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Total Synthesis, Mechanism of Action, and Antitumor Efficacy of Camptothecin and Some of its Analogues

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Abstract: Over the past 55 years of research, various experimental methods have been developed for the total synthesis of the anticancer camptothecin, a potent antitumor antibiotic, and its numerous active derivatives. The discoveries made in synthetic pathways of the camptothecin heterocyclic core have contributed significantly to the theory and strategy of directed organic synthesis aimed at finding effective anticancer drugs. The synthetic, medicinal chemistry of camptothecin, the development of structures of anticancer camptothecin analogues, and the mechanism of their activity in inhibiting the growth of different types of cancers, such as lung, ovarian, breast, pancreas, and stomach cancers are analyzed. Various structural modifications in the **A**, **B**, **C**, **D,** and **E**-rings of the camptothecin molecule have been thoroughly studied to improve bioavailability and diminish toxicity. Modern synthetic approaches to the camptothecin analogues and several semi-synthetic methods are reviewed.

Keywords: Camptothecin, analogues, total synthesis, DNA Topoisomerase I, inhibition, anticancer drugs, structural modifications.

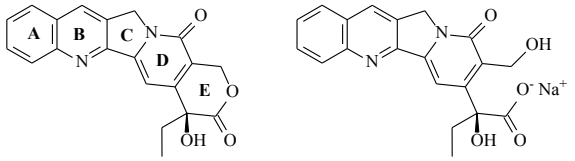
1. INTRODUCTION

In 1966, more than 50 years ago, Wall and Wani reported [1] the isolation of a new alkaloid from *Camptotheca acuminata* (*Nyssaceae*), namely 20(*S*)-camptothecin (**1**, CPT), which showed antitumor activity. Later several new minor analogues of camptothecin were found in the roots, bark and fruits of *C. acuminata* and some other plants [2].

Camptotheca acuminata is known by various names in China, including Happy tree, Dragon tree, Heaven wood tree, and Fine tree. Chinese folk medicine has used the Happy tree for thousands of years in the traditional treatment of psoriasis, leukemia and the stomach, liver, gallbladder, and spleen diseases [3] (Fig. **1**).

The CPT molecule, which has been isolated from the Camptotheca tree, is a pentacyclic (**ABCDE**) pyranoindolisino[1,2-*b*]quinoline alkaloid and has drawn attention because of its significant antitumor activity [1, 2, 4, 5]. Subsequent research also showed that the Na salt of CPT (**2**), which has an antileukemic activity of the same order as **1** [6] and can convert to **1** by acidification [1], demonstrated the low efficacy and excessive non-specific toxicity to bone marrow and bladder cells [2]. CPT was found to rapidly block both DNA replication and RNA transcription in treated cells, and it has become a potent anticancer compound [4, 7, 8].

In the review of 2017, which covered more than 350 published studies concerning the biological activity of CPT derivatives, Li *et al.* discussed various aspects of the mechanism of the action (MOA), including new MOAs for CPT and analogues, antitumor activity of the substituted camptothecins, SARs of CPT analogues and their molecular targets [9]. Based on the data reviewed, the authors concluded that this area of research has great potential and should be aimed at developing analogues of the next generation of CPT with new mechanisms of activity, high efficacy, and low toxicity for the effective treatment of human diseases, including cancer.



20(*S*)-Camptothecin (1) **20(*S*)-Camptothecin Na-salt (2) (water soluble)**

Fig. (1). Chemical structures of 20(*S*)-camptothecin (**1**) and Na salt of **1**.

2. MECHANISM OF 20(*S*)-CAMPTOTHECIN ANTI-TUMOR ACTIVITY

20(*S*)-Camptothecin and its synthetic analogues are chemotherapeutic agents that covalently and reversibly bind DNA Topoisomerase I (Topo-I) to the DNA intermediate, causing the formation of double-strand DNA breaks (DSB) during replication and translation, resulting in the accumulation of a covalent reaction intermediate, the cleavable complex [10, 11]. Only 20(*S*)-isomer of natural CPT was found to be active against Topo-I [12] (Fig. **2**).

Eukaryotic Topo-I has been shown to be the sole intracellular target for CPT and its derivatives [13, 14]. *In vitro* studies revealed that CPT and its analogues inhibited plasmid relaxation by human Topo-I and increased the yield of covalent intermediates when the reaction was stopped with Sodium Dodecyl Sulfate (SDS) [7, 8, 15]. Based on the Topo-I crystallographic information with SAR, a hypothetical CPT binding model was proposed by Redinbo *et al*. [16]. It was demonstrated that a hydrogen-bonding network could be established between the Asp^{533} and Arg^{364} side chains and the 20(*S*)-OH and the lactone moiety of CPT. Mutation of the Phe^{361} and Gly^{363} residues is likely to mediate CPT resistance by disrupting the conformation of the loop holding Arg^{364}. The structural bases for these two activities of CPT were studied and reviewed

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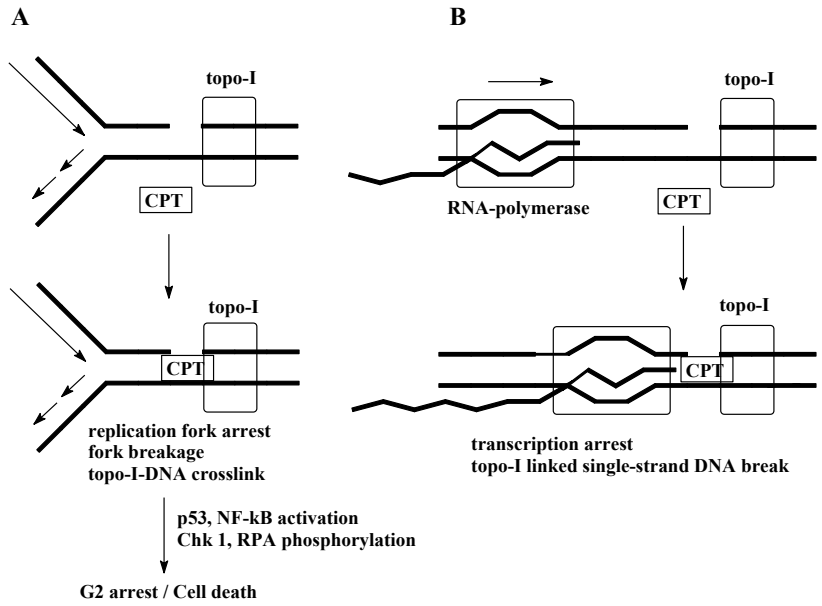


Fig. (2). Mechanism of 20(*S*)-camptothecin action. Cellular processing of Topo-I cleavable complexes. Collision models [11]. (**A**) A replication fork collision model. (**B**) An RNA polymerase collision model.

concerning the published crystal structure of the enzyme with bound DNA [17, 18].

Subsequent studies have confirmed that the **CDE** rings of CPT cannot be altered without affecting its ability to block relegation by Topo-I [19, 20]. In contrast, the modification of the AB-rings, especially at carbon atoms, C-7, C-9, and C-10, was generally well tolerated and, in many cases, enhanced the potency of the CPT analogues in both *in vitro* and *in vivo* studies [21]. Furthermore, substantial evidence indicates that CPT binds reversibly only after the enzyme is cleaved and covalently attached to DNA [22]. Recent studies have shown that CPT changes the cellular localization of the Werner syndrome protein (WRN, RecQ helicase) and induces its degradation by the ubiquitin-mediated proteasome pathway. The study found that the extent of CPT-induced WRN degradation correlated with increased sensitivity of breast cancer cells to CPT and could be used to identify tumors that may be sensitive to CPT and its derivatives [23]. A recent review discussed the main molecular mechanisms of action of Topo-I inhibitors, including CPT and its derivatives, their current applicability and limitations as antineoplastic agents [24].

3. TOTAL SYNTHESIS OF CAMPTOTHECIN

Due to the high anticancer activity of CPT, the total synthesis of this compound and confirmation of its structure are of foremost importance. In addition, owing to the rather complex heterocyclic structure of this alkaloid, including five cycles of different nature and the presence of an asymmetric carbon atom, the synthesis of CPT has become and remains a priority task of synthetic organic medicinal chemistry.

The first successful total synthesis of a CPT molecule was reported by Stork and Schultz (Scheme **1**), which confined the preparation of racemic form because the synthesis of the ring **E** and the chiral lactone presented the greatest complexity [25].

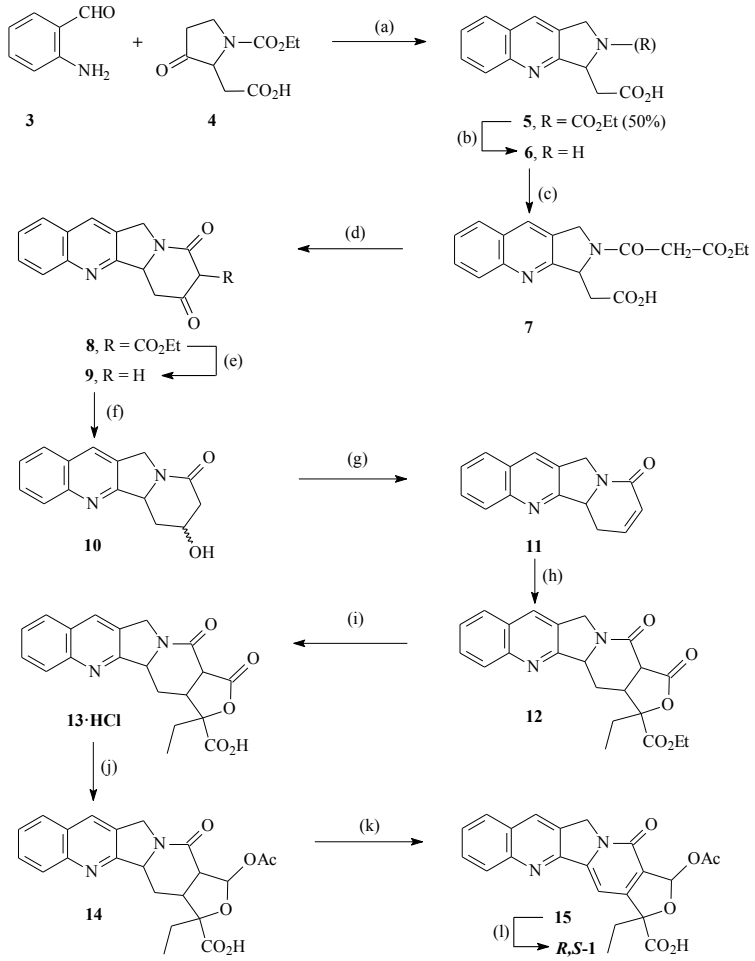
The authors started by condensing 2-aminobenzaldehyde **3** with known pyridone **4**, which gave them a conjugated **ABC** cycle system in compound **5**. Intermediate compound **5** was endowed with suitable substituents for the formation of the cycle **D**. In the following three stages involving replacement of the substituent at the N atom of the **C**-ring and cyclization, the **D**-ring was created in derivative **8**. The next three stages were aimed at modifying the **D**-ring and forming unsaturated lactam **11** with a double bond in the cycle **D**, which was needed for the addition of carbanion generated from ethyl α-hydroxybutyrate. The successful addition was performed at low temperature to yield pentacyclic lactone **12**. In the following stages, the ester residue on cycle **E** was hydrolyzed to the acid; the lactone moiety was reduced, and then acylated to acylhemiacetal **14**, then cycle **D** was dehydrogenated with DDQ to give pyridone **15**. The transformation of **15** to **1** was accomplished by successive treatment with NaOH, $NaBH_4$, and diluted hydrochloric acid.

In general, this strategy was implemented in 12 stages (Scheme **1**) and can be described as follows:

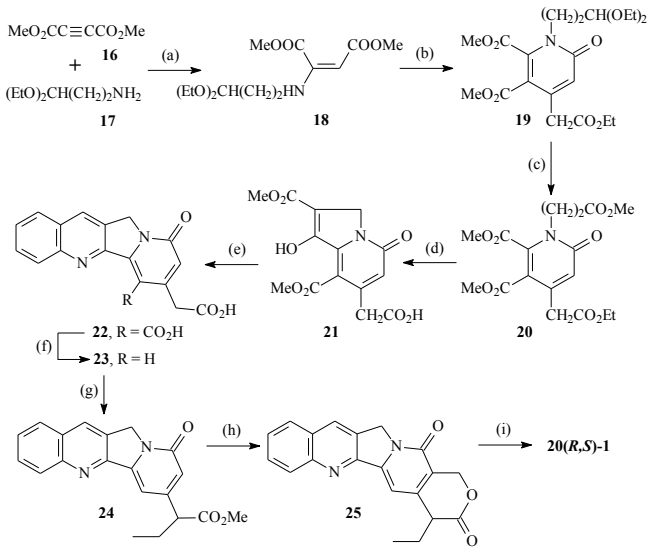
A + C → ABC + D → ABCD + E → ABCDE = 1.

After the publication of Stork and Schultz, Volkmann and coworkers reported the total synthesis of 20(*R,S*)-**1** using a new approach to pyridone synthesis developed in their laboratory [26]. Danishefsky`s synthesis was carried out in 9 steps beginning with the preparation of the tetra substituted pyridone cycle **D** (**19**) by Michael's addition of 1,3-dicarbethoxyallene to enamine **18**, which was obtained by the addition of 3,3-diethoxypropan-1-amine (**17**) to dimethylbut-2-ynedioate (**16**) (Scheme **2**). In the following steps, the **C**-ring in intermediate **21** was created by the hydrolysis-decarboxylation and intramolecular Dieckmann cyclization of **20** to provide **C**, **D**-fragment **21**. Friedländer condensation of **21** with o-aminobenzaldehyde in the sodium hydroxide media yielded the **A**, **B**, **C**, **D**-tetracyclic diacid **22**. Decarboxylation of the carboxyl group at position 5 of the pyridone **D**-ring by pyrolysis and ethylation of the benzylic type carbon with sodium hydride-ethyl iodide provided tetracyclic intermediate of **24**. 20-Deoxycamptothecin (**25**) was isolated after methyl hydroxylation **24** with paraformaldehyde in an acid medium, thereby completing the creation of **A**,**B**,**C**,**D**,**E**-pentacyclic scaffold of CPT. The authors found that intermediate **25** was easily oxidized to **1** by air, indicating a possible bioconversion stage. In the preparative process, H_2O_2, which was used as an oxidant, gave desired 20(*R,S*)-**1** in 20% yield.

In the same year, Winterfeldt published a study on approaches to the biomimetic synthesis of CPT [27, 28], which was based on the discovered transformation of some natural tetrahydro-β-carboline alkaloids into pyrrolo[3,4-b]quinoline by the



Scheme 1. First synthesis of racemic 20(*R,S*)-**1** (12 stages) [25].
Reaction conditions: (**a**) NaOH (dil.), 50%; (**b**) i) HI (50%), 14 h, reflux; ii) C_2H_5OH/HCl; (**c**) $ClCOCH_2CO_2Et/NaHCO_3$ (aq)/C_6H_6; (**d**) NaOEt/EtOH-PhMe (1:5), heating; (**e**) 10% AcOH, reflux, 4 h, (overall 72% from **5**); (**f**) $NaBH_4$/EtOH, 95%; (**g**) $(AcO)_2O$/NaOAc, reflux; (**h**) THF/ -70 °C, LDA/$EtCH(OCO_2Et)CO_2Et$, 85%; (**i**) HCl (conc), reflux, 2 h; (**j**) i) $NaBH_4$/EtOH, ii) $(AcO)_2O$/Py; (**k**) DDQ/1,4-dioxane, reflux, 4 h; (**l**) i) 0.1M NaOH (aq.), 0.5 h; ii) $NaBH_4$, r.t., 6 h; iii) HCl (dil.)

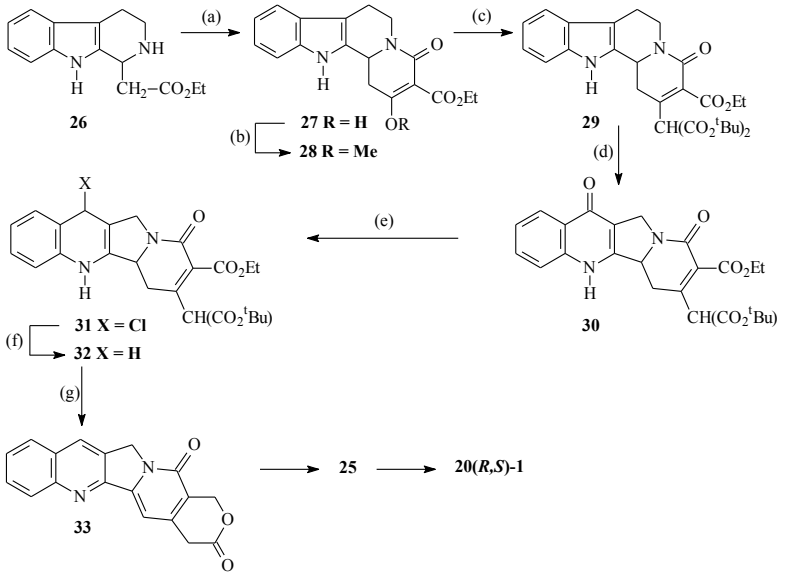


Scheme 2. Total synthesis of 20(*R,S)*-**1** by Volkmann and coworkers [26].
Reaction conditions: (**a**) r.t., ether, 67%; (**b**) 1,3-dicarbethoxyallene, TEA, EtOH(anh), r.t., 45%; (**c**) i) HCl/acetone/water, quant.; ii) CrO_3/H_2SO_4/acetone/H_2O; iii) MeOH/HCl, r.t., 86%; (**d**) i) NaOMe (3 equiv.)/MeOH, reflux, 12 h, 81%; ii) 4% aq. HCl, reflux, 3 h; (**e**) i) NaOH (3 equiv.)/o-aminobenzaldehyde (2 equiv.)/H_2O, reflux, 36 h; ii) MeOH/HCl, r.t.; (**f**) CuO (0.3 equiv.), 29% (from **21**); (**g**) NaH (1 equiv.)/DME/EtI (excess), r.t. 20%; (**h**) CH_2O/H_2SO_4 (trace)/1,4-dioxane, 100 °C, 16 h; (i) t-BuOK/DMSO-BuOH/H_2O_2 (1 equiv.), 20%.

