Electronic Supplementary Information for

Thiyl Chemistry: Cysteine-Catalyzed Maleate Isomerization via Aqueous Thiyl Radical Processes

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S1. Materials and Methods

S1.1 Samples

All chemicals were of reagent-grade quality and were used as received unless otherwise specified, with the exception of *S*-methyl cysteine. Maleic acid reagents of various grades were procured from FUJIFILM Wako Pure Chemical, Kanto Chemical, and Sigma-Aldrich (NMR grade). d_2-Maleic acid was purchased from Toronto Research Chemicals, while fumaric acid, *L*-cysteine, *L*-alanine, 2-aminoethanethiol hydrochloride, and mercaptoacetic acid were purchased from FUJIFILM Wako Pure Chemical. Similarly, *L*-cysteine methyl ester hydrochloride, *S*-methyl cysteine, *D*-penicillamine, 2-mercaptoethanol, 3-mercaptopropionic acid, tiglic acid, angelic acid, and *trans*,*trans*-muconic acid were purchased from Tokyo Chemical Industry. *S*-methyl cysteine was recrystallized twice before use. *N*-acetylcysteine was purchased from Nacalai Tesque, while *N*-acetylcysteine methyl ester and *cis*,*cis*-muconic acid were purchased from Sigma-Aldrich, and acetylcysteinamide was from CEREC Biotech. 2,2'-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) and 2,2'-azobis(2,4-dimethylvaleronitrile) as an azo-type initiator were purchased from Tokyo Chemical Industry. 2,2'-azobis[2-(2-imidazoline-2-yl)propane] dihydrochloride (AIPH) was from FUJIFILM Wako Pure Chemical. HPLC-grade acetonitrile and heavy water manufactured by Sigma-Aldrich were used for reactions and analyses. Ultra-pure water (>18.0 MΩ•cm, Merck Millipore, MA, USA, Milli-Q® Advantage A10) was used for the preparation of standard solutions and mobile phase. All chemical reactions were performed in anoxic environments under Ar atmosphere unless otherwise noted.

S1.2 Catalytic Investigation

Reaction for Cysteine-Catalyzed Isomerization

Due to the relatively high dissociation constant of maleic acid in water ($K_1 = 1.14 \times 10^{-2}$),[S1] the as-prepared solution has a considerably low pH. Therefore, 1.8 equivalents of sodium hydroxide were first added to an aqueous solution of maleic acid (0.1 M). The pH was fine-tuned to 6.0 using a 1 M NaOH aqueous solution. This reaction solution was thoroughly degassed with Ar (0.1 L min^{-1} for 5 min) prior to use. The reaction vial (30 mL, HSH-10VA, AS ONE, Osaka, Japan), equipped with stirring speed and temperature controllers, was added to an aqueous solution containing maleic acid (0.4 mmol) and cysteine catalyst (0.01 mmol). The mixture was kept under a steady flow of Ar (0.1 L min^{-1}) at 315 K and stirred at 400 rpm. The reaction was initiated by adding an aqueous solution of AAPH (0.04 mmol), making the total volume 20 mL. Ar degassing/purge was continued for the subsequent 5 min after starting the reaction, and the vial was sealed with a Teflon stopper. The same procedure was employed for angelic acid/tiglic acid and muconic acid.

Photochemical Reaction for Cysteine-Catalyzed Isomerization

The isomerization reaction under visible light irradiation was conducted using an LED light source, LA-HDF158A (Hayashi-Repic Co., Ltd., Tokyo, Japan). Tris(2,2'-bipyridyl)ruthenium dichloride hexahydrate (18.0 mg, 0.024 mmol) was added to 3 mL of 80 mM maleic acid aqueous solution (pH 7) in a transparent glass vial and then made up to a total volume of 11.4 mL using pure water. The aqueous solution was degassed in the vial with Ar, as described above. A previously prepared and degassed aqueous solution of

10 mM thiol catalyst (0.6 mL) was carefully added into the vial, and degassing was continued for an additional 5 min. The reaction was started by irradiation of white light using an LED light source through an optical fiber. The light intensity was set to 26 W, which is the maximum value of this light source. The reaction was continued at room temperature with stirring using a stir bar.

HPLC Analysis

All samples from the reaction vial were analyzed and quantified using HPLC in a GL Sciences chromatographic system (GL-7400, Tokyo, Japan). The system was equipped with a high-pressure pump that forced the mobile phase (5 vol% of 1% phosphoric acid aqueous solution and 95 vol% of acetonitrile) to flow at 1 mL min^{-1}, an oven to maintain the temperature of the column at 313 K, and a UV detector to monitor the eluted components at 230 nm. An InertSustain C18 column (GL Sciences, 25 cm × 4.6 mm i.d., 5 μm) was used to separate maleic acid, fumaric acids, AAPH, cysteine catalysts, and their adducts. For muconic acid, UV signals at 260 nm were monitored for the analysis of the eluted components, and a CapcellPak C18 column (Osaka Soda, 25 cm × 4.6 mm i.d., 5 μm) was used as the stationary phase.

LC-MS Analysis

A thermo Fischer Scientific ICS-3000 was used for HPLC, while mass spectrometry was recorded on a Thermo Fischer Scientific TSQ Qantis Plus. It was basically equipped with a high-pressure pump that forced the mobile phase (5 vol% of acetonitrile and 95 vol% of 0.1 M formic acid aqueous solution) to flow at 0.2 mL min^{-1} and an oven to maintain the temperature of the column at 313 K. An InertSustain C18 column (GL Sciences, 15 cm × 2.1 mm i.d., 3 μm) was used to separate the products. The aliquot of the 10 μL solution was used for analysis. Mass spectrometry was conducted in ESI-positive/negative mode, with a capillary temperature of 275 ºC and a capillary voltage of 40 V for the positive mode and –40 V for the negative mode.

Bulk Synthesis

Gram-scale synthesis of fumaric acid from maleic acid was carried out under the optimized conditions using the thiyl radical at a 0.25 M scale of maleic acid (2.32 g, 20.0 mmol) which is based on the condition previously reported.[S2] Cysteine (242 mg, 2.0 mmol) and AIPH initiator (388 mg, 1.2 mmol) were added to a neutral aqueous solution of 0.25 M of maleic acid (80 mL in total; the pH was adjusted to 7 using 5 M NaOH) degassed with Ar (99.9999%) at 100 mL min^{-1}. The mixture was reacted at 42 ºC for 1 h. After the reaction, the pH of the reaction solution was adjusted to <1 using concentrated hydrochloric acid to precipitate the product for 3 h in a refrigerator. Owing to the low solubility of fumaric acid (0.63 g in 100 g of water at 25 ºC, corresponding to ~54 mM) as compared with that of maleic acid (78.9 g in 100 g of water at 25 ºC),[S3] the obtained product was efficiently precipitated. The obtained product was dried at 40 ºC under reduced pressure until there was no further weight loss to give fumaric acid as a white solid (1.81 ± 0.03 g, 15.6 mmol). The melting point of fumaric acid was reported to be 286–287 ºC.[S1] The melting point of the obtained product was measured on a melting point apparatus (Yanaco MP-J3, micro melting point apparatus), using phenolphthalein as an external standard (263.4–264.2 ºC).

S1.3 DFT Calculations

Density functional theory (DFT) calculations were conducted to determine the radical reaction paths of *L*-cysteine-catalyzed maleate isomerization with Gaussian 16, Revision C.01.[S4] The B3LYP/6-311G(d,p) level of theory was employed. The solvent effect of water was incorporated using the polarizable continuum model. The thiyl radical of *L*-cysteine was employed as the molecular catalyst. Geometry optimizations and vibrational analyses were performed for the reactants, transition states, intermediates, and products. For comparison, the thermodynamic stabilities of the intermediates were also calculated in the non-radical case using the thiolate of *L*-cysteine as the catalyst at the same level of theory.

S2. Supplementary Figures, Tables, and Discussions

S2.1 Preliminary Investigation under Non-radical Conditions with Excess Thiols

Our preliminary investigation indicated that thiol-based molecules were generally able to convert maleate to fumarate to a certain extent when excess amounts of thiols were used. Later, we found that cysteine and its analogs showed much better activity toward the isomerization reactions, as shown in Figure S1. We recognized the gap between the decrease of maleate and the increase of fumarate (Figure S2), and this could be partly rationalized by the additional formation of adducts between maleate and thiols, which limited the quantitative conversion of maleate to fumarate (Figures S2–S4).

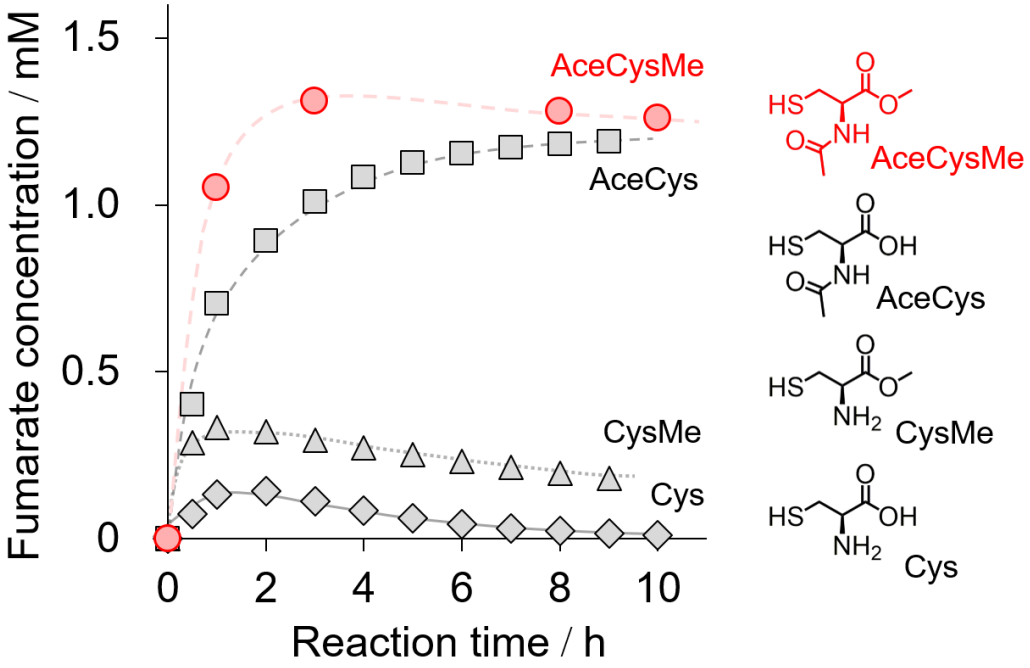

Figure S1. Time-course of quantified compounds during isomerization reactions from maleate (2.5 mM) to fumarate using excess amounts of various thiol-based molecules (15 mM), *N*-acetyl cysteine methyl ester (AceCysMe, red circle), *N*-acetyl cysteine (AceCys, gray square), cysteine methyl ester (CysMe, gray triangle), and pristine cysteine (Cys, gray rotated square). Initial pH = 6.8–7.1; reaction temperature = 42 ºC. The concentration of each compound was determined by HPLC.

