Supporting Information

Contents

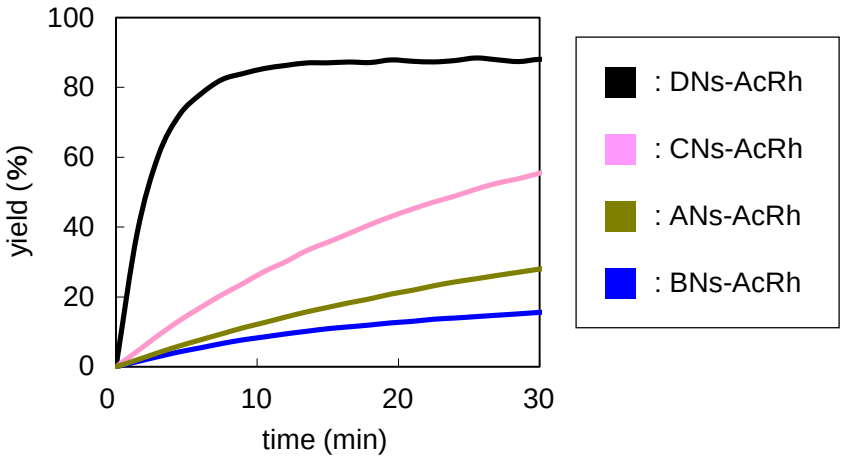

Figure S1. Time course of the yield of AcRh in the reaction between 1 μM probe and 1 mM GSH with 2 μg/ ml GST in 10 mM PBS buffer (pH7.4). Reaction was monitored by excitation at 490 nm and emission at 522 nm.

