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Design and Synthesis of a Selective EP4-receptor Agonist. Part 4: Practical Synthesis and Biological Evaluation of a Novel Highly Selective EP4-Receptor Agonist

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Abstract—A practical method of synthesizing a highly selective EP4-receptor agonist **1** using Corey lactone **2** as a key intermediate was developed. Selective methanesulfonylation of the primary alcohol of the diol **8** under the newly devised conditions followed by the protection of the remaining secondary alcohol are key reactions in this new method. Further biological evaluation of **1a**–**b** is also reported.

Introduction

The receptor of PGE_2 has been known to be divided into four subtypes EP1, EP2, EP3 and EP4.[1] Among them, the EP4-receptor, whose cDNA was already cloned, has been discovered to distribute in thymus, lung, heart, kidney, bone, womb, liver and other organs.[2] Stimulation of the EP4-receptor by PGE_2 leads to an increase of intracellular cAMP. As such, the cytoprotective action of PGE_2, which is thought to mediate EP4-receptor, is derived from the improvement of blood flow and the regulation of inflammatory cytokine production.[3,4] Therefore, an EP4-receptor agonist would be a useful drug for the treatment of several deseases, mediated by inflammatory cytokines, such as shock, rheumatoid arthritis, hepatitis, osteoporosis and others. However, the physiological and pathological roles of the EP4-receptor have not been clarified yet because of a lack of a highly selective EP4-receptor agonist. In a preceding paper,[5] we reported the discovery of highly selective EP4-receptor agonists, which will be useful to clarify the patho-physiological roles of the EP4-receptor subtype. We report here a practical method for the synthesis and further biological evaluation of a newly discovered EP4-receptor selective agonists **1a**–**b** (Chart 1).

Chemistry

The synthesis of 5-thiaPGE_1 using a three-component coupling process, was first reported[6] in 1985. It was not a practical method for large-scale synthesis because of the multi-step reaction process, toxic reagent and poor reproducibility.

A newly developed method starting with the THP-lactone **2**,[7] large amounts of which are commercially available, enabled us to synthesize many analogues of 5-thiaPGE_1.

The synthesis of **1a**–**b** is outlined in Scheme 1. Oxidation of **2** to an aldehyde **3** followed by a Horner–Emmons reaction of the aldehyde **3** with the phosphonate **4**, which was prepared from (3-methoxymethyl)phenylacetic acid in two steps, afforded an enone **5**. Using ethyl acetate as a co-solvent, the oxidation reaction of **2** could be carried out at a lower temperature. As a result, the yield of **3** was remarkably improved while **3** was chemically unstable in the presence of a base.[8] Stereoselective reduction of the enone **5** with SBN reagent[9] provided

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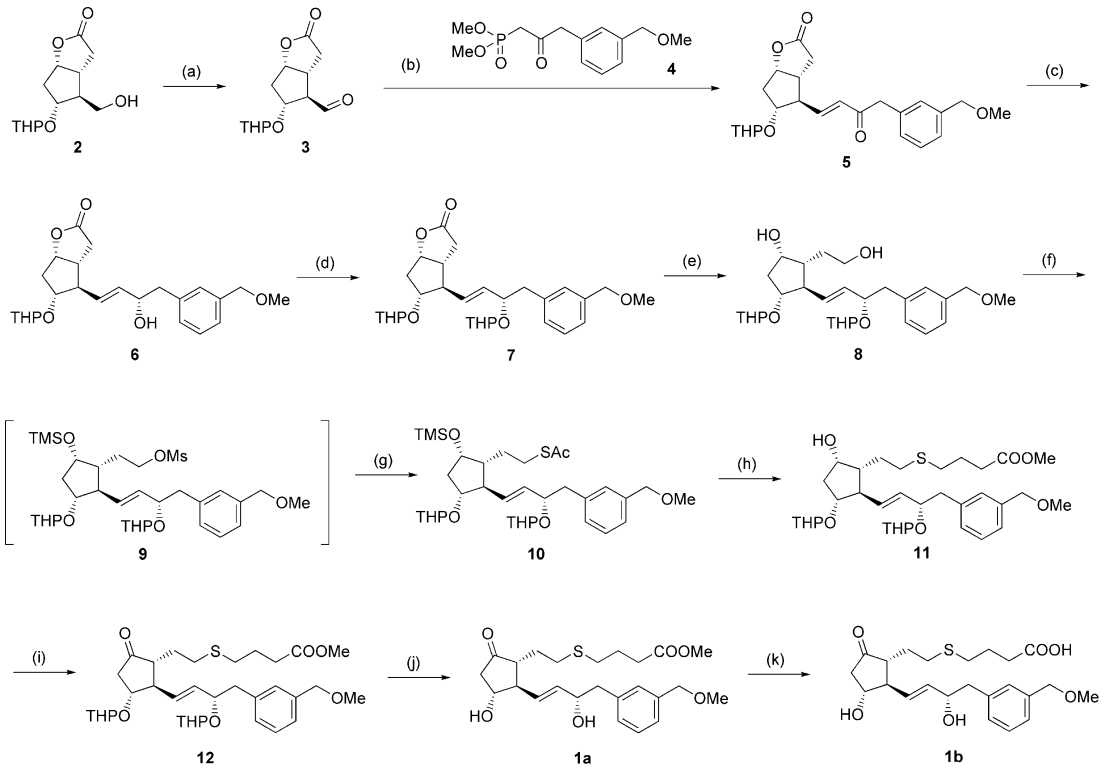
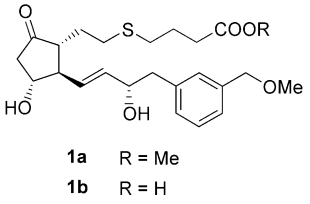
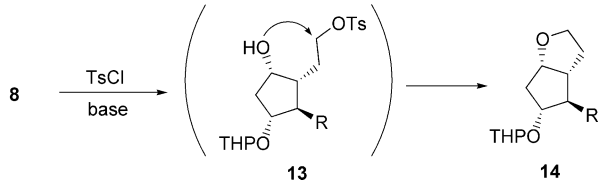


Scheme 1. Synthesis of **1a**–**1b**. Reagents and conditions: (a) SO_3Py, Et_3N, EtOAc, DMSO; (b) phosphonate **4**, NaH, THF; (c) (*S*)-BINOL, $LiAlH_4$, EtOH, THF; (d) DHP, *p*-TsOH–H_2O, toluene; (e) $LiAlH_4$, THF; (f) MsCl, *i*-Pr_2NEt, MeOH, TMSCl, THF; (g) AcSK, K_2CO_3, DMF; (h) methyl 4-iodobutyrate, K_2CO_3, MeOH; (i) SO_3Py, *i*-Pr_2NEt, EtOAc, DMSO; (j) 0.1N HCl, CH_3CN, MeOH; (k) PLE, EtOH, phosphate buffer.



