Discovery of an 8-Aza-5-thiaProstaglandin E1 Analog as a Highly Selective EP4 Receptor Agonist

Tohru Kambe,* Toru Maruyama, Atsushi Naganawa, Masaki Asada, Akiteru Seki, Takayuki Maruyama, Hisao Nakai, and Masaaki Toda

Minase Research Institute, Ono Pharmaceutical Co., Ltd.; Shimamoto, Mishima, Osaka 618–8585, Japan.

Received August 9, 2011; accepted September 21, 2011; published online September 27, 2011

For the purpose of discovering an orally available EP4 subtype-selective agonist, a series of 8-aza prostaglandin E1 (PGE1) analogs were synthesized and evaluated for their affinity for PGE1 receptor subtypes. Additionally, the structure–activity relationships of these compounds were studied. Among the tested compounds, the 8-aza PGE1 analog 6 and 8-aza-5-thiaPGE1, analog 12 had highly potent EP4 receptor affinity, good functional activity, and excellent subtype-selectivity. Furthermore, these analogs demonstrated good stability in human liver microsomes. As a result, we concluded that these two series of 8-aza PGE1 analogs could be promising chemical leads for an orally available EP4 subtype-selective agonist.

Key word prostaglandin; agonist; EP4 receptor

Prostaglandin E2 (PGE2) is one of the oxidative metabolites of arachidonic acid produced by cyclooxygenase, and is involved in a number of significant physiological processes. Receptors for PGE2 (EPs) are classified into four subtypes: EP1, EP2, EP3 and EP4. Each subtype mediates different effects in various tissues and cells.1) The EP4 receptor is found to be distributed in the thymus, lungs, heart, kidneys, bone, uterus and other organs, and mediates the production of intracellular cyclic adenosine monophosphate (cAMP). Various biological actions of PGE2, such as its cytoprotective action, improvement of blood flow, regulation of inflammatory cytokine production and bone resorption/formation, are thought to be mediated via the EP4 subtype.2—4) Accordingly, a highly potent EP4 selective agonist is expected to have potential therapeutic effects on rheumatoid arthritis and other diseases without the adverse effects that occur with other EP subtypes.

We previously reported on the discovery of 3,7-dithia-16-phenyl-PGE1, 1 (Fig. 1), which is often used as a probe, specifically a highly selective EP4 agonist, in investigating the role of EP4 receptors.5) 5-ThiaPGE1, 2 was also found to be an optimized structure that functions as a highly selective EP4 receptor agonist (Fig. 1). As a result, the 3,7-dithia and 5-thia moieties were considered to be structural requirements of EP4 subtype-selectivity. However, the chemical instability, primarily due to their easily enolizable α-alkylthiocyclopentanone and/or β-hydroxy cyclopentanone, which is susceptible to dehydration, is a major drawback for compounds 1 and 2 in regards to their drug candidacy. Thus, the chemical instability of compounds 1 and 2 needs to be modified so as to identify a drug candidate.

According to our previous report,5) 3,7-dithia-16-(3-methoxymethylphenyl)methylPGE1, 1 was found to be an excellent EP4 selective agonist that had an improved EP3/EP4 selectivity (Ki: EP3/EP4 = 124). Additionally, 5-thia-16-(3-methoxymethylphenyl)methylPGE1, 2 was also found to have potent affinity for the EP4 subtype and a good EP3/EP4 selectivity (Ki: EP3/EP4 = 80), however it had potent binding affinity for both the EP3 and EP4 subtypes. Based on this observation, the 3,7-dithia moiety of 1 and the 5-thia moiety of 2 were hypothesized to play an important role in the subtype selectivity of EP3 and EP4. In the molecular design process, we first focused on the easily enolizable α-alkylthiocyclopentanone moiety of 3,7-dithiaPGE1, 1, since compound 1 had an equilibrium mixture with its 8-epimer 1’ (1/1’ = 7/3), as determined with 1H-NMR. Based on the analysis described above, the 3,7-dithiaPGE1, analog 1 was predicted to occupy a planar enol form during the epimerization process (Fig. 2). Specifically, the sulfur atom at position-7 may occupy the same plane, which consists of three carbon atoms (C-8, C-9, C-10) of the cyclopentanone ring. To confirm our hypothesis, 7,8-unsaturated PGE1 analog 3 and 8,9-unsaturated cyclopentene PG analog 4 (Fig. 3), with four carbon atoms (C-7, C-8, C-9, C-10) occupying a single plane, were synthesized and biologically evaluated.

![Fig. 1. Reported Structures of Potent and Selective EP4 Agonists](Image of Figure 1)

![Fig. 2. An Easily Enolizable Structure](Image of Figure 2)

![Fig. 3. Structural Mimetics 3 and 4 of the Enol Form of 1](Image of Figure 3)

* To whom correspondence should be addressed. e-mail: kanbe@ono.co.jp © 2011 Pharmaceutical Society of Japan
Herein, we report the discovery of 8-aza-16-phenyl-PGE₁ analogs that are more chemically stable than compounds 1 and 2, as well as function as highly selective EP₄ agonists.

**Chemistry**

Analogs 3 and 4 were synthesized as described in Charts 1 and 2, respectively. A Michael addition reaction of the vinyl iodide 20 with a commercially available enone 16, and the trapping of the resulting enolate anion with an aldehyde 21 yielded 17. Compound 17 was then converted into analog 3 by the following sequential reactions: (1) mesylation of the hydroxyl group followed by the elimination with a methanesulfonyl chloride and 4-dimethylaminopyridine (DMAP), (2) deprotection with (HF)₂-Py, and (3) enzymatic hydrolysis with porcine liver esterase (PLE).

Furthermore, as shown in Chart 2, the Michael addition reaction of the vinyl iodide 20 with a commercially available enone 22, followed by the trapping of the resulting enolate anion with N,N-trifluoromethanesulfonylanilene, yielded an enol triflate 23. Palladium-catalyzed removal of the trifluoromethanesulfonyloxy moiety from compound 23, followed by the deprotection with (HF)₂-Py, and then the enzymatic hydrolysis with PLE, resulted in analog 4.

The synthesis of 11-deoxy analog 5 was described in Chart 3. Acidic dehydration of 24 in acetic acid followed by reduction with lithium aluminum hydride in the presence of copper iodide produced 25. Enzymatic hydrolysis of 25 gave 5.

The synthesis of analogs 6 and 7 is outlined in Chart 4. tert-Butyldimethylsilyl ether (R)-26 and (S)-26 were prepared from commercially available (R)/(S)-5-(hydroxymethyl)-2-pyrrolidinone, respectively, as previously described. N-Alkylation of (R)-26 with ethyl 7-bromohetanoate using sodium hydride in N,N-dimethylformamide (DMF) produced (R)-27. Then, the deprotection of TBS with tetra-butylammonium fluoride (TBAF) produced (R)-28. (R)-28 was then oxidized with a SO₃-Py complex and dimethylsulfoxide in the presence of disopropylethylamine. Then, following a Horner-Emmons olefination and a reaction with 3-[(3-methoxymethyl)phenyl]-2-oxopropanoic acid 31 resulted in an (12R)-eneone 29. A borane reduction of the enone, which was catalyzed by (R)-methyl-Corey–Bakshi–Shibata (CBS)-oxazaborolidine, stereoselectively created an (15S)-isomer 30. Alkaline hydrolysis of the ethyl ester 30 resulted in the production of carboxylic acid 6. Subsequently, analog 7 was synthesized from the (S)-26, based on the same procedure used to prepare analog 6 from (R)-26.

Analogs 8 and 9 were prepared, as shown in Chart 5. N-Acylation of 7-n-prolinol 32 with ethyl 6-(chloroformyl)hexanoate in aqueous sodium hydroxide and dioxane produced 33. Compound 33 was the converted to 8 using the sequential reactions described above. O-Protection of 2-N-acetyl amino-ethanol 34 was achieved by using a tetrahydropyranol ether. The product was then N-alkylated with an ethyl 7-bromohetanoate in the presence of sodium hydride, which resulted in the production of 36. Deprotection of 36 under acidic conditions produced alcohol 37, which was then transformed into analog 9, as described in Chart 4.

The synthesis of a sulfonamide analog 10 is outlined in Chart 6. A palladium-catalyzed carbon monoxide insertion into a vinyl iodide 38, in the presence of methanol, yielded a methyl ester 39. The methyl ester 39 was then reduced with disobutylaluminum hydride (DIBAL), and resulted in the following products:

*Chart 1.* Synthesis of 7,8-Unsaturated PGE₁, Analog 3

*Chart 2.* Synthesis of Cyclopentene PG Analog 4

*Chart 3.* Synthesis of 11-Deoxy PG Analog 5
production of an allyl alcohol 40. Methanesulfonylation of 40 yielded a methanesulfonate, which was then used in the N-alkylation of a sulfonamide 43, in the presence of sodium hydride, resulting in 41. Deprotection of TBS with tetra-n-butylammonium fluoride (TBAF), followed by alkaline hydrolysis, produced analog 10.

