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Discovery of novel prostaglandin analogs of PGE_2 as potent and selective EP_2 and EP_4 receptor agonists

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Abstract—Analogs of PGE_2 with introduction of diene groups at the ω-side chain have been synthesized and evaluated for their binding affinity for EP_2 and EP_4 receptors. An optimized analog (compound **9b**) showed high potency and selectivity for the EP_4 receptor over other known receptors.
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Prostaglandins are lipid mediators derived from arachidonic acid.[1] Prostaglandin E_2 (PGE_2, Fig. 1) is the most well-known prostanoid derivative and exhibits a broad range of biological actions in diverse tissues through the binding to specific receptors present in the plasma membranes. It is well known that prostaglandin E_2 (PGE_2) is a potent agonist of the four subtypes of PGE receptors, designated EP_1, EP_2, EP_3, and EP_4, which mediate a wide variety of biological activities, including bronchodilation, fertility, bone resorption, and inflammation.[1] Of these four receptors, three are involved in modulation of cAMP levels.[2] EP_1 receptor is involved in regulating intracellular calcium levels. Activation of EP_3 receptor results in a reduction of intracellular cAMP level, and EP_2 receptor and EP_4 receptor increase the intracellular cAMP level, which is linked to the treatment of infertility. EP_2 and EP_4 receptor agonists have been proved to be beneficial for the treatment of preterm labor by suppressing uterine contraction and inducing oophorus maturation required for fertilization during and after ovulation.[1b]

PGE_2 easily undergoes acid- or base-catalyzed elimination of the 11-hydroxy to give the more stable α,β-unsaturated ketone system. PGE_2, metabolized by enzymes, undergoes rapid oxidation of the 15-hydroxy group to a ketone, β-oxidation of the carboxylic acid chain to generate acetic acid and the dinor PG acid, and ω-oxidation at C-20 to produce the 20-hydroxy and carboxylic acid.[3] The metabolites of PGE_2, though inactive against the PGE receptors, produce numerous side effects.[3] PGE_2 also shows no selectivity for the EP_{1-4} receptors (in-house binding affinity data shown in Table 1). Until now, efforts to improve the selectivity and chemical stability of PGE_2 have been focused on only two general

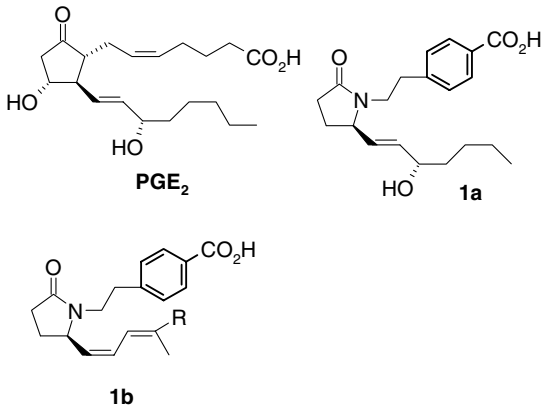

Figure 1. PGE_2 and prostaglandin diene derivative.

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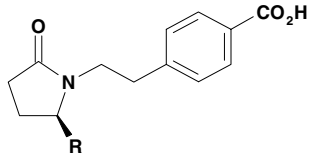
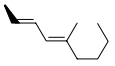
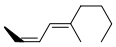
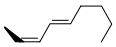
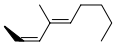
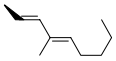
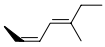
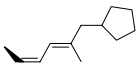
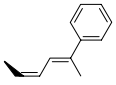
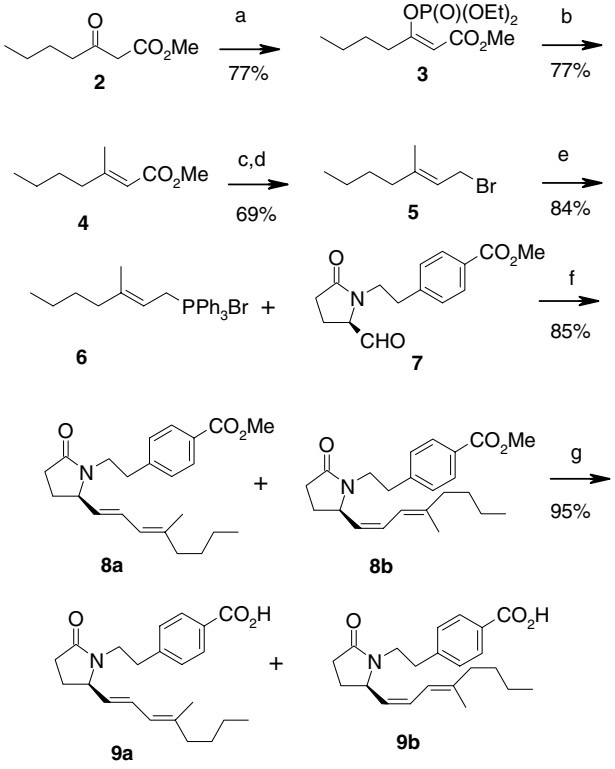
Table 1. γ-Lactam diene derivatives