1a R = Me
1b R = H

Chart 1. Highly selective EP4-receptor agonists.



Scheme 2. Formation of undesired bicyclic ether **14**.

15(*S*)-isomer **6** (84% d.e.) in good yield. Protection of the newly formed hydroxy group of **6** as a tetrahydropyranyl (THP) ether followed by the reduction of the γ-lactone moiety of **7** with lithium aluminum hydride gave a diol **8** in a nearly quantative yield.

An efficient conversion of **8** to **11** was accomplished by selective substitution of the primary alcohol group of **8** with a sulfur moiety. The following reaction conditions were studied. A selective monosubstitution of the primary alcohol group of **8** with thioacetic acid under Mitsunobu reaction conditions was attempted to afford an undesired product **14** (Scheme 2) because of the presumed formation of an intermediate such as **13**. As an alternative, aryl- or alkyl sulfonylation of the primary alcohol followed by the introduction of the *S*-alkyl moiety was investigated. But a selective monotosylation of the primary alcohol of **8** by a conventional method again produced a bicyclic ether **14**. Success was achieved with the application of a selective monomethanesulfonylation of a primary alcohol followed by a substitution reaction with potassium thioacetate. Selective monomethanesulfonylation of **8** by the reported method[10] was carried out as described below.

A highly selective methanesulfonylation reaction took place in a good yield at the lower temperature using MsCl/*i*-Pr_2NEt in THF while it was not successful in dichloromethane or toluene because of the formation of the cyclized product **14**. Et_3N and DMAP were not acceptable as a base to this reaction because of the presumed formation of a reactive intermediate different from that of the successful reaction described above.[11]

The addition of methanol to decompose any excess of the active species followed by the addition of trimethylsilyl chloride (TMSCl) enabled us to protect the secondary alcohol as a trimethylsilyl ether. Decomposition of the excess reagent prior to the addition of potassium thioacetate was needed to carry out the sequential reactions in a one-pot manner. The resulting thiol acetate **10** was converted to **11** by the simultaneous methanolysis of the thiol acetate and the trimethylsilyl ether followed by *S*-alkylation with methyl 4-iodobutanoate in the presence

of K_2CO_3/MeOH. Oxidation of the 9-hydroxy group of **11** with SO_3·Py complex in DMSO-*i*Pr_2NEt afforded 9-keto derivative **12** in good yield. The Swern oxidation and Corey–Kim oxidation of **11** were not successful because of a presumed intramolecular interaction of the 9-sulfoxonium moiety with the 5-thia moiety as illustrated in Chart 2. This type of intramolecular interaction was estimsted to be avoided by using SO_3·Py in DMSO-Et_3N as an oxidative reagent because of its low reactivity, while excess reagent and a relatively long reaction time were needed to complete the reaction. Replacement of triethylamine with a stronger base (*i*-Pr_2NEt) was again effective in completing the oxidative reaction at a lower temperature in a shorter reaction time. Undesired elimination of the 11-THPOH and a Pummerer type rearrangement were successfully avoided under these improved oxidation conditions. In the final deprotection reaction of PGE synthesis, the degradation of PGEs to PGAs has been a serious problem. To solve this problem, mild deprotection conditions were investigated. The deprotective reaction proceeded without the formation of PGA on addition of a catalytic amount of diluted HCl in MeOH-CH_3CN. As such, cyclodextrin clathrates of **1a**, prepared according to the conventional procedure, are available as a stable formulation.

Biological Results and Discussion

Binding assay and intracellular signal transduction assay (Table 1 and Fig. 1)

Results of a binding assay in CHO cells, which express prostanoid receptors mEP1-EP4, hEP4, mFP, hTP and hIP, are demonstrated in Table 1. Using the membrane fractions of the cells, the binding assay of **1b** was performed by the reported method[12] with minor modification. **1b** demonstrated potent EP4-receptor affinity and subtype selectivity although it showed some affinity also to the other subtypes mEP3 and mEP2. The potent mEP4-receptor affinity of **1b** was reproduced in the hEP4-receptor (K_ihEP4 = 1.3 nM). **1b** did not show affinity to the other prostanoid receptors such as mFP, mDP, hTP and hIP at 10 μM.

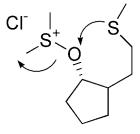


Chart 2. Presumed intramolecular interaction.

Table 1. Binding affinity (K_i) and agonist activity (EC_{50}) of **1b**[a]

	mEP1	mEP2	mEP3	mEP4	hIP	mDP	mFP	hTP
K_i (nM)	$>10^4$	620	56	0.70	$>10^4$	$>10^4$	$>10^4$	$>10^4$
EC_{50} (nM)	$>10^4$	230	400	1.6	$>10^4$	$>10^4$	$>10^4$	$>10^4$

[a]Using membrane fractions of CHO cells expressing the prostanoid receptors, K_i values were determined by competitive binding assay, which was performed according to the method of Kiriyama with some modifications.[12] With regard to the subtype-receptor agonist activity, EC_{50} values were determined based on the effects of the test compounds on the increase in the intracellular c-AMP production in EP4, EP2, IP and DP receptor and in the intracellular Ca^{2+} production in EP1, EP3, FP and TP receptor.

