



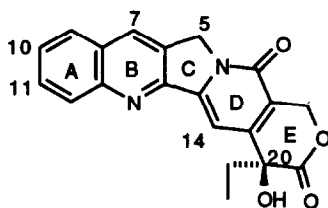
## THE SYNTHESIS AND EVALUATION OF FLEXIBLE ANALOGUES OF THE TOPOISOMERASE I INHIBITOR, CAMPTOTHECIN

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**Abstract:** A convenient synthesis of camptothecin derivatives has been developed in which the D, or D and C rings are incomplete. This approach includes a general route to quinolines such as **12**, and a short synthesis of the key lactone **3**. The two components are easily coupled to give camptothecin derivatives **17-22**. The lack of activity of these compounds confirms the importance of the rigid camptothecin nucleus.

The DNA topoisomerases are ubiquitous enzymes which are responsible for the relief of topological problems arising in the DNA helix, and as such are essential enzymes for the successful completion of nuclear functions such as DNA replication, transcription, recombination and chromatid segregation.<sup>1</sup> Interest in these enzymes was focused by the recognition that many clinically used, anti-tumor drugs e.g., anthracyclines, epipodophyllotoxins, acridines and ellipticine exert their effects via interaction with topoisomerase II.<sup>2</sup> Subsequent to this observation it was found that topoisomerase I is the site of action of the anti-tumor plant alkaloid camptothecin (**1**).<sup>3</sup> The precise mechanism by which camptothecin exerts its anti-tumor activity remains unclear, however the compound appears to induce protein-linked DNA breaks by stabilizing a complex of DNA and topoisomerase I (ternary complex) in both isolated systems and in cultured cells. In addition, studies of camptothecin analogues have suggested a correlation between the ability to cause DNA breaks and anti-tumor effects.<sup>4</sup> Despite its unique mechanism of action, interest in camptothecin as a clinically useful anti-tumor agent was thwarted by the finding that the active form of the compound (closed lactone form)<sup>5</sup> has an extremely poor solubility profile.



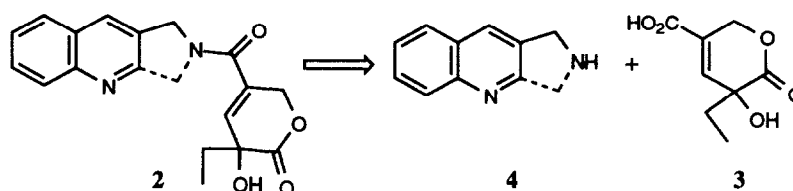
**1** Camptothecin

While a good deal is known about the effects of modification of the A and E rings, relatively little is known about the requirements of the C and D rings.<sup>6</sup> Several key points emerge from a study of the SAR, in particular the A ring is tolerant of substitution (9-amino, 9-hydroxy, 10-hydroxy and 10,11 methylenedioxcamptothecins are more potent than the parent compound) while the lactone ring (E) is very sensitive to alteration (removal of the 20-hydroxyl abolishes activity). We became interested in examining

compounds such as **2** in which the D ring, or C and D rings, are incomplete. The rationale for making such a radical disconnection was the hope that such compounds should be less planar and more flexible and so perhaps might adopt a conformation which could more readily stabilize the ternary complex.

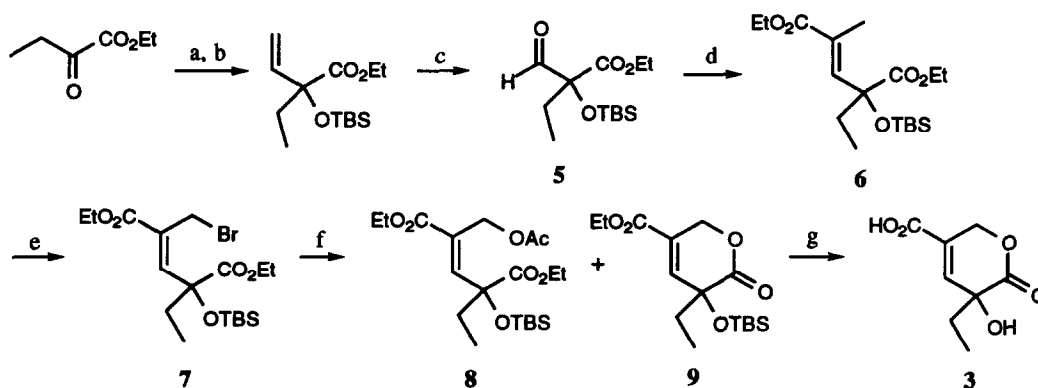
The initial design for the synthesis of this 'bond-deleted' class of compounds involved the simple coupling of an acid such as **3** with an appropriately substituted amine such as **4** via an amide bond (Scheme 1). We therefore required an approach to acid **3** which ideally would be concise, amenable to the preparation of multigram quantities of **3**, and have the potential to afford **3** in optically active form since it is well established that 20(S) camptothecin is more than 20 times more potent than its antipode.<sup>7</sup>

