filter. After irradiation of the solution of 1-(bromoacetyl)pyrene for 100 min, the solvent was removed by evaporation, and the resulting brown solid was adsorbed on a small amount of silica and subsequently chromatographed on a silica column with dichloromethane as the eluent, yielding 0.15 g of 1-(bromoacetyl)pyrene (0.46 mmol, 15%), 0.05 g of 1-acetylpyrene (0.21 mmol, 7%), and 0.29 g of cyclopenta[*cd*]pyren-3(4*H*)-one (120 mmol, 39%). The spectral data are in agreement with those reported by Tintel et al.:[21] ^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ 3.99 (d, 2 H, H(4,4), J = 1.4 Hz), 8.01 (t, 1 H, H(5), J = 1.4 Hz), 8.09 (t, 1 H, H(7), J = 7.7 Hz), 8.18 (d, 1 H, H(9 or 10), J = 9.2 Hz), 7.24–7.37 (m, 5 H, H(1, 2, 6, 8, and 10 or 9)); MS (150 °C), *m/z* (rel intensity) 242 (100), 214 (71), 213 (66); UV (cyclohexane) λ_{max} (relative ϵ) 400 (0.12), 392 (0.79), 384 (0.17), 371 (0.47), 352 (0.65), 342 (0.41), 337 (0.21), 307 (0.21), 285 (0.75), 275 (0.57), 249 (100); melting point 213–214 °C (lit.[21] mp 214 °C). Determination of a mixed melting point with a sample prepared by the alternate[21] route showed no significant depression.

Acknowledgment. We gratefully acknowledge the assistance of B. Boere.

Registry No. 1, 129-00-0; 2, 80480-15-5; 3, 69795-70-6; 5, 27208-37-3; $BrCH_2C(O)Br$, 598-21-0; 1-acetylpyrene, 3264-21-9.

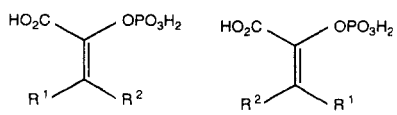
Synthesis of (*E*)- and (*Z*)-3-Deuteriophosphoenolpyruvate

Makarand P. Gore, Palaniappagownder Nanjappan, Geoffrey C. Hoops, and Ronald W. Woodard*

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The University of Michigan, Ann Arbor, Michigan 48109-1065

Received March 28, 1989

Phosphoenolpyruvate (PEP), a compound with a high phosphate group transfer potential ($\Delta G^{\circ\prime}$ = −14.8 kcal/mol),[1] is a very important biological intermediate.[2] Enzymes that utilize PEP as a substrate may be generally divided into three groups according to the formal chemical reaction catalyzed: (1) reactions that involve the simple hydration of the double bond, (2) reactions that involve an addition of a positively charged atom (H^+ or a carbonyl carbon) to the C-3 position of PEP, which is coupled to the transfer of a phosphate to a nucleophile such as ADP, hexose, or water, and (3) reactions that involve displacement of the phosphate by a nucleophile at the C-2 position of PEP with retention of the double bond.[3] The stereochemical mechanism of only a few of these reactions has been probed due to the limited availability of the PEP stereospecifically labeled at the 3 position with some combination of hydrogen, deuterium, and/or tritium (1 and 2).[4-7] The stereospecifically tritiated analogues are



