47.[illegible]), 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 [illegible] The [illegible] reaction [illegible] 3 mL of satura[illegible] cture was extr[illegible] dried (MgSO₄[illegible] d by chromat[illegible] gave 14 mg (1[illegible] ct 5: mp 65–6[illegible] NMR (400 MH[illegible] H, *J* = 4.40 H[illegible] 12.94 Hz), 2.0[illegible] , *J* = 12.94 H[illegible] 5.87, 195.03, [illegible] 3.27, 32.17, 2[illegible]

Anal. [illegible] 76.14; H, 7.95.

3,3a,[illegible] formyl-[illegible] l-6α- [illegible] (8.47 mmol) [illegible] nL of benzene [illegible] action mixture [illegible] r 11 h and cool[illegible] e had been sti[illegible] urated aqueous [illegible] d with Et_2O. [illegible] 4) and concent[illegible] phy on silica ge[illegible] (92%) of the [illegible] er); IR ($CHCl_3$ [illegible] .78 (d, 1 H, *J* [illegible] z), 2.72 (d, 1 H[illegible] 5, 1.10 Hz), 2.4[illegible] 20, 2.20 Hz), 2.[illegible] 14 (d, 1 H, J_{gem} [illegible] s, 3 H), 0.95 (s, [illegible] 8 67.77, 60.10, [illegible] 70.

Anal [illegible] C, 70.32; H, 8.08

Ack[illegible] t is extende[illegible] Fund, admin[illegible] to Research[illegible] and to the Ar[illegible] rded to C.W.M[illegible] search. High-[illegible] the National [illegible] r at the Unive[illegible] 723).

Reg[illegible] 1-78-1; 6, 80695-33-6.

TLC Mesh Column Chromatography[1]

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Received July 6, 1981

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 $\Delta R_f = 0.05$ by TLC are routinely separated) and easily scaled up.

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

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]

(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.

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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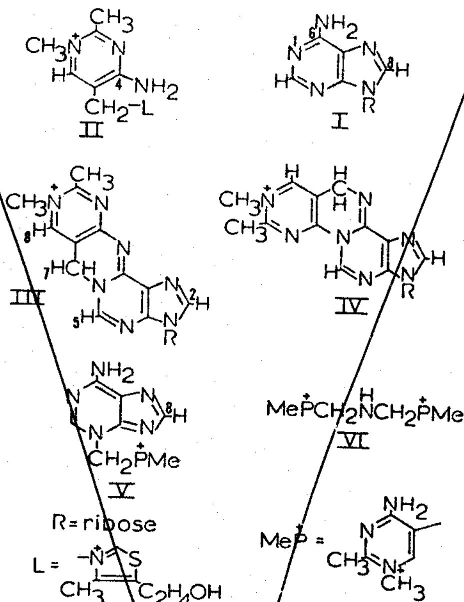
Received September 30, 1981

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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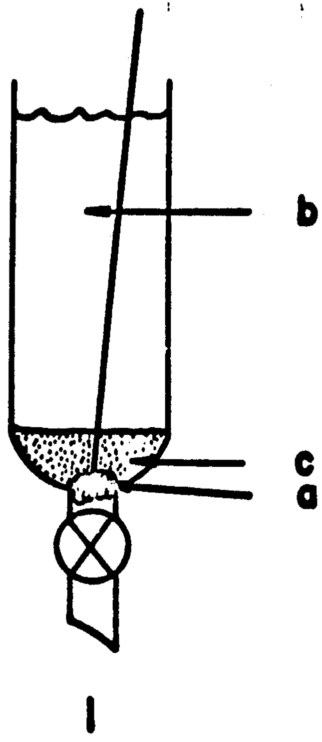
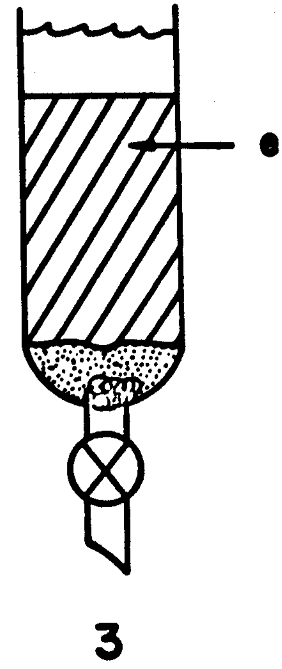
G-1352-H,

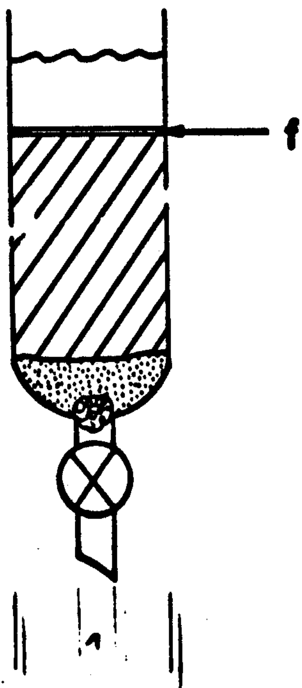
Figure 1.

