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Chemical Transformation of Prostaglandin-A_2: A Novel Series of C-10 Halogenated, C-12 Hydroxylated Prostaglandin-A_2 Analogues

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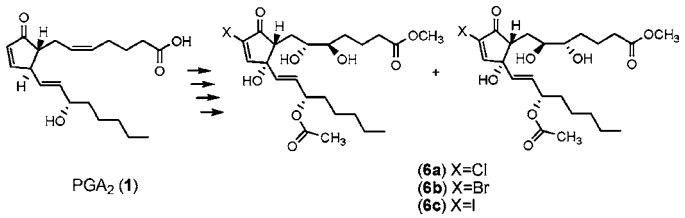
ABSTRACT



Synthesis of a novel class of C-10 halogenated and C-12 oxygenated prostaglandin-A_2 derivatives (6a–6c) has been accomplished. (15*S*)-Prostaglandin-A_2 (1), from the gorgonian *Plexaura homomalla*, served as the starting material for the synthesis. The absolute configuration was determined using NMR.

Δ^{12}-Prostaglandin J_2 (Δ^{12}-PGJ_2), a cross-conjugated enone, is known to inhibit ubiquitin-specific isopeptidase activity causing apoptosis.[1] Mechanistic studies have indicated that inhibition results from Michael addition of an isopeptidase cysteine residue to the endocyclic β-carbon of the cyclopentenone.[2] Punaglandins (PNGs), C-10 chlorinated and C-12 oxygenated prostanoids, are more potent inhibitors of isopeptidase activity than Δ^{12}-PGJ_2 and the PGA series (Figure 1).[3] On the basis of these results, increasing the electrophilicity of the endocyclic β-carbon (C-10) should increase reactivity. To test this hypothesis, a new series of halogenated PGA_2 derivatives were synthesized (**4a**−**4c**) by substituting electron-withdrawing groups (Cl, Br, I) at the α-position of PGA_2 (Scheme 1).

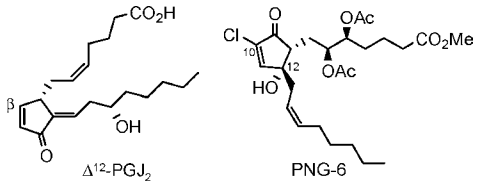


Figure 1. Electrophilic prostanoids.

Preliminary work with these α-halogenated PGA_2 analogues (**4a**−**4c**) indicated that the potency of isopeptidase inhibition for the series is I ≫ Br ≥ Cl.[4] Additionally, iodo-PGA_2 was a more potent inhibitor of ubiquitin-specific isopeptidase activity compared to Δ^{12}-PGJ_2, but less potent than the PNGs. Thus, it was recognized that halogenation and electrophilicity do play a role in the ability of prosta-

(1) (a) Mullally, J. E.; Moos, P. J.; Edes, K.; Fitzpatrick, F. A. *J. Biol. Chem.* **2001**, *276*, 30366. (b) Mullally, J. E.; Fitzpatrick, F. A. *Mol. Pharmacol.* **2002**, *62*, 351.

(2) Masaaki, S.; Mori, M.; Niwa, T.; Hirata, R.; Furuta, K.; Ishikawa, T.; Noyori, R. *J. Am. Chem. Soc.* **1997**, *119*, 2376.

(3) Verbitski, S. M.; Mullally, J. E.; Fitzpatrick, F. A.; Ireland, C. M. *J. Med. Chem.* **2004**, *47*, 2062.

(4) A paper thoroughly describing the pharmacology (outcome of the SAR studies and details of the biological assays data) will be published elsewhere.

