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Synthetic approaches to homocamptothecin antitumor agents

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Abstract

Homocamptothecins are proving to be an especially interesting class of anti-cancer agents because they resemble standard camptothecins in cytotoxicity but have very different pharmacodyanamic properties. This review summarizes synthetic approaches to the parent homocamptothecin and its analogs. Three powerful and general routes—the Lavergne–Comins route, the cascade radical annulation route, and the Friedlander route—have been put into place to make homocamptothecins. These routes are compared and contrasted. Together, they have driven the SAR, preclinical, and clinical development of this class of anti-cancer agents. ***To cite this article: D. P. Curran, C. R. Chimie 11 (2008).***

Keywords: Camptothecin; Homocamptothecin; Topoisomerase inhibitors; Anti-cancer agents; Tandem radical reactions

1. Introduction

The camptothecins constitute one of the most important classes of antitumor agents in use and development today [1]. The parent camptothecin **1** was isolated from the leaves of the *Camptotheca acuminata* tree by Wani and Wall (Fig. 1), but development was stopped because of problems with solubility and toxicity. Nonetheless, camptothecin retains more than historical importance because it is commercially available at reasonable prices. Second-generation camptothecins were extensively produced by both semi-synthesis and total synthesis during the late 1980s and early 1990s. This work culminated in the introduction of two commercial drugs, topotecan **2** (trade name, Hycamtin) and irinotecan **3a** (trade name, Camptosar). Irinotecan, also commonly known as CPT-11, is a pro-drug whose active component is the 10-hydroxy analog, SN-38 **3b**. These drugs have shown both promise and problems in the clinic, and that combination has spurred an ongoing search for third-generation derivatives. A substantial pipeline of such derivatives now exists [1].

The molecular mechanism of action of the camptothecin class of anti-cancer agents is now relatively well understood [2]. The compounds are classed as ‘‘topoisomerase 1 inhibitors’’ or ‘‘topoisomerase 1 poisons’’. The latter name might be better since the camptothecins do not competitively inhibit topoisomerase 1, a key enzyme that helps to unwind DNA during cell replication. Instead, they bind to a covalent intermediate of the enzyme topoisomerase 1 and DNA. The resulting termolecular complex is stable, DNA replication is blocked, and cell death eventually ensues.

The pharmacodynamics of the camptothecins are fundamentally different from most other drugs. Generally, drugs are single small molecules with associated dynamic features such as bond rotations,

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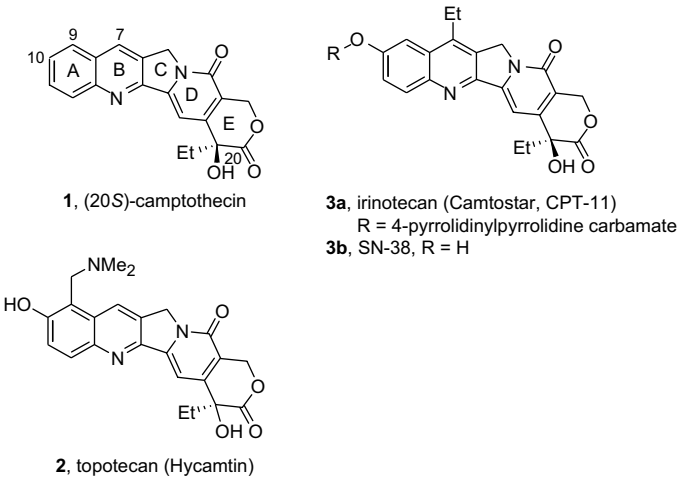


Fig. 1. Structures of camptothecin, topotecan, irinotecan (CPT-11) and SN-38.

ring inversions, etc. These single molecules are then gradually metabolized to other molecules. Camptothecin, in contrast, is a chemically dynamic drug. Under physiological conditions, it exists in relatively rapid chemical equilibrium with its open chain hydroxy acid salt **4** [3] (Fig. 2). This hydroxy acid is not thought to be an active anti-cancer agent. The relative concentration of the two forms depends on many features, some of which (pH, lipophilicity, presence of serum albumin, among others) are now understood. Thus, unlike most drugs, which are gradually metabolized after administration, camptothecins are subject to chemical equilibration both before and during metabolism. This added level of complexity provides opportunities and poses problems at the same time.

Because of the potential problems posed by the dynamic nature of the camptothecins, it would be of interest to have camptothecin analogs that maintain biological activity but that lack the signature equilibrium. For a long time, the search for biologically active E-ring analogs of camptothecin did not bear fruit, and some even speculated that equilibrium and activity were linked. However, in recent years, a few E-ring analogs of camptothecins have shown activity either in cell assays or *in vitro* in topoisomerase assays (Fig. 3). These include the unusual ether **5** (which shows rather low activity) [4], and the cyclopentanone **6** [5] and the natural product luotonin **7** [6], which show significantly higher levels of activity.

