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CHAPTER FOUR

Cephalotaxus Alkaloids

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Abstract

Cephalotaxus alkaloids represent a family of plant secondary metabolites known for 60 years. Significant activity against leukemia in mice was demonstrated for extracts of *Cephalotaxus*. Cephalotaxine (CET) (**1**), the major alkaloid of this series was isolated from *Cephalotaxus drupacea* species by Paudler in 1963. The subsequent discovery of promising antitumor activity among new *Cephalotaxus* derivatives reported by Chinese, Japanese, and American teams triggered extensive structure elucidation and biological studies in this family. The structural feature of this cephalotaxane family relies mainly on its tetracyclic alkaloid backbone, which comprises an azaspiranic 1-azaspiro[4.4]nonane unit (rings C and D) and a benzazepine ring system (rings A and B), which is linked by its C3 alcohol function to a chiral oxygenated side chain by a carboxylic function alpha to a tetrasubstituted carbon center. The botanical distribution of these alkaloids is limited to the *Cephalotaxus* genus (*Cephalotaxaceae*). The scope of biological activities of the *Cephalotaxus* alkaloids is mainly centered on the antileukemic activity of

homoharringtonine (HHT) (**2**), which in particular demonstrated marked benefits in the treatment of orphan myeloid leukemia and was approved as soon as 2009 by European Medicine Agency and by US Food and Drug Administration in 2012. Its exact mechanism of action was partly elucidated and it was early recognized that HHT (**2**) inhibited protein synthesis at the level of the ribosome machinery. Interestingly, after a latency period of two decades, the topic of *Cephalotaxus* alkaloids reemerged as a prolific source of new natural structures. To date, more than 70 compounds have been identified and characterized. Synthetic studies also regained attention during the past two decades, and numerous methodologies were developed to access the first semisynthetic HHT (**2**) of high purity suitable for clinical studies, and then high grade enantiomerically pure CET (**1**), HHT (**2**), and analogs.

Abbreviations

acac	Acetylacetonate
AChE	Acetyl choline esterase
ACN 1,1′	Azobis(cyclohexanecarbonitrile)
ADP	Adenosine 5′-diphosphate
AIBN 2,2′	Azobisisobutyronitrile
AML	Acute myeloid leukemia
APL	Acute promyelocytic leukemia
Ara-C	Cytosine arabinoside
ATRA	All-trans-retinoic acid
AY	Average yield
BCR-ABL	Breakpoint cluster region and ABL (Abelson)
BID	Bis in die (twice daily)
Boc *tert*	Butyloxycarbonyl
BP	Blast phase
brsm	Based on recovered starting material
CBz	Carbobenzoxy
CCDC	Cambridge Crystallographic Data Center
CDI	Carbonyldiimidazole
CET	Cephalotaxine
CGAF	*Cephalotaxus griffithi* alkaloid fraction
CHR	Complete hematologic response
CMC	Cell membrane chromatography
CML	Chronic myeloid leukemia or chronic myelogenous leukemia
CP	Chronic phase
Cp	Cyclopentadiene
CSA	Camphorsulfonic acid
CSD	Cambridge Structural Database
CSE	Conventional solvent extraction
CTA	Cephalotaxinamide
dba	Dibenzylideneacetone
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC *N,N′*	Dicyclohexylcarbodiimide
de	Diastereoisomeric excess
DEPC	Diethyl pyrocarbonate

DIAD	Diisopropyl azodicarboxylate
DIBAL-H	Diisobutylaluminum hydride
DIPEA *N,N*	Diisopropylethylamine
DMAP 4	Dimethylaminopyridine
DMDO	Dimethyldioxirane
DMF	Dimethylformamide
DMP	Dess—Martin periodinane
DMPU 1,3	Dimethyl-1,3-propylurea (1,3-Dimethyl-3, 4,5,6-tetrahydro-2(1H)-pyrimidinone)
DMSO	Dimethyl sulfoxide
DNR	Daunorubicin
DOX	Doxorubicin
dppe	1,3-Bis(diphenylphosphino)ethane
DTBMP 2,6	Di-*tert*-butyl-4-methylpyridine
EDC *N*-(3-Dimethylaminopropyl)-*N*′	ethylcarbodiimide hydrochloride
ee	Enantiomeric excess
EMA	European Medicines Agency
EMP	Embden—Meyerhof pathway
FDA	Food and Drug Administration
G-CSF	Granulocyte colony-stimulating factor
GHI	G-CSF HHT and Imatinib
GMP	Good manufacturing practice
HAA	HHT, aclacinomycin, and Ara-c
HAD	HHT, Ara-c and DNR
Hfc 3	(Heptafluoropropyl-hydroxymethylene)-D-camphorate
HHT	Homoharringtonine, Omacetaxine
H1R	Histamine 1 receptor
HMDS	Hexamethyldisilazane
HMPA	Hexamethylphosphoramide
HOBt	Hydroxybenzotriazole
HPLC	High-performance liquid chromatography
HSP	Heat shock protein
HT	Harringtonine
IBX 2	Iodoxybenzoic acid
IM	Imatinib
Im	Imidazole
IFN-α	Interferon alpha
INN	International nonproprietary name
IUCN	International Union for Conservation of Nature
KHMDS	Potassium hexamethyldisilazide
LDA	Lithium diisopropylamide
LiHMDS	Lithium hexamethyldisilazide
LCL	Long-circulating liposomes
LLS	Longest linear sequence
LOQ	Limit of quantitation
***m*CPBA**	*meta*-Chloroperoxybenzoic acid
Mcl-1	Myeloid cell leukemia-1
MCyR	Major cytogenetic response

MERS-CoV	Middle East respiratory syndrome coronavirus
MDS	Myelodysplastic syndrome
MHR	Major hematologic response
MJ	Methyl jasmonate
MM	Multiple myeloma
MS	Mass spectrometry
Ms	Methanesulfonyl
MTX	Mitoxantrone
MWE	Microwave extraction
NBS	*N*-Bromosuccinimide
NCI	National Cancer Institute
NCS	*N*-Chlorosuccinimide
n.d.	Not determined
n.r.	Not reported
NIS	*N*-Iodosuccinimide
NMM	*N*-Methylmorpholine
NMO	*N*-Methylmorpholine *N*-oxide
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser effect spectroscopy
NS	Number of steps
NsCl 4	Nitrobenzenesulfonyl chloride
OM	Omacetaxine
OY	Overall yield
PARP	Poly(ADP-Ribose) polymerase
PCC	Pyridinium chlorochromate
PDC	Pyridinium dichromate
PEG	Polyethyleneglycol
Ph	Philadelphia (biology) or phenyl (chemistry)
Ph+	Philadephia-positive chromosome
Piv	Pivaloyl
PPA	Polyphosphoric acid
PPL	Porcine pancreas lipase
***p*-TsCl**	*p*-Toluenesulfonic chloride
***p*-TsOH**	*p*-Toluenesulfonic acid
pyr	Pyridine
QCC	Quaternary carbon center
RCM	Ring closing metathesis
re	Regioisomeric excess
Red-Al	Sodium bis(2-methoxyethoxy)aluminum hydride
RI	Resistance index
rt	Room temperature
SAR	Structure–activity relationship
SC	Subcutaneous
SNP	Single nucleotide polymorphism
ssHTT	Semisynthetic HHT
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutylammonium iodide
TBAT	Tetrabutylammonium difluorotriphenylsilicate
TBDMSCl	*tert*-Butyldimethylsilyl chloride
TBDPSCl	*tert*-Butyldiphenylsilyl chloride
TCM	Traditional Chinese medicine

TEA	Triethylamine
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxy
Tf	Triflate
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic acid anhydride
TfOH	Triflic acid
THF	Tetrahydrofuran
TMEDA	Tetramethylethylenediamine
TMG	1,1,3,3-Tetramethylguanidine
TMS	Trimethylsilyl
Ts	*p*-Toluenesulfonyl

1. INTRODUCTION

This contribution is an updated and expanded version of the two preceding chapters published in this series in 1984[1] and 1998.[2] Other reviews focused on the structure and synthesis of these compounds,[3–6] and several reviews are available that cover the structure–activity relationships (SARs), biological, and clinical properties of these alkaloids.[7–19] The literature is reviewed from the last update in this series, which was covering the field till mid-1997, to early 2016. Because they usually exist under the form of unique enantiomer, we numbered the natural products by an arabical digit irrespective to their optical rotation sign, e.g., cephalotaxine (CET) (**1**), their antipode by *ent*-digit, e.g., *ent*-cephalotaxine *ent*-(**1**), and the corresponding racemic compound usually obtained by chemical synthesis *rac*-digit, e.g., *rac*-cephalotaxine *rac*-(**1**). In the asymmetric synthesis section, we have precised the sign of the optical rotation, e.g., CET (−)-(**1**), *ent*-CET (+)-(**1**), and *rac*-CET (±)-(**1**).

For more than 50 years, chemists have studied the extraction, structure determination, biogenesis, and synthesis of CET (**1**) (Fig. 2), the major alkaloid of this series, and related alkaloids, because of the reported biological activity of its natural esters **2**–**5**, in view of their therapeutical use in the treatment of leukemia. Homoharringtonine (HHT) (**2**) and harringtonine (HT) (**3**), or a mixture of both, have been used in traditional Chinese medicine (TCM) since the 1970s to treat a variety of malignancies. HHT (**2**) being the easiest to purify and the most abundant in the plant was selected by the National Cancer Institute (NCI) for clinical development. HHT (**2**), also known as omacetaxine, a simple ester of CET (**1**), probably holds the record for the longest time to reach the market[20] after having experienced increasing interest since its first use in TCM, and efforts by pharmaceutical

companies for its development as an antileukemic agent. Despite its clinical efficacy, natural HHT (**2**) was not recorded until now, simply because normal extract formulations have raised several unresolved problems, such as the reproduction of a constant product from defined extraction procedure, the purity profile, the main source of the raw material in China, and biodiversity conservation issues. The *Cephalotaxus* shrubs with extremely slow growth are mostly protected and endangered species. Moreover, CET (**1**) and its active esters are present in small quantities in the plant (roots, bark, heartwood, and leaves), which hardly makes possible to exploit it to obtain HHT (**2**) or its related esters. Semisynthetic HHT (ssHHT) (**2**) (Myelostat, Ceflatonin, or Omacetaxine mepesuccinate (OM)) is manufactured by a semisynthetic process developed by Robin from naturally occurring alkaloid CET (−)-(**1**) extracted from the dry leaves of *Cephalotaxus harringtonia*, and a synthetic precursor side chain.[21] Although HHT (**2**) was investigated for the treatment of chronic myeloid leukemia (CML) in the 1990s with good results, the advent of Imatinib (IM) mesylate, a breakpoint cluster region and Abelson (BCR-ABL)1 tyrosine kinase inhibitor (TKI) at that time delayed the interest for HHT (**2**). However, de novo or acquired resistance to TKIs[22] renewed the interest for this alternative first-in-class simple natural ester of CET (**1**). HHT (**2**) is currently approved in Europe and in the United States, due to its reported activity in patients with CML resistant to currently available TKIs. As a result, on September 2, 2004, orphan designation (EU/3/04/224) was granted by the European Union for OM (International nonproprietary name (INN), trade name Synribo)/HHT (**2**) for the treatment of CML to Stragen France SAS, France, for the treatment of CML. Exploitation rights were transferred to ChemGenex Europe SAS, France, in January 2009 and subsequently to Teva Pharma GmbH, Germany, in December 2012. Due to their different mechanisms of action, combinations with TKIs represent attractive treatment options. Recently, on October 26, 2012, HHT (**2**), Synribo (formerly Ceflatonin, Omapro), received an accelerated approval by the US Food and Drug Administration (FDA) for adult patients with chronic-phase (CP) or accelerated-phase (AP) CML with resistance or intolerance to two or more TKIs. The US FDA has granted full approval to HHT (**2**) (drug substance INN OM, Synribo) for injection, based on the final analysis of two phase II trials evaluating the efficacy and tolerability data of omacetaxine in 2014. This renewed interest for isolation, characterization, biological evaluation, and total synthesis of *cephalotaxus* alkaloids and analogs to suppress the dependance on natural source supply.

2. NATURAL OCCURRENCE, ISOLATION, AND STRUCTURAL ASSIGNMENT

As early as 1954, Wall brought to light the presence of alkaloids in plants of the genus *Cephalotaxus*,[23] the only genus of the *Cephalotaxaceae* family composed of 11 species of evergreen small and only slowly growing conifers, which are mostly indigenous to China (Fig. 1). The first alkaloid characterized from *Cephalotaxus harringtonia* var. *drupacea* (*Cephalotaxus drupacea*) and *Cephalotaxus fortunei* was named CET by Paudler in 1963[24] and its original benzazepine nucleus fused to an azaspirononane unit established by Powell et al (Fig. 2).[25]

Although its biological activity and partial structure are known since 1959,[26] the elucidation of the structure of natural HT (**3**) (Fig. 2) isolated from *C. harringtonia* var. *drupacea* and antitumor activity against L1210 and P388 leukemia in mice (1 mg/kg) in 1969[25] stimulated the continuous search for active compounds and particularly for alkaloids from the *Cephalotaxus* genus. Apart from the CET series focused in this chapter, the other alkaloid part in *Cephalotaxus* species belongs to the class of homoerythrinane alkaloids, isolated as minor compounds with the exception of *Cephalotaxus wilsoniana*. Biogenesis of CET (**1**) postulated by Parry in 1977 showed a common origin for these alkaloids.[1,2,27]

Cephalotaxanes (**6**) is the generic name for the particular alkaloids of the *Cephalotaxaceae* family, which have a general formula derived from the

Scientific classification

Kingdom: Plantae
Division: Pinophyta
Class: Pinopsida
Order: Pinales
Family: Cephalotaxae
Genus: *Cephalotaxus* Siebold & Zucc ex Endl
Species:
Cephalotaxus fortunei
Cephalotaxus griffithii
Cephalotaxus hainanensis
Cephalotaxus harringtonia
Cephalotaxus koreana
Cephalotaxus lanceolata
Cephalotaxus latifolia
Cephalotaxus mannii
Cephalotaxus oliveri
Cephalotaxus sinensis
Cephalotaxus wilsoniana

C. fortunei (fruits and leaves) (Fortune's Plum Yew), Paris

Figure 1 Scientific classification of *Cephalotaxus genus* and picture (from F. Dumas) of the fruits and leaves of *Cephalotaxus fortunei* at Parc Montsouris, Paris (France).

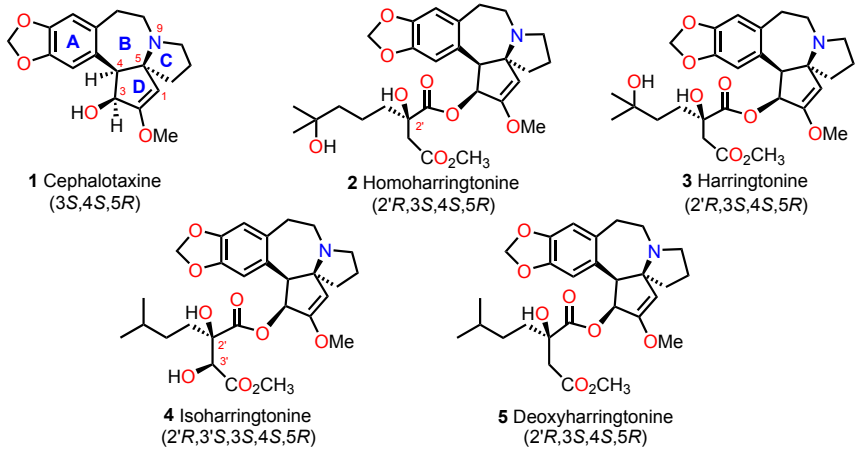


Figure 2 Cephalotaxine (**1**) and its natural esters (**2**–**5**) early characterized in *Cephalotaxus harringtonia* var. *drupacea*.

cephalotaxane skeleton with either one or two units covalently linked forming dimers having two identical or different units (Fig. 3). Cephalotaxanes (**6**) include neutral compounds or salts having this basic skeleton in common and may contain various oxygenated substituents (aliphatic ethers or aromatic units, free or esterified alcohols, substituted or free enols, phenol, and others). Cephalotaxanes include CETs characterized by a free alcohol function on ring D (R = H), and HTs where the 3-OH group is esterified (R ≠ H). Isolation of cephalotaxanes and homoerythrinane alkaloids [skeleton (**7**)] from the same plant suggested that they might be formed through similar biogenetic routes.[1,2] Hainanensine (**8**) is a structurally unique *Cephalotaxus* alkaloid devoid of optical activity and lacking the spiranic tetrasubstituted carbon center. It was isolated from *Cephalotaxus hainanensis* and *C. fortunei* (Fig. 3) and displays a type of single rearranged CET skeleton.[28]

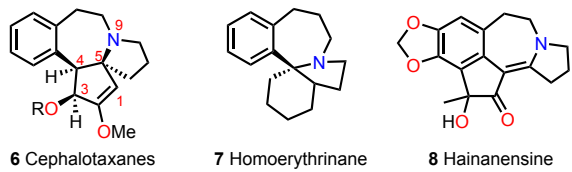


Figure 3 Cephalotaxanes (**6**), the skeleton (**7**) of minor homoerythrinane alkaloids, and the structurally unique *Cephalotaxus* alkaloid hainanensine (**8**).

Until 1997, 36 compounds related to the CET series (Tables 1 and 2), were described, of which 23 are natural esters and 1 is dimeric.

CET (**1**) and HHT (**2**) were also isolated conventionally from fruits, leaves, and branches of *Cephalotaxus sinensis*.[29] Among the alkaloids derived from CET (**1**), compounds possess different D rings such as cephalotaxinone (**10**), demethylcephalotaxine (**11**), and isocephalotaxinone (**13**) and ring C such as cephalotaxinamide (**18**). Oxygenated compounds with modifications on their B ring as 11-hydroxycephalotaxine (**14**) and 4-hydroxycephalotaxine (**15**) or compounds having an oxygen bridge between the rings B and D such as drupacine (**16**) were also described (Fig. 4).[30–32] 11-Hydroxycephalotaxine (**14**) was converted to drupacine (**16**) in acidic medium.[31] Among the minor alkaloids isolated from the bark of *C. hainanensis* Li (in addition to 11 known alkaloids), CET analogs were identified as cephalotaxinone (**10**), cephalotaxinamide (**18**), and demethylneodrupacine (**17**), which possesses an ether bridge between C11 and C3, while drupracine (**16**) exhibits an ether bridge between C11 and C2. The structure of desmethylcephalotaxinone (**12**), isolated from *Cephalotaxus harringtonia*

Table 1 *Cephalotaxus* alkaloids that have been described from natural sources till mid-1997[1,2]

Cephalotaxine analogs
Cephalotaxine (**1**), $C_{18}H_{21}NO_4$, mp 132–133°C, $[\alpha]_D$ −183 (c 0.22, EtOH)
Hainanensine (**8**), $C_{17}H_{17}NO_4$, mp 240–244°C, $[\alpha]_D$ 0 (CH_3OH)
Epicephalotaxine (**9**), $C_{18}H_{21}NO_4$, mp 136–137°C, $[\alpha]_D$ −150 (c 0.8, $CHCI_3$)
Cephalotaxinone (**10**), $C_{18}H_{19}NO_4$, mp 198–200°C, $[\alpha]_D$ −146 (c 0.63, $CHCI_3$)
Desmethylcephalotaxine (**11**), $C_{17}H_{19}NO_4$, mp 109–111°C, $[\alpha]_D$ −110 (c 0.28, CH_3OH)
Demethylcephalotaxinone (**12**), $C_{17}H_{17}NO_4$, mp 102–107°C, $[\alpha]_D$ +2.3 (c 0.52, CH_3OH)
Isocephalotaxinone (**13**), $C_{18}H_{19}NO_4$, mp 130.5–132°C, $[\alpha]^{21}{}_D$ −47 (c 0.5, $CHCI_3$)
Oxyoenated cephalotaxines
11-Hydroxycephalotaxine (**14**), $C_{18}H_{21}NO_5$, mp 235–242°C, $[\alpha]_D$ −139 (c 0.56, $CHCI_3$)
4-Hydroxycephalotaxine (**15**), $C_{18}H_{21}NO_5$, m. 135–137°C, $[\alpha]^{27}{}_D$ +120 (c 0.025, CH_3OH)
Drupacine (**16**), $C_{18}H_{21}NO_5$, mp 70–72°C, $[\alpha]_D$ −137°C (c 0.79, $CHCI_3$)
Demethylneodrupacine (**17**), $C_{17}H_{19}NO_5$, mp 197–200°C
Cephalotaxinamide (**18**), $C_{18}H_{19}NO_5$, mp 230°C

Table 2 Natural *Cephalotaxus* alkaloid esters that have been described up to May 1997[1,2]

Harringtonines

Homoharringtonine (**2**), $C_{29}H_{39}NO_9$, mp 144–146°C, $[\alpha]_D$ −119 (c 0.96, $CHCI_3$)
Harringtonine (**3**), $C_{28}H_{37}NO_9$, mp 73–75°C, $[\alpha]_D$ −104.6 (c 1.0, $CHCI_3$)
Isoharringtonine (**4**), $C_{28}H_{37}NO_9$, mp 69–72.5°C, $[\alpha]_D$ −99.6 (c 1.06, $CHCI_3$)
Deoxyharringtonine (**5**), $C_{28}H_{37}NO_8$, amorphous, $[\alpha]_D$ −125.4 (c 1.76, $CHCI_3$)
Neoharringtonine (**19**), $C_{30}H_{33}NO_8$, oil, $[\alpha]_D$ −148 (c 0.48, MeOH)
Anhydroharringtonine (**20**) $C_{28}H_{35}NO_8$,$[\alpha]_D$ −144 (c 1.08, $CHCI_3$)
Nordeoxyharringtonine (**21**), $C_{27}H_{36}NO_8$, oil, $[\alpha]_D$ −90 (c 0.07, MeOH)
Homodeoxyharringtonine (**22**), $C_{29}H_{39}NO_8$, oil, $[\alpha]_D$ −122 (c 1.00, MeOH)
Bishomodeoxyharringtonine (**23**), $C_{30}H_{41}NO_8$, oil, $[\alpha]_D$ −74 (c 0.27, $CHCI_3$)
Homoneoharringtonine (**24**), $C_{31}H_{36}NO_8$, oil, $[\alpha]_D$ −114 (c 0.07, MeOH)
(2′*R*,3′*S*)-Hydroxyneoharringtonine (**25**), $C_{30}H_{33}NO_9$, oil, $[\alpha]_D$ −126 (c 0.20, MeOH)

Other esters of cephalotaxine

(+)-Acetylcephalotaxine (+)-(**26**), $C_{20}H_{23}NO_5$, mp 140°C, $[\alpha]_D$ +102 ($CHCI_3$), $[\alpha]_D$ +130 (EtOH)
(−)-Acetylcephalotaxine (−)-(**26**), $C_{20}H_{23}NO_5$, mp 141–143°C, $[\alpha]_D$ −186 (c 0.43, EtOH)
5′-Des-*O*-methylisoharringtonine (Isoharringtonic acid) (**27**), $C_{27}H_{35}NO_9$, mp 224–228°C, $[\alpha]_D$ −113(c 0.33, DMSO)
3′*S*-Hydroxy-5′-des-*O*-methylharringtonine (Cephalozemic C acid) (**28**), $C_{27}H_{35}NO_{10}$, amorphous solid, $[\alpha]_D$ −91 (c 1.00, DMSO)
Deoxyharringtonic acid (**29**), $C_{27}H_{35}NO_8$, mp 219–220 °C, $[\alpha]_D$ −91 (c 1.00, DMSO)
5′-Des-*O*-methylharringtonine (harringtonic acid) (**30**), $C_{27}H_{35}NO_9$, amorphous solid,$[\alpha]_D$ −113 (c 0.43, DMSO)
5′-Des-*O*-methylhomoharringtonine (homoharringtonic acid) (**31**), $C_{27}H_{37}NO_9$, amorphous solid, $[\alpha]_D$ −172 (c 0.50, MeOH)
Homoharringtonamide (**32**), $C_{29}H_{37}NO_9$

Esters of oxygenated cephalotaxine analogs

Drupangtonine (**33**), $C_{28}H_{37}NO_9$, oil, $[\alpha]_D$ −24 (c 0.06, MeOH)
11α-Hydroxyhomodeoxyharringtonine (**34**), $C_{29}H_{39}NO_9$, oil, $[\alpha]^{25}{}_D$ −115 (c 0.07, MeOH)
11β-Hydroxyhomodeoxyharringtonine (**35**), $C_{29}H_{39}NO_9$, oil, $[\alpha]^{25}{}_D$ −153 (c 0.10, MeOH)
11-Hydroxydeoxyharringtonine (**36**), $C_{28}H_{37}NO_9$, oil, $[\alpha]^{25}{}_D$−77 (c 0.0085, MeOH)

Dimeric ester

Cephalotaxidine (**37**), $C_{58}H_{74}N_2O_{19}$, Amorphous solid, $[\alpha]_D$ −172 (c 0.10, MeOH)

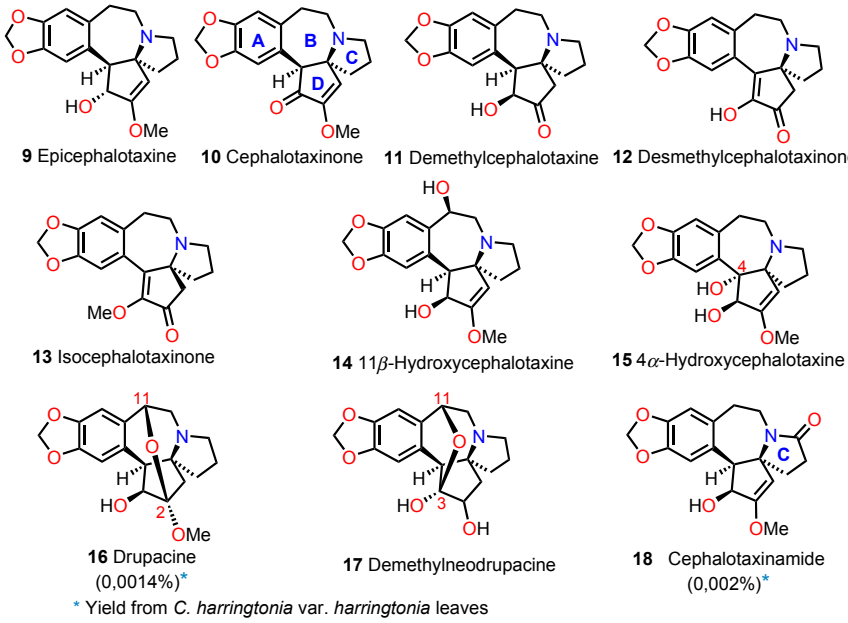


Figure 4 Oxidized cephalotaxines.

(Forbes) K. Koch var. *harringtonia* cv. fastigiata, was determined by semisynthesis from CET (**1**) via oxidation to cephalotaxinone (**10**) and cleavage of the enol ether thus confirming desmethyl CET (**12**) as a natural metabolite and biosynthetic intermediate.[33]

Repeated chromatography of an alkaloidal fraction of the ethanolic extract of *Cephalotaxus fortunei* Hook f.[34] afforded as minor component (+)-4-hydroxycephalotaxine (+)-(**15**) recently also identified in *Cephalotaxus koreana* Nakai.[35] Epicephalotaxine (**9**) is another minor metabolite of *C. fortunei* Hook f. accompanied by CET (**1**) (50%−54% of the total alkaloids), cephalotaxinone (**10**), (+)-acetylCET (+)-(**26**), demethylCET (**11**), HT (**3**), and HHT (**2**).[36]

Molecules coupled with side chains bearing an ester function as in nordeoxyharringtonine (nordeoxyHT) (**21**), homodeoxyharringtonine (homodeoxyHT or deoxyHHT) (**22**), and bishomodeoxyharringtonine (bishomodeoxyHT) (**23**) are found among HTs (Fig. 5).[37]

Including esters **2**−**5**, these compounds are esters of CET (**1**) having its polycyclic core coupled to different side chains deriving from (*R*)-citramalic acid. Although occurrence of enantiomeric pairs in the same family of plants is unusual,[38] acetylcephalotaxine (acetylCET) (**26**) was reported under both

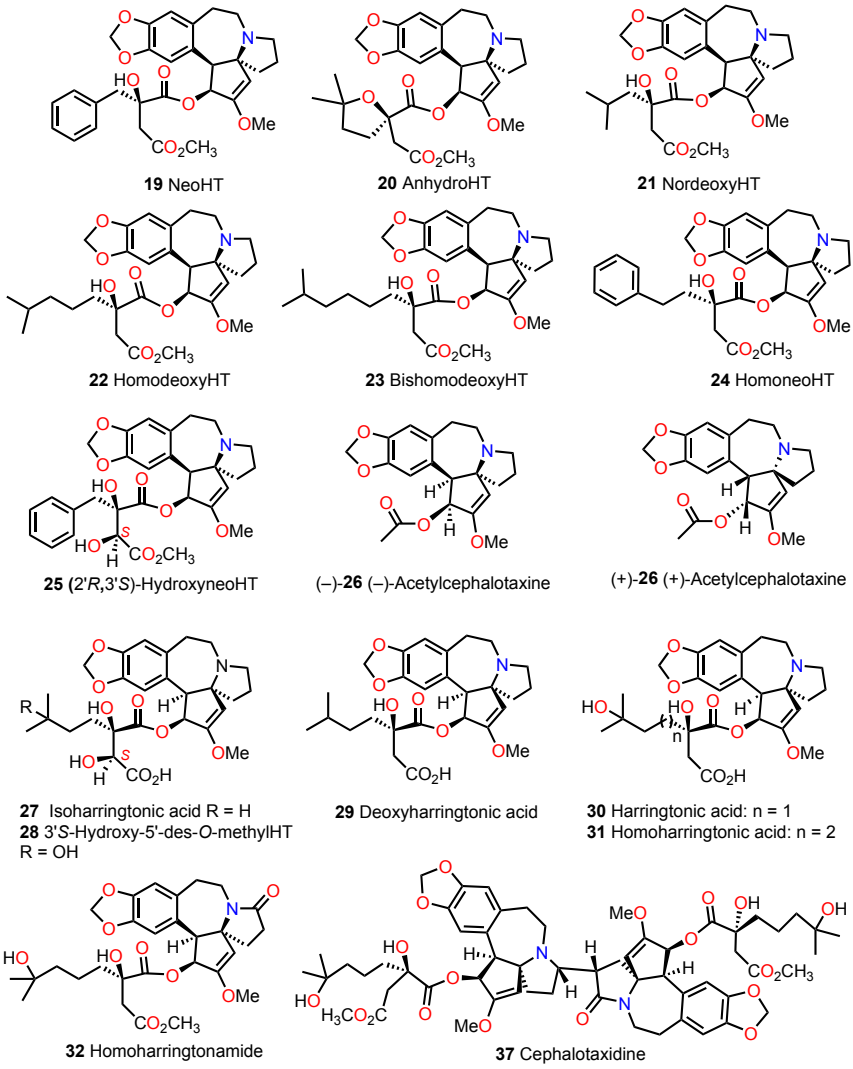


Figure 5 Natural esters of cephalotaxine and cephalotaxinamide.

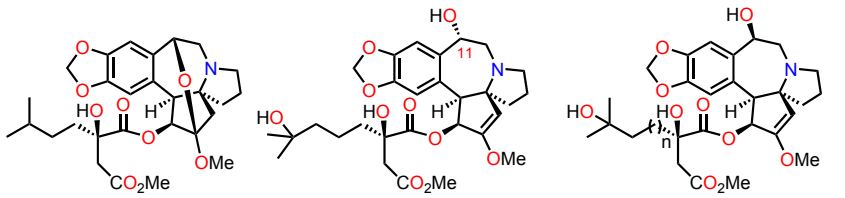
enantiomeric forms. (−)-AcetylCET (−)-(**26**) was obtained by semisynthesis through acetylation of natural CET (−)-(**1**)[24] and was isolated from *C. fortunei*[30] and *C. wilsoniana*.[39,40]

The opposite enantiomer (+)-acetylCET (+)-(**26**) was isolated in *C. fortunei*[41] and *C. hainanensis*.[42] Although an error cannot be excluded in literature data, these findings need to be confirmed since this natural

product (+)-**26** is the only one portraiting an opposite absolute configuration (AC) of alkaloid core.

Anhydroharringtonine (**20**) bearing an additional heterocycle in the side chain is the only natural ester of this type.[43] It induced 98% growth inhibition of P388 leukemia cell lines at 1 μg/mL, a level comparable to that of deoxyHT (**5**). There are also molecules coupled with side chains carrying a free acid group (Fig. 5): 5′-des-*O*-methylHT (**30**) (harringtonic acid), (2′*R*,3′*S*)-3′-hydroxy-5′-des-*O*-methylHT (**28**) (cephalozemic acid C), 5'-des-*O*-methylHHT (**31**) (homoharringtonic acid, also identified as a metabolite of HHT (**2**) in rats and rabbits) and 5′-des-*O*-methylisoHT (**27**) (isoharringtonic acid).[1,2] Others possess an ester function and an aromatic ring, as in homoneoHT (**24**) and (2′*R*,3′*S*)-hydroxyneoHT (**25**).[44] Some of these esters deliver biological activities comparable to those of the first four isolated esters **2**—**5**, but they are available in much lower quantities in the plant. The first dimeric *Cephalotaxus* alkaloid, cephalotaxidine (**37**), was isolated from *C. harringtonia* var. *drupacea* (*C. drupacea*) in 1996.[45] It is a dimer of HHT (**2**) and homoharringtonamide (**32**) with an IC_{50} of 1.8 μg/mL against P-388 murine leukemic cell lines. Drupangtonine (**33**)[46] was the most active (IC_{50} 7 nM against P-388 murine leukemic cell lines) of the four esters of oxygenated CET analogs, 11α-hydroxy-homodeoxyHT (**34**), 11β-hydroxyhomodeoxyHT (**35**), and 11β-hydroxydeoxyHT (**36**) (IC_{50} 0.38, 0.33, and 0.17 μg/mL, respectively, against P-388 murine leukemic cell lines) identified from *C. harringtonia* var. *drupacea* (Fig. 6).[47]

Since the last reviews in this series,[1,2] more than 30 *Cephalotaxus* alkaloids have been isolated from various *Cephalotaxus* sp. These new alkaloids include the following compounds.



33 Drupangtonine

34 11α-Hydroxyhomodeoxyht

35 11β-Hydroxyhomodeoxyht n = 2
36 11β-Hydroxydeoxyht n = 1

Figure 6 Natural esters of oxygenated cephalotaxines.

2.1 Cephalotaxine and Related Alkaloids

N-oxides of CET, CET α *N*-oxide (**38**), CET β *N*-oxide (**39**), and 11β-hydroxycephalotaxine β *N*-oxide (**40**), together with isocephalotaxine (**41**) were identified and characterized in the ethyl acetate extract of *C. fortunei* fruits by Jossang et al. in 2001 (Fig. 7).[48] Although it was described as a synthetic compound earlier,[49] the relative configuration of isocephalotaxine (**41**) isolated during this work remains unknown due to the lack of Nuclear Overhauser effect spectroscopy (NOESY) correlations. These alkaloids exhibited low cytotoxicity against nasopharynx KB cells with IC_{50} values of 30, 14, 31, and 15 μg/mL, respectively. ACs of cephalezomines G (**42**) and H (**43**) described by Kobayashi and Morita[50] from the leaves of *Cephalotaxus harringtonia* var. *harringtonia* were determined by circular dichroism (CD) as 2*S*,3*R* for cephalezomine G (**42**) and 2*R*,3*R* for cephalezomine H (**43**). However, the structure of cephalezomine H (**43**), initially assigned as a *trans* diol was revised to a *syn*-β,β-diol after total synthesis (Fig. 7).[51] Some uncertainty remained, however, due to the divergent optical rotations of the synthetic and the natural compounds.

A new stereoisomer (**44**) of desmethylcephalotaxinone (**12**) has been isolated from the leaves and heartwood of Formosan *C. wilsoniana* (Fig. 8).[52] Although the UV, 1H NMR, and $[\alpha]^{23}_{D}$ of compound **44** were similar to those of natural desmethylcephalotaxinone (**12**), C1 to C7 and C10 signals in the ^{13}C NMR spectrum differ from the one of the

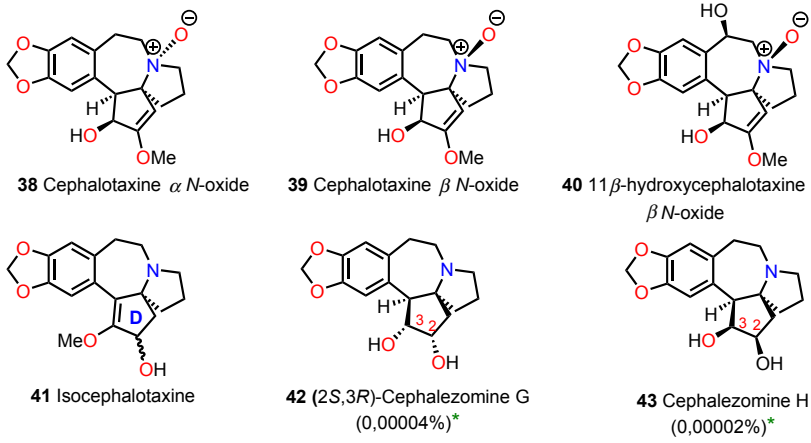
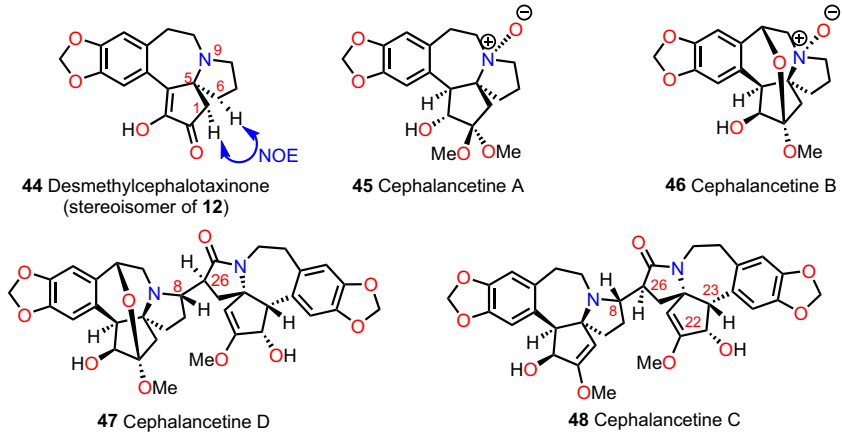


Figure 7 New *N*-oxides and ring D analogs of cephalotaxine.



44 Desmethylcephalotaxinone (stereoisomer of **12**) **45** Cephalancetine A **46** Cephalancetine B

47 Cephalancetine D **48** Cephalancetine C

Figure 8 Desmethylcephalotaxinone stereoisomer and cephalancetines A–D.

synthetic sample of **12**.[100] Evidence that compound **44** was a stereoisomer of desmethylcephalotaxinone (**12**) was also based on H1α and H6α cross-peak in the NOESY correlation chart of **44**. While this correlation seems doubtful, since only one stereogenic carbon center is present in structures **12** and **44**, these findings imply that the second stereogenic center is the nitrogen atom that lost its fast inversion properties, as observed in the X-ray studies showing the rigid structure of CET (**1**).[53] Compound **44** showed no cytotoxic activity against the Hep G2, MCF-7, Hep 3B, and HT-29 human cancer cell lines.

A phytochemical investigation of the branches and leaves of *Cephalotaxus lanceolata*, a *Cephalotaxus* species native to Yunnan Province, China, resulted in the isolation of three new *Cephalotaxus* alkaloids, cephalancetines A (**45**) and B (**46**), and unsymmetrical dimer cephalancetine D (**47**) together with known cephalancetine C (**48**) isolated for the first time from this plant, along with nine other known alkaloids (Fig. 8).[54] The structure of cephalancetine C (**48**) was unambiguously confirmed by single-crystal X-ray diffraction as consisting of CET (**1**) linked at C8 to C7 (C26) of cephalotaxinamide (**18**) thus establishing its AC as (3*S*,4*S*,5*R*,8*S*,22*S*,23*S*,24*R*,26*S*). Cephalancetine D (**47**) is a dimer consisting of drupacine (**16**) linked at C8 to C7 (C26) of cephalotaxinamide (**18**). Cephalancetine C (**48**) was identical to the saponification product of bis-cephalezomines (see Fig. 12) with LiOH.[55]

All these isolated alkaloids were tested for their cytotoxicities against four human tumor cell lines, A549, HCT116, SK-BR-3, and HepG2 that evidenced that HT (**3**) and HHT (**2**) can strongly inhibit the proliferation

of these human tumor cell lines as well, however with limited potency against SK-BR-3 cells (Table 3).[54]

HT (**3**) showed remarkable cytotoxicity against A549, HCT116, and HepG2 cell lines with IC_{50} values of 0.15, 0.054, and 0.38 μg/mL, respectively. HHT (**2**) also exhibited potent cytotoxicity against A549, HCT116, and HepG2 cell lines with IC_{50} values of 0.085, 0.001 and 0.087 μg/mL, respectively, 85 times stronger than that of doxorubicin (DOX) against HCT116 cell line. Comparing the relationship between the structures with their cytotoxicities, the ester side chains at C3 of the CET skeleton seem to play the most prominent role in the cytotoxic activity of these alkaloids.

Four new *cephalotaxus* alkaloids, cephalotines A–D (**49**)–(**52**) were isolated from the leaves and twigs of *C. lanceolata* and *Cephalotaxus fortunei* var. *alpina* (Yunnan province, P.R. China) along with 24 known alkaloids (Fig. 9). None of these compounds showed significant activity against HeLa, SGC-7901 gastric cancer, and A-549 lung cancer cell lines ($IC_{50} > 20$ μM).[56]

2.2 Natural *Cephalotaxus* Esters

In 2000, Kobayashi et al. isolated from the leaves of *Cephalotaxus harringtonia* var. *nana* new cytotoxic alkaloids, cephalezomines A (**53**) and B (**54**), which are esters of drupacine (**16**), as well as cephalezomines C–F (**55**)–(**58**), which are esters of CET (**1**) (Fig. 10).[57] These new compounds were found together with seven known alkaloid esters, HT (**3**), isoHT (**4**), deoxyHT (**5**), homodeoxyHT (**22**), CET (**1**), and related CET alkaloids, demethylCET (**11**),[30] 11β-hydroxyCET (**14**), and drupacine (**16**). Their structure and stereochemistry were elucidated by spectroscopic data, CD, and X-ray diffraction analysis.

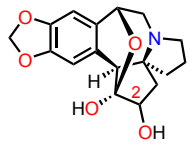
Among the new *Cephalotaxus* alkaloids possessing a CET type backbone with various side chains at C3, cephalezomine D (**56**) is the first one with (2′*R*,3′*R*) AC. In this study[57] the team of Kobayashi also described the cytotoxicity of these alkaloids against L1210 and KB cell lines (Table 4). Two years later, Kobayashi and Morita reported the isolation of five new alkaloids, cephalezomines G, H, and J–L from the leaves of *C. harringtonia* var. *nana* (Sapporo, Japan). Among them, cephalezomines K (**59**) and L (**60**) are esters of CET (**1**) epimers at C4" (Fig. 10).[58]

In 2010, Morita et al. characterized from the leaves of *Cephalotaxus harringtonia* forma *fastigiata* a new alkaloid, cephastigiamide A (**61**),[59] $[\alpha]^{23}_{D}$ −55 (c 0.1, MeOH), along with CET (**1**), cephalotaxinamide (**18**),

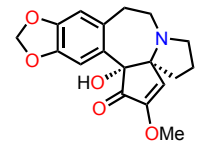
Table 3 Cytotoxic activities {means ± S.D}[54]

Compounds	IC_{50} (μg/mL)			
	A549	HCT116	SK-BR-3	HepG2
Cephalancetine A (**45**)	>100	>100	>100	>100
Cephalancetine B (**46**)	>100	93.06 ± 13.15	>100	>100
Cephalancetine C (**48**)	>100	>100	74.15 ± 8.65	>100
Cephalancetine D (**47**)	>100	>100	>100	>100
CET(**1**)	85.67 ± 16.75	22.65 ± 13.86	>100	71.94 ± 12.30
HHT (**2**)	0.085 ± 0.018	0.001 ± 0.002	>100	0.087 ± 0.024
HT (**3**)	0.15 ± 0.07	0.054 ± 0.016	>100	0.38 ± 0.09
Cephalotaxinone (**10**)	>100	93.06 ± 5.02	>100	>100
4-HydroxyCET (**15**)	>100	>100	>100	>100
Drupacine (**16**)	28.29 ± 7.50	2.61 ± 0.78	>100	3.95 ± 0.85
Cephalotaxinamide (**18**)	>100	45.47 ± 4.57	>100	>100
Cephalotaxine α-*N*-oxide (**38**)	>100	>100	>100	>100
Cephalotaxine β-*N*-oxide (**39**)	>100	51.41 ± 7.31	61.31 ± 15.37	>100
Adriamycin (doxorubicin)	0.079 ± 0.007	0.085 ± 0.008	0.039 ± 0.007	0.014 ± 0.004

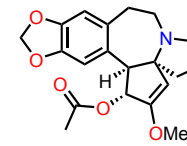
CET, cephalotaxine; *HT*, harringtonine; *HHT*, homoharringtonine.



49 Cephalotine A 2α-OH
50 Cephalotine B 2β-OH

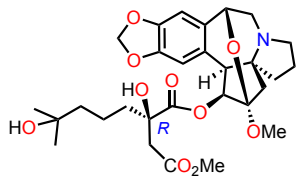


51 Cephalotine C

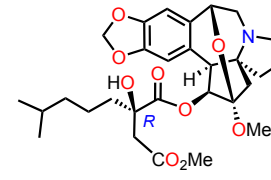


52 Cephalotine D

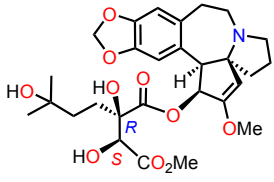
Figure 9 Cephalotines A–D.



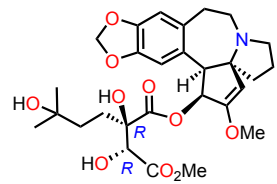
53 Cephalezomine A (0.0001%)*



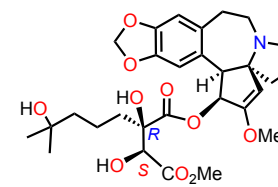
54 Cephalezomine B (0.00005%)*



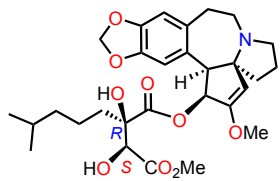
55 Cephalezomine C (0.0003%)*



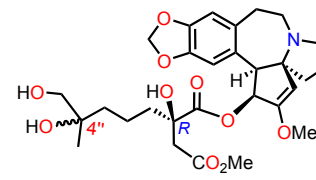
56 Cephalezomine D (0.002%)*



57 Cephalezomine E (0.0001%)*



58 Cephalezomine F (0.002%)*



59 Cephalezomine K (0.00003%)*
60 Cephalezomine L (0.00004%)*

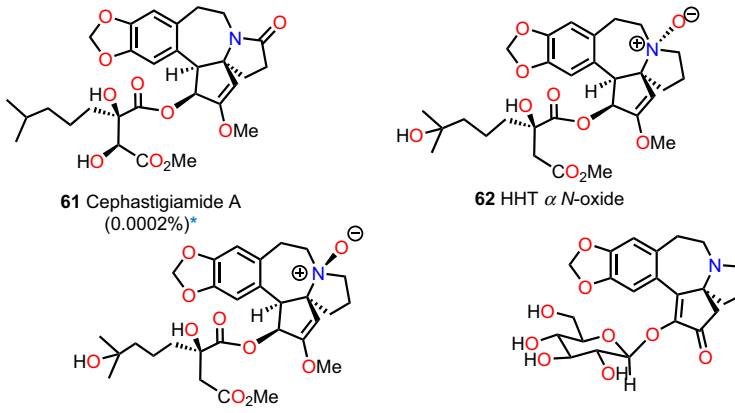
*: Yields from *Cephalotaxus harringtonia* var. *nana*, *C. hainanensis* or *C. fortunei*

Figure 10 Cephalozemines A–F and K–L and their yield from the natural sources in brackets.

bis-cephalezomine A (**65**), homodeoxyHT (**22**), and two HHT *N*-oxides, HHT α *N*-oxide (**62**) and HHT β *N*-oxide (**63**), previously prepared by Takano[60,61] for SAR studies (Fig. 11). HHT (**2**) and HT (**3**) showed pronounced antiplasmodial activity against *Plasmodium falciparum* 3D7 but to a lesser extent against *Leishmania major* (Table 5), while deoxyHT (**5**) and homodeoxyHT (**22**) were active in the two tests.

