Camptothecin

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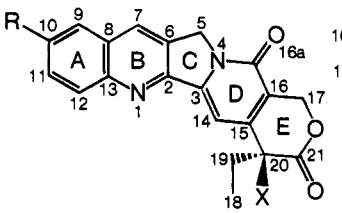
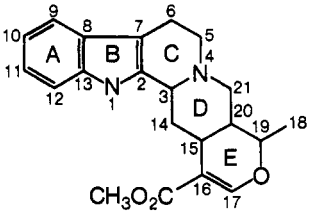
Received November 27, 1972 (Revised Manuscript Received March 1, 1973)

Contents

I. Introduction

An early examination of stem wood extracts from the tree *Camptotheca acuminata* Nyssaceae showed promising antileukemic and antitumor properties in animals.[1] Isolation of the active constituent by Wall and his associates in 1966 led to the assignment of structure **1** for the quinoline alkaloid (+)-camptothecin.[2-4] Further fractionation of *Camptotheca acuminata* resulted in the isolation of two minor and related components 10-hydroxycamptothecin (**2**) and 10-methoxycamptothecin (**3**).[5]





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1, R = H; X = OH
2, R = OH; X = OH
3, R = OCH_3; X = OH
4, R = H; X = Cl
5, R = H; X = $OCOCH_3$
6, R = H; X = $OCOCH_2Cl$
7, R = H; X = $OCOCH_2I$
8, R = H; X = H

The genus *Camptotheca* includes a single species native to mainland China, an area presently off limits to American plant explorers. Although the plant was originally introduced to the United States in 1911,[6] specimens continue to be in short supply. The rarity of the plant source, coupled with the potential chemotherapeutic properties, made a chemical synthesis of camptothecin extremely desirable. Consequently, numerous laboratories embarked on approaches toward the total synthesis of camptothecin.

The initial hopes with respect to the possible clinical utility of camptothecin as an anticancer agent largely have been abandoned because of the drug's very high toxicity.[7] However, the remarkable effect of camptothecin in the inhibition of macromolecular synthesis (*vide infra*, section V) suggest that a high degree of interest in this alkaloid and its analogs will continue for some time.

This article proposes to review all phases of the literature dealing with camptothecin up to July 1972. Particular attention has been devoted to the chemistry associated with this alkaloid. The arrangement of topics in this review hopefully places individual work in perspective with the large body of experimental data that has rapidly accumulated since Wall's characterization of camptothecin in 1966. Although many successful camptothecin syntheses have appeared, it is difficult to assess the practicality of the various approaches because almost all the synthetic literature has been in communication form.

The *Le Men–Taylor* numbering system[8] has been sug-

(1) Camptothecin when treated against leukemia L1210 in mice (on a daily dosage of 0.25–1.0 mg/kg) gave life prolongation as high as 100%. Significant growth inhibition occurred when Walker 256 rat tumors were treated with camptothecin (1.25 mg/kg); see ref 2.

(2) M. E. Wall, M. C. Wani, C. E. Cook, K. H. Palmer, A. T. McPhail, and G. A. Sim, *J. Amer. Chem. Soc.*, **88**, 3888 (1966).

(3) M. E. Wall, K. H. Palmer, M. C. Wani, C. E. Cook, G. A. Sim, and A. T. McPhail, Abstracts, 4th International Symposium on the Chemistry of Natural Products (IUPAC), Stockholm, 1966, p 103.

(4) A. T. McPhail and G. A. Sim, *J. Chem. Soc. B*, 923 (1968).

(5) M. C. Wani and M. E. Wall, *J. Org. Chem.*, **34**, 1364 (1969).

(6) R. E. Perdue, M. E. Wall, J. L. Hartwell, and B. J. Abbott, *Lloydia*, **31**, 229 (1968).

(7) H. B. Wood, Jr., Chief, Drug Research and Development, Chemotherapy, NCI, private communication.

(8) J. Le Men and W. I. Taylor, *Experientia*, **21**, 508 (1965).

gested[9] for camptothecin and will be used in the review. The choice of this nomenclature was based on the probable biogenetic relationship between camptothecin and the indole alkaloids, here represented by ajmalicine (**9**).[10] The pyridone carbonyl carbon in **1** has been designated 16a, although this atom was not assigned a number in the Le Men–Taylor scheme.[9]

II. Chemistry Associated with Camptothecin

A. Structure Determination and Chemical Studies

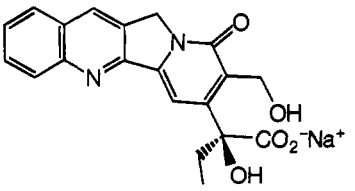
Wall and his coworkers isolated[2] camptothecin from the stem wood of the parent tree by an extraction method. Silica gel chromatography of the extract followed by crystallization from methanol–acetonitrile gave camptothecin as pale yellow needles, mp 264–267° dec. The empirical formula $C_{20}H_{16}N_2O_4$ was established by high-resolution mass measurements and supported by satisfactory elemental analysis. The reported ultraviolet, infrared, and nuclear magnetic resonance spectra were in accord with the proposed structure.[2]

A limited number of chemical transformations on **1** have been reported.[2,11] Chlorocamptothecin (**4**) is formed in poor yield by the reaction of **1** with thionyl chloride and pyridine in benzene. Hydrogenation of **4** in the presence of palladium on carbon gave deoxycamptothecin (**8**).

Camptothecin does not form stable salts with acids but does react with acetic anhydride and chloroacetic anhydride to give the acetate **5** and the chloroacetate **6**, respectively. The iodoacetate **7** was prepared by treatment of **6** with sodium iodide in acetone.[2]

An X-ray analysis was performed on the iodoacetate **7**, which by inference conclusively established that camptothecin has structure **1**.[4] As would be expected, rings A–D and their immediate substituents (atoms C_{17} and C_{20} as well as the pyridone oxygen) are very nearly coplanar. Atoms C_{21} and the lactone ring oxygen deviate from the plane by 0.69 and 0.73 Å, respectively; thus ring E has a boat conformation.[4]

The unusual reactivity of the lactone in **1** was demonstrated by the immediate (reversible) formation of the sodium salt **10** with base and the rapid reduction of **1** with sodium borohydride to the lactol **11**.[11] This reactivity has been ascribed in part to the possibility of intramolecular hydrogen bonding in **1**, which is to some extent supported by the fact that the acetate **5** would not form a sodium salt under the same conditions as **1**.[2]



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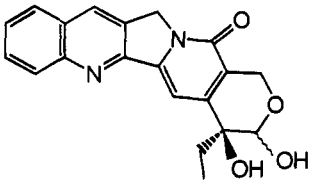

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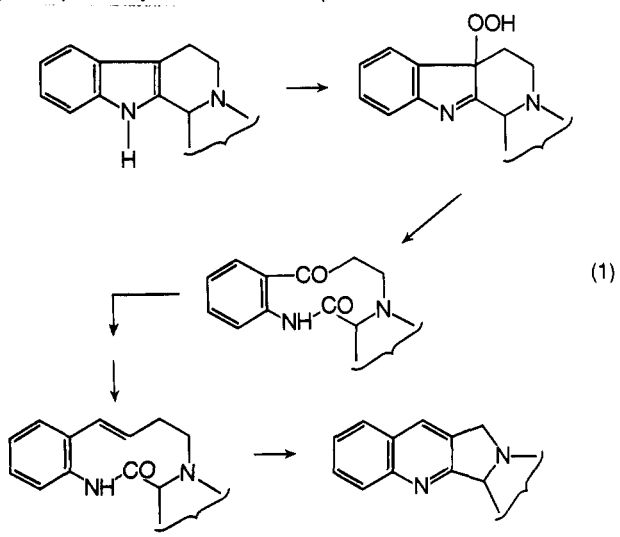
A CNDO/2 study of camptothecin, deoxycamptothecin (**8**), camptothecin acetate (**5**), and numerous substructures containing two or more rings has been performed by Flurry and Howland.[12,13] The calculated dipole moment of camptothecin is 7.1 D, while deoxycamptothecin (**8**) and camptothecin acetate (**5**) have moments of 8.7 and 9.0 D, respectively.[13]

B. Proposed Biogenesis

In 1967 Wenkert suggested that a possible biosynthetic precursor to the quinoline alkaloid camptothecin might be an indole alkaloid of the corynantheidine type.[10] Thus, conversion of an indole to a quinoline alkaloid could occur by the pathway indicated in eq 1. Similar chemical transformations involving tetrahydrocarbazoles,[14] various yohimbine alkaloids,[15] and most recently ajmalicine[16] (*vide infra*, section II) have been reported. Whether or not this sequence of reactions occurs in the plant is currently an open question; however, recently camptothecin has been shown to indeed be derived from an indole alkaloid. Administration of labeled tryptophan to *Camptotheca acuminata* seedlings gave labeled camptothecin when the alkaloid was isolated.[17]



(1)

The nontryptophan portion of camptothecin incorporates a well-recognized ten-carbon fragment, which in the case of the indole alkaloids has been shown to be of terpenoidal origin.[18-23] Thus, the remaining structural features of camptothecin could originate from a dehydro

(9) M. Shamma, *Experientia*, **24**, 107 (1968).

(10) E. Wenkert, K. G. Dave, R. G. Lewis, and P. W. Sprague, *J. Amer. Chem. Soc.*, **89**, 6741 (1967).

(11) M. E. Wall and M. C. Wani, Abstracts, 153rd National Meeting of the American Chemical Society, Miami Beach, Fla., 1967, No. M6.

(12) R. L. Flurry, Jr., and J. C. Howland, Abstracts, 162nd National Meeting of the American Chemical Society, Washington, D. C., 1971, No. MEDI-30.

(13) R. L. Flurry, Jr., and J. C. Howland, *J. Amer. Chem. Soc.*, submitted for publication.

(14) B. Witkop and J. B. Patrick, *J. Amer. Chem. Soc.*, **73**, 2196 (1951).

(15) M.-M. Janot, R. Goutarel, A. Le Hir, G. Tsatsas, and V. Prelog, *Helv. Chim. Acta*, **38**, 1073 (1955); B. Witkop and S. Gqodwin, *J. Amer. Chem. Soc.*, **75**, 3371 (1953).

(16) J. Wier and M. E. Wall, private communication.

(17) E. Winterfeldt, *Justus Liebigs Ann. Chem.*, **745**, 23 (1971).

(18) A. R. Battersby, R. T. Brown, R. S. Kapil, A. O. Plunkett, and J. B. Taylor, *Chem. Commun.*, 46 (1966).

(19) A. R. Battersby, R. T. Brown, J. A. Knight, J. A. Martin, and A. O. Plunkett, *Chem. Commun.*, 346 (1966).

(20) P. Loew, H. Goeggel, and D. Arigoni, *Chem. Commun.*, 347 (1966).

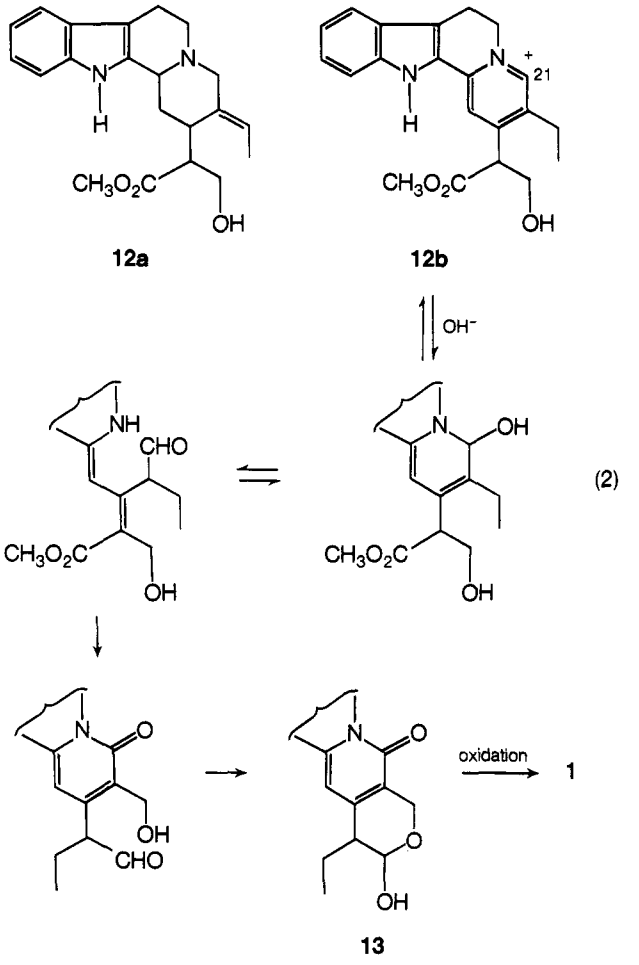
(21) E. S. Hall, F. McCapra, T. Money, K. Fukumoto, J. R. Hanson, B. S. Mootoo, G. T. Phillips, and A. I. Scott, *Chem. Commun.*, 348 (1966).

(22) E. Leete and J. N. Wemple, *J. Amer. Chem. Soc.*, **88**, 4743 (1966).

(23) A. I. Scott, *Accounts Chem. Res.*, **3**, 151 (1970).

modification **12b** of isositsirikine (**12a**) or of a related alkaloid by a ring D hydroxylation, opening, and reclosing as shown in eq 2.[10]

An analogous hydroxylation at C_{21} has been shown to occur in the conversion of deoxyajmaline to ajmaline,[24] and Djerassi has postulated a similar biogenetic transformation of geissoschizine to vallesiachotamine.[25] Oxidation of **13** to camptothecin would complete the sequence.

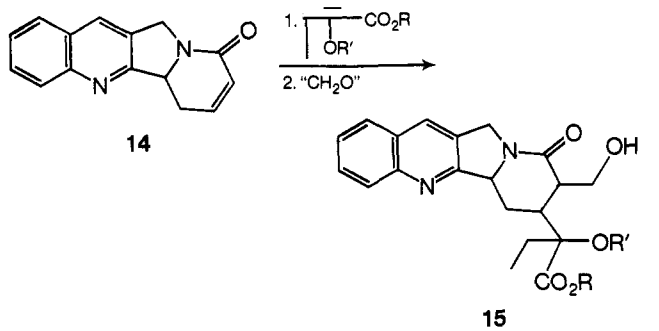


III. Chemical Synthesis of Camptothecin and Analogs

A. Ring E Oriented Approaches

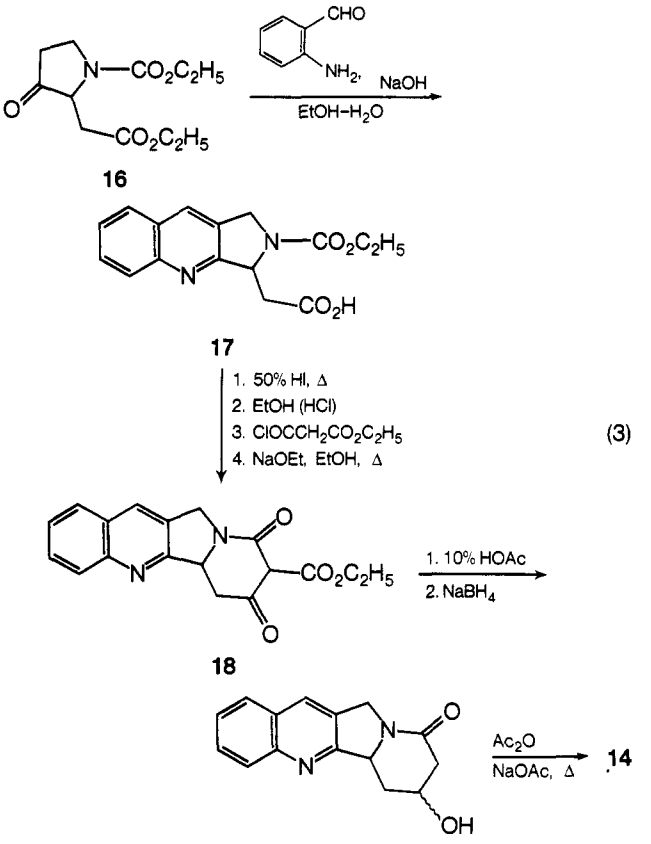
1. Construction of Ring E via Annelation

The first successful total synthesis of *dl*-camptothecin (**1**) was reported by Stork and Schultz in 1971.[26] The initial approach toward the synthesis of **1** required the preparation of the tetracyclic α,β-unsaturated lactam **14.**



From this intermediate, the remaining components of the E ring were to be assembled by (1) the conjugate addition of the anion derived from an α-hydroxybutyric ester equivalent to **14,** followed by (2) incorporation of a hydroxymethyl group at C_{16} to give **15.** Conversion of **15** into **1** would essentially require only a dehydrogenation of **15** to the corresponding pyridone.

To this end, the construction of **14** began at ring C with the pyrrolidone **16.**[27] A base-catalyzed Friedländer condensation of **16** with *o*-aminobenzaldehyde[28] gave the tricyclic quinoline acid **17** (previously prepared by Wall and coworkers),[29] which was converted to the tetracyclic β-keto ester **18** as shown in eq 3.[30]



Hydrolysis and decarboxylation of **18** followed by sodium borohydride reduction of the resulting β-ketoamide gave the β-hydroxy lactam **19.** Treatment of **19** with refluxing acetic anhydride saturated with sodium acetate gave the desired dihydropyridone **14** (eq 3).

The addition of the lithium anion of a protected α-hydroxybutyric ester to **14** did not stop at the desired intermediate **20,** but instead underwent further addition to a second molecule of the α,β-unsaturated lactam **14** to give **21** as the major product, eq 4. An internal dissipation of the anionic charge in **20** seemed to be required; therefore, two generalized solutions to this problem were considered and are shown in eq 5 and 6.

