Typical column sizes used are shown in Table I. The five smaller columns are packed and run as described in Figure 1. For the four smallest, commercial[7] columns are used as received. Air pressure is introduced through a glass tube inserted through a one-hole rubber stopper in the top of the column (Figure 2). It is convenient to maintain column pressure with laboratory compressed air, delivered through a length of Tygon tubing having a small syringe needle inserted in it for a bleed.[3] The same procedure is followed for the 50-g column, except that the top of the commercial column is modified by attaching to it a female 35/20 ball joint. The male joint is necked down to a tubing connector for the air line and secured to the female joint with a screw clamp (Figure 2). The three largest columns[8] are also packed and run as described, except that aspirator vacuum[9] is substituted for air pressure. Fractions are collected in Erlenmeyer flasks by using a vacuum adapter as shown in Figure 3. Again, it is important to close the stopcock at the bottom of the column before releasing the vacuum to change fractions.

The procedure described here, besides using a less costly grade of silica gel, appears to offer substantially better resolution than is claimed for the obvious alternative, flash chromatography.[10,11] This is not a minor consideration, even for "one spot" reactions. We have routinely observed[6] that samples purified as outlined here, followed by bulb-to-bulb distillation to remove traces of solvent residue, are satisfactory for elemental analysis.

Acknowledgment. This work was supported by the National Institutes of Health (Grant No. GM 15431). We are grateful to Professor W. C. Still for sharing his procedures with us.

Supplementary Material Available: Figures 1–3, with accompanying legends describing details of column preparation and operation (4 pages). Ordering information is given on any current masthead page.

(7) Commercial chromatography columns were purchased from Ace Glass, Inc.

(8) As the larger columns are run under vacuum, additional solvent can be run in as needed. Thus, the column need only be tall enough to contain the initial silica gel slurry. The 120-mm-diameter column is 210 mm long, and the 170-mm-diameter column is 270 mm long.

(9) Use of vacuum-driven column chromatography has previously been described: Targett, N. M.; Kilcoyne, J. P.; Green, B. *J. Org. Chem.* **1979**, *44*, 4962.

(10) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(11) The procedure described here is adequate for most routine separations. It clearly does not have the inherent resolving power of medium-pressure liquid chromatography: Meyers, A. I.; Slade, J.; Smith, R. K.; Mihelich, E. D. *J. Org. Chem.* **1979**, *44*, 2247.

(12) Kolar, A. J. *Aldrichimica Acta* **1980**, *13*, 42.

New Highly Fluorescent Derivative of Adenosine. Cyclization of Adenosine with 1′-Methylthiaminium Ion

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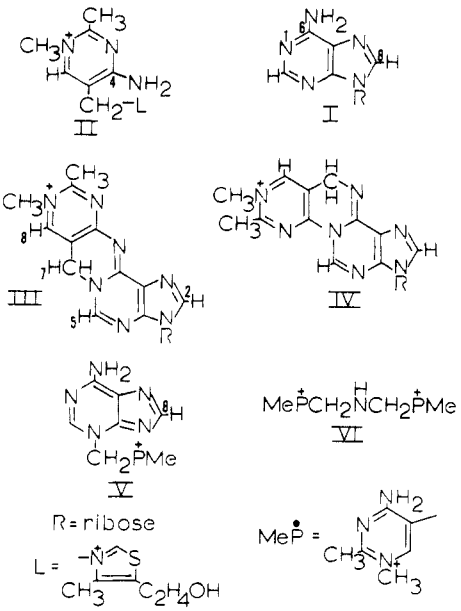
Considerable effort has been expended to convert adenosine (I, Chart I) into fluorescent derivatives. Such conversions not only provide an ultrasensative method of detecting I but also furnish fluorophores which are useful bioprobes.[1]

Chart I



Successful transformations largely include those which fuse a five-membered ring onto I by incorporating N-1 and the 6-amino group along with a reagent such as chloroacetaldehyde[2,3] or glyoxal.[4] Emphasis now is being placed on the synthesis of new fluorescent derivatives of heteroaromatic components of nucleic acids by annulation to give six-membered rings.[1]

We report the preparation of a novel, highly fluorescent derivative of I. Two heterocyclic rings are fused onto I, both six membered, by treatment with 1′-methylthiaminium ion (II),[5] a derivative of vitamin B_1.