An 8-aza-PGE₂ analog 11 was synthesized, as illustrated in Chart 7. First, a dimagnesium salt of 44 was prepared from hex-5-inoic acid and ethylmagnesium bromide. The dimagnesium salt of 44 underwent an addition reaction with paraformaldehyde, which was then followed by esterification with trimethylsilyldiazomethane, and produced compound 45. Bromination of 45 with carbon tetrabromide and triphenylphosphine yielded a bromide 46. N-Alkylation of (R)-26 with the bromide 46 in the presence of sodium hydride produced 47, which was then transformed into 48 by the following sequential reactions: (1) catalytic partial hydrogenation with a Lindlar catalyst in the presence of cyclohexene,
The synthesis of analogs was achieved with potassium carbonate in methanol and in dimethylsulfoxide in the presence of triethylamine, followed by an S-alkylation of compound 51. Deprotection of compound 54, via 53, with tetrabutylammonium fluoride (TBAF) resulted in compound 55, which was used in the synthesis of compound 60, which was used in the synthesis of compound 63. The preparation of methyl 7-iodo-(5S)-methylheptanoate 60, which was used in the synthesis of compound 63, is described in Chart 10. O-Protection of (−)-β-citronellol with a TBS ether, followed by ozonolysis, resulted in an aldehyde 66. A sodium borohydride reduction of compound 66 produced an alcohol 67. p-Toluenesulfonylation of compound 67 with p-toluenesulfonyl chloride in pyridine, followed by a substitution reaction with sodium cyanide in dimethyl sulfoxide (DMSO), resulted in a nitrile 68. The nitrile 68 was then converted into a hydroxyl ester 70 in three steps. Methanesulfonylation of 70, followed by nucleophilic substitution with sodium iodide, produced an iodide 60.

The preparation of methyl 7-iodo-(5R)-methylheptanoate 61 is outlined in Chart 11. Compound 67, which was used as a common intermediate to prepare both enantiomers 60 and 61, was transformed into 71 by the following sequential reactions: a) oxidation with SO$_3$-Py and triethylamine in dimethylsulfoxide, b) an addition of methylmagnesium bromide, and c) another oxidation reaction. C-2 homologation of compound 71, which produces compound 72, was achieved via the following sequential reactions: d) deprotection with TBAF, e) oxidation with pyridinium chlorochromate (PCC), f) a Horner–Emmons olefination with methyl diethylphosphonate in the presence of sodium hydride, and g) a catalytic hydrogenation. A Baeyer–Villiger oxidation of compound 72 with m-chloroperbenzoic acid (m-CPBA) yielded compound 73, which was converted into compound 61 via the following three step reaction: i) methanolysis with methanol in the presence of potassium carbonate, j) methanesulfonylation via the conventional method, and k) a substitution reaction with sodium iodide in acetone. Methyl 5,5-dimethylene-7-iodo-heptanoate 62 was prepared, as illustrated in Chart 12. O-Acetylation of homo-

---

[Diagram and text from the document]
Reagents: a) Boc₂O, DMAP, THF; b) TBAF, THF, 75% in 2 steps; c) SO₃-Py, i-Pr₂NEt, DMSO, AcOEt; d) phosphonate 31, NaH, THF, 65% in 2 steps; e) (R)-Me-CBS, BH₃-THF, THF, 85%; f) TBSCI, imidazole, DMF; g) Mg(OMe)₂, MeOH; 71% in 2 steps.

Chart 9. Synthesis of 8-Aza-PGE₁ Analogs

Reagents: a) TBSCI, imidazole, DMF; b) O₃, Me₂S, MeOH, CH₂Cl₂, 70%; c) NaBH₄, MeOH, 72%; d) TiCl₄, pyridine; e) NaCN, DMSO, 93%; f) TBAF, THF, 77%; g) KOH, EtOH, H₂O; h) CH₂N₂, Et₂O, 91%; i) MsCl, Et₃N, CH₂Cl₂; j) NaI, acetone; 67%.

Chart 10. Preparation of Methyl 7-iodo-(5S)-methylheptanoate

Reagents: a) SO₃-Py, Et₃N, DMSO, EtOAc; b) MeMgBr, THF; c) SO₃-Py, Et₃N, DMSO, EtOAc, 62%; d) TBAF, THF, 83%; e) PCC, CH₂Cl₂; f) MeO₂CCH₂PO(OEt)₂, NaH, THF, 82%; g) H₂, Pd/C, MeOH, 95%; h) m-CPBA, CH₂Cl₂; i) K₂CO₃, MeOH, 65%; j) MeCl, Et₃N, CH₂Cl₂; k) NaI, acetone, 65%.

Chart 11. Preparation of 7-iodo-(5R)-methylheptanoate

Reagents: a) Ac₂O, pyridine; b) NaI, TMSCl, MeCN, H₂O, 47%; c) I₇Zn(CH₃)₂CO₂Me, Pd(PPh₃)₃, DMF, 68%; d) K₂CO₃, MeOH, 90%; e) Et₂Zn, CH₂I₂, CH₂Cl₂; f) NaI, 75% in 2 steps.

Chart 12. Preparation of Methyl 5,5-Dimethylene-7-iodoheptanoate
propargyl alcohol, followed by iodination with sodium iodide, produced a vinyl iodide. A cross-coupling reaction with compound and 3-methoxycarbonylpropyl zinc iodide in the presence of tetrakis(triphenylphosphine)palladium, followed by deacetylation, yielded compound. Lastly, a cyclopropanation reaction with compound under Simmons–Smith conditions, followed by a substitution reaction with iodine in the presence of triphenylphosphine, resulted in an iodide.

**Results and Discussion**

The compounds listed in Tables 1—3 were evaluated for their binding affinity for mouse EP receptor subtypes. The agonist activities of these compounds on each of the EP4 receptor subtype were also evaluated (Table 3).

As shown in Table 1, analog demonstrated excellent EP4 selectivity, while its potency was significantly decreased. The structure–activity relationship (SAR) also demonstrated that the cyclopentene analog had improved EP3/EP4 selectivity compared with compound. On the other hand, compound, which is 11-deoxy derivative of, showed quite good EP3/EP4 selectivity maintaining good EP4 receptor affinity (Fig. 4). Thus, the 11-hydroxy residue, which is one of the reasons for chemical instability, was suggested to be removable without reduction of EP4 subtype-selectivity.

Based on these observations, we synthesized and evaluated 8-aza-lactam analogs, such as (Table 2), which had four atoms (i.e. C-7, N-8, C-9, C-10) corresponding with the C-7, C-8, C-9 and C-10 of analog that occupied a single planar conformation, as a result of the double bond character of the amide C–N bond.

The lactam analog, which has been previously evaluated, exhibits potent binding affinity, agonist activity (compound EC$_50$=24 nM), and excellent EP4 subtype selectivity. Its corresponding 12-$S$ isomer was found to be 160-fold less potent in its binding affinity for EP4 receptor, however it still demonstrated subtype selectivity. Thus, the 12-$R$-configuration of analog is necessary for its potent binding affinity to EP4 receptor.

The $N$-acyl pyrrolidine analog had a 53-fold lower EP4 binding affinity relative to analog because of the presumed steric and/or electronic factors of the 7-carbonyl moiety of although the planarity of the amide moiety still remains. The sec-analog also demonstrated weak affinity for the EP4 receptor, while the corresponding sulfonamide analog did not demonstrate EP4 affinity (i.e. up until a concentration of 10$^4$ nM) possibly due to the non-planarity of its sulfonamide moiety.Fig. 4—17) These findings are consistent with the abovementioned SAR.

The $\alpha$-chain of analog was further optimized so as to improve the activity profiles (Table 3). The introduction of a 5,6-cis-double bond into the 8-aza-PGE$_1$ analog yielded an 8-aza-PGE$_2$ analog, which had a 4-fold lower EP4 binding affinity and a 20-fold lower agonist activity.

Based on the abovementioned activity profiles of 5-thiaPGE$_1$, 8-aza-5-thiaPGE$_1$ was designed and synthe-

---

**Table 1. Binding Affinity of Analogs 1—5 for Each of EP Receptor Subtypes**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Binding affinity $K_i$ (nM)</th>
<th>mEP1</th>
<th>mEP2</th>
<th>mEP3</th>
<th>mEP4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$&gt;10^4$</td>
<td>2100</td>
<td>1200</td>
<td>9.7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>$&gt;10^4$</td>
<td>620</td>
<td>56</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>$&gt;10^4$</td>
<td>$&gt;10^4$</td>
<td>$&gt;10^4$</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>$&gt;10^4$</td>
<td>1600</td>
<td>$&gt;10^4$</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>$&gt;10^4$</td>
<td>470</td>
<td>190</td>
<td>2.4</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Activity Profiles of Various PGE$_1$ Frameworks Possessing an Optimized $\alpha$-Chain**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Binding affinity $K_i$ (nM)</th>
<th>mEP1</th>
<th>mEP2</th>
<th>mEP3</th>
<th>mEP4</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td></td>
<td>$&gt;10^4$</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>$&gt;10^4$</td>
<td>1600</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>$&gt;10^4$</td>
<td>530</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>$&gt;10^4$</td>
<td>250</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>$&gt;10^4$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\text{Ar}=3\text{-}(\text{Methoxymethyl})\text{phenyl}$. 

---
sized. Analog 12 was found to have an excellent EP4 selectivity with good agonist activity. Thus, the C-5 of analog 6 may be replaced by a sulfur atom without reducing EP4 affinity, agonist activity and subtype selectivity. Furthermore, analogs 13—15 were synthesized and evaluated to investigate the detailed SAR of the C-5-substitutions, specifically the 5S-methyl, 5R-methyl and 5,5-dimethylene residues. An introduction of the 5S methyl and 5-R methyl groups into compound 6 yielded compounds 13 and 14. Compounds 13 and 14 retained their EP4 selectivity and EP4 receptor affinity; however, they had a 41-fold and 10-fold lower agonist activity, respectively. An introduction of a 5,5-dimethylene residue into compound 6 produced compound 15, which also retained its EP4 selectivity, but had a 30-fold lower agonist activity. Both compounds 11 and 13—15 demonstrated an unexpected weak agonist activity for their relatively potent EP4 binding affinity. However, the reduction in agonist activity was especially remarkable in analogs 13 (35-fold) and 15 (55-fold). Remarkable reduction of the agonist activity for their potent receptor affinity was considered to be due to their increased protein binding and/or steric factors based on the newly introduced lipophilic alkyl moieties.

