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A Practical Synthesis and Biological Evaluation of 9-Halogenated PGF Analogues

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Abstract—A series of 9-halo PGF analogues **1**–**2** and **5**–**13** were synthesized and biologically evaluated. Among the compounds, **2** was the best EP2-receptor agonist. A practical method of synthesizing **2** via the Julia olefination of an aldehyde **3** with an optically active sulfone **4**, which was prepared by Sharpless asymmetric epoxidation of **15**, was developed. Other 9-halogenated PGF analogues were synthesized essentially by the same procedure and evaluated. The absolute configuration of 16-OH of **2** was determined as *S* by the X-ray analysis of a salt consisting of a 1/1 molar ratio of **2** and L-lysine.

Introduction

In the preceding publications,[1] we reported the development of the highly selective EP2-receptor agonists **1** and **2**, which demonstrate high receptor affinity to the EP2-receptor (Chart 1). Also in functional studies, **2** showed nearly the same potency as that of PGE_2. Compound **2** suppressed spontaneous uterine motility in anesthetized rats in a late-term pregnancy, while PGE_2 stimulated uterine motility. [1a,1c] These findings suggest that **2** is a promising clinical candidate as a tocolytic with a new mechanism of action. The information mentioned above prompted us to develop a practical synthetic method of the newly discovered EP2-receptor selective agonist **2**. According to the reported method,[1c] the undesired 16(*R*)-isomer of **2** has to be removed by column chromatography on silica gel, while this laborious procedure could be successfully avoided by this new synthesis (Scheme 1). Additionally, removal of the $\Delta^{8,9}$-olefinic compound, which was formed as a by-product in the chlorination reaction of the new method, was found to be easier. We report here full details on the practical synthesis of 9-halogenated PGF analogues and their biological evaluations.

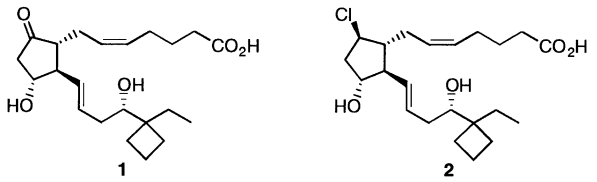
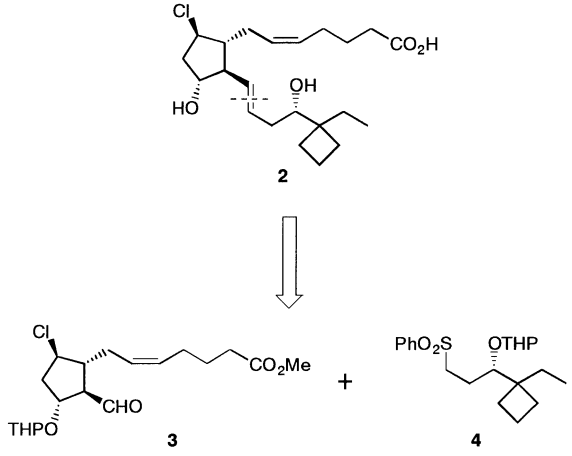


Chart 1. Structures of EP2-receptor agonists **1** and **2**.

Chemistry

A highly selective EP2-receptor agonist **2** was synthesized by the magnesium-mediated Julia olefination[2] of



Scheme 1. Retrosynthesis of **2**.

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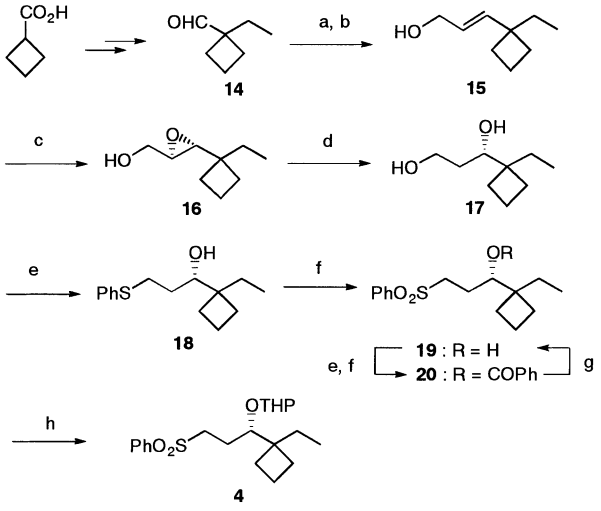
an aldehyde **3** with an optically active sulfone **4** as outlined in Scheme 1.

Preparation of 4

As described in Scheme 2, **4** was prepared from **14**, which was obtained from cyclobutane carboxylic acid in three steps[1b] (1) alkylation; (2) reduction; (3) oxidation. The compound **14** was converted to an allylic alcohol **15** by a Horner–Emmons reaction with diethylethylphosphono acetate followed by the reduction of an α,β-unsaturated ester with diisobutylaluminum hydride. Enantioselective epoxidation of **15** was conducted by Sharpless asymmetric epoxidation[3] to afford an optically active epoxy alcohol **16** (94% ee).[4] A ring-opening reaction of the epoxide **16** with Red-Al® afforded a diol **17**. Selective monotosylation of the primary alcohol in **17** followed by a S_N2 reaction with a sodium thiophenoxide provided **18**. Oxidation of **18** with Oxone® afforded a sulfone **19**, which was converted to a benzoate **20** for recrystallization. After purification, **20** was again converted to **19** (99% ee).[5] The hydroxy group of **19** was protected as a tetrahydropyranyl (THP) ether prior to its use for the Julia olefination.



Scheme 2. Synthesis of an optically active ω chain **4**. Reagents: (a) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF; (b) *i*-Bu_2AlH, THF, 0 °C; (c) *t*-BuOOH, $Ti(OiPr)_4$, D-(−)-DIPT, CH_2Cl_2, −20 °C; (b) $Na(MeOCH_2CH_2O)_2AlH_2$, toluene; (c) TsCl, *n*-Bu_4NBr, NaOHaq, toluene, then PhSH; (d) Oxone®, MeOH, H_2O; (e) PhCOCl, DMAP, pyridine; (f) Recrystallization from EtOH; (g) NaOHaq, MeOH; (h) DHP, *p*-TsOH, CH_2Cl_2.

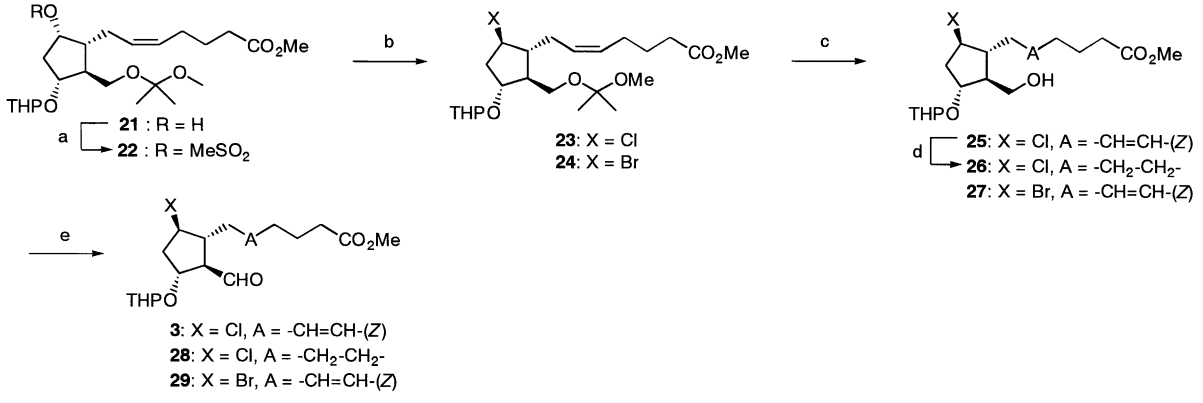
Preparation of 3, 28 and 29

The synthesis of aldehydes **3**, **28** and **29** is outlined in Scheme 3. Methanesulfonylation of the 9α-hydroxy group of **21**[6] followed by the S_N2 substitution reaction with tetrabutylammonium chloride[7] afforded 9β-chloride **23**. Selective deprotection of the 1-methyl-1-methoxyethyl group[6] of **23** provided **25**, which was easily separated from the by-product ($\Delta^{8,9}$-olefinic product) by column chromatography on silica gel. The ratio of the desired 9β-chloro product and the $\Delta^{8,9}$-olefinic side-product was approximately 5.4/1.[8] The compound **25** was converted to the aldehyde **3** by Swern oxidation. The corresponding 5,6-dihydro aldehyde **28** was prepared by oxidation of **26**, which was obtained by the catalytic hydrogenation of **25**.

The 9β-bromide **29** was obtained from **24**, which was prepared by S_N2 substitution of **22** with tetrabutylammonium bromide instead of tetrabutylammonium chloride according to the procedure described for the preparation of **28** from **23**.

Preparation of 38

The synthesis of 9β-chloro-5(*E*) aldehyde **38** from the Corey lactone **30**[9] is outlined in Scheme 4. Conversion of **30** to **31** was carried out by (1) protection of the primary alcohol as a *t*-butyldimethylsilyl ether and (2) reduction with lithium aluminum hydride. Compound **31** was converted to **32** by (1) selective monoacetylation of a primary hydroxy group, (2) tosylation of the α-hydroxy group of **31**, (3) a S_N2 substitution reaction with tetrabutylammonium chloride, and (4) alkaline hydrolysis with sodium hydroxide. Oxidation of **32** to the corresponding aldehyde followed by the addition reaction of a vinyl magnesium bromide provided an allylic alcohol **33**, which was converted to 9β-5(*E*) chloride **34** by the rearrangement reaction. One-carbon homologation of **34** to obtain a nitrile **35** was carried out in three steps: (1) hydride reduction of **34** with lithium aluminum hydride, (2) tosylation with *p*-toluenesulufo-



Scheme 3. Synthesis of 9β-halo aldehyde **3**, **28** and **29**. Reagents: (a) MsCl, Et_3N, CH_2Cl_2; (b) *n*-Bu_4NCl or *n*-Bu_4NBr, Et_3N, toluene, 60 °C; (c) HClaq, THF; (d) Pd/C, H_2, MeOH; (e) SO_3–pyridine, Et_3N, DMSO.

