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Synthesis of Chemically Stable PGI_2 Analogs II -Synthesis of Halogen Substituted PGI_2 Analogs-†‡

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Abstract--Syntheses of several stable PGI_2 analogs substituted by halogen atoms(s) at C-5 or(and) C-7 are described. Reaction of protected PGI_2 methyl ester (1a) with N-chlorosuccinimide gave 5-chloro-Δ^6-PGI_1 derivative (2a), which was transformed into 5-chloro- and 5,7-dichloro-PGI_2 [(7) and (5)] by subsequent isomerization or chlorination. Similarly, reaction of 1b with N-bromosuccinimide gave 5-bromo-Δ^6-PGI_1 derivative (3b), which was further transformed into 7-fluoro-PGI_2 (16) by silver fluoride treatment. These halogenated PGI_2 analogs were found to be much more stable than PGI_2.

Prostacyclin (PGI_2) is a useful therapeutic agent in cardiovascular field because of its powerful vasoactive properties.[1] However its chemical instability against hydrolysis[2] is disadvantageous for its practical use, and chemically stable PGI_2 analogs have been proposed as more practical and useful agents.[3]

The instability of PGI_2 molecule is due to the unstable enol ether linkage in the molecule. Since this enol ether group constitutes a part of the strained bicyclo[3.3.0]octane ring system, it is hydrolyzed much easier than an ordinary enol ether group.[4] In fact, PGI_2 is easily hydrolyzed to result in the formation of biologically less active 6-oxo-$PGF_{1\alpha}$.[2] PGI_2 analogs with an electron-withdrawing group on or adjacent to the enol ether double bond were considered to be resistant against the hydrolysis since the electron-withdrawing group would reduce the electron density of the enol ether double bond to prevent it from the attack of the hydronium ion.[5] These analogs should be chemically more stable than naturally occuring PGI_2. Along with this strategy, 5-phenylthio-PGI_2[6] was synthesized by the electrophilic reaction of benzenesulfenyl chloride to the enol ether linkage of PGI_2, and 7-hydroxy-[6(b),7] and 7-fluoro-PGI_2[6(b),7] were also synthesized in our laboratory. Other stable PGI_2 analogs such as 7-oxo-PGI_2,[8] 4-oxo-PGI_2,[9] 5-oxo-Δ^6-PGI_1,[10] 5-cyano-PGI_2,[11] 5-carboxy-PGI_2,[12] 5-formyl-PGI_2,[13]

†Prostaglandin chemistrty XXX. For Part XXIX; T. Tanaka, N. Okamura, K. Bannai, A. Hazato, S. Sugiura, K. Manabe, and S. Kurozumi, Tetrahedron Lett., 26, 5575 (1985).

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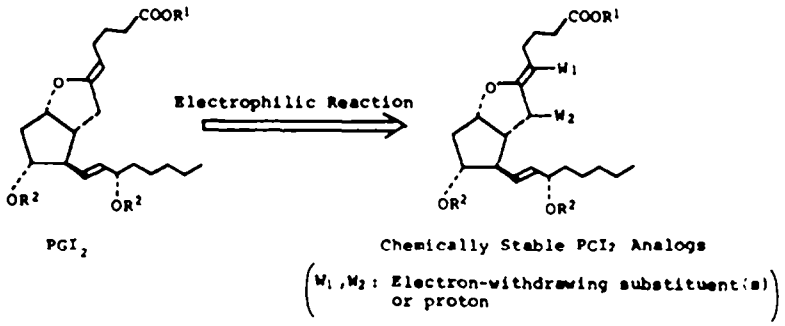
10,10-difluoro-PGI_2[14] etc.[3] are also included in this category. In this report, electrophilic halogenation to the PGI_2-enol ether was examined, and syntheses of 5-chloro-, 5,7-dichloro-, and 7-fluoro-PGI_2 derivatives are described.

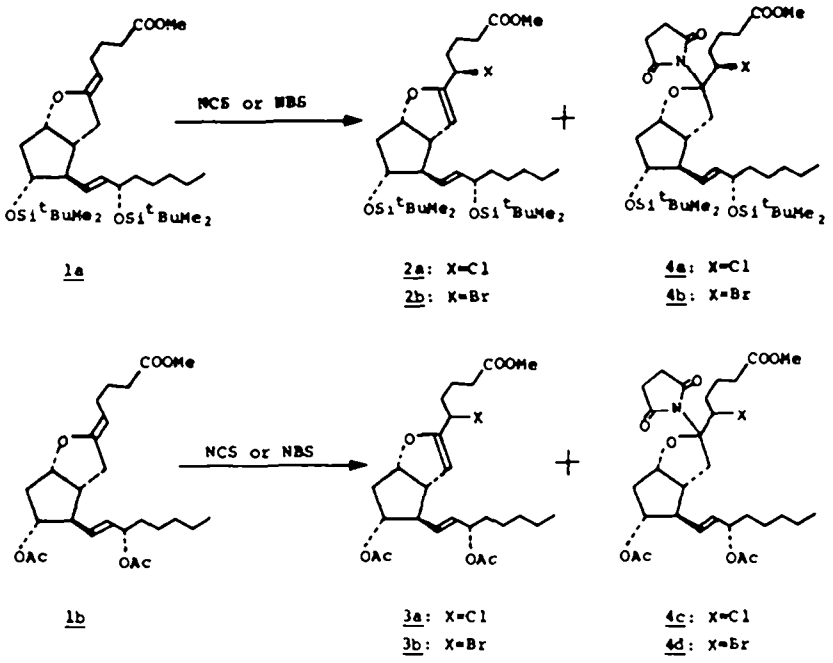
(I) Electrophilic halogenation of PGI_2 methyl ester

Previously we reported the synthesis of stable 5-phenylthio-PGI_2,[6] where the electron-withdrawing phenylthio group on the enol ether double bond participated in the stabilization of the molecule against hydrolysis. However, the phenylthio substitution resulted in the decreased biological activity. As it was considered that the decreased activity might be due to the bulkiness of the phenylthio group, halogen atoms smaller than phenylthio function were chosen as electron-withdrawing groups for the chemical stabilization of PGI_2.



