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Development of a Highly Selective EP2-Receptor Agonist. Part 1: Identification of 16-hydroxy-17,17-trimethylene PGE₂ Derivatives

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Abstract—Design and synthesis of an EP2-receptor selective agonist began with the chemical modification of α - and ω -chains of butaprost **1a**, which exhibits an affinity for the IP-receptor. Two series of prostaglandin (PG) analogues with a 16-hydroxy-17,17-trimethylene moiety as an ω -chain were identified. Among those tested, **4a,b,e,f,h** and **6a,b,e,f,h** were found to be highly selective EP2-receptor agonists. Structure–activity relationships are discussed. © 2002 Elsevier Science Ltd. All rights reserved.

Introduction

Prostanoid receptors are members of the G-protein coupled receptor superfamily. Recently, eight prostanoid receptors were cloned and characterized.^{1,2} The study of the receptor level of prostanoids has resulted in renewed interest in the field because the identification of a subtype of selective ligands might enable the development of a clinically useful drug without side effects such as hypotension, diarrhea or uterine contractions. In fact, therapeutic application of most of the launched prostanoids is limited because of their poorly selective agonist activity.3 Receptors of PGE2 have been classified into four subtypes EP1, EP2, EP3 and EP4.1 The diverse biological activities of PGE₂ have been considered to be expressed as a hybrid of the activities which mediate these four EP-receptor subtypes. Among them, the EP2-receptor subtype^{4,5} has been characterized with a relaxation of blood vessels, the gastrointestinal tract, the trachea and uterine smooth muscle⁶ and has been suggested to play an important role in the production and control of cytokines⁷ and bone metabolism.⁸ Development of a highly selective EP2-receptor agonist has been expected as one of the attractive approaches to develop a therapeutically useful drug. For example, butaprost $1a^9$ (Fig. 1, Table 1) has long been used as a selective EP2-receptor agonist. However, the corresponding carboxylic acid **1b**, which is produced by the metabolic hydrolysis of **1a**, exhibits an affinity also for the IP-receptor, and demonstrates less potent EP2-receptor agonist activity than PGE₂ (Table 2). Compound **2** (AH-13205)¹⁰ is also reported to show an affinity for the EP2-receptor, while its selectivity was very poor according to our internal evaluation (Fig. 1, Table 1). As a result, there has been no report of a highly selective EP2-receptor agonist. In this report, we describe the identification and biological evaluation of 16-hydroxy-17,17-trimethylene PGE₂ derivatives as new selective EP2-receptor agonists. Structure–activity relationships (SARs) are also discussed.



Figure 1.

Table 1. K_i Values of the reported EP2-receptor agonists

Compound	Binding K_i (nM)							
	mEP1	mEP2	mEP3	mEP4				
1a	> 10 ⁴	790	> 10 ⁴	> 10 ⁴				
2	2800	320	49	2200				

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Chemistry

Synthesis of the PG analogues listed in Tables 2–4 began with the preparation of their ω -chain moieties.

Vinyl iodides **19a–o**, **20** and **21** were prepared as described in Scheme 2. Alkylation of the cycloalkyl carboxylic acids **10–12** followed by hydride reduction with lithium



Scheme 1. Discovery of highly selective EP2-receptor agonists 4a,b,e, f,h and 6a,b,e,f,h.

Table 2. Optimization of α -chain

aluminum hydride yielded the alcohols 13a-f,h,j,l,n,o, 14 and 15. Compounds 13g, 13i, 13k and 13m were also prepared as follows. Cyclobutane carboxylic acid 10 was converted to 22 by the sequential reactions: (1) alkylation with 2-tetrahydropyranyloxyethyl iodide; (2) reduction with diborane. After acylation of 22 with benzoyl chloride, deprotection of the tetrahydropyranyl (THP) group under acidic conditions followed by iodination with iodine and triphenylphosphine yielded 23. Elimination reaction followed by alkaline hydrolysis gave 13g. Compounds 13i, 13k and 13m were prepared from 13h. Protection of 13h with dihydropyran followed by hydroboration with diborane yielded 24. Swern oxidation of 24 followed by the Wittig reaction and subsequent deprotection under acidic conditions gave 13i. Tosylation of 24 followed by fluorination with tetra-nbutylammonium fluoride and subsequent acidic deprotection gave 13k. O-Methylation of 24 followed by the acidic deprotection yielded 13m.

Sequential reactions: (1) Swern oxidation of 13a–o, 14 and 15; (2) addition reaction with propargylmagnesiumbromide; 3) protection of the formed hydroxyl group with *t*-butyldimethylsilyl (TBS) group gave alkynes 16a–o, 17 and 18, respectively. Hydrozirconation^{11,12} or hydrostannation¹³ of the resulting alkynes followed by treatment with iodine yielded vinyl iodides 19a–o, 20 and 21.

As described in Scheme 3, conjugate addition^{14,15} of the ω -chain moieties **19a–o**, **20** and **21** to the enone **25**¹⁴ was carried out in the presence of *t*-butyl lithium and 2-thi-enylcyanocuprate¹⁶ to produce **26a–o**, **27** and **28**,



Compd	R			EC ₅₀ (nM) ^b				
		mEP1	mEP2	mEP3	mEP4	hIP	mEP2	hIP
3a 3b	PGE ₂ PGE ₁	18 100	38 87	5.0 5.0	3.1 3.3	> 10 ⁴ 150	2.0 3.0	260 2.0
1b	.,, ¹ , COOH	$> 10^4$	73	$> 10^{4}$	$> 10^4$	870	23	25
4b		$> 10^4$	92	$> 10^{4}$	$> 10^4$	$> 10^4$	43	$> 10^{4}$
5	COOH	$> 10^4$	1100	$> 10^{4}$	$> 10^{4}$	N.T.°	N.T.	N.T.
6b	СООН	> 10 ⁴	25	> 10 ⁴	> 10 ⁴	> 10 ⁴	54	$> 10^4$

^aUsing membrane fractions of Chinese hamster ovary (CHO) cells expressing the prostanoid receptors, K_i values were determined by the competitive binding assay, which was performed according to the method of Kiriyama et al.³ with some modifications. The K_i values of the test compounds, which demonstrated less than 50% inhibition at 10⁴ nM, are expressed > 10⁴.

 ${}^{b}EC_{50}$ values were determined based on the effect of the test compounds on the increase in intracellular cAMP production in the mouse (m) EP2 receptor or human (h) IP-receptor.

^cNot tested.

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Scheme 2. Synthesis of vinyl iodides 19a–o, 20, 21. Reagent: (a) LDA, RX, THF, 0°C; (b) LiAlH₄, THF, reflux; (c) (COCl)₂, DMSO, Et₃N, CH₂Cl₂, -78°C; (d) propargylmagnesium bromide, ether, 0°C; (e) TBSOTf, 2,6-lutidine, CH₂Cl₂, 0°C; (f) Cp₂ZrClH, THF-toluene (Method A) or *n*-Bu₃SnH, AIBN (Method B) then I₂; (g) LDA, I(CH₂)₂OTHP, THF; (h) BH₃–THF, THF; (i) BzCl, pyridine; (j) *p*-TsOH, MeOH; (k) PPh₃, imidazole, I₂, benzene; (l) DBU, benzene; (m) aqueous NaOH, MeOH; (o) DHP, *p*-TsOH, CH₂Cl₂; (p) BH₃–THF, THF; (q) Swern oxdn; (r) MePPh₃Br, BuLi, THF; (s) *p*-TsOH, MeOH; (t) TsCl, pyridine; (u) *n*-Bu₄NF, THF; (v) MeI, NaH, THF.



Scheme 3. Synthesis of compounds 4a–o, 7, 8, 9. Reagent: (a) *t*-BuLi, 2-thienylcyanocuprate, ether, -78 °C; (b) aqueous HF, CH₃CN, 0 °C; (c) separation; (d) porcine liver esterase, phosphate buffer (pH=7.4), EtOH, room temperature.



Scheme 4. Synthesis of compounds 6a,b,e,f and 6h. Reagent: (a) *t*-BuLi, 2-thienylcyanocuprate, ether, -78 °C; (b) 32 CuCN, LiCl, THF, -78 °C; (c) aqueous HF, CH₃CN, 0 °C; (d) separation; (e) porcine liver esterase, phosphate buffer (pH = 7.4), EtOH, room temperature.



Scheme 5. Synthesis of compound 5. Reagent: (a) 19b, 35, *t*-BuLi, 2-thienylcyanocuprate, ether, -78 °C; (b) aqueous HF, CH₃CN, 0 °C; (c) separation; (d) porcine liver esterase, phosphate buffer (pH = 7.4), EtOH, room temperature.

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respectively. Deprotection of the TBS ethers with aqueous hydrogen fluoride¹⁷ followed by separation of C16epimers and subsequent enzymatic hydrolysis with porcine liver esterase (PLE)¹⁸ yielded 4a-o, 9, 7 and 8.

As shown in Scheme 4, conjugate addition of 19a,b,e,f and 19h to the enone 29¹⁴ gave 30a,b,e,f and 30h, which were converted to 31a,b,e,f and 31h, respectively, by another conjugate addition of 32.^{19,20} Deprotection of the TBS groups in 31a,b,e,f and 31h with aqueous hydrogen fluoride followed by separation of C16-epimers and subsequent hydrolysis with PLE yielded 6a,b,e,f and 6h, respectively.

Synthesis of the alkyne-containing PG analogue 5 is described in Scheme 5.²¹ Conjugate addition reaction of



 33^{21} with the cuprate prepared from 19b followed by trapping of the resulting enolate with an iodoalkynoate 35^{21} gave 34. Acidic deprotection of 34 followed by separation of C16-epimers and subsequent enzymatic hydrolysis with PLE yielded 5.

Results and Discussion

Two series of PG analogues 4a-o,6a,b,e,f and 6h with a unique ω-chain 16-hydroxy-17,17-trimethylene moiety were evaluated for their selective affinity for the EP2receptor and potency of agonist activity. K_i values were determined by competitive binding assays which were performed according to the method of Kiriyama et al.³ with some modifications. With regards to agonist activ-

> EC50 (nM) mEP2

> > 11

43

220

580

 $> 10^4$

N.T.