Table 4 Cytotoxicity of cephalotaxines (CETs), harringtonines (HTs), and cephalezomines against L1210 and KB cell lines[57,58]

	IC_{50} (μg/mL)	
Compounds	**L1210**	**KB**
Cephalotaxine (**1**)	3	0.90
11-hydroxyCET (**14**)	2.4	0.75
DemethylCET (**11**)	3.8	0.87
Drupacine (**16**)	0.84	0.99
HT (**3**)	2	0.74
IsoHT (**4**)	0.14	0.22
DeoxyHT (**5**)	0.0082	0.0079
HomodeoxyHT (**22**)	0.014	0.010
Cephalezomine A (**53**)	0.067	0.020
Cephalezomine B (**54**)	0.030	0.024
Cephalezomine C (**55**)	0.88	0.078
Cephalezomine D (**56**)	7.6	0.40
Cephalezomine E (**57**)	0.68	0.18
Cephalezomine F (**58**)	0.10	0.084
Cephalezomine G (**42**)	8	>30
Cephalezomine H (**43**)	8.6	>30
Cephalezomine J (**52**)	12	5.6
Cephalezomine K (**59**)	1.2	0.036
Cephalezomine L (**60**)	3.6	0.044



*: Yields from *C. harringtonia* forma *fastigiata* or *C. harringtonia* var. *nana*

Figure 11 Oxygenated *Cephalotaxus* esters and Cephalozemine J.

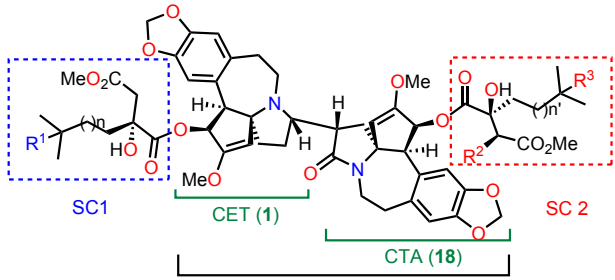
Table 5 Biological evaluation of *Cephalotaxus* alkaloids against *Plasmodium falciparum* 3D7, *Leishmania major*, and human epidermoid carcinoma cells A549[59]

	IC_{50} (μg/mL)			
Compounds	***P. falciparum* 3D7**	***L. major***	**A549**	**SI**
CET (**1**)	17.08	67.90	>31.75	>1.9
Cephalotaxinamide (**18**)	0.67	3.43	17.80	26.6
Cephalezomine B (**54**)	0.040	NT	1.78	44.5
Cephalezomine E (**57**)	0.071	NT	4.79	67.5
Cephalezomine F (**58**)	0.66	NT	4.41	6.7
Cephastigiamide A (**61**)	17.82	117.36	>18.35	>1
Cephalocyclidine A (**72**)	>31.55	NT	>63.00	>2
Bis-cephalezomine A (**65**)	1.99	NT	>18.30	>9.2
HHT (**2**)	0.012	1.36	0.084	7.0
HT (**3**)	0.0043	2.00	0.14	32.6
isoHT (**4**)	0.045	2.00	3.88	86.2
deoxyHT (**5**)	0.0014	0.039	0.021	15.0
HHT α *N*-oxide (**62**)	0.53	28.97	5.20	9.8
HHT β *N*-oxide (**63**)	0.27	27.54	4.30	15.9
HomodeoxyHT (**22**)	0.0018	0.095	0.068	37.8
CD	0.011	–	–	–
AmB	–	0.14	–	–
Taxol	–	–	0.0014	–

AmB, Amphotericin B, Chloroquine, and Amb are positive control for the assays of antiplasmodial and antileishmanial activity respectively; *CD*, chloroquosine diphosphate; *CET*, cephalotaxine; *HT*, harringtonine; *HHT*, homoharringtonine; *NT*, not tested; *Taxol*, positive control for cytotoxicity assay, SI: selectivity index (a measure of the IC_{50} values obtained against human A549 cell divided by the IC_{50} against *P. falciparum* 3D7).

HHT α *N*-oxide (**62**) and HHT β *N*-oxide (**63**) reported for the first time as natural products, did not display any antileishmanial or cytotoxic activity against A549 cell line, despite of an esterified 3-OH function in their structures (Table 5). SAR directed toward antiplasmodial activity of isolated *Cephalotaxus* alkaloids was also discussed. Cephalezomine J (**64**) from the leaves of *C. harringtonia* var. *nana* (Fig. 11) is the unique CET conjugate with a sugar type side chain (D-glucose).[50] Although the data collected in this study are cytotoxicity values (Table 5), a selectivity index was calculated to rate this effect on human versus parasitic cell lines. In all the cases, the cytotoxicity was greater against parasites, rather on tumor cells (SI > 1).

In 2009, Kobayashi et al. isolated five new dimeric alkaloids from *C. harringtonia* var. *nana*, especially heterodimers, named bis-cephalezomines A–E (**65**)–(**69**) (Fig. 12).[55] The alkaloids that constitute the dimers (HHT (**2**), HT (**3**), deoxyHT (**5**), and cephalezomine A (**53**)) showed potent cytotoxic



SC: Side chain	SC 1- CET- CTA-SC 2
(**37**) Cephalotaxidine: n = n' = 2, R^1 = R^3 = OH, R^2 = H	HHTsc- CET- CTA - HHTsc
(**65**) Bis-cephalezomine A: n = 2, n' = 1, R^1 = R^3 = OH, R^2 = H	HHTsc- CET- CTA - HTsc
(**66**) Bis-cephalezomine B: n = 1, n' = 2, R^1 = R^3 = OH, R^2 = H	HTsc- CET- CTA- HHTsc
(**67**) Bis-cephalezomine C: n = n' = 1, R^1 = R^3 = OH, R^2 = H	HTsc- CET- CTA- HTsc
(**68**) Bis-cephalezomine D: n = 2, n' = 1, R^1 = R^2 = OH, R^3 = H	HHTsc- CET- CTA- isoHTsc
(**69**) Bis-cephalezomine E: n = 1, n' = 2, R^1 = R^2 = R^3 = H	deoxyHTsc- CET- CTA- HTsc

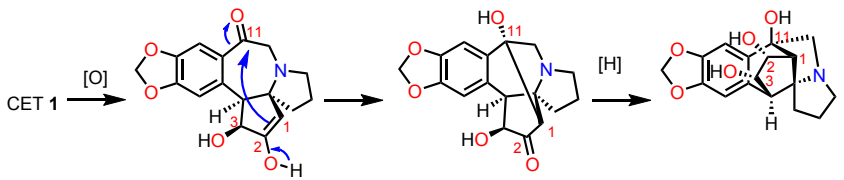
HTsc: HT side chain; HHTsc: HHT side chain; isoHTsc isoHT side chain; deoxyHTsc deoxyHT side chain.

Figure 12 Dimeric *Cephalotaxus* esters.

activity, whereas dimers were far less active against L1210 murine lymphoma cells, with IC_{50} values in the range of 1.9−3.7 μg/mL.

2.3 Other Alkaloids

The new alkaloid cephalocyclidin A (**72**) with an unprecedented polycyclic rearranged skeleton derived from CET (**1**) and six contiguous asymmetric centers was isolated from fruits of *C. harringtonia* var. *nana*.[62] ACs at C2 and C3 were assigned by the exciton chirality method as 2*R* and 3*S*, respectively, indicating 1*R*,2*R*,3*S*,4*S*,5*R*,11*S* configuration, compatible with the absolute stereochemistry of the hydrochloride of CET (**1**) deduced through the Flack parameter of 0.01(5), in the X-ray analysis. It could be derived biogenetically by formation of the C1−C11 link of **71** by an intramolecular aldol reaction of the putative intermediate 11-oxodemethylcephalotaxine (**70**) followed by reduction (Scheme 1). The in vitro cytotoxicity (IC_{50})



Scheme 1 Proposed biosynthetic pathway to cephalocyclidine A.

of cephalocyclidin A (**72**) against the L1210 mouse lymphoma and human epidermoid carcinoma cells KB is 0.85 and 0.80 μg/mL respectively.

2.4 Production and Purification Studies

The proportion of the four major natural esters **2**–**5** with antitumor activity is between 0.7% in the needles of *C. fortunei* and 36% in the roots of *C. harringtonia* var. *harringtonia* of the total amount of alkaloids. This plant is the best natural source for these four compounds of pharmacological interest. At present, the plants of the genus *Cephalotaxus* are endangered species and face the threat of extinction. Only the renewable parts of the plant are used for the extraction of alkaloids. For example, the use of modern methods of extraction provides up to 2 kg of pure CET (**1**) from a ton of dry leaves, that is 0.2% of dry matter. Robin et al. studied a method of preparation of HHT (**2**) by semisynthesis from natural CET (**1**) by attachment of the side chain (see Section 3). Purification provided a product, the purity of which was 99.8% against 98.5% for NCI products and 95%–97% for products of Chinese origin (Table 7).[63] For example, 550 g of HHT (**2**) was injected into a preparative high-performance liquid chromatography (HPLC) system (diameter 45 cm, height 1 m containing 48 kg of ODS stationary phase). The elution was performed using a gradient of buffered solution of pH 3 methanol-water as mobile phase (flow rate 540 L/h, 1200 psi). The collected fractions (20–50 L) were separated by UV detection and analyzed by HPLC. After eliminating fractions with about 0.5% of the total content of HHT (**2**), fractions suitable for drug specification (purity > 99.8%) were combined. HPLC analysis of the organic phase after HHT recovery (basified to pH 8.5 and extracted with dichloromethane) showed a level of impurities less than 0.5%. HHT (**2**) (210 g) was then recrystallized by dissolution in 240 mL of methanol at 30°C and filtered at 0.25 microns for sterilization, and then deionized water (2.4 L) was added and methanol was distilled off. The aqueous solution of HHT (**2**) DS (quality "drug substance") or "drug substance" quality (DS) was held in a decontaminated rotary evaporator until the appearance of white crystals of pure HHT (**2**). After filtration and drying under vacuum at 60°C for 2 days, 88% overall yield (OY) of HHT (**2**) was obtained when compared to its potential content in the semisynthetic gross HHT (**2**). This corresponds to a clinical batch allowing for the preparation of 40,000 therapeutic dosage units containing 5 mg containing less than 0.03% of impurities.[63]

Numerous methods were described for the extraction and purification of CET (1), its natural esters, and related analogs. Extraction of fruits, leaves, or

branches of *C. sinensis* with 90% ethanol under refluxing conditions followed by acid base treatment and purification on alumina allowed for the isolation of CET (**1**) and HHT (**2**).[64]

High speed countercurrent chromatography, a form of liquid–liquid partition chromatography in which the stationary liquid phase is retained in the apparatus without the use of a solid support, was performed with a pH gradient elution to shorten the duration of the separation and improve resolution, allowing a very efficient separation of crude alkaloid extract (800 mg) from *C. fortunei* to yield drupacine (**16**) (9.3 mg), wilsonine (15.9 mg), CET (**1**) (130.4 mg), *epi*-wilsonine (64.8 mg), fortuneine (12.8 mg), and acetylCET (**26**) (35.6 mg) with respective purities of 81.2%, 85.7%, 95.3%, 97.5%, 89.1%, and 96.2%.[65] The recovery of each alkaloid was greater than 90%. The extraction step plays an important role in the overall purification process of HHT (**2**). Traditional methods of isolation and purification of the chemical constituents of plant tissues have some disadvantages. These approaches require very long extraction times and usually a large amount of solvent with sometimes low efficiency. Furthermore, many natural products are thermally unstable and may degrade during hot extraction. To overcome these drawbacks, a new method of extracting HHT (**2**) from *Cephalotaxus koreana* using microwave extraction (MWE) was developed by Kim et al.[66] The MWE process of HHT (**2**) recovery (biomass/MeOH 1/6 m/v, 250 rpm) gave a performance of the first biomass extraction 25% higher than the conventional solvent extraction (CSE) method for which at least four extractions were required. It was possible to recover more than 99% of HHT (**2**) extracting once the biomass by MWE at 40°C for 15 min or 50°C for 5 min. Because the content of tars and waxy compounds were also increased, an adsorbent was added to the biomass (1/1.5 m/m) during the MWE extraction. The use of the MWE process for the extraction of HHT (**2**) when compared to a CSE one has many advantages such as shorter extraction time, reduced amount of solvent, and higher yield resulting in a less costly production system for a superior product. Ultrasonic-MWE of *C. fortunei* produced CET (**1**) in a simple, highly efficient, and low cost process, suitable for its industrial production in high purity after two recrystallizations from anhydrous ethanol.[67]

Using a prepurification method with adsorption prior chromatography, HHT (**2**) with over 52% purity can be obtained simply from *C. koreana* biomass in good yield, minimizing the use of solvents, scale, and complexity of operations for the final HPLC purification of HHT (**2**) (Table 6).[68]

Table 6 The prepurification summary (Steps 2–4) of homoharringtonine (HHT) biomass (16 g)[64]

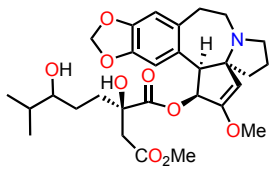
Steps	HHT (2) (g)	Purity (%)	Yield/step (%)	Global yield (%)
Biomass	0.055	–	100	100.0
1. MeOH extraction	0.055	0.5	99	99.0
2. Liquid–liquid extraction	0.049	8.0	90	89.1
3. Adsorption	0.044	10.0	90	80.2
4. Low pressure chromatography	0.038	52.0	85	68.2

This process allows increasing the purity of the raw HHT (**2**) from 0.5% to over 52% with three simple steps and inexpensive pretreatment of the MeOH extract.

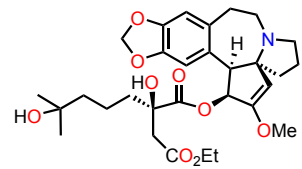
A recent study by Robin related a method for isolation and purification of CET (**1**) from the crude alkaloid extract from 10 kg dry leaves (not from the bark) of *C. fortunei* var *alpina* (Sichuan, China).[69] CET (**1**) purification was performed for the first time by reverse phase HPLC. The crude base (24.5 g) containing 71% of CET (**1**) was dissolved in a mobile phase consisting of *ortho*-phosphoric acid and deionized water containing 1.55% triethylamine (pH 3). The acid additive constrained the alkalization of the obtained aqueous phase and its extraction with dichloromethane. CET (**1**) (18 g) was fully recovered from the crude alkaloid extract as a white solid with an HPLC purity greater than 95%. The limited supply of promising secondary metabolites from their natural sources is a major hurdle to their pharmaceutical development; however, strategies to overcome this problem were developed. For instance, to increase the yield of natural anticancer drug alkaloids from natural sources, methods of extraction are developed. Recovering CET (**1**) from *C. harringtonia*, var. *fastigiata* with vegetable oil in the presence of an aqueous carbonate aqueous phase yielded 5% CET (**1**) with a purity of 26%.[70] The extraction performed with a mixture of $scCO_2$ (supercritical CO_2) with MeOH-10% Et_2NH yielded 0.0029% CET (**1**) of undescribed purity.[71] HHT (**2**) was also obtained from *C. koreana*.[72] He et al. have developed an HPLC analysis method for HHT (**2**) determination,[73] allowing to reveal three congeners present as impurities in the HHT (**2**) samples. Two impurities are new: α-isoHHT (**73**) is an isomer of HHT (**2**) in which the hydroxyl group is alpha to the isopropyl group and ethyl-HHT (**74**), the ethyl ester of HHT (**2**), is an isolation artifact of HHT (**2**) with ethyl acetate, the third being HT (**3**)

Table 7 Impurities of commercial batches of homoharringtonine (HHT) (2) detected by He's high-performance liquid chromatography method[73]

	Impurities %				
Batch number	**HT(3)**	**α-isoHHT (73)**	**Ethyl-HHT (74)**	**Total impurities**	**HHT (2) purity %**
NCI 800528	0.1	1.3	0	1.4	98.6
NCI 971203	0	1.0	0	1.0	99.0
NCI 921115	0.3	3.0	0.8	4.1	95.9
NCI 960625	0	1.8	0.3	2.1	97.9
NCI 800722	0.1	0.9	0.2	1.2	98.8
NCI KS-22-130-2	0	1.5	0.9	2.4	97.6
NCI batch average	0.1	1.6	0.4	2.0	98.0
Oncopharm batch average	0.00	0.00	0.00	<0.05	>99.95



73 α-isoHHT



74 Ethyl-HHT

HT, harringtonine; *NCI*, National Cancer Institute.

(Table 7). α-IsoHHT (**73**) was found concentrated in the crystallization mother liquors of HHT (**2**) (10.7% against 1%–3% in crude extracts). The HPLC analysis according to He's protocol therefore allows the determination of HHT (**2**) in crude samples with a linear response from 4 to 12 μg and a limit of quantitation (LOQ) of 27 μg. It was used to assess the purity of several HHT (**2**) commercial batches.[63]

A tandem HPLC-electron-spray mass spectrometry (MS–MS) method was developed for the analysis of alkaloids contained in the leaves of *C. harringtonia*. Nine alkaloids bearing an ester group were detected with a good sensitivity.[74] Content in HT (**3**) was higher than in HHT (**2**) in the needles of *Cephalotaxus griffithii* alkaloid fraction (CGAF) (122.14 and 16.79 mg/g of CGAF, respectively) by HPLC analysis.[75]

Another major concern is the enantiomeric purity of CET (**1**), which has a direct effect on the diastereoisomeric purity of its natural esters. A process for purification of HHT (**2**) was early developed in view of the clinical studies,[63] although recently, a new process for the purification of CET (**1**) allowed the resolution of racemic or partially racemized samples through

formation of various CET salts protonated at N9 with organic anions either chiral such as (2*R*)- and (2*S*)-malate, (2*R*,3*R*)- and (2*S*,3*S*)-tartrate, or nonchiral such as benzoate, hydrogenfumarate, hydrogensuccinate, hydrogenitaconate, hydrogenmaleate, hydrogenmalonate, hydrogentartrate, hydrogen *meso*-tartrate, hydrogenglutarate, or dihydrogencitrate.[69] This study also pointed out that the cristalline structure of HHT (**2**) deposited at the Cambridge Crystallographic Data Centre (CCDC) exhibits the reverse AC to the commonly accepted one. Eventually, selection of a *Cephalotaxus* cultivar grown in Europe allowed high recovering of CET (**1**) (up to 113−139 g of highly pure cristalline CET (**1**) from 25 kg of dry leaves).[69] Similarly, ammonium salts of HT (**3**) and HHT (**2**) were characterized and, as expected, protonation occurred at N9,[76] a finding in accordance with biological studies of *N*-oxides by Takano[61] and mechanistic studies by Steitz (see Section 6).

Apart from the extraction of the natural source, plant cell cultures represent alternative, efficient, and sustainable chemical factories for the production of biologically important secondary metabolites, especially when the natural source become endangered. The production is limited by the low yields associated with harvest and the high costs are associated with complex chemical synthesis. *Cephalotaxus* evergreen trees are somewhat difficult to propagate and they are relatively rare. Some of them have been listed in the red list of IUCN (International Union for Conservation of Nature) as national plant of second-grade protection in China and are "near threatened" (*Cephalotaxus latifolia*), "vulnerable" (*Cephalotaxus oliveri*, *Cephalotaxus mannii*) or rare and "endangered" (*C. lanceolata* or *Cephalotaxus fortunei* Hook. var. *lanceolata* (K.M.Feng) Silba, *C. hainanensis*) species.[77] A variety of strategies are being developed to overcome the limitation of low product yields. A renewed interest in the study of *Cephalotaxus* crops[78] and the development of methods for analyzing the composition of different plants have been stimulated by the need for raw materials and the search for natural sources rich in CET (**1**). An HPLC protocol was used to determine the content of CET (**1**), HT (**3**), and HHT (**2**) in crop roots and calluses of *C. harringtonia*. As expected, CET (**1**) is the major alkaloid (10 mg/kg dry matter) while HT (**3**) and HHT (**2**) are more present in the roots (6.6 and 7.5 mg/kg, respectively).[79]

In plant cell and tissue culture, cell growth and product formation are not necessarily correlated. Cell culture studies have sought to identify factors that tend to yield low yields and slow metabolic rates. Zhang et al. proposed the

technique of periodic oscillation in temperature between 10 and 25°C every 12 h for 45 days to overcome this key problem.[80] The total production of HT (**3**), HHT (**2**), and isoHT (**4**) (1.22 mg/L) in solid cultures (SoC) of *C. fortunei* was enhanced by 1.8- and 1.3-fold compared to the controls at constant temperatures of 10 or 25°C. When subjected to such an oscillation every 24 h for 30 days, suspension cultures (SuC) gave a total alkaloid production of 0.18 mg/L, a 2.0- and 1.05-fold improvement compared to the SuC controls at 10 or 25°C, respectively. HT (**3**) represents the main alkaloid (72%−92% for SoC in 45 days, and 50%−67% for SuC for 30 days) while the HHT (**2**) content is only 4%−21% in SoC and 23%−40% in SuC, the remaining being isoHT (**4**).

In 2014, Li showed that the combination of 10 mg/L sodium fluoride (NaF) as glycometabolic regulator and 100 μmol/L methyl jasmonate (MJ) as the elicitor both promoted the biosynthesis of CET (**1**) in *C. mannii* suspension cells.[81] NaF was more effective to increase cell membrane permeability and product secretion compared to MJ. The yield of HT (**3**) in the cells was 4.8 greater under NaF + MJ conditions than in the control, 1.7-fold greater under NaF and 1.6-fold under MJ conditions, respectively (Table 8). No HHT (**2**) was found in NaF + MJ−treated cells. The product release rates were 0%, 78%, 24%, and 62% in control, NaF, MJ, and NaF + MJ treatment, respectively. The combined NaF and MJ treatment on cells provided an efficient strategy for producing CET (**1**).

Another study of the effects and action mechanisms of NaF on the growth and CET (**1**) production of *C. mannii* suspension cells indicated that NaF acted as an inhibitor of the Embden−Meyerhof pathway (EMP), not as an elicitor to promote CET (**1**) production.[82]

Table 8 Harringtonines (HTs) production in *Cephalotaxus mannii* culture[81]

	HT (3) content (mg/mL)				HHT (2)
Treatment	**Intracellular**	**Extracellular**	**Total**	**Release rate % (mg/mL)**	
Control	1.506 ± 0.059	0	1.506 ± 0.059	0	−
NaF	0.917 ± 0.076	3.203 ± 0.197	4.12 ± 0.27	78 ± 0.47	−
MJ	3.37 ± 0.201	1.088 ± 0.074	4.458 ± 0.275	24 ± 0.17	−
NaF + MJ	2.735 ± 0.177	4.51 ± 0.215	7.245 ± 0.392	62 ± 0.42	0.491 ± 0.032

HHT, homoharringtonine.

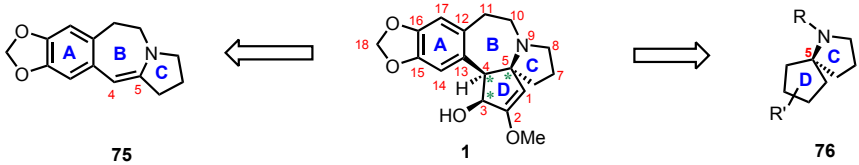
3. SYNTHESIS OF THE ALKALOID CORE

The *Cephalotaxus* alkaloids have drawn the attention of many chemists due to their unique structure and potent antileukemic activities of certain ester derivatives they contain. Excellent review articles on this subject have been published in this series by Huang and Xue,[1] Miah et al.[2], and more recently among others, by Nay et al.[6] Since 1997, 41 syntheses of CET (**1**) have been reported, 18 of which being enantioselective.

3.1 Overview

CET (**1**) can be described as the superposition of two structural units relevant for synthetic strategies, namely the pyrrolobenzazepine core ABC **75** and the synthetically challenging azaspiro unit CD **76** (Scheme 2). The three stereogenic centers (C3, C4, and C5) are contiguous on the D ring. Weinreb showed that the stereochemistry of the B/D ring fusion (C4/C5) is thermodynamically favored. In addition, carbonyl reduction of cephalotaxinone (**10**) delivers CET (**1**) with the natural configuration at C3.[83]

33 (23 since 1997 are described in this review) syntheses of CET (**1**) concerned its racemic form (Table 9). The number of asymmetric syntheses has grown considerably from only 3 before 1997 to 18 until 2015, inflecting the development of asymmetric synthesis, and the efforts to provide a fully synthetic access to the drug (Table 10). The present section describes the recent synthetic strategies organized according to the main carbon–carbon bond disconnections of the central azepine ring B (Fig. 13). Asymmetric syntheses are also classified based on the type of asymmetric center control. The key steps are highlighted for each strategy. Most of these syntheses are formal; nevertheless, the OY of each synthesis up to CET (**1**) was evaluated. Eight racemic and five asymmetric syntheses of CET (**1**) are total syntheses, with only one racemic and three asymmetric routes described since mid-1997, with experimental OY and enantiomeric excess (ee). The OY and



Scheme 2 Main strategies to access cephalotaxine (**1**).

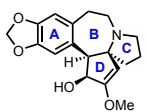
Table 9 Overview of the racemic syntheses of *rac*-CET (±)-(1) arranged according to the main strategic disconnections of ring B. Formal syntheses targeting Dolby–Weinreb's enamine are grouped

Author[a] (year)	Ring B cut	NS	OY%	TNS	AY%	Starting material (ring A)	References
Weinreb (1972)	**a**	11	2.3	–	–	Piperonal (**82**)	2,103
Dolby (1972)[b]	**d**	12	0.7	–	–	Piperonylic acid	106
Weinstein (1976)[b]	**a**	13	1.9	–	–	Benzodioxole (**330**)	2,134
Snieckus (1976)[b]	**a**	14	1.6	–	–	Piperonal (**82**)	2
Danishefsky (1990)[b]	**b**	18	0.2	–	–	Piperonal (**82**)	2
Li (2005)[b]	**a**	17	0.4	–	–	Piperonal (**82**)	105
Semmelhack (1972)	**a**	12	12.7	18	2.0	Piperonal (**82**)	2,83
Hanaoka (1986)	**a**	12	6.5	–	–	Homoveratric acid (**143**)	98
Hanaoka (1988)	**a**	15	3.0	–	–	3,4-Methylenedioxyacetophenone	96
Kuehne (1988)[c]	**a**	12	17.1	14	11.6	Piperonal (**82**)	94
Tietze (1997)	**a**	12	5.2	15	2.5	Piperonal (**82**)	84
Suga and Yoshida (2002)	**a**	14	2.2	17	1.5	Homopiperonylic acid (**89**)	86,87
Nagasaka (2002)	**a**	20	3.1	22	1.8	Piperonal (**82**)	95
Stoltz (2007)	**a**	13	3.2	17	2.3	Homopiperonylic acid (**89**)	90
Li (2007)	**a**	13	4.0	–	–	Homoveratric acid (**143**)	97
Yang and Liu (2009)	**a**	15	7.1	–	–	Homoveratric acid (**143**)	99
Bubnov(2008)	**a**	16	3.8	–	–	Homoveratric acid (**143**)	104
Zhang and Liu (2013)	**a**	17	2.2	–	–	Homoveratryl amine (**158**)	102
Zhang and Liu (2013)	**a**	16	3.7	–	–	Homopiperonylic acid (**89**)	102
Huang (2013)	**a**	14	5.4	–	–	Homopiperonylic acid (**89**)	92
Huang and Wang (2015)	**a**	12	6.2	15	4.3	Homopiperonylic acid (**89**)	93

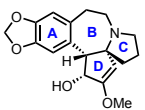
Hong (2015)	**b**	12	23.5	–	–	Homopiperonylic acid (**89**)	115
Hong (2015)	**b**	12	26.2	–	–	Homopiperonylic acid (**89**)	115
Fuchs (1988)	**c**	21	6.2	24	4.8	Piperonyl alcohol	2,147
Mariano (1994)	**c**	18	5.3	–	–	Homopiperonylic acid (**89**)	113
Tu and Zhang (2009)	**c**	23	1.9	24	1.9	Homopiperonylic acid (**89**)	108
Li (2011)	**c**	13	0.4	–	–	Piperonal (**82**)	111
Jiang (2013)	**c**	15	4.3	–	–	Homopiperonylic acid (**89**)	112
Li (2003)	**c**	18	1.8	–	–	Piperonal (**82**)	100
Li (2005)	**c**	21	0.2	–	–	Piperonal (**82**)	105
Li (2005)	**c**	16	0.8	–	–	Piperonal (**82**)	107
Ishibashi and Ikeda (1990)	**d**	19	15.6	–	–	Piperonal (**82**)	138
Chandrasekhar (2016)	**e**	16	6.4	18	4.7	5-Benzodioxolol (**254**)	120

a, disconnection C4-C13; **b**, disconnection C4-C5; **c**, disconnection C5-N9; **d**, disconnection C11-C12; **e**, disconnection N9-C10.
AY, average yield accounting for the TNS; *NS*, number of steps (longest linear sequence); *OY*, overall yield; *TNS*, total number of steps from boths precursors to CET.
[a]Name in bold style, total synthesis; name in plain style, formal synthesis.
[b]Formal synthesis through Weinreb's enamine.
[c]Preparative HPLC of intermediate required.

Table 10 Overview of the asymmetric syntheses of (−)- and (+)-cephalotaxine



(−)-**1**



(+)-**1**

Author[a] (year)	B Cycle cut	NS	OY% (ee%)	TNS	AY%	SM[b]	Chiral source	References
Weinreb-Merk (1972)[c]	**a**	15	5.3 (98.5)	—	—	**82**	L-tartaric acid	2
Zhong (1994)[c]	**a**	16	(nr)	—	—	**82**	L-tartaric acid	2
Mori (1995)[c]	**a**	18	1,6 (86)	19	1,6	**143**	D-proline (*R*)-(**140**)	85
Nagasaka (1997)[c,d,e]	**a**	16	5.8 (99)	18	4.5	**89**	(2*R*,3*R*)-butanediol (**404**)	133
Ikeda (1999)[c]	**d**	19	5.1 (88)	—	—	**330**	D-proline (*R*)-(**140**)	137
Tietze (1999)[c]	**a**	13	3.1 (87)	16	1.7	**82**	Oxazaborolidine **267**	121
El Bialy (2002)[c]	**d**	20	<7.5% (89)	—	—	**82**	L-malic acid (**409**)	140
El Bialy (2002)[c]	**a**	14	12.2 (89)	20	4.3	**330**	D-proline (*R*)-(**140**)	140
Royer (2004)[c]	**a**	16	7.5 (98)	18	5.8	**89**	(*S*)-1-Naphtylethylamine (**298**)	132
Dumas, d'Angelo (2005)[c]	**a**	20	5.1 (>95)	22	3.9	**317**	(*R*)-1-Phenylethylamine (**309**)	135
Stoltz (2007)[c,d]	**a**	14	0.2 (97.5)	19	0.03	**82**	(−)-Ephedrine (**412**)	90

Mariano 1 (2006)[c]	**a**	19	0.2 (51)	–	–	**143**	AchE (electric eel)	127
Mariano 2 (2006)[c]	**a**	19	0.5 (95)	23	0.3	**89**	AchE (electric eel)	127
Hayes 1 (2008)[c]	**a**	23	0.2 (87)	–	–	**89**	L-proline (*S*)-(**140**)	141
Djaballah, Gin (2008)[c]	**a**	25	1.0 (nr)	28	0.7	**330**	D-ribose (**365**)	144
Ishibashi (2008)[c]	**a**	17	1.3 (99.6)	22	0.7	**82**	Diethyl D-tartrate (**384**)	145
Hayes 2 (2008)[c]	**a**	20	0.5 (91)	22	0.45	**89**	L-proline (*S*)-(**140**)	142
Tu (2012)[c]	**a**	14	1.7 (99) or 3.3 (80)	18	1.3	**82**	Silver phosphate (*R*)-**273**	122
Renaud (2012)[c]	**a**	26	0.9 (97)	29	0.6	**89**	Noyori catalyst (*S*,*S*)-**281**	124
Renaud(2012)[c]	**a**	18	1.8 (96.5)	20	1.4	**89**	PPL	124
Trost (2012)[c]	**d**	19	13.9 (91)	21	8.4	**82**	Phosphoramidite **296**	129

(**143**), homoveratric acid; (**317**), safrole; (**330**), benzodioxole; (**82**), piperonal; (**89**), homopiperonytic acid.
AchE, Acetyl choline esterase; *nr*, not reported; *PPL*, Porcine Pancreatic Lipase.
[a]Name in bold, total synthesis; name in plain style, formal synthesis.
[b]For ring A.
[c]Synthesis of (−)-1.
[d]Synthesis of (+)-1.
[e]Including one step of separative HPLC.

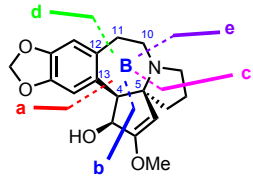


Figure 13 Main disconnections used for the construction of cephalotaxine ring B.

the number of steps (NS) are given from commercially available reagents for comparison purpose. In addition, for convergent syntheses, total number of steps (TNS), which represents the total efforts to be accomplished up to the target molecule, and the corresponding average yield (AY) are given. Synopses of the syntheses of CET (**1**) reported so far are presented in Table 9 (racemic) and Table 10 (asymmetric). Besides formal syntheses targeting Weinreb's enamine grouped together in Table 9, racemic syntheses are classified according to the type of disconnection of ring B (**a**—**e**, Fig. 13), the type of intermediate in the formal syntheses and then in a chronological order. The asymmetric syntheses are presented in chronological order in Table 10.

It is apparent from Table 9 that most of the syntheses of *rac*-CET (±)-(**1**) are linear (only 10 being convergent) and proceed predominantly (19 of 33) by the C4—C13 disconnection **a**. The shortest access to CET (**1**) was described in the first synthesis of CET (**1**) by Weinreb et al.[103] in 11 steps including the preparation of homopiperonylic acid (**89**) in two steps from piperonal (**82**).[134] The most effective total syntheses were described by Kuehne et al.[94] in only 11 steps and 11.2% OY (average 82% yield per step) or 12 steps and 17.1% OY, by Ishibashi and Ikeda et al.[138] in 19 steps and 15.6% OY (average >90% yield per step), and by Chandrasekhar et al.[120] (6.4% OY in 16 steps, 4.7% AY in 18 TNS). The recent formal synthesis by Hong et al. in 12 steps and 23.5% OY is the most efficient route.[115] The favorite choices as ring A precursors are piperonal (**82**) and homopiperonylic acid (**89**) with C11 and the methylenedioxy group already at the right position.

Table 10 shows that proline (**140**) is a widely used chiral source (5 over 21 syntheses), either in its natural L form, or in its unnatural D one. Herein, 15 syntheses are convergent and 6 are linear. The most efficient total syntheses of CET (−)-(**1**) (OY > 5%) were reported by Weinreb et al.[103] (5.3% OY in 15 steps, 98.5% ee) and Royer et al.[132] (7.5 OY in 16 steps, 98% ee; 5.8% AY in 18 TNS). Formal syntheses by Nagasaka et al.[133] (5.8%

OY and 4.5% AY in 16–18 steps, 99% ee), Ikeda et al.[137] (5.1% OY and AY in 19 steps, 88% ee), Dumas and d'Angelo[135] (5.4% OY in 20 steps, >95% ee; 4.1% AY in 22 TNS), El Bialy et al.[140] (12.2% OY in 14 steps, 89% ee; 4.3% AY in 20 TNS) and Trost et al.[129] (13.9% OY in 19 steps, 91% ee; 8.4% AY in 21 TNS) were also efficient.

3.2 Racemic Syntheses

3.2.1 C4–C13 Ring Closure a

The ring closure C4–C13 **a** (Fig. 14) to form the azepine cycle was frequently adopted after Semmelhack's early extensive developments in the second synthesis of *rac*-CET (±)-(**1**).[83]

3.2.1.1 Tietze's Formal Synthesis (1997)

In 1997, Tietze et al.[84] reported an efficient synthesis of Mori's intermediate (±)-**88**[85] in which rings B and C were constructed by two consecutive intramolecular palladium-catalyzed reactions (Scheme 3). The precursor of the D ring and C6–C7–C8 fragment was prepared from 1,3-cyclopentanedione (**77**) via its enol ether **78**. Addition of the Grignard reagent (±)-**81** and elimination of ethanol followed by tosylation, reduction, and acetylation gave the allylic acetate (±)-**81**. Alkylation of the bromoamine **84** prepared in four steps from piperonal **82** with the intermediate iodide derivative generated in situ from tosylate (±)-**81** provided the secondary amine (±)-**85** bearing the AD ring units, which contain all carbon atoms and functionalities required for synthesis completion.

A palladium-catalyzed Tsuji–Trost allylation furnished the spiro compound (±)-**86** containing the A and CD ring units. The B ring was formed by a highly stereoselective intramolecular Heck reaction using Herrmann–Beller catalyst **87**. The selective formation of Mori's tetracyclic intermediate (±)-**88** can be explained by an oxidative addition of the palladium complex to the double bond *syn* to the nitrogen atom followed by a *syn* elimination of PdH. Mori's procedure[85] would allow to complete the synthesis of *rac*-CET (±)-(**1**) in 12 steps (longest linear sequence (LLS))

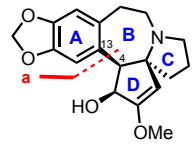
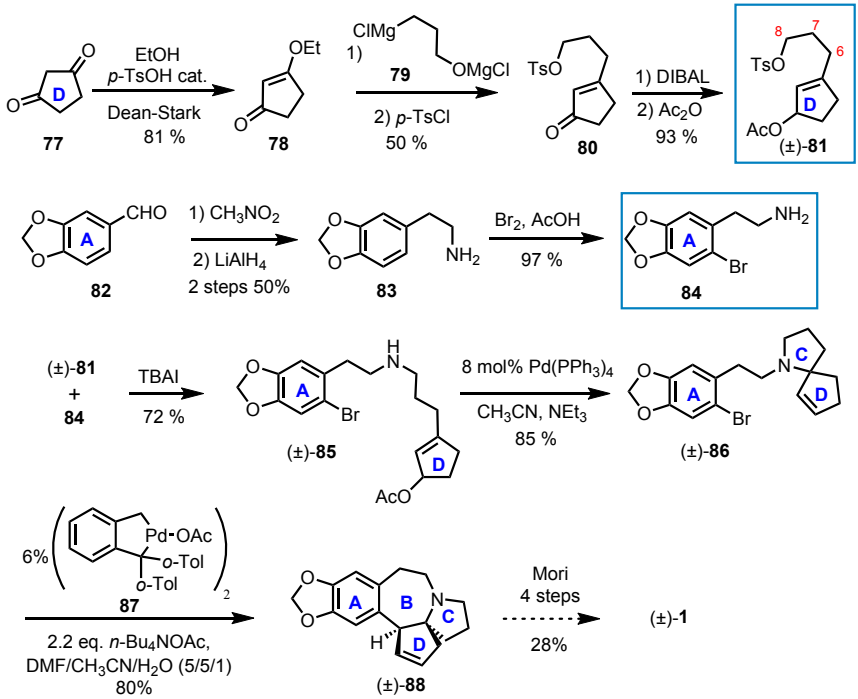


Figure 14 C4–C13 bond disconnection **a** for elaboration of cephalotaxine (**1**) B ring.



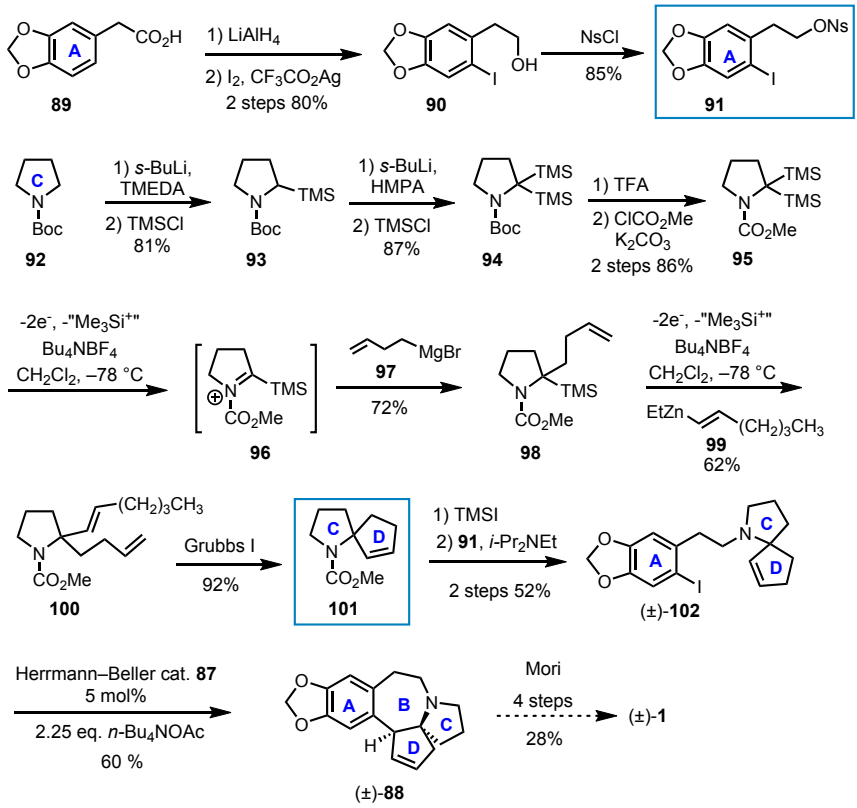
Scheme 3 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) by Tietze (1997).

and 5.2% OY from 1,3-cyclopentanedione (**77**) (2.5% AY in 15 TNS). It should be mentioned that Mori's protocols differ for the synthesis of racemic or optically active CET (**1**). Indeed, the transformation of desmethyl CET (**12**) into cephalotaxinone (**10**) using 2,2-dimethoxypropane and *p*-TsOH led to racemization of the product (76% yield). On the contrary, when *p*-TsOH and methyl orthoformate were used for this transformation at room temperature (rt), limited racemization occurred (ee 88%), but the yield went down to 47%.

3.2.1.2 Suga and Yoshida's Formal Synthesis (2002, 2006)

Suga and Yoshida developed an approach to the azaspiro-compound **101** via ring-closing metathesis (RCM) of a functionalized pyrrolidine **100** generated by electrochemical oxidation of α,α-bis(trimethylsilyl)pyrrolidine **95** (Scheme 4).[86,87]

First, homopiperonylic acid (**89**) was used to prepare the A unit **91** bearing a nosylate and an aromatic iodide suitable for ring B annulation, relying on Semmelhack's procedure.[83,88] The two silyl groups were



Scheme 4 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) by Suga and Yoshida (2002).

introduced by Beak's methodology[89] (deprotonation with *sec*-BuLi and quenching with TMSCl) furnishing compound **94**, which was protected into *N*-methoxycarbonyl compound **95**. Its anodic oxidation at low temperature yielded *N*-acyliminium ion **96**, reacting in situ with homoallylmagnesium bromide (**97**) furnishing pyrolidine **98**. This latter compound was next submitted to a second anodic oxidation followed by direct trapping with the organozinc reagent **99** to provide the diolefin **100**. Diene **100** was converted to azaspiro CD unit **101** via ring closing metathesis promoted by Grubbs I catalyst. After deprotection using TMSI, the spiranic amine intermediate reacted with nosylate **91** obtained from homopiperonylic acid (**89**) following Semmelhack's procedure.[88]

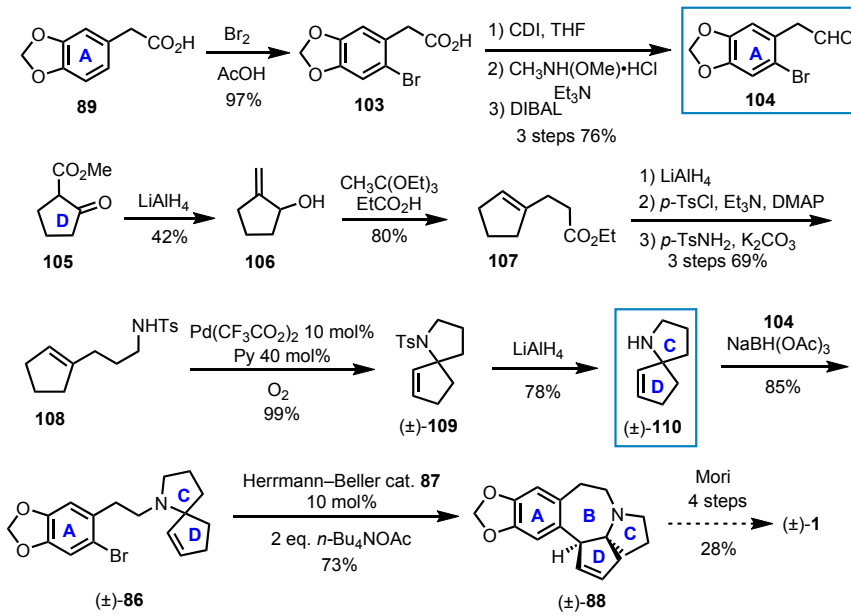
The so-formed aryl iodide (±)-**102** was submitted to an intramolecular Heck-type ring closing reaction according to Tietze[84] to give Mori's intermediate (±)-**88**. Applying Mori's procedure to complete the synthesis

from intermediate (±)-**88** would provide an access to *rac*-CET (±)-(**1**) as a linear sequence of 14 steps and 2.2% OY from pyrrolidine **92**.

3.2.1.3 Stoltz's Formal Synthesis (2007)

Stoltz et al. proposed a concise formal route to *rac*-CET (±)-(**1**) in which the key spirocyclic amine (±)-**110** was built through palladium(II)-catalyzed aerobic oxidation (Scheme 5).[90] This oxidative cyclization and condensation with ring A-containing aldehyde **104** by reductive amination were the basis to elaborate both enantiomers of CET (**1**) and (−)-drupacine (**16**) (see Section 3.3.4.3, Scheme 40).