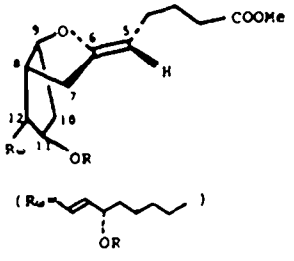
Scheme I

In order to introduce halogen atom into the PGI_2 skeleton, the electrophilic reaction of N-halosuccinimide with PGI_2-enol ether linkage was examined. Electrophilic chlorination of PGI_2 bis(t-butyldimethylsilyl ether) methyl ester (1a) with N-chlorosuccinimide (1.05 equiv.) was carried out in carbon tetrachloride, and (5R)-5-chloro-Δ^6-PGI_1 bis(t-butyldimethylsilyl ether) methyl ester (2a) was obtained in 60% yield accompanied with the formation of succinimido-adduct 4a (20%). Similarly, the reaction of 1a with N-bromosuccinimide gave (5R)-5-bromo-Δ^6-PGI_1 bis(t-butyldimethylsilyl ether) methyl ester (2b) in 57% yield accompanied also with succinimido-adduct 4b (20%). Both allylic halides 2a and 2b were found to be homogeneous by the examinations of their ^{13}C-NMR spectra, and their stereochemistry at C-5 was tentatively assigned as 5R (vide infra). On the other hand, the similar electrophilic halogenations of PGI_2 methyl ester diacetate (1b) with N-chlorosuccinimide or N-bromosuccinimide gave a diastereomeric mixture[15] of 5-chloro-Δ^6-PGI_1 diacetate methyl ester (3a) or 5-bromo-Δ^6-PGI_1 diacetate methyl ester (3b) in 58% or 60% yield, respectively. Succinimido-adduct 4c or 4d was also obtained in these reactions in 20% or 13% yield, respectively. ^{13}C-NMR spectra of the products 3a and 3b exhibited several pairs of peaks corresponding to the several carbons with the peak ratio of ca. 5:3 and ca. 7:3, respectively. These observations suggested that acetyl-protected allylic halides 3a or 3b were epimeric mixtures of (5R)- and (5S)-isomers in the respective ratios.



Scheme II

The exclusive formation of 5R-isomer of silyl-protected allylic halide 2a or 2b from silyl-protected PGI_2 methyl ester 1a could be accounted for the bulkiness of the t-butyldimethylsilyl ether substituent at C-11. From the molecular model study, the C-11 substituent was found to be close to the enol ether double bond as depicted in Fig. 1.[16] The t-butyldimethylsilyl group is bulky enough to hinder



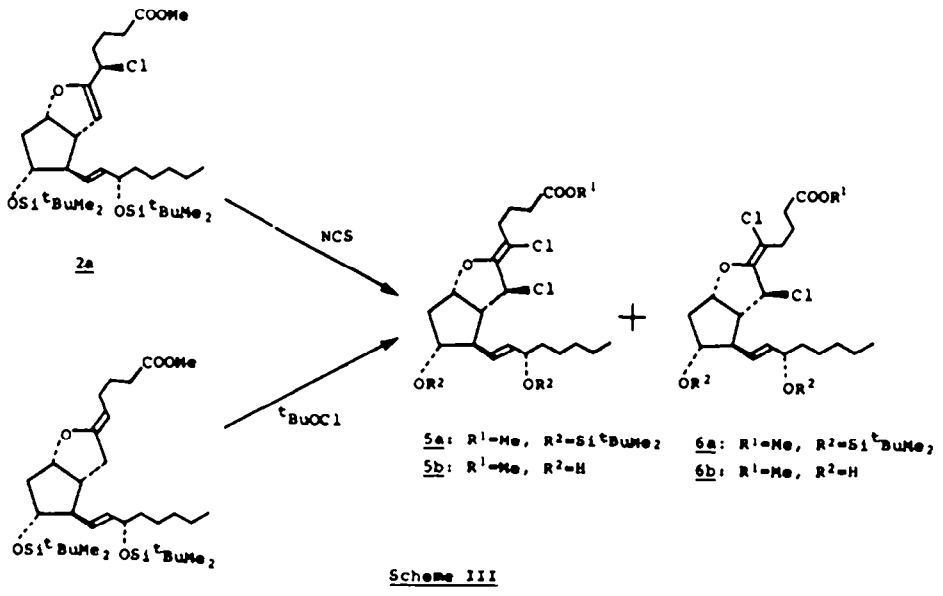
1a: R=Si^{t}BuMe$_2$
1b: R=Ac

Fig. 1

the lower side of the enol ether group from the attack of the halogenating agent, and consequently the halogenating agent could only approach from the less hindered upper side of the enol ether linkage to result in the exclusive formation of (5R)-isomers 2a or 2b. On the other hand, in the case of less hindered acetyl-protected PGI_2 1b, the halogenating agent could approach from the both sides to give the epimeric mixture of (5R)- and (5S)-allylic halides. The stereochemistry at C-5 of silyl-protected succinimido-adducts 4a and 4b were tentatively assigned to 5R, whereas that of acetyl-protected adducts 4c and 4d were assumed to be mixtures of 5R and 5S.

Further chlorination of silyl-protected (5R)-allylic chloride 2a was feasible with N-chlorosuccinimide. When allylic chloride 2a was treated with N-chlorosuccinimide in carbon tetrachloride-methylene chloride, geometric isomers, (5E,7S)- and (5Z,7S)-5,7-dichloro-PGI_2 bis(t-butyldimethylsilyl ether) methyl esters (5a) and (6a), were obtained in 17% and 20% yield, respectively. These dichlorides 5a and 6a were also obtained directly from silyl-protected PGI_2 methyl ester 1a by treatment with t-butyl hypochlorite[17] (8 equiv.) in methylene chloride in 17% and 5% yield, respectively. The 7S-stereochemistry of 5a and 6a was determined from the ^{1}H-NMR study where the 7α-methine protons of the products coupled with their 8β-methine protons by less than one Hz suggesting the 7S-configuration of 5a and 6a.[7] The Δ^5-olefin geometry of 5a and 6a was also tentatively assigned from their ^{1}H-NMR studies as follows. The 7α-methine proton of more polar dichloride 5a appeared in a field lower by 0.10 ppm than that of less polar 6a. By analogy with the case of 5-chloro-PGI_2 derivatives 7b and 8b (vide infra) where 7α-methine proton of 5E-isomer 7b appeared in a field lower by 0.11 ppm than that of 5Z-isomer 8b, the more polar dichloride 5a and the less polar 6a were tentatively assigned to 5E and 5Z, respectively.

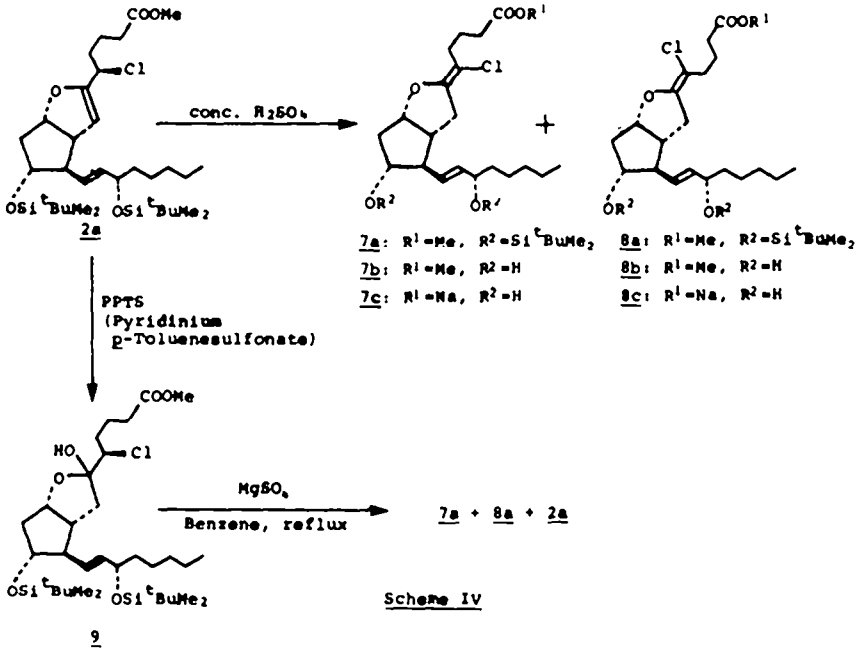
Removal of the silyl-protecting groups of 5a and 6a with hydrogen fluoride-pyridine in acetonitrile afforded (5E,7S)- and (5Z,7S)-5,7-dichloro-PGI_2 methyl ester (5b) and (6b) in 80% and 76% yield, respectively.



