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HIGHLY OXIDIZED γ-LACTAM-CONTAINING NATURAL PRODUCTS: TOTAL SYNTHESIS AND BIOLOGICAL EVALUATION

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Abstract – γ-Lactam is a ubiquitous structure found in the natural products. A number of highly oxidized γ-lactam-containing natural products are produced by various fungi. These compounds often show a wide range of biological activities because their multiple internal reaction sites, which arise from the high oxidation state of the compounds, can react with biological nucleophiles. Due to their high reactivity and dense functionality, total syntheses of these molecules require strict control of the inherent reactivity and the appropriate design of synthetic intermediates. This review focuses on the recent total syntheses of some highly oxidized γ-lactam-containing natural products, including fused bicyclic (epolactaene, NG-391, lucilactaene, L-755,807), spirocyclic (azaspirene, pseurotin A, E, and F_2, cephalimysin A–C, FD-838, and berkeleyamide D), and tricyclic (rubrobramide and talaramide A) skeletons, and on the structure-activity relationship studies of related molecules.

CONTENTS

INTRODUCTION

Lactam-containing compounds are widely used in various areas of contemporary science owing to their useful chemical and physical properties. For example, ε-lactams are a common fiber feedstock[1-3] and β-lactam is a partial structure in a wide range of antibacterial agents, including penicillin and cephalosporin.[4,5] Functionalized γ-lactam structures are frequently found in natural products. In particular, highly oxidized γ-lactam-containing molecules show a diverse range biological activities, such as anticancer, antibacterial, neuroprotective, and anti-Alzheimer activities. The attractive chemical structures

of these compounds and their promising biological activity have made them synthetic targets in chemistry research worldwide. Several total syntheses have been achieved, particularly by Japanese synthetic organic chemists. Furthermore, given the potential of these natural products as pharmaceutical agents, structure-activity relationship (SAR) studies have also been conducted as part of drug discovery research. In this review, we summarize the efficient total syntheses of some natural products containing a highly oxidized γ-lactam structure and SAR studies from the last two decades.

2. SYNTHESIS OF γ-LACTAM-CONTAINING NATURAL PRODUCTS WITH FUSED BICYCLIC RING SYSTEMS

Some natural products, including epolactaene (**1**),[6] NG-391 (**2**),[7] lucilactaene (**3**),[8] and L-755,807 (**4**),[9] that contain a fused bicyclic highly oxidized γ-lactam connected to a long conjugated lipophilic side chain exhibit promising bioactivity against cancer and Alzheimer's disease (Figure 1). However, the absolute stereostructures of these molecules were not determined when they were isolated, and thus they have required structural determination by chemical synthesis, making them valuable synthetic targets.

Epolactaene (**1**) was first isolated as a diastereomeric mixture at the hemiaminal position (*ca*. 5:1 ratio) from the culture broth of *Penicillium* sp. BM 1689-P by Osada *et al*.[6] Epolactaene (**1**) consists of a distinctive epoxy-γ-lactam and a side chain containing a conjugated (*E*,*E*,*E*)-triene and (*E*)-α,β-unsaturated ketone, and the compound induces neurite outgrowth and arrests the cell cycle at the G0/G1 phase in the SH-SY5Y human neuroblastoma cell line.[10] Subsequently, **1** has been found to inhibit the growth of human T-lymphoma Jurkat cells,[11,12] inhibit mammalian DNA polymerases and human DNA topoisomerase II,[13,14] induce apoptosis in the BALL-1 human leukemia B-cell line,[15,16] and have anti-inflammatory activity.[14,17] In addition, epolactaene has several electrophilic reactive sites, such as the α,β-unsaturated ketone, epoxide, and hemiaminal carbon, that can react with biological nucleophiles, and thus, it may exhibit other attractive bioactivities. Due to its interesting chemical structure and promising biological activity, several research groups have reported total syntheses of **1** via various synthetic strategies.

NG-391 (**2**) was isolated from *Fusarium* sp. TF-0452,[2] and lucilactaene (**3**) was isolated from *Fusarium* sp. RK 97-94 together with NG-391, and **3** exhibited an optical rotation of 0 in two different solvents.[3] Compounds **2** and **3** were expected to show bioactivities related to those of epolactaene, which exhibits a variety of biological properties, owing to structural similarities among these compounds. Indeed, **2** shows neurotrophic activity and affects neurite outgrowth, and **3** inhibits the cell cycle in p53-transfected cancer cells.[3]

L-755,807 (**4**), isolated from an endophytic fungus, *Microsphaeropsis* sp., is a bradykinin B_2 antagonist.[4] The compound contains a labile tetraene side chain with two stereocenters and a characteristic

epoxy-γ-lactam, similar to epolactaene and NG-391. Therefore, L-755,807 (**4**) is also expected to show neurite outgrowth activity.

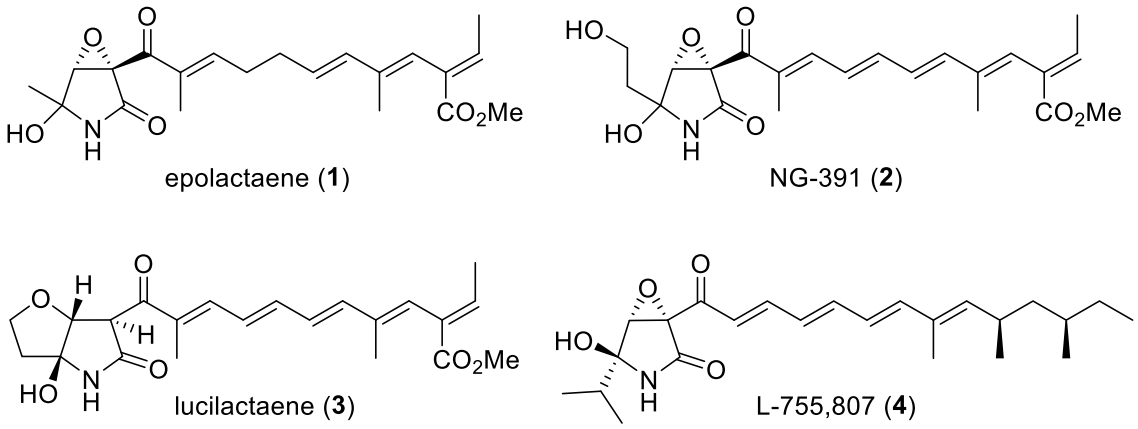
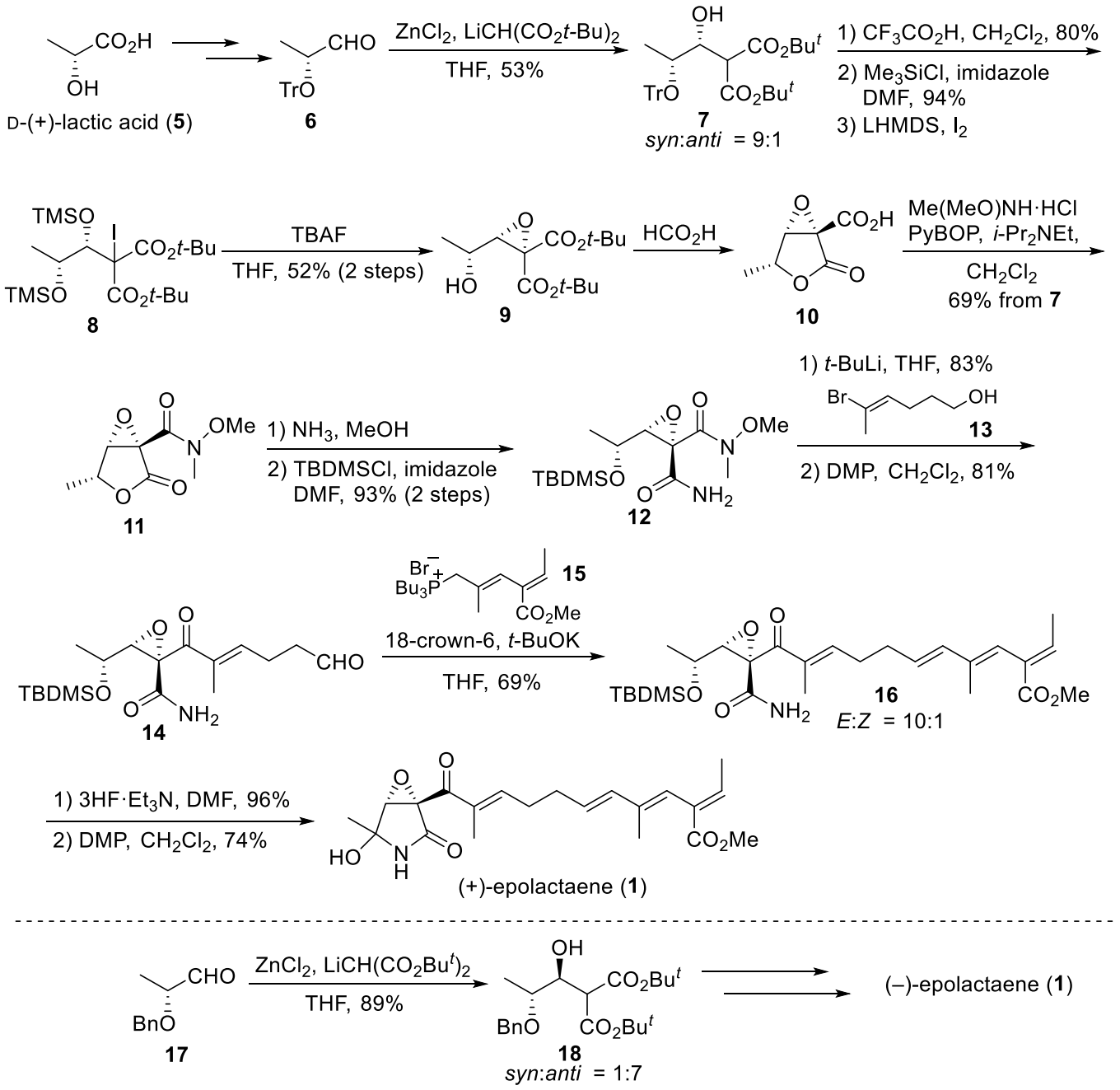


Figure 1. γ-Lactam-containing Natural Products with Fused Bicyclic Ring Systems

2-1-1. Kogen's Total Syntheses of Epolactaene

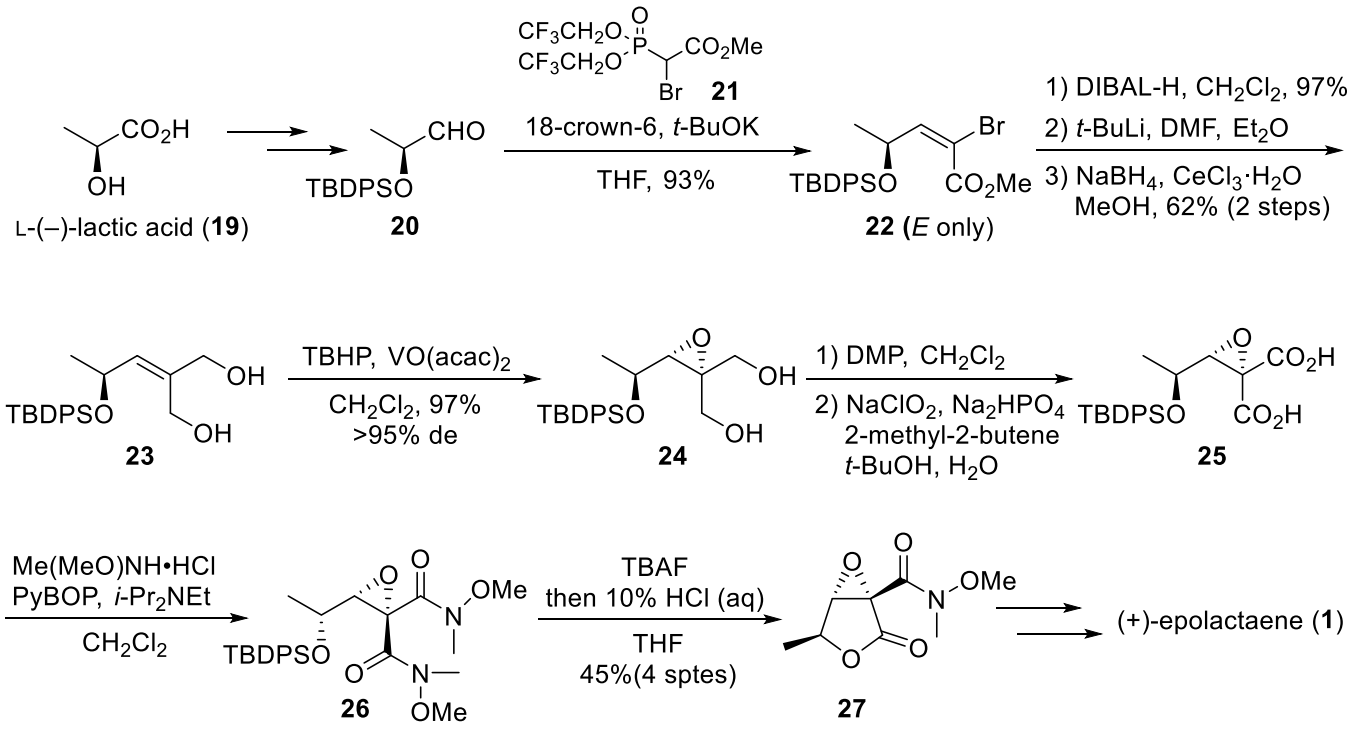
Kogen and co-workers reported the first total synthesis of epolactaene (**1**) by a highly convergent synthetic strategy (Scheme 1).[18,19] They developed a protecting group-controlled diastereoselective aldol reaction[20] to synthesize both enantiomers of epolactaene from D-(+)-lactic acid (**5**) as a common starting material. Thus, aldehyde **6**, which was prepared from **5**, underwent the aldol reaction with di-*tert*-butyl malonate. The bulky trityl protecting group on the hydroxy group provided desirable aldol adduct **7** with good *syn* selectivity. Aldol adduct **7** was transformed to epoxide **9** via removal and reintroduction of the protecting group, iodination of the methine proton, and TMS cleavage and spontaneous epoxidation. After further transformation of epoxide **9** into diamide **12** via lactones **10** and **11**, the vinyllithium species generated from **13** was reacted with Weinreb amide **12** to afford an (*E*)-α,β-unsaturated ketone. Wittig reaction of aldehyde **14** gave conjugated (*E*,*E*,*E*)-triene **16** with good stereoselectivity. They completed the total synthesis of (+)-epolactaene (**1**) after desilylation and spontaneous oxidative cyclization. In addition, substrate **17** with a small benzyl protecting group preferentially afforded the *anti*-aldol adduct, **18**.[20] (–)-Epolactaene (**1**) could be easily accessed from **18** via a similar synthetic scheme to the one used for the (+)-enantiomer.[17]



Scheme 1. Kogen's First-Generation Total Synthesis of Epolactaene

In 2014, Kogen's group reported its second-generation formal synthesis of epolactaene (**1**) (Scheme 2).[21] They realized that the first-generation synthesis was still unsatisfactory because of the moderate stereoselectivity and yield, especially in the key aldol reaction. Additionally, to circumvent the problematic synthetic sequence, they planned an alternative strategy, using a highly diastereoselective epoxidation of an olefinic diol. Hence, TBDPS-protected aldehyde **20**, prepared from L-lactic acid (**19**), was reacted with Horner–Wadsworth–Emmons (HWE) reagent **21** to give bromoacrylate derivative **22** with complete stereoselectivity.[22] Based on their synthetic plan, bromoacrylate **22** was converted to

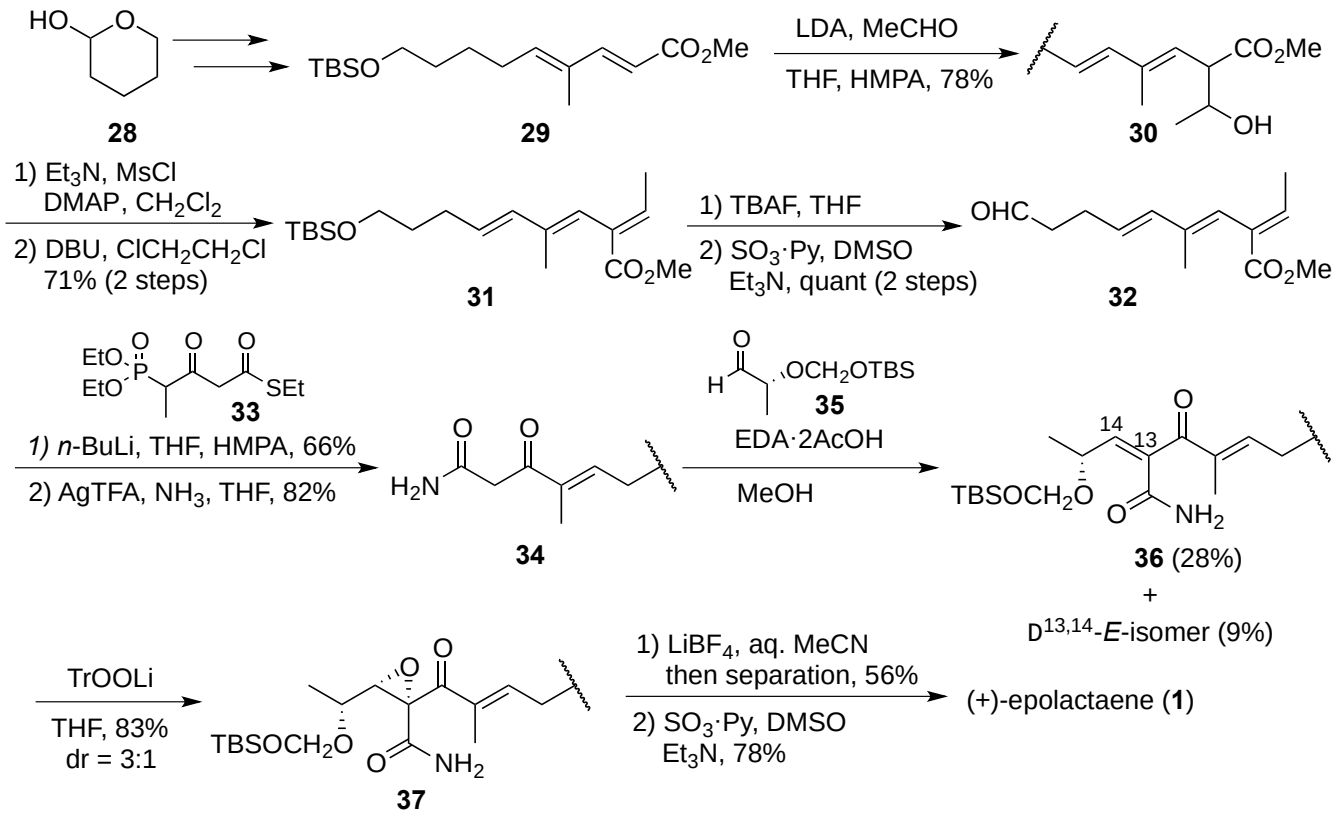
olefinic diol **23**, which was subjected to epoxidation with $VO(acac)_2$ and *tert*-butyl hydroperoxide (TBHP) to deliver desired epoxide **24** in excellent yield and diastereoselectivity. Diol epoxide **24** could be converted to lactone **27**, a known synthetic intermediate, via unstable dicarboxylic acid **25** and diamide **26**, completing the formal synthesis of (+)-epolactaene (**1**).



