methyl sulfate should provide a highly sensitive technique for detection of this type of alkylation of DNA.

Note Added in Proof. Since submission of this manuscript, Professor K. Nakanishi of Columbia University kindly provided us with preprints of studies on the binding of the isomeric 9α,10α-epoxide (**2**) to poly G: I. B. Weinstein, A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, R. G. Harvey, H. Kasai, and K. Nakanishi, *Science,* **193,** 592 (1976), and A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, I. B. Weinstein, F. A. Beland, R. G. Harvey, H. Kasai, I. Miura, and K. Nakanishi, *J. Am. Chem. Soc.,* **98,** 5714 (1976). These studies showed that the 2-amino group of quanine adds to **2** to form a trans adduct as well as other unidentified products. In our hands, diol epoxide **2** behaves much like diol epoxide **1** in that alkylation of phosphate also occurs with this diastereomer of BP 7,8-diol-9,10-epoxide. In addition, A. M. Jeffrey, S. H. Blobstein, I. B. Weinstein, F. A. Beland, R. G. Harvey, H. Kasai, and K. Nakanishi, *Proc. Natl. Acad. Sci., U.S.A.,* **73,** 2311 (1976), have shown that DMBA 5,6-oxide alkylates the N-2 amino group of quanine in poly G.

Acknowledgments. This work was supported by Grant CA 18580 from the National Cancer Institute of the U.S. Public Health Service (to M.K.).

