SYNTHESIS OF PALMEROLIDE A, SACROLIDE A, AND ASPERGILLIDE D

A SYNOPSIS

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in

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By

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SYNOPSIS

The Thesis entitled "**SYNTHESIS OF PALMEROLIDE A, SACROLIDE A, AND ASPERGILLIDE D**" has been divided into five chapters.

CHAPTER I: This chapter describes the introduction, previous synthetic approaches and present work on the synthesis of the C1–C15 fragment of Palmerolide A *via* protecting group dependent RCM reaction.

CHAPTER II: This chapter describes the introduction, previous synthetic approaches and present work on formal total synthesis of Palmerolide A.

CHAPTER III: This chapter includes introduction, Nozaki-Hiyama-Kishi reaction as strategies of macrocyclizations for natural product synthesis and the total synthesis of Sacrolide A by following a Nozaki-Hiyama-Kishi macrocyclization strategy.

CHAPTER IV: This chapter covers introduction, employment of Shiina macrocyclization reaction as the pivotal step in natural product synthesis and present work on a second generation total synthesis of Sacrolide A.

CHAPTER V: This chapter covers introduction, biological enhancement of isolated natural products and the current work on a total synthesis of the proposed structure of aspergillide D.

CHAPTER I: Synthesis of the C1–C15 fragment of palmerolide A via protecting group dependent RCM reaction.

Baker and co-workers isolated Palmerolide A (Figure 1), a complex polyunsaturated macrolide with an impressive molecular architecture

and biological profile, from the circumpolar marine tunicate *Synoicum* adareanum, which is commonly found in the shallow waters around Anvers Island on the Antarctic peninsula. This marine natural product found to exhibit excellent antitumor activity against a number of cell lines in the 60 cell panel of the National Cancer Institute (NCI). Specifically, it exhibits potent cytotoxicity against the melanoma cell lines UACC-62 (LC_{50} = 18 nM). Palmerolide A appears to act on melanoma cells through inhibition of vacuolar ATPase with an IC_{50} of 2 nM. The remarkable 10^3 *in vitro* selectivity index for the melanoma cells over other most sensitive cell lines tested prompted further biological evaluation of the compound. The impressive biological properties of Palmerolide A, along with its extremely limited supply

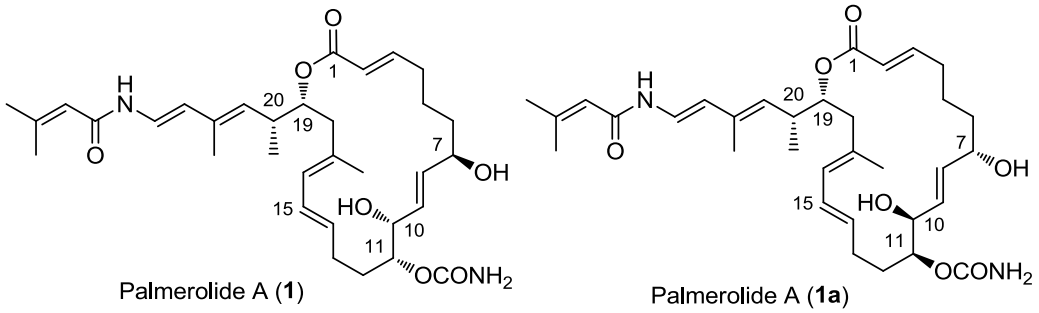


Figure 1. Structures of originally proposed (**1**) and revised (**1a**) Palmerolide A

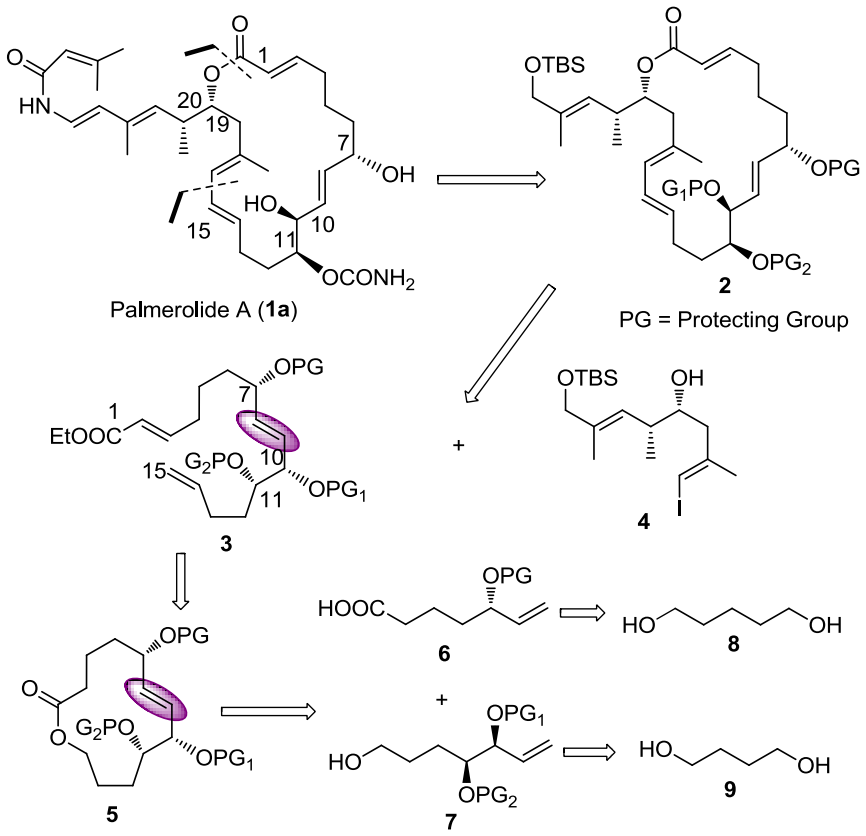
and for further structure-activity studies, prompted us to undertake its chemical synthesis. Considering all the previous synthetic reports, we envisioned a distinct retrosynthesis of fragment **3** (Scheme 1) that would take advantage of protecting group dependent ring-closing metathesis reaction developed in our group.

As part of our ongoing research program on the synthesis of

biologically active natural and unnatural products using protecting group dependent ring-closing metathesis approach, we became interested in the synthesis of C1-C15 fragment of Palmerolide A.

Retrosynthetic analysis:

According to our retrosynthetic analysis of Palmerolide A (**1a**) shown in Scheme 1, macrolactone core **2** could be constructed through

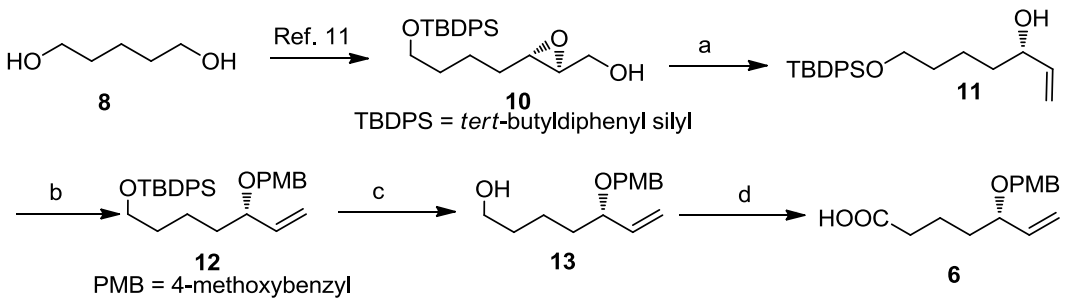


Scheme 1: Retrosynthetic analysis of Palmerolide A (**1a**).

esterification of **3** and **4**, followed by intramolecular Heck coupling. Fragment **3** could be obtained from the 13-membered macrolactone **5**, which in turn could be prepared from **6** and **7** via coupling, followed by ring-closing metathesis reaction of the resulting diene compound. Both the coupling partners **6** and **7** could be prepared from 1,5 pentane diol and 1,4 butane diol respectively.

Synthesis of the acid fragment 6:

The synthesis of acid fragment **6** was achieved following four synthetic steps from a known starting epoxide alcohol **9** as illustrated in Scheme 2.

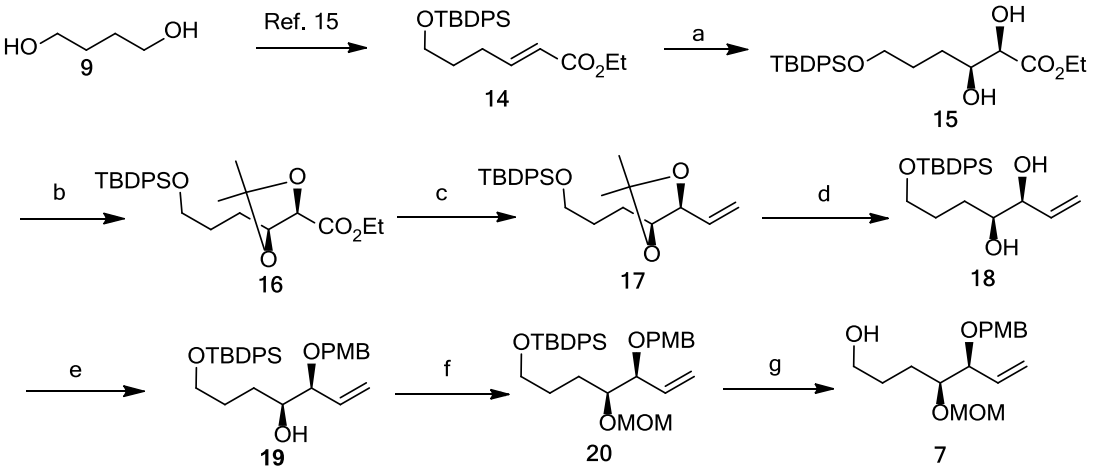


Scheme 2: Synthesis of the acid fragment **6.**

Scheme 2. Reagents and conditions: (a) (i) I_2, PPh_3, imidazole, THF, 0 ^oC, 10 min, 92%; (ii) Activated Zn, NaI, EtOH, reflux, 2 h, 89%; (b) PMB-Br, NaH, THF, 0 ^oC, 4 h, 91%; (c) TBAF, THF, rt, 4 h, 94%; (d) (i) IBX, DMSO, THF, rt, 3 h; (ii) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH, H_2O, rt, 2 h, 92%.

