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# Molecular Design of Glutathione-derived Biochemical Probes targeting the *GS-X* Pump †

**Kyoji Furuta,[a] Keiichiro Tomokiyo,[a] M. Tien Kuo,[b] Toshihisa Ishikawa,[c‡] and Masaaki Suzuki*[a]**

[a] Department of Biomolecular Science, Faculty of Engineering, Gifu University, Yanagido 1-1, Gifu 501-1193, Japan

[b] Department of Molecular Pathology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030, USA

[c] Section of Molecular Therapeutics, Department of Experimental Pediatrics, The University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030, USA

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**Abstract:** The syntheses of novel glutathione derivatives as photoaffinity and fluorescent biochemical probes for the *GS-X* pump are described. GIF-0017, an *S*-alkylated glutathione derivative with a benzophenone photophore, competitively inhibits the transport of leukotriene $C_4$ by the *GS-X* pump with an $IC_{50}$ value of 0.40 μM, indicating the existence of strong interaction between the probe molecule and the *GS-X* pump. 

## INTRODUCTION

The *GS-X* pumps, including MRP1, cMOAT(MRP2), YCF1 and AtMRP, play a physiologically significant role in inflammation, oxidative stress, and xenobiotic metabolism by mediating the export of a variety of organic anions such as glutathione disulfide, glutathione–metal complexes, glutathione *S*-conjugates, and glucuronic and sulfuric acid conjugates from the cells in an ATP-dependent manner.[1] Recently, we have proven that *MRP1/GS-X* pump participates in the drug resistance of cancer cells to antitumor prostaglandins (PGs) (Figure 1), as supported by the following observations: (1) significant resistance of cisplatin-resistant HL-60 (HL-60/R-CP) cancer cells to an antitumor prostaglandin, $\Delta^7$-$PGA_1$ methyl ester (**1**); (2) actual ATP-dependent transport of the PG–glutathione conjugate **2** by *MRP1/GS-X* pump in inside-out vesicles prepared from the resistant cells.[2] Furthermore, we have hypothesized that the resistance of *MRP1*-overexpressing cells to an antitumor agent doxorubicin could also be associated with a similar export mechanism mediated by *MRP1/GS-X* pump.[3] As accumulates evidence for important cellular functions of *GS-X* pumps, great interest is directed toward the elucidation of molecular mechanisms of *GS-X* pump function involved in such biological events. This paper describes the synthesis of novel glutathione derivatives as photoaffinity and fluorescent biochemical probes with high affinity to the *MRP1/GS-X* pump protein.

† Dedicated to Professor Ryoji Noyori on the occasion of his 60th birthday.

‡ Present address: Pfizer Inc. Central Research, Taketoyo 5-2, Aichi 470-2393, Japan

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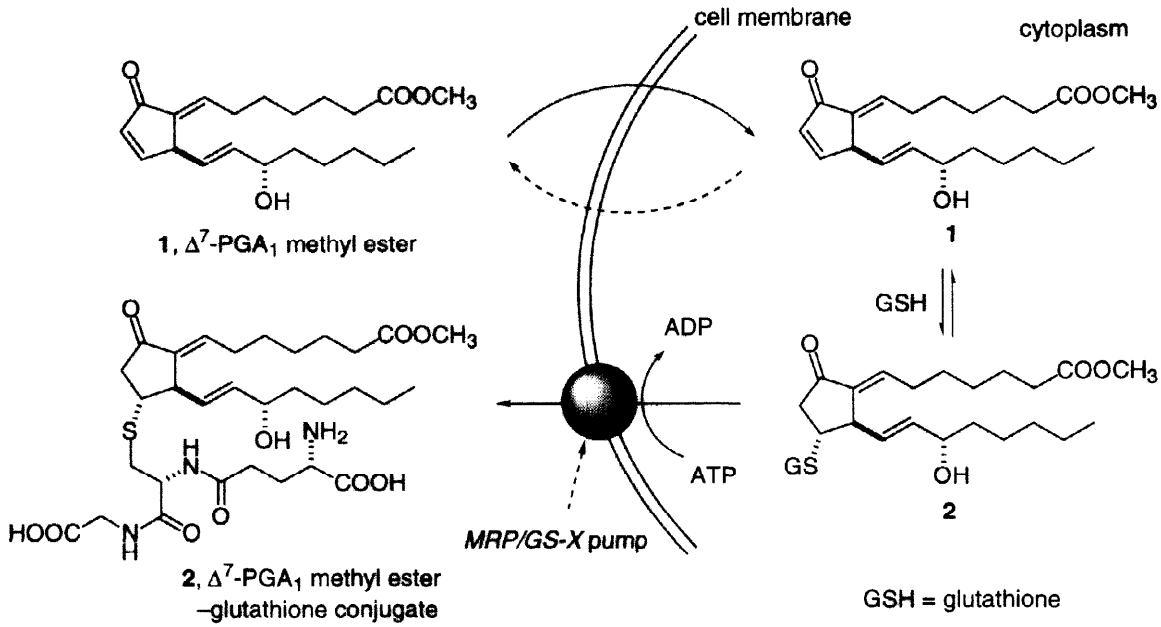
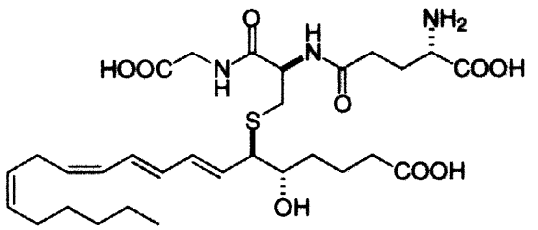