References and Notes

(1) H. Yagi, O. Hernandez, and D. M. Jerina, *J. Am. Chem. Soc.,* **97,** 6881 (1975).
(2) D. M. Jerina, H. Yagi, and O. Hernandez in "Reactive Intermediates: Formation, Toxicity, and Inactivation", D. Jollow, J. Kocsis, R. Snyder, and H. Vainio, Ed., Plenum Press, New York, N.Y., in press.
(3) P. Sims, P. L. Grover, A. Swaisland, K. Pal, and A. Hewer, *Nature (London),* **252,** 326 (1974).
(4) D. J. McCaustland and J. F. Engel, *Tetrahedron Lett.,* 2549 (1975).
(5) D. R. Thakker, H. Yagi, A. Y. H. Lu, W. Levin, A. H. Conney, and D. M. Jerina, *Proc. Natl. Acad. Sci. U.S.A.,* in press. See also ref 6c.
(6) (a) A. H. Conney, A. W. Wood, A. Y. H. Lu, R. L. Chang, P. G. Wislocki, G. M. Holder, P. M. Dansette, H. Yagi, and D. M. Jerina, ref 2; (b) P. G. Wislocki, A. W. Wood, R. L. Chang, W. Levin, H. Yagi, O. Hernandez, D. M. Jerina, and A. H. Conney, *Biochem. Biophys. Res. Commun.,* **68,** 1006 (1976); (c) E. Huberman, L. Sachs, S. K. Yang, and H. V. Gelboin, *Proc. Natl. Acad. Sci. U.S.A.,* **73,** 607 (1976); (d) R. F. Newbold and P. Brookes, *Nature (London),* **261,** 52 (1976); (e) A. W. Wood, P. G. Wislocki, R. L. Chang, W. Levin, H. Yagi, O. Hernandez, D. M. Jerina, and A. H. Conney, *Cancer Res.,* **36,** 3358 (1976).
(7) (a) P. Daudel, M. Duquesne, P. Vigny, P. L. Grover, and P. Sims, *FEBS Lett.,* **57,** 250 (1975); (b) D. W. Nebert, R. E. Kouri, H. Yagi, D. M. Jerina, and A. R. Boobis, ref 2; (c) T. Meehan, D. Warshawsky, and M. Calvin, *Proc. Natl. Acad. Sci. U.S.A.,* **73,** 1117 (1976); (d) M. R. Osborne, M. H. Thompson, E. M. Tarmy, F. A. Beland, R. G. Harvey, and P. Brookes, *Chem.-Biol. Interact.,* **13,** 343 (1976); (e) H. W. S. King, M. H. Thompson, E. M. Tarmy, P. Brookes, and R. G. Harvey, *ibid.,* **13,** 349 (1976); (f) A. Borgen, H. Darvey, N. Castagnoli, T. T. Crocker, R. E. Rasmussen, and I. Y. Wang, *J. Med. Chem.,* **16,** 502 (1973).
(8) W. Levin, A. W. Wood, H. Yagi, P. Dansette, D. M. Jerina, and A. H. Conney, *Proc. Natl. Acad. Sci. U.S.A.,* **73,** 243 (1976).
(9) B. Singer, *Prog. Nucleic Acid Res. Mol. Biol.,* **15,** 219 (1975), and references cited therein. See also P. L. Grover and P. Sims, *Biochem. Pharmacol.,* **22,** 661 (1973), and S. Blobstein.[23,24]
(10) H. Yagi, D. R. Thakker, O. Hernandez, M. Koreeda, and D. M. Jerina, *J. Am. Chem. Soc.,* in press.
(11) S. A. Lesko, Jr., A. Smith, P. O. P. Ts'o, and R. S. Umans, *Biochemistry,* **7,** 434 (1968).
(12) When the polymer was heated for an additional 4 h, no significant further release of hydrocarbon was observed. After removal of initially released tetraols, an equivalent amount of tetraols was added to the modified poly G solution in ^{18}O enriched water. No incorporation of solvent water into the tetraols was detected after identical heating and recovery. Identification of the tetraols was as described.[5,10]
(13) When the modified polymer was first heated in water and the resulting tetraols removed by extraction, no further amount of tetraols was observed upon alkaline hydrolysis of the polymer.
(14) The four nucleoside–hydrocarbon adducts (uv spectra similar to diol epoxide **1,** Figure 1) were separated by high pressure liquid chromatography on a DuPont 5μ, ODS column (7.8 mm × 0.25 m) eluted with 65% methanol in water at a constant flow of 1.6 ml/min; retention times were 13.7, 15.2, 16.8, and 21.2 min. When the poly G was modified at pH 7, the nucleoside adducts were formed in a ratio of 1:2:1:2, respectively, based on absorption at 344 nm. This ratio approached 1:1:1:1 as the pH of the binding experiment was decreased. The first and third compounds ($\Delta\epsilon_{250}$ −90 and +90, respectively) and the second and fourth compounds ($\Delta\epsilon_{250}$ −92 and +92, respectively) to elute from the column constitute two diasteriomeric pairs. Calculations were based on an extinction coefficient (344 nm) of 55 000 (see Figure 1). Mirror image CD spectra among the two pairs indicate that the absolute stereochemistry of the tetrahydro benzo[a]pyrene moiety plays a predominant role in the CD spectra presumably through its chiral interaction with the guanine base.
(15) (a) A. Holy and K. H. Scheit, *Biochim. Biophys. Acta,* **138,** 230 (1967); (b) D. M. Brown, D. I. Magrath, and S. R. Todd, *J. Chem. Soc.,* 4396 (1955); (c) P. D. Lawley, and S. A. Shah, *Biochem. J.,* **128,** 117 (1972). See also ref 9.
(16) M. Tomasz, *Biochim. Biophys. Acta,* **199,** 18 (1970).
(17) Reaction of **2** with DNA containing [8-^{3}H]guanine also failed to cause a release of tritium.[7d] Further chemical evidence for the structure of the adduct(s) was not presented.
(18) R. Shapiro and S. H. Pohl, *Biochemistry,* **7,** 448 (1968).
(19) R. Shapiro and S. J. Shiuey, *Biochim. Biophys. Acta,* **174,** 403 (1969).
(20) Conditions for HPLC were the same as in note 14 except that the column was eluted with 80% methanol in water. Retention times of 17 min for the major and 22 min for the minor product were observed. A small uncharacterized peak was also observed at 11 min.
(21) Spectra were run on a Finnigan 1015D mass spectrometer by C.I. with CH_4 gas. Major fragments observed were *m/e* 534 ($M^+ + 1 - 60$), 474 ($M^+ + 1 - 2 \times 60$), and 332 (474 − 42). Tetraacetates of the tetraols show a similar pattern of fragmentation.
(22) The Fourier transform NMR spectra (220 MHz, $CDCl_3$) of **4a** and **4b** were compared with the spectra of the acetates of the trans-aniline adduct (10α-NHC_6H_5 in **4**) and the cis-phenol adduct (10β-OC_6H_5 in **4**) of diol epoxide **1**.[10] The coupling constants for the methyl guanine adducts, **4a** and **4b,** were within 1 Hz of those for the model compounds: **4a** (trans adduct) H_7 δ 6.71, H_8 5.52, H_9 5.58, H_{10} 6.14, O-Ac 1.98, 2.04, and 2.25, N_7-Me 3.38, guanine H_8 and aromatic hydrogens 7.95–8.28 with $^3J_{7eq,8eq}$ = 5.2, $^3J_{8eq,9eq}$ = 5.4, $^3J_{9eq,10eq}$ = 2.6 Hz; **4b** (cis adduct) H_7 δ 6.93, H_8 6.18, H_9 5.61, H_{10} 6.29, O-Ac 1.95, 2.00, and 2.13, N_7-Me 3.93, guanine H_8 7.95, aromatic hydrogens 8.0–8.4 with $^3J_{7ax,8ax}$ = 8.0, $^3J_{8ax,9ax}$ = 12.0, $^3J_{9ax,10eq}$ = 4.0 Hz.
(23) Although not a potential metabolite, A. Dipple, P. Brookes, D. S. Macintosh, and M. P. Rayman, *Biochemistry,* **10,** 4323 (1971), suggested that 7-bromomethylbenzo[a]anthracene alkylates guanine at N-2. In contrast, S. H. Blobstein, I. B. Weinstein, D. Grunberger, J. Weisgras, and R. G. Harvey, *ibid.,* **14,** 3451 (1975), suggested that 7,12-dimethylbenzo[a]anthracene 5,6-oxide alkylates guanine on the imidazole ring of GMP.
(24) S. H. Blobstein, I. B. Weinstein, P. Dansette, H. Yagi, and D. M. Jerina, *Cancer Res.,* **36,** 1293 (1976).
(25) B. Singer and H. Fraenkel-Conrat, *Biochemistry,* **14,** 772 (1975), have pointed out the importance of alkylation at phosphate in the expression of biological activity.