Scheme 2. Kogen's Second-Generation Total Synthesis of Epolactaene

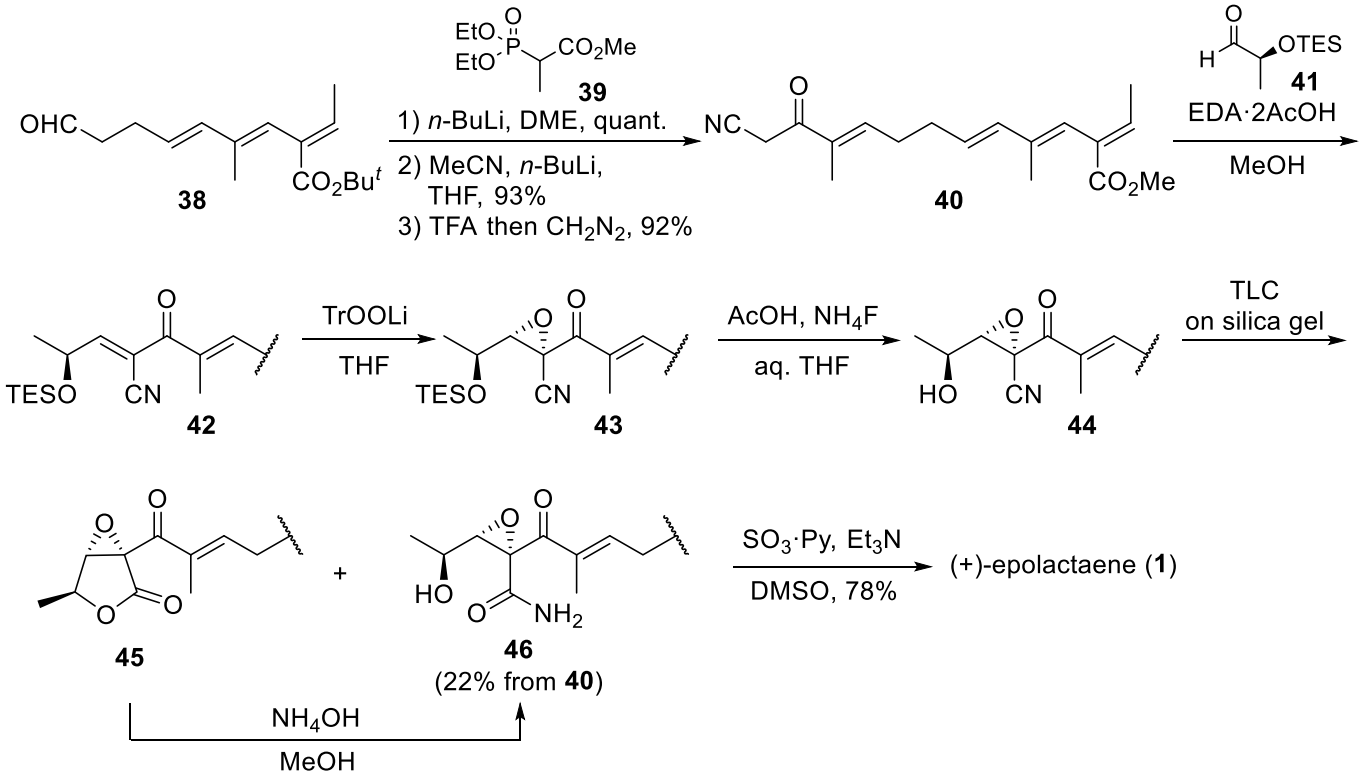
2-1-2-1. Hayashi's Total Synthesis of Epolactaene

Hayashi's group published the total synthesis of epolactaene (**1**) almost simultaneously with the first report from Kogen's group (Scheme 3).[23] In their synthetic route, (*E*,*E*,*E*)-triene ester portion of epolactaene was efficiently constructed in the beginning of the synthesis. Thus, (*E*,*E*)-dienecarboxylate **29**, derived from tetrahydropyran-2-ol (**28**), was initially reacted with acetaldehyde via kinetic deprotonation to give α-adduct **30** with high yield and regioselectivity, and (*E*,*E*,*E*)-triene **31** was created by subsequent dehydration and isomerization. Then, aldehyde **32** underwent HWE olefination using β-ketothioester diethyl phosphonate derivative **33** to provide an γ,δ-unsaturated β-ketothioester. The key Knoevenagel condensation between amide **34** and (*R*)-2-(*tert*-butyldimethylsiloxymethoxy)propanal (**35**) gave desired products **36**, albeit in low yield. Nucleophilic epoxidation with TrOOLi yielded epoxide **37** in moderate stereoselectivity. The total synthesis of epolactaene (**1**) was completed by deprotection, separation of the epoxide isomers, and oxidation of the resultant alcohol.



Scheme 3. Hayashi's First-Generation Total Synthesis of Epolactaene

To overcome the drawbacks in the Knoevenagel condensation and epoxidation steps, Hayashi's group developed a modified approach to epolactaene (**1**) by optimizing the substrate in each step (Scheme 4).[24] Using β-ketonitrile **40** instead of β-ketoamide **34** as the substrate in the Knoevenagel condensation improved the stereoselectivity and yield considerably. They assumed that the improvement was due to the cyano group decreasing steric repulsion and the increased thermodynamic stability of the product. Additionally, incorporating the bulkier triethylsilyl group dramatically increased the stereoselectivity in the epoxidation step (**42**→**43**). They completed the improved total synthesis by the appropriate transformation to epolactaene from epoxide **43**.

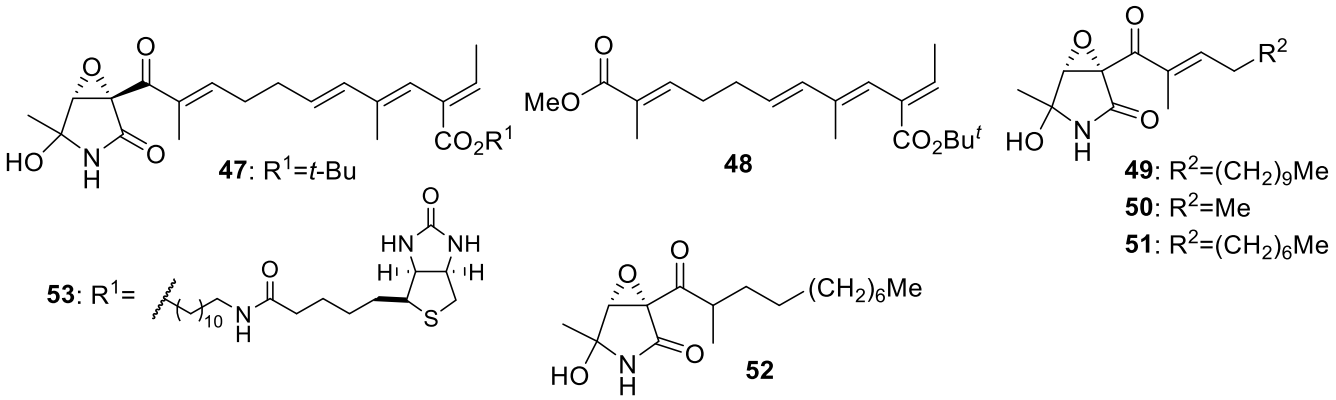


Scheme 4. Hayashi's Second-Generation Total Synthesis of Epolactaene

2-1-2-2. Hayashi's SAR Studies of Epolactaene

After completing the total synthesis of epolactaene (**1**), Hayashi's and Osada's groups launched joint broad biological studies to investigate the inhibitory activity against the growth of SH-SY5Y and Jurkat cells, the target protein of epolactaene, and the interaction mode of the compounds with the protein.[11] Based on their synthetic scheme for epolactaene (**1**), they prepared several epolactaene analogs, including side-chain analog **48** without a γ-lactam, **49**–**52** with simplified lipophilic side chains, and biotin analog **53**. They biologically evaluated the inhibition ability of the synthetic compounds against the growth of SH-SY5Y and Jurkat cells (Table 1). The results suggested that the γ-lactam, α,β-saturated ketone, and length of the alkyl side chain were crucial for the potent biological activities, whereas the triene and ester moieties may not be essential. Furthermore, biotin-tagged analog **53** was used as a chemical probe to identify human heat shock protein (Hsp) 60 as the binding target of epolactaene (**1**), which interacted with Cys^{442} of Hsp60 via the terminal Michael acceptor moiety in the side chain.[11,12]

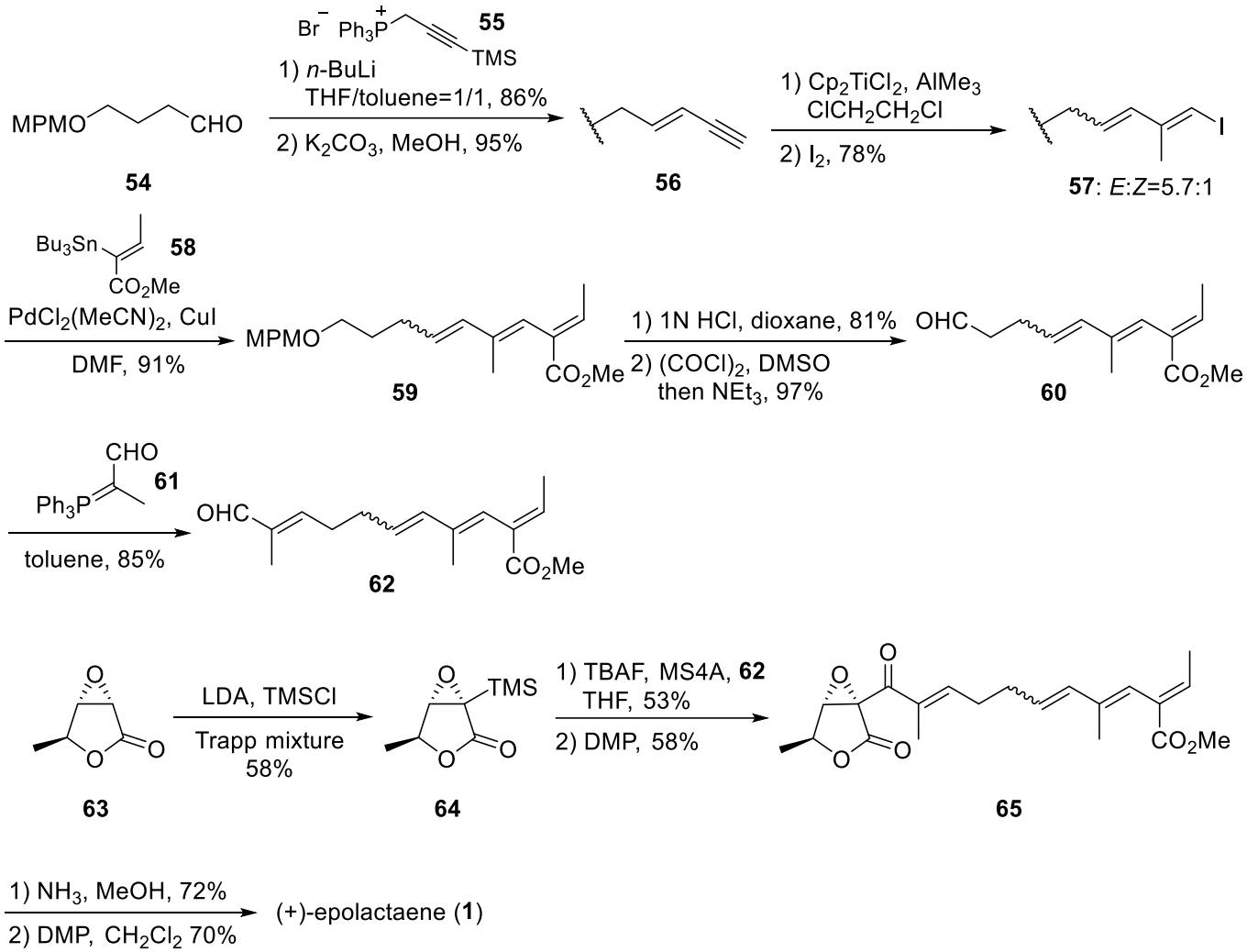
Table 1. IC_{50} Acting Against SH-SY5Y and Jurkat Cell Viability by Epolactaene Derivatives



	IC_{50} (μM)	
Compound	SH-SY5Y cells	Jurkat cells
(+)-epolactaene (**1**)	3.9	1.5
47	1.1	2.0
48	>300.0	90.0
45	>130.0	20.0
46	38.0	20.0
49	5.7	1.2
50	18.0	8.0
51	13.0	1.5
52	n.d.	3.0
53	6.5	3.0

2-1-3-1. Kobayashi's Total Synthesis of Epolactaene

Kobayashi's group also reported the total synthesis of epolactaene (**1**) (Scheme 5).[25] Their convergent synthetic strategy divided **1** into the epoxy-γ-lactam and side-chain segments. A Wittig reaction between aldehyde **54** and phosphonium salt **55** was followed by stereoselective carbometallation with Cp_2TiC1_2-Me_3Al and subsequent iodination to afford dienyl iodide **57**, which was connected with vinylstannane **58** by Stille coupling to construct the triene portion of the epolactaene side chain. Side-chain segment **62** was formed by Wittig homologation, and α-trimethylsilyl epoxylactone **64** was prepared as the epoxy-γ-lactam segment. To couple synthetic segments **62** and **64**, they used the distinctive nucleophilic addition of an oxiranyl anion, generated from **64** with catalytic TBAF, to aldehyde **62** to obtain a coupling product. This was the first report of using an oxiranyl anion generated from epoxylactone.[26] Finally, the lactone moiety in **65** was converted to a lactam to complete the total synthesis of epolactaene (**1**).



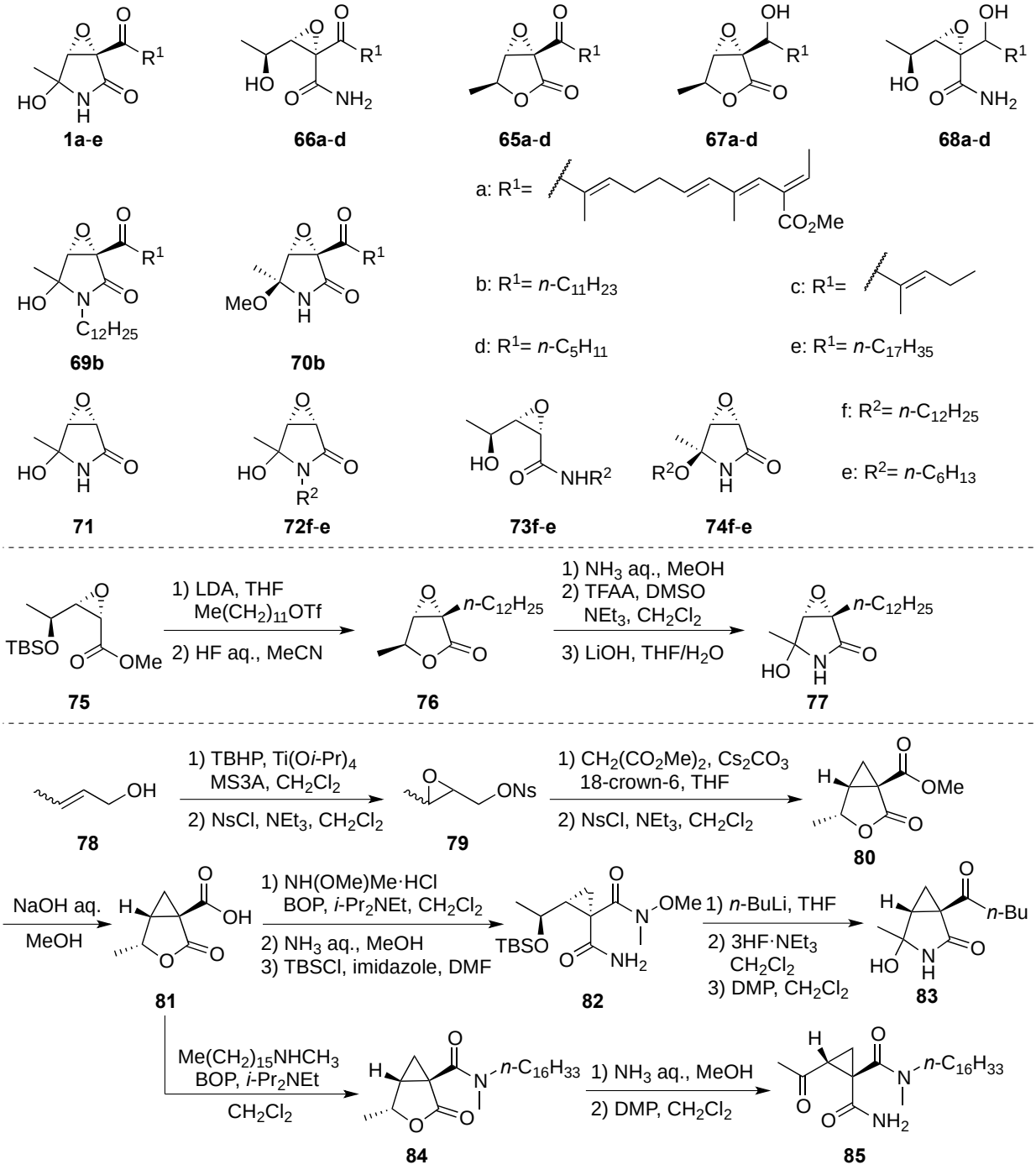
Scheme 5. Kobayashi's Total Synthesis of Epolactaene

2-1-3-2. Kobayashi's SAR Studies of Epolactaene

Kobayashi's and Ikekita's groups investigated the action of epolactaene and its derivatives on DNA metabolic enzymes during their studies of biologically active molecules affecting DNA replication, repair, and recombination.[13-17] Thus, they prepared and biologically evaluated various epolactaene analogs.

Initial screening of the compounds for inhibition of mammalian DNA polymerases[14] and human DNA topoisomerase II[13] indicated the importance of the α,β-epoxy-γ-lactam and the long alkyl chain, implying that hydrophobicity was crucial for the activities. This hypothesis was supported by the correlation of the activities with octanol/water partition coefficient (ClogP) values (Table 2).[14] They evaluated the apoptosis-inducing effect of the synthetic analogs on BALL-1 cells,[16] and the results suggested that the α-acyl-α,β-epoxy-γ-lactam structure, along with the long side chain with high ClogP values, were required for the activity, which was attributed to permeation across the cell membrane.[15,16] However, the stereochemistry of the epoxide had no effect on the activity.

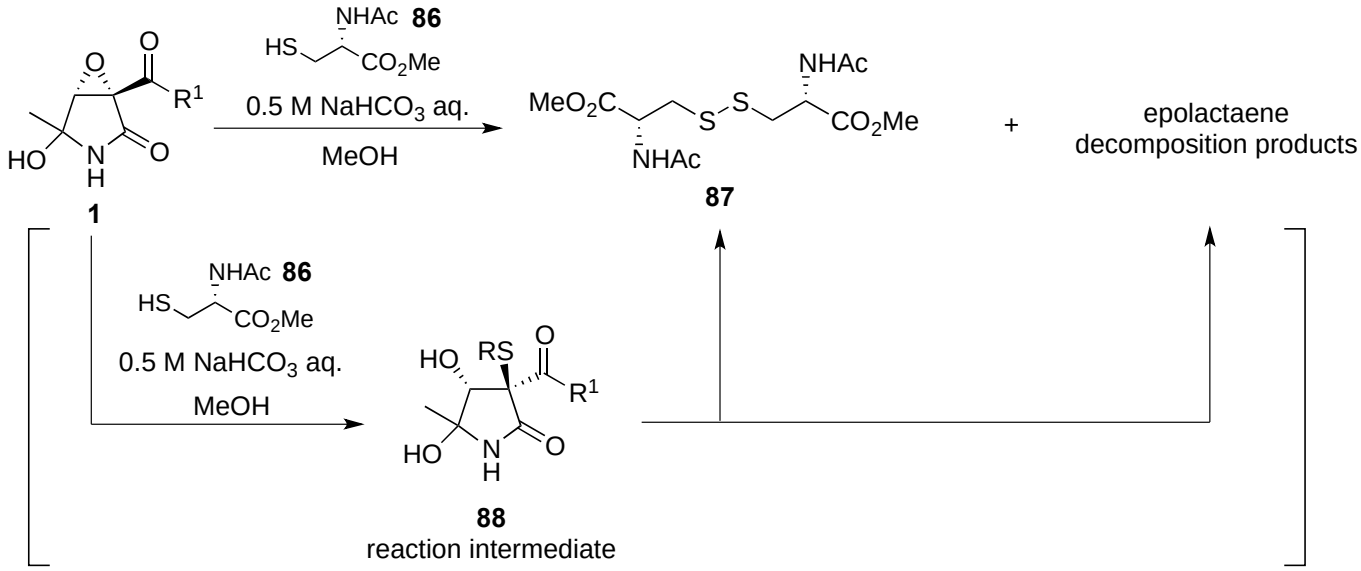
Table 2. Biological Evaluations and ClogP Values of Epolactaene and Its Derivatives



	IC_{50} (μM)				ClogP
Compound	Polymerase α	Polymerase β	Topoisomerase II	BALL-1 cells	
(+)-**1a**	25	94	10	3.82	1.85
1b	26	98	20	1.65	2.80
1c	>200	>200	>200	5.04	0.20
1d	>200	>200	>200	7.40	0.29
1e	13	78	nt[a]	0.70	5.30
(-)-1a	nt[a]	nt[a]	nt[a]	3.26	1.85
65a-d	>200	>200	nt[a]	>10	nd[b]
66a-d	>200	>200	nt[a]	>10	nd[b]
67a-d	nt[a]	nt[a]	nt[a]	>10	nd[b]
68a-d	nt[a]	nt[a]	nt[a]	>10	nd[b]
69b	27	99	nt[a]	6.51	7.61
70b	33	98	nt[a]	3.50	3.16
71	>200	>200	nt[a]	>10	−1.13
72f	81	>200	nt[a]	3.96	3.69
72e	>200	>200	nt[a]	8.34	1.18
73f	>200	>200	nt[a]	>10	nt[a]
73e	>200	>200	nt[a]	>10	nt[a]
74f	27	95	nt[a]	>10	3.81
74e	>200	>200	nt[a]	>10	1.31
77	nt[a]	nt[a]	nt[a]	>10	3.96
83	nt[a]	nt[a]	nt[a]	>10	0.78
85	nt[a]	nt[a]	nt[a]	>10	5.28

[a] Not tested. [b] Not determined.

