. Mol. Biol.*, **10**, 63 (1964).
(3) J. N. Porter, R. I. Hewitt, C. W. Hesseltine, G. Krupka, J. A. Lowrey, W. S. Wallace, N. Hohonos, and J. A. Williams, *Antibiot. Chemother.*, **2**, 409 (1952).
(4) R. I. Hewitt, W. S. Wallace, A. R. Gumble, E. R. Gill, and J. A. Williams, *Am. J. Trop. Med. Hyg.*, **2**, 254 (1953).
(5) E. J. Tobie, *Am. J. Trop. Med. Hyg.*, **3**, 852 (1954).
(6) M. D. Young, *Antibiot. Med. Clin. Ther.*, **6**, 222 (1959).
(7) M. D. Young and J. E. Freed, *South. Med. J.*, **49**, 537 (1956).
(8) D. Nathans, *Antibiotics (N.Y.)*, **1**, 259–277 (1967).
(9) B. A. Borowsky, D. M. Kessner, and L. Recant, *Proc. Soc. Exp. Biol. Med.*, **97**, 857–860 (1958).
(10) R. F. Derr, C. S. Alexander, and H. T. Nagasawa, *Proc. Soc. Exp. Biol. Med.*, **125**, 248 (1967).
(11) N. Dickie, L. Norton, R. F. Derr, C. S. Alexander, and H. T. Nagasawa, *Proc. Soc. Exp. Biol. Med.*, **123**, 421 (1967).
(12) E. Kmetic and A. Tirpack, *Biochem. Pharmacol.*, **19**, 1493 (1970).
(13) R. Vince, R. G. Almquist, C. L. Ritter, F. N. Shirota, and H. T. Nagasawa, *Life Sci.*, **18**, 345 (1976).

0022-2623/81/1824-1511$01.25/0 © 1981 American Chemical Society

Scheme II

PAN

$SOCl_2$, $(C_2H_5O)_3P=O$

1 + tBOC-NH-CH(COOH)-CH_2-C6H4-OMe (2) → NHS/DCC

3 → CF_3COOH → 4

Scheme III

1 → $(C_4H_9)_3SnH$ → 5 → Ref. 14 → 6

of azobisisobutyronitrile. The 5′-deoxyaminonucleoside (**5**) was converted to 5′-deoxypuromycin (**6**) as previously described.[14]

Biology. A desirable approach to improve the selective toxicity of puromycin would involve the replacement or elimination of the 5′-OH of the antibiotic in order to prevent nucleotide formation from the PAN moiety. However, such modifications must not prevent the altered antibiotic from participating as a substrate at the ribosomal peptidyltransferase site. In addition, it must be demonstrated that the altered aminonucleoside moiety is devoid of nephrotoxic activity. In a previous communication, we reported that 5′-deoxy-PAN was not nephrotoxic to rats, while the corresponding 5′-deoxypuromycin was an excellent substrate for the transpeptidation reaction.[13]

1/V vs. [SUBSTRATE MOLARITY]$^{-1}$ × 10^{-4}

Figure 1. Double-reciprocal plot for the peptidyltransferase reaction with Ac-L-[^{14}C]Phe-tRNA as a donor substrate with puromycin (●), 5′-deoxypuromycin (○), and 5′-chloro-5′-deoxypuromycin (△) as acceptor substrates. The Ac-L-[^{14}C]-Phe-tRNA was bound to the ribosomes in a reaction mixture containing 130 mM Tris-Cl (pH 7.5), 130 mM NH_4Cl (pH 7.6), 20 mM $Mg(OAc)_2$, 0.84 mM dithiothreitol, 2.78 A_{260} units of washed *E. coli* ribosomes, 1.50 mM GTP, 63 μg of FWR, 0.35 A_{260} units of poly(U), and 21 pmol of Ac-L-[^{14}C]Phe-tRNA (464 pCi/pmol) in a total volume of 80 μL. The binding mixture was incubated at 28 °C for 8 min, and the peptidyltransferase reaction was initiated by the addition of 20 μL of puromycin analogue in water. Reaction mixtures were incubated for a specified time, and product formation was measured as described in ref 16. Counting efficiency was approximately 90%. All counts were corrected by blanks in which substrate was absent. The kinetic parameters, K_M and V_{max}, were determined using a Wilkinson analysis.[18] All plotted points represent an average of duplicate determinations. The standard deviation of the obtained values averaged ±5%.