**Figure 1.** Export of a PG–glutathione conjugate through *MRP/GS-X* pump.

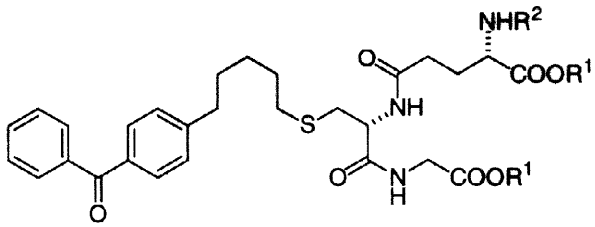
## RESULTS AND DISCUSSIONS

In principle, the biochemical probes require a high binding affinity toward target molecules. The *GS-X* pump recognizes substrates with both glutathione and lipophilic moieties in the molecular structure.[1] Actually, leukotriene $C_4$ ($LTC_4$, **3**) comprising a glutathione residue and a $C_{20}$ fatty acid is an endogeneous substrate for the *GS-X* pump.[4] *S*-(2,4-dinitrophenyl)glutathione and *S*-alkylglutathiones inhibit the transport of $LTC_4$ mediated by the *GS-X* pump in a competitive manner.[4] The affinity of the *GS-X* pump toward substrates well correlates with the length of alkyl chain, whereas the structure of the lipophilic part seems to allow wide tolerance for binding.[4] Based on such information, we have designed three kinds of potential *GS-X* pump-targeting probes, i.e., *S*-alkylated glutathione derivatives, *S*-[5-(4-benzoylphenyl)pentyl]glutathione (**4a**), *S*-[7-(3-azidophenyl)heptyl]glutathione (**5a**), and *S*-[4-(1-pyrenyl)butyl]glutathione (**6a**), referred to GIF-0017, GIF-0068, and GIF-0069, respectively. These compounds commonly carry a glutathione residue and alkyl chain with a photophore at the end. The compounds **4a** and **5a** have been originally designed as probes for the photoaffinity labeling of *GS-X* pump proteins.[5] The benzophenone group has superior characteristics as a photophore, compared with other functional groups, such as azides or diazirines. This is mainly due to its chemical stability, intrinsic ability of photoactivation at 350–360 nm without damaging proteins, and preferential reactivity toward C–H bonds.[6] In addition, the high lipophilicity arising from its bis-aromatic structure is greatly advantageous as a substrate for the *GS-X* pump. The fluorescent compound **6a** was also designed as a non-radioactive tracer for the analysis of *GS-X* pump function. The corresponding esterified derivatives, GIF-0019 (**4b**), GIF-0072 (**5b**), and GIF-0071 (**6b**), are probe molecules with high membrane-permeability. The *GS-X* pump recognizes its substrates at the active site located in the intracellular space and, therefore, the probe molecules to be used for intact cells or *in vivo* system must be so designed as they can be brought into the cell from the extracellular medium.[7] Highly polarized glutathione *S*-conjugates do not meat the criteria. However, ester derivatives with carboxylate and carbamate moieties are lipophilic and neutral in electrical charge.

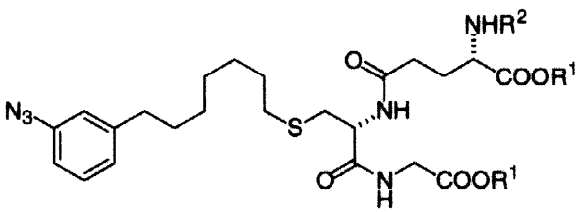
Therefore, they are expected to penetrate the plasma membrane to enter cells. In the cell, these ester derivatives readily undergo hydrolysis catalyzed by esterases to generate the corresponding active probe molecules.[8]



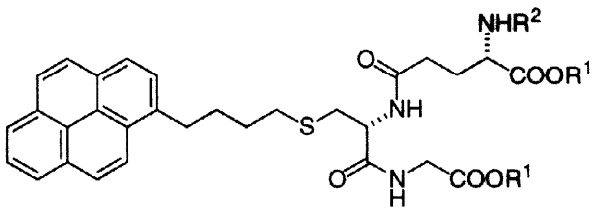
**3**, $LTC_4$



**4a**: $R^1 = R^2 = H$, GIF-0017
**4b**: $R^1 = CH_3$, $R^2 = COOCH_3$, GIF-0019



**5a**: $R^1 = R^2 = H$, GIF-0068
**5b**: $R^1 = CH_3$, $R^2 = COOCH_3$, GIF-0072



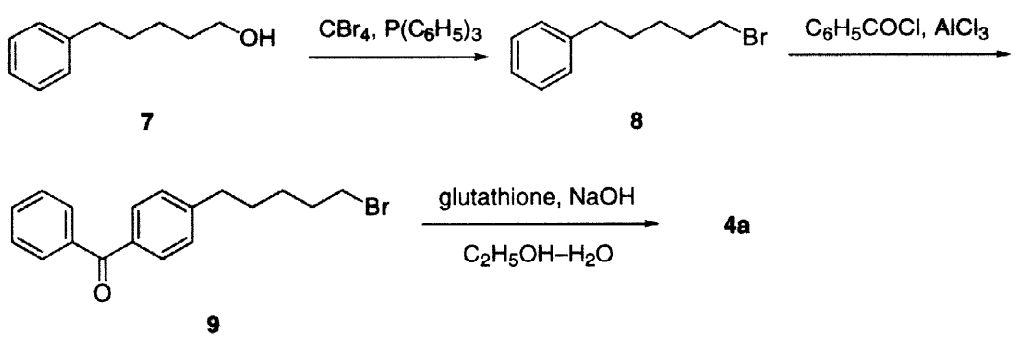
**6a**: $R^1 = R^2 = H$, GIF-0069
**6b**: $R^1 = CH_3$, $R^2 = COOCH_3$, GIF-0071

*Synthesis*

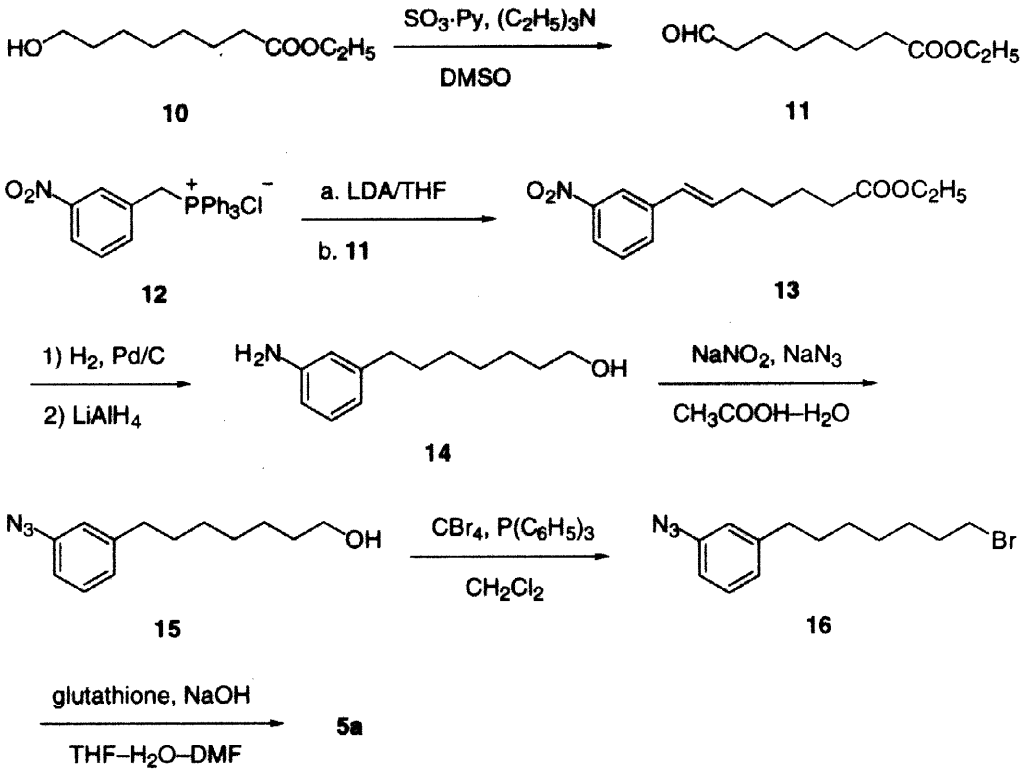
The synthesis of **4a** was accomplished by the sequence shown in scheme 1. Thus the alcohol **7** was first converted to the bromide **8** by treatment with $CBr_4$ and triphenylphosphine. The benzophenone photophore was simply constructed by Friedel-Crafts benzoylation of **8**, and the resulting **9** was reacted with glutathione under basic conditions to afford the desired glutathione conjugate **4a**.

