Design, Synthesis, and Structure−Activity Relationships of Haloenol Lactones: Site-Directed and Isozyme-Selective Glutathione *S*-Transferase Inhibitors

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Overexpression of glutathione *S*-transferase (GST), particularly the GST-π isozyme, has been proposed to be one of the biochemical mechanisms responsible for drug resistance in cancer chemotherapy, and inhibition of overexpressed GST has been suggested as an approach to combat GST-induced drug resistance. 3-Cinnamyl-5(*E*)-bromomethylidenetetrahydro-2-furanone (**1a**), a lead compound of site-directed GST-π inactivator, has been shown to potentiate the cytotoxic effect of cisplatin on tumor cells. As an initial step to develop more potent and more selective haloenol lactone inactivators of GST-π, we examined the relationship between the chemical structures of haloenol lactone derivatives and their GST inhibitory activity. A total of 16 haloenol lactone derivatives were synthesized to probe the effects of (1) halogen electronegativity, (2) electron density of aromatic rings, (3) molecular size and rigidity, (4) lipophilicity, and (5) aromaticity on the potency of GST-π inactivation. The inhibitory potency of each compound was determined by time-dependent inhibition tests, and recombinant human GST-π was used to determine their inhibitory activity. Our structure−activity relationship studies demonstrated that (1) reactivity of the halide leaving group plays a weak role in GST inactivation by the haloenol lactones, (2) aromatic electron density may have some influence on the potency of GST inactivation, (3) high rigidity likely disfavors enzyme inhibition, (4) lipophilicity is inversely proportional to enzyme inactivation, and (5) an unsaturated system may be important for enzyme inhibition. This work facilitated understanding of the interaction of GST-π with haloenol lactone derivatives as site-directed and isozyme-selective inactivators, possibly potentiating cancer chemotherapy.

Introduction

Glutathione *S*-transferase (GST) plays an important role in the deactivation of drugs, toxicants, and carcinogens. A number of alkylating agents used in cancer chemotherapy have been shown to be deactivated by GST. Nitrogen mustards such as melphalan[1−3] and chlorambucil[4] have both been shown to form glutathione (GSH) conjugates. GST has been found to catalyze denitrosation of the nitrosourea BCNU by formation of an *S*-nitroso GSH conjugate.[5,6] The enzyme also catalyzes the conjugation of GSH with acrolein, a major reactive metabolite of cyclophosphamide.[7]

Cancer chemotherapy often fails because of acquired drug resistance. One of the most critical biochemical changes observed in drug-resistant tumor cells is overexpression of GST. The overexpression of three GST isozymes (α, μ, and π) has been found in cultured tumor cells resistant to antineoplastic agents. The GST-π isozyme is often overexpressed in tumor cells, particularly in drug-resistant cancer cells. The human GST-π isozyme has been reported to be overexpressed in a variety of malignancies including carcinoma of the colon, lung, kidney, ovary, pancreas, esophagus, stomach, and breast.[7−]

Glutathione *S*-transferase inhibitors, such as ethacrynic acid,[18] piriprost,[19] indomethacin,[19] gossypol,[20] tributyltin acetate,[21] and antimalarial agents,[22] have been used as potentiating agents for chemotherapeutic drugs. A number of glutathione analogues have been found to be GST inhibitors[23] and to potentiate chlorambucil toxicity toward selected cancer cell lines.[24] Among those GST inhibitors, only ethacrynic acid has been extensively investigated for possible employment as a potentiating chemotherapeutic agent. Ethacrynic acid and its GSH conjugate have been found to be reversible inhibitors of GST.[25−27] Pretreatment of mammalian cancer cell lines with ethacrynic acid increases the cytotoxicity of mitomycin,[28,29] chlorambucil,[30] and melphalan.[31] These in vitro observations suggest that ethacrynic acid could be useful in combination with chemotherapeutic agents. However, the diuretic function of ethacrynic acid limits its clinical use for chemotherapy.

Recently we reported haloenol lactone **1a** as a site-directed GST inactivator, producing a time-dependent inhibitory effect on the mouse GST-π isozyme but weak inhibition of α and μ isozymes.[32] We proposed that enzymatic hydrolysis of the haloenol lactone may be the

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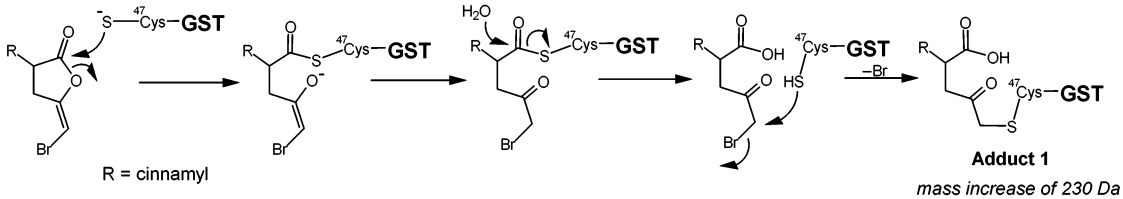
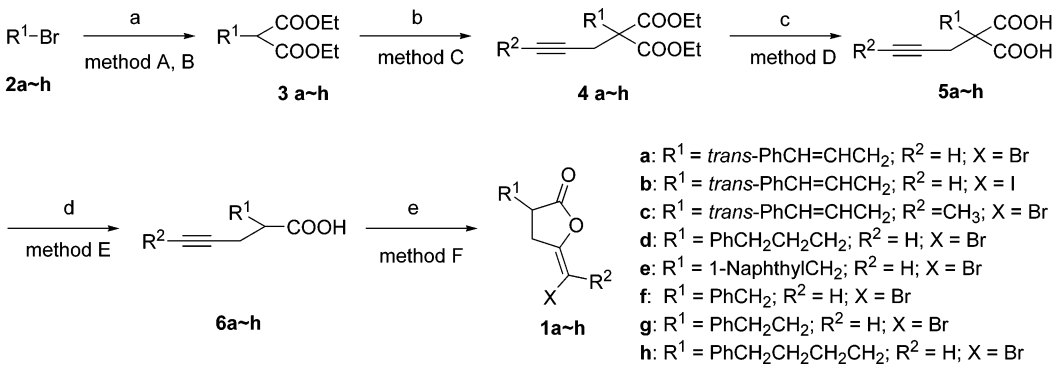
Scheme 1



Scheme 2[a]



[a] (a) EtONa, EtOH, diethyl malonate; or NaH, diethyl malonate, THF, reflux; (b) EtONa, EtOH, propargyl bromide or 1-bromo-2-butyne, 40 °C; (c) EtOH, 2 N NaOH, reflux, 3 h; (d) 120−180 °C, 3 h; (e) NXS, $KHCO_3$, Bu_4NOH, CH_2Cl_2, H_2O, 30 min.

initial step of GST-π chemical modification resulting in the inactivation of the enzyme. Mass spectrometric analysis of proteolytic fragments from haloenol lactone modified GST-π indicated that the haloenol lactone is covalently attached to the protein at Cys-47.[33] In addition, kinetic studies employing recombinant GST-π with replacement of cysteine by serine at Cys-47 and Cys-101 demonstrated that rapid inactivation occurs only when residue 47 is cysteine. As shown in Scheme 1, it is likely that enzyme inactivation is initiated through addition of Cys-47 to the lactone ring, which is opened in the process to form an α-bromoketone adduct. The acidity of Cys-47 confers good leaving group properties, and rapid hydrolysis occurs to generate an α-bromoketoacid intermediate. The reaction may proceed via alkylation of the transient thioester to form a six-membered ring episulfonium ion intermediate that would be yet more reactive toward hydrolysis, with either process leading to the observed mass increase of 230 Da.[32]

Haloenol lactone **1a** has also shown time-dependent inhibition of GST activity in supernatants of UOK130 cells, a kidney cancer cell line overexpressing the GST-π isozyme. In addition, our in vitro study demonstrated the ability of this compound to potentiate cisplatin-induced cytotoxicity toward the UOK130 cell line.[34] As an initial step to develop more potent haloenol lactone inactivators of GST-π, we investigated the relationship between the chemical structures of a series of haloenol lactone derivatives and their GST inhibitory activity. We describe herein the structure−activity relationships of the haloenol lactone derivatives as site-directed and isozyme-selective GST inhibitors.

Results and Discussion

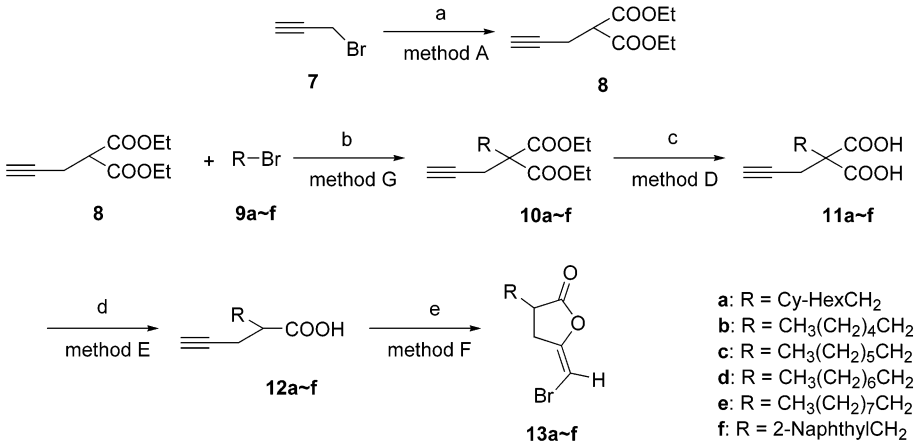
A total of 15 haloenol lactone derivatives besides the lead compound (**1a**) were synthesized through the halolactonization of the corresponding pentynoic acid derivatives by either *N*-bromosuccinimide or *N*-iodosuccinimide[35,36] (see Schemes 2−6). However, we failed to halolactonize the pentynoic acids by *N*-chlorosuccinimide and were unable to obtain the corresponding chloroenol lactones.

Pentynoic acid derivatives **6a**−**h** and **12a**−**f** were prepared as shown in **Schemes** 2 and 3. The synthesis was initiated with alkylation of malonic ester by the corresponding halides, followed by alkaline hydrolysis in NaOH and decarboxylation at 120 °C or higher. However, pentynoic acids substituted with methoxy and trifluoromethyl groups at the phenyl ring are heat-labile. To prepare compounds **25** and **33**, a modified synthetic approach was applied as shown in Schemes 5 and 6. Monoesters **24** and **32** were obtained by direct de-ethoxycarbonylation from the corresponding malonic esters (**23** and **31**) in NaCl solution (method N).[37,38] Synthesis of pentynoic acid **16**, the precursor for haloenol lactone, was started with phenylacetic acid instead of molonic ester (Scheme 4). However, we failed in alkylation of phenylacetic acid by propargyl bromide (**7**). Fortunately, alkylation of phenylacetic acid by 3-bromo-1-(trimethylsilyl)-1-propyne and trimethylsilyl protected propargyl bromide followed by deprotection with Bu_4NF allowed us to obtain the desired pentynoic acid.[35]

The inhibitory effect of haloenol lactones on GST-π was determined by time-dependent inhibition studies. Figures 1−4 show the time-course inactivation of human recombinant GST-π by the haloenol lactones synthesized, and all showed similar time-dependent GST inhibition, although their rates of inhibition varied at the same molar concentrations.

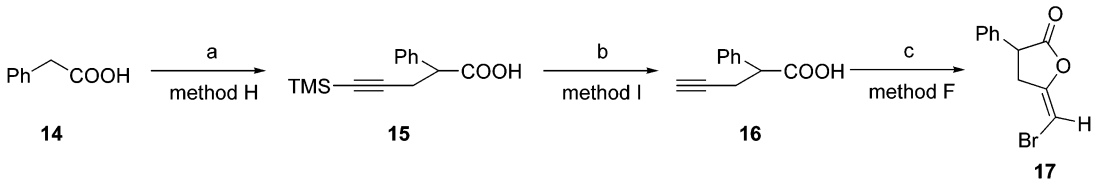
Observed rate constants (k_{obs}) for GST inactivation by the haloenol lactones synthesized were calculated from the slopes of the linear regression lines of semilogarithmic plots of the remaining activity against the preincubation time. A loss of GST activity ($k_{obs} = 0.0604$) was found after exposure to the lead compound (see Figure 1 and Table 1), and in comparison with the lead

Scheme 3. Synthesis of Compound **13a**−**f**[a]



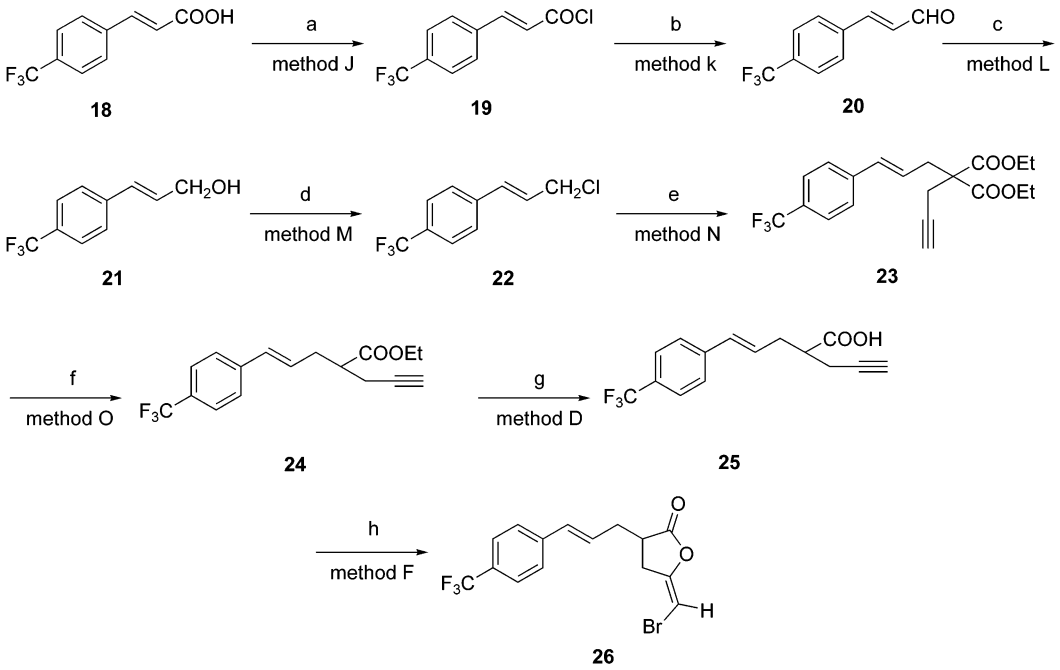
[a] (a) EtONa, EtOH, diethyl malonate; (b) EtONa, EtOH, reflux, 6 h; (c) EtOH, 2 N NaOH, reflux, 3 h; (d) 120−180 °C, 3 h; (e) NBS, $KHCO_3$, Bu_4NOH, CH_2Cl_2, H_2O, 30 min.