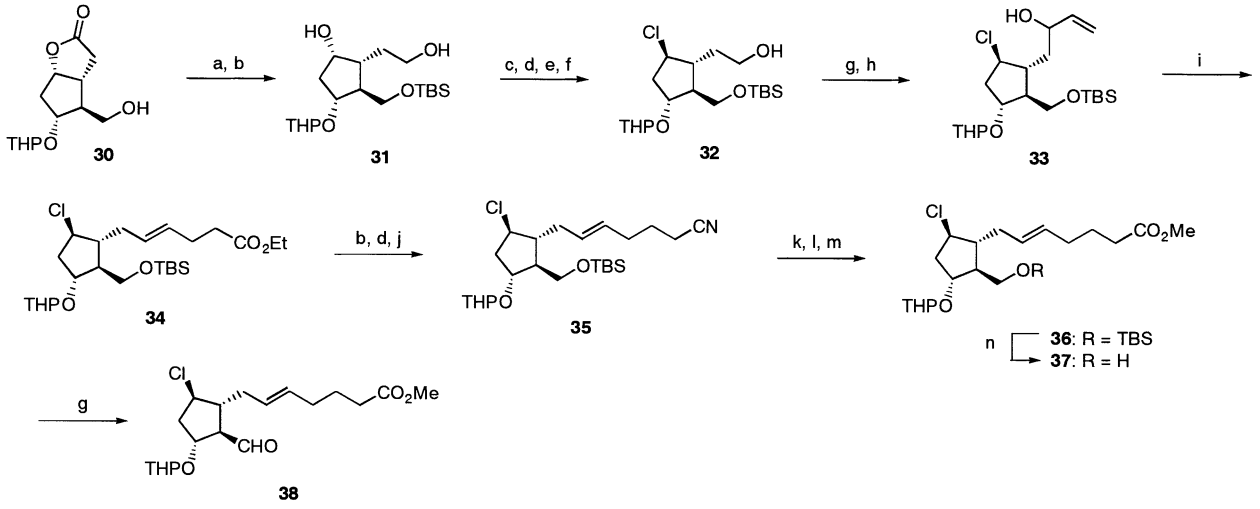
nyl chloride in pyridine, and (3) substitution reaction with sodium cyanide in dimethyl sulfoxide. The nitrile **35** was converted to a methyl ester **36** by (1) reduction with diisobutylaluminum hydride; (2) oxidation with cromium trioxide, and (3) esterification with diazomethane. Deprotection of **36** followed by oxidation afforded **38**.

Synthesis of 9β-halo PG analogues 2, 6, 7, 9, 10 and 12

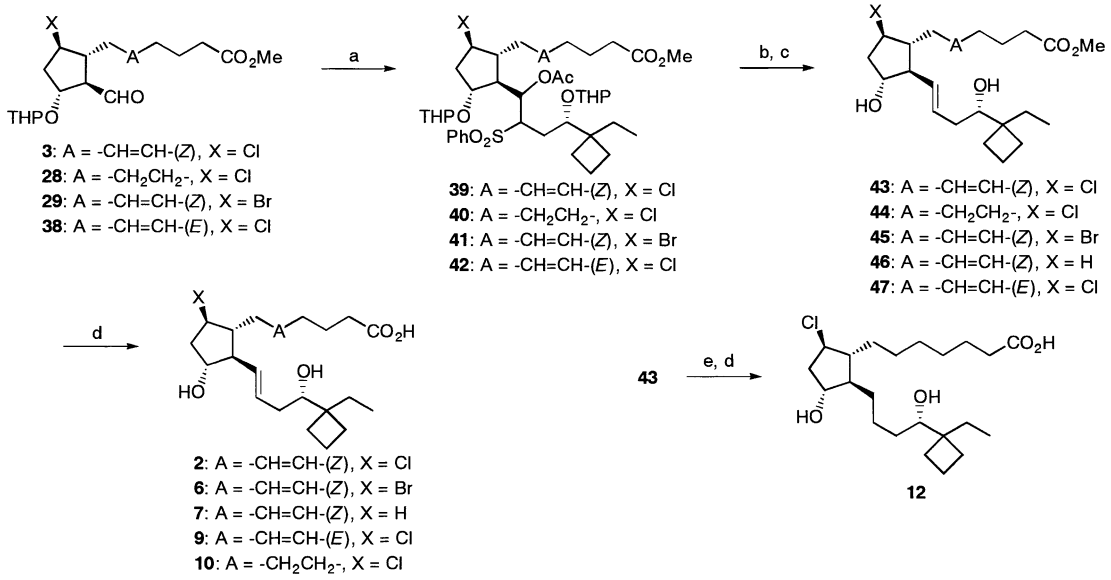
Julia olefination of **3** with **4** using powdered magnesium[2] was conducted as shown in Scheme 5. Addition reaction of the carbanion, which was generated from **4**, to the aldehyde **3** afforded **39** as a diastereomeric mixture. Reductive elimination of **39** followed by acidic deprotection afforded 13(*E*)-olefin **43**, the corresponding 13(*Z*)-isomer of which was produced as a by-product ($E/Z = 5.0/1$)[10] and easily removed by silica gel column chromatography. Alkaline hydrolysis of **43** provided **2**. Catalytic hydrogenation of **43** followed by alkaline hydrolysis afforded **12**. Compounds **6**, **9** and **10** were synthesized from **29**, **38** and **28**, respectively, according to the same procedures used for the preparation of **2** from **3**. The compound **7** was produced as a by-product in the reductive elimination of **41**.

The absolute configuration of the 16-OH in **2** was determined by X-ray analysis. X-Ray analysis of a salt consisting of a 1/1 molar ratio of **2** and L-lysine was carried out in our laboratory. As shown in Figure 1, 16-OH was found to be of the *S*-configuration based on the absolute configuration of L-lysine in the crystal lattice.

Inversion of the 9α-hydroxy group of **48** by the Mitsunobu reaction followed by aminolysis with aqueous



Scheme 4. Synthesis of 9β-chloro 5(*E*)-aldehyde **38**. Reagents: (a) TBSCl, imidazole, DMF; (b) $LiAlH_4$, THF; (c) Ac_2O, collidine, CH_2Cl_2; (d) TsCl, pyridine; (e) *n*-Bu_4NCl, toluene; (f) NaOHaq, MeOH; (g) SO_3–pyridine, Et_3N, DMSO, CH_2Cl_2; (h) CH_2=CHMgBr, THF; (i) $CH_3CH(OEt)_3$, $EtCO_2H$, 140 °C; (j) NaCN, DMSO, 100 °C; (k) *i*-Bu_2AlH, CH_2Cl_2, then HClaq; (l) CrO_3, H^+, H_2O; (m) CH_2N_2; (n) *n*-Bu_4NF, THF.



Scheme 5. Synthesis of 9β-halo PG analogues **2**, **6**, **7**, **9**, **10** and **12**. Reagents: (a) **4**, *n*-BuLi, THF, −78 °C; (b) Mg (powdered, −50 mesh), TMSCl, MeOH; (c) *p*-TsOH, MeOH; (d) NaOHaq, MeOH; (e) Pd/C, H_2, MeOH; (f) L-lysine, EtOH, EtOAc.

ammonia afforded **49**, which was converted to **52** by (1) tosylation of the 9β-hydroxy group of **49**, (2) a substitution reaction with tetrabutylammonium chloride, and (3) deprotection under acidic conditions.

Conversion of **48** to **51** was carried out by (1) tosylation of the 9α-hydroxy group of **48** and (2) a S_N2 substitution reaction with tetrabutylammonium fluoride.

The 13,14-dihydro derivative **50** was converted to a 9β-chloro derivative **53** by the tosylation of **50** followed by a S_N2 substitution reaction with tetrabutylammonium chloride. Alkaline hydrolysis of **51**, **52** and **53** afforded **5, 8** and **11**, respectively.

Results and Discussion

A series of 9-halo-15-deoxy-16-hydroxy-17,17-trimethylene PGF analogues, which were prepared according to the magnesium-mediated Julia olefination described in Schemes 1–6, were biologically evaluated for their affinity to the mouse (m) EP1–4-receptors and their ability to increase the intracellular cAMP concentration. Among the compounds tested, **2** demonstrated the best profile in the EP2-receptor affinity, EP2-receptor selectivity and functional activity (EC_{50}). The EC_{50} value of **2** was similar to that of PGE_2. The compound **2** was nearly 10-fold more potent in EP2-receptor affinity and had nearly 3-fold greater EC_{50} value than the corresponding 9-keto analogue **1**.

