

Synthesis and Biological Properties of Novel Fluoroprostaglandin Derivatives: Highly Selective and Potent Agonists for Prostaglandin Receptors

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Abstract: Synthesis of novel 7,7-difluoroprostacyclin and 15-deoxy-15,15-difluoro-PGF_{2α} derivatives will be presented. These compounds show high affinity to prostaglandin receptors and potent biological activities.

Keywords: Antiplatelet · Fluorination · Glaucoma · Prostaglandin · Receptor

Introduction

Fluorine-containing molecules have attracted much attention recently in medicinal chemistry because fluorine has unique physical properties such as small atomic size, strong electron negativity, and high carbon–fluorine bond energy [1]. Introduction of fluorine atoms into a variety of biologically important substances, *e.g.* steroids [2], amino acids and peptides [3], and nucleosides [4], often brought significant improvement in their pharmacological profiles. A series of prostaglandin (PG) derivatives are particularly attractive targets for us because of their biological importance and a broad range of therapeutic applicability as medicines [5]. Recent advance in mo-

lecular biology unveils novel physiological functions of PG receptors [6]. According to cDNA cloning and structural elucidation of PG receptors, exploratory research of new PG receptor agonists has been largely accelerated [7]. In this article synthesis and some biological properties of novel difluorinated PG derivatives will be presented. It gives an impression of the large applicability and possibilities of a geminal difluoride moiety as one of the key units in drug design to transform physical and biological properties.

Fluorinated Prostacyclin Derivatives

Since the discovery of prostacyclin (PGL₂) by Vane and coworkers in 1976 [8], which is an unstable metabolite of arachidonic acid in the vascular cell wall, research related to prostacyclin has been extensively developed. Prostacyclin, a member of the PG family, has powerful actions as an antiplatelet agent which prevents platelet aggregation, and as a vasodilator which increases blood flow and produces hypotension. However, therapeutic applications of natural prostacyclin are very limited due to its inherent chemical and metabolic instability (Fig. 1). A large number of stabilized analogs have been reported, and some have been used in clinical trials or marketed as powerful agents for ischaemic peripheral vascular disease, Raynaud's disease, and primary pulmonary hypertension [5]. Recently, the structures and main functions of

PG receptors have been characterized [6]. It is reported that natural prostacyclin and its agonists such as iloprost [9] and beraprost [10] have strong affinities not only for the IP receptor (prostacyclin receptor) but also for the EP1 receptor (one of the four PGE receptor (EP) subtypes) [11]. Further research seeking for more selective prostacyclin agonists is needed to elucidate the diverse functions of these receptors and the overall mechanism of signal transduction at the molecular level, and also to obtain potentially valuable drugs without undesirable side effects.

Our study has been focused on novel prostacyclin derivatives fluorinated at the 7-position, particularly 7α-fluoroprostacyclin (**1**) and 7,7-difluoroprostacyclin (AFP-07) (**2**) (Fig. 1). The strong electron-withdrawing effect of fluorine atoms adjacent to the acid-sensitive enol ether should be particularly effective in protecting against hydrolysis.

The monofluoroprostacyclin **1** bearing a cyclobutyl group was synthesized by a three-component coupling approach [12] or by use of methylenecyclopentanone [13]. It exerted potent antiplatelet and anti-anginal activities in animal models in oral administration [14]. Next, we targeted a difluoroprostacyclin **2** to study the contribution of the second fluorine atom to the stability and biological activity. Retrosynthesis of AFP-07 in both Route A and B was featured by difluorination of the Corey lactone and Wittig reaction of the difluoro lactone (Scheme 1).