However, the most important class of E-ring analogs to emerge to date has been the homocamptothecins. This short review focuses on the synthesis of homocamptothecins while at the same time including key biological and medicinal chemistry information to illustrate why the class is unique and important. The structure of (*R*)-homocamptothecin **8** is shown in Fig. 3.[1]

2. Discovery of homocamptothecin

Homocamptothecin **8** was first described by a multidisciplinary team led by Lavergne and Bigg of the Institut Henri Beaufour [7]. To make the initial sample of the racemate (Scheme 1), the lactone ring of (*S*)-camptothecin **1** was first disassembled by reduction to the hydroxylactol and oxidative cleavage to give achiral ketoformate **9** [8]. This effectively excised one carbon (C21) from the ring. Next, a Reformatsky reaction on the liberated ketone added back two carbons to give ester **10** in 31% yield. Finally, acid treatment removed the formate and closed the seven-membered ring lactone of racemic hydroxycamptothecin **8** in 55% yield. Despite the low yield of the Reformatsky reaction, the sequence is short, so substantial quantities of (*rac*)-**8** were produced.

Racemic homocamptothecin showed remarkable properties [9]. Despite being a β-hydroxy lactone rather than an α-hydroxy lactone, it exhibited potent antiproliferative activity in assorted cell lines. It also cleaved DNA in the presence of toposiomerase **1** in the signature assay for ‘‘camptothecin-like’’ molecules, although with a different sequence selectivity from camptothecin. However, unlike camptothecin, it was quite stable in plasma and other fluids, opening slowly *but irreversibly* to the derived β-hydroxy carboxylate **11**.

[1] Camptothecin numbering is used for the homocamptothecins in this review to facilitate comparison.

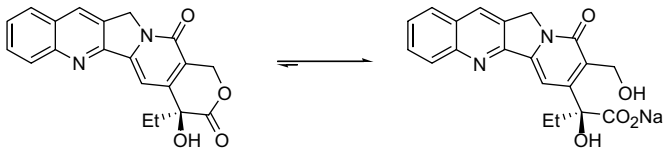


Fig. 2. The lactone/hydroxy acid equilibrium.

Both enantiomers of homocamptothecin were then produced by chemical resolution of the derived hydroxy acid, and the activity was shown to reside in the dextrorotatory (+)- enantiomer. This suggested that (+)-homocamptothecin had the *R* configuration, a suggestion that was later confirmed by asymmetric total synthesis (see below). Because of a change in Cahn–Ingold–Prelog (CIP) priorities of the attached groups, (*S*)-camptothecin is analogous to (*R*)-homocamptothecin.

These preliminary biological and pharmacological studies spurred significant interest in the synthesis of homocamptothecin and its analogs.

3. The "Lavergne–Comins route" to homocamptothecins

The group from Institut Henri Beaufour prepared one analog of homocamptothecin (the 7-ethyl derivative) by the semi-synthetic route from the analogously substituted camptothecin. But recognizing the limitations of the semi-synthesis for structure–activity studies, they also put in place a total synthesis of racemic homocamptothecin and several interesting analogs in the first paper [10]. This was based on the

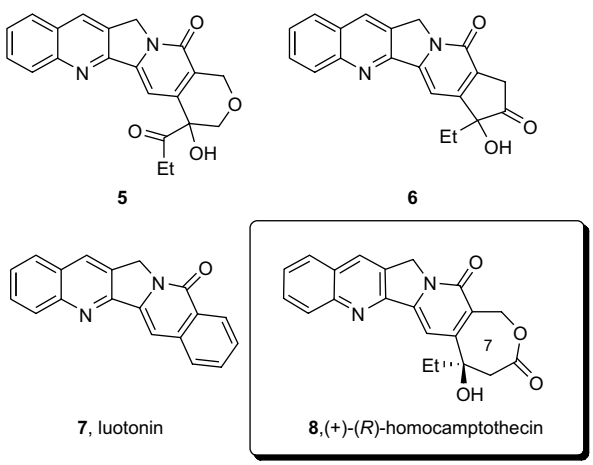


Fig. 3. Selected E-ring Analogs of camptothecin with biological activity, including homocamptothecin.

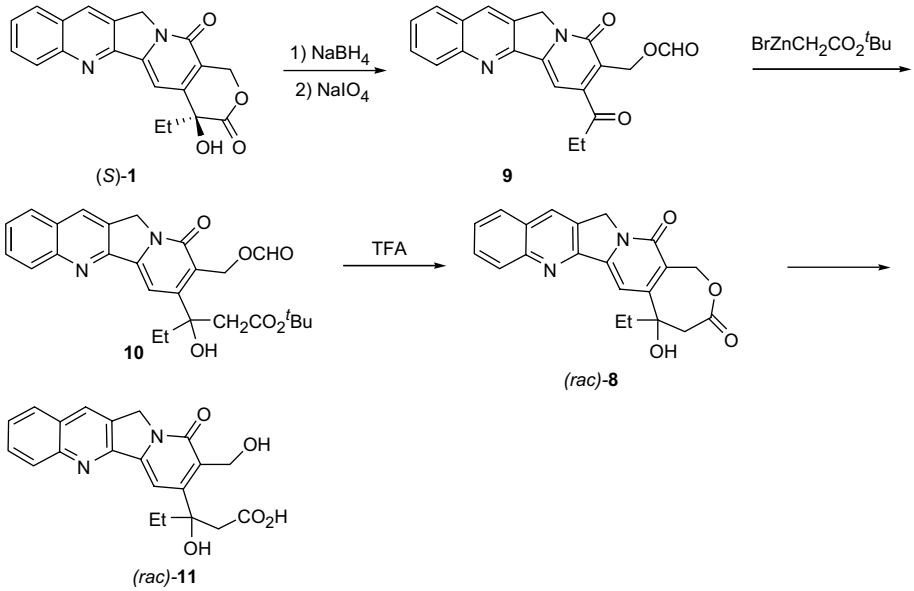
powerful Comins route to camptothecin, whose key penultimate and ultimate steps are summarized in Scheme 2. *N*-Alkylation unites AB fragment **12** with DE fragment **13** to give **14**. Subsequent Heck reaction closes the C ring to give camptothecin **1**.

