47.20, 44.95, 28.04, 27.92, 10.20.

Anal. Calcd for $C_{13}H_{16}O_2$: C, 76.44; H, 7.89. Found: C, 76.20; H, 8.08.

3a,4,5,6-Tetrahydro-3a-acetyl-5,5-dimethyl-1*H*-inden-1-one (5). A solution of 103 mg (0.46 mmol) of 1 and 2 mg of *p*-toluenesulfonic acid in 17 mL of dry benzene was heated at reflux for 4.5 h with azeotropic removal of the water produced. The reaction mixture was then cooled to room temperature, and 3 mL of saturated aqueous sodium bicarbonate was added. The mixture was extracted with Et_2O, and the combined ether layers were dried ($MgSO_4$) and concentrated. The crude product was purified by chromatography on silica gel. Elution with 2:1 hexanes–ether gave 14 mg (15%) of 4 and 43 mg (45%) of the crystalline product 5: mp 65–67 °C; R_f 0.81 (ether); IR (CCl_4) 1710, 1657 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.13 (d, 1 H, J = 5.90 Hz), 6.78 (t, 1 H, J = 4.40 Hz), 6.43 (d, 1 H, J = 5.90 Hz), 2.65 (d, 1 H, J_{gem} = 12.94 Hz), 2.08 (d, 2 H, J = 4.40 Hz), 2.02 (s, 3 H), 1.16 (d, 1 H, J = 12.94 Hz), 0.99 (s, 3 H), 0.90 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 205.87, 195.03, 158.67, 140.10, 136.79, 134.58, 62.25, 40.88, 40.22, 33.27, 32.17, 29.84, 25.04.

Anal. Calcd for $C_{13}H_{16}O_2$: C, 76.44; H, 7.89. Found: C, 76.14; H, 7.95.

3,3a,4,5,6,6aα-Hexahydro-3aα-acetyl-5,5-dimethyl-6α-formyl-2(1*H*)-pentalenone (6). To a solution of 1.88 g (8.47 mmol) of 1 and 1.47 mL (2 equiv) of morpholine in 200 mL of benzene was added 1 mg of *p*-toluenesulfonic acid. The reaction mixture was heated at reflux under a Dean–Stark trap for 11 h and cooled, and 10 mL of H_2O was added. After the mixture had been stirred 1 h at room temperature, it was poured into saturated aqueous sodium bicarbonate, and the product was extracted with Et_2O. The combined ether extracts were dried ($MgSO_4$) and concentrated. The product was purified by chromatography on silica gel. Elution with 3:1 ether–hexanes provided 1.73 g (92%) of the crystalline product 6: mp 68–70 °C; R_f 0.47 (ether); IR ($CHCl_3$) 1742, 1713 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.78 (d, 1 H, J = 2.2 Hz), 3.64 (td, 1 H, J = 11.20, 10.17, 1.65 Hz), 2.72 (d, 1 H, J_{gem} = 18.67 Hz), 2.60 (ddd, 1 H, J = 19.25, 9.35, 1.10 Hz), 2.40 (d, 1 H, J_{gem} = 13.75 Hz), 2.33 (dd, 1 H, J = 11.20, 2.20 Hz), 2.26 (dd, 1 H, J = 18.67, 1.65 Hz), 2.25 (s, 3 H), 2.14 (d, 1 H, J_{gem} = 19.25 Hz), 1.78 (d, 1 H, J_{gem} = 13.75 Hz), 1.31 (s, 3 H), 0.95 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 215.07, 208.45, 201.98 67.77, 60.10, 53.02, 48.93, 43.31, 42.43, 40.64, 29.02, 25.53, 23.70.

Anal. Calcd for $C_{13}H_{18}O_3$: C, 70.25; H, 8.16. Found: C, 70.32; H, 8.08.

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TLC Mesh Column Chromatography[1]

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In a usual laboratory day, the greatest share of working time is devoted to sample isolation and purification. Usually, chromatographic separation plays a central role in this effort. We outline here a procedure for column chromatography that is both efficient (mixtures showing ΔR_f = 0.05 by TLC are routinely separated) and easily scaled up.

There are two central concerns in column chromatography: packing of the absorptive bed and sample application. This procedure, a modification of the short-column technique,[2] effectively addresses both of these concerns.

