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Concise review on isolation, biological activity, structure elucidation, and total synthetic approaches of 16-membered C2-symmetric macrolide pyrenophorol

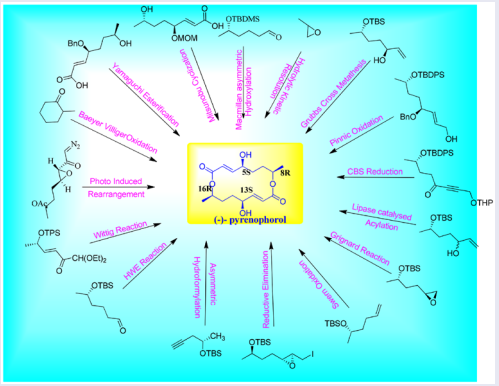
Bheemreddy Anusha[a], Raveendra Babu Kothapalli[b], Miriyala Victor Prem Sagar[c], Bairla Surendra[c] and Ummareddy Venkata Subba Reddy[c]

[a]Department of Chemistry, Silver Jubilee Govt. Degree College (A), Cluster University, Kurnool, Andhra Pradesh, India; [b]Department of Chemistry, SCIM Govt. College, Tanuku, Andhra Pradesh, India; [c]Department of Chemistry, DRG Govt. Degree College, Tadepalligudem, Andhra Pradesh, India

ABSTRACT

This review article provided isolation, biological activity, structural elucidation, and summary of the comprehensive synthesis of the sixteen-membered C2-symmetric macrolide dilactone pyrenophorol. Pyrenophorol has powerful antifungal activity against both *Microbotryum violaceum* and *Saccharomyces cerevisiae*, as well as potent antimicrobial activity against *Microbotryum violaceum*, *Chlorella fusca, Escherichia coli*, and *Bacillus megaterium*. It possesses selective phytotoxicity toward wild oats as well as notable anthelmintic properties. Due to the prominent biological activity of this natural product and its attractive C2-symmetric structure, numerous research groups have been attracted and reported the total synthesis of this natural product *via* vital organic reactions such as photo-induced rearrangement of an *α*-epoxy diazomethyl ketone to 4-hydroxy-2-alkenoate, intramolecular Wittig reaction, Sharpless asymmetric epoxidation, Grubb's cross metathesis, reductive elimination of iodoepoxide, Rh-catalysed Asymmetric Hydro-Formylation (AHF) tandem reaction, the Novozym 435-catalyzed acrylation, hydrolytic kinetic resolution, Grignard reaction, Swern oxidation, CBS reduction, Pinnick oxidation, Photo Induced rearrangement, Yamaguchi esterification, Baeyer-Villager oxidation, McMillan asymmetric hydroxylation, Horner–Wadsworth–Emmons olefination and Mitsunobu cyclization.

GRAPHICAL ABSTRACT



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CONTACT Ummareddy Venkata Subba Reddy ummareddyvsr@gmail.com Department of Chemistry, DRG Govt. Degree College, Tadepalligudem, Andhra Pradesh, India.

Introduction

Nature is a source of countless natural products with complex, fascinating structures and useful properties. Macrodiolides are one of the considerable classes of natural products, displaying interesting biological properties and chemical structures.[1–4] Naturally occurring macrodiolides, especially those isolated from various fungi and marine sponges, are an interesting family of secondary metabolites with pronounced biological activity. According to the basic skeleton, macrodiolides can be roughly divided into homodimers with C2 symmetry, which are constructed from head-to-tail dimerization of two identical fragments, and heterodimers with two different monomeric units. Amphodinolide X **1**, colletodiol **2**, colletol **3**, colletoallol **4**, grahamimycin A **5**, and grahamimycin A1 **6** are some examples of heterodimer macrodiolides (Figure 1), and vermiculine **7**, elaiophyline **8**, elaiolide **9**, helmidiol **10**, pyrenophorine **11** and (-)-pyrenophorol **12** are some examples of homodimer macrodiolides (Figure 2).

Amphidinolide X (**1**) is a 16-membered non-symmetric (heterodimer) macrodiolide isolated from a marine dinoflagellate *Amphidinium* sp. (strain Y-42)[5] and it showed strong cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells *in vitro* with IC_{50} values of 0.6 and 7.5 μgmL respectively. Colletodiol (**2**) was isolated as a metabolite from the plant fungi *Colletotrichum capsici, Chaetomium funicola*[6] and possesses mild antibiotic activity. Colletol (**3**), colletoallol (**4**) and colletoketol (**5**) were isolated from *C. capsici*.[7] Grahamimycin A (**6**) was isolated from the aerobic fermentation of cultures of *Cytospora* sp. ATCC 20502[8] showed significant activity against various pathogenic microorganisms (Figure 1).

(-)-Vermiculine (**7**) was isolated from the culture of *Penicillium vermiculatam*.[9] It showed potent inhibitory effects on the growth of gram-positive bacteria and some protozoa. Elaiophylin (**8**) was isolated from cultures of *Streptomyces melanosporus,*[10] and it showed potent anthelminthic activity against *Trichonomonas vaginalis*. (-)-Helmidiol (**10**) isolated from the *Alternaria alternate* showed anthelmintic properties.[11]

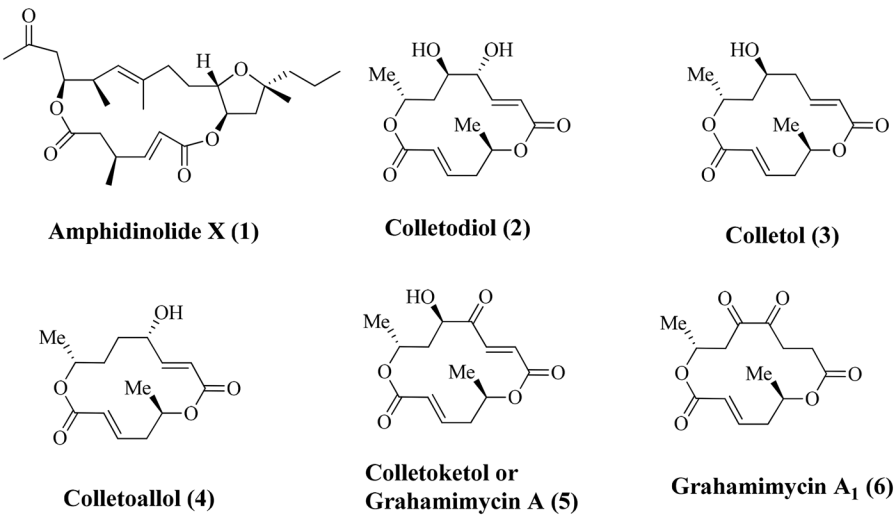


Figure 1. Heterodimer macrodiolides natural products.

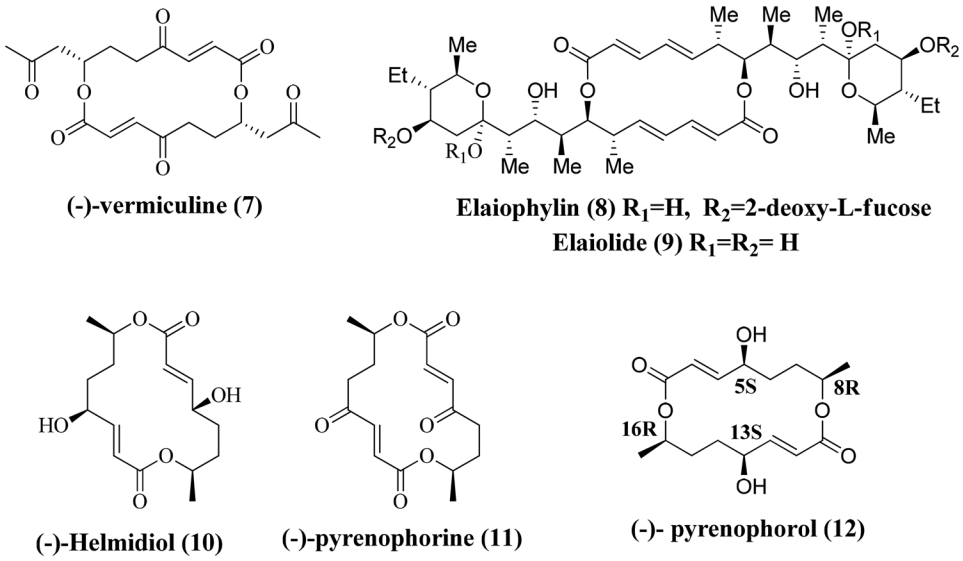


Figure 2. Homodimer macrodiolides natural products.

Isolation and bioactivity of (-)-pyrenophorol

16-Membered C_2-symmetric macrodiolide *i.e.,* (-)-pyrenophorol (**12**), an important secondary metabolite that has high-profile biological activity. Pyrenophorol is a fungal metabolite that has been found in Alternaria and has diverse biological activities. It inhibits human topoisomerase II α when used at concentrations of 75 and 100 µM. It is active against S. cerevisiae (MIC = 4 µM) and M. violaceum. Pyrenophorol induces leaf necrosis and chlorophyll retention in wild oats when used at a concentration of 64 µM.[2–4,11] Pyrenophorol induces leaf necrosis and chlorophyll retention in wild oats when used at a concentration of 64 µM. Pyrenophorol (**12**) was isolated from various sources by many research groups. It was first isolated from the fungi *Pyrenophora avenae*[2] and subsequently found in *Byssochlamys niveah,*[12] *Stemphylium radicinum,*[13] the imperfect fungus *Alternaria alternate,*[11] and later from the *Drechslera avenae* pathotype,[14] *Byssochlamys nivea,*[15] and from *Phoma sp.*,[16] an endophytic fungus that was isolated from *Lycium intricatum* from Gomera. Recently it was also isolated from *CBS 127938*[17] and within the crude extract of *L.nitens DAOM 250027.*[18]

The isomer (5*S*,8*R*,13*S*,16*R*)-(-)-pyrenophorol, which has been isolated from cultures of a Drechslera avenae pathotype with host specificity for wild oat (Avena sterilis), was found to be phytotoxic to A.*sterilis* but not to other related plant species.[14] It showed antimicrobial activity against *Microbotryum violaceum*, *Chlorella fusca*, *Escherichia coli*, and *Bacillus megaterium*.[16,19] In addition, it is a promising nootropic and antidepressant agent.[3,11] It also exhibits pronounced anthelmintic properties and is moderately active against the fungus *Microbotryum violaceum*.[3,11]

The structure and absolute configuration of (-)-pyrenophorol **12** were established by the Zwanenberg group through the first total synthesis.[20] Later, the absolute stereochemistry of (-)-pyrenophorol was confirmed by X-ray diffraction analysis (Figure 3).[15]

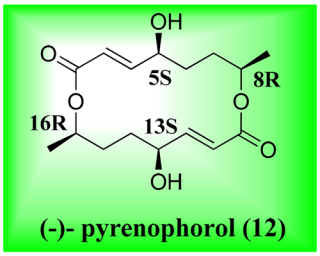


Figure 3. Absolute stereochemistry of (-)-pyrenophorol **12**.

Total synthesis and synthetic organic chemistry are the most prevalent areas in the industry, as the skills of making molecules can be utilized for designing an infinite array of translational applications in medicines, agrochemicals, and materials. From a fundamental perspective, total synthesis is a barometer and proving ground for new methodologies and new strategies or ways of thinking.[21]

Due to the high-profile biological activity of this natural product and its attractive C2-symmetric structure, many research groups have been attracted, and to date, fifteen approaches have been reported for the total synthesis of (-)-pyrenophorol through vital chemical reactions such as photo-induced rearrangement of an α-epoxy diazomethyl ketone to 4-hydroxy-2-alkenoate, intramolecular Wittig reaction, Sharpless asymmetric epoxidation, Grubbs cross-metathesis, reductive elimination of iodoepoxide, Rh-catalysed asymmetric hydroformylation (AHF) tandem reaction, the Novozym 435-catalyzed acrylation, hydrolytic kinetic resolution, Grignard reaction, Swern oxidation, CBS reduction, Pinnick oxidation, photo Induced rearrangement, Yamaguchi esterification, Baeyer-Villiger oxidation, MacMillan asymmetric hydroxylation, Horner–Wadsworth–Emmons olefination and Mitsunobu cyclization as key steps.

