THF solvent. *n*-Octane (0.6 mL) was added as an internal standard for GLC analyses. Several minute samples were removed and analyzed by GLC (a 0.25 in. × 6 in. 20% *N,N,N',N'*-tetrakis(2-hydroxyethyl)-ethylenediamine (THEED) column to remove boranes, followed in series by a $^1/_8$ in. × 72 in. 30% adiponitrile column) for the initial flame ratios of 1-heptene/*n*-octane and 1-hexyne/*n*-octane. The samples were injected directly onto the THEED column, which was inserted into the injection port and maintained at 70–80 °C. The column oven containing the adiponitrile column was maintained at room temperature. The reaction was initiated by injecting 8.7 mL of $(9\text{-BBN})_2$ solution (0.29 M, 2.5 mmol) in THF into the stirred reaction mixture.

The reaction mixture was stirred 4 h at 25 °C to ensure complete reaction. A longer reaction time was needed for less reactive alkynes. A sample (0.5 mL) of the reaction mixture was withdrawn via a syringe and injected into a vial containing 0.5 mL of a 5% solution of glacial acetic acid in methanol. After standing for at least 1 h, the quenched sample was analyzed by GLC for the 1-heptene/*n*-octane and 1-hexyne/*n*-octane flame ratios. The relative reactivity between 1-heptene and 1-hexyne was calculated by using the Ingold–Shaw equation (eq 18)[16]

$$\frac{k_y}{k_x} = \frac{\log y_\infty - \log y_0}{\log x_\infty - \log x_0} \tag{18}$$

in which y_0 = mmol of 1-heptene before reaction, y_∞ = mmol of 1-heptene after reaction, and x_0 and x_∞ = initial and final mmol of 1-hexyne. It was found that 1.24 mmol of 1-heptene and 4.07 mmol of 1-hexyne remained after the reaction. Consequently, the relative reactivity can be calculated as 6.8.

Several other pairs were studied to examine the influence of structure on the relative reactivity. These pairs include 1-decyne/1-octene, 3-methyl-1-butyne/1-hexene, cyclohexylethyne/1-octene, 3,3-dimethyl-1-butyne/1-heptene, phenylethyne/cyclooctene, 3-hexyne/2-methyl-2-butene, 4,4-dimethyl-2-pentyne/*cis*-3-hexene, and diphenylethyne/2,3-dimethyl-2-butene. The results are summarized in Table II.

Relative Rates (k_2/k_2') between Mono- and Dihydroboration of Alkynes with $(9\text{-BBN})_2$. The relative rates between mono- and dihydroboration of alkynes with $(9\text{-BBN})_2$ were also studied by an indirect route. An example of the relative reactivity between *B*-(*trans*-1-hexen-1-yl)-9-BBN and 3,3-dimethyl-1-butene is given as follows: To a 100-mL reaction flask was added 10 mL of THF, 0.45 mL (0.317 g) of *n*-octane (GLC internal standard), 1.18 mL (1.01 g at 0 °C, 5.0 mmol) of *B*-(*trans*-1-hexen-1-yl)-9-BBN, and 0.65 mL (0.42 g, 5.0 mmol) of 3,3-dimethyl-1-butene. *B*-(*trans*-1-Hexen-1-yl)-9-BBN was prepared as described elsewhere.[3] To the rapidly stirred reaction mixture maintained at 25 °C was added 8.7 mL of $(9\text{-BBN})_2$ solution (0.29 M, 2.5 mmol) in THF solvent. The reaction mixture was stirred 4 h at 25 °C to ensure complete reaction. A sample (0.5 mL) of the reaction mixture was withdrawn via a syringe and injected into a vial containing 0.5 mL of a 5% solution of glacial acetic acid in methanol. After standing for at least 1 h, the quenched sample was analyzed by GLC using the same conditions as just described. There were found 1.65 mmol of 3,3-dimethyl-1-butene and 3.44 mmol of 1-hexene [quenched product of *B*-(*trans*-1-hexen-1-yl)-9-BBN]. The relative reactivity between 3,3-dimethyl-1-butene and *B*-(*trans*-1-hexen-1-yl)-9-BBN was calculated by using the Ingold–Shaw equation (eq 18) as 2.96. Therefore, the relative reactivity (k_2/k_2') between 1-hexyne and *B*-(*trans*-1-hexen-1-yl)-9-BBN can be calculated as 2.96/1.54 = 1.92 (Table III).[1b]

The relative reactivity between *B*-(*cis*-3-hexen-3-yl)-9-BBN and 1-methylcyclohexene was also studied and found to be 0.302. Using this result, we calculated the relative reactivity (k_2/k_2') between 3-hexyne and *B*-(*cis*-3-hexen-3-yl)-9-BBN to be 193. These results are summarized in Table III.

Mono- and Dihydroboration of 1-Hexyne with 1 Equiv of 9-BBN in Terms of the Monomer. The amounts of mono- and dihydroboration of 1-hexyne during the reaction with 1 equiv of 9-BBN in terms of the monomer in THF at 25 °C were determined as follows: A 100-mL reaction flask was added with 2.63 mL of THF, 0.57 mL of 1-hexyne (0.41 g, 5.0 mmol), and 0.6 mL of *n*-octane (0.41 g, GLC internal standard). Several minute samples were removed and analyzed for the initial flame ratio of 1-hexyne/*n*-octane. The analytical conditions by GLC were the same as just described. The reaction was initiated by adding 8.7 mL of $(9\text{-BBN})_2$ solution (0.29 M, 2.5 mmol) in THF solvent to the rapidly stirred solution maintained at 25 °C. At appropriate intervals of time, aliquots (0.5 mL) were withdrawn and injected into vials containing 0.5 mL of a 5% solution of glacial acetic acid in methanol to quench the reaction. After standing for at least 1 h, the quenched sample was analyzed by GLC for the flame ratios of 1-hexyne/*n*-octane and 1-hexene/*n*-octane [1-hexene is the quenched product of the monohydroborated adduct, *B*-(*trans*-1-hexen-1-yl)-9-BBN].[3] The amounts of dihydroborated adduct were calculated according to eq 19. A summary of the results is given in Figure 2.

mmol of *gem*-dibora-9-BBN = (initial mmol of 1-hexyne) − (residual mmol of 1-hexyne) − (mmol of 1-hexene) (19)

Mono- and Dihydroboration of Excess Amounts of 1-Hexyne with $(9\text{-BBN})_2$. The percentages of mono- and dihydroboration of excess amounts of 1-hexyne with $(9\text{-BBN})_2$ in THF at 25 °C were also studied by the analytical procedure described above. After the reaction was complete, an aliquot (0.5 mL) of the reaction mixture was removed, quenched, and analyzed. The percentages of mono- and dihydroboration were then calculated.

Numerical Calculations of Mono- and Dihydroboration. The relationship between the percentages of mono- and dihydroboration and the relative reactivity (k_2/k_2') of an alkyne toward $(9\text{-BBN})_2$ was established by the numerical analysis. The differential eq 16 and 17 were integrated by the use of the Runge–Kutta method.[13] A computer program was written, and the calculations and the plots were carried out on a Hewlett-Packard 9820A calculator and Hewlett-Packard 9862A calculator plotter, respectively. For each set of parameters, the calculations were carried out to 99.9% completion of reaction. A detailed discussion of the calculations has been given in the text.

Registry No. $(9\text{-BBN})_2$, 70658-61-6; 1-hexene, 592-41-6; *cis*-3-hexene, 7642-09-3; 1-hexyne, 693-02-7; 1-decyne, 764-93-2; 3-methyl-1-butyne, 598-23-2; 3,3-dimethyl-1-butyne, 917-92-0; cyclohexylethyne, 931-48-6; phenylethyne, 536-74-3; 3-hexyne, 928-49-4; 4,4-dimethyl-2-pentyne, 999-78-0; diphenylethyne, 501-65-5; B-(trans-1-hexen-1-yl)-9-BBN, 69322-45-8; 3,3-dimethyl-1-butene, 558-37-2; B-(cis-3-hexen-3-yl)-9-BBN, 69322-51-6; 1-methylcyclohexene, 591-49-1; hexane-1,1-bis[9-BBN], 79919-22-5.

(16) Ingold, C. K.; Shaw, F. R. *J. Chem. Soc.* **1927**, 2918–2926.

Total Synthesis of the Antitumor Antibiotic Streptonigrin[1]

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Abstract: Full details of the previously reported total synthesis of streptonigrin (**1**) are presented. The successful synthetic strategy outlined in Scheme I involves use of an imino Diels–Alder cycloaddition for construction of the pyridine C ring of **1** and a modified Friedlander synthesis for annulation of the AB quinoline quinone ring system.

A report which appeared approximately 20 years ago by workers at Chas. Pfizer and Co. described the isolation of a dark brown, crystalline compound named streptonigrin from cultures of *Streptomyces flocculus*.[3,4] Streptonigrin was of particular interest

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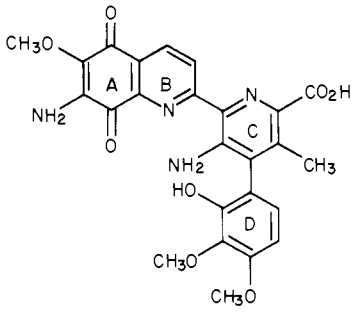
since it showed antibiotic activity against both gram-positive and gram-negative bacteria and since it had pronounced anticancer activity. Subsequently, the same compound was isolated in the Soviet Union under the name bruneomycin from a strain of *Actinomyces albus var bruneomycini*[5] and in France as rufochromomycin from *Streptomyces rufochromogenes* and *Streptomyces echinatus*.[6]

In 1963 Rao, Biemann, and Woodward established tetracyclic pyridylquinoline quinone structure **1** for streptonigrin based upon



