# Chemical Implications for Antitumor and Antiviral Prostaglandins: Reaction of $\Delta^7$-Prostaglandin $A_1$ and Prostaglandin $A_1$ Methyl Esters with Thiols

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**Abstract:** Prostaglandins (PGs) such as $\Delta^{12}$-$PGJ_2$ and $\Delta^7$-$PGA_1$ methyl ester that possess a cross-conjugated dienone unit exhibit unique antitumor and antiviral activities independent of intracellular cAMP levels. These compounds are transported reversibly into cultured cells and accumulate in nuclei via covalent interaction, eliciting growth inhibition. $PGA_1$ methyl ester, a simple cyclopentenone analog, is less potent. The unique cellular behavior of the dienone PGs correlates well with their chemical properties. The PGs react specifically with thiol nucleophiles such as glutathione. Michael addition of thiols to $\Delta^7$-$PGA_1$ methyl ester, an alkylidenecyclopentenone derivative, occurs facilely at the endocyclic C(11) position rather than at the C(7) position. This process is reversible, and in solution phase, the adducts are in equilibrium with considerable amounts of free PG and thiols. However, the reaction of this PG with Sepharose-bound thiols, regarded to be plausible mimics of protein thiols, is irreversible, and the resulting adducts are dissociated only by alkali treatment. On the other hand, $PGA_1$ methyl ester reacts with soluble or polymer-anchored thiols at lower rates than $\Delta^7$-$PGA_1$ methyl ester, but the resulting thiol adducts are substantially more stable than those of the dienone PGs. This tendency of the $PGA_1$ methyl ester causes its equilibrium to shift to the adduct formation. The reversibility of the Michael reaction of PGs with thiols is consistent with their intracellular behavior and biological activities. Since glutathione adducts of PGs have no antiproliferative activities for cancer cells, the intracellular free PGs are presumed to interact with target molecules to cause cell growth inhibition. The involvement of the ATP-dependent glutathione *S*-conjugate export pump (GS-X pump) in the efflux of PGs is discussed. Thus, the marked difference in potency of the dienone and enone PGs is explained by considering the combined kinetic and thermodynamic properties and the action of the GS-X pump.

Recently, intense attention has been focused on the antineoplastic and antiviral properties of prostaglandins (PGs) and related compounds.[1,2] Certain cyclopentenone prostaglandins are known to be involved in the regulation of the cell cycles[3] and cellular defenses against viral infection.[4] PGs express their activities slowly after dosing,[3] resulting in cell cycle arrest at the G1 phase,[3] and effect inhibition of viral replication at noncytotoxic doses,[4] providing possible new therapeutic strategies.[1,2] Some PGs of the A and J series indeed show potent antitumor effects not only in vitro but also in vivo.[5] Particularly, $\Delta^7$-$PGA_1$ methyl ester (**1**), an unnatural dienone PG easily prepared by the three-component synthesis,[6] is now under preclinical study for the treatment of chemotherapeutically

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resistant ovarian cancer by intraperitoneal administration.[7] Since the activities do not affect intracellular cAMP levels,[3] these PGs differ from the PGs known as local hormones.[1,2] This paper discloses the correlation of the fascinating cellular behavior and chemical properties of antitumor PGs.[8]

## Background

Certain cyclopentenone PGs act on intracellular targets to inhibit the growth of cancer cells. An extensive investigation of the structure−activity relationship[5] indicated that (1) the cross-conjugated dienone system is essential for potent antitumor activity (simple enone PGs such as 2-cyclopentenone or 2-alkylidenecyclopentanone derivatives are less effective); (2) the length of the $\omega$-side chain is also important, the analogs possessing short side chains are less effective; and (3) the absolute configuration of C(12) and C(15) as well as the presence or absence of the hydroxyl group at C(15) do not influenece the activity. Among such unconventional PGs,[9] **1** and $\Delta^{12}$-PGJ$_2$ (**2**),[7c,10] the ultimate active metabolite of PGJ$_2$, are ideal examples. These two PGs display common biological

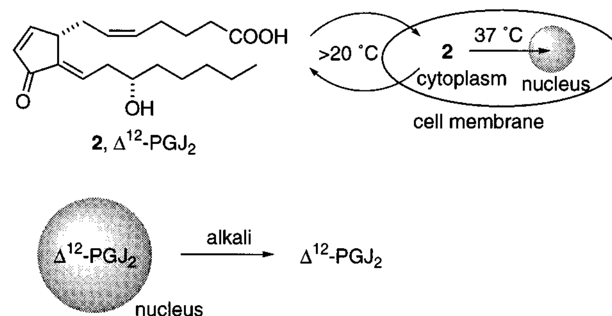
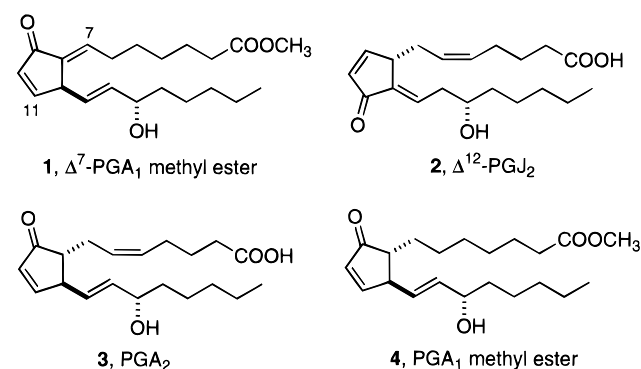



**Figure 1.** Cellular behavior of $\Delta^{12}$-PGJ$_2$.

effects. IC$_{50}$ values of **1** and **2** toward L1210 cells are 0.3 and 0.7 $\mu$g/mL,[5a] respectively, while ID$_{50}$ in inhibiting primary transcription of HSV-2 are 0.35 and 1.8 $\mu$g/mL, respectively.[4c,e] These PGs cause inhibition of *c-myc* gene expression together with the induction of heat-shock proteins and hemoxygenases[11] prior to cell cycle arrest in the G1 phase.




It is also known that these PGs are actively and selectively transported into cultured cells and accumulate in their nuclei.[12] Detailed pharmacokinetic studies on the behavior of the A or J type PGs using L1210 murine leukemia cells revealed the presence of a unique cellular traffic mechanism comprising cellular uptake and nuclear accumulation.[12] The feature is schematically illustrated in Figure 1, where several characteristics are seen: (1) Carrier-mediated incorporation of PG **2** into the cells[13] is very rapid but this process is reversible above 20 °C, establishing an influx−efflux system. (2) There is a considerable medium/cell concentration gradient, where cellular uptake is favored by a factor of 20. (3) The intracellular PG is incorporated into nuclei at 37 °C without metabolism. (4) The PG eventually binds to nuclear proteins. This binding is covalent in nature and irreversible under physiological conditions; the PG does not dissociate with hypotonic washing or by treatment with Triton-X. However, treatment with alkali such as a 0.05 M NaOH solution can break this interaction to liberate the free PGs. The structurally related compounds **1** and **2** show the same characteristics.

No cellular uptake was seen with cyclopentanone PGs, such as PGD$_2$ or PGE$_2$. PGA$_2$ (**3**), a simple enone analog, is transported into the cell and its nuclei in a similar fashion but is more weakly bound to the nuclei by a factor of >3. Recently,

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it was claimed that the efflux phenomenon may be mediated by the ATP-dependent glutathione *S*-conjugate export pump (GS-X pump), probably associated with the multidrug resistance of cancer cells.[14] In any event, such cellular uptake and extrusion mechanisms are closely correlated to growth inhibition caused by the PGs.

## Results

The dienone PGs **1** and **2** act directly in cell nuclei to express their growth inhibitory effects. We have been interested in the chemical implications of such biological properties. Our major concerns were the cellular behavior, the nature of the target molecules, and the origin of the difference in potency between the dienone and enone compounds. Thus, $\Delta^7$-PGA$_1$ methyl ester (**1**) and PGA$_1$ methyl ester (**4**), both readily accessible by the three-component PG synthesis,[6] were selected as representative substrates, and their chemical reactions were studied.

**1. Reaction with Thiols in Solution.** The susceptibility of $\alpha,\beta$-unsaturated ketones[15] to nucleophilic addition prompted us to screen nucleophilic compounds. Thiols are particularly strong nucleophiles and certain unsaturated A- and J-type PGs are, indeed, known to react enzymatically or nonenzymatically with thiols such as gluthathione and cysteine to give the thiol conjugates.[16,17] Therefore, we first examined chemical reactions of the dienone **1** with various thiols under conditions that mimic the physiological system. NMR monitoring revealed that **1** reacts readily with methanethiol, butanethiol, mercaptoethanol, methyl mercaptoacetate, cysteine, and glutathione, giving the conjugate addition products of type **5** (**1**/thiol = 1:0.5−1.0, 2:1 $CD_3OD$−deuterio phosphate buffer, pH 7.4, 20 °C, 5 h). $^1$H NMR signals of the vinylic protons of C(10) and C(11) observed at $\delta$ 6.34 and 7.36, respectively, diminished as the reaction

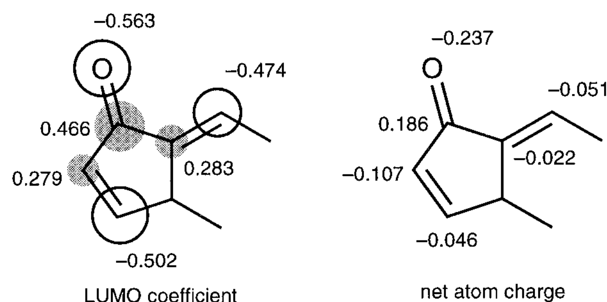
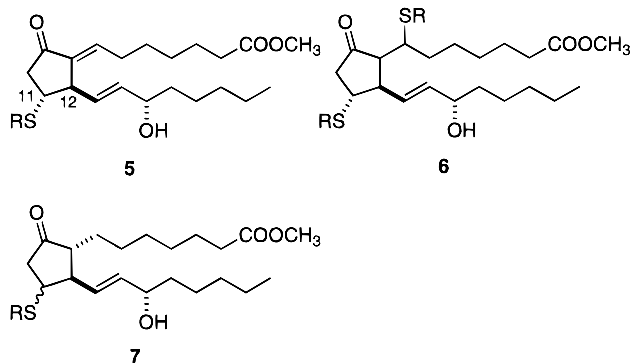