			Hồ	\mathbb{C}^{H}				
Compound	R	п	Binding K _i (nM)					
			mEP1	mEP2	mEP3	mEP4	hIP	
4a	\sim	1	> 10 ⁴	30	$> 10^{4}$	> 10 ⁴	> 10 ⁴	
4b	\sim	1	$> 10^{4}$	92	$> 10^4$	$> 10^{4}$	$> 10^4$	
9 (16-epimer of 4b)	\sim	1	$> 10^{4}$	330	1200	$> 10^{4}$	$> 10^4$	
7	\sim	2	> 10 ⁴	370	$> 10^{4}$	$> 10^{4}$	$> 10^{4}$	
8	\sim	3	> 10 ⁴	3300	> 10 ⁴	$> 10^{4}$	$> 10^4$	
4c	\sim	1	780	43	2000	$> 10^{4}$	$> 10^4$	
4d	\sim	1	1600	20	830	2100	$> 10^{4}$	
4e	\sim	1	> 10 ⁴	40	> 10 ⁴	$> 10^{4}$	$> 10^{4}$	
4f	\sim	1	> 10 ⁴	13	> 10 ⁴	$> 10^{4}$	$> 10^4$	
4g	\sim	1	> 10 ⁴	580	2100	$> 10^{4}$	$> 10^4$	
4h		1	> 10 ⁴	32	> 10 ⁴	$> 10^{4}$	$> 10^4$	
4i	$\sim\sim$	1	> 10 ⁴	36	$> 10^{4}$	> 10 ⁴	> 10 ⁴	

CO₂H

8	\sim	3	> 10 ⁴	3300	> 10 ⁴	> 10 ⁴	> 10 ⁴	$> 10^4$
4c	\sim	1	780	43	2000	$> 10^4$	> 10 ⁴	71
4d	\sim	1	1600	20	830	2100	> 10 ⁴	130
4 e	\sim	1	> 10 ⁴	40	> 10 ⁴	$> 10^4$	> 10 ⁴	45
4f	\sim	1	> 10 ⁴	13	> 10 ⁴	> 10 ⁴	> 10 ⁴	6.0
4g		1	> 10 ⁴	580	2100	> 10 ⁴	> 10 ⁴	N.T.
4h	~⁄/	1	> 10 ⁴	32	> 10 ⁴	> 10 ⁴	> 10 ⁴	12
4i	$\sim \sim$	1	> 10 ⁴	36	> 10 ⁴	$> 10^4$	> 10 ⁴	36
4j		1	> 10 ⁴	360	> 10 ⁴	$> 10^4$	N.T.	N.T.
4k	F	1	> 10 ⁴	410	> 10 ⁴	> 10 ⁴	N.T.	N.T.
41	CI	1	870	140	> 10 ⁴	> 10 ⁴	N.T.	N.T.
4m	ОМе	1	> 10 ⁴	470	> 10 ⁴	> 10 ⁴	N.T.	N.T.
4n	$\widehat{}$	1	2100	2300	1800	$> 10^{4}$	> 10 ⁴	N.T.

 $> 10^{4}$

1200

1300

 $> 10^{4}$

1

ity, EC_{50} values were investigated for the analogues ability to increase cyclic AMP (cAMP) production in Chinese hamster overy (CHO) cells expressing mouse (m) EP2-receptors.⁴ Among those tested, PG analogues **4a,b,e,f,h,6a,b,e,f** and **6h** were found to be potent selective EP2-receptor agonists.

In the process of our screening for an EP2 agonist, we focused on butaprost 1a. This compound is known to be a selective agonist. However, the corresponding carboxylic acid 1b, which is an active metabolite, was found to have an affinity for IP-receptor other than EP2-receptor. The IP-receptor agonist activity was potent (EC₅₀ = 25 nM) in spite of its weak binding affinity. First, we tried chemical modification to reduce its affinity for IP-receptor. We focused on the biological profiles of PGE_2 (3a) and PGE_1 (3b). Although these compounds demonstrate similar EP2-receptor agonist activities, there was a marked difference in the agonist activities for IP-receptor. Compound 4b was designed on the basis of structural hybridization of 1a and 3a (Scheme 1). This compound showed excellent selectivity (Table 2). Figure 2 shows the effects of 1b, 2, 4b and PGE₂ (3a) on intracellular cAMP using mouse EP2receptor expressing cells. Compound 4b stimulated cAMP production in a dose-dependent manner, and the EC_{50} value was 43 nM. The agonist activity was more potent than that of 2 and less potent than PGE₂.

Based on this information, modification of the α -chain in **1b** was conducted. Compound **5**, in which the double bond of **4b** was replaced by a triple bond, demonstrated a lesser affinity for the EP2-receptor ($K_i = 1100 \text{ nM}$) while the EP2-receptor selectivity seemed to be retained. Another successful optimization of α -chain was accomplished via the introduction of a *p*-substituted phenylene moiety into the α -chain of **1b**. The 1,6-inter-*p*-phenylene derivative,²² which is used to block β -oxdative breakdown of the α -chain, **6b** also demonstrated excellent

Table 4. Optimization of the alkyl group at position-17

EP2-receptor selectivity in the binding affinity and potent agonist activity. Although **6b** showed a higher K_i value than **4b**, they showed the nearly same potency in agonist activity (Table 2).

Using one of the optimized α -chains, further optimization of the 17,17-trimethylene moiety was attempted. As shown in Table 3, the activity was maximized in the smaller cycloalkyl derivative **4b**, while EP2-receptor selectivity was retained even by the larger cycloalkyl derivatives **7** and **8**. The importance of the configuration of the hydroxy group at C-16²³ (PG-numbering) of **4b** on activity and EP2-receptor selectivity was also investigated. 16-Epimer **9** exhibited less potent activity compared to **4b**. EP2-receptor selectivity of **9** was also decreased since **9** exhibited weak affinity to the EP3receptor ($K_i = 1.2 \mu M$).

Optimization of the 17-alkyl moiety of both **4b** and **6b** was attempted. As illustrated in Table 3, the agonist activity was maximized in the shortest alkyl chain derivative **4a** among the compounds **4a–d**, while their K_i



Figure 2. Effect of compounds on cAMP accumulation in CHO cells expressing mouse EP2-receptor.



		Binding <i>K</i> _i (nM)						
mEP1	mEP2	mEP3	mEP4	hIP	mEP2			
$> 10^4$	19	$> 10^{4}$	$> 10^{4}$	$> 10^4$	24			
$> 10^{4}$	25	> 10 ⁴	$> 10^4$	> 10 ⁴	54			
> 10 ⁴	42	$> 10^4$	> 10 ⁴	> 10 ⁴	260			
> 10 ⁴	6.1	$> 10^4$	> 10 ⁴	> 10 ⁴	26			
> 10 ⁴	9.7	> 10 ⁴	> 10 ⁴	> 10 ⁴	37			
	mEP1 > 104 > 104 > 104 > 104 > 104	mEP1 mEP2 > 10^4 19 > 10^4 25 > 10^4 42 > 10^4 6.1 > 10^4 9.7	mEP1 mEP2 mEP3 > 10^4 19 > 10^4 > 10^4 25 > 10^4 > 10^4 42 > 10^4 > 10^4 6.1 > 10^4 > 10^4 9.7 > 10^4	mEP1 mEP2 mEP3 mEP4 > 10^4 19 > 10^4 > 10^4 > 10^4 25 > 10^4 > 10^4 > 10^4 42 > 10^4 > 10^4 > 10^4 6.1 > 10^4 > 10^4 > 10^4 9.7 > 10^4 > 10^4	mEP1mEP2mEP3mEP4hIP> 10^4 19> 10^4 > 10^4 > 10^4 > 10^4 25> 10^4 > 10^4 > 10^4 > 10^4 42> 10^4 > 10^4 > 10^4 > 10^4 6.1> 10^4 > 10^4 > 10^4 > 10^4 9.7> 10^4 > 10^4 > 10^4			

values did not always correspond to the potency of their agonist activity. The 17-isobutyl derivative 4e demonstrated nearly the same K_i value as the 17-butyl derivative 4c, while the agonist activity of 4e was somewhat more potent than that of 4c. Compounds 4c and 4d were less selective EP2-receptor agonists compared with 4a and 4b (Table 3), because these exhibited weak affinity for the other receptors (EP1 and EP3). The 17cyclopropylmethyl derivative 4f exhibited the most potent K_i value among this series of compounds. The agonist activity of **4f** was also the most potent among the tested compounds. Terminal alkene derivatives 4g-iwere prepared and evaluated. The 17-vinyl derivative 4g exhibited an unexpectedly weak K_i value. The K_i values of 4h and 4i were nearly same, while the agonist activity of 4h was 3 times more potent than that of 4i. The alkyne derivative 4j demonstrated weak affinity for the EP2-receptor. Introduction of fluoro, chloro and methoxy groups into the terminal position (C-20) in 4c produced 4k, 4l and 4m, respectively, with a marked loss of EP2-receptor affinity. Replacement of the cyclopropyl moiety of 4f with a phenyl group and a cyclohexyl group yielded the 17-phenylmethyl analogue 4n and the 17-cyclohexylmethyl analogue 40, respectively, with a marked reduction in EP2-receptor affinity.

Chemical modification of the ω -chain in another series of 1,6-inter-*p*-phenylene analogues **6b** was carried out and biologically evaluated (Table 4). Replacement of the 17-propyl moiety in **6b** with ethyl, isobutyl, allyl, and cyclopropylmethyl groups yielded **6a,e,f** and **6h**, respectively. Although the 17-ethyl derivative **6a** exhibited the most potent agonist activity (EC₅₀=24 nM) among them, its EP2-receptor affinity did not always correspond to its agonist activity.

Summary

We have found a new series of highly selective EP2receptor agonists with a 16-hydroxy 17,17-trimethylene moiety derived from butaprost **1a**. A number of derivatives that consist of two different types of α -chain, most notably **4a,b,e,f,h**, **6a,b,e,f** and **6h** were highly selective EP2-receptor agonists. The findings from this study confirm the importance of the *cis* double bond or aromatic ring such as those illustrated in **4a–o**, **6a,b,e,f** and **6h** for the EP2-receptor selectivity. These novel highly selective EP2-receptor agonists are suitable for in vivo studies.

Experimental

General directions

All ¹H NMR spectra were obtained using a Varian Gemini-200, VXR-200s or Mercury300 spectrometer. Mass spectra were obtained on a Hitachi M1200H, JEOL JMS-DX303HF or PerSeptive Voyager Elite spectrometer. IR spectra were measured on a Perkin-Elmer FT-IR 1760X or Jasco FT/IR-430 spectrometer. Elemental analyses for carbon, hydrogen, nitrogen and

sulfur were carried out on a Perkin-Elmer PE2400 SeriesII CHNS/O analyzer. Optical rotations were measured using a Jasco DIP-1000 polarimeter. Column chromatography was carried out on silica gel [Merck silica gel 60 (0.063–0.200 mm) or Wako Gel C200]. Thin layer chromatography was performed on silica gel (Merck TLC or HPTLC plates, silica gel 60 F_{254}).

2,2-Trimethylene-1-pentanol (13b). To a stirred solution of lithiumdiisopropylamide (200 mmol) in THF (200 mL) was added cyclobutanecarboxylic acid (9.6 mL, 100 mmol) at $0 \,^{\circ}$ C under an argon atmosphere, and the stirring was continued for 2 h at room temperature. After the addition of *n*-propyliodide (11.4 mL, 100 mmol) at $0 \,^{\circ}$ C, the reaction mixture was stirred for 12 h at room temperature, then poured into 2 mol/L HCl (300 mL) and extracted with EtOAc. The aqueous layer was extracted with BtioAc. The combined organic layers were washed with brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated to give 2,2-trimethylene pentanoic acid as an oil.

To a stirred suspension of lithium aluminum hydride (7.60 g, 200 mmol) in ether (250 mL) was added 2,2-trimethylene pentanoic acid (100 mmol) described above in ether (100 mL) at 0 °C under an argon atmosphere. After stirring for 3.5 h at room temperature, the reaction mixture was diluted with ether (200 mL) and quenched with saturated aqueous sodium sulfate (40 mL) under cooling. The resulting mixture was stirred for additional 1 h at room temperature. Insoluble substances were removed by filtration and the filtrate was evaporated to give a crude product, which was purified by column chromatography on silica gel (EtOAc/n-hexane) to give 13b (100 mmol, quant) as an oil. TLC R_f 0.53 (*n*-hexane/EtOAc, 4/1); ¹H NMR (200 MHz, $CDCl_3$) δ 3.54 (d, J = 5.4 Hz, 2H), 1.95–1.15 (m, 11H), 0.92 (t, J=6.9 Hz, 3H). Compounds 13a, 13c-f, 13h, 13j, 13l, 13n, 13o, 14 and 15 were synthesized from 10, 11 and 12, respectively, according to the same procedure described above.