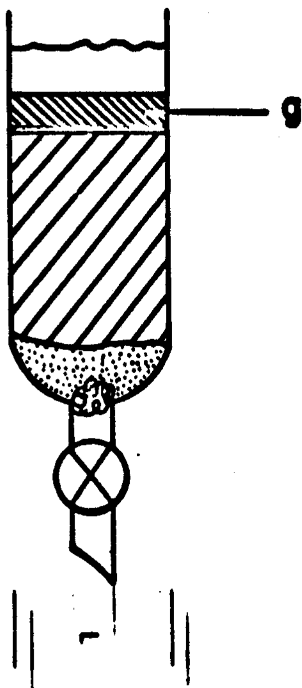
Columns used are described in the text. 1). A glass wool plug (a) is gently inserted in the bottom of the column and held in place with a wire plunger (b). Column eluant is added, and the glass wool plug is covered with an even layer of sand. The sand is gently tapped to remove trapped air bubbles, and the wire plunger is removed. 2). The appropriate weight of TLC mesh silica gel is added as a slurry (d) in the column eluant. The stopcock is opened, and the solvent is allowed to drain (5-10 min), partially compacting the column. 3). Air pressure (5-15 psig) is applied to the top of the column with the stopcock still open, completing (5 min) column compaction (e). The stopcock is then closed, and the pressure is released. Note that it is essential after the column has been pressurized to always close the stopcock at the bottom of the column before releasing the pressure. 4). A circular piece of filter paper, cut to fit the inside of the column, is added. It will slowly settle through the eluant on to the top of the silica gel bed. 5). The material to be chromatographed is taken up in an appropriate volatile solvent (CH_2Cl_2 is usually satisfactory), coarse silica gel (ref. 5) (10% of TLC mesh silica gel dry weight) is added, and the solvent is removed in vacuo to give a free flowing powder. The use of a glass frit adapter (ref. 12) between the flask containing the silica gel and the vacuum line is recommended. The eluant is run through the column by the application of air pressure or removed by pipette so that it stands 1-2 cm above the filter paper, and the impregnated coarse silica gel (g) is added. 6). A layer of sand (h) is gently added. The eluant is run down to the level of the sand (air pressure), the sides of the column are rinsed down with a little eluant, and that is run (air pressure) down to the level of the sand. The column is gently filled with eluant, air pressure is applied, and fractions are collected.

Figure 1. Column Preparation G-1352-M2









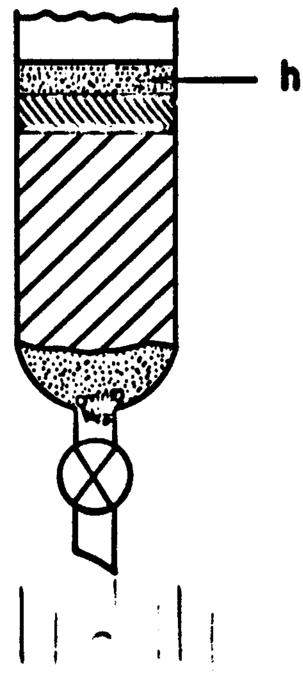
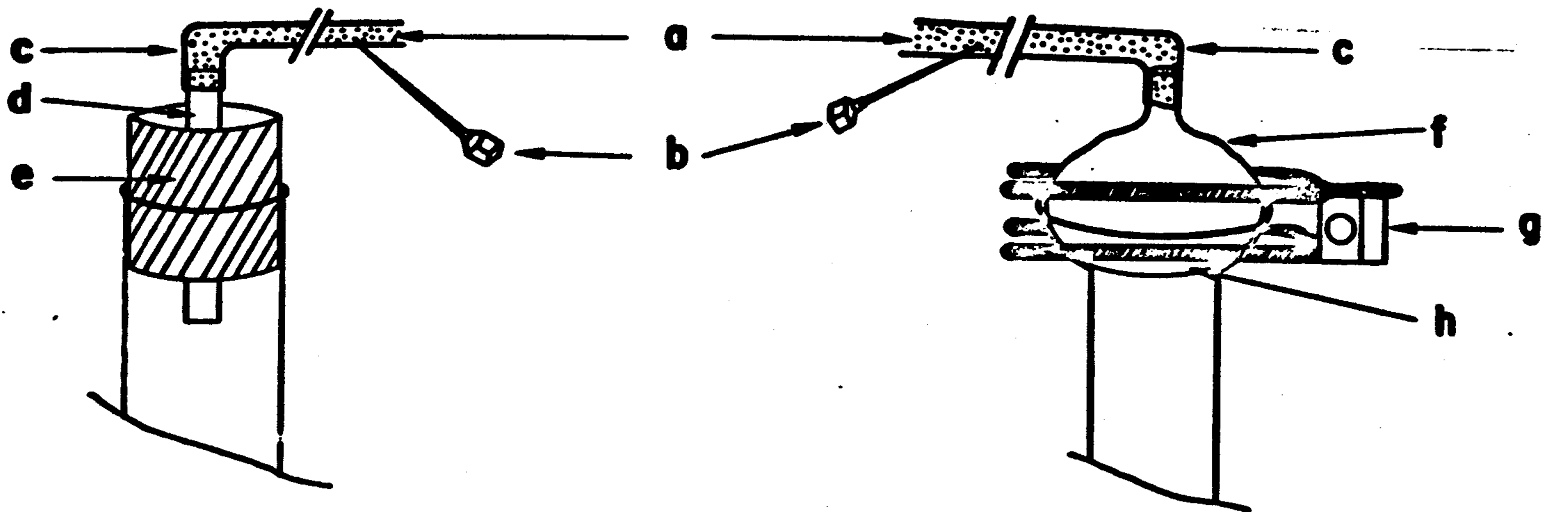