**Figure 2.** LUMO coefficients and the charge distribution of 4-methyl-5-ethylidene-2-cyclopentenone.

proceeded. The signal intensity of the vinylic proton at C(7) was also reduced, but the compensatory new vinylic signal appeared adjacently at $\delta$ 6.75 which was assigned to the C(7) proton of the adduct **5**. These results confirmed that the addition reaction occurs selectively at the C(11) position. NOE measurements in the methanethiol adduct **5a**, which gave a 5% increase of the C(12) and C(13) proton signals ($\delta$ 3.53 and 5.68) upon irradiation at $SCH_3$ ($\delta$ 2.13) and the C(11) proton ($\delta$ 3.17), respectively, indicates that the adduct has the 11,12-*trans* stereochemistry. Other thiol adducts also gave a single set of signals, suggesting stereochemical homogeneity, probably the 11,12-*trans* relationship. The reaction with glutathione, a common endogenous thiol in cells, also occurred at C(11) and afforded a single monoadduct under the given conditions. In the presence of excess thiol, such as methanethiol or glutathione, the monoadduct **5** reacted slowly with thiols at its C(7) position to form the 7,11-bis-thiol adduct of type **6**.[16a]




The MO characteristics of (*E*)-4-methyl-5-ethylidene-2-cyclopentenone, a model for dienone PG **1**, are consistent with the regioselectivity of the thiol addition (Figure 2). The LUMO has a higher coefficient at the 3-position compared to the exocyclic $\beta$-carbon, while a positive charge develops more at the endocyclic $\beta$-position. The difference in the $^{13}$C NMR chemical shift of C(11) and C(7), $\delta$ 160.4 vs 137.0, may reflect such electronic characteristics.[18] This argument is also applicable to the reaction of **2** with thiols, which has the same dienone component as **1** in its upside-down structure.[16a]

The dienone **1**, however, is totally inert to other biomolecules having a phosphate, hydroxyl, carboxylate, or amino function. Inactive compounds examined include nucleotides (CMP, AMP, GMP, UMP, dGMP, and dAMP), carbohydrates (glucose and 2′-deoxyglucose), and amino acids (lysine, histidine, and glutamine).[17g,i]

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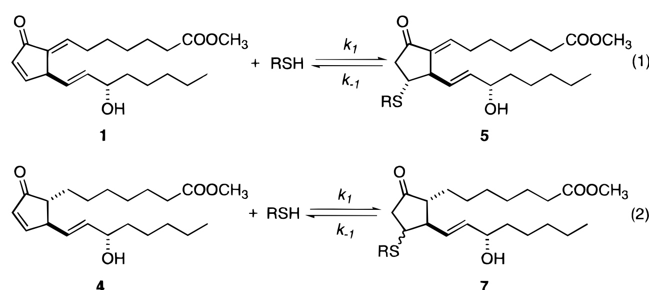
The simple cyclopentenone **4** also reacts with thiols to afford the adducts of type **7** as a mixture of epimers at C(11).[16c,f] For example, **4** and methanethiol formed a 9:1 mixture of the trans and cis isomers. In the NMR spectrum of the trans isomer, a +9% NOE effect was seen with the C(11) proton upon irradiation at the C(13) proton. Reaction with mercaptoethanol affords a 1:1 mixture of the trans and cis adducts. The decrease or absence of stereoselectivity in the formation of **7** is presumably due to the destabilization of the 11α-isomer caused by the 1,3-interaction between the C(8) and C(11) substituents.

The PG−thiol adducts were fully characterized by spectral analyses (see Experimental Section). The authentic monothiol adducts **5** and **7** were prepared by the reaction of equimolar amounts of thiols with **1** or **4** in alkaline phosphate buffer (pH 7.4).

**2. Kinetics and Thermodynamics.** Significantly, the Michael additions depicted in eqs 1 and 2 are reversible under homogeneous conditions such as these, establishing an equilibrium with the free PG and thiol.[17a]




**(a) Rates.** The reaction was conducted at 25 °C in a 2:1 $CH_3OH$/0.1 M phosphate buffer (pH 7.4) under argon atmosphere.[19] The reaction rates of **1** and **4** were found by monitoring the decrease of the UV absorptions at 270 and 220−230 nm, respectively. The second-order rate constant, $k_1$, could be determined when the substrate consumption was as low as 10%.

Notably, addition of thiols to the dienone **1** takes place 6−8 times faster than to the simple enone **4**. With mercaptoethanol, for example, the $k_1$ values for **1** and **4** were determined to be 5.3 and 0.92 $M^{-1}$ $s^{-1}$, respectively. Similarly, reaction with methanethiol gave the rate constant of 4 vs 0.5 $M^{-1}$ $s^{-1}$ for **1** and **4**, respectively. The higher reactivity of **1** correlates well with its lower reduction potential of $E_p = -1.64$ eV, vs $E_p = -1.96$ eV for **4**, obtained by cyclic voltammetry.

The addition of mercaptoethanol to the dienone **1** in a temperature range of 15−30 °C gave $\Delta H^{\ddagger} = 0.13$ kcal mol$^{-1}$ and $\Delta S^{\ddagger} = -24$ eu. In a similar manner, the reaction of the enone **4** proceeded with $\Delta H^{\ddagger} = 0.09$ kcal mol$^{-1}$ and $\Delta S^{\ddagger} = -35$ eu. Thus, the activation energies $\Delta G^{\ddagger}$ at 25 °C for **1** and **4** were calculated to be 7.39 and 10.8 kcal mol$^{-1}$, respectively.

**(b) Equilibria.** The equilibrium constants of the reactions of eqs 1 and 2 were determined by $^1$H NMR measurements of a mixture of a free PG, thiol, and a PG−thiol adduct in a methanol phosphate buffer solution (pH 7.4) in a sealed tube under argon atmosphere. Equilibration was determined by the absence of change of the signal intensities of the PG and its thiol adduct caused by saturation. The concentrations of **1** and the adduct **5** were determined by the comparison of the intensities of their C(7) vinylic protons. The amounts of **4** and **7** were determined by comparing signal intensities of the C(10) or C(11) vinylic protons of **4** and total vinylic protons at C(13) and C(14) of **4** and **7**. The formation of the bis-thiol adduct was negligible. The equilibrium constants, $K$ = [adduct]/[PG][RSH], thus obtained are summarized in Table 1, with the dissociation constants, $K_d = 1/K$, given in parentheses.

(19) Reactions were performed under pseudo-second-order conditions with a large excess of phosphate buffer.

**Table 1.** Equilibrium Constants for the Reaction of PGs with Thiols[a]

| PG | RSH | addudt | equilibrium constant ($M^{-1}$) $10^{-3}K$ ($K_d = 1/K$, mM) |
|---|---|---|---|
| **1** | $CH_3SH$ | **5a** | 1.6 (0.63) |
| | $n$-$C_4H_9SH$ | **5b** | 1.4 (0.71) |
| | $HOCH_2CH_2SH$ | **5c** | 0.3 (3.3) |
| | $CH_3OOCCH_2SH$ | **5d** | 0.5 (2.0) |
| | cysteine | **5e** | 0.24 (4.2) |
| | glutathione | **5f** | 0.35 (2.9) |
| **4** | $CH_3SH$ | **7a** | 10.2 (0.10) |
| | $n$-$C_4H_9SH$ | **7b** | 10.8 (0.09) |
| | $HOCH_2CH_2SH$ | **7c** | 9.3 (0.11) |
| | $CH_3OOCCH_2SH$ | **7d** | 10.0 (0.11) |
| | cysteine | **7e** | 9.3 (0.11) |
| | glutathione | **7f** | 6.1 (0.16) |

[a] The reactions were performed in a 2:1 mixture of $CD_3OD$ and deuterio phosphate buffer (0.1 M, pH 7.4) at 25 °C. The equilibrium constants are the mean values of 3−5 experiments conducted with different initial concentrations of the substrates.

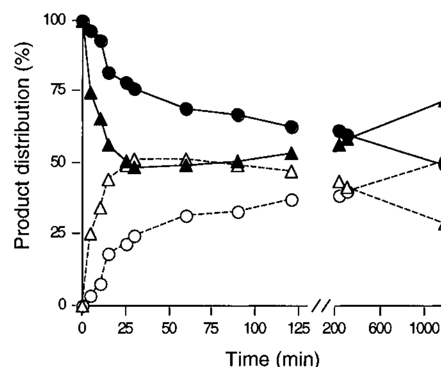



**Figure 3.** Competitive reaction of **1** and **4** with mercaptoethanol. An equimolar mixture of PGs and mercaptoethanol was stirred at 25 °C in a 2:1 mixture of $CD_3OD$ and deuterio phosphate buffer (0.1 M, pH 7.4). The change of the product distribution (▲, **1**; ●, **4**; △, **5c**; ○, **7c**) with the passage of time was monitored by NMR.

Interestingly, the dienone−thiol adducts **5** are substantially more labile than the enone−thiol adducts **7**. The $K_d$ values of the mercaptoethanol adducts **5c** and **7c**, for example, are 3.3 and 0.11 mM, respectively. The same tendency was seen for the glutathione−PG adducts **5f** and **7f**; $K_d$ = 2.9 vs 0.16 mM. For a given thiol, the $K$ value for the dienone **1** appears to always be smaller than that for the enone **4**. The formation of the thiol adducts of **1** and **4** is 3.5−4.4 and 5.2−5.5 kcal mol$^{-1}$ exothermic, respectively.