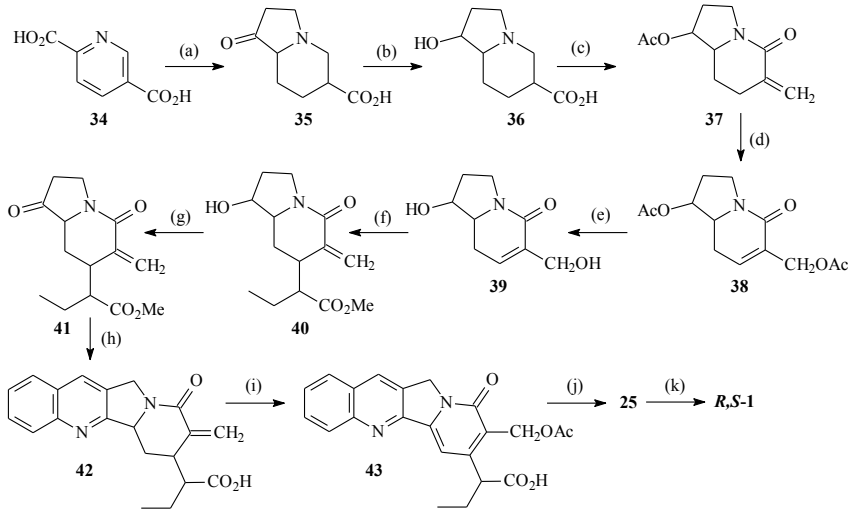
base-catalyzed autoxidation. They applied this reaction as a key step in the synthesis of 20(*R,S*)-**1** (Scheme **3**) [27-30].

Winterfeldt started with ethyl 2-(2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)acetate (**26**) (Scheme **3**), which served as the basis for the **A**,**B**,**C**-moiety CPT. Compound **26** reacted with monoethyl malonate and cyclocondensed to tetracyclic intermediate **27**, in which the hydroxyl group was continuously transformed to the methoxy group of derivative **28**, then to the di-tert-butyl malonyl residue of compound **29**. Intermediate **29** was autoxidized in the presence of t-BuOK/DMF to pyrrolo[3,4-*b*]quinolone (**30**), which contained **A**,**B**,**C**,**D**-cycles and the other necessary atoms to form the cycle **E**. The quinolone moiety was deoxygenated and aromatized to compound **32** and then transformed to 1,12-dihydro-4*H*-2-oxa-6,12*a*-diaza-dibenzo[*b,h*]fluorene-3,13-dione (**33**). From derivative **33,** 20(*R,S*)-**1** can be obtained by consecutive ethylating with sodium hydride and ethyl bromide (iodide) and oxidation. Winterfeldt`s research was very important because it showed how indole alkaloids, including carbolines, as tryptophan derivatives, could be biochemically transformed to CPT.

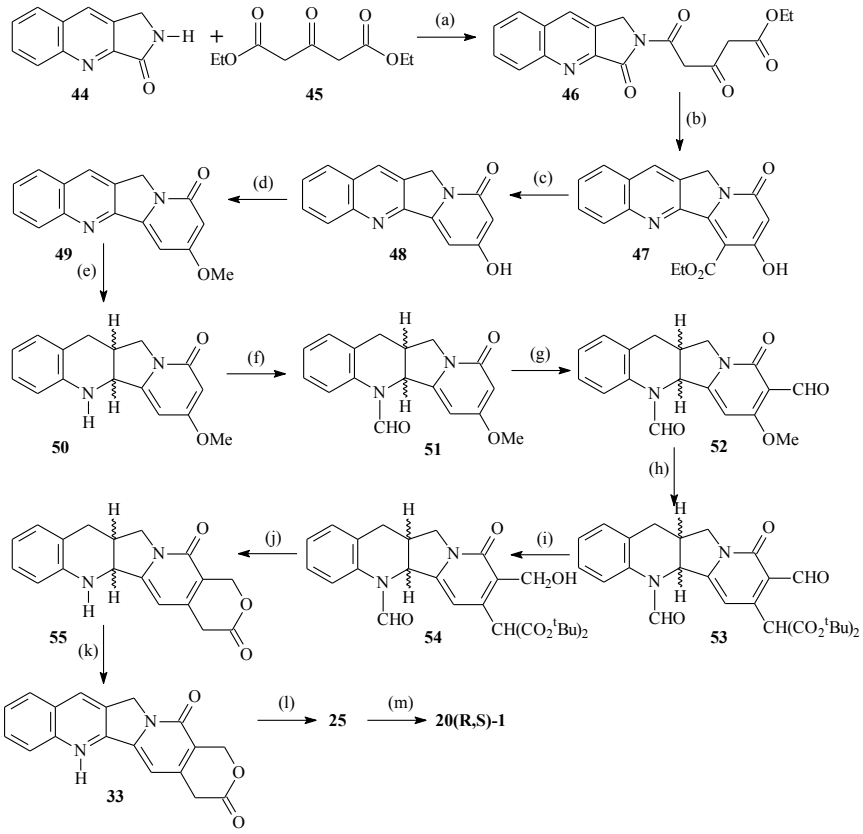
The following year after Danishefsky's report, Plattner *et al.* published research on 20(*R,S*)-**1** synthesis, which was preceded by the studies on the synthesis of CPT analogues. The authors began with pyridine-2,5-dicarboxylic acid **34** [31] and prepared bicyclic **C**, **D**-moiety **35** in the first step (Scheme **4**) [32]. The following steps were aimed at forming an α-carbonyl group (stages *b* and *c*) and a 3,4-double bond (stages *d* and *e*) in the pyrimidine cycle, suitable for the addition of an α-butyrate side chain by Claisen rearrangement. The transformation of hydroxyl at C-1 indolizine **40** into a carbonyl group yielded intermediate **41**, which was subjected to the Friedländer quinoline synthesis for the formation of tetracy-



Scheme 3. Total biomimetic synthesis of 20(*R,S*)-**1** developed by Winterfeldt *et al*. [27-30].
Reaction conditions: (**a**) $CH_2(CO_2Et)CO_2H$/t-BuOK, 86%; (**b**) CH_2N_2/ether, 83%; (**c**) NaH/$CH_2(CO_2t\text{-}Bu)_2$, dioxane, 68%; (**d**) t-BuOK/DMF/O_2, r.t., 2h, 68%; (**e**) $SOCl_2$/DMF, r.t., (**f**) $CuCl_2$/DMF/Sparteine/O_2, 1 h, r.t.; (**g**) i) DIBAL; ii) KBH_4; iii) TFA, 56%.



Scheme 4. Total synthesis of 20(*R,S*)-1 by Tang and Rapoport [32].
Reaction conditions: (**a**) i) Ethyl 3-bromopropionate, 75%; ii) tBuOK/toluene; iii) 6N HCl, 105 °C, 5 h, quant. Yield i-iii) 85%; (**b**) $NaBH_4$/MeOH-H_2O, 0 °C, 18 h, 86%; (**c**) Ac_2O, 145 °C, 2.5 h, 84%; (**d**) SeO_2/AcOH, 70 °C, 1 h, 58%; (**e**) K_2CO_3/MeOH, 20 °C, 0,5 h, quant.; (**f**) i) n-PrCH(OMe)$_3$/$EtCO_2H$ (cat.), 145 °C, 3 h, 75%; ii) K_2CO_3/MeOH, 20 °C, 1 h, quant.; (**g**) DCC/DMSO/H_3PO_4 (cat.), 20 °C, 30 h, 76%; (**h**) i) NaOH/ *N*-(2-aminobenzylidene)-*p*-toluidine/H_2O, reflux; ii) MeOH/HCl, r.t., 75%; (**i**) SeO_2/AcOH, 80 °C, 0.5 h, 58%; (**j**) 2*N* H_2SO_4/glyme, 50 °C, 5 h, 72% from **42**; (**k**) $CuCl_2$/DMF/O_2, quant.



Scheme 5. Total synthesis of 20(*R,S*)-1 by Sugasawa *et al.* [35].
Reaction conditions: (**a**) 160-165 °C, 95%; (**b**) MeCN/piperidine, reflux, 87%; (**c**) $HCl_{conc.}$, 150 °C, glass tube; (**d**) $(MeO)_2SO_2/K_2CO_3$, acetone, reflux, 85% (for c, d); (**e**) Adams cat./MeOH-dioxane/HCl; (**f**) HCOOH, heating; (**g**) $POCl_3$/DMF, 63%; (**h**) $CH_2(CO_2{}^tBu)_2$/NaH, dioxane, reflux, 60%; (**i**) $NaBH_4$/MeOH-H_2O; (**j**) $HCl_{conc.}$, r.t.; (**k**) DDQ, dioxane, reflux, 32% (for i, j); (**l**) EtI/NaH, DMF; (**m**) O_2/$Cu(OAc)_2$, DMF-MeOH, 8% (for l, m).

clic **A**,**B**,**C**,**D**-fragment **42**. The aromatization, hydrolysis, and lactonization to form desoxycamptothecin **25** were carried out in two steps, and the final oxidation of **25** gave desired 20(*R,S)*-**1** in the overall yield of 11% starting from **34**. Three years later, the same authors developed an improved pathway for CPT synthesis [33]. The achievement of these studies was also the high overall yield of the final product, *i.e*., 15%.

The first analysis of methods for the CPT synthesis was carried out by Schultz [34]. In the review article, the author considered in detail the achievements in synthesizing **1** and some of its derivatives, hypotheses on the biogenesis of CPT molecules, and discussed the existing approaches to its chemical synthesis. Since then, the most widespread pathway of CPT synthesis has been scheme using differently substituted pyridones as intermediates, which served as the basis of the cycle **D.** Such a strategy was first proposed in the studies of Volkmann [26] and Tang and Rapoport [32]. By this time, the main pathways **D → CD → ABCD → ABCDE**, **D → DE → ABCDE,** and **ABC → ABCD → ABCDE** had been implemented by various synthetic methods.

In the same year, 1972, Sugasawa *et al.* published a new synthesis of 20(*R*,*S*)-**1** [35]. Their approach was based on the condensation of 1,2-dihydro-3H-pyrrolo[3,4-b]quinolin-3-one [1] (**44**) like an **A**, **B**, **C**-moiety with diethyl 1,3-acetonedicarboxylate (**45**) as a base to form the cycle **D** from derivative **46** (Scheme **5**). The cycle **D** was formed by condensation of **46** in the presence of piperidine as the base to give intermediate **47**.

In the next steps, the researchers attempted to create the necessary substituents to build cycle **E**. The unnecessary ethoxy carbonyl group was removed by heating intermediate **47** in a sealed tube with concentrated HCl to yield the phenolic derivative **48**. The hydroxy group in molecule **48** was protected as methyl ether by the action of dimethyl sulfate to yield compound **49**.

Then, the authors hydrogenated tetracyclic derivative **49** to N-tetrahydroquinolin-pyridone **50** because the corresponding pyridone showed better selective reactivity in the model reaction [35]. Compound **50** was formylated with HCO_2H to provide intermediate **51**, which was subjected to the Vilsmeier–Haack reaction to form aldehyde **52**.

Synthesized aldehyde **52** was treated with di-t-butyl malonate, and during this reaction, the methoxy group was replaced with di-t-butyl malonyl substituent to yield derivative **53**. In the molecule of intermediate **53**, the aldehyde group was reduced by sodium borohydride to a hydroxymethyl group to provide compound **54**. Deformylation, hydrolysis, decarboxylation, and lactonization of **54** to derivative **55** were accomplished in a one-pot reaction in the presence of concentrated HCl under mild reaction conditions. In the next stage, the conjugation between the **A**, **B**- and **D**-moieties was fulfilled using dehydrogenation with DDQ to yield intermediate **33**, which had previously been obtained by Winterfeldt (Scheme **3**) [28]. Ethylation of **33** and oxidation of the obtained 20-deoxycamptothecin **25** resulted in the isolation of target 20(*R,S)*-**1**. A special feature of this synthetic route was the initial synthesis of the intermediate consisting of **A**,**B**,**C**,**D**-rings and its further modifi-

[1]In the article cited, the authors proposed the name of 1,2-dihydro-3*H*-pyrrolo[3,4-b]quinolin-3-one for compound (**44**). 35. Sugasawa, T.; Toyoda, T.; Sasakura, K., A total synthesis of dl-camptothecin. *Tetrahedron Lett.* **1972,** *13* (50), 5109-5112.

cation. In addition, the removal of the conjugation between the **A**,**B** and **D**-cycles made it possible to carry out all the necessary transformations for creating the **E**-ring and the corresponding substituents on it. It should be noted that the lack of conjugation among the **A**, **B**-, and **D**-cycles was also a decisive condition, facilitating the implementation of the targeted transformations in the biomimetic synthesis developed by Winterfeldt *et al.* [28].

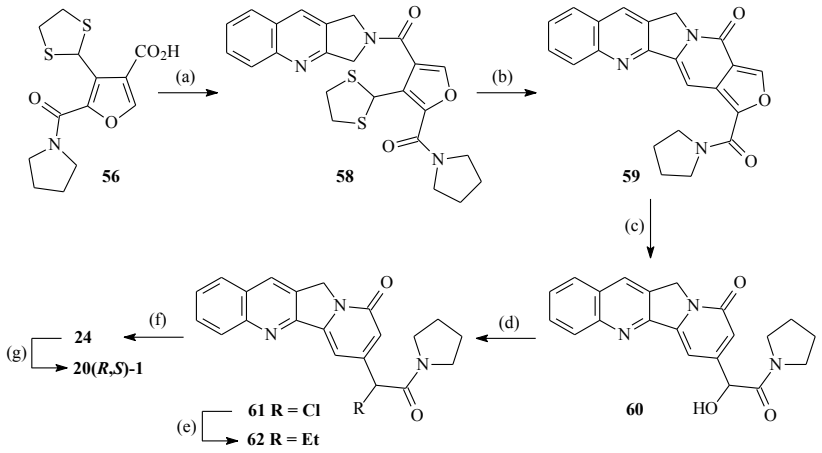
Kende and coworkers [36] obtained substituted furan-3-carboxylic acid **56** from furfural in 12 steps. Acid **56** was condensed with 2,3-dihydro-1*H*-pyrrolo[3,4-*b*]quinoline (**57**) to yield synthon **58**, which was then cyclized, providing the pentacyclic intermediate **59** (Scheme **6**).

The furan ring of **59** was opened in the basic media to provide compound **60**, in which benzylic hydroxyl was appropriate for the substitution with chlorine to yield derivative **61**, and the following reaction with Lithium diethylcuprate gave **62** with an ethyl substituent at the corresponding position.

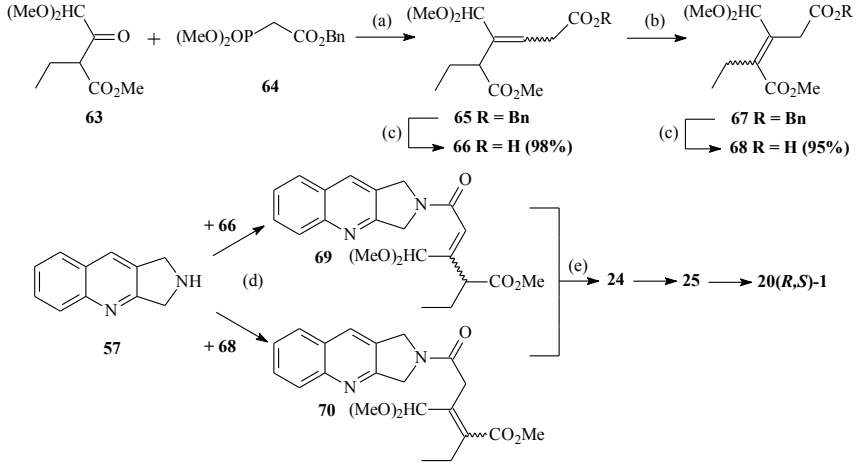
Hydrolysis of pyrrolidin-1-yl substituent of **62** and esterification of the obtained acid with methanol provided derivative **24**, from which the cycle **E** was obtained in four steps according to the methods of Volkmann and coworkers [26] in just thirteen synthetic operations (Scheme **6**). Thus, intermediates **24** and **25** became key compounds due to the easy ability of their transformation into 20(*R,S*)-**1**. This approach corresponds to the **ABC** → **ABC-E** → **ABCDE** pathway, where the substituted furan cycle plays the role of a latent **E**-cycle.

Bradley and Büchi [37] focused on studying the synthetic routes to 20-Deoxycamptothecin **25**. They started with the readily available methyl 2-ethyl-4,4-dimethoxy-3-oxobutanoate (**63**, Scheme **7**), which was subjected to a Wittig reaction with benzyl 2-(dimethoxyphosphoryl)acetate (**64**). The product of the reaction, 6-benzyl-1-methyl-3-(dimethoxymethyl)-2-ethylhex-3-enedioate (**65**, mixture of *E,Z*-isomers), was smoothly transformed to acid **66** by the catalytic removal of the benzyl function or could be isomerized in the presence of tert-butoxide in tetrahydrofuran, yielding diester **67** followed by catalytic debenzylation to give acid **68**. Acids **66** and **68** were reacted with pyrrolo[3,4-*b*]quinoline **57** to give intermediates **70** or **71**, and both of them produced known intermediate **24**. These investigators then carried out the known method of obtaining 20(*R,S*)-**1** (Scheme **7**).

Several other successful attempts were made to synthesize **1** in subsequent years, which were later discussed in the review [2], including Winterfeldt`s biomimetic synthesis [27] and other developments of synthesis **1** made in Winterfeldt's group (See references in [2]).



