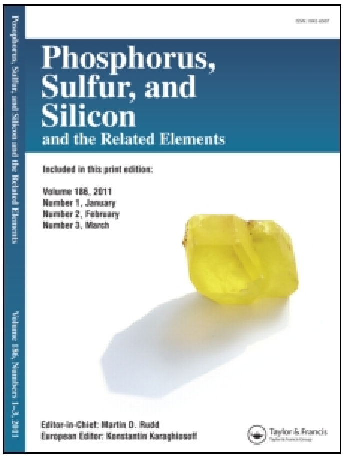

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

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To cite this Article Scovill, John P. , Thigpen II, Don L. and Lemley, Paul V.(1993) 'A CONVENIENT METHOD FOR THE SYNTHESIS AND RANEY NICKEL DESULFURIZATION OF 5'-DEOXY-5' -M ETHYLTHIOADENOSINE', Phosphorus, Sulfur, and Silicon and the Related Elements, 85: 1, 149 — 152

To link to this Article: DOI: 10.1080/10426509308038193

URL: http://dx.doi.org/10.1080/10426509308038193

Phosphorus, Sulfur, and Silicon, 1993, Vol. 85, pp. 149–152
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A CONVENIENT METHOD FOR THE SYNTHESIS AND RANEY NICKEL DESULFURIZATION OF 5′-DEOXY-5′-METHYLTHIOADENOSINE

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(Received April 8, 1993; in final form October 18, 1993)

A convenient procedure for the preparation of 5′-deoxy-5′-methylthioadenosine is reported. Chlorination of adenosine with thionyl chloride yielded 5′-chloro-5′-deoxyadenosine. Reaction of 5′-chloro-5′-deoxyadenosine with aqueous methylmercaptide anion yielded 5′-deoxy-5′-methylthioadenosine. Hydrogenolysis of 5′-deoxy-5′-methylthioadenosine over Raney nickel in water produced 5′-deoxyadenosine. This procedure affords a high yield of readily purified 5′-deoxyadenosine while avoiding the use of anhydrous solvents and pyrophoric reagents. The procedure illustrates the utility of sulfur reagents to accomplish high value added transformations in nucleoside chemistry.

Key words: 5′-Deoxyadenosine; 5′-deoxy-5′-methylthioadenosine; 5′-chloro-5′-deoxyadenosine; Raney nickel desulfurization.

As part of a program aimed at the discovery of substrate-based inhibitors of the plant cytotoxin, ricin, we have undertaken the design and synthesis of adenosine-like nucleosides capable of preventing the ribosome inactivating action of the toxin's A-chain. The cytotoxic effect of ricin is due to the *N*-glycosidase action of the ricin A-chain against a specific adenosine residue in ribosomal RNA.[1] In this regard, we were guided by a three-dimensional x-ray crystal structure of the enzyme's active site bound to the nucleoside antibiotic, formycin-A 5′-monophosphate (FMP), an isomer of adenosine 5′-monophosphate (cf. Figure 1).[2] As it appeared that the phosphate moiety of FMP did not make strong, specific interactions with the protein, we hoped that this portion of the molecule might be unnecessary in an inhibitor and that 5′-deoxypurine nucleosides might form the basis for the design of an inhibitor. This would simplify the structural complexity of nucleosides considered as potential inhibitors. Furthermore, we anticipated a substantially reduced toxicity

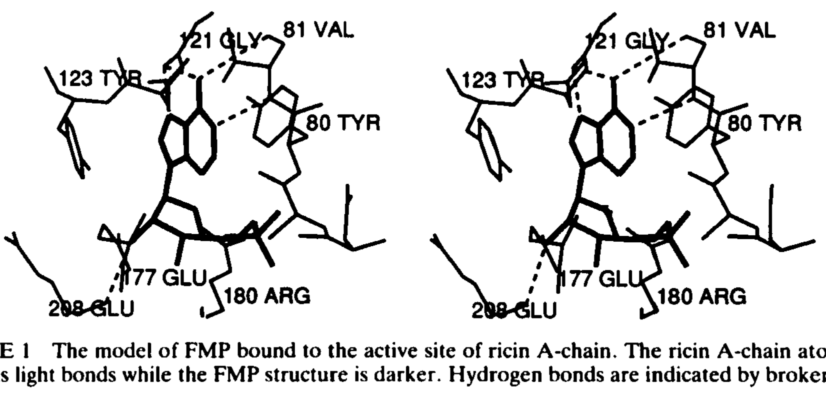
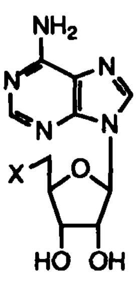