Figure 3 illustrates the profile of a competitive experiment. When a 1:1:1 mixture of **1**, **4**, and mercaptoethanol in $CD_3OD$ and 0.1 M deuterio phosphate buffer (pH 7.4) was allowed to stand at 25 °C, the product distribution varied as the reaction proceeded. At a very early stage of the reaction, the dienone−thiol adduct **5c** was prevalent in the mixture, while after a prolonged reaction the enone adduct **7c** became dominant. *Thus, the dienone **1** reacts with thiols faster than the enone **4**, but under thermodynamic conditions, the former exists in its free state to a greater extent than does the latter.*

The PG−thiol adducts could generally be isolated by flash chromatography or preparative HPLC on a silica gel column. The PG−glutathione adduct **5f** was isolated by combination of the Sep-Pak and reversed-phase TLC under carefully controlled conditions.[16] The stability of the PG−glutathione conjugate was

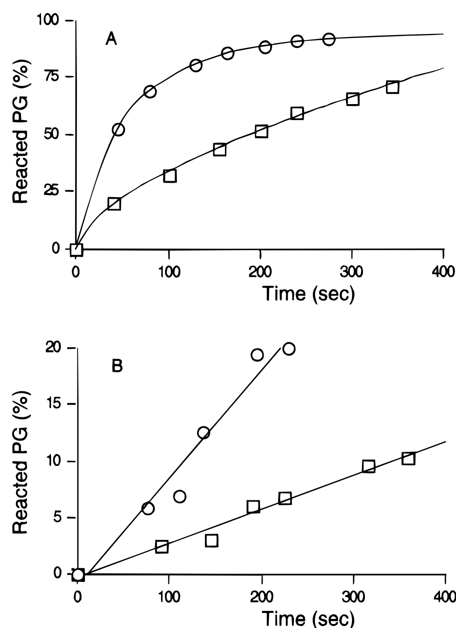
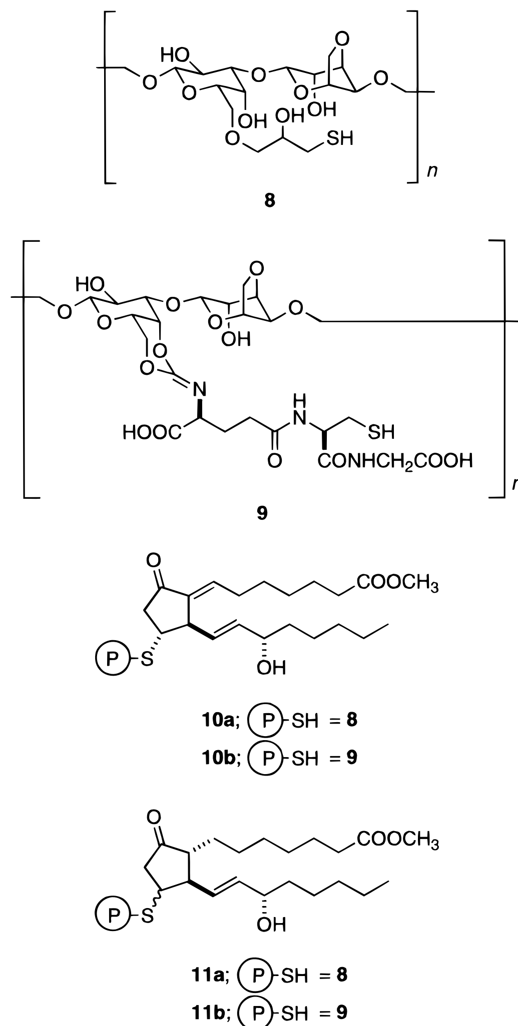



**Figure 4.** Reaction of PGs with polymer-supported thiols in phosphate buffer (pH 7.4) at 25 °C. (A) Reaction with the reduced form of Thiopropyl-Sepharose 6B (**8**) (initial concentrations of the PG and thiol function are 0.89 and 1.78 mM, respectively): ○, $\Delta^7$-PGA$_1$ methyl ester (**1**); □, PGA$_1$ methyl ester (**4**). (B) Reaction with the reduced form of activated Thiol-Sepharose 4B (**9**) (initial concentration of the PG and thiol function are 0.26 and 0.33 mM, respectively): ○, $\Delta^7$-PGA$_1$ methyl ester (**1**); □, PGA$_1$ methyl ester (**4**).




studied from pH 6.0 to 7.4 by dissolving the conjugate (3−6 mM) into a 1:1 mixture of 0.1 M deuterio phosphate buffer/ $CD_3OD$. Consequently, the glutathione adduct **5f** readily liberated a free PG and glutathione at pH 7.4 as described above, and equilibrium was established after approximately 15 min. Although the dissociation rate at pH 7.0 decreased slightly compared to the behavior at pH 7.4, the equilibrium was established after 60 min, giving a 59:41 mixture of the adduct and free PG as judged by $^1$H NMR measurement. At pH 6.0, the adduct **5f** was fairly stable and only 7% of the PG was dissociated after 20 h.

**3. Reaction with Solid Thiols.** Polymer-anchored thiols may mimic biological thiols (proteins) in nuclei.[20] Reduced forms of thiopropyl-Sepharose 6B (**8**) and activated thiol-Sepharose 4B (**9**) were selected as representative models for macromolecular thiols. The gels **8** and **9** contain 20 and 1 $\mu$mol of SH group/mL, respectively.[21] When a mixture of the cyclopentenone PGs and **8** or **9** was stirred in a methanol phosphate buffer mixture at 25 °C, the PGs were smoothly incorporated into the gel. Thus, the reaction of **1** with the solid thiols gave the corresponding adducts of type **10** and PG **4** gave **11**.

The alkylidenecyclopentanone structures of the adducts **10** were verified by FT-IR spectroscopy using a diffuse reflectance measurement technique.[22] Thus, **10a** displayed the carbonyl stretching frequency due to the 9-keto function at 1730 cm$^{-1}$, which is close to the value of **5a** (1725 cm$^{-1}$). In comparison, the starting dienone **1** and its bis-thiol adducts **6a** exhibited ketonic carbonyl absorption at 1702 and 1740 cm$^{-1}$, respectively. This analysis led us to conclude that the dienone binds covalently to the solid thiol as a form of the mono-Michael adduct. The presence of amide moieties in **9** disturbs structural elucidation of **10b** by the FT-IR technique, but it can be presumed that the latter could also have a similar structure.

The rate of the 1,4-addition was examined by measuring the concentration of unreacted PG that remained in aqueous media. The results, given in Figure 4, indicate that, as seen in solution phase reactions, the dienone **1** reacted with the solid thiols approximately 3 times faster than the enone **4**. The rate constants, $k$, of the reaction between **8** and **1** or **4** are 7.7 and 2.7 M$^{-1}$ s$^{-1}$, respectively. In a similar manner, the reaction with **9** gave the $k$ value of 3.7 and 1.2 M$^{-1}$ s$^{-1}$ for **1** and **4**, respectively.

The stability of the resin-anchored products is shown in Figure 5. According to the $K$ values in Table 1, it is expected that free **1** would exist in 92 and 97% in the reactions with mercaptoethanol and glutathione, respectively, under the given concentrations. The same calculation for **4** resulted in 35 and 68%. However, both the dienone and enone adducts **10** and **11** are stable at pH 7.4 (0.1 M phosphate buffer, 20 °C) and do not liberate free PGs, as judged by UV analysis. *They dissociate into free thiols and PGs only under basic conditions at pH > ca. 9.5.*[12] Such behavior is in sharp contrast to the above described properties of the small thiol adducts **5** and **7**, and this is probably due to the decreased molecular motion in the solid state.

## Discussion

The cellular behavior displayed by anticancer PGs can be projected from the above chemical phenomena. Table 2 shows the correlation of the chemical reactions of the PGs **1** and **4** in

(20) For involvement of sulfhydryl components in the binding with the target protein in the nuclei, see: Ohno, K.; Hirata, M. *Biochem. Pharmacol.* **1993**, *46*, 661.
(21) *Affinity Chromatography−Principles and Methods*; Pharmacia Biotech.
(22) Spragg, R. A. *Appl. Spectrosc.* **1984**, *38*, 604.

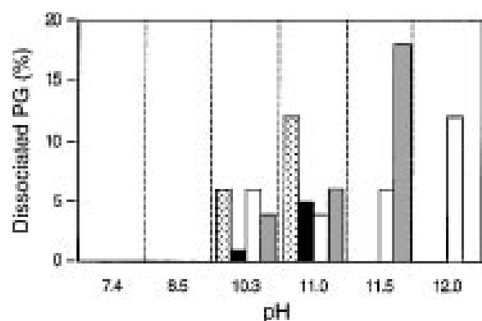



**Figure 5.** Dissociation of PGs from polymer-supported thiol−PG adducts, **10a** (spotted bar), **10b** (white bar), **11a** (solid bar), and **11b** (gray bar), under alkaline conditions. The results of the reaction of **10a** at pH 8.4 were drawn in the column of pH 8.5 for convenience.

a mixture of methanol phosphate buffer at pH 7.4 and the cellular behavior of these and related PGs.

Chemical investigation suggests that the cross-conjugated dienone PGs $\Delta^7$-$PGA_1$ (**1**) and $\Delta^{12}$-$PGJ_2$ (**2**) in cells easily undergo Michael reaction with small, soluble thiols such as glutathione or cysteine in a reversible manner to give an equilibrium mixture.[17a,23,24] Glutathione is the major nonprotein cellular thiol with concentrations of 1−10 mM[14b] and plays important roles in a variety of physiological functions such as oxidative stress, detoxification, inflammation, and drug resistance.[14] Glutathione reacts with a wide range of electrophiles including alkylating anticancer drugs to form glutathione conjugates.[14] In relation to this, there is accumulating evidence that the active export of anticancer drugs as glutathione conjugates from cells via an ATP-dependent export pump (MRP/GS-X pump) is one of the major mechanisms of drug resistance.[14] The fact that the enone PGs can react with glutathione to form PG−glutathione conjugates is important for probing the actions of anticancer PGs in cells. The intracellular state of PGs in L1210 cells were estimated using the equilibrium constants determined by the chemical reactions. When 0.01 mM of dienone PG **1** is incubated at 37 °C to L1210 cells, its intracellular concentration may approach a maximum of 0.2 mM.[12a] Since the cytoplasm of L1210 cells contains about 2.8 mM of glutathione,[12a,25] it is calculated that 52% of **1** exists in a free state and the remaining 48% as the Michael adduct **5f**.[26] The intracellular free PG is thought to bind to target proteins in nuclei (or cytosol) to reveal biological actions leading to the growth inhibition of cancer cells accompanying the suppression of *c-myc* gene expression, the induction of heat-shock proteins and hemoxygenases,[11] and the cell cycle arrest in the G1 phase. The free PGs shift to nuclei directly or by the aid of transport proteins in the cytosol[20] and interact with specific nuclear proteins there. The newly formed high molecular weight thiol adduct of the PG **1**, in which the 11-position is bound covalently to the biomatrix, in turn remains stable. This solid state Michael reaction is barely reversible compared with the solution phase,[27] due to the decrease in the molecular motion leading to the accumulation of PG into the nuclei. The accumulated PG can be dissociated only by alkali treatment. This situation was mimicked by the chemical models forming **10**. The PG is obviously not metabolized during these processes.