Masato Koreeda,* Patrick D. Moore
Department of Chemistry, The Johns Hopkins University Baltimore, Maryland 21218

Haruhiko Yagi, Herman J. C. Yeh, Donald M. Jerina*
Laboratory of Chemistry National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health Bethesda, Maryland 20014

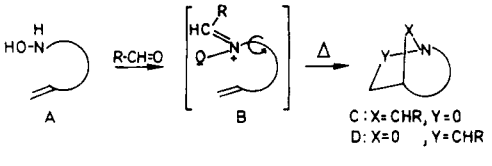
Received July 6, 1976

A Total Synthesis of *d,l*-Luciduline by a Regioselective Intramolecular Addition of an *N*-Alkenylnitrone

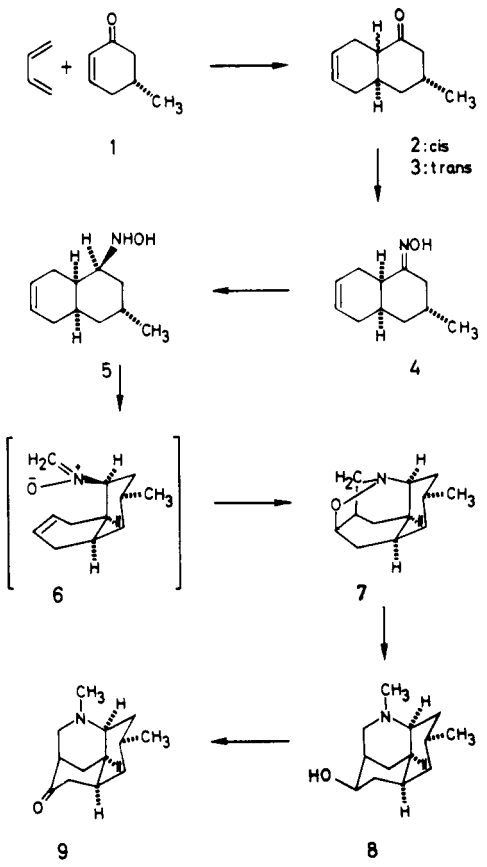
Sir:

Although several studies have been made of intramolecular thermal additions of *C*-alkenylnitrones[1] the corresponding reaction of *N*-alkenylnitrones has received only scant attention.[2] We now wish to report an application of the unexplored thermal reaction of an *N*-alkenylhydroxylamine, A, with an aldehyde (Scheme I)[3] to afford a simple total synthesis of racemic luciduline (**9**). The natural *d*-alkaloid, isolated from *Lycopodium lucidulum,* has been shown by chemical and x-ray evidence[4] to have structure **9.** Its racemate was synthesized recently by a multistep approach involving an internal Mannich reaction.[5]

Scheme I



Scheme II



The first step of our synthesis (Scheme II) consisted in the reaction of butadiene in the presence of 1.0 equiv of $SnCl_4$ in dry CH_3CN[6a] with 5-methylcyclohexenone, **1**.[7] Addition took place exclusively from the side opposite to the methyl substituent to give a 3:2 mixture of the *cis*-octalone, **2**, together with its trans-isomer **3**.[8-10] Oximation of this mixture (26 mmol) with hydroxylamine hydrochloride (31 mmol) in aqueous ethanol, followed by chromatography on silica gel furnished the cis-oxime **4**[9] (mp 143–145 °C; 40%). It was noticed that the *cis*-octalone **2** reacted faster with hydroxylamine than its trans isomer **3**. Consequently the reaction of the mixture of **2** and **3** with a stoichiometric amount (relative to **2**) of hydroxylamine hydrochloride and NaOAc in methanol enabled the pure cis-oxime **4** to be separated from unchanged trans-ketone **3**[11] by simple crystallization from isopropyl alcohol. Reduction[12] of the oxime **4** with 2 equiv of $NaBH_3CN$ in methanol[6b] afforded exclusively the hydroxylamine **5**[9] (mp 133–135 °C; 100%). Heating of **5** with 5 equiv of paraformaldehyde in the presence of molecular sieve in toluene[6c] gave the bridged isoxazolidine **7**[9] as an oil (70%). This transformation presumably involves a transient nitrone, **6**, which undergoes a highly regioselective intramolecular addition to a nonpolarized olefinic bond. Not even a trace of the corresponding positional isomer (isomer D in Scheme I) was found in the reaction mixture. Methylation of the adduct **7** with 1.5 equiv of methyl fluorosulfonate in ether,[6d] followed by reduction of the resulting salt with $LiAlH_4$[6e] gave the alcohol **8**[10,13] (mp 75–77 °C; 97%). Oxidation of **8** with Jones' reagent furnished the hydrochloride of the racemic alkaloid **9** (mp 238–240 °C, sealed capillary, reported mp 171–172 °C;[5] 98%). The free base **9** was identified by comparison of its ir, ^1H NMR, and mass spectra as well as its TLC and GC behavior with those of natural *d*- and synthetic *d,l*-luciduline.

A key feature of our approach is that during the conversion of **1** to **9** the original chiral center largely controls the developing configurations of the four other chiral centers. It may be further pointed out that this synthesis nicely illustrates the utility of intramolecular additions of *N*-alkenylnitrones as an equivalent of the Mannich reaction. The scope of the thermal reaction of *N*-alkenylhydroxylamines with aldehydes is presently being explored by using a variety of model compounds.

Acknowledgment. We are grateful to Professor D. A. Evans, California Institute of Technology, for kindly providing a sample of *d,l*-luciduline. We thank the Fonds National Suisse de la Recherche Scientifique, Sandoz Ltd, Basel, and Givaudan SA, Vernier for financial support of this work.