Results and Discussion

Compounds I and II readily react in refluxing methanol containing 2,4,6-trimethylpyridine catalyst.[6] Proton and ^{13}C NMR show that the product does not contain the thiazole ring (L) from II. In view of the many facile nucleophilic substitution reactions which II undergoes,[7] I must be bonded to II at its CH_2 group in place of the thiazole ring. Elemental analyses reveal that the substitution product is cyclic, cyclization proceeding by the loss of an amino group as ammonia. Therefore, the product is likely to have structure III or IV, both containing four fused heterocyclic rings having a total of seven annular nitrogen atoms.

Regioisomers III and IV differ by having the orientations of the two reactants reversed on cyclization. Isomer III has the CH_2 group of II bonded to N-1 of I. One of the two amino groups is incorporated into the new ring, the other is lost as ammonia. Isomer IV has the CH_2 chain attached to the 6-amino group of I; N-1 of I is bonded to position 4 of II in place of its amino substituent.

Differentiation between these two isomers was achieved by means of a nuclear Overhauser effect (NOE) involving

(1) For a recent list of leading references see: Hosmane, R. S.; Leonard, N. J. *J. Org. Chem.* **1981**, *46*, 1457–1465.

(2) Sattsangi, P. D.; Barrio, J. R.; Leonard, N. J. *J. Am. Chem. Soc.* **1980**, *102*, 770–774.

(3) Arigad, G.; Damle, S. *Anal. Biochem.* **1972**, *50*, 321–326.

(4) Yiki, H.; Sempuku, C.; Park, M.; Takiura, K. *Anal. Biochem.* **1972**, *46*, 123–128.

(5) Zoltewicz, J. A.; Baugh, T. D. *Synthesis* **1980**, 217–218.

(6) Catalyst influences the pH of the solution.

(7) Zoltewicz, J. A. *Synthesis* **1980**, 218–219.

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aromatic protons. Irradiation of the CH_2 signal is expected to enhance the signals of *two* adjacent protons in III but only *one* in IV. Other aromatic protons in IV are located too far away. Our crucial observation in that two signals demonstrate substantial enhancement when the signal due to CH_2 is irradiated. The product is III, a new ring system. A systematic name for it is 9,10-dimethyl-3-ribosyl-3,7-dihydropyrimido[4′,5′:4,5]pyrimido[2,1-*i*]purinium perchlorate.[8]

Independent assignment of the three aromatic proton signals to sites in the cyclized product supports structure III and the observed NOE. Warming III in D_2O causes the signal at lowest field to decrease in intensity as a result of hydrogen–deuterium exchange.[11] Irradiation of the 1′-proton of ribose induces an NOE in this low-field hydrogen signal. Both observations suggest that this signal may be assigned to H-2 which is bonded to the imidazole ring of III. The highest field aromatic proton signal is slightly broadened by coupling with the CH_2 group and is, therefore, H-8 of III, the pyrimidine proton from II.[12] The remaining aromatic hydrogen signal must be associated with H-5, the pyrimidine proton from I. As required by structure III, NOE enhancements due to irradiation of the CH_2 signal are found in the two signals assigned to the two pyrimidine protons. The product must have the cyclopentenoanthracene geometry of III and not the steroid geometry of IV.

The scope of the heterocyclization reaction is indicated by a consideration of the structure of the product from II and adenine, potentially a tetracyclic substance similar to III or IV. However, NMR and elemental analyses clearly show that N-alkylation, not cyclization, is the major pathway. Structure proof reduces to the classical problem, solved now in several ways, of determining the site of N-alkylation of a purine. In a separate experiment, position 8 of adenine was deuterated.[13] This material on N-alkylation with II no longer showed a signal at δ 7.8; therefore, this signal must be associated with H-8 of the product. Of the four possible ring N-alkylated isomers only the N-3 product shows a signal at such high field.[14,15] Our product must be N-3 isomer V. Adenine methylates at the same position.[16]

In keeping with the formation of V, we conclude that III probably forms by alkylation of N-1 of I in the first step followed by cyclization. Had the first step been substitution with the loss of an amino group, both adenosine and adenine would have yielded tetracyclic products by subsequent cyclization.