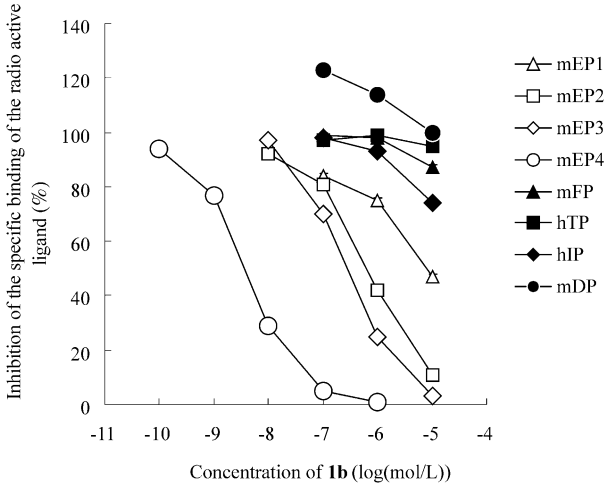


Figure 1. Binding affinity of **1b** in the receptor subtypes.

With regard to the agonist activities of **1b** in the EP1, EP3, FP and TP subtypes, the ability of **1b** to increase the intracellular Ca^{2+} concentration was evaluated using fura-2. The agonist activities of **1b** in the EP2, EP4, DP and IP subtypes were evaluated from its ability to increase the intracellular cAMP level. The subtype selectivity of **1b** was also evaluated based on EC_{50} values. The EP4-receptor selectivity of **1b** was retained also in the functional assay. Interestingly, the ratio of EC_{50}EP3/EC_{50}EP4 was increased 250-fold while the ratio of K_iEP3/K_iEP4 was increased 80-fold. Compound **1b** also demonstrated potent agonist activity in both mEP4- and hEP4-receptors (EC_{50}hEP4 = 1.7 nM) while it did not show significant EC_{50} values for the other subtypes mFP, mDP, hTP and hIP.

LPS-induced changes of cytokine production in human whole blood cells (Fig. 2a and b)

As shown in Figure 2a and b, **1b** was evaluated to elucidate whether it regulates LPS-induced cytokine production in human whole blood cells. The selective EP4-receptor agonist **1b** suppressed TNF-α production and augmented IL-10 production at 10^{-9} M. Based on the experimental results obtained with this highly selective agonist, cytokine regulation was strongly suggested to be one of the EP4-receptor-mediated biological activities.[13] The compound **1b** suppressed TNF-α production and increased IL-10 production at a lower concentration than PGE_1 while it exhibited the same potency as PGE_1. The EP3-receptor decreased the concentration of intracellular cAMP coupled with Gi.[12] Thus the EP3-receptor plays an opposite role in the regulation of the intracellular cAMP level. Accordingly, PGE_1 was thought to suppress the EP4-receptor-mediated activity through the EP3-receptor mediated activity. As a result of the increased EP4-receptor selectivity, **1b** exhibited a regulatory effect on the cytokines at such a low concentration. At higher concentrations, the suppression of

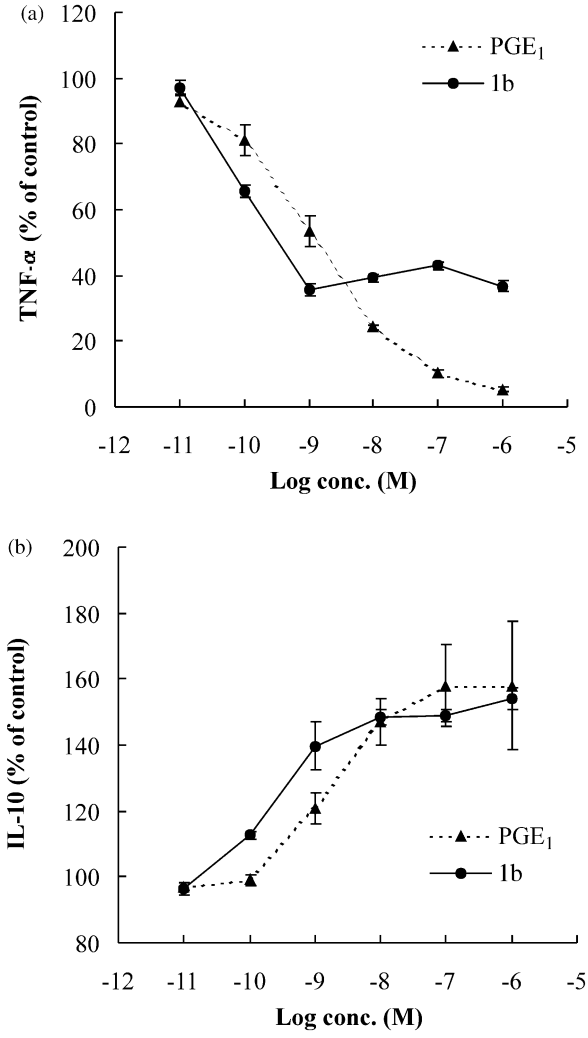


Figure 2. (a) Inhibition of LPS-induced TNF-α production in human whole blood; (b) increase of LPS-induced IL-10 production in human whole blood.