1

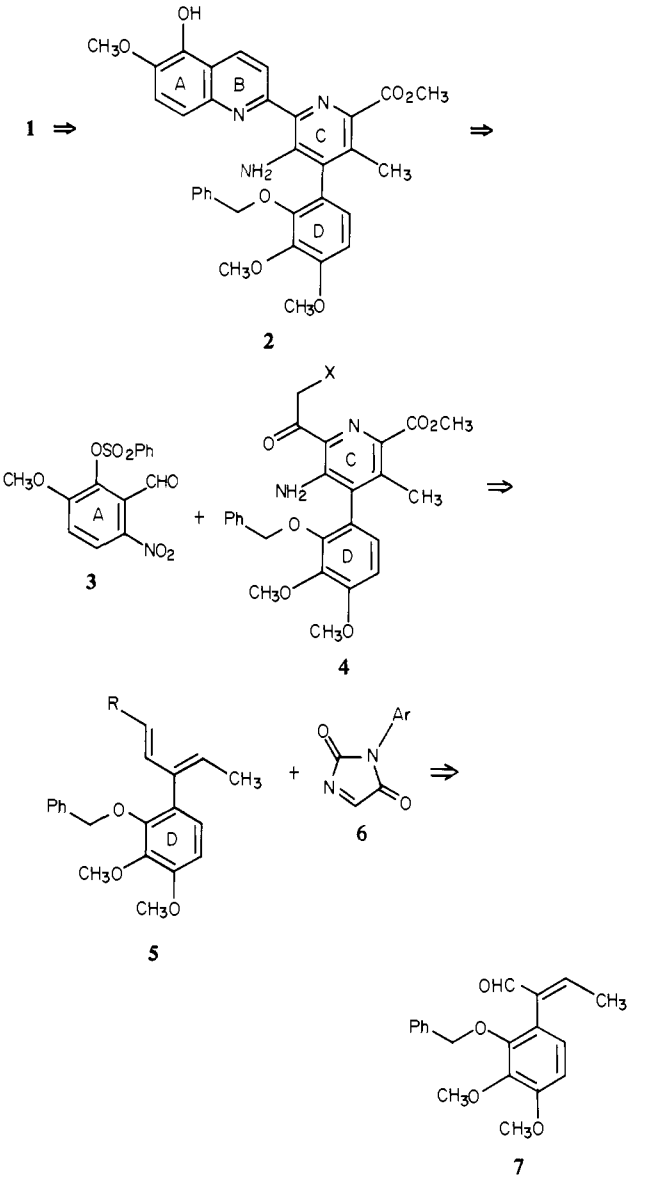
a brilliant combination of degradative and spectral studies.[7] Their original formulation was recently confirmed by X-ray crystallography.[8] In addition to having a unique heterocyclic structure, streptonigrin has also been found to originate in the microorganisms by a novel biosynthetic pathway.[4,9]

Streptonigrin has been investigated clinically as an anticancer drug, and although quite promising, severe bone marrow depression in treated patients has limited its wide application in cancer chemotherapy.[4] Considerable effort has been made to prepare streptonigrin analogues, with the ultimate goal being the preparation of a molecule having high activity but showing attenuated toxicity.[4] Several studies on streptonigrin structure-activity relationships have appeared.[4] Also, good progress has been made recently on elucidating the mechanism of cytotoxicity of **1**.[4,10]

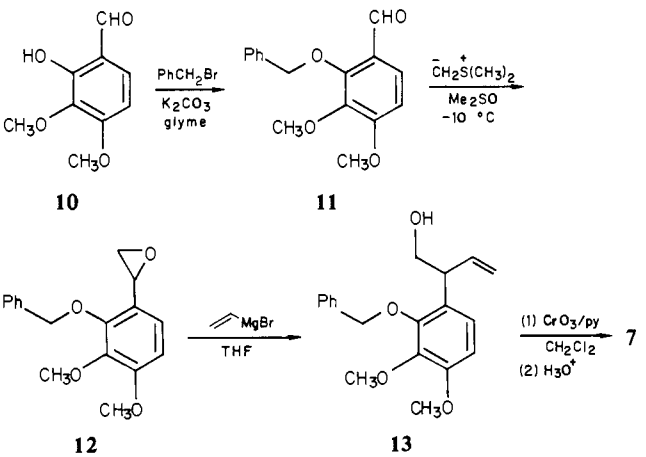
As a target for total synthesis, streptonigrin presents a major challenge with its high degree of functionalization and tightly linked array of aromatic rings. In 1980 we reported the first total synthesis of this complex molecule,[1,11] and now we describe the details of this work.

Our planned synthetic strategy, developed during extensive model studies,[12] is outlined in Scheme I. Streptonigrin (**1**) might

Scheme I



Scheme II



be prepared by A-ring elaboration of tetracyclic intermediate **2**, followed by cleavage of the D-ring benzyl ether and the C-ring methyl ester protecting groups. Compound **2** might in turn be constructed by some variation of the Friedlander quinoline synthesis between the A-ring nitroaldehyde **3** and a CD fragment **4**.[13] We expected that the desired pyridine precursor **4** might

(1) A preliminary account of this research has appeared: Basha, F. Z.; Hibino, S.; Kim, D.; Pye, W. E.; Wu, T.-T.; Weinreb, S. M. *J. Am. Chem. Soc.* **1980**, *102*, 3962.

(2) Fellow of the A. P. Sloan Foundation 1975–1979; Recipient of a Research Career Development Award (HL-00541) from the National Institutes of Health, 1975–1980. Correspondence should be addressed to The Pennsylvania State University.

(3) Rao, K. V.; Cullen, W. P. *Antibiot. Annu.* **1959–1960**, 950.

(4) For an up-to-date review of all aspects of the chemistry, biosynthesis and mechanism of action of streptonigrin see: Gould, S. J.; Weinreb, S. M. *Fortschr. Chem. Org. Naturst.* in press. See also: Hibino, S. *Heterocycles* **1977**, *6*, 1485.

(5) Kudrina, E. S.; Olkhovatova, O. L.; Muravieva, L. I.; Gauze, G. F. *Antibiotiki (Moscow)* **1966**, *11*, 400. Brazhnikova, H. G.; Ponomarenko, V. I.; Kovsharova, I. N.; Kruglyak, E. B.; Proshlyakova, V. V. *Ibid.* **1968**, *13*, 99.

(6) Societe des usines chemiques Rhone-Poulenc, *British Patent* 872261, July 5, 1961 (*Chem. Abstr.* **1961**, *55*, 25158a).

(7) Rao, K. V.; Biemann, K.; Woodward, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2532.

(8) Chiu, Y.-Y.; Lipscomb, W. N. *J. Am. Chem. Soc.* **1975**, *97*, 2525.

(9) Gould, S. J.; Chang, C. C. *J. Am. Chem. Soc.* **1980**, *102*, 1702. Gould, S. J.; Chang, C. C.; Darling, D. S.; Roberts, J. D.; Squillacote, M. *Ibid.* **1980**, *102*, 1707.

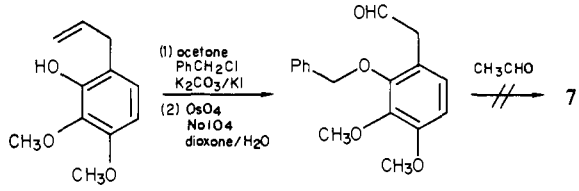
(10) Hajdu, J. *J. Am. Chem. Soc.* **1981**, *103*, 232 and references cited.

(11) Kende, A. S.; Lorah, D. P.; Boatman, R. J. *J. Am. Chem. Soc.* **1981**, *103*, 1271, subsequently reported another total synthesis of streptonigrin.

(12) (a) Hibino, S.; Weinreb, S. M. *J. Org. Chem.* **1977**, *42*, 232. (b) Kim, D.; Weinreb, S. M. *Ibid.* **1978**, *43*, 121. (c) *Ibid.* **1978**, *43*, 125.

be prepared via an imino Diels–Alder route from diene **5** and imine **6**, as successfully tested in a model system.[12b,c] Unsaturated aldehyde **7** appeared to be the precursor of choice for diene **5**, and thus initial work was directed toward synthesis of this intermediate.

The most direct approach to **7** appeared to be through a mixed aldol condensation between aldehyde **9** and acetaldehyde.[14] Aldehyde **9** was prepared in two steps from the known allylphenol **8**[15] as shown. Many attempts were made to effect the desired

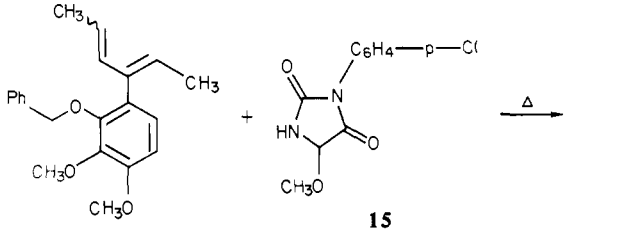


8 9

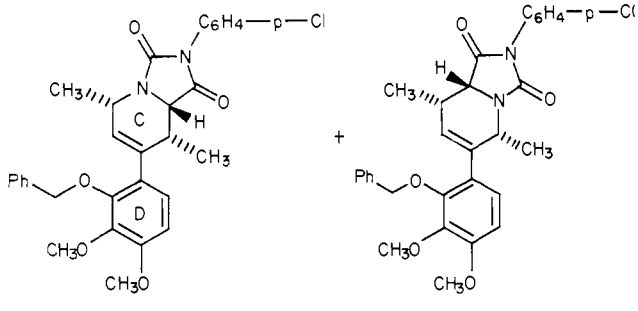
aldol condensation using a variety of reaction conditions, but α,β-unsaturated aldehyde **7** could never be produced in acceptable yield. Thus, an alternative route to **7** had to be found, and the sequence which ultimately was successful is shown in Scheme II.

Readily available 2-hydroxy-3,4-dimethoxybenzaldehyde (**10**)[16] was O-benzylated to afford aldehyde ether **11**,[17] which on treatment with dimethylsulfonium methylid[18] afforded epoxide **12** in good overall yield. Addition of this epoxide to an excess of cold vinylmagnesium bromide in THF led to the homoallylic alcohol **13**. Oxidation of **13** with chromic acid/pyridine in methylene chloride gave a complex mixture of aldehydes, which upon stirring overnight with dilute hydrochloric acid was converted cleanly to a single α,β-unsaturated aldehyde assumed to have the more stable "tiglate" geometry shown in structure **7**.

Wittig chemistry served to convert **7** to the desired diene **14**.



14 15



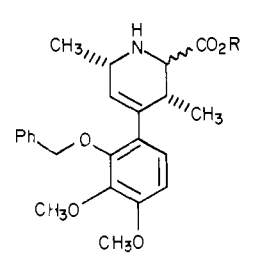
16a 16b

Since we knew from our previous work on the imino Diels–Alder reaction[12b] that diene systems such as **14** are unreactive if they possess a cis-disubstituted double bond, the Schlosser modification[20] of the Wittig reaction was used in order to maximize the amount of trans diene formed. Thus, treatment of aldehyde **7** with ethylidene triphenylphosphorane at −78 °C, followed by addition of *n*-butyllithium and then potassium *tert*-butoxide in *tert*-butyl alcohol, afforded diene **14** as an inseparable mixture of trans and cis isomers in about a 2.5:1 ratio, respectively, as determined by ^{1}H NMR. No attempt was made to improve this ratio since, as it turned out, both geometric isomers were useful in the next step (vide infra).