The PG−glutathione adducts have no antiproliferative activities for cancer cells and cannot be transported into nuclei. Our recent study has suggested that the glutathione adducts of **1** might be effluxed from the cells through the membrane protein MRP/GS-X pump.[28] This view is consistent with the fact that **5f** and **6f** strongly inhibit the ATP-dependent leukotriene $C_4$ ($LTC_4$) transport through this pump in a competitive manner with $IC_{50}$ values of 0.70 and 0.56 $\mu$M, respectively; the experiments were conducted by incubating plasma membrane vesicles with the glutathione adducts and 10 nM $LTC_4$ in the presence of 1 mM ATP at 37 °C.[28] Thus, the PG−glutathione conjugate is considered to be a good substrate for the MRP/GS-X pump and may be eliminated from the cell,[29] resulting in the reduction of intracellular accumulation of the anticancer PG. Actually, the action of the MRP/GS-X pump was clearly demonstrated as a drug resistance phenomenon of human leukemia-60/R-CP (cisplatin-resistant) cells[30] after being incubated with **1**.[28] The decreasing antiproliferative activity of PGs with increasing cellular glutathione concentration is also consistent with this molecular mechanism.[16b,16l,25a,31]

On the other hand, the simple enone PGs such as **3** or **4** incubated to the cells also react with thiols, but the equilibrium favors the adduct formation. Thus, in cytoplasm, the glutathione adducts of such PGs are expected to be dominant and only 6% of **4** could be free, for example, in L1210 cells. The glutathione conjugate of PGs could be effluxed by the MRP/GS-X pump,[28] and the minor unbound PG could react irreversibly with thiols in nuclei but at a lower rate than **1**. This interpretation is consistent with the cellular behavior of $PGA_2$ using L1210 murine leukemia cells:[20] (1) $PGA_2$ was incorporated into cells over 20 °C and existed as a mixture of the glutathione conjugate form (predominant), the protein-bound form[32] (second major component), and the free form (only small portions) in cytosol; (2) $PGA_2$ was transported into nuclei at 37 °C, but the PG−glutathione conjugate and the free PG were also detected in the medium during such incubation and the PG−glutathione conjugate increased time-dependently in the medium; (3) when the glutathione level was lowered by treating cells with

(23) For the reversible reaction of Navelbine, an antitumor vinblastine analog, with the nucleophilic entity, see: (a) Potier, P. *Pure Appl. Chem.* **1986**, *58*, 737. (b) Potier, P. *Semin. Oncol.* **1989**, *16*, 2. For the reaction of leucodaunomycins, the intermediary compounds in the redox chemistry of an antitumor daunomycin, with cysteine derivatives, see: (c) Bird, D. M.; Gaudiano, G.; Koch, T. H. *J. Am. Chem. Soc.* **1991**, *113*, 308. See also: (d) Ishikawa, T.; Akimaru, K.; Kuo, M. T.; Priebe, W.; Suzuki, M. *J. Natl. Cancer Inst.* **1995**, *87*, 1639.

(24) The dienone PGs should be substantially different from the common alkylating anticancer agents, which react with cellular nucleophiles in an irreversible manner.

(25) (a) Ohno, K.; Hirata, M.; Narumiya, S.; Fukushima, M. *Eicosanoids* **1992**, *5*, 81. See also: (b) Kosower, N. S.; Kosower, E. M. *Int. Rev. Cytol.* **1978**, *54*, 109. (c) Meister, A.; Anderson, M. E. *Annu. Rev. Biochem.* **1983**, *52*, 711.

(26) At this concentration of glutathione, the formation of the bis-glutathione adduct **6f** can be excluded on the basis of the kinetic and thermodynamic data.

(27) This type of stabilization is reminiscent of the "message−address" concept which has been used for designing ligand molecules: (a) Schwyzer, R. *Ann. N.Y. Acad. Sci.* **1977**, *297*, 3. (b) Chavkin, C.; Goldstein, A. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 6543. (c) Sayre, L. M.; Larson, D. L.; Takemori, A. E.; Portoghese, P. S. *J. Med Chem.* **1984**, *27*, 1325. (d) Takayanagi, I.; Konno, F.; Goromaru, N.; Koike, K.; Kanematsu, K.; Fujii, I.; Togame, H. *Arch. Int. Pharmacodyn.* **1988**, *294*, 71. For a review, see: (e) Kanematsu, K. *Kagaku* **1990**, *45*, 385, (in Japanese, Kagakudojin, Kyoto).

(28) (a) Akimaru, K.; Kuo, M. T.; Furuta, K.; Suzuki, M.; Noyori, R.; Ishikawa, T. *Cytotechnology* **1996**, *19*, 221. (b) Akimaru, K.; Nakanishi, M.; Suzuki, M.; Furuta, K.; Noyori, R.; Ishikawa, T. In *Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation, and Radiation Injury*; Honn, K. V., Nigam, S., Jones, R., Marnett, L. J., Wong, P. Y-K., Eds.; Plenum Press: New York, 1996.

(29) ATP-dependent transport of the PG−glutathione conjugate by the MRP/GS-X pump across the plasma membrane was verified by using the inside-out vesicles prepared from cisplatin-resistant HL-60 cells: Ishikawa, T.; Suzuki, M.; Furuta, K.; Noyori, R. Manuscript in preparation

(30) The MRP/GS-X pump is overexpressed in this cell 4−5 times more than in usual HL-60 cells: (a) Ishikawa, T.; Wright, C. D.; Ishizuka, H. *J. Biol. Chem.* **1994**, *269*, 29085. (b) Ishikawa, T.; Bao, J.-J.; Yamane, Y.; Akimaru, K.; Frindrich, K.; Wright, C. D.; Kuo, M. T. *J. Biol. Chem.* **1996**, *271*, 14981.

(31) Koizumi, T.; Negishi, M.; Ichikawa, A. *Biochem. Pharmacol.* **1992**, *44*, 1597.

(32) The transport of $PGA_2$ into nuclei is supposed to be mediated by the PG-binding proteins in cytosol. Two proteins of 100−150 and 25−35 kDa were identified so far.[20]

**Table 2.** Correlation of Chemical Reactions and Cellular Behavior of PGs

| chemical reaction[a] | behavior of PG in cells |
|---|---|
| ⟨reversible reaction with thiols including glutathione⟩ | ⟨transport into nuclei in the presence of excess glutathione and/or other soluble thiols⟩ |
| $K_d(1)/K_d(4) \approx 10{:}1$ | effective transport of free $\Delta^{12}$-PGJ$_2$ into nuclei |
| ⟨higher reactivity of **1** to thiols than that of **4**⟩ | |
| small thiols: $k(1)/k(4) \approx 8{:}1$ | |
| polymer thiols: $k(1)/k(4) \approx 3{:}1$ | faster accumulation of $\Delta^{12}$-PGJ$_2$ into nuclei |
| ⟨formation of stable adducts with polymer supported thiols⟩ | ⟨irreversible accumulation in nuclei through covalent binding with nuclear proteins⟩ |

[a] $K_d(1)$, dissociation constant of the type **5** compounds; $K_d(4)$, dissociation constant of the type **7** compound; $k(1)$, rate constant for the reaction of **1** with thiols; $k(4)$, rate constant for the reaction of **4** with thiols.

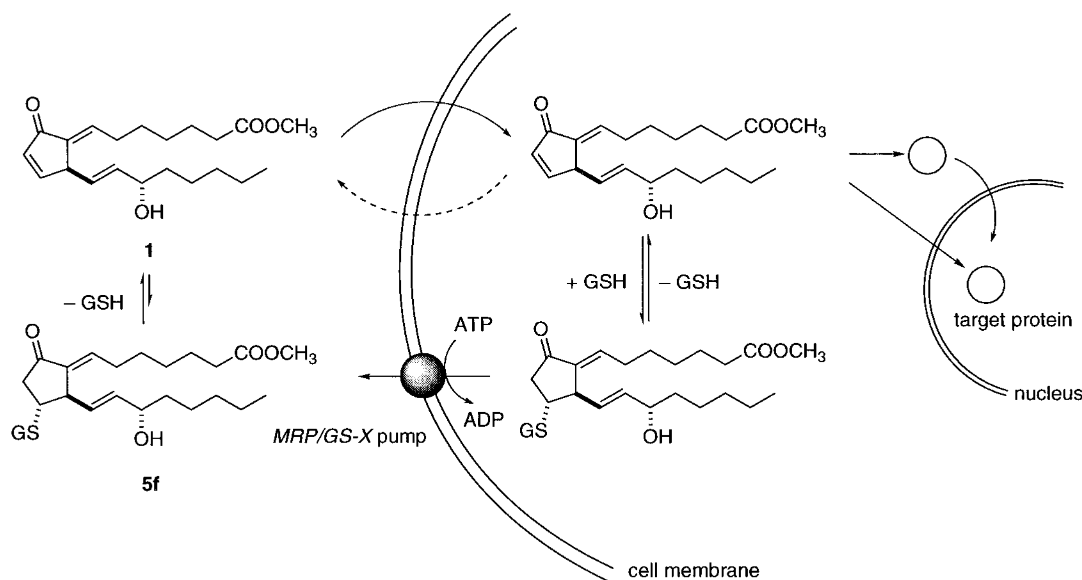



**Figure 6.** Molecular mechanism for cellular behavior of anticancer PGs.

buthionine sulfoximine, the PG−glutathione conjugate in cytosol markedly decreased, while the free PG increased, and the PG−glutathione conjugate in medium decreased. These phenomena can be explained by combining the chemical reaction of **4** with glutathione and the operation of the function of the GS-X pump to extrude the PG−glutathione conjugate.

Thus, the combined thermodynamic/kinetic properties derived from pure chemical reactions and the GS-X pump clearly illustrate the circumstances under which the simple enone PGs demonstrate insufficient potency for tumor cell growth inhibition compared with the cross-conjugated dienone analogs. The mode of action of PGs for viruses may be interpreted in a similar manner.[4] Overall, a tentative molecular mechanism of anticancer PGs influx−efflux phenomena and subsequent transport into nuclei are illustrated in Figure 6. Next, the target proteins of anticancer PGs in the nuclei must be identified, which would reveal the molecular mechanisms involved in the regulation of c-*myc* expression[33] and the induction of specific proteins such as heat-shock proteins and hemoxygenases.[11]

The cellular behaviors of anticancer PGs can be explored using the above chemical implications, which will enable the rational design of effective anticancer drugs. The synthetic anticancer PG, $\Delta^7$-PGA$_1$ methyl ester (**1**), described here has a structure that differs considerably from natural PGs; therefore, the expression of local hormone activity can be avoided which arises when it binds to cell membrane receptors.[33] $\Delta^7$-PGA$_1$ methyl ester (**1**) and its analogs are now under preclinical study for the treatment of chemotherapeutically resistant ovarian cancer as a promising anticancer drug at noncytotoxic doses.[7,34]

## Experimental Section

**Chemicals.** Unless otherwise noted, the reagents were commercial grade. Mercaptoethanol, *n*-butanethiol, and methyl thioglycolate were freshly distilled under argon atmosphere prior to use. Tetrahydrofuran (THF) was freshly distilled from sodium−benzophenone ketyl. Thiopropyl-Sepharose 6B and activated thiol-Sepharose 4B were purchased from Pharmacia Fine Chemicals and were used after the reduction to thiol forms according to the reported procedure.[21] $\Delta^7$-PGA$_1$ methyl ester (**1**) and PGA$_1$ methyl ester (**4**) were synthesized by the three-component coupling process described previously.[6]

