Total Synthesis of α-Pyrone Meroterpenoids, Novel Bioactive Microbial Metabolites

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1. Introduction

Bioactive natural products produced by microbes have limitless potential in pharmaceutical applications, and the organic synthesis of such products as lead compounds will result in the creation of new and widely useful pharmaceutical products.

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With a focus on the drug discovery process, the Kitasato Institute is using cutting-edge, unique screening techniques to discover useful bioactive natural products from microbial metabolites. These novel natural products have distinctive structures and attractive bioactivities.[1,2]

The key challenge in synthetic organic chemistry is how to more efficiently synthesize target compounds with unique, novel molecular skeletons using short process pathways. The construction of novel molecular skeletons necessitates the development of new synthetic strategies and key reactions, which leads to further progress in synthetic organic chemistry (Figure 1).

If efficient synthetic methods can be established, it will be possible to quantitatively supply natural products that are presently only naturally available in trace amounts, and this will contribute to a more thorough elucidation of their bioactivities. In addition, it will become possible to determine, using synthetic techniques, the relative and absolute configuration and structures of compounds for which only trace amounts can be extracted from natural sources. Furthermore, use of newly developed molecular skeleton construction methods will allow the creation of a wide range of analogues, thereby leading to the production of compounds with properties that surpass those found in nature and heralding the promise of bioactivity.

Hence, the discovery of bioactive natural products with novel molecular skeletons will lead to advances in synthetic organic chemistry that are dynamically related to the elucidation and development of bioactive materials (Figure 1).

Target compounds of new natural products may only be available from natural sources in trace amounts, so the Kitasato Institute has built on the above concepts to develop efficient, rational, and highly flexible construction methods for the production of compounds with novel molecular skeletons and useful bioactivities. To date, 20 types of bioactive natural products have been successfully synthesized. The research program also calls for the application of established methods to synthesize related compounds, elucidating their structure−activity relationships and contributing to the creation of improved bioactive compounds. In this article, which provides an overview of this research to date, the total synthesis of acyl-CoA/cholesterol acyltransferase (ACAT)-inhibiting pyripyropenes and acetylcholinest-

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erase (AChE)-inhibiting arisugacins is discussed and a number of issues concerning their biosynthesis are addressed.

During the screening for bioactive compounds from microbial origin, pyripyropenes A−R as ACAT inhibitors and arisugacins A and B as AChE inhibitors have been discovered. These are both fungal metabolites, biosynthesized from terpenoid and polyketide, and are called "meroterpenoids".

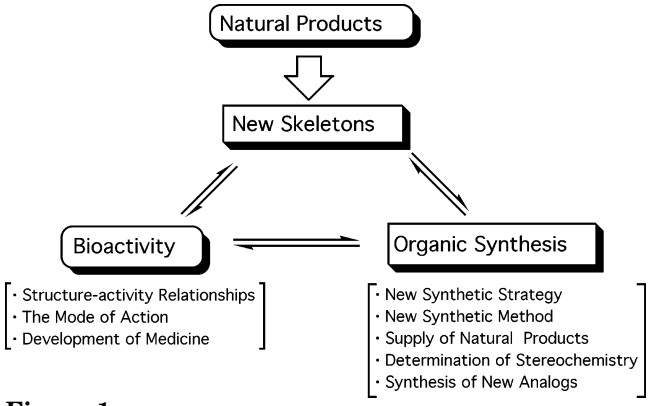


Figure 1.

Meroterpenoids were initially proposed by Cornforth as "Compounds containing terpenoid elements along with structures of different biosynthetic origin".[3] Recently, Simpson defined meroterpenoids as a more limited group: "Compounds of mixed polyketide-terpenoid origin".[4]

Furthermore, very unique structures of α-pyrone meroterpenoids have been discovered: pyripyropenes, phenylpyropenes, oxalicines, arisugacins, territrems, and terreulactones.

The biosynthetic scheme for α-pyrone meroterpenoids proposes that an aryl-α-pyrone moiety is produced via condensation of a primer aromatic acid with two acetates via a polyketide pathway and an all trans farnesyl pyrophosphate is produced via the mevalonate pathway. These two moieties are then linked and cyclized to form the core skeleton.

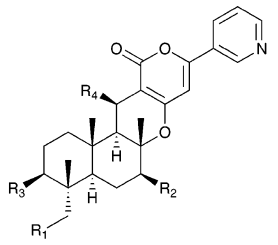
2. Naturally Occurring α-Pyrone Meroterpenoids

Naturally occurring α-pyrone meroterpenoids were discovered by different screening methods.

During the screening program of ACAT inhibitors, the potent inhibitors pyripyropenes A (**1**)−R (**18**) were obtained from a culture broth of *Asperigillus fumigatus* FO-1289.[5−8] Pyripyropenes commonly consist of three parts, a pyridine ring, an α-pyrone, and a sesquiterpene motif, to form a steroid-like structure (Figure 2). Among these, pyripyropene A (**1**) was found to be the most potent ACAT inhibitor (IC_{50} 16 nM).

GERI-BP001, a new ACAT inhibitor (IC_{50} 50 μM), was isolated from a culture broth of *Aspergillus fumigatus* F37, and the structure was elucidated on the basis of spectroscopic data. GERI-BP001 was identical to pyripyropene E (**5**) (Figure 2).[9,10]

Two newly isolated potent compounds, phenylpyripenes A (**19**) and B (**20**), obtained from a fermentation broth of *Penicillum griseofulvum* F1959, are believed to be potential ACAT inhibitors.[11] Their structures share the α-pyrone and sesquiterpene moieties with pyripyripenes A (**1**) and E (**5**), and the pyridine ring of pyripyripenes A and E is replaced by a phenyl ring. Moreover, phenylpyropene C (**21**) was isolated from a fermentation broth of *Penicillium griseofulvum* F1959. Although the structure of this



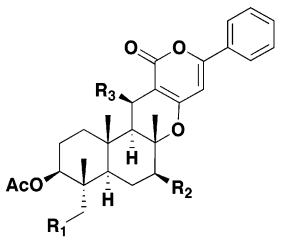
Pyripyropenes

A (**1**) $R_1 = R_2 = R_3 = OAc$, $R_4 = OH$
B (**2**) $R_2 = R_3 = OAc$, $R_1 = OPr$, $R_4 = OH$
C (**3**) $R_1 = R_3 = OAc$, $R_2 = OPr$, $R_4 = OH$
D (**4**) $R_1 = R_2 = OAc$, $R_3 = OPr$, $R_4 = OH$
E (**5**) $R_1 = R_2 = H$, $R_3 = OAc$, $R_4 = H$
F (**6**) $R_1 = R_2 = H$, $R_3 = OPr$, $R_4 = H$
G (**7**) $R_1 = R_2 = H$, $R_3 = OAc$, $R_4 = OH$
H (**8**) $R_1 = R_2 = H$, $R_3 = OPr$, $R_4 = OH$
I (**9**) $R_1 = R_2 = R_3 = OPr$, $R_4 = OH$
J (**10**) $R_1 = OAc$, $R_2 = R_3 = OPr$, $R_4 = OH$
K (**11**) $R_2 = OAc$, $R_1 = R_3 = OPr$, $R_4 = OH$
L (**12**) $R_3 = OAc$, $R_1 = R_2 = OPr$, $R_4 = OH$
M (**13**) $R_1 = R_3 = OAc$, $R_2 = OPr$, $R_4 = H$
N (**14**) $R_1 = R_3 = OPr$, $R_2 = H$, $R_4 = OH$
O (**15**) $R_1 = R_3 = OAc$, $R_2 = R_4 = H$
P (**16**) $R_1 = OPr$, $R_3 = OAc$, $R_2 = R_4 = H$
Q (**17**) $R_1 = OPr$, $R_3 = OAc$, $R_2 = H$, $R_4 = OH$
R (**18**) $R_1 = OAc$, $R_3 = OPr$, $R_2 = R_4 = H$

*GERI-BP001 was identical with pyripyropene E (**5**)

Figure 2. Structures of pyripyropenes.

compound is similar to that of pyripyropene, the pyridine ring of pyripyropene E (**5**) was replaced by a benzene ring in phenylpyropene C (Figure 3).[12]



	R_1	R_2	R_3
Phenylpyropene A (**19**)	OAc	OAc	OH
Phenylpyropene B (**20**)	OAc	H	H
Phenylpyropene C (**21**)	H	H	H

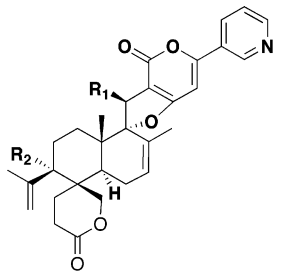
*S14-95 was identical with phenylpyropene C (**21**)

Figure 3. Structures of phenylpyropenes.

Moreover, S14-95, a novel inhibitor of the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway, was isolated from a culture broth of *Penicillium* species. S14-95 was identical to phenylpyropene C (**21**).[13,14]

Oxalicines A (**22**) and B (**23**) and 15-deoxyoxalicine B (**24**) were isolated from a fermentation broth of *Penicillium oxalicum*. These are alkaloidal meroterpenes with a new carbon skeleton, established from spectral data and single-crystal X-ray analysis. Oxalicine A (**22**) is biogenetically related to the pyripyropenes and appears to be derived from an unprecedented pathway involving nicotinic acid (ring A), polyketide (ring B), and diterpenoid (rings C, D, E, and F) moieties (Figure 4).[15,16] They exhibited antiinsecticidal activity against the fall armyworm.

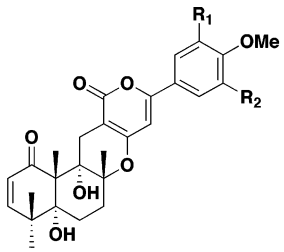
On the other hand, in the course of screening for selective acetylcholinesterase inhibitors from microbial metabolites, arisugacins A (**25**)−H (**32**), architecturally interesting inhibitors of AChE derived



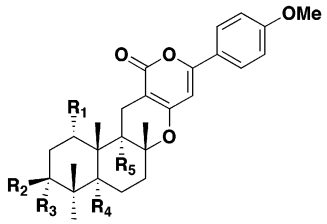
	R_1	R_2
Oxalicine A (**22**)	OH	H
Oxalicine B (**23**)	H	OH
15-Deoxyoxalicine B (**24**)	H	OH

Figure 4. Structures of oxalicines.

from a culture broth of *Penicillium* sp. FO-4259,[17−20] were isolated, together with a structurally related known compound, territrem B (**34**). The arisugacin structure is comprised of a highly oxygenated *trans*-decalin system and an α-pyrone moiety (Figure 5).



	R_1	R_2	R_3
Arisugacin A (**25**)	OMe	OMe	H
Arisugacin B (**26**)	H	OMe	H
Territrem A (**33**)	— OCH_2O —		OMe
Territrem B (**34**)	OMe	OMe	H
Territrem C (**35**)	OMe	OH	OMe



	R_1	R_2	R_3	R_4	R_5
Arisugacin C (**27**)	H	=O		OH	H
Arisugacin D (**28**)	H	H	OAc	OH	OH
Arisugacin E (**29**)	H	H	OH	OH	H
Arisugacin F (**30**)	H	OH	H	H	H
Arisugacin G (**31**)	H	=O		H	H
Arisugacin H (**32**)	OH	H	OAc	OH	OH

Figure 5. Structures of arisugacins and territrems.

The tremorgenic mycotoxins isolated from *Aspergillus terreus* were given the trivial names territrems A (**33**), B (**34**), and C (**35**). They were partially characterized by UV, IR, ^{1}H NMR, and mass spectroscopy. The spectroscopic evidence indicated that their chemical structures were very similar (Figure 5).[21,22]

Yoo isolated a new meroterpenoid containing a unique bridged lactone named terreulactone A (**36**), along with terreulactones B (**37**), C (**38**), and D (**39**), from the solid-state fermentation of *Aspergillus ter-*

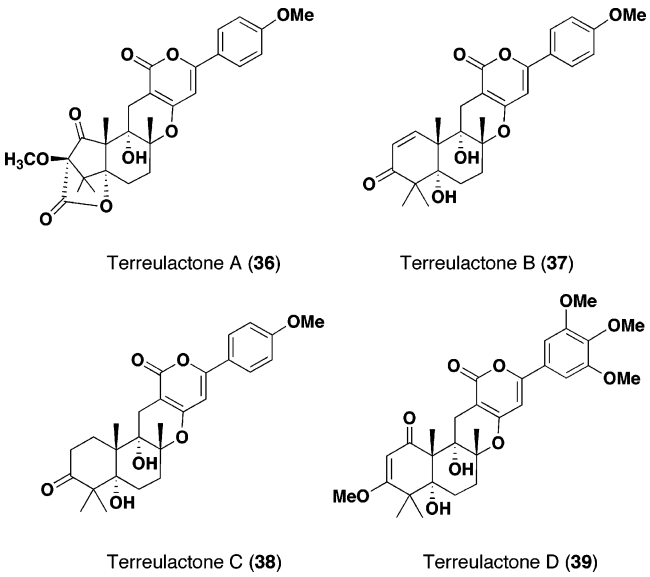


Figure 6. Structures of terreulactones.

reus Fb000501 (Figure 6).[23] Some derivatives of arisugacin B (**26**) were also detected in the same culture, so terreulactone A (**36**) seems to be biogenetically related to arisugacin isolated from *Penicillium* sp. Terreulactone A (**36**) is the first sesquiterpene lactone type meroterpenoid of microbial origin. It inhibited acetylcholinesterase in a dose-dependent manner with an IC_{50} (μM) value of 0.2, which showed higher activity than that (IC_{50} (μM) 0.42) of a methoxylated derivative of arisugacin B (**26**) (Figure 6).

In this review, the main focus is on the synthetic studies of α-pyrone meroterpenoids, pyripyronenes, selective ACAT inhibitors, and also arisugacins, selective AchE inhibitors, in addition to their isolation from nature, structure determination, biosynthesis, and structure−activity relationships.

