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Development of a Highly Selective EP2-Receptor Agonist. Part 1: Identification of 16-hydroxy-17,17-trimethylene PGE_2 Derivatives

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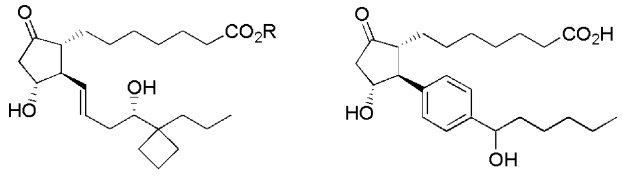
Abstract—Design and synthesis of an EP2-receptor selective agonist began with the chemical modification of α- and ω-chains of butaprost **1a**, which exhibits an affinity for the IP-receptor. Two series of prostaglandin (PG) analogues with a 16-hydroxy-17,17-trimethylene moiety as an ω-chain were identified. 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.

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 PGE_2 have been classified into four subtypes EP1, EP2, EP3 and EP4.[1] The diverse biological activities of PGE_2 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 muscle[6] and has been suggested to play an important role in the production and control of cytokines[7] 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 PGE_2 (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 PGE_2 derivatives as new selective EP2-receptor agonists. Structure–activity relationships (SARs) are also discussed.



1a: R = Me, (Butaprost)
1b: R = H

2: AH-13205

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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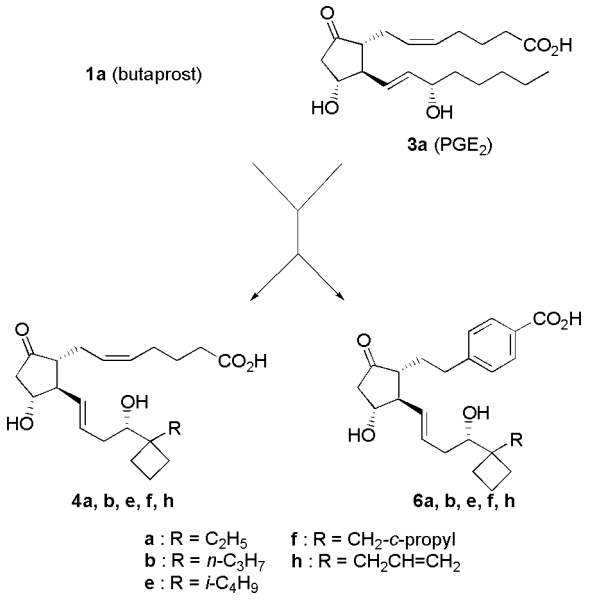
Chemistry

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 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-*n*-butylammonium fluoride and subsequent acidic deprotection gave **13k**. *O*-Methylation of **24** followed by the acidic deprotection yielded **13m**.

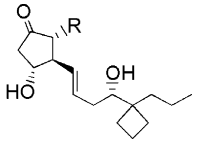


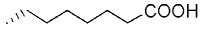
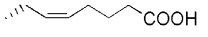
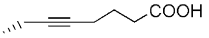
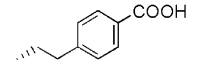
Scheme 1. Discovery of highly selective EP2-receptor agonists **4a**,**b**,**e**, **f**,**h** and **6a**,**b**,**e**,**f**,**h**.

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 hydrostannation[13] of the resulting alkynes followed by treatment with iodine yielded vinyl iodides **19a**–**o**, **20** and **21**.