An excess of II must be employed to achieve high conversions of I. The ammonia liberated in the cyclization step serves as a nucleophile which acts in competition with I and II to give nonfluorescent secondary amine VI having two pyrimidine rings. Amine VI may be independently synthesized from II by using NH_4ClO_4 as a source of low concentrations of ammonia.

The fluorescence properties of III are remarkable. Concentrated solutions show little fluorescence due to self-quenching. A 2.4×10^{-3} M solution in pH 9.2 aqueous buffer shows excitation (323 nm) and emission spectra (429 nm) which are different from those for more dilute samples. Dilution causes excitation and emission peaks to shift position and to increase in intensity initially. A 500-fold dilution shifts the main excitation to longer and the emission to shorter wave lengths. (The excitation and ultraviolet absorption spectra are quite similar.) Further dilution lowers both intensities; a 1×10^{-7} M solution has its main excitation band at 385 nm and the emission band at 408 nm. We could easily detect the fluorescence of a 5×10^{-10} M sample of III in water in spite of the small Stokes shift, with excitation at the 385-nm maximum and detection at the 408-nm maximum. The Raman band of water appears as an emission at 440 nm under these conditions and does not interfere. Detection of III by fluorometry appears to be on sensitivity levels comparable to those for other adenosine derivatives.[3,4]

No doubt other substrates can be converted to fluorescent analogues with II. Our fluorescent derivative and synthetic method await exploration.

Experimental Section

Preparation of Fluorescent Derivative III. A suspension of 2.50 g (9.36 mmol) of adenosine, 6.7 g (14 mmol) of 1′-methylthiaminium diperchlorate,[5] 4 mL (30 mmol) of 2,4,6-trimethylpyridine, and 125 mL of methanol was heated at reflux with stirring for 17.25 h. The creamy white filter cake was washed with ethyl acetate to give 3.05 g (5.8 mmol, 62%) of raw product, mp 200–208 °C dec. A sample in D_2O showed the presence of a very small amount of unreacted adenosine (1′-ribose doublet of adenosine is about 10 Hz upfield from that of III) along with a minor amount of secondary amine VI (CCH_3 singlet falls about 8 Hz upfield from that of III). Recrystallization from ethanol–water (product has a tendency to supersaturate) gave the analytical sample (mp 216–219 °C dec) which was dried at room temperature under vacuum: UV (2.39×10^{-5} M in pH 9.2 borate buffer) 206 nm (ϵ 2.51×10^4), 245 (1.15×10^4), 383 (3.60×10^4); H NMR (D_2O, DSS) δ 8.65, 8.55, 8.3 (broadened, each 1 H), 6.15 (ribose 1′), 5.7 (CH_2, broadened), 5.54–4.9 (ribose), 3.95 (NCH_3), 2.7 (CCH_3); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 163.4, 163.0, 151.2, 148.5 (4 s), 147.5, 144.8, 143.1 (3 d), 124.3, 110.9 (2 s), 87.7, 85.5, 74.4, 70.1 (4 d), 61.0 (t, CH_2OH), 46.2 (t, CH_2N), 42.6 (q, NCH_3), 21.9 (q, CCH_3). Anal. Calcd for $C_{17}H_{20}N_7ClO_8 \cdot 2H_2O$: C, 39.12; H, 4.63; N, 18.78. Found: C, 39.09; H, 4.57; N, 18.77. Heating a sample in D_2O at 100 °C for 3 h resulted in at least 80% deuteration of the lowest field aromatic proton and about 50% deuteration of the CCH_3 group.

