Synthesis of Mycalolides, Actin-depolymerizing Trisoxazole Macrolides

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Table of contents

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List of publications

1. Synthesis and biological activities of the tris-oxazole macrolactone analogs of mycalolides
   M. Kita, H. Oka, A. Usui, T. Ishitsuka, Y. Mogi, H. Watanabe, H. Kigoshi
   Tetrahedron **2012**, *68*, 8753–8760.

2. Total synthesis of mycalolides A and B through olefin metathesis
   M. Kita, H. Oka, A. Usui, T. Ishitsuka, Y. Mogi, H. Watanabe, M. Tsunoda, H. Kigoshi
   Angew. Chem. Int. Ed. **2015**, *54*, 14174–14178.

List of abbreviations and acronyms

Ac	acetyl
Ala	alanine
aq.	aqueous
Bn	benzyl
Boc	*tert*-butoxycarbonyl
br	broad
Bu	butyl
c	concentration
ca.	circa
calcd	calculated
CM	cross metathesis
cm^{-1}	wavenumber(s)
Cy	cyclohexyl
d	doublet
DAST	*N*,*N*-diethylaminosulfur trifluoride
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
°C	degrees Celsius
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DMAP	*N*,*N*-dimethyl-4-aminopyridine
DMBOM	(3,4-dimethoxybenzyloxy)methyl
DME	1,2-dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
dr	diastereomeric ratio
EC_{50}	effective concentration 50%
EDC·HCl	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
ee	enantiomeric excess
Et	ethyl
et al.	et alia
eq	equivalent
ESI	electrospray ionization
g	gram(s)
Glu	glutamic acid
Gly	glycine
h	hour(s)
HOBt	1-hydroxybenzotriazole
HPLC	high performance liquid chlomatography
HRMS	high resonance mass spectroscopy
HWE	Horner–Wadsworth–Emmons
i	iso
IC_{50}	inhibitory concentration 50%
Ile	isoleucine
IR	infrared
L	liter(s)
Leu	leucine
LHMDS	lithium bis(trimethylsilyl)amide
M	molar
m	multiplet
µ	micro
*m*CPBA	*meta*-chloroperoxybenzoic acid
Me	methyl
Mes	mesityl
Met	methionine
min	minute(s)
mmu	milli mass unit
MNBA	2-methyl-6-nitrobenzoic anhydride
MS3A	molecular sieve 3 Å
MTT	3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
m/*z*	mass-to-charge ratio
n	normal
NHK	Nozaki–Hiyama–Kishi
NMO	*N*-methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance
PDB	protein data bank
Ph	phenyl
Phe	phenylalanine
Piv	pivaloyl
PMB	*para*-methoxybenzyl
ppm	parts per million
PPTS	pyridinium *para*-toluenesulfonate
Pr	propyl
PT	phenyltetrazole
q	quart = quartet
quant.	quantitative
RCM	ring-closing metathesis
ref.	reference
R_f	rate of flow
rt	room temperature
s	singlet
sat.	saturated
Ser	serine

sm	starting material
sp.	species
t	triplet
t	*tert* = tertiary
TBAF	tetra-*n*-butylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TCE	trichloroethyl
temp.	temperature
TES	triethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Thr	threonine
TLC	thin layer chlomatography
Tr	trityl = triphenylmethyl
Tyr	tyrosine
v/v	volume per unit volume
Val	valine
w/w	weight per unit weight

1. General introduction

1-1 Natural products chemistry

Nature is very fascinating even if we only look at it. The bright green of plants delights our eyes, and lovely behavior of animals heals our hearts. Furthermore, they bring us precious gifts to make our life more wealthy. We always have used the blessings of nature obtained from animals and plants as drugs, fragrances and dyes since long ago. Natural products chemistry began when we tried to elucidate active components of these materials from a viewpoint of chemistry. It has developed greatly from the early 19th century, then new pharmaceutical drugs were born from natural products. For example, an anti-inflammatory agent acetylsalicylic acid was synthesized based on the chemical structure of salicin isolated from willow, and an antibiotic penicillin G was discovered from the *Penicillium* fungi (Figure 1-1).[1]

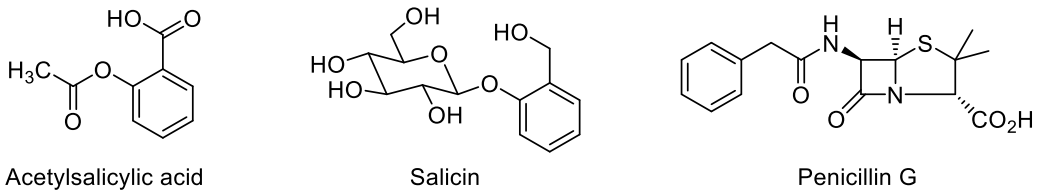


Figure 1-1. Structures of acetylsalicylic acid, salicin, and penicillin G.

Now, the field of natural products chemistry has diversified and progressed, which has enabled the discovery and synthesis of more complex molecules and the elucidation of functional mechanism of bioactive compounds. Representative results include the total synthesis of an anticancer drug paclitaxel[2a] isolated from the Pacific yew, elucidation of the activation mechanism of T-cells using an immunosuppressive drug FK506[2b] produced by a soil bacterium, and the functional elucidation of the sodium channel using a pufferfish neurotoxin tetrodotoxin[2c] (Figure 1-2). A lot of natural products have been continuously discovered from a variety of organisms. These compounds possess structural and functional diversity. Natural products chemistry takes an important role especially in bioscience.

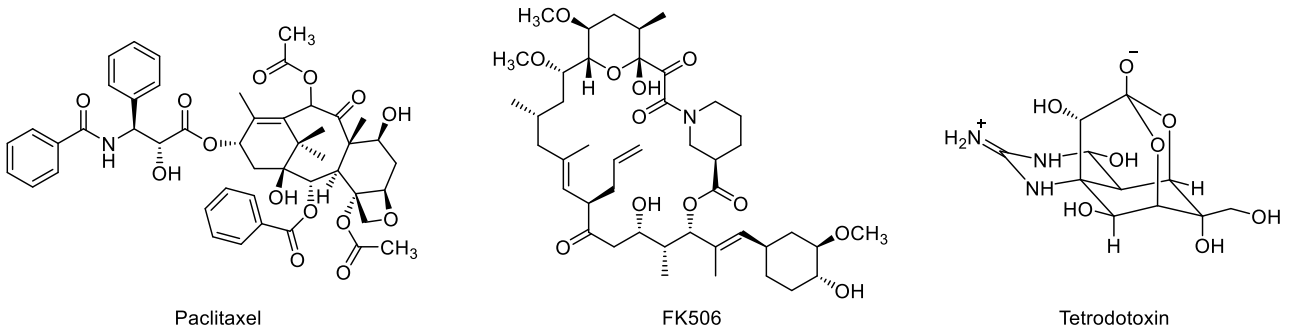


Figure 1-2. Representative natural products contributed to the development of bioscience.

1-2. Trisoxazole macrolides

Trisoxazole macrolides are cytotoxic and antifungal natural products discovered in marine invertebrate. These macrolides have a characteristic macrolactone ring (C1–C24) including three continuous oxazole units and a side-chain (C25–C35) with an *N*-methyl enamide terminus as common structures. The earliest report of these kinds of compounds were ulapualides, which were isolated by Scheuer et al. from the egg masses of a nudibranch *Hexabranchus sanguineus* collected at Pupukea, O'ahu in 1986 (Figure 1-3).[3] Around the same time, kabiramides were also reported by Fusetani et al. from the egg masses of the conspecific nudibranch *Hexabranchus* sp. collected at Kabira Bay, Ishigaki Island (Figure 1-4).[4] After these studies, halichondramides from the marine sponge *Halichondria* sp. and mycalolides from the marine sponge *Mycale* sp. were discovered (Figure 1-5 and 1-6).[5,6] Since then, more than 40 kinds of natural trisoxazole macrolide analogs have been found from the nudibranch *H. sanguineus*, and sponges of the genera *Halichondria*, *Jaspis*, and *Mycale*, and a stony coral of the genera *Tubastrea* (Figure 1-7 and 1-8).[7,8] These molecules have slight differences in oxidation patterns, alkyl substituent groups, and so on. For example, a part of halichondramides and mycalolides have C5–C7 α,β-unsaturated ketone and others are saturated ketones or Michael adducts. At the C30 position, these macrolides have ketone or secondary alcohols, or esters. Additionally, there are derivatives whose oxazole rings are oxidized and/or cleaved like kabiramides H and I. These structural differences provide the diversity of trisoxazole macrolides family.

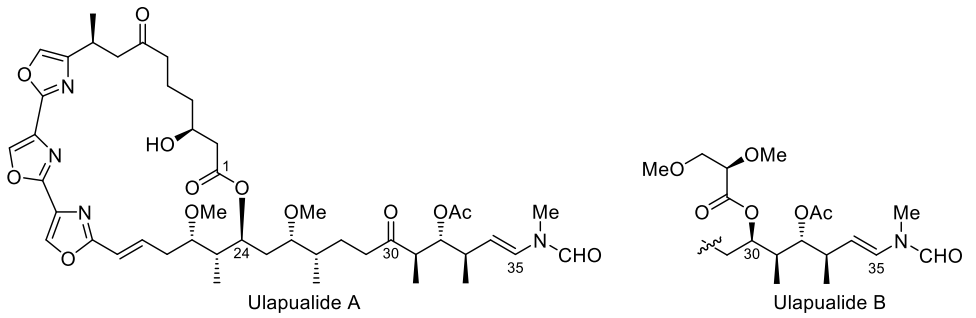


Figure 1-3. Structures of ulapualides.

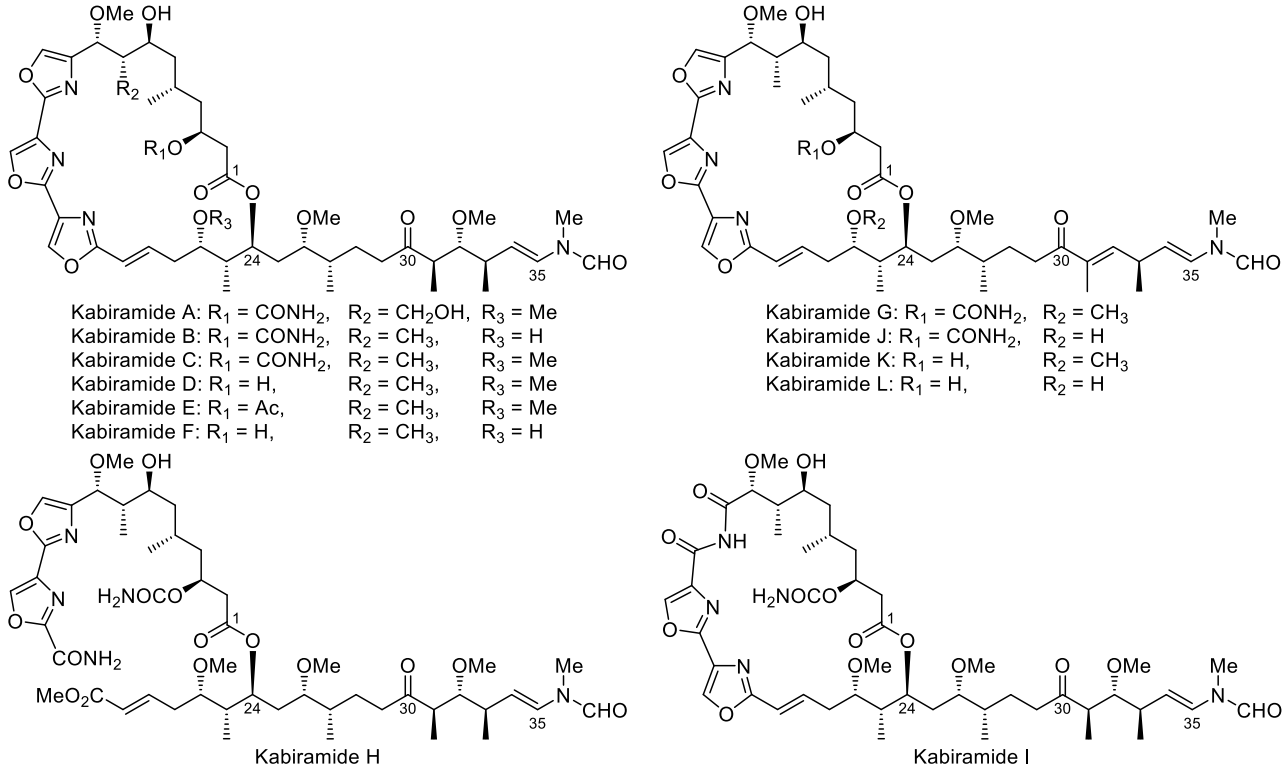
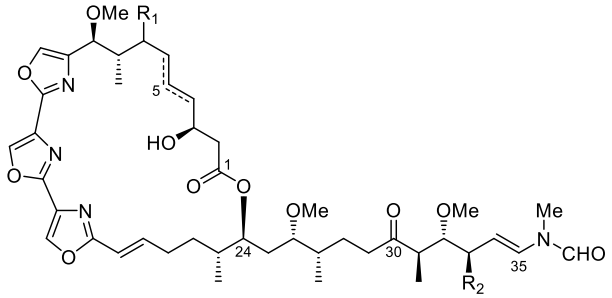
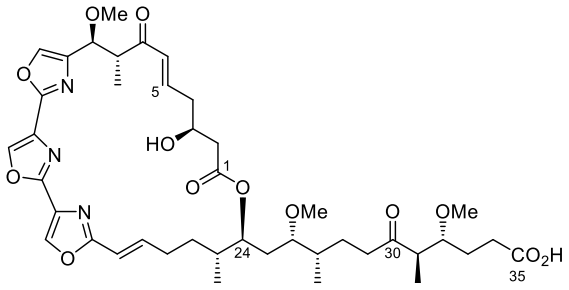


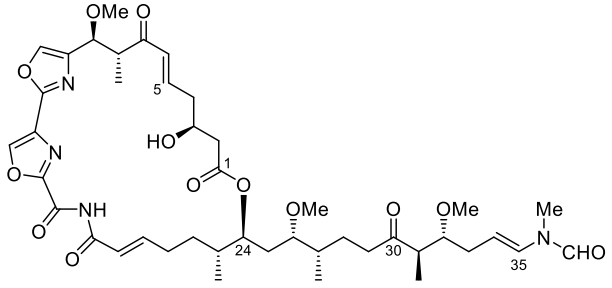
Figure 1-4. Structures of kabiramides.



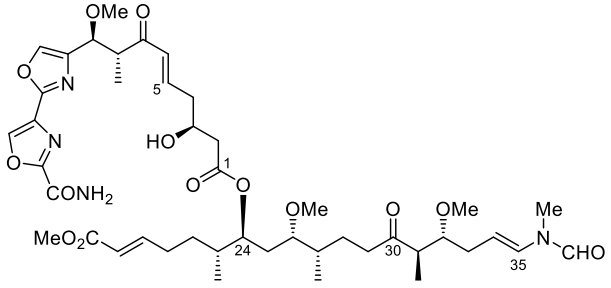
Halichondramide: R_1 = O, R_2 = H, $\Delta_{5,6}$
Isohalichondramide: R_1 = O, R_2 = H, (Z)-$\Delta_{5,6}$
Neohalichondramide: R_1 = O, R_2 = H, $\Delta_{4,5}$
Dihydrohalichondramide: R_1 = O, R_2 = H
Tetrahydrohalichondramide: R_1 = OH, R_2 = H
33-Methyldihydrohalichondramide: R_1 = O, R_2 = Me



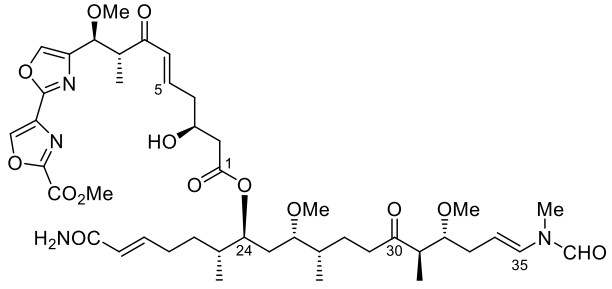
Halichondramide acid



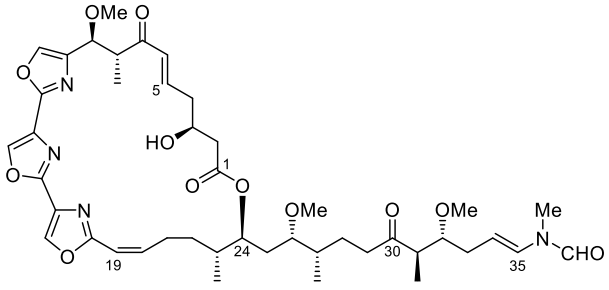
Halichondramide imide



Halichondramide ester



Secohalichondramide



(19Z)-Halichondramide

Figure 1-5. Structures of halichondramides.

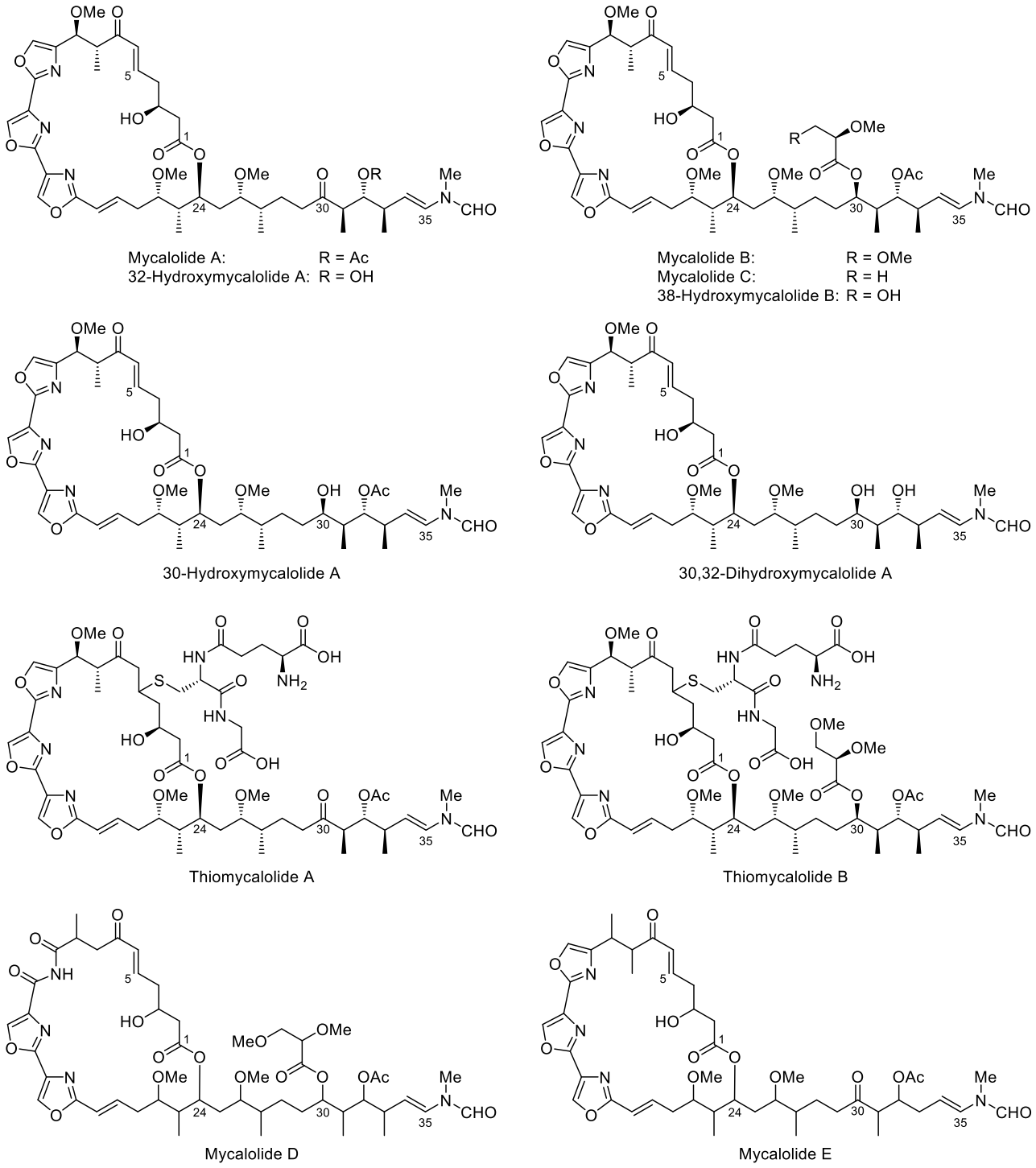


Figure 1-6. Structures of mycalolides.

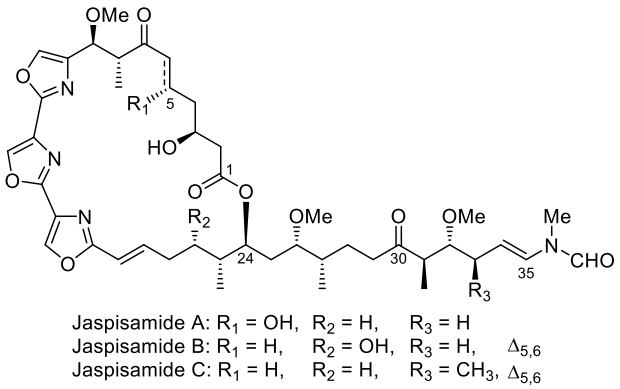


Figure 1-7. Structures of jaspisamides.

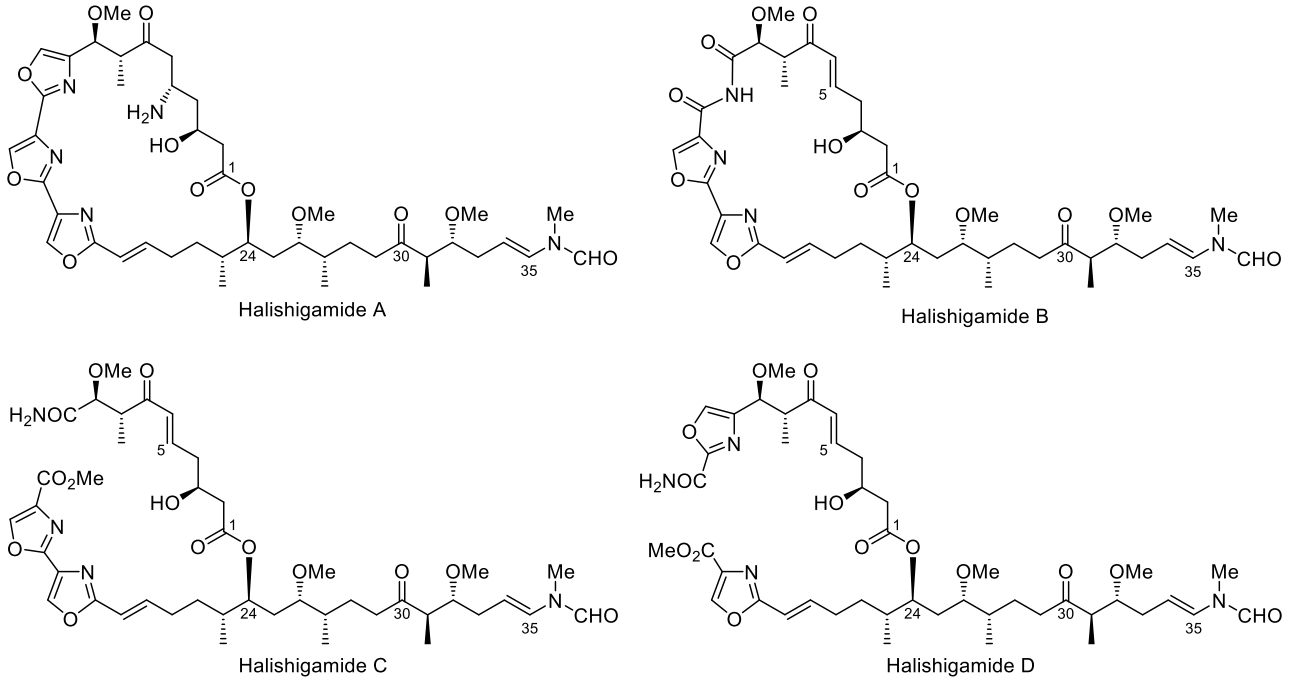


Figure 1-8. Structures of halishigamides.

In addition to unique chemical structures, trisoxazole macrolides show various and potent biological activities, such as antitumor, antifungal, and actin-depolymerizing activity. In particular, actin-depolymerizing activity of these compounds have been well studied.[9] Actin is a globular protein having four subdomains 1–4 (Figure 1-9).[10a] It exists universally in eukaryotic cells and is one of the three major components of the cytoskeleton. In cells, actin forms two structures that is a monomer called G-actin and a polymer called F-actin (Figure 1-10). The assembly of G-actin to form F-actin is reversible. G-actin assembles into F-actin at the barbed end in the part of subdomains 1 and 3, and F-actin dissociates at the pointed end in the part of subdomains 2 and 4. This actin dynamics repeating polymerization and depolymerization plays an important role in cellular functions such as cell motility, cell adhesion, and cytokinesis.[11]

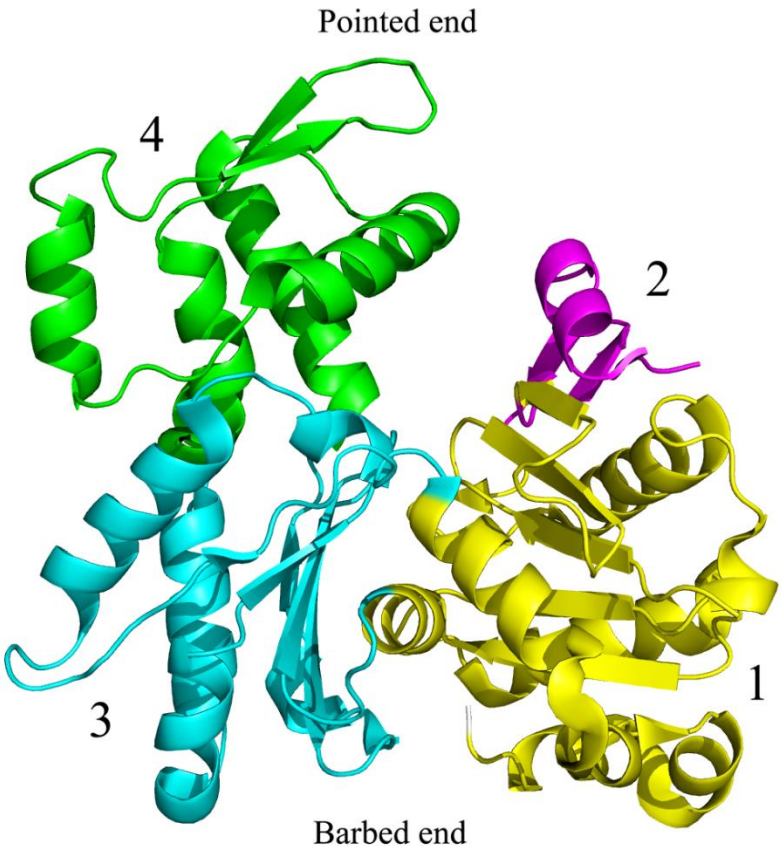


Figure 1-9. Structure of G-actin from a rabbit muscle (PDB[10b] ID: 3HBT). The subdomains are labelled: 1 (yellow), 2 (magenta), 3 (cyan), and 4 (green).

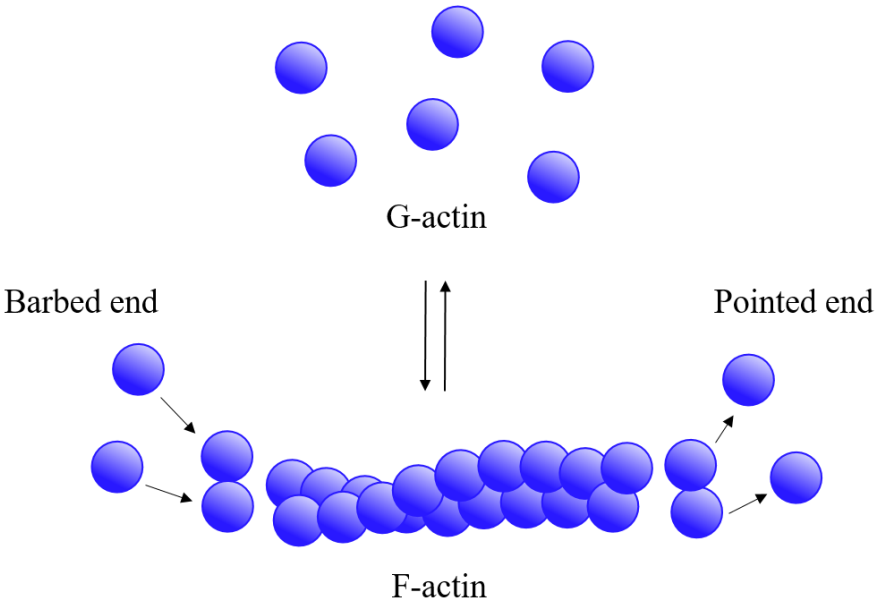


Figure 1-10. The model for actin dynamics.

As for the interaction of trisoxazole macrolides with actin, their binding mode and an actin-depolymerizing mechanism were clarified by the observation of their behavior against fluorescent-labeled actin and X-ray analyses of their actin complexes with several these macrolides.[9f–h] Trisoxazole macrolides highly specifically bind to actin by intercalating their side-chain moieties into the hydrophobic cleft between subdomains 1 and 3, and form a 1:1 complex. For example, the X-ray crystal structure of the actin–kabiramide C is shown in Figure 1-11.[9f] Because of this binding ability at the barbed end, trisoxazole macrolides strongly inhibit the polymerization of G-actin. Some trisoxazole macrolides also sever filamentous F-actin and cause depolymerization after they bind to a protomer in F-actin (Figure 1-12).[9] Due to such interactions with actin, it is thought that trisoxazole macrolides disturbs actin dynamics and finally cause cell death.[11c]

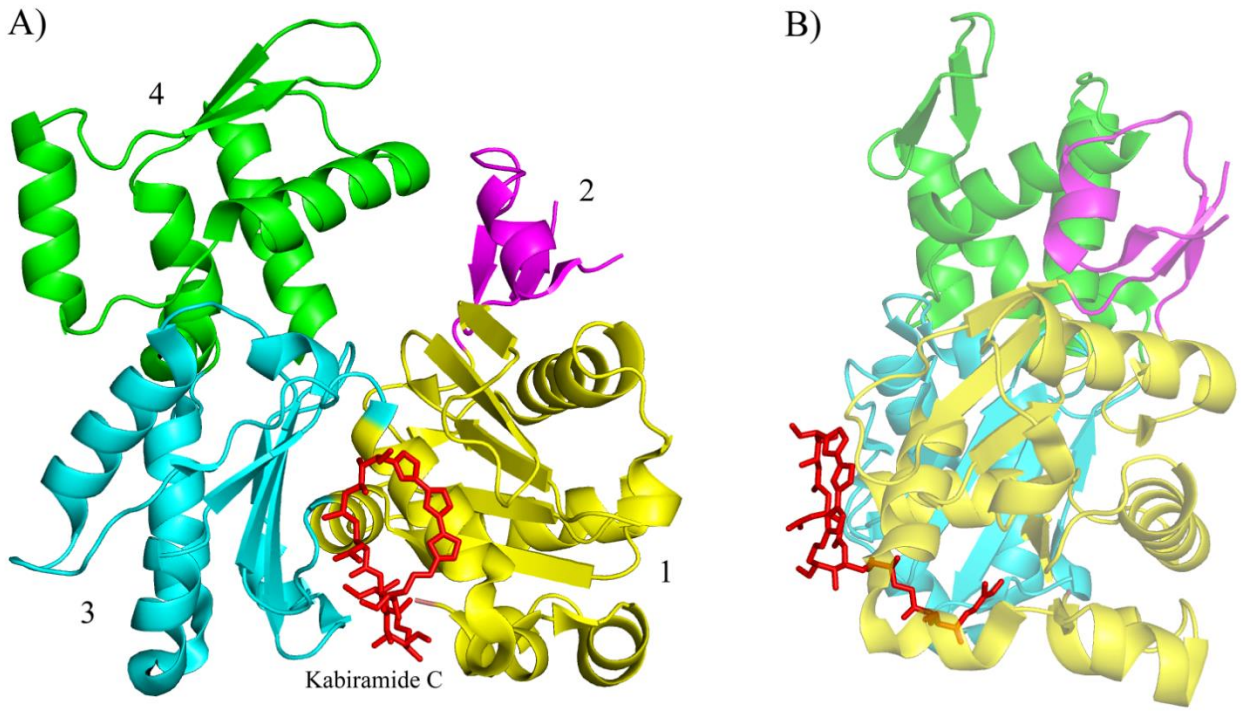


Figure 1-11. Crystal structure of the actin–kabiramide C complex (PDB ID: 1QZ5). Kabiramide C is shown as a stick model in red. A) Kabiramide C is binding to the cleft between the subdomains 1 and 3. B) The macrolactone ring is located on actin surface and the side-chain is inserted into the cleft.

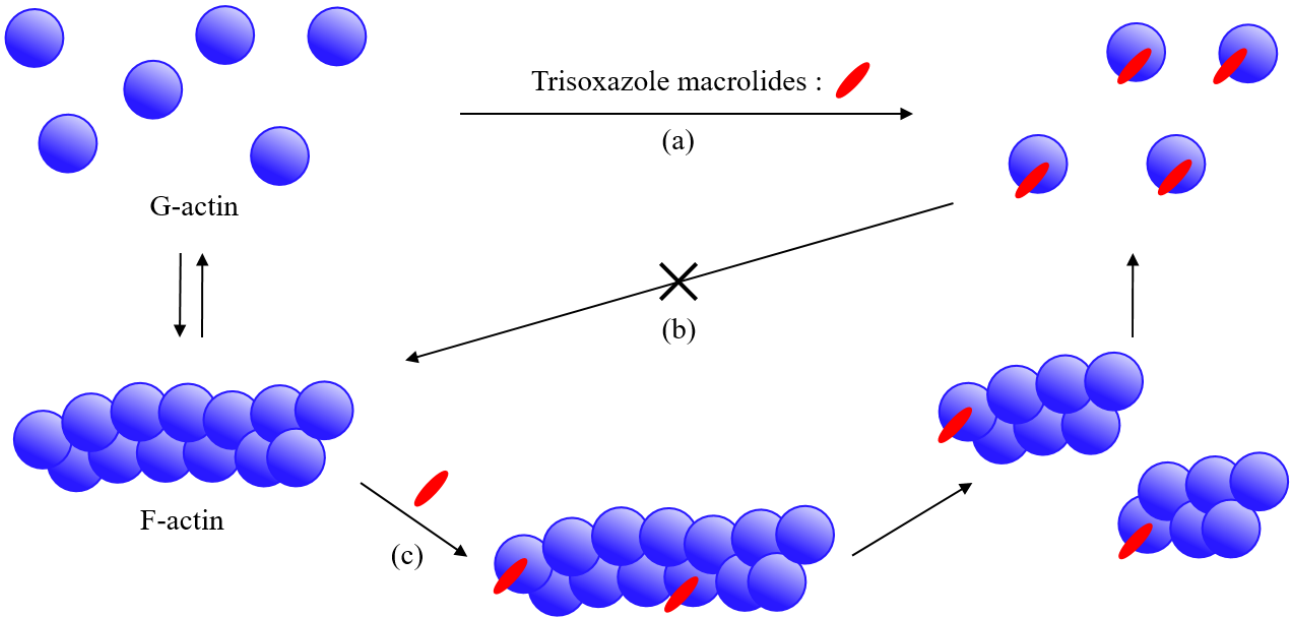


Figure 1-12. The model of the actin-depolymerizing mechanism caused by trisoxazole macrolides. (a) Trisoxazole macrolides bind to G-actin and form a 1:1 complex. (b) The complex is not incorporated in the polymerization. (c) Trisoxazole macrolides severs F-actin and caps the barbed end.

As shown in Figures 1-3–1-8, trisoxazole macrolides have various functional groups, and these differences are shown to affect the ability to interact with actin. For example, Allingham and co-workers reported that actin binding ability of ulapualide A is weaker than that of kabiramide C.[9h] There are differences in ulapualide A and kabiramide C on the C24–C35 side-chain, such as the substituent at C32 (acetyl or methoxy groups). They suggested that the difference changes the electron density of the C31–C35 region and diminishes an interaction with actin. Additionally, Oh and co-workers reported that halichondramide and (19*Z*)-halichondramide show potent F-actin severing activity, however jaspisamide A and neohalichondramide do not, which are the water adduct (C5) and the double bond migrated (C5–C6 to C4–C5) analogs of halichondramide.[12]

Trisoxazole macrolides have variety structures and biological activities. In several trisoxazole macrolides, their structural difference affects the affinity with actin. But, the relationships between structures and the interaction with actin remain unclear. Clarifying the relationships might lead to the development of useful artificial molecules for actin-related biological and biochemical studies.

1-3. Mycalolides

Mycalolides belong to trisoxazole macrolides and have been isolated from the marine sponge of the genus *Mycale* and the stony coral *Tubastres faulkneri* (Figure 1-6).[6] Mycalolides are mainly categorized in two group by the oxidation pattern at C30 position, one is the ketone like mycalolide A, and the other is the secondary alcohol or the ester like mycalolide B. The absolute stereochemistry of mycalolides had remained obscure for a decade after their planar structure had been reported. In 1998, Fusetani and co-workers determined the absolute stereochemistry of mycalolides A–C, 30-, 32-, 30,32-hydroxymycalolide A, and 38-hydroxymycalolide B on the basis of chemical degradation and derivatization.[6d,6e,13] After the total synthesis of mycalolide A was accomplished by Panek and co-workers, its structure was confirmed.[14]

Mycalolides show various biological activities likewise other related trisoxazole macrolides. It was initially reported that mycalolides A-C exhibit potent antifungal activity against a wide range of pathogenic fungi and cytotoxicity against B-16 melanoma cells with IC_{50} values of 0.5-1.0 ng/mL.[6a] The use of mycalolides directly for cancer therapy was expected, but it was unsuccessful due to their high toxicity.[5b,6a,15] The actin-depolymerizing activity of mycalolide B was reported.[9a–d] Mycalolide B severs F-actin to G-actin, forms 1:1 complex with G-actin, and inhibits polymerization by sequestering of the barbed end in actin. Applying this specific nature, mycalolide B is used as a tool to elucidate the functions of actin and actin-related cell systems.[16]

Due to interesting biological activities of mycalolides, several synthetic studies of mycalolides have been reported, since the Panek's total synthesis of mycalolide A[14]; the fragment synthesis of mycalolide A by Cossy,[17] and the fragment and an artificial analog synthesis of mycalolide B by Kigoshi.[18] A number of trisoxazole macrolides have been reported, but most of these were not synthesized. So, It is thought that establishment of various synthetic approaches toward trisoxazole macrolides is important. If an effective synthetic route of these molecules could be established, the elucidation of bioactivity becomes easier. Based on the synthetic route established by a total synthesis, effective design and synthesis of artificial analogs make possible for further structure-activity relationship studies. By the chemical modification of trisoxazole macrolides to reduce its natural toxicity, a novel class of drugs that regulates actin dynamics in cells might be developed. Therefore, further studies are needed to understand entire functions of mycalolides. It is expected that synthetic studies of mycalolides contribute to an application for pharmaceutical and bioscience researches.

In this research, the author carried out the synthesis and biological activities of trisoxazole macrolactone analogs of mycalolides, and the total synthesis of mycalolides A and B.

2. Synthesis and biological activities of the trisoxazole macrolactone analogs of mycalolides

2-1. Introduction

Trisoxazole macrolides including mycalolides possess attractive biological activities, such as potent cytotoxicity against tumor cells and specific interaction with actin, as described in chapter 1. By the X-ray crystal structure analyses of actin complexes with several trisoxazole macrolides, it was revealed that these macrolides interact with actin to insert the C25–C35 side-chain into the hydrophobic cleft (Tyr133, Ala135, Val139, Tyr143, Gly146, Thr148, Glu167, Gly168, Tyr169, Leu346, Leu349, Thr351, Phe352, Met355 and Phe375) between the subdomains 1 and 3 of actin and to put the C1–C24 macrolactone ring on the hydrophobic patch (Gly23, Asp25, Ala144, Ser145, Glu334, Ile341, Ile345, Ser348 and Leu349) adjacent the cleft such as Figures 1-11 and 2-1.[9f,9g] The side-chain analog **1** of mycalolides was synthesized by Suenaga et al., and **1** shows actin-depolymerizing activity (Figure 2-2).[18a,18b] The importance of the side-chain part for the actin-depolymerizing activity was established, but **1** has little cytotoxicity. On the other hand, the macrolactone ring part interacts with actin surface in the X-ray crystal structures (Figure 2-1).[9f,9g] But, there is no report about the biological activity of the macrolactone ring part alone. Because of the interest that the macrolactone ring part might be important for the cytotoxicity of parent molecules, the author decided to synthesis the trisoxazole macrolactone ring analog **2** of mycalolides and evaluate its activity (Figure 2-2).

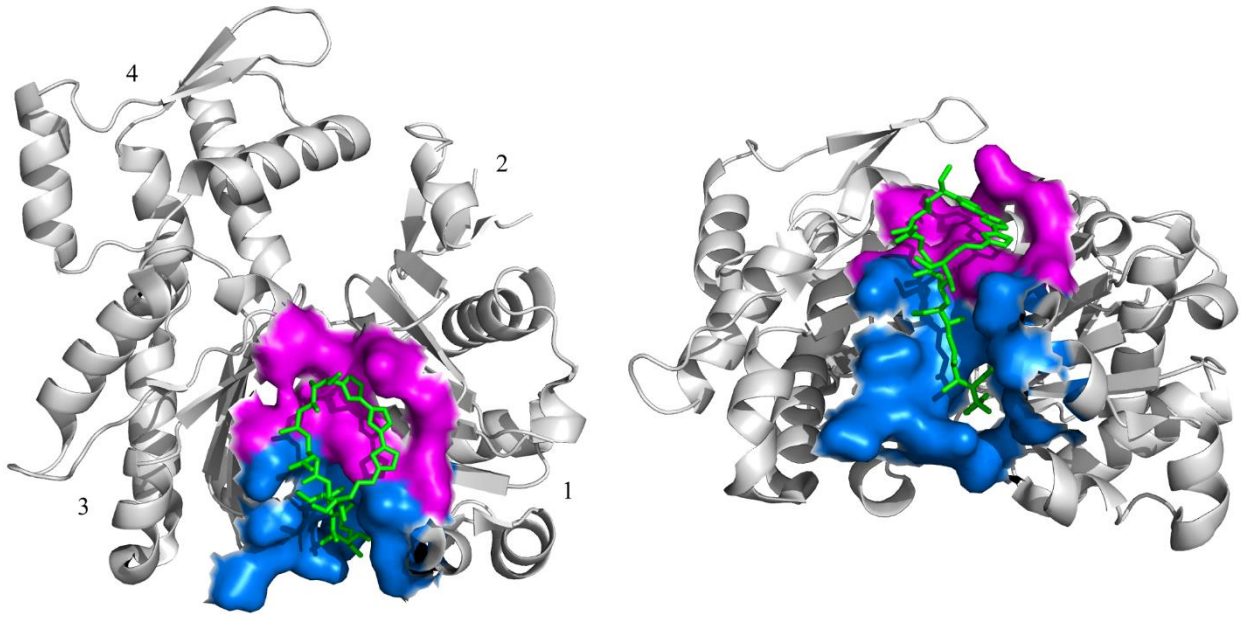


Figure 2-1. Kabiramide C binding site on actin. Kabiramide C is shown as stick representation in green. Actin surfaces contacting the macrolactone part and the side-chain part are shown in magenta and blue, respectively.

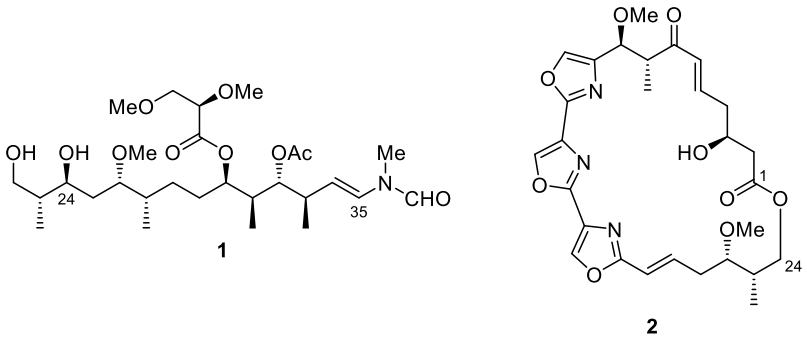
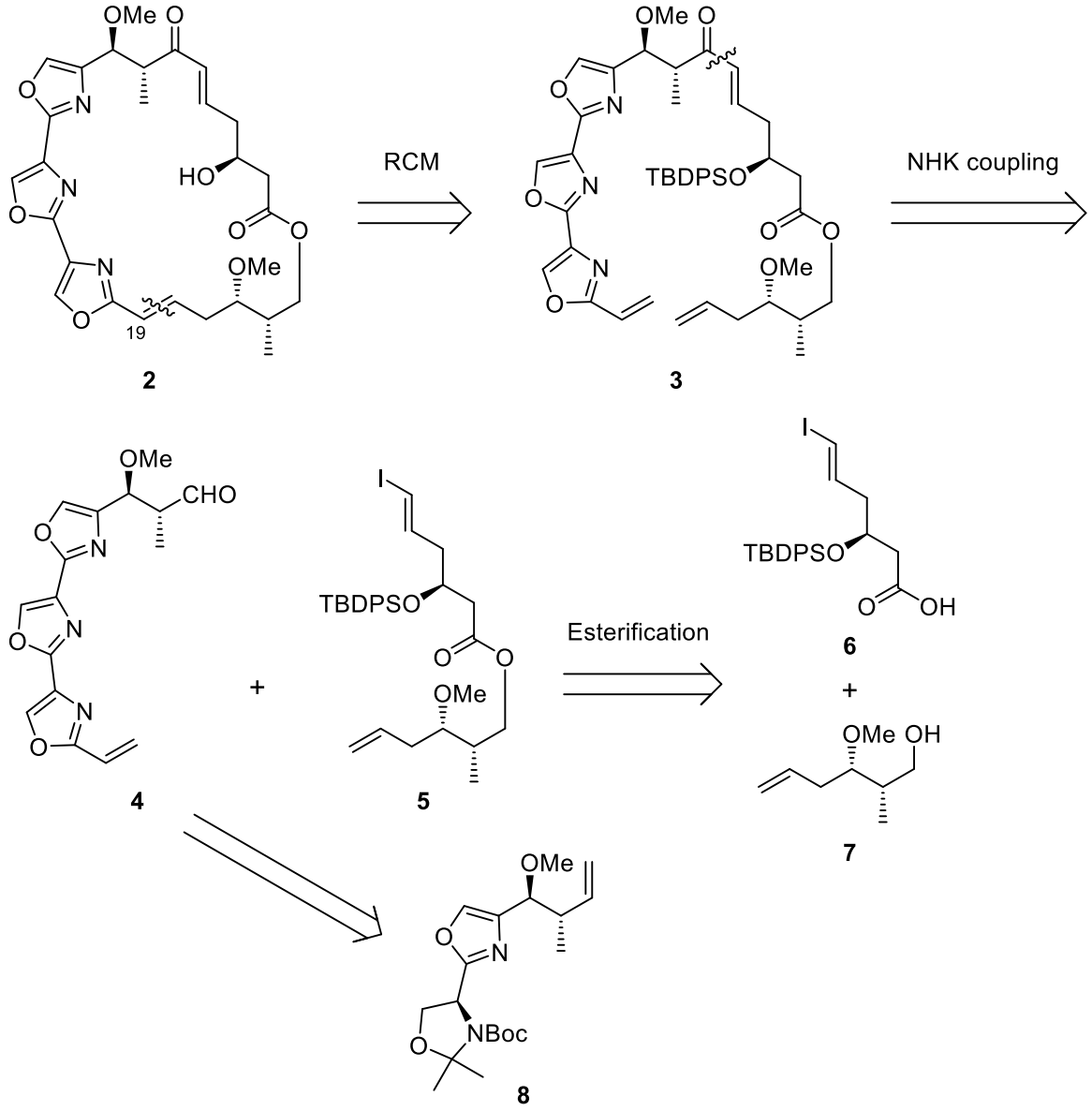


Figure 2-2. Structures of mycalolides analogs.

2-2. Synthetic plan

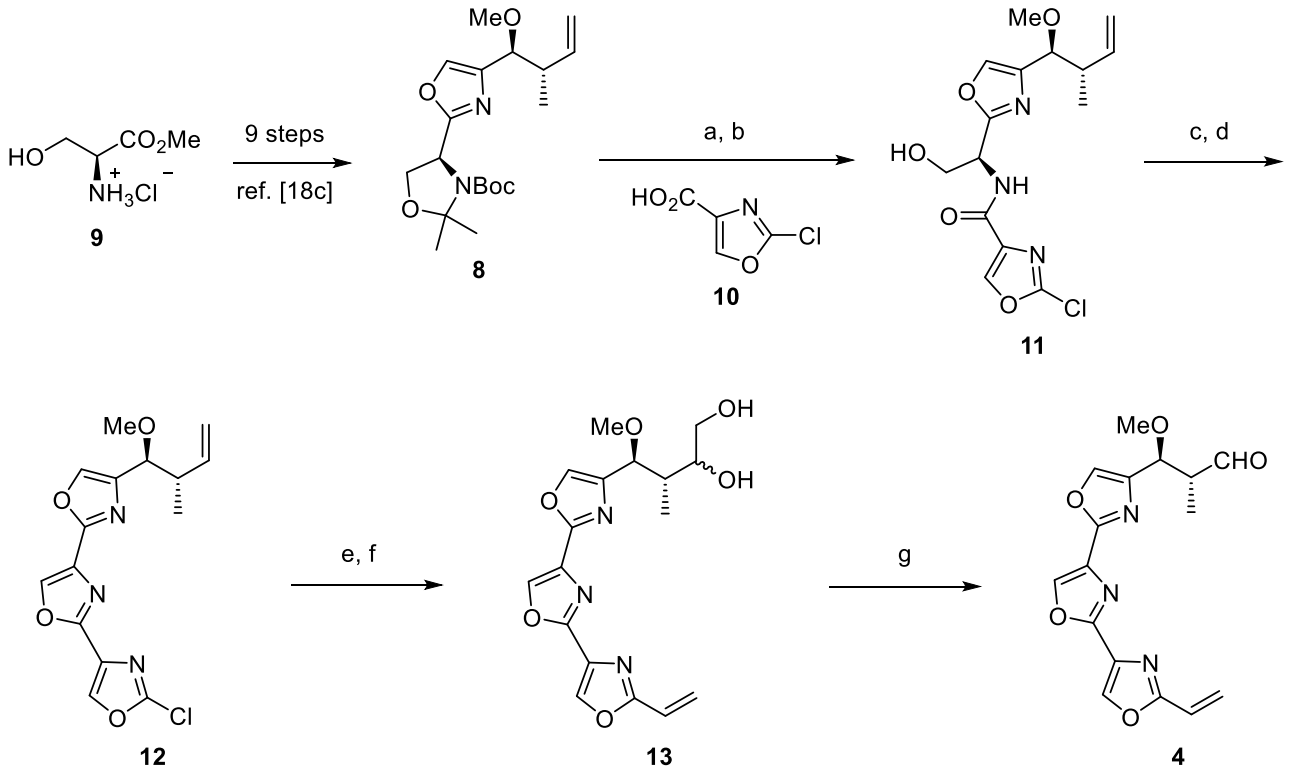
Synthetic plan of the trisoxazole macrolactone analog **2** is shown below (Scheme 2-1). In the synthetic studies of mycalolide B, the usefulness of metathesis reactions for connecting the C19–C20 double bond in mycalolides was reported.[18c,18d] According to the findings, the author decided to synthesize the macrolactone **2** from diene **3** through ring-closing metathesis (RCM) for the construction of the C19–C20 double bond. The diene **3** could be assembled by connecting aldehyde **4** and iodoolefin **5** with the use of Nozaki–Hiyama–Kishi (NHK) coupling.[19] Iodoolefin **5** could be synthesized by the condensation of carboxylic acid **6** and alcohol **7**. Aldehyde **4** could be prepared from methyl ether **8** based on the procedure established by collaborators.



Scheme 2-1. Synthetic plan for the trisoxazole macrolactone analog **2**.