In the adaptation to the analog 10,11-difluorohomocamptothecin **18** (diflomotecan), linking of the two fragments **15** and **16** by a Mitsunobu reaction gave **17** in 35% yield. Then Heck cyclization of **17** provided racemic **18** in 18% yield. Later, the resolution of the hydroxy acid derived by hydrolysis of **16** was described, and this then provided access to enantioenriched intermediates for the preparation of the active *R* enantiomers [11]. Yields for the key coupling and cyclization steps were also improved.

The synthesis of the requisite DE fragment for making homocamptothecin is summarized in Scheme 3. Conversion of **19** to a ketal followed by displacement of the chloride by methoxide provided **20** in 80% yield. Ortholithiation and in situ formylation followed by reduction provided alcohol **21** (62%). Protection of the alcohol followed by removal of the acetal provided a ketone **22** (70%) ready for the same sequence of reactions that was used in the semi-synthesis. Reformatsky reaction of **22** gave β-hydroxyester **23** in 95% yield. In the end game, hydrogenolytic removal of the benzyl group, acid-promoted lactonization and demethylate provided the target lactone pyridone **16** in 40% overall yield.

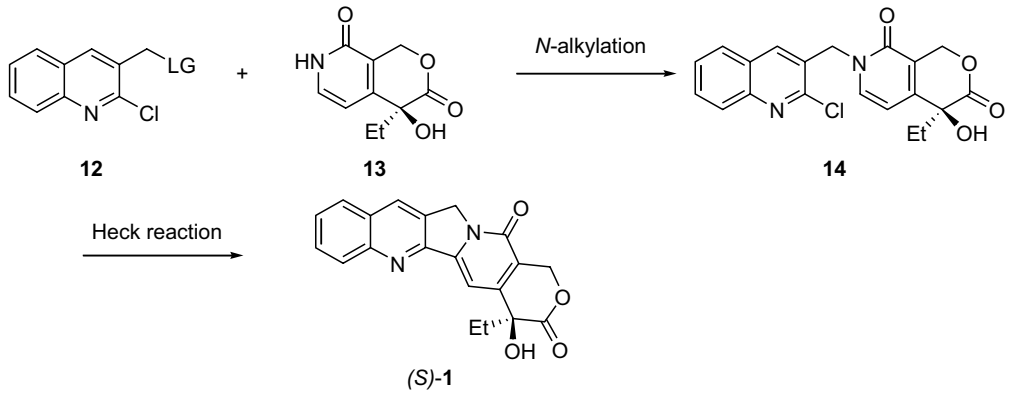
Assorted derivatives of homocamptothecin with one or two substituents on the A ring were also made by this route. The precursors are made starting from appropriately substituted anilides by Vilsmeier-type double formylation and reduction of the resulting alcohol. Especially important among these analogs are fluorinated derivatives, including BN80915 **18** (10,11-difluorohomocamptothecin), which is a clinical candidate for cancer chemotherapy called diflomotecan [12]. The synthesis of the requisite AB fragment **15** for BN80915 from 3,4-difluoroacetanilide **24** is shown in Scheme 4.

Practical asymmetric routes to BN80915 based on asymmetric aldol reactions have recently been reported, as summarized in Scheme 5 [13]. Streamlined

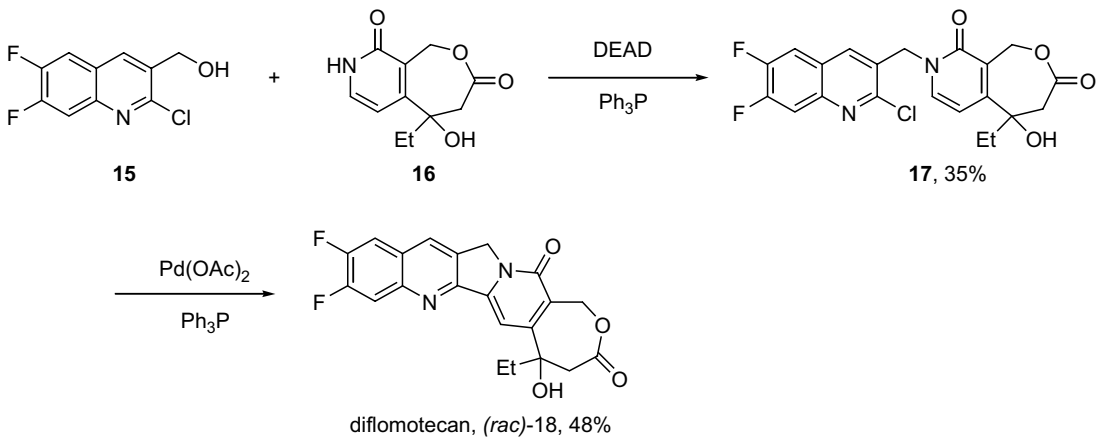


Scheme 1. Semi-synthesis of racemic homocamptothecin from camptothecin.