	R	h-EP$_1$ K_i (nM)	h-EP$_2$ K_i (nM)	h-EP$_2$ EC_{50} (nM)	h-EP$_3$ K_i (nM)	h-EP$_4$ K_i (nM)	h-EP$_4$ EC_{50} (nM)
1a PGE2		>10,000 9.1	125.5 4.9	16	9000 0.33	2.5 0.79	0.002
9a		10,000	134	222	10,000	181	15
9b		10,000	60	35	10,000	4	0.1
16			88	197		25	2
17			1850			45	4
18			802			443	
19			29	23		145	21
20			173			24	3
21			60			117	
22			749	>10,000		29	0.5

chemical modifications,[4] i.e., Replacement of the α-alkenyl side chain with the more chemically stable phenylethyl group and substitution of γ-lactam ring for the 11-hydroxy cyclopentanone (structure **1a** in Fig. 1).[4]

Our goal was to develop PGE_2 analogs with different selectivity and high potency for EP_2 receptor or EP_4 receptor. This allowed an investigation of the new PGE_2 analogs in the treatment of infertility. In this report, we describe the synthesis and structure–activity relationship of a novel PGE_2 analog of general structure **1b** consisting of a phenylethyl moiety in the α-side-chain, a γ-lactam ring as a backbone, and a third, unprecedented modification: use of a diene as the ω-side chain. The diene moiety was selected to circumvent oxidation of the hydroxy group at the ω-side chain.

Synthesis of a representative γ-lactam diene of general formula **1b** is shown in Scheme 1. Construction of the key intermediate, γ-lactam aldehyde **7**, from 4-formyl benzoic acid has been reported.[4c–f] Synthesis of the triphenylphosphonium bromide salt **6** started from commercially available methyl 3-oxoheptanoate. Treatment of 3-oxoheptanoate **2** with sodium hydride, followed by quenching with diethyl chlorophosphate, provided the only one isomer (*Z*) of enol phosphate **3** in 77% yield.[5] The regioselective coupling of this enol phosphate **3** with 3 equiv of lithium dimethylcuprate at −35 °C smoothly introduced a methyl group in the desired product **4** in 77% yield.[5] Reduction of ester **4** with DIBALH offered the allylic alcohol, which was converted into bromide **5** with treatment of the resulting alcohol with phosphorus tribromide in 69% yield in 2 steps. Reflux of bromide **5** with triphenylphosphine in toluene provided the triphenylphosphonium bromide salt **6** in 84% yield.[6] Treatment of triphenylphosphonium bromide salt **6** with *n*-butyllithium (2.5 N) at 0 °C generated the phosphorus yield to react with the aldehyde **7**, to produce inseparable diene **8a** and **8b** in a 1:1 ratio of the two isomers (**8a**: 13*E*,15*E* and **8b**: 13*Z*,15*E*). After saponification of ester **8a** and **8b** into



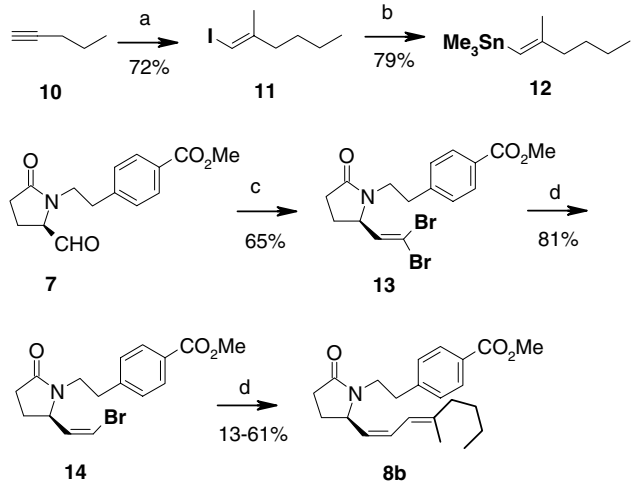
Scheme 1. Reagents and conditions: (a) NaH, $ClP(O)(OEt)_2$, THF, 0 °C; (b) MeMgCl, CuI, MeLi, THF, −35 °C; (c) DIBALH, ether, −78 °C; (d) PBr_3, petroleum ether 0 °C; (e) PPh_3, PhMe, refux; (f) *n*-BuLi (2.5 N), THF, 0 °C.; (g) NaOH (1 N), $MeOH/H_2O$, then reverse phase HPLC.

carboxylic acid with aqueous NaOH (1 N) in MeOH/H_2O, the two carboxylic acid isomers (**9a** and **9b**)[7] were separated through preparative reverse phase HPLC.