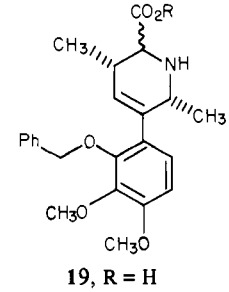
When dienes **14** are refluxed with methoxyhydantoin **15**[21] in xylene for 3 days, an inseparable mixture of adducts **16a** and **16b** in about a 3:1 ratio (estimated by ^{1}H NMR) was formed. For some reason unknown to us, this cycloaddition will not go to completion. Longer reaction times, higher temperatures, and larger excesses of hydantoin **15** had little effect upon the amount of adducts formed. Acid catalysis of the cycloaddition was not at all successful. The best solution to this problem was to recycle unreacted diene **14**. After one recycle the yield of adducts **16a** and **16b** was 56%, and this yield could be improved by continued reuse of recovered **14**. Interestingly, the recovered diene appeared enriched in the cis isomer but always contained a significant amount of the trans compound. It appears that the long reflux periods and high temperature required for the cycloaddition step are sufficient to allow partial thermal isomerization of the cis to the trans diene, and thus the cis isomer was ultimately useful in the cycloaddition.

The stereochemistry shown for adducts **16a** and **16b** was not actually established for these compounds but is postulated by analogy with the model systems which we had previously investigated in detail.[12b] The major Diels–Alder adduct was assumed to be regioisomer **16a**, also based upon information from this earlier model work, and subsequent conversion to streptonigrin, eventually established the validity of this assumption.

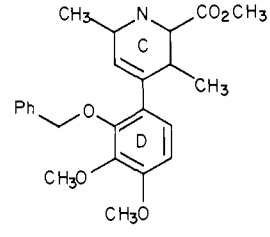
The mixture of adducts **16a** and **16b** was hydrolyzed with aqueous barium hydroxide to produce a mixture of amino acids **17** and **19**, respectively. Without purification, this mixture was



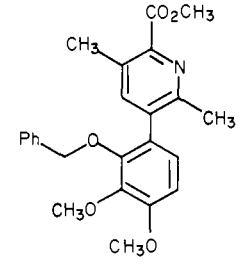
17, R = H
18, R = CH_3



19, R = H
20, R = CH_3



21



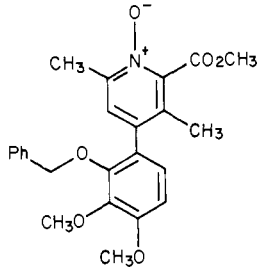
22

converted to methyl esters **18** and **20** with $SOCl_2$/methanol. The crude mixture of esters was then heated with 5% Pd/C in toluene overnight to afford pyridine **21** (~20% from adduct mixture **16a** and **16b**) along with a small amount of the undesired isomeric pyridine **22** separable by chromatography. It is not at all clear just why so little of this isomeric pyridine is produced here, and the necessity of working with such complex regio- and stereoisomeric mixtures at this stage has unfortunately made it impossible to intelligently analyze this reaction sequence.

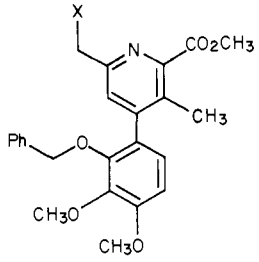
(13) Application of a classical Friedlander condensation between the *o*-aminoaldehyde corresponding to **3** and acetyl pyridine **4** (X = H) was ruled out here, based upon poor yields in related model systems.[12a]

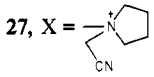
(14) Cf. Kuhn, R.; Michel, J. *Chem. Ber.* **1938**, *67*, 696.
(15) Trikojus, V. M.; White, D. E. *J. Chem. Soc.* **1949**, 436.
(16) Reichstein, T.; Oppenauer, R.; Grüssner, A.; Hirt, R.; Rhyner, L.; Glatthaar, C. *Helv. Chim. Acta* **1935**, *18*, 816.
(17) Kametani, T.; Kozuka, A.; Tanaka, S. *Yakugaku Zasshi* **1970**, *90*, 1574; Kametani, T.; Kano, S.; Watanabe, Y. *Ibid.* **1966**, *86*, 417.
(18) Corey, E. J.; Chaykovsky, M. *J. Am. Chem. Soc.* **1965**, *87*, 1353.
(19) Ratcliffe, R.; Rodehorst, R. *J. Org. Chem.* **1970**, *35*, 4000.
(20) Schlosser, M.; Christmann, K. F. *Justus Liebigs Ann. Chem.* **1967**, *708*, 1.
(21) Ben-Ishai, D.; Goldstein, E. *Tetrahedron* **1971**, *27*, 3119.

Our next subgoal was the introduction of the necessary 5-amino substituent into the pyridine C ring of **21**, and once again a strategy developed in earlier model studies[12c] was employed. Thus, pyridine **21** was transformed to *N*-oxide **23** with *m*-chloroperbenzoic acid.

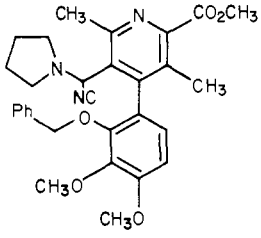


23

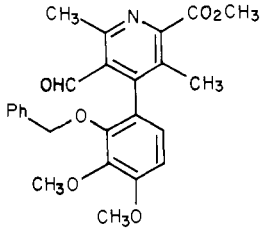


24, X = $OCOCH_3$
25, X = OH
26, X = Cl
27, X = 

Heating **23** in acetic anhydride produced acetate **24** through a well-known pyridine *N*-oxide rearrangement.[22] The acetate ester of **24** was cleaved with methanolic potassium carbonate, yielding alcohol **25**, which produced chloride **26** on treatment with thionyl chloride. Reaction of chloride **26** with *N*-(cyanomethyl)-pyrrolidine[23] in Me_2SO at 45 °C gave the quaternary salt **27**. With use of the carefully controlled reaction conditions which we developed in a related system[12c] (i.e., KO-*t*-Bu, Me_2SO/THF, −12 °C, deoxygenated argon, 10 min), salt **27** could be transformed into amino nitrile **28** via a [2,3]-sigmatropic rearrangement.[24]



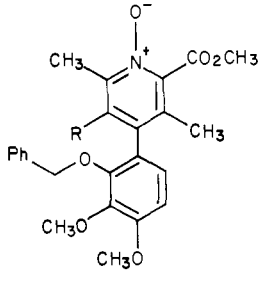
28



29

Without isolation, **28** was hydrolyzed with aqueous oxalic acid, affording aldehyde **29**. The conversion of acetate **24** to aldehyde **29** was done without any purification of intermediates and could be effected in an overall yield of 35%.

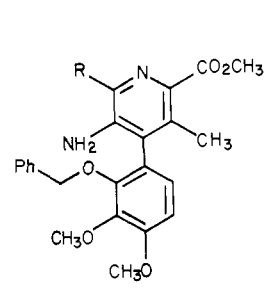
Several attempts to convert pyridine **29** to the corresponding *N*-oxide **30** with *m*-chloroperbenzoic acid were unsuccessful,



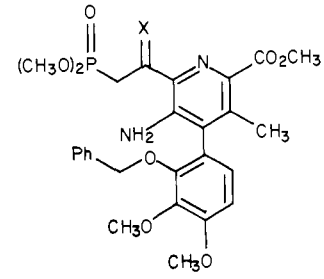
30, R = CHO
31, R = CO_2H
32, R = NH_2

perhaps due to the two electron-withdrawing carbonyl groups resulting in a low basicity of the pyridine nitrogen in this system. However, trifluoroperacetic acid was sufficiently strong to effect this conversion. Further oxidation of **30** with potassium permanganate in acetone led to carboxylic acid **31**. Finally, the desired amino group was introduced by applying the Yamada modification[25] of the Curtius rearrangement to acid **31** ($(PhO)_2PON_3/NEt_3$) followed by hydrolysis of the intermediate isocyanate, giving **32**.

We next turned our attention toward devising a convenient high-yield method for attaching our CD-ring fragment to a suitable AB-ring precursor (Scheme I). Based upon some preliminary work,[12a,26] we elected to couple the appropriate segments via a "nitrochalcone" modification of the Friedlander quinoline synthesis,[13,27] and we have developed a new variation of this process based upon modern phosphonate carbanion chemistry. Thus, *N*-oxide **32** was heated in acetic anhydride,[22] and the crude product of this reaction was stirred with methanolic potassium carbonate at room temperature, yielding alcohol **33**. Activated manganese



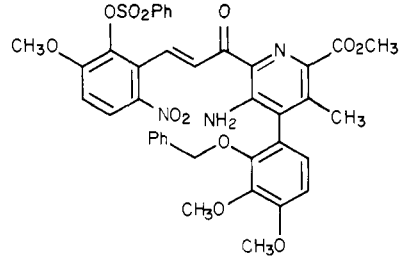
33, R = CH_2OH
34, R = CHO



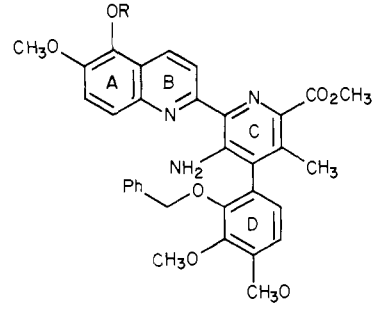
35, X = H, OH
36, X = O

dioxide served to oxidize **33** to aldehyde **34**. It was possible to add dimethyl (lithiomethyl)phosphonate to this aldehyde in THF/HMPA to obtain β-hydroxyphosphonate **35**.[28] Manganese dioxide oxidation of **35** cleanly afforded the β-ketophosphonate **36**.

A Wadsworth–Emmons–Horner condensation of **36** with the known, readily available nitroaldehyde **3**[29] was effected with potassium hydride in benzene to give the nitrochalcone **37**.



37

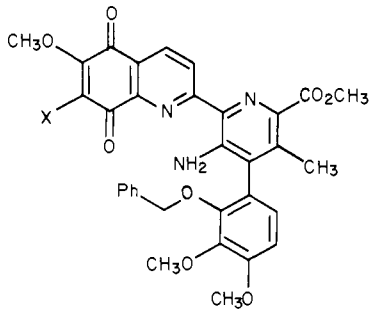


38, R = SO_2Ph
39, R = H

(22) For a review of this rearrangement see: Oae, S.; Orino, K. *Heterocycles* **1977**, *6*, 583.
(23) Lespagnol, A.; Cuingnet, E.; Debaert, M. *Bull. Soc. Chim. Fr.* **1960**, 383.
(24) Sanders, E. B.; Secor, H. V.; Seeman, J. I. *J. Org. Chem.* **1978**, *43*, 324.
(25) Shiori, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203; *Tetrahedron* **1974**, *30*, 2151; *Chem. Pharm. Bull.* **1974**, *22*, 1398.
(26) D. Kim, Ph.D. Thesis, Fordham University; Bronx, NY, 1979.
(27) "The Chemistry of Heterocyclic Compounds. Quinolines"; Jones, G., Ed.; Interscience: New York, Vol. 32, Part 1, Chapter 2.
(28) Corey, E. J.; Kwiatkowski, G. T. *J. Am. Chem. Soc.* **1966**, *88*, 5654.
(29) Reid, W.; Schiller, H. *Chem. Ber.* **1952**, *85*, 216.