$R^1 \neq R^2 = {}^1H, {}^2H, \text{or } {}^3H$

R^1 higher priority than R^2

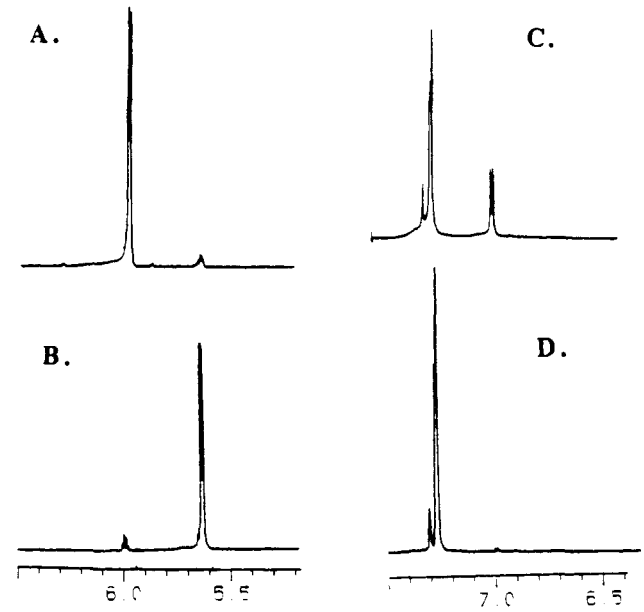


Figure 1. ^{1}H NMR spectra (270 MHz) of (A) ethyl (*Z*)-3-deuterio-2-[(dimethoxyphosphinyl)oxy]propenoate (**5a**); (B) ethyl (*E*)-3-deuterio-2-[(dimethoxyphosphinyl)oxy]propenoate (5b); (C) 72:28 mixture of (*Z*)-**4a** and (*E*)-**4b**; (D) ethyl (*Z*)-3-bromo-2-[(dimethoxyphosphinyl)oxy]propenoate (**4a**). The peak at $\delta \approx$ 7.29 ppm is the residual proton of $CHCl_3$ in the commercial $CDCl_3$.

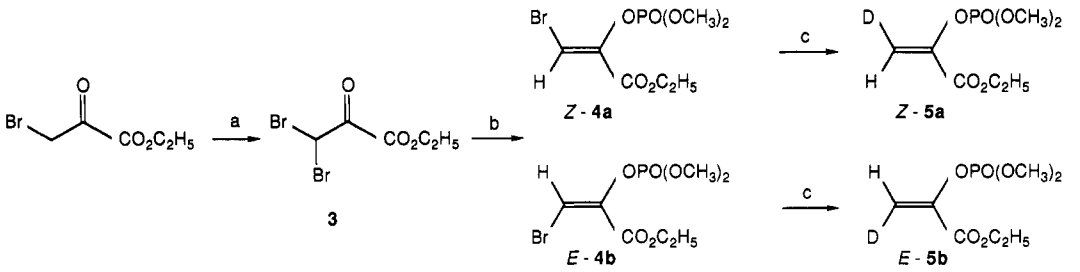
available by the enzymatic route described by Rose et al.[8,9] and the stereospecifically deuteriated analogues by an elegant synthesis reported by Bartlett.[10] The Bartlett procedure, a rather laborious seven-step synthesis, requires the preparation of special reagents such as 3,5-dinitroperoxybenzoic acid and introduces the hydrogen isotope at a very early stage in the synthesis, making the synthesis of the tritium-labeled analogues quite tedious. Studies recently initiated in our laboratory dealing with the stereochemical mechanism of the formation of UDP-*N*-acetylmuramic acid, 3-deoxy-D-*manno*-octulosonate 8-phosphate, and 3-deoxy-D-*arabino*-heptulosonate 7-phosphate have required PEP stereospecifically labeled in the C-3 position with various hydrogen isotopes. We report here an efficient methodology for the preparation of analogues 1 and 2.

Our synthesis of stereospecifically labeled PEP begins with commercially available ethyl bromopyruvate, which is converted to ethyl 3,3-dibromopyruvate (3) in 80% yield by using *N*-bromosuccinimide in chloroform (see Scheme I). A Perkow-type reaction[11-15] of the dibromo ester with trimethyl phosphite produces a stereoisomeric mixture of ethyl bromophosphoenol pyruvates in which the *Z* to *E*