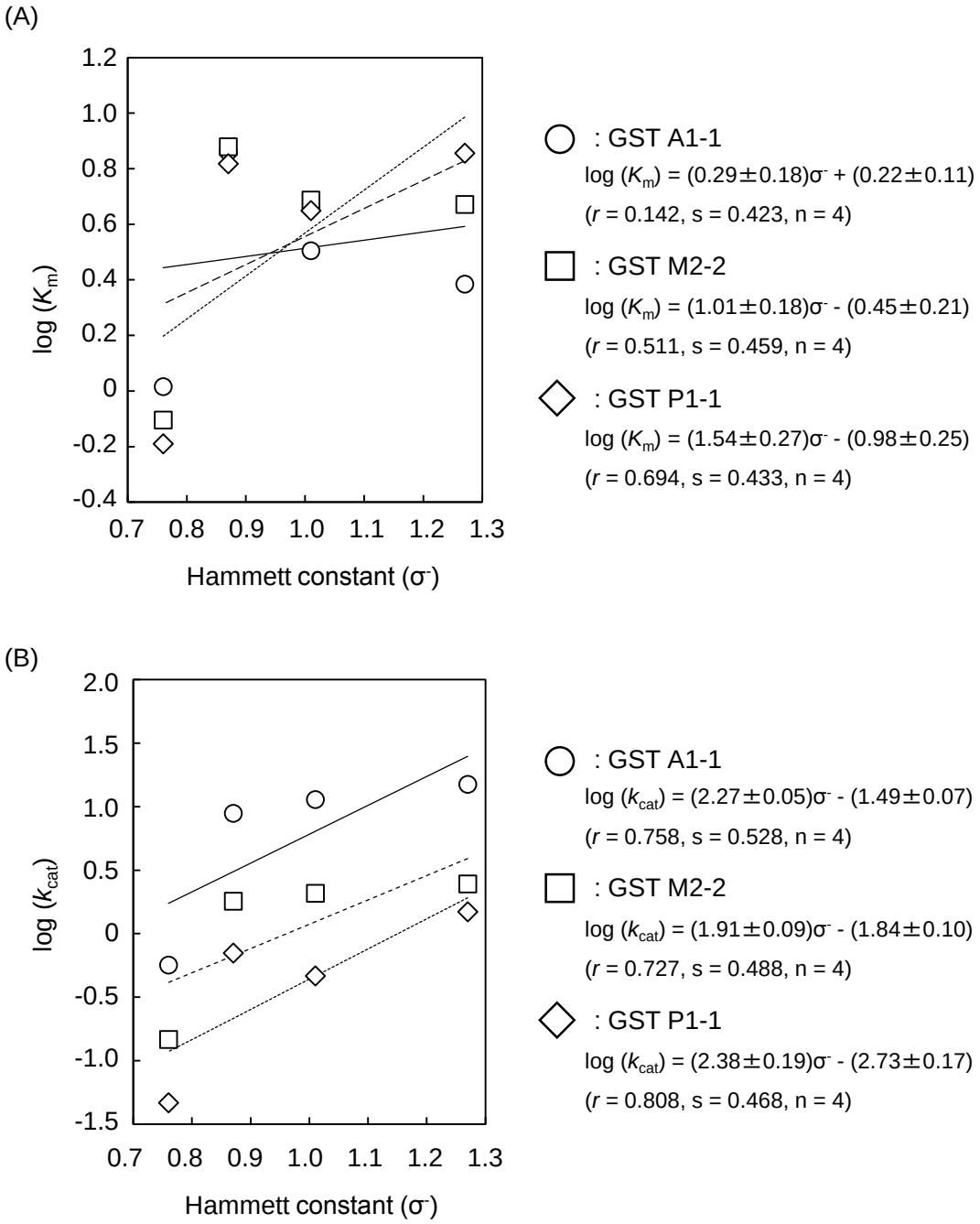

Figure S2. Hammett plot of (A) log (K_m) and (B) log (k_{cat}) vs σ for enzyme-catalyzed reaction. Hammett constants are from Figure 1 and value of K_m and k_{cat} are from Table. Line are least-square fits to the data points by Microsoft Excel.

R =	*F*	*R*	*f*	*L*	B_5
NO_2	0.65	0.13	-0.039	3.44	2.44
CN	0.51	0.15	-0.155	4.23	1.6
$COCH_3$	0.33	0.17	-0.252	4.06	3.13
$COO(CH_2)_3CH_3$	0.34	0.11	1.738	8	5.85

Hammett constant σ^- and field parameter *F*

no GST: $\log (k_{non\text{-}cat}) = (0.79\pm1.27)\sigma^- + (1.26\pm4.76)F - (1.01\pm1.10)$ (r = 0.910, s = 0.394, n = 4)

GSTA1-1: $\log (k_{cat}/K_m) = (1.88\pm2.30)\sigma^- + (0.36\pm3.31)F + (4.29\pm0.90)$ (r = 0.956, s = 0.406, n = 4)

GSTM2-2: $\log (k_{cat}/K_m) = (0.31\pm1.02)\sigma^- + (0.84\pm1.47)F + (4.81\pm0.39)$ (r = 0.959, s = 0.174, n = 4)

GSTP1-1: $\log (k_{cat}/K_m) = (1.63\pm0.40)\sigma^- - (1.21\pm0.56)F + (4.01\pm0.16)$ (r = 0.992, s = 0.163, n = 4)

Field component *F* and resonance parameter *R*

no GST: $\log (k_{non\text{-}cat}) = (3.27\pm0.03)F + (10.06\pm0.21)R - (2.62\pm0.04)$ (r = 0.999, s = 0.458, n = 4)

GSTA1-1: $\log (k_{cat}/K_m) = (3.06\pm0.16)F + (6.55\pm0.88)R + (3.96\pm0.15)$ (r = 0.998, s = 0.409, n = 4)

GSTM2-2: $\log (k_{cat}/K_m) = (1.34\pm0.17)F + (2.19\pm1.07)R + (4.58\pm0.19)$ (r = 0.991, s = 0.179, n = 4)

GSTP1-1: $\log (k_{cat}/K_m) = (1.08\pm0.58)F + (1.91\pm3.51)R + (4.30\pm0.63)$ (r = 0.895, s = 0.153, n = 4)

Hammett constant σ^- and hydrophobic fragment constant *f*

no GST: $\log (k_{non\text{-}cat}) = (1.43\pm0.30)\sigma^- - (0.27\pm0.16)f - (1.02\pm0.38)$ (r = 0.985, s = 0.450, n = 4)

GSTA1-1: $\log (k_{cat}/K_m) = (1.78\pm0.61)\sigma^- - (0.12\pm0.13)f + (4.59\pm0.62)$ (r = 0.976, s = 0.406, n = 4)

GSTM2-2: $\log (k_{cat}/K_m) = (0.80\pm0.33)\sigma^- - (0.03\pm0.08)f + (4.73\pm0.35)$ (r = 0.953, s = 0.175, n = 4)