3. Total Synthesis of Pyripyropenes

3.1. Isolation

The regulation of cholesterol (Ch) levels in humans remains a major focus of drug development. It is widely accepted that hypercholesterolemia is one of the major risk factors leading to coronary heart disease.[24] Interference either with the dietary absorption of cholesterol-containing foodstuffs or with the de novo biosynthesis of cholesterol has been used to lower plasma cholesterol content. Early strategies for containment of hypercholesterolemia involved the use of a bile acid sequestrant cholestyramine resin.[25] However, the difficulties associated with this form of medication prompted a search for other approaches. A milestone in the contemporary management of cholesterol levels arose from the discovery of lovastatin and sinvastatin, powerful inhibitors against β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) reductase.[26,27] This enzyme mediates the rate-limiting enzymatic step in cholesterol biosynthesis. Agents based on the concept of HMG-CoA inhibition have proven to be effective in the treatment of hypercholesterolemic patients. Furthermore, squalene synthase inhibitors, such as the zaragozic acids, have also been identified as possible therapeutic agents but have not yet found clinical application because of their apparent toxicity and difficult availability.[28]

It is believed that the progression from abnormally high cholesterol levels to myocardial infarction begins with the accumulation of intracellular esterified cholesterol in macrophages. This is followed by subsequent foam cell formation and ultimately by the appearance of atherosclerotic plaques in arteries. A promising and fundamentally new approach to the prevention and treatment of atherosclerosis is based upon inhibition of ACAT, the enzyme that catalyzes intracellular esterification of cholesterol. This strategy may permit suppression of three distinct ACAT-dependent steps in the pathology of atherosclerosis: absorption of dietary cholesterol in the gut, hepatic synthesis of lipoproteins, and deposition of oily cholesteryl esters (CE) within the developing arterial lesions. Therefore, inhibitors of ACAT may be promising new types of antiatherosclerotic agents.[29]

Most ACAT inhibitors reported to date are synthetic compounds such as amide, urea, or imidazole derivatives.[30]

To this end, approximately 20 000 soil isolates (containing predominantly actinomycetes and fungi) have been screened for the inhibition of mouse ACAT at the Kitasato Institute. During this process, pyripyropenes A−D (**1**−**4**) (Figure 2) were isolated from a culture broth of *Aspergillus fumigatus* FO-1289. These novel, polyoxygenated, and mixed polyketide−terpenoid (meroterpenoid) metabolites contain a fused pyridyl α-pyrone moiety and eight contiguous stereocenters.[5−8]

The pyripyropenes A−D rank as the most effective naturally occurring in vitro ACAT inhibitors with IC_{50} values of 58, 117, 53, and 268 nM, respectively. Importantly, pyripyropene A (**1**) proved to be orally active in hamsters, reducing cholesterol absorption by 32−46% after single doses of 25−75 mg/kg.[5] Furthermore, pyripyropenes E−R (**5**−**18**, Figure 2) were also isolated from the same strain.[8]

ACAT inhibitory activities (IC_{50}) of pyripyropenes A (**1**) and C (**3**) were approximately 0.15 μM, and they were the most potent ACAT inhibitors of natural origin. ACAT inhibitory activities of pyripyropenes A−R suggested that *O*-acyl residues of R_1 and R_2 were essential for ACAT inhibition, that *O*-acetyl residues of R_1 and R_3 were more effective than *O*-propionyl residues, and that a hydroxyl residue of R_4 was more effective for inhibition (Figure 2).

3.2. Structure Determination

Structures were elucidated using NMR, and the absolute stereochemistry was confirmed by X-ray crystallography and Mosher's method, using an α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) derivative of pyripyropene A. Pyripyropenes have a common structure consisting of naphtho[2,1-*b*]pyrano[3,4-*e*]pyran and pyridine (Figure 2).[31]

The relative stereochemistry at C(4), C(6), C(7), and C(10) in **1** initially emerged from nuclear Overhauser effect (NOE) measurements, which established the syn dispositions of Me(12), Me(14), Me(15), and the OAc at C(7) (Figure 7a). Thus, irradiation of the Me(15) resonance at δ 0.89 led to a 3.0% enhance-

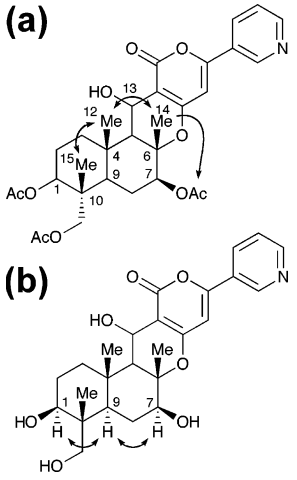


Figure 7. NOE enhancements for (a) pyripyropene A (250 MHz in $CDCl_3$) and (b) tris(desacetyl)pyripyorpene A (**40**) (500 MHz in CD_3OD).

ment of Me(12) (δ 1.44). Irradiation of Me(12) likewise gave NOE enhancements of 3.9% and 3.4% for Me(14) (δ 1.69) and Me(15), respectively, and irradiation of Me(14) enhanced both Me(12) (3.4%) and OAc(7) (δ 2.15 ppm, 1.6%). Unfortunately, irradiation at H(1) (δ 4.79) was inconclusive, and overlap of H(9) with H(8a), and of H(7) with H(13), precluded analysis of the relative configurations at C(1), C(9), and C(13).

The preparation of tris(desacetyl)pyripyropene A (**40**) by hydrolysis allowed the establishment of the syn relationships among H(1), H(7), and H(9) (Figure 7b). Irradiation of H(1) at δ 3.61 resulted in a NOE enhancement of H(9) (δ 1.46) of 3.8%. Irradiation at H(7) (δ 3.73), in turn, afforded a 4.7% enhancement of H(9), and irradiation at H(9) led to 6.4% and 6.7% enhancements for H(1) and H(7), respectively. All of these findings were ultimately verified, and the relative configurations at C(5) and C(13) were elucidated via single-crystal X-ray analysis of **1** (Figure 8).

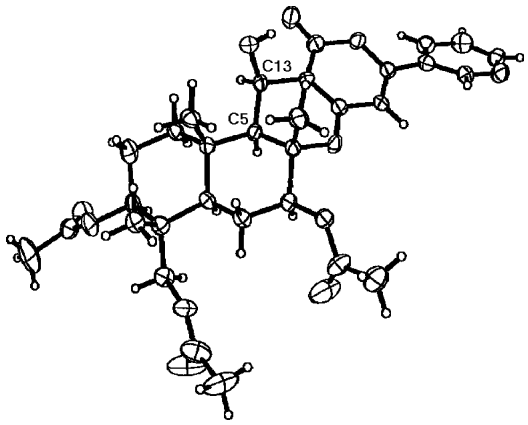
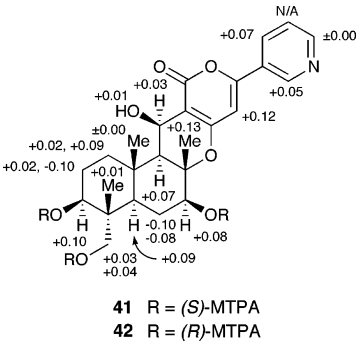


Figure 8.

The complete relative stereochemistry of **1** having been defined, the determination of the absolute configuration via a modified the Mosher NMR method was sought next. The tris-Mosher ester derivatives **41** and **42** were prepared by treatment of **40** with (*S*)-(−)- and (*R*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetic (MTPA) acid in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) (tetrahydrofuran (THF), room temperature).[32] The ^{1}H NMR spectra of **41** and **42** could be completely assigned via selective ^{1}H decoupling. Comparison of the ^{1}H chemical shifts in **41** and **42** (Figure 9) and application of the Kakisawa−Kashman test[33] revealed that the absolute configurations at C(1) and C(7) are *S* (Figure 9).



41 R = *(S)*-MTPA
42 R = *(R)*-MTPA

Figure 9. Absolute stereochemistry determination: $\Delta\delta$ values (ppm, 500 MHz, $\Delta\delta = \delta_S - \delta_R = \delta_{41} - \delta_{42}$) for the tris-Mosher ester derivatives **41** and **42**.

In view of the common biosynthetic origin of the pyripyropenes, we presume that congeners B−D (**2**−**4**) share the relative and absolute stereochemistry of **1**; the structurally related territrems A−C may also embody the same absolute stereochemistry.

3.3. Biosynthesis

The biosynthesis of pyripyropene A (**1**) was studied using feeding experiments with [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]acetate, D,L-[2-^{13}C]mevalonolactone, and [carboxyl-^{14}C]nicotinic acid.[34] ^{13}C NMR and degradation experiments of ^{13}C- and ^{14}C-labeled **1** established that **1** is derived from three mevalonates, five acetates, and one nicotinic acid (Scheme 1).

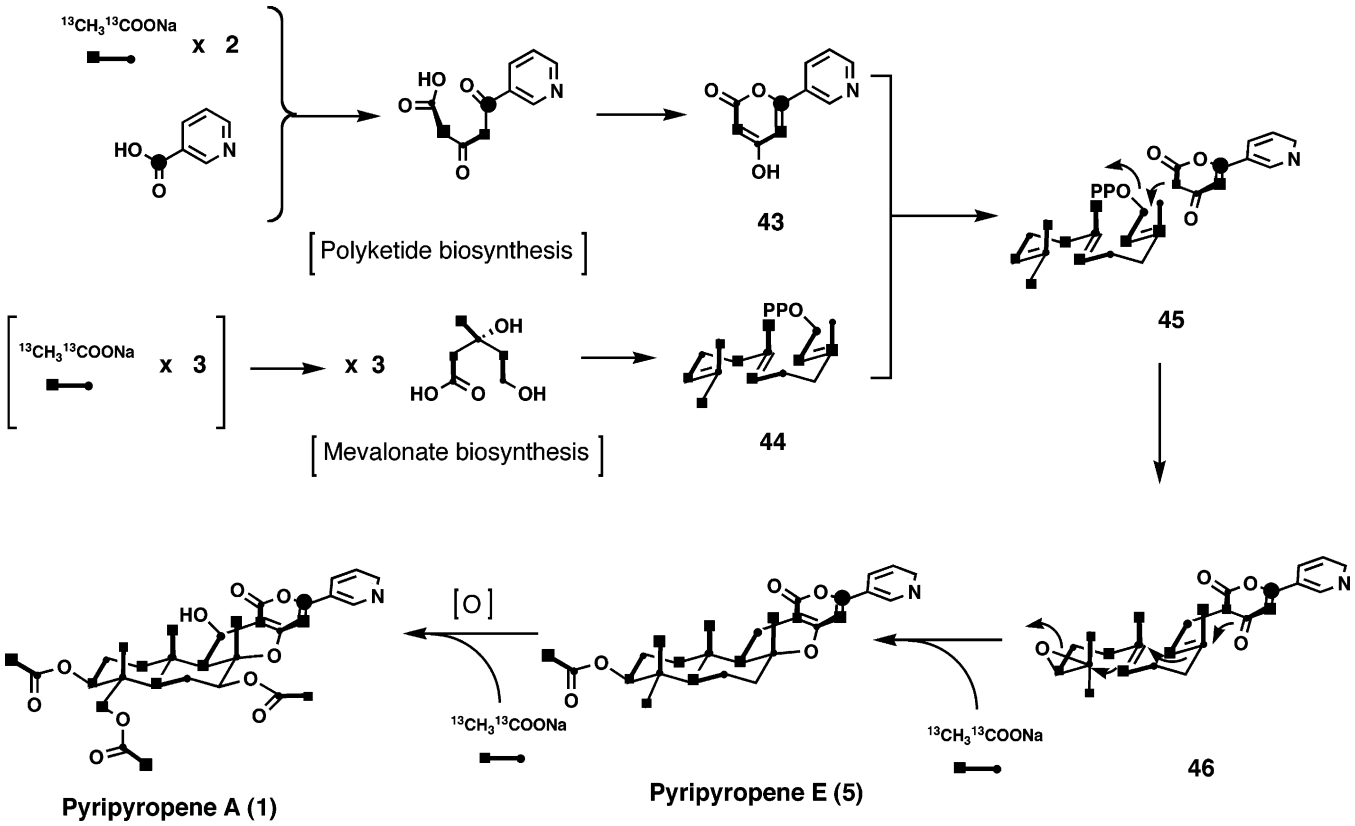
As a result, a biosynthetic pathway was proposed for pyripyropenes A (**1**) and E (**5**) as shown in Scheme 1; (1) a pyridine-α-pyrone moiety (**43**) is produced via condensation of nicotinic acid with two acetates, (2) an all-trans farnesyl pyrophosphate (**44**) is produced via the mevalonate pathway, (3) the two parts are linked to form **45**, followed by epoxidation, and then polyene cyclization of **46** to form the core skeleton **5**, and (4) then the skeleton is oxidized and acetylated to yield pyripyropene A. Therefore, pyripyropenes belonging to the meroterpenoid group were derived via mevalonate and polyketide biosyntheses.[3]

3.4. Total Synthesis of Pyripyropene A

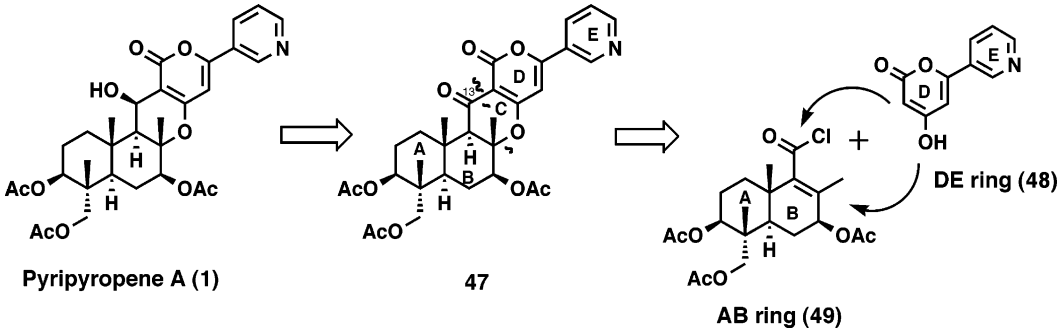
Continuing studies have focused on both the total synthesis and further biological analysis of pyripyropenes and analogues thereof. The intriguing structures and significant pharmacological potential of these substances have stimulated considerable interest.

The first total synthesis is described for the most active member of this family, (+)-pyripyropene A (**1**), via a flexible, concise, and highly efficient route[35] designed to afford easy access to the natural products and a variety of analogues; the biomimetic total synthesis of pyripyropene E (**5**) and structure−activity relationships are also described.[36]

Scheme 1



Scheme 2



3.4.1. Retrosynthetic Analysis of Pyripyropene A (1)

A potentially serviceable disconnection of the pyripyropene A structure is shown in Scheme 2.

The convergent construction of advanced ketone **47** via O-acylation of the known hydroxy α-pyrone **48** (DE ring)[37] with α,β-unsaturated acid chloride **49** (AB ring) was envisioned in the presence of an acid catalyst; isomerization to the C-acylation and ring closure would then deliver **47** with the requisite anti geometry at the BC ring fusion derived from conjugate addition and enolate protonation trans to the C(12) angular methyl group shown in Scheme 3. The final stage would then entail stereoselective reduction of the ketone (**47**) at C(13) (Scheme 2).