**General.** Analytical and preparative thin-layer chromatography (TLC) were performed on precoated silica gel plates (silica gel 60 F$_{254}$, 0.25 mm, Merck 5715) or reversed-phase silica gel plates (RP-18 F$_{254S}$, 0.25 mm, Merck 15685). Visualization of the products, developed as the chromatogram, was performed by UV light, an ethanolic phosphomolybdic acid solution, or an ethanolic solution of *p*-anisaldehyde containing sulfuric acid. Column chromatography was performed on Merck silica gel 60 (No. 9385, 230−400 mesh), Fuji Devison BW-820-MH (70−200 mesh), or Fuji Devison BW-300 (200−400 mesh). Recycle preparative high-performance liquid chromatography (HPLC) was conducted on a GPC column (JAIGEL-2H, 20 mm × 60 cm) or a GS column (JAIGEL-GS310, 20 mm × 50 cm) with a JAI LC-908 instrument. Nuclear magnetic resonance ($^1$H and $^{13}$C) spectra were recorded on a JEOL JNM-A400, FX-90Q, GX-270, or GSX-270 spectrometer. Chemical shifts are reported in parts per million ($\delta$) with tetramethylsilane (in CDCl$_3$) or the deuterium lock signal of the solvent (CDCl$_3$, CD$_3$OD, D$_2$O) as an internal standard. The signal assignment of a glutathione adduct was done on the basis of the H,H-COSY measurement. Mass spectra (MS and HRMS) were measured on a

(33) Elucidation of the molecular mechanism of the cancer cell growth inhibition effect by anticancer PGs, based on the use of **1** as an efficient biochemical probe for HL-60 human leukemia cells and cisplatin resistant HL-60/R-CP cells, has recently been reported. See: Reference 28b. Ishikawa, T.; Akimaru, K.; Nakanishi, M.; Suzuki, M.; Furuta, K.; Noyori, R. Submitted.

(34) The combined use of cisplatin and lipo-$\Delta^7$-PGA$_1$ methyl ester reveals additive antitumor effects on ovarian cancer cells resistant to cisplatin in vivo.[1c]

JEOL TMS-DX 300 (EI) and a SHIMADZU GCMS-QP1000A (EI) instruments. Fast atom bombardment mass spectra (FAB MS) were recorded on a JEOL JMS-DX705L instrument (Xe, 5 keV). Infrared spectra (IR) were taken on a JASCO IRA-1, IR-810, or DR-81 (FT-IR) spectrometer. Ultraviolet (UV) spectra were obtained on a Hitachi 228 model spectrometer. Cyclic voltammetry was performed with a Yanako Polarographic Analyzer P-1100 equipped with the platinum wire counter electrodes, the C-glassy working electrode, and the SCE reference electrode in DMF (0.08 M tetrabutylammonium perchlorate as a supporting electrolyte).

All reactions were performed under a positive pressure of argon with deoxygenated solvents in the dark. Deoxygenation of organic solvents and phosphate buffer was conducted by freeze−pump−thaw cycles using argon as an inert gas.

The MO calculations were performed at Nagoya University Computation Centre with FACOM VP-200 with Monstergauss 81[35] (STO-3G[36]). Geometry of model compounds was decided on the basis of the X-ray date of the compound Shikoccin.[37]

**Screening of Biomolecules Reactive to PGs. Typical procedure.** A mixture of $\Delta^7$-$PGA_1$ methyl ester (**1**) (2.0 mg, 5.74 $\mu$mol) and glutathione (1.76 mg, 5.73 $\mu$mol) placed in an NMR tube was dissolved with $CD_3OD$ (0.4 mL) and deuterio phosphate buffer (0.1 M, pH 7.4, 0.2 mL) under argon atmosphere. The solution was allowed to stand at 20 °C for 5 h and then subjected to the NMR measurement. The formation of adducts was judged by analysis of the NMR spectra (see text). Screening of other biomolecules was conducted by a similar procedure using 0.5−1.0 equiv of a biomolecule for **1** or $PGA_1$ methyl ester (**4**).

**Kinetics and Thermodynamics.** The initial rate of the reactions was measured by monitoring the changes of UV absorbance at 220−230 nm for $PGA_1$ methyl ester and at 270 nm for $\Delta^7$-$PGA_1$ methyl ester, respectively. The reaction of a PG with a thiol was performed in a 2:1 mixture of methanol and phosphate buffer (0.1 M, pH 7.4) under the pseudo-second-order condition ([phosphate buffer] $\gg$ [PG], [RSH]) over a period of 10−15 min at 25 °C with various initial concentrations of 0.1−0.15 mM (PG) and 0.01−0.24 mM (thiol). The rate constant was determined from the slope of the plot of UV absorbance vs time.

The enthalpy and entropy of activation, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, respectively, were calculated by the Eyring equation, $\ln k_1/T = -\Delta H^{\ddagger}/R(1/T) + \Delta S^{\ddagger}/R + \ln k_B/h$, where $R$, $k_B$, and $h$ are the gas constant, the Boltzmann constant, and the Planck constant, respectively.

Decision of the establishment of equilibrium was conducted as follows. In an NMR tube were placed a mixture of PG (3−20 $\mu$mol) and thiol (1−20 $\mu$mol) dissolved in $CD_3OD$ (0.4 mL) and deuterio phosphate buffer (0.1 M, pH 7.4, 0.2 mL) under argon. The course of the reaction was monitored by the NMR measurement of the solution at 25 °C (see text).

The competitive reaction of mercaptoethanol with **1** and **4** was performed using equimolar amounts (2.9 $\mu$mol) of **1**, **4**, and mercaptoethanol dissolved into a mixture of $CD_3OD$ (0.5 mL) and deuterio phosphate buffer (0.1 M, pH 7.4, 0.25 mL) in an NMR tube under argon. The product distribution with the passage of the time was monitored by the $^1$H NMR measurement at 25 °C.

**Synthesis of PG−Thiol Adducts.** The PG−thiol adducts were synthesized and fully characterized by spectral analyses. The authentic thiol adducts **5** and **7** were prepared by the reaction of the corresponding PGs and thiols in THF or methanol in the presence of phosphate buffer (pH 7.4). The glutathione adducts **5f** and **6f** were isolated and purified by combination of the Sep-Pak and reversed-phase TLC technique under carefully controlled conditions.[16a,b]

**Preparation of 10,11-Dihydro-11α-butylthio-Δ$^7$-prostaglandin A$_1$ Methyl Ester (5b).** To a solution of **1** (19.5 mg, 56.0 $\mu$mol) in THF (0.5 mL) and phosphate buffer (0.1 M, pH 7.4, 0.1 mL) was added butanethiol (11.2 $\mu$L, 0.105 mmol) at 15 °C. The solution was stirred for 30 min at the same temperature, and then the reaction was quenched with diluted hydrochloric acid (0.1 M, 1 mL). The product was extracted with ether, and the organic layer was dried over magnesium sulfate. The solvent was removed under reduced pressure, and the residue was subjected to recycle preparative HPLC using chloroform as eluant to give the adduct **5b** (17.9 mg, 73%): TLC $R_f$ = 0.35 (2:1 hexane/ethyl acetate); $^1$H NMR ($CDCl_3$) $\delta$ 0.85−0.94 (m, 6H, $CH_3$), 1.2−1.7 (m, 19H, $CH_2$ and OH), 2.14 (q, $J$ = 7.4 Hz, 2H, C(6)$H_2$), 2.30 (t, $J$ = 7.4 Hz, 2H, C(2)$H_2$), 2.35 (dd, $J$ = 2.5, 18.3 Hz, 1H, C(10)$H_aH_b$), 2.54 (t, $J$ = 7.4 Hz, 2H, $SCH_2$), 2.75 (dd, $J$ = 6.9, 18.3 Hz, 1H, C(10)$H_aH_b$), 3.25 (dt, $J$ = 6.9, 2.5 Hz, 1H, C(11)H), 3.51 (br d, $J$ = 6.4 Hz, 1H, C(12)H), 3.66 (s, 3H, $OCH_3$), 4.09 (m, 1H, C(15)H), 5.51 (dd, $J$ = 5.9, 15.3 Hz, 1H, vinyl), 5.66 (dd, $J$ = 6.4, 15.3 Hz, 1H, vinyl), 6.75 (dt, $J$ = 2.0, 7.4 Hz, 1H, C(7)H); $^{13}$C NMR ($CD_3OD$) $\delta$ 14.0, 14.4, 23.0, 23.7, 25.8, 26.3, 29.1, 29.9, 30.0, 31.6, 32.7, 33.0, 34.6, 38.4, 44.2, 44.6, 49.8, 52.0, 73.0, 130.6, 136.1, 139.1, 141.2, 175.8, 205.9; HRMS $m/z$ calcd for $C_{25}H_{42}O_4S$ 438.2804, found 438.2794 ($M^+$).

Unless otherwise stated, the following monothiol adducts of $\Delta^7$-$PGA_1$ methyl ester were synthesized by a similar procedure to the synthesis of **5b**.

**5a.** Reaction of sodium thiomethoxide and **1** gave **5a** in 33% yield: TLC $R_f$ = 0.44 (1:1 hexane/ethyl acetate); $^1$H NMR ($CDCl_3$) $\delta$ 0.88 (t, $J$ = 6.4 Hz, 3H, $CH_3$), 1.2−1.7 (m, 15H, $CH_2$ and OH), 2.13 (s, 3H, $SCH_3$), 2.15 (q, $J$ = 7.6 Hz, 2H, C(6)$H_2$), 2.30 (t, $J$ = 7.4 Hz, 2H, C(2)$H_2$), 2.38 (dd, $J$ = 3.0, 18.5 Hz, 1H, C(10)$H_aH_b$), 2.75 (dd, $J$ = 7.3, 18.3 Hz, 1H, C(10)$H_aH_b$), 3.17 (dt, $J$ = 7.3, 3.0 Hz, 1H, C(11)H), 3.53 (br d, $J$ = 6.3 Hz, 1H, C(12)H), 3.67 (s, 3H, $OCH_3$), 4.15 (m, 1H, C(15)H), 5.52 (ddd, $J$ = 1.0, 6.6, 15.5 Hz, 1H, vinyl), 5.68 (ddd, $J$ = 0.8, 6.3, 15.3 Hz, 1H, vinyl), 6.75 (dt, $J$ = 2.0, 7.6 Hz, 1H, C(7)H); MS $m/z$ 396 ($M^+$).