**Scheme 1**



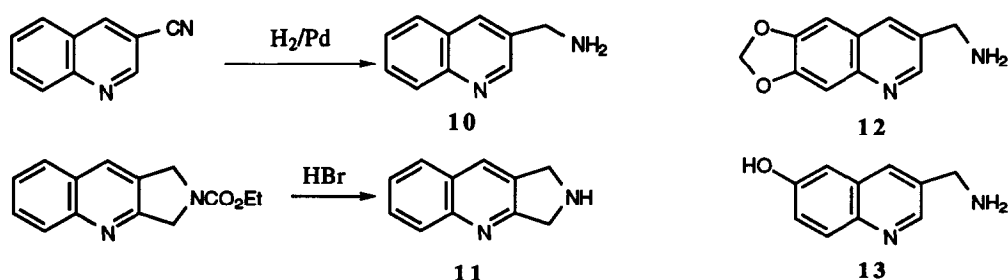
The approach which ultimately afforded **3** is outlined in Scheme 2. The addition of vinylmagnesium bromide to ethyl 2-ketobutyrate gave the hydroxy ester which was protected as a *tert*-butyldimethylsilyl ether.<sup>8</sup> The addition of nucleophiles to optically active esters or amides of  $\alpha$ -keto acids is a well precedented method for the enantioselective synthesis of  $\alpha$ -hydroxy acids,<sup>9</sup> so this approach should be amenable to the preparation of **3** in enantiomerically pure form if needed. Ozonolytic cleavage of the olefin followed by a reductive work-up with dimethyl sulfide cleanly gave the aldehyde **5**. When the aldehyde **5** was reacted with the sodium salt of triethyl 2-phosphonopropionate an equal mixture of the *E*- $\alpha,\beta$ -unsaturated ester, **6**, and the corresponding *Z* isomer resulted. Fortunately, however, use of the corresponding triphenylphosphonium ylide led to the desired *E* isomer, **6**, exclusively.<sup>10</sup> Allylic functionalization was achieved smoothly by the use of N-bromosuccinimide in carbon tetrachloride to give the bromide **7** which, on treatment with silver acetate, was transformed into the allylic acetate **8**. Interestingly, the major product **8** was accompanied by significant amounts (33%) of the lactone **9**. This compound is probably the result of intramolecular attack by the ester carbonyl on the highly polarized carbon-bromine bond followed by (nucleophile assisted) dealkylation. These two compounds could be readily separated by silica gel chromatography however they were more conveniently carried forward to the next step as a mixture. When this mixture was subjected to basic hydrolysis conditions followed by acidification a very pleasing series of transformations occurred such that the desired lactone **3** was isolated directly as a clean product in 66% yield from the bromide **7**.

Scheme 2



With acid **3** now secured, attention now turned to the quinoline portion of the molecule. In particular, four quinolines were targeted which had structural similarities to known potent camptothecin derivatives. The 'parent' aminomethylquinoline **10** was readily prepared from 3-cyanoquinoline,<sup>11</sup> while the tricyclic compound **11** was available by hydrolysis of the corresponding ethyl carbamate (Scheme 3).<sup>12</sup> The substituted quinolines **12** and **13** were chosen as targets since the 10,11-methylenedioxy and 10-hydroxycamptothecins are significantly more potent than camptothecin itself.<sup>6</sup>

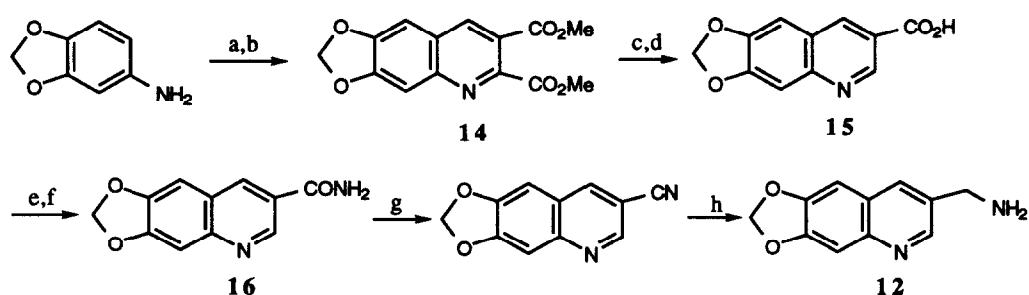
Scheme 3



Central to the design of a synthesis of quinolines of this type was the need for potential flexibility toward the preparation of compounds bearing a variety of substitutions. The successful synthesis developed