2,2-Trimethylene-4-(tetrahydro-2*H*-pyran-2-yloxy)-1-butanol (22). To a stirred solution of lithiumdiisopropylamide (200 mmol) in THF (200 mL) was added cyclobutanecarboxylic acid (9.6 mL, 100 mmol) at 0 °C under an argon atmosphere, and the stirring was continued for 2 h at room temperature. After the addition of 2-iodo ethyl tetrahydropyranyl ether (24.3 g, 95.0 mmol) at 0 °C, the reaction mixture was stirred for 12 h at room temperature, then poured into 2 mol/L HCl (300 mL) and extracted with EtOAc. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo to give 2,2-trimethylene-4-(tetrahydro-2*H*-pyran-2-yloxy)butanoic acid as an oil.

To a stirred solution of the above-described 2,2-trimethylene-4-(tetrahydro-2*H*-pyran-2-yloxy)butanoic acid (95.0 mmol) in 100 mL of THF was added borane-tetrahydrofuran complex (1.0 mol/L in THF, 160 mL, 160 mmol) at 0 °C under argon atmosphere. After stirring

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for 6 h at room temperature, the reaction mixture was quenched with aqueous hydrochloric acid and then extracted with EtOAc. The organic layer was washed with water, then brine, dried over magnesium sulfate and concentrated in vacuo to yield 11.2 g (55% yield in two steps) of 2,2-trimethylene-4-(tetrahydro-2*H*-pyran-2-yloxy)-1-butanol **22** as an oil. TLC R_f 0.19 (*n*-hexane/EtOAc, 4/1); ¹H NMR (200 MHz, CDCl₃) δ 4.62–4.56 (m, 1H), 3.90–3.35 (m, 4H), 3.56 (d, J=7.5 Hz, 2H), 1.95–1.47 (m, 14H).

2,2-Trimethylene-1-benzoyloxy-4-iodobutane (23). To a stirred solution of 22 (5.33 g, 24.9 mmol) and pyridine (6.04 mL, 74.6 mmol) in 20 mL of methylenechloride was added benzoyl chloride (3.83 mL, 33.0 mmol) at 0°C under argon atmosphere. After stirring for 12 h at room temperature, the reaction mixture was diluted with ether and washed sequentially with 1 N HCl aq, saturated aqueous sodium bicarbonate and water. The organic layer was dried over magnesium sulfate and concentrated in vacuo to afford a benzoyl ester. To a stirred solution of this benzoyl ester in methanol (50 mL) was added *p*-toluenesulfonic acid (380 mg) and the stirring was continued for 2 h at room temperature. The resulting mixture was quenched with saturated aqueous sodium bicarbonate and extracted with EtOAc. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a crude product, which was purified by column chromatography on silica gel to give 1-benzoyloxy-2,2-(trimethylene)butan-4-ol (4.82 g, 83%) as an oil. TLC R_f 0.48 (*n*-hexane/ EtOAc, 1/1); ¹H NMR (200 MHz, CDCl₃) δ 8.04 (d, J = 6.5 Hz, 2H), 7.60–7.40 (m, 3H), 4.33 (s, 2H), 3.77 (t, J = 7.0 Hz, 2H), 2.03–1.80 (m, 8H).

To a stirred solution of 1-benzoyloxy-2,2-(trimethylene)butan-4-ol (4.82 g, 20.6 mmol), imidazole (3.41 g, 50.0 mmol) and triphenylphosphine (13.1 g, 50.0 mmol) in 100 mL of benzene was added iodine (12.7 g, 50.0 mmol) at 0 °C under argon atmosphere. After stirring for 15 min at room temperature, the reaction mixture was quenched with saturated aqueous sodium thiosulfate and extracted with *n*-hexane. The organic layer was washed with saturated aqueous ammonium chloride, dried over magnesium sulfate and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel to yield **23** (6.73 g, 95%) as an oil. TLC R_f 0.67 (*n*-hexane/EtOAc, 4/1); ¹H NMR (200 MHz, CDCl₃) δ 8.05–8.01 (m, 2H), 7.65–7.40 (m, 3H), 4.30 (s, 2H), 3.21–3.13 (m, 2H), 2.16–1.90 (m, 8H).

2,2-Trimethylene-3-butene-1-ol (13g). A solution of **23** (6.73 g, 19.6 mmol) and diazabicycloundecene (3.80 mL, 25.4 mmol) in 20 mL of benzene was stirred for 4 h at 80 °C under argon atmosphere. After cooling in an icebath, the reaction mixture was quenched with 2 N HCl aq and extracted with ether. The organic layer was washed with saturated aqueous ammonium chloride, dried over magnesium sulfate and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel to give 1.70 g of olefin as an oil. To a stirred solution of this olefin in 20 mL of methanol, 2 N aqueous sodium hydroxide was

added. After stirring for 30 min at room temperature, the reaction was quenched with saturated aqueous ammonium chloride and extracted with ether. The organic layer was washed with saturated aqueous ammonium chloride, dried over magnesium sulfate and concentrated in vacuo to yield 13g as an oil.

4,4-Trimethylene-5-(tetrahydro-2H-pyran-2-yloxy)pentan-1-ol (24). To a stirred solution of 13h (12.6 g, 100 mmol), 2,3-dihydropyran (10.9 mL, 120 mol) in dichloromethane (100 mL) was added *p*-toluenesulfonic acid (190 mg, 1.0 mmol) at 0°C, and the stirring was continued for 1 h. Saturated aqueous sodium bicarbonate was added to the resulting mixture. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel (*n*-hexane to 20% EtOAc/*n*-hexane) to afford tetrahydropyranyl (THP) ether (21.0 g, quant) as an oil. TLC R_f 0.57 (*n*-hexane/EtOAc, 9/1); ¹H NMR $(200 \text{ MHz}, \text{ CDCl}_3) \delta 5.81 \text{ (ddt, } J = 16.4, 11.0, 7.4 \text{ Hz},$ 1H), 5.12–4.95 (m, 2H), 4.59 (t, J = 3.3 Hz, 1H), 3.95– 3.80 (m, 1H), 3.68 (d, J=9.6 Hz, 1H), 3.58–3.45 (m, 1H), 3.21 (d, J=9.6 Hz, 1H), 2.26 (d, J=7.4 Hz, 2H), 2.00–1.40 (m, 12H).

To a stirred solution of the resulting THP ether in THF (100 mL) was slowly added borane-tetrahydrofuran complex (1.0 mol/L in THF, 100 mL, 100 mmol) at 0 °C under argon atmosphere, and the stirring was continued for 1.5 h at room temperature. To the resulting mixture, 20 mL of water, 25 mL of 2 N aqueous sodium hydroxide and then 10 mL of 31% hydrogen peroxide at 0°C were slowly added. After stirring for 1 h at room temperature, the reaction mixture was extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo to give a crude oil, which was purified by column chromatography on silica gel (20% EtOAc/*n*-hexane to 50% EtOAc/n-hexane) to yield 24 (16.6 g, 73% yield) as a colorless oil. TLC R_f 0.18 (*n*-hexane/ EtOAc, 4/1); ¹H NMR (200 MHz, CDCl₃) δ 4.62–4.55 (m, 1H), 3.95– 3.80 (m, 1H), 3.71 (d, J=9.6 Hz, 1H), 3.70–3.60 (m, 2H), 3.60–3.46 (m, 1H), 3.25 (d, J=9.6 Hz, 1H), 2.00– 1.40 (m, 17H).

2,2-Trimethylene-5-hexen-1-ol (13i). To a stirred solution of oxalyl chloride (3.49 mL, 40.0 mmol) in methylene chloride (50 mL) was slowly added a solution of dimethylsulfoxide (5.68 mL, 80.0 mmol) in methylene chloride at -78 °C under argon atmosphere. After stirring for 10 min, a solution of **24** (4.56 g, 20.0 mmol) in methylene chloride (30 mL) was added to the reaction mixture, which was warmed up to -40 °C over 30 min and then treated with triethylamine (16.2 mL, 160 mmol). After stirring for 1 h at 0 °C, the reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with water, then brine, dried over magnesium sulfate and concentrated in vacuo to yield the corresponding aldehyde as an oil.

To a stirred solution of methyl triphenyl phosphonium bromide (11.8 g, 33.0 mmol) in THF (80 mL) was added

n-butyllithiun (1.6 mol/L in *n*-hexane, 18.8 mL, 30.0 mmol) at 0 $^{\circ}$ C under argon atmosphere. After stirring for 0.5 h, a solution of the above-described aldehyde in THF (20 mL) was added to the reaction mixture. After stirring for another 0.5 h, the resulting mixture was treated with saturated aqueous ammonium chloride and extracted with EtOAc. The organic layer was dried over magnesium sulfate and concentrated in vacuo. The residue was purified by short column chromatography on silica gel to give an olefin as an oil.

To a stirred solution of this olefin in methanol (30 mL) was added *p*-toluenesulfonic acid (150 mg) and the stirring was continued for 2 h at room temperature. The resulting mixture was quenched with saturated aqueous sodium bicarbonate, evaporated to remove methanol and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified by column chromatography on silica gel to yield **13i** (2.25 g, 80% yield in three steps) as a colorless oil. TLC R_f 0.38 (*n*-hexane/EtOAc, 4/1); ¹H NMR (200 MHz, CDCl₃) δ 5.85 (ddt, J=17.1, 10.3, 6.2 Hz, 1H), 5.10–4.90 (m, 2H), 3.56 (bs, 2H), 2.10–1.53 (m, 11H).

2,2-Trimethylene-5-fluoropentan-1-ol (13k). To a stirred solution of 24 (4.57 g, 20 mmol) in pyridine (20 mL) was added p-toluene sulfonyl chloride (4.77 g, 25 mmol) and the stirring was continued for 2 h at room temperature under argon atmosphere. The reaction was quenched with water (1 mL) and stirred for 5 min. After addition of saturated aqueous sodium bicarbonate, the reaction mixture was extracted with n-hexane/EtOAc. The organic layer was washed with water, then brine, dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a tosylate as an oil. A mixture of the tosylate and a solution of tetra-n-butylammonium fluoride in THF (1.0 mol/l, 40 mL) was stirred for 12 h. The resulting mixture was diluted with EtOAc, washed with water, dried over anhydrous magnesium sulfate and concentrated in vacuo to give a crude product. To a stirred solution of the crude product in methanol (50 mL), p-toluenesulfonic acid (200 mg) was added. Stirring was continued for 3 h at room temperature. The reaction was quenched with saturated aqueous sodium bicarbonate. The mixture was extracted with EtOAc after removal of methanol by evaporation. The organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel to yield 13k (2.07 g, 71%) as a colorless oil. TLC R_f 0.36 (*n*-hexane/EtOAc, 3/1); ¹H NMR (200 MHz, CDCl₃) δ 4.46 (dt, J=47.4, 6.0 Hz, 2H), 3.57 (brs, 2H), 2.00–1.53 (m, 11H).