Synthesis of the alcohol fragment 7:

Preparation of alcohol fragment **7** involved ten steps sequences from a



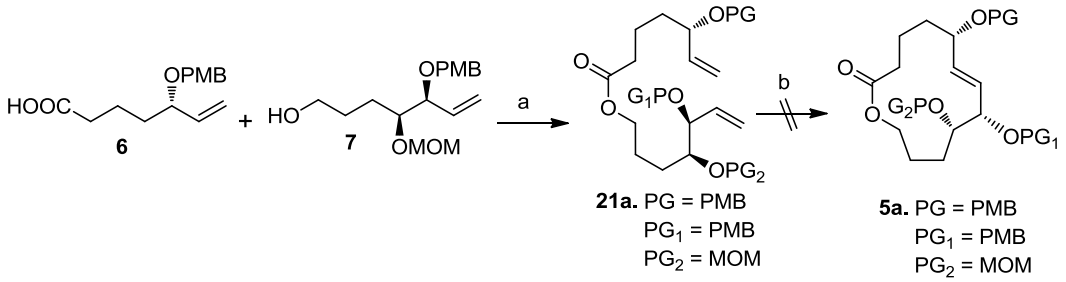
Scheme 3: Synthesis of the alcohol fragment **7.**

Scheme 3: Reagents and conditions: (a) AD-mix-α, $MeSO_2NH_2$, K_2CO_3, $K_3Fe(CN)_6$, *t*-BuOH:H_2O (2:1), OSO_4, 24 h, 0 °C, 89%; (b) 2,2-DMP, CH_2Cl_2, CSA, rt, 3 h, 92%; (c) (i) DIBAL-H, CH_2Cl_2, 0 °C, 30 min; (ii) DMP, CH_2Cl_2, rt, 2 h; (iii) $(PPh_3\text{-}CH_3)^+Br^-$, NaHMDS, THF, −78 °C to 0 °C, 4 h, 70% yield over three steps; (d) AcOH:H_2O (3:2), 50 °C, 3 h, 87%; (e) PMBBr, NaH, THF, 0 °C, 4 h, 91%; (f) MOMCl, *i*-Pr_2EtN, rt, 2 h, 85%; (g) TBAF, THF, rt, 4 h, 95%.

known starting α,β-unsaturated ester **14** via Sharpless asymmetric dihydroxylation, chemoselective deprotection of acetonide group in preference to TBDPS group and regioselective masking of secondary allylic alcohol (Scheme 3). Protecting group manipulation of compound **19** afforded the requisite alcohol fragment **7** in quantitative yield.

Unsuccessful attempt of the RCM reaction:

The resulting diene synthesized from the both coupling partners was failed to produce the requisite 13-membered macrolactone due to



Scheme 4: Formation of the coupling product **21**a followed by unsuccessful attempt of RCM reaction (**5a**).

Scheme 4: Reagents and conditions: (a) EDCI, DMAP, CH_2Cl_2, 0 °C to rt, 10 h, 86%; (b) Grubbs' 2nd generation catalyst, CH_2Cl_2, reflux, no reaction.

steric congestion due to bulky PMB-protecting group around the reacting centers which requires manipulation in protecting group.

At this juncture, we thought of investigating the above reaction with a set of ring-closing metathesis precursors by only changing the protecting group of allylic alcohol. The outcome of the reactions were

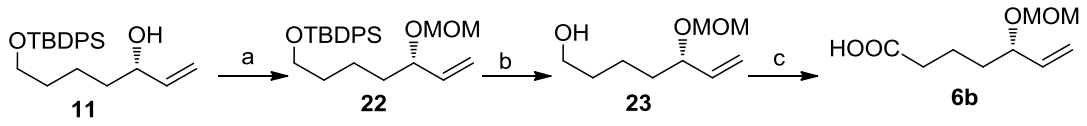
Table 1: Protecting group dependent RCM for 13-membered lactone

Sl. No.	PG	PG_1	PG_2	Duration (in hour)	RCM (Yield %)	Starting Material Recovery (%)
5a	PMB	PMB	MOM	24	0	100
5b	Bn	Bn	MOM	24	0	100
5c	TBS	TBS	MOM	24	0	100
5d	MOM	MOM	MOM	12	70	20
5e	MOM	MOM	MOM	36	78	0
5f	H	H	MOM	12	82	

presented in Table 1. The excellent yield in case of **5f**, could be due to forbearance of Grubbs' catalyst with activated nucleophile in the form of free allylic alcohol group (Table 1).

Protecting group manipulation in the synthesis of acid fragment 6b:

The synthesis of modified acid fragment **6b** involved the same synthetic sequences starting with MOM-protection of the allylic alcohol **10**.

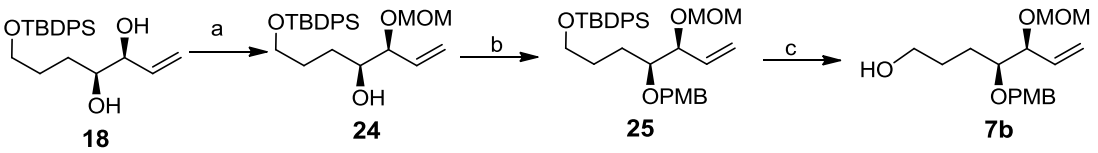


Scheme 5: Synthesis of the acid fragment **6b.**

Scheme 5: Reagents and conditions*: (*a) MOMCl, *i*-Pr_2Et N, 2 h, rt, 91%; (b) TBAF, THF, rt, 4 h, 93%; (c) (i) IBX, DMSO, THF, rt, 3 h; (ii) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH, H_2O, rt, 2 h, 81% (over two steps).

Protecting group manipulation in the synthesis of alcohol fragment 7b:

The synthesis of the modified alcohol fragment **7b** involved the same



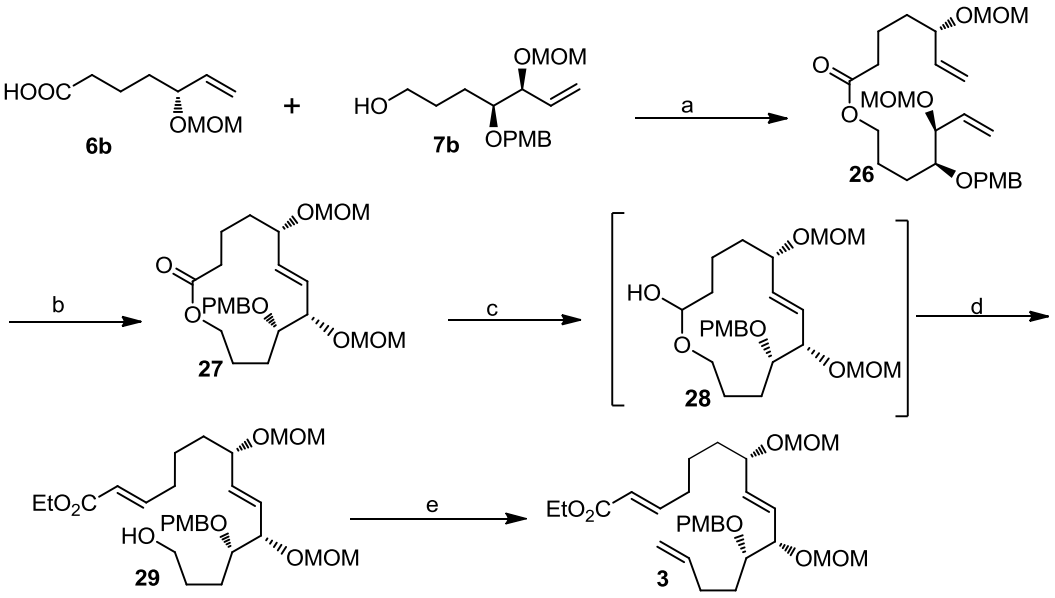
Scheme 6: Synthesis of the alcohol fragment **7b.**

Scheme 6: Reagents and conditions : (a) MOMCl, *i*-Pr_2EtN, 2 h, 0 ^oC, 85%; (b) PMB-Br, NaH, THF, 0 ^oC, 6 h, 91%; (c) TBAF, THF, rt, 3 h, 94%.

synthetic sequences starting with regioselective protection of the allylic alcohol with MOM protecting group of the diol **18**.

Synthesis of the required C1-C15 fragment (3) of palmerolide A (1a):

The synthesis of C1-C15 fragment **3** of Palmerolide A (**1**) has been achieved by following another four step sequences from acid **6** and alcohol **7** via EDCI mediated esterification, ring-closing metathesis followed by one carbon homologation (Scheme 7).



Scheme 7: Synthesis of the C1–C15 fragment of Palmerolide A

Scheme 7: Reagents and Conditions: (a) EDCI, DMAP, CH_2Cl_2, 0 ^oC to r.t.,10 h, 84%; (b) Grubbs' 2nd generation catalyst, CH_2Cl_2, reflux, 32 h, 75%; (c) DIBAL-H, CH_2Cl_2, −78 ^oC, 15 min; (d) $Ph_3P{=}CHCO_2Et$, benzene, reflux, 2 h, 77% over two steps; (e) (i) DMP, CH_2Cl_2, $NaHCO_3$, 0 ^oC to rt, 2 h; (ii) $(PPh_3\text{-}CH_3)^+Br^-$, *n*BuLi, THF, −78 ^oC to 0 ^oC, 3 h, 78% over two steps.