2-3. Synthesis of the trisoxazole macrolactone analogs

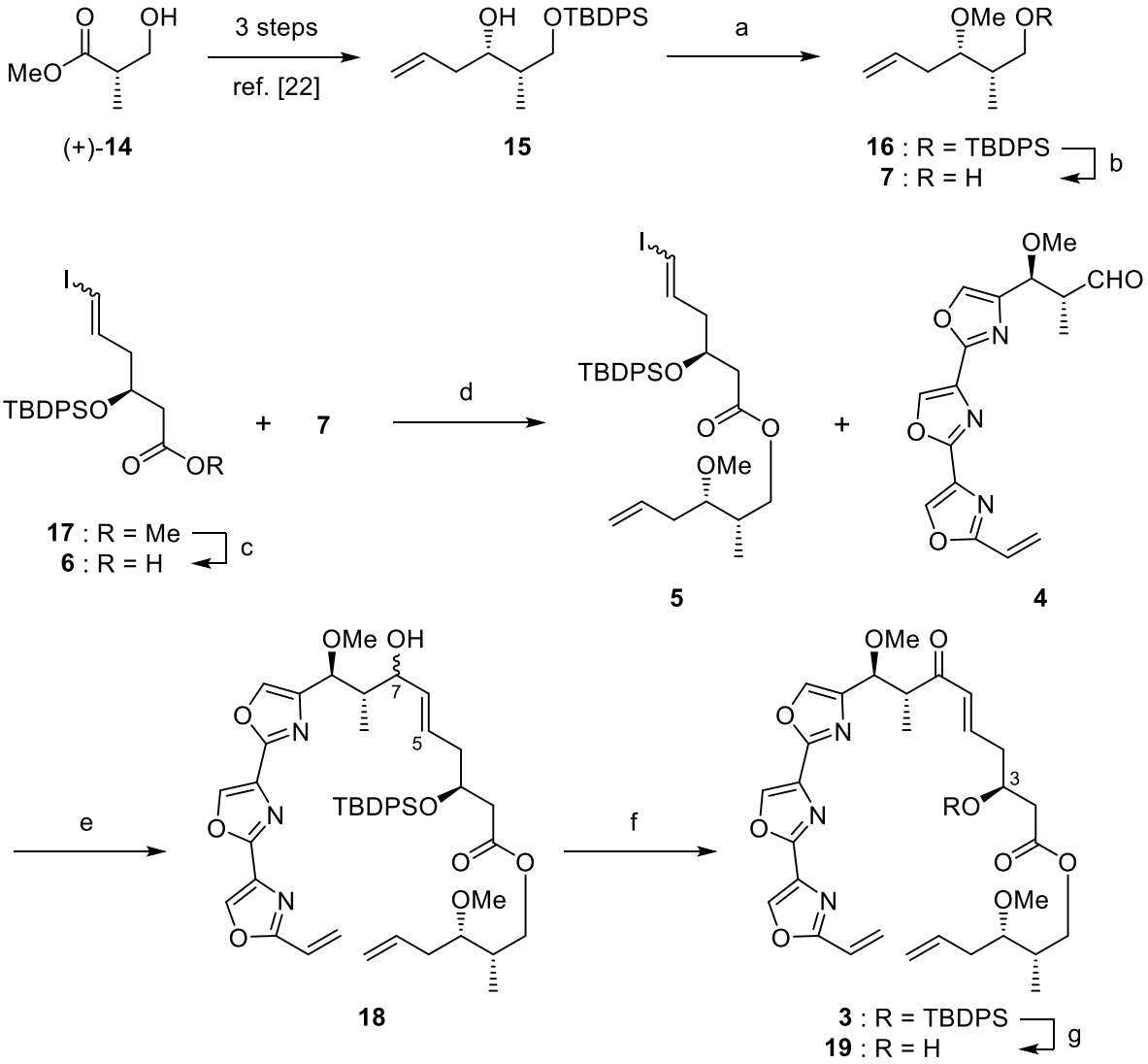
First, (−)-aldehyde **4**[18d] was prepared (Scheme 2-2). (−)-Methyl ether **8** was prepared in 9 steps[18c] from L-serine methyl ester hydrochloride (**9**), which was hydrolyzed with hydrochloric acid and condensed with 2-chlorooxazole-4-carboxylic acid (**10**)[20] to give amide **11**. Cyclodehydration by *N*,*N*-diethylaminosulfur trifluoride (DAST) and subsequent oxidative aromatization with bromotrichloromethane and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)[21] afforded trisoxazole **12**. Dihydroxylation of the olefin moiety in **12** followed by vinylation using Stille coupling gave **13**. Oxidative cleavage of the diol **13** with sodium periodate gave aldehyde **4**.



Scheme 2-2. Synthesis of (−)-aldehyde **4**. Reagents and conditions: a) 3 M HCl aq., EtOAc, rt; b) **10** (1.1 eq), EDC·HCl, HOBt, Et_3N, CH_2Cl_2, rt, 78% in 2 steps; c) Et_2NSF_3, CH_2Cl_2, –78 °C, 85%; d) $BrCCl_3$, DBU, MeCN, rt, 54%; e) OsO_4, NMO, THF–H_2O (4/1 [v/v]); f) tributylvinyltin, $PdCl_2(PPh_3)_2$, 1,4-dioxane, reflux, 83%; g) $NaIO_4$, EtOH–H_2O (4/1 [v/v]), rt, 98%.

Next, synthesis of iodoolefin **5** and segment coupling were carried out (Scheme 2-3). Homoallylic alcohol **15** (*syn*/*anti* = 93:7) was prepared from methyl (*S*)-(+)-3-hydroxy-2-methylpropionate ((+)-**14**, >99%*ee*) in 3 steps[22]. Methylation of **15** by methyl trifluoromethanesulfonate (MeOTf) and removal of the *tert*-butyldiphenylsilyl (TBDPS) group by tetra-*n*-butylammonium fluoride (TBAF) gave primary alcohol **7**.

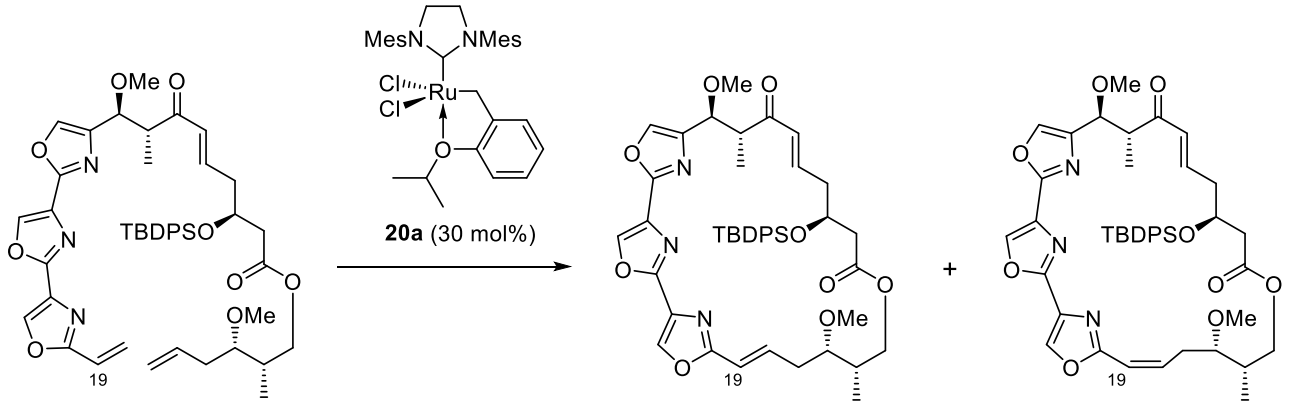
An *E*/*Z* mixture of (+)-methyl ester **17** (99%*ee*, *E*/*Z* = 6.7/1)[18d] with LiOH afforded carboxylic acid **6**. Then, **6** and **7** were condensed by Yamaguchi procedure[23] to give iodoolefin **5**. Segment assembly between **5** and aldehyde **4** by NHK coupling gave allylic alcohol **18** as a single *E* isomer with a 3:2 diastereomeric mixture at C7. Oxidation of **18** with Dess–Martin periodinane[24] yielded a RCM precursor diene **3**. Additionally, removal of the TBDPS group of **4** by TBAF and acetic acid gave a C3 hydroxy RCM precursor **19**.



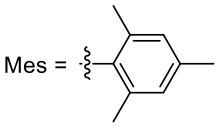
Scheme 2-3. Synthesis of iodoolefin **5** and RCM precursors. Reagents and conditions: a) MeOTf, 2,6-di-*tert*-butylpyridine, CH_2Cl_2, rt, 90%; b) $^nBu_4NF$, THF, rt, 96%; c) 1 M LiOH aq., THF, 40 °C, 80%; d) 2,4,6-trichlorobenzoyl chloride, Et_3N, THF, rt, then *N*,*N*-dimethyl-4-aminopyridine (DMAP), benzene, rt, 92%; e) $CrCl_2$–$NiCl_2$ (99/1 [w/w]), THF–DMF (3/1 [v/v]), rt, 73%; f) Dess–Martin periodinane, CH_2Cl_2, 0 °C, 94%; g) $^nBu_4NF$–AcOH (1/1), THF, rt, 72%.

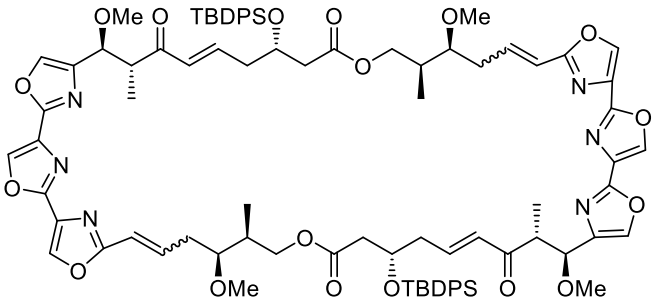
With the key intermediates **3** in hand, RCM reactions were examined (Table 2-1). Treatment of **3** with 30 mol% of the second generation Hoveyda–Grubbs catalyst (**20a**)[25] in refluxing toluene under high dilution conditions (1 mM) gave macrocycle **21** as a separable 1.0/1.9 mixture of *E* and *Z* isomers at C19 position in 61% yield along with dimer **22** (entry 1). Similarly, the reaction at 40 °C in hexane preferred the 19*Z* isomer more (*E*/*Z* = 1.0/2.5, entry 2). With the use of α,α,α-trifluorotoluene which is more polar solvent than toluene and hexane, the product ratio of 19*E* isomer slightly increased (entry 3). Interestingly, in the same solvent at lower temperature, the *E*/*Z* product ratio was reversed and 19*E* isomer was given preferentially (entry 4). The use of ethyl acetate as a solvent also gave the similar selectivity (entry 5). At the refluxing conditions in dichloromethane, the RCM progressed with the most 19*E* selective manner and the highest yield (82%, *E*/*Z* = 1.8/1.0, entry 6). These results suggested that both the solvent polarity and the reaction temperature affected the stereoselectivity of the RCM reaction of **3**.

Table 2-1. RCM of diene **3**.



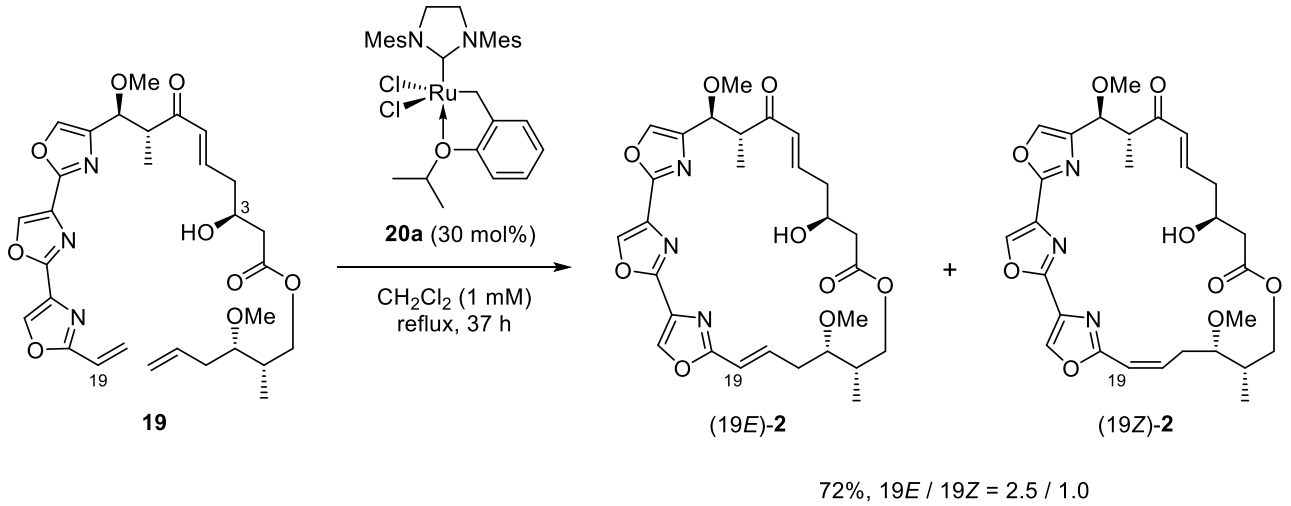
entry	solvent (1 mM)	temp.	time (h)	yields (%)	
				21 (19*E* / 19*Z*)	**22**
1	toluene	reflux	1.5	61 (1.0 / 1.9)	5
2	hexane	40 ℃	24	73 (1.0 / 2.5)	9
3	$C_6H_5CF_3$	reflux	1.0	69 (1.0 / 1.3)	7
4	$C_6H_5CF_3$	40 ℃	24	77 (1.3 / 1.0)	6
5	EtOAc	40 ℃	9	78 (1.2 / 1.0)	9
6	CH_2Cl_2	reflux	9	82 (1.8 / 1.0)	5





dimer **22** (*E*/*Z* ratio was not determined)

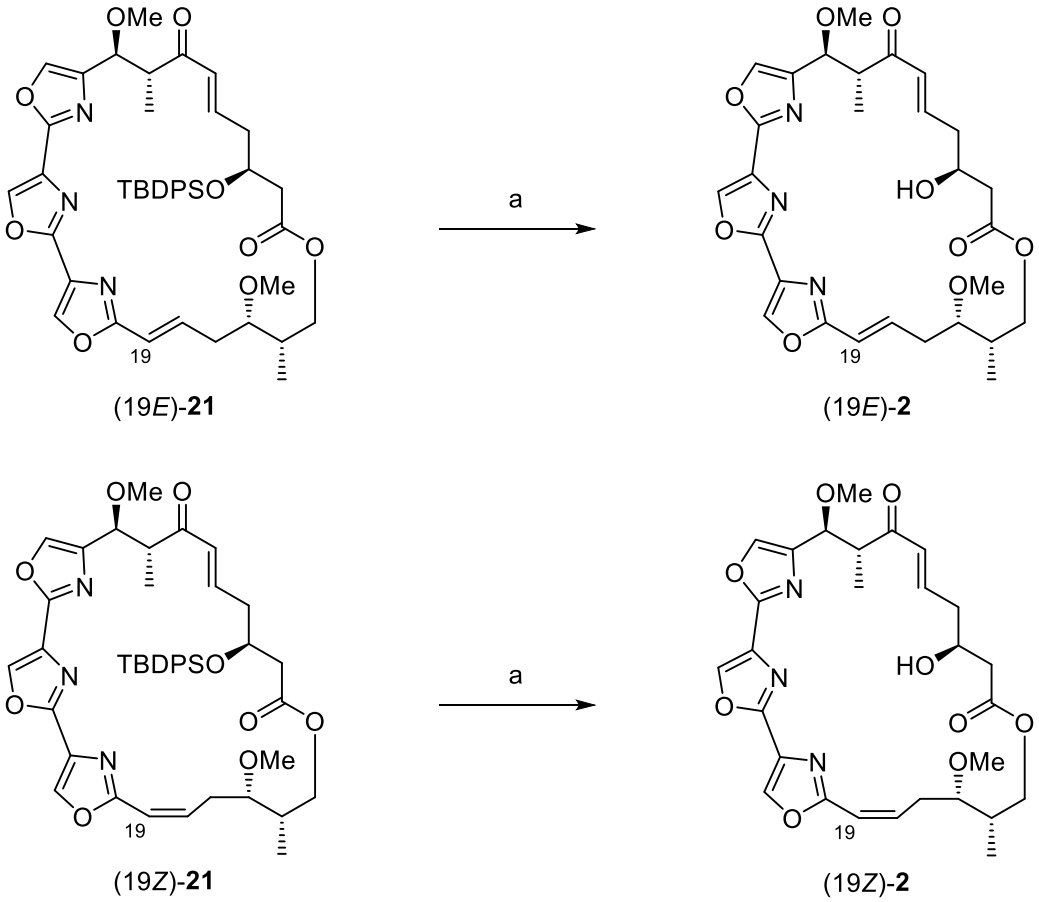
Next, the author examined the effect of the bulky TBDPS group at C3 for RCM stereoselectivity. Therefore the RCM reaction of C3 hydroxy analog **19** was examined (Scheme 2-3). Treatment of **19** with 30 mol% of catalyst **20a** in refluxing dichloromethane (1 mM) gave macrolactone **2** (72%, *E*/*Z* = 2.5/1.0). While the reaction was slower and the yield was lower than the case of the RCM of **3** (see Table 2-1, entry 6), the stereoselectivity was slightly improved. This result suggested that C3 hydroxyl analog **19** would have a specific conformation to prefer the formation of the 19*E* isomer with smaller steric hindrance at C3 position.



19

Scheme 2-3. RCM of C3 hydroxy analog **19**.

Both of the stereoisomers of the trisoxazole macrolactone (19*E*)- and (19*Z*)-**2** were also afforded by removal of the TBDPS groups of (19*E*)- and (19*Z*)-**21** by TBAF and AcOH (Scheme 2-4).



Scheme 2-4. Synthesis of the trisoxazole macrolactone analogs. Reagents and condition: a) $^{n}Bu_4NF$–AcOH (1/1), THF, rt, 87% for (19*E*)-**2**, and 91% for (19*Z*)-**2**.

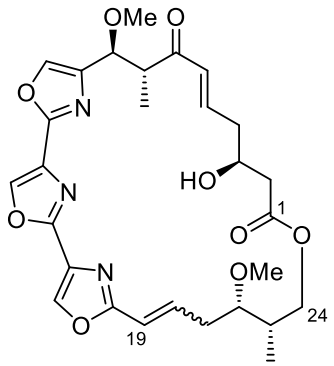
2-4. Biological activity

Biological activities of the synthesized trisoxazole macrolactone analogs were examined, which include cytotoxic, actin-depolymerizing, and antifungal activities (Table 2-2). Both of the (19*E*)- and (19*Z*)-**2** exhibited moderate cytotoxicity against HeLa S3 cells with IC_{50} values of 2.4 and 1.9 μg/mL, respectively, which were approximately 100 times less than that of mycalolide B. However, they showed no actin-depolymerizing effects at 30 μM nor antifungal activity against several pathogenic fungi at 30 μg/mL. Considering these results and the fact that the side chain analog **1** has actin-depolymerizing activity without cytotoxicity, both the side-chain and the macrolactone moieties were suggested to be essential for the potent cytotoxicity or antifungal activity of mycalolides.

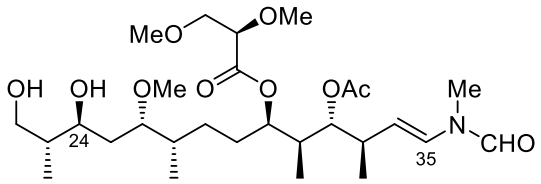
Table 2-2. Biological activities of mycalolide B and its analogs.

compound	cytotoxicity (HeLa S3) IC_{50} (μg/mL)	actin-depolymerizing activity [a] EC_{50} (μM)	antifungal activity [d]
(19*E*)-**2**	2.4	>30	no activity
(19*Z*)-**2**	1.9	>30	no activity
mycalolide B	0.020	1.4 [b]	– [e]
1	>10 [c]	2.7 [c]	– [e]

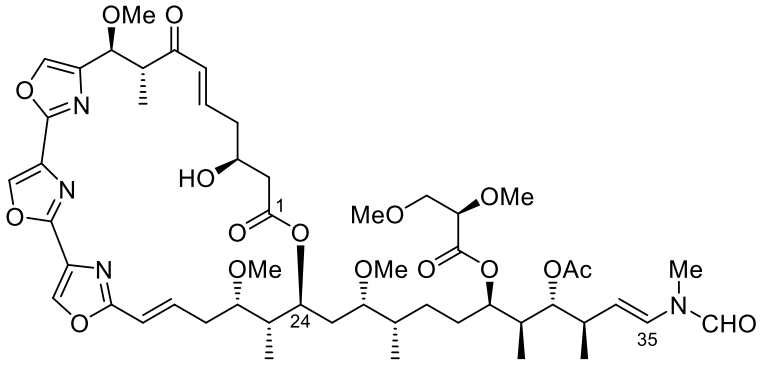
a) Activity was monitored by measuring the fluoresence intensity of pyrenylactin. Values indicate the concentrations required to depolymerize F-actin (3 μM) to 50% of its control amplitude.
b) See ref. [9b,26].
c) See ref. [18a,18b]
d) Against pathogenic fungi (*Astergillus fumigatus, Candida albicans, Trichophyton mentagrophytes*) or nomal fungi (*Mucor hiemalis, Rhizopus nigricans*) at 30 μg/mL.
e) Not examined.



2



1



Mycalolide B

2-5. Conclusion

The trisoxazole macrolactone analogs of mycalolides were synthesized through the use of NHK coupling connecting at C6–C7 bond and RCM for the construction of C19 double bonds. The stereoselectivity at C19 in the RCM was affected by reaction solvent polarity, temperature, and the protecting group at C3 (*E*/*Z* = 2.5/1.0–1.0/2.5).

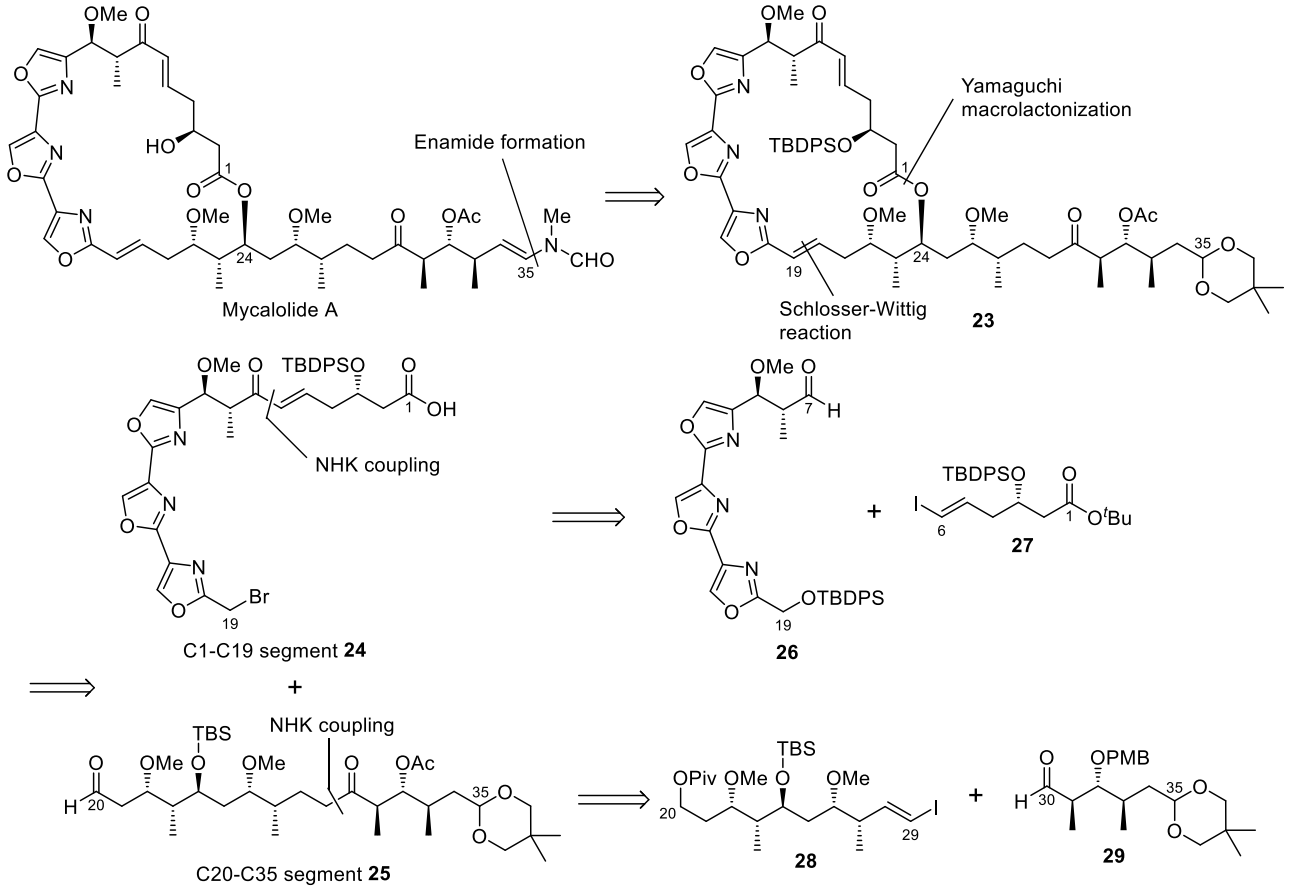
Both of the 19*E* and 19*Z* macrolactone analogs exhibited cytotoxicity against tumor cells, but they were approximately 100 times less cytotoxic than mycalolide B. Furthermore, it was clarified that the macrolactone analogs showed no actin-depolymerizing effects and no antifungal activity. Thus, both the side-chain and the macrolactone moiety were suggested to be essential for the potent biological activities of mycalolides.

3. Total synthesis of mycalolides A and B

3-1. Introduction

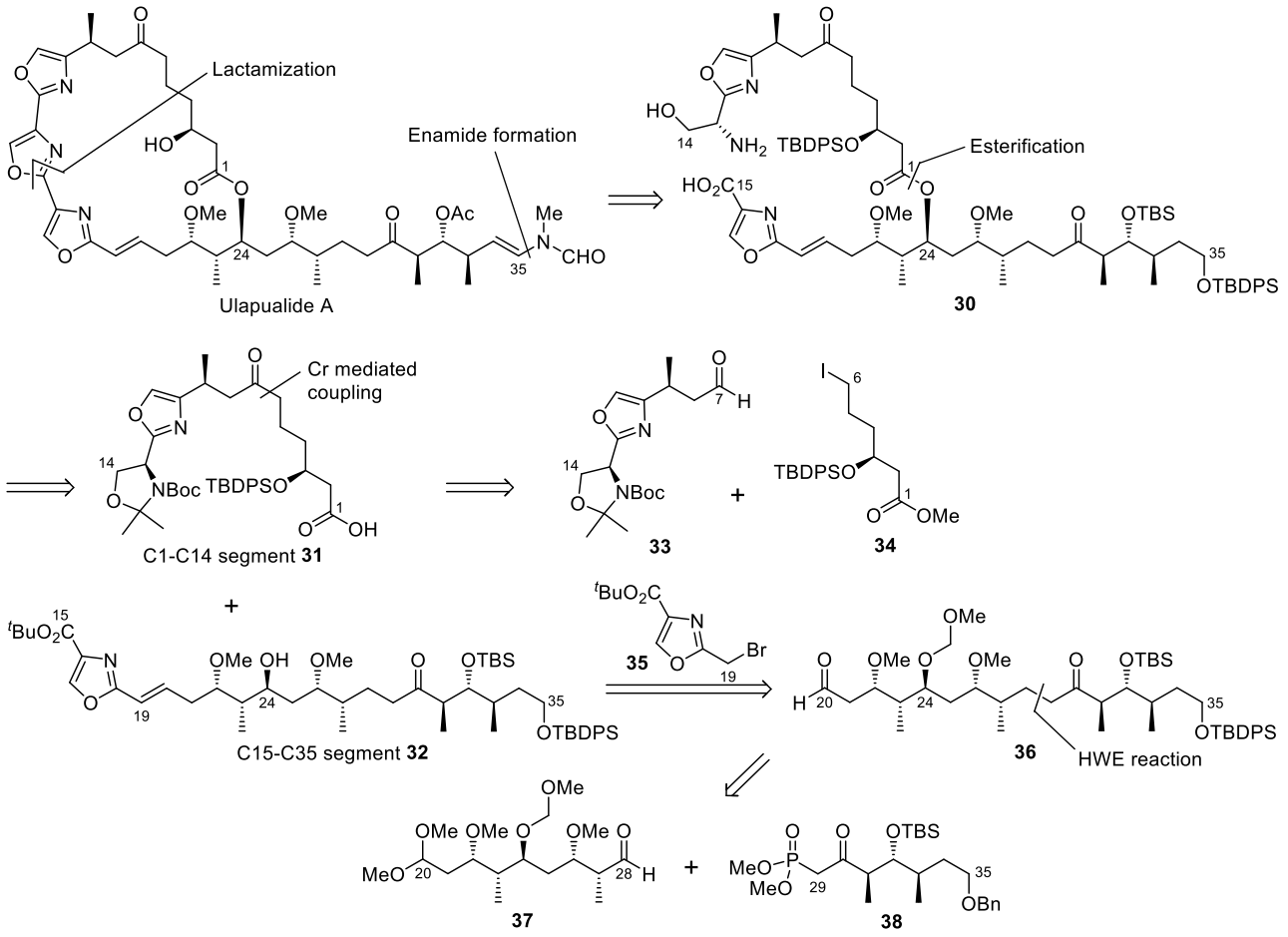
Mycalolides and related trisoxazole macrolides have attracted attention as synthetic targets because of their extraordinary structures and biological activities. To date, total synthesis of mycalolide A and ulapualide A has been accomplished.

In 2000, Panek et al. reported the total synthesis of mycalolide A by the convergent assembly of the C1–C19 segment **24** and the C20–C35 segment **25** (Scheme 3-1).[14] Both of the segments were synthesized by using NHK coupling of aldehydes **26** and **29** with iodoolefins **27** and **28** as key steps. After the segments were connected at the C19–C20 double bond by Schlosser–Wittig reaction, Yamaguchi macrolactonization was employed to furnish macrocyclic structure. Finally, macrolactone **23** was converted to mycalolide A by the installation of enamide and removal of protecting group. This synthesis was the first report of all trisoxazole macrolides, and confirmed the absolute stereochemistry of (−)-mycalolide A.



Scheme 3-1. Strategy for the total synthesis of mycalolide A developed by Panek et al.

In 2007, Pattenden et al. achieved the total synthesis of ulapualide A (Scheme 3-2).[27] Ulapualide A was divided into C1–C14 and C15–C35 segments, and the C1–C14 segment **31** was synthesized from aldehyde **33** and alkyliodide **34** through a Cr(II)-mediated coupling. The C15–C35 segment **32** was synthesized by the connection of the aldehyde **37** and phosphonate **38** by using Horner–Wadsworth–Emmons (HWE) reaction before the installation of monooxazole **35** for the aldehyde **36** by using Schlosser–Wittig reaction. These segments were condensed by using Yamaguchi procedure, and the macrocyclic core was constructed by the oxidative cyclization of the middle oxazole ring in **30**. Enamide formation and the removal of protecting groups accomplished the total synthesis of (−)-ulapualide A.



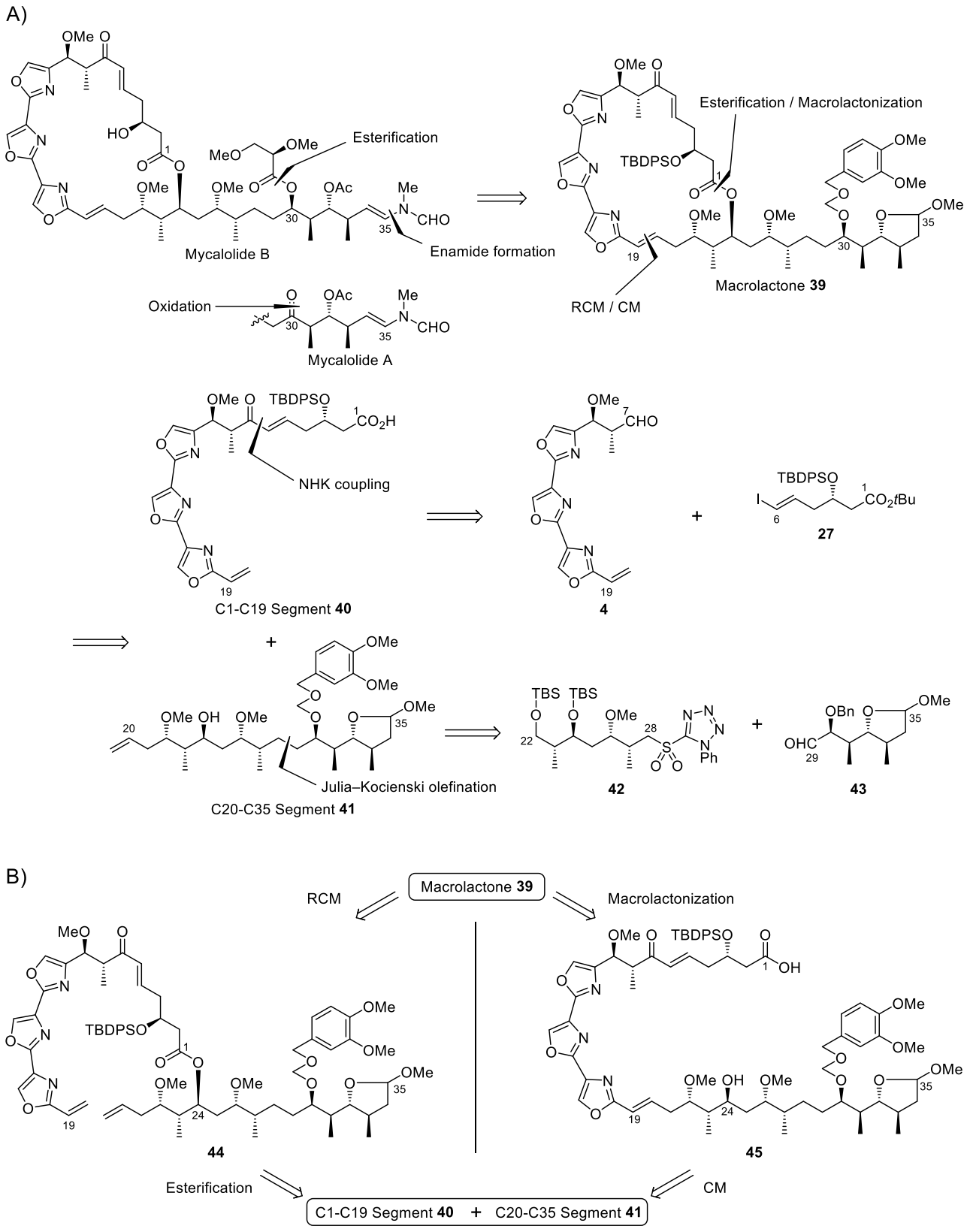
Scheme 3-2. Strategy for the total synthesis of ulapualide A developed by Pattenden et al.

As mentioned above various synthetic routes that provide trisoxazole macrolides have been established. These synthetically provided compounds are expected to be useful for structure-activity relationship studies. The author have challenged the total synthesis of mycalolides A and B. Through the syntheses, the author aimed to develop a new synthetic route for the related trisoxazole macrolides and their artificial analogs.

3-2. Synthetic plan

Synthetic strategies toward mycalolides A and B are illustrated in Scheme 3-3. Mycalolides A and B could be synthesized from macrolactone **39** by the formation of C35 *N*-methyl enamide moiety, and the C30 functionalities (ester or ketone). Based on the finding that olefin metathesis is a useful method for connecting the C19 double bond in mycalolide analogs described in previous reports[18c,18d] and chapter 2, the author decided to synthesize macrolactone **39** from the C1–C19 segment **40** and the C20–C35 segment **41** on two ways using esterification/RCM or CM/macrolactonezation for efficient segment assembly (Scheme 3-3 B). An esterification/RCM approach could easily reach macrolactone **39**, although it might have difficulty regarding the stereoselectivity at C19 double bond, as with the RCM of C1–C24 model compound **3** in chapter 2. As for CM/macrolactonization approach, it was predicted that both segments need to protect their carboxy or hydroxy groups for CM since the yield of RCM of **19** having a free hydroxy group was low. Therefore, the author decided to examine both approaches.

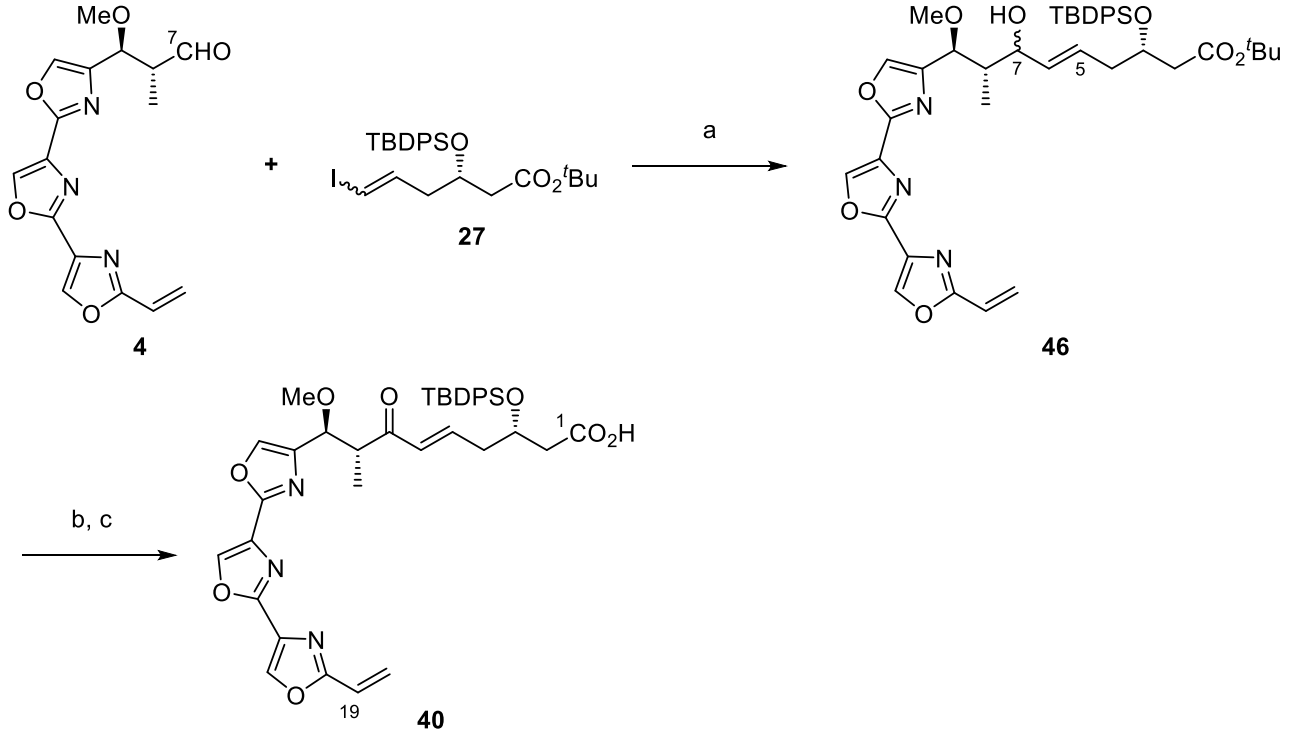
The C1–C19 segment **40** could be prepared from aldehyde **4** and iodoolefin **27** by using NHK coupling. The C20–C35 segment **41** was planned to use Julia–Kocienski olefination[28] between phenyltetrazole- (PT-) sulfone **42** and aldehyde **43**.



Scheme 3-3. Strategies for the total synthesis of mycalolides A and B. A) Entire synthetic plan; B) Two synthetic pathways for macrolactone **39** from the C1–C19 segment **40** and the C20–C35 segment **41**.

3-3. Synthesis of C1–C19 segment

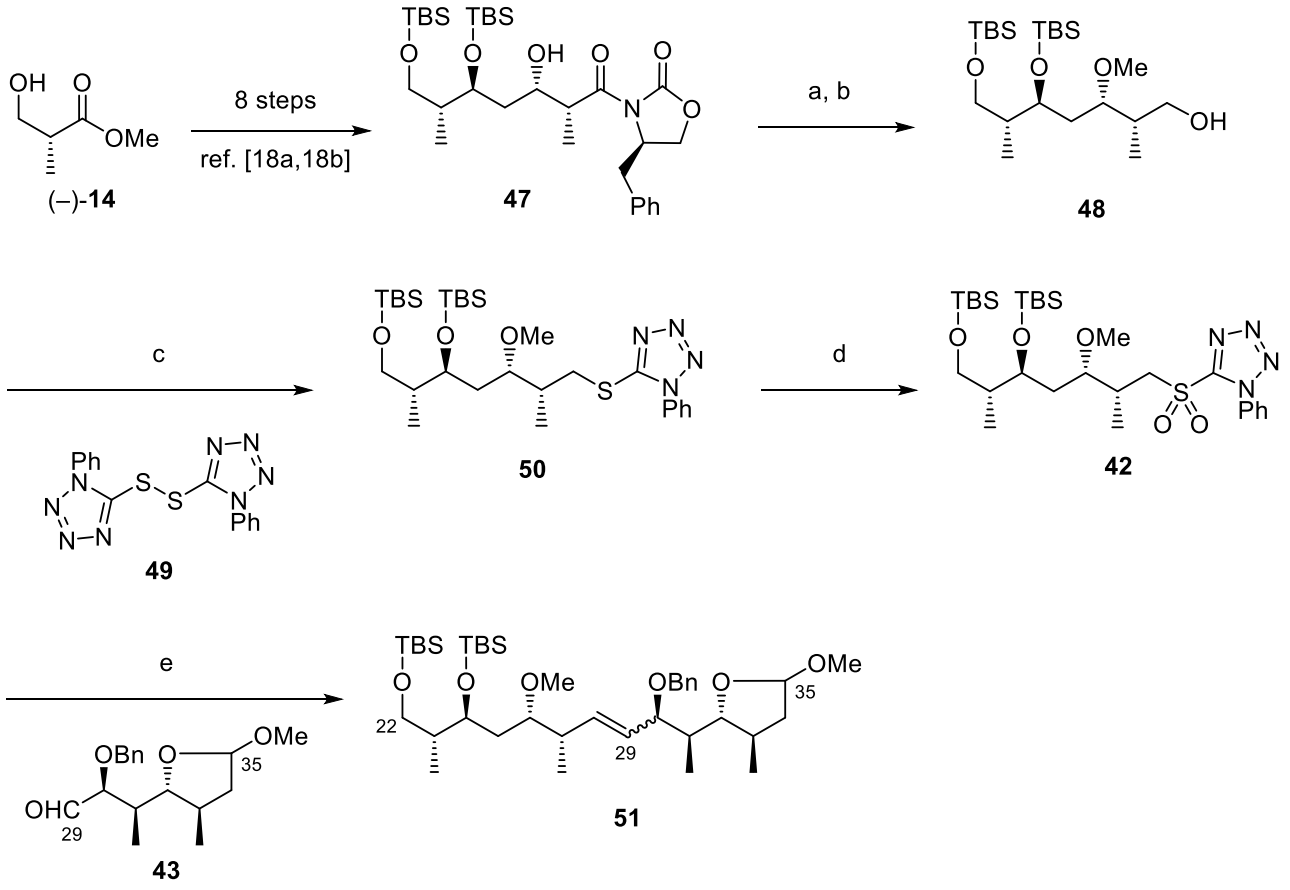
First, the author started to prepare the C1–C19 segment **40** for the asymmetric total synthesis of mycalolides (Scheme 3-4). NHK coupling between (−)-aldehyde **4** and (+)-iodoolefin **27** (*E*/*Z* = 5/1)[14] gave allylic alcohol **46** as a single *E* isomer at C5 with a 1:1 diastereomeric mixture of the hydroxy group at C7. The allylic alcohol was oxidized with Dess–Martin periodinane and subsequent removal of *tert*-butyl group in ester afforded the C1–C19 segment **40**.



Scheme 3-4. Synthesis of the C1–C19 segment **40**. Reagents and conditions: a) **27** (2.0 eq, *E*/*Z* = 5/1), $CrCl_2$–$NiCl_2$ (99:1 [w/w]), DMSO, rt, 97%; b) Dess–Martin periodinane, pyridine, CH_2Cl_2, 0 °C, 93%; c) TFA, CH_2Cl_2, 0 °C, 100%.

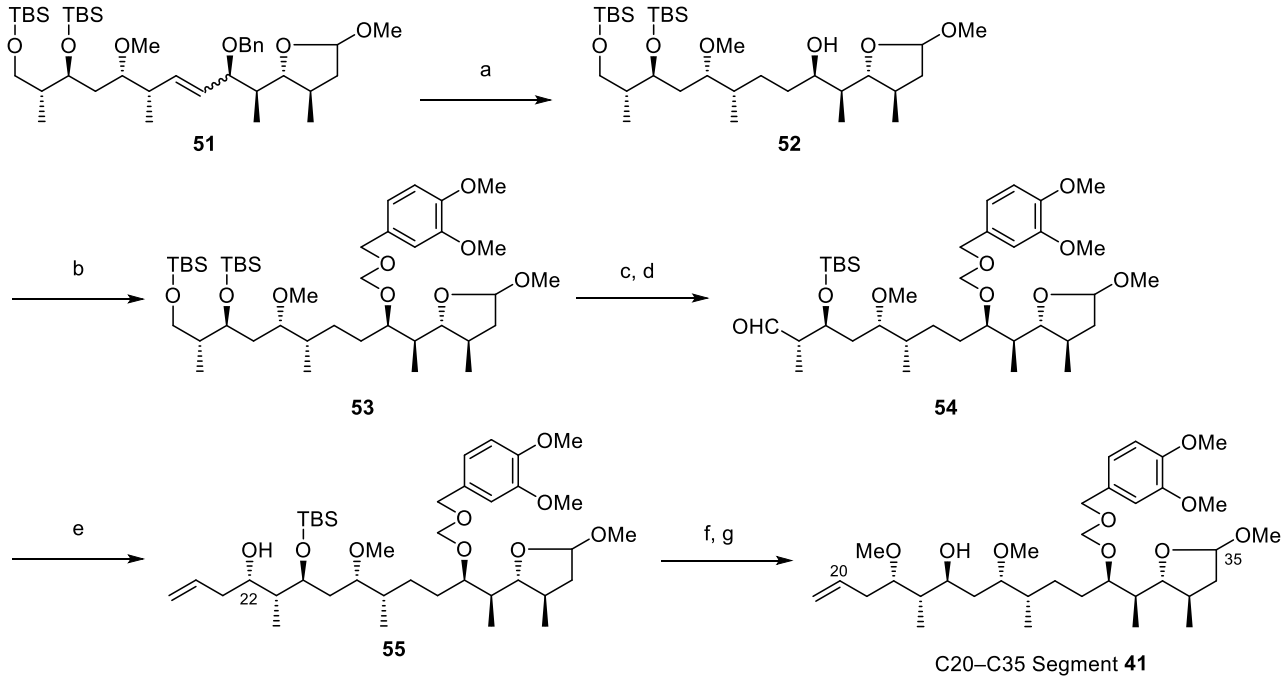
3-4. Synthesis of C20-C35 segment

Next, the author synthesized the C20–C35 segment **41** through the use of Julia–Kocienski olefination as a key step. Preparation of PT-sulfone **42** and its use for olefination are shown in Scheme 3-5. Aldol **47** was prepared from commercially available methyl (*R*)-(−)-3-hydroxy-2-methylpropionate ((−)-**14**, >99%*ee*) in 8 steps[18a,18b]. Methylation of **47** with MeOTf and removal of the chiral auxiliary with $LiBH_4$ gave primary alcohol **48**. After **48** was converted into PT-sulfide **50** with PT-disulfide **49** and $^nBu_3P$, oxidation of the sulfide with *meta*-chloroperbenzoic acid (*m*CPBA) afforded PT-sulfone **42**. Then, Julia–Kocienski olefination of the PT-sulfone **42** and known (+)-aldehyde **43**[29] was performed. Treatment of **42** with lithium hexamethyldisilazide (LHMDS) followed by the addition of aldehyde **43** in 1,2-dimethoxyethane (DME) at −55 °C to rt afforded olefin **51** in 92% yield (*E*/*Z* = 1/1.5). Although an excess amount of PT-sulfone **42** (2.5 eq) was required to complete the coupling reaction, the excess of **42** was quantitatively recovered and reused.



Scheme 3-5. Synthesis of olefin **51**. Reagents and conditions: a) MeOTf, 2,6-di-*tert*-butylpyridine, CH_2Cl_2, rt, 88%; b) $LiBH_4$, Et_2O, −10 °C, 97%; c) **49**, $^nBu_3P$, THF, rt, 100%; d) *m*CPBA, $NaHCO_3$, CH_2Cl_2, rt, 93%; e) **42** (2.5 eq), **43** (1.0 eq), LHMDS, DME, −55 °C to rt, 92% (*E*/*Z* = 1/1.5).

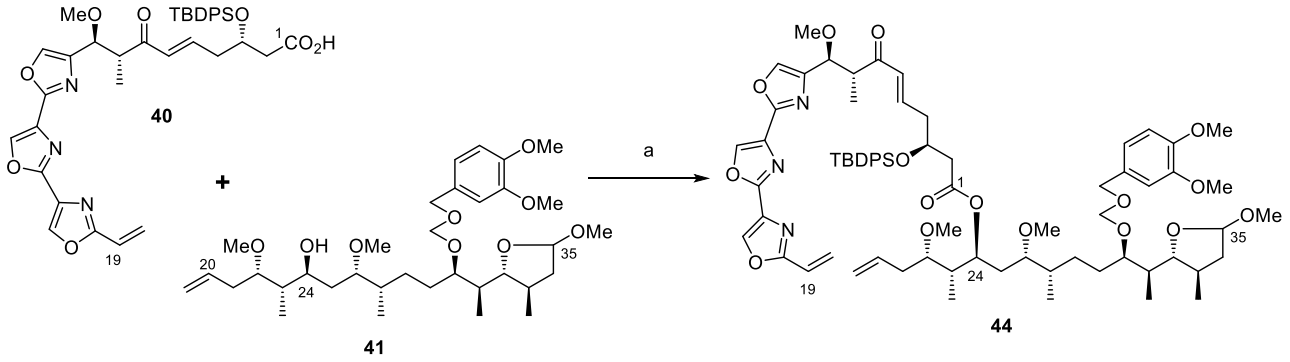
Further transformation toward the C20–C35 segment **41** is illustrated in Scheme 3-6. Catalytic hydrogenation of the double bond and hydrogenolysis of the benzyl group in the *E*/*Z* mixture of **51** were completed with palladium (II) hydroxide on carbon in single step to give secondary alcohol **52**. Protection of the hydroxy group in **52** with (3,4-dimethoxybenzyloxy)methyl (DMBOM) chloride afforded the previously reported DMBOM ether **53**.[18a–18c] As a result, the overall yield of **53** from aldol **47** was improved by the use of Julia–Kocienski olefination. Selective deprotection of the primary *tert*-butyldimethylsilyl (TBS) group in **53** and oxidation of the resulting primary alcohol with Dess–Martin periodinane provided aldehyde **54**. The Grignard reaction of **54** with allylmagnesium bromide gave the secondary alcohol **55** as a diastereomeric mixture at C22 (*dr* = 2.7/1). The desired C22-(*S*) configuration was preferred and their stereoselectivity satisfied the Felkin–Ahn rule. After these diastereomers were separated by a column chromatography, methylation of secondary alcohol **55** with methyl iodide and sodium hydride followed by the removal of TBS group with TBAF gave the C20–C35 segment **41**.



Scheme 3-6. Synthesis of the C20–C35 segment **41**. Reagents and conditions: a) H_2, $Pd(OH)_2/C$ (20 mol%), $NaHCO_3$, EtOH, rt, 89%; b) DMBOM chloride, $^{i}Pr_2NEt$, CH_2Cl_2, rt; c) NH_4F, MeOH, 40 °C, 100%; d) Dess–Martin periodinane, pyridine, CH_2Cl_2, rt, 96%; e) CH_2=$CHCH_2MgBr$, THF–Et_2O (3.4/1 [v/v]), 72% for C22-(*S*), 27% for C22-(*R*); f) MeI, NaH, THF, rt, 98%; g) $^{n}Bu_4NF$, THF, 40 °C, 100%.

3-5. Synthesis of the macrolactone though RCM

With C1–C19 and C20–C35 segments in hand, the author initially made an attempt to assemble these segments through the use of esterification/RCM approaches. As shown in Scheme 3-7, condensation of the segments **40** and **41** using Shiina reagent (2-methyl-6-nitrobenzoic anhydride, MNBA)[30] afforded the RCM precursor **44**.