The next target, phenylazide derivative **5a** was synthesized as follows (scheme 2). Wittig olefination of the aldehyde **11** derived from **10** with the phosphorane generated from the phosphonium salt **12** gave the nitro compound **13** having a long alkyl chain ester moiety. Hydrogenation and subsequent $LiAlH_4$ reduction of **13** afforded the aniline derivative **14**. The amino group of **14** was readily converted to the photosensitive azide function by Sandmeyer reaction. Conversion of the azidophenyl alcohol **15** to the glutathione conjugate **5a** was conducted via the bromide **16** in a similar manner to the preparation of **4a** described above.

**Scheme 1**

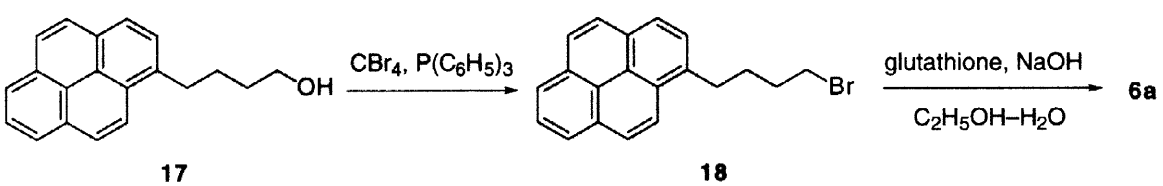


**Scheme 2**




The fluorescent probe **6a** was similarly prepared by the coupling of the bromide **18** derived from commercially available **17** with glutathione in the presence of aqueous base (scheme 3). The esterified derivatives, **4b–6b**, were synthesized by the treatment of the corresponding glutathione conjugates with trimethylsilyldiazomethane in benzene–methanol followed by the reaction with methyl chloroformate.

**Scheme 3**




*Biological Properties*

Our preliminary biological experiments using inside-out vesicles prepared from HL-60/R-CP cells[9] showed that compounds **4a** and **5a** competitively inhibited the ATP-dependent transport of $LTC_4$ via the *GS-X* pump in a dose-dependent manner with an $IC_{50}$ value of 0.40 and 3.1 μM, respectively (Figure 2A). Thus, it is assumed that these compounds and $LTC_4$ share the same binding site of the *GS-X* pump protein. On the other hand, the pyrene derivative **6a** was unexpectedly less effective in inhibition of [$^3$H]$LTC_4$ uptake by the *GS-X* pump ($IC_{50}$ > 10 μM). The reason for the lower inhibitory activity of **6a** is not clear at present, however the molecular size of **6a** may not match with the substrate binding site of the *GS-X* pump. Non-specific binding to membrane lipids due to the high hydrophobicity of the pyrene ring of **6a** may also be a possible reason.[10] The

ATP-dependent transport of glutathione–$\Delta^7$-$PGA_1$ methyl ester conjugate (**2**)[2] by the *GS-X* pump was also competitively inhibited by **4a** ($IC_{50}$ = 0.52 μM).[11] This result inspired a hint of the possibility that the cellular resistance of HL-60/R-CP cells to antitumor $\Delta^7$-$PGA_1$ methyl ester (**1**) could be attenuated by the coexistence of **4a**.[12] In fact, when the HL-60/R-CP cells were incubated with the PG **1** (5 μM) in the presence of **4b** (10 μM), a cell membrane-permeable derivative of **4a**, the cell proliferation was suppressed over 96 h (Figure 2B).[11] Thus, it is strongly suggested that **4b** permeated into the cell and subsequently hydrolyzed by esterase to form **4a** which competitively inhibits the extrusion of the GS-PG conjugate **2** from the cells through the *GS-X* pump (Figure 3). The inhibition of the *GS-X* pump by **4b** in cultured cells was verified by recently developed GS–bimane assay,[9b] where the *GS-X* pump activity was evaluated by measuring the relative fluorescence intensity of cells in a flow cytometer. In this assay, the efflux of the fluorescent GS-bimane conjugate **19** from cells mediated by the *GS-X* pump is characterized as a function of the decrease in the fluorescence intensity of the GS–bimane conjugate remaining in cells. Figure 4 depicts the time course of the fluorescence intensity of the GS–bimane conjugate pre-loaded cells during incubation with or without **4b**. In the absence of **4b**, the fluorescence intensity in HL-60/R-CP cells decreased along the incubation period. On the other hand, in the presence of 10 μM **4b**, the fluorescence intensity of cells decreased very slowly during the same incubation period, suggesting that most part of the GS–bimane conjugate remained in cells due to inhibition of the *GS-X* pump. Since **4b** itself does not show any inhibitory activity for the transport of $LTC_4$ via the *GS-X* pump in isolated membrane vesicles, these results support our idea that **4b** inside the cell readily undergoes hydrolysis catalyzed by esterase and the resulting **4a** blocks the *GS-X* pump (Figure 3). In the cell, GSH and $\Delta^7$-$PGA_1$ establish a thermodynamic equilibrium with the GS–PG conjugate[2] and the free PG can be transported into nuclei to induce p21, a cyclin-dependent kinase inhibitor, to induce cell cycle arrest in the G1 phase.[11,13,14] **4b** dose-dependently manifested its effect on the G1 arrest caused by the antitumor PG. Application of **4a** and **5a** to photoaffinity labeling of the *GS-X* pump and its family, as well as *in vivo* study on anticancer effect of PGs using the *GS-X* pump inhibitor **4b**, are in progress.