CHAPTER II: Formal total synthesis of Palmerolide A

Baker and co-workers in 2006 reported the isolation and structural determination of Palmerolide A (Figure 1), the most prominent

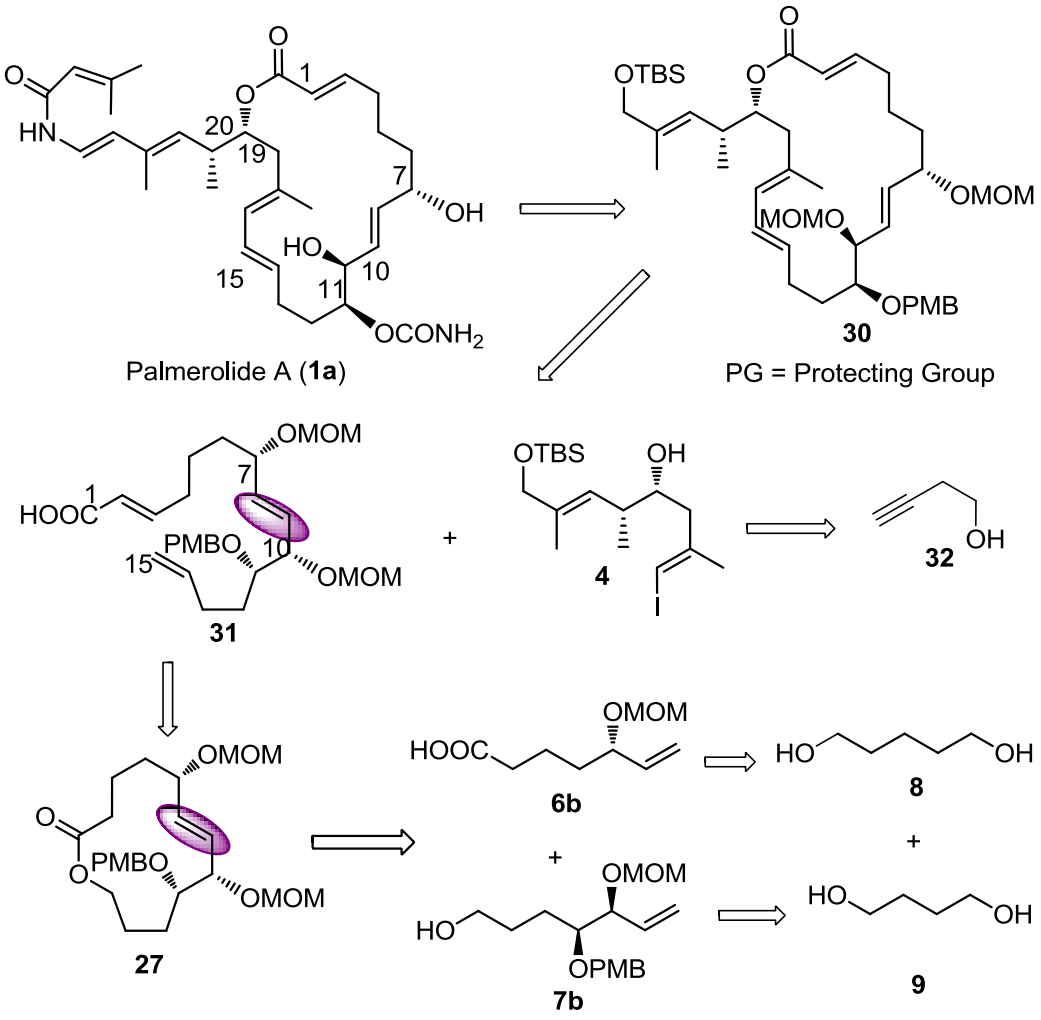
member of Palmerolide group, which is mainly due to its cytotoxicity, from the Antarctic marine tunicate *Synoicum adareanum*. Palmerolide A is a complex natural product comprising of a side chain containing an enamide, a 1,3-diene system, a carbamate moiety, five stereogenic centers, seven unsaturations with *E*-configuration and 20-membered macrolide ring. This natural product demonstrated extraordinary cytotoxic activity against the melanoma cell lines UACC-62 (LC_{50} = 18 nM) by inhibiting the proliferation of vacuolar ATPase with an IC_{50} of 2 nM. The remarkable 10^3 in vitro selectivity index for the melanoma cell lines prompted further biological evaluation of the compound. These findings coupled with the scarce natural abundance of Palmerolide A generated considerable interest in its chemical synthesis.

Considering all these synthetic reports, a distinct retrosynthesis of macrolactone core **30** including intramolecular Heck coupling, intermolecular Yamaguchi reaction, protecting group dependent ring-closing metathesis reaction for the building of fragment **31**, regioselective reductive opening of epoxide by Me_3Al and Takai olefination on methyl ketone for the construction of C16-C23 fragment was envisioned.

Retrosynthetic analysis:

According to the retrosynthetic analysis of Palmerolide A (**1a**) as shown in Scheme 1, macrolactone core **30** could be constructed through esterification of **31** and **4**, followed by intramolecular Heck coupling. Fragment **31** could be obtained from the 13-membered

macrolactone **27**, which in turn could be prepared from **6b** and **7b** via coupling, followed by ring-closing metathesis reaction of the resulting diene compound.



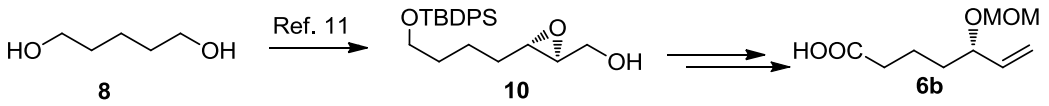
Scheme 8: Retrosynthetic analysis of Palmerolide A.

Synthesis of major acid fragment of Palmerolide A:

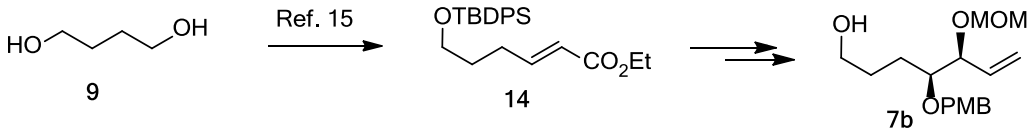
The synthesis of the major acid fragment **31** of Palmerolide A (**1a**) has been achieved by following another five steps sequences from acid **6b** and alcohol **7b** via EDCI mediated esterification, ring-closing metathesis and Wittig olefination. Synthesis of both the coupling partners acid **6b** and alcohol **7b** involved the same synthetic sequence, which was earlier discussed in chapter 1. The ester

functionality of **3** was saponified to produce the required acid **31**, which completes the synthesis of major acid fragment (Scheme 9).

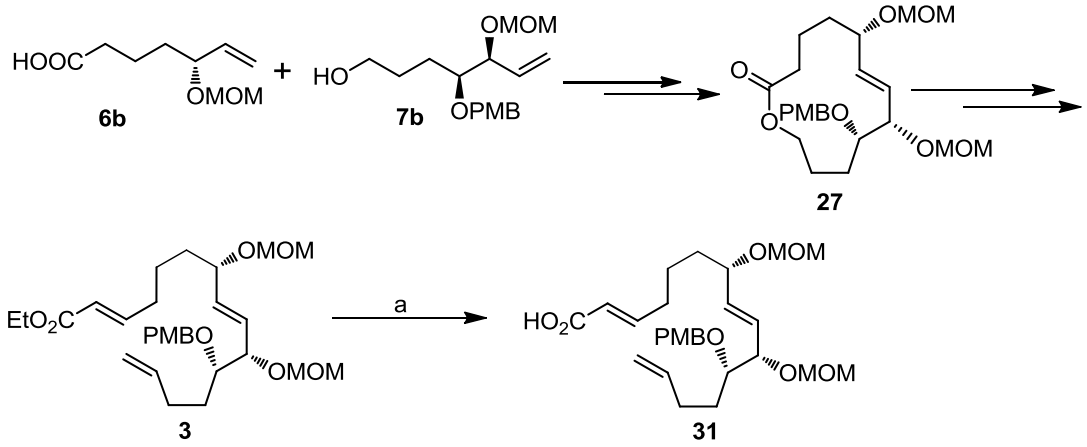
Synthesis of the acid fragment (6b)



Synthesis of the alcohol fragment (7b)



Synthesis of major acid fragment of palmerolide A (31)



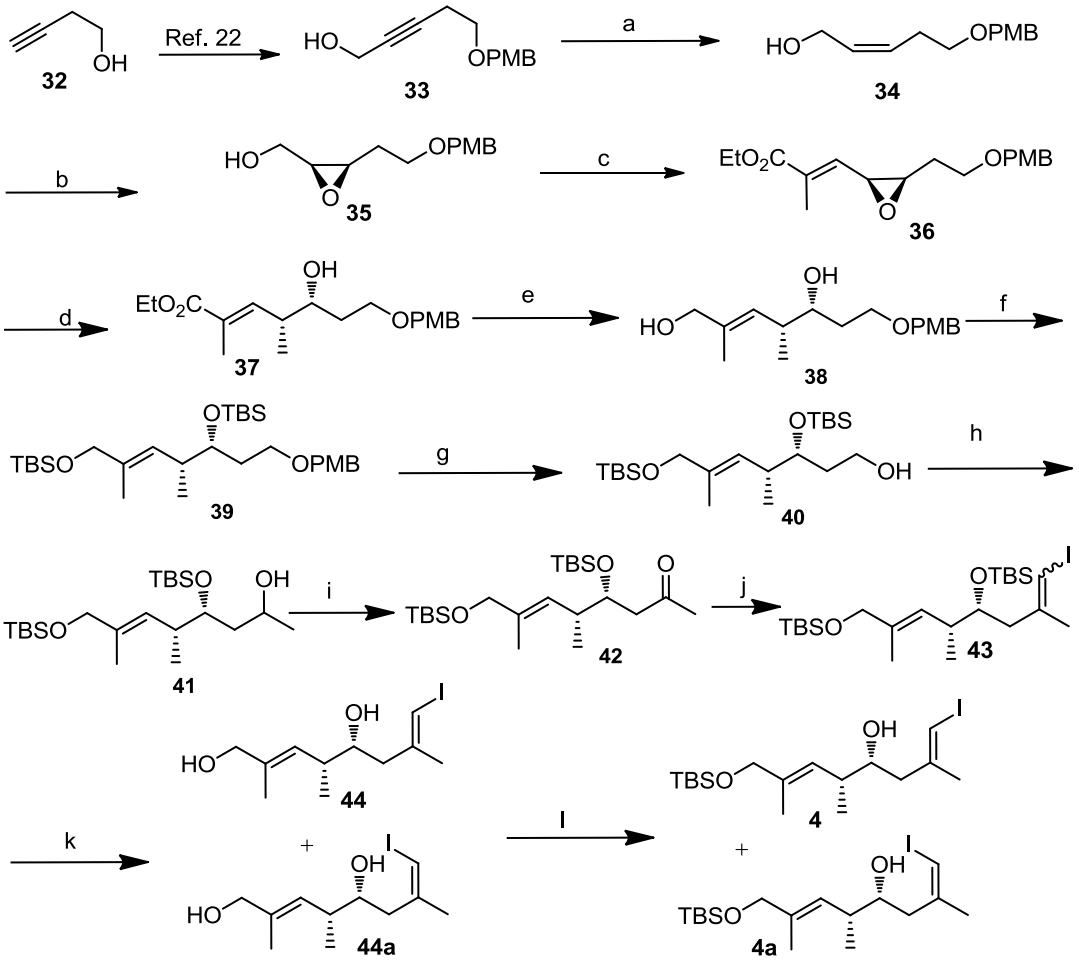
Scheme 9: Synthesis of the major acid fragment **31**.