Scheme 6. Total synthesis of 20(*R,S)*-**1** by Kende *et al.* [36].
Reaction conditions: (**a**) 2,3-dihydro-1H-pyrrolo[3,4-*b*]quinoline (**57**)/β-morpholinoethyl cyclohexyl-carbodiimide metho-*p*-tosylate (MCCD), 65%; (**b**) i) HgO/BF_3; ii) AcOH/AcOK, 80%; (**c**) OH^-, 74%; (**d**) $SOCl_2$, DMF; (**e**) $LiEt_2Cu$, 0 °C, 44%; (**f**) $MeOH/H_2SO_4$, 5 d, reflux; (**g**) Stages (**h**, **i**) on Scheme **2**.



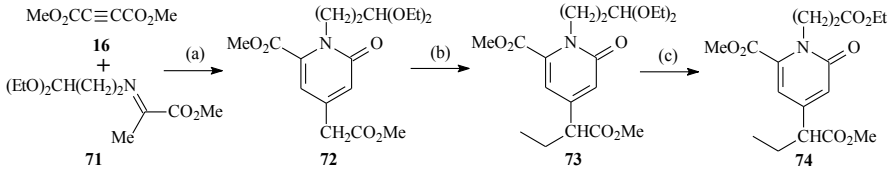
Scheme 7. Total synthesis of 20(*R,S*)-**1** by Bradley, and Büchi. [37].
Reaction conditions: (**a**) i) NaH/DME, 60 °C, 1 h; ii) **64**, reflux, 24 h, 82% (Z:E=3:1); (**b**) i) t-BuOK/THF, -65 °C to -30 °C, ii) H_2O, 96% (Z:E) (**c**) 10% Pd(C), MeOH, r.t.; (**d**) DCC/CH_2Cl_2, r.t., 12 h, 56%; (**e**) i) $BF_3 \bullet Et_2O$, -65 °C to -30 °C, 20 min; ii) TFA/Tol, reflux, 14 h, 41%.

Six years later, Quick suggested avoiding the inefficient decarboxylation of tetracyclic diacid **22** (Stage (f), Scheme **2**), using imine **71** as a starting material for this synthesis to obtain pyridone **72** (Scheme **8**) [38]. In this work, successful ethylation of **72** was accomplished, and pyridone **73** was obtained in a 63% yield. Acetal hydrolysis, oxidation of the aldehyde to acid, and esterification gave triester **74**, which was different from tetraester **20** in the absence of a carboxymethyl group at C-5 of the pyridone moiety. Intermediate **74** was then transformed to **1** according to Danishefsky's pathway (Scheme **2**) [26].

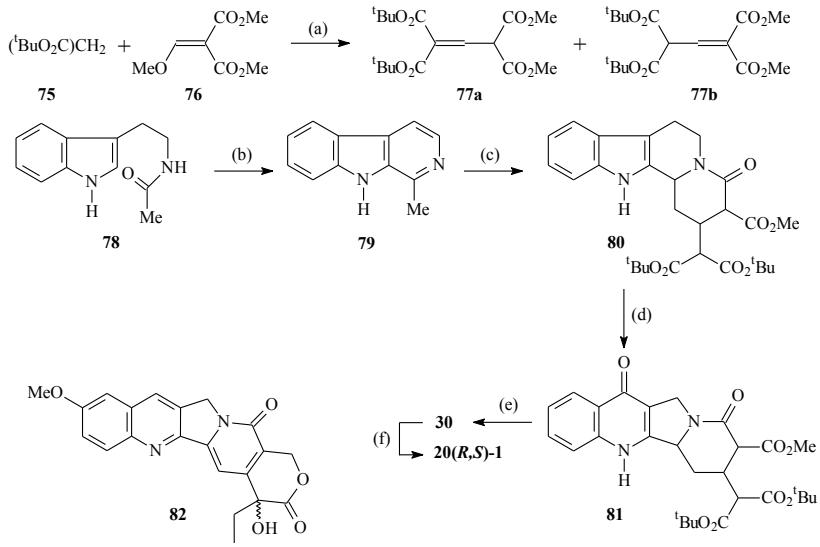
In 1980, Kametani *et al.* discovered an enamine annulation of 3,4-dihydro-l-methylisoquinoline and 3,4-dihydro-l-methyl-β-carboline, which were suitable intermediates for the synthesis of 20(*R,S*)-**1** [39, 40]. They started with acetyl tryptamine (**78**, Scheme **9**), which, in principle, could be the beginning of the biochemical pathway of the CPT synthesis and subjected intermediate **78** to intramolecular cyclization to form 1-methyl-4a*H*-pyrido[3,4-b]indole (**79**). Intermediate **79** was reacted through the enamine annulation with the mixture of two isomeric tetraesters (**77**) prepared from di-t-butyl malonate (**75**) and dimethyl 2-(methoxymethylene)malonate (**76**) so that a derivative (**80**) with the cycle **D** having suitable substituents for creating the cycle **E** was obtained. Photooxygenation of derivative **80** and the treatment with aq. $NaCO_3$ provided quinolone (**81**), which was chlorinated and dechlorinated by hydrogenolysis over a Pd-catalyst to yield quinoline (**30**), previously isolated by Winterfeldt ([28], see Scheme **3**). 20(*R,S*)-10-methoxycamptothecin (**82**) was synthesized by Kametani *et al*. in the same way [40].

The classical integration pathway for the synthesis of the natural asymmetric CPT, in which the **ABC**-moiety was condensed with the preliminary prepared chiral **D**, **E**-ring precursor, was applied by Corey (Scheme **10**) [41]. The convergent pathway was started with diethyl furan-3,4-dicarboxylate **83a** or the corresponding acid **83b**, desymmetrised by partial saponification, followed by a reduction in borane to hydroxy ester **84**. This laid the foundation of the **D**-ring. In the following step, the hydroxy group was protected as a stable tetrahydropyranyl ether (THP, **85**), and the THP protection remained intact until the E-ring was formed. Several steps were followed to form the carbon chain of the **E**-ring, where the key stages were the formation of ketone **86e**, its transformation to protected TBDMS-hydroxynitrile **87a**, hydrolysis of the nitrile to dihydroxy acid **88,** and resolution of compound **89** through a crystalline solid by the composition of **89**-quinine-water in the ratio of 1:5:2. The salt **89b** was obtained after three recrystallizations from benzene-heptane. Lactonization to **90** was followed by photooxidation to pseudo-acid **91a**, **b** and reaction with thionyl chloride, providing pseudo-acid chlorides **92a**, **b** (1:2.5, respectively). At this stage, the formation of the **D**, **E**-ring fragment was completed.

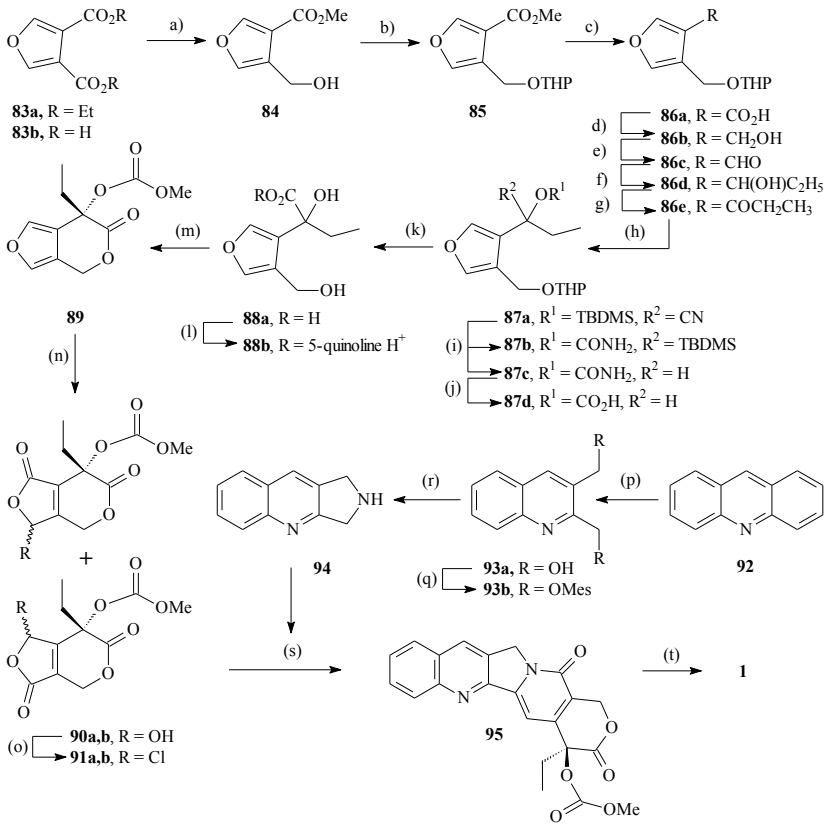
ABC-ring fragment was obtained from acridine **92** in three steps by sequential ozonation to **93a**, mesylation to **93b** and cyclic ammonolysis to yield 2,3-dihydro-1*H*-pyrrolo[3,4-b]quinoline **94**, which provided another component of the convergent pathway. A mixture of lactones **91a** and **b** was combined with tricyclic diamine **94** in a pyridine-acetonitrile medium at room temperature, and then an intermediate amide mixture (not shown) was cyclized using 10% sodium acetate–acetic acid as a medium. Afterward, chromatographic isolation of the pure camptothecin 20-methoxycarbonyl derivative **95** was performed, and the methoxycarbonyl group was removed by reacting with lithium mercaptide in HMPA, yielding 20(*S*)-camptothecin **1**. Among the achievements of the method are



Scheme 8. Total synthesis of 20(*R,S*)-**1** by Quick. [38].
Reaction conditions: (**a**) TEA, -78 °C to r.t., DME, 37%; (**b**) t-BuOK/EtI, DME, 63%; (**c**) i) 5% HCl/MeOH; ii) Jones oxidation; iii) MeOH/HCl, 65%.



Scheme 9. Total synthesis of 20(*R,S*)-**1** by Kametani *et al.* [40].
Reaction conditions: (**a**) NaH, C_6H_6, r.t., 2 d, 76.6%; (**b**) $POCl_3$/MeCN, reflux, 0.5 h; (**c**) i) **77a,b**, THF, 2 d, r.t.; ii) $NaBH_4$/MeOH, 0.5 h, then AcOH, 80.1%; (**d**) O_2, MeOH, Rose Bengal, 500 W halogen lamp, 20-25 °C, 2 h, 56.9%; (**e**) i) $SOCl_2$/DMF, 0 °C, 10 min; ii) H_2/5% Pd/$BaSO_4$, r.t., 5 h, 85.5%; iii) DDQ, dioxane, reflux, 10 min, 43%; (**f**) According to Winterfeld`s procedure. [28].



Scheme 10. Total synthesis of natural 20(*S*)-camptothecin by Corey *et al.* [41].
Reaction conditions: (**a**) i) **83a**, NaOMe (1 equiv), 5% MeOH aq., 25 °C, 2 h (78%) or **83b**, MeOCOCl (1.06 equiv.), TEA (exc.), THF, 25 °C, 3 d (75%); ii) BH_3 (1.2 equiv), THF, 3 h, 0°, (65%); (**b**) DHP, p-TSA, C_6H_6; (**c**) 11% KOH (MeOH) (40%); (**d**) BH_3 (1.05 equiv), THF, 30 h, 0°, (quant.); (**e**) MnO_2; (**f**) EtMgBr; (**g**) Collins' reagent, (69% from the acid **86a**); (**h**) TBDMSCN (4 equiv), 110° (neat), 10 d, dicyclohexano-18-crown-6/KCN (cat. 0.4 equiv); (**i**) H_2O_2 (30%, 10 equiv), K_2CO_3 (1.2 equiv), 1-heptene (10 equiv, O_2-acceptor), MeOH, r.t., then quenching with $NaHSO_3$ (aq.)/pyrrolidine/K_2CO_3, 25 °C; (**j**) H_2O-KOH-CH_3OH (1.5:14:100), reflux, 4 d, (73% from the acid **86a**); (**k**) CH_3COOH (30% aq.), 4 h, 45 °C, (65%); (**l**) quinine trihydrate (5 equiv), recrystallizations from benzene-heptane (1:1)×3, (76%), $[\alpha]^{22}_D = -145°$ (EtOH); (**m**) TEA (34 equiv), MeOCOCl (34 equiv), CH_2Cl_2, 4 h, r.t., then MeI, $MeNO_2$, 18 h, r.t., (quant.), $[\alpha]^{22}_D = +1.10°$ (MeOH); (**n**) hν, 30% 2,6-lutidine, t-BuOH, O_2, eosin, (quant., **90a,b** =1:2.5); (**o**) $SOCl_2$, $ClCH{=}N(CH_3)_2Cl$ (cat), 50 °C, 7 h, (quant., **91a,b** =1:2.5); (**p**) O_3, (2.2 equiv), CH_3OH, – 40 °C, then $NaBH_4$ (4.4 equiv), (43%), m.p. 115-118 °C; (**q**) $MeSO_2Cl$, (3 equiv), C_6H_6/TEA, 0 °C, 1 h, (85%); (**r**) NH_4OH (15%), CH_3OH, 2 h, r.t., (48%), m.p. 92.5-95 °C; (**s**) **91a,b**, **93** (0.85 equiv), 10% Py/CH_3CN, 10 h, r.t., then 10% CH_3CO_2Na/CH_3CO_2H, 20 h, 25 °C, $[\alpha]^{22}_D = +31.7°$ ($CHCl_3$); (**t**) CH_3SLi, HMPA, (90%).

the extensive use of protecting groups and especially THP, as well as an effective method of enantiomer resolution [41].

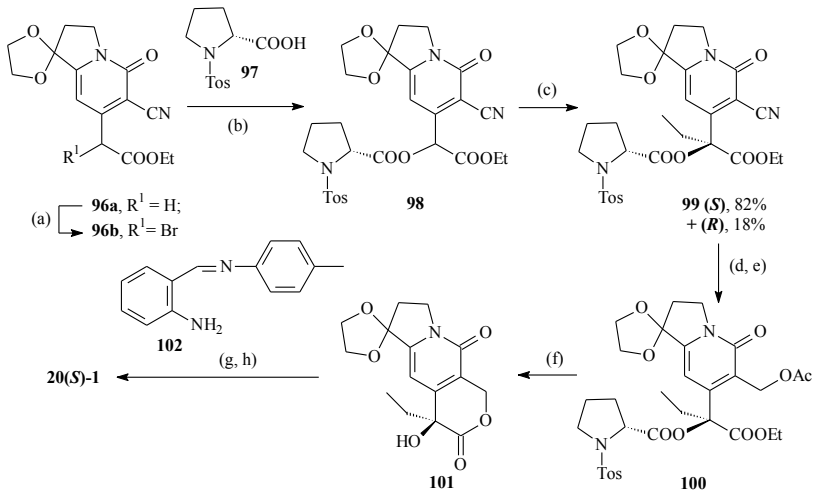
Asymmetric total synthesis of the CPT molecule was reported by Ejima *et al.* [42]. The authors started with the well-known compound **96a** (Scheme **11**), which was conjugated with *N*-Tos-(*R*)-proline **97** after α–bromination to introduce a stereo-controlled unit into the molecule. This facilitated asymmetric induction with the preferable formation of the (*R*,*S*)-diastereomer **99**.

In the following steps, the methylene hydroxyl group was obtained from the CN group, playing the role of a latent function of CH_2OH. Transformation of molecule **100** by cleavage of the hydrolysable functions under basic conditions, followed by weak acid medium, led to derivative **101,** representing **C**,**D**,**E**-fragment of **1**. Condensation of **101** with N-(2-aminobenzylidene)-4-methylaniline (**102**) in the presence of strong acid yielded target 20(***S***)-**1**.

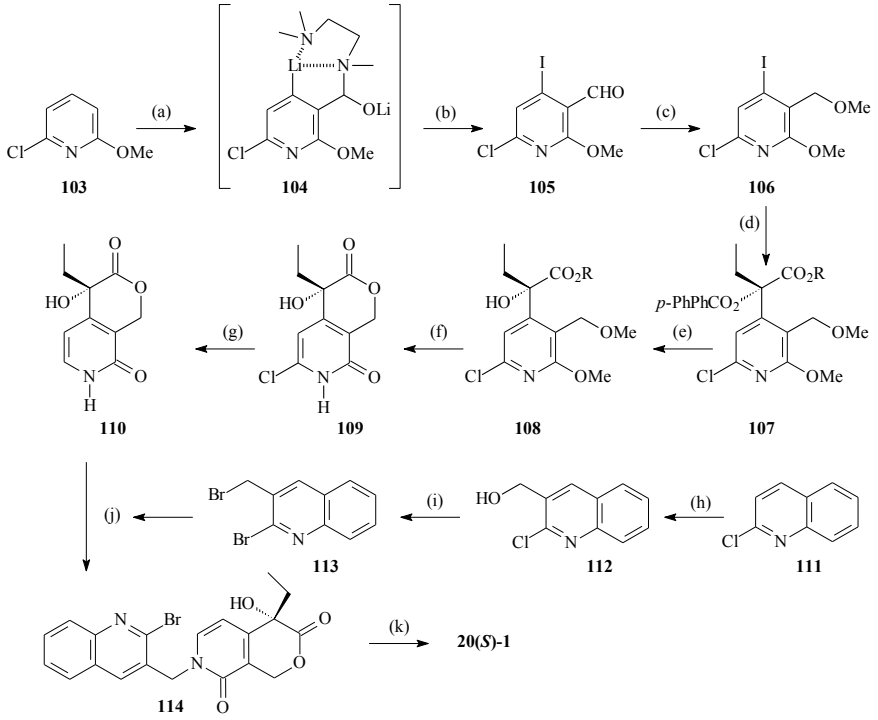
Research by Tang *et al.* demonstrated the synthesis of (*S*)-**1** based on the proposed scheme **A + CDE* = ABCDE***, where derivative **101**, carrying a hydroxyl group instead of a carbonyl on the five-member ring, was used as a **CDE***-intermediate, leading to a more efficient and flexible way of the synthesis of the natural CPT [43].