GSTP1-1: $\log (k_{cat}/K_m) = (0.77\pm0.29)\sigma^- - (0.02\pm0.07)f + (4.31\pm0.30)$ (r = 0.961, s = 0.159, n = 4)

Hammett constant σ^- and sterimol parameter B_5

no GST: $\log (k_{non\text{-}cat}) = (1.41\pm0.03)\sigma^- - (0.14\pm0.01)B_5 - (0.64\pm0.04)$ (r = 0.999, s = 0.457, n = 4)

GSTA1-1: $\log (k_{cat}/K_m) = (1.49\pm0.33)\sigma^- - (0.10\pm0.04)B_5 + (5.16\pm0.41)$ (r = 0.995, s = 0.407, n = 4)

GSTM2-2: $\log (k_{cat}/K_m) = (0.67\pm0.26)\sigma^- - (0.04\pm0.03)B_5 + (4.97\pm0.34)$ (r = 0.977, s = 0.178, n = 4)

GSTP1-1: $\log (k_{cat}/K_m) = (0.86\pm0.33)\sigma^- + (0.01\pm0.04)B_5 + (4.18\pm0.42)$ (r = 0.962, s = 0.157, n = 4)

Figure S3 Multiple regression analysis between kinetic and physicochemical parameters. The values of k_{cat} / K_m and $k_{non\text{-}cat}$ are from Table1. Field parameter *F* and resonance parameter *R* were taken from *Ref 1*. Hydrophobic fragment constant *f* were taken from *Ref 2*. Sterimol parameters *L* and B_5 were taken from *Ref 3*.

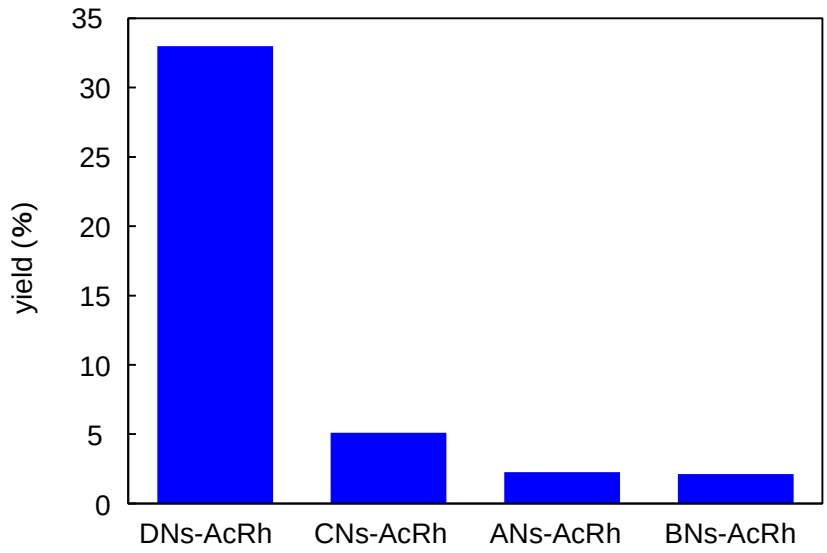

Figure S4. The yield of AcRh in the reaction between 1 μM probe and 1 mM GSH in 10 mM PBS buffer (pH 7.4). Reaction was monitored by excitation at 490 nm and emission at 522 nm.

Experimental details

Materials and General Instrumentation. General chemicals were purchased from Wako Pure Chemical, Tokyo Chemical Institute, or Sigma-Aldrich or Kanto Chemical and were used without further purification. NMR spectra were recorded on a JEOL instrument at 300 MHz for ^{1}H NMR and 75.5 MHz for ^{13}C NMR. ESI-Ms spectra were recorded by JEOL LC-MS/TOF.

2-Nitro-4-cyanobenzensulfonyl chloride. To a stirred solution of thioacetic acid (0.30 mL 4.22 mmol, 1.5 equiv.) and glycylglycine (475.7 mg, 3.60 mmol, 1.3 equiv.) in DMF (10 mL) was added cesium carbonate (2.68 g, 8.24 mmol, 3.0 equiv.) at 0 °C. After 10 min, a solution of 4-chloro-3-nitrobenzonitrile (502.9 mg, 2.75 mmol) in DMF (5.0 mL) was added slowly after which the reaction mixture was stirred for 21 h at room temperature. The reaction mixture was diluted with ethyl acetate and washed with 1 N HCl, water and brine, dried over Na_2SO_4, filtered and the solvent was evaporated under vacuum to give the corresponding thiol compound.