Scheme 4[a]



[a] (a) LDA, HMPA, 3-bromo-1-(trimethylsilyl)-1-propyne, THF, 0 °C; (b) Bu_4NF, THF, 3 h; (c) NBS, $KHCO_3$, Bu_4NOH, CH_2Cl_2, H_2O, 30 min.

Scheme 5[a]

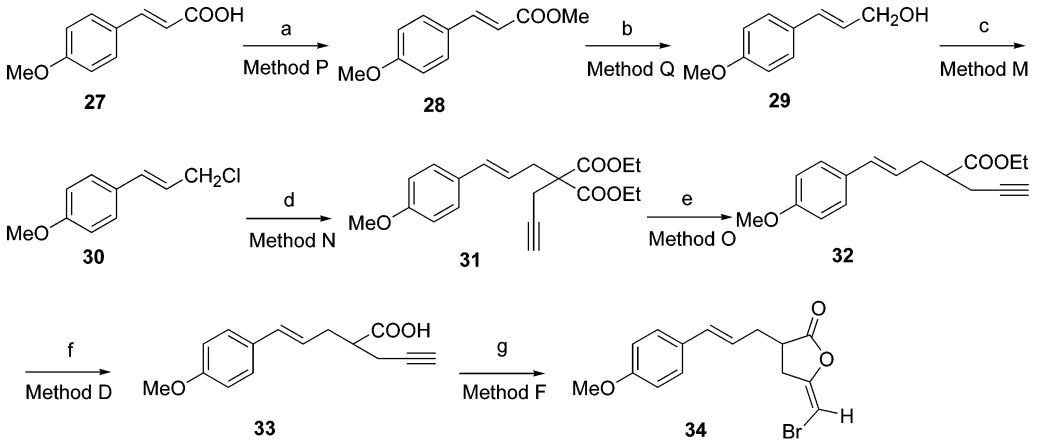


[a] (a) $SOCl_2$, benzene, reflux; (b) $LiAlH[OC(CH_3)_3]_3$, THF, −78 °C; (c) $NaBH_4$, MeOH, 1 h; (d) $SOCl_2$; (e) EtONa, EtOH, compound **8**, reflux, 7 h; (f) NaCl, H_2O, DMSO, reflux, 17 h; (g) EtOH, 2 N NaOH, reflux, 3 h; (h) NBS, $KHCO_3$, Bu_4NOH, CH_2Cl_2, H_2O, 30 min.

compound a mild decrease in GST inhibition (k_{obs} = 0.0421) was observed for iodoenol lactone **1b** (Table 1). This indicates that halogens may not play a critical role in GST inactivation by the haloenol lactones. Iodide (I^-) and bromide (Br^-) are known as leaving groups in S_N1 and/or S_N2 reactions, and the order of reactivity of the halides as leaving groups is $I^- > Br^-$. Replacement of Br^- by I^- as a better leaving group did not facilitate the nucleophilic attack by Cys-47 at the α-carbon of the resulting α-bromoketone to form a protein adduct. This implies that the lactone ring opening may be the rate-limiting step in the process of enzyme inactivation. It is likely that by electron-withdrawing effects halogens stabilize the resulting enolates in lactone ring opening, since bromine is known to be more electronegative than iodine.

Interestingly, compound **1c**, an analogue of the lead compound with an additional methyl group at the

Scheme 6[a]



[a] (a) $BF_3 \cdot Et_2O$, MeOH, reflux; (b) DIBAL-H, THF, −78 °C; (c) $SOCl_2$; (d) EtONa, EtOH, compound **8**, reflux, 7 h; (e) NaCl, H_2O, DMSO, reflux, 17 h; (f) EtOH, 2 N NaOH, reflux, 3 h; (g) NBS, $KHCO_3$, Bu_4NOH, CH_2Cl_2, H_2O, 30 min.

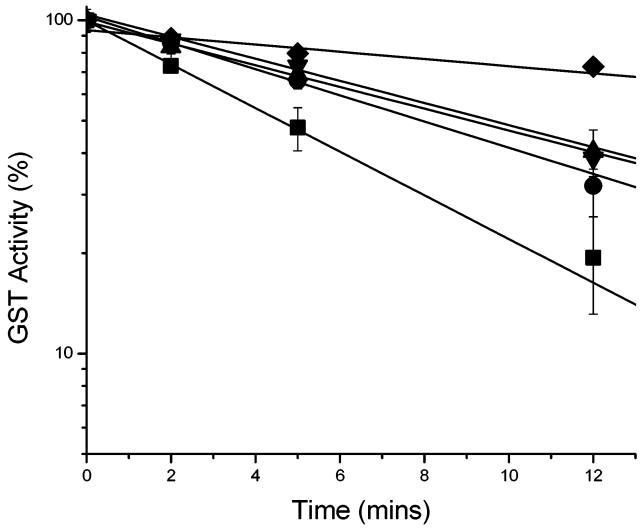


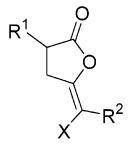
Figure 1. Recombinant human GST-π (0.1 mg/mL) was incubated in phosphate buffer (pH 7.4) with vehicle (◆), **1a** (■), **1b** (●), **1c** (▲), and **26** (▼) (170 μM) at 30 °C. The GST activity was determined periodically by the Habig method.[40]

position of methylidene, was found to be a weak inactivator of GST-π (k_{obs} = 0.0322; Table 1 and Figure 1). It is likely that the methyl substitute as an electron-donating group destabilizes the developing enol oxy anion as a leaving group and slows down the ring opening.

Compounds **26** and **34** were designed and synthesized to probe the role of electron density of the aromatic ring in GST inactivation by the haloenol lactones. Compound **26** was designed and synthesized to investigate the electron-withdrawing effect of the trifluoromethyl group on GST inactivation. The inhibition study showed that compound **26**, substituted with a trifluoromethyl group, produced a significant decrease in GST inactivation in comparison with the lead compound (Table 1 and Figure 1). Compound **34** was designed and synthesized to probe the electron-donating effect of the methoxy group on GST inactivation. Unfortunately this compound was not stable enough to be tested for its inhibitory effect on GST, preventing our effort to investigate the effects of aromatic electron density on GST inactivation.

Configuration and conformation are important issues in enzyme inhibitor design. Compounds **1a**, **1d**, and **13f** were designed and synthesized to test whether molecular rigidity and conformation influence the interaction of haloenol lactones with GST-π resulting in changes in the potency of GST inactivation. As shown in Figure 2 and Table 1 among these three lactones, the lead compound was found to be the most potent in GST inactivation (k_{obs} = 0.0604), followed by compound **13f** (k_{obs} = 0.0499) and compound **1d** (k_{obs} = 0.0439). The order of molecular rigidity is **13f** > **1a** > **1d**. This indicates that high rigidity of the flat naphthalene ring of compound **13f** disfavors GST inactivation. However, conformational flexibility does not necessarily facilitate potency for GST inhibition, since the less rigid compound **1d** did not show improved potency. Because of the limited availability of the haloenol lactone derivatives, we are unable to draw a solid conclusion on whether flexibility plays a significant role in GST inactivation.

Table 1. Inactivation of GST-π by Haloenol Lactone Derivatives[a]



compd	R^1	R^2	X	k_{obs} (r^2)
1a	*trans*-PhCH=CHCH$_2$	H	Br	0.0604 (0.998)
1b	*trans*-PhCH=CHCH$_2$	H	I	0.0421 (0.996)
1c	*trans*-PhCH=CHCH$_2$	CH_3	Br	0.0322 (0.997)
1d	$PhCH_2CH_2CH_2$	H	Br	0.0439 (0.999)
1f	$PhCH_2$	H	Br	0.1014 (0.994)
1g	$PhCH_2CH_2$	H	Br	0.0632 (1.000)
1h	$PhCH_2CH_2CH_2CH_2$	H	Br	0.0366 (0.996)
13a	Cy-HexCH_2	H	Br	0.0438 (1.000)
13b	$CH_3(CH_2)_4CH_2$	H	Br	0.0847 (0.990)
13c	$CH_3(CH_2)_5CH_2$	H	Br	0.0608 (0.977)
13d	$CH_3(CH_2)_6CH_2$	H	Br	0.0382 (0.993)
13e	$CH_3(CH_2)_7CH_2$	H	Br	0.0359 (0.996)
13f	2-naphthyl-CH_2	H	Br	0.0499 (0.999)
17	Ph	H	Br	0.1504 (0.993)
26	*trans*-4-CF_3PhCH=CHCH$_2$	H	Br	0.0364 (0.988)
34	*trans*-4-CH_3OPhCH=CHCH$_2$	H	Br	NA

[a] Recombinant human GST-π (0.1 mg/mL) was preincubated in phosphate buffer (pH 7.4) with individual haloenol lactone (0.17 mM) at 30 °C. Aliquots were withdrawn at 0, 2, 5, and 12 mins, and the remaining GST activity was determined by the Habig method.[40]

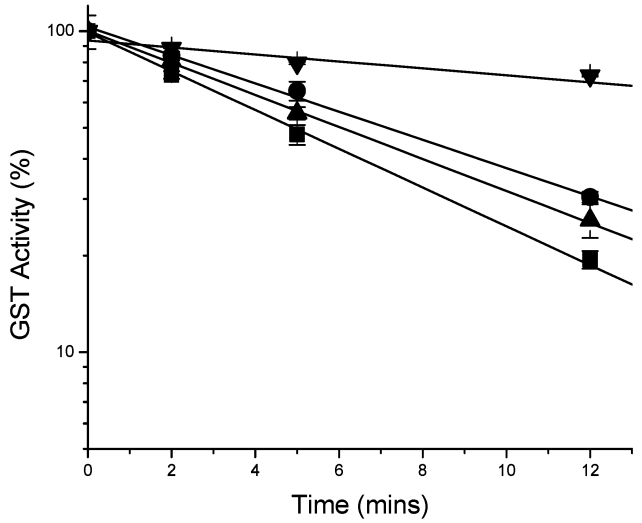


Figure 2. Recombinant human GST-π (0.1 mg/mL) was incubated in phosphate buffer (pH 7.4) with vehicle (▼), **1a** (■), **1d** (●), and **13f** (▲) (170 μM) at 30 °C. The GST activity was determined periodically.

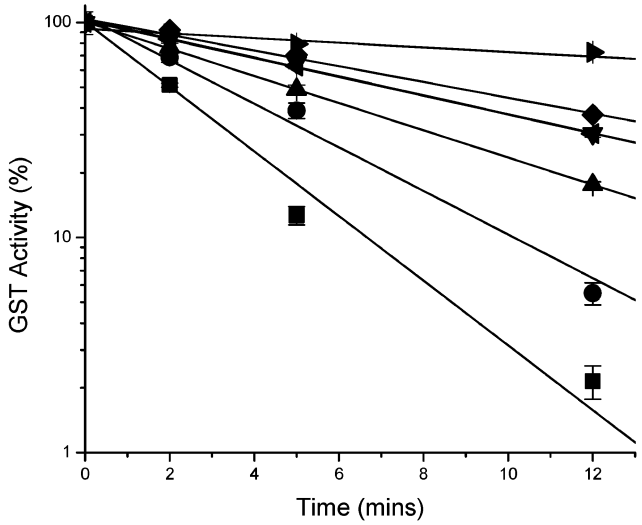


Figure 3. Recombinant human GST-π (0.1 mg/mL) was incubated in phosphate buffer (pH 7.4) with vehicle (triangle solid right), **17** (■), **1f** (●), **1g** (▲), **1d** (▼), **1h** (◆), and **13a** (triangle solid left) (170 μM) at 30 °C. The GST activity was determined periodically.

Compounds **1f** and **13a** were designed and synthesized to test whether aromaticity is required for the inhibitory activity of the haloenol lactones. As shown in Table 1 and Figure 3, the aromatic haloenol lactone **1f** demonstrated much higher inhibitory potency than the saturated haloenol lactone **13a**. Inhibitory activity decreased from k_{obs} = 0.1014 to k_{obs} = 0.0438 after saturation of the phenyl ring of lactone **1f**. This indicates that the phenyl ring (i.e., unsaturation) is important to the enzyme inactivation.

Compounds **13b**−**e** were designed and synthesized to investigate the role of lipophilicity of the haloenol lactone derivatives in their inactivation of GST. As shown in Figure 4 and Table 1, inactivation potency decreased with an increase in length of the aliphatic side chain. The order of lipophilicity is **13e** > **13d** > **13c** > **13b**. This indicates that lipophilicity is not necessarily needed for GST inactivation. However, a

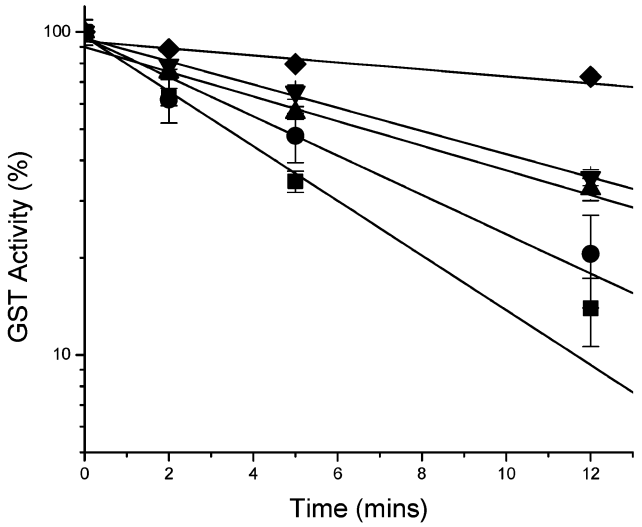


Figure 4. Recombinant human GST-π (0.1 mg/mL) was incubated in phosphate buffer (pH 7.4) with vehicle (◆), **13b** (■), **13c** (●), **13d** (▲), and **13e** (▼) (170 μM) at 30 °C. The GST activity was determined periodically.

correlation with log *P* values of all compounds listed is needed to solidify the conclusion.