Another route for asymmetric 20(*S*)-camptothecin, involving only 10 steps, was suggested by Comins *et al*. [44]. The researchers started with 2-chloro-6-methoxypyridine (**103**), as shown in Scheme **12**. Double-directed lithiation of pyridine derivative **103** formed dianion **104**, which was transformed into aldehyde **105**. Reductive etherification (methoxylation) in the presence of TFA yielded methyl ether **106,** and the lithium halogen exchange in the molecules **106** allowed the introduction of a chiral substituent *via* the diastereoselective addition to the (-)-8-phenylmenthol ester of α-ketobutyric acid. The treatment of the lithium alkoxide intermediate with 4-phenylbenzoyl chloride was provided after recrystallization of a diastereomerically pure pyridine derivative **107**, which was saponified to yield α-hydroxy ester **108**. In the next stage, the action of TMSCl-NaI on compound **108** in the presence of highly nucleophilic DABCO formed an **E**-ring precursor with the correct chiral center configuration together with simultaneous demethylation of the **D**-ring precursor to provide lactone **109**.

The C-6 chloro group in intermediate **109** was substituted by catalytic hydrogenation to provide intermediate **110** intended for condensation with the **A**, **B**-fragment. 2-Bromo-3-(bromomethyl)quinoline **113**, representing the **A**, **B**-fragment, was obtained successively by lithiation of 2-chloroquinoline (**111**), followed by formaldehyde alkylation to give (2-chloroquinolin-3-yl)methanol (**112**). Quinoline **112** was involved in a substitution reaction with PBr_3 to give the target **A**, **B**-intermediate **113**. Intermediates **110** (**A**,**B**-part) and **113** (**D**,**E**-part) were combined under a



Scheme 11. Asymmetric total synthesis of natural 20(*S*)-**1**. [42].
Reaction conditions: (**a**) NaH, Br_2, DME, r.t., (85%); (**b**) **97**, Na_2CO_3, DMF, 70°C, (97%); (**c**) NaH, EtI, DMF, r.t., (100%); (**d**) H_2/Raney-Ni, Ac_2O, r.t., (quant.); (**e**) i) $NaNO_2$, AcOH, 0 °C, ii) CCl_4, reflux, (74% 2 steps); (**f**) i) LiOH, 67% EtOH, r.t., ii) AcOH, r.t., (90% 2 steps); (**g**) 80% TFA, r.t., (79%); (**h**) **102**, *p*-TsOH, toluene, reflux, (73%).

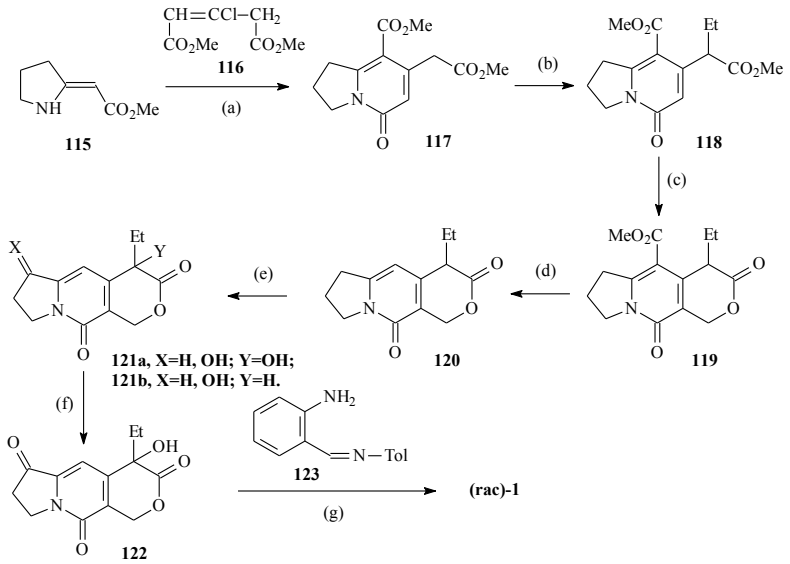


Scheme 12. Asymmetric 10-step synthesis of natural 20(*S*)-**1** by Comins *et al*. [44].
Reaction conditions: (**a**) i) t-BuLi, THF, -78 °C, 1 h, ii) $Me_2NCH_2CH_2NHCHO$, iii) n-BuLi, - 23 °C, 2 h; (**b**) I_2, 78% of **105**; (**c**) MeOH, Et_3SiH, TFA, 92%; (**d**) i) THF, -78 °C, 1 min, ii) (-)-8-phenylmenthol ester of alpha-ketobutyric acid, iii) p-PhPhCOCl, r.t., 36 h, (87% de), iv) recr., petroleum ether, 60% (100% de); (**e**) 2N NaOH/EtOH, ether recovery of (-)-8-phenylmenthol, 100%, H^+, 76%; (**f**) i) TMSCI/NaI, CH_3CN, DABCO, reflux, 5 h, ii) 6 N HC1, 100 °C, 4 h, 77%; (**g**) Pd/C, NaOAc, MeOH, 95%; (**h**) i) LDA, ii) H_2CO; (**i**) PBr_3, high yield; (**j**) t-BuOK, DME, reflux, 48 h, 87%, (**k**) $Pd(OAc)_2$, $Bu_4N^+Br^-$, KOAc, DMF, 90 °C, 3 h, 59%.

strong basic N-alkylation condition to give tetracyclic **114**. The **C**-ring was formed using the Heck reaction, thus completing the 10-stage asymmetric synthesis of 20(*S*)-**1**. This pathway (Scheme **12**, [44]) was an example of a convergent economic strategy **AB** + **DE** = **ABCDE** and efficient use of the advantages of organolithium synthesis.

Interest in the synthesis of **1** never waned in the 1990s and was stimulated by the search for new camptothecin derivatives that would be more active, selective, have better bioavailability, and have less toxicity. Danishefsky and coworkers reported the synthesis of racemic **1** and several derivatives [26, 45].

In their second approach, Volkmann group started with the condensation reaction of enamine **115** and the readily available dimethyl 3-chlorogluconate (**116**, Scheme **13**) [45]. The authors suggested that intermediate **115** was converted into the correspond-

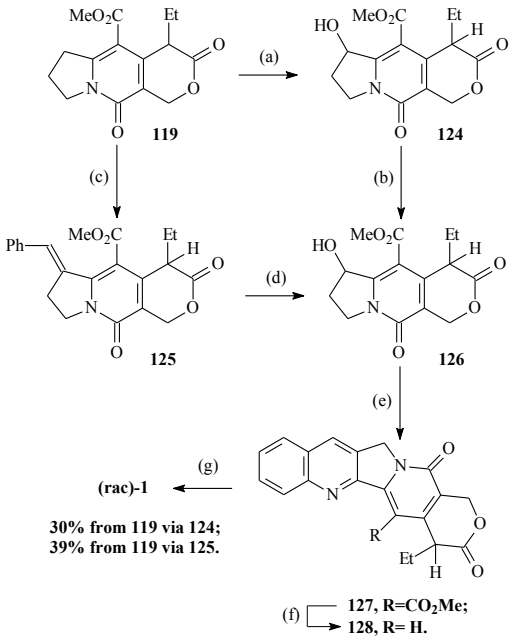


Scheme 13. Concise total syntheses of *dl*-camptothecin. [45].
Reaction conditions: (**a**) **116** (1.1 equiv), TEA (1.2 equiv), EtOH, r.t., 65 h (92%); (**b**) t-BuOK (1.05 equiv), DME, -70 °C, 20 min, then EtI (2 equiv), r.t., 30 h, (91%); (**c**) $(CH_2O)_n$ (5.85 equiv), dioxane:$H_2SO_{4(conc)}$:H_2O=25:1:1, sealed thick-walled tube, 107 °C, 30 h, (95%); (**d**) HBr (48%), sealed thick wall tube, 105 °C, 18 h, (53%); (**e**) SeO_2 (2 equiv), dioxane (95%), sealed thick-walled tube, 160 °C, 4 h, (43%); (**f**) PDC (2 equiv), 4A molecular sieves (activated powder), CH_2Cl_2, 0 °C, 3.5 h, (45%); (**g**) **122** (1.2 equiv), toluene, reflux, 0.5 h, then p-TsOH (0.05 equiv), reflux (Dean-Stark trap), 3.5 h, (80%).

ing allene in the presence of triethylamine, which led to a significant simplification of the pathway to camptothecin. α–Ethylation of the obtained ester **117** with a strong base and subsequent hydroxymethylation of the derivative **118** with paraformaldehyde in a sealed tube yielded compound **119** with a **C**,**D**,**E**-ring framework. Decarboxylation of the ancillary carboxymethyl substituent and insertion of the hydroxyl and carbonyl group into the C6-position of the indolizine fragment (**C**-ring) were achieved in three steps using conc. HBr to decarboxylate into derivative **120**. Furthermore, upon oxidation with SeO_2, it yielded two products, **121a, b**, followed by gentle oxidation of **121a** with pyridinium dichromate alone yielded a compound lacking **AB**-rings, the so-called 7-oxo-de-***AB***-camptothecin (**122**). In the last step, derivative **122**, as a **C**,**D**,**E**-fragment with fully equipped substituents, condensed with N-(2-aminobenzylidene)-4-methylbenzenesulfonamide (**123**) and afforded *d,l*-CPT. Compound **119** became a key intermediate in the development of alternative synthetic routes (Scheme **14**) [45].

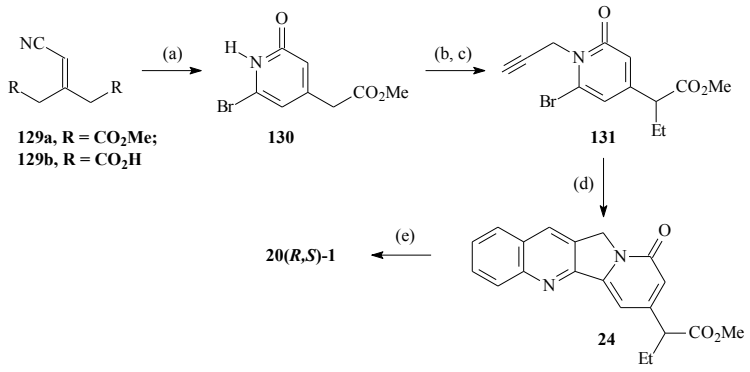
An improved method was characterized in a way that the hydroxyl group at the C20 atom was introduced only at the last stage, which increased the stability of the intermediate products and generally led to a higher yield of the desired racemic **1**.

Previous achievements in synthesis **1** and successes in (4+1) radical annulations allowed D. P. Curran and Liu to develop a rather short way of CPT synthesis (Scheme **15**) [46]. The research began with nitrile **129a**, which was prepared by the well-known Doebner condensation of dimethyl-1,3-acetonedicarboxylate and cyanoacetic acid [47], and obtained product **129a** was saponified to **129b** diacid. Thereafter, the modified cyclisation reaction of **129b** led to pyridone **130**, which was subjected to N-propargylation in the presence of sodium hydride and then α-ethylation to yield substituted pyridone **131** as a precursor for 4+1 radical annulation. The interaction with phenyl isocyanide in the presence of hexamethylditin gave Danishefsky's intermediate **24**, which could be converted into **1** in two known steps [26]. Thus, the synthesis of intermediate **24** was carried out in six steps [47]. In subsequent years, more than a hundred CPT derivatives were obtained by Curran's research group utilizing [4+1] radical annulation [48-53].

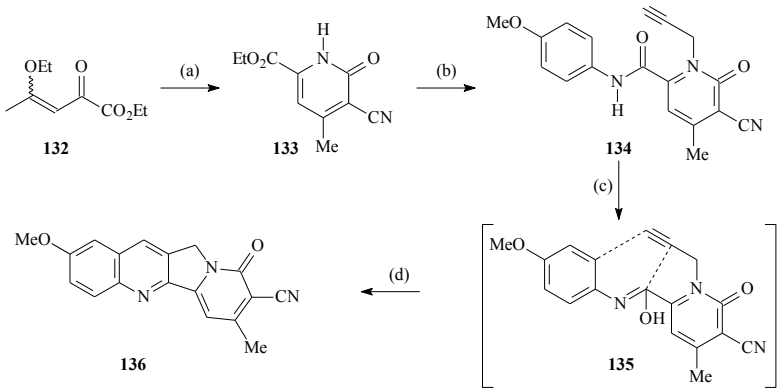


Scheme 14. Improved concise total syntheses of dl-camptothecin [45].
Reaction conditions: (**a**) O_2, $P(OEt)_3$ (2 equiv), NaHMDS (1.1 equiv), –70 °C to r.t., 7 h, NH_4OH aq., (75%); (**b**) f) PDC (3 equiv), 4A molecular sieves (activated powder), CH_2Cl_2, 0 °C, 4 h, (83%); (**c**) benzaldehyde, NaHMDS (1.1 equiv), –70 °C to r.t., 16 h, 5% HC1, 2 h, (90%); (**d**) TMSCHNz (1.3 equiv, 2 M in hexane), C_6H_6:MeOH=3:1, (94%); (**e**) **123** (1.2 equiv), Tol, p-TsOH (0.05 equiv), reflux (Dean-Stark trap), 4 h, (75%); (**f**) 48% HBr aq., sealed thick-walled tube, 140 °C, 15 h, (71%); (**g**) O_2, $CuC1_2$ (0.6 equiv), $(CH_3)_2NH$ (10 equiv), DMF, 7 h, (91%).

Several successful approaches to the synthesis of CPT and its analogues were suggested by Fortunak *et al.* using intramolecular [4+2] cycloadditions (Scheme **16**) [54, 55]. Key pyridone **133**, which was a **D**-ring precursor, was obtained by condensation of (*Z,E*)-ethyl 4-ethoxy-2-oxopent-3-enoate (**132**) with cyanoacetam-



Scheme 15. Total syntheses of *dl*-camptothecin by Curran and Liu [46].
Reaction conditions: (**a**) PCl_5, then HBr (gas, 10 equiv), MeOH (66%); (**b**) NaH, $CHCCH_2Br$, DME, LiBr (70%); (**c**) t-BuOK (1.05 equiv), DME, -70 ºC, 20 min, then EtI (2 equiv), r.t., 30 h (95%) [52, 53]; (**d**) PhNC, $(Me_3Sn)_2$ (45%); (**e**) 2 steps [26].



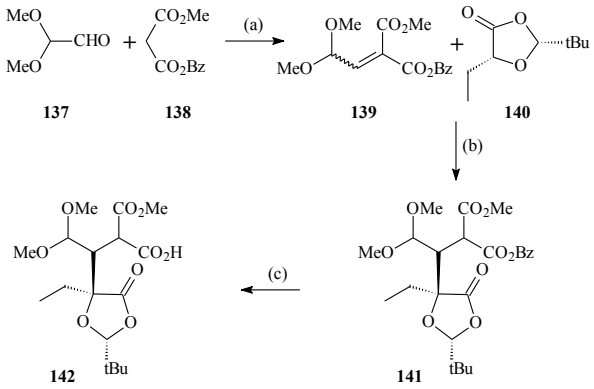
Scheme 16. Total syntheses of the **ABCD**-ring precursor of dl-10-methoxycamptothecin by Fortunak *et al*. [54].
Reaction conditions: (**a**) cyanoacetamide, K_2CO_3, acetone, reflux, 82%; (**b**) i) propargyl bromide, DMF, K_2CO_3 (65%); ii) NaOH, aq. DMF (95%); iii) p-anisidine, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide/HOBT, CH_2Cl_2 (83%); (**c**) i) CH_2Cl_2, Me_3OBF_4, r.t.; ii) CH_3CN, reflux (82%).

ide, followed by cyclization. Replacement of the hydrogen at the N-atom of the pyridine ring of intermediate **133** with a propargyl substituent followed by saponification of the ester group and amidation with *p*-anisidine resulted in the formation of 5-cyano-4-methyl-6-oxo-1-(prop-2-ynyl)-N-p-tolyl-1,6-dihydropyridine-2-carboxamide (**134**). Derivative **134** was a subject for intramolecular [4+2] cycloaddition, which was carried out in the presence of trimethyloxonium tetrafluoroborate as a methylating agent for the enol form of **134**, giving, as the author suggested, the intermediate (**135**). The alkyne substituent as dienophile and 2-aza-1,3-diene, in which the aryl ring served as a component of 2π diene, were involved in the intramolecular formation of the **ABCD** intermediate. Refluxing the reaction mixture in acetonitrile provided **ABCD**-tetracyclic quinoline (**136**) in an 82% yield of **135**. Using this method, the authors obtained 10-methoxy, 10-hydroxy, and 10,11-dihydroxyethylene-7-methyloxyacetyl **ABCD** derivatives [54].

However, the method suggested by Fortunak *et al.* was only a formal total synthesis of (+)10-methoxycamptothecin [54]. A 9-step convergent total synthesis of 20(S)-camptothecin alkaloids was described in their next work (Schemes **17** and **18**) [55].