For the approach shown in eq 5, the vinyl chloride **22** was prepared by the reaction of β-keto ester **18** with phosphorus trichloride. However, treatment of **22** with the anion derived from the protected α-hydroxy ester gave

(24) D. H. R. Barton, G. W. Kirby, R. H. Prager, and E. M. Wilson, *J. Chem. Soc.*, 3990 (1965).

(25) C. Djerassi, H. J. Monteiro, A. Walser, and L. J. Durham, *J. Amer. Chem. Soc.*, **88,** 1792 (1966).

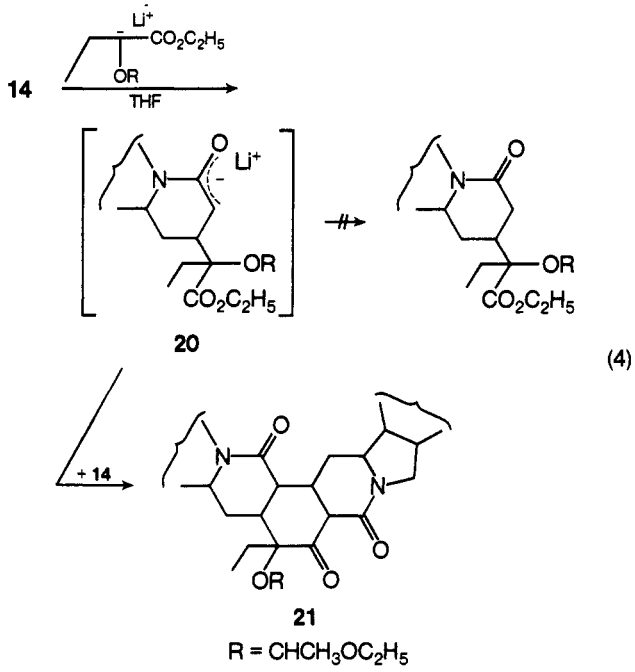
(26) G. Stork and A. G. Schultz, *J. Amer. Chem. Soc.*, **93,** 4074 (1971).

(27) J. W. Clark-Lewis and P. I. Mortimer, *J. Chem. Soc.*, 189 (1961).

(28) L. I. Smith and J. W. Opie, *Org. Syn.*, **28,** 11 (1948).

(29) J. A. Kepler, M. C. Wani, J. M. McNaull, M. E. Wall, and S. G. Levine, *J. Org. Chem.*, **34,** 3853 (1969).

(30) T. K. Liao, W. H. Nyberg, and C. C. Cheng, *J. Heterocycl. Chem.*, **8,** 373 (1971). These workers report an improved preparation of **17** by the acid-catalyzed condensation of pyrrolidone **16** with *N*-(2-aminobenzylidene)-*p*-toluidine in 76.3% yield.



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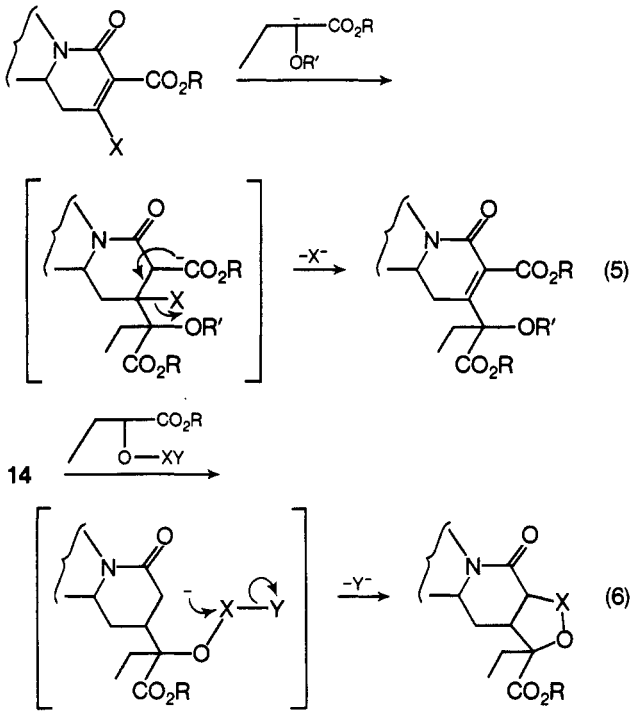
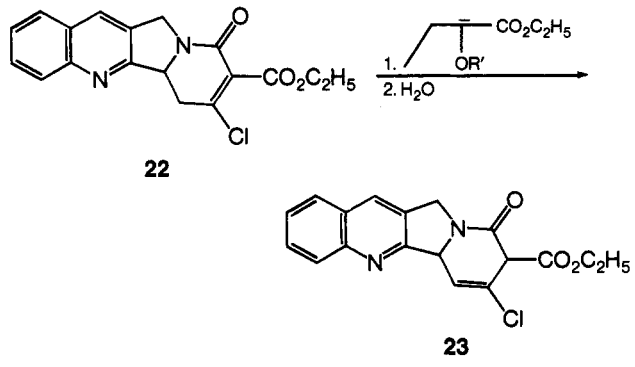


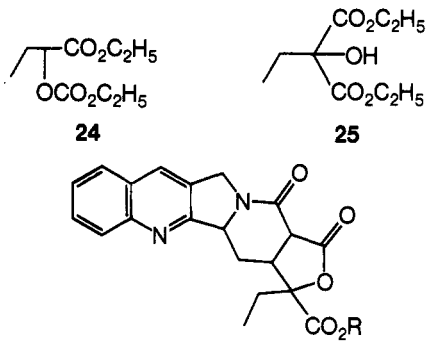
(5)

(6)

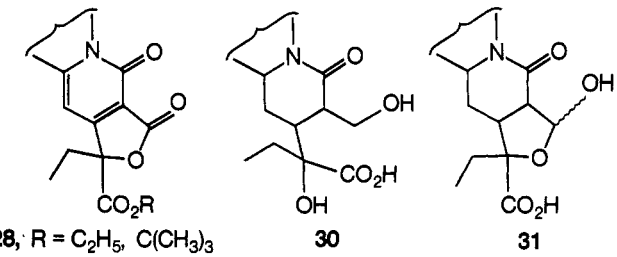
the isomeric vinyl chloride **23.** Apparently the C_{14} hydrogen atom in **22** is sufficiently acidic to allow its abstraction by the ester anion. Protonation of the resulting enolate during aqueous work-up at the carbon atom of highest electron density would be expected to give the isomerized vinyl chloride **23.**



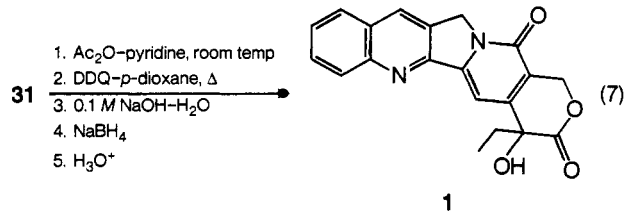
The approach depicted in eq 6 ultimately proved to be successful and represents a new annelation of α,β-unsaturated carbonyl compounds. In the present case, the carbonate ester **24** was chosen to function as the annelating reagent. Formation of the anion of **24,** using 1 equiv of lithium diisopropylamide in tetrahydrofuran (THF) at room temperature, resulted in an expected facile rearrangement to the tartronic ester **25.** The anion of **24** proved, however, sufficiently stable at Dry Ice–acetone temperature so that rapid addition of a solution of the unsaturated lactam **14** in THF to a solution of 5 equiv of the lithium salt of **24,** generated in THF at −70°, gave yields as high as 85% of the pentacyclic lactone **26.** The overall yield from diethyl maleate (the precursor of **16**) to the lactam **26** approached 20%.



Dehydrogenation of **26** with either dichlorodicyanoquinone (DDQ) in refluxing benzene or lead tetraacetate in glacial acetic acid at room temperature gave the pyridone **28.** Attempted reduction of **28** with strongly coordinating metal hydrides, such as diborane or diisobutylaluminum hydride, resulted only in decomposition. Sodium or lithium borohydride did reduce the lactone in **28,** but the ester group also reacted at a comparable rate. The more desirable free acid **29** could not be isolated because its attempted preparation led only to decarboxylation.



Metal hydride reduction of the lactone ester **26** or the corresponding lactone acid **27** directly to the diol **30** generally proved to be difficult as reduction stopped at the hemiacetal **31.** However, prolonged reaction of **27** with sodium borohydride in refluxing ethanol, followed by a mild acidic treatment and dehydrogenation with DDQ, afforded *dl*-camptothecin.



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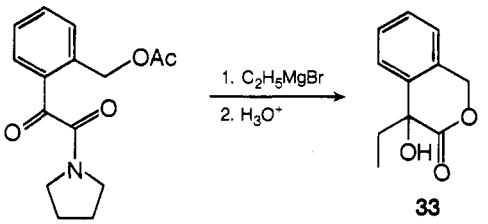
Alternatively, hemiacetal **31** could be converted to *dl*-camptothecin as shown in eq 7. The yield of camptothecin from the pentacyclic lactone **26** (by either route) was 5 to 10% which made the overall yield 1 to 2%.

2. *Construction of Ring E via Grignard Reaction with a Substituted α-Ketoamide*

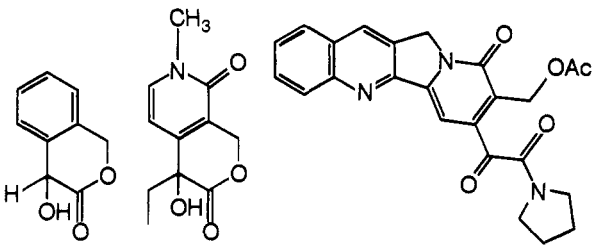
In a model study, Wall and his associates demonstrated that reaction of the α-ketoamide **32** with ethylmagnesium bromide gave the camptothecin-like lactone **33** (eq 8).[31] This method has been successfully applied



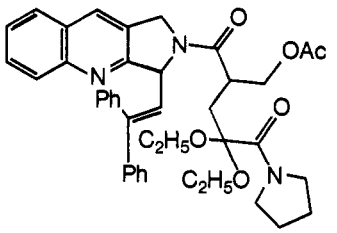
(8)

32 **33**

to the synthesis of **34** and **35**, but all reported attempts to prepare the required tetracyclic α-ketoamide **36** from either **37**[29-32] or **38**[33] have failed.



34 **35** **36**



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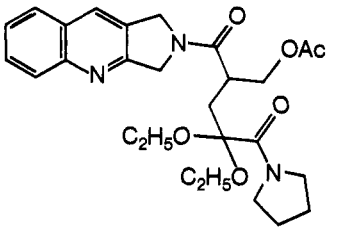

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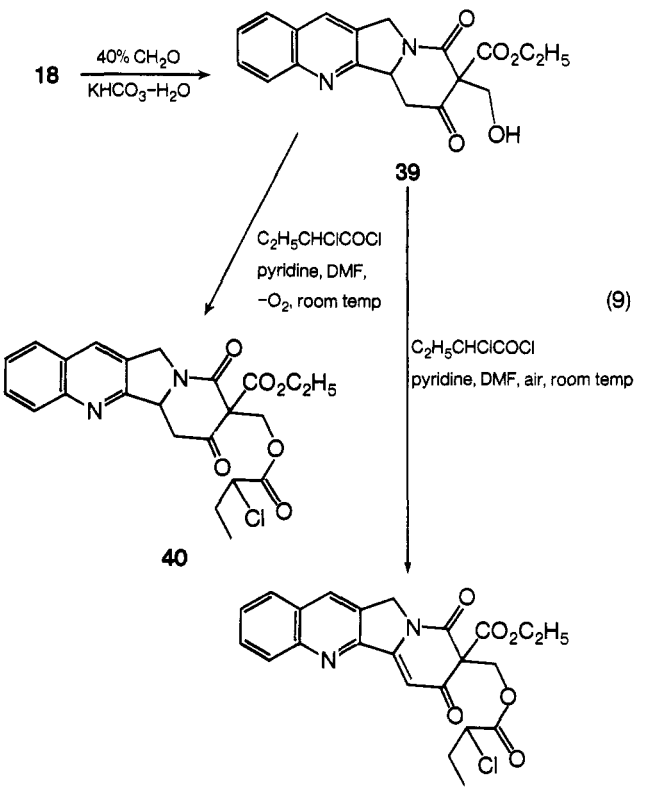
3. *Ring E Formation via a Proposed Internal Cyclization of an α-Chlorobutyric Acid Derivative*

Utilizing an independent but similar synthesis of the previously described β-keto ester **18**, Liao, Nyberg, and Cheng have prepared the potential camptothecin intermediate **41**.[30]

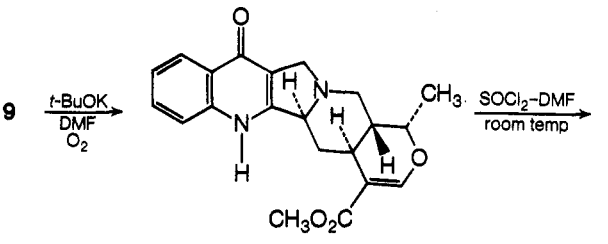
Treatment of **18** with 40% formaldehyde and potassium bicarbonate yielded the hydroxymethyl compound **39** (eq 9). Acylation of **39** with α-chlorobutyryl chloride in the absence of air gave the α-chloro ester **40**, while the same reaction exposed to the air gave the dehydrogenated product **41**. The conversion of either **40** or **41** to camptothecin has not been reported.



(9)

B. Biogenetic-Like Synthesis

Winterfeldt recently has shown that indoles are autoxidized to quinolones in the presence of potassium *tert*-butoxide in dimethylformamide (DMF).[17,34] For example, ajmalicine (**9**) was converted to the quinolone **42** which on treatment with thionyl chloride in DMF gave the chloroquinoline **43**.



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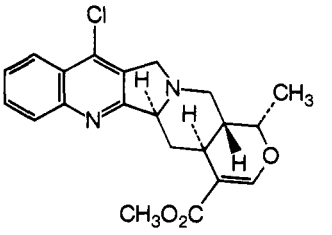

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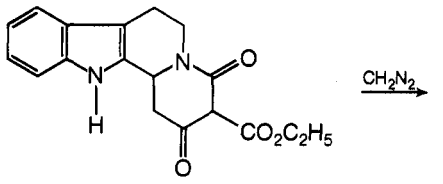
(31) M. E. Wall, F. I. Carroll, J. A. Kepler, M. C. Wani, and M. L. Honjoh, Abstracts, 156th National Meeting of the American Chemical Society, Atlantic City, N. J., 1968, No. MEDI-17.

(32) J. A. Kepler, M. C. Wani, and M. E. Wall, ref 31, No. ORGN-27.

(33) M. C. Wani, J. A. Kepler, and M. E. Wall, ref 31, No. MEDI-16.

(34) E. Winterfeldt and H. Radunz, *Chem. Commun.*, 374 (1971), and J. Warneke and E. Winterfeldt, *Chem. Ber.*, **105**, 2120 (1972).

Using ajmalicine as a model, Winterfeldt has devised a successful camptothecin synthesis incorporating the indole to quinoline interconversion. Thus, indole **44** was prepared and subjected to reaction with diazomethane which resulted in the formation of the α,β-unsaturated lactam **45** together with the enone **46.**



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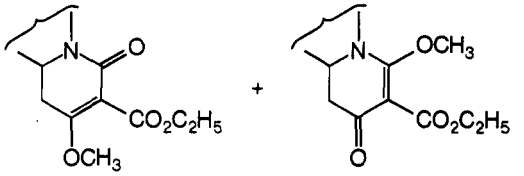
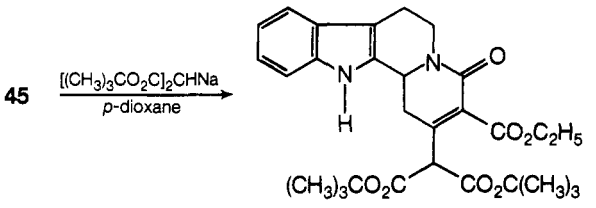


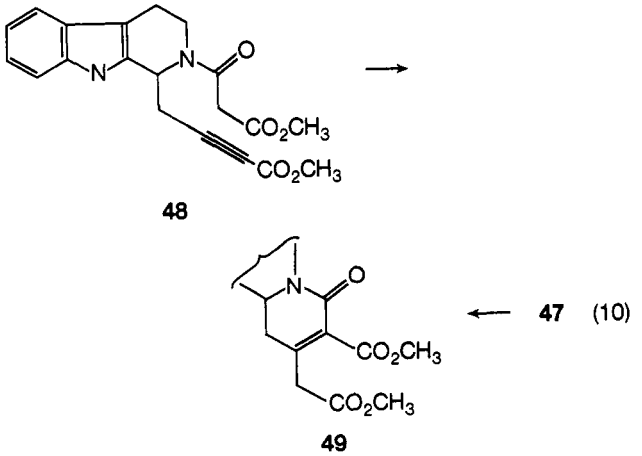
45 **46**

The E ring was elaborated further by the conjugate addition of anion derived from di-*tert*-butyl malonate to **45** to give the triester **47** in a reaction related to the scheme considered in eq 5. The success of the present example is undoubtedly the result of the lower basicity of the anion of a malonate relative to that of an α-alkoxy ester.



