Cephalotaxus Alkaloids

Joelle Pérard-Viret, Laith Quteishat, Rana Alsalim, Jacques Royer, Françoise Dumas

CEPHALOTAXUS ALKALOIDS

Joëlle Pérard-Viret[a], Laith Quteishat[b], Rana Alsalim[b], Jacques Royer[a] and Françoise Dumas[b]*

a COMETE UMR CNRS 8638, Faculty of Pharmaceutical and Biological Sciences, Université Paris Descartes, Sorbonne Paris Cité, 75006 Paris, France
b BioCIS laboratory, UMR CNRS 8076, School of Pharmacy, Université Paris Sud, Université Paris Saclay, 5, rue Jean-Baptiste Clément, Châtenay-Malabry 92290, France

CONTENT

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* 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 marketed as soon as 2009 by EMA (European Medicine Agency) and was approved by US Food and Drug Administration (FDA) 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 2 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 two past decades, and numerous methodologies were developed to access the first semisynthetic HHT (ssHHT) (**2**) of high purity suitable for clinical studies, and then high grade enantiomerically pure CET (**1**), HHT (**2**) and analogs.

Key words:

Alkaloid, *Cephalotaxus*, Cephalotaxine, Harringtonine, Homoharringtonine, Cancer, Leukemia, Protein synthesis inhibitor, Antiviral, Antiparasitic agent.

Abbreviations

acac Acetylacetonate

AChE Acetyl choline esterase

ACN 1,1’-Azobis(cyclohexanecarbonitrile)

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

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

CP Chronic phase

Cp Cyclopentadiene

CSA Camphorsulfonic acid

CSD Cambridge Structural Database

CSE Conventional solvent extraction

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 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

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

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

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

INN International nonproprietary name

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

I. Introduction

This contribution is an updated and expanded version of the two previous chapters published in this series in 1984[1] and 1998.[2] Other reviews focused on the structure and synthesis of these compounds,[3,4,5,6] and several reviews are available that cover the structure–activity relationships, biological and clinical properties of these alkaloids.[7,8,9,10,11,12,13,14,15,16,17,18,19] 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 fifty years, chemists have studied the extraction, structure determination, biogenesis and synthesis of CET (**1**) (Figure 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 therapeutically use in the treatment of leukemia. Homoharringtonine (HHT) (**2**) and harringtonine (HT) (**3**), or mixtures 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 in order to obtain HHT (**2**) or its related esters. Semisynthetic HHT (ssHHT) (**2**) (Myelostat®, Ceflatonin® or Omacetaxine mepesuccinate) is manufactured by a semi-synthetic process developed by Robin from naturally occurring alkaloid (–)-CET (**1**) extracted from the dry leaves of *C. 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 mesylate, a BCR-ABL1

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 USA, due to its reported activity in patients with chronic myelogenous leukemia (CML) resistant to currently available TKIs. As a result, on 2nd September 2004, orphan designation (EU/3/04/224) was granted by the EU for omacetaxine mepesuccinate® (INN, trade name Synribo) /homoharringtonine (HHT) (**2**) for the treatment of chronic myeloid leukemia to Stragen France SAS, France, for the treatment of chronic myeloid leukemia. 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 26th October 2012, HHT (**2**), Synribo® (formerly Ceflatonin, Omapro), received an accelerated approval by the U.S. Food and Drug Administration (FDA) for adult patients with chronic-phase or accelerated-phase CML with resistance or intolerance to two or more TKIs. The U.S. FDA has granted full approval to HHT (**2**) (drug substance international nonproprietary name (INN) omacetaxine mepesuccinate, 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, characterisation, 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 eleven species of evergreen small and only slowly growing conifers which are mostly indigenous to China (Figure 1). The first alkaloid characterized from *C. harringtonia* var. *drupacea* (*C. drupacea*) and *C. fortunei* was named cephalotaxine by Paudler in 1963[24] and its original benzazepine nucleus fused to an azaspirononane unit established by Powell et al. (Figure 2).[25]

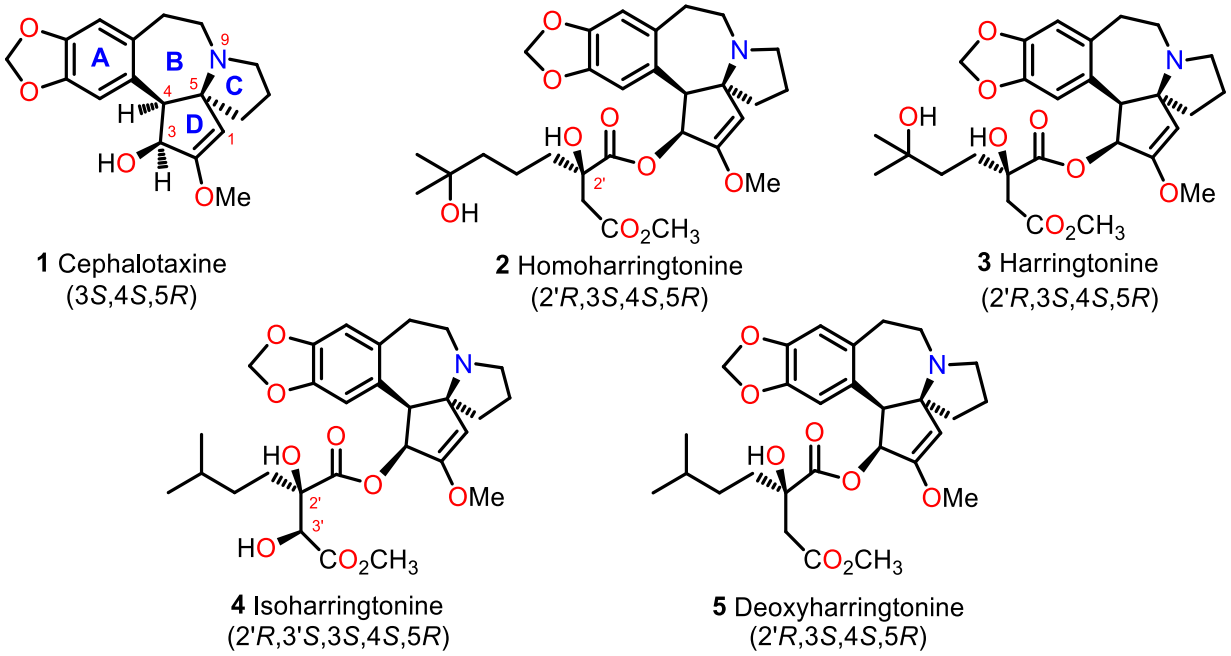
Scientific classification

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

C. haringtonia var *drupacea*
(fruits and leaves)

Figure 1. Scientific classification of *Cephalotaxus genus* and picture (from A. Barra, Wikimedia Commons) of the fruits and leaves of C. *harringtonia* var *drupacea.*

Although its biological activity and partial structure are known since 1959,[26] the elucidation of the structure and antitumor activity of natural harringtonine (HT) (**3**) (Figure 2) isolated from *Cephalotaxus harringtonia* var. *drupacea* 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 cephalotaxine 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 *C. wilsoniana.* Biogenesis of CET (**1**) postulated by Parry in 1977 showed a common origin for these alkaloids.[1,2,27]



1 Cephalotaxine
(3*S*,4*S*,5*R*)

2 Homoharringtonine
(2'*R*,3*S*,4*S*,5*R*)

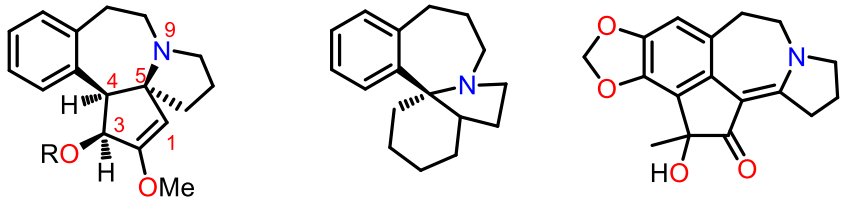
3 Harringtonine
(2'*R*,3*S*,4*S*,5*R*)

4 Isoharringtonine
(2'*R*,3'*S*,3*S*,4*S*,5*R*)

5 Deoxyharringtonine
(2'*R*,3*S*,4*S*,5*R*)

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

Cephalotaxanes (**6**) is the generic name for the particular alkaloids of the *Cephalotaxaceae* family which have a general formula derived from the cephalotaxane skeleton with either 1 or 2 units covalently linked forming dimers having two identical or different units (Figure 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 cephalotaxines characterized by a free alcohol function on ring D (R = H), and harringtonines 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 *C. hainanensis* and *C. fortunei* (Figure 3) and displays a type of single rearranged cephalotaxine skeleton.[28]



6 Cephalotaxanes **7** Homoerythrinane **8** Hainanensine

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

Until 1997, thirty six compounds related to the cephalotaxine series (Tables 1 and 2), were described, of which twenty three are natural esters and one is dimeric.

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, $CHCl_3$)
Cephalotaxinone (**10**), $C_{18}H_{19}NO_4$, mp 198–200 °C, $[\alpha]_D$ –146 (c 0.63, $CHCl_3$)
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, $CHCl_3$)
Oxygenated cephalotaxines
11-Hydroxycephalotaxine (**14**), $C_{18}H_{21}NO_5$, mp 235–242 °C, $[\alpha]_D$ –139 (c 0.56, $CHCl_3$)
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 0.79, $CHCl_3$)
Demethylneodrupacine (**17**), $C_{17}H_{19}NO_5$, mp 197–200 °C
Cephalotaxinamide (**18**), $C_{18}H_{19}NO_5$, mp 230 °C

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**), 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 like drupacine (**16**) were also described (Figure 4).[30,31,32] 11-Hydroxycephalotaxine (**14**) was converted to drupacine **(16**) in acidic medium.[31] Among the minor alkaloids isolated from the bark of *Cephalotaxus hainanensis* Li (in addition to eleven known alkaloids), cephalotaxine 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* (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 desmethylCET (**12**) as a natural metabolite and biosynthesic 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 *C. 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 harringtonines (Figure 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 occurence of enantiomeric pairs in the same family of plants is unusual,[38] acetylcephalotaxine (acetylCET) (**26**) was reported under both 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]

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, $CHCl_3$)

Harringtonine (**3**), $C_{28}H_{37}NO_9$, mp 73–75 °C, $[\alpha]_D$ –104.6 (c 1.0, $CHCl_3$)

Isoharringtonine (**4**), $C_{28}H_{37}NO_9$, mp 69-72.5 °C, $[\alpha]_D$ –99.6 (c 1.06, $CHCl_3$)

Deoxyharringtonine (**5**), $C_{28}H_{37}NO_8$, amorphous, $[\alpha]_D$ –125.4 (c 1.76, $CHCl_3$)

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, $CHCl_3$)

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, $CHCl_3$)

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 ($CHCl_3$), $[\alpha]_D$ +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)

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 (Figure 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 cephalotaxine analogs, 11α-hydroxy-homodeoxyHT (**34**), 11β-hydroxyhomodeoxyHT (**35**) and 11β-hydroxydeoxyHT (**36**) (IC_{50} 0.38, 0.33 and 0.17µg/mL respectively against P-388 murine leukemic cell lines) identified from *C. harringtonia* var. *drupacea* (Figure 6).[47]

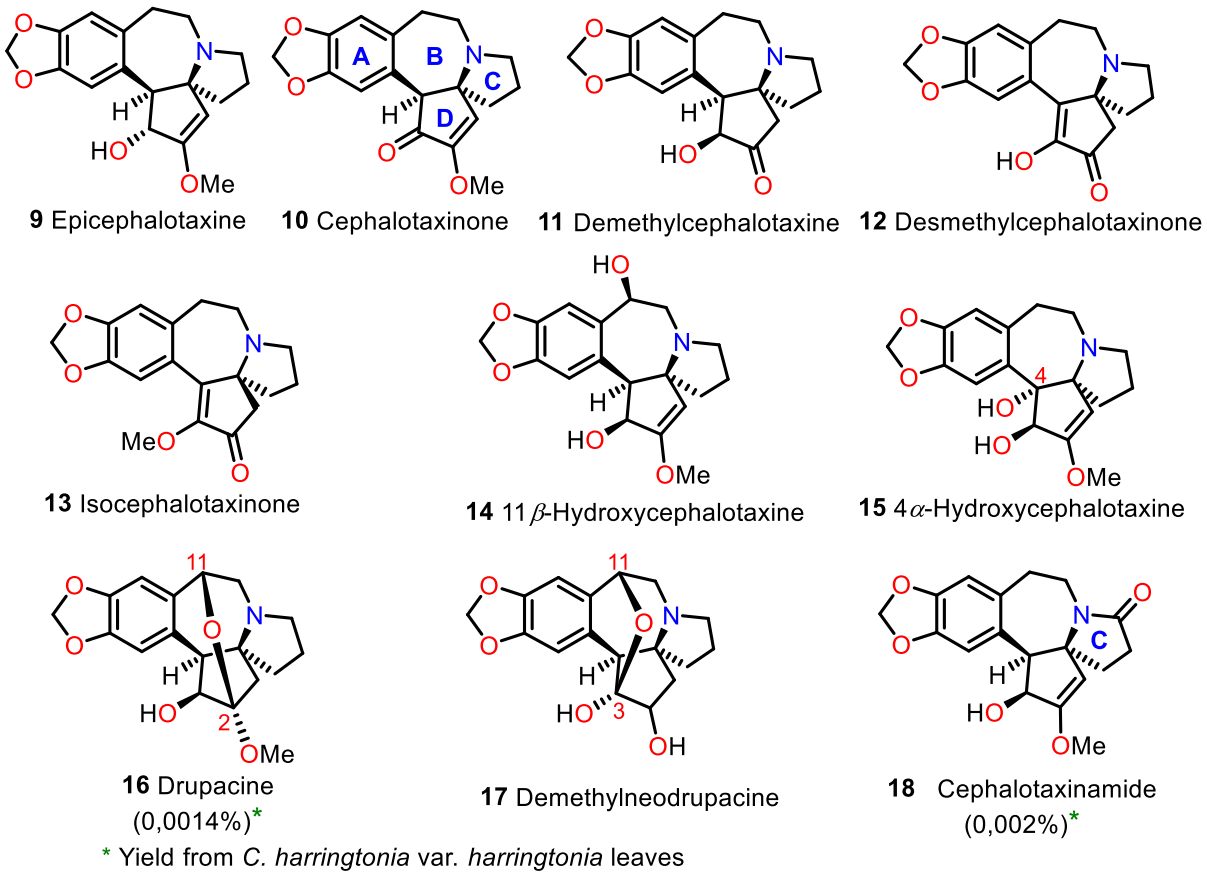


Figure 4. Oxydized cephalotaxines

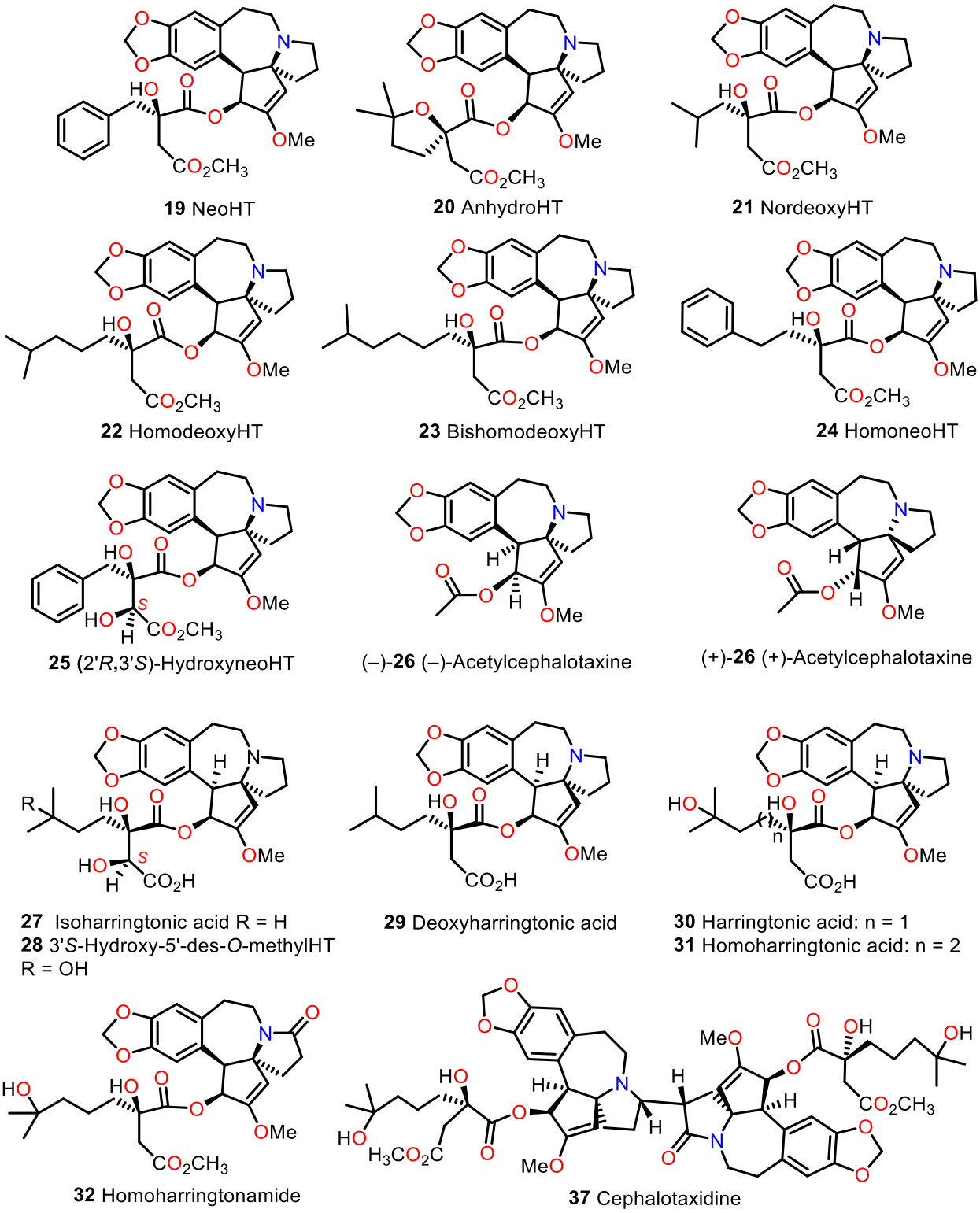


Figure 5. Natural esters of cephalotaxine and cephalotaxinamide

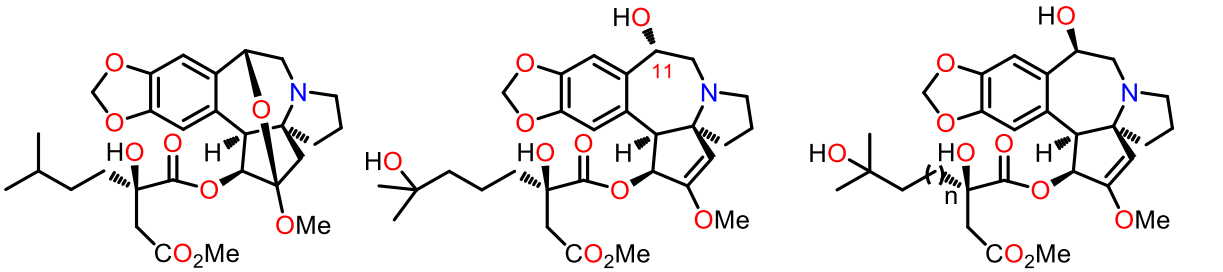


Figure 6 Natural esters of oxygenated cephalotaxines

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

2.1 Cephalotaxine and Related Alkaloids

N-oxides of cephalotaxine, CET *α N*-oxide (**38**), CET *β N*-oxide (**39**) and 11*β*-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 (Figure 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 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 *C. 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 (Figure 7).[51] Some uncertainty remained, however, due to the divergent optical rotations of the synthetic and the natural compounds.

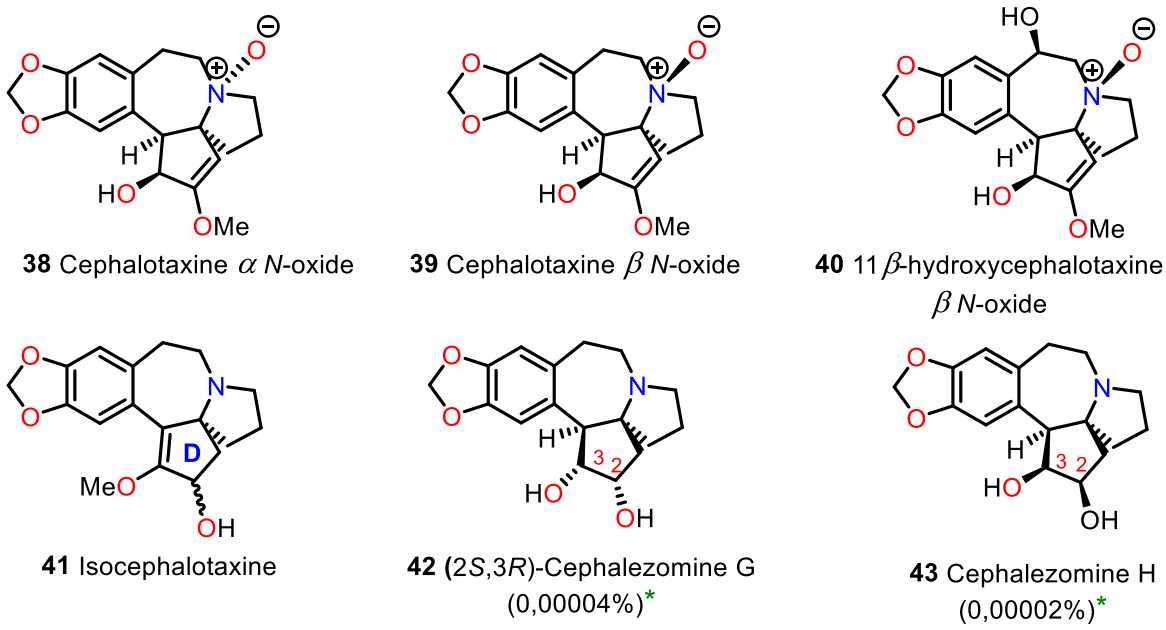


Figure 7. New *N*-oxides and ring D analogs of cephalotaxine

A new stereoisomer (**44**) of desmethylcephalotaxinone (**12**) has been isolated from the leaves and heartwood of Formosan *Cephalotaxus wilsoniana* (Figure 8).[52] Although the UV, ^{1}H 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 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 *C. 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 (Figure 8).[54] The structure of cephalancetine C (**48**) was unambiguously confirmed by single-crystal X-ray diffraction as consisting of cephalotaxine (**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 Figure 12) with LiOH.[55]

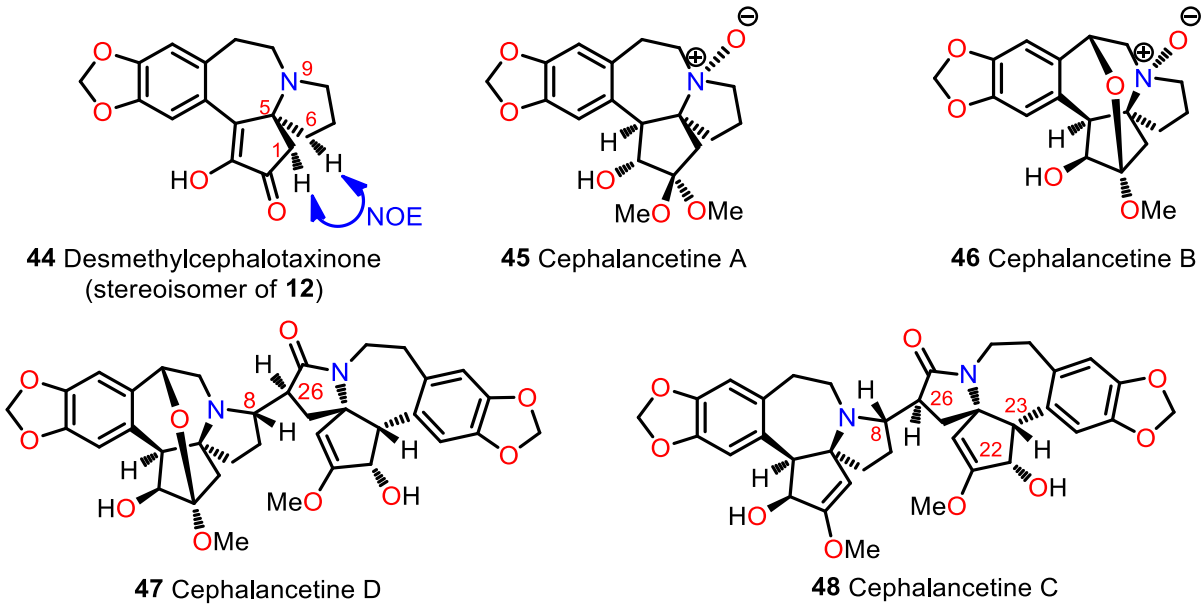


Figure 8 Desmethylcephalotaxinone stereoisomer and cephalancetines A–D

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]

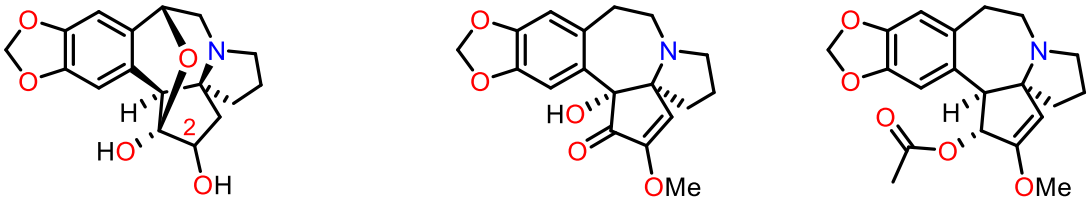
Table 3. Cytotoxic Activities (means ± S.D).[54]

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

Harringtonine (**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 against HCT116 cell line. Comparing the relationship between the structures with their cytotoxicities, the ester side chains

at C3 of the cephalotaxine 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 *Cephalotaxus lanceolata* and *C. fortunei* var. *alpina* (Yunnan province, P.R. China) along with 24 known alkaloids (Figure 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]



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

51 Cephalotine C

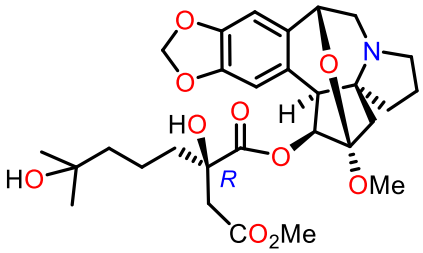
52 Cephalotine D

Figure 9 Cephalotines A-D

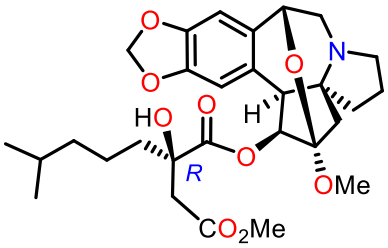
2.2 Natural *Cephalotaxus* esters

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**) (Figure 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 cephalotaxine alkaloids, demethylCET (**11**),[30] 11β-hydroxyCET (**14**) and drupacine (**16**). Their structure and stereochemistry were elucidated by spectroscopic data, circular dichroism and X-ray diffraction analysis.

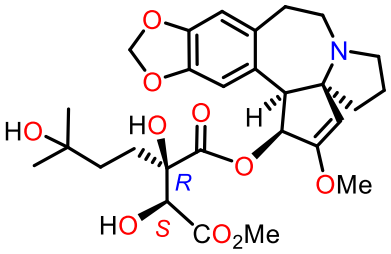
Among the new *Cephalotaxus* alkaloids possessing a cephalotaxine type backbone with various side chains at C3, cephalezomine D (**56**) is the first one with (2'*R*,3'*R*) AC. In this study[57] the team of Kobayashi also described the cytotoxicity of these alkaloids against L1210 and KB cell lines (Table 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" (Figure 10).[58]



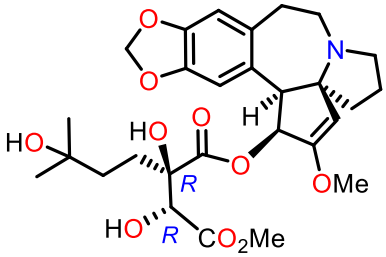
53 Cephalezomine A (0.0001%)*



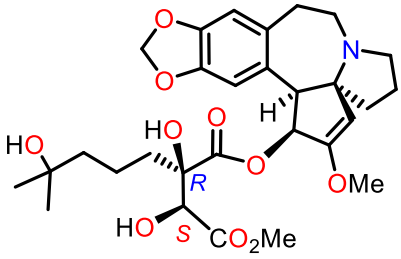
54 Cephalezomine B (0.00005%)*



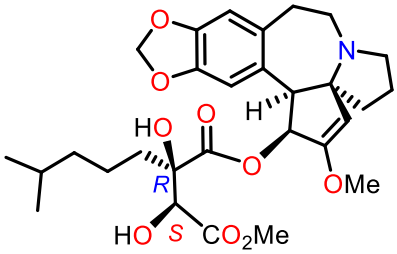
55 Cephalezomine C (0.002%)*



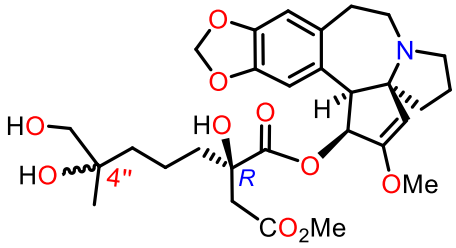
56 Cephalezomine D (0.0003%)*



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 brakets

Table 4: Cytotoxicity of cephalotaxines, harringtonines and cephalezomines against L1210 and KB cell lines[56,57]

Compounds	IC_{50} (µg/mL) 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

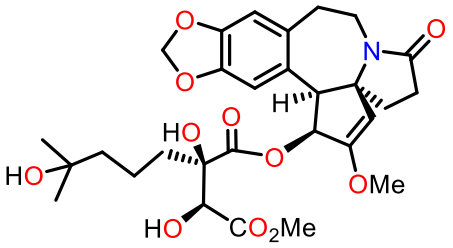
In 2010, Morita et al. characterized from the leaves of *C. harringtonia* forma *fastigiata* a new alkaloid, cephastigiamide A (**61**),[59] $[\alpha]^{20}_{D}$ –55 (c 0.1, MeOH), along with CET (**1**), cephalotaxinamide (**18**), bis-cephalezomine A (**65**), homodeoxyHT (**22**), and two HHT *N*-oxides, HHT α *N*-oxide (**62**) and HHT β *N*-oxide (**63**), previously prepared by Takano[60,61] for SAR studies (Figure 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.

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 the presence an esterified 3-OH function in their structures (Table 5). Structure-activity relationship directed toward antiplasmodial activity of isolated *Cephalotaxus* alkaloids was also discussed. Cephalezomine J (**64**) from the leaves of *Cephalotaxus harringtonia* var. *nana* (Figure 11) is the unique cephalotaxine 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).

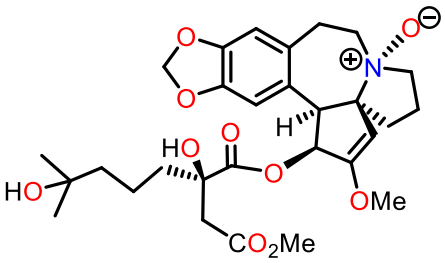
Table 5: Biological evaluation of *Cephalotaxus* alkaloids against *P. falciparum* 3D7, *L.major* and human epidermoid carcinoma cells A549.[58]

Compounds	IC_{50} (µg/mL) *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[a]	1.78	44. 5
Cephalezomine E (**57**)	0.071	NT[a]	4.79	67.5
Cephalezomine F (**58**)	0.66	NT[a]	4.41	6.7
Cephastigiamide A (**61**)	17.82	117.36	> 18.35	> 1
Cephalocyclidine A (**72**)	> 31.55	NT[a]	> 63.00	> 2
Bis-cephalezomine A (**65**)	1.99	NT[a]	> 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 [b]	0.011	-	-	-
AmB [c]	-	0.14	-	-
Taxol [d]	-	-	0.0014	-

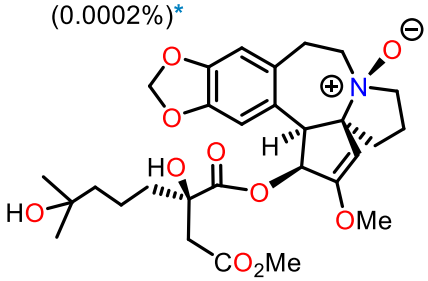
[a] NT = not tested, [b] CD = chloroquine diphosphate, [c] AmB = Amphotericin B, Chloroquine and Amb are positive control for assay of antiplasmodial and antileishmaniale activity respectively. d Positive control for cytotoxicity assay, SI: selectivity index (a measure of the IC_{50} values obtained against human A549 cell divided by the IC_{50} against *P. falciparum* 3D7).



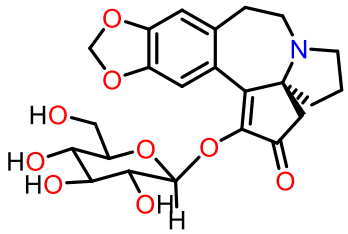
61 Cephastigiamine A (0.0002%)*



62 HHT α *N*-oxide



63 HHT β *N*-oxide



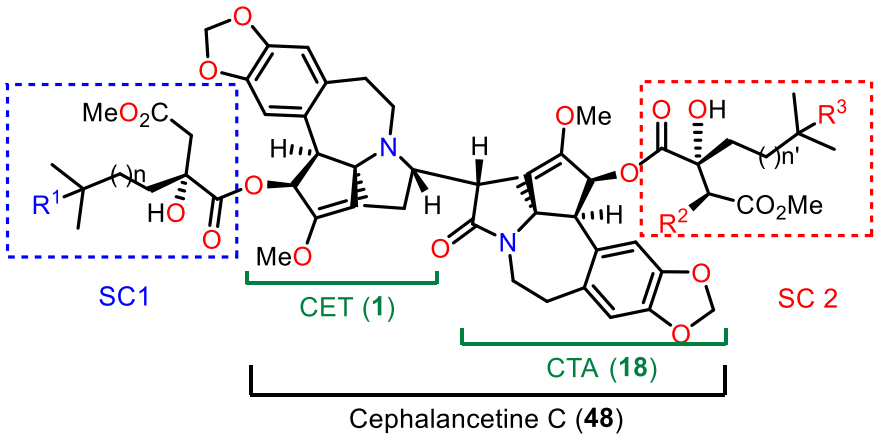
64 Cephalezomine J (0.00002%)*

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

Figure 11. Oxygenated *Cephalotaxus* esters and Cephalozemine J

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

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



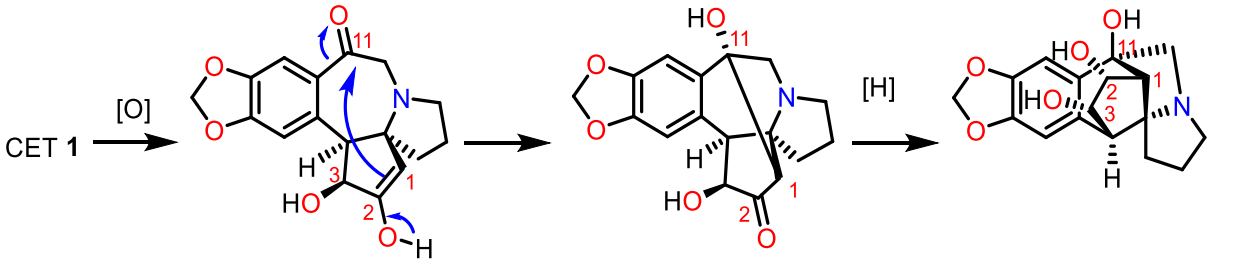
SC: Side chain	SC 1- CET- CTA-SC 2
(**37**) Cephalotaxidine: n = n' = 1, R^1 = R^3 = OH, R^2 = H	HHTsc- CET- CTA - HHTsc
(**65**) Bis-cephalezomine A: n = 2, n' = 1, R^1 = R^3 = OH, R^2 = H	HHTsc- CET- CTA - HTsc
(**66**) Bis-cephalezomine B: n = 1, n' = 2, R^1 = R^3 = OH, R^2 = H	HTsc- CET- CTA- HHTsc
(**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' = 1, 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

2.3 Other alkaloids

The new alkaloid cephalocyclidin A (**72**) with an unprecedented polycyclic fused rearranged skeleton derived from CET (**1**) and six contiguous asymmetric centers was isolated from fruits of *Cephalotaxus 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}) of cephalocyclidin A (**72**) against the L1210 mouse lymphoma and human epidermoid carcinoma cells KB is 0.85 and 0.80 µg/mL respectively.



Scheme 1. Proposed biosynthetic pathway to cephalocyclidine A

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 semi-synthesis from natural CET (**1**) by attachment of the side chain (see section 3). Purification provided a drug whose purity 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**) were injected into a preparative 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 to 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 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, filtration at 0.25 microns for sterilization, 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 to less than 0.03% of impurities.[63]

Numerous methods were described for the extraction and purification of cephalotaxine, its natural esters and related analogs. Extraction of CET (**1**) and HHT (**2**) with 90% ethanol under refluxing conditions allowed for the isolation from fruits, leaves, or branches of *Cephalotaxus sinensis* after acid base treatment and chromatographic purification on alumina.[64]

High speed countercurrent chromatography (HSCC), 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 a hot extraction. To overcome these drawbacks, a new method of extracting HHT (**2**) from *C. koreana* assisted by microwave (MWE) was developed by the team of Kim.[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 4 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 microwave extraction process for the extraction of HHT (**2**) when compared to a CSE 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. A method for ultrasonic-microwave extraction of *Cephalotaxus 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 pre-purification method with adsorption prior chromatography, HHT (**2**) with over 52% purity can be obtained simply from *Cephalotaxus 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] This process allows increasing the purity of the raw HHT (**2**) from 10% to over 52% with 3 simple steps and inexpensive pretreatment of the MeOH extract.

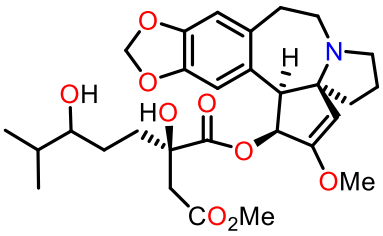
Table 6 The pre-purification summary (Steps 2-4) of HHT biomass (16 g).[64]

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

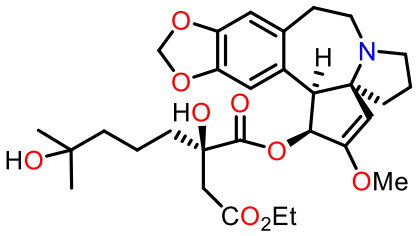
A recent study by Robin related a method for isolation and purification of CET (**1**) from the crude alkaloids extract from 10 kg dry leaves (not from the bark) of *C. fortunei* var *alpina* (Sichuan, China).[69] CET (**1**) purification was performed for the first time by reverse phase HPLC. The crude 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 necessitated the alkalization of the obtained aqueous phase and its extraction with dichloromethane. Thus, 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 anti-cancer drug alkaloids from natural sources, methods of extraction are developed. Methods to recover 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 a 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). *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]

Table 7 Impurities of commercial batches of HHT detected by He's HPLC 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

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 cephalotaxine, which could be translated into diastereoisomeric purity in its natural ester HHT (**2**). 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 racemised 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 non chiral such as benzoate, hydrogenfumarate, hydrogensuccinate, hydrogenitaconate, hydrogenmaleate, hydrogenmalonate, hydrogentartronate, hydrogenmesotartrate, 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 Seitz (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" (*C. latifolia*), "vulnerable" (*C. oliveri*, *C. mannii*) or rare and "endangered" (*C. lanceolate* or *C. 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. In the quest of CET raw material, the study of *Cephalotaxus* crops,[78] and development of analysis methods of the composition of the different plants were stimulated. A HPLC protocol was used to determine the content in CET (**1**), HT (**3**) and HHT (**2**) of 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, tending to give low product 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 *Cephalotaxus 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 HHT (**2**) 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. HT (**3**) yield in 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 combination of NaF and MJ on cells provided an efficient strategy for producing CET (**1**).

Table 8: Harringtonines production in *C. 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

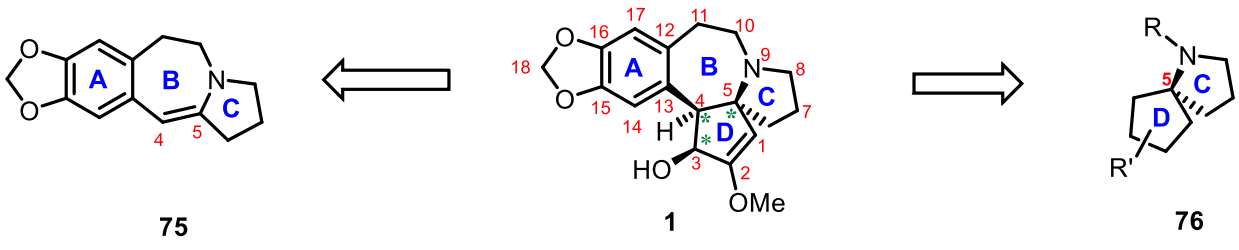
Another study of the effects and action mechanisms of NaF on the growth and CET (**1**) production of *Cephalotaxus mannii* suspension cells indicated that NaF acted as an inhibitor of the Embden-Meyerhof pathway (EMP), not as an elicitor to promote CET (**1**) production.[82]

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 (1984),[1] Miah et al. (1998)[2] and more recently, among others, by Nay et al. (2012).[6] Since 1997, thirty nine syntheses of CET (**1**) have been reported, half of which being asymmetric.

3.1 Overview

CET (**1**) can be described as the superposition of two structural units relevant for synthetic strategy choices, 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 favoured. In addition, carbonyl reduction of cephalotaxinone (**10**) delivers CET (**1**) with the-natural configuration at C3.[83]



75 **1** **76**

Scheme 2 Main strategies to access CET (**1**)

Thirty-two (twenty-one since 1997 described in this review) syntheses of CET (**1**) concerned its racemic form (Table 9). The number of asymmetric syntheses has grown considerably from only three before 1997 to eighteen 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 (Figure 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 overall yield (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 overall yield (OY) and ee. The OY and 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, Figure 13), then in a chronological order. The asymmetric syntheses are presented in chronological order in Table 10.

It is apparent from Table 9 that the syntheses of *rac*-CET (±)-(**1**) proceed-predominantly (twenty of thirty-three) by the C4–C13 disconnection **a**. Most of them are linear, nine only being convergent. 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 twelve 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 twelve steps and 26.2% OY is the most efficent 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.

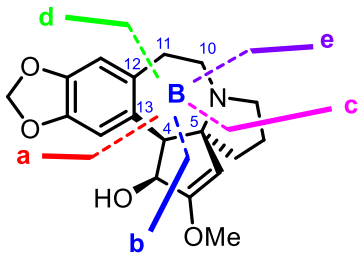


Figure 13 Main disconnexions used for the construction of CET ring B

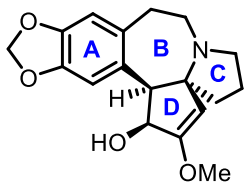
Table 9. Overview of the racemic syntheses of CET (±)-(**1**) arranged according to the main strategic disconnections of ring B and intermediate of the formal synthesis targeting Dolby-Weinreb's enamine.

Author[a] (year)	Ring B cut	NS	OY%	TNS	AY%	Starting material (ring A)	Ref
Weinreb (1972)	a	11	2.3	-	-	Piperonal (**82**)	2,104
Dolby (1972)[b]	d	12	0.7	-	-	Piperonylic acid	106
Weinstein (1975)[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	16	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)	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	17	2.3	-	-	Piperonal (**82**)	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	1.6	-	-	Piperonal (**82**)	100
Li (2005)	c	21	0.7	-	-	Piperonal (**82**)	105
Li (2005)	c	16	0.7	-	-	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

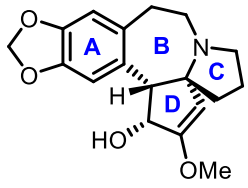
[a]: name in bold style: total synthesis, name in plain style: formal synthesis; [b] formal synthesis through Weinreb's enamine. **NS**: number of steps (longest linear sequence); **OY**: overall yield; **TNS**: total number of steps from boths precursors to CET; **AY**: average yield accounting for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

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, fifteen syntheses are convergent and six 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 by Royer et al.[132] (7.5 OY in 16 steps, 98% ee; 5.8% AY in 18 TNS). Formal syntheses by Ikeda et al.[137] (5.1% OY and AY in 19 steps, 88% ee), by Dumas and d'Angelo[135] (5.4% OY in 20 steps, >95% ee; 4.1% AY in 22 TNS), by El Bialy et al.[140] (12.2% OY in 14 steps, 89% ee; 4.3% AY in 20 TNS) and by Trost et al.[129] (13.6% OY in 19 steps, 91% ee; 8.2% AY in 21 TNS) were also efficient.

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



(–)-**1**



(+)-**1**

Author[a] (year)	B cycle cut	NS	OY% (ee%)	TNS	AY%	SM A[b]	Chiral source	Ref
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	5.0 (99)	18	3.8	**89**	(2*R*,3*R*)-butanediol (**404**)	129
Ikeda (1999)[c]	d	19	5.1 (88)	-	-	**330**	D-proline (*R*)-**(140**)	135
Tietze (1999)[c]	a	13	3.1 (87)	16	1.7	**82**	Oxazaborolidine **267**	117
El Bialy (2002)[c]	d	20	n.r (89)	-	-	**82**	L-malic acid (**409**)	138
El Bialy (2002)[c]	a	14	12.2 (89)	20	4.3	**330**	D-proline (*R*)-(**140**)	138
Royer (2004)[c]	a	16	7.5 (98)	18	5.8	**317**	(*S*)-1-Naphtylethylamine (**298**)	129
Dumas, d'Angelo (2005)[c]	a	20	5.1 (>95)	22	3.9	**317**	(*R*)-1-Phenylethylamine (**309**)	131
Stoltz (2007)[c,d]	a	14	0.2 (97.5)	19	0.03	**82**	(–)-ephedrine (**412**)	90
Mariano 1 (2006)[c]	a	19	0.5 (51)	-	-	**143**	AchE (electric eel)	123
Mariano 2 (2006)[c]	a	19	0.55. (95)	23	0.3	**89**	AchE (electric eel)	124
Hayes 1 (2008)[c]	a	23	0.2 (87)	-	-	**89**	L-proline (*S*)-(**140**)	139
Djaballah, Gin (2008)[c]	a	24	1.5 (nr)	27	1.1	**330**	D-ribose (**365**)	142
Ishibashi (2008)[c]	a	17	1.3 (99.6)	22	0.7	**82**	Diethyl D-tartrate (**384**)	143
Hayes 2 (2008)[c]	a	20	0.5 (91)	22	0.45	**89**	L-proline (*S*)-(**140**)	140
Tu (2012)[c]	a	13	1.7 (99)	17	1.2	**82**	Silver phosphate (*R*)**-273**	118
Renaud (2012)[c]	a	26	0.85 (97)	29	0.6	**89**	Noyori catalyst (*S*,*S*)**-281**	120
Renaud (2012)[c]	a	18	1.8 (96.5)	20	1.4	**89**	PPL	120
Trost (2012)[c]	d	19	13.6 (91)	21	8.2	**82**	Phosphoramidite **296**	125

[a] name in Bold: total synthesis, name in plain style: formal synthesis; [b] For cycle A; [c] synthesis of (-)-**1**; [d] synthesis of (+)-**1**; [e] including one step of separative HPLC; nr: not reported, (**82**): Piperonal, (**89**): homopiperonylic acid, (**317**): safrole, (**330**): Benzodioxole, (**143**)**:** Homoveratric acid. AchE: Acetylcholine esterase. PPL: Porcine Pancreatic Lipase.