S2.2 Preliminary Investigation under Non-radical Conditions with Excess Maleate

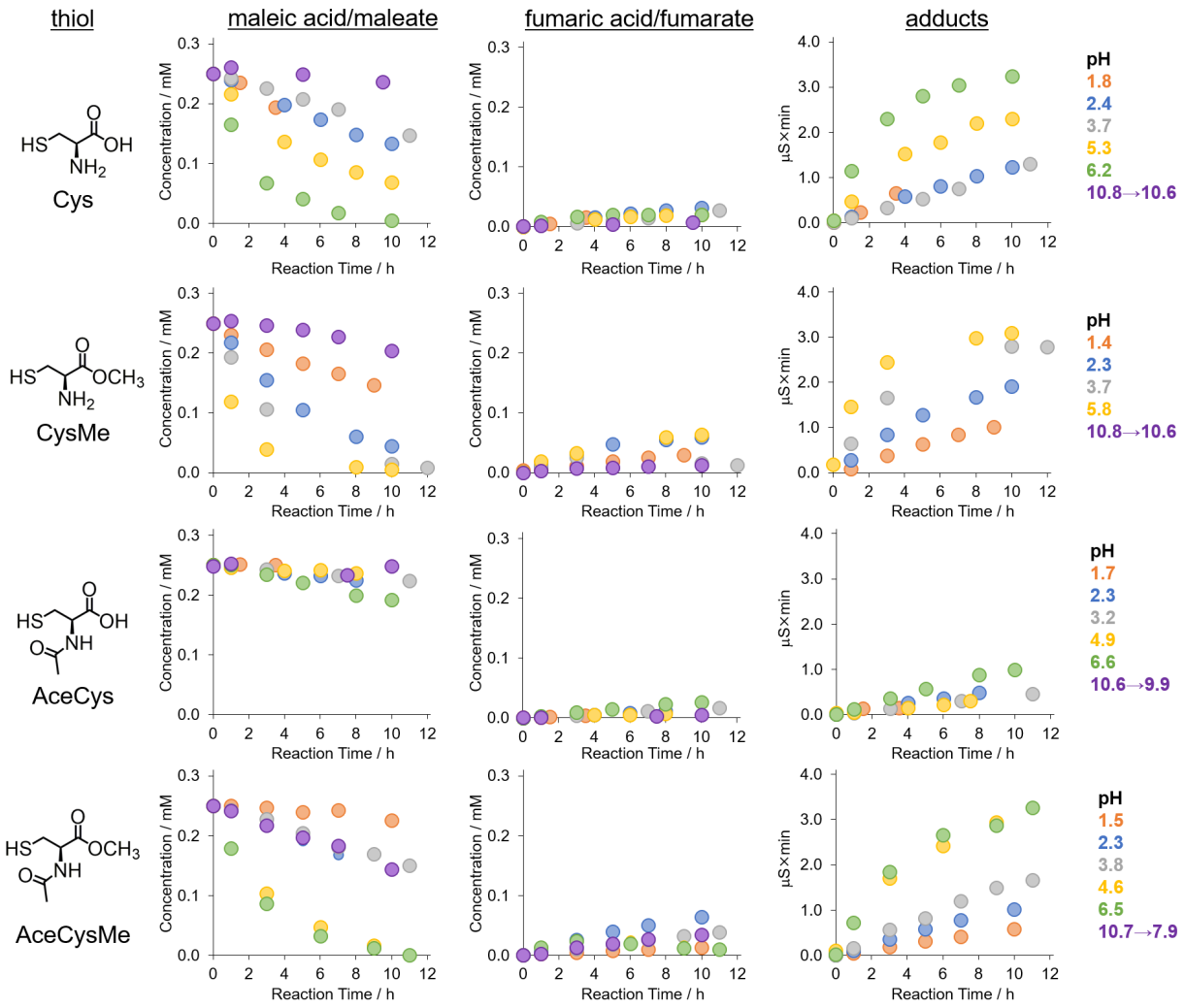

Figure S2. Time-course of quantified compounds during reactions of various thiol-based molecules with maleic acid under various pH conditions. $[Mal]_0$ = 2.5 mM; $[Cys]_0$ = 2.5 mM; reaction temperature = 42 ºC.

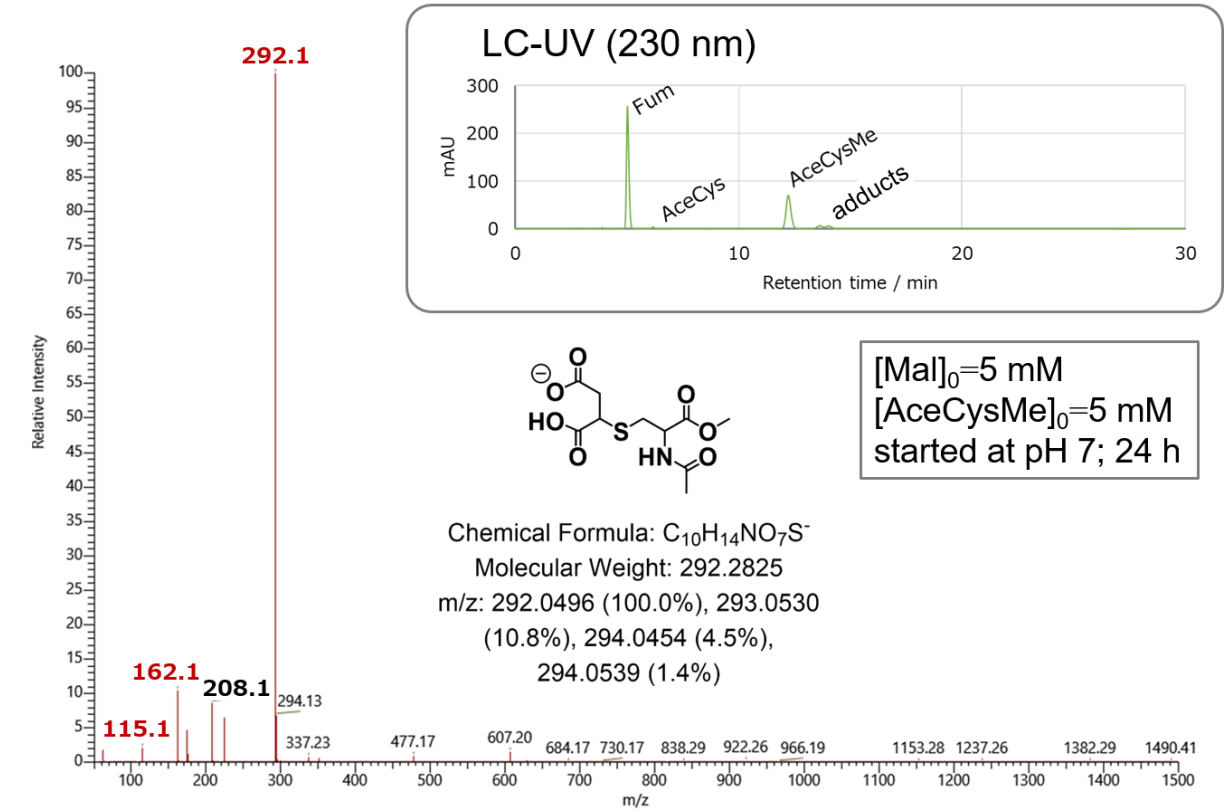

Figure S3. ESI-MS of a reaction product obtained by reacting 5 mM of maleic acid (pH 7) with 5 mM of *N*-acetyl-*L*-cysteine methyl ester (AceCysMe) for 24 h at 42 °C. The solution for analysis was diluted 100-times by acetonitrile.