To a stirred solution of *N*-chlorosuccinimide, (1.50 g, 11.25 mmol, 4.1 equiv.) in 2 N HCl (1.4 mL) and acetonitrile (7.0 mL), the thiol compound was added slowly after which the reaction mixture was stirred for 3 h at 0 °C. This reaction mixture was diluted with ethyl acetate and washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash silica gel column chromatography to give the corresponding sulfonyl chloride (445.8 mg, 1.81 mmol, 66%).

^{1}H-NMR (300 MHz, $CDCl_3$) : δ 8.42-8.40 (1H, d, *J* = 8.4 Hz, Ar), 8.17 (1H, s, Ar), 8.17-8.12 (1H, dd, *J* = 9.9, 1.5 Hz, Ar). ^{13}C-NMR (75.5 MHz, $CDCl_3$) : δ 138.82, 136.27, 131.42, 128.59, 120.37, 114.61. HR-EI-MS *m/z* calcd. for $C_7H_3ClN_2O_4S$ $(M)^+$ 245.9502, Found: 245.9401.

Benzoic acid, 3-nitoro-4-(chlorosulfonyl)-, butyl ester. To a stirred solution of thioacetic acid (0.27 mL 3.80 mmol, 1.5 equiv.) and glycylglycine (430.2 mg, 3.26 mmol, 1.3 equiv.) in DMF (10 mL) was added cesium carbonate (2.43 g, 7.47 mmol, 3.0 equiv.) at 0 °C. After 10 min, a solution of the benzoic acid, 4-chloro-3-nitro-, butyl ester (642.7 mg, 2.49 mmol) in DMF (5.0 mL) was added slowly after which the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with ethyl acetate and washed with 1 N HCl, water and brine, dried over Na_2SO_4, filtered and the solvent was evaporated under vacuum to give thiol compound.

To a stirred solution of *N*-chlorosuccinimide, (1.35 g, 10.11 mmol, 4.1 equiv.) in 2 N HCl (1.4 mL) and acetonitrile (7.0 mL), thiol compound was added slowly after which the reaction mixture was stirred for 4 h at 0 °C. This reaction mixture was diluted with ethyl acetate and washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash silica gel column chromatography to give the corresponding sulfonyl chloride (589.3 mg, 1.83 mmol, 73%).

^{1}H-NMR (300 MHz, $CDCl_3$) : δ 8.47-8.44 (2H, m, Ar), 8.35-8.32 (1H, d, *J* = 9.0 Hz, Ar), 4.46-4.41(2H, t, *J* = 6.6 Hz, CH_2), 1.84-1.75 (2H, m, CH_2), 1.54-1.42 (2H, m, CH_2), 1.02-0.97 (3H, t, *J* = 7.4 Hz, CH_3).^{13}C-NMR (75.5 MHz, $CDCl_3$) : δ 163.87, 150.79, 145.54, 134.57, 130.79, 125.91, 125.32, 67.69, 30.16, 19.02, 13.62. HR-ESI-MS *m/z* calcd. for $C_{11}H_{12}ClNNaO_6S$ $(M+Na)^+$ 343.9972, Found: 343.9967.

CNs-AcRh. 2-Nitro-4-cyanobenzensulfonyl chloride (20.6 mg, 0.08 mmol, 1.8 equiv.) was added to a solution of AcRh (22.5 mg, 0.05 mmol) in pyridine/CH_2Cl_2 (= 1:3, 1.2 ml) at 0 °C. After 20 h at room temperature, the reaction mixture was diluted with ethyl acetate, washed with sat. $NaHCO_3$ aq. The organic layer was dried over Na_2SO_4 and evaporated in vacuum. The residue was purified by flash column chromatography to give the probe (22.7 mg, 0.04 mmol, 84%).
^{1}H-NMR (300 MHz, CD_3OD): δ 8.32 (1H, s, $ArSO_2$), 8.19-8.17 (1H, d, *J* = 8.4 Hz, $ArSO_2$), 8.07-8.04 (1H, d, *J* = 8.1 Hz, $ArSO_2$), 8.00-7.98 (1H, d, *J* = 6.9 Hz, Ar), 7.78-7.68 (3H, m, Ar), 7.21 (1H, ds, *J* = 2.4, Ar), 7.15-7.08 (2H, m, Ar), 6.94-6.90 (1H, dd, *J* = 2.1, 8.7 Hz, Ar), 6.71-6.65 (2H, m, Ar), 2.13 (3H, s, Ac). ^{13}C-NMR (75.5 MHz, CD_3OD) : δ 171.95, 171.04, 154.18, 153.12, 152.63, 149.58, 147.44, 139.95, 137.09, 136.90, 136.81, 133.17, 131.39, 130.31, 129.88, 129.32, 127.48, 126.00, 125.08, 119.27, 117.70, 117.01, 116.80, 116.63, 114.97, 109.75, 108.43, 83.65, 23.98. HR-ESI-MS *m/z* calcd. for $C_{29}H_{17}N_4O_8S$ $(M-H)^-$ 581.0767, Found: 581.0751.