3.2 Racemic syntheses

3.2.1 C4–C13 ring closure a

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

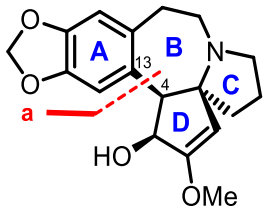
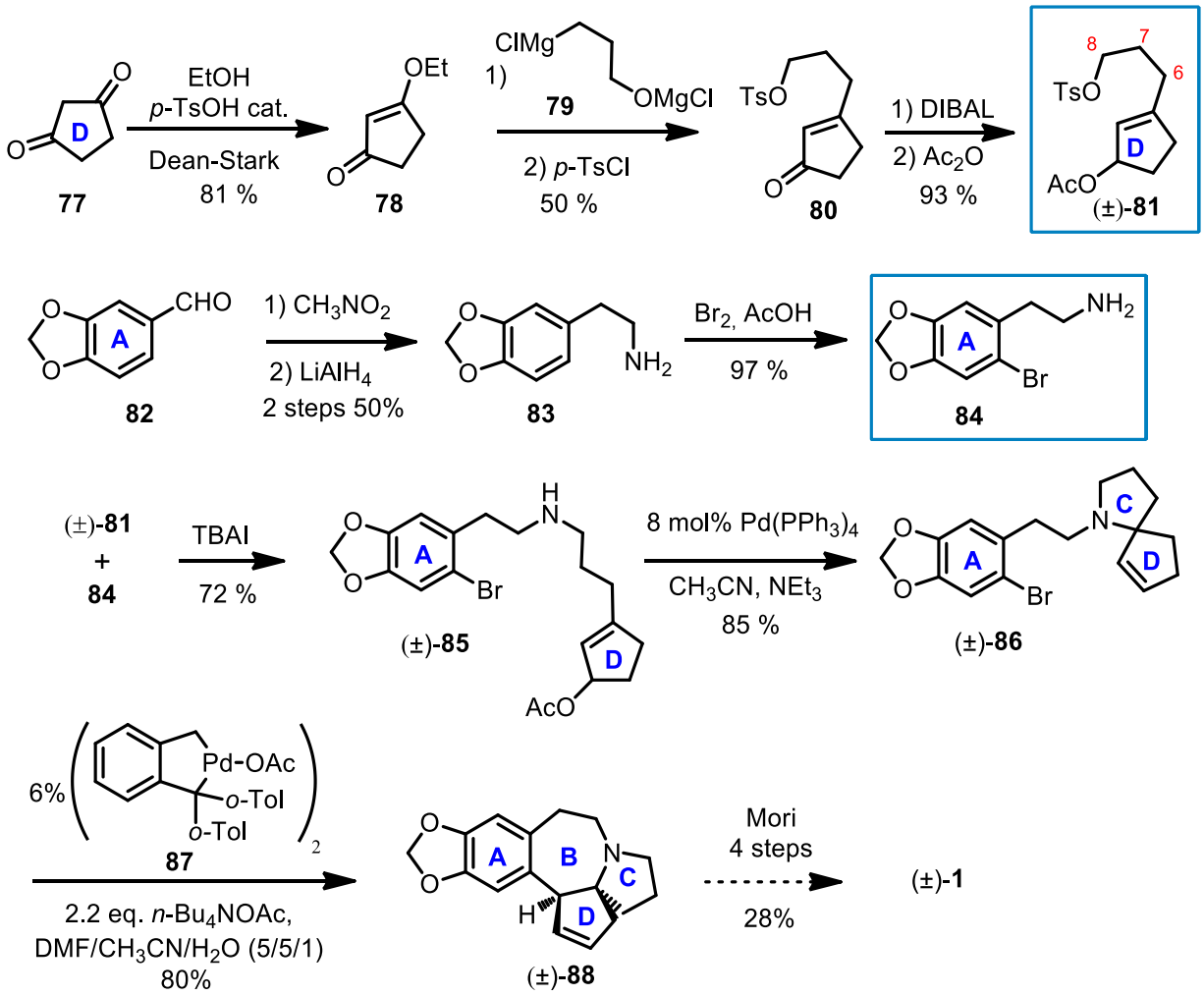


Figure 14 C4–C13 bond disconnection **a** for elaboration of CET (**1**) B ring

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.

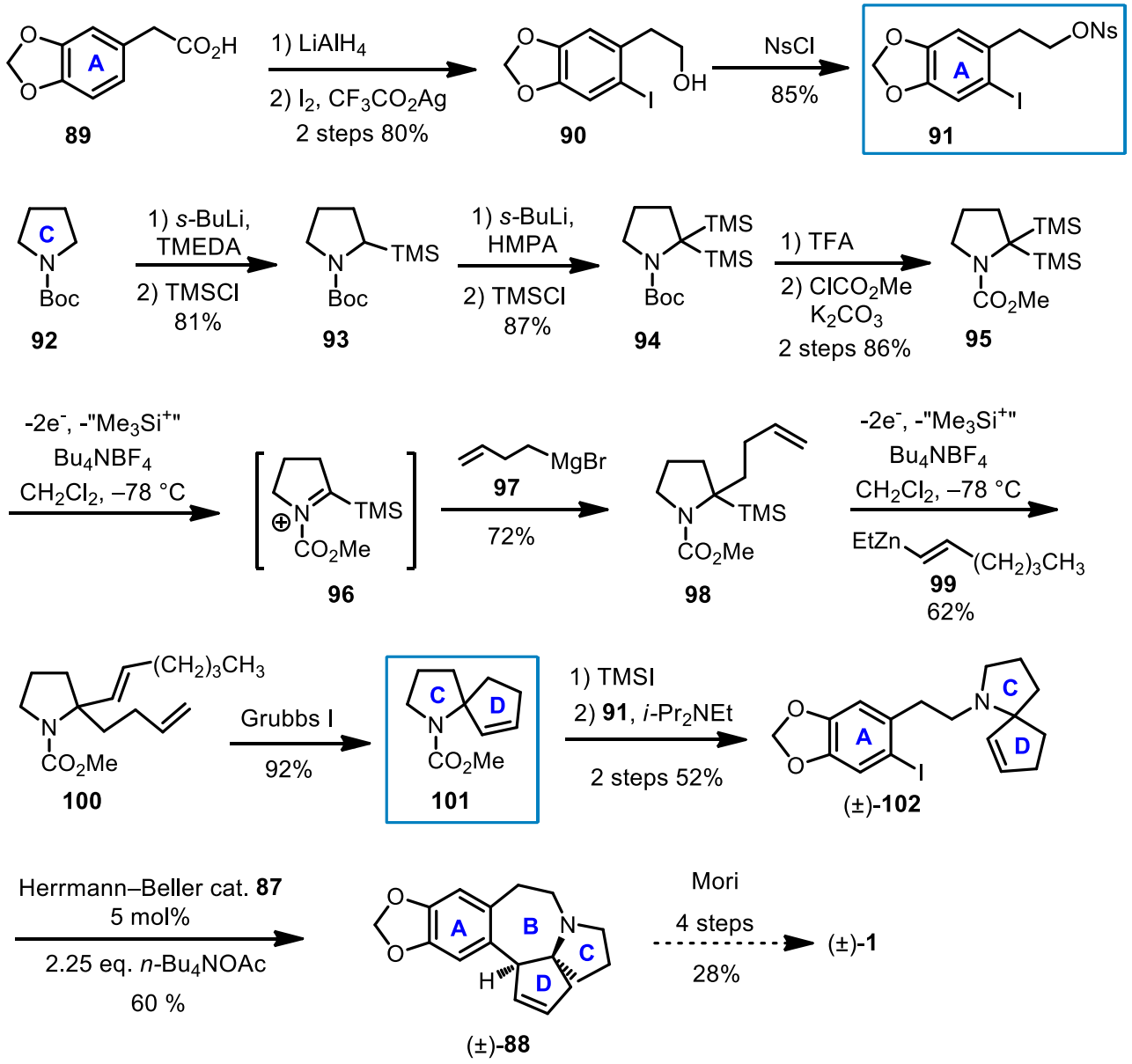


Scheme 3. Formal synthesis of *rac*-CET (±)-(**1**) by Tietze (1997)

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 twelve steps (longuest linear sequence) and 5.2% OY from 1,3-cyclopentanedione (**77**) [2.5% AY in fifteen total number of steps (TNS)]. It should be mentioned that Mori's protocols differ for the synthesis of racemic or optically active CET (**1**). Indeed, the transformation of desmethylCET (**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 orthoformiate 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 of a functionalized pyrrolidine **100** generated by electrochemical oxidation of *α,α*-bis(trimethylsilyl)pyrrolidine **95** (Scheme 4).[86,87]



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

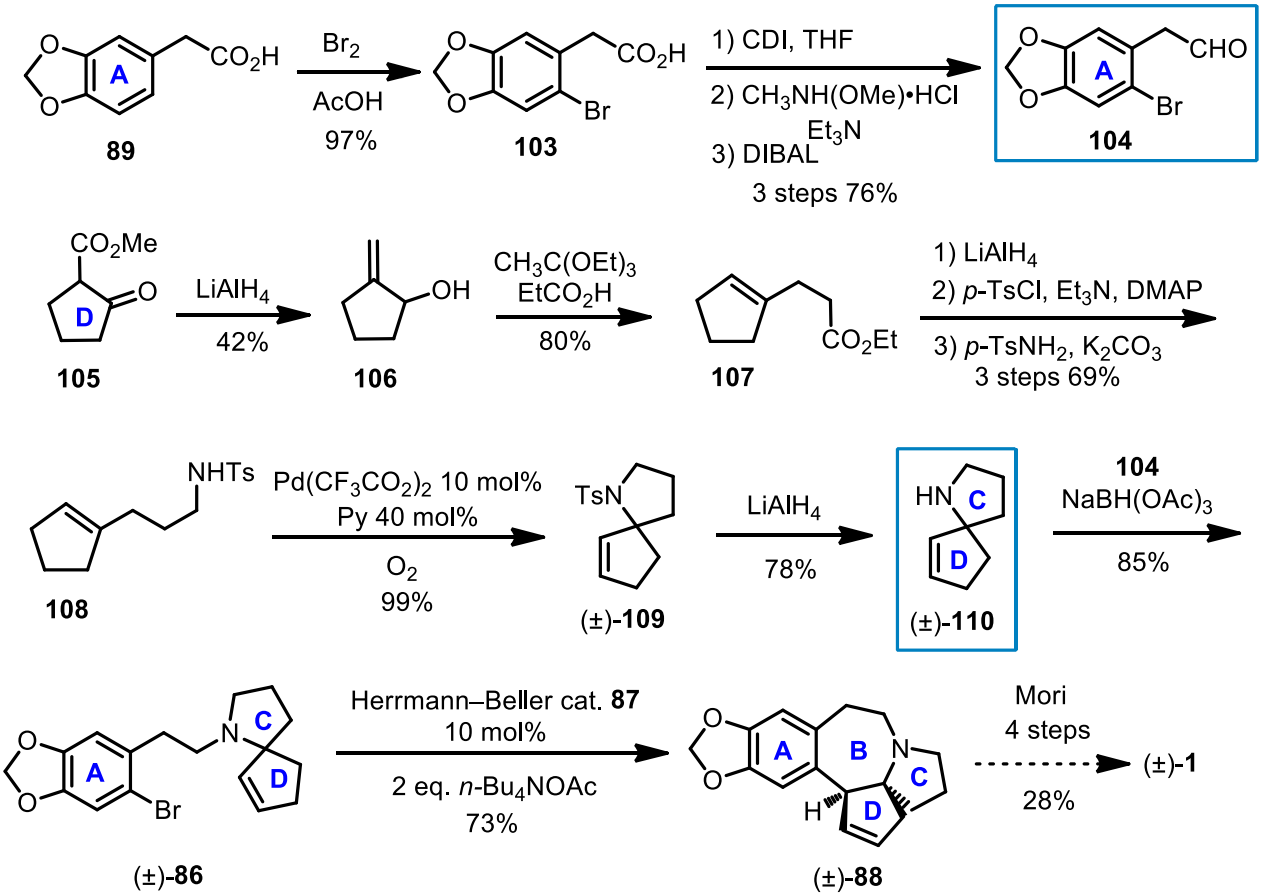
First, homopiperonylic acid (**89**) was used to prepare the A unit **91** bearing a nosylate and an aromatic iodide suitable for ring B annulation, relying on Semmelhack's procedure.[83,88] The two silyl groups were introduced by Beak's methodology[89] (deprotonation with *sec*-BuLi and quenching with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

3.2.1.4 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).

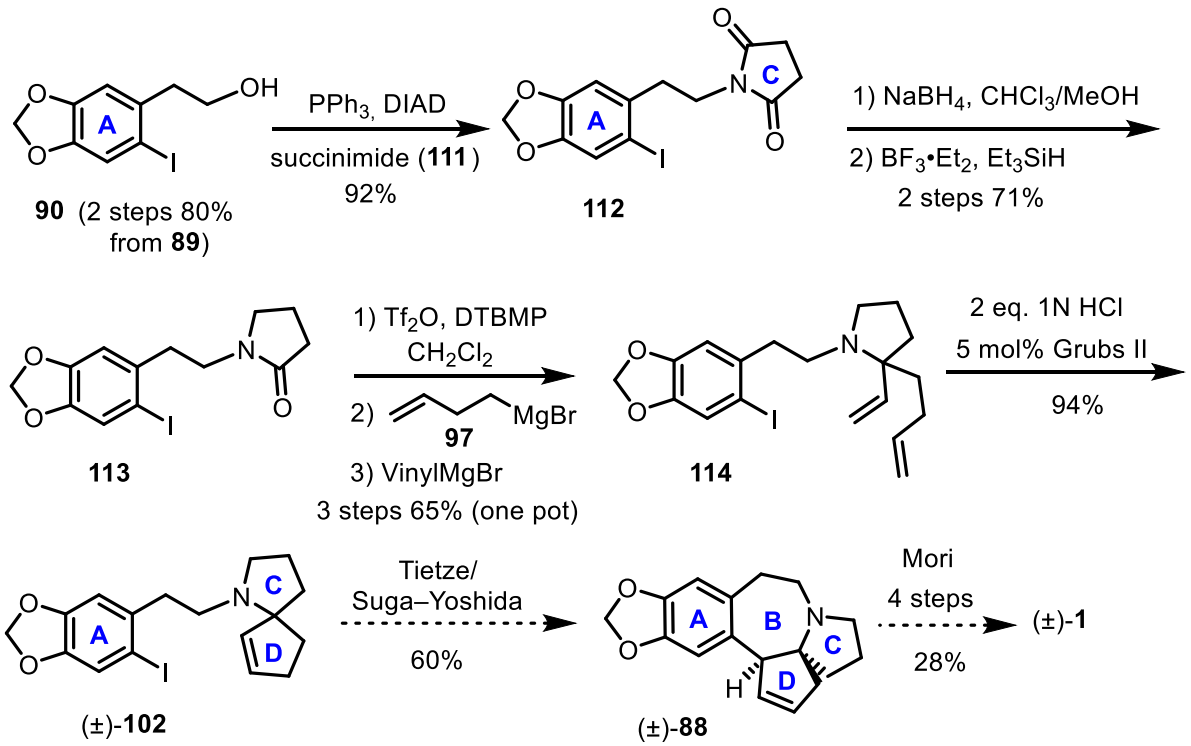


Scheme 5. Formal synthesis of *rac*-CET (±)-(**1**) by Stoltz (2007)

Regioselective bromination of homopiperonylic acid (**89**) yielded bromocarboxylic acid **103** converted to Weinreb's amide,[2,83] 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** tranformed by reduction to allylic alcohol **106**[91] which was treated with triethyl orthoacetate under Johnson orthoester-Claisen conditions to give ethyl ester **107**. Afterwards, ester **107** was converted in three steps into sulfonamide **108** by successive reduction by $LiAlH_4$, tosylation and nucleophilic displacement by tosylamine. The 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 seventeen steps-in 1.6% OY from **89**.

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).

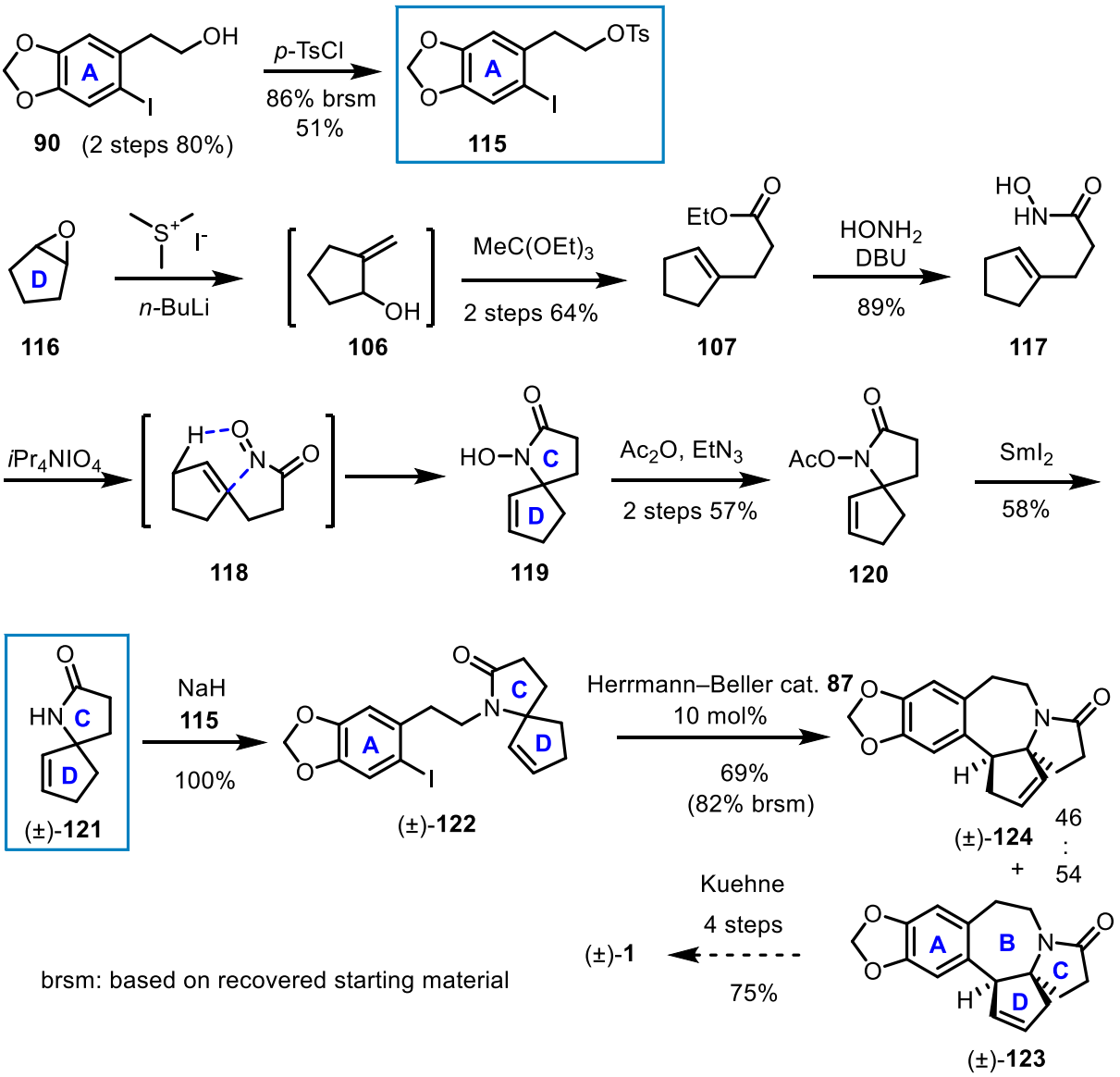


Scheme 6. Formal synthesis of *rac*-CET (±)-(**1**) by Huang (2013)

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 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 ring-closing metathesis (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**), in order 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]



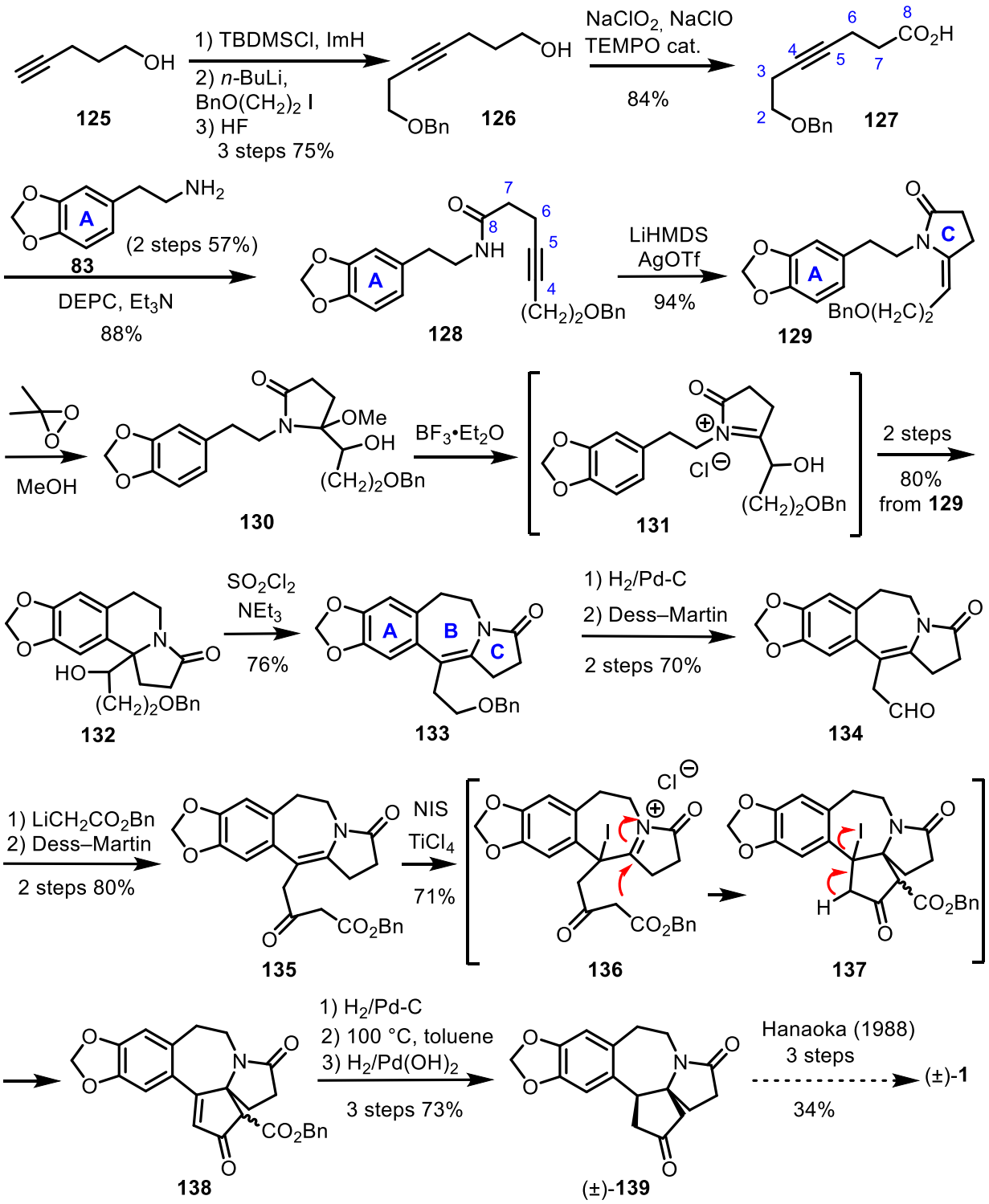
Scheme 7. Formal synthesis of *rac*-CET (±)-(**1**) by Huang and Wang (2015)

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 upon 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] upon 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 twelve steps from cyclopentene

oxide (**116**) and 6.2% OY provided that separation of the regiosiomers 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)

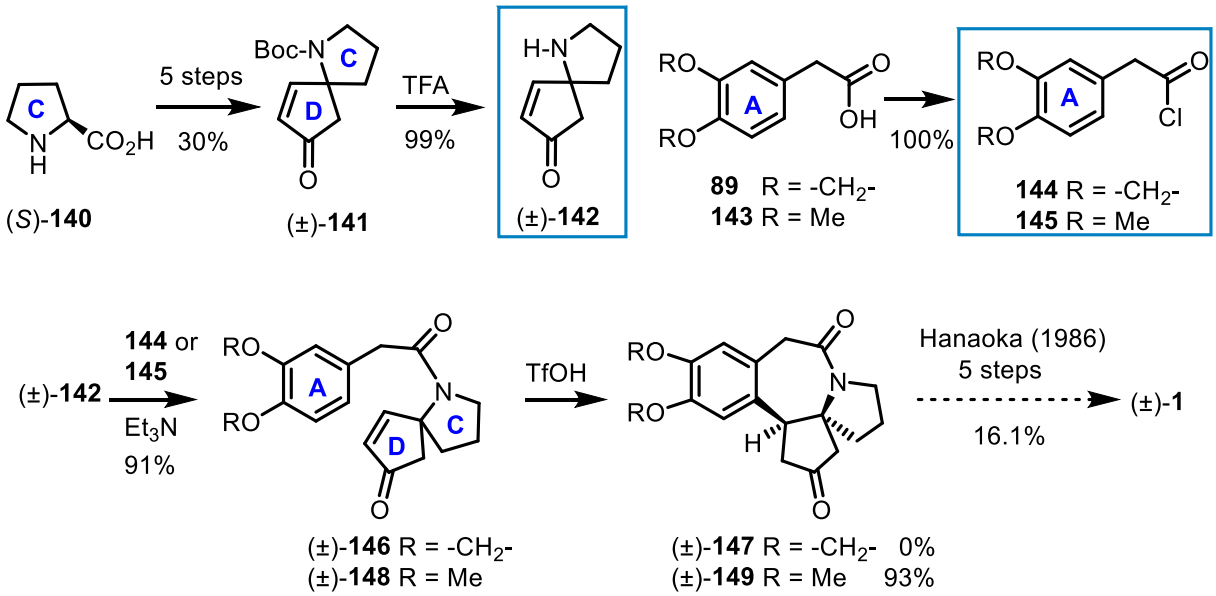
In 2002, Nagasaka et al. reported a formal synthesis of CET (**1**),[95] through a new access to Hanaoka's precursor[96] (±)-**139** (Scheme 8), building successively rings C and B. Ring B was installed by ring expansion of pyrroloisoquinoline **132** which resulted from the cyclization of N-acyliminium ion intermediate **131**. Alkyne **127** which 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 2 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•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 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 cyclisation 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 twenty steps and 3.1% OY from 4-pentyn-1-ol **125**.



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

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]

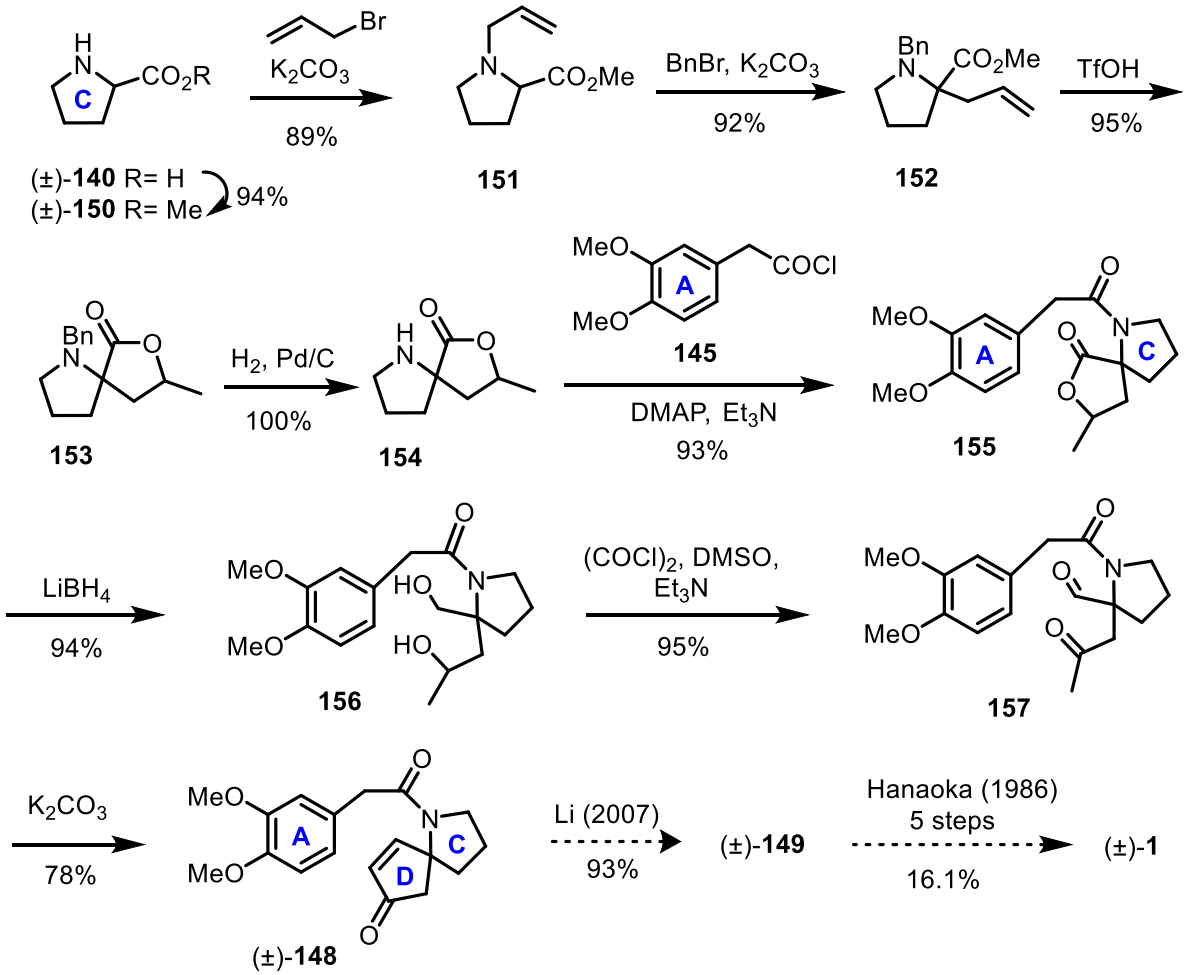


Scheme 9. Formal synthesis of *rac*-CET (±)-(**1**) by Li (2007)

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 thirteen 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-acid and an acid lactonization sequence as key transformations.[99] We describe here the best route which led to Li's intermediate[97] (±)-**148** via the spirolactone **154** (Scheme 10).



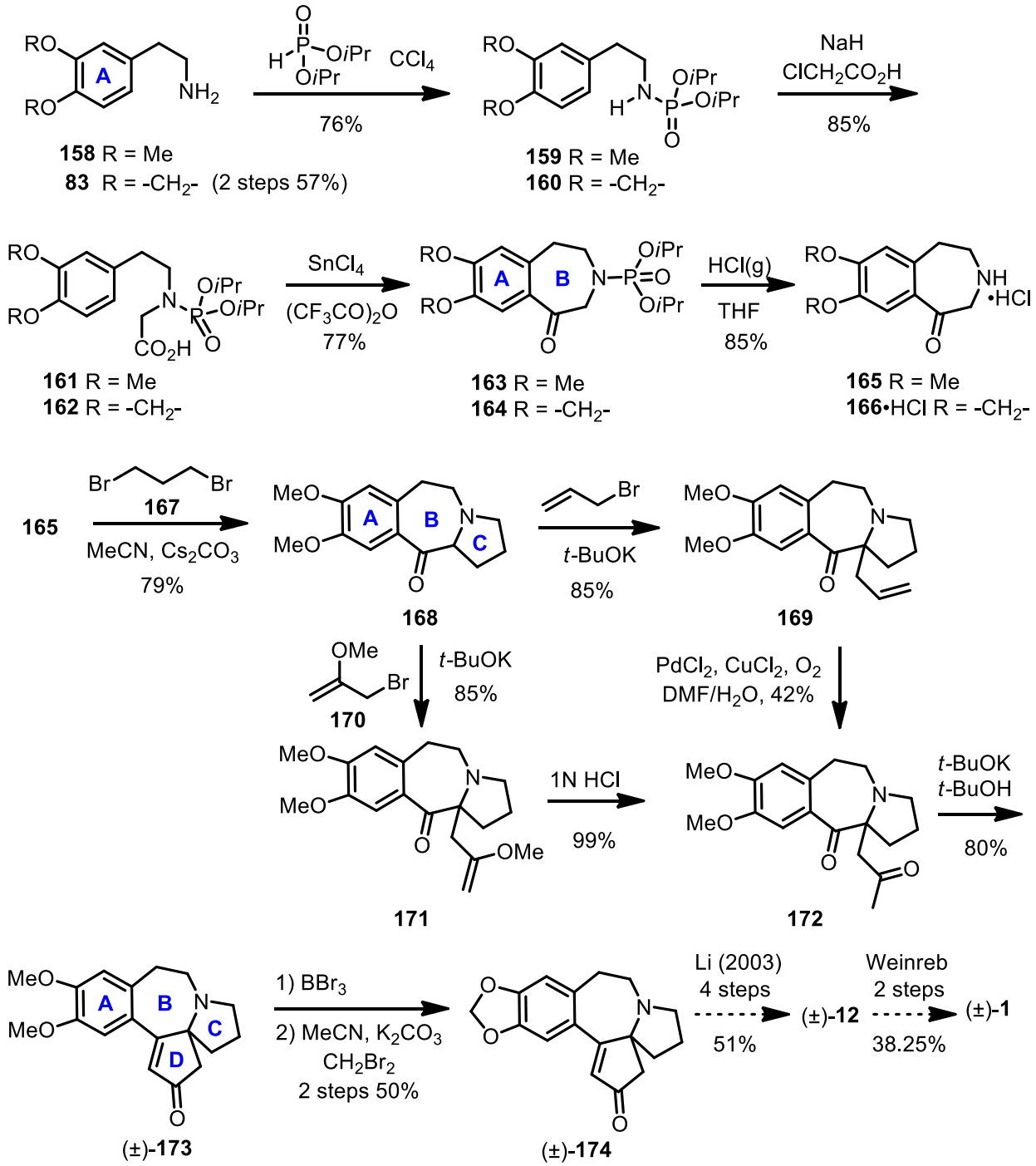
Scheme 10. Formal synthesis of *rac*-CET (±)-(**1**) by Yang and Liu (2009)

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 fifteen 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 4 steps from aryl amine **83** (bearing a methylenedioxy substituent) or **158** (bearing two methoxy substituents), respectively (Scheme 11).[102]

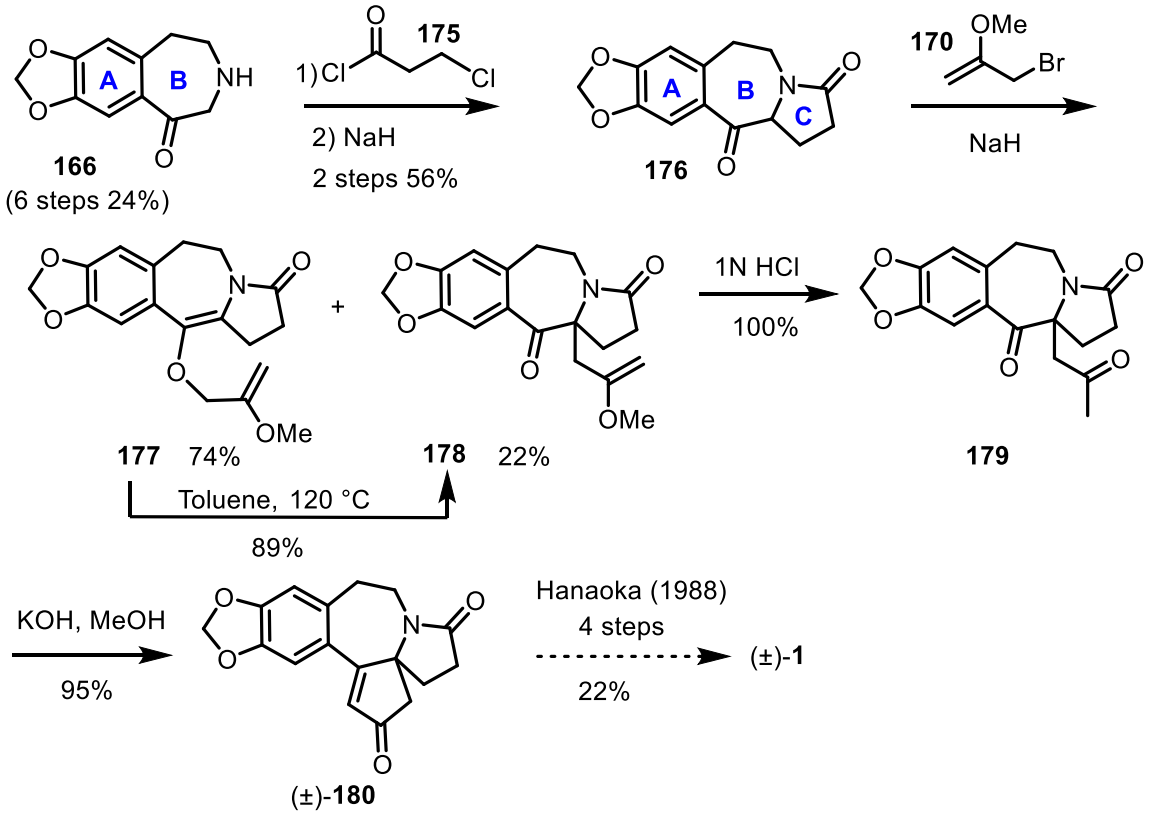


Scheme 11. Formal synthesis of *rac*-CET (±)-(**1**) by Zhang and Liu, the "dimethoxy" route (2013)

The C ring was elaborated via a N- and C-alkylating annulation by treatment of benzazepine **165** with 1,3-dibromopropane (**167**) and 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 seventeen steps from homoveratryl amine (**158**) and 2.2% calculated OY.

These authors also studied the use of methylenedioxybenzazepine **166** as starting material (Scheme 12).

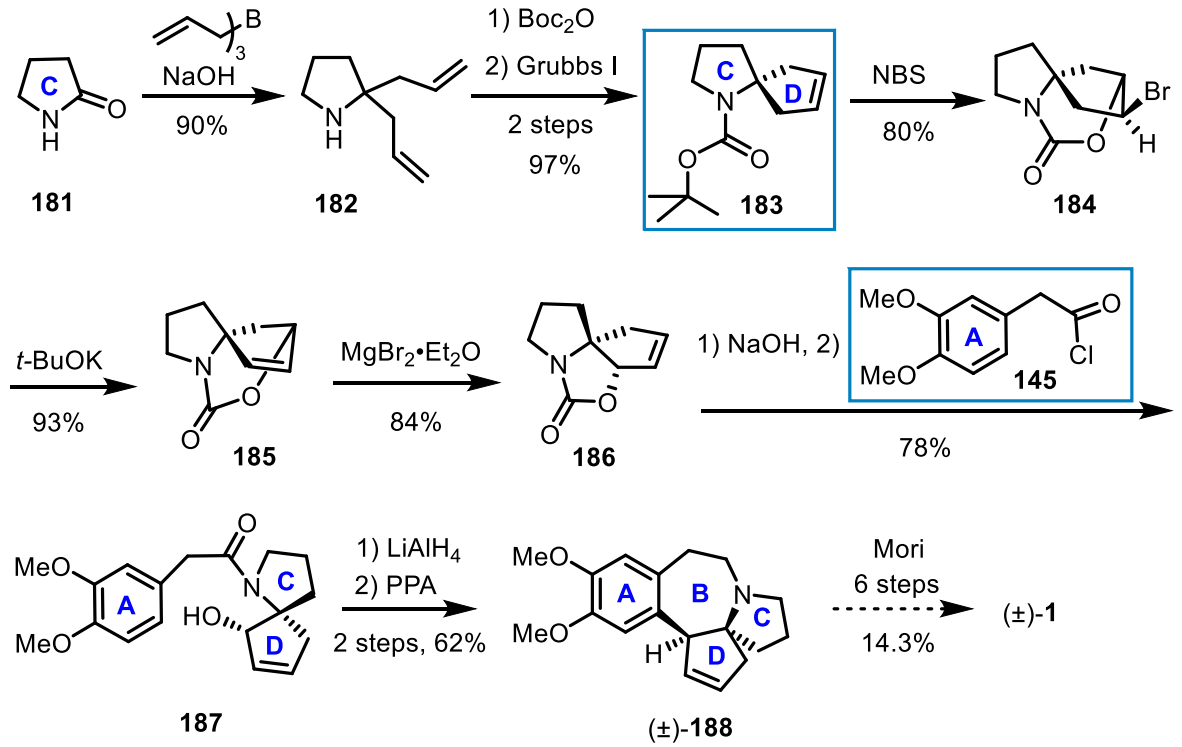


Scheme 12. Formal synthesis of *rac*-CET (±)-(**1**) by Zhang and Liu, the "methylenedioxy" route (2013)

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 following Hanaoka' synthesis[96] in a total of sixteen 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]



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

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 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 cyclise into ABCD compound (±)-**188** by treatment with PPA. Then, the synthesis of *rac*-CET (±)-(**1**) in sixteen steps from 2-pyrrolidone (**181**) and 3.8% estimated OY could be completed using Mori's sequence[85] as the final steps.

3.2.2 N9–C5 ring closure c

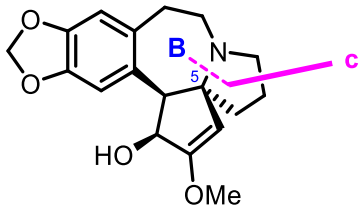
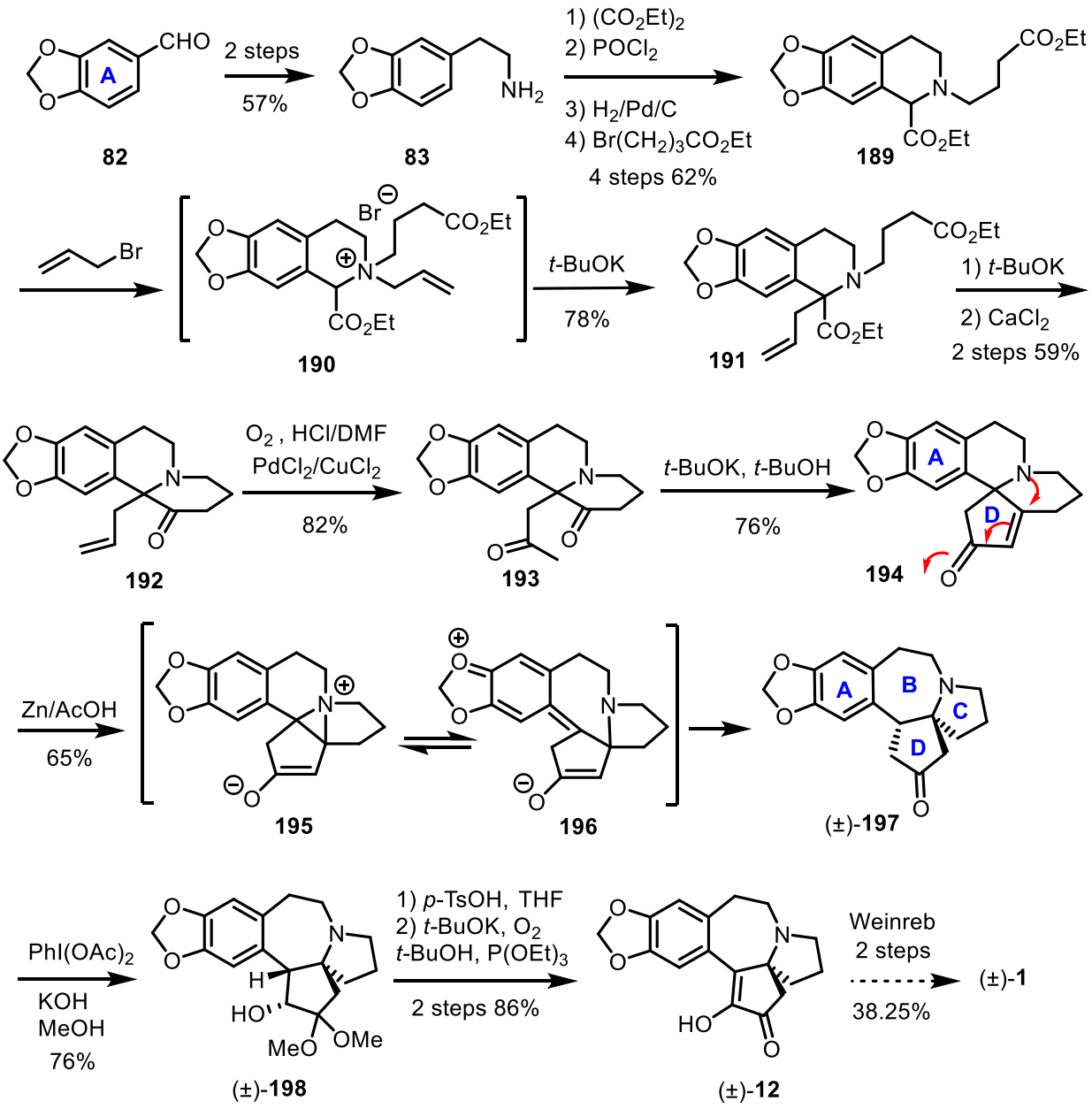


Figure 15. C5-N9 bond disconnection **c** for construction of ring B of CET (**1**)

3.2.2.1 Li's formal syntheses (2003-2005)

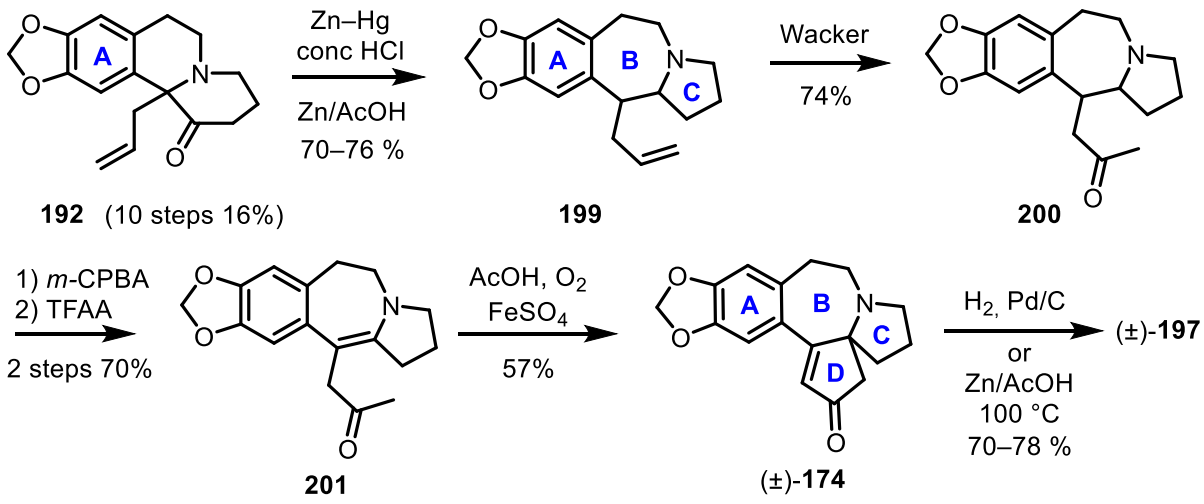
From 2003 to 2005, Li et al. reported three formal syntheses, two of them via the same intermediate **192** (Scheme 14).[100,105] Formation of ring B and D was achieved by transannular reductive skeletal rearrangements.



