Synthesis and Glutathione *S*-Transferase Structure−Affinity Relationships of Nonpeptide and Peptidase-Stable Glutathione Analogues

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A series of nonpeptidic glutathione analogues where the peptide bonds were replaced by simple carbon−carbon bonds or isosteric *E* double bonds were prepared. The optimal length for the two alkyl chains on either side of the mercaptomethyl group was evaluated using structure−affinity relationships. Affinities of the analogues **14a**−**f**, **23**, and **25** were evaluated for a recombinant GST enzyme using a new affinity chromatography method previously developed in our laboratory. Analysis of these analogues gives an additional understanding for GST affinity requirements: (a) the carbon skeleton must conserve that of glutathione since analogue **14a** showed the best affinity (IC_{50} = 5.2 μM); (b) the GST G site is not able to accommodate a chain length elongation of one methylene group (no affinity for analogues **14c**,**f**); (c) a one-methylene group chain length reduction is tolerated, much more for the "Glu side" (**14d**, IC_{50} = 10.1 μM) than for the "Gly side" (**14b**, IC_{50} = 1800 μM); (d) the mercaptomethyl group must remain at position 5 as shown from the null affinity of the 6-mercaptomethyl analogue **14e**; (e) the additional peptide isosteric *E* double bond (**25**) or hydroxyl derivative (**23**) in **14e** did not help to retrieve affinity. This work reveals useful information for the design of new selective nonpeptidic and peptidase-stable glutathione analogues.

Introduction

Glutathione (GSH) is an important natural tripeptide (γ-Glu-Cys-Gly) with multifunctional properties.[1−3] Its implication in detoxification, oxidation and radiation protection, cancer genesis, and immune phenomena increased the general interest in GSH research over the last several years. Although a great number of peptidic GSH analogues have been synthesized,[4−7] to our knowledge, nonpeptidic GSH analogues of type **1** are unknown in the literature. We replaced the two sensitive peptide bonds of the glutathione moiety by simple carbon−carbon bonds or *E* olefinic double bonds. In the latter case the olefinic linkage is bioisosteric with the amide bond,[8] as was previously reported for the leukotriene (LT) D_4 series.[9,10]

Adang et al.[11] gave some important structural requirements concerning the design of peptidic GSH analogues having glutathione *S*-transferase (GST) enzyme activity. On this basis and considering our analogues of type **1** (Figure 1), we kept the two carboxylic acid groups of GSH intact and deleted the γ-glutamyl amino group. To further determine the structural requirements for the best GSH mimic, we first wanted to identify the optimal length for the two alkyl chains on either side of the mercaptomethyl group in **1**. Therefore, we initiated a structure−affinity investigation of a series of synthetic saturated derivatives of

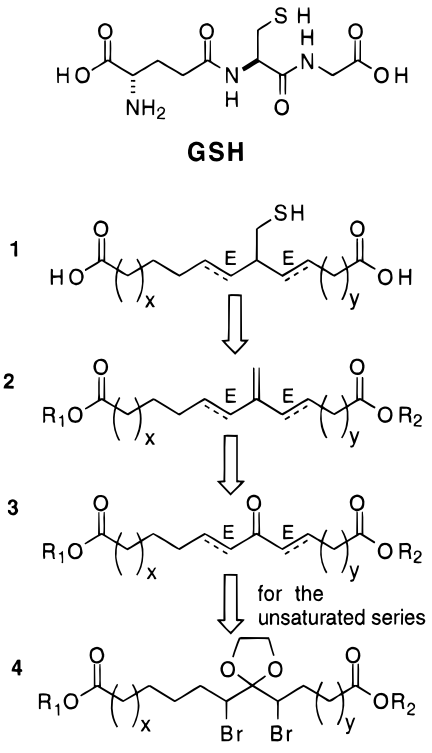

Figure 1. Retrosynthesis of mercaptomethyl diacids **1**.

