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Synthesis of 14-Azacamptothecin, a Water-Soluble Topoisomerase I Poison

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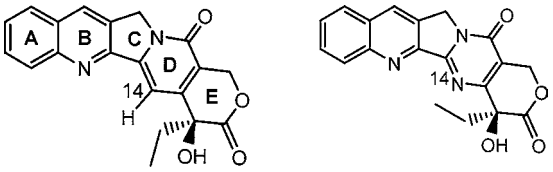
ABSTRACT



14-Azacamptothecin, a potent, water-soluble analogue of the antitumor agent camptothecin, has been prepared by a convergent synthesis. The key condensation of the AB and DE rings with concomitant formation of ring C of 14-aza CPT was carried out in two stages, the latter of which involved a radical cyclization strategy.

Camptothecin (CPT, **1**) (Figure 1) is an alkaloid first isolated from *Camptotheca acuminata*.[1] While exhibiting potent antitumor activity, early clinical trials of CPT were complicated by difficulties in formulation of the compound, which has very poor aqueous solubility.[2] Subsequently, CPT was shown to function by stabilizing the covalent binary complex formed between DNA topoisomerase I and its DNA substrate, a complex that is normally a transient intermediate involved in DNA relaxation during replication and transcription.[3] This permitted the optimization of the antitumor properties of this class of agents; two members of the class are presently employed clinically for the treatment of solid tumors[4] and several analogues are in clinical trials.[5]

Optimization of the CPTs as topoisomerase I poisons revealed the importance of the E ring of CPT to stabilization of the topoisomerase I−DNA covalent binary complex.[6] Simple alteration of the stereochemistry of the 20-OH group, or deoxygenation to afford 20-deoxy CPT (**2**), resulted in complete loss of covalent binary complex stabilization.[7]

Given the importance of the E-ring α-hydroxylactone moiety of CPT to its properties as a topoisomerase I poison,

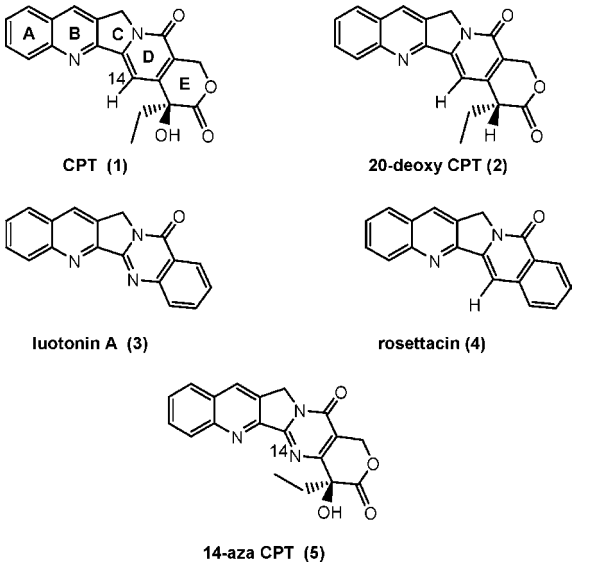


Figure 1. Structures of CPT, luotonin A, and key analogues thereof.