Regioselective bromination of homopiperonylic acid (**89**) yielded bromocarboxylic acid **103** converted to Weinreb's amide,[2,83] and then reduced by DIBAL to furnish aldehyde **104**. Different routes were proposed to prepare the ACD ring—containing aryl bromide **86**. The best one starts from β-ketoester **105** transformed by reduction to allylic alcohol **106**,[91] which was treated with triethyl orthoacetate under Johnson orthoester—Claisen conditions to give ethyl ester **107**. Afterward, ester **107** was converted in three steps into sulfonamide **108** by successive reduction by $LiAlH_4$, tosylation, and nucleophilic displacement by tosylamine. The



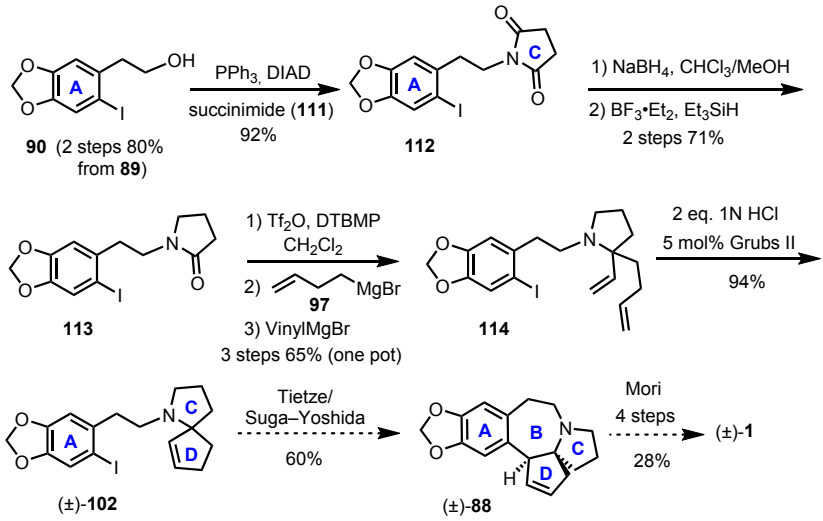
Scheme 5 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) by Stoltz (2007).

oxidative palladium(II)-catalyzed heterocyclization of **108** provided efficiently the spirocyclic tosylamide **109** (scale up to 1.1 g) whose reductive cleavage of the tosyl group with $LiAlH_4$ furnished the desired spirocyclic amine **110**. This compound and the aldehyde **104** were coupled by reductive amination. The resulting tertiary amine (±)-**86** was converted to the target intermediate (±)-**88** using stereoselective Heck conditions described by Tietze.[84] Including the four steps of Mori's procedure, this formal synthesis of *rac*-CET (±)-(**1**) would consist in 17 steps and 3.1% OY from **89** (AY 2.3% in 17 TNS).

3.2.1.4 Huang's Formal Synthesis (2013)

An efficient synthesis of 1-azaspiro[4.4]nonenes in four steps from γ-butyrolactams (e.g., **113** → **102**, Scheme 6) was developed by Huang et al. in 2013[92] and applied to the synthesis of *rac*-CET (**1**), involving Tietze's Heck ring closure[84] of aromatic iodide (±)-**102**[86] (Scheme 6).

The γ-butyrolactam **113** was prepared from 2-arylethanol **90** in 65% OY by Mitsunobu's substitution of the primary alcohol, followed by partial reduction of the crude succinimide **112** with $NaBH_4$ and reductive dehydroxylation of the resulting hemiaminal. Huang's methodology, namely activation of compound **113** by triflic anhydride (2,6-di-tert-butyl-4-methylpyridine (DTBMP)) was the best base for this reaction, followed by successive addition of buten-1-yl Grignard (**97**) and vinyl

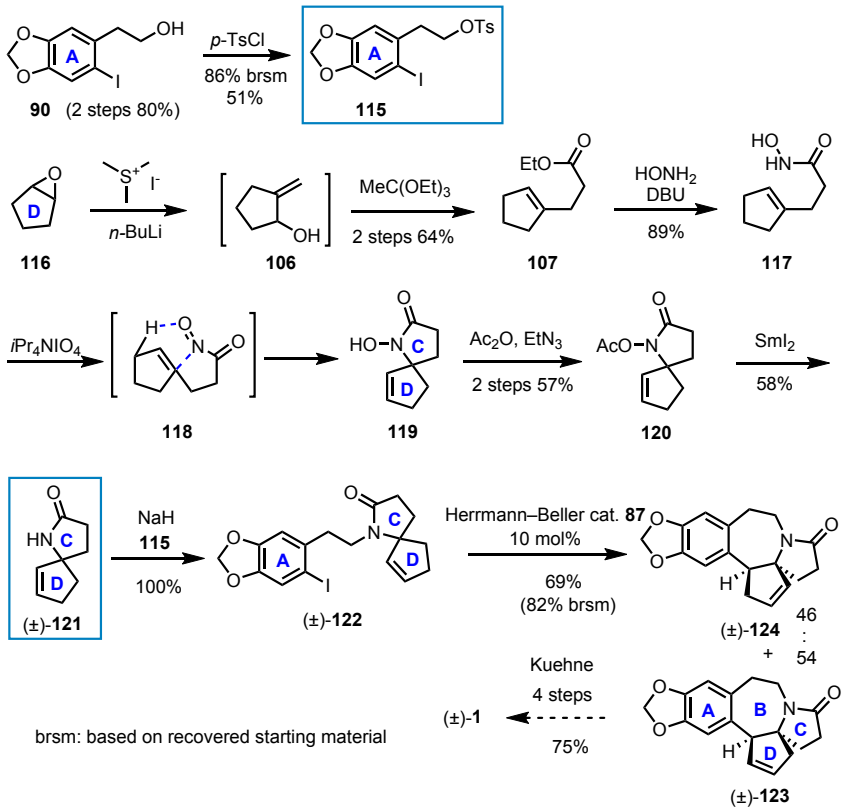


Scheme 6 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) by Huang (2013).

Grignard reagents provided the desired pyrrolidine **114**. The hydrochloride salt of the dienyl pyrrolidine **114** was next transformed into the known spiro-pyrrolidine (±)-**102** under RCM conditions (Grubbs's generation II catalyst). The formal synthesis by Huang et al. was achieved with the synthesis of Mori's intermediate (±)-**88** using the palladium-catalyzed Heck-type cyclization of aryl iodide (±)-**102** developed by Tietze[84] and employed by Suga and Yoshida.[86] Once again, four steps (Mori's protocol)[85] have to be performed to obtain *rac*-CET (±)-(**1**), to give a 14-steps pathway in 5.4% estimated OY from homopiperonylic acid (**89**).

3.2.1.5 Huang and Wang's Formal Synthesis (2015)

In 2015, Huang and Wang proposed a rapid construction of 1-azaspiro[4.4] nonenone (±)-**121** via a nitroso-ene cyclization (Scheme 7).[93]

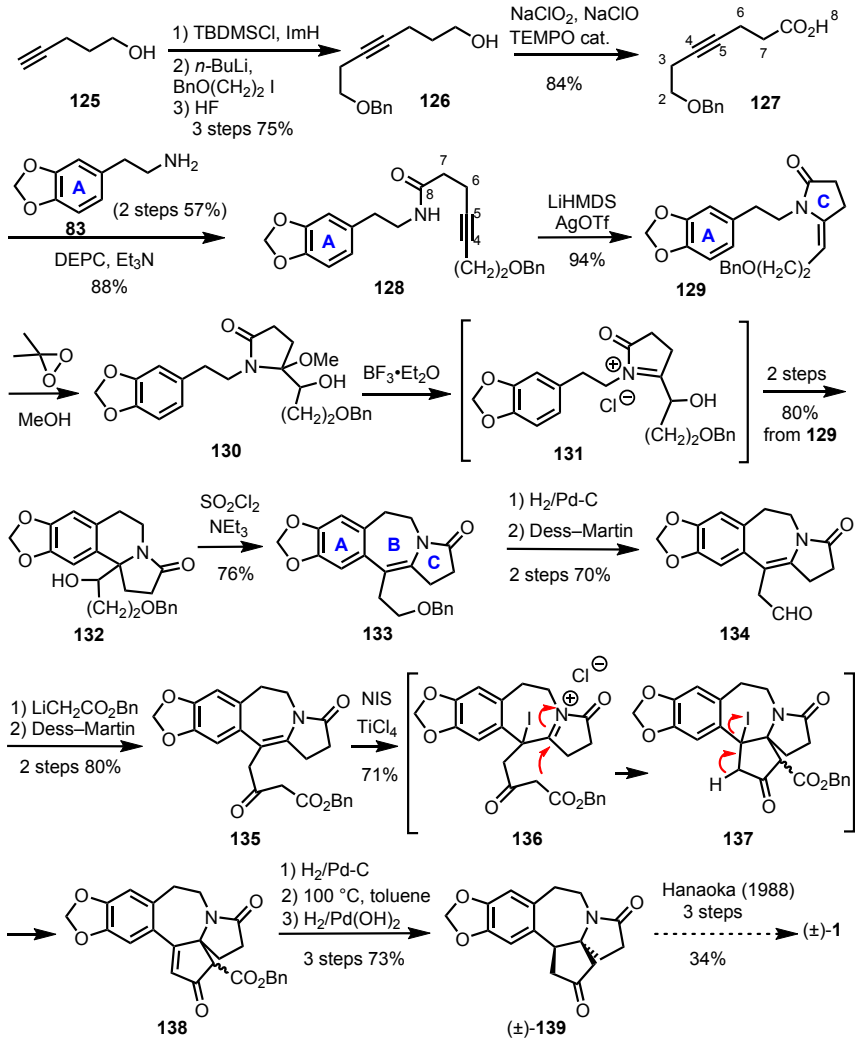


Scheme 7 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) by Huang and Wang (2015).

Reaction of 1,2-epoxycyclopentane **116** with in situ generated dimethylsulfonium methylide gave allylic alcohol **106**, which was directly used for the subsequent Johnson−Claisen rearrangement. The resulting ester **107** was subjected to amidation reaction conditions with hydroxylamine to afford hydroxamic acid **117**. The nitroso-ene cyclization was accomplished on oxidation with tetrapropylammonium periodate via a highly reactive acylnitroso intermediate **118** delivering *N*-hydroxy-1-azaspiro [4.4]non-2-one **119**, which was trapped as an acetate **120** yielding in moderate yield spiro-lactam (±)-**121**[90] on SmI_2 reduction. Alkylation of the sodium salt of (±)-**121** with iodoaryl tosylate **115** gave quantitatively (±)-**122**. The subsequent Heck reaction promoted by Herrmann−Beller catalyst **87** proceeded smoothly to deliver the ABCD Kuehne's intermediate[94] (±)-**123**, and the corresponding regioisomeric product (±)-**124** in 82% combined yield, based on recovered starting material (brsm) as an inseparable 54:46 mixture. *rac*-CET (±)-(**1**) could be obtained in 12 steps from cyclopentene oxide (**116**) and 6.2% OY provided that separation of the regioisomers is effective and four supplementary steps applied to transform the ABCD intermediate (±)-**123** (TNS 15 steps, AY 2.5%, 4.3% brsm). Although Huang and Wang described a rapid access to the CD spirocyclic amide (±)-**121**, Heck ring closure of ring B led to a mixture of two regioisomers and decreasing dramatically the yield.

3.2.1.6 Nagasaka's Formal Synthesis (2002)

In 2002, Nagasaka et al. reported a formal synthesis of CET (**1**),[95] through a new access to Hanaoka's precursor[96] (±)-**139** (Scheme 8), building successively rings C and B. Ring B was installed by ring expansion of pyrroloisoquinoline **132**, which resulted from the cyclization of N-acyliminium ion intermediate **131**. Alkyne **127** that contains C5, C6, C7, and C8 carbon atoms of ring C and C2, C3, and C4 carbon atoms of the CET skeleton was prepared in four steps (63% OY) from 4-pentyn-1-ol **125**. Coupling of **127** with homopiperonylamine **83** (which could be obtained from piperonal **80** in two steps and 64% OY), gave amide **128** whose treatment with LiHMDS and catalytic amounts of silver triflate gave the AC enamide **129** by intramolecular N-heterocyclization. Oxidation of **129** with dimethyldioxirane delivered the unstable methoxylactam **130** immediately treated with $BF_3 \cdot OEt_2$ producing pyrroloisoquinoline **132** via the N-acyliminium ion **131**. Ring-expansion reaction of the six-membered ring elaborated the seven-membered ring B (**132** → **133**). Aldehyde **134** was isolated after debenzylation of **133** followed by Dess−Martin



Scheme 8 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) by Nagasaka (2002).

oxidation. The missing C1 carbon atom in aldehyde **134** was introduced by aldol reaction with the lithium enolate of benzyl acetate. Subsequent oxidation of the resulting aldol gave β-keto-ester **135**. Formation of ring D was accomplished via Mannich cyclization of N-acyliminium ion **136** and elimination of hydroiodic acid to produce the enone **138**. The final compound of this formal synthesis, spiroketone (±)-**139** reported by Hanaoka[96] was isolated after decarbobenzyloxylation followed by

catalytic reduction. Application of Hanaoka's procedure (3 steps, 34% OY) to complete the synthesis from compound (±)-**139** would provide an access to *rac*-CET (±)-(**1**) in 20 steps and 3.1% OY from 4-pentyn-1-ol **125**.

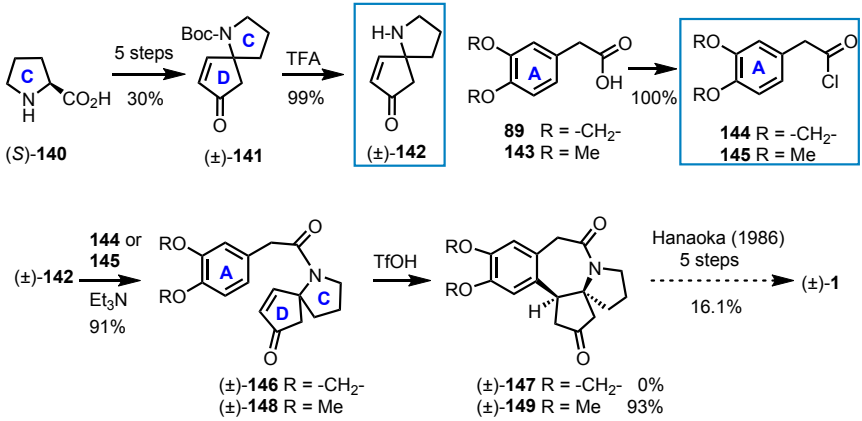
3.2.1.7 Li's Formal Synthesis (2007)

In 2007, Li developed a formal synthesis of *rac*-CET (±)-(**1**) based on a facile Friedel–Crafts cyclization of the amido-spiro-cyclopentenone precursor **148** mediated by triflic acid (Scheme 9).[97]

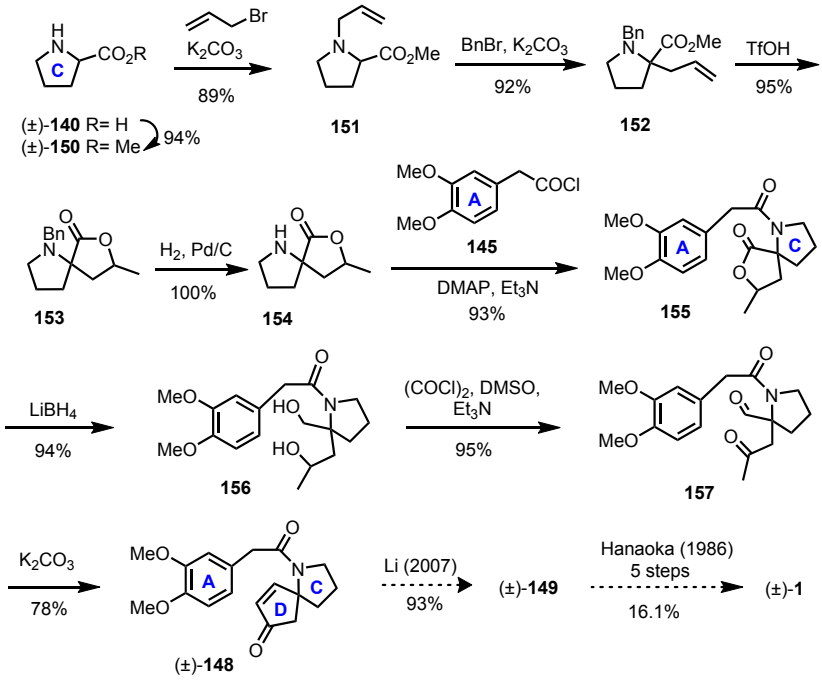
Reaction of acid chlorides **144** and **145** prepared from homopiperonylic acid **86** and homoveratric acid **143**, with proline-derived spirocyclic amino enone (±)-**142**, led to the amido spirocyclopentenones (±)-**146** and (±)-**148**, respectively. Friedel–Craft cyclization of the methylenedioxy precursor (±)-**146** did not led to compound (±)-**147** under various acidic conditions due to an electron-deficient aryl system. On the contrary, an efficient Friedel–Craft cyclization of the dimethoxy derivative (±)-**148** was observed on treatment with triflic acid yielding Hanaoka's intermediate[98] (±)-**149** isolated in 93% yield. In summary, Li described here a concise formal synthesis of *rac*-CET (±)-(**1**) in 13 steps from L-proline (*S*)-**140** and 4% evaluated OY.

3.2.1.8 Yang and Liu's Formal Synthesis (2009)

Yang and Liu reported two strategies to access to intermediate (±)-**148** involving a [2,3]-Stevens rearrangement and an acid lactonization sequence



Scheme 9 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) by Li (2007).



Scheme 10 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) by Yang and Liu (2009).

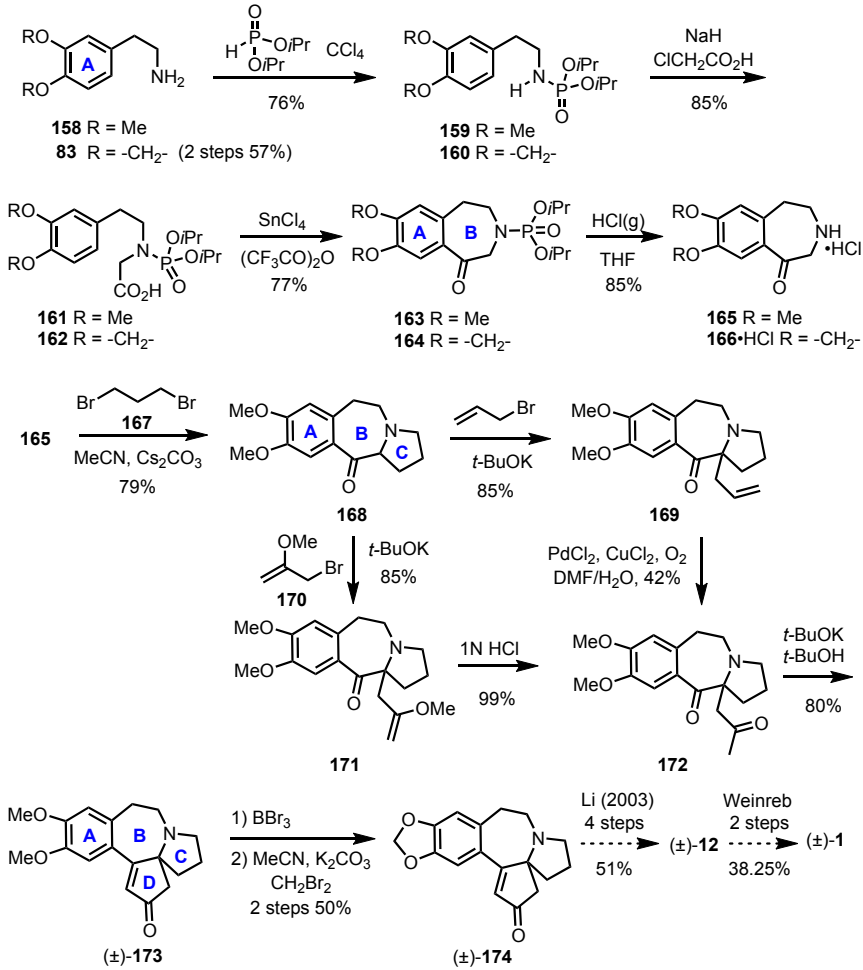
as key transformations.[99] We describe here the best route that led to Li's intermediate[97] (±)-**148** via the spirolactone **154** (Scheme 10).

Spirolactone **154** was obtained from methyl prolinate (±)-**150** by N-allylation with allyl bromide, leading to allylamine **151**, which underwent a [2,3]-Stevens rearrangement of the resulting quaternary ammonium salt by reaction with benzyl bromide providing *N*-benzyl pyrrolidine **152**. After lactonization of **152** under acidic conditions followed by debenzylation through catalytic hydrogenation, spirolactone **154** was acylated with 3,4-dimethoxyphenacetyl chloride (**145**) to produce the amide spirolactone **155** containing the AC ring units, which was selectively reduced with $LiBH_4$ to diol **156** in 63.9% OY. Swern oxidation of diol **156** furnished the amido keto-aldehyde **157**, which was treated with potassium carbonate to give the amido spirocyclopentenone (±)-**148** with the ACD ring units. This formal synthesis is especially suitable for the production of (±)-**148** at a scale up to 10−50 g. Li[97] and Hanaoka's[98]procedures (6 steps, 15% OY) would allow to complete the synthesis of *rac*-CET (±)-(**1**) in 15 steps and 7.1% OY from *rac*-proline (±)-(**140**).

3.2.1.9 Zhang and Liu's Formal Synthesis (2013)

In 2013, Zhang et al. proposed two pathways for the formal synthesis of *rac*-CET (±)-(**1**) targeting Li's enone (±)-**174**[100] and Hanaoka's enone (±)-**180**[96] based on conventional alkylations and aldol condensations from known benzazepines[101] **165** and **166** obtained in four steps from aryl amine **83** (bearing a methylenedioxy substituent) or **158** (bearing two methoxy substituents), respectively (Scheme 11).[102]

The C ring was elaborated via an N- and C-alkylating annulation by treatment of benzazepine **165** with 1,3-dibromopropane (**167**) and

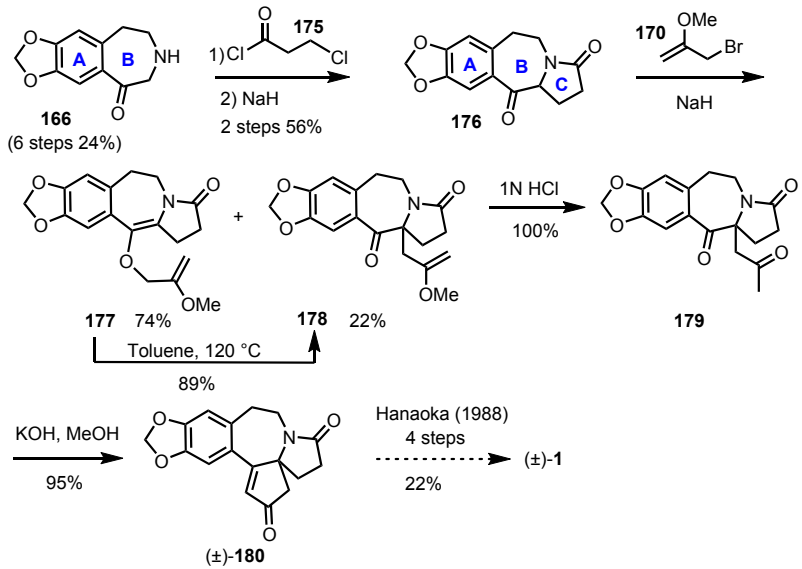


Scheme 11 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) by Zhang and Liu, the "dimethoxy" route (2013).

Cs_2CO_3 giving product **168** in one step. ABC dione **172** was prepared by alkylation either with allyl bromide followed by Wacker oxidation or more efficiently with 2-methoxyallyl bromide (**170**) followed by acidic hydrolysis (Scheme 11). Aldol condensation efficiently constructed the CET (**1**) core in (±)-**173**. The dimethoxy groups were transformed into the required methylenedioxy group by the usual protocol[85] to afford ABCD enone (±)-**174** reported by Li in 2003.[100] Application of Li and Weinreb's[103] procedures would end up a synthesis of *rac*-CET (±)-(**1**) in 17 steps from homoveratryl amine (**158**) and 2.2% calculated OY.

In an alternative synthesis, these authors also studied the use of methylenedioxybenzazepine **166** as starting material (Scheme 12).

Ring C was elaborated by acylation/alkylation of benzazepine **166** with 3-chloropropanoyl chloride (**175**) to furnish the ABC ketone **176**. Treatment with sodium hydride and 2-methoxyallyl bromide (**170**) produced the enol ether **177** (74%) as a major O-alkylated product along with the desired C-alkylation derivative **178** (22%). Claisen rearrangement allowed conversion of **177** to **178**, which was exposed to acid hydrolysis to give dione **179**. Intramolecular aldol condensation provided the known amido enone (±)-**180**[96] in 95% yield. Formally, *rac*-CET (±)-(**1**) could be obtained



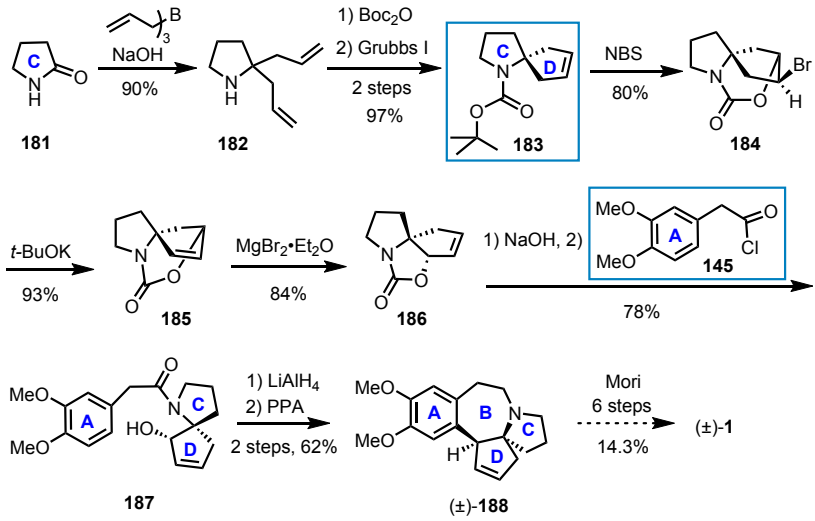
Scheme 12 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) by Zhang and Liu, the "methylenedioxy" route (2013).

following Hanaoka' synthesis[96] in a total of 16 steps from homopiperonylic acid (**89**) and 2.5% estimated OY.

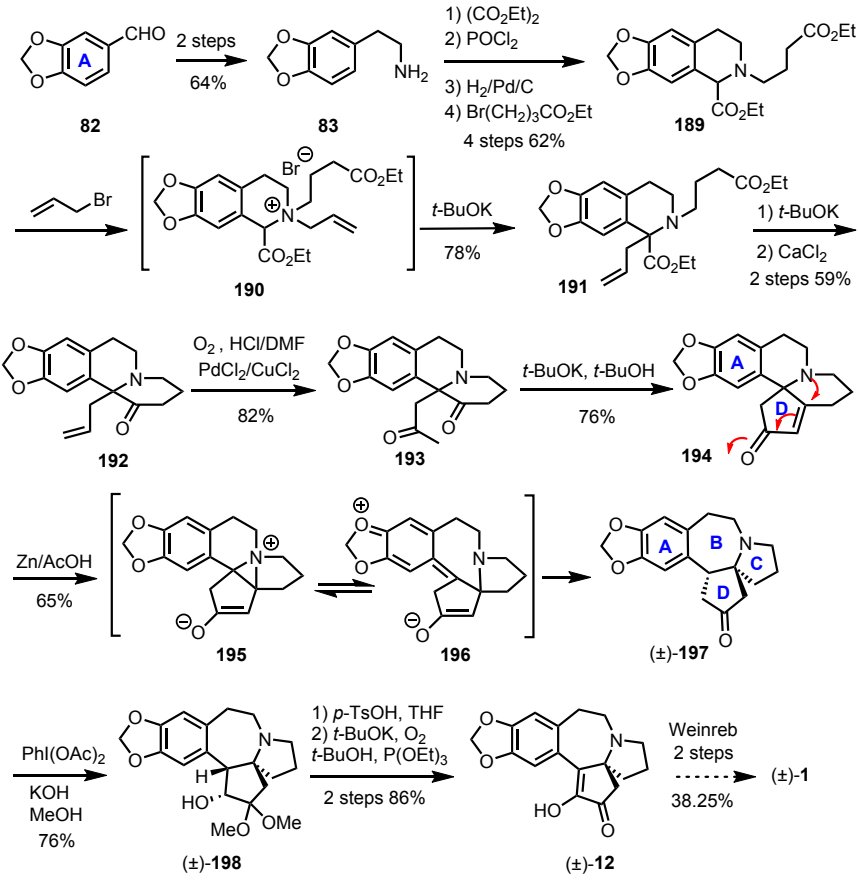
3.2.1.10 Bubnov's Formal Synthesis (2008)

Bubnov et al. developed a convenient and practical methodology for the preparation of various spiro-β-amino alcohols. This procedure was applied to a formal synthesis of *rac*-CET (±)-(**1**) involving allylboration and RCM to prepare the CD ring containing azaspirobicyclic compound **183** (Scheme 13).[104]

2-Pyrrolidone (**181**) was converted to 2,2-diallylated pyrrolidine **182** by addition of triallylborane. After amine protection, RCM was accomplished with Grubbs I catalyst providing azaspirocyclic carbamate **183** in high yield (three steps, 87% OY). Treatment of **183** with *N*-bromosuccinimide (NBS) produced tricyclic bromoamide **184**. After *t*-butoxide mediated dehydrobromination affording the spiro olefin **185**, an allylic isomerization in the presence of $MgBr_2$ gave **186** as the sole isomer. Hydrolysis of **186** and acylation with acid chloride **145** were performed in a one-pot procedure leading to **187**. Mori's ACD intermediate, generated by reduction of amide **187** with $LiAlH_4$, was allowed to cyclize into ABCD compound (±)-**188** by treatment with polyphosphoric acid (PPA). Then, the synthesis of *rac*-CET (±)-(**1**) in 16 steps from 2-pyrrolidone (**181**) and 3.8% estimated OY could be completed using Mori's sequence[85] as the final steps.



Scheme 13 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) by Bubnov (2008).



Scheme 14 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) by Li (2003).

3.2.2 N9—C5 Ring Closure c

3.2.2.1 Li's Formal Syntheses (2003—2005)

From 2003 to 2005, Li et al. reported three formal syntheses, two of them via the same intermediate **192** (Scheme 14).[100,105] Formation of ring B and D was achieved by transannular reductive skeletal rearrangements leading to the formation of N9—C5 bond (Fig. 15).

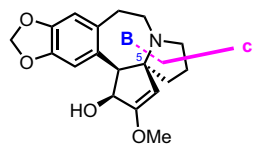
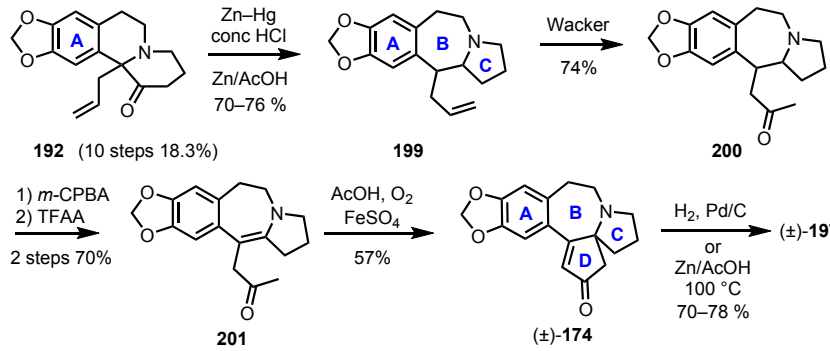


Figure 15 C5—N9 bond disconnection **c** for construction of ring B of cephalotaxine (**1**).

Starting from piperonyl amine **83**, diester **189** was obtained in four steps via a Bishler—Napieralski cyclization followed by hydrogenation and N-alkylation with ethyl 4-bromobutyrate. A second N-alkylation with allyl bromide led to the ammonium salt intermediate **190**, which was transformed via a 2,3-sigmatropic Stevens rearrangement into diester **191**. Eventually, compound **193** was obtained by a Dieckmann cyclization with subsequent decarboxylation followed by Wacker oxidation. Aldol condensation of the dione **193** provided the spirocyclic AD enone **194** (Scheme 14). CET (**1**) B and C rings were formed by reductive rearrangement in acetic acid via the aziridinium ion **195**. The obtained ABCD ketone (±)-**197** (revised stereochemistry at C4 as drawn) was oxidized with phenyliodonium diacetate to give acetal (±)-**198**, which, after hydrolysis, was submitted to autoxidative dehydrogenation with potassium *t*-butoxide and oxygen to provide the desmethylcephalotaxinone (±)-**12**. Formally synthesis of *rac*-CET (±)-(**1**) could be achieved according to Weinreb[103] using two additional steps, leading to a formal total synthesis in 1.8% calculated OY over 18 steps from piperonal (**82**).

An alternative approach to ketone (±)-**197** was described from allyl ketone **192**. Clemmensen reductive rearrangement of this ketone first led to benzazepine derivative **199** (Scheme 15).[100]

After Wacker oxidation of compound **199**, application of Polonovski—Potier reaction conditions to **200** gave the enamine **201**. It was previously reported that cyclization of this intermediate to (±)-**174** (formally an endocyclic enamine annulation) could only be realized in low yield. Li found that an aerobic treatment of **201** with Fe^{2+} salt in acetic acid promoted the cyclization into ABCD enone (±)-**174** in good yield. This cyclization can be seen as an unusual azo-Nazarov-type cyclization.



Scheme 15 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) by Li (2003): alternative synthesis of ketone (±)-**197**.

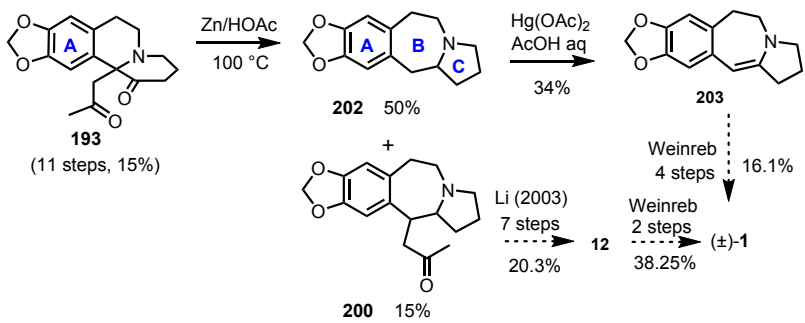
A catalytic hydrogenation eventually gave ketone (±)-**197**. This synthesis in six steps and 14.5%–17% OY from **192** is not advantageous when compared to the three steps and 40.5% OY version presented in Scheme 14. Thus, formal synthesis of *rac*-CET (±)-(**1**) following this procedure could be theoretically achieved in 21 steps and 0.7%–0.8% OY from piperonal (**82**).

In 2005, Li et al. described a formal synthesis of the Dolby–Weinreb enamine[103,106] **203** from dione **193**, with a mild Clemmensen–Clemo–Prelog–Léonard reductive rearrangement of **193** as the key step (Scheme 16).[105]

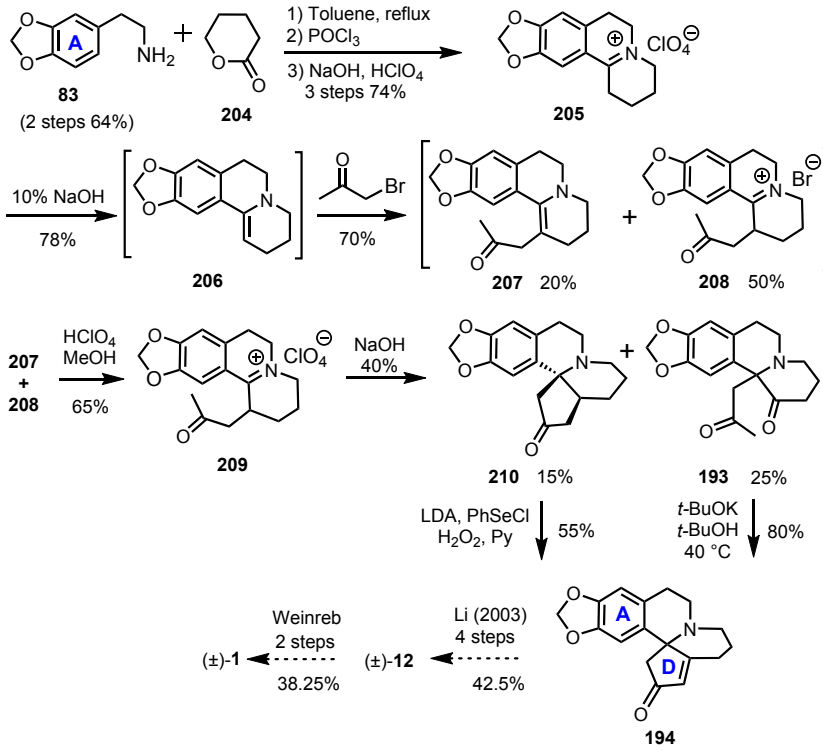
Dehydrogenation of the benzazepine **202** by $Hg(OAc)_2$ gave enamine **203**, which could be likely transformed in *rac*-CET (±)-(**1**) by Weinreb's strategy[103] in expected 0.4% OY for 17 steps from piperonal (**82**). Reductive rearrangement of aminodione **193** also produced aminoketone **200** in 15% yield, which could be processed into demethylcephalotaxinone (**12**) and then into CET (**1**) through a nine-stage sequence. This constitutes another formal synthesis of CET (**1**) with an expected OY of 0.17% in 21 steps from piperonal (**82**).

In 2005, Li reported a novel synthesis of the amino enone **194** involving annulation of the readily available aminodione **193** (Scheme 17).[107]

Bischler–Napierralski condensation of amine **83** with δ-valerolactone (**204**) mediated by $POCl_3$ gave the iminium ion **205** in 74% yield. Treatment of **205** with aqueous NaOH generated a labile enamine **206**, which was alkylated with bromoacetone to give the keto enamine **207** (20%) and the iminium bromide **208** (50%). Both were transformed by treatment with 70% perchloric acid into iminium perchlorate **209**, which on exposure to NaOH furnished the unexpected dione **193** (25%) and annulated *cis*-cyclopentanone **210**. Both compounds can be transformed to **194** by standard procedures (aldol condensation from **193**, selenoxide elimination



Scheme 16 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) via Dolby–Weinreb enamine **203** by Li (2005).



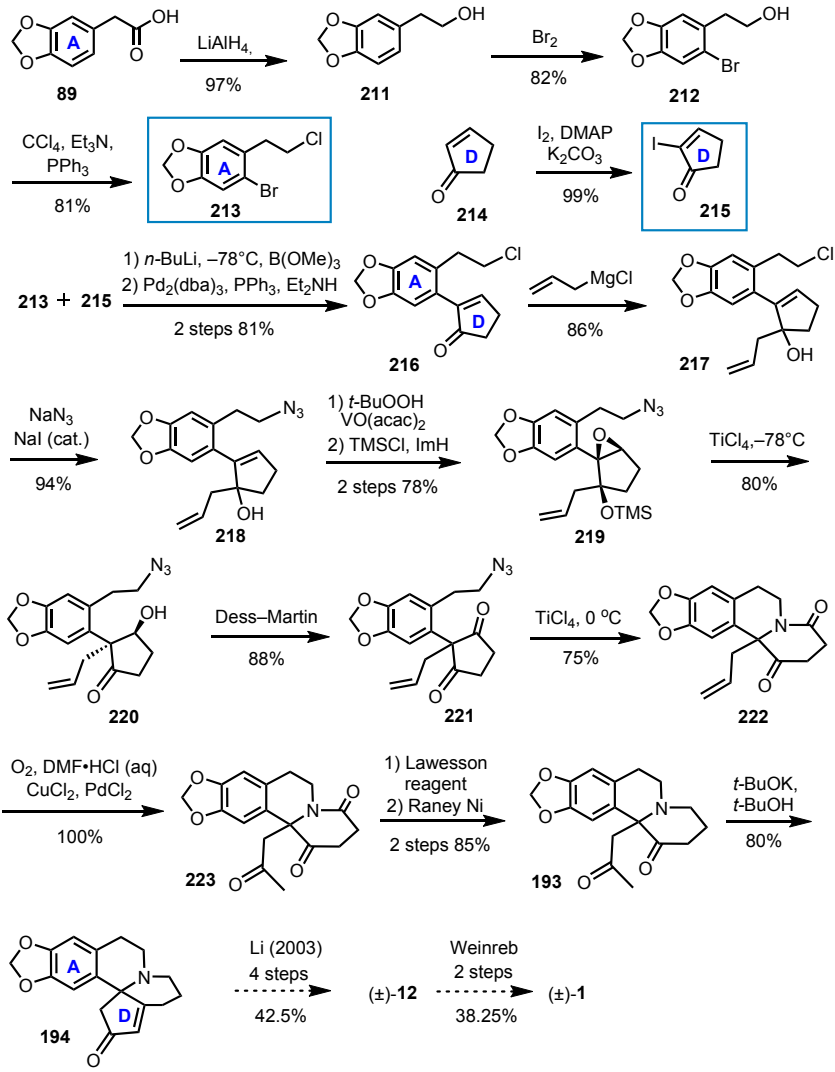
Scheme 17 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) via amino enone **194** by Li (2005).

from **210** (15%). Formally, *rac*-CET (±)-(**1**) could be obtained according to Li's and Weinreb's protocols[100,103] with a calculated 0.8% OY for 16 steps from piperonal (**82**). The ABCD ring construction by rearrangement can be regarded as a biomimetic synthesis.

3.2.2.2 Tu and Zhang's Formal Synthesis (2009)

In 2009, Tu and Zhang developed a synthesis of Li's intermediate dione[100] **193** based on an intramolecular Schmidt reaction of symmetric azido dione **221** (Scheme 18).[108]

The synthesis started with compound **213** (ring A precursor) obtained from homopiperonylic acid (**89**) by reduction, bromation, and chlorination,[109] and iodocyclopentenone **215** (ring D precursor) prepared by iodination of cyclopentenone (**214**) (Scheme 18).[110] Suzuki–Kumada coupling of an aryl boronic acid prepared from **213** with 2-iodocyclopentenone (**215**) afforded enone **216** in 81% yield. Alcohol **217** obtained by Grignard addition on enone **216** was transformed to the azide **220** on treatment with



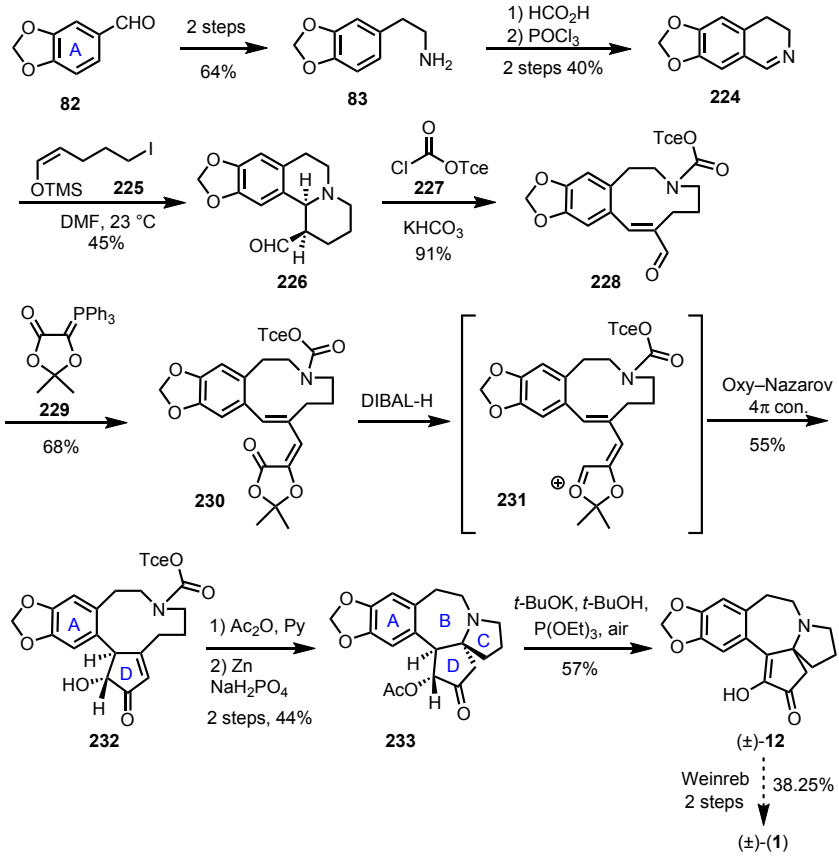
Scheme 18 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) by Tu and Zhang (2009).

sodium azide in the presence of sodium iodide. Epoxidation and protection with TMSCl led to siloxy epoxy azide **219** in 63% OY from **216**. Treatment of epoxide **219** with $TiCl_4$ afforded β-hydroxy ketone **220**, which was oxidized with Dess—Martin's reagent (70% yield, two steps). The key intramolecular Schmidt reaction was applied to dione **221** giving the amide **222**. The terminal double bond in **222** was converted into a methylketone by Wacker oxidation. Selective reduction of an intermediate thiolactam, prepared from amide **223** with Lawesson's reagent, using Raney Ni afforded

the expected tertiary amine **193** described by Li.[100,107] An aldol cyclization gave the key enone **194** in 80% yield. *rac*-CET (±)-(**1**) could be obtained by Li's and Weinreb's procedures in 23 steps from homopiperonylic acid (**89**) and 1.9% evaluated OY.

3.2.2.3 Li's Formal Synthesis (2011)

The new synthetic strategy described by Li et al. in 2011 was based on a facile reductive oxy-Nazarov (RON) cyclization as a key step (Scheme 19).[111] Condensation of norhydrastinine (**224**), prepared in four steps from piperonal (22.8% OY), with iodo enolsilane **225** afforded the aldehyde **226**, which was exposed to 2,2,2-trichloroethyl chloroformate (**227**) to give the macrocyclic enal **228**. Wittig olefination with triphenylphosphoranylidene dioxolanone **229** furnished the 10-membered amide **230** with the exocyclic (*E*)-olefin in 68% isolated yield, along with the separable

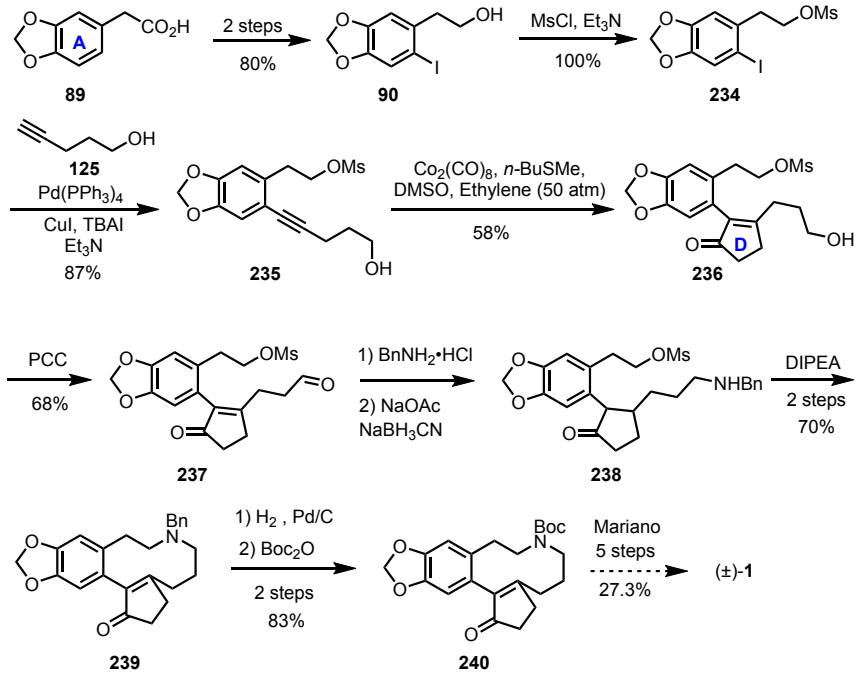


Scheme 19 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) by Li (2011).