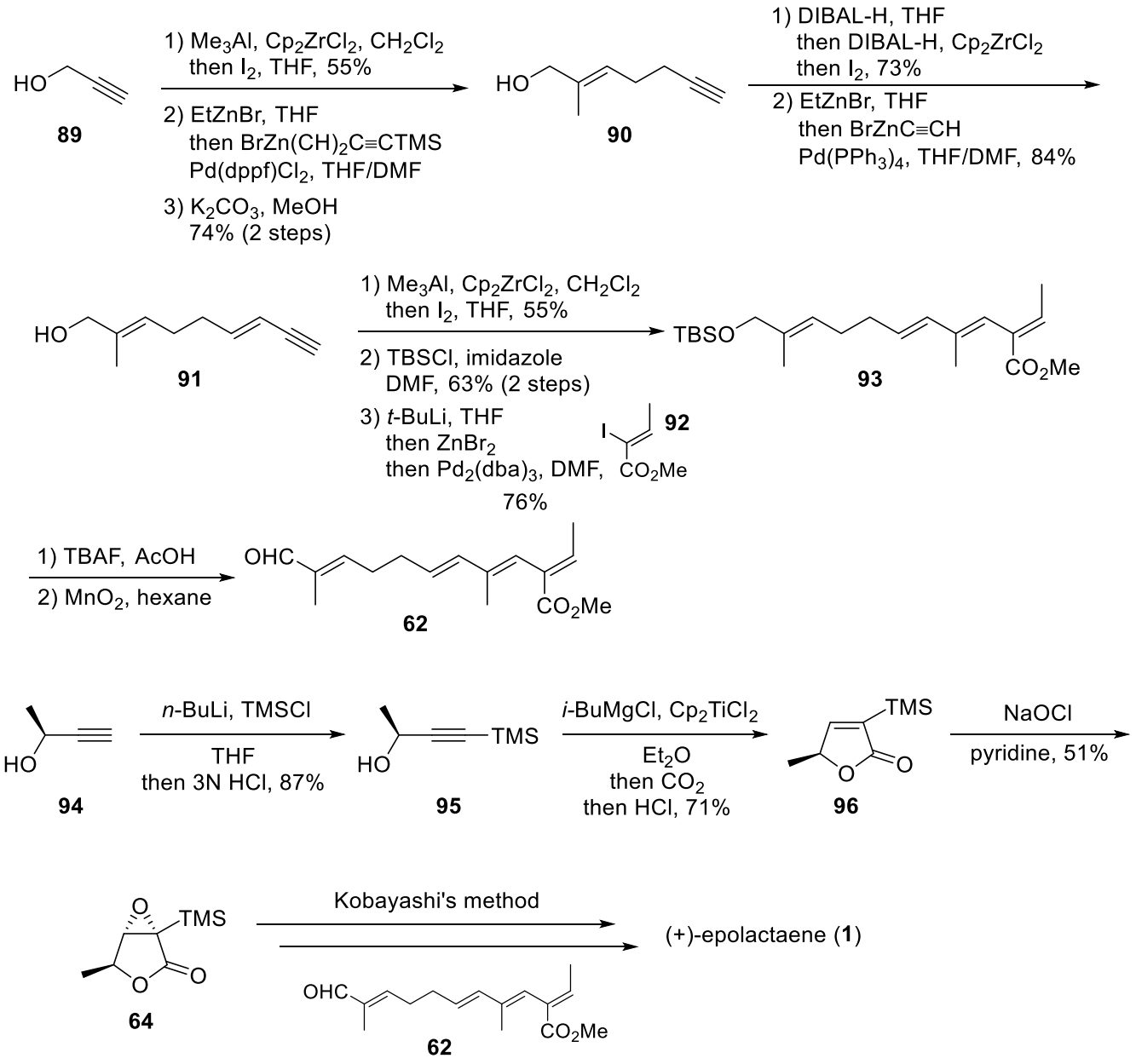
To clarify the reaction site and effects on the target protein, epolactaene and its analogs were reacted with *N*-acetylcysteine methyl ester **86**, which mimicked cysteines in the protein (Scheme 6).[27,28] The reaction proceeded at the α-position of the epoxy-γ-lactam core structure to give corresponding intermediate **88**, which led to the formation of a disulfide bond. Thus, they concluded that epolactaene and its analogs induced intramolecular or intermolecular disulfide formation with cysteines in the protein.[28]



Scheme 6. Disulfide Formation from **86** Induced by Epolactaene

2-1-4. Negishi's Total Synthesis of Epolactaene

In 2006, Negishi's group accomplished the total synthesis of epolactaene (**1**) by a Pd-catalyzed homoallyl-alkenyl coupling and a late-stage coupling between the epoxy-γ-lactam and side-chain segments inspired by Kobayashi's procedure (Scheme 7).[29] Side-chain segment **62** was synthesized from propargyl alcohol by two types of carboalumination using Cp_2TiCl_2-Me_3Al or Cp_2TiCl_2-DIBAL-H and Pd-catalyzed cross-coupling reactions with organozinc reagents, whereas epoxy-γ-lactam segment **64** was constructed by a Ti-catalyzed *trans*-hydromagnesation followed by CO_2 fixation, acid-catalyzed lactonization, and nucleophilic epoxidation. Completion of the total synthesis of epolactaene (**1**) was accomplished by following Kobayashi's protocol.[25]

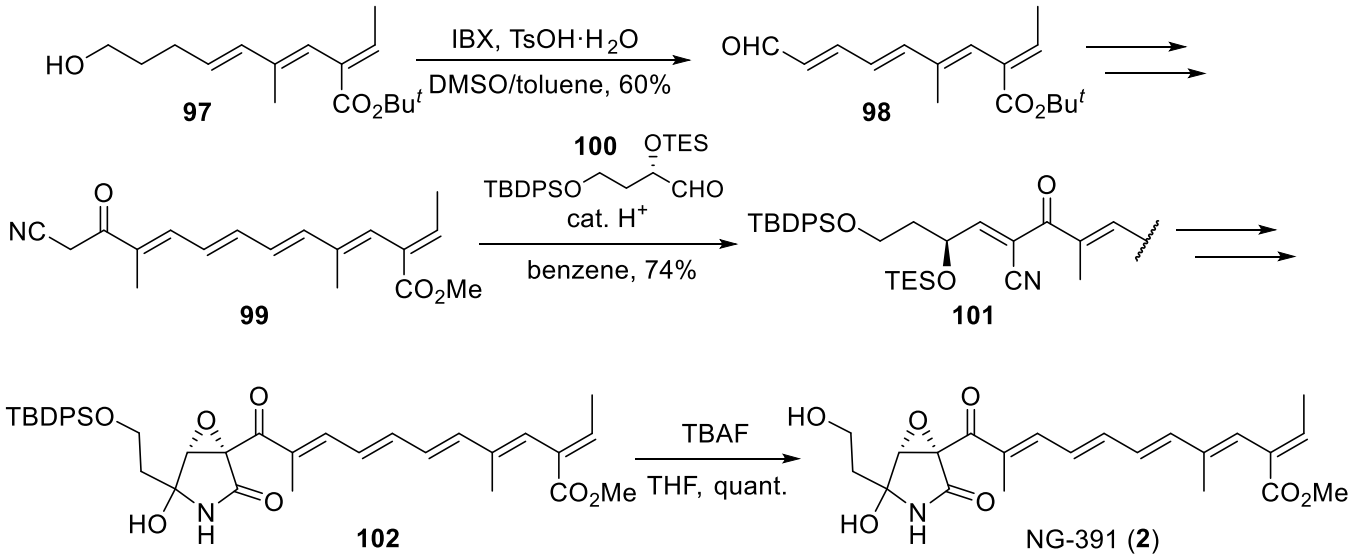


Scheme 7. Negishi's Total Synthesis of Epolactaene

2-2. Hayashi's Total Synthesis of Lucilactaene from NG-391

Hayashi et al. completed the asymmetric total synthesis of lucilactaene (**3**) via NG-391 (**2**)[30] by modifying their synthetic scheme for epolactaene[24] and using a new protecting group (Scheme 8).[31]

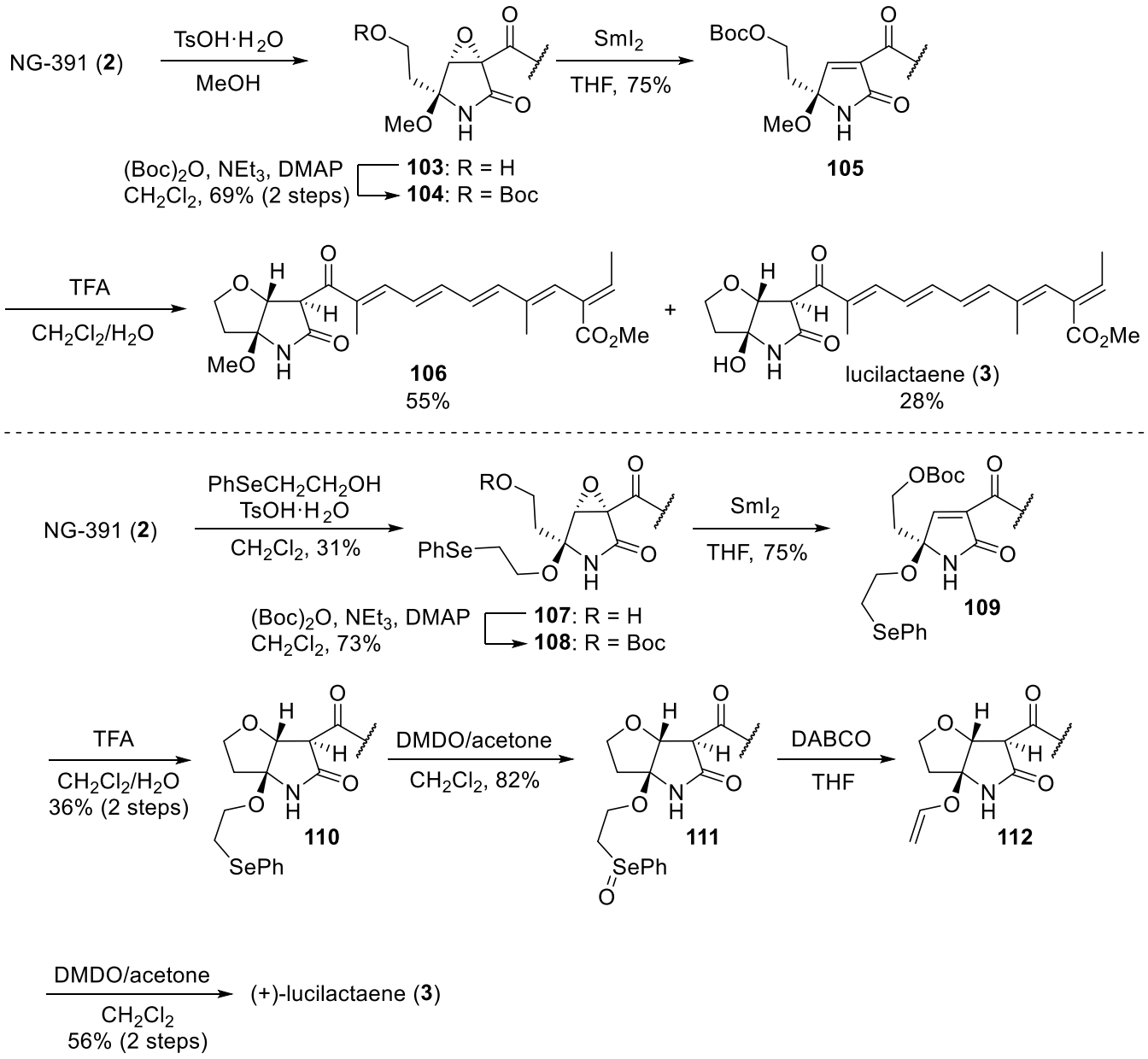
To prepare NG-391 (**2**),[30] (*E*,*E*,*E*)-alcohol **97**, a synthetic intermediate for epolactaene, was first oxidized to (*E*,*E*,*E*,*E*)-aldehyde **98** with IBX (Scheme 8). According to the synthetic protocol for epolactaene, they synthesized NG-391 (**2**) through the condensation of β-ketonitrile **99** and (*S*)-malic acid-derived aldehyde **100**.



Scheme 8. Hayashi's Total Synthesis of NG-391

Next, they investigated the synthesis of lucilactaene (**3**) from NG-391 (**2**) (Scheme 9). NG-391 (**2**) was initially converted to β-methoxide **103** as a single isomer. After Boc protection, the epoxide portion in **104** was reduced with SmI_2, and α,β-unsaturated ketoamide **105** was treated with TFA in CH_2Cl_2/H_2O to form lucilactaene (**3**). The optical rotation of synthetic **3** was 0, identical to that of natural lucilactaene. However, compound **106**, which was obtained in the final step along with **3**, had a value of $[\alpha]_D$ +36.6 (*c* 0.17, MeOH). Therefore, they hypothesized that synthetic **3** was racemized during the final step by treatment with acid. Hence, to obtain optically pure lucilactaene, they decided to develop an alternative synthetic route that would require a new protecting group that was easily removable without an acid.

Synthetic NG-391 (**2**) was transformed to phenylselenylethyl ether **107** by using 2-(phenylselenyl)ethan-1-ol (Scheme 9). Boc protection, reduction of **108** with SmI_2, and Boc deprotection of **109** gave requisite bicyclic lactam **110**, which was oxidized to selenoxide **111** with dimethyldioxirane (DMDO). DABCO-promoted β-elimination gave vinyl ether **112**, and final oxidative treatment with DMDO furnished lucilactaene (**3**). Thus, phenylselenylethyl ether performed well as a new protecting group to give optically pure lucilactaene ($[\alpha]_D$ +39.5 (*c* 0.10, MeOH)).



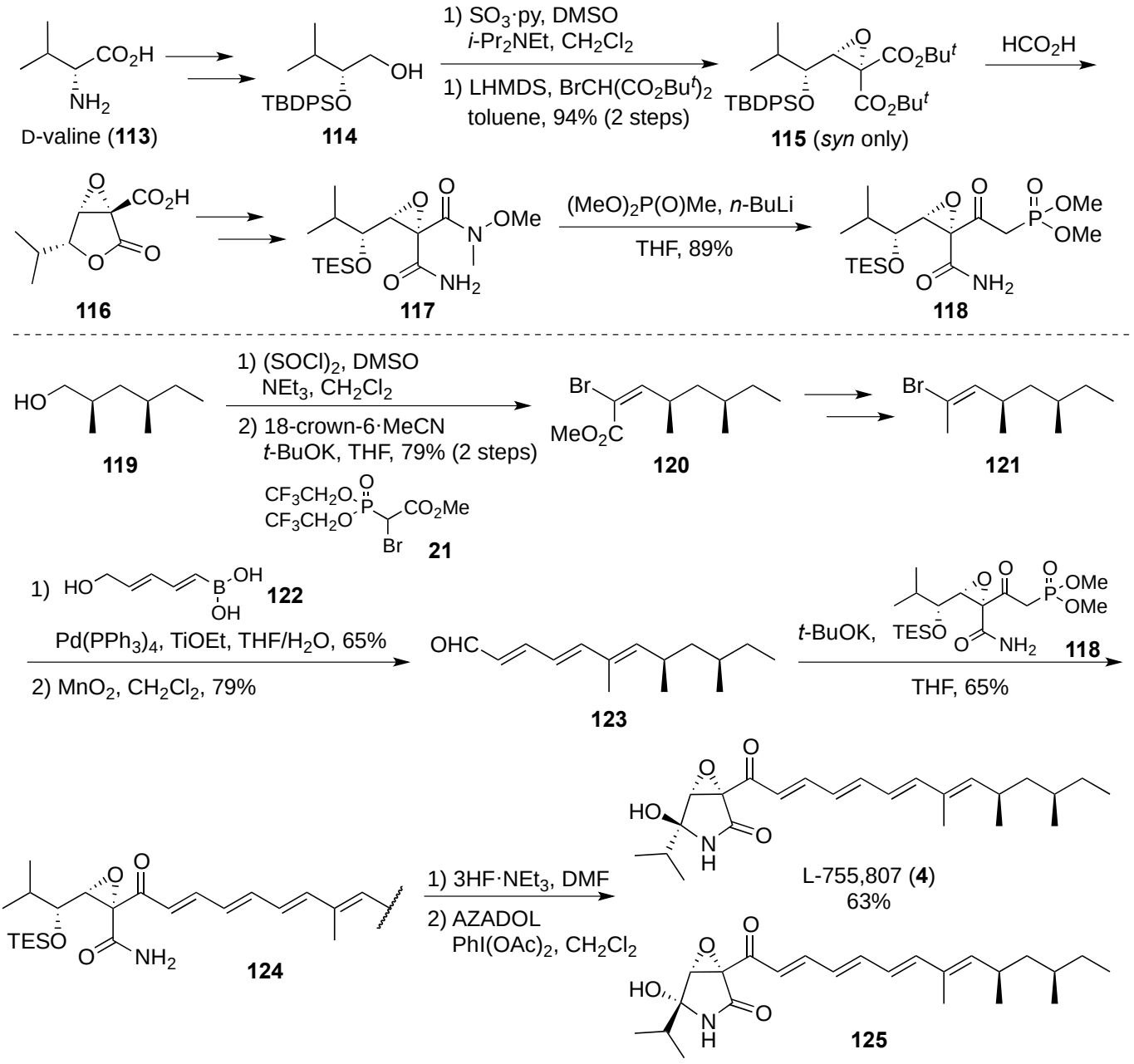
Scheme 9. Hayashi's Total Synthesis of Lucilactaene

2-3-1. Kobayashi and Kogen's Total Synthesis of L-755,807

In 2016, Kobayashi and Kogen achieved the first total synthesis of L-755,807 (**4**) and three stereoisomers to establish the relative and absolute configurations of the natural product (Scheme 10).[32-34] The key reactions were a diastereoselective Darzens condensation[35] to synthesize the epoxy-γ-lactam segment and an HWE reaction[22] to construct the trisubstituted olefin in the side chain.

Darzens condensation of the aldehyde prepared from D-valine (**113**) with *tert*-butyl bromomalonate gave epoxide **115** with high diastereoselectivity, and **115** was immediately converted to phosphonate **118** as the epoxy-γ-lactam segment via lactone **116** and diamide **117**.

For the side-chain segment, known alcohol **119**[36] was converted to α,β-unsaturated ester **120** with high stereoselectivity by an HWE reaction, and then the ester group was reduced to a methyl group to afford vinyl bromide **121**. The Suzuki−Miyaura coupling of vinyl bromide **121** with known boronic acid **122**[37] and subsequent oxidation with MnO_2 yielded side-chain segment **123**. Triene aldehyde **123** was coupled with **118** to deliver tetraene ketone **124**, and then desilylation and AZADOL oxidation furnished L-755,807 (**4**) and its isomer **125** at the hemiaminal position, establishing the relative and absolute configurations of natural **4**.

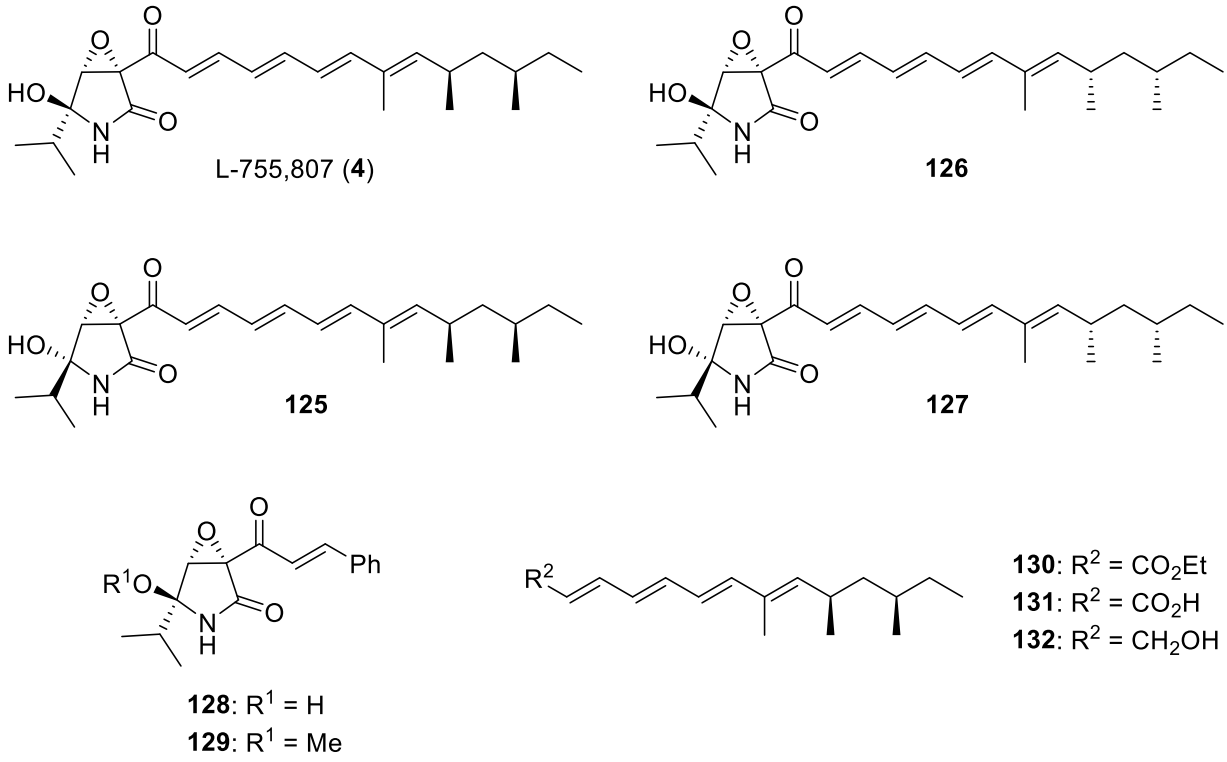


Scheme 10. Kobayashi and Kogen's Total Synthesis of L-755,807

2-3-2. Kobayashi and Kogen's SAR Studies of L-755,807

After completion of the synthesis of L-755,807 (**4**), Kobayashi and Kogen evaluated the inhibitory activity of the synthetic compounds against amyloid-β aggregation for drug discovery in Alzheimer's disease (Table 3). Synthetic L-755,807 and its stereoisomers showed potent inhibitory activity. Isomer **127**, which had the opposite configurations to the natural product at C5, C18, and C20, exhibited the most potent activity.[33,34] Toward pharmacophore identification, they also prepared and biologically evaluated analogs **128**–**132**, which revealed that an epoxy-γ-lactam moiety was not essential for the activity, whereas the amphiphilicity of the compounds, as shown by **131** and **132**, was crucial for potent inhibitory activity.[38]

Table 3. Aβ Aggregation Inhibitory Activity of L-755,807 and Its Derivatives



Compound	Aβ aggregation inhibitory activity, IC_{50} (μM)
4 (L-755,807)	21
125	17
126	12
127	4
128	601

129	448
130	>1000
131	30
132	7.1
Myricetin[a]	4.1

[a]Myricetin was used as a positive control.