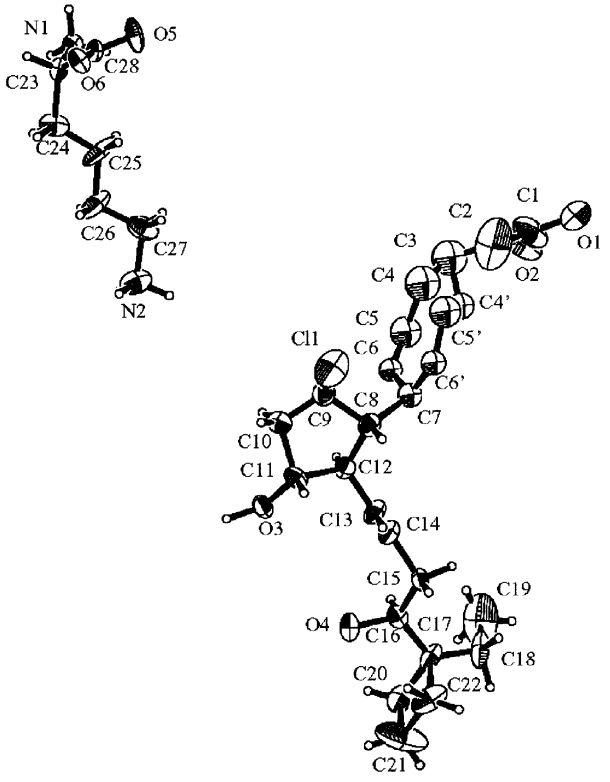
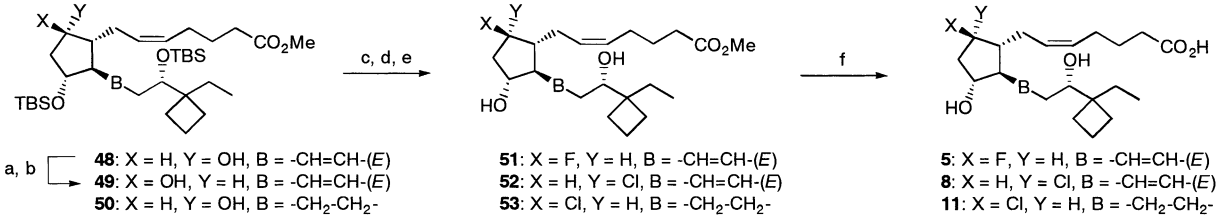


Fig 1. X-ray crystallographic structure of **2Ly**.

Results of the biological evaluation of the other 9-halo-16-hydroxy-17,17-trimethylene PG analogues are shown in Table 1. Replacement of the 9β-chloro group in **2** with a fluorine atom and a bromine atom afforded a 9β-fluoro analogue **5** and a 9β-bromo analogue **6** which retained the EP2-receptor affinity and selectivity, respectively, while losing some of the EP2-receptor agonistic activity (EC_{50} value). Removal of the 9-chloro group from **2** produced **7** with a marked reduction in agonistic activity although it still showed EP2-receptor affinity and selectivity. These results clearly indicate that the 9β-chloro group of **2** plays an important role in both the receptor affinity and agonistic activity. The corresponding 9α-chloro analogue **8** was also found to be a selective EP2-receptor agonist while in receptor affinity and agonistic activity it was less potent than 9β-chloro analogue **2**.

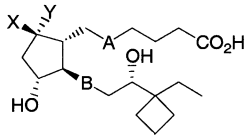
Conversion of the 5(*Z*)-double bond of **2** to a 5(*E*)-double bond and saturated ethylene moiety provided **9** and **10**, respectively, also with a reduction in receptor affinity and agonistic activity. Saturation of the 13(*E*)-double bond of **2** afforded **11** with a fair reduction in activity though its EP2-receptor affinity was nearly 4 times less potent than that of **2**. Saturation of both of the two double bonds of **2** gave **12** with a marked reduction in agonistic activity. Compounds **10**, **11** and **12**, the intramolecular double bonds of which were partially or thoroughly reduced, showed weak affinity to other receptors such as EP1, EP3 and EP4. Therefore, both of the intramolecular 5(*Z*)- and 13(*E*)-double bonds of **2** were indispensable for potent receptor affinity, agonistic activity and EP2-receptor selectivity.

The 16(*R*)-diastereomer **13** had nearly a 100-fold lower EC_{50} value than **2** while its EP2-receptor affinity was 4.8-fold less potent than that of **2**. Besides, **13** exhibited weak affinity to the EP1-receptor ($K_i = 2200$ nM) and the EP3-receptor ($K_i = 170$ nM).



Scheme 6. Synthesis of other 9-halo PG analogues **5**, **8** and **11**. Reagents: (a) diethylazodicarboxylate, PPh_3, HCOOH, THF; (b) NH_3aq; (c) TsCl, pyridine; (d) *n*-Bu_4NF or *n*-Bu_4NCl, toluene; (e) HFaq, MeCN; (f) NaOHaq, MeOH.

Table 1. Biological evaluation of 9-halo-16-hydroxy-17,17-trimethylene PG analogues



Compds	X, Y	A	B	Binding K_i (nM)[a]				EC_{50}[b]
				mEP1	mEP2	mEP3	mEP4	mEP2
1	X = Y = O	–CH=CH– (*Z*)	–CH=CH– (*E*)	$>10^4$	30	$>10^4$	$>10^4$	11
2	X = Cl, Y = H	–CH=CH– (*Z*)	–CH=CH– (*E*)	$>10^4$	3.3	$>10^4$	6100	3.8
5	X = F, Y = H	–CH=CH– (*Z*)	–CH=CH– (*E*)	$>10^4$	7.3	$>10^4$	$>10^4$	10
6	X = Br, Y = H	–CH=CH– (*Z*)	–CH=CH– (*E*)	$>10^4$	2.4	$>10^4$	$>10^4$	25
7	X = Y = H	–CH=CH– (*Z*)	–CH=CH– (*E*)	5100	11	$>10^4$	$>10^4$	1400
8	X = H, Y = Cl	–CH=CH– (*Z*)	–CH=CH– (*E*)	$>10^4$	13	$>10^4$	$>10^4$	39
9	X = Cl, Y = H	–CH=CH– (*E*)	–CH=CH– (*E*)	$>10^4$	8.1	$>10^4$	$>10^4$	31
10	X = Cl, Y = H	$–CH_2–CH_2–$	–CH=CH– (*E*)	5700	8.1	$>10^4$	3100	66
11	X = Cl, Y = H	–CH=CH– (*Z*)	$–CH_2–CH_2–$	$>10^4$	13	2300	$>10^4$	79
12	X = Cl, Y = H	$–CH_2–CH_2–$	$–CH_2–CH_2–$	3500	17	$>10^4$	$>10^4$	590
13(16*R*-OH)	X = Cl, Y = H	–CH=CH– (*Z*)	–CH=CH– (*E*)	2200	16	170	$>10^4$	330
PGE_2				18	38	5.0	3.1	2.0

[a]Using membrane fractions of Chinese hamster ovary (CHO) cells expressing the mouse prostanoid receptors, K_i values were determined by the competitive binding assay, which was performed according to the method of Kiriyama et al.[13] with some modifications. When the test compound did not displace binding of radioligands by 50% even at a concentration of 10^4 nM, the K_i value was not determined (expressed $>10^4$).
[b]With regard to the subtype-receptor agonistic activity, EC_{50} values were determined based on the effect of the test compounds on the increase in the intracellular cAMP production in the mouse EP2 receptor.

Summary

The enantioselective synthesis of the ω-chain segment **4** followed by a magnesium-mediated Julia olefination with **3** provided a practical route to **2**. Compounds **6**, **7**, **9**, **10** and **12** were prepared essentially as described above. Other 9-halo-PGF analogues, **5**, **8** and **11**, were also prepared as described in Scheme 6. All of the synthesized 9-halo PGF analogues, **1**–**2** and **5**–**13**, were biologically evaluated. Among the compounds, **2** was the most effective EP2-receptor agonist with regard to subtype selectivity and agonistic activity.

Experimental

General directions

Analytical samples were homogeneous as confirmed by TLC, and afforded spectroscopic results consistent with the assigned structures. 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}).

4,4-Trimethylene-2-hexen-1-ol (15). To a stirred suspension of sodium hydride (306 g, 7.65 mol) in 6 L of THF was added ethyl diethylphosphonoacetate (1869 g, 8.34 mol) in 2 L of THF at 0 °C and the stirring was continued for 30 min at room temperature under an argon atmosphere. To the resulting solution was added the aldehyde **14**[1b] (780 g, 6.95 mol) at 0 °C and the stirring was continued for 30 min at room temperature. The reaction mixture was treated 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 afford a crude oil, which was purified by column chromatography on silica gel to give ethyl 4,4-trimethylene-2-hexenoate (1134 g, 81%) as a colorless oil. TLC $R_f = 0.53$ (*n*-hexane/EtOAc, 9/1); IR (neat) 2963, 2934, 2855, 1721, 1651, 1463, 1366, 1305, 1267, 1178, 1096, 1041, 986, 861, 721 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 6.98 (d, $J = 15.8$ Hz, 1H), 5.77 (d, $J = 15.8$ Hz, 1H), 4.20 (q, $J = 7.2$ Hz, 2H), 2.10–1.80 (m, 6H), 1.63 (q, $J = 7.4$ Hz, 2H), 1.31 (t, $J = 7.2$ Hz, 3H), 0.77 (t, $J = 7.4$ Hz, 3H); MS (APCI, Pos, 12V) $m/z = 183$ $(M + H)^+$.

To a stirred solution of ethyl 4,4-trimethylene-2-hexenoate (1134 g, 6.22 mol) in 10 L of THF was slowly added diisobutylaluminum hydride (25% in toluene, 7793 g, 13.7 mol) at 0 °C under an argon atmosphere. After stirring for 30 min at 0 °C, the reaction mixture was treated with 1 L of methanol and 24 L of 2 N aqueous hydrochloric acid and extracted with EtOAc. The organic layer was washed with water, then brine, dried over anhydrous magnesium sulfate and concentrated in vacuo to afford a crude oil, which was purified by column chromatography on silica gel to give 4,4-trimethylene-2-hexen-1-ol **15** (678 g, 78%) as a colorless oil.

TLC R_f = 0.39 (*n*-hexane/EtOAc, 4/1); IR (neat) 3322, 2962, 2933, 2875, 2853, 1461, 1377, 1089, 1012, 971 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.71 (d, J = 15.6 Hz, 1H), 5.58 (dt, J = 15.6, 5.0 Hz), 4.18 (m, 2H), 2.00–1.70 (m, 6H), 1.54 (q, J = 7.5 Hz, 2H), 1.30 (br, 1H), 0.75 (t, J = 7.5 Hz, 3H); MS (APCI, Pos, 12V) m/z = 123 $(M+H-H_2O)^+$.