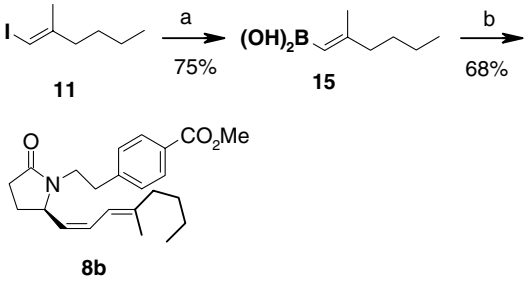
The original synthetic route, as shown in Scheme 1, involving the Wittig reaction of the aldehyde **7** with the triphenylphosphonium bromide salt **6** unfortunately provided a 1:1 ratio of two isomers and the final product was inconveniently separated through reverse phase HPLC. In order to avoid the inconvenient separation and to quickly scale-up compound **9b** for the animal toxic study, we sought a regioselective formation of *cis* C=C double bond at C13–C14. Preliminary attempts to explore the utility of different bases (BuLi, KHMDS), solvents (THF, PhMe), and temperatures (−78 °C, 0 °C, ambient temperature) failed to improve the regioselectivity of Wittig reaction.

Our efforts to selectively form the *cis* C=C double bond at C13–C14 then shifted from the Wittig reaction to the cross-coupling reactions, such as Stille reaction (in Scheme 2) and Suzuki cross coupling reaction (in Scheme 3).

Stille coupling partner **12** was prepared from 1-pentyne in 2 steps with an overall yield of 57%.[8] Conversion of the aldehyde **7** with carbon tetrabromide and triphenylphosphine smoothly furnished vinyl dibromide **13** in 65% yield. Palladium-catalyzed stereoselective hydrogenolysis of **13** with *n*-Bu_3SnH yielded the desired *Z*-vinyl



Scheme 2. Reagents and conditions: (a) i—Cp_2ZrCl_2, $AlMe_3$, DCM, −10 °C; ii—I_2, THF; (b) $Me_3SnSnMe_3$, $Pd(PPh_3)_4$, THF; (c) CBr_4, PPh_3, DCM; (d) Bu_3SnH, $Pd(PPh_3)_4$, toluene; (e) $Pd_2(dba)_3CHCl_3$, PPh_3, reflux, THF, 3 h.



Scheme 3. Reagents and conditions: (a) BuLi, $B(OiPr)_3$, THF, −78 °C; (b) $Pd(PPh_3)_4$, Ag_2CO_3, KOH, THF, 2 h, rt.

bromide **14** in 81% yield.[9] The key cross coupling reaction of vinyl tin **12** and vinyl bromide **14** was carried out between PPh_3 and $Pd_2(dba)_3CHCl_3$ in THF, yielding 13–61% of the desired product **8b** (from 8 mmol scale to 0.6 mmol scale).[10]

Because of the low yield at Stille cross-coupling conditions on the large scale (over 8 mmol), the Suzuki cross coupling reaction was investigated next. Vinyl boronic acid **15** was obtained from iodide **11** by an exchange of iodo with lithium, and then trapping with triisopropyl borate in a 75% yield.[11] Only the desired product **8b** was obtained by this method in 27–68% yield (14–1.4 mmol scale).[12]

Receptor binding assays were performed on membranes prepared from HEK293 expressing the EP_1, EP_2, EP_3, and EP_4 receptors. A 100 μl reaction mixture containing 20 μg of membrane was mixed with [5,6,8,11,12,14,15(*n*)-^{3}H]prostaglandin E_2([^{3}H]PGE_2) (Perkin-Elmer), along with increasing concentrations of test compounds in a final concentration of 1% DMSO. The compounds were diluted in 25 mM MES, 10 mM $MgCl_2$, 1 mM EDTA, pH 6 (binding buffer), containing 4% DMSO. [^{3}H]PGE_2 was added at concentrations equal to previously determined K_d values for EP_1

(3 nM), EP_2 (8 nM), EP_3 (2 nM), and EP_4 (2 nM). For the EP_2 and EP_4 receptor membrane reactions, 500 μg of wheatgerm agglutinin SPA (Scintillation Proximity Assay, Amersham) beads was added to the wells. All reaction mixtures were incubated at room temperature, with shaking, for 1 h. The EP_2 and EP_4 reactions were quantitated on a Topcount reader, whereas the EP_1 and EP_3 reactions were terminated by filtration through glass fiber (GF/C) unifilter plates (Whatman, catalog # 7700-4301) that were previously soaked in 0.5% PEI (polyethylinamine, Sigma). Unifilter plate wells were then washed 4 times with 200 μl of binding buffer and dried for 30 min at 50 °C. After sealing the bottom of the plates, 100 μl of scintillation cocktail (Ultima gold™ XR, Packard # 6013119) was added in the wells and filters were incubated for 1 h at rt and radioactivity remaining on filters was measured using a Topcount plate counter (Packard).