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

Starting from piperonyl amine **83**, diester **189** was obtained in four steps via a Bishler–Napieralski cyclisation 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 (PIDA) 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 1.6% calculated OY over eighteen 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]

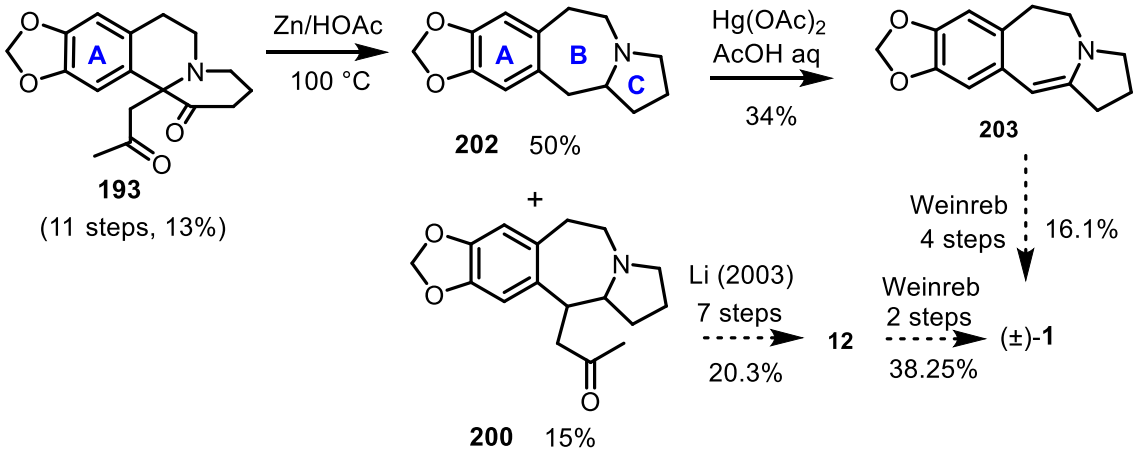


Scheme 15. Formal synthesis of *rac*-CET (±)-(**1**) by Li (2003): alternative synthesis of ketone (±)-**197**

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. 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 twenty-one steps and 0.6–0.7% OY from piperonal (**82**).

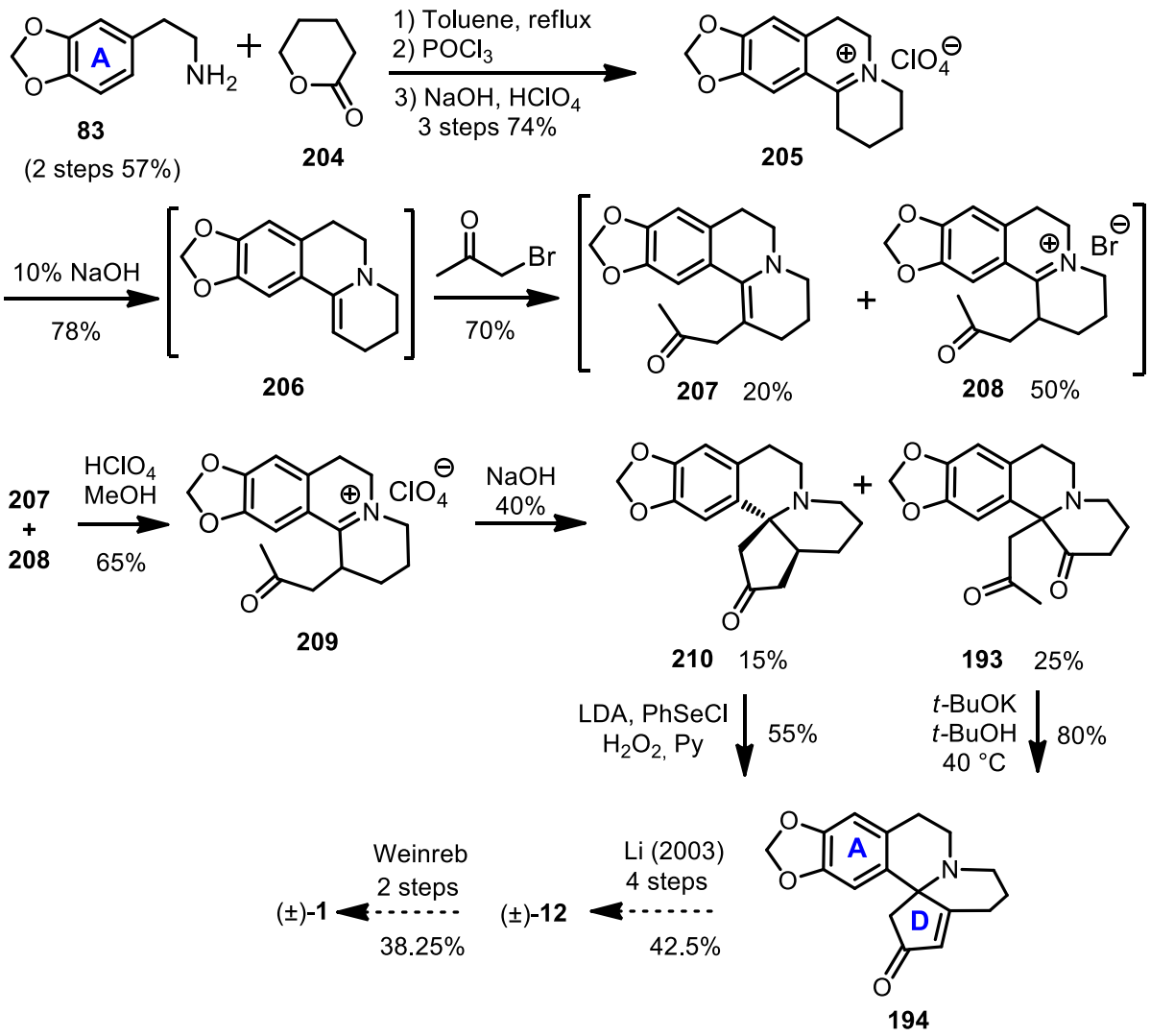
In 2005, Li et al. described a formal synthesis of the Dolby-Weinreb enamine[103,106] **203** from dione **193**, with a mild Clemmensen–Clemo–Prelog–Léonard reductive rearrangement of **193** as the key step (Scheme 16).[105]



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

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 seventeen steps from piperonal (**82**). Reductive rearrangement of amino dione **193** also produced amino ketone **200** in 15% yield, which could be processed into demethylcephalotaxinone (**12**) then into CET (**1**) through a nine-stage sequence. This constitutes another formal synthesis of CET (**1**) with an expected OY of 0.15% in twenty three 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]



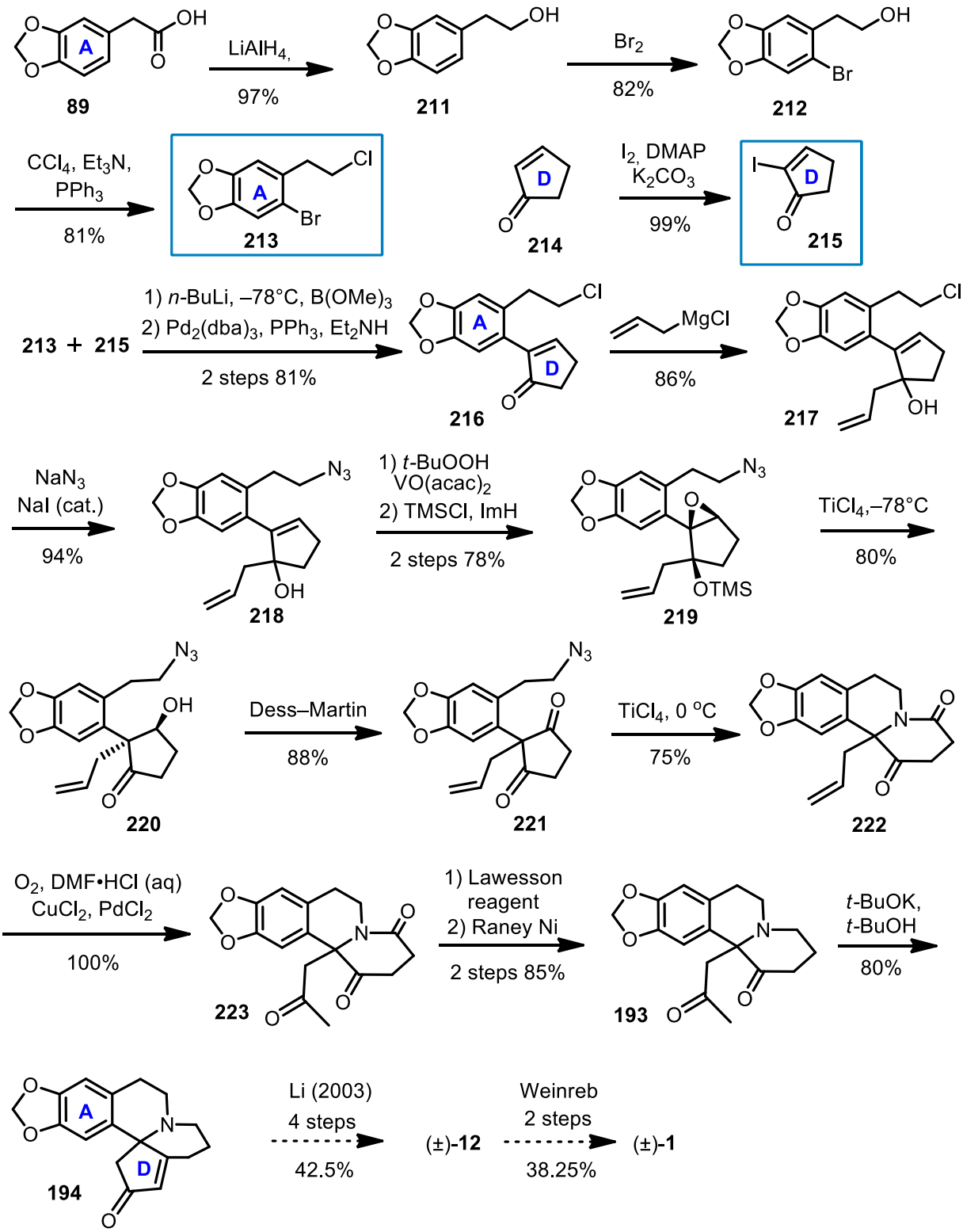
Scheme 17. Formal synthesis of *rac*-CET (±)-(**1**) via amino enone **194** by Li (2005)

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 upon 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 from **210** (15%). Formally, *rac*-CET (±)-(**1**) could be obtained according to Li's and Weinreb's protocols[100,103] with a calculated 0.7% OY for sixteen 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 iodo cyclopentenone **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 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, 2 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 methyl ketone 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 cyclisation gave the key enone **194** in 80% yield. *rac*-CET (±)-(**1**) could be obtained by Li's and Weinreb's procedures in twenty three steps from homopiperonylic acid (**89**) and 1.9% evaluated OY.

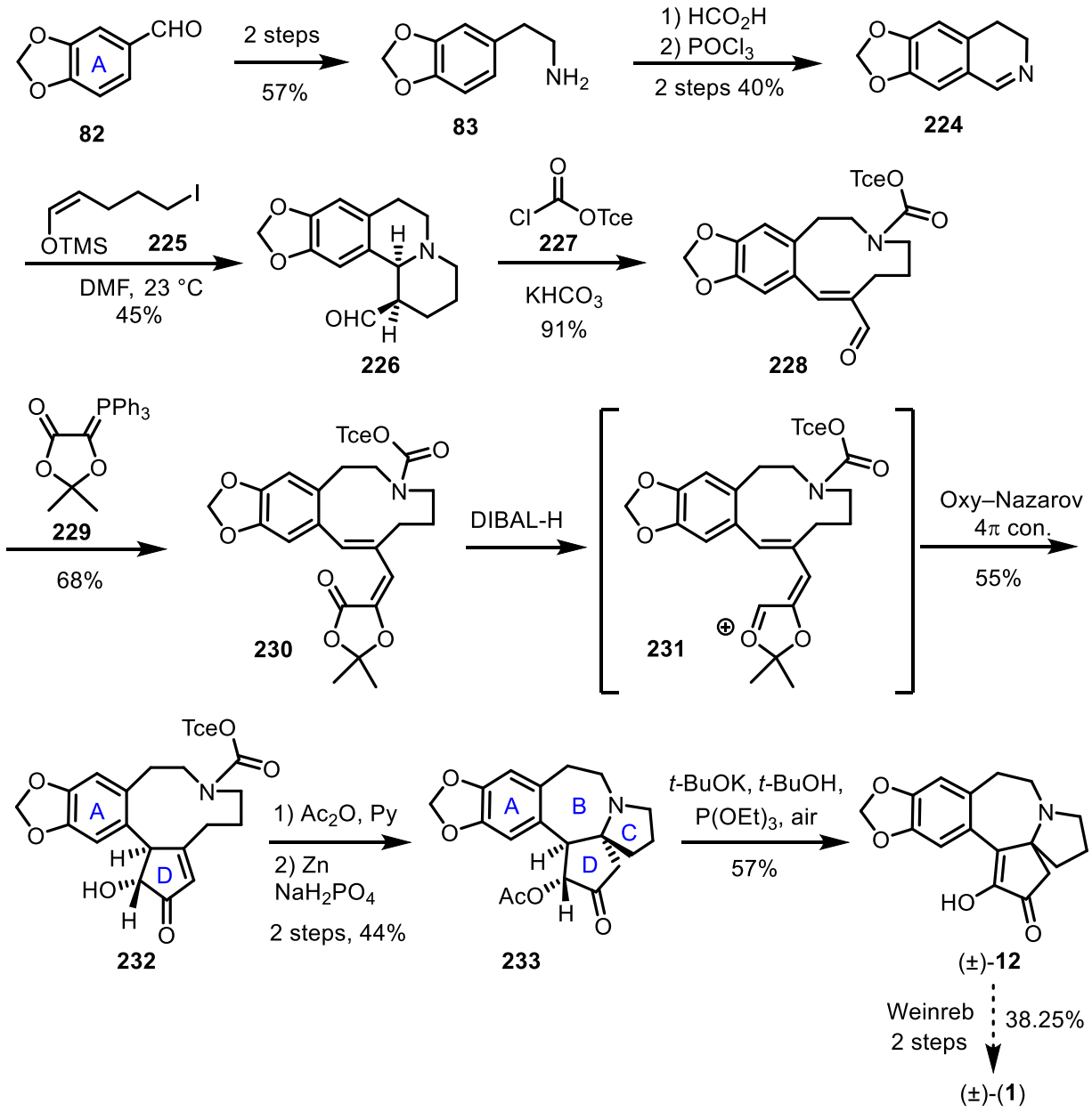


Scheme 18. Formal synthesis of *rac*-CET (±)-(**1**) by Tu and Zhang (2009)

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

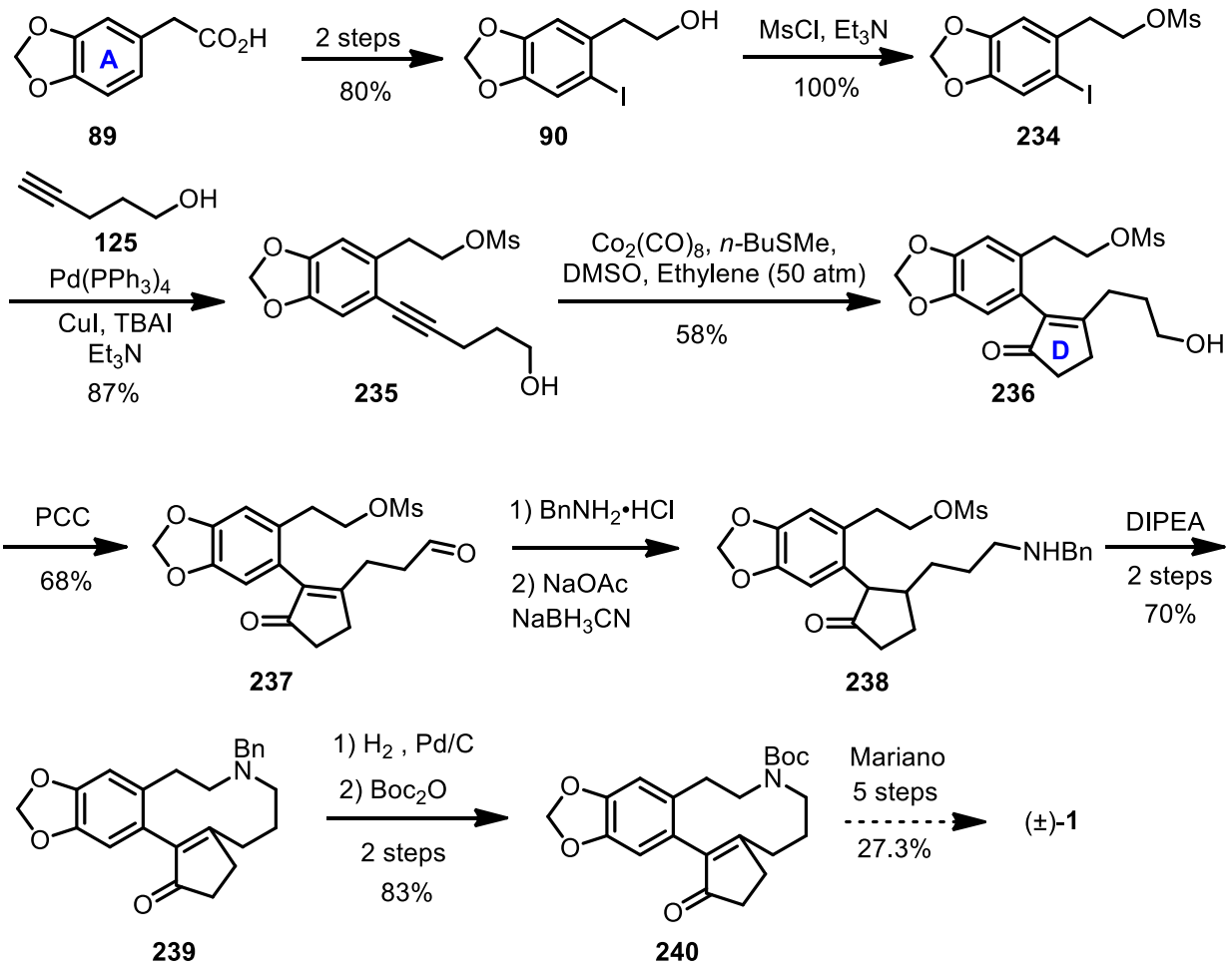
macrocyclic enal **228**. Wittig olefination with triphenylphosphoranylidene dioxolanone **229** furnished the ten-membered amide **230** with the exocyclic (*E*)-olefin in 68% isolated yield, along with the separable (*Z*)-isomer (14%). Mild hydride reduction led to hydroxy enone **232** presumably via the oxonium ion **231** which could 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 synthesise *rac*-CET (±)-(**1**) in thirteen steps from piperonal (**82**) and 0.4 OY.



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

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 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 ten-membered ring compound **239** by intramolecular N-alkylation. Mariano's intermediate[113,114] **240** was prepared successively by deprotection and re-protection with a *t*-butoxycarbonyl group.



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

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 fifteen 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)

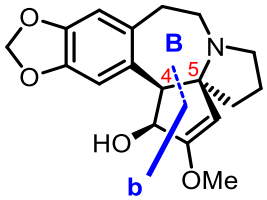
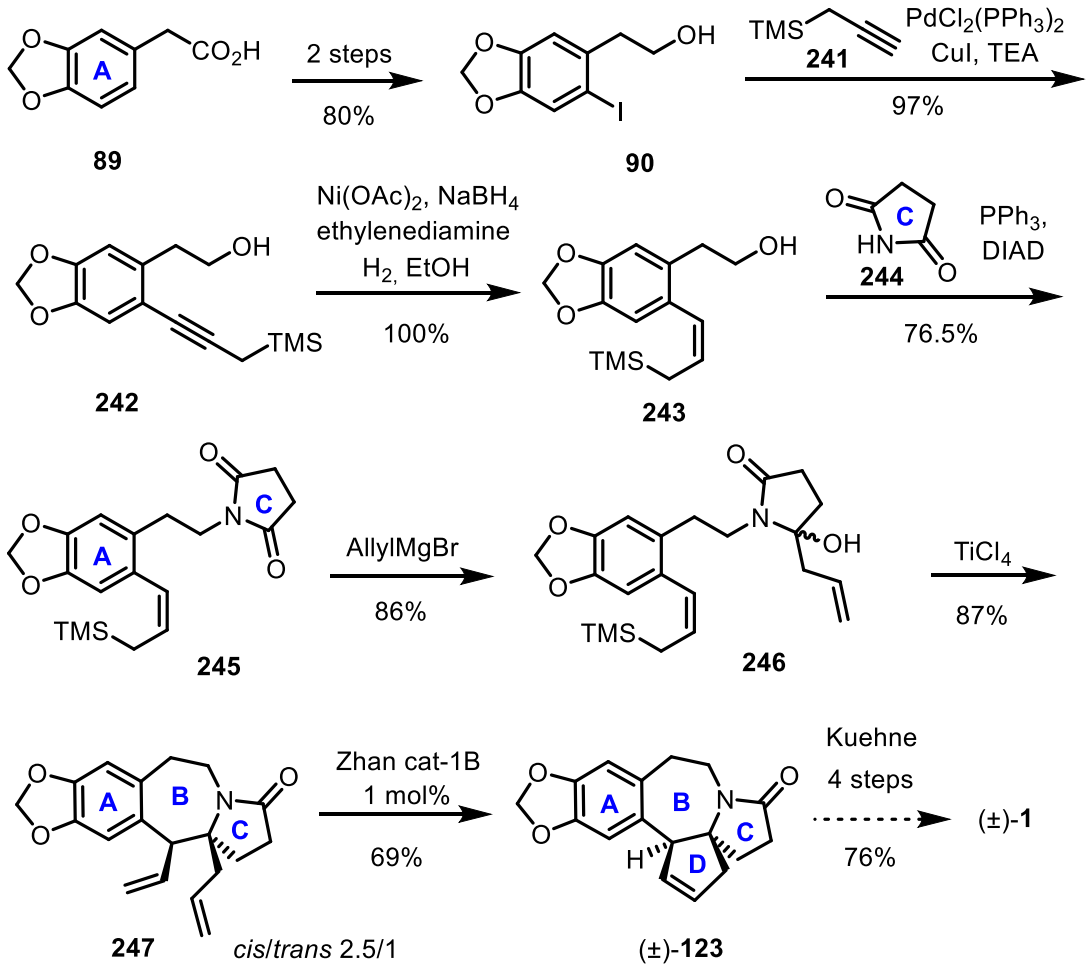


Figure 16. C4–C5 bond disconnection **b** for elaboration of ring B of *rac*-CET (±)-(**1**)

Recently, Hong et al. reported two formal syntheses[115] involving an unusual C4–C5 disconnection[116,117,118] for ring B formation. The key steps were first an *N*-acyliminium ion cyclization which established stereogenic centers C5 and C4 and the formation of B ring. Then a RCM furnished the spiro-ring D (Scheme 21). The two syntheses started from iodo alcohol **90** prepared from homopiperonylic acid (**89**) in two steps.

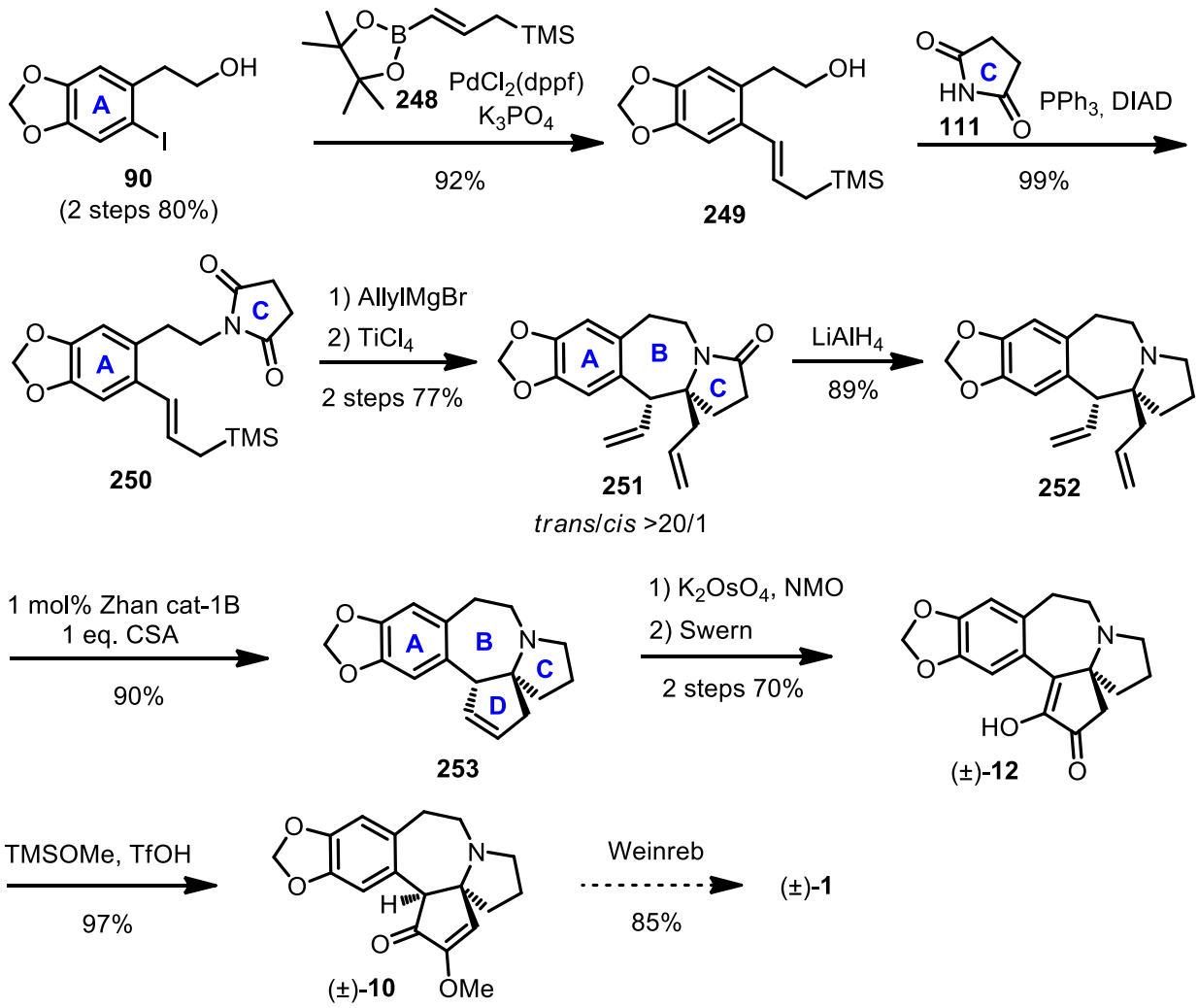


Scheme 21. Formal synthesis of *rac*-CET (±)-(**1**) by Hong via Kuehne's intermediate (±)-**123** (2015)

Sonogashira coupling of aryl iodide **90** with propargyl trimethylsilane catalyzed by palladium(II) and copper(I) iodide delivered alkyne **242** in excellent yield (97%). 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 the hemiaminal **246** which, treated with $TiCl_4$ at low temperature in mesitylene, allowed for the generation of an N-acyliminium ion which 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 twelve steps from homopiperonylic acid (**89**) and 23.5% calculated OY.

Since iminium ion cyclisation 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 *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**).



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

3.2.3.2 N9-C10 ring closure e: Chandrasekhar's total synthesis (2016)

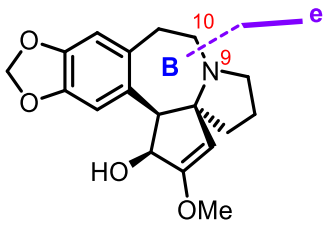
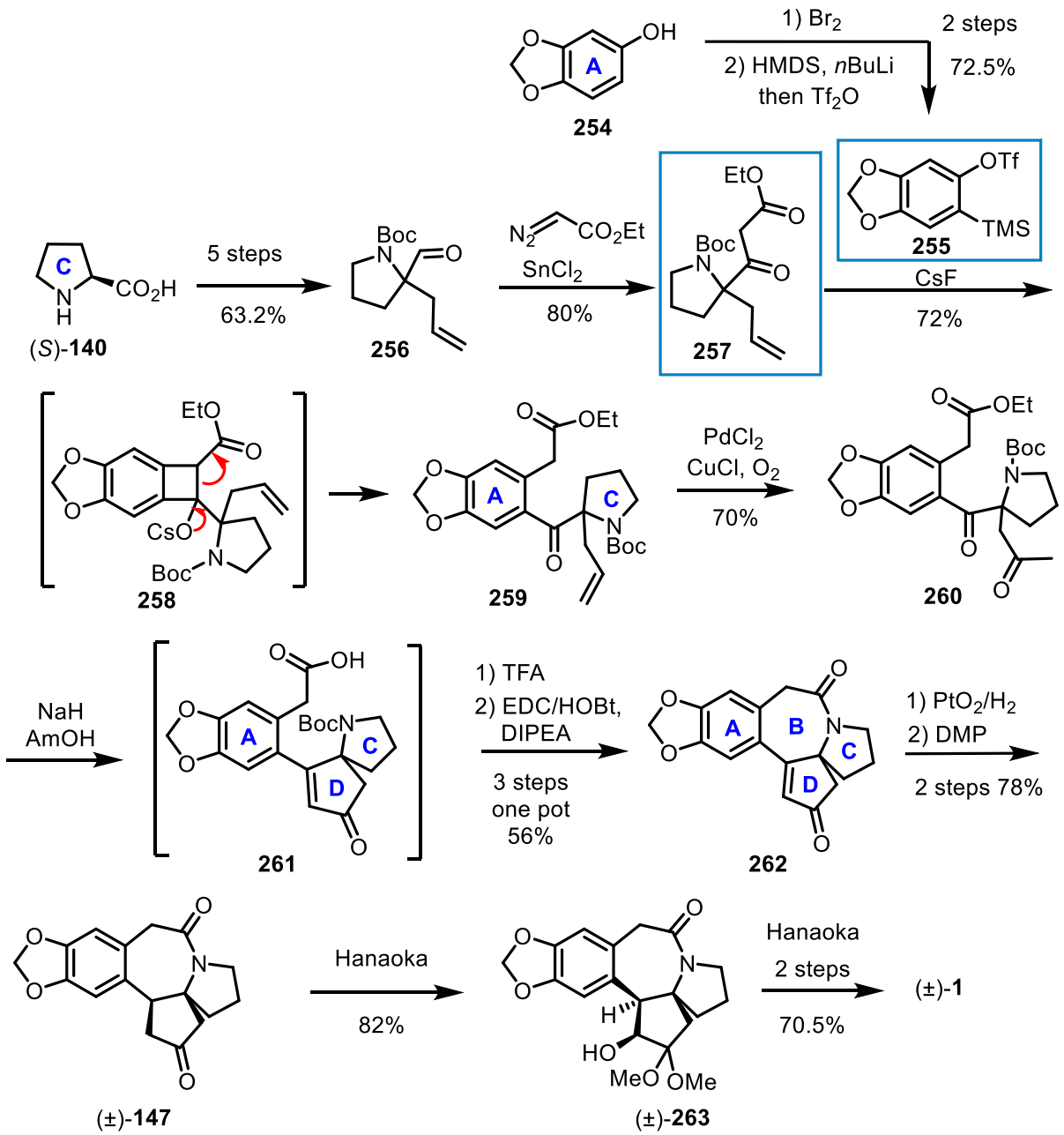


Figure 17. N9–C10 bond disconnection **e** for construction of CET (**1**) B ring

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]

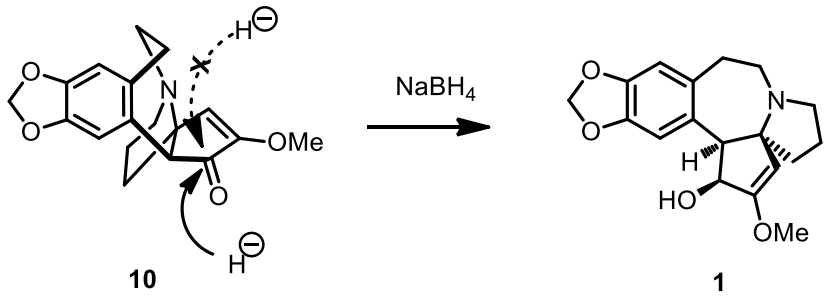


Scheme 23. Total synthesis of *rac*-CET (±)-(**1**) by Chandrasekhar (2016)

Aldehyde **256** derived in five steps from L-proline (**140**) was transformed into β-ketoester **257** by Roskamp conversion. Then, β-ketoester **257** was submitted to [2+2] cycloadditon with benzyne generated from aryne precursor **255**. 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, 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 litterature conditions[98] in a total of sixteen steps and 6.4% OY. Including the preparation of aromatic unit **255** from 5-benzodioxolol (**254**), *rac*-CET (±)-(**1**) was obtained in eighteen total number of steps and 4.7% AY.

3.3 Syntheses of enantioenriched CET

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 cyclisation step, the thermodynamically more stable BD ring junction is *cis*, which is the relative C4–C5 configuration found in natural cephalotaxine. 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**.



10 **1**

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

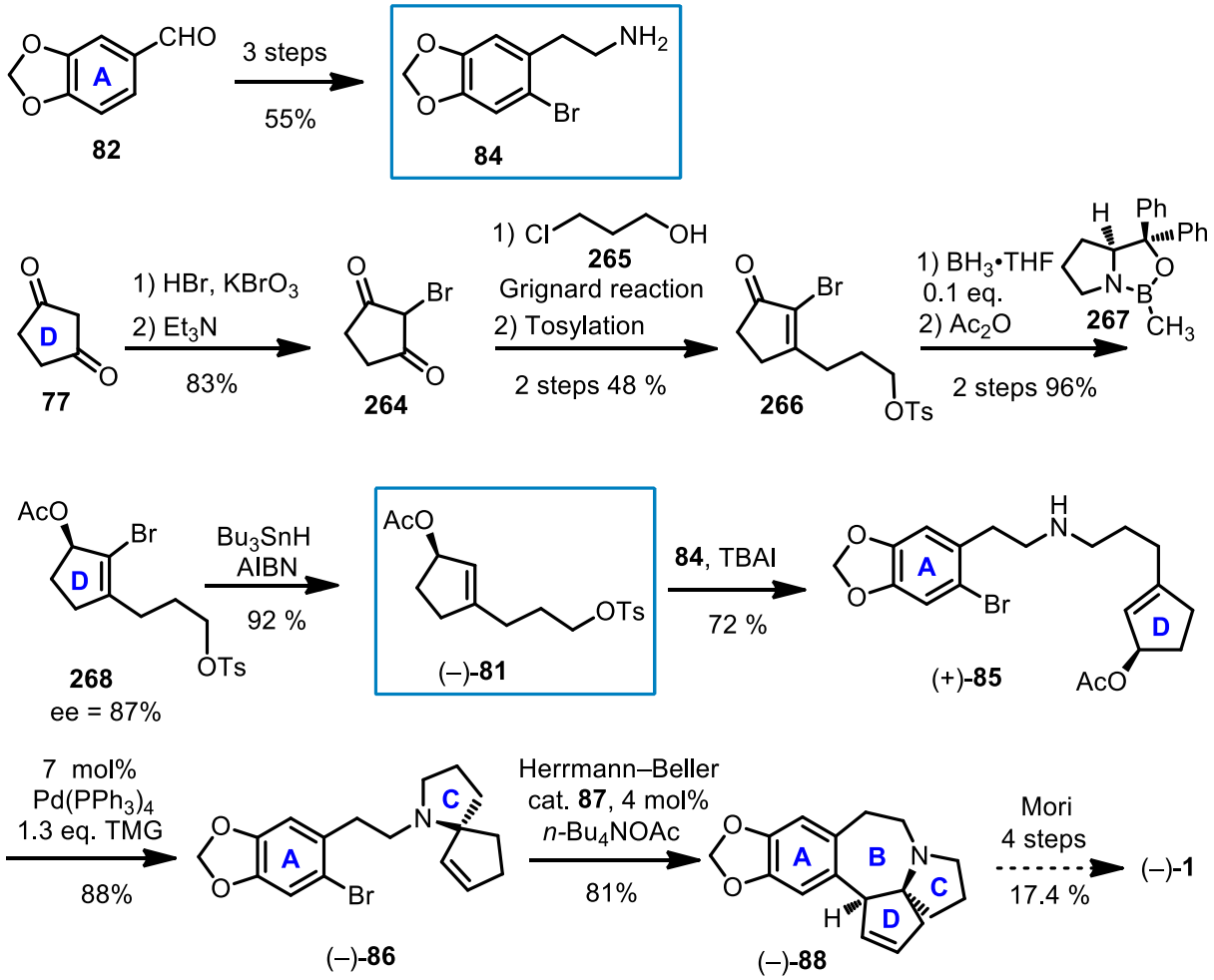
It is thus possible to control successively the C4 center via the C5 tetrasubstituted center then the C3 center via the C4 stereocenter, and consequently, control of the tetrasubstituted C5 stereocenter is the only necessary one to synthetize enantiomerically pure CET (**1**). Most of asymmetric syntheses are based on the control of this spiro center followed by a stereoselective cyclisation 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 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

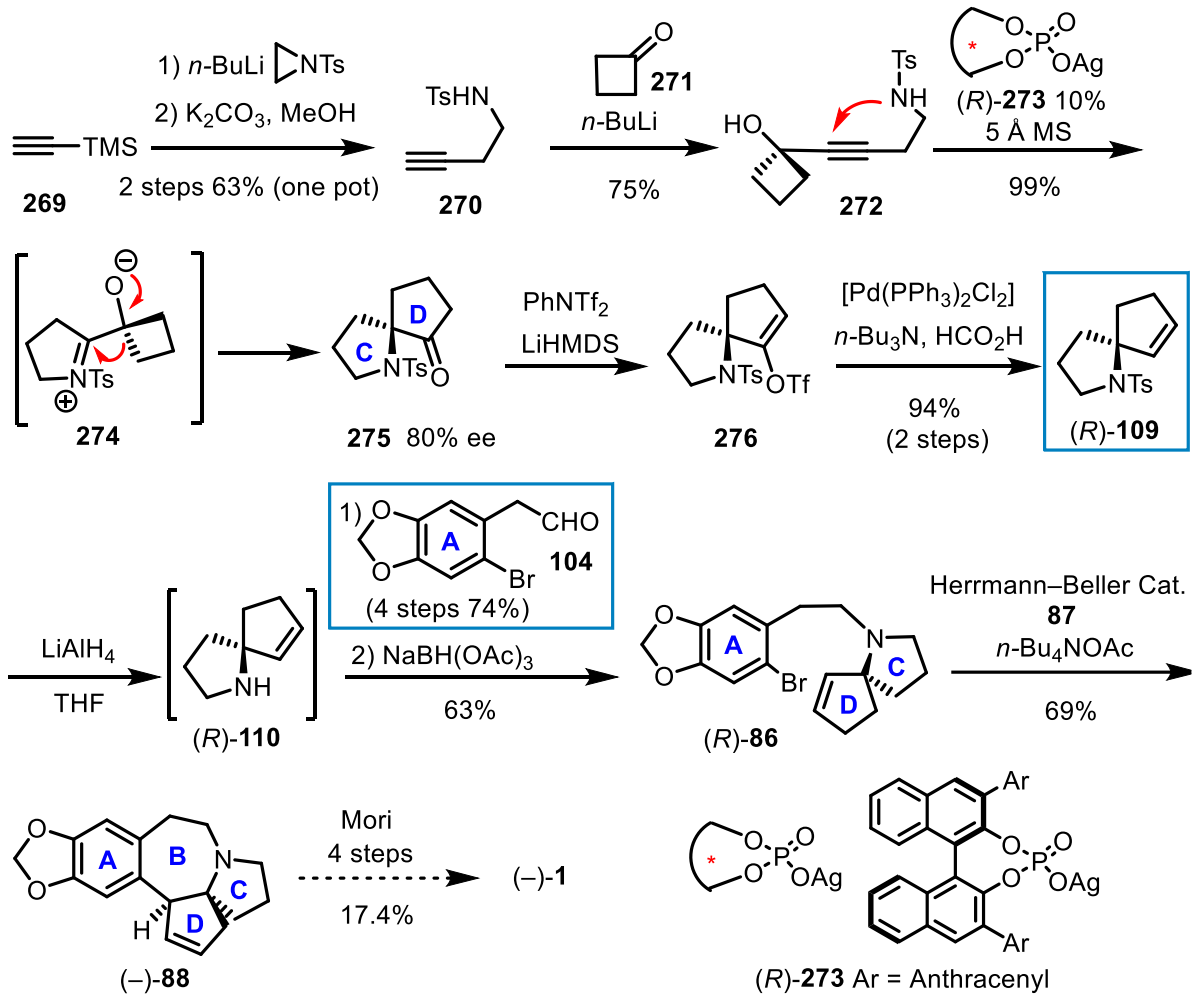
treatment with a large excess of Grignard reagent prepared from chloroalcohol **265** and tosylation (three steps, 40% OY). Chiral bromo-acetate **268** corresponding to the future D ring was obtained by reduction with diborane in the presence of catalytic amounts of the chiral oxazaborolidine **267**, followed by acetylation of the allylic alcohol. A 87% enantiomeric excess (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 thirteen steps linear sequence, 3.1% calculated OY and 87% expected ee from cyclopentane-1,3-dione (**77**).



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

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. At the same time, chirality was introduced using a chiral silver phosphate (*R*)-**273** as catalyst (Scheme 26).[122]



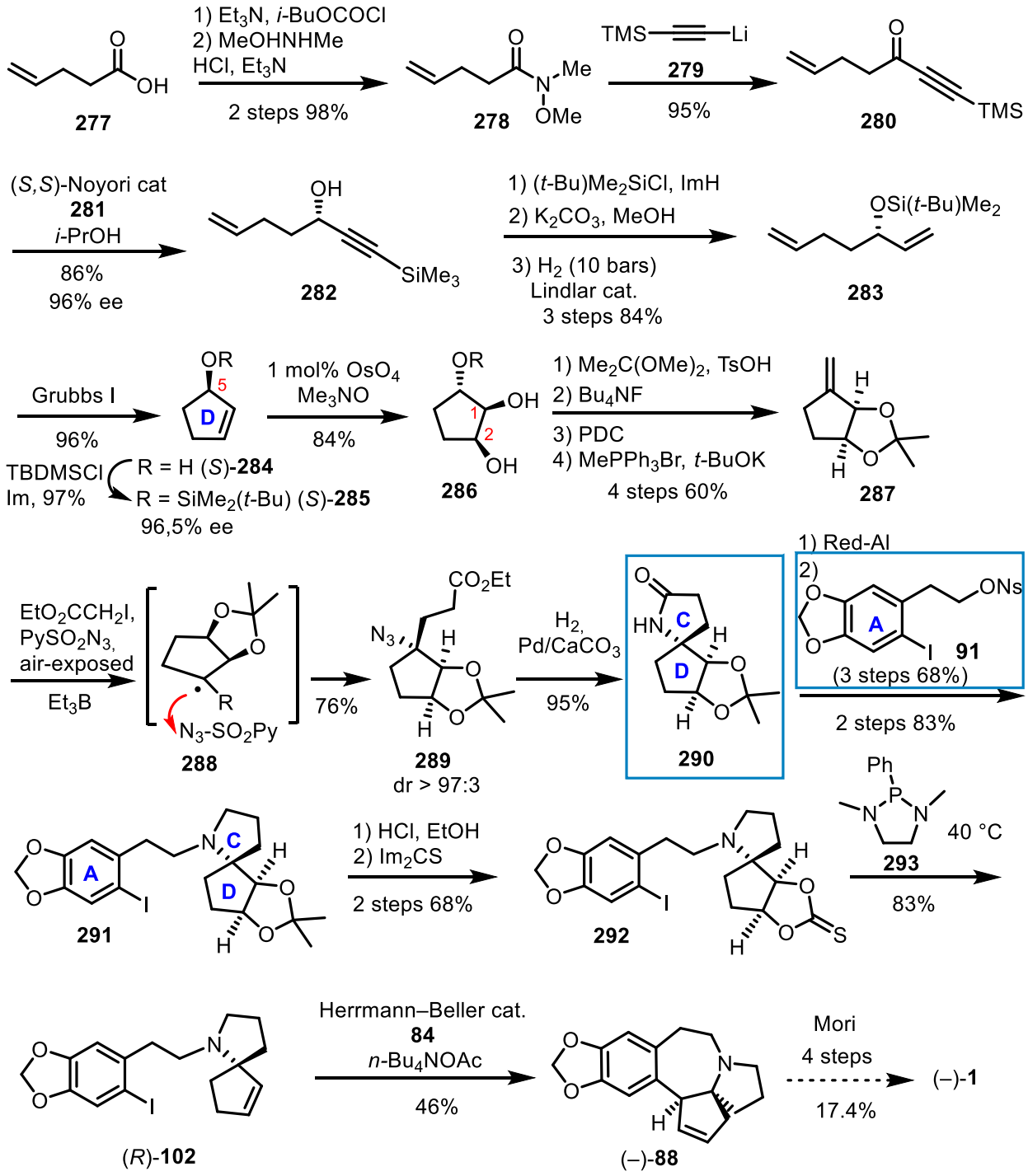
Scheme 26. Formal asymmetric synthesis of CET (–)-(**1**) by Tu (2012)

The anion of butyne **270** prepared from trimethylsilylacetylene (**269**) reacted with cyclobutanone to give **272** which was submitted to a cyclisation-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 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 thirteen steps, 3.3% calculated OY and 80% ee. The high purity material (estimated 99% ee) could be obtained in 1.7% calculated OY from trimethylsilylacetylene (**269**).