(2*R*,3*R*)-2,3-Epoxy-4,4-trimethylene-1-hexanol (16). To a stirred suspension of 480 g of powdered 3A, activated molecular sieves in 8 L of CH_2Cl_2 were added sequentially with titanium tetraisopropoxide (275 g, 0.968 mol) and a solution of D-(−)-diisopropyltartarate (272 g, 1.16 mol) in 1 L of CH_2Cl_2 at −20 °C under an argon atmosphere. After stirring for 1 h at −20 °C, a solution of the allyl alcohol **15** (678 g, 4.84 mol) in 1 L of CH_2Cl_2 was added and the resulting mixture was stirred for 0.5 h, whereupon cumyl hydroperoxide (80% in cumene, 1381 g, 7.26 mol) was added. Stirring was maintained for 3 h at −20 °C. The resulting reaction mixture was treated with dimethyl sulfide (1064 mL, 14.5 mol) and stirred for 3 h at −20 °C. A 10% aqueous solution of tartaric acid was then added and the resulting heterogeneous mixture was filtered to remove molecular sieves. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with aqueous sodium chloride, dried over anhydrous magnesium sulfate and concentrated in vacuo to afford a crude epoxy alcohol **16** (4.84 mol, quant) as a colorless oil. TLC R_f = 0.20 (*n*-hexane/EtOAc, 4/1); IR (neat) 3402, 2975, 2935, 2877, 1446, 1377, 1256, 1175, 1102, 1074, 1030, 955, 862, 763, 700 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.02–3.90 (m, 1H), 3.72–3.58 (m, 1H), 2.95–2.88 (m, 1H), 2.82 (d, J = 2.2 Hz, 1H), 2.10–1.40 (m, 9H), 0.87 (t, J = 7.4 Hz, 3H); MS (APCI, Pos, 12V) m/z = 157 $(M+H)^+$.

(3*R*)-4,4-(Trimethylene)hexane-1,3-diol (17). To a stirred solution of bis(2-methoxyethoxy)aluminum hydride (72% in toluene, 6814 g, 24.2 mol) in 13 L of THF was slowly added the epoxyalcohol **16** (756 g, 4.84 mol) in THF under an argon atmosphere. After stirring for 4 h, the reaction mixture was cooled in an ice-bath and treated with 1400 mL of methanol and 25 L of 2 N aqueous sodium hydroxide. The mixture was then extracted with *t*-butyl methyl ether repeatedly. The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo to afford a crude oil, which was purified by column chromatography on silica gel to give the diol **17** (730 g, 95%) as a white solid. TLC R_f = 0.27 (*n*-hexane/EtOAc, 1/1); IR (neat) 3360, 2962, 2936, 2879, 1463, 1430, 1379, 1055 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.00–3.76 (m, 3H), 2.52 (br, 1H), 2.28 (br, 1H), 2.05–1.30 (m, 10H), 0.92 (t, J = 7.5 Hz, 3H); MS (APCI, Pos, 20V) m/z = 159 $(M+H)^+$, 141, 123.

(3*R*)-1-Phenylthio-4,4-(trimethylene)hexan-3-ol (18). To a stirred heterogeneous mixture of diol **17** (730 g, 4.61 mol), tetrabutylammonium bromide (148 g, 0.461 mol) in 4 L of toluene and 7 L of 2 N aqueous sodium hydroxide, was slowly added *p*-toluenesulfonyl chloride (922 g, 4.84 mol) in 1 L of toluene in an ice-bath. After stirring for 1 h at room temperature, thiophenol (559 g, 5.07 mol) in 1 L of toluene was added to the reaction mixture. After stirring for additional 1 h, the aqueous layer was separated. The organic layer was washed with water three times and concentrated in vacuo to afford a sulfide **18** (1144 g, 99%) as a colorless oil. TLC R_f = 0.52 (*n*-hexane/EtOAc, 4/1); IR (neat) 3441, 2962, 1584, 1481, 1439, 1310, 1272, 1071, 1026, 737, 690 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.40–7.10 (m, 5H), 3.80–3.65 (m, 1H), 3.28–2.94 (m, 2H), 2.00–1.20 (m, 11H), 0.88 (t, J = 7.5 Hz, 3H); MS (APCI, Pos, 12V) m/z = 233 $(M+H-H_2O)^+$.

(3*R*)-1-Benzenesulfonyl-4,4-(trimethylene)hexan-3-ol (19). To a stirred solution of **18** (1144 g, 4.57 mol) in 19 L of methanol was added a solution of Oxone® (4211 g, 13.7 mol as $KHSO_5$) in 19 L of water at 0–10 °C. After stirring for 3 h at below 20 °C, the reaction mixture was diluted with 15 L of water and extracted with EtOAc. The organic layer was washed with water, then brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo to afford a crude sulfone **19** (1291 g, quant) as an orange oil.

To a stirred mixture of the sulfone **19** (1291 g, 4.57 mol) and 4-(dimethylamino)pyridine (56 g, 0.457 mol) in 12 L of pyridine was added benzoyl chloride (964 g, 6.86 mol) at room temperature under an argon atmosphere. After stirring for 6 h at 50 °C, the reaction mixture was treated with water and extracted with EtOAc. The organic layer was washed successively with 2 N aqueous hydrogen chloride, water, saturated aqueous sodium bicarbonate, water, and finally brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo to afford a crude crystal, which was purified by recrystallization from 13 L of EtOH to give an optically pure (>99%ee) benzoate **20** (1250 g, 71%) as a pale yellow crystal. TLC R_f = 0.58 (*n*-hexane/EtOAc, 2/1); IR (KBr) 2983, 2962, 2940, 1710, 1320, 1278, 1153, 1113, 1087, 715, 598, 531 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.05–7.93 (m, 2H), 7.93–7.82 (m, 2H), 7.70–7.40 (m, 6H), 5.28–5.18 (m, 1H), 3.26–2.98 (m, 2H), 2.20–1.40 (m, 10H), 0.95 (t, J = 7.6 Hz, 3H); MS (APCI, Pos, 20V) m/z = 387 $(M+H)^+$, 265, 123.

To a solution of the benzoate **20** (1250 g, 3.23 mol) in 4.5 L of THF and 9 L of methanol was added 4 L of 2 N aqueous sodium hydroxide at room temperature. After stirring for 2 h at 50 °C, the reaction mixture was diluted with 15 L of water and extracted with 10 L of *t*-butyl methyl ether twice. The combined organic layers were washed with water, then brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo to afford the sulfone **19** (912 g, quant) as a colorless oil. TLC R_f = 0.32 (*n*-hexane/EtOAc, 2/1); IR (neat) 3525, 2963, 2936, 2878, 1447, 1304, 1148, 1087, 1072, 748, 689, 599, 533 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.00–7.90 (m, 2H), 7.70–7.50 (m, 3H), 3.66–3.55 (m, 1H), 3.50–3.10 (m, 2H), 2.00–1.30 (m, 11H), 0.88 (t, J = 7.5 Hz, 3H); MS (APCI, Pos, 12V) m/z = 283 $(M+H)^+$, 265 $(M+H-H_2O)^+$.

(3*R*)-1-Benzenesulfonyl-3-(tetrahydro-2*H*-pyran-2-yl)oxy-4,4-trimethylenehexane (4). To a stirred solution of the sulfone **19** (1005 g, 3.55 mol) in 7.5 L of CH_2Cl_2 was added *p*-toluene sulfonic acid (10 g, 0.04 mol) under an argon atmosphere. The mixture was cooled to below 10 °C, and then 3,4-dihydro-2*H*-pyran (359 g, 4.26 mmol) was slowly added. After the solution had been stirred for 1 h at 20–30 °C, the reaction was quenched with saturated aqueous sodium bicarbonate and the mixture was extracted with EtOAc. The organic layer was washed with water, then brine, dried over anhydrous magnesium sulfate and concentrated in vacuo to afford a crude oil, which was purified by column chromatography on silica gel to give the compound **4** (1244 g, 96%) as a colorless oil. TLC R_f = 0.42 and 0.37 (*n*-hexane/EtOAc, 4/1); IR (neat) 2939, 2864, 1446, 1305, 1150, 1075, 1031, 987 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.00–7.90 (m, 2H), 7.70–7.50 (m, 3H), 4.40 (m, 1H), 3.94–3.70 (m, 1H), 3.62–3.28 (m, 3H), 3.17–3.04 (m, 1H), 2.24–1.20 (m, 16H), 0.85 (t, J = 7.4 Hz, 3H); MS (APCI, Pos, 20V) m/z = 265 $(M+H-C_5H_{10}O_2)^+$, 123.

(5*Z*)-7-{(1*R*,2*S*,3*R*,5*S*)-5-Methanesulfonyloxy-2-[(1-methoxy-1-methylethoxy)methyl]-3-(tetrahydro-2*H*-pyran-2-yloxy)cyclopentyl}hept-5-enoic acid methyl ester (22). To a stirred mixture of **21** (90.0 g, 0.210 mol), triethylamine (42.5 g, 0.420 mol) and potassium carbonate (48.5 g, 0.351 mol) in 420 mL of CH_2Cl_2, was slowly added methanesulfonyl chloride (36.1 g, 0.315 mol) at −5 °C under an argon atmosphere. After stirring for 30 min, the reaction mixture was quenched with saturated aqueous sodium bicarbonate and layers were separated. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were washed with water, dried over anhydrous magnesium sulfate, and concentrated in vacuo to afford a mesylate **22** (111 g, quant) as a pale yellow oil. TLC R_f = 0.60 (CH_2Cl_2/EtOAc, 4/1); IR (neat) 2990, 2942, 2872, 1736, 1455, 1174 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.55–5.37 (m, 2H), 5.12–5.02 (m, 1H), 4.71–4.58 (m, 1H), 4.25–4.07 (m, 1H), 3.97–3.79 (m, 1H), 3.67 (s, 3H), 3.67–3.30 (m, 3H), 3.20 (s, 3H), 3.05 and 3.01 (2s, 3H), 2.37–1.40 (m, 18H), 1.33 (s, 6H).