Production of cAMP in response to prostanoid compounds was measured in HEK293 cells transfected with EP_2 or EP_4 receptor, respectively. The cells were plated at a density of 20,000 cells/well in 96-well plates, one day prior to the assay. Stimulation was carried out in assay buffer (phenol red-free DMEM/F12, containing 0.1% BSA, 0.1 mM isobutylmethyl-xanthine, and 1% penicillin–streptomycin) for 60 min with increasing doses of test molecules. Following stimulation, cells were lysed and cAMP in the lysate was measured using a cAMP chemiluminescent assay kit (Tropix, Bedford, MA, USA) as per manufacturers' instructions.

The binding affinity data for the selected compounds listed in Table 1 revealed that compounds **9a** (the 13*E*,15*E* isomer) and **9b** (the 13*Z*,15*E* isomer) were coselective for EP_2 and EP_4 receptors. Notably, Compound **9b** exhibited greater selectivity and potency for the EP_4 receptor (K_i = 4 nM, EC_{50} = 0.1 nM) than the EP_2 receptor (K_i = 60 nM, EC_{50} = 35 nM). Similarly, isomer **18** (13*Z*,15*E*) showed selectivity for the EP_4 receptor, while its isomer, compound **19** (13*E*,15*E*), showed selectivity for EP_2 receptor. It means that the *cis* C=C double bond geometry at C13–C14 is a major factor contributing to improving the selectivity for EP_4 receptor over the EP_2 receptor. Compound **18**, a methyl group at C-15, showed decreased binding affinity and potency for the EP_4 receptor by 100-fold, in comparison with compound **9b**, a methyl group at C-16. It indicates that having a methyl group in the C-16 plays a crucial role in improving the EP_4 activity. Compound **16,** which lacks a methyl group at either C-15 or C-16, almost retains EP_2 and EP_4 receptor activity, but showed decreased selectivity. Introduction of a shorter alkyl chain at C-16 (compounds **17** and **20**) sharply decreased the binding affinity and potency for the EP_2 receptor. The replacement of an *n*-alkyl chain at C-16, with cycloalkyl and simple phenyl groups, (e.g., compounds **21** and **22**) led to a decrease in EP_2 and EP_4 activity as well.

Compound **1a** wherein the hydroxy cyclopentanone ring has been replaced by a lactam was found to exhibit potent and selective agonist activity at EP_4 receptor and lack of the 11-hydroxy group in these lactam analogs might be responsible for the improvement of the selectivity for EP_4 receptor over the other receptors (**1a** vs PGE_2, **9b** vs PGE_2).[4b,d] The diene analogs (**9b** vs **1a**) without the 15-hydroxy group still maintain the similar selectivity. It can be explained that the 15-hydroxy group or diene at ω-side chain residing in the hydrophobic pocket might have different flexibility and the different binding motifs.[4d]

The rat pharmacokinetics data showed **9b** underwent slow degradation. The iv clearance rate of **9b** was measured to be 0.15 L/kg/h, which was a remarkable decrease in clearance rate in comparison with **1a** (iv in rat, CL: 2.5 L/kg/h, half-life time 0.78 h).[4d] This slow clearance rate resulted in its prolonged half-life time (7.7 h). Its bioavailability was calculated to be 72.6% (rat, dosing with 1 mg/kg of **9b**). We also studied the utility of **9b** in animal models of the ovulation induction, Asthma, and Ulcerative Colitis. Preliminary result of diene **9b** showed very good efficacy in an in vivo mice ovulation induction model (CD-1 adult mice) with ED_{50} = 1 mg/kg when administered orally.[4e] The other discoveries will be reported in due course.

Conclusions. Analogs of PGE_2, wherein the hydroxy cyclopentanone ring has been replaced by a γ-lactam and a 15-hydroxy alkenyl group of the ω-chain side has been substituted by a diene, were found to be potent and selective agonist of the EP_2 and EP_4 receptors. In particular, it was determined that the C-15 hydroxy group present at the ω-side chain was not necessary. Furthermore, we found that incorporation of a methyl group at C-16 and *cis* C=C bond geometry at C13–C14 of the ω-side chain played important roles in the improvement of potency and selectivity for the specific subtypes of the EP_2 and EP_4 receptors. The rat PK data exhibited that compound **9b** had more stability during enzyme metabolization.

Acknowledgments

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