Scheme 9: Reagents and conditions: (a) LiOH, THF:H_2O (3:2), reflux, 15 h, 95%.

Synthesis of the major alcohol fragment of Palmerolide A:

The major alcohol fragment **4** has been synthesized from a known compound **33** in 14 steps via Sharpless asymmetric epoxidation, regioselective reductive opening of epoxide in presence of Me_3Al and

introduction of *trans*-vinyl iodide under Takai olefination (Scheme 10).



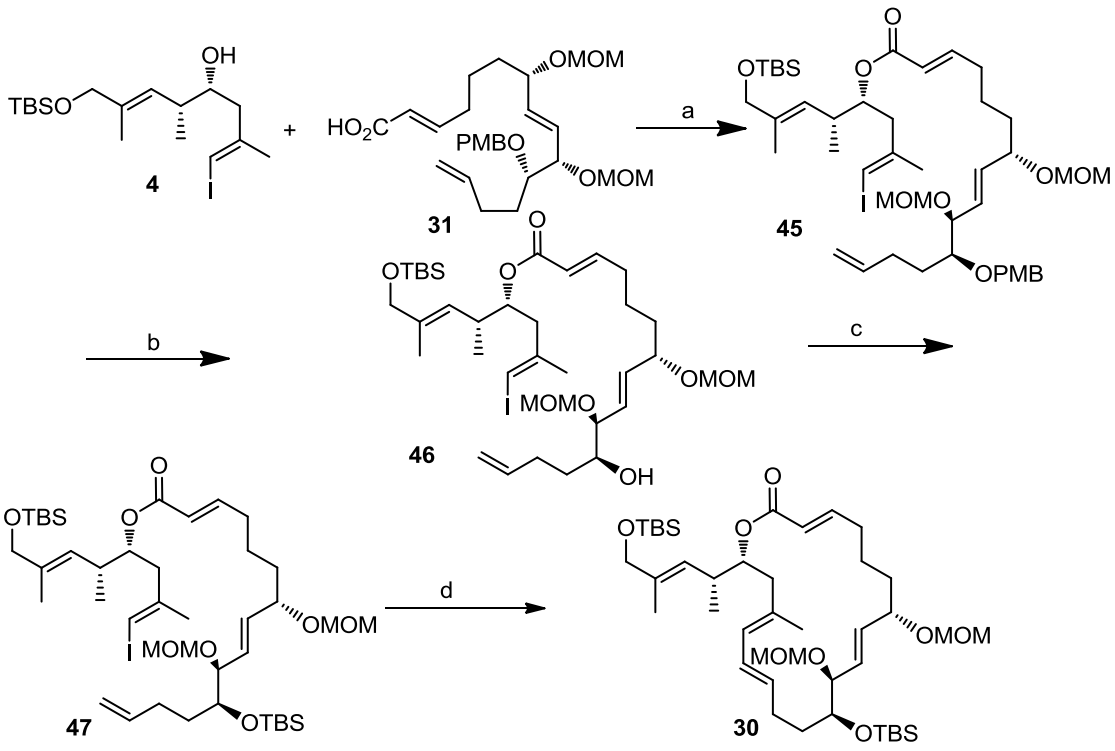
Scheme 10: Synthesis of the major alcohol fragment **4**.

Scheme 10: Reagents and conditions: (a) $Ni(OAc)_2$, $NaBH_4$, $NH_2(CH_2)_2NH_2$, EtOH, H_2, 2 h, 89%; (b) $Ti(O\textit{i}Pr)_4$, (+)DIPT, TBHP, MS (4 Å), CH_2Cl_2, 24 h, −23 °C, 96%; (c) (i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C, 4 h; (ii) $PPh_3{=}C(CH_3)CO_2Et$, benzene, 90 °C, 3 h, 68% over two steps; (d) Me_3Al, H_2O, CH_2Cl_2, −40 °C, rt, 3 h, 91%; (e) DIBAL-*H*, CH_2Cl_2, −78 °C, 1 h, 93%; (f) TBSOTf, 2,6-lutidine, CH_2Cl_2, 30 min, 0 °C, 93%; (g) DDQ, CH_2Cl_2:H_2O (19:1), pH = 7 buffer, rt, 1 h, 92%; (h) (i) DMP, CH_2Cl_2, 0 °C-r.t., 2 h; (ii) MeMgBr, THF, 0 °C, 2 h, 79% yield over two steps; (i) DMP, CH_2Cl_2, 0 °C-r.t., 3 h, 91%; (j) CHI_3, $CrCl_2$,

THF, rt, >3:2 (*E*:*Z*), 10 h, 78%; (k) TBAF, THF, rt, 6 h, 89%; (l) TBSCl, imidazole, CH_2Cl_2, 0 °C, 1 h, 92%.

Completion of the formal total synthesis of Palmerolide A.

The formal total synthesis of Palmerolide A (**1a**) has been accomplished by following four-step sequences from acid **31** and alcohol **4** via intermolecular Yamaguchi esterification and Heck macrocyclization (Scheme 11).

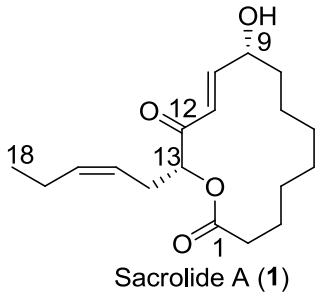


Scheme 11: Completion of the formal total synthesis of Palmerolide A.

Scheme 11: Reagents and conditions: (a) 2,4,6-Cl_3H_2COCl, Et_3N, DMAP, toluene, 4 h, 93%; (b) DDQ, CH_2Cl_2:H_2O (19:1), pH = 7 buffer, r.t., 30 min, 95%; (c) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 1 h, 96%; (d) $Pd(OAc)_2$, K_2CO_3, DMF, 80 °C, 2 h, 60%.

CHAPTER III: Total Synthesis of Sacrolide A by Following a Nozaki-Hiyama-Kishi Macrocyclization Strategy.

Sacrolide A (Figure 2), a new cytotoxic oxylipin 14-membered macrolide was isolated from the cyanobacterium *Aphanothece sacrum* by Igarashi and co-workers in 2014. *Aphanothece sacrum* is an endemic species, which is a grandeur ingredient for Japanese cuisine found in the Aso water system in the Kyushu District, Japan. Edible cyanobacteria *A. Sacrum*, which is a bioactive secondary metabolite possesses a wide antimicrobial spectrum and cytotoxicity. Compound **48** was shown to be a potent inhibitor for the growth of some species of Gram-positive bacteria, yeast *Saccharomyces cerevisiae* and fungus *Penicillium chrysogenum*. It also exhibits cytotoxic activity against 3Y1 rat fibroblasts with the GI_{50} 4.5 μM. Sacrolide A (**48**) contains two unsaturations that include vinylic ketone, a *cis*-configured double bond in the side chain, and two chiral centers. Sacrolide A, which was blessed with impressive molecular structure and interesting biological



Sacrolide A (**1**)

Figure 2. Structure of sacrolide A (**1**).

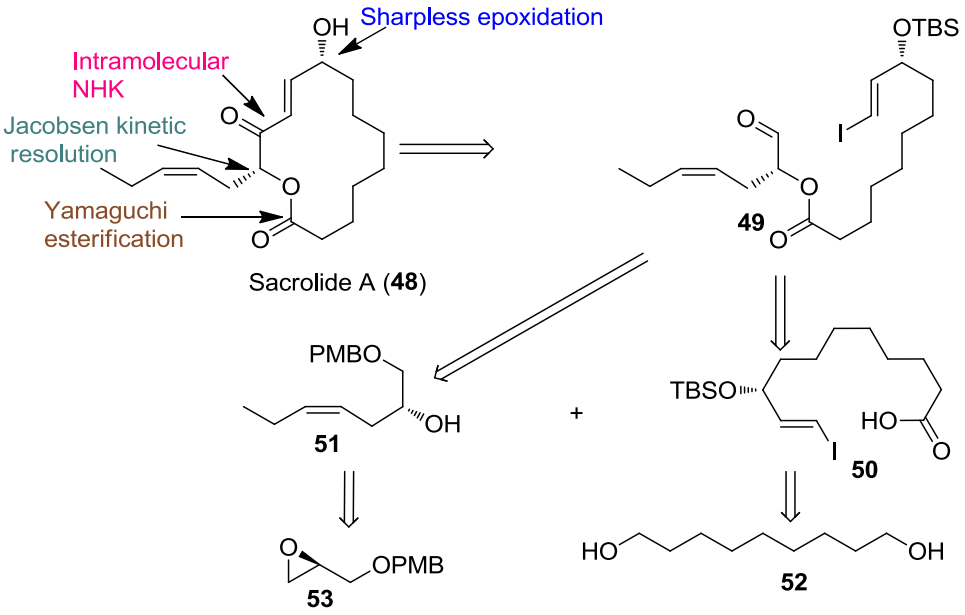
profile coupled with the scarce natural abundance of this macrolide (Class IA endangered species by the Ministry of Environment) has prompted us to undertake its chemical synthesis. Herein, we report

the first enantioselective total synthesis of Sacrolide A.