(*Z*)-isomer (14%). Mild hydride reduction led to hydroxy enone **232** presumably via the oxonium ion **231**, which could have underwent rapid and effective 4π electrocyclization. Acetylation of the hydroxyl function of the resulting compound **232** followed by selective cleavage of the *N*-Troc group by zinc reduction led to the spontaneous formation of ABCD unit **233** as a single diastereomer by an intramolecular aza-Michael addition. Air-oxidation in the presence of potassium *t*-butoxide gave *rac*-demethylcephalotaxinone (±)-(**12**). Application of Weinreb's method[103] would allow to synthesize *rac*-CET (±)-(**1**) in 13 steps from piperonal (**82**) and 0.4% OY.

3.2.2.4 Jiang's Formal Synthesis (2013)

In 2013, Jiang et al. described the construction of the cyclopentenone D ring of *rac*-CET (±)-(**1**) by an intermolecular Pauson–Khand reaction (Scheme 20).[112] Iodo alcohol **90** was first protected as a mesylate and



Scheme 20 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) by Jiang (2013).

submitted to mild Sonogashira conditions to afford the alkyne **235**. The intermolecular Pauson–Khand reaction of alkyne **235** with ethylene could generate two regioisomeric products. Only the desired isomer **236** (58% yield) was obtained under optimized conditions using *n*-butyl methyl sulfide as a promoter with dimethyl sulfoxide as the oxidant. Oxidation of alcohol **236** followed by reductive amination produced the amine **238**, which was advanced to the 10-membered ring compound **239** by intramolecular N-alkylation. Mariano's intermediate[113,114] **240** was prepared successively by deprotection and reprotection with a *t*-butoxycarbonyl group.

This strategy led quickly and efficiently to macrocyclic precursor **240** in eight steps and 20% OY. With this compound **240** in hand, a formal synthesis of *rac*-CET (±)-(**1**) was thus attained, and additional five-step sequence[113,114] would give *rac*-CET (±)-(**1**) over 15 steps from homopiperonylic acid (**89**) and 4.3% estimated OY.

3.2.3 Other Strategies for Ring B Formation

3.2.3.1 C4–C5 Ring Closure b: Hong's Formal Synthesis (2015)

Recently, Hong et al. reported two formal syntheses[115] involving an unusual C4–C5 disconnection **b**[116–118] for ring B formation (Fig. 16). The key steps were first an *N*-acyliminium ion cyclization, which established stereogenic centers C5 and C4 and the formation of B ring. Then an RCM furnished the spiro-ring D. The two syntheses started from iodo alcohol **90** prepared from homopiperonylic acid (**89**) in two steps.

Sonogashira coupling of aryl iodide **90** with propargyl trimethylsilane catalyzed by palladium(II) and copper(I) iodide delivered alkyne **242** in excellent yield (97%) (Scheme 21). After a catalyst screening, partial hydrogenation was carried out with "P-2 Nickel" and ethylenediamine (Brown catalyst) leading to (*Z*)-**243** in quantitative yield (dr > 15/1). Mitsunobu reaction with succinimide, followed by allylation next gave

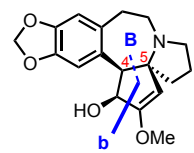
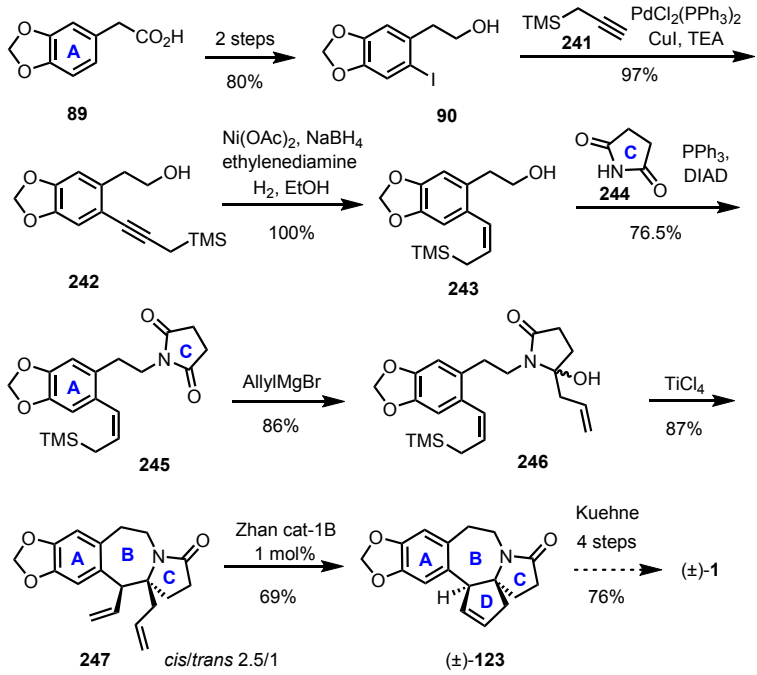


Figure 16 C4–C5 bond disconnection **b** for elaboration of ring B of *rac*-cephalotaxine (±)-(**1**).

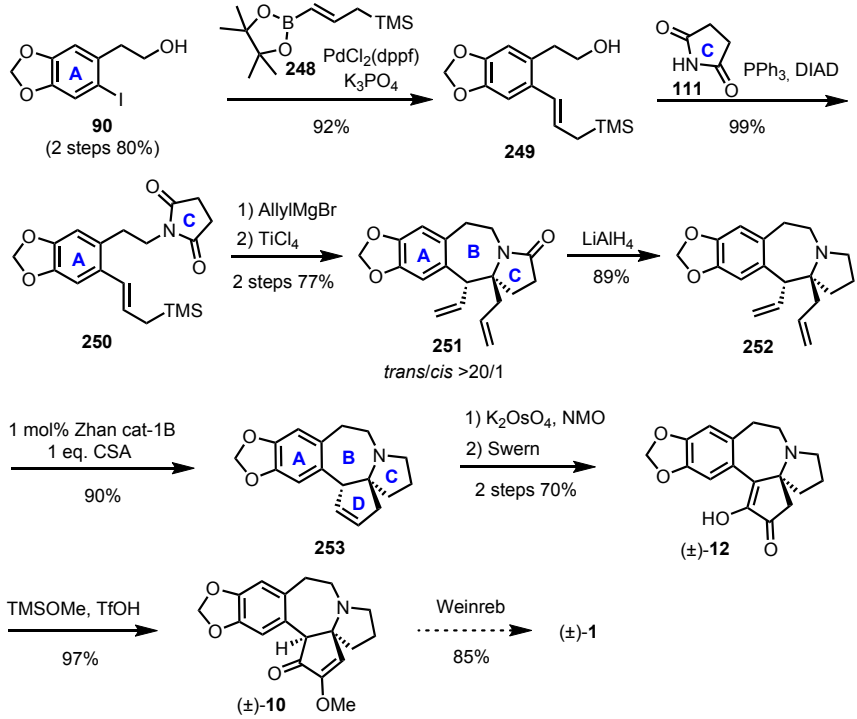


Scheme 21 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) by Hong via Kuehne's intermediate (±)-**123** (2015).

the hemiaminal **246**, which, treated with $TiCl_4$ at low temperature in mesitylene, allowed for the generation of an N-acyliminium ion that cyclized to furnish the ABC unit **247** (*cis/trans* 2.5/1). Zhan's catalyst-1B[119] was the most effective for the RCM reaction to deliver Kuehne intermediate[94] (±)-**123**. Formally, synthesis of *rac*-CET (±)-(**1**) could be achieved by Kuehne's protocol[94] in 12 steps from homopiperonylic acid (**89**) and 23.5% calculated OY.

Since iminium ion cyclization showed a moderate diastereoselectivity and the separation of the two diastereomers by silica gel chromatography failed, this route was less attractive. To improve it, authors investigated cyclization from the *E*-allylsilane **249** obtained by Suzuki coupling between compound **248** and aryl iodide **90** catalyzed by palladium (Scheme 22). As previously seen, a Mitsunobu reaction gave **250**. Subsequently, **250** was treated with a Grignard reagent and cyclized by a Hosomi–Sakurai reaction to **251**.

Through a $TiCl_4$-promoted iminium ion cyclization, the ABC pyrrolobenzazepine **251** was obtained with a ratio of 20/1 in favor of the



Scheme 22 Formal synthesis of *rac*-Cephalotaxine (±)-(**1**) by Hong via Weinreb's intermediate (±)-**12** (2015).

trans-isomer. The RCM reaction of the *trans*-isomer of amide **251** did not happen whatever the catalysts used. Reduction of the amide led to a less rigid tertiary amine **252**, suitable for the desired cyclization. Metathesis proceeded smoothly in the presence of camphor sulfonic acid (1 equiv.) to protonate the amine and Zhan-1B's catalyst (1 mol %). The ABCD ring system **253** was obtained with inverted relative stereochemistry at C4. Dihydroxylation and Swern oxidation afforded the known demethylcephalotaxinone (±)-(**12**). Application of an improved Weinreb's procedure[103] would generate *rac*-CET (±)-(**1**) in 12 steps and 26.2% expected OY from homopiperonylic acid (**89**).

3.2.3.2 N9—C10 Ring Closure e: Chandrasekhar's Total Synthesis (2016)

In 2016, Chandrasekhar et al. developed a total synthesis of *rac*-CET (±)-(**1**) via an aryne insertion from the benzyne precursor **255** into β-ketoester **257** as a key step to build the complete set of carbon atoms and functionalities

to end up the synthesis of *rac*-CET (±)-(**1**) (Scheme 23).[120] An unusual N9—C10 bond formation through an amidation reaction closed B ring (Fig. 17) .

Aldehyde **256** derived in five steps from L-proline (**140**) was transformed into β-ketoester **257** by Roskamp conversion. Then, β-ketoester **257** was

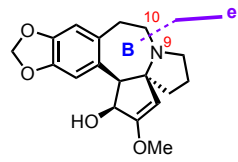
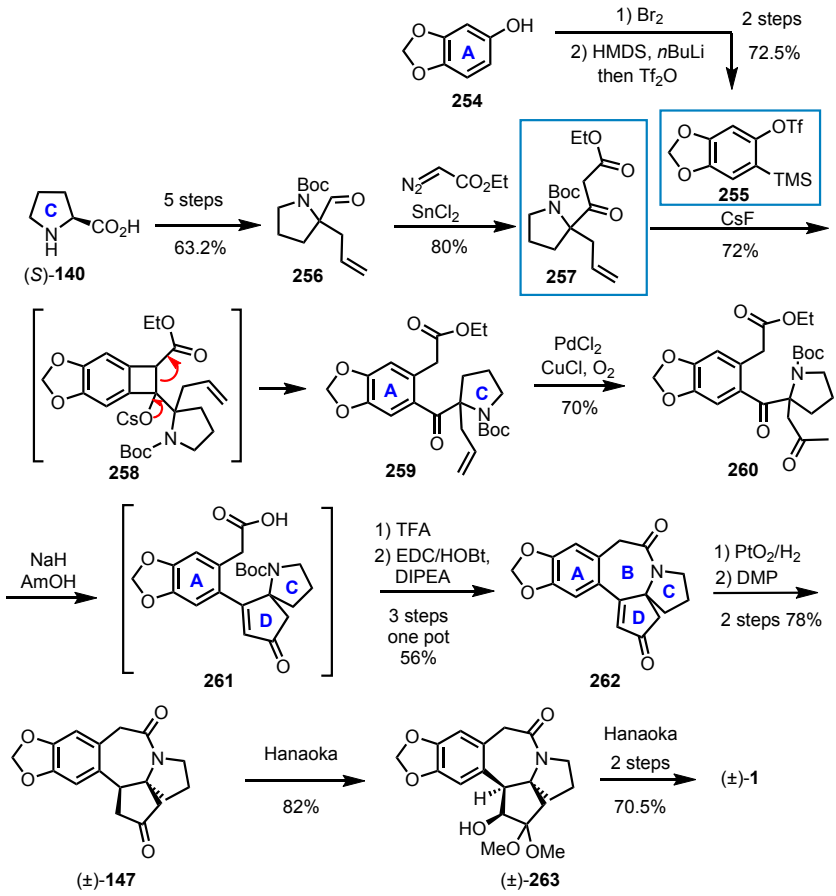


Figure 17 N9—C10 bond disconnection **e** for construction of cephalotaxine (**1**) B ring.



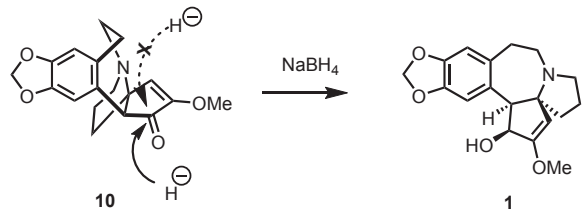
Scheme 23 Total synthesis of *rac*-cephalotaxine (±)-(**1**) by Chandrasekhar (2016).

submitted to [2 + 2] cycloadditon with benzyne generated from aryne precursor **255** easily prepared in a one pot sequence involving involving O-silylation, metal−halogen exchange, O- to C-silyl migration, and entrapment of the phenoxide with triflic anhydride. The unstable benzocyclobutane **258** underwent C−C bond cleavage leading to compound **259**. At this point, all carbons atoms of the CET (**1**) skeleton were introduced. The synthesis of ABCD enone **262** was accomplished by Wacker oxidation, aldol condensation, ester hydrolysis, Butyloxycarbonyl (Boc) deprotection and amidation. Reduction followed by oxidation gave Hanaoka's intermediate[98] (±)-**147**. From proline (**140**), *rac*-CET (±)-(**1**) was obtained through intermediate **147** applying literature conditions[98] in a total of 16 steps and 6.4% OY. Including the preparation of aromatic unit **255** from 5-benzodioxolol (**254**), *rac*-CET (±)-(**1**) was obtained in 18 TNS and 4.7% AY.

3.3 Syntheses of Enantioenriched Cephalotaxine

Synthesis of optically pure CET (**1**) remains a challenge for organic chemists. Three contiguous asymmetric centers including a tetrasubstituted one on D ring have to be controlled. It was early noticed by Weinreb[103] that racemic syntheses led to only one diastereoisomer without any required separation. This means that the configurations of the three asymmetric centers are mutually controlled. Strategies using disconnection C4−C5 for B ring formation exemplify this phenomenon. During the cyclization step, the thermodynamically more stable BD ring junction is *cis*, which is the relative C4−C5 configuration found in natural CET. Moreover, Weinreb also showed that $NaBH_4$ reduction of cephalotaxinone (**10**) is stereoselective (Scheme 24), delivering the required relative stereochemistry of the hydroxyl function at C3 of CET (**1**), which is assumed to be formed as a result of hydride attack from the convex face of **10**.

It is thus possible to control successively the C4 center via the C5 tetrasubstituted center; then the C3 center via the C4 stereocenter, and



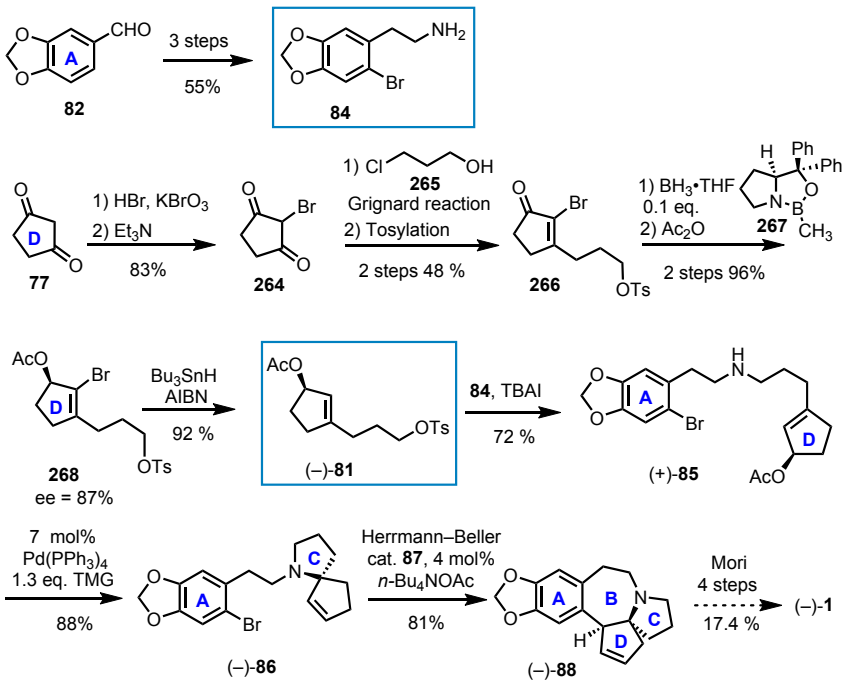
Scheme 24 Stereoselective reduction of cephalotaxinone (**10**).

consequently, control of the tetrasubstituted C5 stereocenter is the only necessary one to synthetize enantiomerically pure CET (**1**). Most of asymmetric syntheses are based on the control of this spiro center followed by a stereoselective cyclization affording the B ring and C4 control, a final reduction establishing the desired configuration at C3. The asymmetric syntheses of CET (**1**) described below are classified according to the type of stereochemical control (asymmetric catalysis, use of chiral auxiliaries, incorporation of chiral building blocks, and resolution strategies) and deconnection of ring B, related strategies then in chronological order in each section.

3.3.1 Asymmetric Catalysis

3.3.1.1 Tietze's Formal Synthesis (1999)

In 1999, Tietze et al. reported an asymmetric version of their previous synthesis[84] using Corey's oxazaborolidine **267** to reduce enantioselectively ketone **266** (Scheme 25).[121] Ketone **266** was prepared by bromination of cyclopentane-1,3-dione (**77**) with potassium bromate followed by



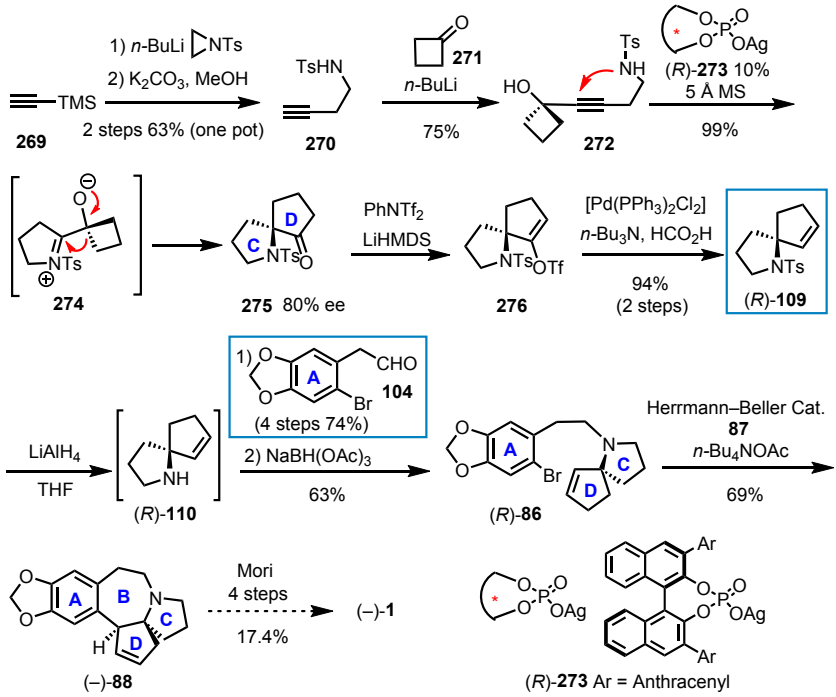
Scheme 25 Formal asymmetric synthesis of cephalotaxine (–)-(**1**) by Tietze (1999).

treatment with a large excess of Grignard reagent prepared from chloroalcohol **265** and tosylation (three steps, 40% OY). Chiral bromoacetate **268** corresponding to the future D ring was obtained by reduction with diborane in the presence of catalytic amounts of the chiral oxazaborolidine **267**, followed by acetylation of the allylic alcohol. A 87% ee was measured by NMR using $Eu(hfc)_3$. Formation of the B and C rings was achieved by the strategy described for *rac*-CET (±)-(**1**), employing two successive Pd-catalyzed transformations. The optically active form of Mori's intermediate[85] (−)-**88** was isolated. The synthesis of CET (−)-(**1**) could be likely achieved in 13-steps linear sequence, 3.1% calculated OY, and 87% expected ee from cyclopentane-1,3-dione (**77**).

3.3.1.2 Tu's Formal Synthesis (2012)

In 2012, Tu et al. developed a formal synthesis of CET (−)-(**1**) targeting Mori's intermediate[85] (−)-**88**, which was built from 1-azaspirocycle (−)-**109** by a tandem hydroamination/semipinacol rearrangement reaction.[122] At the same time, chirality was introduced using a chiral silver phosphate (*R*)-**273** catalyst (Scheme 26).

The anion of butyne **270** prepared from trimethylsilylacetylene (**269**) reacted with cyclobutanone to give **272**, which was submitted to a cyclization rearrangement. The key reaction was readily performed using chiral silver phosphate **273** to give azaspirocycle **275** in very good yield and 80% ee. The ketone **275** was transformed into vinyl triflate **276**, followed by reduction to lead to the key intermediate (*R*)-**109** reported by Stoltz et al. (see Section 3.2.1.3).[90] Elimination of the tosyl group generated the amine (*R*)-**110**, which reacted with bromo aldehyde[90] **104** (see Scheme 5) under reductive amination conditions to give Tietze's intermediate[121] (*R*)-**86**. Application of the Tietze's procedure led to Mori's alkene (−)-**88** in 80% ee determined by chiral HPLC. Surprisingly, during its purification by column chromatography on silica gel, authors found that enantiomers could be separated leading to (−)-**88** in 35.5% yield with >99% ee. This phenomenon called "self-dispropornation of enantiomers" results from different affinities of aggregates of compound **88** on silica gel.[123] Tu thus accomplished a formal synthesis of CET (−)-(**1**) in 14 steps, 3.3% calculated OY and 80% ee (18 TNS, 2.5% OY). The high purity material (estimated 99% ee) could be obtained in 1.7% calculated OY from trimethylsilylacetylene (**269**).

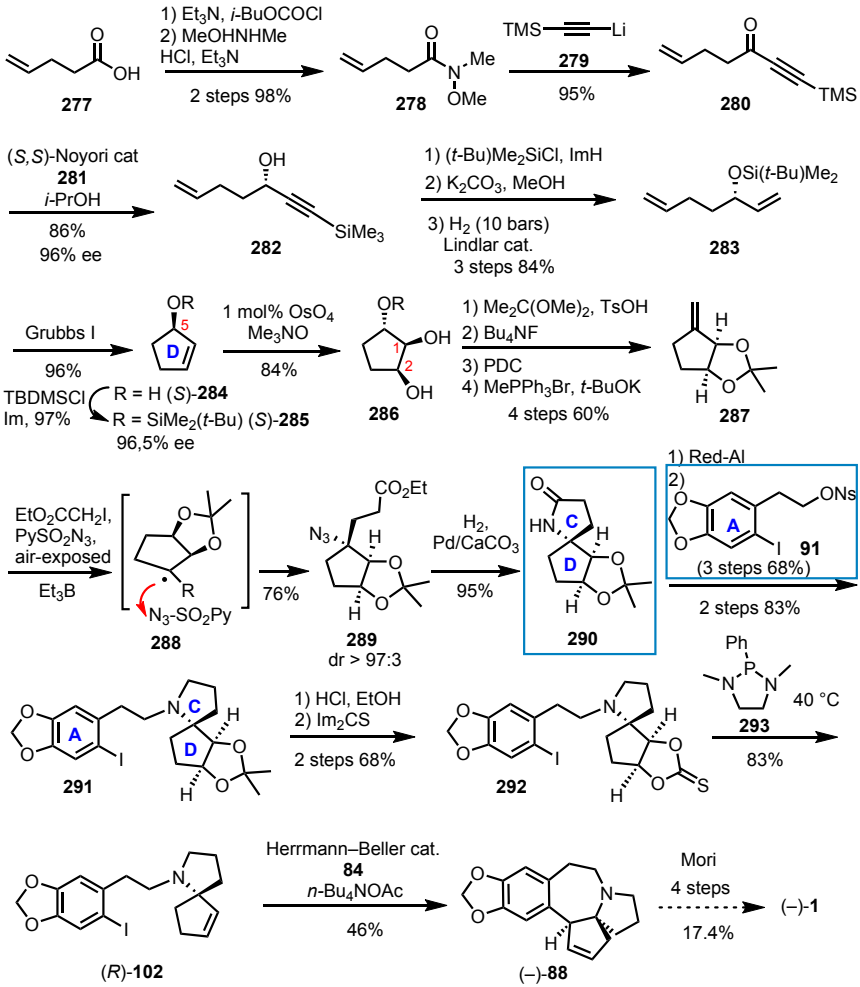


Scheme 26 Formal asymmetric synthesis of cephalotaxine (–)-(**1**) by Tu (2012).

3.3.1.3 Renaud's Formal Synthesis (2012)

In 2012, Renaud et al. reported two variants for the formal synthesis of CET (–)-(**1**).[124] Both syntheses were based on a stereoselective carboazidation of methylene cyclopentanol derivatives. In the first approach, which will be described later (see Section 3.3.5.2, Scheme 43), the starting material was obtained from the racemate by resolution. The second approach was more efficient and started with (*S*)-cyclopentenol (**284**) obtained in 96% ee set up via enantioselective Noyori's hydrogenation of propargylic ketone **280** using (*S,S*)-1,1′-bi-2-naphthol **281** to give **282**. Compound **282** was obtained in 64.5% yield from 4-pentenoic acid (**277**) (Scheme 27).[125]

Dihydroxylation of silyl enolate (*S*)-**285** afforded the diol **286** as a single diastereoisomer, which was protected as an acetonide. Desilylation, oxidation, and Wittig methylenation afforded the bicyclic alkene **287**, which was submitted to radical carboazidation. The azido ester **289** was obtained in high diastereoselectivity (dr > 97:3) resulting from *exo*-selective azidation of the bicyclic intermediate radical **288**. The spirolactam **290** was obtained



Scheme 27 Alternative formal asymmetric synthesis of cephalotaxine (−)-(**1**) by Renaud (2012).

by reduction of the azide. First, the stereogenic center at C5 (CET numbering) of (*S*)-cyclopentenol **284** was used to control the configuration of the newly formed diol at C1−C2, and then it was destroyed on double bond formation in **287**. This chiral center was eventually controlled during the carboazidation step leading to **289**. This sequence corresponds to the concept of self-regeneration of stereogenic centers.[126] Reduction of the lactam **290** gave an amine, which was N-alkylated with nosylate **91** (see Scheme 4), to afford the ACD aryl iodide **291**. After hydrolysis of the

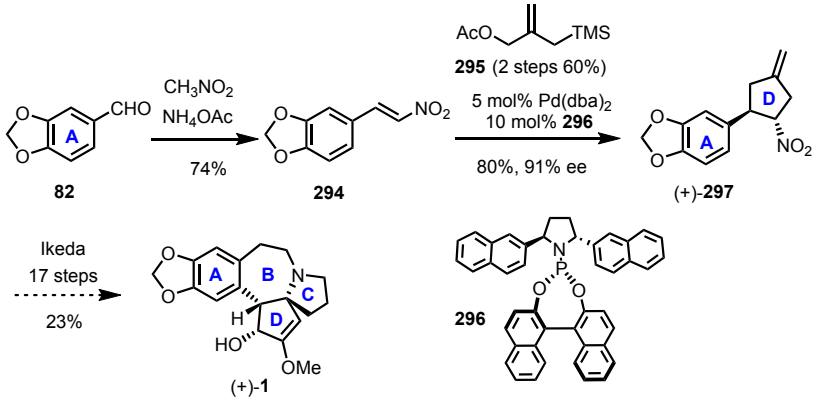
acetonide, the diol was converted to thionocarbonate **292**, which on treatment with Corey–Hopkins' reagent afforded the desired alkene (*R*)-**102**. It was noteworthy that no racemization took place during the synthesis of alkene (*R*)-**102**. Optically active alkene (*R*)-**102** was already prepared by Mariano[127,128] (Scheme 42) and, in its racemic form, by Suga and Yoshida[86] (Scheme 4).

Formation of ring B via Heck intramolecular reaction according to Tietze's procedure afforded Mori's intermediate[85] (−)-**88**. Synthesis of CET (−)-(**1**) could be likely achieved in 97% ee within 26 steps from 4-pentenoic acid (**277**) and 0.9% expected OY.

3.3.1.4 Trost's Formal Synthesis (2012)

In 2012, Trost et al. developed an enantioselective palladium-catalyzed [3 + 2] cycloaddition of trimethylenemethane with a nitroalkene and described an application to the synthesis of the enantiopure AD unit (+)-**297** (Scheme 28),[129] which racemic form is an intermediate in Ikeda's synthesis.[130]

The phosphoramidite ligand **296** provided the chirality during the [3 + 2] cycloaddition, delivering compound (+)-**297** in 91% ee (chiral HPLC). Transformation of compound (±)-**297** into *rac*-CET (**1**) had been previously reported by Ikeda, and formally, *ent*-CET (+)-(**1**) (enantiomer of the natural product) could be obtained in 91% expected ee and 14% calculated OY over a 19 steps in the LLS from piperonal (**82**).



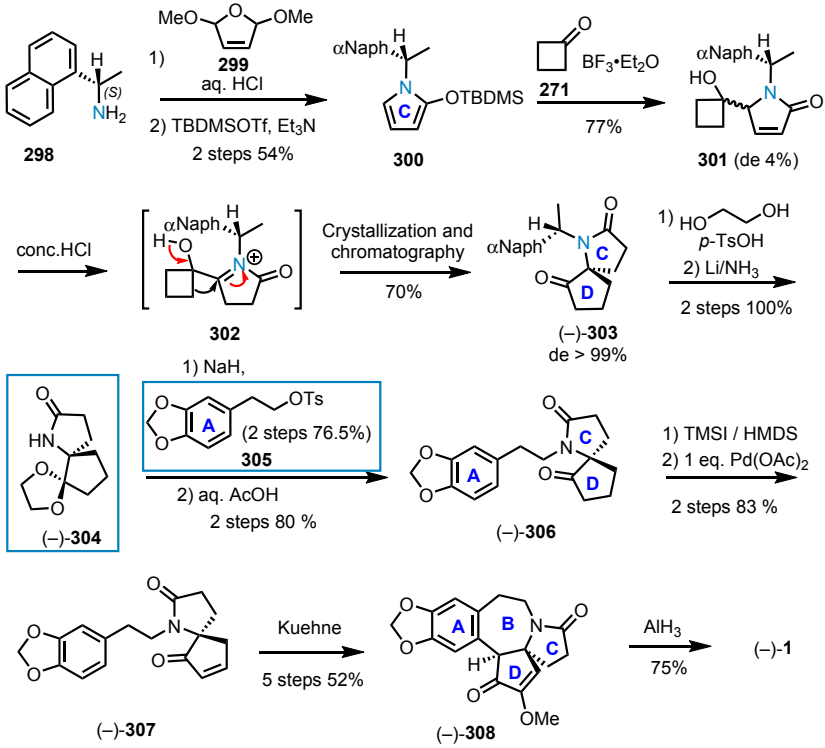
Scheme 28 Formal asymmetric synthesis of *ent*-cephalotaxine (+)-(**1**) by Trost (2012).

3.3.2 Use of Chiral Auxiliaries

3.3.2.1 Royer's Total Synthesis (2004)

In 2002, Royer et al. described an asymmetric synthesis of azaspiro compound (−)-**303**, containing the CD ring fragment exploiting a stereoselective semipinacolic reaction as the key step.[131] In 2004, these authors developed an enantioselective synthesis of (−)-CET (−)-(**1**) from spirolactam ketal (−)-**306**,[132] relying on Kuehne[94] and Nagasaka's[133] procedures (Scheme 29). The immolative removal of the chosen chiral inducer, (*S*)-1-(1-naphthyl)ethylamine (**298**), did not allow the recovery of this chiral material.

The silyloxypyrrole **300** prepared from 2,5-dimethoxy-2,5-dihydrofuran (**299**) over two steps using (*S*)-1-(1-naphthyl)ethylamine (**299**) as the chiral source, was subjected to a vinylogous Mukaiyama aldol reaction with cyclobutanone (**271**) affording the α,β-unsaturated γ-lactam **301**. On treatment with concentrated HCl, a semipinacolic rearrangement of



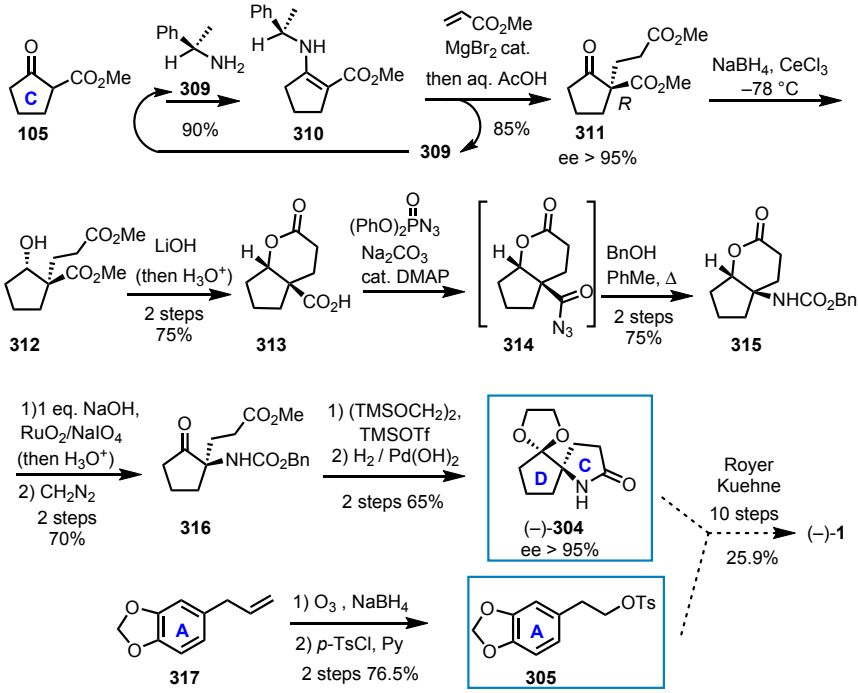
Scheme 29 Asymmetric synthesis of cephalotaxine (−)-(**1**) by Royer (2004).

301 furnished the desired spiro compound (−)-**303**. The *N*-acyliminium ion **302** was attacked preferably *anti* to the naphtyl group, delivering compound (−)-**303** with a good diastereoisomeric excess (de 80%). The major diastereoisomer was recrystallized and also easily recovered by chromatography, and thus azaspirononanedione (−)-**303** was obtained in 70% yield and de surpassing 99%. The ketone function of (−)-**303** was protected as a cyclic ketal, and then reductive cleavage of the *N*-benzylic bond provided spirolactam ketal (−)-**304**. N-alkylation of its sodium salt with the tosylate **305** of 3,4-methylenedioxypheny-2-ethanol[133,134] followed by ketal hydrolysis afforded the enantiopure ACD intermediate (−)-**306** described by Kuehne in its racemic form.[94] Following Kuehne's procedure, compound (−)-**306** was transformed into (−)-CET (**1**) with improved formation of enone (−)-**307** using a stoichiometric amount of $Pd(OAc)_2$ without benzoquinone (83% yield). A further modification consisted in the use of AlH_3[96] at 0°C instead of $LiAlH_4$ in refluxing tetrahydrofuran (THF) for the reduction of (−)-**308** to avoid any racemization of sensitive CET (**1**). CET (−)-(**1**) was thus obtained in 98.7% ee and 7.5% OY over a 16-steps linear sequence from 2,5-dimethoxy-2,5-dihydrofuran (**299**) using (*S*)-1-(1-naphthyl)ethylamine (**298**) as a chiral source.

3.3.2.2 Dumas and d'Angelo's Formal Synthesis (2005)

After having explored ABC → ABCD synthetic strategies,[116,117] in 2005, Dumas and d'Angelo et al. reported a novel enantioselective synthesis of the azaspiro unit (−)-**304** containing CD rings described by Royer[132] based on an asymmetric Michael reaction involving a chiral enamine prepared from cheap and recyclable enantiopure (*R*)-1-phenylethylamine **309** (Scheme 30).[135] As a consequence of stereogenic control of the quaternary carbon center in the resulting (*R*)-ketodiester **311**, the two asymmetric centers C3 and C4 of CET (−)-(**1**) were formed with their natural ACs. Similarly, use of the antipodal (*S*)-1-phenylethylamine (**309**) could afford *ent*-CET (+)-(**1**), although it would lead to biogolically inactive esters.[136] The key step was a Curtius rearrangement of acyl azide **314**, which allowed installation with perfect stereochemical fidelity and high efficiency of an α-nitrogen substituent at the tetrasubstituted center in ketodiester **311** to set the tetrasubstituted spirocyclic center.

The ketodiester (*R*)-**311** was elaborated in 85% yield and an ee $\geq$ 95% through Michael addition of enamino ester (*R*)-**310** to methyl acrylate. Sequential highly stereoselective Luche reduction of the ketone at



Scheme 30 Formal asymmetric synthesis of cephalotaxine (–)-(**1**) by Dumas and d'Angelo (2005).

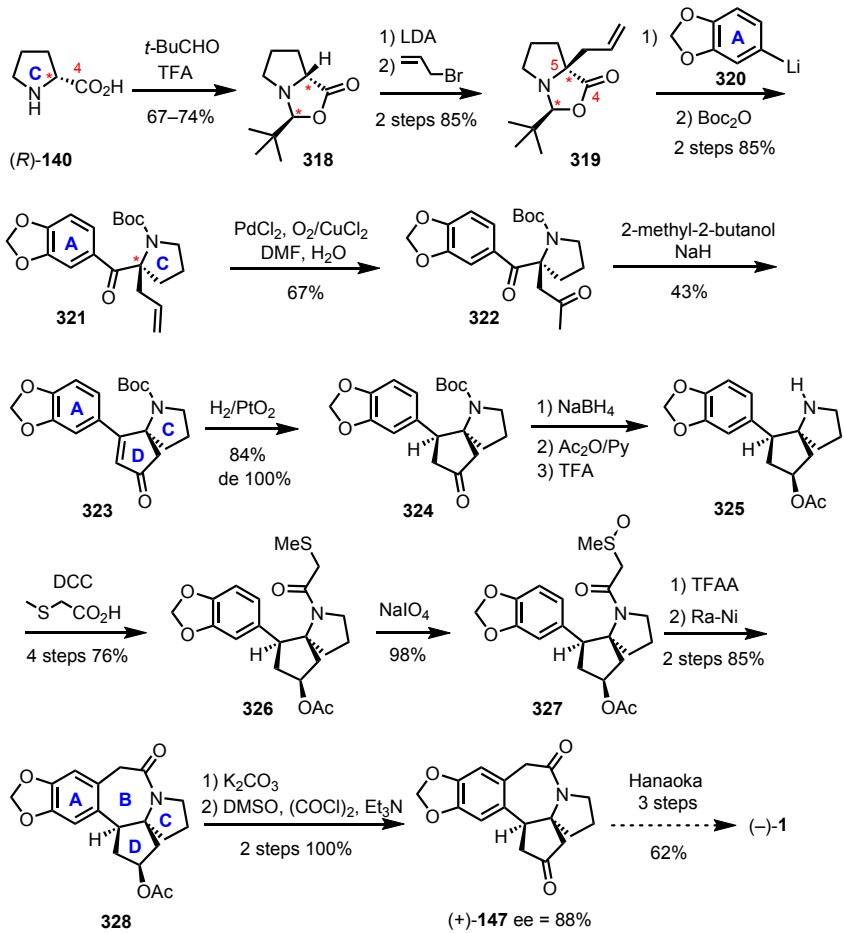
low temperature followed by saponification and acidification provided an intermediary hydroxy diacid, which on spontaneous lactonization generated the diastereomerically pure lactone acid **313** obtained in 75% yield. The two ester functions of **311** were differentiated at this stage. The acid **313** was first converted into acyl azide **314**, which was directly rearranged into the benzyl carbamate **315** by treatment with benzyl alcohol at 110°C. Saponification followed by oxidation with in situ-generated ruthenium tetroxide, and then esterification of the intermediate acid by diazomethane afforded the ketoester (*R*)-**316**. This compound was converted into ketal and then submitted to hydrogenolysis to deliver the target spiroketal amide (–)-**304**. By relying on Royer[132] and Kuehne's procedures,[94] using tosylate **305** obtained in two steps and 76.5% OY from safrole (**317**)[117,118] via 2-arylethanol **211**, the synthesis of CET (–)-(**1**) could be achieved in expected ee ≥ 95%, 20 steps from *β*-ketoester **105** and calculated 5.1% OY (Scheme 30).

3.3.3 Incorporation of Chiral Sources

3.3.3.1 Ikeda's Formal Synthesis (1999)

In 1999, Ikeda et al. developed an asymmetric version[137] of their racemic synthesis.[130,138] The intermediate ABCD ketone (+)-**149** was prepared from unnatural D-proline (*R*)-**140** used to furnish the C ring with C4 atom and to control the C5 tetrasubstituted stereogenic center set in **319** (Scheme 31).

Compound **318** obtained from D-proline (*R*)-**140** according to Seebach[139] was stereoselectively allylated to give oxalolidinone **319**, which reacted with 5-lithiobenzodioxole (**320**), generated from 4-bromobenzodioxole (itself prepared from benzodioxole **330**) and *n*-BuLi at −78°C.



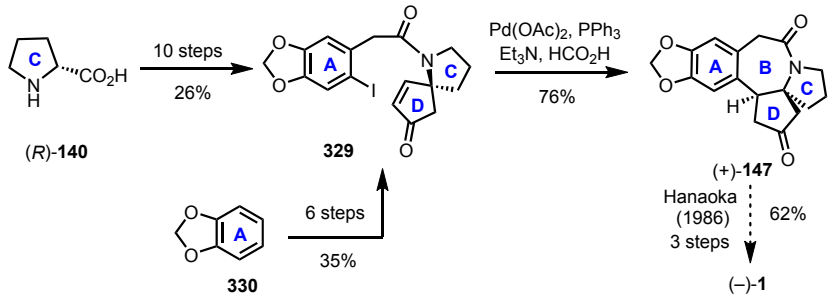
Scheme 31 Formal asymmetric synthesis of cephalotaxine (−)-(**1**) by Ikeda (1999).

Then the intermediate aminoketone was protected to give the AC ring unit found in **321**. Wacker oxidation, followed by aldol condensation of dione **322** generated the D ring of CET (**1**) characteristic of the α,β-unsaturated ketone **323**. Catalytic diastereoselective hydrogenation provided the cyclopentanone **324** with the desired AC at C4 and C5. Eventually, Ikeda applied his racemic strategy[130,137] to build the B ring in six steps using a Pummerer reaction for closure of B ring (**327** → **328**). Hanaoka's intermediate (+)-**147** was obtained in two steps by treatment with K_2CO_3 and Swern oxidation. Ikeda proposed a formal synthesis of CET (−)-(**1**) in 88% estimated ee (HPLC), 19 steps from D-proline (*R*)-**140** and 5.1% expected OY.

3.3.3.2 El Bialy's Formal Synthesis (2002)

In 2002, El Bialy described a formal synthesis of CET (−)-(**1**), where formation of the B ring involved a palladium-catalyzed cyclization leading to Hanaoka's[98] ketolactam intermediate (+)-**147** (Scheme 32).[140] The optically active ACD iodoaryl enone **329** was prepared from D-proline (*R*)-**140** by Seebach's method[139] relying on Ikeda's procedure. Using 20 mole % $Pd(OAc)_2$, PPh_3, Et_3N, and formic acid in THF allowed the intramolecular reductive Heck reaction to furnish the ketolactam (+)-**147** in 76% yield, a noticeable improvement compared to Ikeda's method[130,137] whose catalysis implying $Pd(OAc)_2$ in the presence of PBu_3, 1,3-bis(diphenylphosphino)propane (dppp), Ag_2CO_3 in DMF led to (+)-**147** in a modest 7% yield.

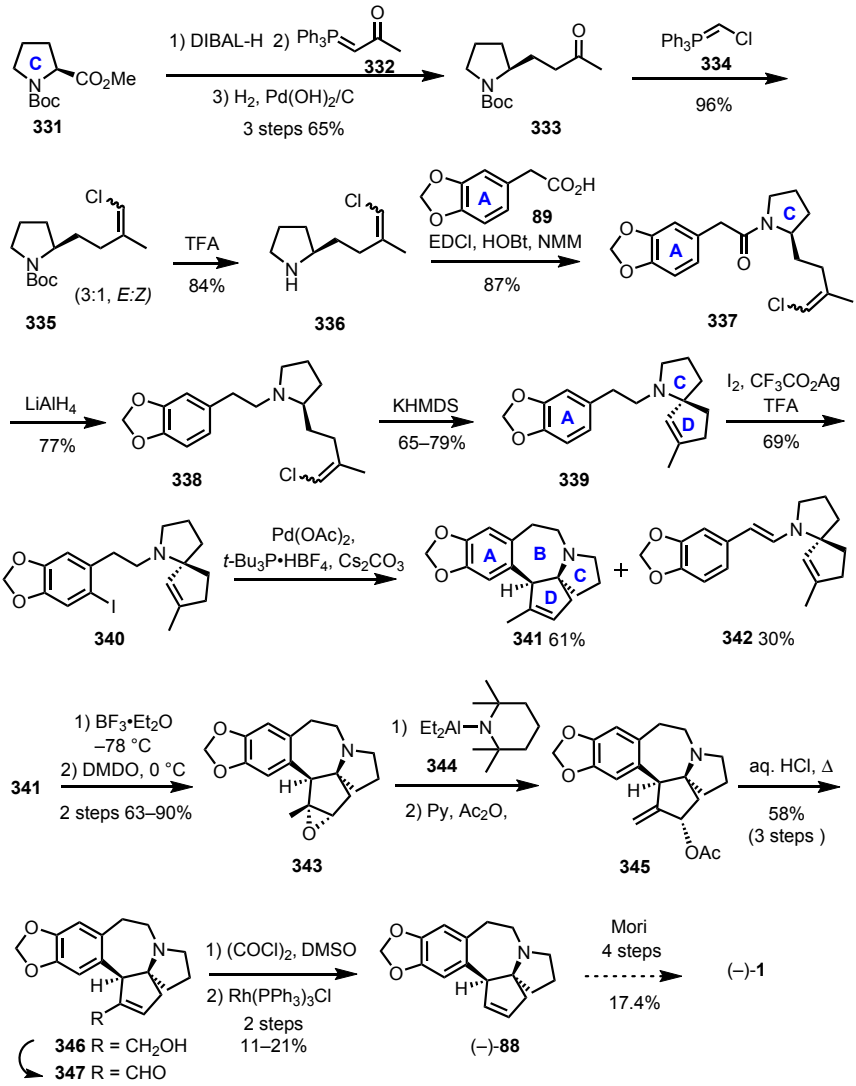
This formal synthesis would deliver CET (−)-(**1**) in expected 89.3% ee over a 14-steps linear sequence from D-proline (*R*)-**140** and an evaluated 12.2% OY.



Scheme 32 Asymmetric formal synthesis of cephalotaxine (−)-(**1**) by El Bialy (2002).