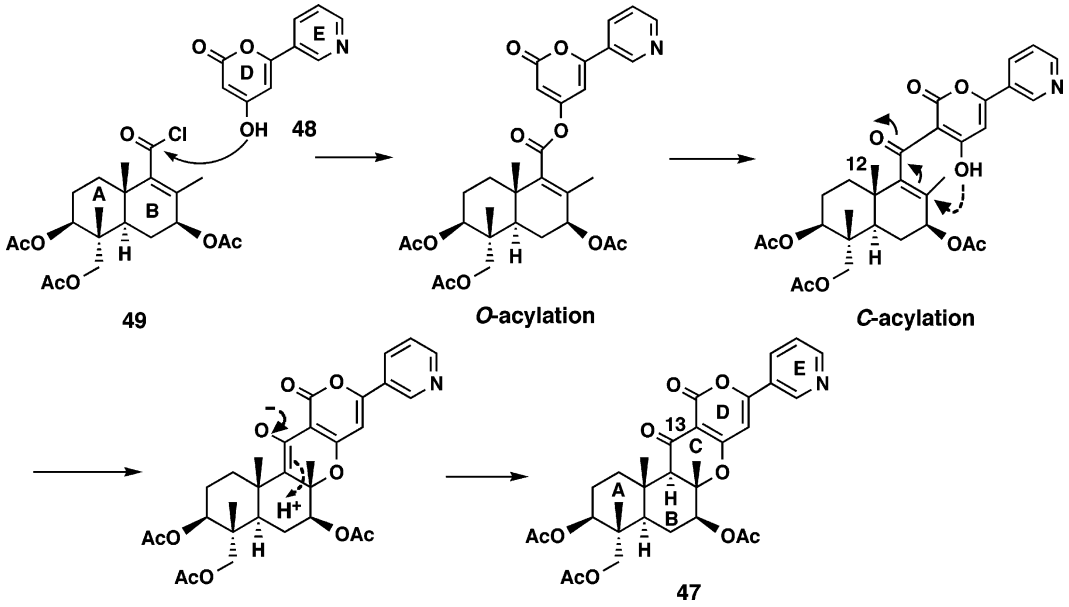
3.4.2. Construction of a Sesquiterpene Moiety (AB-ring)

The sesquiterpene subunit **49** was anticipated to be derived from a known alcohol (**54**),[38] an intermediate, readily available from (+)-Wieland−Miescher ketone (WMK) via stereoselective reduction of **54**, palladium-associated carbonylation of **52**, and allylic oxidation of **50** (Scheme 4).

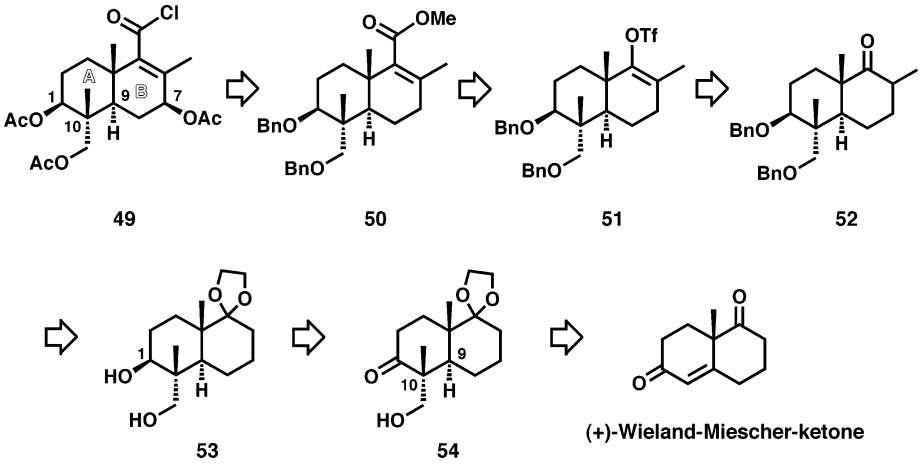
As the point of departure, stereoselective reduction of **54** with tetramethylammonium triacetoxyborohydride[39] in AcOH−CH_3CN furnished *trans*-diol **53** (95% yield, >95% de), which was treated with Ac_2O and DMAP in pyridine to yield a diacetate with identical ^1H NMR spectra as a minor material of an intermediate in the total synthesis of aphidicholine by Trost.[40] Dibenzylation and deketalization of **53** yielded **55** (85%).

An unanticipated difficulty was encountered at this stage. All attempts to alkylate **55**, by employing the standard lithium amide technology led to recovery of the starting material, and alkylation employing potassium diisopropylamide (KDA) led to a complex mixture of starting material and mono- and dialkylated ketones. To circumvent this problem, Kuwajima's alkylation protocol was exploited.[41] Treatment of the enol silyl ether, derived from **55** (KDA/Me_3SiCl), with methyl iodide and benzyltrim-

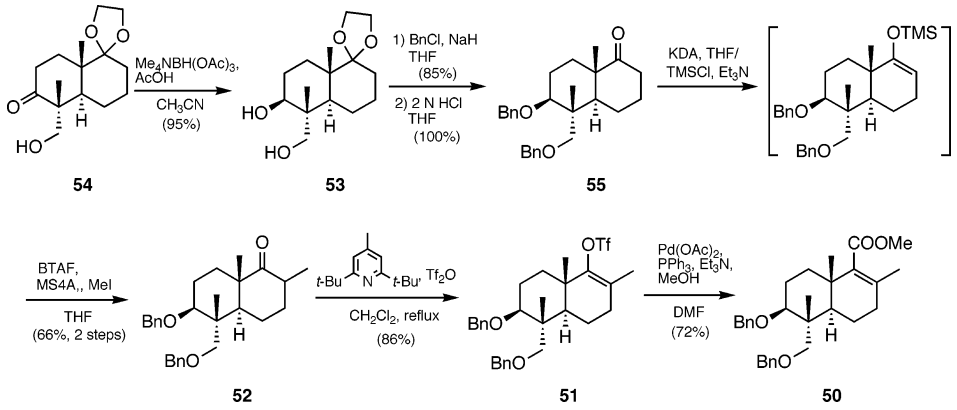
Scheme 3



Scheme 4



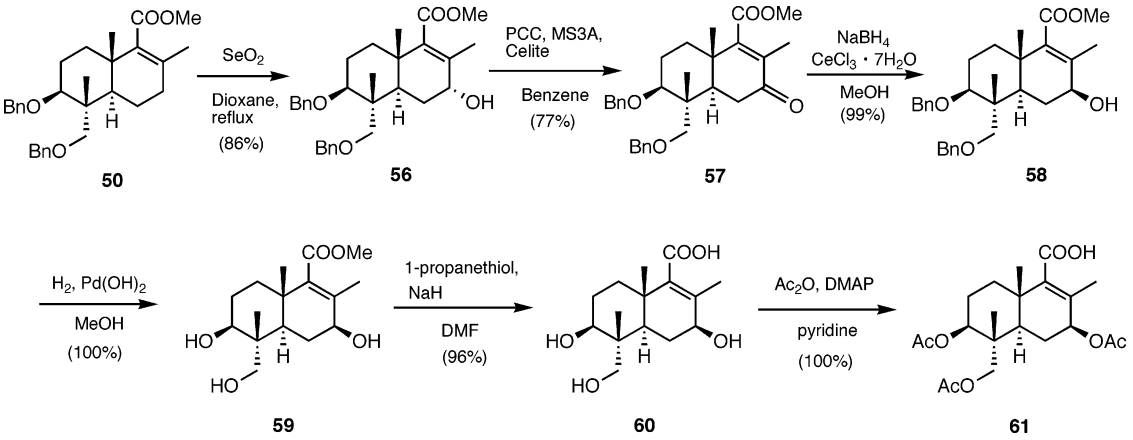
Scheme 5



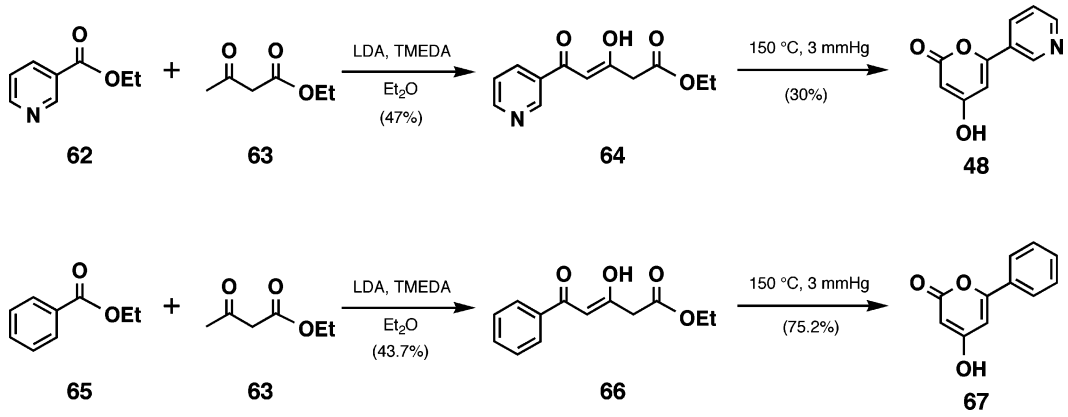
ethylammonium fluoride (BTAF) afforded the requisite monoalkylated ketone **52** as a 5:1 mixture of α and β diastereomers in 66% yield from **55**. Conversion of the monoalkylated ketone into its enolate ion, followed by trapping [KDA/Tf_2NPh][42] to yield enol triflate failed, but treatment of **52** with triflic anhydride (Tf_2O) in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (CH_2Cl_2, reflux)[43] produced the enol triflate **51** in 86% yield, which in turn underwent palladium-catalyzed carbonylation [CO atmosphere, $Pd(OAc)_2$, PPh_3, Et_3N, MeOH, DMF, reflux] as described by Cacchi[44,45] to produce methyl ester **50** in 72% yield (Scheme 5).

Attempts at allylic oxidation of **50** with a variety of chromium reagents produced only traces of dibenzoyl ketone.

Scheme 6



Scheme 7



Installation of the C(7) β-hydroxyl group first entailed SeO_2 oxygenation (dioxane, reflux)[46] and oxidation of the resultant C(7) alcohol **56** to enone **57** (pyridinium chlorochromate (PCC), Celite, benzene (PhH), 3 Å molecular sieves) in 66% yield over two steps. Then a stereoselective Luche reduction[47] provided **58** quantitatively. Hydrogenolysis of the benzyl ether gave **59** in 100% yield, and the observed NOE in **59** clearly supported the assigned structure.

A number of unsuccessful methods (alkaline hydrolysis, halide (I^-),[48] amine (DBU),[49] alkoxide (tBuO$^-$))[50] were investigated to effect the cleavage of ester **59** to acid **60**. Only cleavage of the methyl ester, using sodium thiopropoxide,[51] afforded the acid **60** in 96% yield. Triacetylation of **60** (Ac_2O, DMAP, pyridine) afforded triAc-acid **61** in 100% yield (Scheme 6).

3.4.3. Preparation of α-Pyrones

6-Pyridyl-4-hydroxy-2-pyrone (**48**) and 6-phenyl-4-hydroxy-2-pyrone (**67**) were synthesized in a two-step sequence analogous to a related procedure.[37] The diketo ester **64** was prepared by an addition of the dianion of ethyl acetoacetate **63**, generated with 2.5 equiv of lithium diisopropylamide (LDA), 1.0 equiv of **63**, and 1.0 equiv of tetramethylethylenediamine (TMEDA), to ethyl nicotinate **62** in diethyl ether at 0 °C. This Claisen condensation proceeded relatively slowly and required 18 h. The pyrone **48** was obtained by heating the oily diketoester **64** in a 150 °C oil bath at a pressure of 3.0 mmHg for 45 min. A brown solid was immediately formed along with a dark red oil, and the solid was isolated by vacuum filtration and rinsed with hexane. This simple filtration method provided spectroscopically pure α-pyrone **48**. Similarly, benzyl-α-pyrone **67** was synthesized from ethyl benzoate **65**. Hence, the diketo ester **66** was obtained from a Claisen condensation, followed by thermal cyclization of **66**, which produced **67** in moderate yield (Scheme 7).

3.4.4. Preliminary Experiment for the Lewis Acid Promoted Coupling Reaction

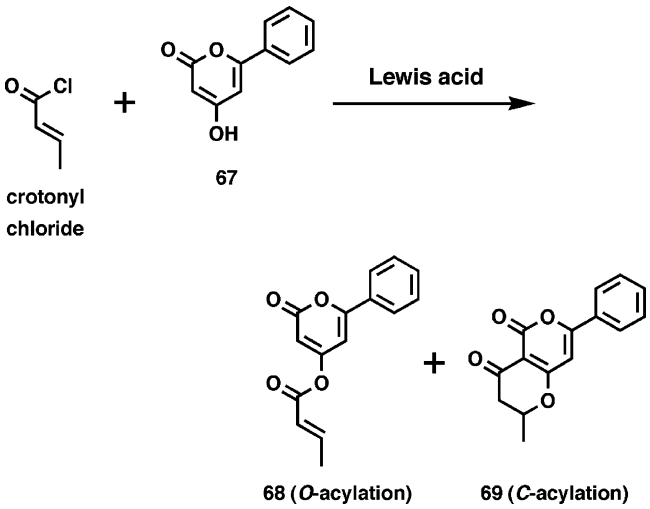
The effects of a Lewis acid and temperature on the coupling reaction were examined. The preliminary experiments performed using benzyl α-pyrone, which was afforded by the Narasinham protocol,[37] and commercially available crotonyl chloride, as a model system, are showen in Table 1. The best result was obtained when the reaction was performed in the presence of $AlCl_3$ at room temperature, and the reaction performed in the presence of TFA at 80 °C also produced a moderate yield.

3.4.5. Completion of the Total Synthesis of Pyripyropene A

The acid **61** was converted to the α,β-unsaturated acid chloride **49** [NaH (1.05 equiv), PhH, room temperature, $(COCl)_2$ (14 equiv)], which was subjected to the next reaction without purification.