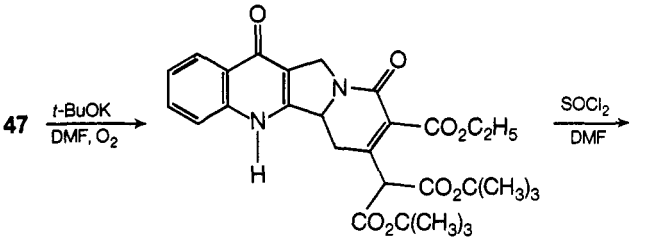
47

The structure of **47** and thus the relative identities of isomers **45** and **46** could be confirmed as shown in eq 10. Isomerization of the acetylenic indole **48** gave the cyclized product **49**,[35] which was identical with the product obtained by saponification, decarboxylation, and esterification of **47**.[36]

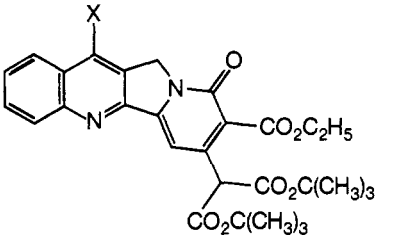


48 **49**

Autoxidation of **47** produced the quinolone **50** which could be converted to the camptothecin-like chromophore **51** on treatment with thionyl chloride in DMF at room temperature.[36,37] The required unsubstituted quinoline **52** was obtained by hydrogenolysis of **51.** Alternatively, **51** served as a precursor of the 7-chlorocamptothecin system.

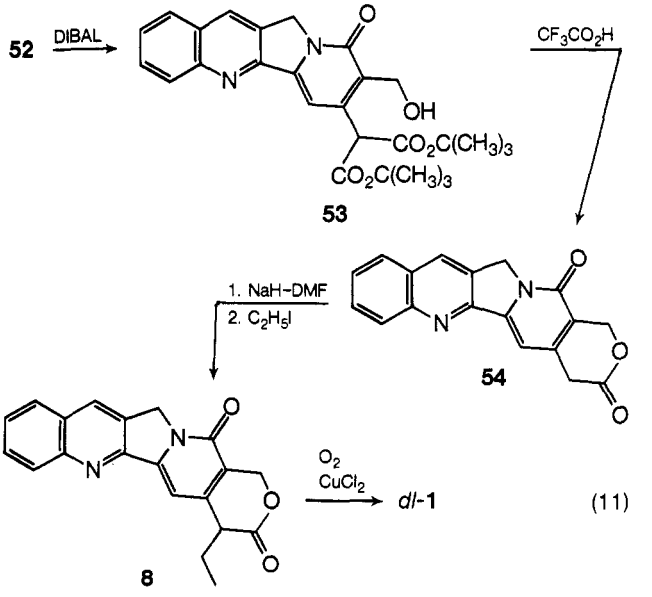


50



51, X = Cl
52, X = H

The reduction of **52** to the hydroxymethyl derivative **53** (eq 11) was accomplished by the use of diisobutylaluminum hydride (DIBAL). It is remarkable that while compound **52** was amenable to a metal hydride reduction, the closely analogous lactone **28** was not. Reduction of the ester function in **52** most probably occurs *via* a conformation, inaccessible in **28,** in which the ester carbonyl is not coplanar with the pyridone ring.



53 **54** **8**

Spontaneous lactonization and decarboxylation occurred when **53** was treated with trifluoroacetic acid at room temperature to give **54.** Alkylation of **54** with ethyl iodide gave deoxycamptothecin which could be converted to *dl*-camptothecin in a manner closely related to that of the Danishefsky synthesis (*vide infra*), by treatment with cupric chloride in the presence of oxygen. Yields are generally satisfactory for this imaginative route.

(35) W. Franzischka, Dissertation, Berlin, 1968.

(36) M. Boch, T. Korth, J. M. Nelke, D. Pike, H. Radunz, and E. Winterfeldt, *Chem. Ber.*, **105,** 2126 (1972).

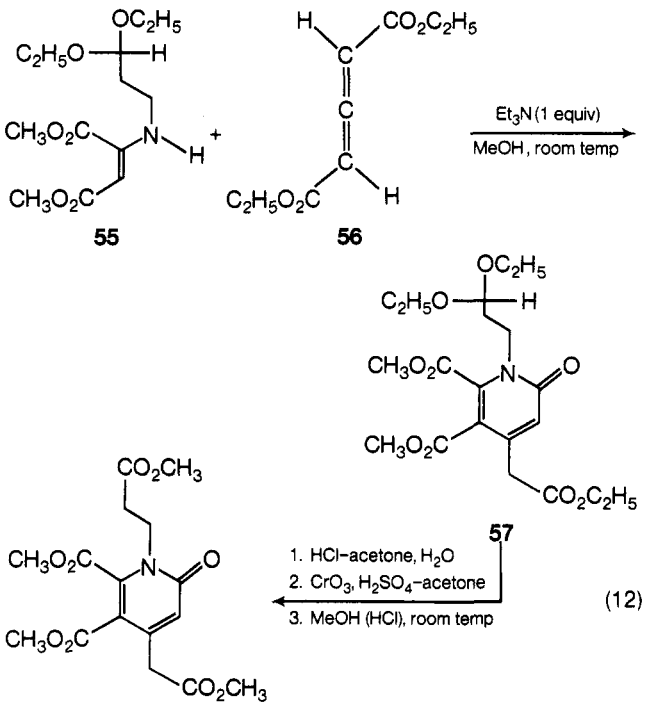
(37) E. Winterfeldt, T. Korth, D. Pike, and M. Boch, *Angew. Chem.*, **84,** 265 (1972); *Angew. Chem., Int. Ed. Engl.*, **11,** 289 (1972).

C. Syntheses Involving a Substituted Pyridone as a Key Intermediate.

1. *Pyridones via Nucleophilic Addition to 1,3-Dicarboalkoxyallenes*

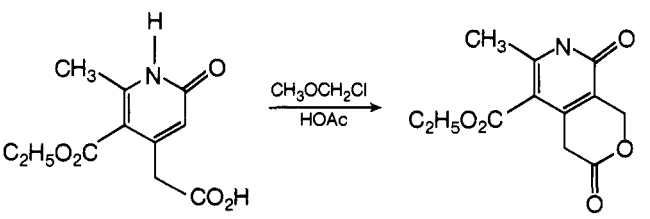
Chronologically, the second reported total synthesis of *dl*-camptothecin was that of Danishefsky and coworkers.[38-40] The focal point of their approach involves an interesting new pyridone synthesis.

Condensation of the enamine diester **55** with 1,3-dicarbethoxyallene (**56**) gave the pyridone triester **57** which was converted to the pyridone **58** in 38% overall yield, eq 12. Other pyridones such as **59** have been prepared by



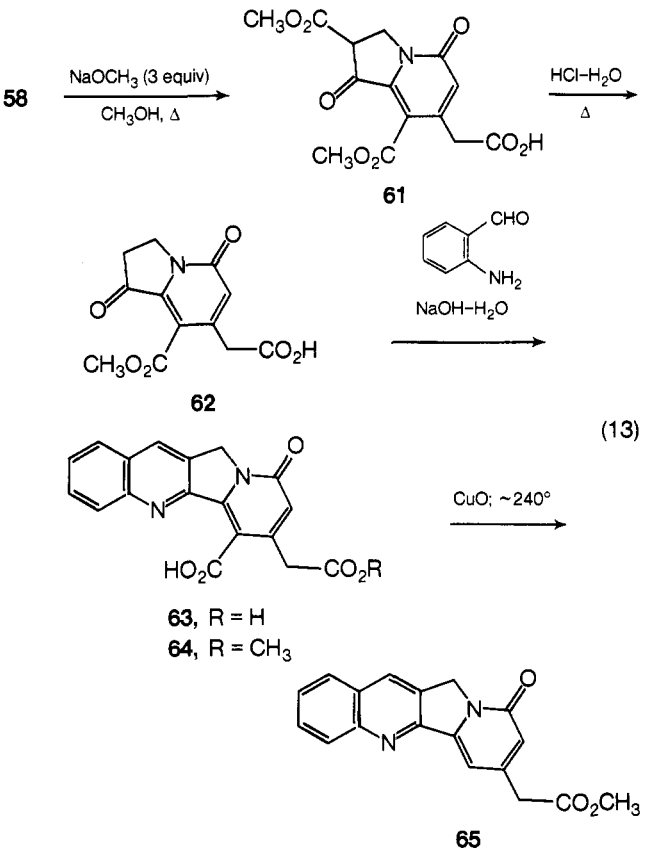
55 56 57 58 (12)

this method. Interestingly, treatment of **59** with chloromethyl methyl ether in acetic acid resulted in electrophilic substitution of the pyridone ring and formation of the camptothecin-like lactone **60.**



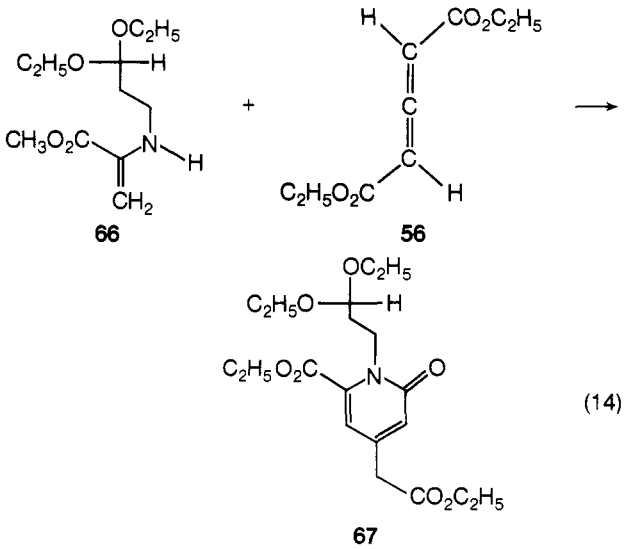
59 60

The camptothecin C ring was formed by a base-catalyzed cyclization of **58** to give the enolic acid ester **61,** eq 13. Hydrolysis and selective decarboxylation of **61** led to the keto acid **62** which could be subjected directly to Friedländer condensation with *o*-aminobenzaldehyde. Under these conditions, the tetracyclic diacid **63** was formed and subsequently monoesterified to the acid ester **64.** Decarboxylation of **64** proved to be difficult; pyrolysis (239–244°, 4 min) over cuprous oxide gave the tetracyclic methyl ester **65** in 23% yield from **58.**



58 61 62 63, R = H 64, R = CH3 65 (13)

The decarboxylation step (*e.g.*, **64** → **65**) could be avoided by suitable modification of the pyridone synthesis. Indeed, pyridone **67** which might lead directly to **65** recently has been synthesized *via* **66** (eq 14).[41]



66 56 67 (14)

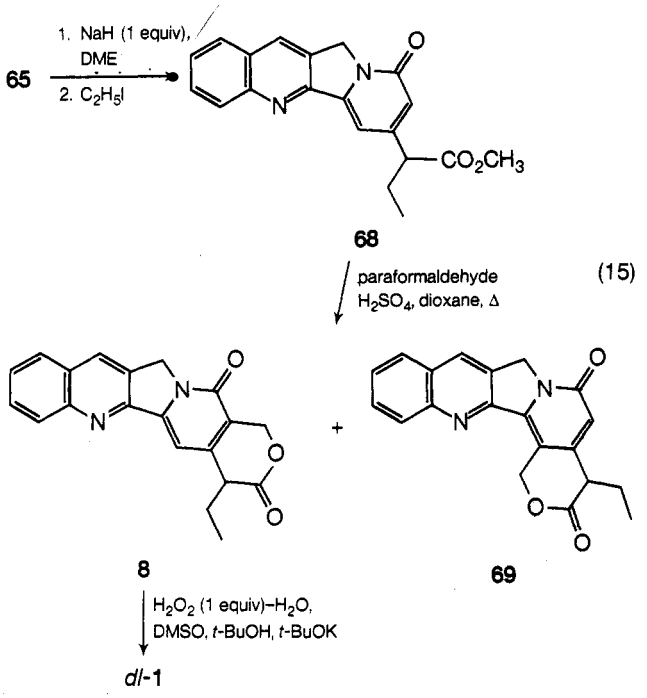
The tetracyclic ester **65** was converted to camptothecin by the route shown in eq 15. Reaction of **65** with sodium hydride followed by ethyl iodide in dimethoxyethane (DME) gave the alkylated material **68** in 20% yield. Hydroxymethylation and subsequent lactonization of **68** with paraformaldehyde gave *dl*-deoxycamptothecin (**8**) along with small amounts of what appears to be the isodeoxycamptothecin **69.** The identity of **8** could be verified by comparison with a sample of deoxycamptothecin derived from the palladium-catalyzed hydrogenolysis of chlorocamptothecin (**4**).[11]

(38) S. Danishefsky, S. J. Etheredge, R. Volkmann, J. Eggler, and J. Quick, *J. Amer. Chem. Soc.*, **93**, 5575 (1971).

(39) R. Volkmann, S. Danishefsky, J. Eggler, and D. M. Solomon, *J. Amer. Chem. Soc.*, **93**, 5576 (1971).

(40) T. A. Bryson, Dissertation, University of Pittsburgh, 1970.

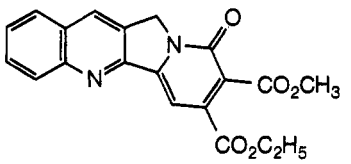
(41) S. Danishefsky, unpublished results.



Deoxycamptothecin (**8**) was converted to *dl*-camptothecin in 20% yield by oxidation with basic hydrogen peroxide.

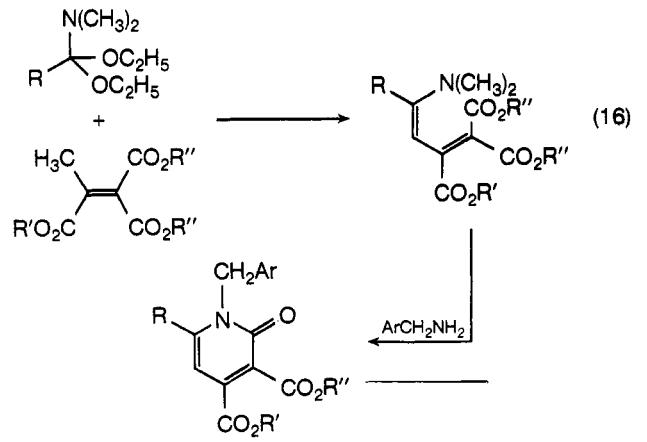
2. *Pyridones via Nucleophilic Addition to 3-Dimethylaminopentadienoic Esters*

Borch and his associates have reported the preparation of the potential camptothecin intermediate **70** by a route that incorporates a novel pyridone synthesis.[42]



70

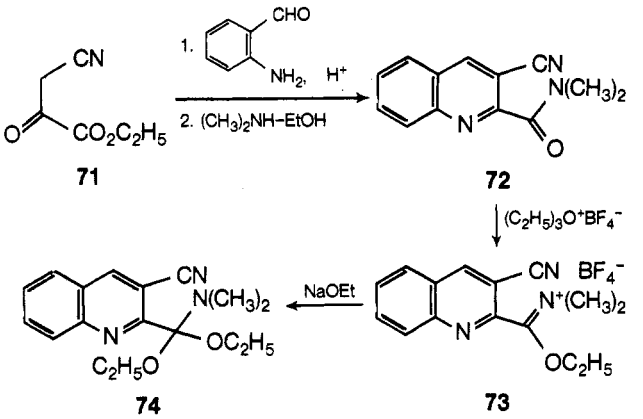
The condensation of an acetal derived from an alkyl- or arylamide with an alkylidene malonic ester was expected to afford a vinylogous urethane, eq 16. Enamine exchange with a primary amine would make possible an internal cyclization to a substituted pyridone. In the examples cited, this addition does in fact occur and represents an efficient synthesis of 2,3-dicarboalkoxy-6-substituted-2-(1*H*)-pyridones. Because of the instability of



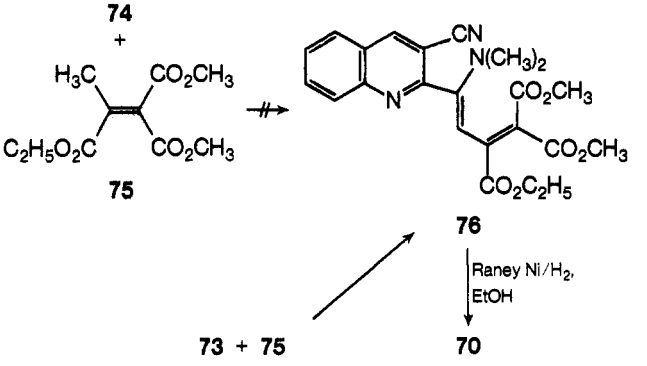
(42) R. F. Borch, C. V. Grudzinskas, D. A. Peterson, and L. D. Weber, *J. Org. Chem.*, **37** 1141 (1972).

those amide acetals, which can undergo elimination of alcohol to give the related enamine $C{=}C(OEt)NR_2$,[43] the investigation was restricted to the hydrogen- and aryl-substituted systems (R = H or Ar).

The amide **72** was prepared by an acid-catalyzed condensation between *o*-aminobenzaldehyde and ethyl 3-cyanopyruvate[44] followed by exchange with diethylamine in ethanol. Formation of the required acetal **74** was accomplished by sequential treatment of **72** with triethyloxonium fluoroborate and sodium ethoxide.

