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Efficient synthesis of a novel *m*-phenylene derivative as a selective EP_4 agonist inducing follicular growth and maturation in the ovary

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ABSTRACT

An efficient and straightforward synthesis of a novel *m*-phenylene derivative has been developed. The optically pure dibromo compound was selected as a starting material. Through a protocol involving the Prins reaction and two steps of the Horner–Wadsworth–Emmons reaction, the basic skeleton was constructed with appropriate alpha and omega side chains. The compound proved to be a highly selective EP_4 agonist and a possible drug candidate for maturation of the uterine cervix.

Prostaglandin E_2 (PGE_2) exhibits a wide range of physiological actions, including uterine constriction, suppression of gastric acid secretion, protection of the gastric mucous membrane, stimulation of digestive peristalsis, and induction of fever and diarrhea. In particular, it plays a crucial role in ovulation. PGE_2 receptors can be classified into four subtypes, namely, EP_1, EP_2, EP_3, and EP_4.[1] It has been revealed that the physiological actions of PGE_2 are mediated by a specific receptor,[2] and it has also been elucidated that each receptor subtype mediates different physiological functions of PGE_2.[3]

The EP_4 receptor is present in various organs including the heart, kidney, liver, intestine, lung, and bones. EP_4 receptor functions include relaxation of smooth muscle, differentiation and proliferation of lymphocytes, proliferation of mesangial cells, and collagen production in fibroblasts. Therefore, EP_4 receptor agonists and antagonists can serve as preventives of or remedies for the above pharmacological effects. For example, Ono showed that both EP_4 agonists and antagonists were useful drugs in the treatment of bone diseases.[4] Kanayama and co-workers revealed the following: (1) localization of the EP_4 receptor in the ovary plays a role in follicular growth, (2) PGE_2 induces ovarian follicular growth, and development is mediated at least in part by the EP_4 receptor, (3) the action of an EP_4 agonist is mediated through IL-8 up-regulation, and (4) the new EP_4 agonist could be a promising reagent for various systems used to induce follicular maturation in clinical or agricultural fields.[5]

Selectivity of EP_4 agonism is necessary for developing a useful drug without side effects, such as constriction of the uterus. Ono's strategy to develop a selective EP_4 agonist could be regarded as a modification of alpha and omega side chains in natural PGE_1 or PGE_2. Although it seems to be a practical means to determine a selective EP_4 agonist starting with these natural products, Ono's EP_4 agonists inevitably exhibited undesirable chemical instability, which could not be corrected and thus proved a problem.[6]

In contrast, we have developed a chemically stable PGI_2 analog called 'Beraprost Sodium' using the *m*-phenylene skeleton instead of the unstable enol ether structure.[7] During the course of the study, some nonselective compounds that could be classified as EP_4 agonists were found. Therefore, we attempted to find a novel selective and chemically stable EP_4 agonist lead; we began by rescreening the *m*-phenylene library containing analogs with a variety of alpha and omega side chains. Finally, we found a potent and selective EP_4 agonist 3-((1*R*,2*R*,3a*S*,8b*S*)-1-((*S*,*E*)-4-cyclohexyl-3-hydroxybut-1-enyl)-2-hydroxy-2,3,3a,8b-tetrahydro-1*H*-cyclopenta[*b*][1]benzofuran-5-propanoic acid, 'APS-856' (**2**), which was derivatized to a more potent and selective EP_4 agonist, (1*R*,2*R*,3a*S*,8b*S*)-5-(2-(1*H*-tetrazol-5-yl)ethyl)-1-((*S*,*E*)-4-cyclohexyl-3-hydroxybut-1-enyl)-2,3,3a,8b-tetrahydro-1*H*-cyclopenta[*b*][1]benzofuran-2-ol, 'APS-999' (**1**)[8] (Scheme 1).

Here we report the efficient synthesis and brief pharmacology of the novel *m*-phenylene EP_4 receptor agonist **1**.

The first synthesis of compound **1** was conducted by functional group transformation starting from methyl ester intermediate **3** of

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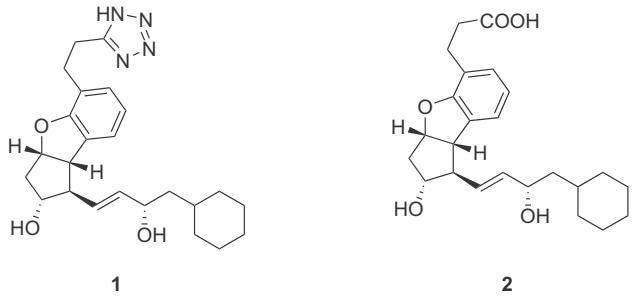


Scheme 1. Structure of APS-999 (**1**) and APS-856 (**2**).

our *m*-phenylene library compound **2**, which was prepared by a method described in a patent.[9] Scheme 2 shows the synthetic route to intermediate **3**, which is a methyl ester prepared from optically active cyclopenta[*b*][1]benzofuran derivative **4** over 13 steps.