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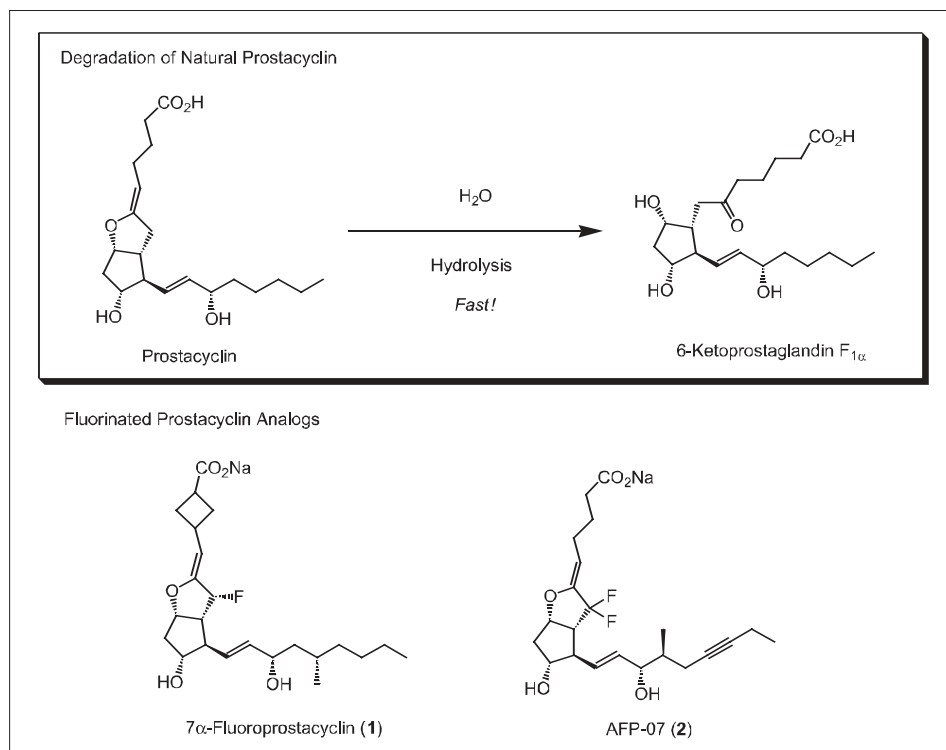
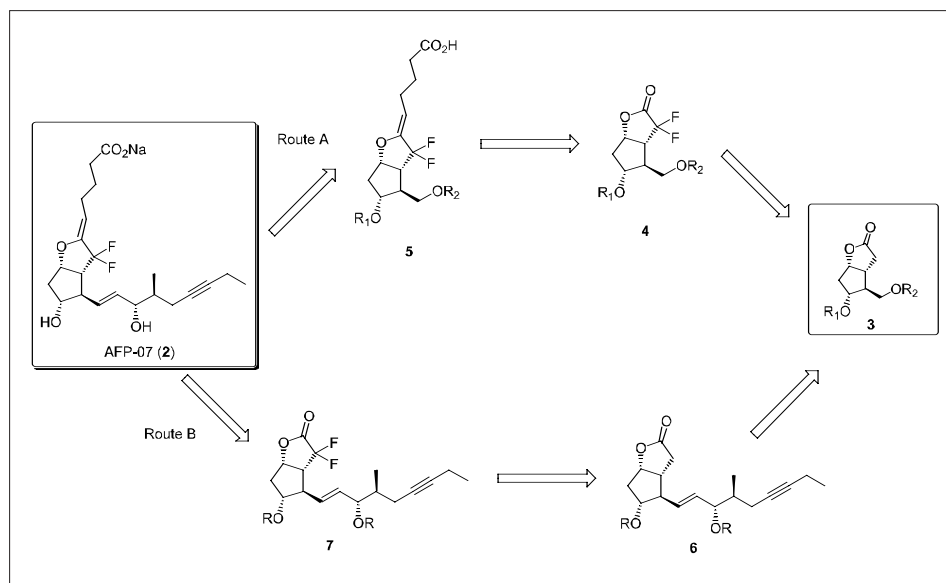


Fig. 1. Degradation of natural prostacyclin and fluorinated prostacyclin analogs



Scheme 1. Retrosynthesis of AFP-07

Electrophilic fluorination is an attractive method to synthesize fluorinated building blocks from rather complicated molecules. Several new electrophilic fluorinating agents have been developed [15], however, selective difluorination of the α -position of carbonyl compounds remains an unsolved issue. We examined various fluorinating agents and found *N*-fluorobenzenesulfonimide [11b] with manganese dibromide to be suitable for the reaction of ketones and esters (Table 1). Applying this method, the Corey lactone **3** was converted to the difluorolactone **4** in 57% yield and the vinyl ether **5** was subsequently obtained by Wittig reaction as a 87:13 mixture of the (*Z*) and (*E*) isomers according to the Route A [16].