Compounds **17**, **1d**, **1f**, **1g**, and **1h** were designed and synthesized to test the role of the linkage length between the phenyl group and the haloenol lactone ring in GST inactivation. Compound **17**, with no spacer between the phenyl and lactone ring, was found to be the most potent GST inactivator among the haloenol lactones synthesized (Figure 3 and Table 1). A decrease in GST inactivation was observed with an increase in spacer length (n = 1−4), suggesting that the spacer is not necessarily required for GST inactivation. This provides additional evidence that an increase in lipophilicity may disfavor the potency for GST inhibition by haloenol lactone derivatives.

Significant spontaneous enzyme activity loss was observed even at 30 °C. Solvent may play a certain role in the spontaneous enzyme inactivation. In addition, the recombinant enzyme for the inhibition studies may not be as stable as a natural one. Fortunately, k_{obs} of spontaneous enzyme activity loss is significantly smaller than that of the inactivation by compound **1c**, the weakest inhibitor. However, we cannot completely exclude the possibility that the spontaneous enzyme inactivation might confound the analysis of those weak inhibitors.

In addition to k_{obs}, we determined K_I and k_{inact} for GST inactivation by the lead compound and compound **17**, the most potent GST inactivator among the 16 haloenol lactone derivatives we synthesized. First, we measured k_{obs} of GST inactivation by **1a** and **17** at a selection of concentrations (Figures 5 and 6). K_I and k_{inact} were calculated by Wilson's plot, or double-reciprocal plot of k_{obs} and haloenol lactone concentrations, as shown in Figure 7. As expected, compound **17** showed a k_{inact} (0.097 min^{-1}) higher than that of lead compound **1a** (k_{inact} = 0.025 min^{-1}). Interestingly, the lead compound was found to have a 20-fold affinity toward GST higher than that of compound **17** (K_I = 6.1 μM for **1a** and K_I = 123.9 μM for **17**). This indicates lipophilicity of haloenol lactones facilitates their binding to GST, but it does not necessarily increase irreversible inactivation. Covalent

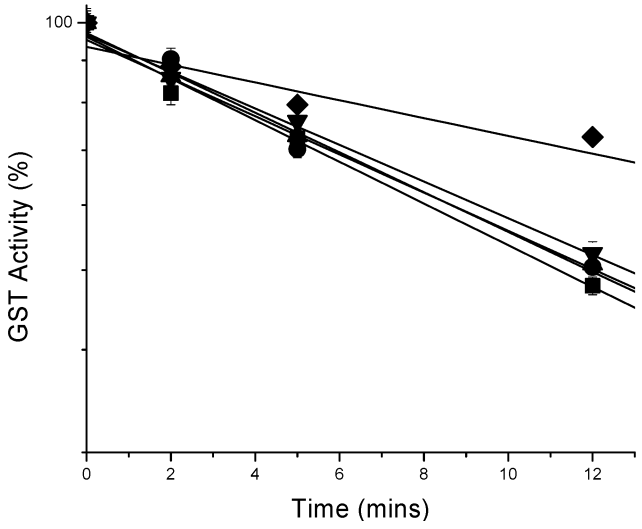


Figure 5. Recombinant human GST-π (0.1 mg/mL) was incubated in phosphate buffer (pH 7.4) with **1a** at concentrations of 340 μM (■), 170 μM (●), 85 μM (▲), and 42.5 μM (▼) and with vehicle (◆) at 30 °C. The GST activity was determined periodically.

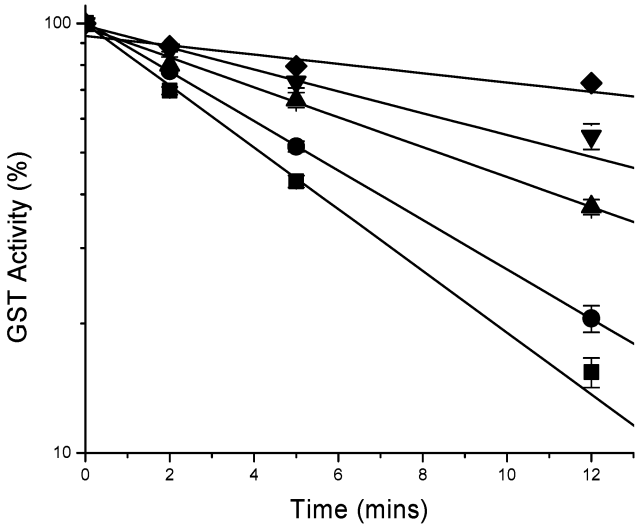


Figure 6. Recombinant human GST-π (0.1 mg/mL) was incubated in phosphate buffer (pH 7.4) with **17** at concentrations of 255 μM (■), 170 μM (●), 85 μM (▲), and 42.5 μM (▼) and with vehicle (◆) at 30 °C. The GST activity was determined periodically.

modification of enzymes by irreversible inhibitors such as affinity labeling agents and mechanism-based inactivators depends on not only the affinity (reversible binding) between enzymes and inhibitors but also the reactivity of the electrophilic center approaching that of the nucleophilic amino acid residues in the active site. In addition, the distance between electrophile and nucleophile of the target enzyme is critical for covalent binding. We speculate that the electrophilic center derived from **17** may be located in an appropriate distance suitable for the nucleophilic replacement reaction, although its affinity to GST is lower than that of **1a**.

Our early study showed that a mass addition of 230 Da was incorporated into mice GST-π after exposure to the lead compound (**1a**).[32] In this study, we used baculovirus *E. coli* expressed human GST-π for our structure−activity relationship investigation. Similar

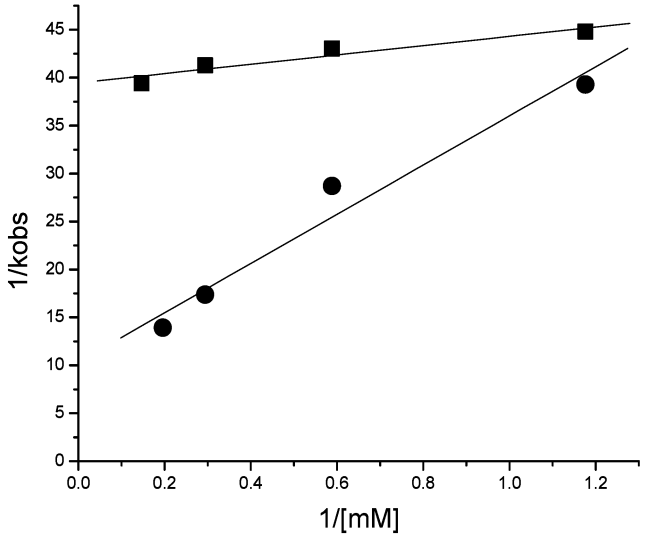


Figure 7. Wilson's plot: (■) **1a**; (●) **17**.

mass analysis study was performed to determine if the same molecular mass was incorporated into human GST-π as for mice GST-π. As expected, exact molecular mass (230 Da) addition was found in the human GST-π after it was incubated with compound **1a** (Figure 8b). This indicates that there is no species difference in GST inactivation by the lead compound, although mice and human GST-π enzymes have different molecular mass.

Compound **17** was found to be the most potent GST inactivator among the haloenol lactones we designed and synthesized, and it has a structure similar to that of the lead compound with the exact haloenol lactone moiety as a precursor of α-bromoketone. To ensure that compound **17** inactivates GST by the same mechanism as the lead compound, we examined the molecular mass of GST after exposure to compound **17**. A molecular mass addition of 190 Da was observed in the modified GST by compound **17** as shown in Figure 8c. The mass addition matches the molecular mass of the protein adduct shown in Scheme 1 if GST is exposed to compound **17**. Taken together, it is most likely that compound **17** inactivates GST-π by the same mechanism as the lead compound.

In conclusion, haloenol lactones are site-directed irreversible inhibitors of GST-π isozyme. The halogen of the enol lactone plays a weak role in enzyme inactivation. In addition, the unsaturated phenyl system is important for the enzyme investigation. Lipophilicity favors the binding of the haloenol lactones to GST, but it does not necessarily increase GST inactivation.

Experimental Section

Synthesis. Solvents and reagents were purchased from commercial sources and were used without further purification except as noted below. Melting points were uncorrected on a Büchi-510. Thin-layer chromatography was carried on glass precoated with silica gel. ^{1}H NMR spectra were determined on a Bruker AMX-400 spectrometer and referenced to Me_4Si. Mass spectra were recorded on a MAT-95 spectrometer. All air-sensitive experiments were carried out under nitrogen with freshly distilled dried solvents. THF was distilled from sodium benzophenone. Benzene and ethyl ether were dried by standing over sodium wire. Ethanol was distilled after reaction with sodium. Chloroform and methylene chloride were distilled from CaH_2. The petroleum ether used in column chromatography had a boiling point range of 60−90 °C.

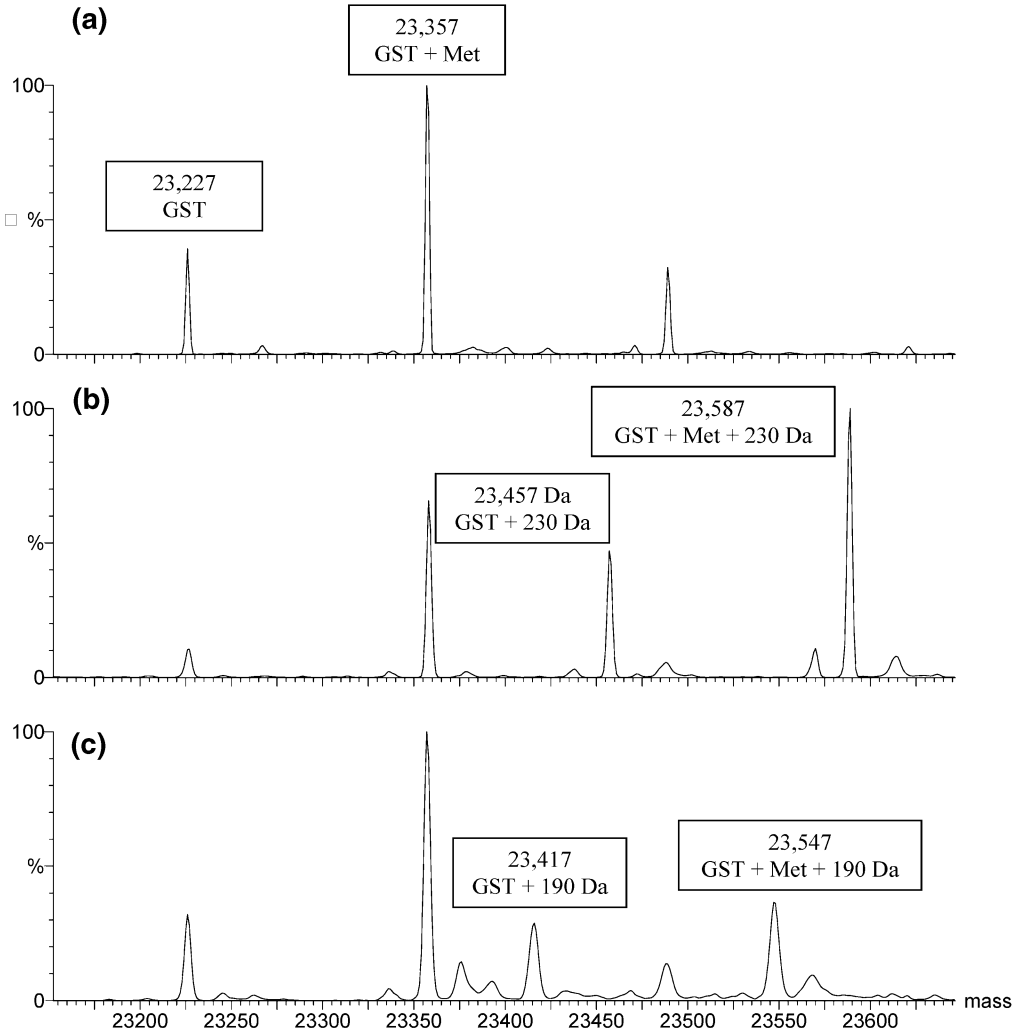


Figure 8. (a) Electrospray ionization MaxEnt transformed spectrum of native recombinant human GST-π. (b) Electrospray ionization MaxEnt transformed spectrum of recombinant human GST-π incubated with **1a**. (c) Electrospray ionization MaxEnt transformed spectrum of native recombinant human GST-π incubated with **17**. Molecular mass addition relative to native protein (GST and GST + Met) of 230 Da corresponds to adduct **1** (Scheme 1). Molecular mass addition relative to native protein of 190 Da corresponds to protein modification by **17**.

Method A. 2-Cinnamylmalonic Acid Diethyl Ester (3a). To a solution of EtONa (750 mg of Na dissolved in 25 mL of EtOH) was sequentially added 4.8 g (30 mmol) of diethyl malonate and a solution of 5.88 g (30 mmol) of cinnamyl bromide (**2a**) in 25 mL of EtOH dropwise. The resulting mixture was stirred overnight at room temperature. The solvent was removed by rotary evaporation, followed by addition of 20 mL of water. The crude mixture was extracted with chloroform, and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in a vacuum. Flash chromatography (petroleum ether/ethyl acetate, 100:1) over silica gel afforded the pure **3a** (2.2 g, 26.7% in yield): ^{1}H NMR ($CDCl_3$) δ 7.33−7.20 (m, 5H), 6.48 (d, J = 15.74 Hz, 1H), 6.16 (dt, J_1 = 7.32 Hz, J_2 = 15.74 Hz, 1H), 4.21 (q, J = 7.32 Hz, 4H), 3.50 (dt, J_1 = 1.1 Hz, J_2 = 7.50 Hz, 1H), 2.80 (dd, J_1 = 7.32 Hz, J_2 = 7.50 Hz, 2H), 1.27 (t, J = 7.32 Hz, 6H); EIMS m/z 276 (M^+), 129 (100).

2-Naphthalen-1-ylmethylmalonic Acid Diethyl Ester (3e). Compound **3e** was prepared as for **3a** from 1-chloromethylnaphthalene (**2e**) (5.3 g, 30 mmol) in 13.3% yield: ^{1}H NMR ($CDCl_3$) δ 8.07−7.36 (m, 7H), 4.00 (q, J = 7.33 Hz, 4H), 3.87 (t, J = 7.63 Hz, 1H), 3.73 (d, J = 7.63 Hz, 2H), 1.20 (t, J = 7.33 Hz, 6H); EIMS m/z 300 (M^+), 149 (100).