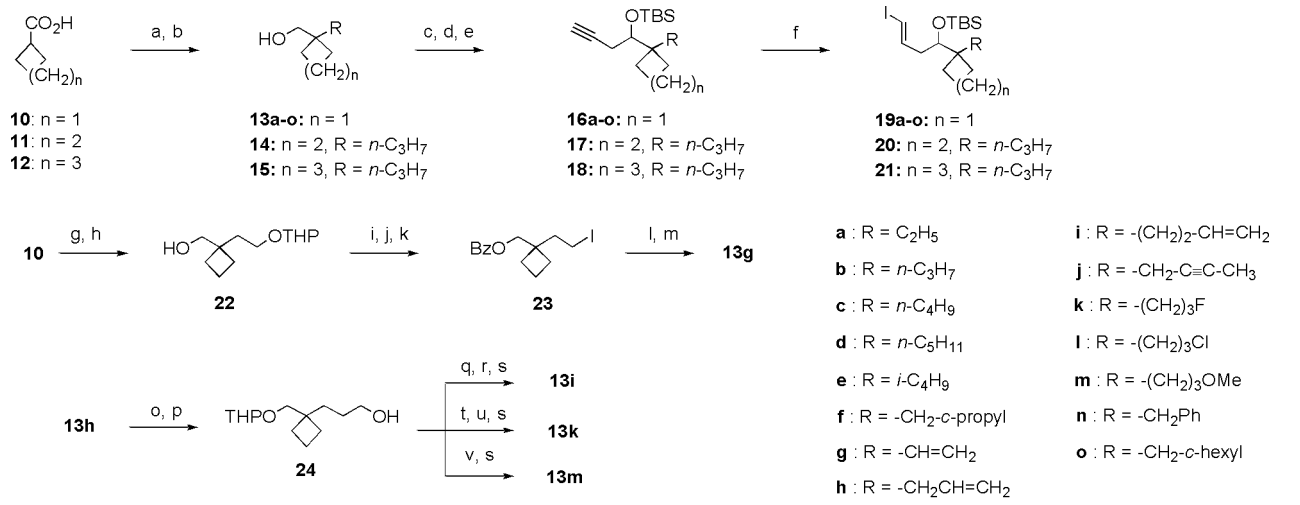
As described in Scheme 3, conjugate addition[14,15] of the ω-chain moieties **19a**–**o**, **20** and **21** to the enone **25**[14] was carried out in the presence of *t*-butyl lithium and 2-thienylcyanocuprate[16] to produce **26a**–**o**, **27** and **28**,

Table 2. Optimization of α-chain

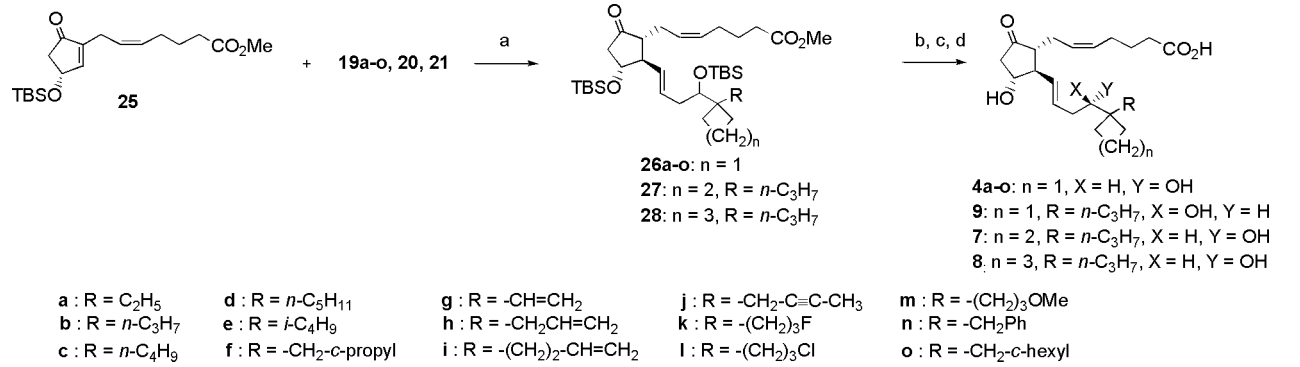


Compd	R	Binding K_i (nM)[a]					EC_{50} (nM)[b]	
		mEP1	mEP2	mEP3	mEP4	hIP	mEP2	hIP
3a	PGE_2	18	38	5.0	3.1	$>10^4$	2.0	260
3b	PGE_1	100	87	5.0	3.3	150	3.0	2.0
1b		$>10^4$	73	$>10^4$	$>10^4$	870	23	25
4b		$>10^4$	92	$>10^4$	$>10^4$	$>10^4$	43	$>10^4$
5		$>10^4$	1100	$>10^4$	$>10^4$	N.T.[c]	N.T.	N.T.
6b		$>10^4$	25	$>10^4$	$>10^4$	$>10^4$	54	$>10^4$