Scheme III

(II) Isomerization of 5-chloro-Δ^6-PGI_1 into 5-chloro-PGI_2

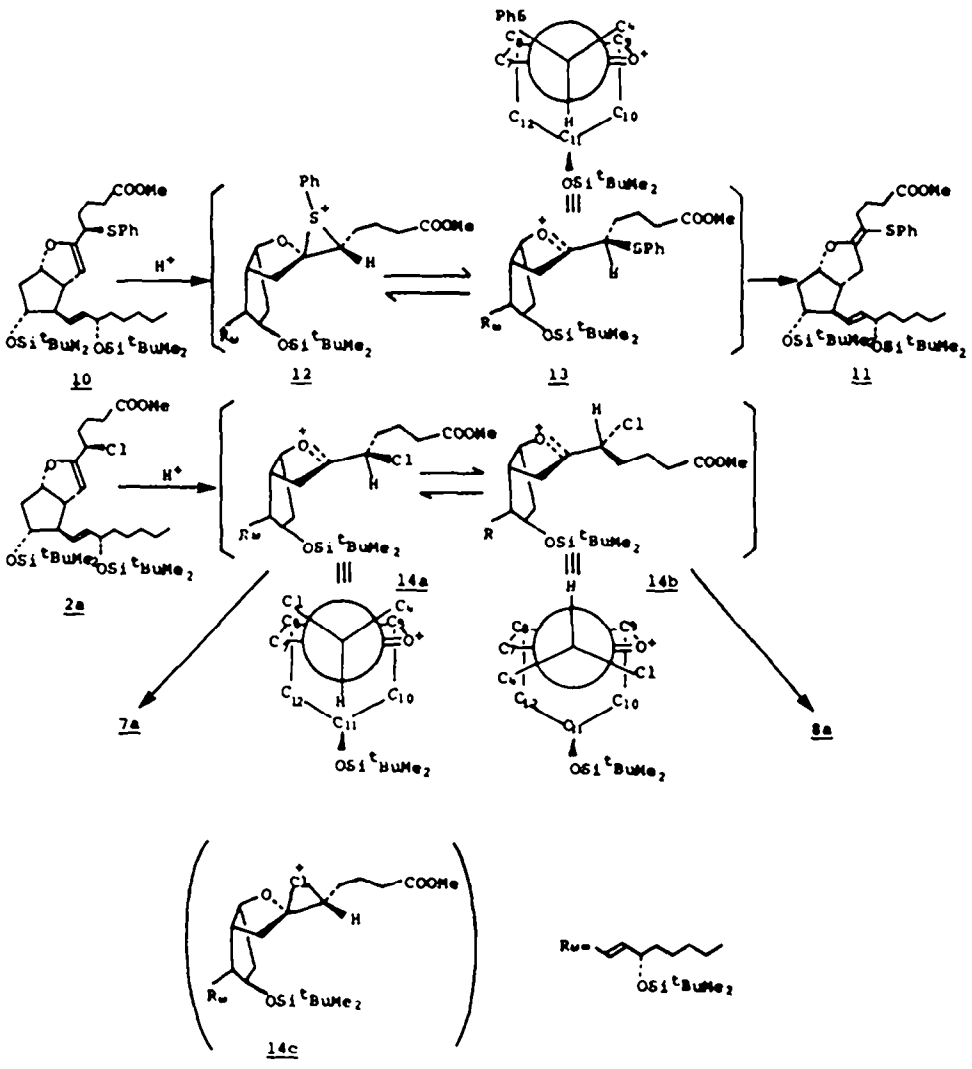
Isomerization of the endo-double bond (Δ^6) of allylic chloride 2a into the exo-double bond (Δ^5) is an important transformation to get a stable PGI_2 analog such as 5-chloro-PGI_2 (7). Previously, acid catalyzed stereospecific isomerization of silyl-protected 5-phenylthio-Δ^6-PGI_1 methyl ester 10 into (5E)-5-phenylthio-PGI_2 derivative 11 was reported.[6] Similar treatment of silyl-protected (5R)-5-chloro-Δ^6-PGI_1 2a with a trace amount of concentrated sulfuric acid gave a mixture of 5E- and 5Z-vinylic chlorides 7a and 8a only in low yield (both in 10% yields) after



Scheme IV

chromatographic separation. The difference in the stereochemical results would be attributed to the different intermediate formations as follows. In the acid catalyzed isomerization of 5-phenylthio derivative 10, there was a proposal of an exclusive formation of episulfonium ion intermediate 12 which could lead only to (5E)-5-phenylthio derivative via oxonium ion intermediate 13.[6(b)] The type 14b conformer of 13 would be excluded probably due to the restricted bond rotation between C-5 and C-6 by the contribution of episulfonium ion 12 or by the bulkiness of the phenylthio group. On the other hand, in the case of the isomerization of 5-chloro derivative 2a, two possible conformational isomers of oxonium ion intermediates 14a and 14b were considered to be more plausible than three membered ring intermediate such as epichloronium intermediate 14c. These two intermediates 14a and 14b would lead to the formation of 7a and 8a, respectively.

The alternative formation of 5-chloro-PGI_2 derivative 7a was achieved by stepwise transformation of 2a via hemiacetal 9. When allylic chloride 2a was treated with a catalytic amount of pyridinium p-toluenesulfonate in moistured benzene, 5-chloro-6-hydroxy-PGI_1 derivative 9 was obtained as an epimeric mixture at C-6. Subsequent dehydration of the crude hemiacetal 9 with excess anhydrous magnesium sulfate in refluxing benzene resulted in the formation of 7a and 8a along with the starting allylic chloride 2a in 20%, 28% and 18% yields, respectively. Removal of the silyl protecting groups of 7a and 8a with tetrabutylammonium fluoride in tetrahydrofuran afforded (5E)-5-chloro-PGI_2 methyl ester (7b) and



Scheme V

(5Z)-5-chloro-PGI_2 methyl ester (8b) in 95% and 80% yields, respectively. Hydrolysis of 8b and 9b with sodium hydroxide in ethanol-water gave sodium salt solution of (5E)-5-chloro-PGI_2 7c and (5Z)-5-chloro-PGI_2 8c, which were used directly for pharmacological tests.