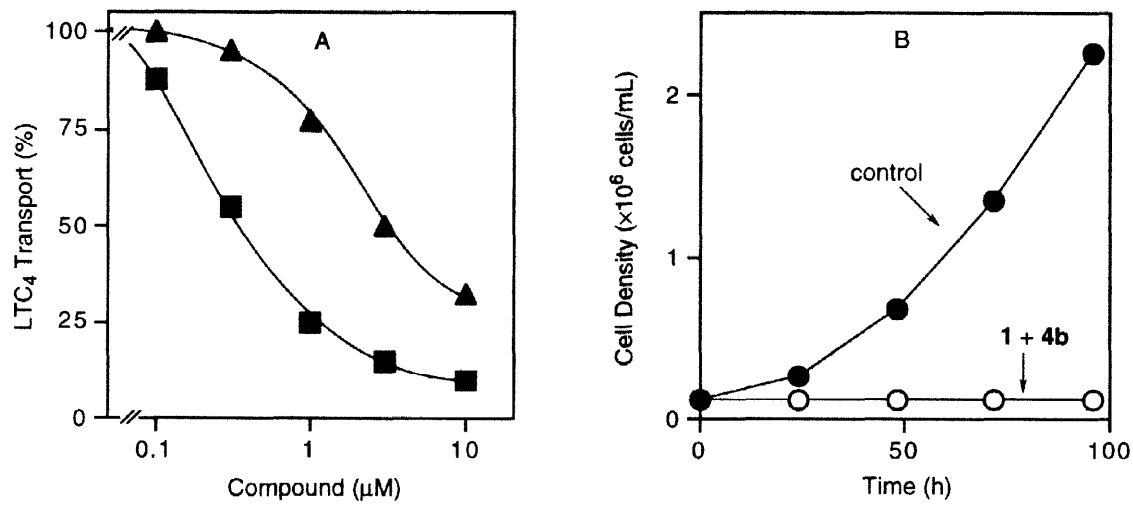



**Figure 2.** (A) Inhibition of ATP-dependent transport of $^3$H-labeled $LTC_4$ by GIF-0017 (**4a**) (■) and GIF-0068 (**5a**) (▲) in plasma membrane vesicles prepared from HL-60/R-CP cells. (B) The increased antiproliferative effect of $\Delta^7$-$PGA_1$ methyl ester (**1**) (5 μM) on HL-60/R-CP cells in the presence of GIF-0019 (**4b**) (10 μM).

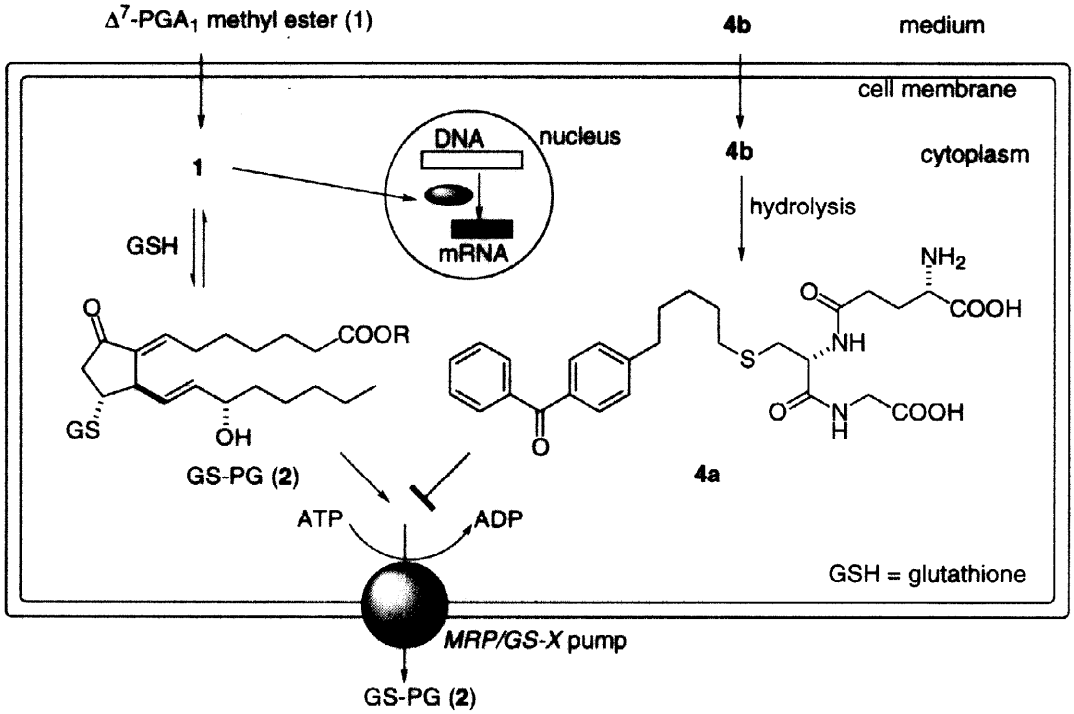
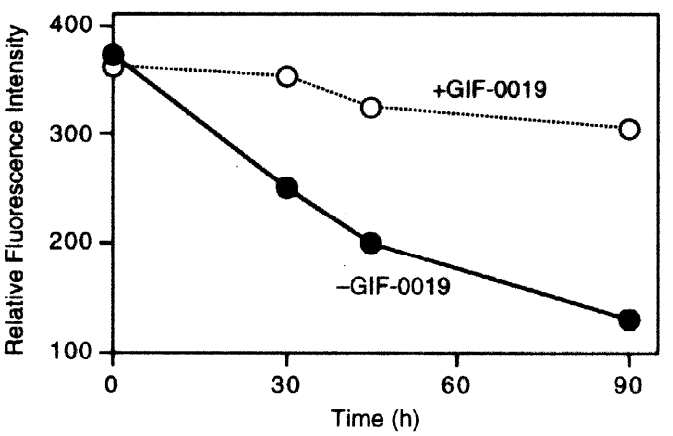
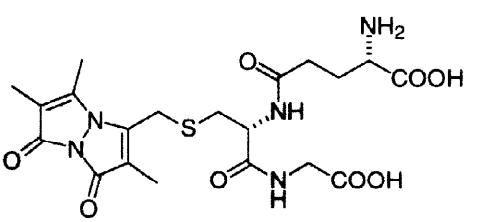



**Figure 3.** Molecular mechanism underlying the sensitization of HL-60/R-CP cells to $\Delta^7$-$PGA_1$ methyl ester (**1**) by co-incubation with the *GS-X* pump inhibitor GIF-0019 (**4b**).