2,2-Trimethylene-5-methoxypentan-1-ol (13m). To a stirred solution of **24** (4.57 g, 20 mmol) in THF (50 mL) was added sodium hydride (60% oil dispersion, 960 mg, 24 mmol) in several portions at $0 \,^{\circ}$ C under argon atmosphere, and the resulting suspension was stirred for 2 h at room temperature. After the addition of methyl iodide (1.87 mL, 30 mmol) at $0 \,^{\circ}$ C, the reaction mixture

was stirred for 12 h, quenched with saturated aqueous ammonium chloride and extracted with EtOAc. The organic layer was washed with water, then brine, dried over anhydrous magnesium sulfate and concentrated in vacuo to give a methyl ether as an oil. To a stirred solution of the above oily product in methanol (50 mL) was added *p*-toluenesulfonic acid (100 mg). The reaction mixture was stirred for 3 h at room temperature and quenched with saturated aqueous sodium bicarbonate. After removal of methanol by evaporation, the reaction mixture was extracted with EtOAc. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel to yield 13m (3.00 g, 95%) as a colorless oil. TLC R_f 0.18 (*n*-hexane/EtOAc, 3/1); ¹H NMR (200 MHz, CDCl₃) δ 3.55 (brs, 2H), 3.44-3.34 (m, 2H), 3.34 (s, 3H), 2.00-1.50 (m, 11H).

4-(t-Butyldimethylsiloxy)-5,5-trimethylene-1-octyne (16b). To a stirred mixture of oxalyl chloride (13.1 mL, 150 mmol) in methylene chloride (300 mL) was added a solution of dimethylsulfoxide (21.3 mL, 300 mmol) in methylene chloride (50 mL) at -78 °C under an argon atmosphere, and stirring was continued for 10 min. To the mixture, 2,2-trimethylene-1-pentanol 13b (100 mmol) in methylene chloride (50 mL) was added. After being warmed up to $-40 \,^{\circ}$ C over 30 min, the reaction mixture was treated with triethylamine (74 mL, 531 mmol), warmed to 0 °C over 1 h, and diluted with aqueous ether. The organic layer was washed with diluted hydrochloric acid. The aqueous layer was repeatedly extracted with ether. The combined organic layers were washed with water, then brine, dried over anhydrous magnesium sulfate and concentrated under reduced pressure to give a crude product, which was distilled (63-66 °C/26-23 mmHg) to give 2,2-trimethylene-1-pentanal (7.38 g, 59%) as an oil. ¹H NMR (200 MHz, CDCl₃) δ 9.58 (s, 1H), 2.38–2.18 (m, 2H), 2.00–1.66 (m. 6H), 1.30–1.10 (m, 2H), 0.91 (t, J = 7.2 Hz, 3H).

To a stirred suspension of magnesium turnings (3.13 g, 128.7 mmol) and mercuric chloride (32 mg, 0.117 mmol) in ether (10 mL) was slowly added propargyl bromide (10.4 mL, 117 mmol) in ether (90 mL) at 0 °C, and stirring was continued for 10 min under argon atmosphere. The mixture was treated with 2,2-trimethylene-1-pentanal (7.38 g, 58.5 mmol) in ether (50 mL), stirred for 1 h at room temperature and then quenched with 2 N HCl aq and extracted with EtOAc. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate and concentrated to give a crude product, which was purified by column chromatography (7%) EtOAc/n-hexane to 10% EtOAc/n-hexane) to yield 4-hydroxy-5,5-trimethylene-1-octyne (8.23 g, 85%) as a yellow oil. TLC R_f 0.69 (*n*-hexane/EtOAc, 4/1); ¹H NMR (200 MHz, $CDCl_3$) δ 3.72 (dt, J=9.0, 3.2 Hz, 1H), 2.37 (ddd, J = 16.8, 3.2, 2.6 Hz, 1H), 2.21 (ddd, J = 16.8, 9.0, 2.6 Hz, 1H), 2.15–1.95 (m, 3H), 2.05 (t, J = 2.6 Hz, 1H), 1.95–1.20 (m, 8H), 0.93 (t, J = 6.9 Hz, 3H).

To a stirred solution of 4-hydroxy-5,5-trimethylene-1octyne (6.23 g, 37.5 mmol) and 2,6-lutidine (8.7 mL, 75.0 mmol) in methylene chloride (100 mL) was slowly added *t*-butyldimethylsilyloxy trifluoromethanesulfonate (10.3 mL, 45.0 mmol) at 0°C under an argon atmosphere, and stirring was continued for 10 min at room temperature. The reaction mixture was quenched with saturated aqueous sodium bicarbonate and extracted with EtOAc. The organic layer was dried over anhydrous magnesium sulfate and concentrated to give a crude product, which was purified by column chromatography (n-hexane to 3% EtOAc/n-hexane) to yield 4-(*t*-butyldimethylsiloxy)-5,5-trimethylene-1-octyne **16b** (9.89 g, 94%) as a colorless oil. TLC R_f 0.64 (*n*-hexane); ¹H NMR (200 MHz, CDCl₃) δ 3.76 (t, J=5.3 Hz, 1H), 2.30 (ddd, J=17.2, 5.0, 2.8 Hz, 1H), 2.17 (ddd, J=17.2, 6.1, 2.8 Hz, 1H), 2.12–1.20 (m, 10H), 1.93 (t, J = 2.8 Hz, 1H), 0.98–0.85 (m, 3H), 0.90 (s, 9H), 0.13 (s, 3H), 0.08 (s, 3H).

(E)-1-Iodo-4-(t-butyldimethylsiloxy)-5.5-trimethylene-1octene (19b). Method A. To a stirred solution of 4-(tbutyldimethylsiloxy)-5,5-trimethylene-1-octyne 16b (3.79 g, 13.5 mmol) in toluene (30 mL) and THF (30 mL) was added zirconocene chloride hydride (3.48 g, 13.5 mmol). Stirring was continued for 15 min at room temperature. To the stirred reaction mixture, zirconocene chloride hydride (3.48 g, 13.5 mmol) and then a solution of iodine (3.43 g, 13.5 mmol) in THF (15 mL) 15 min later were added. After 10 min, the resulting red solution was diluted with *n*-hexane (100 mL). Insoluble substances were removed by filtration. The filtrate was concentrated in vacuo to give a crude oil, which was purified by column chromatography on silica gel using *n*-hexane as an eluent to yield (E)-1-iodo-4-(t-butyldimethylsilyloxy)-5,5-trimethylene-1-octene **19b** (4.73 g, 86%) as a colorless oil.

Method B. To a stirred mixture of 16b (1.64 g, 5.85 mmol) and tributyltin hydride (1.70 mL, 6.44 mmol) was added azobisisobutylonitrile (47 mg, 0.29 mmol) and the reaction mixture was stirred with heating at 80 °C for 2 h under an argon atmosphere. After cooling in an ice-bath, the reaction mixture was diluted with ether (20 mL) and washed with saturated aqueous sodium bicarbonate (20 mL). To the reaction mixture, iodine (1.63 g, 6.44 mmol) in ether (40 mL) was added at 0°C and the mixture was stirred for 10 min. The reaction mixture was diluted with EtOAc. The organic layer was washed with saturated aqueous sodium thiosulfate, 3% aqueous potassium fluoride, then brine, dried over anhydrous magnesium sulfate and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel using *n*-hexane as an eluent to yield **19b** (2.01 g, 76%) as a colorless oil. TLC R_f 0.77 (*n*-hexane); ¹H NMR $(200 \text{ MHz}, \text{CDCl}_3) \delta 6.50 \text{ (dt}, J = 14.8, 7.4 \text{ Hz}, 1\text{H}), 5.97$ (dt, J = 14.8, 1.3 Hz, 1H), 3.59 (dd, J = 6.0, 4.6 Hz, 1H),2.20-1.20 (m, 12H), 0.98-0.85 (m, 3H), 0.91 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H). Compounds 19a, 19c-o, 20 and 21 were synthesized from 13a, 13c-o, 14 and 15, respectively, according to the same procedure as described above.

(*E*)-1-Iodo-4-(*t*-butyldimethylsiloxy)-5,5-trimethylene-1heptene (19a). Method B. TLC R_f 0.86 (*n*-hexane); ¹H NMR (200 MHz, CDCl₃) δ 6.50 (ddd, J=14.4, 8.2, 7.6 Hz, 1H), 5.97 (d, J=14.4 Hz, 1H), 3.60 (dd, J=6.0, 5.0 Hz, 1H), 2.30–1.20 (m, 10H), 0.95–0.85 (m, 3H), 0.91 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H).

(*E*)-1-Iodo-4-(*t*-butyldimethylsiloxy)-5,5-trimethylene-1nonene (19c). Method B. TLC R_f 0.84 (*n*-hexane); ¹H NMR (200 MHz, CDCl₃) δ 6.50 (dt, J=14.4, 8.2 Hz, 1H), 5.97 (dt, J=14.4, 1.2 Hz, 1H), 3.59 (dd, J=6.0, 4.6 Hz, 1H), 2.30–1.20 (m, 14H), 1.00–0.80 (m, 3H), 0.91 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H).

(*E*)-1-Iodo-4-(*t*-butyldimethylsiloxy)-5,5-trimethylene-1decene (19d). Method B. TLC R_f 0.84 (*n*-hexane); ¹H NMR (200 MHz, CDCl₃) δ 6.50 (dt, J=14.4, 8.0 Hz, 1H), 5.97 (dt, J=14.4, 1.2 Hz, 1H), 3.59 (dd, J=6.0, 5.0 Hz, 1H), 2.30–1.20 (m, 16H), 1.00–0.80 (m, 3H), 0.91 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H).

(*E*)-1-Iodo-4-(*t*-butyldimethylsiloxy)-7-methyl-5,5-trimethylene-1-octene (19e). Method B. MS (FAB, Pos) m/z365 (M+H-t-C₄H₉)⁺; ¹H NMR (200 MHz, CDCl₃) δ 6.51 (ddd, J=14.4, 8.0, 7.4 Hz, 1H), 5.97 (dt, J=14.4, 1.2 Hz, 1H), 3.69 (dd, J=6.4, 4.2 Hz, 1H), 2.30–1.20 (m, 11H), 0.95–0.85 (m, 6H), 0.92 (s, 9H), 0.08 (s, 6H).

(E)-1-Iodo-4-(t-butyldimethylsiloxy)-6-cyclopropyl-5,5trimethylene-1-hexene (19f). Method A. TLC R_f 0.70 (nhexane); Pos, 20V) m/zMS (APCI, 289 $(M+H-TBSOH)^+$; ¹H NMR (200 MHz, CDCl₃) δ 6.49 (dt, J=14.4, 7.6 Hz, 1H), 5.97 (dt, J=14.4, 1.2 Hz, 1H), 3.71 (dd, J=6.4, 4.6 Hz, 1H), 2.20-1.60 (m, 8H), 1.62 (dd, J=14.2, 6.2 Hz, 1H), 1.18 (dd, J = 14.2, 7.1 Hz, 1H), 1.00–0.70 (m, 1H), 0.90 (s, 9H), 0.52-0.42 (m, 2H), 0.10-0.00 (m, 2H), 0.07 (s, 3H), 0.06 (s, 3H).