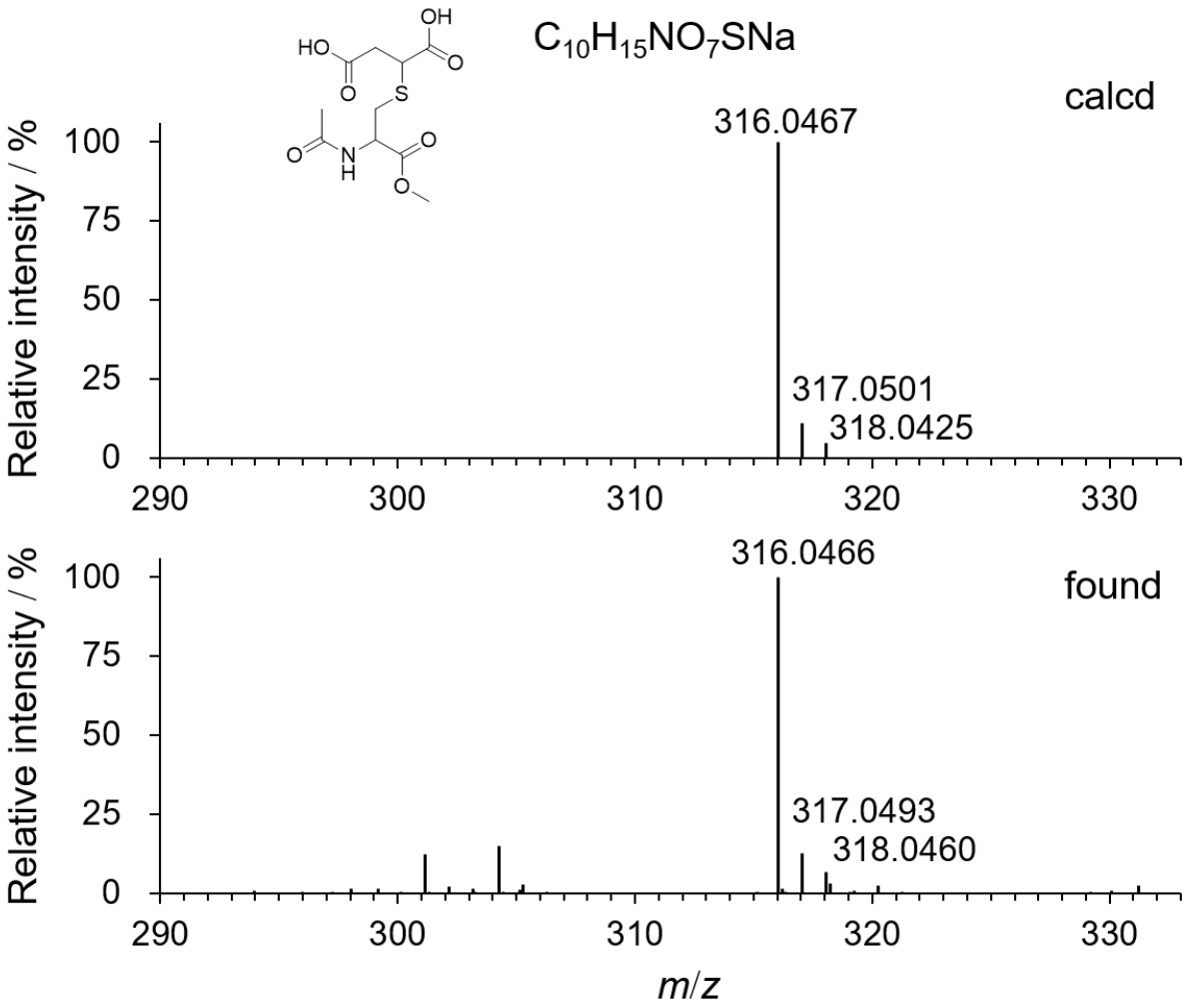

Figure S4. High-resolution TOF-MS of the reaction by-products obtained by reacting 5 mM of maleic acid (pH 7) with 5 mM of *N*-acetyl-*L*-cysteine methyl ester (AceCysMe) followed by conducting conventional LC separation seven times and drying under Ar flow.

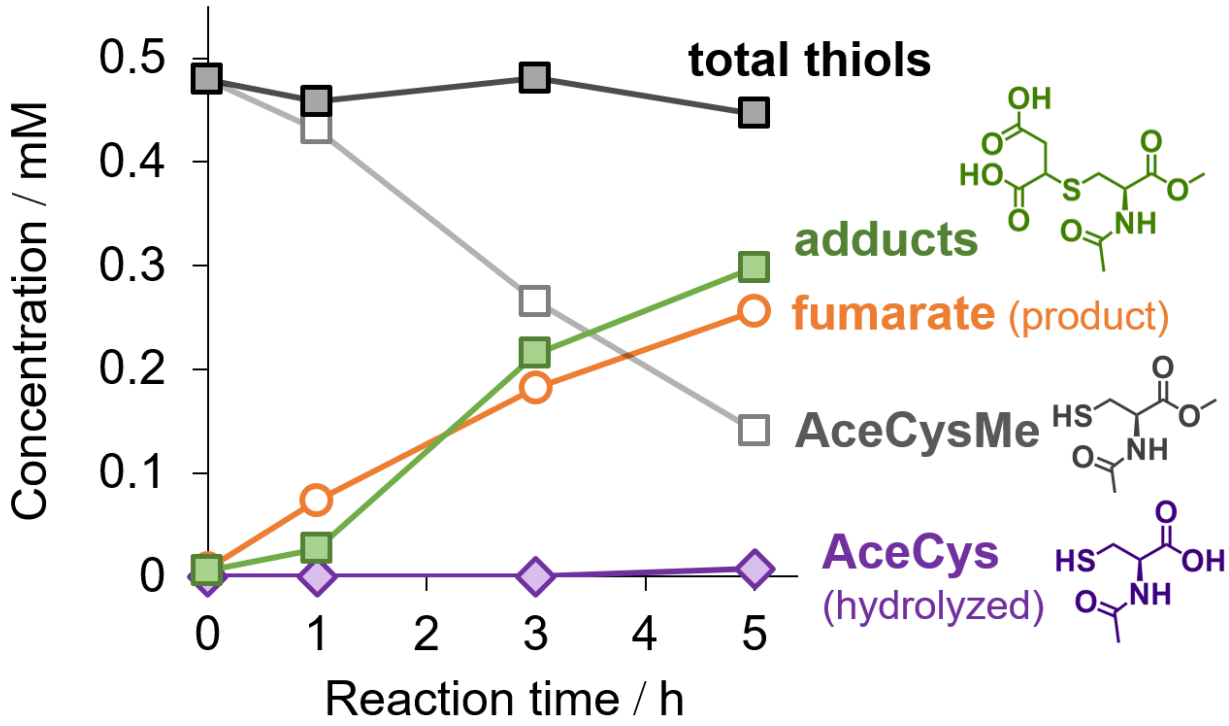

Figure S5. Time-course of the quantified compounds during isomerization reactions using AceCysMe (0.5 mM) with MeCN/H_2O 1+1 as the solvent. The "total thiols" is the sum of adducts, AceCysMe, and AceCys at each time. $[Mal]_0$ = 20 mM; initial pH = 7.1–7.2; reaction temperature = 42 ºC.

Table S1. Summary of molecular thiol- and thiyl-mediated isomerization reactions of C=C bonds.

catalyst		reactant (conc/mM)	solvent/irradiation	pH	Temp	yield	TON	references
2-mercaptoethylamine	20 mM[a]	oleic acid (0.5)	phosphate buffer	7.0	37 ºC	53%	<0.025	Niehaus[S5]
dithiothreitol	10 mM[a]	oleic acid (0.5)	phosphate buffer	7.0	37 ºC	27%	<0.050	Niehaus[S5]
2-mercaptoethanol	20 mM[a]	oleic acid (0.5)	phosphate buffer	7.0	37 ºC	16%	<0.025	Niehaus[S5]
	20 mM+tBuOH	methyl linoleate (50)	IPA+UV irradiation	-	20 ºC	49%	1.2	Chatgilialoglu[S6]
	20 mM	methyl linoleate (50)	IPA+2 kGy[g]	-	22 ºC	49%	0.11	Chatgilialoglu[S6]
	7 mM	DOPC[h] (150)	IPA+26.6 kGy[g]	-	22 ºC	78%	17	Chatgilialoglu[S7]
	75 mM	methyl linoleate (150)	*t*-BuOH+23.3 kGy[g]	-	22 ºC	42%	0.8	Ferreri[S8]
	7 mM	PC[j] (150)	IPA+8 kGy[g]	-	22 ºC	12%	2.5	Ferreri[S8]
	75 mM	oleic acid (150)	*t*-BuOH+3.6 kGy[g]	-	-	85%	1.7	Chatgilialoglu[S9]
	0.75 M	methyl oleate (150)	*t*-BuOH +UV	-	20 ºC	83%	1.7	Chatgilialoglu[S10]
	7 mM	PC[j] (150)	IPA	-	22 ºC	-	-	Ferreri[S11]
	320 µM	9-*cis*-RA[k] (8.5 nM)	methanol+triton X-	7.6	37 ºC	69	<0.001	Shih[S12]
	25 nM	9-*cis*-RA[k] (60 µM)	100/tris-HCl buffer	7.6	37 ºC	16	0.056	Shih[S12]
2,3-dimercaptopropanol	10 mM[a]	oleic acid (0.5)	phosphate buffer	7.0	37 ºC	7%	<0.050	Niehaus[S5]
thiols, 5–30 mM	with sat. N_2O	PUFAs[c] (< 5)	mixture[d]	5.0	-	-	<1	Schöneich[S13]
dodecanethiol	1.0 M	methyl oleate	none	-	89 ºC	45%	0.5[b]	Biermann[S14]
	1.0 M / AIBN	methyl oleate	none	-	89 ºC	81%	0.9[b]	Biermann[S14]
benzenethiol	75 mM / AIBN	methyl oleate (150)	*t*-BuOH	-	71 ºC	87%	1.7	Chatgilialoglu[S7]
	75 mM / AMVN[i]	methyl linoleate (150)	*t*-BuOH	-	54 ºC	5.3%	1.1	Ferreri[S8]
glutathione (GSH)	7 mM	DOPC[h] (150)	IPA+26.6 kGy[g]	-	22 ºC	51%	11	Chatgilialoglu[S7]
	7 mM	PC[j] (150)	IPA+24.7 kGy[g]	-	22 ºC	16%	3.4	Ferreri[S8]
$(PhS)_2$	0.25 M	dimethyl maleate (50)	hexane+UV	-	69 ºC	93%	0.19	Harrowven[S15]
	0.25 M	diethyl maleate (50)	hexane+UV	-	69 ºC	99%	0.20	Harrowven[S15]
L-cysteine	75 mM	DOPC[h] (150)	phosphate buffer	7.2	37 ºC	2.7%	0.05	Chatgilialoglu[S7]
	7 mM	DOPC[h] (150)	IPA+26.6 kGy[g]	-	22 ºC	14%	3.1	Chatgilialoglu[S7]
	15 mM	maleate (2.5)	H_2O	7	42 ºC	5.3%	0.06	*This work*
	0.5 mM / AAPH[f]	maleate (20)	H_2O	7	42 ºC	**98%**	**39**	*This work*
	0.5 mM / AAPH[f]	maleate (20)	phosphate buffer	7	42 ºC	64%	26	*This work*
N-acetyl cysteine methyl ester	15 mM	maleate (2.5)	H_2O	7	42 ºC	52%	0.52	*This work*
	0.5 mM	maleate (20)	H_2O	7	42 ºC	3.3%	1.3	*This work*
	0.5 mM / AIBN[f]	maleate (20)	mixture[e]	7	42 ºC	6.6%	2.7	*This work*
	0.5 mM / AAPH[f]	maleate (20)	H_2O	7	42 ºC	**98%**	**39**	*This work*

[a] Original data: 20 meq L^{-1}; [b] No results for the control experiments were demonstrated in the literature, and therefore, it is not clear whether this reaction is genuinely catalytic; [c] various polyunsaturated fatty acids; [d] The solvent was a 50vol% ethanol aqueous solution; [e] The solvent was a 50vol% CH_3CN aqueous solution. [f] 2.0 mM; [g] γ-radiolysis with saturated N_2O; [h] dioleoyl phosphatidyl choline; [i] AMVN: 2,2'-azobis(2,4-dimethylvaleronitrile); [j] *L*-α-phosphatidylcholine; [k] 9-*cis*-retinoic acid.

