Syntheses and bioactivities of oxygenated lipid derivatives

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Contents

Chapter 1

General Introduction

Lipids exert a wide variety of biological functions, like storing energy and signaling. Since the membrane of cell, which is the basic unit of all organs, is consisted of lipid bilayer, the importance of lipids admits of no doubt. Recently, the gene arrangement of a large variety of organism have been revealed, and advancement of technology of proteomics[1,2] enables us to understand the functions, structures and interactions of proteins. On the other hand, lipids still remain largely unknown, therefore they receive a lot of attention and many studies are performed to investigate their functions and biological roles. The difficulties of studying lipids would come from their low solubility to water and low stability of their natural ligands to light and oxygen. Therefore the establishment of new synthetic method that is efficient and flexible to synthesize natural ligands and their analogues, and development of stable ligands are necessary to promote lipids research and understand the biological and pharmacological roles of lipids.

Prostaglandins are one of the most well-known lipid mediators. A fair amount of researches have been already reported relating to prostaglandins, one of the most important research was performed by Corey et al. in 1969[3]. They established efficient, diverse and economical synthetic method of prostaglandins and his works made an enormous step forward of prostaglandin research. Actually, $PGF_{2\alpha}$, PGE_2 and PGE_1 were on the market in 1974 as labor induction agents or drugs for improvement of blood circulation. Moreover, his synthetic strategy is still widely applied in prostaglandin research.

Therefore the author aimed to develop new synthetic methods of lipid mediators and discover useful tool compounds for further development of lipid research. In this thesis, the author will report discovery of G protein-biased and highly subtype selective novel EP2 receptor agonists and the first total syntheses of resolvin E2 and haterumalide NA methyl ester.

1) Discovery of G protein-biased EP2 agonists.

Prostaglandin E_2 is an oxygenated metabolite of arachidonic acid, which is a polyunsaturated ω-6 fatty acid, and exerts a wide variety of biological actions through four receptor subtypes, EP1-EP4, in various tissues. EPs are a family of prostanoid recepters of progtaglandin E type and belong to G protein-coupled recepter (GPCR) which are also known as seven taransmembrane receptors and control the biological action through G protein-mediated signaling. EP1-4 receptors have been already cloned and subtype selective ligands (agonists or anatgonists) of EPs have been developed and used to evaluate a biological role of each receptors. EP2 receptor has been characterized by a relaxation of blood vessels.[4] Furthermore, EP2 receptor plays important roles in production of cytokines and bone metabolisms by the production of cyclic adenosine monophosphate (cAMP).[5,6] A number of EP2 agonists have been previously reported. PGE_2 analogue, butaprost (**1**)[7] is well-known as a selective EP2 agonist and widely utilized as a chemical tool compound in many studies for investigation of pharmacological activities mediated by EP2 receptor. In the previous studies, ONO Pharmaceutical developed a highly selective and chemically stable EP2 agonist as **2**[8], which is also a good tool compound for EP2 receptor. A number of non-prostanoid scaffolds of EP2 agonists were also reported to show potent EP2 agonist activities (i.e. PF-4217329 (**3**)[9] and **4**[10]) (Figure 1-1).

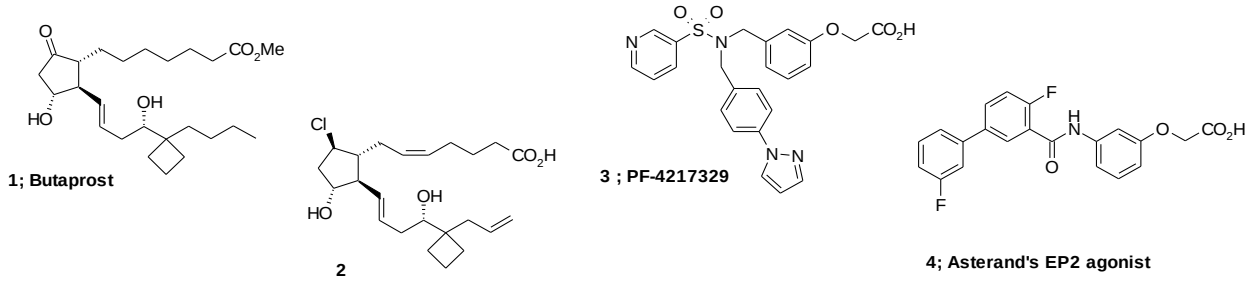


Figure 1-1. Reported EP2 agonists

Up to the present time, however, there is no EP2 agonist that is approved for clinical use. Although the true reasons of suspension of clinical trials of EP2 agonists were not clear, the author assume that variety biological actions induced by EP2 agonists cause crucial side effects for crinical use. Therefore, the author focused on the biased ligand of GPCRs to develop the next generation EP2 agonists.

Recently, biased ligands receive a fair amount of attention in drug discovery,[11] because they have a potential to remove on-target adverse effect and also enhance efficacy. In addition to G protein signaling, GPCRs can also activate other distinct signaling pathways, like β arrestin-mediated signaling. GPCR biased ligands are compounds that selectively engage some signals withour activation of other signals mediated by the same receptor.

A number of studies were performed to understand biological roles of G protein- and β arrestin-mediated signalings of EP2 receptor. In the brain, EP2 receptor modulates beneficial neuroprotective effects in acute models of excitoxicity though G protein-mediated cAMP-PKA signaling.[12-15] On the other hand, the activation of β arrestin-mediated signaling of EP2 receptor led to deleterious effects, like tumorigenesis and angiogenesis.[16-18] Therefore, the author supposed that G protein-biased ligands of EP2 receptor have a potential to be a new generation of EP2 agonists that particularly increase the efficacy and avoid deleterious effect of EP2 receptor.

To identify G protein-biased and highly subtype-selective EP2 agonists, a series of bicyclic prostaglandin analogues were designed and synthesized. Structural hybridization of EP2/4 dual agonist **5** and prostacyclin analog **6**, followed by simplification of ω chain led the author to find novel EP2 agonists with a unique prostacyclin-like scaffold. Further optimization of ω chain was performed to improve EP2 agonist activity and subtype selectivity. Phenoxy derivative **7** showed potent agonist activity and excellent subtype selectivity, furthermore a series of compounds were identified as G protein-biased EP2 receptor agonists. The discovery of novel G protein-biased EP2 agonists as well as structure functional selectivity relationship will be discussed in Chapter 2-1.

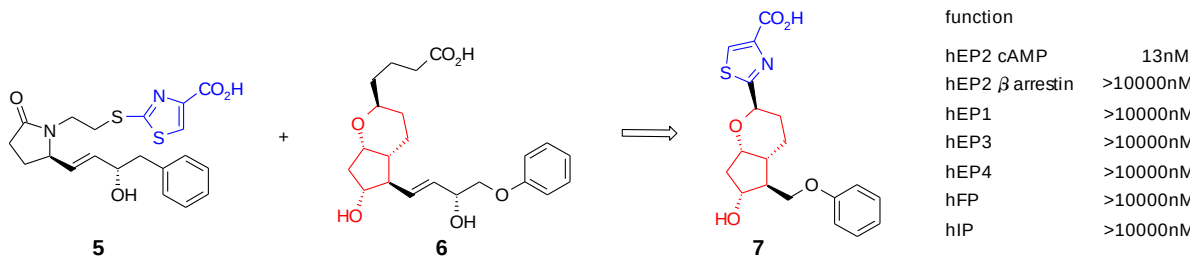


Figure 1-2. Discovery of G protein-biased ligand **7**

Further optimization of novel G protein-biased EP2 agonist **7** was undertaken to improve G protein activity and investigate structure functional selectivity relationship (SFSR). Optimization of substituents on the phenyl group, followed by modification of 11-OH led the author find **8** with 100-fold increase in G protein signaling without increase of β arrestin activity relative to **7**. Furthermore, SFSR studies revealed that the combination of *meta* and *para* substituents on the phenyl moiety was crucial to regulate its functional selectivity. The synthesis of a series of these derivatives and detail structure-activity relationship and structure functional selectivity relationship will be also discussed in Chapter 2-2.

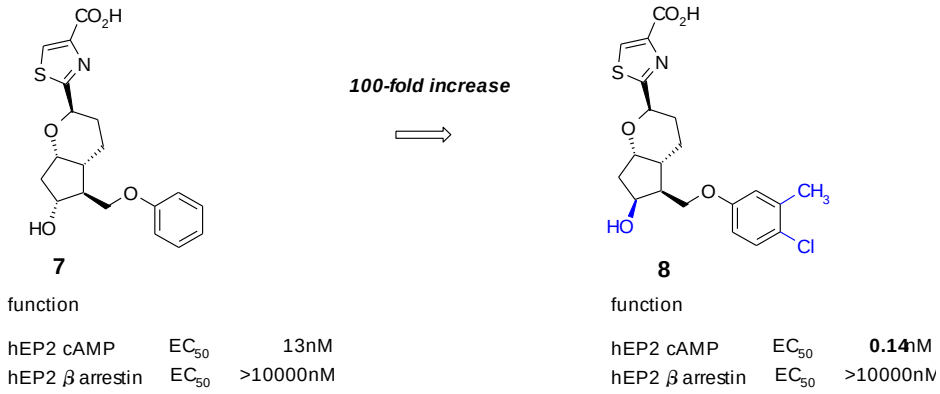


Figure 1-3. Optimization of G protein-biased ligands

2) Total synthesis and bioactivity of Resolvin E2

Prostaglandins derived from arachidonic acid (AA), ω-6 polyunsaturated fatty acids, are well-known as inflammatory mediators. On the other hand, resolvins are new family of lipid mediators derived from ω-3 polyunsaturated fatty acids, like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and they are generated during the resolution phase of acute inflammation.[19] A number of resolvins have been identified and they exert a wide variety of biological actions in various tissues. Resolvin E1 is biosynthesized from EPA via cyclooxygenase (COX)-2- and 5-lipoxygenase-mediated conversion and has been shown to possess significant anti-inflammatory and proresolution properties, thereby protecting organs from collateral damage.[20] Another E series resolvin, namely, resolvin E2 (**1**), is formed via reduction of 5*S*-hydroperoxy-18*R*-hydroxy-EPE, an intermediate in the biosynthesis of resolvin E1, and exhibits potent anti-inflammatory properties in murine peritonitis.[21] It has been hypothesized that these E series resolvins contribute to the beneficial actions

that have been attributed to EPA in certain human diseases, particularly those in which inflammation is suspected as a key component in pathogenesis. Motivated by their therapeutic potential for new treatment of human disorders associated with aberrant inflammation, the author launched the synthetic studies of resolvins as well as other lipid mediators. In Chapter3, the author will report an efficient total synthesis of resolvin E2 by taking advantage of its intrinsic pseudoenantiomeric substructures and using a torquoselective thermal electrocyclic ring-opening reaction of cyclobutene aldehydes **4** and **5** for constructing the *E,Z*-olefins at C6 of **2** and C17 of **3** (Figure 1-4). The biological activities of synthetic resolving E2 will be also shown in this Chapter.

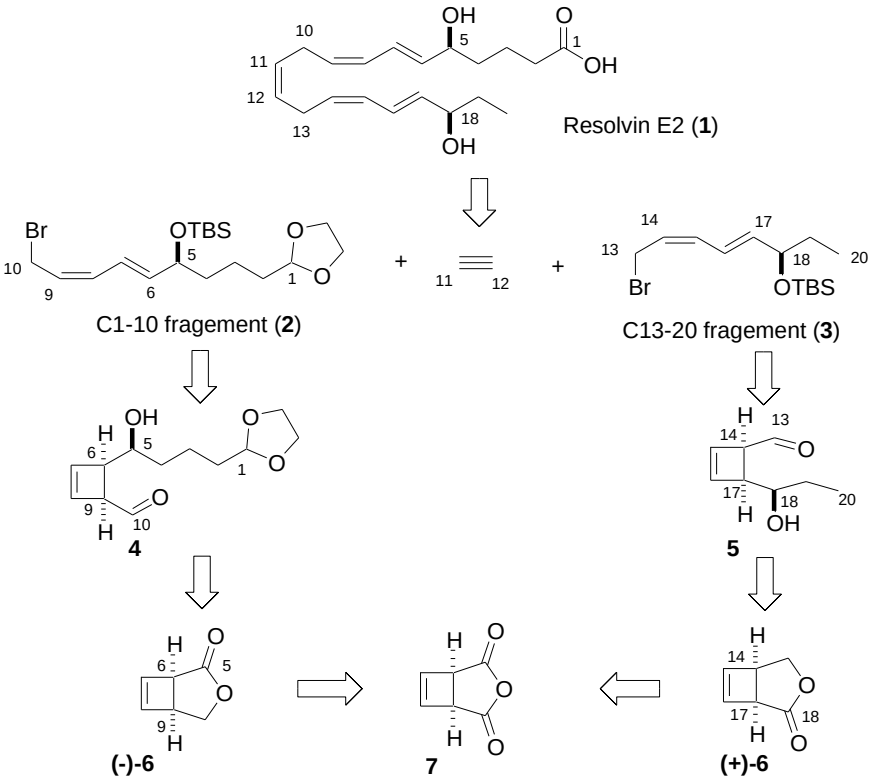
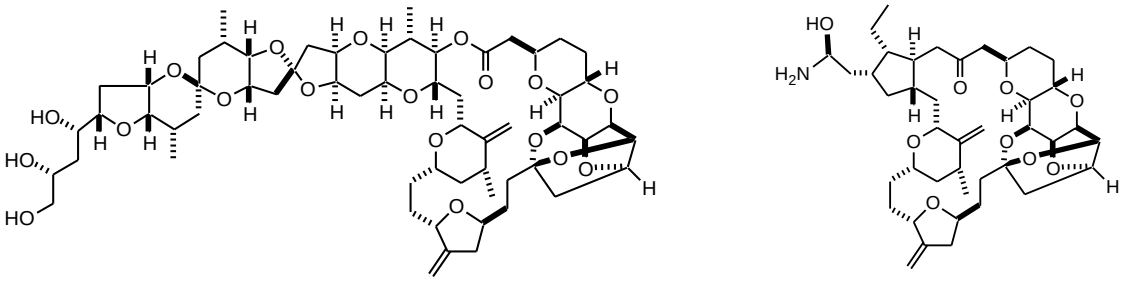


Figure 1-4. Synthetic strategy of resolvin E2

3) Enantioselective Synthesis of Haterumalide NA Methyl Ester and Revised Structure of Haterumalide NA.

A number of bioactive natural products isolated from marine organisms have received a fair amount of attentions for drug discovery because of their potent and unique biological activities. Recently, exploratory research using halichondrin B[22], which was isolated from *Halichondrail okadai*, as a lead compound was

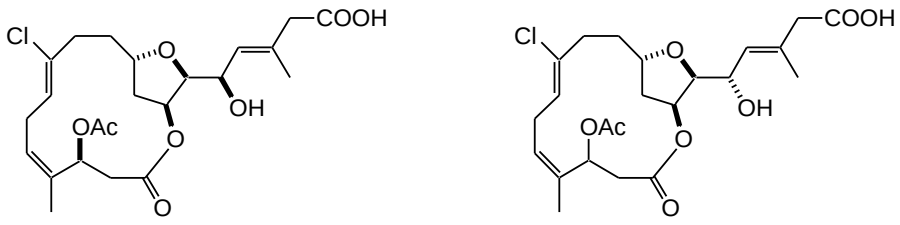
performed to discovery a new anti-cancer drug by Kishi et al. and Eisai Co. Precise SAR studies of halichondrin B led to discovery of Halaven, which is used as anti-cancer agent in clinical from 2010.[23-25]



Halichondrin B Halaven

Figure 1-5. Structure of halichondrin B and halaven

The author focused on haterumalide NA[26] which was isolated from the Okinawan sponge *Ircinia* sp. Haterumalide NA has a 14-membered macrolide moiety (long fatty acid derivative) and exerts moderate cytotoxicity to mouse P388 leukemia cell and acute toxicity to ddY mouse. The structurally-related haterumalide B[27] and oocydin A[28] were isolated from an Okinawan ascidian and a South American epiphyte, respectively, and their stereostructures have not been fully established.



Haterumalide NA Oocydin A

Figure 1-6. Structure of haterumalide NA and oocydin A

The author launched the synthetic study of haterumalide NA to identify the absolute stereochemistry and supply samples to perform further biological studies. The enantioselective synthesis of haterumalide NA methyl ester by using the stereoselective construction of a chloroolefin unit and the intramolecular Reformatsky-type reaction will be discussed in Chapter 4.

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Chapter 2-1

Discovery of G protein-biased EP2 receptor agonists

Abstract:

To identify G protein-biased and highly subtype-selective EP2 receptor agonists, a series of bicyclic prostaglandin analogues were designed and synthesized. Structural hybridization of EP2/4 dual agonist **5** and prostacyclin analog **6**, followed by simplification of the ω chain enabled the author to discover novel EP2 agonists with a unique prostacyclin-like scaffold. Further optimization of the ω chain was performed to improve EP2 agonist activity and subtype selectivity. Phenoxy derivative **27a** showed potent agonist activity and excellent subtype selectivity. Furthermore, a series of compounds were identified as G protein-biased EP2 receptor agonists. These are the first examples of biased ligands of prostanoid receptors.

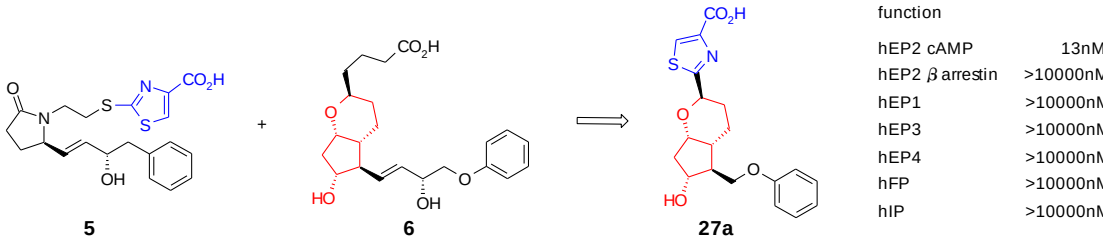


Figure 2-1-1. Outline of Chapter2-1

Introduction

Prostaglandin E_2 (PGE_2) is an oxygenated metabolite of arachidonic acid that exerts a wide variety of biological actions through four receptor subtypes, EP1–EP4, in various tissues, which are G protein-coupled recepter (GPCR), in which their ligands induce signaling through G protein activation. The EP2 receptor has been characterized by relaxation of blood vessels.[1] Furthermore, EP2 receptor plays important roles in cytokine production and bone metabolism.[2,3] It has also been reported that activation of EP2 receptor led to neuroprotective effects in ischemic stroke models.[4-8] EP2 receptor receives a lot of attention as a therapeutic target for various diseases.

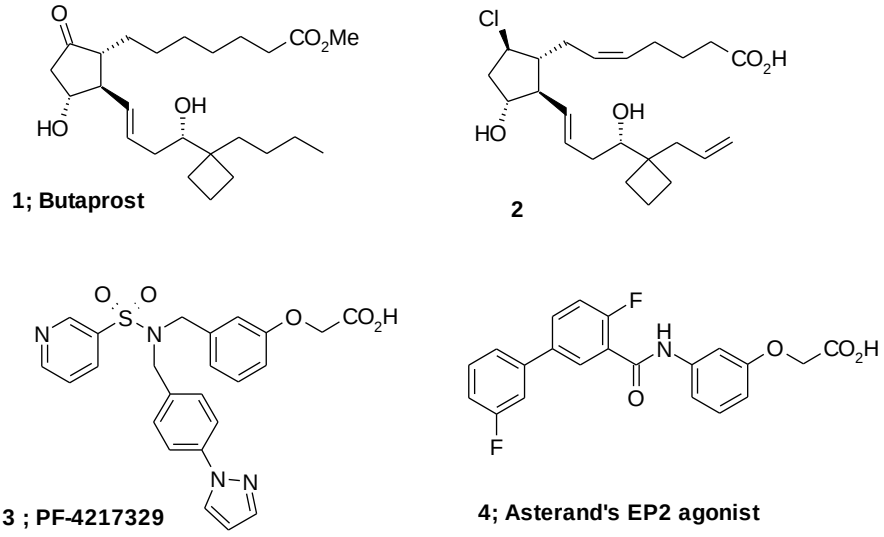


Figure 2-1-2. Reported EP2 agonists

A number of EP2 agonists have previously been reported.[9–15] The PGE_2 analogue, butaprost (**1**), is well known as a selective EP2 agonist, and is widely used as a chemical tool compound in many studies on pharmacological activities mediated by EP2 receptor. In previous studies, ONO Pharmaceutical developed the highly selective and chemically stable EP2 agonist, **2**,[10] which is a good tool compound for EP2 receptor. A number of non-prostanoid scaffolds of EP2 agonists have also been reported to show a potent EP2 agonist

activity (for example, PF-4217329 (**3**)[13] and **4**[15]). In recent studies by Pfizer, PF-4217329 (**3**), an isopropyl ester, showed remarkable intraocular pressure-lowering effects in primary open-angle glaucoma and ocular hypertension.[16] To date, however, there is no EP2 agonist that is approved for clinical use. Although the true reasons for the suspension of clinical trials of EP2 agonists are not clear, the author assumes that a variety of biological actions induced by EP2 agonists caused crucial side effects for clinical use.

Recently, biased ligands have received a fair amount of attention in drug discovery,[17-22] because they have the potential to suppress on-target adverse effects and enhance efficacy. In addition to G protein signaling, G protein-coupled receptors (GPCRs) can activate other distinct signaling pathways, like β arrestin-mediated signaling. GPCR-biased ligands are compounds that selectively engage some signals without activation of other signals mediated by the same receptor. A number of studies have been performed to investigate the biological roles of G protein- and β arrestin-mediated EP2 receptor signaling. In the brain, EP2 receptor modulates beneficial neuroprotective effects in acute models of excitotoxicity through G protein-mediated cAMP-PKA signaling.[4,5,23,24] Conversely, activation of β arrestin-mediated EP2 receptor signaling led to deleterious effects, like tumorigenesis and angiogenesis.[25-27] Therefore, the author hypothesized that G protein-biased ligands of EP2 receptor have the potential to be next generation EP2 agonists that will overcome the clinical problems of previously reported EP2 agonists. To the best of the author's knowledge, there is no report of biased ligands of prostanoid receptors. Moreover, the author performed screening of in-house EP2 agonists and failed to identify G protein-biased agonists. As the compounds the author evaluated have a similar structure to PGE_2, the author aimed to discover G protein-biased EP2 agonists by design and investigation of a new scaffold. In this report, the author describes the discovery of novel, highly selective EP2 agonists with a unique bicyclic scaffold, which were identified as G protein-biased EP2 agonists. The functional selectivity and signaling bias of the compounds are also discussed.

Results and discussion

Identification of initial lead compound

First, to identify novel subtype-selective EP2 agonists with a new scaffold, the author focused on EP2/EP4 dual agonist **5**. In ONO's previous study[28], the thiazole group of **5** was one of the key substructures to increase EP2 agonist activity. Introduction of a thiazole group into various reported scaffolds seemed to contribute to the development of novel and potent EP2 agonists. Chemically stable prostacyclin analogue **6**,[29] which has been reported by the Upjohn group in the 1970s, showed very weak EP2 agonist activity (EC_{50} = 8900 nM). The author designed and synthesized compound **7** with a bicyclic scaffold by hybridization of **6** and the thiazole moiety of **5** (Figure 2-1-3). The resulting **7** exhibited remarkably potent EP2 agonist activity as the author expected, however, it also showed potent agonist activity toward the other receptor subtypes, especially EP1 and EP3 (Table 2-1-1). To increase the subtype selectivity, the author next focused on the ω chain of **7**.

Because all the natural prostanoids (for example, PGE_2, PGI_2, and $PGF_{2\alpha}$) have a hydroxyl group at a particular position in the ω chain, which is supposed to be a crucial moiety for exerting agonist activity toward PG receptors. However, a number of non-prostanoid scaffolds of EP2 agonists without a hydroxyl group have been reported to show potent EP2 agonist activity (for example, **3**[13] and **4**[15]). The author hypothesized that the removal of the 15-hydroxyl group from compound **7** would be effective for decreasing the agonist activity toward all of the receptor subtypes except for EP2. As expected, the dehydroxylated derivative **8** dramatically improved the subtype selectivity without any loss of EP2 agonist activity. As a result of the preliminary modification, compound **8** was identified as an initial lead compound that is a highly selective EP2 agonist.

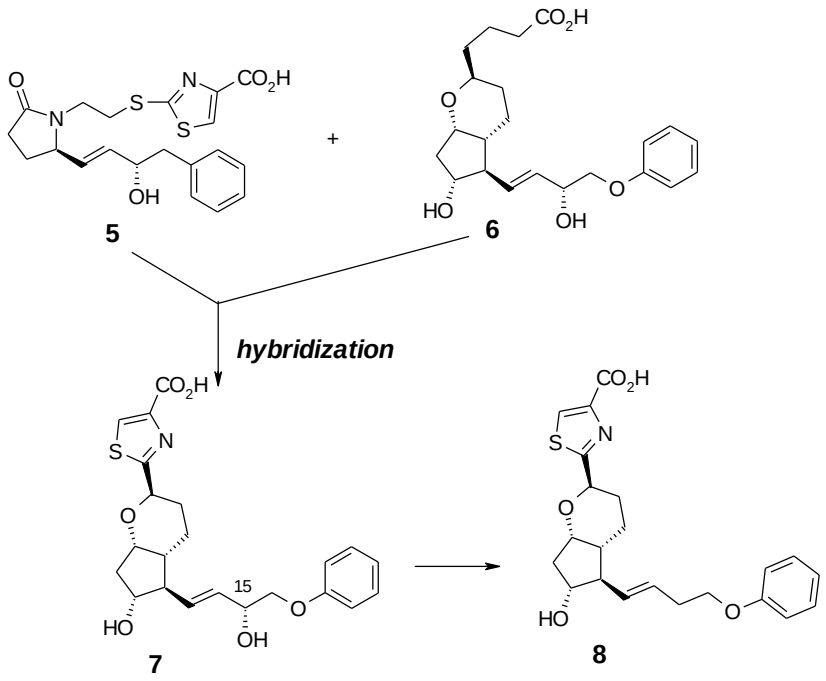


Figure 2-1-3. Design of novel EP2 agonists with unique bicyclic scaffold

cmpd	EC_{50}(nM)[a]					
	hEP1	hEP2	hEP3	hEP4	hFP	hIP
5	N.T.	5.6	3000	0.5	N.T.	N.T.
6	N.T.	8900	N.T.	4600	N.T.	47
7	1.4	7.9	0.8	33	32	11
8	160	3.9	260	1900	380	2500

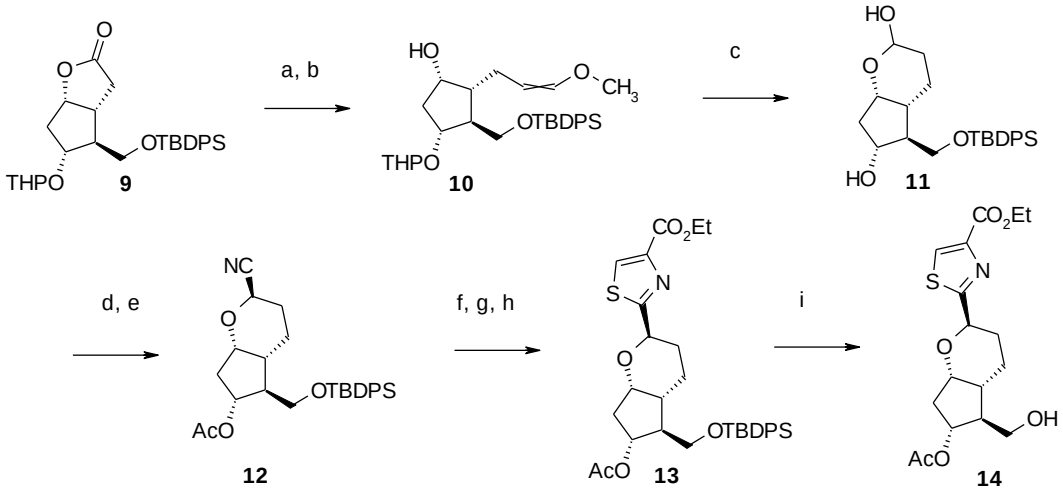
[a] Assay protocols are provided in the Supporting Information. EC_{50} values represent the mean of at two experiments.

Table 2-1-1. Subtype selectivity of initial lead compounds

Syntheses of bicyclic derivatives

All test compounds in Tables 2-1-1, 2 and 3 were synthesized as outlined in Schemes 2-1-1, 2 amd 3.

Synthesis of common intermediate **14** is outlined in Scheme 2-1-1. The protected Corey lactone **9** was reduced to a lactol by DIBAL, Wittig olefination of which afforded the vinyl ether **10**. The vinyl ether **10** was transformed to lactol **11** under acidic hydrolysis conditions. The lactol **11** was treated with acetic anhydride, and the resulting diacetate was transformed to **12** by introducing a cyano group in the presence of Lewis acid catalyst as a diastereomeric mixture (β/α ratio = 5/1). Thioamidation, condensation with bromopyruvate and cyclization by treatment with TFAA generated thiazole **13**. Deprotection of the silyl group with TBAF afforded the common intermediate **14**.

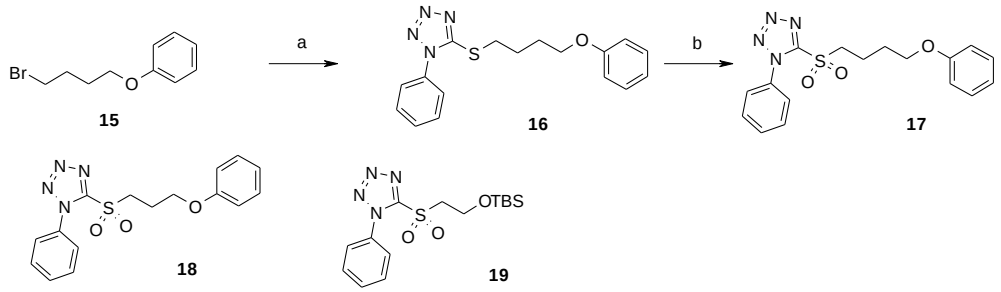


Reagents and conditions: (a) DIBAL, toluene, −78°C, (b) (methoxymethyl)(triphenyl)phosphonium chloride, KO*t*Bu, THF, −78°C, 86% (2 steps), (c) AcOH, THF, H_2O, 55°C, 63%, (d) Ac_2O, Py, rt, (e) TMSCN, $SnCl_4$, MeCN, (f) $(NH_4)_2S$, Py, 10°C, 55% (3 steps), (g) ethyl bromopyruvate, $KHCO_3$, DME, −25°C, (h) TFAA, Py, −25°C, 97% (2 steps), (i) TBAF, AcOH, THF, rt, 84%.

Scheme 2-1-1. Synthesis of common intermediate **14**

Julia-Kocienski reagent **17** was synthesized as outlined in Scheme 2-1-2. Commercially available halide **15** was treated with potassium carbonate and 1-phenyl-1*H*-tetrazole-5-thiol, and oxidation of the resulting sulfide

16 afforded compound **17**. Compounds **18** and **19** were synthesized in a similar manner using the corresponding halides.



Reagents and conditions: (a) 1-phenyl-1*H*-tetrazole-5-thiol, K_2CO_3, acetone, 60°C, 94%, (b) 30%H_2O_2aq., Na_2WO_4 $2H_2O$, $PhPO(OH)_2$, $(C_8H_{17})_3NMe$ HCl, rt, 71%

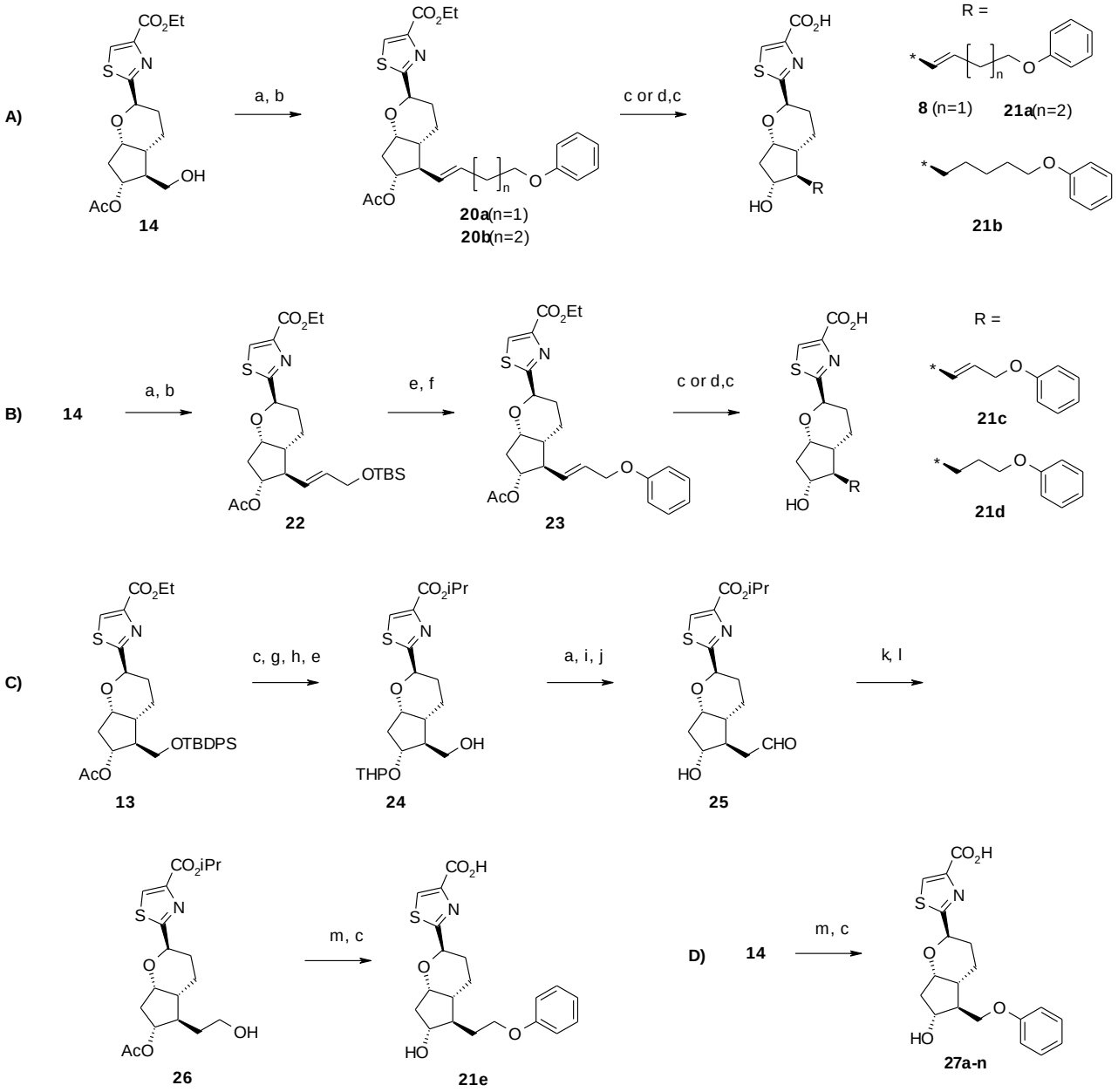
Scheme 2-1-2. Synthesis of Julia-Kocienski reagents **17–19**

Syntheses of compounds **8, 21a** and **21b** are outlined in Scheme 2-1-3A. Oxidation of the common intermediate **14**, followed by the Julia-Kocienski reaction with reagent **17** and **18** gave compound **20a** and **20b**, respectively. Hydrolysis of the both compounds provided compounds **8** and **21a**. Reduction of the double bond of **20b**, followed by hydrolysis gave compound **21b**.

Syntheses of compounds **21c** and **21d** are outlined in Scheme 2-1-3B. Oxidation of the common intermediate **14**, followed by the Julia-Kocienski reaction using reagent **19** gave compound **22**. Deprotection of the TBS group, followed by the Mitsunobu reaction gave compound **23**. Hydrolysis provided compound **21c**. Reduction of the double bond of **23** and hydrolysis gave compound **21d**.

Synthesis of compound **21e** is outlined in Scheme 2-1-3C. Hydrolysis of **13**, esterification and protection of the hydroxyl group by a THP moiety, followed by deprotection of the TBDPS gave alcohol **24**. The resulting alcohol **24** was treated with Dess-Martin reagent to give an aldehyde, which was transformed to a vinyl ether by treatment with a phosphoylide. Acidic hydrolysis of the vinyl ether gave compound **25**. Acetylation of the hydroxy group, followed by reduction of the aldehyde gave compound **26**. Introduction of a phenoxy group by the Mitsunobu reaction and hydrolysis provided compound **21e**.

Syntheses of compounds **27a–n** were started from commercially available phenols as outlined in Scheme 2-1-3D. Phenol was introduced into **14** by the Mitsunobu reaction, and the product was hydrolyzed under basic conditions to give **27a**. Compounds **27b–n** were synthesized in a similar manner using the corresponding phenols.



Reagents and conditions: (a) Dess-Martin periodinane, CH_2Cl_2, 0°C, 77%, (b) **17**, **18** or **19**, KHMDS, DME, 0°C, 37–66%, (c) 2 mol/L NaOHaq, DME, MeOH, 56–96%, (d) $TsNHNH_2$, NaOAc, EtOH, H_2O, 80°C, 55–71%, (e) TBAF, THF, rt, 96% (f) DEAD, Ph_3P, THF, rt, 82%, (g) *i*-PrI, K_2CO_3, DMF, rt, 54%, (h) PPTS,

CH_2Cl_2, DHP, (i) (methoxymethyl)triphenylphospine chrolide, KO*t*Bu, THF, rt, 64%, (j) TsOH, acetone, H_2O, rt, 78%, (k) Ac_2O, Py, 82%, (l) $NaBH_4$, THF, rt, 61% (m) phenol analogues, TMAD, Bu_3P, THF, rt, 61–92%

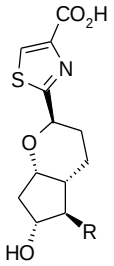
Scheme 2-1-3. Syntheses of compounds 8, 21a–e and 27a–n

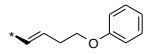
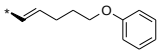
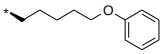
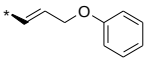
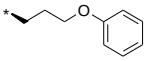
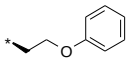
Optimization of omega chain and evaluation of functional selectivity

Chemical modification of the ω chain was performed to further improve subtype selectivity of the initial lead compound **8**. As described in Table 2-1-2, **21a–e** and **27a** were synthesized to adjust the length between the cyclopentane scaffold and the phenoxy moiety, and to investigate the effect of the double bond of the ω chain.

Compound **21a**, which has a longer linker relative to **8**, improved subtype selectivity to EP4 and FP receptors, while it showed a 3.3-fold decreased EP2 agonist activity. Conversely, **21c** with a shorter linker relative to **8** showed potent EP2 agonist activity and improved subtype selectivity. Reduction of the double bond of **21a** and **21c** gave **21b** and **21d** with 2.2 and 2.6-fold decreases in EP2 agonist activity, respectively. Compound **21e**, with a shorter linker relative to **21c**, showed the most potent EP2 agonist activity; however, it also had a potent EP1 agonist activity. The shortest ω chain derivative **27a** exhibited an excellent selectivity to all other receptor subtypes with favorable G protein activity.

The author next investigated the functional selectivity of the newly identified EP2 agonists **8**, **21a**–**e** and **27a**. The compounds were evaluated by the EP2-mediated β arrestin recruitment Path Hunter assay[30] (DiscoveRX), to determine their functional selectivity. Surprisingly, none of the compounds exerted full agonist activity toward β arrestin recruitment at 10 μM, that is, these compounds were identified as G protein-biased EP2 agonists (see Table 2-1-2). To the author's knowledge, these are the first examples of biased ligands of prostanoid receptors.



cmpd	R	hEP2				hEP1	hEP3	hEP4	hIP	hFP
		G protein (cAMP)		β arrestin						
		EC_{50}(nM)	E_{max}(%)	EC_{50}(nM)	E_{max}(%)	EC_{50}(nM)	EC_{50}(nM)	EC_{50}(nM)	EC_{50}(nM)	EC_{50}(nM)
8		3.9	98	>10,000	38	160	260	1900	2500	380
21a		13	91	>10,000	42	970	360	>10,000	>10,000	8600
21b		28	105	>10,000	35	N.T.	N.T.	N.T.	N.T.	N.T.
21c		3.8	96	>10,000	11	700	7600	>10,000	>10,000	>10,000
21d		10	107	>10,000	10	4200	5800	>10,000	>10,000	>10,000
21e		1.4	77	>10,000	22	97	1400	>10,000	>10,000	>10,000
27a		13	118	>10,000	28	>10,000	>10,000	>10,000	>10,000	>10,000

[a]Assay protocols are provided in the Supporting Information. EC_{50} values represent the mean of two experiments.

Table 2-1-2. Optimization of the ω chain for functional and subtype selectivity.

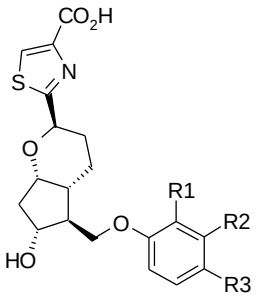
Structure functional selectivity relationship study of 27a

To investigate the structure–functional selectivity relationship[19] and improve G protein agonist activity, the author performed further optimization of compound **27a**. As demonstrated in Table 2-1-3 (**27b–d**), introduction of steric hindering substituents to the *ortho* position on the phenyl moiety improved the β arrestin activity, and the electron nature of the *ortho* substituents had a small effect on its functional selectivity. Introduction of 2-Cl substituent to the phenyl moiety afforded **27b**, which showed a 3.9-fold increase in G protein activity, and it dramatically increased β arrestin recruitment. Compound **27c**, which has a 2-CF_3 substituent on the phenyl moiety, also increased the G protein activity and showed a more than 900-fold

increase in β arrestin recruitment. Conversely, compound **27d**, which possesses a 2-F substituent, showed a partial G protein activity without any change in β arrestin recruitment.

As shown in Table 2-1-3 (**27e–h**), introduction of *meta* substituents into the phenyl moiety generally improved G protein activity. Additionally, steric hindrance of *meta* substituents on the phenyl moiety significantly affected the functional selectivity, that is, bulky substituents enhanced β arrestin recruitment. Compound **27e**, which possesses a 3-Me substituent on the phenyl moiety, was 3.6-fold more potent in G protein activity without an increase of β arrestin activity. Introduction of a 3-Cl substituent gave **27f**, which retained both G protein and β arrestin activity relative to **27a**. However, introduction of 3-OCF_3 and 3-CF_3 substituents gave **27g** and **27h**, respectively, both of which showed a 14-fold increase in G protein activity compared with **27a**. Additionally, **27g** and **27h** showed dramatically increased β arrestin activity (144-fold increase for **27g** and 1111-fold increase for **27h**). Compound **27i** with a 3-F substituent retained both G protein and β arrestin activity relative to **27a**.

Introduction of *para* substituents into the phenyl moiety had little effect on the functional selectivity, namely, all four substituent derivatives were found to be G protein-biased EP2 agonists (see Table 2-1-3, **27j–n**). Introduction of a 4-Me moiety (**27j**) slightly decreased G protein activity with no effect on β arrestin recruitment. 4-Cl derivative **27k** showed a 3.3-fold more potent G protein activity without an increase in β arrestin activity. Compound **27l**, possessing bulky substituents (OCF_3) at the *para* position, showed moderate G protein activity and very weak β arrestin recruitment. In contrast to the *ortho* or *meta* position, introduction of a CF_3 group into the *para* position of the phenyl moiety (**27m**) surprisingly lost the β arrestin activity. Compound **27n**, possessing a less hindered fluoride at the *para* position, showed similar profiles to **27a**



cmpd	R1	R2	R3	hEP2			
				G protein (cAMP)		β arrestin	
				EC_{50}(nM)	E_{max}(%)	EC_{50}(nM)	E_{max}(%)
27a	H	H	H	13	118	>10,000	28
27b	Cl	H	H	3.3	95	203	78
27c	CF_3	H	H	0.5	119	11	121
27d	F	H	H	23	59	>10,000	38
27e	H	Me	H	3.6	100	>10,000	38
27f	H	Cl	H	6.5	65	>10,000	27
27g	H	OCF_3	H	0.9	119	69	79
27h	H	CF_3	H	0.9	112	9	62
27i	H	F	H	5.4	94	>10,000	35
27j	H	H	Me	27	85	>10,000	20
27k	H	H	Cl	3.9	96	>10,000	12
27l	H	H	OCF_3	14	110	4500	57
27m	H	H	CF_3	13	103	>10,000	23
27n	H	H	F	7	99	>10,000	22

[a]Assay protocols are provided in the Supporting Information. EC_{50} values represent the mean of two experiments.

Table 2-1-3. Structure functional selectivity relationship study of phenoxy derivatives

Overall, steric hindrance of the *ortho* and *meta* positions on the phenyl moiety dramatically enhanced β arrestin recruitment and changed the functional selectivity, though the electron characteristics of the substituents did not show any significant difference in functional selectivity among the analogues. These structure activity relationship studies suggest that the functional selectivity is easily controlled by small chemical modifications of the phenyl moiety.

To confirm the G protein-biased agonism of our EP2 agonists, lead compound **27a** and **27k** which was the most potent G protein activity in *para* substituents derivatives were evaluated in an equimolar comparison[31] of G protein and β arrestin responses. Both compounds showed markedly less β arrestin activity with equivalent G protein activity relative to PGE_2, this result indicates **27a** and a series of compounds are G protein-biased agonists of EP2 receptor.

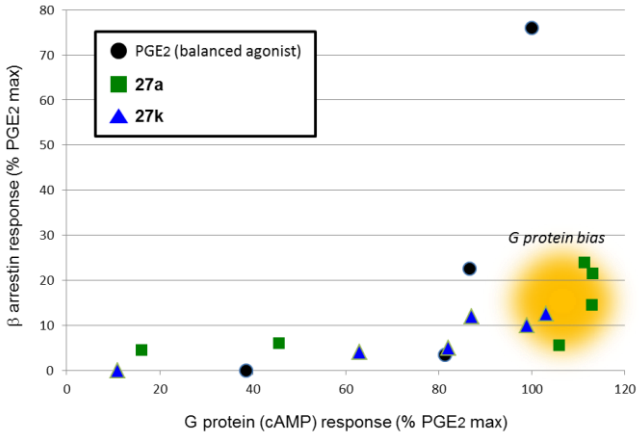


Figure 2-1-4. Equimolar comparison of G protein and β arrestin responses of PGE_2, **27a** and **27k**

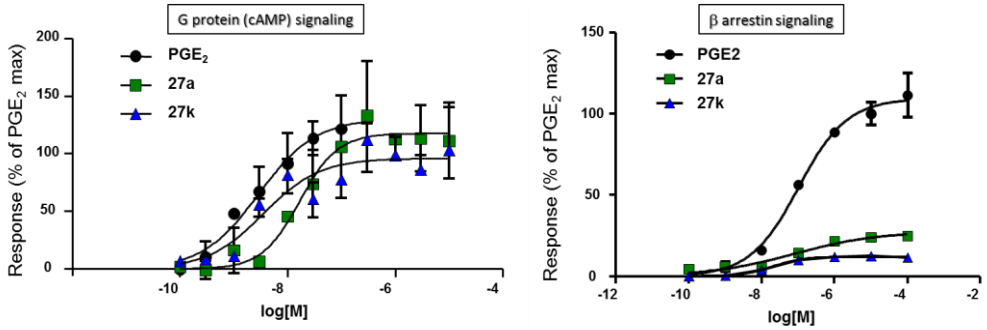


Figure 2-1-5. Concentration-response curves of PGE_2, **27a** and **27k**

In summary, the author designed a novel EP2 agonist **8** by hybridization of the thiazole moiety and the bicyclic scaffold mimicking prostacyclins. Simplification of the ω chain enabled the author to discover the highly selective EP2 phenoxy derivative **27a**, which was identified as a G protein-biased EP2 agonist. The substituents on the phenyl group of **27a** play an important role in modulating the functional selectivity.

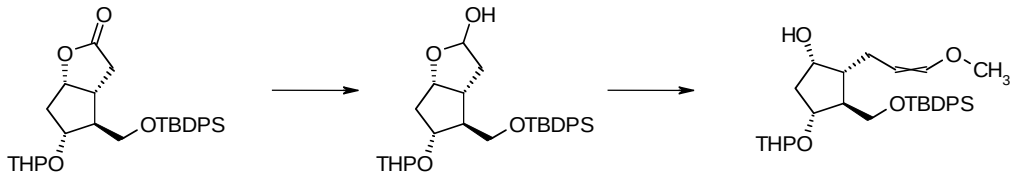
Experimental Section

General Experimental.

Analytical samples were homogeneous as confirmed by TLC, and spectroscopic results were consistent with the assigned structures. NMR spectra were recorded as designated on either a Varian Mercury 300 spectrometer or INOVA-500 spectrometer using deuterated chloroform ($CDCl_3$) or deuterated dimethyl sulfoxide (DMSO-d_6) as the solvent. Mass spectral analyses with fast atom bombardment (FABMS, HRMS) and electron ionization (EI) were performed on a JEOL JMS-DX303HF spectrometer. Purity analysis was carried out by the following LC/MS system. LC/MS: Waters ACQUITY UPLC system fitted by with Waters Micromass ZQ-2000 spectrometer. Column; YMC Triart C18 (2.0 mm × 30 mm). Eluting over 1.5 min with 5−95% acetonitrile(0.1% TFA) in water (0.1%TFA), flow rate of 1.0 mL/min, column temperature of 30 °C, detection with UV (PDA) and ELSD. Column chromatography was performed with silica gel [Merck Silica Gel 60 (0.063−0.200 μm), Wako gel C- 200, or Fuji Silysia PSQ-100B or Fuji Silysia FL60D]. Thin layer chromatography was performed with silica gel (Merck TLC or HPTLC plates, Silica Gel 60 F254). Medium-pressure preparative liquid chromatography was performed with a medium-pressure preparative liquid chromatograph W-prep 2XY (manufactured by Yamazen Corporation; column: main column size S-5L, inject column size SS-2L).

Scheme 2-1-1

(1*S*,2*R*,3*S*,4*R*)-2-(3-methoxy-2-propen-1-yl)-3-({[(2-methyl-2-propanyl)(diphenyl)silyl]oxy}methyl)-4-(tetrahydro-2*H*-pyran-2-yloxy)cyclopentanol (10)



9 **9-2** **10**

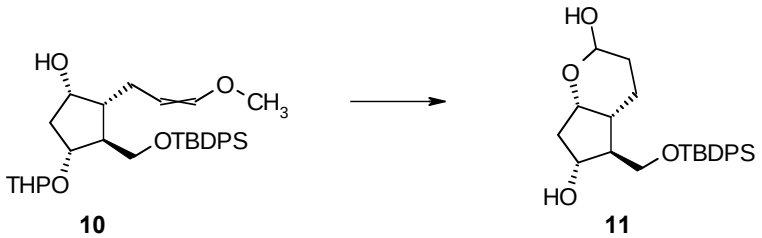
To a solution of **9** (422 g, 853 mmol) in toluene (1.50 L) at –78 °C was added diisobutylaluminium hydride (1.00 M in toluene, 995 mL, 995 mmol). After stirred at –78 °C for 1 h, potassium sodium tartrate (434 g,

1.54 mol) in H_2O (650 mL) was added. The reaction mixture was stirred at room temperature for 20 h and extracted with *tert*-BuOMe. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. Concentration gave **9-2** (462 g, crude), which was directly used in the next reaction.

To a suspension of 85% potassium *tert*-butoxide (298 g, 2.26 mol) in THF (2.30 L) was slowly added (methoxymethyl)triphenylphosphonium chloride (775 g, 2.26 mol). After the mixture was stirred at 0 °C for 30 min, **9-2** (456 g, crude) in THF (600 mL) was added. After stirred at 0 °C for 30 min, the reaction mixture was quenched with H_2O and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration gave crude mixture (1100 g), which was purified by recrystallization from IPA (400 mL) and hexane (400 mL) to remove triphenylphosphine oxide. After filtration of the phosphine oxide, the filtrate was concentrated, and flash column chromatography (Fuji silicia PSQ-100B, hexane/EtOAc 1:0-10:1-5:1-2:1) gave **10** (391 g) in 88% yield over 2 steps.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69-7.60 (m, 4H), 7.46-7.35 (m, 6H), 6.33 (m, 0.7H), 5.90 (m, 0.3H), 4.73 (m, 0.7H), 4.70-4.62 (m, 1H), 4.41 (m, 0.3H), 4.36-4.24 (m, 1H), 4.17-4.00 (m, 1H), 3.92-3.66 (m, 3H), 3.58 (s, 0.45H), 3.57 (s, 0.45H), 3.55-3.44 (m, 1H), 3.43 (s, 1.05 H), 3.42 (s, 1.05 H), 2.39-2.20 (m, 2H), 2.03 (m, 1H), 2.07-1.67 (m, 4H), 1.57-1.47 (m, 5H), 1.054 (s, 4.5H), 1.045 (s, 4.5H) (Peak of O*H* was not observed.).

(4a*R*,5*S*,6*R*,7a*S*)-5-({[(2-methyl-2-propanyl)(diphenyl)silyl]oxy}methyl)octahydrocyclopenta[*b*]pyran-2,6-diol (11)

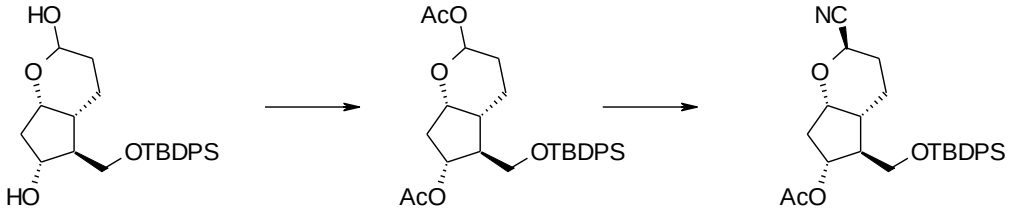


To a solution of **10** (410 g, 781 mmol) in THF (1.50 L) and H_2O (600 mL) was added acetic acid (1.20 L). After stirred at 55 °C for 3 h, the reaction mixture was extracted with toluene. The organic layer was washed with H_2O, 1.0 M hydrochloric acid and brine, dried over Na_2SO_4, and concentrated. The crude product (372 g) was purified by flash column chromatography (Fuji silicia PSQ-100B, hexane/EtOAc 1:0-4:1-2:1-1:3) to give

11 (211 g) in 63% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67-7.62 (m, 4H), 7.43-7.35 (m, 6H), 5.27 (m, 0.4H), 4.90 (m, 0.6H), 4.65 (m, 0.6H), 4.40 (m, 0.4H), 4.19-4.09 (m, 1H), 4.02 (m, 0.4H), 3.79 (dd, *J* = 9.9, 4.5 Hz, 1H), 3.65-3.60 (m, 1H), 3.54 (m, 0.6H), 2.92 (d, *J* = 6.0 Hz, 0.6H), 2.81 (d, *J* = 9.6 Hz, 0.4H), 2.74 (d, *J* = 9.6 Hz, 0.6H), 2.66 (d, *J* = 6.0 Hz, 0.4H), 2.15-2.00 (m, 3H), 1.87-1.72 (m, 3H), 1.63-1.46 (m, 1H), 1.05 (s, 9H).

(2*R*,4a*R*,5*S*,6*R*,7a*S*)-2-cyano-5-({[(2-methyl-2-propanyl)(diphenyl)silyl]oxy}methyl)octahydrocyclopenta[*b*]pyran-6-yl acetate (12)



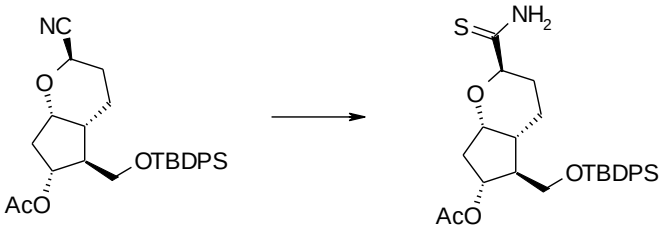
11 **11-2** **12**

To a solution of **11** (211 g, 494 mmol) in pyridine (900 mL) at 0 °C was added acetic anhydride (182 g, 1.78 mol). After stirred at room temperature for 14 h, the reaction mixture was diluted with toluene (500 mL) and H_2O (1.8 L) and extracted with toluene. The organic layer was washed with H_2O, 1.0 M hydrochloric acid, saturated aqueous $NaHCO_3$ and brine, successively, and dried over Na_2SO_4. Concentration gave **11-2** (268 g crude), which was directly used in the next reaction.