Double-reciprocal plots of initial velocities of product formation vs. substrate concentrations were used to compare the kinetic parameters of puromycin, 5′-chloro-5′-deoxypuromycin (**4**), and 5′-deoxypuromycin (**6**). Figure 1 confirms the previously observed activity of **6**[13] and also shows that **4** is an acceptor for the donor acetylphenylalanyl-tRNA in the peptidyltransferase reaction. Further examination of Figure 1 reveals that although both **4** and **6** are excellent substrates, their kinetic parameters differ significantly. Thus, replacement of the 5′-OH of puromycin with Cl had no effect on K_M (0.15 mM), while the V_{max} was lowered by a factor of 2.5. Replacement of the 5′-OH with H had no effect on V_{max} and raised the K_M to 0.30 mM. The above data clearly indicate that the 5′-OH of puromycin can be replaced with H or Cl without destroying its ability to act as a substrate at the peptidyltransferase site of ribosomes and inhibit protein synthesis. Nephrotoxicity studies were conducted to ensure that the corresponding aminonucleosides released by the in vivo hydrolysis of **4** and **6** were devoid of toxic manifestations. Figure 2 shows that PAN causes severe proteinuria in rats due to nephrotoxicity beginning after 8 days of treatment. At the same dose level, no nephrotoxicity was observed with **1** and **5**, even after 28 days of treatment, and urine

(14) R. G. Almquist and R. Vince, *J. Med. Chem.*, 16, 1396 (1973).

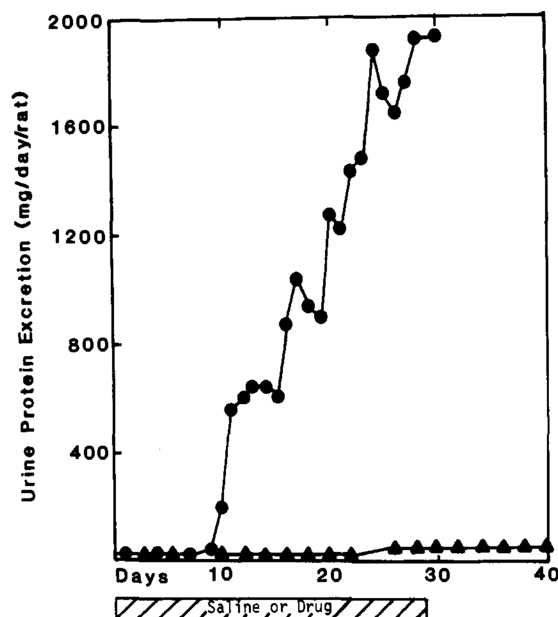


Figure 2. Nephrotoxicity of PAN. Male Sprague–Dawley rats, 6 per group, weighing 60.9 ± 1.8 g, were administered subcutaneously with equimolar doses (0.051 mmol/kg) of the aminonucleosides or with saline daily for 28 days, and the total 24-h urine protein excretion[19] was measured for each rat for a period of 40 days. The daily urinary proteins excreted by rats treated with 5′-deoxy-PAN or 5′-chloro-5′-deoxy-PAN were comparable and did not exceed the levels of protein excreted by control rats given saline (△), whereas PAN (●) administration resulted in the induction of a nephrotic syndrome characterized by excessive proteinuria, ascites, and imminent death.

protein was not different from saline controls up to day 40. Gross examination at sacrifice of the kidney, liver, and spleen indicated that they were normal. The inability of the 5′-deoxy-PAN derivatives to induce a nephrotic syndrome strongly confirms the involvement of the 5′-OH group in toxicity at the aminonucleoside level. Thus, 5′-chloro-5′-deoxypuromycin represents a new puromycin analogue prepared in such a way that the structural features required for antimicrobial activity at the ribosomal level have been retained with concomitant elimination of the molecular moiety responsible for conversion to a toxic metabolite.

Antitrypanosomal testing of the puromycin derivatives 4 and 6 was conducted at the Walter Reed Army Institute. Compounds were administered in a single dose to ICR/HA Swiss mice infected with the Wellcome CT strain of *Trypanosoma rhodesiense* as previously described.[15] The chloro derivative 4 was highly active with curative doses of 26 mg/kg subcutaneously and 13 mg/kg orally. Surprisingly, 5′-deoxypuromycin (6) was not active against trypanosome-infected mice at the maximum dose of 106 mg/kg. Metabolic studies of the puromycin derivatives (4 and 6) and their nucleosides (1 and 6) are presently being conducted to explain their differences in in vivo activities.