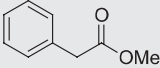
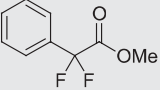
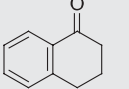
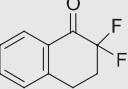
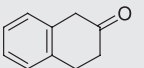
Because separation of the (*E*)-**5** isomer became a problem for scale-up, we further studied the Wittig reaction of the difluorolactone **7** in the Route B. It was supposed that the steric bulkiness of the side chain should affect the selectivity of the Wittig reaction. The synthesis of the difluorolactone **11** is shown in Scheme 2.

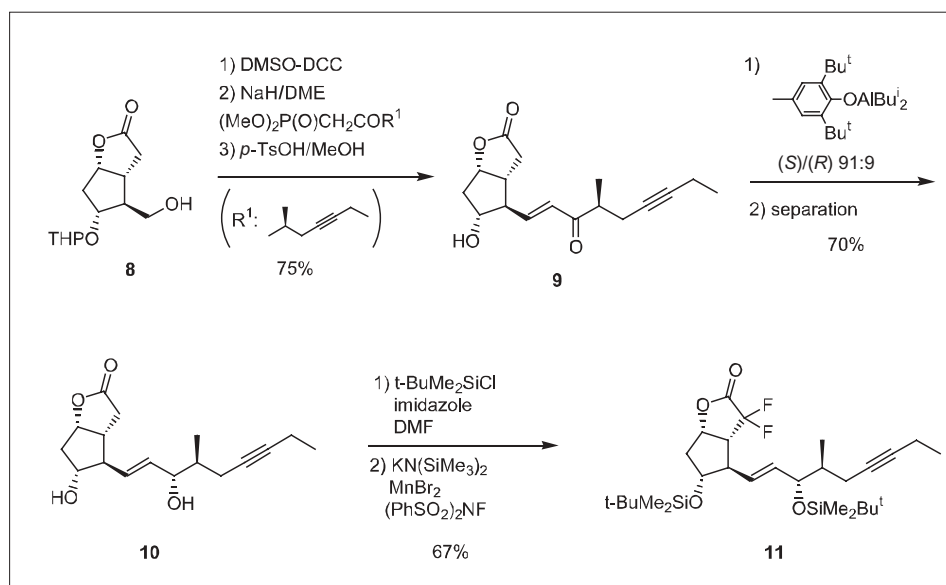
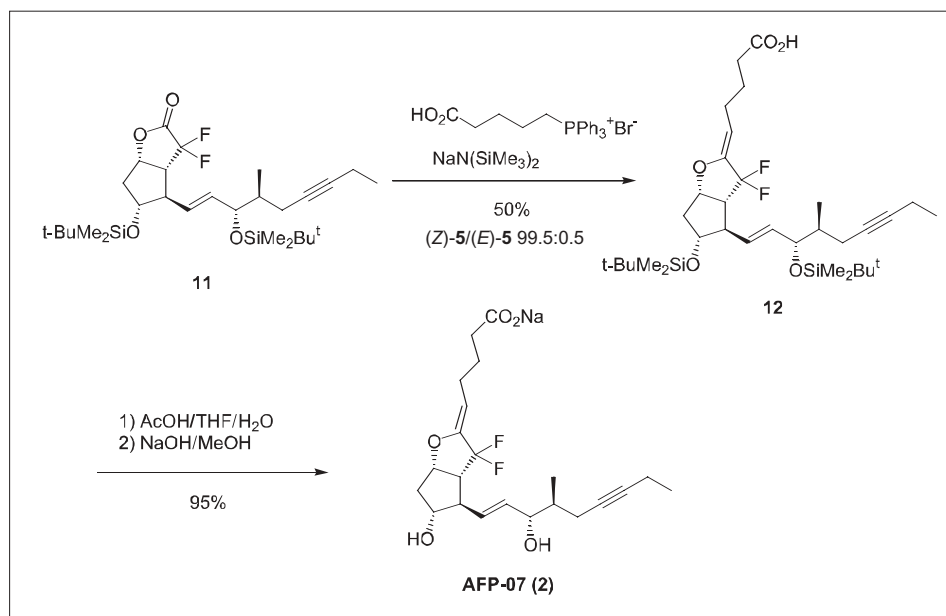
The Corey lactone **8** was transformed to the enone **9** in 75% yield by Moffatt oxidation, Horner-Emmons reaction with the known phosphonate [17], and deprotection of the THP group. Reduction with diisobutylaluminum 2,6-di(*tert*-butyl)-4-methylphenoxide [18] gave a 91:9 mixture of the (*S*) and (*R*) diastereomers. After protection of the diol with *tert*-butyldimethylsilyl groups, fluorination of the