7-{(1*R*,2*S*,3*R*,5*R*)-5-Chloro-2-[(1-methoxy-1-methylethoxy)methyl]-3-(tetrahydro-2*H*-pyran-2-yloxy)cyclopentyl}hept-5-enoic acid methyl ester (23). To a stirred mixture of tetrabutylammonium chloride (104.1 g, 0.374 mol) and triethylamine (105.2 g, 1.04 mol) in 520 mL of toluene was added **22** (105.4 g, 0.208 mol) under an argon atmosphere. After stirring for 20 h at 50 °C, the reaction mixture was cooled to room temperature and treated with water. The mixture was extracted with EtOAc and the organic layer was washed with water repeatedly, and then with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo to afford **23** (90.5 g, 97%) as a colorless oil. The ratio of the desired 9β-chloro product to the $\Delta^{8,9}$-olefinic by-product was approximately 5.4/1. This by-product was removed by column chromatography on silica gel in the next step. TLC R_f = 0.86 (CH_2Cl_2/EtOAc, 4/1); IR (neat) 2943, 2871, 2738, 1740, 1438, 1380, 1367 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.58–5.28 (m, 2H), 4.70–4.58 (m, 1H), 4.20–3.78 (m, 3H), 3.67 (s, 3H), 3.60–3.20 (m, 3H), 3.20 (s, 3H), 2.38–1.40 (m, 18H), 1.32 (s, 6H).

(5*Z*)-7-[(1*R*,2*S*,3*R*,5*R*)-5-Chloro-2-hydroxymethyl-3-(tetrahydro-2*H*-pyran-2-yloxy)cyclopentyl]hept-5-enoic acid methyl ester (25). To a stirred mixture of **23** (89.4 g, 0.200 mol) in 357 mL of THF was added 1 N aqueous hydrochloric acid (120 mL, 0.120 mol) below −5 °C. After stirring for 30 min at −5 °C, the reaction mixture was poured into ice-water and treated with saturated aqueous sodium bicarbonate. The mixture was then diluted with 700 mL of EtOAc. The organic layer was separated and 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 afford a crude oil as a mixture of **25** and the corresponding $\Delta^{8,9}$-olefinic by-product. The residue was purified by column chromatography on silica gel to give **25** (45.8 g, 61%) as a colorless viscous oil. TLC R_f = 0.60 (*n*-hexane/EtOAc, 1/1); MS (FAB, Pos.) m/z = 375 $(M+H)^+$, 291; IR (neat) 3460, 2944, 1740, 1440, 1353, 1202, 1136, 1023, 972, 870, 812 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.60–5.33 (m, 2H), 4.70 (m, 0.35H), 4.57 (m, 0.65H), 4.32–3.40 (m, 9H), 2.40–1.33 (m, 18H). The corresponding bromo-derivative **27** was synthesized from **22** according to the procedure described above, except that tetrabutylammonium bromide was used instead of tetrabutylammonium chloride.

7-[(1*R*,2*S*,3*R*,5*R*)-5-Chloro-2-hydroxymethyl-3-(tetrahydro-2*H*-pyran-2-yloxy)cyclopentyl]heptanoic acid methyl ester (26). A mixture of **25** (500 mg, 1.33 mmol) and 5% Pd/C in 10 mL of ethanol was stirred for 3 h at ambient temperature under a hydrogen atmosphere. The resulting reaction mixture was filtered and concentrated in vacuo to afford **26** (500 mg, quant) as an oil. TLC R_f = 0.40 (*n*-hexane/EtOAc, 1/1); ^1H NMR (200 MHz, $CDCl_3$) δ 4.78–4.54 (m, 1H), 4.35–4.30 (m, 6H), 3.67 (s, 3H), 2.31 (t, J = 7.5 Hz, 2H), 2.30–2.10 (m, 2H), 2.00–1.20 (m, 19H).

(5*Z*)-7-[(1*R*,2*S*,3*R*,5*R*)-5-Chloro-2-formyl-3-(tetrahydro-2*H*-pyran-2-yloxy)cyclopentyl]hept-5-enoic acid methyl ester (3). To a stirred solution of **25** (45.2 g, 0.121 mol) and triethylamine (73.2 g, 0.727 mol) in 358 mL of dimethyl sulfoxide was slowly added a solution of sulfur trioxide-pyridine complex (57.6 g, 0.362 mol) in 215 mL of dimethylsulfoxide at 20 °C under an argon atmosphere. After stirring for 0.5 h at 20 °C, the reaction mixture was poured into ice-cold aqueous ammonium chloride and extracted with EtOAc repeatedly. The combined organic layers were washed successively with ice-cold 0.2 N aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate, water, and finally brine, before being dried over anhydrous magnesium sulfate and concentrated in vacuo to afford **3** (46.9 g, quant) as a dark-brown oil. TLC R_f = 0.60 (*n*-hexane/EtOAc, 2/1); IR (neat) 2947, 2868, 2736, 1439, 1352, 1323, 1247, 1201, 1154, 1133, 1077, 1034, 1021, 970 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.76 and 9.73 (2×d, J = 2.0 Hz, 1H), 5.60–5.30 (m, 2H), 4.65–4.50 (m, 2H), 4.15–3.95 (m, 1H), 3.90–3.70 (m, 1H), 3.67 (s, 3H), 3.60–3.40 (m, 1H), 2.80–1.40 (m, 18H). The compounds

28 and **29** was synthesized from **26** and **27**, respectively, according to the procedure described above.

7-[(1*R*,2*S*,3*R*,5*R*)-5-Chloro-2-formyl-3-(tetrahydro-2*H*-pyran-2-yloxy)cyclopentyl]heptanoic acid methyl ester (28). Oil. TLC R_f = 0.34 (*n*-hexane/EtOAc, 2/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.77 and 9.74 (2×d, J = 2.0 Hz, 1H), 4.70–4.50 (m, 2H), 4.20–4.00 (m, 1H), 3.90–3.70 (m, 1H), 3.67 (s, 3H), 3.60–3.40 (m, 1H), 2.80–2.00 (m, 6H), 2.00–1.20 (m, 16H).

(1*S*,2*R*,3*S*,4*R*)-3-(*t*-Butyldimethylsiloxy)methyl-2-(2-hydroxyethyl)-4-(tetrahydro-2*H*-pyran-2-yloxy)cyclopentanol (31). To a solution of the lactone **30** (21.0 g, 81.9 mmol) and imidazole (10.0 g, 147 mmol) in 50 mL of dimethyl formamide was added *t*-butyldimethylsilyl chloride (14.8 g, 98.3 mmol) at ambient temperature. After stirring overnight, the reaction mixture was poured into ice-water and extracted with hexane. The organic layer was washed with water, then brine, dried over anhydrous sodium sulfate, and concentrated in vacuo to afford a TBS ether (30.3 g, quant) as an oil. To a stirred suspension of lithium aluminum hydride (3.07 g, 81.0 mmol) in 100 mL of THF was slowly added the TBS ether (30.3 g) in 150 mL of tetrahydrofuran over 2 h at ambient temperature. After the mixture had been stirred for 1 h at ambient temperature, the reaction was quenched with a small amount of methanol and saturated aqueous sodium sulfate. The resulting insoluble substance was removed by filtration and the filtrate was concentrated in vacuo to afford **31** (30.4 g, 99%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.69 (m, 1H), 4.30–4.08 (m, 2H), 3.96–3.36 (m, 6H), 3.00–2.20 (br, 2H), 2.15–1.42 (m, 12H), 0.90 (m, 9H), 0.08 (s, 6H).

(1*R*,2*S*,3*R*,5*R*)-[2-(*t*-Butyldimethylsiloxy)methyl-5-chloro-3-(tetrahydro-2*H*-pyran-2-yloxy)cyclopentyl]ethanol (32). To a stirred solution of **31** (30.4 g, 81.0 mmol) and 2,4,6-trimethylpyridine (21.4 mL, 162 mmol) in 200 mL of dichloromethane was added acetyl chloride (7.91 mL, 111 mmol) at −78 °C under an argon atmosphere. After the mixture had been stirred for 1 h at −78 °C, the reaction was quenched with 1.86 mL of methanol. The mixture was poured into ice-water and extracted with EtOAc. The organic layer was washed with water, then brine, dried over anhydrous sodium sulfate, and concentrated in vacuo to afford a crude oil, which was purified by column chromatography on silica gel to give an acetate (26.3 g, 67.2 mmol) as a colorless oil.

To a stirred solution of the acetate (25.9 g, 62.2 mmol) and 180 mL of pyridine was added *p*-toluenesulfonyl chloride (119 g, 622 mmol) at 0 °C and the mixture was stirred for 16 h at ambient temperature. The reaction was quenched with 10 mL of water and the mixture was poured into ice-water, and extracted with EtOAc. The organic layer was washed first with water, and then with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo to afford a tosylate (35.5 g, quant). A mixture of this tosylate (35.5 g) and tetrabutylammonium chloride (34.4 g, 124 mmol), potassium carbonate (25.7 g, 186 mmol) and 500 mL of toluene was stirred for 4 h at 50 °C. The reaction mixture was poured into ice-water and extracted with 25% EtOAc–hexane. The organic layer was washed with water, then brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo to afford a crude oil, which was purified by column chromatography on silica gel to give a chloro compound (21.0 g, 78%) as a colorless oil.