2-Benzylmalonic Acid Diethyl Ester (3f). Compound **3f** was synthesized as for **3a** from benzyl chloride (**2f**) (1.27 g, 10 mmol) in 72.0% yield: ^{1}H NMR ($CDCl_3$) δ 7.27−7.18 (m, 5H), 4.15 (q, J = 7.07 Hz, 4H), 3.63 (t, J = 7.82 Hz, 1H), 3.20 (d, J = 7.82 Hz, 2H), 1.20 (t, J = 7.07 Hz, 6H).

2-Propargylmalonic Acid Diethyl Ester (8). Compound **8** was prepared as for **3a** from propargyl bromide (**7**) (3.6 g, 30 mmol) in 28.5% yield: ^{1}H NMR ($CDCl_3$) δ 4.18 (q, J = 7.33 Hz, 4H), 3.52 (t, J = 7.70 Hz, 1H), 2.74 (dd, J_1 = 2.57 Hz, J_2 = 7.70 Hz, 2H), 1.98 (t, J = 2.57 Hz, 1H), 1.24 (t, J = 7.33 Hz, 6H); EIMS m/z 198 (M^+), 125 (100).

Method B. 2-(3-Phenylpropyl)malonic Acid Diethyl Ester (3d). To a mixture of 4.33 g (27 mmol) of diethyl malonate in 25 mL of THF was sequentially added 1.14 g (28.5 mmol) of 60% NaH/mineral oil and 5.37 g (27 mmol) of (3-bromopropyl)benzene (**2d**). The resulting mixture was refluxed for 18 h, and solvent was removed in a vacuum, followed by addition of 10 mL of water. The mixture was extracted with diethyl ether, and the extracts were dried over anhydrous Na_2SO_4 and concentrated in a vacuum. The crude product was chromatographed over silica gel with petroleum ether/ethyl acetate (100:1) to give 6.6 g of **3d** (88.5%): ^{1}H NMR ($CDCl_3$) δ 7.28−7.12 (m, 5H), 4.00 (q, J = 7.32 Hz, 4H), 3.33 (t, J = 7.69 Hz, 1H), 2.63 (m, 2H), 1.92 (m, 2H), 1.64 (m, 2H), 1.24 (t, J = 7.32 Hz, 6H).

2-Phenethylmalonic Acid Diethyl Ester (3g). Compound **3g** was prepared as for **3d** from (2-bromoethyl)benzene (**2g**) (15.0 g, 81 mmol) in 49.0% yield: ^{1}H NMR ($CDCl_3$) δ 7.29−7.18 (m, 5H), 4.19 (q, J = 7.20 Hz, 4H), 3.34 (t, J = 7.40 Hz, 1H), 2.65 (t, J = 7.60 Hz, 2H), 2.22 (dt, J_1 = 7.60 Hz, J_2 = 7.40 Hz, 2H), 1.26 (t, J = 7.20 Hz, 6H).

2-(4-Phenylbutyl)malonic Acid Diethyl Ester (3h). Compound **3h** was synthesized as for **3d** from (4-chlorobutyl)-

benzene (**2h**) (3.4 g, 20 mmol) in 37.7% yield: ^{1}H NMR ($CDCl_3$) δ 7.28−7.19 (m, 5H), 4.19 (q, J = 6.96 Hz, 4H), 3.31 (t, J = 7.50 Hz, 1H), 2.61 (t, J = 7.69 Hz, 2H), 1.94 (m, 2H), 1.64 (m, 2H), 1.37 (m, 2H), 1.25 (t, J = 6.96 Hz, 6H).

Method C. 2-Cinnamyl-2-propargylmalonic Acid Diethyl Ester (4a). Sodium (334 mg, 14.5 mmol) was dissolved in 40 mL of absolute EtOH. To the EtONa solution 2.7 g (9.6 mmol) of compound **3a** was added, followed by addition of 1.3 g (10 mmol) of propargyl bromide in 20 mL of absolute EtOH. The resulting mixture was stirred at 40 °C overnight. The solvent and remaining propargyl bromide were removed by evaporation in a vacuum, followed by addition of 20 mL of H_2O. The resulting aqueous was extracted with chloroform, and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in a vacuum to provide 2.6 g (87.2%) of **4a** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.34−7.22 (m, 5H), 6.52 (d, J = 15.75 Hz, 1H), 6.04 (dt, J_1 = 7.69 Hz, J_2 = 15.75 Hz, 1H), 4.23 (q, J = 7.32 Hz, 4H), 2.97 (dd, J_1 = 1.1 Hz, J_2 = 7.69 Hz, 2H), 2.84 (d, J = 2.57 Hz, 2H), 2.06 (t, J = 2.57 Hz, 1H), 1.26 (t, J = 7.32 Hz, 6H).

2-But-2-ynyl-2-cinnamylmalonic Acid Diethyl Ester (4c). Compound **4c** was synthesized as for **4a** from **3a** (1.3 g, 4.8 mmol) and 0.7 g (5.2 mmol) of 1-bromo-2-butyne in 88.8% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.31−7.19 (m, 5H), 6.49 (d, J = 15.74 Hz, 1H), 6.06 (dt, J_1 = 7.69 Hz, J_2 = 15.74 Hz, 1H), 4.20 (q, J = 7.33 Hz, 4H), 2.93 (d, J = 7.69 Hz, 2H), 2.78 (q, J = 2.20 Hz, 2H), 1.78 (t, J = 2.20 Hz, 3H), 1.26 (t, J = 7.33 Hz, 6H).

2-(3-Phenylpropyl)-2-propargylmalonic Acid Diethyl Ester (4d). Compound **4d** was prepared as for **4a** from **3d** (5.4 g, 19.6 mmol) and 2.5 g (21.1 mmol) of propargyl bromide in 98.4% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.29−7.12 (m, 5H), 4.16 (q, J = 7.32 Hz, 4H), 2.82 (d, J = 2.44 Hz, 2H), 2.65 (m, 2H), 2.10 (m, 2H), 1.98 (t, J = 2.44 Hz, 1H), 1.54 (m, 2H), 1.22 (t, J = 7.32 Hz, 6H).

2-Naphthalen-1-ylmethyl-2-propargylmalonic Acid Diethyl Ester (4e). Compound **4e** was synthesized as for **4a** from **3e** (1.4 g, 4.7 mmol) and 625 mg (5.2 mmol) of propargyl bromide in 86.3% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 8.00−7.37 (m, 7H), 4.10 (q, J = 7.14 Hz, 4H), 3.90 (s, 2H), 2.72 (d, J = 2.57 Hz, 2H), 2.20 (t, J = 2.56 Hz, 1H), 1. (t, J = 7.14 Hz, 6H).

2-Benzyl-2-propargylmalonic Acid Diethyl Ester (4f). Compound **4f** was prepared as for **4a** from **3f** (1.2 g, 4.8 mmol) and 625 mg (5.2 mmol) of propargyl bromide in 73.0% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.27−7.15 (m, 5H), 4.20 (q, J = 7.06 Hz, 4H), 3.39 (s, 2H), 2.68 (d, J = 2.42 Hz, 2H), 2.14 (t, J = 2.42 Hz, 1H), 1.25 (t, J = 7.06 Hz, 6H).

2-Phenethyl-2-propargylmalonic Acid Diethyl Ester (4g). Compound **4g** was synthesized as for **4a** from **3g** (1.26 g, 4.8 mmol) and 625 mg (5.2 mmol) of propargyl bromide in 75.7% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.30−7.18 (m, 5H), 4.21 (q, J = 7.20 Hz, 4H), 2.92 (d, J = 2.56 Hz, 2H), 2.53 (t, J = 7.60 Hz, 2H), 2.38 (t, J = 7.60 Hz, 2H), 2.04 (t, J = 2.56 Hz, 1H), 1.26 (t, J = 7.20 Hz, 6H).

2-(4-Phenylbutyl)-2-propargylmalonic Acid Diethyl Ester (4h). Compound **4h** was prepared as for **4a** from **3h** (2.0 g, 6.85 mmol) and 883 mg (7.4 mmol) of propargyl bromide in 79.6% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.27−7.14 (m, 5H), 4.18 (q, J = 7.33 Hz, 4H), 2.79 (d, J = 2.56 Hz, 2H), 2.60 (t, J = 7.69 Hz, 2H), 2.07 (m, 2H), 1.98 (t, J = 2.56 Hz, 1H), 1.65 (m, 2H), 1.21 (m, 8H).

Method D. 2-Cinnamyl-2-propargylmalonic Acid (5a). To a solution of 2.60 g (8.3 mmol) of **4a** in 20 mL of EtOH, was added 20 mL of 2 N NaOH. The resulting mixture was heated at reflux for 3 h. The ethanol was removed by evaporation, and the remaining aqueous portion was washed with chloroform. The washed aqueous portion was acidified to pH 2 with cold HCl and extracted with chloroform. The extracts were pooled, dried over anhydrous Na_2SO_4, and concentrated to provide 2.08 g of **5a** in 97.4% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 9.77 (broad s, 2H), 7.35−7.21 (m, 5H), 6.59 (d, J = 15.74 Hz, 1H), 6.06 (dt, J_1 = 7.69 Hz, J_2 = 15.74 Hz, 1H), 3.00 (d, J = 7.69 Hz, 2H), 2.88 (d, J = 2.56 Hz, 2H), 2.08 (t, J = 2.56 Hz, 1H).

2-But-2-ynyl-2-cinnamylmalonic Acid (5c). Compound **5c** was synthesized as for **5a** from **4c** (1.30 g, 4.0 mmol) in 82.6% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 10.60 (broad s, 2H), 7.34−7.19 (m, 5H), 6.55 (d, J = 15.75 Hz, 1H), 6.07 (dt, J_1 = 7.69 Hz, J_2 = 15.75 Hz, 1H), 2.95 (d, J = 7.69 Hz, 2H), 2.82 (q, J = 2.20 Hz, 2H), 1.77 (t, J = 2.20 Hz, 3H).

2-(3-Phenylpropyl)-2-propargylmalonic Acid (5d). Compound **5d** was prepared as for **5a** from **4d** (6.08 g, 19.2 mmol) in 97.0% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 10.87 (broad s, 2H), 7.39−7.22 (m, 5H), 2.94 (d, J = 2.40 Hz, 2H), 2.74 (m, 2H), 2.23 (m, 2H), 2.08 (t, J = 2.40 Hz, 1H), 1.70 (m, 2H).

2-Naphthalen-1-ylmethyl-2-propargylmalonic Acid (5e). Compound **5e** was synthesized as for **5a** from **4e** (1.40 g, 4.1 mmol) in 90.7% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 9.27 (broad s, 2H), 8.27−7.36 (m, 7H), 3.94 (s, 2H), 2.76 (d, J = 2.44 Hz, 2H), 2.27 (t, J = 2.44 Hz, 1H).

2-Benzyl-2-propargylmalonic Acid (5f). Compound **5f** was prepared as for **5a** from **4f** (1.03 g, 3.6 mmol) in 98.8% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.85 (broad s, 2H), 7.31−7.19 (m, 5H), 3.40 (s, 2H), 2.73 (d, J = 2.37 Hz, 2H), 2.20 (t, J = 2.37 Hz, 1H).

2-Phenethyl-2-propargylmalonic Acid (5g). Compound **5g** was synthesized as for **5a** from **4g** (3.35 g, 11 mmol) in 89.4% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.28−7.15 (m, 5H), 6.12 (broad s, 2H), 2.94 (d, J = 2.56 Hz, 2H), 2.61 (t, J = 8.42 Hz, 2H), 2.39 (t, J = 8.42 Hz, 2H), 2.08 (t, J = 2.56 Hz, 1H); EIMS m/z 246 (M^+), 91 (100).

2-(4-Phenylbutyl)-2-propargylmalonic Acid (5h). Compound **5h** was prepared as for **5a** from **4h** (1.80 g, 5.5 mmol) in 94.0% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 9.76 (broad s, 2H), 7.30−7.16 (m, 5H), 2.86 (d, J = 2.56 Hz, 2H), 2.64 (t, J = 7.68 Hz, 2H), 2.15 (m, 2H), 2.06 (t, J = 2.56 Hz, 1H), 1.68 (m, 2H), 1.36 (m, 2H).

2-Cyclohexylmethyl-2-propargylmalonic Acid (11a). Compound **11a** was synthesized as for **5a** from **10a** (650 mg, 2.2 mmol) in 97.0% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 9.01 (broad s, 2H), 2.84 (d, J = 2.57 Hz, 2H), 2.04 (t, J = 2.57 Hz, 1H), 1.99 (d, J = 6.06 Hz, 2H), 1.64−0.95 (m, 11H); ESI-MS m/z 239 ($M^+ + 1$).

2-Hexyl-2-propargylmalonic Acid (11b). Compound **11b** was prepared as for **5a** from **10b** (580 mg, 2 mmol) in 99.0% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 8.29 (broad s, 2H), 2.83 (d, J = 2.56 Hz, 2H), 2.00−0.84 (m, 14H); ESI-MS m/z 225 ($M^+ - 1$).

2-Heptyl-2-propargylmalonic Acid (11c). Compound **11c** was synthesized as for **5a** from **10c** (1.03 g, 3.48 mmol) in 98.2% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.88 (broad s, 2H), 2.83 (d, J = 2.57 Hz, 2H), 2.00−0.82 (m, 16 H); ESI-MS m/z 239 ($M^+ - 1$).

2-Octyl-2-propargylmalonic Acid (11d). Compound **11d** was prepared as for **5a** from **10d** (1.21 g, 3.90 mmol) in 97.0% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 8.29 (broad s, 2H), 2.83 (d, J = 2.56 Hz, 2H), 2.04 (m, 3H), 1.24 (m, 12H), 0.86 (t, J = 6.22 Hz, 3H); ESI-MS m/z 253 ($M^+ - 1$).

2-Nonyl-2-propargylmalonic Acid (11e). Compound **11e** was synthesized as for **5a** from **10e** (1.06 g, 3.27 mmol) in 98.2% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 8.31 (broad s, 2H), 2.83 (d, J = 2.56 Hz, 2H), 2.03 (m, 3H), 1.23 (m, 14H), 0.85 (t, J = 6.77 Hz, 3H); ESI-MS m/z 267 ($M^+ - 1$).