The reviews pertaining to the entire synthesis of natural products serve as a critical evaluation of synthetic methodologies, highlighting a wide range of strategies and stimulating innovative approaches. They consolidate complex knowledge, aiding in reproducibility and validation of reported syntheses. Such reviews inform drug discovery, catalyze interdisciplinary collaboration, and guide the development of more efficient and sustainable synthetic routes. In this review, we aim to assess the multiple synthetic pathways to achieve (-)-pyrenophorol. We anticipate that our analysis will provide valuable insights to synthetic chemists working with natural products, catalyzing innovative strategies for the total synthesis of biologically active compounds through diverse approaches.

Total synthetic approaches

To date, there have been fifteen synthetic approaches reported for the total synthesis of pyrenophorol.

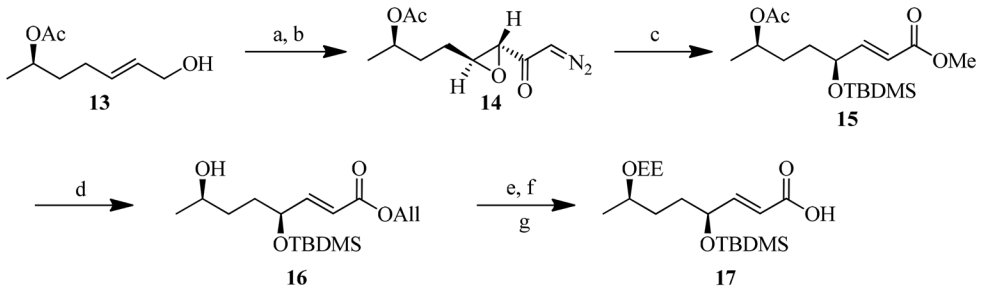
Zwanenburg's approach for total synthesis and structure elucidation of (-)-pyrenophorol 12

Zwanenburg et al.[20] reported the first stereoselective synthesis of (-)-pyrenophorol **12** using the photo-induced rearrangement of an *α, β*-epoxy diazomethyl ketone to

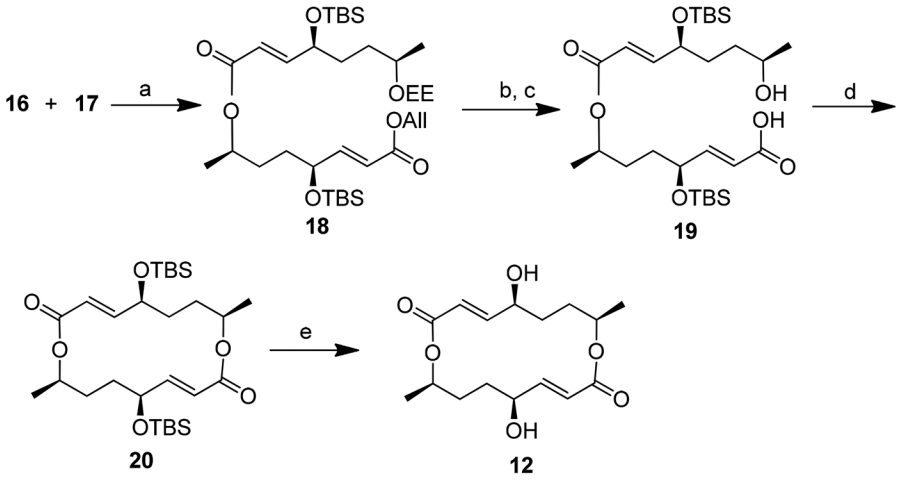
4-hydroxy-2-alkenoate as the key step. The key intermediate **14** was prepared from allyl alcohol **13** employing Sharpless epoxidation and subsequent oxidation to the homochiral 2,3-epoxy carboxylic acid and then conversion into the corresponding epoxydiazomethyl ketone. This *α,β*-epoxy diazomethyl ketone **14** was converted into 4-hydroxy-2-alkenoate through a photo-induced reaction, and secondary alcohol was protected as silyl ether to give compound **15**. The target molecule was obtained by two successive lactonization steps; for this purpose, compound **15** was converted into compounds **16** and **17** with free hydroxyl and carboxylic acid functions, respectively (Scheme 1).

These compounds **16** and **17** were coupled in the presence of the DCC and DMAP reaction conditions, resulting in compound **18**. Then the removal of EE and allyl-protecting groups of hydroxyl and acid functions from compound **18** was carried out to give the *seco*-product **19**. The final step was accomplished with the Yamaguchi macrolactonization of **19** gave the dilactone compound **20**, which, on desilylation, resulted in the target (-)-pyrenophorol **12** with an overall good yield. (Scheme 2).

(-)-Pyrenophorin **11** has also been isolated from culture filtrates of *stemphylium radicinum,*[2] which is structurally related to (-)-pyrenophorol **12**, and both have the *R*-configuration at C_8 and C_{16}. Based on the absolute configuration of pyrenophorin **11**, the authors first synthesized the (5*R*,8*R*,13*R*, 16*R*)-isomer of (-)-pyrenophorol and calculated its melting point, *i.e.,* 145–146 °C and specific rotation $[\alpha]^{D}_{20}$ −147°, which were not matched with the naturally isolated pyrenophorol data, *i.e.,* m. p 135 °C and specific rotation $[\alpha]^{D}_{20}$ −3°. At this stage, a comparison of these physical data reveals clearly that the stereochemistry of the synthesized pyrenophorol differs from that of the natural pyrenophorol. Then, the authors anticipated checking the data on pyrenophorol by changing the configuration at C_5 and C_{13} carbons. Then immediately, they synthesized the (5*S*,8*R*,13*S*,16*R*)-isomer of (-)-pyrenophorol **12**, and, pleasurably, its physical data, *i.e.,* the melting point 134–135 °C, specific rotation $[\alpha]^{D}_{20}$ −2.9°, and spectral data [IR: (KBr): 3382, 2924, 2854, 1713, 1647, 1274, 1173, 1119 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): *δ* 6.83 (dd, *J* = 15.6 Hz, 2H), 5.89 (dd, *J* = 15.6 Hz, 2H), 5.10–5.01 (m, 2H), 4.24–4.16 (m, 2H), 2.69–2.48 (m, 2H), 2.01–1.53 (m, 8H), 1.20 (dd, *J* = 6.8 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): *δ* 165.0, 149.3, 122.0, 70.3, 69.7, 30.4, 28.8, 18.2; MS (ESI): *m/z* = 335 [M + Na]. HRMS (ESI): *m/z* calcd for $C_{16}H_{24}O_6$: 313.1651; found: 313.1656] were in full agreement with those of the natural occurring



Scheme 1. Reagents and conditions a) L-(+)-DET, *t*-BuOOH, $Ti(O^iPr)_4$, molecular sieves, DCM; b) RuO_4, CH_3CN:CCl_4:H_2O = 2:2:3, $ClCOO^iBu$, Et_3N, CH_2N_2; c) hv, MeOH, TBDMSCl, imidazole, DCM; d) K_2CO_3, allyl-alcohol; e) NaOMe, MeOH, 0 °C; f) ethyl vinyl ether (EE), PPTS, DCM; g) LiOH, THF: H_2O = 1:1.



Scheme 2. Reagents and conditions a) DCC, DMAP, DCM; b) $MgBr_2$, Et_2O; c) $Pd(PPh_3)_4$, morpholine, THF; d) 2,6-$Cl_2C_6H_3C(O)Cl$, Et_3N, DMAP, toluene; e) *n*-Bu_4NF, THF.

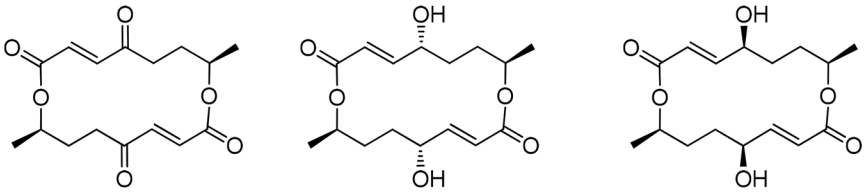


Figure 4. Identification of absolute configuration of (-)-pyrenophorol **12**.

pyrenophorol compound. This establishes the 5*S*,8*R*,13*S*, and 16*R* configurations of the natural product, *i.e.,* (-)- pyrenophorol **12** (Figure 4).

Kibayashi's approach

Kibayashi et al.[22] reported the second stereoselective synthesis of (-)- pyrenophorol **12** utilizing a C2 symmetric (*R*,*R*)-diepoxide **21**[23] as a starting enantiopure chiral building block. Monoepoxide was obtained effectively by the addition of 1 eq. of vitride to the compound **21**. This secondary alcohol was protected as silyl ether using TBDPSCl and DMAP in DCM to afford compound **22.** The addition of vinyl magnesium chloride (Grignard reagent) in the presence of CuI to compound **22** led to the epoxide opening to give the unsaturated alcohol.

The secondary alcohol was then protected with mesyl cholride and the terminal double bond was oxidized with RuO_4 resulting in carboxylic acid compound **23.** Treatment of compound **23** with potassium bicarbonate in aqueous methanol resulted in cyclization to give the *g*-lactone product as a single isomer. In this conversion, the stereochemistry at C-4 was inverted due to the SN2 reaction. This *g*-lactone compound

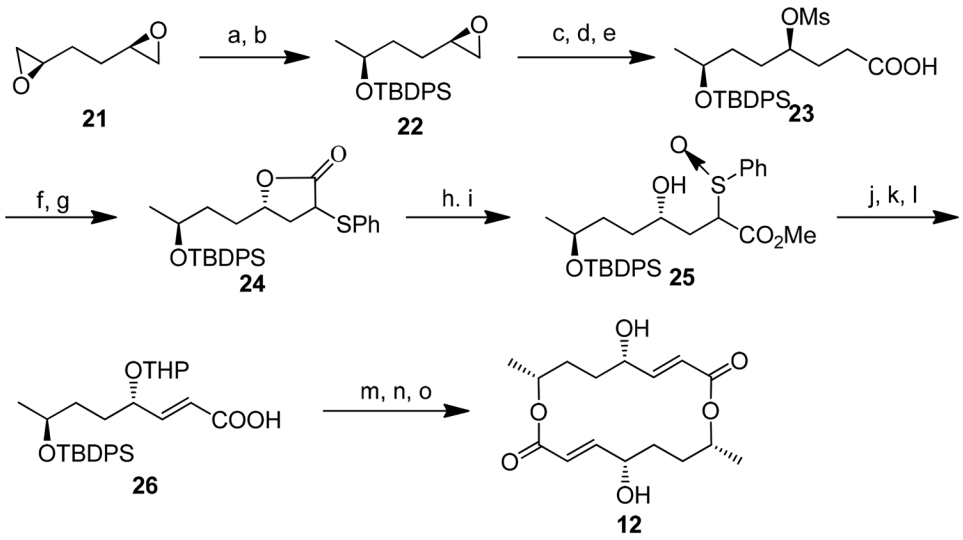
was treated with LHMDS and diphenyl disulfide to afford the α-phenylthio- δ-lactone compound **24** in good yield. It was then subjected to hydrolysis followed by diazomethane esterification to give the α–phenylthio-δ-hydroxyester and then converted to the sulfoxide **25** *via* oxidation with *m*-CPBA.

Compound **25** was treated with pyridine in refluxing toluene to generate the (*E*)-α, β-unsaturated hydroxy ester. After protecting the secondary alcohol as tetrahydropyranyl ether, the ester was hydrolyzed to give the compound **26**. Silyl ether of **26** was removed using TBAF and then hydroxyl carboxylic acid was exposed to the Mitsunobu reaction conditions, during which macro lactonization took place with complete inversion of chirality at C_4 to furnish the dimerized compound. Finally, the removal of the O-protecting groups provided the (-)-pyrenophorol **12** with an overall good yield (95%) (Scheme 3).