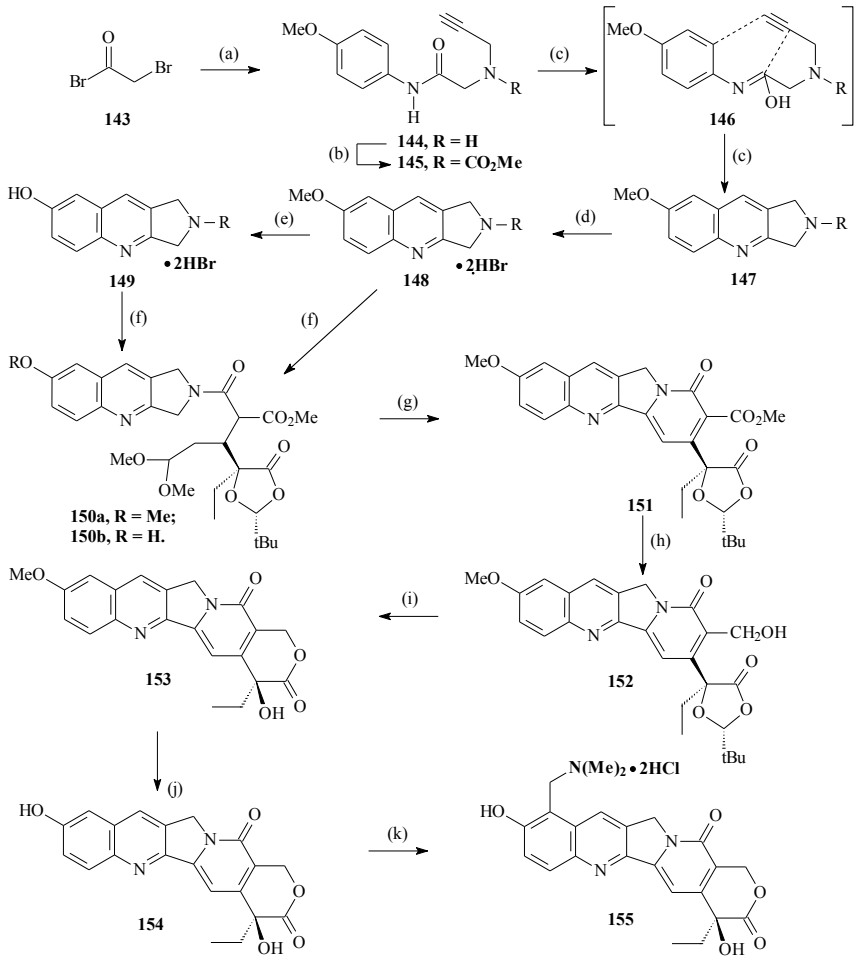
The authors aimed to obtain 20(*S*)-enantiomeric camptothecin derivatives. For this purpose, they developed a method for the synthesis of the chiral precursor (1**42**), starting from the Knoevenagel condensation of glyoxal-1,1-dimethylacetal (**137**) with benzyl methyl malonate (**138**).

The prepared 1-benzyl-3-methyl-2-(2,2-dimethoxyethylidene) malonate (**139**) served as Michael acceptor to introduce the chiral component, namely (*2R,5R*)-2-tert-butyl-5-ethyl-l,3-dioxolan-4-one (**140**, as enolate), thereby providing the chiral precursor **142** in three steps through the formation of benzyl ester intermediate **141**.



Scheme 17. Synthesis of chiral precursor **142** for the concise syntheses of 20(*S*)-10-methoxycamptothecin by J. M. D. Fortunak *et al*. [55].
Reaction conditions: (**a**) piperidine/toluene, reflux, 82%; (**b**) LDA/THF, –78 to –20 ºC; (**c**) 5% Pd/C, H_2, MeOH, quant.

In the parallel pathway (Scheme **18**), the **A**,**B**,**C**-chromophore was obtained starting from the condensation of bromoacetyl bromide, *p*-anisidine, and propargylamine, yielding after the introduction of methoxycarbonyl functionality in an intermediate (**145**) suitable for an intramolecular cycloaddition into the **A**,**B**,**C**-precursor (**147**). Intermediate **147** was transformed into its



Scheme 18. Synthesis of 20(*S*)-10-methoxycamptothecin, 10-hydroxycamptothecin, and Topotecan by Fortunak *et al.* [55].
Reaction conditions: (**a**) CH_2Cl_2/Et_3N, i) p-anisidine); ii) propargylamine, reflux (84%); (**b**) $ClCO_2Me/Et_3N$, TBME (95%); (**c**) Me_3OBF_3/CH_3CN, –40 to 50 °C (72%); (**d**) 30% HBr (5 mol. eq.)/AcOH, r.t., 12 h (70%); (**e**) 30% HBr (10 mol. eq.), r.t., 48 h (65%); (**f**) **142**, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide/1-hydroxybenzotriazole, Et_3N/THF (89%); (**g**) i) TFA/H_2O/$PhCH_3$; ii) DDQ (50% overall); (**h**) DIBAH, CH_2Cl_2; (**i**) $NaBH_4$, MeOH; ii) aq. NaOH (70% overall); (**j**) 48% HBr (72%); (**k**) i) $CH_2(NMe_2)_2$, CH_2Cl_2:n-PrOH; ii) aq. HCl/n-PrOH, azeotrope (92%).

hydrobromide **148** and demethylated to provide **A**,**B**,**C**-precursor **149** with a free OH-group [55].

In the following steps, the authors successfully condensed chiral precursor **142** with **A**,**B**,**C**-precursors **148** and **149** to amide **150**, and created a **D**-cycle by instant intramolecular cyclization of the aldehyde group formed from dimethyl acetal in the presence of trifluoroacetic acid, then aromatized under DDQ to yield the **A**,**B**,**C**,**D**-precursor (**151**). Moreover, the 4-oxodioxolane cycle remained unaffected under those conditions. DIBAH was a suitable reducing agent for transforming the ester group into a hydroxymethyl group, and the conversion to 20(*S*)-10-methoxycamptothecin (**153**) was achieved by the reaction with $NaBH_4$ and NaOH to give a product with an enantiomeric excess of 98.9%. In subsequent steps, the elimination of the methoxy group gave 10-hydroxycamptothecin (**154**), and the Mannich reaction with bis(dimethylamino)methane gave topotecan (**155**, 91% yield of >99.5% e.e.) with a 20(*S*) configuration [55].

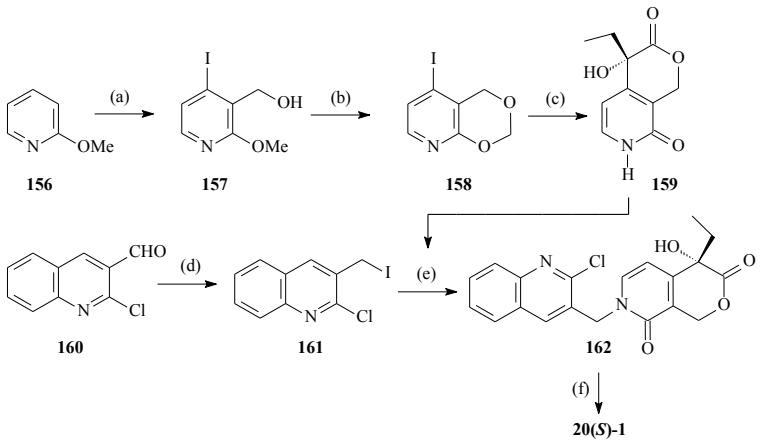
An even more detailed study on the synthetic route to **1** has been recently carried out using the Knoevenagel condensation. Using domino Knoevenagel/hetero-Diels–Alder reactions, the authors investigated methods to synthesize a number of heterocyclic compounds containing the indolizinoquinolinone system, which is the key structure of numerous biologically active alkaloids, including camptothecins [56].

20(*S*)-camptothecin **1**, despite its high anticancer activity, has a number of significant drawbacks, such as poor water solubility and high toxicity. Therefore, since the earliest attempts to synthesize **1**, researchers have studied methods for the synthesis of a number of its derivatives that would have better pharmacological properties. Many methods for the synthesis of analogues can be applied to the synthesis of **1**.

The poor solubility of CPT in aqueous solutions prevents it from exhibiting high biological activity. Considering this, in the early 1980s, Wall *et al.* attempted to obtain better water-soluble analogues both from natural 10-hydroxycamptothecin [57] and by total synthesis [58]. These studies led to the production of analogues with activity comparable to that of CPT or higher if high doses of drugs have been used.

A very efficient route of 20(*S*)-**1** synthesis, which was a continuation of their previous research and included six steps, was developed by Comins and Nolan [59].

The authors [59] started with 2-methoxypyridin (**156**) as a **D**-ring precursor, which was lithiated at C-3 and C-4 positions using mesityllithium and n-BuLi (Scheme **18**). The lithiation allowed the introduction of a hydroxymethyl group and iodine into the molecule, respectively (**157**). The hydroxymethyl group was intramolecularly protected as conjugated 1,3-dioxan (**158**), and the iodine



Scheme 19. Total syntheses of 20(*S*)-camptothecin by Comins and Nolan. [59].
Reaction conditions: (**a**) t-BuLi (1.20 M, 2.6 equiv), 2-bromomesitylene (1.3 equiv), THF, –78 ºC, 1 h; then **156** (1 equiv), up to 0 ºC, 2 h; then N-formyl, N,N´,N´-trimethylethylenediamine (1.1 equiv), –78 ºC, 0.5 h; then –23 ºC, n-BuLi (2.27M in Hex, 0.66 equiv), 3 h; I_2 (1.8 THF), 0.5 h; MeOH, $CeCl_3 \cdot 7H_2O$ (1.1 equiv), 20 min; $NaBH_4$ (1.3 equiv), 0.5 h; H_2O (46%); (**b**) NaI (2.4 equiv, CH_3CN), TMSCl (2.4 equiv), $(CH_2O)_n$ (17.7 equiv), 4 h, r.t. (87%); (**c**) n-BuLi (2.27 M (Hex), 1.1 equiv), (-)TCC-α-ketobutyrate (1.2 equiv) [60], THF, at –78 ºC, 0.5 h, r.t., 0.5 h, MeOH, 5N HCl (iPrOH), then reflux, 12 h, radial PLC (silica gel, EtOAc/hexanes, 1:9), (silica gel, MeOH/$CHCl_3$, 5:95) (71%, e.e. = 89%, recryst. MeOH, 60%, e.e. = 93%); (**d**) Et_3SiH (neat, 3 equiv), TMSI (5 equiv), $CHCl_3$, r.t., 12 h, H_2O (79%); (**e**) **159** (1 equiv), KOtBu (1.0 M, 1.1 equiv), DME, 25 ºC, 0.5 h, r.t., then **161** (neat, 1.3 equiv), reflux, 12 h (88%, recryst. MeOH, 81%, e.e. > 99%); (**f**) KOAc (2.5 equiv), $(PPh_3)_2Pd(II)(OAc)_2$ (0.15 equiv), CH_3CN, 100 °C, 12 h (64%).

function was successfully replaced with a chiral (-)TCC-α-ketobutyrate residue after the one-pot formation of the **D**,**E**-precursor (**159**). Another pathway for the convergent synthesis started with 2-chloroquinoline-3-carbaldehyde (**160**), which was transformed into 2-chloro-3-(iodomethyl)quinoline (**161**), a derivative with all necessary functionalities for the formation of the **C**-ring. Condensation of **159** and **161** to compound (**162**), followed by the intramolecular Heck cyclization, yielded the target 20(*S*)-**1** with a 12.5% overall yield [59]. This method, which corresponds to the pathway **D** → **DE** + **AB** → **ABCDE**, is one of the most effective methods of synthesizing 20(*S*)-**1** (Scheme **19**) [60], because of the ease of obtaining the corresponding synthons and can be used to synthesize numerous derivatives having substituents on cycles **A** and **B** [61].

Similar schemes, where intermediate **160** was chosen as the starting compound, in which the **ABC**-synthons similar to intermediates **147-149** were formed first, followed by the creation of **D** and **E**-rings, were proposed in the work [62]. They have also recently synthesized using the photocatalytic decarboxylative radical addition [63].

Bennasar and coworkers developed the synthesis of 4-substituted dihydropyridones or 2-pyridones by the nucleophilic addition of R-(methylsulfanyl)ester enolates to N-alkyl-2-fluoropyridinium salts, followed by acid hydrolysis or oxidation with concomitant hydrolysis of the intermediate 2-fluoro-1,4-dihydropyridine adducts [64, 65] and used the method to obtain racemic and natural **1**.

Bennasar *et al.* [65] found that 4-substituted dihydropyridones, such as compound (**164)**, or 2-pyridones obtained in good yield were suitable synthons of the **D**-ring formation and easily formed a quaternary salt (**165**) with 2-bromoquinoline-3-yl trifluoromethanesulfonate (**163**) as a precursor to the **A, B**-fragment (Scheme **20**).

In a subsequent process, 2-fluoropyridine (**165**) was converted to 2-pyridone residue, and appropriate substituents were prepared to develop the **E**-ring, affording an intermediate (**166**) that was subjected to intramolecular radical cyclization to form a tetracyclic derivative (**167**). Tetracyclic **167** was transformed in one step into the known 20-deoxycamptothecin **25**, which was easily converted to **1** according to the process developed by Danishefsky and others [26, 66]. Thus, Bennasar *et al.* [65] showed that the nucleophilic addition of ester enolates to 2-fluoropyridinium salts, followed by appropriate manipulation of the resultant 2-fluoro-1,4-dihydropyridine adducts, might be a useful pathway for the synthesis of highly functionalized and substituted dihydro-2-pyridones or 2-pyridones and sequential preparation of various camptothecin derivatives.

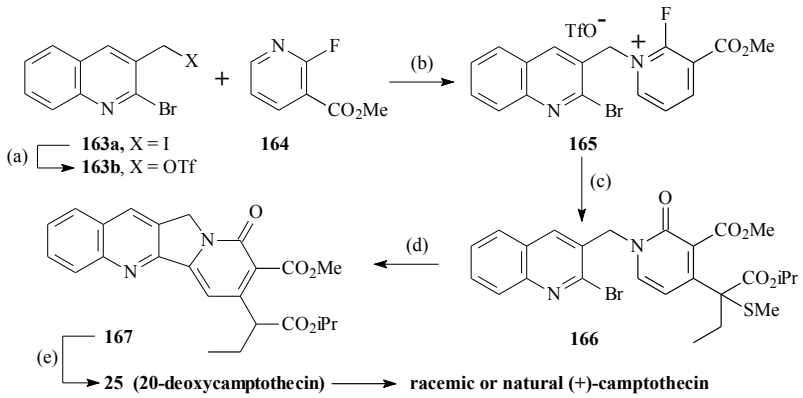
Also, in 2002, Blagg and Boger suggested elaborating the **D**-ring using controlled Diels-Alder cycloaddition (Scheme **21**) [67]. The route also made it possible to formulate the necessary substituents for the synthesis of the **E**-ring, which served as protective groups.

The Diels-Alder adduct (**171**) after removing the methanesulfonic acid and the subsidiary ethoxy group originated from the former dienophile (**170**), and aromatization yielded an intermediate (**172**), in which labile diethyl acetal was transformed into an ethyl ether function (**173**) by hydrosilane reduction. Compound **173** was reacted with ethyl magnesium bromide in the presence of TEA, which stopped the Grignard reaction in the first stage and introduced only one ethyl group, yielding ethyl ketone (**174**), which was an undoubted achievement. Ketone **174** was transformed into enol methyl ether (**175**) to obtain an olefin functionality, which could be subjected to Sharpless asymmetric dihydroxylation. As it turned out, Sharpless asymmetric dihydroxylation of **175** proceeded with high enantioselectivity to form hydroxyaldehyde (**176**) (Scheme **21**) [68]. This stage (*g*) was an excellent application of the Sharpless method.

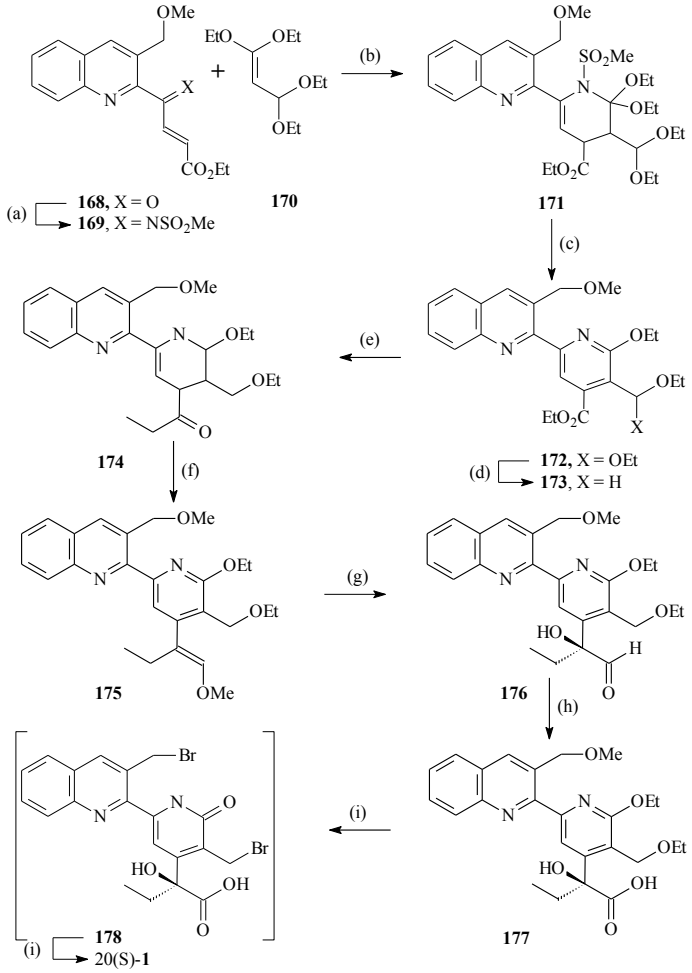
The last three stages of oxidation of aldehyde **176** to acid **177**, removal of protective groups, and simultaneous double cyclization to form cycles **C** and **E** proceeded in high yields, providing the desired product 20(*S*)-**1**. In general, the Blagg and Boger pathway can be characterized as the **AB** – **AB-D** – **AB-D*** - **ABCDE*** scheme in which highly enantioselective Sharpless asymmetric dihydroxylation was used to form chirality (**ABD*** intermediate).

4. SYNTHESIS OF CAMPTOTHECIN CONGENERS

The synthetic methods discussed above have allowed for synthesizing numerous derivatives, some of which have now been introduced into clinical practice. Great achievements have been made in the directed synthesis of the heterocyclic system of camptothecin derivatives. In the review of 2006, Wu Do summarized the



Scheme 20. Formal synthesis of 20(*S*)-camptothecin by Bennasar *et al.* [65].
Reaction conditions: (**a**) AgOTf, CH_2Cl_2, r.t., 30 min; (**b**) CH_2Cl_2, r.t., 30 min; (**c**) i) isopropyl α-(methylsulfanyl)butyrate, LDA, THF, -70 °C, LDA, THF, -78 °C, then -30 °C, 1.5 h; ii) DDQ, THF(3)/MeOH(1), r.t., 4 h, (50% from **165**); (**d**) TTMSS (2 equiv), AIBN, C_6H_6, reflux, 4 h (70%). (**e**) DIBAL–hexane (3 equiv.), DME, -70 °C, 30 min, then $NaBH_4$, iPrOH, r.t., 1 h (65%).