2-Naphthalen-2-ylmethyl-2-propargylmalonic Acid (11f). Compound **11f** was prepared as for **5a** from **10f** (2.30 g, 6.8 mmol) in 99.0% yield as a white solid (mp 156−158 °C, dec): ^{1}H NMR (CD_3OD) δ 7.77 (m, 4H), 7.43 (m, 3H), 3.51 (s, 2H), 2.61 (d, J = 2.48 Hz, 2H), 2.00 (t, J = 2.48 Hz, 1H).

2-[*trans*-4-(Trifluoromethyl)cinnamyl]-4-pentynoic Acid (25). Compound **25** was synthesized as for **5a** from **24** (620 mg, 2 mmol) in 98.0% yield as a pale-yellow oil: ^{1}H NMR ($CDCl_3$) δ 9.05 (broad s, 1H), 7.52 (d, J = 8.06 Hz, 2H), 7.40 (d, J = 8.54 Hz, 2H), 6.50 (d, J = 15.87 Hz, 1H), 6.23 (dt, J_1 = 7.33 Hz, J_2 = 15.87 Hz, 1H), 2.77 (m, 1H), 2.71−2.60 (m, 2H), 2.59−2.54 (m, 1H), 2.51−2.46 (m, 1H), 2.04 (t, J = 2.68 Hz,

1H); EIMS *m/z* 282 (M^+), 167 (100); HREIMS calcd for $C_{15}H_{13}F_3O_2$ 282.0868, found 282.0876.

2-(*trans*-4-Methoxycinnamyl)-4-pentynoic Acid (33). Compound **33** was prepared as for **5a** from **32** (554 mg, 2 mmol) in 99.4% yield as a pale-yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.25 (m, 2H), 6.81 (m, 2H), 6.41 (d, J = 15.63 Hz, 1H), 5.97 (dt, J_1 = 7.33 Hz, J_2 = 15.63 Hz, 1H), 3.77 (s, 3H), 2.73 (m, 1H), 2.61 (m, 1H), 2.56−2.46 (m, 3H), 2.01 (t, J = 2.68 Hz, 1H); EIMS *m/z* 244 (M^+), 147 (100); HREIMS calcd for $C_{15}H_{16}O_3$ 244.1099, found 244.1101.

Method E. 2-Cinnamyl-4-pentynoic Acid (6a). Compound **5a** (2.00 g, 7.8 mmol) was heated at 140 °C for 3 h and the resulting oil was chromatographed (petroleum ether/ethyl acetate/TFA, 30:1:0.1%) to provide 0.96 g of **6a** in 57.9% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 10.50 (broad s, 1H), 7.35−7.21 (m, 5H), 6.51 (d, J = 15.75 Hz, 1H), 6.00 (dt, J_1 = 7.69 Hz, J_2 = 15.75 Hz, 1H), 2.80 (m, 1H), 2.72−2.51 (m, 4H), 2.05 (t, J = 2.56 Hz, 1H).

2-Cinnamyl-4-hexynoic Acid (6c). Compound **5c** (0.89 g, 3.3 mmol) was heated at 170 °C for 3 h and the resulting oil was chromatographed (petroleum ether/ethyl acetate, 10:1) to provide 0.53 g of **6c** in 71.0% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 7.36−7.20 (m, 5H), 6.48 (d, J = 15.74 Hz, 1H), 6.16 (dt, J_1 = 7.32 Hz, J_2 = 15.74 Hz, 1H), 2.75−2.41 (m, 5H), 1.79 (t, J = 2.38 Hz, 3H); ESI-MS *m/z* 227 ($M^+ - 1$).

2-(3-Phenylpropyl)-4-pentynoic Acid (6d). Compound **5d** (4.0 g, 15.4 mmol) was heated at 160 °C for 3 h and the resulting oil was chromatographed (petroleum ether/ethyl acetate/TFA, 50:1:01%) to provide 2.15 g of **6d** in 64.7% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 9.71 (broad s, 1H), 7.29−7.18 (m, 5H), 2.69 (m, 3H), 2.57−2.40 (m, 2H), 2.00 (t, J = 2.40 Hz, 1H), 1.74 (m, 4H).

2-Naphthalen-1-ylmethyl-4-pentynoic Acid (6e). Compound **5e** (1.06 g, 3.76 mmol) was heated at 150 °C for 3 h and the resulting oil was chromatographed (petroleum ether/ethyl acetate/TFA, 50:1:01%) to provide 0.77 g of **6e** in 86.1% yield as a brown oil: ^{1}H NMR ($CDCl_3$) δ 8.07−7.39 (m, 7H), 3.62 (m, 1H), 3.39 (m, 1H), 3.10 (m, 1H), 2.50 (m, 2H), 2.11 (t, J = 2.44 Hz, 1H).

2-Benzyl-4-pentynoic Acid (6f). Compound **5f** (0.82 g, 3.5 mmol) was heated at 145 °C for 3 h and the resulting oil was chromatographed (petroleum ether/ethyl acetate/TFA, 50:1:01%) to provide 0.35 g of **6f** in 53.0% yield as a brown oil: ^{1}H NMR ($CDCl_3$) δ 7.30−7.20 (m, 5H), 3.10 (m, 1H), 2.99−2.84 (m, 2H), 2.44 (m, 2H), 2.06 (t, J = 2.56 Hz, 1H).

2-Phenethyl-4-pentynoic Acid (6g). Compound **5g** (2.14 g, 8.7 mmol) was heated at 145 °C for 3 h and the resulting oil was chromatographed (petroleum ether/ethyl acetate/TFA, 50:1:01%) to provide 1.10 g of **6g** in 62.5% yield as a pale-yellow oil: ^{1}H NMR ($CDCl_3$) δ 8.75 (broad s, 1H), 7.28−7.10 (m, 5H), 2.78−2.60 (m, 3H), 2.60−2.45 (m, 2H), 2.16−1.96 (m, 2H), 2.03 (t, J = 2.61 Hz, 1H).

2-(4-Phenylbutyl)-4-pentynoic Acid (6h). Compound **5h** (1.40 g, 5.1 mmol) was heated at 155 °C for 3 h and the resulting oil was chromatographed (petroleum ether/ethyl acetate/TFA, 50:1:01%) to provide 0.86 g of **6h** in 73.5% yield as a pale-yellow oil: ^{1}H NMR ($CDCl_3$) δ 10.47 (broad s, 1H), 7.31−7.18 (m, 5H), 2.64 (m, 2H), 2.60−2.51 (m, 3H), 2.03 (t, J = 2.56 Hz, 1H), 1.72 (m, 2H), 1.65 (m, 2H), 1.44 (m, 2H).

2-Cyclohexylmethyl-4-pentynoic Acid (12a). Compound **11a** (510 mg, 2.1 mmol) was heated at 125 °C for 3 h and the resulting oil was chromatographed (petroleum ether/ethyl acetate/TFA, 50:1:01%) to provide 210 mg of **12a** in 50.5% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.70 (m, 1H), 2.52−2.35 (m, 2H), 2.00 (t, J = 2.56 Hz, 1H), 1.79−0.83 (m, 13H); ESI-MS *m/z* 195 ($M^+ + 1$).

2-Hexyl-4-pentynoic Acid (12b). Compound **11b** (460 mg, 2 mmol) was heated at 130 °C for 3 h and the resulting oil was chromatographed (petroleum ether/ethyl acetate/TFA, 50:1:01%) to provide 310 mg of **12b** in 83.8% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.60 (m, 1H), 2.55−2.38 (m, 2H), 2.00 (t, J = 2.56 Hz, 1H), 1.76−1.22 (m, 10H), 0.87 (t, J = 6.77 Hz, 3H); ESI-MS *m/z* 183 ($M^+ + 1$).

2-Heptyl-4-pentynoic Acid (12c). Compound **11c** (0.82 g, 3.4 mmol) was heated at 140 °C for 3 h and the resulting oil was chromatographed (petroleum ether/ethyl acetate/TFA, 50:1:01%) to provide 390 mg of **12c** in 58.2% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.60 (m, 1H), 2.55−2.38 (m, 2H), 2.00 (t, J = 2.61 Hz, 1H), 1.72−1.23 (m, 12H), 0.87 (t, J = 6.95 Hz, 3H); ESI-MS *m/z* 197 ($M^+ + 1$).

2-Octyl-4-pentynoic Acid (12d). Compound **11d** (960 mg, 3.78 mmol) was heated at 130 °C for 3 h and the resulting oil was chromatographed (petroleum ether/ethyl acetate/TFA, 50:1:01%) to provide 340 mg of **12d** in 43.0% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 8.30 (broad s, 1H), 2.60 (m, 1H), 2.54−2.38 (m, 2H), 2.00 (t, J = 2.56 Hz, 1H), 1.76−1.25 (m, 14H), 0.87 (t, J = 6.41 Hz, 3H); ESI-MS *m/z* 211 ($M^+ + 1$).

2-Nonyl-4-pentynoic Acid (12e). Compound **11e** (860 mg, 3.2 mmol) was heated at 140 °C for 3 h and the resulting oil was chromatographed (petroleum ether/ethyl acetate/TFA, 50:1:01%) to provide 350 mg of **12e** in 48.6% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.60 (m, 1H), 2.55−2.37 (m, 2H), 2.00 (t, J = 2.57 Hz, 1H), 1.72−1.20 (m, 16 H), 0.86 (t, J = 6.78 Hz, 3H); ESI-MS *m/z* 225 ($M^+ + 1$).

2-Naphthalen-2-ylmethyl-4-pentynoic Acid (12f). Compound **11f** (1.90 g, 6.74 mmol) was heated at 180 °C for 3 h and the resulting oil was chromatographed (petroleum ether/ethyl acetate/TFA, 50:1:01%) to provide 1.02 g of **12f** in 63.8% yield as a pale-yellow solid (mp 119−120 °C): ^{1}H NMR ($CDCl_3$) δ 7.78 (m, 3H), 7.66 (s, 1H), 7.44 (m, 2H), 7.33 (dd, J_1 = 1.65 Hz, J_2 = 8.43 Hz, 1H), 3.26 (dd, J_1 = 6.79 Hz, J_2 = 13.74 Hz, 1H), 3.12 (dd, J_1 = 7.51 Hz, J_2 = 13.74 Hz, 1H), 3.00 (m, 1H), 2.47 (dd, J_1 = 2.66 Hz, J_2 = 6.50 Hz, 2H), 2.08 (t, J = 2.66 Hz, 1H).

Method F. 3-Cinnamyl-5(*E*)-bromomethylidenetetrahydro-2-furanone (1a). To a solution of **6a** (0.428 g, 2 mmol) in 25 mL of CH_2Cl_2 was sequentially added 356 mg (2 mmol) of *N*-bromosuccinimide, 200 mg (2 mmol) of $KHCO_3$, and 0.5 mL of Bu_4NOH (40% in water). After being stirred vigorously at room temperature for 30 min, the mixture was diluted with 10 mL of CH_2Cl_2. The CH_2Cl_2 phase was washed with 5% $Na_2S_2O_3$ and H_2O, dried over anhydrous Na_2SO_4, and concentrated in a vacuum. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 100:1) to afford 0.36 g of **1a** in 61.4% yield as a pale-yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.38−7.24 (m, 5H), 6.52 (d, J = 15.74 Hz, 1H), 6.12 (dt, J_1 = 7.32 Hz, J_2 = 15.74 Hz, 1H), 5.99 (t, J = 2.38 Hz, 1H), 3.12−2.98 (m, 2H), 2.80−2.74 (m, 1H), 2.68−2.62 (m, 1H), 2.58−2.50 (m, 1H); EIMS *m/z* 294 ($M^+ + 2$), 292 (M^+), 149 (100); HREIMS calcd for $C_{14}H_{13}BrO_2$ 292.0098, found 292.0091.

3-Cinnamyl-5(*E*)-iodomethylidenetetrahydro-2-furanone (1b). Compound **1b** was prepared as for **1a** from **6a** (0.428 g, 2 mmol) and 450 mg (2 mmol) of *N*-iodosuccinimide in 50.0% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.37−7.21 (m, 5H), 6.50 (d, J = 15.74 Hz, 1H), 6.10 (dt, J_1 = 7.32 Hz, J_2 = 15.74 Hz, 1H), 5.81 (t, J = 2.38 Hz, 1H), 3.06−2.97 (m, 2H), 2.78−2.71 (m, 1H), 2.65−2.49 (m, 2H); EIMS *m/z* 340 (M^+), 117 (100); HREIMS calcd for $C_{14}H_{13}IO_2$ 339.9960, found 339.9951.

3-Cinnamyl-5(*E*)-(1-bromoethylidene)tetrahydro-2-furanone (1c). Compound **1c** was synthesized as for **1a** from **6c** (228 mg, 1 mmol) in 50.8% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.34−7.21 (m, 5H), 6.49 (d, J = 15.75 Hz, 1H), 6.11 (dt, J_1 = 7.33 Hz, J_2 = 15.75 Hz, 1H), 3.08−2.94 (m, 2H), 2.76−2.70 (m, 1H), 2.65−2.59 (m, 1H), 2.55−2.47 (m, 1H), 2.25 (t, J = 2.56 Hz, 3H); EIMS *m/z* 308 ($M^+ + 2$), 306 (M^+), 117 (100); HREIMS calcd for $C_{15}H_{15}BrO_2$ 306.0255, found 306.0251.

3-(3-Phenylpropyl)-5(*E*)-bromomethylidenetetrahydro-2-furanone (1d). Compound **1d** was synthesized as for **1a** from **6d** (250 mg, 1.16 mmol) in 61.6% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.30−7.15 (m, 5H), 5.94 (t, J = 2.38 Hz, 1H), 3.10−3.02 (m, 1H), 2.84−2.76 (m, 1H), 2.63 (t, J = 6.96 Hz, 2H), 2.53−2.46 (m, 1H), 1.89 (m, 1H), 1.72 (m, 2H), 1.60 (m, 1H); EIMS *m/z* 296 ($M^+ + 2$), 294 (M^+), 91 (100); HREIMS calcd for $C_{14}H_{15}BrO_2$ 294.0255, found 294.0247.

3-Naphthalen-1-ylmethyl-5(*E*)-bromomethylidenetetrahydro-2-furanone (1e). Compound **1e** was prepared as for **1a** from **6e** (476 mg, 2 mmol) in 31.5% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 8.02−7.35 (m, 7H), 5.96 (t, J = 2.44 Hz, 1H), 3.92−3.88 (m, 1H), 3.30 (m, 1H), 3.05−2.99 (m, 1H), 2.88−2.80 (m, 1H), 2.64−2.57 (m, 1H); EIMS *m/z* 318 ($M^+ + 2$), 316 (M^+), 141 (100); HREIMS calcd for $C_{16}H_{13}BrO_2$ 316.0098, found 316.0090.

