Synthesis and Pharmacology of New Camptothecin Drugs

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Abstract: Camptothecin (CPT) drugs exhibit antineoplastic activity against colorectal, breast, lung and ovarian cancers. This review briefly summarizes the pharmacology of CPT drugs, examines four strategies and methods for the synthesis of camptothecins, and finally discusses homocamptothecins and silatecans, two new classes of CPT analog.

Keywords: Camptothecin, Homocamptothecin, Silatecan, Pharmacology, Synthesis.

INTRODUCTION

The camptothecin family of drugs exhibits powerful antineoplastic activity against colorectal, breast, lung and ovarian cancers. In the past two decades substantial progress towards understanding the pharmacology of the parent compound, CPT (**1**), and several analogs has been made [1-3]. Two water-soluble CPT derivatives, Topotecan **2** and Irinotecan **3**, have recently been approved for clinical use. Topotecan hydrochloride (Hycamptin®) is indicated for the treatment of chemoresistant ovarian and small cell lung cancers. Irinotecan (Camptosar®) is a carboxylesterase-dependant prodrug administered in combination with 5-fluorouracil and leucovorin to patients with metastatic colorectal carcinomas (Fig. **1**) [4]. The clinical success of these drugs against recalcitrant cancers has catalyzed the synthesis of new camptothecin compounds. Four distinct approaches have been pursued, namely those in which (1) the C ring is completed by the joining of AB and DE bicycles (2) nascent D and E rings are appended to an AB bicycle (3) the B and C rings are formed in a cascade reaction from an A ring precursor and a DE bicycle (4) a structurally homologous pentacycle derived from known biosynthetic precursors is converted to the natural product. These synthetic advances have enabled the evaluation of homocamptothecins and silatecans (Fig. **2**), two classes of CPT analog employing novel structural modifications (a seven-membered β-lactone E ring and a 7-silylalkyl substituent, respectively) that prevent hydrolysis *in vivo*, more potently inhibit Topo1 and defeat drug resistance mechanisms. This review briefly summarizes the pharmacology of CPT drugs, discusses four new synthetic strategies, and finally places these advances in the context of the development of silatecans and homocamptothecins.

Camptothecin, a pentacyclic quinoline alkaloid, was first isolated from *Camptotheca acuminata* by Wall and co-workers in 1966 and subsequently shown to prolong the lives of mice in L1210 and P388 leukemia cell assays as well as inhibit solid tumor growth [5]. These results, in conjunction with the structural elucidation of the parent compound and several derivatives, created significant interest in CPT drugs as chemotherapeutic agents [6-7]. Unfortunately, early clinical trials employed the ring-opened carboxylic acid salt (**7**) rather than the water-insoluble closed-ring lactone (Fig. **3**). The results were disappointing: biological activity was weak relative to xenograph models and unexpected side effects, leukopenia and hemorrhagic cystitus, limited dosing regiments. [8] Phase II clinical trials were delayed but research on the mechanism of CPT cytotoxicity continued. Camptothecin was known to be an S-phase specific cytotoxin that inhibited oligonucleotide synthesis and induced chromosome fragmentation, but only *in vivo* [9-11]. These observations led to the speculation that another cellular component was necessary. The identification of eukaryotic DNA topoisomerase 1 as the primary vehicle for CPT-mediated DNA damage by Liu *et al*. in 1985 renewed interest in CPT drugs and stimulated research on the biological role of topoisomerases [12].

PHARMACOLOGY

Topoisomerases catalyze the interconversion of DNA topoisomers through the cleavage, manipulation and subsequent religation of DNA strands, thereby opening tightly packaged genetic material to replication and repair machinery [13-14]. Topoisomerases are classified by mechanism. Type 1 topoisomerases, represented in both prokaryotic and eukaryotic organisms, form covalent linkages to either the 3' or 5' ribose sugar backbone by cleaving a single DNA strand; the scissile strand is then unwound by rotation about the intact strand and religated in a microscopically reversible process. Human DNA Topoisomerase 1 (Topo1), the molecular target of CPT, is a monomeric protein of approximately 100 kDa composed of N-terminal, core, linker and C-terminal domains [15-16] that effects the ATP-independent relaxation of supercoiled double-stranded DNA through a "controlled rotation" mechanism [17]. Following non-sequence specific binding of the enzyme to double-stranded DNA, a single strand is clamped between the first and third subdomains of the enzyme core, positioning the 3' phosphate ester bond linking the upstream and downstream nucleotides for nucleophilic attack by the hydroxyl group of enzyme amino acid residue Tyr723. The ensuing transesterification covalently links the enzyme and DNA scissile strand. The phosphotyrosine bond provides an

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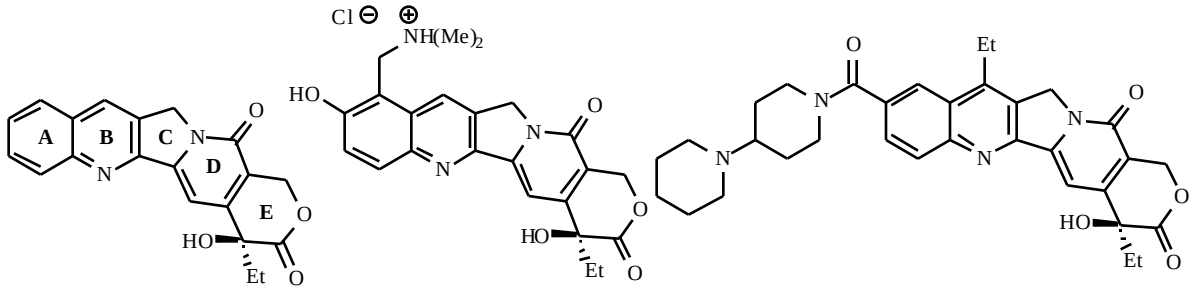


1 **2** **3**

Fig. (1). (S)-Camptothecin (**1**), Topotecan (**2**), Irinotecan (**3**).

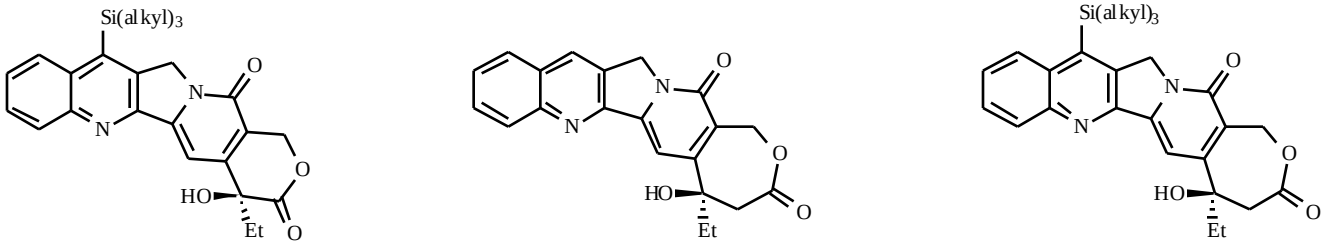
axis about which the free DNA strand may rotate to dissipate torsional force. Rotation of the scissile strand during unwinding is not free: steric interactions between the scissile strand and enzyme (linker domain and nose cone residues) provide an energetic barrier. The scissile strand is religated by 5' hydroxyl attack on the phosphotyrosine bond and the enzyme disassociates.

The binding of CPT to Topo1 midway through the enzymatic cycle, after strand scission but prior to religation, stabilizes the covalent intermediate and results in the formation of long-lived DNA/Topo1/CPT ternary complexes. Efforts to model the ternary complex have sought to unify crystallographic and computational results with known structure/function relationships [18]. Two models were initially proposed [19-20]; both locate CPT near the phosphotyrosine bond and involve AB ring pi-stacking interactions with a +1 guanine nucleotide on the scissile strand and hydrogen bonding between the E-ring and Topo1 residues. Topotecan has been shown to adopt a similar orientation in the ternary complex [21]. The heteroaryl ring system is parallel with the base-pair axis, placing the D and E rings within a binding pocket created by a conformational shift in the non-scissile DNA strand phosphosdiester backbone. A phosphorus ester bond rotation aligns the Topotecan C-20 hydroxyl with a single enzyme amino acid contact, Asp533. As in CPT models, the binding pocket is stabilized by non-covalent bonding interactions. Topotecan binding causes a conformational shift that separates the 5'-OH and phosphotyrosine bond, preventing religation of the broken DNA strand. Following complex formation, the E ring lactone may open and covalently bind to the DNA helix or enzyme directly.

Covalent complex formation occurs mainly at T-G sequences and is readily reversible: lesions disappear rapidly after removal of CPT and are themselves not toxic. However, collision of advancing replication forks with complexes may precipitate cell death by causing enzyme-linked double strand DNA breaks, thus preventing the transcription of proteins crucial for cellular homeostasis or triggering apoptotic pathways [22-23]. A large number of cellular constituents, such as DNA-dependant protein kinase, ATR, and NF-κB are activated in response to replication fork collision [24-25]. Although the mechanism of lesion repair remains unresolved, topoisomerases in covalent complexes are quickly ubiquinated, presumably prior to proteolysis [26-27]. In fact, the destruction of ubiquinated enzymes by proteosome 26S is contemporaneous with the phosphorylation of RNA polymerase II_B [28]; both processes likely precede the functional transcription coupled repair of DNA lesions. Adjuvant therapy with compounds retarding DNA repair, such as PARP-1 [29] inhibitors, have met with success [30-32]. Camptothecins have also been co-administered with docetaxel [33], paclitaxel and cisplatin [34], a metalloproteinase inhibitor [35], an anti-epidermal growth factor receptor antibody, [36] protein kinase C inhibitors [37], and in conjunction with ionizing radiation [38-39]. The elevated concentrations of Topo1 found colorectal, ovarian and other cancers provide a solid therapeutic basis for these CPT-based therapies [40-43].