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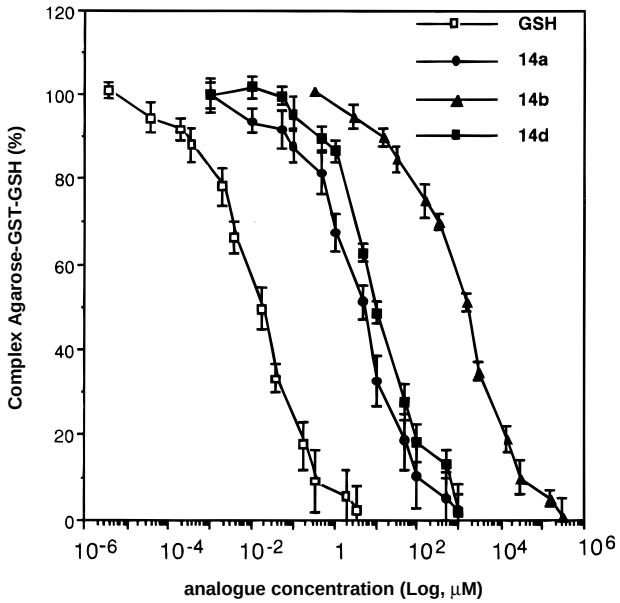


Figure 2. Affinity curves of the GSH analogues. Inhibition of specific binding of GST to glutathione-agarose by GSH and **14a**,**b**,**d**. The dissociation assays were carried out as described in the Experimental Section. Each data point is the mean ± standard deviation of three experiments.

type **1**. Second, we introduced selective *E* double bonds in order to evaluate the affinity improvement in terms of amide bond isosterics. We used a GST enzyme binding experiment as a good way to determine if the analogues of type **1** were actual GSH mimics. We evaluated their affinity with a recombinant GST enzyme, very similar to the mammalian μ class GST. We used a new binding experiment developed in our laboratory based on an affinity chromatocolumn[12] using GSH as reference (Figure 2).

Herein we describe the synthesis and the biological evaluation of the first and original nonpeptidic glutathione analogues as mercapto derivatives **1** and discuss their structure−affinity relationships for the GST enzyme. The final target of our project, after linkage between LTA_4 and nonpeptidic GSH analogues, is the synthesis of LTC_4 stable analogues against enzymatic metabolism. These compouds would therefore be a powerful tool in the characterization of the human LTC_4 receptor (Cys-LT_2 receptors[13−15]), which constitutes a main preoccupation in our laboratory.

Chemistry

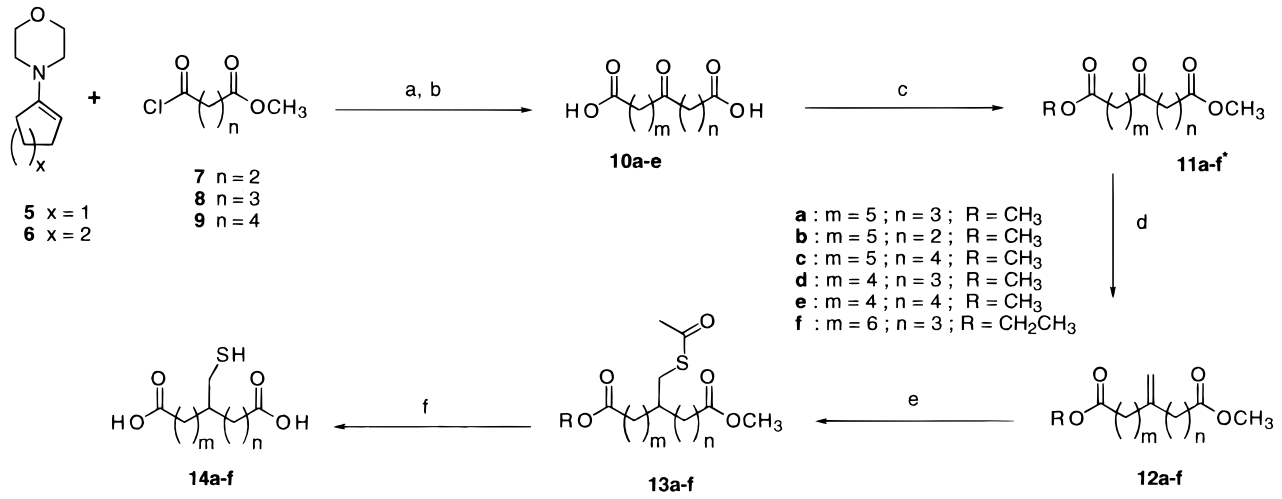
We wanted to develop a synthetic strategy that could be used for the synthesis of either saturated or unsaturated compounds of type **1**. Thus, as shown in Figure 1, we planned the preparation of mercaptomethyl diacids **1** from the functionalization of the ethylene derivatives **2**. These latter compounds were derived from the ketones **3** via carbonyl homologation. Ketones of type **3** with conjugated double bonds were prepared from the selective dehydrohalogenation reaction of the α,α'-dihalogenated protected ketones **4**.