S2.3 Effect of Oxygen under Radical Conditions

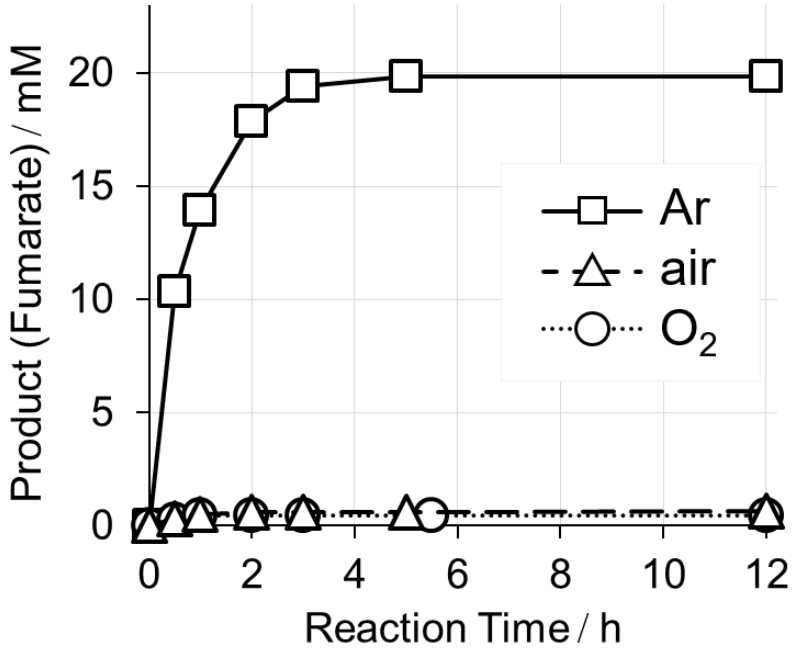

Figure S6. Time-course of the isomerization of maleate to fumarate in the presence of Ar (square, solid line), air (triangle, dashed line), and O_2 (circle, dotted line). $[Mal]_0$ = 20 mM; $[AceCysMe]_0$ = 0.5 mM; $[AAPH]_0$ = 2 mM; initial pH = 7.1; reaction temperature = 42 ℃.

S2.4 Catalysis under Photochemical Conditions

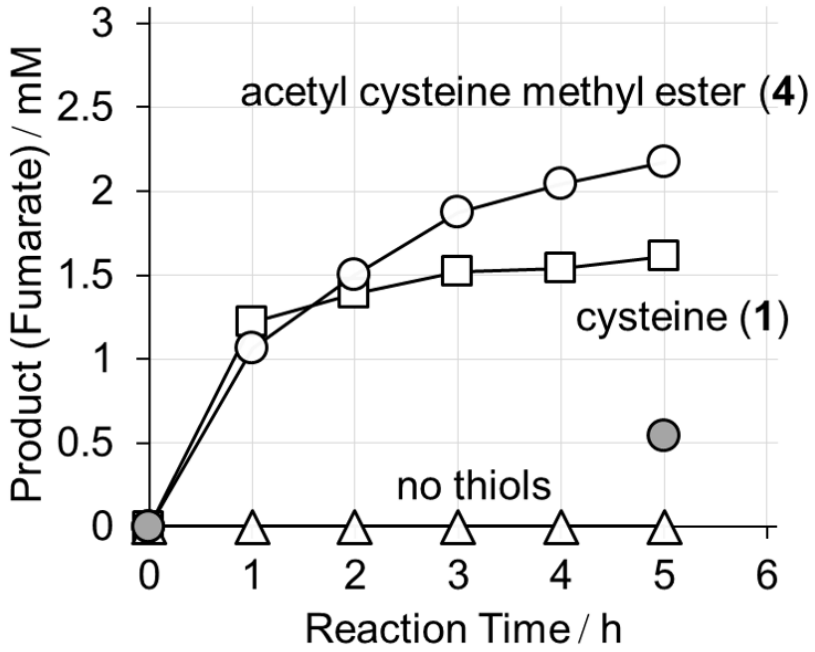

Figure S7. Time-course of the isomerization of maleate to fumarate in the presence of 0.5 mM acetyl cysteine methyl ester (**4**, circle), 0.5 mM cysteine (**1**, square), and in the absence of thiols (triangle) under LED white light illumination (white outline) or in the dark (gray outline). For the dark experiment, only the result at 5 h was measured to exclude accidental irradiation. $[Mal]_0$ = 20 mM; $[Cys]_0$ = $[AceCysMe]_0$ = 0.5 mM; $[Ru(bpy)_3Cl_2]_0$ = 2.0 mM; initial pH = 7; initial reaction temperature = 25 °C.

S2.5 Catalysis under Radical Conditions

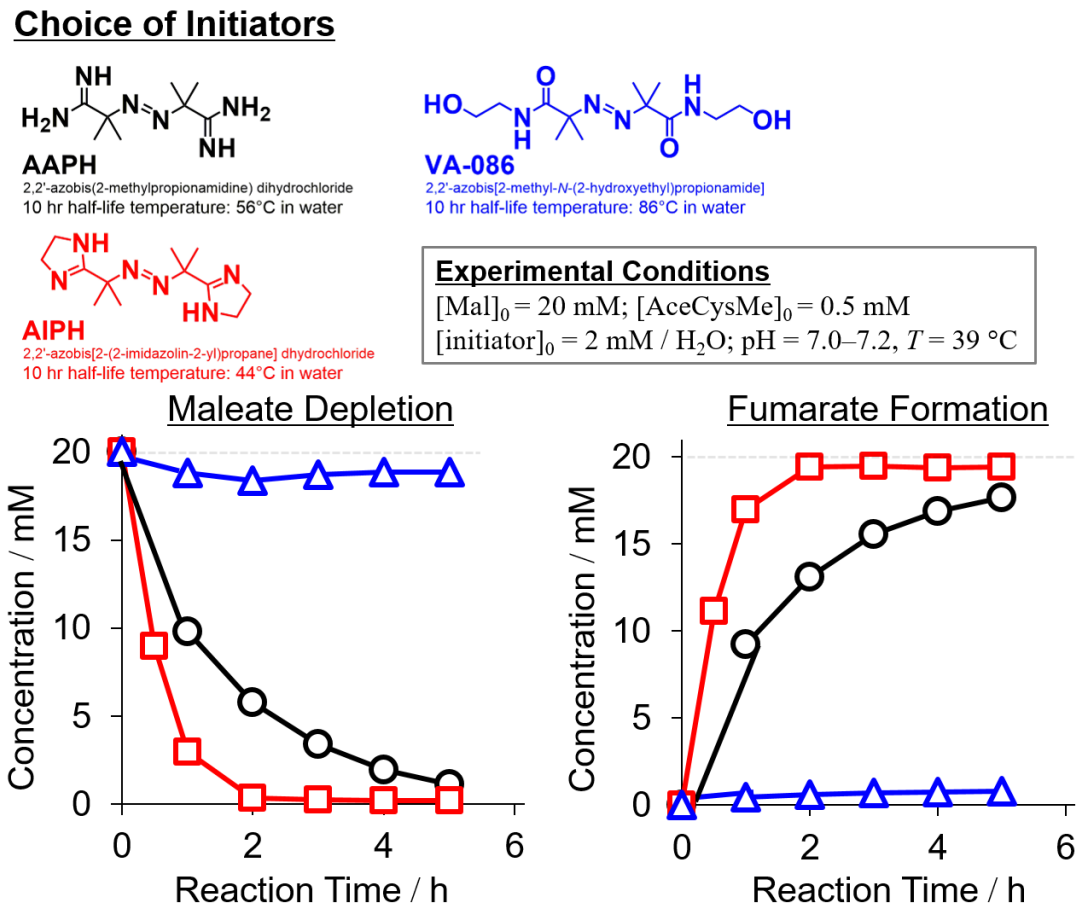

Figure S8. Choice of the radical initiators on the rate of the reactions. AAPH (black circle), AIPH (red square), and VA-086 (blue triangle).

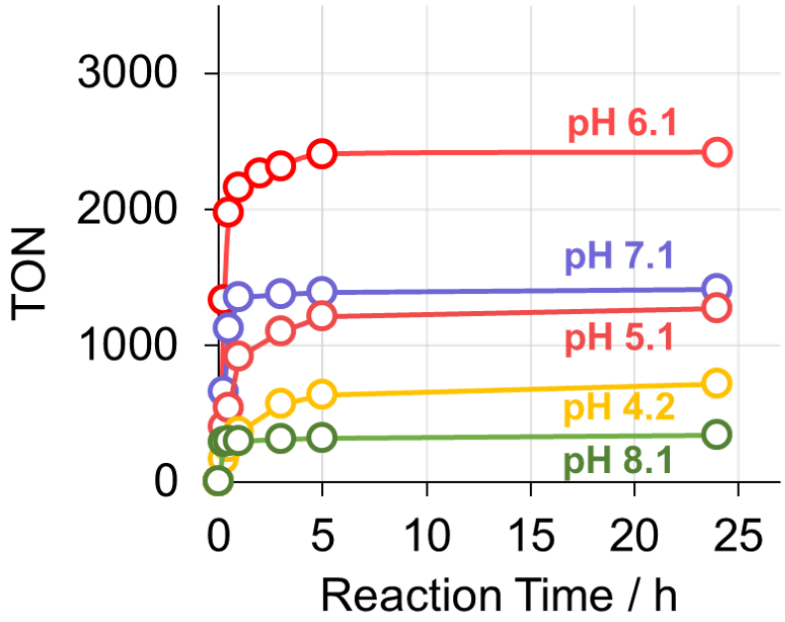

Figure S9. pH dependence of the *cis-trans* isomerization reactions. $[Mal]_0 = 20$ mM; $[\mathbf{1}]_0 = 2$ mM; $[AIPH]_0 = 0.005$ mM; reaction temperature = 39 °C.