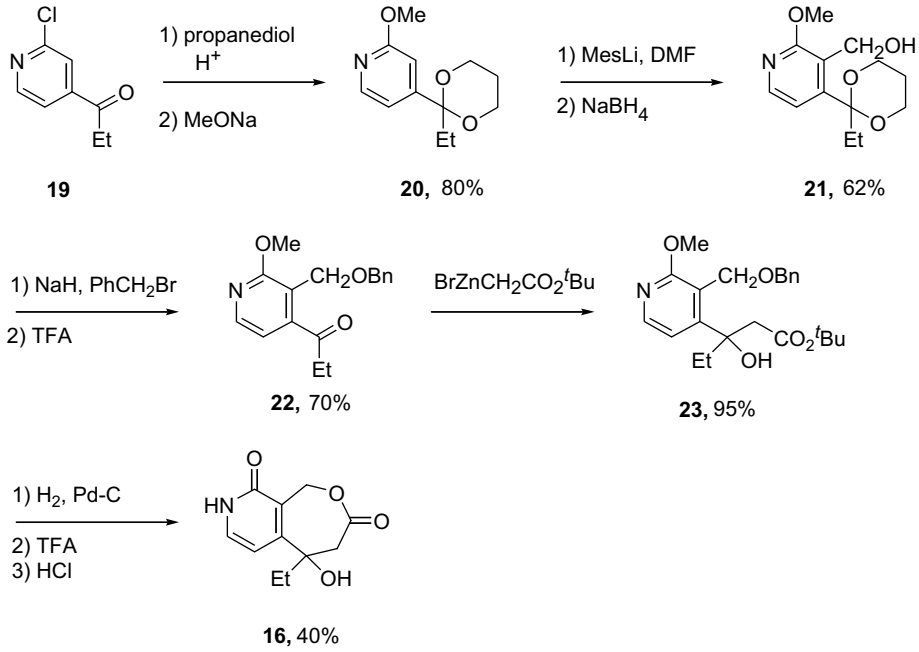
The Comins route to camptothecin (illustrated in the active S-series)



The Lavergne-Comins route to homocamptothecins (illustrated with racemic diflomotecan)



Scheme 2. Key steps in the Comins route to camptothecin and adaptation to homocamptothecins.

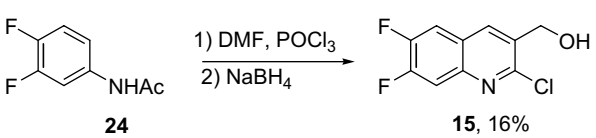


Scheme 3. Initial synthesis of the racemic DE fragment **16**.

routes to benzyl- and TBS-protected ketones **22** and **25** were first developed. Then these intermediates were subjected to asymmetric aldol reactions to give target *R*-isomers **26** and **27** in ratios of about 87/13. These aldol products were readily converted to lactone (*R*)-**16** in comparable enantiomeric ratios. The moderately selective aldol reactions were rendered very practical by the property of (*R*)-**16** to crystallize in very high ee (>99.9%) from the enriched mixtures.

Even more recently, a completely different "*de novo* pyridone" route to enantiopure (*R*)-**16** has been put into place [14]. The *R*-stereocenter at C20 in this route ultimately derives from lactic acid through a self-reproduction of chirality. The authors concluded that this approach could serve as the basis for a technical synthesis of diflomotecan.

Though useful for introducing A-ring substituents, the Lavergne–Comins route is not readily amenable to direct introduction of B-ring substituents a C7. Such substituents have proved very important in the medicinal chemistry of the classical α-hydroxylactones of the camptothecin class.



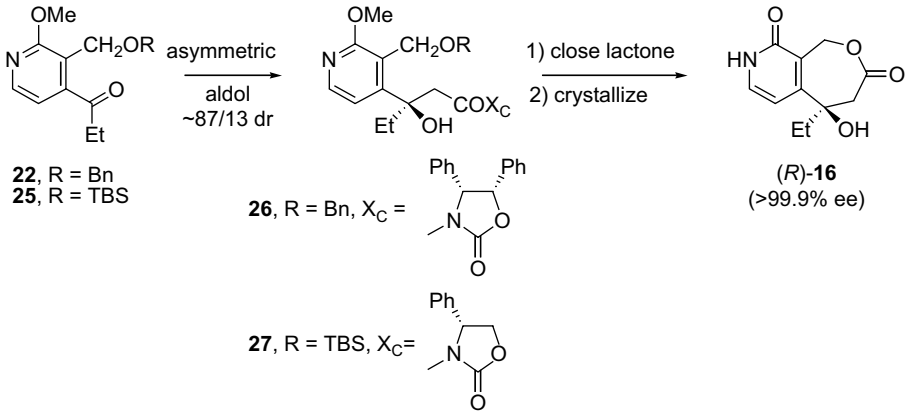
Scheme 4. Synthesis of the AB fragment illustrated with **15**.

4. The cascade radical annulation approach to homocamptothecins

During the 1990s, we developed a route to substituted camptothecins that was notable for its brevity and generality [15]. In the penultimate step, lactone pyridone **28** is *N*-propargylated to give **29**. Finally, cascade radical annulation of **29** and an A-ring isonitrile **30** unites the two building blocks and directly forms rings B and C to give the analogous camptothecin **31** (Fig. 4). Dozens of known and new analogs of camptothecin, including the clinical candidate AR-67 [16] (formerly DB-67), were prepared by this route. AR-67 is a member of a large family of 7-silylcamptothecins that go by the name of silatecans [17].