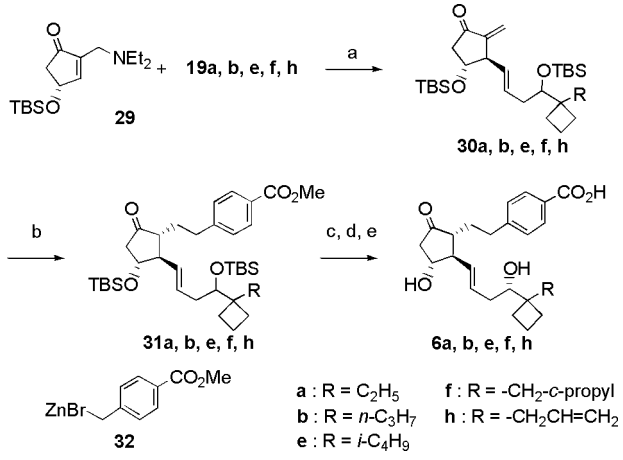
[a]Using membrane fractions of Chinese hamster ovary (CHO) cells expressing the prostanoid receptors, K_i values were determined by the competitive binding assay, which was performed according to the method of Kiriyama et al.[3] with some modifications. The K_i values of the test compounds, which demonstrated less than 50% inhibition at 10^4 nM, are expressed $>10^4$.
[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.
[c]Not tested.



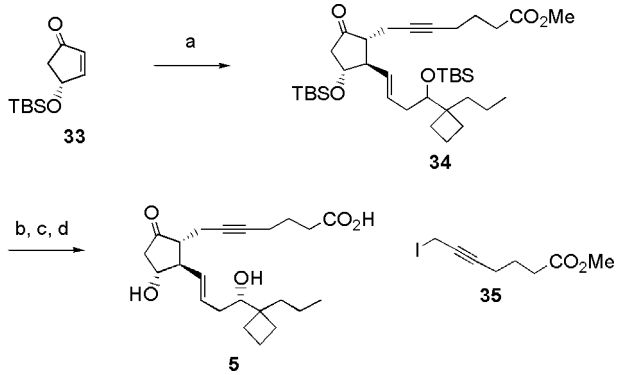
Scheme 2. Synthesis of vinyl iodides **19a–o**, **20**, **21**. Reagent: (a) LDA, RX, THF, 0 °C; (b) $LiAlH_4$, THF, reflux; (c) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C; (d) propargylmagnesium bromide, ether, 0 °C; (e) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C; (f) Cp_2ZrClH, THF–toluene (Method A) or n-Bu_3SnH, AIBN (Method B) then I_2; (g) LDA, $I(CH_2)_2OTHP$, THF; (h) BH_3–THF, THF; (i) BzCl, pyridine; (j) p-TsOH, MeOH; (k) PPh_3, imidazole, I_2, benzene; (l) DBU, benzene; (m) aqueous NaOH, MeOH; (o) DHP, p-TsOH, CH_2Cl_2; (p) BH_3–THF, THF; (q) Swern oxdn; (r) $MePPh_3Br$, BuLi, THF; (s) p-TsOH, MeOH; (t) TsCl, pyridine; (u) n-Bu_4NF, 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, CH_3CN, 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, CH_3CN, 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, CH_3CN, 0 °C; (c) separation; (d) porcine liver esterase, phosphate buffer (pH = 7.4), EtOH, room temperature.

respectively. Deprotection of the TBS ethers with aqueous hydrogen fluoride[17] followed by separation of C16-epimers and subsequent enzymatic hydrolysis with porcine liver esterase (PLE)[18] yielded **4a**–**o**, **9**, **7** and **8**.

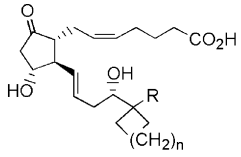
As shown in Scheme 4, conjugate addition of **19a**,**b**,**e**,**f** and **19h** to the enone **29**[14] gave **30a**,**b**,**e**,**f** and **30h**, which were converted to **31a**,**b**,**e**,**f** and **31h**, respectively, by another conjugate addition of **32**.[19,20] Deprotection of the TBS groups 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.