Reduction of **37** with sodium hydrosulfite proceeded nicely to afford tetracyclic pyridylquinoline **38**.[27] Cleavage of the A-ring sulfonyl group with sodium methoxide in methanol provided the phenol **39**.

Elaboration of the A-ring functionality in **39** was investigated next, and the general approach used successfully here was one developed by us[12a] and by others[4,30] for synthesis of streptonigrin quinoline quinone analogues. Fremy's salt[31] oxidation of phenol **39** gave yellow quinone **40** in high yield. The remaining A-ring



40, X = H
41, X = I
42, X = N_3
43, X = NH_2

amino substituent was introduced by a simple three-step sequence. Treatment of **40** with iodine azide in acetonitrile[30] afforded iodoquinone **41**. Displacement of the iodine atom in **41** was effected with sodium azide in DMF, giving azidoquinone **42**. Reduction of azidoquinone **42** with sodium hydrosulfite[12a] led to the desired aminoquinone **43**, along with a small amount of unreacted azidoquinone.

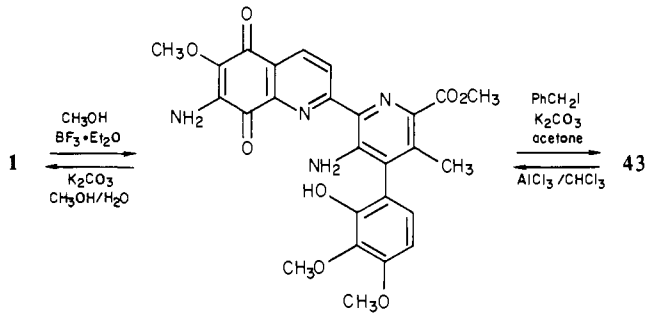
At this point it was possible to correlate our synthetic material with the natural product (Scheme III). Authentic streptonigrin (**1**) was converted to its methyl ester **44** with methanol/BF_3·Et_2O.[32] O-Benzylation of **44** with benzyl iodide/K_2CO_3 in acetone afforded **43** which was identical with the compound prepared by total synthesis.

The final stage of the synthesis involved removal of the two protecting groups of **43**. Cleavage of the benzyl ether function of **43** could be accomplished cleanly at room temperature with excess anhydrous aluminum chloride in chloroform, giving streptonigrin methyl ester (**44**) which was identical with an authentic sample. Lastly, potassium carbonate hydrolysis of **44** afforded synthetic streptonigrin (**1**) identical with the natural product by spectra analysis and by TLC in several solvent systems.

Experimental Section

Melting points were taken on either a Thomas-Hoover "Uni-Melt" capillary melting point apparatus or a Fisher-Johns apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer Model 197 spectrophotometer. Ultraviolet (UV) spectra were recorded on a Cary 14 spectrophotometer using methanol as the solvent. Proton magnetic resonance spectra (60 MHz) were recorded on either a Varian A60-A or an EM-360 NMR spectrometer. ^1H NMR spectra at 100 MHz were obtained on a JEOL-PFT-100 Fourier transform NMR spectrometer at 200 MHz on a Brüker WP 200 spectrometer and at 360 MHz on a Brücker WM-360 instrument. Chemical shifts are reported in δ units, using tetramethylsilane as an internal standard. Spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). All spectra were taken in deuteriochloroform. Carbon-13 magnetic resonance (^{13}C NMR) spectra were obtained on a Varian CFT-20 NMR spectrometer. Mass spectra (MS) were routinely recorded at 70 eV by electron impact (EI) on an Associated Electrical Industries MS-902 double focusing mass spectrometer on which both low and high resolution spectra were obtained. Compounds which did not yield molecular ions (M^+) on this instrument were analyzed by chemical ionization (CI) on a Finnigan 3200 quadrupole mass spectrometer using methane as a carrier gas. Combustion analyses were performed by Micro-Tech Laboratories, Inc., Skokie, IL. Analytical and preparative thin-layer chromatography (TLC) was done on Silica Gel 60 PF-254 (E.M. Merck). Column chromatography was carried out by using 70–230 mesh silica gel 60 (E.M. Merck).

Scheme III



44

3,4-Dimethoxy-2-(phenylmethoxy)benzaldehyde (11). A mixture of 3,4-dimethoxy-2-hydroxybenzaldehyde (**10**) (94.7 g, 520 mmol), benzyl bromide (65 mL, 547 mmol), and anhydrous potassium carbonate (86.3 g, 624 mmol) in ethylene glycol dimethyl ether (530 mL) was refluxed under a nitrogen atmosphere with stirring for 17 h. The cooled reaction mixture was concentrated to approximately one-third of the volume, diluted with water (400 mL), and extracted with benzene (4 × 250 mL). The combined organic layer was washed with 1 M NaOH (3 × 50 mL) and brine (3 × 50 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was distilled to give as a yellow oil 127.46 g (90%) of benzyl ether **11**: bp 176 °C (1 torr) [lit.[17] bp 199–201 °C (3 torr)]; ^1H NMR δ 3.9 (6 H, s), 5.2 (2 H, s), 6.75 (1 H, d, J = 9 Hz), 7.4 (5 H, s), 7.55 (1 H, d, J = 9 Hz), 10.1 (1 H, s); ^{13}C NMR δ 56.2, 61.0, 76.6, 107.8, 124.0, 127.9, 128.5, 136.5, 141.6, 155.2, 159.3, 188.7; IR (film) 1680, 1590, 1400 cm^{-1}.

[3,4-Dimethoxy-2-(phenylmethoxy)phenyl]oxirane (12). To a flame-dried 1-L three-necked round-bottom flask equipped with a mechanical stirrer, reflux condenser, and gas inlet tube under a dry nitrogen atmosphere was added a 50% dispersion of sodium hydride in mineral oil (11.5 g, 0.230 mol). The slurry was washed twice with pentane, and the solvent was decanted. Dry Me_2SO (115 mL, 1.62 mol) was added, and the mixture was heated with stirring in an oil bath at 70–75 °C for 45 min. The greenish mixture was diluted with dry THF (90 mL) and cooled to −10 °C. Trimethylsulfonium iodide (46.9 g, 0.230 mol) in dry Me_2SO (180 mL) was added dropwise to this mixture over 15 min while the temperature was maintained at −10 °C. The mixture was stirred for 5 min at −10 °C and 3,4-dimethoxy-2-(phenylmethoxy)benzaldehyde (**11**, 50.08 g, 0.184 mol) in dry THF (50 mL) was added dropwise over 15 min while the external bath temperature was maintained at −10 °C. The resulting mixture was warmed to room temperature and stirred for 1 h. The solution was poured into an ice–water mixture (1 L) and extracted with ether (5 × 200 mL). The combined organic layer was washed with water (4 × 50 mL) and brine (3 × 100 mL), dried (Na_2SO_4), and evaporated in vacuo to give 52.04 g (99%) of epoxide **12** as an oil which was used in the next step without further purification: ^1H NMR δ 2.5 (1 H, dd, J = 3, 6 Hz), 2.9 (1 H, dd, J = 4, 6 Hz), 3.75 (3 H, s), 3.80 (3 H, s), 3.9 (1 H, m), 5.1 (2 H, s), 6.7 (2 H, m), 7.3 (5 H, m); ^{13}C NMR δ 48.2, 50.2, 55.9, 60.8, 75.7, 107.8, 119.4, 124.1, 128.0, 128.3, 137.4, 142.3, 151.4, 153.4; IR (film) 1600, 1500 cm^{-1}.

β-Ethenyl-3,4-dimethoxy-2-(phenylmethoxy)benzeneethanol (13). To a solution of 1.2 M vinylmagnesium bromide in THF (300 mL, 0.36 mol) and dry THF (100 mL) cooled to −20 °C under a dry nitrogen atmosphere was added epoxide **12** (52.04 g, 0.182 mol) in dry THF (40 mL) dropwise over 30 min. The solution was stirred for 45 min at 0 °C and for 1 h at room temperature. The reaction mixture was cooled to −10 °C and quenched with 10% NH_4Cl solution (400 mL). The aqueous layer was extracted with ether (3 × 150 mL), and the combined organic extract was washed with water (3 × 80 mL) and brine (3 × 100 mL), dried (anhydrous Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on silica gel (500 g) in chloroform affording 55.21 g (97%) of alcohol **13** as an oil: ^1H NMR δ 3.75 (3 H, br m), 3.85 (3 H, s), 3.90 (3 H, s), 5.1 (2 H, s), 5.2–4.9 (2 H, m), 6.2–5.6 (1 H, m), 6.6 and 6.85 (2 H, AB_q, J = 8.5 Hz), 7.45 (5 H, m); ^{13}C NMR δ 44.9, 55.9, 60.8, 65.5, 75.4, 107.8, 116.3, 122.4, 127.3, 127.9, 128.3, 128.4, 137.6, 138.7, 142.6, 150.7, 152.4; IR (film) 3425 (br), 1640, 1600, 1500 cm^{-1}.

(*E*)-α-Ethylidene-3,4-dimethoxy-2-(phenylmethoxy)benzeneacetaldehyde (7). To a mixture of 1.5 L of anhydrous methylene chloride and 93 mL of dry pyridine was added 57.3 g (0.573 mol) of dry CrO_3

(30) Kende, A. S.; Naegely, P. C. *Tetrahedron Lett.* **1978**, 4775.
(31) Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229. We are grateful to Professor L. Hegedus for an improved and reproducible procedure for preparing Fremy's salt.
(32) Kadaba, P. K. *Synthesis* **1972**, 628.

at 0 °C. The suspension was stirred at 0 °C for 5 min and warmed to room temperature over 1 h. A solution of 30 g (0.0968 mol) of alcohol **13** in 16 mL of dry methylene chloride was added to the dark solution, and the resulting mixture was stirred at room temperature for 35 min. The solution was decanted, and the residue was slurried with chloroform several times. The combined organic fraction was washed three times with 5% NaOH, 5% HCl, saturated $NaHCO_3$, and saturated brine. The solution was dried over anhydrous $MgSO_4$, filtered through a pad of Florisil, and evaporated in vacuo. The residue was dissolved in THF, and the solution was stirred with 5% HCl at room temperature for 10 h. Water was added, and the mixture was extracted with methylene chloride. The organic layer was dried over anhydrous $MgSO_4$ and evaporated in vacuo. The residue was chromatographed on silica gel (500 g) in benzene/ethyl acetate (95/5) to afford 16.6 g (55%) of aldehyde **7**: IR (film) 2720, 1690, 1640, 1600, 1500 cm^{-1}; ^1H NMR δ 1.80 (3 H, d, J = 7 Hz), 3.90 (6 H, s), 4.95 (2 H, s), 6.75 (2 H, s), 6.85 (1 H, m), 7.3 (5 H, m), 9.5 (1 H, s); ^{13}C NMR δ 15.7, 55.6, 60.4, 74.6, 107.3, 119.5, 124.8, 127.3, 127.6, 127.8, 137.3, 142.0, 142.3, 150.2, 150.9, 153.4, 192.8, MS, m/e (relative intensity) 312 (9) (M^+), 221 (22), 193 (26), 178 (7), 165 (4), 162 (5), 91 (100). Anal. Calcd for $C_{19}H_{20}O_4$: m/e 312.1350. Found: m/e 312.1356.