Many natural products, including macrolides with different ring size have been synthesized by employing intramolecular NHK coupling as the key macrocyclization step. Considering all these synthetic reports, we envisioned a distinct retrosynthesis of sacrolide A that would validate NHK macrocyclization strategy for 14 membered macrolide.

Retrosynthetic analysis:

According to our retrosynthetic analysis of sacrolide A (**48**) as shown in Scheme 12, macrolactone core of **48** could be constructed through yamaguchi esterification of **50** and **51**, followed by intramolecular Nozaki-Hiyama-Kishi reaction on vinylic iodide **49**. Accordingly, Alcohol fragment **51** would also be achieved by



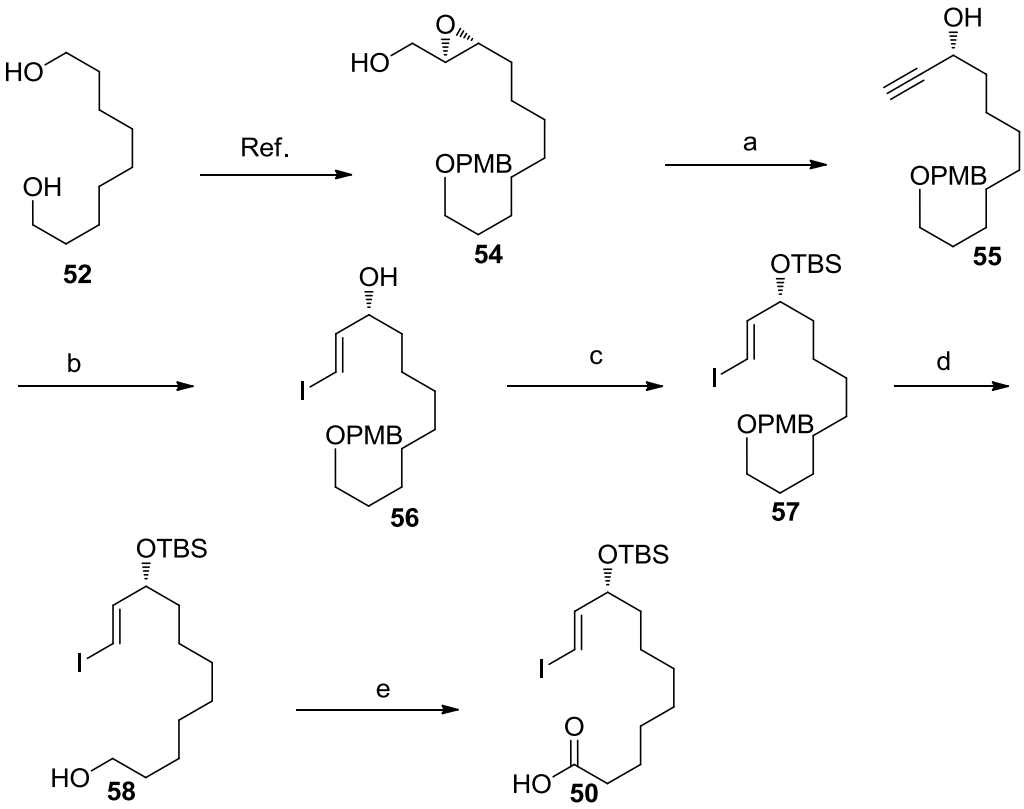
Scheme 12: Retrosynthetic analysis of Sacrolide A

a short sequence involving Jacobsen's hydrolytic kinetic resolution of PMB-protected glycidol (±) to establish the stereogenic centers at C-13. The acid fragment **50** could be obtained from the 1,9-nonane diol

through Sharpless asymmetric epoxidation followed by hydrozirconation-iodination sequence as the key steps. We report here the first enantioselective total synthesis of Sacrolide A highlighted by an intramolecular NHK coupling reaction.

Synthesis of the acid fragment 3:

The synthesis of the acid fragment **6** was achieved by following nine synthetic sequences from the commercially available 1,9-nonane diol



Scheme 13: Synthesis of acid frgament **50**

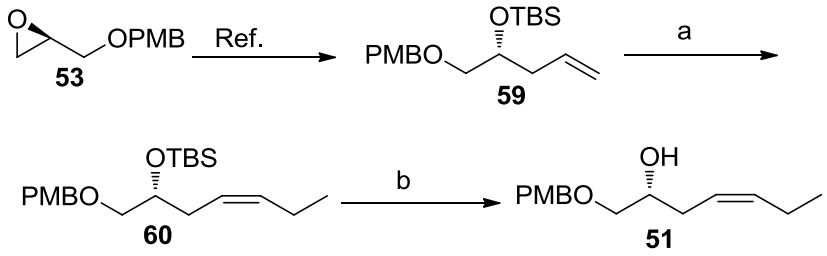
Scheme 13: Reagents and conditions: (a) (i) CCl_4, PPh_3, $NaHCO_3$, THF, reflux, 6 h, 92%; (ii) *n*BuLi, THF, −78 to 0 °C, 2 h, 86%; (b) Cp_2ZrCl_2, DIBAL-H, THF, 0 °C, 1 h, then I_2, THF, −78 to 0 °C, 30 min, 91%; (c) TBSCl, imidazole DMAP, CH_2Cl_2, 0 °C-r.t., 93 %; (d) DDQ, CH_2Cl_2:H_2O (19:1), pH = 7 buffer, r.t., 92%; (e) (i) $(COCl)_2$, DMSO,

Et_3N, CH_2Cl_2, -78 ^oC; (ii) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH, H_2O, rt, 2 h, 78% over two steps.

5 via Sharpless asymmetric epoxidation, *n*-BuLi induced opening of epoxy chloro compound followed by employment of hydrozirconation-iodination sequence on the resulting propargylic alcohol **8** illustrated in Scheme 13.

Synthesis of alcohol fragment 51:

Preparation of alcohol fragment **51** involved two step sequences from a known starting chiral PMB glycidol **53** via Jacobsen's hydrolytic kinetic resolution and incorporation of *cis*-geometry in the side chain with triphenylphosphonium salt of *n*-propylbromide in the presence of *n*BuLi under Wittig reaction condition (Scheme 14).

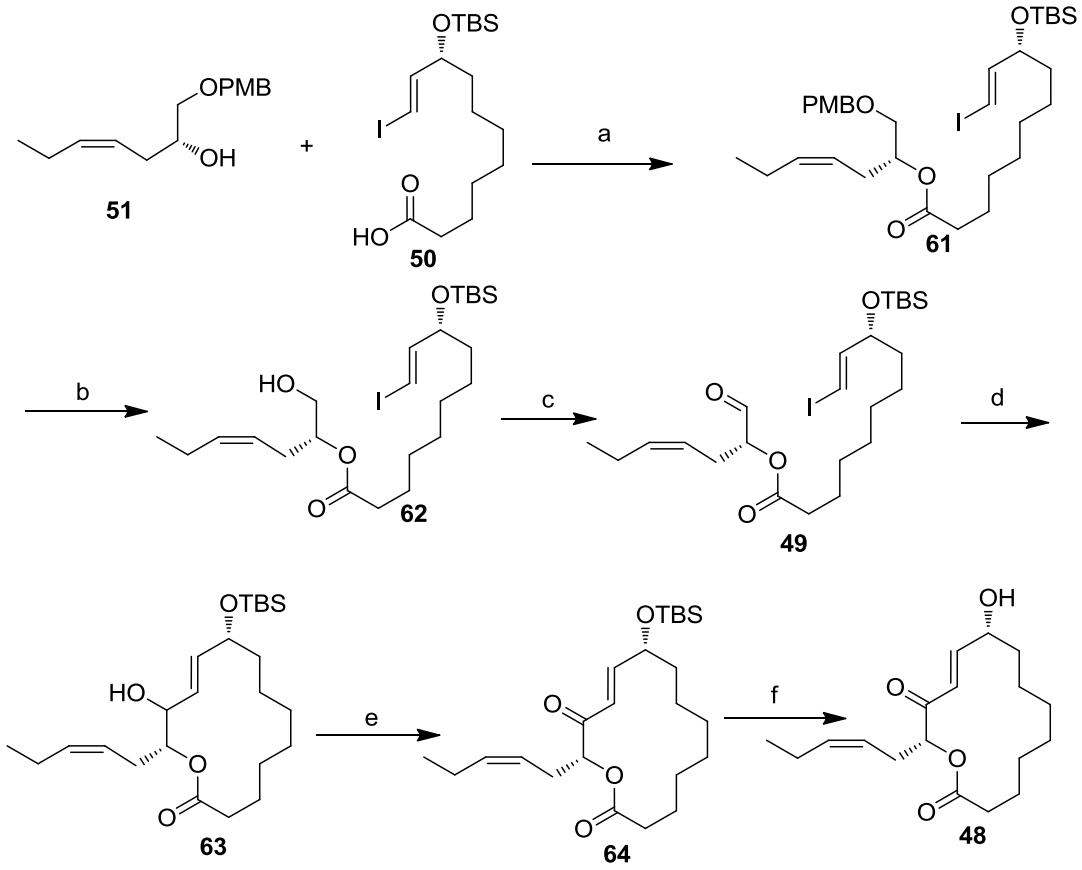


Scheme 14: Synthesis of alcohol frgament **51**

Scheme 14: Reagents and conditions: (a) (i)$NaIO_4$, OsO_4, 2,6-lutidine, 1,4-dioxane–H_2O (3:1), 0 °C, 3 h, 87%; (ii) $(PPh_3\text{-}CH_2CH_2CH_3)^+Br^-$, *n*BuLi, THF, −78 ^oC to 0 ^oC, 2 h, 72% yield over two steps (b) TBAF, THF, rt, 4 h, 90%.

Completion of the total synthesis of Sacrolide A (48): The total synthesis of sacrolide A (**48**) has been achieved by following six step sequences from acid **50** and alcohol **51** via Yamaguchi esterification

and intramolecular Nozaki-Hiyama-Kishi reaction (Scheme 15).



Scheme 15: Completion of the total synthesis sacrolide A (**48**).