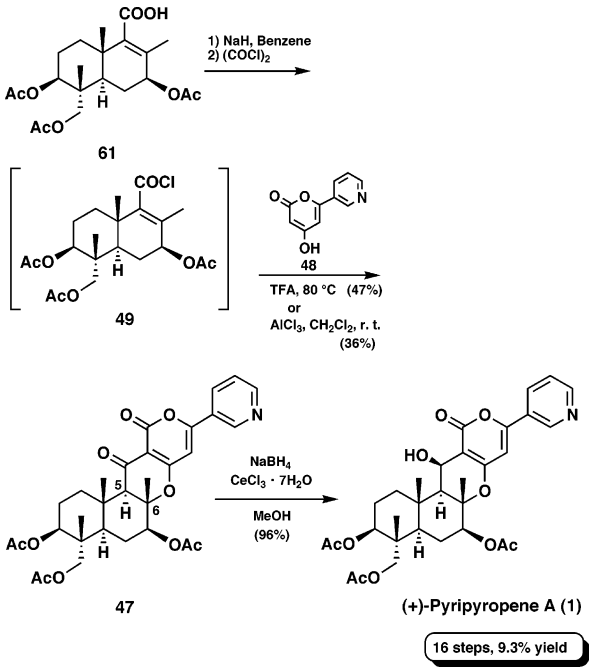
The crucial sequence joining hydroxy pyrone **48** by the Douglas[52] protocol with the AB subunit **49**

Table 1.



	yield (%)					
	−20 °C		rt		80 °C	
Lewis acid	**68**	**69**	**68**	**69**	**68**	**69**
CH_3AlCl_2	7		10		35	
$BF_3{\cdot}OEt_2$	8		52		63	
$TiCl_4$	46			44		20
CF_3COOH	100		52	14		51
$AlCl_3$	41	33		99		80

Scheme 8



proceeded readily in trifluoroacetic acid (TFA) (80 °C, 4 h); O-acylation followed by in situ 1,3-migration and intramolecular Michael addition formed the pentacyclic ketone **47** predominantly in 47% yield for three steps. The treatment of $AlCl_3$ as a Lewis acid afforded **47** in 36% yield.

Stereoselective reduction of **47** ($NaBH_4$, $CeCl_3$, MeOH)[47] then furnished (+)-pyripyropene A (**1**) in 96% yield as colorless needle-like crystals (mp 152−153 °C) (Scheme 8). The synthetic material was identical in all respects with a sample of the natural product (400 MHz ^{1}H NMR and 100 MHz ^{13}C NMR, high-resolution mass spectrometry (HRMS), optical rotation, melting point, and mixed melting point).

The first total synthesis of (+)-pyripyropene A (**1**) has thus been achieved via a convergent and efficient strategy (16 steps, 9.3% overall yield).[35] Importantly, a successful approach is designed to provide flexibility in the construction of congeners B−D and I−L (**2**−**4** and **9**−**12**), in addition to a range of potentially bioactive analogues.

3.5. Biomimetic Total Synthesis of Pyripyropene E

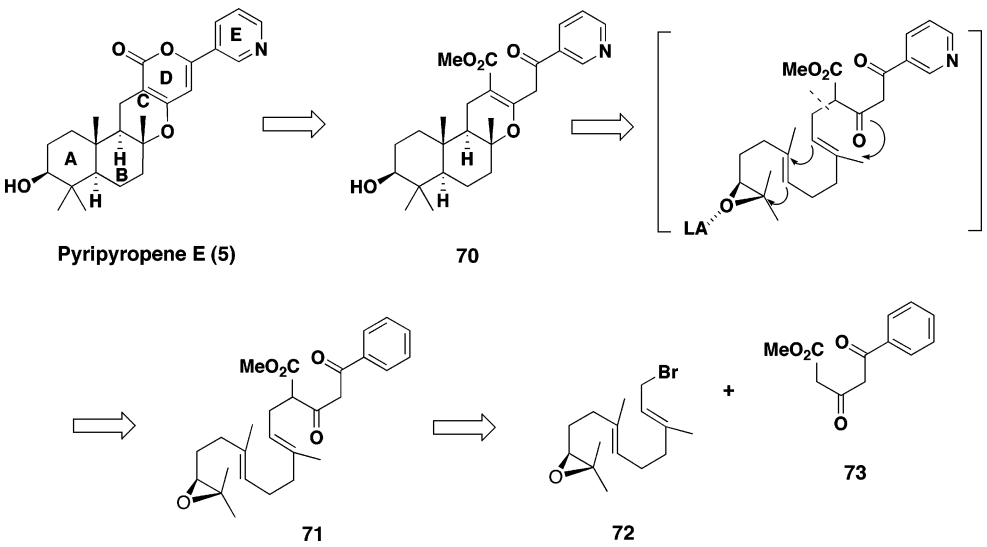
Pyripyropene E (**5**), the simplest member of the family, was proved to be identical with GERI-BP00 M reported by Bok et al.[9,10] Parker and Resnick have completed a biomimetic construction of racemic **5**.[53] Here we describe a similar biomimetic approach to the natural enantiomer of pyripyropene E [(+)-**5**][54] via a cationic polyene cyclization.[55−59]

From the retrosynthetic perspective (Scheme 9), it was envisioned that cyclization of epoxy diene **71**, initiated by a Lewis acid, would provide the requisite pyripyropene skeleton. The substrate **71** in turn would be derivable from the diketo ester **73** and the known epoxy bromide (−)-**72**.

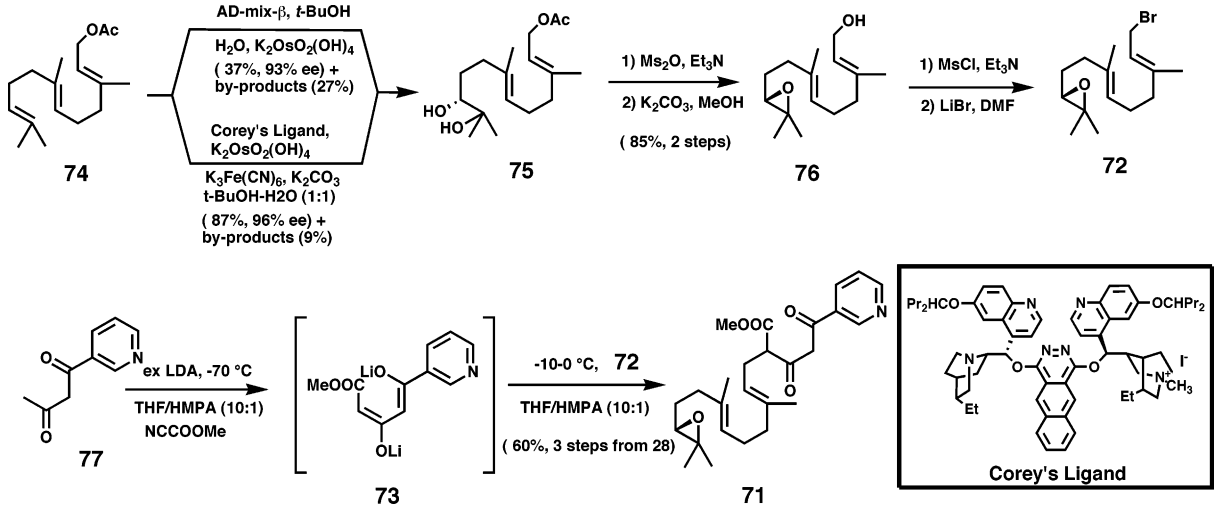
As the point of departure, dihydroxylation of *E*,*E*-farnesyl acetate (**74**) with the Sharpless AD-mix-β[60] under standard conditions furnished diol **75** (22% yield, 37% based on consumed **74** plus the internal diol and tetraol (1:1.5 ratio, 27%). The enantiomeric purity of **75** (ca. 93% ee) was determined by ^{1}H NMR analysis of the C(10) (+)-MTPA esters. As anticipated, Corey's cinchona alkaloid ligand[61] provided distinctly superior results [54% based on consumed **74**, 96% ee, plus 9% (1:2 mixture) of the internal diol and tetraol]. Treatment of diol (+)-**75** with methanesulfonic anhydride (Ms_2O)[62] and then K_2CO_3 in MeOH afforded epoxy alcohol (−)-**76** (85% yield), which was converted to bromide (−)-**72** (MsCl, Et_3N, CH_2Cl_2, LiBr, DMF). After aqueous workup, the unstable bromide was immediately employed in the subsequent alkylation step. Generation of a yellow dienolate of nicotinoylacetone[63] (**77**) (3.3 equiv lithium diisopropylamide (LDA), 10:1, THF/hexamethylphosphoric triamide (HMPA), followed by the treatment with methyl cyanoformate[64] and deprotonation with excess LDA, produced the orange dianion of **73**. Addition of this solution to the epoxy bromide **72** (10:1, THF/HMPA) furnished the substrate for cyclization (−)-**71** (50% from **76**, three steps) (Scheme 10).

The pyripyropene skeleton could be elaborated from **71** via two different sequences (Scheme 11). Exposure to $BF_3{\cdot}Et_2O$ (CH_2Cl_2, 0 °C, 2 h) effected cationic cyclization to give (+)-**78**. Chromatography on silica gel caused partial decomposition, and **78** was therefore carried forward without purification. Closure of the α-pyrone ring (1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), toluene, reflux, 4 h) provided a mixture of the desired tetracycle (+)-**79** and the trans−syn−trans isomer (−)-**80** (ca. 5:1) in 31% overall yield from **71**. Alternatively, initial formation of the α-pyrone (DBU, toluene) gave (+)-**81**; polyene cyclization induced by $BF_3{\cdot}Et_2O$ in CH_2Cl_2 then afforded a mixture of **79** and **80** with improved (>10:1) ratio, albeit

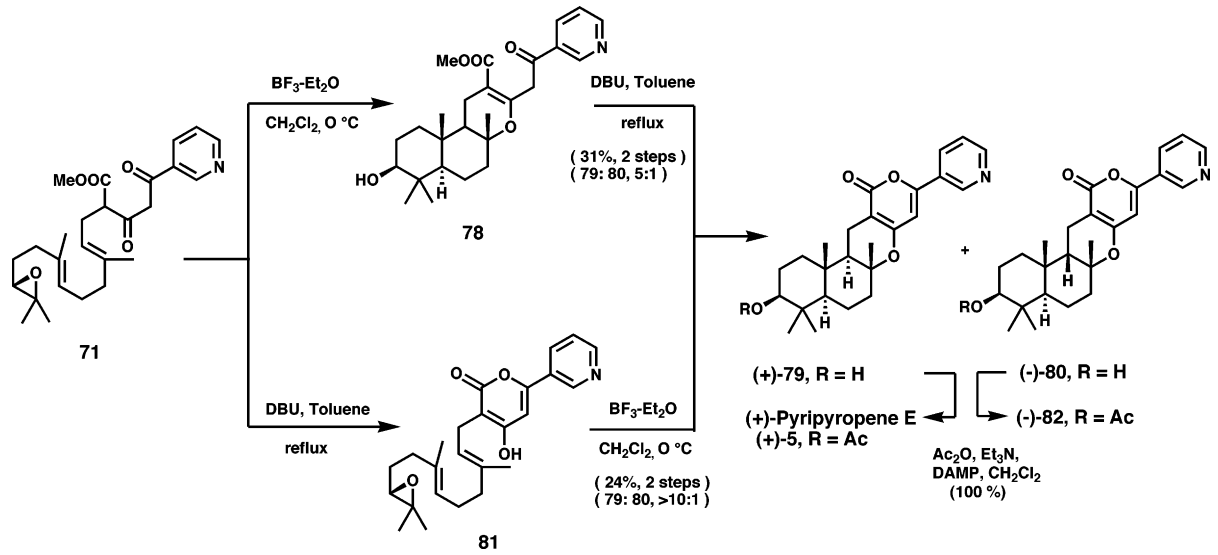
Scheme 9



Scheme 10



Scheme 11



in lower yield (24% from **71**, two steps). Acetylation (Ac_2O, Et_3N, cat. DMAP, CH_2Cl_2, 100%) of the mixture furnished (+)-pyripyropene E (**5**) and (−)-**82** after separation by preparative TLC. Synthetic (+)-**5** was identical in all respects (500 MHz ^1H NMR and 125 MHz ^{13}C NMR, HRMS, optical rotation, melting point, and mixed melting point) with a sample of the natural product. The structure of (−)-**82**, initially

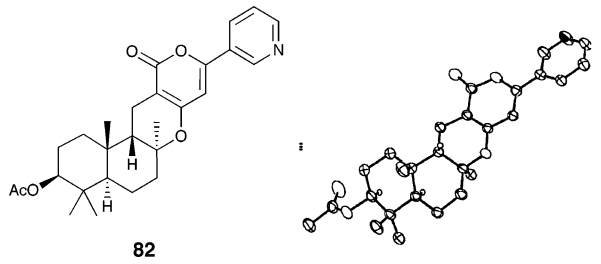


Figure 10.

deduced via extensive NMR studies, was confirmed by single-crystal X-ray analysis (Figure 10).[54]

3.6. Synthesis by Parker et al.

Parker described an efficient preparation of GERI-BP001[9,10] by a scheme using Nishizawa's protocol,[65] which is envisioned as a prototype for the synthesis of the more highly oxygenated and more potent pyripyropenes.[53]

The substrate for cyclization of **85** was prepared in two steps from *trans*,*trans*-farnesyl bromide (**83**). Methyl acetoacetate (**84**) was alkylated under standard conditions (NaH, DMF) to give keto ester **85**. The polyolefin intermediate, methyl ester **85**, was then subjected to Nishizawa's cyclization conditions. The tricyclic organomercurial **86**, uncontaminated by isomers, was easily isolated in 45% yield using silica gel chromatography. This intermediate was converted to a mixture of the α- and β-hydroxylated stereoisomers **87** and **88** via the hydroxylation procedure by Hill and Whitesides[66] (O_2, $NaBH_4$, DMF). Separation by silica gel chromatography provided each alcohol in 35% yield, respectively. The undesired α-hydroxylated isomer **87** was oxidized to the ketone with Jones reagent and then reduced ($NaBH_4$, MeOH) stereoselectively to yield the desired β-isomer **88**.

Completion of the synthesis requires pyrone annelation and acylation. The remaining two steps were accomplished in acceptable yields. Previous attempts to effect pyrone annulations, similar to the conversion desired here, proved to be problematic and required strategies with two or more steps.[67] Nevertheless, in this work, the direct annulation of intermediate **88** was accomplished in 40% yield by a procedure involving 3 equiv of LDA (THF, −78 °C), followed by addition of ethyl nicotinoate (Scheme 12). Acetylation (Ac_2O, Et_3N) of the intermediate **89** proceeded in quantitative yield. The ^1H NMR and ^{13}C NMR spectra of pyripyropene E (**5**) were identical to those obtained by Bok and co-workers[9,10] for the natural product.