1-(1-Ethylidine-2-butenyl)-3,4-dimethoxy-2-(phenylmethoxy)benzene (14). To a stirred suspension of ethyltriphenylphosphonium (13.09 g, 35.26 mmol) in dry THF (65 mL) under a dry nitrogen atmosphere at room temperature was added 2.22 M *n*-butyllithium (15.9 mL, 35.3 mmol) over 2 min. The mixture was stirred for 1.5 h at room temperature, and the orange-red solution was cooled to −78 °C. Aldehyde **7** (10.0 g, 32.05 mmol) in dry THF (45 mL) was added dropwise over 10 min. After the mixture was stirred for 5 min at −78 °C, 2.22 M *n*-butyllithium (14.4 mL, 32.0 mmol) was added over 5 min. After being warmed to −22 °C and stirred for 10 min, to this solution was added potassium *tert*-butoxide (5.40 g, 48.1 mmol) and dry *tert*-butyl alcohol (48 mL) in one portion. The mixture was warmed to room temperature and stirred for 2 h. The reaction mixture was poured into water (125 mL), neutralized with 5% HCl, and extracted with ether (3 × 100 mL). The combined organic layers were washed with brine (3 × 75 mL), dried (Na_2SO_4), and evaporated in vacuo. The residue was chromatographed on silica gel (200 g) in benzene/ether (99/1), affording 7.80 g (75%) of a mixture of dienes **14**: ^1H NMR δ 1.5 (6 H, m), 3.8 (6 H, s), 4.9 (2 H, m), 5.0–6.4 (3 H, m), 6.7 (2 H, m), 7.3 (5 H, m).

(5α,8α,8aβ)-2-(4-Chlorophenyl)-7-[3,4-dimethoxy-2-(phenylmethoxy)phenyl]-8,8a-dihydro-5,8-dimethylimidazo[1,5-*a*]pyridine-1,3-(2*H*,5*H*)-dione (16a) and (5α,8α,8aβ)-2-(4-Chlorophenyl)-6-[3,4-dimethoxy-2-(phenylmethoxy)phenyl]-8,8a-dihydro-5,8-dimethylimidazo-[1,5-*a*]pyridine-1,3(2*H*,5*H*)-dione (16b). A stirred mixture of dienes **14** (7.8 g, 24.1 mmol), 3-(*p*-chlorophenyl)-5-methoxyhydantoin (**15**, 6.37 g, 26.5 mmol), and xylene (100 mL) was refluxed for 3 days. Additional hydantoin (1.2 g, 4.8 mmol) was added every 12 h during this period. The mixture was evaporated to dryness in vacuo, and the residue was chromatographed on silica gel (200 g) in chloroform to yield 5.08 g of recovered dienes **14** and 5.03 g (39%) of a mixture of Diels–alder adducts **16a/16b**. The recovered diene mixture was recycled once and chromatographed as above to afford 2.24 g of recovered dienes **14** and an additional 2.17 g of adduct mixture. The combined yield of adducts after one recycle was 7.20 g (56%): IR ($CHCl_3$) 1770, 1720, 1600, 1500 cm^{-1}.

Hydrolysis of Adducts 16a and 16b. A stirred mixture of 5 g (9.4 mmol) of the mixture of Diels–Alder adducts **16a** and **16b** and 10 g (30 mmol) of $Ba(OH)_2{\cdot}8H_2O$ in 70 mL of dioxane and 50 mL of water was refluxed for 24 h. The reaction mixture was cooled to room temperature and diluted with 20 mL of water, and CO_2 was bubbled through the reaction mixture until no further precipitate formed. The mixture was filtered, and the precipitate was washed with water (20 mL). The precipitate was discarded, and the aqueous filtrate was extracted with ether (2 × 50 mL) to remove neutral impurities. The aqueous layer was evaporated, and the residue was dried in vacuo overnight to give 3.7 g (~100%) of a mixture of white powdery amino acids **17/19**, which was used directly for the next step.

Esterification of Acids 17 and 19. The mixture of amino acids **17/19** (3.7 g, 9.4 mmol) was suspended in anhydrous MeOH (160 mL), and the mixture was cooled to −5 °C. Thionyl chloride (3.6 mL) was added dropwise over 10 min, affording a clear, pale yellow solution. The cooling bath was removed, and the reaction mixture was gently refluxed for 12 h. The reaction mixture was evaporated to dryness in vacuo at room temperature, cooled to 0 °C, and basified with cold, saturated K_2CO_3 solution. The mixture was extracted with ethyl acetate (3 × 100 mL), and the combined organic layer was washed with brine, dried over $MgSO_4$, and evaporated in vacuo to yield 3.3 g of a brown gum partially purified by filtration through a pad of silica gel ($CHCl_3$) to give 2.18 g (57%) of a mixture of esters **18/20**, which was used immediately for the next step.

Methyl 4-[3,4-Dimethoxy-2-(phenylmethoxy)phenyl]-3,6-dimethyl-2-pyridinecarboxylate (21). A mixture of amino esters **18/20** (2.18 g, 5.3 mmol) and 5% Pd/C (1 g) in 80 mL of dry toluene was refluxed for 15 h. The reaction mixture was filtered through a celite pad, and the pad was washed with $CHCl_3$. The filtrate was evaporated to dryness in vacuo, and the residue was purified by column chromatography on silica gel using ethyl acetate/hexane (2/3), affording pure pyridine **21** (0.71 g, 33%): mp 111–112 °C (recrystallized from ethyl acetate/hexane); IR ($CHCl_3$) 1725 cm^{-1}; ^1H NMR δ 2.19 (3 H, s), 2.51 (3 H, s), 3.89 (3 H, s), 3.93 (3 H, s), 3.96 (3 H, s), 4.82 (2 H, s), 6.74 (2 H, s), 6.78–7.38 (6 H, m); MS m/e (relative intensity) 407 (100) (M^+), 392 (17), 348 (10), 256 (47). Anal. Calcd for $C_{24}H_{25}NO_5$: m/e 407.1712. Found: m/e 407.1722.

Isomeric pyridine **22** was isolated in 3–6% yield as an oil: IR ($CHCl_3$) 3000, 2970, 1730, 1600 cm^{-1}; ^1H NMR δ 2.40 (3 H, s), 2.47 (3 H, s), 3.93 (3 H, s), 3.95 (3 H, s), 4.00 (3 H, s), 4.82 (2 H, s), 6.75, 6.80 (2 H, AB_q, J = 8.45 Hz), 6.89–7.22 (6 H, m); MS, m/e (relative intensity) 407 (15) (M^+), 392 (10), 317 (14), 284 (12), 256 (11), 242 (10), 181 (19), 167 (15), 91 (100).

Methyl 4-[3,4-Dimethoxy-2-(phenylmethoxy)phenyl]-3,6-dimethyl-2-pyridinecarboxylate 1-Oxide (23). A mixture of pyridine **21** (1.0 g, 2.4 mmol) and 85% *m*-chloroperbenzoic acid (666 mg, 3.6 mmol) in CH_2Cl_2 (20 mL) was stirred at room temperature overnight. The reaction mixture was diluted with $CHCl_3$, and the organic layer was washed successively with $NaHSO_3$, $NaHCO_3$, water, and brine, dried with $MgSO_4$, and evaporated in vacuo to give 1.04 g (100%) of pyridine *N*-oxide **23**. This compound was used in the next step without further purification: IR ($CHCl_3$) 1740 cm^{-1}; ^1H NMR δ 1.99 (3 H, s), 2.41 (3 H, s), 3.95 (3 H, s), 3.97 (3 H, s), 4.05 (3 H, s), 4.92 (2 H, s), 6.81 (2 H, s), 6.85–7.60 (6 H, m).

Methyl 6-[(Acetyloxy)methyl]-4-[3,4-dimethoxy-2-(phenylmethoxy)-phenyl]-3-methyl-2-pyridinecarboxylate (24). A solution of *N*-oxide **23** (1.04 g, 2.4 mmol) in acetic anhydride (25 mL) was heated at 120 °C for 2 h. The solution was evaporated to dryness under reduced pressure to give crude acetate **24** which was purified by column chromatography on silica gel using ethyl acetate/hexane (2/3) to give 1.03 g (93%) of crystalline acetate **24**. An analytical sample prepared by recrystallization from MeOH had a melting point of 89–90 °C: IR ($CHCl_3$) 1730 cm^{-1}; ^1H NMR δ 2.13 (3 H, s), 2.27 (3 H, s), 3.98 (3 H, s), 4.01 (3 H, s), 4.03 (3 H, s), 4.94 (2 H, s), 5.28 (2 H, s), 6.88 (2 H, s), 6.95–7.42 (6 H, m); MS m/e (relative intensity) 465 (100) (M^+), 449 (12), 433 (9), 405 (9), 373 (11), 314 (34), 300 (19), 284 (12), 256 (23). Anal. Calcd for $C_{26}H_{27}NO_7$: m/e 465.1747. Found: m/e 465.1767.

Methyl 4-[3,4-Dimethoxy-2-(phenylmethoxy)phenyl]-6-(hydroxymethyl)-3-methyl-2-pyridinecarboxylate (25). A mixture of 578 mg (1.24 mmol) of acetate **24** and 400 mg of anhydrous K_2CO_3 in 600 mL of dry methanol was stirred at room temperature for 2 h. The solution was diluted with 1.0 L of methylene chloride, followed by addition of 1.0 L of water at 0 °C. The solution was then partitioned between methylene chloride and water, and the aqueous layer was reextracted with methylene chloride. The combined organic extract was dried over anhydrous $MgSO_4$ and evaporated to dryness in vacuo to give 524 mg (100%) of alcohol **25**. An analytical sample obtained by recrystallization from CH_2Cl_2/hexane had a melting point of 128–128.5 °C: IR (film) 3125–3625, 1730 cm^{-1}; ^1H NMR δ 2.26 (3 H, s), 3.97 (3 H, s), 3.99 (3 H, s), 4.03 (3 H, s), 4.75 (2 H, s), 4.93 (2 H, s), 6.87 (2 H, s), 6.92–7.42 (6 H, m); MS, m/e (relative intensity) 423 (100) (M^+), 407 (17).

Anal. Calcd for $C_{24}H_{25}NO_6$: C, 68.07; H, 5.95. Found: C, 67.99: H, 6.00.