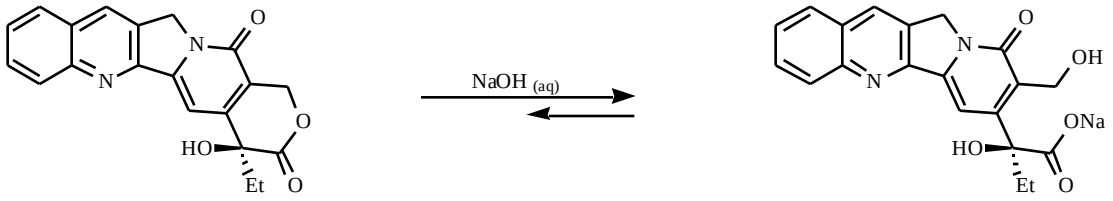
SYNTHESIS

Racemic camptothecin was first synthesized in 1971 by Stork and co-workers through the addition of an α-hydroxy ester carbonate to an α, β unsaturated lactone [44] An asymmetric total synthesis by the Corey group soon followed (Fig. **4**). Pseudoacid chloride **12**, derived from commercially available carboxylic acid **10**, was condensed with diamine **9**. The resulting adduct was transformed to **1**



4 **5** **6**

Fig. (2). Silatecan (**4**), homocamptothecin (**5**), homosilatecan (**6**).



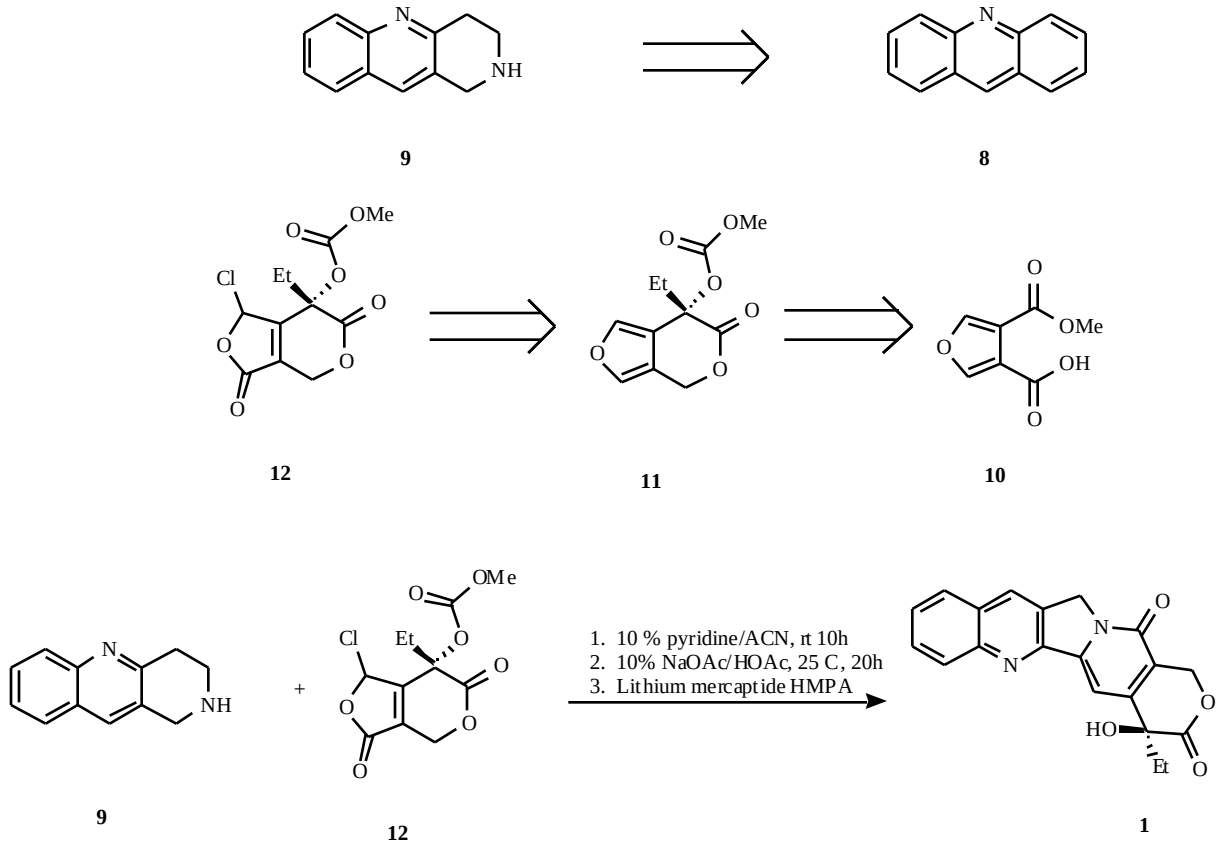
1 **7**

Fig. (3). Conversion of (**1**) to the ring-opened carboxylic acid sodium salt (**7**).

in three synthetic steps. A modular structure, compact functionality and significant chemotherapeutic potential have since made camptothecins perennial target molecules [45-47]. The success of Topotecan **2** and Irinotecan **3** against traditionally recalcitrant cancers has spurred the development of a succedent generation of CPT analogs designed to resist *in vivo* hydrolysis, more effectively inhibit Topo1 and overcome drug-resistance mechanisms. In contrast to Irinotecan and Topotecan, both of which are derived semisynthetically, the structural complexity of these candidates requires the development of total synthetic routes. Four distinct new approaches to CPT drugs have been pursued, those in which (1) the C ring is completed by the joining of AB and DE bicycles (2) a nascent E-ring is appended to an ABCD tetracycle (3) the B and C rings are formed in a cascade reaction from an A ring precursor and a DE bicycle (4) a structurally homologous pentacycle derived from known biosynthetic precursors is converted to the natural product (Fig. **5**.). In the following sections, each approach is briefly examined and the relevant synthetic schemes provided.

INTRAMOLECULAR CARBON-CARBON BOND FORMATION

Comins and co-workers have reported a 6-step synthesis of (S)-CPT from commercially available heterocycles. The AB and DE bicycles are constructed separately, covalently linked, and then the C ring completed through intramolecular carbon-carbon bond formation [48]. The original asymmetric synthesis of CPT required 10 steps [49]. Improvements in the synthetic route, specifically the introduction of chiral auxiliaries and a radical methodology [50-51] led to a 6-step racemic synthesis but the goal of a brief, asymmetric synthesis remained elusive [52]. In the most recent work, an experimentally simple enantioselective synthesis is accomplished (Fig. **6**). The DE ring bicycle is synthesized in three steps. 2-Methoxypyridine **13** is converted to lithium dianion **14** by directed lithiation. Iodination, acetal formation and addition of ketoester **15** yield intermediate **16**. Acid-catalyzed acetal hydrolysis and subsequent lactone cyclization provides **17**. The AB ring precursor **19** is prepared by reductive iodination of 2-chloro-3-quinolinecarboxaldehye **18**, analogs of which are available



9 **8**

12 **11** **10**

9 **12** **1**

Fig. (4). Retrosynthetic scheme for the synthesis of (S)-Camptothecin by Corey *et al*.

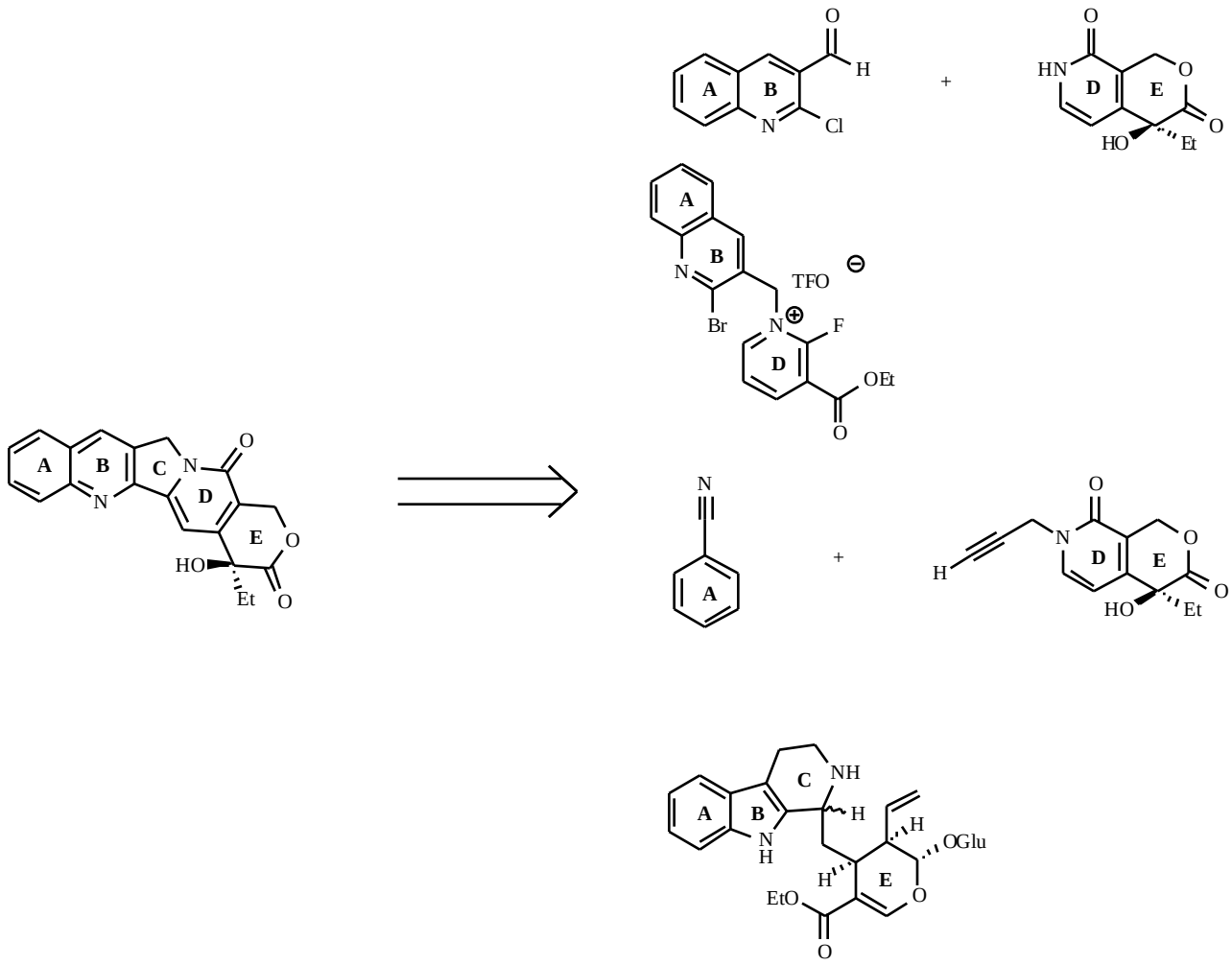


Fig. (5). Four strategies for CPT synthesis: (**1**) the joining of AB and DE bicycles; (**2**) nascent D and E rings are appended to an AB bicycle; (**3**) the B and C rings are formed in a cyclization cascade; (**4**) a structurally homologous indole is converted to the natural product.