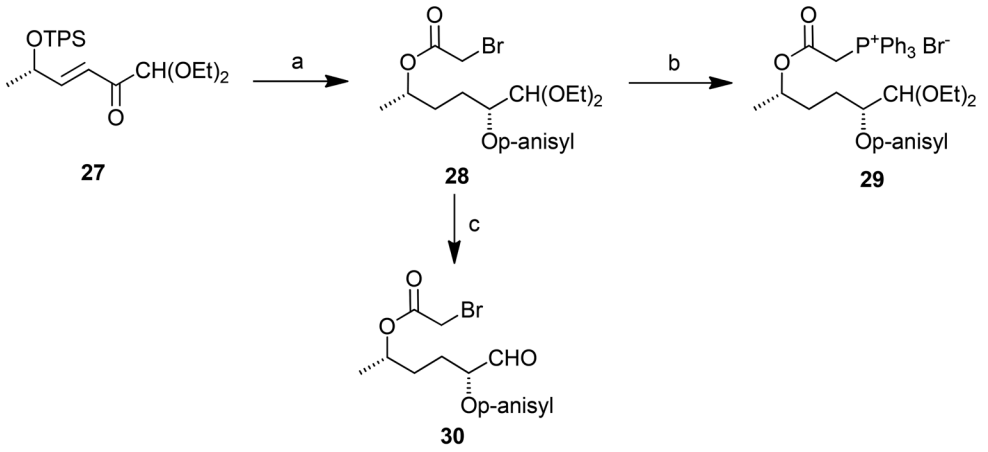
Le Floc'h's approach

The first total synthesis of the *(5R,8S,13R,16S)*-isomer of pyrenophorol **33** was reported by Le Floc'h and coworkers.[24] It is the enantiomer of isolated (-)- pyrenophorol **12**. This synthesis involved a cycle building by two successive Wittig reactions *i.e.*, intermolecular Wittig reaction and intramolecular Wittig reaction, respectively. They started from a key synthon **27** which was prepared from (*S*)-ethyl lactate.[25] Compound **28** was synthesized from **27** in five steps.[26]

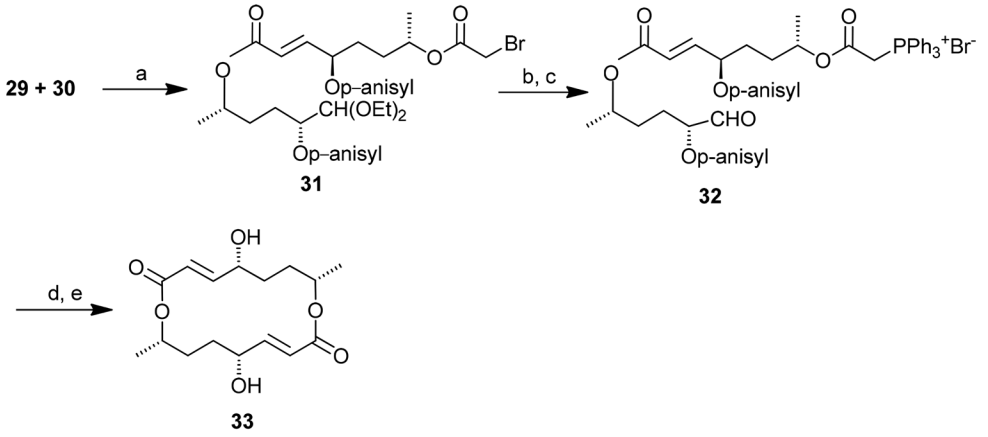
The phosponium salt **29** was obtained by treating compound **28** with triphenylphospine. The aldehyde **30** was obtained by formalizing the acetal function of



Scheme 3. Reagents and conditions: a) vitride (1 mol), THF, 0 °C - rt; b) TBDPSCl, DMAP, DCM, rt; c) vinyl magnesiumchloride, CuI, THF, -15 °C; d) MsCl, Et_3N, DCM, 0 °C; e) RuO_4, CCl_4-CH_3CN-H_2O, rt; f) $KHCO_3$, MeOH-H_2O, rt, 10 min; g) 1 M $LiN(SiMe_3)_2$, HMPA, THF, -78 °C, then PhSSPh, THF, -78 °C; h) 20% *aq*. NaOH, MeOH, rt, then CH_2N_2, Et_2O 0 °C; i) *m*-CPBA, -20 °C; j) Py (2.0 eq.), toluene. reflux; k) 2,3-dihydropyran, CSA. CH_2C1_2; l) 20% *aq*. NaOH, MeOH, rt; m) *t*-Bu_4NF, THF, reflux; n) Ph_3P. DEAD, toluene:THP (10:1). -25 °C, 10 h; o) TsOH-H_2O, MeOH, rt.



Scheme 4. Reagents and conditions: a) Reference;[23] b) PPh_3, CH_3CN, rt, 12h; c) HCOOH, rt, 2h.

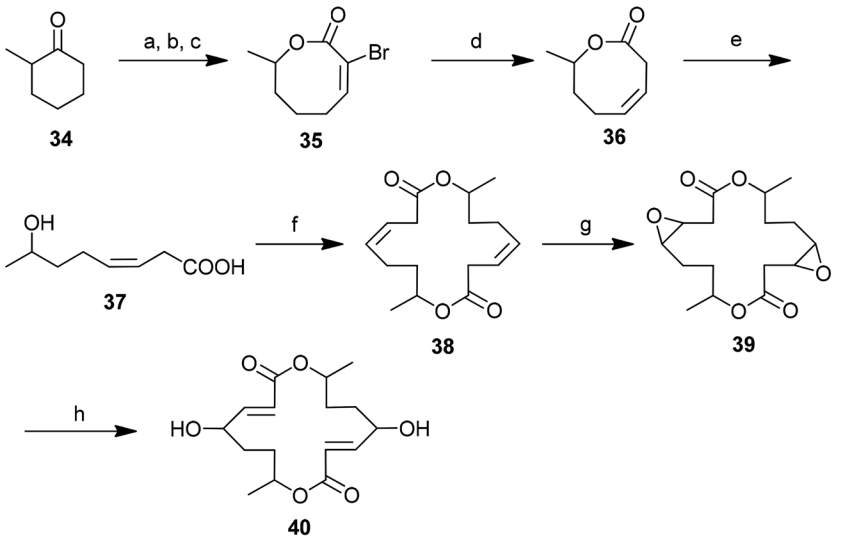


Scheme 5. Reagents and conditions: a) TEA, CH_3CN, 70 °C, 48 h, (75%); b) HCOOH, rt. 2 h; c) PPh_3, CH_3CN, rt, 12 h; d) TEA, CH_3CN, 70 °C, 42 h, (51%); e) CAN, CH_3CN/H_2O, -10 °C, 84%.

compound **28** (Scheme 4). The first Wittig reaction of salt **29** with aldehyde **30** in the presence of triethylamine resulted in the (*E*) olefin **31** in good yield, which comprised all the asymmetric carbons of the target molecule as well as the two ester functions. Again, the formalization of the acetal function of compound **31** followed by the addition of triphenylphospine afforded salt **32** in good yield. Compound **32** was subjected to an intramolecular Wittig reaction under high dilution conditions with triethylamine, which furnished the protected macrodiolide compound. Then, the removal of the ether function by CAN furnished target molecule **33**, which is the enantiomer of natural (-)-pyrenophorol **12** (Scheme 5).

Ohshiro's approach

Yoshiki Ohshiro et al.[27] reported the synthesis of (±) pyrenophorol **40** as an intermediate during the synthesis of (±) pyrenophorin.



Scheme 6. Reagents and conditions: a) *m*-CPBA; b) LDA, TMSCl; c): CBr_2, *t*-BuOK; d) $HPO(OEt)_2$, Et_3N, benzene, 80 °C, 3h; e) KOH, MeOH-H_2O, rt, 1h; f) DEAD, TPP; g) *m*-CPBA, DCM, 0 °C - rt, 3h; h) LDA, THF, -78 0 °C, 1h.

This synthesis started with 2-methyl cyclohexanone **34** which was subjected to Baeyer-Villiger oxidation conditions followed by the addition of dibromocarbene in the presence of LDA to give the compound **35**. Reductive deconjugation of compound **35** was carried out with diethyl phosphonate and triethylamine under reflux conditions resulting in compound **36** as a major product along with some minor products. Hydrolysis of **36** with KOH in a methanol-water medium afforded the *cis*-hydroxyl acid compound **37**. Cyclic dimer **38** was obtained by macro-lactonization of compound **37** under Mitsunobu reaction conditions. Epoxidation of the cyclic dimer with *m*-CPBA gave compound **39**, which was treated with LDA to furnish the (±) pyrenophorol **40** (Scheme 6).

Yadav's approach

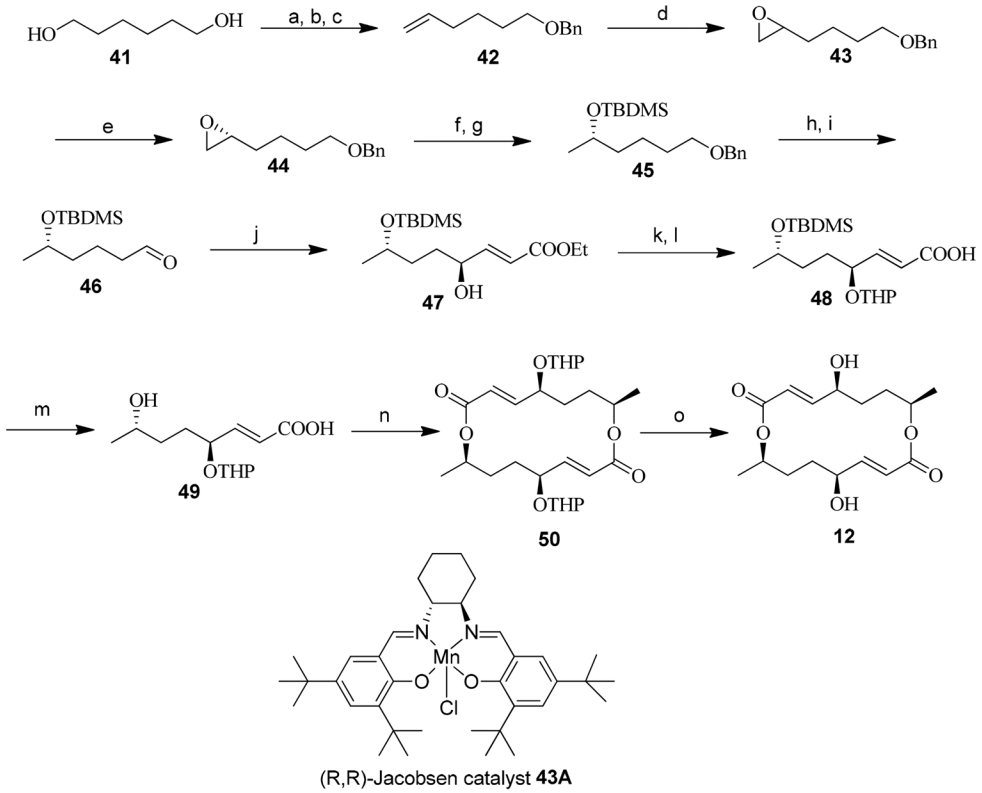
Our synthesis of (-)-pyrenophorol **12**[28] started with 1,6-hexane diol **41**. Accordingly, the selective protection of 1,6-hexanediol **41** with benzyl bromide in the presence of NaH and TBAI gave monobenzyl ether, which in turn was transformed into the iodo compound, followed by treatment with *t*-BuOK in THF to give the 5-hexen-1-ol **42** in good yield. The resulting olefin **42** was treated with *m*-chloroperoxybenzoic acid (*m*-CPBA) to give the racemic oxirane **43**. Compound **43** was hydrolyzed employing (*R, R*)-Salen-Co-(OAc) Jacobsen's catalyst to give the chiral epoxide **44**. The epoxide **44** was reduced with lithium aluminum hydride to generate the secondary alcohol, which was protected as its *tert*-butyldimethylsilyl ether **45**. Removal of the benzyl group with carbon-supported palladium afforded primary alcohol, followed by oxidation under Swern-oxidation conditions, gave the corresponding aldehyde **46**. Treatment of aldehyde **46** with D-proline and nitrosobenzene gave an intermediate α-oxyamino aldehyde with high levels of enantioselectivity utilizing α-oxidation. Olefination using

Horner-Wadsworth-Emmons conditions followed by cleavage of the aminoxy bond gave the γ-hydroxy-α,β-unsaturated ester **47**.