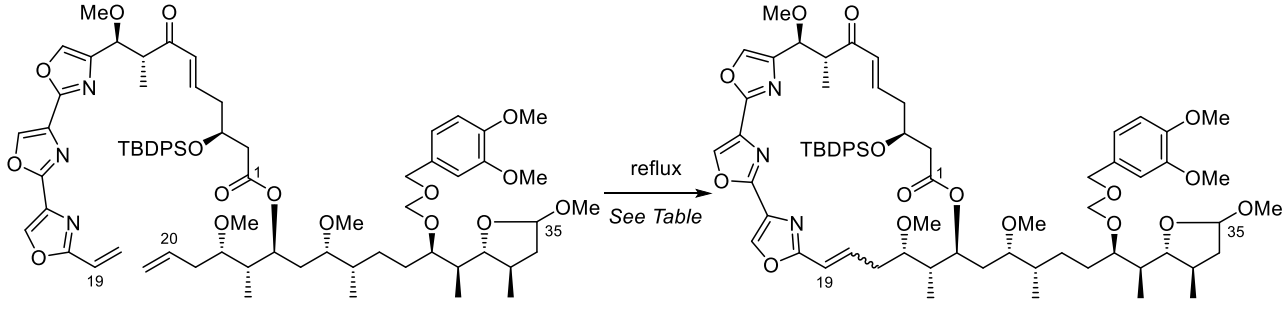


Scheme 3-7. Synthesis of the RCM precursor **44**. Reagents and condition: a) **40** (1.1 eq), **41** (1.0 eq), MNBA, DMAP, Et_3N, CH_2Cl_2, rt, 93%.

The RCM reaction of the precursor **44** was examined (Table 3-1). Kigoshi et al. reported that treatment of **44** with 30 mol% of the second generation Hoveyda–Grubbs catalyst (**20a**) in refluxing toluene afforded macrolactone **39**, but undesired 19*Z* isomer preferred (entry 1).[18d] In the case of the RCM of C1–C24 model compound as described in chapter 2, the reaction temperature and solvent polarity were found to affect the stereoselectivity. So the author examined the similar reaction conditions for the RCM of **44**. First, with the use of the catalyst **20a** in toluene at 40 °C, the ratio of *E* isomer slightly increased (entry 2). Next, by using more polar solvent, dichloromethane, the *E*/*Z* ratio was improved to 2.0/1 (entry 3). But in both cases, the reaction was not completed and the starting material **44** was recovered.

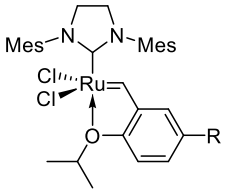
The stereoselectivity of the RCM of **44** was similar with that of C1–C24 model compound, but the reactivity of **44** was decreased. This might be due to the steric hindrance of the C25–C35 side-chain moiety in **44**. To facilitate the initiation of the catalytic cycle at lower temperature, two highly reactive Hoveyda–Grubbs type catalysts **20b** (Grela catalyst)[31] and **20c** (Zhan catalyst 1B)[32] were employed, in which nitro and *N*,*N*-dimethylsulfonamide groups are substituted on the 2-isopropoxybenzylidene ligand, respectively. Because the initiation step of the catalytic cycle for the Hoveyda–Grubbs type catalysts includes the cleavage of the O–Ru coordination bond, the electron withdrawing groups on the aromatic ring in the catalyst **20b** and **20c** lower the electronic density of the O–Ru bond and enhance their reactivity. In fact, the use of both catalysts increased the yield of **39** to 69–75% with similar stereoselectivity as the catalyst **20a** (entries 4 and 5).

Table 3-1. Synthesis of macrolactone **39** through RCM.



44 **39**

entry	catalyst (30 mol%)	solvent (0.9 mM)	time (h)	yields (%) **39** (19*E* / 19*Z*)	recovery of **44** (%)
1 [a]	**20a**	toluene	3	76 (1.0 / 1.2)	–
2 [b]	**20a**	toluene	42	44 (1.0 / 1.0)	30
3	**20a**	CH_2Cl_2	37	37 (2.0 / 1.0)	40
4	**20b**	CH_2Cl_2	24	69 (1.6 / 1.0)	–
5	**20c**	CH_2Cl_2	24	75 (1.7 / 1.0)	–

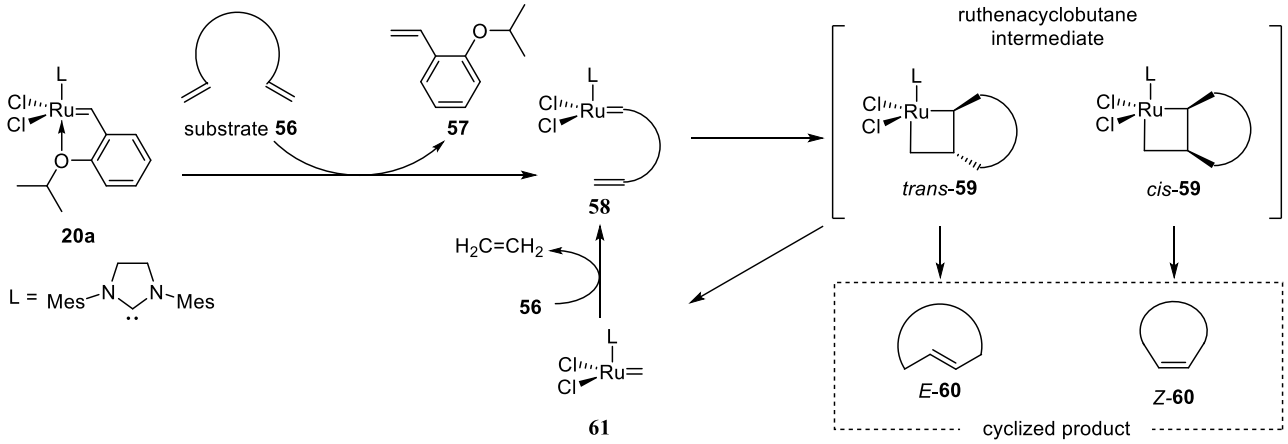


20a : R = H
20b : R = NO_2
20c : R = SO_2NMe_2

[a] See ref. [18d], [b] Reaction was performed at 40 °C.

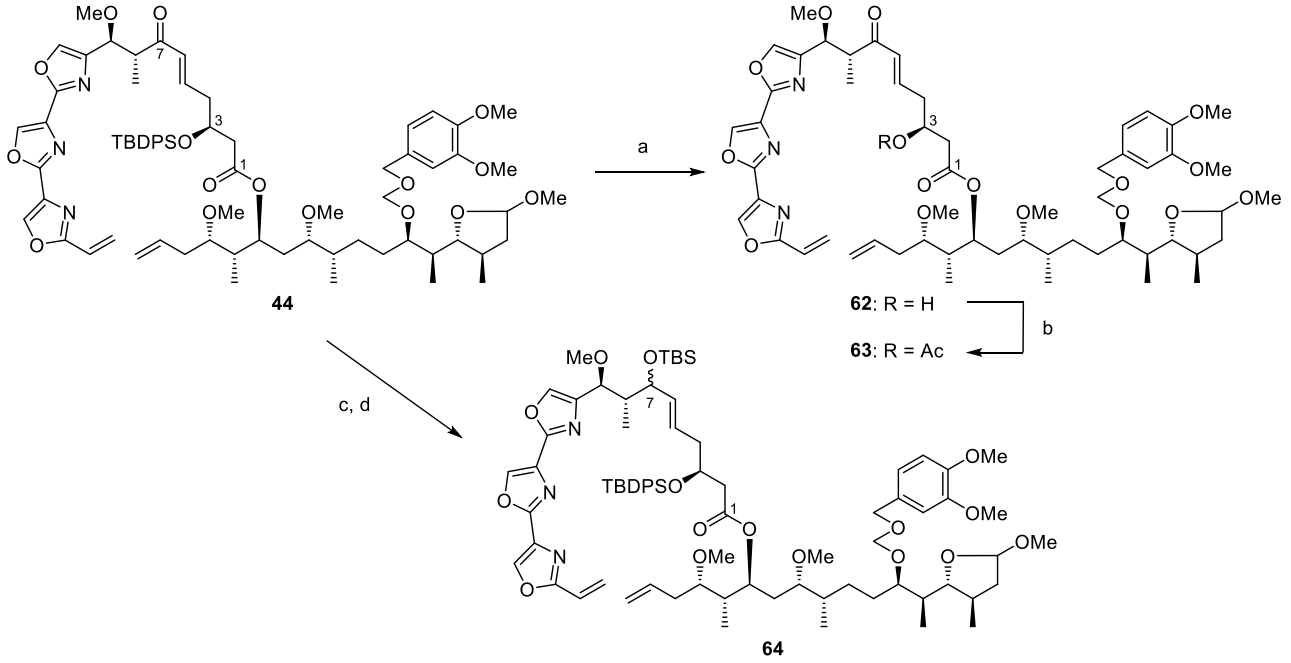
A plausible mechanism of the RCM using the second generation Hoveyda–Grubbs catalyst (**20a**) is shown in Scheme 3-8.[33,34] The reaction of catalyst **20a** with one olefin in substrate **56** results in the release of *O*-isopropoxy styrene (**57**) and the formation of carbene complex **58**. Subsequent intramolecular reaction of the remaining olefin through the ruthenacyclobutane intermediate (*trans*/*cis*)-**59** provides cyclized product (*E*/*Z*)-**60** and carbene complex **61**. The *E* and *Z* isomers of **60** are stereospecifically yielded from the corresponding *trans*- and *cis*-ruthenacyclobutane intermediate **59**, respectively. Carbene complex **61** reacts with substrate **56** and the propagation cycle to generate cyclized product **60** is continued with the release of ethylene.

Generally, the RCM providing medium and large rings tends to result in a mixture of *E* and *Z* isomers.[35] The product stereoselectivity is controlled kinetically and thermodynamically, and this is affected by various reaction conditions including solvent, temperature, catalyst, and substrate. The *E*/*Z* ratio also depends on the product reactivity for catalysts due to essentially reversible metathesis reaction. For example, the *Z* to *E* isomerization of the product was reported during the RCM that provided a 14-membered macrocycle possessing a simple 1,2-disubstituted olefin.[36] Thus the author examined the isomerization of *E* and *Z* isomers of the macrolactone **39** with the use of catalyst **20c** in refluxing dichloromethane, but isomerization was not observed. It was thought that the macrolactone **39** hardly reacts with the catalyst **20c** due to the low reactivity of styrene-like electron deficient oxazolyl olefin. Then, the author considered that the stereoselectivity of **39** depends on the *trans*/*cis* ratio of the ruthenacyclobutane intermediates. So, the author tried to modify the steric hinderance and flexibility in **44**, such as the C3 TBDPS group and the C7 α,β-unsaturated ketone moiety.



Scheme 3-8. The RCM reaction mechanism of the second generation Hoveyda–Grubbs catalyst (**20a**).

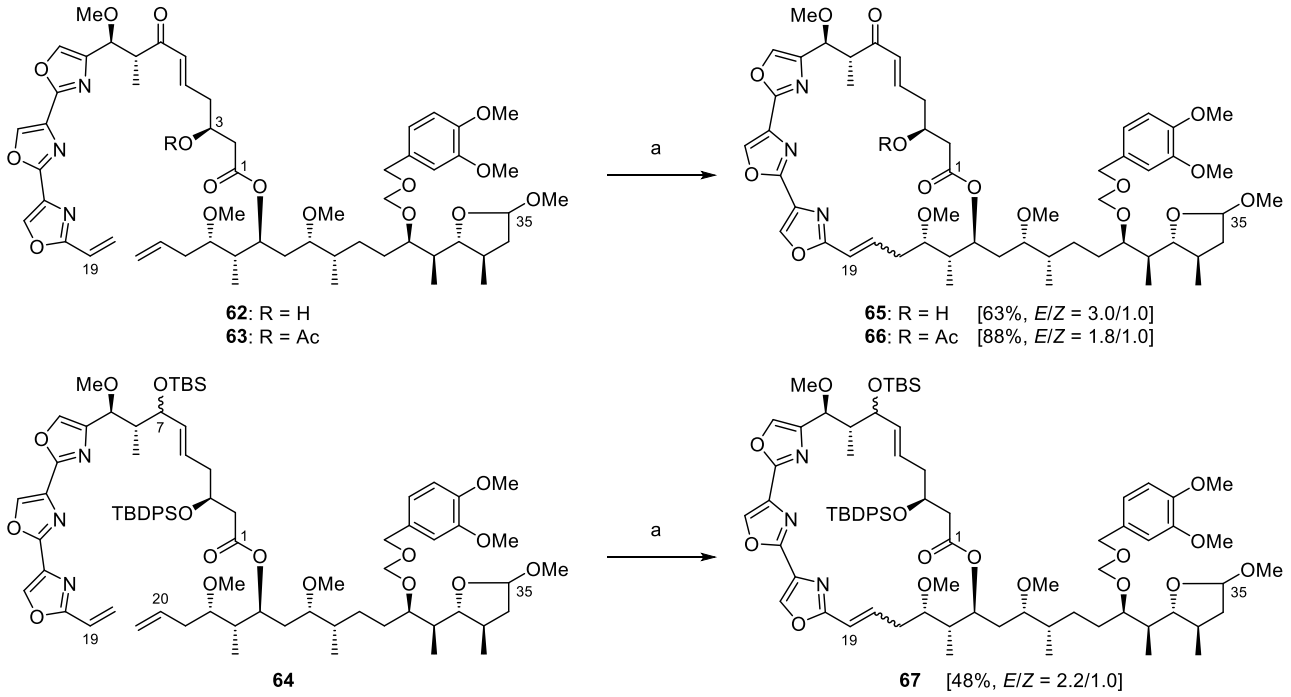
To enhance flexibility of macrocyclic structures, the C3 TBDPS or the C7 ketone groups in **44** were modified (Scheme 3-9). Treatment of **44** with TBAF and acetic acid in THF afforded C3 hydroxy analog **62**. Acetylation of **62** by the treatment with acetic anhydride afforded C3 acetoxy analog **63**. Additionally, Luche reduction of **44** followed by the TBS protection of the resulting allylic alcohol gave C7 silyloxy analog **64** with a 10:1 diastereomeric mixture at C7.



Scheme 3-9. Derivatization at C3 and C7 positions of **44**. Reagents and conditions: a) $^{n}Bu_4NF$–AcOH (1:1), THF, rt, 95%; b) Ac_2O, DMAP, pyridine, rt, 96%; c) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, −20 °C, 91% (*dr* = 10:1); d) TBSCl, imidazole, DMF, rt, 93%.

The RCM reactions for C3 and C7 modified analogs were examined (Scheme 3-10). Treatment of C3 hydroxy analog **62** with 30 mol% Zhan catalyst 1B (**20c**) in dichloromethane at reflux gave C3 hydroxy macrolactone **65**. The stereoselectivity was improved to 3.0/1, but the yield was lower than the case of **44**. On the other hand, the reaction of C3 acetoxy analog **63** in the same condition proceeded in higher yield, but the stereoselectivity was almost the same with the case of **44**. The RCM reaction of C7 silyloxy analog **64** also provided C7 silyloxy macrolactone **67** with a little improvement of *E*/*Z* ratio to 2.2/1, but the yield was low since the C5–C6 olefin was partially cleaved by the catalyst **20c**.

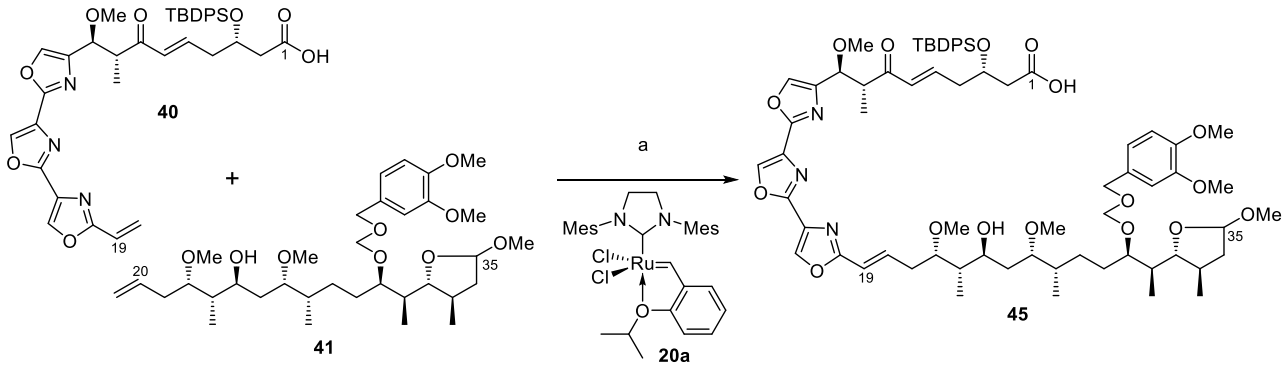
As a result, the stereoselectivity was not significantly improved compared with the RCM of **44**.



Scheme 3-10. RCM of C3 and C7 modified analogs. Reagents and condition: a) **20c** (30 mol%), CH_2Cl_2 (0.9 mM), reflux, 24 h.

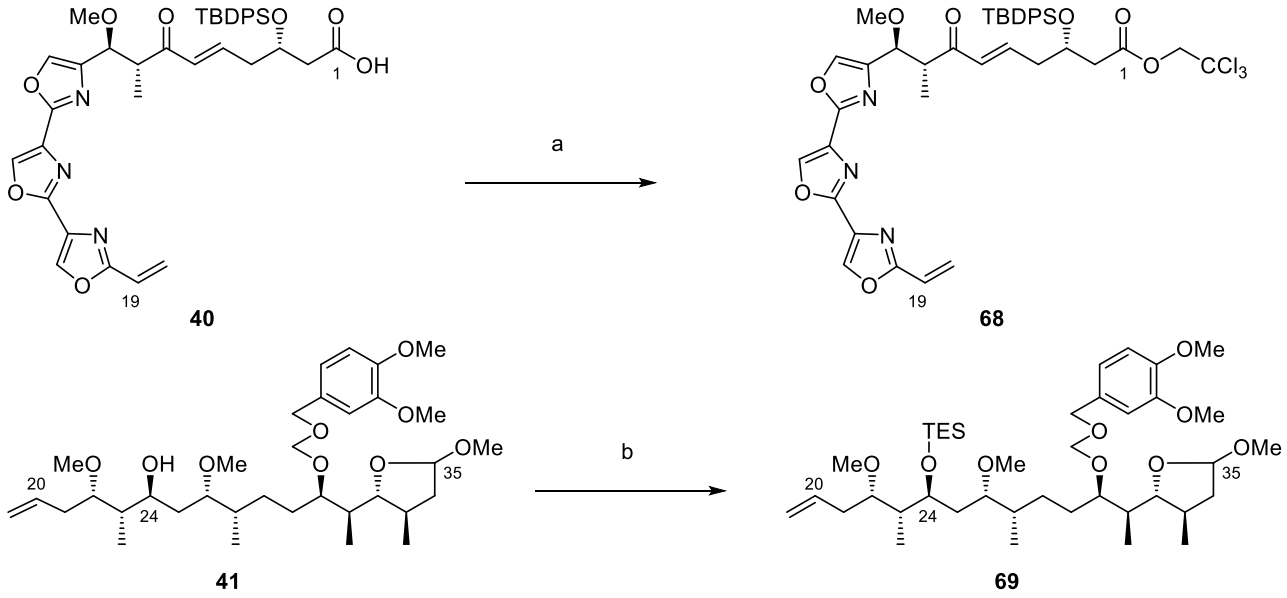
3-6. Synthesis of the macrolactone through CM

Next, the author attempted CM/macrolactonization approaches to synthesize macrolactone **39**. The CM of the C1–C19 segment **40** and the C20–C35 segment **41** was examined (Scheme 3-11). However, the yield of seco acid **45** was low (18%, *E*/*Z* ratio was not determined) and starting materials were recovered (53% for **40**, 64% for **41**).



Scheme 3-11. CM of the C1–C19 segment **40** and the C20–C35 segment **41**. Reagents and condition: a) **20a** (30 mol%), CH_2Cl_2, reflux, 18% .

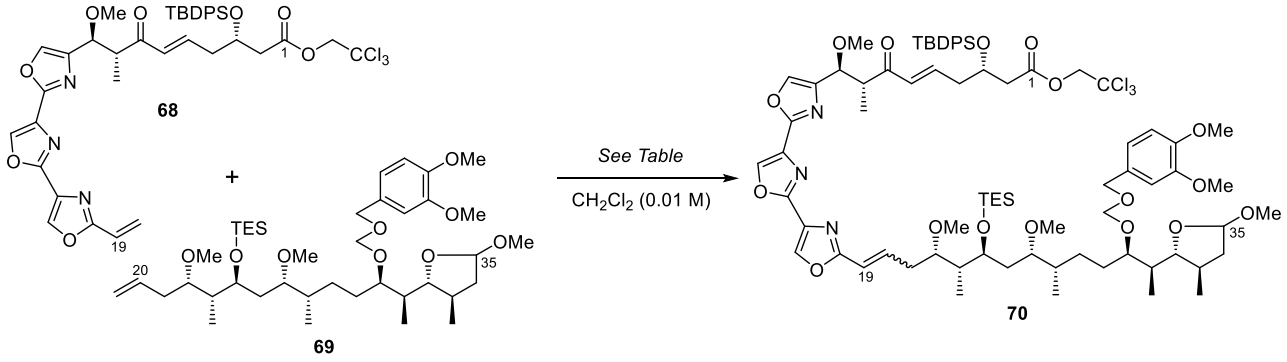
Since polar functional groups in the segments **40** and **41** appeared to quench the metathesis catalyst, then the author decided to protect these functional groups (Scheme 3-12). Condensation of **40** with 2,2,2-trichloroethanol by using EDC·HCl and DMAP afforded trichloroethyl (TCE) ester **68**. The secondary hydroxy group in **41** was protected with the use of triethylsilyl (TES) chloride and imidazole to give TES ether **69**.



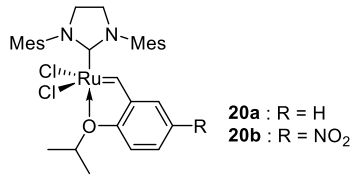
Scheme 3-12. Protection of the C1–C19 segment **40** and the C20–C35 segment **41**. Reagents and conditions: a) 2,2,2-trichloroethanol, EDC·HCl, DMAP, CH_2Cl_2, rt, 89%; b) TESCl, imidazole, DMF, rt, 99%.

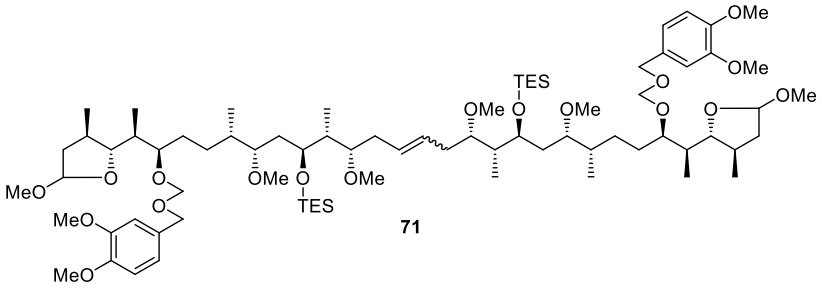
The CM of the protected segments **68** and **69** with 20 mol% of catalyst **20a** in dichloromethane at reflux gave the coupling product **70** in 72% yield with a more *E*-selective manner (*E*/*Z* = 5.5/1.0, Table 3-2, entry 1). An *E*/*Z* mixture of **70** was separated by a column chromatography. In this reaction, the dimer **71** of segment **69** also generated. The terminal olefin in segment **69** is electron rich and more reactive toward metathesis catalysts than that of segment **68** which is an electron deficient styrene-like alkene.[37] When a small excess amount of **69** (1.2 eq) was used to facilitate coupling reaction, **70** was obtained with the highest yield (77%, entry 2). Additionally, the use of Grela catalyst (**20b**) at the same condition in entry 1 shortened the reaction time (entry 3). The CM using catalyst **20b** partially proceeded at rt (entry 4) and **70** was obtained with the highest stereoselectivity (*E*/*Z* = 5.7/1.0).

Table 3-2. Synthesis of coupling product **70** through CM.

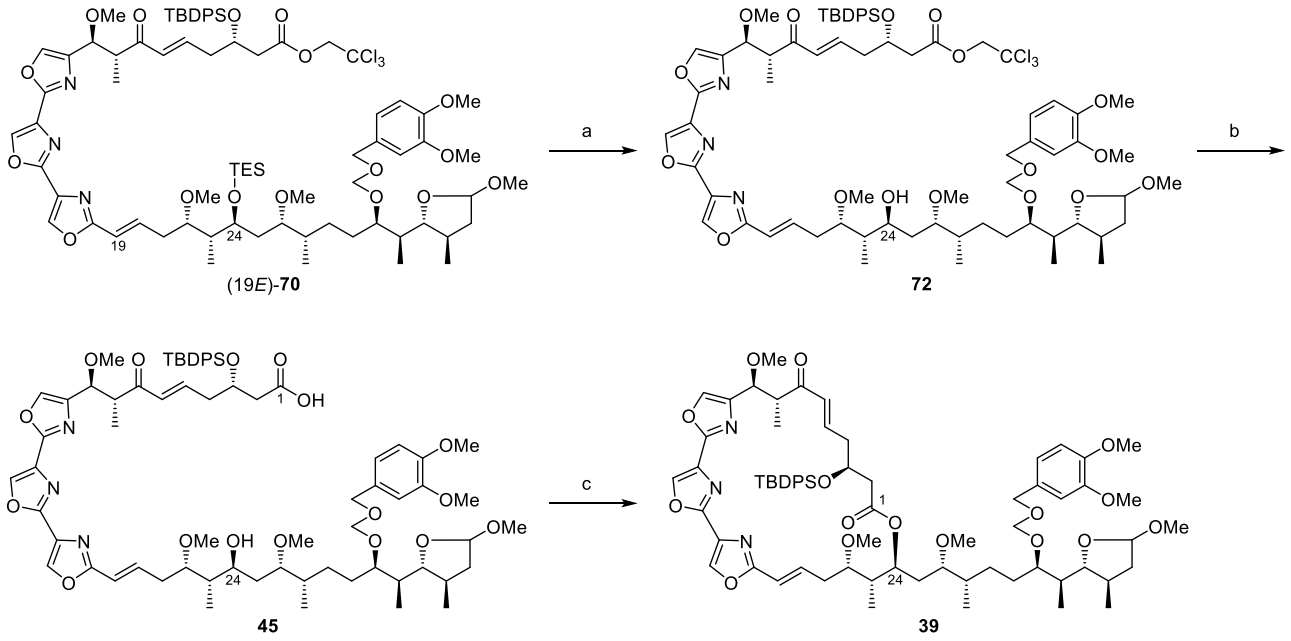


entry	**68** / **69**	catalyst (20 mol%)	temp.	time (h)	yields (%)	recovery of sm (%)		yield of **71** (%)
					70 (19*E* / 19*Z*)	**68**	**69**	
1	1 / 1.0	**20a**	reflux	25	72 (5.5 / 1.0)	20	7	8
2	1 / 1.2	**20a**	reflux	20	77 (5.0 / 1.0)	16	14	19
3	1 / 1.0	**20b**	reflux	9	72 (5.0 / 1.0)	20	10	18
4	1 / 1.0	**20b**	rt	24	43 (5.7 / 1.0)	39	40	10





Next, the author examined macrolactonization (Scheme 3-13). To remove the C24 TES group, 19*E*-**70** was treated with acetic acid to give secondary alcohol **72**. The TCE group in **72** was removed by the treatment with zinc in acetate buffer to afford seco acid **45**. Macrolactonization of **45** using Yamaguchi procedure readily proceeded to give macrolactone **39**.

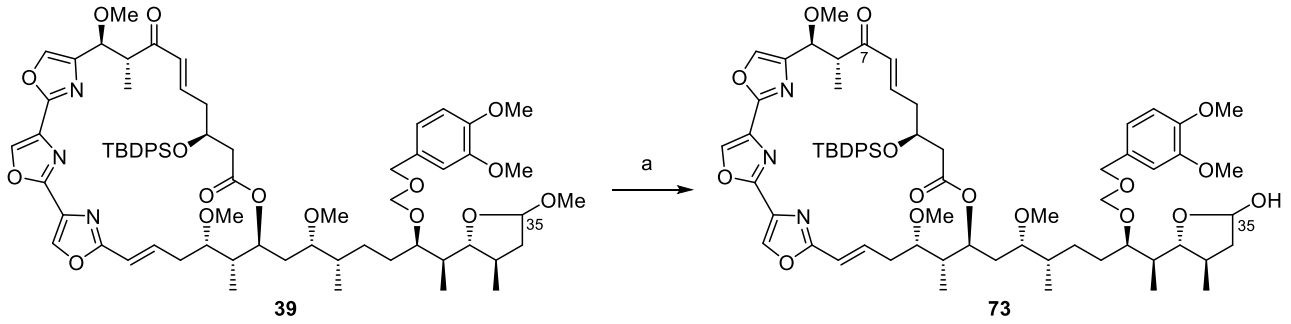


Scheme 3-13. Synthesis of macrolactone **39**. Reagents and conditions: a) AcOH–THF–H_2O (4/4/1 [v/v/v]), rt, 100%; b) Zn, 1 M NH_4OAc aq., THF, rt, 93%; c) 2,4,6-trichlorobenzoyl chloride, $^iPr_2NEt$, benzene, rt, then DMAP, benzene, rt, 77%.

As a result, the CM approach to synthesize macrolactone **39** was preferred to the RCM approach with respect to the stereoselectivity at the C19 double bond. On the other hand, the overall yields of **39** from the C20–C35 segment **41** was almost same in both approach (esterification/RCM approach: 44% in 2 steps from **41**, CM/macrolactonization approach: 45% in 4 steps from **41**). These routes have both advantages and disadvantages. The esterification/RCM approach enabled the author to access macrolactone **39** in 2 steps but showed moderate stereoselectivity at the C19 double bond. Meanwhile, the CM/macrolactonization approach was more selective, but additional protection and deprotection steps were needed.

3-7. Synthesis of mycalolides A and B

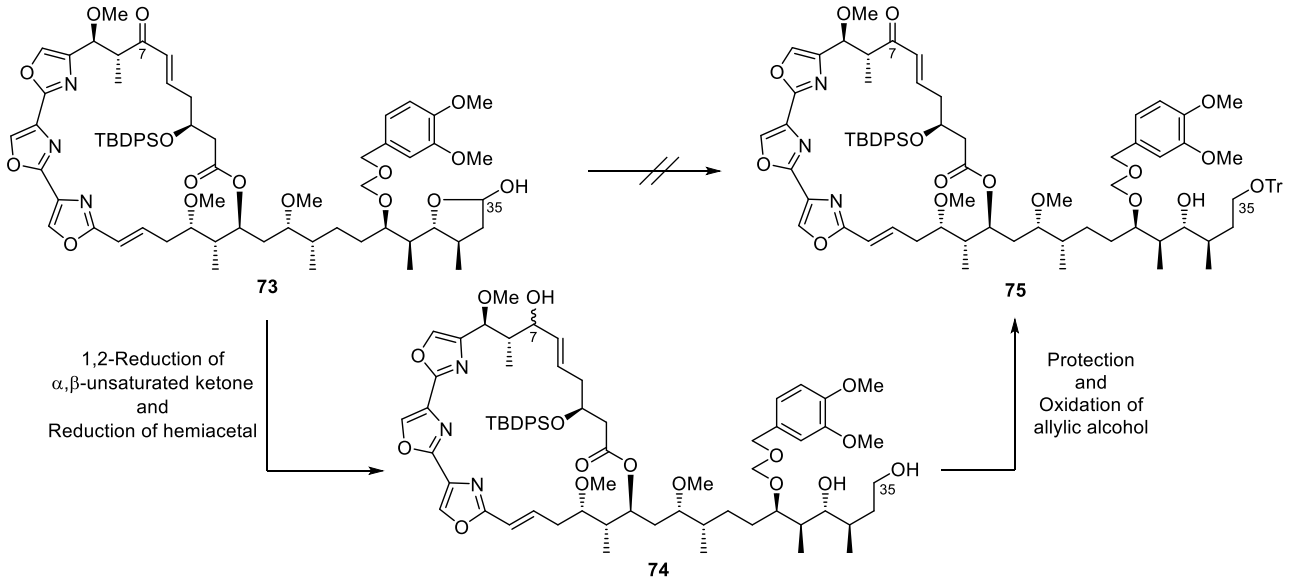
Synthesis of the key macrolactone **39** was completed, so the author attempted the last functionalization toward total syntheses of mycalolides A and B. Acidic hydrolysis of the C35 methyl acetal in **39** afforded hemiacetal **74** (Scheme 3-14).



Scheme 3-14. Synthesis of hemiacetal **73**. Reagents and condition: a) 1 M HCl, DME, rt, 94%.

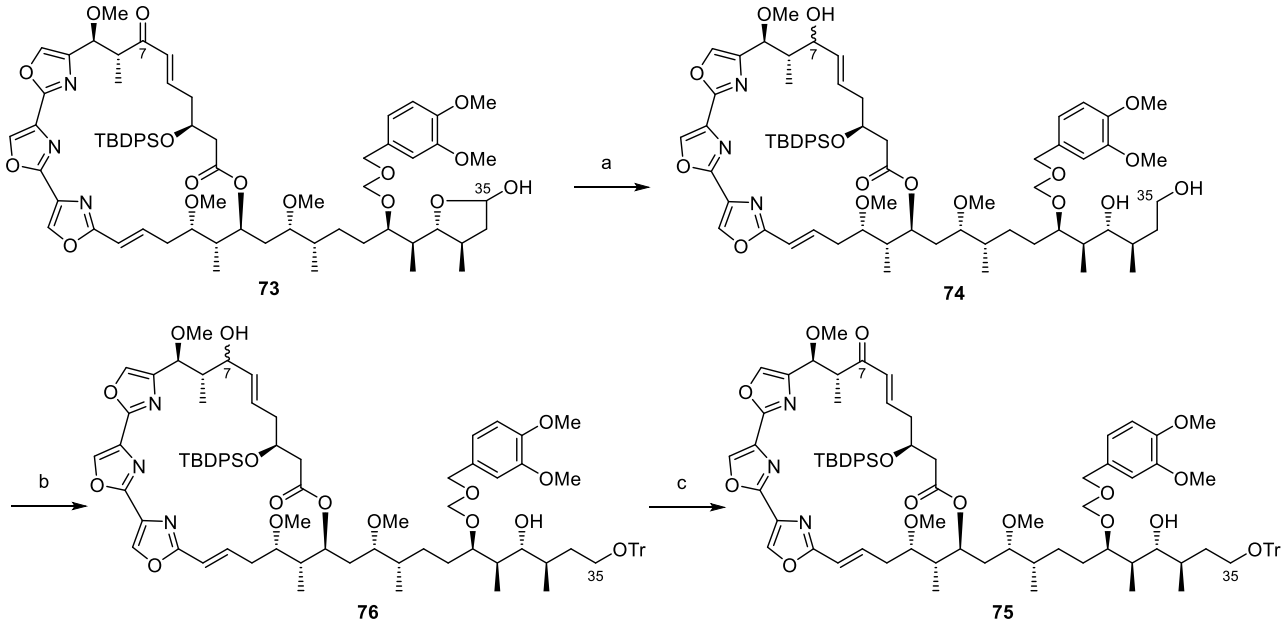
Next, reduction of the five-membered cyclic hemiacetal in **73** using hydride reagents were examined. In the previous studies by the collaborator, sodium trimethoxyborohydride was used, and the hemiacetal in **73** was completely converted into 1,4-diol. However, 1,4-reduction of the C7 α,β-unsaturated ketone in **73** was competed.[38]

In general, hemiacetals are easily reduced by hydride reagents. However, 5- or 6-membered cyclic hemiacetals are relatively stable. Therefore, the author thought the selective reduction of the hemiacetal moiety in **73** was difficult without the reductions of C7 α,β-unsaturated ketone moiety. Then the author planned to synthesize mono-protected 1,4-diol **75** through triol **74** (Scheme 3-15). Triol **74** could be synthesize by 1,2-reduction of the α,β-unsaturated ketone and reduction of the hemiacetal in **73**. After protection of the primary hydroxy group in **74**, oxidation of allylic alcohol could afford **75**.



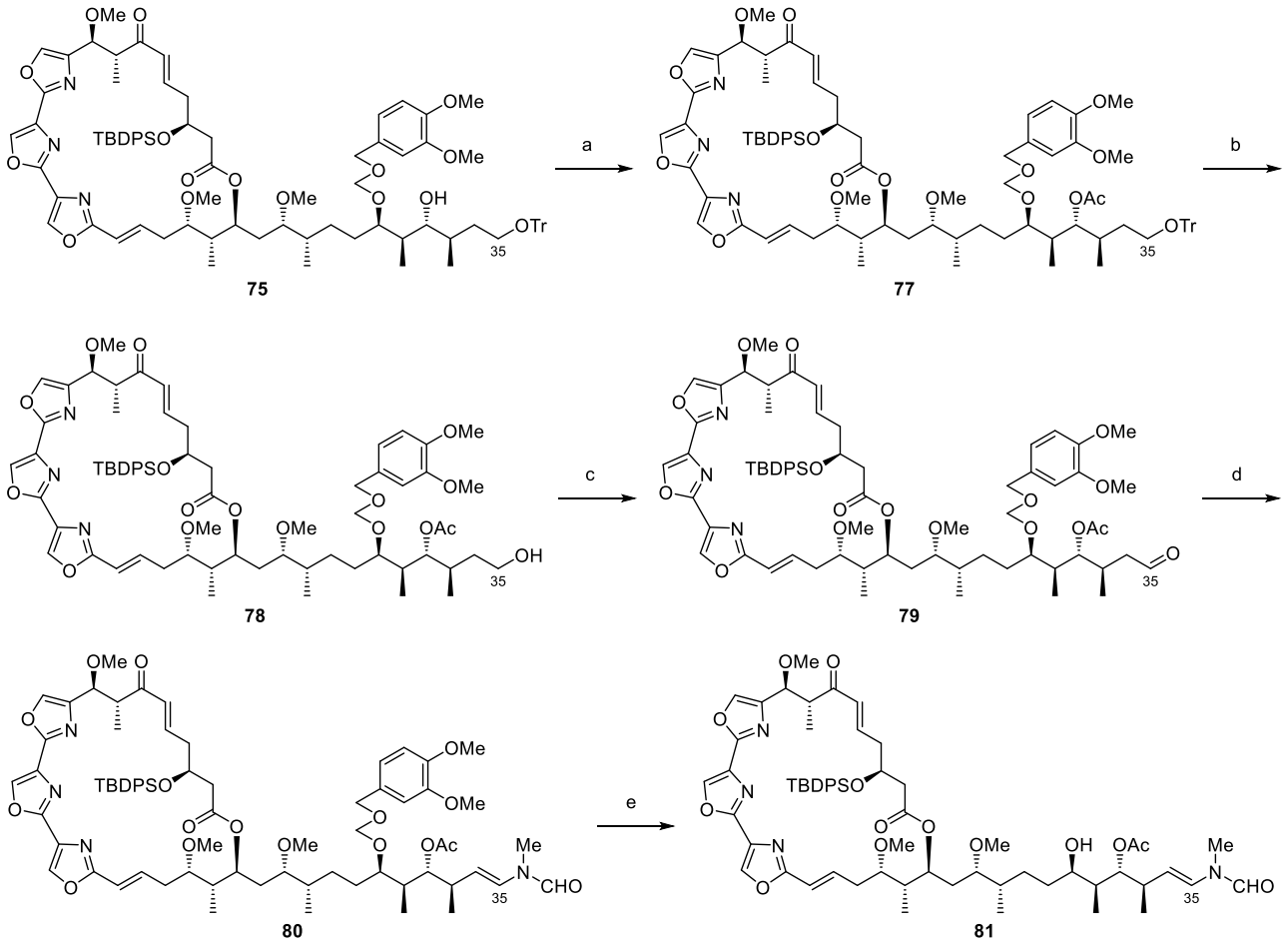
Scheme 3-15. Synthetic route for mono-protected 1,4-diol **75**.

Luche reduction of hemiacetal **73** smoothly proceeded by using cerium(III) chloride heptahydrate and sodium borohydride in methanol at –20 °C to rt to give triol **74** quantitatively. The diastereomeric ratio at the C7 hydroxy group was 10:1. Protection of the primary alcohol in triol **74** with triphenylmethyl (Tr) chloride afforded trityl ether **76**. Then oxidation of the allylic alcohol in **76** with manganese(IV) oxide yielded α,β-unsaturated ketone **75**.



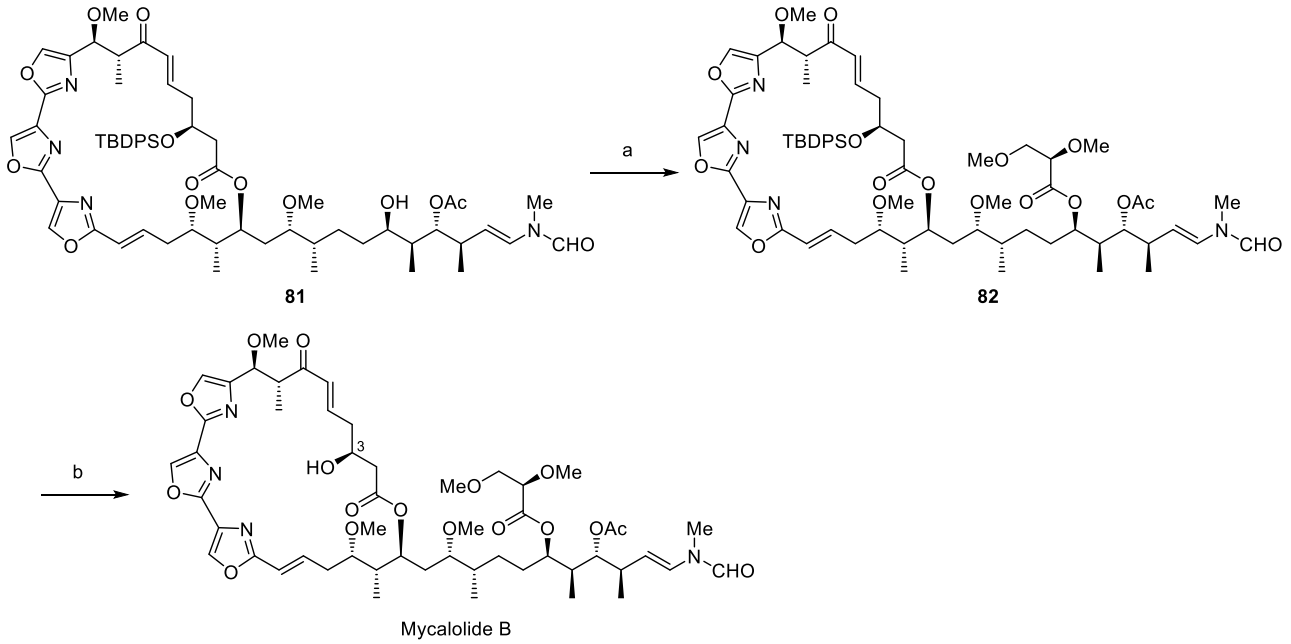
Scheme 3-16. Synthesis of α,β-unsaturated ketone **75**. Reagents and conditions: a) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, –20 °C to rt, 100%; b) TrCl, pyridine, rt, 82%; c) MnO_2, CH_2Cl_2, rt, 80%.

Next, enamide formation is illustrated in Scheme 3-17. Acetylation of the secondary alcohol in **75** with acetic anhydride in pyridine gave acetate **77**. Subsequent removal of the trityl group with formic acid in ether gave primary alcohol **78**, and oxidation of **78** with Dess–Martin periodinane gave aldehyde **79**. Dehydrating condensation of **79** with *N*-methylformamide under acidic conditions gave an enamide **80**. This reaction was stopped before completion due to the competition of enamide formation and elimination of the methoxy, acetoxy, and DMBOM ether groups, and unreacted aldehyde **79** were separated from the product. Treatment of the enamide **80** with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) afforded secondary alcohol **81**.



Scheme 3-17. Synthesis of secondary alcohol **81**. Reagents and conditions: a) Ac_2O, pyridine, rt, 97%; b) HCO_2H, Et_2O, rt, 77%; c) Dess–Martin periodinane, pyridine, CH_2Cl_2, rt, 99%; d) MeNHCHO, PPTS, hydroquinone, benzene, reflux; e) DDQ, ^{t}BuOH, 1.0 M phosphate buffer (pH 6.0), CH_2Cl_2, rt, 29% in 2 steps.

Finally, condensation of **81** with 2,3-di-*O*-methyl-D-glyceric acid by using Yamaguchi procedure, and the subsequent removal of the TBDPS group in **82** by TBAF and acetic acid provided mycalolide B (Scheme 3-18). The ^{1}H and ^{13}C NMR spectra were shown in Figures 3-1 and 3-2. The ^{1}H NMR spectrum of synthetic mycalolide B was consistent with that of natural one.[6a] The 5.47 ppm proton signal (which was not shown in the literature[6a]) was determined as the C3 hydroxyl group based on hydrogen-deuterium exchange experiment. The ^{13}C NMR spectrum and optical rotation of synthetic mycalolide B were identical to those of natural product. TLC and HPLC analyses also revealed that synthetic and natural mycalolide B were completely identical.



Scheme 3-18. Synthesis of mycalolide B. Reagents and conditions: a) 2,3-*O*-dimethyl-D-glyceric acid, 2,4,6-trichlorobenzoyl chloride, Et_3N, DMAP, benzene, rt, 71%; b) $^{n}Bu_4NF$–AcOH (1:1), THF, rt, 98%.

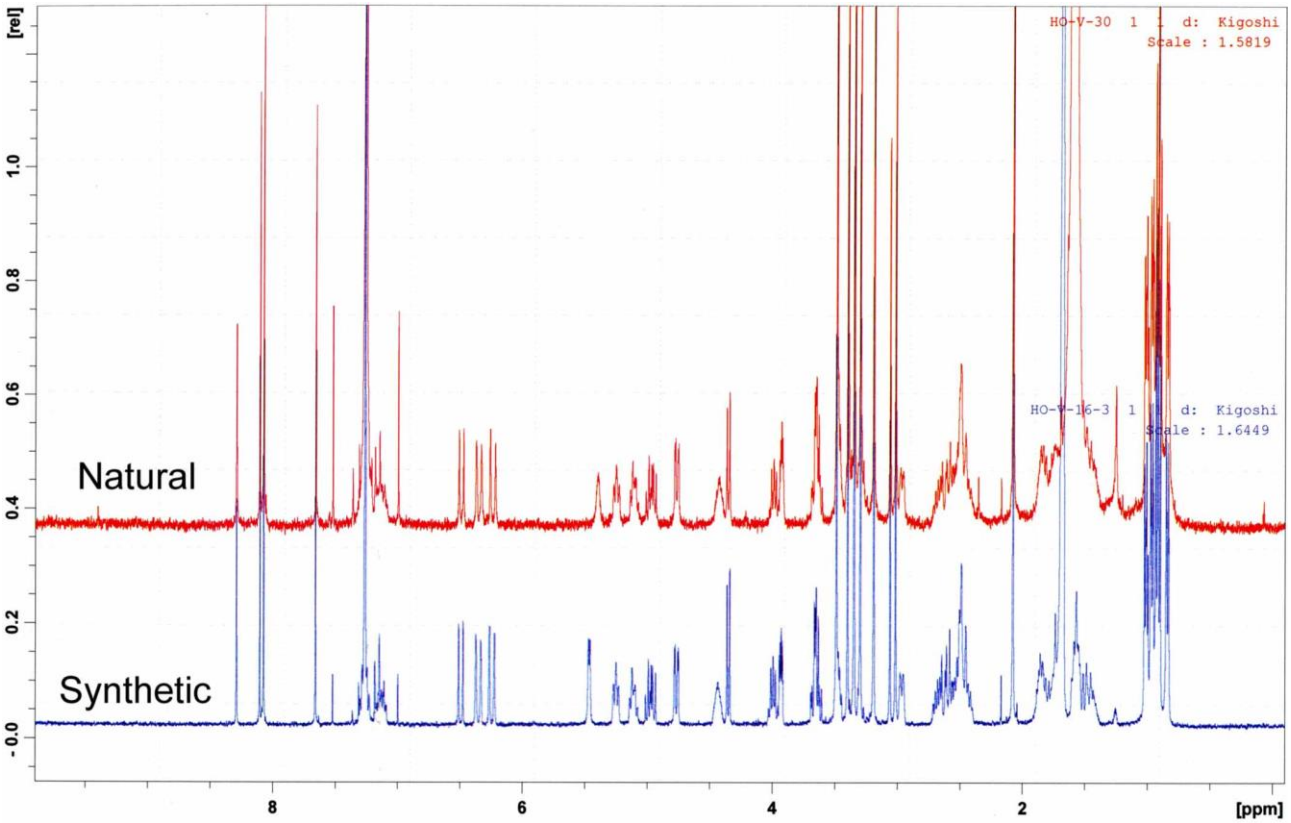


Figure 3-1. ^{1}H NMR spectra of natural and synthetic mycalolide B (400 MHz, $CDCl_3$).

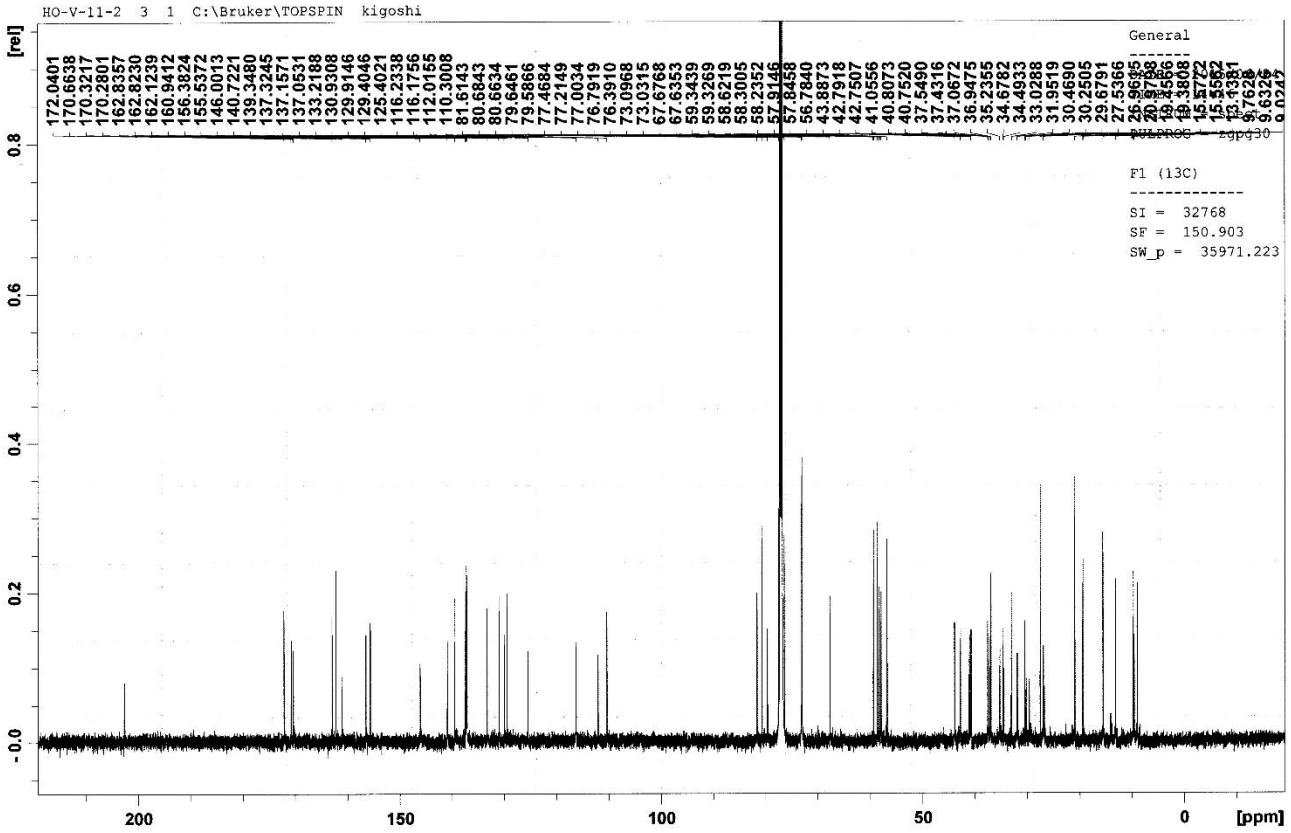
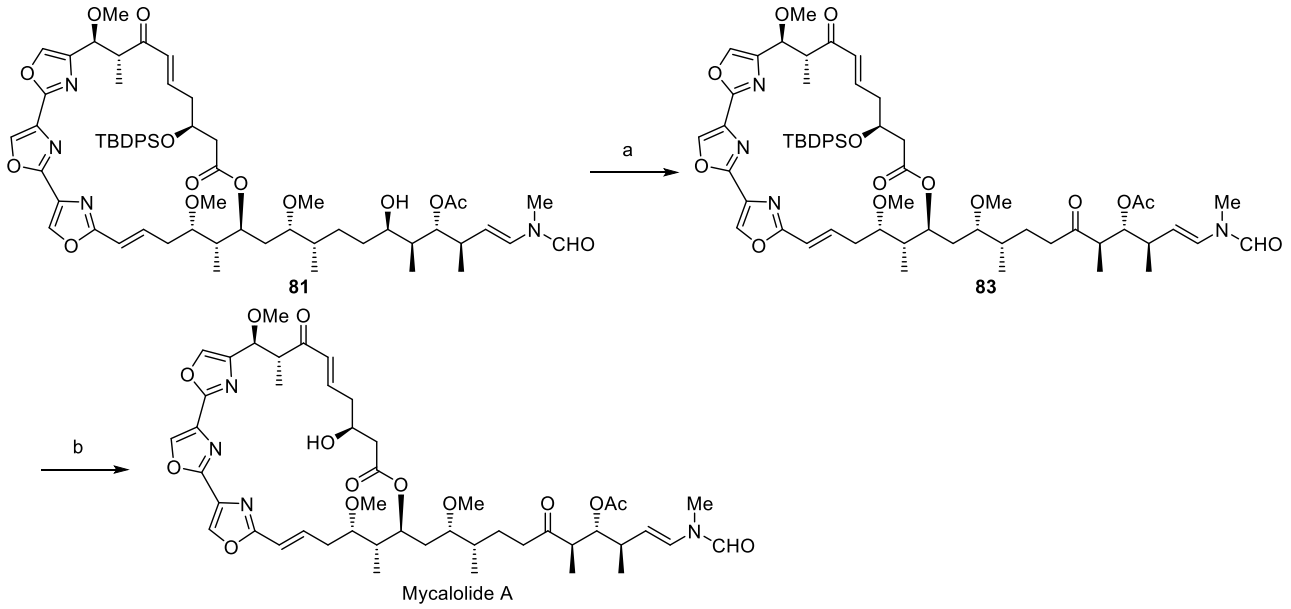


Figure 3-2. ^{13}C NMR spectrum of synthetic mycalolide B (150 MHz, $CDCl_3$).