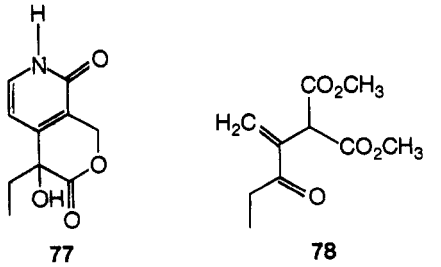


The other necessary component, triester **75,** was prepared by the reaction of dimethyl malonate and ethyl pyruvate. However, all attempts at condensation of **74** with **75** failed, presumably because of the unusual stability of **74.** The problem was solved by treating the corresponding inimium salt **73** with the anion derived from the triester **75** to give the vinylogous urethane **76.** Catalytic reduction of the nitrile in **76** followed by enamine exchange of the resulting amine gave the desired tetracyclic pyridone **70** in 1.2% yield from pyruvate **71.**



3. *Pyridones via Nucleophilic Addition to Methyl 3-Methylene-2-methoxycarbonyl-4-oxohexanoate*

The total synthesis of the DE ring analog **77**[45] as well as *dl*-camptothecin[46] has been reported by Wall, Wani,

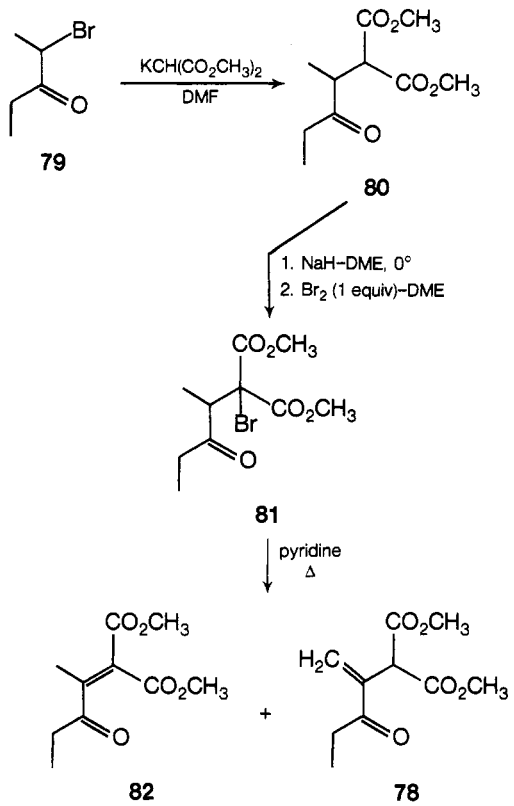


(43) T. Oishi, M. Ochiai, T. Nakayana, and Y. Ban, *Chem. Pharm. Bull.*, **17,** 2314 (1969).

(44) A. Rossey and H. Schinz, *Helv. Chim. Acta*, **31,** 473 (1948).

and associates. The common precursor in both cases was the α,β-unsaturated ketone **78**, methyl 3-methylene-2-carbomethoxy-4-oxohexanoate, the synthesis of which is shown in eq 17.[45]

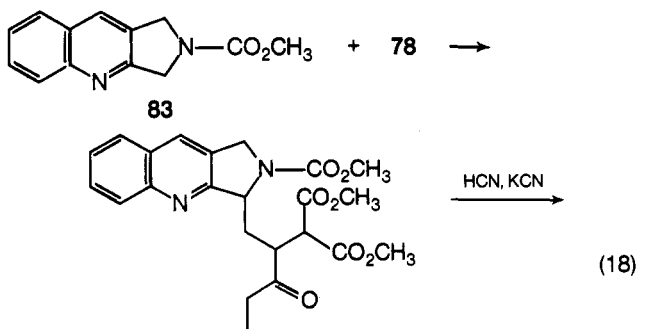
Reaction of 2-bromo-3-pentanone (**79**)[47] with dimethyl malonate in dimethylformamide (DMF) gave the keto diester **80.** Bromination of the sodium anion of **80** in dimethoxyethane (DME) yielded the 2-bromo ketone **81** which was dehydrobrominated in refluxing pyridine to give a mixture of the exo olefin **78** and the endo olefin **82** (ratio 65/35), in 43% yield from **79.** Although separation of the isomers proved to be impossible, the olefinic mixture was suitable for the next step.



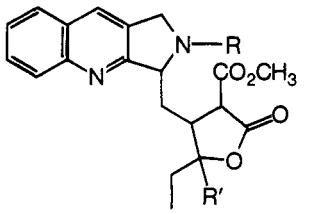
(17)

The uncatalyzed Michael condensation of **78** with **83**[48] gave **84** in 81% yield, eq 18. Treatment of **84** with liquid hydrogen cyanide and a catalytic amount of potassium cyanide gave the cyanolactone **85** which could be hydrolyzed to the amide **86** with methanolic hydrogen chloride in 62% yield from **84.** Selective hydrolysis of the *N*-carbomethoxy group was accomplished by treatment of **86** with glacial acetic acid saturated with hydrogen bromide at room temperature (eq 18).

The resulting amino ester **87** was refluxed in methanol–benzene solution containing sodium methoxide to give a mixture of lactams **88** and **89.** Quantitative lactonization to **89** occurred on refluxing a benzene solution of **88** and *p*-toluenesulfonic acid (*p*-TSA). Dehydrogenation of **89** with DDQ in refluxing *p*-dioxane proceeded in high yield to give the pyridone **90.** Compound **90** is the amide analog of the pentacyclic lactone **28** previously prepared by Stork and Schultz.[26] Whereas selective reduction of **28** proved impossible, the reluctance of a primary amide

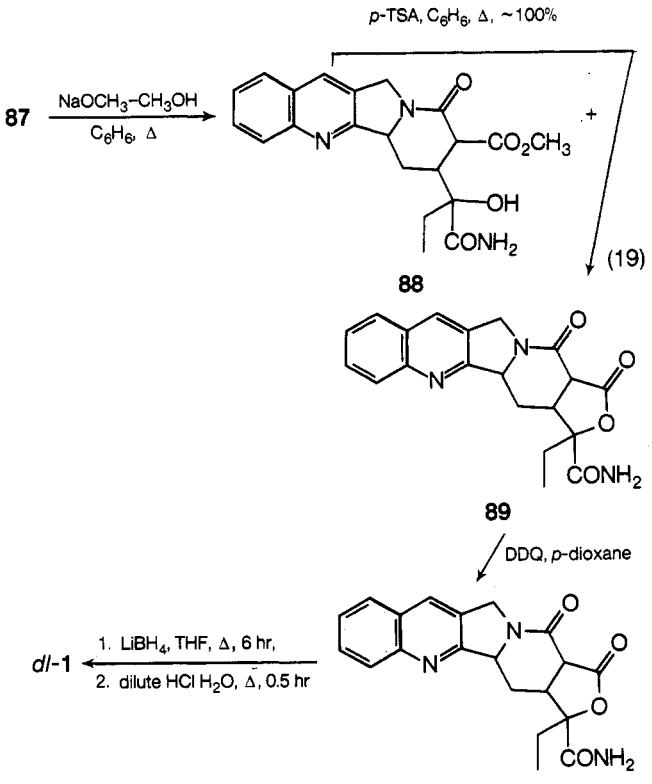


(18)



85, R = CO_2CH_3; R′ = CN
86, R = CO_2CH_3; R′ = $CONH_2$
87, R = H; R′ = $CONH_2$

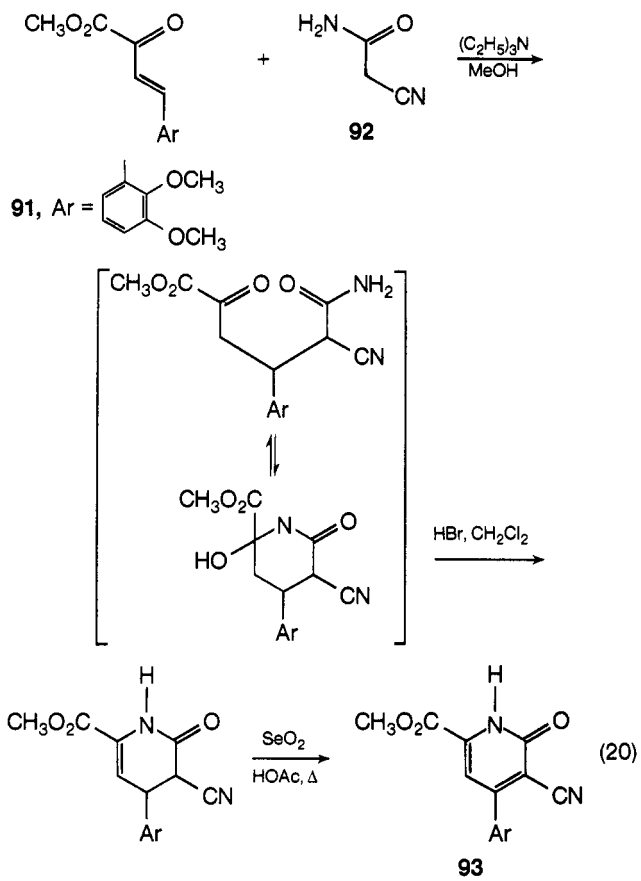
function to undergo reaction with borohydride allowed utilization of this intermediate. Thus, conversion to *dl*-camptothecin was accomplished (unspecified yield) by lithium borohydride reduction of **90** in refluxing THF followed by acidification and heating with dilute hydrochloric acid (eq 19).



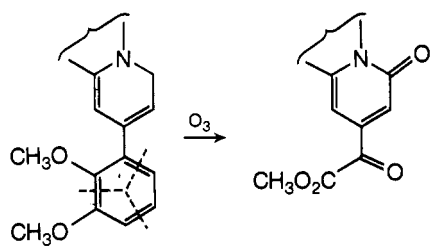
4. *Pyridones via Nucleophilic Addition to a Benzylidene Pyruvate*

Toward a proposed total synthesis of camptothecin, Wojcik has prepared the pyridone **93** in 80% overall yield from 2,3-dimethoxybenzylidene pyruvate (**91**) and 2-cyanoacetamide (**92**) as shown in eq 20.[49] Interestingly,

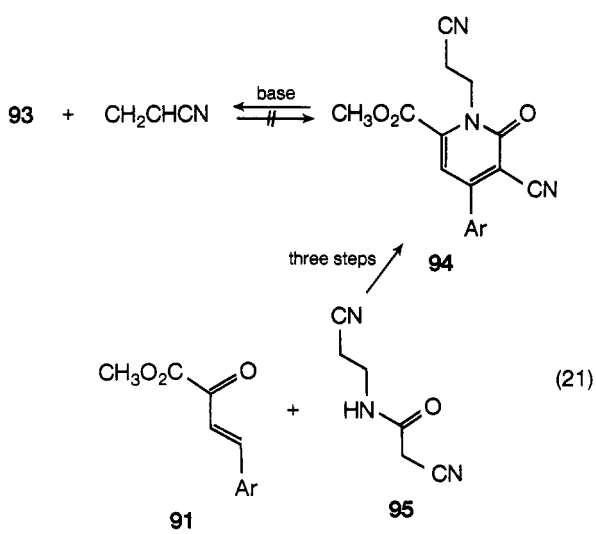
(45) M. E. Wall, H. F. Campbell, M. C. Wani, and S. G. Levine, *J. Amer. Chem. Soc.*, **94**, 3632 (1972).
(46) M. C. Wani, H. F. Campbell, G. A. Brine, J. A. Kepler, M. E. Wall, and S. G. Levine, *J. Amer. Chem. Soc.*, **94**, 3631 (1972).
(47) H. Pauly, *Chem. Ber.*, **34**, 1771 (1901).
(48) M. C. Wani, J. A. Kepler, J. B. Thompson, M. E. Wall, and G. S. Levine, *Chem. Commun.*, 404 (1970).
(49) M. Wojcik, Dissertation, Harvard University, 1970.

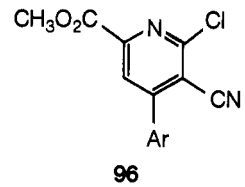


the dimethoxylated phenyl ring was to function as a precursor to a pyruvate system as shown below.



The campothecin C ring was to be established by the addition of pyridone **93** to acrylonitrile, but the anticipated adduct **94** could not be obtained by this method. Reaction of **91** with 2-cyano-(*N*-2-cyanoethyl)acetamide (**95**) did give a low yield of **94**, but all attempts at Dieckmann-type cyclization of **94** resulted only in the elimination of the side chain, eq 21. These results are in accord with Kametani's failure to utilize the analogous cyanopyridone **104** *(vide infra)* as a C,D ring precursor. In the present case, all attempts to utilize either **93** or the 2-chloropyridine **96** (prepared in quantitative yield from **93** and phosphorus oxychloride) in C-ring elaboration sequences failed.

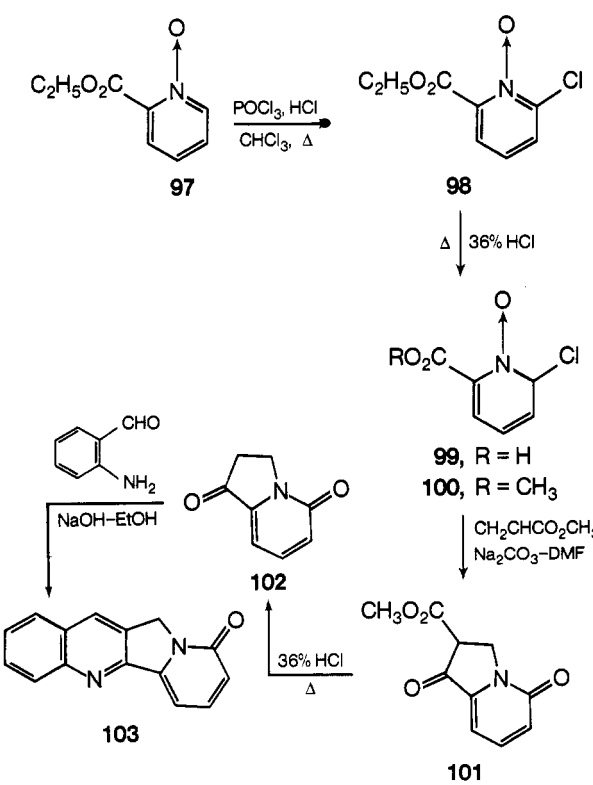




5. *Pyridones from Pyridine Precursors*

a. Hydrolysis of 6-Chloropyridine Oxide Derivatives

Kametani and coworkers have devised a synthesis of the camptothecin chromophore from suitably substituted pyridines. One method utilized in the construction of the pyridone ring involved the hydrolysis of 6-chloropyridine oxide derivatives.[50,51] In a model study ethyl picolinate 1-oxide (**97**) was treated with phosphoryl chloride to give ethyl 6-chloropicolinate 1-oxide (**98**), which on hydrolysis with hydrochloric acid gave 6-oxo-1,6-dihydropicolinic acid (**99**). The ester **100** together with methyl acrylate and sodium carbonate in DMF underwent addition followed by cyclization to give the bicyclic ketoester **101**. Hydrolysis and decarboxylation gave the ketopyridone **102** which underwent base-catalyzed reaction with *o*-aminobenzaldehyde to give the tetracyclic camptothecin analog **103**.[52]

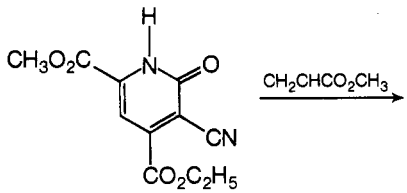


(50) T. Kametani, H. Nemoto, H. Takeda, and S. Takano, *Tetrahedron*, **26**, 5753 (1970).

(51) T. Kametani, H. Nemoto, H. Takeda, and S. Takano, *Chem. Ind. (London)*, 1323 (1970).

(52) The tetracyclic pyridone **103** also has been prepared by air oxidation of the dihydropyridone **14**: A. G. Schultz, unpublished results.

Unfortunately, when this route was tried with the potential camptothecin precursor **104**,[53] reaction with methyl acrylate resulted only in the formation of the cyclohexanone **105**. Similar attempts using acrylonitrile or *tert*-butyl acrylate as the Michael acceptor also failed.[54]



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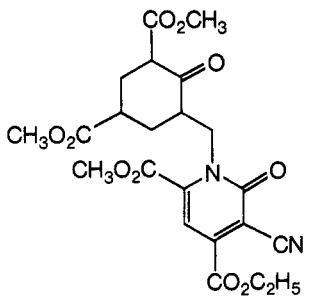
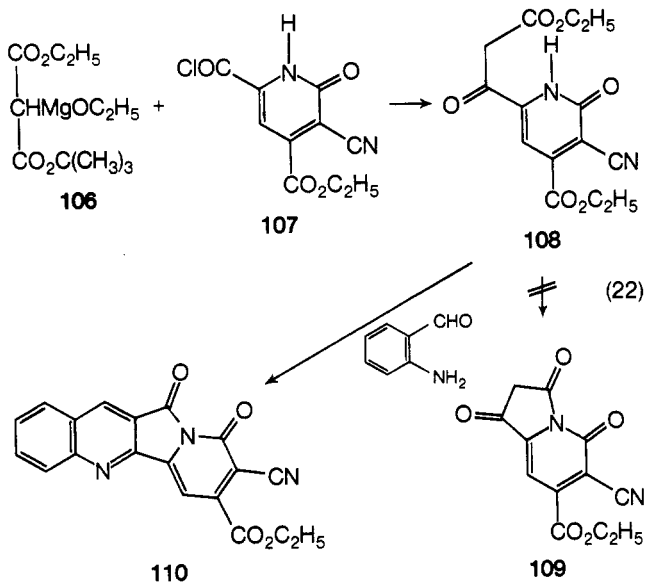


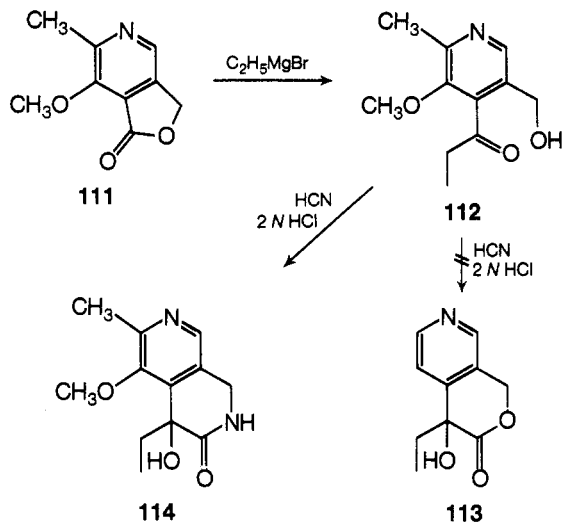
105

The problem of the construction of ring C from a 3-cyano-4-ethoxycarbonylpyridone was to some extent solved as a result of the attempted synthesis of the keto imide **109**, a potential precursor to the tetracycle **110**.[54] Compound **108** was prepared by the reaction of ethoxymagnesium ethyl *tert*-butylmalonate (**106**) and the acid chloride **107** as shown in eq 22. Although cyclization of **108** to the bicyclic keto imide **109** was unsuccessful, reaction of **108** with *o*-aminobenzaldehyde gave the required tetracycle **110** in one experimental step. The conversion of **110** to camptothecin has not been reported.