Methyl 6-(Chloromethyl)-4-[3,4-dimethoxy-2-(phenylmethoxy)-phenyl]-3-methyl-2-pyridinecarboxylate (26). To a solution of 480 mg (1.13 mmol) of alcohol **25** in 47 mL of dry benzene was added 1.53 g of thionyl chloride at 0 °C. The resulting mixture was slowly warmed to room temperature and stirred for 1.5 h. The reaction mixture was evaporated to dryness at 37 °C under reduced pressure to afford 498 mg (100%) of chloride **26**. An analytical sample obtained by recrystallization from CH_2Cl_2/hexane had a melting point of 121–122 °C: IR (film) 1730 cm^{-1}; ^1H NMR δ 2.20 (3 H, s), 3.88 (3 H, s), 3.91 (3 H, s), 3.95 (3 H, s), 4.62 (2 H, s), 4.83 (2 H, s), 6.76 (2 H, s), 6.85–7.33 (6 H, m); MS, m/e (relative intensity) 441 (100) (M^+). Anal. Calcd for $C_{24}H_{24}NO_5Cl$: m/e 441.1339. Found: m/e 441.1341.

Methyl 4-[3,4-Dimethoxy-2-(phenylmethoxy)phenyl]-5-formyl-3,6-dimethyl-2-pyridinecarboxylate (29). A solution of 222 mg (0.501 mmol) of chloride **26** and 0.14 mL of *N*-(cyanomethyl)pyrrolidine[23] in 1.0 mL of dimethyl sulfoxide was heated at 45 °C for 5 days. The solution was evaporated to dryness in vacuo to give quaternary salt **27**, which was used in the next step without further purification.

To a solution of salt **27** in 33.0 mL of Me_2SO/THF (1/2.3) was added 225 mg of potassium *tert*-butoxide at −12 °C under oxygen-free argon.[12c] The solution was stirred for 10 min and quenched with saturated NH_4Cl

solution. The mixture was partitioned between ethyl acetate and water, and the organic fraction was washed with brine, dried over anhydrous Na_2SO_4, and evaporated to dryness in vacuo. A mixture of the residue and 40 mg of oxalic acid in 20 mL of THF/H_2O (2/1) was refluxed for 1 h under a nitrogen atmosphere. The mixture was evaporated to dryness in vacuo, and the residue was purified by preparative TLC in ethyl acetate/hexane (45/55) to give 77 mg (35% from acetate **24**) of aldehyde **29** as a white solid: mp 109–110 °C; IR (film) 1735, 1695 cm^{-1}; ^1H NMR δ 2.12 (3 H, s), 2.78 (3 H, s), 3.98 (3 H, s), 4.00 (3 H, s), 4.06 (3 H, s), 5.04 (2 H, s), 6.80 (2 H, ABq), 6.87–7.42 (5 H, m), 9.52 (1 H, s); MS, *m/e* (relative intensity) 435 (100) (M^+), 406 (54); Anal. Calcd for $C_{25}H_{25}NO_6$: *m/e* 435.1718. Found: *m/e* 435.1700.

Methyl 4-[3,4-Dimethoxy-2-(phenylmethoxy)phenyl]-5-formyl-3,6-dimethyl-2-pyridinecarboxylate 1-Oxide (30). To a stirred mixture of 342 mg (0.786 mmol) of aldehyde **29** in 250 mL of methylene chloride and 28.7 g of Na_2HPO_4 was added dropwise 7.7 mL of pertrifluoroacetic acid. The mixture was partitioned between methylene chloride and water, and the aqueous layer was extracted with methylene chloride. The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness at room temperature to afford 354.5 mg (100%) of crude *N*-oxide **30**. An analytical sample prepared by recrystallization from CH_2Cl_2/hexane had a melting point of 182.8–183.8 °C: IR (film) 1748, 1705 cm^{-1}; ^1H NMR δ 1.94 (3 H, s), 2.66 (3 H, s), 3.97 (3 H, s), 4.00 (3 H, s), 4.08 (3 H, s), 5.07 (2 H, s), 6.63 and 6.83 (2 H, AB_q, J = 9.0 Hz), 6.88–7.43 (5 H, m), 8.92 (1 H, s); MS, *m/e* (relative intensity) 451 (100) (M^+), 422 (47), 344 (57), 326 (36). Anal. Calcd for $C_{25}H_{25}NO_7$: *m/e* 451.1687. Found: *m/e* 451.1659.

2-Methyl Hydrogen 4-[3,4-Dimethoxy-2-(phenylmethoxy)phenyl]-3,6-dimethyl-2,5-pyridinedicarboxylate 1-Oxide (31). To a stirred solution of aldehyde **30** (512 mg, 1.14 mmol) in 420 mL of acetone/H_2O (2/1) was added dropwise over 3 h a solution of $KMnO_4$ (347 mg, 2.2 mmol) in 78 mL of acetone/H_2O (2/1). A small amount of a saturated solution of sodium bisulfite in water was added to the solution in order to destroy excess $KMnO_4$. The mixture was partitioned between chloroform and water, and the aqueous layer was extracted with chloroform three times. The combined organic layer was evaporated to dryness under reduced pressure to give 536 mg (100%) of acid **31** as a white crystalline solid, which was recrystallized from CH_2Cl_2/hexane: mp 184.5–185.5 °C; IR (film) 1750 cm^{-1}; ^1H NMR δ 1.98 (3 H, s), 2.42 (3 H, s), 3.89 (6 H, s), 3.98 (3 H, s), 4.78 and 5.08 (2 H, AB_q, J = 12 Hz), 6.83 (2 H, AB_q), 6.98–7.50(5 H, m); MS, *m/e* (relative intensity) 467 (100) (M^+), 451 (64), 422 (32), 406 (27), 342 (64), 284 (30), 256 (64). Anal. Calcd for $C_{25}H_{25}NO_8$: *m/e* 467.1574. Found: *m/e* 467.1577.

Methyl 5-Amino-4-[3,4-dimethoxy-2-(phenylmethoxy)phenyl]-3,6-dimethyl-2-pyridinecarboxylate 1-Oxide (32). A solution of 568 mg (1.22 mmol) of acid **31**, 2.5 mL of triethylamine, and 2.5 mL of diphenylphosphoryl azide in 92 mL of dry benzene was refluxed under a nitrogen atmosphere for 1 h.[25] To the solution was added 18.0 mL of water, and the mixture was refluxed for an additional 30 min. The mixture was evaporated under reduced pressure, and the residue was purified by preparative TLC on silica gel using 10% methanol in chloroform to afford 441 mg (83%) of amine **32**. An analytical sample obtained by recrystallization from ethyl ether/hexane had a melting point of 56–56.5 °C: IR (film) 3550–3125, 1740 cm^{-1}; ^1H NMR δ 1.86 (3 H, s), 2.39 (3 H, s), 3.97 (6 H, s), 4.01 (3 H, s), 4.99 (2 H, s), 6.84 (2 H, s), 6.94–7.50 (5 H, m); MS, *m/e* (relative intensity) 438 (100) (M^+), 422 (33), 299 (26), 271 (34). Anal. Calcd for $C_{24}H_{26}N_2O_6$: *m/e* 438.1801. Found: *m/e* 438.1796.

Methyl 5-Amino-4-[3,4-dimethoxy-2-(phenylmethoxy)phenyl]-6-(hydroxymethyl)-3-methyl-2-pyridinecarboxylate (33). A stirred solution of 388 mg (0.89 mmol) of *N*-oxide **32** in 14.0 mL of acetic anhydride was heated at 120–125 °C for 2 h, and the solution was evaporated to dryness in vacuo. The residue and 740 mg of anhydrous K_2CO_3 in 53 mL of dry methanol was stirred at room temperature for 21 h and diluted with 200 mL of methylene chloride. The solution was partitioned between methylene chloride and water, and the aqueous layer was extracted with methylene chloride two times. The combined organic layer was dried over anhydrous $MgSO_4$ and evaporated to dryness in vacuo to give 346 mg (89%) of alcohol **33**, which was recrystallized from CH_2Cl_2/hexane: mp 124–125 °C; IR (film) 3700–3100, 1717 cm^{-1}; ^1H NMR δ 2.22 (3 H, s), 3.97 (9 H, s), 4.73 (2 H, s), 4.94 (2 H, s), 6.85 (2 H, s), 6.93–7.40 (5 H, m); MS, *m/e* (relative intensity) 438 (100) (M^+), 407 (21), 315 (45); Anal. Calcd for $C_{24}H_{26}N_2O_6$: *m/e* 438.1829. Found: *m/e* 438.1810.

Methyl 5-Amino-4-[3,4-dimethoxy-2-(phenylmethoxy)phenyl]-6-formyl-3-methyl-2-pyridinecarboxylate (34). To a stirred solution of 473 mg (1.08 mmol) of alcohol **33** in 160 mL of chloroform was added 3 g of activated manganese dioxide in portions over 2 h. The suspension was diluted with 350 mL of methylene chloride and filtered through a pad of celite. The filtrate was evaporated to dryness in vacuo to yield 416 mg (88%) of aldehyde **34**, which was of sufficient purity to be used in the next step. An analytical sample prepared by recrystallization from ethyl ether/hexane had a melting point of 110–111 °C: IR (film) 3475, 3350, 1720, 1670 cm^{-1}; ^1H NMR δ 2.24 (3 H, s), 3.98 (6 H, s), 4.02 (3 H, s), 4.99 (2 H, s), 6.87 (2 H, AB_q), 6.94–7.40 (5 H, m), 10.16 (1 H, s); MS, *m/e* (relative intensity) 436 (100) (M^+), 407 (11), 405 (8), 376 (36), 375 (15), 348 (12). Anal. Calcd for $C_{24}H_{24}N_2O_6$: *m/e* 436.1654. Found: *m/e* 436.1644.

Methyl 5-Amino-[3,4-dimethoxy-2-(phenylmethoxy)phenyl]-6-[2-(dimethoxyphosphinyl)-1-hydroxyethyl]-3-methyl-2-pyridinecarboxylate (35). To a stirred solution of 92.6 mg (0.724 mmol) of dimethyl methylphosphonate in 1.0 mL of THF was added 0.4 mL (0.64 mmol) of 1.6 M *n*-butyllithium in hexane at −78 °C under an argon atmosphere. The resulting solution was stirred at −75 °C for 8 min, and 0.90 mL of dry HMPA was added. The mixture was stirred at −75 °C for 2 min, and a solution of 54.6 mg (0.125 mmol) of aldehyde **34** in 1.0 mL of dry THF was added dropwise at −75 °C. The solution was warmed slowly with stirring to 15 °C over a period of 40 min. Water was added to quench the reaction, and the mixture was partitioned between methylene chloride and water. The aqueous layer was reextracted with methylene chloride. The combined organic layer was evaporated under reduced pressure to yield an oil which was purified by preparative TLC on silica gel in methylene chloride/methanol (94/6) to give 46.9 mg (67%) of β-hydroxyphosphonate **35**: IR (film) 3130–3700 (br), 1715 cm^{-1}; ^1H NMR δ 2.19 (3 H, s), 2.15–2.43 (2 H, m), 3.74–3.84 (6 H, m), 3.91 (3 H, s), 3.93 (3 H,s), 3.94 (3 H, s), 4.89 (2 H, s), 5.18 (1 H, m), 6.79 and 6.80 (2 H, AB_q, J = 8.55 Hz), 6.94–7.01 (2 H, m), 7.17–7.21 (3 H, m); MS, *m/e* (relative intensity) 560 (14) (M^+), 453 (9), 437 (11), 434 (19), 433 (11), 343 (21), 283 (12), 91 (100). Anal. Calcd for $C_{27}H_{33}N_2O_9P$: *m/e* 560.1865. Found: *m/e* 560.1894.