To a solution of **11-2** (268 g, crude) and trimethylsilyl cyanide (91.9 g, 889 mmol) in CH_3CN (1.40 L) at 0 °C was added $SnCl_4$ (1.0 M in CH_2Cl_2, 494 mL, 494 mmol). After stirred at 0 °C for 40 min, the reaction mixture was poured into a mixture of saturated aqueous $NaHCO_3$ and ice. The mixture was extracted with EtOAc. The organic layer was dried over Na_2SO_4. Concentration gave **12** (230 g) in 97% yield over 2 steps.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64-7.60 (m, 4H), 7.45-7.35 (m, 6H), 5.17 (m, 1H), 4.80 (m, 1H), 4.26 (m, 1H), 3.81 (dd, *J* = 10.8, 3.9 Hz, 1H), 3.63 (dd, *J* = 10.8, 4.2 Hz, 1H), 2.37 (m, 1H), 2.32 (m, 1H), 2.10-1.90 (m, 4H), 2.04 (s, 3H), 1.74-1.63 (m, 2H), 1.04 (s, 9H).

(2*R*,4a*R*,5*S*,6*R*,7a*S*)-2-carbamothioyl-5-({[(2-methyl-2-propanyl)(diphenyl)silyl]oxy}methyl)octahydrocyclopenta[*b*]pyran-6-yl acetate (12-2)

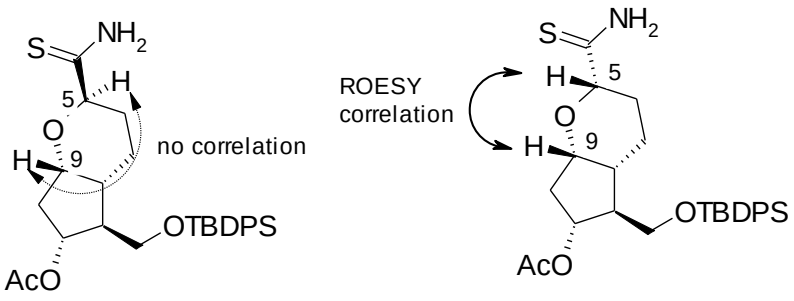


12 12-2

To a solution of **12** (230 g, 482 mmol) in pyridine (1.20 L) at 0 °C was added $S(NH_4)_2$ (20% in aqueous solution, 163 g, 440 mmol). After the mixture was stirred under 10 °C for 22 h, $S(NH_4)_2$ (20% in aqueous solution, 82 g, 240 mmol) was added, and the mixture stirred at 10 °C for 2 h. Ice (300 g) and H_2O (2.0 L) were added, and the mixture was extracted with toluene. The organic layer was washed with H_2O and brine. Concentration and flash column chromatography (Fuji silicia PSQ-100B, hexane/EtOAc 9:1-5:1-4:1-3:1-2:1) gave **12-2** (86.5 g) in 34% yield and diastereomeric mixture (107 g). **12-2** (71.5 g) and its diastereomeric mixture (167 g) were synthesized in the same procedure using **12** (249 g, 523 mmol). Further purification of diastereomeric mixtures (107 g and 167 g) by flash column chromatography (Fuji silicia PSQ-100B, hexane/EtOAc 9:1-5:1-4:1-3:1-2:1) gave **12-2** (124 g), total **12-2** (282g) in 55% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (m, 1H), 7.65-7.60 (m, 4H), 7.52 (m, 1H), 7.45-7.35 (m, 6H), 5.06 (m, 1H), 4.42 (dd, *J* = 7.8, 4.8 Hz, 1H), 4.22 (m, 1H), 3.73 (dd, *J* = 10.2, 4.8 Hz, 1H), 3.63 (dd, *J* = 10.2, 4.2 Hz, 1H), 2.38 (m, 1H), 2.26 (m, 1H), 2.09-2.01 (m, 2H), 2.03 (s, 3H), 1.96-1.84 (m, 4H), 1.05 (s, 9H).

The stereochemistry of C5 position was determined by 2D NMR (ROESY).

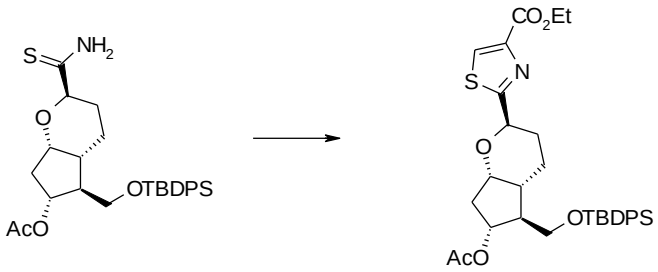


12-2 The diastereomer of 12-2

The diastereomer of **12-2** showed ROESY correlation between H5 and H9.

Ethyl 2-[(2*R*,4a*R*,5*S*,6*R*,7a*S*)-6-acetoxy-5-({[(2-methyl-2-propanyl)(diphenyl)silyl]

-oxy}methyl)octahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylate (13)



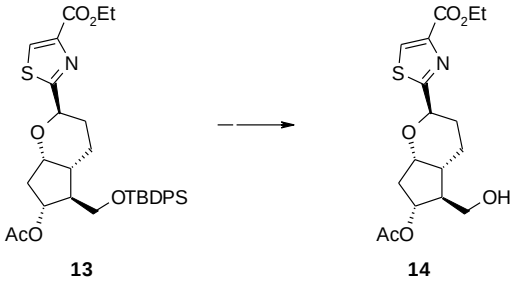
12-2 **13**

To a solution of **12-2** (129 g, 252 mmol) in 1,2-dimethoxyethane (1.10 L) at –25 °C was added $KHCO_3$ (202 g, 2.02 mol). Ethyl bromopyruvate (164 g, 756 mmol) was added at –25 °C, and the mixture was stirred for 4 h. Then, pyridine (160 g, 2.02 mol) and trifluoroacetic anhydride (212 g, 1.01 mol) were added at –25 °C for 35 min. After stirred at –25 °C for 30 min, the mixture was quenched with H_2O and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. Concentration and flash column chromatography (Fuji silicia BW-820MH, hexane/EtOAc 1:0-10:1-6:1-4:1-3:1) gave **13** (150 g) in 97% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.65-7.60 (m, 4H), 7.45-7.31 (m, 6H), 5.14 (t, *J* = 5.7 Hz, 1H), 5.12 (m, 1H), 4.41 (q, *J* = 7.2 Hz, 2H), 4.23 (m, 1H), 3.77 (dd, *J* = 10.2, 4.5 Hz, 1H), 3.65 (dd, *J* = 10.2, 4.8 Hz, 1H), 2.37 (m, 1H), 2.25 (m, 1H), 2.13 (m, 2H), 2.04 (s, 3H), 1.96-1.82 (m, 3H), 1.62-1.50 (m, 1H), 1.40 (t, *J* = 7.2 Hz, 3H), 1.04 (s, 9H).

Ethyl 2-[(2*R*,4a*R*,5*S*,6*R*,7a*S*)-6-acetoxy-5-(hydroxymethyl)octahydrocyclopenta[*b*]

-pyran-2-yl]-1,3-thiazole-4-carboxylate (14)



13 **14**

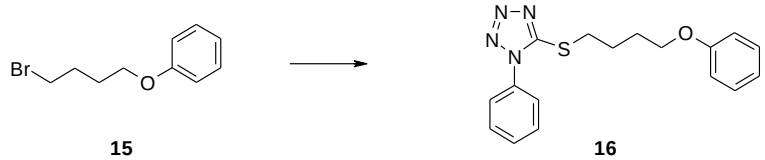
To a solution of **13** (150 g, 247 mmol) in THF (370 mL) and acetic acid (38.5 g, 642 mmol) at room temperature was added tetra-*n*-butylammonium fluoride (1.00 M in THF, 642 mL, 642 mmol). After stirred at

44 °C for 2 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. Concentration and flash column chromatography (Fuji silicia BW-820MH, hexane / EtOAc 1:1-1:2) gave alcohol **14** (77.3 g) in 84% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (s, 1H), 5.18 (t, *J* = 5.4 Hz, 1H), 5.02 (dt, *J* = 8.7, 4.8 Hz, 1H), 4.41 (q, *J* = 7.2 Hz, 2H), 4.23 (m, 1H), 3.70 (m, 1H), 3.61 (m, 1H), 2.70 (m, 1H), 2.35-2.14 (m, 3H), 2.12 (s, 3H), 2.06-1.98 (m, 2H), 1.79-1.61 (m, 3H), 1.40 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.33, 20.79, 21.37, 24.84, 37.14, 38.75, 50.63, 61.40, 62.63, 72.65, 73.49, 76.38, 128.07, 147.10, 161.42, 172.09, 173.52. MS (FAB, Pos.) *m/z* 370 $(M + H)^+$. HRMS (FAB, Pos.) $C_{17}H_{24}NO_6S$ $(M + H)^+$ calc. mass 370.1324, found 370.1331.

Scheme 2-1-2

5-[(4-phenoxybutyl)thio]-1-phenyl-1*H*-tetrazole (16)

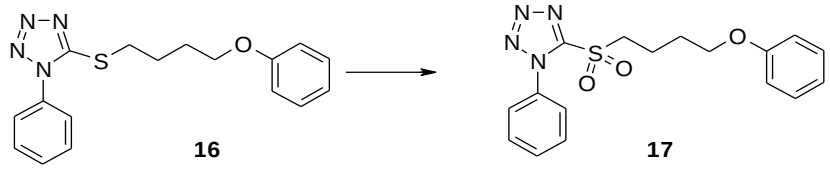


15 **16**

To a solution of **15** (3.00 g, 13.1 mmol) and 1-phenyl-1*H*-tetrazole-5-thiol (2.46 g, 13.8 mmol) in acetone (15.0 mL) at room temperature was added K_2CO_3 (1.90 g, 13.8 mmol). After stirred at 60 °C for 16 h, the reaction mixture was quenched with 1.0 M hydrochloric acid and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography L, hexane/EtOAc 9:1-2:1) gave **16** (4.01 g) in 94% yield.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.60-7.53 (m, 5H), 7.28 (d, *J* = 7.2 Hz, 2H), 6.94 (t, *J* = 7.2 Hz, 1H), 6.87 (d, *J* = 7.2 Hz, 2H), 4.00 (t, *J* = 6.0 Hz, 2H), 3.49 (t, *J* = 7.2 Hz, 2H), 2.05 (m, 2H), 1.95 (m, 2H).

5-[(4-phenoxybutyl)sulfonyl]-1-phenyl-1*H*-tetrazole (17)



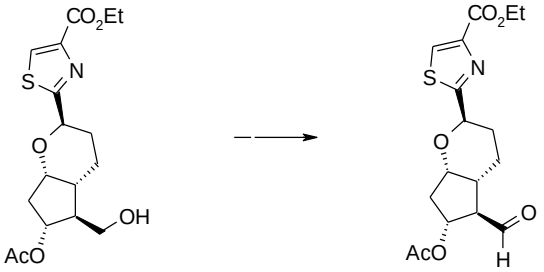
16 **17**

To a solution of **16** (2.00 g, 6.13 mmol) in CH_2Cl_2 (15.0 mL) at 0 °C was added 3-chloroperoxybenzoic acid (3.21 g, 12.1 mmol). After the mixture was stirred at room temperature for 2 h, 3-chloroperoxybenzoic acid (1.66 g, 6.1 mmol) was added. After stirred at room temperature for 2 h, the reaction mixture was quenched with saturated aqueous Na_2SO_3 and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and flash column chromatography (Yamazen W-prep 2XY flash column chromatography L, hexane/EtOAc 9:1-2:1) gave **17** (1.55 g) in 71% yield.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.71-7.68 (m, 2H), 7.64-7.58 (m, 3H), 7.29 (d, J = 6.9 Hz, 2H), 6.96 (t, J = 6.9 Hz, 1H), 6.87 (d, J = 6.9 Hz, 2H), 4.03 (t, J = 5.7 Hz, 2H), 3.87 (t, J = 6.0 Hz, 2H), 2.20 (m, 2H), 2.03 (m, 2H).

Scheme 2-1-3A

Ethyl 2-[(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-acetoxy-5-formyloctahydrocyclopenta[*b*]pyran -2-yl]-1,3-thiazole-4-carboxylate (14-2)



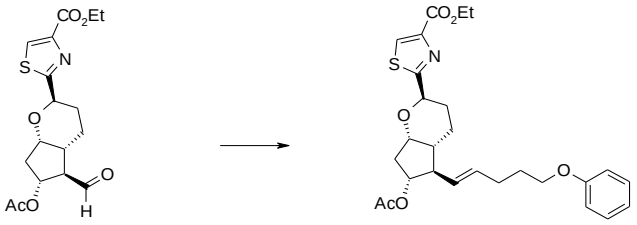
14 **14-2**

To a solution of **14** (3.00 g, 8.12 mmol) in CH_2Cl_2 (30 mL) at 0 °C was added Dess-Martin periodinane (4.00 g, 9.43 mmol). After stirred at room temperature for 2 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography, hexane/EtOAc 1:1-0:1) gave **14-2** (2.30 g) in 77% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.82 (d, J = 2.4 Hz, 1H), 8.17 (s, 1H), 5.29 (m, 1H), 5.18 (t, J = 5.1 Hz, 1H), 4.41 (q, J = 7.2 Hz, 2H), 4.20 (m, 1H), 3.02 (m, 1H), 2.37 (m, 1H), 2.25 (m, 2H), 2.17 (m, 1H), 2.10 (s, 3H), 2.10-2.00 (m, 2H), 1.72 (m, 1H), 1.40 (t, J = 7.2 Hz, 3H).

Ethyl 2-{(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-acetoxy-5-[(1*E*)-5-phenoxy-1-penten-1-yl]

-octahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylate (20b)



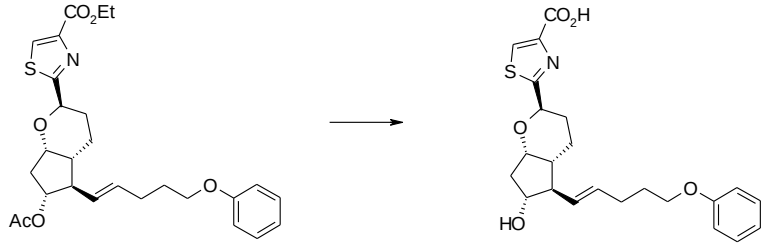
14-2 20b

To a solution of **17** (143 mg, 0.389 mmol) in 1,2-dimethoxyethane (2.0 mL) at –60 °C was added potassium bis(trimethylsilyl)amide (0.50 M in toluene, 0.80 mL, 0.40 mmol). After the mixture was stirred at –60 °C for 10 min, **14-2** (73.4 mg, 0.20 mmol) in 1,2-dimethoxyethane (1.0 mL) was added. After stirred at 0 °C for 30 min, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography M, hexane/EtOAc 9:1-3:1-7:3) gave **20b** (66.0 mg) in 34% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.31-7.24 (m, 2H), 6.93 (t, *J* = 7.2 Hz, 1H), 6.89 (d, *J* = 7.2 Hz, 2H), 5.58 (dt, *J* = 15.6, 6.3 Hz, 1H), 5.31 (dd, *J* = 15.6, 8.4 Hz, 1H), 5.17 (t, *J* = 5.1 Hz, 1H), 4.84 (m, 1H), 4.41 (q, *J* = 6.9 Hz, 1H), 4.13 (m, 1H), 3.96 (t, *J* = 6.6 Hz, 2H), 2.79 (m, 1H), 2.49 (m, 4H), 2.21 (m, 4H), 2.05 (s, 3H), 1.86 (m, 3H), 1.71 (m, 1H), 1.40 (t, *J* = 6.9 Hz, 3H).

2-{(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-hydroxy-5-[(1*E*)-5-phenoxy-1-penten-1-yl]octahydrocyclopenta[b]pyran-2-yl}-1,

3-thiazole-4-carboxylic acid (21a)



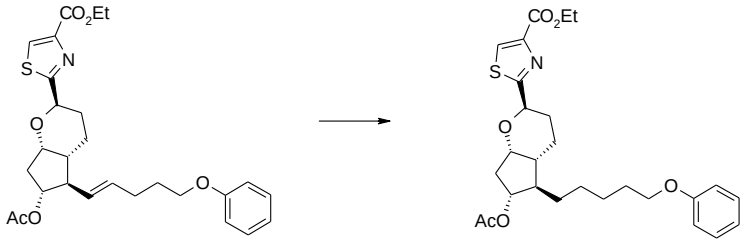
20b **21a**

To a solution of **20b** (30 mg, 0.060 mmol) in 1,2-dimethoxyethane (0.50 mL) and ethanol (0.50 mL) at room temperature was added 1.0 M sodium hydroxide (0.50 mL, 0.50 mmol). After stirred at room temperature for

3 h, the reaction mixture was extracted with *tert*-BuOMe. The aqueous layer was acidified by 1.0 M hydrochloric acid and extracted with EtOAc. The EtOAc layer was washed with brine and dried over $MgSO_4$. Concentration gave **21a** (23.2 mg) in 90% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.27 (t, *J* = 7.5 Hz, 2H), 6.92 (t, *J* = 7.5 Hz, 1H), 6.88 (d, *J* = 7.5 Hz, 2H), 5.62 (dt, *J* = 15.0, 5.4 Hz, 1H), 5.30 (dd, *J* = 15.0, 8.4 Hz, 1H), 5.17 (t, *J* = 4.5 Hz, 1H), 4.13-4.08 (m, 1H), 3.97 (t, *J* = 6.3 Hz, 2H), 3.92-3.86 (m, 1H), 2.61-2.51 (m, 1H), 2.32-2.19 (m, 5H), 1.92-1.85 (m, 3H), 1.83-1.76 (m, 1H), 1.59-1.53 (m, 2H), (Peaks of O*H* and CO_2H were not observed.). LCMS (ELSD) *RT* = 0.94 min (97.3%). MS (FAB, Pos.) *m/z* 430 $(M + H)^+$. HRMS (FAB, Pos.) $C_{23}H_{28}NO_5S$ $(M + H)^+$ calc. mass 430.1688, found 430.1691.

Ethyl 2-[(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-acetoxy-5-(5-phenoxypentyl)octahydrocyclopenta -[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylate (20c)

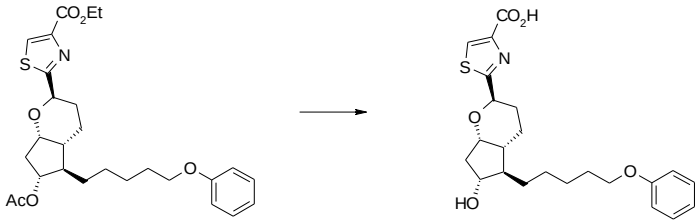


20b **20c**

To a solution of **20b** (32.0 mg, 0.064 mmol) and sodium acetate (105 mg, 1.28 mmol) in EtOH (0.50 mL) and H_2O (1.00 mL) at room temperature was added *p*-toluenesulfonyl hydrazide (119 mg, 0.64 mmol). After the mixture was stirred at 80 °C for 14.5 h, H_2O was added and the reaction mixture was extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. Concentration and preparative thin layer chromatography gave **20c** (17.7 mg) in 55% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.27 (t, *J* = 7.2 Hz, 2H), 6.92 (t, *J* = 7.2 Hz, 1H), 6.87 (d, *J* = 7.2 Hz, 2H), 5.13 (t, *J* = 6.0 Hz, 1H), 4.80 (m, 1H), 4.40 (q, *J* = 6.9 Hz, 2H), 4.21 (m, 1H), 3.95 (t, *J* = 6.6 Hz, 2H), 2.39 (m, 1H), 2.23 (m, 1H), 2.15-1.90 (m, 4H), 2.06 (s, 3H), 1.87-1.74 (m, 4H), 1.75-1.69 (m, 2H), 1.58-1.38 (m, 4H), 1.40 (t, *J* = 6.9 Hz, 3H).

2-[(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-hydroxy-5-(5-phenoxypentyl)octahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylic acid (21b)



To a solution of **20c** (17.5 mg, 0.035 mmol) in 1,2-dimethoxyethane (0.50 mL) and EtOH (0.50 mL) at room temperature was added 1.0 M sodium hydroxide (0.50 mL, 0.50 mmol). After stirred at room temperature for 2 h, the reaction mixture was extracted with *tert*-BuOMe. The aqueous layer was acidified by 1.0 M hydrochloric acid and extracted with EtOAc. The EtOAc layer was washed with brine and dried over $MgSO_4$. Concentration and column chromatography (Wakogel, chloroform/MeOH 1:0-95:5) gave **21b** (8.4 mg) in 56% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.26 (m, 2H), 6.92 (m, 1H), 6.88 (d, *J* = 7.8 Hz, 2H), 5.17 (t, *J* = 5.1 Hz, 1H), 4.15 (m, 1H), 3.96 (t, *J* = 6.3 Hz, 2H), 3.91 (m, 1H), 2.28-2.21 (m, 2H), 2.06-1.91 (m, 4H), 1.81 (m, 2H), 1.67 (m, 1H), 1.55-1.31 (m, 7H) (Peaks of O*H* and CO_2H were not observed.). LCMS (ELSD) *RT* = 0.99 min (>98%). MS (FAB, Neg.) *m/z* 430 $(M - H)^-$. HRMS (FAB, Neg.) $C_{23}H_{28}NO_5S$ $(M - H)^-$ calc. mass 430.1688, found 430.1691.

8 was synthesized in a similar manner by using Julia Kocienski reagent **19**.

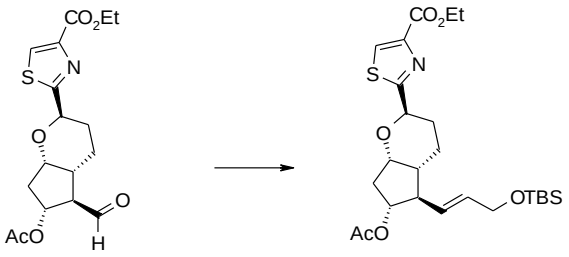
2-{(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-hydroxy-5-[(1*E*)-4-phenoxy-1-buten-1-yl]octahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (8)

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.27 (d, *J* = 7.2 Hz, 2H), 6.93 (t, *J* = 7.2 Hz, 1H), 6.87 (d, *J* = 7.2 Hz, 2H), 5.66 (dt, *J* = 15.0, 6.9 Hz, 1H), 5.41 (dd, *J* = 15.0, 8.7 Hz, 1H), 5.18 (t, *J* = 4.8 Hz, 1H), 4.13 (m, 1H), 4.00 (t, *J* = 6.9 Hz, 2H), 3.99 (m, 1H), 2.64-2.49 (m, 3H), 2.34-2.18 (m, 3H), 1.94 (m, 1H), 1.81 (m, 1H), 1.61 (m, 2H) (Peaks of O*H* and CO_2H were not observed.). LCMS (ELSD) *RT* = 0.89 min (>98%). MS (FAB, Pos.) *m/z* 416 $(M + H)^+$. HRMS (FAB, Pos.) $C_{22}H_{26}NO_5S$ $(M + H)^+$ calc. mass 416.1532, found 416.1531.

Scheme 2-1-3B

Ethyl 2-{(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-acetoxy-5-[(1*E*)-3-{[dimethyl(2-methyl-2-propanyl)

-silyl]oxy}-1-propen-1-yl]octahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylate (22)



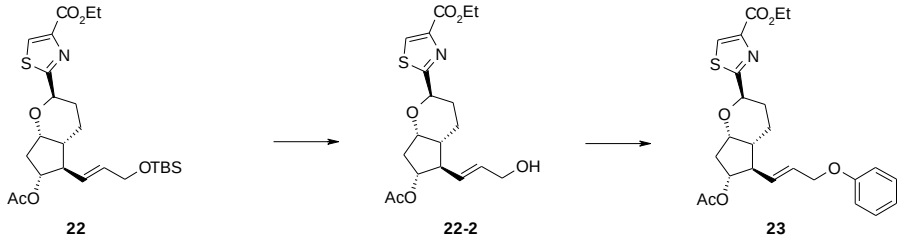
14-2 22

To a solution of **21** (501 mg, 1.36 mmol) and **14-2** (252 mg, 0.689 mmol) in 1,2-dimethoxyethane (6.8 mL) at –60 °C was added potassium bis(trimethylsilyl)amide (0.50 M in toluene, 2.04 ml, 1.02 mmol) slowly. After stirred at –60 °C to –30 °C for 1 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography L, hexane/EtOAc 97:3-85:15-4:1) gave **22** (128 mg) in 18%.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (s, 1H), 5.69 (dt, *J* = 15.3, 4.8 Hz, 1H), 5.51 (dd, *J* = 15.3, 8.7 Hz, 1H), 5.18 (t, *J* = 5.1 Hz, 1H), 4.86 (m, 1H), 4.41 (q, *J* = 7.2 Hz, 2H), 4.17 (m, 2H), 2.86 (m, 1H), 2.52 (m, 1H), 2.24 (m, 2H), 2.06 (s, 3H), 1.93 (m, 1H), 1.70 (m, 1H), 1.64-1.57 (m, 3H), 1.40 (t, *J* = 7.2 Hz, 3H), 0.90 (s, 9H), 0.07 (s, 6H).

Ethyl 2-{(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-acetoxy-5-[(1*E*)-3-phenoxy-1-propen-1-yl]

-octahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylate (23)



22 22-2 23

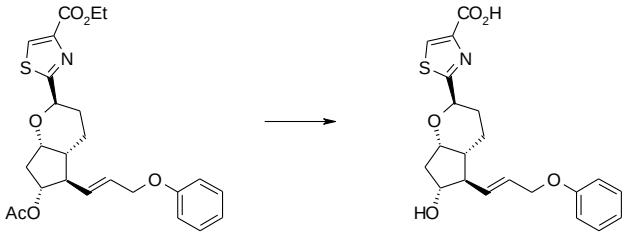
To a solution of **22** (125 mg, 0.245 mmol) in THF (1.5 mL) at 0 °C was added tetra-*n*-butylammonium fluoride (1.0 M in THF, 0.38 mL, 0.38 mmol). After stirred at room temperature for 2.5 h, the reaction mixture was

quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and flash a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography M, hexane/EtOAc 1:1-3:7) gave **22-2**, which was directly used in the next reaction.

To a solution of **22-2**, phenol (31.1 mg, 0.330 mmol) and triphenyl phosphine (86.6 mg, 0.430 mmol) in THF (1.0 mL) at room temperature was added diethyl azodicarboxylate (2.2 M in toluene, 150 μL, 0.430 mmol). After stirred at room temperature for 1 h, the reaction mixture was concentrated. A medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography M, hexane/EtOAc 9:1-3:1-7:3) gave **23** (97.3 mg) in 84% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.27-7.24 (m, 2H), 6.93 (t, *J* = 7.2 Hz, 1H), 6.89 (d, *J* = 7.2 Hz, 2H), 5.81 (dt, *J* = 15.0, 5.4 Hz, 1H), 5.68 (dd, *J* = 15.0, 8.1 Hz, 1H), 5.18 (t, *J* = 5.1 Hz, 1H), 4.89 (m, 1H), 4.51 (d, *J* = 4.5 Hz, 2H), 4.40 (q, *J* = 6.9 Hz, 2H), 4.16 (m, 1H), 2.90 (m, 1H), 2.51 (m, 1H), 2.24 (m, 2H), 2.06 (s, 3H), 1.94 (m, 1H), 1.72 (m, 1H), 1.66-1.54 (m, 2H), 1.40 (t, *J* = 6.9 Hz, 3H).

2-{(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-hydroxy-5-[(1*E*)-3-phenoxy-1-propen-1-yl]octahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (21c)



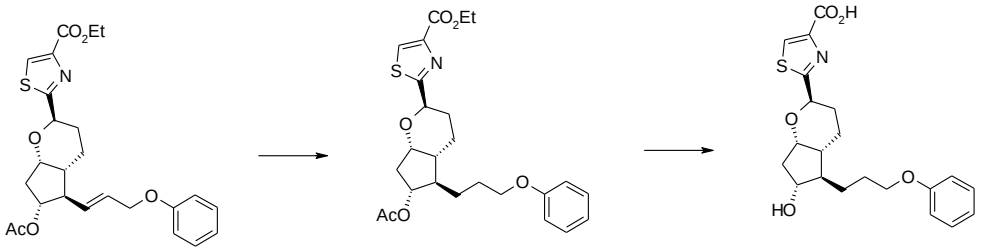
23 21c

To a solution of **23** (95.0 mg, 0.201 mmol) in 1,2-dimethoxyethane (1.50 mL) and EtOH (1.50 mL) at room temperature was added 1.0 M sodium hydroxide (1.50 mL, 1.50 mmol). After stirred at room temperature for 2 h, the reaction mixture was extracted with *tert*-BuOMe. The aqueous layer was acidified by 1.0 M hydrochloric acid and extracted with EtOAc. The EtOAc layer was washed with brine and dried over $MgSO_4$. Concentration gave **21c** (56.6 mg) in 70% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.29 (m, 2H), 6.93 (m, 3H), 5.87 (dt, *J* = 15.3, 5.7 Hz, 1H), 5.70

(dd, *J* = 15.3, 8.4 Hz, 1H), 5.19 (t, *J* = 4.8 Hz, 1H), 4.54 (d, *J* = 4.5 Hz, 2H), 4.13 (m, 1H), 4.00 (m, 1H), 2.69 (m, 1H), 2.31 (m, 1H), 2.26 (m, 2H), 1.95 (m, 1H), 1.85 (m, 1H), 1.69-1.58 (m, 2H) (Peaks of O*H* and CO_2H were not observed.). LCMS (ELSD) *RT* = 0.85 min (>98%). MS (EI, Pos.) *m/z* 401 $(M)^+$. HRMS (EI, Pos.) $C_{21}H_{23}NO5S$ $(M)^+$ calc. mass 401.1297, found 401.1292.

2-[(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-hydroxy-5-(3-phenoxypropyl)octahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylic acid (21d)



23 **23-2** **21d**

To a solution of **23** (75.0 mg, 0.159 mmol) and AcONa (262 mg, 3.20 mmol) in EtOH (1.0 mL) and H_2O (2.0 mL) at room temperature was added *p*-toluenesulphonyl hydrazine (298 mg, 1.60 mmol). After the reaction mixture was stirred at 80 °C for 3 days, H_2O was added and the reaction mixture was extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography M, hexane/EtOAc 92:8-3:1-65:35) gave **23-2** (53.7 mg) in 71% yield.

To a solution of **23-2** (51.0 mg, 0.108 mmol) in 1,2-dimethoxyethane (1.00 mL) and EtOH (1.00 mL) at room temperature was added 1.0 M sodium hydroxide (1.00 mL, 1.00 mmol). After stirred at room temperature for 2 h, the reaction mixture was extracted with *tert*-BuOMe. The aqueous layer was acidified by 1.0 M hydrochloric acid and extracted with EtOAc. The EtOAc layer was washed with brine and dried over $MgSO_4$. Concentration gave **21d** (40.4 mg) in 93% yield.

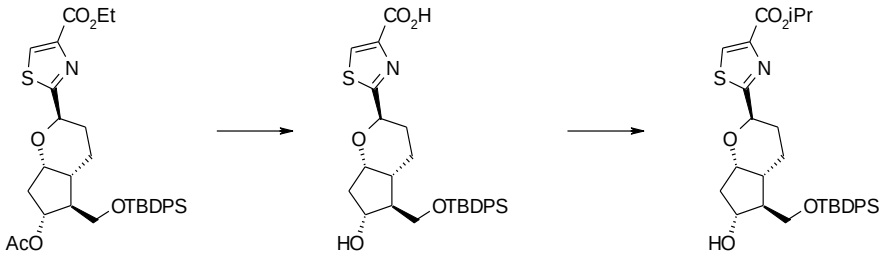
^1H NMR (300 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.27 (t, *J* = 7.5 Hz, 2H), 6.93 (t, *J* = 7.5 Hz, 1H), 6.89 (d, *J* = 7.5 Hz, 2H), 5.17 (t, *J* =4.8 Hz, 1H), 4.16 (m, 1H), 4.00 (t, *J* = 6.3 Hz, 2H), 3.95 (m, 1H), 2.32-2.15 (m, 2H), 2.07-1.90 (m, 6H), 1.76-1.43 (m, 4H) (Peaks of O*H* and CO_2H were not observed.). LCMS (ELSD) *RT* = 0.89 min (>98%). 1.5 min. MS (FAB, Neg.) *m/z* 402 $(M - H)^-$. HRMS (FAB, Neg.) $C_{21}H_{24}NO_5S$ $(M - H)^-$ calc. mass

402.1375, found 402.1372.

Scheme 2-1-3C

Isopropyl 2-[(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-hydroxy-5-({[(2-methyl-2-propanyl)(diphenyl)-silyl]oxy}methyl)octahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylate

(13-3)



13 13-2 13-3

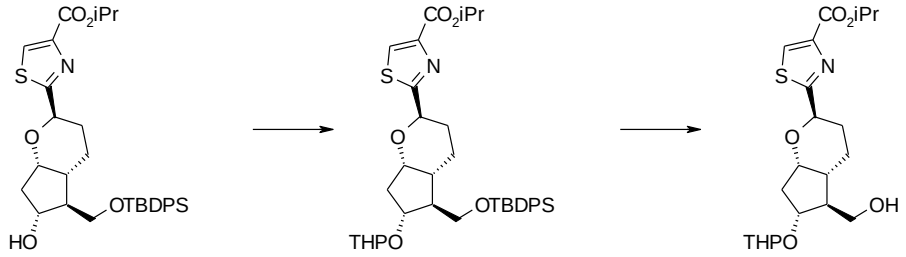
To a solution of **13** (44.2 g, 72.8 mmol) in MeOH (900 mL) at room temperature was added 1.0M sodium hydroxide (180 mL, 180 mmol). After stirred at room temperature for 2 h, the reaction mixture was evaporated. The residue was dissolved in THF (400 mL), 1 M hydrochloric acid (210 mL) and EtOAc (400 mL). The mixture was extracted with EtOAc and the organic layer was washed with brine and dried over Na_2SO_4. Concentration gave **13-2** (40.5 g, crude), which was directly used in the next reaction.

To a solution of **13-2** (40.5 g, crude) and isopropyl iodide (24.7 g, 145 mmol) in DMF (190 mL) at room temperature was added K_2CO_3 (20.1 g, 145 mmol). After the reaction mixture was stirred at 50 ℃ for 14 h, H_2O (200 mL) was added and the mixture was extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and flash column chromatography (Fuji silicia BW-820MH, hexane/EtOAc 4:1-3:1-2:1) gave **13-3** (23.1 g) in 54% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.66-7.61 (m, 4H), 7.43-7.34 (m, 6H), 5.26 (sep, *J* = 6.3 Hz, 1H), 5.16 (t, *J* = 5.4 Hz, 1H), 4.20-4.12 (m, 2H), 3.78 (dd, *J* = 9.9, 4.5 Hz, 1H), 3.61 (dd, *J* = 9.9, 6.3 Hz, 1H), 2.63 (d, *J* = 7.5 Hz, 1H), 2.24-2.06 (m, 4H), 1.95 (m, 1H), 1.90 (m, 1H), 1.79-1.74 (m, 1H), 1.55-1.48 (m, 1H), 1.37 (d, *J* = 6.3 Hz, 6H), 1.04 (s, 9H).

Isopropyl 2-[(2*R*,4a*R*,5*R*,6*R*,7a*S*)-5-(hydroxymethyl)-6-(tetrahydro-2*H*-pyran-2-

yloxy)octahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylate (24)

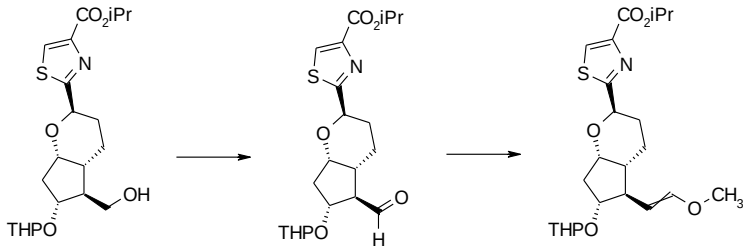


To a solution of **13-3** (54.8 g, 94.5 mmol) and pyridinium *para*-toluenesulfonate (2.30 g, 9.45 mmol) in CH_2Cl_2 (220 mL) at room temperature was added 3,4-dihydro-2*H*-pyran (15.9 g, 189 mmol). After stirred at room temperature for 14 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. Concentration gave **13-4** (64.0 g, crude), which was directly used in the next reaction.

To a solution of **13-4** (64.0 g, crude) in THF (160 mL) at room temperature was added tetra-*n*-butylammonium fluoride (1.00 M in THF, 240 mL, 240 mmol). After stirred at room temperature for 2.5 h, the reaction mixture was evaporated. The residue was dissolved in H_2O (200 mL) and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and flash column chromatography (Fuji silicia BW-820MH, hexane/EtOAc 1:1-1:2) gave **24** (38.8 g) in 96% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (s, 1H), 5.27 (sep, *J* = 6.3 Hz, 1H), 5.14 (t, *J* = 6.3 Hz, 1H), 4.76 (m, 0.5H), 4.62 (m, 0.5H), 4.24-4.02 (m, 2H), 3.96-3.86 (m, 2H), 3.77 (dd, *J* = 10.8, 4.5 Hz, 0.5H), 3.71 (dd, *J* = 10.8, 5.7 Hz, 0.5H), 3.62-3.48 (m, 2H), 2.38-2.23 (m, 3H), 2.19-1.92 (m, 4H), 1.92-1.48 (m, 7H), 1.37 (d, *J* = 6.3 Hz, 6H).

Isopropyl 2-[(2*R*,4a*R*,5*R*,6*R*,7a*S*)-5-(2-methoxyvinyl)-6-(tetrahydro-2*H*-pyran-2-yloxy)octahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylate (24-3)



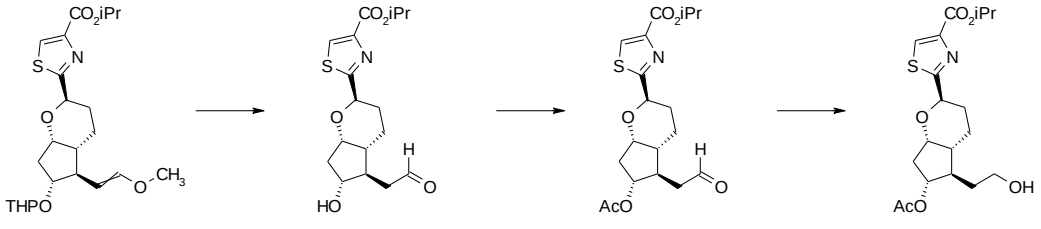
24 24-2 24-3

To a solution of **24** (200 mg, 0.470 mmol) in CH_2Cl_2 (3.0 mL) at 0 °C was added Dess-Martin periodinane (259 mg, 0.611 mmol). After stirred at room temperature for 1 h, the reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$ and extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine and dried over Na_2SO_4. Concentration gave **24-2**, which was directly used in next reaction.

To a suspension of 85%-potassium *tert*-butoxide (79.1 mg, 0.705 mol) in THF (4.70 mL) at 0 °C was slowly added (methoxymethyl)triphenylphosphonium chloride (242 mg, 0.705 mol). After the mixture was stirred at 0 °C for 30 min, **24-2** in THF (1.4 mL) was added and the mixture was stirred at room temperature for 2 h. The reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography L, hexane/EtOAc 9:1-7:3) gave **24-3** (137 mg) in 64% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (s, 1H), 6.40-6.36 (m, 0.8H), 6.03-5.96 (m, 0.2H), 5.26 (sep, *J* = 6.3 Hz, 1H), 5.14 (t, *J* = 5.4 Hz, 1H), 4.71 (m, 1H), 4.58 (m, 1H), 4.16 (m, 1H), 3.95-3.78 (m, 2H), 3.53 (brs, 3H), 3.48 (m, 1H), 2.58 (m, 1H), 2.37 (m, 1H), 2.18 (m, 2H), 1.94-1.81 (m, 3H), 1.74-1.45 (m, 7H), 1.37 (d, *J* = 6.3 Hz, 6H).

Isopropyl 2-[(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-acetoxy-5-(2-hydroxyethyl)octahydro -cyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylate (26)



24-3 25 25-2 26

To a solution of **24-3** (120 mg, 0.266 mmol) in acetone (4.95 mL) and H_2O (50 μL) at room temperature was added *p*-toluene sulfonic acid monohydrate (15.1 mg, 0.0795 mmol). After stirred at room temperature for 3 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography L, hexane/EtOAc 3:2-1:3-0:100) gave **25** (73.0 mg) in 78% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.85 (t, *J* = 1.2 Hz, 1H), 8.12 (s, 1H), 5.26 (sep, *J* = 6.3 Hz, 1H), 5.18 (t, *J* = 5.1 Hz, 1H), 4.14 (m, 1H), 3.89 (m, 1H), 3.08 (d, *J* = 5.1 Hz, 1H), 2.75 (m, 1H), 2.54 (m, 1H), 2.45 (m, 1H), 2.29-2.16 (m, 3H), 2.04 (m, 1H), 1.93 (m, 1H), 1.57 (m, 1H), 1.37 (d, *J* = 6.3 Hz, 6H) (Peak of O*H* was not observed.).

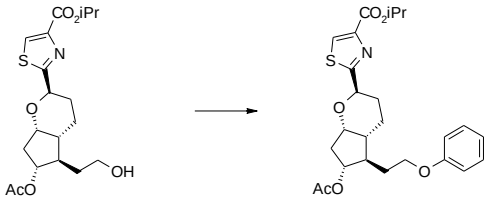
To a solution of **25** (70.0 mg, 0.198 mmol) in pyridine (1.50 mL) at 0 °C was added acetic anhydride (37.4 μL, 0.396 mmol). After stirred at room temperature for 2 h, the reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography L, hexane/EtOAc 3:1-1:1) gave **25-2** (64.0 mg) in 82% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.79 (t, *J* = 1.2 Hz, 1H), 8.12 (s, 1H), 5.27 (sep, *J* = 6.0 Hz, 1H), 5.17 (t, *J* = 5.1 Hz, 1H), 4.84 (m, 1H), 4.19 (m, 1H), 2.68-2.56 (m, 2H), 2.45 (m, 1H), 2.26 (m, 1H), 2.18-1.97 (m, 3H), 2.07 (s, 3H), 1.86 (m, 1H), 1.70-1.53 (m, 2H), 1.37 (d, *J* = 6.0 Hz, 6H).

To a solution of **25-2** (62.0 mg, 0.157 mmol) in THF (1.50 mL) at 0 °C was added $NaBH_4$ (7.1 mg, 0.188 mmol). After stirred at room temperature for 1 h, the reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography L, hexane/EtOAc 3:2-3:7) gave **26** (38.1 mg) in 61% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (s, 1H), 5.26 (sep, *J* = 6.3 Hz, 1H), 5.14 (t, *J* = 5.7 Hz, 1H), 4.86 (m, 1H), 4.24 (m, 1H), 3.73 (m, 2H), 2.42 (m, 1H), 2.23 (m, 2H), 2.14-2.01 (m, 2H), 2.09 (s, 3H), 1.97 (m, 1H), 1.86 (m, 1H), 1.75 (m, 1H), 1.63-1.55 (m, 2H), 1.37 (d, *J* = 6.3 Hz, 6H) (Peak of O*H* was not observed.).

Isopropyl 2-[(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-acetoxy-5-(2-phenoxyethyl)octahydro -cyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylate (26-2)

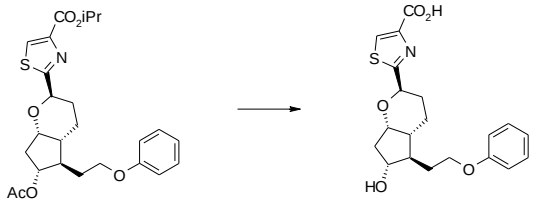


26 26-2

To a solution of **26** (35.1 mg, 0.0883 mmol), phenol (11.3 mg, 0.120 mmol) and 1,1'-azobis(*N*,*N*-dimethylformamide) (31.7 mg, 0.184 mmol) in THF (0.90 mL) at room temperature was added tributylphosphine (45.4 μL, 0.184 mmol). After stirred at room temperature for 16 h, the mixture was concentrated. A medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography M, hexane/EtOAc 9:1-2:3) gave **26-2** (26.4 mg) in 63% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.30-7.24 (m, 2H), 6.93 (t, *J* = 7.5 Hz, 1H), 6.87 (d, *J* = 7.5 Hz, 2H), 5.27 (sep, *J* = 6.6 Hz, 1H), 5.16 (t, *J* = 5.4 Hz, 1H), 4.93 (m, 1H), 4.20 (m, 1H), 4.06-3.97 (m, 2H), 2.50-2.36 (m, 2H), 2.26 (m, 1H), 2.17 (m, 1H), 2.05 (s, 3H), 2.05-1.92 (m, 2H), 1.83 (m, 1H), 1.75 (m, 1H), 1.72-1.64 (m, 2H, 1.37 (d, *J* = 6.6 Hz, 6H).

2-[(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-hydroxy-5-(2-phenoxyethyl)octahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-ca rboxylic acid (21e)



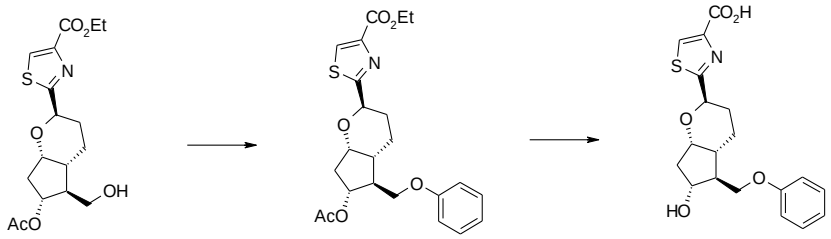
26-2 21e

To a solution of **26-2** (25.0 mg, 0.0528 mmol) in MeOH (1.0 mL) at room temperature was added 2.0 M sodium hydroxide (0.14 mL, 0.28 mmol). After stirred at room temperature for 1.5 h, the reaction mixture was extracted with *tert*-BuOMe. The aqueous layer was acidified by 1.0 M hydrochloric acid and extracted with EtOAc. The EtOAc layer was washed with brine and dried over $MgSO_4$. Concentration gave **21e** (17.5 mg) in 85% yield.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.42 (s, 1H), 7.27 (dd, *J* = 8.7, 7.2 Hz, 2H), 6.90 (m, 3H), 5.06 (t, *J* = 6.6 Hz, 1H), 4.81 (d, *J* = 5.4 Hz, 1H), 4.07 (m, 3H), 3.66 (m, 1H), 2.14 (m, 2H), 1.81 (m, 5H), 1.64 (m, 1H), 1.56 (m, 2H) (Peak of CO_2H was not observed.). LCMS (ELSD) *RT* = 0.85 min (>98%). MS (FAB, Neg.) *m/z* 388 $(M - H)^-$. HRMS (FAB, Neg.) $C_{20}H_{22}NO_5S$ $(M - H)^-$ calc. mass 388.1219, found 388.1215.

Scheme 2-1-3D

2-[(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-hydroxy-5-(phenoxymethyl)octahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylic acid (27a)



14 14-3 27a

To a solution of **14** (50.0 mg, 0.135 mmol), phenol (38.2 mg, 0.406 mmol) and 1,1'-azobis(*N*,*N*-dimethylformamide) (70.0 mg, 0.406 mmol) in THF (1.0 mL) at room temperature was added tributylphosphine (82.5 mg, 0.406 mmol). After the mixture was stirred at 50 °C for 2 h, concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography M, hexane/EtOAc 9:1-2:3) gave **14-3** (55.6 mg) in 92% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.29 (d, *J* = 7.2 Hz, 2H), 6.94 (t, *J* = 7.2 Hz, 1H), 6.87 (d, *J* = 7.2 Hz, 2H), 5.18 (t, *J* = 5.4 Hz, 1H), 5.10 (m, 1H), 4.41 (q, *J* = 7.2 Hz, 2H), 4.40 (m, 1H), 4.13-4.01 (m, 2H), 2.57-2.48 (m, 1H), 2.43 (m, 1H), 2.32-2.23 (m, 1H), 2.18-2.11 (m, 1H), 2.09 (s, 3H), 2.08-1.97 (m, 2H), 1.93 (m, 1H), 1.75 (m, 1H), 1.40 (t, *J* = 7.2 Hz, 3H).

To a solution of **14-3** (44.6 mg, 0.100 mmol) in MeOH (1.0 mL) at room temperature was added 2.0 M sodium hydroxide (0.50 mL, 1.0 mmol). After stirred at room temperature for 16 h, the reaction mixture was quenched with 1.0 M hydrochloric acid and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. Concentration gave **27a** (36.2 mg) in 96 % yield

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (s, 1H), 7.30 (t, *J* = 7.2 Hz, 2H), 6.96 (t, *J* = 7.2 Hz, 2H) 6.89 (d, *J* = 7.2 Hz, 2H), 5.22 (t, *J* = 4.8 Hz, 1H), 4.23 (m, 2H), 4.08 (m, 1H), 3.96 (dd, *J* = 9.0, 6.0 Hz, 1H), 2.46 (m, 1H), 2.29 (m, 2H), 2.16 (m, 1H), 2.11-1.95 (m, 2H), 1.90 (m, 1H), 1.74 (m, 1H) (Peaks of O*H* and CO_2H were not observed.). ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.52, 23.65, 40.01, 40.91, 50.81, 68.91, 72.83, 74.92, 75.91, 114.45(2C), 121.00, 129.50(2C), 129.55, 146.08, 158.81, 163.24, 173.64. LCMS (ELSD) *RT* = 0.82 min (>98%). MS (FAB, Neg.) *m/z* 374 $(M - H)^-$. HRMS (FAB, Neg.) $C_{19}H_{20}NO_5S$ $(M - H)^-$ calc. mass 374.1062, found 374.1070.

All compounds in Table 2-1-3 were synthesized in the same procedure.

2-{(2*R*,4a*R*,5*R*,6*R*,7a*S*)-5-[(2-chlorophenoxy)methyl]-6-hydroxyoctahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (27b)

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.99 (s, 1H), 7.40 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.29 (ddd, *J* = 8.1, 7.5, 1.8 Hz, 1H), 7.40 (dd, *J* = 8.1, 1.8 Hz, 1H), 6.92 (ddd, *J* = 8.1, 7.5, 1.8 Hz, 1H), 5.04 (t, *J* = 6.0 Hz, 1H), 4.18 (m, 2H), 4.02 (m, 1H), 3.92 (m, 1H), 2.23-2.08 (m, 3H), 1.95-1.65 (m, 5H) (Peaks of O*H* and CO_2H were not observed.). LCMS (ELSD) *RT* = 0.87 min (>98%). MS (FAB, Neg.) *m/z* 408 $(M - H)^-$. HRMS (FAB, Neg.) $C_{19}H_{19}{}^{35}ClNO_5S$ $(M - H)^-$ calc. mass 408.0672, found 408.0682.