3.3.3.3 Hayes's Formal Syntheses (2008)

In 2008, Hayes et al. developed a formal synthesis of CET (−)-(**1**) based on an alkylidene carbene 1,5-CH insertion reaction as a key step to construct the 1-azaspiro[4.4]nonane CD ring system (**338** → **339**).[141] In a first approach, vinyl chloride **338** was chosen as a carbene precursor prepared in seven steps and 50% OY from L-proline (*S*)-(**140**) via *N*-Boc-proline methyl ester **331** (Scheme 33).



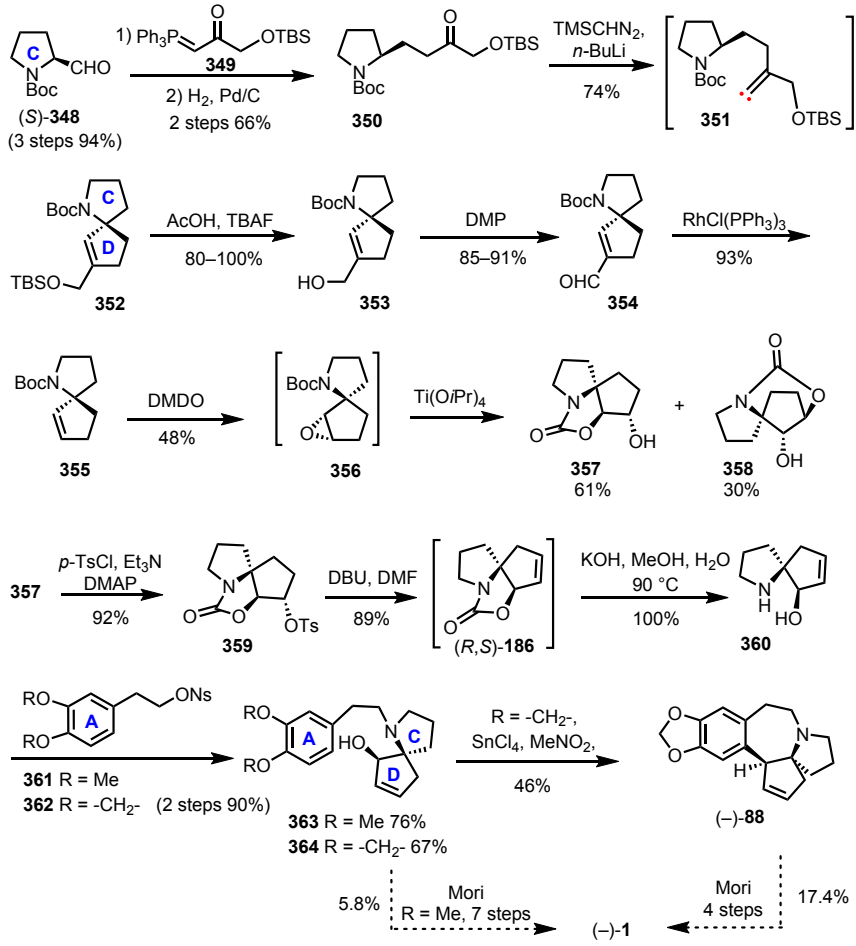
Scheme 33 Formal synthesis of cephalotaxine (−)-(**1**) by Hayes via Mori's intermediate (−)-**88** (2008).

DIBAL-H reduction of **331** followed by Wittig reaction with 1-triphenylphosphoranylidene-2-propanone (**332**) gave an intermediate *E*-enone. Catalytic hydrogenation and a second Wittig reaction with chloromethylenephosphorane (**334**) afforded the vinyl chloride **335**. After deprotection of the amine, amide formation with the carboxylic acid **89** and reduction of the resulting amide **337** led to the pivotal alkylidene carbene precursor **338**. Its treatment with potassium hexamethyldisilazide caused the key alkylidene carbene 1,5-CH insertion reaction leading to the desired azaspiro[4.4]nonane derivative **339** in good yield (65%–79%). Regioselective iodination of the aromatic ring system gave the Heck-cyclization precursor **340**. Its X-ray crystal structure allowed the confirmation of its absolute stereochemistry, thus demonstrating that as expected the 1,5-CH insertion reaction had proceeded with retention of configuration. The Heck cyclization under modified Fu's conditions ($Pd(OAc)_2$, $HBF_4 \cdot PtBu_3$, Cs_2CO_3) delivered the ABCD olefin **341** in 61% yield, together with enamine **342** (30%). The last stage involved removal of the extra carbon atom at C3 on ring D by decarbonylation of aldehyde **347**. Toward this end, alkene **341** was epoxidized using dimethyldioxirane in the presence of $BF_3 \cdot Et_2O$. The resulting epoxide **343** was submitted to a regioselective Yamamoto rearrangement to lead to an allylic alcohol protected as an acetate **345**, which was transposed by treatment with aqueous HCl to primary alcohol **346** and then oxidized (Swern) into aldehyde **347**, which, in the presence of Wilkinson's catalyst, afforded the key Mori's intermediate (−)-**88**. CET (−)-(**1**) could be likely obtained using Mori's protocol[85] in this way in 87% expected ee, 23 steps and 0.06%–0.2% calculated OY from L-proline (*S*)-**140**.

In their second formal synthesis of CET (−)-(**1**), an alkylidene carbene 1,5-CH insertion reaction was also used to establish the key tetrasubstituted stereocenter as previously described (Scheme 34).[142]

However, as there is no additional carbon atom on the phosphorane **349**, the previous tedious sequence to remove this very atom on ring D is no longer required (**341** → (−)-**88**), seven steps, 6.3%–18.3% OY, Scheme 33). The carbene precursor **350** was derived from *N*-Boc prolinal (*S*)-**348**, obtained from L-proline (*S*)-**140** in three steps and 94% yield.[143] The resulting spirocyclic product **352** of carbene insertion was converted by Friedel–Crafts cyclization into Mori's intermediate[85] **363** (R = Me) or (−)-**88** (R = -CH_2-), which were then transformed into CET (−)-(**1**) following Mori's procedure (Scheme 34).

Ketone **350** was prepared by Wittig reaction of aldehyde **348** with phosporane **349**. Protection of hydroxymethyl moiety with TBS was



Scheme 34 Second formal asymmetric synthesis of cephalotaxine (−)-(**1**) by Hayes (2008).

suitable for the carbene insertion, thus treatment of **350** with lithium trimethylsilyldiazomethane gave the azaspirocycle **352**, which was converted to NBoc spiroamine **355** by successive deprotection, oxidation, and decarbonylation in good yield (63.2%—84.6% from **352**). Because racemization occurred at the tetrasubstituted stereocenter on N-deprotection, compound **355** was first subjected to epoxidation. The epoxide **356** was not isolated and immediately treated with Lewis acid to provide **357** as the major product (2:1). Alcohol **357** was transformed into the CD amino alcohol **360** via tosylation, elimination with DBU to deliver the intermediate cyclopentene

186, and hydrolysis of the cyclic carbamate (Scheme 34). Alkylation of **360** with the known nosylates **361** and **362** provided the ACD intermediate allylic alcohols **363** and (−)-**364**, which were subjected to Friedel−Crafts cyclization relying on Mori's procedure[85] (R = Me, PPA, 85%) or in Kuehne's conditions[94] with tin tetrachloride to give the intermediate (−)-**88** although unexpectedly in medium yield. Herein, the relative configuration of the hydroxy group of **364** is very important. Compound **187**, the diastereomer of **363**, with the hydroxy group of opposite configuration, did not undergo the cyclization reaction (see Scheme 13). This sequence would allow access to CET (−)-(**1**) in 91% ee, in 22 steps and 0.3%−0.4% OY (R = Me) or in 20 steps and 0.4%−0.5% OY (R = -CH_2-) from L-proline (*S*)-(**140**).

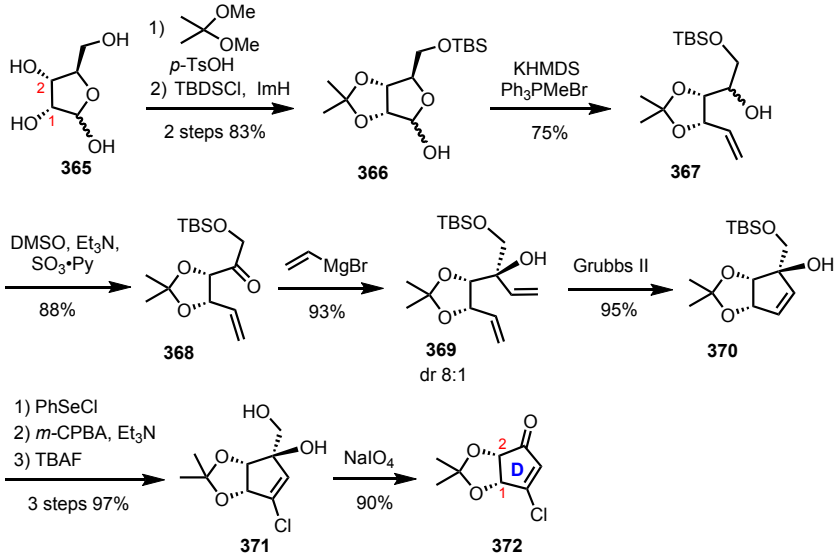
3.3.3.4 Djaballah and Gin's Synthesis (2008)

Three essential steps were used by Djaballah and Gin et al. for their synthesis of CET (−)-(**1**) (Scheme 36).[144] First, the nitrogen atom was introduced from the oxime **374** followed by Neber rearrangement. Secondly, construction of the benzazepine core relied on a strain-releasing rearrangement of *N*-vinyl-2-aryl aziridine **376** to **378**. Eventually, assembly of the spirofused pyrrolidine core occurred via 1,3-dipolar cycloaddition of azomethine ylide derived from vinylogous amide **379**. The chirality was introduced from the optically pure cyclopentenone **372** substituted on C1 and C2 (CET (**1**) numbering), prefiguring the D ring. Compound **372** was prepared from D-ribofuranose (**365**). The isopropylidene group allowed stereocontrol, so that only one diastereomer **381** was obtained.

Synthesis began by double protection of D-ribofuranose (**365**), then C1 olefination followed by Parikh−Doering oxidation furnished ketone **368**, which was alkylated with with vinylmagnesium bromide to provide the allylic alcohol **369** (8:1 dr) (Scheme 35).

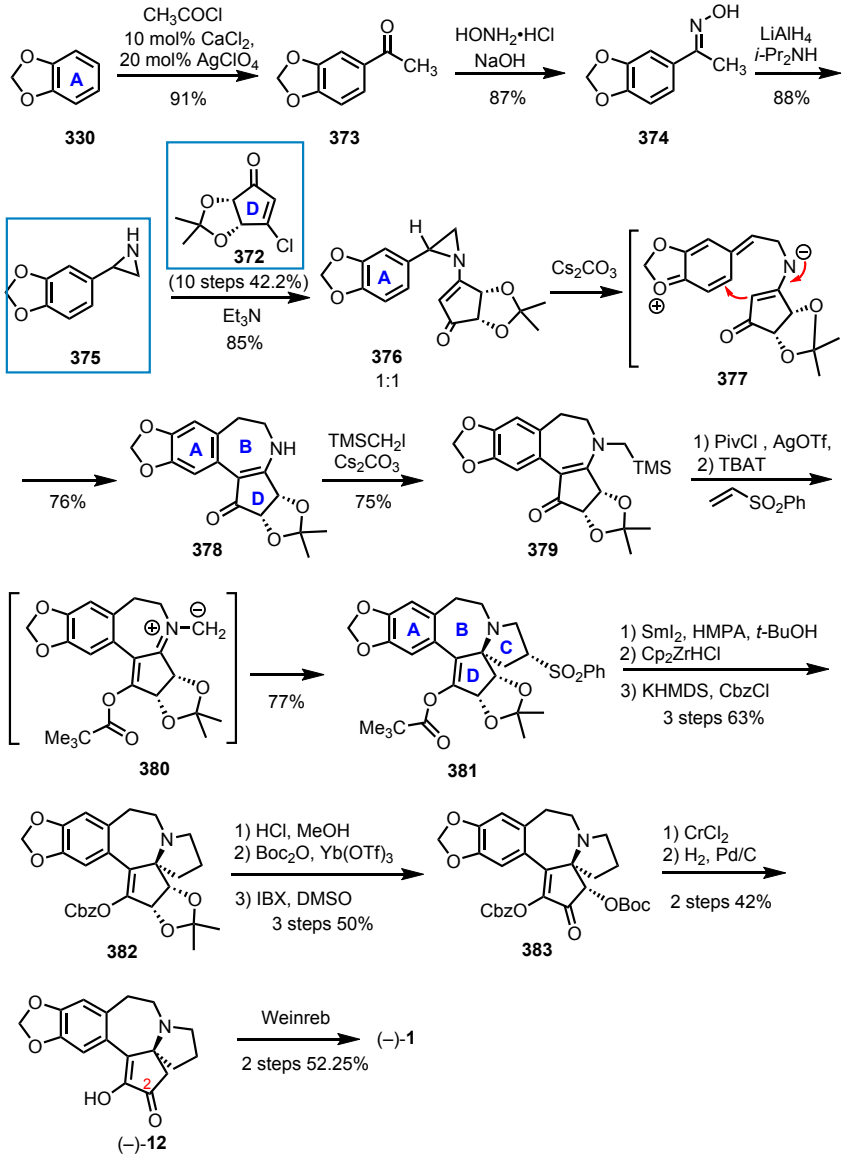
Cyclopentene **370** was prepared by an RCM (Grubbs II). Regioselective chloroselenylation of the alkene followed by selenide oxidation and elimination afforded the chlorocyclopentene and deprotection gave the vicinal diol **371**. Eventually, the chiral β-chloroenone **372** [10 steps, 42.2% OY from D-ribofuranose (**365**)] was obtained by periodate-mediated oxidative cleavage of the diol **371**.

After the synthesis of **372** featuring the D ring of CET (**1**) (Scheme 35), the aziridine **375** was prepared in a few steps (Scheme 36). Condensation of ketone **373** with hydroxylamine chlorhydrate furnished oxime **374**, which was reacted with $LiAlH_4$ and diisopropylamine at 60°C affording the



Scheme 35 Asymmetric synthesis of cephalotaxine (−)-(**1**) by Djaballah and Gin: formation of ring D (2008).

racemic aziridine **375** through a Neber-type reductive rearrangement. An addition−elimination sequence starting with the addition of aziridine **375** onto *β*-chloro-enone **372** furnished the *N*-vinyl aziridine **376** as a 1:1 diastereomeric mixture (Scheme 36). The dihydro-3-benzazepine **378** was prepared by heating the aziridine **376** with Cs_2CO_3, which generated an efficient rearrangement via opening of the aziridine. Compound **378** was N-alkylated with $TMSCH_2I$ to give the tertiary vinylogous amide **379**. O-Activation of vinylogous amide group in **379** was carried out by pivaloyl triflate (generated in situ by combination of pivaloyl chloride and AgOTf), followed by desilylation with TBAT to give azomethine ylide **380**. The dipolar cycloaddition with phenyl vinyl sulfone regioselectively afforded the spirofused pyrrolidine **381**. In unexpected ways, only one diastereoisomer **381** was obtained; the C1−C2 isopropylideneketal was effective as a stereodirecting element. The desired *R* configuration at C5 was determined by single crystal X-ray diffraction analysis. Radical desulfurization followed by hydrolysis of pivalate and reacylation with benzyl chloroformate led to **382**. After diol deprotection, differentiation of the C1 and C2 oxygen substituents was achieved by using Boc_2O and $Yb(OTf)_3$ leading to a selective C1-O-carboxylation. The hydroxy group at C2 was oxidized to ketone using iodoxybenzoic acid (IBX). The enone **383** reacted with



Scheme 36 Total asymmetric synthesis of cephalotaxine (–)-(**1**) by Djaballah and Gin (2008).

$CrCl_2$, which induced reductive deoxygenation of the O-Boc group. Subsequent benzylcarbonate hydrogenolysis provided demethylcephalotaxinone (–)-**12** (Weinreb's intermediate[83]). Sequential methyl enol ether derivatization of the C2 ketone (55%) and stereoselective reduction at C3

with $NaBH_4$ (95%) concluded the synthesis of (−)-CET (**1**) in 25 steps from D-ribofuranose (**365**) and 1.0% OY (ee n.r.).

3.3.3.5 Ishibashi's Total Synthesis (2008)

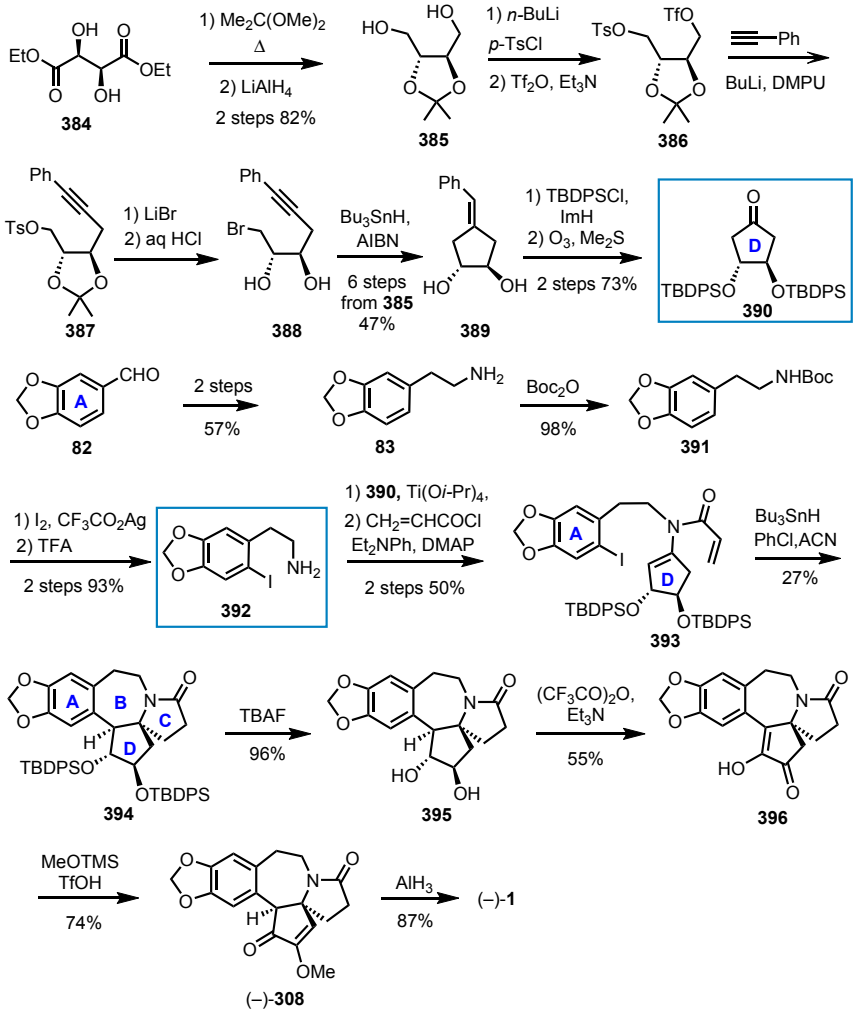
In 2008, Ishibashi et al. reported an asymmetric synthesis[145] based on a previous construction of the CET (1) skeleton using as characteristic feature an elegant radical cascade for the simultaneous construction of the B and C rings.[146] Diethyl D-(−)-tartrate (**384**) was transformed into acetonide diol **385** whose tosylation and reaction with lithium phenylacetylide gave compound **387** (Scheme 37). After bromination, the acetonide group was removed and the intermediate bromodiol **388** was subjected to Bu_3SnH-mediated radical cyclization to afford the cyclopentane diol **389** in 47% yield from **384** (eight steps). The two hydroxyl groups were protected and subsequent ozonolysis afforded the desired chiral cyclopentanone **390**, which was condensed with amine **392** in the presence of $Ti(O\textit{i}\text{-}Pr)_4$ to give an intermediary imine acylated with acryloyl chloride to afford enamide **393** in 50% yield (Scheme 37).

Its treatment with Bu_3SnH in the presence of 1,1′-azobiscyclohexanecarbonitrile (ACN) generated a radical cascade reaction building simultaneously the two rings B and C by a 7-*endo* and 5-*endo* selective cyclization, delivering the desired ABCD compound **394** in 27% yield. Deprotection of silylated hydroxyl groups by tetrabutylammonium fluoride gave diol **395**, which on treatment with trifluoroacetic acid anhydride, dimethyl sulfoxide, and Et_3N treatment afforded compound **396**. Methylation of **394** and ultimate reduction using aluminum hydride[96,132] led to CET (−)-(**1**) in 99.6% ee, ending the total asymmetric synthesis in 1.3% OY and 17 steps from diethyl D-tartrate (−)-(**384**).

3.3.4 Chemical Resolutions

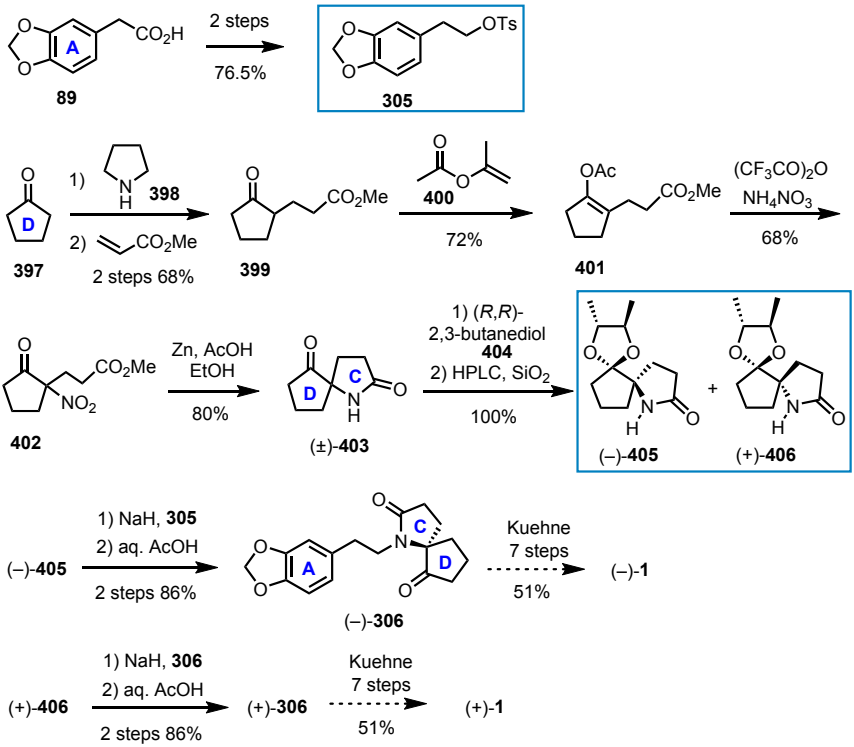
3.3.4.1 Nagasaka's Formal Synthesis (1997)

In 1997, Nagasaka et al. proposed the synthesis of CET (−)-(**1**) and *ent*-CET (+) (**1**) via resolution of chiral actetals of the spiroketone (±)-**403** providing the C and D rings (Scheme 38).[133] This compound was used in the known procedure developed by Kuehne.[94] Alkylation of the Stork enamine of cyclopentanone by methyl acrylate gave the cyclopentanone ester **399**. After formation of the vinyl acetate **401**, a nitro group (nitrogen atom precursor) was introduced using ammonium nitrate and trifluoroacetic anhydride to yield to the nitro compound **402**. Reduction of the nitro group and in situ lactamization allowed formation of the spirolactam (±)-**403**.



Scheme 37 Total asymmetric synthesis of cephalotaxine (−)-(**1**) by Ishibashi (2008).

Acetalization of (±)-**403** with (*R*,*R*)-2,3-butanediol **404** afforded two diastereoisomers (−)-**405** and (+)-**406**, which were easily separated by HPLC (100% combined). The optical purity was checked by chiral HPLC on compounds (−)-**403** and (+)-**403** obtained by hydrolysis of their respective acetals. Each enantiomer was confirmed to be enantiomerically pure. The two diastereoisomers (−)-**405** and (+)-**406** were alkylated with sulfonic ester **305** leading to Kuehne's intermediates (−)-**306** and (+)-**306**. Synthesis of CET (−)-(**1**) and *ent*-CET (+) (**1**) in 99% estimated



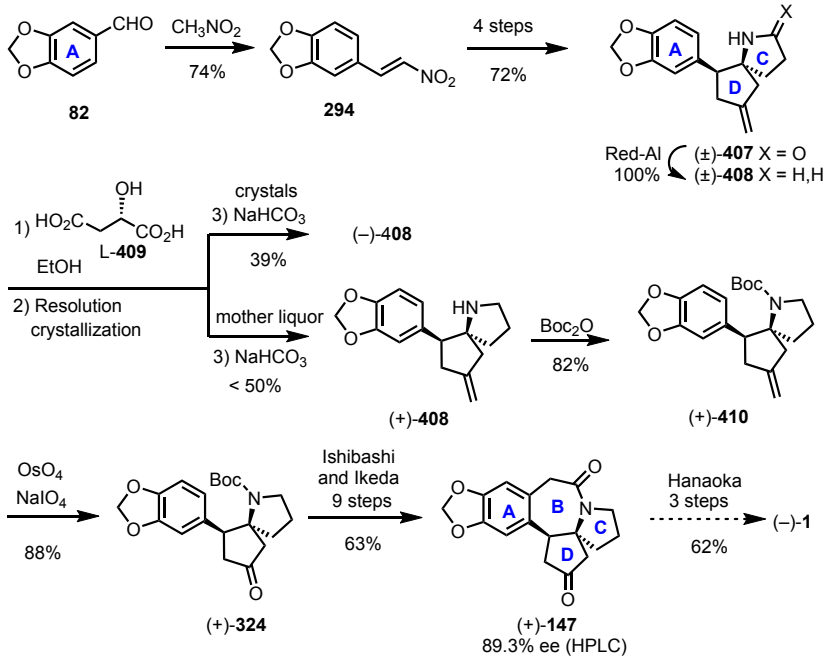
Scheme 38 Formal asymmetric synthesis of cephalotaxine (–)-(**1**) by Nagasaka (1997).

ee could be achieved employing Kuehne's procedure[94] in 16 steps from cyclopentanone (**397**) and 5.8% calculated OY for each enantiomer.

3.3.4.2 El Bialy's Formal Synthesis

In 2002, El Bialy et al.[140] described a resolution of ACD spiroamine (±)-**408**, an intermediate of Ishibashi and Ikeda's syntheses[130,137,138] of *rac*-CET (±)-(**1**), with L-malic acid **409** (Scheme 39). Formal synthesis of CET (–)-(**1**) through Hanaoka's intermediate (+)-**147**[96] is described in Section 3.3.2.2 (Scheme 32).

Recrystallization of the ammonium malate of spiro compound (±)-**408** gave the enantiomer (–)-**408** which could be converted to unnatural *ent*-CET (+)-(**1**). The mother liquor of malate salt afforded Ikeda's intermediate (+)-**408**[130] (yield not reported), which was protected and oxidized to the known ketone (+)-**324** and then transformed according to their sequence[137,138] into intermediate (+)-**149** in 89.3% ee measured by



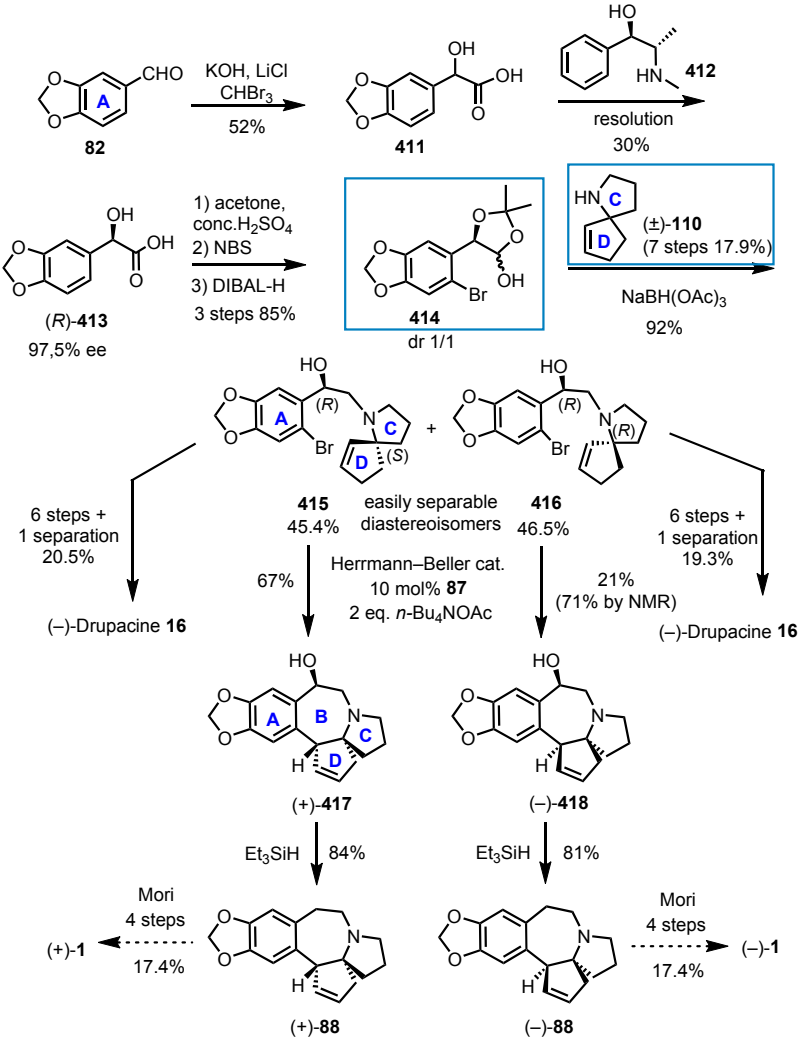
Scheme 39 Formal asymmetric synthesis of cephalotaxine (−)-(**1**) by El Bialy (2002).

HPLC. Applying Hanaoka's protocol would allow the synthesis of CET (−)-(**1**) in 22 steps, <7.5% OY and 89.3% estimated ee from piperonal (**82**).

3.3.4.3 Stoltz's Formal Syntheses (2007)

Stoltz et al. developed a formal asymmetric synthesis of the two enantiomers of CET (**1**) and the first total synthesis of (−)-dupracine (**16**) using the spirocyclic amine (±)-**110** first used in their synthesis of *rac*-CET (±)-(**1**) (Scheme 5) to introduce CD rings.[90] Compound (±)-**110** was coupled with the chiral nonracemic hemiacetal **414** obtained by chemical resolution (Scheme 40).[90]

3,4-Methylenedioxymandelic acid (*R*)-(**413**) (97.5% ee) obtained by chemical resolution with ephedrine (**412**) was protected as a 1,3-dioxolan-4-one prior to bromation then reduction to deliver compound **414** as a 1/1 mixture of diastereomers. This mixture reacted with spirocyclic amine (±)-**110** (see Scheme 5) under reductive amination conditions to give diastereomers **415** and **416** in a 1:1 ratio, which were smoothly separated by chromatography. Both diastereomers were submitted separately to Heck reaction conditions to produce benzazepines (+)-**417** and (−)-**418**.



Scheme 40 Asymmetric syntheses of (−)-drupacine (**16**) and formal asymmetric syntheses of cephalotaxine (−)-(**1**) and *ent*-cephalotaxine (+)-(**1**) by Stoltz (2007).

The benzylic hydroxy group was removed via an ionic deoxygenation procedure to afford the two enantiomers (+)-**88** and (−)-**88** of Mori's intermediate,[85] respectively. Formally, both enantiomers of CET (**1**) could be obtained in 97.5% ee over 14 steps from β-ketoester **105**, precursor of the CD unit (±)-**110** (Scheme 5), in 0.2% OY for natural CET (−)-(**1**) and 0.7% OY for *ent*-CET (+)-(**1**). In their report, Stoltz et al. also completed the first asymmetric total synthesis of (−)-drupacine (**16**) in six steps from

both *anti* and *syn* ABCD units (+)-**417** (20.5% OY) and (−)-**418** (19.3% OY) via a dynamic β-elimination/conjugate addition process of advanced intermediate *a*-hydroxyenones during acetalization (Schemes 5 and 40). Starting from β-ketoester **105**, the sequence consisted in 16 steps via spirocyclic enamine (±)-**110** and delivered natural drupacine (−)-**16** in a combined 1.34% OY. It is worth noting that there remains only one total synthesis of racemic drupacine (±)-(**16**) disclosed by Fuchs in 1990.[147]

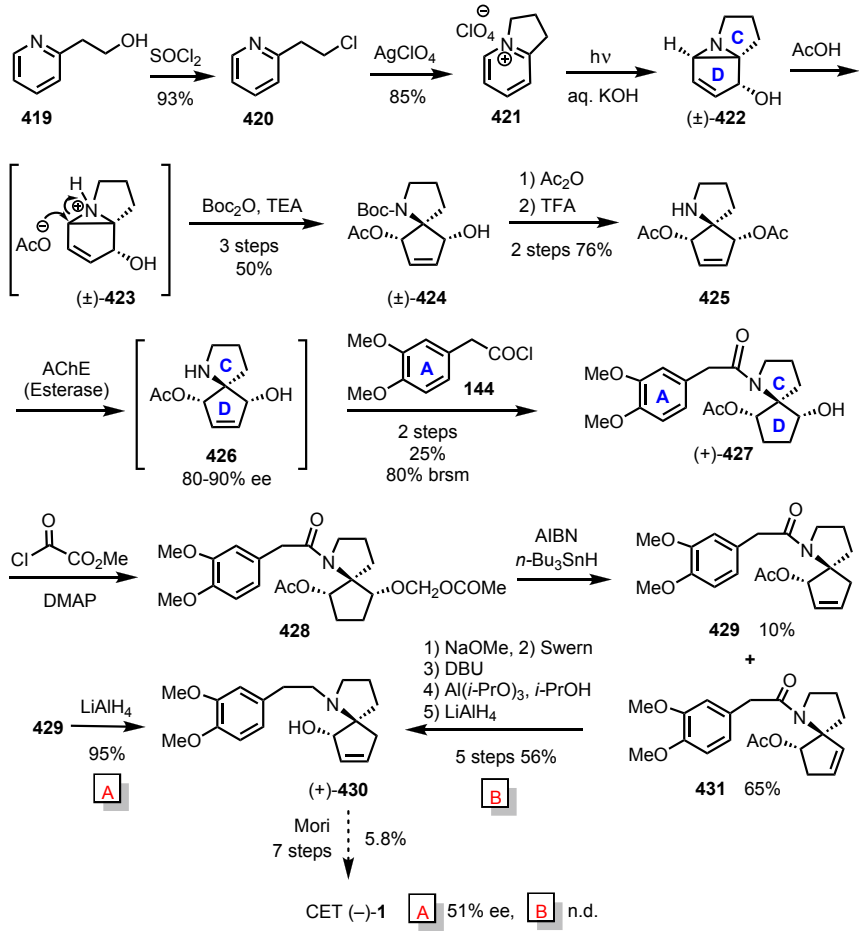
3.3.5 Enzymatic Resolutions

3.3.5.1 Mariano's Formal Syntheses (2006)

In 1994 and 1996 Mariano et al. reported a biomimetic synthesis of *rac*-CET (±)-(**1**) by a transannular cyclization.[113,114] In 2006, they developed two formal syntheses of CET (−)-(**1**) based on photochemistry of pyrrolo-fused pyridinium perchlorate **421** leading to strained aziridine (±)-**422**.[127,128] The first synthesis led to Mori's intermediate[85] **430** (Scheme 41) and the second one to Suga and Yoshida's intermediate[86,87] (−)-**102** (Scheme 42).

The pyrrolo-fused pyridinium salt **421** was irradiated to give the tricyclic aziridine **422**, its regioselective ring opening with acetic acid, amine protection with Boc, then protection of alcohol as acetic ester, at last deprotection of the Boc group with TFA led to *meso* spirocyclic aminodiester **425** in five steps and 38% OY. Desymmetrization by enzymatic hydrolysis with electric eel acetyl cholinesterase afforded the unstable aminomonoalcohol **426** in the range of 80%−90% ee, which reacted with (3,4-dimethoxyphenyl)acetyl chloride (**144**) to furnish amidoalcohol **427**. To remove hydroxy group at C1, compound **427** was transformed into oxalate **428**, which underwent radical reduction by treatment with azobisisobutyronitrile (AIBN)/*n*-Bu_3SnH generating the desired product **429** in a 10% yield along with the isomerized homoallylic alcohol **431** as the major product (65%). Mori's intermediate **430** was obtained by reduction of **429** with $LiAlH_4$. It was also obtained through a five-step sequence (55% OY) from the rearranged homoallylic alcohol **431** involving alcohol deprotection and oxidation, α,β-unsaturated enone formation, and then ketone and amide reduction. This formal synthesis could allow the synthesis of CET (−)-(**1**) in 19/23 steps from **419** via ACD intermediate **430** with 0.2% expected OY (0.5% OY brsm) and 51% estimated ee. This medium ee indicates that some epimerization occurred during transformation of spirocyclic compound **426** into ACD unit **430**.

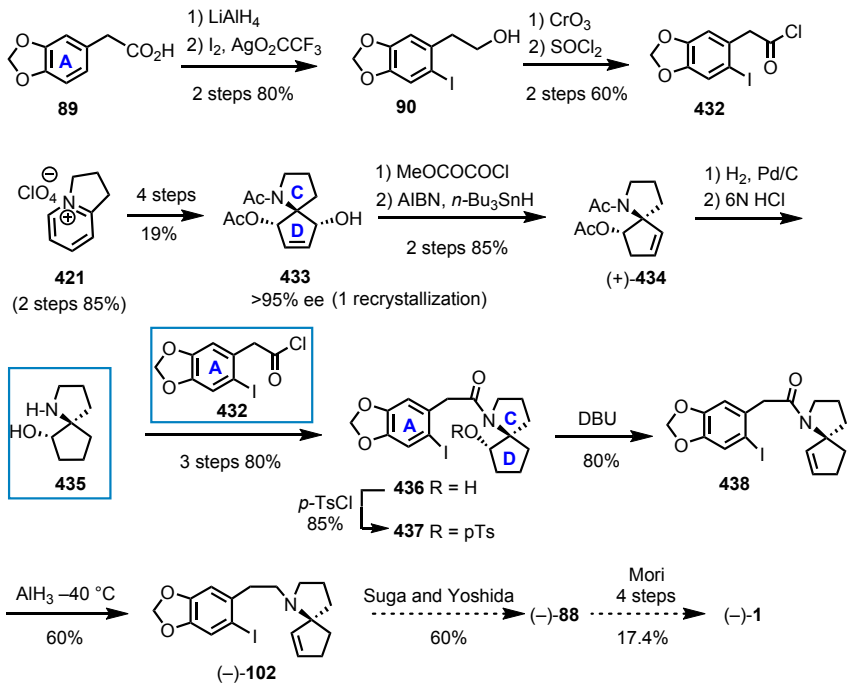
The second approach by Mariano et al. described the synthesis of the ACD unit (−)-**102**, which is the key intermediate described by Suga and



Scheme 41 Formal asymmetric synthesis of cephalotaxine (−)-(**1**) by Mariano via Mori's intermediate **430** (2006).

Yoshida[86,87] (Scheme 42). The azaspiroalcohol **433** was prepared earlier[128] in enantiomerically pure form by using a sequential photocyclization/aziridine ring opening/enzymatic desymmetrization sequence with >95% ee in four steps and 19% OY from pyridinium salt **421**. Radical reduction with tributyltin hydride and AIBN of the mixed oxalate gave the homoallylic ester **434**.

Hydrogenation of **434** with 10% Pd/C followed by hydrolysis with HCl generated the spirocyclic alkanolamine **435**. Reaction with iodoarylacetyl chloride **432** furnished the spirocyclic amidoalcohol **436**, which was transformed into the tosylate **437**. Tosylate elimination with DBU afforded the



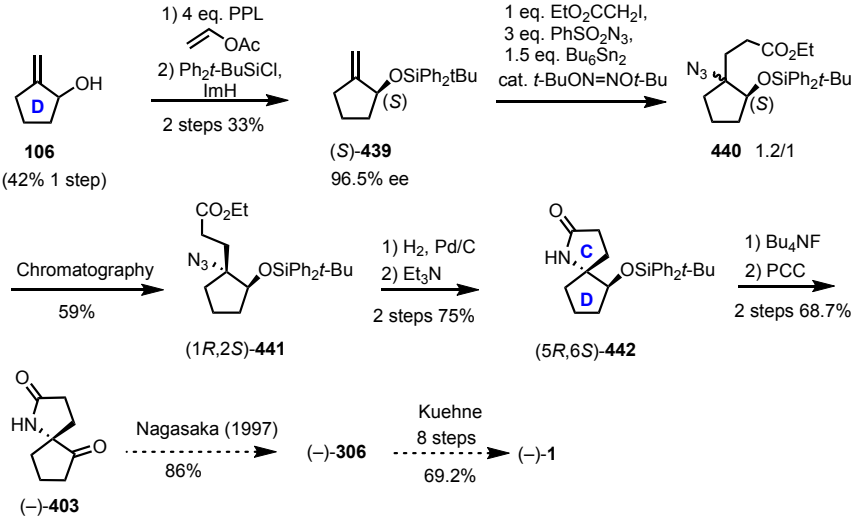
Scheme 42 Formal asymmetric synthesis of cephalotaxine (−)-(**1**) by Mariano via Suga and Yoshida's intermediate (−)-**102** (2006).

unsaturated amide **438**, which was reduced with aluminum trihydride to give the Suga and Yoshida intermediate (−)-**102**. This formal synthesis would give CET (−)-(**1**) in 95% expected ee over 19 steps in 0.5% calculated OY from alcohol **419**.

3.3.5.2 Renaud's Formal Synthesis (2012)

In 2012, another formal synthesis of CET (−)-(**1**) was reported by Renaud.[124] Unlike the alternative approach (see Section 3.3.1.3, Scheme 27), an access to azaspiroamide **404** (Nagasaka's intermediate)[133] was achieved through a stereoselective carboazidation sequence as the key step (Scheme 43).

In this synthesis, the silylated derivative (*S*)-**439** (96.5% ee) was prepared from racemic cyclopentanol[148] **106** via enzymatic enantioselective acetylation with porcine-pancreas lipase (PPL). Carboazidation of (*S*)-**439** afforded azido derivative **440** in 89% yield as a 1.2:1 mixture of isomers and their separation gave the major azido ester (1*R*,2*S*)-**441** in 59% yield. Then,



Scheme 43 Formal asymmetric synthesis of cephalotaxine (−)-(**1**) via Nagasaka's intermediate by Renaud (2012).

reductive lactamization, desilylation, and oxidation gave Nagasaka intermediate (*R*)-**403**. Formally, this intermediate could be converted into CET (−)-(**1**) according to Kuehne[94] and Nagasaka's[133] protocols, in 18 steps, 1.8% calculated OY (alternatively 19 steps and 2.5% OY) and 96.5% estimated ee from methylene cyclopentanol **106** (see Scheme 38).

To conclude this part, it is interesting to compare the target intermediates in the formal syntheses of CET (**1**) as subtle changes in their structure may induce very different reactivities and OY of transformation into CET, either in its racemic form (±)-(**1**) (Fig. 18) or optically active ones, the natural enantiomer (−)-(**1**) and the distomer (+)-(**1**) (Fig. 19). In addition, this schematic view shows in some ways the interconnections between the different approaches.

4. SYNTHESIS AND COUPLING OF THE SIDE CHAINS OF *CEPHALOTAXUS* ESTERS

Side chains of *Cephalotaxus* alkaloids are very important for the biological activity of the corresponding esters since it is well known that CET itself does not exhibit interesting activity. They are characterized by a relatively simple structure with a chiral tertiary alcohol moiety. Nevertheless, coupling of the side chain to CET remains an important challenge due to the steric

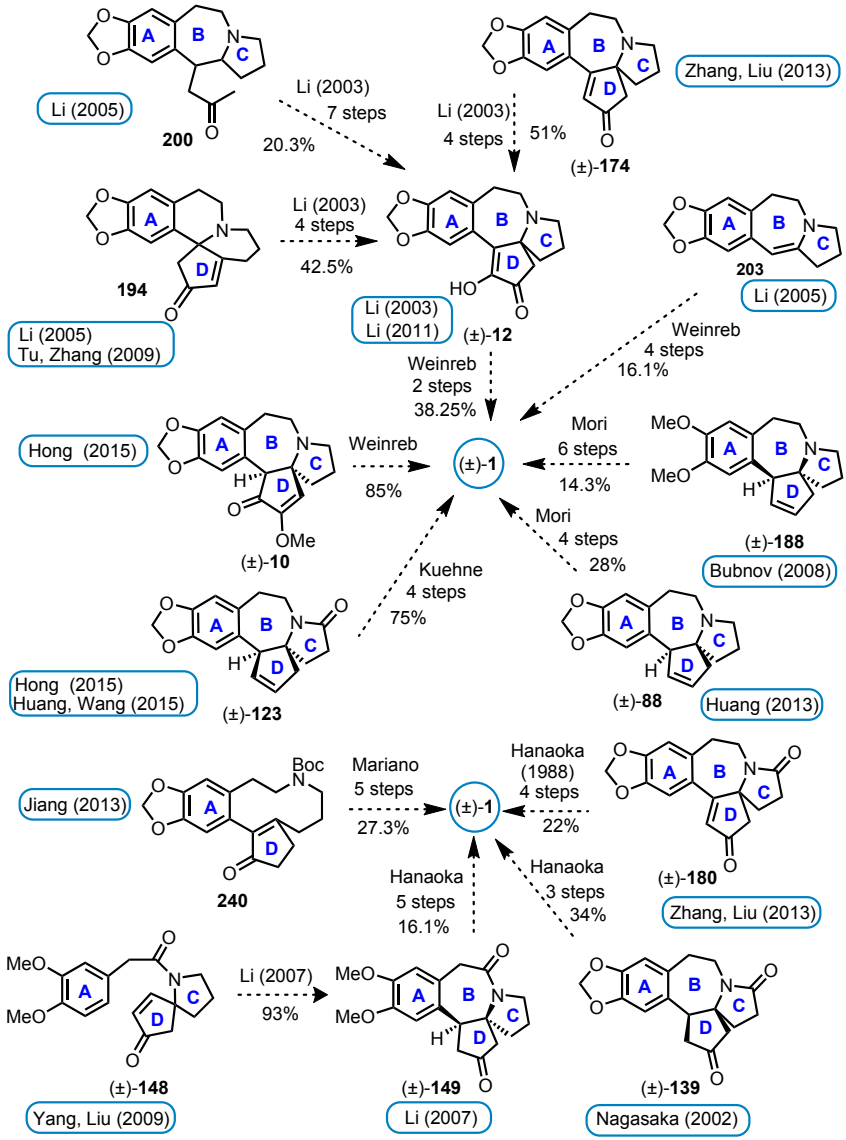


Figure 18 Target intermediates in the formal syntheses of (±)-cephalotaxine (**1**). Names in boxes refer to a formal synthesis.

strain exhibited at both the alcoholic function of CET at C3 and the carboxylic function of the side chains, which precluded a classical esterification process (Scheme 44). Thus, in most of the syntheses of *Cephalotaxus* alkaloids, the esterification is done with a late, activated precursor of the

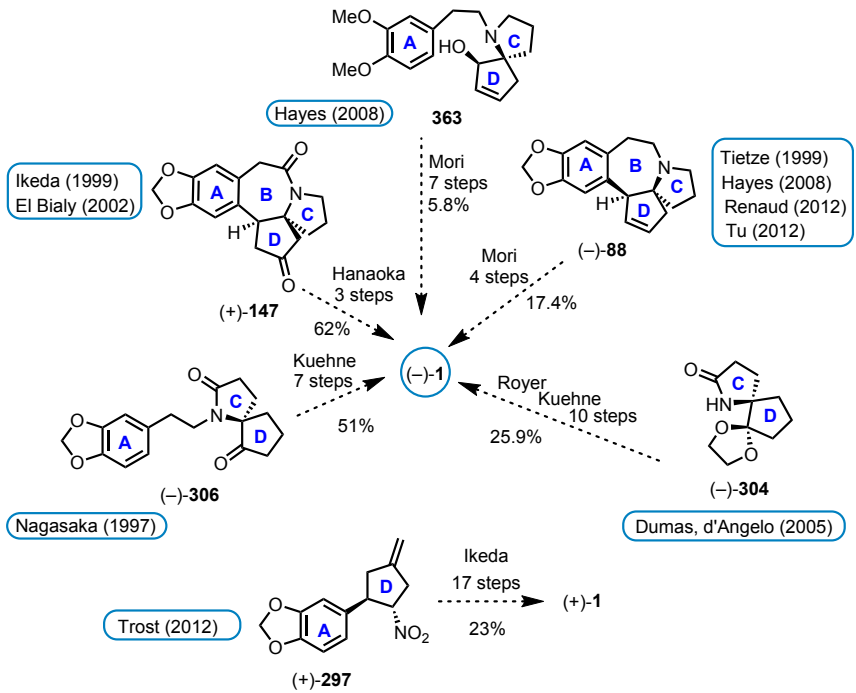
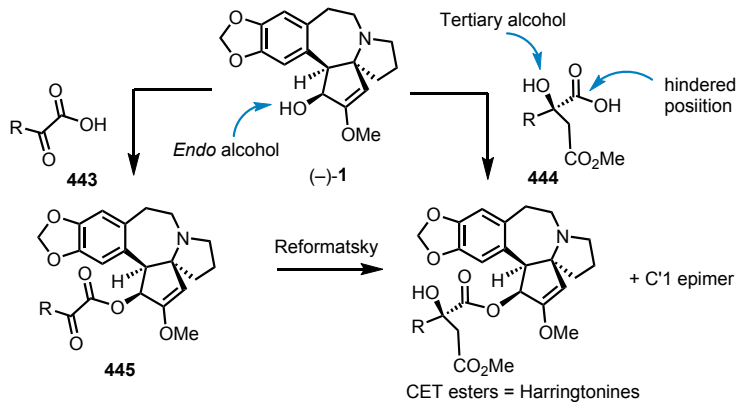


Figure 19 Target intermediates in the formal asymmetric syntheses of cephalotaxine (**1**). Names in boxes refer to a formal synthesis.