(1*E*)-1-Iodo-4-(*t*-butyldimethylsiloxy)-5,5-trimethylene-1,6-heptadiene (19g). Method A. TLC R_f 0.56 (*n*-hexane); MS (FAB, Pos) m/z 335 (M+H-t-C₄H₉)⁺; ¹H NMR (200 MHz, CDCl₃) δ 6.43 (dt, J=14.4, 7.6 Hz, 1H), 5.97 (dd, J=17.0, 11.0 Hz, 1H), 5.93 (dt, J=14.4, 1.2 Hz, 1H), 5.17 (dd, J=11.0, 1.6 Hz, 1H), 5.14 (dd, J=17.0, 1.6 Hz, 1H), 3.60 (dd, J=6.8, 4.6 Hz, 1H), 2.10–1.60 (m, 8H), 0.92 (s, 9H), 0.05 (s, 6H).

(1*E*)-1-Iodo-4-(*t*-butyldimethylsiloxy)-5,5-trimethylene-1,7-octadiene (19h). Method A. TLC R_f 0.70 (*n*-hexane); MS (APCI, Pos, 20V) m/z 275 (M + H-TBSOH)⁺; ¹H NMR (200 MHz, CDCl₃) δ 6.49 (dt, J = 14.4, 7.6 Hz, 1H), 6.00–5.78 (m, 1H), 5.98 (dt, J = 14.4, 1.3 Hz, 1H), 5.15–5.00 (m, 2H), 3.60 (dd, J = 6.0, 4.4 Hz, 1H), 2.45– 2.30 (m, 1H), 2.20–1.50 (m, 9H), 0.91 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H).

(1*E*)-1-Iodo-4-(*t*-butyldimethylsiloxy)-5,5-trimethylene-1,8-nonadiene (19i). Method A. TLC R_f 0.67 (*n*-hexane); MS (APCI, Pos, 20V) m/z 289 (M+H–TBSOH)⁺; ¹H NMR (200 MHz, CDCl₃) δ 6.50 (ddd, J=14.4, 8.2, 7.4 Hz, 1H), 5.99 (dt, J=14.4, 1.3 Hz, 1H), 5.85 (ddt, J=17.0, 10.2, 6.6 Hz, 1H), 5.10–4.90 (m, 2H), 3.62 (dd, *J* = 6.1, 4.7 Hz, 1H), 2.25–1.40 (m, 12H), 0.91 (s, 9H), 0.07 (s, 3H), 0.06 (s, 3H).

(*E*)-1-Iodo-4-(*t*-butyldimethylsiloxy)-5,5-trimethylenenon-1-en-7-yne (19j). Method B. TLC R_f 0.41 (*n*-hexane); MS (APCI, Pos, 20V) m/z 287 (M+H–TBSOH)⁺; ¹H NMR (200 MHz, CDCl₃) δ 6.51 (ddd, J=14.8, 8.0, 6.8 Hz, 1H), 5.99 (dt, J=14.8, 1.3 Hz, 1H), 3.73 (dd, J=6.4, 4.4 Hz, 1H), 2.50–1.55 (m, 10H), 1.80 (t, J=2.6 Hz, 3H), 0.90 (s, 9H), 0.07 (s, 3H), 0.06 (s, 3H).

(*E*)-1-Iodo-4-(*t*-butyldimethylsiloxy)-8-fluoro-5,5-trimethylene-1-octene (19k). Method B. TLC R_f 0.50 (*n*-hexane/EtOAc, 50/1); MS (APCI, Pos, 20V) *m*/*z* 295 (M+H-TBSOH)⁺; ¹H NMR (200 MHz, CDCl₃) δ 6.49 (dt, *J*=14.4, 7.2 Hz, 1H), 6.00 (dt, *J*=14.4, 1.4 Hz, 1H), 4.45 (dt, *J*=47.0, 5.8 Hz, 2H), 3.63 (dd, *J*=6.4, 4.6 Hz, 1H), 2.30–1.40 (m, 12H), 0.90 (s, 9H), 0.07 (s, 3H), 0.06 (s, 3H).

(*E*)-1-Iodo-4-(*t*-butyldimethylsiloxy)-8-chloro-5,5-trimethylene-1-octene (191). Method B. TLC R_f 0.54 (*n*-hexane); MS (APCI, Pos, 20V) m/z 313 (M+H-TBSOH)⁺; ¹H NMR (200 MHz, CDCl₃) δ 6.49 (dt, J=14.4, 7.4 Hz, 1H), 6.01 (d, J=14.4 Hz, 1H), 3.63-3.47 (m, 3H), 2.18–1.42 (m, 12H), 0.90 (s, 9H), 0.05 (s, 6H).

(*E*)-1-Iodo-4-(*t*-butyldimethylsiloxy)-8-methoxy-5,5-trimethylene-1-octene (19m). Method B. TLC R_f 0.31 (*n*-hexane/EtOAc, 50/1); MS (APCI, Pos, 20V) *m*/*z* 307 (M+H-TBSOH)⁺; ¹H NMR (200 MHz, CDCl₃) δ 6.49 (dt, *J*=14.4, 8.0 Hz, 1H), 5.98 (dt, *J*=14.4, 1.0 Hz, 1H), 3.61 (dd, *J*=6.0, 4.6 Hz, 1H), 3.45–3.30 (m, 2H), 3.35 (s, 3H), 2.20–1.30 (m, 12H), 0.90 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H).

(*E*)-1-Iodo-4-(*t*-butyldimethylsiloxy)-6-phenyl-5,5-trimethylene-1-hexene (19n). Method B. TLC R_f 0.45 (*n*-hexane); MS (APCI, Pos, 20V) m/z 325 (M+H-TBSOH)⁺; ¹H NMR (200 MHz, CDCl₃) δ 7.35-7.10 (m, 5H), 6.51 (dt, J=14.2, 6.6 Hz, 1H), 6.00 (d, J=14.2 Hz, 1H), 3.71 (dd, J=7.0, 4.6 Hz, 1H), 2.90 (d, J=13.0 Hz, 1H), 2.55 (d, J=13.0 Hz, 1H), 2.20–1.10 (m, 6H), 0.90 (s, 9H), 0.40 (s, 6H).

(*E*)-1-Iodo-4-(*t*-butyldimethylsiloxy)-6-cyclohexyl-5,5-trimethylene-1-hexene (190). Method B. TLC R_f 0.86 (*n*hexane); ¹H NMR (200 MHz, CDCl₃) δ 6.51 (ddd, J = 14.2, 8.0, 7.4 Hz, 1H), 5.97 (dt, J = 14.2, 1.2 Hz, 1H), 3.67 (dd, J = 6.2, 4.4 Hz, 1H), 2.35–0.80 (m, 21H), 0.92 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H).

(*E*)-1-Iodo-4-(*t*-butyldimethylsiloxy)-5,5-tetramethylene-1-octene (20). Method B. MS (APCI, Pos, 20V) m/z 291 (M+H-TBSOH)⁺; ¹H NMR (200 MHz, CDCl₃) δ 6.54 (ddd, J=14.6, 8.4, 6.8 Hz, 1H), 5.98 (dt, J=14.6, 1.4 Hz, 1H), 3.57 (dd, J=6.4, 3.8 Hz, 1H), 2.20–2.05 (m, 2H), 1.80–1.10 (m, 12H), 0.95–0.80 (m, 3H), 0.89 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H).

(*E*)-1-Iodo-4-(*t*-butyldimethylsiloxy)-5,5-pentamethylene-1-octene (21). Method B. MS (APCI, Pos, 20V) *m*/*z* 177 $(M + H - TBSOH-HI)^+$; ¹H NMR (200 MHz, CDCl₃) δ 6.54 (ddd, J = 14.4, 8.4, 6.8 Hz, 1H), 5.98 (dt, J = 14.4, 1.4 Hz, 1H), 3.54 (dd, J = 7.0, 3.8 Hz, 1H), 2.20–2.00 (m, 2H), 1.60–1.00 (m, 14H), 0.95–0.80 (m, 3H), 0.89 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H).

(16RS)-11-O-(t-Butyldimethylsilyl)-15-deoxy-16-(t-butyldimethylsilyloxy)-17,17-trimethylenePGE₂ methyl ester (26b). To a stirred solution of 19b (681 mg, 1.67 mmol) in 3 mL of dry ether was slowly added t-butyllithium (1.64 M in pentane, 2.04 mL, 3.34 mmol) at -78 °C under argon atmosphere, and stirring was continued for 1 h. To this mixture, lithium 2-thienylcyanocuprate (0.25 M in THF, 8.1 mL, 2.02 mmol) was slowly added. After stirring for 20 min at -78 °C, a solution of cyclopentenone 25 (350 mg, 0.994 mmol) in ether was slowly added. The reaction mixture was warmed up to 0°C over 1 h and then poured into a stirred heterogeneous mixture of *n*-hexane and saturated aqueous ammonium chloride. The organic layer was washed with brine, over anhydrous magnesium dried sulfate and concentrated to give a crude product, which was purified by column chromatography on silica gel (3%) EtOAc/n-hexane to 10% EtOAc/n-hexane) to yield 26b (419 mg, 66%) as a yellow oil. TLC $R_f 0.35$ (*n*-hexane/ EtOAc, 9/1); MS (EI, Pos) 577 (M-t-Bu)⁺, 445; IR (neat) 2955, 2857, 1746, 1405, 1252, 1094, 837, 775 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 5.73–5.50 (m, 1H), 5.42-5.25 (m, 3H), 4.08-3.93 (m, 1H), 3.66 (s, 3H), 3.58 (t, J=5 Hz, 1H), 2.70-1.20 (m, 24H), 1.00-0.85 (m, 21H), 0.10-0.00 (m, 12H).

(16S)-15-Deoxy-16-hydroxy-17,17-trimethylenePGE₂ (4b) and (16R)-15-deoxy-16-hydroxy-17,17-trimethylenePGE₂ (9). To a stirred solution of 26b (1130 mg, 1.78 mmol) in 40 mL of acetonitrile, 47% aqueous hydrogen fluoride (2.0mL) was added at 0 °C and stirring was continued for 1.5 h at room temperature. The resulting mixture was poured into saturated aqueous sodium bicarbonate–EtOAc. The aqueous layer was repeatedly extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo to give a crude product, which was purified by column chromatography (Lobar[®] pre-packed column, size B, 60% EtOAc/n-hexane) to yield a less polar product (344 mg, 48%) and a more polar product (332 mg, 46%). Less polar product (16*R*–OH); Colorless oil; TLC $R_f 0.37$ (*n*hexane/EtOAc, 2/3); MS (APCI, Pos, 20V) 389 $(M+H-H_2O)^+$, 371 $(M+H-2H_2O)^+$; IR (neat) 3436, 2954, 1741, 1436, 1245, 1158, 1077, 972 cm⁻¹; ¹H NMR $(200 \text{ MHz}, \text{ CDCl}_3) \delta 5.71 \text{ (ddd}, J=15.3, 7.6, 6.3 \text{ Hz},$ 1H), 5.54–5.26 (m, 3H), 4.18–4.00 (m, 1H), 3.67 (s, 3H), 3.55 (dd, J = 10.0, 2.4 Hz, 1H), 2.75 (ddd, J = 18.6, 7.2)1.0 Hz, 1H), 2.85-2.65 (br, 1H), 2.50-1.50 (m, 19H), 2.32 (t, J=7.5 Hz, 2H), 1.50–1.20 (m, 3H), 0.94 (t, J = 6.9 Hz, 3H). More polar product (16S–OH); colorless oil; TLC R_f 0.29 (*n*-hexane/EtOAc, 2/3); MS 389 (APCI, Pos. 20V) $(M + H - H_2O)^+$, 371 $(M+H-2H_2O)^+$; IR (neat) 3401, 2954, 1742, 1436, 1245, 1159, 1075, 968 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 5.69 (ddd, J=15.4, 8.2, 5.4 Hz, 1H), 5.49– 5.25 (m, 3H), 4.12-3.98 (m, 1H), 3.67 (s, 3H), 3.65-3.20 (br, 1H), 3.55 (dd, J=10.2, 2.4 Hz, 1H), 2.74 (ddd, J=18.4, 7.4, 1.0 Hz, 1H), 2.50–1.50 (m, 19H), 2.31 (t, J=7.3 Hz, 2H), 1.50–1.20 (m, 3H), 0.94 (t, J=6.9 Hz, 3H).