After protecting the secondary alcohol of enoate as the tetrahydropyranyl ether, the ester was hydrolyzed under basic aqueous conditions, giving compound **48**. Desilylation of **48** provided the hydroxycarboxylic acid **49**. Finally, compound **49** was subjected to Mitsunobu cyclization by Gerlach's procedure for the macrolactonization to take place with complete inversion of chirality at C_4 to furnish **50** in 58% yield. Removal of the THP group gave the target macrolide **12** with 98% yield as a white solid. (Scheme 7).

Young Kang's approach

H. Y. Kang et al.[29] reported the total synthesis of (-)-pyrenophorol **12** *via* regioselective epoxide ring-opening, Wittig reaction, Yamaguchi esterification as key reactions. They, started with vital chiral building block **52**, which was prepared from methyl



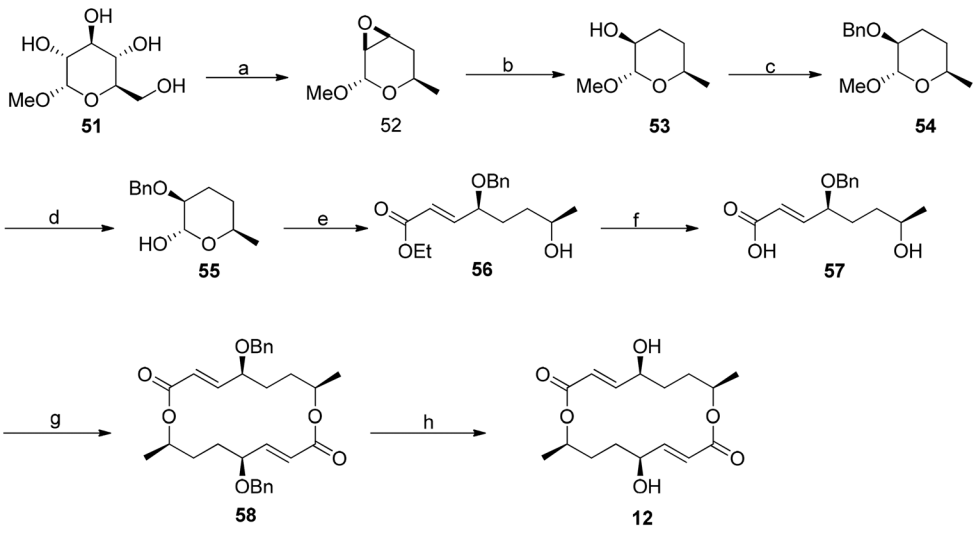
Scheme 7. Reagents and conditions: a) NaH/BnBr, THF, 0 °C – rt, 6h, 82%; b) TPP/I_2, imidazole/THF, 0 °C - rt, 1h, 98%; c) *t*-BuOK, THF, 0 °C - rt, 3h, 90%; d) *m*-CPBA, DCM, 0 °C - rt, 2h, 84%; e) (*R,R*)-jacobsen catalyst **43 A**, H_2O, rt, 12h, 45%: f) LAH, THF, 0 °C - rt, 30 min, 95%: g) TBDMSCl, imidazole, DCM, 0 °C - rt, 1h, 96%; h) 10% Pd/C, H_2, EtOAc, rt, 10h, 92%: i) $(COCl)_2$, DMSO, Et_3N, DCM, -78 °C, 1h, 80%; j) PhNO, D-proline, DMSO, $PO(OEt)_2CH_2CO_2Et$, DBU, LiCl, 0 °C, $Cu(OAC)_2$, NH_4Cl, MeOH, rt, 24h, 55% (one pot); k) 2,3-DHP/CSA, DCM, 0 °C - rt, 1h, 86%; l) 20% *aq*. NaOH, MeOH, rt, 30 min, 85%; m) Bu_4NF, THF, reflux, 2h, 90%; n) TPP/DEAD, toluene-THF(10:1), -25 °C, 10h, 58%; o) *p*-TSA, MeOH, rt, 30 min, 98%.

α-D-glucopyranoside **51**. The compound **51** was synthesized from α-D-glucose, in four steps by following the previous report.[30] Regioselective reductive opening epoxide **52** with LAH results in the alcohol compound **53**. The resulting chiral hydroxyl group was protected as a benzyl ether and then initial methyl acetal was hydrolyzed under acidic conditions providing the hydroxyl compound **55** in overall good yields.

The compound **55** was subjected to the Wittig olefination (Ph_3PCHCO_2Et, benzene) to yield the corresponding hydroxy ester **56** followed by hydrolysis afforded hydroxy acid **57** with good yield. The Yamaguchi esterification of **57** afforded the desired dimeric product **58** in very low yield (30%), along with a substantial amount of a trimeric product as a by-product. The authors tried various methods to improve the yield of desired product but ended with optimum yield when the concentration of **57** was about 0.02 M. The overnight treatment of **58** with DDQ certainly provided the desired (–)-pyrenophorol **12**, but as a mixture with the mono-benzylated product. Finally, they achieved the target **12** as a single product by treating it with $TiCl_4$ in CH_2Cl_2 with good yield (Scheme 8).

Yadav's approach

In 2012, Yadav et al.[31] again reported the total synthesis of (–)-pyrenophorol **12** employing Sharpless asymmetric epoxidation, olefin cross-metathesis, and intermolecular Mitsunobu cyclization. The synthesis started with lactate ester **59**, which was protected as its silyl ether using *tert*-butyldiphenylsilyl chloride (TBDPSCl) and imidazole. The reduction of the TBDPS ether of the lactate ester with diisobutylaluminum hydride (DIBAL-H) at −78 °C offered the corresponding aldehyde, which was then exposed to Wittig olefination with $Ph_3PCHCOOEt$ in benzene under reflux conditions to give the corresponding unsaturated ester **60** in good yield. Reduction of the double bond in **60** using $NaBH_4$ in the presence of $NiCl_2$ gave the saturated ester **61**.

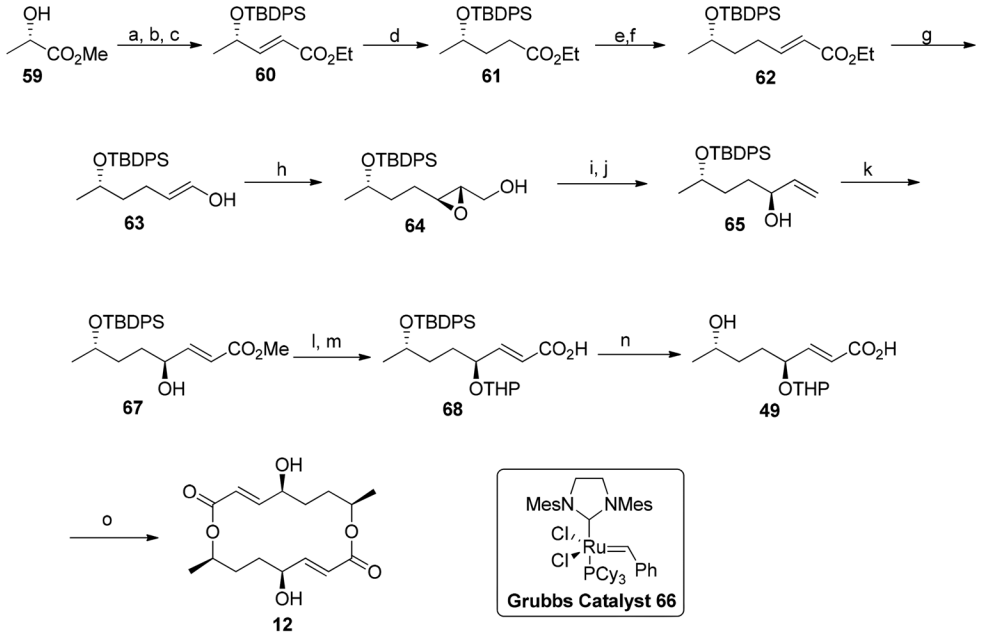


Scheme 8. Reagents and conditions: a) Reference;[30] b) $LiAlH_4$, ether; c) BnBr, NaH, *n*-Bu_4NI; d) H_2SO_4, AcOH, rt; e) Ph_3PCHCO_2Et, benzene; f) LiOH, THF, MeOH, H_2O; g) 2,4,6-$Cl_3C_6H_2COCl$, Et_3N, toluene, then DMAP, rt; h) $TiCl_4$, CH_2Cl_2.

Further reduction of ester **61** with DIBAL-H followed by Wittig olefination offered ester **62** in high yield. Yet again, the reduction of ester **62** using DIBAL-H gave the allylic alcohol **63**, which was then subjected to Sharpless asymmetric epoxidation to give the epoxy alcohol **64** in good yield. Iodination of **64** followed by epoxide opening with metallic zinc powder in refluxing MeOH afforded the secondary allylic alcohol **65**. Olefin cross metathesis of **65** with methyl acrylate gave the enoate **67** in good yield. Protection of the hydroxy group of enoate **67** as its tetrahydropyranyl ether (THP), followed by hydrolysis of the ester moiety under basic conditions gave the carboxylic acid **68**. Desilylation of **68** gave the key intermediate **49**. By following Reference,[28] key intermediate **49** was subjected to an intermolecular Mitsunobu cyclization, and finally, cleavage of the THP ether furnished the target macrolide **12** in overall good yield (Scheme 9).

Saroja's approach

Saroja et al.[32] reported the second total synthesis of an unnatural (5*R*,8*S*,13*R*,16*S*) isomer of pyrenophorol **33** *via* hydrolytic kinetic resolution, Sharpless epoxidation, reductive elimination of chloro-epoxide, and Mitsunobu reaction as key reactions. The authors started with a total synthetic approach with known epoxide **69**. The reaction of **69** with allyl magnesium chloride in ether and subsequent silylation of the resulting secondary

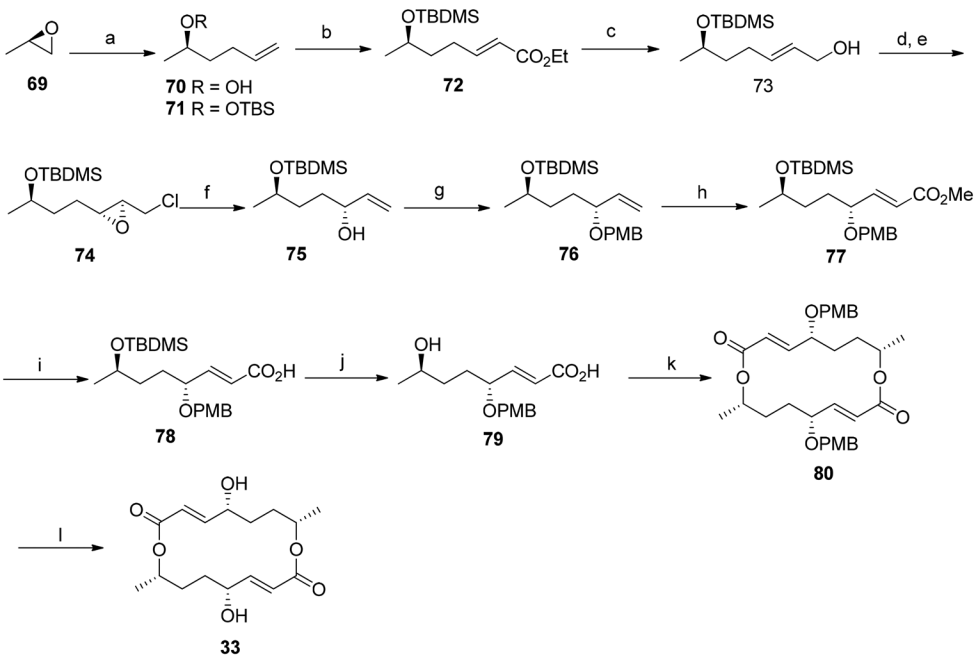


Scheme 9. Reagents and conditions: (a) TBDPSCl, imidazole, CH_2Cl_2, 0 °C to rt, 30 min, 98%; (b) DIBAL-H, CH_2Cl_2, -78 °C, 20 min, 80%; (c) $Ph_3PCHCOOEt$, benzene, reflux, 1 h, 92%; (d) $NiCl_2{\cdot}6H_2O$, $NaBH_4$, MeOH, 0 °C, 1 h, 90%; (e) DIBAL-H, CH_2Cl_2, -78 °C, 15 min, 82%; (f) $Ph_3PCHCOOEt$, benzene, reflux, 1 h, 94%; (g) DIBAL-H, CH_2Cl_2, -78 to 0 °C, 1 h, 92%; (h) L-(+)-DIPT, *t*-BuOOH, $Ti(O^iPr)_4$, CH_2Cl_2, -20 °C, 7 h, 78%; (i) I_2, imidazole, Ph_3P, THF:MeCN (4:1), 0 °C to rt, 30 min, 92%; (j) Zn, MeOH, reflux, 12 h, 82%; (k) methyl acrylate, grubbs catalyst **66**, CH_2Cl_2, reflux, 24 h, 78%; (l) 3,4-dihydropyran, CSA, CH_2Cl_2, r.t, 1h, 88%; (m) 20% aq. NaOH, MeOH, r.t, 30 min, 85%; (n) TBAF, THF, 60 °C, 2 h, 90%; (o) Reference.[28]