Although the cascade reaction is usually run in a radical mode with a tin (or sometimes silicon) reagent, a palladium-mediated variant is also available that starts from the same precursors and gives the same products [18]. The tin- and silicon-mediated reactions involve radicals. Presumably, organopalladium intermediates are involved in the palladium variant, whose mechanism has not been studied in detail.

Within a few months of the report of homocamptothecin activity, we were able to retool the cascade annulation route to make racemic homosilaecan analogs (Scheme 6) [19]. Starting from readily available enol ether **32**, dihydroxylation and ozonolysis provided the requisite ketoformate **33**. Standard



Scheme 5. Asymmetric aldol routes to the DE fragment (*R*)-**16**.

Reformatsky reaction and ring closure, followed by iododesilylation and demethylation, provided the key lactone pyridone **34**, again in racemic form. Now, *N*-propargylation provided the finished DE fragments **35a**,**b**, ready for the radical annulation.

In a preliminary illustration of the usefulness of this approach for making analogs, we prepared four 10-substituted-7-silylhomosilatecans **36a–d** from the appropriate propargyl bromide and isonitrile. These homosilatecans are triply ring-modified (A, D and E) camptothecins that are highly biologically active and are considerably more stable in blood than homocamptothecin (which in turn is already very stable compared to standard camptothecins) [20]. That they can be made so easily is a testament to the power and generality of the cascade radical annulation approach.

After further shortening of the synthesis of **34**, the route in Scheme 6 was parallelized and 115 analogs of homosilatecans were made in individual, pure form on 1–5 mg scale [21]. Simple manual techniques for parallel reactions were coupled with automated purifications (SPE, HPLC) to give high-purity final products. The speed and simplicity of the automated purification protocol more than compensated for yield losses in the synthesis of some analogs relative to traditional flash chromatographic purifications. The ready adaptability of the cascade radical annulation route to parallel synthesis is yet another testament of

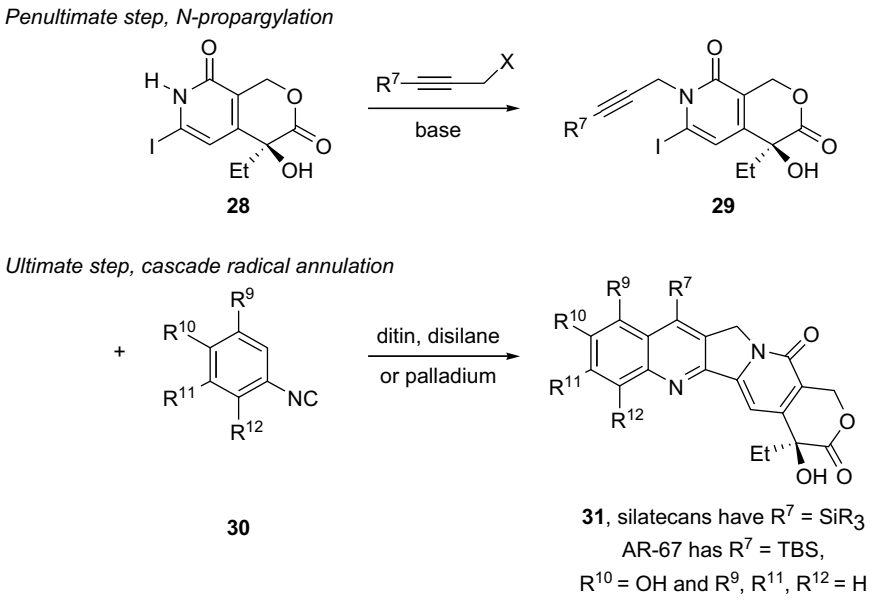
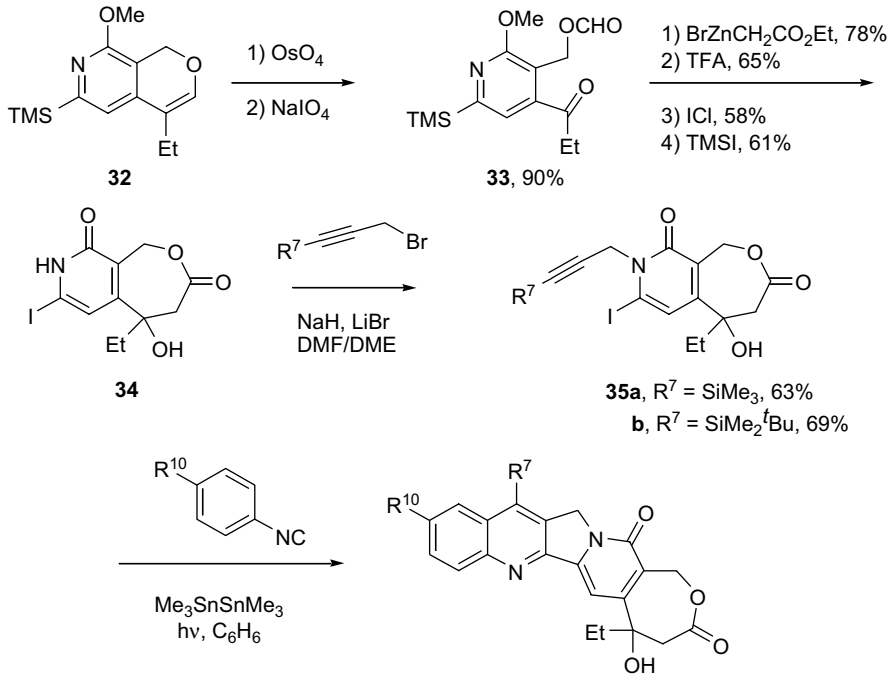


Fig. 4. The cascade radical annulation route to camptothecins.