The steps to follow in packing a column are detailed in Figure 1 (Figures 1–3, with accompanying legends describing details of column preparation and operation, are available as supplementary material). Note that the silica gel bed is first allowed to settle by gravity flow and then further compacted by application of air pressure.[3,4] This assures a dense, evenly packed bed. Then, rather than application of the mixture to be chromatographed in liquid form, it is first evaporated onto coarse silica gel.[5] This assures even application of the sample on to the top of the column and avoids concerns about mixtures that are not soluble in the (usually nonpolar) column solvent.

We find it convenient in running such columns to adjust the air pressure so as to collect about one fraction per minute. Fractions are monitored by TLC. For routine separations, the polarity of the eluant is adjusted so that the first component of the mixture appears in about fraction 10. It is usually then sufficient to collect 20 fractions, with fraction collection and TLC monitoring being effected simultaneously. When components of the mixture are widely separated, it is appropriate to switch to a more polar eluant after the less polar components have come off the column. The entire process of column construction, elution, and fraction analysis usually takes a little less than 1 h.

We have used a variety of solvent mixtures following this procedure. Ethyl acetate in petroleum ether appears to be the most generally satisfactory. For less polar mixtures, CH_2Cl_2 in petroleum ether is effective, and for very polar mixtures we use ethyl acetate in CH_2Cl_2. We have found that if it requires more than 40% ethyl acetate in hexane or less than 5% to give a TLC R_f of 0.4 for the mixture to be separated, it is best to switch to the alternative less polar or more polar solvent system. While it is possible to plot "most effective column eluant" as a function of TLC R_f, derivation of the most effective solvent system for a given separation is still best done empirically.[6]

Table I

column diameter, mm	wt of silica gel, g	sample size, g	fraction collected, mL
10.4	1	0.03	1
14	2.5	0.06	2.5
19	5	0.1	5
24	10	0.2	10
41	50	1	50
60	100	3	100
120	300	8	300
170	500	15	500

(1) EM 7747 silica gel (10–15 μm), purchased from Scientific Products, was used.

(2) (a) Hunt, B. J.; Rigby, W. *Chem. Ind. (London)* **1967**, 1868. (b) Still, W. C., unpublished manuscript, Vanderbilt University.

(3) As an alternative to the use of laboratory compressed air, the columns can conveniently be pressurized by pumping air in with a pipet filling bulb. We thank Dr. Matthew Schlecht for this improvement.

(4) Although we have never experienced any difficulty, prudence dictates the use of a safety shield with such pressurized or evacuated glassware.

(5) Coarse silica gel used for sample preadsorption was 60–200 mesh.

(6) We have used this procedure successfully for several years: (a) Taber, D. F.; Korsmeyer, R. W. *J. Org. Chem.* **1978**, *43*, 4925. (b) Taber, D. F.; Gunn, B. P. *Ibid.* **1979**, *44*, 450. (c) Taber, D. F.; Saleh, S. A. *J. Am. Chem. Soc.* **1980**, *102*, 5085. (d) Taber, D. F.; Saleh, S. A.; Korsmeyer, R. W. *J. Org. Chem.* **1980**, *45*, 4699.

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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.

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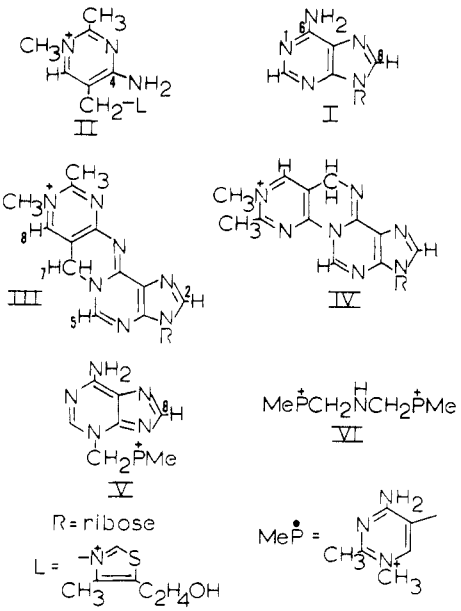
Considerable effort has been expended to convert adenosine (I, Chart I) into fluorescent derivatives. Such conversions not only provide an ultrasensative method of detecting I but also furnish fluorophores which are useful bioprobes.[1]

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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