Experimental Section

Puromycin dihydrochloride and PAN were obtained from ICN Pharmaceuticals, Inc., L-[^{14}C]phenylalanine was obtained from New England Nuclear, and *Escherichia coli* cell past (B, midlog) was purchased from General Biochemicals. The polynucleotides were obtained from Miles Laboratories. Preparation of ribosomes, S-100, factors washable from ribosomes (FWR), and Ac-L-[^{14}C]Phe-tRNA were as previously described.[16]

Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Melting points were determined on a Mel-Temp apparatus and are corrected. Nuclear magnetic resonance spectra were obtained with a Varian T-60A spectrometer, infrared spectra with a Perkin-Elmer 237B spectrophotometer, and ultraviolet spectra with a Beckman 25 recording spectrophotometer. Column chromatography on silica gel 60, 70–230 mesh (E. Merck, Darmstadt), was used for purification of some compounds. Satisfactory elemental analyses (±0.4% of calculated values) were obtained for each compound listed in Schemes II and III.

6-(Dimethylamino)-9-(3′-amino-5′-chloro-3′,5′-dideoxy-β-D-ribofuranosyl)purine (1). To a suspension of PAN (294 mg, 1.0 mmol) in triethyl phosphate (10 mL) was added freshly distilled thionyl chloride (0.3 mL, 4.0 mmol). The reaction mixture was stirred at room temperature for 14 h and then poured into anhydrous ether (250 mL) and allowed to stand at ambient temperature for 1 h. The solid was collected by filtration, dissolved in methanol (25 mL), and neutralized with Dowex 1 resin, 25–50 mesh, OH^- form. The resin was removed by filtration and washed with methanol (50 mL). The combined filtrates were evaporated to dryness in vacuo at 25 °C and gave a white crystalline product. Recrystallization from water gave pure 1: yield 230 mg (74%); mp 174–175 °C; IR (KBr) 2900–3380 (OH, NH), 1610, 1570 (C=C, C=N), 640 (Cl) cm^{-1}; UV λ_{max} ($\epsilon \times 10^{-3}$), at pH 1, 268 nm (19.0); at pH 14, 275 (20.1). Anal. ($C_{12}H_{17}ClN_6O_2$) C, H, N, Cl.

***N*-(*tert*-Butyloxycarbonyl)-L-*p*-methoxyphenylalanine (2).** To an ice-cooled solution of L-*p*-methoxyphenylalanine hydrochloride[17] (2.32 g, 1.0 mmol) in 0.5 N sodium hydroxide (60 mL) and dioxane, (90 mL) was added *tert*-butoxycarbonyl azide (5 mL), and the solution was stirred at room temperature for 48 h. The solution was concentrated in vacuo to 60 mL and adjusted to pH 5 at 0–5 °C with 1 N sulfuric acid. A heavy precipitate of 2 was collected by filtration and dried in vacuo at 50 °C: yield 1.90 g (76%); mp 132–135 °C. Recrystallization from chloroform–hexane gave the pure product: mp 134–135 °C; NMR ($CDCl_3$) δ 12.03 (br, 1 H, COOH), 7.06 and 6.73 (2 d, 4 H, aromatic), 5.33 (br, 1 H, NH), 4.35 (m, 1 H, C_α H), 3.70 (s, 3 H, OCH_3), 3.03 (m, 2 H, C_β H_2), 1.36 (s, 9 H, *t*-Bu). Anal. ($C_{15}H_{21}NO_5$) C, H, N.

6-(Dimethylamino)-9-[3′-[[*N*-(*tert*-butyloxycarbonyl)-L-*p*-methoxyphenylalanyl]amino]-5′-chloro-3′,5′-dideoxy-β-D-ribofuranosyl]purine (3). To a cooled (ice bath) solution of 1 (624 mg, 2.0 mmol), 2 (590 mg, 2.0 mmol), and *N*-hydroxysuccinimide (250 mg, 2.1 mmol) in anhydrous dimethylformamide (5 mL) was added dicyclohexylcarbodiimide (460 mg, 2.20 mmol), and the stoppered mixture was stirred at room temperature for 16 h. The solvent was removed under reduced pressure, and the residue was triturated with ethyl acetate (15 mL). The insoluble dicyclohexylurea (DCU) was removed by filtration. The filtrate was evaporated to dryness, and the residue was dissolved in chloroform and applied to a silica gel column (50 g) packed in chloroform. A small amount of DCU was removed with chloroform, and the desired product was eluted with chloroform containing 1% methanol. Evaporation of the eluate containing 3 gave a white powder: yield 900 mg (80%); mp 180–183 °C dec; NMR ($CDCl_3$) δ 8.16 and 7.96 (2 s, 2, C_2 H and C_8 H), 7.16 and 6.80 (2 d, 4 H, J = 8 Hz, aromatic), 5.75 (d, 1 H, J = 3.0 Hz, $C_{1'}$ H), 3.73 (s, 3 H, OCH_3), 3.5 [s, 6 H, $N(CH_3)_2$], 1.4 (s, 9 H, *t*-Bu). Anal. ($C_{27}H_{36}ClN_7O_6$) C, H, N, Cl.