To a stirred solution of the more polar product (330 mg, 0.813 mmol) in 3 mL of ethanol and 30 mL of phosphate buffer (pH = 7.4), 800 μ L (1880 units) of aqueous (3.2 mol/L ammonium sulfate) suspension of porcine liver esterase (PLE) was added and stirring was continued for 6 h at room temperature. The resulting mixture was diluted with EtOAc. The organic layer was washed with water and brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified by column chromatography on silica gel to yield 4b (275 mg, 86%) as a pale yellow viscous oil. Optical rotation $[\alpha]_D^{25} = -69.4$ (c 0.79, EtOH); TLC R_f 0.36 (EtOAc/n-hexane/AcOH, 16/8/1); IR (neat) 3369, 2956, 2932, 2872, 1741, 1709, 1456, 1431, 1407, 1340, 1240, 1158, 1074, 969, 734 cm⁻¹; MS 40v) 391 (APCI. Neg. m/z $(M-H)^{-}$. 373 $(M-H_2O-H)^-$; ¹H NMR (200 MHz, CDCl₃) δ 5.68 (ddd, J=15.4, 8.2, 6.0 Hz, 1H), 5.50-5.25 (m, 3H),5.20–4.00 (br, 3H), 4.13–3.96 (m, 1H), 3.59 (dd, J=10.4, 2.2 Hz, 1H), 2.73 (ddd, J=18.6, 7.4, 1.0 Hz, 1H), 2.50-1.20 (m, 23H), 0.94 (t, J=6.7 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 214.77, 177.95, 132.41, 131.85, 130.79, 126.70, 75.96, 72.13, 54.63, 53.79, 46.37, 45.25, 38.44, 34.50, 33.19, 27.30, 27.04, 26.34, 24.97, 24.51, 17.42, 15.14, 14.83. Anal. calcd for C₂₃H₃₆O₅; C, 70.38; H, 9.24; Found; C, 69.97; H, 9.37.

Compound **9** was synthesized from the less polar product by the same procedure described above. Pale yellow viscous oil; TLC R_f 0.41 (EtOAc/*n*-hexane/AcOH, 16/8/1); IR (neat) 3418, 2932, 1734, 1406, 1241, 1158, 1075, 971 cm⁻¹; MS (APCI, Neg, 40v) *m*/*z* 391 (M–H)–, 373 (M–H₂O–H)⁻; ¹H NMR (200 MHz, CDCl₃) δ 5.74 (dt, *J*=15.0, 6.0 Hz, 1H), 5.55–5.25 (m, 3H), 4.08 (q, *J*=7.5 Hz, 1H), 3.64 (dd, *J*=10.5, 2.5 Hz, 1H), 2.75 (dd, *J*=18.0, 7.5 Hz, 1H), 2.50–2.20 (m, 7H), 2.20–1.20 (m, 18H), 0.94 (t, *J*=7.0 Hz, 3H). The compounds **4a** and **4c**–**o** were synthesized from **19a** and **19c**–**o** and **25**, respectively, according to the same procedure used for preparation of the compound **4b** described above.

(16*S*) - 15 - Deoxy - 16 - hydroxy - 17,17 - trimethylene - ω norPGE₂ (4a). Colorless oil; TLC R_f 0.39 (EtOAc/ AcOH, 50/1); IR (neat) 3391, 2936, 1740, 1714, 1461, 1431, 1406, 1339, 1241, 1159, 1073, 1028, 969, 914, 875, 734, 649 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 5.71 (ddd, J = 15, 8, 6 Hz, 1H), 5.49–5.29 (m, 3H), 5.20–4.40 (br, 3H), 4.11–3.98 (m, 1H), 3.60 (dd, J = 10, 2 Hz, 1H), 2.73 (ddd, J = 18, 7, 1 Hz, 1H), 2.45–1.35 (m, 19H), 2.33 (t, J = 7 Hz, 2H), 0.92 (t, J = 7 Hz, 3H); MS (FAB, Pos) m/z 379 (M+H)⁺, 361 (M+H–H₂O)⁺, 343, 325.

(16.5)-15-Deoxy-16-hydroxy-17,17-trimethylene-20-methyl-PGE₂ (4c). Pale yellow oil; TLC R_f 0.67 (EtOAc/AcOH, 50/1); IR (neat) 3392, 2931, 1732, 1405, 1242, 1159, 1075 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 5.90–4.80 (m, 7H), 4.10–3.98 (m, 1H), 3.56 (d, J=9 Hz, 1H), 2.72 (dd, J=18, 7 Hz, 1H), 2.47–1.15 (m, 23H), 2.30 (t, J=7 Hz, 2H), 0.90 (t, J=7 Hz, 3H); MS (EI, Pos) m/z 388 (M–H₂O)⁺, 370 (M–2H₂O)⁺, 266, 248.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-20-ethyl-PGE₂ (4d). Yellow oil; TLC R_f 0.73 (EtOAc/AcOH, 50/ 1); IR (neat) 3392, 2931, 1740, 1406, 1244, 1159, 1075 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 5.76–5.61 (m, 1H), 5.49–5.32 (m, 3H), 4.80–4.20 (bs, 3H), 4.11–3.98 (m, 1H), 3.59 (dd, J=10, 1 Hz, 1H), 2.73 (dd, J=18, 8 Hz, 1H), 2.45–1.15 (m, 25H), 2.35 (t, J=7 Hz, 2H), 0.90 (t, J=7 Hz, 3H); MS (FAB, Pos) m/z 421 (M+H)⁺, 403 (M+H–H₂O)⁺, 385, 367.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-19-methyl-PGE₂ (4e). Pale yellow oil; TLC R_f 0.24 (*n*-hexane/ EtOAc/AcOH, 1/2/0.03); IR (neat) 3369, 2952, 1741, 1709, 1407, 1241, 1158, 1075, 968 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 5.72 (ddd, J=15.2, 8.0, 5.8 Hz, 1H), 5.55–5.25 (m, 3H), 5.20–4.20 (br, 3H), 4.14–3.98 (m, 1H), 3.68 (dd, J=10.0, 2.0 Hz, 1H), 2.74 (ddd, J=18.0, 7.2, 1.0 Hz, 1H), 2.50–1.50 (m, 20H), 1.55 (dd, J=14.2, 7.2 Hz, 1H), 1.33 (dd, J=14.2, 6.4 Hz, 1H), 0.92 (d, J=6.4 Hz, 6H); MS (FAB, Pos. *m*/*z* 389 (M+H–H₂O)⁺, 371, 353.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene- ω -dinor-18-cyclopropylPGE₂ (4f). Pale yellow oil; TLC R_f 0.26 (*n*-hexane/EtOAc/AcOH, 1/2/0.03); IR (neat) 3369, 2932, 1741, 1430, 1241, 1158, 1075, 1019, 968 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) & 6.00–4.00 (br, 3H), 5.70 (ddd, J=15.4, 7.8, 5.6 Hz, 1H), 5.50–5.25 (m, 3H), 4.14–3.96 (m, 1H), 3.73 (dd, J=10.0, 2.0 Hz, 1H), 2.74 (dd, J=18.4, 7.6 Hz, 1H), 2.50–1.60 (m, 19H), 1.50 (dd, J=14.2, 6.8 Hz, 1H), 1.37 (dd, J=14.2, 6.3 Hz, 1H), 0.90–0.70 (m, 1H), 0.60–0.45 (m, 2H), 0.17–0.05 (m, 2H); MS (FAB, Pos.) m/z 405 (M+H)⁺, 387 (M+H–H₂O)⁺, 369, 351.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene- ω -nor-18,19-didehydroPGE₂ (4g). Colorless oil; TLC R_f 0.32 (EtOAc/AcOH, 50/1); IR (neat) 3392, 2942, 1732, 1636, 1412, 1242, 1160, 1074, 970, 914 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 5.93 (dd, J=17.2, 10.6 Hz, 1H), 5.65 (ddd, J=15.2, 8.2, 5.6 Hz, 1H), 5.28–5.15 (m, 3H), 5.25 (dd, J=10.6, 1.4 Hz, 1H), 5.16 (dd, J=17.2, 1.4 Hz, 1H), 5.10–4.10 (br, 3H), 4.08–3.95 (m, 1H), 3.63 (dd, J=10.6, 2.0 Hz, 1H), 2.70 (ddd, J=19.2, 7.6, 1.1 Hz, 1H), 2.42–1.60 (m, 19H); MS (FAB, Pos) m/z 377 (M+H)⁺, 359 (M+H–H₂O)⁺, 341, 323.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-19,20-didehydroPGE₂ (4h). Colorless oil; TLC R_f 0.21 (*n*-hexane/EtOAc/AcOH, 1/2/0.03); IR (neat) 3392, 2933, 1740, 1434, 1241, 1159, 1075, 998, 969, 914, 733 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 5.94 (ddt, J=17.2, 10.2, 7.2 Hz, 1H), 5.66 (ddd, J=15.2, 8.0, 5.6 Hz, 1H), 5.53– 5.25 (m, 3H), 5.30–4.50 (br, 3H), 5.20–5.00 (m, 2H), 4.12–3.96 (m, 1H), 3.58 (dd, J=10.2, 1.8 Hz, 1H), 2.72 (dd, J=18.2, 7.2 Hz, 1H), 2.50–1.60 (m, 21H); MS (EI, Pos) m/z 372 (M–H₂O)⁺, 354 (M–2H₂O)⁺, 248, 230. (16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-20-methylenePGE₂ (4i). Pale yellow oil; TLC R_f 0.27 (*n*-hexane/EtOAc/AcOH, 1/2/0.03); IR (neat) 3369, 2932, 1741, 1407, 1242, 1158, 1073, 968, 909 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 5.86 (ddt, J=17.0, 10.2, 6.4 Hz, 1H), 5.78–5.60 (m, 1H), 5.60–4.40 (br, 3H), 5.55–5.25 (m, 3H), 5.10–4.90 (m, 2H), 4.12–3.96 (m, 1H), 3.61 (dd, J=10.2, 1.8 Hz, 1H), 2.74 (dd, J=18.6, 7.4 Hz, 1H), 2.50–1.40 (m, 23H); MS (FAB, Pos) *m*/*z* 405 (M+H)⁺, 387 (M+H–H₂O)⁺, 369, 351.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-19,20tetradehydro-20-methylPGE₂ (4j). Colorless oil; TLC R_f 0.20 (*n*-hexane/EtOAc/AcOH, 1/2/0.03); IR (neat) 3368, 2930, 1741, 1430, 1243, 1158, 1076, 969 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 5.71 (ddd, J=15.0, 7.6, 5.8 Hz, 1H), 5.52–5.28 (m, 3H), 5.30–4.20 (br, 3H), 4.13–3.95 (m, 1H), 3.72 (dd, J=10.2, 2.2 Hz, 1H), 2.74 (ddd, J=18.4, 7.4, 1.0 Hz, 1H), 2.50–1.60 (m, 21H), 1.81 (t, J=2.5 Hz, 3H); MS (EI, Pos) m/z 384 (M–H₂O)⁺, 368 (M–2H₂O)⁺, 248, 230.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-20-fluoroPGE₂ (4k). Pale yellow oil; TLC R_f 0.23 (*n*-hexane/ EtOAc/AcOH, 1/3/0.04); IR (neat) 3369, 2937, 1740, 1709, 1403, 1241, 1158, 1073, 971, 914 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.68 (ddd, J=15.5, 8.0, 5.5 Hz, 1H), 5.46 (dd, J=15.5, 8.5 Hz, 1H), 5.50–4.50 (br, 3H), 5.45–5.33 (m, 2H), 4.55–4.48 and 4.46–4.38 (m, 2H), 4.10–4.02 (m, 1H), 3.61 (dd, J=10.5, 2.0 Hz, 1H), 2.73 (dd, J=18.0, 7.0 Hz, 1H), 2.43–2.25 (m, 6H), 2.20 (dd, J=18.0, 10.0 Hz, 1H), 2.15–1.95 (m, 6H), 1.95–1.62 (m, 9H), 1.57–1.48 (m, 1H); MS (FAB, Pos) m/z 411 (M+H)⁺, 393 (M+H–H₂O)⁺, 375, 357.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-20-chloro-PGE₂ (4l). Pale yellow oil; TLC R_f 0.44 (EtOAc/AcOH, 50/1); IR (neat) 3392, 2937, 1733, 1405, 1244, 1158, 1072, 969, 912, 734, 649 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 5.68 (ddd, J=15, 7, 5 Hz, 1H), 5.50–5.29 (m, 3H), 4.80–4.00 (br, 3H), 4.12–3.99 (m, 1H), 3.63–3.53 (m, 3H), 2.74 (dd, J=18, 7 Hz, 1H), 2.45–1.50 (m, 21H), 2.30 (t, J=7 Hz, 2H); MS (FAB, Pos) m/z 427 (M+H)⁺, 419 (M+H–H₂O)⁺, 391 (M+H–2H₂O), 373.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-20-methoxyPGE₂ (4m). Colorless oil; TLC R_f 0.27 (EtOAc/ AcOH, 100/1); IR (neat) 3369, 2936, 1741, 1396, 1240, 1158, 1076, 969 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 5.68 (ddd, J=15.2, 8.0, 5.0 Hz, 1H), 5.50–5.20 (m, 3H), 5.40–4.20 (br, 3H), 4.13–3.97 (m, 1H), 3.56 (dd, J=10.4, 2.0 Hz, 1H), 3.55–3.35 (m, 2H), 3.38 (s, 3H), 2.75 (dd, J=18.2, 7.4 Hz, 1H), 2.50–1.40 (m, 23H); MS (FAB, Pos.) m/z 423 (M+H)⁺, 405 (M+H–H₂O)⁺, 387 (M+H–2H₂O)⁺, 369, 355.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene- ω -dinor-18-phenylPGE₂ (4n). Pale yellow oil; TLC R_f 0.43 (EtOAc/AcOH, 50/1); IR (neat) 3371, 2933, 1739, 1604, 1495, 1455, 1435, 1406, 1244, 1158, 1075, 969, 911, 760, 733, 705, 649 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 7.37–7.18 (m, 5H), 5.67 (ddd, J=15, 8, 6 Hz, 1H), 5.49– 5.28 (m, 3H), 5.20–4.60 (bs, 3H), 4.18–3.98 (m, 1H), 3.62 (bd, J = 10 Hz, 1H), 2.87 (d, J = 14 Hz, 1H), 2.73 (dd, J = 18, 8 Hz, 1H), 2.65 (d, J = 14 Hz, 1H), 2.45–1.42 (m, 19H); MS (MALDI, Pos) m/z 463 (M + Na)⁺.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene- ω -dinor-18-cyclohexylPGE₂ (40). Yellow oil; TLC R_f 0.26 (*n*-hexane/EtOAc/AcOH, 1/2/0.03); IR (neat) 3369, 2923, 2851, 1741, 1448, 1242, 1158, 1075, 968 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) & 5.73 (ddd, J=15.0, 7.7, 6.1 Hz, 1H), 5.55–5.30 (m, 3H), 4.80–3.60 (br, 3H), 4.15–3.98 (m, 1H), 3.66 (dd, J=10.2, 2.0 Hz, 1H), 2.74 (dd, J=18.2, 6.8 Hz, 1H), 2.50–1.90 (m, 14H), 1.90–1.40 (m, 11H), 1.40–0.80 (m, 7H); MS (FAB, Pos) *m*/*z* 429 (M+H–H₂O)⁺, 411 (M+H–2H₂O)⁺, 393.