Additionally, oxidation of secondary alcohol **81** with Dess–Martin periodinane gave TBDPS-protected mycalolide A (**83**)[14](Scheme 3-19), and removal of the TBDPS group in **83** obtained mycalolide A. The ^{1}H NMR spectrum was shown in Figure 3-3. The ^{1}H NMR spectrum of synthetic mycalolide A was consistent with that of the natural products.[6a]



Scheme 3-19. Synthesis of mycalolide A. Reagents and conditions: a) Dess–Martin periodinane, pyridine, CH_2Cl_2, 0 °C; b) ^{n}Bu$_4$NF–AcOH (1:1), THF, rt, 14% in 2 steps.

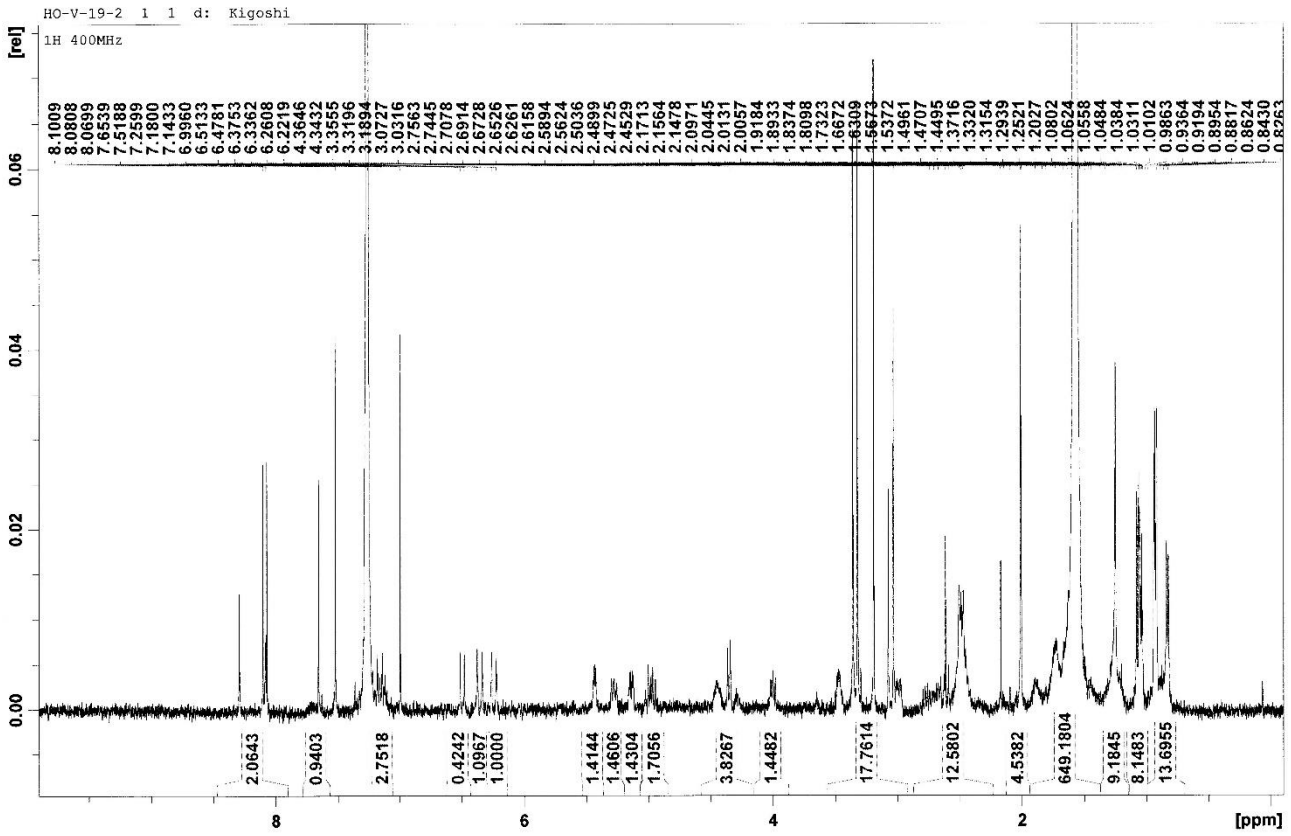
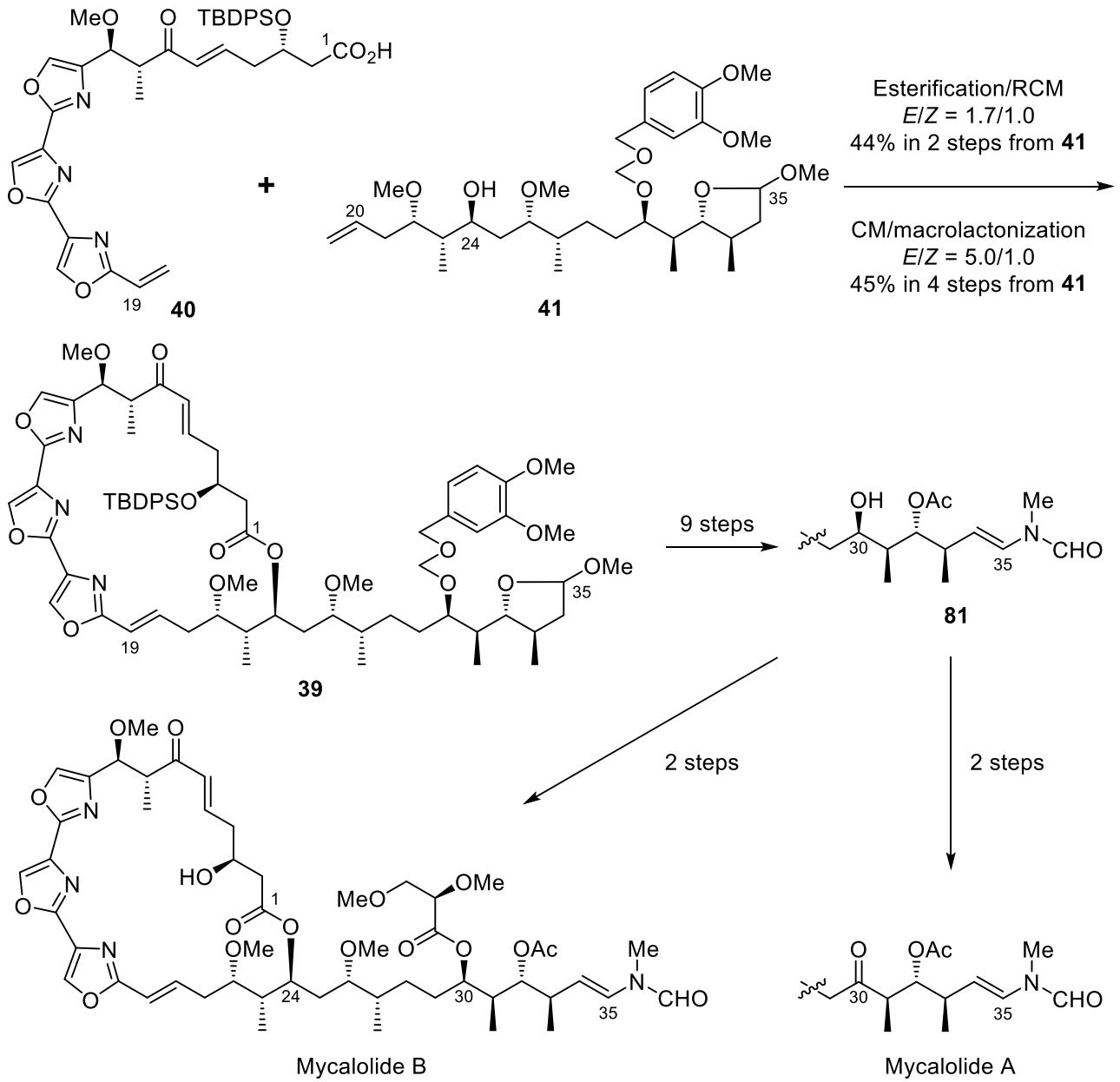


Figure 3-3. ^{1}H NMR spectrum of synthetic mycalolide A (400 MHz, $CDCl_3$).

3-8. Conclusion

An asymmetric total synthesis of trisoxazole marine macrolides, mycalolides A and B, is described (Scheme 3-20). The assembly of the C1–C19 segment **40** and the C20–C35 segment **41** using esterification/RCM or CM/macrolactonization as key steps afforded macrolactone **39**, which has all main carbon-chain in mycalolides. Subsequent functional group transformation including the installation of terminal enamide and dimethyl glyceric ester accomplished the first total synthesis of mycalolide B. Also, oxidation of the C30 hydroxyl group in **81** followed by the removal of protecting group achieved the second total synthesis of mycalolide A.



Scheme 3-20. Total synthesis of mycalolides A and B.

4. Conclusion

In this research, the author described the synthesis and biological activities of the trisoxazole macrolactone analogs of mycalolides, and the total synthesis of mycalolides A and B. The author aimed to develop a new synthetic route for mycalolides and understand their biological activities.

In chapter 1, the author mainly outlined the trisoxazole macrolides from the viewpoint of their various structures and biological activities. In chapter 2, the synthesis of the trisoxazole macrolactone analogs (19*E*)- and (19*Z*)-**2** was accomplished through the use of NHK coupling and RCM as key steps (Figure 4-1). Both of the analogs exhibited moderate cytotoxicity against tumor cells and showed no antifungal and actin-depolymerizing activities. In chapter 3, the first total synthesis of (−)-mycalolide B and the second total synthesis of (−)-mycalolide A were accomplished through the use of olefin metathesis and esterification as well as NHK coupling, Julia–Kocienski olefination and enamide formation as key steps (Figure 4-2).

Through this research, the author showed that both the macrolactone ring and the side-chain part are important for the potent cytotoxicity of mycalolides. The author developed the new synthetic route of mycalolides using olefin metathesis. These findings are expected to contribute to the elucidation of the mode of actions of mycalolides, and the development of useful artificial molecules for actin-related biochemical studies.

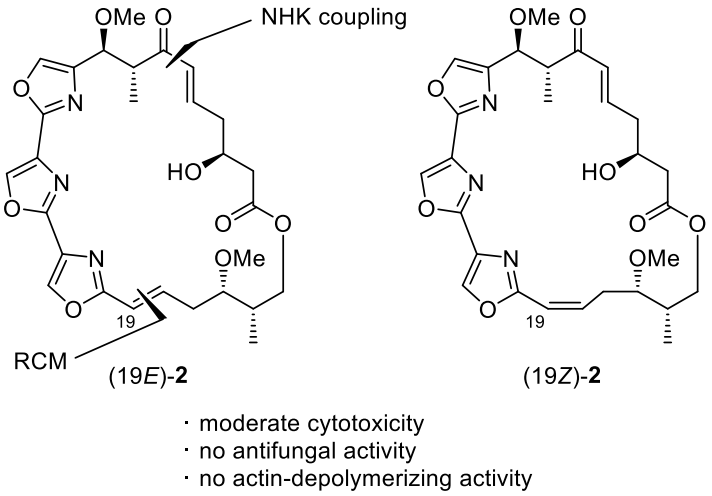


Figure 4-1. Structures of the trisoxazole macrolactone analogs **2** and its biological activities.

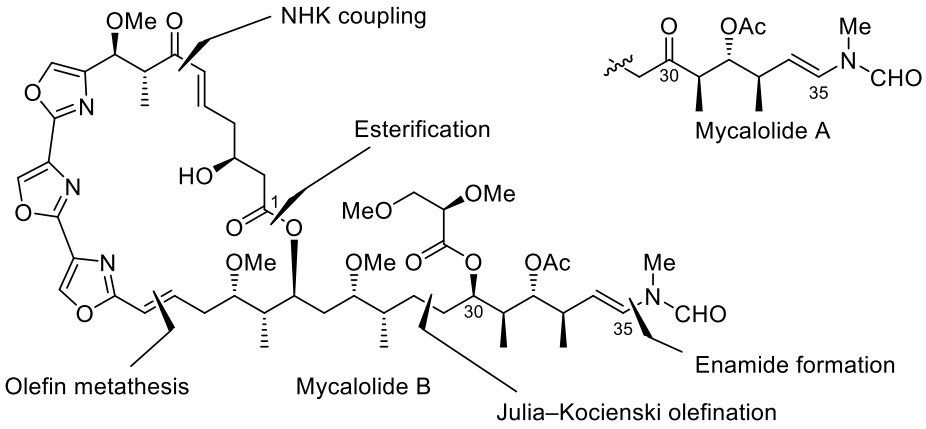


Figure 4-2. Total synthesis of mycalolides A and B.

Experimental Section

General experimental

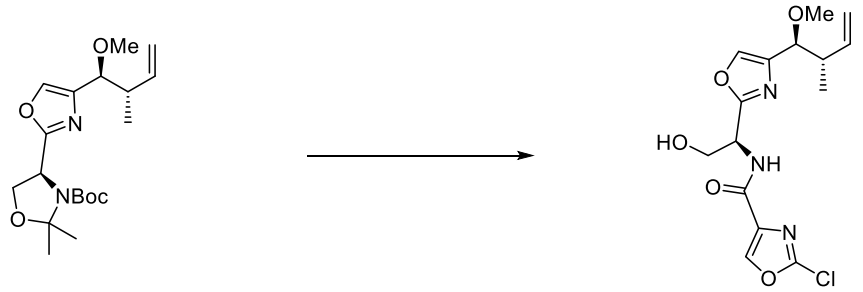
All chemicals were obtained commercially unless otherwise noted. Organic solvents and reagents for moisture-sensitive reactions were distilled by the standard procedure. Anhydrous CH_2Cl_2, THF, Et_2O, benzene, pyridine, EtOAc, α,α,α-trifluorotoluene, DMSO, and DMF were obtained commercially. Column chromatography was performed using silica gel BW-820MH or FL60D (75–200 or 45–75 μm, Fuji Silysia Co., Aichi, Japan) or a Yamazen preparative silica gel (40 μm). All moisture-sensitive reactions were performed under an atmosphere of nitrogen unless otherwise noted, and the starting materials were azeotropically dried with benzene before use. Merck precoated silica gel 60 F254 plates were used for TLC.

Spectroscopic analysis

^1H and ^{13}C NMR spectra were recorded on Bruker Biospin AVANCE 600 spectrometer (600 MHz for ^1H and 150 MHz for ^{13}C), AVANCE 500 spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C), AVANCE 400 spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C), or a JEOL EX-270 spectrometer (270 MHz for ^1H). Chemical shifts were reported in parts per million (ppm) with coupling constants (*J*) in hertz relative to the solvent peaks, δ_H 7.26 (residual $CHCl_3$) and δ_C 77.0 for $CDCl_3$, respectively. Optical rotations were measured with a JASCO DIP-1000 polarimeter using the sodium D line. IR spectra were recorded on a JASCO FT/IR-230 spectrometer. HR-ESIMS were measured on a JEOL AccuTOF CS spectrometer.

Synthesis and spectroscopic data

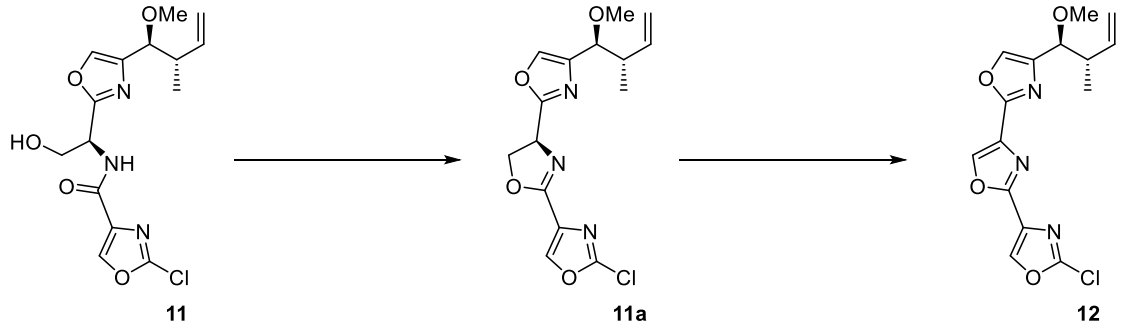
Synthesis and biological activities of trisoxazole macrolactone analogs of mycalolides



8 **11**

Amide 11. A mixture of (–)-methyl ether **8**[18c] (61. 9 mg, 0.169 mml) and 3.0 M HCl aq. (1.1 mL) in EtOAc (1.1 mL) was stirred at room temperature for 29 h under air. The mixture was diluted with Et_2O (2 mL) and extracted with water (2 mL × 3). The combined aqueous layer was concentrated and dried to give crude hydrochloride (50.6 mg) as a brown solid. The crude solid was used for the next reaction without further purification.

To a stirred solution of the crude hydrochloride (50.6 mg) in CH_2Cl_2 (1.7 mL) cooled at 0 °C were added triethyl amine (35.3 µL, 0.254 mmol), EDC·HCl (48.6 mg, 0.254 mmol), HOBt (34.3 mg, 0.254 mmol), and 2-chlorooxazole-4-carboxilic acid (**10**)[20] (27.4 mg, 0.186 mmol). After being stirred at room temperature for 12 h, the reaction mixture was quenched with 10% citric acid aq. (2 mL) and extracted CH_2Cl_2 (3 mL × 3). The combined extracts were washed with sat. $NaHCO_3$ aq. (4 mL) and brine, dried with Na_2SO_4, and concentrated. The crude oil was purified with a SiO_2 column chromatography (BW-820MH 2.0 g, hexane / EtOAc = 1/1) to give amide **11** (47.3 mg, 78%) as a pale yellow solid. **11**: R_f 0.55 ($CHCl_3$ / MeOH = 10:1); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.63 (d, *J* = 8.6 Hz, 1H), 7.54 (s, 1H), 5.85 (ddd, *J* = 7.6, 10.2, 17.5 Hz, 1H), 5.42 (dt, *J* = 8.6, 7.9 Hz, 1H), 5.04 (d, *J* = 10.2 Hz, 1H), 5.03 (d, *J* = 17.5 Hz, 1H), 4.26–4.18 (m, 1H), 4.01 (d, *J* = 6.2 Hz, 1H), 4.01–3.95 (m, 1H), 3.31 (s, 3H), 3.17 (m, 1H), 2.68 (ddq, *J* = 6.2, 7.6, 6.9 Hz, 1H), 0.99 (d, *J* = 6.9 Hz, 3H).

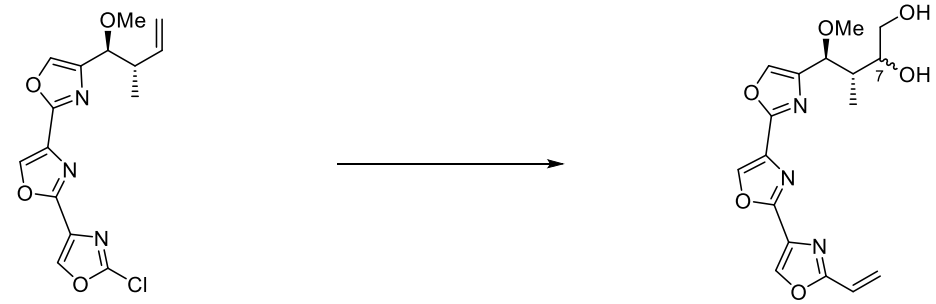


11 **11a** **12**

Ttisoxaxole 12. To a stirred solution of amide **11** (2.30 g, 6.43 mmol) in CH_2Cl_2 (64 mL) cooled at –78 °C was added DAST (1.9 mL, 14.1 mmol). After the reaction mixture was stirred at –78 °C for 7 h, it was wormed to 0 °C and stirred for 12 h additionally. The reaction was quenched with K_2CO_3 (2.38 g) and sat. $NaHCO_3$ aq. (70 mL), and extracted with CH_2Cl_2 (50 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude solid was purified with a SiO_2 column chromatography (BW-820MH 90 g, hexane / EtOAc = 3/2) to give oxazoline **11a** (1.85 g, 85%) as a pale yellow solid. **11a**: R_f 0.33 (hexane / EtOAc = 1:1); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.54 (s, 1H), 5.85 (ddd, *J* = 7.5, 10.8, 16.7 Hz, 1H), 5.51 (dd, *J* = 8.4, 10.3 Hz,

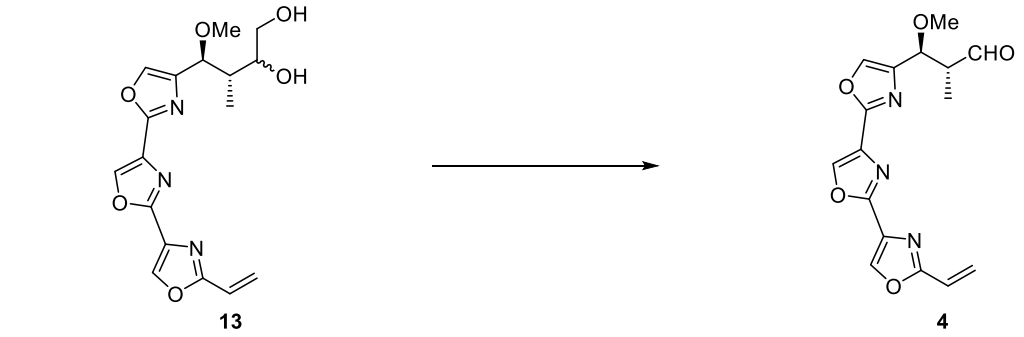
1H), 5.03 (d, *J* = 10.8 Hz, 1H), 5.02 (d, *J* = 16.7 Hz, 1H), 4.88–4.71 (m, 2H), 3.99 (d, *J* = 6.7 Hz, 1H), 3.29 (s, 3H), 2.67 (ddq, *J* = 6.7, 7.5, 6.9 Hz, 1H), 1.04 (d, *J* = 6.9 Hz, 3H).

To a stirred solution of oxazoline **11a** (13.6 mg, 40.3 μmol) in acetonitrile (2.0 mL) cooled at 0 °C were added DBU (19.5 μL, 130 μmol) and $BrCCl_3$ (12.0 μL, 122 μmol). After being stirred at room temperature for 48 h, the reaction mixture was quenched sat. NH_4Cl aq. (3 mL), and extracted with CH_2Cl_2 (3 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude solid was purified with a SiO_2 column chromatography (FL60D 0.5 g, hexane / EtOAc = 5/1 to 1/1) to give trisoxazole **12** (7.4 mg, 54%) as a colorless solid and recovered oxazoline **11a** (6.2 mg, 45%) as a pale yellow solid. **12**: R_f 0.55 (hexane / EtOAc = 1:1); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.30 (s, 1H), 7.63 (s, 1H), 5.87 (ddd, *J* = 7.5, 10.8, 16.7 Hz, 1H), 5.06–4.98 (m, 2H), 4.12 (d, *J* = 5.7 Hz, 1H), 3.34 (s, 3H), 2.74 (ddq, *J* = 5.7, 7.5, 7.0 Hz, 1H), 1.04 (d, *J* = 7.0 Hz, 3H).

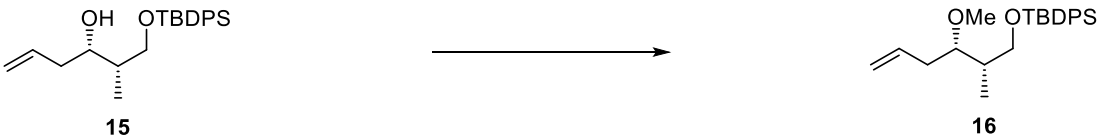


Diol 13. To a stirred solution of trisoxazole **12** (259.7 mg, 0.774 mmol) in THF (7.8 ml) at room temperature under air were added a 0.90 M solution of NMO in water (1.9 mL) and a 0.1 M solution of OsO_4 in ^{t}BuOH (1.55 mL). After being stirred for 15 h, the reaction mixture was cooled to 0 °C, diluted with EtOAc (10 mL), quenched sat. $Na_2S_2O_3$ aq. (10 mL), and stirred at room temperature for 1 h. After the reaction mixture was extracted with EtOAc (20 mL × 2), the aqueous layer was salted out and extracted with EtOAc (20 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated, to give crude diol (291.3 mg) as a pale yellow solid. The crude solid was used for the next reaction without further purification.

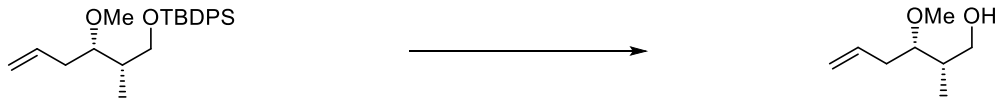
A mixture of crude diol (291.3 mg), bis(triphenylphospine)palladium (II) dichloride (108.7 mg, 0.155 mmol), and tributyl(vinyl)tin (1.1 mL, 3.87 mmol) in 1,4-dioxane (19.0 mL) was stirred at refluxing temperature for 46 h. The mixture was cooled to room temperature and concentrated. The crude oil was purified with a SiO_2 column chromatography (BW-820MH 50 g, $CHCl_3$ / MeOH = 1/0 to 50/1) to give diol **13** (231.4 mg, 83% in 2 steps, ca. 1:1 diastereomeric mixture at C7) as a pale yellow solid. **13**: R_f 0.52 ($CHCl_3$ / MeOH = 10:1); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.31 (s, 1H), 7.69 (s, 1H), 6.68 (dd, *J* = 11.1, 17.7 Hz, 1H), 6.34 (d, *J* = 17.7 Hz, 1H), 5.78 (d, *J* = 11.1 Hz, 1H), 4.40 [4.30] (d, *J* = 7.8 [6.5] Hz, 1H), 4.32–4.28 [4.05–3.95] (m, 1H), 3.80–3.55 [3.38–3.36] (m, 1H), 3.36 [3.38] (s, 1H), 2.40–2.55 (m, 1H), 1.45–1.25 (m, 1H), 0.78 [0.93] (d, *J* = 7.2 [7.0] Hz, 1H) Chemical shifts of the minor isomers are within parentheses (square brackets).



Aldehyde 4. To a stirred solution of diol **13** (29.7 mg, 82.2 μmol) in EtOH (2.1 mL) at room temperature under air was added a 0.14 M $NaIO_4$ in water (2.0 mL). After being stirred for 10 minutes, the reaction mixture was diluted with $CHCl_3$ (5 mL), quenched sat. $Na_2S_2O_3$ aq. (5 mL), and extracted with $CHCl_3$ (5 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude solid was purified with a SiO_2 column chromatography (BW-820MH 0.6 g, $CHCl_3$ only) to give aldehyde **4** (26.6 mg, 98%) as a pale yellow solid. **4**: R_f 0.60 ($CHCl_3$ / MeOH = 10:1); ^{1}H NMR (270 MHz, $CDCl_3$) δ 9.86 (d, *J* = 2.0 Hz, 1H), 8.34 (s, 1H), 8.32 (s, 1H), 7.71 (s, 1H), 6.68 (dd, *J* = 11.0, 17.6 Hz, 1H), 6.34 (d, *J* = 17.6 Hz, 1H), 5.78 (d, *J* = 11.0 Hz, 1H), 4.46 (d, *J* = 7.3 Hz, 1H), 3.35 (s, 3H), 3.04 (ddq, *J* = 2.0, 7.3, 7.0 Hz, 1H), 1.03 (d, *J* = 7.0 Hz, 3H).



Methyl ether 16. To a stirred solution of (–)-homoallylic alcohol **15**[22] (787 mg, 2.14 mmol, >99%ee, *syn*/*anti* = 93/7) and in CH_2Cl_2 (4.8 mL) cooled at 0 °C were added MeOTf (0.72 mL, 6.4 mmol) and 2,6-di-*tert*-butylpyridine (1.5 mL, 6.4 mmol). After the reaction mixture was stirred at room temperature for 20 h, the reaction was quenched with sat. $NaHCO_3$ aq. (15 mL) at 0°C, stirred for 45 min at room temperature, and extracted with CH_2Cl_2 (5 mL × 4). The combined extracts were washed with water and brine, dried with Na_2SO_4, and concentrated. The crude oil was purified with a Yamazen preparative SiO_2 column (30 g, hexane / EtOAc = 100/0 to 95/5) to give methyl ether **16** (737 mg, 90%, *syn*/*anti* = 93/7) as a colorless oil. **16**: R_f 0.41 (hexane / EtOAc = 9:1); $[\alpha]^D_{25}$ +9.0 (*c* 1.09 $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.72–7.65 (m, 4H), 7.46–7.36 (m, 6H), 5.81 (ddt, *J* = 17.2, 10.2, 7.0 Hz, 1H), 5.08 (d, *J* = 17.2 Hz, 1H), 5.05 (d, *J* = 10.2 Hz, 1H), 3.69 (dd, *J* = 10.0, 7.2 Hz, 1H), 3.54 (dd, *J* = 10.0, 6.1 Hz, 1H), 3.45 (dt, *J* = 3.9, 6.8 Hz, 1H), 3.36 (s, 3H), 2.36 (dddt, *J* = 13.9, 7.0, 6.8, 1.2 Hz, 1H), 2.23 (dddt, *J* = 13.9, 7.0, 6.8, 1.0 Hz, 1H), 1.87 (dddq, *J* = 7.8, 6.1, 3.9, 7.0 Hz, 1H), 1.08 (s, 9H), 0.89 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 135.6 (d, 4C), 135.5 (d), 134.0 (s), 133.9 (s), 129.5 (d, 2C), 127.6 (d, 4C), 116.5 (t), 80.6 (d), 65.9 (t), 57.9 (q), 38.7 (d), 35.6 (t), 26.9 (q, 3C), 19.3 (s), 10.9 (q); IR (CHCl3) 3073, 3009, 2962, 2932, 2859, 1640, 1589, 1472, 1428, 1389, 1362, 1217, 1112, 1088, 998, 918, 824, 705 cm^{-1}; HRMS (ESI) *m/z* 405.2207 (calcd for $C_{24}H_{34}NaO_2Si$ $[M+Na]^+$, Δ –1.9 mmu).

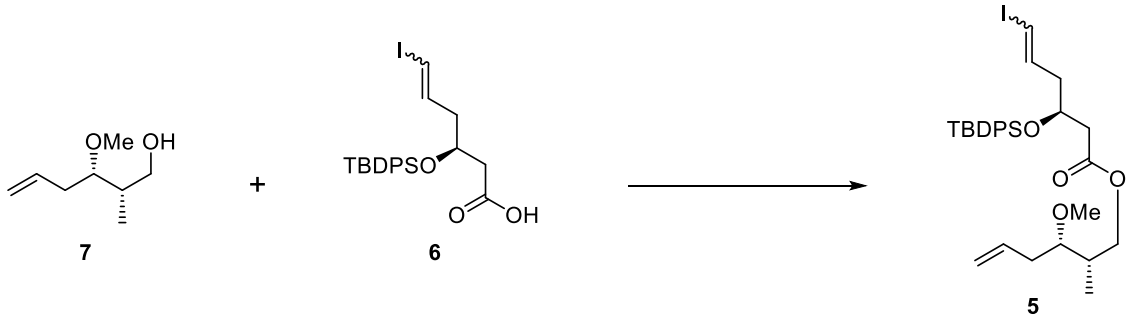


16 7

Primary alcohol 7. To a stirred solution of methyl ether **16** (1.01 g, 2.64 mmol, *syn*/*anti* = 93/7) in dry THF (18 mL) cooled at 0 °C was added a 1.0 M solution of TBAF in THF (3.2 mL, 3.2 mmol). After being stirred for 4 h at room temperature, the reaction was quenched with sat. NH_4Cl aq. (10 mL), and extracted with Et_2O (10 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (BW-820MH 40 g, hexane / Et_2O = 9/1 to 1/1) to give primary alcohol **7** (366 mg, 96%, *syn*/*anti* = 93/7) as a colorless oil. **7**: R_f 0.15 (hexane / Et_2O = 7:3); $[\alpha]^D_{25}$ +17.2 (*c* 1.31, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 5.81 (ddt, *J* = 17.0, 10.7, 7.2 Hz, 1H), 5.10 (d, *J* = 17.0 Hz, 1H), 5.04 (d, *J* = 10.7 Hz, 1H), 3.65 (dd, *J* = 10.7, 7.1 Hz, 1H), 3.58 (dd, *J* = 10.7, 4.6 Hz, 1H), 3.38 (s, 3H), 3.36 (dt, *J* = 3.4, 6.6 Hz, 1H), 2.56 (br s, 1H), 2.38 (dddt, *J* = 14.0, 7.2, 6.6, 1.3 Hz, 1H), 2.20 (dddt, *J* = 14.0, 7.2, 6.6, 1.1 Hz, 1H), 1.95 (m, 1H), 0.89 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 135.2 (d), 116.8 (t), 83.8 (d), 66.3 (t), 57.6 (q), 37.0 (d), 34.6 (t), 11.1 (q); IR ($CHCl_3$) 3632, 3484, 3081, 3009, 2979, 2935, 2832, 1641, 1460, 1428, 1380, 1361, 1236, 1192, 1083, 1032, 918, 780, 773, 726 cm^{-1}; HRMS (ESI) *m/z* 167.1050 (calcd for $C_8H_{16}NaO_2$ $[M+Na]^+$, Δ +0.2 mmu).

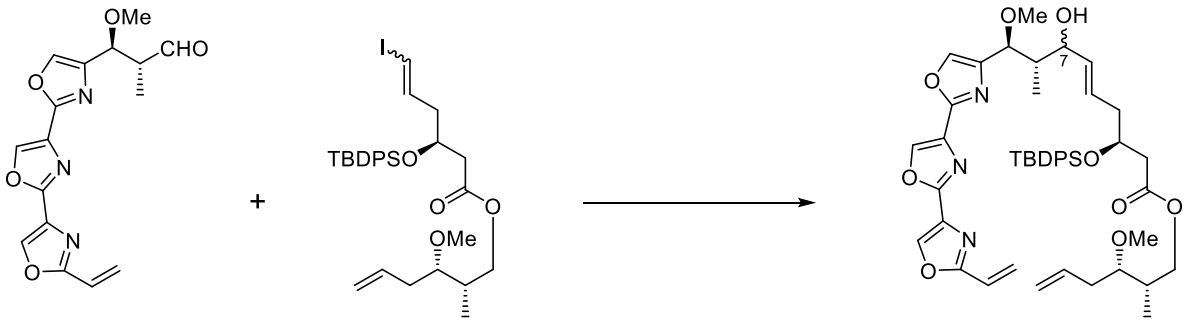
17 6

Carboxylic acid 6. To a stirred solution of (+)-methyl ester **17**[18d] (302 mg, 0.593 mmol, 99%*ee*, *E*/*Z* = 6.7/1) in THF (3 mL) cooled at 0 °C was added 1 M lithium hydroxide aq. (3.6 mL, 3.6 mmol). After being stirred for 23 h at 40 °C, the reaction mixture was acidified with 1 M HCl aq. (5 mL) and extracted with Et_2O (5 mL × 4). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (BW-820MH 10 g, hexane / EtOAc = 9/1 to 5/1) to give carboxylic acid **6** (234 mg, 80%, *E*/*Z* = 6.7/1) as a colorless oil. **6**: R_f 0.42 (hexane / EtOAc =2:1); $[\alpha]^D_{25}$ +33.7 (*c* 1.22, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 7.75–7.64 (m, 4H), 7.50–7.35 (m, 6H), 6.38 (dt, *J* = 14.4, 7.6 Hz, 1H), 5.98 (d, *J* = 14.4 Hz, 1H), 4.18 (tt, *J* = 5.9, 5.9 Hz, 1H), 2.49 (dd, *J* = 6.7, 6.5 Hz, 2H), 2.30–2.16 (m, 2H), 1.06 (s, 9H). COO*H* signal was not observed; ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.1 (s), 141.5 (d), 135.9 (d, 4C), 133.4 (s, 2C), 129.9 (d), 129.8 (d), 127.7 (d, 4C), 78.2 (d), 68.8 (d), 43.1 (t), 41.2 (t), 26.9 (q, 3C), 19.3 (s); IR ($CHCl_3$) 3073, 3054, 3010, 2961, 2933, 2897, 2860, 1712, 1606, 1589, 1472, 1428, 1363, 1221, 1111, 951, 822, 786, 704 cm^{-1}; HRMS (ESI) *m/z* 517.0701 (calcd for $C_{22}H_{27}INaO_3Si$ [M+Na]+, Δ +2.9 mmu).



7 6 5

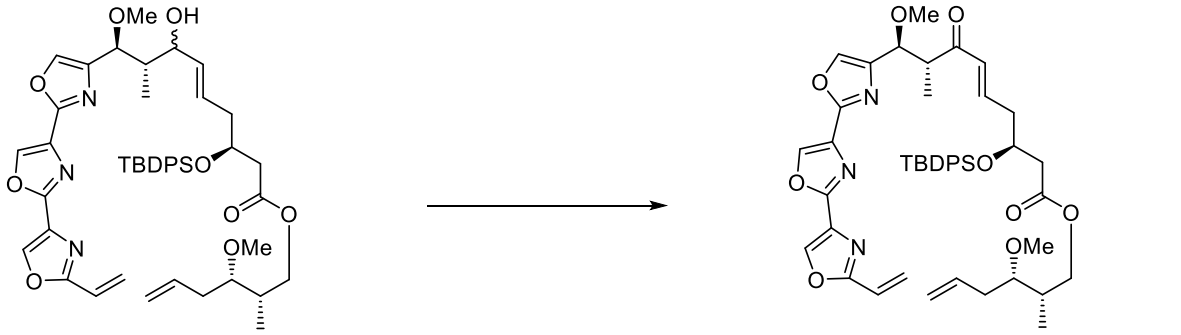
Iodoolefin 5. To a stirred solution of carboxylic acid **6** (234 mg, 0.473 mmol) in dry THF (8.6 mL) were added triethylamine (72 μL, 0.52 mmol) and 2,4,6-trichlorobenzoyl chloride (81 μL, 0.52 mmol). After the mixture was stirred for 5 h at room temperature, the precipitate was removed by centrifugation. After the precipitate was washed with dry THF (4 mL), the combined filtrates and washings were concentrated *in vacuo* and dissolved in dry benzene (11.8 mL). To the activated ester solution was added a solution of primary alcohol **7** (75.3 mg, 0.522 mmol, *syn*/*anti* = 93/7) and *N*,*N*-dimethyl-4-aminopyridine (124 mg, 1.02 mmol) in dry benzene (4 mL). After being stirred for 2 h at room temperature, the reaction mixture was quenched with sat. $NaHCO_3$ aq. (10 mL), and extracted with EtOAc (3 mL × 5). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude oil was purified with a SiO_2 column chromatography (FL60D, 9 g, hexane / EtOAc = 49/1 to 4/1) to give iodoolefin **5** (269 mg, 92%, *syn*/*anti* = 99/1, *E*/*Z* = 6.1/1) as a colorless oil. **5**: R_f 0.60 (hexane / EtOAc = 5:1); $[\alpha]^{D}_{25}$+26.8 (*c* 1.31, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.68–7.64 (m, 4H), 7.46–7.37 (m, 6H), 6.38 (dt, *J* = 14.5, 7.4 Hz, 1H), 5.95 (d, *J* = 14.5 Hz, 1H), 5.76 (ddt, *J* = 17.2, 10.1, 7.1 Hz, 1H), 5.10 (d, *J* = 17.2 Hz, 1H), 5.06 (d, *J* = 10.1 Hz, 1H), 4.20 (tt, *J* = 5.9, 5.9 Hz, 1H), 3.99 (dd, *J* = 10.7, 6.8 Hz, 1H), 3.87 (dd, *J* = 10.7, 6.8 Hz, 1H), 3.31 (s, 3H), 3.16 (dt, *J* = 3.7, 6.5 Hz, 1H), 2.45 (dd, *J* = 6.5, 5.9 Hz, 2H), 2.34 (m, 1H), 2.27–2.15 (m, 3H), 1.90 (m, 1H), 1.04 (s, 9H), 0.87 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.9 (s), 141.8 (d), 135.9 (d, 2C), 135.8 (d, 2C), 134.9 (d), 133.6 (s), 133.5 (s), 129.8 (d, 2C), 127.7 (d, 2C), 127.6 (d, 2C), 117.1 (t), 80.9 (d), 77.8 (d), 69.0 (d), 66.8 (t), 58.0 (q), 43.1 (t), 41.5 (t), 35.4 (d), 35.3 (t), 26.9 (q, 3C), 19.2 (s), 11.0 (q); IR (CHCl3) 3074, 3054, 3010, 2962, 2933, 2897, 2860, 2829, 1729, 1641, 1472, 1428, 1363, 1233, 1190, 1105, 998, 952, 920, 822, 735, 704 cm^{-1}; HRMS (ESI) *m/z* 643.1729 (calcd for $C_{30}H_{41}INaO_4Si$ $[M+Na]^+$, Δ +1.3 mmu).



4 5 18

Allylic alcohol 18. To a stirred solution of aldehyde **4** (23.3 mg, 70.8 μmol) and iodoolefin **5** (65.9 mg, 106 μmol, *E*/*Z* = 6.1/1) in degassed dry THF / DMF (3:1, 1.3 mL) under an argon atmosphere was added a 99:1 (w/w) mixture of chromium chloride (II) – nickel chloride (II) (56.5 mg, $CrCl_2$ 455 μmol and $NiCl_2$ 4.36 μmol). After being stirred for 14 h, the reaction mixture was diluted with EtOAc (4 mL), sat. NH_4Cl aq. (4 mL), and water (1 mL), and extracted with EtOAc (4 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and

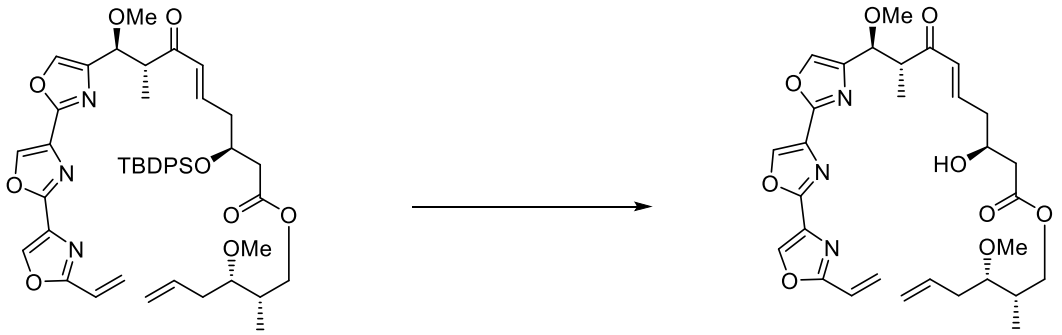
concentrated. The crude oil was purified with a SiO_2 column chromatography (BW-820MH 3.1 g, hexane / $CHCl_3$ / EtOAc = 1/1/0, 3/7/0, 0/1/0, 0/4/1 and 0/3/1) to give allylic alcohol **18** (42.7 mg, 73%, 5*E*-isomer only, ca. 3:2 diastereomeric mixture at C7) as a colorless oil. **18**: R_f 0.50 (hexane / acetone = 7:3); $[\alpha]^{D}_{25}$ +5.7 (*c* 0.93, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.32 (s, 0.4H), 8.32 (s, 0.6H), 8.31 (s, 0.6H), 8.30 (s, 0.4H), 7.72–7.61 (m, 5H), 7.44–7.32 (m, 6H), 6.67 (dd, *J* = 17.7, 11.2 Hz, 1H), 6.34 (d, *J* = 17.7 Hz, 1H), 5.77 (d, *J* = 11.2 Hz, 1H), 5.74 (m, 1H), 5.55 (m, 1H), 5.39 (m, 1H), 5.08 (d, *J* = 17.2 Hz, 1H), 5.03 (d, *J* = 10.2 Hz, 1H), 4.30–4.26 (m, 0.4H), 4.26 (d, *J* = 7.9 Hz, 0.6H), 4.25–4.19 (m, 0.6H), 4.17 (d, *J* = 7.8 Hz, 0.4H), 4.01 (m, 1H), 3.97 (m, 1H), 3.84 (m, 1H), 3.30 (s, 1.2H), 3.30 (s, 1.8H), 3.29 (s, 1.8H), 3.28 (s, 1.2H), 3.16 (m, 1H), 2.51–2.43 (m, 2H), 2.36–2.12 (m, 6H), 1.89 (m, 1H), 1.02 (s, 9H), 0.87 (d, *J* = 6.8 Hz, 1.2H), 0.86 (d, *J* = 6.8 Hz, 1.8H), 0.75 (d, *J* = 7.1 Hz, 1.2H), 0.65 (d, *J* = 6.9 Hz, 1.8H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.34 [171.27], 161.90 [161.89], 156.0, 155.3 [155.0], 141.0 [140.6], 138.96 [138.95], 138.43 [138.39], 136.7 [136.5], 135.8 (4C), 134.9 [134.8], 134.3, 134.0 [133.9], 133.7 (2C), 131.54 [131.50], 130.7, 129.7 [129.6], 127.8, 127.5 (4C), 126.4 [124.2], 122.5, 117.0, 80.9, 80.1 [79.9], 75.7 [73.0], 70.2 [70.1], 66.6, 58.0 [57.5], 57.2 [56.9], 42.5, 42.1, 41.5 [41.4], 40.0, 39.8, 35.3, 26.9, 19.2, 12.0, 11.4, 11.02 [11.01] (sprit signals derived from the C7 diastereomer were shown in parenthesis); IR ($CHCl_3$) 3673, 3462, 3167, 3072, 3029, 3009, 2966, 2933, 2899, 2859, 2828, 1729, 1651, 1542, 1462, 1428, 1380, 1308, 1238, 1218, 1191, 1111, 980, 942, 918, 822 cm^{-1}; HRMS (ESI) *m/z* 846.3741 (calcd for $C_{46}H_{57}N_3NaO_9Si$ $[M+Na]^+$, Δ –2.1 mmu).



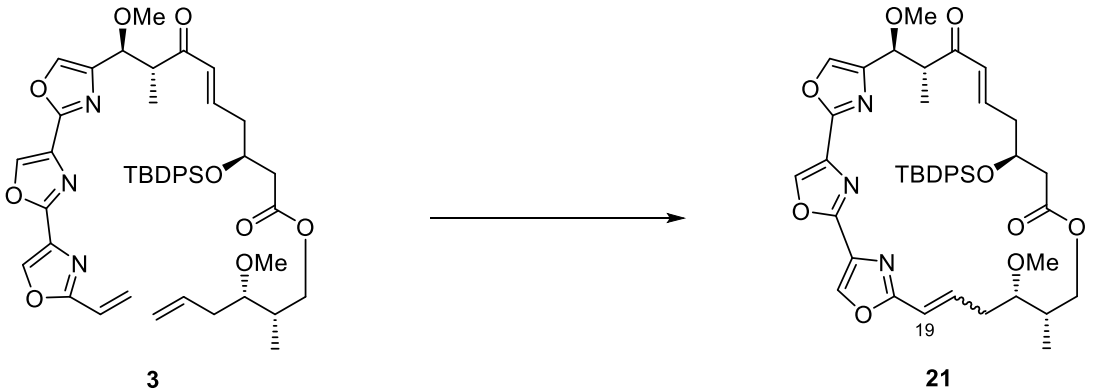
18 **3**

RCM precursor diene 3. To a stirred solution of allylic alcohol **18** (26.1 mg, 28.3 μmol) in dry CH_2Cl_2 (0.63 mL) cooled at 0 °C were added pyridine (26 μL) and Dess–Martin periodinane (20.2 mg, 47.6 μmol). After being stirred for 2.5 h at 0 °C, the reaction mixture was quenched with a 1:1:1 mixture of sat. $Na_2S_2O_3$ aq. – sat. $NaHCO_3$ aq. – water (5 mL), and extracted with CH_2Cl_2 (2 mL × 4). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude oil was purified with a SiO_2 column chromatography (FL60D 2 g, hexane / $CHCl_3$ = 1/1 to 0/1) to give RCM precursor diene **3** (24.6 mg, 94%) as a colorless oil. **3**: R_f 0.42 ($CHCl_3$ / EtOAc = 4:1); $[\alpha]^{D}_{25}$ –5.5 (*c* 0.54, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.332 (s, 1H), 8.327 (s, 1H), 7.70 (s, 1H), 7.70–7.65 (m, 4H), 7.44–7.35 (m, 6H), 6.83 (dt, *J* = 15.8, 7.6 Hz, 1H), 6.67 (dd, *J* = 17.7, 11.3 Hz, 1H), 6.34 (d, *J* = 17.7 Hz, 1H), 6.12 (d, *J* = 15.8 Hz, 1H), 5.78 (d, *J* = 11.3 Hz, 1H), 5.75 (ddt, *J* = 17.2, 10.2, 7.1 Hz, 1H), 5.07 (d, *J* = 17.2 Hz, 1H), 5.04 (d, *J* = 10.2 Hz, 1H), 4.40 (d, *J* = 9.7 Hz, 1H), 4.32 (tt, *J* = 6.1, 5.9 Hz, 1H), 4.01 (dd, *J* = 10.8, 6.8 Hz, 1H), 3.86 (dd, *J* = 10.8, 6.9 Hz, 1H), 3.44 (dq, *J* = 9.7, 7.1 Hz, 1H), 3.31 (s, 3H), 3.21–3.13 (m, 1H), 3.17 (s, 3H), 2.51 (dd, *J* = 15.1, 6.1 Hz, 1H), 2.46 (dd, *J* = 15.1, 6.1 Hz, 1H), 2.47–2.35 (m, 2H), 2.32 (m, 1H), 2.19 (m, 1H), 1.90 (m, 1H), 1.04 (s, 9H), 0.87 (d, *J* = 6.9 Hz, 3H), 0.84 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.7 (s), 170.9 (s), 161.9 (s), 156.0 (s), 155.5 (s), 142.8 (d), 140.0 (s), 139.0 (d), 138.5 (d), 137.2 (d), 135.9 (d, 2C), 135.8 (d, 2C), 134.8 (d), 133.5 (s), 133.4 (s), 132.9 (d), 131.6 (s), 130.7 (s), 129.9 (d), 129.8 (d), 127.7 (d, 4C), 124.2 (t), 122.6 (d), 117.1 (t), 80.9 (d), 77.6 (d), 69.2 (d), 66.8 (t), 58.0 (q), 57.0 (q), 47.0 (d), 41.6 (t), 40.0 (t), 35.3 (d), 35.3 (t), 26.9 (q, 3C), 19.2 (s), 14.1 (q), 11.0 (q); IR (CHCl3) 3163, 3027, 3010, 2933, 2859, 1729, 1694, 1665,

1637, 1542, 1462, 1428, 1376, 1308, 1111, 978, 943, 919, 823, 703 cm^{-1}; HRMS (ESI) *m/z* 844.3618 (calcd for $C_{46}H_{55}N_3NaO_9Si$ $[M+Na]^+$, Δ +1.3 mmu).