3.7. Synthesis of Terpene Moiety by Aggarwal et al.

Aggarwal et al. described an efficient preparation of a decalin subunit, a key intermediate[35] in the first synthesis of (+)-pyripyropene A, which was synthesized from geraniol, utilizing an epoxy-olefin biomimetic cyclization of allylsilane[68] as the key step.[69]

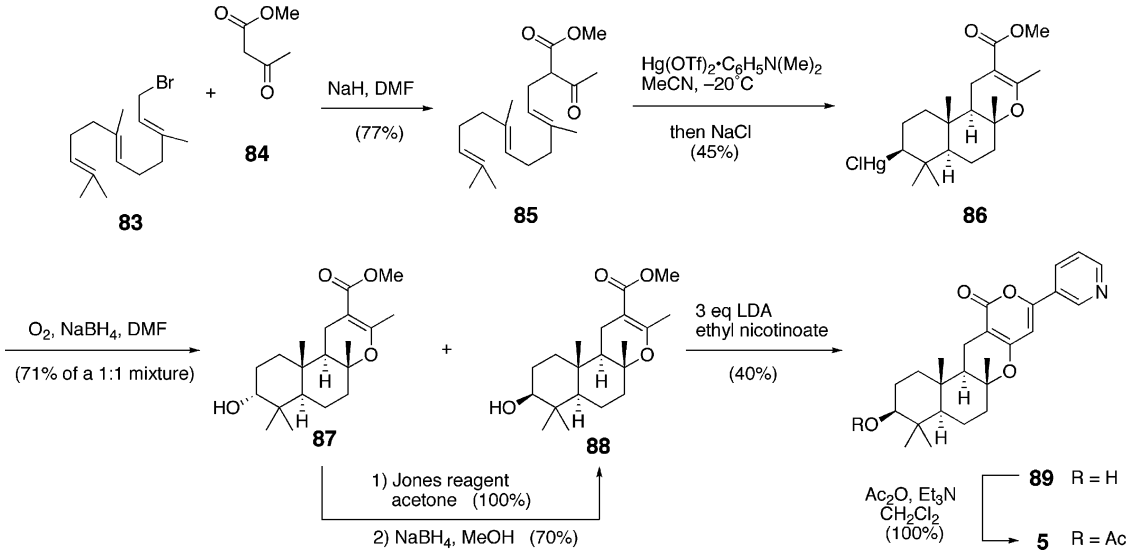
The known allylic alcohol **90**, derived from geraniol in five steps,[70] was converted to bromide **91**. The bromide was employed in the alkylation step with the dianion of methyl acetoacetate[71] to afford the β-keto ester **92**. Conversion of β-keto ester to *Z*-enol phosphate **93**, followed by coupling with $TMSCH_2MgCl$, furnished the cyclization substrate, *Z*-allysilane **94**.[72] Treatment of the allylsilane with $BF_3{\cdot}OEt_2$ produced the required decalin **95** in 54% yield. Finally, benzylation of alcohol, using an excess of NaH and BnBr, resulted in concomitant isomerization of the alkene to provide the desired decalin subunit, a key intermediate **96** (Scheme 13).

This result presents a formal synthesis of (+)-pyripyropene A using a biomimetic epoxy-olefin cyclization.

3.8. Structure–Activity Relationships

Modification and structure−activity relationships of ACAT inhibitor pyripyropenes were examined. Over 300 derivatives of pyripyropenes have been synthesized.[73−76] The pyridine ring of **1** was replaced by the benzene ring (**PR-264**), which proved to be 100-fold less active than **1**. This suggests that the pyridine moiety plays a significant role in binding to

Scheme 12



Scheme 13

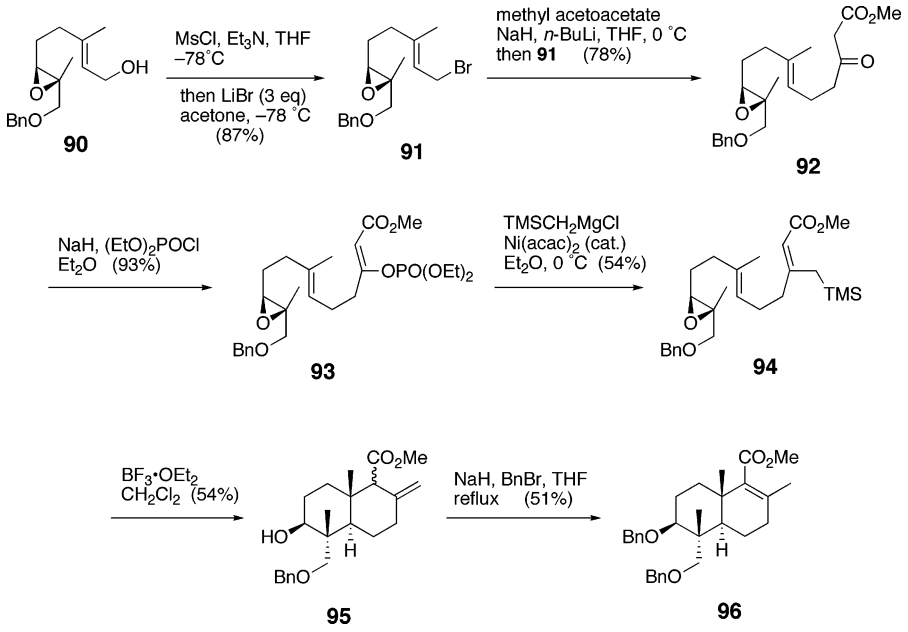
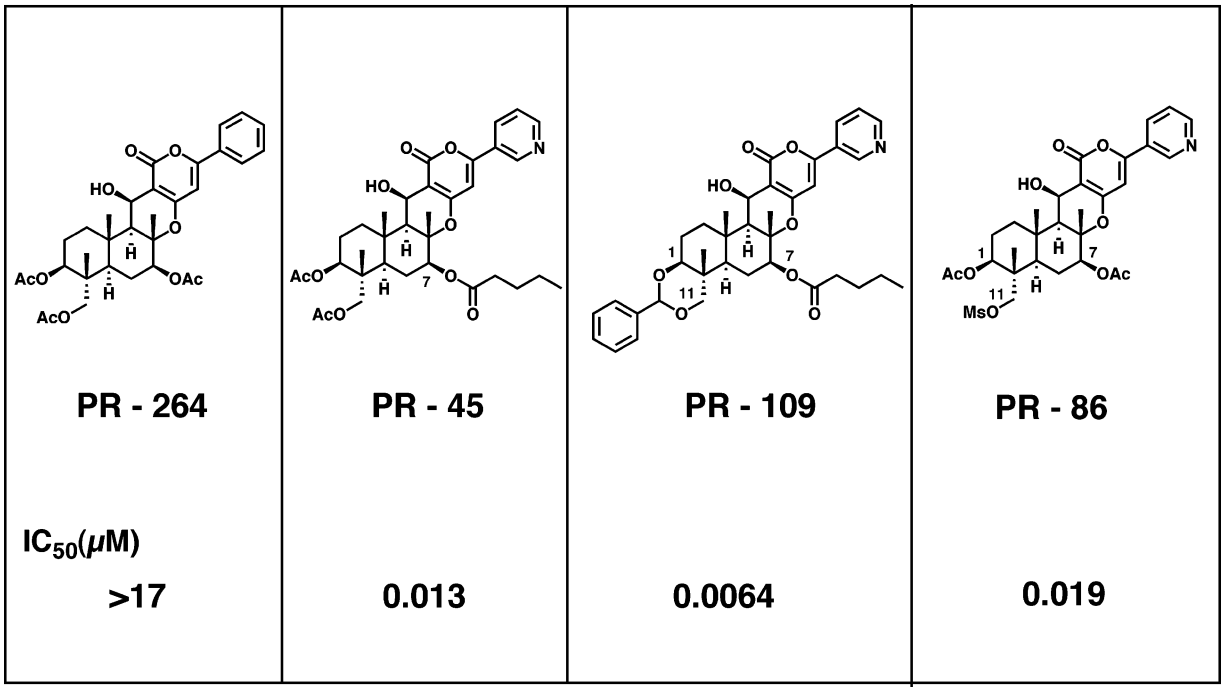


Table 2.



the enzyme. Some of them, such as **PR-86**, **PR-45**, and **PR-109**, have shown inhibitions on the order of nanomolar (Table 2). **PR-109** showed the most potent (IC_{50} 6 nM) in vitro inhibitory activity. **PR-86** also displayed strong ACAT inhibition (IC_{50} 19 nM).

From in vivo experiments using hamsters, **PR-86** (ED_{50}, 10 mg/kg) was found to be approximately 10 times more effective than pyripyropene A (ED_{50}, ca. 100 mg/kg) in the inhibition of cholesterol absorption from intestines.[36]

Based on the derived structure−activity relationships, a binding model to ACAT was proposed for the most potent derivative **PR-109** (Figure 11). The phenyl group of the **PR-109** 11-benzylidene acetal moiety is located in an equatorial position, suitably fitting a hydrophobic site of ACAT. Another hydrophobic pocket might exist for the 7-*O*-valeryl group. 7-*O*-Valeryl, including the carboxyl group, 13-hy-

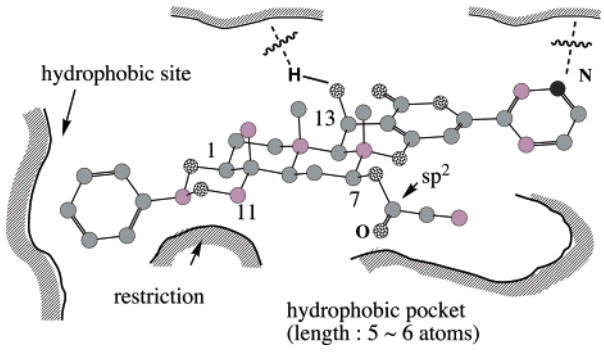
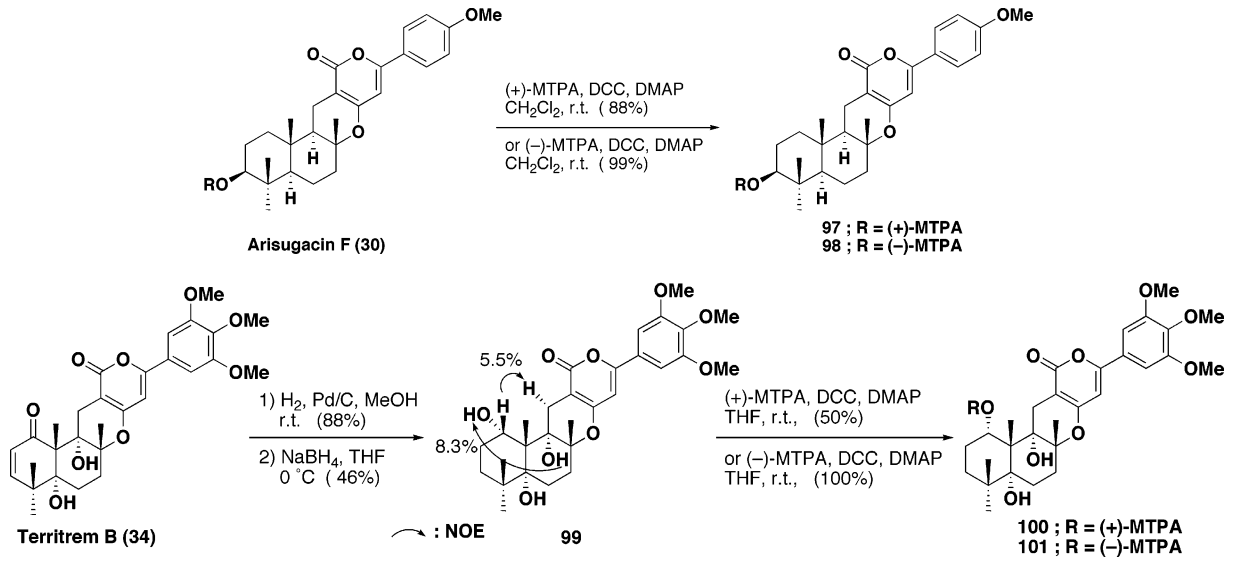


Figure 11.

Scheme 14



droxy, and pyridine moieties, appears to be very important for binding to ACAT.[36]

4. Total Synthesis of Arisugacins

4.1. Isolation

As the elderly population increases, patients with senile dementia are expected to increase similarly if no countermeasures are provided. Alzheimer's disease (AD) is known to be associated with an anomaly in the neurotransmitter system, such as acetylcholinergic, catecholaminergic, and serotonergic neurons, the amino acid system, and the neuropeptide system. Since the acetylcholinergic neuron is closely involved in high-order brain functions such as memory, learning, and cognition, a number of groups have searched for potential substances that can normalize the disorder of the acetylcholinergic neuron.[77] In this context, synthetic inhibitors of acetylcholinesterase (AChE) have recently attracted particular attention, since a synthetic agent, 1-benzyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidine (E2020), was approved by the United States Food and Drug Administration (FDA) for the treatment of AD.[78]

The Kitasato Institute has screened approximately 10 000 soil isolates (containing predominantly actinomycetes and fungi) to find a new class of AChE inhibitor from microbial metabolites. During this process, (+)-arisugacins A and B (**25** and **26**), architecturally interesting inhibitors of AChE derived from a culture broth of *Penicillium* sp. FO-4259,[17−20] were isolated, together with the structurally related known compound territrem B (**34**) (Figure 5). A mutant of *Penicillium* sp. FO-4259 was found to produce a series of metabolites, arisugacins C (**27**), D (**28**), E (**29**), F (**30**), G (**31**), and H (**32**).[20] The structures of **25** and **26** are comprised of a highly oxygenated *trans*-decalin system and an α-pyrone moiety.

Initial biological studies revealed that **25** and **26** possess in vitro inhibitory activity against AChE (from human erythrocyte) with IC_{50} values of 1 and 26 nM, respectively.[17] And the activity against AChE was more than 20 000 times higher than that against butyrylcholinesterase (BChE, from horse serum), inhibition of which is suspected to be a cause of liver disorder. Studies on the effects of **25** in an animal model of scopolamine-induced amnesia showed that **25** protected against amnesia and exhibited very weak effects on mouse salivation and hypothermia, a peripheral cholinergic response and central cholinergic response.[79] This strongly suggests that **25** and **26** could be potentially excellent drugs for the treatment of AD.[1]

4.2. Structure Determination

Structure elucidation was performed by extensive spectroscopic studies, including a combination of 1-D and 2-D NMR techniques.[18] Arisugacins and territrems have a common structure consisting of naphtho[2,1-*b*]pyrano[3,4-*e*]pyran and benzene.