2-[(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-hydroxy-5-{[2-(trifluoromethyl)phenoxy]methyl}octahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylic acid (27c)

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.22 (s, 1H), 7.61 (t, *J* = 7.5 Hz, 1H), 7.59 (d, *J* = 7.5 Hz, 1H), 7.26 (d, *J* = 7.5 Hz, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 5.08 (t, *J* = 5.4 Hz, 1H), 4.21 (dd, *J* = 9.9, 3.0 Hz, 1H), 4.11 (m, 2H), 3.88 (m, 1H), 2.15 (m, 3H), 1.86 (m, 3H), 1.70 (m, 2H) (Peaks of O*H* and CO_2H were not observed.). LCMS (ELSD) *RT* = 0.90 min (>98%). MS (FAB, Neg.) *m/z* 442 $(M - H)^-$. HRMS (FAB, Neg.) $C_{20}H_{19}F_3NO_5S$ $(M - H)^-$ calc. mass 442.0936, found 442.0932

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2-{(2*R*,4a*R*,5*R*,6*R*,7a*S*)-5-[(2-fluorophenoxy)methyl]-6-hydroxyoctahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (27d)

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.34 (s, 1H), 7.17 (m, 3H), 6.94 (m, 1H), 5.07 (t, *J* = 5.4 Hz, 1H), 4.21-4.10 (m, 2H), 4.04 (m, 1H), 3.90 (m, 1H), 2.27-2.04 (m, 3H), 1.96-1.65 (m, 5H) (Peaks of O*H* and CO_2H were not observed.). LCMS (ELSD) *RT* = 0.83 min (>98%). MS (FAB, Neg.) *m/z* 392 $(M - H)^-$. HRMS (FAB, Neg.) $C_{19}H_{19}FNO_5S$ $(M - H)^-$ calc. mass 392.0968, found 392.0964

2-{(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-hydroxy-5-[(3-methylphenoxy)methyl]octahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (27e)

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.42 (s, 1H), 7.13 (t, *J* = 7.8 Hz, 1H), 6.74-6.70 (m, 3H), 5.08 (t, *J* = 6.3 Hz, 1H), 4.88 (d, *J* = 5.1 Hz, 1H), 4.15-4.03 (m, 2H), 3.95-3.82 (m, 2H), 2.26 (s, 3H), 2.23-2.08 (m, 3H), 1.90-1.65 (m, 5H) (Peak of CO_2H was not observed.). LCMS (ELSD) *RT* = 0.88 min (>98%). MS (FAB, Neg.) *m/z* 388 $(M - H)^-$. HRMS (FAB, Neg.) $C_{20}H_{22}NO_5S$ $(M - H)^-$ calc. mass 388.1219, found 388.1212

2-{(2*R*,4a*R*,5*R*,6*R*,7a*S*)-5-[(3-chlorophenoxy)methyl]-6-hydroxyoctahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (27f)

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.42 (s, 1H), 7.28 (dd, *J* = 8.1, 7.8 Hz, 1H), 7.01 (m, 1H), 6.97 (d, *J* = 7.8 Hz, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 5.08 (t, *J* = 6.0 Hz, 1H), 4.89 (d, *J* = 5.4 Hz, 1H), 4.10 (m, 2H), 4.01 (m, 1H), 3.86 (m, 1H), 2.25-2.04 (m, 3H), 1.93-1.60 (m, 5H) (Peak of CO_2H was not observed.). LCMS (ELSD) *RT* = 0.90 min (>98%). MS (FAB, Neg.) *m/z* 408 $(M - H)^-$. HRMS (FAB, Neg.) $C_{19}H_{19}{}^{35}ClNO_5S$ $(M - H)^-$ calc. mass 408.0672, found 408.0663

2-[(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-hydroxy-5-{[3-(trifluoromethoxy)phenoxy]methyl}octahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylic acid (27g)

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.27 (m, 1H), 6.81 (m, 2H), 6.74 (m, 1H), 5.22 (t, *J* = 4.8 Hz, 1H), 4.21 (m, 2H), 4.07 (m, 1H), 3.96 (m, 1H), 2.47 (m, 1H), 2.27 (m, 2H), 2.22-2.12 (m, 1H), 2.11-2.00 (m, 2H), 1.87 (m, 1H), 1.77 (m, 1H) (Peaks of O*H* and CO_2H were not observed.). LCMS (ELSD) *RT* = 0.95 min (>98%). MS (FAB, Neg.) *m/z* 458 $(M - H)^-$. HRMS (FAB, Neg.) $C_{20}H_{19}F_3NO_6S$ $(M - H)^-$ calc. mass

458.0885, found 458.0883

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2-[(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-hydroxy-5-{[3-(trifluoromethyl)phenoxy]methyl}octahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylic acid (27h)

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.38 (dd, *J* = 8.1, 7.8 Hz, 1H), 7.22 (m, 1H), 7.07 (m, 2H), 5.22 (t, *J* = 5.1 Hz, 1H), 4.23 (m, 2H), 4.10 (m, 1H), 3.99 (m, 1H), 2.49 (m, 1H), 2.32 (m, 2H), 2.19 (m, 1H), 2.05 (m, 2H), 1.91 (m, 1H), 1.79 (m, 1H) (Peaks of O*H* and CO_2H were not observed.). LCMS (ELSD) *RT* = 0.92 min (>98%). MS (FAB, Neg.) *m/z* 442 $(M - H)^-$. HRMS (FAB, Neg.) $C_{20}H_{19}F_3NO_5S$ $(M - H)^-$ calc. mass 442.0936, found 442.0930

2-{(2*R*,4a*R*,5*R*,6*R*,7a*S*)-5-[(3-fluorophenoxy)methyl]-6-hydroxyoctahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (27i)

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.19 (m, 1H), 6.68-6.57 (m, 3H), 5.22 (t, *J* = 4.8 Hz, 1H), 4.22 (m, 2H), 4.04 (m, 1H), 3.94 (m, 1H), 2.45 (m, 1H), 2.27 (m, 2H), 2.20-1.98 (m, 3H), 1.87 (m, 1H), 1.75 (m, 1H) (Peaks of O*H* and CO_2H were not observed.). LCMS (ELSD) *RT* = 0.85 min (>98%). MS (FAB, Neg.) *m/z* 392 $(M - H)^-$. HRMS (FAB, Neg.) $C_{19}H_{19}FNO_5S$ $(M - H)^-$ calc. mass 392.0968, found 392.0959

2-{(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-hydroxy-5-[(4-methylphenoxy)methyl]octahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (27j)

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.42 (s, 1H), 7.06 (d, *J* = 8.1 Hz, 2H), 6.81 (d, *J* = 8.1 Hz, 2H), 5.08 (t, *J* = 5.7 Hz, 1H), 4.87 (d, *J* = 5.4 Hz, 1H), 4.11 (m, 1H), 4.03 (m, 1H), 3.88 (m, 2H), 2.21 (s, 3H), 2.15 (m, 1H), 2.07 (m, 2H), 1.91-1.64 (m, 5H) (Peak of CO_2H was not observed.). LCMS (ELSD) *RT* = 0.88 min (>98%). MS (FAB, Neg.) *m/z* 388 $(M - H)^-$. HRMS (FAB, Neg.) $C_{20}H_{22}NO_5S$ $(M - H)^-$ calc. mass 388.1219, found 388.1215

2-{(2*R*,4a*R*,5*R*,6*R*,7a*S*)-5-[(4-chlorophenoxy)methyl]-6-hydroxyoctahydrocyclopenta[*b*]pyran-2-yl}-1,3-th

iazole-4-carboxylic acid (27k)

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.40 (s, 1H), 7.29 (d, *J* = 9.0 Hz, 2H), 6.95 (d, *J* = 9.0 Hz, 2H), 5.08 (t, *J* = 6.0 Hz, 1H), 4.91 (m, 1H), 4.09 (m, 2H), 3.96 (m, 1H), 3.85 (m, 1H), 2.27-2.07 (m, 3H), 1.90-1.63 (m, 5H) (Peak of CO_2H was not observed.). LCMS (ELSD) *RT* = 0.90 min (>98%). MS (FAB, Neg.) *m/z* 408 (M - H)$^-$. HRMS (FAB, Neg.) $C_{19}H_{19}{}^{35}ClNO_5S$ (M - H)- calc. mass 408.0672, found 408.0677

2-[(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-hydroxy-5-{[4-(trifluoromethoxy)phenoxy]methyl}octahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylic acid (27l)

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.40 (s, 1H), 7.27 (d, *J* = 8.4 Hz, 2H), 7.03 (d, *J* = 8.4 Hz, 2H), 5.08 (t, *J* = 6.3 Hz, 1H), 4.90 (m, 1H), 4.10 (m, 2H), 3.99 (m, 1H), 3.87 (m, 1H), 2.19 (m, 1H), 2.10 (m, 2H), 1.90-1.64 (m, 5H) (Peak of CO_2H was not observed.). LCMS (ELSD) *RT* = 0.94 min (>98%). MS (FAB, Neg.) *m/z* 458 (M - H)$^-$. HRMS (FAB, Neg.) $C_{20}H_{19}F_3NO_6S$ (M - H)$^-$ calc. mass 458.0885, found 458.0883

2-[(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-hydroxy-5-{[4-(trifluoromethyl)phenoxy]methyl}octahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylic acid (27m)

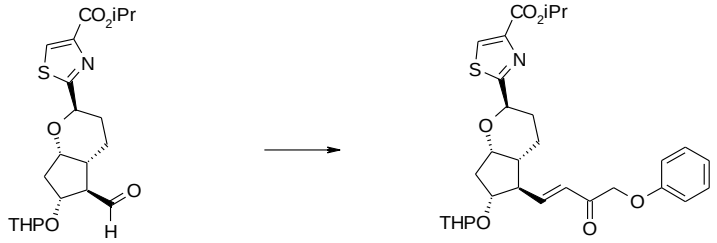
^{1}H NMR (300 MHz, DMSO-d_6) δ 8.39 (s, 1H), 7.63 (d, *J* = 9.0 Hz, 2H), 7.12 (d, *J* = 9.0 Hz, 2H), 5.09 (t, *J* = 6.3 Hz, 1H), 4.92 (m, 1H), 4.14 (m, 2H), 4.09 (m, 1H), 3.87 (m, 1H), 2.21 (m, 1H), 2.12 (m, 2H), 1.92-1.61 (m, 5H) (Peak of CO_2H was not observed.). LCMS (ELSD) *RT* = 0.93 min (>98%). MS (FAB, Neg.) *m/z* 442 (M - H)$^-$. HRMS (FAB, Neg.) $C_{20}H_{19}F_3NO_5S$ (M - H)$^-$ calc. mass 442.0936, found 442.0932

2-{(2*R*,4a*R*,5*R*,6*R*,7a*S*)-5-[(4-fluorophenoxy)methyl]-6-hydroxyoctahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (27n)

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (s, 1H), 6.96 (m, 2H), 6.83 (m, 2H), 5.21 (t, *J* = 4.8 Hz, 1H), 4.22 (m, 2H), 4.04 (m, 1H), 3.91 (m, 1H), 2.43 (m, 1H), 2.29 (m, 2H), 2.18 (m, 1H), 2.04 (m, 2H), 1.90 (m, 1H), 1.76 (m, 1H) (Peaks of O*H* and CO_2H were not observed.). LCMS (ELSD) *RT* = 0.83 min (>98%). MS (FAB, Neg.) *m/z* 392 (M - H)$^-$. HRMS (FAB, Neg.) $C_{19}H_{19}FNO_5S$ (M - H)$^-$ calc. mass 392.0968, found 392.0962

Synthesis of 7

Isopropyl 2-[(2*R*,4a*R*,5*R*,6*R*,7a*S*)-5-[(1*E*)-3-oxo-4-phenoxy-1-buten-1-yl]-6-(tetrahydro-2*H*-pyran-2-yloxy)octahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylate (24-4)

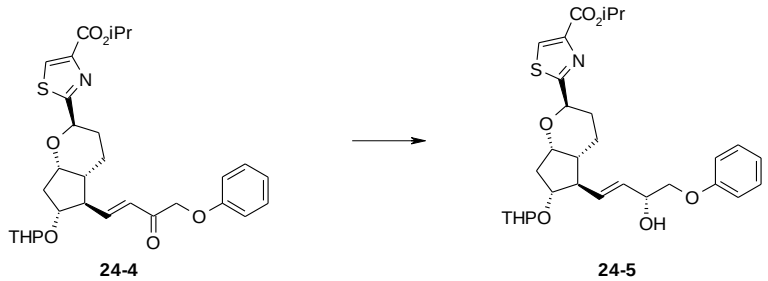


24-2 **24-4**

To a solution of **24-2** (450 mg, 1.06mmol), dimethyl (2-oxo-3-phenoxypropyl)-phosphonate (549 mg, 2.13 mmol) and triethylamine (0.296 mL, 2.13 mmol) in THF (5.0 mL) at room temperature was added LiCl (91 mg, 2.13 mmol). After stirred at room temperature for 16 h, the reaction mixture was quenched with 1.0 M hydrochloric acid and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography L SI50, hexane/EtOAc 3:1-1:1) gave **24-4** (309 mg) in 52% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (brs, 1H), 7.33-7.25 (m, 2H), 7.05-6.98 (m, 2H), 6.90 (d, *J* = 9.0 Hz, 2H), 6.57 (dd, *J* = 15.0, 7.5 Hz, 1H), 5.27 (sep, *J* = 6.3 Hz, 1H), 5.16 (m, 1H), 4.72 (s, 1H), 4.70 (s, 1H), 4.62 (m, 0.5H), 4.51 (m, 0.5H), 4.21-3.96 (m, 2H), 3.84 (m, 0.5H), 3.71 (m, 0.5H), 3.42 (m, 1H), 2.94 (m, 1H), 2.41 (m, 1H), 2.22-2.17 (m, 2H), 1.92 (m, 1H), 1.84-1.69(m, 3H), 1.63-1.45 (m, 6H), 1.37 (d, *J* = 6.3 Hz, 6H).

Isopropyl 2-[(2*R*,4a*R*,5*R*,6*R*,7a*S*)-5-[(1*E*,3*R*)-3-hydroxy-4-phenoxy-1-buten-1-yl]-6-(tetrahydro-2*H*-pyran-2-yloxy)octahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylate (24-5)



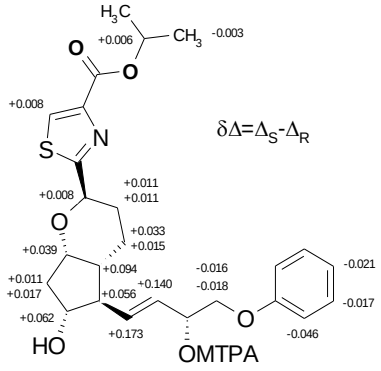
24-4 **24-5**

To a solution of **24-4** (289 mg, 0.520 mmol) and (3aR)-1-methyl-3,3-diphenyl-tetrahydro-3H-pyrrolo[1,2-c][1,3,2]oxazaborole (1.0 M THF solution, 0.182 mL,

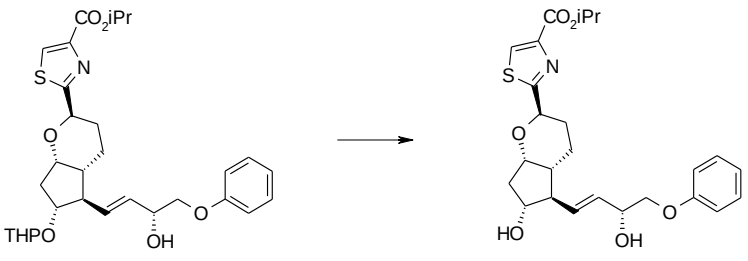
0.182 mmol) in THF (4.0 mL) at 0 °C was added borane-dimethyl sulfide complex (35.6 mg, 0.468 mmol). After stirred at 0 °C for 1 h, the reaction mixture was quenched with MeOH and H_2O and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography L SI50, hexane/EtOAc 3:1-1:1) gave **24-5** (267 mg) in 92% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (brs, 1H), 7.31 (t, *J* = 7.5 Hz, 2H), 7.00 (t, *J* = 7.5 Hz, 1H), 6.92 (d, *J* = 7.5 Hz, 2H), 5.85-5.65 (m, 2H), 5.27 (sep, *J* = 6.0 Hz, 1H), 5.16 (t, *J* = 5.4 Hz, 1H), 4.70 (m, 1H), 4.54 (m, 1H), 4.17 (m, 1H), 4.02 (m, 2H), 3.87 (m, 2H), 3.46 (m, 1H), 2.78 (m, 1H), 2.40 (m, 2H), 2.29-2.10 (m, 2H), 1.97-1.76 (m, 3H), 1.72-1.43 (m, 6H), 1.33 (d, *J* = 6.0 Hz, 6H) (Peak of O*H* was not observed.).

The stereochemistry of C15 position was determined by modified Mosher's method.



Isopropyl 2-{(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-hydroxy-5-[(1*E*,3*R*)-3-hydroxy-4-phenoxy-1-buten-1-yl]octahydro cyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylate (24-6)



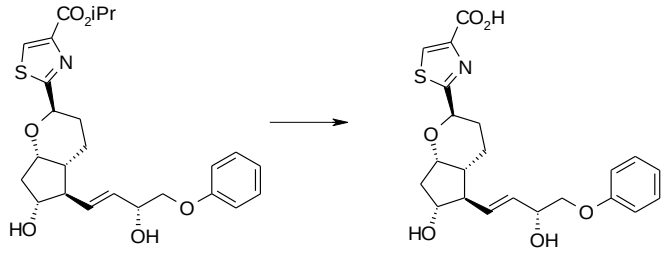
24-5 24-6

To a solution of **24-5** (130 mg, 0.233 mmol) in MeOH (4.0 mL) at room temperature was added *p*-toluene sulfonic acid monohydrate (4.4 mg, 0.023 mmol). After stirred at room temperature for 5 h, the reaction

mixture was quenched with Et_3N. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography L SI50, hexane/EtOAc 3:1-1:1) gave **24-6** (89.0 mg) in 81% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.31 (t, *J* = 7.5 Hz, 2H), 6.98 (t, *J* = 7.5 Hz, 1H), 6.92 (d, *J* = 7.5 Hz, 2H), 5.73 (m, 2H), 5.27 (sep, *J* = 6.0 Hz, 1H), 5.19 (t, *J* = 5.1 Hz, 1H), 4.55 (m, 1H), 4.16 (m, 1H), 4.01 (dd, *J* = 15.3, 3.3 Hz, 1H), 3.97 (m, 1H), 3.89 (dd, *J* = 15.3, 7.8 Hz, 1H), 2.66 (m, 1H), 2.46 (m , 1H), 2.33-2.24 (m, 3H), 1.98 (m, 1H), 1.85 (m, 1H), 1.64 (m, 1H), 1.38 (d, *J* = 6.0 Hz, 6H) (Peaks of O*H* were not observed.).

2-{(2*R*,4a*R*,5*R*,6*R*,7a*S*)-6-hydroxy-5-[(1*E*,3*R*)-3-hydroxy-4-phenoxy-1-buten-1-yl]octahydrocyclopenta[*b*] pyran-2-yl}-1,3-thiazole-4-carboxylic acid (7)



To a solution of **24-6** (64.0 mg, 0.135 mmol) in MeOH (2.0 mL) at room temperature was added 2.0 M sodium hydroxide (1.0 mL, 2.0 mmol). After stirred at room temperature for 2 h, the reaction mixture was quenched with 1.0 M hydrochloric acid and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. Concentration gave **7** (56.2 m) in 96 % yield: colorless viscous oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.28 (m, 2H), 6.99 (t, *J* = 6.6 Hz, 1H), 6.92 (d, *J* = 6.6 Hz, 2H), 5.74 (m, 2H), 5.19 (t, *J* = 5.1 Hz, 1H), 4.56 (m, 1H), 4.14 (m, 1H), 4.01 (m, 2H), 3.91 (m, 1H), 2.68 (m, 1H), 2.33 (m, 1H), 2.25 (m, 2H), 1.96 (m, 1H), 1.85 (m, 1H), 1.69-1.56 (m, 2H) (Peaks of O*H* and CO_2H were not observed.). LCMS (ELSD) *RT* = 0.75 min (>98%). MS (FAB, Neg.) *m/z* 430 $(M - H)^-$. HRMS (FAB, Neg.) $C_{22}H_{24}NO_6S$ $(M - H)^-$ calc. mass 430.1324, found 430.1321.

Biology In vitro assay

EP2, EP4 and IP cAMP assay

Chinese hamster ovary (CHO) cells (1.25×10^5 cells/well) expressing human EP2 or human EP4 or human IP receptor were harvested and suspended in a 96-well 1/2 area plate. cAMP concentrations were measured using a cAMP HTRF HiRange kit (Cisbio Bioassays)* after treatment of compounds. The reaction rate (%) of the compounds relative to the cAMP concentration obtained with PGE_2 treatment at 1μM was calculated. Furthermore, a non-linear regression analysis was performed using the Sigmoid Emax Model to estimate EC_{50} values.

*http://www.cisbio.com/usa/drug-discovery/membrane-based-assays-camp-hirange-assay-kit (accessed Nov 24, 2015)

EP2 β arrestin recruitment assay

PathHunter β-arrestin HEK-293 PTGER2 cell lines (DiscoveRx) were seeded at a density of 5000 cells/well into a 384-well plate and cultured at 37 °C in the presence of 5 % CO_2 for 24 hours. β-arrestin recruitment were measured using a PathHunter Detection Kit (DiscoveRx)* after treatment of compounds. The reaction rate (%) of the compounds relative to the β-arrestin recruitment obtained with PGE_2 treatment at 10μM was calculated. Furthermore, a non-linear regression analysis was performed using the Sigmoid Emax Model to estimate EC_{50} values.

* https://www.discoverx.com/product-data-sheets-3-tab/93-0214c1 (accessed Nov 24, 2015)

EP1, EP3 and FP Ca assay

Chem-1 cells expressing human FP receptor or Chinese hamster ovary (CHO) cells expressing human EP1 or human EP3 were seeded at a density of 1×10^4 cells per well into 96-well plates and cultured at 37℃ in the presence of 5% CO_2 for 2 days. Load buffer (HBSS containing Calcium 5, 10 mM HEPES, 20μM indomethacin, and 2.5 mM probenecid) was added in each well and incubated in the dark at room temperature for 1 hour. After addition of the compounds, intracellular Ca^{2+} concentration was measured using a fluorescence drug screening

system (FDSS-7000 : Hamamatsu Photonics, Tokyo, Japan)*. The reaction rate (%) of the compounds relative to intracellular Ca^{2+} concentration obtained with maximum increases of PGE_2 treatment was calculated. Futhermore, a non-linear analysis was performed using the Sigmoid Emax Model to estimate EC_{50} values.

* http://www.hamamatsu.com/jp/ja/FDSS7000EX.html (accessed Nov 24, 2015)

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Chapter 2-2

Structural optimization and structure-functional selectivity relationship studies of G protein-biased EP2 receptor agonists

Abstract:

Further modification of novel G protein-biased EP2 agonist **1** was undertaken to improve G protein activity and investigate structure-functional selectivity relationship. Optimization of substituents on phenyl group, followed by modification of 11-OH led the author find **9** with 100-fold increase in G protein activity relative to **1** without increase of β arrestin recruitment. Furthermore, SFSR studies revealed that the combination of *meta* and *para* substituents on phenyl moiety was crucial to regulate the functional selectivity.

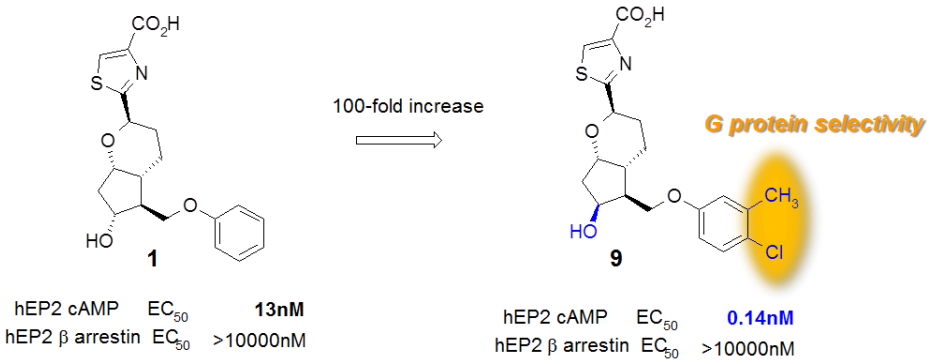

Figure 2-2-1. Outline of Chapter 2-2

Introduction

G protein-coupled receptors (GPCRs) are the one of the most successful targets of drug discovery. A lot of drugs targeting GPCRs have already been launched as therapeutic agents for a variety of diseases. In addition to G protein activation, GPCRs can also activate other distinct signaling pathways like β arrestin signaling. β arrestins were regarded as negative regulators of G protein-mediated signaling. It is suggested that the β arrestin also have a variety of functions by regulating GPCR internalization and promoting intracellular signaling independently. Recently, GPCR biased ligands are identified to engage signals selectively and also inhibit other signals mediated by the same receptor. Biased ligand received a fair amount of attention in drug discovery, because they might have potentials to enhance efficacy or remove on-target adverse effect. A number of GPCR biased ligands have been revealed[1-6] and some of them have already tested in clinical development.

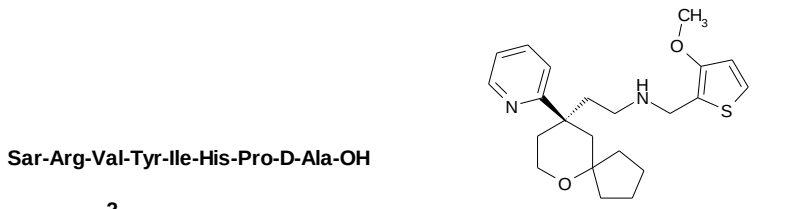

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Figure 2-2-2; Reported GPCR biased ligands

TRV027 (**2**)[6] is a β arrestin-biased agonist of angiotensin II type I receptor. TRV027 exhibited unique pharmacology, distinct from classical angiotensin II receptor blockers (ARBs, i.e. losartan and varsartan). Classical ARBs is known to decrease both blood pressure and cardiac performance in rat. On the other hand, TRV027, which particularly activates β arrestin signaling, exerted potent hypotensive action without decrease of cardiac performance and preserved stroke volume in rat. These findings suggested that TRV027 would be a beneficial therapeutic agent for acute heart failure (AHF).[7] TRV130 (**3**)[5]is reported as G protein-biased agonist of μ opioid receptor to exert desired analgesic effects in mice and rat with reducing morphine's side effects, such

as constipation and respiratory depression. TRV130 is under evaluation in Phase IIb for the treatment of acute severe pain.

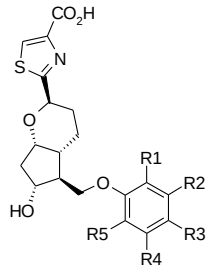
In previous study, the author has reported the identification of novel G protein-biased EP2 agonist **1**[8]. Recently a number of studies of EP2 receptor signaling and their functions were reported. EP2 receptor exerted beneficial neuroprotective effects in the brain via G protein-mediated cAMP-PKA signaling[9-12], on the other hand, the activation of β arrestin signaling of EP2 receptor led to deleterious effects, like tumorigenesis and angiogenesis.[13-15] Therefore, EP2 receptor G protein-biased ligand is expected to be new generation of EP2 agonists that particularly increase the efficacy and avoid deleterious effect of EP2 receptor.

In this chapter, the author describes further optimization of novel G protein-biased agonist **1** to increase G protein activity and functional selectivity. Structure-functional selectivity relationship (SFSR)[4] studies are also reported.

Results and discussion

Optimization of substituents on pheny group

In chapter 2-1[8], the author reported the identification of highly selective EP2 agonists **1** and investigated SFSR study. The SFSR indicated that introduction of *meta* substituent into phenyl group of **1** remarkably improved G protein activity while it also increased β arrestin activity (**4a**). On the other hand, introduction of *para* substituent (**4b**) showed decreased β arrestin recruitment (Emax 28% → 12%) without loss of G protein activity. The author supposed that combination of *meta* substituent and *para* substituent improved both G protein activity and functional selectivity. Therefore, the author synthesized and evaluated di-substituted analogues **4c-j**. The results are shown in Table2-2-1.



Cpmd	R_1	R_2	R_3	R_4	R_5	hEP2			
						G protein (cAMP)		β arrestin	
						EC_{50}(nM)	E_{max} [b]	EC_{50}(nM)	E_{max}
1	H	H	H	H	H	13	118	>10,000	28
4a	H	CF_3	H	H	H	0.90	112	9.0	62
4b	H	H	Cl	H	H	3.9	96	>10000	12
4c	H	CF_3	Cl	H	H	0.11	78	>10,000	41
4d	H	Cl	Cl	H	H	0.57	100	>10,000	23
4e	H	Me	Cl	H	H	0.69	88	>10,000	23
4f	H	Et	Cl	H	H	0.68	95	>10,000	29
4g	H	iPr	Cl	H	H	2.0	122	>10,000	29
4h	H	OMe	Cl	H	H	1.8	83	>10,000	19
4i	H	Me	Me	H	H	2.9	90	>10,000	36
4j	H	Me	CF_3	H	H	3.9	85	>10,000	46
4k	H	Me	Cl	Me	H	0.17	102	2.3	98
4l	H	Cl	Cl	H	Cl	0.20	99	3.7	94
4m	Cl	Cl	Cl	H	H	0.21	102	2.3	98

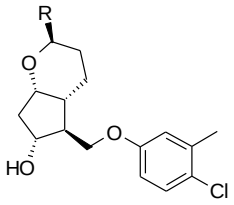
[a]Assay protocols are provided in the Supporting Information. EC_{50} values represent the mean of two experiments. [b]All E_{max} were normalized to PGE_2 results.

Table 2-2-1. Optimization of substituents on the pheny group

As the author expected, compound **4c**, which hybridizes the substituents of **4a** and **4b,** showed a 35-fold increased G protein activity compared to **4b** with decreasing β arrestin activity relative to **4a**. However, **4c** still modulated β arrestin recruitment (Emax 41%) while its G protein activity is partial (78%). Therefore, the author continued further optimization of *meta* substituents of **4c**. Both of 3,4-diCl analogue **4d** and 3-Me-4-Cl analogue **4e** decreased β arrestin efficacy relative to **4c** (Emax 41% to 23%), retaining potent G protein activity. Introductions of more steric hindered substituent 3-Et and 3-*i*Pr group gave **4f** and **4g**, both of which also decreased β arrestin activity (Emax 41% to 29%) without significant loss of G protein activity. Electron donating group 3-MeO analogue **4h** showed less potent G protein activity than **4c** though it decreased β arrestin activity (Emax 41% to 19%). Conversion of para-substituents of **4e** to Me and CF_3 group gave **4i** and **4j**, both of which exerted lower G protein activity and increased β arrestin activity compared to **4e** (Emax 23% to 36% and 46%, respectively). Introduction of 5-Me substituent into another *meta* position of **4e** gave **4k**, which showed significant increase of β arrestin recruitment compared to **4e** (EC_{50} >10,000nM to 2.3 nM). The other tri-substituents analogues **4l** and **4m** also showed highly potent β arrestin activity similar to **4k**. These results suggested that the combination of *meta* and *para* substituent should be essential to satisfy both G protein biased agonist activity and selectivity.

Optimization of α chain of 4e

In next attempts, transformation of heterocyclic group of α chain was carried out to investigate the effect of thiazole group as outlined in Table 2-2-2. Introduction of oxazole group instead of thiazole of **4e** gave **5**, which showed a 21-fold decrease of G protein activity. 2,5-Furan analogue **6** showed slightly decreased G protein activity without loss of functional and receptor-subtype selectivity. On the other hand, 2,4-thiophene analogue **7** and 2,4-furan analogue **8** significantly decreased G protein activity (167-fold and 80-fold). These results indicate that thiazole group should be an optimal moiety of the heterocyclic part for potent EP2 activity.



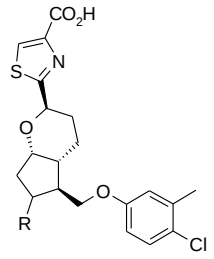
cmpd	R	hEP2				hEP1	hEP3	hEP4	hIP	hFP
		G protein(cAMP)		β arrestin						
		EC_{50}(nM)[a]	E_{max}(%)	EC_{50}(nM)[a]	E_{max}(%)	EC_{50}(nM)[a]	EC_{50}(nM)[a]	EC_{50}(nM)[a]	EC_{50}(nM)[a]	EC_{50}(nM)[a]
4e		0.69	88	>10,000	23	>10,000	>10,000	>10,000	4900	>10,000
5		15	127	>10,000	18	>10,000	>10,000	>10,000	7900	>10,000
6		2.2	96	>10,000	17	>10,000	>10,000	>10,000	5300	>10,000
7		114	102	>10,000	28	>10,000	>10,000	>10,000	>10,000	>10,000
8		55	109	>10,000	21	>10,000	>10,000	>10,000	>10,000	>10,000

[a]Assay protocols are provided in the Supporting Information. EC_{50} values represent the mean of two experiments.

Table 2-2-2. Optimization of α chain

Optimization of 11-hydroxyl group

Chemical modification of 11-OH was performed to improve G protein activity and investigate SFSR as shown in Table 2-2-3. Compound **9** with 11β-hydroxyl moiety exerted 4.9-fold more potent G protein activity than **4e** without any change in β arrestin recruitment. On the other hand, 11β-methoxy analogue **10** and 11β-fluoride analogue **11** decreased G protein activity relative to compound **9**. These results indicate that hydrogen donating profile of 11β-hydroxyl group would be crucial to show potent G protein activity. To the author's best knowledge, there are no reports of potent EP2 agonist which has unnatural 11β-hydroxyl group, therefore the author supposed that hydroxyl moiety of **9** interacts with EP2 receptor in a different manner from 11α-hydroxyl group of PGE_2 or previously reported EP2 agonist (PGE_2 analogues). Furthermore, the interaction of 11β-hydroxyl group with EP2 receptor might cause conformational change of intracellular region to interact with G protein preferentially.



cmpd	R	hEP2				hEP1	hEP3	hEP4	hIP	hFP
		G protein(cAMP)		β arrestin						
		EC_{50}(nM)[a]	E_{max}(%)	EC_{50}(nM)[a]	E_{max}(%)	EC_{50}(nM)[a]	EC_{50}(nM)[a]	EC_{50}(nM)[a]	EC_{50}(nM)[a]	EC_{50}(nM)[a]
4e	HO	0.69	88	>10,000	23	>10,000	>10,000	>10,000	4900	>10,000
9	HO	0.14	97	>10,000	21	>10,000	>10,000	>10,000	>10,000	>10,000
10	MeO	23	73	>10,000	-	>10,000	>10,000	>10,000	>10,000	>10,000
11	F	11	110	>10,000	31	>10,000	>10,000	>10,000	1700	>10,000

[a]Assay protocols are provided in the Supporting Information. EC_{50} values represent the mean of two experiments.

Table 2-2-3; Optimization of 11-hydroxyl group

Recently, a fair number of researches on relationship between the structure of GPCRs and biased signaling have been reported. X-ray crystallography and NMR spectroscopy studies give useful information to consider the differences between biased agonist and full agonist, particularly, binding site of biased ligands[16,17], interactions of receptor with G protein and β arrestin[18], and conformational change of intracellular loop of receptors when biased ligand or unbiased ligands bind to the receptor.[19] Taking into account these reports, the interaction of ligand with transmembrane 5 (TM5) and TM6 of receptor is considered to play a key role to activate G protein-mediated signaling, and the interaction of ligands with TM7 would result in activating β arrestin recruitment. The author supposes that *meta* and *para* substituents of phenyl group obtained a specific hydrophobic or Van der Waals interaction with TM5 and/or TM6 of EP2 receptor, and *ortho* substituents may interact with TM7. The technical innovation in analyzing the structure of GPCRs (i.e. X-ray crystallography and NMR spectroscopy) makes enormous strides forward in these days. These technologies might reveal three- dimensional structure of EP2 receptor and its structure would enable the author to validate the author hypothesis.

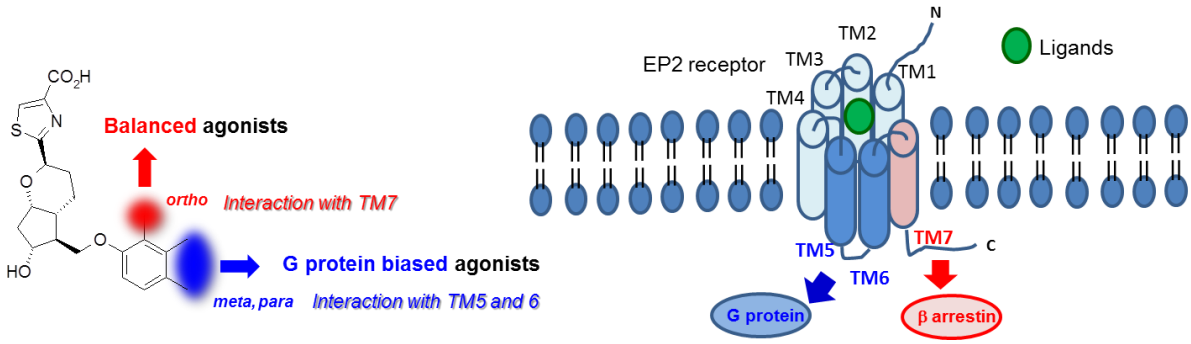


Figure 2-2-3; Proposed interaction between EP2 agonists and receptor.

Syntheses of bicyclic derivatives

All tested compounds in Tables 2-2-1, 2 and 3 were synthesized as outlined in Schemes 2-2-1A-F. Compounds **4c-l** were synthesized by a sequential procedure: step 1; introduction of 3-chloro-4-methylphenol by Mitsunobu reaction, step 2; removal of protecting groups of 11-hydroxyl or carboxylic acid moiety as outlined in Scheme 2-2-1A.

Syntheses of **6**, **7** and **8** were described in Scheme 2-2-1B. Transformations of the thiazole part were performed

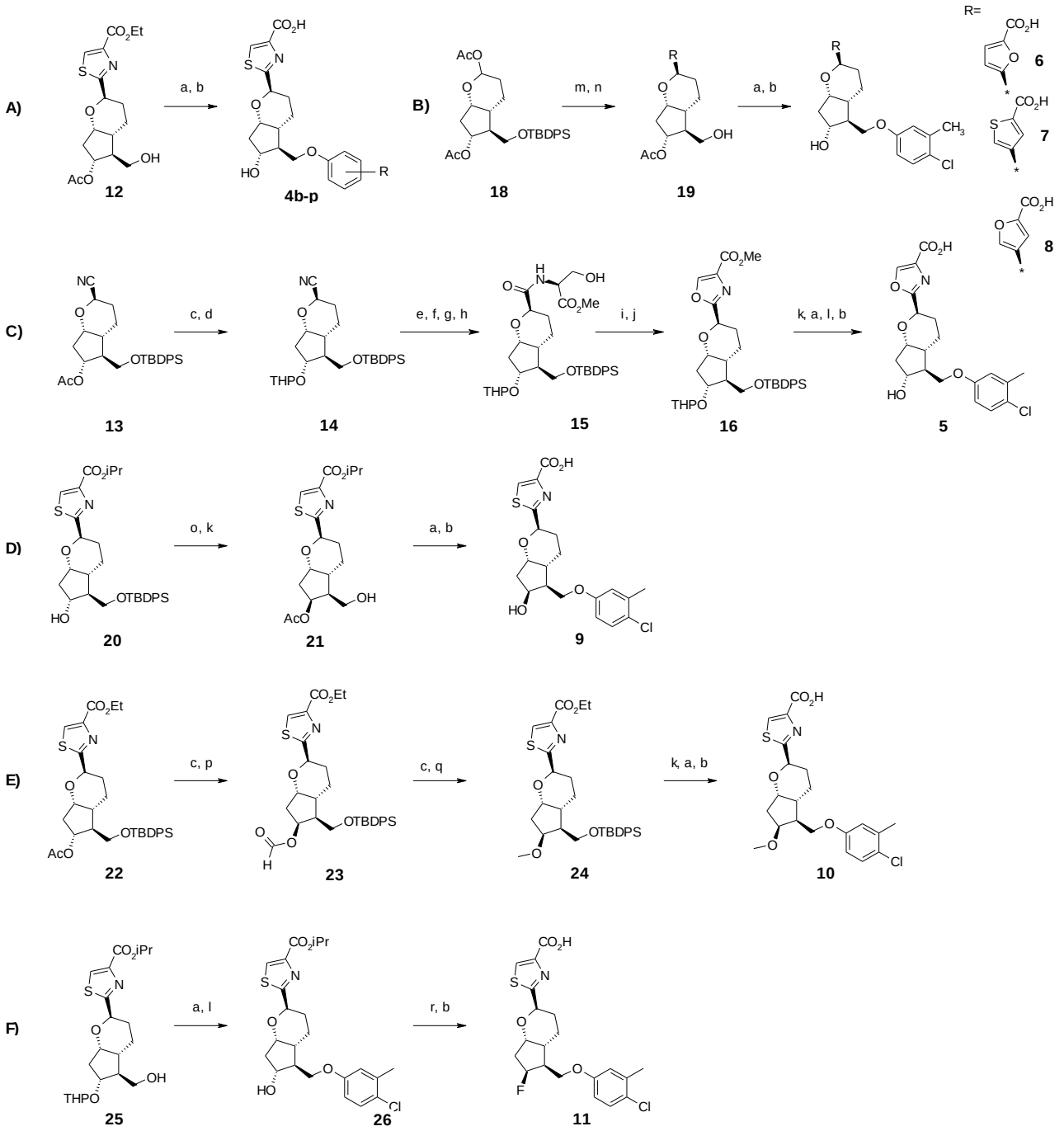
by coupling reaction of **18** with Reformatsky type reagents. Deprotection of the TBS group gave alcohols, followed by a sequential procedure afforded compounds **6-8**.

Compound **5** couldn't be synthesized in similar manner as described in Scheme 2-2-1B due to instability of Reformatsky reagent, therefore oxazole ring was constructed stepwise procedure as described in Scheme 2-2-1C. Firstly, the acetyl protecting group of **13** was converted to THP group, which is tolerable to reducing agent. Nitrile **14** was reduced to an aldehyde, Pinnick oxidation of which afforded the carboxylic acid derivative. The resulting product was transformed to serine ester **15** through an acid anhydride intermediate. The oxazoline ring was constructed under dehydration condition, and aromatization under oxidation condition gave oxazole **16**. Deprotection of the TBS group, followed by a sequential procedure gave **5**.

As described in Scheme 2-2-1D, the 11β-hydroxyl group of **9** was introduced by Mitsunobu reaction with **20**. Deprotection of the TBDPS group afforded **21**, which was transformed to **9** by a sequential procedure.

Synthesis of **10** was outlined in Scheme 2-2-1E. 11β-OH group was introduced in the similar manner of Scheme 1D. Methylation was performed with methyl iodide in the presence of silver oxide.

Synthesis of **11** was shown in Scheme 2-2-1F. A sequential procedure from **25** gave **26**. 11β-fluoride was introduced by using Deoxo-Fluor, and hydrolysis of ester gave **11**.



Reagents & conditions: (a) TMAD, Bu_3P, THF, rt, 32-100%, (b) NaOHaq., DME, MeOH, rt, 86-100%, (c) K_2CO_3, MeOH, rt, 85-93%, (d) DHP, PPTS, CH_2Cl_2, rt, 92%, (e) DIBAL, CH_2Cl_2, –78 °C, (f) NaOCl, NaH_2PO_4, *t*-BuOH, H_2O, THF, rt, (g) isobutyl chloroformate, NMM, THF, –30 °C, (h) L-serine, rt, 42% (4 steps), (i) [bis(2-methoxyethyl)amino]sulfur trifluoride, CH_2Cl_2, –20 °C, 35%, (j) $BrCCl_3$, DBU, CH_2Cl_2, 0 °C, 89%, (k) TBAF, THF, rt, 48-84%, (l) TsOH, MeOH, rt, 81-93%, (m) $BrZnRCO_2Et$, $AlCl_3$, CH_3CN, 0 °C-rt, 31-87%, (n) TBAF, AcOH, THF, 31-86%, (o) AcOH, DEAD, Ph_3P, THF, rt, (p) HCO_2H, DEAD, Ph_3P, rt, 93%, (q) MeI, Ag_2O, MeCN, rt, 22%, (r) diethyl-aminosulfur trifluoride, CH_2Cl_2, –78 °C, 57%,

Scheme 2-2-1. Syntheses of G protein-biased EP2 agonists

Overall, precise optimization of substituents on the phenyl group of **1** and introduction of 11β hydroxyl group led the author find 100-fold more potent G protein-biased EP2 agonist **9**. SFSR studies revealed that structure of ω chain dramatically changes the functional selectivity of EP2 receptor. Particularly, the combination of *meta* and *para* substituents on the phenyl group enhanced G protein-biased signaling of EP2 receptor. A series of G protein-biased EP2 agonists showed potent intraocular lowering effect in rabbit and monkey.[20] Further studies of EP2 biased agonists will be reported in due course.

General Experimental.

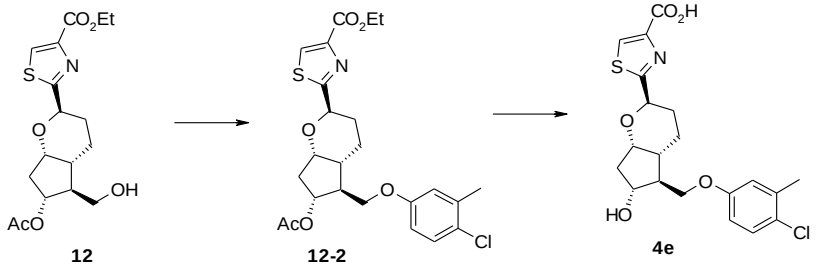
Analytical samples were homogeneous as confirmed by TLC, and spectroscopic results were consistent with the assigned structures. NMR spectra were recorded as designated on either a Varian Mercury 300 spectrometer or INOVA-500 spectrometer using deuterated chloroform ($CDCl_3$) or deuterated dimethyl sulfoxide (DMSO-d_6) as the solvent. Mass spectral analyses with fast atom bombardment (FABMS, HRMS) and electron ionization (EI) were performed on a JEOL JMS-DX303HF spectrometer. Purity analysis was carried out by the following LC/MS system. LC/MS: Waters ACQUITY UPLC system fitted by with Waters Micromass ZQ-2000 spectrometer. Column; YMC Triart C18 (2.0 mm × 30 mm). Eluting over 1.5 min with 5−95% acetonitrile(0.1% TFA) in water (0.1%TFA), flow rate of 1.0 mL/min, column temperature of 30 °C, detection with UV (PDA) and ELSD. Column chromatography was performed with silica gel [Merck Silica Gel 60 (0.063−0.200 μm), Wako gel C- 200, Fuji Silysia PSQ-100B or Fuji Silysia FL60D]. Thin layer chromatography was performed with silica gel (Merck TLC or HPTLC plates, Silica Gel 60 F254). Medium-pressure preparative liquid chromatography was performed with a medium-pressure preparative liquid chromatograph W-prep 2XY (manufactured by Yamazen Corporation; column: main column size S-5L, inject column size SS-2L).

The following abbreviations for solvents and reagents are used: DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EtOH, ethanol; EtOAc, ethyl acetate; MeOH methanol; THF, tetrahydrofuran; CH_2Cl_2, dichloromethane; *tert*-BuOMe, *tert*-butyl methyl ether; iPr_2O, diisopropyl ether; CH_3CN, acetonitrile; Et_3N, triethylamine; TFA, trifluoroacetic acid; IPA, isopropyl alcohol;

Experimental Procedure

Scheme 2-2-1A

2-{(2*R*,4a*R*,5*S*,6*R*,7a*S*)-5-[(4-chloro-3-methylphenoxy)methyl]-6-hydroxyoctahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (4e)



To a solution of **12** (30.0 mg, 0.081 mmol), 3-methyl-4-chlorophenol (15.0 mg, 0.105 mmol) and 1,1'-azobis(*N*,*N*-dimethylformamide) (27.9 mg, 0.162 mmol) in THF (0.8 mL) at room temperature was added tributylphosphine (40 μL, 0.162 mmol)). After the mixture was stirred at room temperature for 16 h, concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography S, hexane/EtOAc 4:1-3:2) gave **12-2** (26.3 mg) in 66% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.20 (d, *J* = 8.7 Hz, 1H), 6.75 (d, *J* = 3.0 Hz, 1H), 6.64 (dd, *J* = 8.7, 3.0 Hz, 1H), 5.17 (t, *J* = 5.4 Hz, 1H), 5.07 (m, 1H), 4.40 (q, *J* = 6.9 Hz, 2H), 4.32 (m, 1H), 4.02 (m, 2H), 2.50-2.43 (m, 1H), 2.41 (m, 1H), 2.33 (s, 3H), 2.32-2.22 (m, 1H), 2.16 (m, 1H), 2.09 (s, 3H), 2.06-1.97 (m, 2H), 1.92 (m, 1H), 1.74 (m, 1H), 1.40 (t, *J* = 6.9 Hz, 3H).

To a solution of **12-2** (26.3 mg, 0.053 mmol) in MeOH (1.0 mL) at room temperature was added 2.0 M sodium hydroxide (0.14 mL 0.28 mmol). After stirred at room temperature for 16 h, the reaction mixture was quenched with 1.0 M hydrochloric acid and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. Concentration gave **4e** (25.0 mg) in 100 % yield.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.35 (s, 1H), 7.27 (d, *J* = 8.7 Hz, 1H), 6.95 (d, *J* = 3.0 Hz, 1H), 6.79 (dd, *J* = 8.7, 3.0 Hz, 1H), 5.07 (t, *J* = 6.6 Hz, 1H), 4.89 (m, 1H), 4.14-4.04 (m, 2H), 3.92 (m, 1H), 3.84 (m, 1H), 2.26 (s, 3H), 2.21-2.04 (m, 3H), 1.90-1.63 (m, 5H). (Peak of CO_2H was not observed.) ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.32, 20.68, 24.19, 38.80, 41.92, 45.65, 67.20, 72.46, 72.79, 73.59, 113.11, 117.08, 126.30, 129.04, 129.70, 137.18, 145.96, 157.12, 162.24, 174.54. LCMS (ELSD) *RT* = 0.95 min (>983%). MS (FAB, Neg.) *m/z* 422 $(M - H)^-$.

HRMS (FAB, Neg.) $C_{20}H_{21}{}^{35}ClNO_5S$ $(M - H)^-$ calc. mass 422.0829, found 422.0829.

All compounds in Table 2-2-3 were synthesized in the same procedure.

2-[(2*R*,4a*R*,5*S*,6*R*,7a*S*)-5-{[4-chloro-3-(trifluoromethyl)phenoxy]methyl}-6-hydroxyoctahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylic acid (4c)

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.36 (s, 1H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.34 (d, *J* = 3.0 Hz, 1H), 7.27 (dd, *J* = 8.4, 3.0 Hz, 1H), 5.08 (t, *J* = 6.0 Hz, 1H), 4.90 (m, 1H), 4.18-4.04 (m, 3H), 3.87 (m, 1H), 2.20 (m, 1H), 2.11 (m, 2H), 1.90-1.64 (m, 5H). (Peak of CO_2H was not observed.) LCMS (ELSD) *RT* = 0.95 min (>98%). MS (FAB, Neg.) *m/z* 476 $(M - H)^-$. HRMS (FAB, Neg.) $C_{20}H_{18}{}^{35}ClF_3NO_5S$ $(M - H)^-$ calc. mass 476.0546, found 476.0549.

2-{(2R,4aR,5S,6R,7aS)-5-[(3,4-dichlorophenoxy)methyl]-6-hydroxyoctahydrocyclopenta[b]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (4d)

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.37 (s, 1H), 6.83 (d, J = 9.0 Hz, 1H), 6.54 (d, J = 2.7 Hz, 1H), 6.42 (dd, J = 9.0, 2.7 Hz, 1H), 5.08 (t, J = 6.6 Hz, 1H), 4.87 (m, 1H), 4.12 (m, 1H), 4.00 (m, 1H), 3.89 (m, 2H), 3.72 (s, 3H), 3.67 (s, 3H), 2.18 (m, 1H), 2.08 (m, 2H), 1.90-1.69 (m, 5H). (Peak of CO_2H was not observed.) LCMS (ELSD) *RT* = 0.96 min (>98%). MS (FAB, Neg.) *m/z* 442 $(M - H)^-$. HRMS (FAB, Neg.) $C_{19}H_{18}{}^{35}Cl_2NO_5S$ $(M - H)^-$ calc. mass 442.0283, found 442.0278.

2-{(2*R*,4a*R*,5*S*,6*R*,7a*S*)-5-[(4-chloro-3-ethylphenoxy)methyl]-6-hydroxyoctahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (4f)

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.39 (s, 1H), 7.25 (d, *J* = 8.7 Hz, 1H), 6.91 (d, *J* = 3.0 Hz, 1H), 6.79 (dd, *J* = 8.7, 3.0 Hz, 1H), 5.08 (t, *J* = 6.3 Hz, 1H), 4.90 (m, 1H), 4.11 (m, 1H), 4.07 (m, 1H), 3.95 (m, 1H), 3.85 (m, 1H), 2.64 (q, *J* = 7.2 Hz, 2H), 2.28-2.04 (m, 4H), 1.95-1.59 (m, 4H), 1.15 (t, *J* = 7.2 Hz, 3H). (Peak of CO_2H was not observed.) LCMS (ELSD) *RT* = 0.98 min (>98%). MS (FAB, Neg.) *m/z* 436 $(M - H)^-$. HRMS (FAB, Neg.) $C_{21}H_{23}{}^{35}ClNO_5S$ $(M - H)^-$ calc. mass 436.0985, found 436.0988.

2-{(2*R*,4a*R*,5*S*,6*R*,7a*S*)-5-[(4-chloro-3-isopropylphenoxy)methyl]-6-hydroxyoctahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (4g)

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 6.80 (d, *J* = 3.0 Hz, 1H), 6.64 (dd, *J* = 8.4, 3.0 Hz, 1H), 5.21 (t, *J* = 5.1 Hz, 1H), 4.21 (m, 2H), 4.04 (m, 1H), 3.93 (m, 1H), 3.35 (sep, *J* = 6.9 Hz, 1H), 2.43 (m, 1H), 2.28 (m, 2H), 2.20-2.05 (m, 2H), 1.95 (m, 1H), 1.87 (m, 1H), 1.76 (m, 1H), 1.22 (d, *J* = 6.9 Hz, 6H). (Peaks of O*H* and CO_2H were not observed.) LCMS (ELSD) *RT* = 1.02 min (>98%). MS (FAB, Neg.) *m/z* 450 $(M - H)^-$. HRMS (FAB, Neg.) $C_{22}H_{25}{}^{35}ClNO_5S$ $(M - H)^-$ calc. mass 450.1142, found 450.1140.

2-{(2*R*,4*aR*,5*S*,6*R*,7a*S*)-5-[(4-chloro-3-methoxyphenoxy)methyl]-6-hydroxyoctahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (4h)

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.24 (d, *J* = 9.0 Hz, 1H), 6.47 (d, *J* = 2.4 Hz, 1H), 6.41 (dd, *J* = 9.0, 2.4 Hz, 1H), 5.22 (t, *J* = 4.8 Hz, 1H), 4.21 (m, 2H), 4.04 (m, 1H), 3.94 (m, 1H), 3.87 (s, 3H), 2.47 (m, 1H), 2.28 (m, 2H), 2.20-2.07 (m, 2H), 1.90 (m, 1H), 1.87 (m, 1H), 1.76 (m, 1H). (Peaks of O*H* and CO_2H were not observed.) LCMS (ELSD) *RT* = 0.87 min (>98%). MS (FAB, Neg.) *m/z* 438 $(M - H)^-$. HRMS (FAB, Neg.) $C_{20}H_{18}{}^{35}ClF_3NO_6S$ $(M - H)^-$ calc. mass 438,0778, found 438.0779.

2-{(2*R*,4a*R*,5*S*,6*R*,7a*S*)-5-[(3,4-dimethylphenoxy)methyl]-6-hydroxyoctahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (4i)

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.33 (s, 1H), 6.99 (d, *J* = 8.4 Hz, 1H), 6.73 (d, *J* = 2.4 Hz, 1H), 6.64 (dd, *J* = 8.4, 2.4 Hz, 1H), 5.07 (t, *J* = 6.3 Hz, 1H), 4.88 (m, 1H), 4.11 (m, 1H), 4.02 (m, 1H), 3.86 (m, 2H), 2.21-2.03 (m, 3H), 2.16 (s, 3H), 2.12 (s, 3H), 1.87-1.61 (m, 5H). (Peak of CO_2H was not observed.) LCMS (ELSD) *RT* = 0.94 min (97.3%). MS (FAB, Neg.) *m/z* 402 $(M - H)^-$ HRMS (FAB, Neg.) $C_{21}H_{24}NO_5S$ $(M - H)^-$ calc. mass 402.1375, found 402.1381.

2-[(2*R*,4a*R*,5*S*,6*R*,7a*S*)-6-hydroxy-5-{[3-methyl-4-(trifluoromethyl)phenoxy]methyl}octahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylic acid (4j)

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.51 (d, *J* = 9.0 Hz, 1H), 6.77 (m, 1H), 6.72 (m, 1H), 5.22 (t, *J* = 4.8 Hz, 1H), 4.24-4.18 (m, 2H), 4.09 (dd, *J* = 9.0 , 5.4 Hz, 1H), 3.98 (dd, *J* = 9.0 , 6.3 Hz, 1H), 2.48 (m, 1H), 2.44 (m, 3H), 2.29 (m, 2H), 2.20-1.98 (m, 3H), 1.88 (m, 1H), 1.72 (m, 1H). (Peaks of O*H* and CO_2H were not observed.) LCMS (ELSD) *RT* = 0.96 min (>98%). MS (FAB, Neg.) *m/z* 456 $(M - H)^-$. HRMS (FAB, Neg.) $C_{21}H_{21}F_3NO_5S$ $(M - H)^-$ calc. mass 456.1093, found 456.1100.

2-{(2R,4aR,5S,6R,7aS)-5-[(4-chloro-3,5-dimethylphenoxy)methyl]-6-hydroxyoctahydrocyclopenta[b]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (4k)

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.14 (s, 1H), 6.79 (s, 2H), 5.05 (t, *J* = 6.6 Hz, 1H), 4.85 (m, 1H), 4.12 (m, 1H), 4.04 (dd, *J* = 9.6, 4.2 Hz, 1H), 3.91 (dd, *J* = 9.6, 6.3 Hz, 1H), 3.84 (m, 1H), 2.29 (s, 6H), 2.22-2.05 (m, 3H), 1.91-1.60 (m, 5H). (Peak of CO_2H was not observed.) LCMS (ELSD) *RT* = 0.98 min (>98%). MS (FAB, Neg.) *m/z* 436 $(M - H)^-$. HRMS (FAB, Neg.) $C_{21}H_{23}{}^{35}ClNO_5S$ $(M - H)^-$ calc. mass 436.0985, found 436.0978.

2-[(2*R*,4a*R*,5*S*,6*R*,7a*S*)-6-hydroxy-5-[(2,4,5-trichlorophenoxy)methyl]octahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (4l)

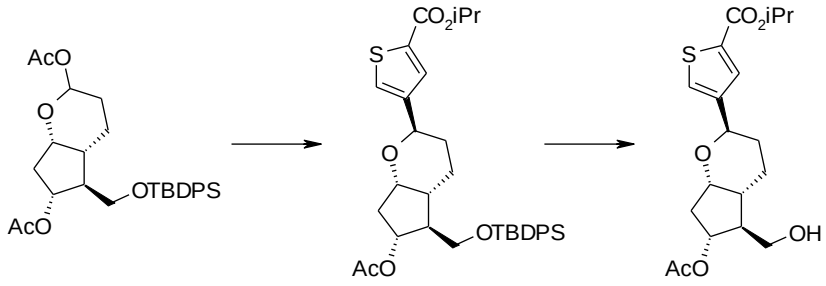
^{1}H NMR (300 MHz, DMSO-d_6) δ 8.36 (s, 1H), 7.79 (s, 1H), 7.46 (s, 1H), 5.09 (t, *J* = 5.7 Hz, 1H), 4.91 (m, 1H), 4.25 (dd, *J* = 9.6, 3.3 Hz, 1H), 4.11 (m, 2H), 3.87 (dt, J = 7.8, 6.6 Hz, 1H), 2.25-2.05 (m, 3H), 1.94-1.65 (m, 5H). (Peak of CO_2H was not observed.) LCMS (ELSD) *RT* = 1.01 min (>98%). MS (FAB, Neg.) *m/z* 476 $(M - H)^-$. HRMS (FAB, Neg.) $C_{19}H_{17}{}^{35}Cl_3NO_5S$ $(M - H)^-$ calc. mass 475.9893, found 475.9897.