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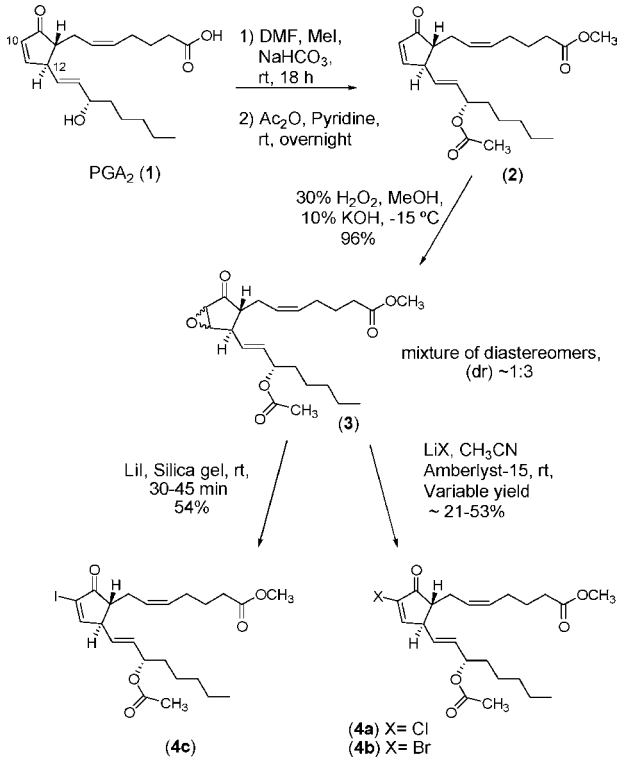
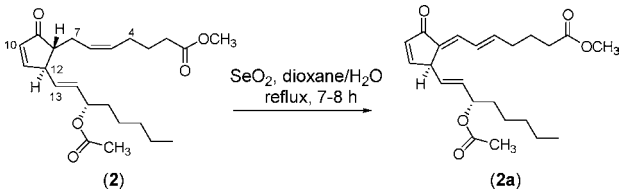
Scheme 1. Synthesis of α-Halogenated PGA_2 Analogues



glandins to inhibit ubiquitin isopeptidase activity but are not the only factors. These results suggested C-12 hydroxylation similar to PNGs may be necessary to achieve optimum activity. To test this hypothesis, a series of C-10 halogenated and C-12 oxygenated PGA_2 derivatives (**6a**−**6c**)[4] have been synthesized (Scheme 3).

Scheme 2. SeO_2 Oxidation of **2**



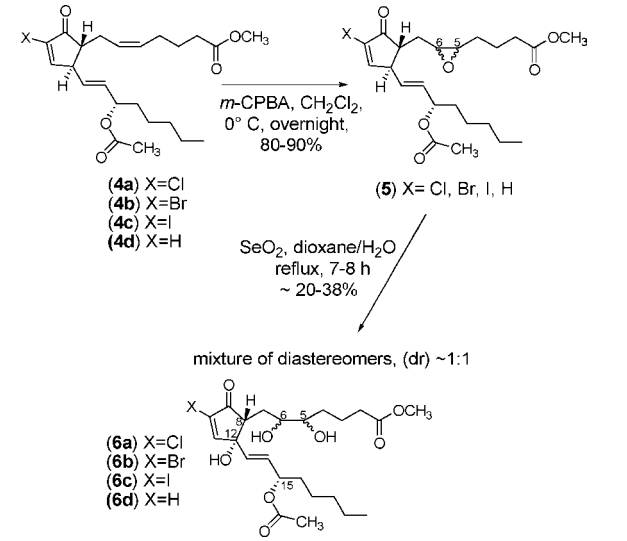
The starting material for the synthesis, (15*S*)-prostaglandin-A_2 (PGA_2), was isolated in abundance (5.7% recovery)[5] from the gorgonian *Plexaura homomalla*.[6] The side-chain functional groups of PGA_2 (the terminal carboxylic acid at C-1 and the alcohol at C-15) were blocked as their methyl ester and acetate, respectively, using standard derivatization conditions (Scheme 1).[7]

(5) See experimental section for details on the isolation of PGA_2 from *P. homomalla*.

(6) Schneider, W. P.; Hamilton, R. D.; Rhuland, L. E. *J. Am. Chem. Soc.* **1972**, *94*, 2122.

(7) Schneider, W. P.; Bundy, G. L.; Lincoln, F. H.; Daniels, E. G.; Pike, J. E. *J. Am. Chem. Soc.* **1977**, *99*, 1222.

Scheme 3. Synthesis of C-12 Oxygenated PGA_2 Derivatives



The 15-*O*-acetyl-PGA_2 methyl ester (**2**) was treated with alkaline hydrogen peroxide at −15 °C to generate a mixture of C(10,11)-α,*β*-epoxides (**3**).[8] The subsequent C-10 halide substitutions[9] were achieved by regioselective ring opening of the C(10,11)-epoxide (**3**) with halide (Cl, Br, I) salts under mildly acidic conditions (Amberlyst 15[10] or silica gel support[11]). Similar transformations of epoxy cyclopentenones into α-haloenones have been shown to occur via the halohydrin intermediates, which are spontaneously dehydrated under acidic conditions to afford the vinylhalide products.[10]