Scheme 15: Reagents and conditions: (a) 2,4,6-$Cl_3C_6H_2COCl$, Et_3N, DMAP, toluene, 89%; (b) DDQ, CH_2Cl_2:H_2O (19:1), pH = 7 buffer, r.t., 92%; (c) DMP, CH_2Cl_2, $NaHCO_3$, 0 ^oC to rt, 2 h; (d) $NiCl_2$,$CrCl_2$, DMSO, r.t., 6 h, 52% over two steps; (e) DMP, CH_2Cl_2, $NaHCO_3$, 0 ^oC to rt, 2 h, 78%; (f) HF.Py, THF, r.t., 7 h, 81%.

CHAPTER IV: A second generation total synthesis of sacrolide A

In 2014, Igarashi and co-workers reported the isolation of 14-membered oxylipin-derived cytotoxic macrolide from the cyanobacterium *Aphanothece sacrum*. *Aphanothece sacrum*, a luxury ingredient for Japanese cuisine exist as a macroscopic gelatinous floating colonies in fresh water discovered in the Kyushu District,

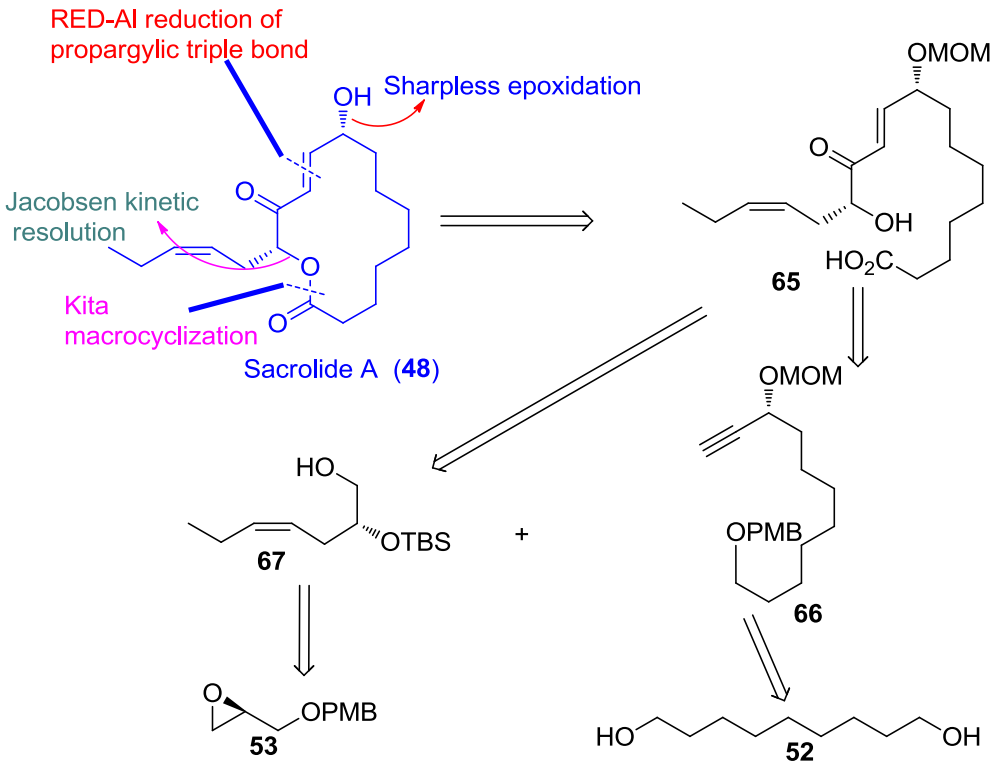
Japan. The absolute structures were confirmed through chiral anisotropy analysis and conformational analysis of different ring-opened derivatives through NOESY and HSQC experiments. Compound **48** possesses a wide antimicrobial spectrum, a potent inhibitor for the growth of few species of Gram-positive bacteria, yeast *Saccharomyces cerevisiae* and fungus *Penicillium chrysogenum* and cytotoxic activity against 3Y1 rat fibroblasts with GI_{50} 4.5 μM. Sacrolide A (**48**) embedded with α,β-unsaturated ketone, a *cis*-configured double bond in the side chain, and two chiral centers. Barred from overharvesting and water pollution due to its economical value coupled with increasing urbanization make Sacrolide A, a scarce source which could be procured by it's synthesis. Impressive molecular structure with sensitive functionality and modification in structure-activity relationship with biological activation of suitable derivatives in order to enhance biological profile coupled with the limited availability of large quantities of Sacrolide A (Class IA endangered species by the Ministry of Environment) has prompted us to undertake its chemical synthesis.

Several methods were addressed for esterification/lactonization strategy towards the formation of macrolide core of natural products on the basis of numerous acid activating reagent in presence of different acid and base catalyst to make the activated ester better electrophile for the alcohol. Yamaguchi and Shiina macrocyclization are the well-known (versatile) methods where high concentration of DMAP as acylation catalyst is required without which, reactions do

not work. High dose of 4-(dimethylamino) pyridine was problematic for base-sensitive substrates by inducting isomerization/epimerization. To circumvent the above problem, macrolactonization developed by Kita constitutes an effective method for unsaturated acids and enolizable alcohol *via* ruthenium-proton two stage esterification in presence of catalytic amount of acid catalyst. Barry M. Trost examined a set of precursors under acid catalyzed macrolactonization showing apt method for 14 and more members lactone. Considering all these synthetic reports, we envisaged a distinct retrosynthesis of Sacrolide A that would validate Kita macrocyclization strategy for 14-membered macrolide.

Retrosynthetic analysis:

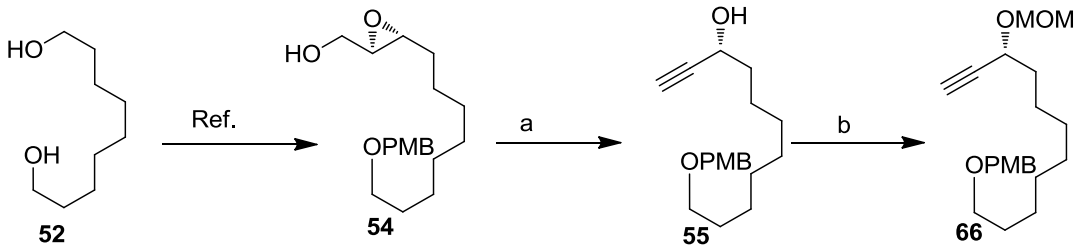
According to our retrosynthetic analysis of Sacrolide A (**48**) as shown



Scheme 16: Retrosynthetic analysis of sacrolide A (**48**)

in Scheme 16, macrolactone core of **48** could be constructed through nucleophilic addition of lithiated MOM-protected propargylic moiety to aldehyde **68**, followed by acid-catalyzed Kita macrocyclization of seco-acid **65**. Accordingly, alcohol fragment **67** would also be achieved by a short sequence involving Jacobsen's hydrolytic kinetic resolution of PMB-protected glycidol (±) **53** to establish the stereogenic center at C-13. The alkyne moiety **66** could be obtained from the 1,9-nonane diol (**52**) through Sharpless asymmetric epoxidation followed by base-induced opening of the epoxide ring as key steps.

Synthesis of the MOM-protected propargylic alcohol fragment 66:



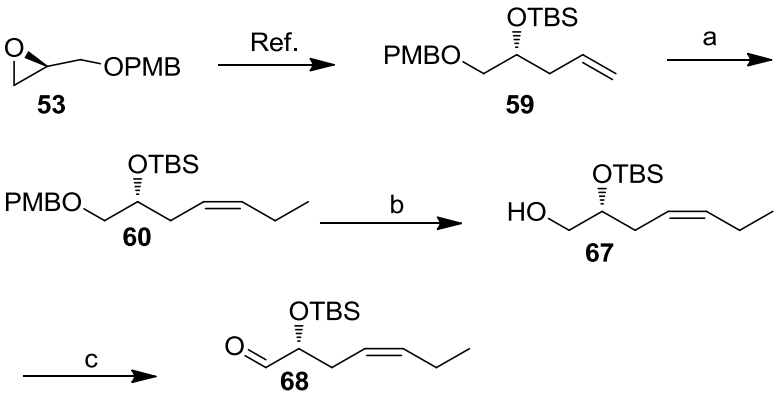
Scheme 17: Synthesis of the MOM-protected propargylic alcohol fragment **66**.

Scheme 17: Reagents and conditions: (a) (i) CCl_4, PPh_3, $NaHCO_3$, THF, reflux, 6 h, 92%; (ii) *n*BuLi, THF, −78 to 0 °C, 2 h, 86%; (b) MOMCl, *i*-Pr_2EtN, 2 h, 0 °C, 85%.

Preparation of MOM-protected propargylic alcohol fragment **66** involved three step sequences from a known starting epoxy alcohol **54** via Sharpless asymmetric epoxidation, formation of propargylic alchol **55** following Yadav's protocol and protection of resulting secondary alcohol as it's MOM-ether (Scheme 17).

Synthesis of aldehyde fragment 2:

Preparation of aldehyde fragment **68** involved three step sequences from a known starting chiral PMB glycidol **53** following Jacobsen's hydrolytic kinetic resolution and Wittig reaction with triphenylphosphonium salt of *n*-propylbromide to introduce *cis*-geometry in the side chain.