ANs-AcRh. 2-Nitro-4-acetylbenzensulfonyl chloride (28.4 mg, 0.11 mmol, 2.5 equiv.) was added to a solution of AcRh (20.9 mg, 0.04 mmol) in pyridine/CH_2Cl_2 (=1:3, 1.2 ml) at 0 °C. After 16 h at room temperature, the reaction mixture was diluted with ethyl acetate, washed with sat. $NaHCO_3$ aq. The organic layer was dried over Na_2SO_4 and evaporated in vacuum. The residue was purified by flash column chromatography to give the probe (24.3 mg, 0.04 mmol, 94%).
^{1}H-NMR (300 MHz, CD_3OD) : δ 8.31 (1H, ds, *J* = 1.8 Hz, $ArSO_2$), 8.24-8.20 (1H, dd, *J* = 1.8, 8.1 Hz, $ArSO_2$), 8.15-8.13 (1H, d, *J* = 7.2 Hz, $ArSO_2$), 8.99-7.97 (1H, d, *J* = 6.6 Hz, Ar), 7.76-7.66 (3H, m, Ar), 7.21-7.20 (1H, ds, *J* = 1.8, Ar), 7.13-7.07 (2H, m, Ar), 6.94-6.91 (1H, dd, *J* = 2.7, 9.0 Hz, Ar), 6.68-6.63 (2H, m, Ar), 2.58 (3H, s, Ac (ANs)), 2.13 (3H, s, Ac).^{13}C-NMR (75.5 MHz, CD_3OD) : δ 196.55, 171.92, 171.03, 154.15, 153.07, 152.62, 149.84, 142.85, 142.41, 140.23, 136.78, 136.15, 133.02, 132.65, 131.36, 130.22, 129.30, 127.48, 125.97, 125.40, 125.07, 117.53, 116.76, 114.96, 109.46, 108.40, 83.68, 26.85, 23.98. HR-ESI-MS *m/z* calcd. for $C_{30}H_{20}N_3O_9S$ $(M-H)^-$ 598.0920, Found: 598.0923.

BNs-AcRh. Benzoic acid, 3-nitoro-4-(chlorosulfonyl)-, butyl ester (33.6 mg, 0.10 mmol, 2.7 equiv.) was added to a solution of AcRh (16.0 mg, 0.04 mmol) in pyridine/CH_2Cl_2 (= 1:3, 1.2 ml) at 0 °C. After 21 h at room temperature, the reaction mixture was diluted with ethyl acetate, washed with sat.