(1) Lehninger, A. L. *Bioenergetics*, 2d ed.; W. A. Benjamin: Menlo Park, CA, 1971; pp 67–68.
(2) Metzler, D. E. *Biochemistry, The Chemical Reactions of Living Cells*; Academic Press: New York, 1977; pp 417–418.
(3) Cassidy, P. J.; Kahan, F. M. *Biochemistry* **1973**, *12*, 1364–1374.
(4) Rose, I. A.; O'Connel, E. L.; Noce, P.; Utter, M. F.; Wood, H. G.; Willard, J. M.; Cooper, T. G.; Benziman, M. *J. Biol. Chem.* **1969**, *244*, 6130–6133.
(5) Floss, H. G.; Onderka, D. K.; Carroll, M. *J. Biol. Chem.* **1972**, *247*, 736–744.
(6) Onderka, D. K.; Floss, H. G. *J. Am. Chem. Soc.* **1969**, *91*, 5894–5896.
(7) Grimshaw, C. E.; Sogo, S. G.; Knowles, J. R. *J. Biol. Chem.* **1982**, *257*, 596–598.
(8) Cohn, M.; Pearson, J. E.; O'Connel, E. L.; Rose, I. A. *J. Am. Chem. Soc.* **1970**, *92*, 4095–4098.
(9) Rose, I. A. *Methods Enzymol.* **1975**, *41b*, 110–115.
(10) Bartlett, P. A.; Chouinard, P. M. *J. Org. Chem.* **1983**, *48*, 3855–3858.
(11) Perkow, W.; Ullrich, K.; Myer, F. *Naturwissenschaften* **1952**, *39*, 353.
(12) Lichtenthaler, F. W. *Chem. Rev.* **1961**, *61*, 607–649.
(13) Clark, V. M.; Kirby, A. J. *Biochim. Biophys. Acta* **1963**, *78*, 732.
(14) Stubbe, J.; Kenyon, G. L. *Biochemistry* **1971**, *10*, 2669–2677.
(15) Stubbe, J.; Kenyon, G. L. *Biochemistry* **1972**, *11*, 338–345.

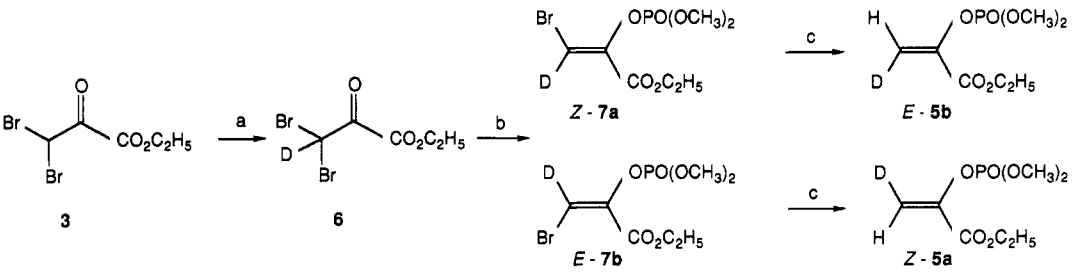
0022-3263/90/1955-0758$02.50/0

Scheme I[a]



[a] (a) *N*-Bromosuccinimide/CCl_4; (b) $P(OCH_3)_3$; (c) Zn/Ag, D_2O/THF.

Scheme II[a]



[a] (a) $D_2O/NaHCO_3$; (b) $P(OCH_3)_3$; (c) Zn/Ag, H_2O/THF.

ratio is dependent on both solvent and reaction temperature. When trimethyl phosphite is added to a solution of **3** in either refluxing dioxane or diethyl ether, the *Z* to *E* ratio is 72:28 [**4a**:**4b**] as determined from the ^1H NMR spectrum[8,14,16,17] of the crude sample, shown in Figure 1C. Addition of trimethyl phosphite to a solution of **3** in diethyl ether at −20 °C, followed by slow warming to room temperature, gives a *Z* to *E* ratio of 52:48 [**4a**:**4b**]. In both cases the purified yield of the combined isomers is ~70%. The separation of ethyl (*Z*)- and (*E*)-3-bromo-2-[(dimethoxyphosphinyl)oxy]propenoate (**4a** and **4b**) is readily accomplished, each in >98% stereochemical purity as determined by ^1H NMR, via medium pressure liquid chromatography (MPLC).[15] The bromo enol compound **4a** is reduced to the corresponding ethyl (*Z*)-3-deuterio-2-[(dimethoxyphosphinyl)oxy]propenoate (**5a**) in quantitative yield with >98% deuterium incorporation using a Zn/Ag couple, prepared by the method of Clark and Heathcock,[18] doped with D_2O. This reduction catalysis has been utilized, although with a considerable loss of stereoselectivity, in the synthesis of chiral acetic acid from ethyl (*Z*)- and (*E*)-2-acetoxy-3-bromoacrylates.[19] The ^1H NMR of **5a** (Figure 1A) shows >95% of the expected stereoisomer present, indicating that 5% or less scrambling has occurred in the reduction reaction. The (*E*)-**5b** prepared from (*E*)-**4b** by this procedure (see Scheme I) is only ≈85% stereochemically pure. The preparation of (*E*)-**5b** in >98% stereochemical purity will be described below.