**5c.** Reaction of mercaptoethanol and **1** gave **5c** in 56% yield: TLC $R_f$ = 0.22 (1:2 hexane/ethyl acetate); IR ($CHCl_3$) 3300−3700, 3000, 2930, 2850, 1720, 1645 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 0.88 (t, $J$ = 6.4 Hz, 3H, $CH_3$), 1.2−1.7 (m, 14H, $CH_2$), 2.0−2.3 (m, 2H, OH), 2.17 (q, $J$ = 7.4 Hz, 2H, C(6)$H_2$), 2.31 (t, $J$ = 7.4 Hz, 2H, C(2)$H_2$), 2.36 (dd, $J$ = 3.5, 18.3 Hz, 1H, C(10)$H_aH_b$), 2.74 (dt, $J$ = 13.9, 5.9 Hz, 1H, $SCH_aH_b$), 2.78 (dd, $J$ = 6.9, 18.3 Hz, 1H, C(10)$H_aH_b$), 2.83 (dt, $J$ = 13.9, 5.9 Hz, 1H, $SCH_aH_b$), 3.27 (dt, $J$ = 3.5, 6.9 Hz, 1H, C(11)H), 3.52 (m, 1H, C(12)H), 3.67 (s, 3H, $OCH_3$), 3.76 (t, $J$ = 5.9 Hz, 2H, $OCH_2$), 4.10 (q, $J$ = 6.4 Hz, 1H, C(15)H), 5.56 (dd, $J$ = 5.9, 15.3 Hz, 1H, vinyl), 5.66 (dd, $J$ = 6.4, 15.3 Hz, 1H, vinyl), 6.75 (dt, $J$ = 2.0, 7.4 Hz, 1H, C(7)H); $^{13}$C NMR ($CD_3OD$) $\delta$ 14.4, 23.8, 25.8, 26.3, 19.1, 30.0, 30.1, 33.0, 33.3, 34.6, 38.4, 44.3, 44.7, 49.9, 52.0, 62.5, 73.0, 130.6, 136.1, 139.0, 141.3, 175.8, 205.8; HRMS $m/z$ calcd for $C_{23}H_{36}O_4S$ (M − $H_2O$) 408.2334, found 408.2327 ($M^+$ − $H_2O$).

**5d.** Reaction of methyl thioglycolate and **1** gave **5d** in 47% yield: TLC $R_f$ = 0.63 (1:1 chloroform/ethyl acetate); $^1$H NMR ($CDCl_3$) $\delta$ 0.88 (t, $J$ = 6.6 Hz, 3H, $CH_3$), 1.2−1.7 (m, 14H, $CH_2$), 1.83 (br, 1H, OH), 2.15 (q, $J$ = 7.1 Hz, 2H, C(6)$H_2$), 2.30 (t, $J$ = 7.4 Hz, 2H, C(2)$H_2$), 2.36 (dd, $J$ = 3.2, 18.5 Hz, 1H, C(10)$H_aH_b$), 2.78 (dd, $J$ = 7.1, 18.5 Hz, 1H, C(10)$H_aH_b$), 3.25 (d, $J$ = 14.7 Hz, 1H, $SCH_aH_b$), 3.33 (d, $J$ = 14.7 Hz, 1H, $SCH_aH_b$), 3.41 (dt, $J$ = 3.2, 7.1 Hz, 1H, C(11)H), 3.54 (br, 1H, C(12)H), 3.67 (s, 3H, $OCH_3$), 3.76 (s, 3H, $OCH_3$), 4.10 (q, $J$ = 6.4 Hz, 1H, C(15)H), 5.56 (dd, $J$ = 6.3, 15.6 Hz, 1H, vinyl), 5.66 (dd, $J$ = 6.4, 15.6 Hz, 1H, vinyl), 6.76 (dt, $J$ = 1.7, 7.1 Hz, 1H, C(7)H); $^{13}$C NMR ($CD_3OD$) $\delta$ 14.0, 22.6, 24.6, 25.1, 28.0, 28.8, 28.9, 31.7, 32.8, 33.8, 37.2, 42.7, 44.0, 48.1, 51.6, 52.6, 72.2, 129.2, 135.3, 136.7, 140.7, 170.4, 174.1, 202.8; MS $m/z$ 454 ($M^+$).

**Preparation of 10,11-Dihydro-11-(2-hydroxyethylthio)prostaglandin A$_1$ Methyl Ester (7c).** Mercaptoethanol (24.2 mg, 0.31 mmol) was added to a solution of **4** (54.2 mg, 0.155 mmol) in THF (1.5 mL) and phosphate buffer (0.1 M, pH 7.4, 0.1 mL) at 15 °C. The mixture was stirred for 1 h and then diluted hydrochloric acid (0.1 M, 4 mL) was introduced to the solution. The product was extracted with ether, and the organic layer was dried over magnesium sulfate. After removal of the solvent under reduced pressure, the residue was subjected to column chromatography on silica gel (hexane/ethyl acetate, 1:1) to afford two stereoisomers of adduct **7c** (1:1, 57.7 mg, 87%). For **7c** (11,12-*trans*): TLC $R_f$ = 0.21 (1:2 hexane/ethyl acetate); $^1$H NMR ($CDCl_3$) $\delta$ 0.88 (t, $J$ = 6.4 Hz, 3H, $CH_3$), 1.1−1.7 (m, 18H, $CH_2$), 2.0−2.5 (m, 2H, OH), 2.00 (dt, $J$ = 11.1, 5.6 Hz, 1H, C(8)H), 2.18 (dd, $J$ = 11.4, 18.3 Hz, 1H, C(10)$H_aH_b$), 2.29 (t, $J$ = 7.4 Hz, 2H, C(2)$H_2$), 2.39 (ddd, $J$ = 8.4, 11.1, 11.4 Hz, 1H, C(12)H), 2.79 (dd, $J$ = 7.9, 18.3 Hz, 1H, C(10)$H_aH_b$), 2.89 (t, $J$ = 7.4 Hz, 2H, $SCH_2$), 2.99

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(dt, $J$ = 7.9, 11.4 Hz, 1H, C(11)H), 3.66 (s, 3H, $OCH_3$), 3.6−3.8 (m, 2H, $OCH_2$), 4.15 (q, $J$ = 6.9 Hz, 1H, C(15)H), 5.54 (dd, $J$ = 8.4, 15.3 Hz, 1H, vinyl), 5.72 (dd, $J$ = 6.9, 15.3 Hz, 1H, vinyl); $^{13}$C NMR ($CD_3OD$) $\delta$ 14.5, 23.8, 26.0, 26.3, 27.7, 28.5, 30.0, 30.6, 33.0, 34.7, 34.8, 38.4, 45.2, 47.4, 52.0, 54.4, 56.4, 62.7, 72.4, 132.3, 137.9, 175.9, 218.2; MS *m/z* 428 ($M^+$). For **7c** (11,12-*cis*): TLC $R_f$ = 0.14 (1:2 hexane/ethyl acetate); $^1$H NMR ($CDCl_3$) $\delta$ 0.88 (t, $J$ = 6.4 Hz, 3H, $CH_3$), 1.1−1.7 (m, 20H, $CH_2$ and OH), 2.25 (dt, $J$ = 6.9, 10.4 Hz, 1H, C(8)H), 2.29 (t, $J$ = 7.9 Hz, 2H, C(2)$H_2$), 2.52 (ddd, $J$ = 1.0, 3.0, 18.8 Hz, 1H, C(10)$H_aH_b$), 2.64 (dd, $J$ = 6.9, 18.8 Hz, 1H, C(10)$H_aH_b$), 2.7−2.9 (m, 3H, $SCH_2$ and C(12)H), 3.55 (dt, $J$ = 3.0, 6.9 Hz, 1H, C(11)H), 3.66 (s, 3H, $OCH_3$), 3.75 (t, $J$ = 6.4 Hz, 2H, $OCH_2$), 4.15 (q, $J$ = 6.4 Hz, 1H, C(15)H), 5.64 (dd, $J$ = 6.4, 15.3 Hz, 1H, vinyl), 5.81 (dd, $J$ = 8.9, 15.3 Hz, 1H, vinyl); $^{13}$C NMR ($CD_3OD$) $\delta$ 14.4, 23.8, 25.9, 26.3, 27.6, 29.1, 29.9, 30.5, 33.0, 34.8, 35.0, 38.4, 46.7, 47.4, 50.8, 52.0, 52.1, 62.4, 73.5, 131.5, 137.3, 175.9, 219.9; MS *m/z* 428 ($M^+$).

Unless otherwise stated, the following monothiol adducts of $PGA_1$ methyl ester were synthesized by a similar procedure to the synthesis of **7c**.

**7a.** Reaction of sodium thiomethoxide and **4** gave **7a** in 85% yield as a mixture of stereoisomers (9:1, 11,12-*trans* form predominant): TLC $R_f$ = 0.46 (1:1 hexane/ethyl acetate); $^1$H NMR ($CDCl_3$) $\delta$ 0.88 (t, $J$ = 6.4 Hz, 3H, $CH_3$), 1.1−1.7 (m, 19H, $CH_2$ and OH), 2.01 (m, 1H, C(8)H), 2.13 (s, 3H, $SCH_3$), 2.18 (dd, $J$ = 10.5, 18.3 Hz, 1H, C(10)$H_aH_b$), 2.29 (t, $J$ = 7.9 Hz, 2H, C(2)$H_2$), 2.39 (ddd, $J$ = 8.0, 10.5, 11.4 Hz, 1H, C(12)H), 2.79 (ddd, $J$ = 1.0, 8.0, 18.3 Hz, 1H, C(10)$H_aH_b$), 2.94 (dt, $J$ = 8.0, 10.5 Hz, 1H, C(11)H), 3.66 (s, 3H, $OCH_3$), 4.14 (m, 1H, C(15)H), 5.56 (dd, $J$ = 8.0, 15.3 Hz, 1H, vinyl), 5.69 (dd, $J$ = 6.2, 15.3 Hz, vinyl); $^{13}$C NMR ($CD_3OD$) $\delta$ 14.1, 14.4, 23.8, 26.0, 26.3, 27.7, 28.6, 29.9, 30.5, 33.0, 34.8, 38.5, 46.3, 46.5, 52.0, 53.5, 56.5, 73.3, 132.2, 137.8, 175.9, 218.3; MS *m/z* 398 ($M^+$).