from substituted acetanilides by the Vilsmeier-Haack reaction. The C ring is completed by coupling AB and DE bicycles under Heck reaction cross-coupling conditions to yield the enantiomerically pure natural product. The late-stage combination of AB and DE bicycles has proven to be of great practical utility. The transition-metal catalyzed method is employed in the total synthesis of homocamptothecin analogs (see below) and a radical-initiated variation utilized as a means to close the ABCD tetracycle in the following strategy.

ADDITION OF ESTER ENOLATES TO N-ALKYL-2-FLUOROPYRIDINIUM SALTS

Bennesar *et al*. have appended nascent D and E rings to an AB bicycle through the addition of ester enolates to N-alkyl-2-fluoropyridinium salts [53]. Two model reactions, the addition of α-methylsulfonyl butyrate **25** to 3-substituted N-benzyl-2-fluoropyridinium salts such as **24** and nucleophilic attack of 2-fluoro-pyridine on quinoline triflates **26**, were first investigated (Fig. **7**).

Enolate addition was found to be sufficiently regioselective in the case of methyl esters, substituents having the same oxidation state as the CPT lactone ring. Pyrolloquinoline construction by nucleophilic attack also proceeded in good yield. Reaction of pyrroloquinoline **28** with α-(methylsulfonyl) butyrate **29** following oxidation, radical cylation as per the method of Commins, and unmasking of the nascent E-ring, provides a formal synthesis of racemic 20-deoxy CPT **31**. (Fig. **9**). (S)-CPT **1** was reached by a similar route using dioxalan-4-one **33** (Fig. **9**). The method has been expanded to encompass indole-containing nucleophiles [54] and applied to the synthesis of (-)-N_a-methylervitsine [55]. Importantly, this strategy allows for the creation and testing of a large number of potentially active E ring congeners such as novel hexacycle **32** from a single intermediate-a distinct advantage given the sensitivity of the CPT E-ring to even minor modification. [56-57].

CYCLIZATION CASCADE REACTION

An alternative strategy is to construct the DE bicycle, then simultaneously form the B and C rings through a cyclization cascade with an A-ring precursor (Fig. **10**.).

The transition-metal catalyzed cascade method developed by Curran *et al*. [58] builds upon an initial report of a [4+1] annulation route to racemic CPT proceeding through the "Danishefsky tetracycle" [59]. The synthesis of a number of

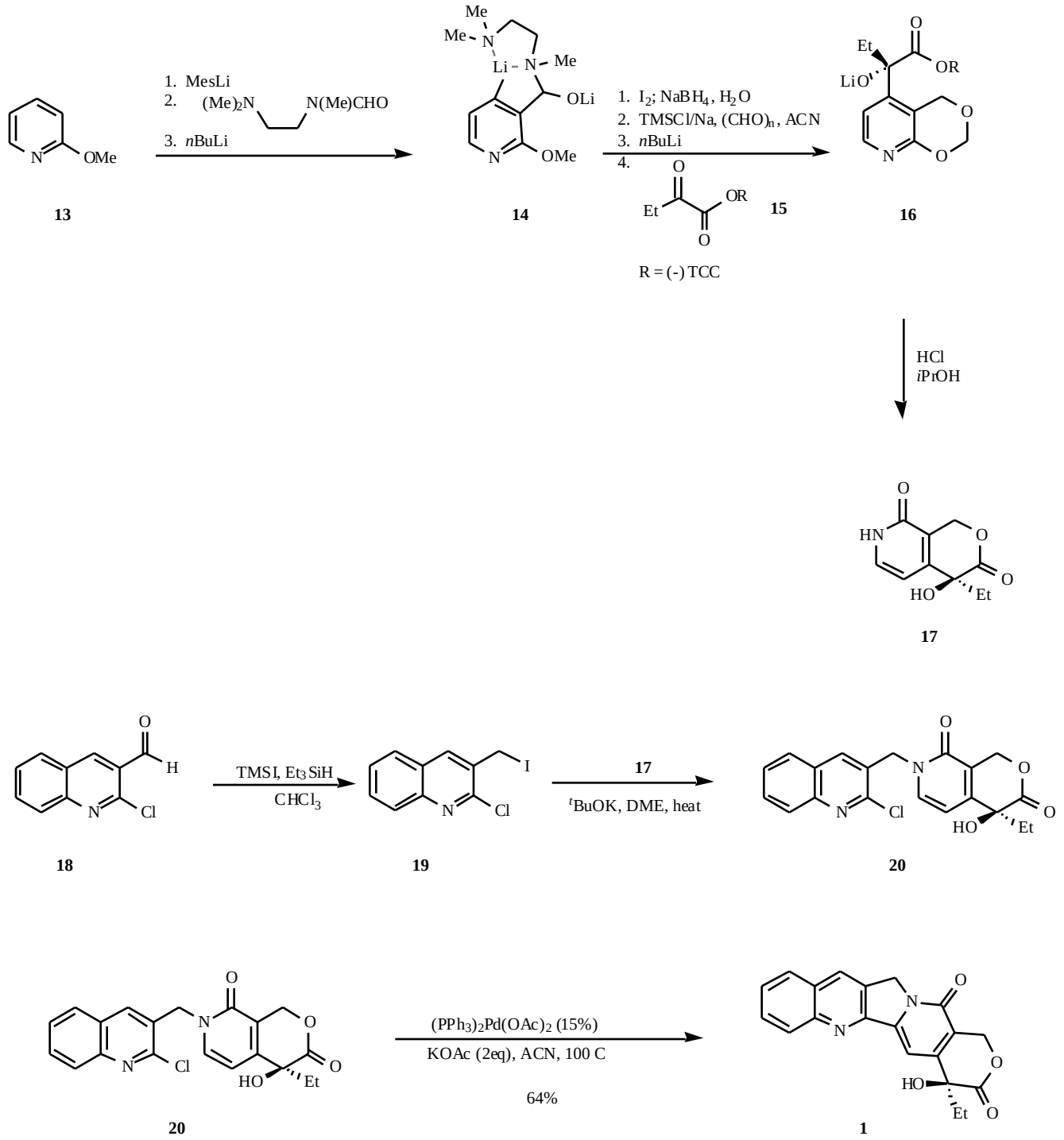


Fig. (6). 6-step asymmetric synthesis of (S)-CPT by intramolecular carbon-carbon bond formation.

Danishefsky tetracycle analogs by the tandem radical reaction of isonitriles with 2-pyridonyl derivatives soon followed (Fig. **11a**) [60]. A second-generation asymmetric, regiocontrolled radical cyclization route to enantiomerically pure CPT and congeners demonstrated the broad substrate scope and substituent tolerance of this method (Fig. **11b**) [61]. The reaction, unfortunately, requires stoichiometric quantities of tin. The introduction of a palladium-catalyzed process has simplified the synthesis of camptothecins, homocamptothecins and mappacines (Fig. **11c**) [62]. The DE bicycle is obtained from 2-bromo-3-methoxy pyridine in a manner analogous to that of Commins and co-workers. The bicycle is then propargylated with an alkyne bearing the desired alkylsilane before cyclization with an isonitrile. Although limited to electron-rich isonitriles, the yield can be raised by recycling material from the cyclization step. This strategy creates complex ring systems from modular staring materials, making it particularly well suited to combinatorial synthesis.