3-Benzyl-5(*E*)-bromomethylidenetetrahydro-2-furanone (1f). Compound **1f** was synthesized as for **1a** from **6f** (359 mg, 1.9 mmol) in 64.7% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.36−7.19 (m, 5H), 5.93 (t, J = 2.38 Hz, 1H), 3.28−3.23 (m, 1H), 3.13 (m, 1H), 3.00−2.93 (m, 1H), 2.89−2.83 (m, 1H), 2.62−2.55 (m, 1H); EIMS *m/z* 266 (M^+), 187 (100); HREIMS calcd for $C_{12}H_{11}BrO_2$ 265.9942, found 265.9912.

3-Phenethyl-5(*E*)-bromomethylidenetetrahydro-2-furanone (1g). Compound **1g** was prepared as for **1a** from **6g** (490 mg, 2.4 mmol) in 71.9% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.33−7.20 (m, 5H), 5.97 (t, J = 2.38 Hz, 1H), 3.15−3.06 (m, 1H), 2.85−2.68 (m, 3H), 2.59−2.53 (m, 1H), 2.25 (m, 1H), 1.89 (m, 1H); EIMS *m/z* 282 ($M^+ + 2$), 280 (M^+), 91 (100); HREIMS calcd for $C_{13}H_{13}BrO_2$ 280.0098, found 280.0089.

3-(4-Phenylbutyl)-5(*E*)-bromomethylidenetetrahydro-2-furanone (1h). Compound **1h** was synthesized as for **1a** from **6h** (470 mg, 2.04 mmol) in 73.0% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.29−7.16 (m, 5H), 5.96 (t, J = 2.56 z, 1H), 3.12−3.03 (m, 1H), 2.80 (m, 1H), 2.63 (m, 2H), 2.55−2.50 (m, 1H), 1.92 (m, 1H), 1.71−1.57 (m, 3H), 1.49−1.41 (m, 2H); EIMS *m/z* 310 ($M^+ + 2$), 308 (M^+), 91 (100); HREIMS calcd for $C_{15}H_{17}BrO_2$ 308.0411, found 308.0387.

3-Cyclohexylmethyl-5(*E*)-bromomethylidenetetrahydro-2-furanone (13a). Compound **13a** was prepared as for **1a** from **12a** (210 mg, 1.08 mmol) in 57.5% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 5.93 (t, J = 2.75 Hz, 1H), 3.12−3.05 (m, 1H), 2.89−2.81 (m, 1H), 2.52−2.45 (m, 1H), 1.82−0.83 (m, 13H); EIMS *m/z* 274 ($M^+ + 2$), 272 (M^+), 193 (100); HREIMS calcd for $C_{12}H_{17}BrO_2$ 272.0407, found 272.0417.

3-Hexyl-5(*E*)-bromomethylidenetetrahydro-2-furanone (13b). Compound **13b** was synthesized as for **1a** from **12b** (310 mg, 1.7 mmol) in 45.1% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 5.94 (t, J = 2.38 Hz, 1H), 3.10−3.03 (m, 1H), 2.82−2.74 (m, 1H), 2.55−2.48 (m,1H), 1.90−1.82 (m, 1H), 1.58−1.49 (m, 1H), 1.40−1.22 (m, 8H), 0.86 (t, J = 6.87 Hz, 3H); EIMS *m/z* 262 ($M^+ + 2$), 260 (M^+), 55 (100); HREIMS calcd for $C_{11}H_{17}BrO_2$ 260.0407, found 260.0394.

3-Heptyl-5(*E*)-bromomethylidenetetrahydro-2-furanone (13c). Compound **13c** was prepared as for **1a** from **12c** (390 mg, 2.0 mmol) in 42.0% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 5.96 (t, J = 2.38 Hz, 1H), 3.12−3.05 (m, 1H), 2.84−2.76 (m, 1H), 2.57−2.50 (m, 1H), 1.92−1.84 (m, 1H), 1.60−1.50 (m, 1H), 1.43−1.27 (m, 10H), 0.88 (t, J = 6.96 Hz, 3H); EIMS *m/z* 276 ($M^+ + 2$), 274 (M^+), 195 (100); HREIMS calcd for $C_{12}H_{19}BrO_2$ 274.0563, found 274.0573.

3-Octyl-5(*E*)-bromomethylidenetetrahydro-2-furanone (13d). Compound **13d** was prepared as for **1a** from **12d** (340 mg, 1.6 mmol) in 60.0% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 5.95 (t, J = 2.38 Hz, 1H), 3.12−3.05 (m, 1H), 2.84−2.76 (m, 1H), 2.57−2.50 (m, 1H), 1.92−1.84 (m, 1H), 1.60−1.50 (m, 1H), 1.43−1.26 (m,12H), 0.88 (t, J = 6.78 Hz, 3H); EIMS *m/z* 290 ($M^+ + 2$), 288 (M^+), 55 (100); HREIMS calcd for $C_{13}H_{21}BrO_2$ 288.0719, found 288.0721.

3-Nonyl-5(*E*)-bromomethylidenetetrahydro-2-furanone (13e). Compound **13e** was synthesized as for **1a** from **12e** (350 mg, 1.56 mmol) in 53.2% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 5.96 (t, J = 2.38 Hz, 1H), 3.13−3.05 (m, 1H), 2.84−2.76 (m, 1H), 2.57−2.50 (m, 1H), 1.92−1.84 (m, 1H), 1.60−1.51 (m, 1H), 1.40−1.26 (m, 14H), 0.88 (t, J = 6.97 Hz, 3H); EIMS *m/z* 304 ($M^+ + 2$), 302 (M^+), 72 (100); HREIMS calcd for $C_{14}H_{23}BrO_2$ 302.0875, found 302.0880.

3-Naphthalen-2-ylmethyl-5(*E*)-bromomethylidenetetrahydro-2-furanone (13f). Compound **13f** was synthesized as for **1a** from **12f** (620 mg, 2.6 mmol) in 53.4% yield as a yellow solid (mp 82−84 °C): ^{1}H NMR ($CDCl_3$) δ 7.83 (m, 3H), 7.65 (s, 1H), 7.49 (m, 2H), 7.32 (dd, J_1 = 1.83 Hz, J_2 = 8.43 Hz, 1H), 5.93 (t, J = 2.56 Hz, 1H), 3.43 (dd, J_1 = 4.59 Hz, J_2 = 13.94 Hz, 1H), 3.25 (m, 1H), 3.05−2.93 (m, 2H), 2.63 (ddd, J_1 = 2.70 Hz, J_2 = 7.70 Hz, J_3=0.41 Hz, 1H); EIMS *m/z* 318 ($M^+ + 2$), 316 (M^+), 141 (100); HREIMS calcd for $C_{16}H_{13}BrO_2$ 316.0100, found 316.0108.

3-Phenyl-5(*E*)-bromomethylidenetetrahydro-2-furanone (17). Compound **17** was prepared as for **1a** from **16** (0.78 g, 4.48 mmol) in 24.4% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.36 (m, 5H), 6.08 (t, J = 2.37 Hz, 1H), 4.05 (dd, J_1 = 7.32 Hz, J_2 = 10.60 Hz, 1H), 3.45 (m, 1H), 3.03 (m, 1H); EIMS *m/z* 254 ($M^+ + 2$), 252 (M^+), 104 (100); HREIMS calcd for $C_{11}H_9BrO_2$ 251.9786, found 251.9799.

3-[*trans*-4-(Trifluoromethyl)cinnamyl]-5(*E*)-bromomethylidenetetrahydro-2-furanone (26). Compound **26** was synthesized as for **1a** from **25** (552 mg, 2 mmol) in 21.5% yield as a pale-yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.57 (d, J = 8.23 Hz, 2H), 7.45 (d, J = 8.23 Hz, 2H), 6.55 (d, J = 15.74 Hz, 1H), 6.23 (dt, J_1 = 7.31 Hz, J_2 = 15.74 Hz, 1H), 6.00 (t, J = 2.56 Hz, 1H), 3.15−3.08 (m, 1H), 3.07−3.00 (m, 1H), 2.82−2.76 (m, 1H), 2.68−2.55 (m, 2H); EIMS *m/z* 362 ($M^+ + 2$), 360 (M^+), 149 (100); HREIMS calcd for $C_{15}H_{12}BrF_3O_2$ 359.9973, found 359.9969.

3-(*trans*-4-Methoxycinnamyl)-5(*E*)-bromomethylidenetetrahydro-2-furanone (34). Compound **34** was prepared as for **1a** from **33** (485 mg, 2 mmol) in 2.3% yield as a yellow oil. But compound **34** was unstable and decomposed during purification with column chromatography: EIMS *m/z* 324 ($M^+ + 2$), 322 (M^+), 149 (100); HREIMS calcd for $C_{15}H_{15}BrO_3$ 322.0204, found 322.0208.

Method G. 2-Cyclohexylmethyl-2-propargylmalonic Acid Diethyl Ester (10a). Sodium (167 mg, 7.2 mmol) was dissolved in 10 mL of absolute EtOH. To the EtONa solution 990 mg (5 mmol) of compound **8** was added, followed by addition of 885 mg (5 mmol) of bromomethylcyclohexane (**9a**). The mixture was refluxed for 6 h, and the resulting precipitates were removed by filtration. The organic solvent was evaporated in a vacuum, followed by addition of H_2O (20 mL). The mixture was extracted with chloroform (3 × 30 mL), and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in a vacuum. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 100:1) to afford 670 mg of **10a** in 45.6% yield as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 4.15 (q, J = 6.97 Hz, 4H), 2.84 (d, J = 2.57 Hz, 2H), 2.00−0.94 (m, 20H); EIMS *m/z* 294 (M^+), 220 (100).

2-Hexyl-2-propargylmalonic Acid Diethyl Ester (10b). Compound **10b** was synthesized as for **10a** from 842 mg (5 mmol) of 1-bromohexane (**9b**) in 41.1% yield as a yellow liquid: ^{1}H NMR ($CDCl_3$) δ 4.19 (q, J = 7.33 Hz, 4H), 2.80 (d, J = 2.57 Hz, 2H), 2.02 (m, 2H), 1.98 (t, J = 2.57 Hz, 1H), 1.33−1.13 (m, 14H), 0.86 (t, J = 6.80 Hz, 3H); EIMS *m/z* 282 (M^+), 198 (100).

2-Heptyl-2-propargylmalonic Acid Diethyl Ester (10c). Compound **10c** was prepared as for **10a** from 904 mg (5 mmol) of 1-bromoheptane (**9c**) in 69.6% yield as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 4. (q, J = 7.33 Hz, 4H), 2.78 (d, J = 2.57 Hz, 2H), 2.00 (m, 2H), 1.96 (t, J = 2.57 Hz, 1H), 1.28−1.09 (m, 16 H), 0.84 (t, J = 6.96 Hz, 3H); EIMS *m/z* 297 ($M^+ + 1$), 198 (100).

2-Octyl-2-propargylmalonic Acid Diethyl Ester (10d). Compound **10d** was synthesized as for **10a** from 975 mg (5 mmol) of 1-bromooctane (**9d**) in 78.1% yield as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 4.19 (q, J = 7.33 Hz, 4H), 2.80 (d, J = 2.57 Hz, 2H), 2.02 (m, 2H), 1.97 (t, J = 2.57 Hz, 1H), 1.33−1.11 (m, 18H), 0.86 (t, J = 6.97 Hz, 3H); EIMS *m/z* 311 ($M^+ + 1$), 198 (100).

2-Nonyl-2-propargylmalonic Acid Diethyl Ester (10e). Compound **10e** was prepared as for **10a** from 1.05 g (5 mmol) of 1-bromononane (**9e**) in 65.4% yield as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 4.18 (q, J = 7.15 Hz, 4H), 2.80 (d, J = 2.57 Hz, 2H), 2.02 (m, 2H), 1.97 (t, J = 2.57 Hz, 1H), 1.23 (m, 20H), 0.86 (t, J = 6.78 Hz, 3H); EIMS *m/z* 324 (M^+), 198 (100).

2-Naphthalen-2-ylmethyl-2-propargylmalonic Acid Diethyl Ester (10f). A solution of 4.74 g (30 mmol) of 2-naph-

thalenemethanol in 60 mL of CH_2Cl_2 was treated sequentially with Et_3N (4.2 mL, 30 mmol), *p*-toluenesulfonyl chloride (6.86 g, 36 mmol), and DMAP (2.20 g, 18 mol). After being stirred at room temperature for 2 h, the mixture was diluted with 120 mL of Et_2O and the precipitated solids were removed by filtration. The organic phase was washed with 10% $CuSO_4$ solution, 10% $NaHCO_3$, and saturated brine, dried over anhydrous Na_2SO_4, and concentrated in a vacuum to provide 3.39 g of 2-chloromethylnaphthalene (**9f**) in 64.0% yield as a white solid (mp 50−51 °C): ^{1}H NMR ($CDCl_3$) δ 7.85 (m, 4H), 7.52 (m, 3H), 4.77 (s, 2H); EIMS *m*/*z* 178 ($M^+ + 2$), 176 (M^+), 141 (100). Compound **10f** was synthesized as for **10a** from 1.76 g (10 mmol) of 2-chloromethylnaphthalene (**9f**) in 74.3% yield as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.78 (m, 3H), 7.67 (d, J = 0.69 Hz, 1H), 7.46 (m, 2H), 7.31 (dd, J_1 = 1.72 Hz, J_2 = 8.44 Hz, 1H), 4.25 (q, J = 7.14 Hz, 4H), 3.59 (s, 2H), 2.73 (d, J = 2.70 Hz, 2H), 2.22 (t, J = 2.70 Hz, 1H), 1.28 (t, J = 7.14 Hz, 6H); EIMS *m*/*z* 338 (M^+), 141 (100).