References and Notes

(1) N. A. LeBel, M. E. Post, and J. J. Whang, *J. Am. Chem. Soc.*, **86**, 3759 (1964); W. Oppolzer and K. Keller, *Tetrahedron Lett.*, 1117, 4313 (1970); for a recent review on intramolecular 1,3-dipolar additions see A. Padwa, *Angew. Chem., Int. Ed. Engl.*, **15**, 123 (1976).
(2) The regio- and stereoselective thermolysis of *N*-but-3-enyl-*C*-phenylnitrone (prepared from butenyl iodide and benzaldoxime) has been reported by W. C. Lumma, Jr., *J. Am. Chem. Soc.*, **91**, 2820 (1969), for an internal addition of 5-allyl-3,3,5-trimethyl-1-pyrroline 1-oxide see J. B. Bapat, D. S. C. Black, R. F. C. Brown, and C. Ichlov, *Aust. J. Chem.*, **25**, 2445 (1972).
(3) For the related reaction of *N*-acyl-*N'*-alkenylhydrazines with aldehydes see W. Oppolzer, *Tetrahedron Lett.*, 1707 (1972).
(4) W. A. Ayer, N. Masaki, and D. S. Nkunika, *Can. J. Chem.*, **46**, 3631 (1968).
(5) W. L. Scott and D. A. Evans, *J. Am. Chem. Soc.*, **94**, 4779 (1972).
(6) (a) The addition was carried out in a silylated Pyrex flask at 25 °C for 6–24 h; (b) at pH 3 at 25 °C for 1 h; (c) in a closed flask at 115 °C for 14 h; (d) at 0 °C for 1 h; (e) in THF at 25 °C for 5 h.
(7) H. O. House and W. F. Fischer, Jr., *J. Org. Chem.*, **33**, 949 (1968).
(8) The addition of butadiene to 5-methylcyclohexenone in presence of BF_3–etherate in benzene has been reported to furnish a 1:5 mixture of **2** and **3**, which are interconvertible by base: T. Harayama, H. Cho, M. Ohtani, and Y. Inubushi, *Chem. Pharm. Bull.*, **22**, 2784 (1974).
(9) Ir, ^1H NMR, and mass spectra are in full agreement with the assigned structure.
(10) Analyzed by GC using a steel column 12 ft × ⅛ in., 5% FFAP on 60–100 mesh chromosorb W; 170 °C; 24 kg of N_2/cm^2; **2**, retention time 14.2 min; **3**, retention time 16.5 min.
(11) For recycling of the trans-ketone **3** to the desired cis-ketone **2** see ref 8.
(12) R. F. Borch, M. D. Bernstein, and H. D. Durst, *J. Am. Chem. Soc.*, **93**, 2897 (1971).
(13) **8** displayed identical ^1H NMR and mass spectra as the alcohol obtained by reduction of natural luciduline with $NaBH_4$; see ref 4.

Wolfgang Oppolzer,* Martin Petrzilka
Département de Chimie Organique, Université de Genève
CH-1211, Genève 4, Switzerland
Received June 2, 1976

A Total Synthesis of Gliotoxin

Sir:

Gliotoxin **1**,[1] an antibiotic produced by various species of *Gladiocladium, Trichoderma, Aspergillus,* and *Penicillium,* presents a formidable challenge to synthetic chemists. Difficulties in controlling stereochemistry as well as functionality are accumulated in this small molecule. Four asymmetric centers in addition to two delicate ring systems—hydrated benzene and epidithiapiperazinedione—are present. We would like to report the first total synthesis of gliotoxin, using a novel solvent-dependent Michael reaction as a key step.

The thioacetal **2**[2,3] (mp 250–252 °C) was synthesized from glycine sarcosine anhydride in six steps[4] in 30% overall yield by the method previously reported.[5] Michael reaction of 4-carbo-*tert*-butoxybenzene oxide **3**[6] (excess) with **2** in methylene chloride containing Triton B at room temperature for a short period afforded the alcohol **4**[3] (mp 217–218 °C dec) as the major product (45% yield) and the epimeric alcohol **5**[3] (mp 255–257 °C dec) as the minor product (15% yield). The ratio of alcohols **4** and **5** produced in this Michael reaction was found to be dependent on the solvent and the time of reaction. A 3:1 ratio (88% yield) favoring the alcohol **5**, the minor