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 by reduction of the azide. First, the stereogenic center at C5 (cephalotaxine numbering) of (*S*)-cyclopentenol **284** was used to control the configuration of the newly formed diol at C1–C2, and then it was destroyed upon double bond formation in **287**. This chiral center was eventually controled 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).



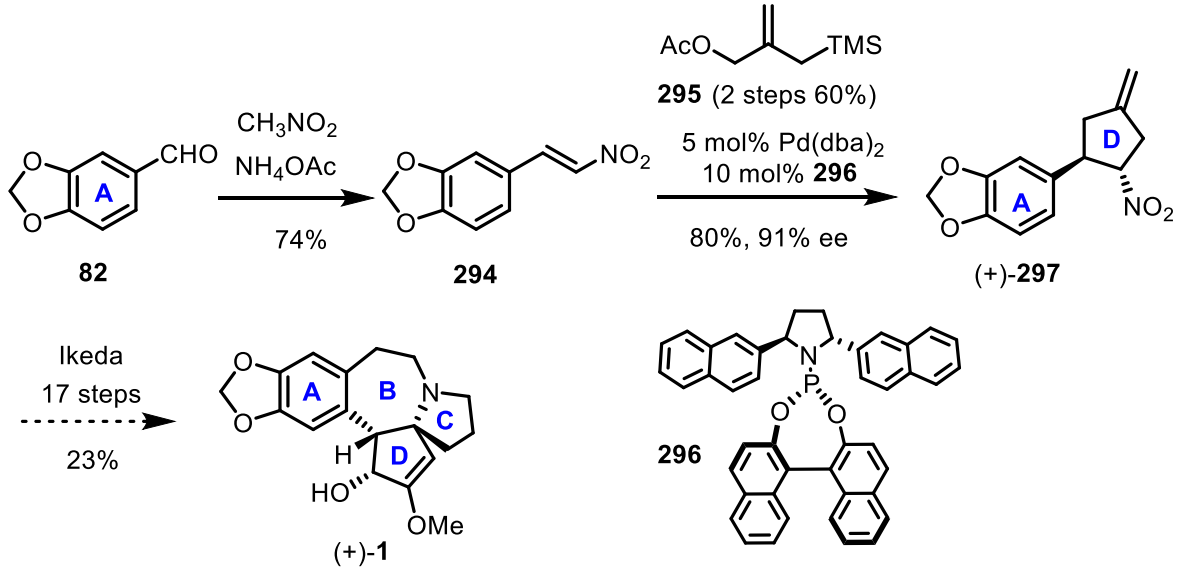
Scheme 27. Alternative formal asymmetric synthesis of CET (–)-(**1**) by Renaud (2012)

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 twenty-six steps from 4-pentenoic acid (**277**) and 0.85% 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 (TMM) 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]



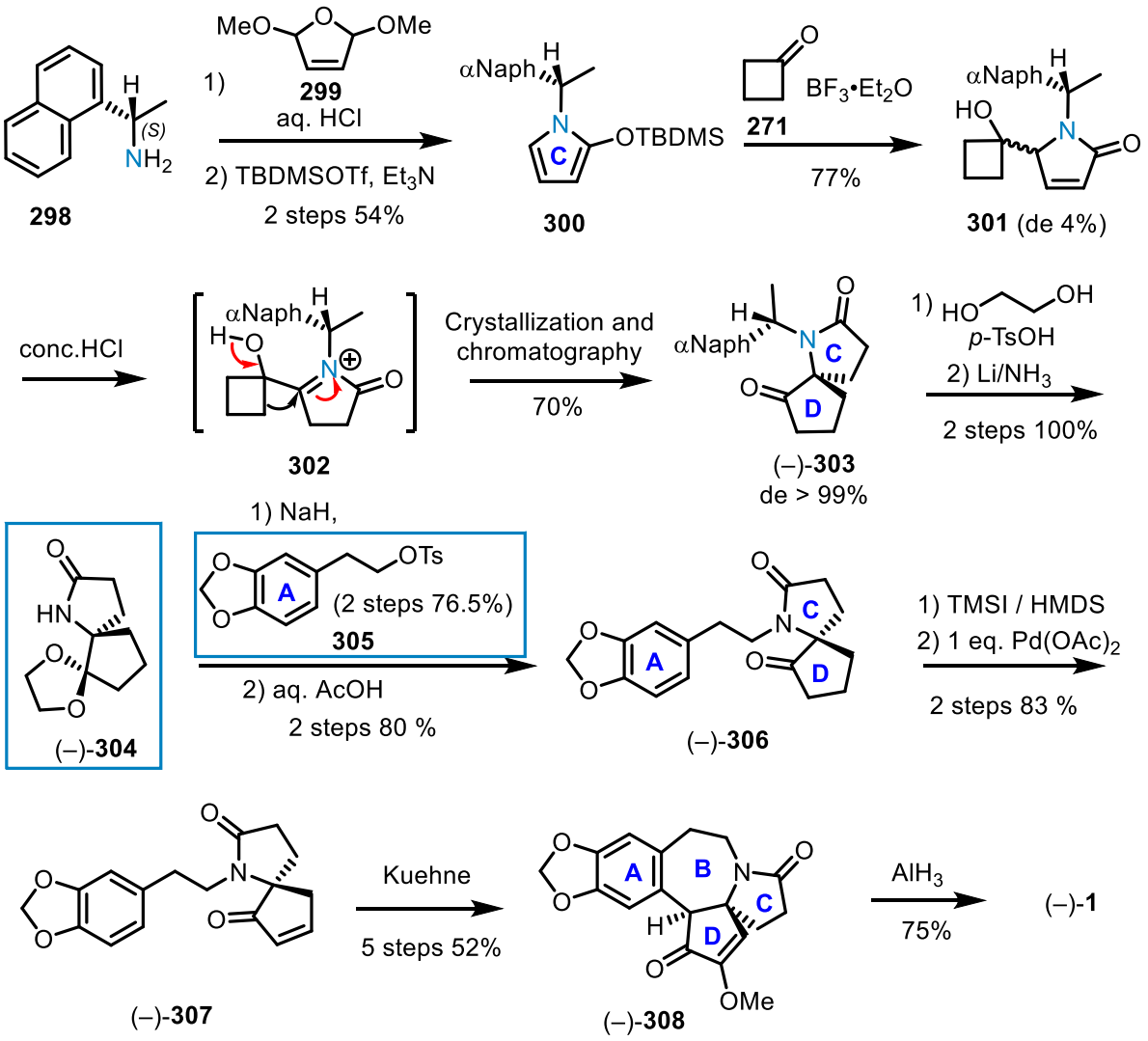
Scheme 28. Formal asymmetric synthesis of *ent*-CET (+)-(**1**) by Trost (2012)

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 13.6% calculated OY over a nineteen steps in the longest linear sequence from piperonal (**82**).

3.3.2 Use of chiral auxiliary

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 semi-pinacolic 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.



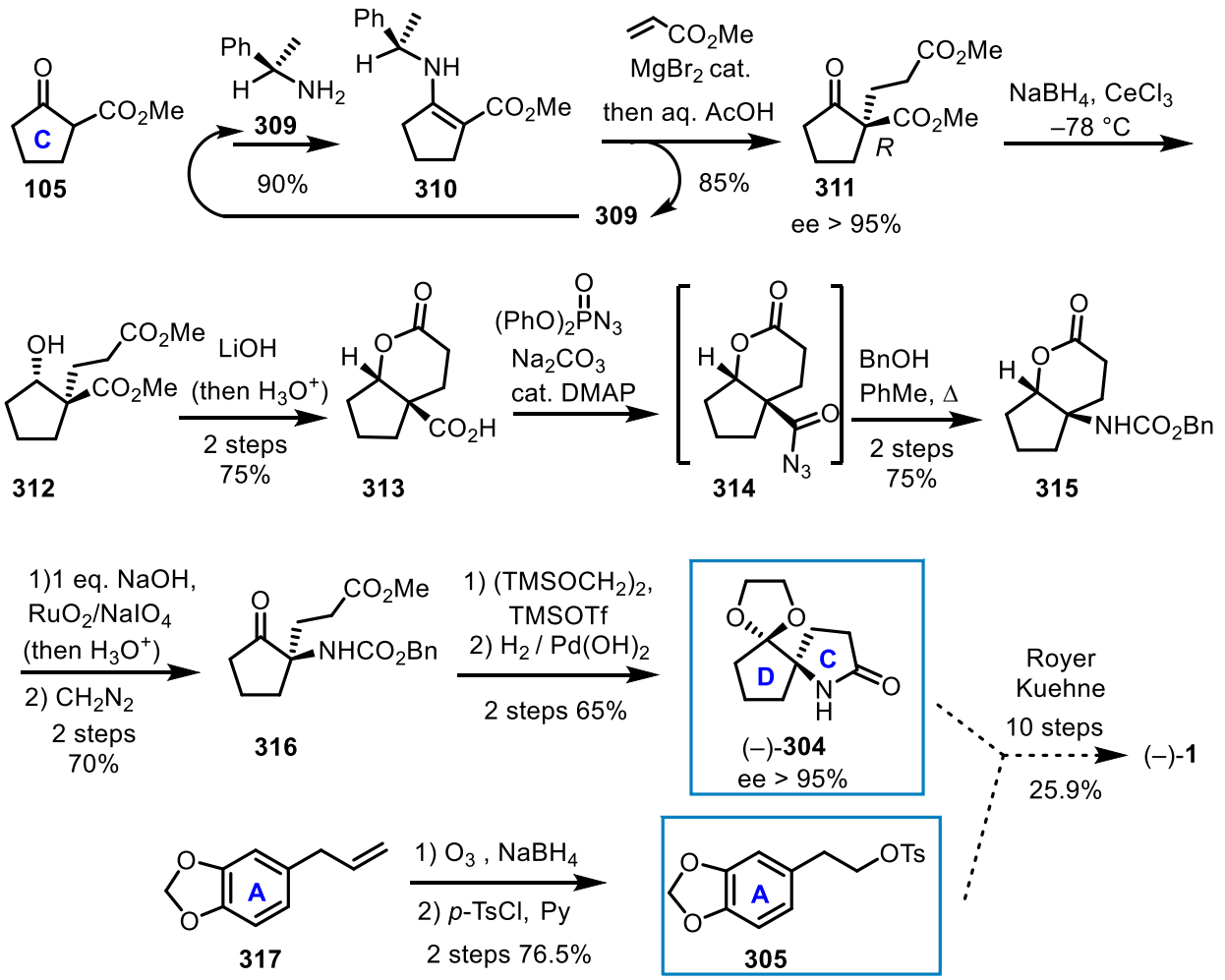
Scheme 29. Asymmetric synthesis of CET (–)-(**1**) by Royer (2004)

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 **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, thus azaspirononanedione (–)-**303** was obtained in 70% yield and de surpassing 99%. The ketone function of (–)-**303** was protected as a cyclic ketal, 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[116,117,118,134] 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 THF for the reduction of (–)-**308** in order to avoid any racemization of sensitive CET (**1**). CET (–)-(**1**) was thus obtained in 98.7% ee and 7.5% OY over a sixteen 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 ring unit CD (–)-**304** described by Royer[132] based on an asymmetric Michael reaction involving a chiral enamine prepared from recyclable enantiopure (*R*)-1-phenylethylamine **309** (Scheme 30).[135] As a consequence of stereogenic control of the quaternary carbon center in the resulting (*R*)-keto diester **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 terasubstituted spirocyclic center.



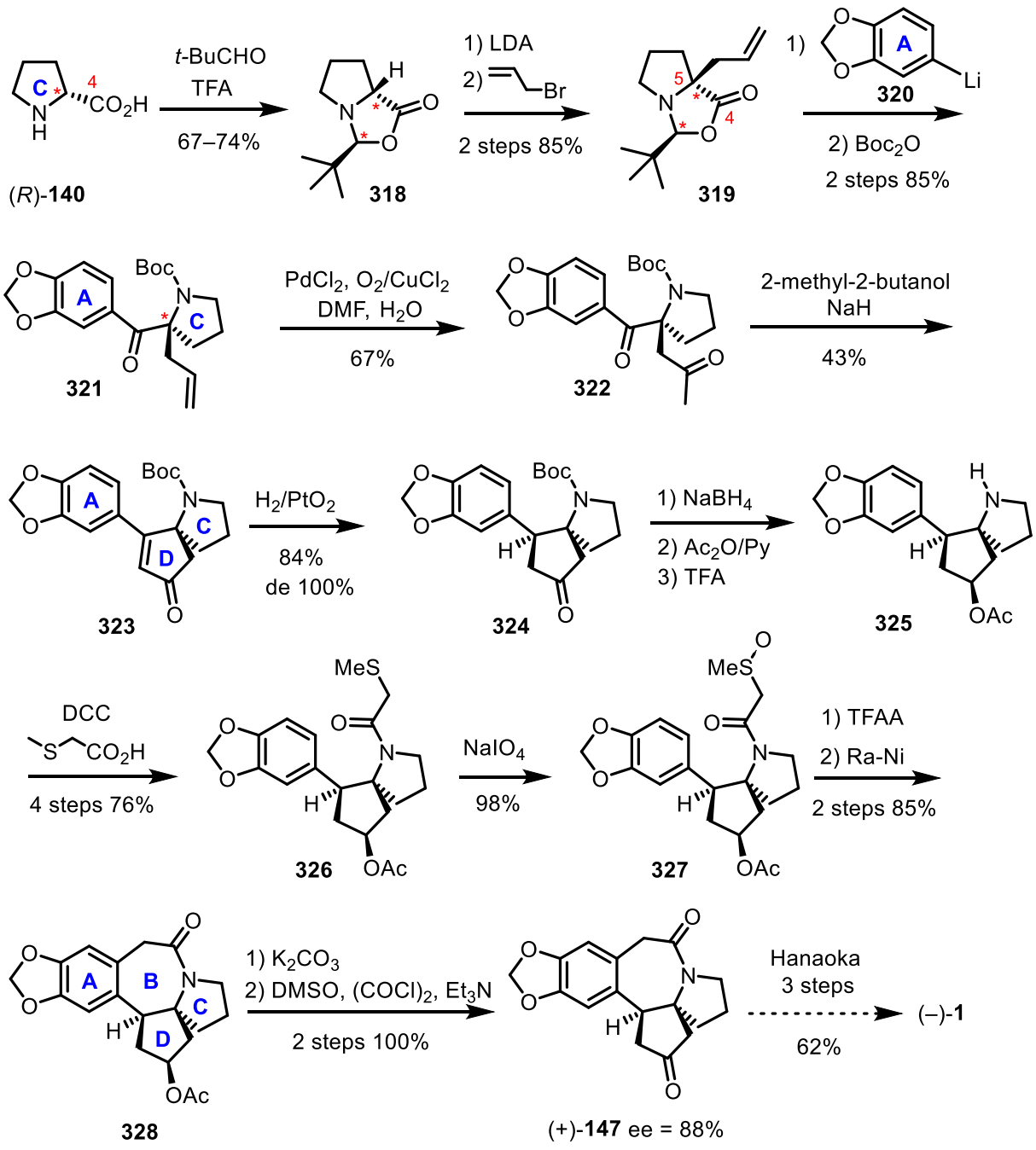
Scheme 30. Formal asymmetric synthesis of CET (–)-(**1**) by Dumas and d'Angelo (2005)

The keto diester (*R*)-**311** was elaborated in 85% yield and an ee ≥ 95% through Michael addition of enamino ester (*R*)-**310** to methyl acrylate. Sequential stereoselective Luche reduction–of the ketone at low temperature followed by saponification, and acidification provided an intermediary hydroxy diacid which upon 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 then esterification of the intermediate acid by diazomethane afforded the keto ester (*R*)-**316**. This compound was converted into ketal 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 2 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%, twenty 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).



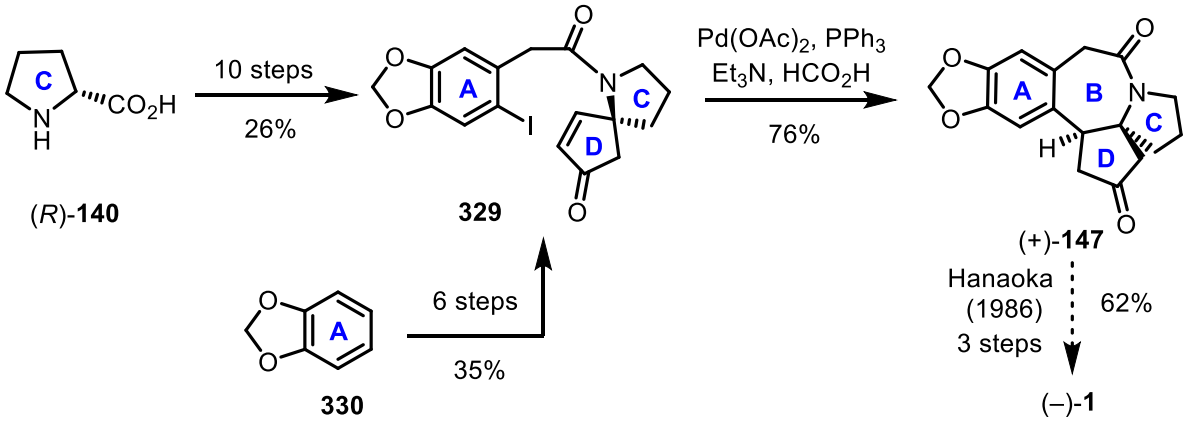
Scheme 31. Formal asymmetric synthesis of CET (–)-(**1**) by Ikeda (1999)

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. 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 absolute configuration 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), nineteen 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, dppp, Ag_2CO_3 in DMF led to (+)-**147** in a modest 7% yield.



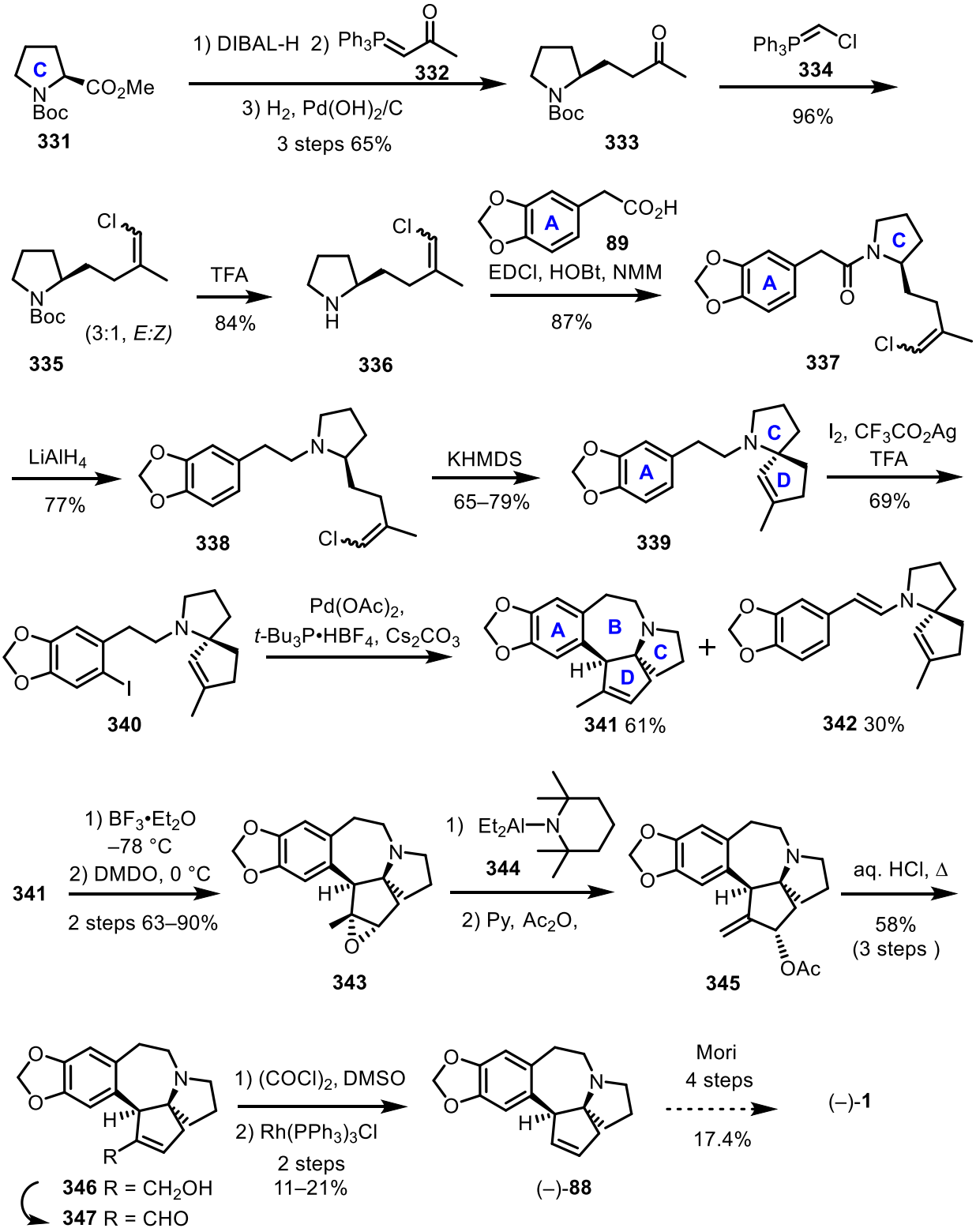
Scheme 32. Asymmetric formal synthesis of CET (–)-(**1**) by El Bialy (2002)

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

3.3.3.3 Hayes's formal syntheses (2008)

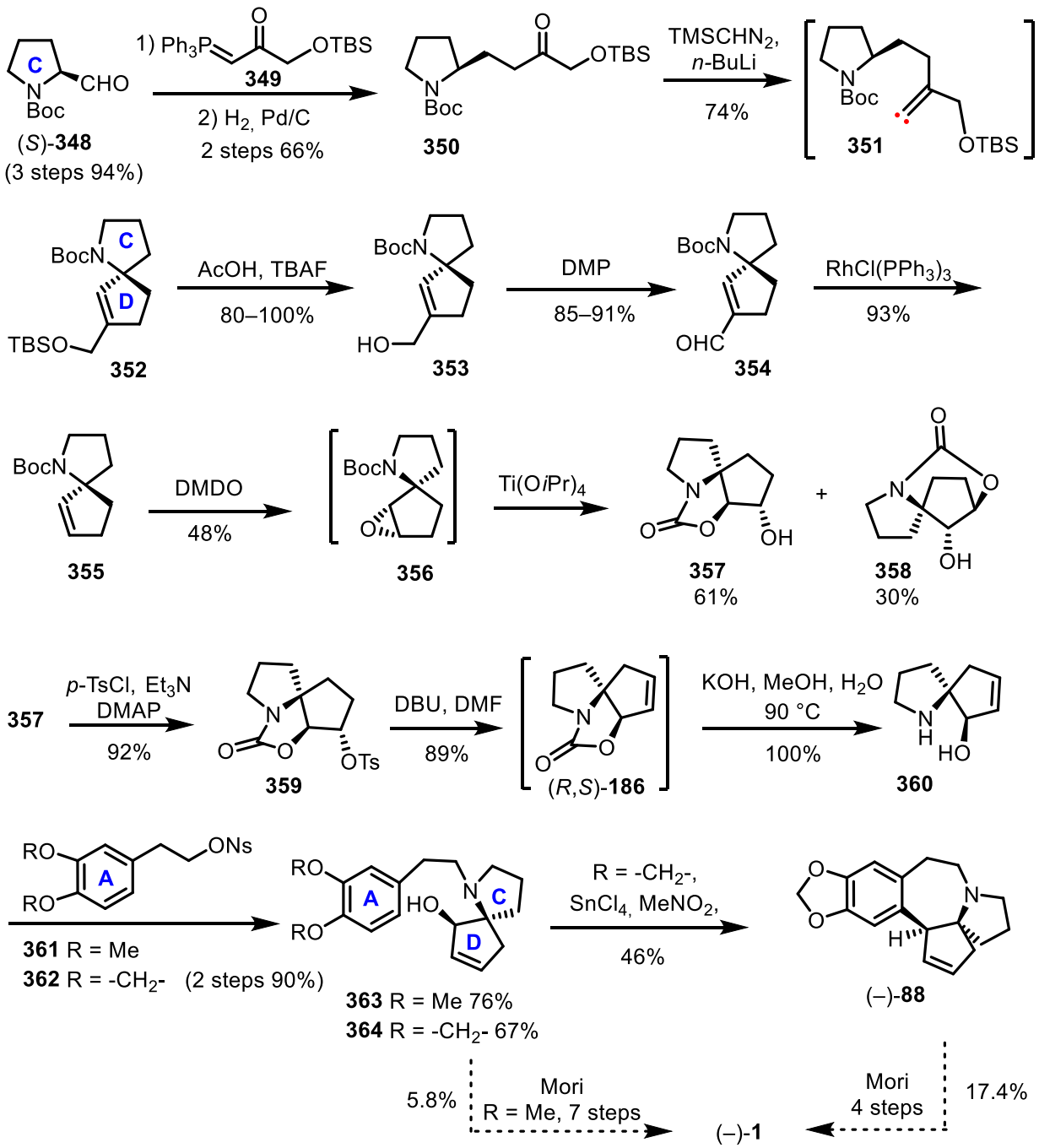
In 2008, Hayes et al. developed a formal synthesis of CET (–)-(**1**) based on an alkylidene carbene 1,5-CH insertion reaction as a key step to construct the 1-azaspiro[4.4]nonane CD ring system (**338** → **339**).[141] In a first approach, vinyl chloride **338** was chosen as a carbene precursor prepared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

DIBAL-H reduction of **331** followed by Wittig reaction with 1-triphenylphosphoranylidene-2-propanone (**332**) gave an intermadiate *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 KHMDS 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•P*t*Bu_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 DMDO in the presence of BF_3•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** 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, twenty-three steps and 0.06-0.2% calculated OY from L-proline (*S*)-**140**.



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

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]



Scheme 34. Formal asymmetric synthesis of CET (–)-(**1**) by Hayes (2008)

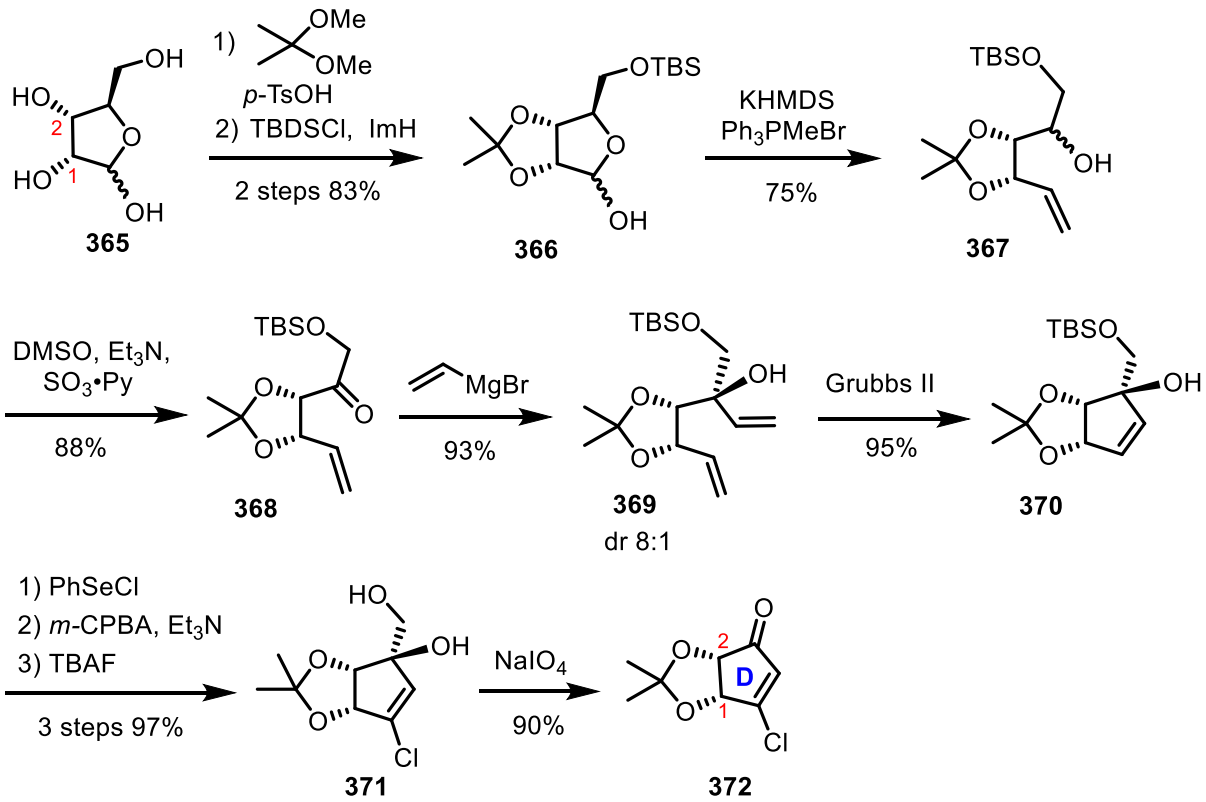
However, as there is no additional carbon atom on the phosphorane **349**, the previous tedious sequence to remove this very atom on ring D is no longer required (**341** → (–)-**88**), seven steps, 6.3 to 18.3% OY, scheme 33). The carbene precursor **350** was derived from *N*-Boc prolinal (*S*)-**348**, 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 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 upon 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**, he diastereomer of **363**, with the hydroxy group of opposite configuration, did not undergo the cyclisation reaction (see scheme 13). This sequence would allow access to CET (–)-(**1**) in 91% ee, in twenty-two steps and 0.3-0.4 % OY (R = Me) or in twenty 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)

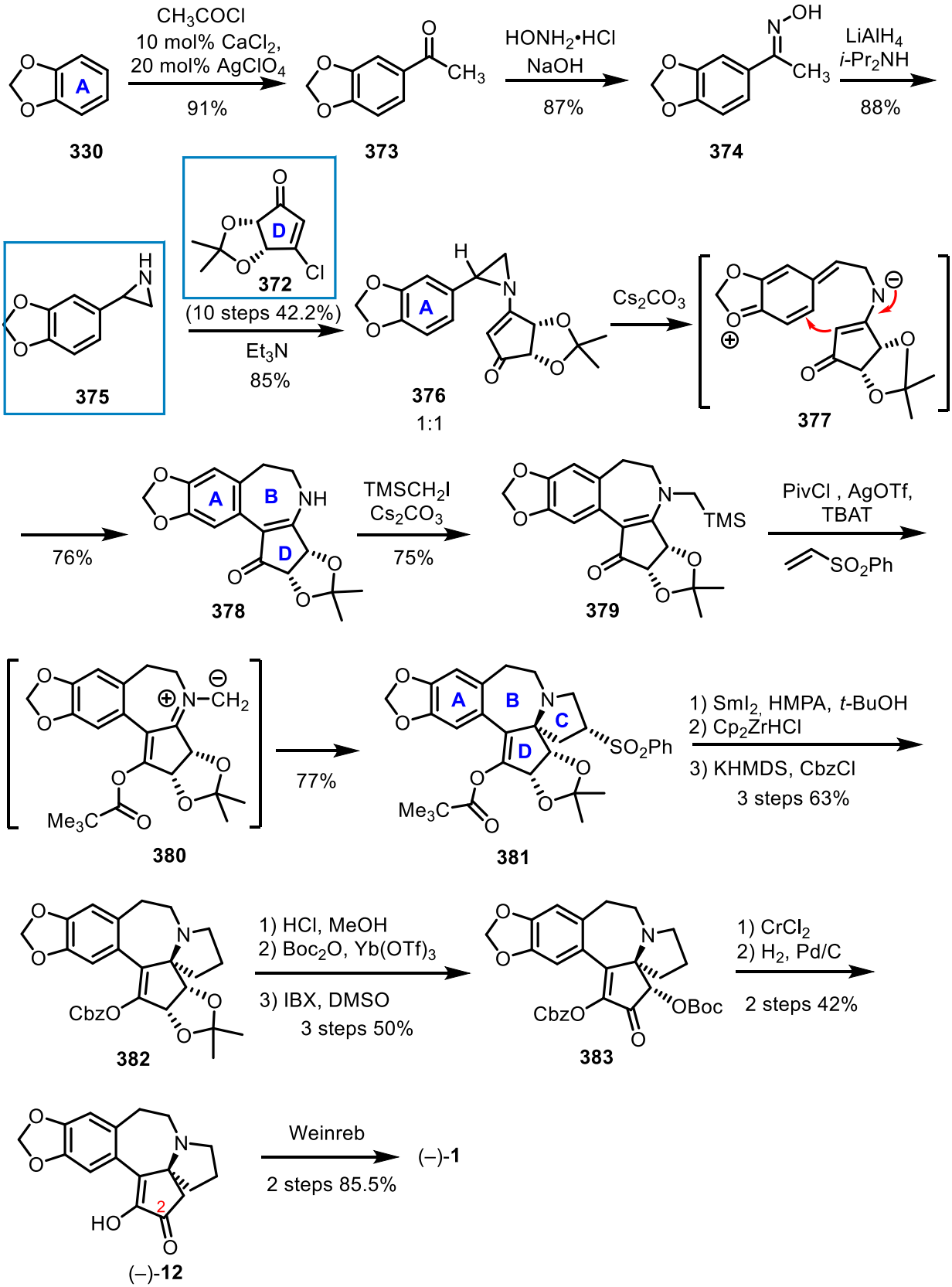
Three essential steps were used by Djaballah and Gin et al. for CET (–)-(**1**) synthesis (Scheme 36).[144] First, the nitrogen atom was introduced from the oxime **374** followed by Neber rearrangement. Secondly, construction of the benzazepine core relied on a strain-releasing rearrangement of *N*-vinyl-2-aryl aziridine **376** to **378**. Eventually, assembly of the spiro-fused 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).



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

Cyclopentene **370** was prepared by a 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** [ten steps, 42.2% OY from D-ribofuranose (**365**)] was obtained by periodate-mediated oxidative cleavage of the diol **371**.



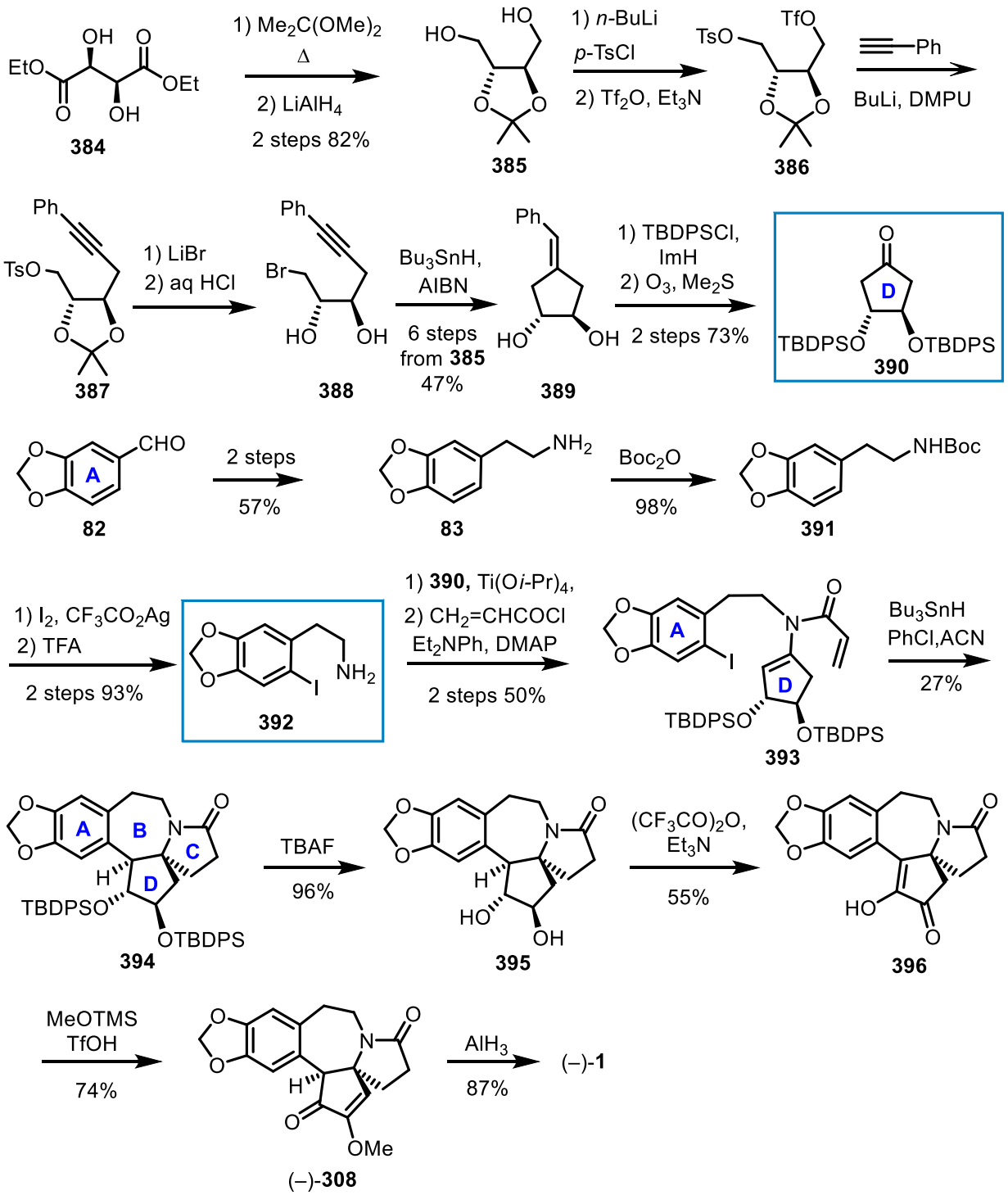
Scheme 36. Total asymmetric synthesis of CET (–)-(**1**) by Djaballah and Gin (2008)

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 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 re-acylation 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 IBX. The enone **383** reacted with $CrCl_2$ 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 and stereoselective reduction at C3 with $NaBH_4$ concluded the synthesis of (–)-CET (**1**) in twenty-four steps from D-ribofuranose (**365**) and 1.5% OY (ee n.d.).

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).



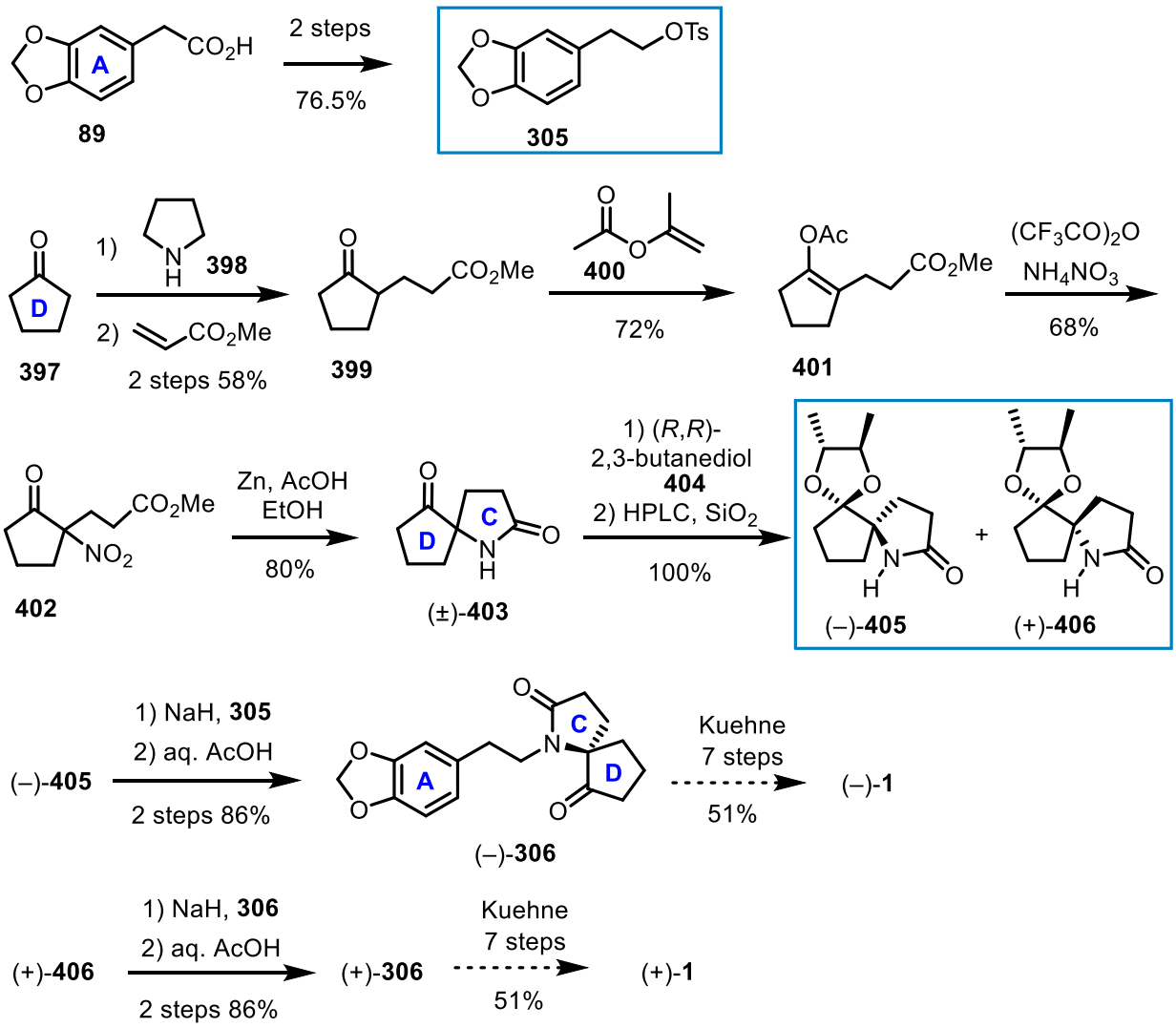
Scheme 37. Total asymmetric synthesis of CET (–)-(**1**) by Ishibashi (2008)

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 TBAF gave diol **395** which upon treatement with TFAA, DMSO 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 seventeen 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 spiro ketone (±)-**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**.

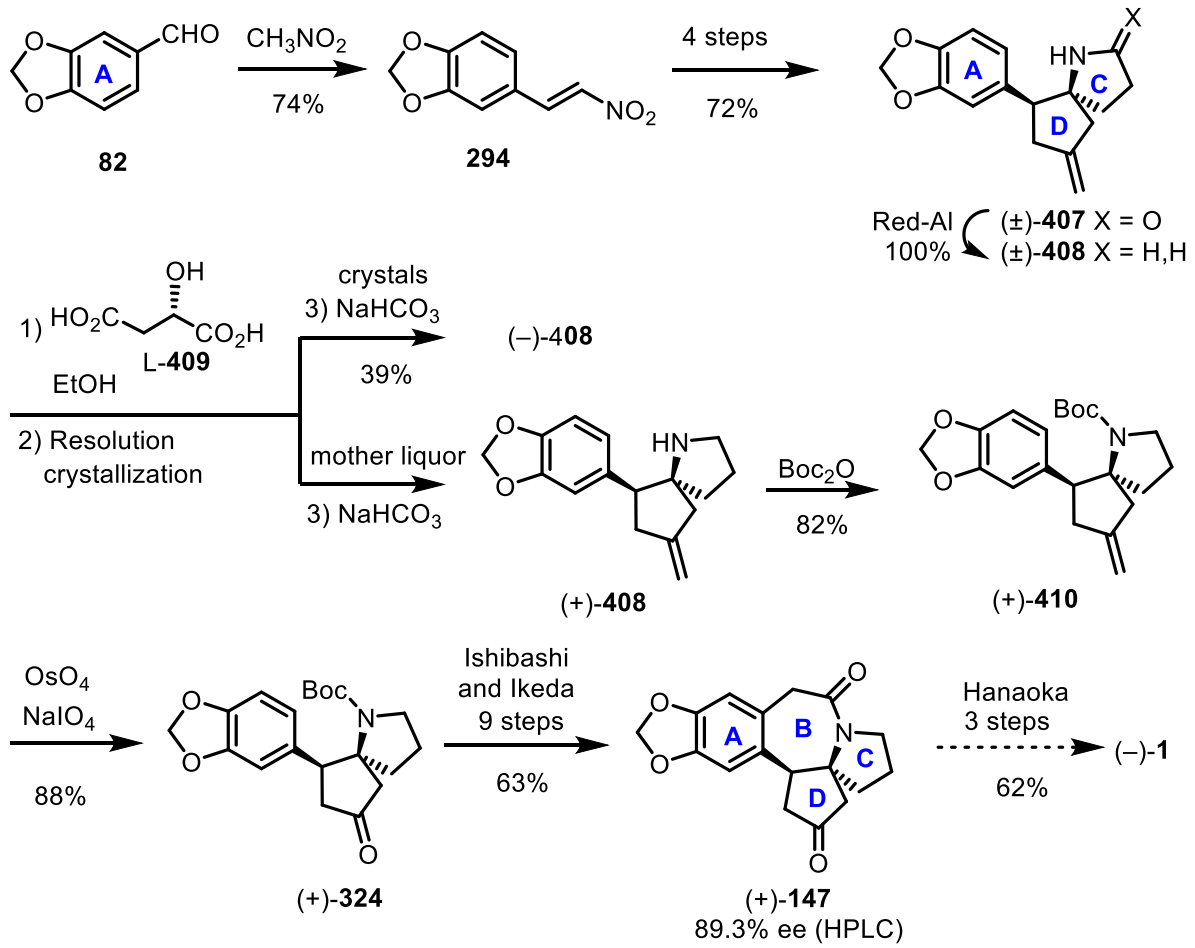


Scheme 38. Formal asymmetric synthesis of CET (–)-(**1**) by Nagasaka (1997)

Acetalization of (±)-**403** with (*R*,*R*)-2,3-butanediol **404** afforded two diastereoisomers (–)-**405** and (+)-**406** which were easily separated by HPLC (100%). 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 ee could be achieved employing Kuehne's procedure[94] in sixteen steps from cyclopentanone (**397**) and 5% 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).