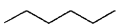
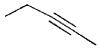
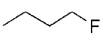
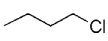
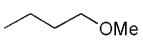
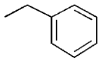
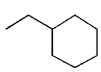
Synthesis of the alkyne-containing PG analogue **5** is described in Scheme 5.[21] Conjugate addition reaction of **33**[21] with the cuprate prepared from **19b** followed by trapping of the resulting enolate with an iodoalkynoate **35**[21] gave **34**. Acidic deprotection of **34** followed by separation 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 EP2-receptor 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-

Table 3. Optimization of the alkyl group at position-17



Compound	R	*n*	Binding K_i (nM)					EC_{50} (nM)
			mEP1	mEP2	mEP3	mEP4	hIP	mEP2
4a		1	$>10^4$	30	$>10^4$	$>10^4$	$>10^4$	11
4b		1	$>10^4$	92	$>10^4$	$>10^4$	$>10^4$	43
9 (16-epimer of **4b**)		1	$>10^4$	330	1200	$>10^4$	$>10^4$	220
7		2	$>10^4$	370	$>10^4$	$>10^4$	$>10^4$	580
8		3	$>10^4$	3300	$>10^4$	$>10^4$	$>10^4$	$>10^4$
4c		1	780	43	2000	$>10^4$	$>10^4$	71
4d		1	1600	20	830	2100	$>10^4$	130
4e		1	$>10^4$	40	$>10^4$	$>10^4$	$>10^4$	45
4f		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		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		1	$>10^4$	410	$>10^4$	$>10^4$	N.T.	N.T.
4l		1	870	140	$>10^4$	$>10^4$	N.T.	N.T.
4m		1	$>10^4$	470	$>10^4$	$>10^4$	N.T.	N.T.
4n		1	2100	2300	1800	$>10^4$	$>10^4$	N.T.
4o		1	$>10^4$	1200	1300	$>10^4$	$>10^4$	N.T.

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 ($EC_{50}=25$ 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 PGE_2 (**3a**) on intracellular cAMP using mouse EP2-receptor 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 PGE_2.

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 ($K_i=1100$ 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 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-16[23] (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 EP3-receptor ($K_i=1.2$ μM).

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

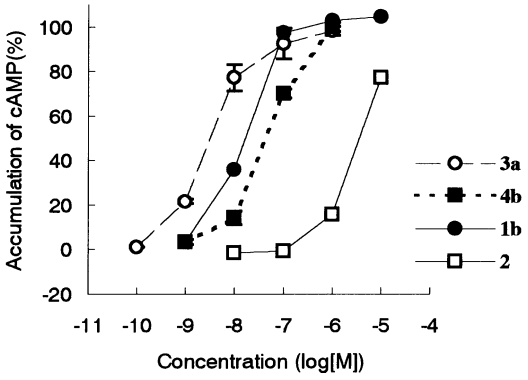
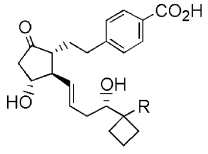


Figure 2. Effect of compounds on cAMP accumulation in CHO cells expressing mouse EP2-receptor.

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



Compound	R	Binding K_i (nM)					EC_{50} (mM)
		mEP1	mEP2	mEP3	mEP4	hIP	mEP2
6a		$>10^4$	19	$>10^4$	$>10^4$	$>10^4$	24
6b		$>10^4$	25	$>10^4$	$>10^4$	$>10^4$	54
6e		$>10^4$	42	$>10^4$	$>10^4$	$>10^4$	260
6f		$>10^4$	6.1	$>10^4$	$>10^4$	$>10^4$	26
6h		$>10^4$	9.7	$>10^4$	$>10^4$	$>10^4$	37

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 17-cyclopropylmethyl 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–i** were 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 **4o**, 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 ($EC_{50} = 24$ 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 EP2-receptor 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 ^{1}H 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 °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 °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. 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); ^{1}H 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