2-[(2*R*,4a*R*,5*S*,6*R*,7a*S*)-6-hydroxy-5-[(2,3,4-trichlorophenoxy)methyl]octahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (4m)

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.27 (s, 1H), 7.60 (d, *J* = 9.0 Hz, 1H), 7.20 (d, *J* = 9.0 Hz, 1H), 5.08 (t, *J* = 6.0 Hz, 1H), 4.25 (dd, *J* = 9.9, 3.6 Hz, 1H), 4.10 (m, 2H), 3.89 (m, 1H), 2.29-2.04 (m, 3H), 1.86 (m, 3H), 1.71 (m, 2H). (Peaks of O*H* and CO_2H were not observed.) LCMS (ELSD) *RT* = 0.99 min (97.3%). MS (FAB, Neg.) *m/z* 476 $(M - H)^-$. HRMS (FAB, Neg.) $C_{19}H_{17}{}^{35}Cl_3NO_5S$ $(M - H)^-$ calc. mass 475.9893, found 475.9897.

Scheme 2-2-1B

Isopropyl4-[(2*R*,4a*R*,5*S*,6*R*,7a*S*)-6-acetoxy-5-(hydroxymethyl)octahydrocyclopenta[*b*]pyran-2-yl]-2-thiophenecarboxylate (19)



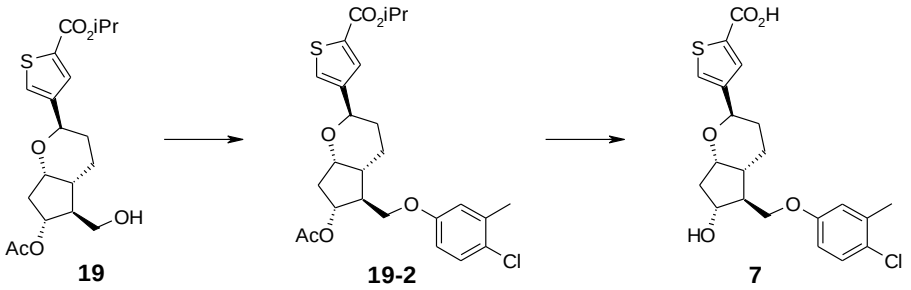
18 **18-2** **19**

To a solution of **18** (490 mg, 0.960 mmol) in acetonitrile (9.0 mL) at 0 °C was added bromo-(5-isopropoxycarbonyl-3-thienyl)zinc (1.00 M in acetonitrile, 1.91 mL, 1.91 mmol). After stirred at 0 °C for 5 min, aluminum chloride (256 mg, 1.91 mmol) was added and stirred at 0 °C for 5 min. The reaction was quenched with saturated aqueous potassium sodium tartrate and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography L, hexane/EtOAc 98:2-85:15) gave **18-2** (519 mg) in 87% yield.

18-2 ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.64-7.60 (m, 4H), 7.42-7.34 (m, 7H), 5.20 (sep, *J* = 6.3 Hz, 1H), 5.13 (m, 1H), 4.93 (m, 1H), 4.05 (m, 1H), 3.78 (dd, *J* = 10.5, 4.5 Hz, 1H), 3.64 (dd, *J* = 10.5, 4.2 Hz, 1H), 2.36 (m, 2H), 2.10 (m, 2H), 2.05 (s, 3H), 1.88-1.77 (m, 4H), 1.35 (d, *J* = 6.3 Hz, 6H), 1.03 (s, 9H).

To a solution of **18-2** (519 mg, 0.836 mmol) in THF (5.0 mL) at 0 °C was added acetic acid (151 mg, 2.51 mmol) and tetra-*n*-butylammonium fluoride (1.0 M in THF, 2.51 mL, 2.51 mmol). After stirred at room temperature for 16 h and stirred at 50 °C for 3h, the reaction mixture was quenched with 1.0M HCl and extracted with EtOAc. The organic layer was washed with saturated aqueous NaHCO3 and brine and dried over Na_2SO_4. Concentration and flash a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography M, hexane/EtOAc 3:2-1:3) gave **19** (317 mg) in 99% yield.

4-{(2*R*,4a*R*,5*S*,6*R*,7a*S*)-5-[(4-chloro-3-methylphenoxy)methyl]-6-hydroxyoctahydrocyclopenta[*b*]pyran-2-yl}-2-thiophenecarboxylic acid (7)



To a solution of **19** (60.0 mg, 0.157 mmol), 3-methyl-4-chlorophenol (67.1 mg, 0.471 mmol) and 1,1'-azobis(*N*,*N*-dimethylformamide) (81.0 mg, 0.471 mmol) in THF (1.0 mL) at room temperature was added tributylphosphine (95.2 mg, 0.471 mmol). After the mixture was stirred at 50 °C for 3 h, concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography S, hexane/EtOAc 9:1-2:3) gave **19-2** (75.2 mg) in 95% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, *J* = 1.5 Hz, 1H), 7.36 (d, *J* = 1.5 Hz, 1H), 7.19 (d, *J* = 8.7 Hz, 1H), 6.74 (d, *J* = 2.7 Hz, 1H), 6.65 (dd, *J* = 8.7, 2.7 Hz, 1H), 5.20 (sep, *J* = 6.3 Hz, 1H), 5.08 (m, 1H), 4.96 (t, *J* = 4.5 Hz, 1H), 4.11 (m, 1H), 4.06-3.98 (m, 2H), 2.53 (m, 1H), 2.39-2.29 (m, 1H), 2.32 (s, 3H), 2.14 (m, 1H), 2.07 (s, 3H), 1.93-1.85 (m, 4H), 1.74 (m, 1H), 1.35 (d, *J* = 6.3 Hz, 6H).

To a solution of **19-2** (24.0 mg, 0.0473 mmol) in MeOH (1.0 mL) at room temperature was added 2.0 M sodium hydroxide (0.20 mL, 0.40 mmol). After stirred at room temperature for 16 h, the reaction mixture was quenched with 1.0 M hydrochloric acid and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. Concentration gave **7** (17.3 mg) in 86 % yield

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (br s, 1H), 7.46 (br s, 1H), 7.18 (m, 1H), 6.75 (s, 1H), 6.64 (dd, *J* = 8.7, 3.0 Hz, 1H), 5.03 (m, 1H), 4.14 (m, 1H), 4.06 (m, 1H), 3.98 (m, 1H), 3.90 (m, 1H), 2.45 (m, 1H), 2.33 (s, 3H), 2.24 (m, 1H), 1.98 (m, 3H), 1.77 (m, 3H). (Peaks of O*H* and CO_2H were not observed.) LCMS (ELSD) *RT* = 1.01 min (>98%). MS (FAB, Neg.) *m/z* 421 $(M - H)^-$. HRMS (FAB, Neg.) $C_{21}H_{22}{}^{35}ClO_5S$ $(M - H)^-$ calc. mass 421.0876, found 421.0870.

Compound **6** and **8** were synthesized in the same procedure.

5-{(2*R*,4a*R*,5*S*,6*R*,7a*S*)-5-[(4-chloro-3-methylphenoxy)methyl]-6-hydroxyoctahydrocyclopenta[*b*]pyran-2-yl}-2-furoic acid (6)

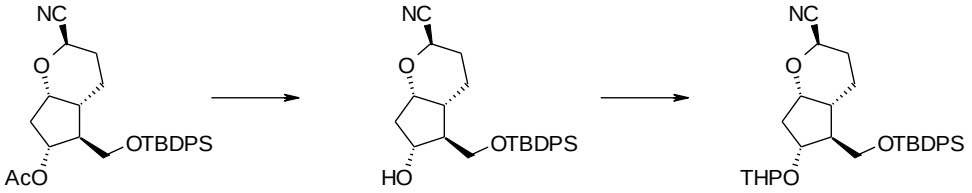
^{1}H NMR (300 MHz, DMSO-d_6) δ 7.27 (d, *J* = 8.7 Hz, 1H), 7.14 (d, *J* = 3.6 Hz, 1H), 6.94 (d, *J* = 2.7 Hz, 1H), 6.78 (dd, *J* = 8.7, 2.7 Hz, 1H), 6.57 (d, *J* = 3.6 Hz, 1H), 4.84 (m, 2H), 4.06 (m, 1H), 3.96 (m, 1H), 3.86 (m, 2H), 2.27 (s, 3H), 2.17 (m, 2H), 1.92 (m, 2H), 1.74 (m, 2H), 1.60 (m, 2H). (Peak of CO_2H was not observed.) LCMS (ELSD) *RT* = 0.96 min (>98%). MS (FAB, Neg.) *m/z* 405 $(M - H)^-$. HRMS (FAB, Neg.) $C_{21}H_{22}{}^{35}ClO_6$ $(M - H)^-$ calc. mass 405.1105, found 405.1100.

4-{(2*R*,4a*R*,5*S*,6*R*,7a*S*)-5-[(4-chloro-3-methylphenoxy)methyl]-6-hydroxyoctahydrocyclopenta[*b*]pyran-2-yl}-2-furoic acid (8)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (br s, 1H), 7.30 (br s, 1H), 7.20 (d, *J* = 9.0 Hz, 1H), 6.75 (d, *J* = 2.7 Hz, 1H), 6.64 (dd, *J* = 9.0, 2.7 Hz, 1H), 5.01 (m, 1H), 4.14 (m, 1H), 4.06 (m, 1H), 3.99 (m, 1H), 3.91 (m, 1H), 2.46 (m, 1H), 2.33 (s, 3H), 2.22 (m, 1H), 2.09-1.92 (m, 3H), 1.85-1.60 (m, 3H). (Peaks of O*H* and CO_2H were not observed.) LCMS (ELSD) *RT* = 0.82 min (>98%). MS (FAB, Neg.) *m/z* 405 $(M - H)^-$. HRMS (FAB, Neg.) $C_{21}H_{22}{}^{35}ClO_6$ $(M - H)^-$ calc. mass 405.1105, found 405.1114.

Scheme 2-2-1C

(4a*R*,5*S*,6*R*,7a*S*)-5-({[(2-methyl-2-propanyl)(diphenyl)silyl]oxy}methyl)-6-(tetrahydro-2*H*-pyran-2-yloxy)octahydrocyclopenta[*b*]pyran-2-carbonitrile (14)



13 **13-2** **14**

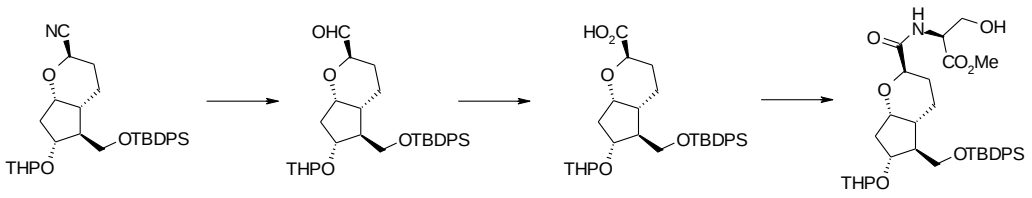
To a solution of **13** (200 mg, 0.42 mmol) in MeOH (2.0 mL) at room temperature was added K_2CO_3 (69 mg, 0.50 mmol). After the reaction mixture was stirred at room temperature for 5 h, H_2O (200 mL) was added and the

mixture was extracted with *tert*-BuOMe. The organic layer was washed with brine and dried over Na_2SO_4. Concentration gave **13-2** (196 mg), which was directly used in the next reaction.

To a solution of **13-2** (196 mg, crude) and pyridinium *para*-toluenesulfonate (10.0 mg, 0.040 mmol) in CH_2Cl_2 (2.0 mL) at room temperature was added 3,4-dihydro-2*H*-pyran (77 μg, 0.84 mmol). After stirred at room temperature for 14 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography S, hexane/EtOAc 97:3-4:1) gave **14** (227 mg) in 100% in 2 steps.

14 ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66-7.60 (m, 4H), 7.43-7.35 (m, 6H), 4.80 (m, 1H), 4.57-4.51 (m, 1H), 4.33-4.21 (m, 2H), 4.12 (m, 1H), 3.91-3.87 (m, 1H), 3.79-3.76 (m, 2H), 2.33-2.18 (m, 3H), 2.05-2.00 (m, 2H), 1.91-1.65 (m, 6H), 1.52-1.43 (m, 3H), 1.04 (s, 9H).

Methyl (2*R*)-3-hydroxy-2-({[(2*R*,4a*R*,5*S*,6*R*,7a*S*)-5-({[(2-methyl-2-propanyl)(diphenyl)silyl]oxy}methyl)-6-(tetrahydro-2*H*-pyran-2-yloxy)octahydrocyclopenta[*b*]pyran-2-yl]carbonyl}amino)propanoate (15)



14 **14-2** **14-3** **15**

To a solution of **14** (227 mg, 0.470 mmol) in CH_2Cl_2 (2.0 mL) at -78 °C was added diisobutylaluminiumhydride (1.00 M in toluene, 0.42 mL, 0.42 mmol). After stirred at -78 °C for 1h, the reaction was quenched with saturated aqueous potassium sodium tartrate and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration gave **14-2**, which was directly used in the next reaction.

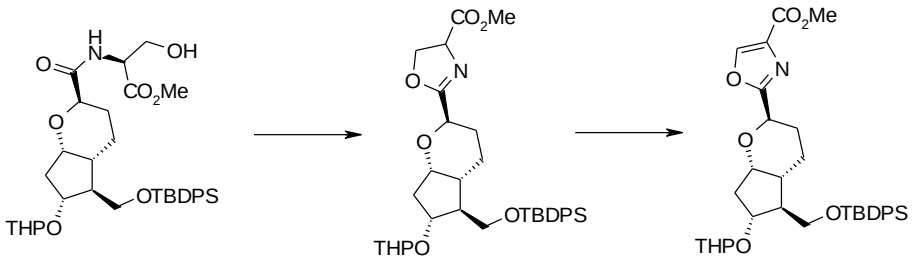
To a solution of **14-2** (166 mg, 0.320 mmol) in *tert*-butanol (1.2 mL), THF (1.0 mL) and H_2O (0.3 mL) at room temperature was added 2-methyl-2-butene (152 μL, 1.4 mmol) and sodium chlorite (72 mg, 0.80 mmol). After stirred for 1h, the reaction was quenched with 1M HCl and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography

(Yamazen W-prep 2XY flash column chromatography S, hexane/EtOAc 9:1-0:100) gave **14-3** (113 mg), including inseparable compound, which was used in the next reaction.

To a solution of **14-3** (109 mg, 0.200 mmol) in THF (2.0 mL) at -30 °C was added N-methyl morpholine (0.46 μL, 0.42 mmol) and isobutyl chloroformate (29 μL, 0.22 mmol). After stirred at -30 °C for 1h, L-serine methyl ester-HCl (34 mg, 0.22 mmol) was added to the reaction mixture. After stirred at room temperature for 14 h, the reaction was quenched with brine and extracted with EtOAc. The organic layer was dried over $MgSO_4$. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography S, hexane/EtOAc 4:1-0:100) gave **15** (78 mg) in 61%.

15 ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67-7.59 (m, 4H), 7.45-7.34 (m, 6H), 4.70 (m, 1H), 4.59-4.49 (m, 1H), 4.25 (m, 2H), 4.18 (m, 1H), 4.05-3.97 (m, 2H), 3.93 (m, 1H), 3.87 (m, 1H), 3.81-3.80 (m, 3H), 3.74 (m, 2H), 2.36 (m, 1H), 2.24-2.18 (m, 2H), 2.03-1.92 (m, 2H), 1.88-1.67 (m, 5H), 1.54-1.40 (m, 4H), 1.05 (s, 9H). (Peaks of O*H* and N*H* were not observed.)

Methyl 2-[(2*R*,4a*R*,5*S*,6*R*,7a*S*)-5-({[(2-methyl-2-propanyl)(diphenyl)silyl]oxy}methyl)-6-(tetrahydro-2*H*-pyran-2-yloxy)octahydrocyclopenta[b]pyran-2-yl]-1,3-oxazole-4-carboxylate (16)



15 **15-2** **16**

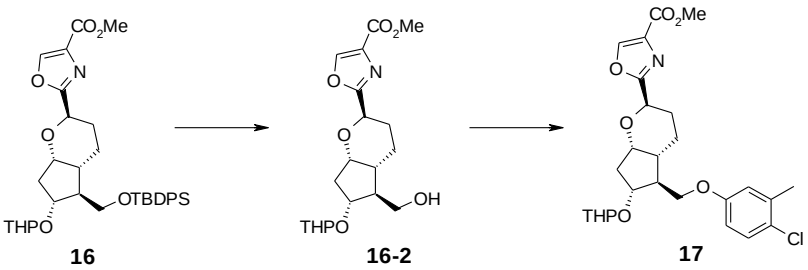
To a solution of **15** (209 mg, 0.327 mmol) in THF (3.0 mL) at room temperature was added Burgess reagent (117 mg, 0.49 mmol). After stirred under reflux for 2.5 h, the reaction mixture was concentrated and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography S, hexane/EtOAc 3:1- MeOH/EtOAc 3:7) gave **15-2** (46 mg) in 22%

To a solution of **15-2** (46 mg, 0.074 mmol) in CH_2Cl_2 (1.0 mL) at 0 °C was added DBU (17 μL, 0.11 mmol) and $BrCl_3$ (11 μL, 0.11 mmol). After stirred at 0 °C for 8h, the reaction was quenched with saturated aqueous NH_4Cl

and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography S, hexane/EtOAc 85:15-65:35) gave **16** (33 mg) in 72%

16 ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.68-7.60 (m, 4H), 7.44-7.35 (m, 6H), 4.64-4.53 (m, 1H), 4.30-4.05 (m, 2H), 3.92 (s, 3H), 3.88 (m, 1H), 3.78-3.65 (m, 2H), 3.48 (m, 2H), 2.34 (m, 1H), 2.24-2.18 (m, 2H), 2.03-1.91 (m, 2H), 1.83-1.67 (m, 5H), 1.54-1.40 (m, 4H), 1.04 (s, 9H).

Methyl 2-[(2*R*,4a*R*,5*S*,6*R*,7a*S*)-5-[(4-chloro-3-methylphenoxy)methyl]-6-(tetrahydro-2*H*-pyran-2-yloxy octa hydrocyclopenta[*b*]pyran-2-yl]-1,3-oxazole-4-carboxylate (17)



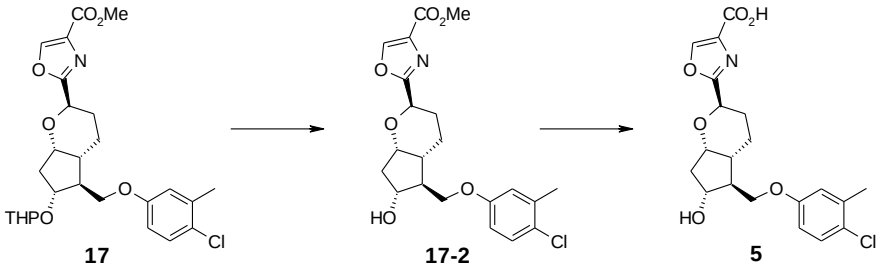
To a solution of **16** (58 mg, 0.090 mmol) in THF (1.0 mL) at room temperature was added tetra-*n*-butylammonium fluoride (1.00 M in THF, 140 μL, 0.140 mmol). After stirred at room temperature for 16 h, the reaction mixture was quenched with H_2O and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and flash column chromatography (Fuji silicia BW-820MH, hexane / EtOAc 15:85-0:100) gave alcohol **16-2** (23 g) in 67% yield.

16-2 ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (s, 1H), 5.04 (m, 1H), 4.76-4.58 (m, 1H), 4.06 (m, 1H), 3.91 (s, 3H), 3.89-3.74 (m, 2H), 3.60 (m, 1H), 3.50 (m, 1H), 3.13 (m, 1H), 2.44-2.23 (m, 3H), 2.20-2.03 (m, 4H), 1.96-1.45 (m, 7H). (Peak of O*H* was not observed.)

To a solution of **16-2** (36.5 mg, 0.0962 mmol), 3-methyl-4-chlorophenol (41.0 mg, 0.288 mmol) and 1,1'-azobis(*N*,*N*-dimethylformamide) (49.0 mg, 0.284 mmol) in THF (1.0 mL) at room temperature was added tributylphosphine (0.071 mL, 0.287 mmol). After the mixture was stirred at 50 °C for 3 h, concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography S, hexane/EtOAc 9:1-2:3) gave **17** (75.2 mg) in 95% yield.

17 ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.20 (m, 1H), 6.77 (m, 1H), 6.60 (m, 1H), 5.05 (m, 1H), 4.65 (m, 1H), 4.25 (m, 1H), 4.06 (m, 2H), 3.96 (m, 1H), 3.91 (s, 3H), 3.44 (m, 2H), 2.58-2.40 (m, 2H), 2.33 (s, 3H), 2.19-2.09 (m, 3H), 1.89-1.68 (m, 4H), 1.68-1.49 (m, 5H).

2-{(2*R*,4a*R*,5*S*,6*R*,7a*S*)-5-[(4-chloro-3-methylphenoxy)methyl]-6-hydroxyoctahydrocyclopenta[*b*]pyran-2-yl} -1,3-oxazole-4-carboxylic acid (5)



To a solution of **17** (46.5 mg, 0.0923 mmol) in MeOH (1.5 mL) at room temperature was added *p*-toluene sulfonic acid monohydrate (3.0 mg, 0.0157 mmol). After stirred at room temperature for 4 h, the reaction mixture was quenched with H_2O and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography S, hexane/EtOAc 4:1-0:100) gave **17-2** (38.9 mg) in 93% yield.

17-2 ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.19 (d, *J* = 8.7 Hz, 1H), 6.75 (d, *J* = 2.7 Hz, 1H), 6.65 (dd, *J* = 8.7, 2.7 Hz, 1H), 5.10 (m, 1H), 4.19-4.12 (m, 2H), 4.09 (m, 1H), 4.02 (m, 1H), 3.92 (s, 3H), 2.63 (m, 1H), 2.45 (m, 1H), 2.33 (s, 3H), 2.19 (m, 3H), 2.09 (m, 1H), 1.94 (m, 1H), 1.84 (m, 1H), 1.74 (m, 1H).

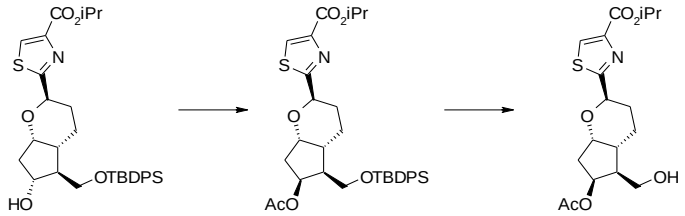
To a solution of **17-2** (10.0 mg, 0.0237 mmol) in MeOH (1.0 mL) at room temperature was added 2.0 M sodium hydroxide (0.20 mL, 0.4 mmol). After stirred at room temperature for 18 h, the reaction mixture was quenched with 1.0 M hydrochloric acid and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. Concentration gave **5** (8.4 mg) in 87 % yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.20 (d, *J* = 8.7 Hz, 1H), 6.75 (d, *J* = 3.0 Hz, 1H), 6.65 (dd, *J* = 8.7, 3.0 Hz, 1H), 5.12 (m, 1H), 4.15 (m, 1H), 4.10 (m, 1H), 4.03 (m, 1H), 3.93 (m, 1H), 2.46 (m, 1H), 2.33 (s, 3H), 2.20 (m, 2H), 2.17-2.06 (m, 2H), 1.95 (m, 1H), 1.87 (m, 1H), 1.77 (m, 1H). (Peaks of O*H* and CO_2H were not observed.) LCMS (ELSD) *RT* = 0.89 min (>98%). MS (FAB, Neg.) *m/z* 406 $(M - H)^-$. HRMS (FAB, Neg.)

$C_{20}H_{21}{}^{35}ClNO_6$ $(M - H)^-$ calc. mass 406.1057, found 406.1051.

Scheme 2-2-1D

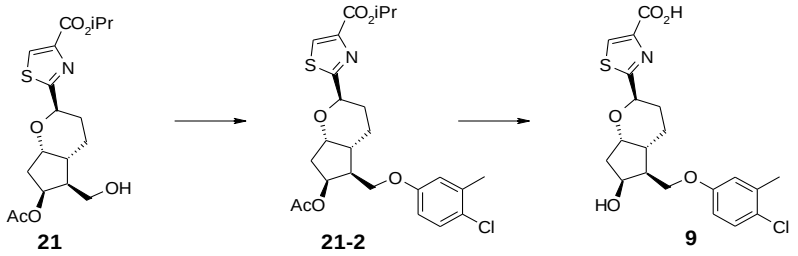
Isopropyl 2-[(2*R*,4a*R*,5*S*,6*R*,7a*S*)-6-acetoxy-5-(hydroxymethyl)octahydrocyclopenta[b]pyran-2-yl]-1,3-thiazole-4-carboxylate (21)



To a solution of **20** (250 mg, 0.431 mmol) and acetic acid (51.8 mg, 0.862 mmol) in THF (2.0 mL) at room temperature was added triphenylphosphine (226 mg, 0.862 mmol) and diethyl azodicarboxylate (2.2 M in toluene, 0.391 mL, 0.862 mmol). After stirred at room temperature for 2 h, the reaction mixture was concentrated. A medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography M, hexane/EtOAc 9:1-7:3-1:1) gave **20-2** (312 mg), including inseparable compound, which was used in the next reaction.

To a solution of **20-2** (268 mg, crude) in THF (2.0 mL) and acetic acid (77.7 mg, 1.29 mmol) at room temperature was added tetra-*n*-butylammonium fluoride (1.00 M in THF,1.29 mL, 1.29 mmol). After stirred at 50 °C for 2 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography M, hexane/EtOAc 3:7-0:1) gave **21** (78.9 mg) in 48% yield.

2-{(2*R*,4a*R*,5*S*,6*R*,7a*S*)-5-[(4-chloro-3-methylphenoxy)methyl]-6-hydroxyoctahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (9)



To a solution of **21** (70.0 mg, 0.182 mmol) and 3-methyl-4-chlorophenol (78.1 mg, 0.548 mmol) and 1,1'-azobis(*N*,*N*-dimethylformamide) (94.2 mg, 0.547 mmol) in THF (2.0 mL) at room temperature was added tributylphosphine (0.140 mL, 0.547 mmol). After the mixture was stirred at room temperature for 3 h, concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography S, hexane/EtOAc 95:5-3:2-1:4) gave **21-2** (75.2 mg) in 100% yield.

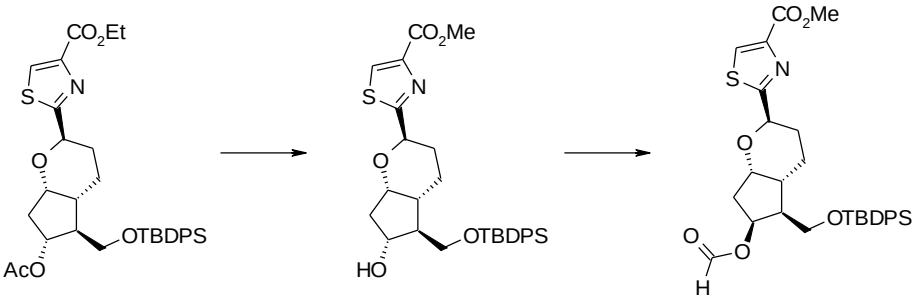
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.20 (d, *J* = 8.7 Hz, 1H), 6.74 (d, *J* = 3.0 Hz, 1H), 6.64 (dd, *J* = 8.7, 3.0 Hz, 1H), 5.51 (m, 1H), 5.26 (sep, *J* = 6.3 Hz, 1H), 5.10 (t, *J* = 6.0 Hz, 1H), 4.30 (m, 1H), 4.03 (m, 1H), 4.04-3.90 (m, 2H), 2.62 (m, 1H), 2.33 (s, 3H), 2.25 (m, 1H), 2.19 (m, 1H), 2.17-2.01 (m, 3H), 2.04 (s, 3H), 1.67 (m, 1H), 1.37 (d, *J* = 6.3 Hz, 6H).

To a solution of **21-2** (36.0 mg, 0.0708 mmol) in MeOH (1.0 mL) at room temperature was added 2.0 M sodium hydroxide (0.071 mL, 0.142 mmol). After stirred at room temperature for 2 h, the reaction mixture was quenched with 1.0 M hydrochloric acid and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. Concentration gave **9** (30.8 mg) in 100 % yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.22 (d, *J* = 9.0 Hz, 1H), 6.79 (d, *J* = 2.7 Hz, 1H), 6.69 (dd, *J* = 9.0, 2.7 Hz, 1H), 5.10 (t, *J* = 6.0 Hz, 1H), 4.63 (m, 1H), 4.31 (m, 1H), 4.10 (m, 2H), 2.49 (m, 1H), 2.34 (s, 3H), 2.24 (m, 1H), 2.20-1.95 (m, 5H), 1.60 (m, 1H). (Peaks of O*H* and CO_2H were not observed.) ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.32, 20.68, 24.19, 38.80, 41.92, 45.65, 67.20, 72.46, 72.79, 73.59, 113.11, 117.08, 126.30, 129.04, 129.70, 137.18, 145.96, 157.12, 162.24, 174.54. LCMS (ELSD) *RT* = 0.95 min (>98%). MS (FAB, Neg.) *m/z* 422 $(M - H)^-$. HRMS (FAB, Neg.) $C_{20}H_{21}{}^{35}ClNO_5S$ $(M - H)^-$ calc. mass 422.0829, found 422.0829.

Scheme 2-2-1E

Ethyl 2-[(2*R*,4a*R*,5*S*,6*R*,7a*S*)-6-(formyloxy)-5-({[(2-methyl-2-propanyl)(diphenyl)silyl]oxy}methyl)octahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylate (23)



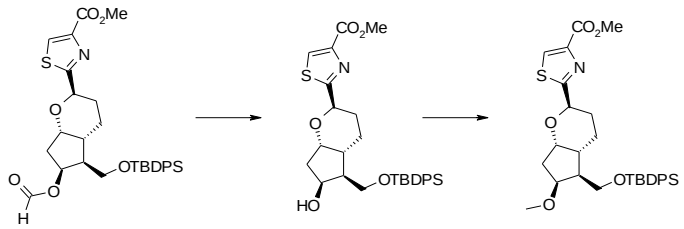
22 **22-2** **23**

To a solution of **22** (3.3 g, 5.40 mmol) in MeOH (27 mL) at room temperature was added K_2CO_3 (1.50 g, 11.0 mmol). After the reaction mixture was stirred at room temperature for 5 h, saturated aqueous NH_4Cl was added and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography L, hexane/EtOAc 19:1-7:3-1:1-0:1) gave **22-2** (2.76 g) in 93% yield.

22-2 ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.65-7.59 (m, 4H), 7.41-7.34 (m, 6H), 5.16 (t, *J* = 5.1 Hz, 1H), 4.20-4.12 (m, 2H), 3.94 (s, 3H), 3.78 (dd, *J* = 10.2, 4.8 Hz, 1H), 3.61 (dd, *J* = 10.2, 6.0 Hz, 1H), 2.62 (m, 1H), 2.12-2.05 (m, 5H), 1.96-1.86 (m, 2H), 1.79 (m, 1H), 1.04 (s, 9H).

To a solution of **22-2**, phenol (2.70 g, 4.89 mmol) and triphenyl phosphine (2.57 g, 9.79 mmol) in THF (20 mL) at room temperature was added diethyl azodicarboxylate (2.2 M in toluene, 4.45 mL, 9.79 mmol). After stirred at room temperature for 1 h, the reaction mixture was concentrated. A medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography L, hexane/EtOAc 19:1-7:3-1:1-0:1) gave **23** (2.99 g), including inseparable compound, which was used in the next reaction.

Ethyl 2-[(2*R*,4a*R*,5*S*,6*R*,7a*S*)-6-methoxy-5-({[(2-methyl-2-propanyl)(diphenyl)silyl]oxy}methyl)octahydrocyclopenta[*b*]pyran-2-yl]-1,3-thiazole-4-carboxylate (24)



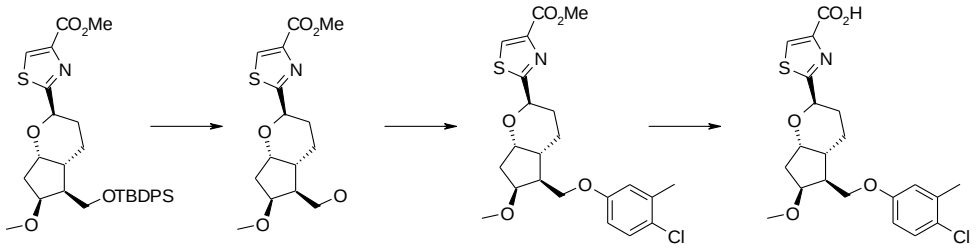
To a solution of **23** (2.84 g, 4.89 mmol) in MeOH (20 mL) at room temperature was added K_2CO_3 (1.35 g, 9.79 mmol). After the reaction mixture was stirred at room temperature for 5 h, saturated aqueous NH_4Cl was added and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography L, hexane/EtOAc 19:1-7:3-1:1-0:1) gave **23-2** (2.43 g) in 90% yield.

23-2 ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.68-7.63 (m, 4H), 7.47-7.36 (m, 6H), 5.03 (t, *J* = 5.7 Hz, 1H), 4.60 (m, 1H), 4.30 (m, 1H), 3.95 (m, 1H), 3.94 (s, 3H), 3.80 (dd, *J* = 10.2, 6.3 Hz, 1H), 3.01 (m, 1H), 2.14-1.94 (m, 7H), 1.78 (m, 1H), 1.06 (s, 9H).

To a solution of **23-2** (400 mg, 0.725 mmol) in acetonitrile (2.0 mL) at room temperature was added methyliodide (206 mg, 1.45 mmol) and Ag_2O (336 mg, 1.45 mmol). After the reaction mixture was stirred at room temperature for 4 days, filterated wit celite pad and the filtrate was concentrated and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography M, hexane/EtOAc 95:5-6:4-0:1) gave **24** (89.5 mg) in 22% yield.

24 ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.69-7.62 (m, 4H), 7.41-7.35 (m, 6H), 5.05 (t, *J* = 6.6 Hz, 1H), 4.25 (m, 1H), 3.95 (m, 1H), 3.93 (s, 3H), 3.89 (m, 1H), 3.64 (m, 1H), 3.25 (s, 3H), 2.25-2.09 (m, 4H), 1.98-1.87 (. (m, 4H), 1.05 (s, 9H).

2-{(2*R*,4a*R*,5*S*,6*R*,7a*S*)-5-[(4-chloro-3-methylphenoxy)methyl]-6-methoxyoctahydrocyclopenta[*b*]pyran-2-yl}-1,3-thiazole-4-carboxylic acid (10)



24 **24-2** **24-3** **10**

To a solution of **24** (86 mg, 0.152 mmol) in THF (2.0 mL) at room temperature was added tetra-*n*-butylammonium fluoride (1.00 M in THF, 0.304 mL, 0.304 mmol). After stirred at room temperature for 2 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography S, hexane/EtOAc 6:4-0:1) gave alcohol **24-2** (32.5 mg), including inseparable compound, which was used in the next reaction.

To a solution of **24-2** (32.0 mg, 0.0977 mmol) and 3-methyl-4-chlorophenol (41.8 mg, 0.293 mmol) and 1,1'-azobis(*N*,*N*-dimethylformamide) (50.5 mg, 0.293 mmol) in THF (1.0 mL) at room temperature was added tributylphosphine (59.3 mg, 0.293 mmol). After the mixture was stirred at room temperature for 3 h, concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography S, hexane/EtOAc 95:5-7:3-1:1) gave **24-3** (40.8 mg) in 59% yield in 2 steps.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.21 (d, *J* = 8.7 Hz, 1H), 6.78 (d, *J* = 2.7 Hz, 1H), 6.69 (dd, *J* = 8.7, 2.7 Hz, 1H), 5.09 (t, *J* = 6.3 Hz, 1H), 4.31 (m, 1H), 4.01-3.89 (m, 2H), 3.94 (s, 3H), 3.25 (s, 3H), 2.45 (m, 1H), 2.34 (s, 3H), 2.33-2.25 (m, 2H), 2.19 (m, 1H), 2.05-1.90 (m, 4H), 1.60 (m, 1H).

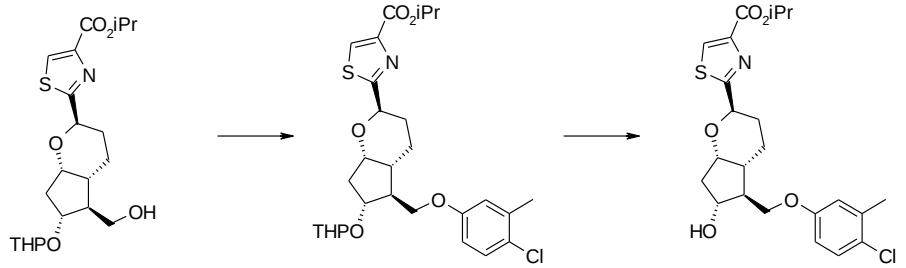
To a solution of **24-3** (16.0 mg, 0.0354 mmol) in MeOH (2.0 mL) at room temperature was added 2.0 M sodium hydroxide (0.035 mL, 0.071 mmol). After stirred at room temperature for 2 h, the reaction mixture was quenched with 1.0 M hydrochloric acid and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. Concentration gave **10** (15.8 mg) in 100 % yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.20 (d, *J* = 9.0 Hz, 1H), 6.78 (d, *J* = 2.7 Hz, 1H), 6.68 (dd, *J* = 9.0, 2.7 Hz, 1H), 5.09 (t, *J* = 6.3 Hz, 1H), 4.26 (m, 1H), 4.16 (dd, *J* = 9.3, 7.5 Hz, 1H), 3.99 (m, 1H), 3.90 (dd, *J* = 9.3, 6.6 Hz, 1H), 3.25 (s, 3H), 2.43 (m, 1H), 2.34 (s, 3H), 2.29 (m, 1H), 2.18 (m, 1H), 2.09-1.92 (m, 4H), 1.60 (m, 1H). (Peaks of CO_2H was not observed.) LCMS (ELSD) *RT* = 1.12 min (>98%). MS (FAB, Neg.) *m/z* 436 $(M - H)^-$.

HRMS (FAB, Neg.) $C_{21}H_{23}{}^{35}ClNO_5S$ $(M - H)^-$ calc. mass 436.0985, found 436.0988.

Scheme 2-2-1F

Isopropyl 2-{(2*R*,4a*R*,5*S*,6*R*,7a*S*)-5-[(4-chloro-3-methylphenoxy)methyl]-6 hydroxyoctahydrocyclopenta [*b*] pyran-2-yl}-1,3-thiazole-4-carboxylate (26)



25 **25-2** **26**

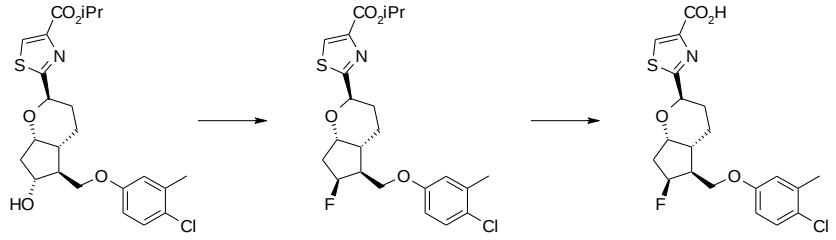
To a solution of **25** (500 mg, 1.175 mmol) and 3-methyl-4-chlorophenol (503 mg, 3.53 mmol) and 1,1'-azobis(*N*,*N*-dimethylformamide) (405 mg, 2.35 mmol) in THF (4.0 mL) at room temperature was added tributylphosphine (0.587 mL, 2.35 mmol). After the mixture was stirred at room temperature for 4 h, concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography L, hexane/EtOAc 95:5-75:25) gave **25-2**, including inseparable compound, which was used in the next reaction.

To a solution of **25-2** (500 mg, crude) in MeOH (8.4 mL mL) at room temperature was added *p*-toluene sulfonic acid monohydrate (19.3 mg, 0.102 mmol). After stirred at room temperature for 2 h, the reaction mixture was quenched with triethylamine. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography L, hexane/EtOAc 3:2-2:3) gave **26** (444 mg) in 81% yield in 2 steps

26 ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.19 (d, *J* = 8.7 Hz, 1H), 6.75 (d, *J* = 2.4 Hz, 1H), 6.65 (dd, *J* = 8.7, 2.4 Hz, 1H), 5.26 (sep, *J* = 6.3 Hz, 1H), 5.20 (t, *J* = 4.8 Hz, 1H), 4.24 (m, 1H), 4.15 (m, 1H), 4.02 (m, 1H), 3.90 (m, 1H), 2.61 (m, 1H), 2.41 (m, 1H), 2.33 (s, 3H), 2.31-2.26 (m, 2H), 2.17-1.96 (m, 3H), 1.85 (m, 1H), 1.71 (m, 1H), 1.38 (d, *J* = 6.3 Hz, 6H).

2-{(2*R*,4a*R*,5*S*,6*R*,7a*S*)-5-[(4-chloro-3-methylphenoxy)methyl]-6-fluorooctahydrocyclopenta[*b*]pyran-2-yl}-1

,3-thiazole-4-carboxylic acid (11)



26 **26-2** **11**

To a solution of diethylaminofsulfur trifluoride (103 mg, 0.643 mmol) in CH_2Cl_2 (1.5 mL) at -78 °C was added **26** (100 mg, 0.215 mmol) in CH_2Cl_2 (0.75 mL). After stirred at -78 °C for 30 min, the reaction was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Concentration and a medium-pressure preparative liquid chromatography (Yamazen W-prep 2XY flash column chromatography L, hexane/EtOAc 97:3-7:3) gave **26-2** (100 mg) in 57% yield.

26-2 ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.20 (d, *J* = 9.0 Hz, 1H), 6.79 (d, *J* = 3.0 Hz, 1H), 6.79 (dd, *J* = 9.0, 3.0 Hz, 1H), 5.53-5.19 (m, 1H), 5.27 (sep, *J* = 6.0 Hz, 1H), 5.10 (t, *J* = 6.0 Hz, 1H), 4.36 (m, 1H), 4.15 (m, 1H), 3.98 (m, 1H), 2.65-2.42 (m, 1H), 2.43-2.24 (m, 2H), 2.34 (s, 3H), 2.22-1.97 (m, 4H), 1.64 (m, 1H), 1.37 (d, *J* = 6.0 Hz, 6H).

To a solution of **26-2** (23.0 mg, 0.0491 mmol) in MeOH (0.30 mL) at room temperature was added 2.0 M sodium hydroxide (0.15 mL, 0.300 mmol). After stirred at room temperature for 2 h, the reaction mixture was quenched with 1.0 M hydrochloric acid and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. Concentration gave **11** (18.8 mg) in 89 % yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.23 (d, *J* = 9.0 Hz, 1H), 6.79 (d, *J* = 3.0 Hz, 1H), 6.70 (dd, *J* = 9.0, 3.0 Hz, 1H), 5.48-5.22 (m, 1H), 5.11 (t, *J* = 6.3 Hz, 1H), 4.34 (m, 1H), 4.18 (m, 1H), 3.99 (m, 1H), 2.69 (m, 1H), 2.49 (m, 1H), 2.34 (s, 3H), 2.34-2.22 (m, 2H), 2.21-2.03 (m, 3H), 1.66 (m, 1H). (Peak of CO_2H was not observed.) LCMS (ELSD) *RT* = 1.10 min (>98%). MS (FAB, Neg.) *m/z* 424 $(M - H)^-$. HRMS (FAB, Neg.) $C_{20}H_{20}{}^{35}ClFNO_4S$ $(M - H)^-$ calc. mass 424.0786, found 424.0792.

Biology

In vitro assay

EP2, EP4 and IP cAMP assay

Chinese hamster ovary (CHO) cells (1.25×10^5 cells/well) expressing human EP2 or human EP4 or human IP receptor were harvested and suspended in a 96-well 1/2 area plate. cAMP concentrations were measured using a cAMP HTRF HiRange kit (Cisbio Bioassays)* after treatment of compounds. The reaction rate (%) of the compounds relative to the cAMP concentration obtained with PGE_2 treatment at 1μM was calculated. Furthermore, a non-linear regression analysis was performed using the Sigmoid Emax Model to estimate EC_{50} values.

*http://www.cisbio.com/usa/drug-discovery/membrane-based-assays-camp-hirange-assay-kit (accessed Nov 24, 2015)

EP2 β arrestin recruitment assay

PathHunter β-arrestin HEK-293 PTGER2 cell lines (DiscoveRx) were seeded at a density of 5000 cells/well into a 384-well plate and cultured at 37 °C in the presence of 5 % CO_2 for 24 hours. β-arrestin recruitment were measured using a PathHunter Detection Kit (DiscoveRx)* after treatment of compounds. The reaction rate (%) of the compounds relative to the β-arrestin recruitment obtained with PGE_2 treatment at 10μM was calculated. Furthermore, a non-linear regression analysis was performed using the Sigmoid Emax Model to estimate EC_{50} values.

* https://www.discoverx.com/product-data-sheets-3-tab/93-0214c1 (accessed Nov 24, 2015)

EP1, EP3 and FP Ca assay

Chem-1 cells expressing human FP receptor or Chinese hamster ovary (CHO) cells expressing human EP1 or human EP3 were seeded at a density of 1×10^4 cells per well into 96-well plates and cultured at 37℃ in the presence of 5% CO_2 for 2 days. Load buffer (HBSS containing Calcium 5, 10 mM HEPES, 20μM indomethacin, and 2.5 mM probenecid) was added in each well and incubated in the dark at room temperature for 1 hour. After addition of the compounds, intracellular Ca^{2+} concentration was measured using a fluorescence drug screening

system (FDSS-7000 : Hamamatsu Photonics, Tokyo, Japan)*. The reaction rate (%) of the compounds relative to intracellular Ca^{2+} concentration obtained with maximum increases of PGE_2 treatment was calculated. Futhermore, a non-linear analysis was performed using the Sigmoid Emax Model to estimate EC_{50} values.

* http://www.hamamatsu.com/jp/ja/FDSS7000EX.html (accessed Nov 24, 2015)

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Chapter 3

Total Synthesis and Bioactivity of Resolvin E2

Abstract:

Resolvin E2 is a potent anti-inflammatory compound, derived from eicosapentaenoic acid. The efficient total synthesis of resolvin E2 by taking advantage of its intrinsic pseudoenantiomeric substructures is reported. The synthetic resolvin E2 proved to be biologically active in blocking neutrophil infiltration and reducing proinflammatory cytokines in the acute peritonitis model.

Introduction

Resolvins are a new family of lipid mediators derived from ω-3 polyunsaturated fatty acids, namely, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are generated during the resolution phase of acute inflammation. [1] Resolvin E1 is biosynthesized from EPA via cyclooxygenase (COX)-2- and 5-lipoxygenase-mediated conversion and has been shown to possess significant anti-inflammatory and proresolution properties, thereby protecting organs from collateral damage.[2] Another E series resolvin, namely, resolvin E2 (**1**), is formed via reduction of 5*S*-hydroperoxy-18*R*-hydroxy-EPE, an intermediate in the biosynthesis of resolvin E1, and exhibits potent anti-inflammatory properties in murine peritonitis.[3] It has been hypothesized that these E series resolvins contribute to the beneficial actions that have been attributed to EPA in certain human diseases, particularly those in which inflammation is suspected as a key component in pathogenesis. Motivated by their therapeutic potential for new treatment of human disorders associated with aberrant inflammation, the author launched the synthetic studies of resolvins as well as other lipid mediators. In this Chapter, the author reports an efficient total synthesis of resolvin E2[4] and its biological activity in reducing neutrophil infiltration and proinflammatory cytokine productions *in vivo*.

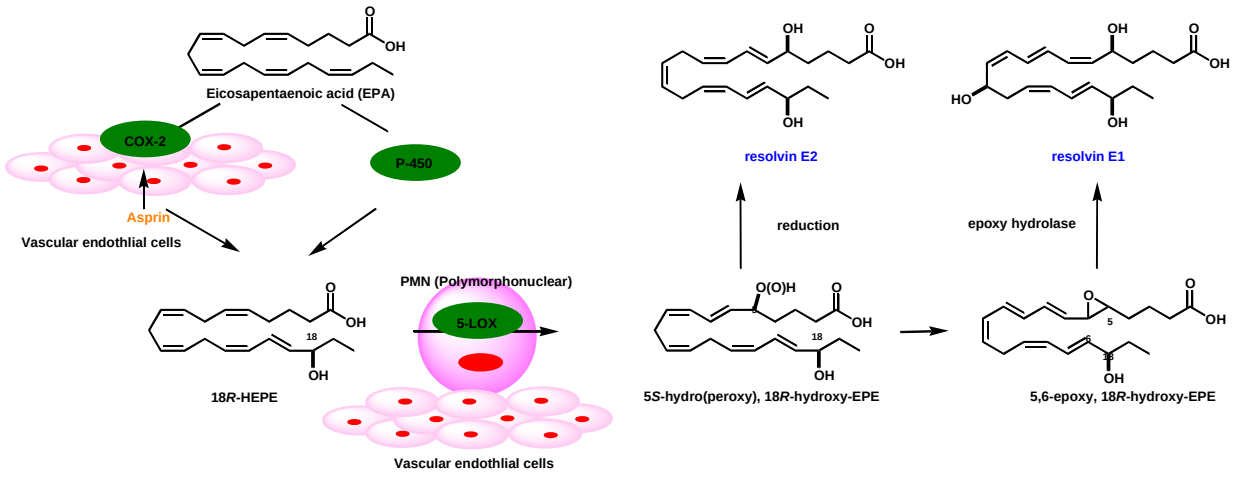
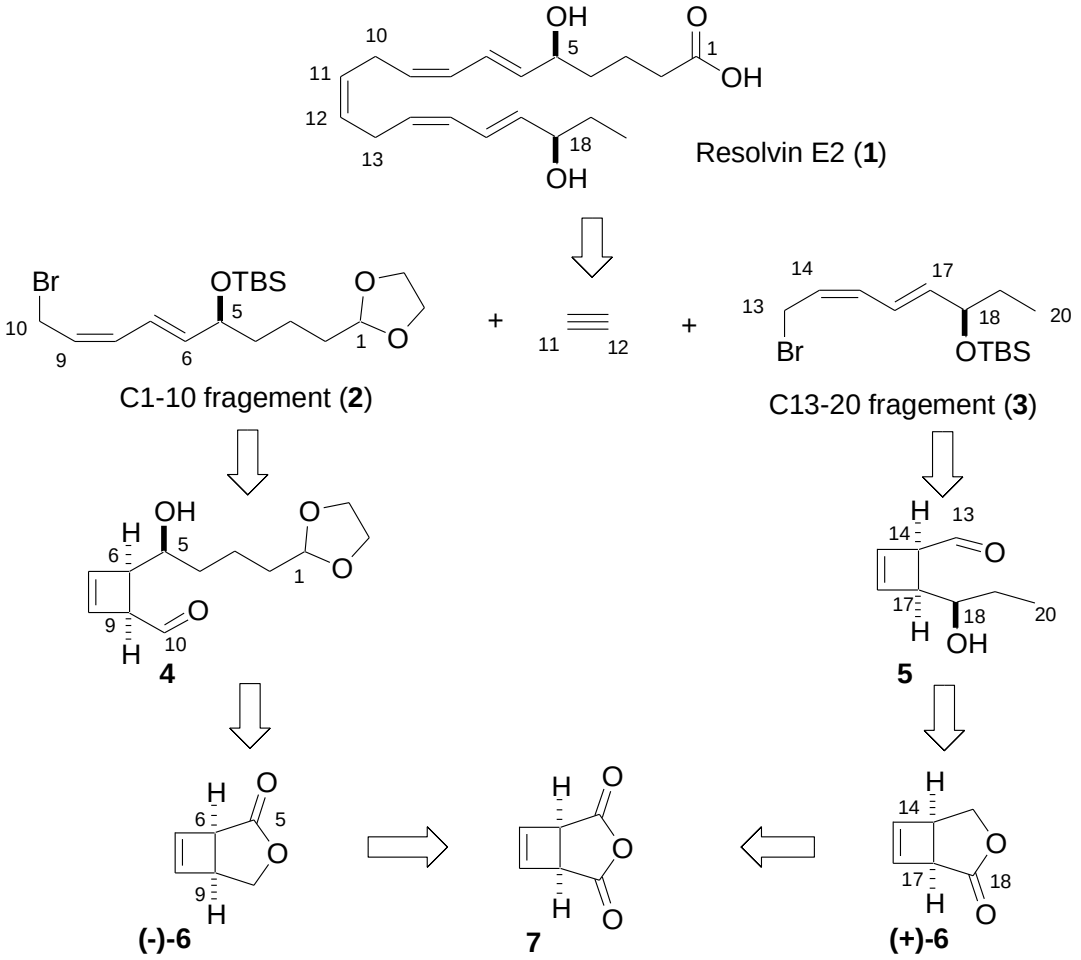


Figure 3-1. Anti-inflamamatory lipid mediators (resolvins) from ω-3 fatty acids.

Results and discussion

Synthetic plan of resolvin E2

The author planned to simplify the synthetic route to resolvin E2 (**1**) by taking advantage of its two symmetric substructures at C5-10 and C13-18 (Scheme 3-1). Retrosynthetic disconnections at C10-11 and C12-13 provided a C11-12 unit together with pseudo-enantiomeric fragments, **2** and **3**, both of which have the *E*,*Z*-conjugated olefin and allylic alcohol groups. Because of their structural similarity, **2** and **3** would be prepared from enantiomers of **6** by applying the same strategy. Specifically, the stereocenters at C5 of **4** and C18 of **5** would be generated by substrate-controlled stereoselective addition of the corresponding carbon nucleophiles, while the *E*,*Z*-olefins at C6 of **2** and C17 of **3** would be constructed using a torquoselective thermal electrocyclic ring-opening reaction[5] of cyclobutene aldehydes **4** and **5**, respectively.[6] Hence, the stereochemistries of the cyclobutane of (-)- or (+)-**6** were envisioned to be transferred to the stereochemistries of the hydroxyl group at C5 or C18 and the diene at C6 or C17. A pair of optically active six-carbon units **6**[7] would be obtained from the known achiral *meso* anhydride **7**[8] by enantioselective desymmetrization.