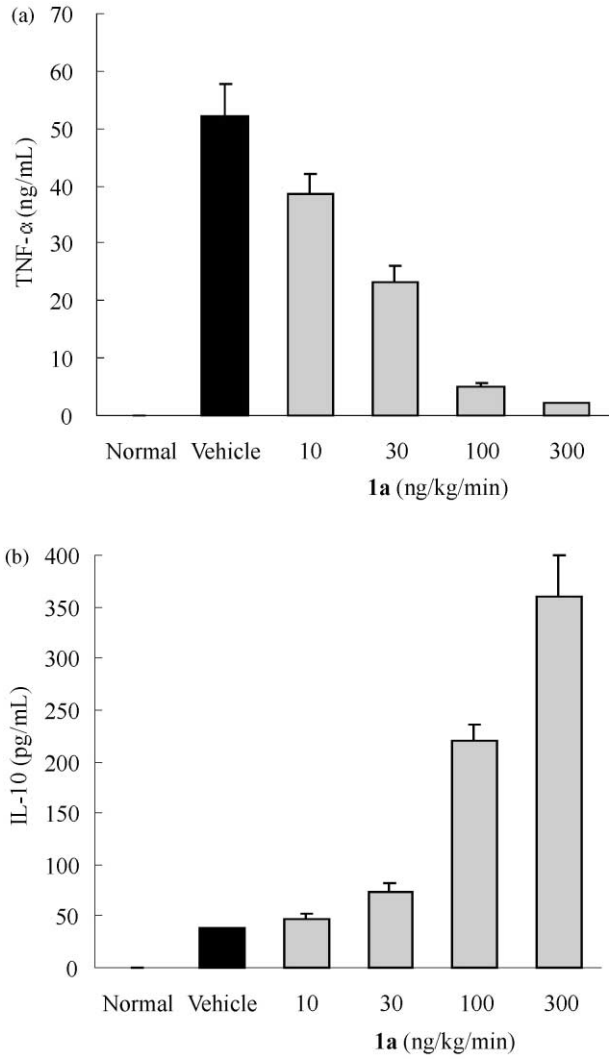


Figure 3. (a) Inhibition of LPS-induced TNF-α production in rats; (b) increase of LPS-induced IL-10 production in rats.

TNF-α production by **1b** was less potent than that by PGE_1 (Fig. 2). The EP2-receptor agonist activity was considered to be one of the reasons. Because the EP2 subtype has been known to regulate cytokine production by increasing the intracellular cAMP concentration like the EP4 subtype, the higher concentration of PGE_1 is estimated to show maximum activity by EP4- and EP2-receptor-mediated activity.

LPS-induced changes of plasma TNF-α and IL-10 levels in rats (Fig. 3a and b)

The effect of **1a**, which was metabolically converted to the free acid form **1b**, on LPS-induced changes of TNF-α and IL-10 levels in the plasma of rats was evaluated. As demonstrated in Figure 3, increased production of the plasma TNF- α after the intraveneous administration of LPS (1 μg/kg) was significantly suppressed by the intraveneous infusion of **1a** (10, 30, 100 and 300 ng/kg/min) in a dose-dependent manner. The plasma IL-10 level after the administration of LPS was augmented by the intraveneous infusion of **1a** (30, 100 and 300 ng/kg/min) in a dose-dependent manner.[14] Based on these results, the EP4-receptor was considered to mediate various cytoprotective effects of PGE_1 and PGE_2 by regulating cytokine production. Selective stimulation of the EP4-receptor with **1a** was suggested to be a therapeutic approach to the treatment of several deseases related to TNF-α and IL-10, such as rheumatoid arthritis,[15] hepatitis,[16] osteoporosis,[17] and others. Besides, the therapeutic dose of **1a** in the model, was found to be lower than that of the non-selective agonist, natural PGE_2.

More details about the role of the EP4-receptor are now being disclosed in our laboratory using the newly discovered selective EP4-receptor agonists **1a**–**b**.

In summary, we have succeeded in the practical synthesis of the EP4-receptor agonist **1a** starting from Corey's lactone **2**, which is available in large quantities. The success was based on complete selectivity in the newly devised selective mono-methanesulfonylation of the diol **8**. As a result, a one-pot reaction to obtain **10** from **8** was accomplished. Further biological evaluations of **1a** and **1b** were performed both in vitro and in vivo. Based on the results of the binding assay and functional assay,

the EP4-receptor selectivity and agonist activity of **1a** and **1b** are expected to be reproduced in humans.

Experimental

General procedures

Analytical samples were homogeneous as confirmed by TLC, and afforded spectroscopic results consistent with the assigned structures. Proton nuclear magnetic resonance spectra (^{1}H NMR) were obtained on a Varian Gemini-200 or VXR-200s spectrometer using deuterated chloroform ($CDCl_3$) or deuterated methanol (CD_3OD) as the solvent. Fast atom bombardment mass spectra (FAB-MS) were obtained on a JEOL JMS-DX303HF spectrometer or a JEOL JMS-AX-500 spectrometer. Atmospheric pressure chemical ionization (APCI) was determined on a Hitachi M1200H spectrometer. Infrared spectra (IR) were measured on a Perkin-Elmer FT-IR 1760X spectrometer or a Jasco Varolar-II spectrometer. Optical rotations were measured using a Jasco DIP-370 polarimeter or a Jasco DIP-1000 polarimeter. Results of elemental analyses were uncorrected. Column chromatography was carried out on silica gel [Merck silica gel 60 (0.063–0.200 mm), Wako gel C-200 or Fuji Silysia BW235]. Thin layer chromatography was performed on silica gel (Merck TLC or HPTLC plates, silica gel 60 F_{254}). The following abbreviations for solvents and reagents are used: ethyl acetate (EtOAc), dimethylsulfoxide (DMSO), tetrahydrofuran (THF), methanol (MeOH), TBME (*t*-butylmethylether), hydrochloride (HCl), sodium bicarbonate (NaHCO3), sulfur trioxide pyridine complex (SO_3·Py).

Synthesis of 1

3-(2H-3,4,5,6-Tetrahydropyran-2-yloxy)(1*S*,3*S*,2*R*,5*R*)-6-oxa-7-oxobicyclo[3,3,0]octane-2-carbaldehyde 3. To a stirred solution of **2** (30 g, 117 mmol) and triethylamine (97.9 mL, 702 mmol) in ethyl acetate (380 mL) was added a solution of SO_3·Py (55.9 g, 351 mmol) in EtOAc (100 mL) and DMSO (189 mL) slowly at a temperature below 10 °C under Ar. After stirring for an additional 1 h, the mixture was combined with 1 N HCl (330 mL) slowly. The two layers were separated and the aqueous layer was extracted with EtOAc (200 mL) and THF (100 mL) repeatedly. The combined organic layer was washed with water (60 mL), saturated aqueous $NaHCO_3$ (60 mL) and brine (60 mL), and dried over $MgSO_4$. The solvent was removed by evaporation to give **3** as a pale yellow oil (29.3 g), which was used in the next reaction without purification.