Since our purpose has been discovery of an orally available EP4 subtype-selective agonist, representative compounds were evaluated for their metabolic stability in the human liver microsomes. Their agonist activity in rat Chinese hamster ovary (CHO) overexpressing EP4-receptor was also evaluated for the scheduled in vivo evaluation in rats. Results are summarized in Table 4.

Compounds 2 and 5 exhibited potent agonist activity while they showed relatively unstable in the human liver microsomes. Compound 6 showed less potent agonist activity relative to 2 and 5 although it showed more stability. The corresponding 5-thia analog 12 exhibited equipotent agonist activity and better stability in the liver microsomes. The 5-thiaPGE1 analog 2 decomposed due to the oxidation of its sulfur atom at 5-position, while the 8-aza-5-thiaPGE1 analog 12 was highly stable in liver microsomes despite its 5-thia moiety. The relatively more polar γ-lactam moiety may play a role in preventing the oxidation of compound 12 via metabolic enzymes. Thus, replacement of the 5-carbon atom with a sulfur atom was reconfirmed to be effective for the increase of the EP4 affinity and agonist activity while analogous results were reported in our previous report. As a result, 8-aza-5-thiaPGE1 12 was considered to be a promising chemical lead for orally active EP4 agonist because of its potent agonist activity with excellent subtype-selectivity.

In summary, a series of 8-aza-16-phenyl-PGE1 analogs, which had a γ-lactam moiety instead of the cyclopentanone moiety of PGEs, were found to have potent EP4 agonist activity with good subtype selectivity. Of the compounds tested, analogs 6 and 12 were found to be chemically and metabolically stable EP4 subtype-selective agonists, which demonstrated highly potent EP4 agonist activity with good subtype-selectivity. Furthermore, the γ-lactam moiety was found to be a biososere of α-alkylthiocyclopentanone moiety of 7-thiaPGE analogs. Further research optimizing these compounds so as to produce orally available EP4 agonists is warranted.

**Experimental**

**mEP1-4 Receptor Binding Assay** Competitive binding studies were conducted using radiolabeled ligands and membrane fractions prepared from CHO cells, which stably express the prostanoid receptors mEP1—4. Membranes from CHO cells expressing prostanoid receptors were incubated with a radiolabeled ligand (i.e. 2.5 nM [3H]PGE1) and test compounds at various concentrations in an assay buffer (i.e. 10 nM KH2PO4–KOH buffer containing 1 nM ethylenediaminetetraacetic acid (EDTA), 10 mM MgCl2, and 0.1 mM NaCl, pH 6.0). Incubation was carried out at 25 °C for 60 min, with the exception of mEP1, which was incubated for 20 min. Incubation was terminated via filtration through a Whatman GF/B filter. The filter was subsequently washed with ice-cold buffer (10 mM KH2PO4–KOH buffer containing 0.1 mM NaCl, pH 6.0), and the radioactivity on the filter was measured in a 6 ml liquid scintillation (ACSII) mixture with a liquid scintillation counter. Non-specific binding was achieved by adding excess amounts of unlabeled PGE1, in the assay buffer. The concentration that causes 50% of inhibition (IC50 value) was estimated from the regression curve. The Ki value (M) was calculated according to the following equation: \[ K_i = IC_{50} (1 + [I]/K_a) \], where \([I]\) is the concentration of radiolabeled ligand and \(K_a\) is the dissociation constant of radiolabeled ligand for the prostanoid receptor of interest.

**Table 3. Activity Profiles of Various α-Chain PG Analogs Possessing an Optimized α-Chain**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Binding affinity (K_i) (nM)</th>
<th>EC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>&gt;10⁴</td>
<td>8400</td>
</tr>
<tr>
<td>12</td>
<td>&gt;10⁴</td>
<td>8500</td>
</tr>
<tr>
<td>13</td>
<td>&gt;10⁴</td>
<td>&gt;10⁴</td>
</tr>
<tr>
<td>14</td>
<td>&gt;10⁴</td>
<td>4900</td>
</tr>
<tr>
<td>15</td>
<td>&gt;10⁴</td>
<td>2900</td>
</tr>
</tbody>
</table>

\(\text{Ar} = 3\text{-}3\text{-}\text{Methoxymethyl}/1\text{phenyl}\).

**Table 4. Metabolic Stability in the Human Liver Microsomes and EP4 Agonist Activity in Rat of Representative Compounds**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Functional assay in rat EC50 (nM)</th>
<th>Stability in liver microsomes % remaining in HLM&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.4</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>1.1</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>72</td>
</tr>
<tr>
<td>12</td>
<td>1.3</td>
<td>94</td>
</tr>
</tbody>
</table>

<sup>a</sup> HLM: human liver microsomes. Concentration of test compounds, 1 μM; liver microsomes, 1 mg/ml.
EP-receptor were cultured in 24-well plates (1 × 10^4 cells/well). After 2 d, the media were removed and cells were washed with 500 µl of minimum essential medium (MEM) and incubated for 10 min in 500 µl of buffer (MEM containing 2 µM of dicyclofenac) at 37 °C. After the removal of buffer via suction, cells were pre-incubated in 450 µl of assay medium (containing 1% of bovine serum albumin (BSA)) for 10 min at 37 °C. 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 adding 500 µl of ice-cold 10% trichloroacetic acid. cAMP production was determined via a cAMP radioimmunoassay kit (Amersham).

Human Microsome Stability Assessments

The test compound (5 µl, 10 mm in DMSO) was diluted in 995 µl of assay buffer. After incubation for 10 min at 37 °C, the reaction mixture was warmed to 37 °C in a water bath, and incubated for 5 min. The mixture was then diluted with 500 µl of assay medium (containing 2 µM of dicyclofenac) at 37 °C for 10 min and centrifuged, and the resulting residue was purified by column chromatography on silica gel (EtOAc:hexane, 1:2) to afford 18 as a pale yellow oil (76 mg, 67%). 1H-NMR (300 MHz, CDCl3, δ): 7.32–7.00 (m, 4H), 6.71 (td, J = 7.5, 1.5 Hz, 1H), 5.51 (dd, J = 15.6, 5.7 Hz, 1H), 5.42 (dd, J = 15.6, 4.2 Hz, 1H), 4.41 (s, 2H), 2.47–4.21 (m, 1H), 4.09 (d, J = 4.8 Hz, 1H), 3.66 (s, 3H), 3.40–3.29 (m, 4H), 2.76–2.62 (m, 2H), 2.43–2.02 (m, 1H), 1.67–1.25 (m, 6H), 0.84 (s, 9H), 0.82 (s, 9H), 0.05 (s, 6H), −0.14 (s, 3H), −0.23 (s, 3H), −0.14 (s, 3H).

(7E)-7-Didehydro-16-(3-methoxyphenyl) methoxytetrahydro-PGE2, Methyl Ester (19)

A solution of 18 (33 mg, 0.049 mmol) and pyridine (0.1 ml) in acetonitrile (1.5 ml) was cooled in an ice-bath and treated with TBAF, py (Aldrich, 0.2 ml). The mixture was stirred for 90 min without cooling and then slowly poured into a heterogeneous mixture of EtOAc and saturated aqueous NaHCO3 under stirring. The mixture was separated and the aqueous layer was extracted with EtOAc a few times. The combined organic layers were washed with 1 M HCl (aq) and saturated aqueous NaHCO3, brine, and dried over Na2SO4. The organic solvent was removed by evaporation and the residue was purified by column chromatography on silica gel (hexane/EtOAc, 1:0–1:0) to give 19 as a pale yellow oil (43 mg, 88%). 1H-NMR (200 MHz, CDCl3, δ): 7.32–7.07 (m, 4H), 6.77 (d, J = 7.5, 2.0 Hz, 1H), 5.63 (dd, J = 15.0, 6.0 Hz, 1H), 5.54 (dd, J = 15.0, 8.0 Hz, 1H), 4.43–4.35 (m, 3H), 4.19–4.02 (m, 1H), 3.65 (s, 3H), 3.48 (m, 1H), 3.41 (s, 3H), 2.81 (d, J = 6.6 Hz, 2H), 2.48 (dd, J = 18.0, 5.4 Hz, 1H), 2.34–2.09 (m, 6H), 1.95–1.85 (m, 11H), 1.70–1.23 (m, 6H).

(7E)-7-Didehydro-16-(3-methoxyphenyl) methoxytetrahydro-PGE2, Methyl Ester (20)

A solution of 19 (43 mg, 0.10 mmol) and porcine liver esterase (PLE) (Sigma, 2000 U, 0.1 ml) in EtOH (0.5 ml) and phosphate buffer (pH 7.4, 2.5 ml) was stirred for 1.5 h at room temperature. The resulting clear solution was poured into saturated aqueous NH4HCO3 and the mixture was extracted with EtOAc twice. The organic layer was dried over Na2SO4 and concentrated. The residue was purified by column chromatography on silica gel (CHCl3/MeOH, 1:0–1:0) to afford 20 as a colorless oil (22 mg, 53%). IR (film cm−1): 3398, 2930, 2859, 1715, 1645, 1385, 1191, 1033, 974, 890, 793, 735, 705. 1H-NMR (300 MHz, CDCl3, δ): 7.32–7.07 (m, 4H), 6.74 (d, J = 7.5, 2.0 Hz, 1H), 5.70–5.56 (m, 2H), 4.3–4.38 (m, 3H), 4.09–4.05 (m, 1H), 3.49–3.43 (m, 1H), 3.42 (s, 3H), 2.89–2.76 (m, 2H), 2.52 (dd, J = 18.0, 5.4 Hz, 1H), 2.38–2.06 (m, 7H), 1.67–1.23 (m, 6H), 0.84 (s, 9H), 0.82 (s, 9H), 0.05 (s, 6H), 0.14 (s, 3H), 0.23 (s, 3H), 0.14 (s, 3H).