6-(Dimethylamino)-9-[3′-[(L-*p*-methoxyphenylalanyl)-amino]-5′-chloro-3′,5′-dideoxy-β-D-ribofuranosyl]purine. 5′-Chloro-5′-deoxypuromycin (4). Trifluoroacetic acid (ice cold) was added dropwise to 3 (200 mg, 0.339 mmol) until a solution was obtained (5 mL). The solution was kept at room temperature for 8 min, and the acid was removed under reduced pressure at 25 °C. The last traces of acid were removed by coevaporation

(15) L. Rane, D. S. Rane, and K. E. Kinnamon, *Am. J. Trop. Med. Hyg.*, **25**, 395 (1976).

(16) P. H. Duquette, C. L. Ritter, and R. Vince, *Biochemistry*, **13**, 4855 (1974).

(17) B. R. Baker, J. P. Joseph, and J. H. Williams, *J. Am. Chem. Soc.*, **77**, 1 (1955).

(18) G. N. Wilkinson, *Biochem. J.*, **80**, 325 (1961).

(19) H. T. Nagasawa, C. S. Alexander, F. N. Shirota, H. Ghobrial, K. F. Swingle, and R. F. Derr, *Toxicol. Appl. Pharmacol.*, **16**, 1 (1970).

with acetonitrile. The oily residue was dissolved in methanol (20 mL) and stirred with excess basic ion-exchange resin (Dowex 1, 20–50 mesh, OH^- form, 5 mL) for 10 min. The resin was removed by filtration and washed with methanol (50 mL). Evaporation of the combined filtrates gave **4** as a white crystalline solid: yield 155 mg (93.3%). Recrystallization from methanol–ether gave an analytical sample: mp 124–125 °C dec; UV λ_{max} ($\epsilon \times 10^{-3}$), in methanol, 275 (20.6); at pH 1, 268 (20.3); at pH 14, 276 (20.9). Anal. ($C_{22}H_{28}ClN_7O_4$) C, H, N, Cl.

6-(Dimethylamino)-9-(3′-amino-3′,5′-dideoxy-β-D-ribofuranosyl)purine. 5′-Deoxy-PAN (5). To a solution of **1** (2.35 g, 8.0 mmol) in anhydrous tetrahydrofuran (100 mL) were added azobisisobutyronitrile (0.50 g, 3.04 mmol) and tributyltin hydride (6.0 g, 21.0 mmol). The reaction mixture was heated under reflux for 18 h under anhydrous conditions. The solvent was removed under reduced pressure, and the residue was triturated with cold petroleum ether (50 mL). A solid product was removed by filtration, washed with cold petroleum ether (100 mL), and crystallized from ethyl acetate to give pure **5**: yield 1.25 g (90.5%); mp 202–203 °C (lit.[14] mp 204–205 °C); IR and NMR spectra were identical with an authentic sample.[14]

Acknowledgment. We thank Jay Brownell for assistance in the biological assays. We are also grateful to Drs. David E. Davidson, Jr., and William Y. Ellis, Walter Reed Army Institute, for providing the antitrypanosomiasis data. This investigation was supported by Grants CA 13592 and CA 23263 from the National Cancer Institute, Department of Health, Education, and Welfare.

Synthesis of *S*-(3-Deazaadenosyl)-L-homocysteine

J. A. Montgomery,* H. J. Thomas, M. C. Thorpe,

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35255

and P. K. Chiang

Division of Biochemistry, The Walter Reed Army Institute of Research, Washington, DC 20012. *Received July 1, 1981*