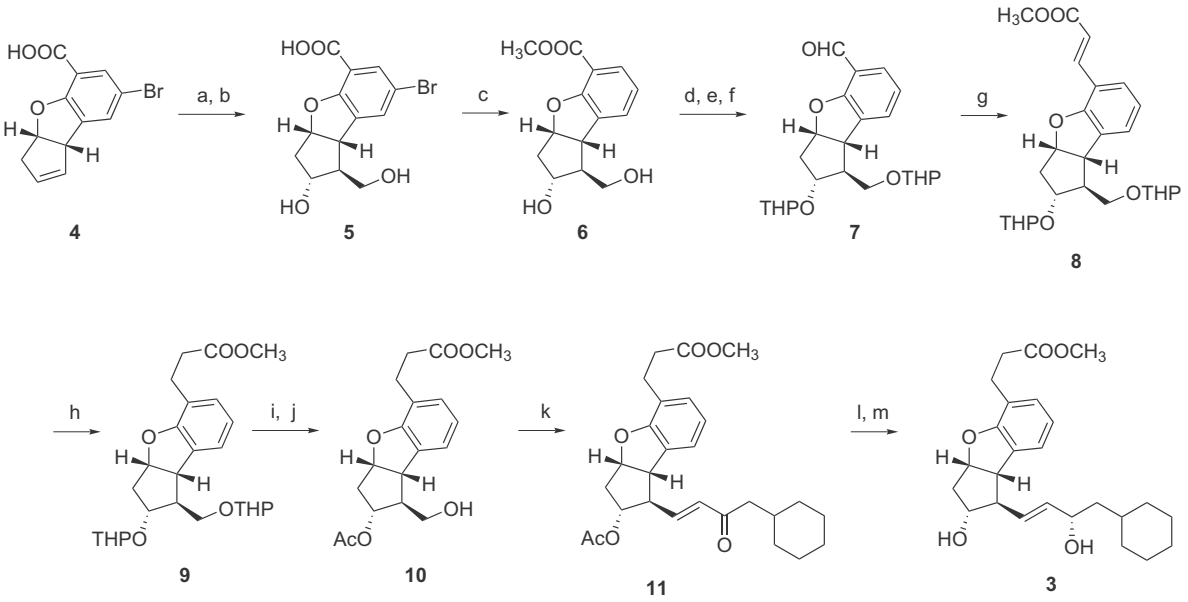
Scheme 3 shows the sequence of transformations from intermediate **3** to target compound **1**. This sequence started with silyl ether protection of two hydroxy groups of methyl ester intermediate **3** (97% yield). A methyl ester group was hydrolyzed to be a carboxyl group under alkaline conditions (95% yield). Oxalyl chloride and ammonia saturated chloroform were used to obtain amide compound **14** (63% yield), which was used as a substrate to yield nitrile compound **15** by reaction with tosyl chloride in pyridine (53% yield). After deprotection of the silyl ethers using tetrabutylammonium fluoride (70% yield), the tetrazole ring, which would be a bioisostere of the carboxyl group of **2**, was constructed by treatment with sodium azide to yield **1** (57% yield).[10] The target **1** was obtained from **3** through six steps in 12.2% overall yield.

Practically, the synthetic route described in Schemes 2 and 3 is not straightforward, and can be simplified by introduction of an appropriate alpha chain during the preparation of the *m*-phenylene intermediates. Accordingly, we have attempted to develop a more efficient method for the preparation of compound **1**.