4.2.1. Determination of Absolute Stereochemistries of Arisugacins

A modified Mosher method[32,33] was used to elucidate the absolute configuration at C(3) of arisugacin F (**30**). Arisugacin F (**30**) was treated with (*R*)-(+)- and (*S*)-(−)-2-methoxy-2-trifluoromethyl-2-phenylacetic (MTPA) acid in the presence of DCC and DMAP to afford the (*R*)-(+)- and (*S*)-(−)-MTPA esters (**97** and **98**, Scheme 14). The $\Delta\delta$ values ($\delta_S - \delta_R$) obtained from ^{1}H NMR data of **97** and **98** are shown in Figure 12. The $\Delta\delta$ values for H_2(1), H_2(2), H(3), H_2(12), and H_3(12b)-OMe are negative, while positive $\Delta\delta$ values are observed for H_6(4α), β-Me, and H(5) thus indicating a 3*S* configuration. Therefore, the absolute configurations at C(3), C(4a), C(6a), C(12a), and C(12b) of **30** were assigned as *S*, *R*, *R*, *R*, and *S*, respectively, on the basis of the relative stereochemistry elucidated previously.[80]

On the other hand, to elucidate the absolute configuration of territrem B (**34**), its MTPA esters were prepared as follows. Territrem B (**34**) was subjected to hydrogenation of the enone moiety, followed by $NaBH_4$ reduction to yield **99**. NOE correlations from H(12a)-OH to H(1)-OH and from

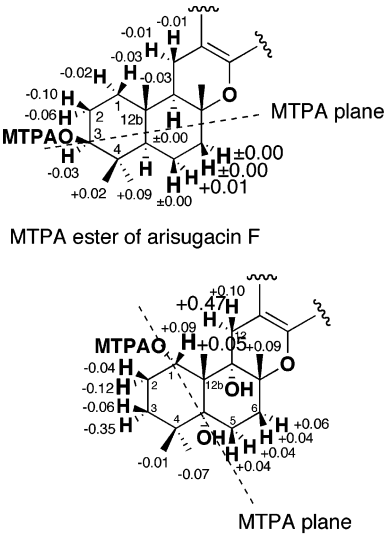


Figure 12. $\Delta\delta$ values of MTPA esters derived from arisugacin F (**30**) and 2,3-dihydro-1α-ol territrem B (**99**).

H(1) to H(12) in **99** indicated that the 1-hydroxyl group was oriented to the α-side (Scheme 14). The calculated $\Delta\delta$ values ($\delta_S - \delta_R$) of the (*R*)-(+)- and (*S*)-(−)-MTPA esters (**100** and **101**) are indicated in Figure 12, and the absolute stereochemistry of **99** was determined as 1-(*S*), 4a-(*R*), 6a-(*R*), 12a-(*R*), and 12b-(*S*). The absolute stereochemistry of **34** was the same as that of **30**. In view of the biosynthetic pathway common to arisugacins and territrems, it was presumed that the relative and absolute stereochemistries of **25** and **26** should also be common to those of **30** and **34**.[80]

4.3. Biosynthesis

Arisugacins biogenetically belong to the mixed polyketide−terpenoid group (meroterpenoid).[2,3] Their biosynthetic pathways via arisugacin F (**30**) as the key intermediate were proposed (Scheme 15): (1) condensation of an acetoacetate with a benzoic acid analogue derived from shikimate, to give a phenyl-α-pyrone moiety, (2) substitution of an all-trans farnesyl group to the α-pyrone moiety, (3) epoxidation of the terminal olefin of the farnesyl group followed by cyclization to give the core skeleton, arisugacin F, and (4) oxidation and further transformation leading to arisugacins A and B and territrem B.[80]

Described herein, is the first total synthesis of (+)-**25** and (+)-**26**, the most active member of this family, via a flexible, concise, and highly efficient route to provide the natural arisugacins, the biomimetic total synthesis of (±)-**30** and (±)-**31**, and the structure−activity relationships. R. P. Hsung et al. reported the total synthesis of this interesting natural product[81−87] using a similar strategy to that we had reported.[88−91]

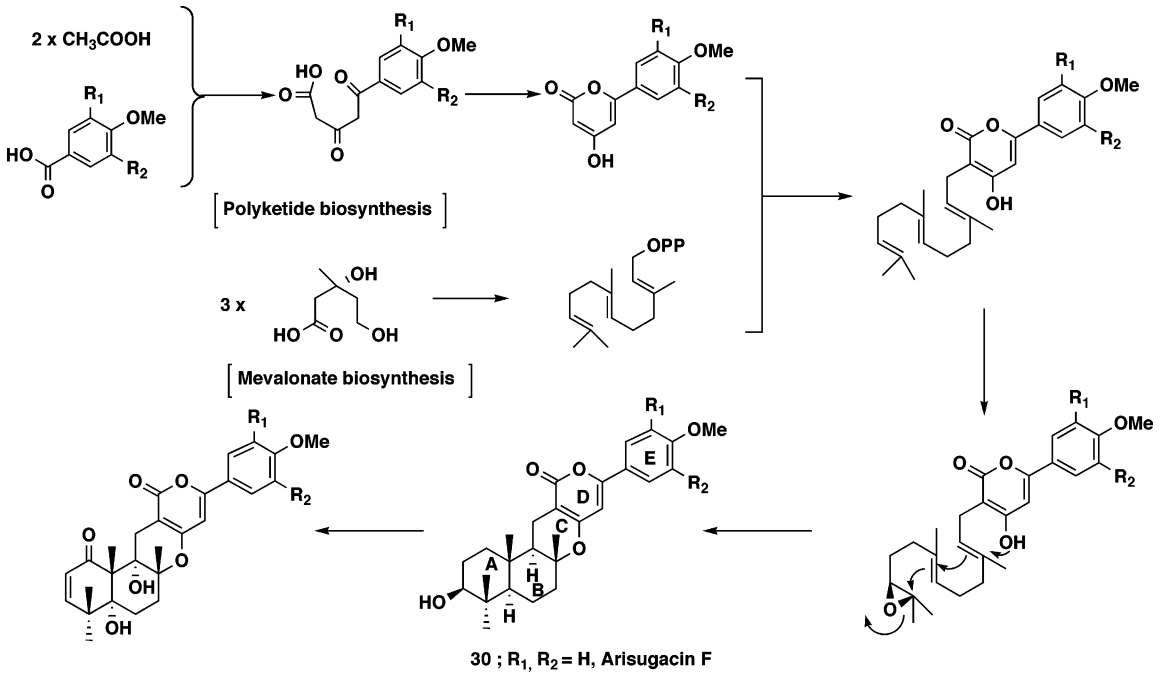
4.4. Total Synthesis of Arisugacins A and B

According to retrosynthetic analyses (Scheme 16), arisugacin A could be accessed by several steps of oxidation reactions from the pentacycle **102**, which would be derived from α,β-unsaturated lactol **105** and the known 4-hydroxy-2-pyrone **104**[37] via a Knoevenagel-type reaction in the presence of L-proline. In this process, condensation of α-pyrone with a Shiff-base formed by lactol and amino acid and subsequent amine elimination followed by 6π-electron electrocyclic ring closure[92] would be performed. The cyclization step should proceed with the requisite geometry at the BC ring fusion, controlled by the steric effect of the C(12b) angular methyl group. The known lactone **106**, an intermediate for the synthesis of forskolin,[93] would then lead to lactol **105**.

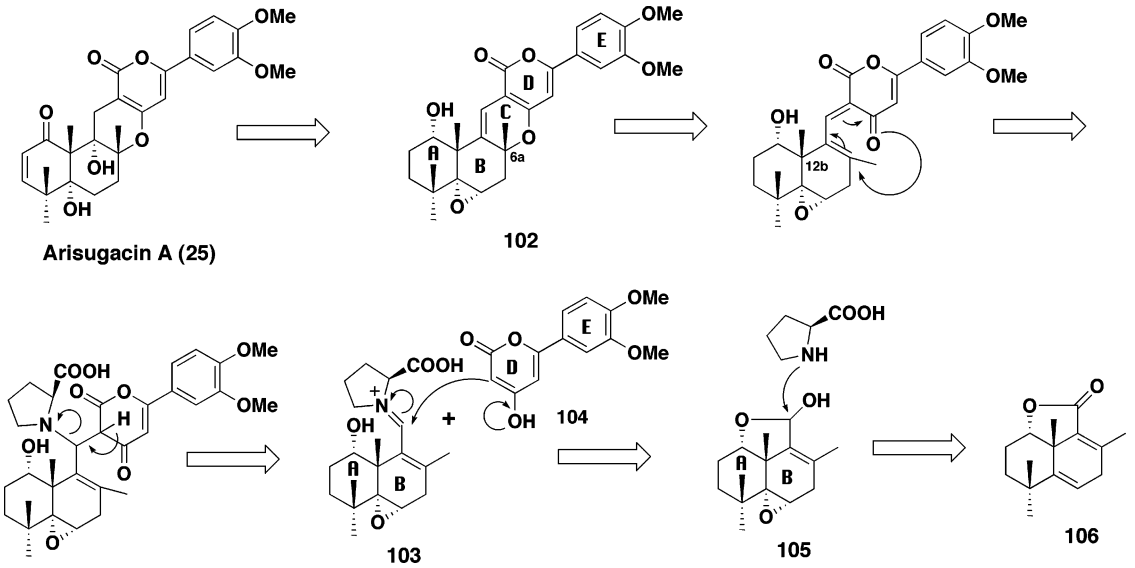
4.4.1. Construction of Arisugacin Skeleton

To synthesize (+)-arisugacin A, the chiral building block **108** was prepared from **107**, which was syn-

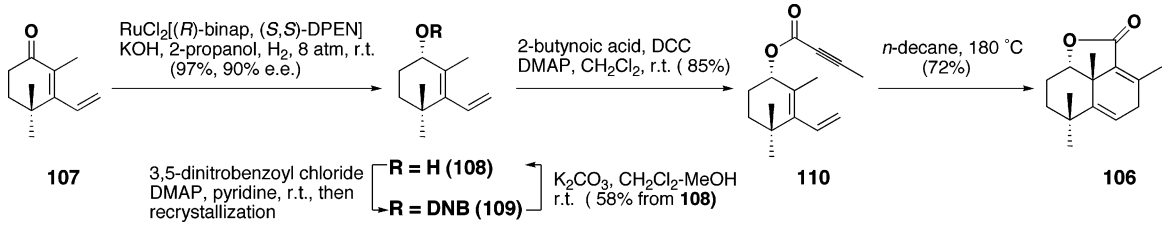
Scheme 15



Scheme 16



Scheme 17

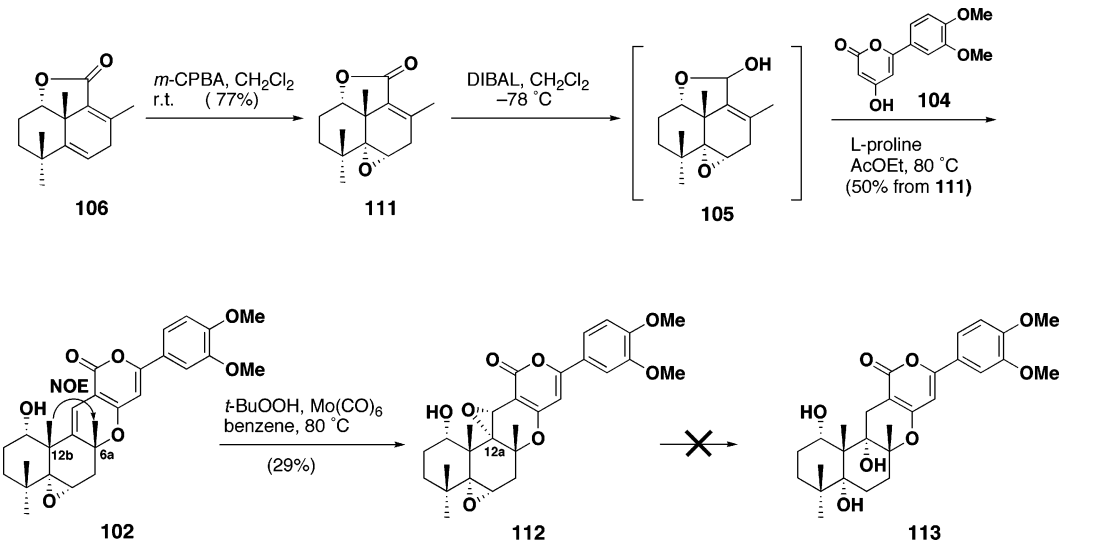


thesized from the commercially available α-ionone by a known procedure.[94] The known CBS reduction procedure was not feasible in this case because of poor reproducibility and the requirement of a large amount of expensive catalyst.[95,96] On the other hand, asymmetric hydrogenation using a ruthenium catalyst can be conducted easily on a preparative scale with a S/C ratio of up to 500 and a substrate concentration as high as 2.5 M in 2-propanol.[97] When the achiral cyclic enone **107** was hydrogenated with a catalyst system containing $RuCl_2$[(*R*)-binap,(*S*,*S*)-DPEN] (binap = [2,2′-bis(diphenylphosphino)-1,1′-binaphthyl]; DPEN = diphenylethylenediamine in 2-propanol containing KOH (**107**/Ru/KOH = 500:1:40, 8 atm, room temperature, 24 h), the allylic alcohol **108** was obtained in 90% ee and 97% yield. The enantiomeric mixture of **108** was converted to the 3,5-dinitrobenzoate **109**, which was purified by recrystallization. Pure **109** was reconverted into **108** (98% e.e.) upon treatment with potassium carbonate in methanol. The enantiomeric purities were determined by NMR analysis of the corresponding MTPA esters. The absolute configuration of (−)-**108** was determined to be the *S* configuration by optical rotation described in the literature (Scheme 17).[95]

The lactol **105**, a coupling unit, was prepared in four steps starting from the chiral building block **108**. Esterification of **108** with 2-butynoic acid, followed by an intramolecular Diels−Alder reaction, afforded **106**, which was treated with *m*-chloroperbenzoic acid (*m*-CPBA) to afford epoxide **111** stereoselectively, and the resulting epoxide **111** was converted into lactol with diisobutylaluminum hydride (DIBAL). Subsequently, the condensation with α-pyrone **104**, using L-proline as a catalyst, was examined to construct the pentacycle **102**. By treatment of lactol with α-pyrone **104** in the presence of a catalytic amount of L-proline[81] in EtOAc at 80 °C, the coupling reaction proceeded smoothly to provide the desired pentacyclic skeleton **102** in 50% yield (two steps). Subsequently, the introduction of a hydroxy group at the 12a position was studied via epoxidation. Epoxidation of **102** with *m*-CPBA or dimethyldioxirane (DMDO) was inefficient, and from the efforts of this work, oxidation with *t*-BuOOH in the presence of $Mo(CO)_6$[98] gave the best result but only in 29% yield. For the following epoxide-opening reaction, various conditions were attempted, such as $LiAlH_4$, Super-H, or Birch reduction, but all of them were unsuccessful (Scheme 18); therefore, the introduction of two hydroxy groups by this strategy was abandoned.