To a stirred solution of this compound (21.0 g) in 50 mL of dimethoxyethane and 150 mL of methanol was added 2 N aqueous sodium hydroxide (50 mL, 100 mmol) at 0 °C. After stirring for 0.5 h at room temperature, the reaction mixture was poured into ice-water and extracted with 25% EtOAc–hexane. The organic layer was washed with water, followed by brine, dried over anhydrous sodium sulfate and concentrated in vacuo to afford a crude oil, which was purified by column chromatography on silica gel to give **32** (15.7 g, 83%) as a colorless oil. TLC R_f = 0.40 (*n*-hexane/EtOAc, 2/1); MS (APCI, pos, 20V) m/z = 309 $(M-THP+H)^+$, 273; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.60 (m, 1H), 4.20–3.94 (m, 2H), 3.95–3.40 (m, 6H), 2.57–1.33 (m, 12H), 0.92 (s, 9H), 0.08 (s, 6H).

(4*E*)-6-[(1*R*,2*S*,3*R*,5*R*)-2-(*t*-Butyldimethylsiloxy)methyl-5-chloro-3-(tetrahydro-2*H*-pyran-2-yloxy)cyclopentyl]-hex-4-enoic acid ethyl ester (34). To a stirred solution of **32** (15.6 g, 39.7 mmol) in 15 mL of dimethyl sulfoxide was added sequentially 30 mL of triethylamine and sulfur trioxide–pyridine complex (18.9 g, 119 mmol) at ambient temperature. After the mixture had been stirred for 1 h, the reaction was quenched with water. Following extraction with 25% EtOAc–hexane, the organic layer was washed with water, then brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo to afford the corresponding aldehyde (15.5 g, quant) as a pale yellow oil. To a stirred solution of the aldehyde (12.9 g, 32.0 mmol) in 200 mL of THF was added a 1.0 M THF solution of vinyl magnesium bromide (64 mL, 64 mmol) at −78 °C under an argon atmosphere. After stirring for 1 h at −78 °C, the reaction mixture was treated with saturated aqueous ammonium chloride and extracted with 60% ether–hexane. The organic layer was washed with water, followed by brine, dried over anhydrous sodium sulfate, and concentrated in vacuo to afford **33** (13.4 g) as an oil. TLC R_f = 0.18 (*n*-hexane/EtOAc, 5/1).

A mixture of **33** (13.4 g, 32 mmol), 80 mL of orthoacetic acid triethyl ester and catalytic amount of propionic acid (ca. 250 mg) was stirred for 2 h at 140 °C. After it had cooled, 0.5 mL of triethylamine was added. The resulting mixture was concentrated in vacuo to afford a crude oil, which was purified by column chromatography on silica gel to give **34** (9.90 g, 63%) as a colorless oil. TLC R_f = 0.44 (*n*-hexane/EtOAc, 5/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.53–5.42 (m, 2H), 4.59 (m, 1H), 4.22–3.40 (m, 8H), 2.46–1.38 (m, 19H), 0.92 (s, 9H), 0.07 (s, 6H).

(5*E*)-7-[(1*R*,2*S*,3*R*,5*R*)-2-(*t*-Butyldimethylsiloxy)methyl-5-chloro-3-(tetrahydro-2*H*-pyran-2-yloxy)cyclopentyl]-hept-5-enenitrile (35). To a stirred suspension of lithium aluminum hydride (1.15 g, 30.4 mmol) in 100 mL of

THF was slowly added **34** (9.90 g, 20.2 mmol) in 150 mL of THF at ambient temperature. After the mixture had been stirred for 0.5 h at ambient temperature, the reaction was quenched with 3.3 mL of methanol and 20 mL of saturated aqueous sodium sulfate. The resulting insoluble substance was removed by filtration and the filtrate was concentrated in vacuo to afford an alcohol (9.03 g, quant) as a colorless viscous oil. A mixture of this alcohol and *p*-toluenesulfonyl chloride (9.65 g, 50.6 mmol) in 70 mL of pyridine was stirred for 5 h at ambient temperature. The reaction was quenched with 0.6 mL of water and the mixture poured into ice-water. After extraction with ether, the organic layer was washed with water, then brine, dried over anhydrous sodium sulfate, and concentrated in vacuo to afford a tosylate (12.1 g, quant.). A mixture of the crude tosylate (12.1 g) and sodium cyanide (1.49 g, 30.3 mmol) in 70 mL of dimethyl sulfoxide was stirred for 0.5 h at 100 °C. The reaction mixture was poured into ice-water and extracted with ether. The organic layer was washed with water, then brine, dried over anhydrous sodium sulfate, and concentrated in vacuo to afford a crude oil, which was purified by column chromatography on silica gel to give **35** (6.02 g, 67% in three steps) as a colorless viscous oil. TLC $R_f = 0.52$ (benzene/EtOAc, 10/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.57–5.47 (m, 1H), 5.43 (dt, $J = 15.5$, 6.5 Hz, 1H), 4.59 (m, 1H), 4.16 (ddd, $J = 6.5$, 3.5, 3.5 Hz, 0.6H), 4.12 (ddd, $J = 6.5$, 2.5, 2.5 Hz, 0.4H), 4.02 (m, 0.4H), 3.97 (ddd, $J = 9.0$, 9.0, 6.5 Hz, 0.6H), 3.89–3.81 (m, 1H), 3.70 (dd, $J = 10.0$, 5.0 Hz, 0.6H), 3.62 (dd, $J = 10.0$, 5.0 Hz, 0.4H), 3.58 (dd, $J = 10.0$, 5.0 Hz, 0.6H), 3.52–3.45 (m, 1.4H), 2.37–2.15 (m, 6H), 0.92 (s, 9H), 0.07 (s, 6H).

(5*E*)-7-[(1*R*,2*S*,3*R*,5*R*)-2-(*t*-Butyldimethylsiloxy)methyl-5-chloro-3-(tetrahydro-2*H*-pyran-2-yloxy)cyclopentyl]-hept-5-enoic acid methyl ester (36). To a stirred solution of **35** (5.98 g, 13.1 mmol) in 150 mL of CH_2Cl_2 was added a 1.01 M toluene solution of diisobutylaluminum hydride (14.3 mL, 14.4 mmol) at −70 °C under an argon atmosphere. The mixture was stirred for 1 h at −40 °C, and the mixture was quenched with a small amount of methanol and 1 N aqueous hydrochloric acid. After extraction with EtOAc, the organic layer was washed successively with water, saturated aqueous sodium bicarbonate, water, and finally brine, dried over anhydrous sodium sulfate, and concentrated in vacuo to afford a crude aldehyde (5.96 g) as an oil. To a stirred solution of the crude aldehyde (5.96 g, ca. 13 mmol) in 150 mL of acetone was added Jones' Reagent (7.5 mL) at −30 °C. After stirring for 1 h at −30 °C, the reaction mixture was treated with a small amount of isopropanol and stirred for 20 min. After the addition of water, the mixture was extracted with EtOAc. The organic layer was washed with water, then brine, dried over anhydrous sodium sulfate, and concentrated in vacuo to afford a carboxylic acid, which was esterified with diazomethane to give a crude methyl ester. This crude ester was purified by column chromatography on silica gel to afford **36** (3.58 g, 56%) as a colorless viscous oil. TLC $R_f = 0.45$ (benzene/EtOAc, 5/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.53–5.37 (m, 2H), 4.60 (m, 1H), 4.23–3.40 (m, 9H), 2.40–1.00 (m, 18H), 0.90 (s, 9H), 0.04 (s, 6H).

(5*E*)-7-[(1*R*,2*S*,3*R*,5*R*)-5-Chloro-2-hydroxymethyl-3-(tetrahydro-2*H*-pyran-2-yloxy)cyclopentyl]hept-5-enoic acid methyl ester (37). To a stirred solution of **36** (3.02 g, 6.17 mmol) in 50 mL of THF was added 1.0 M tetrabutylammonium fluoride in THF (8.0 mL, 8.0 mmol). After stirring for 5 h at ambient temperature, the reaction mixture was poured into ice-water and extracted with 50% EtOAc–hexane. The organic layer was washed first with water, and then with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo to afford a crude oil, which was purified by column chromatography on silica gel to give **37** (1.84 g, 79%) as a colorless viscous oil. MS (FAB, pos) $m/z = 375$ $(M+H)^+$, 291; IR (neat) 3460, 2944, 1740, 1440, 1353, 1202, 1136, 1023, 972, 870, 812 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.60–5.33 (m, 2H), 4.70 (m, 0.35H), 4.57 (m, 0.65H), 4.32–3.40 (m, 9H), 2.40–1.33 (m, 18H). The compound **38** was synthesized from **37** by the same procedure used for the preparation of **3**.

(5*E*)-7-[(1*R*,2*S*,3*R*,5*R*)-5-Chloro-2-formyl-3-(tetrahydro-2*H*-pyran-2-yloxy)cyclopentyl]hept-5-enoic acid methyl ester (38). Colorless viscous oil. TLC $R_f = 0.36$ (*n*-hexane/EtOAc, 3/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.75 and 9.73 (2×d, $J = 0.4$ Hz, 1H), 5.60–5.28 (m, 2H), 4.65–4.50 (m, 2H), 4.17–3.96 (m, 1H), 3.92–3.71 (m, 1H), 3.67 (s, 3H), 3.58–3.39 (m, 1H), 2.74 and 2.58 (2×m, 1H), 2.52–1.97 (m, 9H), 1.90–1.40 (m, 8H).