Scheme 21. Syntheses of 20(*S*)-camptothecin by Blagg and Boger [67].
Reaction conditions: (**a**) **168** [68] (1 eq.), $CH_3SO_2NH_2$ (2 eq.), CH_2Cl_2 (1 mL/10 mg of **168**), $TiCl_4$ (1.0M in CH_2Cl_2, Et_3N (0.7 mL/eq.), -42 °C, 40 min, 25 °C, 60 min, workup; (**b**) **169** (4 eq. to **168**) C_6H_6, 4 h; (**c**) NaOEt (3M in EtOH, 8 eq. to **168**), THF, 0 °C, 75 min (68% from **168**); (**d**) ZnI_2 (2 eq.), Et_3SiH (11 eq.) CH_2Cl_2, 25 °C, 5 d (92%); (**e**) EtMgBr (3M in Et_2O, 2 eq.), Et_3N (40 eq.), toluene (20 mL/eq.), -5 °C, 5 h (66%); (**f**) KHMDS (0.5M in toluene, 5 eq.), $MeOCH_2P(Ph)_3Cl$ (5 eq.), THF (0.3 mL/eq.), 0 °C, 30 min, then **174** (1 eq.) in THF (1 mL/eq.), 25 °C, 10 h, (76%, trans/cis = 74/26); (**g**) $(DHQ)_2$-Pyr$(OMe)_3$ (0.157 eq), K_2CO_3 (3 eq.), $K_3Fe(CN)_6$ (1 eq.), OsO_4 (4% in H_2O, 0.117 eq.) 0 °C, 5 min, then *trans*-**175** (1 eq.)/t-BuOH (10 mL/eq.), 0 °C, 4 h, then $K_3Fe(CN)_6$, (1 eq.), 0 °C, 4 h, Na_2SO_3 (10 eq.) (84%, 86% ee); (**h**) resorcinol (10 eq.), DMSO (25 mL/eq.), NaH_2PO_4 (5 eq. in H_2O (0.04 mL/eq.)), $NaClO_2$ (5 eq.), 0 °C, 1 h (93%); (**i**) HBr(g) (sat. soln in 2,2,2-trifluoroethanol, 90 mL/eq.), 75 °C, 24 h, 25 °C, 14 h, K_2CO_3 (0.1 mmol) 25 °C, 1 h (72%).

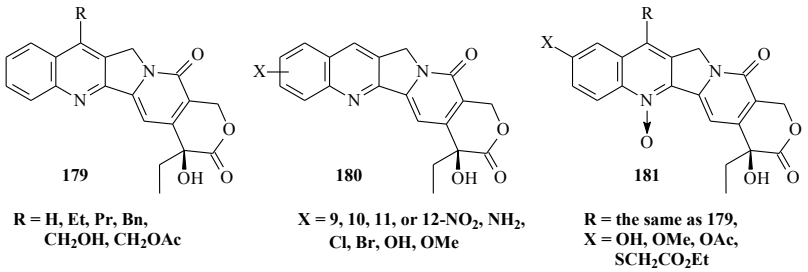
results of a decade of research on the synthesis of camptothecins and related alkaloids due to a significant increase in publications since the late 1980s [69].

In the search for more active and selective derivatives as well as water-soluble agents, numerous successful studies by Sawada *et al.* have focused on the chemical modification of natural or synthetic **1**. [70-76] Thus, derivatives with ethyl, propyl, benzyl, methylhydroxy, and methylacetate groups at C7 (Scheme **22**, **179**), and nitro, amino, hydroxy, chloro, bromo, methoxy groups at C9 and C12 (Scheme **22**, **180**), 10- or 11-nitrocamptothecins and 10-hydroxycamptothecin (**180**) were obtained by conversion of a **B**-ring hydrogenated compound. Several substituted camptothecins **180** were prepared by photoreaction of substituted camptothecin-N-oxides (**181**) [70].

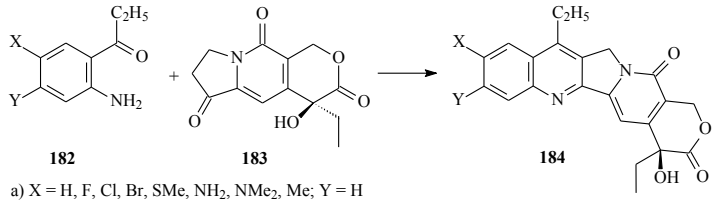
The Friedländer condensation, first suggested for the synthesis of **ABCD** fragment of **1** by Volkmann and coworkers [26], was further modified in the studies of Yaegashi *et al.* [76] to form the **ABCDE** structure with substituents at positions C10 and C11 on the A-ring and C7 position on the B-ring (Scheme **23**).

Among studies on synthetic methods and, consequently, the activity of CPT derivatives, it is relevant to mention the research by Lavergne and coworkers. They prepared a number of substituted CPT analogues, including homocamptothecins (hCPTs), derivatives of seven-membered α-hydroxylactone, combining enhanced plasma stability and potent inhibition of Topo-I and even Topo-II mediated activity [77-79]. Like CPT, hCPT carries asymmetric tertiary alcohol and displays stereoselective inhibition of Topo-I; this molecule was reported to be more hydrolytically stable and more potent than CPT.

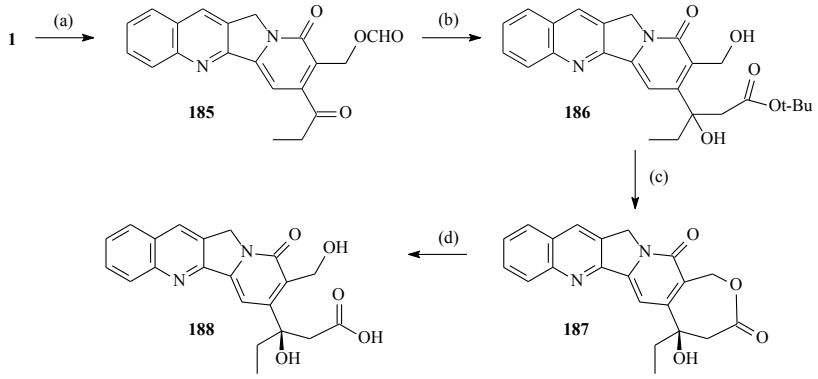
To synthesize the derivatives, Lavergne and coworkers initially used CPT as the starting material and modified the **E**-cycle into a seven-member α-hydroxylactone (Scheme **24**). In starting molecule **1**, the lactone **E**-cycle was easily reduced to the corresponding hydroxylactol with $NaBH_4$, and then the product was oxidized to yield formyloxy-mappicin ketone (**185**). The additional carbon was added by Reformatsky alkylation with t-butyl bromoacetate to give an intermediate compound (**186**). The t-butoxy group was removed by acid hydrolysis and immediate intramolecular cyclization with the formation of a seven-membered lactone provided homo-



Scheme 22. Synthesis of CPT derivatives with substituents on the **A** and **B**-rings.



Scheme 23. Derivatives of 7-ethyl-**1** (**184**) with a variety of substituents on the A-ring prepared by Friedländer condensation [76].
Reagents and conditions: TsOH, Tol, reflux, 18-24 h.



Scheme 24. Preparation of homocamptothecin by Lavergne and coworkers [77].
Reagents and conditions: (**a**) i) $NaBH_4$, MeOH, r.t.; ii) $NaIO_4$, AcOH, r.t., 84% overall; (**b**) Zn, $BrCH_2COOt$-Bu, THF, reflux, 31%; (**c**) CF_3COOH, r.t., 73%; (**d**) 0.05M NaOH, 35°C, then pH 3, 89%.

camptothecin (**187**) and an open **E**-ring derivative (**188**) after saponification and acidification [77].

However, the suggested approach [77] was only suitable for preparing derivatives endowed with modified E-ring because the number of substituted CPTs available from the natural product was limited. Therefore, a method was developed to prepare derivative compounds corresponding to the scheme **AB** + **DE** = **ABCDE**, which allowed the introduction of various substituents into cycles **A** and **B**. The most difficult task was to obtain a **DE**-heterobicycle. In a subsequent study, Lavergne *et al.* utilized the process of obtaining **DE**-cycles starting from the known ketone (**189**) [80], where the ketone function was protected by converting to 1,3-dioxane ketal (**190**), and the chlorine atom was replaced with methoxy group to give methoxypyridine (**191**) (Scheme **25**).

The hydroxyl group in precursor **191** was protected with benzyl, and the 1,3-dioxane protecting group was removed by subsequent acid hydrolysis to give ketone **192**. The carbonyl group in precursor **192** was alkylated with tert-butyl bromoacetate under the Reformatsky reaction conditions to form intermediate **193**. Removing benzyl and tert-butyl protecting groups from **193**, followed by intramolecular cyclization provided bicyclic intermediate **194**. Demethylation of **194** provided the **DE**-heterobicycle **195** as a key substance for the convergent pathway.

With the chiral key **D**, **E**-precursor **195**, a series of 9,10-substituted derivatives were obtained and tested for Topo-I inhibitory and antiproliferative activities. Fluorinated at C9 of the **A**-ring, hCPTs were found to have potent cytotoxicity against A427 and PC3 tumor cell lines and were more efficacious *in vivo* than CPT against HT-29 xenografts. Homologation of the lactone **E**-ring reinforced the stability of the lactone [78]. The benefits of homocamptothecins and silatecans were discussed in a mini-review published in 2005, covering publications up to 2004 [81].

Several attempts have been made to modify the **E**-cycle, either by adding new substituents to the hydroxyl group or by changing the nature of the **E**-ring. Along with the study on substituted CPT derivatives having a six- and seven-membered **E**-cycle, derivatives without a lactone moiety were also investigated. These studies were aimed not only at obtaining active and selective antitumor agents but also at finding more hydrolytically stable derivatives. Thus, modification of the **E**-cycle also leads to an increase in the activity of CPT derivatives. Based on this fact, the authors [82] devised a considerable design of CPT derivatives and investigated the antitumor activity of non-lactone CPT analogues. The lactone-free CPT compounds, which had a five-membered ketone **E**-ring, were found to be potent Topo-I poisons and exhibited promising cytotoxic activities with IC_{50} values in the range of nM [82].

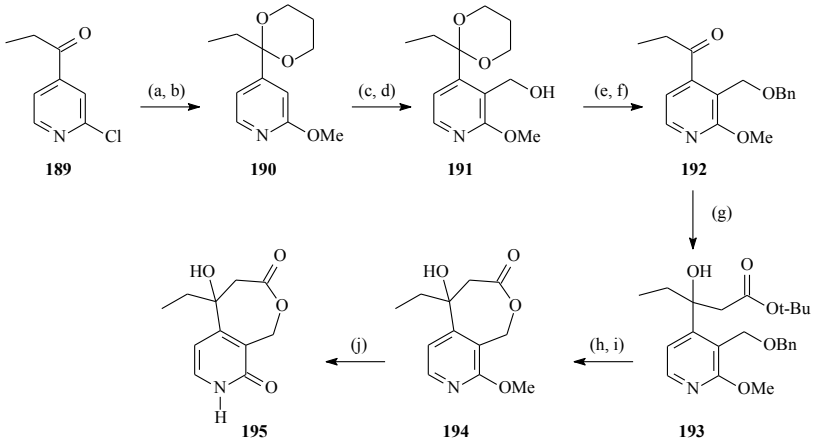
To synthesize these derivatives, the authors used the Comins procedure by the scheme **E** → **ED** + **AB** → **ABCDE**, in which [(2-fluoro-4-iodo)pyrid-3-yl]carboxaldehyde (**196**) [83, 84] was the starting material (Scheme **26**) [82].

The 2-ethoxypropylene group was introduced at the C4 position of pyridine cycle **196** under Stille conditions. The intramolecular cyclization of compound **197** proceeded smoothly, providing a **DE**-precursor as a mixture of two derivatives (**198** and **198**), which after hydrolysis (**198**) and alkylation with Grignard reagent, gave diol (**200**) as a mixture of diastereomers. In the next stages, the oxidation and transformation of **200** to the pyridone intermediate (**201**) were carried out. In the last two steps, pyridone **201** was N-alkylated with bromo-2-bromomethyl-3-quinoline (**113**) using classical Curran's conditions, [85] and the **C**-ring was formed in an intramolecular Heck reaction to yield the target compound (**202**).

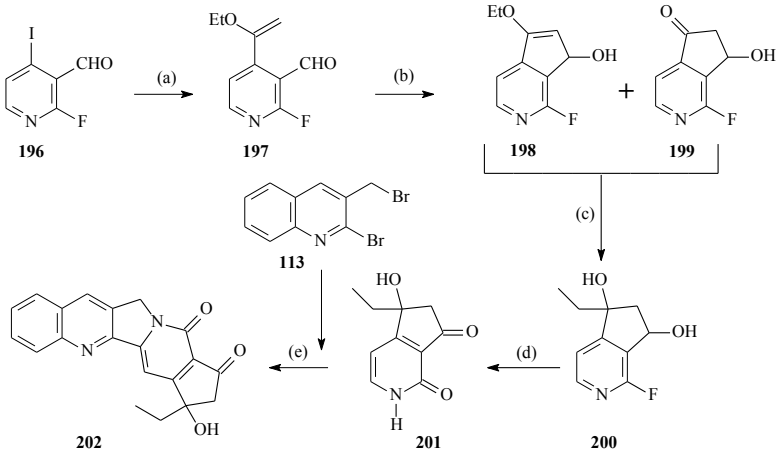
Various non-lactone 5-membered **E**-ring analogues were prepared in accordance with the previous pathway starting with pyridine derivative **203** (Scheme **27**), earlier prepared by Comins [84]. In the second approach, the hydroxyl in molecule **203** was protected as MEM ether. The obtained intermediate (**204**) was subjected to intermolecular ring closure by condensing the ester and activated methyl groups in the presence of an excess strong base. The ketone function of **205** was protected by converting to dioxolan ketal (**206**). The following steps were performed identically to step (*e*) in Scheme **26** using variously substituted bromo-2-bromomethyl-3-quinolines (**207**, for references, see [82]) in Heck conditions. The last stage was the removal of the protective groups, which gave the target camptothecin analogues with a non-lactone 5-membered **E**-ring (**209**) [82].

Using a DNA relaxation assay, the authors found that these CPT analogues with the hydroxypentanone **E**-ring were potent Topo-I poisons despite the absence of a lactone fragment. Among the agents studied, the compound with a dioxomethylene link at R_1-R_2 with an IC_{50} = 60 nM was the most potent against L1210 leukemia cells, which was close to the value for topotecan. The other cancer cells DU145 (prostate) and HT29 (colon) studied were slightly more sensitive than L1210, with an average IC_{50} of 30 nM. [82]

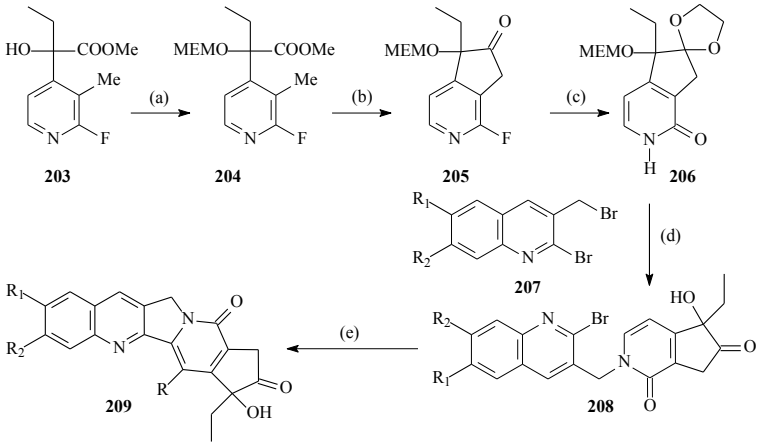
Another pathway for synthesis of **1** and various derivatives to evaluate antitumor activity was adopted by Manikumar *et al*. [86]. The researchers used a previously yielded tricyclic intermediate **212**



Scheme 25. Preparation of DhE-intermediate **195** in the synthesis of homocamptothecin by Lavergne and coworkers [78].
Reagents and conditions: (**a**) $HO(CH_2)_3OH$, pTSA (cat.), toluene, reflux; (**b**) MeONa, MeCN, reflux; (**c**) MesLi, THF, -78 °C to r.t., then DMF, -78 °C to r.t.; (**d**) $NaBH_4$, MeOH, r.t.; (**e**) NaH, $PhCH_2Br$, THF, r.t.; (**f**) TFA, 100 °C; (**g**) Zn, $BrCH_2COO$-t-Bu, THF, reflux; (**h**) H_2 (1 atm), Pd(C), EtOH, r.t.; (**i**) TFA, r.t.; (**j**) 1 N HCl, reflux.



Scheme 26. Syntheses of a non-lactone 5-membered **E**-ring analogue of camptothecin [82].
Reagents and conditions: (**a**) 1-Ethoxy-1-(tributyltin)ethylene, $Pd(PPh_3)_4$; (**b**) silica gel, CH_2Cl_2/MeOH (98/2); (**c**) i) CF_3COOH, H_2O/CH_3CN, r.t.; ii) EtMgBr, Et_2O, –30 °C, 50%, mixture of diastereoisomers (75/25); (**d**) i) PCC, CH_2Cl_2; ii) 2N HCl, reflux, 1 h; (**e**) i) **113**, NaH, LiBr, DME, DMF, [85], 80%; ii) $Pd(OAc)_2$, NBu_4Br, KOAc, CH_3CN, reflux, 25%.