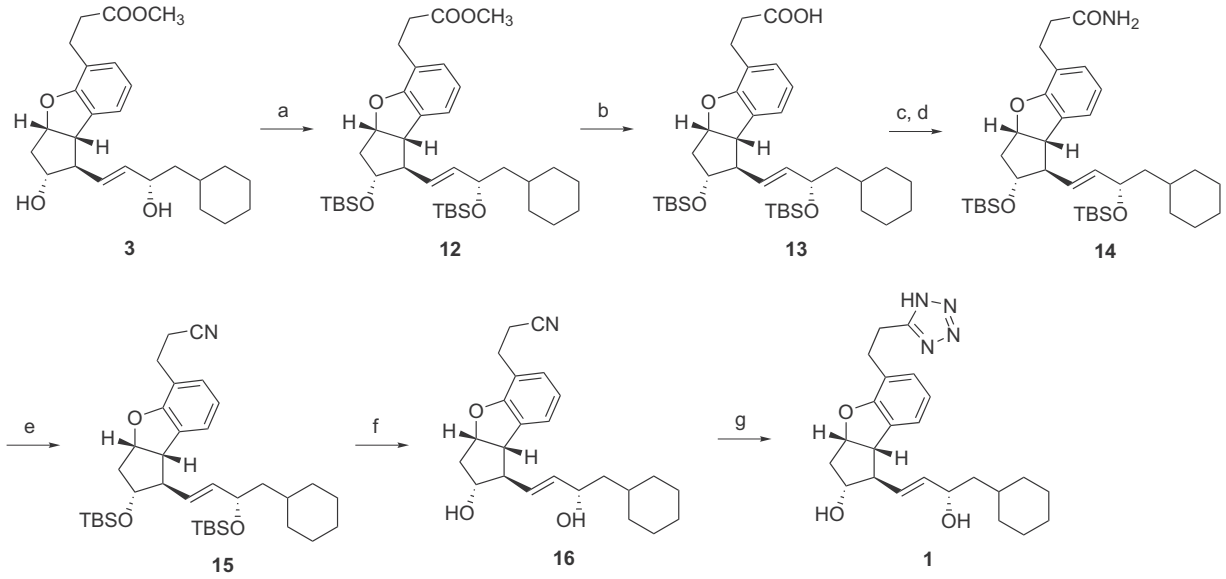
An improved method for the preparation of compound **1** is shown in Scheme 4. The optically pure starting material **17** was prepared by Nishiyama's asymmetric synthesis.[11] In the first step, compound **17** was subjected to Prins reaction conditions and then hydrolyzed to afford diol **18**. The two hydroxy groups of **18** were then protected with TBDPS to afford the disilylether compound **19** (40% yield, 3 steps, after recrystallization). The next step was a halogen-metal exchange reaction of the bromobenzene moiety of disilylether **19** using *i*-PrMgCl; the product was then reacted with dimethylformamide to give the corresponding aldehyde. Without purification, the aldehyde was immediately condensed with the corresponding Wadsworth reagent to afford a *cis*/*trans* mixture of conjugated nitrile **20**. After selective removal of the silyl ether protecting group on the primary alcohol of **20**, the resulting compound was hydrogenated to afford the saturated nitrile **21** (53% yield, 3 steps). Simultaneously, unnecessary bromide on the *m*-phenylene moiety was removed. The corresponding aldehyde, which was obtained by Moffatt oxidation of **21**, was converted to enone **22** by Horner–Wadsworth–Emmons reaction in THF (90% yield after recrystallization). Enone **22** was then stereoselectively reduced by using the BH_3-THF/(*R*)-5,5-Diphenyl-2-methyl-3,4-propano-1,3,2-oxazaborolidine system to yield an alcohol. Desilylation afforded the diols, which were a mixture of stereoisomers at the allylic hydroxy group position (α-alcohol:β-alcohol = 94:6). Purification by column chromatography (silica gel) gave the pure α-alcohol **16** (75% yield, 2 steps). Conversion of the nitrile group to a tetrazole group was performed by treatment with NaN_3 to yield compound **1** (70% yield, after recrystallization). Therefore, a new and straightforward synthetic route with much fewer steps (10 steps) than the corresponding conventional process (25 steps) was developed. The overall yield of compound **1** from compound **17** was 10%.

The prostaglandin EP_4 receptor agonistic activity and selectivity of APS-856 (**2**) and APS-999 (**1**) were evaluated by the Magnus method with appropriate isolated tissue (EP_4: rabbit saphenous vein; EP_{1+2}: guinea pig ileum; EP_3: guinea pig uterus).[12] The results are presented in Table 1. Converting the carboxylate group (**2**) to its bioisosteric tetrazole moiety (**1**) improved EP_4 activity and especially EP_4 selectivity.