FIGURE 1 The model of FMP bound to the active site of ricin A-chain. The ricin A-chain atoms are shown as light bonds while the FMP structure is darker. Hydrogen bonds are indicated by broken lines.

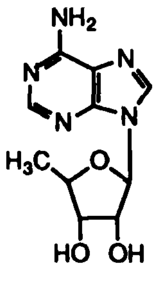
of nucleosides upon their conversion to the 5'-deoxy derivatives.[3] In order to evaluate this hypothesis, we required an inexpensive source of 5'-deoxyadenosine (**2**). Our interest in the application of sulfur reagents to accomplish high value added transformations in nucleoside chemistry prompted us to investigate the utility of the Raney nickel desulfurization of 5'-deoxy-5'-methylthioadenosine (**1c**) as a source of **2**.

Previously, 5'-deoxyadenosine (**2**) and other 5'-deoxynucleosides have been prepared by lengthy procedures and in relatively low yields. Thus, 5'-deoxyadenosine was originally prepared by coupling 5'-deoxyribose with adenine using the method of Davoll and Lowry.[4] Wagner *et al.* obtained a low yield of impure **2** through the Raney nickel desulfurization of 5'-deoxy-5'-thioethyladenosine.[5] Recently, the use of tributyltin hydride and α,α-azobis(isobutyronitrile) to synthesize deoxynucleosides by dechlorination has proven to be effective and simple.[6] More recently, **2** was obtained in 93% yield through reductive dechlorination of 5'-chloro-5'-deoxyadenosine (**1b**) by lithium triethylborohydride.[7] Although these latter two procedures are expedient, using anhydrous solvent (THF), inert atmosphere, and pyrophoric reagents presents some drawbacks. In this note, we describe a convenient method to prepare 5'-deoxyadenosine (**2**) by the Raney nickel desulfurization of 5'-deoxy-5'-methylthioadenosine (**1c**) in aqueous solution.

Adenosine (**1a**) was converted to 5'-chloro-5'-deoxyadenosine (**1b**) by thionyl chloride in acetonitrile (cf. Reference 7). We found that the 5'-chloro group of **1b** was readily replaced by a thiomethyl group in aqueous solution. The methyl mercaptide anion may be generated by bubbling methane thiol through a dilute solution of sodium hydroxide. Adding 5'-chloro-5'-deoxyadenosine to the solution and heating briefly completed the transformation of **1b** to 5'-thiomethyl-5'-deoxyadenosine (**1c**). A serum bottle sealed with a septum was useful for containing this reaction. Hydrogenolysis of **1c** to 5'-deoxyadenosine was effected by heating with a large excess of Raney nickel (7 mL of 50% aqueous slurry to 1 g of **1c**) under hydrogen at 4 atm. These transformations were conveniently monitored by thin-layer chromatography (TLC) (SiO_2, $CHCl_3$/MeOH, 3:1). Raney nickel has an unusual affinity for 5'-deoxyadenosine. However, the deoxynucleoside was readily extracted from the catalyst by 50% aqueous pyridine. Evaporating the pyridine solution under reduced pressure yielded **2** as a colorless, crystalline solid in 62% yield overall.



1a, X=OH
1b, X=Cl
1c, X=CH_3S



2

These reactions serve to illustrate the utility of sulfur reagents in accomplishing high value added transformations in nucleoside chemistry. In the case described above, inexpensive adenosine ($0.50 per g) is readily converted to the more valuable 5'-deoxyadenosine ($1.34 per mg), which is about 3000 times more expensive.