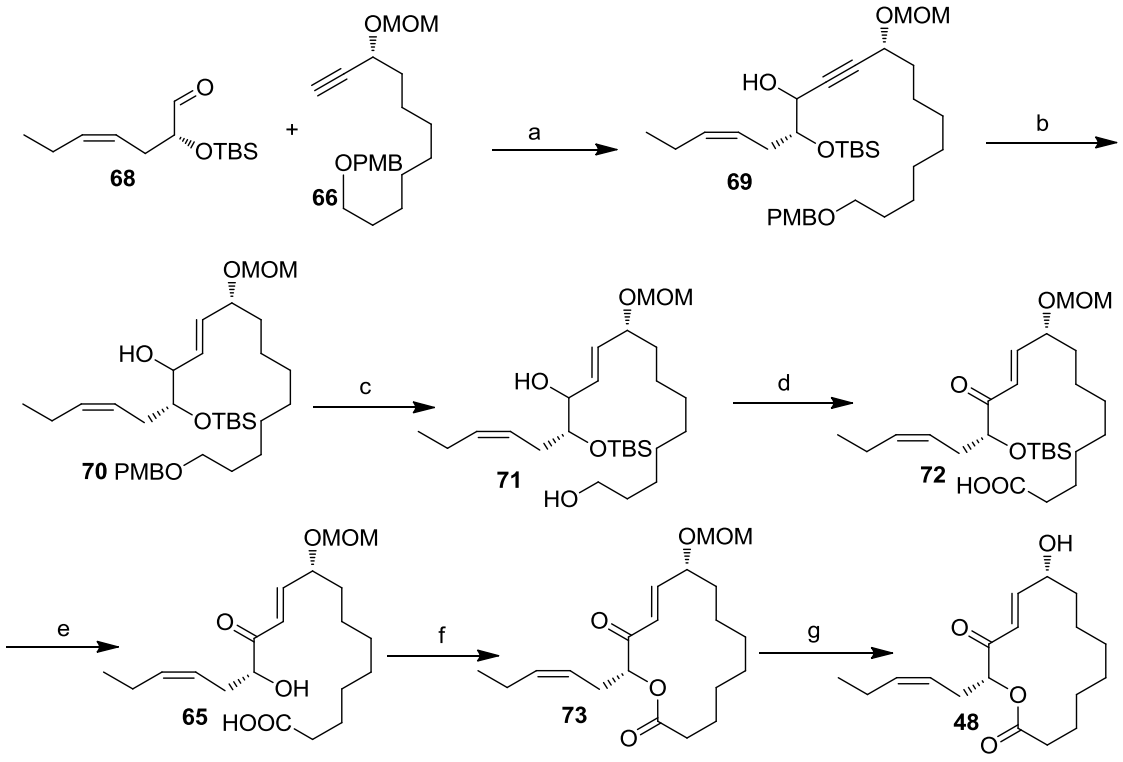


Scheme 18: Synthesis of aldehyde fragment **7.**

Scheme 18: Reagents and conditions: (a) (i) $NaIO_4$, OsO_4, 2,6-lutidine, 1,4-dioxane–H_2O (3:1), 0 °C, 3 h; (ii) $(PPh_3\text{-}CH_2CH_2CH_3)^+Br^-$, *n*-BuLi, THF, −78 °C to 0 °C, 2 h, 72% yield over two steps; (b) DDQ, CH_2Cl_2:H_2O (19:1), pH = 7 buffer, rt, 1 h, 92%; (c) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C, 4 h, 87%.

Completion of the second generation synthesis of Sacrolide A:

The total synthesis of Sacrolide A (**48**) has been achieved by following seven step sequences from both the coupling partners **67** and **68** via nucleophilic addition of lithiated MOM-protected propargylic moiety to aldehyde **68**, Red-Al mediated controlled reduction of internal triple bond to *trans*-double bond, followed by acid-catalyzed Kita



Scheme 19: Completion of the second generation total synthesis sacrolide A.

Scheme 19: Reagents and conditions: (a)) *n*-BuLi, HMPA, dry THF, -78 ^{o}C, 1.5 h, 74%; (b) Red-Al, THF, 4 h, -20 ^{o}C, 76%; (c) DDQ, CH_2Cl_2:H_2O (19:1), pH = 7 buffer, r.t., 92%; (d) (i) DMP, CH_2Cl_2, $NaHCO_3$, 0 ^{o}C to rt, 2 h; (ii) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH, H_2O, r.t., 2 h, 81% over two steps (e) HF.Py, THF, r.t., 8 h, 76%; (f) EtOCCH, [{$RuCl_2$(p-cymene)}$_2$], toluene, 0 ^{o}C to r.t., CSA, 50 ^{o}C, 35%; (g) TFA, CH_2Cl_2, r.t., 5 h, 82%.

macrocyclization of seco-acid **65** (Scheme 19). Macrolactonization of the resulting seco-acid **65** was first attempted under Shiina's protocol by treating with 2-methyl-6-nitrobenzoic anhydride (MNBA) as an acid activating reagent in presence of DMAP led to **13** in quantitative yield

(82%) but poor stereoselectivities (approximately 65:35). At this juncture, we envisioned that acid catalyzed macrolactonization of seco-acid **82** under kita protocol[39] as basic acylating catalyst DMAP inducted isomerisation.

CHAPTER V: Total synthesis of the proposed structure of aspergillide D.

Aspergillide D (**74**) (Figure 3), a 16-membered macrolide was isolated along with two polyketones, (*R*)-semivioxanthin (**75**), (*R*)-semixanthomegnin (**76**) and four alkaloids, 5-(1*H*-indol-3-ylmethyl)-imidazolidine-2,4-dionemarine (**77**), 9α,14-dihydroxy-6β-*p*-nitro-benzoylcinnamolide (**78**), 7α,14-dihydroxy-6β-*p*-nitro-benzoylconfertifolin (**79**), azonazine (**80**) from marine-derived fungus *Aspergillus* sp. SCSGAF 0076. Elucidation of the structure of aspergillide D was based on NMR with *E*-configuration of double bond from ^{1}H nmr (J = 16Hz), relative configuration by NOESY spectrum and mass spectra (HRESIMS: m/z 323.1828 $[M + Na]^{+}$). 14-membered and 16-membered macrolides isolated from fungi and bacteria generally displayed antibacterial activity but this new macrolide exhibited no antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*. Compounds **78** and **79** showed antiviral activity against H1N1 and H3N2, with IC_{50} values of 7.4 and 4.3 μM for **78** and IC_{50} values of 36.0 and 12.0 μM for **79** respectively. The structure-activity relationships revealed that the combination of lactone and side chain parts is important to the strong biological activity of natural products. Much of the structure-activity studies on

such molecules is gathered on the aglycons without attention to the

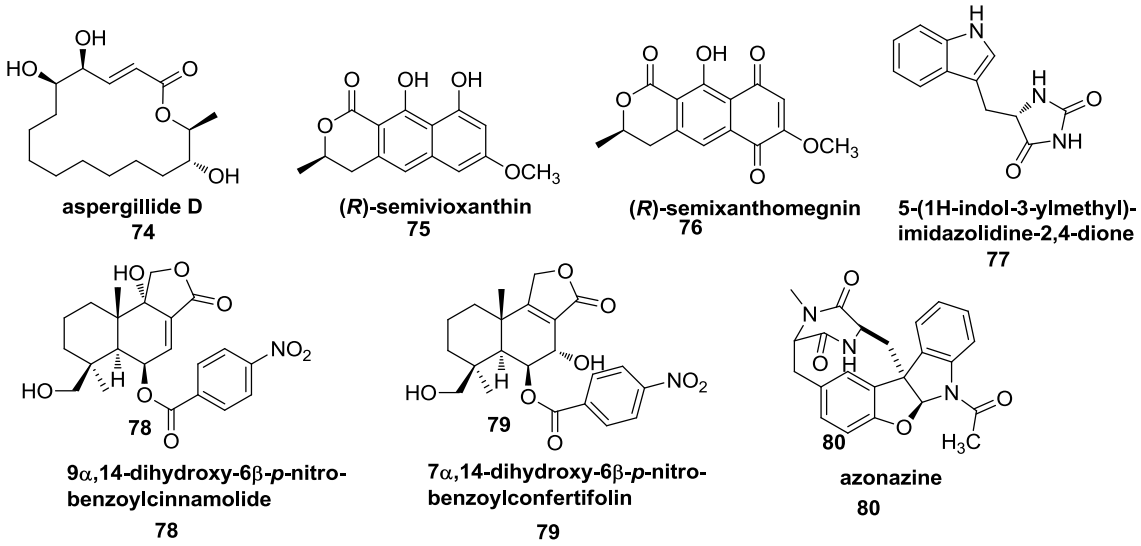
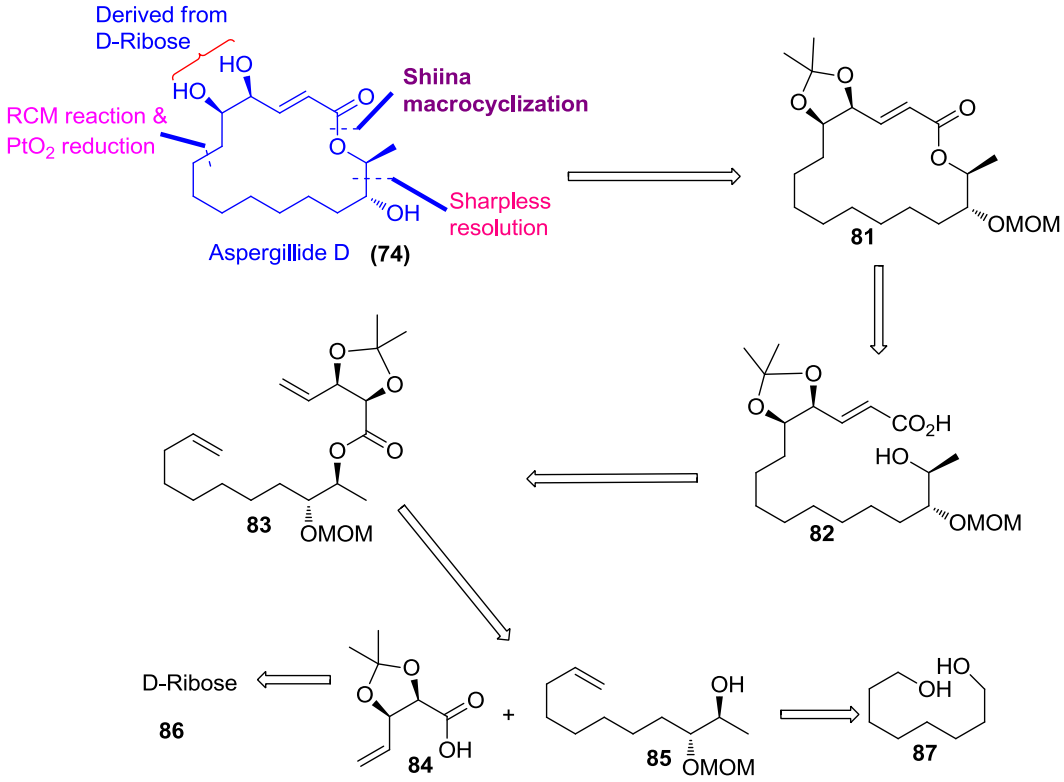


Figure 3. Structure of Aspergillide D (**74**) and different polyketones and alkaloids.

corresponding carbohydrate components. Here in we describe the total synthesis of aspergillide D, a 16-membered macrolactone following Shiina macrolactonization as the key step. The biological activities of aspergillide D has not been extensively explored, presumably due to the scarce natural abundance which can be recouped by total synthesis for further biological evaluation. The quest for improvement of biological significance of aspergillide D with further structure-activity studies, along with its extremely limited supply, prompted us to undertake its chemical synthesis.