3. SYNTHESIS OF γ-LACTAM-CONTAINING NATURAL PRODUCTS WITH SPIRO AND TRICYCLIC RING SYSTEMS

Many highly oxidized γ-lactam containing compounds with a 1-oxa-7-azaspiro[4.4]non-2-ene-4,6-dione core have been isolated as fungal metabolites and also possess various attractive biological activities (Figure 2). The syntheses and biosynthetic pathways of these compounds have been studied extensively and are summarized in the review by Jo and Han.[39] In this section, we introduce compounds for which total synthesis has been achieved or is being investigated.

Azaspirene (**133**) was isolated from the fungus *Neosartorya* sp. by the Osada and Kakeya groups and was identified as a novel angiogenesis inhibitor.[40] They found that natural product **133** inhibited endothelial migration induced by avascular endothelial growth factor (ED_{100} = 27.1 μM), and the *in vivo* antiangiogenic activity was confirmed by a tumor neoangiogenesis assay and a chicken chorioallantoic membrane (CAM) assay.[41] Additionally, they showed that **133** specifically blocks vascular endothelial growth factor-induced phosphorylation of Rik1-associated factor. Based on these results, natural product **133** is widely expected to have clinical utility for the treatment of cancer, diabetic retinopathy, rheumatoid arthritis, and related disease.[42-45]

Pseurotins A (**134**) and E (**135**) were isolated from a culture broth of *Pseudeurotium ovalis* STOLK together with pseurotin analogues (pseurotins B–D).[46-50] The structure of pseurotin A (**134**) was determined by single-crystal X-ray analysis of its 12,13-dibromo derivative.[47] Pseurotin A (**134**) has diverse inhibitory activities against chitin synthase (IC_{50} = 81 mM),[51] MAO (42.7% inhibitory activity at 0.1 mg/mL),[52] and IgE production *in vitro* (IC_{50} =3.6 mM; details are given in Section 3-4-2).[53] Moreover, pseurotin A (**134**) acts on cell types associated with neural diseases or cancer. The compound **134** shows neuritogenic activity in rat pheochromocytoma PC12 cells, cytotoxicity against A2780 human ovarian carcinoma cells (IC_{50} = 12 μg/mL),[54] apomorphine antagonist activity,[55] and cytotoxicity against the HL-60 cell line (IC_{50} = 67.0 mM).[56] Although the biological activity of pseurotin A has been studied extensively, that of pseurotin E (**135**) has not. Pseurotin F_2 (**136**) was isolated from *Aspergillus fumigatus* DSM 6598 together with pseurotin F_1. Natural product **136** exhibited inhibitory activity against chitin synthase (IC_{50} = 192 mM)[50] and apomorphin.[55]

Synerazol (**137**) was isolated from *A. fumigatus* SANK 10588 by the Ando group.[57] The absolute configuration was established by spectroscopic analysis and the modified Mosher's method.[58] Natural product **137** possesses IgE production inhibitory activity (IC_{50} = 0.26 mM); details are given in Section 3-4-2).[53] The fluorinated derivatives showed antiangiogenic activity in a CAM assay and exhibited inhibitory activity against neovascularization.[59] Notably, 19-fluorosynerazol possessed more potent cytotoxicity for several cancer cell lines than natural compound **137**.

Cephalimysins A–C (**138**–**140**) were isolated from a culture broth of *A. fumigatus* from the marine fish *Mugil cephalus* by Yamada group[60-62] and FD-838 (**141**) was isolated from *A. fumigatus* Fresenius F-838 by Omura group.[63] These compounds, except cephalimysin A (**138**), are structurally similar, differing only in the stereochemistry at the aminal and contiguous hydroxy group. The biological activities of those compounds were also similar, and they showed cytotoxicity against murine P388 and human HL-60 leukemia cell lines.[60,61] In addition, FD-838 (**141**) suppressed the growth of certain Gram-positive bacteria and fungi.[63]

Berkeleyamide D (**142**) was isolated from the fungus *Penicillium rubrum* Stoll by Stierle et al. and inhibited matrix metalloproteinase-3 and caspase-1.[64]

Two tricyclic natural products that have the same oxidation state as these azaspiro products and have a characteristic tricyclic framewok incorporating a central γ-lactam structure, and hemiaminal and acetal functionalities have also been identified. Rubrobramide (**143**), isolated from *Cladobotyrum rubrobrunnescens*, shows antifungal, cytotoxic, and nematicidal activites.[65]

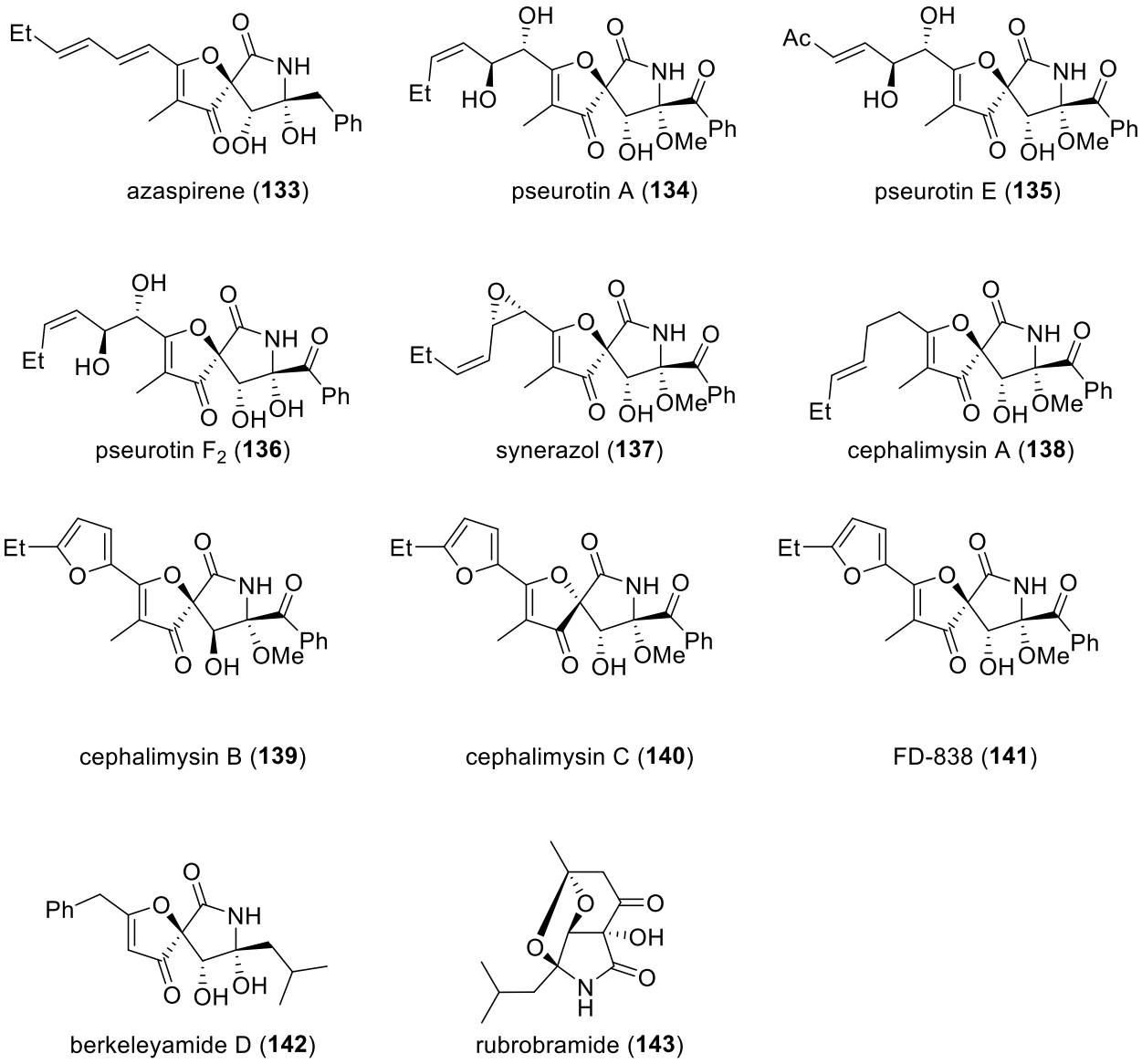
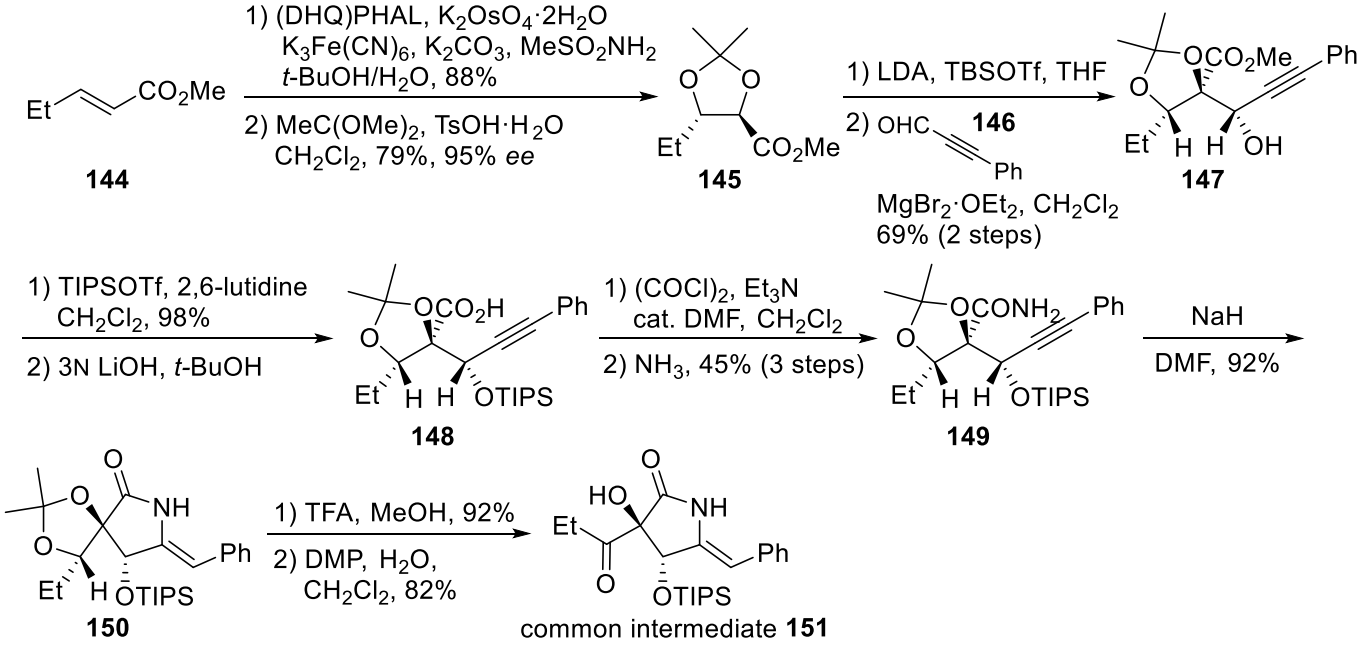


Figure 2. γ-Lactam-containing Natural Products with Spiro and Tricyclic Ring Systems

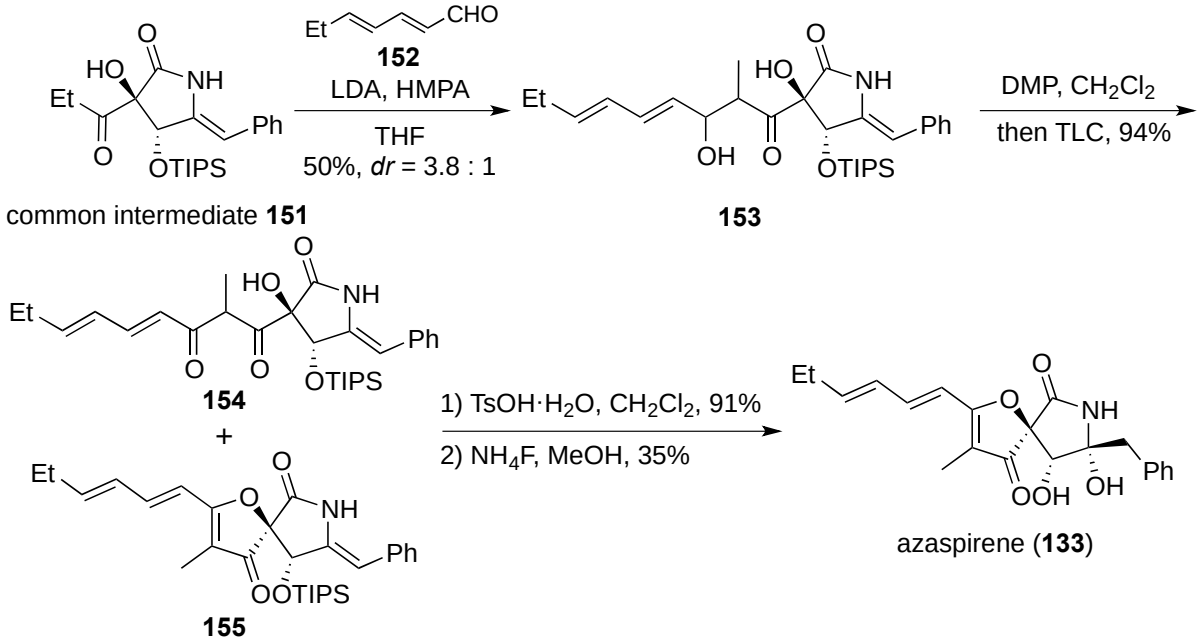
3-1. Hayashi's Total Syntheses of (−)-Azaspirene, Pseurotin A, and Synerazol

Hayashi's group achieved the total syntheses of azaspirene (**133**),[66] pseurotin A (**134**),[67] and synerazol (**137**)[68] using convergent strategies with late-stage coupling between a common intermediate and partners bearing different moieties of the target compounds. The common intermediate **151** was synthesized from 2-pentenoate (**144**) (Scheme 11). Sharpless asymmetric dihydroxylation of **144** was followed by acetonidation to afford acetonide **145**. After conversion to ketene silyl acetal from **145**, a Mukaiyama aldol reaction promoted by $MgBr_2{\cdot}OEt_2$ against phenylpropargyl aldehyde (**146**) generated propargyl alcohol **147** with high diastereoselectivity. The protection of the alcohol with a TIPS group and hydration of the ester delivered carboxylic acid **148**, followed by amidation with an acid chloride to produce amide **149**. Treatment of **149** with NaH under sonication gave lactam **150**, which was deprotected at the

acetonide and oxidized at the secondary alcohol to form common intermediate **151**. The lithium enolate of **151** was reacted with heptadienal **152** to afford coupling product **153** (Scheme 12). After oxidation, acid-mediated cyclization, and deprotection, the azaspirene (**133**) was obtained.

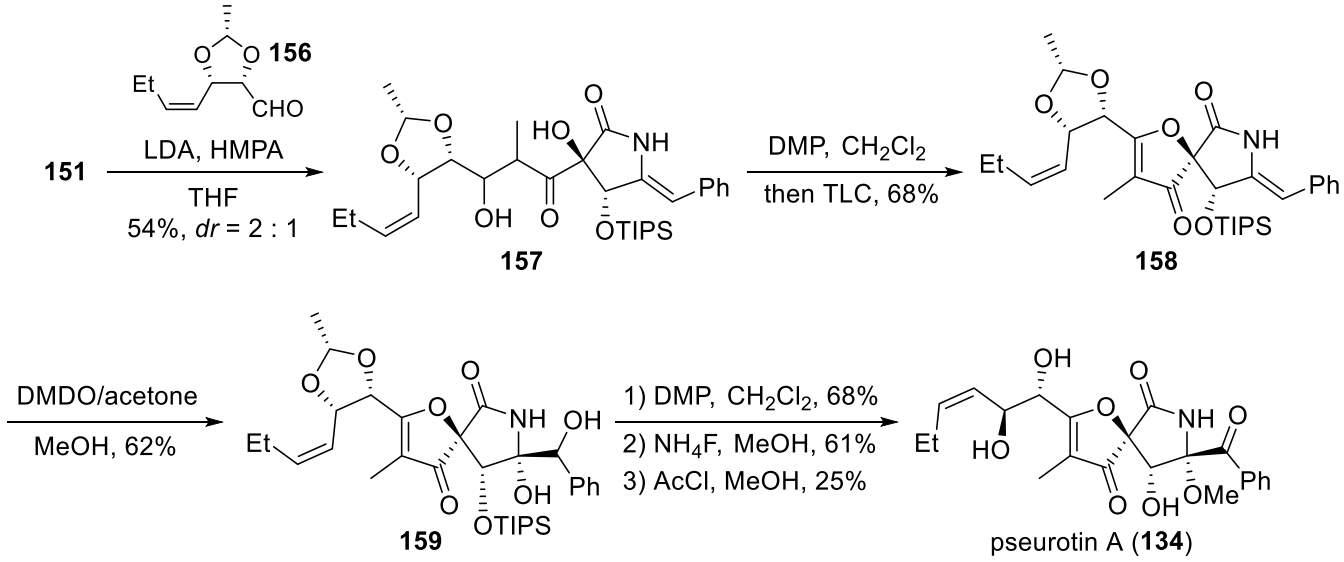


Scheme 11. Synthesis of the Common Intermediate **151**



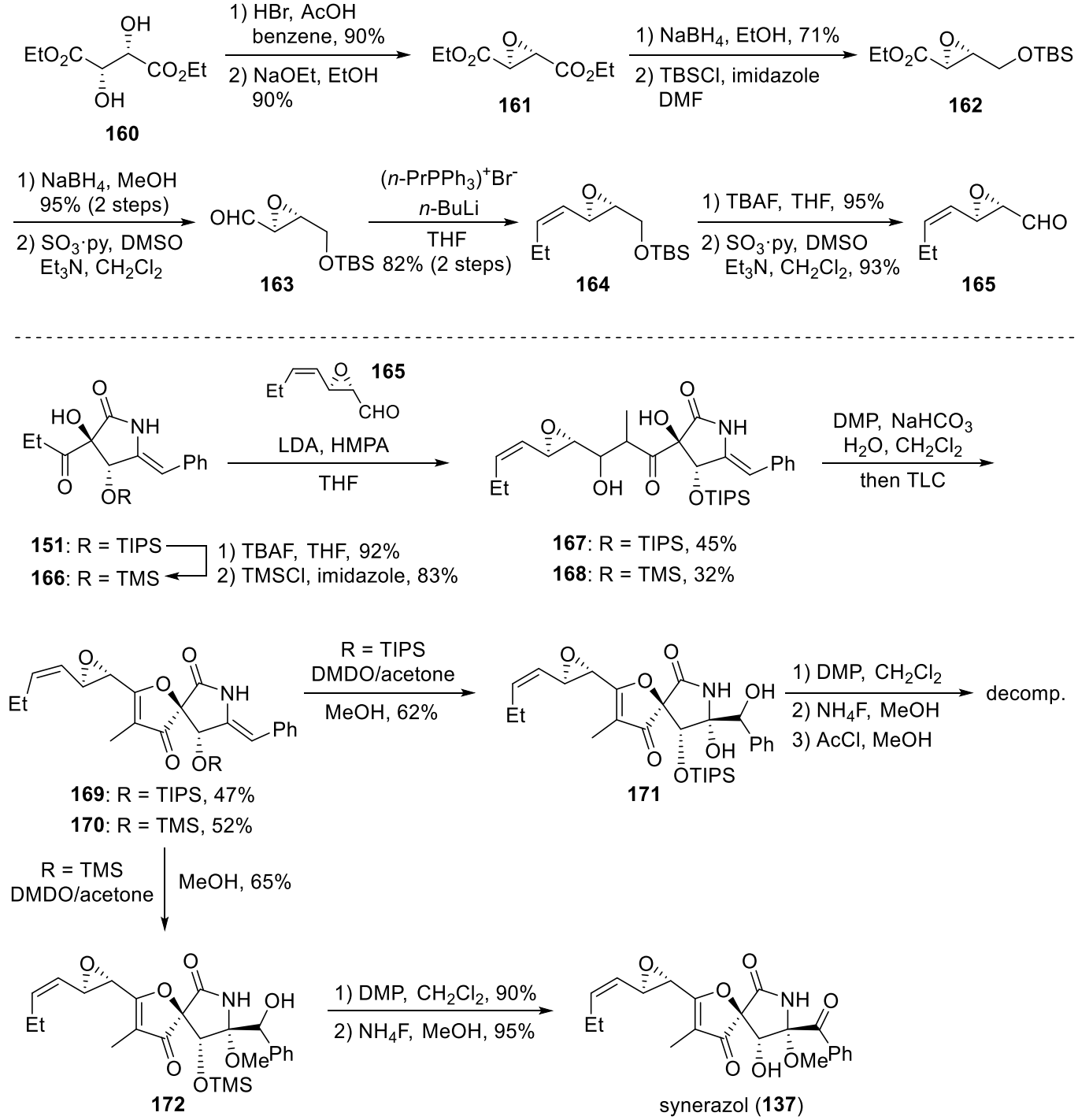
Scheme 12. Hayashi's Total Synthesis of Azaspirene

In the total synthesis of pseurotin A (**134**),[67] the common intermediate **151** was coupled with known aldehyde **156**[68] to afford lactam **157**, and the secondary alcohol was oxidized and cyclized on silica gel during TLC to produce spiro compound **158** (Scheme 13). The selective oxidation of the benzylidene moiety without affecting the other olefin parts was achieved with DMDO and afforded diol **159**. Oxidation of benzyl alcohol, desilylation, and aminalization gave pseurotin A (**134**).