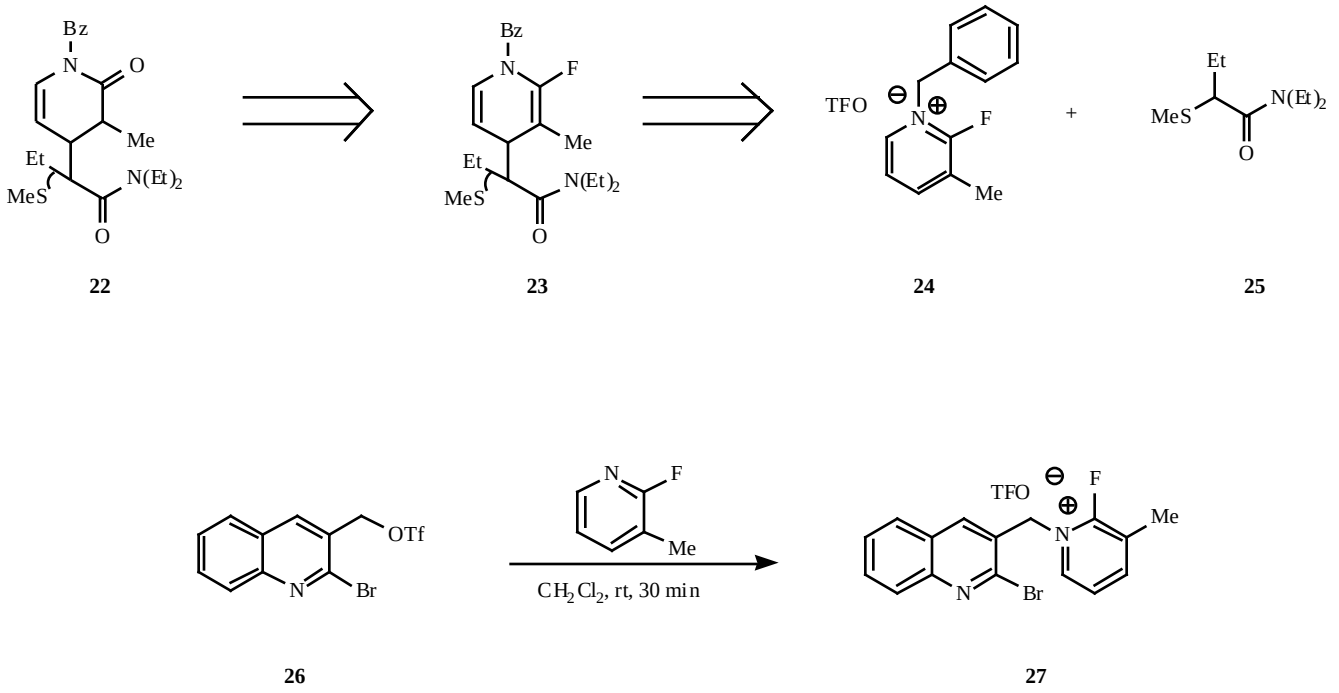


Fig. (7). Model studies of the addition of ester enolates to N-alkyl-2-fluoropyridinium salts.

BIOMIMETIC SYNTHESIS

Biogenically patterned syntheses of CPT utilizing known biosynthetic precursors as starting materials to rapidly access structurally advanced intermediates have also been reported [63-65]. Although classified as an indole alkaloid, CPT is derived via the monoterpene indole alkaloid biosynthesis pathway. The coexpression of three enzymes, tryptophan synthase, tryptophan decarboxylase and strictosidine synthase, implicates strictosidine **44**, a structurally homologous indole, as a biosynthetic intermediate [66-68]. As in the biosynthetic pathway, tryptamine **41** and secologanin **42** are condensed to set the carbon skeleton and install D and E ring functional groups; **44** is reached through E-ring manipulation (Fig. **12**).

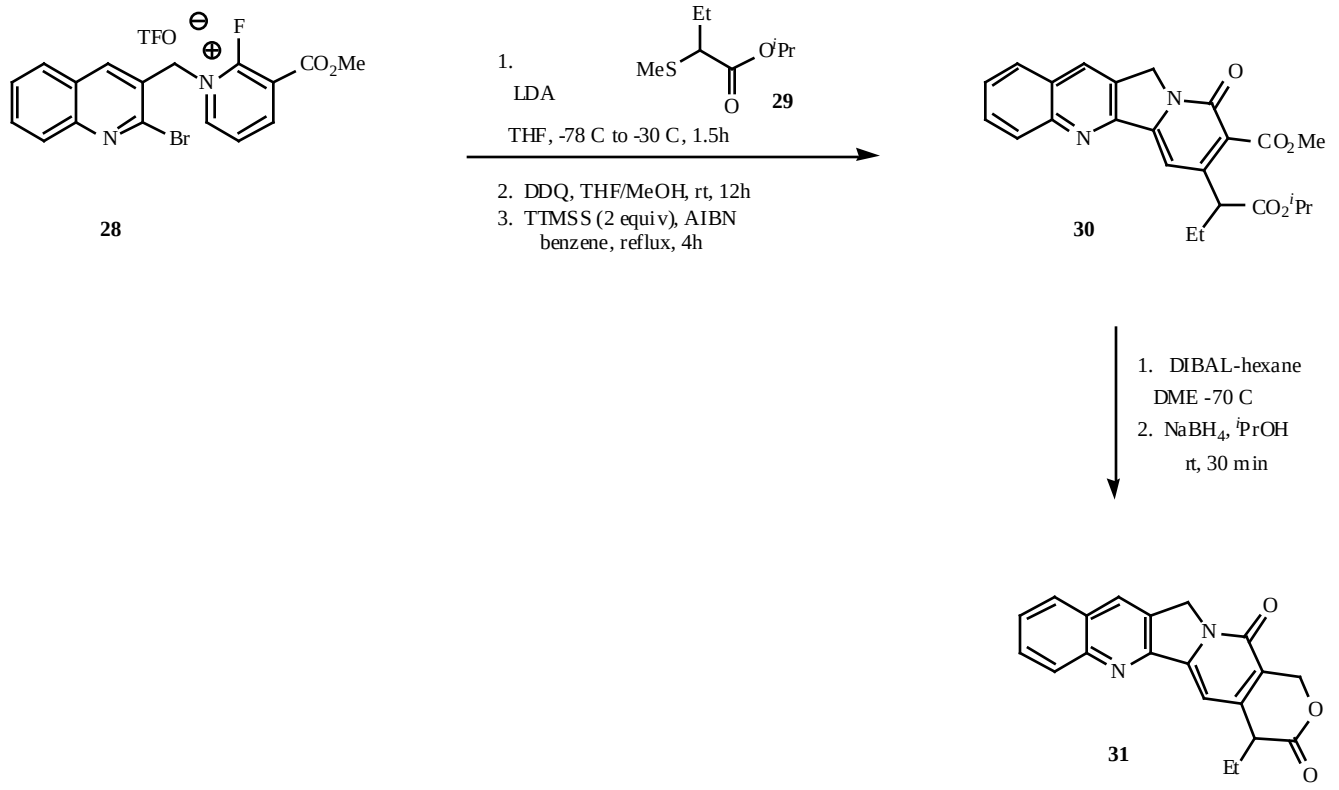


Fig. (8). Synthesis of 20-deoxyCPT (**31**) and hexacycle **32** by the addition of ester enolates to N-alkyl-2-fluoropyridinium salts.

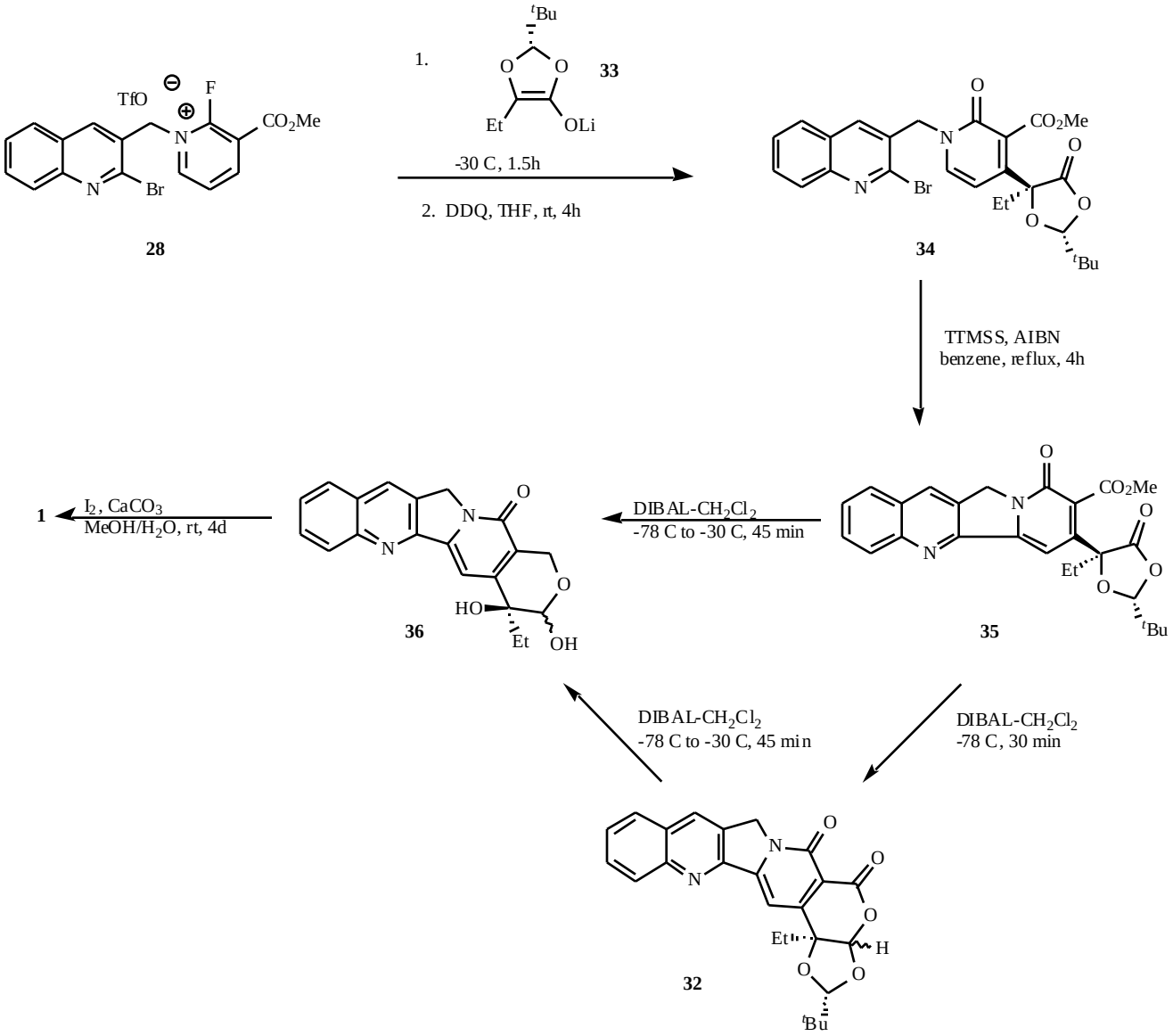


Fig. (9). Synthesis of **1** by the addition of ester enolates to N-alkyl-2-fluoropyridinium salts.