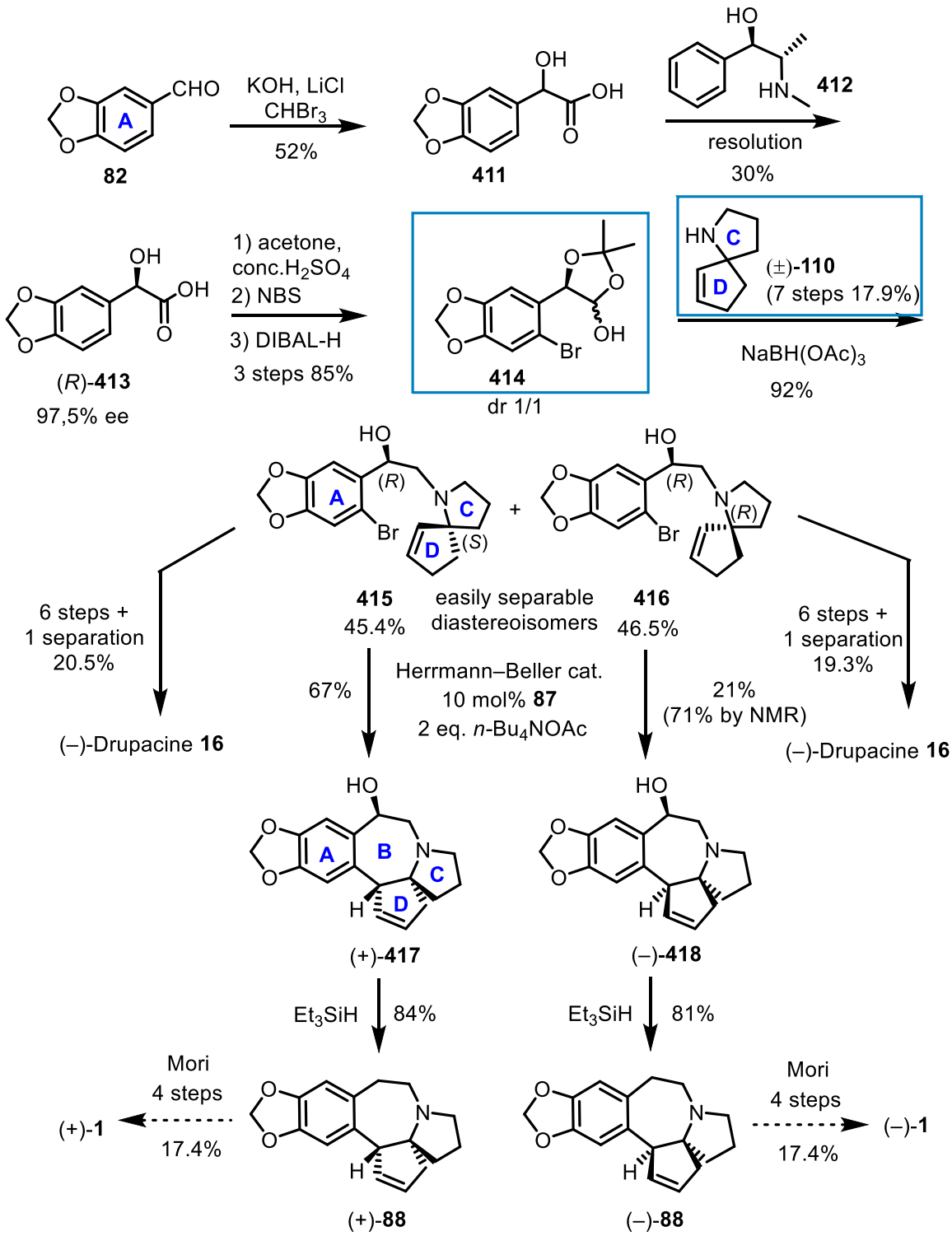


Scheme 39. Formal asymmetric synthesis of CET (–)-(**1**) by El Bialy (2002)

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**, then transformed according to their sequence[137,138] into intermediate (+)-**149** in 89.3% ee measured by HPLC. Applying Hanaoka's protocol would allow the synthesis of CET (–)-(**1**) in twenty steps 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 non racemic hemiacetal **414** obtained by chemical resolution (Scheme 40).[90]



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

5 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**. 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 sixteen 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 acetalisation (Schemes 5 and 40). Starting from *β*-ketoester **105**, the sequence consited in sixteen steps via spirocyclic enamine (±)-**110**, and delivered natural drupacine (–)-**16** in a combined 1.34% OY. It is worthnoting that there remains only one total synthesis of racemic drupacine (±)-(**16**) disclosed by Fuchs in 1990.[147]

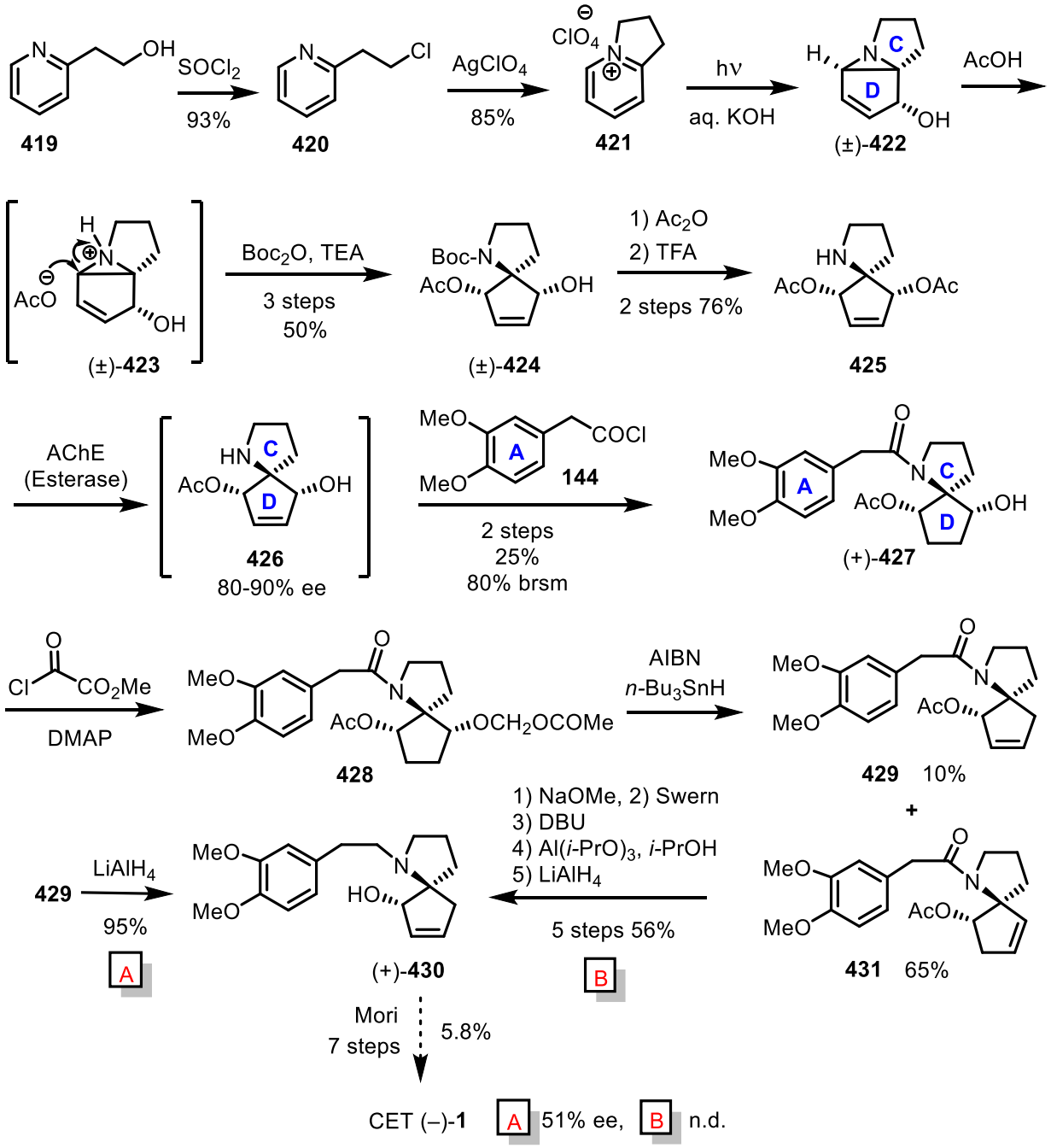
3.3.5 Enzymatic resolution

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 cyclisation.[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 5 steps and 38% OY. Desymmetrisation by enzymatic hydrolysis with electric eel acetyl cholinesterase (EEACE) afforded the unstable aminomonoalcohol **426** in the range of 80-90% ee which reacted with (3,4-dimethoxyphenyl)acetyl chloride (**144**) to furnish amido alcohol **427**. To remove hydroxy group at C1, compound **427** was transformed into oxalate **428** which underwent radical reduction by treatment with 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, then ketone and amide reduction. This formal synthesis could allow the synthesis of CET (–)-(**1**) in nineteen/twenty-three steps from **419** via ACD intermediate **430** with 0.1-0.5% expected OY

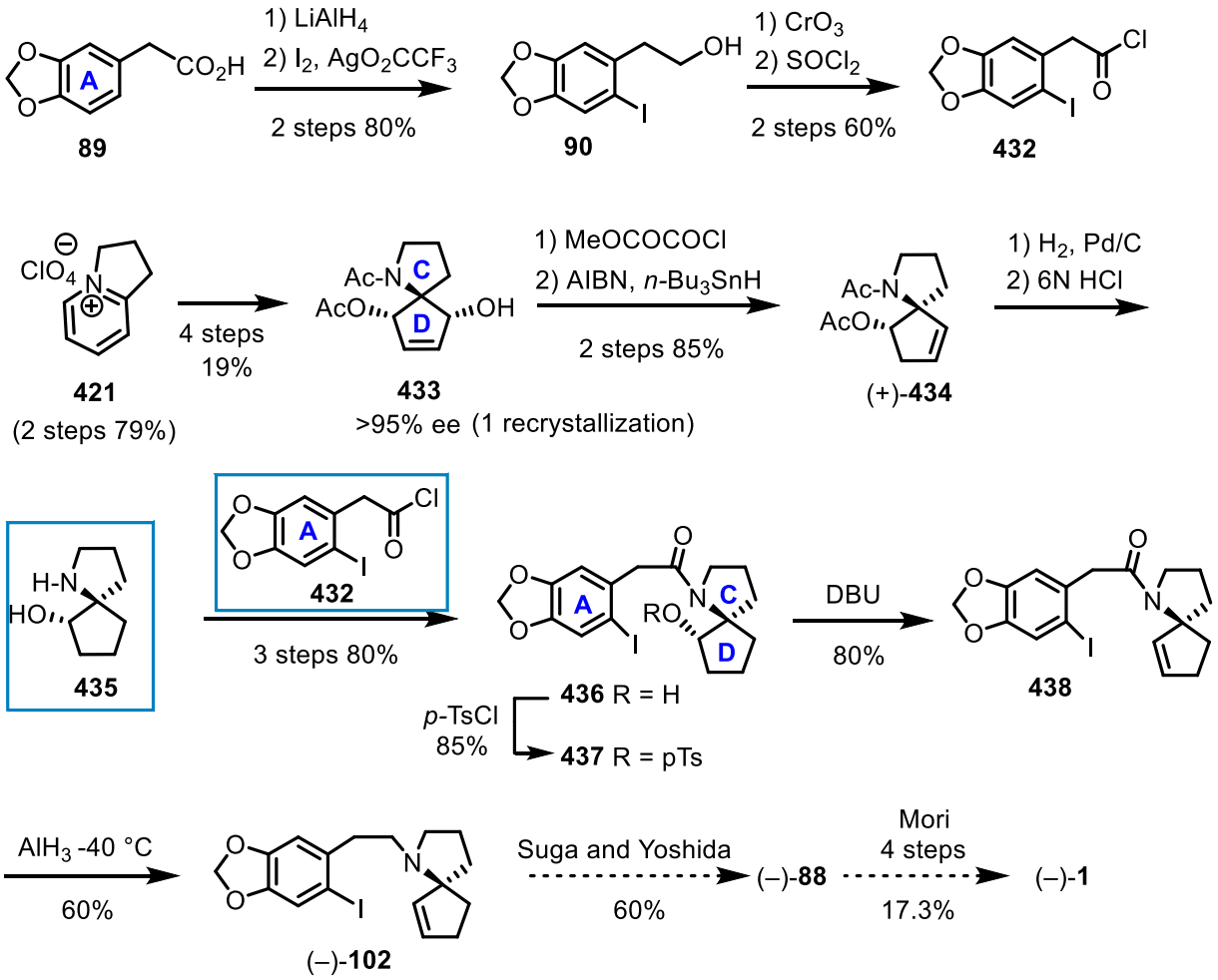
(brsm) and 51% estimated ee. This medium ee indicates that some epimerization occurred during transformation of spirocyclic compound **426** into ACD unit **430**.



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

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



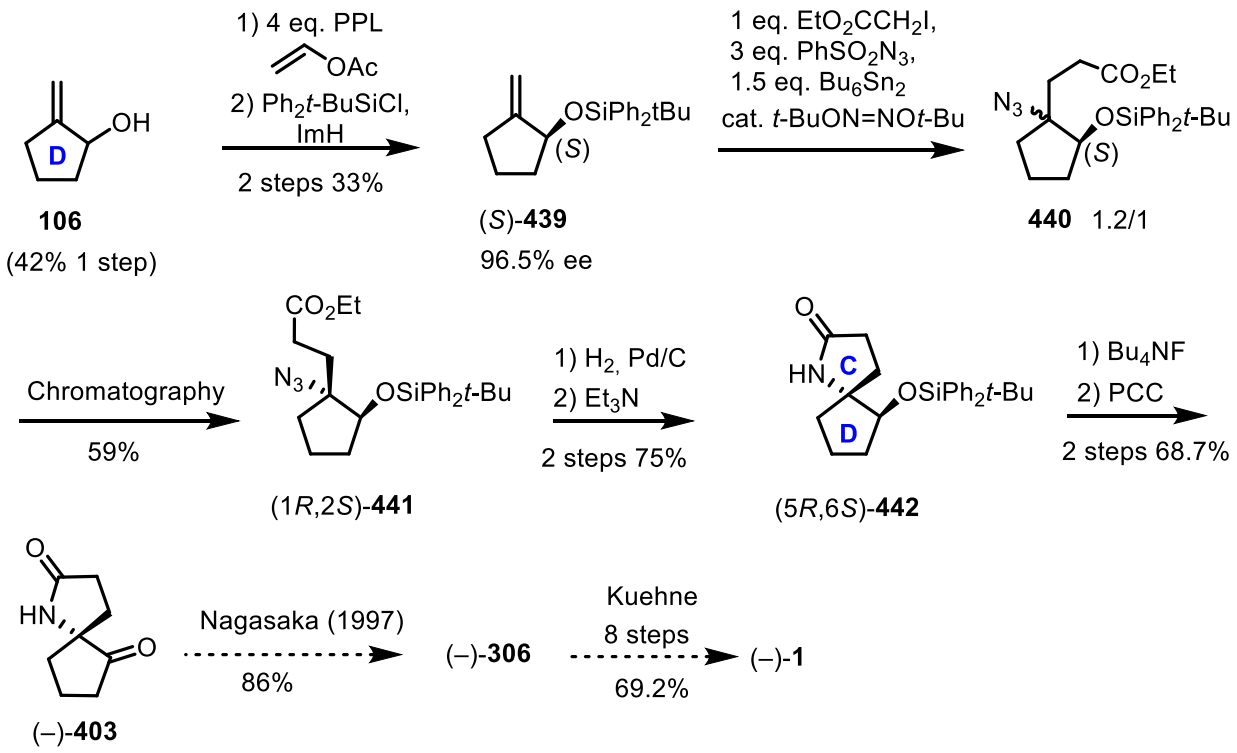
Scheme 42. Formal asymmetric synthesis of CET (–)-(**1**) by Mariano via Suga and Yoshida's intermediate (–)-**102** (2006)

Hydrogenation with 10% Pd/C followed by hydrolysis with HCl of **434** generated the spirocyclic alkaloamine **435**. Reaction with iodoarylacetyl chloride **432** furnished the spirocyclic amidoalcohol **436** which was transformed into the tosylate **437**. Tosylate elimination with DBU afforded the unsaturated amide **438** which was reducted with aluminium trihydride to give the Suga and Yoshida intermediate (–)-**102**. This formal synthesis would give CET (–)-(**1**) in 95% expected ee over nineteen steps in 0.55% 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 part 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).



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

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, 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 eighteen steps, 1.8% calculated OY (alternatively nineteen 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 (Figure 18) or optically active one (Figure 19). In addition, this schematic view shows in some ways the interconnections between the different approaches.

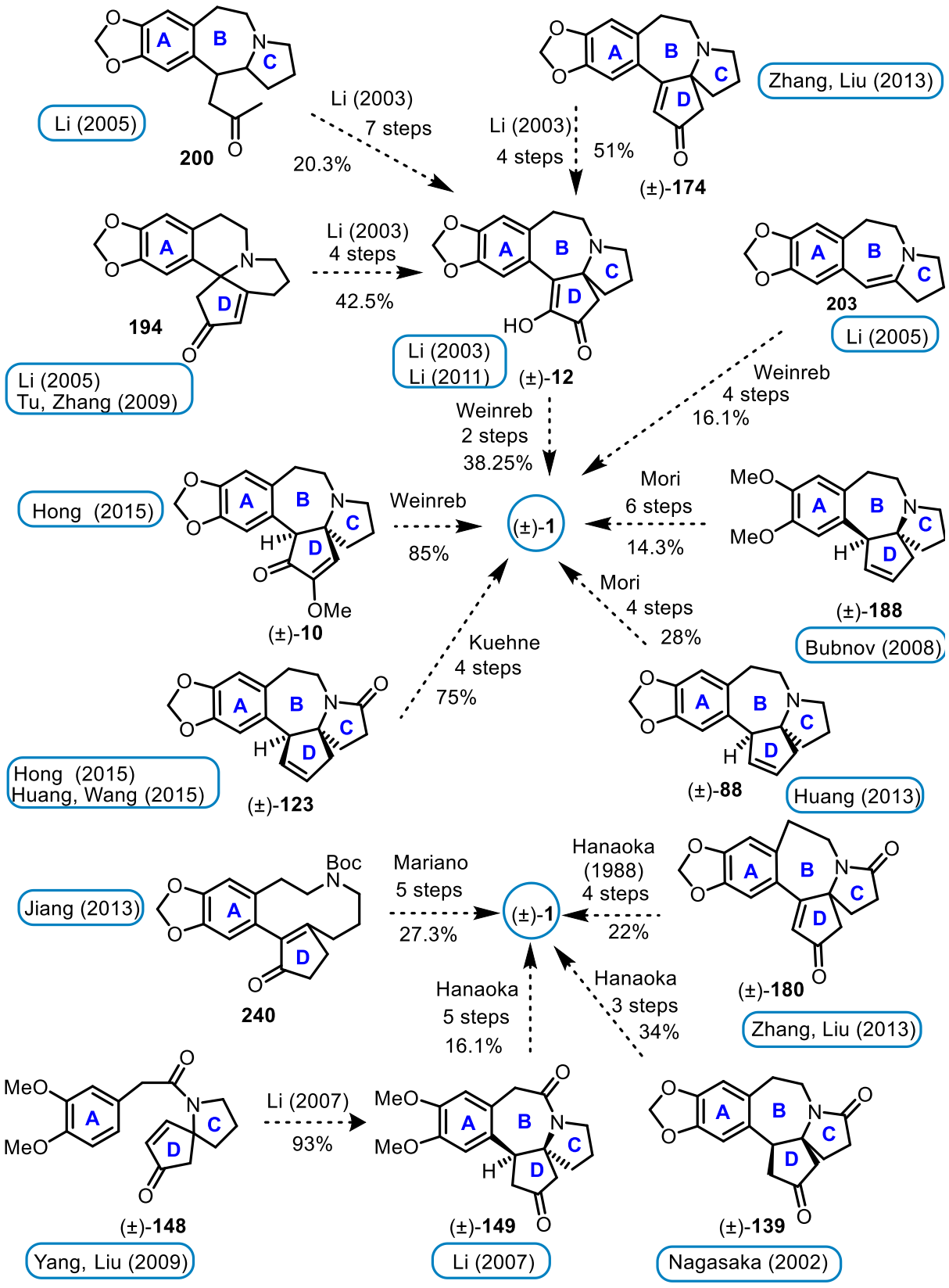


Figure 18 Target intermediates in the formal syntheses of (±)-CET (**1**). Names in boxes refer to a formal synthesis.

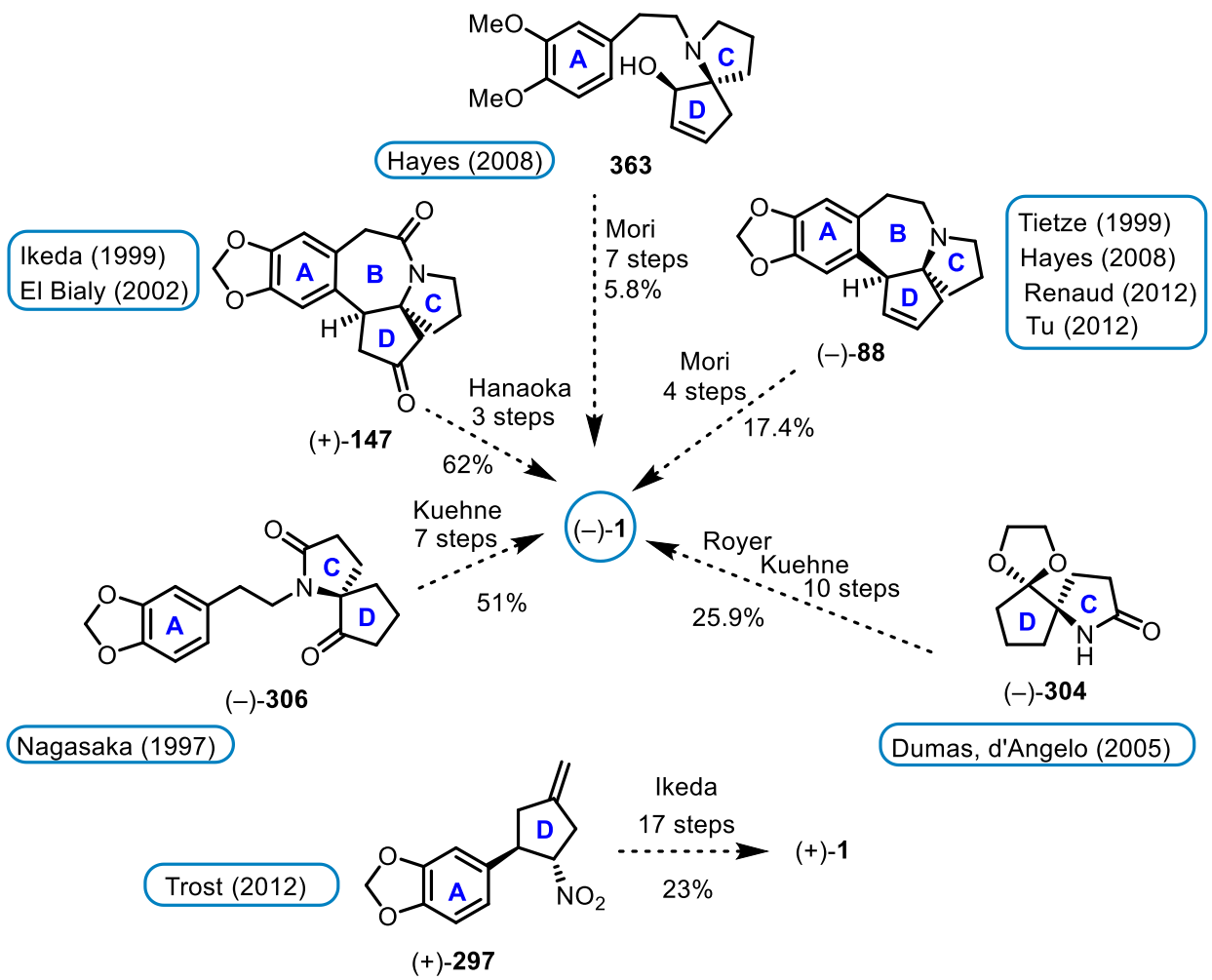


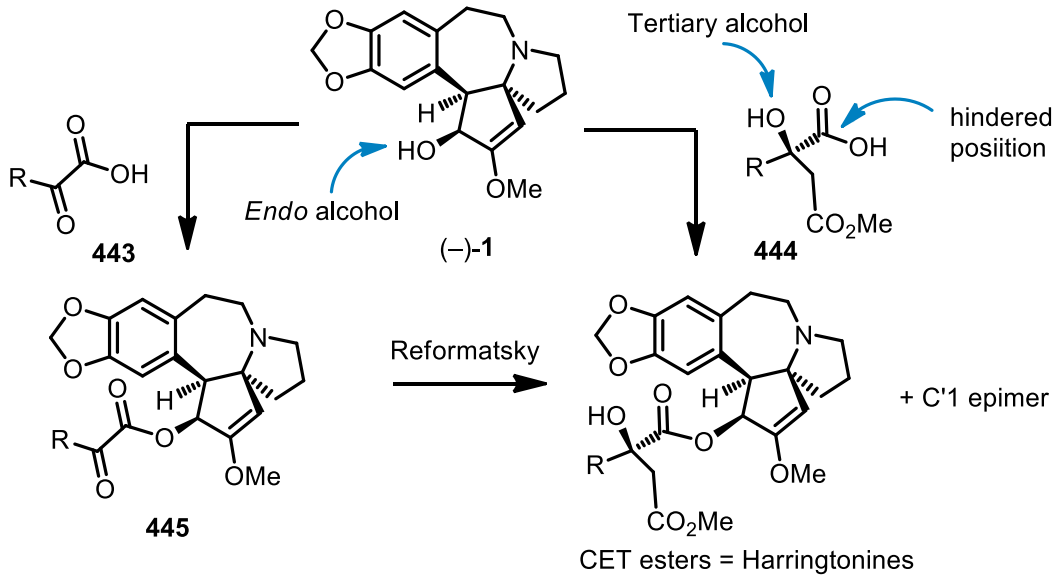
Figure **19** Target intermediates in the formal asymmetric syntheses of CET (**1**). Names in boxes refer to a formal synthesis.

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 cephalotaxine 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 cephalotaxine remains an important challenge due to the steric strain exhibited at both the alcoholic function of cephalotaxine 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 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 cephalotaxine 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 fifteen syntheses of cephalotaxine's esters. Due to the impossible coupling with the entire side chain **444**, most of these syntheses dealed with the esterification of CET (**1**) with a precursor α-keto acid **443** as shown in scheme 44. Formation of the α-keto ester **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 harringtonines such as HT (**3**), HHT (**2**), deoxyHT (**5**) and so on, the sequence exhibits also a major drawback: the Reformatsky reaction on the prochiral keto ester 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 cephalotaxine. 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).



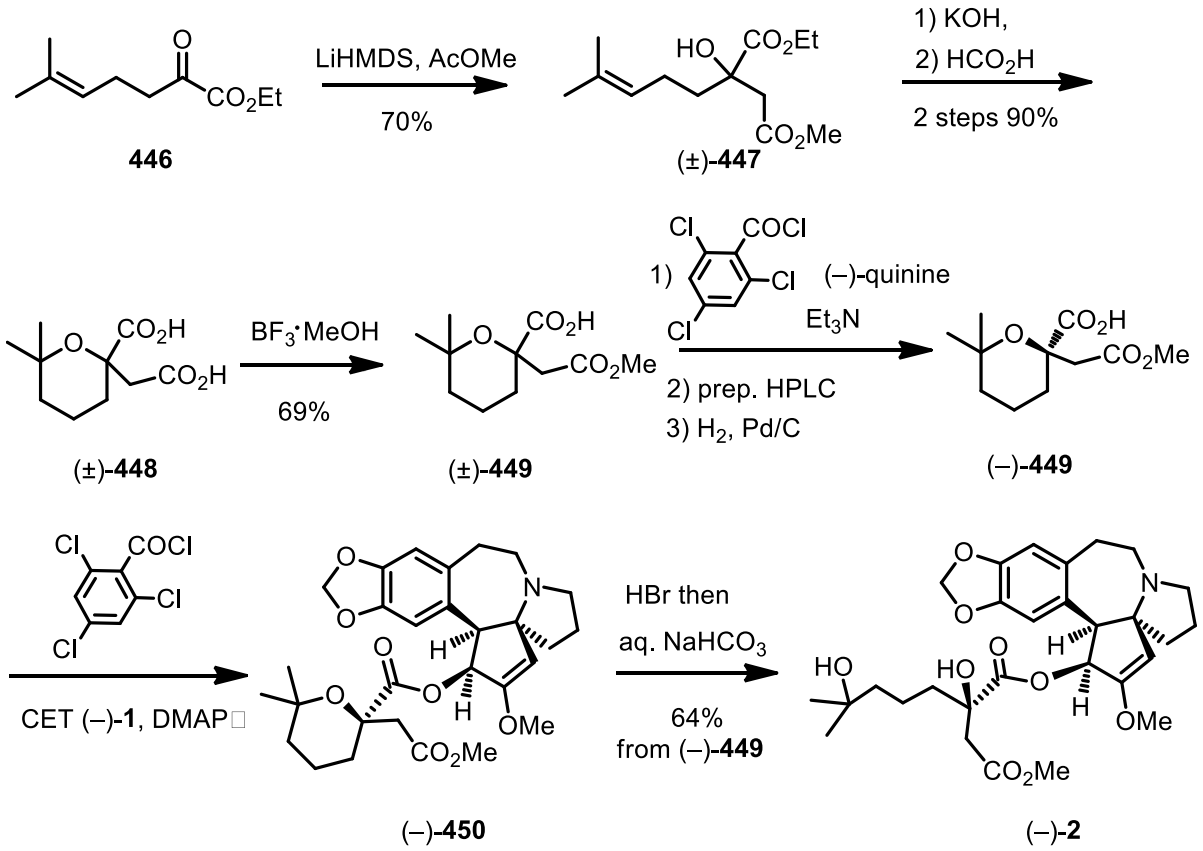
Scheme 44 Coupling of the side chain onto CET (–)-(**1**)

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 HHT (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 4 steps from α-ketoester **446**.



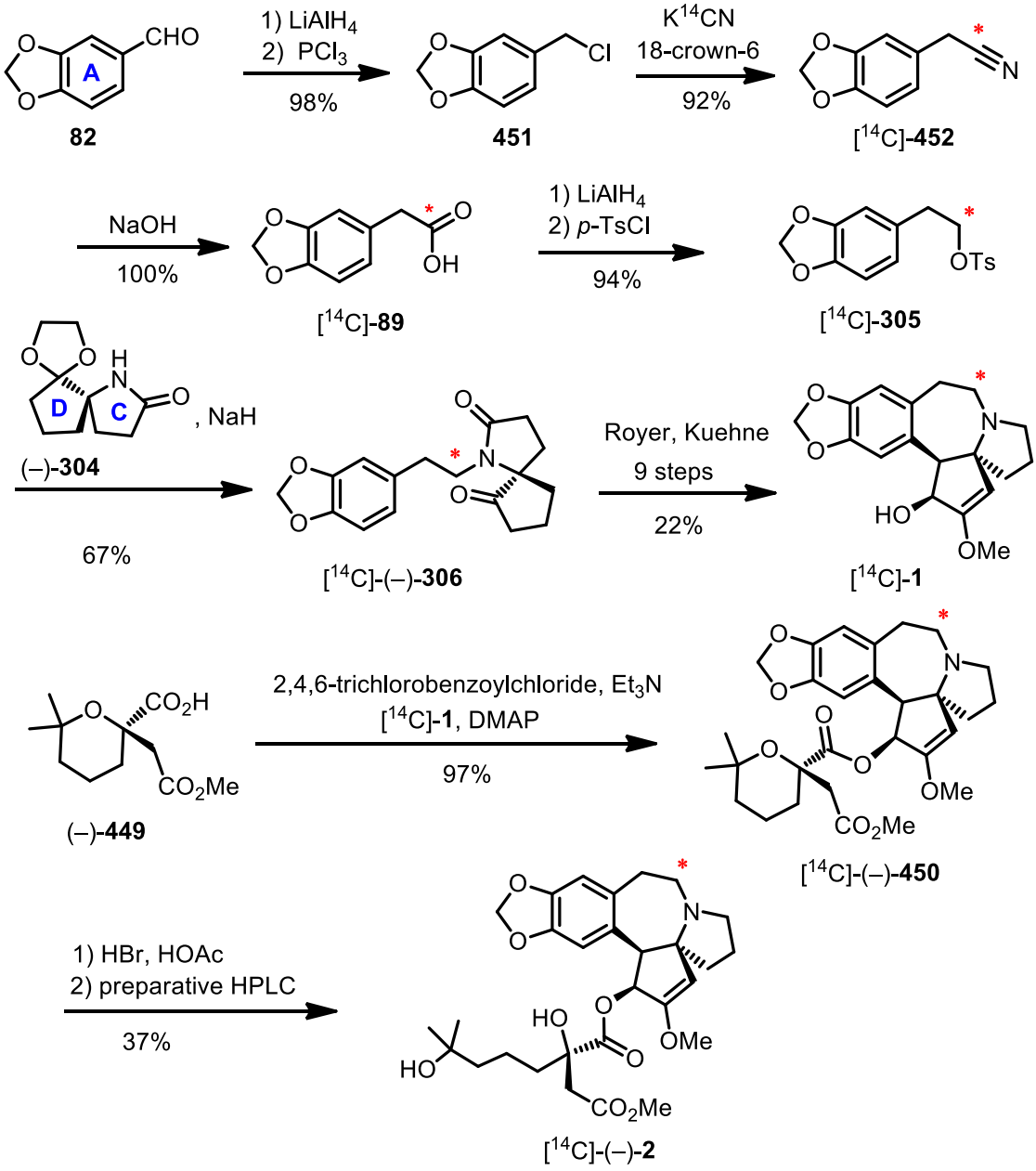
Scheme 45 Hemisynthesis of ssHHT (**2**) by Robin (1999)

Keto ester **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•MeOH to furnish the acid ester **449**. CET (**1**) was coupled with *rac*-**449** using DCC or mixed anhydride method, but once again the 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]-Labelled HHT (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-labelled compound was used in a human mass balance study that was a post-approval commitment to the U.S. FDA.



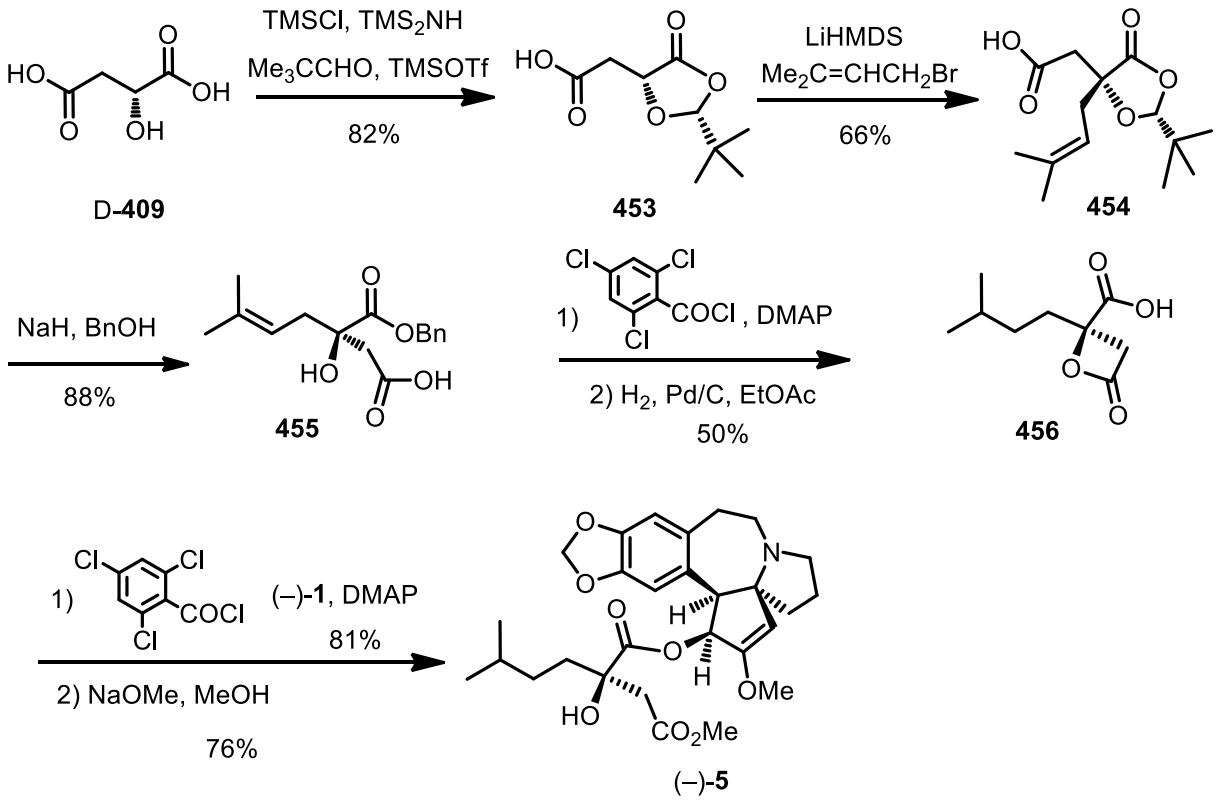
Scheme 46 Synthesis of [^{14}C]-labelled HHT (–)-(2) by Marguerit (2015)

[^{14}C]-Labelled HHT ([^{14}C]-(–)-**2**) was obtained from [^{14}C]-labelled CET ([^{14}C]-(–)-(**1**)) according to an efficient semi-synthesis 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]-labelled tosylate 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** ~~s~~ynthesized 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 seventeen 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 DeoxyHT (2006)

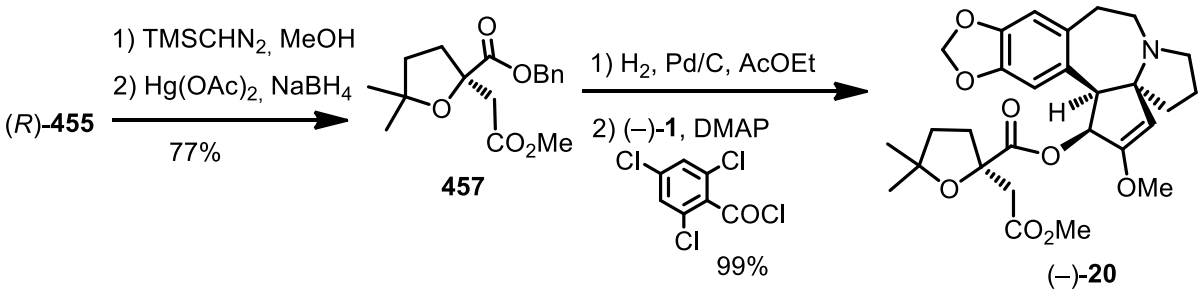
The synthesis of deoxyHT (–)-(**5**) reported by Gin in 2006[153] was the most recent and also the most efficient method of synthesis of CET esters. This synthesis was based on the esterification of a cyclic acid, namely the *β*-lactone **456** obtained in a non-racemic form through an efficient sequence (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**) upon methanolysis.



Scheme 47 Synthesis of deoxyHT (–)-(**5**) by Gin (2006)

4.1.4. Djaballah and Gin's Synthesis of AnhydroHT, HHT and HomodeoxyHT (2008)

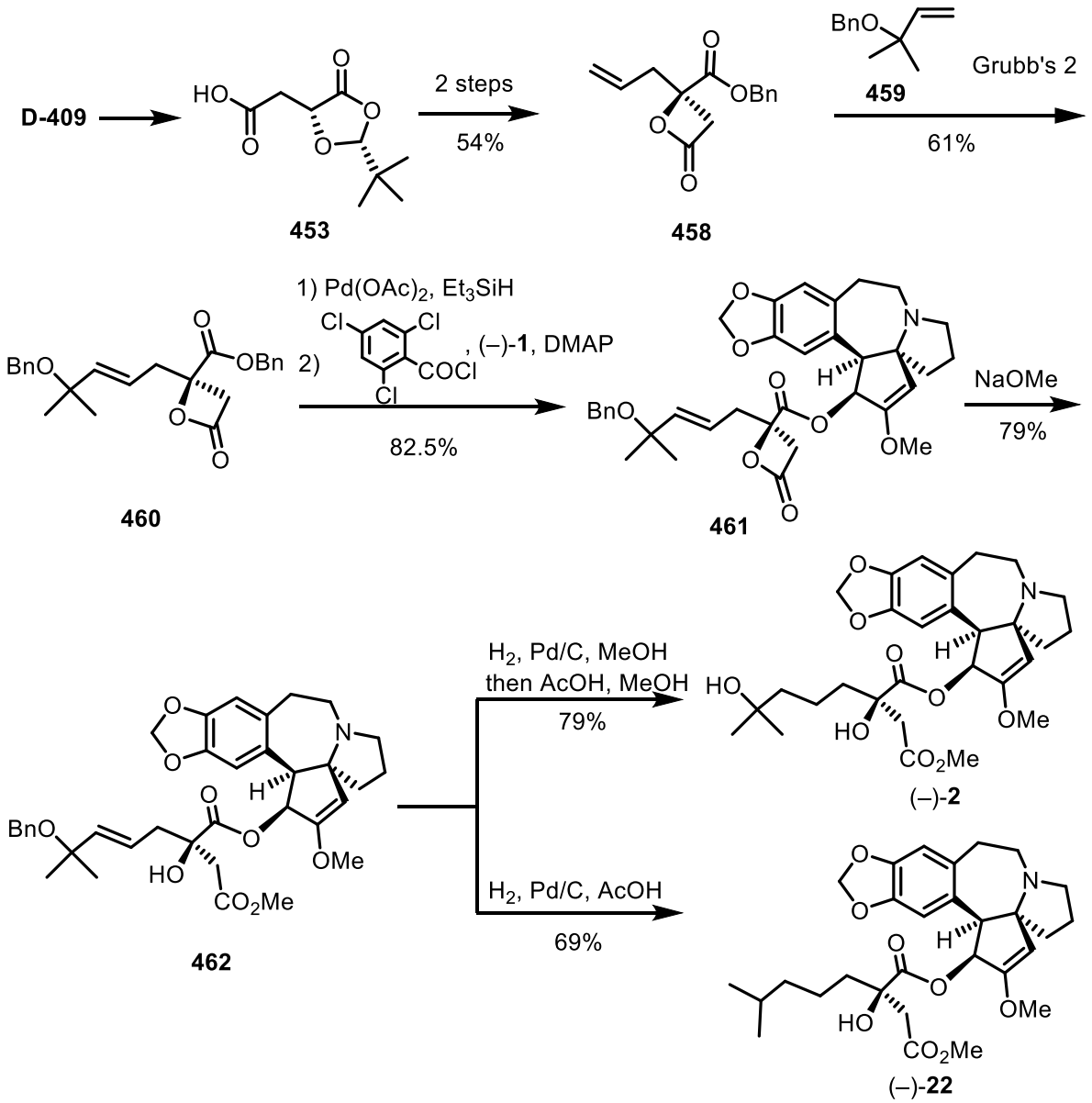
Djaballah, Gin and coworkers extended this study to the syntheses of others CET esters like HHT (–)-(**2**), homodeoxyHT (–)-(**22**) and anhydroHT (–)-(**20**).[144] Preparation of anhydroHT (–)-(**20**) (Scheme 48) involved the intermediate (*R*)-**455** of the previous synthesis[153] (see Scheme 47) 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.



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

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** upon 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.