Figure 2. Top of Column Assembly

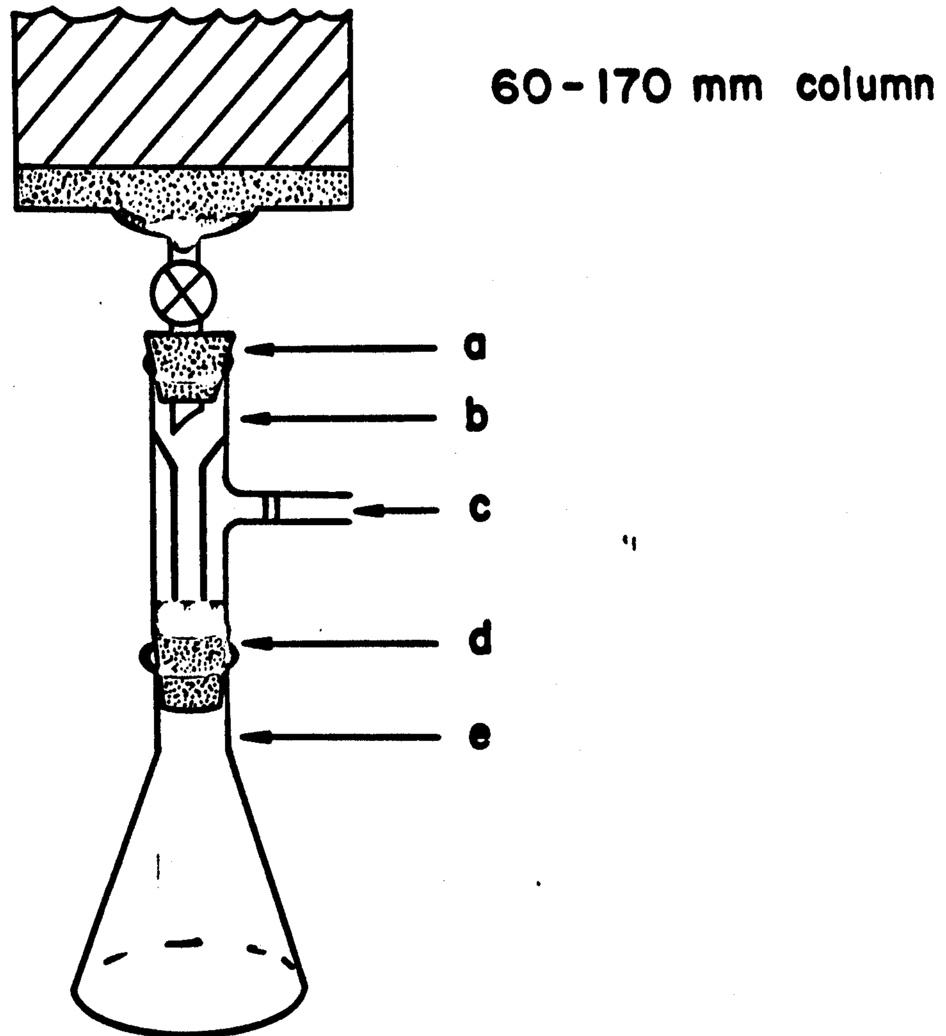


a) Laboratory compressed air. b) Small syringe needle for bleed. c) Tygon tubing. d) Glass tubing. e) Rubber stopper. f) Male 35/20 spherical ground glass joint. g) Screw clamp. h) Female 35/20 spherical ground glass joint.

G-1352-M3

G-1352-My

Figure 3. Vacuum Adapter for Larger Columns



a) One-hole rubber stopper. b) Vacuum adapter, Ace 5260-10. c) Aspirator vacuum. d) Rubber filter funnel adapter. e) Erlenmeyer flask.