**19**: glutathione–bimane conjugate

**Figure 4.** Time course of the fluorescence intensity of GS–bimane remaining in HL-60/R-CP cells during incubation with (○) (10 μM) or without GIF-0019 (**4b**) (●).

## EXPERIMENTAL

*General.* Nuclear magnetic resonance ($^1H$ and $^{13}C$) spectra were recorded on a JEOL JNM-A400 instrument at 400 MHz and 100 MHz, respectively. Chemical shifts are reported in parts per million (δ) with tetramethylsilane or the deuterium lock signal of the solvent ($D_2O$, DMSO-$d_6$) as an internal standard. The signal assignments of glutathione derivatives were done on the basis of the H,H-COSY measurement. Fast atom bombardment mass spectra (FAB MS) were recorded on a JEOL DATUM JMS-700 instrument. Analytical thin-layer chromatography (TLC) was performed on pre-coated 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). Column chromatography was performed on Merck silica gel 60 (Art. 9385, 230–400 mesh). Unless otherwise noted, reagents were used in commercial grade. Dichloromethane and dimethylformamide (DMF) were freshly distilled over $CaH_2$ prior to use. Tetrahydrofuran (THF) was freshly distilled from sodium–benzophenone ketyl. [$^3$H]Leukotriene $C_4$ was obtained from DuPont NEN.

*Synthesis of S-[5-(4-Benzoylphenyl)pentyl]glutathione (**4a**).* To a solution of 5-phenyl-1-pentanol (**7**) (2.70 g, 16.4 mmol) in dichloromethane (100 mL) was added triphenylphosphine (5.16 g, 19.7 mmol) followed by tetrabromomethane (11.0 g, 33.2 mmol) quickly at ambient temperature. After stirring the mixture for a few minutes, saturated aqueous $NaHCO_3$ (80 mL) was added to the solution and the mixture was extracted with dichloromethane (50 mL × 3). The organic phase was washed with brine, dried over anhydrous sodium sulfate, and then concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel using hexane as eluent to give the bromide **8** (3.62 g, 97%) as a colorless oil: TLC $R_f$ = 0.43 (hexane); $^1$H NMR ($CDCl_3$) δ 1.49 (tt, $J$ = 7.4, 7.4 Hz, 2H), 1.67 (tt, $J$ = 7.4, 7.7 Hz, 2H), 1.90 (tt, $J$ = 6.8, 7.4 Hz, 2H), 2.64 (t, $J$ = 7.7 Hz, 2H), 3.41 (t, $J$ = 6.8 Hz, 2H), 7.19 (br d, $J$ = 6.6 Hz, 2H), 7.20 (br t, $J$ = 6.6 Hz, 1H), 7.3 (br t, $J$ = 6.6 Hz, 2H); $^{13}$C NMR ($CDCl_3$) δ 27.8, 30.6, 32.7, 33.7, 35.7, 125.7, 128.3 (2C), 128.4 (2C), 142.3.

Benzoyl chloride (2.22 mL, 19.1 mmol) was added to a solution of anhydrous aluminum chloride (2.57 g, 19.3 mmol) in dichloromethane (60 mL) at 0 °C and the resulting mixture was stirred for 20 min. To this was introduced the bromide **8** (3.62 g, 15.9 mmol) dissolved in dichloromethane (30 mL) gradually at 0 °C and the solution was stirred at ambient temperature for 22.5 h. Then the solution was poured onto an ice–conc. HCl and the mixture was extracted with dichloromethane (50 mL × 3). The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated on a rotary evaporator. The crude product was purified by column chromatography on silica gel (20:1 hexane/ethyl acetate) to afford the desired ketone **9** (4.57 g, 87%) as a colorless oil: TLC $R_f$ = 0.43 (6:1 hexane/ethyl acetate); $^1$H NMR ($CDCl_3$) δ 1.46 (tt, $J$ = 7.4, 7.4 Hz, 2H), 1.64 (tt, $J$ = 7.4, 7.7 Hz, 2H), 1.85 (tt, $J$ = 6.7, 7.4 Hz, 2H), 2.66 (t, $J$ = 7.7 Hz, 2H), 3.36 (t, $J$ = 6.7 Hz, 2H), 7.23 (br d, $J$ = 8.4 Hz, 2H), 7.39–7.45 (2H), 7.49–7.55 (m, 1H), 7.69 (br d, $J$ = 8.4 Hz, 2H), 7.71–7.76 (2H); $^{13}$C NMR ($CDCl_3$) δ 27.8, 30.2, 32.6, 33.6, 35.7, 128.2 (2C), 128.3 (2C), 130.0 (2C), 130.4 (2C), 132.2, 135.3, 137.9, 147.4, 196.4.

A solution of **9** (3.03 g, 9.15 mmol) in ethanol (110 mL) was mixed with glutathione (4.25 g, 13.8 mmol) dissolved in $H_2O$ (40 mL). To this was added aqueous NaOH (2 M, 14 mL) at room temperature and the mixture was stirred for 22.5 h. Then the solution was acidified with oxalic acid to pH 2 at 0 °C and the resulting precipitate was collected by filtration. The solid was washed with water (15 mL) followed by ether (15 mL) and then dried under vacuum to give the product **4a** as a colorless powder (4.12 g, 81% yield): TLC (reversed-phase) $R_f$ = 0.43 (4:1 methanol/water); $^1$H NMR (0.1 M phosphate buffer in $D_2O$, pH 7.4) δ 1.22–1.38 (br, 2H, $CH_2$), 1.40–1.60 (br, 4H, $CH_2$), 2.18–2.38 (br, 2H, Glu($C\beta H_2$)), 2.40–2.70 (br, 6H, $SCH_2$, $ArCH_2$, Glu($C\gamma H_2$)), 2.86–2.92 (dd, $J$ = 8.8, 13.8 Hz, 1H, Cys($SCH_aH_b$)), 3.03–3.08 (dd, $J$ = 5.2, 13.8 Hz, 1H, Cys($SCH_aH_b$)), 3.81 (d, $J$ = 17.2 Hz, 1H, Gly($CH_aH_b$)), 3.87 (t, $J$ = 6.4 Hz, 1H, Glu($C_\alpha H$)), 3.92 (d, $J$ = 17.2 Hz, 1H, Gly($CH_aH_b$)), 4.6 (br, 1H, Cys($C_\alpha H$)), 7.14 (d, $J$ = 8 Hz, 2H), 7.31 (t, $J$ = 7.2 Hz, 2H), 7.44 (t, $J$ = 7.2 Hz, 1H), 7.53 (d, $J$ = 8 Hz, 4H), 7.56 (d, $J$ = 7.2 Hz, 2H); $^{13}$C NMR (DMSO-$d_6$) δ 26.9, 27.9, 28.8, 30.2, 31.2, 31.5, 33.4, 35.0, 41.2, 52.5, 53.1, 128.2 (2C), 128.3 (2C), 129.9 (2C), 130.4 (2C),

132.2, 135.3, 137.9, 147.4, 170.3, 170.8, 170.9, 171.8, 195.5; HRMS (FAB, NBA–NaI) *m/z* calcd for $C_{28}H_{33}N_3O_7SNa_3$ 624.1732, found 624.1750.