$NaHCO_3$ aq. The organic layer was dried over Na_2SO_4 and evaporated in vacuum. The residue was purified by flash column chromatography to give the probe (13.8 mg, 0.02 mmol, 54%).
^{1}H-NMR (300 MHz, $CDCl_3$) : δ 8.43-8.42 (1H, ds, *J* = 1.8 Hz, $ArSO_2$), 8.27-8.23 (1H, dd, *J* = 1.2, 7.8 Hz, $ArSO_2$), 8.07-8.04 (1H, d, *J* = 8.4 Hz, $ArSO_2$), 8.02-7.99 (1H, dd, *J* = 1.5, 6.6 Hz, Ar), 7.68-7.63 (3H, m, Ar), 7.19-7.18 (1H, ds, *J* = 2.1, Ar), 7.11-7.09 (1H, d, *J* = 6.6 Hz, Ar), 7.04-7.00 (1H, dd, *J* = 1.8, 8.4 Hz, Ar), 6.93-6.89 (1H, dd, *J* = 2.4, 9.0 Hz, Ar), 6.71-6.65 (2H, m, Ar), 4.38-4.34 (2H, t, *J* = 6.6 Hz, CH_2), 2.19 (3H, s, Ac), 1.77-1.70 (2H, m, CH_2), 1.48-1.40 (2H, m, CH_2), 0.98-0.93 (3H, t, *J* = 7.4 Hz, CH_3).^{13}C-NMR (75.5 MHz, $CDCl_3$) : δ 169.56, 168.98, 163.00, 152.66, 151.78, 151.30, 148.08, 140.18, 137.54, 136.16, 135.43, 135.21, 133.36, 132.00, 130.10, 129.31, 128.37, 126.21, 126.09, 125.21, 123.88, 117.17, 116.48, 115.59, 109.92, 107.67, 82.22, 66.45, 30.46, 24.57, 19.09, 13.64. HR-ESI-MS *m/z* calcd. for $C_{33}H_{26}N_3O_{10}S$ $(M-H)^-$ 656.1339, Found: 656.1328.

Measurement of Quantum Yield. AcRh, DNs-AcRh, CNs-AcRh, ANs-AcRh and BNs-AcRh measurements were done in sodium phosphate buffer (10 mM, pH 7.4). Fluorescence intensities were measured by fluorescence spectrometry (FP-6500; JASCO). Compounds were excited at 490 nm. Quantum yields are determined by using fluorescein (0.85, 0.1 M NaOH) as standard. The quantum efficiency of fluorescence was obtained from the following equation (F denotes fluorescence intensity at each wavelength and Σ [F] was calculated by summation of fluorescence intensities).

$$\Phi_{fl}^{\ sample} = \Phi_{fl}^{\ standard} \mathrm{Abs}^{\ standard} \Sigma [F^{\ sample}] / \mathrm{Abs}^{\ sample} \Sigma [F^{\ standard}]$$

Fluorescence Measurement of GST Activity. Reactions were performed in 1.2 mL of PBS (pH 7.4, 10 mM) containing 100 nM probe, 2 μg/ml human recombinant GST (20237, Thermo Scientific) and 1 mM GSH for 30 min at 37 °C. The increase in fluorescence intensity produced by the reduction of GSH was monitored continuously at time intervals (excitation, 490 nm; emission, 522 nm). Reactions were observed by fluorescence spectrometry (FP-6500; JASCO).

Expression and purification of human GST. Human GSTs were expressed essentially as described previously[4] and purified from lysate using GSTrap HP (GE Healthcare) according to the manufacturer's instructions. The high purity of the enzyme was confirmed by SDS-PAGE. Protein concentration was determined from the absorbance at 280 nm.

Determination of Steady-State Kinetic Constants. Kinetic parameters K_m, k_{cat}, k_{cat}/K_m for GSTA1-1, GSTM2-2 or GSTP1-1 were determined with DNs-AcRh, CNs-AcRh, ANs-AcRh and BNs-AcRh as substrates. The same assay conditions as those described above were used. The

cytosolic GST (GSTA1-1, GSTM2-2 or GSTP1-1) was assayed in 0.1 M potassium phosphate buffer pH 6.5, at a constant GSH concentration of 5 mM and varying concentrations of DNs-AcRh (1-10 μM), CNs-AcRh (1-10 μM), ANs-AcRh (1-30 μM) or BNs-AcRh (1-50 μM). Reactions were observed by fluorescence spectrometry (Mithras LB940; BERTHOLD). The values of the steady-state kinetic constants were determined by fitting the data to the Michaelis-Menten equation by non-linear regression analysis using Microsoft Excel. In cases where saturation was not reached, k_{cat}/K_m was determined by fitting the Michaelis-Menten equation at low substrate concentration $v = (k_{cat}/K_m)$ [E] [S] to the data, when $K_m >$ [S]. The k_{cat} value was calculated per subunit for cytosolic GSTs (25.5 kDa) since the enzyme displays third of the sites reactivity. In order to assess the rate enhancement ($[k_{cat}/K_m]/ k_{non\text{-}cat}$), the second-order rate constant of the non-enzymatic reaction $k_{non\text{-}cat}$ was determined and compared with the apparent second-order rate constant for the enzyme-catalyzed reaction k_{cat}/K_m (i.e. k_{cat}/K_m divided by $k_{non\text{-}cat}$).