for the preparation of **12** is outlined in Scheme 4. The reaction of 3,4 methylenedioxyaniline with dimethyl acetylenedicarboxylate gives an enamine which is cleanly cyclized using the Vilsmeier conditions to give the quinoline-2,3-dicarboxylic ester **14**.<sup>13</sup> Saponification followed by regioselective decarboxylation of the 2-carboxylate function<sup>14</sup> gave the quinoline 3-carboxylic acid **15** which was routinely transformed to the corresponding amide **16**. All attempts to effect the direct reduction of the amide function to an aminomethyl group were unsatisfactory and it was found that a two step procedure of dehydration<sup>15</sup> followed by hydrogenation readily gave the desired compound. A similar series of transformations were performed using 4-benzyloxyaniline to afford quinoline **13**.

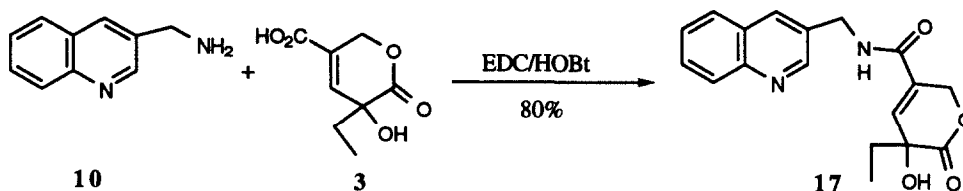
Scheme 4



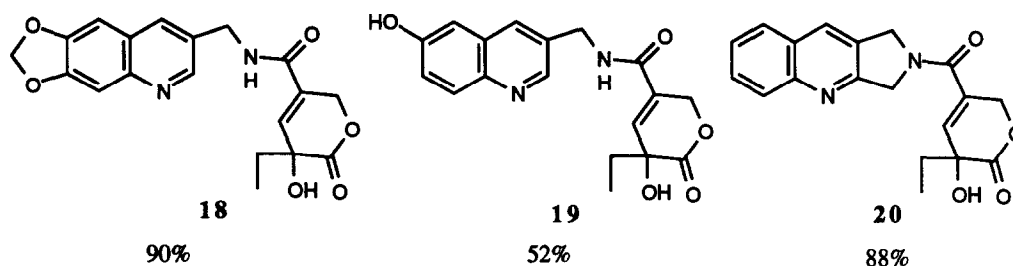
a) dimethyl acetylenedicarboxylate, b) DMF/ $\text{POCl}_3$ , c)  $\text{KOH}/\text{MeOH}$ , d) quinoline/ $180^\circ\text{C}$ , e)  $\text{SOCl}_2$ , f)  $\text{NH}_3/\text{MeOH}$ , g) trifluoroacetic anhydride/pyridine, h)  $\text{H}_2/\text{Pd/C}$

With **3** and an array of quinoline amines now in hand the camptothecin analogues were readily assembled using a standard peptide coupling procedure (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride/1-hydroxybenzotriazole) to give compounds **17-20** in reasonable yields (52-88%) (Scheme 5).

Scheme 5

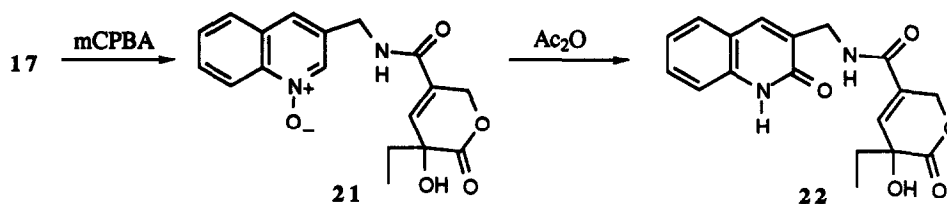


Similarly prepared:



The availability of compound **17** allowed us to further investigate this class of compounds. The quinolone **22** was prepared easily from **17** via a Polonovski type rearrangement<sup>16</sup> of the corresponding quinoline N-oxide **21** (Scheme 6). This compound offered the possibility of yielding a structure intermediate in rigidity between the amides **17** and **20** since intramolecular hydrogen bonding between the quinolone carbonyl and the amide hydrogen might be expected to confer some conformational stability.

**Scheme 6**



The ability of compounds **17-22** to inhibit topoisomerase I was determined by the method described by Hsiang.<sup>3</sup> Specifically, the compounds were incubated with DNA and calf thymus topoisomerase I for 30 min. then the reactions were terminated with SDS and denatured with aqueous base. The degree of DNA nicking, and hence ability of the compound to stabilize a DNA-topoisomerase I-drug ternary complex, was assayed by gel electrophoresis using agarose gel. The amount of fragmentation was standardized to that induced by camptothecin.