3-[(4-Amino-1,2-dimethyl-5-pyrimidinio)methyl]adenine Perchlorate (V). A suspension of 0.500 g (3.07 mmol) of adenine, 3.68 g (6.14 mmol) of 1′-methylthiaminium diperchlorate,[5] 2 mL (15 mmol) of 2,4,6-trimethylpyridine, 40 mL of methanol, and 10 mL of dimethyl sulfoxide was heated at reflux for 1 h. Following filtration of the hot mixture and two washings with 10 mL portions of methanol, 1.00 g of product (mp 280–281 °C dec) was collected. Recrystallization from 90 mL of 90% aqueous ethanol gave 0.725 g (2.0 mmol, 64%) of product, mp 286–287 °C dec. (Another mixture heated for 24 h gave the same product.) An analytical sample was prepared by recrystallization from 50% aqueous acetonitrile; mp 286–287 °C dec. It was dried at 100 °C under vacuum over $MgClO_4$: ^1H NMR (Me_2SO-d_6, Me_4Si) δ 9.30 (NH), 8.50, 8.44 (H-2, H-6′), 8.13 (NH), 7.80 (H-8), 5.40 (CH_2), 3.74 (NCH_3), 2.59 (CCH_3); ^1H NMR (Me_2SO-d_6, D_2O with excess CF_3COOH) δ 8.93, 8.22 (H-2, H-6′), 8.68 (H-8), 5.52 (CH_2), 3.73 (NCH_3), 2.61 (CH_3). Anal. Calcd for $C_{12}H_{15}N_8ClO_4$: C, 33.88; H, 4.09; N, 30.22. Found: C, 38.97; H, 4.11; N, 30.21. The large change in chemical shift of H-8 on protonation is expected; our values are to be compared with those of δ 8.63 (H-2) and 8.58 (H-8)

(8) As a trivial name for III we suggest riboadenichrome. Thiochrome[9] and pyrichrome[10] have long been known. All three have the same two pyrimidine rings; they differ in the identity of other fused rings. The latter two contain a thiazole and a pyridine ring, respectively.

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for 3-methyladeninium ion in aqueous acid.[17] Adenine was deuterated at position 8 by heating in D_2O at 100 °C for 8 h.[13]

Bis[(4-amino-1,2-dimethyl-5-pyrimidinio)methyl]amine Diperchlorate VI. A suspension of 0.950 g (1.98 mmol) of 1'-methylthiaminium diperchlorate[5] 1.4 mL (10 mmol) of 2,4,6-trimethylpyridine, and 0.26 g (2.2 mmol) of ammonium perchlorate in 20 mL of methanol was heated at reflux for 18 h. The filter cake was washed with ethyl acetate to give 0.368 g (0.75 mmol, 76%) of product, mp 259–261 °C dec. Recrystallization from water gives the analytical sample of VI: mp 261–264 °C dec; ^1H NMR (Me_2SO-d_6 Me_4Si) δ 9.0, 8.3 (NH_2), 8.2 (6-H), 3.8 (NCH_3), 3.6 (CH_2), 3.3 (NH and HOD), 2.6 (CCH_3); ^{13}C NMR δ 162.4, 161.4 (C-2 and C-4), 146.1 (C-6), 113.9 (C-5), 44.9 (CH_2), 41.6 (NCH_3), 21.4 (CCH_3). Anal. Calcd for $C_{14}H_{23}N_7Cl_2O_8$: C, 34.44; H, 4.75; N, 20.08. Found: C, 34.30; H, 4.74; N, 19.96.

Nuclear Overhauser Effects. A 50-mg suspension of adenosine derivative III was made to undergo hydrogen–deuterium exchange (OH and some CCH_3) by brief heating in 5 mL of D_2O. Solvent was removed under reduced pressure. The cycle was repeated four times. To a 1% solution of this deuterated III in D_2O was added 0.5 μL of $C_6H_5CH_2OD$ prepared by exchanging the hydroxyl proton in D_2O–DCl, followed by fractional distillation of ether extracts. The solution was filtered through a 1-μm Millipore filter into an NMR tube and degassed by five freeze–thaw–pump cycles. The tube was sealed under vacuum. The NMR tube was soaked in 0.01 M EDTA for 24 h to remove any paramagnetic ions, rinsed with D_2O, and oven dried.