Opening of the epoxide on the B ring of **111** was therefore planned prior to the coupling. Reduction with $LiAlH_4$ afforded a diol, together with the unreacted epoxide. Attempts to reduce using DIBAL or Super-H also failed. Fortunately, $LiAlH_4$ in an addition of $AlCl_3$ furnished the desired triol **114** (Scheme 19). Selective oxidation of the primary alcohol **114** with tetrapropylammonium perrhuthenate (TPAP) (0.02 equiv) and 4-methylmorpholine *N*-oxide (NMO) (1.5 equiv) afforded **115** in 73% yield. Initially, coupling of aldehyde **115** with α-pyrone **104** was attempted under previously used conditions for the formation of **102** and gave the corresponding pentacycle **116** in only a 17% yield. In an effort to improve the step, the reaction conditions were precisely examined. Table 3 summarizes the results obtained by varying the catalyst loading, concentration, and

Scheme 18



Scheme 19

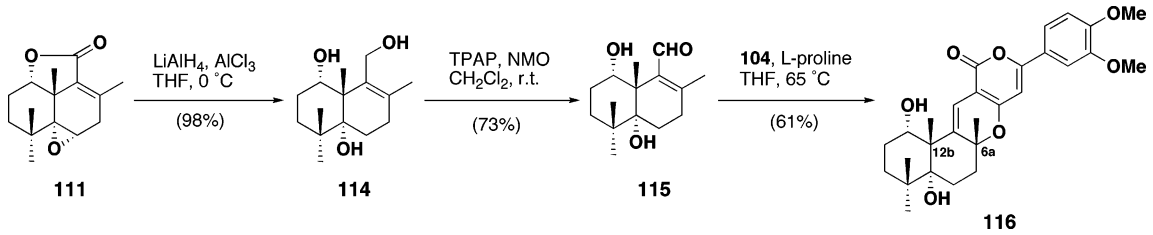


Table 3

entry	L-proline (equiv)	MS 3 Å	solvent	temp (°C)	yield (%)
1	0.5	+	benzene	70	26
2	3.0	+	benzene	80	31
3	3.0	−	benzene	80	29
4	2.0	−	benzene	80	32
5	2.0	−	1,2-dichloroethane	80	16
6	2.0	−	2-propanol	80	20
7	2.0	−	THF	65	61
8	2.0	−	DMF	80	14

solvent. The data indicated that high loading of L-proline and the use of THF as solvent are essential for efficient formation of **116**. The reaction condition using 2.0 equiv of L-proline in THF at 65 °C gave the best result, affording the desired pentacycle **116** in 61% yield.

4.4.2. Arisugacin A Final Stage

Introduction of a hydroxy group at the 12a position in **116**, via epoxidation and epoxide opening, was planned as applied for the preceding case, **102**. Unexpectedly, oxidation of **116** with *t*-BuOOH did not yield the desired epoxide **117**. A series of epoxidation conditions attempted (*m*-CPBA, DMDO, AcOOH, or CF_3COOOH) were all unsuccessful and gave complex mixtures. Dihydroxylation of 12-ene with OsO_4 also failed.[85] These results were surprising because the structural difference between **102** and **116** lies only in the presence of either an epoxy group or a hydroxyl group at the 4a-position. It was presumed that the difficulty may be due to hydrogen bonding between the 1-OH and 4a-OH, which prevents hydrogen bond formation between 1α-OH and peracids. Therefore, inversion of 1α-OH to form 1*β*-OH was attempted (Figure 13). Treatment of **116** with TPAP and NMO, followed by stereoselective reduction involving 4a-OH, furnished *β*-alcohol **118** in 71% yield (two steps).

Epoxidation of **118** with *m*-CPBA provided only *β*-epoxide **120**. During the careful NMR analysis of **120** and its acetate **121**, it was noted that the hexacycle, reported by R. P. Hsung et al.,[85,86] must be revised to **120** (Scheme 21). Treatment of **118** with AcOOH produced a mixture of triol **119** and *β*-epoxide **120**, and treatment with performic acid or trifluoroperacetic acid furnished α-epoxide as the other product. Since the desired α-epoxide could not be obtained as the major product under the condition examined, an attempt to increase the yield of triol was made. The optimum condition was gained by treatment with AcOOH in a CH_2Cl_2−phosphate buffer system to keep lower acidity at room temperature (Scheme 20). The stereochemistry of the introduced hydroxy groups in **119** was confirmed by NOE experiments as shown in Figure 14.

For the mechanism of this dihydroxylation reaction, a process involving acyl migration was assumed, as shown in Scheme 22. The endocyclic olefin in 2*H*-pyran fused to 2-pyrone has unique reactivity, owing to activation from the pyranyl oxygen. The electron-rich C(12a) would attack AcOOH selectively to afford the oxocarbenium intermediate. Stereoselective addition of the AcO^- anion to the initially formed oxocarbenium intermediate occurs exclusively from the bottom face, because the two angular methyl groups shield the top face of the olefin. The acetyl group migrates from the C(12) hydroxy to the C(1) hydroxy,

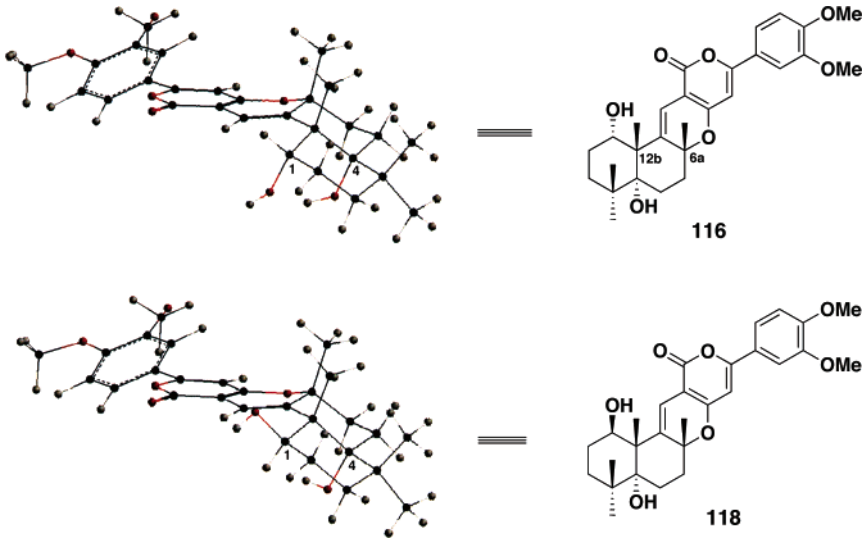
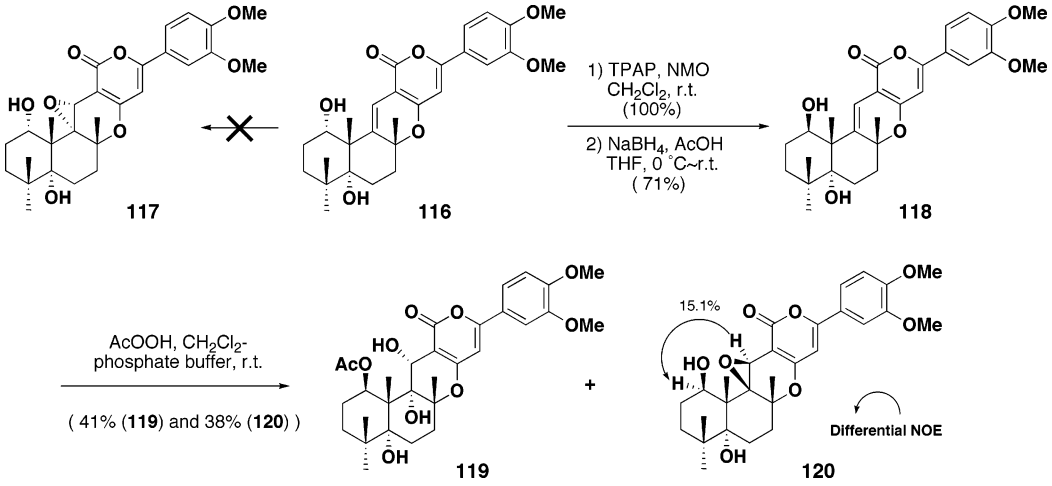
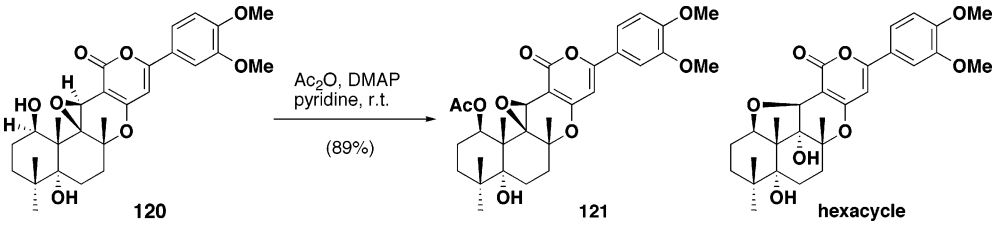


Figure 13. Heat of conformation for **116** and **118**.

Scheme 20



Scheme 21



located at a conformationally close position (Scheme 22).

The next step is the reductive elimination of 12-OH in **119**. Since the formation of xantate and mesylate failed, probably due to the steric hindrance

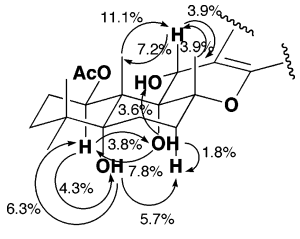
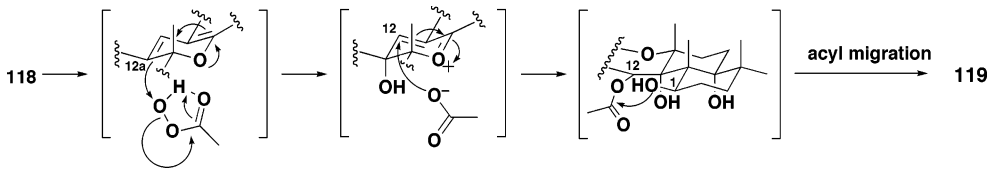


Figure 14. NOE results for **119**.

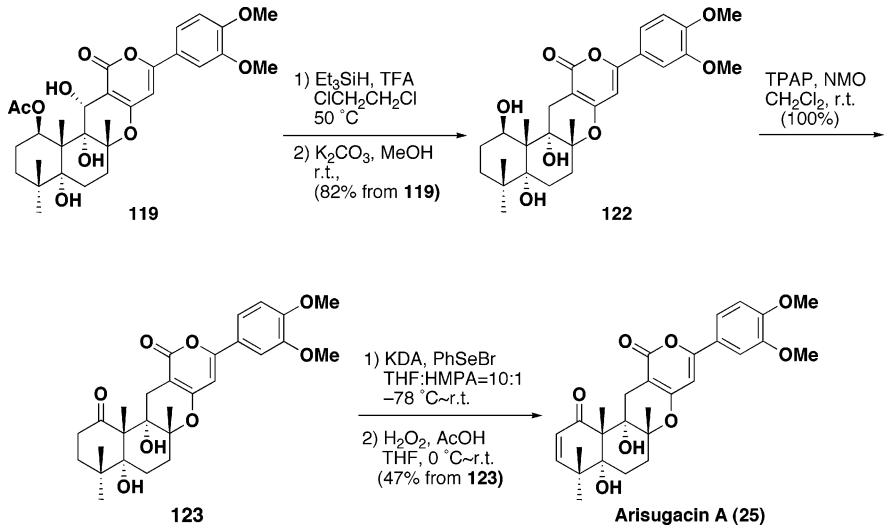
around this functionality, direct reduction of the hydroxy group was attempted. Reaction with BH_3-THF/BF_3-OEt_2, Et_3SiH/BF_3-OEt_2, or $NaBH_3CN$/TFA gave unsatisfactory results. While the adaptation of $NaBH_3CN/ZnI_2$ furnished **122** in low yield, this procedure was not satisfactory in that the starting material remained. Finally, successful results were obtained by the treatment of **119** with Et_3SiH and TFA,[99] affording the desired product in good yield. Successive methanolysis of the C(1) acetate furnished triol **122** in 82% yield (two steps).

In the final step, triol **122** was readily oxidized to ketone **123** in quantitative yield by treatment with TPAP and NMO. To examine the conversion of ketone to enone, 2,3-dihydroterritrem B was used as a model compound. The procedure, via bromina-

Scheme 22



Scheme 23



tion,[100] failed due to attack of α-bromoketone by the 4a-hydroxy group, to form an ether ring. *o*-Iodoxybenzoic acid (IBX)[101] and the Saegusa method[102] were also unsuccessful. Fortunately, the phenylselenenylation−oxidation method, enolization with Schlosser's base,[103] followed by phenylselenenylation, was found to be effective. The phenylselenide was treated with H_2O_2 to give territrem B, although the yield was low. Therefore, several reaction conditions were examined with compound **123** to optimize the yield. As a result, the best condition was found to be enolization at −20 °C and phenylselenenylation at room temperature. Without any retro-aldol−aldol reaction, this protocol furnished arisugacin A (**25**) in 47% yield (Scheme 23). The IR, HRMS, and $^1H/^{13}C$ NMR spectra of this synthetic **25** were in solid agreement with the corresponding data reported for natural **25**. An $[\alpha]_D^{25}$ value of +144.0° (*c* 0.10, $CHCl_3$) was determined for the synthetic sample. Although the reported value for the natural product was $[\alpha]_D^{23}$ +72° (*c* 0.10, $CHCl_3$), the optical rotation was shown to be $[\alpha]_D^{25}$ +150.0° (*c* 0.10, $CHCl_3$) when the natural product sample was reexamined. Therefore, the absolute configurations at C(4a), C(6a), C(12a), and C(12b) were assigned as *R*, *R*, *R*, and *S*, respectively.[104]

4.4.3. Total Synthesis of Arisugacin B

To demonstrate the applicability of our strategy, an analogue, (+)-arisugacin B (**26**), was prepared. The intermediate in the analogous skeleton was obtained in 62% yield by the method described in Scheme 24, using 4-methoxy-α-pyrone **124**[37] instead of 3,4-dimethoxy-α-pyrone **104**. Synthetic procedures from **114** to **26** were performed similarly as those for (+)-arisugacin A (**25**). Spectroscopic data obtained for (+)-arisugacin B (**26**) were in agreement with those published for the natural product, except for the optical rotation. An $[\alpha]_D^{24}$ value of +168.5° (*c* 0.10, $CHCl_3$/MeOH 10/1) was determined for the synthetic isolate. The reported $[\alpha]_D^{23}$ rotation for the natural substance was +26° (*c* 0.10, $CHCl_3$). However, after reexamination, the optical rotation, $[\alpha]_D^{25}$, for the natural product was determined as +139.8° (*c* 0.10, $CHCl_3$/MeOH 10/1). Therefore, the absolute configurations at C(4a), C(6a), C(12a), and C(12b) were assigned as *R*, *R*, *R*, and *S*, respectively.[104]