alcohol **70** with TBDMSCl, imidazole gave **71** in overall good yield. Ozonolysis of **71** and Wittig olefination of the resultant aldehyde yielded **72**, which was reduced with DIBAL-H to produce allylic alcohol **73**. Sharpless epoxidation of **73** with cumene hydroperoxide and $Ti(OiPr)_4$ gave the corresponding epoxy alcohol, which was further treated with Ph_3P, $NaHCO_3$, and CCl_4 under refluxing conditions and yielded **74**. Reductive elimination of chloro-epoxide **74** with Na in dry ether afforded alcohol **75**, further treatment of **75** with NaH and *p*-methoxy benzyl bromide at 0 °C gave the PMB ether **76** in good yield. Ozonolysis of **76** in CH_2Cl_2 gave the corresponding aldehyde, which, on Wittig reaction, gave ester **77** in 76% yield. The ester compound **77** on hydrolysis afforded acid **78**, which on desilylation afforded the hydroxy-acid **79** in good yield. Finally, cyclodimerization of hydroxy-acid **79** under Mitsunobu reaction conditions yielded dimer compound **80**, followed by oxidative deprotection with DDQ, which yielded an enantiomer of pyrenophorol **33** in good yield (Scheme 10).

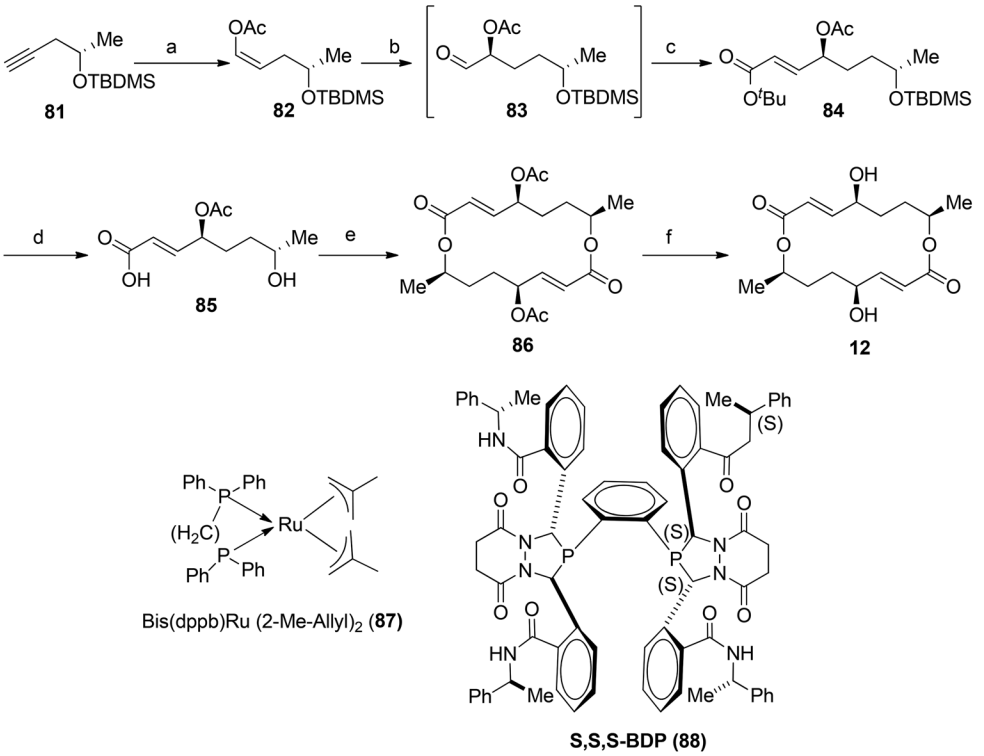
Burke's approach

Steven Bruke et al.[33] established the various Rh-catalysed asymmetric hydroformylation (AHF) tandem reactions in the context of the total synthesis of various natural products in a short and efficient route. The authors have achieved the total synthesis of (-)-pyrenophorol **12** in just five steps using Ru (II)-catalysed *Z*-selective *anti*-Markovnikov



Scheme 10. Reagents and conditions: (a) i) allyl chloride, Mg, dry ether, -78 °C, 2 h; ii) TBDMSCl, imidazole, CH_2Cl_2, rt, 4 h; (b) i) O_3, CH_2Cl_2, -78 °C, 30 min; ii) $Ph_3P{=}CHCOOEt$, CH_2Cl_2, rt, 4 h; (c) DIBAL-H, CH_2Cl_2, -78 °C, 2 h; (d) (*L*)-DIPT, MS (4 A°), cumene hydroperoxide, $Ti(O^iPr)_4$, CH_2Cl_2, -20 °C, 3 h; e) Ph_3P, $NaHCO_3$, CCl_4, reflux, 30 min; f) Na, ether, rt, 12 h; (g) PMBBr, NaH, THF, 0 °C to rt, 8 h; (h) i) O_3, CH_2Cl_2, -78 °C, 15 min; ii) $Ph_3P{=}CHCOOMe$, benzene, reflux, 2 h. (i) LiOH, THF:MeOH:H_2O (3:1:1), rt, 4 h; (j) TBAF, THF, 0 °C to rt, 3 h; (k) Ph_3P, DEAD, toluene:THP (10:1) -25 °C, 10 h; (l) DDQ, CH_2Cl_2:H_2O (19:1), rt, 3 h.

hydroacetoxylation of terminal alkynes followed by asymmetric hydroformylation (AHF) and Wittig olefination sequences to rapidly establish functionality and stereogenicity. They began with Ru (II)-catalysed hydroacetoxylation of known alkyne **81**, which resulted in high yields and outstanding stereoselectivity (97%) of *Z*-enol acetate **82**. A novel selective route to (*Z*)-alk-1-en-1-yl esters is provided by the direct addition of carboxylic acids to terminal alkynes in the presence of catalytic quantities of (bis(diphenylphosphino)butane)Ru(2-Me-allyl)$_2$ **87** complexes.[34] The asymmetric hydroformylation (AHF) of **82** with Rh(acac)(CO)$_2$ complex with (*S*,*S*,*S*)-BDP **88** in THF, and MeCN under 150 psi CO/H$_2$ afforded *α*-acetoxy aldehyde **83** as the sole product, which was confirmed by ^{1}H NMR. The unsaturated *tert*-butyl ester **84** was obtained in 98% yield by direct Wittig olefination of crude aldehyde **83** with *tert*-butyl (triphenylphosphoranylidene) acetate. Simultaneous deprotection of both the TBDMS ether and *tert*-butyl ester of **84** was achieved with Montmorillonite K-10 Clay50 in refluxing MeCN to give hydroxy acid **85** with excellent yield. Head-to-tail Mitsunobu dimerization of the hydroxy acid **85** afforded diacetylpyrenophorol **86** with a moderate yield by slow addition of the hydroxy acid **85** to a solution of *di-tert*-butylazodicarboxylate (DBAD) and Ph$_3$P at a 5 mM concentration. Finally, enzymatic hydrolysis of the acetates with *Pseudomonas fluorescens* lipase under neutral conditions produced (-)-pyrenophorol **12** in good yield (Scheme 11).



Scheme 11. Reagents and conditions: (a) 5.3 mol% (dppb)Ru(2-Me-allyl)2, AcOH/PhMe, 92% (97% *Z*); (b) 0.28 mol% Rh(acac)(CO)2, 0.37 mol% (*S*,*S*,*S*)-BDP 88, THF, MeCN, 150 psi CO/H2, 67 °C; (c) Ph3P = CHCO2*t*Bu, DCM, rt, 98% (95% *E*); (d) Montmorillonite K-10, MeCN, reflux, 97%; (e) DBAD, PPh3, THF, 48%; (f) *Pseudomonas fluorescens* lipase, PH = 7 buffer, 77%.

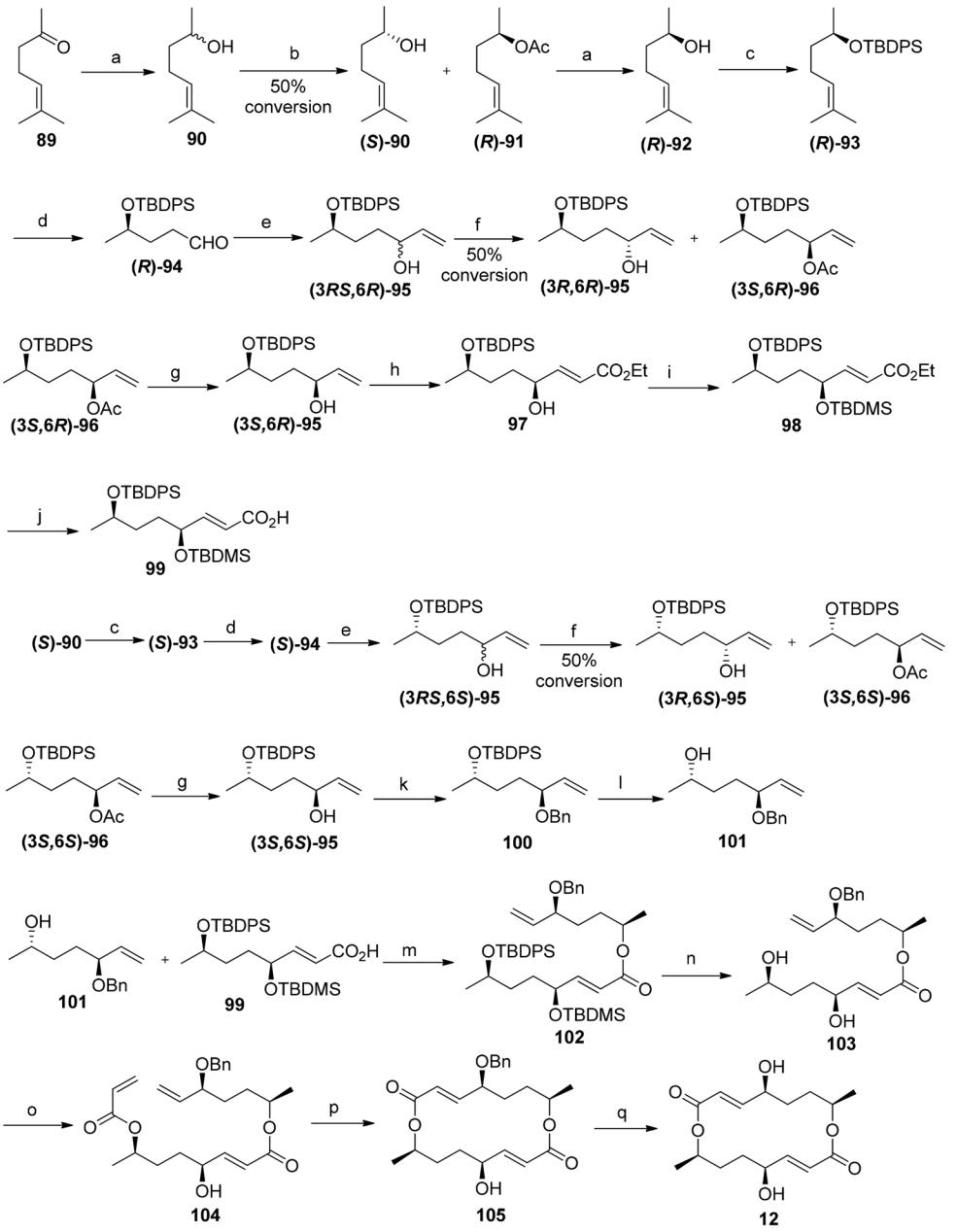
Chatopadhyay's approach

Chatopadhyay et al.[35] synthesized the hept-6-ene-2,5-diol derivatives using lipase-catalysed acylation reactions as the key intermediates in the total synthesis of (-)-pyrenophorol **12**. They started synthesis with the reduction of commercially available 6-methyl-5-hepten-2-one **89** with $LiAlH_4$ to furnish the racemic alcohol **90**. The racemic alcohol **90** was efficiently resolved by the Novozyme 435 enzyme. The (*R*)-acetate **91** was obtained in >98% enantiomeric excesses after 50% conversion, along with minor (*S*)-**90**. The compounds **90** and **91** were easily separated by column chromatography, followed by deacetylation of **91** with $LiAlH_4$, which yielded the alcohol **92**. The alcohol **92** was silylated with *tert*-butyldiphenylsilyl chloride (TBDPSCl) in the presence of imidazole and DMAP-furnished **93**. The olefin function of **93** was subjected to reductive ozonolysis (O_3/Ph_3P) to get the aldehydes (*R*)-**94**. The reaction of (*R*)-**94** with commercially available vinyl magnesium bromide furnished the allylic alcohol (3*RS*,6*R*)-**95** as a 1:1 mixture of C-3 epimers. Again, the Novozym 435-enzyme-catalyzed acetylation of **95** produced (3*R*,6*R*)-**95** and (3*S*,6*R*)-**96** in >98% *ee* at 50% conversion, which were easily separated by column chromatography. Deacetylation of **96** under basic conditions gave (3*S*, 6*R*)-**95**. The isomer (3*S*, 6*R*)-**95** was subjected to a cross-metathesis reaction with ethyl acrylate in the presence of Hoveyda Grubbs' II catalyst to furnish **97**. In the presence of imidazole and DMAP, the alcohol group was silylated with *tert*-butyldimethylsilyl chloride (TBDMSCl) to make **98**. The basic-mediated hydrolysis of **98** yielded the acid compound **99**.