Scheme 44 Coupling of the side chain onto cephalotaxine (–)-(**1**).

side chain. It thus appeared that syntheses of the acidic side chains themselves are not useful processes to attain the *Cephalotaxus* esters. Nevertheless, numerous interesting syntheses of the acidic side chains have been reported. We will first report on the synthesis of the CET esters and then on the synthesis of the side chains. In each part, syntheses will be presented in chronologic order.

4.1 Synthesis of Cephalotaxine Esters

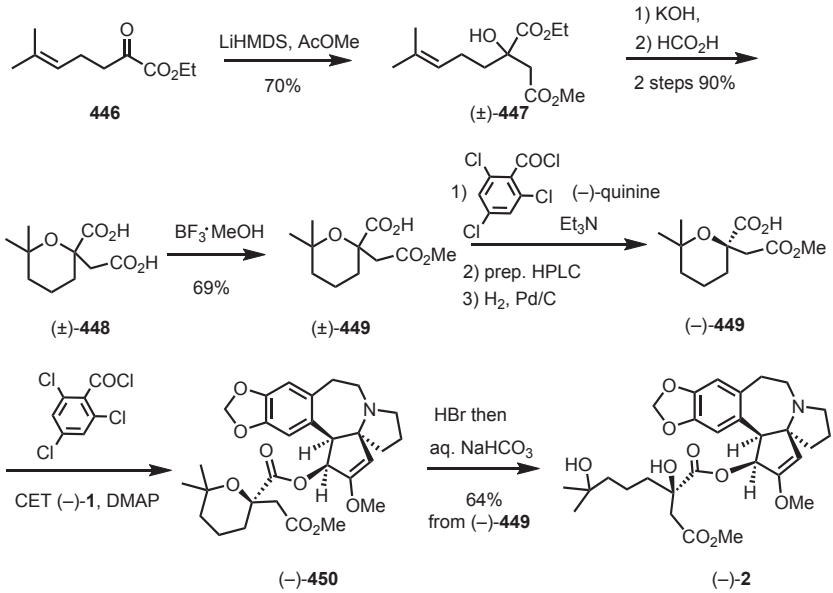
The previous review in this series[2] reported more than 15 syntheses of CET's esters. Due to the impossible coupling with the entire side chain **444**, most of these syntheses dealt with the esterification of CET (**1**) with a precursor α-keto acid **443** as shown in Scheme 44. Formation of the α-ketoester **445** was followed by the introduction of the acetyl group through a Reformatsky reaction. Although this esterification/Reformatsky alkylation sequence is interesting, proceeded well and allowed the preparation of various HTs such as HT (**3**), HHT (**2**), deoxyHT (**5**), and so on, the sequence also exhibits a major drawback: the Reformatsky reaction on the prochiral ketoester part of **445** continuously gave a mixture of two epimers in a 1/1 mixture leading thus to the loss of half part of the CET. This major inconvenience was avoided in only two syntheses of HHT (**2**): the synthesis reported by Hudlicky[149] (where the presence of the second keto group allowed a chelation with the Reformatsky reagent leading to the formation of a single stereomer) and the synthesis of Cheng[150] (using a chiral sulfinyl ester).

Since the previous review in this series,[2] only four articles dealing with syntheses of CET esters have been published.

4.1.1 Robin's Synthesis of Semisynthetic Homoharringtonine (1999)

The first one by Robin[21] in 1999 is quite interesting in opening new routes to these alkaloids. This strategy was based on the esterification of CET (**1**) with a cyclic precursor of the side chain, the tetrahydropyran *rac*-**449** (Scheme 45), which was easily prepared in four steps from α-ketoester **446**.

Ketoester **446** was treated with the lithium methyl acetate to give diester **447**, which was fully saponified and then cyclized with formic acid to the diacid **448**. The latter was selectively monoesterified with $BF_3 \cdot MeOH$ to furnish the acid ester **449**. CET (**1**) was coupled with *rac*-**449** using dicyclohexylcarbodiimide or mixed anhydride method, but once again the



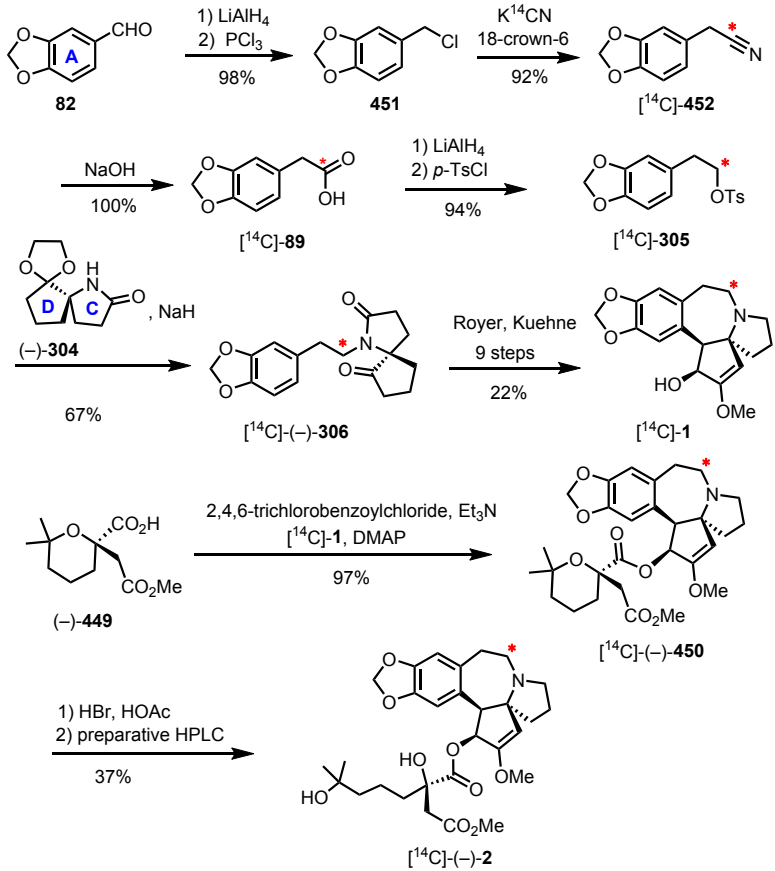
Scheme 45 Hemisynthesis of semisynthetic homoharringtonine (**2**) by Robin (1999).

obtained ester was a mixture of two epimers in a 6/4 ratio and had to be separated by chromatography. While the authors showed that highly enantiomerically pure HHT (**2**) (99.8% ee) could be obtained in a large scale process, they also developed a diastereoselective sequence. For this purpose, *rac*-**449** was resolved through its quinine ester. Eventually, coupling of CET (−)-(**1**) and (−)-**449** gave anhydroHHT (**450**), which can also be hydrolyzed to enantiopure HHT (−)-(**2**). This methodology using tetrahydropyran **449** was patented in 2005 and applied to synthetize various analogs.[151]

4.1.2 Marguerit's Synthesis of [^{14}C]-Labeled Homoharringtonine (2015)

In 2015, Marguerit et al. achieved the first radiolabelled synthesis of HHT (−)-(**2**) (Scheme 46).[152] This synthetic process enabled the production of Good Manufacturing Practice (GMP) compliant [^{14}C]-HHT (−)-(**2**). This ^{14}C-labeled compound was used in a human mass balance study that was a postapproval commitment to the US FDA.

[^{14}C]-Labeled HHT ([^{14}C]-(−)-**2**) was obtained from [^{14}C]-labeled CET ([^{14}C]-(−)-(**1**)) according to an efficient semisynthesis described in 1999 using enantiopure Robin's acid **449**.[21] The synthetic strategy to obtain [^{14}C]-CET (−)-(**1**) is based on the coupling of [^{14}C]-labeled tosylate



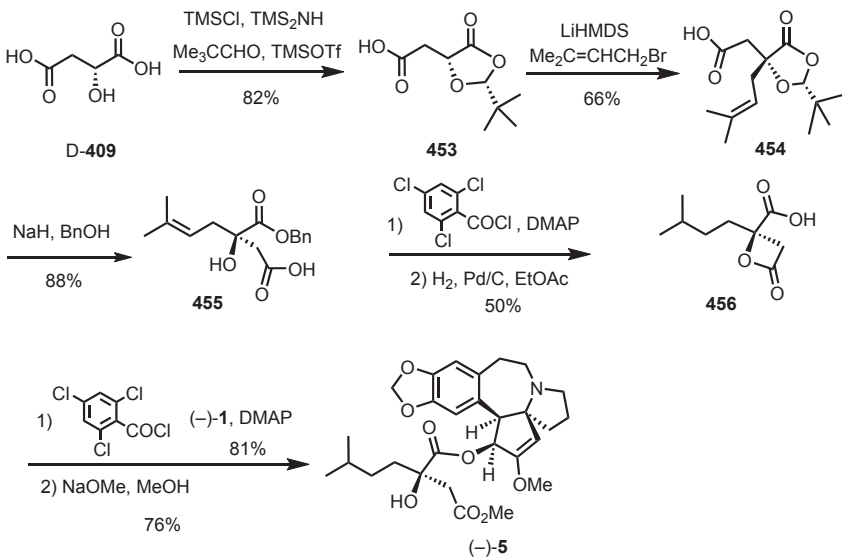
Scheme 46 Synthesis of [^{14}C]-labeled homoharringtonine (−)-(2) by Marguerit (2015).

intermediate [^{14}C]-**305** with enantiopure spirolactam (−)-**304** developed by Royer[132] for the synthesis of CET (−)-(**1**). For their purpose, introduction of the [^{14}C] label was done by reaction of benzyl chloride intermediate **451** with potassium [^{14}C]-cyanide, a readily available source of carbon-14, yielding compound [^{14}C]-**452**, followed by transformation into [^{14}C]-tosylate **305**. This convergent approach proceeds via two key unlabeled intermediates **304** and **451** synthesized in parallel. Alkylation of spirolactam (−)-**304** with the tosylate [^{14}C]-**305** afforded ACD unit [^{14}C]-(−)-**306**, which was transformed into radiolabelled [^{14}C]-CET (−)-(**1**). Eventually, [^{14}C]-HHT (−)-(**2**) was obtained using Robin's protocol by coupling [^{14}C]-CET (−)-(**1**) with Robin's acid (−)-(**449**) and hydrolysis of the side chain of intermediate anhydroHT [^{14}C] (−)-(**450**).[21] From piperonyl

alcohol obtained from piperonal (**82**), the 17-steps sequence provided after preparative reverse phase HPLC a 100% enantiopure product with a radiochemical purity of 98.9% and a chemical purity of 98.5% in 4.475% OY.

4.1.3 Gin's Synthesis of Deoxyharringtonine (2006)

The synthesis of deoxyHT (−)-(**5**) reported by Gin in 2006[153] was the most recent and also the most efficient method of synthesis of CET esters. This synthesis was based on the esterification of a cyclic acid, namely the β-lactone **456** obtained in a nonracemic form through an efficient sequence (Scheme 47). It started with acetal derivatization of D-malic acid D-(**409**) with pivalaldehyde to give dioxolanone **453**. Diastereoselective alkylation of dioxolanone **453** with prenyl bromide was accomplished after deprotonation with LiHMDS to give the prenylated dioxolanone **454** in 66% yield. This sequence allowed the introduction of the chiral tertiary alcohol with complete diastereoselectivity following the studies of D. Seebach[126] and Tietze (see Scheme 51).[154] Transesterification with acetal cleavage of **454** using benzyl alcoholate provided the tertiary alcohol **455**, which was cyclized to the β-lactone followed by hydrogenolysis and hydrogenation to give the acid **456**. Activation of the carboxylic function with 2,4,6-trichlorobenzoyl chloride allowed the acylation of CET (−)-(**1**) followed by transformation into deoxyHT (−)-(**5**) on methanolysis.



Scheme 47 Synthesis of deoxyharringtonine (−)-(**5**) by Gin (2006).

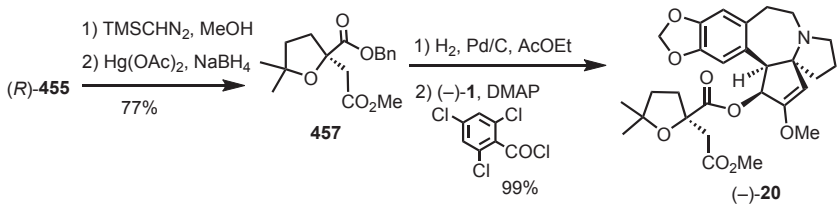
4.1.4 Djaballah and Gin's Synthesis of Anhydroharringtonine, Homoharringtonine, and Homodeoxyharringtonine (2008)

Djaballah, Gin, and coworkers extended this study to the syntheses of others CET esters such as HHT (−)-(**2**), homodeoxyHT (−)-(**22**), and anhydroHT (−)-(**20**).[144] Preparation of anhydroHT (−)-(**20**) (Scheme 48) involved the intermediate (*R*)-**455** of the previous synthesis[153] (see Scheme 47), which, after transformation into the corresponding methyl ester, was cyclized to tetrahydrofuran **457** through alkoxymercuration. Deprotection of the benzyl ester and activation as a mixed anhydride (Yamaguchi's reagent) allowed the coupling with CET (−)-(**1**) to give anhydroHT (−)-(**20**) in excellent yield.

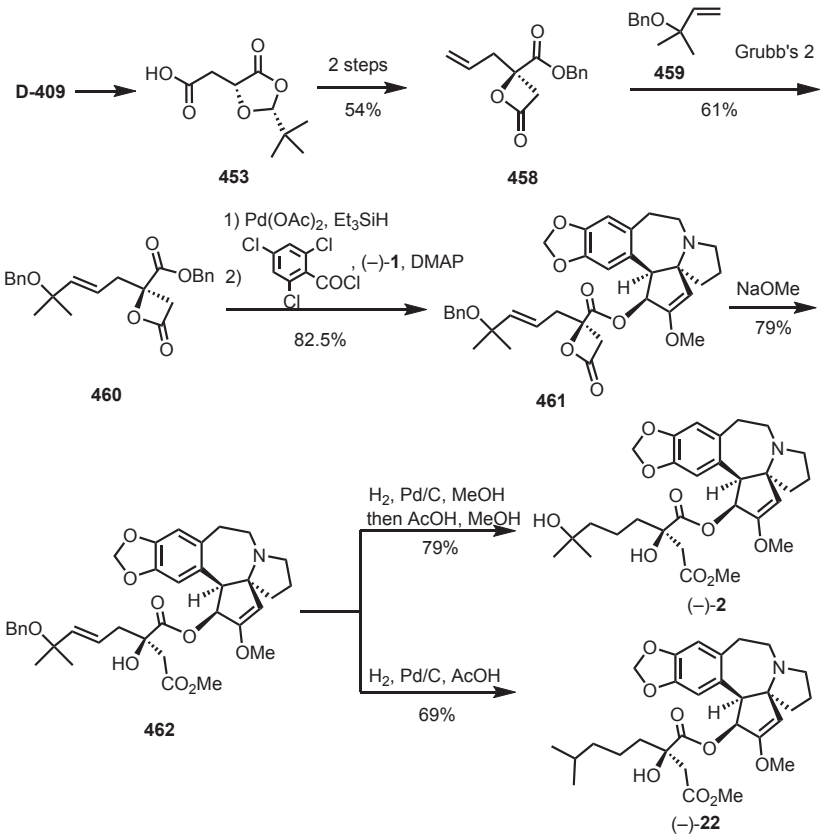
Preparation of HHT (−)-(**2**) and homodeoxyHT (−)-(**22**) (Scheme 49) involved a synthesis based on β-lactone **458** obtained through a sequence, which mimicked the synthesis described in Scheme 47. A cross-metathesis provided the disubstituted alkene **460** in 61% yield (a dimeric bislactone was also formed). After cleavage of the benzyl ester, the activation of the carboxyl group (Yamaguchi's reagent) allowed the coupling with CET (−)-(**1**) to furnish **461**, which was transformed into **462** on methanolysis. Preparation of each natural compound HHT (−)-(**2**) and homodeoxyHT (−)-(**22**) from this common intermediate is interesting. When allyl benzyl ether **462** was treated with H_2 (Pd/C) in MeOH followed by addition of AcOH at the last stage of the reaction, HHT (−)-(**2**) was isolated in 79% yield. On the other hand, **462** gave homodeoxyHT (−)-(**22**) in 69% yield when submitted to Pd/C catalyzed hydrogenation in glacial AcOH.

4.2 Synthesis of Side Chains of *Cephalotaxus* Esters

Though it has been clearly shown that coupling of CET (**1**) with entire side chains was not possible, syntheses of these side chains have been continuously prolific. All the syntheses reported since 1998 were asymmetric and



Scheme 48 Synthesis of anhydroHT (−)-(**20**) by Djaballah and Gin (2008).

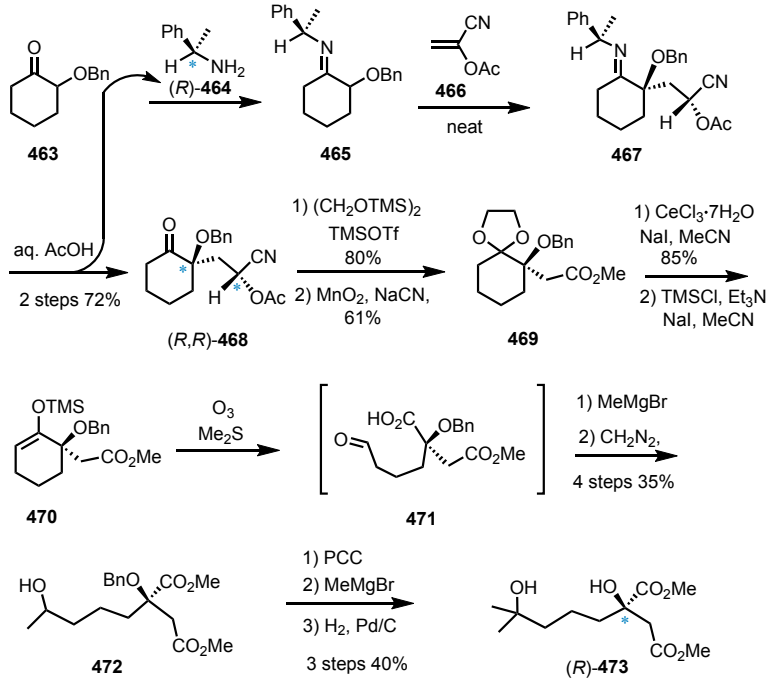


Scheme 49 Synthesis of homoharringtonine (−)-(**2**) and homodeoxyharringtonine (−)-(**22**) by Djaballah and Gin (2008).

are of interest due to their diversity and originality. Some of them included the preparation of already described intermediates in the synthesis of *Cephalotaxus* esters and then could be considered as formal syntheses of the natural products.

4.2.1 Dumas and d'Angelo's Synthesis (2001)

The first asymmetric synthesis of the side chain ester **473** of HHT (**2**) was reported by Dumas and d'Angelo.[155,156] In this work, the formation of the chiral tertiary alcohol center was accomplished in a highly diastereoselective manner in an early stage of the sequence. The synthesis started with the preparation of the known chiral imine **465** (Scheme 50), which was subjected to a Michael reaction with acetoxyacrylonitrile (**466**), an equivalent

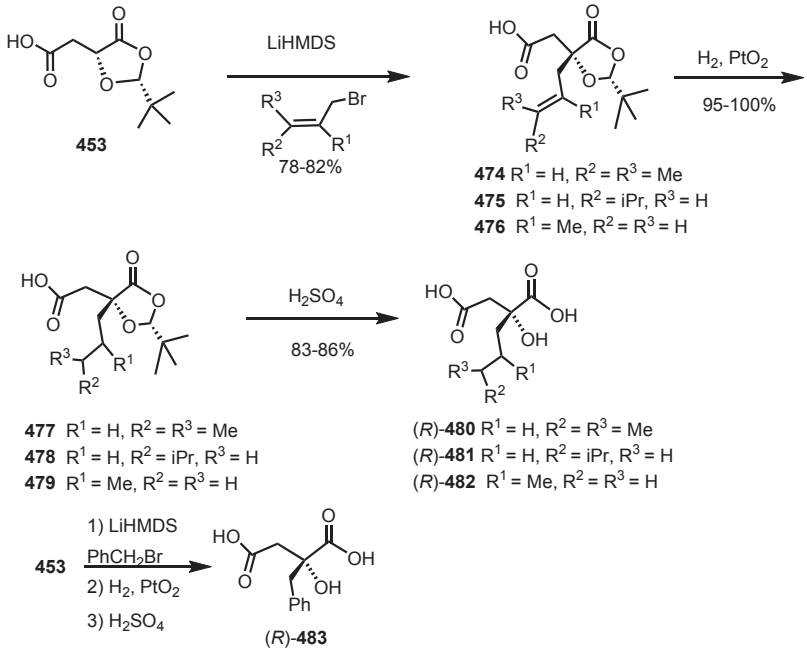


Scheme 50 Synthesis of homoharringtonine side chain (*R*)-**473** by Dumas and d'Angelo (2001).

of the acid side chain. The adduct **467** was proved to be stereochemically homogeneous and was hydrolyzed without purification to give the more stable ketone (*R*,*R*)-**468**. The keto group was protected as a dioxolane and the potential aldehyde function was directly transformed into the ester **469** on treatment with MnO_2 in MeOH in the presence of NaCN. The ketal of ester **469** was cleaved using $CeCl_3 \cdot 7H_2O$ and NaI, and the so-formed keto group was converted to the silylvinyl ether **470**. Ozonolysis of **470** gave the sensitive aldehyde **471**. Addition of MeMgBr to the crude aldehyde and esterification of the acid furnished alcohol **472**. Oxidation of the alcohol **472** to a methylketone followed by new addition of MeMgBr gave the tertiary alcohol, which eventually on hydrogenolysis led to (*R*)-methyl 3,7-dihydroxy-3-methylcarbonyl-7-methoxyoctanoate (**473**), the methyl derivative of HHT side chain.

4.2.2 Tietze's Synthesis (2005)

In 2005, Tietze reported the preparation of the chiral side chain acids **480**–**483** of deoxyHT (**5**), homodeoxyHT (**22**), nordeoxyHT (**21**), and neoHT

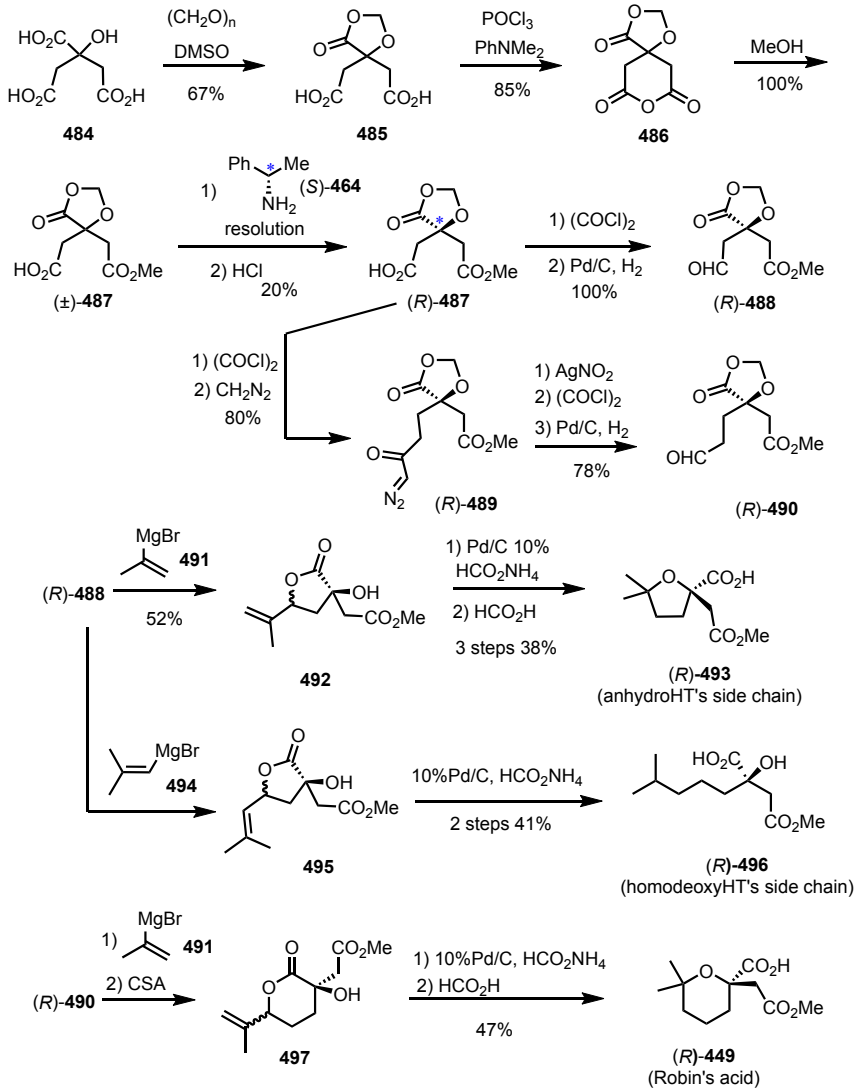


Scheme 51 Synthesis of side chain acids of harringtonines by Tietze (2005).

(**19**), respectively (Scheme 51)[154] based on Seebach's procedure for the alkylation of D-malic acid with self-regeneration of stereogenic centers.[126] The strategy was used later by Gin for the preparation of CET esters.[153] The dioxolanone **453** prepared from D-malic acid (D-**409**) was treated with two equivatents of LiHMDS followed by bromoallyl derivatives to give allylated dioxolanones **474**–**476** in high yield and diastereoselectivity. On hydrogenation on PtO_2, these compounds furnished dioxolanones **477**–**479** whose acid hydrolysis permitted the isolation of **480**–**482**, side chains of deoxyHT (**5**), homodeoxyHT (**22**), and nordeoxyHT (**21**), respectively. When **453** was alkylated with benzyl bromide, the same sequence allowed the obtention of **483,** the side chain of neoHT (**19**).

4.2.3 Russel's Synthesis (2006)

An original approach was reported by Russel et al. in 2006.[157] The synthesis started with chiral nonracemic aldehydes (*R*)-**489** or (*R*)-**491** (Scheme 52) whose preparation in large scale was reported by the authors from citric acid (**484**) and resolution of dioxolanone (±)-**487**.[158]



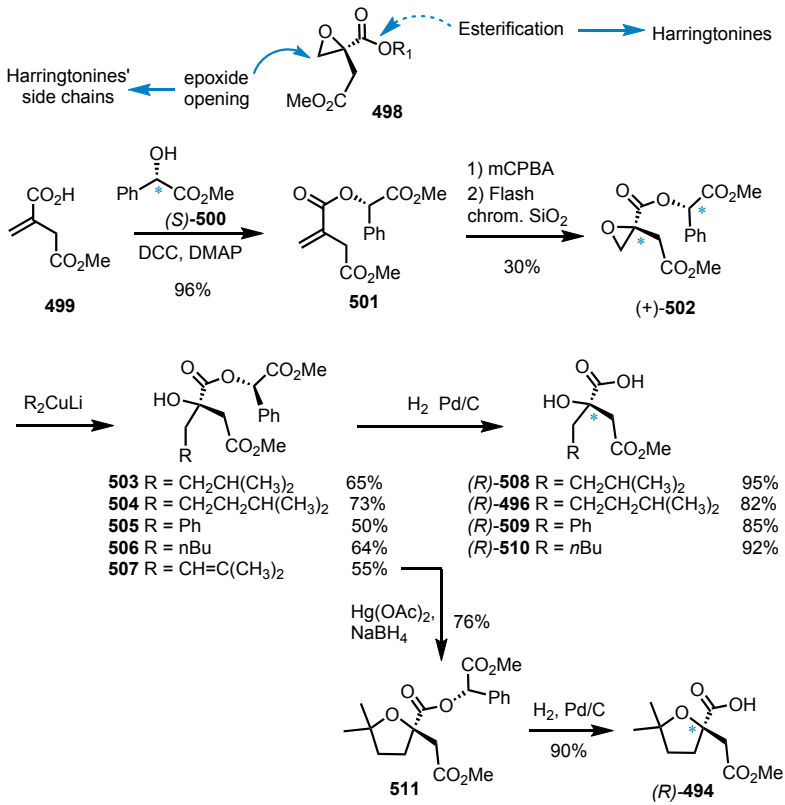
Scheme 52 Synthesis of harringtonines' side chains by Russel (2006).

Three compounds were described in this study: (*R*)-**493** and (*R*)-**496**, the anhydroHT and HHT's side chains respectively, and (*R*)-**449**, the intermediate used by Robin for the synthesis of HHT (**2**) (see Scheme 46) thus representing a formal synthesis of HHT (**2**). Dioxolanone (*R*)-**487** was obtained in three steps and resolution in 11.4% OY from citric acid (**484**).[158] The acid function of (*R*)-**487** was selectively reduced under

Rosenmund conditions to aldehyde, which reacted with a vinyl Grignard reagent (**491** or **494**) to give lactones **492** or **495**. Lactone **492** was reduced with rearrangement to (*R*)-**493**, the side chain of anhydroHT (**20**) obtained in 2.3% OY (nine steps from **484**), while reduction of lactone **495** gave (*R*)-**496**, the side chain of homodeoxyHT (**22**) in 4.7% OY over eight steps from **484**. The same sequence was applied to aldehyde (*R*)-**490** allowing preparation of Robin's intermediate (*R*)-**449** useful for the synthesis of HTs, in 3.3% OY and 12 steps from citric acid (**484**).

4.2.4 Royer's Synthesis (2009)

Several *Cephalotaxus* alkaloid side chains were prepared by Royer through a rapid synthesis (four—five steps).[159] The central point of this synthesis is a chiral nonracemic epoxide **498** (Scheme 53). Side chains were obtained on epoxide ring opening with appropriate nucleophiles. On the other



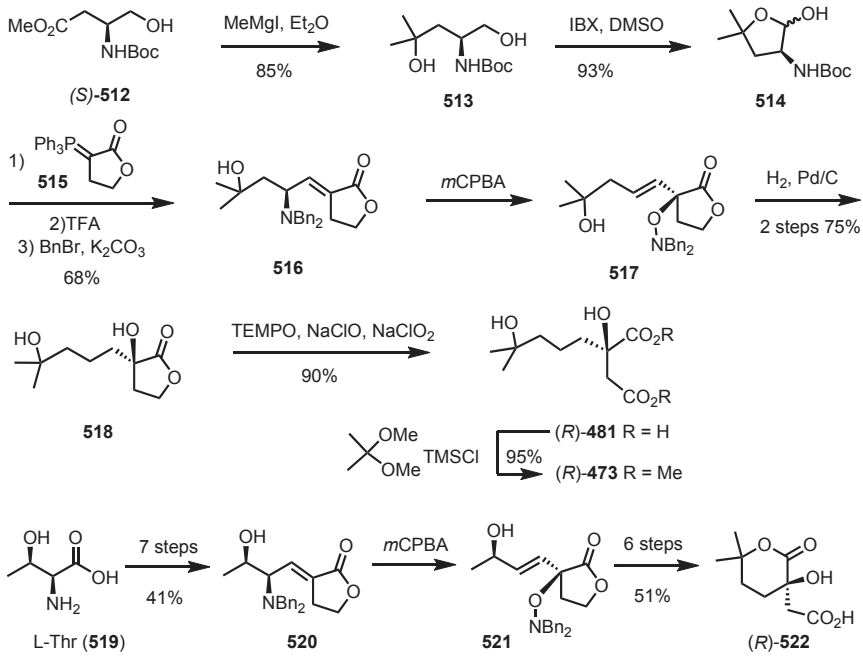
Scheme 53 Synthesis of harringtonines' side chains by Royer (2009).

hand, it was postulated that this epoxide could be a good candidate for esterification with CET (**1**) while no report of such coupling was available. The diester **501**, easily obtained from commercially available monomethyl itaconate (**499**) and methyl mandelate (**500**), was epoxydized with *m*CPBA to give a 1/1 diastereomeric mixture of **502** in good yield. Flash chromatography permitted the isolation of (+)-**502** with the required (*S*,*S*) absolute configuration. Then, epoxide (+)-**502** was reacted with various organocuprates to give compounds **503**−**507**. Hydrogenolysis of **503**−**507** allowed the obtention of (*R*)-**508**, (*R*)-**496**, (*R*)-**509**, side chains of deoxyHT (**5**), homodeoxyHT (**22**), and neoHT (**19**), respectively and analog (*R*)-**510** in 12.2%−17.8% OY. Hydroxymercuration of **507** followed by spontaneous cyclization and then hydrogenolysis of the chiral appendage delivered (*R*)-**494**, the side chain of anhydroHT (**20**) in 10.8% OY.

4.2.5 Yang's Synthesis (2013)

In 2013, Yang reported the [2,3]-Meisenheimer rearrangement as a general strategy for the construction of chiral tertiary alcohols.[160] As an illustration of this method, the synthesis of HT and HHT's side chains is shown in Scheme 54. The synthesis began with known *N*-Boc ester (*S*)-**512** of L-aspartic acid obtained through a two-step process (94%). The *gem*-dimethyl group was first installed by a Grignard reaction to give **513**, which was transformed into the lactol **514** by IBX oxidation and concomitant cyclization. The Wittig reaction with phosphorane **515** followed by deprotection and N-bisbenzylation of the amino group furnished key allylamine (*S*)-**516**. The Meisenheimer rearrangement occurred on treatment with *m*CPBA on the latter to give hydroxylamine **517**. This compound led to **518** on treatment with H_2 on catalytic Pd/C via double bond saturation and then N−O bond cleavage. Eventually, on oxidation of **518** the HHT's side chain acid (*R*)-**481** was obtained whose methylation yielded the corresponding dimethyl ester (*R*)-**473**. Its optical rotation indicated a perfect stereochemical outcome of the rearrangement. A similar process allowed synthesis of a cyclic form of the HT's side chain. Starting from L-threonine (**519**), amino alkene **520** was prepared in seven steps. The Meisenheimer rearrangement occurred on treating **520** with *m*CPBA to give the sensitive hydroxylamine **521**. Six steps were then needed to obtain (*R*)-**522**, a cyclic form of the HT's side chain acid.

These authors patented the preparation of HHT's side chain acid (*R*)-**481** by this methodology.[161] Through the same sequence, Yang and



Scheme 54 Synthesis of harringtonines' side chains by Yang (2013).

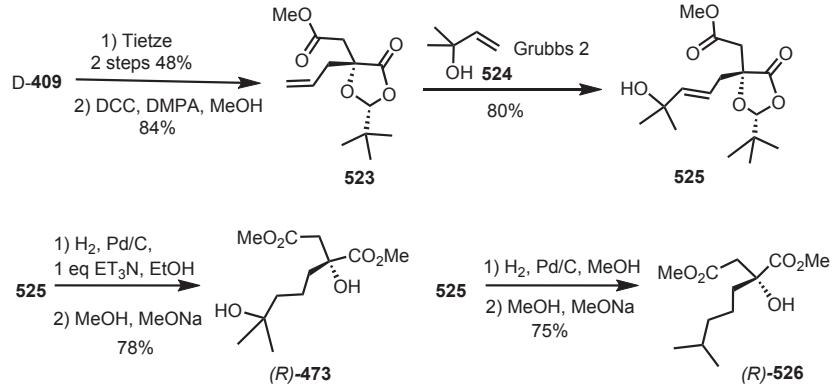
coworkers have also described the synthesis of the diacid side chain of deoxyHT (**5**) and homodeoxyHT (**22**) starting from L-valine and L-leucine respectively.[162]

4.2.6 Hung's Synthesis (2014)

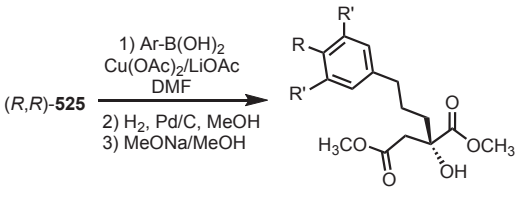
Hung et al.[163] recently reported the synthesis of the esters (**473**) and (**524**) of the side chains of HHT (**2**) and homodeoxyHT (**22**), respectively, by using Tietze[154] and Gin's methods[144] (Scheme 55). Allylation of **453** (see Scheme 51), esterification, and then cross-metathesis (see Scheme 49) with allyl alcohol **524** allowed the preparation of dioxolanone **523**. The latter was subjected to different hydrogenation conditions to give after methanolysis (*R*)-**473** or (*R*)-**526**, side chains' esters of HHT (**2**) and homodeoxyHT (**22**).

4.2.7 Mac's Synthesis (2016)

In 2016, Tietze's method[154] (see Scheme 51) was also used by Mac et al. for the synthesis of a series of side chains analogs of HHT (**2**) (Scheme 56).[164] The dioxolanone **453** was diastereoselectively allylated to furnish compound



Scheme 55 Synthesis of homoharringtonine and homodeoxyharringtonine side chains by Hung (2014).



527	yield from 523
a R = R' = H	83%
b R = Cl, R' =H	70%
c R = F, R' =H	81%
d R = CN, R' =H	71%
e R = CF_3, R' = H	56%
f R = $COCF_3$, R' = H	85%
g R = R' = F	65%
h R =  , R' = H	63%

Scheme 56 Synthesis of homoharringtonine side chain's analogs by Mac (2016).

(*R*,*R*)-**523**. A Mizoroki—Heck type reaction with various phenylboronic acid derivatives followed by hydrogenation and methanolysis gave arylated ester chain analogs **527a—h** of HHT (**2**) in moderate to good yield.

5. SYNTHETIC ANALOGS AND THEIR BIOLOGICAL PROPERTIES

The synthesis of analogs of bioactive natural products remains a broad area of research that can lead to very interesting new structures with improved activity. In the case of *Cephalotaxus* alkaloids, the exchange of the ester side chain appears to be the simplest way to access analogs and this strategy has been used and described in some patents. The structural modifications of the CET core seem more difficult but have been also scarcely addressed giving rise to interesting compounds and synthetic pathways. We will describe below the most significant reports. Works describing

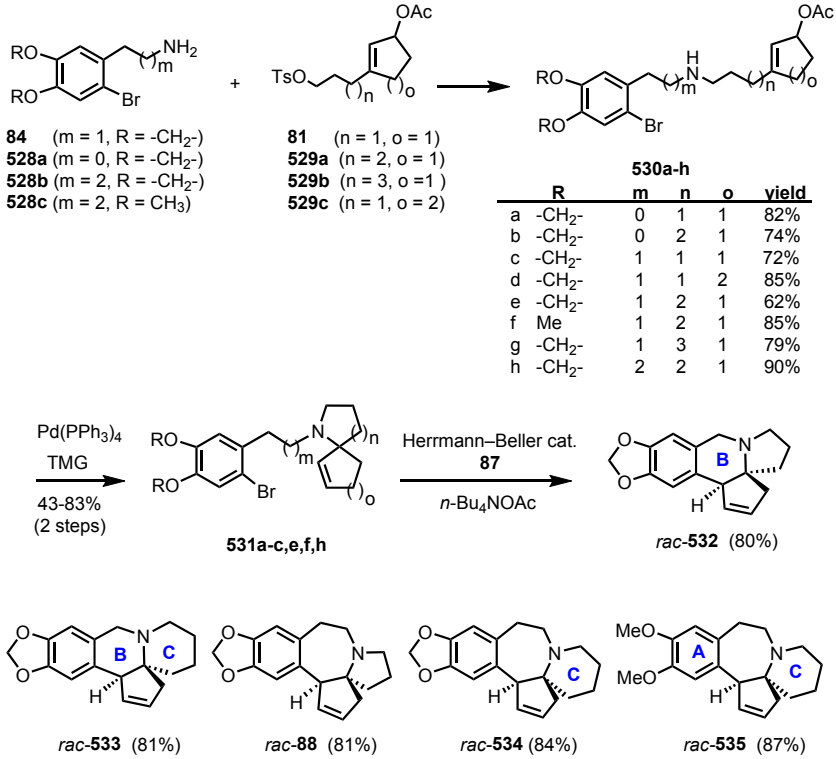
attempts to synthesize CET (**1**) or the preparation of compounds with a passing structural resemblance will not be considered in this section.

5.1 Cephalotaxine Analogs

5.1.1 Tietze's Cephalotaxine Analogs (2000, 2007)

Since 1997, several reports on the synthesis of CET's analogs have appeared. Tietze et al. in 2000[165] described the preparation of four analogs *rac*-**532**–**535**. For these compounds, variation of the size of the different rings B and C of the polycyclic core of CET (**1**) and ring A substitution was explored. These authors used their strategy developed for the synthesis of the natural product (Scheme 57).

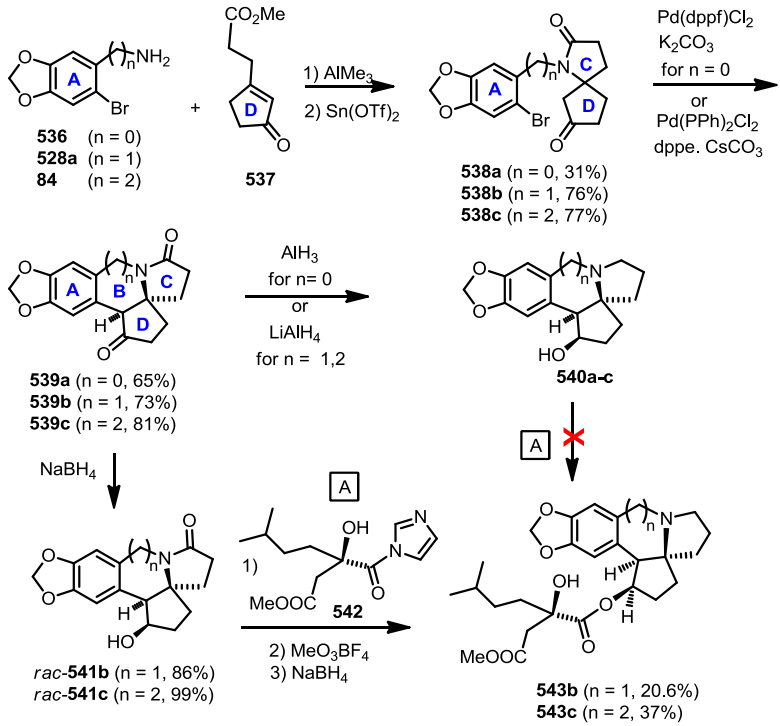
Compounds **530a**–**h**, with various chain lengths and ring sizes, were easily prepared in a few steps by alkylation of **84** and **528a**–**c** with **81** and **529a**–**c** using a standard S_N reaction. Compounds **530a**–**h** were then submitted to a Pd-catalyzed Tsuji–Trost cyclization to give **531a**–**c,e,f,h**



Scheme 57 Synthesis of cephalotaxine analogs by Tietze (2000).

in satisfactory yields (compounds **530d,g** did not cyclize under these conditions). The final Heck cyclization required the use of Hermann–Beller's palladacycle **87** and allowed the preparation of compounds **532–535** and **88**. In this study, the final compounds required further reactions to install at least an alcoholic function on ring D to allow the coupling with side chains. This drawback was eliminated in a following study published in 2007 by the same authors.[166] Furthermore, *Cephalotaxus* esters analogs were prepared by coupling the CET analogs with homodeoxyHT side chain (Scheme 58). The synthesis was now based on a domino reaction giving a rapid and efficient access to ACD compounds **538** from amines **536, 528a, 84** and cyclopentenone ester **537**.

The Pd-catalyzed reaction of **538a–c** allowed the formation of the tetracycle **539a–c**. While $LiAlH_4$ reduction of **539a–c** gave efficient access to amino alcohols **540a–c**, which are analogs of CET (**1**), these compounds did not couple with an activated form of the side chain of homodeoxyHT (**22**). In contrast, it was observed that $NaBH_4$ reduction



Scheme 58 Synthesis of deoxyharringtonine analogs by Tietze (2007).

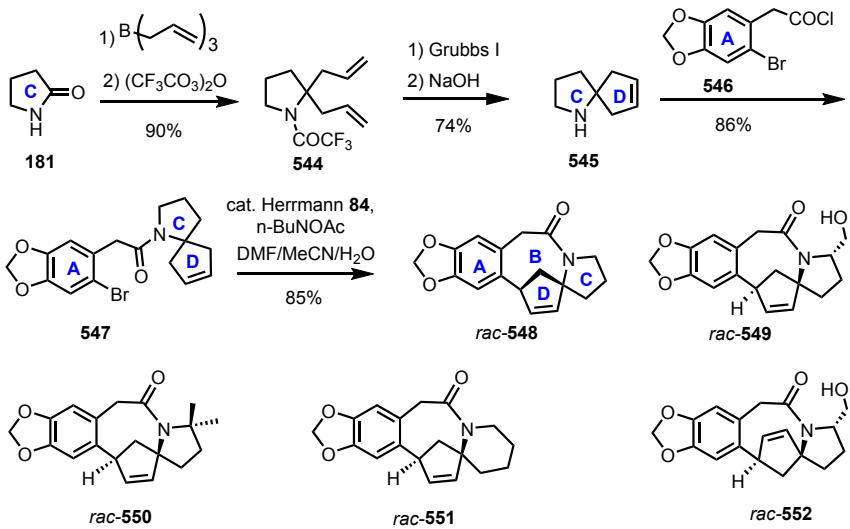
of **539b,c** gave the amidoalcohols *rac*-**541b,c**, which allowed coupling with the imidazolide **542** of homodeoxyHT side chain furnishing after further reduction the esters **543b,c**, which are analogs of homodeoxyHT(**22**). It should be noted that the presented synthesis is racemic and that optically pure side chains were used leading to a mixture of two diastereomers, which were separated.