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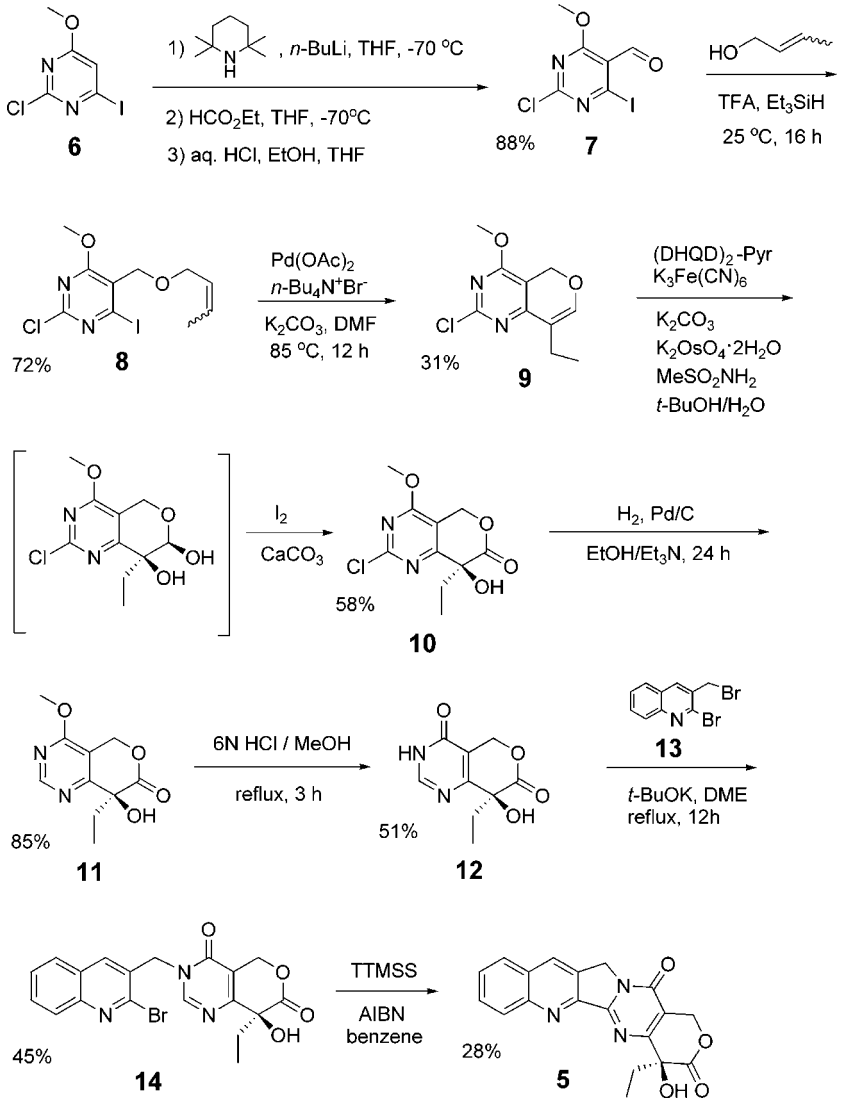
Scheme 1. Route Employed for the Synthesis of 14-Azacamptothecin



it was surprising to find that the pyrroloquinazolinoquinoline alkaloid luotonin A (**3**) exhibited reasonable potency as a topoisomerase I poison and topoisomerase I dependent cytotoxic agent, and stabilized enzyme-linked DNA breaks with the same sequence selectivity as CPT itself.[8] An extensive study of effects of modification of the E-ring of luotonin A have facilitated an understanding of those structural features essential to support the function of luotonin A as a topoisomerase I poison but have not completely explained how luotonin A can function in the absence of structural elements analogous to those apparently essential for the action of CPT, such as the 20 (*S*)-OH group.[9]

Luotonin A differs from CPT primarily within ring E but also at position 14 within ring D (Figure 1). To explore the possible role of this latter structural element, we prepared the luotonin A having the same functionality at position 14 as CPT itself, i.e., analogue **4**. This compound had been prepared previously and reported to have weak activity as a topoisomerase I poison.[10] While we were able to verify the ability of **4** to stabilize the enzyme−DNA covalent binary complex, testing of the analogue in a yeast strain that expressed human topoisomerase I under the control of a galactose promotor indicated that the cytotoxicity of **4** did **not** result from poisoning of topoisomerase I.[11] Thus the presence of a CH group at position 14 of luotonin A actually eliminated the effect of luotonin A as a topoisomerase I dependent cytotoxin.

The foregoing experiments prompted the synthesis and evaluation of 14-azacamptothecin (**5**) to determine the role

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of the 14 CH functional group in CPT. The synthesis of 14-aza CPT was accomplished as outlined in Scheme 1. Thus commercially available 2-chloro-6-methoxypyrimidine was iodinated as described to afford 4-iodopyrimidine **6**,[12] the latter of which was lithiated (*n*-BuLi, tetramethylpiperidine) and treated with ethyl formate to provide 2-chloro-4-iodo-6-methoxypyrimidine-5-carboxaldehyde (**7**) in 88% yield. Reductive etherification with crotyl alcohol (Et_3SiH, CF_3-COOH) afforded crotyl ether **8** in 72% yield. When compound **8** was heated in DMF for 12 h at 85 °C in the presence of K_2CO_3, $Bu_4N^+Br^-$, and catalytic $Pd(OAc)_2$, cyclized olefin **9** was obtained in 31% yield.[13]

Asymmetric dihydroxylation of **9** was carried out in analogy with the work of Fang et al.[14] by treatment with K_2OsO_4 in the presence of the chiral ligand ($(DHQD)_2$-PYR) (AD-system);[15] product **10** was obtained initially in 58% yield after I_2-mediated oxidation of the lactol to the corresponding lactone but was found by chiral HPLC[16] to consist only ~90% of the desired enantiomer. Optically pure **10** was obtained by crystallization from hexane/CH_2Cl_2. Compound **10** was then dechlorinated to afford **11** by hydrogenolysis over 10% palladium-on-carbon in absolute EtOH (85% yield) and demethylated hydrolytically (6 N HCl, MeOH) to afford pyrimidone **12** in 51% yield.