Scheme 13. Hayashi's Total Synthesis of Pseurotin A

Finally, they reported total synthesis and structural determination of synerazol (Scheme 14).[69] The coupling partner for **151** was synthesized from L-tartaric acid diethyl ester (**160**). The diol moiety of **160** was converted to an epoxide **161** in two steps. TBS ether **162** was obtained by reducing the ester and installing protecting groups. Transformation of the ester **162** to an aldehyde **163** followed by a Wittig reaction afforded olefin **164**, which was deprotected and oxidized to give coupling partner **165**. Lactams **151** and **166** were prepared by protecting the secondary alcohol with silyl groups. After coupling with **165** via an aldol reaction, the resultant alcohol was oxidized on silica gel during TLC to form spiro compounds **169** and **170**. The hydroxy group on TIPS-protected substrate **171** could not be converted to a methoxy group by the same method as in the pseurotin A synthesis[67] because of acid-mediated decomposition of the epoxide moiety. In contrast, DMDO oxidation in MeOH at the benzylidene moiety on TMS-protected substrate **170** afforded methyl ether **172**, although the TIPS-protected substrate produced only diol **171** under the same conditions. Oxidation and deprotection of **172** afforded synerazol (**137**).

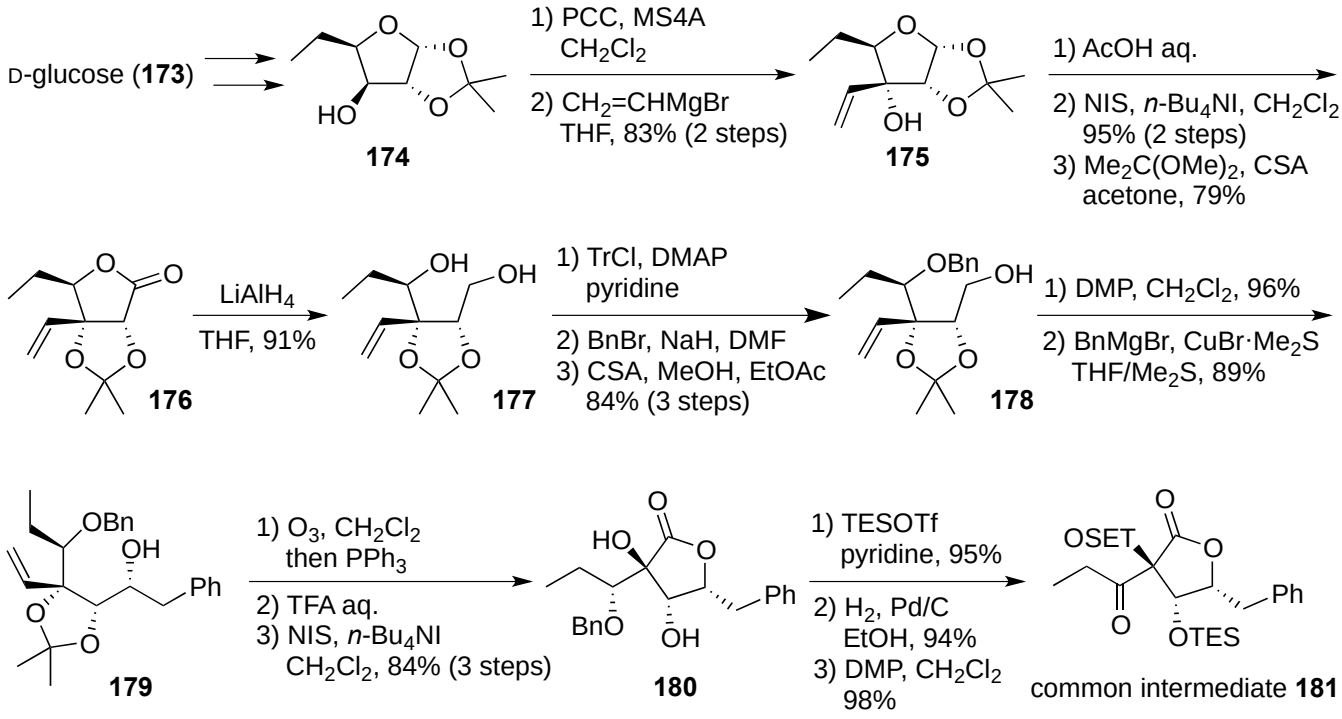


Scheme 14. Hayashi's Total Synthesis of Synerazol

3-2. Tadano's Total Syntheses of Pseurotin A and F_2 and Azaspirene

Tadano's group reported the total syntheses of pseurotin A (**134**) and F_2 (**136**), and azaspirene (**133**).[70,71] They prepared two segments consisting of a lactam moiety, which was a common intermediate **181**, and the side-chain moiety stereoselectively and accomplished the total syntheses via coupling of those compounds. In the total synthesis of pseurotins A and F_2, both segments were synthesized from D-glucose

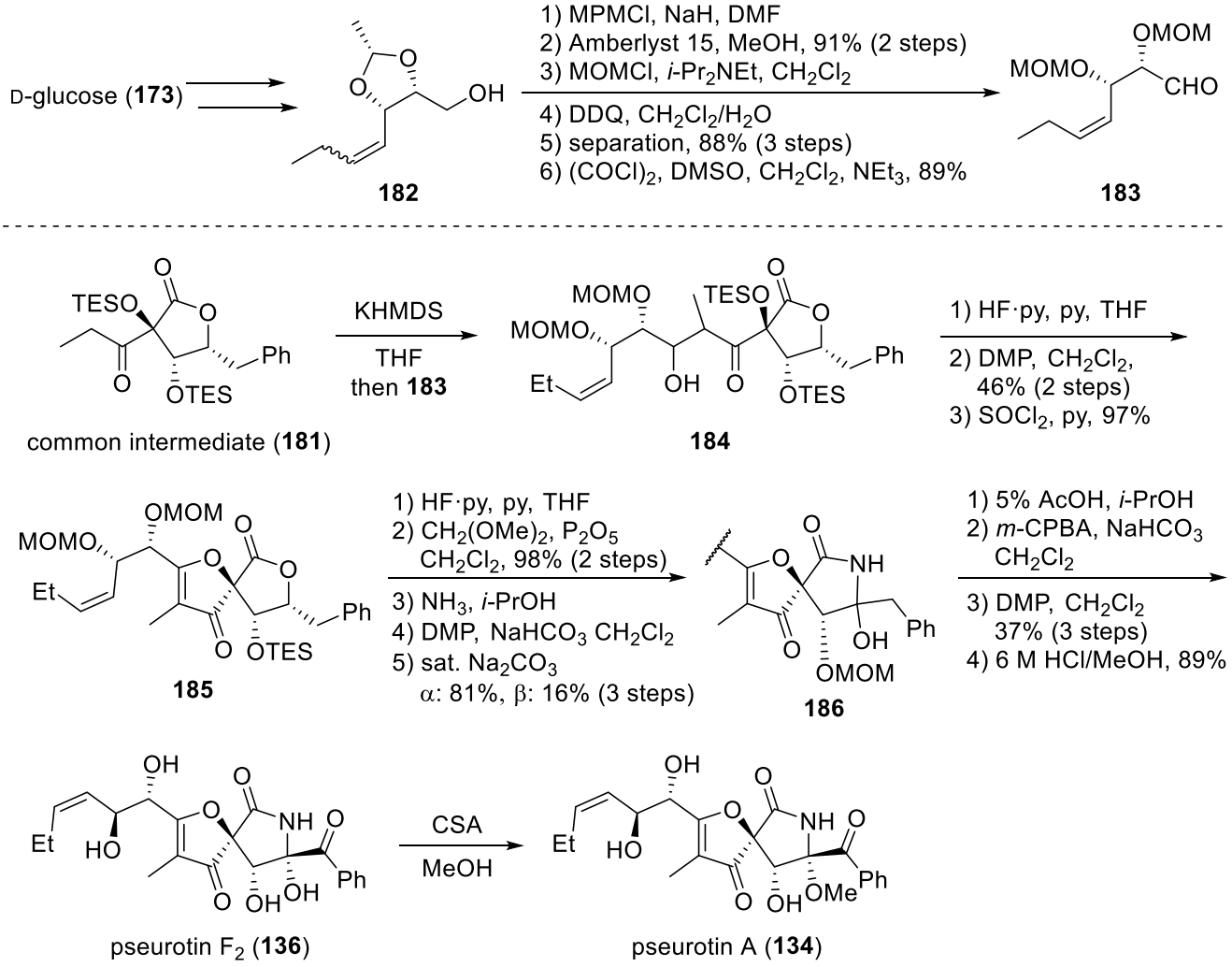
(**173**). Oxidation of known hexafuranose **174**[72] and the addition of a vinyl Grignard reagent provided **175** as a single isomer (Scheme 15). Replacement of the acetonide and chemoselective oxidation with NIS afforded lactone **176**, and the lactone moiety was reduced with $LiAlH_4$ to obtain diol **177**. The secondary alcohol of **177** was protected with a benzyl group through a sequence involving protection and deprotection of the primary alcohol with a trityl group to afford alcohol **178**. After oxidation of primary alcohol, the benzyl Grignard reagent was reacted with aldehyde to afford alcohol **179**. In this reaction, using $CuBr{\cdot}Me_2S$ as an additive greatly improved the stereoselectivity. Cyclization was achieved by ozonolysis of the methylene moiety and treatment with TFA, followed by oxidation of hemiacetal to produce lactone **180**. Protection of two alcohols with TES, deprotection of the Bn group, and oxidation produced common intermediate **181**.



Scheme 15. Synthesis of the Common Intermediate **181** for Pseurotin A and F_2 and Azaspirene

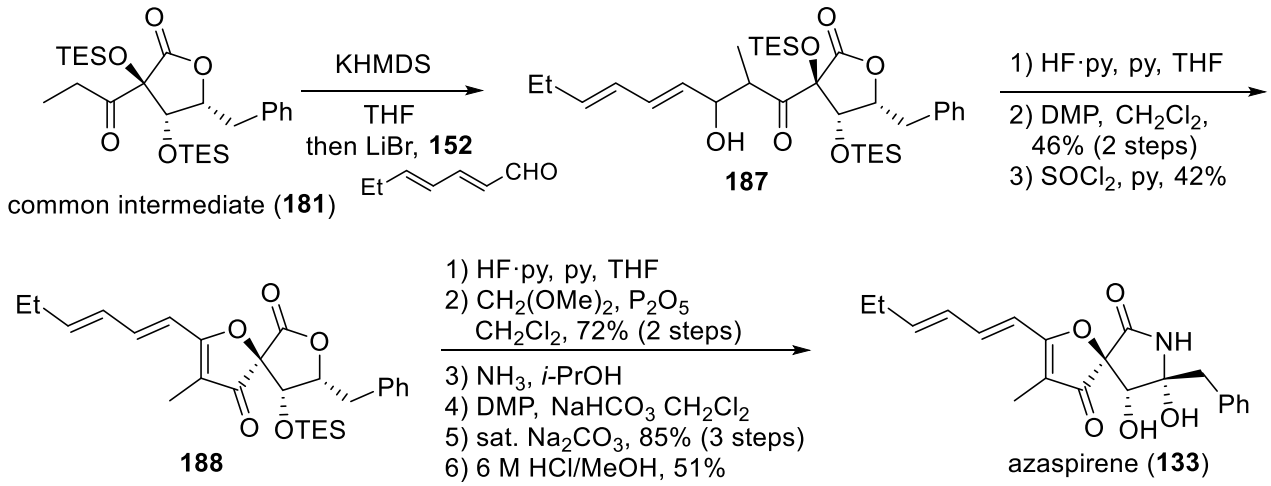
In the syntheses of pseurotin A (**134**) and F_2 (**136**), the diol segment was synthesized from D-glucose (**173**) (Scheme 16). Alcohol **182**, prepared by a known procedure[73] from D-glucose (**173**), was converted to aldehyde **183** by careful protection and deprotection. An aldol reaction between ketone **181** and aldehyde **183** produced adduct **184**, which was converted to spiro lactone **185** via deprotection, oxidation, and dehydration. The TES protecting group on the alcohol of lactone **185** was replaced with a MOM group, and the product was treated with NH_3 followed by oxidizing the resultant alcohol and

lactamization to afford lactam **186**. Sequential dehydration, oxidation, and deprotection delivered pseurotin F_2 (**136**), which was treated with camphorsulfonic acid (CSA) in MeOH to obtain pseurotin A (**134**).



Scheme 16. Tadano's Total Synthesis of Pseurotin A and F_2

In contrast, in the total synthesis of azaspirene, coupling ketone **181** and (2*E*,4*E*)-2,4-heptadienal (**152**) produced **187** (Scheme 17). The same synthetic route to that of pseurotin A and F_2 was followed up to aminal formation, and then the deprotection of the TES group delivered azaspirene (**133**).

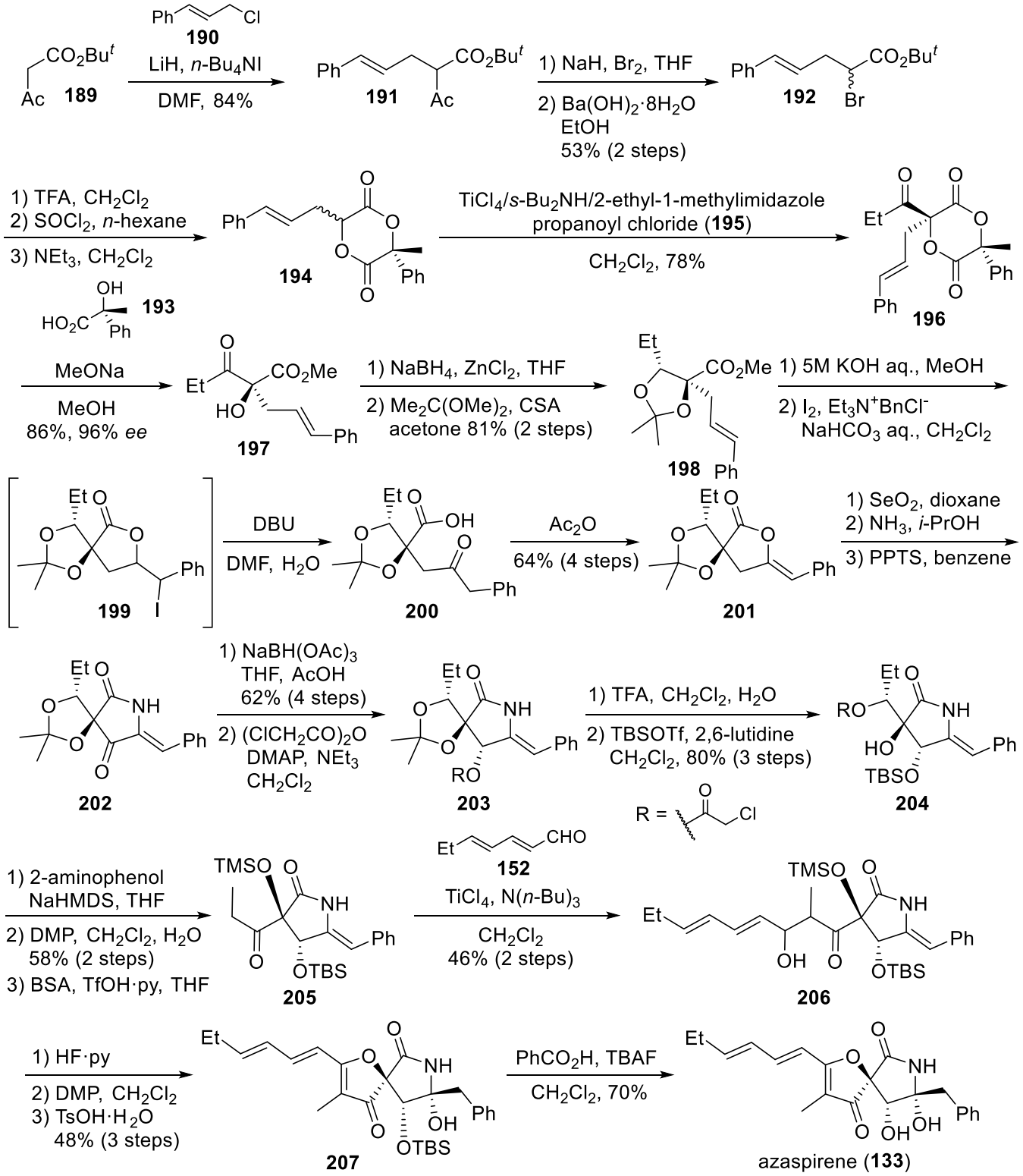


Scheme 17. Tadano's Total Synthesis of Azaspirene

3-3. Misaki and Tanabe's Total Synthesis of Azaspirene

Misaki and Tanabe's group accomplished the asymmetric total synthesis of azaspirene by using the Ti-Claisen condensation and Ti-direct aldol reaction as key reactions (Scheme 18).[74] First, the chiral template for the Ti-Claisen condensation was prepared. The reaction of *tert*-butyl acetoacetate (**189**) with cinnamyl chloride (**190**) afforded ester **191**, which was brominated and deacetylated to give α-bromo ester **192**. The *tert*-butyl moiety of ester **192** was converted into the acid chloride over two steps, and then condensation and cyclization with (*R*)-atrolacic acid (**193**) produced 1,4-dioxane derivative **194**. The $TiCl_4$/*s*-Bu_2NH/2-ethyl-1-methylimidazole-mediated[75] asymmetric Ti-crossed-Claisen condensation between chiral template **194** and propanoyl chloride (**195**) afforded ester **196**, followed by methanolysis to furnish ester **197**. Diastereoselective reduction of the ketone moiety of ester **197** with $NaBH_4$ and $ZnCl_2$ and acetonidation of the resultant diol produced acetonide **198**. After ester hydrolysis, the resultant carboxylic acid was subjected to iodolactonization, and then treated with DBU in the presence of small amount of water to afford lactone-opened carboxylic acid **200**. The lactone **201** was prepared by treating carboxylic acid **200** with Ac_2O. Lactam **202** was obtained after oxidation of the vinyl position with SeO_2, treatment with NH_3, and dehydration with acid. Lactam **202** was reduced with $NaBH(OAc)_3$ with high diastereoselectivity, and the resultant secondary alcohol was protected with an α-chloro-acetyl group. Treatment with TFA produced the diol **204** by acetonide deprotection and caused unexpected migration of the α-chloro-acetyl group. After protection and deprotection, oxidation of the secondary alcohol was followed by the protection of the tertiary alcohol with TMS by using *N*,*O*-bis(trimethylsilyl)acetamide (BSA) to produce ketone **205**, which was subjected to the $TiCl_4$/Bu_3N-mediated aldol reaction with (2*E*,4*E*)-heptadienal (**152**) to yield aldol adduct **206**. Deprotection of the TMS group, oxidation of the

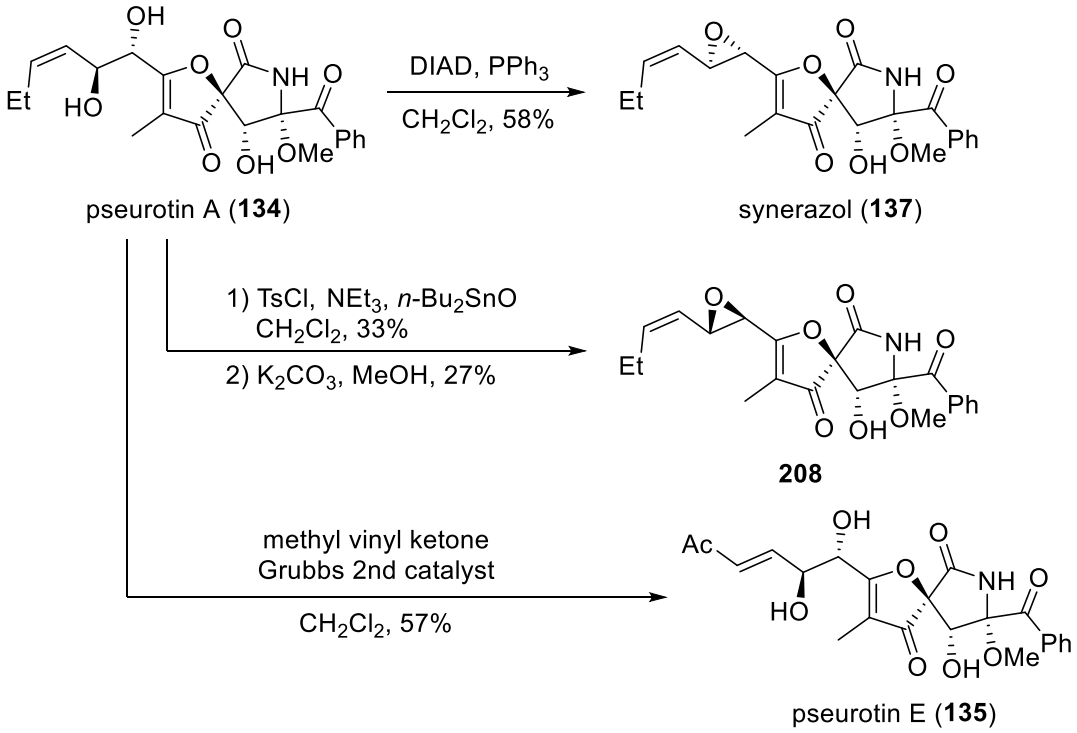
secondary alcohol, and treatment with acid formed spiro compound **207**, and then the TBS group was removed to afford azaspirene (**133**).