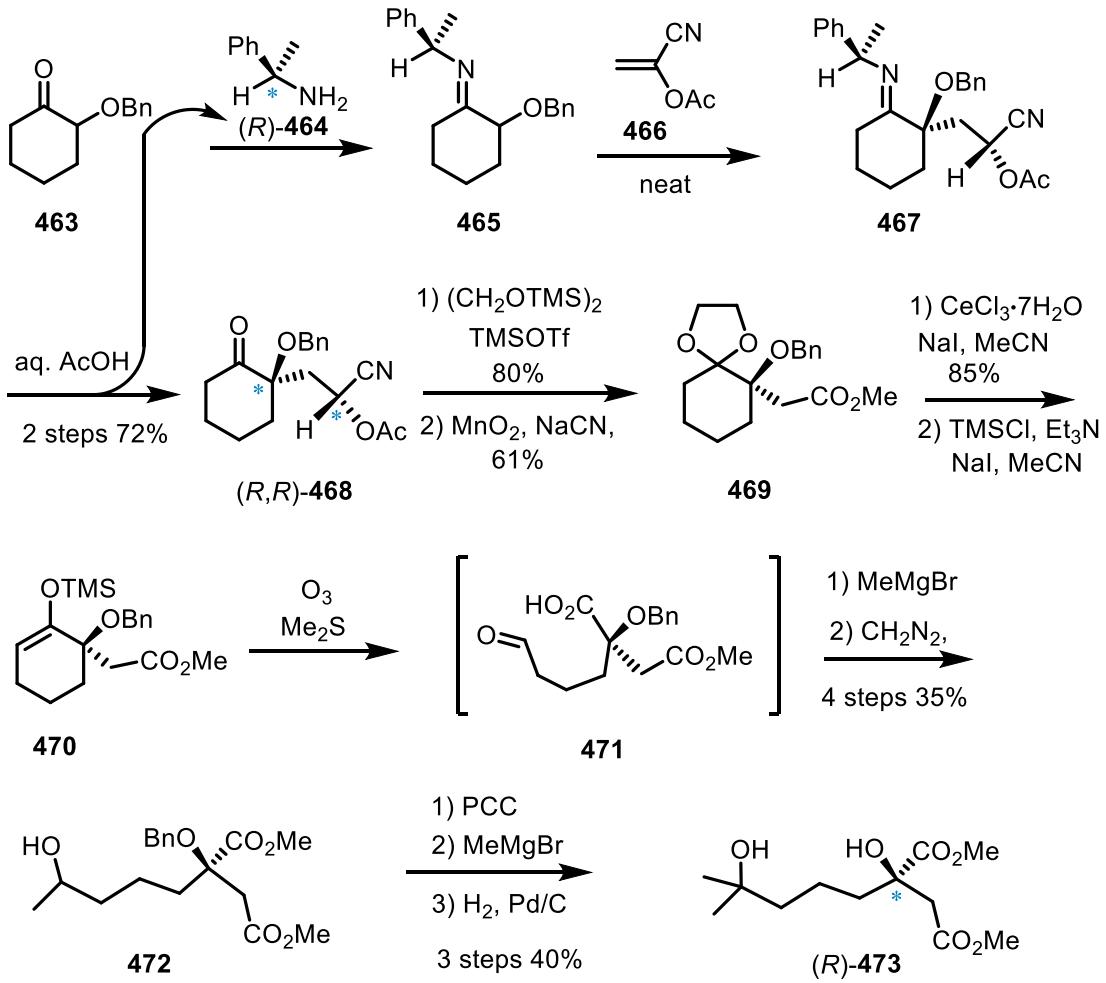
Scheme 49 Synthesis of HHT (–)-(**2**) and homodeoxyHT (–)-(**22**) by Djaballah and Gin (2008)

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 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)

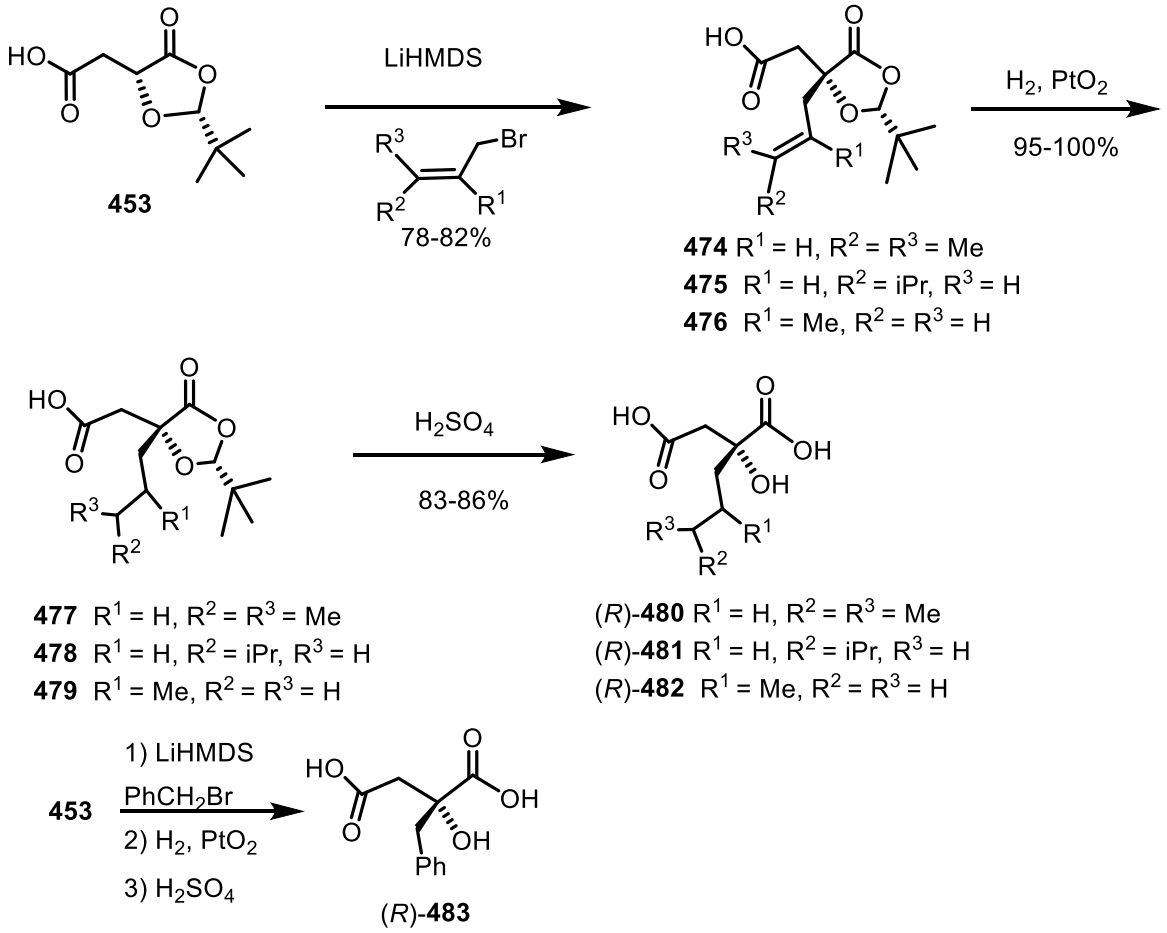
The first asymmetric synthesis of the side chain ester **473** of HHT (**2**) was reported by Dumas and d'Angelo.[155,156] In this work, the formation of the chiral tertiary alcohol center was accomplished in a highly diastereoselective manner in an early stage of the sequence. The synthesis started with the preparation of the known chiral imine **465** (Scheme 50) which was subjected to a Michael reaction with acetoxyacrylonitrile (**466**), an equivalent 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** upon treatment with MnO_2 in MeOH in the presence of NaCN. The ketal of ester **469** was cleaved using $CeCl_3 \bullet 7H_2O$ 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 upon hydrogenolysis led to (*R*)-methyl 3,7-dihydroxy-3-methylcarbonyl-7-methoxyoctanoate (**473**), the methyl derivative of HHT side chain.



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

4.2.2 Tietze's Synthesis (2005)

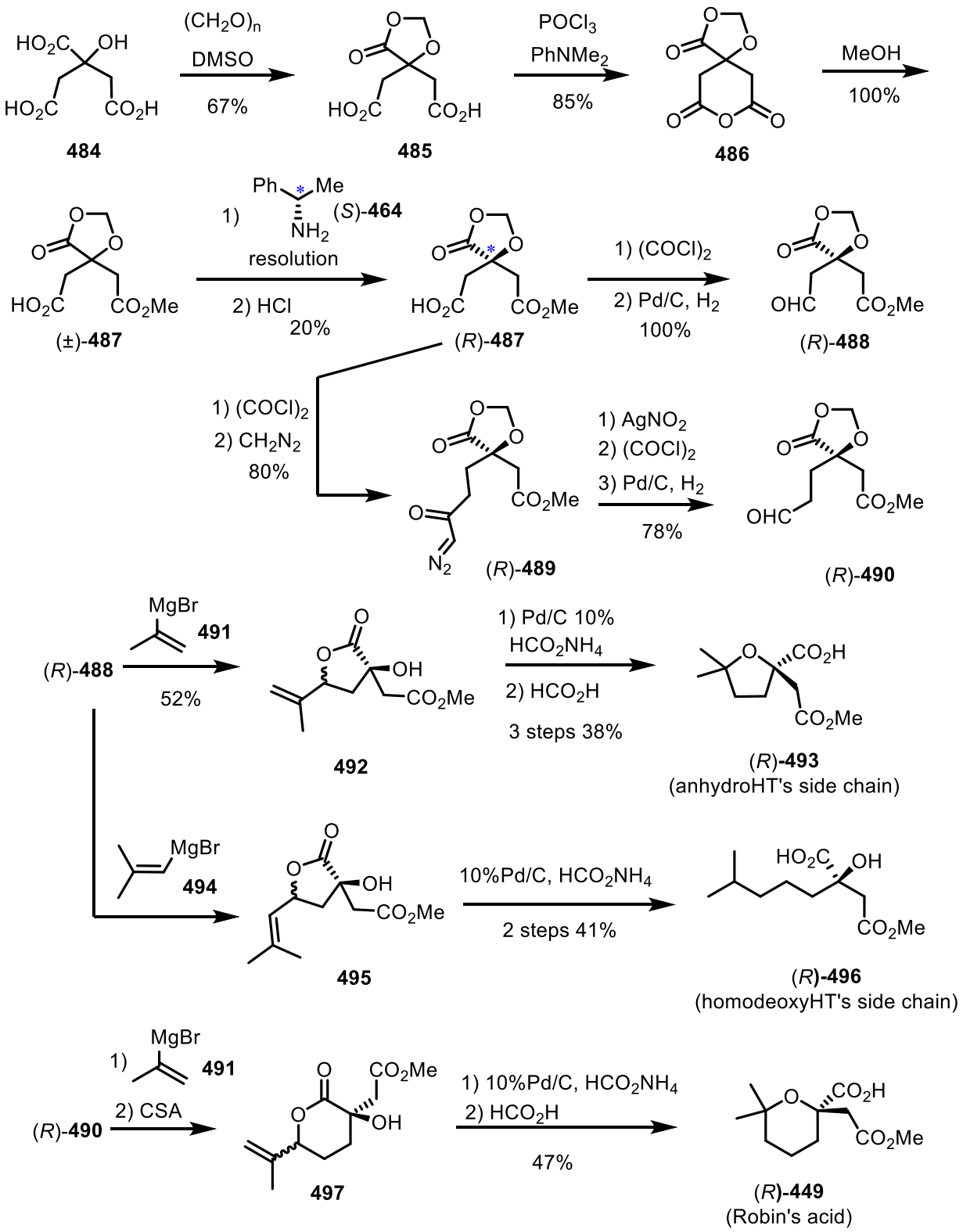
In 2005, Tietze reported the preparation of the chiral side chain acids **480**–**483** of deoxyHT (**5**), homodeoxyHT (**22**), nordeoxyHT (**21**) and neoHT (**19**), respectively (Scheme 51)[154] based on Seebach's procedure for the alkylation of D-malic acid with self-regeneration of stereogenic 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 2 equiv. of LiHMDS followed by bromoallyl derivatives to give allylated dioxolanones **474**–**476** in high yield and diastereoselectivity. Upon 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**).



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

4.2.3 Russel's synthesis (2006)

An original approach was reported by Russel et al. in 2006.[157] The synthesis started with chiral non-racemic 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]



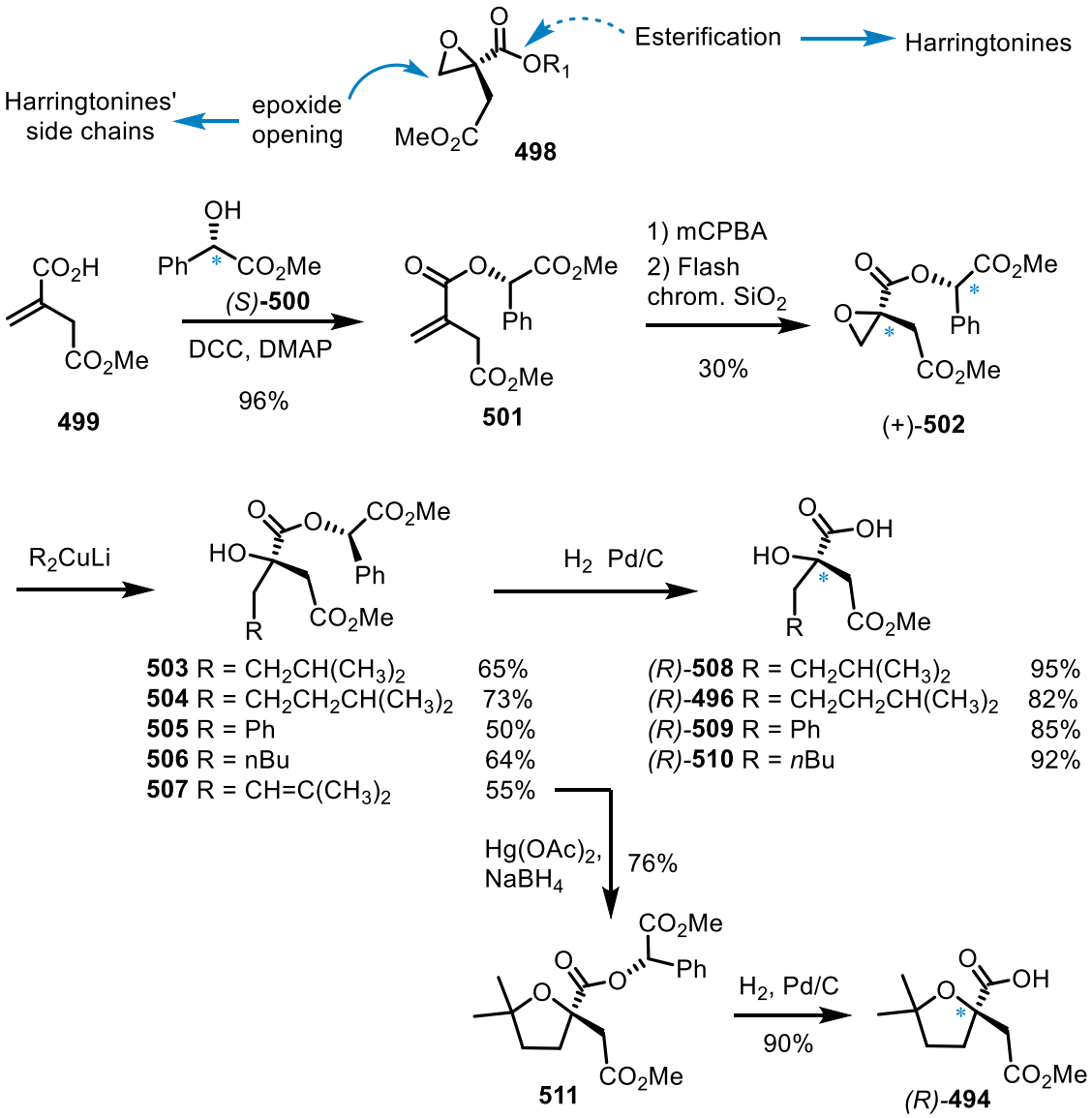
Scheme 52 Synthesis of harringtonines' side chains by Russel (2006)

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 harringtonines, in 3.3% OY and twelve 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 non-racemic epoxide **498** (Scheme 53). Side chains were obtained upon epoxide ring-opening with appropriate nucleophiles. On the other 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 then hydrogenolysis of the chiral appendage delivered (*R*)-**494**, the side chain of anhydroHT (**20**) in 10.8% OY.

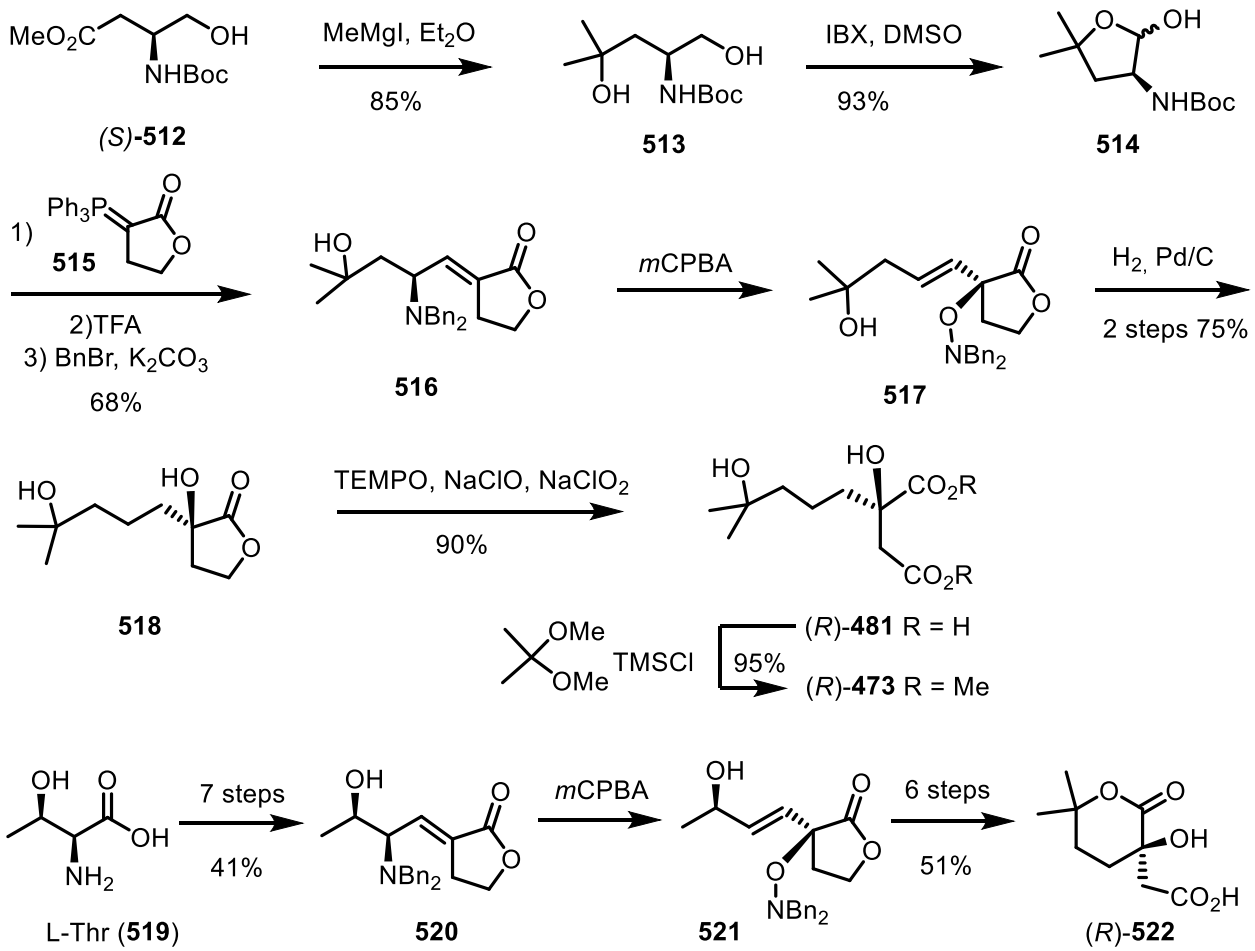


Scheme 53 Synthesis of harringtonines' side chains by Royer (2009)

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 upon treatment with *m*CPBA on the latter to give hydroxylamine **517**. This compound led to **518** upon treatment with H_2 on catalytic Pd/C via double bond saturation then N-O bond cleavage. Eventually, upon 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 upon 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.



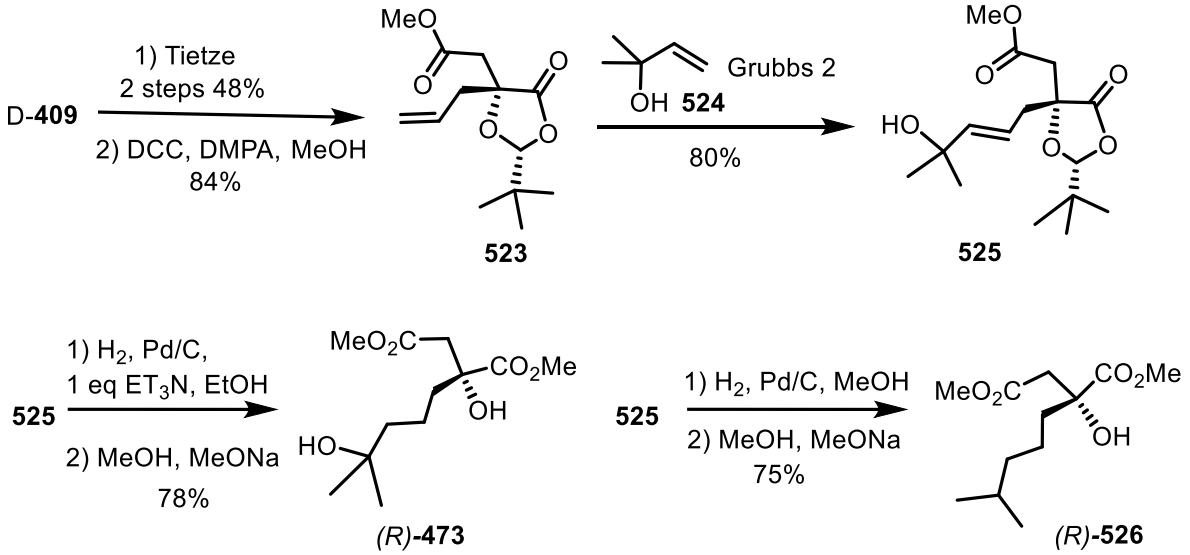
Scheme 54 Synthesis of harringtonines' side chains by Yang (2013)

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

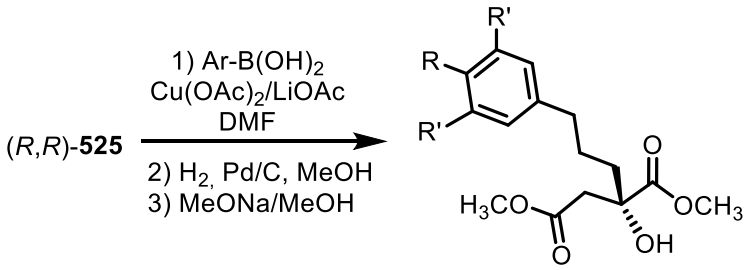
Scheme 49) with allyl alcohol **524** allowed the preparation of dioxolanone **523**.The latter was subjected to different hydrogenation conditions to give after methanolysis (*R*)-**473** or (*R*)-**526**, side chains' esters of HHT (**2**) and homodeoxyHT (**22**).



Scheme 55 Synthesis of HHT and homodeoxyHT side chains by Hung (2014)

4.2.7 Mac's synthesis

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 (*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.



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 HHT side chain's analogs by Mac (2016)

5. Synthetic Analogs and their Biological Properties

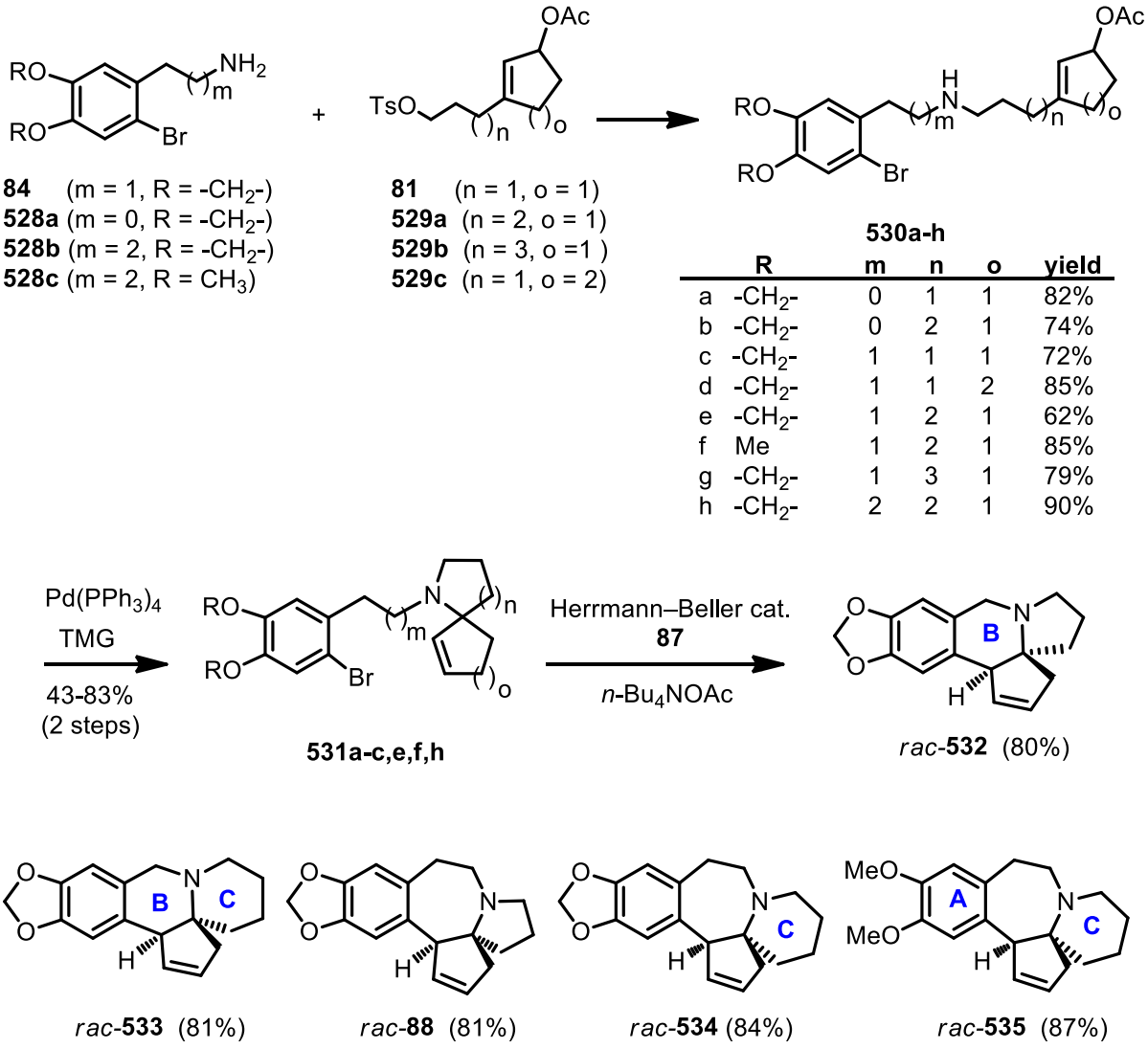
The synthesis of analogs of bioactive natural products remains a broad area of research that can lead to very interesting new structures with improved activity. In the case of *Cephalotaxus*

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 cephalotaxine 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

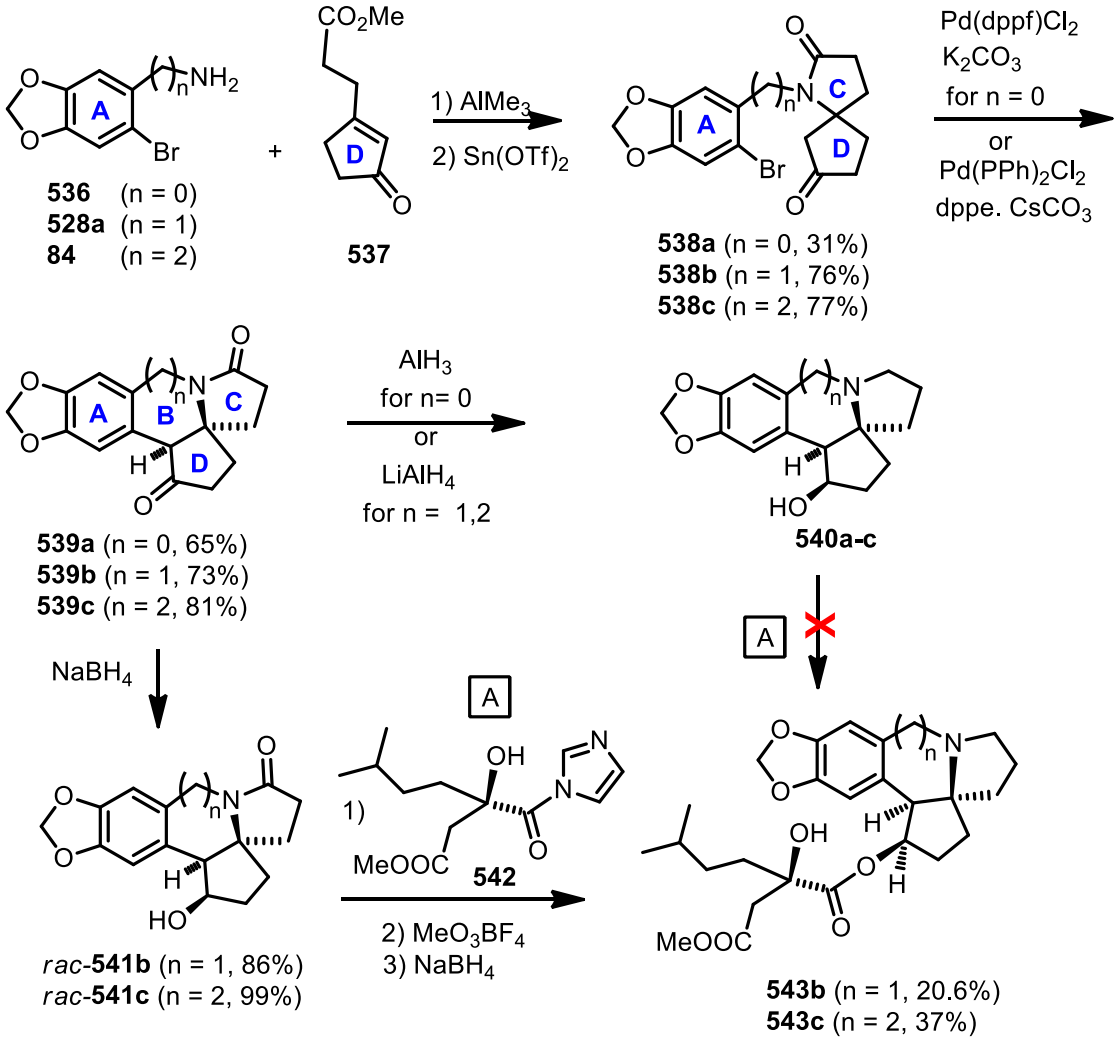
5.1.1. Tietze's CET analogs (2000, 2007)

Since 1997, several reports on the synthesis of CET's analogs have appeared. Tietze *at al*. in 2000[165165] 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 developped for the synthesis of the natural product (Scheme 57).



Scheme 57 Synthesis of CETanalogs by Tietze (2000)

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** 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**.

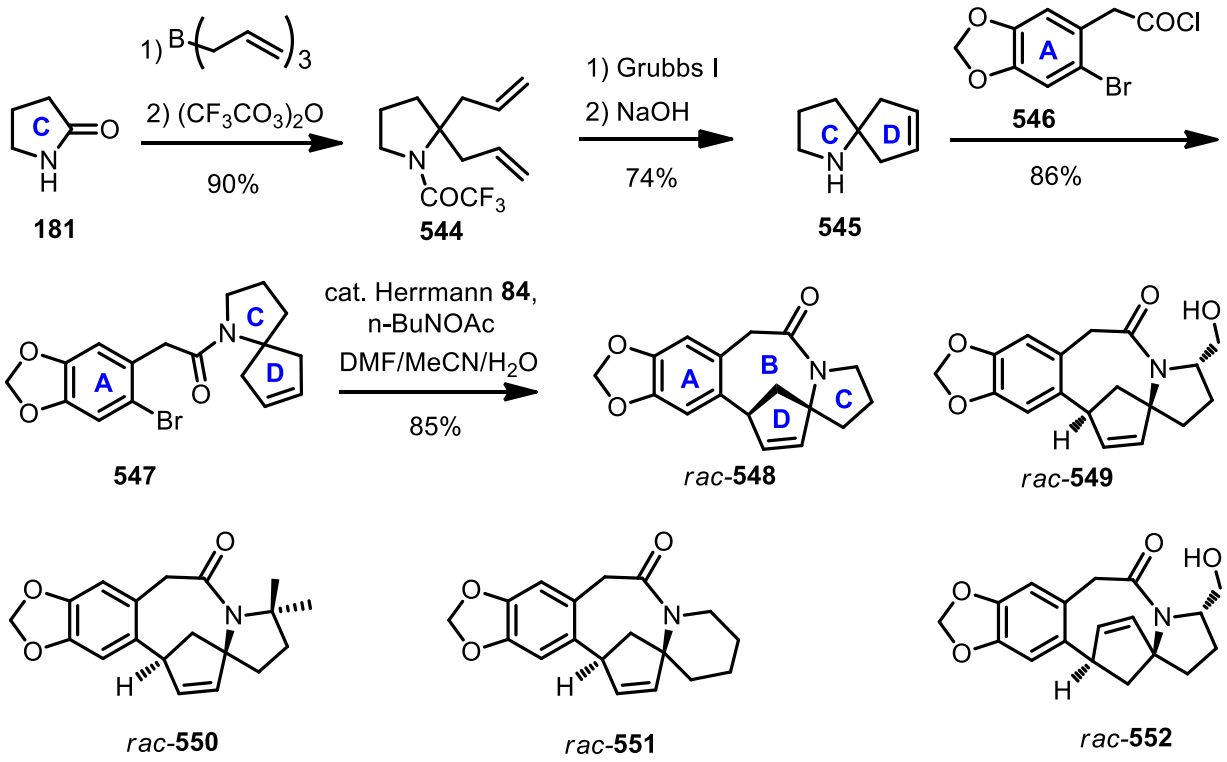


Scheme 58 Synthesis of deoxyHT analogs by Tietze (2007)

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 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 CET analogs (2005-2010)

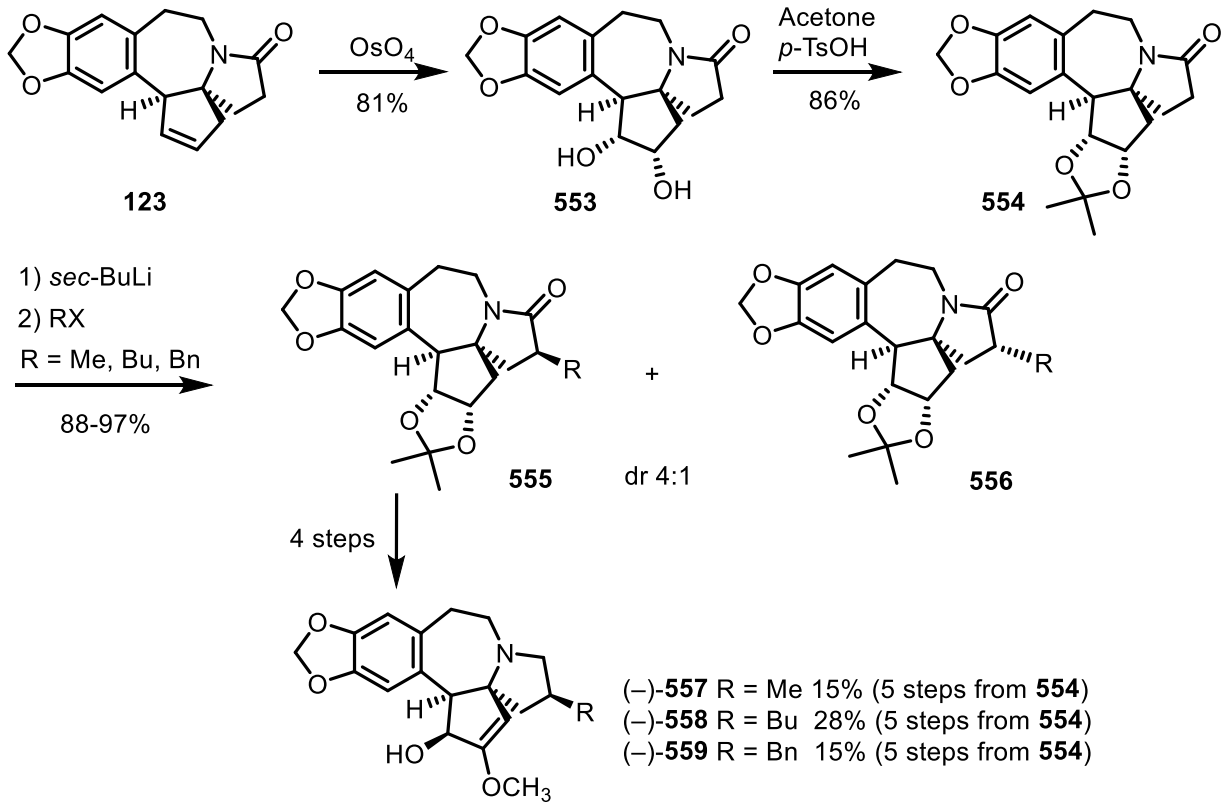
In 2005, Bubnov et al.[167] published the synthesis of compound *rac*-**548** exhibiting some structural analogies to CET (**1**) with ring B and D being bridged and not fused (Scheme 59). The synthetic strategy was simple and started with 2-pyrrolidone (**181**) which was diallylated, ~~N~~ N-protected 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 cyclisation. The same type of work was pursued by the authors in 2010[168] allowing the preparation of new products **549–552**.



Scheme 59 CET's analog synthesized by Bubnov (2005-2010)

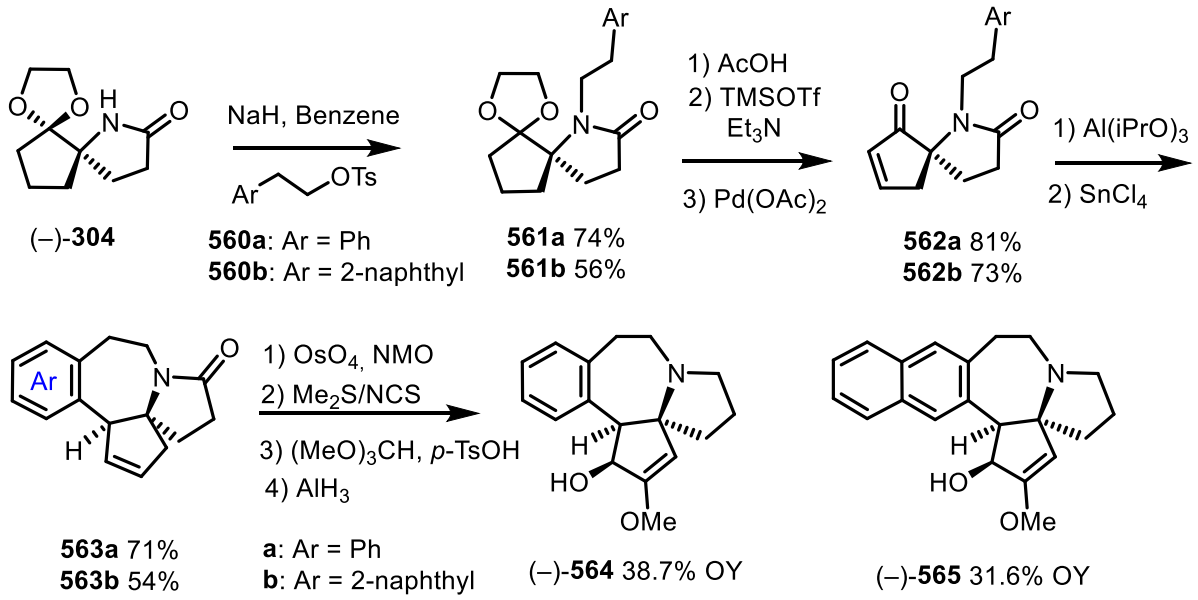
5.1.3. Royer's CET analogs (2004-2010)

Exploiting their early developed strategy, Royer et al. (see Scheme 29) prepared three C7-alkylated CET analogs from chiral non-racemic Kuehne's intermediates **123** and **553** (Scheme 60).[132] Alkylation was carried out on 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 cephalotaxines **557**–**559** in a four-step sequence in 15-28% OY.



Scheme 60 Synthesis of CET alkylated analogs by Royer (2004)

In 2010, these authors reported a different type of cephalotaxine 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]

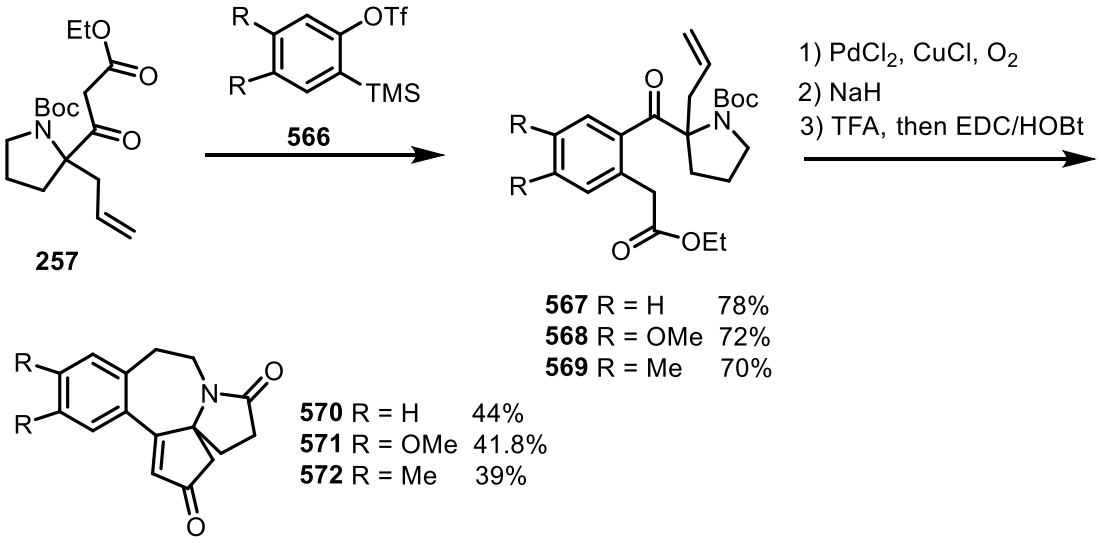


Scheme 61 Synthesis of CET "A" analogs by Royer (2010)

N-alkylation of the spirolactam (–)-**304** with the appropriate ~~a~~rylethyl derivative **560a,b** gave **561a,b**. The end of the synthesis paralleled those of the cephalotaxine 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 CET 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.



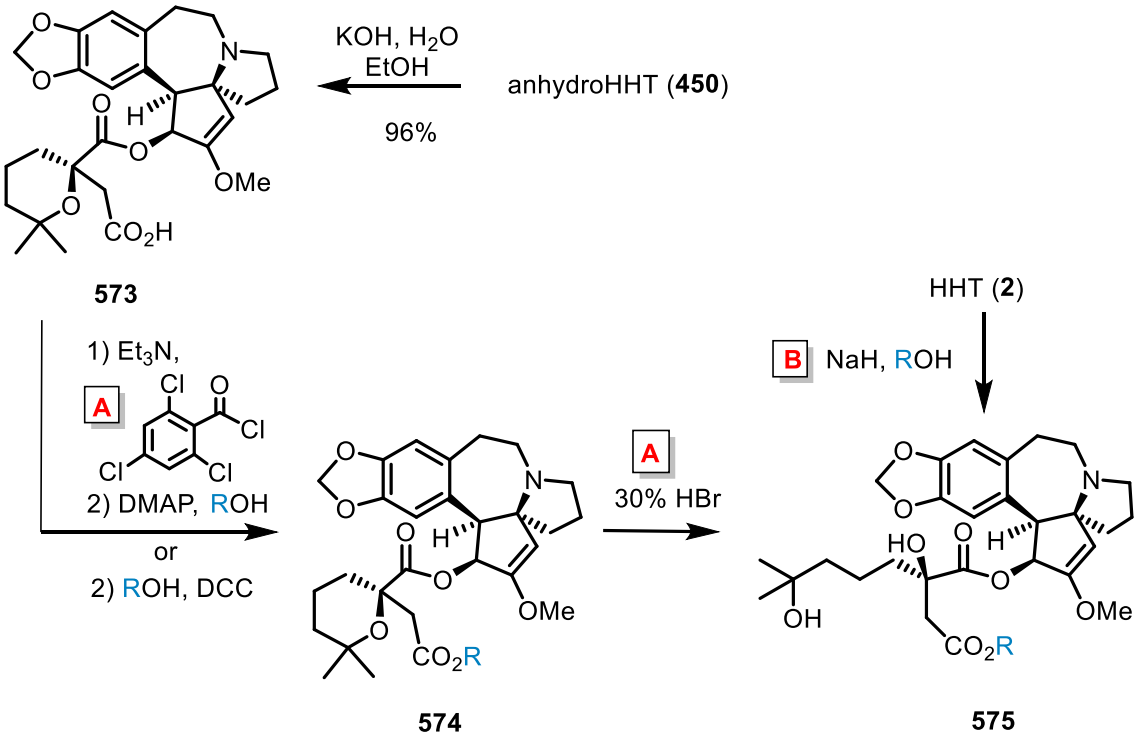
Scheme 62 Formal synthesis of CET "A analogs" by Chandrasekhar (2016)

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.

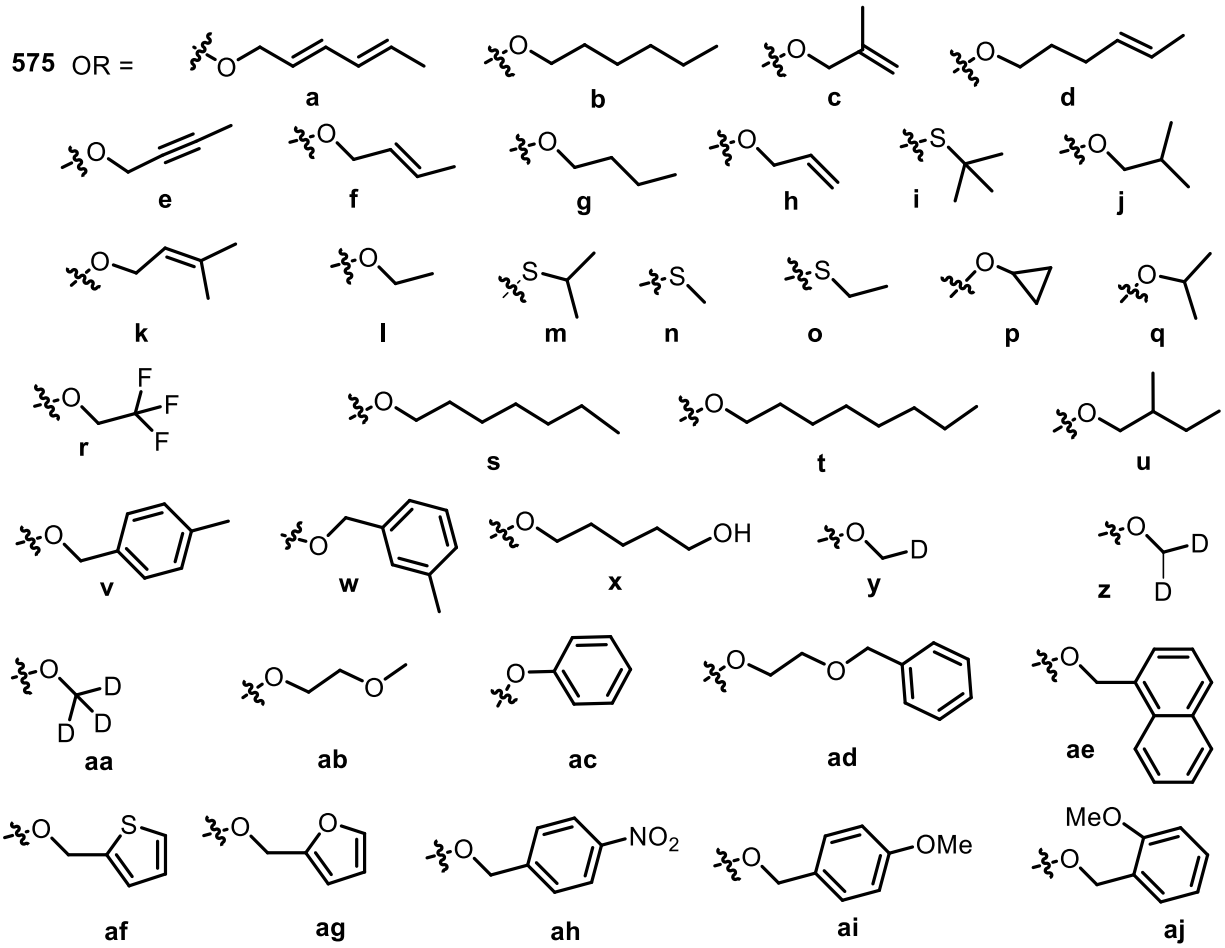
5.2 *Cephalotaxus* esters with side chain analogs

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 to 82%. For example, HHT (**2**) dissolved in (*E*,*E*)-hexa-2,4-dien-1-ol was 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).