Silylation of (S)-**90** with *tert*-butyldiphenylsilyl chloride (TBDPSCl) gave (S)-**93**, which was further subjected to reductive ozonolysis (O_3/Ph_3P) to get the aldehyde (*S*)-**94**. The reaction of (*S*)-**94** with commercially available vinyl magnesium bromide furnished the allylic alcohol (3*RS*,6*S*)-**95** as a 1:1 mixture of C-3 epimers. Again, Novozym 435-enzyme-catalyzed acetylation of (3*RS*,6*S*)-**95** furnished (3*R*,6*S*)-**95** and (3*S*,6*S*)-**96** in high *ee* at 50% conversion, which were easily separated by column chromatography. Deacetylation of (3*S*,6*S*)-**96** in basic conditions yielded (3*S*,6*S*)-**95**, which was benzylated with BnBr in the presence of NaH as the base to furnish **100**, which, on desilylation with Bu_4NF, furnished the alcohol **101**. Finally, esterification of **101** with **99** under the Mitsunobu reaction conditions afforded the ester **102**. Desilylation of **102** with aqueous HF in CH_3CN afforded the diol **103**. The Novozym 435 enzyme-catalyzed acrylation of **103** with ethyl acrylate furnished the desired acrylate **104** exclusively as a single compound. In conclusion, the RCM reaction of **104** with Grubbs' II catalyst to obtain **105** in moderate yield as a single isomer with E-olefin Lastly, $TiCl_4$-catalysed debenzylation of **105** completed the total synthesis of the target (-)-pyrenophorol **12** in an overall good yield (Scheme 12).

Raju's approach

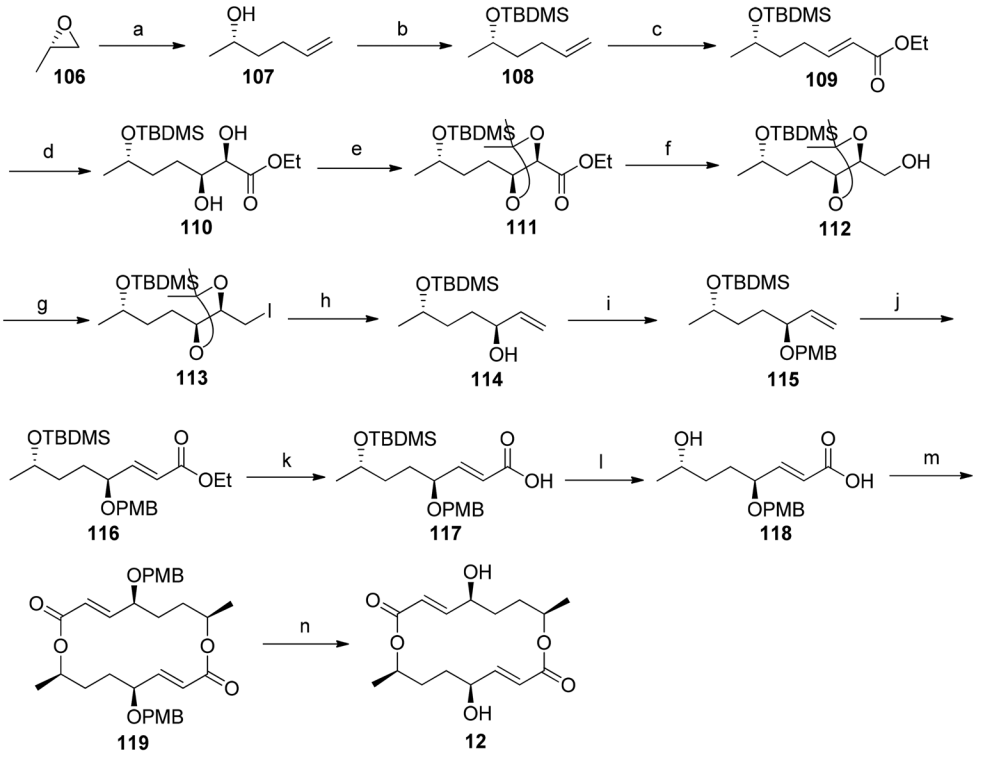
Raju et al.[36] reported the total synthesis of (-)-pyrenophorol **12** beginning with propylene oxide *via* the essential reaction of cross-metathesis. In ether, they combine allyl magnesium chloride and epoxide **106** to initiate the synthesis. The alcohol **107** is then silylated with TBDMSCl and imidazole to produce **108**. Then, **108** was ozonolyzed at −78 °C for 30 minutes in DCM to produce the corresponding aldehyde. This aldehyde was then



Scheme 12. Reagents and conditions: (a) $LiAlH_4$, Et_2O, 0 °C, 2h; (b) novozym 435, vinyl acetate, hexane, 50 min; (c) TBDPSCl, imidazole, DMAP, CH_2Cl_2, 25 °C, 7 h; (d) O_3, CH_2Cl_2, -78 °C, 1.5 h; Ph_3P, -78 to 25 °C, 18 h; (e) CH_2=$CHMgBr$, THF, -78 °C, 3 h, (f) Novozym 435, vinyl acetate, 25 °C, 6 h; (g) K_2CO_3, MeOH, 25 °C, 6 h; (h) CH_2=$CHCO_2Et$, hoveyda grubbs' II catalyst, CH_2Cl_2, 25 °C, 3 h; (i) TBDMSCl, imidazole, DMAP, CH_2Cl_2, 25 °C, 7 h; (j) aqueous 20% NaOH, MeOH, 25 °C, 2 h; (k) NaH, BnBr, Bu_4NI, THF, reflux, 4 h; (l) Bu_4NF, THF, 0 to 25 °C, 8 h; (m) Ph_3P, DIAD, THF, 0 to 25 °C, 18 h; (n) aqueous HF, MeCN, 25 °C, 16 h; (o) CH_2=$CHCO_2Et$, novozyme 435, diisopropyl ether, 25 °C, 30 h; (p) grubbs' II catalyst, CH_2Cl_2, reflux, 72 h; (q) $TiCl_4$, CH_2Cl_2, 0 to 25 °C, 0.5 h.

olefinated with (ethoxycarbonylmethylene) triphenylphosphorane in DCM at 25 °C to produce **109** with a high yield. The sharpless asymmetric dihydroxylation of ester **109** with AD-mix- α in the presence of methanesulfonamide in *t*-BuOH/H_2O (1:1) at 0 °C afforded diol **110** in high yield, which was subsequently treated with 2,2-dimethoxypropane in the presence of catalytic amounts of *p*-TSA to afford acetonide **111** in good yield.

The ester **111** was reduced with DIBAL-H in anhydrous DCM at 0 °C to produce the primary alcohol **112**, which was then converted to the iodide **113** using I_2, PPh_3, and imidazole. Reductive fragmentation of iodide **113** in ethanol with zinc powder produced *S*-allylic alcohol **114**, which was then protected at 0 °C with *p*-methoxybenzyl bromide to produce PMB ether **115**. The olefin **115** was subjected to cross-metathesis with ethyl acrylate using Grubb's II catalyst in DCM at reflux temperature for 12 hours, resulting in the formation of the *trans*-unsaturated ester **116**, which was then hydrolyzed under basic conditions to produce the acid **117**. The hydroxy acid **118** was produced in high yield by desilylation of **117** with TBAF in anhydrous THF. Therefore, cyclodimerization of hydroxy-acid **118** under Mitsunobu reaction conditions with Ph_3P and DEAD at −25 °C for 10 hours produced **119** with a moderate yield. The oxidative deprotection of PMB groups of **119** with DDQ in aqueous DCM: H_2O (19:1) yielded (5*S*,8*R*,13*S*,16*R*)-pyrenophorol **12** as a white solid (Scheme 13).



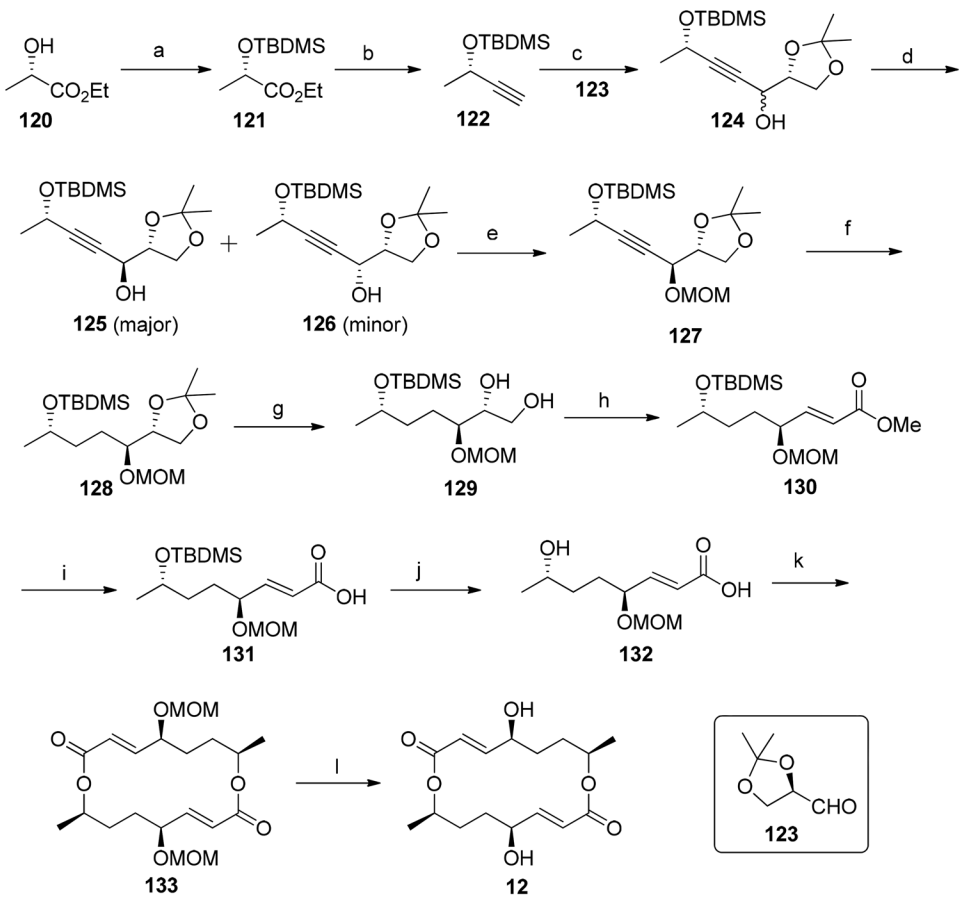
Scheme 13. Reagents and conditions: (a) allyl chloride, Mg, dry ether, -78 °C, 2 h; (b) TBDMSCl, imidazole, CH_2Cl_2, 25 °C, 4 h; (c) i) O_3, CH_2Cl_2, -78 °C, 30 min; ii) $Ph_3P{=}CHCO_2CH_2CH_3$, CH_2Cl_2, 25 °C, 4 h; (d) AD-mix-*α*, *t*-BuOH/H_2O, 0 °C, 24 h; (e) *p*-TSA, 2,2-DMP, CH_2Cl_2, 1.5 h; (f) DIBAL-H, CH_2Cl_2, 0 °C to 25 °C, 2 h; (g) I_2, imidazole, THF, -40 °C to 25 °C, 1 h; (h) Zn, EtOH, reflux, 3 h; (i) PMBBr, NaH, THF, 0 °C to 25 °C, 8 h; (j) Grubbs-II catalyst (10 mol%), ethyl acrylate, CH_2Cl_2, reflux, 12 h; (k) LiOH, THF:MeOH:H_2O (3:1:1), 25 °C, 4 h; (l) TBAF, THF, 0 °C to 25 °C, 3 h; (m) Ph_3P, DEAD, toluene:THP (10:1) -25 °C, 10 h; (n) DDQ, CH_2Cl_2: H_2O (19:1), 25 °C, 3 h.