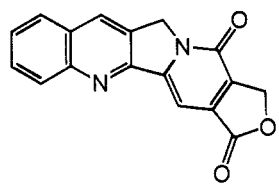
106 **107** **108** **110** **109** (22)

At this stage in the model studies, Kametani turned his attention toward elaboration of the E-ring lactone system.[55] Reaction of the known lactone **111**[56] with ethylmagnesium bromide gave the ketone **112**. However, treatment of **112** with hydrogen cyanide in hydrochloric acid gave the lactam **114** instead of the desired lactone **113**.



111 **112** **114** **113**

In connection with the choice of an intermediate such as lactone **111** for the synthesis of camptothecin, attention should be drawn to the pentacyclic lactone **115**, reported by Wall and coworkers.[48] Reaction of *o*-aminobenzaldehyde with *N*-ethoxycarbonyl-3-pyrrolidone (**116**)[57,58] gave a mixture of two tricyclic quinolines **83** and **117** in unspecified yield.



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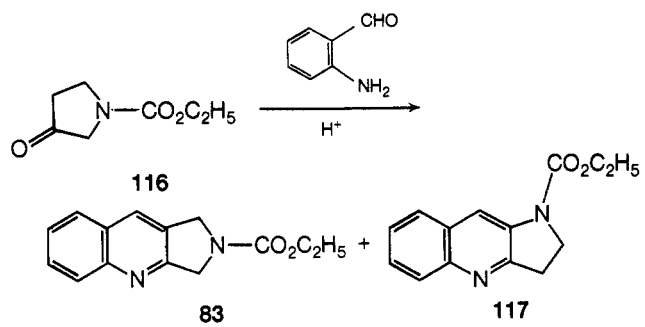
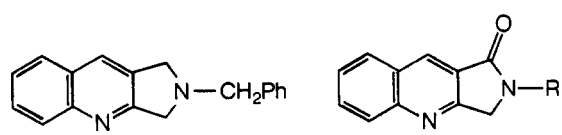


116 **83** **117**

It is noteworthy, however, that a completely regiospecific synthesis of **83** from **116** in 88% yield[59,60] has been described as well as an analogous preparation of the benzyl derivative **118**.[61] Neither **83** nor **118** could be prepared from the lithium aluminum hydride reduction of the lactams **119** or **120**, respectively. The isolated product in



118 **119**, R = H **120**, R = CH_2Ph

(53) L. Rateb and G. Soliman, *J. Chem. Soc.*, 1430 (1960).

(54) T. Kametani, S. Takano, and H. Takeda, *Yakugaku Zasshi*, **92**, 743 (1972), and unpublished results.

(55) T. Kametani, S. Takano, H. Nemoto, and H. Takeda, *Yakugaku Zasshi*, **91**, 966 (1971).

(56) D. Heyl, *J. Amer. Chem. Soc.*, **70**, 3434 (1948).

(57) R. Kuhn and G. Osswald, *Chem. Ber.*, **89**, 1423 (1956).

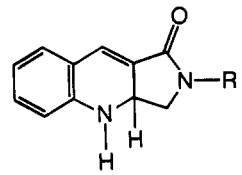
(58) M. Viscontini and H. Buhler, *Helv. Chim. Acta*, **50**, 1289 (1967).

(59) L. H. Zalkow, J. B. Nabors, K. French, and S. C. Bisarya, *J. Chem. soc. C*, 3551 (1971).

(60) J. B. Nabors, Dissertation, Georgia Institute of Technology, 1970.

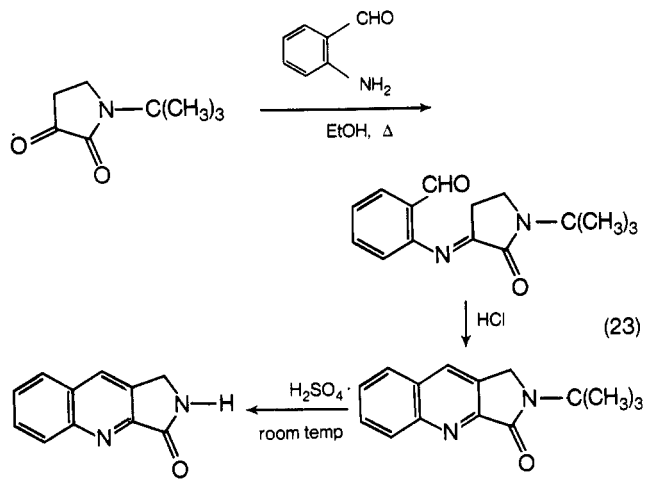
(61) M. Shamma and L. Novak, *Collect. Czech. Chem. Commun.*, **35**, 3280 (1970).

both cases resulted from the reduction of the quinoline ring to give **121** or **122.**



121, R = H
122, R = CH_2Ph

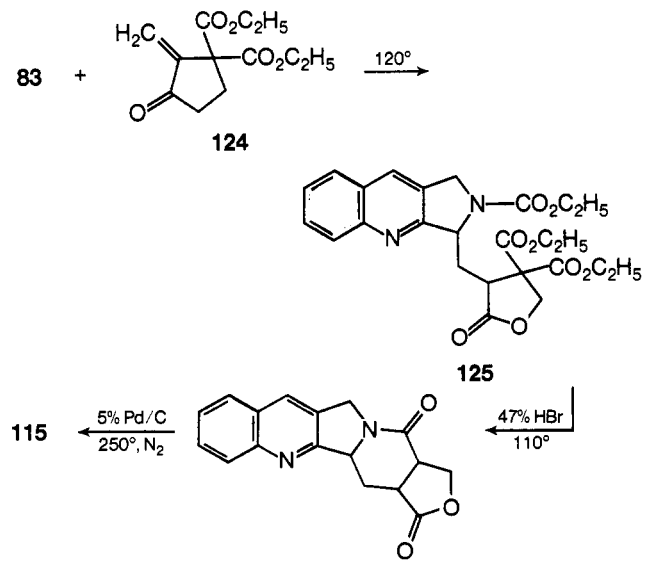
The tricyclic lactone **123** has been prepared as shown in eq 23.[49,62]



(23)

123

Returning to the synthesis of **115,** Michael addition of **83** to α-methylene-β,β-diethoxycarbonyl-γ-butyrolactone (**124**)[63] without added catalyst gave **125** as a mixture of two diastereomers. Treatment of **125** with aqueous hydrobromic acid, followed by neutralization with sodium bicarbonate, effected hydrolysis, decarboxylation, and cyclization in one operation to give the pentacyclic lactone **126.** Dehydrogenation of **126** to the pyridone **115** completed the sequence. Although no further reports have appeared concerning the conversion of **115** into camptothecin, the success of this route is not assured in the light of Kametani's results with the model lactone **111.**



124

125

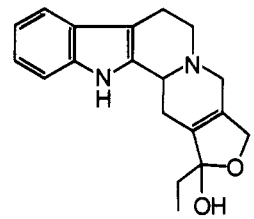
126

(62) T. Sugasawa, T. Toyoda, K. Sasakura, and T. Hidaka, *Chem. Pharm. Bull.*, **19,** 1971 (1971).

(63) V. B. Piskov, *Zh. Obshch. Khim.*, **30,** 1390 (1960); V. B. Piskov, *J. Gen. Chem. USSR,* **30,** 1421 (1960).

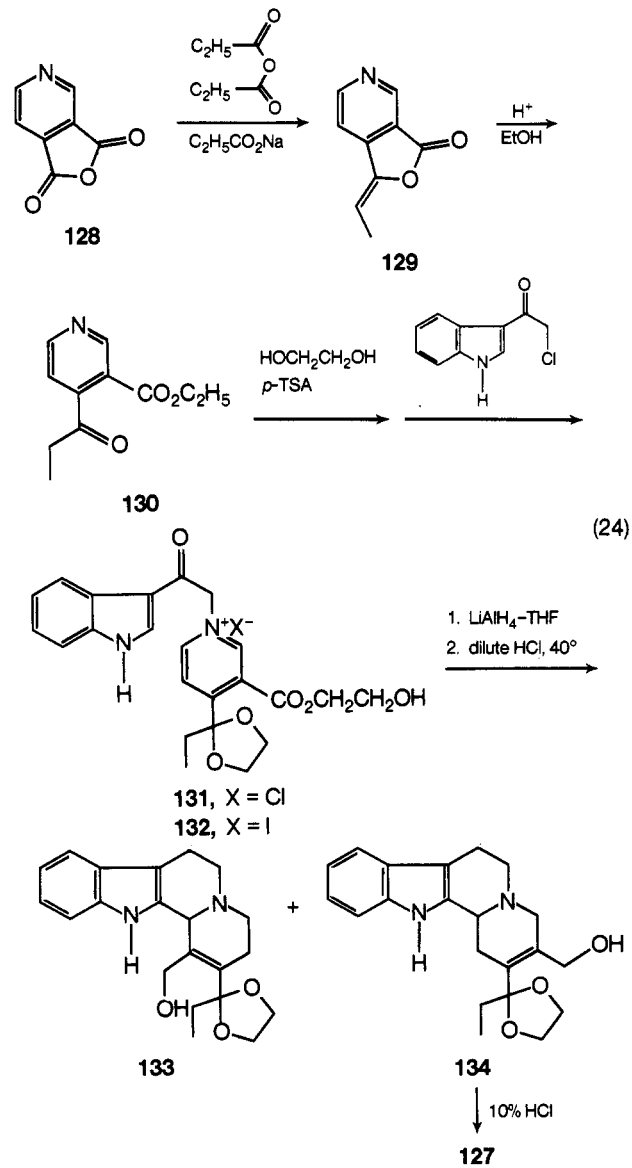
b. Reduction of Quaternary Pyridinium Salts

Encouraged by Winterfeldt's success in the synthesis of camptothecin by the conversion of an indole base to a quinoline,[36,37] Kametani has prepared the corynantheidine-type intermediate **127.**[54] In this approach, the D ring again assumes the form of a substituted pyridine.



127

Perkin reaction of cinchomeronic anhydride (**128**), using conditions similar to those described by Fels,[64] afforded the ethylidene derivative **129,** which was converted to ethyl 4-propionylnicotinate (**130**) (eq 24). Ketalization of **130** followed by alkylation with 3-chloroacetylindole gave the quaternary pyridinium salt **131.** Lithium aluminum hydride ($LiAlH_4$) reduction of the corresponding iodo salt **132** followed by treatment with dilute acid, gave a 50:50 mixture of two cyclized products **133** and **134** in



(24)

128

129

130

131, X = Cl
132, X = I

133

134

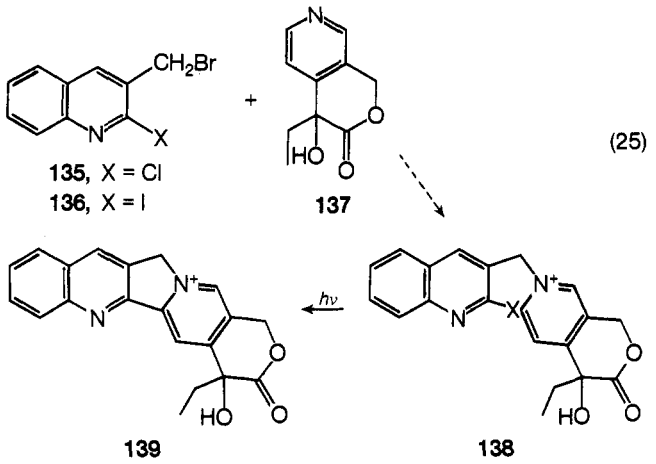
127

(64) B. Fels, *Chem. Ber.*, **37,** 2137 (1904).

low yield. Reaction of the desired tetracyclic material **134** with 10% aqueous hydrochloric acid gave **127.**

c. Photochemical Cyclization of a Pyridine Derivative

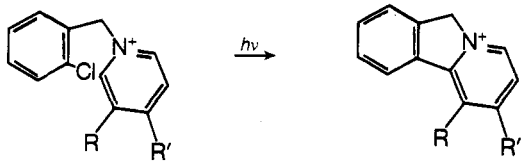
Lyle and coworkers have proposed a synthesis of camptothecin by the combination of the quinoline derivative **135** or **136** and the substituted pyridine **137** (eq 25).[65] The pyridinium salt **138** so formed might be photo-



135, X = Cl
136, X = I
137
139
138

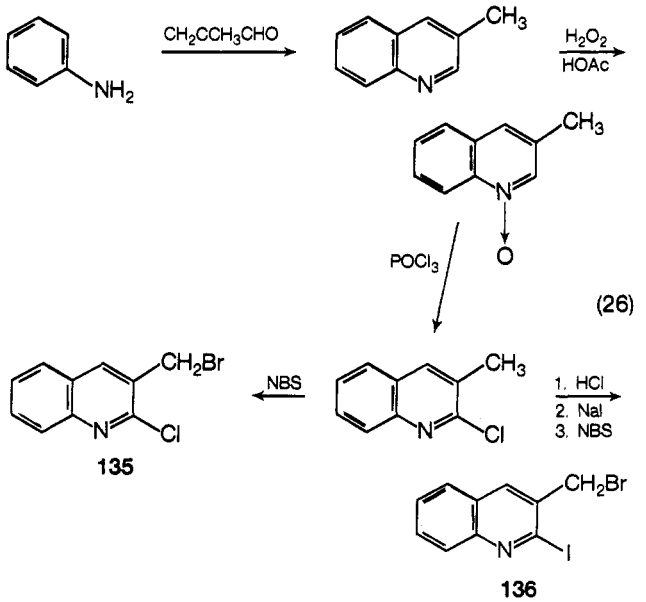
(25)

cyclized to the pentacyclic material **139.** The photocyclization step is based on Lyle's work on the conversions **140** → **142** and **141** → **143.**[65]



140, R = H; R′ = CH_3
141, R = CH_3; R′ = H
142, R = H; R′ = CH_3
143, R = CH_3; R′ = H

Quinolines **135** and **136** have been prepared in 33 and 15% overall yield from propionaldehyde by a Skraup-type synthesis as shown in eq 26.



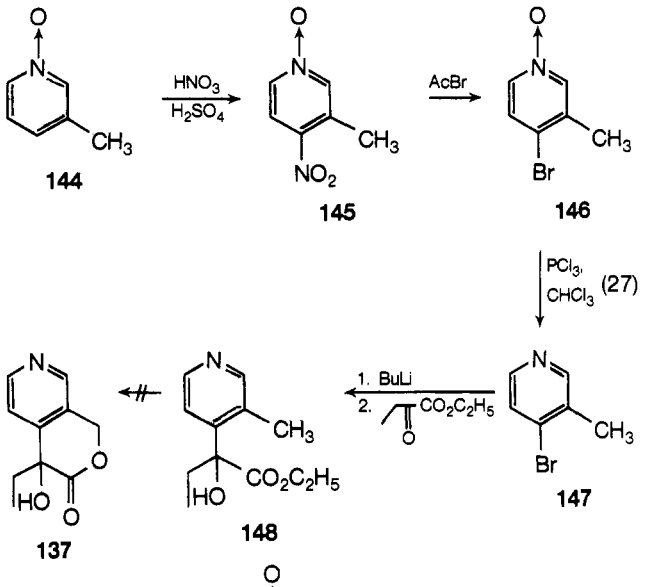
135
136

(26)

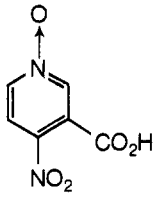
For the pyridone portion of the molecule, β-picoline 1-oxide (**144**) was utilized as starting material (eq 27).

(65) J. A. Bristol, M. J. Kane, and R. E. Lyle, Abstracts, XXIIIrd International Congress of Pure and Applied Chemistry, Boston, Mass., 1971, p 67, and unpublished results.

Thus, nitration of **144** gave the 4-nitro derivative **145** which could be converted to **146** on treatment with acetyl bromide. Removal of the oxide oxygen was accomplished with phosphorus trichloride in chloroform to give the pyridine **147.** The remaining components of the E ring were introduced by converting **147** to a 4-lithiopyridine followed by addition to ethyl 2-ketobutyrate to give the α-hydroxy ester **148** in 30% overall yield from **144.** Unfortunately, all attempts to convert **148** to **137** by means of bromination or oxidation have failed.