**7b.** Reaction of butanethiol and **4** gave two stereoisomers of adduct **7b** in 90% yield (1:1). For **7b** (11,12-*trans*): TLC $R_f$ = 0.52 (2:1 hexane/ethyl acetate); IR ($CHCl_3$) 3300−3600, 3015, 2950, 2925, 2850, 1735 $cm^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 0.88 (t, $J$ = 6.4 Hz, 3H, $CH_3$), 1.2−1.7 (m, 21H, $CH_2$ and OH), 2.00 (br dt, $J$ = 5.4, 10.9 Hz, 1H, C(8)H), 2.18 (dd, $J$ = 11.4, 18.3 Hz, 1H, C(10)$H_aH_b$), 2.29 (t, $J$ = 7.4 Hz, 2H, C(2)$H_2$), 2.37 (dt, $J$ = 8.4, 10.9 Hz, 1H, C(12)H), 2.57 (t, $J$ = 5.4 Hz, 2H, $SCH_2$), 2.81 (br dd, $J$ = 7.9, 18.3 Hz, 1H, C(10)$H_aH_b$), 2.99 (dt, $J$ = 7.9, 10.6 Hz, C(11)H), 3.68 (s, 3H, $OCH_3$), 4.14 (q, $J$ = 6.4 Hz, 1H, C(15)H), 5.54 (dd, $J$ = 7.9, 15.3 Hz, 1H, vinyl), 5.68 (dd, $J$ = 5.9, 15.3 Hz, 1H, vinyl); $^{13}$C NMR ($CD_3OD$) $\delta$ 14.1, 14.5, 23.0, 23.8, 26.0, 26.3, 27.7, 28.5, 30.0, 30.6, 32.1, 33.1, 33.1, 34.8, 38.5, 45.2, 47.4, 52.0, 54.0, 56.4, 73.2, 132.0, 137.7, 175.9, 218.3; HRMS *m/z* calcd for $C_{25}H_{44}O_4S$ 440.2960, found 440.2982 ($M^+$). For **7b** (11,12-*cis*): TLC $R_f$ = 0.44 (2:1 hexane/ethyl acetate); IR ($CHCl_3$) 3300−3600, 3010, 3000, 2950, 2925, 2850, 1740 $cm^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 0.8−1.0 (m, 6H, $CH_3$), 1.2−1.7 (m, 21H, $CH_2$ and OH), 2.2−2.4 (m, 3H, C(8)H and C(2)$H_2$), 2.5−2.7 (m, 4H, C(10)$H_2$ and $SCH_2$), 2.79 (ddd, $J$ = 5.4, 8.4, 10.4 Hz, 1H, C(12)H), 3.51 (dt, $J$ = 3.5, 5.4 Hz, 1H, C(11)H), 3.66 (s, 3H, $OCH_3$), 4.14 (q, $J$ = 6.9 Hz, 1H, C(15)H), 5.60 (dd, $J$ = 6.9, 15.3 Hz, 1H, vinyl), 5.81 (dd, $J$ = 8.4, 15.3 Hz, 1H, vinyl); $^{13}$C NMR ($CD_3OD$) $\delta$ 14.0, 14.4, 23.0, 23.8, 25.9, 26.3, 27.6, 29.1, 29.9, 30.5, 32.6, 32.8, 33.0, 34.8, 38.5, 46.5, 47.3, 50.8, 52.0, 52.0, 73.5, 131.5, 137.1, 175.9, 220.1; MS *m/z* 440 ($M^+$).

**Preparation of 10,11-Dihydro-11α-glutathion-*S*-yl-$\Delta^7$-prostaglandin $A_1$ Methyl Ester (5f).** To a solution of $\Delta^7$-$PGA_1$ methyl ester (18.1 mg, 51.9 $\mu$mol) in methanol (0.5 mL) was added glutathione (14.4 mg, 46.8 $\mu$mol) dissolved in phosphate buffer (0.1 M, pH 7.4, 0.5 mL), and the mixture was stirred at room temperature for 3.5 h in the dark. Then, aqueous acetic acid (1.75 M, 0.8 mL) was added to the solution followed by water (4 mL). The resulting mixture was applied to a C-18 Sep-Pak (Waters Associates, WAT020515), which was preconditioned by rinsing with methanol (2 mL) and water (10 mL). After loading the sample, the Sep-Pak was rinsed with water (10 mL) and then with methanol (3 mL). The methanol eluate was concentrated under reduced pressure and subjected to reversed-phase preparative thin-layer chromatography with methanol/phosphate buffer (0.1 M, pH 7.4) (3:1, v/v) as eluent. The band at a $R_f$ value of 0.49 was scraped, and the adsorbate was washed out with methanol. After the solvent was removed under reduced pressure, the residue was dissolved in methanol (0.5 mL), diluted with water (5 mL), and then applied to a C-18 Sep-Pak cartridge again. The cartridge was washed with water (10 mL), and the desired compound was eluted with methanol (3 mL). Evaporation of the solvent in vacuo afforded the PG−glutathione adduct as a colorless oil (10.1 mg, 33%). Analysis of the compound by FAB MS and NMR confirmed the structure of the monoglutathione adduct of $\Delta^7$-$PGA_1$ methyl ester at the C(11) position. For **5f**: TLC (reversed-phase) $R_f$ = 0.49 (3:1 methanol/phosphate buffer (0.1 M, pH 7.4)); $^1$H NMR ($CD_3OD$) $\delta$ 0.89 (t, $J$ = 6.8 Hz, 3H, $CH_3$), 1.23−1.42 (m, 6H, $CH_2$), 1.4−1.55 (m, 6H, $CH_2$), 1.61 (tt, $J$ = 7.4, 7.7 Hz, 2H, C(3)$CH_2$), 2.02−2.2 (m, 2H, Glu($C_\beta H_2$)), 2.19 (dt, $J$ = 7.6, 7.3 Hz, 2H, C=CHC$H_2$), 2.31 (t, $J$ = 7.4 Hz, 2H, C$H_2$COOCH$_3$), 2.34 (m, 1H, COC$H_aH_b$), 2.53 (m, 2H, Glu($C_\gamma H_2$)), 2.82 (dd, $J$ = 6.5, 19 Hz, 1H, COC$H_aH_b$), 2.86 (dd, $J$ = 8.9, 14.0 Hz, 1H, Cys(SC$H_aH_b$)), 3.07 (dd, $J$ = 5.0, 14.0 Hz, 1H, Cys(SC$H_aH_b$)), 3.41 (br m, 1H, SCH), 3.55 (br m, 1H, C(12)H), 3.64 (s, 3H, $COOCH_3$), 3.65 (m, 1H, Glu($C_\alpha$H)), 3.90 (br s, 2H, Gly($CH_2$COO)), 4.02 (br dt, $J$ = 6.2, 6.8 Hz, 1H, C*H*OH), 4.58 (br dd, $J$ = 5.0, 8.9 Hz, 1H, Cys($C_\alpha$H)), 5.51 (dd, $J$ = 6.8, 15.4 Hz, 1H, CH=C*H*CHOH), 5.70 (dd, $J$ = 6.9, 15.4 Hz, 1H, C*H*=CHCHOH), 6.69 (dt, $J$ = 1.6, 7.6 Hz, 1H, C=CH); $^{13}$C NMR ($CD_3OD$) $\delta$ 14.4, 23.7, 25.8, 26.3, 27.7, 29.1, 30.0, 30.0, 32.9, 33.0, 33.7, 34.7, 38.4, 42.2, 44.2, 44.9, 49.8, 52.0, 54.4, 55.3, 73.1, 130.6, 136.3, 138.9, 141.4, 173.0, 173.1, 173.7, 175.1, 175.8, 205.6; MS (FAB, glycerol matrix) *m/z* 656.3 ($MH^+$).

**Preparation of 10,11-Dihydro-7,11-bis(glutathion-*S*-yl)prostaglandin $A_1$ Methyl Ester (6f).** The bis-glutathione adduct was synthesized in a similar manner to the synthesis of **5f** except that an excess amount of glutathione to $\Delta^7$-$PGA_1$ methyl ester was used. A solution of glutathione (39.8 mg, 130 $\mu$mol) in phosphate buffer (0.1 M, pH 7.4, 0.5 mL) was added to a solution of $\Delta^7$-$PGA_1$ methyl ester (15.0 mg, 43.0 $\mu$mol) in methanol (0.5 mL) at room temperature. After the mixture was stirred for 6 h in the dark, a solution of acetic acid in water (1.75 M, 0.8 mL) and then water (4 mL) was added. The mixture was loaded on a C-18 Sep-Pak column washed in advance with methanol (2 mL) and water (10 mL). The column was rinsed with water (10 mL) and then treated with methanol (3 mL). The methanol eluate was concentrated under reduced pressure, and the semisolid residue was triturated in the presence of methanol (2 mL). The resulting solid material was filtered off, washed with methanol (2 mL), and dried in vacuo to give the desired product as a colorless solid (16.7 mg, 40%). Analysis of the compound by FAB MS and NMR confirmed the structure of the bis-glutathione adduct of $\Delta^7$-$PGA_1$ methyl ester at C(7) and C(11) positions. For **6f**: TLC (reversed-phase) $R_f$ = 0.59 (2:1 methanol/phosphate buffer (0.1 M, pH 7.4)); $^1$H NMR (1:1 $CD_3OD$/$D_2O$) $\delta$ 0.87 (t, $J$ = 6.8 Hz, 3H, $CH_3$), 1.23−1.63 (br m, 16H, $CH_2$), 2.15 (m, 4H, double Glu($C_\beta H_2$)), 2.25 (dd, $J$ = 11.6, 18.6 Hz, 1H, COC$H_aH_b$), 2.33 (t, $J$ = 7.3 Hz, 2H, C$H_2$COOCH$_3$), 2.53 (m, 4H, double Glu($C_\gamma H_2$)), 2.61−2.67 (br, 1H, COCH), 2.68−2.77 (m, 1H, C(12)H), 2.82−3.0 (m, 4H, Cys(SC$H_aH_b$), Cys′($SCH_2$), CO$H_aH_b$), 3.07−3.15 (m, 2H, Cys(SC$H_aH_b$), C(7)H), 3.19 (m, 1H, C(11)H), 3.66 (s, 3H, $COOCH_3$), 3.74 (m, 2H, double Glu($C_\alpha$H)), 3.92 (br, 4H, double Gly($CH_2$COO)), 4.06 (dt, $J$ = 6.2, 6.9 Hz, 1H, C*H*OH), 4.45 (dd, $J$ = 4.7, 8.7 Hz, Cys′($C_\alpha$H)), 4.56 (dd, $J$ = 5.4, 8.5 Hz, Cys($C_\alpha$H)), 5.59 (dd, $J$ = 8.2, 15.2 Hz, C(13)*H*=CH), 5.66 (dd, $J$ = 6.9, 15.2 Hz, CH=C(14)*H*); MS (FAB, glycerol matrix) *m/z* 963.3 ($MH^+$).