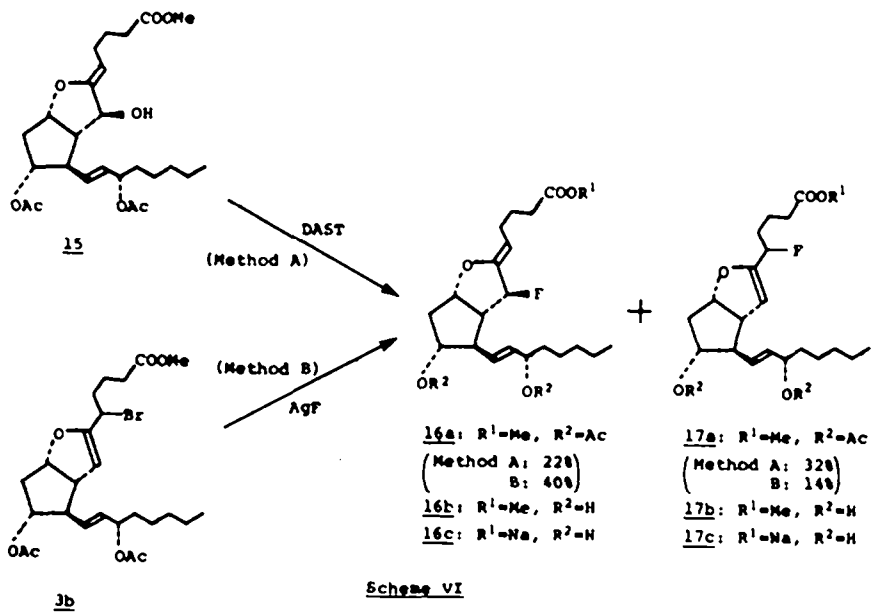
The Δ^5-olefin geometries of the products 7b and 8b were determined on the basis of their ^{1}H-NMR (400 MHz) spectra with the aid of decoupling studies. The C-4 methylene protons of less polar component 7b appeared in a field lower by 0.21 ppm (1H) and 0.11 ppm (1H) than those of more polar component 8b. Since allylic methylene protons cis to oxygen atom are known to appear lower field than those of trans,[18] the Δ^5-olefin geometry of less polar isomer 7b was assinged to 5E, and more polar 8b, to 5Z.

(III) Fluorination of 5-halo-Δ^6-PGI_1 into 7-fluoro-PGI_2

Fluorine atom substituted in the vicinity of the enol ether group of PGI_2 molecule should stabilize the enol ether linkage effectively because of the strong electron-withdrawing character.[19] Moreover, it is known that the substitution by fluorine atom of biologically active substances often leads to their enhanced biological activities.[20] In these respects, we have already reported the synthesis of chemically stable 7-fluoro-PGI_2 (16c),[7] which also retained its biological activity. However the previous method involving the fluorination of 7-hydroxy-PGI_2 derivative 15 with diethylaminosulfur trifluoride (DAST) is not satisfactory because of the low yield.[6(b)] The more convenient synthesis of 7-fluoro-PGI_2 (16) is the use of the present allylic bromide 3b.

Treatment of acetyl protected 5-bromo-Δ^6-PGI_1 methyl ester (3b) with silver fluoride (10 equiv.) in acetonitrile gave (5Z,7S)-7-fluoro-PGI_2 methyl ester diacetate (16a) (40%) and 5-fluoro-Δ^6-PGI_1 methyl ester 11,15-diacetate (17a) (20%) after chromatographic separation. Similar reactions of allylic chloride 3a with silver fluoride or of allylic bromide 3b with cecium fluoride (in DMF, r.t. ~160°C) gave poor results to obtain the expected allylic fluoride 16a. The Δ^5-double bond geometry and the C-7 stereochemistry of 7-fluoro-PGI_2 derivative 16a thus obtained was determined as (5Z,7S) by comparison with the authentic sample synthesized previously.[6(b)] The isolated 5-fluoro-Δ^6-PGI_1 derivative 17a was an inseparable mixture of epimers at C-5.

Removal of the acetyl groups of 16a by treatment with sodium methoxide in absolute methanol afforded (7S)-7-fluoro PGI_2 methyl ester (16b) in 82% yield, and hydrolysis of 16b with sodium hydroxide in ethanol-water gave sodium salt solution of (7S)-7-fluoro-PGI_2 (16c). The present new method is a convenient way to synthesize 7-fluoro-PGI_2 (16c) from PGI_2 methyl ester diacetate (1b).



Scheme VI

Halogenated PGI_2 analogs thus obtained were found much more stable than PGI_2. The chemical half life of 5-chloro- and 5,7-dichloro-PGI_2 methyl esters 7b, 8b, 5b and 6b in pH 2.4 buffer solution was 4h, 6h, 12h and 2.5 h, respectively, while that of PGI_2 methyl ester was less than one minute. Moreover 7-fluoro-PGI_2 16c had a half-life more than one month in pH 7.4 buffer solution,[6(b)] while that of PGI_2 in pH 7.46 has been reported to be only 15 minutes. 7-Fluoro-PGI_2 16c and (5E)-5-chloro-PGI_2 7c showed inhibitory activities on platelet aggregation (Rabbit, IC_{50}; 0.05 and 0.14 μg/ml, respectively), while (5Z)-5-chloro-PGI_2 8c and 5,7-dichloro-PGI_2 methyl esters (5b and 6b) were less active (>10 μg/ml).

Experimental

IR spectra were recorded on a JASCO A102 spectrometer ^1H- and ^{13}C-NMR spectra were obtained on a JEOL JNM-GX400 (400 MHz), JEOL JNM-PS-100 (100 MHz) or a Varian EM360A (60 MHz) spectrometer. Chemical shifts are reported as parts per million (ppm) relative to internal tetramethylsilane. Mass spectra were taken at 70 or 20 ev on a HITACHI M-80B or a LKB-9000 mass spectrometer. Optical rotations were measured on a Union Giken PM-101 automatic polarimeter. For high pressure liquid chromatography (HPLC) analysis, a Shimadzu Model LC-3A liquid chromatography equipped with a Shimadzu SPD-2A UV detector, and a YMC-Pack A-312 ODS (5 μm) column (15 cm × 6 mm I.D.) were employed. Thin layer chromatography (TLC) was performed using Merck silica gel (Kieselgel 60 F_{254}) analytical or preparative plates. All reactions were carried out under argon or nitrogen. Solvents for reactions were purified if necessary before use by distillation from suitable drying agents. Solvents for extraction and chromatography were GR grades.