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); ^{1}H NMR (200 MHz, $CDCl_3$) 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); ^{1}H NMR (200 MHz, $CDCl_3$) 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); ^{1}H NMR (200 MHz, $CDCl_3$) 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 ice-bath, 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-2*H*-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); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.81 (ddt, $J=16.4$, 11.0, 7.4 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); ^{1}H NMR (200 MHz, $CDCl_3$) 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 °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); ^{1}H NMR (200 MHz, $CDCl_3$) 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); ^{1}H NMR (200 MHz, $CDCl_3$) 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 °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 °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); ^{1}H NMR (200 MHz, $CDCl_3$) 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 °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. ^{1}H NMR (200 MHz, $CDCl_3$) 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); ^{1}H 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-1-octyne (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); ^{1}H NMR (200 MHz, $CDCl_3$) 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-1-octene (19b). Method A. To a stirred solution of 4-(*t*-butyldimethylsiloxy)-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); ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.50 (dt, J = 14.8, 7.4 Hz, 1H), 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-1-heptene (19a). Method B. TLC R_f 0.86 (*n*-hexane); ^{1}H NMR (200 MHz, $CDCl_3$) 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-1-nonene (19c). Method B. TLC R_f 0.84 (*n*-hexane); ^{1}H NMR (200 MHz, $CDCl_3$) 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-1-decene (19d). Method B. TLC R_f 0.84 (*n*-hexane); ^{1}H NMR (200 MHz, $CDCl_3$) 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/z 365 $(M+H-t\text{-}C_4H_9)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) 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-5,5-trimethylene-1-hexene (19f). Method A. TLC R_f 0.70 (*n*-hexane); MS (APCI, Pos, 20V) m/z 289 $(M+H-TBSOH)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) 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 $(M+H-t\text{-}C_4H_9)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) 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 $(M+H-TBSOH)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) 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 $(M+H-TBSOH)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) 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, $CDCl_3$) 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, $CDCl_3$) 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 (19l). Method B. TLC R_f 0.54 (*n*-hexane); MS (APCI, Pos, 20V) *m*/*z* 313 $(M+H-TBSOH)^+$; ^1H NMR (200 MHz, $CDCl_3$) 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, $CDCl_3$) 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, $CDCl_3$) 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 (19o). Method B. TLC R_f 0.86 (*n*-hexane); ^1H NMR (200 MHz, $CDCl_3$) 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, $CDCl_3$) 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, $CDCl_3$) 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).

(16*RS*)-11-*O*-(*t*-Butyldimethylsilyl)-15-deoxy-16-(*t*-butyldimethylsilyloxy)-17,17-trimethylenePGE_2 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, dried over anhydrous magnesium 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 $(M-t\text{-}Bu)^+$, 445; IR (neat) 2955, 2857, 1746, 1405, 1252, 1094, 837, 775 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 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).

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylenePGE_2 (4b) and (16*R*)-15-deoxy-16-hydroxy-17,17-trimethylenePGE_2 (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 MHz, $CDCl_3$) δ 5.71 (ddd, J = 15.3, 7.6, 6.3 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 (16*S*–OH); colorless oil; TLC R_f 0.29 (*n*-hexane/EtOAc, 2/3); MS (APCI, Pos, 20V) 389 $(M+H-H_2O)^+$, 371 $(M+H-2H_2O)^+$; IR (neat) 3401, 2954, 1742, 1436, 1245, 1159, 1075, 968 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 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 μL (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 (APCI, Neg, 40v) *m/z* 391 $(M-H)^-$, 373 $(M-H_2O-H)^-$; ^1H NMR (200 MHz, $CDCl_3$) 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); ^{13}C NMR (125 MHz, $CDCl_3$) 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 $C_{23}H_{36}O_5$; 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 $(M-H_2O-H)^-$; ^1H NMR (200 MHz, $CDCl_3$) 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-norPGE$_2$ (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, $CDCl_3$) 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 $(M+H-H_2O)^+$, 343, 325.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-20-methyl-PGE$_2$ (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, $CDCl_3$) 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 $(M-H_2O)^+$, 370 $(M-2H_2O)^+$, 266, 248.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-20-ethyl-PGE$_2$ (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, $CDCl_3$) 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 $(M+H-H_2O)^+$, 385, 367.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-19-methyl-PGE$_2$ (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, $CDCl_3$) 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 $(M+H-H_2O)^+$, 371, 353.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-ω-dinor-18-cyclopropylPGE$_2$ (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, $CDCl_3$) δ 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 $(M+H-H_2O)^+$, 369, 351.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-ω-nor-18,19-didehydroPGE$_2$ (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, $CDCl_3$) 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 $(M+H-H_2O)^+$, 341, 323.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-19,20-didehydroPGE$_2$ (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, $CDCl_3$) 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 $(M-H_2O)^+$, 354 $(M-2H_2O)^+$, 248, 230.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-20-methylenePGE$_2$ (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}; ^{1}H NMR (200 MHz, $CDCl_3$) 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 $(M+H-H_2O)^+$, 369, 351.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-19,20-tetradehydro-20-methylPGE$_2$ (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}; ^{1}H NMR (200 MHz, $CDCl_3$) 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 $(M-H_2O)^+$, 368 $(M-2H_2O)^+$, 248, 230.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-20-fluoroPGE$_2$ (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}; ^{1}H NMR (500 MHz, $CDCl_3$) 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 $(M+H-H_2O)^+$, 375, 357.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-20-chloroPGE$_2$ (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}; ^{1}H NMR (200 MHz, $CDCl_3$) 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 $(M+H-H_2O)^+$, 391 $(M+H-2H_2O)$, 373.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-20-methoxyPGE$_2$ (4m). Colorless oil; TLC R_f 0.27 (EtOAc/AcOH, 100/1); IR (neat) 3369, 2936, 1741, 1396, 1240, 1158, 1076, 969 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) 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 $(M+H-H_2O)^+$, 387 $(M+H-2H_2O)^+$, 369, 355.