Following this strategy, we prepared the saturated analogues **14a**−**f** in a synthesis depicted in Scheme 1. A Stork[16] condensation between enamine **5** or **6** and commercially available acid chlorides **7**−**9** gave us, after alkaline treatment, the keto diacids **10a**−**e**. Reactions were performed with freshly prepared enamines **5** and **6**, by known methods.[17] The diacids **10a**−**e** were then easily esterified with methanol in the presence of DCC and DMAP, to afford the dimethyl esters **11a**−**e** in good to excellent yields (80−95%). Methyl ethyl diester **11f** was prepared differently as explained below. Transformation of the ketonic group of compounds **11a**−**f** into a methylene double bond was completed by treatment with methylenetriphenylphosphorane in 75%−85% yields.[18] Double-bond functionalization of **12a**−**f** was then performed by reaction with thiolacetic acid under free-radical conditions. This reaction is usually initiated with light irradiation,[19] but for easier large-scale preparation, we modified the protocol and replaced the light radical initiation by AIBN. The thiolacetates **13a**−**f** were obtained from exclusive anti-Markovnikov addition. They were difficult to purify, and several silica gel column chromatographies were necessary for correct elemental analysis. Subsequent treatment of **13a**−**f** with KOH in a water−ethanol mixture afforded, after acidification, the mercapto diacids **14a**−**f** in good yields (59−85%). Reaction at 90 °C for only 30 min produced exclusively the thiols **14a**−**f** (on TLC analysis), whereas longer reaction times (>2 h) led to mixtures containing the oxidized dithio compounds of **14a**−**f**. Reactions were therefore performed under argon, and final mercapto derivatives **14a**−**f** were protected against oxygen. Total functional identification between reduced thiols and their oxidized dithio forms was possible with the synthesis of the oxidized dithio compound of **14a**. This was performed by oxidation of **14a** with iodine in aqueous methanol containing NaOH. The structure of **14a** was established by comparison with the prepared dithio compound using ^1H and ^{13}C NMR. This synthetic route is attractive because it is short, the overall yields are good (around 25%), and the starting materials are commercially available and inexpensive. Depending on the starting enamine and acid chloride, a great number of chain length variations can be obtained.

For the requisite mercapto diacid **14f**, the corresponding keto diester **11f** cannot be synthesized through the Stork enamine acylation. To overcome this limitation, we used another strategy for the preparation of **11f** as outlined in Scheme 2. Ethyl 7-iodoheptanoate (**16**) was first prepared from its commercially available bromide derivative **15**. The iodide **16** was then converted to the corresponding zinc iodide organometallic, with the zinc−copper couple, and reacted with acid chloride **8** in a palladium(0) coupling reaction inspired from Tamaru et al.[20] In this way, we obtained the keto diester **11f** in high yield (80%). Compound **11f** was further functionalized as described above in Scheme 1.