Chlorination of PGI_2 bis(t-butyldimethylsilyl ether) methyl ester (1a) with N-chlorosuccinimide(NCS)——(5R)-5-Chloro-Δ^6-PGI_1 bis(t-butyldimethylsilyl ether) methyl ester (2a) and 5-chloro-6-succinimido-PGI_1 bis(t-butyldimethylsilyl ether) methyl ester (4a)

N-Chlorosuccinimide (17 mg, 0.13 mmol) was added at room temperature to a stirred solution of 1a (71 mg, 0.12 mmol) in carbontetrachloride (4 ml) in the presence of solid potassium carbonate (25 mg). After stirring the mixture at room temperature for 1 hr, water (10 mL) was added and the mixture was extracted with ether (2 × 20 mL). The combined extracts were washed with brine, dried over $MgSO_4$-K_2CO_3, and concentrated in vacuo. The residue was chromatographed on Florisil eluting with n-hexane-ethyl acetate (99.5 : 0.5) containing 0.1% of triethylamine to give allylic chloride 2a (45 mg, 60%) as an oil; $[\alpha]_D^{23}$ + 31° (c, 0.17, $CHCl_3$); IR (film) 1742, 1658, 1255, 1122, 1090, 1002, 970, 838, 775 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 2.85-3.2(1H, br), 3.66(3H, s), 3.7-4.2(2H, m), 4.38(1H, t, J=7 Hz), 4.7-5.0(1H, br), 5.00(1H, d, J=3 Hz), 5.52 (2H, m); ^{13}C-NMR ($CDCl_3$) δ 33.3(C_2), 22.0(C_3), 34.9(C_4), 55.4(C_5), 154.8(C_6), 101.6(C_7), 50.5(C_8), 83.3(C_9), 42.7(C_{10}), 76.1(C_{11}), 57.9(C_{12}); MS m/e 630, 628(M^+), 592, 573, 571, 535. Calc. for $C_{33}H_{61}NO_5Si_2\ ^{35}Cl$ 628.3743, Found 628.3780.
Further elution with n-hexane-ethyl acetate (8 : 2) containing 0.1% of triethylamine gave succinimido-adduct 4a (17.5 mg, 20%) as an oil; IR ($CHCl_3$) 1730, 1708, 1338, 1255, 972, 838 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 0.03(12H, s), 0.87(9H, s), 0.88(9H, s), 2.66(4H, s), 3.66(3H, s), 3.7-4.2(2H, m), 4.45-4.9(2H, m), 5.46(2H, m); MS m/e 672, 670(M-^{t}Bu), 630, 628, 592, 573, 571, 535. Calc. for $C_{33}H_{57}NO_7Si_2\ ^{35}Cl$ 670.3358, Found 670.3322.

Chlorination of PGI_2 diacetate methyl ester (1b) with N-chlorosuccinimide (NCS)——5-Chloro-Δ^6-PGI_1 diacetate methyl ester (3a) and 5-chloro-6-succinimido-PGI_1 diacetate methyl ester (4c)

Chlorination of 1b (125 mg, 0.28 mmol) with NCS (39 mg, 0.29 mmol) was carried out according to the same method described above. The crude product was purified by Florisil column chromatography to isolate two compounds; 3a (78 mg, 58%) (eluting solvent : n-hexane-ethyl acetate, 87.5 : 12.5, 0.1% triethylamine) and 4c (32 mg, 20%) (eluting solvent : ethyl acetate, 0.1% triethylamine); 3a IR (film) 1735, 1660, 1370, 1240, 1060, 1018, 968 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 0.88(3H, t, J=5 Hz), 1.98(3H, s), 2.00(3H, s), 2.95-3.4(1H, m), 3.69(3H, s), 4.39(1H, bt, J=6 Hz), 4.7-5.35(4H, m), 5.53(2H, m); ^{13}C-NMR ($CDCl_3$) δ 33.2(C_2), 22.1 and 22.0 (C_3), 34.3(C_4), 54.3 and 54.5 (C_5), 155.0 and 155.4(C_6), 101.3 and 101.0(C_7), 51.5 (C_8), 84.9 and 84.6(C_9), 38.7(C_{10}), 78.03 and 77.79(C_{11}), 54.9(C_{12}); MS m/e 486, 484(M^+), 448, 426, 424, 388, 366, 364, 329, 328. Calc. for $C_{25}H_{37}O_7{}^{35}Cl$ 484.2226, Found 484.2192. 4c; ^1H-NMR ($CDCl_3$) δ 0.88(3H, t, J=5 Hz), 2.00(6H, s), 2.65(4H, s), 2.65(4H, s), 3.67(3H, s), 4.4-5.3(4H, m), 5.50(2H, m).

Bromination of PGI_2 bis(t-butyldimethylsilyl ether) methyl ester (1a) with N-bromosuccinimide (NBS)——(5R)-5-bromo-Δ^6-PGI_1 bis(t-butyldimethylsilyl ether) methyl ester (2b) and 5-bromo-6-succinimide-PGI_1 bis(t-butyldimethylsilyl ether) methyl ester(4b)

Bromination of 1a (60 mg, 0.10 mmol) with NBS (18.7 mg, 0.105 mmol) was carried out by the similar method described above. The crude product was purified by Florisil column chromatography (n-hexane-ethyl acetate, 99.5 : 0.5, 0.1% triethylamine) to give allylic bromide 2b (38 mg, 57%) as an oil; $[\alpha]_D^{24}$ + 59° (c, 0.32, $CHCl_3$), IR (film) 1742, 1254, 1122, 968, 835, 775 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.04(12H, s), 0.86(9H, s), 0.89(9H, s), 2.92(1H, t, J=8 Hz), 3.68(3H, s), 3.65-3.9(1H, m), 3.9-4.15(1H, m), 4.47(1H, t, J=7 Hz), 4.7-5.0(1H, m), 5.01(1H, d, J=2 Hz), 5.47(2H, m); ^{13}C-NMR ($CDCl_3$) δ 33.2(C_2), 23.3(C_3), 35.5(C_4), 46.1(C_5), 155.3(C_6), 101.6(C_7), 50.6(C_8), 83.1(C_9), 42.6(C_{10}), 76.0(C_{11}), 57.8(C_{12}); MS m/e 674, 672(M^+), 617, 615, 592, 537, 535, 460. Calc. for $C_{33}H_{61}O_5Si_2{}^{79}Br$ 672.3238, Found 672.3181.
Further elution with n-hexane-ethyl acetate (8 : 2) containing 0.1% of triethylamine gave an oily succinimido-adduct 4b (15.5 mg, 20%) IR (film) 1740, 1712, 1258, 975, 838, 778 cm^{-1}; ^{1}H-NMR ($CHCl_3$) δ 0.03(12H, s), 0.84(21H, bs), 2.63(4H, s), 3.65(3H, s), 3.6-4.2(2H, m), 4.3-5.0(2H, m), 5.48(2H, m); MS m/e 716, 714 (M-^{t}Bu), 674, 672, 634, 617, 615, 553, 535. Calc. for $C_{33}H_{57}NO_7Si_2{}^{79}Br$ (M-^{t}Bu) 714.2854, Found 714.2939.