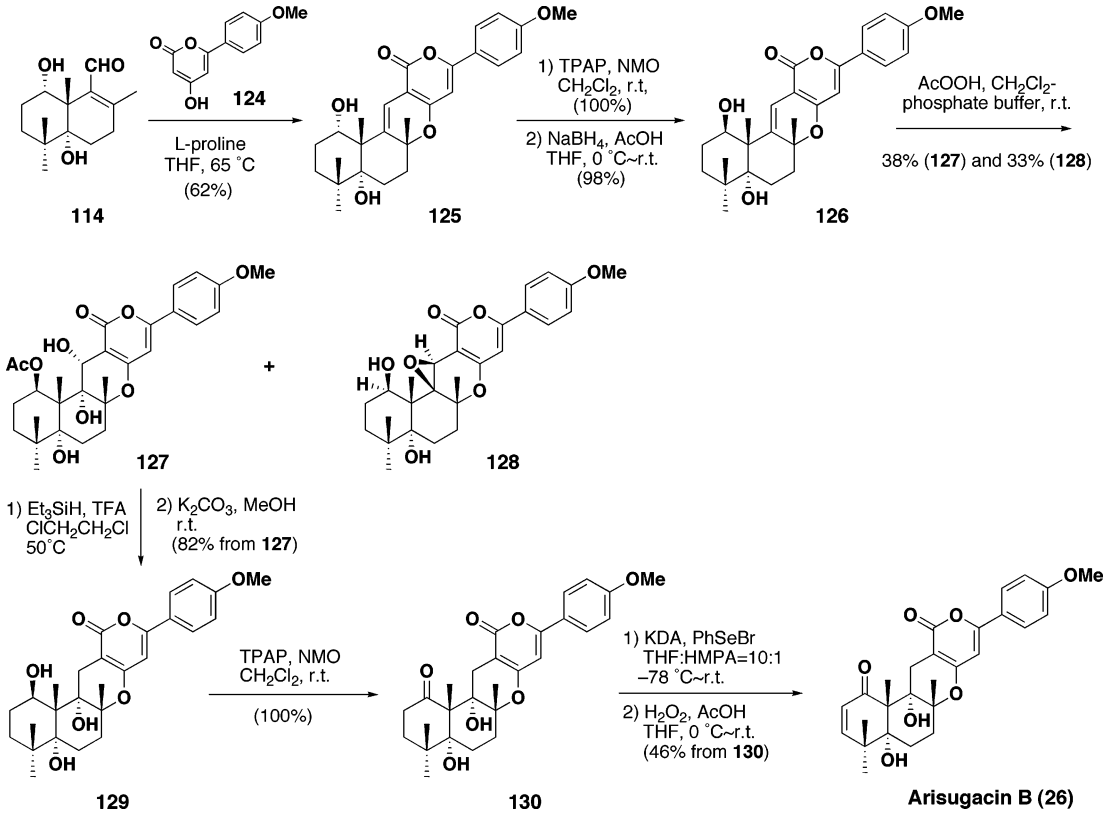
The first total syntheses of arisugacin A (**25**) and arisugacin B (**26**) have been achieved via an efficient and convergent strategy through 22 steps, starting from α-ionone (total yield 0.9% and 1.1% for arisugacin A and arisugacin B, respectively). The absolute stereochemistry of arisugacin A and arisugacin B was thus determined. This successful synthetic approach provides prospects for further supply of arisugacin derivatives and potentially bioactive analogues.

4.5. Biomimetic Total Synthesis of Arisugacins F and G

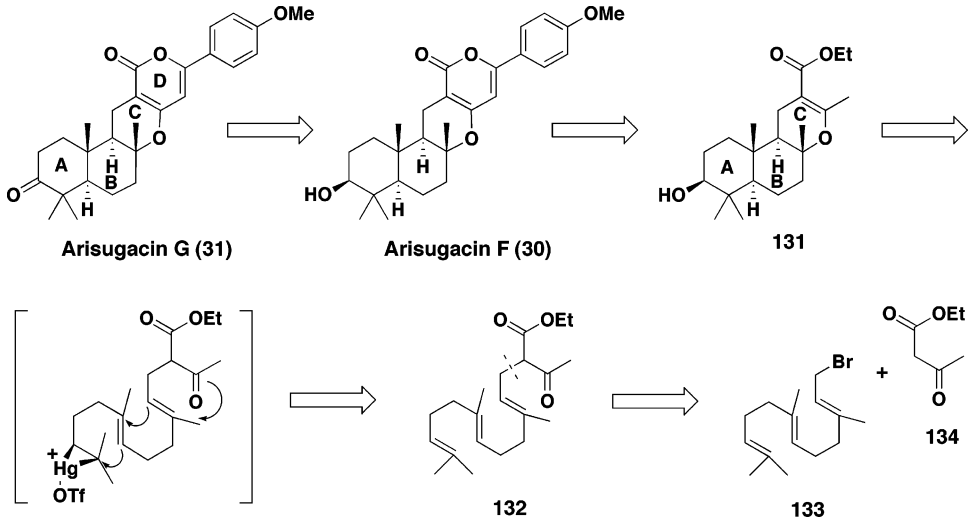
Arisugacins F (**30**) and G (**31**), the simplest members of the family, are supposed to be the biosynthetic intermediates. The stereoselective and efficient total syntheses of arisugacins F (**30**) and G (**31**) are described here.[105]

The ring system of arisugacin suggests that a synthetic strategy through biomimetic cyclization is possible. From a retrosynthetic perspective (Scheme 25), it was envisioned that the mercuric ion-induced cyclization[65] of a polyolefin substrate **132** would provide the requisite ABC-ring system of the arisugacin skeleton **131**. The tricyclic ester **131** would then be converted to **30** via pyrone annulation. The substrate **132**, in turn, could be derived from *trans*,*trans*-farnesyl bromide (**133**) and β-keto ester (**134**).

Scheme 24



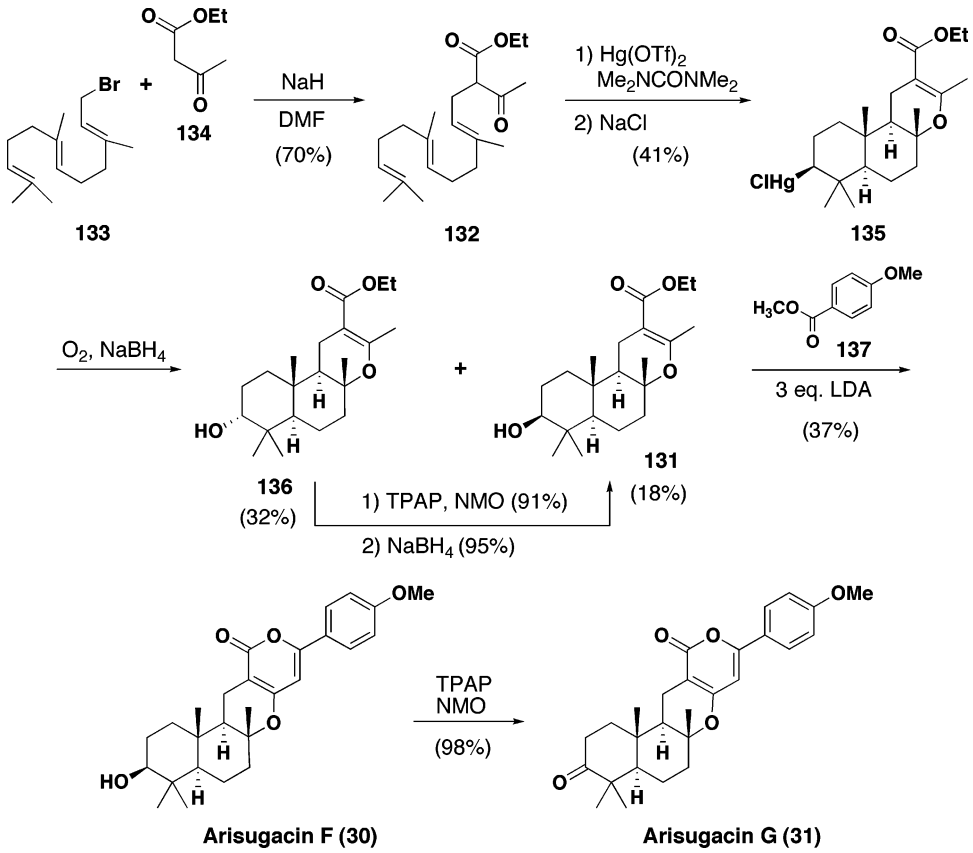
Scheme 25



Ethyl acetoacetate (**134**) was alkylated with *trans*,*trans*-farnesyl bromide (**133**) under a standard condition (NaH, DMF, room temperature) to give keto ester **132** in 70% yield. The keto ester **132** was then treated with mercury(II) trifluoromethanesulfonate ($Hg(OTf)_2$) and tetramethylurea (TMU) in CH_3CN, followed by saturated aqueous NaCl, to afford the desired tricyclic organomercurial **135** as a single isomer.[65,106] This intermediate was converted to a mixture of the α- and β-hydroxylated stereoisomers **136** (32% yield) and **131** (18% yield) by means of the hydroxylation procedure ($NaBH_4$, O_2, DMF).[66] The undesired α-hydroxylated isomer **136** was oxidized to a ketone (TPAP, NMO, CH_2Cl_2), followed by the stereoselective reduction of the ketone to give the desired β-isomer **131**. Next, dienolate γ-acylation and in situ cyclization[53,107] of **131** by treatment with 3 equiv of LDA and TMEDA, followed by addition of methyl *p*-methoxybenzoate (**137**), afforded the desired (±)-arisugacin F (**30**) in 37% yield. Finally, oxidation of **30** (TPAP, NMO, CH_2Cl_2) afforded the desired (±)-arisugacin G (**31**) in 98% yield (Scheme 26). These synthetic arisugacins F and G were identical in all respects with natural **30** and **31** (400 MHz ^1H NMR and 100 MHz ^{13}C NMR, HRMS).

In conclusion, the highly convergent, first total syntheses of the AChE inhibitors (±)-arisugacins F (**30**) and G (**31**) were completed by a very short sequence from commercially available starting materials, ethyl acetoacetate (**134**), *trans*,*trans*-farnesyl bromide (**133**), and methyl *p*-methoxybenzoate (**137**). The key transformation, a polyolefin cyclization,

Scheme 26



provides rapid access to the vinylogous ester (**135**), establishing the ABC-ring system, which includes all five stereocenters of the target. Annulation of the α-pyrone ring in one step provided the natural product **30**, and then oxidation afforded another natural product **31**. The efficiency of this general approach suggests its exploitation in the synthesis of other members of the arisugacin class.

4.6. Structure–Activity Relationships

Due to the interest in bioactivities of the synthetic intermediates, the activities of **1-keto-116**, **118**, **119**, **120**, **122**, **123**, **125**, **126**, **127**, **128**, **129**, and **130** against AChE were tested using our previously published method.[18] It was found that only **123** showed activity, and this was 80-fold lower than that for **25**. It was also found that **1-keto-116**, lacking both an enone moiety on the A ring and a 12a-α-hydroxy group, and **129** and **130**, lacking the enone moiety, no longer inhibited AChE. This suggests that these functionalities are essential for the inhibition of AChE by arisugacins. In addition, Peng reported that 1β-hydroxy-territrem B was 300-fold less potent than territrem B for inhibition of AChE.[108] Furthermore, it was found that **25** was 25-fold more potent than **26**.[17] Consequently, it is suggested that the enone moiety in ring A, the hydroxy group at position 12a, and the E ring substituents play important roles in the inhibition of AChE by arisugacins.

Considering the structure–function relationship between arisugacins A and B, territrem B, and territrem C, they have different ring E structures (Figure 5). The compounds can be arranged in descending order of selectivity for AChE, that is, arisugacin A, territrem C, territrem B, and arisugacin B. For the arisugacins, it may be argued that the presence of the R_1 or the R_3 methoxy group in ring E is at least associated with the selective inhibition of AChE. Regarding territrem B, Peng suggested the importance of the double bond, the enone group in ring A, and the pyrone group in ring D, for expressing selectivity for AchE, as judged by comparison between several synthetic derivatives of territrem B.[108]

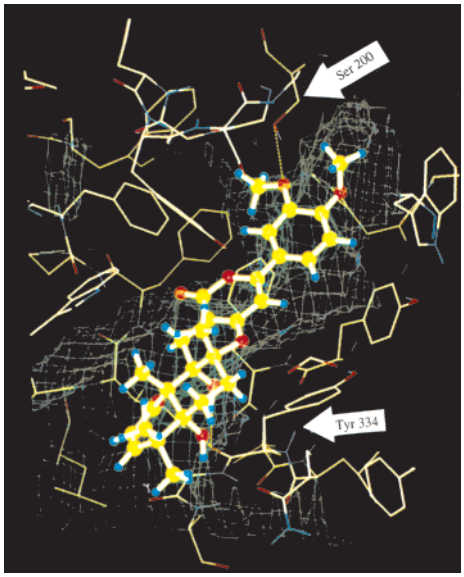


Figure 15. Computer simulation of arisugacin A docking with AchE.

Recently, in collaboration with Dr. Itai of the Institute of Medicinal Molecular Design, a three-dimensional view of arisugacin A docking with AChE could be simulated using an automated computer docking program, ADAM (Figure 15, Mizutani et al., 1994), in which a basket-like structure in blue represents the putative cavity in the AChE protein, to which the substrate or inhibitory molecule binds. As is evident in Figure 15, the long stretched arisugacin A molecule is well buried along the long and narrow cavity of the enzyme, and hydrogen bondings are also apparent.

5. Concluding Remarks

A collection of microorganisms has been screened for metabolites that selectively inhibit ACAT and AChE, which has led to the isolation and identification of novel α-pyrone meroterpenoids, designated as pyripyropenes and arisugacins, from strains of *Aspergillus fumigatus* FO-1289 and *Penicillium* sp. FO-4259, respectively.

The efficient and concise total synthesis of bioactive α-pyrone meroterpenoids, pyripyropenes A and E and arisugacins A, B, F, and G, has been successfully performed and their structure−activity relationships qualified. It is believed that further studies would enable the development of novel therapeutic drugs for life-style related diseases, such as hypercholesterolemia, and Alzheimer's disease.

6. Acknowledgments

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