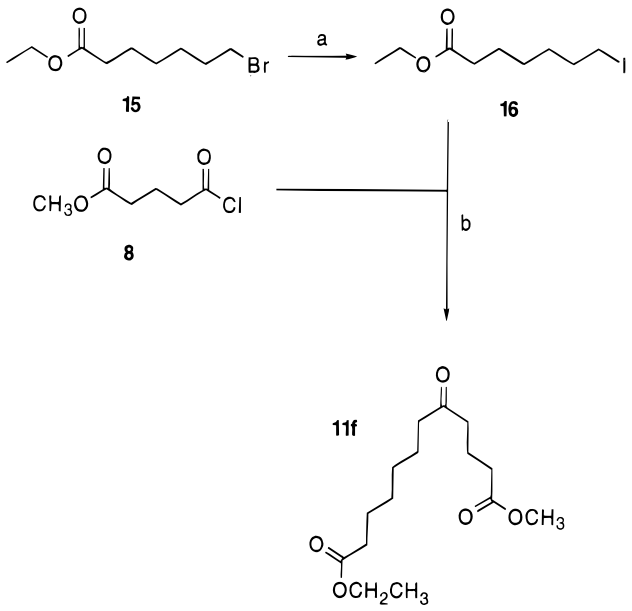
We then prepared compounds **23** and **25** containing exclusively *E* double bonds derived from symmetrical keto diester **11e** as depicted in Scheme 3. For clean bromination−dehydrobromination reactions, ketone **11e** was first converted to its ethylene ketal **17** using classical methods.[21] Monobromination of **17** was carried out with slow addition of bromine (1 equiv) in ethyl ether at room temperature. Monobrominated ethylene ketal **18** was obtained in high yield (90%). Dehydrobromination was optimal when using DBU in warm DMSO. These conditions produced clean dehydrohalo-

Scheme 1. Synthesis of Saturated Mercaptomethyl Diacids **14a**−**f**[a]



[a] Legend: (a) (1) CH_2Cl_2, Et_3N, reflux, (2) HCl (18%), reflux; (b) (1) KOH, 100 °C, (2) HCl (concd); (c) DCC, DMAP, MeOH, CH_2Cl_2; (d) *t*-BuOK, Ph_3PCH_3Br, THF; (e) CH_3COSH, AIBN, 90 °C; (f) (1) KOH, ethanol/water, reflux, (2) HCl. *For the synthesis of compound **11f**, see Scheme 2.

Scheme 2. Synthesis of Compound **11f**[a]



[a] Legend: (a) NaI, acetone, reflux, 87%; (b) Zn(Cu), $(Ph_3P)_4Pd(0)$, benzene/DMA, 80%.

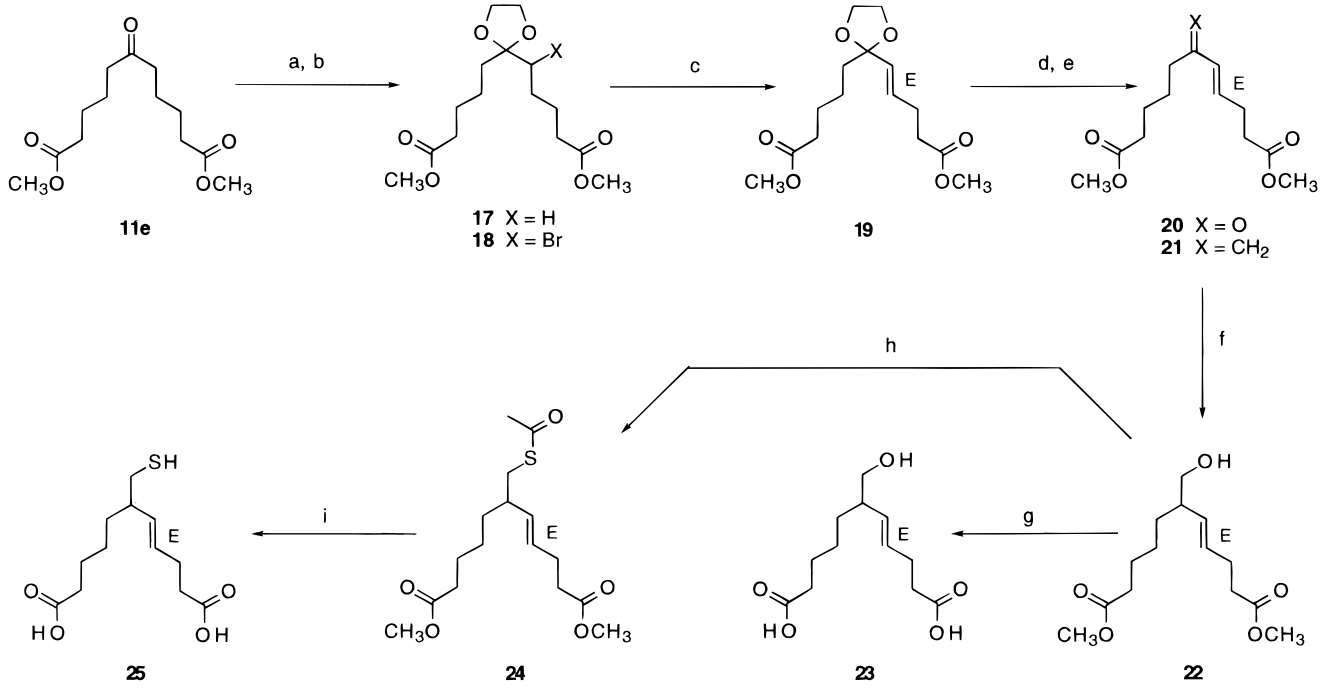
genation with exclusive formation of the *E* double-bond isomer **19** (^{1}H NMR: J_{trans} = 15.5 Hz) in good yield (86%). Deprotection of the ethylene ketal with TFA, water, and silica gel afforded the α,β-unsaturated ketone **20**. Wittig functionalization with methylenetriphenylphosphorane then gave the diene **21**. Regioselective reaction of the terminal double bond could not be achieved with thiolacetic acid as for the saturated series (Scheme 1). This reaction was realized through a selective hydroboration step using 9-BBN−H.[22] After oxidative cleavage, we isolated the homoallylic alcohol **22**, which was saponified with KOH to the final diacid alcohol **23**. The conversion of the alcohol function to thiol **25** was achieved using the Mitsunobu reaction.[23] The thiolacetate **24** was first prepared and obtained in high yield (93%) but required tedious purification. Saponification and acetyl cleavage with KOH gave the desired homoallylic thiol **25**. The product was kept refrigerated and protected against oxygen to avoid oxidation.