(11R,12R,13E,15S)-9-[(Trifluoromethyl)sulfonyloxy]-(11,15-bis-( tert-butylidimethylsilyloxy)-16-(3-methoxyphenyl)-17,18,19,20-tetranorprostan-8,13-dioic Acid Methyl Ester (23) To a stirred solution of 3-(3S)-tert-butylidimethylsilyloxy-1-iodo-4-(3-methoxyphenyl)benzyl-1-butene 20 (600 mg, 1.4 mmol) in freshly distilled dry Et2O (6 ml) was slowly added tert-butyl lithium (1.47 M in pentane, 1.9 ml, 2.8 mmol) at −70 °C under argon atmosphere and stirring was continued for 1 h at the same temperature. To the resulting suspension was added lithium 2-thienylcyanacurate (0.25 m in THF, 3.1 ml, 0.76 mmol). The yellow suspension was stirred for 20 min and then a solution of 4-(R)-tert-butylidimethylsilyloxy-2-cyclopentene 16 (147 mg, 0.69 mmol) in THF (1 ml) was added dropwise in 3 min. After being stirred for additional 30 min at −70 °C, the resulting yellowish reaction mixture was treated with methyl 7-oxoepox- tanoate 21 (121 mg, 0.76 mmol) and then stirred for 90 min and the reaction was quenched with saturated aqueous NH4Cl. The mixture was vigorously stirred for 30 min without cooling and then extracted with diethyl ether repeatedly. The combined organic layers were washed with H2O, brine, dried over MgSO4, and the organic solvent was removed by evaporation. The residue was purified by column chromatography on silica gel (hexane/ EtOAc, 5:1–2:1) to give 17 as colorless oil (119 mg, 25%). 1H-NMR (300 MHz, CDCl3, δ): 7.37–7.06 (m, 4H), 5.63 (dd, J = 15.6, 6.0 Hz, 1H), 5.51 (dd, J = 15.6, 8.0 Hz, 1H), 4.42 (s, 2H), 4.3–4.12 (m, 1H), 4.06–4.01 (m, 1H), 3.72–3.63 (m, 4H), 3.38 (s, 3H), 3.27–3.25 (m, 1H), 2.74 (d, J = 6.6 Hz, 2H), 2.70–2.50 (m, 2H), 2.35–2.18 (m, 4H), 2.05–1.97 (m, 1H), 1.70–1.30 (m, 7H), 0.88 (s, 9H), 0.84 (s, 9H), 0.06 (s, 6H), −0.12 (s, 3H), −0.26 (s, 3H), −0.23 (s, 3H).
3H).

\((11R,12R,13E,15S)-11.15-Dihydroxy-16-(3-methoxymethylphenyl)-17,18,19,20-tetranorprost-8,13-dienoic Acid (4)\) A mixture of 23 (400 mg, <0.49 mmol), formic acid (0.038 ml, 1.0 mmol), triethylamine (0.21 ml, 1.5 mmol), triphenylphosphine (53 mg, 0.2 mmol) and palladium acetate (22 mg, 0.1 mmol) in DMF was stirred at 60 °C for 3 h under argon atmosphere. The resulting solution was cooled to ambient temperature and purified by a short column chromatography on silica gel to remove the residual metal. The collected fractions were concentrated to result in crude olefin as a brown oil.

A solution of the above-described olefin and pyridine (0.3 ml) in CH\(_2\)CN (5 ml) was cooled to 0 °C and treated with (HF\(_2\))\(_3\)Py (0.6 ml). The reaction mixture was stirred for 2 h without cooling and then slowly poured into a heterogeneous stirred mixture of EtOAc and saturated aqueous NaHCO\(_3\). The aqueous phase was extracted with EtOAc again. The combined organic layers were washed with water, 1 M HCl, water, saturated aqueous NaHCO\(_3\), brine and dried over Na\(_2\)SO\(_4\). The solvent was removed by evaporation and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 1/0—1/0) to give an as a pale yellow oil (71 mg, <3%). A heterogeneous mixture of ester (52 mg, <0.13 mmol) and porcine liver esterase (PSE) (Sigma, 20000 U, 0.1 ml) in EtOH (5 ml) and phosphate buffer (pH 7.4, 1 ml) was stirred for 2 h at room temperature. The resulting clear solution was poured into saturated aqueous Na\(_2\)SO\(_4\), and extracted with EtOAc. The combined organic layers were dried (Na\(_2\)SO\(_4\)) and concentrated, and the residue was purified by column chromatography on silica gel (hexane/EtOAc, 1/0—1/0) and then EtOAc/AcOH, 99/1) to give 4 as a pale brown oil (38 mg, 76%). IR (film cm\(^{-1}\)) 3364, 2928, 2856, 1710, 1447, 1194, 1087, 1033, 971, 791, 756, 703. 1H-NMR (300 MHz, CDCl\(_3\)): \(\delta\) 7.30—7.12 (m, 4H), 5.62 (dd, \(J = 16.0, 6.9, 1H\)), 5.41 (dd, \(J = 16.0, 9.9, 1H\)), 5.32 (d, \(J = 12.9, 1H\)), 4.43 (s, 2H), 4.38 (m, 3H), 4.12—4.05 (m, 4H), 3.52 (s, 3H), 2.98 (m, 1H), 2.91—2.78 (m, 2H), 2.65 (m, 1H), 2.53 (t, \(J = 7.0, 2H\)), 2.26—2.14 (m, 1H), 2.05—1.83 (m, 2H), 1.69—1.52 (m, 2H), 1.50—1.22 (m, 6H). MS (APCI m/z): 401.2324 (Calcd for C\(_{24}\)H\(_{33}\)O\(_5\): 401.2328).

To a stirred solution of 4 (52 mg, 0.13 mmol) and porcine liver esterase (PSE) (Sigma, 20000 U, 0.1 ml) in 1 M HCl (0.35 ml) was stirred at 80 °C under argon atmosphere. Stirring was continued at room temperature for 1 h, and 60 °C for additional 1 h. To the resulting suspension was added ethyl 7-bromohexanoate (0.6 ml, 3.13 mmol) at 0 °C, and stirring was continued at 80 °C for additional 1.5 h. The resulting pale brown solution was poured into saturated aqueous NaCl, extracted with EtOAc, washed with H\(_2\)O, brine, and dried over MgSO\(_4\). The organic solvent was removed by evaporation, and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 2/0—1/0) to give (R)-27 as a colorless oil (620 mg, 63%). 1H-NMR (300 MHz, CDCl\(_3\)): \(\delta\) 4.11 (q, \(J = 7.2, 2H\)), 3.71—3.55 (m, 4H), 3.02—2.90 (m, 1H), 2.50—2.22 (m, 4H), 2.13—1.99 (m, 1H), 1.88—1.86 (m, 1H), 1.66—1.44 (m, 5H), 1.40—1.25 (m, 3H), 1.12 (t, \(J = 7.2, 2H\)), 0.88 (s, 9H), 0.95 (s, 6H).

**Ethyl 7-[[2R]-2-(Hydroxymethyl)-5-oxo-1-pyrrolidinyl]heptanoate (R)-28** A solution of (R)-27 (620 mg, 1.61 mmol) in THF (2.0 ml) was treated with a solution of TBAB (tetrabutylammonium fluoride, 1.0 mol in THF, 1.93 ml, 1.93 mmol) at room temperature under argon atmosphere for 1 h. The reaction was quenched with H\(_2\)O. The reaction mixture was extracted with EtOAc three times. The combined organic layers were washed with brine, and dried over Na\(_2\)SO\(_4\). The organic solvent was removed by evaporation, and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 1/0—1/0) to give an as a pale yellow oil (220 mg, 30%). To a stirred solution of dimethyl 3-[[3-methoxymethyl]phenyl]-2-oxopropanephosphonate (584 mg, 2.04 mmol) in THF (20 ml) was added sodium hydride (60% in mineral oil, 68.0 mg, 1.75 mmol) in several portions at 0 °C under argon atmosphere. After being stirred at ambient temperature for 90 min, to this stirred suspension was added a solution of the above-described aldehyde in THF (2 ml) at 0 °C and stirring was continued for 2.5 h. The reaction mixture was quenched with acetic acid. The resulting yellow solution was diluted with EtOAc, washed with water, then brine, and dried over MgSO\(_4\). The solvent was removed by evaporation and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 1/1—0/1) to give an as a pale yellow oil (220 mg, 30%). 1H-NMR (300 MHz, CDCl\(_3\)): \(\delta\) 7.38—7.10 (m, 4H), 6.65 (dd, \(J = 15.6, 6.6, 1H\)), 4.41 (s, 2H), 4.21—4.03 (m, 3H), 3.85 (s, 2H), 3.05 (m, 1H), 1.34 (s, 3H), 2.70 (m, 1H), 2.43—2.19 (m, 5H), 1.83—1.75 (m, 1H), 1.68—1.56 (2H), 1.50—1.18 (2H).