Retrosynthetic analysis: According to the retrosynthetic analysis of aspergillide D (**74**) as shown in Scheme 20, the macrolactone core of aspergillide D could be synthesized from seco-acid **82** via intramolecular Shiina esterification. Seco-acid **82** could be obtained

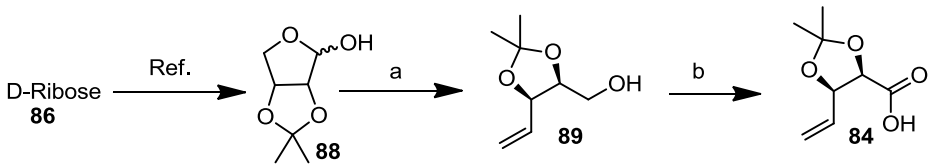


Scheme 20: Retrosynthetic analysis of aspergillide D (**74**).

from α,β-unsaturated ester **98** through two carbon homologation on the corresponding lactol of the 14-membered macrolactone **97** with controlled DIBAL-H reduction. 14-membered saturated macrolactone **97** could be constructed by ring-closing metathesis reaction of the resulting diene compound followed by PtO_2 reduction wich in turn could be prepared from **84** and **85** via Yamaguchi coupling. Acid fragment **84** would be obtained from lactol **88** by Wittig olefination, which in turn could be prepared from D-ribose by following the previous report whereas alcohol fragment **85** could be acessed from 1,8-octane diol **87** through Sharpless asymmetric resolution.

Synthesis of the acid fragment 84: The synthesis of acid fragment **84** was achieved following two synthetic sequences from a known starting material 2,3-acetonide **88** via Wittig olefination followed by

Swern and Pinnick oxidations as illustrated in Scheme 21.

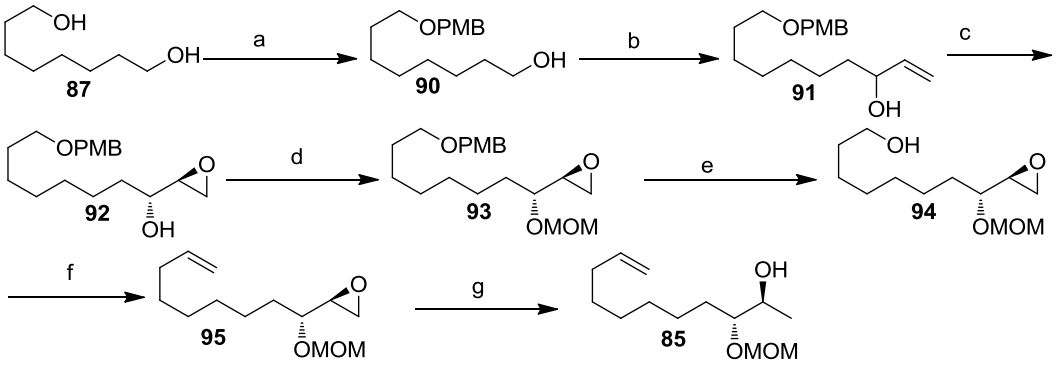


Scheme 21: Synthesis of the acid fragment **84.**

Scheme 21: Reagents and Conditions: (a) $(PPh_3\text{-}CH_3)^+Br^-$, *n*-BuLi, THF, 0 °C to 50 °C, 3 h, 81%; (b) TEMPO, BAIB, CH_2Cl_2:H_2O, r.t., 3 h, 82% over two steps.

Synthesis of the alcohol fragment 85:

The synthesis of alcohol fragment **85** was achieved following seven synthetic sequences from the commercially available 1,8-octane diol (**87**) via Sharpless asymmetric resolution, Wittig olefination and $LiAlH_4$



Scheme 22: Synthesis of the alcohol fragment **85.**

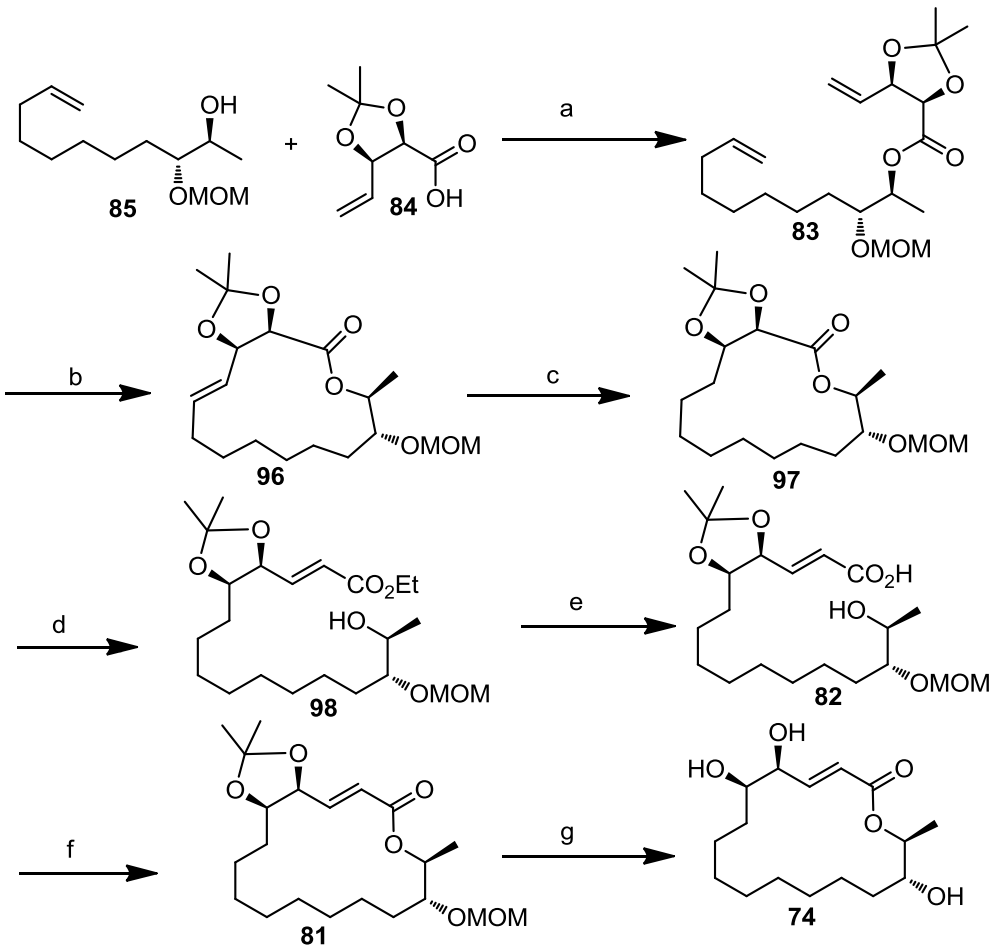
Scheme 22: Reagents and conditions: (a) PMB-OH, Amberlyst-15, CH_2Cl_2, reflux, 5 h, 89%; (b) (i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C, 4 h; (ii) CH_2=CHMgBr, THF, −20 °C, 3 h, 74% over two steps; (c) Ti(O*i*Pr)$_4$, (+)DIPT, TBHP, MS (4 Å), CH_2Cl_2, 24 h, −23 °C, 43%; (d) MOMCl, *i*-Pr_2EtN, 2 h, 0 °C, 85%; (e) DDQ, CH_2Cl_2:H_2O (19:1), pH = 7 buffer, rt, 1 h, 92%; (f) (i) DMP, CH_2Cl_2, 0 °C-r.t., 2 h; (ii) (PPh_3-

$CH_3)^+Br^-$, *n*-BuLi, THF, −78 °C 3 h, 65%; (g) $LiAlH_4$, THF, −10 to 0 °C, 1 h, 79%.

assisted region-selective epoxide opening as illustrated in Scheme 22.

Completion of the total synthesis of aspergillide D (74):

The total synthesis of aspergillide D (**74**) has been achieved by



Scheme 23: Completion of the total synthesis of aspergillide D (**74**).

Scheme 23: Reagents and Conditions: (a) 2,4,6-$Cl_3C_6H_2COCl$, Et_3N, DMAP, toluene, 65%; (b) Grubbs' 2nd generation catalyst, CH_2Cl_2, reflux, 10 h, 73%; (c) PtO_2, H_2, MeOH, 3 h, 81%; (d) (i) DIBAL-H, CH_2Cl_2, −78 °C, 15 min; (ii) $Ph_3P{=}CHCO_2Et$, benzene, reflux, 2 h, 61% over two steps; (e) LiOH, THF:H_2O (3:2), reflux, 10 h, 77%; (f) MNBA, DMAP, toluene, r.t., 12 h, 66%; (g) (i) $CuCl_2.2H_2O$, CH_3CN, r.t., 2 h,

70%; (ii) 4N HCl, THF, r.t., 6 h, 42%.

following another seven step sequences from acid **84** and alcohol **85** via Yamaguchi esterification, ring-closing metathesis, DIBAL-H mediated controlled reduction of lactone followed by Wittig olefination and Shiina macrolactonization reaction (Scheme 23). The protecting group of macrolactone core **81** was deproteced stepwise to complete the synthesis of aspergillide D (**74**).