Method H. 2-Phenyl-5-trimethylsilyl-4-pentynoic Acid (15). Lithium diisopropylamide solution (10 mL of a 2.0 M solution in THF/heptane/ethylbenzene, 20 mmol) was diluted with dry THF (10 mL), and this solution was stirred at 0 °C for 20 min. 2-Phenylethanoic acid (1.36 g, 10.0 mmol) in 15 mL of THF was added dropwise to the lithium diisopropylamide solution while maintaining the solution at 0 °C, and the resulting turbid mixture was stirred for 1 h. HMPA (2 mL) was added to solubilize the dianion precipitated. To the resulting yellow homogeneous solution was added dropwise 1.91 g (10 mmol) of 3-bromo-1-(trimethylsilyl)-1-propyne in THF (5 mL), and the reaction mixture was allowed to warm to 25 °C. After being stirred for 4 h, the reaction was quenched with cold 6 N HCl, and the mixture was extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with 1 N HCl, water, and saturated brine and dried over anhydrous Na_2SO_4. The solvent was evaporated in a vacuum and the residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 20:1) to afford 2.1 g of **15** in 85.4% yield as a pale-yellow solid (mp 87−89 °C): ^{1}H NMR ($CDCl_3$) δ 8.50 (broad s, 1H), 7.31 (m, 5H), 3.80 (t, J = 7.70 Hz, 1H), 2.92 (m, 1H), 2.64 (m, 1H), 0.06 (s, 9H); EIMS *m*/*z* 246 (M^+), 75 (100).

Method I. 2-Phenyl-4-pentynoic Acid (16). To a solution of **15** (1.23 g, 5.0 mmol) in 30 mL of THF was added tetrabutylammonium fluoride trihydrate (3.15 g, 10 mmol). After being stirred for 3 h at room temperature, the solution was acidified with 1 N HCl to pH 3 and 60 mL of diethyl ether was added. The organic layer was separated, washed with saturated brine, dried over anhydrous Na_2SO_4, and concentrated to give 0.78 g of **16** in 89.7% yield as a yellow solid (mp 89−91 °C): ^{1}H NMR ($CDCl_3$) δ 8.70 (broad s, 1H), 7.34 (m, 5H), 3.83 (t, J = 7.70 Hz, 1H), 2.94 (m, 1H), 2.65 (m, 1H), 1.97 (t, J = 2.57 Hz, 1H); EIMS *m*/*z* 174 (M^+), 129 (100).

Method J. *trans*-4-(Trifluoromethyl)cinnamoyl Chloride (19). To a mixture of 9.90 g (45.8 mmol) of *trans*-4-(trifluoromethyl)cinnamic acid (**18**) and 60 mL of dry benzene was added thionyl chloride (7.7 g, 64.7 mmol). After the mixture was stirred at reflux for 10 h, the solvent and excess thionyl chloride were removed by evaporation under reduced pressure. Recrystallization of the mixture from benzene/cyclohexane (3:1) afforded 10.53 g of **19** in 98.0% yield as white flakes (mp 61−63 °C).

Method K. *trans*-4-(Trifluoromethyl)cinnamaldehyde (20). To a 500 mL round-bottom flask containing 10.53 g (45.0 mmol) of **19** and 90 mL of dry THF at −78 °C was added dropwise a solution of 11.76 g (45 mmol) of lithium tri-*tert*-butoxyaluminohydride in 220 mL of THF. After being stirred for an additional 30 min, the reaction mixture was allowed to warm to room temperature and poured over crushed ice (250 g). The solids were filtered off and washed thoroughly with 400 mL of diethyl ether. The organic layer was separated, washed with 5% NaOH and brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 50:1) to afford 6.60 g (73.5%) of **20** in 73.5% yield as a yellow solid (mp 59−61 °C): ^{1}H NMR ($CDCl_3$) δ 9.75 (d, J = 7.33 Hz, 1H), 7.69 (m, 4H), 7.51 (d, J = 16.11 Hz, 1H), 6.77 (dd, J_1 = 7.69 Hz, J_2 = 16.11 Hz, 1H); EIMS *m*/*z* 199 ($M^+ - 1$, 100).

Method L. *trans*-4-(Trifluoromethyl)cinnamyl Alcohol (21). To a solution of 5.3 g (26.5 mmol) of **20** in 110 mL of absolute methanol was added dropwise a solution of 2.0 g (53 mmol) of sodium borohydride in 60 mL of absolute methanol. After being stirred at room temperature for 1 h, the reaction mixture was neutralized with 10% sulfuric acid and filtered to remove inorganic salts. The filtrate was concentrated, and the residue was dissolved in 100 mL of diethyl ether, washed with 5% aqueous sodium carbonate, and dried over anhydrous Na_2SO_4. Purification of the crude product by flash column chromatography (petroleum ether/ethyl acetate, 20:1) afforded 5.3 g of **21** in 99.05% yield as a reddish solid (mp 62−64°C): ^{1}H NMR ($CDCl_3$) δ 7.56 (d, J = 8.05 Hz, 2H), 7.46 (d, J = 8.05 Hz, 2H), 6.66 (d, J = 16.11 Hz, 1H), 6.45 (dt, J_1 = 5.49 Hz, J_2 = 16.11 Hz, 1H), 4.36 (d, J = 5.49 Hz, 2H), 1.76 (s, 1H); EIMS *m*/*z* 202 (M^+), 160 (100).

Method M. *trans*-4-(Trifluoromethyl)cinnamyl Chloride (22). To a chilled solution of thionyl chloride (32 mL) was slowly added 5.3 g (26.2 mmol) of **21** neat in portions. The mixture was stirred for 30 min at 0 °C and allowed to warm to room temperature followed by stirring for an additional hour. The resulting mixture was poured into ice/water (500 mL) and extracted with methylene chloride (3 × 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4, and the solvent was removed in a vacuum. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 50:1), affording 4.40 g of **22** in 76.0% yield as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.59 (d, J = 8.05 Hz, 2H), 7.48 (d, J = 8.05 Hz, 2H), 6.69 (d, J = 15.74 Hz, 1H), 6.41 (dt, J_1 = 7.14 Hz, J_2 = 15.74 Hz, 1H), 4.25 (dd, J_1 = 2.38 Hz, J_2 = 7.32 Hz, 2H).

***trans*-4-Methoxycinnamyl Chloride (30).** To a chilled solution of **29** (3.28 g, 20 mmol) in 20 mL of dry diethyl ether was added dropwise 2.38 g (20 mmol) of thionyl chloride, followed by immediate removal of the solvent. The resulting crude solid was purified by recrystallization from petroleum ether (30−60 °C) to provide 2.20 g of **30** in 60.3% yield as a white solid (mp 61−62 °C): ^{1}H NMR ($CDCl_3$) δ 7.33 (m, 2H), 6.86 (m, 2H), 6.60 (d, J = 15.58 Hz, 1H), 6.18 (dt, J_1 = 7.33 Hz, J_2 = 15.58 Hz, 1H), 4.24 (dd, J_1 = 1.10 Hz, J_2 = 7.33 Hz, 2H), 3.81 (s, 3H); EIMS *m*/*z* 184 ($M^+ + 2$), 182 (M^+), 147 (100).

Method N. 2-Propargyl-2-[*trans*-4-(trifluoromethyl)-cinnamyl]malonic Acid Diethyl Ester (23). Sodium (735 mg, 32 mmol) was dissolved in 45 mL of absolute EtOH. To the EtONa solution 4.40 g (22.2 mmol) of compound **8** was added, followed by addition of 4.90 g (22.2 mmol) of **22**. The mixture was stirred at room temperature for 2 h and refluxed for 7 h to provide a brown suspension. The resulting precipitated inorganic salts was removed by filtration. The filtrate was concentrated in a vacuum, followed by addition of H_2O (50 mL). The resulting mixture was extracted with chloroform (3 × 40 mL), and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in a vacuum. Purification by flash column chromatography (petroleum ether/ethyl acetate, 100:1) afforded 4.85 g of **23** in 57.1% yield as a pale-yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.51 (d, J = 8.43 Hz, 2H), 7.38 (d, J = 8.05 Hz, 2H), 6.52 (d, J = 15.74 Hz, 1H), 6.13 (dt, J_1 = 7.69 Hz, J_2 = 15.74 Hz, 1H), 4.21 (q, J = 7.14 Hz, 4H), 2.96 (d, J = 7.69 Hz, 2H), 2.81 (d, J = 2.56 Hz, 2H), 2.04 (t, J = 2.56 Hz, 1H), 1.23 (t, J = 7.14 Hz, 6H); EIMS *m*/*z* 382 (M^+), 297 (100).

2-Propargyl-2-(*trans*-4-methoxycinnamyl)malonic Acid Diethyl Ester (31). Compound **31** was synthesized as for **23** from **30** (4.88 g, 26.7 mmol) and **8** (5.30 g, 26.7 mmol) in 37.2% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.25 (m, 2H), 6.83 (m, 2H), 6.45 (d, J = 15.77 Hz, 1H), 5.86 (dt, J_1 = 7.70 Hz, J_2 = 15.76 Hz, 1H), 4.22 (q, J = 6.96 Hz, 4H), 3.79 (s, 3H), 2.93 (dd, J_1 = 1.10 Hz, J_2 = 7.70 Hz, 2H), 2.83 (d, J = 2.57 Hz, 2H), 2.05 (t, J = 2.57 Hz, 1H), 1.25 (t, J = 6.97 Hz, 6H); EIMS *m*/*z* 344 (M^+), 147 (100).

Method O. 2-[*trans*-4-(Trifluoromethyl)cinnamyl]-4-pentynoic Acid Ethyl Ester (24). To a 100 mL round-bottom flask containing 4.77 g (12.5 mmol) of **23** was added NaCl (913 mg, 15.6 mmol), H_2O (844 mg, 46.9 mmol), and DMSO (20 mL), and the mixture was refluxed for hours. After cooling to room temperature, the resulting dark-brown mixture was poured into 200 mL of H_2O and extracted with diethyl ether (6 × 30 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in a vacuum. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 100:1) to afford 1.87 g of **24** in 48.3% yield as a pale-yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.52 (d, J = 8.06 Hz, 2H), 7.40 (d, J = 8.06 Hz, 2H), 6.47 (d, J = 15.76 Hz, 1H), 6.22 (dt, J_1 = 7.33 Hz, J_2 = 15.76 Hz, 1H), 4.16 (q, J = 7.15 Hz, 2H), 2.71 (m, 1H), 2.62 (m, 2H), 2.56−2.41 (m, 2H), 2.02 (t, J = 2.57 Hz, 1H), 1.23 (t, J = 7.15 Hz, 3H); EIMS m/z 310 (M^+), 236 (100); HREIMS calcd for $C_{17}H_{17}F_3O_2$ 310.1180, found 310.1179.

2-(*trans*-4-Methoxycinnamyl)-4-pentynoic Acid Ethyl Ester (32). Compound **32** was synthesized as for **24** from **31** (2.57 g, 7.5 mmol) in 31.4% yield as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.26 (m, 2H), 6.83 (m, 2H), 6.40 (d, J = 15.77 Hz, 1H), 5.97 (dt, J_1 = 7.33 Hz, J_2 = 15.77 Hz, 1H), 4.00 (q, J = 7.15 Hz, 2H), 3.80 (s, 3H), 2.71 (m, 1H), 2.63−2.42 (m, 4H), 2.02 (t, J = 2.57 Hz, 1H), 1.25 (t, J = 7.15 Hz, 3H); EIMS m/z 272 (M^+), 147 (100); HREIMS calcd for $C_{17}H_{20}O_3$ 272.1412, found 272.1412.

Method P. *trans*-4-Methoxycinnamic Acid Methyl Ester (28). A mixture of *trans*-4-methoxycinnamic acid (10.68 g, 60 mmol), boron trifluoride diethyl etherate (8.52 g, 60 mmol), and absolute methanol (19.2 g, 600 mmol) was refluxed for 21 h. After the mixture was cooled to room temperature, 150 mL of 5% aqueous Na_2CO_3 was added and the resulting mixture was stirred for 30 min. The precipitated white solid was filtered, washed with water, and dried to provide 10.96 g of **28** in 95.1% yield (mp 85−87 °C): ^{1}H NMR ($CDCl_3$) δ 7.65 (d, J = 15.95 Hz, 1H), 7.48 (m, 2H), 6.91 (m, 2H), 6.31 (d, J = 15.95 Hz, 1H), 3.83 (s, 3H), 3.79 (s, 3H); EIMS m/z 192 (M^+), 161 (100).

Method Q. *trans*-4-Methoxycinnamyl Alcohol (29). To a flame-dried 500 mL round-bottom flask containing **28** (10.9 g, 56.8 mmol) in 230 mL of dry THF at −78 °C was added dropwise DIBAL-H (136 mL of a 1 M solution in hexane, 136 mmol). After being stirred at −78 °C for 2 h, the reaction was quenched with saturated NH_4Cl solution and the mixture was stirred at room temperature for 30 min. The resulting precipitated inorganic salts were removed by filtration and washed with diethyl ether. The aqueous layer was extracted with diethyl ether (3 × 100 mL), and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in a vacuum. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 5:1) to afford 8.35 g of **29** in 89.7% yield as a white solid (mp 79−81 °C): ^{1}H NMR ($CDCl_3$) δ 7.32 (d, J = 8.61 Hz, 2H), 6.85 (d, J = 8.79 Hz, 2H), 6.55 (d, J = 15.95 Hz, 1H), 6.23 (dt, J_1 = 5.96 Hz, J_2 = 15.76 Hz, 1H), 4.78 (broad s, 1H), 4.28 (dd, J_1 = 1.10 Hz, J_2 = 5.87 Hz, 2H), 3.80 (s, 3H); EIMS m/z 164 (M^+), 121 (100).

Enzyme. The cDNA encoding human GSTP 1-1, the most common allelic form in most human populations, was subcloned into a bacterial expression vector, and the protein was expressed in *Escherichia coli* as described in ref 39. Cell pellet was homogenized by sonication, followed by centrifuge at 1500*g* at 4 °C for 30 min. The resulting supernatant was collected for enzyme inhibition studies. The crude GST enzyme was homogenized in buffer A (20 mM potassium phosphate buffer, pH 7.0, containing 2 mM EDTA and 1.4 mM β-mercaptoethanol). Centrifugation and all subsequent purification steps were carried out at 4 °C. The homogenate was centrifuged at 5000*g* for 20 min. The supernatant fraction was then collected and centrifuged at 14000*g* for 30 min. The supernatant was loaded onto a glutathione−agarose column (Sigma, St. Louis), which had been preequilibrated in buffer A. The column was washed with buffer B (20 mM potassium phosphate buffer, pH 7.0, containing 1.4 mM β-mercaptoethanol) using a peristaltic pump at a flow rate of 1.0 mL/min (total volume of 800−1000 mL), followed by 100 mL of buffer C (20 mM potassium phosphate buffer, pH 7.0, containing 1.4 mM β-mercaptoethanol and 0.5 M sodium chloride). The column was eluted with 100 mL of buffer D (50 mM Tris buffer, pH 9.6, containing 5 mM GSH and 1.4 mM β-mercaptoethanol). The chromatography was monitored by UV at 280 nm using an Isco-UA6 detector. The eluted GST enzyme was dialyzed against Nanopure water (4 L × 4). The dialyzed protein was lyophilized and stored at −80 °C.