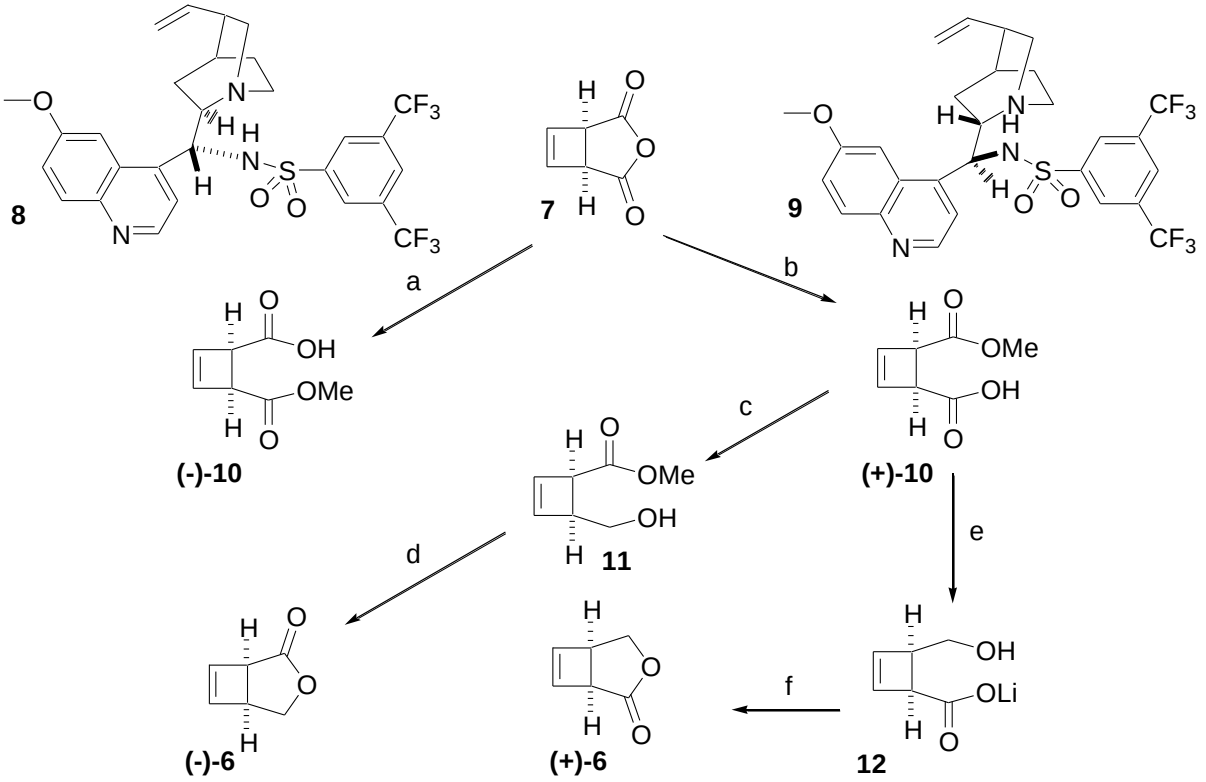


Scheme 3-1. Retrosynthesis of resolvin E2

Syntheses of chiral lactones (+)-6 and (-)-6

Both enantiomers of **6** were prepared from methyl ester (+)-**10** (Scheme 3-2).[9] The critical desymmetrization of *meso*-**7** into (+)-**10** was realized using a catalytic amount of the quinine derivative **9**, according to the conditions developed by Song.[10,11] Namely, 1 mol % of **9** and 10 equiv of methanol were applied to **7** in Et_2O to generate (+)-**10** in highly enantioselective fashion (95% yield, 87% ee). Interestingly, methanolysis of the same **7** using the quinidine derivative **8**, the pseudo-enantiomer of **9**, indeed gave the enantiomeric (-)-**10**, albeit in lower enantioselectivity (64% ee). Due to this, the author decided to synthesize (-)- and (+)-**6** from the same (+)-**10** using chemoselective reduction of either the carboxylic acid or the ester. Lactone (-)-**6** was prepared from (+)-**10** in three steps: conversion of the carboxylic acid of (+)-**10** into an acid chloride, followed by chemoselective

$NaBH_4$ reduction,[12] and subsequent acid-mediated cyclization of methyl ester **11**. The enantiomer (+)-**6** was in turn synthesized by $LiEt_3BH$ reduction[13] of the methyl ester of (+)-**10** and subsequent cyclization of carboxylic acid **12** under acidic conditions.



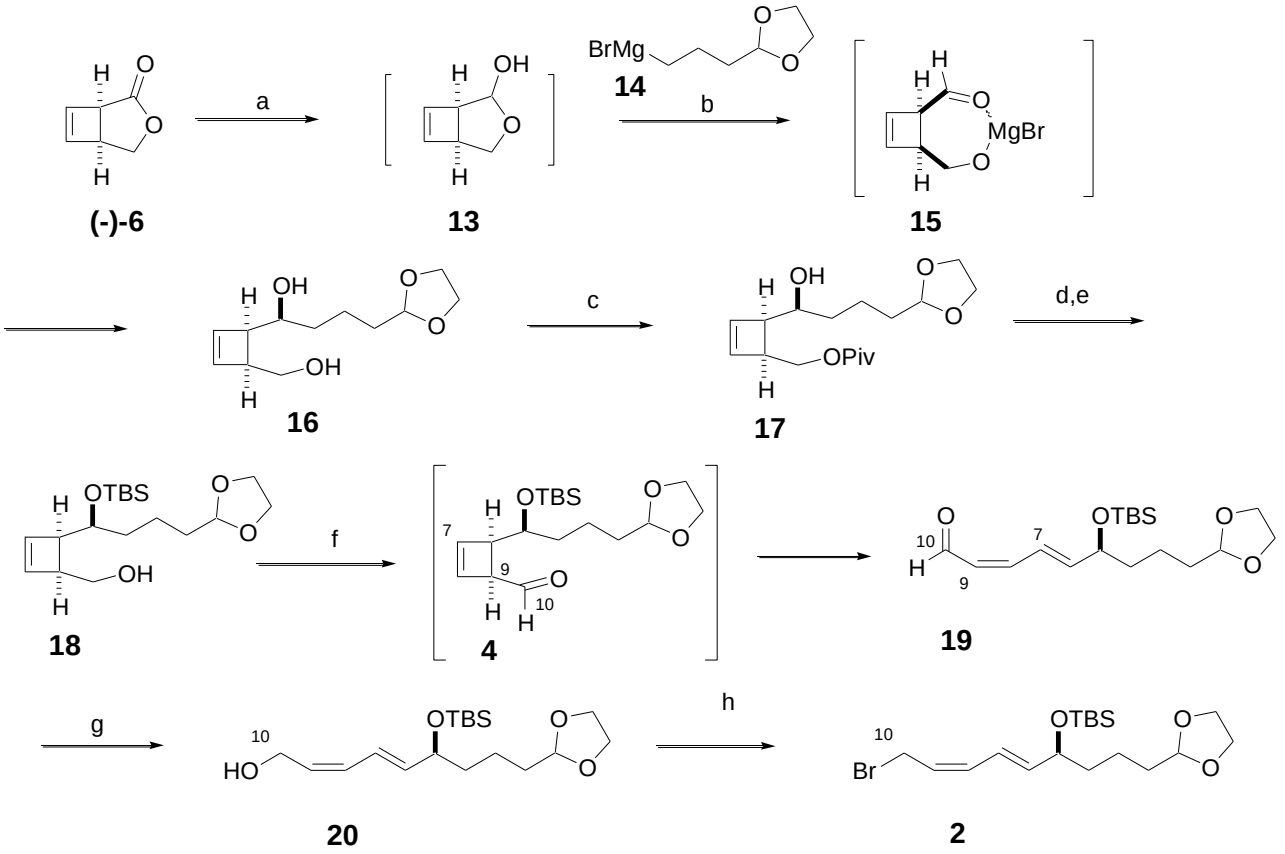
Reagents and conditions: (a) **8** (1mol%), Et_2O, MeOH(10 eq) (100%, 64%ee). (b) **9**(1mol%), Et_2O, MeOH(10 eq) (95%, 87%ee). (c) (i) $(COCl)_2$, DMF, CH_2Cl_2 (ii) $NaBH_4$, DMF. (d) TFA, CH_2Cl_2 (61%, 3 steps). (e) $LiEt_3BH$, THF. (f) TsOH, benzene (66%, 2 steps).

Scheme 3-2. Synthesis of both enantiomers of **6.**

Synthesis of C1-10 fragment

Synthesis of the C1-10 fragment **2** started with reduction of (-)-**6** by DIBAL-H, followed by addition of Grignard reagent of **14** in one pot,[14] resulting in stereoselective introduction of the C5-hydroxy group of **16** (dr = 6:1, Scheme 3-3).[15] The high diastereoselectivity is attributable to chelation between the magnesium alkoxide and the aldehyde and subsequent nucleophilic attack from the convex face of the 4/7-fused ring system **15**.[6b,d] Next, 1,4-diol **16** was transformed to alcohol **18** by a protection/deprotection procedure: stepwise introduction of Piv

and TBS groups to the primary and the secondary hydroxy groups, respectively, and subsequent reductive removal of the Piv ester from **17**. Swern oxidation of alcohol **18** at -78 °C generated aldehyde **4**, which underwent the crucial torquoselective electrocyclic ring-opening reaction even at room temperature to deliver *E*,Z-diene **19** as a sole isomer.[5] Stereoselective formation of the diene of **19** would originate from strong preference of the electron-accepting aldehyde of **4** for the inward rotation (Figure 3-2)[6]. Because of its chemical instability, the resulting α, β, γ, δ-unsaturated aldehyde **19** was immediately subjected to $NaBH_4$ reduction without purification to give allylic alcohol **20**.[16] Finally, bromination of the chemically-sensitive allylic alcohol was realized by the action of CBr_4 and $(CH_2PPh_2)_2$ to give the C1-10 fragment **2**.[17]



Reagents and conditions: (a) DIBAL, THF (b) **14**. (c) PivCl, pyridine, CH_2Cl_2 (68%, 2 steps). (d) TBSOTf, lutidine, CH_2Cl_2. (e) DIBAL, CH_2Cl_2 (98%, 2 Steps). (f) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2. (g) $NaBH_4$, EtOH (86%, 2 steps). (h) CBr_4, $(Ph_2PCH_2)_2$, CH_2Cl_2 (100%).

Scheme 3-3. Synthesis of C1-10 fragment **2**

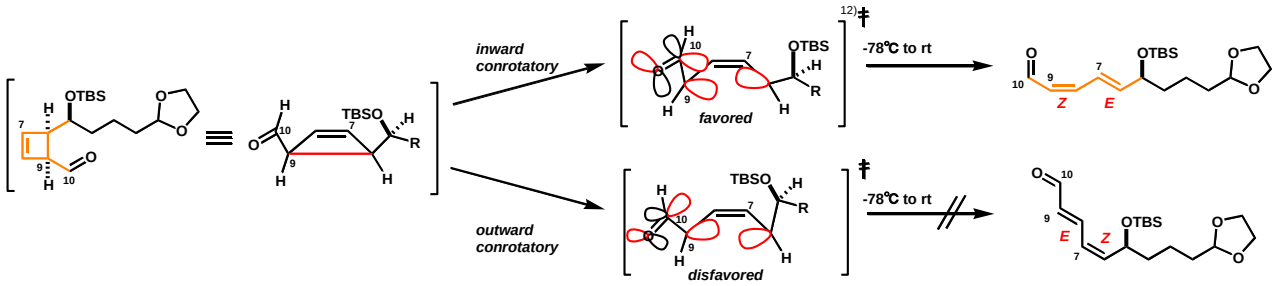
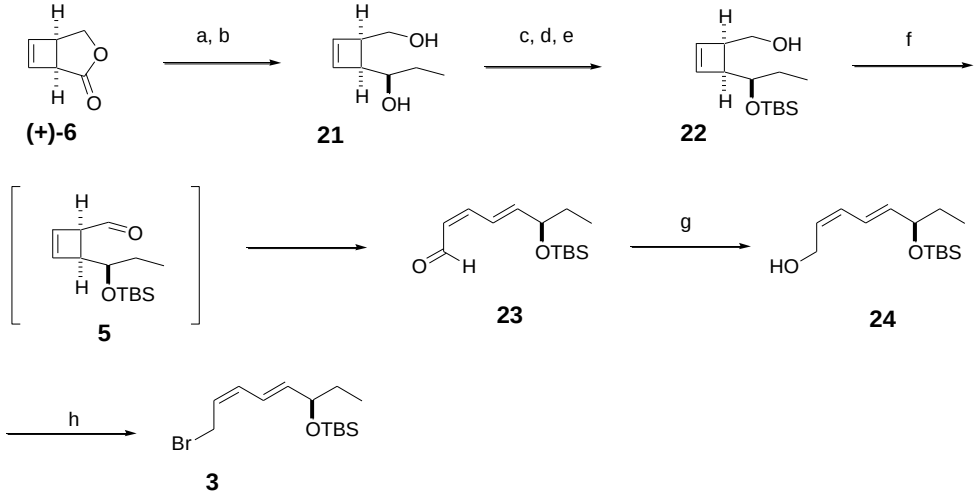


Figure 3-2. Torquoselective electrocyclic ring-opening reaction.

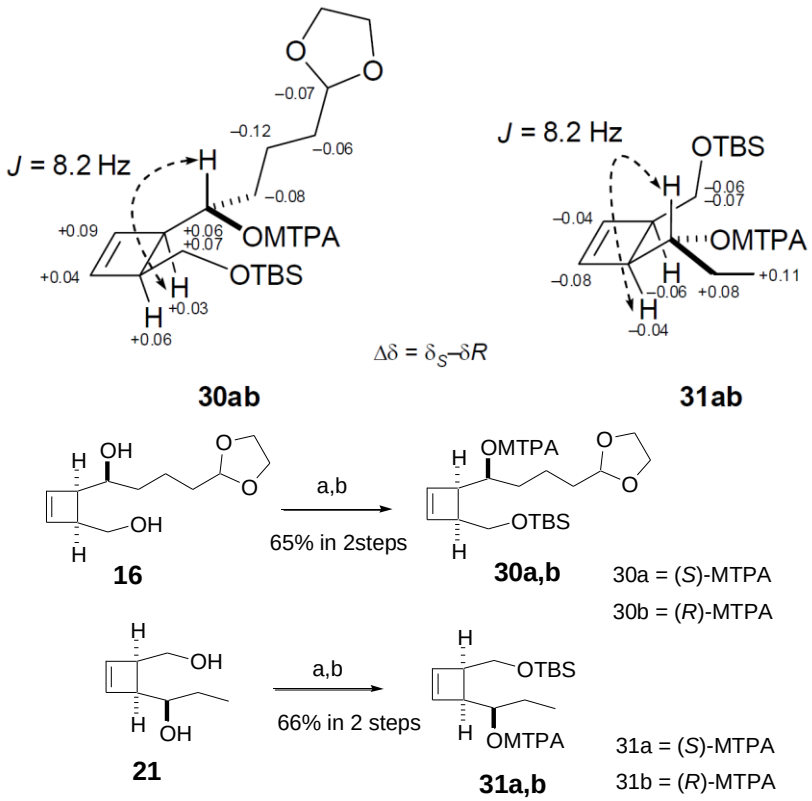
Synthesis of C13-20 fragment

The C13-20 fragment **3** was synthesized from (+)-**6** similarly to the C1-10 fragment **2** (Scheme 3-4). Reduction of (+)-lactone **6** with DIBAL was followed by stereoselective addition of ethyl magnesium bromide in the presence of zinc bromide to afford **21** with the desired C18-stereochemistry (dr = 7:1). The stereochemistries of **16** and **21** were identified by the modified Mosher's method (Figure 3-3). After protecting group manipulations from **21** to **22** in two steps, Swern oxidation of the primary alcohol of **22** to aldehyde **5** accelerated the thermal ring-opening reaction to produce *E*,*Z*-diene **23** as a single isomer. Reduction of the resulting aldehyde of **23** into alcohol **24**, followed by bromination, led to the C13-20 fragment **3**.



Reagents and conditions: (a) DIBAL, toluene. (b) EtMgBr (68%). (c) PivCl, pyridine, CH_2Cl_2 (68%, 2 steps). (d) TBSOTf, lutidine, CH_2Cl_2. (e) DIBAL, CH_2Cl_2 (100%, 2 steps). (f) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2. (g) $NaBH_4$, EtOH (81%, 2 steps). (h) CBr_4, $(Ph_2PCH_2)_2$, CH_2Cl_2 (100%).

Scheme 3-4. Synthesis of C13-20 fragment **3**

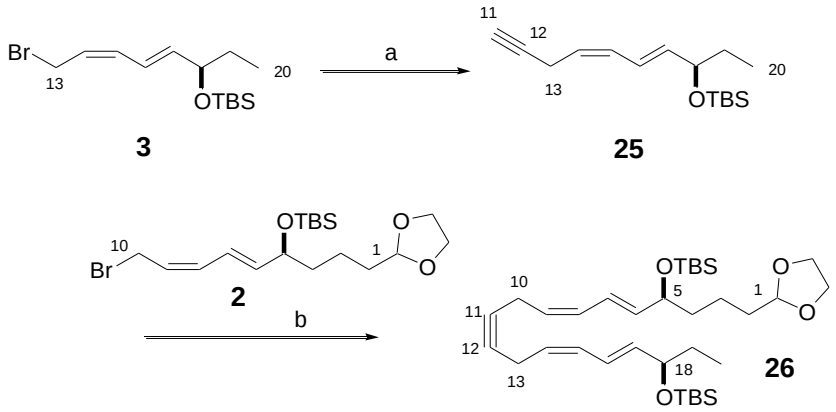


Reagents and conditions: (a) TBSCl, imidazole, DMAP, DMF. (b) (*S*) or (*R*) MTPACl, Et_3N, DMAP, CH_2Cl_2

Figure 3-3. Syntheses of (*S*)- and (*R*)-Mosher esters **30a,b** and **31a,b** and their ^{1}H NMR data

Coupling of each fragments

Final convergent assemblies of the three partial structures utilized two copper-mediated couplings (Scheme 3-5). The bromide of **3** was first displaced with the copper acetylide, generated from ethynyl magnesium bromide and CuCl,[18] delivering the C11-20 fragment **25**. Deprotonation of the C11-proton of **25** by *n*-BuLi in the presence of CuBr-SMe_2[19] at -78 °C then afforded the corresponding copper acetylide, which was treated with the C1-10 fragment **2**, giving rise to the entire structure **26**. Intriguingly, only this particular condition produced a sufficient amount of the adduct **26**. For instance, use of copper iodide instead of copper bromide for the coupling only gave a mixture of byproducts, in which the C6-*E*,*Z*-olefins were reacted or isomerized.

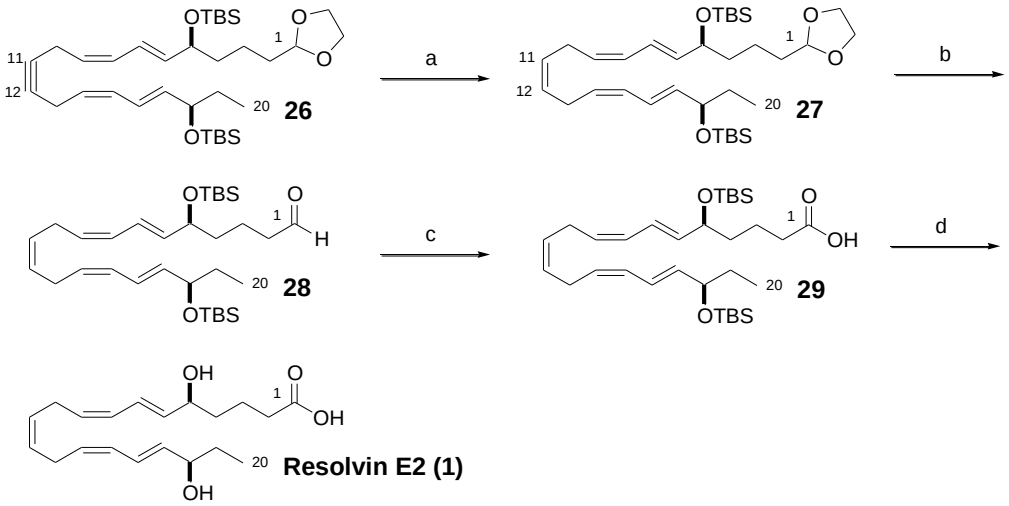


Reagents and conditions: (a) ethynylmagnesium bromide, CuCl, THF (84%). (b) **2**, *n*-BuLi, CuBr・Me_2S, HMPA, THF (54%)**.**

Scheme 3-5. Coupling of each fragments.

Synthesis of resolvin E2 (1).

Four transformations from **26** led to the targeted resolving E2 (Scheme 3-6). Lindlar conditions[20] enabled partial reduction of the alkyne of **26** into alkene **27** without reduction and/or isomerization of the reactive C6- and C17-*E*,*Z*-conjugated olefins. Acid-mediated removal of the ketal of **27** was troublesome because of the presence of the acid-labile allylic TBS ethers. After many attempts, the author found that Kita's conditions[21] were effective for selective reaction of the cyclic ketal. Treatment of **27** with an excess amount of TMSOTf and lutidine followed by aqueous work-up provided aldehyde **28** in high yield. Lastly, $NaClO_2$-mediated oxidation of the obtained aldehyde **28** into a carboxylic acid and subsequent desilylation with TBAF gave rise to resolvine E2 (**1**).



Reagents and conditions: (a) H_2, 5% $Pd/BaSO_4$, quinoline, EtOAc (54%). (b) TMSOTf, lutidine, CH_2Cl_2 then H_2O. (c) $NaClO_2$, $NaH_2PO_4 \cdot 2H_2O$, 2-methyl-2-butene, *t*-BuOH/H_2O. (d) TBAF, THF (67%, 3 steps).

Scheme 3-6. Synthesis of resolvin E2(**1**)**.**

Biological evaluation of synthetic resolvin E2 (1).

The author evaluated the bioactivity of synthetic **1** using the *in vivo* inflammation model (Figures 3-4 and 3-5).[3] Zymosan A, a glucan from the yeast cell wall, was used to induce sterile inflammation characterized by local neutrophil infiltration and proinflammatory cytokine productions. In acute peritonitis with zymosan A, intravenous administration of synthetic resolvin E2 as low as 0.1 or 1.0 μg significantly blocked neutrophil infiltrations at 2 h in the inflamed peritoneal cavity (Figure 3-4). The potency of resolvin E2's anti-inflammatory action was comparable to that of a higher dose of dexamethasone at 10 μg (data not shown). Also **1** (1.0 μg *i.v.*/mouse) markedly reduced production of proinflammatory cytokines such as tumor necrosis factor (TNF)-α (123.2 pg vs 56.7 pg, $p < 0.005$) and interleukin (IL)-6 (3.40 ng vs 2.05 ng, $p < 0.02$) (Figure 3-5).

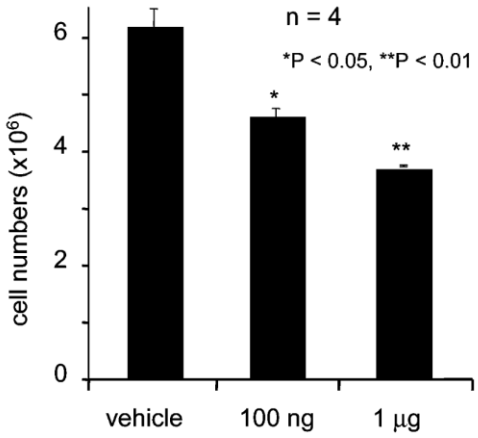


Figure 3-4. Synthetic resolvin E2 reduced neutrophil infiltrations.

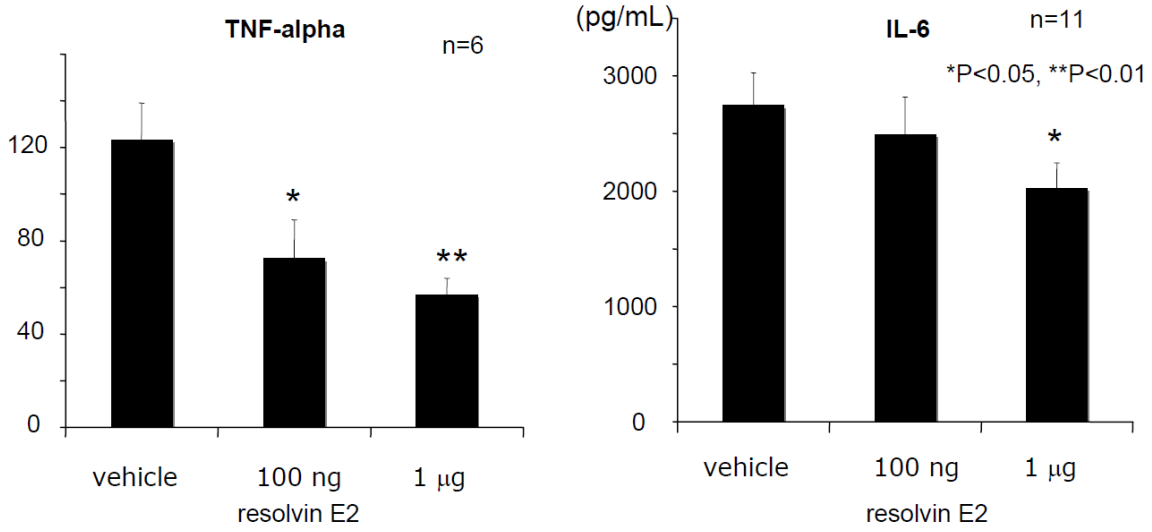


Figure 3-5. Resolvin E2 reduced productions of TNF-α and IL-6 in zymosan-induced peritonitis.

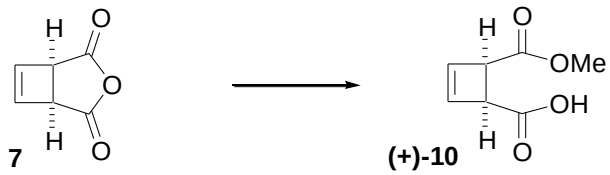
In summary, the efficient total synthesis of resolvin E2 (**1**) was achieved by utilizing the intrinsic pseudoenantiomeric nature of the key fragments **2** and **3**. Most importantly, the two stereochemistries of **6** introduced via the desymmetrization step were effectively transferred to those of the hydroxyl group and the diene for preparing **2** and **3**. The obtained fragments were assembled into **1** in a convergent fashion. Furthermore, the author confirmed synthetic resolving E2 blocked neutrophil infiltrations in the inflamed peritoneal cavity in acute peritonitis with zymosan A. And it also markedly reduced production of proinflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6.

Experimental Section

General Methods.

All reactions sensitive to air or moisture were carried out under argon atmosphere in dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. THF was distilled from sodium/benzophenone, pyridine, triethylamine (Et_3N) and 2,6-lutidine from calcium hydride under reduced pressure. All other reagents were used as supplied unless otherwise stated. Analytical thin-layer chromatography (TLC) was performed using E. Merck Silica gel 60 F254 pre-coated plates. Column chromatography was performed using 75-150 μm BW-820MH (Fuji Silysia Co., Inc.). Flash column chromatography was performed using 40-63 μm Silica Gel 60 (Merck Co., Inc.) or 32-53 μm BW-300 (Fuji Silysia Co., Inc.). ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM ECA-500 (500 MHz) or JEOL JNM ECX-500 (500 MHz) spectrometer. Chemical shifts are reported in δ (ppm) with reference to solvent signals [^{1}H NMR: $CHCl_3$ (7.26), C_6D_6 (7.16), $(CD_3)_2CO$ (2.05), CD_3OD (3.30); ^{13}C NMR: $CHCl_3$ (77.0), C_6D_6 (128.06), $(CD_3)_2CO$ (29.84), CD_3OD (49.00). Signal patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak. IR spectra were recorded on a JASCO FT/IR-4100 spectrometer. FAB-MS and EI-MS were on JEOL JMS-700. ESI-MS were on BRUKER DALTONICS Bio TOF-Q. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. Melting points were measured on a Yanaco MP-J3 micro melting point apparatus and uncorrected.

Experimantal procedure

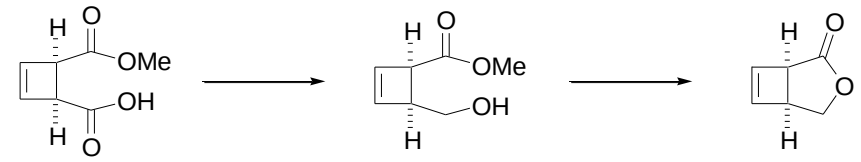


Methyl ester (+)-10.

Methanol (0.16 mL, 4.0 mmol) was added to a solution of *meso*-**7** (50 mg, 0.40 mmol) and quinine derivative **9** (2.4 mg, 4.0 μmol) in Et_2O (4 mL) at room temperature. The reaction mixture was stirred at room temperature for 20 h and 1M HCl solution was added. The mixture was extracted with EtOAc and the organic layer was dried

over Na_2SO_4. Concentration gave methyl ester (+)-**10** (60 mg, 0.38 mmol) in 95% yield. The enantiomeric excess was determined to be 87% from ^{1}H NMR analysis of Mosher ester of alcohol **11.**

pale yellow oil $[\alpha]_D^{25}$ = 3.3 (*c* 0.87, $CHCl_3$); IR (neat) ν 3475, 2956, 1728, 1573, 1210 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.65 (3H, s, Me), 3.93 (2H, s, C*H*-CO_2Me, C*H*-CO_2H), 6.22 (1H, d, *J* = 2.8 Hz, C*H*A=CHB), 6.24 (1H, d, *J* = 2.8 Hz, CHA=C*H*B), 11.20 (1H, br s, CO_2*H*). ^{13}C NMR (125 MHz, $CDCl_3$) δ 48.66, 48.74, 52.0, 136.2, 136.9, 171.1, 176.8. HRMS (FAB), calcd for $C_7H_8O_4Cs$ 288.9477 $(M+Cs)^+$, found 288.9470.

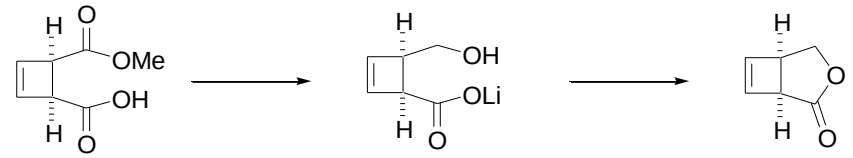


(+)-10 **11** **(-)-6**

(–)-Lactone 6.

To a solution of methyl ester (+)-**10** (2.12 g, 13.7 mmol) in CH_2Cl_2 (60 mL) and DMF (0.3 mL) at 0 °C was added $(COCl)_2$ (1.7 mL, 21 mmol). The reaction mixture was stirred at 0 °C for 1.5 h and was transferred to a suspension of $NaBH_4$ (3.11 g, 82.2 mmol) in DMF (120 mL) at 0 °C. The reaction mixture was stirred for further 1.5 h at 0 °C and 1M HCl solution was added. The mixture was extracted with CH_2Cl_2 and the organic layer was dried over Na_2SO_4. Concentration gave methyl ester **11**, which was directly used in the next reaction. Trifluoroacetic acid (2.9 mL, 39 mmol) was added to a solution of crude methyl ester **11** in CH_2Cl_2 (70 mL) at 0 °C and the reaction mixture was stirred at room temperature for 15 h. Concentration and flash column chromatography (CH_2Cl_2/MeOH 99:1-97:3 then hexane/EtOAc 4:1-2:1) gave (–)-lactone **6** (919 mg, 8.35 mmol) in 61% yield over 3 steps.

Colorless oil; $[\alpha]_D^{24}$ = –272.2 (*c* 0.62, $CHCl_3$); IR (neat) ν 2974, 1759, 1170, 984 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.59 (1H, ddd, *J* = 6.7, 3.9, 2.2 Hz, OCH_2C*H*), 3.62 (1H, d, *J* = 3.9 Hz, COC*H*), 4.24 (1H, dd, *J* = 9.6, 2.3 Hz, OC*H*AHB), 4.27 (1H, dd, *J* = 9.6, 6.7 Hz, OCHA*H*B), 6.28 (1H, d, *J* = 2.8 Hz, C*H*A=CHB), 6.33 (1H, d, *J* = 2.8 Hz, CHA=C*H*B). ^{13}C NMR (125 MHz, $CDCl_3$) δ 41.8, 46.5, 67.9, 138.9, 141.5, 175.4. HRMS (EI), calcd for $C_6H_6O_2$ 110.0368 (M^+), found 110.0368.



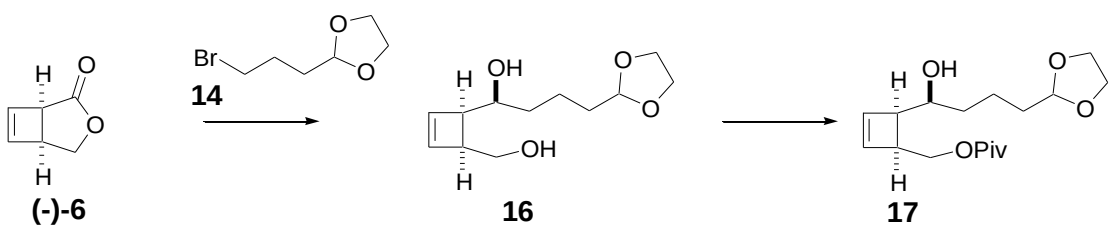
(+)-10 **12** **(+)-6**

(+)-Lactone 6.

To a solution of methyl ester (+)-**10** (85 mg, 0.55 mmol) in THF (5 mL) at 0 °C was added $LiEt_3BH$ (1.0 M in THF, 1.8 ml, 1.8 mmol). The reaction mixture was stirred at 0 °C for 10 min and additional $LiEt_3BH$ (1.0 M in THF, 2.4 ml, 2.4 mmol) was added. The reaction mixture was stirred for further 10 min at 0 °C and additional $LiEt_3BH$ (1.0 M in THF, 0.82 ml, 0.82 mmol) was added again. The reaction mixture was stirred at 0 °C for 10 min, and then 1M HCl solution was added. The resulting solution was neutralized by careful addition of 0.5 M LiOH solution. Concentrated gave carboxylic acid **12**, which was directly used in the next reaction.

p-Toluenesulfonic acid monohydrate (400 mg, 2.11 mmol) was added to a solution of crude carboxylic acid **12** in benzene (40 mL) at 0 °C. The reaction mixture was heated at 100 °C for 1 h and cooled to room temperature, and then H_2O was added. The mixture was extracted with CH_2Cl_2 and the organic layer was dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc 6:1-4:1) gave (+)-lactone **6** (37 mg, 0.33 mmol) in 66% yield over 2 steps.

Colorless oil; $[\alpha]_D^{27}$ 276.7 (*c* 0.22, $CHCl_3$); IR (neat) ν 2974, 1758, 1171, 983 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.60 (1H, ddd, *J* = 6.8, 3.4, 2.3 Hz, OCH_2CH), 3.64 (1H, d, *J* = 3.9 Hz, COC*H*), 4.26 (1H, dd, *J* = 9.7, 2.3 Hz, OC*H*AHB), 4.29 (1H, dd, *J* = 9.7, 6.8 Hz, OCHA*H*B), 6.31 (1H, d, *J* = 2.8 Hz, C*H*A=CHB), 6.35 (1H, d, *J* = 2.8 Hz, CHA=C*H*B). ^{13}C NMR (125 MHz, CDCl3) δ 41.8, 46.5, 68.0, 139.0, 141.6, 175.4. HRMS (EI), calcd for $C_6H_6O_2$ 110.0368 (M^+), found 110.0363.



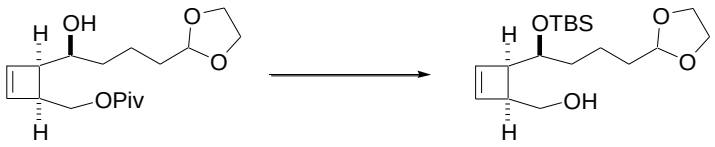
(-)-6 **14** **16** **17**

Piv ester 17.

To a solution of (–)-lactone **6** (239 mg, 2.17 mmol) in THF (31 mL) at –78 °C was added DIBAL (1.0 M in hexane, 2.4 ml, 2.4 mmol). The reaction mixture was stirred at –78 °C for 20 min and a solution of Grignard reagent **14** (ca. 0.6 M in THF, 31 mL, 18 mmol) was added at –78 °C. The reaction mixture was stirred at room temperature for further 2 h and saturated aqueous potassium sodium tartrate was added. The mixture was extracted with EtOAc and the organic layer was washed with brine and dried over anhydrous Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc 1:1-1:2-1:3) gave 1,4-diol **16** (508 mg), and its diastereomer (46.2 mg, 0.204 mmol) in 9% yield.

To a solution of the impure 1,4-diol **16** and pyridine (1.8 mL, 22 mmol) in CH_2Cl_2 (100 mL) at 0 °C was added PivCl (1.1 ml, 8.9 mmol). The reaction mixture was stirred at room temperature for 4 h and saturated aqueous NH_4Cl solution was added. The mixture was extracted with CH_2Cl_2 and the organic layer was dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc 9:1-3:1-1:1) gave Piv ester **17** (463 mg, 1.48 mmol) in 68% yield over 3 steps.

Pale yellow oil; $[\alpha]_D^{25}$ = –19.9 (*c* 0.59, $CHCl_3$); IR (neat) ν 3493, 2958, 2908, 2880, 1726, 1480, 1461, 1288, 1149, 967, 945 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 1.18 (9H, s, *t*-Bu of Piv), 1.40-1.58 (2H, m, H-4), 1.56-1.72 (4H, m, H-2, H-3), 2.93 (1H, dd, *J* = 10.1, 3.9 Hz, H-6), 3.19 (1H, ddd, *J* = 9.5, 5.0, 3.9 Hz, H-9), 3.68 (1H, ddd, *J* = 10.1, 8.4, 2.8 Hz, H-5), 3.79-3.86 (2H, m, OC*H*$_2$CH$_2$O), 3.91-3.98 (2H, m, OCH$_2$C*H*$_2$O), 4.11 (1H, dd, *J* = 11.8, 9.5 Hz, H10), 4.45 (1H, dd, *J* = 11.8, 5.0 Hz, H10), 4.84 (1H, t, *J* = 4.5 Hz, H1), 6.02 (1H, d, *J* = 2.8 Hz, H7), 6.06 (1H, d, *J* = 2.8 Hz, H8). ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.9, 27.1, 33.7, 34.9, 38.7, 44.7, 52.6, 64.1, 64.8, 70.9, 104.5, 137.1, 138.1, 178.0. HRMS (FAB), calcd for $C_{17}H_{29}O_5$ 313.2015 $(M+H)^+$, found 313.2000.



17 **18**

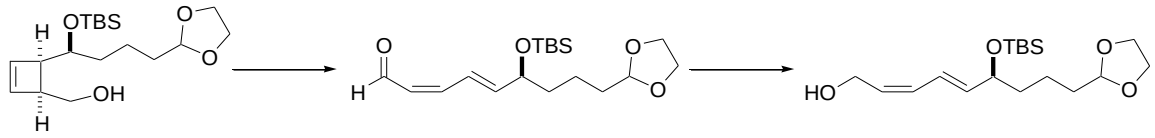
Alcohol 18.

To a solution of Piv ester **17** (406 mg, 1.30 mmol) and 2,6-lutidine (1.2 mL, 10 mmol) in CH_2Cl_2 (100 mL) at

0 °C was added TBSOTf (890 μL, 3.90 mmol). The reaction mixture was stirred at room temperature for 2 h and H_2O was added. The mixture was extracted with Et_2O and the organic layer was washed with H_2O, saturated aqueous $NaHCO_3$ and brine, and dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc 100:0-9:1) gave TBS ether (694 mg), including inseparable compound, which was used in the next reaction.

To a solution of the impure TBS ether (694 mg) in CH_2Cl_2 (20 mL) at –78 °C was added DIBAL solution (1.0 M in hexane, 2.60 mL, 2.60 mmol). The reaction mixture was stirred at –78 °C for 10 min and saturated aqueous Na_2SO_4 and saturated aqueous $NaHCO_3$ were added. The resulting suspension was stirred at room temperature for 1 h and was filtrated through a pad of Celite with CH_2Cl_2. Concentration and flash column chromatography (hexane/EtOAc 2:1-1:1) gave alcohol **18** (435 mg, 1.27 mmol) in 98% yield over 2 steps.

Pale yellow oil; $[\alpha]_D^{27}$ = 6.9 (*c* 0.41, $CHCl_3$); IR (neat) ν 3421, 2952, 2931, 2886, 2859, 1468, 1254, 1140, 1098, 1031, 939, 836 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.09 (3H, s, Me of TBS), 0.10 (3H, s, Me of TBS), 0.88 (9H, s, *t*-Bu of TBS), 1.46-1.52 (2H, m, H-3), 1.59-1.67 (4H, m, H-2, H-4), 3.11 (1H, dd, *J* = 5.0 Hz, H-6), 3.17-3.20 (1H, m, H-9), 3.75-3.81 (2H, m, H-10), 3.83-3.87 (2H, m, OC*H*$_2$CH$_2$O), 3.91-3.96 (2H, m, OCH$_2$C*H*$_2$O), 3.96-4.00 (1H, m, H-5), 4.83 (1H, t, *J* = 5.0 Hz, H-1), 5.96 (1H, d, *J* = 2.8 Hz, H-7) , 6.07 (1H, d, *J* = 2.8 Hz, H-8). ^{13}C NMR (125 MHz, $CDCl_3$) δ –4.1, –3.7, 18.2, 19.9, 25.9, 33.8, 35.5, 49.6, 51.3, 61.8, 64.82, 64.84, 72.7, 104.2, 137.0, 137.6. HRMS (FAB), calcd for $C_{18}H_{35}O_4Si$ 343.2305 $(M+H)^+$, found 343.2307.



18 **19** **20**

Allylic alcohol 20.

Dimethyl sulfoxide (0.95 M in CH_2Cl_2, 7.4 ml, 6.2 mmol) was added to a solution of $(COCl)_2$ (0.21 mL, 2.5 mmol) in CH_2Cl_2 (15 mL) at –78 °C. The resulting mixture was stirred for 10 min then a solution of alcohol **18** (423 mg, 1.24 mmol) in CH_2Cl_2 (7.0 mL) was added at -78 °C. The reaction mixture was stirred at –78 °C for 2 h and was treated with Et_3N (1.1 mL, 7.9 mmol). The resulting solution was stirred at –78 °C for further 1 h,

warmed to room temperature over 1.5 h, and stirred for further 1 h, and then H_2O (8.0 mL) was added. The mixture was extracted with CH_2Cl_2 (8.0 mL) and the organic layer was poured into a suspension of $NaBH_4$ (233 mg, 6.18 mmol) in EtOH (30 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and saturated aqueous NH_4Cl was added. The mixture was extracted with CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc 4:1-2:1) gave allylic alcohol **20** (363 mg, 1.06 mmol) in 86% yield.

Colorless oil; $[\alpha]_D^{27}$ = 4.9 (*c* 0.39, $CHCl_3$); IR (neat) ν 3420, 2952, 2929, 2884, 2857, 1685, 1643, 1254, 1141, 1034, 957, 837 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.01 (3H, s, Me of TBS), 0.04 (3H, s, Me of TBS), 0.89 (9H, s, *t*-Bu of TBS), 1.37-1.60 (6H, m, H-2, H-3, H-4), 3.82-3.85 (2H, m, OC*H*$_2$CH$_2$O), 3.91-3.98 (2H, m, OCH$_2$C*H*$_2$O), 4.17 (1H, dt, *J* = 5.7, 5.7 Hz, H-5), 4.30 (2H, d, *J* = 6.8 Hz, H-10), 4.83 (1H, t, *J* = 5.0 Hz, H-1), 5.57 (1H, dt, *J* = 11.3, 6.8 Hz, H-9), 5.71 (1H, dd, *J* = 15.3, 5.7 Hz, H-6), 6.06 (1H, t, *J* = 11.3 Hz, H-8), 6.41 (1H, dd, *J* = 15.3, 11.3 Hz, H7). ^{13}C NMR (125 MHz, $CDCl_3$) δ –4.8 –4.4, 18.2, 19.7, 25.8, 33.8, 38.0, 58.8 (2C), 64.8, 72.7, 104.5, 123.6, 128.9, 130.3, 139.1. HRMS (FAB), calcd for $C_{18}H_{34}O_4SiCs$ 475.1281 $(M+Cs)^+$, found 475.1274.

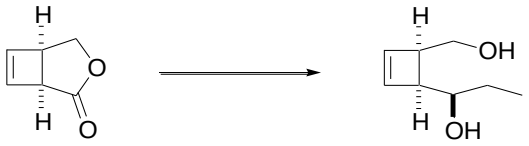
20 **2**

C1-10 Fragment (2).

Tetrabromomethane (406 mg, 1.27 mmol) and DPPE (504 mg, 1.27 mmol) were successively added to a solution of allylic alcohol **20** (144 mg, 0.422 mmol) in CH_2Cl_2 (4.0 mL) at 0 °C. The reaction mixture was stirred for 10 min at 0 °C and was directly subjected to flash column chromatography (hexane/EtOAc 9:1) to give C1-10 fragment (**2**) (170 mg, 0.422 mmol) in 100% yield.

$[\alpha]_D^{24}$ = 37.8 (*c* 0.24, $CHCl_3$); IR (neat) ν 2952, 2928, 2856, 1652, 1472, 1458, 1252, 1124, 949, 836, 775 cm^{-1}. ^1H NMR (500 MHz, C_6D_6) δ 0.06 (3H, s, Me of TBS), 0.08 (3H, s, Me of TBS), 1.00 (9H, s, *t*-Bu of TBS), 1.43-1.70 (4H, m, H-3, H-4), 1.74-1.79 (2H, m, H-2), 3.34-3.37 (2H, m, OC*H*$_2$CH$_2$O), 3.52-3.55 (2H, m, OCH$_2$C*H*$_2$O), 3.81 (1H, dd, *J* = 11.8, 8.4 Hz, H-10), 3.90 (1H, dd, *J* = 11.8, 7.8 Hz, H-10), 4.06 (1H, dt, *J* = 6.1,

5.5 Hz, H-5), 4.81 (1H, t, *J* = 5.0 Hz, H1), 5.36 (1H, br dt, *J* = 10.7, 7.8 Hz, H9), 5.61 (1H, dd, *J* = 15.1, 6.1 Hz, H6), 5.90 (1H, br dd, *J* = 11.2, 10.7 Hz, H8), 6.46 (1H, dd, *J* = 15.1, 11.2 Hz, H7). ^{13}C NMR (125 MHz, C_6D_6) δ –4.6 –4.2, 18.5, 20.2, 26.1, 34.4, 38.4, 39.3, 64.9(2C), 73.0, 104.8, 123.1, 125.5, 132.6, 140.9.

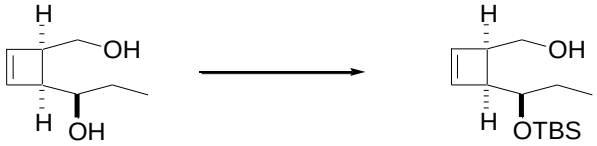


(+)-6 **21**

1,4-Diol 21.

To a solution of (+)-lactone **6** (242 mg, 2.19 mmol) in THF (20 mL) at –78 °C was added DIBAL (1.0 M in hexane, 2.2 ml, 2.2 mmol). The reaction mixture was stirred for 20 min at –78 °C, then $ZnBr_2$ (493 mg, 2.19 mmol) and EtMgBr (0.50 M in THF, 13.1 ml, 6.57 mmol) were added successively. The reaction mixture was warmed to room temperature and stirred for 16 h, and then 1M HCl solution was added. The mixture was extracted with Et_2O and the organic layer was dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc 3:1-2:1-1:1-1:2) gave 1,4-diol **21** (213 mg, 15.0 mmol) in 68% yield and a mixture of 1,4-diol **21** and its diastereomeric isomer in 1:3 ratio in 13% yield.

White solid; m.p. 53-55 °C; $[\alpha]_D^{24}$ = 28.7 (*c* 0.31, $CHCl_3$); IR (neat) ν 3317, 3044, 2964, 2922, 1461, 1290, 1029, 967 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.00 (3H, t, *J* = 7.4 Hz, H-20), 1.37-1.46 (1H, m, H-19), 1.61-1.70 (1H, m, H-19), 2.94 (1H, dd, *J* = 10.8, 4.0 Hz, H17), 3.20 (1H, dt, *J* =11.3, 4.0 Hz, H14), 3.63-3.68 (1H, m, H18), 3.69 (1H, dd, *J* = 11.3, 11.3 Hz, H13), 3.75-3.94 (2H, m, O*H* x2), 3.82 (1H, dd, *J* = 11.3, 4.0 Hz, H13), 6.01 (1H, d, *J* = 2.8 Hz, H15 or 16), 6.02 (1H, d, *J* = 2.8 Hz, H15 or 16). ^{13}C NMR (125 MHz, $CDCl_3$) δ 9.7, 27.9, 48.2, 52.4, 62.3, 72.6, 137.2, 137.8; HRMS (FAB), calcd for $C_8H_{14}O_2Cs$ 275.0048 $(M+Cs)^+$, found 275.0039.



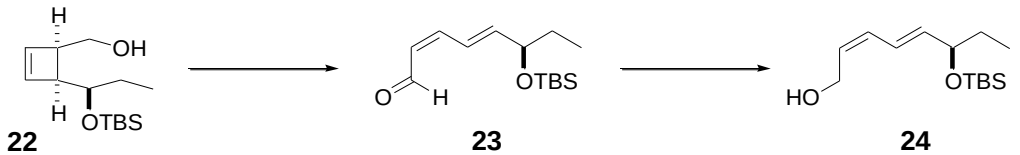
21 **22**

TBS ether 22.

To a solution of 1,4-diol **21** (291 mg, 2.04 mmol) and 2,6-lutidine (2.2 mL, 20 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added PivCl (0.6 ml, 6.1 mmol). The reaction mixture was stirred at room temperature for 2 h and additional PivCl (0.2 mL, 2.0 mmol) was added. The reaction mixture was stirred at room temperature for further 1 h and cooled to 0 °C, and then TBSOTf (0.9 mL, 4.1 mmol) was added. The reaction mixture stirred for 1 h, and additional TBSOTf (0.9 mL, 4.1 mmol) was added. The reaction mixture was stirred for further 10 min and H_2O was added. The mixture was extracted with Et_2O and the organic layer was washed with H_2O, saturated aqueous $NaHCO_3$ and brine, and dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc 100:0-9:1) gave product (811 mg), including inseparable compound, which was used in the next reaction.

To a solution of crude product (811 mg) in CH_2Cl_2 (100 mL) at –78 °C was added DIBAL (1.0 M in hexane, 4.0 ml, 4.0 mmol). The reaction mixture was stirred at –78 °C for 10 min and additional DIBALn (1.0 M in hexane, 0.60 ml, 0.60 mmol) was added. The reaction mixture was stirred for further 10 min and a mixture of saturated aqueous Na_2SO_4 and saturated aqueous $NaHCO_3$ was added. The suspension was stirred at room temperature for 1 h and was filtrated through a pad of Celite with CH_2Cl_2. Concentration and flash column chromatography (hexane/EtOAc 9:1-2:1) gave TBS ether **22** (529 mg, 2.05 mmol) in 100% yield over 2 steps.

Pale yellow oil; $[\alpha]_D^{26}$ = –8.8 (*c* 0.42, CHCl3); IR (neat) ν 3343, 3045, 2957, 2931, 2887, 2858, 1467, 1384, 1254, 1102, 1007, 836 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.108 (3H, s, Me of TBS), 0.112 (3H, s, Me of TBS), 0.90 (9H, s, *t*-Bu of TBS), 0.93 (3H, t, *J* = 7.4 Hz, H-20), 1.59-1.70 (2H, m, H-19), 3.13 (1H, m, H-17), 3.19 (1H, m, H-14), 3.77 (1H, dd, *J* = 11.3, 6.2 Hz, H-13), 3.84 (1H, dd, *J* = 11.3, 3.4 Hz, H-13), 3.93 (1H, dt, *J* = 6.2, 5.6 Hz, H-18), 5.97 (1H, d, *J* = 2.8 Hz, H-16), 6.09 (1H, d, *J* = 2.8 Hz, H-15). ^{13}C NMR (125 MHz, $CDCl_3$) δ –4.1, –3.7, 9.9, 18.3, 25.9, 28.4, 49.6, 51.0, 61.9, 74.1, 137.1, 137.5. HRMS (FAB), calcd for $C_{14}H_{29}O_2Si$ 257.1937 ($M+H^+$), found 257.1933.

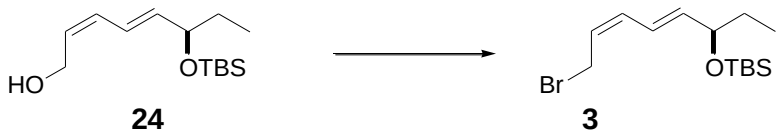


22 **23** **24**

Allylic alcohol 24.

Dimethyl sulfoxide (0.95 M in CH_2Cl_2, 18.2 ml, 17.4 mmol) was added to a solution of $(COCl)_2$ (0.59 mL, 7.0 mmol) in CH_2Cl_2 (100 mL) at –78 °C. The mixture was stirred at –78 °C for 10 min, then a solution of TBS ether **22** (891 mg, 3.48 mmol) in CH_2Cl_2 (17 mL) was added. The reaction mixture was stirred at –78 °C for 2 h and treated with Et_3N (3.1 mL, 22 mmol). The resulting solution was stirred at –78 °C for additional 1 h, warmed to room temperature over 1.5 h, and stirred for further1 h, and then H_2O (17 mL) was added. The mixture was extracted with CH_2Cl_2 (17 mL) and the organic layer was poured into a suspension of $NaBH_4$ (659 mg, 17.4 mmol) in EtOH (85 mL) at 0 °C. The reaction mixture was stirred for 30 min and saturated aqueous NH_4Cl was added. The resulting solution was extracted with CH_2Cl_2 and the organic layer was dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc 9:1-4:1) gave allylic alcohol **24** (751 mg, 2.93 mmol) in 84% yield.

Colorless oil; $[\alpha]_D^{25} = 2.7$ (*c* 0.37, $CHCl_3$); IR (neat) ν 3357, 2956, 2932, 2858, 1679, 1642, 1467, 1362, 1255, 1065, 1042, 1009, 837 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.03 (3H, s, Me of TBS), 0.05 (3H, s, Me of TBS), 0.87 (3H, t, *J* = 7.4 Hz, H-20), 0.90 (9H, s, *t*-Bu of TBS), 1.51 (2H, qd, *J* = 7.4, 6.2 Hz H-19), 4.11 (1H, td, *J* = 6.2, 5.7 Hz, H-18), 4.31 (1H, dd, *J* = 13.6, 6.8 Hz, H-13), 4.33 (1H, dd, *J* = 13.6, 6.8 Hz, H-13), 5.57 (1H, dt, *J* = 11.3, 6.8 Hz, H-14), 5.72 (1H, dd, *J* = 14.8, 5.7 Hz, H-17), 6.09 (1H, dd, *J* = 11.3, 11.3 Hz, H15), 6.43 (1H, dd, *J* = 14.8, 11.3 Hz, H16). ^{13}C NMR (125 MHz, $CDCl_3$) δ –4.8, –4.4, 9.6, 18.3, 25.9, 31.0, 58.8, 73.9, 123.4, 128.8, 130.4, 139.2. HRMS (ESI), calcd for $C_{14}H_{28}O_2SiNa$ 279.1756 $(M+Na)^+$, found 279.1765.



24 **3**

C13-20 Fragment (3).

Tetrabromomethane (129 mg, 0.391 mmol) and DPPE (156 mg, 0.391 mmol) were successively added to a

solution of allylic alcohol **24** (100 mg, 0.391 mmol) in CH_2Cl_2 (3 mL) at 0 °C. The reaction mixture was stirred at room temperature for 20 min and was directly subjected to flash column chromatography (hexane/EtOAc 9:1) to give C13-20 fragment (**3**) (122 mg, 0.384 mmol) in 98% yield.

Yellow oil ; $[\alpha]_D^{24}$ = –72.2 (*c* 0.47, $CHCl_3$); IR (neat) ν 2957, 2929, 2884, 2857, 1651, 1472, 1362, 1255, 1200, 1044, 837, 775 cm^{-1}. ^1H NMR (500 MHz, C_6D_6) δ 0.06 (3H, s, Me of TBS), 0.07 (3H, s, Me of TBS), 0.86 (3H, t, *J* = 7.3 Hz, H-20), 1.01 (9H, s, *t*-Bu of TBS), 1.38-1.50 (2H, m, H-19), 3.69 (1H, dd, *J* = 9.5, 8.3 Hz, H-13), 3.80 (1H, dd, *J* = 9.5, 9.5 Hz, H-13), 3.96 (1H, td, *J* = 6.2, 5.6 Hz, H-18), 5.40 (1H, br ddd, *J* = 10.6, 9.5, 8.3 Hz, H14), 5.60 (1H, dd, *J* = 15.1, 5.6 Hz, H17), 5.87 (1H, dd, *J* = 11.2, 10.6 Hz, H15), 6.51 (1H, dd, *J* = 15.1, 11.2 Hz, H16). ^{13}C NMR (125 MHz, C_6D_6) δ –4.6, –4.2,9.7, 18.5, 26.1, 26.9, 31.2, 74.1, 123.1, 125.5, 132.9, 140.7.

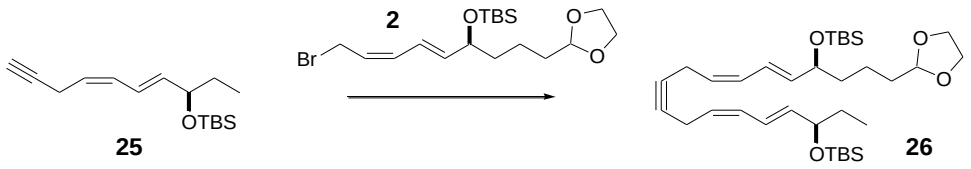
3 **25**

C11-20 Fragment (25).