**Ethyl 7-[[2R]-2-[[1(E)-4-[3-(methoxymethyl)phenyl]-3-oxo-1-butenyl-1-yl]-5-oxo-1-pyrrolidinyl]heptanoate (R)-29** A solution of (R)-28 (620 mg, 1.61 mmol) in THF (2.0 ml) was treated with a solution of TBAF (tetrabutylammonium fluoride, 1.0 mol in THF, 1.93 ml, 1.93 mmol) at room temperature under argon atmosphere for 1 h. The reaction was quenched with H\(_2\)O. The reaction mixture was extracted with EtOAc three times. The combined organic layers were washed with brine, and dried over Na\(_2\)SO\(_4\). The organic solvent was removed by evaporation, and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 1/0—1/0) to give an as a pale yellow oil (220 mg, 30%). To a stirred solution of dimethyl 3-[[3-methoxymethyl]phenyl]-2-oxopropanephosphonate (351 mg, 2.04 mmol) in TMF (20 ml) was added sodium hydride (60% in mineral oil, 68.0 mg, 1.75 mmol) in several portions at 0 °C under argon atmosphere. After being stirred at ambient temperature for 20 min, the reaction mixture was quenched with 1 M HCl. The reaction mixture was extracted with EtOAc three times, washed with saturated aqueous NaHCO\(_3\), brine, and dried over MgSO\(_4\). The organic solvent was removed by evaporation to yield an aldehyde as a pale yellow oil.
Na₂SO₄. The organic solvent was removed by evaporation. The resulting residue was purified by column chromatography on silica gel (CHCl₃/MeOH, 50/1—20/1) to afford 5 as a colorless oil (104 mg, 98%). IR (film cm⁻¹): 3410, 2925, 1734, 1639, 1444, 1405, 1381, 1219, 1157, 1091, 1029, 971, 793, 756, 704. ¹H-NMR (300 MHz, CDCl₃) δ: 7.36—7.12 (m, 4H), 5.72 (d, J = 1.5 Hz, 1H), 5.68—5.64 (m, 2H), 4.47 (s, 2H), 4.46—4.34 (m, 1H), 3.74 (s, 3H), 3.13 (s, 2H), 2.91—2.80 (m, 2H), 2.60—2.30 (m, 4H), 2.23 (d, J = 1.5 Hz, 3H), 2.22—2.05 (m, 3H), 2.00—1.78 (m, 2H), 1.75—1.57 (m, 2H). ¹³C-NMR (125 MHz, CDCl₃) δ: 177.2, 175.1, 138.5, 137.6, 130.4, 128.1, 128.6, 74.7, 72.3, 60.4, 58.3, 43.9, 40.5, 33.8, 30.1, 28.5, 26.9, 26.3, 25.7, 24.5. MS (APCI) m/z: 431 (M⁺—H⁻). HR-MS-FAB (m/z): 402.2285 (Calcd for C₂₃H₂₉NO₄; 402.2280).

Ethyl 7-(2-(Butyldimethylsilyloxy)methyl-5-oxo-1-pyrrolidinyl)heptanoate (S-(27)) Compound 27 was prepared from (S)-26 according to the same procedure as described of (R)-27 as (R)-26 as a pale yellow oil (54% yield). ¹H-NMR (300 MHz, CDCl₃) δ: 4.11 (q, J = 7.2 Hz, 2H), 3.72—3.52 (m, 4H), 2.96 (m, 1H), 2.35—2.28 (m, 2H), 2.04 (m, 1H), 1.81 (m, 1H), 1.72—1.18 (m, 9H), 1.24 (t, J = 7.2 Hz, 3H), 0.87 (s, 9H), 0.04 (s, 3H).

Ethyl 7-(2S)-2-(Hydroxymethyl)-5-oxo-1-pyrrolidinyl)heptanoate (S-(28)) Compound 28 was prepared from (S)-27 according to the same procedure as described of (R)-28 as (R)-27 as a pale yellow oil (78% yield). ¹H-NMR (200 MHz, CDCl₃) δ: 4.12 (q, J = 7.2 Hz, 2H), 3.87—3.53 (m, 4H), 2.98 (m, 1H), 2.60—1.84 (m, 1H), 1.80—1.20 (m, 8H), 1.26 (t, J = 7.2 Hz, 3H).

Ethyl 7-(2S)-2-(1E,3S)-3-Hydroxy-4-[3-(methoxymethyl)phenyl]-1-buten-1-yl]-5-oxo-1-pyrrolidinyl)heptanoate (S-(29)) Compound 29 was prepared from (S)-28 according to the same procedure as described of (R)-29 as (R)-28 as a pale yellow oil. This crude oil was used for the next reaction without further purification.

Ethyl 7-(2S)-2-(1E,3S)-3-Hydroxy-4-[3-(methoxymethyl)phenyl]-1-buten-1-yl]-5-oxo-1-pyrrolidinyl)heptanoate (6) Compound 30 was prepared from (S)-29 according to the same procedure as described of (R)-30 from (R)-29 as a colorless oil (42% from (S)-28). ¹H-NMR (300 MHz, CDCl₃) δ: 7.34—7.09 (m, 4H), 5.75 (dd, J = 15.5, 5.9 Hz, 1H), 5.47 (dd, J = 15.6, 6.1 Hz, 1H), 4.44 (s, 2H), 4.40 (m, 1H), 4.11 (q, J = 7.2 Hz, 2H), 4.03 (m, 1H), 3.48 (m, 1H), 3.40 (s, 3H), 2.94—2.72 (m, 3H), 2.44—1.84 (m, 6H), 1.76—1.18 (m, 9H), 1.25 (t, J = 7.2 Hz, 3H).

(7S)-(2S)-2-(1E,3S)-3-Hydroxy-4-[3-(methoxymethyl)phenyl]but-1-enyl]-5-oxo-1-pyrrolidinyl)heptanoic Acid (7) Compound 7 was prepared from (S)-30 according to the same procedure as described of 6 from (R)-30 as a pale yellow oil (76% yield). IR (KBr cm⁻¹): 3403, 2930, 2729, 1660, 1455, 1388, 1215, 1093, 1086, 793, 724, 607. ¹H-NMR (300 MHz, CDCl₃) δ: 7.43—7.09 (m, 4H), 5.76 (dd, J = 15.6, 6.1 Hz, 1H), 5.47 (dd, J = 15.6, 8.2, 1.7 Hz, 1H), 4.45 (s, 2H), 4.40 (m, 1H), 4.03 (m, 1H), 3.50—3.35 (m, 4H), 3.00—2.62 (m, 3H), 2.50—2.02 (m, 6H), 1.78—1.10 (m, 11H); MS (APCI) m/z: 402 (M⁺—H⁻). HR-MS-FAB (m/z): 402.2274 (Calcd for C₂₃H₂₉NO₄; 402.2278).

Ethyl 7-(2R)-2-(Hydroxyethyl)-1-pyrrolidinyl)-7-oxoheptanoate (33) To a stirred solution of p-nitrololeic acid (320 mg, 434 mmol) in dioxane (15 ml) and 2 NaOH (7.4 ml) was added a solution of ethyl 6-chloroformyl-hexanoate (1.53 g, 741 mmol) in dioxane (2 ml) at 0°C. The reaction mixture was stirred for 30 min, and extracted with EtOAc. The organic layer was washed with H₂O, brine, and dried over Na₂SO₄. The organic solvent was removed by evaporation and the resulting residue was purified by column chromatography on silica gel (EtOAc) to give amide alcohol 33 as a colorless oil (615 mg, 46%). ¹H-NMR (300 MHz, CDCl₃) δ: 5.28 (m, 1H), 4.27—4.19 (m, 3H), 3.71—3.40 (m, 3H), 2.40—2.20 (m, 4H), 2.10—1.78 (m, 4H), 1.75—1.55 (m, 5H), 1.45—1.19 (m, 5H).

7-(2R)-2-(1E,3S)-3-Hydroxy-4-[3-(methoxymethyl)phenyl]but-1-enyl]-5-oxo-1-pyrrolidinyl)heptanoic Acid (8) Compound 8 was prepared from 33 according to the same procedure as described of 6 from (R)-28 as a colorless oil (38% from 33). IR (film cm⁻¹): 3397, 2922, 1729, 1614, 1454, 1384, 1193, 1094, 1038, 971, 793, 753, 705. ¹H-NMR (300 MHz, CDCl₃) δ: 7.35—7.10 (m, 4H), 5.66—5.40 (m, 2H), 4.67—4.31 (m, 4H), 3.60—2.75 (m, 9H), 2.42—2.13 (m, 4H), 2.12—1.57 (m, 8H), 1.53—1.22 (m, 2H). MS (APCI) m/z: 402 (M⁺—H⁻). HR-MS-FAB (m/z): 402.2277 (Calcd for C₂₃H₂₉NO₄; 402.2280).