Accordingly, the method of choice for the formation of vinyl chloride (**4a**) was LiCl/Amberlyst/CH_3CN system, where as the vinylbromide (**4b**) was formed by NaBr under otherwise identical conditions.[12] However, attempts to form the vinyl iodide (**4c**) under similar conditions (LiI or NaI/Amberlyst/CH_3CN) were unsatisfactory and resulted in the re-formation of the alkene (**2**). A subsequent literature survey of alternative methods of vinyliodide formation[13] from epoxides revealed that silica gel could be employed as an efficient acid catalyst in the nucleophilic ring opening of epoxides under solvent-free conditions.[11] Accordingly, the solvent-free iodination of epoxide (**3**) was carried out using LiI supported on silica gel. The reaction proceeded smoothly

(8) ^{1}H NMR and HPLC analysis of crude mixture (**3**) showed no evidence of epimerization at C-8 during the epoxidation reaction.

(9) (a) Dominguez, J. N.; Taddei, A.; Cordero, M.; Blanca, I. J. *J. Pharm. Sci.* **1994**, *83*, 472. (b) Iguchi, K.; Kaneta, S.; Tsune, C.; Yamada, Y. *Chem. Pharm. Bull.* **1989**, *37*, 1173.

(10) Righi, G.; Bovicelli, P.; Sperandio, A. *Tetrahedron Lett.* **1999**, *40*, 5889.

(11) Kotsuki, H.; Shimanouchi, T.; Ohshima, R.; Fujieara, S. *Tetrahedron* **1998**, *54*, 2709.

(12) In a few instances during scale-up, the intermediate chloro- and bromohydrins were isolated. These halohydrins can be readily dehydrated to form the vinyhalides, by heating with glacial acetic acid on a steam bath for 2−5 h.

(13) (a) Johnson, C. R.; Adams, J. P.; Braun, M. P.; Senanayake, C. B. W. *Tetrahedron Lett.* **1992**, *33*, 917. (b) Konaklieva, M. I.; Dahl, M. L.; Turos, E. *Tetrahedron Lett.* **1992**, *33*, 7093. (c) Palumbo, G.; Ferreri, C.; Caputo, R. *Tetrahedron Lett.* **1983**, *24*, 1307.

to give 54% of the vinyliodide (**4c**) along with 13% of the alkene byproduct (**2**).

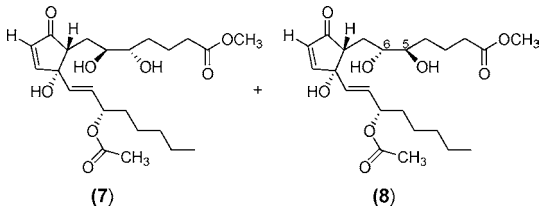
The next task of the synthesis was the conversion of the C-10 halogenated PGA_2 series (**4a**−**4c**) to the corresponding C-12 hydroxylated derivatives (**6a**−**6c**). This novel series of C-12 hydroxylated derivatives was believed to be accessible via allylic oxidation mediated by selenium dioxide[14] because the C-12 position of PGA_2 is bisallylic to C-10 and C-13 double bonds (Scheme 2). However, the likely formation of byproducts due to multiple potential oxidation sites was foreseen as a possible drawback of this method.

To test the feasibility of the allylic oxidation reaction, PGA_2−acetate methyl ester (**2**) was employed. As speculated, compound **2** with SeO_2 gave several oxidation byproducts. One major byproduct was identified as the conjugated diene (**2a**) possibly arising from allylic hydroxylation at C-7 followed by dehydration (Scheme 2). Shorter reaction times, lower reflux temperatures (95% EtOH), or reduced amounts of the oxidant did not facilitate formation of the desired tertiary alcohol product (**6**).

It was now evident that in order to selectively hydroxylate the bisallylic position (C-12) of PGA_2 and suppress byproduct formation during SeO_2 oxidation, the other allylic positions (C-4 and C-7) needed to be removed or masked in some manner. This was achieved by selective epoxidation of the C(5,6) double bond in **4** using *m*-CPBA (Scheme 3). The resulting C(5,6)-α,*β*-epoxide mixture (**5**) was treated as an intermediate and used without further purification for the SeO_2 oxidation. As anticipated, allylic hydroxylation at the C-12 position proceeded smoothly and was accompanied by C(5,6)-epoxide ring opening to yield the novel series of prostaglandin-A_2 analogues (**6a**−**6c**)[15] in moderate yields (Scheme 3).