NMR spectra were recorded on a FT JEOL FX-100Q spectrometer. Data were recorded when the CH_2 group was irradiated and also when the sample was irradiated off resonance with a second signal located 500 Hz upfield from the CH_2 signal. The cycle of irradiation on and off resonance was repeated four times; the data were averaged. Signals from the three aromatic protons and the phenyl group of the benzyl alcohol internal standard were processed according to peak heights and areas. The height of the lowest field imidazole-bonded proton essentially was the same during resonance and off resonance. But the height of the signal associated with the proton bonded to the pyrimidine ring of adenosine showed a large NOE enhancement of 33%. The remaining aromatic signal sharpened due to decoupling and possibly increased in intensity due to an NOE, but evaluation is difficult due to the large change in line width associated with decoupling.

The areas of the peaks of interest also were determined by planimetry after the peaks were recorded at a 66.4-Hz sweep width. Areas of the two low-field aromatic protons depend on how a base line is drawn because they do not show base-line separation. The area of the lowest field imidazole signal increased by <3% on irradiation of the CH_2 group, that for the pyrimidine ring of adenine increased by 17–18%, and that for the remaining proton showing decoupling increased by 9–12%. With the exception of the latter signal, line-width changes for the other two aromatic protons were no more than 2%.

Noteworthy is our preliminary experiment which in a few minutes demonstrated an NOE and gave us a proof of structure. A sample of III in D_2O was routinely prepared. Irradiation of the CH_2 signal with a Varian 360L spectrometer showed that the intensity of the proton bonded to the pyrimidine ring of adenosine increased while that of the imidazole did not; the remaining aromatic proton was decoupled. The more elaborate experiment was then performed as a rigorous check.

In order to check assignments for the aromatic protons in III the H-1' signal of ribose was irradiated. Because the signal due to CH_2 is close, the irradiating power was systematically varied in order to minimize irradiating this neighbor as well. The signal at lowest field showed NOE enhancement first and others as the irradiating power increased. This lowest field aromatic proton signal must be associated with the imidazole proton. The same conclusion was reached from a deuteration experiment.

Fluorescence Measurements. Fluorescence spectra were recorded on a Perkin-Elmer MPF-44A; they are uncorrected. A 2.4×10^{-3} M solution of III in pH 9.2 borate buffer was prepared by weight. Dilutions with buffer and/or water gave other samples. Spectral data are reported in the text. A 5×10^{-10} M sample which stood under air at room temperature for 1 week showed about a 3-fold reduction in intensity.

Several reaction mixtures were checked to determine whether fluorescent materials are formed. (a) 1'-Methylthiaminium diperchlorate (0.2 M) was heated at 65 °C for 30 h in 80% methanol–20% Me_2SO. No fluorescence was detected. (b) Repetition with prior addition of 2,4,6-trimethylpyridine gave a solution which on 10^3-fold dilution with water had excitation and emission bands at 361 and 413 nm, respectively. (c) A third sample containing the thiamine, the pyridine, and adenine was heated under the same conditions. Following 10^6-fold dilution with water, excitation (391 nm) and emission [410, 428 (shoulder)] were observed. Adenine may form a tetracyclic product related to III in a small amount.

Acknowledgment. This work was kindly supported in part by Grant AM 17442 from the National Institute of Arthritis, Metabolism, and Digestive Diseases and by a University of Florida Biomedical Research Grant. Dr. G. Uray and Dr. B. Langhammer kindly provided the systematic name for III.

Registry No. I, 58-61-7; II, 73333-47-8; III, 80584-80-1; V, 80584-82-3; VI, 80584-84-5; adenine, 73-24-5.

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Bis(trialkylsilyl) Chalcogenides. 1. Preparation and Reduction of Group 6A Oxides

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(Alkyl- and arylchalcogeno)silanes (**1**) are compounds of current interest in the literature.[1-3] With the scrutiny that the silicon–chalcogen bond has received with respect to these compounds, it is somewhat surprising that the much older bis(trimethylsilyl) chalcogenides (**2**) have not

$ArYSiR_3$	$Me_3SiYSiMe_3$
1	2a, Y = S
	b, Y = Se
	c, Y = Te

received more study, particularly of their applications to organic chemistry.[3d,4-6] Herein, we report the facile and high-yield preparation of the compounds **2** and their

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