(16*S*)-15-Deoxy-16-hydroxy-17,17-tetramethylenePGE₂ (7). Yellow oil; TLC R_f 0.26 (*n*-hexane/EtOAc/AcOH, 2/3/0.05); IR (neat) 3369, 2954, 2870, 1741, 1709, 1455, 1407, 1240, 1158, 1075, 967, 911, 734 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 5.75–5.57 (m, 1H), 5.50–5.30 (m, 3H), 5.80–4.80 (br, 3H), 4.12–3.94 (m, 1H), 3.51 (d, J=9.4 Hz, 1H), 2.73 (dd, J=18.0, 7.0 Hz, 1H), 2.50–1.95 (m, 11H), 1.80–1.10 (m, 14H), 0.90 (t, J=6.4 Hz, 3H); MS (EI, Pos) m/z 388 (M–H₂O)⁺, 370 (M–2H₂O)⁺.

(16*S*)-15-Deoxy-16-hydroxy-17,17-pentamethylenePGE₂ (8). Yellow oil; TLC R_f 0.28 (*n*-hexane/EtOAc/AcOH, 2/3/0.05); IR (neat) 3392, 2931, 2867, 1741, 1713, 1456, 1243, 1158, 1074, 968, 911, 734 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 5.75-5.57 (m, 1H), 5.50–5.30 (m, 3H), 5.80–5.00 (br, 3H), 4.11–3.95 (m, 1H), 3.50 (d, J=10.0 Hz, 1H), 2.73 (dd, J=18.4, 7.0 Hz, 1H), 2.50–1.90 (m, 11H), 1.80–1.10 (m, 16H), 0.90 (t, J=6.4 Hz, 3H); MS (EI, Pos) m/z 402 (M–H₂O)⁺, 384 (M–2H₂O)⁺.

(3R,4R)-2-Methylene-3-[(1E)-4-(t-butyldimethylsiloxy)-5,5-trimethylene-1-octenyl]-4-(t-butyldimethylsiloxy)cyclopentane-1-one (30b). To a stirred solution of 19b (490 mg, 1.20 mmol) in 4 mL of dry ether was slowly added *t*-butyllithium (1.64 M in pentane, 1.46 mL, 2.40 mmol) at -78 °C under argon atmosphere and stirring was continued for 1 h. To the stirred mixture, lithium 2-thienvlcyanocuprate (0.25 M in THF, 5.80 mL, 1.44 mmol) was slowly added and then a solution of 29 (255 mg, 1.00 mmol) in 3 mL of ether after 15 min. The reaction mixture was warmed up to 0°C over 1 h and then poured into a stirred heterogeneous mixture of *n*-hexane and saturated aqueous ammonium chloride. The organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/n-hexane, 1/100 to 1/50) to yield 30b (308 mg, 61%) as a yellow oil. MS (APCI, Pos, 20V) m/z375 (M+H-TBSOH)⁺, 311; IR (neat) 2956, 2930, 2858, 1734, 1642, 1472, 1362, 1256, 1092, 836, 775 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 6.12–6.06 (m, 1H), 5.78– 5.54 (m, 1H), 5.40–5.15 (m, 2H), 4.12–3.98 (m, 1H), 3.63– 3.54 (m, 1H), 3.32–3.18 (m, 1H), 2.70–2.50 (m, 1H), 2.40– 1.20 (m, 13H), 1.00–0.80 (m, 21H), 0.10–0.00 (m, 12H).

(2*R*,3*R*,4*R*)-2-[2-(4-Carbomethoxyphenyl)ethyl]-3-[(1*E*)-4-(*t*-butyldimethylsiloxy)-5,5-trimethylene-(*t*-butyldimethylsiloxy)-cyclopentane-1-one (31b). To a

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stirred solution of the 4-(carbomethoxy)benzylzincbromide 32 (0.58 mL, 0.577 mmol, prepared from 4-(carbomethoxy)benzylbromide and activated zinc powder in THF) was added a solution of CuCN-2LiCl in THF (0.72 mL, 0.721 mmol) at -78 °C under argon atmosphere. After stirring for 30 min, a solution of chlorotrimethylsilane (66 µL, 0.519 mmol) and 31b (150 mg, 0.288 mmol) in THF (2 mL) was added to the reaction mixture at -78 °C. The resulting mixture was stirred for 1 h at -78 °C, then stirred for additional 2 h at -20 °C, quenched with saturated aqueous ammonium chloride and extracted with EtOAc. The organic layer was washed with water, then brine, dried over magnesium sulfate and concentrated in vacuo. The residue was dissolved in methanol (2 mL)-ether (1 mL). After the addition of pyridinium *p*-toluenesulfonate (4 mg), the reaction mixture was stirred for 1 h at room temperature, then quenched with saturated aqueous sodium bicarbonate and extracted with EtOAc. The organic layer was washed with water, then brine, dried over magnesium sulfate and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/n-hexane, 1/20 to 1/4) to yield 31b (53 mg, 28%) as a colorless oil. TLC R_f 0.60 (EtOAc/ *n*-hexane, 1/5); ¹H NMR (200 MHz, CDCl₃) δ 7.94 (d, J = 8.5 Hz, 2H), 7.23 (d, J = 8.5 Hz, 2H), 5.80–5.50 (m, 1H), 5.40–5.20 (m, 1H), 4.96 (dd, J=7.5, 2.5 Hz, 1H), 3.70 (s, 3H), 3.57 (t, J=5.0 Hz, 1H), 2.90-1.10 (m, 20H), 1.00–0.75 (m, 21H), 0.10–0.00 (m, 12H).