Ethynyl magnesium bromide (0.50 M in THF, 4.70 ml, 2.35 mmol) was added to a solution of CuCl (23 mg, 0.23 mmol) in THF (4.0 mL) at room temperature, and the mixture was stirred for 15 min. A solution of C13-20 fragment (**3**) (61 mg, 0.19 mmol) in THF (3.0 mL) was added to the mixture. The reaction mixture was stirred at 60 °C for 14 h and cooled to room temperature, and then saturated aqueous NH_4Cl solution was added. The mixture was extracted with Et_2O and the organic layer was washed with brine and dried over anhydrous Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc 100:0-98:2) gave C11-20 fragment (**25**) (43 mg, 0.16 mmol) in 84% yield.

Pale brown oil; $[\alpha]_D^{28}$ = –21.8 (*c* 0.33, CHCl3); IR (neat) ν 3311, 2957, 2931, 2857, 2122, 1466, 1254, 1065, 837 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.03 (3H, s, Me of TBS), 0.05 (3H, s, Me of TBS), 0.87 (3H, t, *J* = 7.4 Hz, H-20), 0.91 (9H, s, *t*-Bu of TBS), 1.51 (2H, qd, *J* = 7.4, 5.6 Hz, H-19), 2.00 (1H, t, *J* = 2.8 Hz, H-11), 3.07 (2H, ddd, *J* = 6.8, 2.8, 2.8 Hz, H-13), 4.11 (1H, dt, *J* = 6.2, 5.6 Hz, H-18), 5.42 (1H, dt, *J* = 10.2, 6.8 Hz, H-14), 5.71 (1H, dd, *J* = 15.3, 6.2 Hz, H-17), 6.06 (1H, ddd, *J* = 11.3, 10.2, 2.8 Hz, H-15), 6.38 (1H, dd, *J* = 15.3, 11.3 Hz, H-16). ^{13}C NMR (125 MHz, $CDCl_3$) δ –4.8 –4.4, 9.6, 17.2, 18.3, 25.9, 31.0, 68.3, 74.0, 82.4, 123.2, 124.1, 129.9,

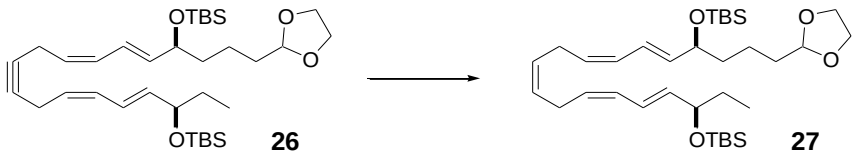
138.7.



Coupling product 26.

n-Buthyl lithium (1.6 M in hexane, 0.21 mL, 0.34 mmol) was added to a solution of $CuBr \cdot SMe_2$ (70 mg, 0.34 mmol) and C11-20 fragment (**25**) (100 mg, 0.379 mmol) in THF (2.0 mL) at –78 °C. The solution was stirred at –78 °C for 5 min and then HMPA (2.0 mL) was added. The resulting mixture was dropped into a solution of C1-10 fragment (**2**) (76.2 mg, 0.189 mmol) in THF (3.0 mL) at 0 °C *via* cannular. The reaction mixture was stirred at room temperature for 17 h and saturated aqueous NH_4Cl was added. The mixture was extracted with CH_2Cl_2 and the organic layers were washed with brine and dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc 99:1-98:2-97:3) gave **26** (59 mg, 0.10 mmol) in 53% yield.

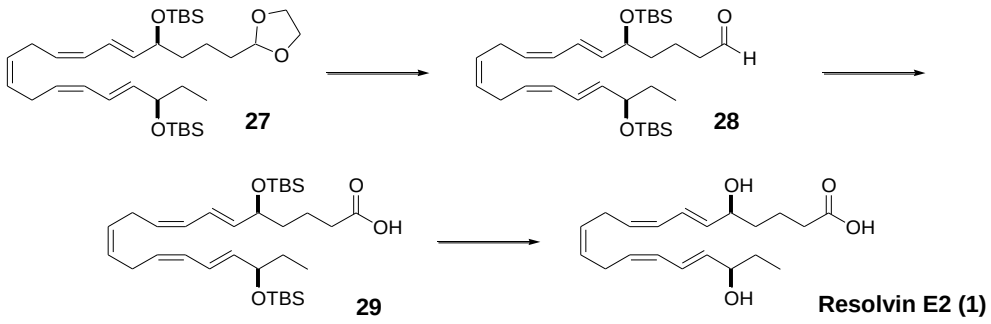
Colorless oil; $[\alpha]_D^{24} = -2.2$ (*c* 0.35, $CHCl_3$); IR (neat) ν 2954, 2929, 2857, 1472, 1463, 1254, 1063, 950, 836 cm^{-1}. ^1H NMR (500 MHz, C_6D_6) δ 0.07 (9H, s, Me of TBS x 3), 0.09 (3H, s, Me of TBS), 0.88 (3H, t, *J* = 7.3 Hz, H-20), 1.00 (18H, s, *t*-Bu of TBS x 2), 1.40-1.57 (3H, m, H-3. H-19), 1.55-1.60 (2H, m, H4), 1.60-1.70 (1H, m, H-3), 1.74-1.77 (2H, m, H2), 2.95-3.08 (4H, m, H10, H13), 3.35-3.37 (2H, m, C*H*$_2$ of acetal), 3.52-3.55 (2H, m, OC*H*$_2$CH$_2$O), 4.00 (1H, dt, *J* = 6.2, 6.2 Hz, H18), 4.08 (1H, dt, *J* = 6.2, 5.0 Hz, H5), 4.81 (1H, t, *J* = 4.5 Hz, H1), 5.43-5.49 (2H, m, H9, H14), 5.60 (2H, dd, *J* = 15.1, 6.2 Hz, H6, H17), 5.96 (1H, dd, *J* = 11.2, 11.2 Hz, H8 or 15), 5.98 (1H, dd, *J* = 10.6, 10.6 Hz, H8 or 15), 6.49 (1H, dd, *J* = 15.1, 11.2 Hz, H7 or 16), 6.50 (1H, dd, *J* = 15.1, 11.2 Hz, H7 or 16). ^{13}C NMR (125 MHz, C_6D_6) δ –4.60, –4.57, –4.1, –4.0, 9.8, 18.0 (2C), 18.5 (2C), 20.4, 26.13, 26.15, 31.5, 34.4, 38.6, 64.8 (2C), 73.3, 74.5, 78.5, 78.6, 104.8, 124.1, 124.2, 126.3 (2C), 129.5 (2C), 138.4, 138.6. HRMS (EI), calcd for $C_{34}H_{60}O_4Si_2$ 588.4030 (M^+), found 588.4044.



Alkene 27.

A suspension of compound **26** (22 mg, 38 μmol), quinoline (38 μL, 0.31 mmol) and 5% Pd-$BaSO_4$ (22 mg) in EtOAc (6.0 mL) was exposed to H_2 atmosphere (1 atm) and stirred for 10 h at room temperature. Additional 5% Pd-$BaSO_4$ (22 mg) was added and the reaction mixture was stirred for further 4 h at room temperature. Additional 5% Pd-$BaSO_4$ (11 mg) was added again and the reaction mixture was stirred for further 2 h at room temperature. The suspension was filtrated through a pad of Celite with EtOAc. Concentrated and purification by HPLC (Inertsil, SIL 100A, 250 x 10 mm, UV 254 nm, hexane/EtOAc 96/4, 3.0 mL/min, *TR* = 23 min) gave alkene **27** (13 mg, 22 μmol) in 54% yield.

Pale yellow oil; $[\alpha]_D^{23}$ = 1.4 (*c* 0.37, $CHCl_3$); IR (neat) ν 2955, 2928, 2856, 1471, 1463, 1255, 1142, 1063, 952, 836 cm^{-1}. ^1H NMR (500 MHz, C_6D_6) δ 0.10 (3H, s, Me of TBS), 0.11 (6H, s, Me of TBS x 2), 0.12 (3H, s, Me of TBS), 0.92 (3H, t, *J* = 7.3 Hz, H20), 1.04 (18H, s, *t*-Bu of TBS x 2), 1.50-1.58 (2H, m, H19), 1.60-1.73 (4H, m, H3, H4), 1.75-1.82 (2H, m, H2), 2.94-3.04 (4H, m, H10, H13), 3.35-3.40 (2H, m, CH_2 of acetal), 3.53-3.56 (2H, m, CH_2 of acetal), 4.08 (1H, dt, *J* = 6.2, 5.6 Hz, H18), 4.17 (1H, dt, *J* = 6.2, 5.6 Hz, H5), 4.82 (1H, t, *J* = 4.5 Hz, H1), 5.36-5.43 (2H, m, H9, H14), 5.45 (2H, t, *J* = 5.0 Hz, H11, H12), 5.67 (2H, dd, *J* = 15.1, 6.2 Hz, H6, H17), 6.06 (1H, dd, *J* = 11.2, 10.1 Hz, H8 or 15), 6.08 (1H, dd, *J* = 11.2, 10.1 Hz, H8 or 15), 6.65 (1H, dd, *J* = 15.1, 11.2 Hz, H7 or 16), 6.67 (1H, dd, *J* = 15.1, 11.2 Hz, H7 or 16). ^{13}C NMR (125 MHz, $(CD_3)_2CO$) δ –4.55, –4.51, –4.1, –4.0, 9.9, 18.8 (2C), 20.6, 26.29, 26.31, 26.7 (2C), 31.8, 34.7, 39.0, 65.3 (2C), 73.7, 74.9, 105.0, 125.21, 125.24, 128.8 (2C), 129.2 (2C), 129.74, 129.83, 138.1, 138.2. HRMS (EI), calcd for $C_{34}H_{62}O_4Si_2$ 590.4187 (M^+), found 590.4165.



Resolvin E2 (1).

To a solution of alkene **27** (7.0 mg, 12 μmol) and 2,6-lutidine (33 μL, 270 μmol) in CH_2Cl_2 (1.5 mL) at –20 °C was TMSOTf (33 μL, 180 μmol). The reaction mixture was stirred at –20 °C for 1 h and H_2O was added. The mixture was stirred at room temperature for 1 h and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc 9:1) gave the aldehyde **28** (7.0 mg, 13 μmol).

A solution of $NaClO_2$ (8.7 mg, 96 μmol) and $NaH_2PO_4 \cdot 4H2O$ (16 mg, 0.10 mmol) in H_2O (0.5 mL) was added to a solution of the crude aldehyde **28** in 2-methyl-2-butene (0.50 mL) and *t*-BuOH (0.50 mL) at 0 °C was added. The reaction mixture was stirred at room temperature for 1 h and brine was added. The mixture was extracted with EtOAc and the organic layer was dried over Na_2SO_4. Filtration, concentration gave the carboxylic acid **29** (8.2 mg), which was directly used in the next reaction. To a solution of the crude carboxylic acid **29** in THF (3.0 mL) at 0 °C was added TBAF (1.0 M in THF, 240 μL, 0.24 mmol). The reaction mixture was stirred at room temperature for 11 h and additional TBAF (60 μL, 0.06 μmol) was added. The reaction mixture was stirred at room temperature for further 2 h and brine and saturated aqueous NH_4Cl were added. The mixture was extracted with Et_2O and the organic layer was dried over Na_2SO_4. Concentration, chromatography (silica gel; BW-820, CH_2Cl_2/MeOH 99:1-97:3-10:1), and further purification by HPLC (Inertsil, ODS-3, 150 x 4.6 mm, UV 254 nm, MeOH/H_2O/AcOH 60/40/0.01-90/10/0.01, 1.0 mL/min, *TR* = 17 min) gave resolvin E2 (**1**) (2.60 mg, 7.8 μmol) in 67% yield over 3 steps.

Colorless oil; $[\alpha]_D^{24}$ = –2.1 (*c* 0.25, MeOH); IR (neat) ν 3384, 3012, 2963, 2933, 1715, 1244, 1034, 985, 954 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ 0.91 (3H, t, *J* = 7.3 Hz, H2), 1.48-1.73 (6H, m, H-3. H-4. H-19), 2.03 (2H, t, *J* = 8.4 Hz, H2), 2.98 (4H, m, H10, H13), 4.02 (1H, dt, *J* = 6.8, 6.7 Hz, H5 or 18), 4.11 (1H, dt, *J* = 6.8, 6.2 Hz, H5 or

18), 5.34-5.42 (4H, m, H9, H11, H12, H14), 5.65 (1H, dd, *J* = 15.2, 6.8 Hz, H6 or 17), 5.66 (1H, dd, *J* = 15.2, 6.8 Hz, H6 or 17), 6.00 (2H, dd, *J* = 11.2, 10.6 Hz, H8, H15), 6.55 (1H, dd, *J* = 15.2, 11.2 Hz, H7 or 16), 6.56 (1H, dd, *J* = 15.2, 11.2 Hz, H7 or 16). ^{13}C NMR (125 MHz, CD_3OD) δ 10.2, 22.3, 27.0 (2C), 31.2, 35.0, 37.8, 72.9, 74.7, 126.4 (2C), 129.09, 129.11, 129.4, 129.5, 130.5, 130.6, 137.7 (2C), 177.7. HRMS (FAB), calcd for $C_{20}H_{29}O_4$ 333.2066 $(M-H)^-$, found 333.2072.

Bioassay

Murine peritonitis was carried out using 7- to 8-week old C57BL/6 male mice (CLEA Japan). Resolvin E2 or vehicle alone was injected into the tail vein followed by 1 mL of zymosan A (1 mg/mL; Sigma) injected into the peritoneum. At 2 h, peritoneal lavages were collected and cells were enumerated via light microscopy. Differential leukocyte counts were performed using Wright Giemsa stain. Cytokine levels were determined from peritoneal cell-free exudates using BD OptEIA Kit for mouse TNFα and IL-6 (BD Biosciences)

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Chapter 4

Enantioselective Synthesis of Haterumalide NA Methyl Ester and Revised Structure of Haterumalide NA

Abstract:

The enantioselective synthesis of the enantiomer of the haterumalide NA methyl ester, a cytotoxic macrolide from an Okinawan sponge, was achieved from the threitol derivative in 26 steps. The key steps are the stereoselective construction of a chloroolefin unit and the intramolecular Reformatsky-type reaction. This synthesis revised the absolute stereochemistry of haterumalide NA.

Introduction

Haterumalide NA is a macrolide isolated from the Okinawan sponge *Ircinia* sp. This compound exhibited a cytotoxicity against P388 cells with an IC_{50} of 0.32 μg/mL.[1] The gross structure and stereochemistry were elucidated by the spectroscopic analysis and the modified Mosher's method as structural formula **1**. The structural features of this compound are a 14-membered macrolide (long chain fatty acid derivative) involving a *trans* disubstituted tetrahydrofuran ring, a *Z*-chloroolefin, and a β,γ-unsaturated acid moiety. The structurally related haterumalide B[2] and oocydin A[3] were isolated from an Okinawan ascidian and a South American epiphyte, respectively, and their stereostructures have not been fully established. It is noteworthy that haterumalide NA was recently isolated from a soil bacterium.[4] The author describes in this chapter the enantioselective synthesis of the *ent*-haterumalide NA methyl ester, which revises the initially assigned stereostructure.

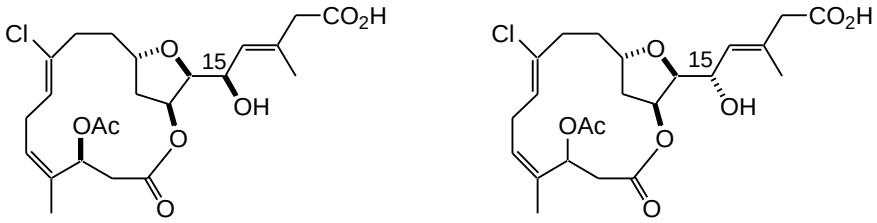
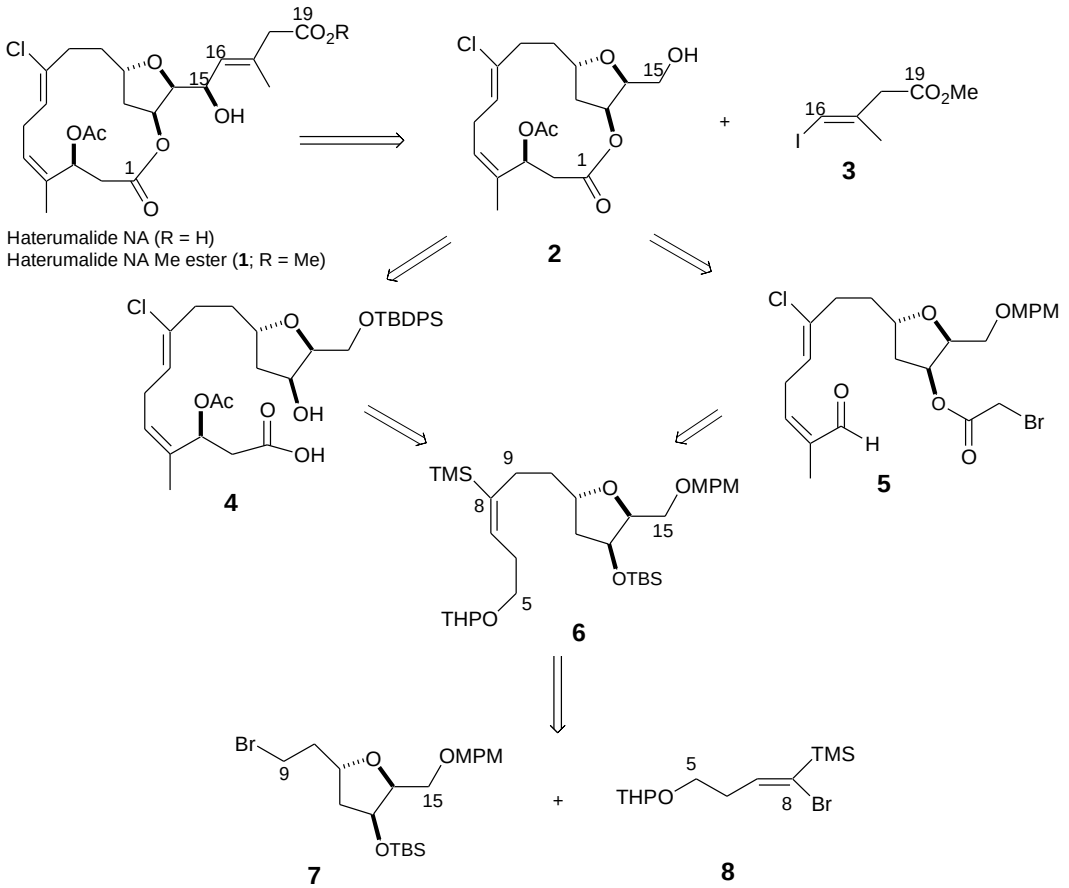


Figure 4-1. Structure of haterumalide NA and oocydin A

Results and discussion

Retrosynthesis of haterumalide NA

Retrosynthetic analysis of haterumalide NA is outlined in Scheme 4-1. The haterumalide NA methyl ester (**1**: R = Me) can be logically divided into the macrolide unit **2** and the side chain unit **3**. The side chain unit **3** can be easily prepared from 3-butyn-1-ol **18** (see Scheme 4-8).[5] The macrocyclic structure of **2** can be established by lactonization of the seco acid **4** or by the intramolecular Reformatsky-type reaction of the bromo ester derivative **5**. These precursors, **4** and **5**, can be synthesized from a common intermediate **6**, which can be prepared from the tetrahydrofuran unit **7** by a coupling reaction with **8**.

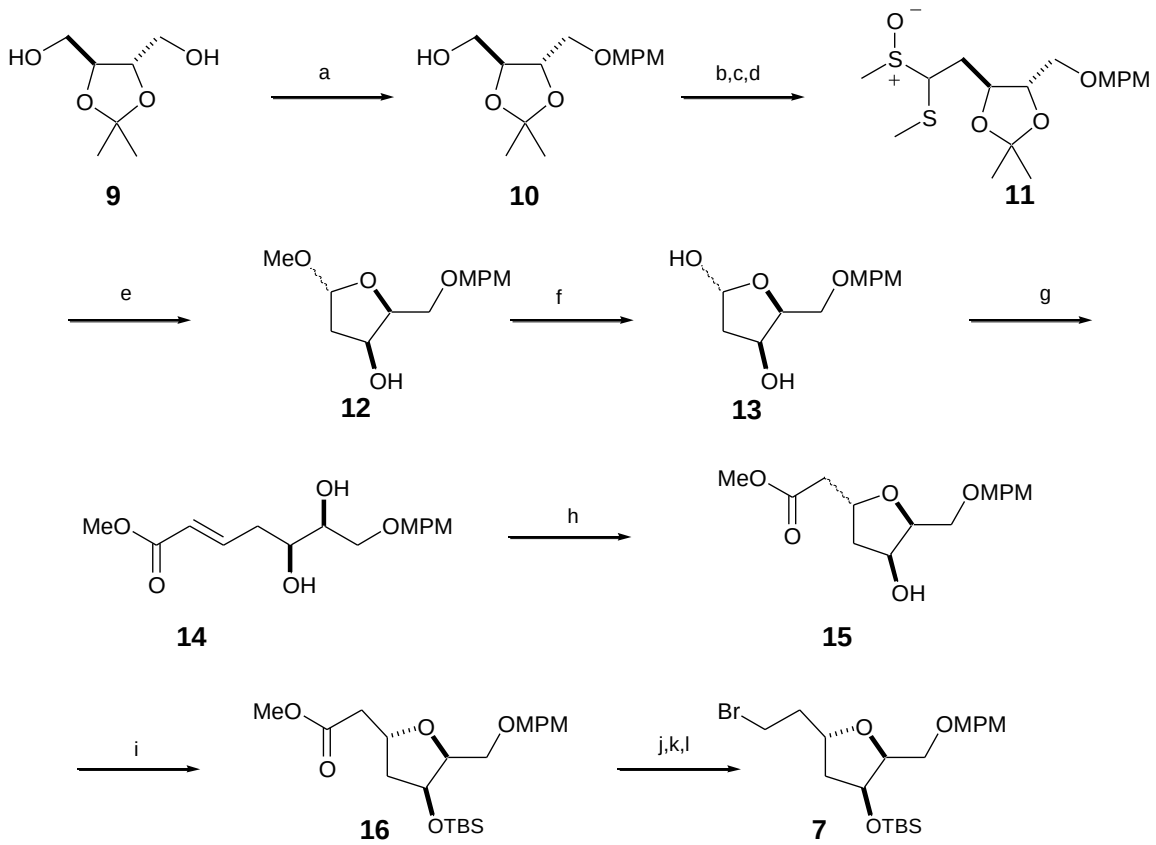


Scheme 4-1. Retrosynthesis of haterumalide NA methyl ester

Synthesis of C9-C15 fragment

Scheme 4-2 summarizes the synthesis of the tetrahydrofuran unit **7**. The mono-MPM ether **10** was synthesized

from commercially available (+)-2,3-*O*-isopropylidene-L-threitol **9**.[6] After transformation into the corresponding iodide, C1 homologation was effected by using the FAMSO[7] carbanion to afford sulfoxide **11** (69%). Sequential acidic methanolysis[8] and hydrolysis afforded the hemiacetal **13** in 41% yield. Wittig reaction of **13** and cyclization provided the 5.3:1 diastereomeric mixture of tetrahydrofurans, which could be separated after silylation to afford the desired *trans*-tetrahydrofuran **16** (41%) and the *cis*-isomer **17**. The latter could be isomerized into the former as a 1:1 mixture (isolation yield of **14** 42%). The stereochemistry of **16** and **17** were determined by the coupling constants from their ^{1}H NMR data and the NOE experiments (Figure 4-2). The desired *trans*-tetrahydrofuran **16** was quantitatively converted into the bromide **7** in 3 steps.



Reagents and conditions: (a) MPMCl, NaH, DMF (63%). (b) *p*-TsCl, pyridine. (c) NaI, $CaCO_3$, acetone (89%, 2 steps). (d) FAMSO, *n*-BuLi, THF-hexanes (69%). (e) conc. HCl, MeOH (8:92) (41%). (f) 1 M HCl aq, THF (99%). (g) Ph_3PCHCO_2Me, MeCN. (h) NaOMe, MeOH. (i) TBSCl, imidazole (41%, 3 steps). (j) $LiAlH_4$, THF (100%). (k) *p*-TsCl, pyridine. (l) LiBr, DMF (100%, 2 steps).

Scheme 4-2. Synthesis of C9-C15 fragment **7**

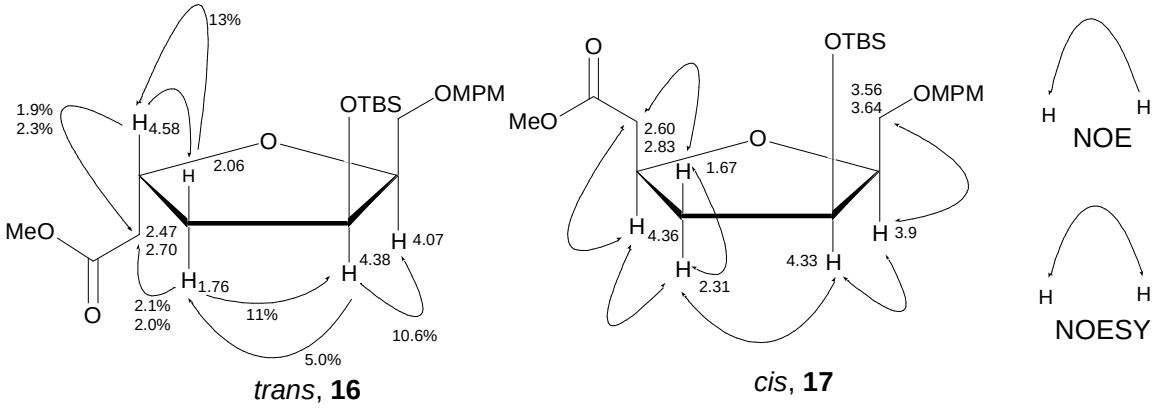
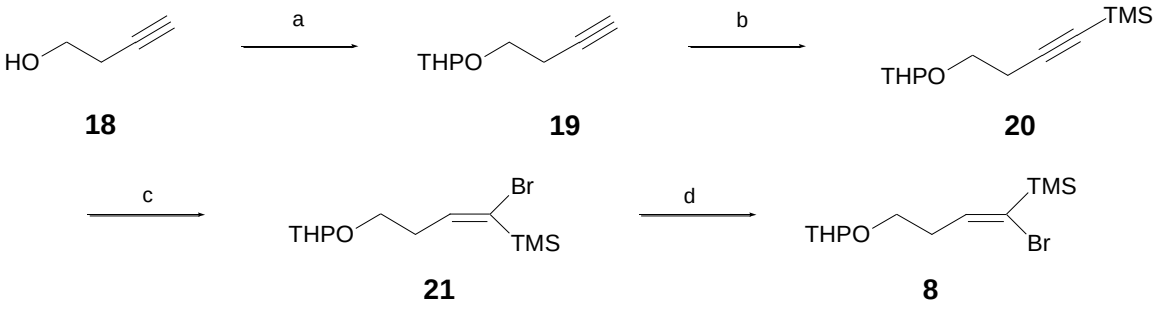


Figure 4-2. ^{1}H NMR data and the observed NOEs of **16** and **17**

Synthesis of C5-C8 fragment

Synthesis of C5-C8 fragment was started with 3-butyn-1-ol **18** (Scheme 4-3). 3-Butyn-1-ol (**18**) was transformed into the *E*-alkenylsilane **21** (52%) following a reported procedure[9]. The *E*-alkenylsilane **21** was photochemically isomerized to the *Z*-isomer **8** in 99% yield. The stereochemistry of **8** was determined by the NOE experiments (Figure 4-3).



Reagents and conditions: (a) DHP, *p*-TsOH (86%). (b) TMSCl, *n*-BuLi, ether-hexanes (67%). (c) (i) DIBAL, ether-hexanes; (ii) pyridine, ether; (iii) Br_2, CH_2Cl_2 (91%). (d) *hv,* Br_2 , pyridine, CH_2Cl_2 (100%).

Scheme 4-3. Synthesis of C5-C8 fragment

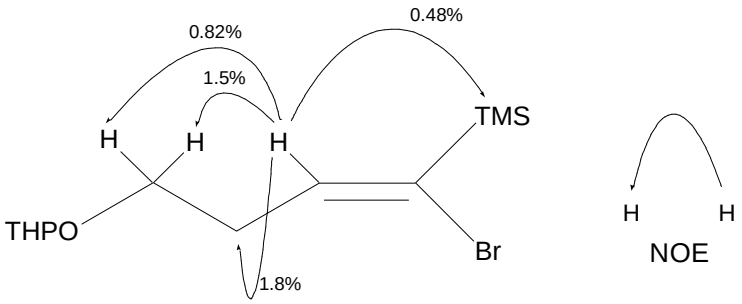
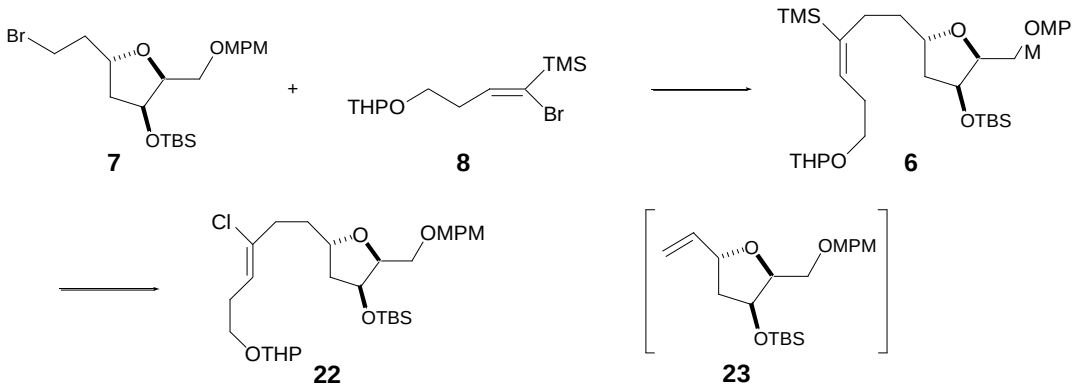


Figure 4-3. NOE experiment of **8**

Synthesis of *Z*-chloroolefin 22

The coupling reaction between the tetrahydrofuran unit **7** and the carbanion generated from the *Z*-alkenylsilane **8** and *sec*-butyllithium was accomplished to give compound **6** in 68% yield. There are only a few published procedures for the stereoselective preparation of chloroolefins. The author modified the procedure[10] for conversion of an alkenylsilane to a bromoolefin for preparation of the chloroolefin **22** (Scheme 4-4). After several attempts, the author found that the addition of a catalytic amount of water was important for the reaction to be reproducible.



Reagents and conditions: (e) (i) **8**, *s*-BuLi, THF-hexanes; (ii) **7**, HMPA, THF (68%). (f) NCS, H_2O, DMF (45%)**.**

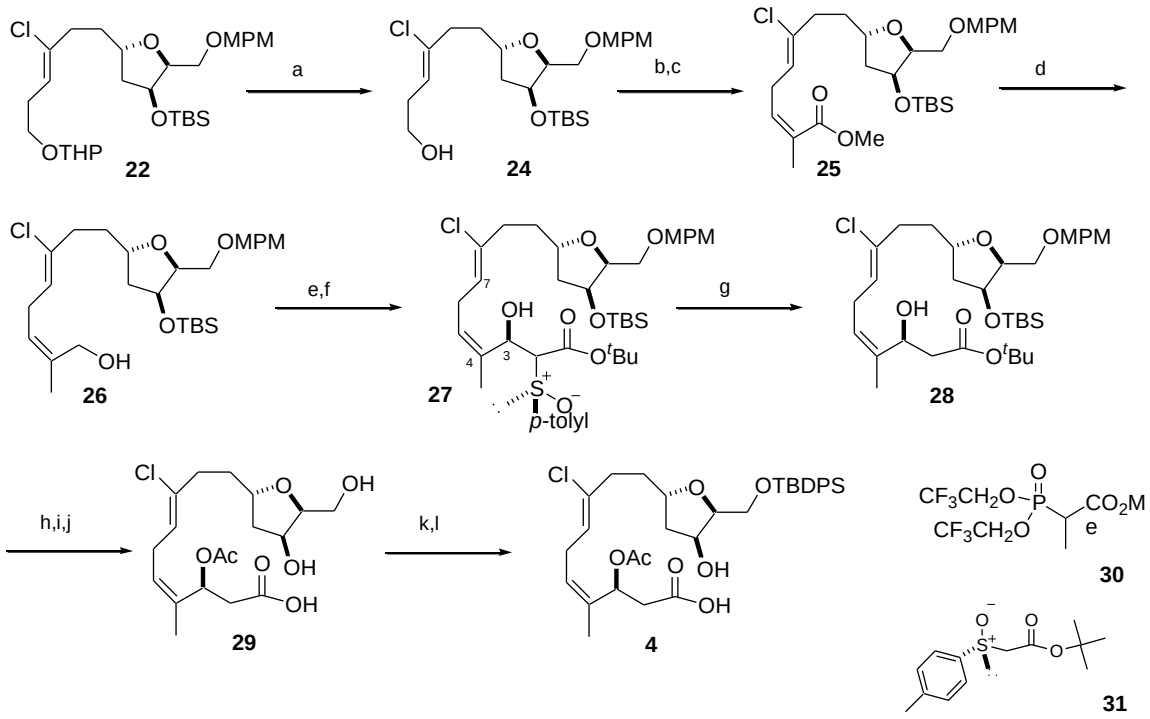
Scheme 4-4. Synthesis of **22**

Synthesis of seco acid 4 and macrolactonization

Scheme 4-5 summarizes the synthesis of the seco acid **4**. Acidic hydrolysis of **22** gave **24** and subsequent Dess Martin oxidation afforded a labile aldehyde, which was converted into the *Z*-conjugated ester **25** by using the Still's modified Horner-Emmons reaction[11] (62%, three steps). The regiochemistry at C4 double bond of **25** was

determined by the NOE experiment (Figure 4-4). The DIBAL reduction of **25** gave the allylic alcohol **26** (100%), which was oxidized to a conjugated aldehyde. The asymmetric aldol reaction[12] with Corey's sulfoxide **31** provided a hydroxysulfoxide, amalgam reduction of which gave the desired hydroxyl ester **28** (49%, three steps). The absolute stereochemistry of the C-3 hydroxyl group in **28** was established by the modified Mosher's method.[14] After acetylation, the protecting groups were removed to give the dihydroxyl acid **29** (84%, two steps), the primary hydroxyl group of which was protected as the TBDPS ether to afford the seco acid **4** (47%, two steps).

Thus, the precursors for the macrolide unit **2** were in hand. However, all attempts at macrolactonization of the dihydroxyl acid **29** or the seco acid **4** to **2** failed under the Yamaguchi, Keck, and Mukaiyama-Corey conditions (Scheme 4-6).



Reagents and conditions: (a) AcOH, THF, H_2O (80%). (b) Dess-Martin periodinane, CH_2Cl_2. (c) **30**, KHMDS, 18-crown-6, TH, toluene (77%, 2 steps). (d) DIBAL, toluene (100%). (e) Dess-Martin periodinane, CH_2Cl_2. (f) **31**, *t*-BuMgCl, THF (57%, 3*S*:3*R*) 19:1, 2 steps). (g) Al-Hg, THF-H_2O (86%). (h) Ac_2O, pyridine (86%). (i) HF-py, pyridine, THF (93%). (j) TMSOTf, 2,6-lutidine, CH_2Cl_2 (90%). (k) TBDPSCl, DMAP, Et_3N, CH_2Cl_2.(l) AcOH, THF, H_2O (47%, 2 steps).

Scheme 4-5. Synthesis of seco acid **4**

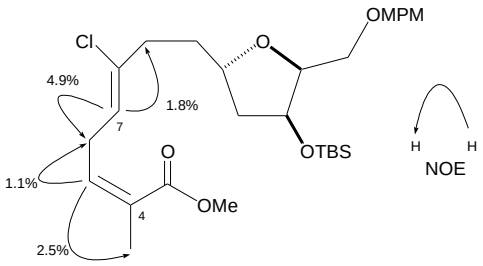
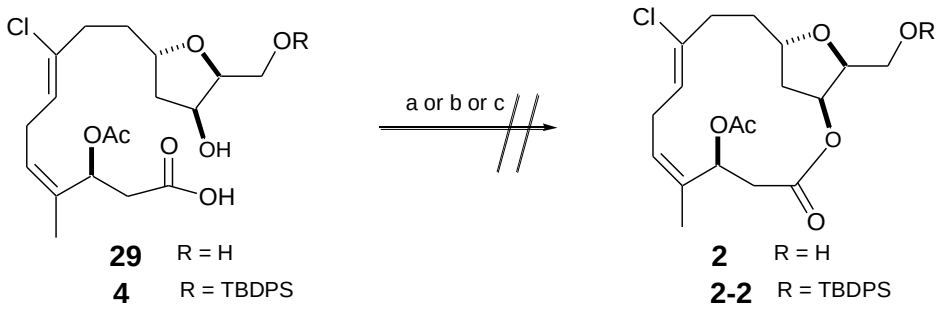


Figure 4-5. NOE experiment of **25**



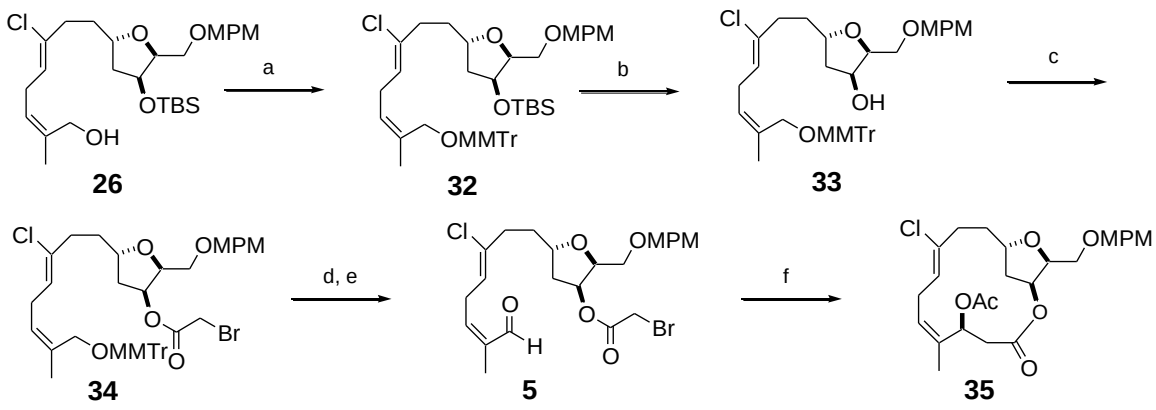
Reagents and conditions: (a) 2,4,6-trichlorobenzoylchloride, DMAP, Et_3N, CH_2Cl_2 (b) DCC, DMAP, CSA, CH_2Cl_2 (c) dipyridyl disulfide, PPh_3, benzene

Scheme 4-6. Macrolactonization with seco acid **4** and **29**

Synthesis of macrolactone 35

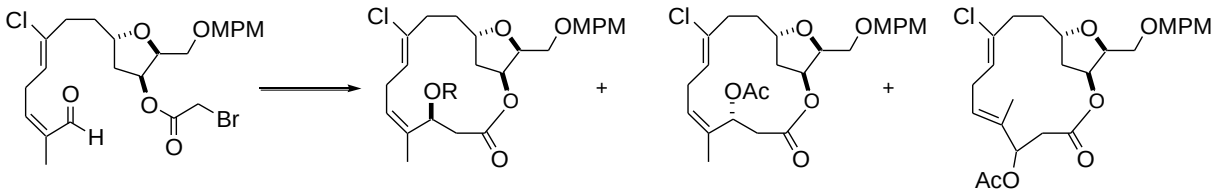
The author next tried to cyclize the 14-membered ring using the intramolecular Reformatsky-type reaction (Scheme 4-7). The hydroxy group of the allylic alcohol **26** was protected as an MMTr ether to quantitatively afford compound **32**. The silyl group in **32** was removed, and the resulting alcohol **33** (99%) was converted into the bromo ester **34**. The MMTr group was removed to give allylic alcohol intermediate (82%, two steps), which was oxidized to afford the conjugated aldehyde **5**, a precursor of the intramolecular Reformatsky-type reaction, in 93% yield. Attempts toward the intramolecular Reformatsky-type reaction are summarized in Table 4-5. The cyclization with SmI_2[15] provided the cyclic compounds in good yields (86%, 3*S*:3*R* = 1:1); however, the stereochemistry of the C-4 double bond was totally isomerized into *trans* (entry 1). The reaction at lower temperature also gave the same *trans*-products in a lower yield (entry 2). The molecular mechanics calculation indicated that the desired *cis* compound **34** was less stable (7.5 kJ/mol) than the *trans* compound. (The

calculations were executed by MacroModel (Version 6.0) with the MM2* force field.) This isomerization might be due to the allylic radical nature of the transition state and/or the reactive intermediates. Therefore, the author investigated the cyclization with zinc reagents apt to effect the two-electron reduction. The reactions under the standard conditions[16,17] afforded no cyclized compounds (entries 3 and 4). The reaction under Honda's conditions[18] with Et_2Zn-$RhCl(PPh_3)_3$ resulted in the decomposition of the starting material; however, the author found by TLC monitoring the generation of an intermediate, the β-hydroxy lactone **36**, which decomposed upon workup (entry 5). The addition of Ac_2O to trap the reactive products allowed the author to isolate the desired cyclized product (3*S*,4*Z*)-**35** in 9% yield along with the (3*R*,4*Z*)- and (4*E*)-isomers (entry 6).



Reagents and conditions: (a) MMTrCl, pyridine (100%). (b) TBAF, THF (99%). (c) $BrCH_2COBr$, pyridine, CH_2Cl_2. (d) AcOH, THF-H_2O (82% in 2 steps). (e) Dess-Martin periodinane, CH_2Cl_2 (93%). (f) (i) Et_2Zn, $RhCl(PPh_3)_3$, THF-hexane; (ii) Ac_2O (9%).

Scheme 4-7. Synthesis of macrolactone **35.**



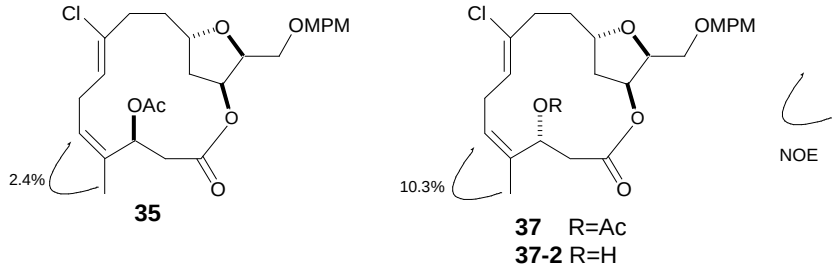
5 **35** R=Ac, **36** R=H **37** **38**

entry	conditions	product	(3*S*,4*Z*)/(3*R*,4*Z*)/(4*E*) (yeild)
1	SmI_2, 0 ℃	**36**	-/-/100 (86%)
2	SmI_2, -100 ℃	**36**	-/-/100 (7%)
3	Zn, $B(OMe)_3$, rt	**36**	_[b]
4	Zn, CuBr, Et_2AlCl, -20 ℃	**36**	_[c]
5	Et_2Zn, $RhCl(PPh_3)_3$, -20 ℃	**36**	_[b]
6[a]	Et_2Zn, $RhCl(PPh_3)_3$, -20 ℃	**35**	20/72/8 (45%)

a After 4 h, Ac_2O was added to trap the reactive product **36**. *b* Complex mixture. *c* Noncyclized reduced products were obtained: debromo-**5** (65%) and the corresponding debromo-allyl alcohol (23%).

Table 4-5. Intramolecular Reformatsky type reaction of bromoesrter **5**

The stereochemistry at the C-4 double bond was easily determined by the NOE experiments (Figure 4-6). On the other hand, the stereochemistry at C-3 was determined by the modified Mosher's method. The minor isomer, (3*S*,4*Z*)-**35**, could not be transformed into the corresponding MTPA esters because of the instability during the methanolysis of the acetyl group. However, the modified Mosher's method could be applied to (3*R*,4*Z*)-**37-2** that was obtained by methanolysis of the major isomer, (3*R*,4*Z*)-**37**, establishing that the major isomer possessed the undesired stereochemistry 3*R*, i.e., the minor isomer was the desired (3*S*)-compound (Figure 4-7).



35 **37** R=Ac, **37-2** R=H

Figure 4-6. NOE studies of **35** and **37**

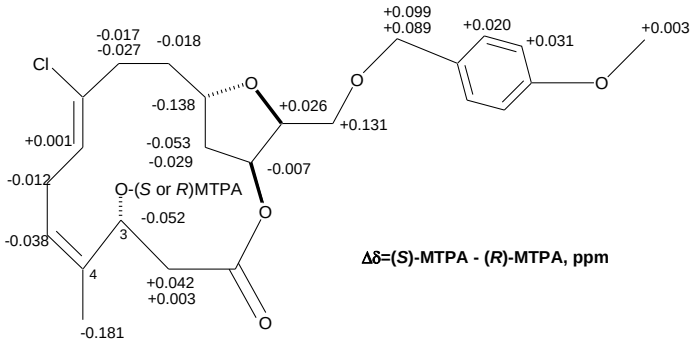
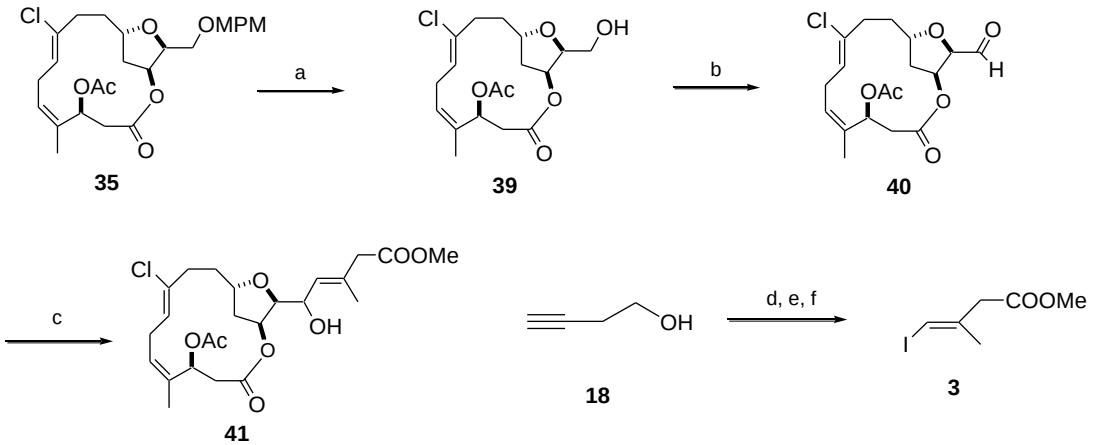


Figure 4-7. Determination of the stereochemistry of **37** by modified Mosher's method

Synthesis of haterumalide NA methyl ester

Scheme 4-8 summarizes the synthesis of the haterumalide NA methyl ester **41**. The MPM group in (3*S*,4*Z*)-**35** was removed to give the alcohol **39** in 88% yield, which was oxidized with the Dess-Martin periodinane to afford an unstable aldehyde **40**. The Nozaki-Hiyama-Kishi coupling reaction[19] of the aldehyde and iodide **3**, prepared from **18**,[5] afforded the coupling product **41** (57%, *S*:*R* = 11:1), and the diastereomers were separated with HPLC. The major isomer, (15*S*)-**41**, was found to be identical with the naturally occurring sample upon comparison of their spectral data (Figure 4-8) and chromatographic behavior except for the sign of the CD spectrum (Figure 4-9).



Reagents and conditions: (a) DDQ, CH_2Cl_2-phosphate buffer (pH 5.9) (88%). (b) Dess-Martin periodinane, CH_2Cl_2. (c) **3**, $CrCl_2$, $NiCl_2$, DMSO (57%, 15*S*:15*R* = 11: 1, 2 steps). (d) Cp_2ZrCl_2, Me_3Al, then I_2, CH_2Cl_2 (89%). (e) CrO_3, H_2SO_4, acetone (68%). (f) $TMSCHN_2$, MeOH, benzene (74%).

Scheme 4-8. Synthesis of haterumalide NA methyl ester

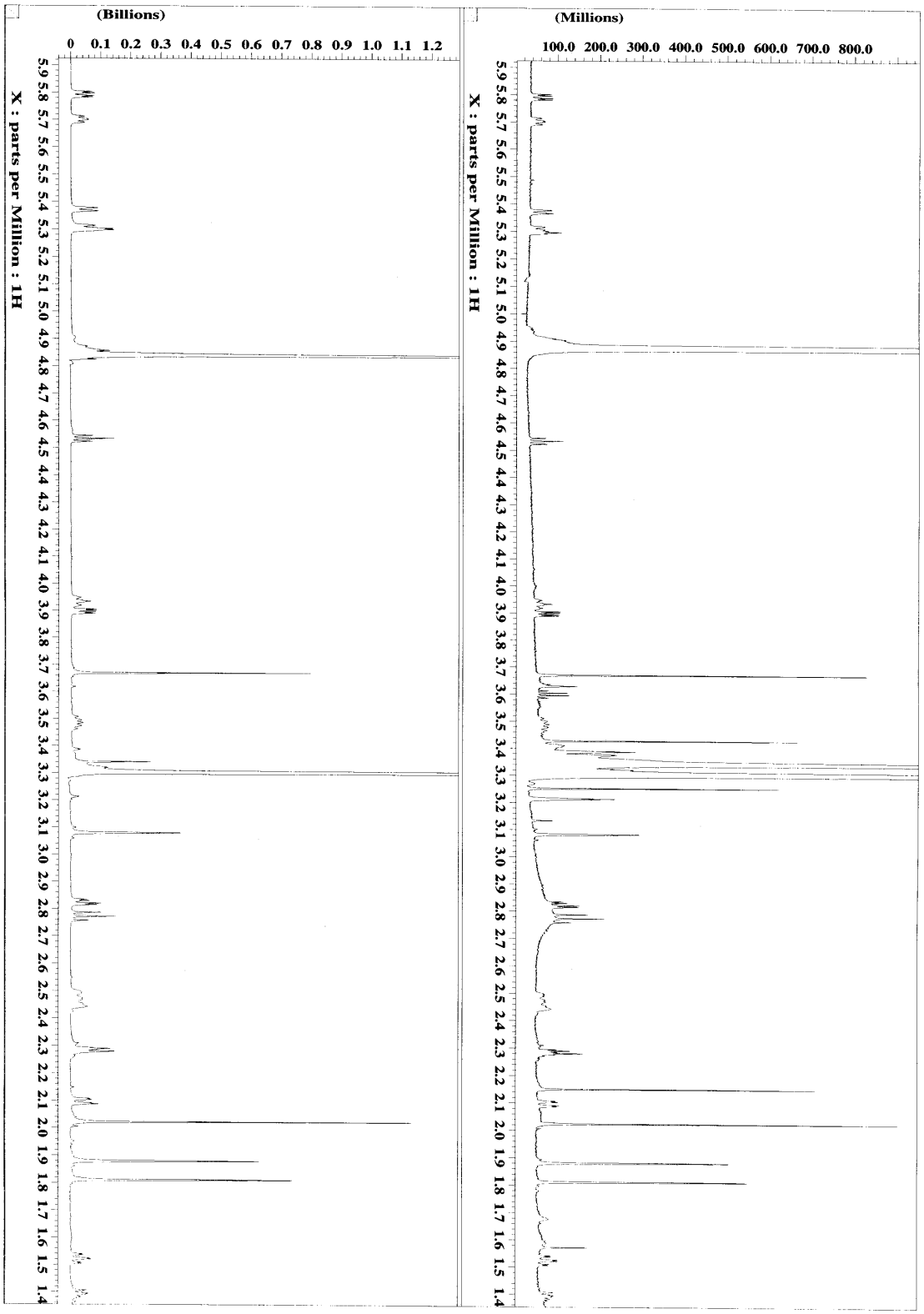


Figure 4-8. Comparison of ^{1}H NMR spectrum of synthetic haterumalide NA methyl ester (**41**) and natural haterumalide NA methyl ester **1** (800 MHz, CD_3OD)

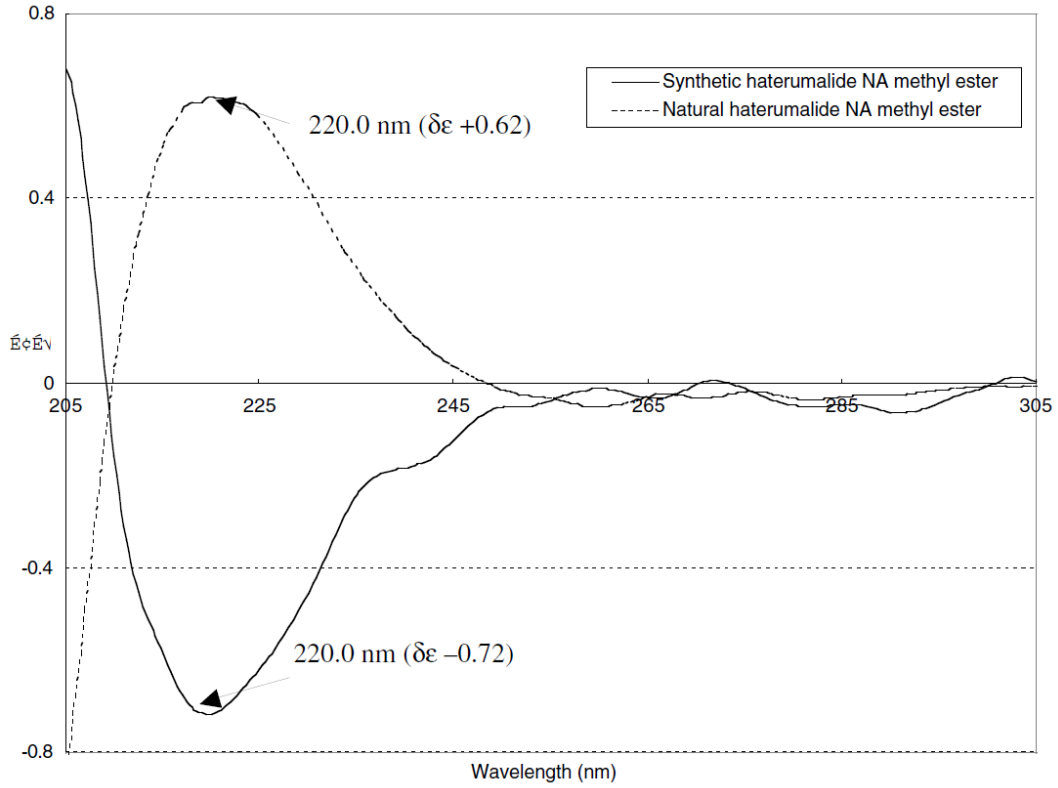


Figure 4-9 Comparison of CD spectrum of synthetic haterumalide NA methyl ester (**41**) and natural haterumalide NA methyl ester **1**

Revised the absolute stereochemistry of Haterumalide NA

The author revised the absolute stereochemistry of haterumalide NA **1** (Figure 4-10). The stereochemistry of the C-3, C-11, C-13, and C-14 in synthetic **41** was undoubtedly constructed by the organic synthetic method, and the final product was the enantiomer of haterumalide NA methyl ester. As a result, the author unambiguously determined the absolute stereochemistry of C-3, C-11, C-13, and C-14 in natural **1** to be *R*, *R*, *R*, and *R*. On the other hand, since the absolute stereochemistry of C-15 in natural **1** was already determined to be *R* by the modified Mosher's method, the total absolute stereochemistry of haterumalide NA was revealed, which revised the previously reported structure.[1] To confirm these results, **41** was converted into the (*R*)-MTPA ester, which was found to be the enantiomer of the (*S*)-MTPA ester of the natural haterumalide NA methyl ester on comparison of their ^{1}H NMR spectra. In a previous paper,[1] in light of the abnormal Δδ values in the experiments of the modified Mosher's method for **1** (R = Me), Uemura et al. postulated the folded conformation of the side chain in

the Mosher esters of **1**, which led the author to the wrong conclusion that the stereochemistry at C14-C15 was *threo* (Figure 4-11). The generally accepted zigzag conformation of the side chain in the Mosher esters of **1** is consistent with the revised stereostructure, in which the stereochemistry at C14-C15 is *erythro*, from the viewpoint of the coupling constants and the NOESY correlations of **1**.