*Synthesis of S-[7-(3-Azidophenyl)heptyl]glutathione (**5a**).* To a solution of ethyl 8-hydroxyoctanoate (**10**) (2.00 g, 12.5 mmol) in dimethylsulfoxide (70 mL) was added triethylamine (28 mL, 0.2 M) followed by pyridine sulfur trioxide (14.7 g, 92.4 mmol) at ambient temperature, and the solution was stirred for 1 h. The solution was poured into water (100 mL) and the mixture was extracted with ether (100 mL × 4). The organic extract was washed sequentially with saturated aqueous ammonium chloride, saturated aqueous sodium bicarbonate, and then brine. The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was distilled under reduced pressure to give the aldehyde **11** (1.2 g, 61%): bp 74 °C (14 mmHg); $^1$H NMR ($CDCl_3$) δ 1.26 (t, *J* = 7.2 Hz, 3H), 1.66–1.69 (4H), 2.31–2.35 (m, 2H), 2.44–2.49 (m, 2H), 4.13 (q, *J* = 7.2 Hz, 2H), 9.77 (t, *J* = 1.6 Hz, 1H).

A solution of lithium diisopropylamide prepared from diisopropylamine (129 mg, 1.27 mmol) and butyllithium (1.66 M in hexane, 760 μL, 1.26 mmol) in THF (20 mL) was added to a solution of 3-nitrobenzyltriphenylphosphonium chloride (12) (550 mg, 1.27 mmol) in THF (20 mL) at –20 °C under argon. After stirring at –20 °C for 20 min, the mixture was added to a solution of the aldehyde **11** (104 mg, 0.66 mmol) in THF (20 mL) and the stirring was continued for 3 h. The reaction was quenched with saturated aqueous ammonium chloride (50 mL) and the mixture was extracted with ether (50 mL × 3). The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and then concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel (9:1 hexane/ethyl acetate) to give **13** (110 mg, 60%) as a mixture of olefinic isomers (E/Z = 6:4): TLC $R_f$ = 0.58 (4:1 hexane/ethyl acetate); $^1$H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.2 Hz, 3H, *Z*-isomer), 1.26 (t, *J* = 7.2 Hz, 3H, *E*-isomer), 1.48–1.60 (2H), 1.62–1.76 (2H), 2.25–2.40 (4H), 4.12 (q, *J* = 7.2 Hz, 2H, *Z*-isomer), 4.14 (q, *J* = 7.2 Hz, 2H, *E*-isomer), 5.81 (dt, *J* = 7.4 and 11.6 Hz, 1H, *Z*-isomer), 6.36 (dt, *J* = 6.6 and 15.8 Hz, 1H, *E*-isomer), 6.45 (d, *J* = 15.8 Hz, 1H, *E*-isomer), 6.46 (d, *J* = 11.6 Hz, 1H, *Z*-isomer), 7.45 (t, *J* = 8 Hz, 1H, *E*-isomer), 7.50 (t, *J* = 8 Hz, 1H, *Z*-isomer), 7.56 (d, *J* = 8 Hz, 1H, *Z*-isomer), 7.62 (d, *J* = 8 Hz, 1H, *E*-isomer), 8.02–8.05 (m, 1H, *E*-isomer), 8.06–8.1 (m, 1H, *Z*-isomer), 8.11 (br, 1H, *Z*-isomer), 8.19 (br, 1H, *E*-isomer).

A mixture of the olefin **13** (674 mg, 2.43 mmol), methanol (7 mL), and benzene (7 mL) was stirred under $H_2$ (1 atom) in the presence of Pd (10% on char coal, 400 mg) at ambient temperature for 3 h. Then the mixture was filtered through celite and concentrated under reduced pressure. The residue was dissolved in THF (13 mL) and treated with $LiAlH_4$ (139 mg, 3.67 mmol) at room temperature under argon. After stirring the mixture for 2 h, sodium sulfate decahydrate (1.17 g) was added and the stirring was continued for 18 h. The resulting mixture was dried over sodium sulfate, filtered through celite, and then evaporated. The residue was subjected to column chromatography on silica gel (3:1 hexane/ethyl acetate) to afford the amino alcohol **14** (469 mg, 93%): TLC $R_f$ = 0.28 (1:1 hexane/ethyl acetate); $^1$H NMR ($CDCl_3$) δ 1.30–1.40 (6H), 1.50–1.64 (4H), 2.51 (t, *J* = 7.7 Hz, 2H), 3.63 (t, *J* = 6.7 Hz, 2H), 3.50–3.70 (br, 2H), 6.48–6.55 (2H), 6.59 (d, *J* = 7.7 Hz, 1H), 7.03–7.09 (m, 1H).

To a solution of the amine **14** (370 mg, 1.78 mmol) in aqueous acetic acid (10 %, 20 mL) was added sodium nitrite (255 mg, 3.70 mmol) at 0 °C and the solution was stirred for 10 min. Then sodium azide (357 mg, 5.49 mmol) was added to the reaction mixture in the dark and stirring was continued for 15 min at the same temperature. To this were added saturated $NaHCO_3$ (50 mL) and solid $NaHCO_3$ sequentially until foaming

ceased. The mixture was extracted with ethyl acetate (50 mL) and the extract was washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated. The residue was subjected to column chromatography on silica gel (2:1 hexane/ethyl acetate) to give the azide **15** (381 mg, 92%): TLC $R_f$ = 0.69 (1:1 hexane/ethyl acetate); $^1$H NMR ($CDCl_3$) δ 1.26–1.42 (6H), 1.52–1.68 (4H), 2.56 (t, $J$ = 7.7 Hz, 2H), 3.63 (q, $J$ = 6.1 Hz, 2H), 6.81–6.88 (2H), 6.95 (d, $J$ = 7.7 Hz, 1H), 7.25 (t, $J$ = 7.7 Hz, 1H).