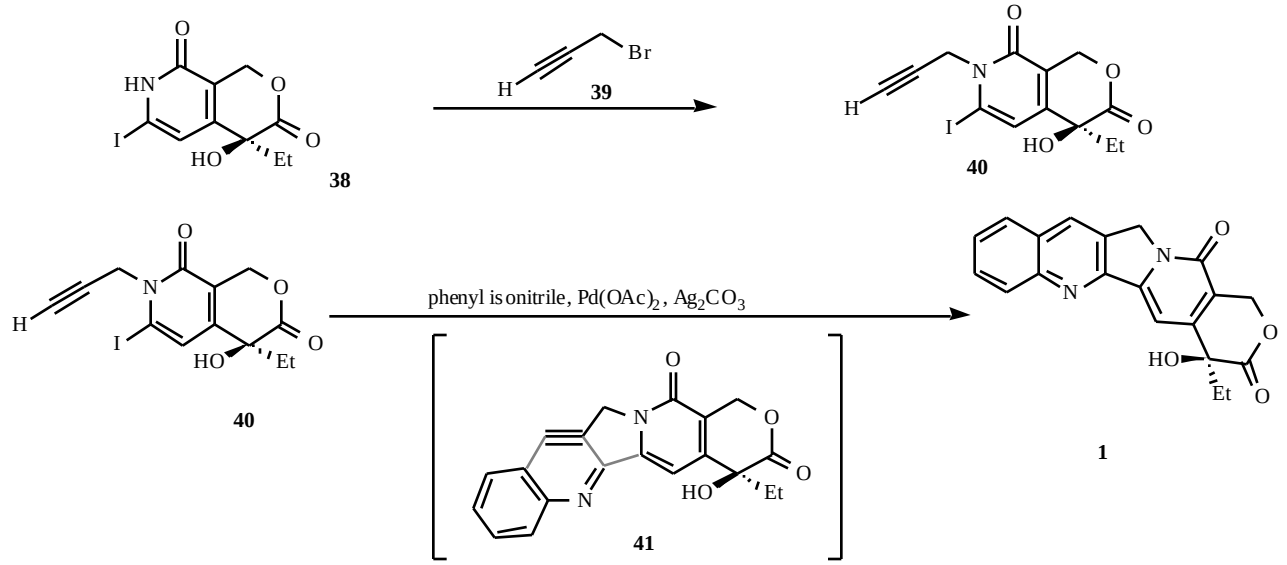


Fig. (10). Carbon atom connectivity in the transition metal catalyzed cyclization reaction.

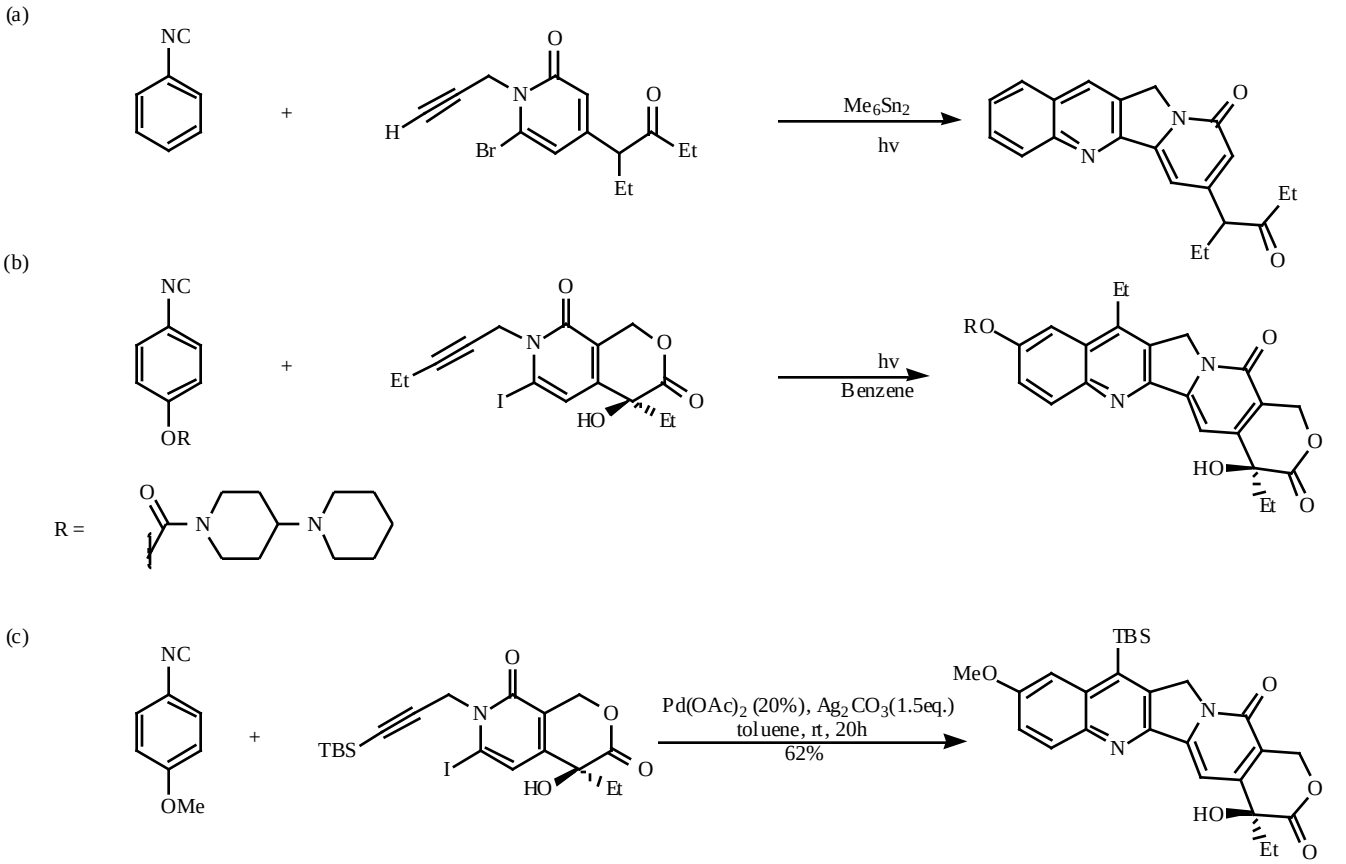


Fig. (11). Synthesis of mappacines and camptothecins by radical cyclization. (a) Radical-initiated cyclization of mappacines (b) Enantioselective synthesis of **3** (c) Transition metal catalyzed cyclization.

The transformation of the AB rings from an indole to a quinoline system mimics that of the biosynthetic pathway, although the mechanism is almost certainly different. Treatment of **45** with thinoyl chloride, Raney Nickel and DDQ yields intermediate **46**; Completion of the α-hydroxylactone by enzymatic hydrolysis and oxidation provides 20-deoxy CPT **31**. Oxidation yields racemic **1**. Reaching a structurally advanced intermediate such as strictosidine quickly and simply is an attractive means to generate a library of related and potentially biologically active compounds.

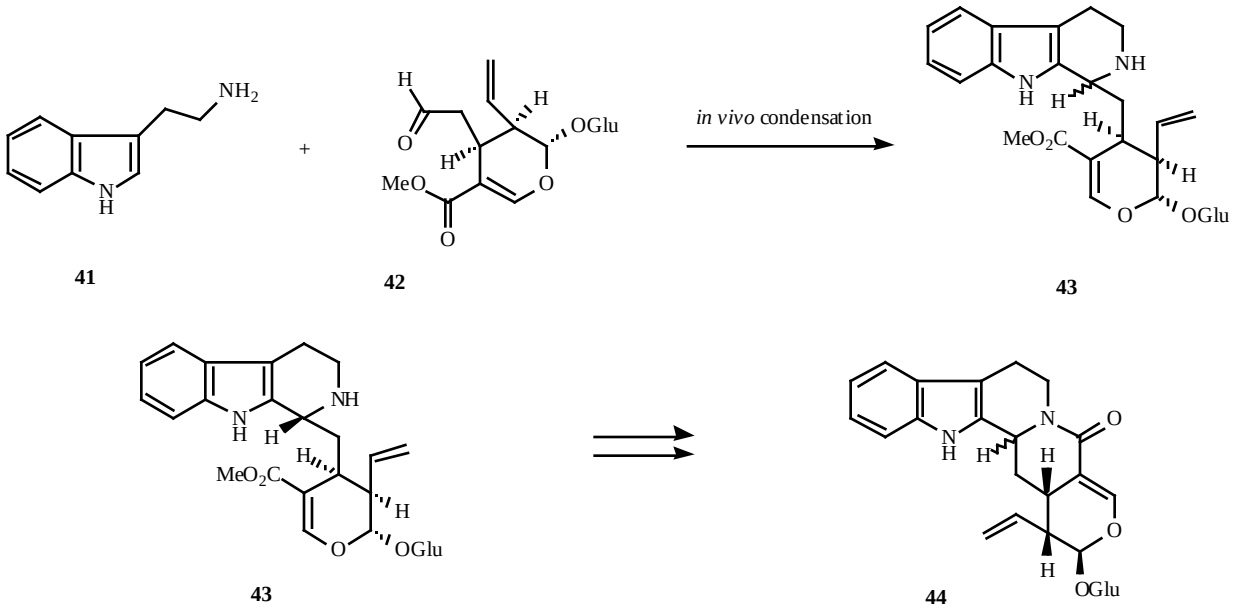


Fig. (12). Biomimetic synthesis of camptothecin. Condensation of tryptamine **41** and secologanin **42** yields intermediate 4**3**, which is subsequently converted to the structurally homologous indole strictosidine **44**.