Scheme 63 Robin synthesis of HHT analogs at the side chain



Scheme 63 (continued) Robin synthesis of HHT analogs at the side chain

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

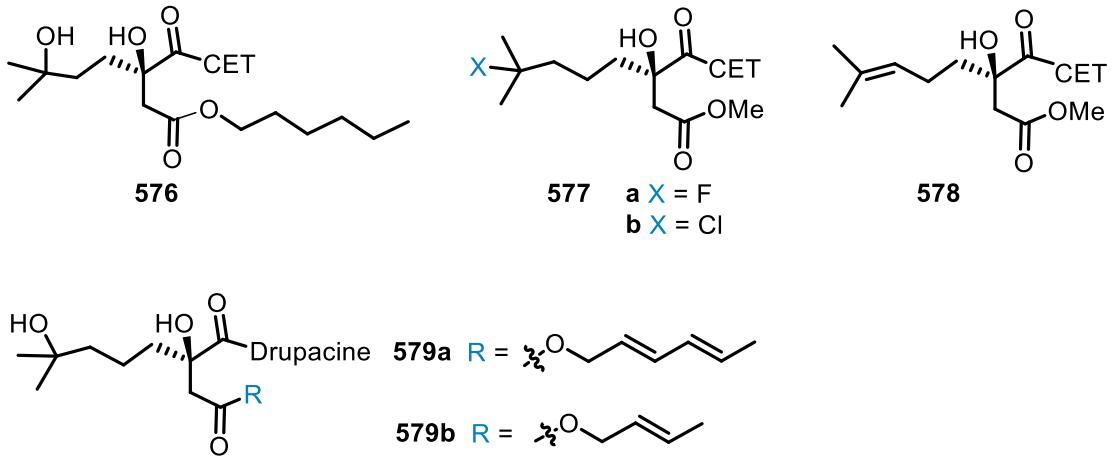


Figure 20 Harringtonines analogs at the side chain (HHT (top) and drupangtonine (bottom) analogs) synthesized by Robin (2002-2004)

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).

Table 11: Amount and yield of Robin's harringtonines analogs (see Scheme 63 and Figure 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	**575z**	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	
575l	A: 125	55	41	**575q**	A:103	50	80
	B: 298	97			B: 143	39	
575ad	100	55	25				

5.2.2 Djaballah & 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** (Figure 21), using the protocol described in section 4.1.3 (Scheme 47).[144] They exemplified up to forty three *Cephalotaxus* esters analogs, some of which being dimers of CET (**1**) linked by a central oxygenated chain by the C3 hydroxyl group.[172]

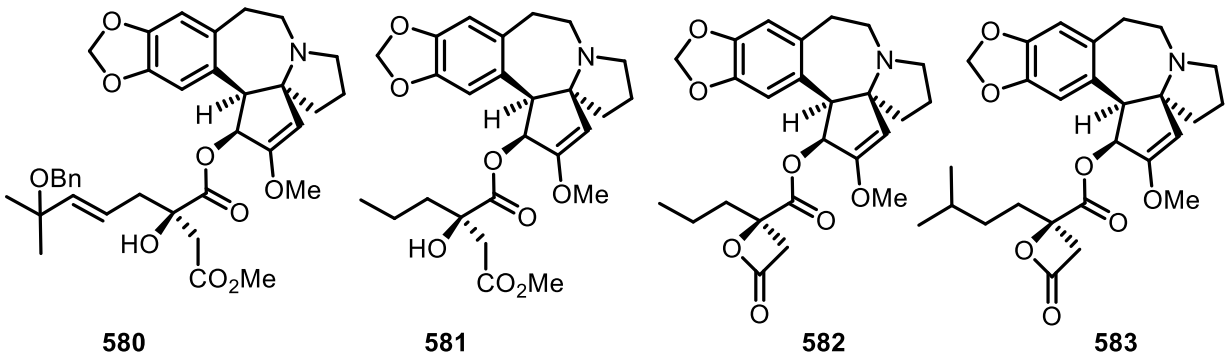


Figure 21 Djaballah and Gin's CET-ester analogs (2009)

These harringtonine 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.

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

Compound	IC_{50} (µg/mL)		Resistance index	ClogP
	HL-60	HL-60/RV$^+$		
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 (**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 MDR and tumor cell lines can therefore be 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.

Table 13. Cytotoxicity of deoxyHT (**5**), anhydroHT (**20**) and CET 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

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] (Figure 20) and the other on acylated HHT derivatives **585** and **586** (Figure 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 to 44% yield (Figure 22). Groups R^1 and R^2 could be H, $C_{1–18}$ alkyl, $C_{2–18}$ alkenyl, $C_{2–18}$ alkynyl, $C_{3–7}$ 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**).

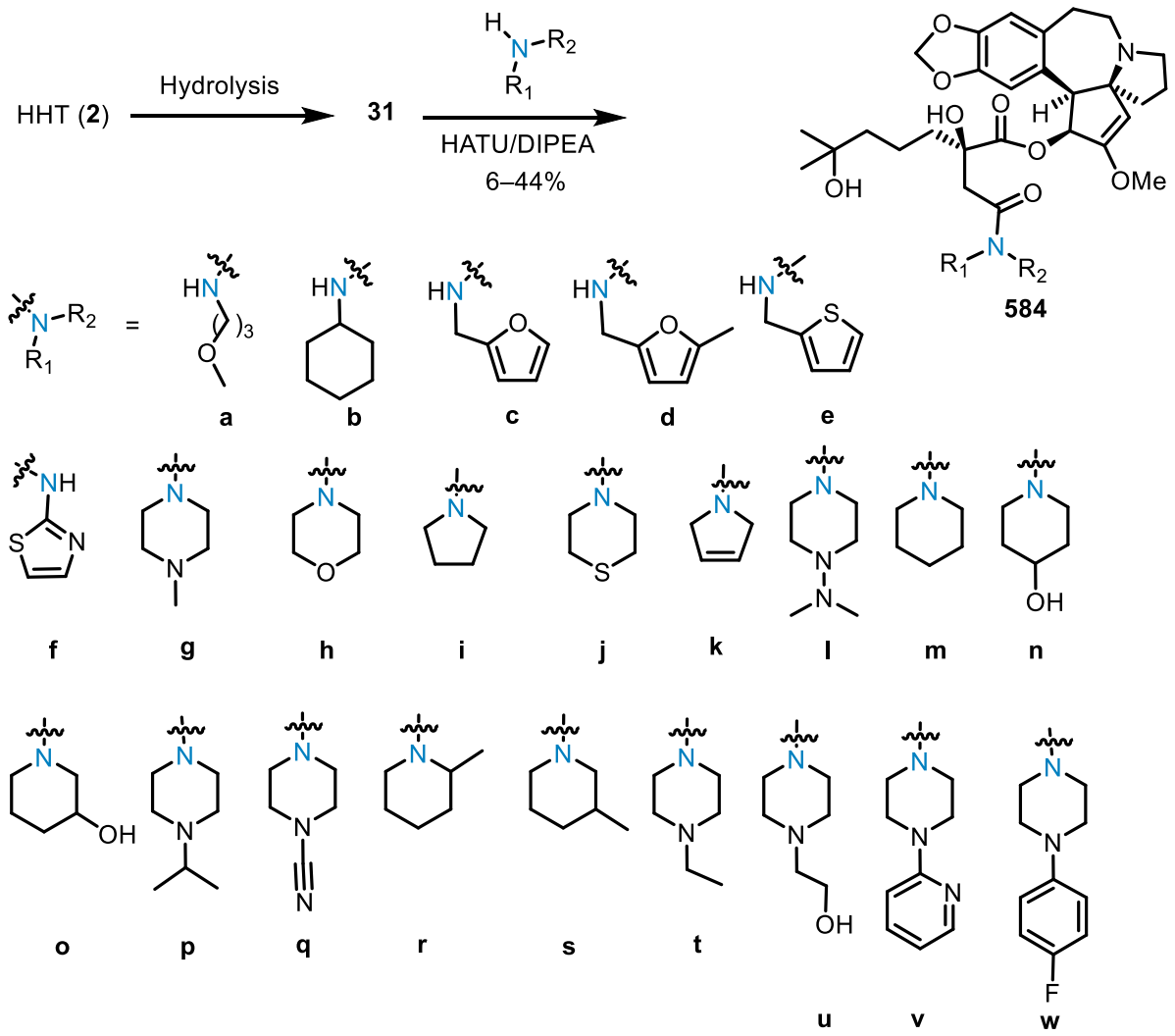


Figure 22 Lai's HHT (**2**) aza analogs (2013)

Table 14. Cytotoxicity (IC_{50} and IC_{90} in µg/mL) of Lai's 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
584l	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

Table 15. Cytotoxicity (IC_{50} and IC_{90} in µg/mL) of Lai's 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
584l	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 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
584l	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 (Figure 23).

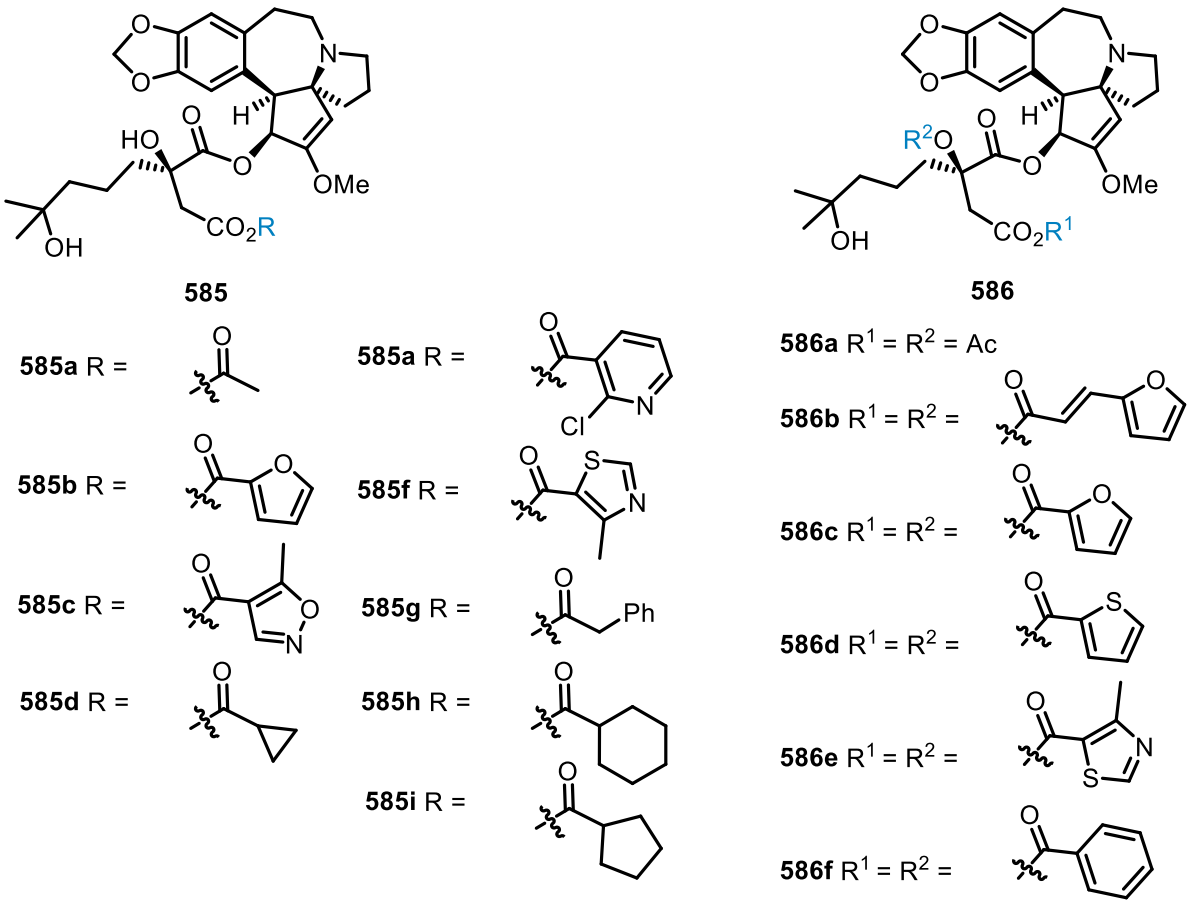


Figure 23 Lai's HHT (**2**) acylated analogs (2013)

The acylated analogs were compared to HHT (**2**) and tested against against human hematopoietic (Table 17) and solid tumor cell lines (Tables 18–19) including K562/adr (myelogenous leukemia resistant to Doxorubicin), Kasumi-1 (acute myeloid leukemia), NB4 (acute promyelocytic

leukemia), Jurkat (childhood T acute lymphoblastic leukemia), 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), U87 MG (glioma), HeLa (cervical), CaES-17 (esophageal), CNE (nasopharyngeal), Hep2 (liver), PC-3 (prostate), SK-OV-3 (ovary) 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 17–19). Compound **585f** and **585g** were more potent than HHT (**2**) against eighteen and seventeen over the twenty cell lines tested, respectively.

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

Table 18. Cytotoxicity (IC_{50} and IC_{90} in µg/mL) of Lai's 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,

Table 19. Cytotoxicity (IC_{50} and IC_{90} in µg/mL) of Lai's 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.

5

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 (>thirty syntheses of fifty). The cyclization reactions used (electrophilic aromatic substitutions, organometallic coupling ...) 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. 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 Figures 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 compangy Oncopharm SA later based in Houston (USA). A number of HHT 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 HHT

Molecules like HHT (**2**) which works on several targets are increasingly attractive. Protein synthesis inhibitors could affect the translation of mRNA into proteins at several different stages, such as, initiation, elongation and termination as illustrated in Figure 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 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 twelve eukaryote-specific inhibitors and four broad-spectrum inhibitors in *Saccharomyces cerevisiae*.[177]

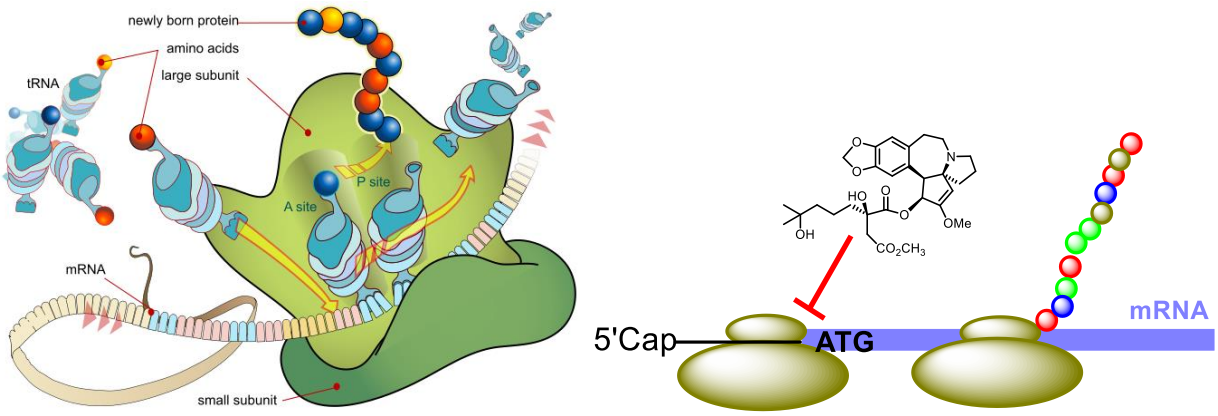


Figure 24: Simplified diagram of protein synthesis showing the A site where HHT binds to inhibit protein synthesis.[178] In the cytoplasm of the cells, ribosomes (green) bind to the messenger RNA (mRNA) containing the code translated from the switched on genes into the DNA. The twenty different amino 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.

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 (Figure 25). Its six-membered aromatic ring stacks on the base of Cytosine 2452 for *H. marismortui* (2487 for *E. 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 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 ° 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 ° 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 (Figure 25). HHT (**2**) has a different mechanism of action from that of tyrosine kinase inhibitors, a class of drugs used in for the treatement of chronic myloid leukemia (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 systematic 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.

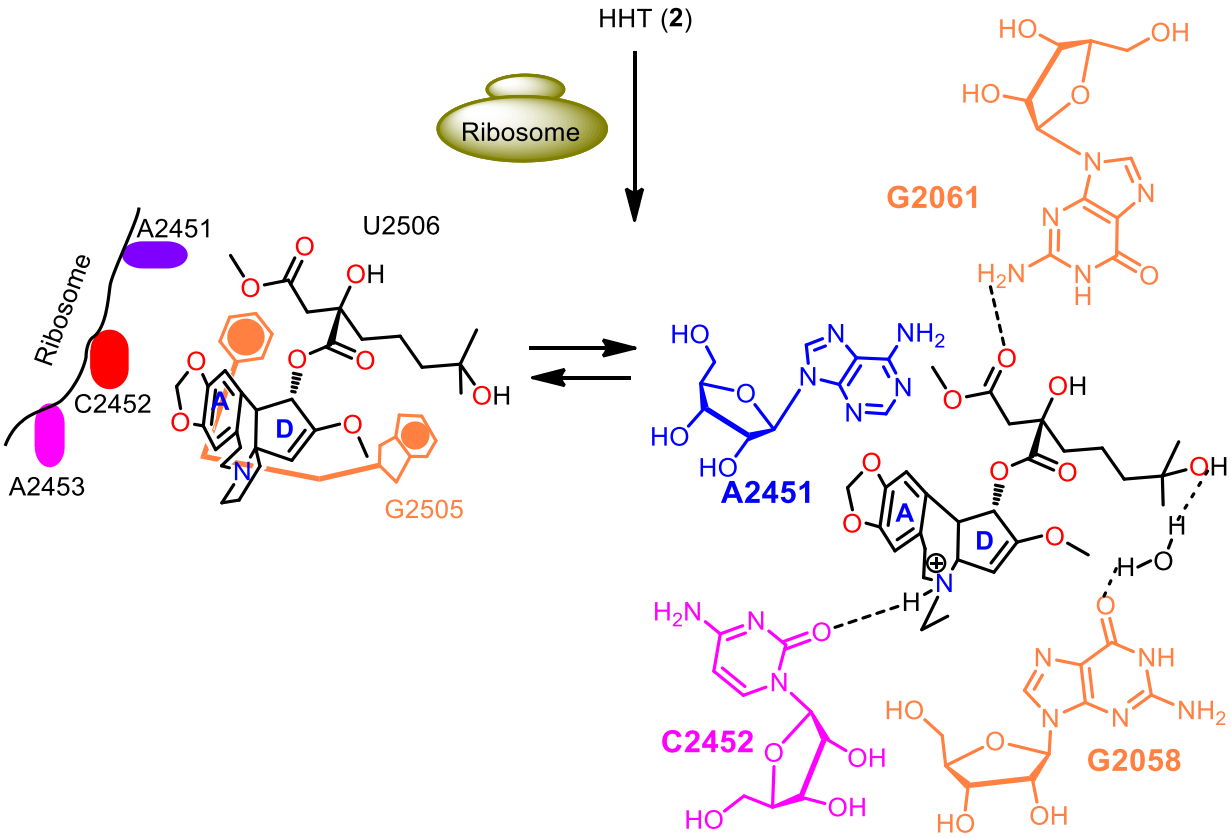


Figure 25: Interaction of HHT (**2**) with the ribosome of *H. marismortui* and the conformational changes associated with this binding.

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 induces also apoptosis through death receptors (extrinsic pathway). The intrinsic pathway is induced by cleaving the pro-active 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 seventy-eight 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, 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 upon treatment with HHT (**2**), this was measured by increased annexin V binding capacity, caspase-3 activation, and the cleavage of poly(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] Upon 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 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 pro-apoptotic 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 mitochondrial membrane potential 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 anti-apoptotic activity (Figure 26).[187] HHT (**2**) inhibited the telomerase content of HL-60 cells effectively and induced apoptosis.[188]

HHT (**2**) inhibits synthesis of anti-apoptotic 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]

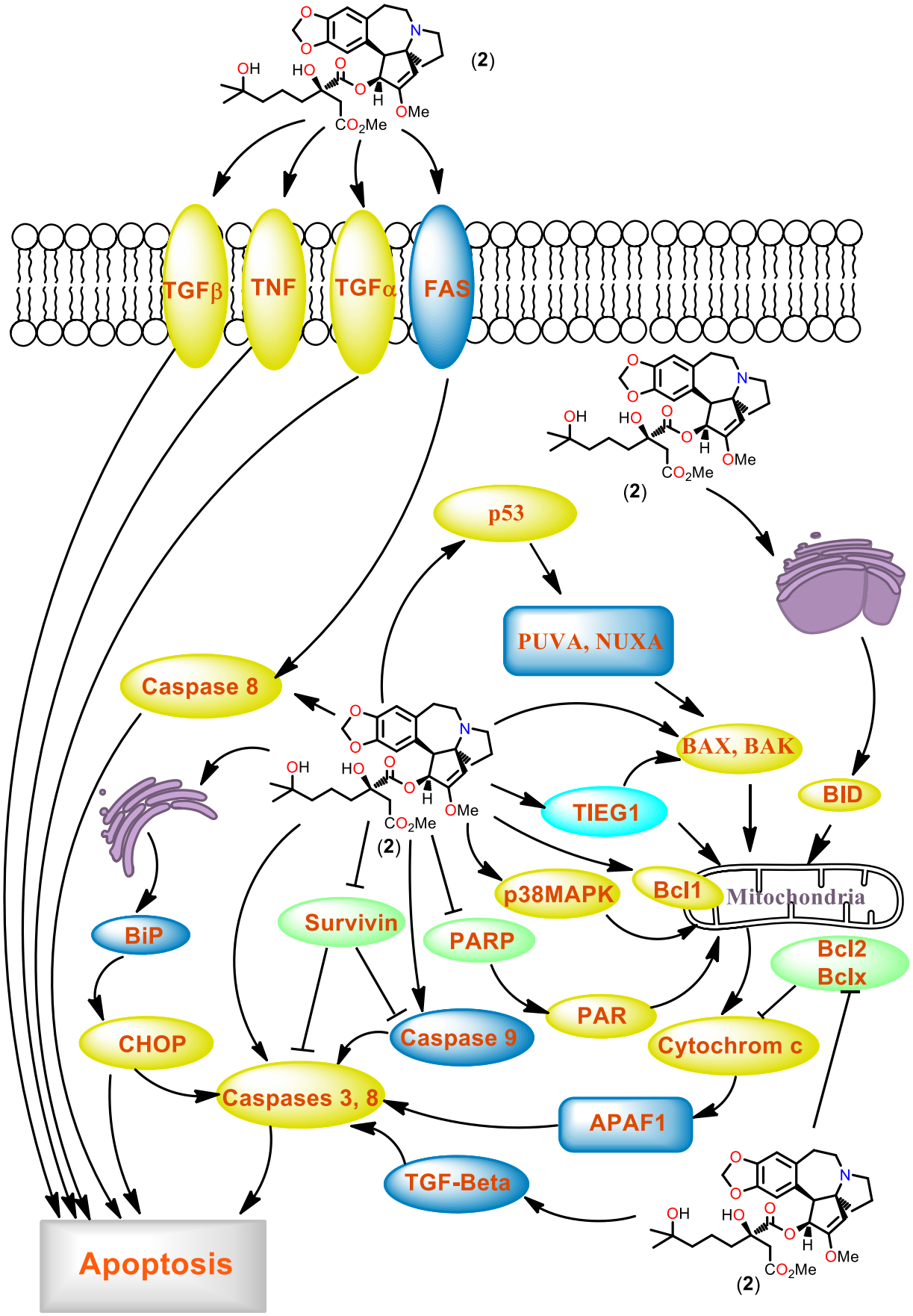


Figure 26: Main pathways involved in apoptosis mechanism induced by HHT (**2**). Yellow: apoptosis factor; Blue: apoptosis intermediator; Turquoise: Dual role as intermediator and factor; Green: Apoptosis inhibitor

5

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 up-regulation of Bax and Bim and down-regulation 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 seventeen genes were up-regulated and twenty seven were down-regulated. 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 1 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 tumor necrosis factor 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 up-regulated. Other genes, such as *BRD2*, *ACVRL1* and *RBM4* were also up-regulated 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 poly(adenosine 5'-diphosphate-ribose) polymerase (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 reduced the expression level of the anti-apoptotic protein Bcl-2 and of c-Myc. HHT (**2**) was examined on ponatinib-resistant primary Ph-positive ALL and chronic-phase 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 pro-oncogenic or pro-survival proteins (important proteins for tumorigenesis) having short half-lives, like 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 acute myeloid lymphoma (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 mitochondrial membrane potential (Dwm). U937, K562, KU812, and KCL22 cells that expressed B-cell lymphoma-extra-large (Bcl-xL) (anti-apoptotic 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 VEGF (vascular endothelial growth factor), an important mediator of angiogenesis in the bone marrow. This work thus demonstrated a possible anti-angiogenic 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 (AUC). 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 Imatinib (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 ($P<0.05$). From day 1 to day 8 after HHT (**2**) treatment, the BCL-2 expression level declined first and then went up ($P<0.05$). 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 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 down regulated and four up-regulated. 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 heat shock proteins (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 poly (ADP-ribose) polymerase 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 myelodysplastic syndrome (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-dimerisation 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 systemic mastocytosis (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 effecitively 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 (VHL) gene was revealed by high-throughput simultaneous screen and counterscreen.[210] In addition, HHT (**2**) has the potential of treatment 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 chronic myelogenous leukemia (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 imatinib (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 doxorubicin (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 co-delivery of DOX and HHT (**2**) could synergistically potentiate antitumor effects [213]

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

A synergistic effect of Sulforaphane (SF) in human CML cell line K562 with chemotherapy drugs-HHT (**2**) and cytosine arabinoside (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]

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a pro-apoptotic 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 anti-tumor agent. HHT (**2**) was found to be a very effective, (in nano-molar dose) enhancer of TRAIL-mediated apoptosis/growth

suppression. Co-treatment 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 HHT

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 sub-lethal 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**), doxorubicin and curcumin was decreased alongside the decreased expression of MRD1. The study indicated that shRNA recombinant plasmids could modulate MDR in vitro.[223]

Human multi-drug resistance (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 over express the conferring P-glycoprotein/MDR1 (CEM/ADR5000), that 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 over-expressed H1R should be aware of possible allergic reaction when applying HHT (**2**) injection.[226]

6.1.6 Other Pharmacological applications

From the 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 doxorubicine 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 post-entry 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, 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 (**2**) 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 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 two hundred and ninety 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 acute lymphoblastic leukemia) 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 Omacetaxine mepesuccinate (International nonproprietary name (INN)) (OM) 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 omacetaxine mepesuccinate allowing to confirm its efficacy in CML and put the first EMA application in 2004 leading to its orphan drug status in 2009, and in the US with a stable source of hydrosoluble omacetaxine reported in 2001 by Brown (ChemGenex founder).[12,237,238,239,240]

6.2.1 Approved clinical applications

HHT (**2**) was approved in 2009 by the European medicine agency (EMA) for t-Philadelphia chromosome positive chronic myeloid leukaemia 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 chronic or accelerated phase 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 high-performance liquid chromatography (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 omacetaxine mepesuccinate. 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% to 90% of patients with CML in the early chronic phase 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% to 4%, and the mortality is 1% to 2%.[244]

In 2001 a study was conducted on 37 patients ≤ 21 years old with recurrent refractory AML, twenty-eight 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 remission (CHR) ten 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 achieved a CHR after five months.[246]

The outcomes of one hundred seventy six patients of accelerated-phase CML who received treatment with imatinib were reviewed and compared with the outcomes of 213 historic control patients treated with other drugs including HHT (**2**). Treatments included INF-α (hundred patients), HHT (**2**) (thirty five patients), DNR plus cytarabine (twenty three patients), decitabine (forty seven patients), and other therapies (eight patients). Complete hematologic responses were 82% with IM and 35% with HHT (**2**).[247]

In 2010, eight TKI-resistant chronic-phase 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 re-sensitized 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 six patients with CML received 1.5 mg/m^2 of HHT (**2**) alone for seven to nine days every four weeks. Seventy nine patients were in the control group, thirty one were treated with interferon alpha (IFN-α) and forty eight with hydroxycarbamide. Seventeen patients who were treated with IFN-α failed to achieve cytogenetic response within twelve months and subsequently received HHT (**2**) as a salvage treatment. After twelve 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 seventeen patients who received HHT (**2**) as salvage treatment, six achieved cytogenetic response (3 major). At the forty eight 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 months-98 months) and forty nine months (12 months-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 fourteen days every twenty eight days followed by seven days every twenty eight days) in chronic phase (CP) and accelerated phase (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 twelve 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 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. Fifty-one patients in AP-CML and forty four in myeloid blast phase (BP-CML) received subcutaneous omacetaxine 1.25 mg/m^2 twice daily days 1-14 every 28 days until hematologic response/improvement or any cytogenetic response, then days 1-7 every 28 days until disease progression. A major hematologic response

(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 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-chronic phase (CP) who had been treated with 2 or more TKIs. This Data was analyzed from two phase II clinical trials of eighty one 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 eight weeks. The overall survival was 34 months.[253]

In 2015, a study was carried on thirty one CML patients with complete cytogenic response to compare the immunological effect on T lymphocytes between HHT (**2**) (fifteen patients) and IM (sixteen 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 subcutaneous (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 Figure 25) and Takano's[60] results implying that N9 should be available for protonation to ensure its biological activity. Therefore, harringtonines 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 phycobniliprotein nanoparticles.[258] HHT (**2**) can be given as aqueous solution preferably at a pH of about 4. Also it can be provided orally.[259,260,261,262,263] The protonated form of HHT (**2**) was patented for potential application in oral and intravenous 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 High-performance liquid chromatography mass spectrometry (MS) (high resolution) in combination with off-line 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 in order to synergize and potentiate the activities of these drugs and improve therapy outcome. In some cases, the associated drugs can be efficiently co-delivered in the form of liposomes.[269]

6.2.4.1 Combination therapy for treatment chronic myeloid leukemia (CML)

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

In 2009, a clinical trial phase II carried on forty four 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/m2 per day). Both drugs were given together via continuous intravenous infusion for seven days. Thirty six of forty four patients (82%) achieved a complete hematologic remission. Although HHT/cytarabine was generally well tolerated, no significant amelioration as compared to interferon-refractory CML and was not nearly as high as association with IM in newly diagnosed patients.[271]

In 2010 in China, twelve 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 chronic phase received the triple regimen IFN-α, ara-C, and HHT. After a median duration of 16.5 months of therapy, seventy-eight patients had their therapy changed to IM, eighty five patients (94%) achieved complete hematologic response and sixty seven patients (74%) had a cytogenetic response (Ph suppression to ≤ 90%).[273]

Fourteen 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 treatment acute myeloid leukemia (AML)

In 2009, eighty-seven AML patients were treated with HHT (**2**), cytarabine (Ara-C) and DNR (HAD) regimen, the complete remission rate of the eighty-seven cases was eighty/eighty-seven (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 eighteen months and estimated survival at three years was 30.8%. The third year relapse rate was 55.1%.[276]

A group of two hundred and fifty four 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/d 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. Post remission 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, intravenously 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 granulocyte colony-stimulating factor (G-CSF) on patients with relapsed or refractory AML: thirty five out of sixty seven (52.2%) patients achieved complete remission and eight out of sixty seven (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 twelve pediatric patients, nine 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 (HHT (**2**), Ara-C and aclarubicin), was evaluated in forty six 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**), cytarabine (Ara-C) and granulocyte colony-stimulating factor (G-CSF) (HAG). All the patients were treated with HAG regimen. Thirty five patients (52.2%) achieved complete remission and eight (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 cytosine arabinoside (HA) based induction therapy composed of three chemotherapeutic drugs (HAD/M, D-DNR, M-MTX). The complete response rate was one hundred and fifty three of all cases (78.1%).[283]

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

Fourty eight patients median aged thirty five (14–57) years were enrolled into a clinical study to evaluate the efficacy and toxicity of HAA regimen (HHT (**2**), Ara-C (cytarabine), aclarubicin) 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 (fifty two cases) and HAG (HHT (**2**) and cytarabine (Ara-C) combined with G-CSF) group (thirty eight cases). In the CAG group were thirty eight cases with complete remission (73.08%) and total efficiency was 84.62%; forty 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). Up dated results showed good overall survival.[288]

A recent meta-analysis provided an overview of the effectiveness of HHT (**2**) combination regimens to treat AML. Twenty one studies were analyzed including one thousand three hundred and ten patients, two hundred twenty nine pediatric, and two hundred sixteen 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 to 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 to 7.[289] The output was as following: thirteen of thirty one (41.9%) patients had complete remission, nine (29.0%) had partial remission and nine (29.0%) had no response.

A forty-five year old female patient was reported with de novo acute megakaryoblastic leukemia (AMKL) (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. Upon HAD induction chemotherapy, the patient achieved partial hematological remission. The patient was then switched to Imatinib (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 forty 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 HHT, cytarabine and aclarubicin (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, twenty-one patients with high-risk MDS and twelve with MDS–AML to evaluate the efficacy and toxicity of CHG 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 nineteen patients achieved complete remission (57.6%) and three patients achieved partial remission (9.1%).[292]

In a long trial of nine years, fifty-three 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 acute promyelocytic leukemia (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 one hundred and thirty-two patients with higher risk myelodysplastic syndrome (MDS) was done to compare two treatment combination therapy of

low-dose CHG (cytarabine, HHT (**2**) and G-CSF) 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 ninety-eight 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 forty-nine 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 HHT (**2**) and Cytarabine regimen for CML-MBC, overall improvement cytogenetic response was 60.1%, seven 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 *Cephalotaxus griffithii* inhibited proliferation and migration of human cervical cancer cells.[298] The oil from *Cephalotaxus griffithii* needle (CGN) 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 cardiac insufficiency,[316] treating liver-depression qi-stagnation type breast

cancer,[317]spleen-kidney-qi-deficiency bladder cancer,[318] lung cancer.[319] A preparation that 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).

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

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]

Acknowledgements

Authors are thankful to BioCIS, Centre National de la Recherche Scientifique (CNRS), Labex LERMIT (ANR-10-LABX-33), Université Paris-Sud, Université Paris Descartes, Sorbonne Paris Cité and Université Paris-Saclay (France) for their financial support during the time devoted to this contribution. A.R. thanks ISSAT (Syria) for financial support.

During the preparation of this manuscript, additional studies were published[326,327]

References

[1] Huang, L.; Xue, Z. in "*The Alkaloids*" (R. H. F. Manske, ed.), Vol. 23, Academic Press, New York, 1984, pp. 157–226.

[2] Miah, M. A. J.; Hudlicky, T.; Reed, J. W. in "*The Alkaloids-Chemistry and Biology*," *Vol. 51*, ed. by Cordell G.A., Academic Press, San Diego, 1998 pp 199–269.

[3] Mei, W.; Wu, J.; Dai, H. *Zhongcaoyao* **2006**, *37*, 452–458.

[4] Itokawa, H.; Wang, X.; Lee, K-H. In *Anticancer agents from natural products*; Cragg, G.M., Kingston, D.G.I., Newman, D., Eds; Boca Raton, Florida, Brunner-Routledge Psychology Press, Taylor & Francis Group, 2005, pp 47–70.

[5] Itokawa, H.; Hitotsuyanagi, Y.; Lee, K.-H.; Homoharringtonine and related compounds In *Anticancer Agents from Natural Products (2nd Edition)*, Cragg, G.M.; Kingston, D.G.I.; Newman, D.J Eds, CRC Press/Taylor & Francis Group: Boca Raton, FL. **2012**, pp 65–93.

[6] Abdelkafi, H.; Nay, B. *Nat. Prod. Rep.* **2012**, *29*, 845–869.

[7] Lu, D.; Cao, J.; Xu, B. *Tianran Chanwu Yanjiu Yu Kaifa* (*Natural Product Res. Dev.*) **2000**, *12*, 70–73.

[8] Kantarjian, H.M.; Talpaz, M.; Santini, V.; Murgo, A.; Cheson, B.; O'Brien, S.M. *Cancer* **2001**, *92*, 1591–1605.

[9] Luo, C.Y.; Tang, J.Y.; Wang, Y.P. *Hematology* **2004**, *9*, 259–270.

[10] Ye, X.; Lin, M. *Zhonghua Xueyexue Zazhi* **2004**, *25*, 296–298.

[11] Efferth, T.; Li, P.C.H.; Konkimalla, V.S.B.; Kaina, B. *Trends Mol. Med.* **2007**, *13*, 353–361.

[12] Quintas-Cardama, A.; Kantarjian, H.; Cortes, J. *Cancer* **2009**, *115*, 5382–5393.

[13] Kantarjian, H.M.; O'Brien, S.; Cortes, J. *Clin. Lymphoma Myeloma Leuk.* **2013**, *13*, 530–533.

[14] Al Ustwani, O.; Wetzler, M. *Leuk. Lymphoma* **2013**, *54*, 2100–2102.

[15] Jiang, X.; Tang, Y.-M.; Huang, H.-F. *Yixue Zongshu* **2014**, *20*, 3345–3347.

[16] Lu, S.; Wang, J. *J. Hemat. Oncol.* **2014**, *7*, 2/1–2/10.

[17] Tian, H.-P.; Yang, P.; Liu, Y.; Tao, R.; Zhang, J. *Zhongguo Yaofang* **2014**, *25*, 1485–1489.

[18] Tian, H.-P.; Wang, F.; Tao, R.; Yang, P.; Zhang, J. *Shijie Linchuang Yaowu* **2015**, *36*, 19–25.

[19] Badgujar, V. B.; Ansari, M. T.; Abdullah, M. S.; Badgujar, S. V. *World J. Pharm. Pharm. Sci.* **2016**, *5*, 421–432.

[20] The ASCO Post, March 15, 2014. http://www.ascopost.com/issues/march-15-2014/omacetaxine-mepesuccinate-receives-full-fda-approval-for-cml/

[21] Robin, J.-P., Dhal, R., Dujardin, G., Girodier, L., Mevellec, L., Poutot, S. *Tetrahedron Lett.* **1999**, *40*, 2931–2934.

[22] Quintas-Cardama, A.; Kantarjian, H. M.; Cortes, J. E. *Cancer Control* **2009**, 16, 122–131.

[23] Wall, M. E.; Eddy, C. R.; Willaman, J. J.; Correll, D. S.; Schubert, B. G.; Gentry, H. S. *J. Am. Pharm. Assos.* **1954**, *43*, 503–505.

[24] Paudler, W. W.; Kerley, G. I.; McKay, J. *J. Org. Chem.* **1963**, *28*, 2194–2197.

[25] Powel, D.; Weisleider, R.G.; Smith Jr., C. R.; Wolff, I. A. *Tetrahedron Lett*. **1969**, 4081–4084.

[26] Powell, R. G.; Weisleder, D.; Smith, Jr., C. R.; Wolff. I. A. Abtracts of Papers, 158th National ACS Meeting, New York, September 7-12, 1959.

[27] Parry, R. I.; Chang, M. N. T.; Schwab, J. M.; Foxman, B. M. *J. Am. Chem. Soc.* **1980**, *102*, 1099–1111.

[28] N. J. Sun, X. T. Liang, *Acta Pharmacol. Sin*. **1981**, *16*, 24–26. *Chem. Abstr.* **1981**, *9, 5*, 175622t.

[29] He, H. *Zhongcaoyao* **2001**, *32*, 201–202.

[30] Paulder, W. W.; McKay, J. *J. Org. Chem.* **1973**, *38*, 2110–2112.

[31] Powell, R. G.; Madrigal, R. V.; Smith Jr., C. R.; Mikolajczak, K. L. *J. Org. Chem.* **1974**, *12*, 676–680.

[32] Lin, W.; Chen, R.; Xue, Z. *Yaoxue Xuebao* **1985**, *20*, 283–287.

[33] Powell, R. G.; Mikolajczak, K. L. *Phytochemistry* **1973**, *12*, 2987–2991.

[34] Ma, G.-E.; Sun, G.-Q.; El Sohly, M. A.; Turner, C. E. *J. Nat. Prod.* **1982**, *45*, 585–589.

[35] K. D. Yoon, Y.-W. Chin, J. Kim, *Bull. Korean Chem. Soc.* **2010**, *31*, 495–496.

[36] Yin, W. *Linchan Huaxue Yu Gongye* **1986**, *6*, 36. *Chem. Abst*. 105, 222807u.

[37] Takano, I.; Yasuda, I.; Nishijima, M.; Hitotsuyanagi Y.; Takeya, K.; Itokawa, H. *J. Nat. Prod.* **1996**, *59*, 965–967.

[38] Finefield, J. M.; Sherman, D. H.; Kreitman, M.; Williams, R. M. *Angew. Chem. Int. Ed.* **2012**, *51*, 4802–4836.

[39] Powell, R. G.; Mikolajczak, K. L.; Weisleder, D.; Smith, Jr., C. R. *Phytochemistry* **1972**, *11*, 3317–3320.

[40] Furukawa, H.; Itogawa, M.; Haruna, M.; Jinno, Y.; Ito, K.; Lu**,** S. T. *Yakugaku Zasshi* **1976**, 1373-1377.

[41] Ma, G. E.; Lin, L. T.; Chao, T. Y.; Fan, H. C. *Acta Chim. Sin.* **1977**, *35*, 201–208.

[42] Xue, Z.; Xu, L. Z.; Chen, D. H.; Huang, L. *Acra Pharm. Sin.* **1981**, *16*, 752–756.

[43] Wang, D. Z.; Ma, G. E.; Xu, R. S. *Acta Pharm. Sin.* **1992**, *27*, 178–184.

[44] Takano, I.; Yasuda, I.; Nishijima, M.; Hitotsuyanagi, Y.; Takeya, K.; Itokawa, H. *Phytochemistry* **1997**, *44*, 735-738.

[45] Takano, I.; Yasuda, I.; Nishijima, M.; Hitotsuyanagi, Y.; Takeya, K.; Itokawa, H. *Tetrahedron Lett.* **1996**, *37*, 7053–7054.

[46] Takano, I.; Yasuda, I.; Nishijima, M.; Hitotsuyanagi, Y.; Takeya, K.; Itokawa, H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1689–1690.

[47] Takano, I.; Yasuda, I.; Nishijima, M. *J. Nat. Prod.* **1996**, *59*, 1192–1195.

[48] Bocar, M.; Jossang, A.; Bodo, B. *J. Nat. Prod*. **2003**, *66*, 152–154.

[49] Parry, R. J.; Chang, M. N. T.; Schwab, J. M.; Foxman, B. M. *J. Am. Chem. Soc*. **1980**, *102*, 1099–1111.

[50] Morita, H.; Yoshinaga, M.; Kobayashi, J.'i. *Tetrahedron* **2002**, *58*, 5489–5495.

[51] Taniguchi, T.; Yokoyama, S'i.; Ishibashi, H. *J. Org. Chem.* **2009**, *74*, 7592-7594.

[52] Wang, L.-W.; Su, H.-J.; Yang, S.-Z.; Won, S.-J.; Lin, C.-N. *J. Nat. Prod.* **2004,** *67*, 1182–1185.

[53] Arora, S. K.; Bates, R. B.; Grady, R. A.; Powell, R. G. *J. Org. Chem.* **1974**, *39*, 1269–1271.

[54] He, Y.-R.; Shen, Y.-H.; Li, B.; Li, B.; Lu, L.; Tian, J.-M.; Zhang, W.-D. *Chem Biodiv.* **2013**, *10*, 584–595.

[55] Yoshinaga, M.; Morita, H.; Dota, T.; Kobayashi, J.'i. *Tetrahedron* **2004**, *60*, 7861–7868.

[56] Ni, L.; Zhong, X.-H.; Cai, J.; Bao, M.-F.; Zhang, B.-J.; Wu, J.; Cai, X.-H. *Nat. Prod. Bioprospect.* **2016**, *6*, 149–154.

[57] Morita, H.; Arisaka, M.; Yoshida, N.; Kobayashi, J. *Tetrahedron* **2000**, *56*, 2929–2934.

[58] Morita, H.; Yoshinaga, M.; Kobayashi, J. *Tetrahedron* **2002**, *58*, 5489–5495.

[59] Morita, H.; Nagahura, Y.; Hosoya, T.; Ekasari, W.; Widyawaruyanti, A.; Mori-Yasumoto, K.; Sekita, S.; Hirasawa, Y. *Heterocycles* **2010**, *81*, 441–450.

[60] Takano, I.; Yasuda, I.; Nishijima, M.; Hitotsuyanagi, Y.; Takeya, K.; Itokawa, H. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu*, **1997**, *39*, 535-540.

[61] Takano, I.; Yasuda, I.; Nishijima, M.; Hitotsuyanagi, Y.; Takeya, K.; Itokawa, H. *J. Org. Chem.* **1997**, *62*, 8251–8254.

[62] Kobayashi, J.'i.; Yoshinaga, M.; Yoshida, N.; Shiro, M.; Morita, H. *J. Org. Chem.* **2002**, *67*, 2283–2286.

[63] Robin, J-P.; Blanchard, J.; Dhal, R.; Marie, J-P.; Radosevic, N. US 2004/0186095, 2004.

[64] He, H. *Zhongcaoyao* **2001**, *32*, 201-202.

[65] Liu, Z.; Du, Q.; Wang, K.; Xiu, L.; Song, G. *J. Chromatogr. A* **2009**, *1216*, 4663–4667.

[66] Kim, W.-K.; Chae, H.-J.; Kim, J.-H. *Biotechnol. Bioproc. Eng.* **2010**, *15*, 481–487.

[67] Zhang, J.f.; Yang, C. Faming Zhuanli Shenqing CN 104193755, 2014.