The relative and absolute configurations of the five stereocenters (C-5/6/8/12/15) in (**6a**−**6d**) were deduced as follows. The absolute stereochemistry of C-8 was assumed to be (*R*), based on literature precedent for coral-derived PGA_2.[16] The assignment of the 15(*S*) configuration of **6** was based on the application of Mosher's method[17] to (*S*)- and (*R*)-MPA derivatives of PGA_2−methyl ester.[18] The *trans* (*R*,*R*) relationship between the two side chains (C-8/C-12) was established based on a strong NOESY cross-peak observed between H-8 and H-13. Subsequent molecular modeling studies with energy-minimized conformations for each of the C-12 epimers supported this observation. The *trans* (8*R*,12*R*) relationship was further corroborated by heteronuclear coupling constant analysis ($^2J_{H8/C12}$ = small)[19] and dihedral angle measurements (−135.7°) for the (8*R*,12*R*) diastereomer of **6**.[20]

The *cis* orientation of the C(5,6) epoxide mixture [(5*S*,6*R*) and (5*R*,6*S*) diastereomers] in **5** was supported by the homonuclear coupling constant value of $^3J_{H5/H6}$ = 4.3 Hz.[21] It was presumed that the C(5,6) diol in **6**, generated during the allylic oxidation (SeO_2/dioxane/H_2O/reflux) of **5**, occurs via nucleophilic opening of the C(5,6) epoxide by water. Accordingly, nonregioselective attack of water on the (5*S*,6*R*) and (5*R*,6*S*) *cis*-epoxides in **5** would generate two secondary alcohol diastereomers, (5*R*,6*R*) and (5*S*,6*S*). This was supported by the ^{1}H NMR spectra of (**6a**−**6d**), which showed doubling of resonances due to the formation of two diastereomers in a ∼1:1 ratio. Accordingly, HPLC analysis of **6d** showed two peaks eluting at 11.5 (**7**) and 13 min (**8**). The two peaks (**7** and **8**) were separated[22] and were employed for absolute stereochemical assignments of the C(5,6) centers by *J*-based analysis,[19] and Mosher's method[17] in conjunction with molecular modeling studies (Figure 2).



(**7**) (**8**)

Figure 2. C(5,6)-Diol diastereomers of **6d**.

Initial attempts focused on utilizing *J*-based analysis[19] to relate the configuration of the vicinal diol segment C(5,6) to that of the known absolute configuration at C-8. However, diastereomer **7** proved undesirable for *J* analysis due to overlap of resonances for H-5 and H-6 (*δ* 3.51−3.57, m, 2H) as well as for H-7_h and H-7_l (*δ* 1.98, m, 2H). Therefore, the viable alternative was to attempt the *J*-based analysis on the diastereomer **8**.

For diastereomer **8**, sufficient chemical shift dispersion of all proton resonances was observed in $CDCl_3$ at 0 °C. While all of the couplings required for *J* analysis were determined successfully (Table 1), the information failed to fit any of the typical rotamers with certainty.[19]

The reason for the occurrence of atypical coupling values in **8** could be explained based on a molecular modeling study, which revealed that the cyclopentenone carbonyl (C-9) is involved in an intramolecular H-bond with C-6(OH) adopting a seven-membered cyclic conformation (Figure 3). The measured *J* values and NOESY data were in accordance with such a seven-membered conformation.

(14) (a) Sharpless, K. B.; Lauer, R. F. *J. Am. Chem. Soc.* **1972**, *94*, 7154. (b) Groszek, G.; Tyrlik-K, A.; Wicha, J. *Tetrahedron* **1989**, *45*, 2223.

(15) NMR data were recorded at 0 °C. Compounds stored at rt tend to decompose slowly over 3−5 days.

(16) (a) Baker, B. J.; Okuda, R. Y.; Yu, P. T. K.; Scheuer, P. J. *J. Am. Chem. Soc.* **1985**, *107*, 2976. (b) Weinheimer, A. J.; Spraggins, R. L. *Tetrahedron Lett.* **1969**, *10*, 5185.

(17) (a) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092. (b) Seco, J. M.; Quiñoà, E.; Riguera, R. *Tetrahedron: Asymmetry* **2001**, *12*, 2915.