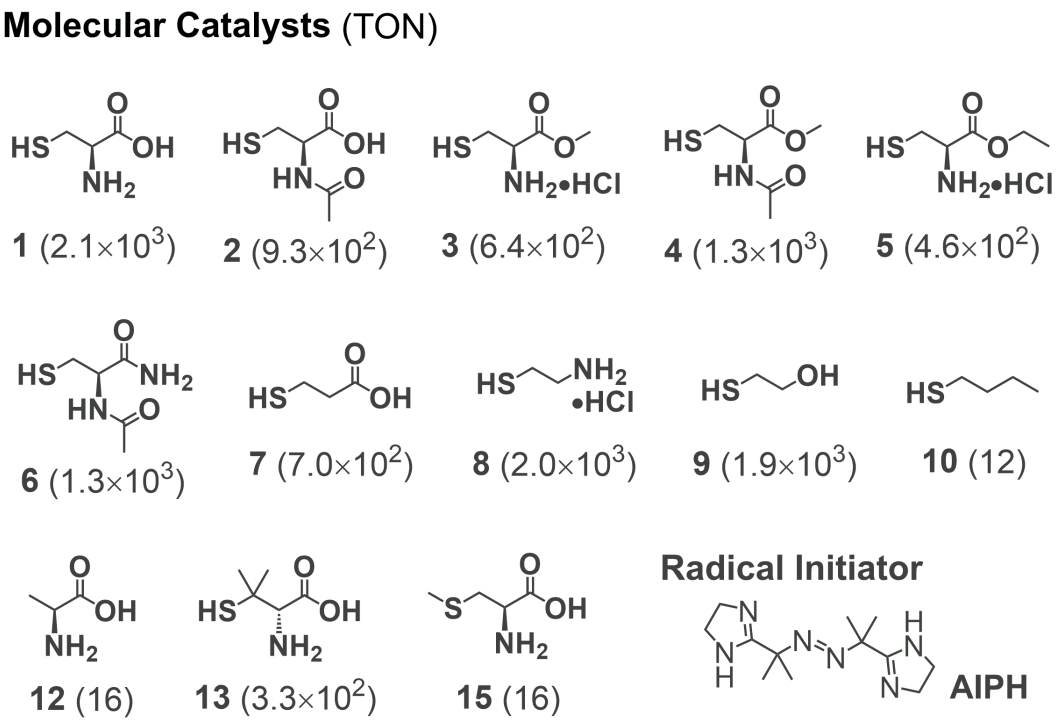

Figure S10. Catalytic investigation using various thiol-based molecular catalysts for the radical-based *cis-trans* isomerization reactions. The values in parentheses are the catalyst-molecule-based TON of the reactions at 30 min. AIPH, instead of AAPH, was used as the initiator based on the optimization as shown in Figure S8. Initial pH = 6.1; reaction temperature = 39 °C.

Table S2. Summary of the time-course of the thiyl-based catalytic isomerization reactions using cysteine (**1**) as the molecular catalyst in the presence of various concentrations of the radical initiator (AIPH). $[Mal]_0$ = 20 mM, $[Cat]_0$ = 0.005 mM; initial pH = 6.1; reaction temperature = 39 ºC.

initiator (AIPH) concentration c / mM	ln(c / mM)	time elapsed					
		0.5 h	1 h	2 h	3 h	5 h	24 h
0.05	-2.9957	116	203	235	245	250	259
0.16	-1.8326	375	546	578	606	633	664
0.28	-1.2730	1014	1263	1341	1368	1415	1455
0.5	-0.6931	1990	2188	2327	2380	2429	2445
2.0	0.6931	2015	2246	2392	2445	2489	2521
5.0	1.6094	2303	2499	2613	2658	2689	2621
9.0	2.1972	2006	2145	2205	2247	2261	2223
16	2.7726	968	1027	1056	1083	1119	1232
50	3.9120	312	328	346	361	384	472

Table S3. Summary of catalytic isomerization from maleate to fumarate.

catalyst	additives			maleate	T / ºC	pH	t / h	TON	TOF / s^{-1}	references
thiourea	1.86%	-	-	30-31%	50	-[b]	2	49[c]	-[b]	Schliesser (1962)[S16]
1-allyl-2-thiourea	2%	-	-	30-31%	50	-[b]	2	40	-[b]	Schliesser (1962)[S16]
bromine	24 mM	H_2SO_4	1.0 M	1.17 M	25	-[b]	-[b]	43	-[b]	Jwo (1983)[S17]
N-bromosuccinimide	67 mM	H_2SO_4	1.2 M	1.0 M	25	-[b]	-[b]	12	-[b]	Jwo (1983)[S18]
NH_4Br	19.6 mM	$(NH_4)_2S_2O_8$	16.8 mM	3.07 M[a]	60	-[b]	1	148	>0.04	González-Velasco (1991)[S2]
	6.2 mM	$(NH_4)_2S_2O_8$	5.3 mM	3.07 M[a]	65	-[b]	1	459	>0.13	González-Velasco (1991)[S2]
	3.9 mM	$(NH_4)_2S_2O_8$	16.8 mM	3.07 M[a]	75	-[b]	1	720	>0.20	González-Velasco (1991)[S2]
CH_2Br_2	saturated	ultrasonic assist		1.0 M	-	-		~0	-[b]	Troia (2006)[S19]
poly(4-vinylpyridine)	1 g/1 L	-	-	6.9 mM	60	1.5	26	-	-[b]	Shuang (2014)[S20]
thiourea	4%	-		0.5 M	40			<1	-[b]	Kartnaller (2020)[S21]
$BiVO_4/WO_3$/FTO + NaBr (100 mM) + $h\nu$ (100 mW cm^{-2})[d]				15 mM	-	-	1.5	0.13	-[b]	Sayama (2021)[S22]
N-acetyl cysteine methyl ester (**4**)	0.5 mM	-	2 mM	20 mM	42	7		1.3	0.0003	*This work*
	0.5 mM	AAPH	2 mM	20 mM	42	7		39	0.006	*This work*
	5 µM	AIPH	2 mM	20 mM	39	6.1		**1,600**	0.72	*This work*
L-cysteine (**1**)	5 µM	AIPH	2 mM	20 mM	39	8.1		340	0.16	*This work*
	5 µM	AIPH	2 mM	20 mM	39	7.1		**1,400**	0.63	*This work*
	5 µM	AIPH	2 mM	20 mM	39	6.1		**2,500**	**1.1**	*This work*
	5 µM	AIPH	2 mM	20 mM	39	5.1		**1,300**	0.30	*This work*
	5 µM	AIPH	2 mM	20 mM	39	4.2		720	0.14	*This work*

[a] The reaction was carried out with a 0.2 L scale; [b] the information is unknown; [c] the value is based on another publication.[S21] ; [d] illumination using a solar-simulator (calibrated to AM 1.5, 100 mW cm^{-2})

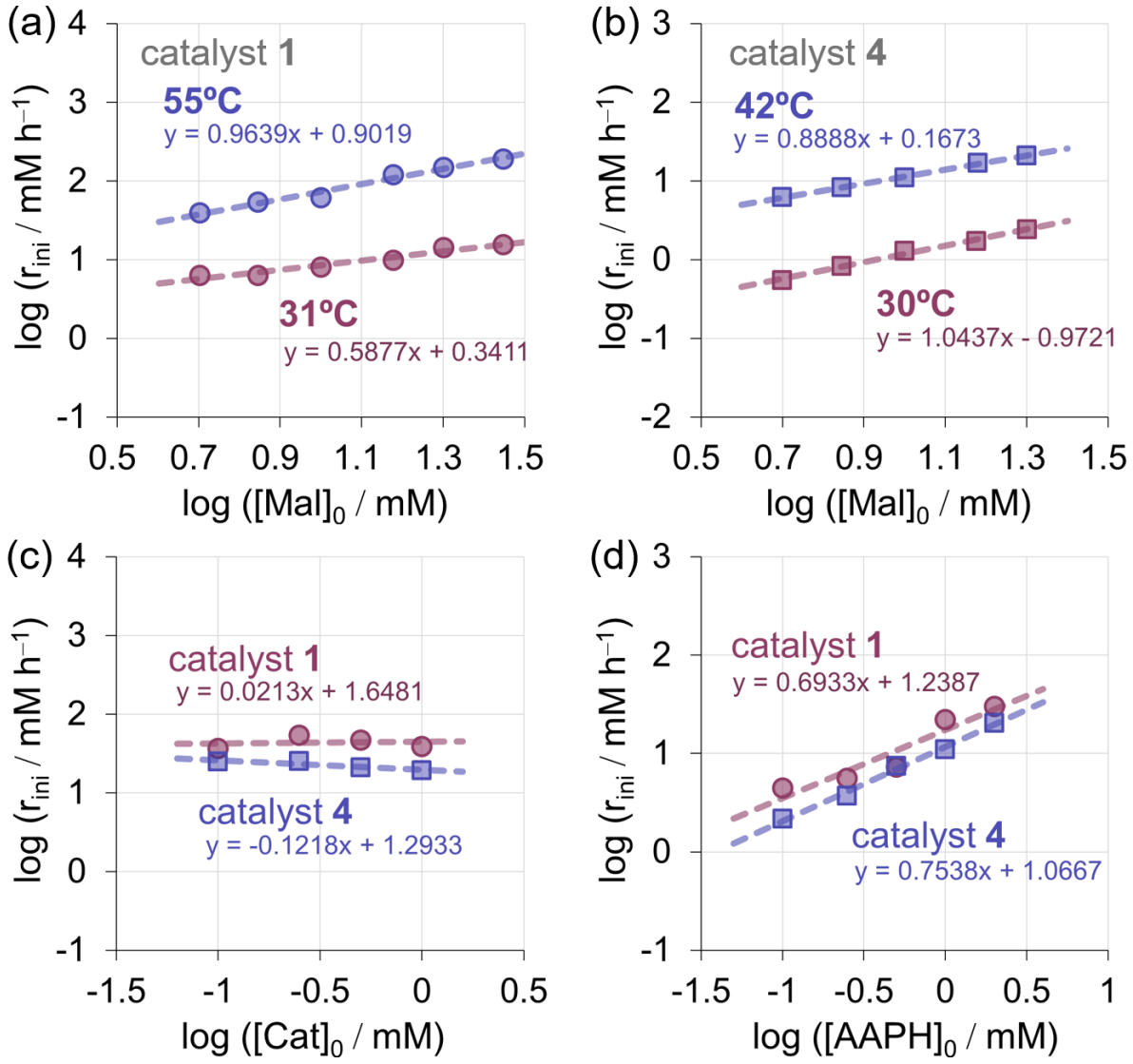

Figure S11. Dependence of the rate of reactions on the concentration of (a,b) maleic acid, (c) catalysts, and (d) radical initiators, with thiol **4** (square) or **1** (circle) as the molecular catalyst. Initial pH = 6.1; reaction temperature = 42 ºC.