[N-(2-Tetrahydro-2F-pyran-2-yloxy)ethylacetamide (35) To a stirred solution of 2-N-acetyl aminoalkan 34 (1.03 g, 10.0 mmol) in CH₂Cl₂ (7 ml) were added a solution of Et₂O in CH₂Cl₂ (1.00 mmol) and the mixture was stirred for 30 min in 0°C for additional 25 min. The reaction mixture was poured into saturated aqueous NH₄Cl, extracted with Et₂O, washed with H₂O, and dried over Na₂SO₄. The organic solvent was removed by evaporation to afford 35 as a yellow oil (455 mg).]
A solution of 41 (455 mg) in THF (1.5 ml) was treated with a solution of tetrabutylammonium fluoride (1.0 mmol in THF, 0.98 ml, 0.98 mmol) at room temperature under argon atmosphere for 30 min. The reaction mixture was diluted with Et2O, washed with H2O, and dried over Na2SO4. The organic solvent was removed by evaporation, and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 1/1−1/2) to give 42 as a pale yellow oil (248 mg, 84% from 39). 1H-NMR (200 MHz, CDCl3): δ: 7.33 (m, 4H), 5.79 (dd, J = 16.5, 5.9 Hz, 1H), 5.68 (d, J = 15.6, 6.1 Hz, 1H), 4.44 (s, 2H), 4.40 (m, 1H), 4.12 (q, J = 7.1 Hz, 2H), 3.82 (d, J = 6.4 Hz, 2H), 2.67 (dd, J = 13.5, 3.8 Hz, 1H), 2.80 (dd, J = 13.7, 7.4 Hz, 1H), 2.78 (s, 3H), 2.29 (t, J = 7.3 Hz, 2H), 1.73−1.47 (m, 4H), 1.43−1.19 (m, 4H), 1.25 (s, J = 7.3 Hz, 3H).

7-{[(2E,4S)-4-Hydroxy-5-{3-(methoxymethyl)phenyl}-2-enyl](methylsulfonyl)amino}linoacetonic Acid (10) A solution of 42 (226 mg, 0.47 mmol) in MeOH (5 ml) and 2Na (2.5 ml) was stirred at room temperature for 1.5 h. After acidification with 2N H2SO4 (3 ml) under cooling, the reaction mixture was extracted with EtOAc three times, washed with brine, and dried over Na2SO4. The combined organic layers were evaporated to a dry residue, which was purified by column chromatography on silica gel (hexane/EtOAc 4/1−2/1) to afford 43 (460 mg, 83%). 1H-NMR (300 MHz, CDCl3): δ: 7.30−7.09 (m, 4H), 5.79 (dd, J = 15, 5.5 Hz, 1H), 5.69 (d, J = 15, 6.0 Hz, 1H), 4.45 (s, 2H), 4.41 (m, 1H), 3.81 (d, J = 5.1 Hz, 2H), 2.57 (dd, J = 12.9, 5.4 Hz, 1H), 2.79 (s, 3H), 2.33 (t, J = 7.2 Hz, 2H), 1.70−1.49 (m, 4H), 1.42−1.22 (m, 4H). MS (APCI): m/z: 426 (M+H+).

7-Hydroxy-5-htpyrene-4(1H)-one (45) A solution of 7-hydroxy-5-htpyrene-4(1H)-one (46) in THF (5 ml) was treated with triphenylphosphine (680 mg, 2.60 mmol) in CH2Cl2 (5 ml) and a solution of potassium thioacetate (188 mg, 1.65 mmol) in MeOH (5 ml) was added sodium borohydride (450 mg, 11.9 mmol) at 0°C under argon atmosphere. After being stirred at room temperature for additional 1 h, the reaction mixture was quenched with H2O. The reaction mixture was extracted with EtOAc, washed with water, and dried over MgSO4. The organic solvent was removed by evaporation and the residue was purified by column chromatography on silica gel (hexane/EtOAc, 4/1−1/1) to obtain 47 (540 mg, 60%). 1H-NMR (200 MHz, CDCl3): δ: 4.24 (dt, J = 6.2 Hz, 2H), 3.63 (s, 3H), 2.44 (tt, J = 7.0 Hz, 2H), 2.30 (tt, J = 7.0, 2.0 Hz, 1H), 1.90−1.75 (m, 2H), 1.56 (t, J = 6.0 Hz, 1H).

7-{[(2Z)-2-(tert-Butyldimethylsilyloxy)methyl]-5-oxo-1-pyrrolidinyl}-5-htpyneone (47) To a stirred solution of 46 (340 mg, 2.18 mmol) and triphenylphosphineoxide (680 mg, 2.60 mmol) in CH2Cl2 (5 ml) was added tetrabromomethane (860 mg, 2.60 mmol) at 0°C under argon atmosphere and stirring was further continued at room temperature for 30 min. The reaction mixture was poured into 1N HCl, extracted with EtOAc, washed with water twice, and dried over Na2SO4. The organic solvent was removed by evaporation, and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 4/1−1/1) to give 45 (940 mg, 60%). 1H-NMR (200 MHz, CDCl3): δ: 7.33−7.09 (m, 4H), 5.79 (dd, J = 15, 5.6 Hz, 1H), 5.69 (d, J = 15, 6.1 Hz, 1H), 4.45 (s, 2H), 4.41 (m, 1H), 3.81 (d, J = 5.1 Hz, 2H), 2.57 (dd, J = 12.9, 5.4 Hz, 1H), 2.79 (s, 3H), 2.33 (t, J = 7.2 Hz, 2H), 1.70−1.49 (m, 4H), 1.42−1.22 (m, 4H). MS (APCI): m/z: 426 (M+H+). HR-MS-FAB (m/z): 426.1937 (C24H24NO5S, 426.1950).

Compound 7-{[(2Z)-2-(tert-Butyldimethylsilyloxy)methyl]-5-oxo-1-pyrrolidinyl}-5-htpyneone (47) To a stirred solution of 46 (340 mg, 2.18 mmol) and triphenylphosphineoxide (680 mg, 2.60 mmol) in CH2Cl2 (5 ml) was added tetrabromomethane (860 mg, 2.60 mmol) at 0°C under argon atmosphere and stirring was further continued at room temperature for 30 min. The reaction mixture was poured into 1N HCl, extracted with EtOAc, washed with water twice, and dried over Na2SO4. The organic solvent was removed by evaporation, and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 4/1−1/1) to give 45 (940 mg, 60%). 1H-NMR (200 MHz, CDCl3): δ: 7.33−7.09 (m, 4H), 5.79 (dd, J = 15, 5.6 Hz, 1H), 5.69 (d, J = 15, 6.1 Hz, 1H), 4.45 (s, 2H), 4.41 (m, 1H), 3.81 (d, J = 5.1 Hz, 2H), 2.57 (dd, J = 12.9, 5.4 Hz, 1H), 2.79 (s, 3H), 2.33 (t, J = 7.2 Hz, 2H), 1.70−1.49 (m, 4H), 1.42−1.22 (m, 4H). MS (APCI): m/z: 426 (M+H+). HR-MS-FAB (m/z): 426.1937 (C24H24NO5S, 426.1950).

A solution of 41 (455 mg) in THF (1.5 ml) was treated with a solution of tetrabutylammonium fluoride (1.0 mmol in THF, 0.98 ml, 0.98 mmol) at room temperature under argon atmosphere for 30 min. The reaction mixture was diluted with Et2O, washed with H2O, and dried over Na2SO4. The organic solvent was removed by evaporation, and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 1/1−1/2) to give 42 as a pale yellow oil (248 mg, 84% from 39). 1H-NMR (200 MHz, CDCl3): δ: 7.33 (m, 4H), 5.79 (dd, J = 16.5, 5.9 Hz, 1H), 5.68 (d, J = 15, 6.0 Hz, 1H), 4.44 (s, 2H), 4.40 (m, 1H), 4.12 (q, J = 7.1 Hz, 2H), 3.82 (d, J = 6.4 Hz, 2H), 2.67 (dd, J = 13.5, 3.8 Hz, 1H), 2.80 (dd, J = 13.7, 7.4 Hz, 1H), 2.78 (s, 3H), 2.29 (t, J = 7.3 Hz, 2H), 1.73−1.47 (m, 4H), 1.43−1.19 (m, 4H), 1.25 (s, J = 7.3 Hz, 3H).
The organic solvent was removed by evaporation and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 1/1) to afford a thioacetate 51 as a pale brown oil (186 mg, 71% in 2 steps). 1H-NMR (300 MHz, CDCl3): δ: 3.81 (m, 2H), 3.78—3.62 (m, 2H), 3.62 (m, 1H), 3.23 (m, 1H), 3.14—2.94 (m, 2H), 2.45 (m, 1H), 2.34 (s, 3H), 2.28 (m, 1H), 2.16—2.02 (m, 1H), 1.85 (m, 1H), 0.88 (s, 3H), 0.06 (s, 3H), 0.05 (s, 3H). 13C-NMR (75.5 MHz, CDCl3): δ: 124.6, 124.4, 122.6, 121.4, 121.0, 118.6, 118.3, 113.7, 113.5, 113.0, 129.0, 128.8, 128.7, 128.3, 74.7, 74.4, 63.7, 40.3, 40.2, 33.5, 27.3, 27.2, 26.9, 26.8, 26.7. MS (APCI) m/z: 420 (M−H). The mixture was stirred at room temperature for 30 min. The reaction was quenched with saturated aqueous NH4Cl. The reaction mixture was diluted with EtOAc, washed with H2O twice, and dried over MgSO4. The organic solvent was removed by evaporation and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 1/1) to give a sulfide 52 as a yellow oil (206 mg, 94%). 1H-NMR (300 MHz, CDCl3): δ: 3.85—3.66 (m, 3H), 3.68 (s, 3H), 3.58 (m, 1H), 3.23 (m, 1H), 2.80—2.56 (m, 4H), 2.50—2.24 (m, 2H), 2.44 (t, J=8.4 Hz, 1H), 4.50—4.40 (m, 1H), 4.46 (s, 2H), 4.20—4.01 (m, 1H), 3.70—3.50 (m, 1H), 3.42 (s, 3H), 3.10 (m, 1H), 2.90—2.80 (m, 2H), 2.80—2.10 (m, 8H), 2.00—1.70 (m, 2H). 13C-NMR (75.5 MHz, CDCl3): δ: 175.5, 175.4, 138.4, 138.7, 136.5, 135.9, 131.0, 129.0, 128.8, 128.7, 126.3, 74.7, 74.4, 63.7, 40.3, 40.2, 33.5, 27.3, 27.1, 26.9, 26.8, 26.7. MS (APCI) m/z: 420 (M−H).