Following our synthetic strategy (Figure 1), we also attempted to prepare the analogues **1** containing two *E* double bonds. Starting from ketones **11a**−**e**, we synthesized the dibromo ethylene ketals **4** (Figure 1) by regioselective dibromination. Subsequent bis-dehydrobromination and deprotection of the ethylene ketal gave the cross-conjugated dienones **3**. Again, the generated double bonds were exclusively in the *E* form (^{1}H NMR, 360 MHz: $J_{trans} \geq 15$ Hz). These unsaturated ketones **3** were shown to be metastable compounds. They are known to undergo Michael type additions and Nazarov cyclizations. However, Wittig olefination gave us the cross-conjugated trienes **2** without isomerization of the *E* double bonds (on NMR analysis). We then attempted the regioselective functionalization of the ethylene terminal double bond in order to obtain the bis-homoallylic thiols **1**, but all attempts failed in our hands. The cross-conjugated trienes **2** were very sensitive compounds and extremely difficult to handle. Isomerization and polymerization were the predominant reactions. We want to stress that *E*,*E*-cross-conjugated trienes of type **2** have not been previously described in the literature. Recently, the synthesis of a reasonably stable *Z*,*Z*-cross-conjugated triene was reported.[24] However this result is apparently not transposable to the *E*,*E* system, as shown by our results. No chemical method is available for the selective and specific formation of (*E*,*E*)-1,4-dienes as in thiols of type **1**. One of our challenges was to introduce a new approach to this synthetic problem through the synthesis of 1,4-dienes. We are currently studying other synthetic strategies for the formation of (*E*,*E*)-1,4-dienes.

Biochemistry

A protein affinity chromatography method[12] was used to evaluate the binding of the glutathione analogues to the recombinant *Schistosoma japonicum* GST protein. The recombinant GST was bound to a glutathione-agarose slurry for 2 h at room temperature in a PBS buffer. The glutathione analogue was then added at increasing concentrations. After a 2 h incubation, the

Scheme 3. Synthesis of Unsaturated Mercaptomethyl Diacids **23** and **25**[a]