Bromination of PGI$_2$ diacetate methyl ester (1b) with N-bromosuccinimide (NBS)—5-bromo-Δ^6-PGI$_1$ diacetate methyl ester (3b) and 5-bromo-6-succinimido-PGI$_1$ diacetate methyl eser (4d)

Bromination of 1b (372 mg, 0.83 mmol) with NBS (155 mg, 0.87 mmol) was carried out by following the same method described above. The crude product was purified by Florisil column chromatography (n-hexane-ethyl acetate, 85 : 15, 0.1% triethylamine) to give allylic bromide 3b (262 mg, 60%) as an oil; $[\alpha]_D^{24}$ + 39.5° (c, 0.51, $CHCl_3$); IR (film) 1740, 1655, 1372, 1240, 1060, 1018, 968 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.88(3H, bt, J=5 Hz), 1.99(3H, s), 2.00(3H, s), 2.9-3.3(1H, m), 3.67(3H, s), 4.41(1H, bt, J=7 Hz), 4.6-5.3(4H, m), 5.48(2H, m); ^{13}C-NMR ($CDCl_3$) δ 33.1(C_2), 23.2(C_3), 35.4 and 35.6(C_4), 45.5(C_5), 155.8 and 155.4(C_6), 101.0 and 101.2(C_7), 51.6(C_8), 84.5 and 84.7(C_9), 38.8(C_{10}), 77.6 and 77.9(C_{11}), 54.4 and 54.2(C_{12}); MS m/e 530, 528(M^+), 470, 468, 449, 448, 389, 388, 329. Calc. for $C_{25}H_{37}O_7{}^{79}Br$ 528.1721, Found 528.1646.
Further elution with ethyl acetate containing 0.1% of triethylamine gave succinimido-adduct 4d (66 mg, 13%) as an oil; IR (film) 1740, 1710, 1438, 1372, 1330, 1250, 1010, 975, 918, 732 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.87(3H, t, J=5 Hz), 2.00(6H, s), 2.68(4H, s), 3.67(3H, s), 4.4-5.3(4H, m), 5.51(2H, m); MS m/e 530, 528(M-succinimide), 448.

Further chlorination of (5R)-5-chloro-Δ^6-PGI$_1$ bis(t-butyldimethylsilyl ether) methyl ester (2a) with NCS—(5E,7S)- and (5Z,7S)-5,7-dichloro-PGI$_2$ bis(t-butyldimethylsilyl ether) methyl esters (5a) and (6a)

N-Chlorosuccinimide (36 mg, 0.27 mmol) was added at room temperature to a stirred solution of 2a (57 mg, 0.091 mmol) in carbontetrachloride-dichloromethane (0.6 ml each) in the presence of solid potassium carbonate (38 mg). After stirring at room temperature (1.5 hr), the reaction mixture was filtered through celite which was washed with ethyl acetate (15 mL). The filtrate was washed with water (10 mL) and the water layer was extracted with ethyl acetate (10 mL) again. The combined organic layer was washed with brine twice, dried over $MgSO_4$-K_2CO_3, and concentrated in vacuo. The residue (61 mg) was separated by preparative TLC developing with 10% ethyl acetate-n-hexane containing 0.1% of triethylamine to give more polar dichloride 5a (9.8 mg, 17%) and less polar dichloride 6a (11.3 mg, 20%); 5a $[\alpha]_D^{23}$ + 64° (c, 0.49, $CHCl_3$); IR (film) 1750, 1682, 1255, 1122, 1180, 970, 835, 775 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.7-1.0(21H), 3.67(3H, s), 3.85(1H, q, J=8 Hz), 4.05-4.3(1H, br), 4.79(1H, s), 4.8-5.1(1H, br), 5.53(2H, m); ^{13}C-NMR ($CDCl_3$) δ 32.7(C_2), 22.3(C_3), 31.6(C_4), 111.4(C_5), 152.5(C_6), 58.5(C_7), 56.1(C_8), 83.9(C_9), 41.2(C_{10}), 77.7(C_{11}), 52.6(C_{12}); MS m/e 607, 605(M-57), 571, 569. Cacl. for $C_{29}H_{51}O_2Si_2{}^{35}Cl$ (M-57) 605.2651, Found 605.2642. 6a $[\alpha]_D^{23}$ + 79° (c, 0.57, $CHCl_3$); IR (film) 1742, 1682, 1252, 1124, 970, 835, 775 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.7-1.0(21H), 3.67(3H, s), 3.86(1H, q, J=7 Hz), 3.9-4.2(1H, br), 4.69(1H, s), 4.85-5.1(1H, br), 5.53(2H, m); ^{13}C-NMR($CDCl_3$) δ 32.7(C_2), 22.6(C_3), 33.3(C_4), 108.7(C_5), 151.0(C_6), 57.6(C_7), 56.9(C_8), 83.2(C_9), 41.0(C_{10}), 77.8(C_{11}), 52.7(C_{12}); MS m/e 607, 605(M-57), 571, 569. Calc. for $C_{29}H_{51}O_5Si_2{}^{35}Cl_2$ (M-57) 605.2651, Found 605.2622.

Dichlorination of 1a with t-butylhypochlorite—5a and 6a

t-Butylhypochlorite (42 μL, 0.37 mmol) was added at -40°C to a solution of 1a (28 mg, 0.047 mmol) in dry methylene chloride (0.8 mL) containing triethylamine (20 μL). After stirring at -40°C for 2 hr, saturated $NaHCO_3$ solution (10 mL) was poured into the reaction mixture and this was extracted with ether (2 × 12 mL). Combined extracts were washed with brine twice, dried over $MgSO_4$-K_2CO_3, and concentrated in vacuo. The residual oil was separated by preparative TLC developing with 8% ethyl acetate-benzene containing 0.1% of triethylamine to isolate more polar component 5a (5.3 mg, 17%) and less polar componet 6a (1.5 mg, 5%).

(5E,7S)-5,7-Dichloro-PGI$_2$ methyl ester (5b)

A solution of pyridine (0.14 ml) and hydrogen fluoride-pyridine (0.28 mL, Aldrich) in acetonitrile (2 mL) was prepared, and 0.4 mL of this solution was added to 5a (18 mg, 0.027 mmol) at room temperature. After stirring at the same temperature for 1 hr, saturated $NaHCO_3$ solution (10 mL) was added and this was extracted

with ethyl acetate (2 × 12 mL). The combined extracts were washed with brine, dried over $MgSO_4$-K_2CO_3 and concentrated in vacuo. The residual oil was chromatographed on Florisil eluting with n-hexane-ethyl acetate (1 : 1) containing 0.1% of triethylamine to give 5b (9 mg, 80%) as an oil; $[\alpha]_D^{22}$ + 98° (c, 0.11, $CHCl_3$); IR (film) 3380, 1738, 1680, 1438, 1215, 1078, 970, 920, 655 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 3.69(3H, s), 3.93(1H, q, J=8 Hz), 4.13(1H, q, J=6 Hz), 4.82(1H, s), 5.00(1H, m), 5.59(1H, dd, J=8, 16 Hz), 5.67(1H, dd, J=6, 16 Hz); MS m/e 436, 434(M^+), 400, 398, 382, 380, 363: Calc. for $C_{21}H_{35}O_5{}^{35}Cl$ 434.1625, Found 434.1648.