R_f 0.60 (EtOAc/AcOH, 100/1); IR (neat) 2944, 2731, 1768, 1724, 1181 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.73 (m, 1H), 5.11–5.00 (m, 1H), 4.81–4.51 (m, 2H), 3.92–3.79 (m, 1H), 3.59–3.35 (m, 2H), 3.20–3.03 (m, 1H), 2.90 and 2.87 (dd, J = 18, 6.3 Hz, 1H), 2.57 and 2.44 (dd, J = 18, 3.2 Hz, 1H), 2.40–2.30 (m, 1H), 1.97–1.42 (m, 7H); MS (FAB, Pos.) m/z: 255 $(M+H)^+$.

6-{(1*E*)-4-[3-(Methoxymethyl)phenyl]-3-oxobut-1-enyl}-7-(2H-3,4,5,6-tetrahydropyran-2-yloxy)(5*S*,7*S*,1*R*,6*R*)-2-oxabicyclo[3,3,0]octan-3-one 5. To a stirred suspension of sodium hydride (61.1% in oil, 4.98 g, 127 mmol) in THF (1.0 L) was added a solution of dimethyl 3-[(3-methoxymethyl)phenyl]-2-oxopropanephosphonate **4** (42.9 g, 138 mmol) at room temperature under Ar. After stirring for 1 h, to the resulting suspension was added a solution of **3** in THF (300 mL). The mixture was stirred for 30 min before the addition of acetic acid (7.3 mL, 127 mmol). The resulting yellow solution was diluted with EtOAc (600 mL), washed with water (600 mL) and brine (300 mL), and dried over $MgSO_4$. The solvent was removed by evaporation and the residue was purified by column chromatography on silica gel (Wako gel C-200, 750 g, EtOAc/hexane, 1/1) to give **5** as a pale yellow oil. (40.3 g, 83.1%).

R_f 0.26 (EtOAc/hexane, 1/1); IR (neat) 2942, 1770, 1695, 1669, 1627, 1444, 1382, 1339, 1185, 1078, 1035, 974, 916, 871, 815, 762, 706 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37–7.09 (m, 4H), 6.78–6.64 (m, 1H), 6.22 (d, J = 15.6 Hz, 1H), 5.03–4.88 (m, 1H), 4.66–4.53 (m, 1H), 4.44 (s, 2H), 4.22–3.94 (m, 1H), 3.88–3.32 (m, 2H), 3.81 (s, 2H), 3.39 (s, 3H), 2.92–2.05 (m, 5H), 1.90–1.35 (m, 7H); MS (FAB, Pos.) m/z: 415 $(M+H)^+$; HRMS (MALDI-TOF) calcd for $C_{24}H_{30}O_6 + Na^+$: 437.1940; found: 437.1916.

6-{(1*E*)(3*S*)-3-Hydroxy-4-[3-(methoxymethyl)phenyl]but-1-enyl}-7-(2H-3,4,5,6-tetrahydropyran-2-yloxy)(5*S*,7*S*,1*R*,6*R*)-2-oxabicyclo[3,3,0]octan-3-one 6. A pellet of lithium aluminum hydride (8.10 g, 213 mmol) in THF (190 mL) was stirred vigorously at 60 °C under Ar for 2 h. The resulting suspension was cooled to room temperature and a solution of ethanol (9.83 g, 213 mmol) in THF (100 mL) was added slowly. After stirring for 30 min, a solution of (*S*)-(−)-1,1′-bi-2-naphthol (61.1 g, 213 mmol) in THF (200 mL) was added to the suspension over 20 min. After stirring overnight, the suspension was cooled with a dry-ice MeOH bath and a solution of **5** (17.7 g, 42.7 mmol) in THF (200 mL) was added slowly at a temperature below −60 °C. After stirring for 2 h, the resulting suspension was quenched with MeOH (25 mL, 617 mmol) and warmed to 0 °C. The mixture was poured into aqueous sodium bitartrate (75 g/1.5 L) and extracted with EtOAc (300 mL×2) repeatedly. The organic layer was washed with brine (200 mL), and dried over $MgSO_4$. The solvent was evaporated and the residual solid was dissolved in 2-propanol (100 mL) and diisopropylether (100 mL) at 60 °C. To this stirred solution was added hexane (250 mL) and the mixture was allowed to cool to room temperature in 30 min. The precipitates were removed by filtration and the filtrate was evaporated. The residue was purified by column chromatography on silica gel (Wako gel C-200, 460 g, EtOAc, hexane, 2/1) to give **6** as a colorless oil (13.9 g, 78.2%, 15α/15β = 92/8).

R_f 0.42 (EtOAc/benxene, 2/1); IR (neat) 3436, 2942, 1772, 1445, 1381, 1243, 1185, 1078, 1034, 974, 916, 871, 811, 705 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34–7.09 (m, 4H), 5.61 (dd, J = 15.6, 6.0 Hz, 1H), 5.52–5.43 (m, 1H), 5.02–4.88 (m, 1H), 4.71–4.66 and 4.66–4.60 (m, 1H), 4.43 (s, 2H), 4.38–4.28 (m, 1H), 4.12–3.89 (m, 1H), 3.89–3.78 (m, 1H), 3.54–3.32 (m, 1H), 3.40 (s, 3H), 2.92–2.24 (m, 5H), 2.18–2.05 (m, 2H), 1.85–1.40 (m,

8H); MS (FAB, Pos.) m/z: 417 $(M+H)^+$; HRMS (MALDI-TOF) calcd for $C_{24}H_{32}O_6+Na^+$: 439.2097; found: 439.2074.