[a] Legend: (a) ethylene glycol, *p*-toluenesulfonic acid, benzene, Dean−Stark, 75%; (b) Br_2, Et_2O, 90%; (c) DBU, DMSO, 120 °C, 86%; (d) SiO_2, TFA, water, CH_2Cl_2, 90%; (e) *n*-BuLi, Ph_3PCH_3Br, THF, 62%; (f) (1) 9-BBN-H, THF, (2) NaOH, H_2O_2, 59%; (g) (1) KOH, ethanol, water, 25 °C, (2) HCl, 73%; (h) DIAD, PPh_3, CH_3COSH, THF, 0−20 °C, 93%; (i) (1) KOH, ethanol, water, reflux, (2) HCl, 64%.

unbound GST protein was recovered and its concentration determined from its UV absorption at 276 nm using the extinction coefficient.[12] The percentage of GST−glutathione-agarose complex was then plotted as a function of the analogue concentration (Figure 2). The dissociation plots obtained were used to determine the IC_{50} values (Table 1). Our affinity chromatography test has been compared with a more typical and sensitive but also more expensive method, i.e., the ELISA-based assay using a commercially available anti-GST monoclonal antibody (Sigma). The observed results are within a similar value.[12]

Results and Discussion

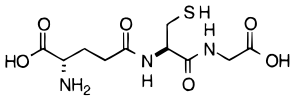
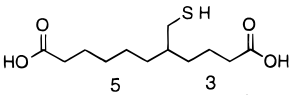
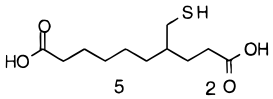
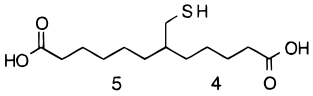
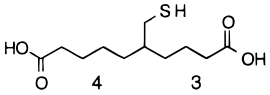
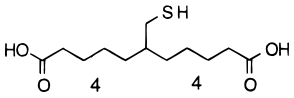
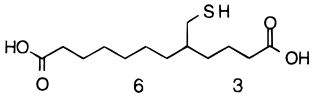
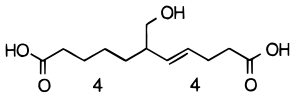
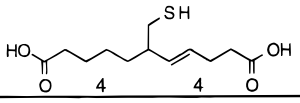
In the current study, we explored structural requirements for new nonpeptidic GSH analogues lacking amide bonds. For this purpose we prepared a series of analogues shown in Table 1. Their affinity for a recombinant GST was evaluated (Figure 2), and we determined their IC_{50} values (Table 1). It is important to note that our binding experiments[12] gave excellent displacement curves (Figure 2) in accordance with known GSH affinity. In the series of compounds studied (Table 1) a first general IC_{50} examination indicates that a substantial structure−affinity relationship exists. Depending on the analogue, the IC_{50} values vary between 5.2 μM and no measurable affinity (NA). Thus our initial question concerning the influence of chain length on the affinity is fully justified. The results clearly indicate that the most potent analogue is **14a** with an IC_{50} value of 5.2 μM. This IC_{50} is substantial and noteworthy when considering the simplicity of structure **14a** in comparison with the GSH structure. Indeed, compound **14a** has only a 250-fold weaker IC_{50} than GSH. This also indicates that the binding interactions are concentrated around the two carboxylic acid moieties and thiol group of **14a**. The importance of these carboxylic acid groups was already emphasized by Adang et al.[11] and is clearly demonstrated here. With only these two carboxylic acid groups present, the IC_{50} value was still 5.2 μM. It is also noteworthy that the carbon structure of **14a** is that of GSH with three and five methylene groups between the terminal carboxylic acid and the mercaptomethyl groups (see Table 1).

The compounds examined in this study were divided into three classes as compared to **14a**: first compounds **14c**,**f** with a one-methylene group extension, then compounds with removal of one methylene group (**14b**,**d**), and finally symmetrical compounds with an equal number of methylene groups on either side (**14e**, **23**, and **25**).

For compounds **14c**,**f**, the extension of one methylene group either on the "Glu side" or on the "Gly side" resulted in a total loss of affinity and no binding was detected. This indicates that the GST G binding site is very selective. Although some distances between different binding regions can be assessed from known GST X-ray data,[25] dynamic interactions of a GSH analogue and its binding site are difficult to predict. Our results suggest that the binding pocket must be very tight and rigid and cannot accommodate a one-carbon elongation of the GSH backbone structure. This could also indicate that the extended analogues cannot fold inside the binding site in order to interact with their enzyme binding region. This differs from the results of Adang et al.,[11] particularly for the Gly-extended analogue **14c**.