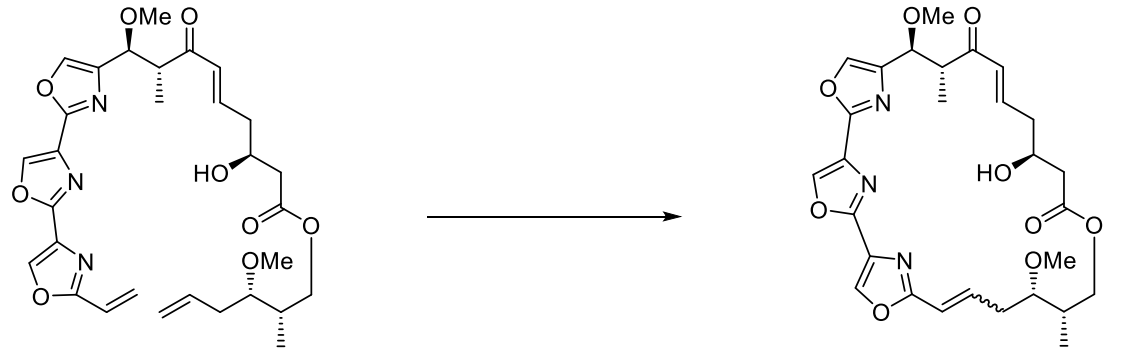


C3 hydroxy RCM precursor 19. To a stirred solution of RCM precursor diene **3** (22.7 mg, 27.6 μmol) in THF (10.2 mL) cooled at 0 °C were added a 1.0 M solution of TBAF/AcOH (1:1) in THF (0.91 mL, 0.91 mmol) [prepared by adding AcOH (114.5 μL, 2.0 mmol) to a 1.0 M solution of TBAF in THF (2.0 mL, 2.0 mmol)]. After being stirred for 18 h at room temperature, the reaction mixture was diluted with sat. $NaHCO_3$ aq. (10 mL) and extracted with EtOAc (5 mL × 4). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude oil was purified with two SiO_2 column chromatographies (FL60D 1.2 g, $CHCl_3$ / EtOAc = 4/1 to 1/1; FL60D 0.4 g, EtOAc) to give C3 hydroxy RCM precursor **19** (11.6 mg, 72%) as a colorless oil. **19**: R_f 0.66 ($CHCl_3$ / MeOH = 10:1);$[\alpha]^{D}_{25}$ –39.7 (*c* 0.46, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.34 (s, 1H), 8.33 (s, 1H), 7.71 (s, 1H), 6.97 (dt, *J* = 15.8, 7.3 Hz, 1H), 6.67 (dd, *J* = 17.7, 11.5 Hz, 1H), 6.34 (d, *J* = 17.7 Hz, 1H), 6.29 (d, *J* = 15.8 Hz, 1H), 5.78 (d, *J* = 11.5 Hz, 1H), 5.77 (ddt, *J* = 17.8, 10.0, 7.3 Hz, 1H), 5.11 (d, *J* = 17.8 Hz, 1H), 5.06 (d, *J* = 10.0 Hz, 1H), 4.42 (d, *J* = 9.6 Hz, 1H), 4.21 (m, 1H), 4.12 (dd, *J* = 10.9, 7.0 Hz, 1H), 4.05 (dd, *J* = 10.9, 6.5 Hz, 1H), 3.56 (dq, *J* = 9.6, 7.1 Hz, 1H), 3.35 (s, 3H), 3.34 (m, 1H), 3.23 (dt, *J* = 3.5, 6.5 Hz, 1H), 3.19 (s, 3H), 2.55 (dd, *J* = 16.6, 3.1 Hz, 1H), 2.48 (dd, *J* = 16.6, 9.2 Hz, 1H), 2.52–2.40 (m, 2H), 2.36 (m, 1H), 2.21 (m, 1H), 2.04 (m, 1H), 0.93 (d, *J* = 7.0 Hz, 3H), 0.90 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 202.0 (s), 172.4 (s), 161.9 (s), 156.0 (s), 155.6 (s), 142.8 (d), 139.8 (s), 139.0 (d), 138.6 (d), 137.2 (d), 134.8 (d), 132.9 (d), 131.6 (s), 130.7 (s), 124.2 (t), 122.6 (d), 117.6 (t), 81.3 (d), 77.7 (d), 67.1 (d), 66.8 (t), 58.0 (q), 57.0 (q), 46.8 (d), 41.0 (t), 39.4 (t), 35.2 (d), 35.1 (t), 14.2 (q), 11.1 (q); IR ($CHCl_3$) 3674, 3481, 3168, 3079, 3010, 2934, 2826, 1720, 1665, 1628, 1542, 1459, 1377, 1233, 1222, 1210, 1187, 1092, 980, 943, 919, 787, 771, 756, 741, 731 cm^{-1}; HRMS (ESI) *m/z* 606.2411 (calcd for $C_{30}H_{37}N_3NaO_9$ $[M+Na]^+$, Δ –1.6 mmu).



Macrocycle 21. To a stirred solution of RCM precursor diene **3** (7.8 mg, 9.5 μmol) in dry CH_2Cl_2 (8.8 mL) was added 2 mM solution of the 2nd generation Hoveyda–Grubbs catalyst (**20a**) in dry CH_2Cl_2 (1.4 mL, 2.8 μmol). After being stirred for 9 h at refluxing temperature, the reaction mixture was concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 0.5 g, hexane / EtOAc = 2/1 to 1/1) to give macrocycle (19*E*)-**21** (4.0 mg, 53%), its stereoisomer (19*Z*)-**21** (2.2 mg, 29%), and its dimer (0.8 mg, 5%) as colorless oils. (19*E*)-**21**: R_f 0.16 ($CHCl_3$ / EtOAc = 4:1); $[\alpha]^{D}_{25}$ –38.1 (*c* 0.63, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.13 (s, 1H), 8.08 (s, 1H), 7.71–7.66 (m, 4H), 7.66 (s, 1H), 7.44–7.35 (m, 6H), 7.07 (td, *J* = 7.6, 15.8 Hz, 1H), 6.94 (td, *J* = 7.2, 16.2 Hz, 1H), 6.35 (d, *J* = 15.8 Hz, 1H), 5.97 (d, *J* = 16.2 Hz, 1H), 4.45 (m, 1H), 4.36 (d, *J* = 9.6 Hz, 1H), 4.13 (qd, *J* = 7.0, 9.6 Hz, 1H), 4.00 (dd, *J* = 6.8, 10.4 Hz, 1H), 3.92 (dd, *J* = 6.8, 10.4 Hz, 1H), 3.39 (m, 1H), 3.35 (s, 3H), 3.09 (s, 3H), 2.77 (m, 1H), 2.72 (dd, *J* = 5.6, 15.6 Hz, 1H), 2.66 (dd, *J* = 6.8, 15.6 Hz, 1H), 2.45 (m, 2H), 2.42 (m, 1H), 2.06 (ddtq, *J* = 2.0, 6.8, 6.8, 6.8 Hz, 1H), 1.02 (s, 9H), 0.88 (d, *J* = 6.8 Hz, 3H), 0.81 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 203.2 (s), 171.1 (s), 162.7 (s), 156.5 (s), 155.6 (s), 143.3 (d), 139.6 (d), 139.0 (s), 137.4 (d), 137.3 (d), 137.2 (d), 135.9 (d, 2C), 135.83 (d, 2C), 135.0 (d), 133.7 (s), 133.6 (s), 131.5 (s), 130.3 (s), 129.9 (d), 129.7 (d), 127.7 (d, 2C), 127.6 (d, 2C), 116.3 (d), 79.7 (d), 76.7 (d), 68.7 (d), 67.5 (t), 57.7 (q), 56.2 (q), 42.6 (d), 41.7 (t), 40.3 (t), 35.0 (d), 32.4 (t), 26.9 (t, 3C), 19.2 (s), 14.9 (q), 9.3 (q); IR ($CHCl_3$) 3160, 3005, 2932, 2856, 1731, 1662, 1560, 1485, 1424, 1220, 1209, 1181, 1104, 997, 979, 909, 821, 790, 721, 703 cm^{-1}; HRMS (ESI) *m/z* 816.3320 (calcd for $C_{44}H_{51}N_3O_9SiNa$ $[M+Na]^+$, Δ +2.8 mmu).

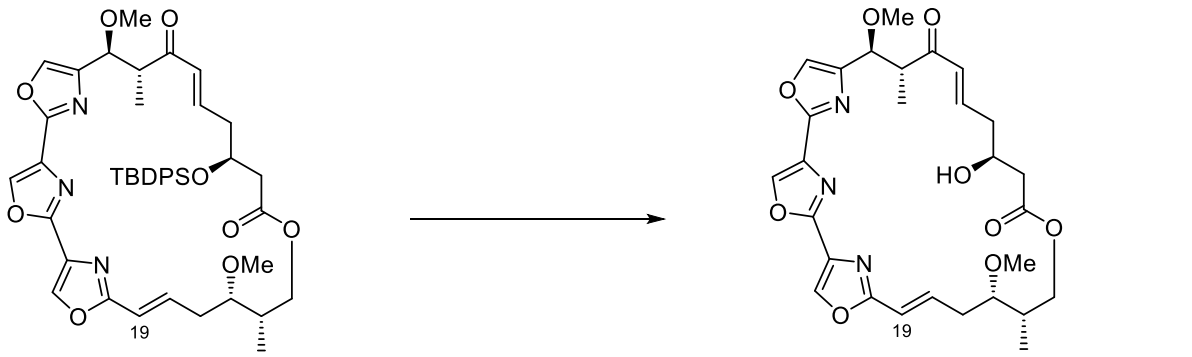
(19*Z*)-**21**: R_f 0.21 ($CHCl_3$ / EtOAc = 4:1); $[\alpha]^{D}_{25}$ –67.8 (*c* 0.57, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.13 (s, 1H), 8.10 (s, 1H), 7.69–7.62 (m, 4H), 7.61 (s, 1H), 7.44–7.35 (m, 6H), 6.84 (td, *J* = 6.8, 16.0 Hz, 1H), 6.43 (td, *J* = 7.6, 11.8 Hz, 1H), 6.33 (d, *J* = 11.8 Hz, 1H), 6.01 (d, *J* = 16.0 Hz, 1H), 4.38–4.29 (m, 1H), 4.35 (d, *J* = 8.4 Hz, 1H), 4.16 (dd, *J* = 5.2, 10.8 Hz, 1H), 3.95 (m, 1H), 3.94 (dd, *J* = 7.6, 10.8 Hz, 1H), 3.50–3.41 (m, 2H), 3.37 (s, 3H), 3.16 (s, 3H), 2.88 (m, 1H), 2.60 (dd, *J* = 6.4, 15.8 Hz, 1H), 2.53 (dd, *J* = 6.4, 15.8 Hz, 1H), 2.40 (m, 2H), 2.19 (m, 1H), 1.07 (d, *J* = 6.8 Hz, 3H), 1.02 (s, 9H), 0.93 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 202.5 (s), 170.9 (s), 161.7 (s), 156.6 (s), 155.7 (s), 142.7 (d), 141.1 (d), 139.3 (s), 137.6 (d), 137.4 (d), 137.0 (d), 135.80 (d, 2C), 135.79 (d, 2C), 134.7 (d), 133.7 (s), 133.5 (s), 131.6 (s), 130.7 (s), 129.83 (d), 129.78 (d), 127.71 (d, 2C), 127.67 (d, 2C), 114.3 (d), 81.7 (d), 77.6 (d), 69.0 (d), 66.9 (t), 58.3 (q), 56.9 (q), 44.0 (d), 41.0 (t), 39.4 (t), 36.8 (d), 31.9 (t), 26.9 (q, 3C), 19.2 (s), 14.0 (q), 12.6 (q); IR ($CHCl_3$) 3162, 3005, 2932, 2856, 1730, 1663, 1558, 1458, 1424, 1179, 1103, 980, 917, 820, 790, 758, 739, 702 cm^{-1}; HRMS (ESI) *m/z* 816.3318 (calcd for $C_{44}H_{51}N_3O_9SiNa$ $[M+Na]^+$, Δ +2.4 mmu).



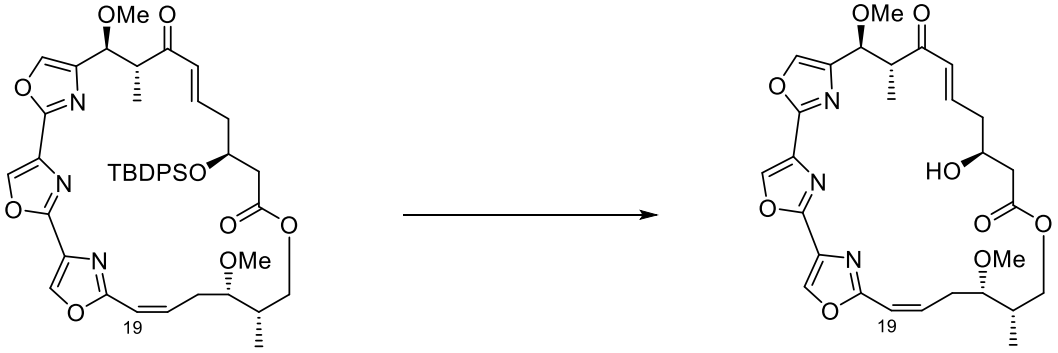
RCM of C3 hydroxy precursor 19. To a stirred solution of C3 hydroxy RCM precursor **19** (5.8 mg, 9.9 μmol) in dry CH_2Cl_2 (9.2 mL) was added a 2.0 mM solution of the 2nd generation Hoveyda–Grubbs catalyst (**20a**) in dry CH_2Cl_2 (1.5 mL, 3.0 μmol). After being stirred for 37 h at refluxing temperature, the reaction mixture was concentrated. The crude material was purified with two SiO_2 column chromatographies (FL60D 0.5 g, $CHCl_3$ / MeOH = 100/1 to 10/1; FL60D 0.5 g, hexane / EtOAc = 3/7 to 1/9) to give macrolactone (19*E*)-**2** (2.9 mg, 72%),

its stereoisomer (19*Z*)-**2** (1.1 mg, 20%), and its dimer (0.8 mg, 7%) as colorless oils. (19*E*)-**2**: R_f 0.30 (hexane/EtOAc = 1/9); $[\alpha]^{D}_{25}$ –54 (*c* 0.38, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.12 (s, 1H), 8.08 (s, 1H), 7.66 (s, 1H), 7.05 (dt, *J* = 15.8, 7.7 Hz, 1H), 7.04 (dt, *J* = 15.9, 7.5 Hz, 1H), 6.35 (dt, *J* = 15.8, 1.6 Hz, 1H), 6.23 (dt, *J* = 15.9, 1.3 Hz, 1H), 4.40 (d, *J* = 8.9 Hz, 1H), 4.34 (m, 1H), 4.16 (dd, *J* = 10.7, 8.2 Hz, 1H), 4.10 (m, 1H), 4.04 (dd, *J* = 10.7, 5.9 Hz, 1H), 4.00 (dt, *J* = 8.9, 7.0 Hz, 1H), 3.53 (ddd, *J* = 10.2, 4.7, 2.6 Hz, 1H), 3.39 (s, 3H), 3.15 (s, 3H), 2.78 (dddd, *J* = 14.8, 7.7, 4.7, 1.6 Hz, 1H), 2.71 (dd, *J* = 15.8, 2.9 Hz, 1H), 2.61 (dd, *J* = 15.8, 10.0 Hz, 1H), 2.57–2.54 (m, 2H), 2.41 (dddd, *J* = 14.8, 10.2, 7.7, 1.6 Hz, 1H), 2.05 (m, 1H), 0.93 (d, *J* = 6.9 Hz, 3H), 0.88 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 202.6, 172.3, 162.7, 156.5, 155.5, 143.6, 139.8, 139.2, 137.4, 137.3, 137.2, 134.2, 131.3, 130.1, 116.3, 79.2, 77.1, 67.2, 66.9, 57.9, 56.6, 43.6, 41.5, 40.4, 35.7, 33.2, 13.8, 9.5; IR (CHCl3) 3690, 3167, 3026, 3003, 2929, 2846, 1719, 1661, 1609, 1563, 1458, 1386, 1261, 1215, 1091, 1100, 1023, 977, 918 cm^{-1}; HRMS (ESI) *m/z* 578.2087 (calcd for $C_{28}H_{33}N_3NaO_9$ $[M+Na]^+$, Δ –2.7 mmu).

(19*Z*)-**2**: R_f 0.39 (hexane/EtOAc = 1/9); $[\alpha]^{D}_{25}$ –71 (*c* 0.36, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.17 (s, 1H), 8.14 (s, 1H), 7.63 (s, 1H), 6.91 (dt, *J* = 16.1, 6.7 Hz, 1H), 6.42 (dt, *J* = 11.7, 7.5 Hz, 1H), 6.35 (d, *J* = 11.7 Hz, 1H), 6.18 (d, *J* = 16.1 Hz, 1H), 4.37 (d, *J* = 7.5 Hz, 1H), 4.18 (m, 1H), 4.16 (dd, *J* = 10.9, 5.3 Hz, 1H), 4.09 (dd, *J* = 10.9, 5.6 Hz, 1H), 3.89 (qd, *J* = 7.1, 7.5 Hz, 1H), 3.75 (br s, 1H), 3.51 (dt, *J* = 4.7, 6.6 Hz, 1H), 3.40 (s, 3H), 3.36 (dddd, *J* = 16.2, 7.5, 4.7, 1.4 Hz, 1H), 3.24 (s, 3H), 3.08 (dddd, *J* = 16.2, 7.5, 6.6, 1.4 Hz, 1H), 2.60 (dd, *J* = 16.0, 3.2 Hz, 1H), 2.51 (dd, *J* = 16.0, 9.5 Hz, 1H), 2.46–2.41 (m, 2H), 2.15 (m, 1H), 1.08 (d, *J* = 7.0 Hz, 3H), 1.01 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 202.2, 172.2, 161.8, 156.6, 155.6, 143.4, 140.4, 139.7, 137.9, 137.7, 136.8, 133.9, 131.4, 130.6, 114.6, 81.0, 78.2, 67.0, 66.8, 58.1, 57.3, 44.3, 41.2, 39.6, 37.0, 31.4, 13.5, 12.7; IR ($CHCl_3$) 3675, 3167, 3026, 3007, 2931, 2846, 1724, 1663, 1557, 1459, 1377, 1279, 1261, 1220, 1182, 1100, 1013, 975, 918 cm^{-1}; HRMS (ESI) *m/z* 578.2091 (calcd for $C_{28}H_{33}N_3NaO_9$ $[M+Na]^+$, Δ –2.3 mmu).



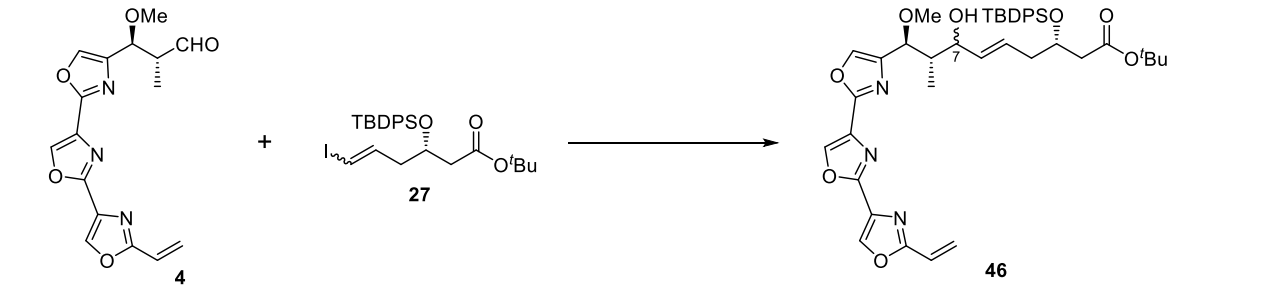
Removal of the TBDPS group of (19*E*)-21. To a stirred solution of macrocycle (19*E*)-**21** (7.7 mg, 9.7 μmol) in THF (3.7 mL) cooled at 0 °C were added a 1.0 M solution of TBAF/AcOH (1:1) in THF (0.29 mL, 0.29 mmol) [prepared by adding AcOH (114.5 μL, 2.0 mmol) to a 1.0 M solution of TBAF in THF (2.0 mL, 2.0 mmol)]. After being stirred for 60 h at room temperature, the reaction mixture was diluted with sat. $NaHCO_3$ aq. (5 mL), and extracted with EtOAc (5 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude oil was purified with a SiO_2 column chromatography (FL60D 0.5 g, hexane / EtOAc = 3/7 to 1/9) to give macrolactone (19*E*)-**2** (4.7 mg, 87%) as a colorless oil.



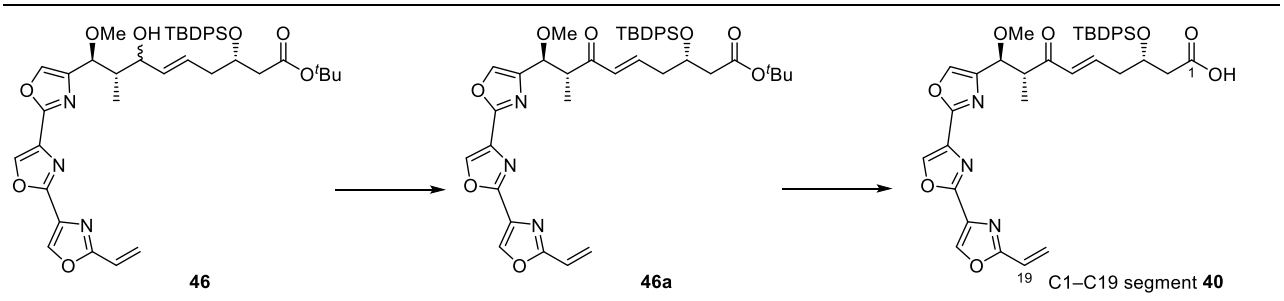
(19*Z*)-**21** (19*Z*)-**2**

Removal of the TBDPS group of (19*Z*)-21. To a stirred solution of macrocycle (19*Z*)-**21** (3.3 mg, 4.2 μmol) in THF (1.6 mL) under a nitrogen atmosphere at 0 °C were added a 1.0 M solution of TBAF/AcOH (1:1) in THF (0.14 mL, 0.14 mmol) [prepared by adding AcOH (114.5 μL, 2.0 mmol) to a 1.0 M solution of TBAF in THF (2.0 mL, 2.0 mmol)]. After being stirred for 48 h at room temperature, the reaction mixture was diluted with sat. $NaHCO_3$ aq. (5 mL), and extracted with EtOAc (5 mL × 4). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude oil was purified with a SiO_2 column chromatography (FL60D 0.5 g, hexane / EtOAc = 1/9) to give macrolactone (19*Z*)-**2** (2.1 mg, 91%) as a colorless oil.

Bioassay of mycalolide B and its synthetic analogs. The cytotoxicities of mycalolide B and its synthetic analogs were measured by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) method. The actin-depolymerizing activities of the compounds were measured based on their ability to attenuate the fluorescence of pyrene-conjugated actin, as previously described.[39] Antimycotic assays against several fungi were conducted at Ricerca Biosciences Inc. (Taipei, Taiwan).



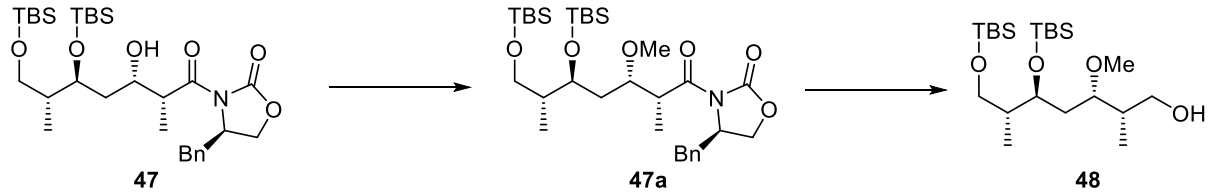
Allylic alcohol 46. To a stirred solution of (−)-aldehyde **4** (31.2 mg, 94.7 μmol) and (+)-iodoolefin **27**[14] (104.3 mg, 189 μmol, *E*/*Z* = 5/1) in degassed dry DMSO (1.9 mL) under an argon atmosphere was added a 99:1 (w/w) mixture of chromium chloride (II)–nickel chloride (II) (69.9 mg, $CrCl_2$ 563 μmol and $NiCl_2$ 5.39 μmol). After being stirred for 12 h, the reaction mixture was diluted with EtOAc (4 mL) and sat. NH_4Cl aq. (4 mL), and extracted with EtOAc (4 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude oil was purified with a SiO_2 column chromatography (FL60D 3.8 g, hexane / EtOAc = 4/1 to 2/1) to give allylic alcohol **46** (59.0 mg, 83%, 5*E*-isomer only, ca. 1:1 diastereomeric mixture at C7) as a colorless oil. **46**: R_f 0.38 (hexane / EtOAc = 1:1); $[\alpha]^{D}_{25}$ +7.7 (*c* 1.09, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 8.31 (s, 1H), 7.70–7.63 (m, 5H), 7.43–7.33 (m, 6H), 6.67 (dd, *J* = 11.2, 17.8 Hz, 1H), 6.34 (d, *J* = 17.8 Hz, 1H), 5.78 (d, *J* = 11.2 Hz, 1H), 5.57 (m, 1H), 5.39 (m, 1H), 4.30–4.25 (m, 1H), 4.21–4.15 (m, 1.5H), 3.97 (m, 0.5H), 3.50 (m, 0.5H), 3.29 (s, 1.5H), 3.28 (s, 1.5H), 3.17 (m, 0.5H), 2.45–2.13 (m, 5H), 1.39 (s, 4.5H), 1.38 (s, 4.5H), 1.03 (s, 9H), 0.75 (d, *J* = 7.2 Hz, 1.5H), 0.66 (d, *J* = 6.8 Hz, 1.5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6 [170.5], 161.9, 156.0, 155.3 [155.0], 141.0 [140.6], 139.0, 138.5 [138.4], 136.7 [136.6], 135.9 (4C), 134.2 [134.10], 134.07, 133.9, 133.5, 131.6 [131.5], 130.7, 129.6 (2C), 127.5 (4C), 124.2, 122.6, 80.3 [80.2], 80.1 [80.0], 77.2 [75.8], 73.1, 70.3 [70.2], 57.2 [56.9], 42.4 [42.1], 39.7 [39.5], 28.1 (3C), 26.9 (3C), 19.3, 12.0 [11.5] (sprit signals derived from the C7 diastereomer were shown in parenthesis); IR ($CHCl_3$) 3511, 3168, 3073, 3052, 3033, 3009, 2983, 2966, 2933, 2860, 1720, 1651, 1542, 1472, 1428, 1368, 1308, 1221, 1216, 1211, 1153, 1112, 979, 909, 822, 726 cm^{-1}; HRMS (ESI) *m/z* 776.3334 (calcd for $C_{42}H_{51}N_3NaO_8Si$ $[M+Na]^+$, Δ –0.9 mmu).



C1–C19 segment 40. To a stirred solution of the allylic alcohol **46** (517.2 mg, 0.686 mmol) in dry CH_2Cl_2 (13.7 mL) cooled at 0 °C were added pyridine (0.55 mL) and Dess–Martin periodinane (581.9 mg, 1.37 mmol). After being stirred for 1 h at 0 °C, the reaction mixture was warmed to room temperature and stirred for 1 h. The reaction mixture was quenched with a 1:1:1 mixture of sat. $Na_2S_2O_3$ aq. – sat. $NaHCO_3$ aq. – water (30 mL), and extracted with CH_2Cl_2 (20 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude oil was purified with a SiO_2 column chromatography (FL60D 15 g, hexane / EtOAc = 4/1 to 2/1) to give ketone **46a** (470.4 mg, 91%) as a colorless oil. **46a**: R_f 0.55 (hexane / EtOAc = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ

8.34 (s, 2H), 7.70 (s, 1H), 7.70–7.67 (m, 4H), 7.44–7.36 (m, 6H), 6.85 (dt, *J* = 15.8, 7.6 Hz, 1H), 6.68 (dd, *J* = 11.2, 17.6 Hz, 1H), 6.35 (d, *J* = 17.6 Hz, 1H), 6.12 (d, *J* = 15.8 Hz, 1H), 5.78 (d, *J* = 11.2 Hz, 1H), 4.41 (d, *J* = 9.6 Hz, 1H), 4.27 (m, 1H), 3.44 (dq, *J* = 9.6, 6.8 Hz, 1H), 3.18 (s, 3H), 2.49–2.34 (m, 4H), 1.39 (s, 9H), 1.05 (s, 9H), 0.85 (d, *J* = 6.8 Hz, 3H).

To a stirred solution of ketone **46a** (189.8 mg, 0.252 mmol) in dry CH_2Cl_2 (5.0 mL) cooled at 0 °C was added trifluoroacetic acid (2.8 mL). After being stirred for 3 h at 0 °C, the reaction mixture was neutralized (pH = 7.0) with sat. $NaHCO_3$ aq. (30 mL), and extracted with EtOAc (20 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude oil was purified with a SiO_2 column chromatography (FL60D 5 g, hexane / EtOAc = 2/1 to 1/1) to give the C1–C19 segment **40** (170.5 mg, 100%) as a colorless oil. **40**: R_f 0.55 (hexane / EtOAc = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.32 (s, 1H), 7.70–7.67 (m, 4H), 7.69 (s, 1H), 7.45–7.36 (m, 6H), 6.78 (dt, *J* = 15.8, 7.6 Hz, 1H), 6.68 (dd, *J* = 11.8, 18.0 Hz, 1H), 6.35 (d, *J* = 18.0 Hz, 1H), 6.12 (d, *J* = 15.8 Hz, 1H), 5.79 (d, *J* = 11.8 Hz, 1H), 4.45 (d, *J* = 9.2 Hz, 1H), 4.31(m, 1H), 3.44 (dq, *J* = 9.2, 7.0 Hz, 1H), 3.20 (s, 3H), 2.58 (dd, *J* = 5.6, 15.2 Hz, 1H), 2.51 (dd, *J* = 6.8, 15.2 Hz, 1H), 2.47–2.43 (m, 2H), 1.06 (s, 9H), 0.86 (d, *J* = 7.0 Hz, 3H) [COO*H* signal was not observed].



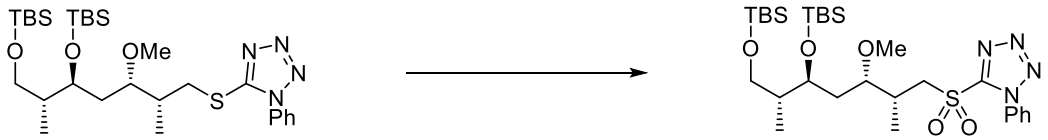
Primary alcohol 48. To a stirred solution of aldol **47**[18a,18b] (2.66 g, 4.48 mmol) in dry CH_2Cl_2 (9.9 mL) cooled at 0 °C were added 2,6-di-*tert*-butylpyridine (3.3 mL, 15 mmol) and MeOTf (1.34 mL, 11.6 mmol). After being stirred for 43 h at room temperature, the reaction mixture was quenched with 10% $NaHCO_3$ aq. (80 mL) at 0°C, and extracted with CH_2Cl_2 (20 mL × 3). The combined extracts were washed with water and brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 100 g, hexane / EtOAc = 49/1 to 19/1) to give methyl ether **47a** (2.40 g, 88%) as a colorless oil. **47a**: R_f 0.58 (hexane / EtOAc = 5/1); $[\alpha]^D_{24}$ –63 (*c* 0.88, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35–7.21 (m, 5H), 4.61 (ddt, *J* = 3.2, 10.0, 4.8 Hz, 1H), 4.27 (dq, *J* = 4.8, 6.8 Hz, 1H), 4.14 (d, *J* = 4.8 Hz, 2H), 4.02 (ddd, *J* = 2.0, 2.4, 10.0 Hz, 1H), 3.54 (ddd, *J* =1.6, 4.8, 9.6 Hz, 1H), 3.43 (dd, *J* = 7.2, 10.0 Hz, 1H), 3.40 (dd, *J* = 6.8, 10.0 Hz, 1H), 3.35 (s, 3H), 3.30 (dd, *J* = 3.2, 13.2 Hz, 1H), 2.77 (dd, *J* = 10.0, 13.2 Hz, 1H), 1.98–1.89 (m, 1H), 1.51 (ddd, *J* = 2.0, 9.6, 14.0 Hz, 1H), 1.41 (ddd, *J* = 1.6, 10.0, 14.0 Hz, 1H), 1.20 (d, *J* = 6.8 Hz, 3H), 0.90 (s, 9H), 0.88 (s, 9H), 0.83 (d, *J* = 7.2 Hz, 3H), 0.08 (s, 6H), 0.02 (s, 3H), 0.01 (s, 3H); 13C NMR (100 MHz, CDCl3) δ 175.6, 153.6, 135.8, 129.8 (2C), 129.3 (2C), 127.6, 80.5, 69.9, 66.2, 65.6, 58.1, 56.3, 42.5, 39.8, 38.1, 34.2, 26.3 (3C), 26.2 (3C), 18.5 (2C), 13.4, 10.8, –3.5, –4.2, –5.1, –5.2; IR ($CHCl_3$) 2956, 2929, 1779, 1697, 1471, 1251, 1220, 1210, 1086, 730, 670 cm^{-1}; HRMS (ESI) *m/z* 630.3629 (calcd for $C_{32}H_{57}NNaO_6Si_2$ $[M+Na]^+$, Δ +0.7 mmu).

To a stirred solution of methyl ether **47a** (27.9 mg, 45.9 μmol) in dry Et_2O (0.9 mL) cooled at –10 °C were added dry EtOH (5 μL, 90 μmol) and a 2.0 M solution of lithium borohydride in THF (28 μL, 56 μmol). After being stirred at –10 °C for 1.5 h, the reaction mixture was quenched by addition of 1 M NaOH aq. (1 mL) and sat. $Na_2S_2O_3$ aq. (1 mL), and extracted with Et_2O (5 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (0.5 g, hexane / EtOAc = 24/1) to give primary alcohol **48** (19.3 mg, 97%) as a colorless oil. **48**: R_f 0.60 (hexane / EtOAc = 3/1); $[\alpha]^D_{24}$ –22 (*c* 0.86, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.07 (ddd, *J* = 2.6, 2.6, 9.2 Hz, 1H), 3.66 (dd, *J* = 9.2, 10.6 Hz, 1H), 3.51 (dd, *J* = 4.8, 10.6 Hz, 1H), 3.47-3.35 (m, 3H), 3.39 (s, 3H), 2.28 (m, 1H), 1.94 (m, 1H), 1.47 (ddd, *J* = 2.6, 9.2,

14.2 Hz, 1H), 1.40 (ddd, *J* = 2.6, 9.2, 14.2 Hz, 1H), 0.89 (s, 18H), 0.81 (d, *J* = 7.2 Hz, 3H), 0.79 (d, *J* = 7.2 Hz, 3H), 0.072 (s, 3H), 0.067 (s, 3H), 0.03 (s, 6H) [O*H* signal was not observed]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 83.1, 70.2, 66.5, 65.5, 57.4, 42.4, 35.4, 31.8, 26.3 (3C), 26.2 (3C), 18.5, 18.4, 13.1, 10.8, –3.6, –4.2, –5.1, –5.2; IR ($CHCl_3$) 3471, 1471, 1463, 1388, 1256, 1220, 1082, 1038, 837, 668 cm^{-1}; HRMS (ESI) *m/z* 457.3152 (calcd for $C_{22}H_{50}NaO_4Si_2$ [M+Na]$^+$, Δ –0.7 mmu).

48 **50**

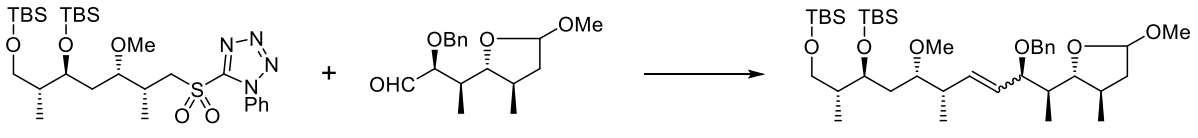
PT-sulfide 50. To a stirred solution of primary alcohol **48** (1.73 g, 3.98 mmol) and 5,5'-dithiobis(1-phenyl-*1H*-tetrazole) (**49**) (2.82 g, 7.96 mmol) in dry THF (13.2 mL) cooled at 0 °C was added tri-*n*-butylphosphine (2.2 mL, 8.8 mmol). After being stirred for 17 h at room temperature, the reaction mixture was diluted with water (15 mL), and extracted with EtOAc (10 mL × 3). The combined extracts were washed with sat. $NaHCO_3$ aq. and brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (BW-820MH 50 g, hexane / EtOAc = 10/1) and a Yamazen preparative silica gel column (90 g, hexane / EtOAc = 20/1) to give PT-sulfide **50** (2.44 g, quant.) as a light yellow oil. **50**: R_f 0.65 (hexane / EtOAc = 5/1); $[\alpha]^D_{25}$ –30 (*c* 0.79, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61–7.51 (m, 5H), 4.04 (ddd, *J* = 3.2, 3.2, 8.8 Hz, 1H), 3.60 (dd, *J* = 6.0, 12.8 Hz, 1H), 3.45 (dd, *J* = 8.0, 10.0 Hz, 1H), 3.41 (m, 1H), 3.40 (dd, *J* = 6.0, 10.0 Hz, 1H), 3.37 (s, 3H), 3.20 (dd, *J* = 8.4, 12.8 Hz, 1H), 2.32 (m, 1H), 1.91 (m, 1H), 1.43 (ddd, *J* = 3.2, 8.4, 14.0 Hz, 1H), 1.37 (ddd, *J* = 2.8, 8.8, 14.0 Hz, 1H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.88 (s, 9H), 0.87 (s, 9H), 0.81 (d, *J* = 6.8 Hz, 3H), 0.06 (s, 3H), 0.05 (s, 3H), 0.03 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.1, 134.2, 130.4, 130.1 (2C), 124.2 (2C), 80.7, 70.4, 65.5, 57.7, 42.4, 36.4, 35.4, 33.0, 26.3 (3C), 26.2 (3C), 18.5, 18.4, 15.3, 11.0, –3.6, –4.2, –5.1, –5.1; IR ($CHCl_3$) 2957, 2929, 2884, 2857, 1598, 1500, 1471, 1463, 1388, 1255, 1089, 837, 686, 666 cm^{-1}; HRMS (ESI) *m/z* 617.3332 (calcd for $C_{29}H_{54}N_4NaO_3Si_2$ [M+Na]$^+$, Δ–2.2 mmu).



50 **42**

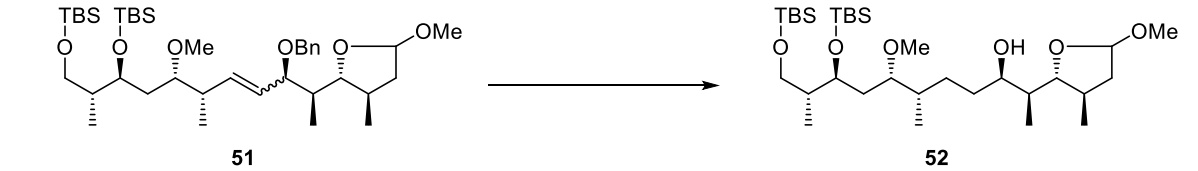
PT-sulfone 42. To a stirred solution of PT-sulfide **50** (92 mg, 0.16 mmol) in dry CH_2Cl_2 (3.1 mL) cooled at 0 °C were added $NaHCO_3$ (69 mg, 0.82 mmol) and *m*CPBA (134 mg, 0.78 mmol). After being stirred for 18 h at room temperature, the reaction was quenched with sat. $Na_2S_2O_3$ aq. (10 mL) at 0 °C. The resulting mixture was stirred for 1 h at 0 °C, and extracted with CH_2Cl_2 (10 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 3.6 g, hexane / EtOAc = 99/1) to give PT-sulfone **42** (88 mg, 91%) as a light yellow oil. **42**: R_f 0.70 (toluene / Et_2O = 20/1); $[\alpha]^D_{25}$ –34 (*c* 0.79, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69–7.58 (m, 5H), 4.04 (ddd, *J* = 2.0, 3.2, 9.6 Hz, 1H), 3.98 (dd, *J* = 2.4, 14.0 Hz, 1H), 3.43 (dd, *J* = 10.0, 14.0 Hz, 1H), 3.42-3.40 (m, 2H), 3.36 (ddd, *J* = 2.0, 3.6, 9.6 Hz, 1H), 3.30 (s, 3H), 2.81 (m, 1H), 1.93 (m, 1H), 1.35 (ddd, *J* = 2.0, 9.6, 13.6 Hz, 1H), 1.19 (ddd, *J* = 2.0, 9.6, 13.6 Hz, 1H), 1.12 (d, *J* = 6.8 Hz, 3H), 0.90 (s, 9H), 0.88 (s, 9H), 0.80 (d, *J* = 7.2 Hz, 3H), 0.07 (s, 3H), 0.051 (s,

3H), 0.046 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.4, 133.4, 131.8, 130.0 (2C), 125.6 (2C), 80.6, 69.8, 65.5, 57.7, 57.1, 42.4, 31.9, 29.1, 26.3 (3C), 26.2 (3C), 18.5, 18.4, 16.3, 10.7, –3.6, –4.2, –5.1, –5.2; IR ($CHCl_3$) 3031, 2956, 2930, 1596, 1498, 1339, 1256, 1154, 1063, 1007, 744, 736, 688, 539 cm^{-1}; HRMS (ESI) *m/z* 649.3277 (calcd for $C_{29}H_{54}N_4NaO_5Si_2$ [M+Na]$^+$, Δ +2.6 mmu).

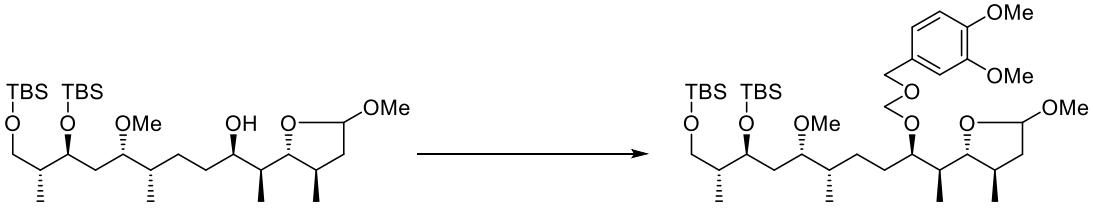


Olefin 51. To a stirred solution of PT-sulfone **42** (139 mg, 0.222 mmol) in dry DME (1.2 mL) cooled at –55 °C was added a 1.0 M solution of lithium hexamethyldisilazide in THF (0.22 mL, 0.22 mmol) dropwise under a nitrogen stream. The mixture was stirred at –55 °C for 30 min, then a solution of (+)-aldehyde **43**[28] (26 mg, 89 μmol) in DME (0.6 mL) was added dropwise, and the resulting mixture was stirred at –55 °C for 2 h and allowed to warm to room temperature for 8 h. The reaction was quenched by addition of brine (5 mL) at 0 °C and extracted with Et_2O (5 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 4.5 g, hexane / acetone = 50/1) to give olefin **51** (56.4 mg, 92%, *E*/*Z* = 1/1.5) and recovered PT-sulfone **42** (86 mg) as light yellow oils. A part of the *E*/*Z*-stereoisomers of **51** was separated by the same SiO_2 column chromatography as above. (*E*)-**51**: R_f 0.70 (hexane / Et_2O = 4/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35–7.22 (m, 5H), 5.64 (dd, *J* = 7.5, 15.6 Hz, 1H), 5.47 (dd, *J* = 7.4, 15.6 Hz, 1H), 4.91 (d, *J* = 4.4 Hz, 1H), 4.61–4.35 (AB quart, *J* = 12.0 Hz, 2H), 4.21 (dd, *J* = 2.6, 7.5 Hz, 1H), 4.03 (m, 1H), 3.70 (dd, *J* = 7.0, 9.0 Hz, 1H), 3.44–3.26 (m, 3H), 3.32 (s, 3H), 3.29 (s, 3H), 2.56 (m, 1H), 2.23 (m, 1H), 2.08 (dd, *J* = 7.2, 12.4 Hz, 1H), 1.90 (m, 1H), 1.68–1.59 (m, 1H), 1.66 (dd, *J* = 4.8, 12.4 Hz, 1H), 1.42–1.38 (m, 1H), 1.32–1.24 (m, 1H), 1.09 (d, *J* = 7.2 Hz, 3H), 0.98 (d, *J* = 7.2 Hz, 3H), 0.94 (d, *J* = 7.2 Hz, 3H), 0.89 (s, 9H), 0.88 (s, 9H), 0.79 (d, *J* = 6.8 Hz, 3H), 0.07 (s, 6H), 0.01 (s, 6H).

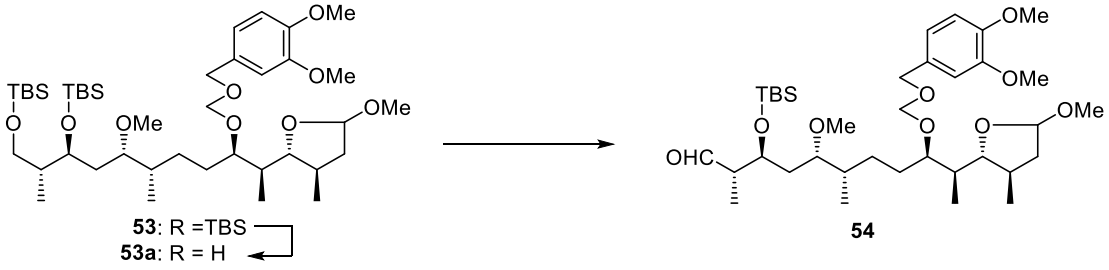
(*Z*)-**51**: R_f 0.75 (hexane / Et_2O = 4/1); $[\alpha]^D_{24}$ +7.6 (*c* 0.79, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.22 (m, 5H), 5.52 (dd, *J* = 8.4, 11.2 Hz, 1H), 5.42 (dd, *J* = 10.6, 11.2 Hz, 1H), 4.92 (dd, *J* = 1.3, 5.0 Hz, 1H), 4.61–4.59 (m, 1H), 4.59–4.39 (AB quart, *J* = 12.0 Hz, 2H), 4.03 (ddd, *J* = 3.0, 3.0, 9.6 Hz, 1H), 3.74 (dd, *J* = 6.8, 10.0 Hz, 1H), 3.43 (dd, *J* = 7.4, 10.2 Hz, 1H), 3.37 (dd, *J* = 6.8, 10.2 Hz, 1H), 3.32 (s, 3H), 3.31 (s, 3H), 3.22 (ddd, *J* = 2.4, 6.6, 9.5 Hz, 1H), 2.70 (ddq, *J* = 6.6, 10.6, 7.2 Hz, 1H), 2.20 (dddq, *J* = 6.8, 7.4, 8.8, 6.8 Hz, 1H), 2.05 (ddd, *J* = 1.3, 7.4, 12.6 Hz, 1H), 1.91 (dddq, *J* = 3.0, 6.8, 7.4, 7.2 Hz, 1H), 1.68 (ddd, *J* = 5.0, 8.8, 12.6 Hz, 1H), 1.71–1.63 (m, 1H), 1.40 (ddd, *J* = 2.4, 9.6, 14.2 Hz, 1H), 1.33 (ddd, *J* = 3.0, 9.6, 14.2 Hz, 1H), 1.09 (d, *J* = 6.8 Hz, 3H), 0.98 (d, *J* = 7.2 Hz, 3H), 0.96 (d, *J* = 7.2 Hz, 3H), 0.90 (s, 9H), 0.89 (s, 9H), 0.79 (d, *J* = 7.2 Hz, 3H), 0.08 (s, 3H), 0.07 (s, 3H), 0.033 (s, 3H), 0.029 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.8, 135.1, 130.7, 128.4 (2C), 127.7 (2C), 127.4, 105.2, 87.8, 81.4, 75.2, 70.8, 70.0, 65.7, 55.3, 57.1, 46.5, 42.7, 42.5, 36.14, 36.10, 34.2, 26.4 (3C), 26.2 (3C), 20.3, 18.5, 18.5, 17.6, 10.8, 10.1, –3.6, –4.1, –5.10, –5.14; IR (CHCl3) 2957, 2930, 1471, 1463, 1256, 1221, 1188, 1094, 1065, 785, 767, 758, 669 cm^{-1}; HRMS (ESI) *m/z* 715.4784 (calcd for $C_{39}H_{72}NaO_6Si_2$ [M+Na]$^+$, Δ +1.9 mmu).



Secondary alcohol 52. A mixture of olefin **51** (44.8 mg, 64.7 μmol, *E*/*Z* = 1/1.5), $NaHCO_3$ (10.9 mg, 0.129 mmol), and 20% $Pd(OH)_2$ on carbon (9.1 mg) in dry EtOH (0.65 mL) was stirred under a hydrogen atmosphere for 45 h at room temperature. The mixture was filtered through a pad of Celite, and the residue was washed with EtOAc. The filtrate and the washings were combined and concentrated. The crude oil was purified with a SiO_2 column chromatography (FL60D 0.8 g, hexane / EtOAc = 20/1 to 10/1) to give secondary alcohol **52** (28.8 mg, 74%) as a colorless oil. **52**: R_f 0.52 (hexane / EtOAc = 3:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.91 (d, *J* = 5.0 Hz, 1H), 3.99 (m, 1H), 3.86 (m, 1H), 3.57 (t, *J* = 7.5 Hz, 1H), 3.48 (dd, *J* = 7.5, 10.2 Hz, 1H), 3.37 (dd, *J* = 6.6, 10.2 Hz, 1H), 3.33 (s, 3H), 3.31 (s, 3H), 3.23 (m, 1H), 2.74 (br d, *J* = 4.1 Hz, 1H), 2.34–2.22 (m, 1H), 2.08 (dd, *J* = 7.0, 12.7 Hz, 1H), 1.94–1.84 (m, 1H), 1.82–1.57 (m, 4H), 1.56–1.39 (m, 2H), 1.33 (m, 2H), 1.06 (d, *J* = 6.5 Hz, 3H), 0.95 (d, *J* = 7.0 Hz, 3H), 0.88 (s, 18H), 0.86 (d, *J* = 7.0 Hz, 3H), 0.81 (d, *J* = 6.8 Hz, 3H), 0.06 (s, 6H), 0.02 (s, 6H).



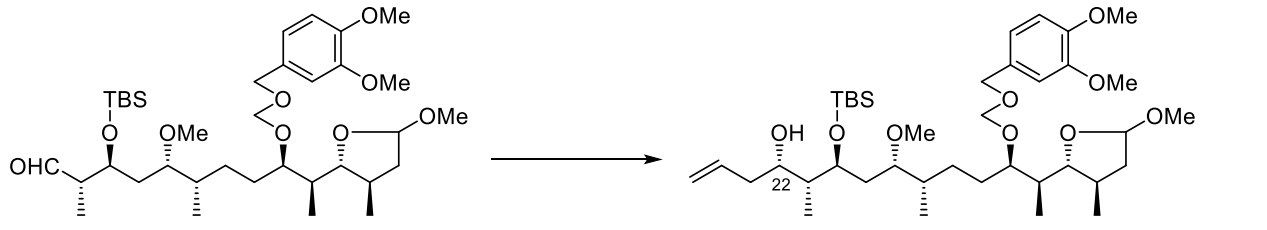
DMBOM ether 53. To a stirred solution of secondary alcohol **52** (28.8 mg, 47.6 μmol) in dry CH_2Cl_2 (0.48 mL) cooled at 0 °C were added diisopropylethylamine (0.33 mL, 1.9 mmol) and a 1.1 M solution of (3,4-dimethoxybenzyloxy)methyl chloride in CH_2Cl_2 (0.43 mL, 0.47 mmol). After being stirred at 0 °C for 18 h, the reaction was quenched by addition of MeOH (2 mL) and $NaHCO_3$ (50 mg). The resulting mixture was stirred at room temperature for 1 h, diluted with water (5 mL) at 0 °C, and extracted with hexane (5 mL × 3). The combined extracts were washed with sat. $NaHCO_3$ aq., water, and brine, successively, dried with Na_2SO_4, and concentrated. The crude oil was purified with a SiO_2 column chromatography (FL60D 1.5 g, hexane / Et_2O = 20/1 to 7/1) to give DMBOM ether **53** (36.5 mg, 98%) as a colorless oil. **53**: R_f 0.61 (benzene / Et_2O = 6:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (m, 2H), 6.82 (d, *J* = 8.0 Hz, 1H), 4.89 (d, *J* = 4.6 Hz, 1H), 4.85 (d, *J* = 7.3 Hz, 1H), 4.82 (d, *J* = 7.3 Hz, 1H), 4.59 (s, 2H), 4.07 (m, 1H), 3.99 (m, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.58 (dd, *J* = 6.5, 9.7 Hz, 1H), 3.44 (dd, *J* = 7.6, 10.0 Hz, 1H), 3.36 (dd, *J* = 6.5, 10.0 Hz, 1H), 3.28 (s, 6H), 3.21 (m, 1H), 2.28–2.19 (m, 1H), 2.09 (dd, *J* = 7.6, 12.7 Hz, 1H), 1.93–1.85 (m, 1H), 1.78–1.69 (m, 1H), 1.68–1.55 (m, 5H), 1.54–1.43 (m, 1H), 1.36–1.23 (m, 2H), 1.10 (d, *J* = 6.8 Hz, 3H), 0.89 (s, 18H), 0.88 (d, *J* = 7.2 Hz, 3H), 0.86 (d, *J* = 6.8 Hz, 3H), 0.81 (d, *J* = 7.0 Hz, 3H), 0.06 (s, 6H), 0.02 (s, 6H).