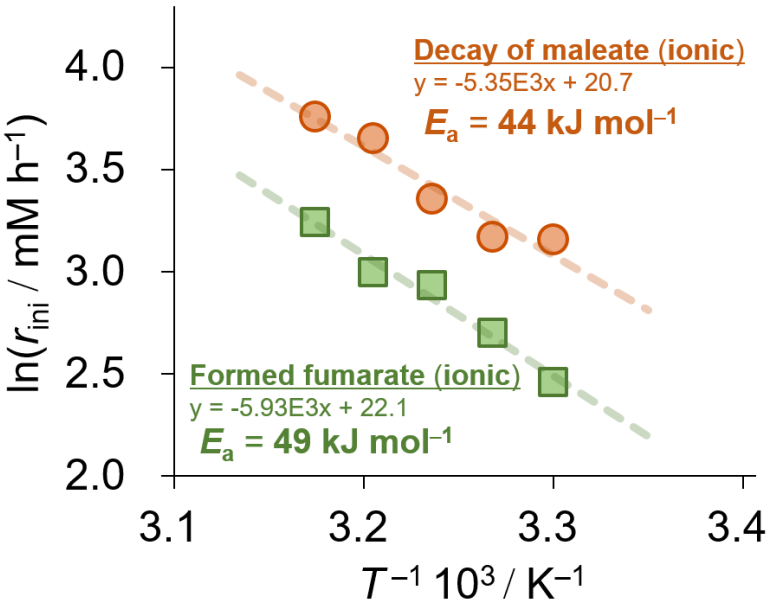

Figure S12. Arrhenius plots for the maleate depletion (orange) and fumarate formation (green) under non-radical conditions. $[Mal]_0$ = 20 mM; $[AceCysMe]_0$ = 20 mM; $[AAPH]_0$ = 0 mM; reaction time = 5 min; initial pH = 7.1; reaction temperature = 42 °C.

Table S4. Summary of the calculated energies of the thiyl process shown in Figure 7a.

species		energy in Hartree			activation energy in eV	
		total energy	enthalpy	Gibbs energy	enthalpy	Gibbs energy
reactants	thiyl radical of **1**	-721.425325314	-721.318228	-721.360702		
	maleic acid	-455.886851851	-455.795930	-455.836575		
	total	-1177.312177165	-1177.114158	-1177.197277		
before insertion		-1177.321994910	-1177.122505	-1177.188460		
TS of insertion		-1177.302942170	-1177.103141	-1177.167348	0.518451	0.574487
intermediate (maleate)		-1177.325263880	-1177.124695	-1177.186263		
TS of isomerization		-1177.304888060	-1177.105770	-1177.167297	0.554454	0.516091
intermediate (fumarate)		-1177.319571890	-1177.119118	-1177.182597		
after elimination		-1177.304766770	-1177.105364	-1177.169150	0.402868	0.365912
products	thiyl radical of **1**	-721.425325314	-721.318228	-721.360702		
	fumaric acid	-455.881946325	-455.791049	-455.833254		
	total	-1177.307271639	-1177.109277	-1177.193956		

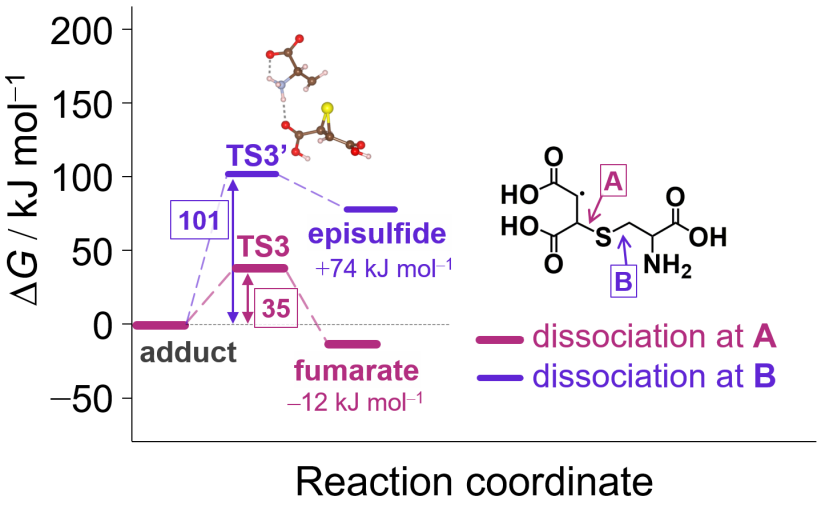

Figure S13. Energy diagram of the elimination steps for **TS3** and **TS3'** with the *L*-cysteine (**1**)-thiyl radical. Calculated using the polarizable continuum model for implicit solvents.

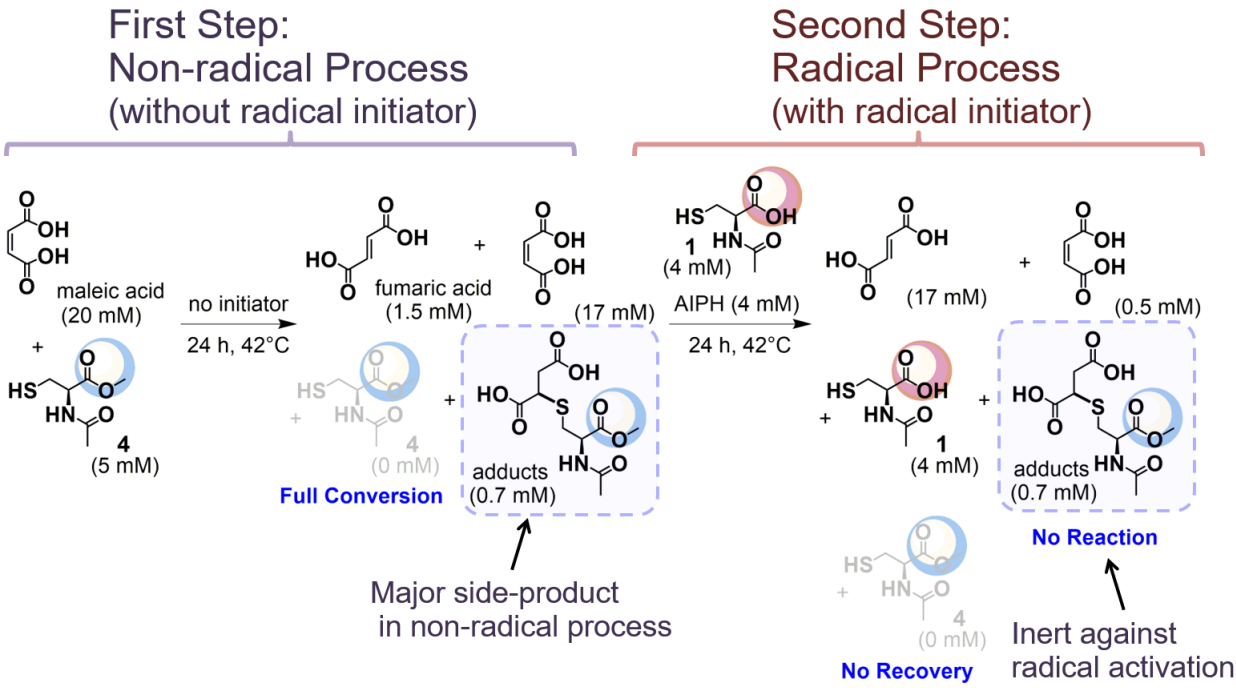

Figure S14. Mechanistic investigation using two-step experiments. This result supports that the formed 1,2-adducts via the first-step non-radical condition are stable against the second-step radical activation.

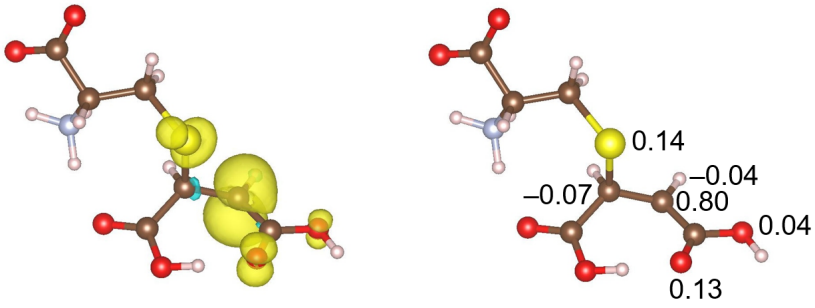

Figure S15. Spin density of the thiyl adduct-(*Z*).

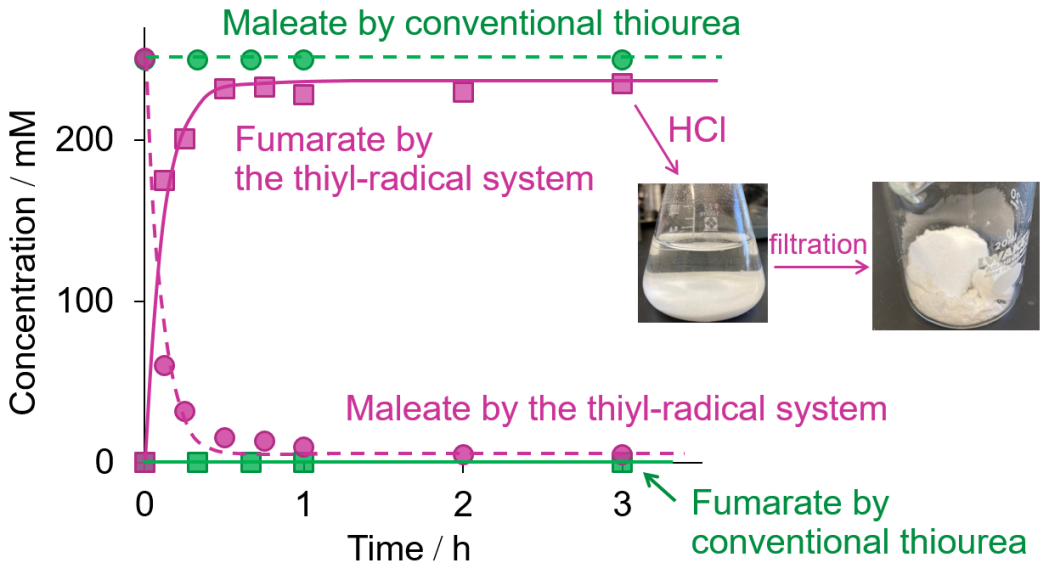

Figure S16. Time-course of the gram-scale synthesis of fumaric acid from maleic acid in the presence of the thiyl-radical (purple) or thiourea (green). $[Mal]_0 = 0.25$ M; $[Cysteine]_0 = 0.025$ M and $[AIPH]_0 = 0.015$ M for the thiyl-radical system; $[Thiourea]_0 = 0.025$ M for the thiourea-based system; total volume: 80 mL; initial pH = 6; reaction temperature: 42 ºC. The time-course of the gram-scale synthesis was obtained independent of other experiments for determining the isolated yield of 78%.