[68] Sungkim, B.-S.; Kim, J.-H. *J. Chem. Technol. Biotechnol.* **2005**, *80*, 1148–1153.

[69] Robin, J.-P.; Blanchard, J.; Radosevic, N.; Roisnel, T.; Bataille, T., EP2015/058833, 2015.

[70] Kallimopoulos, T. DE200910 040381; EP20100754864 and US20120172590, 2012.

[71] Choi, Y. H.; Kim, J.; Kim, J. Y.; Joung, S. N.; Yoo, K. P.; Chang, Y. S. *Arch. Pharm. Res.*, **2000**, *23*, 163–166.

[72] Ju, U. H.; Shin, D. S.; Lee, I.; Lee, Y.; Park, H. I.; Lee, H. C.; Yoon, C. W.; Lee, G. S.; Kwon, G. R. Kongkae Taeho Kongbo KR99000203 A, 1999.

[73] He, J.; Cheung, A. P.; Wang, E.; Struble, E.; Fang, K.; Nguyen, N.; Liu, P. *J. Pharm. Biomed. Anal.* **2000**, *22*, 541–554.

[74] Choi, Y.-H.; Kim, J. *J. Chromatogra. Sci.* **2003**, *21*, 67–72.

[75] Moirangthem, D. S.; Borah, J. C.; Laishram, S.; Kalita, M. C.; Talukdar, N. C. *Nat. Prod. Res.* **2014**, *28*, 1503–1506.

[76] Robin, J.-P.; Blanchard, J.; Radosevic, N.; Roisnel, T.; Bataille, T. WO2015/128463, 2015.

[77] The IUCN Red List of Threatened Species. Version 2016-1, http://www.iucnredlist.org/ accessed on May 7th 2016.

[78] Liu, Y. US20100240887 A1, 2010.

[79] Gao, S.; Lin, L. *Yaowu Fenxi Zazhi* **1999**, *19*, 103–105.

[80] Zhang, W.; Bai, X.; Bu, Z.; Wang, J.; Yu, X.; Yuan, Q. *Biotechnol. Lett.* **1998**, *20*, 63–66.

[81] Li, Y.-C. *Process Biochem.* **2014**, *49*, 2279–2284.

[82] Li, Y.-C.; Jiang, X.-X.; Long, X.-J. *Enz. Microb. Technol.* **2014**, *67*, 77–81.

[83] Weinreb, S. M.; Semmelhack, M. F. *Acc. Chem. Res.* **1975**, *8*, 158–164.

[84] Tietze, L. F.; Schirok, H. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1124–1125.

[85] Isono, N.; Mori, N. *J. Org. Chem.* **1995**, *60*, 115–119.

[86] Suga, S.; Watanabe, M.; Yoshida, J. *J. Am. Chem. Soc.* **2002**, *124*, 14824–14825.

[87] Suga, S.; Watanabe, M.; Song, C.-H.; Yoshida, J. *Electrochem. (Tokyo, Japan)* **2006**, *74*, 672–679.

[88] Semmelhack, M. F.; Chong, B. P.; Stauffer, R. D.; Rogerson, T. D.; Chong, A.; Jones, L. D. *J. Am. Chem. Soc.* **1975**, *97*, 2507–2516.

[89] Beak, P.; Lee, W. K. *Tetrahedron Lett.* **1989**, *30*, 1197–1200.

[90] Liu, Q.; Ferreira, E. M.; Stoltz, B. M. *J. Org. Chem.* **2007**, *72*, 7352–7358.

[91] Dreiding, A. S.; Hartman, J. A. *J. Am. Chem. Soc.* **1953**, *75*, 939–943.

[92] Xiao, K.-J.; Luo, J.-M.; Xia, X.-E.; Wang, Y.; Huang, P.-Q. *Chemistry Eur. J.* **2013**, *19*, 13075–13086.

[93] Huang, S.-H.; Tian, X.; Mi, X.; Wang, Y.; Hong, R. *Tetrahedron Lett.* **2015**, *56*, 6656–6658.

[94] Kuehne, M. E.; Bornmann, W. G.; Parsons, W. H.; Spitzer, T. D.; Blount, J. F.; Zubieta, J. *J. Org. Chem.* **1988**, *53*, 3439–3450.

[95] Koseki, Y.; Sato, H.; Watanabe, Y.; Nagasaka, T. *Org. Lett.* **2002**, *4*, 885–888.

[96] Yasuda, S.; Yamamoto, Y.; Yoshida, S.; Hanaoka, M. *Chem. Pharm. Bull.* **1988**, *36*, 4229–4231.

[97] Li, W.-D. Z.; Wang, X.-W. *Org. Lett.* **2007**, *9*, 1211–1214.

[98] Yasuda, S.; Yamada, T.; Hanaoka, M. *Tetrahedron Lett.* **1986**, *27*, 2023–2026.

[99] Sun, M.; Lu, H.; Wang, Y.; Yang, H.; Liu, H. *J. Org. Chem.* **2009**, *74*, 2213-2216.

[100] Li, W.-D. Z.; Wang, Y.-Q. *Org. Lett.* **2003**, *5*, 2931–2934.

[101] Zhao, Y.; Xi, S.; Song, A.; Ji, G. *J. Org. Chem.* **1984**, *49*, 4549–4551.

[102] Zhang, Z.-W.; Zhang, X.-F.; Feng, J.; Yang, Y.-H.; Wang, C.-C.; Feng, J.-C.; Liu, S. *J. Org. Chem.* **2013**, *78*, 786–790.

[103] Auerbach, J.; Weinreb, S. M. *J. Am. Chem. Soc.* **1972**, *94*, 7172–7173.

[104] Kuznetsov, N. Y.; Kolomnikova, G. D.; Khrustalev, V. N.; Golovanov, D. G.; Bubnov, Y. N. *Eur. J. Org. Chem.* **2008**, 5647–5655.

[105] Ma, B.-C.; Wang, Y.-Q.; Li, W.-D. Z. *J. Org. Chem.* **2005**, *70*, 4528–4530.

[106] Dolby, L. J.; Nelson, S. J.; Senkovich, D. *J. Org. Chem.* **1972**, *37*, 3691–3695.

[107] Li, W.-D. Z.; Ma, B.-C. *J. Org. Chem.* **2005**, *70*, 3277–3280.

[108] Zhao, Y.-M.; Gu, P.; Zhang, H.-J.; Zhang, Q.-W.; Fan, C.-A.; Tu, Y.-Q.; Zhang, F.-M. *J. Org. Chem.* **2009**, *74*, 3211–3213.

[109] Moore, S. P.; Coote, S. C.; O'Brien, P.; Gilday, J. *Org. Lett.* **2006**, *8*, 5145–5148.

[110] Krafft, M. E.; Cran, J. W. *Synlett* **2005**, 1263–1266.

[111] Li, W.-D. Z.; Duo, W.-G.; Zhuang, C.-H. *Org. Lett.* **2011**, *13*, 3538–3541.

[112] Xing, P.; Huang, Z.; Jin, Y.; Jiang, B. *Synthesis* **2013**, *45*, 596–600.

[113] Lin, X.; Kavash, R. W.; Mariano, P. S. *J. Am. Chem. Soc.* **1994**, *116*, 9791–9792.

[114] Lin, X.; Kavash, R. W.; Mariano, P. S. *J. Org. Chem.* **1996**, *61*, 7335–7347.

[115] Liu, H.; Yu, J.; Li, X.; Yan, R.; Xiao, J.-C.; Hong, R. *Org. Lett.* **2015**, *17*, 4444–4447.

[116] Pizzonero, M.; Keller, L.; Dumas, F.; Ourevitch M.; Morgant G.; Spasojević-de Biré, A.; Bogdanović, G.; Ghermani, N. E.; d'Angelo, *J. Org. Chem.* **2004**, *69*, 4336–4350.

[117] de Oliveira, E. R.; Dumas, F.; d'Angelo, J. *Tetrahedron Lett.* **1997**, *38*, 3723–3726.

[118] Quteishat, L.; Panossian, A.; Le Bideau, F.; Alsalim, R.; Retailleau P.; Troufflard, C.; Rose, E.; Dumas, F. *J. Organomet. Chem.* **2015**, *776*, 35-42.

[119] Cai, Q.; Zhao, Z. A.; You, S. L. *Angew. Chem. Int. Ed*. **2009**, *48*, 7428–7431.

[120] Gouthami, P.; Chegondi, R.; Chandrasekhar, S. *Org. Lett.* **2016**, *18*, 2044–2046.

[121] Tietze, L. F.; Schirok, H. *J. Am. Chem. Soc.* **1999**, *121*, 10264–10269.

[122] Zhang, Q.-W.; Xiang, K.; Tu, Y.-Q.; Zhang, S.-Y.; Zhang, X.-M.; Zhao, Y.-M.; Zhang, T.-C. *Chem. Asian J.* **2012**, *7*, 894–898.

[123] Wzorek, A.; Sato, A.; Drabowicz, J.; Soloshonok, V. A.; Klika, K. D. *Helv. Chim. Acta* **2015**, *98*, 1147–1159.

[124] Gonçalves-Martin, M. G.; Sigmantas, S.; Renaud, P. *Helv. Chim. Acta* **2012**, *95*, 2502–2514.

[125] Goncalves-Martin, M. G.; Saxer, A.; Renaud, P. *Synlett* **2009**, 2801–2802.

[126] Seebach, D.; Sting, A. R.; Hoffmann, M. *Angew. Chem. Int. Ed. Eng.* **1996**, *35*, 2708–2748.

[127] Zhao, Z.; Mariano, P. S. *Tetrahedron* **2006**, *62*, 7266–7273.

[128] Zhao, Z.; Duesler, E.; Wang, C.; Guo, H.; Mariano, P. S. *J. Org. Chem.* **2005**, *70*, 8508–8512.

[129] Trost, B. M.; Bringley, D. A.; Seng, P. S. *Org. Lett.* **2012**, *14*, 234–237.

[130] Ikeda, M.; Okano, M.; Kosaka, K.; Kido, M.; Ishibashi, H. *Chem. Pharm. Bull.* **1993**, *41*, 276–281.

[131] Planas, L.; Perard-Viret, J.; Royer, J.; Selkti, M.; Thomas, A. *Synlett* **2002**, 1629–1632.

[132] Planas, L.; Pérard-Viret, J.; Royer, J. *J. Org. Chem.* **2004**, *69*, 3087–3092.

[133] Nagasaka, T.; Sato, H.; Saeki, S.; Science, L. *Tetrahedron: Asymmetry* **1997**, *8*, 191–194.

[134] Weinstein, B.; Craig, A. R. *J. Org. Chem.* **1976**, *41*, 875–878.

[135] Pizzonero, M.; Dumas, F.; d'Angelo, J. *Heterocycles* **2005**, *66*, 31–37.

[136] Milolajczak, K. L.; Smith Jr., C. R.; Weisleder, D. J. *J. Med. Chem.* **1977**, *20*, 328–332.

[137] Ikeda, M.; El Bialy, S. A. A.; Hirose, K.-I.; Kotake, M.; Sato, T.; Bayomi, S. M. M.; Shehata, I. A.; Abdelal, A. M.; Gad, L. M.; Yakura, T. *Chem. Pharm. Bull.* **1999**, *47*, 983–987.

[138] Ishibashi, H.; Okano, M.; Tamaki, H.; Maruyama, K; Yakura, T.; Ikeda, M. *J. Chem. Soc. Chem. Commun.* **1990**, 1436–1437.

[139] Seebach, D.; Naef, R.; Calderari, G. *Tetrahedron* **1984**, *40*, 1313–1324.

[140] El Bialy, S. A. A.; Ismail, M. A.; Gad, L. M.; Abdelal, A. M. M. *Med. Chem. Res.* **2002**, *11*, 293–300.

[141] Esmieu, W. R.; Worden, S. M.; Catterick, D.; Wilson, C.; Hayes, C. J. *Org. Lett.* **2008**, *10*, 3045–3048.

[142] Hameed, A.; Blake, A. J.; Hayes, C. J. *J. Org. Chem.* **2008**, *73*, 8045–8048.

[143] Reed, P. E.; Katzenellenbogen, J. A. *J. Org. Chem.* **1991**, *56*, 2624–2634.

[144] Eckelbarger, J. D.; Wilmot, J. T.; Epperson, M. T.; Thakur, C. S.; Shum, D.; Antczak, C.; Tarassishin, L.; Djaballah, H.; Gin, D. Y. *Chem. Eur. J.* **2008**, *14*, 4293–4306.

[145] Taniguchi, T.; Ishibashi, H. *Org. Lett.* **2008**, *10*, 4129–4131.

[146] Taniguchi, T.; Ishita, A.; Uchiyama, M.; Tamura, O.; Muraoka, O.; Tanabe, G.; Ishibashi, H. *J. Org. Chem.* **2005**, *70*, 1922–1925.

[147] Burkholder, T. P.; Fuchs, P. L. *J. Am. Chem. Soc.* **1990**, *112*, 9601–9613.

[148] Dreiding, A. S.; Hartman, J. A. *J. Am. Chem. Soc.* **1953**, *75*, 939–943.

[149] Hiranuma, S.; Hudlicky, T. *Tetrahedron Lett.* **1982**, *23*, 3431–3434.

[150] Cheng, J.; Zhang, J.; Zhang, C.; Yang, J.; Huang, L. *Acta Pharm. Sinica* **1984**, *19*, 178–187.

[151] Robin, J.-P.; Blanchard, J.; Chauviat, L.; Dhal, R.; Marie, J.-P.; Radosevic, N. U.S. Patent 20050090484 A1, 2005.

[152] Marguerit, M.; Little, G.; Wang, Y.; He, L.; Allwein, S.; Reif, J.; Rossi, J.; Roemmele, R.; Bakale, R. *Eur. J. Org. Chem.* **2015**, *2015*, 8003–8010.

[153] Eckelbarger, J. D.; Wilmot, J. T.; Gin, D. Y. *J. Am. Chem. Soc.* **2006**, *128*, 10370–10371.

[154] El Bialy, S. A. A.; Braun, H.; Tietze, L. F. *Eur.J. Org. Chem.* **2005**, 2965–2972.

[155] Keller, L.; Dumas, F.; d'Angelo, J. *Tetrahedron Lett.* **2001**, *42*, 1911–1913.

[156] Keller, L.; Dumas, F.; d'Angelo, J. *Eur. J. Org. Chem.* **2003**, 2488–2497.

[157] Ancliff, R. A.; Russell, A. T.; Sanderson, A. J. *Chem. Commun.* **2006**, 3243–3245.

[158] Ancliff, R. A.; Russell, A. T.; Sanderson, A. J. *Tetrahedron: Asymmetry* **1997**, *8*, 3379–3382.

[159] Berhal, F.; Tardy, S.; Pérard-Viret, J.; Royer, J. *Eur. J. Org. Chem.* **2009**, 437–443.

[160] Yang, H.; Sun, M.; Zhao, S.; Zhu, M.; Xie, Y.; Niu, C.; Li, C. *J. Org. Chem.* **2013**, *78*, 339–346.

[161] Sun, M.; Yang, H.; Zhou, H.; Gao, K.; Cao, Q. Faming Zhuanli Shenqing CN 102976924 A, 2013.

[162] Sun, M.; Xie, Y.; Gu, J.; Yang, H. *Can. J. Chem.* **2013**, *91*, 738–740.

[163] Hue, V. T.; Nhung, N. T. H.; Hung, M. D. *Arkivoc* **2014**, *(vi)*, 206–212.

[164] Nguyen, T. H. N.; Bui, T. T. T.; Pham, P. V.; Mac, D. H. *Tetrahedron Lett.* **2016**, *57*, 216–218.

[165] Tietze, L.F.; Schirok, H.; Wöhrmann, M.*Chem. Eur. J.* **2000**, *6*, 510–518.

[166] Tietze, L. F.; Braun, H.; Steck, P. L.; El Bialy, S. A. A. Tölle, N.; Düfert, A. *Tetrahedron* **2007**, *63*, 6437–6445.

[167] Kuznetsov, N. Y.; Zhun, I. V.; Khrustalev, V. N. ; Bubnov Y. N. *Russ. Chem. Bull.* **2005**, *54*, 2229–2232.

[168] Kuznetsov, N. Y.; Khrustalev, V. N.; Bubnov Y. N, *Russ. Chem. Bull*. **2010**, *59*, 1393–1399

[169] Berhal, F.; Pérard-Viret, J.; Royer, J. *Tetrahedron: Asymmetry* **2010**, *21*, 325–332.

[170] Robin, J.-P.; Dhal, R.; Drouye, F.; Marie, J.-P.; Radosevic, N.; Robin, J.; Souchaud, K.; Bataille, P. PCT Int. Appl. WO 2002032904 A1, 2002.

[171] Robin, J.-P.; Dhal, R.; Drouye, F.; Marie, J.-P.; Radosevic, N.; Robin, J.; Souchaud, K.; Bataille, U.S. Pat. Appl. US20040006064, 2004.

[172] Gin, D. Y.; Wilmot, J. T.; Djaballah, H. PCT WO 2009/148654 A2, 2009.

[173] Rong, F.; Xu, R.; Xie, F.; Lai, H. PCT WO2013023620 A1, 2013.

[174] Xu, R.; Rong, F.; Xie, F.; Lai, H. PCT WO2013023622 A1, 2013.

[175] Robert, F.; Carrier, M.; Rawe, S.; Chen, S.; Lowe, S.; Pelletier, J. *PloS One*, **2009**, *4*, 1–12

[176] Gürel, G.; Blaha, G.; Moore, P.B.; Steitz,T. A. *J. Mol. Biol*. **2009**, *389*, 146–156.

[177] Loubresse, N. G.; Prokhorova, I.; Holtkamp, W.; Rodnina M. V.; Yusupova, G.; Yusupov, M. *Nature* **2014,** *513*, 517–522.

[178] This work has been released into the public domain by its author, LadyofHats https://en.wikipedia.org/wiki/Translation_(biology)#/media/File:Ribosome_mRNA_translation_en.svg. Acessed on December, 10, 2016.

[179] Preudhomme, C.; Cayuela, J.-M.; Chomel, J.-C.; Corm, S.; Hayette, S.; Mahon, F.-X.; Nicolini, F. E.; Réa, D.; Roche-Lestienne, C.; Guilhot, F. *Hématologie* **2010**, *16*, 65–79.

[180] Zhao, Y.; Yang, M. *Anhui Yike Daxue Xuebao*, **2014**, *49*, 1080–1084.

[181] Jin, W.; Qu, L.-F.; Chen, Q.; Chang, X.-Z.; Wu, J.; Shao, Z. M. *Acta Pharmacol. Sin.* **2007**, *28*, 859–868.

[182] Efferth, T.; Sauerbrey, A.; Halatsch M.E.; Ross, D. D.; Gebhart, E. G. *Naunyn Schmiedebergs Arch. Pharmacol*. **2003,** *367*, 56–67.

[183] Yinjun, L.; Jie, J.; Weilai, X.; Xiangming, T. *Am. J. Hematology* **2004**, *76*, 199–204.

[184] Cai, Z.; Hu, J.; He, D.; Wu, W.; Gao, L.; Bao, H. *Blood* **2005**, 106, 4917–4917.

[185] Jie, H.; Donghua, H.; Xingkui X.; Liang, G.; Wenjun W.; Xiaoyan, H.; Zhen, C. *Leuk. Lymphoma* **2007**, *48*, 964–977.

[186] Cai, Z.; Bao, H. Y.; Lin, M.-F. *Chin. Med. J.* **2005**, *118*, 548–554.

[187] Tang, R., Faussat; A. M., Majdak, P.; Marzac, C.; Dubrulle, S.; Marjanovic, Z.; Legrand, O.; Marie J. P. *Mol. Cancer Ther.* **2006**, *5*, 723–731.

[188] Wan-Zhuo, X.; Mao-Fang, L.; He, H.; Zhen, C. *Am. J. Chinese Med.* **2006**, 34, 233–244.

[189] Allan, E. K.; Holyoake, T. L.; Craig, A. R.; Jørgensen, H. G. *Leukemia* **2011**, *25*, 985–994.

[190] Wei, J.; Genhong, D.; Junjie, L.; Ying, C.; Wenfeng, L.; Jiong, W.; Tiewei C.; Ming Y.; Zhimin, S. *FEBS Lett.* **2007**, *581*, 3826–3832.

[191] Jin, W.-J., Wu, J.; Zhuan, Z.; Li, J.; Fei, F.; Di, G.; Chen, Y.; Yao, M.; Shao, Z. *Acta Biochim. Biophys. Sin.* **2007**, *39*, 982–991.

[192] Jin, W.; Qu, L.-F.; Min, P.; Chen, S.; Li, H.; Lu, H.; Hou, Y.-T. *Acta Pharm. Sin.* **2004**, *25*, 319–326.

[193] Okabe, B. S.; Tauchi, T.; Tanaka, Y.; Katagiri, S.; Kitahara, T.; Ohyashiki, K. *Blood* **2013**, *122*, 3086–3088.

[194] Gu, Y.; Zhou, H.; Gan, Y.; Chen, J.; Zheng, W.; Xu, X.; Lu, X.; Liang, Y.; Zhang, X.; Xu, R. *Oncotarget* **2015**, *6*, 15111–15121.

[195] Yin, S.; Wang, R.; Zhou, F.; Zhang, H.; Jing, Y. *Mol. Pharmacol.* **2011**, *79*, 1072–1083.

[196] Ye, X.-J.; Lin, M.-F. *J. Zhejiang Univ. Sci.* **2004**, *5*, 230–234.

[197] Tong, Y.; Niu, N.; Jenkins, G.; Batzler, A.; Li, L.; Kalari, Krishna R.; Wang, L. *Front. Genet.* **2014**, *8*, 95–98.

[198] Huang, B-T.; Zhao, W.; Xiao, Z.; Gao, D. *Zhongguo Zhongliu Linchuang* **2014**, *41*, 1017–1020.

[199] Huang, B.; Zeng, Q.-C.; Zhao, W.-H.; Tan, Y. *Med. Oncol.* **2014**, *31*, 836–844.

[200] Lu, X.-Y.; Cao, W.-k.; Ye, L.-L.; Deng, Z.-K.; Zhang, X.-H.; Li, Y.-F. *Zhongguo Shiyan Xueyexue Zazhi* **2014**, *22*, 712–716.

[201] Sun, X.; Kong, W.; Shi, W.; Shen, Q. *Jisuanji Yu Yingyong Huaxue* **2014**, *31*, 388–392.

[202] Li, H.; Zhang, S.; Zhou, J.; Yang, X. *Int. J. Mass Spectrom.* **2015**, *379*, 157–164.

[203] Zhou, X.; Xu, N.; Li, R.; Xiao, Y.; Gao, G.; Lu, Q.; Ding, L.; Li, L.; Li, Y.; Du, Q.; Liu, X. *Leuk. Lymphoma* **2015**, *56*, 2162–2169.

[204] Brown, D. M. U.S. Patent 20107642252, 2010.

[205] Brown, D. M. U.S. Patent 20090270368, 2009.

[206] Lou, Y.-J.; Qian, W.-B.; Jin, J. *Leuk. Lymphoma* **2007**, *48*, 1400–1406.

[207] Kuroda, J.; Kamitsuji, Y.; Kimura, S.; Ashihara, E.; Kawata, E.; Nakagawa, Y.; Takeuichi, M.; Murotani, Y.; Yokota, A.; Tanaka, R.; Andreeff, M.; Taniwaki, M.; Maekawa, T. *Int. J. Hematol.* **2008**, *87*, 507–515.

[208] Zhang, J.; Chen, B.; Wu, T.; Wang, Q.; Zhuang, L.; Zhu, C.; Fan, N.; Qing, W.; Ma, Y.; Xu, X. *PloS One* **2015**, *10*, 1–15.

[209] Jin, Y.; Lu, Z.; Cao, K.; Zhu, Y.; Chen, Q.; Zhu, F.; Qian, C.; Pan, J. *Mol. Cancer Ther.* **2010**, *9*, 211–223.

[210] Wolff, N. C.; Pavia-Jimenez, A.;Tcheuyap V. T.; Alexander S.; Christie, A.; Xie, X.-J.; Williams, N. S.; Kapur, P.; McKay, R. M.; Brugarolas, J. *Oncotarget* **2015**, *6*, 16951–16962.

[211] Cao, W.; Liu, Y.; Zhang, R.; Zhang, B.; Wang, T.; Zhu, X.; Mei, L.; Chen, H.; Zhang, H.; Ming, P. *Sci. Rep.* **2015**, *5*, 8477–8494.

[212] Chen, R.; Gandhi, V.; Plunkett, W. *Cancer Res*. **2006**, *66*, 10959–10966.

[213] Shim, G.; Lee, S.; Choi, J.; Lee, S.; Kim, C.-W.; Oh, Y.-K. *Pharm. Res.* **2014**, *31*, 2178–2185.

[214] Jiang, X.; Wang, Z.; Ding, B.; Yin, C.; Zhong, Q.; Yu, G.; Jiang, L.; Ye, J.; Dai, M.; Zhang, Y.; Liang, S.; Zhao, Q.; Liu, Q.; Meng, F. *Oncotarget* **2015**, *6*, 33612–33622.

[215] Xu, X.; Zhang, X.; Rubin, C.; G., Ruilan, *Zhonghua Zhongyiyao Xuekan* **2014**, *32*, 121–124

[216] Feng, W.; Zhou, Y.; Liu, Z. *Linchuang Xueyexue Zazhi* **2015**, *28*, 209–211.

[217] Beranova, L.; Pombinho, A. R.; Spegarova, J.; Koc M.; Klanova, M.; Molinsky, J.; Klener P.; Bartunek P.; Andera, L. *Apoptosis* **2013**, *18*, 739–750.

[218] Philipp, S.; Sosna, J.; Plenge, J.; Kalthoff, H.; Adam, D. *Cell Commun. Signal* **2015**, *13*(25) 1–16.

[219] Brown, D. M. U.S. Patent 0207855, 2012.

[220] Han, Q.; Wu, L.; Ye, J.; Jiang, X.; Zhu, J.; Wang, H.; Tao, L.; Sun, M. *Baixuebing Linbaliu* **2015**, *24*, 224–226.

[221] Han, Q.; Fu, Y.; Tao, L.; Ye, J.; Wu, Li.; Chen, H.; Chen, L.; Jiang, X.; Sun, M. *Zhonghua Xueyexue Zazhi* **2015**, *36*, 433–435.

[222] Zhou, F.; Tan, W.; Ma, Y.; Chen, B.; Zhu, P.; Xu, X.; Lin, G. *Zhonghua Xueyexue Zazhi* **2012**, 33, 433–438.

[223] Shaoa, S.-L.; Suna,Y.-Y.; Lib, X.-Y.; Zhang, W.-W.; Fu, B.; Yun, D.-Z.; Zuo, M.-X. *Cell Biol. Int*. **2008**, *32*, 893–898.

[224] Bao, S.; Jiang, L.; Zhang, X.; Li, Z.; Zhenzhen; S., Shao, X.; Qi, H.; Yang, Y.; Xiong, D. *Baixuebing Linbaliu* **2014**, *23*, 144–147.

[225] Efferth, T.; Sauerbrey, A.; Halatsch, M.-E.; Ross, D.D.; Gebhart, E. *Naunyn Schmiedebergs Arch. Pharmacol.* **2003**, *367*, 56–67.

[226] Guo, Y.; Han, S.;Cao, J.; Liu, Q.; Zhang, T. *Biomed. Chromatogr.* **2014**, *28*, 1607–1614.

[227] Abd El Shakour, Z. T. *Nat. Sci.* **2014**, *12*, 74–78.

[228] Kaur P.; Thiruchelvan, M.; Lee R. C. H.; Chen, H.; Chen, K. C.; Ng, M.L.; Chu, J. J. H. *Antimicrob. Agents Chemother.* **2013**, *57*, 155–167.

[229] Bessoff, K.; Sateriale, A.; Lee, K.K.; Hustona, C. D. *Antimicrob. Agents Chemother.* **2013**, *57*, 1804–1814.

[230] Romero, M.; Serrano, M.; Efferth, T.; Alvarez, M.; Marin, J.J.G. *Planta Med*. **2007**, *73*, 552–558.

[231] Dyall, J.; Coleman, C. M.; Hart, B. J.; Venkataraman, T.; Holbrook, M. R.; Kindrachuk, J.; Johnson, R. F.; Olinger, G. G. Jr.; Jahrling, P. B.; Laidlaw, M.; Johansen, L. M; Lear-Rooney, C. M.; Glass, P. J.; Hensley, L. E.; Frieman, M. B. *Antimicrob. Agents Chemother*. **2014**, *58*, 4885–4893.

[232] Cai, J.; Wei, R.; Ma, X.Y.; Zhu, H.; Li, Y.; *Int. Ophthalmol.* **2001**, *24*, 225–23.

[233] Chen, Y.; Hu, Y.; Michaels, S.; Segal, D.; Brown, D.; Li, S. *Leukemia* **2009**, *23*, 1446–1454.

[234] Cao, J.; Craig, J. F.; Zhang, X. *Antiviral Res.* **2015**, *114*, 1–10.

[235] Krstin, S.; Peixoto, H. S.; Wink, M. *Antimicrob. Agents Chemother.* **2015**, *59*, 7011–7017.

[236] Lipton, J. H.; Kim, D. *Expert Opin. Orphan Drugs* **2013**, *1*, 745–754.

[237] Chen, Y.; Li, S. *OncoTargets Ther.* **2014**, *7*, 177–186.

[238] Heiblig, M.; Sobh, M.; Nicolini, F.E. *Leukemia Res.* **2014**, *38*, 1145–1153.

[239] Lü, S.; Wang, J. *J. Hematol. Oncol.* **2014**, *7*, 1–10. http://www.jhoonline.org/content/7/1/2.

[240] Narayanan, V.; Gutman, J. A.; Pollyea, D. A.; Jimeno, A. *Drugs of Today* **2013**, *49*, 447–456.

[241] EMA-000484-PIP01-08.

[242] Alvandi, F.; Kwitkowski, V. E.; KO, C.-W.; Rothmann, M.; Dricci, S.; Saber, H.; Ghosh, D.; Brown, J.; Pfeiler, E.; Chikhale, E.; Grillo, J.; Bullock, J.; Kane, R.; Askaminskas, E.; Farrell, A. T.; Pazdur, R.; *Oncologist* **2014**, *19*, 94–99.

[243] Nijenhuis, C. M.; Lucas, L.; Rosing, H.; Schellens, J. H. M.; Beijnen, J. H.; Gorman, S. H.; Burke, S. M.; Campbell, D. A.; Chapple, M. W.; Yousey, T. H.; Mulvana, D. E. *J. Chromatogr. B* **2015**, *1002*, 152–159.

[244] O'Brien, S. G.; Guilhot, F.; Larson, R. L.; Gathmann, I.; Baccarani, M.; Cervantes, F.; Cornelissen, J. J.; Fischer, T.; Hochhaus, A.; Hughes, T.; Lechner, K.; Nielsen, J.; Rousselot, P., Reiffers, J.; Saglio, G.; Shepherd, J.; Simonsson, B.; Gratwohl, A.; Goldman, J. M.; Kantarjian, H.; Taylor, K.; Verhoef, G.; Bolton, A. E.; Capdeville, R.; Druker, B. J. *N. Engl. J. Med.* **2003**, *348*, 994–1004.

[245] Bell, B. A.; Chang, M. N.; Weinstein, H. J. *Med. Pediatr. Oncol.* **2001**, *37*, 103–107.

[246] De Lavallade, H.; Khorashad, J. S.; Davis, H. P.; Milojkovic, D., Kaeda, J. S.; Goldman, J. M.; Apperley, J. F.; Marin D., *Blood* **2007**, *110*, 2779–2780.

[247] Kantarjian, H., Talpaz, M.; O'Brien, S.; Giles, F.; Faderl, S.; Verstovsek, S.; Garcia-Manero, G.; Shan, J.; Rios, M. B.; Champlin, R.; De Lima, M.; Cortes, J. *Cancer* **2005**, *103*, 2099–2108.

[248] Nicolini, F. E.; Chomel, J.-C., Roy, L.; Legros, L.; Chabane, K.; Ducastelle, S.; Nicolas-Virelizier, E.; Michallet, M.; Tigaud, I.; Magaud, J.-P.; Turhan, A.; Guilhot, F.; Hayette, S. *Clin. Lymphoma, Myeloma Leuk.* **2010**, *10*, 394–399.

[249] Li, Y.-F.; Deng, Z.-K.; Xuan, H.-B.; Zhu, J.-B.; Ding, B.-H., Liu X.-N.; Chen, B.-A *Chin. Med. J.* **2009**, *122*, 1413–1417.

[250] Khoury, H. J.; Cortes, J.; Baccarani, M.; Wetzler, M.; Masszi, T.; Digumarti, R.; Craig, A.; Benichou, A.-C.; Akard, L. *Leuk. Lymphoma* **2015**, *56*, 120–127.

[251] Khoury, H. J.; Cortes, J.; Baccarani, M.; Wetzler, M.; Masszi, T.; Digumarti, R.; Craig, A.; Benichou, A.-C.; Akard, L. *Leuk. Lymphoma* **2015**, *56*, 120–127.

[252] Legros, L.; Hayette, S.; Nicolini, F. E.; Raynaud, S.; Chabane, K.; Magaud, J.-P.; Cassuto, J.-P.; Michallet, M. *Leukemia* **2007**, *21*, 2204–2206.

[253] Cortes, J. E.; Nicolini, F. E.; Wetzler, M.; Lipton, J. F.; Akard, L.; Craig, A.; Nanda, N.; Benichou, A.-C., Leonoudakis, J.; Khoury, J.; Hochhaus, A.; Baccarani, M.; Kantarjian, H. M. *Clin. Lymphoma Myeloma Leuk.* **2013**, *13*, 584–591.

[254] Deng, Z.; Li, Y.; Li, Y.-F. *Tumori* **2015**, *101*, 323–327.

[255] Nemunaitis, J.; Mita, A.; Stephenson, J.; Mita, M. M.; Sarantopoulos, J.; Padmanabhan-Iyer, S.; Nanda, N.; Gleich, L.; Benichou, A.-C.; Craig, A. *Cancer Chemother. Pharmacol.* **2013**, *71*, 35–41.

[256] Brown, D. M. U.S. Patent 20040082565, 2004.

[257] Lévy, V.; Zohar, S.; Bardin, C.; Vekhoff, A.; Chaoui, D.; Rio, B.; Legrand, O.; Sentenac, S.; Rousselot, P.; Raffoux, E.; Chast, F.; Chevret, S.; Marie. J.P. *Br. J. Cancer* **2006**, *95*, 253–259.

[258] Chen, T; Zheng, W.; Huang, Y. Faming Zhuanli Shenqing CN 104667289 A, 2015.

[259] Brown, D. M. U.S. Patent 20120058991, 2012.

[260] Brown, D.; Michaels, S. U.S. Patent 2011/0293676, 2011.

[261] Brown, D. U.S. Patent 20110009388, 2011**.**

[262] Brown, D.M. U.S. Patent 7683050, 2010**.**

[263] Brown, D.; Michaels, S. U.S. Patent 20090068236, 2009**.**

[264] Robin, J. P.; Blanchard, J.; Radosevic, N.; Roisnel, T.; Bataille, T. WO 2015128463, 2015.

[265] Li, M.; Fei, X.; Shi, F.; Dou, J.; Wu, S.; Wu, D.; Zhang, Y.; Pan, M.; Luo, S.; Gu, N. *Am. J. Transl. Res.* **2016**, *8*, 1355–1368.

[266] Nijenhuis , C. M.; Lucas, L.; Rosinga, H.; Schellens J. H. M.; Beijnena, J. H.; Gormand, S. H.; Burkee, S. M.; Campbell D. A.; Chapplee, M. W.; Youseye, T.H.; Mulvana, D.E. *J. Chromatogr. B* **2015**, 1002, 152–159.

[267] Nijenhuis, C. M.; Lucas, L.; Rosing, H.; Schellens, J. H. M.; Beijnen, J. H.; Gorman, S. H.; Burke, S. M.; Campbell, D. A.; Chapple, M. W.; Yousey, T. H. *J. Chromatogr. B Analyt. Technol. Biomed Life Sci.* **2015**, *1002*, 152–159.

[268] Nijenhuis, C. M.; Lucas, L.; Rosing, H.; Robertson, P. Jr.; Hellriegel, E. T.; Schellens, J. H.; Beijnen, A. J. *Xenobiotica* **2016**, 1–11.

[269] Shim, G.; Lee, S.; Choi, J.; Lee, S.; Kim, C.-W.; Oh, Y.-K. *Pharm. Res*. **2014**, *31*, 2178–85.

[270] O'Brien, S.; Talpaz, M.; Cortes, J.; Shan, J.; Giles, F.J.; Faderl,S.; Thomas, D.; Garcia-Manero, G.; Mallard, S.; Rios, B.; Koller, C.; Kornblau, S.; Andreeff, M.; Murgo, A.; Keating, M.; Kantarjian H. M. *Cancer* **2002**, *94*, 2024–2032.

[271] Stone, R.M.; Donohue, K.A.; Stock ,W.; Hars, V.; Linker, C. A.; Shea, T.; DeAngelo, D. J.; Marcucci, G.; BloomWeld, C.D.; Larson, R.A. *Cancer Chemother. Pharmacol.* **2009**, *6*, 859–864.

[272] Fang, B.; Li, N.; Song Y.; Han, Q.; Zhao, C. R. *Ann. Hematol.* **2010**, *89*, 1099–1105.

[273]O'Brien, S.; Giles, F.; Talpaz, M.; Cortes, J.; Rios, M.B.; Shan, J.; Thomas, D.; Andreeff , M.; Kornblau, S.; Faderl, S.; Garcia-Manero, G.; White, K.; Mallard, S.; Freireich, E.; Kantarjian, H. M. *Cancer* **2003**, *98*, 888–893.

[274] Li, Y.-F.; Liu, X.; Liu, D.-S.; Din, B.-H.; Zhu, J.-B. *Leuk. Lymphoma* **2009**, *50*, 1889–1891.

[275]Liu, J.; Mi, Y.; Fu, M.; Yu, W.; Wang, Y.; Lin, D.; Bian, S.; Wang, J. *Am. J. Hematol.* **2009**, *84*, 422–427.

[276] Ma, Y.; Wang, X.; Xu X.; Lin, G. *J. Int. Med. Res.* **2009**, *37*, 1191–1201.

[277] Xiaoa, Z.; Yanga, L.; Xub, Z.; Zhanga, Y.; Liua, L.; Niea, L.; Lia, L.; Wanga, J.; Hao, Y. *Leukemia Res.* **2008**, *32*, 1288–1291.

[278] Wu, L.; Li, X.; Su, J.; He, Q.; Zhang, X.; Chang, C.; Pu; Q. *J. Cancer Res. Clin. Oncol.* **2011**, *137*, 997–1003.

[279] Tang, J.; Xue, H.; Pan, MD, C.; Chen, J.; Gu, L.; Zhao, H. *Med. Pediatr. Oncol.* **2003**, *41*, 70–72.

[280] Yu, W.; Mao L.; Jiejing Qian, J.; Qian, W.; Meng, H.; Mai, W.; Tong, H.; Tong, Y.; Jin, J. *Ann. Hematol.* **2013**, *92*, 1091–1100.

[281] Zheng, C.; Cai, X.; Wu, S.; Liu, Z.; Shi, Y.; Zhou, W. *Pak. J. Med. Sci.* **2014**, *30*, 1270-1272.

[282] Gu, L.-F.; Zhang, W.-G.; Wang, F.-X.; Cao, X.-M.; Chen, Y.-X.; He, A.-L.; Liu, J.; Ma, X.-R. *J. Cancer Res. Clin. Oncol*. **2011**, *137*, 997–1003.

[283] Meng, H.; Yang, C.; Jin, J.; Zhou, Y.; Qian, W. *Leuk. Lymphoma*, **2008**, *49*, 524–530.

[284] Tang, J.; Liu, Y.; Chen, J.; Xue, H.; Pan, C.; Gu, L. *Int. J. Hematol.* **2011**, *93*, 610–617.

[285] Jin, J.; Jiang, D.-Z.; Mai, W.-Y.; Meng, H.-T.; Qian, W.-B.; Tong, H.-Y., Huang, J.; Mao, L.-P., Tong, Y.; Wang, L.; Chen, Z.-M., Xu, W.-L. *Leukemia* **2006**, *20*, 1361–1367.

[286] Xu, G.; Mao, L.; Liu, H.; Yang, M.; Jin, J.; Qian, W. *Int. J. Clin. Exp. Med.* **2015**, *8*, 19891–19894.

[287] Su, J. *Zhongguo Yaoye*-**2014**, *23*, 114–116.

[288] Chen, C.; Xu, W.; Yang, J.; *Leuk. Lymphoma* **2015**, *56*, 141–146.

[289] Tao, J.; Huang, Y.; Li H.-Q.; Wang,T.-T.; Wang, X.-Y.; Ji, L-X.; Yang, R.-C. *Chin. Med. J-Peking* **2007**, *120*, 1643–1646.

[290] Min, X.; Na, Z.; Yanan, L.; Chunrui, L. *J. Hematol. Oncol.* **2011**,*4*, 1–7.

[291] Xu, W.-L.; Jin, J.; Qian, W.-B. *Chin. Med. J.-Peking* **2010**,123, 108–110.

[292] Gu, L.-F.; Zhang, W.-G.; Wang, F.-X; Cao, X.-M.; Chen, Y.-X.; He, A.-L.; Liu, J.; Ma, X.-R. *J. Cancer Res. Clin. Oncol.* **2011**, *137*, 1563–1569.

[293] Wang, Y.; Lin, D.; Wei, H.; Li, W.; Liu, B.; Zhou, C.; Liu, K.; Mi, Y.; Wang, J. *Int. J. Hematol.* **2015**, *101*, 279–285.

[294] Wu, L.; Li, X.; Chang, C.; Xu, F.; He, Q.; Wu, D.; Zhang, Z.; Su, J.; Zhou, L.; Song, L.; Chaoa, X.; Zhao, Y. *Leuk. Lymphoma* **2015**, *57*, 1367–1374.

[295] Xiao, X.; Si, J.; Chen, S.; Gu, Y.; Zhu, L. *Zhonghua Shiyan He Linchuang Ganranbing Zazhi, Dianziban* (*Chinese J. Exp. Clin. Virol.*) **2014**, *8*, 95–98.

[296] Li, Y.; Deng, Z.; Zhu, J.; Ding, B.; Shi, Y.; Li, Y. *Haematologica* **2014**, *132*, 172–176.

[297] Akihiro, W.; Maki, H.; Kiyohito, Y.; Masuo, K. *Eur. J. Pharmaceut. Biopharmaceut.* **2015**, *89*, 232–238.

[298] Moirangthem, D. S.; Laishram, S.; Rana, V. S.; Borah, Jagat, C.; Talukdar, N. C. *Nat. Prod. Res.* **2015**, *29*, 1161–1165.

[299] Moirangthem, D. S.; Laishram, S.; Borah, J. C.; Kalita, M. C.; Talukdar, N. C. *BMC Compl. Altern. Med.* **2014**, 14:305, 1–10.

[300] Teng, J. *Faming Zhuanli Shenqing* CN 104306792, 2015.

[301] Wang, Y. *Faming Zhuanli Shenqing* CN 103990002, 2014.

[302] Gu, Y. *Faming Zhuanli Shenqing* CN 103721167 A 20140416, 2014.

[303] Zhao, L. *Faming Zhuanli Shenqing* CN 103610743 A 20140305, 2014.

[304] Sun, Z. *Faming Zhuanli Shenqing* CN 103550547 A 20140205, 2014.

[305] Fan, Y. Faming Zhuanli *Shenqing* CN 103520324 A 20140122, 2014.

[306] Dong, L. *Faming Zhuanli Shenqing* CN 105147940 A 20151216, 2015.

[307] Xia, F. *Faming Zhuanli Shenqing* CN 105193994 A 20151230, 2015.

[308] Xia, F *Faming Zhuanli Shenqing* CN 105106469 A 20151202, 2015.

[309] Li, F. *Faming Zhuanli Shenqing* CN 104324356 A 20150204, 2015.

[310] Wang, X. *Faming Zhuanli Shenqing* CN 104800457 A 20150729, 2015.

[311] Wang, X. *Faming Zhuanli Shenqing* CN 104784401 A 20150722, 2015.

[312] Ma, J. *Faming Zhuanli Shenqing* CN 104784351 A 20150722, 2015.

[313] Li, Y. *Faming Zhuanli Shenqing* CN 104474475, 2015.

[314] Huang, Y. *Faming Zhuanli Shenqing* CN 104784406, 2015.

[315] Huang, Y. *Faming Zhuanli Shenqing* CN 104784423, 2015.

[316] Zhu, J.; Kong, C.; Liu, F.; Wang, J.; Zhao, Z. *Faming Zhuanli Shenqing* CN 104758572, 2015.

[317] Li, M. *Faming Zhuanli Shenqing* CN 104721400, 2015.

[318] Chen, B. *Faming Zhuanli Shenqing* CN 104667025, 2015.

[319] Chu, L.; Jiang, Y.; Liu, X. *Faming Zhuanli Shenqing* CN 104623242, 2015.

[320] Yue, L.; Zhang, H.; Sui, A.; Zhang, Q.; Zheng, H.; Dong, Q. *Faming Zhuanli Shenqing* , CN 104888115, 2015.

[321] Wang, L. *Faming Zhuanli Shenqing* CN 105012613, 2015.

[322] Ha, S.E.; Ryu, J. S.; Jin, Mu H.; Jang, M. Y. *Repub. Korean Kongkae Taeho Kongbo* KR 2014092544, 2014.

[323] Wang, L. *Faming Zhuanli Shenqing* CN 105012651, 2015.

[324] Zhang, Y.*Faming Zhuanli Shenqing* CN 103947691, 2014.

[325] Zheng, Q. *Faming Zhuanli Shenqing* CN 105016437, 2015.

[326] Diastereoselective Synthesis of Cephalotaxus Esters via Asymmetric Mukaiyama Aldol Reaction, Ma, G.-Z.; Li, P.-F.; Liu, L.; Li, W.-D. Z.; Chen, L. Org. Lett. 2017, 19, 2250−2253.

[327] Chang, Y.; Meng, F.-C.; Wang, R.; Wang, C.M.; Lu, X.-Y.; Zhang, Q.-W. In *Studies in Natural Products Chemistry*, Atta-ur-Rahman Ed., Elsevier B.V. Volume 53, Chapter 10 - Chemistry, Bioactivity, and the Structure-Activity Relationship of Cephalotaxine-Type Alkaloids From *Cephalotaxus* sp. pp 339–373.