Aldehyde 54. DMBOM ether **53** (963 mg, 1.23 mmol) was dissolved in a 0.5 M solution of NH_4F in dry MeOH (24.6 mL, 12.3 mmol). The resulting mixture was stirred for 20 h at 40 °C, SiO_2 (0.8 g) was added, and concentrated. The residue was suspended in EtOAc, filtered through a small plug of cotton, and washed with EtOAc. After the

filtrate and washings were concentrated, the crude material was purified with a SiO_2 column chromatography (BW-820MH 12 g, hexane / EtOAc = 3/1 to 1/1) to give primary alcohol **53a** (828 mg, quant.) as a colorless oil. **53a**: R_f 0.43 (hexane / EtOAc = 1/1); $[\alpha]^{D}_{21}$ –0.70 (*c* 1.1, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.94–6.80 (m, 3H), 4.88 (d, *J* = 4.6 Hz, 1H), 4.83 (s, 2H), 4.58 (s, 2H), 4.05 (m, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 3.87 (m, 1H), 3.68 (dd, *J* = 4.6, 11.1 Hz, 1H), 3.56 (dd, *J* = 6.8, 10.1 Hz, 1H), 3.47 (dd, *J* = 6.5, 11.1 Hz, 1H), 3.29 (s, 3H), 3.28 (s, 3H), 3.15 (m, 1H), 2.65 (br s, 1H), 2.21 (m, 1H), 2.09 (dd, *J* = 7.6, 12.7 Hz, 1H), 1.89–1.79 (m, 3 H), 1.79–1.38 (m, 7H), 1.10 (d, *J* = 6.5 Hz, 3H), 0.94 (d, *J* = 7.3 Hz, 3H), 0.89 (s, 9H), 0.88 (d, *J* = 6.5 Hz, 3H), 0.86 (d, *J* = 7.3 Hz, 3H), 0.08 (s, 3H), 0.08 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.0, 148.6, 130.7, 120.5, 111.4, 110.9, 104.7, 94.5, 87.2, 82.5, 78.5, 73.0, 69.4, 64.9, 56.8, 55.9, 55.8, 54.5, 43.5, 42.4, 40.1, 35.9, 34.5, 34.1, 30.6, 27.1, 25.9 (3C), 20.1, 18.0, 15.4, 12.9, 8.8, –4.3, –4.4; IR ($CHCl_3$) 3448, 1606, 1512, 1464, 1383, 1259, 1032, 955, 935, 835 cm^{-1}; HRMS (ESI) *m/z* 693.4366 (calcd for $C_{36}H_{66}NaO_9Si$ $[M+Na]^+$, Δ –0.8 mmu).

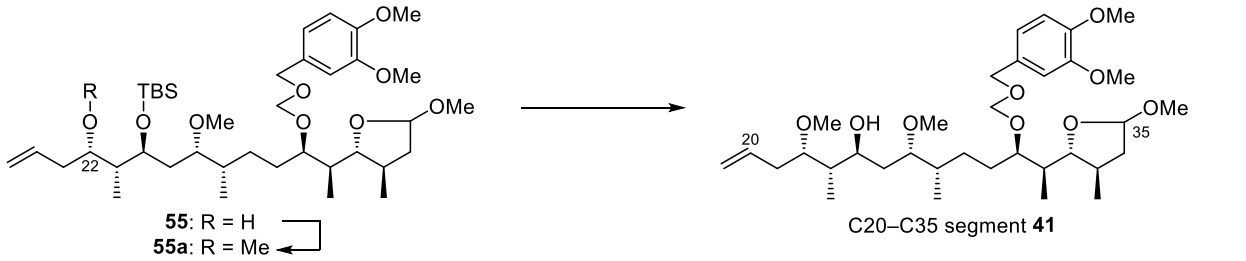
To a stirred solution of primary alcohol **53a** (194 mg, 0.289 mmol) in dry CH_2Cl_2 (2.9 mL) were added dry pyridine (0.23 mL, 2.9 mmol) and Dess–Martin periodinane (194 mg, 0.46 mmol). After being stirred for 2 h at room temperature, the resulting mixture was diluted with a mixture of sat. $Na_2S_2O_3$ aq. – sat. $NaHCO_3$ aq. – water (10 mL, 1:1:1 [v/v/v]) at 0 °C and extracted with EtOAc (10 mL × 2). The combined extracts were washed with water and brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (BW-820MH 5 g, hexane / EtOAc = 5/1) to give aldehyde **54** (184 mg, 96%) as a light yellow oil. **54**: R_f 0.70 (hexane / EtOAc = 1/1); $[\alpha]^{D}_{20}$ +18 (*c* 1.0, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 9.70 (d, *J* = 1.6 Hz, 1H), 6.93–6.81 (m, 3H), 4.88 (d, *J* = 4.6 Hz, 1H), 4.83 (AB quart, *J* = 6.8 Hz, 2H), 4.58 (s, 2H), 4.20 (dt, *J* = 8.4, 3.5 Hz, 1H), 4.05 (m, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.56 (dd, *J* = 6.8, 10.0 Hz, 1H), 3.28 (s, 3H), 3.27 (s, 3H), 3.23–3.17 (m, 1H), 2.53 (m, 1H), 2.24 (m, 1H), 2.09 (dd, *J* = 7.6, 12.4 Hz, 1H), 1.79 (m, 1H), 1.65–1.21 (m, 8H), 1.10 (d, *J* = 7.0 Hz, 6H), 0.90–0.86 (m, 12H), 0.85 (d, *J* = 7.0 Hz, 3H), 0.10 (s, 3H), 0.08 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.1, 148.9, 148.5, 130.8, 120.5, 111.3, 110.9, 104.7, 94.5, 87.1, 81.4, 78.5, 70.2, 69.4, 56.5, 55.9, 55.8, 54.5, 52.6, 43.5, 42.5, 35.9, 35.1, 34.4, 30.7, 27.0, 25.8 (3C), 20.1, 18.0, 15.4, 9.2, 8.8, –4.3, –4.4; IR ($CHCl_3$) 3690, 3447, 3025, 3020, 2957, 2935, 1718, 1602, 1516, 1465, 1382, 1260, 1224, 1158, 1140, 1095, 1029, 938, 852, 840, 800, 793, 770 cm^{-1}; HRMS (ESI) *m/z* 691.4221 (calcd for $C_{36}H_{64}NaO_9Si$ $[M+Na]^+$, Δ +0.4 mmu).



Secondary alcohol 55. To a stirred solution of aldehyde **54** (179 mg, 0.268 mmol) in dry THF (2.7 mL) cooled at –78 °C was added a 1.0 M allylmagnesium bromide in Et_2O (0.8 mL, 0.8 mmol). After being stirred for 1 h at –78 °C, a 1.0 M allylmagnesium bromide in Et_2O (0.8 mL, 0.8 mmol) was added, stirred for 1 h, a 1.0 M allylmagnesium bromide in Et_2O (0.8 mL, 0.8 mmol) was added, and stirred for 2 h. The resulting mixture was quenched with sat. NH_4Cl aq. (20 mL) and extracted with EtOAc (10 mL × 3). The combined extracts were washed with water and brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 7 g, hexane / EtOAc = 6/1 to 4/1) to give secondary alcohol **55** (137 mg, 72%) and its 22*R* isomer (52 mg, 27%) as colorless oils. (22*S*)-**55**: R_f 0.28 (hexane / EtOAc = 2/1); $[\alpha]^{D}_{26}$ +4.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.93–6.81 (m, 3H), 5.79 (m, 1H), 5.13–5.03 (m, 2H), 4.89 (d, *J* = 4.6 Hz, 1H), 4.83 (AB quart, *J* = 7.3 Hz, 2H), 4.59 (s, 2H), 4.11–4.02 (m, 2H), 3.94–3.81 (m, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.57 (dd, *J* =

6.5, 10.0 Hz, 1H), 3.48 (br s, 1H), 3.29 (s, 3H), 3.26 (s, 3H), 3.06 (m, 1H), 2.36–2.06 (m, 3H), 2.10 (dd, *J* = 7.8, 13.5 Hz, 1H), 1.79–1.42 (m, 10H), 1.11 (d, *J* = 6.5 Hz, 3H), 1.01 (d, *J* = 7.0 Hz, 3H), 0.89–0.88 (m, 12H), 0.87 (d, *J* = 7.3 Hz, 3H), 0.10 (s, 3H), 0.09 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.0, 148.6, 135.5, 130.8, 120.5, 116.9, 111.4, 110.9, 104.7, 94.5, 87.2, 82.1, 78.7, 76.8, 70.5, 69.5, 56.8, 56.2, 55.9, 54.5, 43.9, 42.6, 39.6, 39.5, 35.9, 35.2, 34.7, 30.7, 27.6, 26.3, 20.1, 18.0, 15.0, 10.9, 8.9, –4.2, –4.4; IR (CHCl3) 3460, 3025, 3019, 3010, 2934, 1596, 1516, 1465, 1421, 1382, 1262, 1225, 1158, 1140, 1095, 1028, 837, 797, 781, 771 cm^{-1}; HRMS (ESI) *m/z* 733.4686 (calcd for $C_{39}H_{70}NaO_9Si$ [M+Na]$^+$, Δ –0.1 mmu).

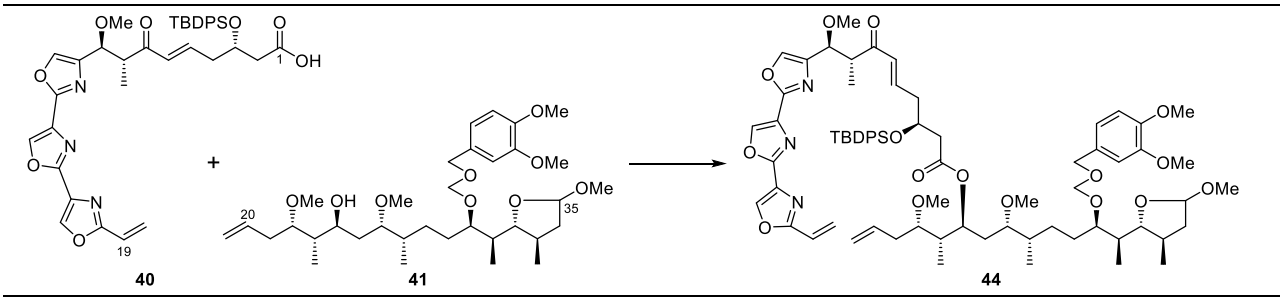
(22*R*)-**55**: R_f 0.20 (hexane / EtOAc = 2/1); $[\alpha]^D_{26}$ –12 (*c* 0.86, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93–6.81 (m, 3H), 5.86 (m, 1H), 5.14–5.07 (m, 2H), 4.88 (d, *J* = 4.9 Hz, 1H), 4.83 (s, 2H), 4.58 (s, 2H), 4.13 (m, 1H), 4.06 (br t, *J* = 6.4 Hz, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 3.57 (dd, *J* = 6.8, 10.0 Hz, 1H), 3.40-3.14 (m, 2H), 3.32 (s, 3H), 3.28 (s, 3H), 2.86 (br s, 1H), 2.39 (m, 1H), 2.23 (m, 1H), 2.15–2.01 (m, 2H), 1.89–1.21 (m, 10H), 1.10 (d, *J* = 6.8 Hz, 3H), 0.884 (s, 9H), 0.878 (d, *J* = 6.8 Hz, 3H), 0.86 (d, *J* = 6.8 Hz, 3H), 0.81 (d, *J* = 7.0 Hz, 3H), 0.07 (s, 3H), 0.06 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 148.9, 148.5, 135.4, 130.8, 120.5, 117.3, 111.4, 110.9, 104.6, 94.4, 87.1, 83.4, 78.4, 72.9, 70.3, 69.4, 56.9, 55.9, 55.8, 54.5, 44.7, 43.4, 42.5, 39.5, 35.8, 34.5, 32.7, 30.5, 26.7, 25.9 (3C), 20.1, 18.0, 15.7, 10.2, 8.9, –4.1, –4.6; IR ($CHCl_3$) 3464 (br), 1593, 1518, 1464, 1381, 1261, 1032, 993, 955, 935 cm^{-1}; HRMS (ESI) *m/z* 733.4677 (calcd for $C_{39}H_{70}NaO_9Si$ [M+Na]$^+$, Δ –1.0 mmu).



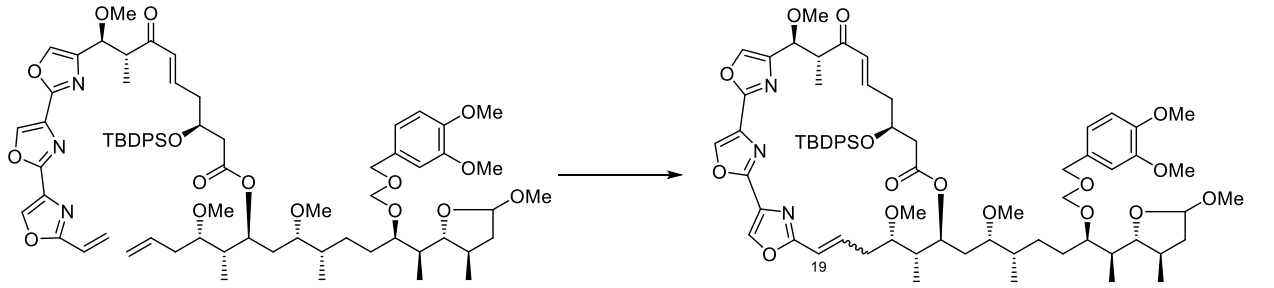
C20–C35 segment 41. To a stirred solution of secondary alcohol (22*S*)-**55** (2.16 g, 3.04 mmol) in dry THF (30 mL) cooled at 0 °C were added iodomethane (5.7 mL, 91 mmol) and sodium hydride (1.34 g of 60% dispersion in mineral oil, 33 mmol). After being stirred for 14 h at room temperature, the resulting mixture was quenched with cold water (50 mL) and extracted with Et_2O (25 mL × 3). The combined extracts were washed with sat. $Na_2S_2O_3$ aq., water, and brine, successively, dried with Na_2SO_4, and concentrated. The crude material was purified with a Yamazen preparative silica gel column (90 g, hexane / EtOAc = 10/1) to give methyl ether **55a** (2.14 g, 97%) as a colorless oil. **55a**: R_f 0.55 (hexane / EtOAc = 2/1); $[\alpha]^D_{25}$ –4.0 (*c* 0.73, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.93–6.81 (m, 3H), 5.84 (m, 1H), 5.16–5.04 (m, 2H), 4.88 (d, *J* = 4.6 Hz, 1H), 4.83 (s, 2H), 4.59 (s, 2H), 4.06 (m, 1H), 3.93–3.85 (m, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.57 (dd, *J* = 6.8, 10.0 Hz, 1H), 3.32 (s, 6H), 3.28 (s, 3H), 3.19 (m, 1H), 2.93 (m, 1H), 2.40–2.18 (m, 3H), 2.11 (m, 1H), 1.82–1.15 (m, 10H), 1.10 (d, *J* = 6.8 Hz, 3H), 0.93–0.84 (m, 9H), 0.88 (s, 9H), 0.04 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.2, 148.8, 134.8, 131.1, 120.7, 117.3, 111.6, 111.2, 104.8, 94.6, 87.4, 83.0, 82.2, 78.7, 70.4, 69.6, 57.5, 57.3, 56.1, 56.0, 54.6, 43.6, 42.7 (2C), 36.1, 35.1, 34.9, 33.0, 30.8, 27.3, 26.1 (3C), 20.3, 18.2, 15.9, 9.0, 8.9, -3.7, -4.5; IR ($CHCl_3$) 1518, 1464, 1381, 1257, 1095, 1032, 993, 935, 835 cm^{-1}; HRMS (ESI) *m/z* 747.4858 (calcd for $C_{40}H_{72}NaO_9Si$ [M+Na]$^+$, Δ +1.5 mmu).

To a stirred solution of methyl ether **55a** (14.0 mg, 19.3 μmol) in dry THF (0.36 mL) was added a 1.0 M solution of TBAF in THF (97 μL, 97 μmol). After being stirred for 14 h at room temperature and for 10 h at 40 °C, the reaction mixture was quenched with sat. NH_4Cl aq. (2 mL) and water (1 mL), and extracted with EtOAc (5 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 0.5 g, hexane / EtOAc = 5/1 to 2/1) to give the C20–C35 segment **41** (11.8 mg, quant.) as a colorless oil. **41**: R_f 0.43 (hexane / EtOAc = 1/1); $[\alpha]^D_{24}$ +4.1 (*c* 0.71, $CHCl_3$); ^{1}H

NMR (270 MHz, $CDCl_3$) δ 6.93–6.81 (m, 3H), 5.79 (m, 1H), 5.14–5.03 (m, 2H), 4.89 (d, *J* = 4.6 Hz, 1H), 4.84 (AB quart, *J* = 6.8 Hz, 2H), 4.59 (s, 2H), 4.06 (m, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.75 (m, 1H), 3.62 (m, 1H), 3.57 (dd, *J* = 6.5, 10.0 Hz, 1H), 3.54 (dt, *J* = 2.7, 7.0 Hz, 1H), 3.38 (s, 3H), 3.37 (m, 1H), 3.36 (s, 3H), 3.29 (s, 3H), 2.44 (m, 1H), 2.29-2.12 (m, 2H), 2.09 (dd, *J* = 7.6, 12.4 Hz, 1H), 1.80–1.35 (m, 10H), 1.11 (d, *J* = 6.5 Hz, 3H), 0.92–0.87 (m, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.2, 148.8, 135.4, 131.1, 120.7, 117.0, 111.6, 111.2, 104.9, 94.8, 87.4, 83.0, 82.4, 78.7, 71.5, 69.6, 58.2, 57.6, 56.1, 56.0, 54.7, 43.7, 42.6 (2C), 40.1, 36.5, 36.1, 34.9, 30.7, 28.1, 20.3, 15.5, 11.7, 9.0; IR ($CHCl_3$) 3481, 2934, 1516, 1457, 1380, 1265, 1095, 1030 cm^{-1}; HRMS (ESI) *m/z* 633.3980 (calcd for $C_{34}H_{58}NaO_9$ $[M+Na]^+$, Δ +0.1 mmu).



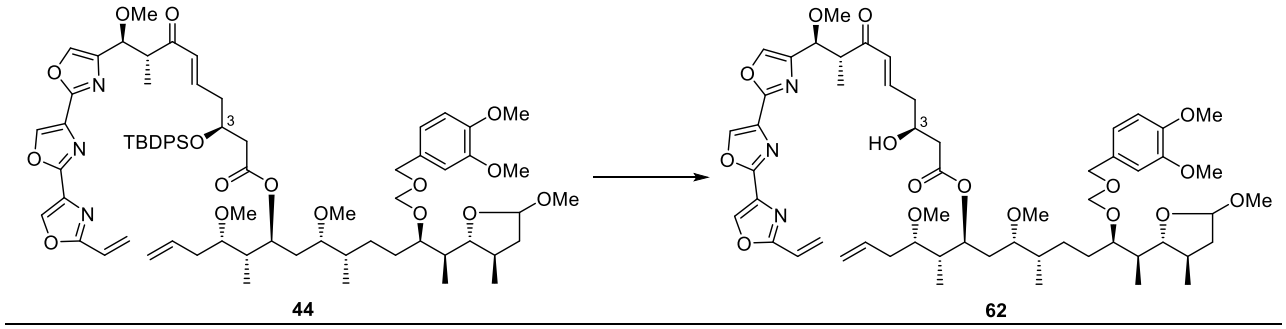
RCM precursor 44. To a stirred solution of the C1–C19 segment **40** (7.2 mg, 10 μmol) and the C20–C35 segment **41** (5.2 mg, 8.7 μmol) in dry CH_2Cl_2 (0.12 mL) cooled at 0 °C were added triethylamine (5 μL, 36 μmol), 2-methyl-6-nitrobenzoic anhydride (MNBA) (4.5 mg, 13 μmol), and a 0.5 M solution of *N*,*N*-dimethyl-4-aminopyridine in dry CH_2Cl_2 (4.5 μL, 2.3 μmol). After being stirred for 34 h at room temperature, the reaction mixture was quenched with sat. $NaHCO_3$ aq. (2 mL) and extracted with EtOAc (3 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude oil was purified with a SiO_2 column chromatography (FL60D 0.6 g, hexane / acetone = 10/1 to 9/1) to give the RCM precursor **44** (10.5 mg, 93%) as a colorless oil. **44**: R_f 0.52 (hexane / EtOAc = 1/1); $[\alpha]^D_{25}$ –8.3 (*c* 1.26, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 8.33 (s, 2H), 7.71 (s, 1H), 7.70–7.65 (m, 4H),7.42–7.33 (m, 6H), 6.97–6.80 (m, 4H), 6.68 (dd, *J* = 11.2, 17.8 Hz, 1H), 6.34 (d, *J* = 17.8 Hz, 1H), 6.14 (d, *J* = 15.9 Hz, 1H), 5.78 (d, *J* = 11.2 Hz, 1H), 5.72 (m, 1H), 5.10–5.01 (m, 3H), 4.87 (d, *J* = 4.6 Hz, 1H), 4.82 (s, 2H), 4.57 (s, 2H), 4.42 (d, *J* = 9.7 Hz, 1H), 4.32 (m, 1H), 4.05 (m, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 3.56 (m, 1H), 3.46 (m, 1H), 3.26 (s, 3H), 3.22 (s, 3H), 3.21 (s, 3H), 3.17 (s, 3H), 3.03 (m, 1H), 2.80 (m, 1H), 2.54–2.04 (m, 8H), 1.79–1.37 (m, 10H), 1.09 (d, *J* = 6.2 Hz, 3H), 1.04 (s, 9H), 0.88–0.79 (m, 12H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.6, 170.1, 161.9, 156.0, 155.4, 149.0, 148.6, 143.2, 140.0, 139.0, 138.5, 137.2, 135.8 (4C), 134.6, 133.5 (2C), 132.9, 131.7, 130.8 (2C), 129.9 (2C), 127.7 (4C), 124.2, 122.6, 120.5, 117.2, 111.4, 110.9, 104.7, 94.4, 87.2, 81.8, 81.1, 78.4, 77.6, 73.6, 69.4, 69.0, 57.8, 57.5, 57.0, 55.9, 55.8, 54.5, 47.0, 43.5, 42.5, 41.6, 39.7 (2C), 35.9, 35.6, 35.2, 31.5, 30.6, 27.04, 27.00 (3C), 20.1, 19.3, 15.6, 14.2, 8.9, 8.8; IR ($CHCl_3$) 2931, 1730, 1669, 1627, 1516, 1462, 1380, 1264, 1103, 1029, 755, 704 cm^{-1}; HRMS (ESI) 1310.6534 (calcd for $C_{72}H_{97}N_3NaO_{16}Si$ $[M+Na]^+$, Δ –0.2 mmu).



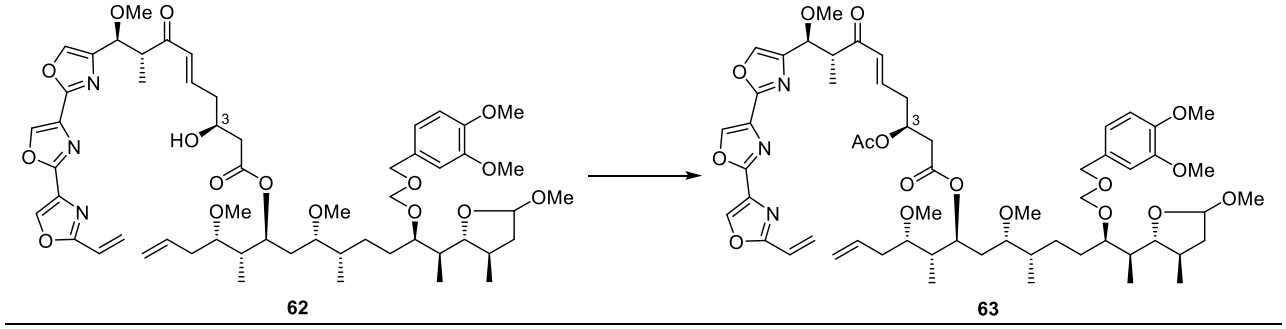
44 **39**

Macrolactone 39. To a stirred solution of the RCM precursor **44** (137 mg, 0.106 mmol) in dry CH_2Cl_2 (101 mL) was added a 6.4 mM solution of Zhan catalyst 1B (**20c**) in dry CH_2Cl_2 (5 mL, 32 μmol). After being stirred for 24 h at reflux temperature, the reaction mixture was concentrated. The crude material was purified with two SiO_2 column chromatographies (FL60D 6 g, hexane / EtOAc = 3/1 to 1/1; FL60D 0.6 g, hexane / EtOAc = 3/1 to 1/1) to give macrolactone (19*E*)-**39** (62.5 mg, 47%) and its stereoisomer (19*Z*)-**39** (37.1 mg, 28%) as colorless oils. (19*E*)-**39**: R_f 0.21 (hexane / EtOAc = 2/3); $[\alpha]^D_{25}$ –33 (*c* 1.15, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 8.06 (s, 1H), 7.74–7.62 (m, 4H), 7.67 (s, 1H), 7.45–7.32 (m, 4H), 7.18–7.03 (m, 2H), 6.91 (s, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 6.32 (d, *J* = 16.0 Hz, 1H), 5.90 (d, *J* = 16.4 Hz, 1H), 5.13 (m, 1H), 4.86 (d, *J* = 4.4 Hz, 1H), 4.81 (d, *J* = 6.8 Hz, 1H), 4.79 (d, *J* = 6.8 Hz, 1H), 4.56 (s, 2H), 4.41 (ddt, *J* = 3.2, 5.2, 5.2 Hz, 1H), 4.37 (d, *J* = 9.6 Hz, 1H), 4.19 (dq, *J* = 9.6, 6.8 Hz, 1H), 4.02 (m, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.54 (dd, *J* = 10.0, 6.8 Hz, 1H), 3.29 (m, 1H), 3.26 (s, 3H), 3.24 (s, 3H), 3.22 (s, 3H), 3.10 (s, 3H), 2.99 (m, 1H), 2.79–2.61 (m, 3H), 2.42 (ddd, *J* = 8.8, 8.8, 14.8 Hz, 1H), 2.27 (m, 1H), 2.20 (m, 1H), 2.06 (dd, *J* = 7.4, 12.6 Hz, 1H), 1.83–1.22 (m, 11H), 1.08 (d, *J* = 6.8 Hz, 3H), 1.03 (s, 9H), 0.85 (m, 6H), 0.84 (d, *J* = 6.8 Hz, 3H), 0.78 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.0, 170.4, 162.5, 156.5, 155.5, 148.9, 148.5, 143.7, 139.6, 139.1, 137.2, 136.9 (2C), 135.9 (4C), 135.1, 133.7, 133.5, 131.5, 130.8, 130.2, 128.8, 129.7, 127.6 (4C), 120.5, 116.4, 111.3, 110.9, 104.6, 94.4, 87.1, 81.7, 79.8, 78.4, 77.1, 73.6, 69.4, 68.8, 57.6, 57.4, 56.2, 55.9, 55.8, 54.4, 43.4, 42.8, 42.4, 40.9, 39.7, 39.5, 35.8, 35.0, 32.7, 32.6, 30.7, 27.1 (3C), 26.9, 20.1, 19.2, 15.6, 14.7, 8.8, 8.1; IR ($CHCl_3$) 3008, 2961, 2934, 1729, 1659, 1609, 1593, 1561, 1427, 1263, 1240, 1177, 1158, 1138, 1097, 1029, 980, 918, 840, 728, 704 cm^{-1}; HRMS (ESI) *m/z* 1282.6223 (calcd for $C_{70}H_{93}N_3NaO_{16}Si$ $[M+Na]^+$, Δ –2.0 mmu).

(19*Z*)-**39**: R_f 0.33 (hexane / EtOAc = 2/3); $[\alpha]^D_{25}$ –35 (*c* 0.037, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 (s, 1 H), 8.09 (s, 1 H), 7.67–7.65 (m, 4 H), 7.61 (s, 1 H), 7.44–7.34 (m, 6 H), 7.00–6.94 (m, 1 H), 6.92–6.81 (m, 3 H), 6.38–6.30 (m, 2 H), 5.82 (d, *J* = 16.1 Hz, 1 H), 5.26 (m, 1 H), 4.88 (d, *J* = 4.7 Hz, 1 H), 4.82 (d, *J* = 6.8 Hz, 1 H), 4.80 (d, *J* = 6.8 Hz, 1 H), 4.57 (s, 2 H), 4.35 (d, *J* = 8.9 Hz, 1 H), 4.04–3.98 (m, 2 H), 3.88 (s, 3 H), 3.87 (s, 3 H), 3.58–3.51 (m, 3 H), 3.37 (s, 3 H), 3.29 (s, 3 H), 3.25 (s, 3 H), 3.23–3.18 (m, 2 H), 3.14 (s, 3 H), 2.83 (m, 1 H), 2.61 (m, 2 H), 2.23 (m, 2 H), 2.10–2.03 (m, 2 H), 1.70–1.20 (m, 7 H), 1.15–1.12 (m, 1 H), 1.09 (d, *J* = 6.6 Hz, 3 H), 1.03 (s, 9 H), 1.01 (d, *J* = 6.9 Hz, 3 H), 0.95 (m, 1 H), 0.89 (m, 1 H), 0.87 (d, *J* = 7.0 Hz, 3 H), 0.85 (d, *J* = 7.0 Hz, 3 H), 0.76 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 202.7, 170.2, 161.8, 156.5, 155.7, 149.0, 148.6, 143.6, 141.0, 139.3, 137.4, 137.3, 136.9, 135.8 (4C), 134.7, 133.6, 133.5, 131.5, 130.8, 130.7, 129.9 (2C), 127.7 (4C), 120.5, 114.1, 111.4, 110.9, 104.7, 94.5, 87.2, 81.8, 81.1, 78.4, 76.6, 74.2, 70.6, 69.8, 69.4, 58.2, 58.0, 56.8, 55.9, 55.8, 54.5, 43.5, 43.4, 42.5, 41.5, 40.3, 39.0, 35.9, 35.2, 33.3, 31.8, 30.7, 27.0 (3C), 20.1, 19.2, 15.5, 14.3, 9.7, 8.8; IR ($CHCl_3$) 2929, 1726, 1670, 1516, 1458, 1379, 1263, 1099, 1029, 754, 703 cm^{-1}; HRMS (ESI) *m/z* 1282.6224 (calcd for $C_{70}H_{93}N_3NaO_{16}Si$ $[M+Na]^+$, Δ –1.9 mmu).

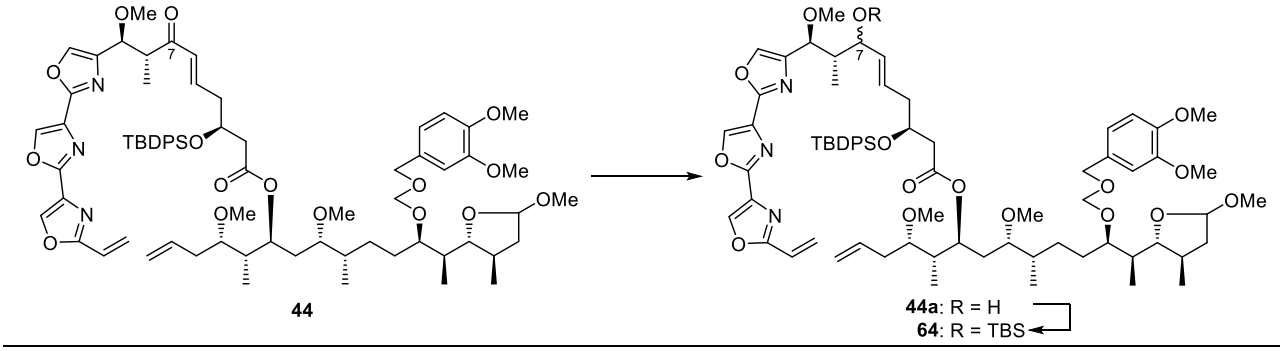


C3 hydroxy analog 62. To a stirred solution of the RCM precursor **44** (9.7 mg, 7.53 μmol) in dry THF (1.0 mL) was added a 1.0 M solution of TBAF/AcOH (1:1) in THF (0.23 mL, 0.23 mmol) [prepared by adding AcOH (114.5 μL, 2.0 mmol) to a 1.0 M solution of TBAF in THF (2.0 mL, 2.0 mmol)]. After being stirred for 21 h at room temperature, the reaction mixture was diluted with sat. $NaHCO_3$ aq. (7 mL), and extracted with EtOAc (4 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude oil was purified with a SiO_2 column chromatography (FL60D 0.4 g, hexane / acetone = 5/1 to 3/1) to give C3 hydroxy analog **62** (7.5 mg, 95%) as a colorless oil. **62**: R_f 0.19 (hexane / acetone = 2:3); $[\alpha]^{D}_{25}$ –26 (*c* 0.58, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 8.33 (s, 1H), 7.71 (s, 1H), 6.98 (dt, *J* = 12.7, 5.8 Hz, 1H), 6.92 (s, 1H), 6.90 (d, *J* = 6.7 Hz, 1H), 6.82 (d, *J* = 6.7 Hz, 1H), 6.67 (dd, *J* = 9.1, 14.1 Hz, 1H), 6.34 (d, *J* = 14.1 Hz, 1H), 6.28 (d, *J* = 12.7 Hz, 1H), 5.77 (d, *J* = 9.1 Hz, 1H), 5.74 (m, 1H), 5.20 (m, 1H), 5.11 (d, *J* = 13.7 Hz, 1H), 5.06 (d, *J* = 8.1 Hz, 1H), 4.88 (d, *J* = 3.8 Hz, 1H), 4.83 (d, *J* = 5.5 Hz, 1H), 4.81 (d, *J* = 5.5 Hz, 1H), 4.57 (s, 2H), 4.42 (d, *J* = 7.7 Hz, 1H), 4.18 (m, 1H), 4.05 (m, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.55 (m, 3H), 3.29 (s, 6H), 3.27 (s, 3H), 3.19 (s, 3H), 2.96 (m, 1H), 2.55–2.38 (m, 5H), 2.27–2.14 (m, 2H), 2.08 (dd, *J* = 5.9, 10.2 Hz, 1H), 1.88–1.81 (m, 1H), 1.80–1.65 (m, 3H), 1.65–1.45 (m, 6H), 1.09 (d, *J* = 5.2 Hz, 3H), 0.89 (d, *J* = 5.9 Hz, 3H), 0.88 (d, *J* = 5.5 Hz, 3H), 0.87 (d, *J* = 4.8 Hz, 3H), 0.85 (d, *J* = 5.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.0, 171.7, 161.9, 156.0, 155.5, 148.9, 148.5, 143.0, 139.8, 139.0, 138.6, 137.3 (2C), 134.6, 132.8, 131.6, 130.7, 124.2, 122.5, 120.5, 117.3, 111.3, 110.9, 104.7, 94.5, 87.1, 81.7, 80.7, 78.4, 77.2, 74.0, 69.4, 66.9, 57.8, 57.5, 57.0, 55.9, 55.8, 54.5, 46.8, 43.4, 42.4, 41.5, 39.6, 39.3, 35.9, 35.4, 35.3, 32.3, 30.5, 27.4, 20.1, 15.5, 14.2, 9.4, 8.8; IR ($CHCl_3$) 3632, 3009, 2937, 2836, 1721, 1665, 1630, 1516, 1464, 1420, 1380, 1264, 1222, 1216, 1211, 1157, 1139, 1096, 1029, 980, 945, 919, 788, 784, 774 cm^{-1}; HRMS (ESI) *m/z* 1072.5383 (calcd for $C_{56}H_{79}N_3NaO_{16}$ $[M+Na]^+$, Δ +2.5 mmu).



C3 acetoxy analog 63. To a stirred solution of C3 hydroxy analog **62** (4.7 mg, 4.48 μmol) in dry pyridine (0.45 mL) cooled at 0 °C were added acetic anhydride (130 μL, 1.4 mmol) and *N*,*N*-dimethyl-4-aminopyridine (1.2 mg, 9.0 μmol). After being stirred for 8 h at room temperature, the reaction was quenched with sat. $NaHCO_3$ aq. (3 mL) at 0 °C, and extracted with EtOAc (3 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 0.4 g, hexane /

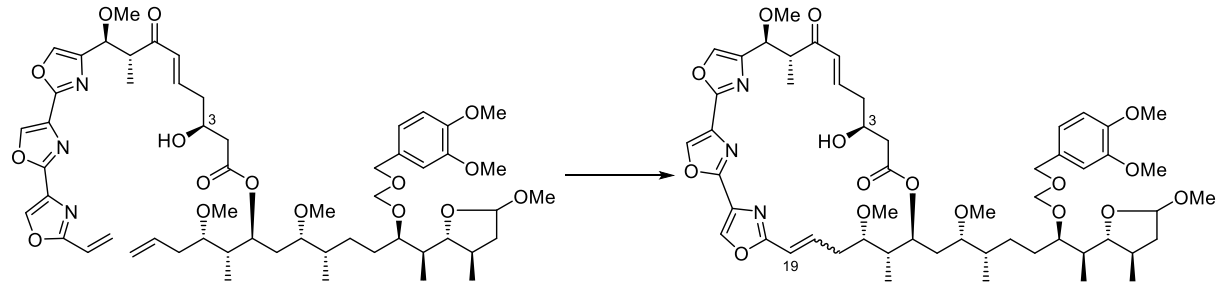
EtOAc = 1/1 to 2/3) to give C3 acetoxy analog **63** (4.7 mg, 96%) as a colorless oil. **63**: R_f 0.40 (hexane / EtOAc = 2/3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.33 (s, 1H), 7.72 (s, 1H), 6.94–6.80 (m, 4H), 6.67 (dd, *J* = 11.4, 17.7 Hz, 1H), 6.34 (d, *J* = 17.7 Hz, 1H), 6.27 (d, *J* = 15.6 Hz, 1H), 5.83–5.71 (m, 1H), 5.78 (d, *J* = 11.4 Hz, 1H), 5.38 (m, 1H), 5.16 (m, 1H), 5.09 (d, *J* = 17.5 Hz, 1H), 5.06 (d, *J* = 10.5 Hz, 1H), 4.88 (d, *J* = 4.7 Hz, 1H), 4.83 (d, *J* = 7.0 Hz, 1H), 4.81 (d, *J* = 7.0 Hz, 1H), 4.58 (s, 2H), 4.42 (d, *J* = 9.7 Hz, 1H), 4.05 (m, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 3.59–3.47 (m, 3H), 3.29 (s, 3H), 3.27 (s, 6H), 3.18 (s, 3H), 3.13 (m, 1H), 2.88 (m, 1H), 2.70–2.51 (m, 4H), 2.45–2.35 (m, 1H), 2.28–2.15 (m, 2H), 2.08 (dd, *J* = 7.5, 12.6 Hz, 1H), 2.03 (s, 3H), 1.86–1.44 (m, 9H), 1.09 (d, *J* = 6.6 Hz, 3H), 0.89–0.84 (m, 12H); HRMS (ESI) *m/z* 1114.5444 (calcd for $C_{58}H_{81}N_3NaO_{17}$ $[M+Na]^+$, Δ −2.0 mmu).



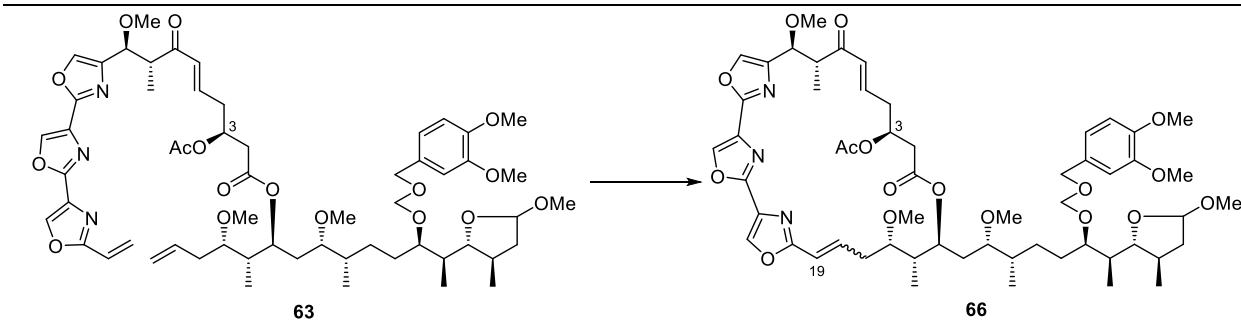
C7 silyloxy analog 64. To a stirred solution of the RCM precursor **44** (18.3 mg, 14.2 μmol) in dry MeOH (2.8 mL) cooled at –20 °C was added cerium (III) chloride heptahydrate (95.2 mg, 0.26 mmol). The mixture was stirred for 5 min, and sodium borohydride (8.1 mg, 0.21 mmol) was added. After being stirred at –20 °C for 1 h, the reaction was quenched with acetone (130 μL), and stirred for 5 min. The resulting mixture was diluted with EtOAc (2 mL) and sat. NH_4Cl aq. (7 mL), and extracted with EtOAc (5 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (BW-820MH 0.5 g, hexane / acetone = 3/1) to give C7 hydroxy analog **44a** (16.6 mg, 91%, a 10:1 diastereomeric mixture at C7) as a colorless oil. **44a**: R_f 0.45 (hexane / acetone = 2/1); $[\alpha]^D_{25}$ +2.0 (*c* 1.35, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 8.31 (s, 1H), 7.71–7.63 (m, 4H), 7.65 (s, 1H), 7.44–7.31 (m, 6H), 6.92 (s, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 6.82 (d, *J* = 8.1 Hz, 1H), 6.67 (dd, *J* = 11.3, 17.7 Hz, 1H), 6.34 (d, *J* = 17.7 Hz, 1H), 5.77 (d, *J* = 11.3 Hz, 1H), 5.78–5.60 (m, 2H), 5.43 (dd, *J* = 5.7, 15.5 Hz, 1H), 5.05 (m, 3H), 4.88 (d, *J* = 4.7 Hz, 1H), 4.83 (d, *J* = 6.9 Hz, 1H), 4.80 (d, *J* = 6.9 Hz, 1H), 4.58 (s, 2H), 4.31 (m, 1H), 4.24 (m, 1H), 4.18 (d, *J* = 8.0 Hz, 1H), 4.04 (m, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.56 (dd, *J* = 6.7, 9.8 Hz, 1H), 3.30–3.13 (m, 3H), 3.28 (s, 3H), 3.27 (s, 3H), 3.23 (s, 6H), 3.05 (m, 1H), 2.83 (m, 1H), 2.55–2.40 (m, 2H), 2.39–2.30 (m, 2H), 2.30–2.13 (m, 4H), 2.08 (dd, *J* = 7.4, 12.6 Hz, 1H), 1.84–1.38 (m, 9H), 1.09 (d, *J* = 6.6 Hz, 3H), 1.03 (s, 9H), 0.87 (d, *J* = 7.0 Hz, 3H), 0.83 (d, *J* = 7.0 Hz, 3H), 0.81 (d, *J* = 6.9 Hz, 3H), 0.74 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.5, 162.0 [160.4], 156.0, 155.3, 149.0, 148.6, 141.0 [140.7], 139.0, 138.4 [138.1], 136.6, 135.9 (2C), 135.8 (2C), 134.6, 134.0, 133.9, 133.8, 131.6, 130.8, 130.7 [130.6], 129.7, 129.6, 127.6 (2C), 127.5 (2C), 126.5, 124.2, 122.6 [121.8], 120.5, 117.2, 111.4, 111.0, 104.7, 94.4, 87.2, 81.8, 81.2, 79.8, 78.4, 73.4, 73.0, 69.9 [69.7], 69.4, 57.9, 57.5, 57.1, 55.9, 55.8, 54.5, 43.5, 42.4, 42.1, 41.2, 39.7, 39.4, 35.9, 35.7, 35.4, 31.6, 30.6, 27.3, 27.0 (3C), 20.1, 19.3 [19.0], 15.5, 11.4, 8.9, 8.8. Chemical shifts of the minor isomers are within parentheses (square brackets); IR ($CHCl_3$) 3492, 3008, 2962, 2934, 2860, 1724, 1641, 1592, 1516, 1465, 1427, 1382, 1308, 1264, 1240, 1223, 1217, 1210, 1190, 1157, 1139, 1103, 1029, 980, 942, 918, 822, 774, 766, 744 cm^{-1}; HRMS (ESI) *m/z* 1312.6681 (calcd for $C_{72}H_{99}N_3NaO_{16}Si$ $[M+Na]^+$, Δ –1.1 mmu).

To a stirred solution of C7 hydroxy analog **44a** (5.5 mg, 4.26 μmol) in dry DMF (0.10 mL) were added 1.0 M solution of *tert*-butyldimethylsilyl chloride in DMF (43 μL, 43 μmol) and imidazole (5.8 mg, 85 μmol). After being

stirred for 24 h at room temperature, the resulting mixture was diluted with EtOAc (0.5 mL) and sat. $NaHCO_3$ aq. (2 mL), and extracted with EtOAc (2 mL × 3). The combined extracts were washed with water and brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 0.4 g, hexane / acetone = 5/1) to give C7 silyloxy analog **64** (5.6 mg, 93%) as a colorless oil. **64**: R_f 0.51 (hexane / acetone = 2/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 2H), 7.71–7.66 (m, 4H), 7.64 (s, 1H), 7.42–7.33 (m, 6H), 6.92 (s, 1H), 6.91 (d, *J* = 8.2 Hz, 1H), 6.82 (d, *J* = 8.2 Hz, 1H), 6.67 (dd, *J* = 11.3, 17.7 Hz, 1H), 6.34 (d, *J* = 17.7 Hz, 1H), 5.77 (d, *J* = 11.3 Hz, 1H), 5.76–5.68 (m, 1H), 5.61 (dt, *J* = 15.2, 7.6 Hz, 1H), 5.42 (dd, *J* = 6.4, 15.2 Hz, 1H), 5.09–5.01 (m, 3H), 4.87 (d, *J* = 4.7 Hz, 1H), 4.82 (d, *J* = 6.9 Hz, 1H), 4.80 (d, *J* = 6.9 Hz, 1H), 4.73 (d, *J* = 6.1 Hz, 1H), 4.57 (s, 2H), 4.23 (m, 1H), 4.04 (m, 3H), 3.87 (s, 3H), 3.86 (s, 3H), 3.56 (dd, *J* = 6.7, 9.8 Hz, 1H), 3.26 (s, 3H), 3.22 (s, 3H), 3.21 (s, 3H), 3.06 (m, 1H), 2.82 (m, 1H), 2.56–1.95 (m, 9H), 1.82–1.38 (m, 9H), 1.09 (d, *J* = 6.6 Hz, 3H), 1.04 (s, 9H), 0.91 (s, 9H), 0.86 (d, *J* = 6.6 Hz, 3H), 0.83 (d, *J* = 7.0 Hz, 3H), 0.79 (d, *J* = 6.8 Hz, 3H), 0.62 (d, *J* = 6.8 Hz, 3H), 0.06 (s, 3H), −0.01 (s, 3H).



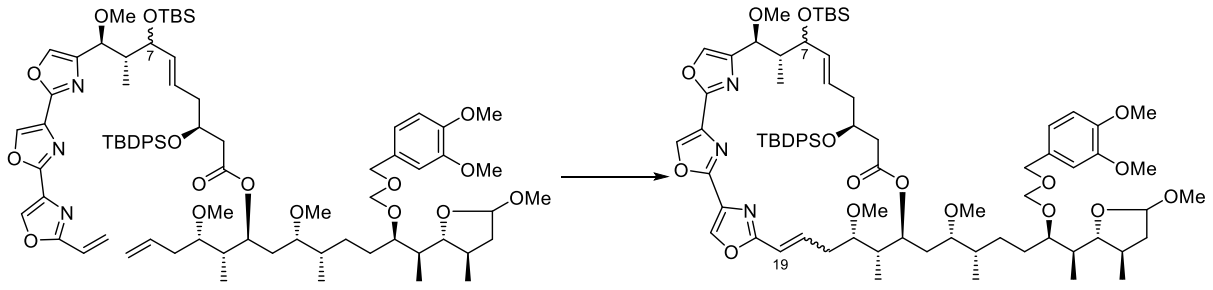
RCM of C3 hydroxy analog 62. To a stirred solution of C3 hydroxy analog **62** (7.2 mg, 6.86 μmol) in dry CH_2Cl_2 (7.1 mL) was added a 4.1 mM solution of Zhan catalyst 1B (**20c**) in dry CH_2Cl_2 (0.5 mL, 2.1 μmol). After being stirred for 24 h at reflux temperature, the reaction mixture was concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 0.4 g, hexane / EtOAc = 1/1 to 1/3) to give an *E*/*Z* mixture of C3 hydroxy macrolactone **65** (4.4 mg, 63%, *E*/*Z* = 3.0/1.0) as a colorless oil. **65**: R_f 0.30 (hexane / EtOAc = 1/3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 [8.16] (s, 1H), 8.04 [8.13] (s, 1H), 7.65 [7.63] (s, 1H), 7.25 [7.02] (dt, *J* = 15.8, 7.2 Hz, 1H), 7.14 [6.36] (dt, *J* = 16.0, 7.5 Hz, 1H), 6.94–6.80 (m, 3H), 6.35 [6.33] (d, *J* = 15.8 Hz, 1H), 6.23 [6.15] (d, *J* = 16.0 Hz, 1H), 5.41 (m, 1H), 5.27 (m, 1H), 4.88 [4.87] (d, *J* = 4.5 Hz, 1H), 4.83 [4.81] (m, 2H), 4.58 [4.57] (s, 2H), 4.44 [4.27] (m, 1H), 4.35 (d, *J* = 8.4 Hz, 1H), 4.08–3.89 (m, 2H), 3.88 (s, 3H), 3.87 (s, 3H), 3.56 [3.63] (m, 1H), 3.34 [3.44] (s, 3H), 3.33–3.25 (m, 1H), 3.31 [3.30] (s, 3H), 3.28 [3.27] (s, 3H), 3.19 [3.21] (s, 3H), 2.98 (m, 1H), 2.72–2.38 (m, 4H), 2.28–2.16 (m, 2H), 2.12–1.86 (m, 3H), 1.77–1.45 (m, 9H), 1.09 [1.088] (d, *J* = 6.7 [6.3] Hz, 3H), 1.01–0.81 (m, 12H). Chemical shifts of the *Z* isomers are within parentheses (square brackets).



RCM of C3 acetoxy analog 63. To a stirred solution of C3 acetoxy analog **63** (4.7 mg, 4.30 μmol) in dry CH_2Cl_2 (4.3 mL) was added a 2.6 mM solution of Zhan catalyst 1B (**20c**) in dry CH_2Cl_2 (0.5 mL, 1.3 μmol). After being

stirred for 24 h at reflux temperature, the reaction mixture was concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 0.5 g, hexane / EtOAc = 1/1 to 1/3) to give C3 acetoxy macrolactone (19*E*)-**66** (2.6 mg, 57%) and its stereoisomer (19*Z*)-**66** (1.4 mg, 31%) as colorless oils. (19*E*)-**66**: R_f 0.28 (hexane / EtOAc = 1/3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 8.06 (s, 1H), 7.65 (s, 1H), 7.18 (dt, *J* = 15.9, 7.4 Hz, 1H), 7.07 (dt, *J* = 16.1, 6.8 Hz, 1H), 6.92 (s, 1H), 6.91 (d, *J* = 7.8 Hz, 1H), 6.82 (d, *J* = 7.8 Hz, 1H), 6.32 (d, *J* = 15.9 Hz, 1H), 6.17 (d, *J* = 16.1 Hz, 1H), 5.48 (m, 1H), 5.26 (m, 1H), 4.88 (d, *J* = 4.8 Hz, 1H), 4.84 (d, *J* = 6.5 Hz, 1H), 4.81 (d, *J* = 6.5 Hz, 1H), 4.58 (s, 2H), 4.33 (d, *J* = 8.7 Hz, 1H), 4.05 (m, 2H), 3.88 (s, 3H), 3.87 (s, 3H), 3.56 (dd, *J* = 6.5, 9.6 Hz, 1H), 3.34–3.22 (m, 1H), 3.31 (s, 3H), 3.28 (s, 6H), 3.13 (s, 3H), 3.02–2.80 (m, 3H), 2.73–2.51 (m, 2H), 2.48–2.38 (m, 1H), 2.28–2.17 (m, 1H), 2.13 (s, 3H), 2.12–2.00 (m, 3H), 1.93–1.45 (m, 9H), 1.09 (d, *J* = 6.5 Hz, 3H), 0.92–0.84 (m, 12H); HRMS (ESI) *m/z* 1086.5144 (calcd for $C_{56}H_{77}N_3NaO_{17}$ $[M+Na]^+$, Δ –0.6 mmu).