(16*S*)-9-Deoxy-9β-chloro-15-deoxy-16-hydroxy-17,17-trimethylene-20-norPGF$_2$ methyl ester (43). To a stirred solution of **4** (19.8 g, 54.0 mmol) in 125 mL of dry THF was added *n*-butyllithium (1.6 M in hexane, 33.8 mL, 54.0 mmol) at −78 °C under an argon atmosphere. The reaction mixture was stirred for 1 h at −78 °C and then added to a stirred solution of **3** (16.0 g, 41.5 mmol) in 125 mL of dry THF at −78 °C. After stirring for 1 h at −78 °C, the resulting mixture was treated with acetic anhydride (7.85 mL, 83.0 mmol) and allowed to warm up to ambient temperature over 1 h. The reaction mixture was treated with saturated aqueous ammonium chloride and extracted with EtOAc repeatedly. The combined organic layers were washed with water, then brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo to afford a crude **39** (40.1 g) as a dark-brown viscous oil. TLC $R_f = 0.41$ (*n*-hexane/EtOAc, 3/1); IR (neat) 2943, 2863, 1740, 1447, 1371, 1307, 1228, 1150, 1131, 1075, 1031 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.00–7.45 (m, 5H), 5.70–5.20 (m, 2H), 5.00–3.20 (m, 14H), 2.60–1.20 (m, 37H), 1.10–0.80 (m, 3H); MS (MALDI, Pos) $m/z = 803$ $(M+Na)^+$.

To a stirred solution of the crude **39** (10.6 g, 11.0 mmol) in 100 mL of methanol was added powdered magnesium (−50 mesh, 2.40 g, 100 mmol) under an argon atmosphere. The mixture was again stirred (15 min), and a catalytic amount of chloro trimethylsilane (0.1 mL) was added. After stirring for 1 h at ambient temperature, the reaction mixture was poured into ice-cold 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 afford a crude oil, which was purified by column

chromatography on silica gel to give a mixture of 13*E*- and 13*Z*-olefinic compounds (3.0 g, 47% yield from aldehyde **3**) as a pale yellow oil, in which the ratio of 13*E* to 13*Z* was approximately 5 to 1. TLC R_f = 0.66 (*n*-hexane/EtOAc, 3/1); IR (neat) 2942, 2875, 1741, 1440, 1352, 1200, 1115, 1077, 1032, 977 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.80–5.25 (m, 4H), 4.60 (m, 2H), 4.15–3.75 (m, 4H), 3.57 (s, 3H), 3.55–3.35 (m, 3H), 2.50–1.40 (m, 34H), 1.00–0.85 (m, 3H); HRMS calcd for $C_{33}H_{53}ClO_6Na$ $[M+Na]^+$: 603.3428, found 603.3446.

To a stirred solution of the olefinic compounds (13.8 g, 23.8 mmol) in 120 mL of methanol was added *p*-toluenesulfonic acid (226 mg, 1.14 mmol) at 0 °C. After stirring for 3 h at ambient temperature, the reaction mixture was treated with saturated aqueous sodium bicarbonate and extracted with EtOAc repeatedly. The combined organic layers were washed with water, then brine, dried over anhydrous magnesium sulfate and concentrated in vacuo to afford a crude oil as a mixture of **43** (13*E*) and the corresponding 13*Z* isomer. The residue was purified by column chromatography on silica gel to give **43** (7.64 g, 78%) as a pale yellow oil. TLC R_f = 0.47 (*n*-hexane/EtOAc, 1/1); IR (neat) 3369, 2932, 1739, 1437, 1220, 1157, 1070, 968 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.60 (ddd, *J* = 15.0, 8.0, 5.6 Hz, 1H), 5.52–5.35 (m, 3H), 4.18–3.95 (m, 2H), 3.67 (s, 3H), 3.54 (dd, *J* = 10.0, 2.6 Hz, 1H), 2.45–1.30 (m, 22H), 2.33 (t, *J* = 7.6 Hz, 2H), 0.92 (t, *J* = 7.5 Hz, 3H); MS (APCI, Pos, 20V) *m*/*z* = 395 $(M+H-H_2O)^+$, 377 $(M+H-2H_2O)^+$, 341 $(M+H-2H_2O-HCl)^+$; HRMS calcd for $C_{23}H_{37}ClO_4Na$ $[M+Na]^+$: 435.2278, found 435.2307.

(16*S*)-9-Deoxy-9β-chloro-15-deoxy-16-hydroxy-17,17-trimethylene-20-norPGF$_2$ (2). To a stirred solution of **43** (7.40 g, 17.9 mmol) in 90 mL of methanol, 18 mL of 2 N sodium hydroxide was added at room temperature. After stirring for 4 h, the reaction mixture was diluted with water and the aqueous layer was washed with *n*-hexane/ether (1/1). The resulting aqueous layer was acidified with aqueous hydrochloric acid 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 to yield **2** (5.79 g, 81%) as a pale yellow viscous oil. Optical rotation $[\alpha]_D^{25} = -24.4$ (*c* 1.00, EtOH); TLC R_f = 0.33 (EtOAc/*n*-hexane/AcOH, 60/30/1); IR (neat) 3351, 2936, 1709, 1432, 1243, 1069, 968, 866 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.56 (ddd, *J* = 15.4, 8.2, 5.2 Hz, 1H), 5.55–5.30 (m, 3H), 5.60–5.00 (br, 3H), 4.20–3.96 (m, 2H), 3.56 (dd, *J* = 10.2, 2.0 Hz, 1H), 2.42–1.30 (m, 20H), 2.35 (t, *J* = 7.0 Hz, 2H), 0.92 (t, *J* = 7.4 Hz, 3H); MS (FAB, Neg) *m*/*z* = 397 $(M-H)^-$, 361 $(M-H-HCl)^-$; HRMS calcd for $C_{22}H_{35}ClO_4Na$ $[M+Na]^+$: 421.2122, found 421.2117. Compounds **6**, **9** and **10** were synthesized from **29**, **38** and **28**, respectively, by the procedure described above. The compound **7** was synthesized from **46**, which was obtained as a by-product in the reductive elimination reaction of **41**, according to the procedure outlined above.

(16*S*)-9-Deoxy-9β-bromo-15-deoxy-16-hydroxy-17,17-trimethylene-20-norPGF$_2$ (6). Pale yellow oil. TLC R_f = 0.40 (EtOAc/*n*-hexane/AcOH, 8/4/0.1); IR (neat) 3369, 2931, 1709, 1434, 1242, 1154, 1069, 968, 735 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.62 (dt, *J* = 15.0, 6.0 Hz, 1H), 5.55–5.30 (m, 3H), 4.22–4.00 (m, 2H), 3.61 (dd, *J* = 10.0, 2.5 Hz, 1H), 2.37 (t, *J* = 7.0 Hz, 2H), 2.50–1.30 (m, 20H), 0.93 (t, *J* = 7.5 Hz, 3H); MS (APCI, Neg, 20 V) *m*/*z* = 441, 443 $(M-H)^-$; HRMS calcd for $C_{22}H_{35}BrO_4Na$ $[M+Na]^+$: 465.1616, found 465.1640.

(16*S*)-9-Deoxy-15-deoxy-16-hydroxy-17,17-trimethylene-20-norPGF$_2$ (7). Pale yellow oil; TLC R_f = 0.36 (EtOAc/*n*-hexane/AcOH, 8/4/0.1); IR (neat) 3368, 2932, 1709, 1430, 1261, 1071, 1029, 968, 873, 799 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.61 (dt, *J* = 15.0, 6.5 Hz, 1H), 5.55–5.25 (m, 3H), 3.88 (q, *J* = 7.5 Hz, 1H), 3.63 (dd, *J* = 10.0, 2.5 Hz, 1H), 2.34 (t, *J* = 7.0 Hz, 2H), 2.40–1.40 (m, 22H), 0.93 (t, *J* = 7.5 Hz, 3H); MS (APCI, Neg, 20 V) *m*/*z* = 363 $(M-H)^-$; HRMS calcd for $C_{22}H_{36}O_4Na$ $[M+Na]^+$: 387.2511, found 387.2546.

(16*S*)-9-Deoxy-9β-chloro-15-deoxy-16-hydroxy-5,6-*trans*-17,17-trimethylene-20-norPGF$_2$ (9). Colorless viscous oil; TLC R_f = 0.44 (EtOAc/*n*-hexane/AcOH, 6/3/0.1); IR (neat) 3368, 2916, 1715, 1435, 1048, 773 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.65–5.28 (m, 4H), 5.27–4.40 (br, 3H), 4.19–3.97 (m, 2H), 3.55 (dd, *J* = 10, 1.8 Hz, 1H), 2.48–1.30 (m, 22H), 0.92 (t, *J* = 7.6 Hz, 3H); MS (APCI, Neg. 20 V) *m*/*z* = 397 $(M-H)^-$; HRMS calcd for $C_{22}H_{35}ClO_4Na$ $[M+Na]^+$: 421.2122, found 421.2141.

(16*S*)-9-Deoxy-9β-chloro-15-deoxy-16-hydroxy-17,17-trimethylene-20-norPGF$_1$ (10). Colorless oil. TLC R_f = 0.47 ($CHCl_3$/MeOH, 9/1); IR (neat) 3368, 2931, 1713, 1463, 1271, 1069, 969 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.63–5.53 (m, 1H), 5.42 (dd, *J* = 15, 8 Hz, 1H), 4.16–4.08 (m, 1H), 4.04–3.98 (m, 1H), 3.56 (dd, *J* = 10, 2 Hz, 1H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.30–1.20 (m, 24H), 0.92 (t, *J* = 7.5 Hz, 3H); MS (FAB, Pos) *m*/*z* = 401 $(M+H)^+$, 365, 329; HRMS calcd for $C_{22}H_{37}ClO_4Na$ $[M+Na]^+$: 423.2278, found 423.2284. The compound **5** was synthesized from **48** according to a procedure reported previously, except that tetrabutylammonium fluoride was used instead of tetrabutylammonium chloride. Compounds **8** and **11** were synthesized from **49** and **50** according to the same procedure.