In a modification of the above procedure (Scheme II) designed to test the feasibility of synthesizing both isomers of [^2H,^3H]PEP and preparing (*E*)-**5b** in higher stereochemical purity, compound **3** is deuteriated by treatment with D_2O containing a catalytic amount of sodium bicarbonate to give ethyl 3-deuterio-3,3-dibromopyruvate (**6**) in quantitative yield with >98% deuteriation. Treatment of **6** with trimethyl phosphite in refluxing dioxane, followed by flash chromatography and MPLC, gave the two isomers **7a** and **7b** (the *Z* to *E* ratio of the stereoisomeric mixture is 72:28), which after separation are reduced by using Zn/Ag doped with H_2O, as described above, to give the corresponding isomers **5b** and **5a**, respectively. The minor isomer **7b** gave **5a** with ≈15% loss in stereochemistry. The reduction of both the deuteriated and undeuteriated *E* isomers gives products that have undergone ≈15% scrambling. One should therefore select the proper (*Z*)-vinyl bromide isomer for reduction if one wishes to obtain PEP with the highest possible stereochemical purity. Based on the above observation, if the Zn/Ag couple is doped with ^3H_2O, then one should be able to obtain the double-labeled PEP in which the tritium atom has been introduced in the penultimate step. Analogues **5a** and **5b** are deprotected in quantitative yield to **2** and **1**, respectively, using the procedure reported by Bartlett.[4]

In conclusion all six possible labeled stereoisomers of PEP are available via a simple four-step synthesis (overall yield > 50%) that utilizes readily available, inexpensive starting materials. The hydrogen isotope is available, in all cases, from the corresponding isotopically labeled water and the heavy isotope, in cases involving only one heavy isotope, can be introduced in the penultimate step.

Experimental Section

The ^1H NMR spectra are recorded on an IBM-Bruker 270 MHz FT instrument in the solvent specified. Mass spectra are recorded on a VG analytical 70-250-S and/or a Finnigan 4021 instrument. Flash chromatography is performed via the method of Still et al.,[20] using columns fabricated in the University of Michigan Chemistry Department Glass Blowing Shop, packed with E. Merck silica gel 60 (230–400 μm), and MPLC via the method of Michel and Miller[21] using LP-HPLC columns purchased from ACE Glassware, packed with Analtech Sorbent silica gel without binder (10 ± 4

(16) Duffy, T. H.; Nowak, T. *Biochemistry* **1984**, *23*, 661–670.
(17) Wirsching, P.; O'Leary, M. H. *Biochemistry* **1988**, *27*, 1355–1360.
(18) Clark, R. D.; Heathcock, C. H. *J. Org. Chem.* **1976**, *41*, 636–643.
(19) Fryzuk, M. D.; Bosnich, B. *J. Am. Chem. Soc.* **1979**, *101*, 3043–3049.
(20) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.
(21) Michel, K. H.; Miller, R. F. Lilly Research Lab, Indianapolis, IN, U.S. Patent 4,131,547.

μm). All solvents used were purified and/or dried by standard methods.[22]

Ethyl 3,3-Dibromopyruvate (3). A solution of ethyl bromopyruvate (5.00 g, 25.6 mmol), *N*-bromosuccinimide (4.56 g, 25.6 mmol), and azobis(isobutyronitrile) (50 mg, catalytic) in CCl_4 (100 mL) was heated at reflux for 4 h with intermittent UV irradiation from a Minerallight UVSL-25 UV lamp. The 254 μm light was held directly on the side of the flask for 2 min every 10 min during the first 30 min of the reaction. The mixture was cooled and filtered to remove succinimide, and the filtrate was evaporated to give a yellow oil. Flash chromatography (35% EtOAc/hexanes) of the crude product gave 5.60 g (80% yield) of **3** as a colorless oil: ^{1}H NMR (270 MHz, D_2O) δ 6.03 (s, 1 H), 4.24 (q, J = 7.15 Hz, 2 H), 1.22 (t, J = 7.15 Hz, 3 H); exact mass 273.87 (273.87 calcd for $C_5H_6O_3{}^{79}Br^{81}Br$).