To a solution of **15** (116 mg, 0.5 mmol) in dichloromethane (5 mL) was added tetrabromomethane (442 mg, 1.33 mmol) followed by triphenylphosphine (232 mg, 0.89 mmol) quickly at ambient temperature. After stirring the mixture for 10 minutes, saturated aqueous $NaHCO_3$ (10 mL) was added to the solution and the mixture was extracted with dichloromethane (10 mL × 3). The organic phase was washed with brine, dried over anhydrous sodium sulfate, and then concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel (20:1 hexane/ethyl acetate) to give the bromide **16** (141 mg, 96%): TLC $R_f$ = 0.89 (4:1 hexane/ethyl acetate); $^1$H NMR ($CDCl_3$) δ 1.28–1.38 (4H), 1.38–1.46 (2H), 1.52–1.64 (2H), 1.83 (q, $J$ = 6.8 Hz, 2H), 2.57 (t, $J$ = 7.8 Hz, 2H), 3.38 (t, $J$ = 6.8 Hz, 2H), 6.81–6.88 (2H), 6.95 (d, $J$ = 7.7 Hz, 1H), 7.25 (t, $J$ = 7.7 Hz, 1H).

A solution of **16** (311 mg, 1.05 mmol) in ethanol (16 mL) was mixed with glutathione (653 mg, 2.12 mmol) dissolved in $H_2O$ (6 mL). To this was added aqueous NaOH (2 M, 2.10 mL) at room temperature and the mixture was stirred for 15 h. Then the solution was acidified with oxalic acid to pH 2 at 0 °C and the resulting precipitate was collected by filtration. The solid material was washed with water (50 mL) followed by ether (50 mL) and then dried under vacuum to give the desired product **5a** (494 mg, 90% yield): TLC (reversed-phase) $R_f$ = 0.61 (6:1 ethanol/water); $^1$H NMR (DMSO-*d*6) δ 1.2–1.35 (br, 6H), 1.4–1.5 (br, 2H), 1.5–1.58 (br, 2H), 1.78–1.97 (m, 2H), 2.22–2.37 (m, 2H), 2.43–2.52 (3H), 2.56 (t, $J$ = 7.2 Hz, 1H), 2.60 (dd, $J$ = 9 and 13.6 Hz, 1H), 2.86 (dd, $J$ = 4.3 and 13.6 Hz, 1H), 3.31 (t, $J$ = 6.8 Hz, 1H), 3.68 (m, 2H), 4.39 (dt, $J$ = 4.3 and 9 Hz, 1H), 6.91 (d, $J$ = 7.6 Hz, 1H), 6.92 (s, 1H), 7.01 (d, $J$ = 7.6 Hz, 1H), 7.30 (t, $J$ = 7.6 Hz, 1H), 8.36 (d, $J$ = 9 Hz, 1H), 8.67 (t, $J$ = 5.8 Hz, 1H); $^{13}$C NMR (DMSO-$d_6$) δ 26.9, 28.1, 28.4, 28.5, 29.0, 30.7, 31.2, 31.5, 33.3, 34.8, 41.1, 52.5, 53.0, 116.4, 118.8, 125.2, 129.8, 139.1, 144.7, 170.1, 170.8, 170.9, 171.7; HRMS (FAB, NBA–NaI) *m/z* calcd for $C_{23}H_{32}N_6O_6SNa_3$ 589.1797, found 589.1823.

*Synthesis of S-[4-(1-Pyrenyl)butyl]glutathione (**6a**).* To a solution of 4-pyrenylbutanol (**17**) (800 mg, 2.92 mmol) in dichloromethane (20 mL) was added triphenylphosphine (920 mg, 3.51 mmol) followed by tetrabromomethane (1.94 g, 5.85 mmol) quickly at ambient temperature. After stirring the mixture for a few minutes, saturated aqueous $NaHCO_3$ (30 mL) was added to the solution and the mixture was extracted with dichloromethane (30 mL × 3). The combined organic phase was washed with brine, dried over anhydrous sodium sulfate, and then concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel (96:4 hexane/ethyl acetate) to give the bromide **18** (889 mg, 90%) as colorless powder: TLC $R_f$ = 0.35 (96:4 hexane/ethyl acetate); $^1$H NMR ($CDCl_3$) δ 1.97–2.09 (4H), 3.38 (t, $J$ = 7.2 Hz, 2H), 3.47 (t, $J$ = 6.5 Hz, 2H), 7.86–8.29 (9H).

A solution of **18** (300 mg, 0.890 mmol) in THF (9 mL) was mixed with glutathione (658 mg, 2.14 mmol) dissolved in $H_2O$ (0.6 mL). To this were added aqueous NaOH (1 M, 8.8 mL) and DMF (2 mL) at room temperature and the mixture was stirred for 44 h. Then the solution was cooled to 0 °C and acidified with oxalic acid to pH 2. The resulting precipitate was collected by filtration, washed with a saturated oxalic acid solution (5 mL) followed by THF (5 mL), and then dried under vacuum to give the product **6a** as a colorless powder (262 mg, 49% yield): TLC (reversed-phase) $R_f$ = 0.42 (9:1 methanol/water); $^1$H NMR (DMSO-*d*6) δ 1.60–1.75

(2H), 1.76–2.0 (4H), 2.20–2.42 (m, 2H), 2.57–2.68 (3H), 2.90 (dd, $J$ = 4.4, 13.0 Hz, 1H), 3.25–3.35 (3H), 3.62–3.76 (m, 2H), 4.38–4.47 (m, 1H), 7.92–8.40 (10H, aromatic and NH), 8.70 (br t, $J$ = 5.8 Hz, 1H); HRMS (FAB, diethanolamine–NaI) *m/z* calcd for $C_{30}H_{31}N_3O_6SNa_3$ 630.1627, found 630.1609.