6-{(1E)(3S)-3-(2H-3,4,5,6-tetrahydropyran-2-yloxy)-4-[3-(methoxymethyl)phenyl]but-1-enyl}-7-(2H-3,4,5,6-tetrahydropyran-2-yloxy)(5S,7S,1R,6R)-2-oxabicyclo[3,3,0]octan-3-one 7. To a stirred solution of **6** (17.9 g, 43.0 mmol) and 3,4-dihydropyran (4.71 mL, 51.6 mmol) in toluene (172 mL) was added a solution of *para*-toluenesulfonic acid monohydrate (81.8 mg, 0.430 mmol) in THF (1 mL) at room temperature under Ar. After stirring for 45 min, the solution was treated with triethylamine (0.090 mL, 0.645 mmol) and then concentrated to give **7** as a colorless oil (21.5 g), which was used for the next reaction without purification.

R_f 0.79 (EtOAc/hexane, 2/1); IR (neat) 2942, 1778, 1442, 1353, 1243, 1201, 1158, 1117, 1078, 1022, 977, 917, 870, 815, 705 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.28–7.05 (m, 4H), 5.60–5.26 (m, 2H), 4.98–4.46 (m, 3H), 4.41 (s, 2H), 4.30–3.72 (m, 2H), 3.54–3.20 (m, 4H), 3.38 (s, 3H), 3.04–2.23 (m, 5H), 2.14–1.90 (m, 2H), 1.90–1.30 (m, 13H); MS (FAB, Pos.) m/z: 501 $(M+H)^+$; HRMS (MALDI-TOF) calcd for $C_{29}H_{40}O_7+Na^+$: 523.2672; found: 523.2712.

11(*R*),15(*S*)-Bis(tetrahydro-2-pyranyloxy)-5,9(*S*)-dihydroxy-16-(3-methoxymethyl)phenyl-1,2,3,4,17,18,19,20-octanorprostanoic acid 8. To a stirred suspension of $LiAlH_4$ (1.54 g, 40.5 mmol) in anhydrous THF (80 mL), cooling in an ice-brine bath, was added dropwise a solution of **7** (21.1 g, 42.2 mmol) over 15 min. After stirring for 45 min, to the reaction mixture was added MeOH (4.5 mL) slowly. The mixture was poured into aqueous sodium bitartrate (14 g/280 mL) and extracted with EtOAc (100 mL) repeatedly. The combined organic layer was washed with brine (100 mL), and dried over $MgSO_4$. The solvent was removed by evaporation to give **8** as a colorless oil (21.2 g), which was used for the next reaction without purification.

R_f 0.35 (EtOAc); IR (neat) 3402, 2941, 1441, 1383, 1352, 1201, 1184, 1134, 1114, 1077, 1021, 976, 913, 869, 813, 704 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.28–7.07 (m, 4H), 5.64–5.24 (m, 2H), 4.78–4.43 (m, 2H), 4.41 (s, 2H), 4.38–4.08 (m, 2H), 4.04–3.26 (m, 7H), 3.39 (s, 3H), 3.10–1.15 (m, 22H); MS (FAB, Pos.) m/z: 505 $(M+H)^+$; HRMS (MALDI-TOF) calcd for $C_{29}H_{44}O_7+Na^+$: 527.2985; found: 527.3008.

11(*R*),15(*S*)-Bis(tetrahydropyran-2-yloxy)-16-(3-methoxymethyl)phenyl-1,2,3,4,17,18,19,20-octanor-5-acetylthio-9(*S*)-trimethylsilyloxyprostanoic acid 10. To a stirred solution of MsCl (2.0 mL, 25.9 mmol) in anhydrous THF (70 mL) was added dropwise diisopropylethylamine (4.5 mL, 25.9 mmol) in 6 min at −15 °C under Ar atmosphere. The resulting slightly suspended solution was stirred at that temperature for 20 min. To the reaction mixture was added a mixed solution of **8** (10.9 g, 21.6 mmol) and diisopropylethylamine (6.4 mL, 36.7 mmol) in anhydrous THF (40 mL) dropwise over 17 min (−15 to −10 °C). The resulting suspension was stirred at that temperature for an additional 30 min and then treated with MeOH (0.35 mL, 8.63 mmol). After stirring for 30 min, to the reaction mixture was added TMSCl (4.1 mL, 32.4 mmol) dropwise in 5 min. The mixture was stirred at a temperature between −10 and −5 °C for another 10 min. Then it was diluted with anhydrous DMF (165 mL), and potassium carbonate (8.6 g, 63 mmol) and potassium thioacetate (4.9 g, 43.2 mmol) was added successively. The reaction mixture was stirred at 50 °C for 4 h. It was cooled to room temperature and diluted with TBME (160 mL). The mixture was washed with water (×2) and brine, and dried over $MgSO_4$. Removal of the solvent by rotary evaporator gave **10** as a brown oil (14.2 g), which was used for the next reaction without purification.

R_f 0.74 (EtOAc/hexane, 1/4); IR (neat) 2942, 1693, 1488, 1441, 1383, 1353, 1323, 1251, 1201, 1183, 1134, 1113, 1078, 1021, 975, 915, 885, 870, 842, 815, 749, 703 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.35–7.05 (m, 4H), 5.50–5.30 (m, 2H), 4.80–4.40 (m, 2H), 4.34 (s, 2H), 4.35–4.20 (m, 1H), 4.20–4.00 (m, 1H), 3.95–3.60 (m, 3H), 3.50–3.30 (m, 1H), 3.37 (s, 3H), 3.30–3.15 (m, 1H), 3.15–3.00 (m, 1H), 3.00–2.70 (m, 3H), 2.60–2.40 (m, 1H), 2.31 (s, 3H), 1.90–1.10 (m, 17H), 0.10 (s, 9H).

16-(3-Methoxymethyl)phenyl-ω-tetranor-5-thiaPGF_1 methyl ester 11,15-bis(tetrahydropyran-2-yl ether) 11. To a stirred solution of **10** (14.2 g, 21.6 mmol) and methyl 4-iodobutyrate (5.91 g, 25.9 mol) in anhydrous MeOH (88 mL) was added potassium carbonate (7.16 g, 51.8 mol) in one portion at room temperature under Ar. After stirring for 2 h, the mixture was diluted with TBME (90 mL) and poured into aqueous NH_4Cl. The organic layer was washed with water (90 mL) and brine (90 mL). The solution was dried over $MgSO_4$ and decolorized with activated carbon powder (Takeda, Shirasagi A, 2.0 g). The mixture was filtered through a Pad of Celite and the filtrate was concentrated. The residue was purified by column chromatography on silica gel (BW235, 220 g, EtOAc/hexane, 2/3∼1/1) to give **11** as a pale yellow oil (10.5 g, 78.4% in three steps).