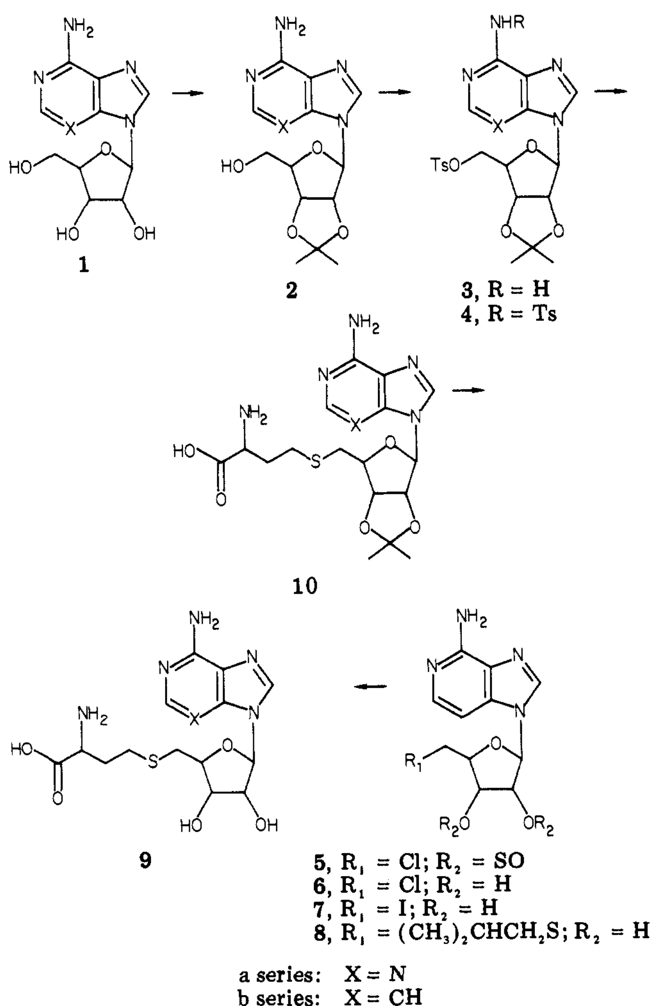
A satisfactory procedure for the preparation of *S*-(3-deazaadenosyl)-L-homocysteine, a metabolite of 3-deazaadenosine, which is a potent inhibitor of *S*-adenosyl-L-homocysteine hydrolase with antiviral activity, is described. For the first time this compound is completely characterized and its optical purity established.

S-Adenosyl-L-homocysteine (**9a**), the biochemical byproduct of methylation reactions involving adenosylmethionine, was first synthesized by displacement of the tosyloxy group of 2′,3′-*O*-isopropylidene-5′-*O*-tosyladenosine (**3a**) with the sodium salt of L-homocysteine, followed by removal of the isopropylidene group from the product.[1,2] This procedure has been applied to the synthesis of *S*-(3-deazaadenosyl)-L-homocysteine monohydrate (**9b**) in an overall yield of 12%; the intermediates were not characterized, and no information on the specific rotation or optical purity of **9b** was provided.[3]

Because of the recent interest in 3-deazaadenosine (**1b**) as a competitive inhibitor of and alternate substrate for *S*-adenosyl-L-homocysteine hydrolase,[4,5] we have reinvestigated the chemical synthesis of the enzymatic product **9b** (Scheme I). Tosylation[3] of 2′,3′-*O*-isopropylidene-3-deazaadenosine (**2b**), most satisfactorily prepared by the procedure of Zderic et al.,[6] gave a mixture of 61% unreacted **2b**, 31% 5′-*O*-tosyl-2,3-*O*-isopropylidene-3-deazaadenosine (**3b**), and a small amount of material identified by UV and mass spectral data as a ditosyl derivative of **2b** (**4b**).

The rather unsatisfactory results of the tosylation reaction led us to explore the conversion of the sulfite ester (**5**) of 5′-chloro-5′-deoxy-3-deazaadenosine (**6**) directly to **9b**, since we previously prepared the 5′-(isobutylthio)-5′-deoxy-3-deazaadenosine (**8**) from **6** itself,[7] and Borchardt et al. have prepared other analogues of *S*-adenosyl-L-homocysteine in a similar manner.[8] Unfortunately, **5**

Scheme I



(1) Baddiley, J.; Jamieson, G. A. *J. Chem. Soc.* **1955**, 1085.
(2) Sakami, W. *Biochem. Prep.* **1961**, *8*, 8.
(3) Borchardt, R. T.; Huber, J. A.; Wu, Y. S. *J. Med. Chem.* **1974**, *17*, 868.
(4) Chiang, P. K.; Richards, H.; Cantoni, G. L. *Mol. Pharmacol.* **1977**, *13*, 939.
(5) Guranowski, A.; Montgomery, J. A.; Cantoni, G. L.; Chiang, P. K. *Biochemistry* **1981**, *20*, 110.
(6) Zderic, J. A.; Moffatt, J. G.; Kau, D.; Gerzon, K.; Fitzgibbon, W. E. *J. Med. Chem.* **1965**, *8*, 275.
(7) Chiang, P. K.; Cantoni, G. L.; Bader, J. P.; Shannon, W. M.; Thomas, H. J.; Montgomery, J. A. *Biochem. Biophys. Res. Commun.* **1978**, *82*, 417.

0022-2623/81/1824-1514$01.25/0 © 1981 American Chemical Society