*Synthesis of Esterified Derivative* ***4b***. To a solution of 4 (1.00 g, 1.79 mmol) in a mixture of methanol and benzene (1:4, 50 mL) was added (trimethylsilyl)diazomethane (2 M in hexane, 2.00 mL, 4.00 mmol) at ambient temperature, and the resulting mixture was stirred for 1.5 h. The solution was concentrated under reduced pressure to dryness, and the residue was dissolved in dichloromethane (60 mL). To this was added methyl chloroformate (210 μL, 2.72 mmol) and the mixture was stirred at ambient temperature for 4 h. Then, saturated aqueous sodium bicarbonate (50 mL) was added to the solution, and the mixture was extracted with dichloromethane (50 mL × 3). The combined organic extract was dried over anhydrous sodium sulfate and evaporated. the residue was subjected to column chromatography on silica gel (17:2 haxane/ethyl acetate) to give **4b** (882 mg, 77% yield): TLC $R_f$ = 0.42 (9:1 hexane/ethyl acetate); $^1$H NMR ($CDCl_3$) δ 1.38–1.50 (m, 2H), 1.54–1.70 (4H), 1.95–2.05 (1H), 2.17–2.42 (3H), 2.58 (dt, $J$ = 2, 7.6 Hz, 2H), 2.68 (t, $J$ = 7.6 Hz, 2H), 2.78–2.85 (m, 1H), 2.94 (dd, $J$ = 5.8, 14 Hz, 1H), 3.63 (s, 3H), 3.72 (s, 3H), 3.73 (s, 3H), 3.98 (dd, $J$ = 5.4, 18 Hz, 1H), 4.07 (dd, $J$ = 5.6, 18 Hz, 1H), 4.4 (br, 1H), 4.53 (q, $J$ = 7.2 Hz, 1H), 5.56 (br, 1H), 6.65–6.72 (br, 1H), 7.05 (br, 1H), 7.26 (d, $J$ = 8.2 Hz, 2H), 7.46 (t, $J$ = 7.6 Hz, 2H), 7.56 (t, $J$ = 7.6 Hz, 1H), 7.72 (d, $J$ = 8.2 Hz, 2H), 7.77 (d, $J$ = 7.6 Hz, 2H); $^{13}$C NMR ($CDCl_3$) δ 28.1, 28.3, 29.3, 30.6, 32.0, 32.2, 33.6, 35.7, 41.2, 52.2, 52.3, 52.4, 52.5, 53.3, 128.1 (2C), 128.3 (2C), 129.9 (2C), 130.3 (2C), 132.1, 135.1, 137.8, 147.6, 156.8, 169.9, 170.6, 172.1, 172.5, 196.5; HRMS (FAB, NBA–NaI) *m/z* calcd for $C_{32}H_{41}N_3O_9SNa$ 666.2461, found 666.2456.

***5b***. The esterified derivative **5b** was synthesized from **5a** according to the procedure for the synthesis of **4b**. **5b**: 91% yield; TLC $R_f$ = 0.28 (9:1 hexane/ethyl acetate); $^1$H NMR ($CDCl_3$) δ 1.29–1.42 (6H), 1.55–1.64 (4H), 1.95–2.1 (m, 1H), 2.18–2.44 (3H), 2.59 (t, $J$ = 7.6 Hz, 4H), 2.81–2.87 (m, 1H), 2.97 (dd, $J$ = 6, 14 Hz, 1H), 3.68 (s, 3H), 3.75 (s, 3H), 3.76 (s, 3H), 4.0 (dd, $J$ = 5.2, 18.4 Hz, 1H), 4.09 (dd, $J$ = 5.6, 18.4 Hz, 1H), 4.39–4.49 (br, 1H), 4.55 (q, $J$ = 7 Hz, 1H), 5.54–5.62 (br, 1H), 6.68–6.77 (br, 1H), 6.83 (s, 1H), 6.85 (d, $J$ = 8 Hz, 1H), 6.95 (d, $J$ = 8 Hz, 1H), 7.04–7.09 (br, 1H, NH), 7.25 (t, $J$ = 8 Hz, 1H); $^{13}$C NMR ($CDCl_3$) δ 27.9, 28.5, 28.9, 29.0, 29.3, 31.0, 31.9, 32.3, 33.7, 35.6, 41.1, 52.2, 52.2, 52.3, 52.4, 53.3, 116.2, 118.8, 125.0, 129.4, 139.7, 144.7, 156.8, 169.9, 170.7, 172.2, 172.5; HRMS (FAB, NBA–NaI) *m/z* calcd for $C_{27}H_{40}N_6O_8SNa$ 631.2526, found 631.2529.

***6b***. The esterified derivative **6b** was synthesized from **6a** according to the procedure for the synthesis of **4b**. **6b**: 82% yield; TLC $R_f$ = 0.58 (1:4 acetone/ethyl acetate); $^1$H NMR ($CDCl_3$) δ 1.73–1.9 (m, 2H), 1.93–2.1 (3H), 2.15–2.4 (3H), 2.68 (t, $J$ = 6.6 Hz, 2H), 2.8–2.9 (m, 1H), 2.97 (dd, J = 5.8, 14.2 Hz, 1H), 3.37 (t, $J$ = 7.6 Hz, 2H), 3.65 (s, 3H), 3.70 (s, 3H), 3.71 (s, 3H), 3.96 (dd, J = 5.0, 18 Hz, 1H), 4.05 (dd, J = 5.6, 18 Hz, 1H), 4.4–4.44 (m, 1H), 4.53 (q, $J$ = 6.7 Hz, 1H), 5.57 (br, 1H), 6.7 (br, 1H), 7.0 (br, 1H), 7.83–8.34 (9H); HRMS (FAB, NBA–NaI) *m/z* calcd for $C_{34}H_{39}N_3O_8SNa$ 672.2356, found 672.2372.

*Inhibition of ATP-Dependent Transport of $^3$H-labeled $LTC_4$ by S-alkylated glutathione derivatives.* Plasma membrane vesicles were prepared from HL-60 and HL-60/R-CP cells as described previously.[9] The vesicles were incubated with $^3$H-labeled $LTC_4$ (10 nM) in the absence or presence of *S*-alkylated glutathione derivatives (0.1, 0.3, 1, 3, and 10 μM) at 37 °C. The amount of [$^3$H]$LTC_4$ incorporated into the vesicles was measured by the rapid filtration technique.[4a] ATP-dependent transport was calculated from the difference between radioactivities incorporated into membrane vesicles incubated with and without ATP.

*Effect of GIF-0019 (**4b**) on Antiproliferative Effect of $\Delta^7$-PGA$_1$ Methyl Ester (**1**).* HL-60/R-CP cells were incubated with $\Delta^7$-PGA$_1$ methyl ester (5 μM) in the absence or presence of **4b** (10 μM), and the time course of cell proliferation was followed. In the control, cells were incubated in the absence of $\Delta^7$-PGA$_1$ methyl ester and **4b**.

*Inhibition of the GS-X pump activity by GIF-0019 (**4b**) in HL-60/R-CP cells (GS-bimane assay).* The GS–bimane conjugate was pre-loaded by incubating HL-60/R-CP cells with 20 μM monochlorobimane at 37 °C for 20 min and subsequently cells were washed with the incubation medium.[9a] The resulting cells were incubated with (10 μM) or without **4b** in the RPMI 1640 culture medium at 37 °C for 0, 30, 45, and 90 min. The relative fluorescence intensity of cells was measured in an EPICS Elite flow cytometer equipped with an air-cooled Helium-Cadmium laser for excitation and a 470-nm long-pass filter for emission. At every observation, at least $1 \times 10^4$ cells were subjected to the flow cytometry.

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