None of the compounds (**17-22**) showed any evidence of ternary complex stabilization, as determined by the procedure described above, when tested at concentrations up to ca. 200  $\mu\text{M}$ . Under the assay conditions used, camptothecin was found to have an  $\text{IC}_{50}$  of 0.7  $\mu\text{M}$ .<sup>17</sup>

#### Acknowledgement

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## References and Notes

1. Osheroff, N. *Pharmac. Ther.* **1989**, *41*, 223-241.
2. Rose, K. M. *FASEB*, **1988**, 2474-2478. D'Arpa, P.; Liu, L. F. *Biochimica et Biophysica Acta.* **1989**, *989*, 163-177. A comprehensive review of the topoisomerases and their inhibitors can be found in *DNA Topoisomerases in Cancer*; Potmesil, M.; Kohn, K.W., Eds.; Oxford University Press: New York, 1991.
3. Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. *J. Am. Chem. Soc.* **1966**, *88*, 3888-3890. Hsiang, Y. H.; Hertzberg, R. P.; Hecht, S.; Liu, L. F. *J. Biol. Chem.*, **1985**, *260*(27), 14873-14878.
4. Horwitz, S. B. In *Antibiotics III. Mechanism of Action of Antimicrobial and Antitumor Agents*; Corcoran, J. W.; Hahn, F. E., Eds.; Springer: New York, 1975; pp 48-57.
5. Hsiang, Y. H.; Liu, L. F.; Wall, M. E.; Wani, M. C.; Nicholas, A. W.; Manikumar, G.; Kirschenbaum, S.; Silber, R.; Potmesil, M. *Cancer Research*, **1989**, *49*, 4385-4389.
6. Wani, M. C.; Nicholas, A. W.; Wall, M. E. *J. Med. Chem.* **1986**, *29*, 2358-2363. Wani, M. C.; Ronman, P. E.; Lindley, J. T.; Wall, M. E. *J. Med. Chem.* **1980**, *23*, 554-560. Nicholas, A. W.; Wani, M. C.; Manikumar, G.; Wall, M. E.; Kohn, K. W.; Pommier, Y. *J. Med. Chem.* **1990**, *33*, 972-978, and references cited therein. For a recent description of the effects of D-ring substitution on camptothecin analogues see: Crow, R. T.; Crothers, D. M. *J. Med. Chem.* **1992**, *35*, 4160-4164.
7. Wani, M. C.; Nicholas, A. W.; Wall, M. E. *J. Med. Chem.* **1987**, *30*, 2317.
8. Corey, E. J.; Cho, H.; Rucker, C.; Hua, D. H. *Tetrahedron Lett.* **1981**, *22*, 3455-3458.
9. Whitesell, J. K.; Bhattacharya, A.; Henke, K. J. *J. Chem. Soc., Chem. Commun.* **1982**, 988. Comins, D. L.; Baevsky, M. F.; Hong, H. *J. Am. Chem. Soc.* **1992**, *114*, 10971-10972.
10. An excellent review of the Wittig olefination can be found in: Maryanoff, B. E.; Reitz, A. B. *Chem. Rev.* **1989**, *89*, 863-927.
11. Kametani, T.; Kigasawa, K. *Chem. Pharm. Bull.* **1966**, *14*, 566-571.
12. Compound **11** was a key component in the enantioselective synthesis of 20-(S)-camptothecin reported by Professor Corey: Corey, E. J.; Crouse, D. N.; Anderson, J. E. *J. Org. Chem.* **1975**, *40*, 2140-2141. The ethyl carbamate of compound **11** was kindly supplied by Professor Andrew Kende (University of Rochester).
13. The preparation of quinoline dicarboxylic acids from anilines is described in: Los, M. UK Patent Application GB 2174395A, 1986.
14. The relative reactivity of 2-vs 3-pyridinecarboxylates is well established, for example see: Hoogewerff, S.; van Dorp, W. A. *Chem. Ber.* **1880**, *13*, 61.
15. Campagna, F.; Carotti, A.; Casini, G. *Tetrahedron Lett.* **1977**, 1813-1816.
16. Markgraf, J. H.; Brown, H. B.; Mohr, S. C.; Peterson, R. G. *J. Am. Chem. Soc.* **1963**, *86*, 958.
17. The 'cleavable complex' assay is performed under a limiting concentration of DNA, such that an EC<sub>50</sub> represents the drug concentration required to capture 50% of the DNA as a ternary complex.

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