Fluorescence Microscopy. For live cell imaging studies, HL-60 cells were grown in RPMI1640 containing 10% FBS in an atmosphere containing 5% CO_2 at 37 °C. The cells were incubated at 37°C in the presence of 10 μM probe (2.5% DMSO in PBS (-), v/v) for 60 min. For the control experiment, the cells were treated with 50 μM ethacrynic acid or 1 mM *N*-methylmaleimide in PBS (-) buffer for 60 min at 37 °C. And then, the cells were incubated in the presence of 10 μM probe (2.5% DMSO in PBS (-), v/v) for 60 min at 37 °C.

Fluorescence images were obtained through a fluorescence microscope (Axiovert 200M; Carl Zeiss) with mercury lamp, using digital camera (Cool Snap HQ; Roper Scientific) and imaging software (MetaMorph; Molecular Devices). Microscope settings were as follows: ex. 470/40 bandpass filter; em. 525/50 bandpass filter.

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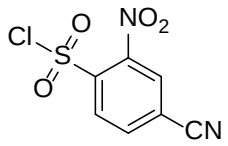
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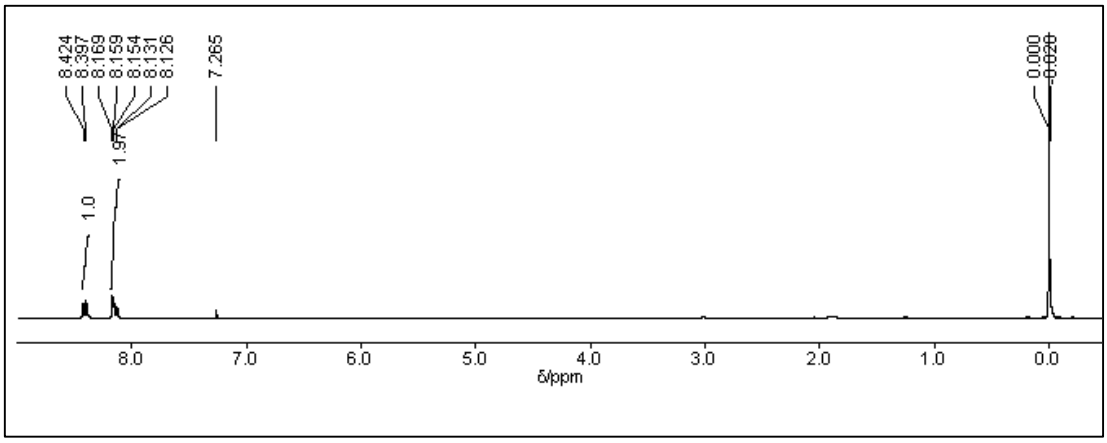
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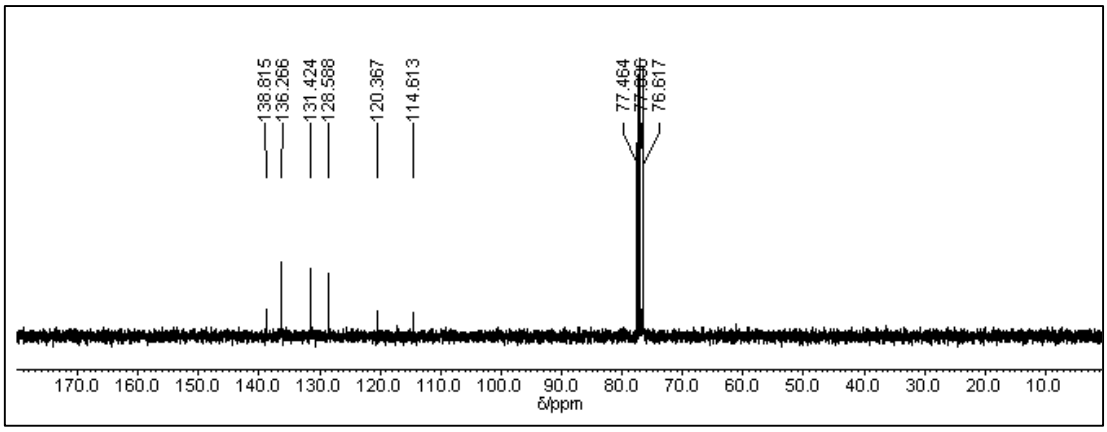
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