(5Z,7S)-5,7-Dichloro-PGI$_2$ methyl ester (6b)

The silyl-protecting groups of 6a (20 mg, 0.030 mmol) were removed by the same method described above (as in the case of the desilylation of 5a) to give 10 mg (76%) of 6b as an oil; $[\alpha]_D^{22}$ + 107° (c, 0.10, $CHCl_3$); IR (film) 3400, 1738, 1680, 1438, 1215, 1132, 972, 912, 652 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 3.68(3H, s), 3.95(1H, q, J=8 Hz), 4.13(1H, q, J=6 Hz), 4.76(1H, s), 5.05(1H, m), 5.58(1H, dd, J=8, 16 Hz), 5.68(1H, dd, J=6, 16 Hz); MS m/e 436, 434(M^+), 400, 398, 380, 363, 345: Calc. for $C_{21}H_{31}O_5{}^{35}Cl$ (M-H ^{35}Cl) 398.1858, Found 398.1882.

Stepwise transformation of 2a into 7a and 8a via hemiketal 9

Pyridinium p-toluenesulfonate (20 mg) was added to a stirred solution of silyl-protected 5-chloro-Δ^6-PGI$_1$ 2a (55 mg, 0.088 mmol) in 1 mL of moistured benzene. After stirring the mixture at room temperature overnight, saturated sodium bicarbonate solution was added and the mixture was extracted with ethyl acetate twice. The combined extracts were washed with brine, dried over $MgSO_4$-K_2CO_3 and concentrated in vacuo to give 47 mg of crude 9. The thin layer chromatogram of 9 showed two spots due to the epimers at C-6; IR ($CDCl_3$) 3570, 3350, 1730, 1460, 1360, 1122, 1002, 972, 835 cm^{-1}; NMR ($CDCl_3$) δ 0.7-1.0(21H), 3.67(3H, s), 3.7-4.1(3H, m), 4.5-4.8(1H, m), 5.43(2H, m); MS m/e 630, 628 (M-18), 592, 591, 589, 573, 571.

Anhydrous magnesium sulfate (1.2 g) was added to the crude hemiketal 9 (47 mg) in dry benzene (4 mL), and the mixture was stirred with reflux for 3 hr. After removing the magnesium sulfate with filtration, the filtrate was partitioned between ether and saturated sodium bicarbonate solution. The water layer was extacted with ether again, and the combined organic layer was washed with brine twice, dried over $MgSO_4$-K_2CO_3, and concentrated in vacuo. The residue (41 mg) was separated by preparative TLC developing with 8% ethyl acetate-n-hexane containing trace amount of triethylamine giving three components. 8a (15 mg, 28%), 2a (9 mg, 18%), 7a (11 mg, 20%) in the order of polarity. The least polar 7a; $[\alpha]_D^{23}$ + 11° (c, 0.22, $CHCl_3$); IR ($CHCl_3$) 1736, 1696, 1462, 1260, 1122, 978, 838 cm ; H-NMR ($CDCl_3$), δ 0.86(9H, s), 0.89(9H, s), 3.66(3H, s), 3.84(1H, q, J=8 Hz), 4.07(1H, m), 4.62(1H, m), 5.49(2H, m); ^{13}C-NMR ($CDCl_3$) δ 33.6 (C_2), 22.6(C_3), 32.9(C_4), 106.0(C_5), 153.2(C_6), 31.4(C_7), 44.8(C_8), 85.6(C_9), 41.7(C_{10}), 78.0(C_{11}), MS m/e 630, 628(M^+), 573, 571, 535, 439. Calc. for $C_{33}H_{61}O_5Si_2{}^{35}Cl$ 628.3743, Found 628.3734.

The most polar 8a; $[\alpha]_D^{23}$ + 17° (c, 0.24, $CHCl_3$); IR ($CHCl_3$) 1734, 1690, 1460, 1252, 1122, 975, 838 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 0.86(9H, s), 0.89(9H, s), 3.66(3H, s), 3.85(1H, q, J=7.6 Hz), 4.07(1H, m), 4.70(1H, m), 5.48(2H, m); ^{13}C-NMR ($CDCl_3$) 33.4(C_2), 22.6(C_3), 32.7(C_4), 100.9(C_5), 151.2(C_6), 32.6(C_7), 45.5(C_8), 85.1(C_9), 41.6(C_{10}), 78.1(C_{11}), 54.6(C_{12}); MS m/e 630, 628(M^+), 573, 571, 535, 439. Calc. for $C_{33}H_{61}O_5Si_2{}^{35}Cl$ 628.3743, Found 628.3712.

Acid catalyzed isomerization of 2a into 7a and 8a

Trace amount of concentrated sulfuric acid was added by means of a thin glass capillary into a NMR sample tube containing a solution of 2a (20 mg, 0.032 mmol) in chloroform-d (0.3 mL). As soon as the C-7 vinylic proton of the starting material was disappeared in its NMR spectrum, this mixture was poured into aqueous $NaHCO_3$ solution and extracted with methylene chloride twice. The combined extracts were washed with brine, dried over $MgSO_4$-K_2CO_3 and concentrated in vacuo. The residue was separated by preparative TLC developing with n-hexane-ethyl acetate (9 : 1) containing 0.5% of triethylamine giving less polar 7a (2.0 mg, 10%) and more polar 8a (2.0 mg, 10%).