(16*S*)-15-Deoxy-16-hydroxy-17,17-trimethylene-ω-dinor-18-phenylPGE$_2$ (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}; ^{1}H NMR (200 MHz, $CDCl_3$) 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-cyclohexylPGE$_2$ (4o). 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}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 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 $(M+H-H_2O)^+$, 411 $(M+H-2H_2O)^+$, 393.

(16*S*)-15-Deoxy-16-hydroxy-17,17-tetramethylenePGE$_2$ (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}; ^{1}H NMR (200 MHz, $CDCl_3$) 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 $(M-H_2O)^+$, 370 $(M-2H_2O)^+$.

(16*S*)-15-Deoxy-16-hydroxy-17,17-pentamethylenePGE$_2$ (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}; ^{1}H NMR (200 MHz, $CDCl_3$) 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 $(M-H_2O)^+$, 384 $(M-2H_2O)^+$.

(3*R*,4*R*)-2-Methylene-3-[(1*E*)-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-thienylcyanocuprate (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/z 375 $(M+H-TBSOH)^+$, 311; IR (neat) 2956, 2930, 2858, 1734, 1642, 1472, 1362, 1256, 1092, 836, 775 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) 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- 1-octenyl]-4-(*t*-butyldimethylsiloxy)-cyclopentane-1-one (31b). To a

stirred solution of the 4-(carbomethoxy)benzylzinc-bromide **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); ^{1}H NMR (200 MHz, $CDCl_3$) 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).

(2*R*,3*R*,4*R*)-2-[2-(4-Carboxyphenyl)ethyl]-3-[(1*E*)-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}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.99 (d, J = 8.5 Hz, 2H), 7.28 (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 $(M-H_2O-H)^-$. 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}; ^{1}H NMR (200 MHz, $CDCl_3$) 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}; ^{1}H NMR (200 MHz, $CDCl_3$) 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 $(M-H-H_2O)^-$.

(2*R*,3*R*,4*R*)-2-[2-(4-Carboxyphenyl)ethyl]-3-[(1*E*)-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}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.99 (d, J = 8.3 Hz, 2H), 7.25 (d, J = 8.3 Hz, 2H), 5.71 (ddd, J = 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}; ^{1}H NMR (200 MHz, $CDCl_3$) 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 $(M+H-H_2O)^+$, 377 $(M+H-2H_2O)^+$.

(16*RS*)-5,6-Didehydro-11-(*t*-butyldimethylsilyl)-15-deoxy-16-(*t*-butyldimethylsiloxy)-17,17-trimethylenePGE_2 methyl ester (34). To a stirred solution of (*E*)-1-iodo-4-(*t*-butyldimethylsilyloxy)-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 (4*R*)-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, $CDCl_3$) 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-trimethylenePGE_2 (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, $CDCl_3$) 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 $(M+H-H_2O)^+$, 355 $(M+H-2H_2O)^+$.

Prostanoid EP and IP receptor binding assay

Membranes from CHO cells expressing the prostanoid receptors were incubated with radioligand (2.5 nM of $[^3H]PGE_2$ for EP1-4 or 5.0 nM of [^{3}H]Iloprost for IP) and the test compounds at various concentrations in assay buffer [10 mM Kpi (KH_2PO_4, 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 $MgCl_2$ 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 (KH_2PO_4, 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 PGE_2 (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 μL of ice-cold 10% trichloroacetic acid. The cAMP production was measured by radio-immunoassay using a cAMP assay kit (Amersham).

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