4-[(2R)-2-[(1E,3S)-3-Hydroxy-4-[(3-methoxymethyl)phenyl]but-1-enyl]-5-oxopyrrolidin-1-yl]ethylsulfanyl]butanoic Acid (12) Compound 12 was prepared from 53 according to the same procedure as described for the preparation of 6 from (R)-29 as a colorless oil (78% from 53). IR (film) cm⁻¹: 3398, 2925, 1726, 1666, 1447, 1419, 1383, 1236, 1159, 1096, 1035, 974, 791, 731, 704, 667, 569. 1H-NMR (200 MHz, CDCl3): δ: 7.40—7.10 (m, 4H), 6.67 (dd, J=15.8, 8.0 Hz, 1H), 5.68 (t, J=15.6, 6.0 Hz, 1H), 2.60—2.38 (m, 2H), 2.26 (m, 1H), 1.78 (m, 1H), 1.51 (s, 9H). 13C-NMR (50 MHz, CDCl3): δ: 139.0, 137.9, 134.0, 128.7, 127.7, 127.3, 126.2, 126.0, 125.8, 123.9, 119.6, 109.5, 108.8, 100.5, 74.7, 74.4, 57.9, 40.3, 40.2, 33.5, 27.3, 27.2, 26.9, 26.8, 26.7. MS (APCI) m/z: 420 (M−H). The mixture was stirred at room temperature for 30 min. The reaction was quenched with satuared aqueous NH4Cl. The reaction mixture was diluted with EtOAc, washed with H2O twice, and dried over MgSO4. The organic solvent was removed by evaporation and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 1/1—0/1) to give an aldehyde as a yellow oil. To a stirred solution of 56 as a pale yellow oil (195 mg, 52% in 2 steps). 1H-NMR (300 MHz, CDCl3): δ: 7.39—7.10 (m, 4H), 6.83 (dd, J=15.6, 6.0 Hz, 1H), 6.21 (dd, J=15.6, 1.2 Hz, 1H), 4.74 (m, 1H), 4.43 (s, 3H), 3.83 (s, 2H), 3.39 (s, 3H), 2.59—2.40 (m, 2H), 2.29 (m, 1H), 1.79 (m, 1H), 1.41 (s, 9H). 13C-NMR (75 MHz, CDCl3): δ: 141.2, 136.9, 129.3, 128.8, 128.7, 128.3, 128.1, 127.7, 126.8, 126.5, 125.5, 124.3, 119.7, 118.9. MS (APCI) m/z: 420 (M−H).

To a stirred solution of dimethyl 3-[(3-methoxymethyl)phenyl]-2-oxopropanephosphonate 31 (390 mg, 1.36 mmol) in THF (14 ml) was added sodium hydride (62% in mineral oil, 46.0 mg, 1.17 mmol) at 0°C under argon atmosphere and stirring was further continued at ambient temperature for 90 min. To the stirred suspension was added a solution of the above-described aldehyde in THF (2 ml) at 0°C and stirring was continued for 1 h. The reaction mixture was diluted with EtOAc, washed with water, then brine, and dried over MgSO4. The organic solvent was removed by evaporation and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 2/1—0/1) to give an enone 57 as a pale yellow oil (195 mg, 52% in 2 steps). 1H-NMR (200 MHz, CDCl3): δ: 7.39—7.10 (m, 4H), 6.83 (dd, J=15.6, 6.0 Hz, 1H), 6.21 (dd, J=15.6, 1.2 Hz, 1H), 4.74 (m, 1H), 4.43 (s, 3H), 3.83 (s, 2H), 3.39 (s, 3H), 2.59—2.40 (m, 2H), 2.29 (m, 1H), 1.79 (m, 1H), 1.41 (s, 9H). 13C-NMR (75 MHz, CDCl3): δ: 141.2, 136.9, 129.3, 128.8, 128.7, 128.3, 128.1, 127.7, 126.8, 126.5, 125.5, 124.3, 119.7, 118.9. MS (APCI) m/z: 420 (M−H).

tert-Butyl (2R)-2-[(1E,3S)-3-Hydroxy-4-[(3-methoxymethyl)phenyl]but-1-enyl]-5-oxopyrrolidin-1-carboxylate (55) A solution of 54 (385 mg, 1.30 mmol) in THF (1 ml) was treated with a solution of TBDMS (1.0 ml in THF, 1.56 ml, 1.56 mmol) at room temperature under argon atmosphere for 90 min. The reaction mixture was diluted with CHCl3, washed with H2O, brine, and dried over Na2SO4. The organic solvent was removed by evaporation, and the resulting residue was purified by column chromatography on silica gel (EtOAc/MeOH, 1/0—95/5) to give 55 as a pale yellow oil (209 mg, 75% in 3 steps). 1H-NMR (200 MHz, CDCl3): δ: 4.30—4.19 (m, 1H), 3.93—3.69 (m, 2H), 2.70 (m, 1H), 2.51—1.85 (m, 4H), 1.70—1.45 (m, 1H).
moved by evaporation to give a TBS ether as a yellow oil.

A stirred solution of the above-described ether in MeOH (70 ml) and CH2Cl2 (70 ml) was cooled to −78 °C. Gaseous ozone was bubbled through the reaction mixture at the same temperature for 3 h. The reaction mixture was treated with dimethyl sulfide (2.95 ml, 40 mmol) and then warmed up to 0 °C in 1 h. The organic solvent was removed by evaporation, and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 2/1) to give an aldehyde 66 as a colorless oil (5.20 g, 70% in 2 steps). 1H-NMR (300 MHz, CDCl3): δ: 7.98—7.95 (m, 1H), 2.50—2.40 (m, 2H), 1.76—1.35 (m, 5H), 0.93—0.87 (m, 12H), 0.04 (s, 6H).

(4S)-6-(tert-Butyldimethylsilyl)-4-methyl-1-hexanol (67) To a stirred solution of 66 (437 mg, 16.2 mmol) in MeOH (30 ml) was added sodium borohydride (307 mg, 8.10 mmol) at 0 °C under argon atmosphere, and the stirring was continued at room temperature for 1 h. The reaction was quenched with H2O. The reaction mixture was diluted with EtOAc, and successively washed with 2N HCl, saturated aqueous NaHCO3, brine, and dried over MgSO4. The organic solvent was removed by evaporation, and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 4/1) to give an alcohol as a colorless oil (3.16 g, 72%).

1H-NMR (300 MHz, CDCl3): δ: 3.72—3.58 (m, 4H), 1.70—1.09 (m, 8H), 0.98—0.72 (m, 12H), 0.05 (s, 3H), 0.02 (s, 3H).

(5S)-7-(tert-Butyldimethylsilyl)-5-methylheptanenitrile (68) To a stirred solution of an aldehyde 66 (700 mg, 2.84 mmol) in pyridine (5 ml) was added p-tolyl chloroide (1.10 g, 5.68 mmol) at room temperature under argon atmosphere. The resulting mixture was stirred for 1 h, diluted with EtOAc, washed with 1N HCl, H2O, and dried over MgSO4. The organic solvent was removed by evaporation to give a p-toluenesulfonate. To a stirred solution of p-toluenesulfonate in DMSO (5 ml) was added sodium cyanide (209 mg, 4.26 mmol) at room temperature, then warmed up to 70 °C. Gaseous ozone was bubbled through the reaction mixture at the same temperature for 3 h. The reaction mixture was diluted with EtOAc, and successively washed with 2N HCl, saturated aqueous NaHCO3, brine, and dried over MgSO4. The organic solvent was removed by evaporation, and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 1/1—1/2) to give 69 as a colorless oil (210 mg, 77%).

1H-NMR (300 MHz, CDCl3): δ: 7.30—7.05 (m, 4H), 5.67 (dd, J = 15.2, 7.4 Hz, 1H), 5.53 (dd, J = 15.2, 8.4 Hz, 1H), 4.50—4.30 (m, 1H), 4.44 (s, 2H), 4.06 (m, 1H), 3.66 (s, 3H), 3.55 (m, 1H), 3.41 (s, 3H), 3.00—2.70 (m, 3H), 2.50—2.10 (m, 5H), 2.00—1.00 (m, 9H), 0.90 (d, J = 6.2 Hz, 3H).

A solution of the above-described ester (55 mg, 0.127 mmol) in MeOH (4 ml) and 2N NaOH (1 ml) was stirred at room temperature for 3 h. After acidification with 2N HCl (2 ml under cooling, the reaction mixture was extracted with EtOAc three times, and the organic layer was washed with brine, and dried over Na2SO4. The combined organic layers were evaporated. The resulting residue was purified by column chromatography on silica gel (CHCl3/MeOH, 10/1—10/1) to afford 13 as a pale yellow oil (54 mg, 100%).