5.1.2 Bubnov's Cephalotaxine Analogs (2005–2010)

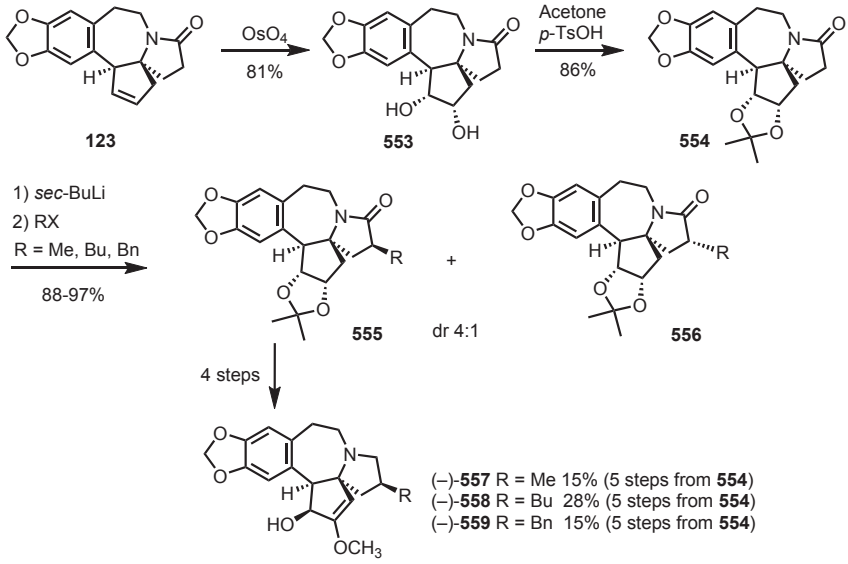
In 2005, Bubnov et al.[167] published the synthesis of compound *rac*-**548** exhibiting some structural analogies to CET (**1**) with ring B and D being bridged and not fused (Scheme 59). The synthetic strategy was simple and started with 2-pyrrolidone (**181**), which was diallylated, *N* N-protected, and transformed into spiro-pyrrolidine **545** through ring closure metathesis. Condensation of **545** with acyl chloride **546** gave access to **547**, which was transformed to the desired product *rac*-**548** by Heck cyclization. The same type of work was pursued by the authors in 2010[168] allowing the preparation of new products **549–552**.

5.1.3 Royer's Cephalotaxine Analogs (2004–2010)

Exploiting their early developed strategy, Royer et al. (see Scheme 29) prepared three C7-alkylated CET analogs from chiral nonracemic Kuehne's intermediates **123** and **553** (Scheme 60).[132] Alkylation was carried out on



Scheme 59 Cephalotaxine's analog synthesized by Bubnov (2005–2010).



Scheme 60 Synthesis of Cephalotaxine alkylated analogs by Royer (2004).

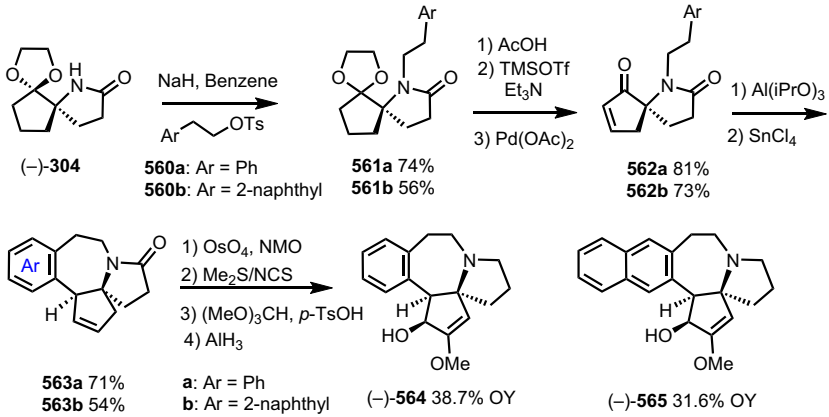
protected lactam **554** through deprotonation using *sec*-BuLi and addition of MeI, BuI, or $PhCH_2Br$ to provide diastereomeric alkylated lactams **555** and **556** in a 4/1 ratio. The new chiral center of the major isomer has the (*S*)-configuration. Transformation of acetonide **555** led to alkylated CETs **557**–**559** in a four-step sequence in 15–28% OY.

In 2010, these authors reported a different type of CET analogs in which the A ring was changed for a phenyl or a naphthyl group (Scheme 61) using the same strategy as for CET's synthesis (see Scheme 34).[169]

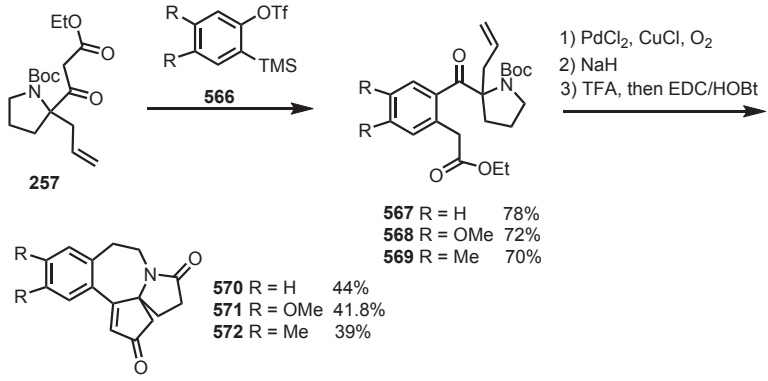
N-alkylation of the spirolactam (−)**-304** with the appropriate arylethyl derivative **560a,b** gave **561a,b**. The end of the synthesis paralleled those of the CET synthesis: functional group manipulations of **561a,b** led to **562a,b**. These spiro compounds **562a,b** were cyclized to the ABCD compounds **563a,b**, which were eventually transformed to the phenyl **564** and naphthyl **565** CET analogs.

5.1.4 Chandrasekhar's Cephalotaxine Analogs (2016)

In 2016, Chandrasekhar et al. used their aryne insertion methodology to synthetize three CET analog intermediates (Scheme 62).[120] In these compounds **570**–**572**, the A ring is changed for a phenyl, dimethylphenyl, or dimethoxyphenyl ring.



Scheme 61 Synthesis of cephalotaxine "A" analogs by Royer (2010).



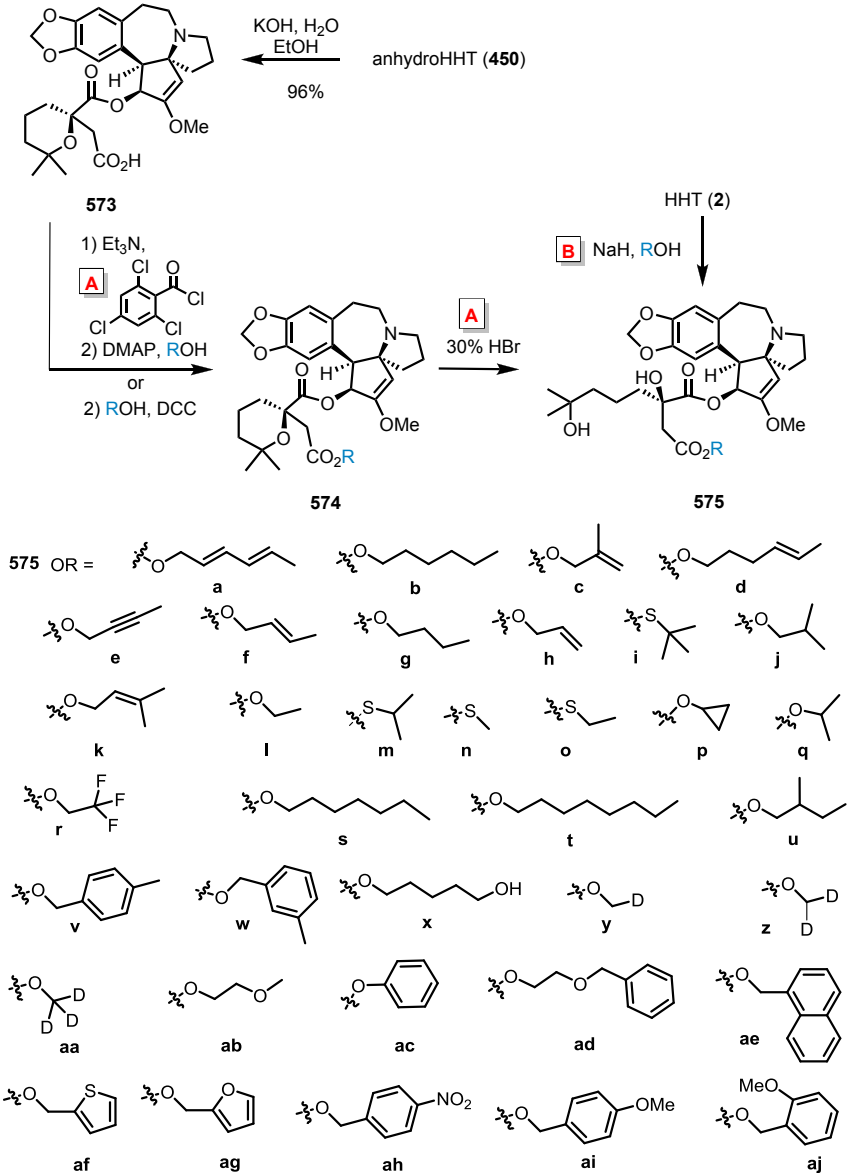
Scheme 62 Formal synthesis of cephalotaxine "A analogs" by Chandrasekhar (2016).

To conclude, it is noteworthy that if several analogs of CET (**1**) have been designed and synthesized, they have been coupled with side chains for only two products (in two reports), although their biological activity was not reported.

5.2 *Cephalotaxus* Esters With Side Chain Analogs

5.2.1 Robin's Analogs (2002–2004)

Robin et al. patented *Cephalotaxus* esters **575** with various side chains.[170,171] They were prepared by transesterification of HHT (**2**) (path B) or ring opening of intermediate **574** (Scheme 63) with yields between 21% and 82%. For example, HHT (**2**) dissolved in (*E*,*E*)-hexa-2,4-dien-1-ol was



Scheme 63 Robin's synthesis of homoharringtonine analogs at the side chain.

treated with sodium hydride to yield synthetic CET ester **575a** in 30% yield. This compound was the most active of the tested analogs in this study with nanomolar IC_{50} (3.5 ng/mL) while HHT (**2**) was less potent (IC_{50} 14 ng/mL).

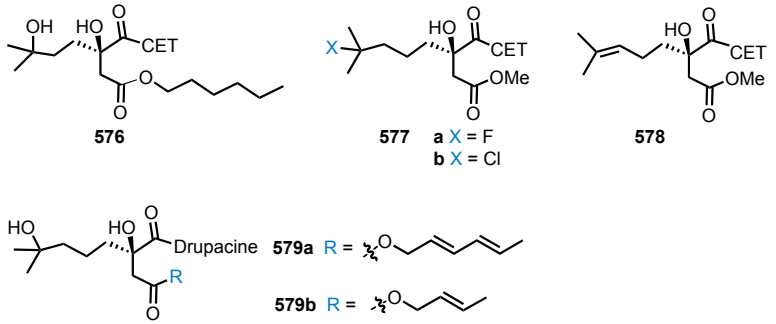


Figure 20 Harringtonines analogs at the side chain (homoharringtonine (top) and drupangtonine (bottom) analogs) synthesized by Robin (2002–04).

Analogs **576** of HT (**3**), **579** of drupangtonine (**33**), and **577** and **578** of HHT (**2**) were also synthetized by conventional methods (Fig. 20).

This work delineated the ester groups that reduced the cytotoxic activity against the K562 leukemic cell line (Table 11). Nevertheless, a few ones gave rise to improved activity as esters **575a** (*n*-hexadienyl), **575b** (*n*-hexyl), **575s** (*n*-hepthyl), **575v** (4-methylbenzyl), **575w** (3-methylbenzyl), **575af** (2-methylthiophene), **575ag** (2-methylfurane).

5.2.2 Djaballah and Gin's Analogs (2008)

In 2008, Djaballah and Gin synthesized four CET-ester analogs, benzyldehydroHHT **580**, bisnorhomodeoxyHT **581**, bisnorhomodeoxyHT β-lactone **582**, and deoxyHT *β*-lactone **583** (Fig. 21), using the protocol described in Section 4.1.3 (Scheme 47).[144] They exemplified up to 43 *Cephalotaxus* esters analogs, some of which being dimers of CET (**1**) linked by a central oxygenated chain by the C3 hydroxyl group.[172]

These HT structural analogs were tested against human hematopoietic and solid tumor cell lines (Tables 12 and 13), and compared to HHT (**2**), deoxyHT (**5**), homodeoxyHT (**22**), and anhydroHT (**20**), which are among the most active *Cephalotaxus* alkaloid esters.

HHT (**2**) shows decreased activity by a factor of 125 (resistance index (RI) = 125), whereas esters deoxyHT **5**, homodeoxyHT (**22**), benzyldehydroHHT (**580**), and bisnordeoxyHT (**581**) have much lower RI of 11, 3, 19, and 12 respectively, indicating that they are significantly less sensitive to multidrug resistance (MDR) and tumor cell lines can therefore be

Table 11 Amount and yield of Robin's harringtonines (HTs) analogs (see Scheme 63 and Fig. 18) and their cytotoxicity (K562 cell line derived from CML cells)

Compound	Quantity mg	Yield %	IC_{50} (ng/mL)	Compound	Quantity mg	Yield %	IC_{50} (ng/mL)
CET(**1**)	—	—	2000	**575ae**	62	34	8
HT (**3**)	—	—	30	**575af**	85	49	5
576	30	22	7	**575ag**	47	28	3
577a	11	22	13.5	**575ah**	100	58,6	18
578	20	21	17.5	**575ai**	90	54	20
HHT (**2**)	—	—	14	**575aj**	147	82	27
575a	175	30	3.5	**579a**	50	23,5	9
575a	158	47	5	**579b**	132	22	13.5
575c	171	46,5	8.5	**575u**	146	44	29
575d	226	54	8.5	**575v**	141	31	6
575e	171	46,5	10.5	**575w**	213	45,5	4
575f	122	30	12.5	**575x**	245	43,3	24
575g	107	44	13	**575y**	56,5	56	18
575h	289	54	14.5	**5752**	51,7	50	21
575i	70	31,5	20	**575aa**	110	60	25
575j	254	61	24	**575ab**	174	78	12
575k	266	48	28	**575r**	122	23	100
575m	118	43	50	**575s**	183	40	3
575n	18	76	50	**575t**	249	51	9
575o	120	46	80	**575ac**	A: 54	47	10
575p	107	44	70		B: 46	40	
575I	A: 125	55	41	**575q**	A: 103	50	80
	B: 298	97			B: 143	39	
575ad	100	55	25				

CET, cephalotaxine.

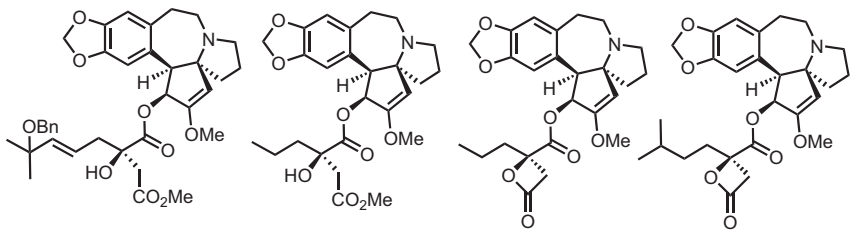


Figure 21 Djaballah and Gin's cephalotaxine-ester analogs (2009).

Table 12 Comparative antitumor effect of cephalotaxine esters against HL60 lines sensitive (HL-60) or resistant (HL-60/RV+) to vincristine

	IC_{50} (μg/mL)			
Compound	**HL-60**	**HL-60/RV$^+$**	**Resistance index**	**ClogP**
DeoxyHT (**5**)	0.02	0.22	11	1.93
HHT (**2**)	0.02	2.50	125	0.95
HomodeoxyHT (**22**)	0.03	0.10	3.3	2.33
580	0.01	0.19	19	2.80
581	0.08	1.00	12	1.21

HHT, homoharringtonine.

Table 13 Cytotoxicity of deoxyharringtonine (**5**), anhydroharringtonine (**20**) and cephalotaxine ester analogs **580**–**583**

	IC_{50} (μg/mL)					
Cell lines	**DeoxyHT (5)**	**583**	**AnhydroHT (20)**	**580**	**582**	**581**
HL-60	0.02	2.68	22.7	0.01	5.73	0.08
HL-60/RV+	0.22	21.8	–	0.19	40.30	0.80
JURKAT	0.04	5.71	42.99	0.03	12.01	0.19
ALL3	<0.1[b]	1.47	>100[a]	<0.01	4.24	0.16
NCEB1	0.07	8.62	>100[a]	0.06	39.24	0.50
JEKO	0.08	10.48	>100[a]	0.08	25.1	0.56
MOLT-3	0.02	2.68	26.83	0.01	6.41	0.06
SKNLP	<0.1[b]	6.46	5,34	<0.01	10.04	0.11
Y79	70.59	0.095	>100[a]	>1.00	>1.00	>100[a]
PC9	0.03	0.039	29.08	0.04	11.29	0.13
H1650	0.04	>1.00[a]	n.a.	n.a.	n.a.	n.a.
H1975	0.06	4.23	n.a.	n.a.	n.a.	n.a.
H2030	0.10	4.53	n.a.	n.a.	n.a.	n.a.
H3255	0.08	8.42	n.a.	n.a.	n.a.	n.a.
A431	0.06	n.a.	n.a.	n.a.	n.a.	n.a.
HeLa	0.04	n.a.	n.a.	n.a.	n.a.	n.a.
TC71	0.06	12	>100[a]	0.03	24	0.20
HTB-15	0.20	52	>100[a]	0.10	58	0.50
WD0082	0.10	5	>100[a]	0.05	11	0.20

n.a., not active.
[a]Highest tested concentration.
[b]Lowest tested concentration giving 100% cellular death.

considered to be sensitive to these products. One possible explanation for the greater susceptibility of HHT (**2**) to MDR is its low lipophilicity due to the structure of the side chain, making it a good substrate for the efflux pumps.

Compounds having a ClogP value greater than 1.2 generally lead to a low susceptibility to MDR (i.e., IR = 19 to the esters **5**, **22**, **580**, and **581**). The exception is HHT (**2**), which has a relatively low value of cLogP (0.95, relatively polar) reflecting its susceptibility to MDR (i.e., IR = 125). All these compounds show a broad spectrum of cytotoxic activity, with the exception of the retinoblastoma cell line Y79 (Table 13), which can be attributed to the overexpression of the MDR gene in this line.

5.2.3 Lai's Analogs (2013)

In 2013, Lai et al. applied for two patents, one on preparation of aminated HHT derivatives **584**[173] (Fig. 20) and the other on acylated HHT derivatives **585** and **586** (Fig. 22).[174]

Compounds **584a-w** were prepared from HHT (**2**) via saponification of the methyl ester to HHT acid (**31**) and amidation with various amines R^1R^2NH in the presence of HATU/DIPEA in 6%–44% yield (Fig. 22). Groups R^1 and R^2 could be H, C_{1-18} alkyl, C_{2-18} alkenyl, C_{2-18} alkynyl,

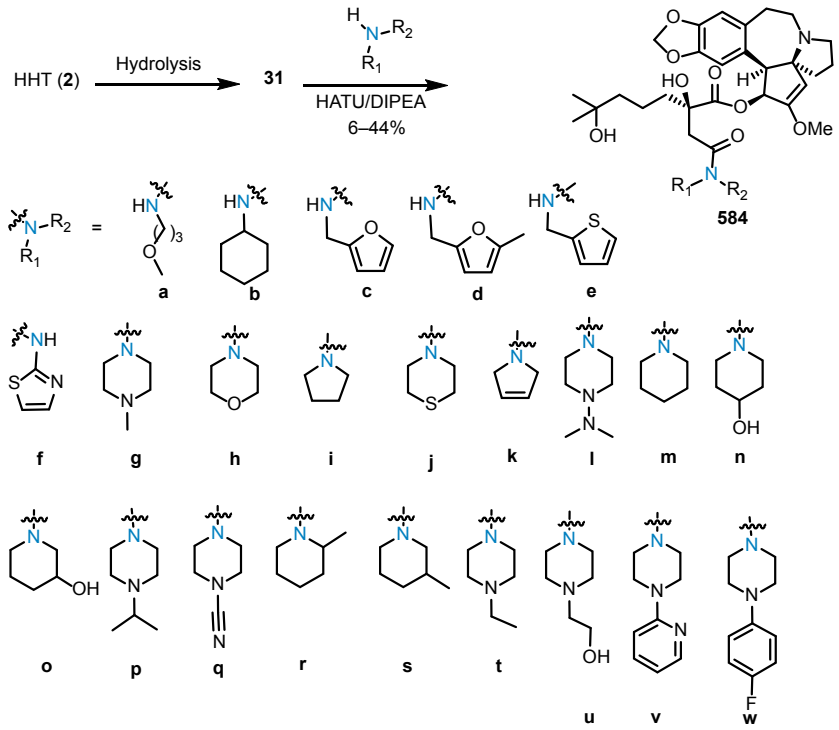


Figure 22 Lai's homoharringtonine (**2**) aza analogs (2013).

Table 14 Cytotoxicity (IC_{50} and IC_{90} in μg/mL) of Lai's homoharringtonine (HHT) aza analogs **584a-w**

	K562/adr		Kasumi-1		NB4		Jurkat		H9	
Compound	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
HHT (**2**)	0.035	0.98	0.005	0.024	0.006	0.012	0.007	16	0.02	0.046
584a	>16	>16	2.75	13	2.59	7.52	3.56	15.04	6.52	>16
584b	3.2	16	0.048	0.21	0.06	0.13	0.037	16	0.04	0.1
584c	>16	>16	11.06	>16	15	>16	13	>16	>16	>16
584d	0.66	4.4	0.053	0.19	0.09	0.2	0.11	16	0.17	0.43
584e	6.24	16	1.3	6.68	2.38	7.61	1.58	8	4	10.64
584f	>16	>16	5	>16	5.62	12.83	4.69	16	13.43	>16
584g	>16	>16	8.54	>16	>16	11.2	3	>16	8	>16
584h	>16	>16	8.28	>16	3.22	9.54	6.7	>16	>16	>16
584i	0.65	9.87	0.038	0.14	0.08	0.23	0.058	16	0.27	3.49
584j	>16	>16	1.33	8.61	1.29	3.7	2	>16	8.25	>16
584k	7.77	>16	0.47	2.32	0.38	1	0.88	16	2.69	>16
584I	0.72	8.99	0.041	0.18	0.08	0.17	0.12	16	0.13	0.35
584m	>16	>16	2	16	1.5	16	1.3	>16	7.26	>16
584n	>16	>16	14.58	>16	8	16	13.4	>16	>16	>16
584o	>16	>16	>16	>16	8.31	>16	>16	>16	>16	>16
584p	4.53	16	0.31	1.77	0.48	2.3	0.46	16	0.6	2.7
584q	14.1	>16	0.8	2.96	0.72	6.3	0.87	>16	5.49	>16
584r	>16	>16	1.58	16	0.7	5.08	0.41	>16	3.69	>16
584s	>16	>16	2	12.02	0.32	4.24	1.47	16	7.37	>16
584t	>16	>16	2.79	14.99	1.85	16	0.89	>16	3.2	>16
584u	>16	>16	>16	>16	7.46	15.79	15.14	>16	>16	>16
584v	5.18	16	0.38	2.25	0.25	0.64	0.48	16	1.41	16
584w	14.5	>16	0.26	2.26	0.19	0.5	0.32	15.21	0.5	7.84

C_{3-7} cycloalkyl or cycloalkenyl, aryl, heteroaryl, or saturated heterocyclic ring systems, etc. Twenty-three HHT aza analogs were tested against human hematopoietic and solid tumor cell lines (Table 14), and compared to HHT (**2**). Analogs **584c**, **f**, **n**, **o**, and **u** were inactive ($IC_{50} \geq 16$ μg/mL). Compound **584b** and **584i** were the most active of the tested analogs in this study with nanomolar IC_{50} (up to 42 and 57 ng/mL) while HHT (**2**) was more potent (IC_{50} 6 ng/mL). The most promising analogs were tested against HeLa (cervical), CaES-17 (esophageal), CNE (nasopharyngeal), Hep2 (liver), MGC 803 (gastric), PC-3 (prostate), and SK-OV-3 (ovary) human cancer cell lines (Tables 15 and 16), and however, none of these compounds were more active than HHT (**2**).

Table 15 Cytotoxicity (IC_{50} and IC_{90} in μg/mL) of Lai's homoharringtonine (HHT) aza analogs **584**

	RPMI8226		A549	PANC-1	Becap37	MG63	Huh7	RKO	
Compound	IC_{50}	IC_{90}	IC_{50}	IC_{50}	IC_{50}	IC_{50}	IC_{50}	IC_{50}	IC_{90}
HHT (**2**)	0.006	0.027	0.03	0.035	0.01	0.01	0.004	0.003	0.009
584b	0.042	0.24	0.45	0.13	0.17	0.12	0.044	0.17	0.029
584d	0.092	0.24	0.72	0.15	0.14	0.12	0.12	0.14	6.97
584i	0.057	0.36	0.45	0.26	0.26	0.18	0.2	0.26	0.43
584I	0.1	0.38	0.7	0.23	0.26	0.19	0.1	0.053	0.23
584p	0.47	1.74	1.74	1.94	0.81	0.32	1.05	0.18	1.25
584v	0.48	1.76	1.91	1.91	0.71	0.8	0.94	0.16	2.37
584w	0.17	0.84	0.45	0.45	0.4	0.23	1.37	0.054	0.93

Table 16 Cytotoxicity (IC_{50} and IC_{90} in μg/mL) of Lai's homoharringtonine (HHT) aza analogs **584**

	U87 MG		HeLa	CaES-17	CNE	Hep2	PC3	SK-OV-3	MGC 803	
Compound	IC_{50}	IC_{90}	IC_{50}	IC_{50}	IC_{50}	IC_{50}	IC_{50}	IC_{50}	IC_{50}	IC_{90}
HHT (**2**)	0.004	0.018	0.019	0.037	0.038	0.014	0.004	0.003	0.016	0.2
584b	0.12	13.03	0.45	0.29	0.13	0.97	0.31	0.21	0.096	0.25
584d	0.32	16	0.3	1.37	0.15	0.23	0.46	0.47	0.15	6
584i	0.24	1.13	0.35	0.43	0.13	0.15	0.32	0.41	0.24	6.84
584I	0.24	1.06	0.46	0.46	0.15	0.31	0.45	0.57	0.18	0.48
584p	0.89	5.74	1.97	1.91	0.49	1.29	0.91	2.81	0.81	11.81
584v	0.64	16	1.91	1.63	0.76	1.23	0.78	3.97	0.74	>16
584w	0.25	>16	1.56	0.98	0.3	0.27	0.78	1.89	0.39	8.28

Monoacyl-HHT analogs **585a–i** and diacyl-HHT analogs **586a–f** were prepared with activated HHT and carboxylic acids, anhydrides, or acyl chlorides via esterification in 6%–99% yield (Fig. 23).

The acylated analogs were compared to HHT (**2**) and tested against human hematopoietic (Table 17) and solid tumor cell lines (Tables 18 and 19) including K562/adr (myelogenous leukemia resistant to DOX), Kasumi-1 (acute myeloid leukemia (AML)), NB4 (acute promyelocytic leukemia (APL)), Jurkat (childhood T acute lymphoblastic leukemia (ALL)), H9 (cutaneous T-cell lymphoma), RPMI8226 (plasma cell myeloma), Huh7 (hepatoma), A549 (lung adenocarcinoma), PANC-1 (exocrine pancreas carcinoma), Becap37 (breast), MG63 (osteosarcoma), RKO (colon), U87MG (glioma), HeLa (cervical), CaES-17 (esophageal), CNE (nasopharyngeal), Hep2 (liver), PC-3 (prostate), SK-OV-3 (ovary),

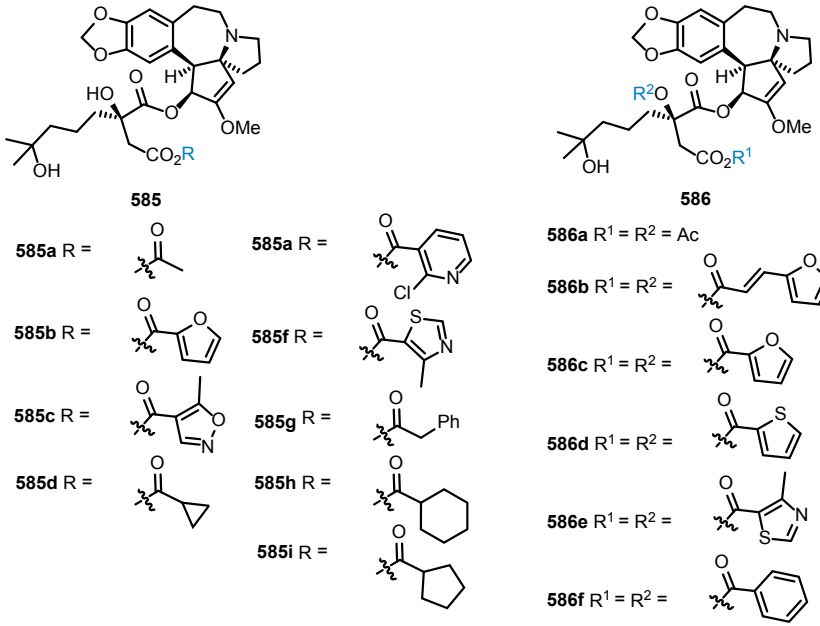


Figure 23 Lai's homoharringtonine (**2**) acylated analogs (2013).

Table 17 Cytotoxicity (IC_{50} and IC_{90} in μg/mL) of Lai's ester analogs **585** and **586**

	K562/adr		Kasumi-1		NB4		Jurkat		H9		RPMI8226	
Compound	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
HHT (**2**)	0.035	0.98	0.005	0.024	0.006	0.012	0.007	16	0.02	0.046	0.006	0.027
586a	0.062	1.18	0.006	0.021	0.01	0.022	0.012	16	0.02	0.05	0.009	0.12
585a	0.015	0.31	0.002	0.007	0.002	0.006	0.003	16	0.004	0.01	0.003	0.018
586b	0.41	1.84	0.063	0.22	0.08	0.25	0.088	10.13	0.19	0.4	0.12	0.63
586d	0.32	16	0.065	0.4	0.12	0.4	0.11	7.5	0.21	0.48	0.155	1.13
585b	0.03	0.35	0.005	0.012	0.006	0.012	0.003	16	0.007	0.02	0.006	0.039
585c	0.24	12.57	0.062	0.23	0.07	0.14	0.071	16	0.14	0.25	0.062	0.25
585d	0.06	0.75	0.002	0.005	0.004	0.007	0.012	16	0.008	0.02	0.007	0.029
585e	0.03	3.18	0.0007	0.002	0.002	0.004	0.005	16	0.003	0.01	0.006	0.033
585f	0.01	0.05	0.002	0.004	0.003	0.005	0.002	16	0.003	0.009	0.002	0.014
586e	0.9	3.72	0.14	0.47	0.2	0.49	0.3	16	0.47	1.24	0.29	1.82
586f	2.5	6.5	1.35	3.56	1.19	2.47	1.4	6.4	1.3	3.9	1.15	3.84
585g	0.007	14.1	0.001	0.005	0.003	0.007	0.0038	16	0.004	0.01	0.003	0.015
585h	0.02	0.21	0.006	0.023	0.009	0.022	0.006	6.24	0.01	0.04	0.007	0.034
585i	0.01	0.05	0.004	0.012	0.009	0.022	0.001	11.37	0.01	0.03	0.004	0.024

HHT, homoharringtonine

Table 18 Cytotoxicity (IC_{50} and IC_{90} in μg/mL) of Lai's homoharringtonine (HHT) acylated analogs **585** and **586**

	Huh7		A549[a]	PANC-1[b]	Becap37[c]	MG63		RKO	
Compound	IC_{50}	IC_{90}	IC_{50}	IC_{50}	IC_{50}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
HHT (**2**)	0.004	0.049	0.03	0.035	0.01	0.01	1.2	0.003	0.009
586a	0.016	0.13	0.03	0.036	0.015	0.02	2.4	0.007	0.02
585a	0.0021	0.018	0.013	0.012	0.005	0.006	0.42	0.002	0.01
586b	0.13	0.99	0.23	0.69	0.23	0.24	3.25	0.044	0.35
586d	0.17	1.7	0.48	0.43	0.2	0.2	4.75	0.12	0.56
585b	0.012	0.081	0.03	0.029	0.007	0.02	3.08	0.001	0.015
585c	0.056	0.34	0.52	0.21	0.17	0.093	10.76	0.039	0.24
585d	0.004	0.049	0.04	0.044	0.014	0.012	0.77	0.005	0.025
585e	0.0016	0.023	0.03	0.026	0.02	0.0032	0.2	0.003	0.023
585f	0.0022	0.038	0.01	0.009	0.003	0.006	0.44	0.0009	0.005
586e	0.66	7.6	0.89	0.83	0.47	0.21	9.43	0.22	0.87
586f	4.8	14.5	5.4	5.8	2.9	1.34	9.88	1.4	5.24
585g	0.003	0.05	0.007	0.014	0.003	0.006	1.18	0.002	0.006
585h	0.008	0.096	0.03	0.042	0.01	0.022	1.72	0.008	0.02
585i	0.016	0.036	0.02	0.055	0.007	0.021	5.14	0.006	0.02

[a] $IC_{90} > 16$.
[b] $IC_{90} > 12.56$.
[c] IC_{90} 5.53–16.

and MGC 803 (gastric) cell lines. Contrary to aza analogs **584** and diacylated analogs **586a–f**, the monoacetate analog **585a** was at least as effective as HHT (**2**) against all the investigated cell lines (Tables 17, 18 and 19). Compounds **585f** and **585g** were more potent than HHT (**2**) against 18 and 17 over the 20 cell lines tested, respectively.

In conclusion, the study of many syntheses of CET (**1**) published to date can draw a number of conclusions about the key points of its structure and the way chemists have overcome the problems posed by its synthesis. The formation of the B ring often remains a crucial point in building the CET skeleton. The C4–C13 disconnection remains by far the most studied (>30 syntheses of 50). The cyclization reactions used (electrophilic aromatic substitutions, organometallic coupling, etc.) perform generally with good yields but seem susceptible to cyclization intermediates and electronic richness of the aromatic ring. This disconnection is also extremely interesting from a stereochemical point of view providing the correct relative configuration at C4 and C5, avoiding difficult separations. Other disconnections do not always give such good results and are not completely diastereoselective.

Table 19 Cytotoxicity (IC_{50} and IC_{90} in μg/mL) of Lai's homoharringtonine (HHT) acylated analogs **585** and **586**

	U87 MG		HeLa[a]	CaES-17[b]	CNE[c]	Hep2[d]	PC3[d]	SK-OV-3[d]	MGC 803	
Compound	IC_{50}	IC_{90}	IC_{50}	IC_{50}	IC_{50}	IC_{50}	IC_{50}	IC_{50}	IC_{50}	IC_{90}
HHT (**2**)	0.004	0.018	0.019	0.037	0.038	0.014	0.08	0.09	0.016	0.2
586a	0.018	0.11	0.026	0.062	0.062	0.026	0.029	0.12	0.03	0.66
585a	0.002	0.012	0.0077	0.02	0.024	0.0037	0.013	0.05	0.008	0.056
586b	0.16	0.59	0.33	0.61	0.21	0.22	1.37	1.33	0.16	0.76
586d	0.23	0.99	0.45	0.74	0.35	0.23	1.84	0.98	0.24	1.62
585b	0.023	0.18	0.014	0.029	0.031	0.015	0.08	0.08	0.021	0.11
585c	0.31	0.96	0.31	0.38	0.42	0.39	3.15	0.4	0.058	0.16
585d	0.02	0.1	0.026	0.058	0.028	0.015	0.11	0.12	0.015	0.07
585e	0.007	0.048	0.021	0.049	0.03	0.025	0.1	0.051	0.005	0.052
585f	0.008	0.07	0.005	0.014	0.0037	0.004	0.04	0.022	0.006	0.019
586e	0.67	3.42	0.87	1.24	0.77	0.48	0.82	1.94	0.5	12
586f	2	8.54	2.61	3.93	3.91	3.46	3.97	6.71	2.7	9.92
585g	0.012	0.08	0.0034	0.015	0.0014	0.006	0.0077	0.027	0.006	0.055
585h	0.009	0.067	0.011	0.039	0.029	0.012	0.03	0.031	0.025	0.098
585i	0.035	0.16	0.014	0.031	0.012	0.007	0.039	0.034	0.021	0.087

[a]IC_{90} 8.37−16.
[b]IC_{90} 16 or >16.
[c]IC_{90} 14.28−>16.
[d]$IC_{90} > 16$.

The formation of the spiro CD system is also a key point in these syntheses; in fact, the control of center C5 at the start of synthesis greatly simplified stereocontrolled strategies and enabled enantioselective approach to CET (**1**) by asymmetric synthesis of the CD system. Subsequent stereoinduction of the C5 center on C4 center and C4 center on the C3 center facilitate the production of optically active CET (**1**). However, the operating conditions used after the synthesis of the spiro system must be chosen carefully because of the risk of racemization of CET (**1**) and certain synthetic intermediates by breaking the N9−C5 bond (See Figs. 18 and 19, the conversion of Mori's intermediates (±)-**88** and (−)-**88**). Last but not least, from an industrial point of view, initially the Merck Group took over the Weinreb racemic synthesis improving performance (11.7% by eliminating some purification steps). The optically active CET (**1**) in its natural configuration may be obtained by resolution of racemic cephalotaxine *rac*-CET (±)-(**1**) with tartaric acid, by incorporation of chiral sources, or by enantioselective synthesis. The high-purity HHT (**2**) used for clinical trials was obtained by extraction

(NCI batches) and by semisynthesis (ssHHT) from natural CET (**1**) then purified by HPLC to remove impurities and minor diastereoisomers first by the French company Oncopharm SA later based in Houston (USA). A number of HHT (**2**) analogs were prepared; however only the monoacylated analogs **585a**, **585f**, and **585g** showed slight increase in activity agains both hematopoietic and solid caner cell lines. The main advantage was to prepare hydrosoluble stable forms.

6. PHARMACOLOGY AND CLINICAL STUDIES

6.1 Cellular Pharmacology

6.1.1 Molecular Mechanism of Action of Homoharringtonine

Molecules such as HHT (**2**), which works on several targets, are increasingly attractive. Protein synthesis inhibitors could affect the translation of messenger RNA (mRNA) into proteins at several different stages, such as initiation, elongation, and termination as illustrated in Fig. 24. The main activity of HHT (**2**) is blocking protein synthesis during the initiation step. Mainly, it inhibits the synthesis of short-lived proteins[175] by binding to the A-site cleft of tRNA A-site in the peptidyl-transferase center of the ribosome. The aminoacyl-tRNAs binding site is universally conserved over prokaryotes and eukaryotes. The Nobel Prize in Chemistry 2009 was awarded jointly to Ramakrishnan, Steitz, and Yonath "for studies of

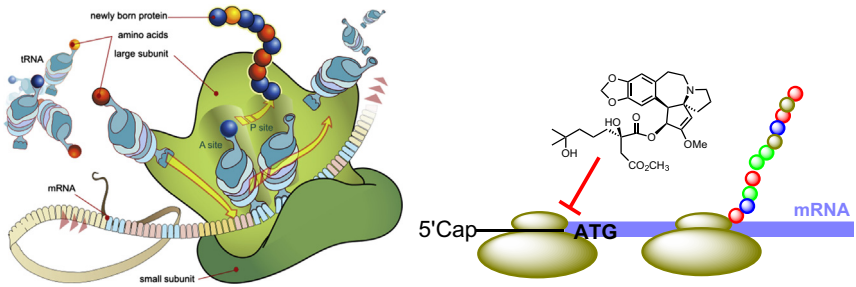


Figure 24 Simplified diagram of protein synthesis showing the A site where homoharringtonine (HHT) binds to inhibit protein synthesis.[178] In the cytoplasm of the cells, ribosomes (green) bind to the mRNA containing the code translated from the switched on genes into the DNA. The 20 different amino acids carried by transfer RNA (tRNA) are recruited according to a three nuclear base code into the ribosome A site. The protein chain elongation includes the aminoacyl tRNA entry, its proofreading, the peptidyl transfer, and the ribosomal translocation along the tRNA chain. The cartoon on the right explains how HHT (**2**) inhibits protein synthesis through inhibition of initiation elongation step.

the structure and function of the ribosome." They have all generated 3D models that have shown how different antibiotics, including HHT (**2**),[176] bind to the ribosome. Also, they demonstrated by X-ray (at high resolution up to 2.9 Å) the crystals of the 80S ribosome in complexes with 12 eukaryote-specific inhibitors and four broad-spectrum inhibitors in *Saccharomyces cerevisiae*.[177]

Both the ring system and the tail of HHT (**2**) participate in interactions with the ribosome that contribute to drug binding. HHT (**2**) interaction with the ribosome is stabilized by hydrophobic interactions and hydrogen bonds (Fig. 25). Its six-membered aromatic ring stacks on the base of Cytosine 2452 for *Haloarcula marismortui* (2487 for *Escherichia coli*), and it fills the A-site cleft completely, the five-membered dioxolane ring attached to it. When HHT (**2**) is bound to the ribosome, 73% of its surface area becomes water inaccessible. The tertiary amine of HHT (**2**), which is protonated at neutral pH, establishes hydrogen bond with the O2 of Cytosine 2452 *H. marismortui* (2487 for *E.coli*). The hydroxyl group at the side chain forms a hydrogen bonding to the O6 of Guanine 2058 *H. marismortui* (2099 for *E. coli*) via intermediate water molecule, and the carbonyl group at the other end of the side chain establishes hydrogen bond with the N2 of Guanine

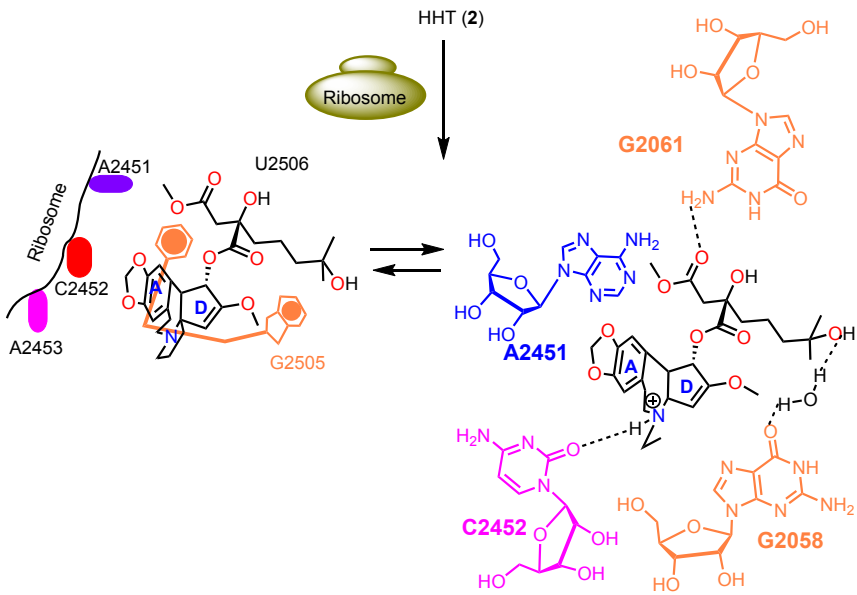


Figure 25 Interaction of homoharringtonine (**2**) with the ribosome of *Haloarcula marismortui* and the conformational changes associated with this binding.

2061 for *H. marismortui* (2102 for *E. coli*). The binding of HHT (**2**) to the large ribosomal subunit induces several conformational changes. The most powerful of them is roughly 50 degrees reorientation of the base of Uracil 2506 for *H. marismortui* (2541 for *E. coli*), which enables it to stack on top of the drug. In the drug complex, Uracil 2506 for *H. marismortui* (2541 for *E. coli*) forms a symmetric O4—N3/N3—O4 base pair with Uracil 2585 for *H. marismortui* (2620 for *E. coli*). In all other crystal structures of the large ribosomal subunit from *H. marismortui*, Uracil 2506 for *H. marismortui* (2541 for *E. coli*) forms a Guanine—Uracil base pair with G2583(2618). A number of more subtle conformational changes are seen in the A-site cleft region; C2452 of *H. marismortui* (2487 for *E. coli*) undergoes a slight rotation away from A2451 for *H. marismortui* (2486 for *E.coli*), and the base of A2453 for *H. marismortui* (2488 for *E. coli*) rotates by approximately 40 degrees about its glycosidic bond. This rotation allows A2453 for *H. marismortui* (2488 for *E. coli*) to form a base pair with U2500 *H. marismortui* (2535 for *E. coli*) and improves its stacking on C2452 for *H. marismortui* (2487 for *E. coli*).[176]

One of the main actions of HHT (**2**) is induction of apoptosis (Fig. 25). HHT (**2**) has a different mechanism of action from that of tyrosine kinase inhibitors, a class of drugs used in for the treatment of CML. Accordingly its activity in CML is independent of the mutation state of BCR-ABL cells (particular CML cells that contain a fusion gene called BCR-ABL1, generated by translocation of sequences from the c-ABLgene on chromosome 9 into the BCR gene on chromosome 22).[179]

The mechanism of apoptosis induced by HHT (**2**) was studied on different leukemic cell lines: U937, HL-60, HL60/MRP, HEL, THP, K562, MUTZ-1, CD34+/38^{lo}, CD34+, U-87MG, ΔEGFR, NB4, KU812, and KCL22 and on other diseases including systemic mastocytosis (SM) cell lines: HMC-1.2 and P815, multiple myeloma (MM) cell lines AMO-1, IM9, RPMI8226, EJM, U266, BA/F3, hematopoietic cell lines, OPM-2, and human hepatoma QGY-7703 cells.