lactone **10** with *N*-fluorobenzenesulfonimide in the presence of manganese dibromide gave the desired difluoride **11** in 67% yield. Wittig reaction of **11** with the phosphonium salt and sodium *N,N*-bis(trimethylsilyl)amide in THF at 0 °C furnished the vinyl ether **12**, as a 99.5:0.5 mixture of the desired (*Z*) and (*E*) isomers in 50% yield (Scheme 3). After removal of *tert*-butyldimethylsilyl group in AcOH/THF/H₂O and salt formation with NaOH, the desired AFP-07 (**2**) was obtained in 95% yield.

The half lives of prostacyclin derivatives in an aqueous solution are shown in Table 2. The stability of fluorinated prostacyclin derivatives, especially AFP-07 (**2**) is extraordinarily increased compared to nat-

Table 1. Difluorination of ketones and esters

| $\text{R}^1-\text{C}(=\text{O})-\text{CH}_2-\text{R}^2 \xrightarrow[\text{THF, -78 to 0 } ^\circ\text{C}]{\begin{array}{l} \text{MnBr}_2 \text{ (4.0 equiv.)} \\ (\text{PhSO}_2)_2\text{NF (4.0 equiv.)} \\ \text{KN}(\text{SiMe}_3)_2 \text{ (3.0 equiv.)} \end{array}} \text{R}^1-\text{C}(=\text{O})-\text{CF}_2-\text{R}^2 + \text{R}^1-\text{C}(=\text{O})-\text{CF}-\text{R}^2$ | | | |
|---|---|-----------|-----------------------|
| substrate | difluorinated product | yield [%] | selectivity (di:mono) |
|  |  | 93 | >99:1 |
|  |  | 80 | >98:2 |
|  | complex mixture | | |

Scheme 2. Synthesis of difluorolactone **11**

Scheme 3. Stereoselective Wittig reaction

ural prostacyclin. Protonation of the double bond in the vinyl ether, the rate-limiting step in the hydrolysis, should be strongly retarded by the adjacent difluoromethylene group of **2** [19].

The inhibitory activity of the derivatives on ADP-induced human platelet aggregation *in vitro* is indicated as a relative potency to PGE₁ in Table 2. The activity of **2** is much higher than that of natural prostacyclin. In the binding study to PG receptors, **2** shows a very strong affinity for IP receptor with little affinity for four kinds of the PGE receptor (EP) subtype [20].

As for animal models *in vivo*, in the microcirculation of the hamster cheek pouch oral administration of **2** at 1 or 10 µg/kg produced a maximum percentage inhibition of ADP-induced thrombi of 27% and 39%, respectively [21]. Oral dosage of AFP-07 (**2**) at 40 µg/kg twice a day prevented chronic hypoxic pulmonary hypertension in rats exposed to a simulated altitude of 17,000 ft for 28 days [22].