(19*Z*)-**67**: R_f 0.40 (hexane / EtOAc = 1/3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 8.11 (s, 1H), 7.63 (s, 1H), 6.94–6.80 (m, 4H), 6.45–6.36 (m, 1H), 6.32 (d, *J* = 11.5 Hz, 1H), 6.13 (d, *J* = 16.1 Hz, 1H), 5.34–5.21 (m, 2H), 4.87 (d, *J* = 4.3 Hz, 1H), 4.83 (d, *J* = 6.9 Hz, 1H), 4.80 (d, *J* = 6.9 Hz, 1H), 4.57 (s, 2H), 4.35 (d, *J* = 8.6 Hz, 1H), 4.08–3.91 (m, 2H), 3.88 (s, 3H), 3.87 (s, 3H), 3.61–3.46 (m, 2H), 3.41 (s, 3H), 3.28 (s, 3H), 3.26 (s, 3H), 3.20 (s, 3H), 2.99 (m, 1H), 2.86–2.67 (m, 2H), 2.62–2.47 (m, 3H), 2.30–1.94 (m, 4H), 2.06 (s, 3H), 1.75–1.46 (m, 9H), 1.09 (d, *J* = 6.3 Hz, 3H), 1.00 (d, *J* = 7.5 Hz, 3H), 0.95 (d, *J* = 6.9 Hz, 3H), 0.86 (d, *J* = 6.9 Hz, 3H), 0.83 (d, *J* = 6.9 Hz, 3H); HRMS (ESI) *m/z* 1086.5167 (calcd for $C_{56}H_{77}N_3NaO_{17}$ $[M+Na]^+$, Δ +1.6 mmu).

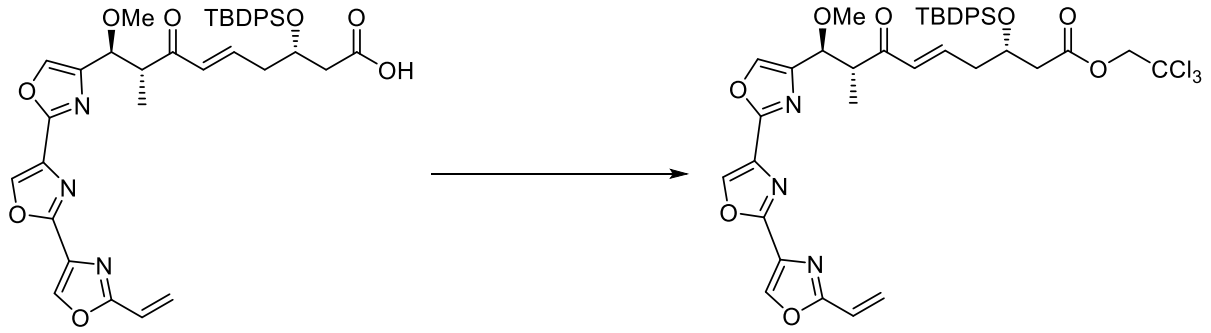


64 **67**

RCM of C7 silyloxy analog 64. To a stirred solution of C7 silyloxy analog **64** (5.6 mg, 3.99 μmol) in dry CH_2Cl_2 (3.9 mL) was added a 2.4 mM solution of Zhan catalyst 1B (**20c**) in dry CH_2Cl_2 (0.5 mL, 1.2 μmol). After being stirred for 24 h at reflux temperature, the reaction mixture was concentrated. The crude material was purified with two SiO_2 column chromatographies (FL60D 0.4 g, hexane / EtOAc = 3/1 to 1/2; FL60D 0.4 g, hexane / EtOAc = 3/1 to 1/1) to give C7 silyloxy macrolactone (19*E*)-**67** (1.8 mg, 33%) and its stereoisomer (19*Z*)-**67** (0.8 mg, 15%) as a colorless oils. (19*E*)-**67**: R_f 0.50 (hexane / EtOAc = 1/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 8.03 (s, 1H), 7.77–7.62 (m, 4H), 7.56 (s, 1H), 7.42–7.31 (m, 6H), 7.11 (m, 1H), 6.92 (s, 1H), 6.90 (d, *J* = 7.2 Hz, 1H), 6.81 (d, *J* = 7.2 Hz, 1H), 6.31 (d, *J* = 15.4 Hz, 1H), 5.68 (dt, *J* = 15.8, 7.1 Hz, 1H), 5.38 (dd, *J* = 6.7, 15.8 Hz, 1H), 5.15 (m, 1H), 4.87 (d, *J* = 4.6 Hz, 1H), 4.81 (d, *J* = 6.9 Hz, 1H), 4.79 (d, *J* = 6.9 Hz, 1H), 4.57 (s, 2H), 4.36 (m, 1H), 4.21 (m, 1H), 4.07 (d, *J* = 7.1 Hz, 1H), 4.04 (m, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.55 (m, 1H), 3.33–3.17 (m, 2H), 3.27 (s, 3H), 3.25 (s, 6H), 3.19 (s, 3H), 3.02 (m, 1H), 2.76–1.87 (m, 9H), 1.74–1.44 (m, 9H), 1.09 (d, *J* = 6.6 Hz, 3H), 1.04 (s, 9H), 0.90–0.83 (m, 6H), 0.84 (s, 9H), 0.77 (d, *J* = 7.4 Hz, 3H), 0.71 (d, *J* = 6.6 Hz, 3H), −0.05 (s, 3H), −0.11 (s, 3H).

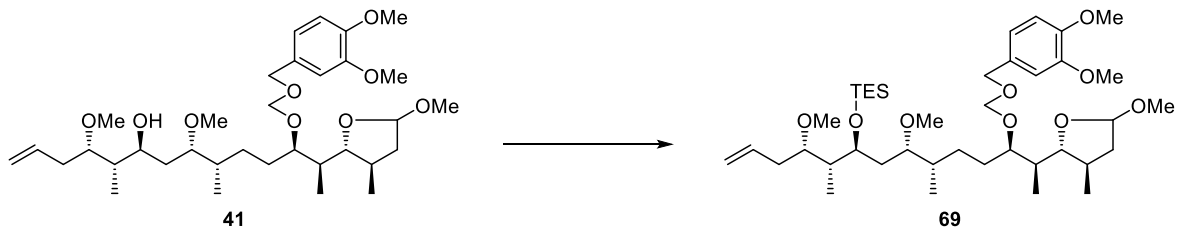
(19*Z*)-**67**: R_f 0.55 (hexane / EtOAc = 1/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 8.06 (s, 1H), 7.72–7.66 (m, 4H), 7.56 (s, 1H), 7.43–7.33 (m, 6H), 6.92 (s, 1H), 6.91 (d, *J* = 7.5 Hz, 1H), 6.81 (d, *J* = 7.5 Hz, 1H), 6.40–6.32 (m, 1H), 6.29 (d, *J* = 11.5 Hz, 1H), 5.51–5.40 (m, 2H), 5.20 (m, 1H), 4.87 (d, *J* = 4.1 Hz, 1H), 4.81 (d, *J* = 7.0 Hz, 1H), 4.79 (d, *J* = 7.0 Hz, 1H), 4.57 (s, 2H), 4.53 (m, 1H), 4.17 (m, 1H), 4.04 (m, 1H), 4.02 (d, *J* = 8.5 Hz, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.55 (dd, *J* = 6.6, 9.5 Hz, 1H), 3.46 (m, 1H), 3.33 (s, 3H), 3.29–3.19 (m, 1H), 3.24 (s, 6H), 3.17

(s, 3H), 2.97 (m, 1H), 2.64–2.30 (m, 5H), 2.26–2.01 (m, 4H), 1.72–1.46 (m, 9H), 1.09 (d, *J* = 6.4 Hz, 3H), 1.06 (d, *J* = 7.0 Hz, 3H), 1.03 (s, 9H), 0.88 (s, 9H), 0.85 (d, *J* = 7.0 Hz, 3H), 0.76 (d, *J* = 6.8 Hz, 3H), 0.69 (d, *J* = 6.8 Hz, 3H), −0.01 (s, 3H), −0.08 (s, 3H).



40 **68**

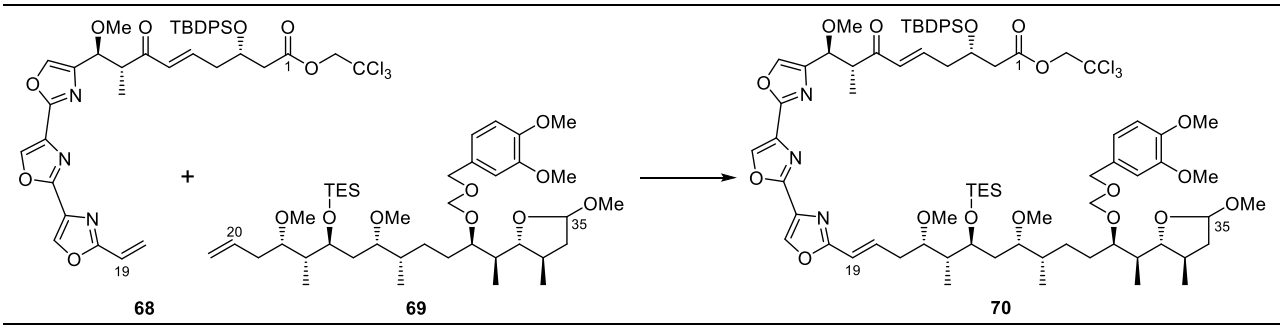
TCE ester 68. To a stirred solution of the C1–C19 segment **40** (10.0 mg, 14 μmol) and 2,2,2-trichloroethanol (6 μL, 57 μmol) in dry CH_2Cl_2 (0.7 mL) cooled at 0 °C were added *N*,*N*-dimethyl-4-aminopyridine (3.5 mg, 29 μmol) and EDC·HCl (6.1 mg, 32 μmol). After being stirred at room temperature for 11.5 h, the reaction mixture was diluted with 5% citric acid aq. (4 mL) and extracted with EtOAc (3 mL × 3). The combined extracts were washed with water and brine, dried with Na_2SO_4, and concentrated. The crude oil was purified with a SiO_2 column chromatography (FL60D 1.5 g, hexane / EtOAc = 6/1 to 4/1) to give TCE ester **68** (10.6 mg, 89%) as a colorless oil. **68**: R_f 0.63 (hexane / EtOAc = 1/1); $[\alpha]^D_{23}$ –10 (*c* 0.32, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.332 (s, 1H), 8.330 (s, 1H), 7.73–7.70 (m, 4H), 7.70 (s, 1H), 7.48–7.34 (m, 6H), 6.83 (dt, *J* = 15.9, 7.6 Hz, 1H), 6.68 (dd, *J* = 11.1, 17.7 Hz, 1H), 6.35 (d, *J* = 17.7 Hz, 1H), 6.15 (d, *J* = 15.9 Hz, 1H), 5.78 (d, *J* = 11.1 Hz, 1H), 4.69–4.55 (AB quart, *J* = 12.0 Hz, 2H), 4.40 (d, *J* = 9.9 Hz, 1H), 4.36 (m, 1H), 3.45 (dq, *J* = 9.9, 7.0 Hz, 1H), 3.18 (s, 3H), 2.66–2.41 (m, 4H), 1.05 (s, 9H), 0.85 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.9, 169.4, 162.1, 156.1, 155.7, 142.5, 140.0, 139.1, 138.7, 137.4, 136.0 (2C), 136.0 (2C), 133.5, 133.4, 133.3, 131.8, 130.9, 130.1, 130.1, 127.9 (2C), 127.9 (2C), 124.4, 122.7, 94.9, 77.8, 74.1, 69.1, 57.1, 47.2, 41.3, 40.1, 27.1 (3C), 19.4, 14.3; IR ($CHCl_3$) 3167, 3032, 3007, 2933, 2859, 1752, 1693, 1665, 1628, 1541, 1462, 1428, 1377, 1308, 1269, 1223, 1209, 1112, 978, 942 cm^{-1}; HRMS (ESI) *m/z* 848.1715 (calcd for $C_{40}H_{42}{}^{35}Cl_3N_3NaO_8Si$ $[M+Na]^+$, Δ +1.1 mmu).



41 **69**

TES ether 69. To a stirred solution of the C20–C35 segment **41** (88.0 mg, 0.144 mmol) in dry DMF (1.4 mL) cooled at 0 °C were added imidazole (196 mg, 2.88 mmol) and chlorotriethylsilane (0.24 mL, 1.4 mmol). After being stirred for 21 h at 40 °C, the reaction mixture was diluted with sat. $NaHCO_3$ aq. (10 mL) and extracted with EtOAc (10 mL × 2). The combined extracts were washed with water and brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 6 g, hexane / EtOAc = 9/1 to 0/1) to give TES ether **69** (104 mg, 99%) as a colorless oil. **69**: R_f 0.83 (hexane / EtOAc = 1/1); $[\alpha]^D_{25}$ –6.2 (*c* 0.80, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.93–6.90 (m, 2H), 6.82 (d, *J* = 8.6 Hz, 1H), 5.82 (ddt, *J* = 9.7, 17.0, 7.3 Hz, 1H),

5.15–5.04 (m, 2H), 4.88 (d, *J* = 4.9 Hz, 1H), 4.83 (s, 2H), 4.59 (s, 2H), 4.06 (m, 1H), 3.90 (m, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.57 (dd, *J* = 6.8, 9.7 Hz, 1H), 3.31 (s, 3H), 3.31 (s, 3H), 3.28 (s, 3H), 3.20 (m, 1H), 2.96 (dt, *J* = 5.8, 5.8 Hz, 1H), 2.33–2.17 (m, 3H), 2.09 (dd, *J* = 7.8, 12.4 Hz, 1H), 1.82–1.44 (m, 8H), 1.38–1.21 (m, 2H), 1.10 (d, *J* = 6.5 Hz, 3H), 0.95 (t, *J* = 7.7 Hz, 9H), 0.91 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 7.0 Hz, 3H), 0.86 (d, *J* = 6.8 Hz, 3H), 0.58 (q, *J* = 7.7 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.2, 148.8, 134.8, 131.1, 120.7, 117.2, 111.6, 111.2, 104.8, 94.6, 87.4, 82.8, 82.3, 78.7, 70.9, 69.6, 57.5, 57.2, 56.1, 56.0, 54.6, 43.6, 43.0, 42.7, 36.1, 35.3, 35.2, 33.3, 30.8, 27.4, 20.3, 15.8, 9.0, 8.8, 7.2 (3C), 5.5 (3C); IR ($CHCl_3$) 3510, 3091, 3072, 3037, 3008, 2960, 2937, 2911, 2877, 2832, 1516, 1465, 1420, 1382, 1264, 1239, 1157, 1139, 1094, 1029, 855, 681 cm^{-1}; HRMS (ESI) *m/z* 747.4841 (calcd for $C_{40}H_{72}NaO_9Si$ $[M+Na]^+$, Δ –0.2 mmu).

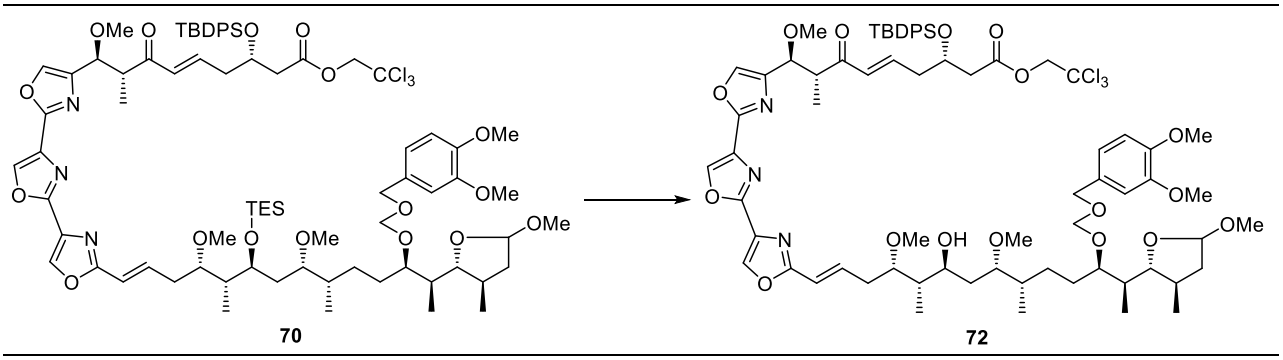


Coupling product 70. To a stirred solution of TCE ester **68** (10.4 mg, 12.6 μmol) and TES ether **69** (11.0 mg, 15.2 μmol) was added a solution of the 2nd generation Hoveyda–Grubbs catalyst (**20a**) (1.6 mg, 2.6 μmol) in dry CH_2Cl_2 (0.97 mL). After being stirred for 20 h at reflux temperature, the reaction mixture was concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 1 g, hexane / EtOAc = 9/1, 4/1, 2/1, 1/1 to 1/2) to give the coupling product **70** (14.8 mg, 77%, *E*/*Z* = 5.0/1.0), and TES ether homodimer **71** (4.0 mg, 19%, *E*/*Z* = 2/1) as colorless oils, and to recover **68** (1.7 mg) and **69** (1.5 mg). (19*E*)-**70**: R_f 0.13 (hexane / EtOAc = 2/1); $[\alpha]^D_{25}$ –8.1 (*c* 0.28, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 8.28 (s, 1H), 7.71–7.65 (m, 4H), 7.70 (s, 1H), 7.47–7.37 (m, 6H), 6.95–6.87 (m, 2H), 6.91 (ddd, *J* = 6.8, 7.6, 16.0 Hz, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 6.82 (dt, *J* = 15.8, 7.6 Hz, 1H), 6.44 (d, *J* = 16.0 Hz, 1H), 6.15 (d, *J* = 15.8 Hz, 1H), 4.89 (d, *J* = 4.4 Hz, 1H), 4.85–4.81 (AB quart, *J* = 6.8 Hz, 2H), 4.67–4.56 (AB quart, *J* = 12.0 Hz, 2H), 4.59 (s, 2H), 4.39 (d, *J* = 9.8 Hz, 1H), 4.35 (m, 1H), 4.07 (m, 1H), 3.91 (m, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 3.57 (dd, *J* = 6.6, 9.6 Hz, 1H), 3.44 (dq, *J* = 9.8, 7.2 Hz, 1H), 3.35 (s, 3H), 3.33 (s, 3H), 3.30 (s, 3H), 3.19 (m, 1H), 3.17 (s, 3H), 3.06 (m, 1H), 2.65 (dd, *J* = 6.4, 15.2 Hz, 1H), 2.60 (dd, *J* = 6.0, 15.2 Hz, 1H), 2.59–2.40 (m, 4H), 2.23 (m, 1H), 2.09 (dd, *J* = 7.2, 12.4 Hz, 1H), 1.84–1.72 (m, 2H), 1.64–1.46 (m, 5H), 1.61 (ddd, *J* = 4.8, 7.6, 12.4 Hz, 1H), 1.36–1.16 (m, 2H), 1.10 (d, *J* = 6.4 Hz, 3H), 1.05 (s, 9H), 0.95 (d, *J* = 7.2 Hz, 3H), 0.89 (t, *J* = 7.8 Hz, 9H), 0.884 (d, *J* = 6.8 Hz, 3H), 0.877 (d, *J* = 6.8 Hz, 3H), 0.84 (d, *J* = 7.2 Hz, 3H), 0.53 (q, *J* = 7.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.1, 169.6, 162.3, 156.5, 155.9, 149.3, 148.9, 142.7, 140.2, 139.0, 139.0, 138.8, 137.5, 136.24 (2C), 136.16 (2C), 133.6, 133.6, 133.5, 131.9, 131.1, 130.9, 130.32, 130.28, 128.13 (2C), 128.08 (2C), 120.9, 118.3, 111.7, 111.2, 105.0, 95.1, 94.7, 87.5, 82.7, 82.4, 78.8, 77.9, 74.3, 70.4, 69.8, 69.3, 57.9, 57.5, 57.3, 56.3, 56.1, 54.8, 47.4, 43.8, 42.8, 41.4, 40.3, 36.2, 35.1, 34.3, 33.4, 31.0, 30.0, 27.3, 27.2 (3C), 20.5, 19.6, 16.1, 14.5, 9.5, 9.2, 7.3 (3C), 5.6 (3C); IR ($CHCl_3$) 3021, 3006, 2958, 2934, 1751, 1696, 1664, 1628, 1592, 1544, 1517, 1464, 1427, 1380, 1263, 1239, 1156, 1138, 1096, 1029, 919 cm^{-1}; HRMS (ESI) *m/z* 783.8092 (calcd for $(C_{78}H_{110}{}^{35}Cl_3N_3Na_2O_{17}Si_2)/2$ $[M+2Na]^{2+}$, Δ –2.5 mmu).

(19*Z*)-**70**: R_f 0.20 (hexane / EtOAc = 2/1); $[\alpha]^D_{26}$ –5.1 (*c* 0.49, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.33 (s, 1H), 7.71–7.66 (m, 4H), 7.70 (s, 1H), 7.47–7.37 (m, 6H), 6.93–6.90 (m, 2H), 6.83 (dt, *J* = 15.8, 7.5 Hz, 1H), 6.82 (d, *J* = 7.6 Hz, 1H), 6.44 (d, *J* = 11.8 Hz, 1H), 6.25 (dt, *J* = 11.8, 7.2 Hz, 1H), 6.15 (d, *J* = 15.8 Hz, 1H), 4.88 (d, *J* = 5.2 Hz, 1H), 4.85– 4.81 (AB quart, *J* = 7.0 Hz, 2H), 4.68–4.56 (AB quart, *J* = 11.8 Hz, 2H), 4.59 (s, 2H),

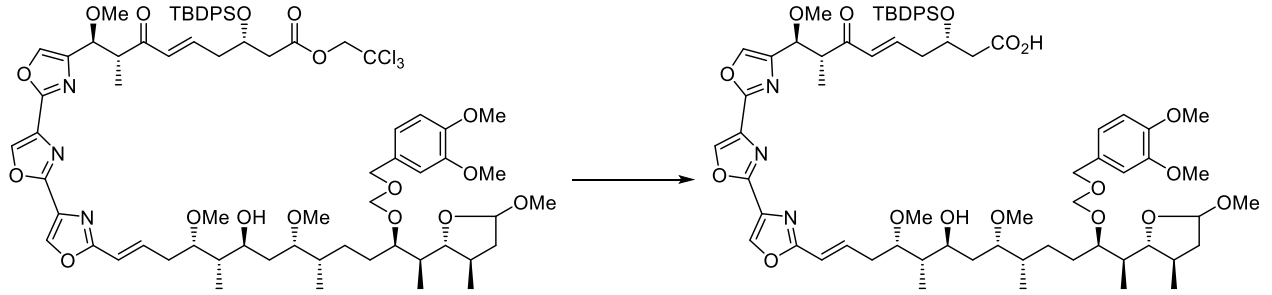
4.40 (d, *J* = 9.6 Hz, 1H), 4.35 (m, 1H), 4.06 (br t, *J* = 6.4 Hz, 1H), 3.88 (s, 3H), 3.87 (m, 1H), 3.87 (s, 3H), 3.57 (dd, *J* = 6.6, 9.8 Hz, 1H), 3.44 (dq, *J* = 9.6, 7.2 Hz, 1H), 3.36 (s, 3H), 3.28 (s, 3H), 3.23 (s, 3H), 3.17 (s, 3H), 3.05–3.02 (m, 2H), 2.65 (dd, *J* = 6.0, 15.6 Hz, 1H), 2.60 (dd, *J* = 6.4, 15.6 Hz, 1H), 2.55–2.40 (m, 2H), 2.23 (m, 1H), 2.09 (dd, *J* = 7.6, 12.8 Hz, 1H), 1.83–1.73 (m, 2H), 1.67–1.45 (m, 6H), 1.38–1.16 (m, 4H), 1.10 (d, *J* = 7.2 Hz, 3H), 1.05 (s, 9H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.91 (t, *J* = 7.8 Hz, 9H), 0.88 (d, *J* = 6.0 Hz, 3H), 0.854 (d, *J* = 6.8 Hz, 3H), 0.847 (d, *J* = 7.2 Hz, 3H), 0.54 (q, *J* = 7.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.0, 169.6, 162.5, 156.5, 155.9, 149.3, 148.9, 142.7, 140.3, 139.1, 138.9, 138.8, 137.5, 136.25 (2C), 136.17 (2C), 133.7, 133.4, 131.9, 131.1, 130.9, 130.33, 130.29, 128.14 (2C), 128.09 (2C), 120.9, 119.1, 116.0, 111.7, 111.2, 105.0, 95.1, 94.7, 87.5, 82.3 (2C), 78.8, 78.0, 74.3, 71.0, 69.7, 69.3, 57.7, 57.35, 57.33, 56.3, 56.2, 54.8, 47.5, 44.0, 42.8, 41.5, 40.3, 36.3, 35.3, 33.6, 31.3, 31.0, 30.0, 27.5, 27.3 (3C), 20.5, 19.6, 16.0, 14.5, 9.25, 9.19, 7.4 (3C), 5.6 (3C); IR ($CHCl_3$) 3007, 2958, 2934, 1750, 1696, 1663, 1628, 1517, 1464, 1427, 1381, 1263, 1240, 1220, 1156, 1139, 1096, 1029, 952, 785, 767, 758, 747, 731 cm^{-1}; HRMS (ESI) *m/z* 1544.6328 (calcd for $C_{78}H_{110}{}^{35}Cl_3N_3NaO_{17}Si_2$ $[M+Na]^+$, Δ –0.9 mmu).

TES ether homodimer **71**: R_f 0.34 (hexane / acetone = 4/1); $[\alpha]^D_{25}$ –7.4 (*c* 1.60, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93–6.90 (m, 4H), 6.82 (d, *J* = 8.4 Hz, 2H), 5.51–5.47 (m, 2H), 4.88 (d, *J* = 4.4 Hz, 2H), 4.83 (AB quart, *J* = 7.2 Hz, 4H), 4.58 (s, 4H), 4.08–4.04 (m, 2H), 3.91–3.83 (m, 2H), 3.88 (s, 6H), 3.87 (s, 6H), 3.57 (dd, *J* = 6.6, 9.8 Hz, 2H), 3.31 [3.30] (s, 6H), 3.290 [3.3295] (s, 6H), 3.27 (s, 6H), 3.22–3.17 (m, 2H), 2.93 [3.00] (dt, *J* = 5.6, 5.4 Hz, 2H), 2.35–2.18 (m, 6H), 2.08 (dd, *J* = 7.6, 12.8 Hz, 2H), 1.83–1.56 (m, 14H), 1.55–1.46 (m, 2H), 1.37–1.20 (m, 4H), 1.10 (d, *J* = 6.8 Hz, 6H), 0.94 (t, *J* = 7.8 Hz, 18H), 0.89 (d, *J* = 7.2 Hz, 6H), 0.88 (d, *J* = 6.8 Hz, 6H), 0.86 (d, *J* = 6.8 Hz, 6H), 0.57 (q, *J* = 8.0 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.9 [148.5], 130.8, 128.8 [127.3], 120.5, 111.3, 110.9, 104.6, 94.3, 87.1, 82.8 [82.6], 82.0 [81.9], 78.4, 77.2, 71.0 [71.4], 69.3, 57.2, 57.0 [56.9], 55.9, 55.8, 54.4, 43.3, 42.6 [42.9], 42.4, 35.9, 35.01 [34.96], 33.8, 33.1 [33.3], 30.5, 27.2 [28.7], 20.1, 15.6, 8.8, 8.14 [8.10], 7.0, 5.2 [5.3]. Chemical shifts of the *Z*-isomer are within parentheses (square blankets); IR ($CHCl_3$) 3092, 3072, 3037, 3011, 2959, 2937, 2912, 2878, 2832, 1961, 1819, 1594, 1517, 1479, 1465, 1420, 1382, 1264, 1240, 1157, 1139, 1095, 1030, 855, 810, 758, 740, 723, 666 cm^{-1}; HRMS (ESI) *m/z* 1443.9495 (calcd for $C_{78}H_{140}NaO_{18}Si_2$ $[M+Na]^+$, Δ +1.7 mmu).

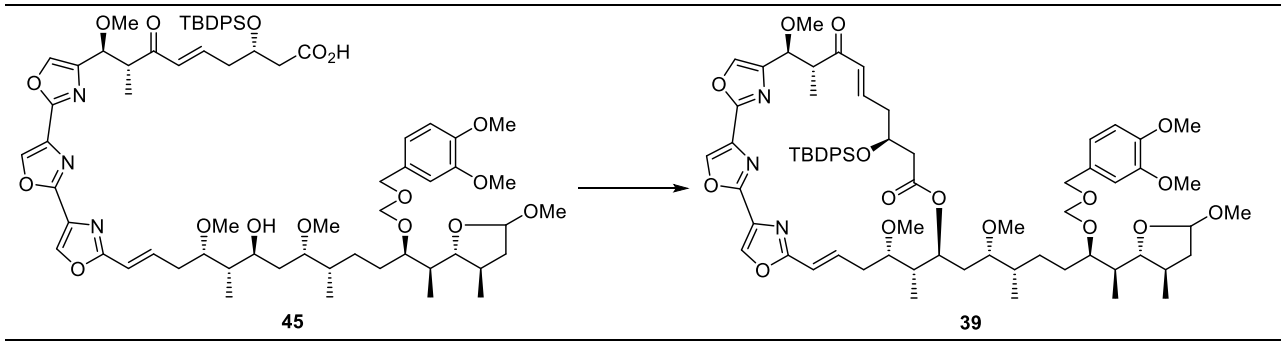


Secondary alcohol 72. A solution of coupling product (19*E*)-**70** (35.9 mg, 23.6 μmol) in a mixture of AcOH–THF–H_2O (4.5 mL, 4:4:1 [v/v/v]) was stirred for 3 h at room temperature. The resulting mixture was quenched with sat. $NaHCO_3$ aq. (20 mL) at 0 °C and extracted with EtOAc (10 mL × 4). The combined extracts were washed with sat. $NaHCO_3$ aq., water, and brine, successively, dried with Na_2SO_4, and concentrated. The crude oil was purified with a SiO_2 column chromatography (FL60D 1.7 g, hexane / EtOAc = 1/1 to 1/2) to give secondary alcohol **72** (33.3 mg, quant.) as a colorless oil. **72**: R_f 0.15 (hexane / EtOAc = 2/3); $[\alpha]^D_{26}$ –11 (*c* 0.20, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 8.29 (s, 1H), 7.70–7.65 (m, 4H), 7.69 (s, 1H), 7.46–7.36 (m, 6H), 6.92–6.90 (m, 2H), 6.88 (m, 1H), 6.83 (dt, *J* = 15.8, 7.6 Hz, 1H), 6.82 (d, *J* = 7.6 Hz, 1H), 6.44 (d, *J* = 16.4 Hz, 1H), 6.15 (d, *J* = 15.8 Hz, 1H), 4.88 (d, *J* = 5.0 Hz, 1H), 4.85–4.81 (AB quart, *J* = 6.8 Hz, 2H), 4.68–4.55 (AB quart, *J* = 12.0 Hz, 2H), 4.58

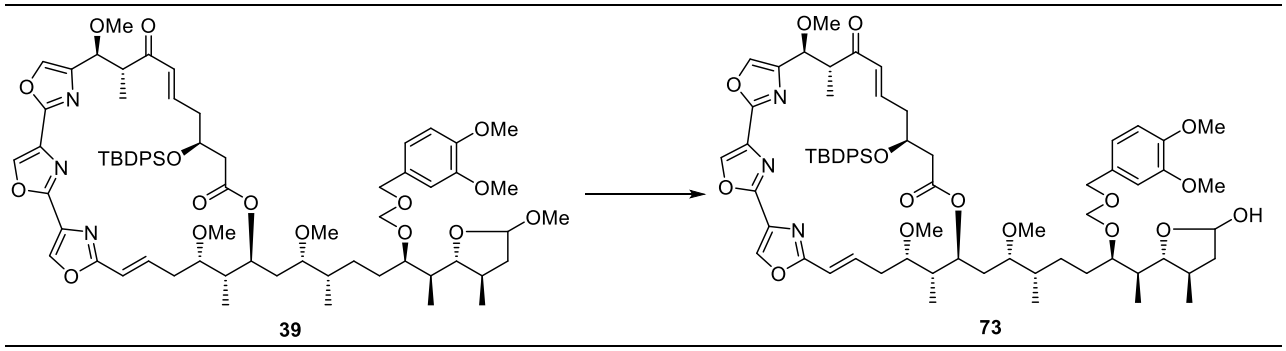
(s, 2H), 4.39 (d, *J* = 9.6 Hz, 1H), 4.35 (ddt, *J* = 5.2, 6.4, 6.4 Hz, 1H), 4.06 (br t, *J* = 6.4 Hz, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.76 (br t, *J* = 8.4 Hz, 1H), 3.70–3.64 (m, 2H), 3.57 (dd, *J* = 6.6, 9.8 Hz, 1H), 3.44 (dq, *J* = 9.6, 7.2 Hz, 1H), 3.42 (s, 3H), 3.37 (m, 1H), 3.35 (s, 3H), 3.28 (s, 3H), 3.17 (s, 3H), 2.68 (m, 1H), 2.64 (dd, *J* = 5.8, 15.6 Hz, 1H), 2.60 (dd, *J* = 6.0, 15.6 Hz, 1H), 2.55–2.40 (m, 3H), 2.23 (m, 1H), 2.09 (dd, *J* = 7.8, 12.8 Hz, 1H), 1.81–1.40 (m, 9H), 1.62 (ddd, *J* = 5.0, 10.0, 12.8 Hz, 1H), 1.10 (d, *J* = 6.4 Hz, 3H), 1.04 (s, 9H), 0.90 (d, *J* = 7.2 Hz, 3H), 0.89 (d, *J* = 7.2 Hz, 3H), 0.88 (d, *J* = 6.8 Hz, 3H), 0.84 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.0, 169.5, 162.3, 156.4, 155.9, 149.3, 148.9, 142.7, 140.2, 139.3, 139.0, 138.8, 137.5, 136.23 (2C), 136.15 (2C), 133.6, 133.5, 133.4, 131.9, 131.1, 130.9, 130.30, 130.26, 128.11 (2C), 128.07 (2C), 120.9, 117.9, 111.7, 111.2, 105.0, 95.1, 94.9, 87.5, 82.7, 82.6, 78.8, 77.9, 74.3, 71.3, 69.8, 69.3, 58.3, 58.2, 57.3, 56.3, 56.1, 54.9, 47.4, 43.8, 42.8, 41.4, 40.8, 40.3, 36.3, 36.05, 35.95, 34.5, 30.7, 28.0, 27.2 (3C), 20.5, 19.6, 15.8, 14.4, 11.9, 9.2; IR ($CHCl_3$) 3459, 3029, 3006, 2934, 2361, 1752, 1665, 1592, 1517, 1465, 1427, 1381, 1263, 1240, 1212, 1156, 1139, 1096, 1029, 992, 952, 919 cm^{-1}; HRMS (ESI) *m/z* 1430.5455 (calcd for $C_{72}H_{96}{}^{35}Cl_3N_3NaO_{17}Si$ [M+Na]$^+$, Δ –1.7 mmu).



Seco acid 45. To a stirred solution of secondary alcohol **72** (72.7 mg, 51.6 μmol) in THF (5.2 mL) were added an activated Zn powder (1.52 g, 23.2 mmol) and a 1.0 M solution of NH_4OAc aq. (0.39 mL). After being stirred for 6 h at room temperature, the mixture was filtered through a pad of Celite, and the residue was washed with EtOAc (50 mL). The filtrate and the washings were combined and concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 3 g, $CHCl_3$ / MeOH = 200/1 to 100/1) to give seco acid **45** (61.2 mg, 93%) as a colorless oil. **45**: R_f 0.03 (hexane / EtOAc = 1/5); $[\alpha]^D_{25}$ –19 (*c* 0.15, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 8.28 (s, 1H), 7.70–7.65 (m, 5H), 7.46-7.34 (m, 6H), 6.92–6.90 (m, 2H), 6.86 (m, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 6.79 (dt, *J* = 15.4, 7.5 Hz, 1H), 6.44 (d, *J* = 16.4 Hz, 1H), 6.12 (d, *J* = 15.4 Hz, 1H), 4.88 (d, *J* = 4.8 Hz, 1H), 4.85–4.81 (AB quart, *J* = 6.8 Hz, 2H), 4.62–4.56 (AB quart, *J* = 12.2 Hz, 2H), 4.44 (d, *J* = 8.8 Hz, 1H), 4.29 (m, 1H), 4.06 (br t, *J* = 6.4 Hz, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 3.77 (m, 1H), 3.66 (m, 1H), 3.57 (dd, *J* = 6.8, 10.0 Hz, 1H), 3.49–3.33 (m, 3H), 3.42 (s, 3H), 3.35 (s, 3H), 3.28 (s, 3H), 3.19 (s, 3H), 2.67 (m, 1H), 2.60–2.40 (m, 5H), 2.23 (m, 1H), 2.09 (dd, *J* = 7.2, 12.6 Hz, 1H), 1.81–1.69 (m, 2H), 1.67–1.39 (m, 7H), 1.62 (ddd, *J* = 4.8, 9.6, 12.6 Hz, 1H), 1.10 (d, *J* = 6.4 Hz, 3H), 1.05 (s, 9H), 0.90 (d, *J* = 6.8 Hz, 3H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 6.8 Hz, 3H), 0.86 (d, *J* = 7.2 Hz, 3H) [COO*H* signal was not observed.]; ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.7, 170.3, 162.3, 156.4, 155.8, 149.2, 148.8, 142.9, 140.0, 139.3, 139.0, 138.9, 137.5, 136.2 (2C), 136.1 (2C), 133.7, 133.6, 133.1, 131.7, 131.1, 130.8, 130.24, 130.23, 128.1 (2C), 128.0 (2C), 120.8, 117.9, 111.6, 111.1, 105.0, 94.8, 87.5, 82.7, 82.6, 78.8, 77.8, 71.3, 69.8, 69.5, 58.24, 58.16, 57.3, 56.2, 56.1, 54.8, 47.3, 43.8, 42.8, 40.8, 40.7, 36.2, 36.0, 35.9, 34.5, 30.7, 30.0, 28.0, 27.2 (3C), 20.5, 19.6, 15.7, 13.9, 11.9, 9.1; IR ($CHCl_3$) 3566, 3502, 3026, 3007, 2935, 1715, 1664, 1517, 1464, 1427, 1264, 1220, 1210, 1157, 1139, 1097, 1029, 991, 822, 549 cm^{-1}; HRMS (ESI) *m/z* 1300.6322 (calcd for $C_{70}H_{95}N_3NaO_{17}Si$ [M+Na]$^+$, Δ –0.6 mmu).

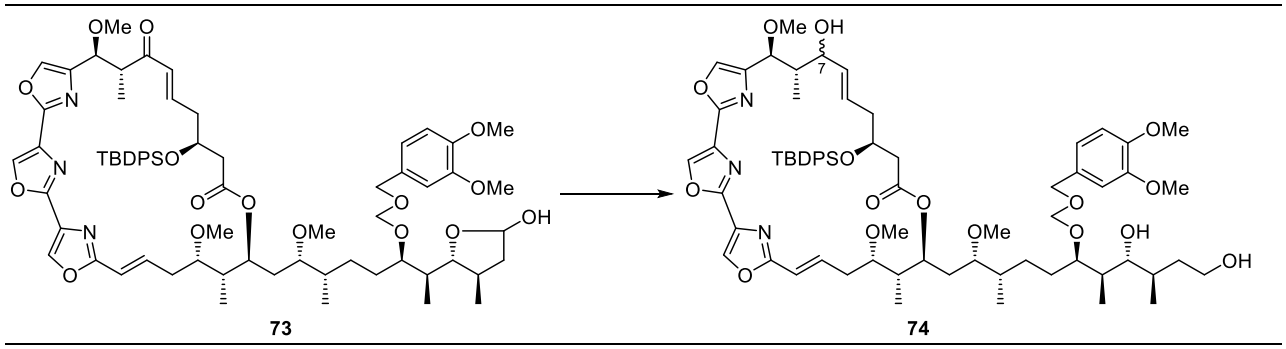


Macrolactonization of seco acid 45. To a stirred solution of seco acid **45** (21.8 mg, 17.0 μmol) in dry benzene (3.5 mL) under a nitrogen stream were added diisopropylethylamine (0.14 mL, 0.77 mmol) and 2,4,6-trichlorobenzoyl chloride (66 μL, 0.41 mmol). After being stirred for 12 h at room temperature, the reaction mixture was diluted with dry benzene (14 mL) and loaded in a syringe. The activated ester solution was slowly added using a syringe pump over 13.5 h to a stirred solution of *N*,*N*-dimethyl-4-aminopyridine (48 mg, 0.39 mmol) in dry benzene (23 mL) under a nitrogen stream. The remaining activated ester in syringe was rinsed with dry benzene (7 mL) to the above reaction mixture over 1 h. After being stirred for 11 h at room temperature, the reaction mixture was quenched with sat. NH_4Cl aq. (40 mL) and extracted with EtOAc (20 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 2.2 g, hexane / EtOAc = 5/1, 1/1 to 0/1) to give macrolactone **39** (16.5 mg, 77%) as a colorless oil.



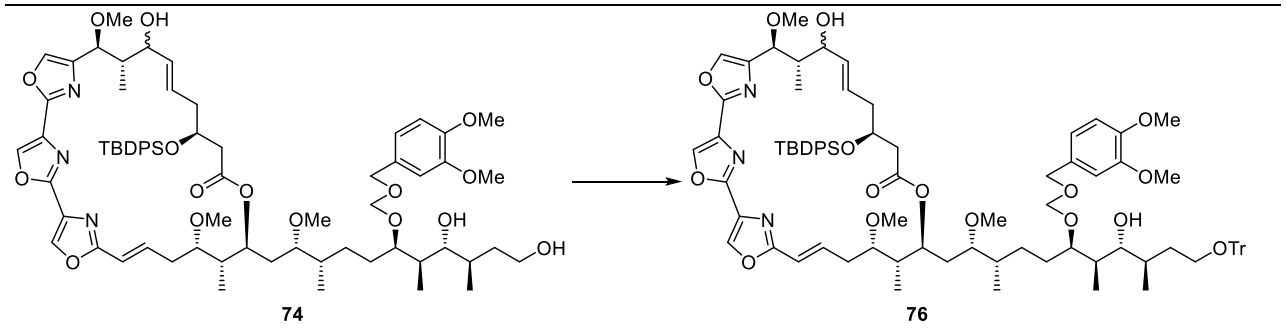
Hemiacetal 73. A mixture of macrolactone **39** (147 mg, 0.116 mmol) in 1,2-dimethoxyethane (23 mL) and 1 M HCl aq. (7 mL) was stirred for 7 h at 25 °C. The resulting mixture was neutralized with sat. $NaHCO_3$ aq. (25 mL) at 0 °C, and extracted with EtOAc (20 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 4 g, hexane / acetone = 9/2 to 7/3) to give hemiacetal **73** (145 mg, quant., a 1.8:1 diastereomeric mixture at C35) as a colorless oil. **73**: R_f 0.40 ($CHCl_3$ / MeOH = 20/1); $[\alpha]^{D}_{25}$ –44 (*c* 0.92, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 8.06 (s, 1H), 7.72–7.65 (m, 4H), 7.67 (s, 1H), 7.44–7.30 (m, 6H), 7.16–7.03 (m, 2H), 6.92–6.87 (m, 2H), 6.80 [6.81] (d, *J* = 8.0 Hz, 1H), 6.33 (d, *J* = 15.6 Hz, 1H), 5.91 (d, *J* = 16.8 Hz, 1H), 5.42 [5.40] (dd, *J* = 1.6, 4.4 Hz, 1H), 5.12 (m, 1H), 4.76 (s, 2H), 4.58–4.50 [4.62–4.53] (AB quart, *J* = 11.6 Hz, 2H), 4.42 (m, 1H), 4.37 (d, *J* = 9.6 Hz, 1H), 4.24 (m, 1H), 3.94 (m, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.48 [3.37] (dd, *J* = 6.8, 9.6 Hz, 1H), 3.32 (s, 3H), 3.29 (m, 1H), 3.23 (s, 3H), 3.10 (s, 3H), 2.99 (m, 1H), 2.80–2.63 (m, 3H), 2.43 (m, 1H), 2.35–2.18 (m, 2H), 2.08 (dd, *J* = 7.6, 12.4 Hz, 1H), 1.81–1.23 (m, 12H), 1.09 [1.17] (d, *J* = 6.4 Hz, 3H), 1.03 (s, 9H), 0.94–0.84 (m, 9H), 0.76 [0.78] (d, *J* = 6.8 Hz, 3H). Chemical shifts of the minor isomers are within parentheses (square brackets); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.0, 170.6, 162.5, 156.5, 155.6, 148.9 [149.0], 148.5, 143.9, 139.7, 139.1, 137.3, 137.0 (2C), 135.9 (4C), 135.0, 133.8, 133.5, 131.4, 130.7 [130.8], 130.2, 129.8, 129.7, 127.65 (2C), 127.59 (2C), 120.5 [120.2],

116.4, 111.3 [111.2], 110.87 [110.93], 98.2 [98.1], 93.9 [94.2], 87.9 [85.9], 82.5 [81.8], 79.8, 77.2, 73.5 [73.6], 69.3 [69.4], 68.8, 57.7 [57.6], 57.5, 56.3, 55.9, 55.78 [55.83], 43.5, 43.1, 42.8, 42.0, 41.2, 40.9 [41.0], 39.7, 39.46 [39.54], 36.0 [36.2], 34.1 [34.5], 32.7 [32.5], 32.1, 30.0 [30.2], 27.1 [26.1] (3C), 20.0 [21.1], 19.3, 16.2 [15.6], 14.7 [14.2], 9.8 [9.3], 8.09 [8.14]; IR ($CHCl_3$) 3167, 3007, 2961, 2936, 2860, 1729, 1661, 1517, 1464, 1378, 1263, 1219, 1157, 1103, 1028, 981, 918, 822, 761, 703, 666, 611 cm^{-1}; HRMS (ESI) *m/z* 1268.6042 (calcd for $C_{69}H_{91}N_3NaO_{16}Si$ $[M+Na]^+$, Δ –2.4 mmu).



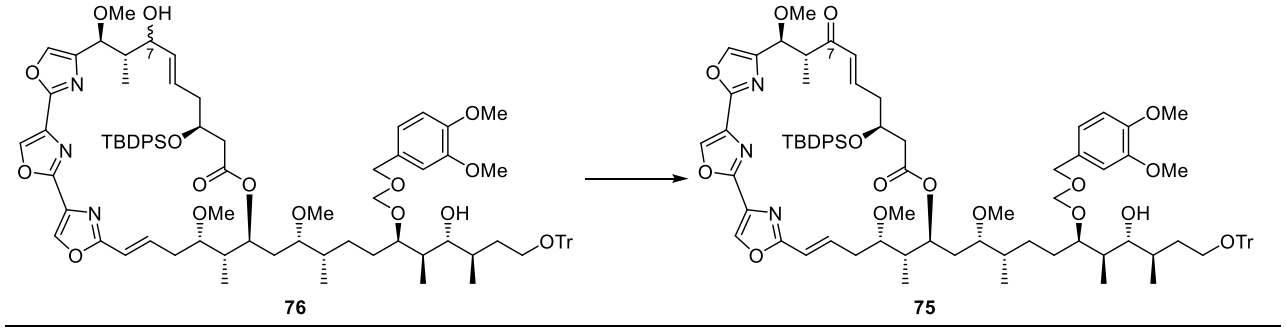
73 74

Triol 74. To a stirred solution of hemiacetal **73** (11.9 mg, 9.6 μmol) in dry MeOH (2.3 mL) cooled at –20 °C was added cerium (III) chloride heptahydrate (64 mg, 0.17 mmol). The mixture was stirred for 10 min, and sodium borohydride (5.4 mg, 0.14 mmol) was added. After being stirred at –20 °C for 1 h and at 0 °C for 5.5 h, the resulting mixture was quenched with acetone (0.1 mL), diluted with sat. NH_4Cl aq. (10 mL), and extracted with EtOAc (5 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (BW-820MH 0.4 g, hexane / acetone = 4/1, 2/1, 1/1) to give triol **74** (12.0 mg, 100%, a 10:1 diastereomeric mixture at C7) as a colorless oil. **74**: R_f 0.55 (hexane / acetone = 1/1); $[\alpha]^{D}_{25}$ –18 (*c* 0.66, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 8.05 (s, 1H), 7.74–7.68 [7.80–7.74] (m, 4H), 7.59 [7.55] (s, 1H), 7.39–7.29 [7.43–7.35] (m, 6H), 7.13 (ddd, *J* = 5.7, 9.5, 15.6 Hz, 1H), 6.91–6.79 (m, 3H), 6.32 (d, *J* = 15.6 Hz, 1H), 5.74 (dt, *J* = 15.5, 7.4 Hz, 1H) [5.81 (dt, *J* = 15.3, 7.5Hz)], 5.47 (dd, *J* = 15.5, 6.1 Hz, 1H) [5.37 (dd, *J* = 15.3, 6.5 Hz)], 5.11 (t, *J* = 7.8 Hz, 1H) [5.15 (t, *J* = 8.9 Hz)], 4.76 (AB quart, *J* = 6.9 Hz, 2H), 4.56 (AB quart, *J* = 11.6 Hz, 2H), 4.45–4.43 (m, 1H), 4.34 (d, *J* = 3.0 Hz, 1H), 4.30–4.25 (m, 1H), 4.01 (br s, 1H), 3.89–3.81 (m, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.75–3.66 (m, 1H), 3.59–3.49 (m, 1H), 3.48–3.37 (m, 1H), 3.39 (s, 3H), 3.36–3.25 (m, 1H), 3.290 (s, 3H), 3.286 (s, 3H), 3.18 (br s, 2H), 3.03–2.95 (m, 1H), 2.74–2.30 (m, 5H), 2.28–2.18 (m, 1H), 2.15–2.06 (m, 1H), 2.00–1.78 (m, 4H), 1.74–1.38 (m, 8H), 1.04 (d, *J* = 8.0 Hz, 3H), 1.01 (s, 9H), 0.99 (d, *J* = 7.0 Hz, 3H), 0.89 (d, *J* = 6.9 Hz, 3H), 0.84 (d, *J* = 7.0 Hz, 3H), 0.77 (d, *J* = 6.8 Hz, 3H). Chemical shifts of the minor isomers are within parentheses (square brackets); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 162.5, 156.5, 154.9, 149.1, 148.8, 141.0, 139.6, 137.2, 137.1, 136.3, 136.0 (4C), 135.0, 134.4, 134.0, 131.5, 130.4, 129.8, 129.53, 129.47, 127.5 (2C), 127.4 (2C), 127.2, 120.4, 116.7, 111.1, 111.0, 94.3, 81.5, 80.7, 80.6, 80.2, 77.2, 73.0, 72.0, 70.3, 70.2, 59.6, 58.1, 57.9, 57.5, 55.9, 55.8, 42.2, 40.7, 39.5, 39.3, 37.6, 35.3, 33.1, 32.5, 32.2, 29.6, 27.6, 27.11, 27.07 (3C), 19.3, 17.2, 15.5, 11.2, 9.4, 8.5; IR ($CHCl_3$) 3168, 3073, 3008, 2963, 2936, 1731, 1655, 1594, 1560, 1517, 1464, 1427, 1383, 1263, 1210, 1158, 1105, 1028, 977, 916, 856, 822, 771, 749, 739, 705, 668, 612 cm^{-1}; HRMS (ESI) *m/z* 1272.6355 (calcd for $C_{69}H_{95}N_3NaO_{16}Si$ $[M+Na]^+$, Δ –2.4 mmu).