Scheme 18. Misaki and Tanabe's Total Synthesis of Azaspirene

3-4-1. Ishikawa's Synthesis of Synerazol and Pseurotin E

The Ishikawa group reported the one-pot syntheses of synerazol (**137**) and pseurotin E (**135**) from pseurotin A (**134**) and determined the absolute stereochemistry of pseurotin E (Scheme 19).[76] Pseurotin A (**134**) was obtained from the fermentation broth of *Aspergillus* sp. The Mitsunobu reaction of **134** gave synerazol (**137**), and selective tosylation in the presence of dibutyltin oxide was followed by epoxidation under basic conditions to afford **208**, in which the epoxide moiety was stereoinverted compared with synerazol. Lastly, olefin cross-metathesis using the second-generation Grubbs catalyst with **134** and methyl vinyl ketone produced pseurotin E (**135**).



Scheme 19. Ishikawa's Total Synthesis of Synerazol and Pseurotin E

3-4-2. Ishikawa's SAR Studies of Pseurotin A

The Ishikawa group was interested in the inhibitory activity of pseurotin A (**133**) against IgE production, which is involved in the pathogenesis of atopy, asthma, rhinitis, and dermatitis. They conducted SAR studies by synthesizing various derivatives of pseurotin A, including pseurotin E (**135**), pseurotin F_2 (**136**), and synerazol (**137**).[53] The known compounds obtained from fermentation or precursor-directed biosynthesis were 12,13-dihydropseurotin A (**209**),[49,50] 12,13-dibromopseurotin A (**210**),[46] 10,11-acetonide **211**,[46] aldehyde **212**,[46,49] (10*R*,11*S*)-diastereomer of synerazol **208**,[76] triacetylpseurotin A (**214**),[46] tetraacetylpseurotin A (**215**),[46] 17-dihydropseurotin A (**216**),[50] and fluorinated pseurotin A (**217**,

218) (Figure 3).[40] Other compounds were prepared by synthetic derivatization of pseurotin A (**134**) from fermentation (Scheme 20). Compound **134** was subjected to oxidative cleavage to form aldehyde **212**,[46] which was converted to olefins **219**–**224** through the Witting reaction and amines **225**–**230** by reductive amination. Next, olefin metathesis, as described in Section 3.4.1,[76] of compound **134** gave olefins **231**–**236**, in which the olefin moiety was hydrogenated to produce saturated side chain compounds **237**–**240**. Various modifications of the hydroxy groups, methoxy group, and lactam moiety were performed to produce compounds **241**–**244**. Deoxygenation via compounds **245** and **247** obtained by selective thioacylation afforded compounds **246** and **248**. Finally, epoxidation and modified Simmons−Smith cyclopropanation of compound **134** afforded epoxide **249** and cyclopropane **250**.

The evaluation of both known and newly synthesized pseurotin analogues revealed that compounds **133**, **137**, **227**, and **248** exhibited IgE inhibitory activity. In particular, compound **248** showed 50-fold higher activity than natural pseurotin A (**133**). The SAR study indicated that the epoxide stereochemistry, C12-C13 (*Z*)-olefin, 11-hydroxy group, spiro skeleton, and benzoyl moiety were important for the inhibitory activity.

The specificity of the four compounds showing potent inhibitory activity was investigated further. The concentrations at which IgE production was inhibited were much lower than those at which K562 cytotoxicity was observed or B-cell viability was affected. Furthermore, the most potent inhibitory compound (**248**) did not inhibit phytohemagglutinin-induced T-cell proliferation (10 μM). Compound **248** also possessed high IgE selectivity (IgM/IgE: 44, IgG2a/IgE: 23, IgG1/IgE: 3.5), whereas the positive control, prednisolone, which is a corticosteroid, showed no selectivity (IgM/IgE: 0.4, IgG2a/IgE: 0.8, IgG1/IgE: 1.0). These data suggest that **248** may be a potent, specific inhibitor of IgE production.

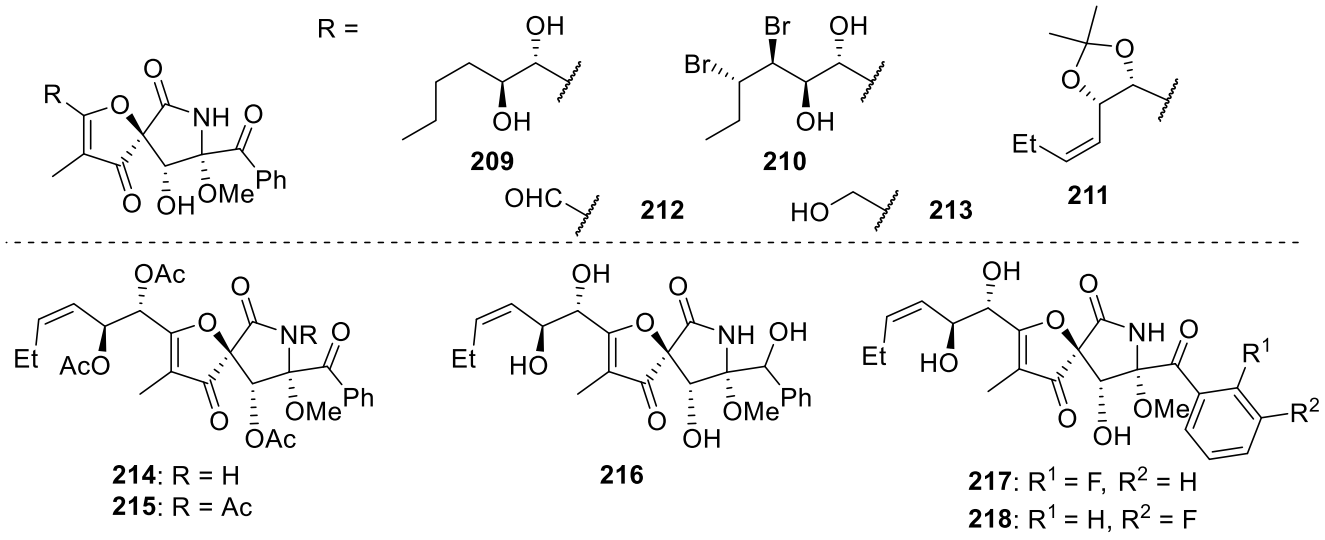
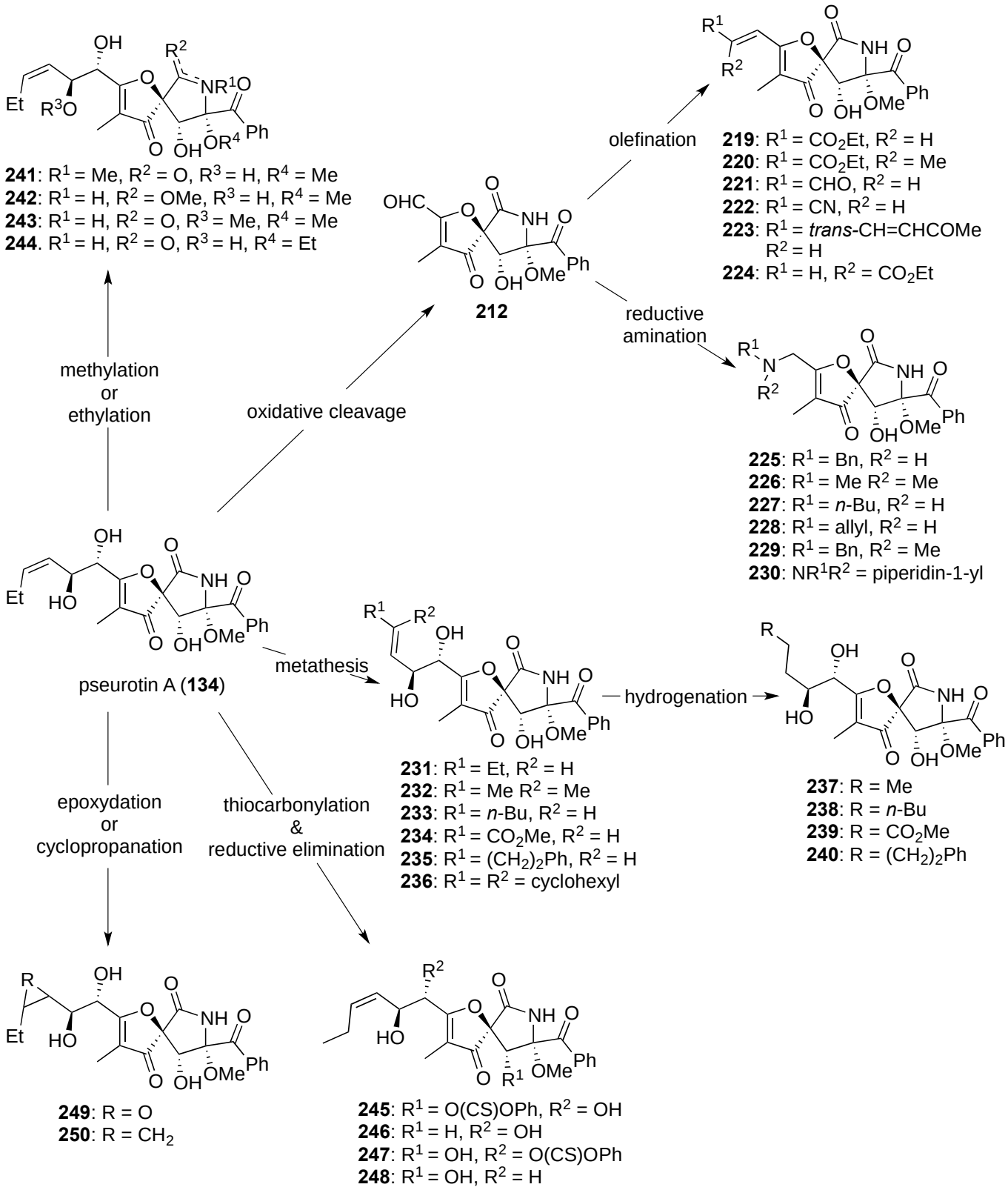


Figure 3. Pseurotin A Derivatives



Scheme 20. Ishikawa's Chemical Modification of Pseurotin A

Table 4. IgE Inhibitory Activity of Pseurotin Analogs

Compound	IgE production, IC_{50} (μM)	Compound	IgE production, IC_{50} (μM)
133	3.6	**229**	>10
135	>10	**230**	>10
136	>10	**231-236**	>10
137	0.26	**237-240**	>10
208-213	>10	**241**	>10
214,**215**	>10	**242**	>10
216	>10	**243**	>10
217,**218**	>10	**244**	>10
219-224	>10	**246**	>10
225	>10	**248**	0.066
226	>10	**249**	>10
227	3.1	**250**	>10
228	>10		

Table 5. Specificity of IgE Production Inhibitors

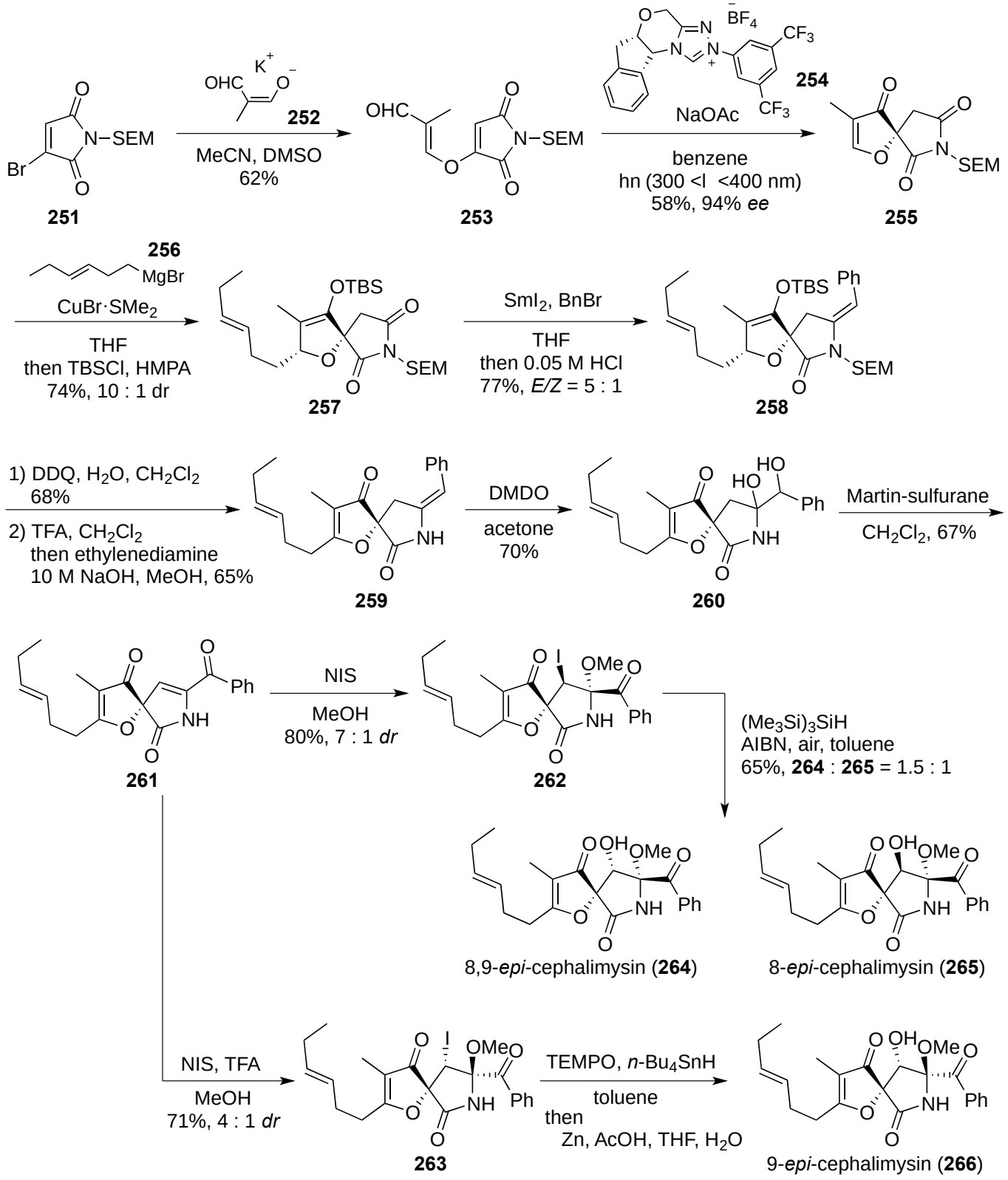
Compound	IgE production, IC_{50} (μM)	K562 cell cytotoxicity, IC_{50} (μM)	B-cell viability, IC_{50} (μM)	T cell proliferation, % inhibition at 10 μM	MLR[b], IC_{50} (μM)
133	3.6	>10	>10	0%	>10
137	0.26	>10	>10	54%	3.1
227	3.1	>10	>10	2%	nt[a]
248	0.066	>10	4.4	0%	nt[a]
Prednisolone	0.0059	nt[a]	>10	84%	0.078

[a] Not tested.

[b] MLR: Mixed lymphocyte reaction

3-5. Rovis' Syntheses of Three Diastereomers of Cephalimysin A

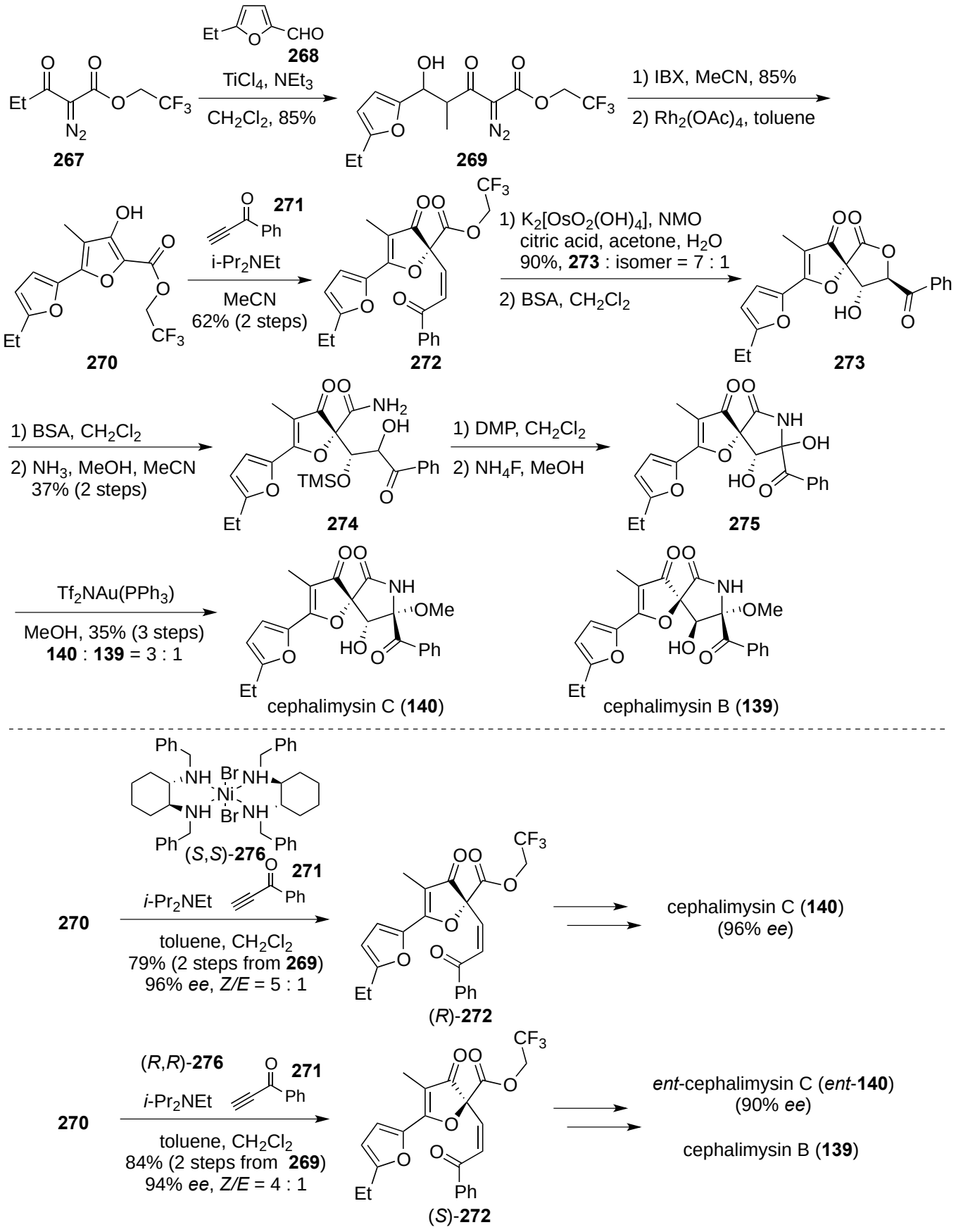
Rovis' group attempted total syntheses of three isomers of cephalimysin A (**138**) by constructing a spiro ring system with a photoisomerization-coupled asymmetric Stetter reaction using their *N*-heterocyclic carbenes (**254**)[77] (Scheme 21). The syntheses began with preparation of substrate for the Stetter reaction in two steps from known bromomaleimide **251**[78] with the potassium salt of dialdehyde **252**. Although they obtained undesired olefin isomer **253**, the problem was solved by *in situ* olefin photoisomerization to the desired isomer. Aldehyde **253** was isomerized under photoirradiation and reacted with known NHC catalyst **254**[79] to form spiro compound **255** with high enantioselectivity. The conjugate addition of Grignard reagent **256** in the presence of CuBr to spiro compound **255** generated the enolate intermediate, which was trapped by TBSCl to produce silyl enolate **257**. Silyl enolate **257** was treated with benzyl bromide and SmI_2, followed by dehydration with acid to afford benzylidene-lactam **258**. Oxidation of the silyl enol ether and deprotection of the SEM group delivered lactam **259**, and the benzylidene moiety was oxidized by DMDO to afford diol **260**. Treatment with Martin's sulfurane dehydrated the *tert*-alcohol and unexpectedly oxidized the secondary alcohol to produce enone **261**. The condition-controlled stereoselective haloetherification yielded products **262** and **263** with opposite stereoselectivity. These compounds were treated with a radical to obtain compounds **264**, **265** and **266**, respectively. Although they did not obtain cephalimysin A, these synthetic studies provide important knowledge about spiro compound reactivity and spectroscopic data.