Methyl 5-Amino-4-[3,4-dimethoxy-2-(phenylmethoxy)phenyl]-6-[(dimethoxyphosphinyl)acetyl]-3-methyl-2-pyridinecarboxylate (36). To a stirred solution of 332.0 mg (0.59 mmol) of β-hydroxyphosphonate **35** in 200 mL of chloroform was added 6.3 g of activated manganese dioxide in small portions over 1 h. The mixture was diluted with 200 mL of methylene chloride and filtered through a pad of celite. The filtrate was evaporated to dryness in vacuo to give 260.5 mg (79%) of crude β-ketophosphonate **36**, which was used in the next step without further purification. A purified sample prepared by preparative TLC on silica gel using 7% methanol in methylene chloride had the following spectral data: IR (film) 3480, 3349, 1720, 1660 cm^{-1}; ^1H NMR δ 2.17 (3 H, s), 3.81 (6 H, d, J = 11.2 Hz), 3.93 (3 H, s), 3.94 (3 H, s), 3.95 (3 H, s), 4.02–4.21 (2 H, m), 4.92 (2 H, AB_q), 6.74 and 6.84 (2 H, AB_q, J = 8.46 Hz), 6.96–7.16 (2 H, m), 7.19–7.24 (3 H, m); MS, *m/e* (relative intensity) 558 (100) (M^+), 451 (30), 498 (27). Anal. Calcd for $C_{27}H_{31}N_2O_9P$: *m/e* 558.1797. Found: *m/e* 558.1782.

Methyl 5-Amino-4-[3,4-dimethoxy-2-(phenylmethoxy)phenyl]-6-[3-[3-methoxy-6-nitro-2-[(phenylsulfonyl)oxy]phenyl]-1-oxo-2-propenyl]-3-methyl-2-pyridinecarboxylate (37). To a stirred suspension of 136 mg of KH (24.9% in mineral oil) in 15.0 mL of dry benzene was added dropwise a solution of 137.0 mg (0.246 mmol) of β-ketophosphonate **36** in 6.5 mL of dry benzene. The resulting mixture was stirred under a nitrogen atmosphere at room temperature for 15 min. A solution of 205 mg of nitroaldehyde **3**[29] in 15.0 mL of dry benzene was added dropwise to the mixture, and the resulting mixture was stirred at room temperature for 75 min. Distilled water was added, the mixture was partitioned between benzene and water, and the aqueous layer was extracted with methylene chloride. The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo, and the residue was purified by preparative TLC on silica gel in methanol/chloroform (1/99) to yield 151 mg (80%) of nitrochalcone **37**, which was crystallized from CH_2Cl_2/hexane: mp 168–169 °C; IR (film) 3490, 3335, 1720, 1659 cm^{-1}; ^1H NMR δ 2.23 (3 H, s), 3.96 (9 H, s), 3.97 (3 H, s), 4.97 (2 H, s), 6.78 and 6.87 (2 H, AB_q, J = 8.64 Hz), 7.04–8.15 (14 H, m); MS, *m/e* (relative intensity) 769 (56) (M^+), 752 (100), 737 (26), 628 (29), 610 (35), 597 (51), 447 (36), 433 (49), 408 (50), 371 (63), 285 (40).

Methyl 5-Amino-4-[3,4-dimethoxy-2-(phenylmethoxy)phenyl]-6-[6-methoxy-5-[(phenylsulfonyl)oxy]-2-quinolinyl]-3-methyl-2-pyridinecarboxylate (38). A mixture of 180 mg (0.234 mmol) of nitrochalcone **37**, 120 mL of methanol, 60 mL of water, and 750 mg of sodium hydrosulfite was refluxed under a nitrogen atmosphere for 3 h. The reaction mixture was cooled to room temperature and partitioned between methylene chloride and water, and the aqueous layer was reextracted twice with methylene chloride. The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo. The residue was purified by preparative TLC on silica gel in 1% MeOH/CH_2Cl_2 to give 101 mg (60%) of tetracyclic quinoline **38**. An analytical sample prepared by recrystallization from CH_2Cl_2/hexane had a melting point of 185.5–186.5 °C: IR (film) 3470, 3255, 1715 cm^{-1}; ^1H NMR δ 2.26 (3 H, s), 3.74 (3 H, s), 3.96 (3 H, s), 3.97 (3 H, s), 4.00 (3 H, s), 4.92

(2 H, s), 6.87 (2 H, s), 7.02–8.85 (14 H, m); MS, *m/e* (relative intensity) 721 (89) (M^+), 614 (49), 581 (2), 580 (50), 563 (34), 520 (43), 504 (31), 474 (100), 414 (74), 281 (37), 207 (63). Anal. Calcd for $C_{39}H_{35}N_3O_9S$: *m/e* 721.2092. Found: *m/e* 721.2094.

Methyl 5-Amino-4-[3,4-dimethoxy-2-(phenylmethoxy)phenyl]-6-(5-hydroxy-6-methoxy-2-quinolinyl)-3-methyl-2-pyridinecarboxylate (39). To a stirred solution of 21.2 mg (0.0294 mmol) of sulfonate **38** in 74 mL of dry methanol at 40 °C was added 300 mg of sodium methoxide in portions over 3 h. The reaction mixture was cooled to room temperature and diluted with 200 mL of methylene chloride followed by addition of 200 mL of water at 0 °C. The mixture was partitioned between water and methylene chloride, and the aqueous layer was reextracted with methylene chloride. The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo to give 17.1 mg (100%) of phenol **39**. This material was used in the next step without further purification.

Methyl 5-Amino-6-(5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolinyl-4-[3,4-dimethoxy-2-(phenylmethoxy)phenyl]-3-methyl-2-pyridinecarboxylate (40). A solution of phenol **39** (17.1 mg, 0.0294 mmol) in methanol (75 mL) was added to a stirred solution of Fremy's salt (2.2 g in 74 mL of 0.05 M KH_2PO_4).[31] The solution was stirred at room temperature for 10 min and diluted with methylene chloride. The reaction mixture was partitioned between water and methylene chloride. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo to give 17.5 mg (100%) of quinoline quinone **40**, which was recrystallized from CH_2Cl_2/hexane: mp 187–188 °C: IR (film) 3440, 3250, 1710, 1680, 1660 cm^{-1}; ^1H NMR δ 2.24 (3 H, s), 3.95 (6 H, s), 3.97 (3 H, s), 4.40 (3 H, s), 4.89 and 4.97 (2 H, AB_q, J = 11.0 Hz), 6.30 (1 H, s), 6.83 and 6.88 (2 H, AB_q, J = 8.5 Hz), 6.98–7.14 (5 H, m), 8.51 and 9.04 (2 H, AB_q, J = 8.64 Hz): MS, *m/e* (relative intensity) 595 (100) (M^+), 535 (20), 207 (96). Anal. Calcd for $C_{33}H_{29}N_3O_8$: *m/e* 595.1951. Found: *m/e* 595.1998.

Methyl 5-Amino-6-(7-iodo-5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolinyl)-4-[3,4-dimethoxy-2-(phenylmethoxy)phenyl]-3-methyl-2-pyridinecarboxylate (41). To a suspension of 1.0 g (15.4 mmol) of NaN_3 in 36 mL of CH_3CN in MeOH/ice bath (−10 °C) was added 714 mg (4.39 mmol) of ICl, and the resulting mixture was swirled at −10 °C for 10 min. The suspension was filtered, and the yellow filtrate was used immediately. To a solution of 84 mg (0.141 mmol) of quinoline quinone **40** in 72 mL of acetonitrile in a MeOH/ice bath was added dropwise 22.8 mL of the above IN_3 solution. The resulting solution was stirred at room temperature for 3 h and diluted with methylene chloride. The organic solution was washed with water and 5% sodium thiosulfate solution, dried over anhydrous Na_2SO_4, and evaporated to dryness in vacuo. The residue was purified by preparative TLC on silica gel using 1% methanol in methylene chloride as eluant, giving 92.8 mg (91%) of iodoquinone **41**. An analytical sample obtained by recrystallization from CH_2Cl_2/hexane had a melting point of 218.5–219.5 °C dec: IR (film) 3450, 3260, 1712, 1670 cm^{-1}; ^1H NMR δ 2.23 (3 H, s), 3.95 (3 H, s), 3.97 (3 H, s), 3.99 (3 H, s), 4.38 (3 H, s), 4.88 and 4.97 (2 H, AB_q, J = 11.0 Hz), 6.82 and 6.88 (2 H, AB_q, J = 9.0 Hz), 6.99–7.12 (5 H, m), 8.45 and 9.06 (2 H, AB_q, J = 8.0 Hz); MS, *m/e* (relative intensity) 721 (14) (M^+), 720 (15), 594 (14), 207 (12), 142 (66), 128 (47), 127 (45), 91 (100). Anal. Calcd for $C_{33}H_{28}N_3O_8I$: *m/e* 721.0923. Found: *m/e* 721.0984.

Methyl 5-Amino-6-(7-azido-5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolinyl)-4-[3,4-dimethoxy-2-(phenylmethoxy)phenyl]-3-methyl-2-pyridinecarboxylate (42). A suspension of 498 mg of NaN_3 in 91 mL of dry THF was stirred at room temperature for 20 min. The suspension was allowed to stand at room temperature for 5 h and was filtered. To 80.7 mg (0.112 mmol) of iodoquinone **41** was added 18 mL of the above NaN_3 solution, and the mixture was stirred at room temperature for 10 min with protection from light. The reaction mixture was diluted with methylene chloride and partitioned between methylene chloride and water. The aqueous layer was reextracted with methylene chloride several times. The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo. The residue was purified by preparative TLC on silica gel using 1% methanol in methylene chloride to give 41 mg (58%) of azidoquinone **42**. An analytical sample prepared by recrystallization from CH_2Cl_2/hexane and a melting point of 137.4–139.1 °C dec; IR (film) 3450, 3270, 2120, 1714, 1660 cm^{-1}; ^1H NMR δ 2.24 (3 H, s), 3.95 (3 H, s), 3.96 (3 H, s), 3.99 (3 H, s), 4.27 (3 H, s), 4.89 and 4.97 (2 H, AB_q, J = 11.5 Hz), 6.82 and 6.88 (2 H, AB_q, J = 8.5 Hz), 7.02–7.13 (5 H, m), 8.44 and 9.05 (2 H, AB_q, J = 8.6 Hz); MS (CI), *m/e* 637 ($M^+ + 1$).