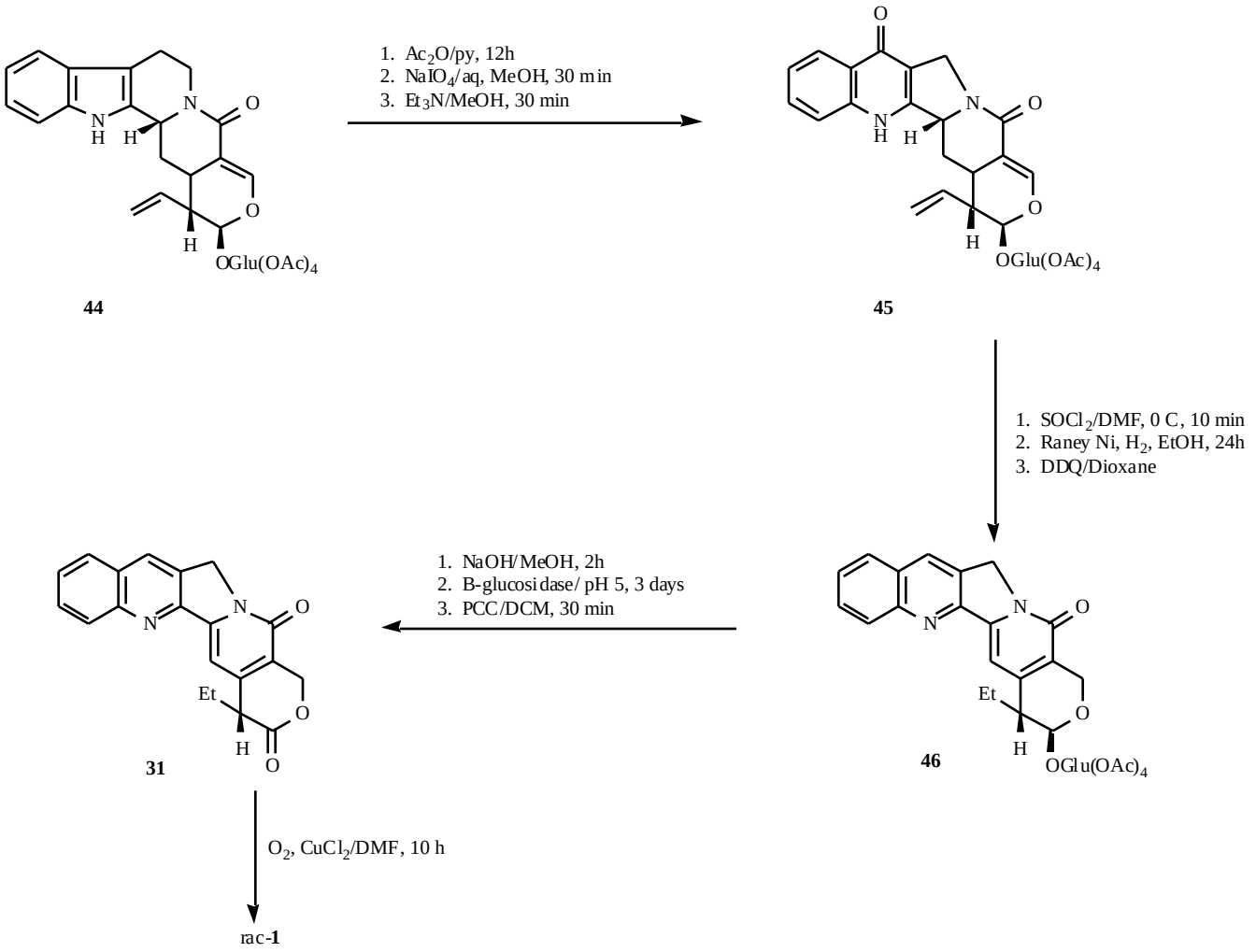


Fig. (13). **1** is derived from **44** through a biomimetic rearrangement of the indole system.

HOMOCAMPTOTHECINS AND SILATECANS

The discrepancy between the effectiveness of CPT drugs *in vitro* and *in vivo* is caused by rapid and reversible hydrolysis of **1** to **7** at pH 7 and above. Early clinical trials employing the sodium salt of the carboxylic acid as a means to address the water-insolubility of CPT itself discovered the acid to be toxic and ineffective. Homocamptothecins (hCPT) and silatecans, two new classes of CPT analog, contain complementary structural modifications, a seven-membered β-lactone E-ring and a 7-silylalkyl substituent, respectively that limit E ring hydrolysis *in vivo*. The seven-membered β-hydroxy lactone E-ring of hCPT is less prone to open through nucleophilic attack than the parent α-hydroxy lactone E-ring. The silylalkyl group of silatecans allows silatecans to partition into phospholipid bilayers of blood stream components, thereby avoiding interactions with physiological nucleophiles.

The advantage of E-ring homologation is three-fold. The methylene placer prevents activation of the lactone to nucleophilic attack by (1) eliminating hydrogen-bonding between the C-20 alcohol and the lactone carbonyl; (2) attenuating inductive electron withdrawal from the electrophilic C-20 alcohol. Additionally, seven-membered ring opening is slow relative to that of six-membered rings [69]. Lactone homologation was found to have unexpected beneficial effects. Homocamptothecins stabilize the ternary complex more effectively than CPT, and thereby induce a greater number of DNA lesions. Double-stranded DNA cleavage, normally observed only at TG sites, also occurs at AAC/G sequences [70]. Fluorinated derivates of hCPT have a higher activity against Topo1 than CPT and are not substrates for P-glycoprotein and MRP multidrug resistant proteins [71].

The ring-opened carboxylic acid form of CPT is preferentially bound over the lactone form by human serum albumin (HAS), an effect that shifts the concentration-dependant equilibrium towards the carboxylate [72-73]. By partitioning into the phospholipid bilayers of erythrocytes, silatecans avoid this interaction and thus accelerated hydrolysis. The partition constant for silatecans can be quite large. DB-67, a 7-*tert*-butyl-dimethyl-silyl-10-hydroxy analog is 25 to 50-fold more lipophilic than CPT [74]. Silatecans display cytotoxicity superior or comparable to that of CPT against colon, prostate, leukemia and non-small cell lung cancer cells *in vitro* and against intercranial gliomas *in vivo* [75].

Homocamptothecins are obtained either by a four-step semisynthetic route (Fig. **14**) or a convergent total synthesis

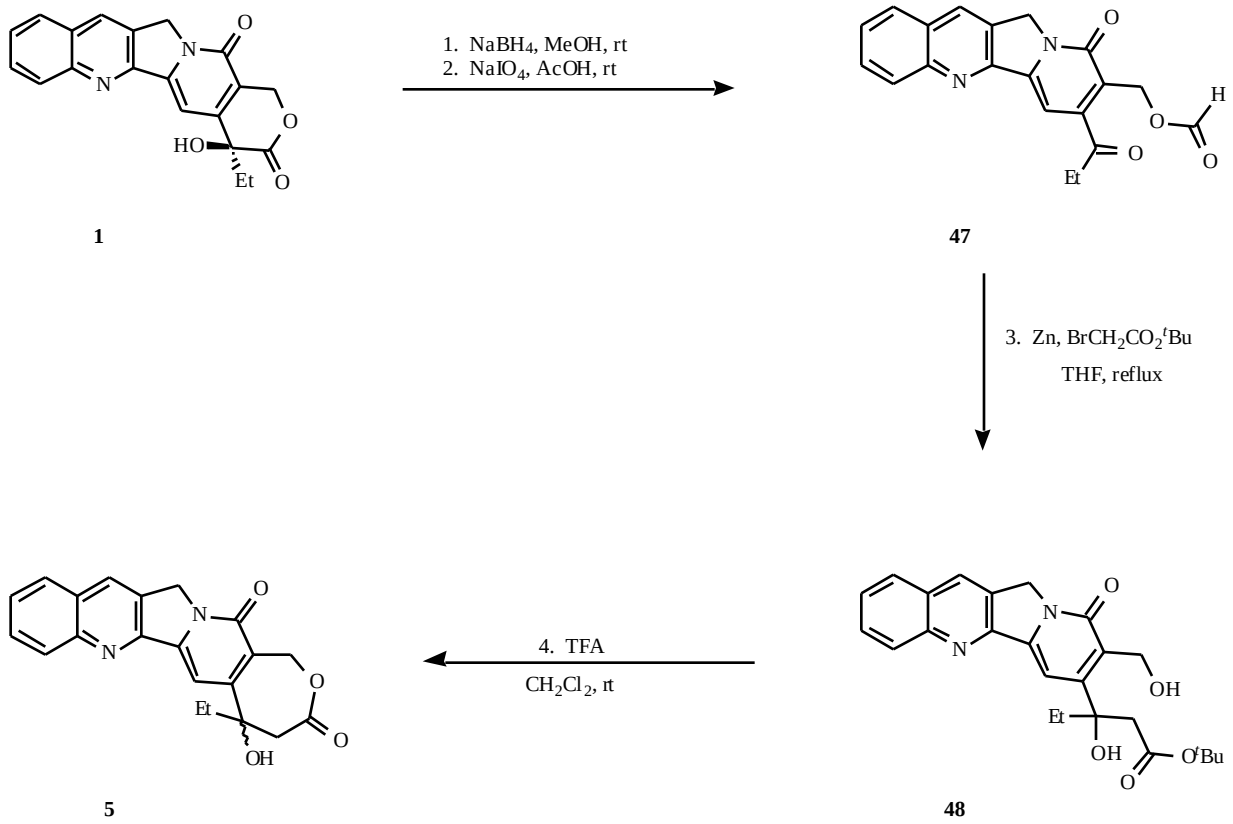


Fig. (14). Semisynthesis of homocamptothecin.