(5E)-5-Chloro-PGI$_2$ methyl ester (7b) and its sodium salt solution (7c)

Tetrabutylammonium fluoride trihydrate (139 mg, 0.44 mmol) was added at room temperature to a stirred mixture of 7a (18 mg, 0.028 mmol) and triethylamine (67 μL) in 2.5 mL of tetrahydrofuran. The reaction mixture was stirred at room temperature for 4 hr and concentrated in vacuo. Saturated $NaHCO_3$ solution (10 mL) was poured into the residue and this was extracted with ethyl acetate (2 × 12 mL). The combined extracts were washed with brine, dried over $MgSO_4$-K_2CO_3 and concentrated in vacuo. Chromatography of the crude product with Florisil eluted with n-hexane-ethyl acetate (3 : 1 ~ 1 : 1) containing 0.1% of triethylamine furnished 10.5 mg of 7b (95%) as an oil; IR ($CHCl_3$) 3420, 1730, 1592, 1438, 1158, 1079, 972, 916 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 2.16(1H, q, J=8 Hz; C_{12}-H), 2.325(2H, t, J=7 Hz; C_2-H_2), 2.37(1H, q, J=8 Hz; C_3-H), 2.38(1H, dt, J=14, 7 Hz; C_4-H), 2.48(1H, dt, J=14, 7 Hz; C_8-H), 2.48(1H, dt, J=14, 7 Hz; $C_{10\alpha}$-H), 2.63(1H, d, J=17 Hz; $C_{7\alpha}$-H), 2.70(1H, dd, J=17, 8 Hz; C_9-H), 3.68(3H, s), 3.90(1H, br; C_{13}-H), 4.10(1H, q, J= 6 Hz, C_{14}-H), 4.66(1H, dt, J=3, 8 Hz; C_9-H), 5.53(1H, dd, J=8, 15 Hz; C_{13}-H), 5.64(1H, dd, J=6, 15 Hz; C_{14}-H); MS m/e 402, 400(M^+), 384, 382, 366, 365, 364, 347: Calc. for

$C_{21}H_{33}O_5{}^{35}Cl$ 400.2014, Found 400.1998.
To the solution of 7b (8.7 mg, 0.022 mmol) in ethanol (1.3 ml) was added 1.3 ml of 0.1 M aqueous sodium hydroxide. The mixture was stirred at room temperature until the starting ester 7b disappeared on TLC (6 h). This solution was used for pharmacological assay as a solution of 7c.

(5Z)-5-Chloro-PGI_2 methyl ester (8b) and its sodium salt solution (8c)

The silyl-protecting groups of 8a (22 mg, 0.035 mmol) were removed by the same method described above (as in the case of the desilylation of 7a) to give 11 mg (80%) of 8b as an oil; IR ($CHCl_3$) 3420, 1730, 1690, 1437,1135, 1082, 975, 908 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.17(1H, q, J=8 Hz; C_{12}-H), 2.27(2H, t, J=7 Hz; C_4-H_2), 2.33(2H, t, J=7 Hz; C_2-H_2) 2.44(1H, q, J=8 Hz; C_8-H), 2.52(1H, d, J=16 Hz, $C_{7\alpha}$-H), 2.53(1H, dt, J=14, 8 Hz, C_{10}-H), 2.67(1H, dd, J=16, 8 Hz; $C_{7\beta}$-H), 3.67(3H, s), 3.91(1H, q, J=8 Hz; C_{11}-H), 4.10(1H, q, J=6 Hz; C_{15}-H), 4.74(1H, dt, J=3, 8 Hz; C_9-H), 5.52(1H, dd, J=8, 16 Hz; C_{13}-H), 5.64(1H, dd, J=6, 16 Hz; C_{14}-H); MS m/e 402, 400(M^+), 384, 382, 366, 365, 364, 347; Calc. for $C_{21}H_{33}O_5{}^{35}Cl$ 400.2014, Found 400.1980.
To the solution of 8b (3.5 mg, 0.009 mmol) in ethanol (0.52 ml) was added 0.52 ml of 0.1 M aqueous sodium hydroxide. The mixture was stirred at room temperature until the starting ester 8b disappeared on TLC (6 h). This solution was used for pharmacological assay as a solution of 8c.

Fluorination of 3b with silver fluoride—(7S)-7-fluoro-PGI_2 diacetate methyl ester (16a) and 5-fluoro-δ^6-PGI_1 diacetate methyl ester (17a)

Silver fluoride (610 mg, 4.8 mmol) was added at room temperature to a stirred solution of 3b (253 mg, 0.48 mmol) in acetonitrile (12 mL) containing potassium carbonate (199 mg, 1.44 mmol) and the mixture was stirred at room temperature for 4 hr. Benzene (12 mL) was added and the mixture was filtered through Celite. After washing the Celite with benzene, combined filtrate was concentrated in vacuo. The resulting residue was purified by Florisil column chromatography to isolate two compounds; 16a (90 mg, 40%) (eluting solvent : n-hexane-ethyl acetate, 9 : 1, 0.1% triethylamine) and 17a (45 mg, 20%) (eluting solvent : n-hexane-ethyl acetate, 87.5 : 12.5, 0.1% triethyl amine). 16a; IR (film) 1740, 1700, 1438, 1370, 1240, 970 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.01(3H, s), 2.05(3H, s), 3.67(3H, s), 4.77(1H, t, J=7 Hz), 4.7-5.1(2H, m), 4.95(1H, d, J=55 Hz), 5.1-5.3(1H, m), 5.55(2H, m); ^{13}C-NMR ($CDCl_3$) δ 33.5(C_2), 24.8(C_3), 24.8(C_4), 104.5(d, J=10.7 Hz; C_5), 153.3(d, J=15.3 Hz; C_6), 94.4(d, J=177.0 Hz: C_7), 52.1(d, J=22.9 Hz: C_8), 83.2(C_9), 37.9(C_{10}), 78.2(C_{11}), 48.3(d, J=6.1 Hz: C_{12}), MS m/e 468 (M^+), 448, 408, 348, 328. Calc. for $C_{23}H_{33}O_5F$ (M-AcOH) 408.2311, Found 408.2344. 17a, IR (film) 1735, 1665, 1365, 1240, 970 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.99(3H, s), 2.04(3H, s), 3.14(1H, m), 4.6-5.3(5H, m), 5.51(2H, m); ^{13}C-NMR ($CDCl_3$) δ 33.5(C_2), 20.3(d, J=3.7 Hz: C_3), 33.0 and 32.8(d, J=24.4 Hz; d, J=24 Hz, respectively: C_4), 87.54 and 87.47 (d, J=168 Hz; d, J=172 Hz, respectively: C_5), 154.5 and 154.4 (d, J=20.8 Hz; d, J=20 Hz, respectively: C_6), 101.2 and 101.7(d, J=6.1 Hz; d, J=7.3 Hz, respectively: C_7), 51.5, 54.5(C_8 or C_{12}), 84.7 and 84.9(C_9), 38.7(C_{10}), 77.9(C_{11}); MS m/e 468(M^+), 448, 408, 348, 328.

Measurement of chemical stability in pH 2.35 buffer solution

A buffer solution was prepared by mixing 0.2 M-KCl (50 mL), 0.2M-HCl (4 mL) and ethanol (29 mL). The pH of this buffer solution was 2.35 at 23°C. Ethanol solutions (25 µL, 2 mg/ml) of chlorinated PGI_2 methyl esters 5b, 6b, 7b and 8b were added to the buffer solution (975 µL) at 23°C. The hydrolysis was monitored at the same temperature by following the peak area change of the compounds on high pressure liquid chromatography (HPLC) charts (Mobile phase, CH_3CN : H_2O = 1 : 1; Wave length, 235 nm for 5b and 6b and 210 nm for 7b and 8b). The half lives of PGI_2 methyl ester was measured in the same condition and was found to be less than one minute.

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