74 76

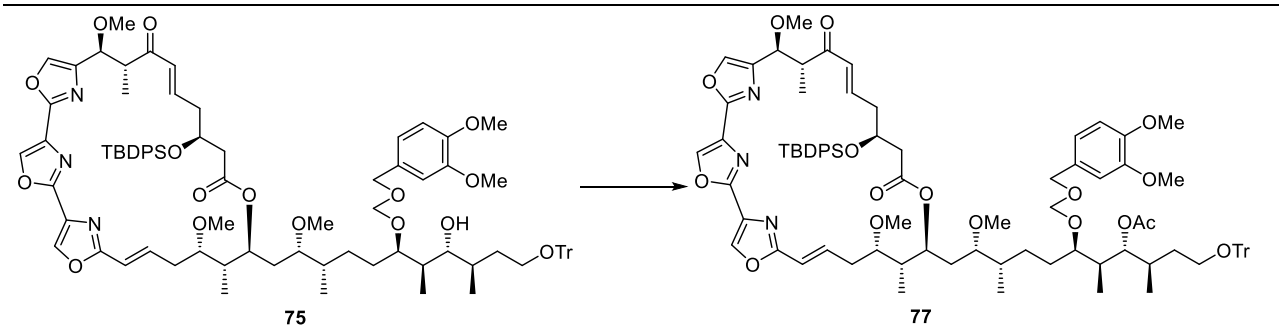
Trityl ether 76. To a stirred solution of triol **74** (12.6 mg, 10.1 μmol) in dry pyridine (1.3 mL) was added trityl chloride (108 mg, 0.39 mmol) at room temperature. After being stirred for 48 h at room temperature, the resulting mixture was diluted with EtOAc (3 mL), quenched with sat. $NaHCO_3$ aq. (10 mL), and extracted with EtOAc (5 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified with two SiO_2 column chromatographies (BW-820MH 1 g, $CHCl_3$ / MeOH = 1/0 to 10/1; FL60D 0.5 g, hexane / acetone = 4/1, 3/1 to 1/1) to give trityl ether **76** (12.4 mg, 82%, a 10:1 diastereomeric mixture at C7) as a colorless oil. **76**: R_f 0.65 (hexane / acetone = 2/1); $[\alpha]^{D}_{25}$ –7.6 (*c* 0.63, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 8.05 (s, 1H), 7.73–7.69 [7.79–7.75] (m, 4H), 7.60 (s, 1H), 7.50–7.19 (m, 21H), 7.14 (ddd, *J* = 15.9, 9.3, 5.6 Hz, 1H), 6.90–6.76 (m, 3H), 6.31 (*d*, *J* = 15.9 Hz, 1H), 5.75 [5.83] (dt, *J* = 15.4, 7.4 Hz, 1H), 5.47 [5.35] (dd, *J* = 15.4, 6.1 Hz, 1H), 5.12 [5.17] (t, *J* = 7.7 Hz, 1H), 4.77 (AB quart, *J* = 7.0 Hz, 2H), 4.56 (AB quart, *J* = 11.5 Hz, 2H), 4.44 (t, *J* = 4.6 Hz, 1H), 4.34 (d, *J* = 3.5 Hz, 1H), 4.27 (m, 1H), 3.86–3.80 (m, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.48–3.38 (m, 2H), 3.40 [3.43] (s, 3H), 3.33–3.25 (m, 1H), 3.29 (s, 3H), 3.27 (s, 3H), 3.23–3.16 (m, 1H), 3.07 (br s, 1H), 3.05–2.95 (m, 2H), 2.72–2.46 (m, 4H), 2.45–2.36 (m, 1H), 2.27–2.19 (m, 1H), 2.15–2.05 (m, 1H), 2.02–1.77 (m, 4H), 1.74–1.35 (m, 8H), 1.02 [1.05] (s, 9H), 0.90 (d, *J* = 7.0 Hz, 3H), 0.86 (d, *J* = 7.0 Hz, 3H), 0.84–0.82 (m, 6H), 0.79 [0.62] (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 162.5, 156.5, 154.9, 149.0, 148.7, 144.4 (3C), 141.0, 139.5 (2C), 137.13, 137.09, 136.2, 136.0 (4C), 135.0, 134.4, 134.0, 131.5, 130.4, 129.9, 129.53, 129.47, 128.6 (6C), 127.7 (6C), 127.5 (2C), 127.4 (2C), 126.8 (3C), 120.5, 116.8, 111.1, 111.0, 94.5, 86.4, 81.6, 81.4, 80.7, 80.3, 77.2, 72.9, 72.0, 70.21, 70.16, 61.9, 57.99, 57.96, 57.4, 55.9, 55.8, 42.2, 40.7, 39.5, 39.3, 37.9, 35.2, 33.0, 32.0, 31.8, 29.5, 29.4, 27.6, 27.1 (3C), 19.3, 17.4, 15.5, 11.5, 9.5, 8.5; IR ($CHCl_3$) 3167, 3027, 3020, 3007, 2937, 1732, 1655, 1517, 1463, 1427, 1379, 1261, 1157, 1109, 1027, 977, 916, 824, 797, 707, 666 cm^{-1}; HRMS (ESI) *m/z* 1514.7504 (calcd for $C_{88}H_{109}N_3NaO_{16}Si$ $[M+Na]^+$, Δ +2.9 mmu).



76 75

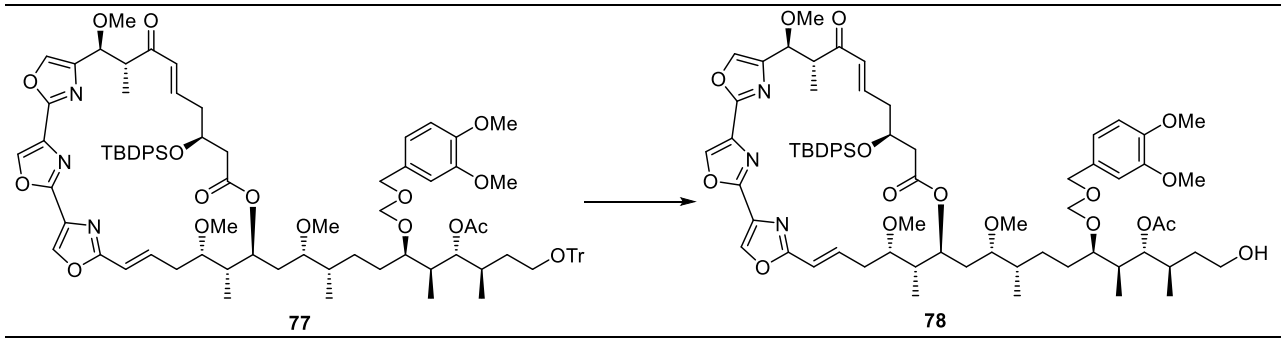
Ketone 75. To a stirred solution of trityl ether **76** (12.4 mg, 8.3 μmol) in dry CH_2Cl_2 (0.4 mL) was added activated manganese dioxide (58 mg, 0.66 mmol) [Cat. No. CMD-100, Chuo Denki Kogyo Co. Ltd] at room temperature. After being stirred for 48 h at room temperature, the resulting mixture was diluted with EtOH (1 mL) and further stirred for 1.5 h at room temperature. The resulting mixture was filtered on Celite, and the residue was sufficiently washed with EtOH (30 mL) and EtOAc (30 mL). The combined filtrate and washings were concentrated. The crude

material was purified with a SiO_2 column chromatography (FL60D 0.5 g, benzene / acetone = 10/1 to 5/1) to give ketone **75** (9.8 mg, 80%) as a colorless oil. **75**: R_f 0.38 (benzene / acetone = 5/1); $[\alpha]^D_{25}$ –30 (*c* 0.49, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 8.12 (s, 1H), 8.06 (s, 1H), 7.72–7.67 (m, 4H), 7.67 (s, 1H), 7.45–7.33 (m, 12H), 7.30–7.18 (m, 9H), 7.15–7.06 (m, 2H), 6.88–6.85 (m, 2H), 6.79 (d, *J* = 8.6 Hz, 1H), 6.32 (d, *J* = 15.9 Hz, 1H), 5.91 (d, *J* = 16.1 Hz, 1H), 5.14 (dt, *J* = 1.6, 8.2 Hz, 1H), 4.76 (AB quart, *J* = 7.0 Hz, 2H), 4.55 (AB quart, *J* = 11.5 Hz, 2H), 4.42–4.40 (m, 1H), 4.38 (d, *J* = 9.5 Hz, 1H), 4.18 (dq, *J* = 9.4, 7.0 Hz, 1H), 3.87–3.80 (m, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.42–3.39 (m, 2H), 3.32–3.26 (m, 1H), 3.29 (s, 3H), 3.23–3.17 (m, 1H), 3.22 (s, 3H), 3.10 (s, 3H), 3.04–2.97 (m, 2H), 2.80–2.64 (m, 4H), 2.46–2.38 (m, 1H), 2.32–2.24 (m, 1H), 1.93–1.37 (m, 12H), 1.03 (s, 9H), 0.855 (d, *J* = 6.8 Hz, 3H), 0.849 (d, *J* = 7.0 Hz, 3H), 0.845 (d, *J* = 6.7 Hz, 3H), 0.82 (d, *J* = 6.8 Hz, 3H), 0.79 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 203.0, 170.5, 162.5, 156.6, 155.6, 149.0, 148.7, 144.4 (3C), 143.9, 139.7, 139.1, 137.3, 137.0 (2C), 135.9 (4C), 135.1, 133.8, 133.6, 131.5, 130.3, 129.94, 129.86, 129.7, 128.6 (4C), 127.7 (12C), 126.8 (3C), 120.5, 116.4, 111.2, 111.0, 94.5, 86.4, 81.7, 81.4, 79.8, 77.2, 73.5, 70.6, 70.2, 68.9, 61.9, 57.7, 57.4, 56.3, 55.9, 55.8, 42.9, 41.0, 39.8, 39.6, 37.9, 34.8, 32.8, 32.5, 31.8, 29.7, 29.6, 29.4, 27.1 (3C), 19.3, 17.4, 15.7, 14.7, 11.4, 8.1; IR ($CHCl_3$) 3446, 3008, 2961, 2933, 2879, 2858, 1729, 1658, 1517, 1464, 1378, 1263, 1241, 1158, 1102, 1028, 980, 918, 822, 767, 742, 707, 633, 612 cm^{-1}; HRMS (ESI) *m/z* 1512.7328 (calcd for $C_{88}H_{107}N_3NaO_{16}Si$ $[M+Na]^+$, Δ +1.0 mmu).



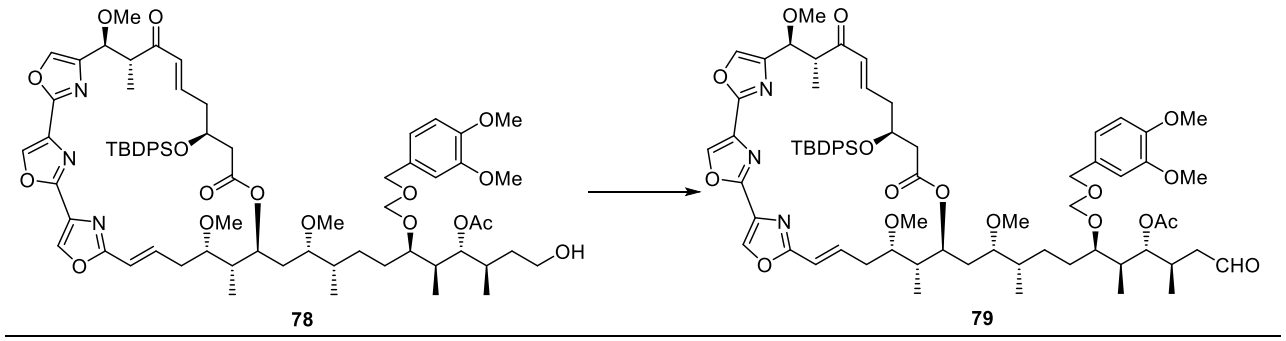
Acetate 77. A mixture of ketone **75** (1.4 mg, 0.94 μmol) and *N*,*N*-dimethyl-4-aminopyridine (ca. 0.2 mg) in dry pyridine (0.1 mL) and acetic anhydride (0.1 mL) was stirred at room temperature for 20.5 h. The resulting mixture cooled at 0 °C was quenched with EtOAc (1 mL) and sat. $NaHCO_3$ aq. (3 mL) and extracted with EtOAc (3 mL × 4). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 0.5 g, hexane / acetone = 9/1 to 9/2) to give acetate **77** (1.4 mg, 97%) as a light yellow oil. **77**: R_f 0.44 (benzene / acetone = 5/1); $[\alpha]^D_{25}$ –23 (*c* 0.51, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 8.06 (s, 1H), 7.72–7.65 (m, 4H), 7.67 (s, 1H), 7.46–7.16 (m, 21H), 7.15–7.05 (m, 2H), 6.90–6.75 (m, 3H), 6.31 (d, *J* = 15.9 Hz, 1H), 5.97 (d, *J* = 16.1 Hz, 1H), 5.15 (t, *J* = 8.0 Hz, 1H), 4.96 (dd, *J* = 9.7, 2.3 Hz, 1H), 4.69 (AB quart, *J* = 7.0 Hz, 2H), 4.52 (AB quart, *J* = 11.6 Hz, 2H), 4.50–4.37 (m, 1H), 4.38 (d, *J* = 9.5 Hz, 1H), 4.18 (dq, *J* = 9.4, 7.0 Hz, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.40 (t, *J* = 6.6 Hz, 1H), 3.35–3.15 (m, 2H), 3.28 (s, 3H), 3.23 (s, 3H), 3.10 (s, 3H), 3.05–2.94 (m, 2H), 2.83–2.62 (m, 4H), 2.48–2.35 (m, 1H), 2.33–2.24 (m, 1H), 1.97 (s, 3H), 1.93–1.40 (m, 12H), 1.03 (s, 9H), 0.91 (d, *J* = 7.0 Hz, 3H), 0.87 (d, *J* = 6.9 Hz, 3H), 0.85 (d, *J* = 6.9 Hz, 3H), 0.80 (d, *J* = 6.9 Hz, 3H), 0.69 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.0, 170.8, 170.4, 162.5, 156.6, 155.6, 149.0, 148.5, 144.3 (3C), 143.7, 139.7, 139.1, 137.3, 137.01, 136.96, 135.9 (4C), 135.1, 133.8, 133.5, 131.5, 130.8, 130.3, 129.9, 129.7, 128.6 (6C), 127.70 (6C), 127.60 (4C), 126.9 (3C), 120.4, 116.4, 111.2, 110.9, 95.3, 86.5, 81.8, 79.8, 78.7, 78.6, 77.2, 73.6, 69.5, 68.8, 61.4, 57.6, 57.4, 56.3, 55.9, 55.8, 42.9, 41.0, 39.8, 39.6, 36.7, 34.4, 32.8, 32.3, 31.0, 30.7, 29.6, 27.1 (3C), 26.4, 21.1, 19.3, 16.9, 15.7, 14.8, 9.6, 8.1; IR ($CHCl_3$) 3020, 2963, 2935, 2880, 1725, 1659, 1516, 1464, 1383, 1257, 1178, 1158, 1139, 1102, 1030, 980, 918, 707 cm^{-1}; HRMS

(ESI) *m/z* 1554.7417 (calcd for $C_{90}H_{109}N_3NaO_{17}Si$ $[M+Na]^+$, Δ –0.7 mmu).



77 78

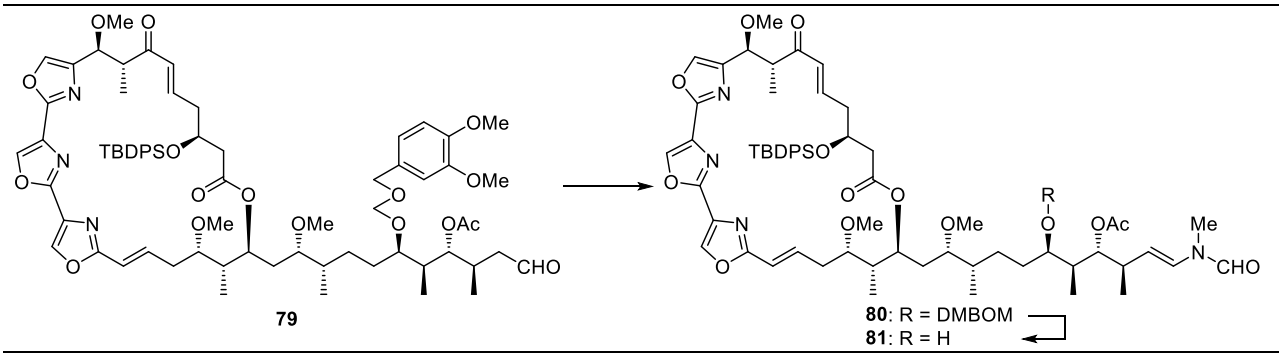
Primary alcohol 78. To a stirred solution of acetate **77** (3.6 mg, 2.3 μmol) in dry Et_2O (0.6 ml) cooled at 0 °C was added formic acid (0.4 mL, 11 μmol). After being stirred for 1 h at room temperature, the resulting mixture cooled at 0 °C was quenched with EtOAc (1 mL) and 10% $NaHCO_3$ aq. (15 mL), and extracted with EtOAc (6 mL × 3). The combined extracts were washed with 10% $NaHCO_3$ aq. and brine, dried with Na_2SO_4 and concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 0.5 g, hexane / acetone = 7/1 to 2/1) to give primary alcohol **78** (2.3 mg, 77%) as a colorless oil. **78**: R_f 0.42 (benzene / acetone = 3/1); $[\alpha]^{D}_{25}$ –29 (*c* 0.30, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 8.06 (s, 1H), 7.75–7.65 (m, 4H), 7.67 (s, 1H), 7.45–7.33 (m, 6H), 7.18–7.05 (m, 2H), 6.87–6.79 (m, 3H), 6.33 (d, *J* = 16.1 Hz, 1H), 5.91 (d, *J* = 16.1 Hz, 1H), 5.12 (t, *J* = 7.5 Hz, 1H), 4.95 (dd, *J* = 9.6, 2.6 Hz, 1H), 4.68 (AB quart, *J* = 7.0 Hz, 2H), 4.52 (AB quart, *J* = 11.6 Hz, 2H), 4.49–4.41 (m, 1H), 4.38 (d, *J* = 9.6 Hz, 1H), 4.19 (dq, *J* = 9.4, 7.0 Hz, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.81–3.70 (m, 1H), 3.67–3.58 (m, 1H), 3.44 (t, *J* = 6.6 Hz, 1H), 3.37–3.25 (m, 1H), 3.29 (s, 3H), 3.24 (s, 3H), 3.10 (s, 3H), 3.04–2.97 (m, 1H), 2.86–2.65 (m, 4H), 2.49–2.38 (m, 1H), 2.33–2.24 (m, 1H), 2.01 (s, 3H), 2.06–1.20 (m, 13H), 1.03 (s, 9H), 0.89 (d, *J* = 6.5 Hz, 3H), 0.87 (d, *J* = 6.2 Hz, 3H), 0.86 (d, *J* = 7.0 Hz, 3H), 0.85 (d, *J* = 6.6 Hz, 3H), 0.79 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.1, 170.8, 170.5, 162.5, 156.6, 155.6, 148.9, 148.5, 143.8, 139.8, 139.1, 137.3, 137.04, 137.00, 135.0 (4C), 135.1, 133.8, 133.5, 131.4, 130.7, 130.2, 129.9, 129.7, 127.7 (2C), 127.6 (2C), 120.4, 116.3, 111.2, 110.9, 95.2, 81.7, 79.7, 78.4, 78.3, 77.2, 73.6, 69.6, 68.7, 60.6, 57.8, 57.5, 56.3, 55.9, 55.8, 42.8, 41.0, 39.7, 39.6, 36.5, 34.6, 32.9, 32.7, 30.9, 30.5, 29.7, 27.1 (3C), 26.8, 21.1, 19.3, 16.9, 15.6, 14.8, 9.5, 8.1; IR ($CHCl_3$) 3168, 3073, 3026, 3008, 2961, 2935, 2860, 1726, 1659, 1517, 1464, 1378, 1258, 1178, 1158, 1139, 1103, 1030, 980, 918, 822, 772, 704, 669 cm^{-1}; HRMS (ESI) *m/z* 1312.6356 (calcd for $C_{71}H_{95}N_3NaO_{17}Si$ $[M+Na]^+$, Δ +2.8 mmu).



78 79

Aldehyde 79. To a stirred solution of primary alcohol **78** (18.6 mg, 14.4 μmol) in dry CH_2Cl_2 (1.4 mL) were added dry pyridine (12 μL, 150 μmol) and Dess–Martin periodinane (9.2 mg, 22 μmol). After being stirred for 30 min at room temperature, the resulting mixture was diluted with EtOAc (1 mL) and a mixture of sat. $Na_2S_2O_3$ aq. – sat.

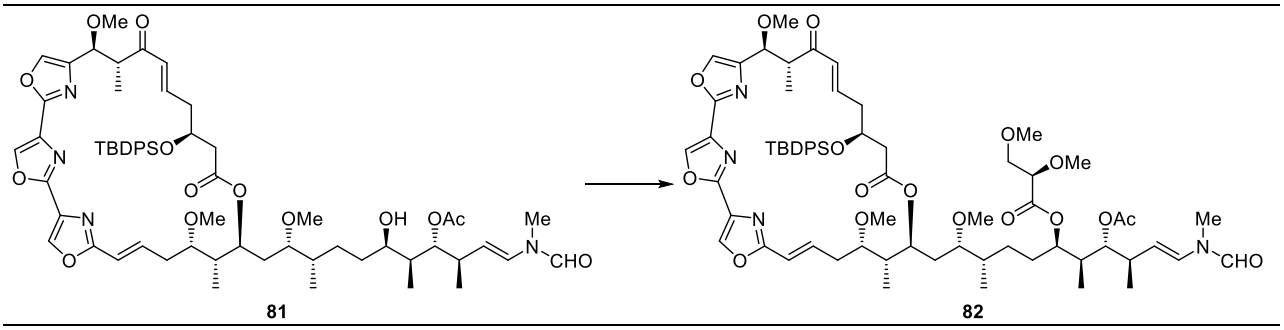
$NaHCO_3$ aq. – water (15 mL, 1:1:1 [v/v/v]) at 0 °C, and extracted with EtOAc (10 mL × 3). The combined extracts were washed with water and brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 0.5 g, hexane / acetone = 9/1 to 7/1) to give aldehyde **79** (18.4 mg, 99%) as a colorless oil. **79**: R_f 0.50 (benzene / acetone = 1/1); $[\alpha]^{D}_{25}$ –37 (*c* 0.62, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (br s, 1H), 8.11 (s, 1H), 8.06 (s, 1H), 7.54–7.65 (m, 4H), 7.67 (s, 1H), 7.45–7.33 (m, 6H), 7.16–7.03 (m, 2H), 6.89–6.78 (m, 3H), 6.33 (d, *J* = 16.2 Hz, 1H), 5.91 (d, *J* = 16.2 Hz, 1H), 5.11 (t, *J* = 8.0 Hz, 1H), 4.98 (dd, *J* = 9.2, 3.0 Hz, 1H), 4.68 (AB quart, *J* = 7.0 Hz, 2H), 4.51 (AB quart, *J* = 11.6 Hz, 2H), 4.49–4.40 (m, 1H), 4.38 (d, *J* = 9.6 Hz, 1H), 4.18 (dq, *J* = 9.5, 7.0 Hz, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.45–3.42 (m, 1H), 3.36–3.25 (m, 1H), 3.28 (s, 3H), 3.23 (s, 3H), 3.10 (s, 3H), 3.05–2.97 (m, 1H), 2.83–2.65 (m, 4H), 2.52–2.37 (m, 3H), 2.33–2.22 (m, 2H), 2.02 (s, 3H), 1.86–1.37 (m, 9H), 1.03 (s, 9H), 0.94 (d, *J* = 6.7 Hz, 3H), 0.86 (d, *J* = 6.9 Hz, 6H), 0.85 (d, *J* = 6.9 Hz, 3H), 0.78 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.0, 201.7, 170.5 (2C), 162.5, 156.5, 155.6, 148.9, 148.5, 143.6, 139.7, 139.0, 137.3, 137.0 (2C), 135.9 (4C), 135.1, 133.7, 133.5, 131.4, 130.6, 130.2, 129.9, 129.7, 127.7 (2C), 127.6 (2C), 120.4, 116.3, 111.1, 110.9, 95.1, 81.8, 79.6, 78.3, 77.6, 77.3, 73.6, 69.6, 68.7, 57.6, 57.4, 56.2, 55.9, 55.8, 44.8, 42.8, 40.9, 39.7, 39.6, 36.9, 34.4, 32.7, 32.6, 30.6, 29.6, 27.1 (3C), 26.4, 21.0, 19.3, 18.1, 15.7, 14.8, 9.7, 8.1; IR ($CHCl_3$) 3027, 3007, 2962, 2935, 2860, 1726, 1660, 1595, 1562, 1517, 1464, 1427, 1380, 1242, 1210, 1177, 1158, 1134, 1103, 1030, 980, 917, 789, 745, 728 cm^{-1}; HRMS (ESI) *m/z* 1310.6193 (calcd for $C_{71}H_{93}N_3NaO_{17}Si$ $[M+Na]^+$, Δ +2.1 mmu).



Secondary alcohol 81. A solution of aldehyde **79** (10.7 mg, 8.3 μmol), *N*-methylformamide (150 μL, 2.6 mmol), hydroquinone (1.8 mg, 16 μmol), and pyridinium *p*-toluenesulfonate (4.2 mg, 17 μmol) in dry benzene (17 mL) was stirred at reflux temperature for 8 h under a nitrogen stream with continuous removal of water using MS3A. The resulting mixture was diluted with triethylamine (12 μL) and sat. $NaHCO_3$ aq. (10 mL) at 0 °C, and extracted with EtOAc (10 mL × 4). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude material was partially purified with two SiO_2 column chromatographies (FL60D 0.5 g, benzene / MeOH = 40/1; FL60D 0.5 g, benzene / MeOH = 7/1 to 3/1) to give crude enamide **80** (6.5 mg) and recovered aldehyde **80** (3.5 mg, 33%) as colorless oils.

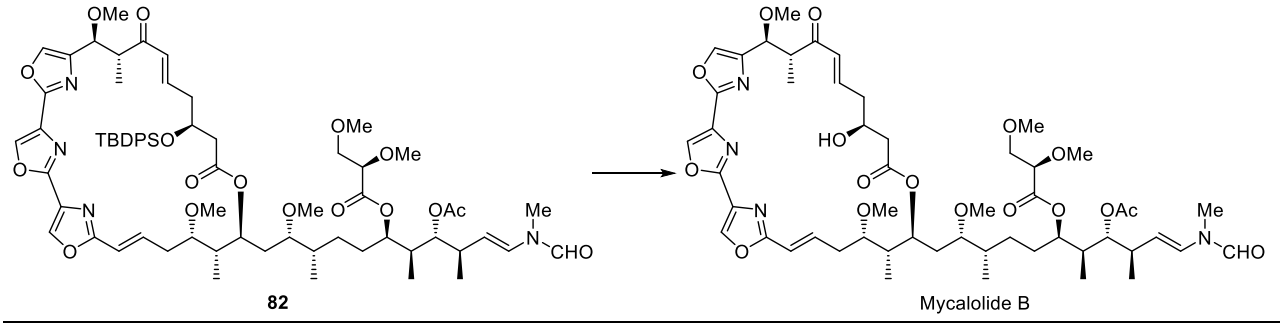
To a stirred solution of the crude enamide **80** (6.5 mg) in CH_2Cl_2 (0.98 mL), *tert*-butyl alcohol (50 μL), and 1.0 M phosphate buffer (pH 6.0, 50 μL) was added 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (3.9 mg, 17 μmol) at 0 °C. After being stirred at room temperature for 1 h, DDQ (2.0 mg, 8.8 μmol) and 1.0 M phosphate buffer (pH 6.0, 25 μL) was further added, and the mixture was stirred at room temperature for 2 h. After being diluted with EtOAc (2 mL) and 1.0 M phosphate buffer (pH 6.0, 3 mL), the mixture was stirred for 1.5 h and extracted with EtOAc (5 mL × 3). The combined extracts were washed with 5% $NaHCO_3$ aq. and brine, dried with Na_2SO_4, and concentrated. The crude material was purified with a SiO_2 column chromatography (FL60D 0.4 g, benzene / acetone = 7/1 to 3/1) to give secondary alcohol **81** (2.8 mg, 29% from aldehyde **79**) as a colorless oil. **81**: R_f 0.47 (benzene / acetone = 2/1); $[\alpha]^{D}_{25}$ –55 (*c* 0.72, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 [8.05] (s, 1H), 8.12 (s, 1H), 8.06

(s, 1H), 7.73–7.65 (m, 4H), 7.67 (s, 1H), 7.44–7.34 (m, 6H), 7.15–7.04 (m, 2H), 6.48 [7.16] (d, *J* = 14.0 [14.6] Hz, 1H), 6.34 (d, *J* = 15.8 Hz, 1H), 5.91 (d, *J* = 16.1 Hz, 1H), 5.12 (dt, *J* = 1.7, 8.2 Hz, 1H), 4.99 [5.02] (dd, *J* = 13.9, 9.5 Hz, 1H), 4.80 [4.79] (dd, *J* = 9.8, 3.1 Hz, 1H), 4.43 (br s, 1H), 4.38 (d, *J* = 9.5 Hz, 1H), 4.18 (dq, *J* = 9.2, 7.1 Hz, 1H), 3.42 (br s, 1H), 3.36–3.35 (m, 1H), 3.281 [3.277] (s, 3H), 3.237 [3.243] (s, 3H), 3.102 [3.105] (s, 3H), 3.04–2.97 (m, 1H), 3.00 [3.04] (s, 3H), 2.79–2.67 (m, 4H), 2.63–2.54 (m, 1H), 2.48–2.39 (m, 2H), 2.32–2.25 (m, 1H), 2.13 [2.12] (s, 3H), 1.84–1.20 (m, 9H), 1.03 (s, 9H), 0.90–0.80 (m, 12H), 0.77 [0.76] (d, *J* = 7.3 [6.8] Hz, 3H). Chemical shifts of the minor rotamer at the *N*-methylenamide moiety (2/1) are within parentheses (square blankets); ^{13}C NMR (150 MHz, $CDCl_3$) δ 203.0, 172.3, 170.5, 162.5, 162.1 [161.0], 156.6, 155.6, 143.8, 139.7, 139.1, 137.3, 137.0 (2C), 136.0 (4C), 135.1, 133.8, 133.5, 131.4, 130.2, 129.8, 129.7, 129.4, 127.7 (2C), 127.6 (2C), 116.4, 110.2 [112.0], 81.6, 79.7, 79.4, 79.2, 73.5, 70.0, 68.7, 57.6, 57.5, 56.3, 42.8, 41.0, 39.6 [39.7], 39.5 [39.4], 36.4, 36.2, 34.8 [34.7], 32.7, 32.5, 29.7, 27.7, 27.5 [33.0], 27.1 (3C), 26.9, 20.9, 19.3 [19.4], 15.7, 14.8, 8.5 [8.4], 8.2; IR ($CHCl_3$) 3676, 3025, 3005, 2959, 2932, 1731, 1697, 1657, 1603, 1559, 1458, 1375, 1253, 1211, 1179, 1103, 1047, 980, 917, 779, 745, 732 cm^{-1}; HRMS (ESI) *m/z* 1171.5641 (calcd for $C_{63}H_{84}N_4NaO_{14}Si$ $[M+Na]^+$, Δ –1.0 mmu).

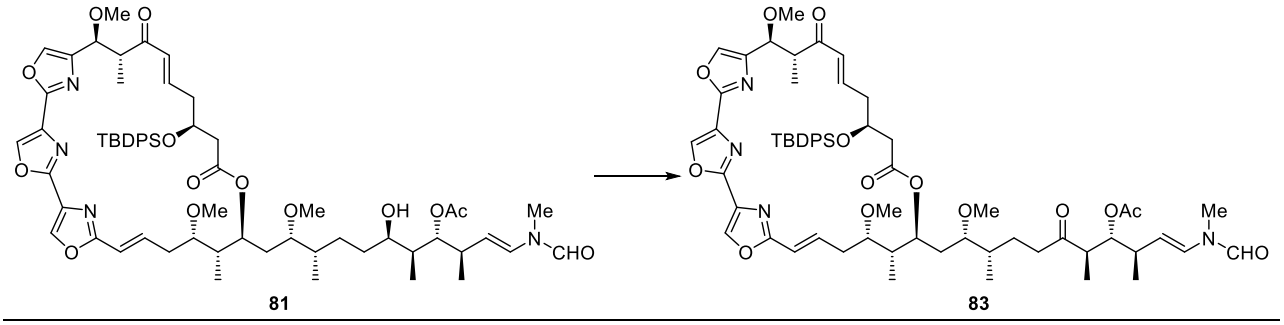


2,3-Di-*O*-methylglyceric ester 82. To a stirred solution of secondary alcohol **81** (1.6 mg, 1.4 μmol) and 2,3-di-*O*-methyl-D-glyceric acid (4.5 mg, 34 μmol) in dry benzene (0.5 mL) were added a 0.65 M solution of triethylamine in benzene (0.1 mL, 65 μmol), a 0.51 M solution of 2,4,6-trichlorobenzoyl chloride in benzene (0.1 mL, 51 μmol), and a 0.34 M solution of 4-(dimethylamino)pyridine in benzene (0.1 mL, 34 μmol). The mixture was stirred at room temperature for 1 h, diluted with EtOAc (1 mL) and 10% citric acid aq. (10 mL), and extracted with EtOAc (5 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified with two SiO_2 column chromatographies (FL60D 0.5 g, benzene / acetone = 10/1 to 3/1; FL60D 0.3 g, benzene / acetone = 5/1) to give 2,3-di-*O*-methylglyceric ester **82** (1.3 mg, 71%) as a colorless oil. **82**: R_f 0.51 (benzene / acetone = 2/1); $[\alpha]^{D}_{25}$ –50 (*c* 0.56, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 [8.04] (s, 1H), 8.12 (s, 1H), 8.07 (s, 1H), 7.73–7.66 (m, 4H), 7.67 (s, 1H), 7.45–7.34 (m, 6H), 7.15–7.03 (m, 2H), 6.47 [7.14] (d, *J* = 14.2 [14.6] Hz, 1H), 6.34 (d, *J* = 15.8 Hz, 1H), 5.91 (d, *J* = 16.2 Hz, 1H), 5.14–5.05 (m, 2H), 4.96 (dt, *J* = 14.2, 9.6 Hz, 1H), 4.74 (dd, *J* = 10.1, 2.8 Hz, 1H), 4.45–4.39 (m, 1H), 4.38 (d, *J* = 9.5 Hz, 1H), 4.18 (dq, *J* = 9.5, 6.9 Hz, 1H), 3.910 (dd, *J* = 6.6, 3.4 Hz, 1H), 3.69–3.57 (m, 2H), 3.46 (s, 3H), 3.38 (s, 3H), 3.34–3.26 (m, 1H), 3.264 [3.261] (s, 3H), 3.22 [3.23] (s, 3H), 3.10 (s, 3H), 3.02–2.95 (m, 1H), 2.99 [3.04] (s, 3H), 2.80–2.65 (m, 4H), 2.60–2.49 (m, 1H), 2.46–2.37 (m, 1H), 2.31–2.23 (m, 1H), 2.06 [2.05] (s, 3H), 1.85–1.20 (m, 9H), 1.03 (s, 9H), 1.00 [0.99] (d, *J* = 6.9 Hz, 3H), 0.94 [0.93] (d, *J* = 6.9 [7.0] Hz, 3H), 0.85 (d, *J* = 6.8 Hz, 3H), 0.84 (d, *J* = 6.8 Hz, 3H), 0.76 (d, *J* = 6.8 Hz, 3H). Chemical shifts of the minor rotamer at the *N*-methylenamide moiety (2/1) are within parentheses (square blankets); ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.0, 170.7, 170.6, 170.2, 162.6, 162.1 [161.0], 156.5, 155.6, 144.0, 139.7, 139.0, 137.3, 137.1 (2C), 135.9 (4C), 135.0, 133.8, 133.5, 131.3, 130.1, 129.8, 129.7, 129.4 [125.4], 127.6 (4C), 116.5, 110.3 [112.0], 81.6, 80.7, 79.8, 77.3, 76.4, 73.4, 73.0 (2C), 68.8, 59.3, 58.6, 57.5, 57.4, 56.3, 42.8, 40.9, 39.7, 39.5, 37.5 [37.4], 36.9 [37.0], 34.0 [33.8], 32.7, 32.1 [31.9], 30.5 [30.3], 29.7, 27.5 [33.0], 27.1 [26.9] (3C), 26.4 [26.3], 20.9, 19.3 [19.4], 15.8, 14.6, 9.7 [9.6], 8.2; IR ($CHCl_3$) 3072, 3032, 2999, 2932, 1732, 1692,

1656, 1461, 1375, 1243, 1221, 1181, 1106, 980, 918, 787, 782, 774, 728 cm^{-1}; HRMS (ESI) *m/z* 1287.6107 (calcd for $C_{68}H_{92}N_4NaO_{17}Si$ $[M+Na]^+$, Δ –1.7 mmu).

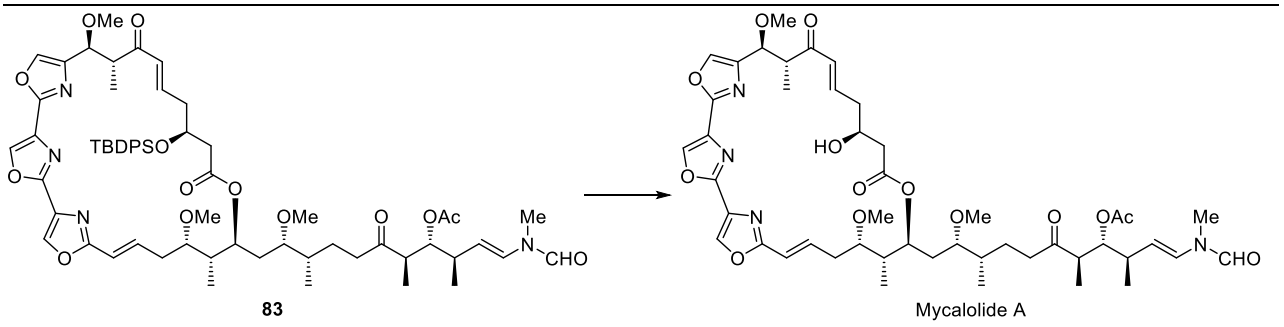


Mycalolide B. To a stirred solution of 2,3-di-*O*-methylglyceric ester **82** (6.9 mg, 5.5 μmol) in dry THF (1.4 mL) cooled at 0 °C were added a 1.0 M solution of TBAF/AcOH (1:1) in THF (0.164 mL, 0.164 mmol) [prepared by adding AcOH (114.5 μL, 2.0 mmol) to a 1.0 M solution of TBAF in THF (2.0 mL, 2.0 mmol)]. After being stirred for 60 h at room temperature, the reaction mixture was diluted with EtOAc (1 mL) and sat. $NaHCO_3$ aq. (5 mL) at 0 °C, and extracted with EtOAc (5 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude oil was purified with a SiO_2 column chromatography (BW-820MH 0.5 g, $CHCl_3$ / MeOH = 40/1) to give mycalolide B (5.5 mg, 98%) as a colorless oil. Synthetic mycalolide B: R_f 0.43 ($CHCl_3$ / MeOH = 40:1); $[\alpha]^{D}_{25}$ –55 (*c* 0.55, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 [8.07] (s, 1H), 8.10 (s, 1H), 8.07 (s, 1H), 7.66 (s, 1H), 7.29 (m, 1H), 7.14 (m, 1H), 6.49 [7.16] (d, *J* = 14.0 [14.5] Hz, 1H), 6.35 (d, *J* = 15.8 Hz, 1H), 6.24 (d, *J* = 16.0 Hz, 1H), 5.47 (d, *J* = 4.5 Hz, 1H, O*H*), 5.25 (br t, *J* = 8.1 Hz, 1H), 5.12 [5.09] (br t, *J* = 6.1 [8.4] Hz, 1H), 4.96 [4.99] (dd, *J* = 14.0, 9.4 [14.5, 9.6] Hz, 1H), 4.77 (dd, *J* = 10.0, 2.8 Hz, 1H), 4.44 (m, 1H), 4.35 (d, *J* = 8.3 Hz, 1H), 4.00 (dq, *J* = 7.1, 7.1 Hz, 1H), 3.93 (dd, *J* = 6.6, 3.4 Hz, 1H), 3.69–3.60 (m, 2H), 3.49 (s, 3H), 3.46 (m, 1H), 3.40 (s, 3H), 3.35 (s, 3H), 3.30 (s, 3H), 3.19 (s, 3H), 3.02 [3.06] (s, 3H), 2.97 (m, 1H), 2.68 (m, 1H), 2.66–2.45 (m, 5H), 2.43 (m, 1H), 2.079 [2.076] (s, 3H), 1.89–1.79 (m, 2H), 1.77–1.72 (m, 1H), 1.70–1.22 (m, 6H), 1.01 [1.00] (d, *J* = 6.8 [6.7] Hz, 3H), 0.97 [0.96] (d, *J* = 6.9 [6.8] Hz, 3H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.91 (d, *J* = 7.0 Hz, 3H), 0.844 [0.838] (d, *J* = 6.9 [6.8] Hz, 3H). Chemical shifts of the minor rotamer at the *N*-methylenamide moiety (2/1) are within parentheses (square blankets). O*H* signal was assigned based on deuterium exchange experiments; ^{13}C NMR (150 MHz, $CDCl_3$) δ 202.5, 172.0, 170.7, 170.3, 162.8, 162.1 [161.0], 156.4, 155.5, 146.0, 140.7, 139.3, 137.3, 137.2, 137.1, 133.2, 131.0, 129.9, 129.4 [125.4], 116.2, 110.3 [112.0], 81.6, 80.7, 79.6, 77.5, 76.4, 73.1 (2C), 73.0, 67.6, 59.3, 58.6, 58.2 [58.3], 57.9 [57.8], 56.8, 43.9, 42.8, 41.1, 40.8, 37.5 [37.4], 36.9 [37.1], 35.2 [35.4], 34.7 [34.5], 32.0 [31.9], 30.5 [30.3], 27.5 [33.0], 27.0 [27.8], 21.0, 19.4 [19.5], 15.6, 13.1, 9.8 [9.6], 9.0; IR ($CHCl_3$) 3689, 3503, 3022, 3008, 2934, 1711, 1656, 1603, 1558, 1457, 1418, 1362, 1222, 1192, 1092, 979, 918, 767, 746, 739 cm^{-1}; HRMS (ESI) *m/z* 1049.4954 (calcd for $C_{52}H_{74}N_4NaO_{17}$ $[M+Na]^+$, Δ +0.7 mmu).



TBDPS-protected mycalolide A (83).[14] To a stirred solution of secondary alcohol **81** (0.50 mg, 0.43 μmol) in dry

CH_2Cl_2 (0.42 mL) were added a 1.0 M solution of pyridine in CH_2Cl_2 (4.3 μL, 4.3 μmol) and a 0.3 M solution of Dess–Martin periodinane in CH_2Cl_2 (6 μL, 1.8 μmol). After being stirred for 1 h at room temperature, the resulting mixture was diluted with a mixture of sat. $Na_2S_2O_3$ aq. – sat. $NaHCO_3$ aq. – water (2.5 mL, 1:1:1 [v/v/v]) at 0 °C, and extracted with EtOAc (3 mL × 3). The combined extracts were washed with water and brine, dried with Na_2SO_4, and concentrated. The crude material was partially purified with a SiO_2 column chromatography (FL60D, 0.2 g, benzene / acetone = 5/1 to 3/1) to give crude TBDPS-protected mycalolide A (**83**) (ca. 0.3 mg) as a colorless oil, which was used for the next step without further purification. **83**: R_f 0.60 (benzene / acetone = 2/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 8.11 (s, 1H), 8.07 (s, 1H), 7.73–7.66 (m, 4H), 7.67 (s, 1H), 7.45–7.34 (m, 6H), 7.15–7.03 (m, 2H), 6.48 [7.15] (d, *J* = 13.4 [14.6] Hz, 1H), 6.33 (d, *J* = 15.9 Hz, 1H), 5.92 (d, *J* = 16.2 Hz, 1H), 5.17–5.08 (m, 2H), 4.97 (dt, *J* = 14.2, 10.2 Hz, 1H), 4.47–4.39 (m, 1H), 4.38 (d, *J* = 9.4 Hz, 1H), 4.18 (m, 1H), 3.34–3.26 (m, 1H), 3.29 (s, 3H), 3.25 (s, 3H), 3.11 (s, 3H), 3.02–2.95 (m, 1H), 3.02 [3.06] (s, 3H), 2.77–2.65 (m, 6H), 2.57–2.38 (m, 2H), 2.33–2.25 (m, 1H), 2.01 [2.00] (s, 3H), 1.87–1.17 (m, 5H), 1.04–1.01 (m, 6 H), 1.03 (s, 9H), 0.87 (d, *J* = 7.0 Hz, 3H), 0.86 (d, *J* = 6.9 Hz, 3H), 0.74 (d, *J* = 6.8 Hz, 3H). Chemical shifts of the minor rotamer at the *N*-methylenamide moiety (2/1) are within parentheses (square blankets); HRMS (ESI) *m/z* 1169.5516 (calcd for $C_{63}H_{82}N_4NaO_{14}Si$ $[M+Na]^+$, Δ +2.1 mmu).



Mycalolide A. To a stirred solution of the above crude TBDPS-protected mycalolide A (**83**) (ca. 0.3 mg) in dry THF (110 μL) cooled at 0 °C were added a 1.0 M solution of TBAF/AcOH (1:1) in THF (13 μL, 13 μmol) [prepared by adding AcOH (114.5 μL, 2.0 mmol) to a 1.0 M solution of TBAF in THF (2.0 mL, 2.0 mmol)]. After being stirred for 53 h at room temperature, the reaction mixture was diluted with EtOAc (2 mL) and sat. $NaHCO_3$ aq. (3 mL) at 0 °C, and extracted with EtOAc (2 mL × 3). The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated. The crude oil was purified with a reversed-phase HPLC [Develosil ODS-HG-5 (ϕ 20 × 250 mm), 70% MeOH, 5 mL/min, UV254 nm] to give mycalolide A (62 nmol, quantified by ^{1}H NMR spectrum, 14% from secondary alcohol **83**) as a colorless oil. Synthetic mycalolide A: R_f 0.45 (benzene / acetone = 2/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 [8.08] (s, 1H), 8.10 (s, 1H), 8.07 (s, 1H), 7.65 (s, 1H), 7.30 (m, 1H), 7.17 (m, 1H), 6.50 [7.16] (d, *J* = 14.0 Hz, 1H), 6.36 (d, *J* = 15.6 Hz, 1H), 6.24 (d, *J* = 15.9 Hz, 1H), 5.44 (m, 1H, O*H*), 5.28 (t, *J* = 8.2 Hz, 1H), 5.14 (m, 1H), 4.97 [5.00] (t, *J* = 9.1 [9.4] Hz, 1H), 4.44 (m, 1H), 4.35 (d, *J* = 8.5 Hz, 1H), 3.99 (t, *J* = 7.8 Hz, 1H), 3.48 (m, 1H), 3.36 (s, 3H), 3.32 (s, 3H), 3.19 (s, 3H), 3.03 [3.07] (s, 3H), 3.00 (m, 1H), 2.81–2.56 (m, 3H), 2.53–2.42 (m, 7H), 2.014 [2.006] (s, 3H), 1.90 (m, 1H), 1.79–1.37 (m, 4H), 1.25 (m, 1H), 1.07 (d, *J* = 7.1 Hz, 3H), 1.05 [1.04] (d, *J* = 7.0 [6.9] Hz, 3H), 0.932 (d, *J* = 6.8 Hz, 3H), 0.928 (d, *J* = 6.8 Hz, 3H), 0.834 (d, *J* = 6.7 Hz, 3H) Chemical shifts of the minor rotamer at the *N*-methylenamide moiety (2/1) are within parentheses (square blankets); HRMS (ESI) *m/z* 931.4332 (calcd for $C_{47}H_{64}N_4NaO_{14}$ $[M+Na]^+$, Δ +1.5 mmu).

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Table. The list of trisoxazole macrolides.

[ref.]	Compounds	Natural source	Cytotoxicity IC_{50} (against tumor cells)
	Ulapualides		
[3]	Ulapualide A	nudibranch, *H. sanguineus*	0.01-0.03 μg/mL (L1210)
[3]	Ulapualide B	nudibranch, *H. sanguineus*	0.01-0.03 μg/mL (L1210)
	Kabiramides		
[4b]	Kabiramide A	nudibranch, *Hexabranchus* sp.	0.03 μg/mL (L1210)
[4b]	Kabiramide B	nudibranch, *Hexabranchus* sp.	0.03 μg/mL (L1210)
[4a,4b]	Kabiramide C	nudibranch, *Hexabranchus* sp.	0.01 μg/mL (L1210)
[4b]	Kabiramide D	nudibranch, *Hexabranchus* sp.	0.02 μg/mL (L1210)
[4b]	Kabiramide E	nudibranch, *Hexabranchus* sp.	0.02 μg/mL (L1210)
[4c]	Kabiramide F	sponge, *Pachastrissa nux*	unkown
[4c]	Kabiramide G	sponge, *Pachastrissa nux*	unkown
[4c]	Kabiramide H	sponge, *Pachastrissa nux*	unkown
[4c]	Kabiramide I	sponge, *Pachastrissa nux*	unkown
[4d]	Kabiramide J	sponge, *Pachastrissa nux*	0.020 μg/mL (MCF-7)
[4d]	Kabiramide K	sponge, *Pachastrissa nux*	0.060 μg/mL (MCF-7)
[4e]	Kabiramide L	sponge, *Pachastrissa nux*	unkown
	Halichondramides		
[5a]	Halichondramide	sponge, *Halichondria* sp.	0.19 μg/mL (K562)[5c], 0.038 μg/mL (A549)[4e]
[5b]	Isohalichondramide	sponge, *Halichondria* sp.	unkown
[5c]	Neohalichondramide	sponge, *Chondrosia corticata*	0.38 μg/mL (K562)[5c], 3.1 μg/mL (A549)[4e]
[5b,4b]	Dihydrohalichondramide	sponge, *Halichondria* sp., nudibranch, *Hexabranchus* sp.	0.03 μg/mL (L1210), 0.32 μg/mL (K562)[5c]
[5b]	Tetrahydrohalichondramide	sponge, *Halichondria* sp.	unknown
[4b]	33-Methyldihydrohalichondramide	nudibranch, *Hexabranchus* sp.	0.05 μg/mL (L1210)
[5b]	Halichondramide acid	sponge, *Halichondria* sp.	unknown
[5b]	Halichondramide imide	sponge, *Halichondria* sp.	unknown
[5b]	Halichondramide ester	sponge, *Halichondria* sp.	unknown
[5c]	Secohalichondramide	sponge, *Chondrosia corticata*	>500 μg/mL (K562)[5c]
[5c]	(19*Z*)-Halichondramide	sponege, *Chondrosia corticata*	0.90 μg/mL (K562)[5c], 0.020 μg/mL (A549)[4e]
	Mycalolides		
[6a]	Mycalolide A	sponge, *Mycale* sp.	0.5-1.0 ng/mL (B16 melanoma)
[6a]	Mycalolide B	sponge, *Mycale* sp.	0.5-1.0 ng/mL (B16 melanoma)
[6a]	Mycalolide C	sponge, *Mycale* sp.	0.5-1.0 ng/mL (B16 melanoma)
[6b]	Mycalolide D	coral, *Tubastrea faulkneri*	average 0.6 μg/mL (NIC 60-human-tumor cell line)
[6b]	Mycalolide E	coral, *Tubastrea faulkneri*	unknown
[6c]	Thiomycalolide A	sponge, *Mycale* sp.	0.018 μg/mL (P388)
[6c]	Thiomycalolide B	sponge, *Mycale* sp.	0.018 μg/mL (P388)
[6d]	30-hydroxymycalolide A	sponge, *Mycale magellanica*	0.019 μg/mL (L1210)
[6d]	32-hydroxymycalolide A	sponge, *Mycale magellanica*	0.013 μg/mL (L1210)
[6d]	38-hydroxymycalolide B	sponge, *Mycale magellanica*	0.015 μg/mL (L1210)
[6e]	30,32-dihydroxymycalolide A	sponge, *Mycale izuensis*	2.6 ng/mL (HeLa)
[6f]	Secomycalolide A	sponge, *Mycale* sp.	unknown
	Jaspisamides		
[7]	Jaspisamide A	sponge, *Jaspis* sp.	0.015 μg/mL (KB), 0.31 μg/mL (K562)[5c] 28 μg/mL (A549)[4e]
[7]	Jaspisamide B	sponge, *Jaspis* sp.	0.006 μg/mL (KB)
[7]	Jaspisamide C	sponge, *Jaspis* sp.	0.013 μg/mL (KB)
	Halishigamides		
[8]	Halishigamide A	sponge, *Halichondria* sp.	0.0036 μg/mL (L1210), 0.012 μg/mL (KB)
[8]	Halishigamide B	sponge, *Halichondria* sp.	4.4 μg/mL (L1210), 7.5 μg/mL (KB)
[8]	Halishigamide C	sponge, *Halichondria* sp.	5.2 μg/mL (L1210), 6.5 μg/mL (KB)
[8]	Halishigamide D	sponge, *Halichondria* sp.	1.1 μg/mL (L1210), 1.8 μg/mL (KB), 92 μg/mL (K562)[5c], 1.5 μg/mL (A549)[4e]