(Fig. **15**) [76-77]. In the semisynthesis, **1** is first reduced to the diol, then oxidatively cleaved by sodium periodate. The resulting formoxy-mappacine ketone **47** is subjected to a Reformatsky reaction using α-bromo-*tert*butylacetate. Trifluoroacetic acid-catalyzed lactonization provides racemic hCPT **5** from β-hydroxy ester **48**. Enantiopure material is available by recrystallization with α-methylbenzylamine. The total synthesis of homocamptothecins is accomplished by the method of Comins. The AB **50** and DE **51** bicycles are synthesized separately and then attached through nucleophilic attack. Intramolecular carbon-carbon bond formation completes the C ring of **52**. This strategy has allowed the evaluation of numerous racemic and enantiomerically pure A-ring derivatives [78]. One such drug candidate, BN80915 (10, 11-difluoro-hCPT), **53** is more potent inhibitor of Topo1 and more effectively induces apoptosis in HL-60 cells than hCPT itself [79].

The large-scale evaluation of silatecans has been made possible by the radical cyclization method of Curran (Fig. **16**) [80]. The DE bicycle **54** is propargylated with an alkyne bearing the desired alkylsilane **55**, then cyclized with an isonitrile. Synthesis of the DE bicycle, originally prepared in a somewhat lengthy linear sequence, has been simplified by the introduction of a method to asymmetrically cyanosilate ketone intermediates (Figs. **17**, 1**8**) [81]. The method utilizes samarium or gallium triisopropoxide in addition to a chiral phosphine oxide ligand **62** to simultaneously activate the ketone substrate through complexation with the metal center and position the cyanide anion for nucleophilic attack. Ligand screening of phosphine aryl groups identified difluorocatechol containing ligands as superior- both yield and enantiomeric excess exceed 90 percent. The method has been adapted to a catalytic enantioselective synthesis of both Curran **38** and Corey **11** intermediates on a gram scale [82-83].

Modification of the C-7 silylalkyl chain significantly alters the partition coefficients of silatecan drugs. This observation, in addition to the convergent nature of the cyclization strategy, created interest in combinatorial syntheses of both silatecans and homosilatecans, CPT analogs combining the 7-silylakyl group of silatecans with the expanded β-lactone of homocamptothecins [84-86]. Although homosilatecans were indeed found to manifest both the lipophilicity of silatecans and the stability of hCPTs, they are less effective than CPT against breast carcinoma cells *in vitro*.

The semisynthesis of silatecans directly from CPT via a radical silylation holds much promise. The position of addition is temperature-dependant, with low temperatures favoring the 7-silylalkyl isomer and higher temperatures the 12-silylalkyl isomer. The addition of a thiol greatly improves the reaction [87]. Although yields are low, the method obviates a costly total synthesis.

SUMMARY

To summarize, camptothecin drugs have enjoyed a renaissance since the identification of human DNA topoisomerase 1 as the molecular vehicle for CPT-mediated DNA damage. The emergence of homocamptothecins and silatecans, two new classes of CPT analog that employ unique structural modifications to resist *in vivo* hydrolysis,

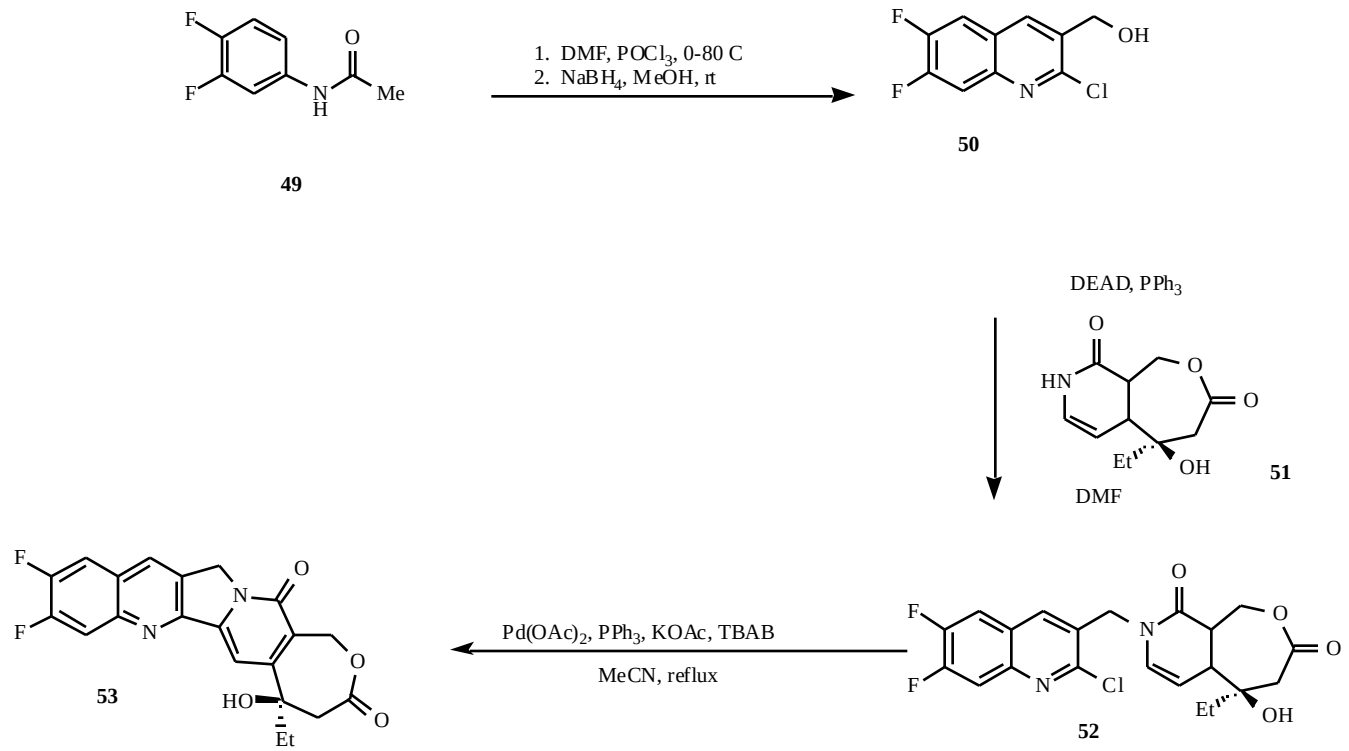


Fig. (15). Total synthesis of enantiomerically pure homocamptothecin.

more effectively inhibit Topo1 and overcome drug-resistance mechanisms, results directly from the introduction of novel synthetic routes and methods. The future for these and other drug candidates appears bright. The elevated concentration of Topo1 in a variety of tumors provides a solid therapeutic basis for intervention, either with CPT drugs alone or in conjunction with adjuvants. Furthermore, the success of CPT has legitimated Topo1 as a drug target; the large-scale screening of molecular libraries has produced a number of previously unknown non-CPT inhibitors of Topo1 [88-90]. The abundance of new analogs may well result in the clinical advancement of improved CPT drugs.

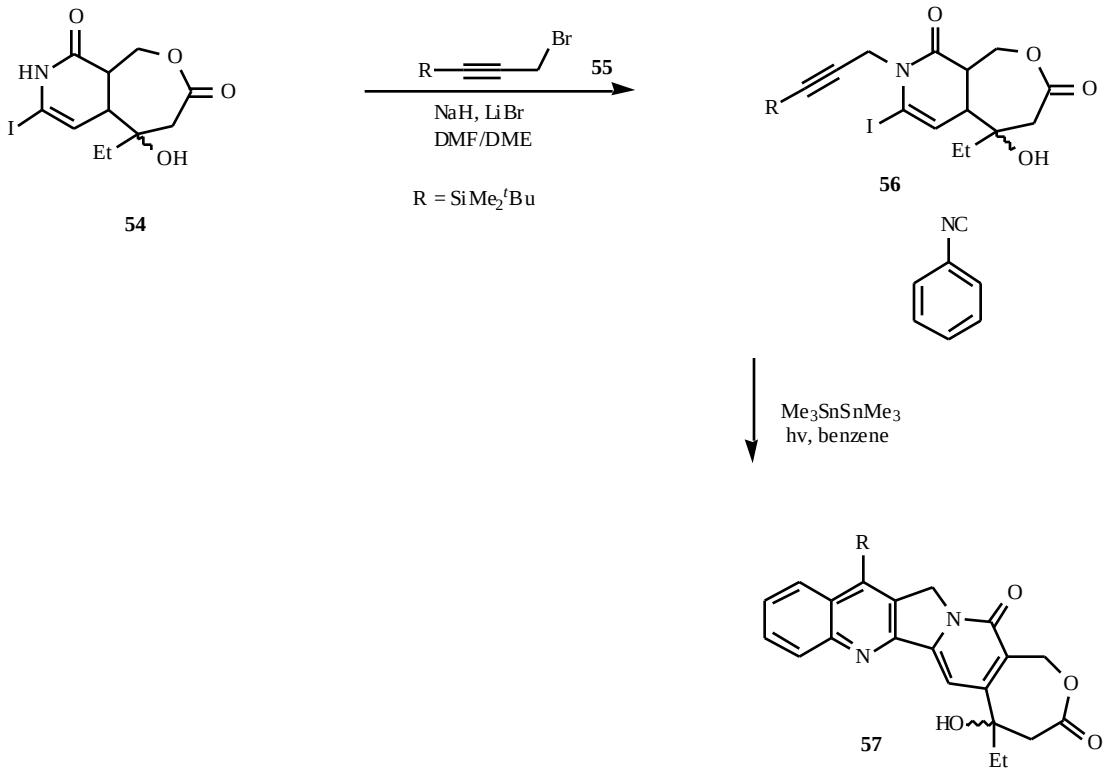
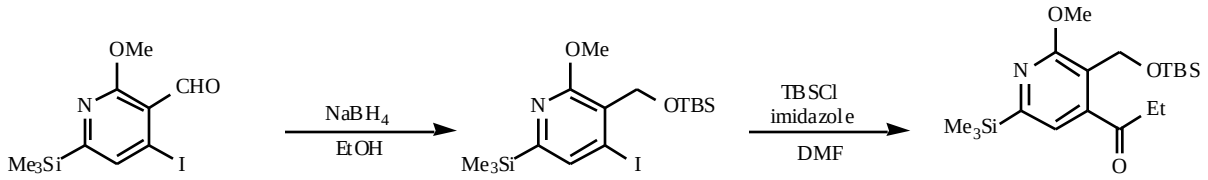
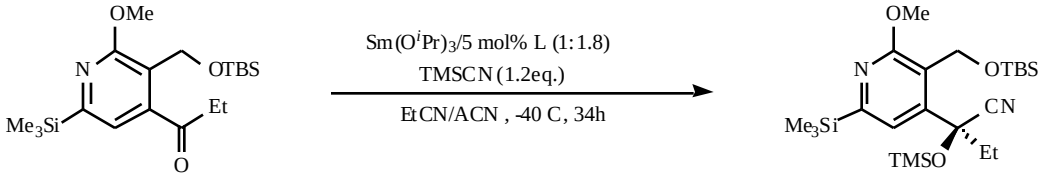