(18) An attempt to determine the absolute stereochemistry by a variable temperature ^{1}H NMR experiment with a single MPA derivative, (15*R*)-MPA, in $CDCl_3$ at −1 and 25 °C was not successful: Latypov, S. K.; Seco, J. M.; Quiñoà, E.; Riguera, R. *J. Am. Chem. Soc.* **1998**, *120*, 877.

(19) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibaba, K. *J. Org. Chem.* **1999**, *64*, 866.

(20) Contreras, R. H.; Peralta, J. E. *Prog. NMR Spectrosc.* **2000**, *37*, 321.

(21) Due to the overlap of H-5 and H-6, a combination of ^{1}H NMR, HOMODEC, and 1D TOCSY experiments was employed to extract the coupling constants with certainty.

(22) See experimental section for details of the separation procedure.

Table 1. $^{2,3}J$ Values of **8** for the C-6/C-7 Segment Measured in $CDCl_3$

$^{2,3}J$ values[a]	J (Hz)	classification[b]
^3J (H-6, H-7$_h$)	+6.3	medium
^3J (H-6, H-7$_l$)	+6.3	medium
^3J (H-6, C-8)	+2.2	small
^3J (C-5, H-7$_h$)	+4.4	medium
^3J (C-5, H-7$_l$)	+2.2	small
^2J (C-6, H-7$_h$)	−4.3	medium
^2J (C-6, H-7$_l$)	−1.1	small

[a] H-7$_h$ and H-7$_l$ represent the high- and low-field H-7 protons, respectively. [b] Classification for magnitude of the coupling constants; values between large and small are regarded as medium.[19]

Having failed to unequivocally determine the C(5,6) stereochemistry by *J*-based analysis, Mosher's method was attempted on **8**. Accordingly, reaction of **8** with (*R*)- and (*S*)-MPA acids[23] was expected to yield the corresponding C(5,6)-bis-MPA esters. However, ^{1}H NMR data confirmed that the C-5 MPA derivative was the sole product. Comparison of the chemical shift differences ($\Delta\delta = \delta_R - \delta_S$) in the ^{1}H NMR spectra of the C-5 (*R*)- and (*S*)-MPA derivatives of **8** at 0 °C indicated the absolute configuration was (*R*) (Figure 4).

Thus, the absolute configuration of all five stereocenters (C-5/6/8/12/15) in **8** was established as (5*R*,6*R*,8*R*,12*R*) and

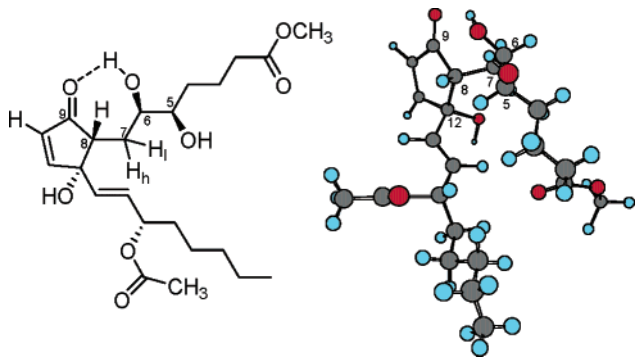


Figure 3. The MM2/AM1-optimized model for **8**.

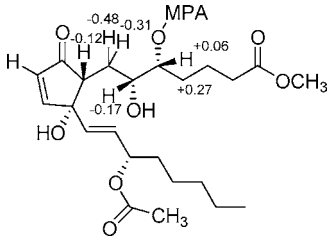


Figure 4. The $\Delta\delta$ values for **8** ($\Delta\delta = \delta_R - \delta_S$, 500 MHz, $CDCl_3$, 0 °C).

(15*S*). Consequently, the absolute configuration of the diol diastereomer **7** was assigned as (5*S*,6*S*,8*R*,12*R*) and (15*S*). The absolute configuration assignments of **7** and **8** were in good agreement with their ^{1}H NMR data, coupling constant analysis, and molecular modeling studies employing MM2 and AM1.[24]

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Note Added after ASAP Publication. There were two errors in Table 1 in the version published ASAP April 15, 2006; the corrected version was published April 21, 2006.

Supporting Information Available: Experimental details and spectroscopic data for synthetic compounds **2**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) 2 equiv of MPA per OH was employed. Reaction was carried out at room temperature for ~16 h, until complete conversion to the product, as indicated by TLC and NMR.

(24) *CS Chem3D Pro*; ChembridgeSoft Corporation: Cambridge, MA.