144
145
146
147
148
137

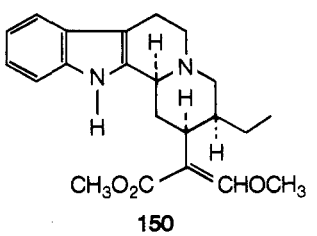


149

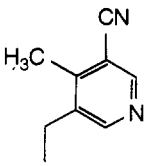
To avoid the problem of oxidation of the 3-methyl group in **148,** the utilization of 4-nitronicotinic acid 1-oxide (**149**) in eq 27 is currently under investigation.[65] Lactone **137** has recently been prepared by Myers and Hansen (*vide infra,* eq 31), using a different approach.

6. *Pyridones Formed on Cyclization of an α-Keto Ester Nitrile*

The reduction–acid-catalyzed cyclization of β-(3-indoyl) ethylpyridinium salts leading to compounds such as **134** has been used by Wenkert in the construction of indole alkaloids of the tetrahydrocarboline type.[66] Most recently this approach was utilized in the synthesis of *dl*-corynantheidine (**150**).[10] To Wenkert, however, the precursor, 4-methyl-5-ethylnicotinonitrile (**151**), not only represented a potential D ring equivalent in the construction of corynantheidine, but also incorporated functionality necessary for



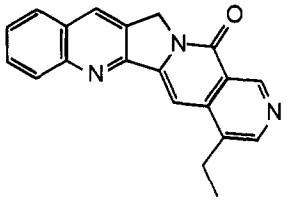
150



151

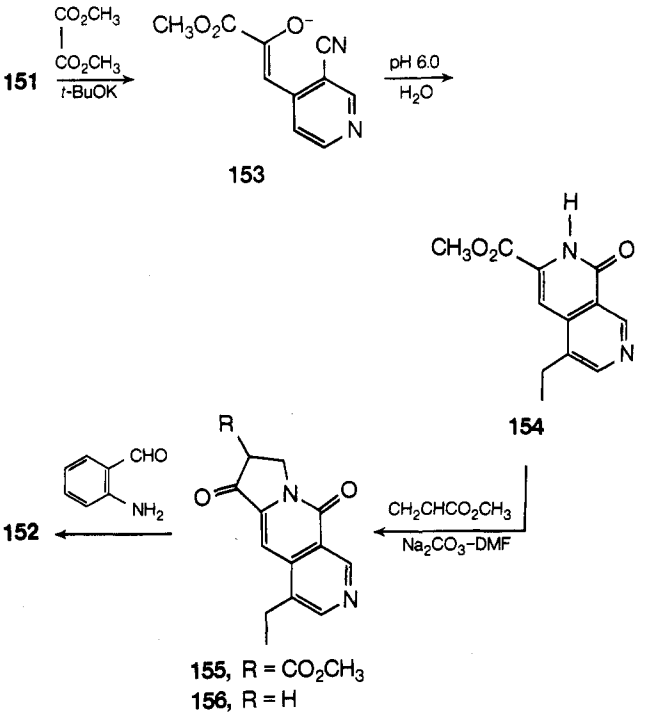
(66) E. Wenkert, *Accounts Chem. Res.,* **1,** 78 (1968), and references cited therein; E. Wenkert, D. B. R. Johnston, and K. G. Dave, *J. Org. Chem.,* **29,** 2534 (1964).

the construction of the heteropentacyclic substance **152**, structurally related to camptothecin.



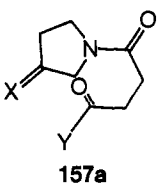
152

The method employed for the construction of **152** makes use of a pyridone synthesis that originally was reported by Wenkert in 1964.[66] Condensation of **151** with dimethyl oxalate followed by exposure of the resulting enolate salt **153** to mild acid yielded the pyridone **154**. Reaction of **154** with methyl acrylate produced the β-keto ester **155**, which could be hydrolyzed and decarboxylated to give the tricyclic ketone **156**. Condensation with *o*-aminobenzaldehyde gave the camptothecin analog **152** in good overall yield.

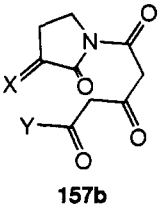


7. *Pyridones via Cyclization of Active Methylene Compounds*

Cyclization of an intermediate such as **157** would result in a potential pyridone ring synthesis. This technique has been utilized in the formulation of a variety of approaches toward the synthesis of camptothecin.



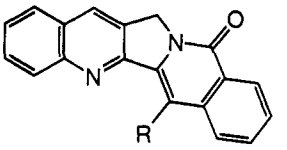
157a



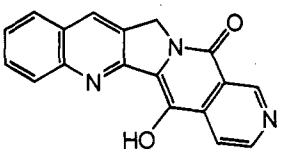
157b

a. Cyclization of Amido Esters (Camptothecin Analogs)

Shamma and Novak have reported the preparation of the pentacyclic camptothecin analogs **158**, **159**, and **160**.[67] Condensation of 3-pyrrolidine ethylene ketal

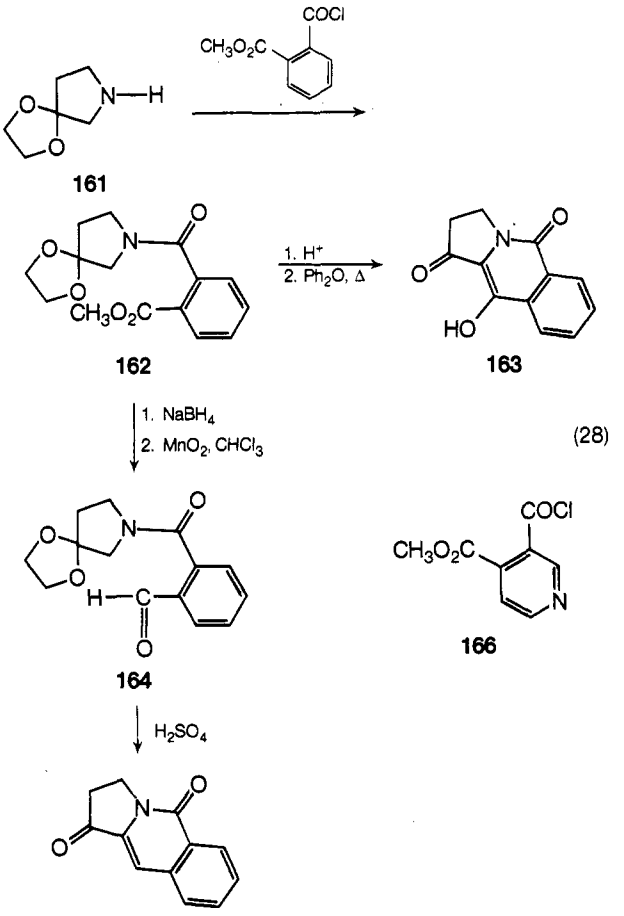


158, R = OH
159, R = H



160

(**161**)[58] with the acid chloride of phthalic acid monomethyl ester gave the ketal ester **162** (eq 28). Deketalization of **162** with aqueous oxalic acid followed by refluxing the resulting keto ester in diphenyl ether gave the cyclized hydroxypyridone **163** in good overall yield. Alternatively, the ketal ester **162** could be converted to the ketal aldehyde **164** by sodium borohydride reduction, followed by oxidation with manganese dioxide.



(28)

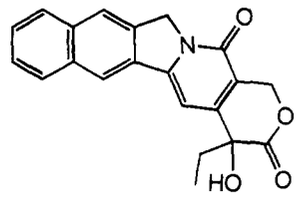
Deketalization as well as cyclization to the tricyclic ketopyridone **165** occurred on treatment of **164** with concentrated sulfuric acid. Both **163** and **165** could be converted to the corresponding pentacycle **158** or **159** by Triton B catalyzed condensation with *o*-aminobenzaldehyde. Substitution of the acid chloride of cinchomeronic acid γ-methyl ester **166**[68] for phthaloyl chloride in the above sequence led to the synthesis of **160**, a structural analog of **152**.

The attempted synthesis of the naphthalene analog **167** of camptothecin has been reported.[69]

b. Cyclization of an Amido Ketone

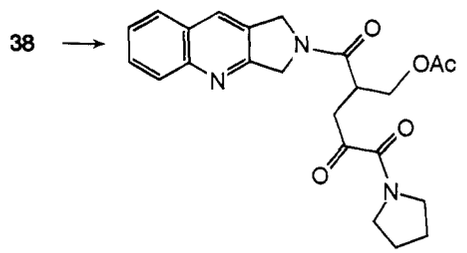
At this juncture it is instructive to note that an unwanted cyclization inadvertently occurred to give the tricycle

(67) M. Shamma and L. Novak, *Tetrahedron*, **25**, 2275 (1969).
(68) H. Meyer, *Monatsh. Chem.*, **22**, 583 (1901).
(69) H. J. Teague, Dissertation, North Carolina State University, 1970.

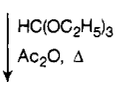


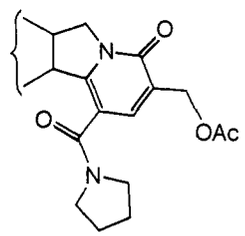
167

169 when the α-ketoamide **168** was refluxed with triethyl orthoformate and acetic anhydride. The desired ketoamide **36** was not formed.[33]



168

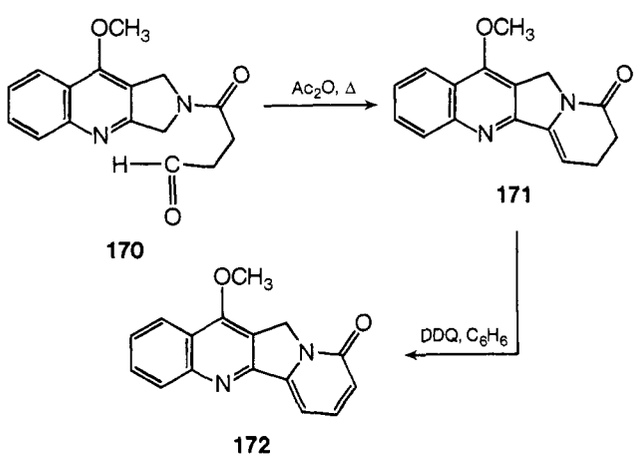




169

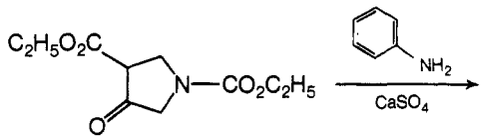
c. Cyclization of Amido Aldehydes

i. Preparation of a Tetracyclic Camptothecin Analog. Tanaka and coworkers used a cyclization reaction in the conversion of the amido aldehyde **170** to the dihydropyridone **171** in 42% yield.[70] Dehydrogenation with DDQ in benzene gave the tetracyclic pyridone **172.**



170 171 172

In the preparation of **170,** Tanaka used a method alternative to the Friedländer condensation with *o*-aminobenzaldehyde for the direct construction of the A,B ring system. Thus, reaction of aniline with 1,4-diethoxycarbonyl-3-oxopyrrolidine **173**[58] gave the anil **174** which could be converted to the tricycle **175** in high overall yield by refluxing its solution in diphenyl ether. Treatment of **175** with phosphorus oxychloride in pyridine gave the chloro analog **176** which could be catalytically reduced with palladium on carbon and hydrolyzed to the amine **177.**



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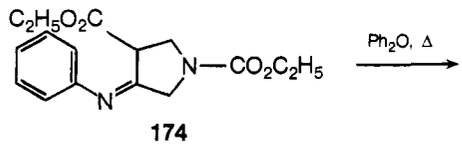
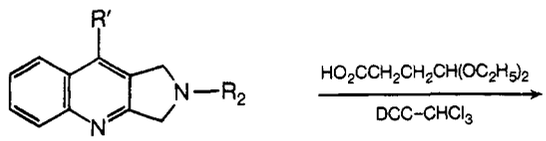
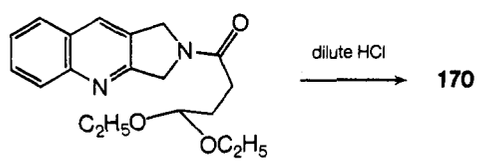


174



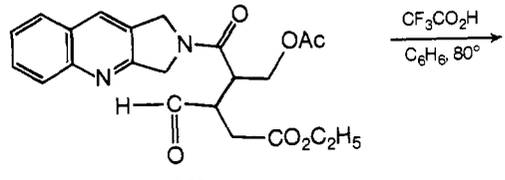
175, R_1 = OH; R_2 = $CO_2C_2H_5$
176, R_1 = Cl, R_2 = $CO_2C_2H_5$
177, R_1 = H; R_2 = H
178, R_1 = OCH_3; R_2 = H



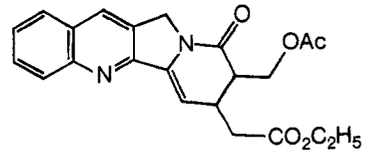
179

The reaction of **176** with sodium methoxide and subsequent hydrolysis gave the free amine **178.** Condensation of **178** with β-formylpropionic acid diethyl acetal using dicyclohexylcarbodiimide (DCC) in chloroform at room temperature afforded the amide acetal **179.** Deacetalization with dilute aqueous hydrochloric acid gave the required aldehyde **170** in unspecified yield. The analogous conversion of the unsubstituted amine **177** to **103** has not been reported.

ii. Total Synthesis of Camptothecin and a Bicyclic Analog. In their synthesis of camptothecin, Meyers and coworkers obtained the tetracyclic dihydropyridone **181** in 25% yield by the acid-catalyzed cyclization of the amidoaldehyde **180.**[71] Compound **180** was prepared from the tricyclic amine **177** and furfural (**182**) as shown in eq 29.



180



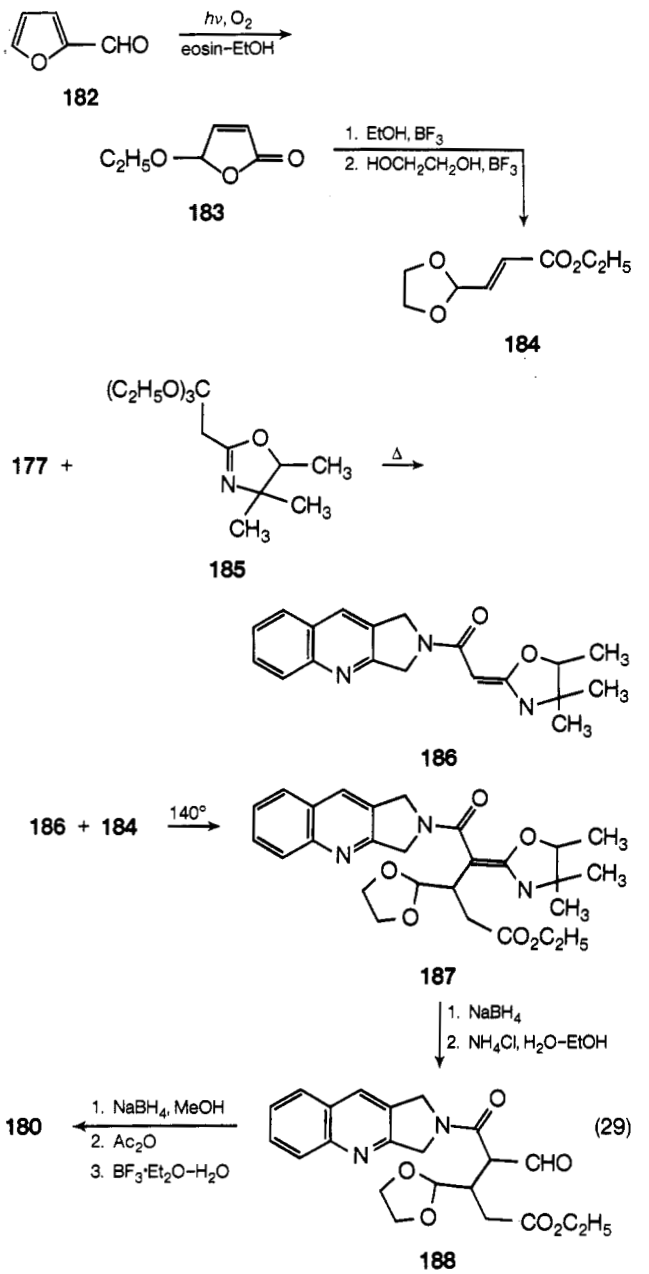
181

Reaction of photochemically generated singlet oxygen with furfural (**182**) in ethanol gave **183** which was converted to the unsaturated acetal ester **184** as shown in

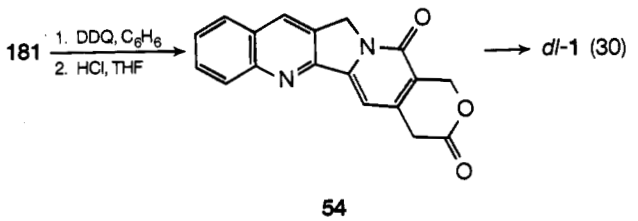
(70) T. Tanaka, K. Mashimo, and M. Wagatsuma, *Tetrahedron Lett.*, 2803 (1971).

(71) A. I. Meyers, R. L. Nolen, E. W. Collington, R. Strickland, and T. A. Narwid, unpublished results.

36% yield. Amine **177** when treated with the oxazoline ortho ester **185** gave **186**, which underwent condensation with **184** to give **187** in good yield. Borohydride reduction of **187** followed by treatment with aqueous ethanolic ammonium chloride gave aldehyde **188** which could be converted to **180** (11% yield from **177**, eq 29).