In summary, the enantioselective synthesis of haterumalide NA methyl ester (**41**) has been achieved from the threitol derivative **10** in 26 steps. This synthesis revises the absolute stereochemistry of haterumalide NA.

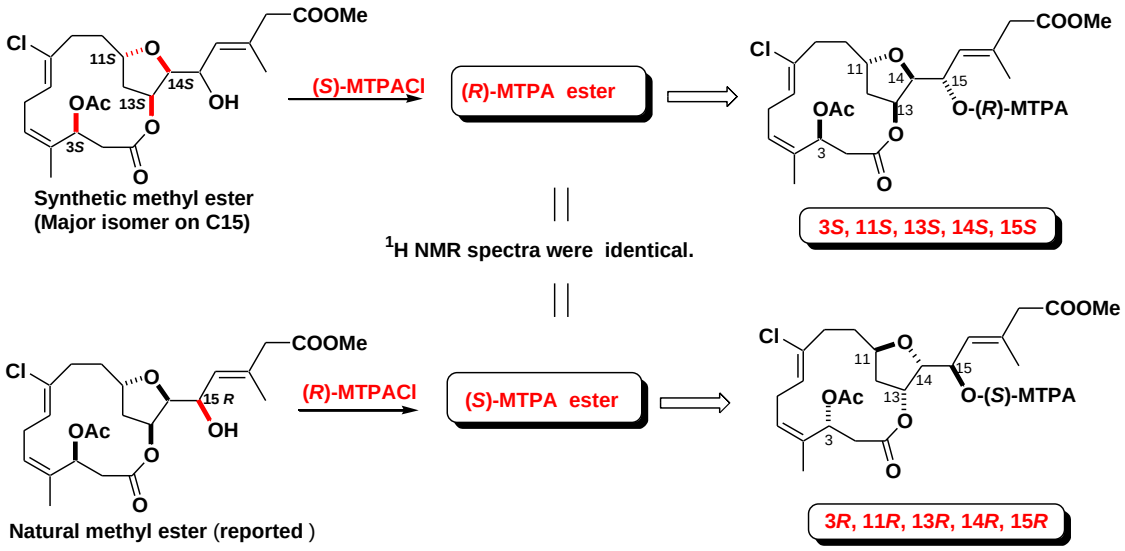


Figure 4-10. Determination of complete structure of haterumalide NA

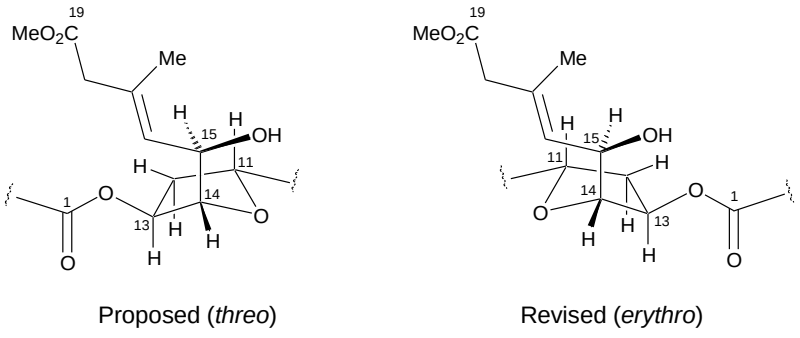


Figure 4-11. Relative stereochemistry of the C14-C15 part of haterumalide NA

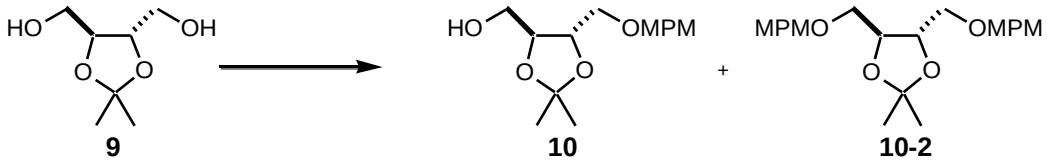
Experimental Section

General Methods.

All reactions sensitive to air or moisture were carried out under argon atmosphere in dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. THF was distilled from sodium/benzophenone, pyridine, triethylamine (Et_3N) and 2,6-lutidine from calcium hydride under reduced pressure. All other reagents were used as supplied unless otherwise stated. Analytical thin-layer chromatography (TLC) was performed using E. Merck Silica gel 60 F254 pre-coated plates. Column chromatography was performed using BW-820MH or FL60D (Fuji Silysia Co., Inc.). ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM ECP800 (800 MHz), JNM A600 (600 MHz), JNM A400 (400 MHz) or JNM EX270 (270 MHz) spectrometer. Chemical shifts are reported in δ (ppm) with reference to solvent signals [^{1}H NMR: $CHCl_3$ (7.26), C_6D_6 (7.16), $(CD_3)_2CO$ (2.05), CD_3OD (3.30); ^{13}C NMR: $CHCl_3$ (77.0), C_6D_6 (128.06), $(CD_3)_2CO$ (29.84), CD_3OD (49.00). Signal patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak. IR spectra were recorded on a JASCO FT/IR-230 spectrometer. FAB-MS and EI-MS were on JEOL JMS-LG2000 or JMS-700. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. CD spectra were recorded on a JEOL J-720WN. FT/IR-230 spectrometer.

Experimental procedure

Synthesis of C9-C15 fragment



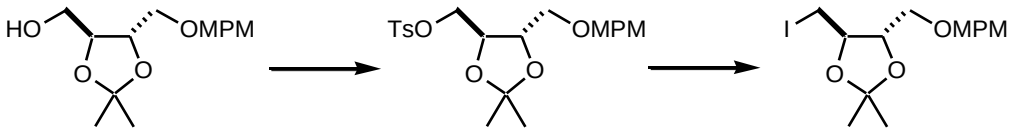
9 **10** **10-2**

Mono MPM ether 10

To a suspension of 60% sodium hydride (1.54 g, 38.5 mmol) in dry DMF (51 mL) at 0 °C was added **9** (3.0 g, 18.5 mmol) in dry DMF (33 mL) slowly. MPMCl (2.77 mL, 20.4 mmol) was added and stirred at 0 °C for 2 h and stirred at room temperature for 2h. The reaction mixture was cooled to 0 °C and quenched with H_2O (140

mL) and saturated aqueous NH_4Cl. The mixture was extracted with Et_2O and the organic layer was washed with brine and dried over $MgSO_4$. Concentration and flash column chromatography (hexane/Et_2O 3:1-2:1-3:4-1:2) gave **10** (3.28 g) in 69% yield.

R_f = 0.38 (1:1 hexane/EtOAc). $[\alpha]_D^{29}$ +13.2°(*c* 0.28, $CHCl_3$). IR ($CHCl_3$) ; 3590, 3460, 1615, 1515, 1460, 1375, 1245, 1080, 1040, 845 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 7.25 (br d, *J* = 8.2 Hz, 2H), 6.88 (br.d, *J* = 8.2 Hz, 2H), 4.51 (s, 2H), 4.03 (m, 1H), 3.91 (m, 1H), 3.81 (s, 3H), 3.78-3.62 (m, 2H), 3.66 (dd, *J* = 9.6, 4.6 Hz, 1H), 3.52 (dd, *J* = 9.6, 5.9 Hz, 1H), 2.27 (dd, *J* = 8.3, 4.6 Hz, 1H), 1.41 (s, 6H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 159.4, 129.6, 129.4 (2C), 113.9 (2C), 109.3, 79.8, 77.2, 73.4, 70.0, 62.5, 55.3, 26.9 (2C). MS (FAB) *m/z* 305 $(M + Na)^+$. HRMS (FAB) $C_{15}H_{22}NaO_5$ $(M + Na)^+$ calc.305.1365, observed 305.1353 (Δ -1.2 mmu).



10 **10-2** **10-3**

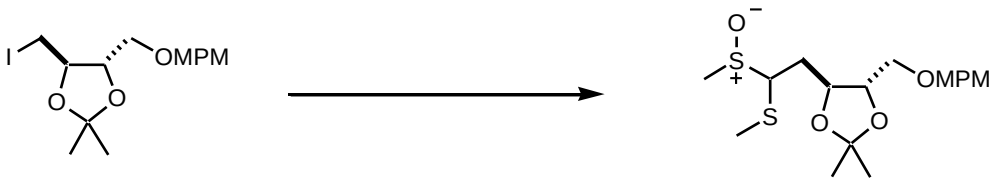
Iodide 10-3

To a solution of **10** (3.28 g, 11.6 mmol) in pyridine (6.3 mL) at 0 °C was added *p*-toluenesulfonyl chloride (4.01 g, 21.0 mmol). After stirred at 0 °C for 7.5 h, the reaction mixture was quenched with H_2O and extracted with Et_2O. The organic layer was washed with brine and dried over $MgSO_4$. Concentration gave **10-2** (4.95 g) which was directly used in the next reaction.

To a solution of **10-2** in acetone (24.4 mL) at room temperature was added K_2CO_3 (2.15 g, 21.5 mmol) and NaI (4.75 g, 31.7 mmol). After stirred for 16.5 h under reflux, the reaction mixture was diluted with H_2O and extracted with Et_2O. The organic layer was washed with brine and dried over $MgSO_4$. Concentration and column chromatography (silicagel, hexane/Et_2O, 11:1-5:1) gave **10-3** (4.04 g) in 89% yield.

R_f = 0.63 (1:1 hexane/Et_2O). $[\alpha]_D^{30}$ -9.3°(*c* 0.35, $CHCl_3$). IR ($CHCl_3$) ; 1615, 1515, 1460, 1375, 1245, 1190, 1135, 820 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 7.25 (br d, *J* = 8.9 Hz, 2H), 6.88 (br d, *J* = 8.9 Hz, 2H), 4.52 (s, 2H), 3.95 (ddd, *J* = 7.6, 5.0, 5.0 Hz, 1H), 3.84 (ddd, *J* = 7.6, 5.3, 5.3 Hz, 1H), 3.81 (s, 3H), 3.64 (dd, *J* = 12.9, 5.3 Hz, 1H), 3.58 (dd, *J* = 12.9, 5.3 Hz, 1H), 3.41 (dd, *J* = 10.6, 5.0 Hz, 1H), 3.27 (dd, *J* = 10.6, 5.0 Hz, 1H), 1.46 (s,

3H), 1.41 (s, 3H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 159.3, 129.9, 129.3 (2C), 113.9 (2C), 109.8, 80.1, 77.8, 73.3, 70.2, 55.3, 27.4, 27.3, 6.4. MS (FAB) *m/z* 415 $(M + Na)^+$. HRMS (FAB) $C_{15}H_{21}Na\ IO_4$ $(M + Na)^+$: calc.415.0382, found 415.0357 (Δ -2.5 mmu).

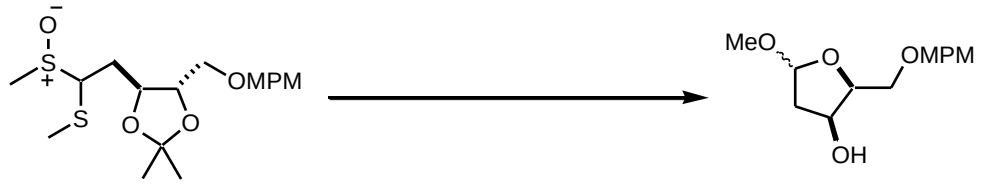


10-3 **11**

FAMSO 11

To a solution of formaldehyde dimethyl dithioacetal S-Oxide (2.15 mL, 20.6 mmol) in THF (26 mL) at 0 °C was added *n*-butyllithium (13.6 mL, 1.51 M THF solution, 20.6 mmol). **10-3** (4.04 g, 10.3 mmol) in THF (13.0 mL) was added to the reaction mixture. After stirred at room temperature for 12.5 h, the reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. Concentration and column chromatography (silicagel, hexane/EtOAc, 1:1-1:5) gave **11** (2.77 g) in 69% yield. Diastereomer ratio of **11** was 3 : 3 : 2 : 2.

R_f = 0.21 (1:5 hexane/EtOAc). IR ($CHCl_3$) ; 1615, 1515, 1460, 1375, 1245, 1080, 1040, 845 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.26 (br d, *J* = 8.6 Hz, 2H), 6.88 (br d, *J* = 8.6 Hz, 2H), 4.56-4.44 (m, 2H), 4.20 (m, 1H), 3.96 (m, 1H), 3.80 (s, 3H), 3.77 (m, 1H), 3.65 (m, 1H), 3.52 (m, 1H), 2.70 (s, 0.9H), 2.56 (s, 0.6H), 2.53 (s, 0.9H), 2.53 (s, 0.6H), 2.27 (s, 0.9H), 2.17 (s, 0.6H), 2.16 (s, 0.6H), 2.10 (s, 0.9H), 2.09-1.73 (m, 2H), 1.43-1.39 (m, 6H). MS (FAB) *m/z* 411 $(M + Na)^+$. HRMS (FAB) $C_{18}H_{28}NaS_2O_5$ $(M + Na)^+$ calc. : 411.1276, found : 411.1269 (Δ -0.7 mmu).

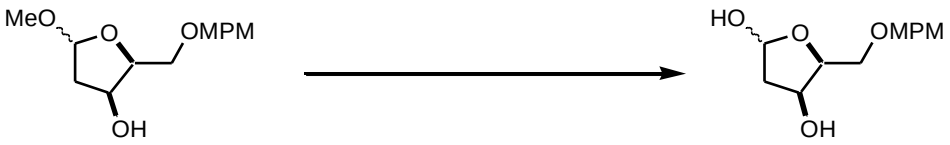


11 **12**

Methyl acetal 12

To a solution of **11** (638 mg, 1.64 mmol) in MeOH (33 mL) at 0 °C was added conc. HCl (2.6 mL). After stirred at room temperature for 14 h, the reaction mixture was quenched by saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. Concentration and column chromatography (silcagel, hexane/EtOAc, 3:1-2:1-1:1) gave **12** (182 mg) in 41% yield.

R_f = 0.55 (1:5 hexane/EtOAc). IR ($CHCl_3$) ; 3500 (br), 1615, 1515, 1440, 1360, 1250, 1090, 1055, 830 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 7.26 (br d, *J* = 8.6 Hz, H), 6.88 (br d, *J* = 8.6 Hz, 2H), 5.16 (dd, *J* = 5.0, 3.0 Hz, 0.75H), 5.05 (br d, *J* = 3.3 Hz, 0.25H), 4.51 (s, 2H), 4.52-4.47 (m, 0.75H), 4.33-4.23 (m, 0.25H), 4.11 (dt, *J* = 5.0, 4.6 Hz, 1H), 3.81 (s, 3H), 3.81 (m, 0.25H), 3.77 (d, *J* = 5.0 Hz, 1.5H), 3.64 (dd, *J* = 10.2, 6.9 Hz, 0.25H), 3.36 (s, 3H), 2.87 (d, *J* = 10.6 Hz, 0.25H), 2.78 (d, *J* = 5.6 Hz, 0.75H), 2.14 (m, 2H). MS (FAB) *m/z* 291 $(M + Na)^+$. HRMS (FAB) $C_{14}H_{20}NaO_5$ $(M + Na)^+$ calc. : 291.1208, found : 291.1225 (Δ+1.7 mmu).



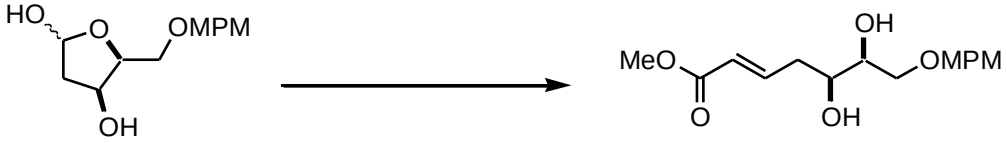
12 **13**

Hemiacetal 13

To a solution of **12** (1.36 g, 5.07 mmol) in THF (46 mL) at room temperature was added 1M HCl (46 mL, 46 mmol). After stirred at room temperature for 4 h, the reaction mixture was quenched by saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. Concentration and column chromatography (silcagel, hexane/EtOAc, 5:1-3:1-2:1-1:1-1:1-1:5) gave **13** (1.27 g) in 90% yield.

Melting point; 70-73 °C. R_f = 0.36 (1:2 hexane/EtOAc). IR ($CHCl_3$) 3590, 3360, 1615, 1515, 1460, 1440, 1360, 1250, 1105, 1065, 1035 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 7.27 (br d, *J* = 8.6 Hz, 2H), 6.89 (br d, *J* = 8.6 Hz, 2H), 5.71 (dt, *J* = 4.0, 3.3 Hz, 0.3H), 5.44 (ddd, *J* = 8.6, 3.3, 1.7 Hz, 0.7H), 4.54 (s, 1.4H), 4.52 (s, 0.6H), 4.49 (m, 0.3H), 4.42 (m, 0.7H), 4.27 (dd, *J* = 10.9, 5.0 Hz, 0.3H), 4.07 (dd, *J* = 9.6, 5.6 Hz, 0.7H), 3.84-3.80 (m, 1H), 3.81 (s, 3H), 3.77 (d, *J* = 5.0 Hz, 0.6H), 3.63 (d, *J* = 8.6 Hz, 1.4H), 3.09 (d, *J* = 5.6 Hz, 0.7H), 2.71 (d, *J* = 5.6

Hz, 0.3H), 2.66 (d, *J* = 3.3 Hz, 0.3H), 2.17 (t, *J* = 4.3 Hz, 0.6H), 2.11 (m, 1.4H). MS (FAB) *m/z* 277 $(M + Na)^+$. HRMS (FAB) $C_{13}H_{18}NaO_5$ $(M + Na)^+$ calc.: 277.1052, found: 277.1074 (Δ +2.2 mmu).

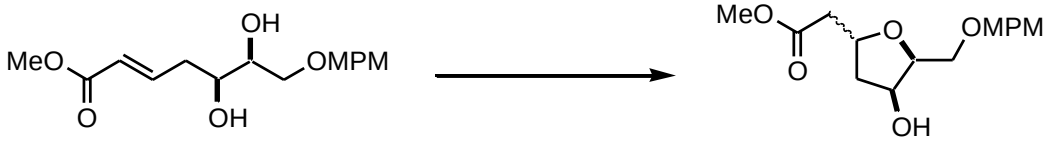


13 **14**

α,β–unsaturated ester 14

To a solution of **13** (113 mg, 0.446 mmol) in CH_3CN (2.2 mL) at room temperature was added methyl (triphenylphosphoranylidene) acetate (247 mg, 0.738 mmol). After stirred at room temperature for 2.5 h, the reaction mixture was evaporated and column chromatography (silcagel, hexane/acetone, 5:1-3:1-2:1-1:1 and silcagel FL60D, hexane/acetone, 5:1-2:1) gave **14** (128 mg) in 92% yield.

R_f = 0.53 (1:1 hexane/acetone). IR ($CHCl_3$) ; 3560, 1715, 1660, 1615, 1515, 1305, 1280, 1250, 1120, 1035 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 7.24 (br d, *J* = 8.8 Hz, 2H), 6.99 (dt, *J* = 15.6, 7.3 Hz, 0.75H), 6.89 (br d, *J* = 8.8, 2H), 6.38 (dt, *J* = 11.5, 7.8 Hz, 0.25H), 5.91 (br d, *J* = 15.6 Hz, 1H), 4.51 (d, *J* = 11.5 Hz, 1H), 4.46 (d, *J* = 11.5 Hz, 1H), 3.82 (s, 2.25H), 3.80 (s, 0.75H), 3.72 (s, 2.25H), 3.71 (s, 0.75H), 3.68-3.56 (m, 3H), 2.92 (m, 1H), 2.70 (d, *J* = 6.1 Hz, 1H), 2.61 (d, *J* = 4.4 Hz, 1H), 2.45 (m, 2H). MS (FAB) *m/z* 333 $(M + Na)^+$. HRMS (FAB) $C_{16}H_{22}NaO_6$ $(M + Na)^+$ calc. : 333.1314, found : 333.1307 (Δ -0.7 mmu).



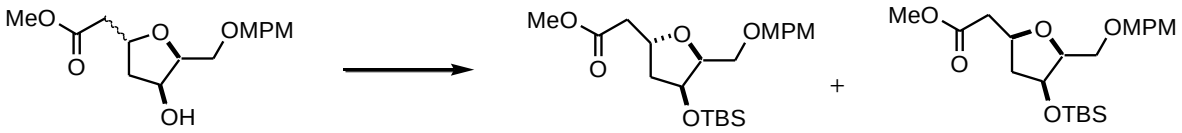
14 **15**

Tetrahydrofurna 15

To a solution of **14** (122 mg, 0.393 mmol) in MeOH (4.0 mL) at 0 °C was added sodium methoxide (28.2 mg, 0.520 mmol). After stirred at -20 °C for 38 h, the reaction mixture was quenched by saturated aqueous NH_4Cl and extracted with Et_2O. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. Concentration and column chromatography (silcagel, hexane/acetone, 5:1-4:1-1:1) gave **15** (68.3 mg) in 56%

yield.

R_f = 0.44 (3:2 hexane/acetone). IR ($CHCl_3$) ; 3460, 1735, 1615, 1515, 1300, 1250, 1170, 1135, 825 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 7.26 (br d, *J* = 8.6 Hz, 2H), 6.89 (br d, *J* = 8.6 Hz, 2H), 4.65 (m, 0.9H), 4.51 (s, 2H), 4.51 (m, 0.1H), 4.49 (m, 0.9H), 4.39 (m, 0.1H), 4.27 (m, 0.1H), 4.09 (m, 0.9H), 3.81 (s, 3H), 3.75 (d, *J* = 5.3 Hz, 2H), 3.67 (s, 3H), 2.93 (d, *J* = 4.0 Hz, 1H), 2.63 (dd, *J* = 15.2, 6.9 Hz, 0.9H), 2.72 (m, 0.1H), 2.50 (dd, *J* = 15.2, 5.4 Hz, 0.9H), 2.39 (m, 0.1H), 2.18 (m, 1H), 1.82 (ddd, *J* = 13.9, 9.2, 5.0 Hz, 1H). MS (FAB) *m/z* 447 $(M + Na)^+$. HRMS (FAB) $C_{16}H_{22}NaO_6$ $(M + Na)^+$ calc. : 333.1314, found : 333.1331 (Δ +1.7 mmu).



15 **16** **17**

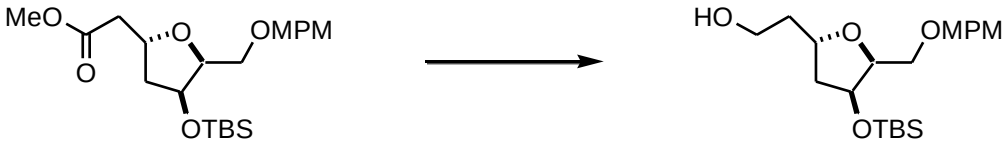
TBS 16

To a solution of **15** (444 mg, 1.43 mmol) and imidazole (390 mg, 5.78 mmol) in DMF (10.6 mL) at room temperature was added *tert*-butyldimethylsilyl chloride (431 mg, 2.8 mmol). After stirred at room temperature for 10 h, the reaction mixture was quenched by H_2O and extracted with Et_2O. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. Concentration and column chromatography (silcagel, hexane/acetone, 5:1-3:1-1:1-1:3 and silcagel FL60D, hexane/acetone, 5:1-1:1) gave **16** (494 mg) in 81% yield and **17** (93 mg) in 15%.

R_f = 0.65 (1:1 benzene/Et_2O). $[\alpha]_D^{26}$ +22.7°(*c* 0.42, $CHCl_3$). IR ($CHCl_3$) ; 1735, 1615, 1515, 1300, 1250, 1170, 835 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 7.25 (br d, *J* = 8.5 Hz, 2H), 6.86 (br d, *J* = 8.5 Hz, 2H), 4.58 (m, 1H), 4.50 (d, *J* = 11.7 Hz, 1H), 4.43 (d, *J* = 11.7 Hz, 1H), 4.38 (br t, *J* = 3.4 Hz, 1H), 4.07 (m, 1H), 3.80 (s, 3H), 3.68 (s, 3H), 3.57 (m, 2H), 2.70 (dd, *J* = 15.4, 6.6 Hz, 1H), 2.47 (dd, *J* = 15.4, 6.6 Hz, 1H), 2.07 (ddd, *J* = 12.9, 5.6, 1.3 Hz, 1H), 1.76 (ddd, *J* = 12.9, 9.8, 4.4 Hz, 1H), 0.88 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 171.5, 159.1, 130.4, 129.6 (2C), 113.7 (2C), 81.8, 73.9, 73.1, 73.0, 68.8, 55.3, 51.6, 41.9, 40.4, 25.7 (3 C), 18.0, -4.7, -5.2 MS (FAB) *m/z* 447 $(M + Na)^+$; HRMS (FAB) $C_{22}H_{36}NaO_6Si(M + Na)^+$ calc.: 447.2179, found : 447.2207 (Δ +2.8 mmu).

17 R_f = 0.59 (1:1 benzene/Et_2O). $[\alpha]_D^{30}$ +23.4° (*c* 0.42, $CHCl_3$). IR ($CHCl_3$) ; 3500, 1615, 1515, 1460, 1440,

1360, 1250, 1180, 1070, 1035, 835 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 7.26 (br d, *J* = 8.6 Hz, 2H), 6.86 (br d, *J* = 8.6 Hz, 2H), 4.51 (d, *J* = 11.5 Hz, 1H), 4.44 (d, *J* = 11.5 Hz, 1H), 4.42-4.30 (m, 2H), 3.90 (ddd, *J* = 6.6, 5.3, 4.6 Hz, 1H), 3.80 (s, 3H), 3.68 (s, 3H), 3.63 (dd, *J* = 9.9, 5.3 Hz, 1H), 3.57 (dd, *J* = 9.9, 6.6 Hz, 1H), 2.83 (dd, *J* = 15.5, 6.3 Hz, 1H), 2.59 (dd, *J* = 15.5, 7.6 Hz, 1H), 2.32 (ddd, *J* = 13.5, 7.9, 5.6 Hz, 1H), 1.67 (ddd, *J* = 13.5, 5.0, 2.3 Hz, 1H), 0.88 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 171.8, 159.1, 130.3, 129.5 (2C), 113.7 (2C), 82.3, 74.2, 73.1, 72.6, 69.1, 55.3, 51.5, 41.5, 41.0, 25.7 (3 C), 18.0, -4.7, -5.3. MS (FAB) *m/z* 447 $(M + Na)^+$

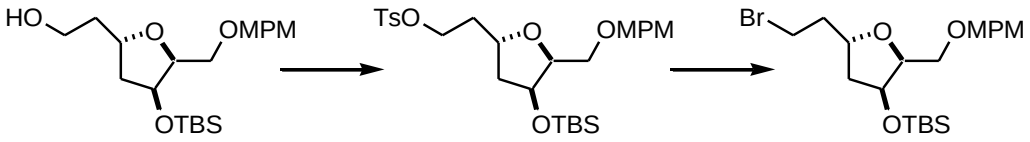


16 **16-2**

Alcohol 16-2

To a solution of **16** (491 mg, 1.16 mmol) in THF (7.2 mL) at room temperature was added lithiumaluminium hydride (1.0M, THF solution, 2.31 mL, 2.31 mmol). After stirred at room tempareture for 50 min, the reaction mixture was quenched by MeOH and 0.5M potassium sodium tartartrate (30 mL). After stirred at room temperature for 30 min, the mixture was extracted with Et_2O. The organic layer was washed with brine and dried over $MgSO_4$. Concentration and column chromatography (silcagel, hexane/acetone, 5:1-3:1-1:1 and silcagel FL60D, hexane/acetone, 3:1-2:1-1:1-1:3) gave **16-2** (458 mg) in 100% yield.

R_f = 0.39 (1:1 hexane/EtOAc) $[\alpha]_D^{30}$ +17.4° (*c* 0.34, $CHCl_3$). IR ($CHCl_3$) ; 3500, 1615, 1515, 1460, 1440, 1360, 1250, 1180, 1070, 1035, 835 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 7.25 (br d, *J* = 8.6 Hz, 2H), 6.87 (br d, *J* = 8.6 Hz, 2H), 4.46 (s, 2H), 4.43-4.34 (m, 2H), 4.08 (m, 1H), 3.80 (s, 3H), 3.84-3.76 (m, 2H), 3.62 (dd, *J* = 9.9, 5.3 Hz, 1H), 3.53 (dd, *J* = 9.9, 6.3 Hz, 1H), 2.65 (t, *J* = 5.6 Hz, 1H), 1.97 (ddd, *J* = 12.9, 5.6, 1.3 Hz, 1H), 1.83-1.66 (m, 3H), 0.87 (s, 9H), 0.07 (s, 3H), 0.04 (s, 3H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 159.2, 130.4, 129.5 (2C), 113.7 (2C), 81.7, 77.7, 73.1, 72.6, 68.8, 61.5, 55.3, 42.4, 37.5, 25.7 (3C), 18.0, -4.7, -5.2 MS (FAB) *m/z* 419 $(M + Na)^+$; HRMS (FAB) $C_{21}H_{36}NaO_5Si$ $(M + Na)^+$ calc.: 419.2230, found : 419.2207 (Δ -2.3 mmu).



16-2 **16-3** **7**

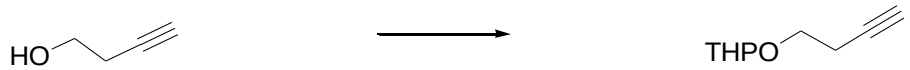
Bromide 7

To a solution of **16-2** (48.1 mg, 0.121 mmol) in pyridine (0.6 mL) at 0 °C was added TsCl (46.9 g, 0.246 mmol). After stirred at 0 °C for 7h, the reaction mixture was quenched with H_2O and extracted with Et_2O. The organic layer was washed with brine and dried over $MgSO_4$. Concentration gave **16-3** (69.0 mg) which was directly used in the next reaction.

To a solution of **16-3** in DMF (1.0 mL) at room tempareture was added lithium bromide (52.5 m g, 0.605 mmol). After stirred for 1 h under reflux, the reaction mixture was dilluted with Et_2O and washed with H_2O and brine and dried over $MgSO_4$. Concentration and column chromatography (silicagel, hexane /Et_2O 5:1-3:1) gave **7** (48.6 mg) in 87% yield in 2 steps.

R_f = 0.60 (1:1 hexane/Et_2O). $[\alpha]_D^{26}$ +11.1°(*c* 0.43, $CHCl_3$). IR ($CHCl_3$) ; 1610, 1515, 1460, 1440, 1360, 1250, 1170, 1080, 935, 835 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 7.26 (br d, *J* = 8.6 Hz, 2H), 6.87 (br d, *J* = 8.6 Hz, 2H), 4.52 (d, *J* = 11.6 Hz, 1H), 4.44 (d, *J* = 11.6 Hz, 1H), 4.39-4.27 (m, 2H), 4.03 (ddd, *J* = 6.6, 5.0, 3.6 Hz, 1H), 3.80 (s, 3H), 3.59 (dd, *J* = 9.9, 5.0 Hz, 1H), 3.59 (m, 1H), 3.55-3.47 (m, 2H), 2.19-1.79 (m, 3H), 1.67 (ddd, *J* = 12.5, 9.9, 4.6 Hz, 1H), 0.88 (s, 9H), 0.15 (s, 3H), 0.05 (s, 3H), ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 159.1, 129.6, 129.6 (2C), 113.7 (2C), 81.6, 75.9, 73.1, 73.0, 68.9, 55.3, 41.8, 39.3, 30.0, 25.7 (3C), 18.0, -4.7, -5.2. MS (FAB) *m/z* 481 $(M + Na)^+$. HRMS (FAB) $C_{21}H_{35}{}^{79}BrNaO_4Si(M + Na)^+$ calc.: 481.1386, found : 81.1361 (Δ -2.5 mmu).

Synthesis of C5-C8 fragment

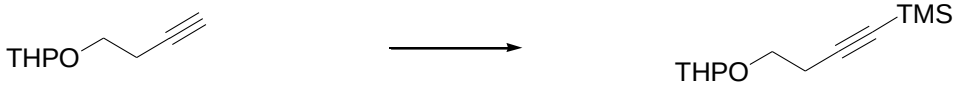


18 **19**

THP 19

To a mixture of **18** (9.71 g, 139 mmol) and *p*-toluenesulfonic acid (270 mg, 1.42 mmol) at 0 °C was added 3,4-dihydro-2*H*-pyrane (15.2 mL, 166 mmol). After stirred at room temperature for 30 min, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with Et_2O. The organic layer was washed with brine and dried over $MgSO_4$. Concentration and distillation gave **19** (18.3 g) in 86% yeild.

R_f = 0.61 (3:1 hexane/Et_2O). boiling point; 86-89 °C / 19 mmHg. IR ($CHCl_3$) ; 3310, 2120, 1455, 1440, 1350 , 1130, 1030, 980, 905, 870, 815 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 4.65 (t, *J* = 3.6 Hz, 1H), 3.91 (m, 1H), 3.84 (dt, *J* = 9.9, 6.9 Hz, 1H), 3.58 (dt, *J* = 9.9, 6.9 Hz, 1H), 3.52 (m, 1H), 2.50 (dt, *J* = 2.6, 6.9 Hz, 2H), 1.98 (t, *J* = 2.6 Hz, 1H), 1.91-1.48 (m, 6H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 98.7, 81.4, 69.2, 65.5, 62.2, 30.5, 25.4, 19.9, 19.4. HRMS (ESI) $C_9H_{14}NaO_2$ $(M + Na)^+$ calc.: 177.0891, found : 177.0866 (Δ -2.5 mmu).



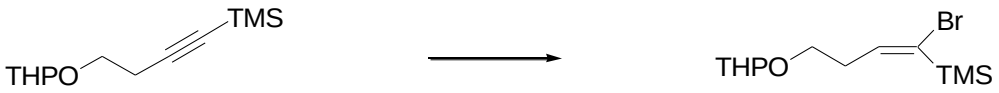
19 **20**

TMS 20

To a solution of **19** (4.99 g, 32.4 mmol) in Et_2O (32.0 mL) at -78 °C was added *n*-butyl lithium (1.58M, hexane solution, 24.6 mL, 38.8 mmol). Trimethylsilyl chloride (4.93 mL, 38.8 mmol) was added to the reaction mixture. After stirred at room temperature for 2.5 h, the reaction mixture was quenched with saturated aqueous NH_4Cl and extractedvwith Et_2O. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. Concentration and column chromatography (silicagel, hexane /Et_2O 59:1-39:1-1:1) gave **20** (4.90 g) in 67% yield.

R_f = 0.52 (19:1 hexane/EtOAc) IR ($CHCl_3$) 2170, 1455, 1440, 1355, 1250, 1220, 1180, 1120, 1080, 1030, 905, 845 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 4.66 (t, *J* = 3.6 Hz, 1H), 3.91 (m, 1H), 3.82 (dt, *J* = 9.6, 7.3 Hz, 1H), 3.55 (dt, *J* = 9.6, 7.3 Hz, 1H), 3.51 (m, 1H), 2.53 (t, *J* = 7.3 Hz, 2H), 1.90-1.47 (m, 6H), 0.13 (s, 9H). ^{13}C NMR

(67.8 MHz, $CDCl_3$) δ 104.0, 98.2, 85.1, 65.6, 60.3, 30.6, 25.7, 20.2, 19.4, 0.3 (3C) HRMS (ESI) $C_{12}H_{22}O_2Si$ (M + Na)$^+$ calc. : 249.1278, found : 249.1259 (Δ -1.9 mmu).



20 **21**

Vinyl bromide 21

To a solution of **20** (4.91 g, 21.6 mmol) in Et_2O (47.2 mL) at 0 °C was added diisobutylaluminium hydride (0.95M, THF solution, 25.0 mL, 23.8 mmol). After stirred at 40 °C for 3 h, the reaction mixture was cooled to 0 °C and Et_2O (22 mL) and pyridine (3.48 mL, 43.2 mmol) were added. The reaction mixture was cooled to -78 °C and bromine (2.2 M, CH_2Cl_2 solution, 14.0 mL, 32.4 mmol) was added and stirred for 2 h. The reaction mixture was quenched with 1M NaOH and H_2O and extracted with hexane. The organic layer was washed with 1M HCl, saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. Concentration and column chromatography (silicagel, hexane /Et_2O, 99:1-49:1) gave **21** (6.07 g) in 91% yield.

R_f = 0.19 (1:1 hexane/benzene) IR ($CHCl_3$) ; 1605, 1455, 1440, 1250, 1140, 1120, 1070, 1030, 985 905, 845 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.79 (t, *J* = 7.9 Hz, 1H), 4.60 (t, *J* = 4.0 Hz, 1H), 3.85 (m, 1H), 3.81 (dt, *J* = 9.6, 6.6 Hz, 1H), 3.51 (m, 1H), 3.42 (dt, *J* = 9.6, 6.6 Hz, 1H), 2.41 (dt, *J* = 7.9, 6.6 Hz, 2H),1.90-1.47 (m, 6H), 0.28 (s, 9H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 144.3, 129.4, 98.8, 66.1, 62.1, 32.8, 30.6, 25.4, 19.4, 0.3 (3C) HRMS (ESI) $C_{12}H_{23}{}^{79}BrNaO_2Si$(M + Na)$^+$ calc. : 329.0548, found : 329.0527 (Δ -2.1 mmu).

21 **8**

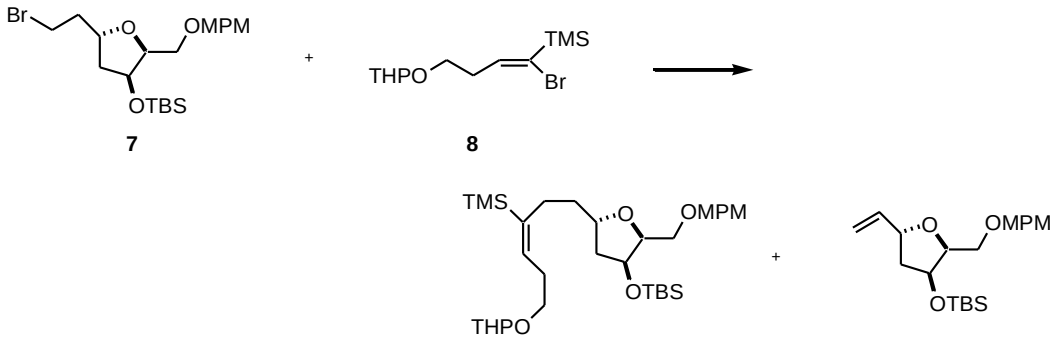
***Z*-alkenylsilane 8**

To a solution of **21** (10.5 g, 34.2 mmol) in Et_2O (35.0 mL) at 20-30 °C was added pyridine (0.97 mL, 12.0 mmol) and bromine (1.92M, CH_2Cl_2 solution, 0.88 mL, 0.18 mmol). The reaction mixture was stirred and irradiated at 20-30 °C for 3 h, the reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$ and extracted with hexane. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. Concentration and column

chromatography (silicagel, hexane /benzene 1:1-1:2) gave **8** (10.6 g) in 100% yield.

R_f = 0.65 (3:1 hexane/Et_2O). IR ($CHCl_3$) ; 1615, 1455, 1440, 1350, 1250, 1135, 1120, 1075, 1030, 980 890, 840 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 6.33 (t, *J* = 6.6 Hz, 1H), 4.63 (t, *J* = 4.0 Hz, 1H), 3.81 (m, 1H), 3.81 (dt, *J* = 9.6, 6.9 Hz, 1H), 3.72 (m, 1H), 3.51 (dt, *J* = 9.6, 6.9 Hz, 1H), 2.58 (dt, *J* = 6.6, 6.9, Hz, 2H), 1.90-1.46 (m, 6H), 0.18 (s, 9H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 149.6, 138.5, 98.6, 65.3, 62.1, 33.1, 30.6, 25.5, 19.4, -1.9 (3C) HRMS (FAB) $C_{12}H_{23}{}^{79}BrNaO_2Si(M + Na)^+$ calc.: 329.0548, found : 329.0527 (Δ -2.1 mmu).

Synthesis of *Z*-chloroolefin 22



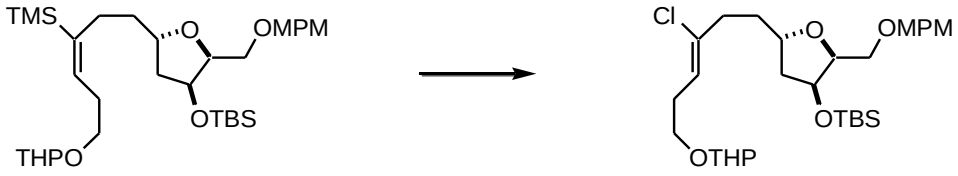
Coupling product 6

To a solution of **8** (1.28 g, 4.23 mmol) in Et_2O (21.2 mL) at -78 °C was added *sec*-butyl lithium (1.01M, THF solution, 8.37 mL, 8.45 mmol). After stirred at -78 °C for 2 h, **7** (485 mg, 1.06 mmol) in THF (4.5 mL) and hexamethylphosphoric triamide (1.47 mL, 8.45 mmol) were added at -78 °C. After stirred at -78 °C for 20.5 h and the reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with Et_2O. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. Concentration and column chromatography (silicagel, hexane/Et_2O, 23:2-11:1-9:1-7:1-5:1) gave **6** (435 mg) in 68% yield and **23** (115 mg) in 29%yield.

6 R_f = 0.41 (2:1 hexane/Et_2O) $[\alpha]_D{}^{26}$ +17.2° (*c* 0.23, $CHCl_3$) IR ($CHCl_3$) ; 1610, 1585, 1515, 1465, 1440, 1340, 1250, 1170, 1120, 1030, 835 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 7.26 (br d, *J* = 8.9 Hz, 2H), 6.86 (br d, *J* = 8.9 Hz, 2H), 5.74 (t, *J* = 6.6 Hz, 1H), 4.61 (t, *J* = 4.0 Hz, 1H), 4.52 (d, *J* = 11.6 Hz, 1H), 4.44 (d, *J* = 11.6 Hz, 1H), 4.36 (m, 1H), 4.17 (m, 1H), 4.04 (m, 1H), 3.80 (s, 3H), 3.90-3.69 (m, 2H), 3.65-3.37 (m, 4H), 2.42 (dt, *J* = 6.8, 7.3 Hz, 2H), 2.29-2.03 (m, 2H), 1.95 (m, 1H), 1.82 (m, 1H), 1.71-1.47 (m, 7H), 1.43 (m, 1H), 0.88 (s, 9H),

0.15 (s, 3H), 0.05 (s, 12H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 159.1, 142.6, 136.1, 130.5, 129.5 (2C), 113.7 (2C), 98.6, 81.4, 73.2, 73.1, 69.0, 66.9, 62.1, 55.2, 42.0, 36.4, 30.7, 30.0, 29.0, 26.0, 25.8 (3 C), 25.5, 19.5, 18.0, -1.2 (3C), -4.7, -5.2 MS (FAB) *m/z* 629 $(M + Na)^+$. HRMS (FAB) $C_{33}H_{58}NaO_6Si_2(M + Na)^+$ calc. : 629.3670, found : 629.3667 (Δ -0.3 mmu).

23 R_f = 0.77 (2:1 hexane/Et_2O) $[\alpha]_D^{26}$ +15.2° (*c* 0.72, $CHCl_3$). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.26 (br d, *J* = 8.9 Hz, 2H), 6.86 (br d, *J* = 8.9 Hz, 2H), 5.85 (ddd, *J* = 17.2, 10.2, 6.6 Hz, 1H), 5.25 (ddd, *J* = 17.2, 1.7, 1.3 Hz, 1H), 5.09 (ddd, *J* = 10.2, 1.7, 1.0 Hz, 1H), 4.63 (m, 1H), 4.52 (d, *J* = 11.6 Hz, 1H), 4.44 (d, *J* = 11.6 Hz, 1H), 4.39 (m, 1H), 4.09 (m, 1H) , 3.81 (s, 3H), 3.59 (dd, *J* = 9.9, 5.0 Hz, 1H), 3.63 (m, 1H), 2.01 (ddd, *J* = 12.9, 5.6, 1.7 Hz, 1H), 1.79 (m, 1H), 0.89 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H)



6 **22**

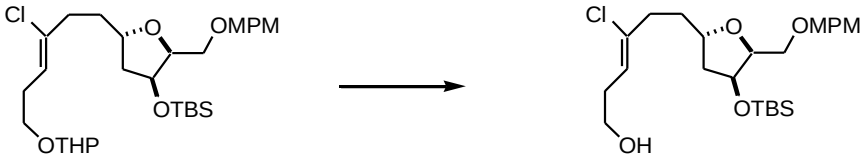
Chloroolefin 22

To a solution of **6** (970 mg, 1.60 mmol) in DMF (40 mL) was added H_2O (16M, DMF solution, 0.02 mL, 0.272 mmol) and *N*-chlorosuccinimide (427 mg, 3.20 mmol). After stirred at 50 °C for 6 h under shading condition, the reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$ and extracted with Et_2O. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. Concentration and column chromatography FL60D (hexane /Et_2O, 11:1-5:1-1:1) gave **22** (407 mg) in 45% yield.

R_f = 0.51 (3:2 hexane/Et_2O). $[\alpha]_D^{26}$ +15.1°(*c* 0.54, $CHCl_3$) IR ($CHCl_3$) ; 1610, 1515, 1460, 1360, 1250, 1170, 1140, 1120, 1030, 835 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.26 (br d, *J* = 8.6 Hz, 2H), 6.87 (br d, *J* = 8.6 Hz, 2H), 5.57 (t, *J* = 6.1 Hz, 1H), 4.60 (m, 1H), 4.52 (d, *J* = 11.6 Hz, 1H), 4.44 (d, *J* = 11.6 Hz, 1H), 4.36 (m, 1H), 4.20 (m, 1H), 4.05 (m, 1H), 3.80 (s, 3H), 3.85 (m, 1H), 3.75 (m, 1H), 3.65-3.38 (m, 4H), 2.29-2.01 (m, 4H), 1.93 (ddd, *J* = 12.7, 5.7, 1.7 Hz, 1H), 1.91-1.63 (m, 9H), 1.05 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 159.1, 135.9, 130.4, 129.5 (2C), 122.0, 113.7 (2C), 98.7, 81.4, 77.2, 73.1, 73.1, 69.0, 65.9, 62.3,

55.3, 42.0, 36.3, 33.9, 30.7, 29.7, 25.7 (3C), 25.4, 19.5, 18.0, -4.7, -5.2 MS (FAB) *m/z* 591 $(M + Na)^+$; HRMS(FAB) $C_{30}H_{49}{}^{35}ClNaO_6Si(M + Na)^+$ calc. : 591.2885, found : 591.2864 (Δ -2.1 mmu).

[+]Synthesis of seco acid

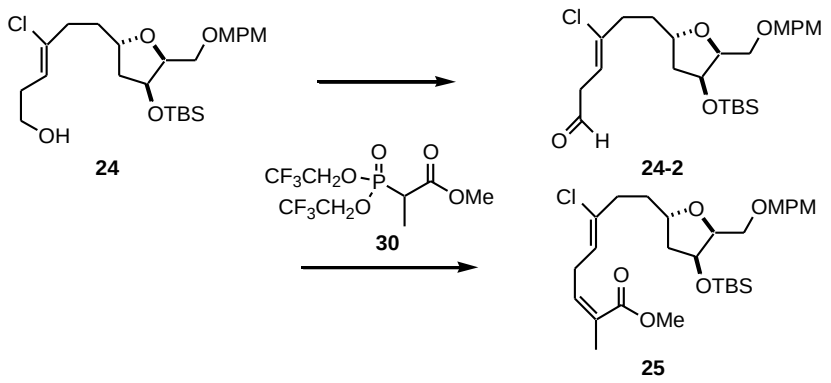


22 **24**

Alcohol 24

To a solution of **22** (150 mg, 0.263 mmol) in THF (2.8 mL) and H_2O (1.4 mL) was added acetic acid (5.6 mL). After stirred at 45 °C for 3 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. Concentration and column chromatography FL60D (hexane /Et_2O 7:1-5:1) gave **24** (102 mg) in 80% yield.

R_f = 0.25 (2:1 hexane/EtOAc) $[\alpha]_D{}^{26}$ +18.0°(*c* 0.45, $CHCl_3$). IR ($CHCl_3$) ; 3610, 3460, 1610, 1515, 1460, 1360, 1250, 1170, 1080, 835 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 7.26 (br d, *J* = 8.3 Hz, 2H), 6.87 (br d, *J* = 8.3 Hz, 2H), 5.57 (br t, *J* = 6.9 Hz, 1H), 4.53 (d, *J* = 11.5 Hz, 1H), 4.44 (d, *J* = 11.5 Hz, 1H), 4.36 (m, 1H), 4.19 (m, 1H), 4.03 (m, 1H), 3.80 (s, 3H), 3.71-3.50 (m, 4H), 2.61-2.30 (m, 5H), 1.93 (br dd, *J* = 12.5, 5.6 Hz, 1H), 1.84-1.54 (m, 3H), 0.87 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 159.1, 136.8, 130.4, 129.6 (2C), 121.6, 113.7 (2C), 81.4, 76.7, 76.5, 73.1, 69.0, 61.6, 55.3, 41.9, 36.4, 33.8, 32.1, 25.7 (3C), 18.0, -4.7, -5.1 MS (FAB) *m/z* 507 $(M + Na)^+$; HRMS(FAB) $C_{25}H_{41}{}^{35}ClNaO_5Si(M + Na)^+$ calc. : 507.2310, found : 507.2296 (Δ -1.4 mmu).

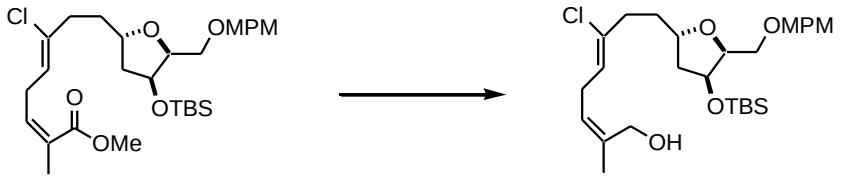


unsaturated ester 25

To a solution of **24** (277 mg, 0.572 mmol) in CH_2Cl_2 (0.6 mL) at 0 °C was added Dess-Martin periodinane (364 g, 0.858 mmol). After stirred at room temperature for 30 min, the reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$ and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. Concentration gave **24-2** (299 mg) which was directly used in the next reaction.

To a solution of **30** (284 mg, 0.858 mmol) and 18-crown-6 ether (226 mg, 2.86 mmol) in THF (1.0 mL) at room tempareture was added potassium hexamethylphosphorictriamide (0.5 M, toluene solution, 1.72 mL, 0.858 mmol). After stirred for 10 min, **24-2** in THF (5.0 mL) was added to the reaction mixture. After stirred at -78 °C for 3h, the reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. Concentration and column chromatography (silicagel, hexane /Et_2O, 5:1-4:1-3:1-1:1) gave **25** (243 mg) in 77% yield in 2 steps.

R_f = 0.60 (1:1 hexane/Et_2O) $[\alpha]_D^{28}$ +13.0°(*c* 0.11, $CHCl_3$) IR ($CHCl_3$) ; 1710, 1615, 1515, 1460, 1250, 1135, 1075, 1035, 835 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$)δ 7.26 (br d, *J* = 8.6 Hz, 2H), 6.86 (br d, *J* = 8.6 Hz, 2H), 5.88 (dt, *J* = 7.6, 1.7 Hz, 1H), 5.51 (t, *J* = 7.6 Hz, 1H), 4.51 (d, *J* = 11.6 Hz, 1H), 4.43 (d, *J* = 11.6 Hz, 1H), 4.35 (m, 1H), 4.19 (m, 1H), 4.04 (m, 1H), 3.80 (s, 3H), 3.75 (s, 3H), 3.59 (dd, *J* = 16.8, 9.3 Hz, 1H), 3.52 (dd, *J* = 16.8, 9.3 Hz, 1H), 3.32 (dd, *J* = 7.6, 7.6 Hz, 2H), 2.47 (m, 1H), 2.36 (m, 1H), 1.89 (d, *J* = 1.7 Hz, 3H), 1.97-1.56 (m, 4H), 0.87 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H). MS (FAB) *m/z* 575 $(M + Na)^+$; HRMS (FAB) $C_{29}H_{45}{}^{35}ClNaO_6Si$ $(M + Na)^+$ calcu: 527.2572, found : 527.2549 (Δ -2.3 mmu).

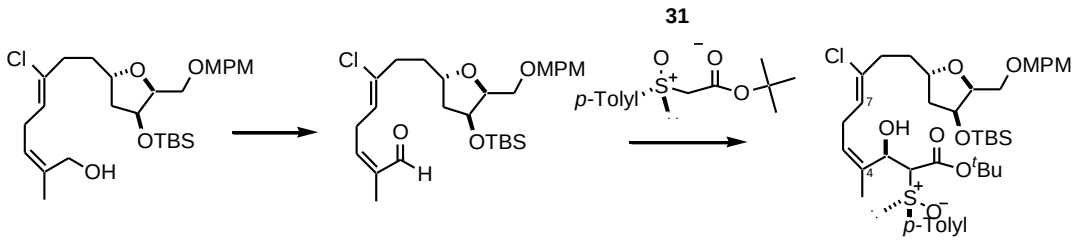


25 **26**

Alcohol 26

To a solution of **25** (23.3 mg, 42.1 μmol) in toluene (0.86 mL) at-78 °C was added diisobutylaluminium hydride (1.0M, hexane solution, 0.126 mL, 0.126 mmol). After stirred at -78 °C for 1.5 h, the reaction mixture was quenched by MeOH. 0.5M potassium sodium tartartrate (4.9 mL) was added to the reaction mixture and stirred at room temperature for 1h and extracted with Et_2O. The organic layer was washed with brine and dried over $MgSO_4$. Concentration and column chromatography (silcagel, hexane/acetone, 5:1-3:1-1:1) gave **26** (24.3 mg) in 100% yield.

R_f = 0.28 (1:1 hexane/Et_2O). $[\alpha]_D^{29}$ +16.9° (*c* 0.20, $CHCl_3$). IR ($CHCl_3$) ; 3600, 3480, 1615, 1585 1515, 1460, 1250, 1070, 1035, 835 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 7.26 (br d, *J* = 8.9 Hz, 2H), 6.86 (br d, *J* = 8.9 Hz, 2H), 5.45 (t, *J* = 6.9 Hz, 1H), 5.27 (br t, *J* = 6.9 Hz, 1H), 4.52 (d, *J* = 11.6 Hz, 1H), 4.44 (d, *J* = 11.6 Hz, 1H), 4.36 (m, 1H), 4.18 (m, 1H), 4.16 (s, 2H), 4.03 (m, 1H), 3.80 (s, 3H), 3.57 (dd, *J* = 16.5, 9.9 Hz, 1H), 3.54 (dd, *J* = 16.5, 9.6 Hz, 1H), 2.92 (br t, *J* = 6.9 Hz, 2H), 2.29-2.54 (m, 2H), 1.93 (ddd, *J* = 12.9, 5.6, 1.3 Hz, 1H), 1.80 (d, *J* = 1.3 Hz, 3H), 1.82-1.56 (m, 4H), 0.87 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 159.1, 135.6, 134.7, 130.4, 129.5, 129.5 (2C), 123.7, 113.7 (2C), 81.4, 77.3, 77.2, 73.1, 69.0, 61.5, 55.3, 41.9, 36.1, 33.8, 27.3, 25.7 (3C), 21.2, 18.0, -4.7, -5.2 MS (FAB) *m/z* 547 $(M + Na)^+$. HRMS (FAB) $C_{28}H_{45}{}^{35}ClNaO_5Si(M + Na)^+$ calc. : 547.2623, found : 547.2596 (Δ -2.7 mmu).