Fluorinated PGF_{2α} Derivatives

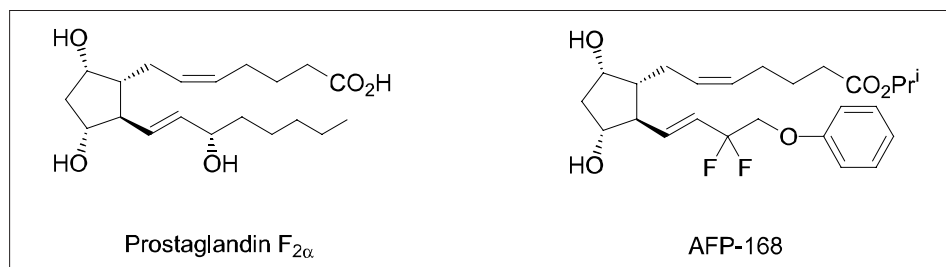
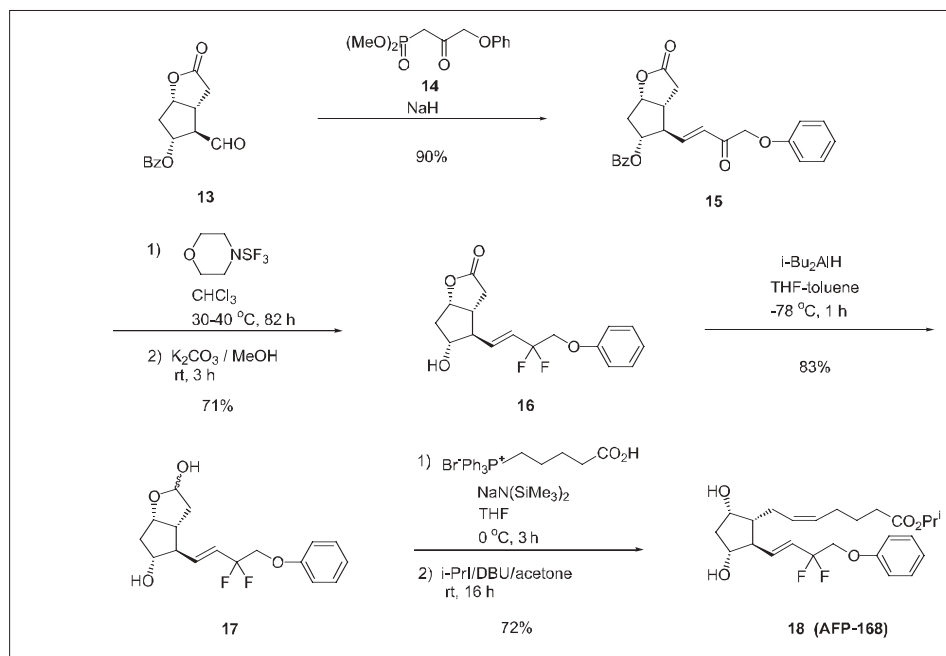
Glaucoma is one of the most common, but serious eye diseases that gradually steals sight without warning and often without symptoms. Vision loss is caused by damage to the optic nerve. The main risk factor is thought to be high intraocular pressure (IOP). Since the discovery that PGF_{2α} reduces intraocular pressure in an animal model [23], extensive efforts have been dedicated to develop PGF receptor (FP) agonists as new antiglaucoma agents [24]. Latanoprost, the representative FP agonist, has been first developed and widely used for the treatment of glaucoma [24b]. However, long-term application of the drug causes chronic adverse effects, such as iris/skin pigmentation [25]. In the search for a new FP agonist having more potent IOP reducing activity and weaker side effects than latanoprost, we have recently found 15-deoxy-15,15-difluoro-PGF_{2α}, AFP-168 which shows highly potent affinity to the prostanoid FP receptor (Fig. 2) [26].