Ethyl (*Z*)-3-Bromo-2-[(dimethoxyphosphinyl)oxy]propenoate (4a) and Ethyl (*E*)-3-Bromo-2-[(dimethoxyphosphinyl)oxy]propenoate (4b). A refluxing solution of **3** (0.274 g, 1.00 mmol) in dioxane (5 mL) was treated all at once with trimethyl phosphite (0.144 g, 1.00 mmol), and the heating was continued for 30 min. The solvent was removed on a rotary vacuum evaporator (water aspirator/35 °C). The residual colorless oil was purified by flash chromatography (70% EtOAc/hexane) followed by MPLC (20% EtOAc/hexanes) to give 0.171 g (52% yield) of **4a** and 0.061 g of **4b** (20%) as colorless oils.[23] **4a**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.24 (d, J = 1.7 Hz, 1 H), 4.30 (q, J = 7.3 Hz, 2 H), 3.95 (d, J = 11.5 Hz, 6 H), 1.33 (t, J = 7.3 Hz, 3 H); exact mass 302.9626 (302.9633 calcd for $C_7H_{12}{}^{79}BrPO_6$). **4b**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.96 (d, J = 2.9 Hz, 1 H), 4.34 (q, J = 7.3 Hz, 2 H), 3.87 (d, J = 11.4 Hz, 6 H), 1.26 (t, J = 7.3 Hz, 3 H).

Ethyl (*Z*)-3-Deuterio-2-[(dimethoxyphosphinyl)oxy]propenoate (5a). Bromopyruvate **4a** (0.080 g, 0.264 mmol) in 20% D_2O/THF (the THF is predried and distilled as previously described)[22] (5 mL) was treated with freshly prepared Zn/Ag couple[18] (200 mg) doped with D_2O. The progress of the reaction was monitored via TLC (silica gel/40% EtOAc/hexanes). After all the starting material has been consumed (24 h), the couple is removed by filtration. The solid was washed with diethyl ether (3 × 5 mL). The ether layer was separated from the filtrate, dried (Na_2SO_4), and evaporated under reduced pressure to give colorless oil **5a** in quantitative yield (0.059 g): ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.96 (d, J = 2.4 Hz, 1 H), 4.28 (q, J = 7.3 Hz, 2 H), 3.88 (d, J = 11.4 Hz, 6 H), 1.33 (t, J = 7.3 Hz, 3 H); exact mass 225.05 (225.05 calcd for $C_7H_{12}DPO_6$).

Ethyl 3-Deuterio-3,3-dibromopyruvate (6). Ethyl dibromopyruvate (**3**) (1.00 g, 3.64 mmol) was dissolved in D_2O (25 mL), sodium bicarbonate (200 mg) was added, and the mixture was stirred overnight. Evaporation of the water layer (0.1 mm/35 °C) gave a yellowish emulsion, which was extracted with diethyl ether (3 × 10 mL). The ether layers were dried (Na_2SO_4) and evaporated to give yellow oil (1.00 g, 100% yield) **6**. The material was *not* purified by chromatography since this results in complete loss of the deuterium atom: ^{1}H NMR (270 MHz, D_2O) δ 4.24 (q, J = 7.1 Hz, 2 H), 1.22 (t, J = 7.1 Hz, 3 H); exact mass 274.88 (274.88 calcd for $C_5H_5DO_3{}^{79}Br^{81}Br$).

Ethyl (*Z*)-3-Bromo-3-deuterio-2-[(dimethoxyphosphinyl)oxy]propenoate (7a). In a procedure analogous to that used to convert **3** to **4a**, compound **6** (0.738 g, 2.68 mmol) was transformed to *Z* isomer **7a** (0.462 g) in 56% yield. The *E* isomer **7b** was obtained in 20% yield. **7a**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 4.30 (q, J = 7.3 Hz, 2 H), 3.95 (d, J = 11.5 Hz, 6 H), 1.33 (t, J = 7.3 Hz, 3 H); exact mass 303.97 (303.97 calcd for $C_7H_{11}D^{79}BrPO_6$). **7b**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 4.34 (q, J = 7.3 Hz, 2 H), 3.87 (d, J = 11.4 Hz, 6 H), 1.26 (t, J = 7.3 Hz, 3 H); exact mass 303.97 (303.97 calcd for $C_7H_{11}D^{79}BrPO_6$).