Ashok's approach

Ashok et al.[37] reported the total synthesis of (-)-pyrenophorol **12** from commercially available (*S*)-ethyl lactate *via* the oxidation-reduction protocol and cyclodimerization under the Mitsunobu reaction conditions as key steps. Protection of the hydroxyl group of commercially available chiral (*S*)-ethyl lactate **120** with TBDMSCl and imidazole in DCM gave ester **121**. Then, reduction of ester **121** with DIBAL-H at − 78 °C in DCM to give the respective aldehyde, which on treatment with dimethyl (2-oxopropylphosphonate), tosyl azide, and K_2CO_3 (acetonitrile, methanol) at 0 °C afforded the known alkyne compound **122**. The reaction of alkyne **122** with *n*-BuLi in THF at −78 °C employed the acetylene anion, which was immediately reacted with aldehyde **123** to give **124** with 20% de. To enhance diastereoselectivity with the desired stereocenter (opposed to the existing one), they have employed an oxidation-reduction protocol. Propargylic alcohol **124** was oxidized using Dess-Martin periodinane in dry DCM at 0 °C yielded the corresponding keto compound, which was selectively reduced using $Zn(BH_4)_2$ furnished alcohols **125** (major) and **126** (minor) in an 82% yield with good diastereoselectivity (74% de). The two alcohols were easily separated by column chromatography. Subsequently, the major isomer **125** was protected as a MOM ether by reacting with MOM-Cl in the presence of DIPEA and DMAP in dry DCM, yielding compound **127** in a high yield. Reduction of compound **127** using H_2/Pd–C in MeOH resulted in the formation of compound **128**. The acetonide protection of **128** was then removed by treating with *aq*. 60% acetic acid yielded the diol **129**. Oxidative cleavage of diol **129** with $NaIO_4$ and $NaHCO_3$ followed by Wittig olefination of the resulting aldehyde afforded **130** in good yield. Ester **130** was subjected to hydrolysis in a basic medium resulting in the formation of acid **131** which was then desilylated utilizing TBAF in dry THF, yielded the hydroxy-acid **132** with a good yield. Hydroxy-acid **132** was subjected to cyclodimerization under the Mitsunobu reaction conditions resulting in cyclized compound **133** with an overall yield of 53%. Finally, the MOM protection on compound **133** was eliminated using 10% HCl, resulting in the formation of (−)-pyrenophorol **12** as a white solid (Scheme 14).

Sridhar's approach

In 2018, Sridhar et al.[38] reported the total synthesis of (-)-pyrenophorol **12**, commencing with the meso (*R, S*)-*di*-epoxide **134** (1-((*R*)-oxirane-2-yl)-2-((*S*)-oxirane-2-yl) ethane).[39] The *di*-epoxide **134** was opened regio-selectively with DIBAL-H in DCM at 0 °C to RT, and silylation of the resulting secondary alcohol **135** with TBDMSCl and imidazole in DCM resulted in a high yield of **136**. Later, the second epoxide ring in compound **136** was also opened with trimethyl sulfonium iodide in the presence of *n*-BuLi in THF at 0 °C to produce the allylic alcohol **114**, which, when treated with NaH and *p*-methoxybenzyl bromide at 0 °C, produced the PMB ether **115** in high yield. Then, ozonolysis of olefin **115**, followed by Wittig olefination of the resulting aldehyde, afforded, *α,β*-unsaturated ester compound **137** with a satisfactory overall yield. After ester **137** was made, the authors used the same sequence of reactions as in the previous report,[34] such as hydrolysis of the ester, desilylation of the

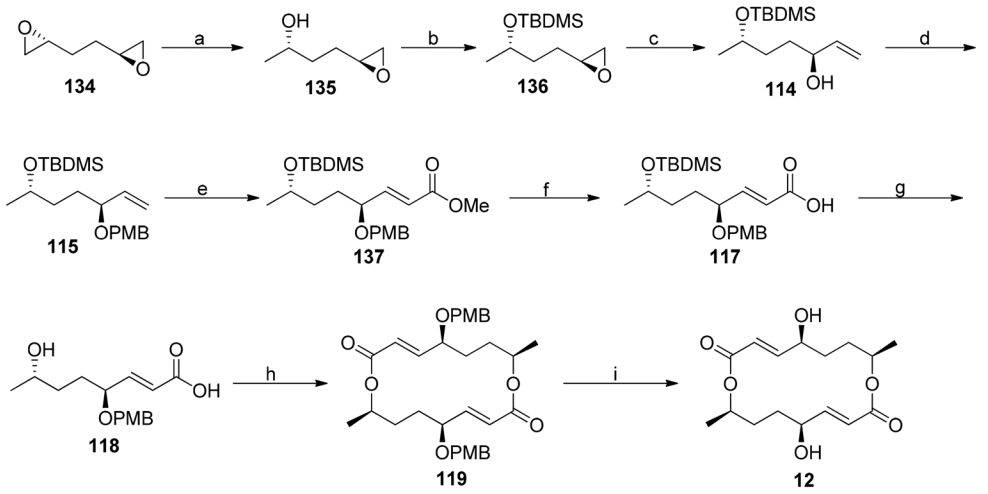


Scheme 14. Reagents and conditions: (a) TBDMSCl, imidazole, CH_2Cl_2, rt, 4h; (b) i) DIBAL-H, CH_2Cl_2, -78 °C, 1 h; ii) dimethyl(2-oxopropyl)phosphonate, tosyl azide, K_2CO_3, acetonitrile: methanol (2:1), 0 °C to rt, 8 h; (c) *n*-BuLi, dry THF, -78 °C, **123**, 3 h; (d) i) dess–martin periodinane, CH_2Cl_2, 0 °C to rt, 4 h; ii) $Zn(BH_4)_2$, ether, -30 °C, 3 h; (e) MOMCl, DIPEA, DMAP, CH_2Cl_2, rt, 6 h; (f) 10% Pd/C, H_2, MeOH, 12 h; (g) *aq*. 60% acetic acid, rt, 12 h; (h) i) $NaIO_4$, sat. $NaHCO_3$ soln., CH_2Cl_2, rt, 5 h; ii) $Ph_3P{=}CHCOOMe$, benzene, reflux, 2 h; (i) LiOH, THF:MeOH:H_2O (3:1:1), rt, 4 h; (j) TBAF, THF, 0 °C to rt, 3 h; (k) Ph_3P, DEAD, toluene: THP (10:1) -25 °C, 10 h; (l) 10% *aq*. HCl, THF, 0 °C to rt, 5 h.

TBS ether with TBAF, cyclodimerization under the conditions of the Mitsunobu reaction. Finally, oxidative deprotection of the PMB groups with DDQ produced the target molecule (-)-pyrenophorol **12** as a white solid with a good overall yield (Scheme 15).

Raju's approach

In 2015, Raju et al.[36] reported the total synthesis of (-)-pyrenophorol **12**, and again, in 2018,[40] the same team reported the total synthesis of (-)-pyrenophorol **12** using propylene oxide as a starting compound. The hydrolytically kinetically resolving epoxide **138** with Jacobson (*S*,*S*)-catalyst **139**, yielding the known (*S*)-epoxide **106** in 43% yield and (*R*)-diol **140** in 39% yield. The epoxide **106** was opened with



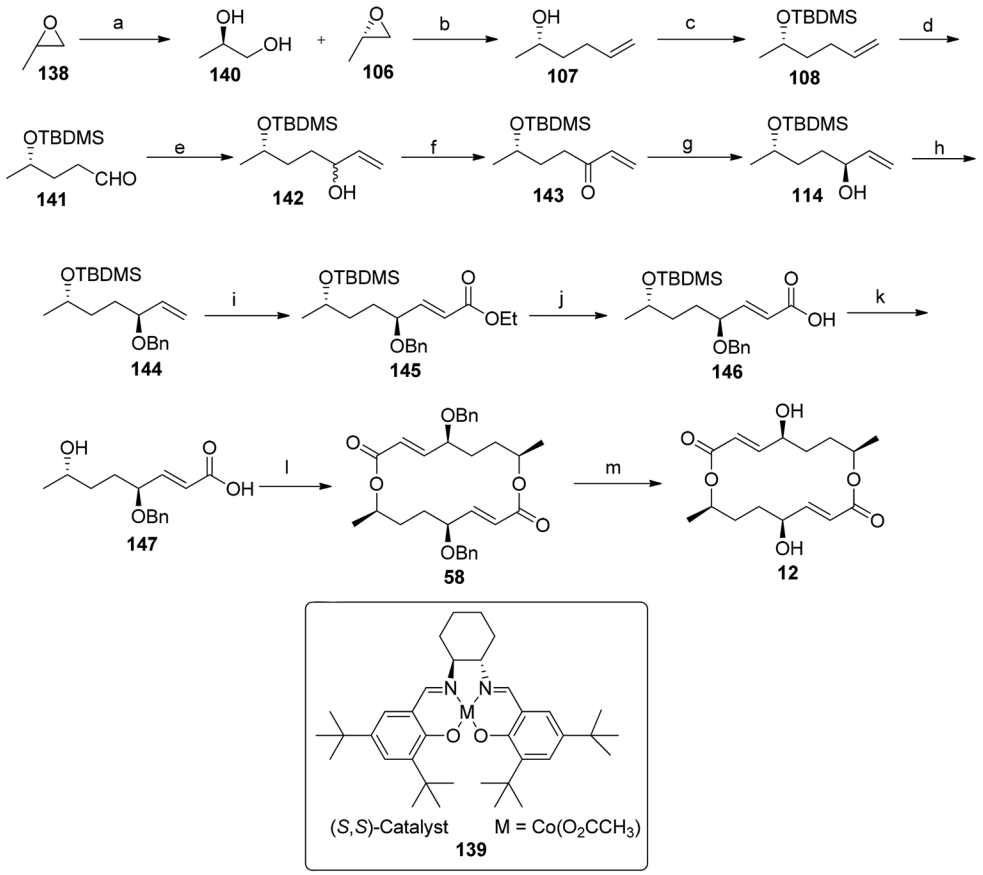
Scheme 15. Reagents and conditions: (a) DIBAL-H, CH_2Cl_2, 0 °C to rt, 2h; (b) TBDMSCl, imidazole, CH_2Cl_2, rt, 4 h; (c) *n*-BuLi, Me_3SI, THF, 0 °C, 6 h (d) PMBBr, NaH, THF, 0 °C to rt, 8 h; (e) i) O_3, CH_2Cl_2, -78 °C, 30 min; ii) $Ph_3P=CHCOOMe$, benzene, reflux, 2 h; (f) LiOH, THF: MeOH: H_2O (3:1:1), rt, 4 h; (g) TBAF, THF, 0 °C to rt, 3 h; (h) Ph_3P, DEAD, toluene:THF (10:1) -25 °C, 10 h; (i) DDQ, CH_2Cl_2: H_2O (19:1), rt, 3 h.

allyl magnesium chloride in ether to give **107**, which was then silylated with TBDMSCl and imidazole to provide **108** in fair yield as a colorless liquid. Ozonolysis of **108** produced aldehyde **141**, which upon rapid treatment with vinyl magnesium bromide yielded an inseparable diastereomeric combination of allylic alcohols **142**. Under Swern reaction conditions, **142** was oxidized to give ketone **143**, which was reduced with (*S*)-CBS catalyst to give alcohol **114** with excellent selectivity and good yield.