R_f 0.42 (EtOAc/hexane, 1/1); IR (neat) 3467, 2942, 1739, 1440, 1368, 1321, 1261, 1201, 1135, 1021, 977, 908, 869, 813, 704 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.31–7.07 (m, 4H), 5.65–5.29 (m, 2H), 4.75–4.59 (m, 2H), 4.43 (s, 2H), 4.31–3.19 (m, 7H), 3.67 (s, 3H), 3.37 (s, 3H), 3.00–2.70 (m, 2H), 2.65–2.03 (m, 10H), 2.01–1.28 (m, 16H); MS (FAB, Pos.) m/z: 621 $(M+H)^+$; HRMS (MALDI-TOF) calcd for $C_{34}H_{52}O_8S+Na^+$: 643.3281; found: 643.3300.

16-(3-Methoxymethyl)phenyl-ω-tetranor-5-thiaPGE_1 methyl ester 11,15-bis(tetrahydro-2-pyranyl ether) 12. To a stirred solution of **11** (10.2 g, 16.4 mmol) and diisopropylethylamine (17.2 mL, 98.6 mmol) in EtOAc (63 mL) and DMSO (43 mL) was added a solution of SO_3·Py (7.85 g, 49.3 mmol) in EtOAc (5 mL) and DMSO (25 mL) slowly at a temperature below 10 °C under Ar. After stirring for 50 min, the reaction mixture was diluted with TBME (165 mL) and quenched with water (33 mL) slowly. The mixture was washed with

ice-cooled 1 N HCl (100 mL) and the aqueous layer was extracted with TBME (50 mL). The combined organic layer was washed with water (30 mL), saturated aqueous $NaHCO_3$ (30 mL) and brine (30 mL), and dried over $MgSO_4$. The solvent was removed by evaporation and the residue was purified by column chromatography on silica gel (BW235, 310 g, EtOAc/hexane, 1/2) to give **12** as a pale yellow oil (9.20 g, 90.7%).

R_f 0.45 (EtOAc/hexane, 1/1); IR (neat) 2942, 1740, 1440, 1353, 1200, 1133, 1077, 1021, 975, 911, 870, 815, 704 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.3–7.1 (m, 4H), 5.75–5.5 (m, 2H), 4.8–4.7 and 4.7–4.6 (m, 1H), 4.7–4.6 and 4.5–4.4 (m, 1H), 4.43 (s, 2H), 4.4–4.25 (m, 1H), 4.2–4.05 and 4.0–3.9 (m, 1H), 3.9–3.7 and 3.55–3.4 (m, 3H), 3.67 (s, 3H), 3.38 (s, 3H), 3.3–3.2 (m, 1H), 3.05–2.9 (m, 1H), 2.9–2.6 (m, 2H), 2.6–2.2 (m, 7H), 2.2–2.0 (m, 1H), 2.0–1.3 (m, 17H); HRMS (MALDI-TOF) calcd for $C_{34}H_{50}O_8S + Na^+$: 641.3124; found: 641.3077.

16-(3-Methoxymethyl)phenyl-ω-tetranor-5-thiaPGE$_1$ methyl ester 1a. To a stirred solution of **12** (10.0 g, 16.2 mmol) in acetonitrile (32 mL) and methanol (16 mL) was added 0.1 N HCl (16 mL, 1.6 mmol) at 35 °C under Ar. After stirring for 3 h, the solution was cooled in an ice-bath and diluted with EtOAc (120 mL). The mixture was washed with aqueous $NaHCO_3$ (2.72 g/90 mL) and the aqueous layer was extracted with EtOAc (60 mL). The combined organic layer was washed with brine (50 mL) and dried over $MgSO_4$. The solvent was removed by evaporation and the residue was purified by column chromatography on silica gel (BW235, 300 g, EtOAc–EtOAc/MeOH, 50/1) to give **1a** as a colorless oil (5.40 g, 74.2%).

R_f 0.39 ($CHCl_3$/MeOH, 9/1); $[\alpha]_D^{25}$ −41.8 (*c* 1.0, $CHCl_3$); IR (neat) 3410, 2923, 1744, 1438, 1366, 1317, 1194, 1158, 1090, 1032, 971, 888, 792, 704 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.30–7.10 (m, 4H), 5.75 (dd, $J=6$, 15 Hz, 1H), 5.53 (dd, $J=8$, 15 Hz, 1H), 4.43 (s, 3H), 4.45–4.35 (m, 1H), 3.96 (q, $J=9$ Hz, 1H), 3.67 (s, 3H), 3.42 (s, 3H), 2.95–2.75 (m, 2H), 2.80–2.00 (m, 10H), 2.00–1.60 (m, 4H); MS (FAB, Pos.) *m/z*: 451 $(M+H)^+$. Anal. calcd for $C_{24}H_{34}O_6S$: C, 63.97; H, 7.61; S, 7.12. Found: C, 63.68; H, 7.90; S, 7.41.

16-(3-Methoxymethyl)phenyl-ω-tetranor-5-thiaPGE$_1$ 1b. A heterogeneous mixture of **1a** (3.03 g, 6.72 mmol) and porcine liver esterase (PLE, Sigma, 20000U, 3.0 mL) in EtOH (30 mL) and phosphate buffer (pH 7.4, 300 mL) was stirred for 1 h at room temperature. The resulting clear solution was poured into saturated aqueous $(NH_4)_2SO_4$ and the mixture was extracted with EtOAc twice. The organic layer was dried (Na_2SO_4) and concentrated, and the residue was purified by column chromatography on silica gel ($CHCl_3$ to $CHCl_3$/MeOH, 30/1) to afford **1b** as a pale yellow oil (2.67 g, 91%).