Table S5. Scalability of the thiyl-radical catalyzed isomerization of maleic acid.

entry	reaction volume	maleic acid		catalyst	initiator	Temp.	LC		isolated yield
		scale	quantity				conv.	yield	
1	20 mL	2.0 mmol (**0.10 M**)	0.23 g	cysteine **1** 0.2 mmol	AIPH 0.12 mmol	42 ºC	98% (1 h)	96% (1 h)	-
2	20 mL	5.0 mmol (**0.25 M**)	0.58 g	cysteine **1** 0.5 mmol	AIPH 0.30 mmol	42 ºC	96% (1 h)	91% (1 h)	-
3	26 mL	7.9 mmol (**0.30 M**)	0.92 g	cysteine **1** 0.79 mmol	AIPH 0.47 mmol	42 ºC	63% (1 h)	60% (1 h)	-
4	20 mL	20 mmol (**1.0 M**)	2.32 g	cysteine **1** 1.0 mmol	AIPH 1.2 mmol	42 ºC	30% (3 h)	27% (3 h)	-
5	**50 mL**	12.5 mmol (0.25 M)	1.45 g	cysteine **1** 1.3 mmol	AIPH 0.75 mmol	40 ºC	86% (3 h)	85% (3 h)	53%
6	**74 mL**	18.6 mmol (0.25 M)	2.16 g	cysteine **1** 1.9 mmol	AIPH 1.1 mmol	42 ºC	91% (1 h)	81% (1 h)	73%
7	**80 mL**	20.0 mmol (0.25 M)	2.32 g	cysteine **1** 2.0 mmol	AIPH 1.2 mmol	42 ºC	99% (1 h)	90% (1 h)	78±1.2%
8	**200 mL**	50.0 mmol (0.25 M)	5.80 g	cysteine **1** 5.0 mmol	AIPH 3.0 mmol	42 ºC	98% (1 h)	81% (1 h)	70%

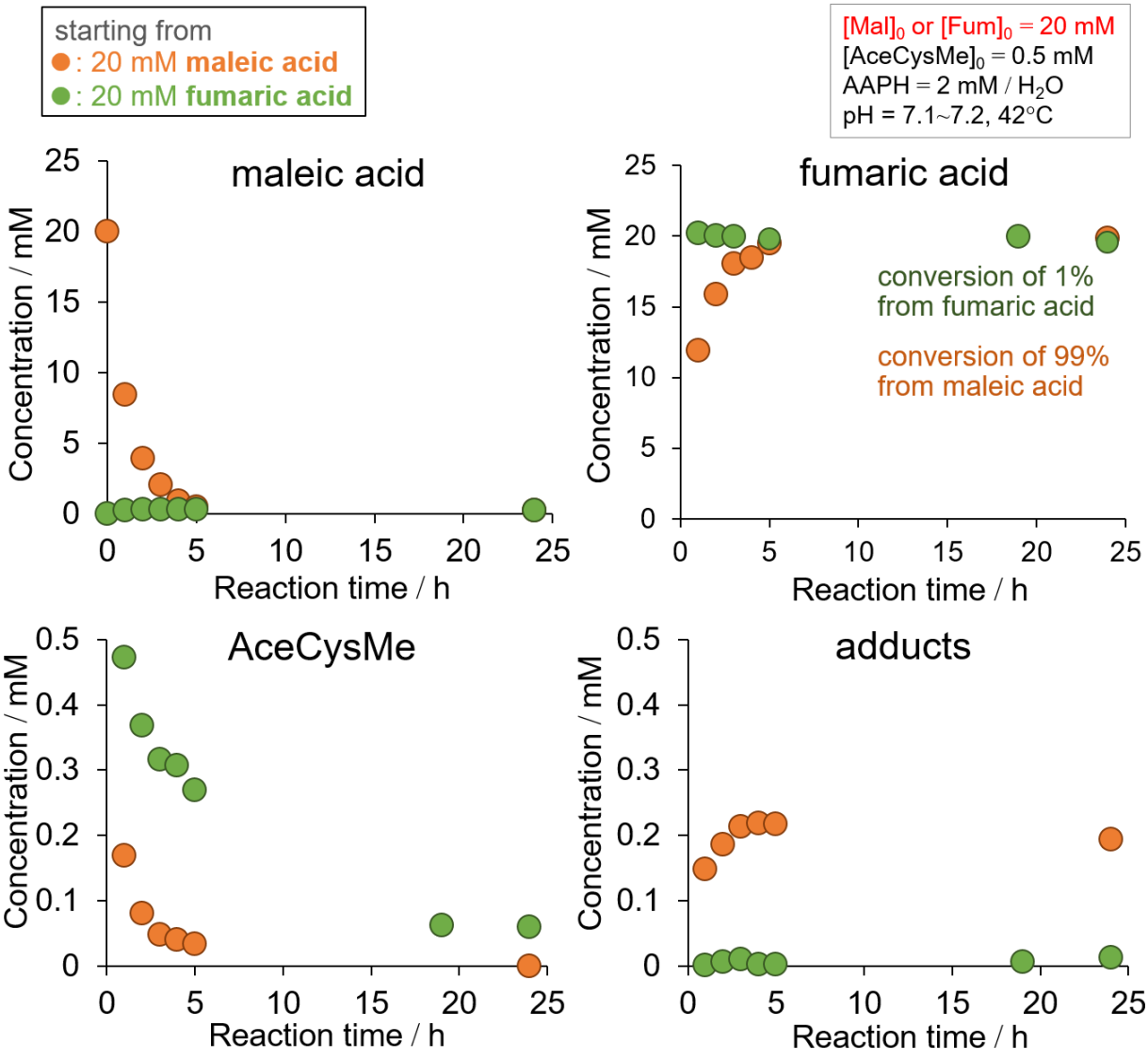

Figure S17. Time-course of the reaction starting from maleate (orange trace) or fumarate (green trace). $[Mal]_0$ or $[Fum]_0$ = 20 mM; $[AceCysMe]_0$ = 0.5 mM; $[AAPH]_0$ = 2 mM; initial pH = 7.1–7.2; reaction temperature = 42 °C.

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S4. Appendix: Optimized Structures in Quantum Chemistry Calculations

Optimized Structures for Figure 7a

Coordinate for maleic acid and thiyl radical of cysteine before insertion:

C	−4.75538495	0.31233817	−0.02903560
C	−3.48827537	0.90446736	0.45834560
C	−2.25765415	0.37293148	0.42870946
C	−1.73556469	−0.93994843	−0.07380559
O	−5.78189379	1.12947154	0.18034646
O	−4.88828126	−0.78015490	−0.56179480
O	−0.54343321	−1.18477886	0.03907334
O	−2.53884047	−1.82244622	−0.62949939
H	−3.60123413	1.89233479	0.88807180
H	−1.46000691	0.97974521	0.84043263
H	2.19968618	−0.17828031	−1.08704852
C	2.79514496	−0.08775192	−0.18018475
N	1.86548337	−0.08852606	0.99475064
H	2.31806501	−0.77762375	1.63671918
H	0.92658398	−0.43992940	0.73244040
C	3.65480628	1.17516727	−0.23746338
H	4.28015289	1.25746629	0.66201010
C	3.71948937	−1.35299315	−0.02254570
O	3.61484434	−1.93716475	1.08654363
O	4.45217414	−1.60084213	−0.99027043
S	2.75927166	2.75141161	−0.32702255
H	4.33720374	1.09070557	−1.08302897
H	1.75703534	0.82979530	1.42753076
H	−3.48481662	−1.50294789	−0.65442777
H	−6.59132611	0.70788873	−0.15290400

Coordinate for **TS1** with maleic acid and thiyl radical of cysteine:

C	−3.54006837	−0.61238681	−0.32857533
C	−2.26186285	−0.44969685	−1.02606691
C	−1.24370348	0.39778010	−0.65690451
C	−1.21453123	1.62778330	0.21645272
O	−4.34438635	−1.47392574	−0.94849170
O	−3.86305208	−0.04970704	0.71291578
O	−0.31323173	2.42673745	0.02075666
O	−2.11650546	1.81733345	1.15580766
H	−2.09733046	−1.10728128	−1.86971009
H	−0.40159316	0.48530191	−1.32728193
H	2.26605879	0.56271296	0.99987167
C	2.42354051	−0.02597977	0.09317080
N	2.06021423	0.86900993	−1.04710199
H	2.05286468	0.40906198	−1.95690128
H	2.85702108	1.52692415	−1.08328266
C	1.58360402	−1.28980815	0.16057890
H	1.50345940	−1.78467534	−0.81086082

C	3.97253243	-0.29008674	0.01935522
O	4.60490018	0.61576466	-0.58912656
O	4.39733146	-1.29998265	0.59295027
S	-0.07681905	-1.10744610	0.89518410
H	2.10658939	-1.98506791	0.82367237
H	1.28131196	1.51972652	-0.86444923
H	-2.82738032	1.11825992	1.11263621
H	-5.17091057	-1.54428642	-0.44348825

Coordinate for **add-(*Z*)**:

C	3.56781214	-0.57164883	0.18148825
C	2.36338082	-0.47370291	0.97303356
C	1.10456852	0.18825260	0.55948586
C	1.07478144	1.57131153	-0.10648858
O	4.49209706	-1.36467822	0.73082776
O	3.75874586	-0.00053640	-0.89636974
O	0.14111085	2.32411334	0.11359938
O	2.03007295	1.91396654	-0.94061866
H	2.33254512	-1.04965664	1.88802763
H	0.46881923	0.25985477	1.43849150
H	-2.21453509	0.42637149	-1.12538824
C	-2.41185890	-0.01411439	-0.14620870
N	-2.28647367	1.10661099	0.84522359
H	-2.17861451	0.75609135	1.79817879
H	-3.22391670	1.55272447	0.80549533
C	-1.46634323	-1.17477185	0.10570669
H	-1.41050795	-1.44793531	1.16167333
C	-3.94120864	-0.42956320	-0.14231800
O	-4.69845763	0.46672294	0.30349182
O	-4.20640877	-1.54622206	-0.60734675
S	0.23351627	-1.04461469	-0.58700921
H	-1.88317514	-2.02940949	-0.43001831
H	-1.50175539	1.75036285	0.63889946
H	2.72334011	1.19668312	-1.03081798
H	5.27278032	-1.36903540	0.15417583