The secondary allyl alcohol **114** was protected as benzyl ether. The olefin **144** was ozonolyzed in DCM, yielding the corresponding aldehyde, which was immediately treated with (ethoxycarbonyl methylene) triphenyl phosphorane in benzene at reflux conditions, yielding trans-Wittig product **145** in 79% yield. After ester **145** was made, the authors used the same sequence of reactions as in the previous report,[36] such as hydrolysis of the ester, desilylation of the TBS ether with TBAF, cyclodimerization under the conditions of the Mitsunobu reaction, and finally oxidative deprotection of the Bn groups with DDQ furnished the target molecule (-)-pyrenophorol **12** with an overall good yield (Scheme 16).

Narasaiah's approach

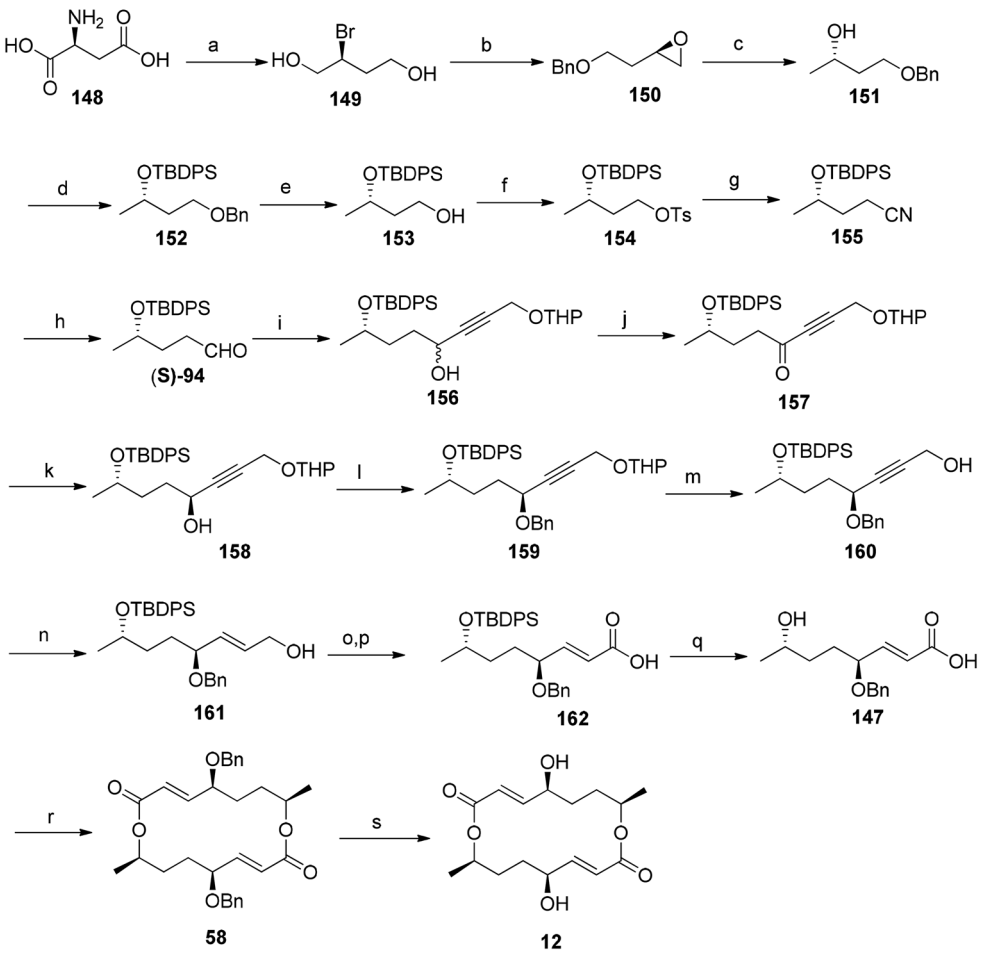
In 2020, Narasaiah et al.[41] reported the synthesis of (-)-pyrenophorol **12** from commercially available *L*-Aspartic acid. Regioselective epoxide opening, CBS reduction, Pinnick oxidation, and Mitsunobu lactonization were the major reactions in this approch. Brominative diazotization of (*L*)-aspartic acid **148** with $NaNO_2$ and KBr, followed by BH_3DMS-mediated reduction of dicarboxylic acid to diol **149**,



Scheme 16. Reagents and conditions: (a) (*S*,*S*)-catalyst, AcOH, H_2O, 15 °C, 14 h; (b) Mg, allyl chloride, ether, -78 °C, 2 h; (c) TBDMSCl, imidazole, CH_2Cl_2, rt, 4 h; (d) O_3, CH_2Cl_2, -78 °C, 30 min; (e) CH_2=CHMgBr, THF, -40 °C, 4 h; (f) $(COCl)_2$, DMSO, CH_2Cl_2, -78 °C; (g) (*S*)-CBS catalyst, BH_3.THF, THF, 25 °C, 30 min; (h) BnBr, NaH, THF, 0 °C to rt, 8 h; (i) i) O_3, CH_2Cl_2, dimethylsulphide, -78 °C, 15 min; ii) Ph_3P=$CHCO_2Et$, benzene, reflux, 2 h; (j) LiOH, THF:MeOH:H_2O (3:1:1), rt, 4 h; (k) TBAF, THF, 0 °C to rt, 3 h; (l) Ph_3P, DEAD, toluene-THP (10:1), -25 °C, 10 h; (m) DDQ, CH_2Cl_2:H_2O (19:1), rt, 3 h.

gave quantitative yield. Bromine is eliminated intramolecularly by a base, resulting in the formation of epoxide, followed by the protection of primary alcohol with benzyl bromide and NaH, resulting in an overall high yield of benzyl ether **150**. Regioselective epoxide opening with $LiAlH_4$ afforded exclusively secondary alcohol **151**, which when protected as TBDPS ether **152** with TBDPS-Cl and imidazole. The selective reduction of benzyl ether to its primary alcohol **153** by lithium naphthalene. Free alcohol group converted to tosyl group, then SN2 substitution with NaCN in the presence of a catalytic amount of NaI in DMSO to produce cyano compound **155** in high yield. Partial reduction of **155** with DIBAL-H at −78 °C gave aldehyde **(*S*)-94**, followed by nucleophilic addition with THP-protected propargyl alcohol using *n*-BuLi at −78 °C to afford compound **156** in high yield. The secondary hydroxyl group was oxidized with IBX in DMSO and DCM mixture

(1:3) gave alkenone **157**, which was then subjected to enantioselective reduction with borane dimethyl sulfide and a Corey-Bakshi-Shibata ligand [(*R*)-methyl-oxazaborolidine] at −40 °C in dry THF to afford compound **158** in good yield with an excellent diastereoselectivity ratio of 92:8. The resulted alcohol was protected as benzyl ether **159** and tetrahedropyranyl ring was selectively deprotected with pyridinium *p*-toluene sulfonate (PPTS) in methanol to give compound **160**. The propargyl alcohol was subjected to Red-Al in dry THF at 0 °C, affording the exclusively *trans*-allylic alcohol **161**. The allylic alcohol **161** was oxidized by using MnO_2 to afford aldehyde, which was further oxidized under



Scheme 17. Reagents and conditions: (a) (i) $NaNO_2$, KBr, H_2SO_4, -10 °C, 5h, 82%; (ii) BH_3.DMS, dry THF, -10 °C to rt, 93%; (b) NaH, BnBr, TBAI, 0 °C, 5h, 70%; (c) LAH, dry THF, rt, 1h, 90%; (d) TBDPS-Cl, imidazole, DMAP, dry CH_2Cl_2, rt, 2h, 90%; (e) Li, naphthalene, dry THF, -40 °C, 1 h, 83%; (f) Ts-Cl, Et_3N, DMAP, dry CH_2Cl_2, 6 h, 69%; (g) NaCN, NaI, dry DMSO, 80 °C, 1.5 h, 88%; (h) DIBAL-H, -78 °C, dry CH_2Cl_2, 1h; (i) $C_8H_{12}O_2$, *n*-BuLi, -78 °C, dry THF, 3 h (73%, over two steps); (j) IBX, DMSO, CH_2Cl_2 (1:3), rt, 1.5 h, 86%; (k) (*R*)-CBS-catalyst, BH_3.DMS, dry THF, -40 °C, 1 h, 81%; (l) NaH, BnBr, dry THF, rt, 4 h, 78%; (m) PPTS, MeOH, rt, 2 h, 89%; (n) Red-Al, dry THF, 0 °C to rt, 1 h, 91%; (o) MnO_2, hexane, rt, 20 hr; (p) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH-H_2O (5:1) 30 min (over two steps 92%); (q) HF.Pyridine, dry THF, 20 h, 85%; (r) TPP, DEAD, THF: H_2O (10:1), -30 °C, 18 h, 55%; (s) $TiCl_4$, dry CH_2Cl_2, 2h, rt, 80%.

Pinnick conditions in the presence of sodium chlorate, sodium dihydrogen phosphate, *t*-BuOH, and 2-methyl-2-butene to obtain *α,β*-unsaturated acid **162**. Deprotection of TBDPS with HF-Pyridine yielded the hydroxy acid **147**. The cyclodimerization of **147** under the conditions of the Mitsunobu reaction produced the cyclic dilactone **58**. Finally, oxidative deprotection of the Bn groups with $TiCl_4$ generated the desired (-)-pyrenophorol **12** (Scheme 17).

Summary

In this review, we demonstrated the isolation, biological activity, structure elucidation and total synthetic approaches of 16-membered C_2-symmetric macrolide dilactone pyrenophorol to date. Pyrenophorol was first isolated from *Byssochlamys niveah*, *Stemphylium radicinum*, the imperfect fungus *Alternaria alternate*, and later from the *Drechslera avenae* pathotype, the fungi *Pyrenophora avenae*, *Byssochlamys nivea*, and recently from *Phoma sp*., an endophytic fungus that was isolated from *Lycium intricatum* from Gomera. Pyrenophorol has strong antifungal activity against both the rust *Microbotryum violaceum* and *Saccharomyces cerevisae* and also has potent antimicrobial activity against the fungus *Microbotryum violaceum*, the alga *Chlorella fusca*, and the bacteria *Escherichia coli* and *Bacillus megaterium*. It has selective phytotoxicity against wild oats and also exhibits pronounced anthelmintic properties. Several research groups have been drawn to this natural product due to its prominent biological activity and its attractive C2-symmetric structure. So far, fifteen methods have been reported for the total synthesis of pyrenophorol. Photo-induced rearrangement of an *α, β*-epoxy diazomethyl ketone to 4-hydroxy-2-alkenoate, intramolecular Wittig reaction, Sharpless asymmetric epoxidation, Grubbs cross-metathesis, reductive elimination of iodoepoxide, Rh-catalysed asymmetric hydroformylation (AHF) tandem reaction, the Novozym 435-catalysed acrylation, hydrolytic kinetic resolution, Grignard reaction, Swern oxidation, CBS reduction, Pinnick oxidation, Photo Induced rearrangement, Yamaguchi esterification, Baeyer-Villager oxidation, MacMillan asymmetric hydroxylation, Horner-Wadsworth-Emmons olefination, and Mitsunobu cyclization were the important reactions employed in their synthetic approaches. We strongly believe that writing a review on the total synthesis of a natural product is a valuable endeavor that offers several important benefits to the scientific community, such as the advancement of science, providing valuable resources for researchers, and promoting critical analysis and innovation within the field of synthetic chemistry.

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Author contributions

Conceptualization, writing-original draft preparation and supervision by U.V.S.R and review and editing by B. A, K. R. B, M.V. P. S and B. S. All authors have read and agreed to publish the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

ORCID

Bheemreddy Anusha http://orcid.org/0000-0002-0041-4890
Raveendra Babu Kothapalli http://orcid.org/0000-0002-4877-561X
Ummareddy Venkata Subba Reddy http://orcid.org/0000-0002-3300-533X

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