Kinetics of Enzyme Inhibition. A solution of GST-π (1.0 μg/μL) was prepared in a 0.1 M potassium phosphate buffer (pH 7.4). To 100 μL of ice-cold 0.1 mM potassium phosphate buffer (pH 7.4) was sequentially added 10 μL of the reconstituted GST solution and 5 μL of a solution containing the haloenol lactone in ethanol. Aliquots were drawn for enzyme assay, and the remaining solution was immediately incubated in a water bath at 30 °C. Aliquots were withdrawn at 2, 5, and 12 mins, and GST activity was determined using a spectrophotometric assay described below.

Enzyme Assays. Glutathione *S*-transferase activity was measured using glutathione and 1-chloro-2,4-dinitrobenzene (CDNB) as substrates according to the method of Habig.[40] The activity of the enzyme was determined in a 0.1 M potassium phosphate buffer (pH 6.5) containing 1.0 mM GSH and 1.0 mM CDNB. The rate of product formation was monitored by measuring the change in absorbance at 340 nm using a 96-well microplate reader (VersaMax, Molecular Devices Co., Sunnyvale, CA). Enzyme activities were calculated after correction for nonenzymatic reaction.

GST Modification. To 5.0 mg of pure recombinant human GST-π in 25 mL of potassium phosphate buffer (pH 7.4) was added **1a** (1.0 mg in 1 mL of absolute ethanol) or **17** (0.1 mg in 1.0 mL of ethanol). The resulting mixtures were incubated at 30 °C for 2 h and dialyzed against distilled water for mass spectrometry analysis.

Mass Spectrometry Analysis. The molecular masses of intact proteins were determined by electrospray ionization mass spectroscopy (ESI-MS) on a ZMD single-quadrupole mass spectrometer equipped with an electrospray ion source (Waters Corp, Milford, MA). Samples were desalted using an on-line HPLC system (2695 separation module, Waters Corp, Milford, MA) with a Vydac C4 cartridge (2 mm × 5 mm). A 20 min 5−80% acetonitrile gradient in 0.03% trifluoroacetic acid was used for desalting. The flow rate was 70 μL/min. All electrospray mass spectral data were processed using the Waters MassLynx data system.

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References

(1) Dulik, D. M.; Fenselau, C.; Hilton, J. Characterization of Melphalan−glutathione Adducts Whose Formation Is Catalyzed by Glutathione Transferases. *Biochem. Pharmacol.* **1986**, *35*, 3405−3409.
(2) Dulik, D. M.; Fenselau, C. Conversion of Melphalan to 4-(Glutathionyl)-phenylalanine. A Novel Mechanism for Conjugation by Glutathione S-Transferases. *Drug Metab. Dispos.* **1987**, *15*, 195−199.
(3) Bolton, M. G.; Colvin, O. M.; Hilton, J. Specificity of Isozymes of Murine Hepatic Glutathione S-Transferase for the Conjugation of Glutathione with L-Phenylalanine Mustard. *Cancer Res.* **1991**, *51*, 2410−2415.
(4) Ciaccio, P. J.; Tew, K. D.; LaCreta, F. P. The Spontaneous and Glutathione S-Transferase-Mediated Reaction of Chlorambucil with Glutathione. *Cancer Commun.* **1990**, *2*, 279−285.
(5) Williams, D. L. H. S-Nitrosation and the Reactions of S-Nitroso Compounds. *Chem. Soc. Rev.* **1985**, *14*, 171−196.
(6) Weber, G. F.; Waxman, D. J. Denitrosation of the Anti-Cancer Drug 1,3-Bis(2-chloroethyl)-1-nitrosourea Catalyzed by Microsomal Glutathione S-Ttransferase and Cytochrome P450 Monooxygenases. *Arch. Biochem. Biophys.* **1993**, *307*, 369−378.
(7) Berhane, K.; Mannervik, B. Inactivation of the Genotoxic Aldehyde Acrolein by Human Glutathione Transferases of Classes Alpha, Mu, and Pi. *Mol. Pharmacol.* **1990**, *37*, 251−254.

(8) Harrison, D. J.; Kharbanda, R.; Bishop, D.; McLellan, L. L.; Hayse, J. D. Glutathione *S*-Transferase Isoenzymes in Human Renal Carcinoma Demonstrated by Immunohistochemistry. *Carcinogenesis* **1989**, *10*, 1257−1260.
(9) Shiratori, Y.; Soma, Y.; Maruyama, H.; Sato, S.; Takano, A.; Sato, K. Immunohistochemical Detection of the Placental Form of Glutathione *S*-Transferase in Dysplastic and Neoplastic Human Uterine Cervix Lesions. *Cancer Res.* **1987**, *47*, 6806−6809.
(10) Di Ilio, C.; Del Boccio, G.; Aceto, A.; Federici, G. Alteration of Glutathione Transferase Isoenzyme Concentrations in Human Renal Carcinomas. *Carcinogenesis* **1987**, *8*, 861−864.
(11) Di Ilio, C.; Del Boccio, G.; Aceto, A.; Casaccia, R.; Mucilli, F.; Federici, G. Elevation of Glutathione Transferase Activity in Human Lung Tumor. *Carcinogenesis* **1988**, *9*, 335−340.
(12) Eimoto, H.; Tsutsumi, M.; Nakajima, A.; Yamamoto, K.; Takashima, Y.; Maruyama, H.; Konishi, Y. Expression of the Glutathione *S*-Transferase Placental Form in Human Lung Carcinomas. *Carcinogenesis* **1988**, *9*, 2325−2327.
(13) Shea, T. C.; Kelley, S. L.; Henner, W. D. Identification of an Anionic Form of Glutathione Transferase Present in Many Human Tumors and Tumor Cell Lines. *Cancer Res.* **1988**, *48*, 527−533.
(14) Moscow, J. A.; Fairchild, C. R.; Madden, M. J.; Ransom, D. T.; Wieand, H. S.; O'Brien, E.; E.; Poplack, D. G.; Crossman, J.; Myers, C. E.; Cowwan, K. H. Expression of Anionic Glutathione *S*-Transferase and P-Glycoprotein Genes in Human Tissues and Tumors. *Cancer Res.* **1989**, *49*, 1422−1428.
(15) Peters, W. H. M.; Nagengast, F. M.; Wobbes, T. Glutathione *S*-Transferases in Normal and Cancerous Human Colon Tissue. *Carcinogenesis* **1989**, *10*, 2371−2374.
(16) Moscow, J. A.; Townsend, A. J.; Goldsmith, M. E.; Whang-Peng, J.; Vickers, P. J.; Poisson, R.; Legault-Poisson, S.; Myers, C. E.; Cowan, K. H. Isolation of the Human Anionic Glutathione *S*-Transferase cDNA and the Relation of Its Gene Expression to Estrogen-Receptor Content in Primary Breast Cancer. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6518−6522.
(17) Howie, A. F.; Miller, W. R.; Hawkins, R. A.; Hutchinson, A. R.; Beckett, G. J. Expression of Glutathione *S*-Transferase B_1, B_2, Mu and Pi in Breast Cancer. *Br. J. Cancer* **1989**, *60*, 834−837.
(18) Tew, K. D.; Bomber, A. M.; Hoffman, S. J. Ethacrynic Acid and Piriprost as Enhancers of Cytotoxicity in Drug Resistant and Sensitive Cell Lines. *Cancer Res.* **1988**, *48*, 3622−3625.
(19) Hall, A.; Robson, C. N.; Hickson, I. D.; Harris, A. L.; Proctor, S. J.; Cattan, A. R. Possible Role of Inhibition of Glutathione S-Transferase in the Partial Reversal of Chlorambucil Resistance by Indomethacin in a Chinese Hamster Ovary Cell Line. *Cancer Res.* **1989**, *49*, 6265−6268.
(20) Benz, C. C.; Keniry, M. A.; Ford, J. M.; Townsend, A. J.; Cox, F. W.; Palayoor, S.; Matlin, S. A.; Hait, W. N.; Cowan, K. H. Biochemical Correlates of the Antitumor and Antimitochondrial Properties of Gossypol Enantiomers. *Mol. Pharmacol.* **1990**, *37*, 840−847.
(21) Asakura, T.; Ohkawa, K.; Takahashi, N.; Takada, K.; Inoue, T.; Yokoyama, S. Glutathione−Doxorubicin Conjugate Expresses Potent Cytotoxicity by Suppression of Glutathione S-Transferase Activity: Comparison between Doxorubicin-Sensitive and -Resistant Rat Hepatoma Cells. *Br. J. Cancer* **1997**, *76*, 1333−1337.
(22) Mukanganyama, S.; Widersten, M.; Naik, Y. S.; Mannervik, B.; Hasler, J. A. Inhibition of Glutathione S-Transferases by Antimalarial Drugs. Possible Implications for Circumventing Anticancer Drug Resistance. *Int. J. Cancer* **2002**, *97*, 700−705.
(23) Lyttle, M. H.; Hocker, M. D.; Hei, H. C.; Caldwell, C. G.; Aaron, D. T.; Engqvist-Goldstein, M.; Flatgaard, J. E.; Bauer, K. Isozyme-Specific Glutathione *S*-Transferase Inhibitors: Design and Synthesis. *J. Med. Chem.* **1994**, *37*, 189−194.
(24) Morgan, A. S.; Ciaccio, P. J.; Tew, K. D.; Kauvar, L. M. Isozyme-Specific Glutathione *S*-Transferase Inhibitors Potentiate Drug Sensitivity in Cultured Human Tumor Cell Lines. *Cancer Chemother. Pharmacol.* **1997**, *37*, 363−370.
(25) Awasthi, S.; Srivastava, S. K.; Ahmad, F.; Ahmad, H.; Ansari, G. A. Interactions of Glutathione S-Transferase-Pi with Ethacrynic Acid and Its Glutathione Conjugate. *Biochim. Biophys. Acta* **1993**, *1164*, 173−178.
(26) Ploemen, J. H.; Van Schanke, A.; Van Ommen, B.; Van Bladeren, P. J. Reversible Conjugation of Ethacrynic Acid with Glutathione and Human Glutathione S-Transferase P1-1. *Cancer Res.* **1994**, *54*, 915−919.
(27) Hoffman, D. W.; Wiebkin, P.; Rybak, L. P. Inhibition of Glutathione-Related Enzymes and Cytotoxicity of Ethacrynic Acid and Cyclosporine. *Biochem. Pharmacol.* **1995**, *49*, 411−415.
(28) Singh, S. V.; Xu, B. H.; Maurya, A. K.; Mian, A. M. Modulation of Mitomycin C Resistance by Glutathione Transferase Inhibitor Ethacrynic Acid. *Biochim. Biophys. Acta* **1992**, *1137*, 257−263.
(29) Xu, B. H.; Gupta, V.; Singh, S. V. Mitomycin C Sensitivity in Human Bladder Cancer Cells: Possible Role of Glutathione and Glutathione Transferase in Resistance. *Arch. Biochem. Biophys.* **1994**, *308*, 164−170.
(30) Yang, W. Z.; Begleiter, A.; Johnston, J. B.; Israels, L. G.; Mowat, M. R. Role of Glutathione and Glutathione S-Transferase in Chlorambucil Resistance. *Mol. Pharmacol.* **1992**, *41*, 625−630.
(31) Hansson, J.; Berhane, K.; Castro, V. M.; Jungnelius, U.; Mannervik, B.; Ringborg, U. Sensitization of Human Melanoma Cells to the Cytotoxic Effect of Melphalan by the Glutathione Transferase Inhibitor Ethacrynic Acid. *Cancer Res.* **1991**, *51*, 94−98.
(32) Zheng, J.; Mitchell, A. E.; Jones, A. D.; Hammock, B. D. Haloenol lactone: A New Isozyme-Selective and Active Site-Directed Inactivator of Glutathione *S*-Transferase (GST). *J. Biol. Chem.* **1996**, *271*, 20421−20425.
(33) Mitchell, A. E.; Zheng, J.; Hammock, B. D.; Lo Bello, M.; Jones, A. D. Structural and Functional Consequences of Haloenol Lactone Inactivation of Murine and Human Glutathione *S*-Transferase. *Biochemistry* **1998**, *37*, 6752−6759.
(34) Zheng, J.; Wurz, G. T.; Cadman, T. B.; DeGregorio, M. W.; Jones, A. D.; Hammock, B. D. Haloenol Lactone: A New Synergist of Cancer Chemotheropy *in Vitro*. *Biochem. Biophys. Res. Commun.* **1997**, *241*, 13−.
(35) Krafft, G. A.; Katzenellenbogen, J. A. Synthesis of Halo Enol Lactones. Mechanism-Based Inactivators of Serine Proteases. *J. Am. Chem. Soc.* **1981**, *103*, 5459−5466.
(36) Dai, W.; Katzenellenbogen, J. A. Stereoselective *Z*- and *E*-Bromo Lactonization of Alkynoic Acids. *J. Org. Chem.* **1991**, *56*, 6893−6896.
(37) Krapcho, A. P. Synthetic Applications of Dealkoxycarbonylations of Malonate Esters, β-Keto Esters, α-Cyano Esters and Related Compounds in Dipolar Aprotic Media−Part I. *Synthesis* **1982**, *10*, 805−822.
(38) Krapcho, A. P. Synthetic Applications of Dealkoxycarbonylations of Malonate Esters, β-Keto Esters, α-Cyano Esters and Related Compounds in Dipolar Aprotic Media−Part II. *Synthesis* **1982**, *10*, 893−914.
(39) Zimniak, P.; Nanduri, B.; Pikula, S.; Bandorowicz-Pikula, J.; Singhal, S. S.; Srivastava, S. K.; Awasthi, S.; Awasthi, Y. C. Naturally occurring human glutathione S-Transferase GSTP1-1 Isoforms with Isoleucine and Valine in Position 104 Differ in Enzymic Properties. *Eur. J. Biochem.* **1994**, *224*, 893−899.
(40) Habig, W. H.; Pabst, M. J.; Jakoby, W. B. Glutathione *S*-Transferases. The First Enzymatic Step in Mercapturic Acid Formation. *J. Biol. Chem.* **1974**, *249*, 7130−7139.

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