IR (film) cm−1: 3376, 2927, 1726, 1656, 1458, 1421, 1380, 1257, 1189, 1159, 1038, 973, 789, 703, 618. 1H-NMR (200 MHz, CDCl3): δ: 7.40—7.10 (m, 4H), 5.78 (dd, J = 15.2, 5.2 Hz, 1H), 5.55 (dd, J = 15.2, 8.4 Hz, 1H), 4.41 (m, 1H), 4.46 (s, 2H), 4.03 (m, 1H), 3.55 (m, 1H), 3.42 (s, 3H), 3.00—2.70 (m, 4H), 2.50—2.10 (m, 5H), 1.80—1.00 (m, 8H), 0.91 (d, J = 5.8 Hz, 3H). MS (APCI m/z): 416 (M+H)+. HR-MS-FAB (m/z): 416.2446 (Calcd for C27H50NaNO3; 416.2437).

(5S)-7-(tert-Butyldimethylsilyl)-5-methyl-2-heptanone (71) To a stirred solution of the alcohol 67 in EtOAc (10 ml) and N,N-diisopropylethylamine (4.96 ml, 28.5 mmol) was added a solution of SO3-Py (2.27 g, 14.2 mmol) in DMSO (10 ml) at 0 °C under argon atmosphere. After being stirred at the same temperature for 30 min, the reaction mixture was added to H2O. The reaction mixture was diluted with EtOAc, washed with 1N HCl, brine, and dried over MgSO4. The organic solvent was removed by evaporation to yield an aldehyde as a colorless oil (1.08 g, 93%). 1H-NMR (200 MHz, CDCl3): δ: 9.78 (t, J = 1.8 Hz, 1H), 6.65 (td, J = 6.2, 3.0 Hz, 2H), 2.50—2.40 (m, 2H), 1.80—1.20 (m, 6H), 0.90—0.80 (m, 2H), 0.81 (s, 9H), 0.04 (s, 6H).

To a stirred solution of the above-described aldehyde (1.08 g, 4.75 mmol) in THF (10 ml) was added a solution of methylmagnesium bromide (0.93 M in THF, 7.70 ml, 7.12 mmol) at 0 °C under argon atmosphere. After being stirred at the same temperature for 15 min, the reaction was quenched with saturated aqueous NaHCl. The reaction mixture was diluted with EtOAc, washed with H2O, and dried over MgSO4. The organic solvent was removed by evaporation to yield an aldehyde as a colorless oil (1.08 g, 93%).

1H-NMR (300 MHz, CDCl3): δ: 7.35 (m, 1H), 3.66 (m, 2H), 1.80—1.10 (m, 7H), 1.91 (d, J = 6.3 Hz, 3H), 1.00—0.80 (m, 3H), 0.89 (s, 3H), 0.05 (s, 6H).

To a stirred solution of the above-described alcohol (1.11 g, 4.26 mmol) in EtOAc (10 ml) and N,N-diisopropylethylamine (4.45 ml, 25.6 mmol) was added a solution of SO3-Py (2.27 g, 14.2 mmol) in DMSO (10 ml) at 0 °C under argon atmosphere. After being stirred at the same temperature for 30 min, the reaction mixture was added to H2O. The reaction mixture was diluted with EtOAc, washed with 1N HCl, brine, and dried over MgSO4. The organic solvent was removed by evaporation, and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 10/1) to give 71 as a colorless oil (760 mg, 69% from 67).
Methyl (5R)-5-Methyl-8-oxononanate (72) A solution of 71 (640 mg, 2.48 mmol) in THF (3 ml) was treated with a solution of tetrabutylammonium fluoride (1.0 x in THF, 4.95 ml, 4.95 mmol) at room temperature under argon atmosphere for 1 h. The reaction mixture was diluted with EtOAc, and successively washed with saturated aqueous NH4Cl, H2O, then brine, and dried over Na2SO4. The organic solvent was removed by evaporation, and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 1:2) to give an alcohol as a colorless oil (297 mg, 83%). 1H-NMR (300 MHz, CDCl 3): δ: 7.30—7.00 (m, 4H), 5.68 (dd, J = 15.0, 5.4 Hz, 1H), 5.41 (dd, J = 15.0, 8.7, 1.5 Hz, 1H), 4.43 (s, 2H), 4.35 (m, 1H), 4.05 (m, 1H), 3.66 (s, 3H), 3.58 (m, 1H), 3.38 (s, 3H), 2.80—2.60 (m, 3H), 2.45—2.10 (m, 5H), 1.70—1.00 (m, 8H), 0.90 (d, J = 6.6 Hz, 3H), 0.84 (s, 9H), —0.11 (s, 3H), —0.21 (s, 3H).

(5R)-7-[(2R)-2-[(1E,3S)-3-(tert-Butyldimethylsilyloxy)-4-[(3-methoxymethyl)phenyl]-1-buten-1-yl]-5-oxo-1-pyrrolidinyl]-5-methylheptanoate (64) Compound 64 was prepared from 59 using iodide 61 according to the same procedure as described for the preparation of 63 from 59 using iodide 60 as a pale yellow oil (51%). 1H-NMR (300 MHz, CDCl 3): δ: 7.30—7.00 (m, 4H), 5.68 (dd, J = 15.0, 5.4 Hz, 1H), 5.41 (dd, J = 15.0, 8.7, 1.5 Hz, 1H), 4.43 (s, 2H), 4.35 (m, 1H), 4.05 (m, 1H), 3.66 (s, 3H), 3.58 (m, 1H), 3.38 (s, 3H), 2.80—2.60 (m, 3H), 2.45—2.10 (m, 5H), 1.70—1.00 (m, 8H), 0.90 (d, J = 6.6 Hz, 3H), 0.84 (s, 9H), —0.11 (s, 3H), —0.21 (s, 3H).
by column chromatography on silica gel (hexane/EtOAc, 4/1—1/1) to give a cyclopentyl alcohol as a colorless oil (980 mg, 90%). 1H-NMR (300 MHz, CDCl3): δ: 3.80—3.70 (m, 2H), 3.68 (s, 3H), 2.32 (t, J=7.0 Hz, 2H), 1.81—1.65 (m, 2H), 1.66 (t, J=7.0 Hz, 2H), 1.45—1.33 (brs, 1H), 1.30—1.20 (m, 2H), 0.35—0.25 (m, 4H).

To a solution of the above-described cyclopentyl alcohol (1.80 mg, 0.97 mmol) and imidazole (95 mg, 1.40 mmol) in benzene (5 mL) was added a solution of iodine (305 mg, 1.20 mmol) in benzene (5 mL) at room temperature under argon atmosphere. After being stirred for 30 min, the reaction was quenched with MeOH. The resulting precipitates were removed by filtration and washed with Et2O. The filtrate was evaporated and the resulting precipitates were removed by filtration again. The filtrate was evaporated again and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 9/1) to afford an iodide 62 as a colorless oil (232 mg, 81%). 1H-NMR (300 MHz, CDCl3): δ: 3.68 (s, 3H), 3.18 (t, J=8.0 Hz, 2H), 2.31 (t, J=7.0 Hz, 2H), 1.85 (t, J=8.0 Hz, 2H), 1.75—1.60 (m, 2H), 1.31—1.20 (m, 2H), 0.41—0.29 (m, 4H).

**Methyl 4-([2R]-2-{[(1E,3S)-3-(tert-Butyldimethysilyloxy)-4-[3-(methoxymethyl)phenyl]-1-buten-1-y1]-5-oxo-1-pyrrolidinyl]ethyl)cyclopropyl)butanoate (65)** Compound 65 was prepared from 59 using iodide 62 according to the same procedure as described for the preparation of 63 from 59 using iodide 60 as a pale yellow oil (69%). 1H-NMR (300 MHz, CDCl3): δ: 7.30—7.06 (m, 4H), 5.69 (dd, J=15.0, 6.0 Hz, 1H), 5.42 (dd, J=15.0, 8.0 Hz, 1H), 4.42 (s, 2H), 4.35 (m, 1H), 4.00 (m, 1H), 3.66 (s, 3H), 3.67 (m, 1H), 3.38 (s, 3H), 2.80—2.70 (m, 2H), 2.43—2.10 (m, 5H), 1.75—1.60 (m, 3H), 1.50—1.22 (m, 4H), 0.82 (s, 9H), 0.35—0.23 (m, 4H), −0.11 (s, 3H), −0.22 (s, 3H).

**1-(2-((2R)-2-{[(1E,3S)-1-Hydroxy-4-{3-(methoxymethyl)phenyl]but-1-enyl}-5-oxopyrroline-1-yl)ethyl](cylopropyl)butanoic Acid (15)** Compound 15 was prepared from 65 according to the same procedure as described for the preparation of 13 from 63 as a colorless oil (100%). IR (film): 3292, 1726, 1659, 1545, 1422, 1383, 1265, 1194, 1160, 1097, 1039, 975, 755. 1H-NMR (300 MHz, CDCl3): δ: 7.33—7.13 (m, 4H), 5.81 (dd, J=15.0, 5.6 Hz, 1H), 5.61 (dd, J=15.0, 8.0 Hz, 1H), 4.46 (s, 2H), 4.42 (m, 1H), 4.08 (m, 1H), 3.54 (m, 1H), 3.43 (s, 3H), 2.98 (m, 1H), 2.90 (dd, J=13.8, 8.8 Hz, 1H), 2.47—2.12 (m, 5H), 1.79—1.52 (m, 4H), 1.36—1.10 (m, 3H), 0.37—0.22 (m, 4H). MS (APCI) m/z: 428 (M+H). HR-MS-FAB (m/z): 428.2439 (Calcd for C25H34NO5: 428.2437).

**References**