A synthetic route to AFP-168 (**18**) is shown in Scheme 4. The synthesis started from the Corey aldehyde **13** [27], which was converted to the enone **15** by Horner-Emmons reaction with the phosphonate **14** [28]. Although a geminal difluoride unit has drawn more attention recently in medicinal chemistry, there is no general method to prepare an allyl difluoride from the corresponding enone efficiently [29]. We studied the fluorination reaction and found that reaction of the enone **15** with morpholinisulfur trifluoride [30] and successive deprotection of the benzoyl group with potassium carbonate in MeOH gave the geminal diflu-

Table 2. Half life in saline and inhibitory effects on human platelet aggregation *in vitro* of protacyclin derivatives

| Compounds | Half life in saline ^a | Inhibitory effects on human platelet aggregation <i>in vitro</i> (PGE ₁ = 1) ^c |
|---|----------------------------------|--|
| Prostacyclin | 10 min | 10 |
| 7 α -Fluoroprostacyclin (1) | 2–3 h ^b | 3–13 |
| AFP-07 (2) | >90 d | 237 |

^a25 °C, pH 7, conc. 10 μ g/ml. ^b37 °C, pH 7, conc. 2 μ g/ml. ^cRelative potency, ADP = 2 μ M

Fig. 2. Prostaglandin F_{2α} derivativesScheme 4. Synthesis of AFP-168 (**18**)

oride **16** in 71% yield. Reduction of the lactone **16** with diisobutylaluminum hydride in THF-toluene afforded lactol **17** in 83% yield.

Wittig reactions of lactol **17** with the ylide prepared from 4-carboxybutyltriphenylphosphonium bromide with various bases yielded the 15-deoxy-15,15-difluoro-PGF_{2α} derivative as a 5-(Z)/5-(E) mixture. Using sodium bis(trimethylsilyl)amide in tetrahydrofuran at 0 °C, the (Z)/(E) stereoselectivity became 99:1. Wittig reaction and the successive esterification of the crude acid treated with isopropyl iodide and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in acetone afforded the desired 15-deoxy-15,15-difluoro-PGF_{2α} isopropyl ester **18** (AFP-168) in 72% yield [31].

The affinity for the FP receptor of the corresponding carboxylic acid of AFP-168 was 0.4 nM [26], which was 12 times higher than that of latanoprost [24b]. It should be noted that substitution of the hydroxyl group at the 15-position of PGF_{2α} by this novel 15,15-difluoro-moiety maintains an inherent nature to bind to the prostaglandin receptor with such high affinity. Because other modifications at 15-position often caused loss or reduction of affinity to prostaglandin receptors, the hydroxyl group seemed to be necessary to show pharmacological activity before [5]. In cultured melanoma cells, a carboxylic acid of latanoprost has been reported to increase melanogenesis [32]. However, a carboxylic

acid of AFP-168 did not show the stimulatory effects on melanin content in cultured B16-F10 melanoma cells [33]. It is supposed the application of AFP-168 may cause less pigmentation than latanoprost. AFP-168 has a potent intraocular pressure reducing effect in animal models [26] and proceeds to clinical trial.

Conclusions

Synthesis and biological properties of novel 7,7-difluoroprostacyclin (AFP-07, **2**) and 15-deoxy-15,15-difluoro-PGF_{2α} (AFP-168, **18**) derivatives are presented. Further studies of these PG agonists are in progress as potential new drugs. New approaches using geminally difluorinated building blocks in drug design are important tools to improve the physical and pharmacological profiles.

Acknowledgments

The authors thank Dr. Yasutaka Takagi, Mr. Tadashi Nakajima, Mr. Atsushi Shimazaki, Dr. Takeshi Matsugi, Ms. Wakana Goto, Dr. Masaki Kageyama, Dr. Hideaki Hara at Research & Development Center, Santen Pharmaceutical Co., Limited for pharmacological evaluation of AFP-168.

Received: December 23, 2003

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