A study was conducted on SHI-1 cell lines to prove that mitochondria are involved in the apoptosis induced by HHT (**2**).[180] Although HHT (**2**) induces apoptosis mediated by mitochondria (intrinsic pathway), it also induces apoptosis through death receptors (extrinsic pathway). The intrinsic pathway is induced by cleaving the proactive forms into active ones. HHT (**2**) activates some apoptosis mediators; it inhibits the apoptotic inhibitors and upregulates the release of other mediators through the action on genetic level. For instance apoptosis was studied in QGY-7703 cell line and 78

individual mRNAs were identified as having their transcription levels significantly changed. Those genes were mostly oncogenes, tumor suppressors, enzymes, kinases, and partially related to apoptosis, 68% being upregulated and 32% were downregulated. The following signaling apoptosis pathways were activated: TGF-β, tumor necrosis factor (TNF), FAS, p38MAPK, and p53.[181] The head protein of intrinsic apoptosis cascade is p53. It was activated by HHT (**2**) in human hepatoma QGY-7703 cells[181], but cell line with p53 mutation was more resistant to HHT (**2**).[182] A study demonstrated rapid apoptosis of U937 and HL60 cell lines on treatment with HHT (**2**); this was measured by increased annexin V binding capacity, caspase-3 activation, and the cleavage of poly(adenosine 5′-diphosphate (ADP)-ribose) polymerase (PARP). Also, the expression of Bax was upregulated during cell death induced by HHT (**2**), while the expression of bcl-2 was only slightly decreased.[183] On treatment of human myelodysplastic syndrome (MDS) MUTZ-1 cell lines with HHT (**2**), Ca^{2+} translocated from endoplasmic reticulum (ER) pool to cytosol, the mitochondrial membrane potential (Dwm) decreased, and Bid protein translocated from ER to mitochondria. Ca^{2+} and Bid proteins were released from ER pool to cytosol. The activation of ER stress-associated proapoptotic factor, CHOP and ER chaperones BiP and XBP1 genes, was followed by cleavage of caspase-3 but not caspase-4.[184,185]

mRNA expression levels of survivin were investigated in various hematopoietic cell lines in relation with apoptosis induced by HHT (**2**). It was concluded that the apoptotic effect of HHT (**2**) on the hematopoietic cell lines is associated with decreased level of survivin expression.[186] In a study of apoptosis using ssHHT (**2**) on HL60 and HL60/MRP (cells lacking Baxes) cell lines, ssHHT (**2**) was effective in a time- and dose-dependent manner, and independently of the expression of Bax. Additionally, ssHHT (**2**) induced apoptosis through the decrease of Dwm and the release of cytochrome c. This was explained by decreased myeloid cell leukemia-1 (Mcl-1) level and cleavage of Bcl-2, one of the proteins that governs the outer membrane of the mitochondria and has antiapoptotic activity (Fig. 26).[187] HHT (**2**) inhibited the telomerase content of HL-60 cells effectively and induced apoptosis.[188]

HHT (**2**) inhibits synthesis of antiapoptotic proteins of the Bcl-2 family, including Mcl-1 cell lines, leading to cell death. HHT (**2**) effectively induced apoptosis in primary CML stem cells ($CD34+/38^{lo}$) by downregulation of Mcl-1 protein.[189]

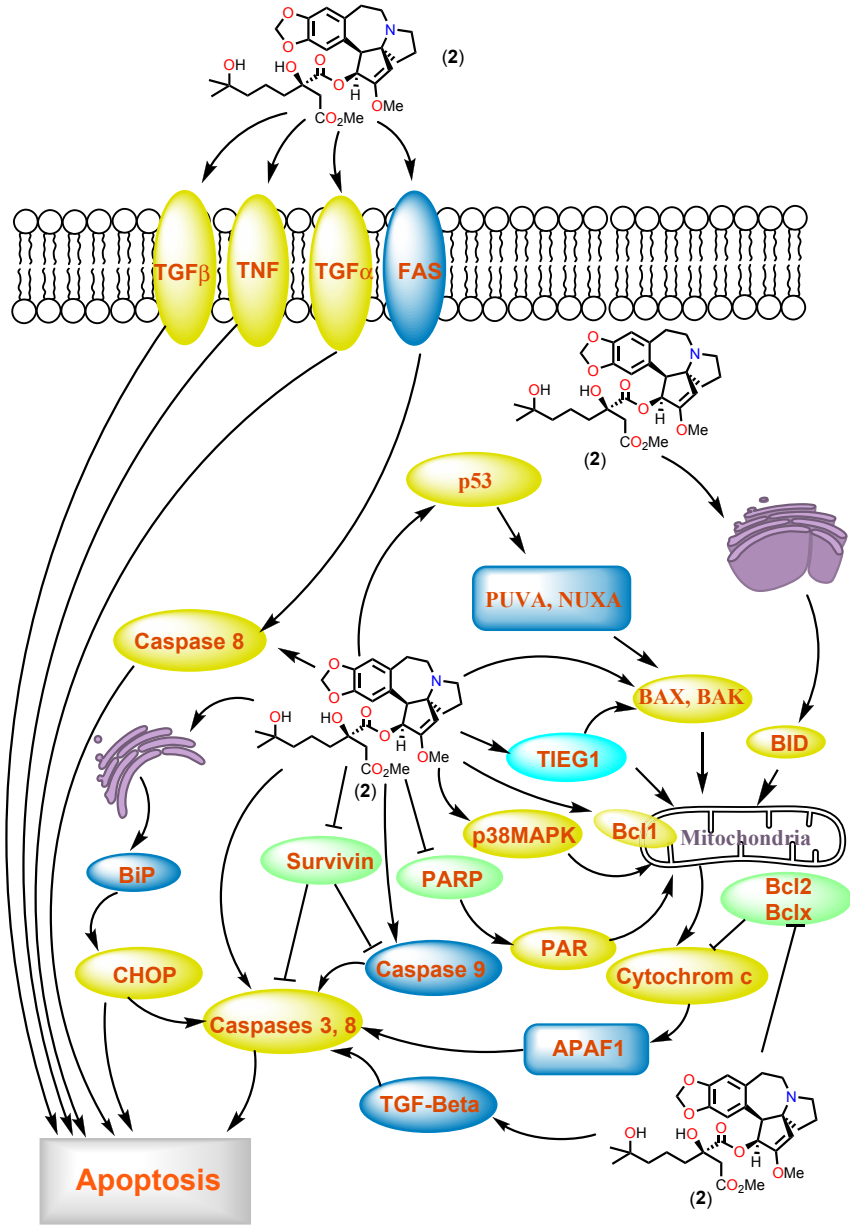


Figure 26 Main pathways involved in apoptosis mechanism induced by homoharringtonine (**2**). Yellow: apoptosis factor; Blue: apoptosis intermediator; Turquoise: dual role as intermediator and factor; Green: apoptosis inhibitor.

The intrinsic apoptosis using transforming subfamily of growth factor-β-inducible Sp1-like proteins called transforming growth factor-beta inducible early protein gene (TIEG1) was studied. TIEG1 induced apoptosis through upregulation of Bax and Bim and downregulation of Bcl-2 and Bcl-XL, release of cytochrome c, and caspase-3 activation.[190] The apoptotic effect of HHT was studied in K562 cell line using gene chip technology, it was found that 17 genes were upregulated and 27 were downregulated. Most of them were found to be related to apoptosis, oncogenes, or tumor suppression. Several genes with altered gene expression were found, such as *TIEG*, vitamin D3 upregulated protein one gene (*VDUP1*), RNA binding motif protein 4 gene (*RBM4*), and v-myc myelocytomatosis viral oncogene homolog (*C-MYC*). The activated transforming growth factor-β and TNF—signaling pathways play an important role in apoptosis according to the dynamic gene expression pattern in these apoptotic cells. TIEG was significantly modified after induction of apoptosis, which is critical for apoptosis signal transmission.[191] A Chinese team tried to identify different genes whose expression is modified during apoptosis induced by HHT (**2**) on leukemic HL-60 cell lines; they demonstrated that TGF-α and TNF apoptosis signaling pathways were initiated but not the Fas signaling pathway. Overexpression of *TIEG* and *VDUP1* could induce apoptosis in some kinds of cells and they were upregulated. Other genes, such as *BRD2*, *ACVRL1*, and *RBM4* were also upregulated and are probably involved in apoptosis signaling pathways.[192]

The efficacy of HHT (**2**) against ponatinib-resistant Ph-positive cells Ba/F3 (Ba/F3 ponatinib-R) was shown by decreased protein expression levels of BCR-ABL and Crk-L. On the other hand, caspase-3 and cleaved PARP levels were significantly increased. HHT (**2**) treatment reduced the expression of BCR-ABL, as well as heat shock protein (HSP) 90, which stabilizes the BCR-ABL protein. It was found that HHT (**2**) reduced the expression level of the antiapoptotic protein Bcl-2 and of c-Myc. HHT (**2**) was examined on ponatinib-resistant primary Ph-positive ALL and CP CML cell lines. The synthesis of BCR-ABL, HSP90, and Bcl-2 was downregulated and was independent of caspase activity.[193] HHT (**2**) antagonizes specifically the *p*-eIF4F function, which is specifically required by cancer cell for their survival and not the eIF4F, which is required for normal cells.[194] HHT (**2**) reduces the levels of prooncogenic or prosurvival proteins (important proteins for tumorigenesis) having short half-lives, such as Mcl-1, cyclin

D1, or c-Myc. This was demonstrated on a well-studied transgenic mice model *Eμ-myc* lymphomas harboring lesions in *Pten*, *Tsc2*, *Bcl-2*, or *eIF4E*.[175]

Cytotoxicity of HHT (**2**) and cell-growth activity were investigated in three AML cell lines, HL-60, NB4, and U937, and in three CML cell lines, K562, KU812, and KCL22. AML cells showed more sensitivity than CML cells to HHT-induced cytotoxicity. Using HL-60 cells, it was revealed that HHT (**2**) decreased the levels of Mcl-1, X-linked inhibitor of apoptosis protein (XIAP), survivin, and B-cell lymphoma 2 (Bcl-2) homology domain 3 (BH3)-only proteins as well as the Dwm. U937, K562, KU812, and KCL22 cells that expressed B-cell lymphoma-extra-large (Bcl-xL) (antiapoptotic transmembrane protein in the mitochondria) were less responsive to HHT-induced apoptosis than HL-60 cells. It was suggested that Bcl-xL plays a more important role than Bcl-2 and Mcl-1 in protecting against HHT (**2**)-induced apoptosis.[195]

In addition, it was demonstrated in leukemic cell lines K562 that HHT (**2**) inhibits the synthesis of vascular endothelial growth factor, an important mediator of angiogenesis in the bone marrow. This work thus demonstrated a possible antiangiogenic activity for HHT (**2**).[196]

A study to identify novel biomarker that might contribute to variation in response to HHT (**2**) by using a panel of various human lymphoblastoid cell lines was performed. Genome-wide association analysis was applied using single nucleotide polymorphism (SNP) and mRNA expression data. The integrated analysis identified four unique SNPs that were associated with both expression and area under the curve. Functional validation using siRNA knockdown in leukemia cell lines showed that knocking down *CCDC88A*, *CTBP2*, *SOCS4* genes in U937 and K562 cell lines significantly altered HHT (**2**) cytotoxicity.[197]

The overexpression of EphB4 contributed to IM resistance in CML line cells. Cell lines from a patient with relapsed CML were established at the time of first diagnosis and after being relapsed. EphB4/RhoA may be a new marker for IM resistance. This is supported by the fact that HHT (**2**) with IM yielded more treatment advantages than IM alone by blocking EphB4/RhoA pathways.[198,199]

Apoptosis varying intervals in K562 cell line treated with HHT (**2**) was studied. The results showed that the viability of K562 cells reduced gradually from day 1 to day 5 and ascended from day 6 to day 8 after HHT (**2**) treatment. At the same time, the cleaved caspase-3 expression level in K562 cell lines increased gradually from day 1 to day 7 but decreased at

the day 8 ($P < .05$). From day 1 to day 8 after HHT (**2**) treatment, the BCL-2 expression level declined first and then went up ($P < .05$). Autophagosome was also increased at day 8 after HHT (**2**) treatment. It is concluded that the apoptosis level of K562 cells after being treated with HHT (**2**) increased at the beginning of the treatment and then it declined, which may be associated by higher autophagy level in the late stage of HHT (**2**) treatment.[200] A recent study demonstrated that HHT (**2**) binds to the DNA with an intercalation mode.[201] Also, HHT (**2**) binds to the telomere G-quadruplex, which makes it a good model for drug design.[202]

Analysis of proteins expression profiles in K562 cell line (a CML cell line) in response to HHT (**2**) treatment showed that nine proteins were downregulated and four upregulated. Aside from alterations in apoptotic proteins and proteins associated with transcription and translation, changes in oxidative stress response and redox reaction—related proteins, such as HSPs, DJ-1, and thioredoxin were revealed. Specifically, these proteins were decreased after HHT (**2**) treatment in K562 and primary CML cell lines.[203] Also, it was found that HHT (**2**) is an effective inhibitor of unwanted angiogenesis.[204,205]

6.1.2 Potential Use for the Treatment of Other Tumors

Beside its use in the treatment of CML, HHT (**2**) is being investigated for other type of tumors alone and in combination with other drugs: HHT (**2**) significantly inhibited the proliferation of human MM cell lines and tumor cells from patients with relapsed refractory MM in a dose-dependent manner. This apoptotic process was associated with the activation of caspase-8, caspase-9, caspase-3, and PARP.[206] The effect of HHT (**2**) was demonstrated on MM, on the following cell lines AMO-1, IM9, RPMI8226, EJM, U266, and OPM-2; HHT (**2**) significantly reduced Mcl-1, a crucial protein involved in myeloma cell survival, in all the myeloma cell lines that were examined. Also, HHT (**2**) reduced the levels of c-FLIPL/S, activated caspase-8, and induced active reduction of Bid. It follows that HHT-induced apoptosis appears to be mediated via both intrinsic and extrinsic apoptosis pathways. The resultant imbalance between BH3-only proteins and Mcl-1 may be pivotal for apoptosis by HHT (**2**). Also, HHT (**2**) treatment resulted in reduced levels of b-catenin and XIAP proteins, which also contribute to disease progression and resistance to chemotherapy in MM. HHT (**2**) enhanced the effects of melphalan, bortezomib, and ABT-737 when given in combinations with these agents. These results suggest that HHT (**2**) could constitute an attractive option

for MM treatment through its ability to simultaneously target multiple tumor-promoting molecules.[207] HHT (**2**) and Bortezomib synergistically inhibit SKM-1 MDS cell proliferation and induce apoptosis in vitro.[208]

Transmembrane receptor of the tyrosine kinase KIT oncoprotein is an important therapeutic target as part of the type III tyrosine kinases. Some mutations in this receptor (V560G, D816V, or D814Y) give it the ability of auto-dimerization and auto-phosphorylation without activation by its ligand, which can eventually lead to uncontrolled cell proliferation and a resistance to apoptosis. The gain of function associated with these mutations of the KIT receptor plays a critical role in the pathogenesis of SM and gastrointestinal stromal tumors (GISTs). The hypothesis that HHT (**2**), as an inhibitor of protein synthesis, decreases the protein level of KIT thereby reduces the level of phospho-KIT and repeals its constitutive signaling downstream. HHT (**2**) is effective both in vitro and in vivo demonstrating that it effectively inhibits tumor growth in mice and induced apoptosis of tumor cells carrying the V560G (sensitive to IM) and D816V (insensitive to IM) mutations of KIT. In addition, HHT (**2**) significantly prolonged the mouse survival time with aggressive SM (35 days instead of 7) though a rapid resistance to HHT (**2**) was observed in this case.[209]

Potential activity of HHT (**2**) against clear cell–type renal cell carcinoma (RCC) with mutant von Hippel–Lindau gene was revealed by high-throughput simultaneous screen and counterscreen.[210] In addition, HHT (**2**) has the potential for treatment of the Gefitinib-resistant non–small cell lung cancer, according to an in vivo study.[211]

6.1.3 Combination With Other Agents

A signal oncogene is important for some tumors for their continued activity of their malignant phenotype. The BCR-ABL fusion protein in CML is the best-studied example. HHT (**2**) that inhibits protein synthesis thus reduces the level of the BCR-ABL protein level. The kinase activity of BCR-ABL is directly inhibited by IM and flavopiridol (a transcription inhibitor). The median-effect method was used to evaluate combinations of flavopiridol/HHT (**2**) and flavopiridol/IM. The resulting analysis indicated synergistic decrease in the clonogenicity. Synergy increased when HHT (**2**) and IM were administered sequentially as opposed to simultaneously. Transfected IM-resistant Ba/F3 cells that expressed the E255K and T315I mutations of BCR-ABL were not cross-resistant to flavopiridol

and HHT (**2**). These results provided a rationale for the combination of inhibitors of transcription and/or translation with specific kinase inhibitors.[212]

The combination of HHT (**2**) and DOX had ratio-dependent synergistic activities, with very strong synergism observed at a molar ratio of 4/1 (DOX/HHT) within double-loaded liposomes. According to this study liposomal codelivery of DOX and HHT (**2**) could synergistically potentiate antitumor effects.[213]

HL60/ADR, Kasumi-1, and primary AML cells were pretreated with Decitabine and then exposed to HAA regimen (HHT (**2**), aclarubicin, and cytarabine (Ara-C)). Decitabine increased cytotoxic effect of HAA regimen and enhanced apoptosis in chemoresistant AML cells.[214]

A synergistic effect of Sulforaphane in human CML cell line K562 with chemotherapy drugs HHT (**2**) and Ara-C was found and indicated clinical potential.[215]

The combination of HHT (**2**) and YM155 was investigated on K562 cell lines, and it was found to be synergistic. The mechanism was related to the apoptosis induced through downregulation of survivin.[216]

TNF-related apoptosis-inducing ligand (TRAIL) is a proapoptotic ligand from the TNF-α family. HHT (**2**) represents a very efficient enhancer of TRAIL-induced apoptosis with potential application in TRAIL-based, anticancer combination therapy. The agonistic anti-TRAIL receptor antibody is a potential antitumor agent. HHT (**2**) was found to be a very effective (in nano-molar dose) enhancer of TRAIL-mediated apoptosis/growth suppression. Cotreatment of TRAIL-resistant RKO or HT-29 cells with HHT (**2**) and TRAIL led to the effective induction of apoptosis and the complete elimination of the treated cells. HHT (**2**) suppressed the expression of the antiapoptotic proteins Mcl-1 and cFLIP and enhanced the TRAIL-triggered activation of JNK and p38 kinases.[217] Another study suggested that HHT (**2**) can replace toxic substances such as cycloheximide because it activates necroptosis through the same signaling pathways (involving RIPK1/RIPK3/MLKL) to sensitize human cancer cells to TRAIL-induced necroptosis.[218]

HHT (**2**) was combined with antiproliferatives such as alkylating agents, intercalating agents, metal coordination complexes, pyrimidine nucleosides, purine nucleosides, inhibitors of nucleic acid—associated enzymes and proteins, and agents affecting structural proteins and cytoplasmic enzymes, for the treatment of a host with a cellular proliferative disease, particularly a neoplasia.[219] When matrine combined with HHT (**2**) was

tested on the proliferation of human AML cells HL-60, a synergistic effect on apoptosis was noticed.[220,221]

6.1.4 Resistance Mechanism for Homoharringtonine

The main mechanism of HHT (**2**) resistance is through a pump that removes HHT (**2**) from tumor cells. This transporter is called drug-efflux pump P-glycoprotein, also known as MDR1. Through the action of this pump the intracellular drug levels may remain sublethal and, in the case of upregulation of MDR1, cancer cells may survive. The HHT-resistant cell line SKM-1/HHT was established and the prominent overexpression of MRD1 may be the main cause for multidrug resistance.[222] ssHHT (**2**) derivatives that bypass MDR1 might improve treatment efficacy.

Short hairpin RNAs (shRNAs) were designed, constructed, and transfected into HT9 leukemia cell line, for specific silencing of the MRD1 gene. Resistance against HT (**3**), DOX, and curcumin was decreased alongside the decreased expression of MRD1. The study indicated that shRNA recombinant plasmids could modulate MDR in vitro.[223]

Human MDR B-cell lymphoma cell line BJAB/ADR was found to be resistant to HHT (**2**), daunorubicin (DNR), etoposide, and mitoxantrone (MTX).[224]

A study on the pharmacological effect of HHT (**2**) on the molecular level was conducted. CET (**1**) and HHT (**2**) were tested for resistance on the MDR cell lines. These were the 55 cell lines of the Developmental Therapeutics Program of the National Cancer Institute (NCI, Bethesda, Md., USA) that overexpress the conferring P-glycoprotein/MDR1 (CEM/ADR5000), which can expel HHT (**2**) of the cell and consequently which can attenuate its activity. In CEM/ADR5000 cells, a threefold increase to HHT (**2**) resistance was observed, though the other MDR cell lines did not show cross-resistance.[225]

6.1.5 Mechanism of Allergic Reaction

HHT (**2**) was screened and identified as a potential allergic factor. Histamine 1 receptor/cell membrane chromatography (H1R/CMC) model was applied for capturing membrane-retained components, which could induce H1R activation. Retention components were enriched and analyzed by H1R/CMC-HPLC/MS. HHT (**2**) was recognized, separated, and identified in HHT (**2**) medication. Ca^{2+} flux assay and p-IP3R (channels that most often mediate Ca^{2+} release from intracellular stores) expression found that HHT (**2**) retained by the H1R/CMC model

increased phosphorylation of IP3R and promoted cytosolic free Ca^{2+} elevation in a dose-dependent manner, which further verified the activity of HHT (2) in activating H1R. This provides an indication that a patient with overexpressed H1R should be aware of possible allergic reaction when applying HHT (**2**) injection.[226]

6.1.6 Other Pharmacological Applications

From the chloroform extract of the aerial parts of the only *Cephalotaxus harringtonia* L. specimen grown in Egypt, five known alkaloids CET (**1**), HHT (**2**), HT (**3**), isoHT (**4**), and deoxyHT (**5**) were identified by HPLC−ESI−MS.[227] This extract was tested in vitro for its cytotoxicity against HCT116, HepG2, and MCF-7 cell lines and resulted with IC_{50} values of 4.77, 12.9, and 17.5 μg/mL, while DOX had IC_{50} values of 3.74, 4.57, and 2.97 μg/mL against these cell lines, respectively.

Chikungunya virus (CHIKV) is a mosquito-transmitted virus that has reemerged as a significant public health threat in the last decade. HT (**3**) displayed potent inhibition of CHIKV infection (EC_{50} 0.24 μM) with minimal cytotoxicity. HT (**3**) acts on the postentry stage of the CHIKV replication cycle and strongly interferes with the process of viral protein synthesis.[228]

Cryptosporidium parasites cause cryptosporidiosis, a diarrheal disease usually, which can result in fulminant diarrhea and death in AIDS patients and chronic infection and stunting in children. A library of drug repurposing candidates were screened against *Cryptosporidium parvum* among which HHT (**2**) showed promising in vitro activity, its IC_{50} activity was 0.86 μM.[229]

HHT (**2**) and CET (**1**) were tested against the hepatitis B virus (HCV), using the bovine viral diarrhoea virus (BVDV) as surrogate for HCV. The activity relative to BVDV was determined by reduction of BVDV-RNA production and protection of infected embryonic bovine trachea (EBTr) cells. Activity against HBV was investigated by HBsAg release and HBV-DNA release from hepatoblastoma HepG2 2.2.15 cells infected with HBV. HHT (**2**) showed high activity against HBV (IC_{50} was 2 μM against HBV, but it has a very low therapeutic index).[230] CET (**1**) and HHT (**2**) induced toxicity in EBTr cells and had no protective effect against BVDV. In contrast, they were able to inhibit HBV production at concentrations 10- to 100-fold lower than other molecules inducing cell toxicity, which suggests that they are potentially useful in combined therapy against hepatitis B.

A library of 290 compounds was screened for antiviral activity against Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV). Selection of compounds for inclusion in the library was dependent on current or previous FDA approval or advanced clinical development. Some drugs that had a well-defined cellular pathway as targets were included. In total, 27 compounds with activity against both MERS-CoV and SARS-CoV were identified. The EC_{50} activity of HHT (**2**) against MERS-CoV was 71.8 nM.[231]

HT (**3**) was extremely effective in inhibiting human retinal glial cell proliferation. Its activity was comparable to other antiproliferative drugs such as colchicine, daunomycin, and 5-fluorouracil. HT (**3**) is therefore a potential drug for the treatment of proliferative vitreoretinopathy (PVR).[232]

In 2009, the inhibitory effects of HHT (**2**) on CML and B-ALL (B cell ALL) cell line was evaluated in vitro and showed that over 90% of leukemic stem cells were killed after treatment with HHT (**2**). In contrast, less than 9% or 25% of leukemic stem cells were killed after treatment with IM or dasatinib, respectively.[233]

HHT (**2**) has demonstrated activity against coronavirus with an IC_{50} ranging from 11 nM to 1.2 μM.[234] Also, HHT (**2**) when combined with the saponin digitonin showed activity against *Trypanosoma brucei brucei*.[235]

6.2 Clinical Application

HHT (**2**) or OM (INN) provides a treatment option with a distinct mechanism of action for patients who have not achieved an optimal outcome with TKIs, including patients with the T315I mutation.[236] Recent reviews compiled clinical trials conducted first in China with mixtures of HHT (**2**) and HT (**3**), then in Western countries using purified high purity ssHHT (**2**) reported in 1998 by Robin (OncoPharm founder) also known as OM allowing to confirm its efficacy in CML and put the first European Medicine Agency (EMA) application in 2004 leading to its orphan drug status in 2009, and in the United States with a stable source of hydrosoluble omacetaxine reported in 2001 by Brown (ChemGenex founder).[12,237–240]

6.2.1 Approved Clinical Applications

HHT (**2**) was approved in 2009 by the EMA for t-Philadelphia chromosome positive CML in patients who have the T315I BCR-ABL kinase domain mutation and who are resistant to prior IM therapy.[241] It was approved

by the FDA in October 2012 for the treatment of CML in CP or AP after failure of two or more TKIs.[242]

6.2.2 Different Clinical Trials for Chronic Myeloid Leukemia

To evaluate the pharmacokinetics of HHT (**2**), sensitive HPLC-tandem mass spectrometry (LC-MS/MS) assays for the quantification of omacetaxine and its inactive 4′-desmethyl (4′-DMHHT) and cephalotaxine metabolites in human plasma and urine were developed and validated, according to the latest FDA and EMA guidelines.[243] Deuterated isotopes were used as internal standards and these assays were considered very suitable to support clinical pharmacologic studies of OM. Since omacetaxine is mainly metabolized by esterases, the plasma samples were immediately stabilized after collection with an esterase inhibitor and stored at a nominal temperature of −80°C. The validated plasma assay quantifies all analytes in the concentration range of 0.1−100 ng/mL and the urine assay in the range of 0.1−50 ng/mL. At all concentrations, the accuracies were within ±15% of the nominal concentrations and precisions were ≤15%. The developed methods have successfully been applied in a human mass balance study of omacetaxine.

Nearly 70%−90% of patients with CML in the early chronic phase (CP) who were treated with IM achieved complete cytogenetic response. With a median follow-up period of 5 years, the annual rate of resistance or progression is 3%−4%, and the mortality is 1%−2%.[244]

In 2001 a study was conducted on 37 patients ≤21 years old with recurrent refractory AML, 28 patients were evaluable for response. Four patients responded completely and one responded partially (response rate 5/28 18%); the median duration was roughly 62 days. This study showed potential use of HHT (**2**) for pediatrics with tolerable toxicity.[245]

Two cases of CML patients acquired the T315I mutation after IM treatment; one was treated with recombinant interferon-alpha (IFN-α), and the patient was in complete hematologic response (CHR) 10 months later, while the other patient treated with HHT (**2**) started at 1.25 mg/m^2 subcutaneously twice daily for 5 consecutive days every month and achieved a CHR after 5 months.[246]

The outcomes of 176 patients of AP CML who received treatment with IM were reviewed and compared with the outcomes of 213 historic control patients treated with other drugs including HHT (**2**). Treatments included INF-α (100 patients), HHT (**2**) (35 patients), DNR plus cytarabine

(23 patients), decitabine (47 patients), and other therapies (8 patients). CHR were 82% with IM and 35% with HHT (**2**).[247]

In 2010, eight TKI-resistant CP CML patients unmutated and T315I-mutated BCR-ABL were enrolled in a clinical trial. They all received HHT (**2**) to investigate if patients will be resensitized to TKIs. Initially a rapid decline and a sustained disappearance of T315I-mutated transcripts were observed in 50% of patients. This result indicates the disappearance of the mutated clone.[248]

In 2009, a year-long study on long-term treatment with HHT (**2**) was carried out. A year-long study on long-term treatment with HHT (**2**) was carried out. One hundred and six patients with CML received 1.5 mg/m^2 of HHT (**2**) alone for 7 to 9 days every 4 weeks. 79 patients were in the control group, 31 were treated with IFN-α, and 48 with hydroxycarbamide. Seventeen patients who were treated with IFN-α failed to achieve cytogenetic response within 12 months and subsequently received HHT (**2**) as a salvage treatment. After 12 months of therapy, cytogenetic response rate of the HHT (**2**), IFN-α, and hydroxycarbamide groups were 39/106, 14/31, and 3/48, and corresponding molecular cytogenetic response rates 6/18, 3/8, and 0, respectively. Of the 17 patients who received HHT (**2**) as salvage treatment, 6 achieved cytogenetic response (3 major). At the 48-months follow-up, cytogenetic response was maintained in 32/39 patients treated with HHT (**2**). Patients who had cytogenetic response in HHT (**2**) group or treated with IFN-α also showed longer median chronic durations, which were 45 months (12−98 months) and 49 months (12−92 months) respectively, indicating a longer survival time.[249]

In 2015, the efficacy and safety data of long-term administration of HHT (**2**) were evaluated. After 24 months of follow-up, the final analysis included additional efficacy and safety analyses to assess the benefit of long-term HHT (**2**) administration (1.25 mg/m^2 twice daily for 14 days every 28 days followed by 7 days every 28 days) in CP and AP CML patients who received >3 cycles. 18% of CP-CML patients achieved a major cytogenetic response (MCyR) with a median duration of 12.5 months. Responses were maintained for 12 months in three of forteen responders, and the median overall survival (OS) was 40.3 months. Among patients with AP-CML, 14% achieved or maintained a major hematologic response (MHR) for a median of 4.7 months, MCyR was not achieved, and the median OS was 14.3 months. In patients with CP-CML and patients with AP-CML who received >3 cycles of treatment (n-50 and n-14, respectively), the median OS was 49.3 and 24.6 months respectively. Grade 3 or higher hematologic

toxicities were the major side effects (79% and 73% for CP-CML and AP-CML, respectively), with discontinuation due to toxicity in 10% of CP patients and in 5% of AP patients.[250]

The efficacy and safety of HHT (**2**) in patients with AP-CML previously treated with tyrosine kinase inhibitors were assessed in two phase II trials. 51 patients in AP-CML and 44 in myeloid blast phase (BP-CML) received subcutaneous (SC) omacetaxine 1.25 mg/m^2 twice daily, days 1−14 every 28 days until hematologic response/improvement or any cytogenetic response, and then days 1−7 every 28 days until disease progression. An MHR was 37% in patients with AP-CML and 9% in patients with BP-CML (22% and 5% in those were with a history of *T315I* mutation).[251]

In 2007, a 63-year-old male CML patient was reported harboring a T315I BCR-ABL mutation while on IM treatment that completely disappeared when he was treated with HHT (**2**).[252]

In 2013, HHT (**2**) produced good clinical responses with acceptable tolerance levels in patients with CML-CP who had been treated with two or more TKIs. These Data were analyzed from two phase II clinical trials of 81 patients with CML-CPs. The median age of those in the analysis was 59 years (range, 26−83 years). Eight patients completely responded. The median duration for the study was 17.7 months. Fifty-six patients (69%) achieved and/or maintained hematologic response for at least 8 weeks. The overall survival was 34 months.[253]

In 2015, a study was carried on 31 CML patients with complete cytogenic response to compare the immunological effect on T lymphocytes between HHT (**2**) (15 patients) and IM (16 patients); the study indicated that both have the same effect.[254]

6.2.3 Administration, Dosage, Metabolites, and Elimination

A recent review discussed the use and effectiveness of HHT (**2**) in the treatment of intractable CML, with coverage of its pharmacology, mode of action, and pharmacokinetics.[237] A SC formulation demonstrated efficacy and safety in phase 1/2 trials. HHT (**2**) at 1.25 mg/m^2 SC was administered (twice daily) BID, days 1−14 every 28 days for two cycles. This mode of administration was developed because in early clinical studies short intravenous (IV) infusions (over 60−90 min) was used, but life-threatening hypotension and tachycardia were observed at dose levels above 3−4 mg/m^2.[255] A stable aqueous solution for injection of HHT (**2**) was prepared with tartaric acid at pH around 4.[256] It is only recently however that the site of protonation, although obvious, was unambiguously seen at N9 of CET (**1**)

or HT (**3**) as established through X-ray diffraction studies of various organic salts,[76] an observation in concordance with Steitz[176] (see Fig. 25) and Takano's[60] results implying that N9 should be available for protonation to ensure its biological activity. Therefore, HTs seem to be active via their protonated form. The maximum tolerated dose was estimated to be 5 mg/m^2.[257] It is provided in vials that contain 3.5 mg HHT (**2**) as a sterile, preservative-free, and white to off-white lyophilized single-use solution for injection.[241] In a recent patent HHT (**2**) was incorporated in phycobiliprotein nanoparticles.[258] HHT (**2**) can be given as aqueous solution preferably at a pH of about 4. Also it can be provided orally.[259–263] The protonated form of HHT (**2**) was patented for potential application in oral and IV dosage forms.[264]

MM remains an incurable disease in most patients. Newly developed high-proportion polyethyleneglycol (H-PEG) of long-circulating liposomes (LCL) loaded with HHT (**2**) (HHT-LCL-H-PEG) may be regarded as a promising nano-device to deliver anti-MM drug HHT (**2**) for treatment of MM patients.[265] This HHT-LCL freeze-dried powder can significantly improve storage stability, can make HHT-LCL freeze-dried powder convenient to dilution administration, make drug in liposome-sustained release, and extend the circulation time in blood, to improve the utilization rate of medicament effective component, and reduce toxic and side effects.

The major metabolites of HHT (**2**) are CET (**1**) and 4'-desmethylHHT (**31**), they are detected by HPLC.[266] In another study using ^{14}C HHT and detection by HPLC mass spectrometry (MS) (high resolution) in combination with offline radioactivity determination, six metabolites were detected in the plasma, urine, and feces, unchanged HHT (**2**) was predominant.[267,268]

6.2.4 Combination Therapy

Trials have been carried on combining HHT (**2**) with other anticancer drugs to synergize and potentiate the activities of these drugs and improve therapy outcome. In some cases, the associated drugs can be efficiently codelivered in the form of liposomes.[269]

6.2.4.1 Combination Therapy for Chronic Myeloid Leukemia

In 2002, the efficacy and safety of a combination regimen of simultaneous HHT (**2**) and IFN-therapy was proved with 37 patients with CP-CML who were not treated previously by either agent. The CHR rate was 85%.[270]

In 2009, a clinical trial phase II carried on 44 previously untreated patients with Philadephia-positive chromosome (Ph+) CP-CML, the treatment consisted of HHT (**2**) (2.5 mg/m^2 per day) and cytarabine (7.5 mg/m^2 per day). Both drugs were given together via continuous IV infusion for 7 days. 36 of 44 patients (82%) achieved a CHR. Although HHT/cytarabine was generally well tolerated, no significant amelioration was observed for patients with interferon-refractory CML and result was not nearly as high as association with IM in newly diagnosed patients.[271]

In 2010 in China, 12 patients with CML in blast crisis who have failed prior single-agent therapy with IM were enrolled in clinical trial to investigate the induction therapy of granulocyte colony-stimulating factor (G-CSF) and low-dose HHT (**2**) as well as standard-dose IM (GHI). The outcome of this study suggested that the GHI regimen may overcome disease-poor response to conventional doses of IM followed by allogeneic hematopoietic stem cell transplantation (allo-HSCT).[272]

Ninety patients with Ph+ CML in early CP received the triple regimen IFN-α, ara-C, and HHT. After a median duration of 16.5 months of therapy, 78 patients had their therapy changed to IM, 85 patients (94%) achieved CHR, and 67 patients (74%) had a cytogenetic response (Ph suppression to $\leq$90%).[273]

14 patients were treated with a regimen that combines HHT (**2**) and IM among which six cases failed and eight cases sub-optimally responded to IM therapy. This trial proved the efficacy and safety of combining both drugs.[274]

6.2.4.2 Combination Therapy for Acute Myeloid Leukemia

In 2009, 87 AML patients were treated with HHT (**2**), Ara-C, and DNR (HAD) regimen, the complete remission rate of the 87 cases was 80/87 (92%).[275]

Six hundred and twenty three newly diagnosed Chinese patients with AML were treated with induction chemotherapeutic regimens based on idarubicin, DNR, and HHT (**2**). Total complete remission rate was 66.5%, median survival was 18 months, and estimated survival at 3 years was 30.8%. The third year relapse rate was 55.1%.[276]

A group of 254 Chinese patients with de novo AML with glutathione S-transferase theta 1 (GSTT1) and glutathione S-transferase mu 1 (GSTM1) mutations were administered in a clinical study. As induction therapy, the patients were treated with HHT (**2**) 2.5 mg/m^2 intravenously on days 1−7, Ara-C 150 mg/m^2 per day continuous infusion on days 1−7,

and DNR 45 mg/m^2 intravenously on days 1−3 (HAD regimen). The second course of HAD induction therapy was applied to patients who were not in complete remission after the initial course of induction therapy. Postremission therapy consisted of HA regimen (HHT (**2**) and Ara-C) (course 1 and 4), DA regimen (DNR and Ara-C) (courses 2 and 5), and MA regimen (MTX and Ara-C) or HAM (Ara-C days 1−4, combined with MTZ 8 mg/m^2, IV on days 5−7) (courses 3 and 6). Overall survival and disease-free survival of complete remission patients in *GSTT1* and *GSTM1* double present group was better than in *GSTT1*- and/or *GSTM1*-group.[277]

A regimen was investigated to explore the effect of low dose of HHT (**2**) and Ara-C combined with G-CSF on patients with relapsed or refractory AML: 35 out of 67 (52.2%) patients achieved complete remission and 8 out of 67 (11.9%) achieved partial remission. The overall response rate was 64.1%.[278]

In 2003 a clinical trial of combined regiments HHT (**2**), Ara-C, and etoposide (VP-16) was conducted on 12 pediatric patients, 9 patients achieved complete remission within 20−45 days after one course of therapy. One patient had partial remission this clinical trial gave good indication for this combination.[279]

HAA regimen, was evaluated in 46 patients with refractory and/or relapsed AML for efficacy and toxicity. 80% of patients achieved complete remission.[280]

β-Elemene emulsion had a curative effect in treatment of refractory/relapsed AML in combination with HAA regimen. This was clinically proven by comparing two groups: one using the HAA alone and one using β-elemene emulsion with HAA. The total effective rate in the β-elemene emulsion plus HAA group was 80.8%. However, the total effective rate in the HAA only group was 52.9%.[281]

Sixty-seven patients with relapsed or refractory AML were enrolled in a clinical trial. They received the following treatment: HHT (**2**), Ara-C, and G-CSF (HAG). All the patients were treated with HAG regimen. 35 patients (52.2%) achieved complete remission and 8 (11.9%) partial remission. The overall response rate was 64.1%[282]

One hundred and ninety six untreated de novo AML patients were recruited in China for a clinical trial using HHT (**2**). Patients were treated with HHT (**2**) and Ara-C (HA)-based induction therapy composed of three chemotherapeutic drugs (HAD/M, D-DNR, M-MTX). The complete response rate was 153 of all cases (78.1%).[283]

One hundred and seventy-one pediatric patients were recruited in a trial. They received a regimen including HHT (**2**), cytarabine and DNR with a median age of 7.58 years. Complete response was obtained in 140 of 171 (81.9%) cases within 60 days. The 5-year event-free survival was 52.75%.[284]

Fourty-eight patients median aged 35 (14−57) years were enrolled into a clinical study to evaluate the efficacy and toxicity of HAA regimen as an induction therapy in the treatment of de novo AML. The HAA regimen was a well-tolerable and effective induction regimen for patients with de novo AML.[285]

A case was reported with primary refractory FLT3-ITD-positive AML was treated with combination of low dose HHT (**2**) and Sorafenib (salvage treatment); the therapy was successful and the patient was in complete remission.[286]

Ninety elderly patients with AML were chosen and divided into CAG (low-dose cytarabine and aclarubicin in combination with G-CSF) group (52 cases) and HAG (HHT (**2**) and Ara-C combined with G-CSF) group (38 cases). In the CAG group were 38 cases with complete remission (73.08%) and total efficiency was 84.62%; 40 cases with complete remission in HAG group (36.84%) and total efficiency was 73.68% in the HAG group. CAG regimen was more efficient than HAG in elderly patients with AML.[287]

In a recent clinical study for elderly patients with de novo AML, 56 patients were enrolled in this study. They were treated with HHT (**2**) and cytarabine in combination with G-CSF (HAG). Updated results showed good overall survival.[288]

A recent metaanalysis provided an overview of the effectiveness of HHT (**2**) combination regimens to treat AML. Twenty-one studies were analyzed including 1310 patients, 229 pediatric, and 216 elderly. HHT (**2**) was given in combination with cytarabine, DNR, idarubicin, aclacinomycin, MTX, or G-CSF. This retroanalysis showed the superiority of HHT (**2**)-containing regimens in AML treatments.

6.2.5 Other Clinical Applications

Clearly, other potential clinical applications are the other subtype of leukemia. Consequently different clinical trials with different regiments took place:

Thirty-one adult patients suffering from idiopathic thrombocytopenic purpura (ITP) were treated with low-dose combination chemotherapy consisting of cyclophosphamide and prednisone on days 1−7, combined

with vincrinstine on day 1, and/or azathioprine on days 1 to 5 or 7 and/or HHT (**2**) on days 1, 3, 5 or on days 1–7 and/or etoposide on days 1–7.[289] The output was as following: 13 of 31 (41.9%) patients had complete remission, 9 (29.0%) had partial remission, and 9 (29.0%) had no response.

A 45-year-old female patient was reported with de novo acute megakaryoblastic leukemia (rare type of AML) with BCR/ABL rearrangement and der(16)t(1; 16)(q21; q23) translocation but negative for t(9; 22) Ph chromosome. On HAD induction chemotherapy, the patient achieved partial hematological remission. The patient was then switched to IM plus one cycle of CAG regimen (low-dose cytarabine and aclarubicin in combination with G-CSF). She achieved complete remission, the disease recurred after 40 days and the patient eventually died of infection.[290]

Thirty-four myelodysplastic syndromes patients with MDS-RAEB and RAEB-T were recruited for a clinical trial of two regimens therapy of either HAA regimen or Idarubicin Ara-C (IA) regimen. It was suggested that the HAA regimen is more effective than IA and well tolerated.[291]

Thirty-three elderly patients were enrolled in a clinical trial, 21 patients with high-risk MDS, and 12 with MDS–AML to evaluate the efficacy and toxicity of CHG (cytarabine, HHT (**2**), and G-CSF) induction chemotherapy (low-dose cytarabine, HHT (**2**) with G-CSF priming) for elderly patients with high-risk MDS or AML transformed from MDS (MDS–AML). As outcome of this study the overall response rate was 66.7% after one course of the CHG regimen with 19 patients achieved complete remission (57.6%) and 3 patients achieved partial remission (9.1%).[292]

In a long trial of 9 years, 53 patients were enrolled to evaluate the effect of combination therapy of HHT (**2**) plus all-trans-retinoic acid (ATRA)–based therapy for the treatment of APL as induction therapy followed by three courses of consolidation chemotherapy and 2 years sequential therapy. All patients achieved complete remission.[293]

A retrospective study on high risk 132 patients with higher risk MDS was done to compare two treatment combination therapy of low-dose CHG and Decitabine. Complete remission was not significantly different between the groups (27.1% with Decitabine vs. 30.6% with CHG).[294]

In a clinical study of total of 98 patients at the acute phase of CML with invasive pulmonary aspergillosis (IPA) were treated with Voriconazole plus a low-dose of HHT (**2**) and IM. This study suggested that this combination could be used as the first-line therapy for IPA patients with CML.[295] The 49 cases in observation group were treated with the combination while the control group was treated with fluconazole, low-dose of HHT (**2**),

and IM. The effective rates of the observation group and the control group were 95.92% and 91.84%, respectively with no significant differences. The study concluded that Voriconazole and low-dose HHT (**2**) and IM could be used as the first-line antifungals for the treatment of IPA patients with CML.

Thirty-three patients were enrolled in a clinical study to investigate the effectiveness of HA regimen for CML-MBC, overall improvement cytogenetic response was 60.1%, 7 patients (21.2%) had partial response. The study concluded that it is an effective treatment and that $CD34^{+}CD7^{+}$ cells may be one of the valuable clinical parameters to assess treatment effectiveness.[296]

6.2.6 Side Effects

The major side effect is myelosuppression (a condition in which bone marrow activity is decreased, resulting in fewer red blood cells, white blood cells, and platelets). Myelosuppression is a side effect of some cancer treatments,[257,271,272,279] with gastrointestinal manifestation including diarrhea occurring as well in some cases.[252] Those side effects were explained due to the effect of HHT (**2**) on the epithelial permeability of intestinal Caco-2 cell monolayers.[297]

6.3 Natural Extracts From *Cephalotaxus* sp. and Natural Chinese Medicine

The essential oil of *C. griffithii* inhibited proliferation and migration of human cervical cancer cells.[298] The oil from *C. griffithii* needle extracted with petroleum ether was tested for its effect on proliferation of cancer cells (MTT assay on HeLa, ZR751, and HepG2 cell lines). The extract showed important phyto-components with multiple cellular targets for control of breast cancer.[299]

Patents based on Chinese traditional medicine were filed containing *Cephalotaxus* as an ingredient combined with different plants' extracts and are used for the following indication: treatment of phlegm stagnation type osteoarthritis,[300] liver cancer,[301] ischemic intestinal colic,[302] for phthisis,[303] hepatitis syndrome,[304] for leukemia,[305] traumatic bleeding consists,[306] hookworm disease,[307] ovarian cancer,[308,309] lump-resolving agent in gastric cancer,[310] treatment of gastric cancer,[311] liver cancer,[312] collateral cancer,[313] rectal cancer,[314,315] phlegm and fluid-retention lung-obstructing type

Table 20 Indications for the plant preparations incorporating *Cephalotaxus* extracts

Entry	Indication	Reference
1	Phlegm stagnation type osteoarthritis	300
2	Liver cancer	301
3	Ischemic intestinal colic	302
4	Phthisis	303
5	Hepatitis syndrome	304
6	Leukemia	305
7	Traumatic bleeding consists	306
8	Hookworm disease	307
9	Ovarian cancer,	308, 309
10	Lump resolving agent in gastric cancer	310
11	Gastric cancer	311
12	Liver cancer	312
13	Collateral cancer	313
14	Rectal cancer,	314, 315
15	Phlegm and fluid-retention lung-obstructing type cardiac insufficiency	316
16	Liver-depression qi-stagnation type breast cancer	317
17	Spleen-kidney-qi-deficiency bladder cancer	318
18	Lung cancer	319
19	Adhesion and migration of breast cancer, colorectal cancer, prostate cancer, lung cancer, gastric cancer, osteosarcoma and other cancer cells	320
20	Liver fats	321

cardiac insufficiency,[316] treating liver-depression qi-stagnation type breast cancer,[317] spleen-kidney-qi-deficiency bladder cancer,[318] and lung cancer.[319] A *Cephalotaxus* plant preparation inhibits the adhesion and migration of breast cancer, colorectal cancer, prostate cancer, lung cancer, gastric cancer, osteosarcoma, and other cancer cells,[320] liver fats.[321] (Table 20).

A patent for dermatological application reported that HHT (**2**) was an active inhibitor of matrix metalloproteinase-1 that can be used for reduction and prevention of wrinkles.[322] Another preparation relates to a veterinary drug for treatment of bovine mastitis.[323] Eventually, patents were filed using preparations that contain HHT (2) for treatment of water and purifying it from cyanobacteria.[324,325]

Note Added In Proof

During the preparation of this manuscript, additional synthetic studies[326,327] and a review were published.[328]

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