Scheme 27. Syntheses of non-lactone five-membered E-ring analogues of **1** [82].
Reagents and conditions: (**a**) NaH, MEMCl, THF; (**b**) i) 5 equiv LDA/HMPA, THF; ii) 3N HCl, reflux, 1 h; (**c**) $HO(CH_2)_2OH$, p-TSA (cat.), toluene, reflux; (**d**) variously substituted bromo-2-bromomethyl-3-quinolines (**207**), [82] NaH, LiBr, DME, DMF; (**e**) i) $Pd(OAc)_2$, NBu_4Br, KOAc CH_3CN, reflux; ii) CF_3COOH/H_2O/reflux.

in the *S*–configuration [57, 58], obtained by removing the cyclic ketal from **101** (Scheme **11**) [42] and a number of substituted aminoacetophenones prepared in turn from substituted aminobenzonitriles (**210**) by the Grignard reaction (Scheme **28**). Friedländer condensation of the corresponding aminoketones **211** with key **212** resulted in the formation of 7,10,11-substituted derivatives **213**, where the substituents at C7 of the bulky, lipophilic CPT analogues, were determined by R_2 substituents in acetophenones **211** (Scheme **28**). This pathway was an implementation of the scheme **A** + **CDE** = **ABCDE**, when the **B**-ring was formed during condensation. The authors studied the antitumor activity of the obtained analogues and concluded that hydrophilicity was not a requirement for the stability of the cleavable Topo-I–DNA complexes as applied to the selected tumor cell lines.

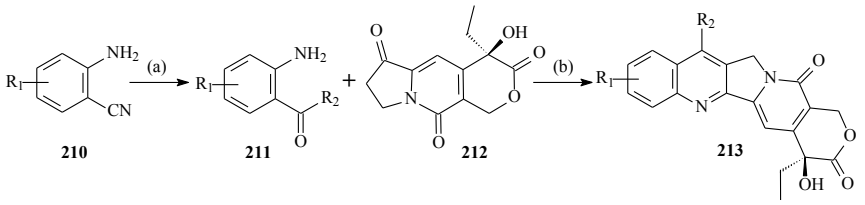
Both *in vitro* and *in vivo* anti-tumor activity studies of alkenyl esters of CPT and 9-nitrocamptothecin showed a wide range and strong antitumor inhibition against 14 various cancer cell lines with low toxicity [87]. These CPT derivatives prepared directly from **1** or 9-nitrocamptothecin were more potent than irinotecan and topotecan against all testing cell lines.

Danishefsky *et al.* [88] synthesized 18-noranhydrocamptothecin analogue (**214**, Scheme **29**) bearing an exocyclic methylene group on the **E**-ring. Compound **214** was designed as an alkylating agent and exhibited CPT-like inhibition of Topo-I. Although the inhibitory activity of the enzyme persisted, the cytotoxicity of **214** was significantly reduced relatively to CPT.

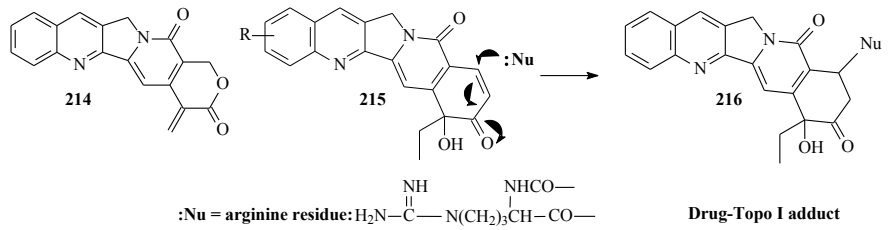
The next attempt to investigate the possibility of obtaining derivatives with the ability to form a covalent bond was undertaken by the research group of T.-L. Su [89]. To design and synthesize CPT analogues with the ability to form a covalent bond, the authors replaced the **E**-ring of CPT with the α,β-cyclohexenone moiety (compound **215**). They hypothesized that the nucleophilic guanidine function of the Arg364 residue of the enzyme active site could attack the α,β-cyclohexenone moiety *via* Michael addition to form the drug-Topo-I non-cleavable adduct (Scheme **30**).

For the total synthesis of **215,** the authors [89] used the following strategy: **AB + D = ABCD → ABCDE**, and the pathway was partially based on the study of K. E. Henegar *et al.* [90] on the synthesis of irinotecan and other camptothecin analogues and the total synthesis of homocamptothecins by O. Lavergne *et al.* [78]. As a result of their study, the authors suggested the pathway shown in Scheme **30** [91].

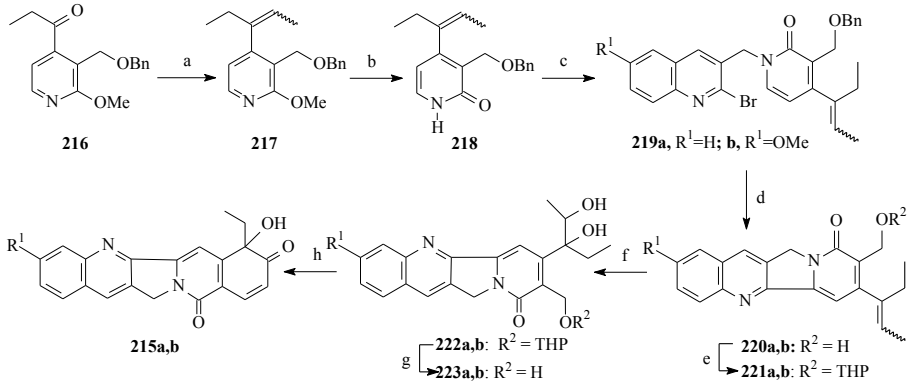
In this path, key ketone **216** [78] reacted in the Wittig reaction with ethyltriphenylphosphonylide to form an isomeric mixture of



Scheme 28. Syntheses of 7,10,11-substituted CPT analogues [86].
Reagents and conditions: (**a**) R_2MgX, CuI, THF, r.t. overnight, then 15% H_2SO_4; (**b**) p-TSA, toluene, reflux, 8 h.; R_1 = 10H, 11H or 10,11-methylenedioxy group (–OCH_2O–).



Scheme 29. Capability of **215** to form a covalent bond with Topoisomerase I [89].



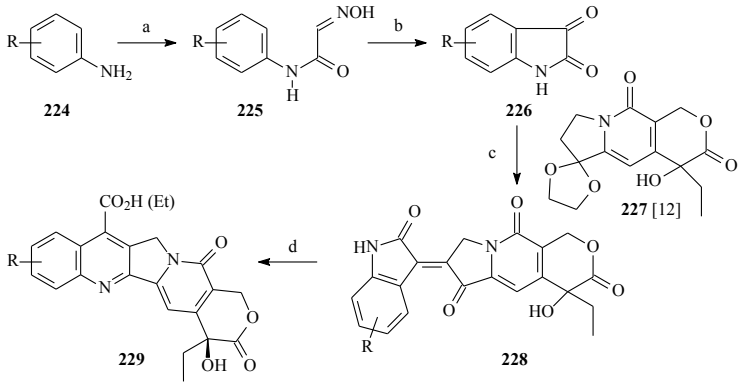
Scheme 30. Synthesis of camptothecin analogues with the **E**-lactone ring replaced by α,β-cyclohexenone [89].
Reagents and conditions: (**a**) Ph_3PEtBr, $KN(SiMe_3)_2$, THF, -85 °C to r.t., 4 h, 96%; (**b**) TMSCl, NaI, MeCN, r.t., 30 h, 84%; (**c**) 2-bromo-3-(bromomethyl)quinoline (**a**), [44], 2-bromo-3-(bromomethyl)-6-methoxyquinoline (**b**) [82], t-BuOK, DME, reflux, **219a**: 5 h, 98%; **219b**: 6 h, 90%; (**d**) i) $Pd(OAc)_2$, PPh_3, KOAc, MeCN, reflux, **a**: 12 h, 99%; **b**: 30 h, 75%; ii) BBr_3, CH_2Cl_2, -80 °C, 3 h, **220a**: 77%, **220b**: 56%; (**f**) OsO_4, $Me_3NO•2H_2O$, THF, r.t., **222a**: 45%, **222b**: 20%; (**g**) p-TSA(cat), CH_3OH, r.t., 5 h, **223a**: 70%, **223b**: 71%; (**h**) MnO_2/C [91], CH_3COOH(cat), CH_2Cl_2, r.t., **215a**: 85%, **215b**: 17%.

olefin **217**. The double bond in olefin **217** was stable enough for further transformations of the substituted pyridine ring into the pyridone ring in substance **218**. Pyridone **218**, as a precursor of the **D**-ring, easily condensed with the intermediate of the **AB**-rings, 2-bromo-3-(bromomethyl)quinoline [44], as an implementation of the path developed by Comins and Nolan. [59], M. L. Bennasar *et al.* [65], and Lavergne *et al*. [78]. In a subsequent reaction, the **C**-ring was formed by the Heck reaction to form the tetracyclic intermediate **219**, in which it was necessary to replace the protecting benzyl group with a tetrahydropyranyl (THP) protecting group (intermediate **221**). Olefin **221** was dihydroxylated (OsO_4/$Me_3NO·2H_2O$) to give diol (**222**). Unfortunately, attempts to carry out this stage under the classical conditions of Sharpless asymmetric catalytic dihydroxylation were unsuccessful, probably because of steric hindrances in the trisubstituted olefin moiety [92]. Subsequent reactions after the removal of the THP protection went surprisingly smoothly. The authors were able to efficiently oxidize the CH_2OH group to the aldehyde using the MnO_2 on carbon as an oxidizing agent, and the presence of a catalytic amount of acetic acid in the reaction mixture ensured a smooth flow of intramolecular aldol condensation, followed by removal of water. The active force of this highly productive transformation was favorable conditions for forming a stable six-membered cycle, and forming a system of conjugated double bonds contributed to the elimination of water.

5. SYNTHESIS OF DIVERSE SUBSTITUTED CPT DERIVATIVES IN SEARCH OF ACTIVE COMPOUNDS

Lackey and co-workers synthesized and conducted an *in vitro* cleavable complex assay of analogues of **1** with rigid, planar geometry, namely the oxodihydroindolylidene derivatives (**228**, Scheme **31**). They disclosed that compound **228** formed a ternary complex with DNA and Topo-I due to its appropriate planar geometry, similar to **1** [93].

They also studied the effect of the stereochemistry of the chiral center on the ability of the compound to stabilize a DNA-Topoisomerase I-drug ternary complex and found that the *S*-isomers were slightly more active than the racemic compounds, and *R*-isomers were totally inactive. Compounds **228** (R = F) and **229** (R = OCH_2CH_2O) were the most active derivatives in this series, with IC_{50} 1.1 and 0.7 μM, respectively [93].



Scheme 31. Synthesis of rigid analogues of 7-carboxy-**1** as DNA Topoisomerase I inhibitors with a variety of substituents on the A ring [93].
Reagents: (**a**) chloral hydrate, hydroxylamine, H_2O/HCl; (**b**) concentrated H_2SO_4, $BF_3 \cdot Et_20$, or $H_4P_2O_7$/heat; (**c**) **227** [12], AcOH, HCl, room temperature; (**d**) AcOH, concentrated HC1, 105 °C.

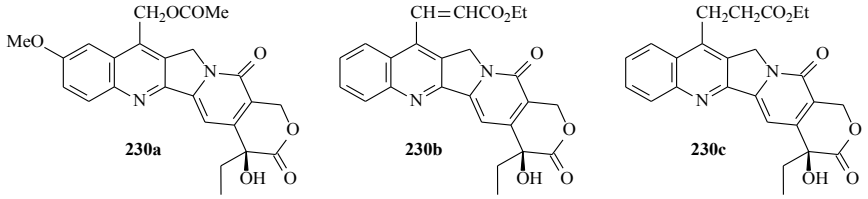


Fig. (3). 7-substituted CPT derivatives active against H460 cell lines *in vitro* [94].

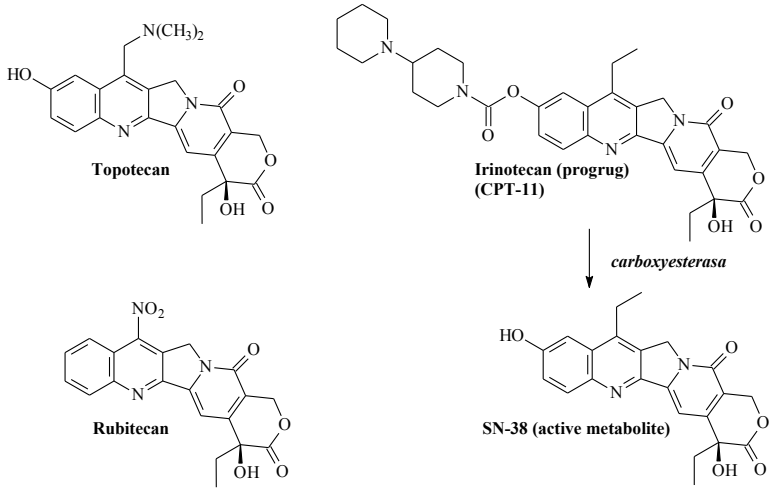


Fig. (4). Water-soluble camptothecin medicines approved by the FDA for IV administration.

By the end of the 20th century, accumulated earlier synthetic successes enabled an increase in the number of new CPT derivatives in order to find more selective agents active against drug-resistant tumors. For example, the previously synthesized 20(*S*)-camptothecin-7-aldehyde [71] served as the starting material for the synthesis of series 7-substituted camptothecin derivatives (**230**, Fig. **3**) [94], and most of the derivatives studied exhibited IC_{50} values in the 0.05-1 μM range. The authors demonstrated that the lipophilicity of the C7 substituent significantly affected antitumor efficacy [94]. In the study, the highest *in vitro* antitumor activity against human non-small-cell lung carcinoma H460 cell line was discovered for 10-methoxy-7-methyl acetate (**230a**, IC_{50} = 0.04 μM), ethyl 7-acrylate (**230b**, IC_{50} = 0.04 μM), and ethyl 7-propionate (**230c**, IC_{50} = 0.06 μM) camptothecins, which was higher than that of topotecan (IC_{50} = 1.38 μM) [94].

Selected strategies for the synthetic construction of camptothecin and its derivatives were discussed in the review of 2006 [95]. By this time, several highly active drugs, such as the water-soluble camptothecin medicines, topotecan and irinotecan (Fig. **4**), had been approved by the FDA for IV administration and introduced into clinical practice. Topotecan (Hycamtin) was used to treat ovarian cancers and small-cell lung cancers (SCLC). A New Drug Application (NDA) for rubitecan (orathecin) was submitted by SuperGen, Inc. to the US FDA in 2004 as an investigational drug to treat pancreatic cancer patients who had failed at least one prior chemotherapy regimen but was later withdrawn [96].

Several active compounds had been found among 10-hydroxycamptothecin derivatives, such as 9-allyl-10-hydroxycamptothecin [97], which inhibited tumor cells by the same mechanism as SN38 and topotecan, but showed greater cytotoxicity

without causing cross-resistance. Recently, two 20-aryloxy derivatives of 9-allyl-10-hydroxycamptothecin were found to inhibit colorectal cancer cell proliferation through induction of cell cycle arrest and apoptosis *in vitro* and *in vivo* [98].

Based on the antitumor activity data of the compounds studied, the structural features of CPT, which were essential for activity, were thoughtfully reconsidered and discussed [21, 22, 99].

Structure-activity relationships were investigated, and Verma and Hanch summarized the basic assumptions regarding the design of the new analogues [99]. They hypothesized that the structural features required to exhibit high anticancer activity were 20(*S*)-hydroxy-group (**a**), the lactone moiety of the **E**-ring (**b**), the pyridone moiety of the **D**-ring system (**c**), and the planarity of the five-member ring system (**d**). Additional factors positively affecting activity were the corresponding substituents at 9, 10, and 11-positions of the **A**-ring and 7-position of the **B**-ring discussed above, as well as the improved ADMET properties (Fig. **5**) [99].

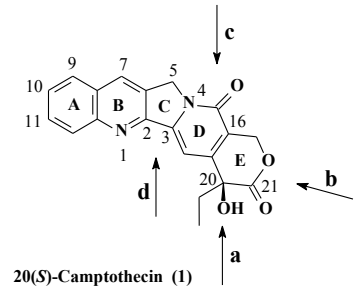


Fig. (5). Positions in the camptothecin molecule affecting the activity.

Several CPT derivatives, such as gimatecan, exatecan, belotecan, and lutrotecal, have been in clinical trials [100]. These derivatives differ in nature, the number of substituents on the **A-** and **B**-rings, but the **E**-ring remains unchanged.

Based on the fact that the substitution at position 7 of the CPT molecule leads to active antitumor analogues (Fig. **6**), many attempts have been made to synthesize various derivatives based on the CPT. More recently, Song *et al*. used **1** as a starting material to synthesize the group of 7-[(N-thioureidopiperazinyl)methyl] derivatives, which were much more active than irinotecan and topotecan with the greatest cytotoxicity against the MDR KB-VIN cell line, and these derivatives were good preclinical candidates for cancer treatment, especially the MDR phenotype [101].

It was also found that derivatives of **1** can be used as insecticides. 7-(N-substituted and N-acylsubstituted- thioureido-, -carbamidohydrazono)-formylcamptothecin derivatives were synthesized starting from **1** and demonstrated good-to-excellent activity against the insect species tested [102].

Modification of the **E**-ring of alkaloid derivatives has been done in several ways. One was the design and synthesis more stable to hydrolysis at the physiological pH lactone ring, like in hCPT [103] or more active diflomotecan (Scheme **32**) [104], with limited (but irreversible) **E**-ring-opening, and the synthesis of CPT analogues without ring opening.

E-ring structure modification is one of the current approaches to structural modification in CPT-based drug design. The main successful and promising CPT analogues in clinical use or clinical trials have been discussed in the review [105].