	R^7	R^{10}	Yield
36a	$SiMe_2{}^tBu$	NH_2	13%
36b	$SiMe_3$	NH_2	24%
36c	$SiMe_2{}^tBu$	OH	16%
36d	$SiMe_2{}^tBu$	H	27%

Scheme 6. Racemic homosilatecans made by co-opting camptothecin intermediate **32**.

its generality. We failed to isolate only one of the designed library products. The entire library was made by a single student over several months.

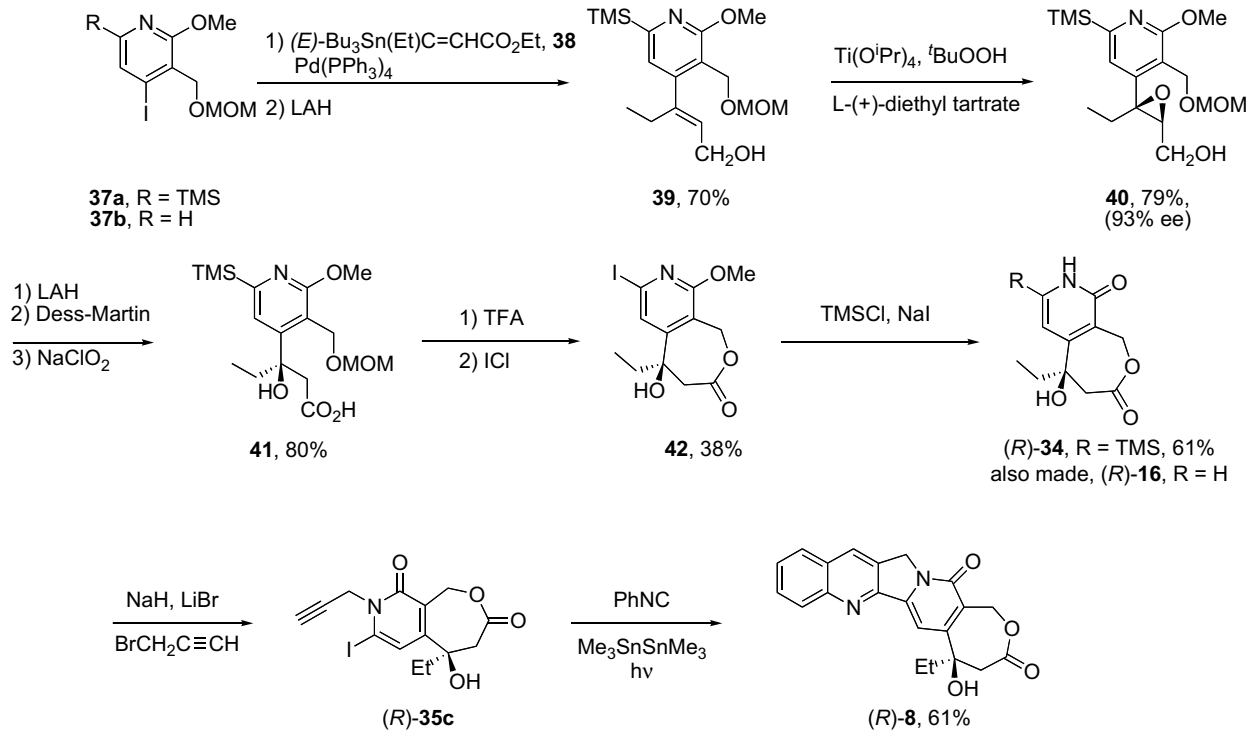
Recognizing the need for an asymmetric synthesis of the DE fragment of homocamptothecins for both the Lavergne–Comins and cascade radical annulation approaches, we put in place the sequence in Scheme 7 based on a classical Sharpless asymmetric epoxidation (SAE). Stille coupling of ready available iodide **37a** (R = TMS) and **38** followed by ester reduction provided **39** as a single alkene stereoisomer. Following the SAE reaction, epoxide **40** was isolated in 79% yield and 93% ee. A standard three-step sequence of reactions converted this intermediate into lactone **41**, which in turn was converted to **42** by acid-promoted lactonization and iododesilylation. Finally, demethylation provided (*R*)-**34** in >90% ee. This synthesis again borrows the starting material **32** and the last several steps from the original camptothecin synthesis. An analogous route starting from the **37b** (now lacking the silyl group, R = H) provided intermediate (*R*)-**16** for the Lavergne–Comins route in high ee.

The conversion of the defined intermediate (*R*)-**34** to (*R*)-**35c** and then onward to (+)-homocamptothecin (*R*)-**8** provided a chemical confirmation of the absolute configuration of homocamptothecin that validated the earlier biological assignment.