EXPERIMENTAL

5'-chloro-5'-deoxyadenosine (**1b**). A suspension of adenosine (13.35 g, 50 mmol) in 50 mL of acetonitrile was treated dropwise with thionyl chloride (11 mL, 17.9 g, 150 mmol), keeping the temperature between 0 to 5°C. Pyridine (8.1 mL) was then added and the mixture was stirred at 0°C for 3 hours. Stirring was continued overnight, and the mixture allowed to warm to room temperature. The precipitate that formed was dissolved by adding 150 mL of water to the reaction mixture. The solution was neutralized with sodium hydrogen carbonate, and the solid that precipitated was collected, washed with cold water, and dried under vacuum to yield 17.3 g (100%) of a mixture of the epimeric 5'-chloro-5'-deoxy-2',3'-*O*-sulfinyladenosines. The sulfinyladenosine mixture converted to 5'-chloro-5'-deoxyadenosine, without further purification, as follows: a solution containing 3.15 g (9.51 mmol) of the sulfinyl ester in 50 mL of methanol was treated with 5 mL of concentrated NH_4OH. Crystals formed upon standing for an hour at room temperature. These were collected, washed with cold methanol and dried under vacuum to yield 2.69 g (99%) of colorless needles of 5'-chloro-5'-deoxyadenosine. The product was identical to an authentic sample (Sigma Chemical Company) by TLC (SiO_2, $CHCl_3$/MeOH, 3:1, rf = 0.57) and infrared spectrum, and was sufficiently pure to be utilized in the subsequent step without further purification.

5'-Deoxy-5'-methylthioadenosine (**1c**). A solution containing 1.20 g of sodium hydroxide (30 mmol) in aqueous methanol (15 mL of methanol and 2 mL of water) was saturated with CH_3SH. This solution was added to a suspension of 2.86 g (10 mmol) of 5'-chloro-5'-deoxyadenosine in 80 mL of water in a 100 mL serum bottle. The bottle was capped and heated at 75°C for 24 hours. The solution was allowed to cool to room temperature and then was vented. The pH was adjusted to 6 with glacial acetic acid, and the reaction mixture was cooled at 5°C overnight. The crystals that formed were collected, washed with cold water, and dried under vacuum. The yield was 2.21 g (75%) of colorless needles of 5'-deoxy-5'-methylthioadenosine, mp 209–211°C (lit[7]: 212°C). The product was identical to an authentic sample (Sigma Chemical Company), by TLC (SiO_2, $CHCl_3$/MeOH, 3:1, rf = .65) and infrared spectrum.

5'-Deoxyadenosine (**2a**). A solution containing 1.00 g (3.36 mmol) of 5'-deoxy-5'-methylthioadenosine (**1c**) in 80 mL of H_2O was hydrogenated over Raney nickel (12 g of a 50% aqueous slurry). After 2 days at 90°C and 60 psi, the reaction was complete, as evidenced by TLC. The pressure vessel was cooled and vented, and the catalyst collected by filtration. Evaporating the solvent under reduced pressure afforded a meager amount of 5'-deoxyadenosine. Extracting the catalyst with boiling water (4 × 225 mL) afforded 220 mg of product. However, extraction of the catalyst with 100 mL of hot (70°C) 50% aqueous pyridine yielded, after evaporation of the solvent at reduced pressure, an additional 600 mg of product. The two fractions were combined and recrystallized from water to yield 750 mg (83%) of colorless crystals of 5'-deoxyadenosine, mp 130–132°C (lit[7]: 134°C). The product was identical to an authentic sample (Sigma Chemical Company) by TLC (SiO_2, $CHCl_3$/MeOH, 3:1, rf = .51) and infrared spectrum. The overall yield of **2** based on **1a** is 62%.

ACKNOWLEDGEMENT

We gratefully acknowledge the assistance of Professor Jon D. Robertus for providing the atomic coordinates of the ricin-formycin-A complex.

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of the 5'-hydroxy group to a halo group or a hydrogen atom. Thus, we observed that the nucleoside antibiotic tubercidin, had an IC_{50} of 50 ng/mL against EL4 mouse leukemia cells in culture, while 5'-chloro-5'-deoxytubercidin lacked toxicity at 10 μg/mL. A similar effect was observed for formycin-A, which also had an IC_{50} of 50 ng/mL, while the 5'-chloro-5'-deoxy derivative was non-toxic at 100 μg/mL.

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