Scheme 21. Rovis's Synthesis of Three Diastereomers of Cephalimysin A

3-6. Ŝvenda's Total Synthesis of Cephalimysin C

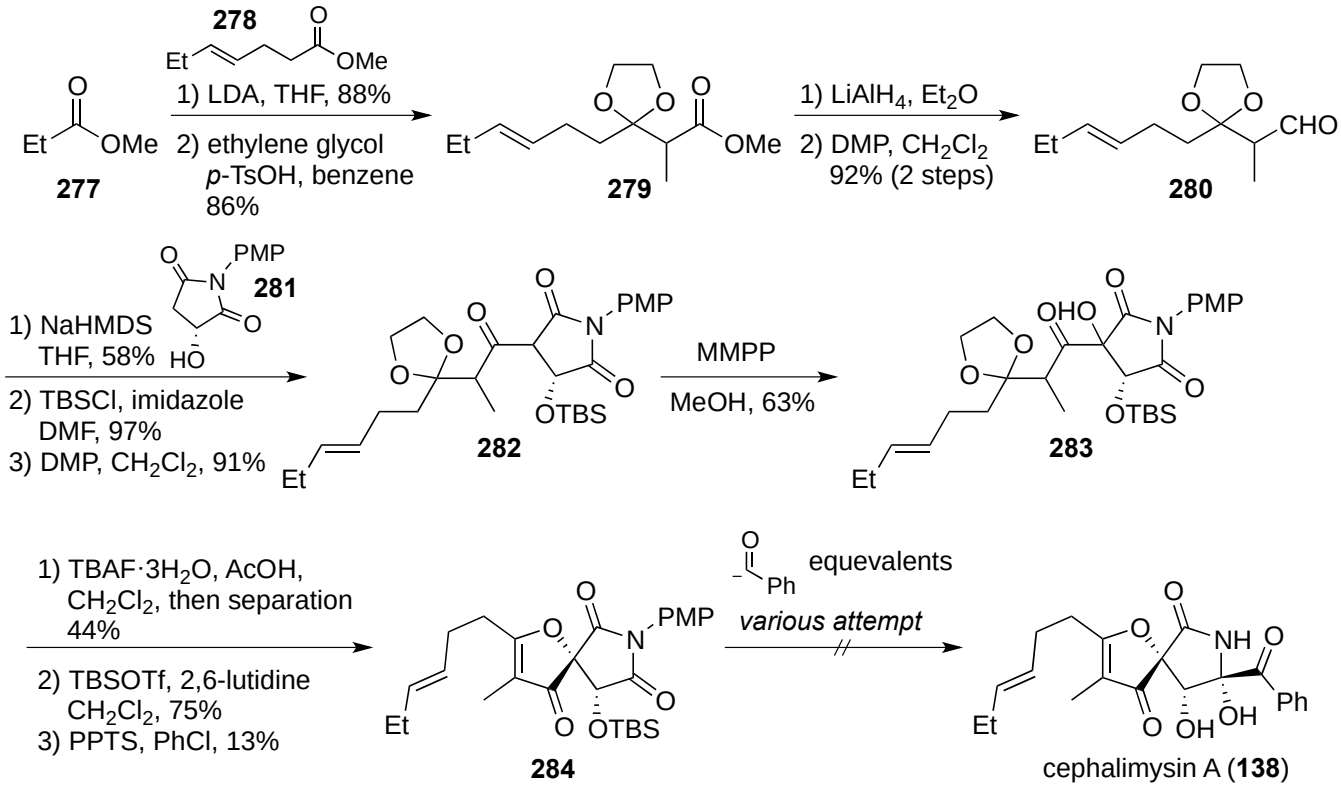
The Ŝvenda group reported the enantioselective total synthesis of both enantiomers of cephalimysin C and B via enantioselective Ni(II)−diamine-catalyzed conjugate addition (Scheme 22).[80,81] Toward the asymmetric synthesis, they initially performed a racemic synthesis. Titanium enolate generated from readily available α-diazo trifluoroethyl ester **267** was reacted with commercially available 5-ethylfurfural (**268**) to produce aldol adduct **269**, in which oxidation of the resultant alcohol was followed by Rh-catalyzed transformation[81] to furanone **270**. Conjugate addition of furanone **270** to alkynyl ketone **271** gave enone **272**, and then Sharpless dihydroxylation of the resultant olefin resulted in spontaneous lactonization and afforded two lactones **273** and isomer from the diastereomers obtained by dihydroxylation. Desired lactone **273** was protected with a TMS group and amide **274** was obtained by ring-opening amidation. The secondary alcohol was oxidized with concomitant heminaminal formation to furnish lactam **275**. Finally, the deprotection of the TMS group and formation of aminal with a gold catalyst gave (±)-cephalimysin B (**139**) and C (**140**). Next, to achieve the asymmetric total synthesis, they performed an asymmetric conjugate addition reaction by using the Ni(II)−diamine complex described by Evans and Seidel.[82,83] Their optimized conditions using Ni catalyst **276** afforded the desired product with high enantioselectivity. Because it is easy to prepare both enantiomers of the diamine ligand, conjugate addition could be performed enantioselectively as desired. After the six-step sequence described above, the total syntheses of cephalimysin C, *ent*-cephalimysin C, and cephalimysin B were accomplished.



Scheme 22. Ŝvenda's Total Synthesis of Cephalimysin B and C

3-7. Xiao and Wang's Synthesis of the Spirofuranone-γ-Lactam Core of Cephalimysin A

The Xiao and Wang group reported the stereoeselective construction of the spirofuranone-γ-lactam core for the total synthesis of cephalimysin A (**138**) (Scheme 23).[84] Methyl propionate (**277**) and known ester **278**[85] were subjected to the Claisen condensation, followed by ketal protection to afford ester **279**, which was converted to aldehyde **280** via reduction and oxidation. The aldol reaction of aldehyde **280** with the dianion generated from phthalimide **281** produced diol, in which one alcohol was selectively protected with TBS, and oxidation of the other alcohol afforded ketone **282**. Oxidation of the methylene moiety with MMPP delivered alcohol **283**. After removal of the TBS group, products were separated by silica gel column chromatography, and reprotection of the alcohol with a TBS group was followed by the construction of the spiro skeleton under acidic conditions to give spirofuranone-γ-lactam **284**. Although various methods were attempted to install the benzoyl moiety, this was not achieved.

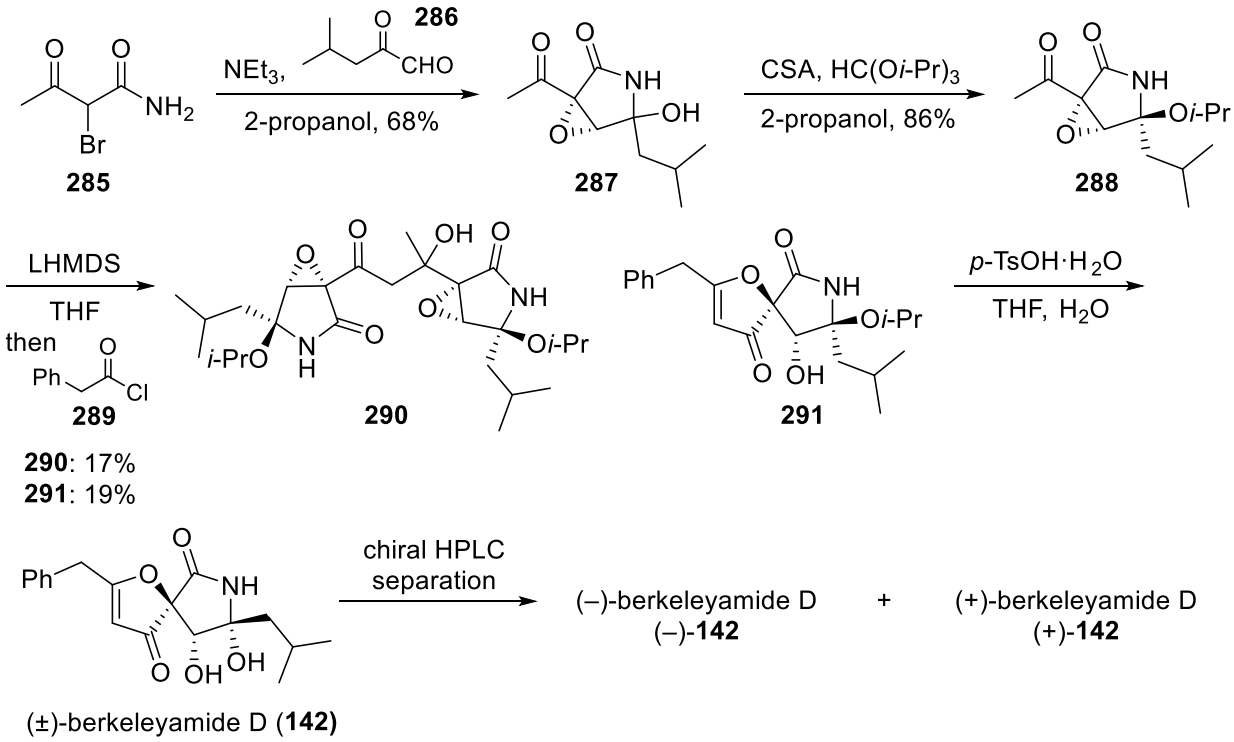


Scheme 23. Xiao and Wang's Synthetic Studies of Cephalimysin A

3-8. Kuramochi and Tsubaki's First- and Second-Generation Total Syntheses of Berkeleyamide D and Rubrobramide

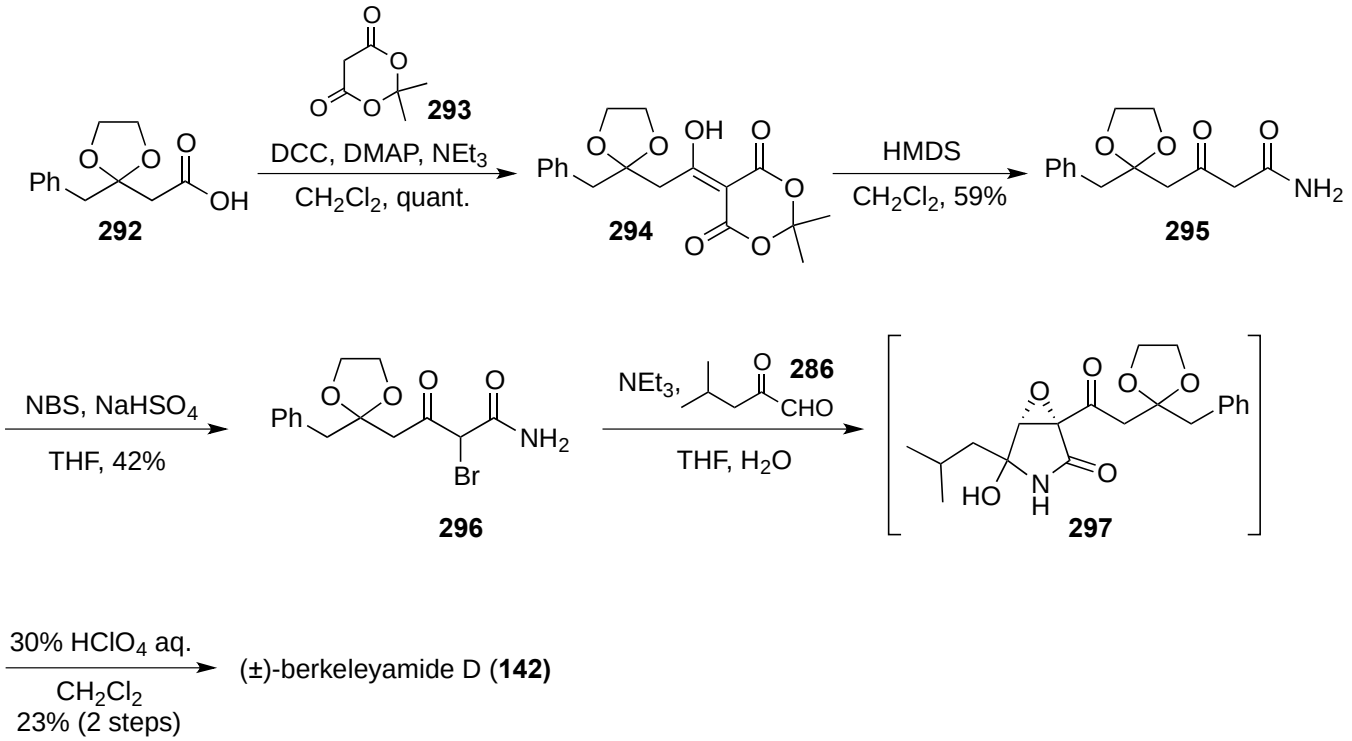
Kuramochi and Tsubaki's group determined the absolute configuration of berkeleyamide D via a short synthesis using their Darzens reaction of α-bromo-β-ketoamide with glyoxals (Scheme 24).[86] The

Darzens reaction of α-bromo-β-ketoamide **285** and isobutyl glyoxal **286** in the presence of a base formed α,β-epoxy-γ-lactam **287** as an intermediate for berkeleyamide D without forming the undesired isomer. The utility of this reaction is also demonstrated in the synthesis of epolactaene with glyoxal. The hemiaminal of lactam **287** was treated with isopropanol and an acid catalyst to produce aminal **288** as a single diastereomer. The ketone in aminal **288** was lithiated and reacted with phenylacetyl chloride (**289**) to afford spiro compound **291** together with dimer **290** in low yield. Hydrolysis of spiro compound **291** gave (±)-berkeleyamide (**142**), which was separated by chiral HPLC into (+)-**142** and (−)-**142**, and the absolute configuration was determined by vibrational circular dichroism (VCD).



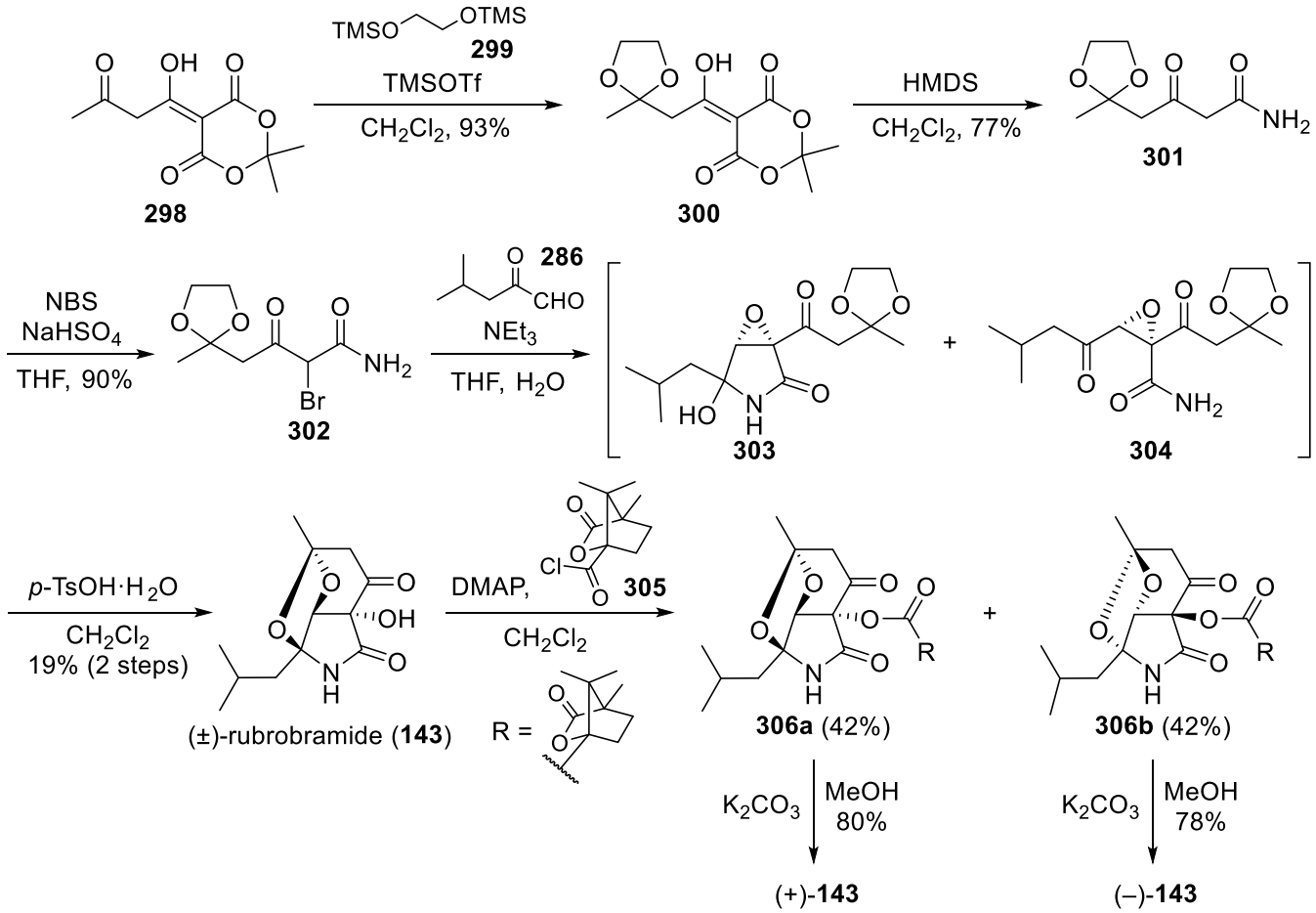
Scheme 24. Kuramochi and Tsubaki's First-Generation Total Synthesis of Berkeleyamide D

In 2016, Kuramochi and Tsubaki's group improved the total yield of berkeleyamide D synthesis by introducing a synthon equivalent to phenylacetyl chloride, used in the previous total synthesis, to the starting material to avoid the problematic acylation (Scheme 25).[87] Carboxylic acid **292** was prepared according to a known procedure from methyl 3-oxo-4-phenylbutyrate[88] and condensation with Meldrum's acid (**293**) afforded ester **294**. Amidation of ester **294** with hexamethyldisilazane (HMDS) generated amide **295**, which was reacted with NBS in the presence of $NaHSO_4$ to afford bromide **296**. The Darzens reaction with bromide **296** and isobutyl glyoxal (**286**) followed by treatment under acidic conditions completed the modified total synthesis of berkeleyamide D (**142**).



Scheme 25. Kuramochi and Tsubaki's Second-Generation Total Synthesis of Berkeleyamide D

Around the same time, they also reported the bioinspired synthesis of rubrobramide (**143**) based on their γ-lactam construction method with the Darzens reaction and absolute configuration determination by VCD analysis (Scheme 26).[89] Known ketone **298**[90] was protected with an acetal using the Noyori method[91] to afford 1,3-dioxolane **300**. The reaction of **300** with HMDS was followed by brominating the methylene with NBS to give substrate **302** for the Darzens reaction. In the presence of triethylamine, **302** and isobutyl glyoxal (**286**) were reacted to produce tautomers **303**/**304**, which were used in the next reaction without purification because of their instability. **303**/**304** were treated with acid in CH_2Cl_2 to obtain rubrobramide (**143**). Esterification with (−)-camphanic chloride (**305**) followed by separation of the diastereomers and methanolysis afforded optically pure (−) and (+)-rubrobramide (**143**).