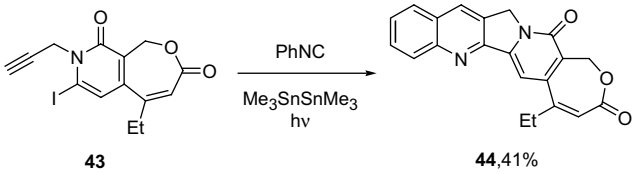
Finally, in a further demonstration of the versatility of this approach, we synthesized a series of unsaturated analogs of homocamptothecins [22]. The synthesis of the parent, dehydrohomocamptothecin **44** from its DE precursor **43** is typical, as summarized in Scheme 8. These compounds had significantly reduced biological activity compared to the analogous homocamptothecins.

5. The Friedlander route to homocamptothecins

The Lavergne–Comins and cascade radical annulation routes to camptothecins are generally considered second-generation syntheses because they were developed during the revival of camptothecins as anti-



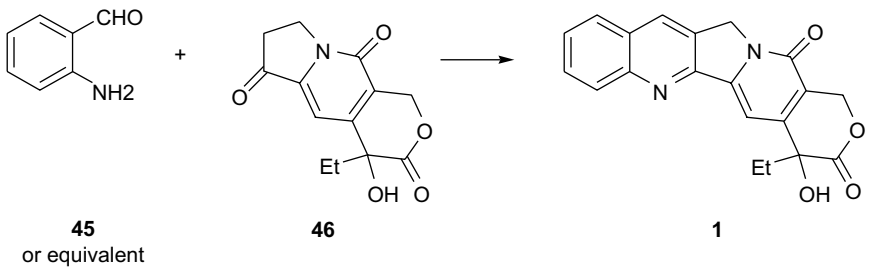
Scheme 7. Asymmetric synthesis of both cascade radical (**34**) and Heck (**16**) DE fragments.



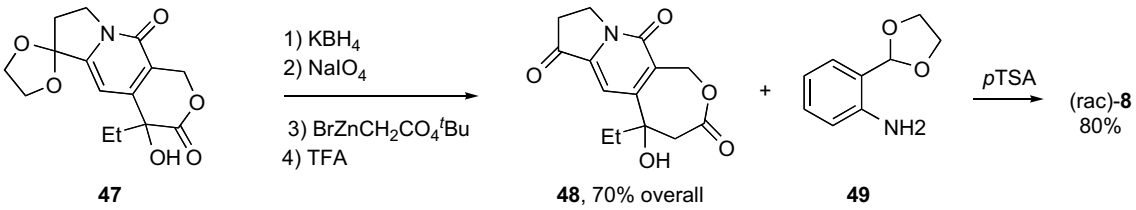
Scheme 8. Synthesis of dehydrohomocamptothecin.

cancer agents. From among the first-generation syntheses [23], the Friedlander approach has commonly been used for preparing analogs for medicinal chemistry purposes. Summarized in Scheme 9, this involves the Friedlander condensation of tricyclic keto pyridone **46** with analino aldehyde **45** (or an equivalent) to provide camptothecin **1**.

Recently, Miao and coworkers adapted this route to make homocamptothecins (Scheme 10). Ketal **47**, the immediate precursor of **46**, was ring expanded by



Scheme 9. The Friedlander approach to camptothecins.



Scheme 10. The Friedlander approach to homocamptothecins.

a series of reactions similar to those above to provide tricyclic ketone **48**, now with the seven-membered lactone, in excellent overall yield. Friedlander condensation of this lactone with **49** provided homocamptothecin **8**. The route was generalized by producing a number of A- and B-ring analogs starting from suitably substituted anilino aldehydes or ketones. To date, only racemic homocamptothecins have been produced by this route.

6. Conclusions

Homocamptothecin and its analogs are proving to be an especially interesting class of camptothecins that merits an independent classification because the lactones of these analogs open slowly and irreversibly, rather than rapidly and reversibly. The discovery of this class of molecules provided unexpected new opportunities to leverage lessons learned during the synthesis of the camptothecins.

Three powerful and general routes—the Lavergne–Comins route, that cascade radical annulation route, and the Friedlander route—have been put into place to make homocamptothecins. While homocamptothecin was initially made in racemic form from camptothecin, substituted homocamptothecins are increasingly remote from the parent, especially considering that the sole stereocenter of camptothecin is destroyed in the semi-synthesis. Thus, total synthesis efforts have dominated the medicinal chemistry development of the homosilatecans. Likewise, both the preclinical and clinical developments of diflomotecan have been driven by increasingly efficient variants of the Lavergne–Comins route.

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References

[1] (a) F. Zunino, S. Dallavalle, D. Laccabue, G. Beretta, L. Merlini, G. Pratesi Recent reviews: Curr. Pharm. Des. 8 (2002) 2505;
(b) H.-G. Lerchen, Drugs Future 29 (2002) 869;
Kim D.-K.; Lee N; Mini Rev. Med. Chem. 2002, 2, 611.(c) H. Ulukan, P. Swaan, Drugs 62 (2002) 2039;
(d) W. Du, Tetrahedron 59 (2003) 8649;
(e) C.J. Thomas, N.J. Rahier, S.M. Hecht, Bioorg. Med. Chem. 12 (2004) 1585;
(f) Q.-Y. Li, Y.-G. Zu, R.-Z. Shi, L.-P. Yao, Curr. Med. Chem. 12 (2006) 2021.