Methyl 5-Amino-6-(7-amino-5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolinyl)-4-[3,4-dimethoxy-2-(phenylmethoxy)phenyl]-3-methyl-2-pyridinecarboxylate (43). A. A mixture of 8.67 mg (0.01667 mmol) of streptonigrin methyl ester (**44**), 5.0 mg (0.0229 mmol) of benzyl iodide, 35.0 mg of potassium carbonate, and 2.0 mL of acetone was stirred under a nitrogen atmosphere at room temperature for 21 h while the reaction mixture was protected from light. Ethyl acetate (5 mL) was added, and the mixture was filtered through a glass wool pad. The filtrate was concentrated in vacuo and was purified by preparative TLC on a pH 7 buffered silica gel plate. Ethyl acetate/hexane (1/1) was used to develop half of the 20 × 20 cm plate, and the plate was developed twice in ethyl acetate/hexane (1/2) to give 6.59 mg (65%) of benzyl ether **43**. A sample was recrystallized from methylene chloride/hexane: mp 170.5–171.5 °C; IR (film) 3450, 3350, 3250, 1710 cm^{-1}; ^1H NMR δ 2.24 (3 H, s), 3.95 (3 H, s), 3.96 (3 H, s), 3.99 (3 H, s), 4.09 (3 H, s), 4.89 and 4.96 (2 H, AB_q, J = 11.2 Hz), 5.10 (br s, exchangeable) 6.83 and 6.88 (2 H, AB_q, J = 8.55 Hz), 6.99–7.13 (5 H, m), 8.44 and 9.00 (2 H, AB_q, J = 8.46 Hz); MS, *m/e* (relative intensity) 610 (100) (M^+). Anal. Calcd for $C_{33}H_{30}N_4O_8$: *m/e* 610.2063. Found: *m/e* 610.2058.

B. A mixture of 36.1 mg (0.0568 mmol) of azidoquionone **42**, 120 mg of sodium hydrosulfite, 30 mL of methanol, and 15 mL of water was refluxed under a nitrogen atmosphere for 5 h with protection from light. The reaction mixture was cooled to room temperature and partitioned between water and methylene chloride. The aqueous layer was reextracted with methylene chloride. The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo. The residue was purified by preparative TLC on silica gel using 1.5% methanol in methylene chloride to give 17.2 mg (50%) of aminoquinone **43** along with 2.2 mg (6%) of recovered starting azide **42**. This product was identical with a sample prepared in part A from natural streptonigrin (**1**).

Methyl 5-Amino-6-(7-amino-5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolinyl)-4-(2-hydroxy-3,4-dimethoxyphenyl)-3-methyl-2-pyridinecarboxylate (Streptonigrin Methyl Ester) (44). A. A mixture of 50.3 mg (0.0995 mmol) of natural streptonigrin (**1**), 0.3 mL of BF_3·Et_2O, and 70 mL of freshly distilled methanol was refluxed under a nitrogen atmosphere for 5 h with protection from light.[32] The mixture was cooled to room temperature, 100 mL of water was added, and methanol was evaporated under reduced pressure. The solution was extracted with methylene chloride twice, and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated to dryness in vacuo. The residue was purified by preparative TLC on silica gel using 15% methanol in chloroform to give 35.8 mg (69%) of streptonigrin methyl ester (**44**): IR ($CHCl_3$) 3510, 3470, 3400, 3250, 1710, 1640, 1620 cm^{-1}; ^1H NMR δ 2.33 (3 H, s), 3.96 (3 H, s), 3.98 (3 H, s), 3.99 (3 H, s), 4.09 (3 H, s), 6.67 and 6.82 (2 H, AB_q, J = 8.65 Hz), 8.43 and 9.00 (2 H, AB_q, J = 8.46 Hz); MS, *m/e* (relative intensity) 520 (100) (M^+), 403 (10), 487 (17), 473 (15), 471 (10), 459 (16), 445 (24), 443 (31), 431 (25); UV λ_{max} 246, 375 nm (ϵ 42 600, 17 600).

B. To a stirred solution of 1.0 mg (0.001 64 mmol) of synthetic benzyl ether **43** in 6.0 mL of chloroform was added 100 mg of anhydrous aluminum chloride in small portions over 2 h. The mixture was diluted with 30 mL of methylene chloride and partitioned between methylene chloride and water, and the aqueous layer was thoroughly reextracted with methylene chloride. The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo. The residue was purified by preparative TLC on pH 7 buffered silica gel[33] using 4% methanol in methylene chloride to give 0.682 mg (80%) of synthetic streptonigrin methyl ester (**44**), which was identical with authentic material prepared in part A from natural streptonigrin (**1**).

5-Amino-6-(7-amino-5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolinyl-4-(2-hydroxy-3,4-dimethoxyphenyl)-3-methyl-2-pyridinecarboxylic Acid (Streptonigrin 1). A mixture of 2.6 mg (0.005 mmol) of streptonigrin methyl ester (**44**), and 63 mg of anhydrous potassium carbonate in 4 mL of methanol/H_2O (2/1) was stirred under an argon atmosphere at room temperature for 45 h. The reaction mixture was concentrated to half of the volume in vacuo at room temperature and was neutralized with 3 N HCl. The mixture was extracted with $CHCl_3$ (3 × 15 mL), and the organic phase was dried (Na_2SO_4) and evaporated. The residue was purified by preparative TLC on pH 7 buffered silica gel[33] (5% MeOH/$CHCl_3$) to give 2.0 mg (79%) of streptonigrin (**1**) identical with authentic natural material.

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Registry No. 1, 3930-19-6; **3**, 2426-60-0; **7**, 74614-86-1; **10**, 19283-70-6; **11**, 6527-13-5; **12**, 74614-84-9; **13**, 74614-85-0; **14** isomer 1,

(33) Buffered preparative TLC plates were prepared from a slurry of 177 g of silica gel 60 (Merck) and 400 mL of 0.05 M phosphate buffer (pH 7) solution (Fisher).

79953-13-2; **14** isomer 2, 79953-14-3; **15**, 30454-96-7; **16a**, 74614-90-7; **16b**, 74614-91-8; **17**, 79953-15-4; **18**, 74614-94-1; **19**, 79953-16-5; **20**, 79953-17-6; **21**, 74614-75-2; **22**, 79953-18-7; **23**, 74614-96-3; **24**, 74614-97-4; **25**, 74614-98-5; **26**, 74614-99-6; **27**, 79953-19-8; **29**, 74615-01-3; **30**, 74615-02-4; **31**, 74615-03-5; **32**, 74615-04-6; **33**, 74615-05-7; **34**, 74615-06-8; **35**, 79953-20-1; **36**, 79953-21-2; **37**, 74615-09-1; **38**, 74615-10-4; **39**, 74615-11-5; **40**, 74615-12-6; **41**, 79953-22-3; **42**, 79953-23-4; **43**, 74615-13-7; **44**, 3398-48-9; vinyl bromide, 593-60-2; ethyltriphenylphosphonium bromide, 1530-32-1; *N*-(cyanomethyl)pyrrolidine, 29134-29-0; dimethyl methlphosphonate, 756-79-6.

Photochemical Epoxidation of Aflatoxin B_1 and Sterigmatocystin: Synthesis of Guanine-Containing Adducts

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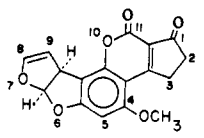
Abstract: Benzil-sensitized photoepoxidation of the mycotoxins aflatoxin B_1 and sterigmatocystin provided the presumed reactive epoxides which have been implicated in mutagenesis and carcinogenesis. These intermediates, after trapping with 3′,5′-di-*O*-butyryldeoxyguanosine and hydrolysis with aqueous acid, led to the N^7-guanine adducts identical with the in vivo natural products. This method provides an alternative to the more complex procedures of organ perfusion and whole-body dosing.

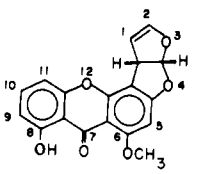
The formation of chemical carcinogen–DNA component adducts leads to the creation of mutations through the misrepair or misreplication of lesions, according to current theory. Because of DNA's central role as the repository of cellular genetic information, the fixation of mutations may represent a crucial factor in neoplastic transformation. As a result, the modes of formation and the structure of DNA adducts have been subjected to increasing scrutiny. The structures of the major adducts of aflatoxin B_1,[2] sterigmatocystin,[3] benzo[*a*]pyrene,[4] and acetylaminofluorene[5] have been identified, but a host of minor unidentified adducts are also formed, in part as the result of the complexity of the metabolic conversion of carcinogens to a variety of reactive species and the multiplicity of binding sites available in DNA. It is not possible currently to state the correlation between an adduct's structure and its efficiency in inducing a mutagenic transformation (if such a correlation indeed exists). Major adducts, in other words, may not necessarily present the highest risk of mutation to a cell if they are more easily excised from DNA than minor adducts or if they do not alter template function. This indeed is the case with alkylating agents, where quantitatively minor adducts are believed to be more significant in causing mutations than relatively more abundant lesions.[6]

The present line of investigation was initiated with the immediate objective of synthesizing adducts of known structure for comparison with the natural products, which often are available in quantities insufficient for full structure elucidation. As an extension of these studies, we have begun to chemically build carcinogens into specific genetic loci of biologically active DNA molecules in order to correlate adduct structure with mutagenic potential.[7]

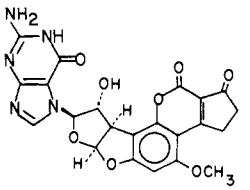
In this paper we describe a simple synthesis of the major adducts of aflatoxin B_1 (AFB_1) (**1**) and sterigmatocystin (ST) (**2**) with DNA, viz., the guanine adducts **3** and **4**. AFB_1 and ST, like many



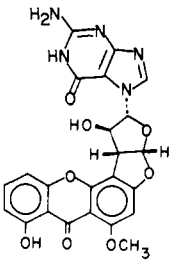
1



2



3



4

other carcinogens, must be metabolically activated in order to exert their toxic effects, in this case principally by epoxidation of the terminal dihydrofuran double bond.[8] Although these highly reactive intermediates have not been isolated, the stereochemistry of the adducts **3** and **4** indicates that epoxidation occurs on the convex face of the molecules prior to concave face nucleophilic attack at the carbons adjacent to the tetrahydrofuryl oxygens.[2] The reactivity of the putative epoxides makes their generation for adduct synthesis by methods such as peroxyacid oxidation[2,9,10]

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