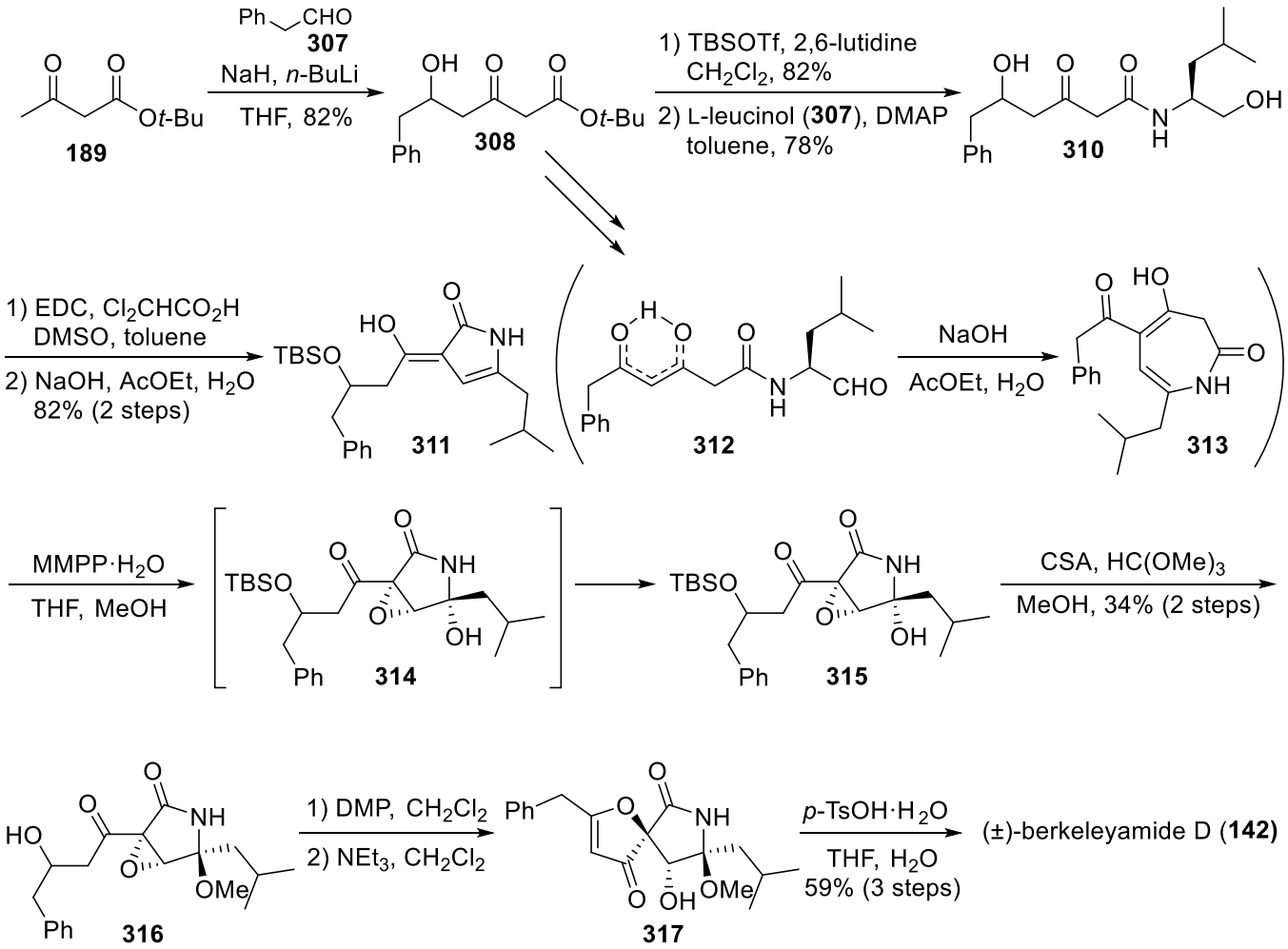


Scheme 26. Kuramochi and Tsubaki's Total Synthesis of Rubrobramide

3-9. Han's Total Syntheses of Berkeleyamide D, Azaspirene, FD-838, and Cephalimysin A

Han's group reported the total syntheses of berkeleyamide D (**142**),[92] azaspirene (**133**),[93] FD-838 (**141**),[94] and cephalimysin A (**138**)[94] based on a strategy inspired by the biosynthetic origin of azaspirene[95] and the biomimetic synthesis of α,β-unsaturated γ-hydroxy-γ-lactam and α,β-epoxy-γ-hydroxy-γ-lactam ring systems reported by the Snider group.[96] Initially, they achieved the total synthesis of berkelyamide D (**142**) (Scheme 27).[92] The reaction of dienolate generated from *tert*-butyl acetoacetate **189** and phenylacetaldehyde **307** produced aldol adduct **308**, in which the secondary alcohol was protected with a TBS group and the *tert*-butyl ester was condensed with L-leucinol (**307**) to afford amide **310**. Oxidation of alcohol **310** under modified Pfitzner-Moffatt conditions was followed by treatment with base to give lactam **311**, although the asymmetric center was lost. In this cyclization, when amide **312**, prepared from **308** in four steps, was used as a substrate, the undesired cyclization proceeded to give 7-membered product **313**. Continuous oxidation, consisting of electrophilic epoxidation followed by nucleophilic epoxidation, of lactam **311** to α,β-epoxy-γ-hydroxy-γ-lactam **315** was performed using magnesium

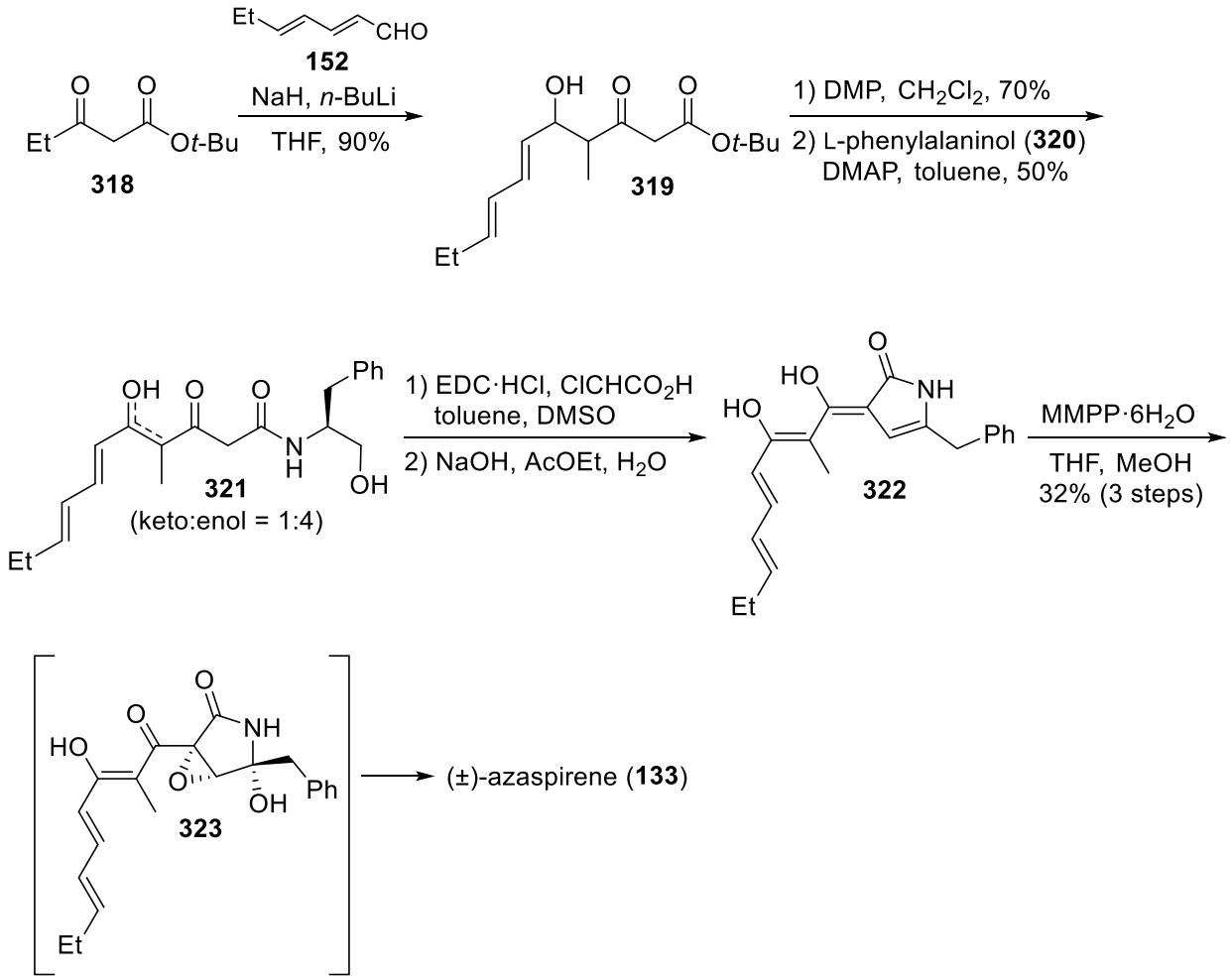
monoperoxyphthalate (MMPP). Subsequently, desilylation of the secondary alcohol and hydroxy to methoxy substitution of **315** by treatment with CSA in methanol provided alcohol **316**. Oxidation of the secondary alcohol followed by treatment with a base generated spiro compound **317**, which was subjected to acidic conditions to produce berkeleyamide D (**142**).



Scheme 27. Han's Total Synthesis of Berkeleyamide D

Han's group used the same synthetic method[92] to achieve the total synthesis of azaspirene from β-ketoester **319**, prepared from α,β,γ,δ-unsaturated aldehyde **152** and β-ketoester **318** (Scheme 28).[93] Oxidation of the secondary alcohol of **319** was followed by condensation with L-phenylalaninol (**320**) to produce amide **321**. Amide **321** was subjected to a modified Pfitzner−Moffatt oxidation and treatment with base to afford lactam **322**. Interestingly, the cyclization of β,δ-diketoamide **321** produced the γ-lactam, although the reaction of β,δ-diketoamide **312** gave the 7-membered lactam (Scheme 27). Because the methyl group at the γ-position in substrate **321** precluded the dehydration reaction that

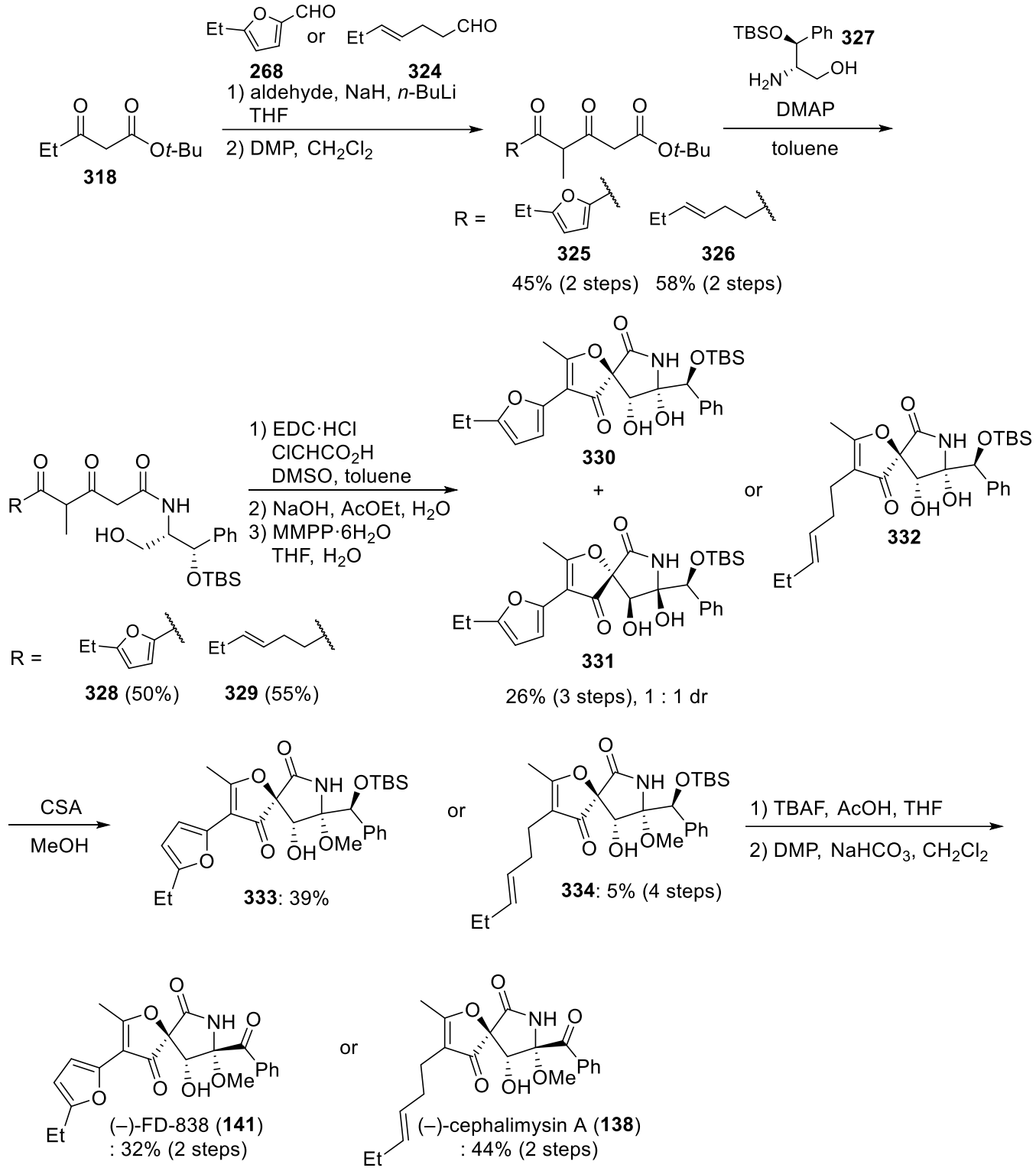
formed the 7-membered lactam, the reactions of β-ketoamide **312** or β-ketoamide **321** proceeded differently. After continuous oxidation using MMPP, spontaneous spiro cyclization delivered (±)-azaspirene (**133**).



Scheme 28. Han's Total Synthesis of Azaspirene

Han's group then attempted the asymmetric syntheses of berkeleyamide D and azaspirene by using an asymmetric epoxidation as part of the continuous oxidation, but this approach did not work. Finally, they achieved the total syntheses of FD-838 (**141**) and cephalimysin A (**138**) by chiral pool synthesis (Scheme 29).[94] These total syntheses used β-ketoester **318**, the appropriate aldehyde **268** or **324**, and amine **327**, which contains two chiral centers. Spiro compounds consisting of an inseparable mixture of **330** and **331** or **332** were obtained in same manner as the previous azaspirene synthesis.[93] Treatment of spiro compounds **330** and **332** with camphorsulfonic acid in methanol produced only methoxy derivatives **333**

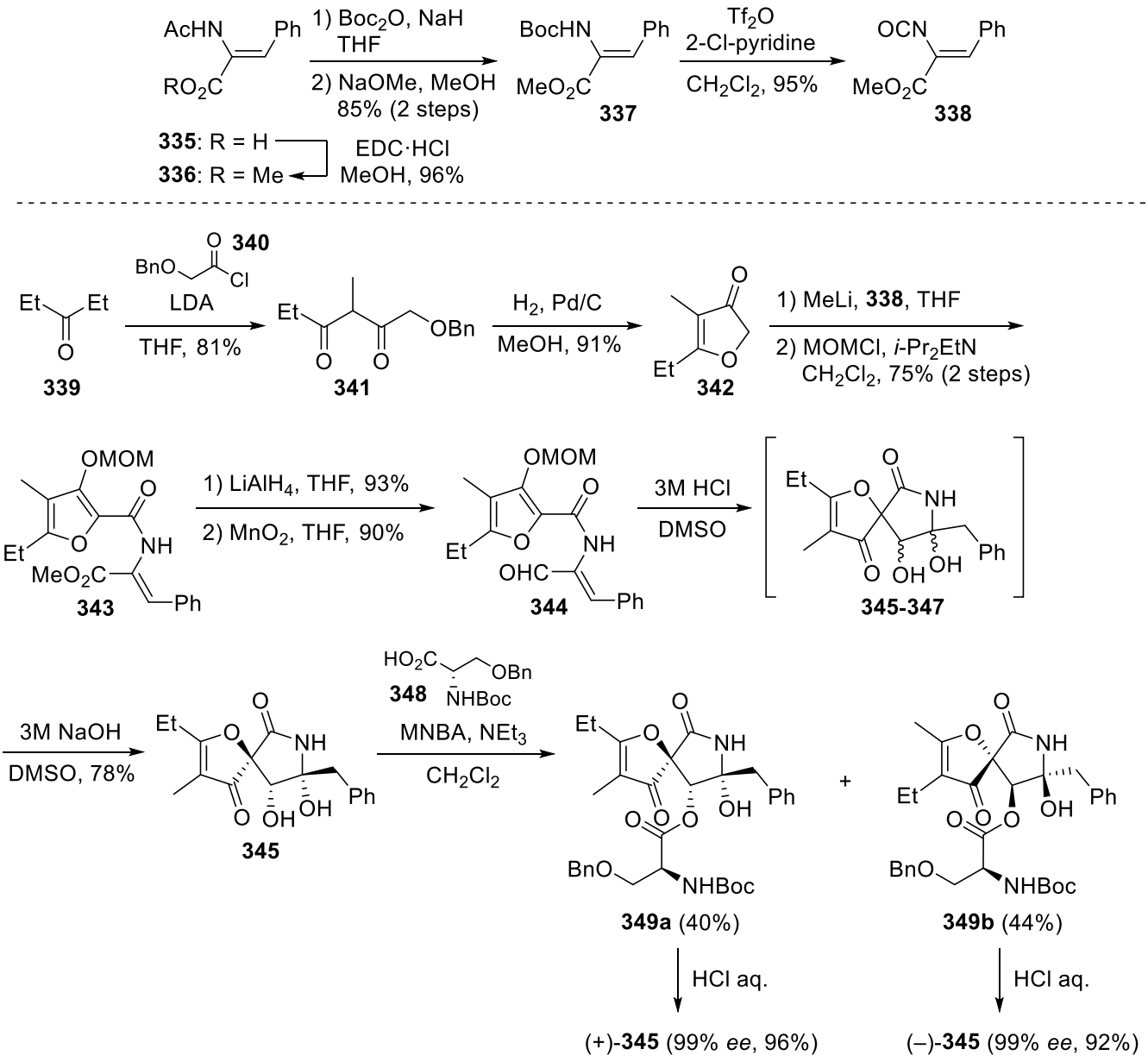
or **334** as single diastereomer. After deprotection of TBS group and oxidation of the resultant alcohol, the total syntheses of FD-838 (**141**) and cephalimysin A (**138**) were completed.



Scheme 29. Han's Total Syntheses of Cephalimysin A and FD-838

3-10. Kanomata and Emoto's Synthesis of the Azaspirene Skeleton

Kanomata and Emoto synthesized the azaspirene skeleton to elucidate its racemization (Scheme 30).[97] The synthesis used a convergent approach with a lactam segment bearing an isocyanate group and a furanone segment. Commercially available α-acetamidocinnamic acid (**335**) was converted to ester **336**, and the acetyl group on the amine was replaced with a Boc group to afford ester **337**. The Kokotos protocol was used to react the ester with trifluoromethanesulfonic anhydride and 2-chloropyridine as base[98] to give isocyanate **338**. Next, the furanone segment was synthesized and used to complete the synthesis of the azaspirene skeleton. Claisen condensation between 3-pentanone (**339**) and benzyloxyacetyl chloride (**340**) gave 1,3 diketone **341**, followed by hydrogenolysis of the benzyl group and spontaneous lactonization to produce furanone **342**. The anion generated from furanone **342** was reacted with isocyanate **338**, and then the resultant product was protected with a MOM group to afford furan **343**. The ester was converted to aldehyde **344** by reduction and oxidation, and then treatment with aqueous hydrochloric acid in DMSO produced diastereomers **345–347**, which were neutralized with aqueous NaOH solution under heating to afford thermodynamically favored **345**. Diastereomeric resolution by Shiina's method[99] which involves installation of a serine derivative and separation, and subsequent hydrolysis afforded enantiomerically pure azaspirene derivatives (+)-**345** and (−)-**345**. Emoto's group reported that compounds (+)-**345** and (−)-**345** have antiangiogenic activity and can inhibit the growth of human uterine carcinosarcoma.[100]



Scheme 30. Kanomata and Emoto's Synthesis of Azaspirene Skelton

4. CONCLUSION

In this review, we have discussed the synthesis and biological studies of highly oxidized γ-lactam-containing natural products and related molecules, focusing on epolactaene; NG-391, lucilactaene; L-755,807; azaspirene; pseurotins A, E, and F_2; synerazol; cephalimysins A-C; FD-838; berkeleyamide D; rubrobramide; and talaramide A. Their complexity and densely packed functional groups allow for a diverse array of synthetic approaches, and many researchers have worked to establish elegant and efficient synthetic routes. Moreover, the wide-ranging biological activities of these molecules, which likely arise from the high reactivity of their highly oxidized multifunctional structures, have

attracted much attention in medical research. These molecules will continue to serve as attractive synthetic and pharmaceutical targets in drug discovery.

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