[2] J.G. Liehr, B.C. Giovanella, C.F. Verschraegen (Eds.), The Camptothecins: Unfolding Their Anticancer Potential, vol. 922, New York Academic of Sciences, NY, 2000;
P. Pantazis, B.G. Giovanella, M.L. Rothenberg (Eds.), The Camptothecins: From Discovery to Patient, vol. 803, New York Academic of Sciences, NY, 2000.

[3] (a) T.G. Burke, A.E. Staubus, A.K. Mishra, H. Malak, J. Am. Chem. Soc. 114 (1992) 8318;
(b) D.Z. Yang, J.T. Strode, H.P. Spielmann, A.H.J. Wang, T.G. Burke, J. Am. Chem. Soc. 120 (1998) 2979.

[4] W. Du, D.P. Curran, R.L. Bevins, S.G. Zimmer, J. Zhang, T.G. Burke, Bioorg. Med. Chem. 10 (2002) 103.

[5] P. Hautefaye, B. Cimetière, A. Pierré, S. Léonce, J. Hickman, W. Laine, C. Bailly, G. Lavielle, Bioorg. Med. Chem. Lett. 13 (2003) 2731.

[6] (a) A. Cagir, S.H. Jones, R. Gao, B.M. Eisenhauer, S.M. Hecht, J. Am. Chem. Soc. 125 (2003) 13628;
(b) Z.Z. Ma, Y. Hano, T. Nomura, Heterocycles 65 (2005) 2203.

[7] O. Lavergne, L. Lesueur-Ginot, F.P. Rodas, P.G. Kasprzyk, J. Pommier, D. Demarquay, G. Prevost, G. Ulibarri, A. Rolland, A.-M. Schiano-Liberatore, J. Harnett, D. Pons, J. Camara, D.C.H. Bigg, J. Med. Chem. 41 (1998) 5410.

[8] J.M.D. Fortunak, A.R. Mastrocola, M. Mellinger, J.L. Wood This compound is the formate derivative of the natural product mappicine ketone, Tetrahedron Lett. 35 (1994) 5763.

[9] C. Bailly, A. Lansiaux, L. Dassonneville, D. Demarquay, H. Coulomb, M. Huchet, O. Lavergne, D.C.H. Bigg, Biochemistry 38 (1999) 15556.

[10] (a) D.L. Comins, M.F. Baevsky, H. Hong, J. Am. Chem. Soc. 114 (1992) 10971;
(b) D.L. Comins, H. Hong, G. Jianhua, Tetrahedron Lett. 35 (1994) 5331;
(c) D.L. Comins, H. Hong, J.K. Saha, J.H. Gao, J. Org. Chem. 59 (1994) 5120;
(d) D.L. Comins, J.M. Nolan, Org. Lett. 3 (2001) 4255.

[11] O. Lavergne, D. Demarquay, C. Bailly, C. Lanco, A. Rolland, M. Huchet, H. Coulomb, N. Muller, N. Baroggi, J. Camara, C. Le Breton, E. Manginot, J.-B. Cazaux, D.C.H. Bigg, J. Med. Chem. 43 (2000) 2285.
[12] N.I. Osheroff, Drugs 7 (2004) 257.
[13] R. Peters, M. Althaus, C. Diolez, A. Rolland, E. Manginot, M. Veyrat, J. Org. Chem. 71 (2006) 7583.
[14] R. Peters, C. Diolez, A. Rolland, E. Manginot, M. Veyrat, Heterocycles 72 (2007) 255.
[15] (a) D.P. Curran, H. Liu, J. Am. Chem. Soc. 114 (1992) 5863;
(b) H. Josien, S.B. Ko, D. Bom, D.P. Curran, Chem. Eur. J. 4 (1998) 67;
(c) D.P. Curran, H. Josien, D. Bom, A. Gabarda, W. Du, in: J.G. Liehr, B.C. Giovanella, C.F. Verschraegen (Eds.), The Camptothecins: Unfolding their Anticancer Potential, vol. 922, New York Academic of Sciences, NY, 2000, pp. 112–121.
[16] D. Bom, D.P. Curran, S. Kruszewski, S.G. Zimmer, J. Thompson Strode, G. Kohlhagen, W. Du, A.J. Chavan, K.A. Fraley, A.L. Bingcang, L.J. Latus, Y. Pommier, T.G. Burke, J. Med. Chem. 43 (2000) 3970.
[17] H. Josien, D. Bom, D.P. Curran, Y.-H. Zheng, T.-C. Chou, Bioorg. Med. Chem. Lett. 7 (1997) 3189.
[18] D.P. Curran, W. Du, Org. Lett. 4 (2002) 3215.
[19] D. Bom, D.P. Curran, A.J. Chavan, S. Kruszewski, S.G. Zimmer, K.A. Fraley, T.G. Burke, J. Med. Chem. 42 (1999) 3018.
[20] D. Bom, W. Du, A. Gabarda, D.P. Curran, A.J. Chavan, S. Kruszewski, S.G. Zimmer, K.A. Fraley, A.L. Bingcang, V.P. Wallace, B.J. Tromberg, T.G. Burke, Clin. Cancer Res. 5 (1999) 560.
[21] A.E. Gabarda, D.P. Curran, J. Comb. Chem. 5 (2003) 617.
[22] R.S. Tangirala, S. Antony, K. Agama, Y. Pommier, B.D. Anderson, R. Bevins, D.P. Curran, Bioorg. Med. Chem. 14 (2006) 6202.
[23] C.R. Hutchinson, Tetrahedron 37 (1981) 1047.