R_f 0.26 (CHCl3/AcOH/MeOH, 20/2/1); IR (neat) 3392, 2922, 1734, 1447, 1240, 1192, 1159, 1085, 1031, 972, 792, 705 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.32–7.11 (m, 4H), 5.76 (dd, $J=16$, 6.3 Hz, 1H), 5.55 (dd, $J=16$, 8.4 Hz, 1H), 4.49–4.39 (m, 3H), 3.95 (m, 1H), 3.42 (s, 3H), 3.32 (br, 3H), 2.90 (dd, $J=14$, 5.6 Hz, 1H), 2.83 (dd, $J=14$, 7.1 Hz, 1H), 2.70 (dd, $J=19$, 7.8 Hz, 1H), 2.60 (t, $J=7.2$ Hz, 2H), 2.55–2.16 (m, 7H), 1.94–1.61 (m, 4H); MS (APCI) *m/z*: 435 $(M-H)^-$; HRMS (MALDI-TOF) calcd for $C_{23}H_{32}O_6S + Na^+$: 459.1817; found: 459.1850.

Prostanoid receptor binding assay

Membranes from CHO cells expressing prostanoid receptors were incubated with radioligand (2.5 nM of $[^3H]PGE_2$ for EP1–4, 2.5 nM of $[^3H]PGF_2\alpha$ for FP, 2.5 nM of [^{3}H]SQ29548 for TP, 5.0 nM of [^{3}H]Iloprost for IP, or 2.5 nM of $[^3H]PGD_2$ for DP) 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 6 mL of liquid scintillation (ACSII) mixture with a liquid scintillation counter. Nonspecific binding was determined by incubation of unlabeled ligand with assay buffer.

Measurement of cAMP production

CHO cells expressing EP4-, EP2-, IP- or DP-receptors were cultured in 24-well plates (1×10^5 cells/well). After 2 days, the medium was 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 the 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).

Measurement of intracellular Ca^{2+} production

The intracellular Ca^{2+} concentration was measured using a Jasco CAM220 Spectrofluorometer. Chinese Hamster Ovary (CHO) cells expressing EP1-, EP3-, TP- or FP-receptors were cultured for two days. After the medium was removed, the cells were washed with PBS and centrifuged at 800 rpm for 3 min. The cells were incubated at 37 °C for 60 min with fura 2-AM in a conditioned medium consisting of MEM containing 20 μM indomethacin, 10% FCS and 10 mM HEPES–NaOH (pH 7.4). The medium containing the cells was centrifuged at 800 rpm for 3 min and the cells were suspended in assay buffer consisting of MEM containing 2 μM indomethacin, 0.1% BSA and 10 mM HEPES-NaOH (pH 7.4). The test compound was added to the suspension of the cells with stirring. Intracellular Ca^{2+} production was calculated from the ratio of the fluorescence intensities at 340 and 380 nm.

LPS-induced changes of cytokine production in human whole blood cells

Heparinized whole blood (from three healthy adult male volunteers) was diluted 1:10 with Iscove's modified Dulbecco's medium supplemented with 0.1% FBS, penicillin (100 U/mL) and streptomycin (100 μg/mL). Diluted whole blood was cultured with lipopolysaccharide (LPS) from WE coli (serotype O55:B5) (100 ng/mL) and the test compound ($10^{-11} \sim 10^{-6}$ M) at 37 °C. After 8 h (TNF-α) or 20 h (IL-10), supernatants were harvested and the levels of several cytokines were determined by commercial ELISA kits (Iwaki Glass, Japan).

LPS-induced changes of plasma TNF-α and IL-10 levels in rats

LPS and the test compound were dissolved in a sterile saline solution. Seven-week-old IGS rats (Charles River, Japan) were injected intravenously with test compounds (10–300 ng/kg/min), followed by an intravenous administration of LPS (10 μg/2 mL/kg) after 30 min. 60 min after the LPS injection, blood samples were withdrawn into heparinized syringes by aorta abdominalis puncture. After centrifugation at 12,000 rpm for 3 min at 4 °C, plasma was recovered and immediately frozen at −80 °C until assayed. Plasma TNF-α and IL-10 concentrations were determined by ELISA kits.

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8. The yield of **3** markedly decreased with an increase in temperature.
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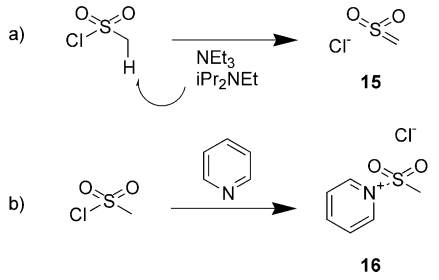
11. To confirm this hypothesis, these reactions were analyzed using ReactIR systems. With regard to methanesulfonylation, two reaction mechanisms are known as shown in Scheme 3a and b. A reactive intermediate **15** is thought to be produced using a trialkylamine as a base (Scheme 3a) while **16** is thought to be produced using pyridine as a base (Scheme 3b).[18] Absorption of **15** (1038 cm^{-1}) was observed with decreased absorption of MsCl (1370, 1177 cm^{-1}) when the reaction was carried out in dichloromethane using Et_3N or *i*-Pr_2NEt as a base. Using THF as a solvent instead of dichloromethane, a completely different result was obtained. In the reaction of MsCl/Et_3N in THF, no absorption of **15** (1038 cm^{-1}) was observed with the decrease in absorption of MsCl (1370 and 1177 cm^{-1}). Using MsCl/*i*-Pr_2NEt in THF, the absorption of MsCl (1370, 1177 cm^{-1}) was retained but the absorption of **15** (1038 cm^{-1}) was not. Based on this information, it was concluded that the above-mentioned reactive intermediates were not produced under these conditions. The methanesulfonylation in THF was considered to proceed via unknown reactive intermediate. *i*-Pr_2NEt was estimated to react with MsCl in the presence of an alcohol with some interaction, which is different from Scheme 3a.



Scheme 3. Formation of the presumed reactive intermediates in the methanesulfonylation.

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