Ethyl (*E*)-3-Deuterio-2-[(dimethoxyphosphinyl)oxy]propenoate (5b). Compound **7a** (0.126 g, 0.45 mmol) was treated with a freshly prepared Zn/Ag couple (250 mg) doped with H_2O, in a manner similar to that reported above for the transformation of **4a** to **5a**, to give **5b** as a colorless oil (0.93 g, quantitative yield): ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.62 (d, J = 2.4 Hz, 1 H), 4.28 (q, J = 7.3 Hz, 2 H), 3.88 (d, J = 11.4 Hz, 6 H), 1.33 (t, J = 7.3 Hz, 3 H).

Acknowledgment. This work was supported by U.S. Public Health Service Grant GM 36184 and an Upjohn Starter Grant from the College of Pharmacy. We are grateful to the U.S.P.H.S. and the College of Pharmacy for their contribution to the purchase of the IBM 270-MHz NMR instrument. We wish to thank Ms. Stacie Tate, a participant in the NIH sponsored College of Pharmacy Summer Minority High School Apprenticeship Program, for her expert technical assistance in the purification of the **3**, **4a**, and **4b**.

Registry No. 1 R_1 = D, R^2 = H, 56585-32-1; 1 R_1 = H, R_2 = D, 87115-15-9; **2**, 76179-25-4; **4a**, 124225-39-4; **4b**, 124225-40-7; **5a**, 87115-22-8; **5b**, 87115-21-7; **6**, 124225-41-8; **7a**, 124225-42-9; **7b**, 124225-43-0; ethyl bromopyruvate, 70-23-5; trimethyl phosphite, 121-45-9.

(22) Gordon, A. J.; Ford, R. A. *The Chemist's Companion: A Handbook of Practical Data, Techniques and References*; John Wiley and Sons: New York, 1972.

(23) The conversion of **3** to **4a** and **4b** when carried out in refluxing diglyme (bp 162 °C) yields and 82:18 *Z*/*E* ratio. The separation of the *Z* and *E* isomers can also be effected via column chromatography using 100 g of *dry* silica gel/1 g of compound. The column is eluted at 100 mL/h with 100 mL of 5% EtOAc/heptanes (vol/vol), 200 mL of 10% EtOAc/heptanes, 300 mL of 15% EtOAc/heptanes, and finally 1 L of 20% EtOAc/heptanes until the product (R_f = 0.2 30% EtOAc/heptanes) was collected in >95% optical purity.

Stereochemistry in a Medium-Sized Ring. Highly Diastereoselective N-Oxidation of a Substituted 3-Benzazonine. X-ray Crystal Structure of an Unusual Complex between an Amine *N*-Oxide and Saccharin

Bruce E. Maryanoff*,†

Chemical Research Department, McNeil Pharmaceutical, Spring House, Pennsylvania 19477

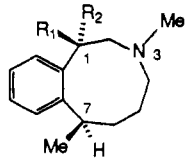
Masood Parvez and R. A. Olofson

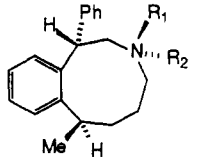
Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802

Received April 11, 1989

Medium-sized cyclic compounds often adopt a wide range of low-energy conformations, which can render the control of reaction stereochemistry unpredictable or difficult.[1-4] However, with the introduction of sp^2 atoms into the ring, certain conformations may be significantly differentiated, so that impressive diastereoselection between remote sites (e.g., 1,3 and 1,4) can be achieved.[1-3] Both Still[1] and Vedejs[2] have drawn attention to local conformational effects as a source of effective stereocontrol in such molecules.

Our interest in this area stems from our work with hexahydro-1-phenyl-3-benzazonine derivatives.[3] An important problem in this earlier paper was the stereochemical assignment of *cis*- and *trans*-**1**, which was predicated





trans-**1** R_1 = H, R_2 = Ph

cis-**1** R_1 = Ph, R_2 = H

2 R_1 = Me, R_2 = O

3 R_1 = O, R_2 = Me

†Current address: Chemical Research Department, R. W. Johnson Pharmaceutical Research Institute, Spring House, PA 19477.

0022-3263/90/1955-0760$02.50/0