Pyrimidone **12** was condensed with 2-bromo-3-bromomethylquinoline (**13**)[17] (*t*-BuOK, dimethoxyethane) to afford key intermediate **14** in 45% yield. Radical mediated cyclization[20] of **14** (tris(trimethylsilyl)silane, AIBN) afforded 14-aza CPT (**5**) as a colorless solid in 28% yield.

As shown in Figure 2, 14-aza CPT inhibited the relaxation of supercoiled pSP64 plasmid DNA more potently than CPT.

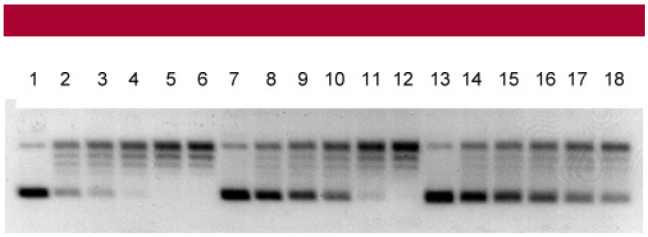

Figure 2. Inhibition of human topoisomerase I mediated DNA relaxation by CPT (**1**) and 14-aza CPT (**5**). Supercoiled DNA plasmid was incubated with 0.1 ng of human topo I as indicated below. Lanes 1−6, topoisomerase I alone for 0, 5, 10, 15, 30, and 60 min, respectively; lanes 7−12, 500 μM CPT (**1**) for 0, 5, 10, 15, 30, and 60 min, respectively; lanes 13−18, 500 μM 14-aza CPT (**5**) for 0, 5, 10, 15, 30, and 60 min, respectively.

As reported previously, 14-aza CPT stabilized DNA cleavage with the same sequence selectivity as CPT itself.[11] Compound **5** also stabilized the topoisomerase I−DNA covalent binary complex almost to the same extent as CPT despite a much faster off-rate (Figure 3). As reported, 14-aza CPT was also strongly cytotoxic toward yeast lacking the homologous topoisomerase I but expressing human topoisomerase I.[11] The structure modification of **5** and in vivo evaluation of appropriate analogues is envisioned and will be reported in due course.

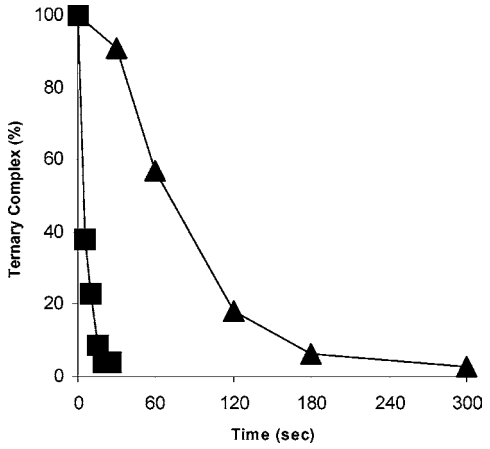

Figure 3. Time course of NaCl-induced dissociation of the topoisomerase I−DNA−inhibitor ternary complexes. Time points were taken after the addition of NaCl to a final concentration of 0.35 M in the presence of CPT (▲) or 14-aza CPT (■).

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Supporting Information Available: Experimental procedures and full characterization for all new compounds shown in Scheme 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) The analysis was carried out by chiral HPLC on a Chirobiotic T column (250 × 4.6 mm); the mobile phase consisted of 10% EtOH in hexane and the column was washed at a flow rate of 1.0 mL/min. The desired product eluted after 43.0 min, while its enantiomer had an elution time of 46.6 min.

(17) Compound **13** was prepared in two steps from 2-chloroquinoline-3-carboxaldehyde by successive treatments with $NaBH_4$[18] and PBr_3;[19] the overall yield was 67%.

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