In contrast, the compounds lacking one methylene group, **14b**,**d**, exhibited interesting binding affinities (Table 1). The most potent was **14d** (IC_{50} = 10.0 μM) which was only 2-fold less selective compared to **14a** and 500-fold less selective compared to glutathione.

Table 1. Affinity of the Analogues for GST Enzyme

n°	structures	IC_{50} (μM)
GSH		0.02 ±0.07
14a		5.2 ± 1.1
14b		1800 ± 380
14c		NA
14d		10.1 ± 0.9
14e		NA
14f		NA
23		NA
25		NA

NA: No Affinity.

These results reveal that shortening the glutathione backbone by one methylene group on the "Glu side" results in only a weak decrease in affinity of **14d** compared to **14a**. This suggests that the Glu carboxylic acid group can orient in the pocket site to interfere with the enzyme binding region. This is not the case for compound **14b** and the Gly carboxylic acid group. Indeed, in **14b**, removing one carbon of the Gly branch showed a significant decrease in affinity (IC_{50} = 1800 μM). It seems that the carboxylic acid group in **14b** cannot interact properly with the enzyme binding region. This weak affinity may be due to the greater distance between the two interacting sites. In this case we could assume that the major coordinating group would be the "Gly side" carboxylic acid group.

The symmetrical compounds **14e**, **23**, and **25** did not show any affinity for GST. As seen for compound **14d**, shortening the "Glu side" branch did not significantly reduce the affinity. This should also be true for the reduced "Glu side" chain in **14e**. The total loss of affinity is likely due to additional elongation of the "Gly side" chain in **14e**. This symmetrical compound cannot interfere properly within the active binding site. Interestingly, **14e** possesses the same number of carbons as the best analogue **14a**. From this point of view, only the mercaptomethyl group is shifted from carbon position 5 to 6. This suggests that the mercaptomethyl group also plays an important role in binding affinity, at least with respect to its position on the carbon chain. A one-carbon displacement of this group (from **14a** to **14e**) resulted in a total loss of affinity, attributed to the presence of unfavorable steric or electronic interactions. The analogues may be rigidly bound to the G site of the protein, and in the case of **14e**, the space available for the mercaptomethyl group in the G active site is not large enough to accommodate this positional variation. Addition of a peptide isosteric *E* double bond gave compound **25**, which also did not show any affinity for the GST enzyme. If we consider that the peptide isosteric *E* double bond can bring some additional binding interactions, it is clearly not strong enough to overcome the unfavorable steric or electronic interactions of **14e**. Changing the bulky thiol group for the more polar hydroxyl group in **23** did not change the affinity for the GST enzyme.

It is important to note that all the compounds were synthesized and biologically evaluated in their racemic forms. It would be interesting to observe any differences in binding activities among the pure enantiomeric forms of these compounds, especially for the more potent **14a**. Indeed, the importance of the chiral Cys center in GSH was previously demonstrated.[11] The affinity enhancement that was observed for the LTD_4 analogues when adding an amide isosteric *E* double bond must also be verified for compound **14a**.

Conclusion

As part of a program concerning the synthesis of peptidostable LTC_4 analogues, a series of original GSH analogues lacking peptide bonds were prepared. Their affinities for a recombinant GST enzyme were evaluated in order to assess the optimal chain length of the analogues **1** and the influence of a peptide isosteric *E* double bond.

The synthesized analogues (Table 1) show significant structure−affinity relationships. Our studies show that synthesis of nonpeptidic GSH analogues with potent affinities is possible with compound **14a**. The structural requirements are the two carboxylic acid groups and perhaps the mercaptomethyl group. It was demonstrated that the carbon skeleton must conserve that of GSH which is an undecane carbon chain with the carboxylic acids in positions 1 and 11. The mercaptomethyl group must remain at position 5 as was deduced from analogue **14e**. Starting from **14a**, it was shown that a chain length elongation of one methylene group leads to a total loss of affinity (**14c**,**f**). Chain length reduction of one methylene group is not detrimental to the affinity. Moreover, chain shortening at the "Glu side" branch (**14d**) is tolerated much more than at the "Gly side" branch (**14b**). Addition of a peptide isosteric *E* double bond in **25** did not recover affinity, even with a less bulky hydroxyl group (**23**).