New ideas in this direction were realized in work by Miao and coworkers, who proposed to replace the carbonyl group of the E-ring with a fluorine atom, which led to improved hydrolytic stability of the obtained drugs. The authors substantiated in detail the advantages of introducing a fluorine-based new bioisostere of lactone into a molecule and synthesized (20*S*, 21*S*)-21-

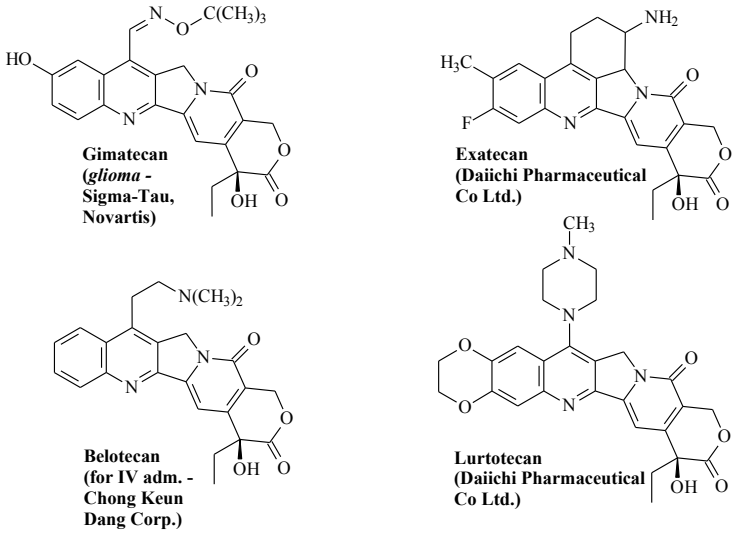
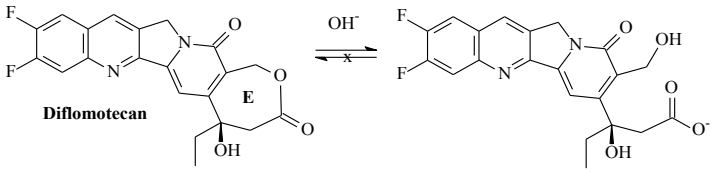


Fig. (6). Semi-synthetic analogues of **1** used in the chemotherapy.



Scheme 32. Diflomotecan irreversible hydrolysis [104].

fluorocamptothecins, which showed excellent *in vitro* and *in vivo* antitumor activities and were hydrolytically stable Topoisomerase I inhibitors (**233**, Scheme **33**) [106].

The derivatives with a 10-carbamate linker were synthesized at the same time, starting with 10-hydroxycamptothecin (**154**), but *in vitro* cytotoxicity against three human tumor cell lines K562, HepG2, and HT-29 was close to CPT, although the authors noted reduced cytotoxicity against normal human cell HEK293 and improved solubility of these derivatives compared to that of **154** (**234**, Scheme **33**) [107]. The most active compound from this group, namely 10-(2-tert-butoxy-2-oxoethoxy)-20(*S*)-camptothecin, was further investigated as an inhibitor of a large group of various cancer cells, markedly inhibiting tumor cell proliferation *via* mitochondrial-mediated apoptosis *in vitro* [108].

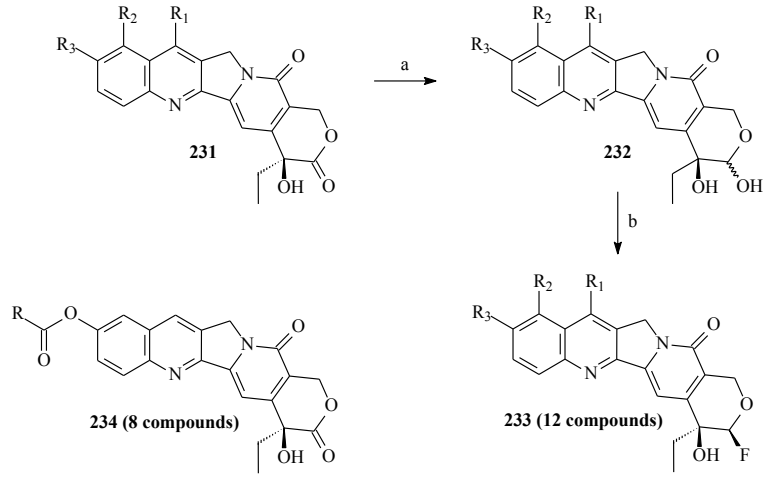
In the past decade, intensive research has focused on the study of 9,10-methylenedioxy-20(*R,S*)-CPT (**235**, Fig. **7**), which was previously obtained by Wani *et al.* in 1986 [109] and was also evaluated as an (*S*)-enantiomer [110] and was recently found to have high antitumor activity. Li *et al.,* using high throughput screening (HTS), discovered that **235**, also known as FL118, selectively inhibited the expression of several antiapoptotic proteins, such as survivin, Mcl-1, XIAP, and cIAP2, from the inhibitor of apoptosis (IAP) and Bcl-2 families, and effectively inhibited cancer cell growth [111-114]. It was also found that **233** overcame the resistance of tumors acquired by exposure to irinotecan and topotecan, was rapidly withdrawn from circulation, accumulated effectively in tumors, and had a long half-life. Studies had shown that the drug demonstrated 25-100 greater activity than irinotecan or topotecan in suppressing tumor cell growth and colony formation but was an ineffective Topo-I inhibitor compared to SN-38 [9, 115, 116].

Based on the high potential of analogue **235**, several novel 20-substituted derivatives coupled to glycosyl-succinic acid esters have recently been obtained and exhibited superior *in vitro* and *in vivo* cytotoxic activity. They were found to be more potent than irinotecan against A549, MDA-MB-231, and prostate cancer RM-1 cells.

Semi-synthetic methodologies for synthesizing new derivative **1** have also been effective in the search for new highly active anticancer agents. Many studies have been focused on highly efficient semi-synthetic methodologies, paving the way for the development of potent new C-20-modified CPT analogues. Researchers in a team led by K.-H Lee applied this methodology to find active compounds among 20-substituted derivatives of **1**, which would be more resistant to hydrolysis of the lactone ring and have better selectivity and less toxicity [117, 118, 120, 121]. Using this approach, a series of spin-labeled antineoplastic derivatives were synthesized and studied [119-121].

The researchers based the drug design on the knowledge that introducing a nitroxyl moiety into molecules of CPT derivatives could reduce toxicity and potentiate antitumor effects to some extent. Stable nitroxyl radical in a pharmaceutical agent allowed the antiproliferative activity to be studied and monitored by electron paramagnetic resonance (EPR) in pharmacological experiments [118, 121]. In addition, the effect of the 20(*S*)-sulfonylamidine residue on the cytotoxic activity of new derivatives of **1** has been extensively studied *in vitro* and *in vivo* [118].

In a study [117], the authors investigated the effect of different substituents at C5, C7, C9, C10, and C12 atoms on the antitumor activity in the large group of 20(*S*)-sulfonylamidine camptothecin derivatives and also varied the substituents in the sulfonylamidine residue (compounds **236**). The original lead compound (**236a**, $R_{1\text{-}6}$ = H, R_7 = 4-MeOPhCH$_2$, R_8 = 4-MePh) was more active than irinotecan in inhibiting the growth of A549, DU-145, KB, and KBv in



Scheme 33. (20*S*, 21*S*)-21-Fluoro- [106] and 10-carbamate camptothecins [107].
Reagents and conditions: **a**) KBH_4, CH_3OH, r.t., 4 h; **b**) DAST, CH_2Cl_2, -78 °C to 0 °C, 2 h.

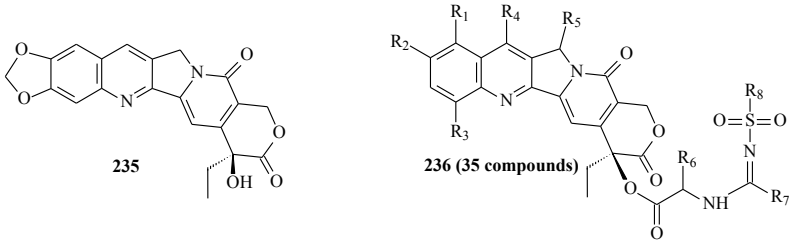


Fig. (7). 9,10-methylenedioxy-20(*S*)-camptothecin (**235**), [109-113] and 20(*S*)-sulfonylamidine camptothecin derivatives (**236**) [117, 118].

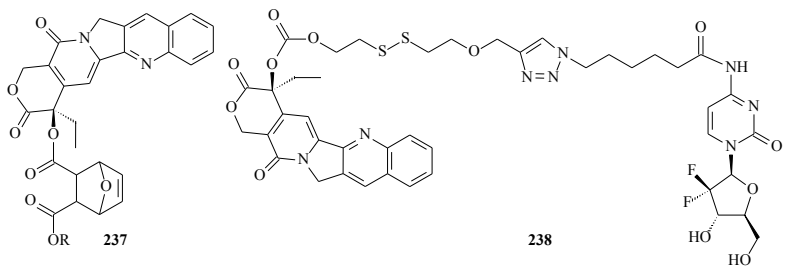


Fig. (8). Antitumor CPT conjugates [125, 126].

cells with IC_{50} values of 0.031, 0.050, 0.14, and 0.026 μM, respectively. Compound **236a** selectively inhibited Topo-I, which caused significant DNA damage and showed no apparent acute toxicity in contrast to **1** (LD_{50} 56.2 mg/kg, i.p.) and irinotecan (LD_{50} 177.5 mg/33 kg, i.p.). According to *in vitro* evaluation data, all synthesized compounds were more potent than irinotecan in cytotoxicity assays. It was also found that the short -CH_2- link, opposite -$(CH_2)_2$- or longer chain, and the presence of an ethyl group at C4 improved cytotoxicity, but the introduction of a nitro group at C9 or C12, or a methoxy group at C5 significantly reduced the antitumor activity. The authors investigated the binding mode of the most active compounds using molecular docking modeling. The docking study showed a high affinity of the studied substances to the DNA-Topo 1 complex with a total free binding energy of 11.56 kcal/mol and the additional formation of π-π stacking interaction with nucleotides using a methyl phenyl group on the sulfonyl amidine side chain. Based on the molecular modeling data obtained, the authors hypothesized that introducing the bulky substituent also established new interactions with Topo-I [117].

Further studies resulted in the synthesis of 10-fluoro substituted derivatives of **235,** which were more potent than the clinically used irinotecan and topotecan, and also suppressed the proliferation of multidrug-resistant (MDR) KB-VIN cells [122]. Several new substituted CPT derivatives containing a 20-acylthiourea fragment had been synthesized and shown promising *in vitro* cytotoxicity against six tumor cell lines (Hep3B, MCF7, A549, MDA-MB-231, KB, and KB-vin) and were not toxic to normal cells in a mouse model [123]. A large group of derivatives has recently been synthesized from the readily available 10-hydroxy-**1** in order to obtain highly soluble compounds [124]. These researchers obtained a derivative of 9-(2-(4-methylpiperidin-1-yl)-2-oxoethoxy), whose water solubility reached 5.73 μg/mL. This drug was demonstrated *in vitro* as a more potent antitumor agent than SN-38 by comparing their inhibitory activities [124].

The frequently used strategy in medicinal chemistry is the synthesis of hybrid molecules. Recently, an attempt has been made to study methods for the synthesis of hybrid molecules, including the CPT core. The simplest pathway was realized by forming esters with a naturally occurring toxin norcantharidin and the 20(*S*)-hydroxyl group of CPT [125]. The obtained compound **237** (Fig. **8**, R = Me or Bn) had activity similar to that of CPT and is probably of theoretical interest.

More complex structures have been presented in recent work, where methods of synthesis and cytotoxicity of the camptothecin−SS−1,2,3-triazole−gemcitabine conjugate (**238**) as an amphiphilic prodrug are investigated [126]. The researchers combined CPT, 2,2'-disulfanediyldiethanolate, 1,2,3-triazole, and cytotoxic gemcitabine in one molecule **238**, where 1,2,3-triazole was an active participant in acid-base equilibrium. The disulfide-containing fragment could be cleaved under reducing conditions, forming two antitumor drugs acting by different mechanisms. The authors demonstrated that these structural features allow molecule **238** to self-assemble with the formation of nanoparticles in an aqueous solution and without the participation of polymer organizers. As a result, self-delivery system **238** demonstrated greater cytotoxicity against HepG2 cells compared to CPT and gemcitabine at 48 h and 72 h incubation [126].

Something similar to previous structures was proposed by the researchers, who conjugated CPT and paclitaxel with a cell-penetrating cyclic peptide $[WR]_5$ as an effective molecular transporter for various hydrophilic and hydrophobic molecules, but the conjugates had less activity than the corresponding parent anticancer drugs [127].

CONCLUSION

The highly active anticancer alkaloid 20(*S*)-camptothecin is of particular scientific interest for synthetic organic chemistry, which is stimulated by the great potential of this substance and by the fact that most of its derivatives have more or less anticancer activity. Chemoselective reactions have been developed and used to synthesize camptothecin and its many derivatives. Many of these reactions have been discovered due to advances in chemical theory and, especially, spectroscopy and chromatography. These advances allowed Comins and Nolan to reduce the synthesis of these remarkably complex molecules to six steps [59]. On the other hand, some studies on methods for synthesizing camptothecin derivatives aimed to explore the possibility of using new or poorly studied reactions, where the final structure was a popular model.

The modern development in methods for designing drugs involves both the use of traditional methods of adding a variety of substituents at different positions in the CPT core and applying new discoveries of organic synthesis, taking into account the already known effects of substituents on anticancer activity. Among such molecules, it is desirable to discover agents with high selectivity and reversible mechanism of action. The second way to modernize effective and highly selective drugs is to create hybrid or conjugated molecules capable of exhibiting dual biological activity. Another newly developed strategy is nanomedicines, in which an active compound is integrated by physico-chemical methods with nanoparticles for efficient drug delivery [128].

Although some CPT derivatives have been used as antitumor drugs for a long time, others are undergoing clinical trials; however, it is hoped that a drug with improved selectivity and less toxicity compared to the medicines used in clinical practice can be found [129]. In addition, the synthesis of CPT and its derivatives is a good example of using and testing the effectiveness of some organic synthetic methods.

The considered discoveries indicate the high potential of CPT derivatives and the excellent possibilities of their diverse structural modification in the search for highly active anticancer drugs.

LIST OF ABBREVIATIONS

Acronym	=	Description
A427	=	Tumor Cell Line

A549	=	Tumor Cell Line
ADMET	=	Absorption, Distribution, Metabolism, and Excretion
AIBN	=	2,2'-Azodi(isobutyronitrile)
CPT	=	20(S)-Camptothecin
DABCO	=	1,4-Diazabicyclo[2.2.2]octane
DAST	=	Diethylaminosulfur Trifluoride
DCC	=	N,N′-Dicyclohexylcarbodiimide
DDQ	=	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DHP	=	3,4-Dihydropyran
$(DHQ)_2PHAL$	=	Hydroquinine 1,4-phthalazinediyl Diether
DME	=	Dimethoxyethane
DMF	=	N,N-Dimethylformamide
DMSO	=	Dimethyl Sulfoxide
DNA	=	Deoxyribonucleic Acid
DSB	=	Double-strand DNA Breaks
DU145	=	Tumor Cell Line
EPR	=	Electron Paramagnetic Resonance
FDA	=	Food and Drug Administration
FL118	=	10,11-methylenedioxy-20(S)-camptothecin
H460	=	Tumor Cell Line
HB	=	Tumor Cell Line
hCPT	=	Homocamptothecin
HEK293	=	Tumor Cell Line
HepG2	=	Tumor Cell Line
HMDS	=	Hexamethyldisilazane
HMPA	=	Hexamethylphosphoramide
HOBT	=	Hydroxybenzotriazole
HT29	=	Tumor Cell Line
HTS	=	High Throughput Screening
IAP	=	Inhibitor of Apoptosis
IC_{50}	=	Half maximal Inhibitory Concentration
K562	=	Tumor Cell Line
KB	=	Tumor Cell Line
KB-VIN	=	Tumor Cell Line
KHMDS	=	Potassium bis(trimethylsilyl)Amide
KOAc	=	Potassium Acetate
L1210	=	Tumor Cell Line
LD_{50}	=	Lethal Dose, 50%
LDA	=	Lithium Diisopropylamide
MCCD	=	β-morpholinoethyl Cyclohexyl-carbodiimide metho-p-tosylate
MCF7	=	Tumor Cell Line
MDA-MB-231	=	Tumor Cell Line
MDR	=	Multidrug-resistant
MEM	=	2-Methoxyethoxymethyl Chloride
MEMC	=	2-Methoxyethoxymethyl Chloride
MOA	=	Mechanism of the Action
NDA	=	New Drug Application
PC3	=	Tumor Cell Line
PCC	=	Pyridinium Chlorochromate
PDC	=	Pyridinium Dichromate
Ph_3PEtBr	=	Ethyltriphenyl Phosphonium Bromide
PLC	=	Preparative Liquid Chromatography
QSAR	=	Quantitative Structure-Activity Relationship
RNA	=	Ribonucleic Acid
SAR	=	Structure-Activity Relationship
SCLC	=	Small-Cell Lung Cancer
SDS	=	Sodium Dodecyl Sulfate
TBDMS	=	Tert-Butyldimethylsilyl Chloride
TBDMSCN	=	tert-Butyldimethylsilyl Cyanide
TBME	=	tert-Butyl Methyl Ether
t-BuOK	=	Potassium Tert-Butoxide
TCC	=	trans-2-(R-cumyl)cyclohexyl
TEA	=	Triethylamine
TFA	=	Trifluoroacetic Acid
THF	=	Tetrahydrofuran
THP	=	Tetrahydropyran
TMSCHN	=	Trimethylsilyl Cyanide
TMSCl	=	Trimethylsilyl Chloride
TMSI	=	Trimethylsilyl Iodide
TSA	=	p-Toluenesulfonic Acid
TTMSS	=	Tris(trimethylsilyl)silane
WRN	=	Werner Syndrome Protein
XIAP	=	X-linked Inhibitor of Apoptosis Protein

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CONFLICT OF INTEREST

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