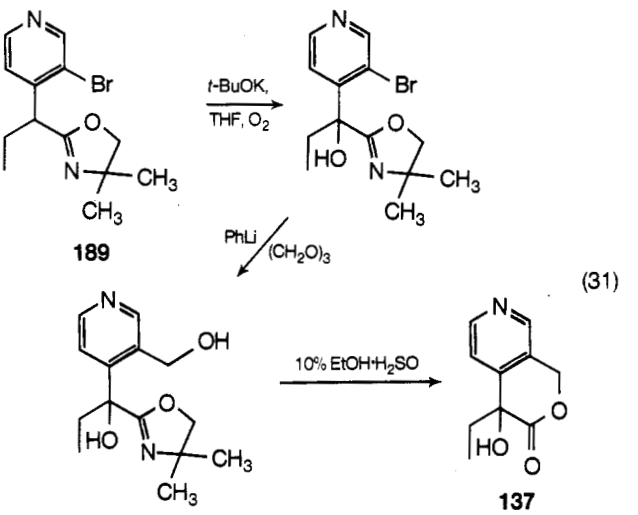


Dehydrogenation of **181** with DDQ in benzene followed by acid-catalyzed lactonization gave the pentacyclic lactone **54**, the intermediate in Winterfeldt's synthesis of camptothecin (eq 30).

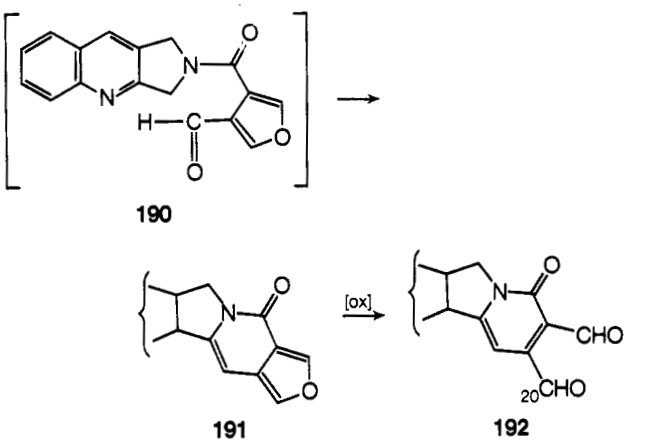


54

In a related study, the bicyclic camptothecin analog **137** was prepared in 45% yield from the 2-oxazoline **189** as shown in eq 31.[72] In this case, the 2-oxazoline system functions as a carboxyl protecting group.



iii. A Furanopyridone Route toward the Total Synthesis of Camptothecin. The potential camptothecin precursor **191** has been prepared by Kende and coworkers by the cyclization of the unstable amidoaldehyde **190**.[73] It was anticipated that oxidative cleavage of the furan ring in **191** would result in the dialdehyde **192**. Selective nucleophilic acylation of the C_{20} carbonyl, *e.g., via* the Passerini reaction,[74] would establish the E ring of camptothecin.



The synthesis of **191** is shown in eq 32. Monosaponification of methyl furan-3,4-dicarboxylate (**193**) followed by reduction with diborane and reoxidation with activated manganese dioxide gave the aldehyde ester **194**. Although an unstable aldehyde acid (**194**, H instead of CH_3) could be obtained by careful acid hydrolysis of the ester **194**, protection of the aldehyde as its hemithioketal **195** proved desirable for subsequent operations. Compound **195** was prepared by the reaction of 2-mercaptoethanol with **194** in the absence of Lewis acid catalysts, followed by mild alkaline hydrolysis.

Condensation of acid **195** with amine **177** using dicyclohexylcarbodiimide (DCC) at 0° yielded the hemithioketal amide **196**.

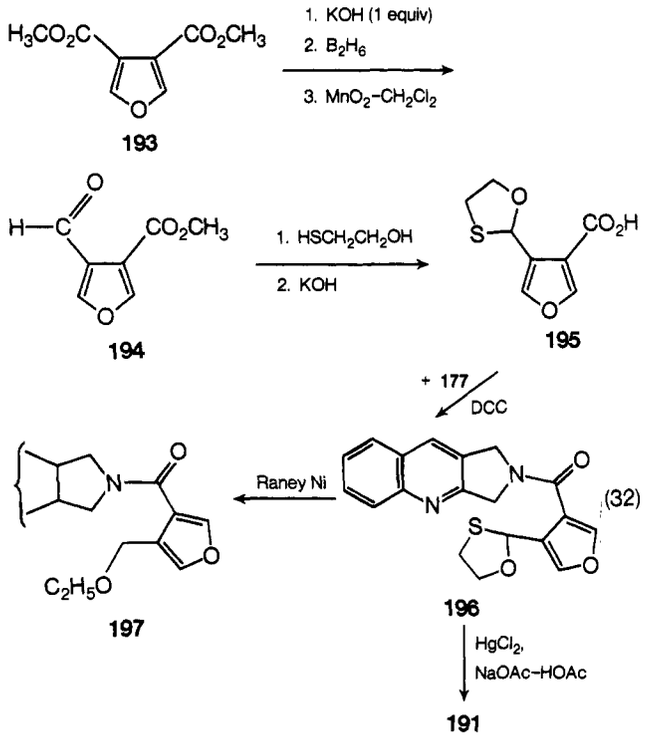
Attempted regeneration of the carbonyl from its hemithioketal in **196** using the Raney nickel method[75] resulted

(72) A. I. Meyers and J. F. Hansen, unpublished results.

(73) A. S. Kende, R. W. Draper, I. Kubo, and M. Joyeux, Abstracts, 160th National Meeting of the American Chemical Society, Chicago, Ill., 1970, No. ORGN-10.

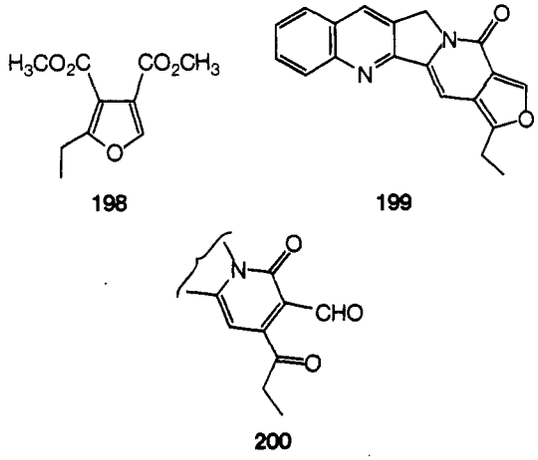
(74) P. Kurtz in Houben-Weyl-Müller, "Die Methoden der organischen Chemie," Vol. 8, Georg Thieme, Liepzig, 1952, p 355.

(75) C. Djerassi, M. Shamma, and T. Y. Khan, *J. Amer. Chem. Soc.*, **80**, 4723 (1958).

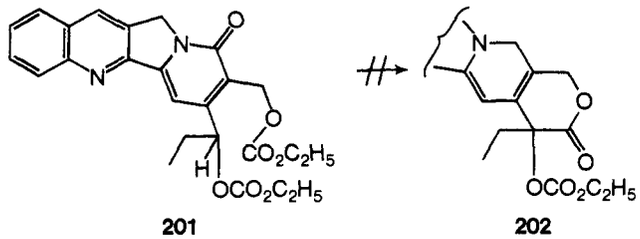


only in the desulfurized product **197**, so hydrolysis was accomplished by warming the hemithioketal **196** with mercuric chloride in acetic acid containing sodium acetate. The isolated product, however, was not the aldehyde **190** but rather the cyclized furanopyridone **191**.

Starting with the ethylfuran derivative **198**, the substituted furanopyridone **199** also was prepared.[76] Although **191** with lead tetraacetate and hydrolysis gave **192**, and **199** could be oxidized with nitric acid to produce some **200**, neither dicarbonyl intermediate could be utilized in

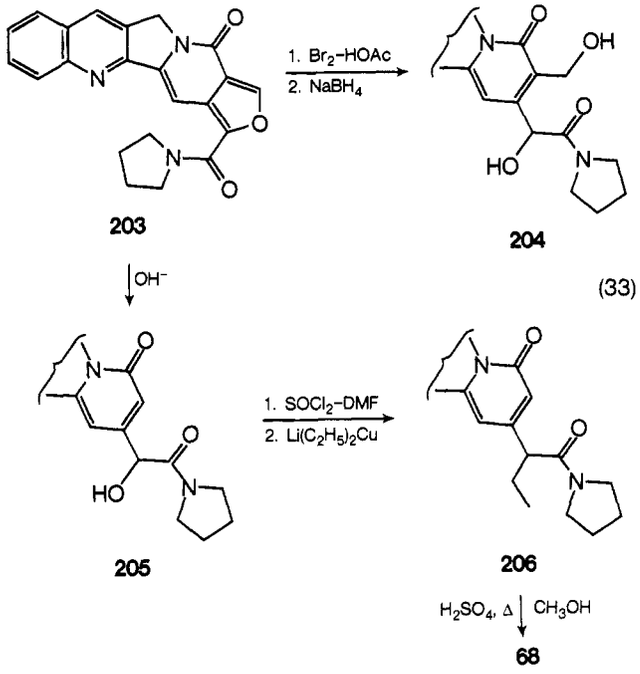


the proposed lactone elaboration sequence. Accordingly, **200** was reduced and cathylated to the diester **201** which, however, failed to undergo the desired base-catalyzed rearrangement to **202**.



(76) A. S. Kende, unpublished results.

In order to circumvent the problems encountered in the introduction of the lactone carbonyl, Kende has prepared the pentacyclic furanoamide **203** by a procedure similar to that described for the construction of **191** and **199**, in a satisfying (10%) overall yield.[76] Amide **203** could be converted to the diol amide **204** in 85% yield (eq 33). The conversion of **204** into camptothecin is currently under investigation.

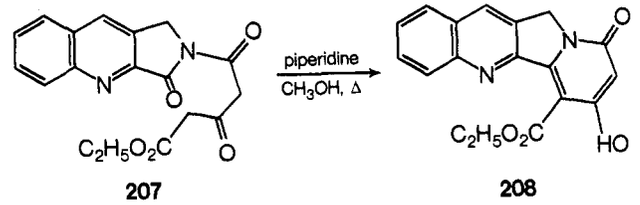


(33)

Recently, Kende has reported that the furanoamide **203** has been converted to ester **68**, an intermediate in Danishefsky's synthesis of camptothecin (eq 33).[77] Of note is the facile hydrolysis with loss of one carbon atom which occurred when **203** was treated with alkali to give **205** in 74% yield.

d. Cyclization of an Imido Ester

Sugasawa, Toyoda, and Sasakura converted the imido ester **207** to the tetracyclic pyridone **208** (87% yield).[78]

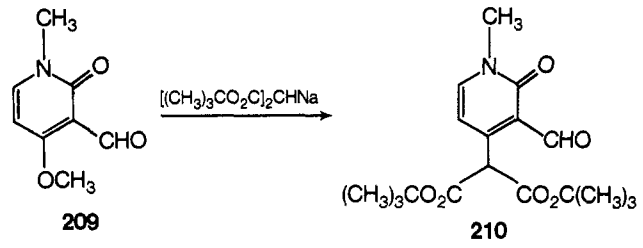


Imido ester **207** was prepared by condensation of the previously reported tricyclic lactone **123**[49,62] with diethyl acetonecarboxylate. That a β-hydroxypyridone such as **208** would serve as a useful intermediate in the synthesis of camptothecin was suggested by the successful addition of a malonic ester to the 3-formylpyridone **209** to give **210**.[78]

Conversion of **208** to the required tetracycle **212** was accomplished in 50% overall yield from **123** (eq 34). Unfortunately, reaction of **212** with di-*tert*-butyl malonate failed to produce a useful reaction product, presumably because of the instability of **212** toward base. Reduction of **211** with Adams' catalyst gave the *N*-tetrahydroquinoline **213**. *N*-Formylation of **213** followed by a Vilsmeier

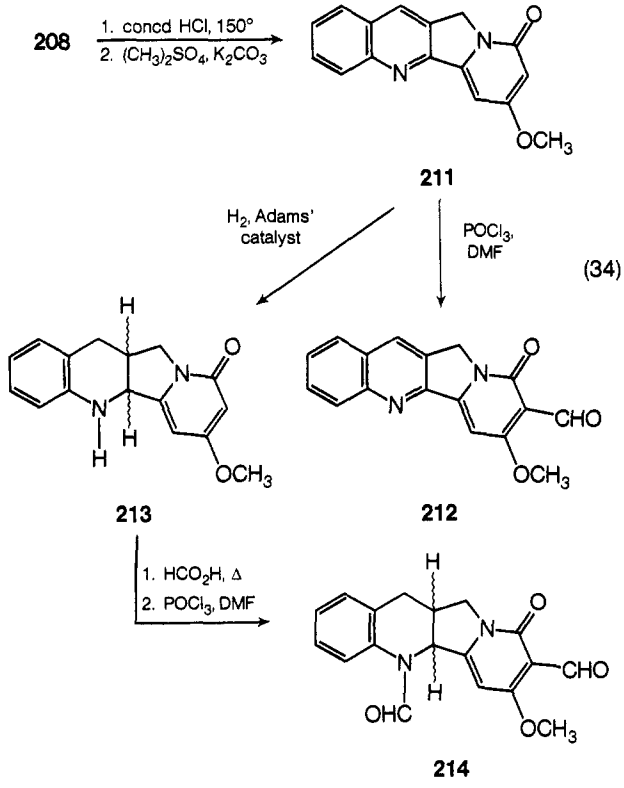
(77) A. S. Kende, T. J. Bentley, R. W. Draper, J. K. Jenkins, M. Joyeux, and I. Kubo, *Tetrahedron Lett.*, 1307 (1973).

(78) T. Sugasawa, T. Toyoda, and K. Sasakura, *Tetrahedron Lett.*, 5109 (1972).

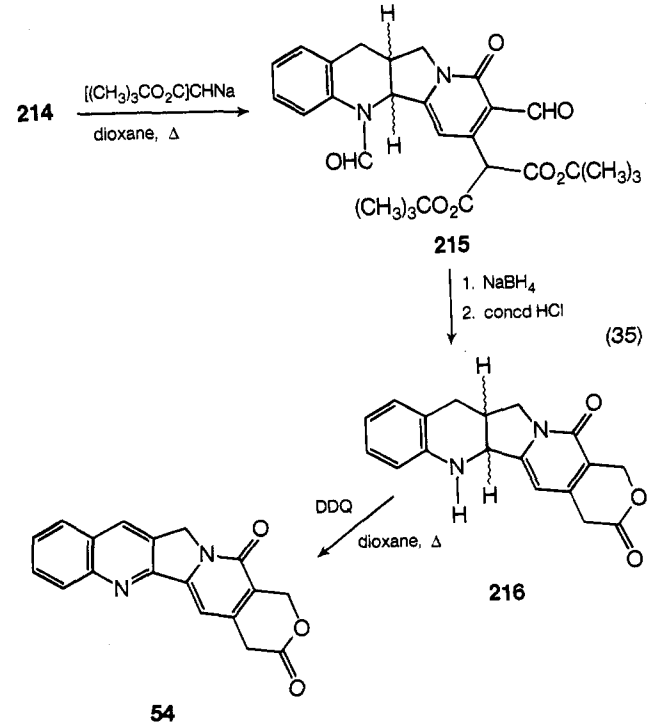


209 210

reaction (phosphoryl chloride in dimethylformamide) afforded a Michael acceptor **214** which more closely resembled the model compound **209.**

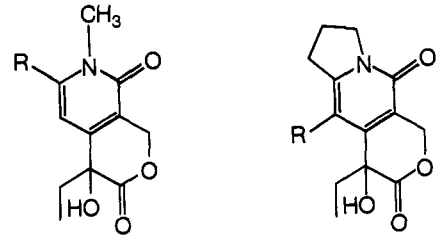


Pyridone **214** underwent addition with di-*tert*-butyl malonate to give **215** in 60% yield (eq 35). Selective borohydride reduction and treatment with concentrated hydrochloric acid gave the pentacyclic lactone **216.** Dehydrogenation of **216** with dichlorodicyanoquinone (DDQ) in refluxing dioxane gave lactone **54** (an intermediate in Winterfeldt's synthesis of camptothecin) in 32% yield from **215.**



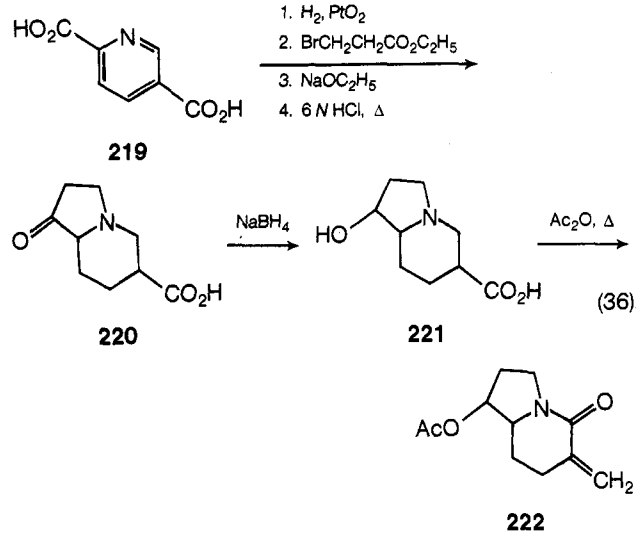
8. *Pyridones via an α-Methylene Lactam Rearrangement of a Nipecotic Acid*

Quite recently, Rapoport and coworkers reported a high yield synthesis of camptothecin[79] and the DE and CDE ring analogs **217** and **218.**[80] An interesting feature



217, R = H; CO_2CH_3; C_6H_5 **218,** R = H; Br

in their approach involves the α-methylene lactam rearrangement[81] of the nipecotic acid **221** (prepared in 73% yield from pyridine-2,5-dicarboxylic acid as shown in eq 36) to give piperidone **222.** Conversion of **222** to the dihy-