Fig. (16). Synthesis of homosilatecan **57** by a radical-initiated cyclization cascade.

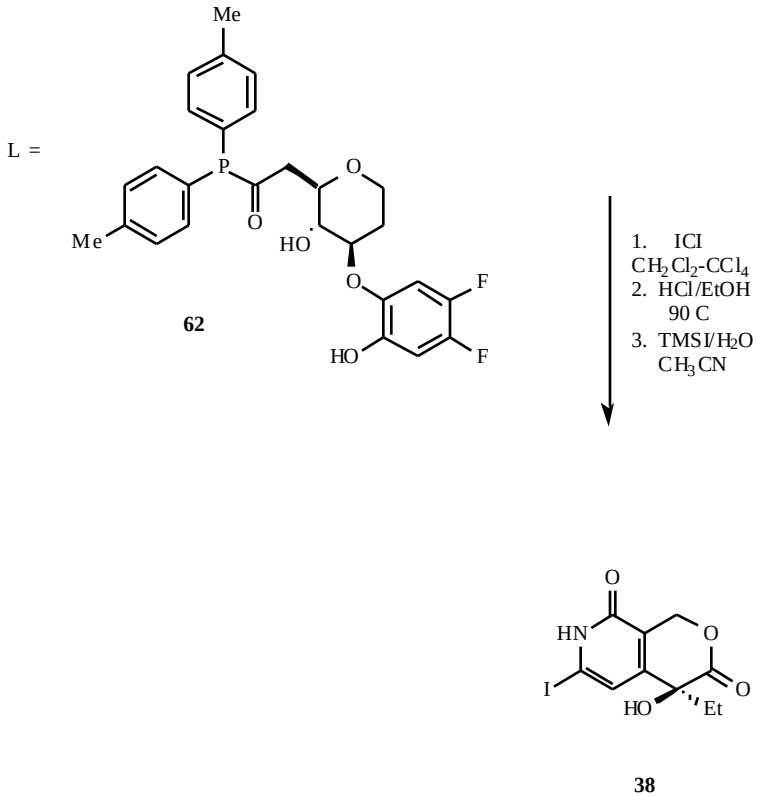


58 **59** **60**



60 **61**

91 %, 90% ee



62 **38**

Fig. (17). Synthesis of Curran's intermediate **38** by enantioselective cyanosilation.

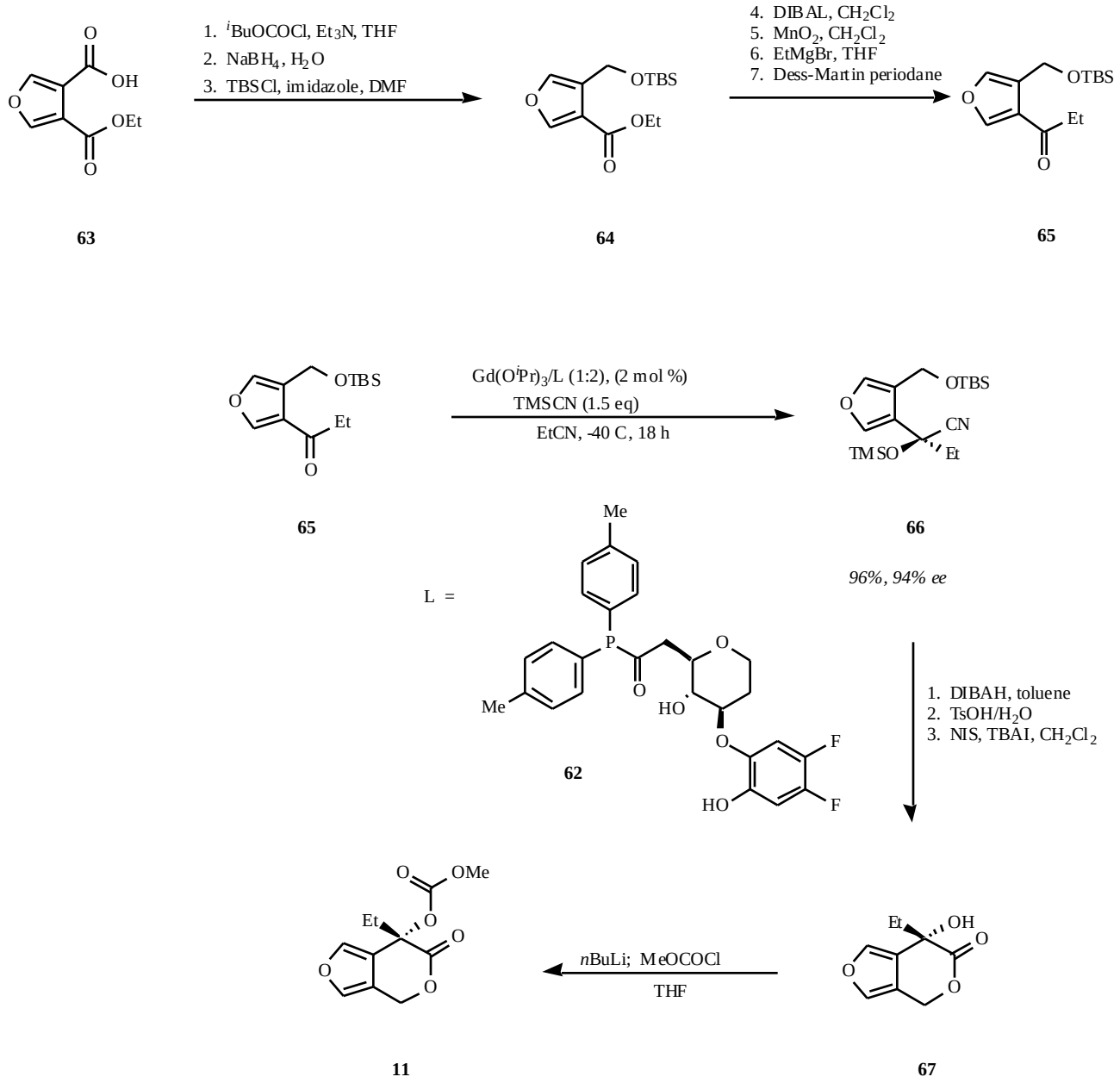


Fig. (18). Synthesis of Corey's intermediate **11** by enantioselective cyanosilation.

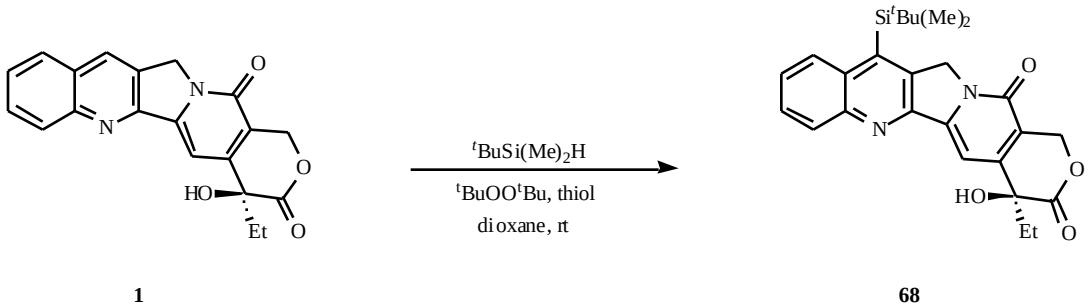


Fig. (19). Semisynthesis of silatecan **69**.

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LIST OF ABBREVIATIONS

CPT = Camptothecin

Topo1 = Human DNA topoisomerase 1

hCPT = Homocamptothecin

PARP = Poly(ADP-ribose) polymerase

TCC = Trans-2-(α-cumyl) cyclohexyl

TMS = Trimethyl silyl

TBS = Te*rt*-butyldimethyl silyl

DMF = N, N dimethylformamide

DDQ = 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone

PCC = Pyridinium chlorochromate

DCM = 4-(dicyanomethylene)-2-methyl-6-(4-dimethyl-aminostyry)-4*H*-pyan

TBAB = Tetrabutylammonium bromide

Bz = Benzyl

Glu = Glucose

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