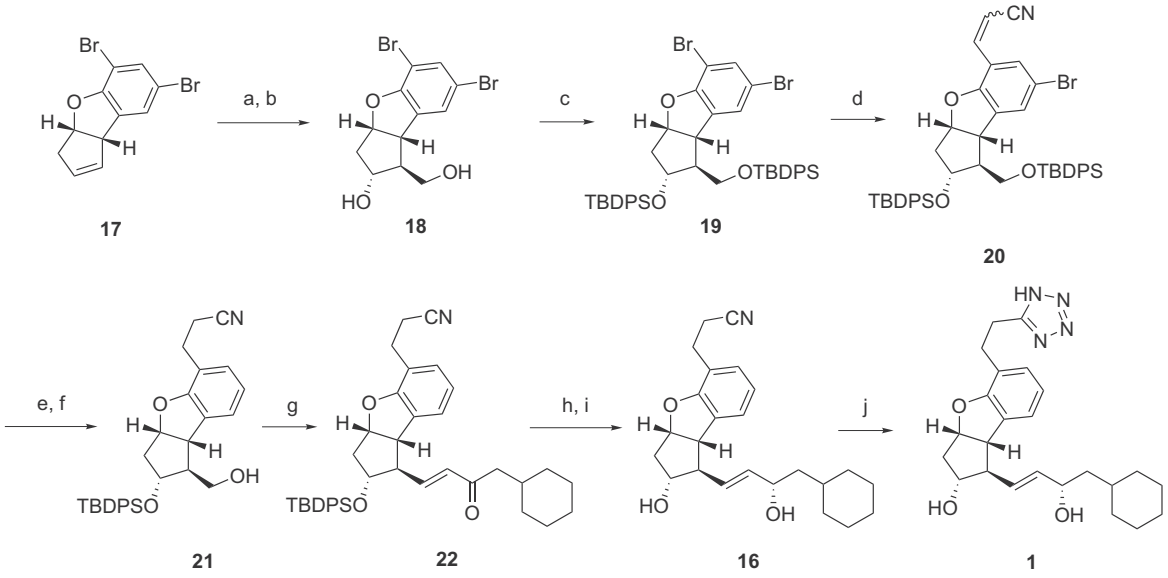
El-Nefiawy, Abdel-Hakim, and Kanayama have used compound **1** as a selective EP_4 receptor agonist in vivo to explore whether this compound has a positive impact on ovarian follicle growth in rats,



Scheme 2. Synthesis of starting material **3**: reaction conditions a Trioxane, H2SO4, AcOH, 80 °C b NaOH, MeOH, reflux c H2, Pd/C, MeOH, rt - reflux d Dihydropyrane, *p*-tolSO3H, THF, 35 °C e LAH, THF, -20 °C f MnO2, CH2Cl2, rt g H3COOCCH2=PPh3, THF, -10 °C h H2, Pd/C, MeOH, rt i *p*-tolSO3H, MeOH, 50 °C j Ph3CCl, Et3 N, THF, reflux then Ac2O, Py, reflux then HCl/MeOH, rt k DMSO, DCC, CF3COOH, Py, THF, rt then NaH, WadsWorth reagent, THF, -20 °C l NaBH4, CeCl3-7H2O, MeOH, −20 °C m NaOMe, MeOH, rt then silica gel column chromatography.



Scheme 3. Functional group transformations of compound **3** to target compound **1**: a TBSCl, Imidazole, DMF, 60 °C, 97% b 2 N NaOHaq, THF-MeOH, rt, 95% c (COCl)2, CH2Cl2, rt d NH3, CHCl3, 0 °C, 63% e TsCl, pyridine, rt, 53% f TBAF, THF, rt, 70% g NaN3, NEt3, N-Me-2-pyrolidone, 150 °C, 57%.



Scheme 4. An efficient and straightforward synthesis of compound **1**. a Trioxane, H2SO4, AcOH, 70 °C b 3 N NaOHaq, THF, 50 °C c TBDPSCl, Imidazole, DMF, rt, 40% (3 steps, after recrystallizaiton) d *i*-PrMgCl, THF then DMF, 60 °C then NaH, (EtO)2P(O)CH2CN, rt e TBAF, THF, −10 °C f H2, Pd/C, NaOAc, MeOH, rt, 53% (3 steps) g DCC, DMSO, TFA, Pyridine, THF, rt then NaH, (MeO)2P(O)CH2C(O)CH2-*c*-Hex, rt, 90% (after recrystallization) h Oxazaborolidine, BH3-THF, THF, 0 °C i TBAF, THF, rt, 75% (2 steps) j NaN3, Et3 N HCl, NMP, 140 °C, 70% (after recrystallization).