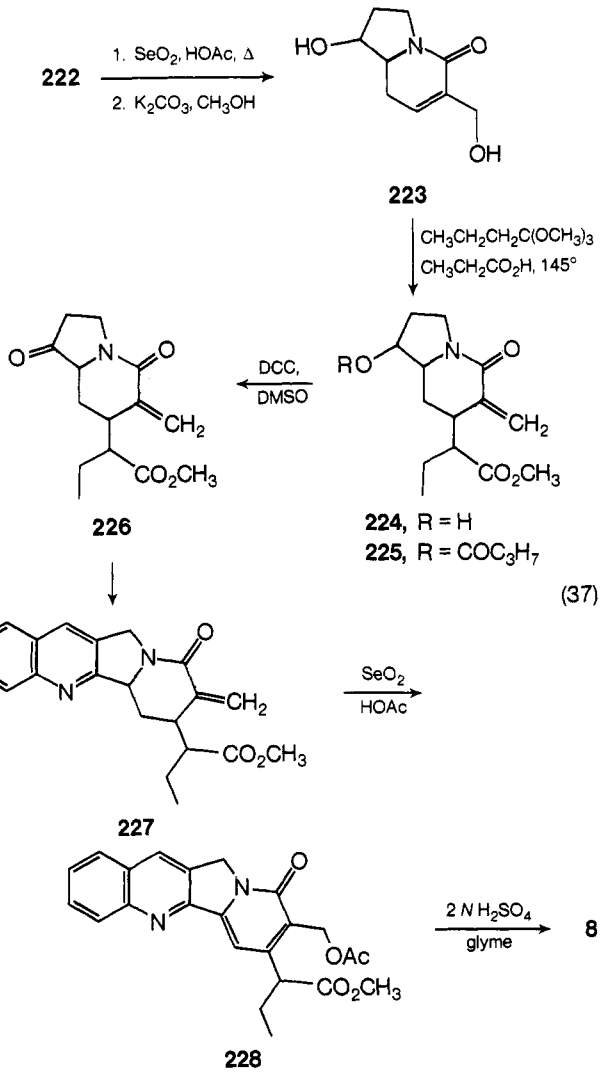
dro pyridone allylic alcohol **223** permitted the remaining ring E carbon atoms to be introduced by a Claisen rearrangement (eq 37). Thus, treatment of diol **223** with excess trimethyl orthobutyrate resulted in a 75% yield of material containing the α-butyrate side chain as a mixture of the free alcohol **224** and its butyrate ester **225.** Hydrolysis of the crude reaction mixture with potassium carbonate in methanol followed by oxidation of the free alcohol with dicyclohexylcarbodiimide (DCC) gave the ketone **226.**

Friedländer condensation of **226** with *N*-(2-aminobenzylidene)-*p*-toluidine[30] gave the tetracyclic α-methylene lactam **227.** Rings AB have been incorporated at earlier stages in the synthetic scheme to provide intermediates that potentially could be converted to camptothecin.

Both aromatization of ring D and formation of the necessary ring E primary allylic alcohol were accomplished in one experimental step by selenium dioxide oxidation of

(79) C. Tang and H. Rapoport, *J. Amer. Chem. Soc.*, **94,** 8615 (1972).
(80) J. J. Plattner, R. D. Gless, and H. Rapoport, *J. Amer. Chem. Soc.*, **94,** 8613 (1972).
(81) M. Ferles, *Collect. Czech. Chem. Commun.*, **29,** 2323 (1964).

227 in glacial acetic acid. The resulting α-acetoxymethylpyridone 228 was converted to deoxycamptothecin (8) by an acid-catalyzed hydrolysis-lactonization. Rapoport and coworkers obtained a quantitative conversion of 8 to *dl*-camptothecin using Winterfeldt's cuprous chloride–dimethylformamide–oxygen reaction system. An impressive 11% overall yield from pyridine-2,5-dicarboxylic acid to *dl*-camptothecin is claimed for this potentially versatile synthesis.



IV. Camptothecin as a Chemotherapeutic Drug

Initial reports of the high activity of camptothecin against animal tumors prompted an intensive investigation of the chemotherapeutic properties of camptothecin and its derivatives in experimental and clinical situations.[7,82-96]

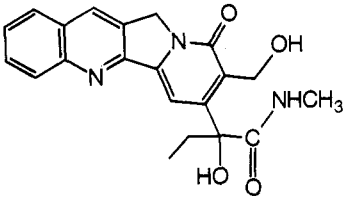
A. Laboratory Studies[97]

Some broad preliminary conclusions with regard to structure–activity relationships in the ability of camptothecin and its analogs to suppress the growth of tumors in experimental animals has been presented.[96] Thus, nuclear substitution in the benzene ring [*i.e.*, 10-hydroxycamptothecin (2) and 10-methoxycamptothecin (3)] has little effect on antitumor activity. Oxidation of the quinoline to the *N*-oxide reduces but does not destroy activity. The lactone group appears to be essential because activity is lost after reduction to the lactol 11.

The 20-hydroxyl group also seems to be essential for antitumor activity. Thus, chlorocamptothecin (4) and camptothecin acetate (5) are inactive.[96] Replacement of the hydroxyl group with hydrogen was claimed to cause no great reduction in activity;[96] however, it now appears that deoxycamptothecin (8) is indeed inactive.[11,98,99]

It is significant that cytotoxicity data indicate that the racemic DE ring analog 77 has about 1% the potency of 1.[45] While the relationship between cytotoxicity and antitumor activity is not well established, the facile synthesis of 77 should certainly encourage further testing.

The activity of the lactone-ring opened derivatives [*i.e.*, camptothecin sodium salt 10 and camptothecin methylamide (229)] is believed to be due to their subsequent cyclization to the lactone after administration to an animal.[96] That camptothecin sodium salt proved to be active was of practical importance because saline solutions of 10 could be administered orally in preclinical pharmacology and clinical studies.



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A review article describing the antitumor and antileukemic activity of camptothecin and numerous derivatives has been prepared by Monroe E. Wall,[98] and the reader is referred to this article for further details.

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(93) U. Schaeppi, D. A. Cooney, and R. D. Davis, *U. S. Clearinghouse Fed. Sci. Tech. Inform.*, PB Rep 1967, PB-180549, 13 pp.

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(95) D. D. Tyrer, I. Kline, M. Gang, A. Goldin, and J. M. Venditti, *Cancer Chemother. Rep.*, **53**, 229 (1969).

(96) J. L. Hartwell and B. J. Abbott in "Advances in Pharmacology and Chemotherapy," Vol. 7, S. Garattini, A. Goldin, F. Hawking, and K. J. Kopin, Ed., Academic Press, New York, N. Y., p 137.

(97) A microbiological assay for estimating concentrations of camptothecin (or cytotoxic equivalents) in mouse tissue (ref 90) and a fluorometric determination of camptothecin in body fluids (ref 89) have been reported.

(98) M. E. Wall, Abstracts, 4th International Symposium on Biochemistry and Physiology of Alkaloids, Halle, 1969, Band a d. Symposium, Ber. 1, Akademie-Verlag, Berlin, 1969, p 77.

(99) In 1966, the first sample of deoxycamptothecin (8) was prepared in M. E. Wall's laboratory. It was shown to be active at a dose about 15 times higher than the dose at which camptothecin is active, and inactive at lower doses. Subsequently, it was found that this sample was contaminated with about 5–10% of camptothecin. Wall and his associates prepared a much purer sample which had a maximum of 1% of camptothecin present. This sample was essentially inactive at high dose levels and is totally inactive at levels where camptothecin always shows high activity: private communication from M. E. Wall.

B. Clinical Studies

Camptothecin has been subjected to preliminary[87] and phase II clinical studies with gastrointestinal cancer.[91,92] Unfortunately, camptothecin has an extremely toxic effect on both animals and man.[93,94] Because of this high toxicity, camptothecin is no longer of prime interest in clinical testing.[7] Hopefully, structural modifications of camptothecin may hold future promise in clinical screening.

V. Effects of Camptothecin on Macromolecular Synthesis

Although camptothecin may prove to be of rather limited value as a chemotherapeutic agent, recent studies indicate that the alkaloid may be a useful probe for the study of macromolecular synthesis in animal cells.

In 1970, Bosmann reported that camptothecin at a concentration of 1 mg per ml greatly inhibits DNA and RNA synthesis in human cervical carcinoma cell culture (HeLa) and mouse lymphoma cell culture (L5178Y).[100,101] Protein synthesis was only slightly inhibited. Interestingly, camptothecin had no effect on rat liver or brain mitochondria and *Escherichia coli* macromolecular synthesis.

The mitochondrion is a subcellular particle providing membrane surfaces upon which energy conversion can occur and is also an organelle capable of autonomous synthesis of nucleic acids, lipids, and glycoproteins. Because of this autonomy, mitochondria are thought to be similar in some respects to bacteria. Mitochondria and *E. coli* have been found to be similar in their response to inhibition of macromolecular synthesis by certain antibiotics, the response in some cases being the opposite of that found in mammalian whole cells or tissues.[100,101] Thus, camptothecin may be important as a tool for inhibiting whole cell nuclear nucleic acid synthesis but not mitochondrial nucleic acid synthesis.

Related studies on camptothecin have utilized very low concentrations of the drug. Treatment of L1210 mouse leukemia cells in culture with camptothecin at concentrations of 10 μg/ml during the S-phase of mitosis arrests subsequent cell division.[82,102,103] While the cells can never divide, removal of the drug allows considerable labeled thymidine incorporation into the DNA.[101]

In similar experiments with L1210 cells, Kessel found that camptothecin (50 μg/ml) inhibited incorporation of labeled uridine into 45S RNA with remarkable rapidity (10 sec, 80% inhibition). The camptothecin-insensitive incorporation of uridine into RNA represents mainly synthesis of tRNA (*i.e.*, 4S and 5S RNA)[104] and mRNA.[105] However, the drug does not inhibit maturation of prelabeled 45S RNA and *removal of the drug immediately restored synthesis of 45S RNA.* In contrast, actinomycin D required 15–20 min to inhibit 80% of RNA synthesis, and the inhibition was irreversible.[104]

When the L1210 cells were treated for longer periods (2 hr), RNA sedimenting at 23 S accumulated within the nucleus.[105,106] This unusual RNA was rapidly lost when the drug was removed and the cells were transferred to a medium containing high levels of actinomycin D.

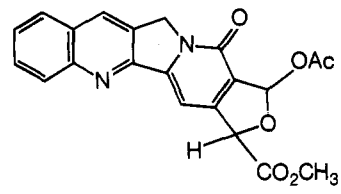
In camptothecin-treated L1210 cells, several hours are required for protein synthesis inhibition.[107] At about this time, drug-induced inhibition of 45S RNA synthesis begins to become irreversible.

Several camptothecin-resistant sublines (L1210/CN) were derived by treatment of animals bearing L1210 with 25 mg per kg of camptothecin.[107] These drug-resistant sublines overcame *in vivo* camptothecin-induced inhibition of nucleic acid synthesis before this became irreversible. Because no evidence to indicate a barrier to drug uptake by the resistant cells was found, it was concluded that L1210/CN cells can detoxify the drug or repair drug-induced damage before drug effects become irreversible.

Treatment of mice with camptothecin leads to considerable toxicity. If camptothecin is administered together with an inhibitor of protein synthesis, such as cycloheximide, the toxicity is considerably reduced. However, the camptothecin–cycloheximide combination is not any less lethal to L1210 cells than is camptothecin alone.[105]

In another study with L1210 cells, Hacker and coworkers have reported that camptothecin sodium salt **10** (50 μg/ml) inhibits DNA and RNA synthesis *in vitro* within 15 min by 40 and 71%, respectively.[108,109] Again, inhibition of protein synthesis was not observed at this or at higher concentration of camptothecin.

In an effort to determine a structure–activity relationship, several intermediates encountered in Kende's camptothecin synthesis were examined by Hacker.[109] *In vitro* testing in L1210 cells indicated the following order for those compounds that exhibited a high degree of nucleic acid biosynthesis inhibition: group I (some substituted dihydropyrroloquinolines, *e.g.*, compound **117** but not **83**) > group II (camptothecin) > group III (pentacyclic furanopyridones, *e.g.*, compound **199**). The pentacyclic pyridones **28** and **230** showed little if any inhibitory activity.



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In vivo treatment of male mice tumored with L1210 cells was performed with camptothecin and selected derivatives.[109] When nucleic acid biosynthesis was measured (incorporation of radioactive thymidine into DNA or uridine into RNA), only camptothecin was active. Inhibition (80%) of DNA synthesis occurred within 1 hr and persisted up to 24 hr, while interference with RNA synthesis appeared to be slower (45% inhibition after 4 hr).

Hacker suggests that the lack of correlation between direct testing of each derivative *in vitro* and their effects *in vivo* indicate the need for such correlation when evaluating new agents. In addition, the present results suggest that camptothecin may undergo some sort of biological transformation to become an active antitumor metabolite, *in vivo*.[109]

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(101) H. B. Bosmann, *J. Biol. Chem.*, **246**, 3817 (1971).

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(107) D. Kessel, *Cancer Res.*, **31**, 1883 (1971).

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(109) B. Hacker, A. S. Kende, and T. C. Hall, *Biochem. Pharmacol.*, in press.

Horwitz, Chang, and Grollman also have studied the effects of camptothecin on nucleic acid and protein synthesis in HeLa cells.[110,111] Their results are consistent with the work of Bosmann[100,101] and Hacker.[108,109] Thus, camptothecin was observed to inhibit both DNA and ribosomal RNA synthesis in treated cells. However, DNA that was isolated from cells inhibited by camptothecin had a sedimentation constant of 40 S in contrast to the high molecular weight DNA (90 S) found in untreated cells. Removing camptothecin from the media completely reversed the inhibition of RNA synthesis while DNA synthesis was only partially reversed.

In vitro testing of camptothecin derivatives by these workers revealed that camptothecin methylamide (**229**), the sodium salt of camptothecin (**10**), 10-methoxycamptothecin (**3**), and deoxycamptothecin (**8**) all have nearly the same inhibitory effect as camptothecin on nucleic acid synthesis in HeLa cells.[111] Only camptothecin lactol (**11**) had a significantly lower activity. These results correspond closely to the ability of these compounds to suppress the growth of tumors in experimental animals.[96] In light of Hacker's results, however, it would be interesting to determine if the *in vivo* effects of the camptothecin derivatives would correlate with these *in vitro* results.

Camptothecin does not significantly inhibit activity of the DNA-dependent RNA polymerase isolated from *E. coli* or of DNA and RNA polymerases prepared from HeLa cells.[111] In another study, camptothecin was found to be only a weak inhibitor of an RNA-dependent DNA polymerase in L5178Y and L1210 cells.[112]

The mechanism by which camptothecin affects nucleic acid synthesis is unclear. It is apparent, however, that both the RNA[105,106] and DNA[111] that is present in camptothecin-treated cells have unusually low sedimentation values. These results suggest that DNA and RNA may be partially degraded in camptothecin-treated cells. A similar degradation of template DNA could account for the observed inhibition of DNA and RNA synthesis in animal cells.[111]

The postulated degradation of DNA could occur as a result of drug inhibition of an enzyme involved in repair of DNA, such as DNA ligase. Alternatively, camptothecin or a metabolic product may loosely bind to DNA, thus rendering it susceptible to the action of nucleases. Preliminary results indicate that camptothecin binds to DNA under certain experimental conditions.[110,111,113]

(110) S. B. Horwitz, C. K. Chang, and A. P. Grollman, *Pharmacologist*, **12**, 283 (1970).

(111) S. B. Horwitz, C. K. Chang, and A. P. Grollman, *Mol. Pharmacol.*, **7**, 632 (1971).

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Acknowledgments. I sincerely wish to thank the many people who supplied me with information prior to publication. I also wish to thank Professor Gilbert Stork for his valuable suggestions during the preparation of this review article.

VI. Addendum

Camptothecin, in yields as high as 0.1%, along with the minor and hitherto unknown 9-methoxycamptothecin have been isolated from *Mappia foetida* Miers, a small tree, abundant in the Western Ghats of India. This discovery is noteworthy, because the only previously known natural source, the rare *Camptotheca acuminata*, is said to contain only about 0.005% camptothecin.[114]

The synthesis of a potential tetracyclic camptothecin intermediate and related compounds has been reported.[115] Breisler has prepared several antitumor inactive tetracyclic camptothecin analogs using the indole to quinoline conversion.[116] A monocyclic δ-lactone which may be useful in evaluating camptothecin ring E structure–activity relationships[117] and a variety of pyrrolo[3,4-*b*]quinolines[118] have been prepared.

The effect of camptothecin on macromolecular synthesis continues to be studied.[119–121] Camptothecin has been found to be active (*in vitro*) against human cytomegalovirus.[122] In an interesting report, Neale and coworkers have demonstrated that camptothecin blocks memory of conditioned avoidance in goldfish, presumably as a result of inhibition of RNA synthesis.[123] Camptothecin appears to be more selective and reversible in its action on nuclear RNA synthesis, and less toxic to animals than actinomycin. Therefore, camptothecin may be a suitable drug for investigation of the role of RNA in brain function.

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(120) H. B. Bosmann, *Biochem. Pharmacol.*, **21**, 1991 (1972).

(121) M. Artico, *Farmaco, Ed. Sci.*, **27**, 683 (1972); a review of natural antiblastic agents.

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(123) J. H. Neale, P. D. Klinger, and B. W. Agranoff, *Science*, **179**, 1243 (1973).