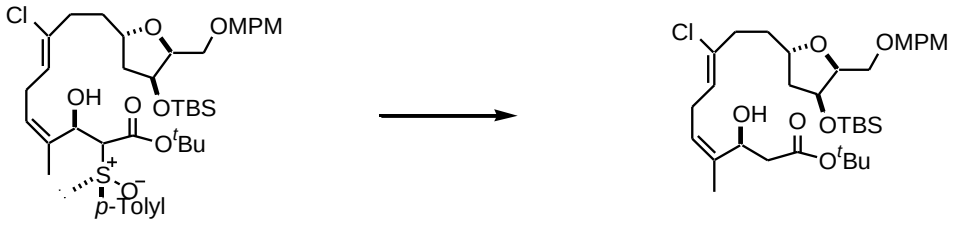
26 **26-2** **27**

Sulfoxide 27

To a solution of **26** (24.6 mg, 46.8 μmol) in CH_2Cl_2 (0.94 mL) at 0 °C was added Dess-Martin periodinane (29.6 mg, 698 μmol). After stirred at room temperature for 30 min, the reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$ and extracted with Et_2O. The organic layer was washed with brine, dried over $MgSO_4$. Concentration gave **26-2** which was directly used in the next reaction.

To a solution of **31** (59.4 mg, 0.234 mmol) in THF (1.2 mL) at -78 °C was added *tert*- butyl magnesium (0.93 M, THF solution, 0.25 mL, 0.234 mmol). **26-2** in THF (0.8 mL) was dropwised to the reaction mixture at -78 °C. After stirred at -78 °C for 3 h, the reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over $MgSO_4$. Concentration and column chromatography (silicagel, hexane /EtOAc 11:1-7:1-5:1-1:1) gave **27** (20.9 mg) in 57% yield in 2 steps.

R_f = 0.56 (3:2 hexane/EtOAc). $[\alpha]_D^{28}$ +105° (*c* 0.33, $CHCl_3$). IR ($CHCl_3$) ; 3595, 3480, 1720, 1515, 1460, 1370, 1250, 1145, 1060, 1035, 835 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 7.53 (br d, *J* = 8.3 Hz, 2H), 7.34 (br d, *J* = 8.3 Hz, 2H), 7.25 (br d, *J* = 8.6 Hz, 2H), 6.86 (br d, *J* = 8.6 Hz, 2H), 5.45 (t, *J* = 6.9 Hz, 1H), 5.38 (br t, *J* = 6.9 Hz, 1H), 5.12 (dd, *J* = 7.6, 5.9 Hz, 1H), 4.51 (d, *J* = 11.5 Hz, 1H), 4.43 (d, *J* = 11.5 Hz, 1H), 4.35 (m, 1H), 4.18 (m, 1H), 4.03 (m, 1H), 3.80 (s, 3H), 3.63-3.51 (m, 2H), 3.47 (d, *J* = 7.6 Hz, 1H), 3.07 (d, *J* = 5.9 Hz, 1H), 2.92 (m, 1H), 2.83 (m, 1H), 2.42 (s, 3H), 2.53-2.27 (m, 2H), 1.93 (ddd, *J* = 12.9, 5.6, 1.3 Hz, 1H), 1.77 (br s, 3H), 1.83-1.58 (m, 3H), 1.31 (s, 9H), 0.87 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 165.8, 159.2, 141.9, 138.7, 135.3, 133.1, 130.5, 129.5, 129.8 (2C), 129.8 (2C) 127.7 (2C), 124.7, 123.0, 113.7 (2C), 83.4, 81.4, 77.3, 76.7, 73.1, 73.1, 72.4, 69.0, 67.7, 55.3, 41.9, 36.1, 33.9, 27.9 (3C), 27.2, 25.7 (3C), 21.4, 18.0, -4.7, -5.2. MS (FAB) *m/z* 799 $(M + Na)^+$.

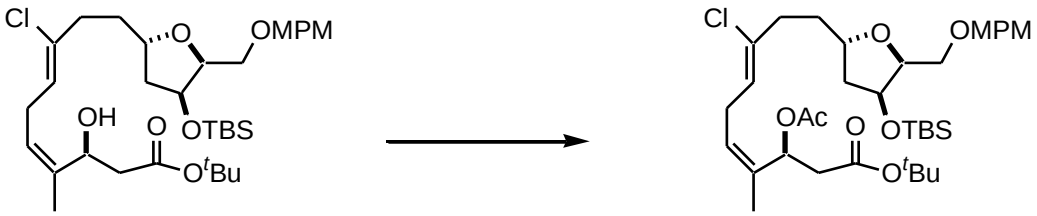


27 **28**

***t*-butyl ester 28**

To a solution of **27** (19.4 mg, 0.0250 mmol) in THF (1.8 mL) and H_2O (0.2 mL) at room temperature was added aluminium amalgam (80 mg). The reaction mixture was sonicated for 1.5h and stirred at room temperature for 14 h. The reaction mixture was filterated though celite pad and washed with $CHCl_3$. Concentration of filtarate and column chromatography (silicagel, hexane /Et_2O 5:1-2:1-1:1) gave **28** (13.7 mg) in 86% yield..

R_f = 0.51 (3:2 hexane/EtOAc). $[\alpha]_D^{28}$ +9.2° (*c* 0.056, $CHCl_3$) IR ($CHCl_3$) ; 3520, 1715, 1595, 1515, 1460, 1370, 1250, 1150, 1075, 1030, 835 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 7.25 (br d, *J* = 8.9 Hz, 2H), 6.85 (br d, *J* = 8.9 Hz, 2H), 5.45 (t, *J* = 7.3 Hz, 1H), 5.21 (br t, *J* = 6.9 Hz, 1H), 4.99 (dd, *J* = 9.9, 3.3 Hz, 1H), 4.51 (d, *J* = 11.6 Hz, 1H), 4.43 (d, *J* = 11.6 Hz, 1H), 4.35 (m, 1H), 4.17 (m, 1H), 4.03 (m, 1H), 3.79 (s, 3H), 3.57 (dd, *J* = 17.2, 9.9 Hz, 1H), 3.55 (dd, *J* = 17.2, 9.9 Hz, 1H), 2.98-2.83 (m, 2H), 2.57 (dd, *J* = 16.2, 9.8 Hz, 1H), 2.41 (dd, *J* = 16.2, 3.3 Hz, 1H), 2.57-2.28 (m, 2H), 1.93 (ddd, *J* = 11.6, 5.6, 1.3 Hz, 1H), 1.87-1.67 (m, 4H), 1.71 (br s, 3H), 1.47 (s, 9H), 0.87 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H) MS (FAB) *m/z* 661 $(M + Na)^+$



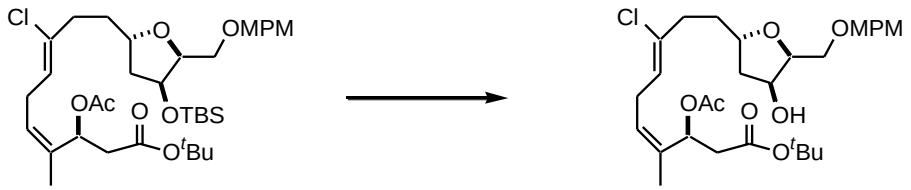
28 **28-2**

Acetate 28-2

To a solution of **28** (7.0 mg, 11.0 μmol) in pyridine (0.60 mL) at room temperature was added acetic anhydride (0.30 mL, 0.32 mmol). After stirred at room temperature for 7.5 h, the reaction mixture was concentrated with toluene. Column chromatography (silicagel, hexane /Et_2O, 7:1-3:1-1:1) gave **28-2** (7.4 mg) in 99% yield..

R_f = 0.38 (3:1 hexane/EtOAc). $[\alpha]_D^{30}$ -31.7° (*c* 0.011, $CHCl_3$) IR ($CHCl_3$) ; 1735, 1590, 1515, 1460, 1370, 1230, 1150, 1080, 1030, 835 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 7.25 (br d, *J* = 8.6 Hz, 2H), 6.88 (br d, *J* = 8.6 Hz, 2H), 5.95 (dd, *J* = 9.6, 5.3 Hz, 1H), 5.47 (t, *J* = 6.9 Hz, 1H), 5.25 (br t, *J* = 6.3 Hz, 1H), 4.51 (d, *J* = 11.6 Hz, 1H), 4.43 (d, *J* = 11.6 Hz, 1H), 4.35 (m, 1H), 4.18 (m, 1H), 4.03 (m, 1H), 3.80 (s, 3H), 3.57 (dd, *J* = 17.5, 9.6 Hz,

1H), 3.55 (dd, *J* = 17.5, 9.6 Hz, 1H), 3.05 (m, 2H), 2.69 (dd, *J* = 15.2, 9.6 Hz, 1H), 2.41 (dd, *J* = 15.2, 5.3 Hz, 1H), 2.53-2.35 (m, 2H), 2.20 (s, 3H), 1.93 (ddd, *J* = 11.6, 5.6, 1.2 Hz, 1H), 1.85-1.70 (m, 3H), 1.66 (br s, 3H), 1.43 (s, 9H), 0.87 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H) MS (FAB) *m/z* 703 $(M + Na)^+$

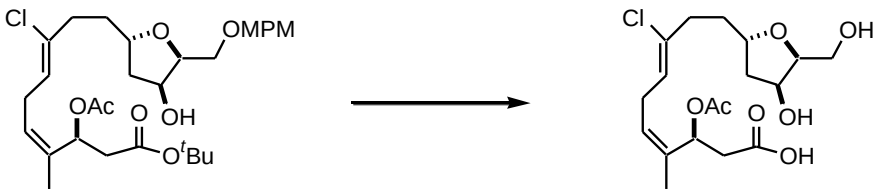


28-2 **28-3**

Secondary alcohol 28-3

To a solution of **28-2** (10.9 mg, 16.0 μmol) in THF (0.80 mL) and pyridine (0.30 mL) at 0 °C was added 70%HF-pyridiene (0.50 mL). After stirred at room temperature for 3 h, the reaction mixture was diluted with EtOAc and poured into saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. Concentration and column chromatography (silicagel, hexane/EtOAc, 3:1-2:1-1:1-1:2) gave **28-3** (8.4 mg) in 93% yield.

R_f = 0.33 (1:1 hexane/EtOAc). $[\alpha]_D^{29}$ +11.3° (*c* 0.043, $CHCl_3$) IR ($CHCl_3$) ; 3480, 1730, 1610, 1510, 1370, 1250, 1160, 1100, 1035, 835 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 7.25 (br d, *J* = 8.9 Hz, 2H), 6.88 (br d, *J* = 8.9 Hz, 2H), 5.95 (dd, *J* = 9.4, 5.3 Hz, 1H), 5.47 (t, *J* = 6.9 Hz, 1H), 5.27 (dt, *J* = 6.3, 1.3 Hz, 1H), 4.51 (s, 2H), 4.46 (m, 1H), 4.23 (m, 1H), 4.04 (m, 1H), 3.81 (s, 3H), 3.73 (d, *J* = 5.6 Hz, 2H) 3.07 (m, 2H), 2.93 (d, *J* = 4.0 Hz, 1H), 2.69 (dd, *J* = 15.2, 9.4 Hz, 1H), 2.51-2.25 (m, 2H), 2.41 (dd, *J* = 15.2, 5.3 Hz, 1H), 2.07 (ddd, *J* = 12.9, 5.6, 1.0 Hz, 1H), 2.02 (s, 3H), 1.67 (d, *J* = 1.3 Hz, 3H), 1.82-1.65 (m, 3H), 1.45 (s, 9H) MS (FAB) *m/z* 589 $(M + Na)^+$



28-3 **29**

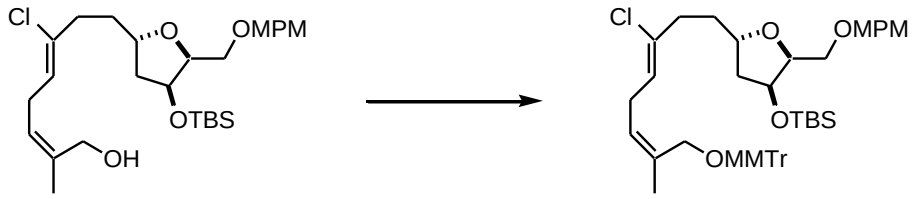
Seco acid 29

To a solution of **28-3** (2.1 mg, 3.8 μmol) in THF (0.50 mL) at 0 °C was added trimethylsilyl

trifuluoromethanesulfonate (20 μL, 0.010 mmol). After stirred at 0 °C for 2 h, the reaction mixture was quenched with 1M HCl and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. Concentration and column chromatography (ODS, MeOH /H_2O 7:1, and silicagel , $CHCl_3$/MeOH 39:1-29:1) gave **29** (1.3 mg) in 90% yeild.

R_f = 0.30 (5:1 $CHCl_3$/MeOH). $[\alpha]_D^{28}$ +13.8° (*c* 0.058, $CHCl_3$). IR ($CHCl_3$) ; 3680, 3420, 1730, 1610, 1460, 1280, 1170, 970 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 6.01 (dd, *J* = 10.3, 5.9 Hz, 1H), 5.69 (dd, *J* = 7.6, 7.6 Hz, 1H), 5.65 (br t, *J* = 7.6 Hz, 1H), 4.45 (m, 1H), 4.25 (m, 1H), 3.96 (d, *J* = 3.7 Hz, 2H), 3.91 (m, 1H), 3.15 (m, 1H), 2.93-2.80 (m, 2H), 2.86 (m, 1H), 2.67 (dd, *J* = 12.9, 10.3 Hz, 1H), 2.57 (m, 1H), 2.33 (m, 1H), 2.20 (ddd, *J* = 13.4, 6.8, 1.7 Hz, 1H), 2.08 (s, 3H), 1.75 (s, 3H), 1.89-1.63 (m, 3H) Peaks of CO_2H and O*H* were not found. MS (FAB) *m/z* 413 $(M + Na)^+$

Synthesis of macrolactone 34



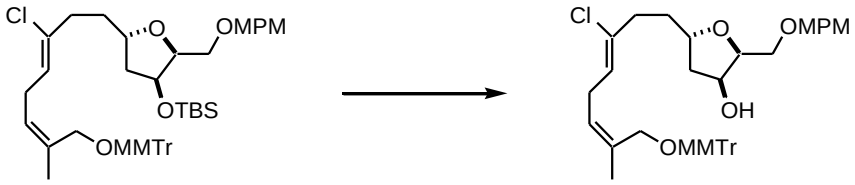
26 **32**

MMTr 32

To a solution of **26** (182 mg, 0.347 mmol) in pyridine (9.70 mL) at room temperature was added 4-methoxytriphenylmethyl chloride (1.49 g, 4.82 mmol). After stirred at 50 °C for 15 min, the reaction mixture was quenched with H_2O and extracted with Et_2O. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. Concentration and column chromatography (ODS, MeOH/H_2O/Et_3N 80:20:1-1:0:0) gave **32** (261 mg) in 94% yield..

R_f = 0.31 (1:1 hexane/Et_2O). $[\alpha]_D^{29}$ +11.4° (*c* 0.090, $CHCl_3$) IR ($CHCl_3$) ; 1615, 1515 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 7.47 (br d, *J* = 7.3 Hz, 4H), 7.35 (br d, *J* = 9.0 Hz, 2H), 7.28 (br dd, *J* = 7.9, 7.3 Hz, 4H), 7.25 (br d, *J* = 8.6 Hz, 2H), 7.21 (br d, *J* = 7.3 Hz, 2H), 6.86 (br d, *J* = 8.6 Hz, 2H), 6.83 (br d, *J* = 9.0 Hz, 2H), 5.33 (br t, *J*

= 7.0 Hz, 1H), 5.21 (br t, *J* = 7.3 Hz, 1H), 4.50 (d, *J* = 11.5 Hz, 1H), 4.44 (d, *J* = 11.5 Hz, 1H), 4.34 (m, 1H), 4.16 (m, 1H), 4.02 (m, 1H), 3.79 (s, 3H), 3.79 (s, 3H), 3.59 (br s, 2H), 3.58 (dd, *J* = 9.7, 5.3 Hz, 1H), 3.53 (dd, *J* = 9.7, 6.6 Hz, 1H), 2.68 (br dd, *J* = 7.3, 7.0 Hz, 2H), 2.40 (m, 1H), 2.38 (m, 1H), 1.93 (br dd, *J* = 11.7, 5.5 Hz, 1H), 1.85 (br s, 3H), 1.75 (m, 1H), 1.69 (m, 1H), 1.62 (m, 1H), 0.87 (s, 9H), 0.04 (s, 3H), 0.02 (s, 3H). MS (FAB) *m/z* 547 $(M + Na)^+$; HRMS (ESI) $C_{48}H_{61}{}^{35}ClNaO_6Si(M + Na)^+$ calc. : 819.3824, found : 819.3814 (Δ -1.0 mmu).

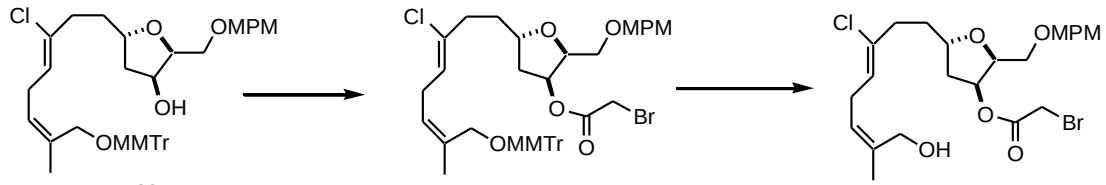


32 **33**

Alcohol 33

To a solution of **32** (299 mg, 0.375 mmol) in THF (10.8 mL) at room temperature was added tetrabutylammonium fluoride (1.0M, THF solution, 0.75 mL, 0.75 mmol). After stirred at room temperature for 2 h, the reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. Concentration and column chromatography (silicagel, hexane/EtOAc, 3:1-2:1-1:1) gave **33** (251 mg) in 98% yield.

R_f = 0.13 (1:1 hexane/Et_2O). $[\alpha]_D{}^{31}$ +0.0° (*c* 0.19, $CHCl_3$). IR ($CHCl_3$) ; 1615, 1515 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 7.47 (br d, *J* = 7.3 Hz, 4H), 7.35 (br d, *J* = 8.8 Hz, 2H), 7.28 (br dd, *J* = 8.0, 7.3 Hz, 4H), 7.24 (br d, *J* = 8.6 Hz, 2H), 7.21 (br d, *J* = 7.3 Hz, 2H), 6.87 (br d, *J* = 8.6 Hz, 2H), 6.83 (br d, *J* = 8.8 Hz, 2H), 5.34 (br t, *J* = 7.0 Hz, 1H), 5.22 (br t, *J* = 8.3 Hz, 1H), 4.51 (d, *J* = 11.5 Hz, 1H), 4.48 (d, *J* = 11.5 Hz, 1H), 4.45 (m, 1H), 4.20 (m, 1H), 4.03 (br dd, *J* = 9.2, 5.3, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 3.73 (d, *J* = 5.3 Hz, 2H), 3.59 (br s, 2H), 2.90 (d, *J* = 4.0 Hz, 1H), 2.68 (br dd, *J* = 8.3, 7.0 Hz, 2H), 2.39 (m, 1H), 2.29 (m, 1 H), 2.05 (br dd, *J* = 13.4, 5.7 Hz, 1H), 1.85 (br s, 3H), 1.76-1.67 (m, 3H). MS (FAB) *m/z* 547 $(M + Na)^{+\cdot}$ HRMS (ESI) $C_{42}H_{47}{}^{35}ClNaO_6$ $(M + Na)^+$ calc. : 705.2959, found : 705.2975 (Δ +1.6 mmu).

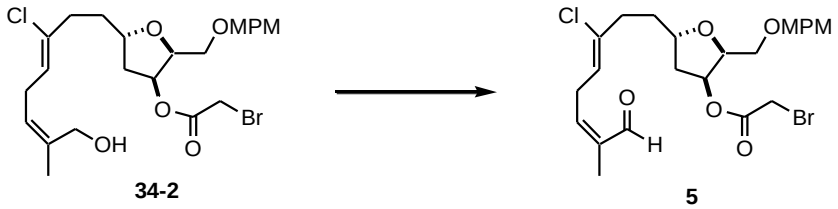


Bromoacetate 34-2

To a solution of **33** (14.7 mg, 21.5 μmol) in pyridine (0.30 mL) at 0 °C was added bromoacetyl bromide (20 μL, 0.230 mmol). After stirred at room temperature for 1 h, the reaction mixture was quenched with H_2O and extractedvwith EtOAc. The organic layer was washed with H_2O, saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. Concentration gave **34** which was directly used in the next reaction.

A solution of **34** (20.0 mg) in acetic acid (1.6 mL) and H_2O (0.40 mL) stirred at room temperature for 4 h, the reaction mixture was evaporated and extracted with Et_2O. The organic layer was washed with H_2O, saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. Concentration and column chromatography (silicagel, hexane/EtOAc 5:1-3:1-2:1) gave **34-2** (8.7 mg) in 89% yield in 2 steps.

R_f = 0.28 (1:1 hexane/Et_2O). $[\alpha]_D^{32}$ +53.4° (*c* 0.027, $CHCl_3$). IR ($CHCl_3$) ; 1735, 1615, 1515 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 7.24 (br d, *J* = 8.8 Hz, 2H), 6.87 (br d, *J* = 8.8 Hz, 2H), 5.46 (m, 2H), 5.27 (br dd, *J* = 9.0, 6.2 Hz, 1H), 4.52 (d, *J* = 11.7 Hz, 1H), 4.41 (d, *J* = 11.7 Hz, 1H), 4.21 (m, 1H), 4.16 (m, 2H), 4.16 (s, 2H), 3.81 (s, 3H), 3.72 (s, 2H), 3.59 (m, 2H), 2.92 (br t, *J* = 7.3 Hz, 2H), 2.47 (m, 1H), 2.38 (m, 1H), 2.14 (br dd, *J* = 13.9, 5.9 Hz, 1H), 1.88-1.75(m, 3H), 1.81 (d, *J* = 1.3 Hz, 3H) MS (FAB) *m/z* 547 $(M + Na)^+$; HRMS (ESI) $C_{24}H_{32}{}^{79}Br^{35}ClO_5$ $(M + Na)^+$ calc. : 553.0968, found : 553.0967 (Δ -0.1 mmu).

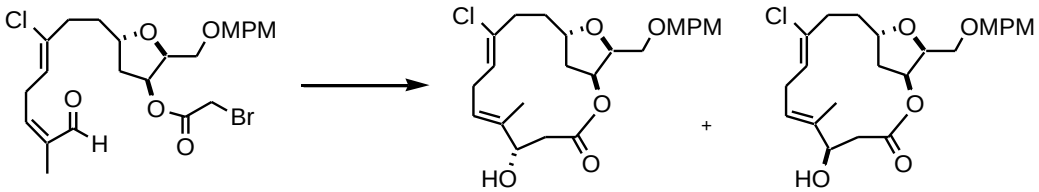


Aldehyde 5

To a solution of **34-2** (1.8 mg, 3.4 μmol) in CH_2Cl_2 (0.5 mL) at 0 °C was added Dess-Martin periodinane (2.9 mg, 6.8 μmol). After stirred at room temperature for 30 min, the reaction mixture was quenched with saturated

aqueous $Na_2S_2O_3$ and extracted with Et_2O. The organic layer was washed with brine and dried over $MgSO_4$. Concentration and column chromatography (silicagel, hexane/Et_2O, 1:1-1:2) gave **5** (1.7 mg) in 95% yield.

R_f = 0.28 (1:1 hexane/Et_2O). $[\alpha]_D^{32}$ +21.0° (*c* 0.053, $CHCl_3$). IR ($CHCl_3$) ; 1740, 1685, 1615, 1515 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 9.40 (s, 1H), 7.24 (br d, *J* = 8.4 Hz, 2H), 6.87 (br d, *J* = 8.4 Hz, 2H), 6.41 (br dd, *J* = 6.8, 6.4 Hz, 1H), 5.56 (br t, *J* = 6.8 Hz, 1H), 5.46 (br t, *J* = 4.2 Hz, 1H), 4.52 (d, *J* = 11.5 Hz, 1H), 4.46 (d, *J* = 11.5 Hz, 1H), 4.22 (m, 1H), 4.18 (m, 1H), 3.80 (s, 3H), 3.72 (br s, 2H), 3.58 (dd, *J* = 6.1, 2.6 Hz, 1H), 3.49 (dd, *J* = 13.9, 7.0 Hz, 1H), 3.21 (br t, *J* = 6.4 Hz, 2H), 2.52 (m, 1H), 2.42 (m, 1H), 2.15 (m, 1H), 1.88-1.78(m, 3H), 1.79 (br s, 3H). MS (FAB) *m/z* 547 $(M + Na)^+$; HRMS (ESI) $C_{24}H_{30}{}^{79}Br^{35}ClNaO_6$ $(M + Na)^+$ calc. : 551.0812, found : 551.0814 (Δ +0.2 mmu).



5 **38-1** **38-2**

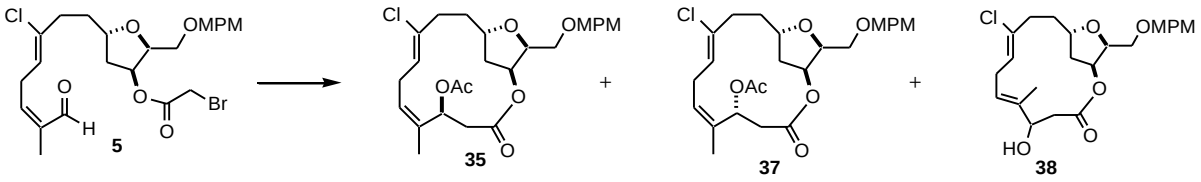
Regioisomer 38-1, 38-2

To a solution of samarium iodide (0.10 M, THF solution, 4.8 mL, 0.48 mmol) at 0 °C was dropwised **5** (4.1 mg, 0.108 μmol) in THF (9.6 mL) for 3.5h. After stirred at room temperature for 30 min, and the reaction mixture was quenched in the air and evaporated. Saturated aqueous $NaHCO_3$ was added to the reaction mixture and extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. Concentration and column chromatography (silicagel, hexane/EtOAc 3:1-2:1-1:1-1:2-0:1) , preparative TLC (hexane / EtOAc 1:2) gave **38-1** (1.52 mg) in 44% yield and **38-2** (1.48 mg) in 42% yield.

38-1 R_f = 0.57 (1:1 hexane/EtOAc). ^1H NMR (400 MHz, $CDCl_3$) δ 7.24 (br d, *J* = 8.5 Hz, 2H), 6.87 (br d, *J* = 8.5 Hz, 2H), 5.53 (br t, *J* = 7.6 Hz, 1H), 5.33 (dd, *J* = 10.7, 4.9 Hz, 1H), 5.14 (br t, *J* = 3.7 Hz, 1H), 4.57 (d, *J* = 11.7 Hz, 1H), 4.39 (d, *J* = 11.7 Hz, 1H), 4.25 (m, 1H), 4.19 (m, 1H), 3.82 (m, 1H), 3.80 (s, 3H), 3.58 (m, 2H), 3.56 (m, 1H), 3.23 (m, 1H), 2.69 (dd, *J* = 14.2, 3.7 Hz, 1H), 2.63 (dd, *J* = 14.2, 6.6 Hz, 1H), 2.59 (m, 1H), 2.52-2.40 (m, 2H), 2.20 (m, 1H), 1.67 (br s, 3H), 1.54 (m, 1H), 1.43 (m, 1H) Peak of O*H* was not found. MS

(ESI) *m/z* 473 $(M + Na)^+$

38-2 R_f = 0.57 (1:1 hexane/EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (br d, *J* = 8.5 Hz, 2H), 6.87 (br d, *J* = 8.5 Hz, 2H), 5.49 (m, 1H), 5.28 (dd, *J* = 11.0, 3.4, 1H), 5.15 (br t, *J* = 3.9 Hz, 1H), 4.58 (d, *J* = 12.0 Hz, 1H), 4.44 (m, 1H), 4.41 (d, *J* = 12.0 Hz, 1H), 4.24 (m, 2H), 3.85 (m, 1H), 3.80 (s, 3H), 3.59 (m, 2H), 3.56 (m, 1H), 3.30 (m, 1H), 2.52-2.46 (m, 3H), 2.18 (m, 1H), 2.12 (m, 1H), 1.73 (br s, 3H),1.69 (m, 1H), 1.40 (m, 2H). MS (ESI) *m/z* 473 $(M + Na)^+$



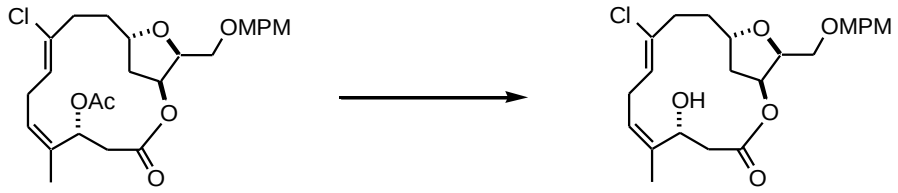
Macrolide 35

To a solution of Wilkinson's catalyst (5.37 mg, 5.38 μmol) in THF (4.0 mL) at 0 °C was added diethyl zinc (1.0M, hexane solution, 2.37 mL, 2.37 mmol). **5** (57.1 mg, 0.108 mmol) in THF (18 mL) was dropwised to the reaction mixture at 0 °C for 4 h then acetic acid anhydride (0.1 mL, 5.29 mmol) was added. After stirred at 0 °C for 1 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and filterated though celite pad with Et_2O. The organic layer was washed with brine and dried over $MgSO_4$. Concentration and column chromatography (silicagel, hexane/EtOAc 11:1-9:1-7:1-5:1-3:1-0:1) , preparative TLC (hexane / Et_2O 1:3) gave **35** (4.5 mg) in 9% and **37** and **38** (18.4 mg) in 33% yield..

35 R_f = 0.58 (1:1 hexane/EtOAc). $[\alpha]_D^{33}$ +43.7° (*c* 0.016, $CHCl_3$). IR ($CHCl_3$) ; 1740, 1615, 1515 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD) δ 7.24 (br d, *J* = 8.8 Hz, 2H), 6.87 (br d, *J* = 8.8 Hz, 2H), 5.70 (dd, *J* = 11.5, 4.4 Hz, 1H), 5.69 (m, 1H), 5.30 (br d, *J* = 6.8 Hz, 1H), 5.24 (br t, *J* = 3.4 Hz, 1H), 4.52 (d, *J* = 11.7 Hz, 1H), 4.38 (d, *J* = 11.7 Hz, 1H), 4.25 (dt, *J* = 6.3, 3.9 Hz, 1H), 3.95 (m, 1H), 3.77 (s, 3H), 3.59 (dd, *J* = 6.3, 2.0 Hz, 2H), 3.47 (m, 1H), 2.68 (dd, *J* = 11.7, 11.5 Hz, 1H), 2.53 (dd, *J* = 11.7, 4.4 Hz, 1H), 2.45 (m, 2H), 2.30 (m, 2H), 2.12 (m, 1H), 2.01 (s, 3H), 1.85 (br s, 3H), 1.52 (m, 1H), 1.42 (m, 1H). MS (FAB) *m/z* 547 $(M + Na)^{+\cdot}$ HRMS (ESI) $C_{26}H_{33}{}^{35}ClNaO_7$ $(M + Na)^+$ calc. : 515.1812, found : 515.1817 (Δ +0.5 mmu).

37 R_f = 0.57 (1:1 hexane/EtOAc). ^{1}H NMR (400 MHz, CD_3OD)δ 7.25 (br d, *J* = 8.3 Hz, 2H), 6.89 (br d, *J* =

8.3 Hz, 2H), 5.76 (m, 2H), 5.54 (br t, *J* = 7.3 Hz, 1H), 5.16 (br t, *J* = 4.2 Hz, 1H), 4.53 (d, *J* = 11.7 Hz, 1H), 4.43 (d, *J* = 11.7 Hz, 1H), 4.30 (m, 1H), 4.08 (dt, *J* = 7.3, 7.1 Hz, 1H), 3.77 (s, 3H), 3.52 (m, 2H), 2.97 (m, 1H), 2.81 (dd, *J* = 12.2, 10.5 Hz, 1H), 2.76 (m, 1H), 2.47 (dd, *J* = 12.2, 3.7 Hz, 1H), 2.39-2.26 (m, 3H), 2.17 (m, 1H), 2.00 (s, 3H), 1.65 (br s, 3H), 1.49 (m, 1H), 1.42 (m, 1H).

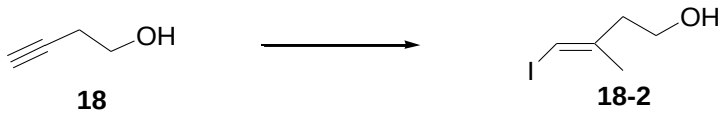


Secondary alcohol 37-2

To a solution of **37** (5.3 mg, 11 μmol) in MeOH (1.8 mL) and H_2O (0.2 mL) at 0 °C was added K_2CO_3 (20 mg, 0.189 mmol). After stirred at 0 °C for 1 h, H_2O and NaCl were added to the reaction mixture and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. Concentration and preparative TLC (hexane / EtOAc 1:1) gave **37-2** (2.2 mg) in 45% yield.

R_f = 0.58 (1:1 hexane/EtOAc). ^{1}H NMR (400 MHz, CD_3OD) δ 7.27 (br d, *J* = 8.8 Hz, 2H), 6.88 (br d, *J* = 8.8 Hz, 2H), 5.53 (br t, *J* = 7.1 Hz, 1H), 5.21 (br t, *J* = 6.6 Hz, 1H), 5.03 (br dd, *J* = 8.3, 5.5 Hz, 1H), 4.51 (d, *J* = 11.5 Hz, 1H), 4.46 (d, *J* = 11.5 Hz, 1H), 4.33 (br t, *J* = 3.9 Hz, 1H), 4.19 (m, 1 H), 4.01 (m, 1H), 3.77 (s, 3H), 3.67 (dd, *J* = 10.2, 4.6 Hz, 1H), 3.65 (m, 1H), 3.57 (dd, *J* = 10.2, 7.0 Hz, 1H), 2.93 (m, 2H), 2.63 (dd, *J* = 14.6, 8.5 Hz, 1H), 2.41 (dd, *J* = 14.6, 5.4 Hz, 1H), 2.42 (m, 2H), 2.01 (ddd, *J* = 13.2, 5.9, 1.2 Hz, 1H),1.75 (m, 2H), 1.69 (br s, 3H) Peak of O*H* was not found.

<u>Synthesis of Haterumalide NA Methyl ester</u>

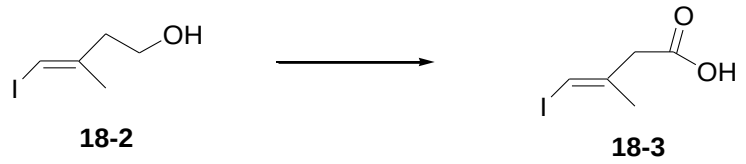


Vinyl iodide 18-2

To a solution of zirconocene dichloride (4.82 g, 16.5 mmol) in 1,2-dichloroethane (130 mL).

Trimethylaluminum (2.0M, touluene solution, 99.0 mL, 198 mmol) was added to the reaction mixture. A solution of 3-butyn-1-ol **18** (4.63 g, 66.0 mmol) in 1,2-dichloroethane (35 mL) was also dropwised to the reaction mixture. After stirred at room temperature for 14 h, the reaction mixture was cooled to 0 °C ans iodine (20.0 g, 79.2 mmol) in THF (65 mL) was added. After stirred at room temperature for 40 min, the reaction mixture was quenched with saturated aqueous K_2CO_3 and filterated with celite pad and washed with Et_2O. The organic layer was concentrated and column chromatography (silicagel, hexane/EtOAc 5:1-3:1) gave **18-2** (8.7 mg) in 89% yield.

R_f = 0.41 (1:1 hexane/Et_2O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.18 (br s, 1H), 3.74 (m, 1H), 3.70 (br t, *J* = 6.3 Hz, 2H), 2.47 (br t, *J* = 6.3 Hz, 2H), 1.88 (br s, 3H).

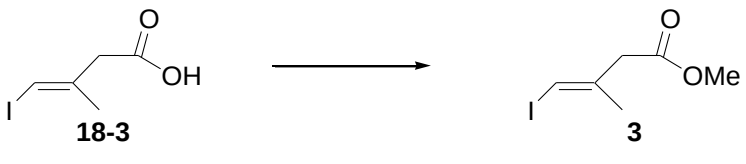


18-2 **18-3**

Carboxylic acid 18-3

To a solution of chromic acid (2.61 g, 26.2 mmol) in 1.5M sulfuric acid (43 mL) at 5-10 °C was dropwised **18-2** (1.46 g, 6.88 mmol) in acetone (80 mL) for 30 min. After stirred at room temperature for 4 h, the reaction mixture was extracted with Et_2O and dried over $MgSO_4$. Concentration gave crude mixture and diluted with Et_2O (30 mL) and extracted with 1M NaOH. Aqueous layer was acidified with 6.0 M HCl and extracted with Et_2O. The organic layer was washed with brine and dried over $MgSO_4$. Concentration and column chromatography (silicagel, hexane/Et_2O 1:0-1:1) gave **18-3** (1.06 g) in 68% yield.

R_f = 0.23 (1:1 hexane/Et_2O). ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.22 (br s, 1H) , 3.25 (br s, 2H), 1.96 (br s, 3H). Peak of CO_2H was not found.

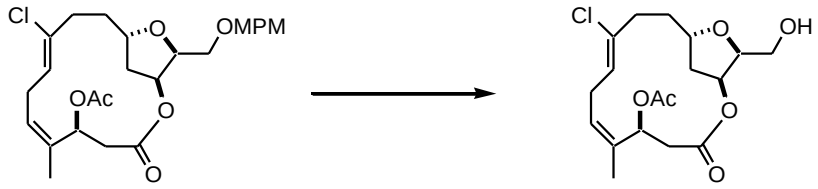


18-3 **3**

C16-19 fragment 3

To a solution of **18-3** (1.06 g, 5.25 mmol) in benzene (50 mL) and MeOH (10 mL) was added (trimethylsilyl) diazomethane (12.0 mL, 6.93 mmol). After stirred at room temperature for 5 min, the reaction mixture was concentrated and column chromatography (silicagel, hexane/Et_2O, 49:1-9:1) gave **3** (0.930 g) in 74% yield.

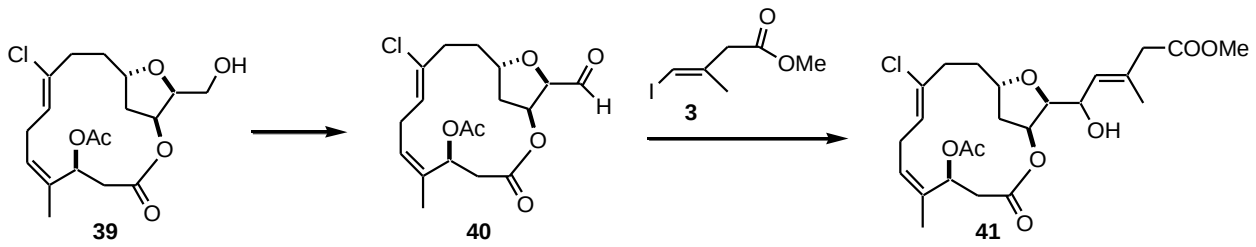
R_f = 0.79 (1:2 hexane/Et_2O). ^1H NMR (270 MHz, $CDCl_3$) δ 6.18 (br s, 1H), 3.70 (s, 3H), 3.21 (br s, 2H), 1.94 (br s, 3H).



Alcohol 39

To a solution of **35** (1.1 mg, 2.2 μmol) in CH_2Cl_2 (1.0 mL), *tert*-butyl alcohol (0.1 mL) and 1.0M phosphate buffer (pH=5.91) at 0 °C was added 2,3-dichloro-5,6-dicyanoquinone (4.0 mg, 17.8 μmol). After stirred at room temperature for 1.5 h, 2,3-dichloro-5,6-dicyanoquinone (4.0 mg, 17.8 μmol) was added again. After stirred at room temperature for 1 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over $MgSO_4$. Concentration and column chromatography (silicagel, hexane/acetone, 5:1-3:1-1:1) gave **39** (1.10 mg) in 100%.

R_f = 0.17 (1:1 hexane/EtOAc). $[\alpha]_D^{33}$ +7.7° (*c* 0.019, $CHCl_3$). IR ($CHCl_3$) ; 3500 (br), 1730 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 5.76 (dd, *J* = 10.1, 6.0 Hz, 1H), 5.70 (m, 1H), 5.30 (br d, *J* = 7.4 Hz, 1H), 5.26 (br t, *J* = 3.4 Hz, 1H), 4.15 (m, 1H), 3.91 (m, 1H), 3.68 (m, 2H), 3.48 (m, 1H), 2.76 (m, 2H), 2.68 (m, 1H), 2.47 (m, 2H), 2.30 (m, 2H), 2.02 (s, 3H), 1.87 (br s, 3H), 1.52 (m, 1H), 1.43 (m, 1H) Peak of O*H* was not found. HRMS (ESI) $C_{18}H_{25}{}^{35}ClNaO_6$ $(M + Na)^+$ calc. : 395.1237, found : 395.1251 (Δ +1.4 mmu).



15-epi-haterumalideNA menthyl ester 41

To a solution of **39** (0.80 mg, 2.15 μmol) in CH_2Cl_2 (1.0 mL) at 0 °C was added Dess-Martin periodinane (5.20 mg, 12.3 μmol). After stirred at room temperature for 30 min, the reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$ and extracted with Et_2O. The organic layer was washed with brine and dried over $MgSO_4$. Concentration gave **40** (0.90 mg) which was directly used in the next reaction.

A solution of **40** (0.90 mg) and **3** (41.0 mg, 0.170 mmol) in DMSO (0.50 mL) was degassed by freeze-pump-thaw. To a reaction mixture was added $CrCl_2$ (58.0 mg, 0.472 mmol) and catalytic amount of $NiCl_2$. After stirred at room temperature for 20 h, the reaction mixture was quenched with H_2O and extractedvwith Et_2O. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. Concentration and column chromatography (silicagel, hexane / Et_2O, 5:1-EtOAc), preparative TLC (hexane/EtOAc, 1:2) gave **41** (15R : 15S = 11 : 1)**.** **41** (15R : 15S = 11 : 1) was purified by HPLC (Develosil ODS HG-5 φ4.6 × 250 mm), MeOH : H2O = 3 : 2) and **40** (0.59 mg) was obtained in 57% yield in 2 steps.

R_f = 0.38 (1:2 hexane/EtOAc). ^{1}H NMR (800 MHz, CD_3OD) δ 5.79 (dd, *J* = 11.4, 4.6, 1H), 5.70 (br dd, *J* = 9.2, 7.4, 1H), 5.37 (br d, *J* = 8.7 Hz, 1H), 5.31 (br d, *J* = 7.3 Hz, 1H), 5.30 (br t, *J* = 3.4 Hz, 1H), 4.53 (br t, *J* = 8.5 Hz, 1H), 3.93 (br tt, *J* = 11.4, 3.6 Hz , 1H), 3.90 (dd, *J* = 8.7, 3.7 1H), 3.67 (s, 3H), 3.49 (m, 1H), 3.08 (s, 2H), 2.82 (dd, *J* = 11.5, 4.2 Hz, 1H), 2.77 (dd, *J* = 11.5, 11.4 Hz, 1H), 2.47 (m, 2H), 2.29 (m, 2H), 2.10 (br dd, *J* = 12.8, 3.2, 1H), 2.02 (s, 3H), 1.88 (br s, 3H), 1.81 (d, *J* = 1.4 Hz, 3H), 1.52 (br dt, *J* = 12.8, 3.2, 1H), 1.39 (m, 1H) HRMS (ESI) $C_{24}H_{33}{}^{35}ClNaO_8$ $(M + Na)^+$ calc. : 507.1762, found : 507.1753 (Δ -0.9 mmu). CD (MeOH) λ_{ext}220 nm (Δε -0.72)

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Chapter 5

Summary

In conclusion, the author identified G protein-biased and highly subtype selective EP2 receptor agonists and reported the first total synthesis of resolvin E2 and haterumalide NA methyl ester.

As described in Chapter 2, the author discovered G protein-biased and highly subtype selective novel EP2 agonist **27a** by hybridization of a thiazole moiety and a bicyclic scaffold of prostacyclin. Optimization of α chain, 11-hydroxyl group and ω-chain led the author to identify compound **9** with 100-fold increase in G protein signaling without increase of β arrestin activity relative to **27a**. Furthermore, structure functional selectivity relationship studies revealed that the combination of *meta* and *para* substituents on the phenyl moiety was crucial to regulate its functional selectivity (Figure 5-1).

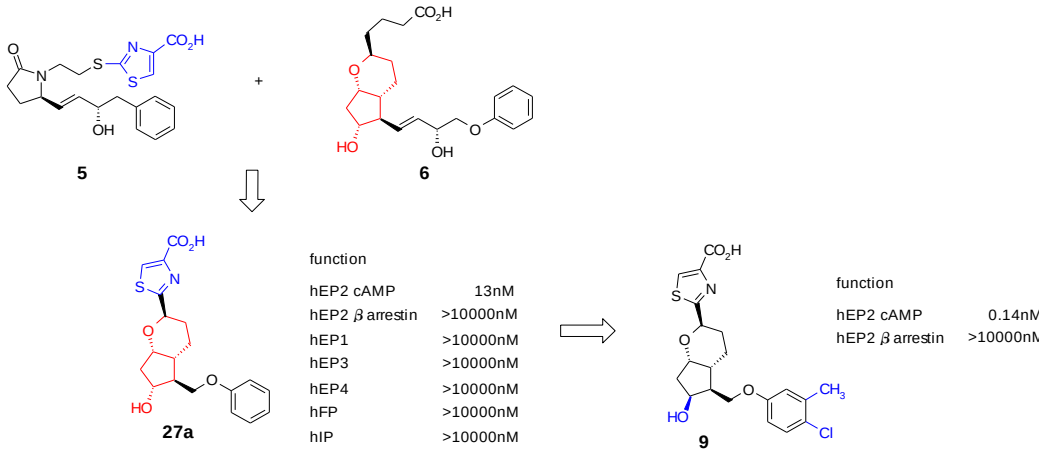

Figure 5-1. Outline of discovery of G protein-biased EP2 agonists.

The author reported the efficient total synthesis of resolvin E2 by using its intrinsic pseudoenantiomeric substructure as shown in Chapter 3 (Figure 5-2). Moreover, biological evaluation in the acute peritonitis model revealed that resolvin E2 exerts potent activity in blocking neutrophil infiltration and reducing proinflammatory cytokines.

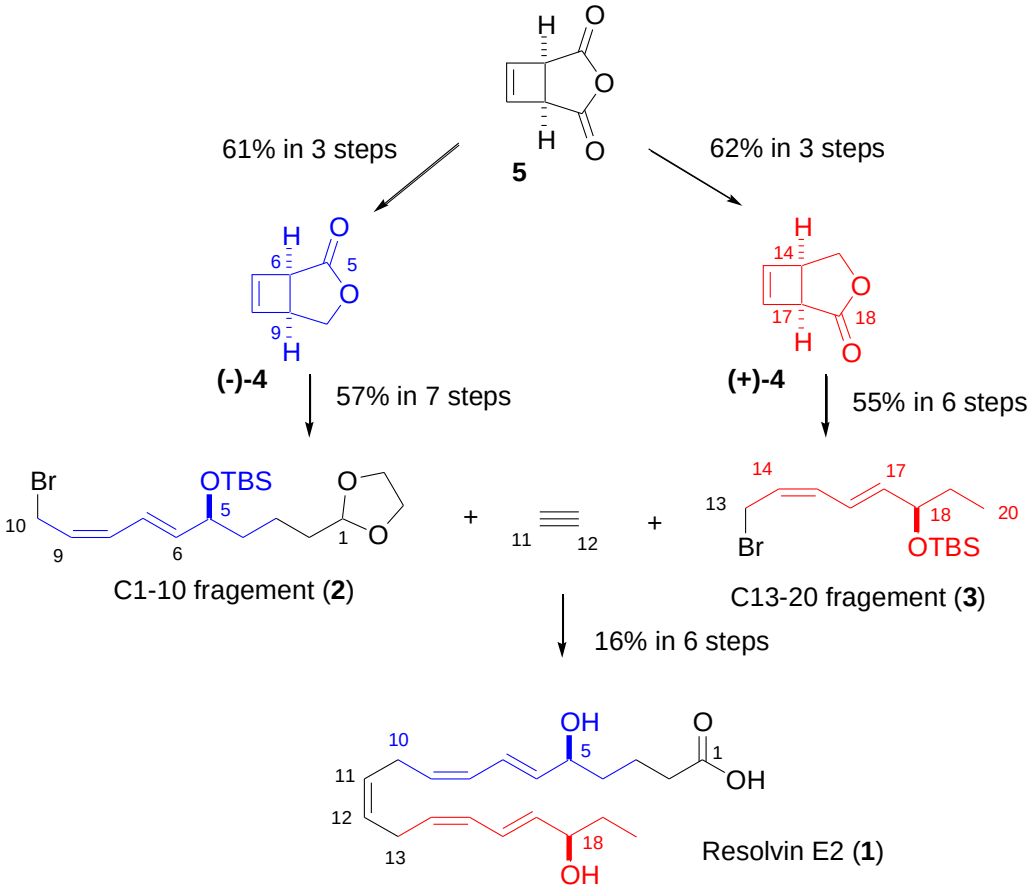

Figure 5-2. Outline of the first total synthesis of Resolvin E2

As described in Chapter 4, the author also reported the first total synthesis of haterumalide NA methyl ester. 14-membered macrolide and *Z*-chloroolefin moieties of haterumalide NA were synthesized by the intramolecular Reformatsky-type reaction and the stereoselective taransformation from *E*-vinylsilane moiety. The author revised the absolute stereochemistry of haterumalide NA.

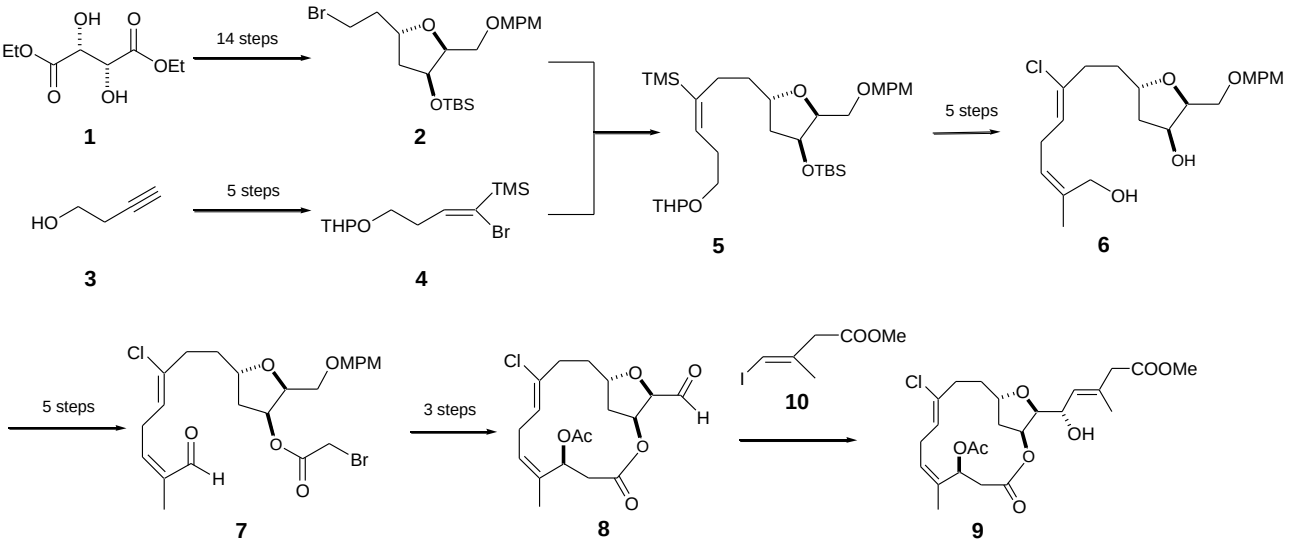

Figure 5-3. Outline of the total synthesis of 15-*epi*-Haterumalide NA methyl ester.

Overall, the author identified the first examples of G protein-biased EP2 receptor agonists and these compounds would be a good tool compounds to investigate the biological role of G protein- and β arrestin-mediated signaling for EP2 receptor. And efficient synthetic routes of lipid mediators (resolvin E2 and haterumalide NA) should be helpful strategy for further investigation of resolvins and haterumalides.

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The studies in this thesis have been performed under the direction of Professor Hideo Kigoshi at Department of Chemistry, Graduate School of Pure and Applied Sciences, University of Tsukuba.

First of all, I am deeply grateful to Professor Kigoshi for his valuable advices and helpful encouragement for carrying out this work and giving me pleasure of organic chemistry when I was a graduate student at Nagoya University. I would like to express my sincere thanks to Professor Daisuke Uemura for his hearty encouragement and teaching me an importance of organic chemistry at Nagoya University. I also appreciate Associate Professor Masaki Kita for his proper advice for this studies and giving me experimental skills of organic chemistry. I would like to offer my heartfelt thanks to Professor Masayuki Inoue for his excellent suggestion and giving me strategies for rational synthesis when I was a visiting researcher at Tokyo University and thank Assistant Professor Daisuke Urabe for his valuable suggestion.

The investigations at ONO Pharmaceutical described in this thesis were performed under the direction of Dr. Kazuhito Kawabata, general manager of research department, Dr Takuya Seko, a former manager of medicinal chemistry research laboratory. I would like to offer them my sincere thanks for their kind direction and encouragement. The collaborations of Dr Toshihide Watanabe, Mr Isamu Sugimoto, Ms Kazumi Moriyuki, Dr Yoshikazu Goto, Mr Shinsaku Yamane, Dr. Akio Watanabe, Mr Kzuma Tsuboi, Dr Atsushi Kinoshita, Dr Kousuke Tani and Dr Toru Maruyama and other members in “EP2 agonist project” are greatly acknowledged. Particularly, I wish to thank to Dr Maruyama and Dr Tani for their kind and excellent suggestion for these studies.

Finally, I would like to dedicate this doctoral dissertation to all my family, my wife Hitomi for her hearty encouragement, my son Hiroya and daughter Sumire for giving power to perform these works.

List of publications and patents included in this thesis

1) Ogawa, S.; Watanabe, T.; Sugimoto, I.; Moriyuki, K.; Goto, Y.; Yamane, S.; Watanabe, A.; Tsuboi, K.; Kinoshita, A.; Kigoshi, H.; Tani, K.; Maruyama, T. Discovery of G protein-biased EP2 receptor agonists. *ACS Med.Chem. Lett.* DOI; 10.1021/acsmedchemlett.5b00455 (in press)

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List of publications and patents not included in this thesis

1) Kambe, T.; Maruyama, T.; Nagase, T.; Ogawa, S.; Minamoto, C.; Sakata, K.; Maruyama, T.; Nakai, H.; Toda, M. Synthesis and evaluation of novel modified γ-lactam prostanoids as EP4 subtype-selective agonists. *Bioorg Med Chem.* **2012**, *20*, 702-713

2) Iwahashi, M.; Naganawa, A.; Kinoshita, A.; Shimabukuro, A.; Nishiyama, T.; Ogawa, S.; Matsunaga, Y.; Tsukamoto, K.; Okada, Y.; Matsumoto, R.; Nambu, F.; Oumi, R.; Odagaki, Y.; Katagi, J.; Yano, K.; Tani, K.; Nakai, H.; Toda M. Discovery of new orally active prostaglandin D2 receptor antagonists. *Bioorg Med Chem.* **2011**, *19*, 6935-6948.

3) Iwahashi, M.; Kawauchi, S.; Kinoshita, A.; Kishida, Y.; Kobayashi, K.; Matsunaga, Y.; Naganawa, A.; Nambu, F.; Ogawa, S.; Okada, Y.; Shimabukuro, A.; Tsukamoto, K.; Yano, K. Carboxylic acid compounds and medicinal compositions containing the same as the active ingredient. PCT Int. Appl., WO 2005028455, 2005