(2R,3R,4R)-2-[2-(4-Carboxyphenyl)ethyl]-3-[(1E)-4-hydroxy-5,5-trimethylene-1-octenyl]-4-hydroxy-cyclopentane-1-one (6b). Compound 6b was synthesized from **31b** according to the same procedure used for the preparation of compound 4b described above. Yellow oil; TLC R_f 0.45 (EtOAc/n-hexane/AcOH, 12/7/1); IR (neat) 3392, 2931, 1739, 1696, 1611, 1576, 1418, 1282, 1178, 1073, 970, 910, 733, 669, 649, 523 cm⁻¹; ¹H NMR $(200 \text{ MHz}, \text{ CDCl}_3) \delta 7.99 \text{ (d, } J = 8.5 \text{ Hz}, 2\text{H}), 7.28 \text{ (d,}$ J=8.5 Hz, 2H), 5.80–5.60 (m, 1H), 5.50–5.30 (m, 1H), 4.05 (q, J = 8.0 Hz, 1H), 3.59 (dd, J = 10.0, 2.5 Hz, 1H),3.00-1.20 (m, 23H), 0.95 (t, J = 7.0 Hz, 3H); MS (APCI,Neg, 20V) m/z 413 (M-H)⁻, 395 (M-H₂O-H)⁻. The compounds 6a, e, f and 6h were prepared from 19a, e, f, h and 29, respectively, according to the same procedure used for the preparation of compound 6b described above.

(2*R*,3*R*,4*R*) - 2 - [2 - (4 - Carboxyphenyl)ethyl] - 3 - [(1*E*) - 4-hydroxy - 5,5 - trimethylene - 1 - heptenyl] - 4 - hydroxy - cyclopentane - 1 - one (6a). Colorless oil; TLC R_f 0.39 (EtOAc/ *n*-hexane/AcOH, 9/3/0.1); IR (neat) 3392, 2932, 1739, 1694, 1611, 1575, 1418, 1282, 1178, 1073, 1020, 969, 910, 860, 733 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 7.99 (d, *J* = 8.0 Hz, 2H), 7.26 (d, *J* = 8.0 Hz, 2H), 5.70 (ddd, *J* = 14.0, 8.5, 5.0 Hz, 1H), 5.39 (dd, *J* = 14.0, 9.0 Hz, 1H), 4.05 (q, *J* = 8.0 Hz, 1H), 3.60 (dd, *J* = 10.0, 2.0 Hz, 1H), 2.90–2.65 (m, 3H), 2.50–2.15 (m, 3H), 2.15–1.30 (m, 15H), 0.93 (t, *J* = 7.5 Hz, 3H); MS (APCI, Neg, 20v) *m*/*z* 399 (M-H)⁻.

(2*R*,3*R*,4*R*)-2-[2-(4-Carboxyphenyl)ethyl]-3-[(1*E*)-4-hydroxy-5,5-trimethylene-7-methyl-1-octenyl]-4-hydroxy-

cyclopentane-1-one (6e). White amorphous; TLC R_f 0.52 (*n*-hexane/EtOAc, 1/4); IR (neat) 3392, 2953, 2360, 1733, 1696, 1611, 1418, 1287, 1179, 1076, 970, 910, 734, 649 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 7.99 (d, J=8.4 Hz, 2H), 7.25 (d, J=8.4 Hz, 2H), 5.71 (ddd, J=15, 9.4, 5.4 Hz, 1H), 5.40 (dd, J=15, 9.0 Hz, 1H), 4.48 (br, 3H), 4.06 (m, 1H), 3.69 (dd, J=9.0, 1.4 Hz, 1H), 2.78 (m, 3H), 2.50–1.62 (m, 14H), 1.56 (dd, J=14, 6.7 Hz, 1H), 1.35 (dd, J=14, 6.3 Hz, 1H), 0.92 (d, J=6.2 Hz, 6H); MS (APCI, Neg, 20V) m/z 427 (M–H)⁻, 409 (M–H–H₂O)⁻.

(2R,3R,4R)-2-[2-(4-Carboxyphenyl)ethyl]-3-[(1E)-4-hydroxy-5,5-trimethylene-6-cyclopropyl-1-hexenyl]-4-hydroxy-cyclopentane-1-one (6f). White amorphous; TLC R_f 0.44 (*n*-hexane/EtOAc = 1/4); IR (neat) 3392, 2930, 2360, 1733, 1696, 1611, 1418, 1284, 1179, 1078, 1019, 970, 910, 734 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 7.99 (d, J=8.3 Hz, 2H), 7.25 (d, J=8.3 Hz, 2H), 5.71 (ddd, Hz, 2H), 5J=15, 9.1, 5.3 Hz, 1H), 5.38 (dd, J=15, 8.7 Hz, 1H), 4.19 (br, 3H), 4.05 (m, 1H), 3.71 (dd, J=9.4, 1.5 Hz, 1H), 2.78 (m, 3H), 2.31 (m, 3H), 2.17–1.61 (m, 10H), 1.52 (dd, J = 14, 6.8 Hz, 1H), 1.38 (dd, J = 14, 6.4 Hz, 1H), 0.78 (m, 1H), 0.51 (m, 2H), 0.11 (m, 2H); MS (APCI, Neg, 20V) m/z 425 (M-H)⁻, 407 $(M-H-H_2O)^{-}$.

(2*R*,3*R*,4*R*)-2-[2-(4-Carboxyphenyl)ethyl]-3-[(1*E*)-4-hydroxy-5,5-trimethylene-1,7-octadienyl]-4-hydroxy-cyclopentane-1-one (6h). Pale yellow amorphous; TLC R_f 0.33 (*n*-hexane/EtOAc/AcOH, 1/2/0.03); IR (neat) 3391, 2932, 1739, 1696, 1611, 1419, 1283, 1179, 1077, 911, 734 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 7.99 (d, *J*=8.2 Hz, 2H), 7.26 (d, *J*=8.2 Hz, 2H), 5.95 (ddt, *J*=17.0, 10.0, 7.4 Hz, 1H), 5.69 (ddd, *J*=15.0, 8.6, 5.2 Hz, 1H), 5.38 (dd, *J*=15.0, 9.0 Hz, 1H), 5.20–5.05 (m, 2H), 5.00– 3.00 (br, 3H), 4.15–3.95 (m, 1H), 3.59 (dd, *J*=10.2, 2.2 Hz, 1H), 2.95–2.65 (m, 3H), 2.50–1.65 (m, 15H); MS (FAB, pos) *m*/*z* 413 (M+H)⁺, 395 (M+H–H₂O)⁺, 377 (M+H–2H₂O)⁺.

(16RS)-5,6-Didehydro-11-(t-butyldimethylsilyl)-15-deoxy-16-(t-butyldimethylsiloxy)-17,17-trimethylenePGE₂ methyl ester (34). To a stirred solution of (E)-1-iodo-4-(tbutyldimethylsilyloxy)-5,5-trimethylene-1-octene 19b (265 mg, 0.65 mmol) in 2 mL of dry ether was slowly added t-butyllithium (1.7 M in pentane, 0.83 mL, 1.30 mmol) at -78 °C under argon atmosphere, and stirring was continued for 1 h and lithium 2-thienylcyanocuprate (0.25 M in THF, 3.12 mL, 0.78 mmol) was slowly added. After 20 min, to the resulting mixture was added a solution of (4R)-4-(t-butyldimethylsilyloxy)-2-cyclopenten-1-one 33 in THF (4 mL) and the mixture was warmed up to -20 °C over 30 min. To the resulting mixture, a solution of methyl-7-iodo-5-heptynoate 35 in THF (5 mL) was added. After stirring for 3 h at -20 °C, the reaction mixture was poured into a stirred heterogeneous mixture of *n*-hexane and saturated aqueous ammonium chloride. The organic layer was washed with water, then brine, dried over anhydrous magnesium sulfate and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel (EtOAc/n-hexane, 1/50 to 1/20) to yield 34 (44 mg, 14%) as an oil. TLC R_f 0.36 (*n*-hexane/EtOAc, 9/1); MS (APCI, Pos, 20V) m/z 501 (M+H–TBSOH)⁺, 369 (M+H–2TBSOH)⁺; IR (neat) 2955, 2931, 1747, 1436, 1362, 1252, 1162, 1092, 837, 775 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 5.78–5.55 (m, 1H), 5.40–5.23 (m, 1H), 4.10–3.95 (m, 1H), 3.66 (s, 3H), 3.63–3.53 (m, 1H), 2.80–2.50 (m, 2H), 2.50–1.20 (m, 22H), 1.00–0.80 (m, 3H), 0.91, 0.90 and 0.88 (3×s, 18H), 0.09, 0.05 and 0.04 (3×s, 12H).

(16*S*)-5,6-Didehydro-15-deoxy-16-hydroxy-17,17-trimethylenePGE₂ (5). Compound 5 was synthesized from 34 according to the same procedure used for the preparation of compound 4b described above. Colorless oil; TLC R_f 0.25 (*n*-hexane/EtOAc/AcOH, 1/3/0.04); IR (neat) 3369, 2956, 2932, 2872, 2253, 1746, 1714, 1429, 1339, 1241, 1162, 1077, 969, 758 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 6.00–4.80 (br, 3H), 5.71 (ddd, *J*=15.0, 9.2, 4.4 Hz, 1H), 5.41 (dd, *J*=15.0, 8.5 Hz, 1H), 4.20– 4.03 (m, 1H), 3.61 (d, *J*=10.0 Hz, 1H), 2.88–2.65 (m, 3H), 2.50 (t, *J*=7.0 Hz, 2H), 2.40–1.20 (m, 19H), 0.94 (t, *J*=6.7 Hz, 3H); MS (FAB, pos) *m*/*z* 391 (M+H)⁺, 373 (M+H–H₂O)⁺, 355 (M+H–2H₂O)⁺.

Prostanoid EP and IP receptor binding assay

Membranes from CHO cells expressing the prostanoid receptors were incubated with radioligand (2.5 nM of [³H]PGE₂ for EP1-4 or 5.0 nM of [³H]Iloprost for IP) and the test compounds at various concentrations in assay buffer [10 mM Kpi (KH₂PO₄, KOH; pH 6.0), 1 mM EDTA and 0.1 mM NaCl, for EP1-4-receptors; 50 mM Tris-HCl (pH 7.5), 1 mM EDTA and 10 mM MgCl₂ for IP-receptor]. Incubation was carried out at 25°C for 60 min except for EP1 (20 min) and IP (30 min) receptors. The incubation was terminated by filtration through Whatman GF/B filters. The filters were then washed with ice-cold buffer [10 mM Kpi (KH₂PO₄, KOH; pH 6.0), 0.1 mM NaCl for EP1-4; 10 mM Tris-HCl (pH 7.5), 0.1 mM NaCl for IP], and the radioactivity on the filter was measured in 6mL of liquid scintillation (ACSII) mixture with a liquid scintillation counter. Nonspecific binding was determined by incubation of 10 µM unlabeled PGE₂ (for EP1-4) or 1 µM unlabeled Iloprost (for IP) with assay buffer.

Measurement of cAMP production

CHO cells expressing EP2- or IP-receptors were cultured in 24-well plates (1×10^5 cells/well). After 2 days, the media were removed and cells were washed with 500 µL of Minimum Essential Medium (MEM) and preincubated for 10 min in 450 µL of assay buffer (MEM containing 1 mM of IBMX, 1% of BSA) at 37 °C. Then reaction was started with the addition of each test compound in 50 µL of assay buffer. After incubation for 10 min at 37 °C, the reaction was terminated by addition of 500 μ L of ice-cold 10% trichloroacetic acid. The cAMP production was measured by radio-immunoassay using a cAMP assay kit (Amersham).

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23. The configuration of C-16 of the more potent isomer **4b** was tentatively assigned to 16*S*.