Table 1
EP_4 agonist activity and selectivity of synthesized compounds

Compound	Rabbit saphenous vein (EP_4) EC_{50} (nM)	Guinea pig ileum (EP_{1+2}) EC_{50} (nM)	Guinea pig uterus (EP_3) EC_{2000} (nM)
1	2.6	3400	2800
2	5.7	150	540

while aiming to acquire a better understanding of the underlying mechanism of action of PGE_2.[5] They concluded that the EP_4 receptor agonist could be a promising reagent for various systems used to induce follicular maturation in clinical or agricultural fields.

In summary, we have developed an efficient and straightforward synthesis of an *m*-phenylene-type EP_4 receptor agonist from an optically pure starting material. This simple approach gave compound **1** in 10 steps. Compound **1** was subsequently proved to be a selective EP_4 receptor agonist and a possible drug candidate for maturation of uterine cervix.

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10. Compound **16** (74 mg, 0.20 mmol) was dissolved in *N*-methyl-2-pyroridinone (3.5 mL). To this solution, sodium azide (87 mg, 1.2 mmol) and triethylamine (92 mg, 0.60 mmol) was added. After stirring for 56 h at 150 °C, sodium azide (87 mg, 1.2 mmol) and triethylamine (92 mg, 0.6 mmol) was added to complete substrate consumption following additional heating at 150 °C for 24 h. The reaction mixture was poured onto ice-cold 1 N hydrochloric acid solution and extracted with ethyl acetate. Silica gel column chromatography (cyclohexane/ethyl acetate: 1:3) was conducted to yield compound **1**. Analytical data: IR (neat, cm^{-1}) 3386, 2922, 2850, 1655, 1560, 1509, 1450, 1257, 1194, 1064, 973, 863, 744. ^1H NMR ($CDCl_3$) δ: 6.98 (1H, d, J = 6.9 Hz), 6.72–6.64 (2H, m), 5.59–5.57 (1H, m), 5.34–5.24 (1H, br s), 4.26–4.20 (2H, m), 4.08–3.42 (2H, m), 3.29–3.16 (2H, m), 2.80–2.72 (1H, m), 2.15–1.99 (3H, m), 1.79–1.68 (6H, m), 1.51–1.11 (6H, m), 0.97–0.90 (2H, m). Three protons (–OH, –NH) were not observed. HRMS (EI) m/z calcd for $C_{24}H_{32}N_4O_3$ 424.2427; found 424.2456.
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Isolated preparation: All animal experiments were approved by our institutional ethics review committee. EP_4 receptor agonistic activity was evaluated according to a previous method with slight modification. Male New Zealand white rabbits (2–4 kg) were sacrificed by overdosing with the anesthetic pentobarbital. Saphenous veins were excised and adherent fat and connective tissue were removed. Vessel rings (4 mm wide) were cut and suspended by stainless steel hooks under 1.0 g tension in 10 ml organ baths containing Krebs solution (NaCl 118.1 mM, KCl 5.31 mM, $CaCl_2$ 3.52 mM, $MgSO_4$ 1.01 mM, NaH_2PO_4 1.09 mM, $NaHCO_3$ 25 mM, glucose 9.99 mM; pH 7.4) at 37 °C and aerated with 95% O_2/5% CO_2. Isometric tension changes were recorded with isometric transducers (Nihon-Kohden, Tokyo, Japan).
Experimental protocols: To reduce intertissue variation, relaxant responses were measured by a paired curve experimental design. After an equilibration period, tissues were initially exposed to 40 mM KCl to obtain a stable contraction. Subsequent tests were performed in the presence of TP antagonist S-145 (1000 nM) to block TP receptor response. A standard concentration-response curve was obtained using PGE_2, and following a washout period, a testing curve was constructed in the presence of the testing agonist. Data are expressed as a percentage of the maximum contraction of 40 mM KCl. The EC_{50} value was calculated from each log concentration-response curve as the concentration that caused a relaxation response at 50% of its maximum value.
EP_1 and EP_2 receptor agonist activities were evaluated using guinea pig ileum contracted with bethanechol (1000 nM) in the presence of TP antagonist S-145 (1000 nM).
EP_3 receptor agonist activity was evaluated using guinea pig uterus. Quantification of uterine contraction was expressed as uterine motility index, which was calculated according to the following equation, where AUC is area under the contraction curve.
Uterine Motility Index = (AUC for 15 min after test article treatment—AUC for 15 min before test article treatment)/contractile height induced with 60 mM KCl.
The EP_3 agonist potency is shown as EC_{2000} which is the concentration of test article reached at number 2000 in Uterine Motility Index.