Coordinate for **TS2**:

C	-3.07585628	1.39761310	0.25662074
C	-1.67311648	1.05282718	0.12074056
C	-1.13956807	-0.32872824	0.23256017
C	-2.15716206	-1.47984724	0.15913597
O	-3.28048962	2.72835926	0.14467881
O	-3.97737203	0.60109738	0.45760841
O	-2.35689040	-2.22007655	1.08246584
O	-2.76106131	-1.69066246	-1.02650377
H	-0.96662868	1.86703822	0.02756815
H	-0.63200671	-0.46538407	1.18881116
H	2.82151094	-1.35944016	-0.32065641
C	2.68837686	-0.34946242	0.06813330

N	2.48517238	-0.48185038	1.55397666
H	1.86614231	0.22852957	1.94386498
H	3.47212235	-0.26840488	1.88436976
C	1.57502760	0.35793790	-0.68542870
H	1.25767154	1.27778424	-0.19112083
C	4.08441795	0.38919239	-0.07786219
O	4.74205908	0.42633563	0.99650345
O	4.37231706	0.80535208	-1.20412080
S	0.10451198	-0.65535161	-1.14129278
H	2.00441186	0.64716362	-1.64449840
H	2.16925866	-1.40053697	1.86076677
H	-2.55226369	-0.99960430	-1.67125494
H	-4.22983613	2.89099448	0.25654307

Coordinate for **add-(*E*)**:

C	-3.44530551	-1.05114231	-0.26628147
C	-2.71161110	0.02008104	0.39348633
C	-1.34454145	0.35732693	-0.06335924
C	-1.04590098	1.85074023	-0.25080730
O	-4.63544138	-1.28942732	0.32204042
O	-3.04532629	-1.66274443	-1.24026469
O	0.05336578	2.33939264	-0.13579667
O	-2.07049133	2.62181064	-0.62051724
H	-3.13437364	0.48470004	1.27606825
H	-1.18270458	-0.12455614	-1.03246084
H	2.77167837	-0.64159705	1.58588407
C	2.58331430	-0.30278335	0.56828332
N	2.59641527	1.20068168	0.55592612
H	3.33667208	1.39408230	-0.16301618
H	2.89217967	1.59264812	1.44809737
C	1.27167481	-0.87613619	0.04889838
H	1.05833880	-0.51478138	-0.95756131
C	3.78193510	-0.76554994	-0.35267328
O	4.38823448	0.17882152	-0.92053756
O	3.96815561	-1.98733594	-0.41176931
S	-0.17603129	-0.46102669	1.12992773
H	1.37461084	-1.95776643	0.01092423
H	1.68825478	1.62463728	0.30134428
H	-2.89544834	2.11531909	-0.67874709
H	-5.07298011	-2.00038606	-0.17137925

Coordinate for **TS3**:

C	-1.07989264	2.23182918	-0.03535040
C	-2.01053307	1.11153624	-0.23851175
C	-1.78943644	-0.07943200	0.41177716
C	-2.84185618	-1.13929834	0.56351676
O	-1.51493079	3.35933137	-0.60153552
O	-0.02427555	2.15809740	0.57571521
O	-3.00948368	-1.70830239	1.60955648

O	−3.61891265	−1.41421824	−0.49935090
H	−2.84726504	1.27159053	−0.90664634
H	−1.06317241	−0.08200561	1.21167457
H	2.09138754	−1.87202157	0.35518160
C	2.06253441	−0.79619809	0.17438971
N	1.53880460	−0.15653781	1.42601786
H	1.06291716	0.73955646	1.23768491
H	2.42199892	0.04581575	1.94671575
C	1.22948612	−0.49309491	−1.05865509
H	1.11726206	0.58207875	−1.21267885
C	3.55877369	−0.32082988	0.02889719
O	4.03975137	0.16709054	1.08772757
O	4.09268041	−0.50765861	−1.07070773
S	−0.41144262	−1.28644929	−1.17737629
H	1.78455930	−0.87110373	−1.92059329
H	0.95237813	−0.77749889	1.98386460
H	−3.33755051	−0.93931070	−1.29379637
H	−0.86415362	4.06005863	−0.43313323

Coordinate for fumaric acid and thiyl radical of cysteine before insertion:

C	−1.42676146	0.92522966	1.36372423
C	−2.77179062	0.51095770	0.89323436
C	−2.91426679	−0.31293020	−0.14601021
C	−4.21644952	−0.84278085	−0.64233458
O	−1.51304308	1.78860181	2.37731207
O	−0.36656637	0.55018368	0.90086346
O	−4.29460088	−1.92393475	−1.17021961
O	−5.31988082	−0.09080675	−0.46689851
H	−3.61109783	0.90964121	1.45131966
H	−2.04537120	−0.71988142	−0.64884701
H	3.21178848	−0.86526639	−1.51623395
C	2.77720922	−0.53544714	−0.57203258
N	1.46413377	−1.25332898	−0.41097662
H	0.73903066	−0.66825929	0.02907367
H	1.73331231	−2.01398845	0.26052977
C	2.64234406	0.97821541	−0.55793595
H	2.12037304	1.32599289	0.33819493
C	3.70275698	−1.06523875	0.58913186
O	3.24557094	−2.06750008	1.19697029
O	4.76806865	−0.45352837	0.74858782
S	1.85339559	1.74063184	−2.00137630
H	3.65578242	1.38981917	−0.50210049
H	1.09566833	−1.63864361	−1.27837708
H	−5.10215265	0.79484619	−0.14628779
H	−0.61523845	2.03677391	2.65199839

Coordinate for maleic acid and cysteine before insertion:

C	−4.71967819	−0.14010504	−0.08354986
C	−3.88131279	0.05071932	1.12859039

C	−2.54520839	−0.01191984	1.22837791
C	−1.46809034	−0.27502982	0.21947422
O	−6.03216763	0.01109567	0.06997380
O	−4.29646951	−0.42401550	−1.19314730
O	−0.30595116	−0.23115629	0.60185842
O	−1.75463275	−0.54705566	−1.03368055
H	−4.42232502	0.26960210	2.04345516
H	−2.11687436	0.16116254	2.20826035
H	3.79970988	0.30889068	−1.25966523
C	3.15671904	−0.05197373	−0.45531573
N	1.93812101	−0.65393071	−1.09619370
H	2.17497203	−1.67265852	−1.07659783
H	1.76958533	−0.32887064	−2.04576162
C	2.82349578	1.08115298	0.50085471
H	2.09855690	0.75633972	1.24861518
C	3.91553076	−1.24369801	0.24778283
O	3.48745085	−2.38442141	−0.06817365
O	4.84799705	−0.92591974	0.99907187
S	2.05823047	2.56942482	−0.27900667
H	3.73619092	1.36754020	1.01595810
H	1.07711214	−0.50717958	−0.53232093
H	3.14401476	2.97002095	−0.97154796
H	−2.74452261	−0.53672578	−1.19664980
H	−6.27350461	0.23550458	0.97920948

Coordinate for **1,2-adduct** in the non-radical insertion of cysteine to maleic acid:
ΔG = –28.25036672 kJ mol^{-1}

C	3.47453837	−0.69964818	0.05167624
C	2.47936451	−0.39197297	1.14493599
C	1.13153956	0.20493659	0.70200688
C	1.10551364	1.64317536	0.18080885
O	4.20035280	−1.77972632	0.30374666
O	3.63353533	−0.01946092	−0.94952195
O	0.15778090	2.36766766	0.43325491
O	2.10136907	2.08140080	−0.56779991
H	2.97591862	0.30729081	1.82897085
H	0.49682274	0.21082249	1.58593951
H	−2.49184611	−0.31109028	−1.60836910
C	−2.51491343	−0.14957826	−0.53223244
N	−2.39277091	1.32798975	−0.27226294
H	−3.23012942	1.49426076	0.34146198
H	−2.51412179	1.87012804	−1.12633930
C	−1.44449896	−0.99427512	0.14681443
H	−1.40170949	−0.80707966	1.22124894
C	−3.93828466	−0.56396252	0.01868978
O	−4.54537813	0.35534637	0.62704600
O	−4.27482286	−1.73514870	−0.19772044
S	0.27125159	−0.89280477	−0.53160288

H	−1.74507401	−2.03172766	0.00746916
H	−1.51224567	1.64310247	0.16821514
H	2.74690928	1.35461938	−0.77994794
H	2.28784587	−1.30233609	1.71107057
H	4.85474986	−1.89172276	−0.40647366

Optimized Structure for Figure S13

Coordinate for **TS3'** leading to the episulfide:

C	−3.56078659	−0.92296949	0.06054848
C	−2.38552516	−0.14181019	0.50238570
C	−1.53749477	0.52239289	−0.52409650
C	−1.01378853	1.90729091	−0.20317871
O	−4.28914737	−1.34186049	1.11336710
O	−3.84000117	−1.17350825	−1.09127431
O	−0.00562371	2.14715138	0.41892286
O	−1.76189301	2.91760415	−0.65796497
H	−2.39237445	0.24058074	1.51380585
H	−1.91776498	0.43023715	−1.53908311
H	2.46221198	−1.47199463	1.03286457
C	2.55343351	−0.61453753	0.36835264
N	2.33533033	0.64652325	1.15789683
H	3.18447994	1.20878019	0.92362756
H	2.33364229	0.46974183	2.16124476
C	1.63991362	−0.71219453	−0.78799309
H	1.49006560	0.17744930	−1.39202339
C	4.08889268	−0.56016395	−0.13676897
O	4.63718755	0.54724484	0.06496391
O	4.50569663	−1.59978488	−0.65091101
S	−0.60924807	−0.91686465	0.04524883
H	1.68208507	−1.64438256	−1.33661772
H	1.46317515	1.14545186	0.91412683
H	−2.54578273	2.60198806	−1.13039927
H	−5.03670094	−1.85650298	0.77086876