(16*S*)-9-Deoxy-9β-fluoro-15-deoxy-16-hydroxy-17,17-trimethylene-20-norPGF$_2$ (5). Colorless oil. TLC R_f = 0.38 (EtOAc); IR (neat) 3388, 2934, 2359, 1708, 1436, 1240, 1027, 968, 734, 669, 418 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.64–5.57 (m, 1H), 5.54–5.36 (m, 3H), 4.90–4.70 (m, 1H), 4.13–4.05 (m, 1H), 3.61 (dd, *J* = 10, 2 Hz, 1H), 2.40–1.40 (m, 24H), 0.93 (t, *J* = 7.5 Hz, 3H); MS (APCI, Neg 20 V) *m*/*z* = 381 $(M-H)^-$; HRMS calcd for $C_{22}H_{35}FO_4Na$ $[M+Na]^+$: 405.2417, found 405.2397.

(16*S*)-11,16-*O*-Bis(*t*-butyldimethylsilyl)-15-deoxy-16-hydroxy-20-norPGF$_{2\beta}$ methyl ester (49). To a stirred solution of **48** (248 mg, 0.399 mmol) in 2.7 mL of THF

was added sequentially triphenylphosphine (601 mg, 2.29 mmol), diethyl azodicarboxylate (0.375 mL, 2.38 mmol) and formic acid (0.09 mL, 2.39 mmol) at 0 °C. After stirring for 1 h at 0 °C, the resulting mixture was treated with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo to afford a formate. A mixture of this formate and 0.3 mL of aqueous ammonia in 3 mL of methanol was stirred for 14 h at room temperature. The resulting reaction mixture was extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo to afford a crude oil, which was purified by column chromatography on silica gel to give **49** (152 mg, 0.243 mmol) as a colorless oil. TLC $R_f = 0.24$ (*n*-hexane/EtOAc, 5/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.58–5.35 (m, 3H), 5.25 (m, 1H), 4.04–3.90 (m, 2H), 3.66 (s, 3H), 3.56 (t, $J = 5.8$ HZ, 1H), 2.36–1.40 (m, 22H), 0.94–0.96 (m, 3H), 0.89 (s, 9H), 0.85 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H), 0.00 (s, 3H), −0.10 (s, 3H).

(16*S*)-9-Deoxy-9α-chloro-15-deoxy-16-hydroxy-17,17-trimethylene-20-norPGF$_2$ (8). Colorless viscous oil. TLC $R_f = 0.40$ (EtOAc); IR (neat) 3391, 2932, 1714, 1434, 1378, 1262, 1073, 1031, 969 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.75–5.31 (m, 3H), 4.41 (m, 1H), 4.02 (m, 1H), 3.64 (m, 1H), 2.68–1.33 (m, 24H), 0.93 (t, $J = 7.4$ Hz, 3H); MS (FAB, Pos.) $m/z = 363$ $(M-2H_2O+H)^+$, 327 $(M-2H_2O-HCl+H)^+$; HRMS calcd for $C_{22}H_{35}ClO_4Na$ $[M+Na]^+$: 421.2122, found 421.2166.

(16*S*)-9-Deoxy-9β-chloro-13,14-dihydro-15-deoxy-16-hydroxy-17,17-trimethylene-20-norPGF$_2$ (11). Colorless viscous oil. TLC $R_f = 0.30$ (*n*-hexane/EtOAc/AcOH, 1/1/0.02); IR (neat) 3369, 2933, 1708, 1459, 1247, 1071 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.58–5.38 (m, 2H), 4.50–3.50 (br, 3H), 4.15–4.00 (m, 2H), 3.58 (d, $J = 8.8$ Hz, 1H), 2.40–1.20 (m, 24H), 2.37 (t, $J = 7.0$ Hz, 2H), 0.91 (t, $J = 7.5$ Hz, 3H); MS (FAB, Pos) $m/z = 401$ $(M+H)^+$, 383 $(M+H-H_2O)^+$, 365 $(M+H-2H_2O)^+$, 329 $(M+H-2H_2O-HCl)^+$; HRMS calcd for $C_{22}H_{37}ClO_4Na$ $[M+Na]^+$: 423.2278, found 423.2291.

(16*S*)-9-Deoxy-9β-chloro-15-deoxy-16-hydroxy-17,17-trimethylene-5,6,13,14-tetrahydro-20-norPGF$_2$ (12). Compound **12** was synthesized from **43**, by the same procedure used for the preparation of compound **26** (catalytic hydrogenation) and compound **2** (alkaline hydrolysis). Colorless oil. TLC $R_f = 0.37$ (*n*-hexane/EtOAc/AcOH, 1/1/0.02); IR (KBr) 3392, 2931, 2857, 1709, 1459, 1071 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.50–2.50 (br, 3H), 4.17–3.98 (m, 2H), 3.57 (d, $J = 9.0$ Hz, 1H), 2.35 (t, $J = 7.0$ Hz, 2H), 2.14 (dd, $J = 7.0$, 5.8 Hz, 2H), 2.00–1.20 (m, 26H), 0.92 (t, $J = 7.5$ Hz, 3H); MS (APCI, Neg., 20V) $m/z = 401$ $(M-H)^-$; HRMS calcd for $C_{22}H_{39}ClO_4Na$ $[M+Na]^+$: 425.2435, found 425.2475.

(16*R*)-9-Deoxy-9β-chloro-15-deoxy-16-hydroxy-17,17-trimethylene-20-norPGF$_2$ (13). Compound **13** was synthesized from the C-16 epimer of compound **48** as reported previously. White solid. TLC $R_f = 0.45$ ($CHCl_3/CH_3OH$, 9/1); IR (neat) 3369, 2934, 1708, 1434, 1245, 1070, 1027, 970, 910, 734 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.65–5.40 (m, 4H), 4.20–4.00 (m, 2H), 3.63 (dd, $J = 2.1$, 10.5 Hz, 1H), 4.00–2.60 (br, 1H), 2.40–1.35 (m, 24H), 0.92 (t, $J = 7.5$ Hz, 3H); MS (FAB, Pos) $m/z = 399$ $(M+H)^+$; HRMS calcd for $C_{22}H_{35}ClO_4Na$ $[M+Na]^+$: 421.2122, found 421.2128.

(16*S*)-9-Deoxy-9β-chloro-15-deoxy-16-hydroxy-17,17-trimethylene-20-norPGF$_2$ L-lysine salt (2Ly). To a stirred solution of **2** (45.9 g, 110 mmol) in 460 mL of ethanol was added L-lysine (16.0 g, 110 mmol). To this suspension was added 1600 mL of ethanol. The mixture was heated at 80 °C until the precipitates were dissolved, and then the insoluble substance was removed by filtration. After cooling, 5 L of EtOAc was added to the filtrate. The resulting precipitates were collected by filtration and dried in vacuo to afford L-lysine salt **2Ly** (54.6 g, 100 mmol, 91% yield) as a colorless crystal. Mp 166–168 °C (dec.); Optical rotation $[\alpha]_D^{25} = -20.4$ (*c* 1.00, H_2O); MS (FAB, Pos) $m/z = 545$ $(M+H)^+$, 421 $(M-Lys+Na)^+$; IR (KBr) 3424, 2934, 1618, 1542, 1406, 1307, 965 cm^{-1}; ^{1}H NMR (200 MHz, CD_3OD) δ 5.70–5.30 (m, 4H), 4.02 (q, $J = 7.1$ Hz, 2H), 3.58–3.45 (m, 2H), 2.92 (t, $J = 7.3$ Hz, 2H), 2.40–1.30 (m, 28H), 0.92 (t, $J = 7.5$ Hz, 3H). Anal. calcd for $C_{28}H_{49}ClN_2O_6$; C, 61.69; H, 9.06; N, 5.14; found; C, 61.72; H, 9.21; N, 5.21.

X-ray crystallography of 2Ly

Colorless platelet crystals of **2Ly** were obtained from a methanol/dioxane (1/3) solution. A suitable crystal of **2Ly** having the approximate dimension of 0.10×0.20×0.40 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC5R diffractometer with graphite monochromated Cu-K_α radiation and a RU-200 rotating anode X-ray generator. Of the 9156 reflections which were collected, 4523 were unique. Equivalent reflections were merged. An empirical absorption correction was applied.

The program package teXsan[11] was used for analysis and drawing figures. The positions of almost all the non-H atoms were determined by the program SHELXS86,[12] and carbon atoms of the α chain of **2Ly** were found in the difference Fourier synthesis. Three methylene atoms of the α chain of **2Ly**, C4, C5 and C6, were disordered over two sites, C4′, C5′ and C6′, respectively. These atoms were refined isotropically with 50% occupancy. C3 and C7 Carbon atoms were also refined isotropically, while the other non-H atoms were refined with anisotropic temperature parameters. The positions of the H atoms were calculated from the coordinates of non-H atoms and confirmed by the difference Fourier synthesis, however the H atoms of the α chain of **2Ly** were not assigned. The assigned H atoms were included in structure factor calculations but not refined. Space group C2, cell constant $a = 32.91$ (2) Å, $b = 5.91(2)$ Å, $c = 17.64(2)$ Å, $\beta = 112.52$ (6)° $V = 3169$ (10) Å^3, 1307 reflections with $I > 3\sigma(I)$ were used for the final structure factor calculation, 2θ max = 130.1°, $R = \Sigma||Fobs|-|Fcalc||/\Sigma|Fobs| = 0.104$, $Rw = ((\Sigma w(|Fobs|-|-|Fcalc|)^2/\Sigma wFobs^2)^{1/2} = 0.119$ where $w = 1/\sigma^2(Fobs)$.

Prostanoid EP1–4 receptor binding assay

Membranes from CHO cells expressing the mouse prostanoid receptors were incubated with radioligand (2.5 nM of [^{3}H]PGE_2) 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]. Incubation was carried out at 25 °C for 60 min except for EP1 (20 min). 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), and the radioactivity on the filter was measured in 6 mL of liquid scintillation (ACSII) mixture with a liquid scintillation counter. Nonspecific binding was determined by incubation of 10 μM unlabeled PGE_2 with assay buffer.

Measurement of cAMP production

CHO cells expressing EP2-receptor 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 the 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 radioimmunoassay using a cAMP assay kit (Amersham).

References and Notes

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