**Preparation of 10,11-Dihydro-7,11-bis(methylthio)prostaglandin $A_1$ Methyl Ester (6a).** To a solution of **1** (12.0 mg, 34.4 $\mu$mol) in THF (0.5 mL) was added an aqueous solution of sodium thiomethoxide (1.55 M, 0.2 mL, 0.31 mmol) at 0 °C, and the mixture was stirred for 30 min at the same temperature. Saturated brine (1 mL) was added to the solution, and the product was extracted with benzene. The organic layer was dried over sodium sulfate and evaporated under reduced pressure. The residue was subjected to column chromatography on silica gel using a 4:1 mixture of hexane and ethyl acetate as eluent, giving the bis-thiol adduct **6a** (10.1 mg, 66%) as a mixture of stereoisomers. For **6a**: TLC $R_f$ = 0.47 (1:1 hexane/ethyl acetate); IR (KBr) 3500, 1740, 1650 $cm^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 0.89 (t, $J$ = 6.6 Hz, 3H, $CH_3$), 1.2−1.7 (m, 16H, $CH_2$), 2.07 and 2.075 (each s, 3H, $CH_3$SC(7) of two isomers), 2.13 and 2.14 (each s, 3H, $CH_3$SC(11) of two isomers), 2.30 and 2.33 (each t, $J$ = 7.4 Hz, 2H, C(2)$H_2$ of two isomers), 2.2−3.2 (m, 6H, C(7)H, C(8)H, C(11)H, C(12)H, and C(10)$H_2$), 3.67 (s, 3H, $OCH_3$), 4.15 (m, 1H, C(15)H), 5.53−5.63 (m, 1H, C(13)H), 5.66−5.80 (m, 1H, C(14)H).

**Synthesis of 7-(Methylthio)prostaglandin $A_1$ Methyl Ester (the monothiol adduct of 1 at the C(7) position).** In order to confirm the

first addition of a thiol to the C(11) position of $\Delta^7$-$PGA_1$ methyl ester (**1**), 7(-methylthio)$PGA_1$ methyl ester was prepared independently. Synthesis of the adduct was accomplished in two steps as follows: To a solution of (7*E*)-7,8-didehydro-11,15-*O*-bis(*tert*-butyldimethylsilyl-)prostaglandin $E_1$ methyl ester[6] (38.5 mg, 64.7 $\mu$mol) in THF (0.5 mL) was added aqueous sodium thiomethoxide (1.55 M, 0.23 mL, 0.356 mmol) at 0 °C, and the mixture was stirred for 1.5 h. Then, saturated brine (4 mL) was added, and the product was extracted with ether. The combined ethereal extracts were dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure. The residue was subjected to column chromatography on silica gel using hexane/ethyl acetate (gradient from 30:1 to 20:1) as eluent, giving 7-(methylthio)-11,15-*O*-bis(*tert*-butyldimethylsilyl)prostaglandin $E_1$ methyl ester (13.9 mg, 33%) as a mixture of stereoisomers: TLC $R_f = 0.35$ (10:1 hexane/ethyl acetate); $^1$H NMR ($CDCl_3$) $\delta$ 0.02, 0.03, 0.04, and 0.05 (each s, 12H, $CH_3Si$ of 4 isomers), 0.81−0.89 (m, 21H, $CH_3$ and $C(CH_3)_3$), 1.2−1.7 (m, 16H, $CH_2$), 2.04, 2.05, 2.06, and 2.07 (each s, 3H, $CH_3S$ of 4 isomers), 2.2−2.42 (m, 2H, $C(10)H_2$), 2.29 (t, $J = 7.6$ Hz, 2H, $C(2)H_2$), 2.5−3.1 (m, 3H, C(7)H), C(8)H, and C(12)H), 3.66 (s, 3H, $OCH_3$), 4.0 (m, 1H, C(11)H), 4.1 (m, 1H, C(15)H), 5.50 (m, 1H, C(13)H), 5.62 (m, 1H, C(14)H). The resulting 7-(methylthio)$PGE_1$ derivative (9.0 mg, 14 $\mu$mol) was dissolved in a mixture of $CH_3COOH$/$H_2O$/THF (6:3:1, 1 mL) and then heated at 65 °C for 21 h. Then, the mixture was concentrated under vacuum, and the residue was subjected to column chromatography on silica gel using hexane/ethyl acetate (4:1) as eluent, affording 7-(methylthio)$PGA_1$ methyl ester (1.5 mg, 27%) as a mixture of four stereoisomers at the C(7) and C(8) positions: TLC $R_f = 0.35$, 0.29, 0.23, and 0.17 (1:2 hexane/ethyl acetate); $^1$H NMR ($CDCl_3$) $\delta$ 0.88 (m, 3, $CH_3$), 1.2−1.8 (m, 17, 8 $CH_2$ and OH), 2.045, 2.068, 2.080, and 2.083 (each s, 3, $CH_3S$), 2.3−2.4 (m, 3, C(8)H, and $C(2)H_2$), 3.13 (m, 1, C(7)H)), 3.58 (m, 1, C(12)H), 3.67 (s, 3, $OCH_3$), 4.0−4.16 (m, 1, C(15)H), 5.64 (m, 2, C(13)H and C(14)H), 6.22 (m, 1, C(10)H), 7.53 (m, 1, C(11)H); HPLC $t_R = 14.1$, 17.07, 17.93, and 18.85 min for four stereoisomers, respectively (YMC Pack A002 + A003, 1:3 hexane/ether, detection 254 nm, flow rate = 1 mL/min).

**Influence of pH on the Stability of the PG−Glutathione Conjugate 5f.** The monoglutathione conjugate **5f** (2.0 to 2.5 mg, 3.0 to 3.8 $\mu$mol) was dissolved independently into a 1:1 mixture of $CD_3OD$ (0.3 mL) and pH 6.0, 7.0, 7.4 deuterio phosphate buffers (0.1 M, 0.3 mL each), respectively, in an NMR tube at 0 °C under argon atmosphere. The sample tube was placed in an NMR probe immediately, and $^1$H NMR spectra were measured at 18.7 °C at certain intervals. The dissociation percentage of glutathione was determined by comparison of the signal intensity of the the vinylic protons at C(7) for **5f** and **1**.

**Preparation of Polymer-Supported Thiols.** Sepharose-anchored thiols **8** and **9** were prepared according to the reported methods.[21] Commercially available thiopropyl-Sepharose 6B (200 mg, 16 $\mu$mol) was placed on a glass filter (G-3 type) and washed with phosphate buffer (0.1 M, pH 7.4, 20 mL) with stirring. The resulting resin was treated with carbonate buffer (0.3 M, pH 8.4, 2 mL) containing mercaptoethanol (0.5 M) and EDTA disodium salt (0.1 mM), and the mixture was stirred at 20 °C for 40 min. The resin was filtered and washed with aqueous acetic acid (0.1 M, 80 mL) containing NaCl (0.5 M) and EDTA disodium salt (0.1 mM) and subsequently with phosphate buffer (0.1 M, 60 mL), giving the swelling gel **8** (0.8 mL, 16 $\mu$mol of SH group). The gel **9** was prepared similarly from the commercially available activated thiol-Sepharose 4B.

**Reaction of Polymer-Supported Thiols with PGs. Reaction with 8.** To a mixture of the reduced form of activated thiopropyl-Sepharose 6B (1 mL, 20 $\mu$mol as SH) and phosphate buffer (0.1 M, pH 7.4, 10 mL) was added a PG solution in methanol (57 mM, 176 $\mu$L, 10 $\mu$mol) at 25 °C under argon. At certain intervals (indicated in Figure 4), 1 mL samples were withdrawn and filtered through a glass filter (3G). The filtrate containing unreacted PG was diluted with phosphate buffer (0.1 M, pH 7.4, 2 mL) and then subjected to UV measurement.

**Reaction with 9.** A methanol solution of PG (57 mM, 70 $\mu$L, 4 $\mu$mol) was added to a mixture of the reduced form of activated thiol-Sepharose 4B (5 mL, 5 $\mu$mol as SH) and phosphate buffer (0.1 M, pH 7.4, 10 mL) under argon at 25 °C. Samples (1 mL) were withdrawn at certain time intervals (indicated in Figure 4) and filtered through a glass filter (3G). The filtrate was diluted with phosphate buffer (0.1 M, pH 7.4, 2 mL) and then subjected to UV measurement.

**FT-IR Spectral Measurement.** After the reaction of **8** (20 $\mu$mol) with $\Delta^7$-$PGA_1$ methyl ester (**1**) (4.5 mg, 12.9 $\mu$mol) as above, the mixture was centrifuged at 3000 rpm for 10 min. The precipitate was separated, washed with phosphate buffer (0.1 M, pH 7.4), and then dried *in vacuo* ($10^{-2}$ mmHg) at room temperature for 18 h, giving **10a**. The resulting resin **10a** was subjected to FT-IR measurement using the diffuse reflectance method.[22] For **10a**: IR (KBr) 1730 and 1650 $cm^{-1}$; the starting polymer thiol **8** indicated 1650 $cm^{-1}$ as a broad small band and the difference spectrum between **10a** and **8** indicated 1730 and 1650 $cm^{-1}$. In a similar manner, the FT-IR spectra of **1** and its derivatives, **5a** and **6a**, were measured for the spectral comparison. For **1**: 1740 (ester), 1702 (ketone), 1655 (double bond) $cm^{-1}$. For **5a**: 1735 (ester), 1725 (ketone), 1650 (double bond) $cm^{-1}$. For **6a**: 1740 (ester and ketone), 1650 (small, double bond) $cm^{-1}$.

**Dissociation of PGs from the Polymer-Supported Thiol−PG Adducts.** The resin **8** (1 mL, 20 $\mu$mol as SH) was mixed with PG **1** (10 $\mu$mol) in phosphate buffer (0.1 M, pH 7.4, 2.0 mL) at 20 °C. After 24 h, the mixture was filtered through a glass filter (3G) to afford the PG−polymer adduct **10a**. The reacton of **4** with **8** gave **11a**. The PG−**9** adducts **10b** and **11b** were also prepared in a similar manner by mixing PGs (0.75 $\mu$mol) with **9** (1.5 mL, 1.5 $\mu$mol as SH) in phosphate buffer (0.1 M, pH 7.4, 2.0 mL).

The effect of pH for the dissociation of PGs from the PG−polymer adducts was investigated as follows: A mixture of the PG−polymer adduct (0.03 mL for **10a** and **11a** and 0.2 mL for **10b** and **11b**) obtained above and phosphate buffer (0.1 M, pH 7.4, 2 mL) was stirred at 25 °C for 5 min and then centrifuged at 3000 rpm for 10 min. The supernatant was removed pipette and subjected to UV analysis to determine the concentration of the dissociated PG from the PG−polymer thiol adduct. Studies on other pH conditions (pH 7.4−11.5) were conducted in a similar manner.

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**Supporting Information Available:** Spectra of **5f** ($^1$H and $^{13}$C NMR, FAB MS) and **6f** ($^1$H NMR, FAB MS) (6 pages). See any current masthead page for ordering and Internet access instructions.

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