From this work compound **14a** emerged as the best GSH analogue. Its high affinity will allow us to develop further peptidostable LTC_4 analogues. Better GSH analogues may be obtained with the synthesis of pure enantiomeric forms of **14a** with or without *E*-unsaturated double bonds. Since LTD_4 is formed before LTE_4,[26,27] the Glu−Cys amide bond is metabolically much more labile then the Cys−Gly amide bond.

Therefore, replacement of only the more labile peptidic bond by a simple carbon−carbon bond or an *E* olefinic double bond is also being considered.

These results should provide additional insight in mapping the length and breadth of the GST G site binding pocket.

Experimental Section

Melting points were determined on a Büchi Tottoli capillary apparatus and are not corrected. ^{1}H and ^{13}C NMR spectra were recorded either on a Bruker AC100 spectrometer at 100 and 25 MHz, respectively, or on a Bruker AMX360 at 360 and 90 MHz, respectively; 2D NMR spectra were recorded on a Bruker AMX360 spectrometer. Chemical shifts (δ) are quoted in ppm, and coupling constants (J) are given in Hz. The residual hydrogenated solvent peak was used as internal reference ($CHCl_3$, δ_H 7.27 ppm, δ_C 77.0 ppm; CH_3OH, δ_H 3.31 ppm, δ_C 49.0 ppm). Mass spectra were recorded on a JEOL JMS DX 300 spectrometer in the FAB$^+$ mode with 2-nitrobenzyl alcohol (NBA) or glycerol-thiol (GT) as matrix. Infrared spectra were obtained on a Beckman AccuLab2 spectrophotometer, and absorption bands (ν) are given in cm^{-1}. Elemental analyses were performed at the Service de Microanalyses de l'ENSCM at Montpellier (France), the maximum error being in the range of ±0.4% of calcd. Analytical TLC was performed on Merck silica gel 60 F_{254}, precoated 0.25-mm glass-backed plates. Visualization methods included UV light, iodine vapors, and phosphomolybdic acid or anisaldehyde dipping. Column chromatography was performed with Merck silica gel Geduran 40−63 or 63−200 μm particle size. All air-sensitive experiments were carried out under argon with freshly distilled dried solvents. Chloroform and thiolacetic acid were distilled prior to use. Methylene chloride (CH_2Cl_2) and *N*,*N*-dimethylacetamide (DMA) were distilled from CaH_2. THF was distilled from sodium-benzophenone. Triethylamine (Et_3N) was dried over KOH. Benzene and ethyl ether were dried by standing over sodium wire. Methanol was distilled after reaction with sodium. Acetone was dried over anhydrous $CaSO_4$. DMSO was purchased anhydrous and kept over 4-Å molecular sieves.

5-Oxo-1,11-undecanedioic Acid (10a). To a stirred solution of enamine **6** (9.15 mL, 54.69 mmol) and Et_3N (9.20 mL, 66.01 mmol) in dry CH_2Cl_2 (70 mL) at room temperature was added the acid chloride **8** (10.0 g, 60.76 mmol) diluted in CH_2Cl_2 (30 mL) dropwise. A white precipitate appeared during the addition. The mixture was stirred at room temperature for 1 h and under reflux for 3 h. The reaction was then carefully hydrolyzed by addition of HCl (18%, 30 mL) and heated under reflux (bath temperature 50 °C) for 4 h. After cooling to room temperature, the water phase was extracted with CH_2Cl_2. The combined organic extracts were washed with 1 N HCl, water and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The brown crude residue was dissolved in a KOH solution (12.75 g, 227 mmol in 13 mL water) and heated at 100 °C for 30 min. After the mixture cooled to room temperature, a minimum amount of water (47 mL) was added to allow the dissolution of the KCl formed in the next acidification step (KCl solubility in water: 1 g/2.8 mL at 20 °C). The reaction was ice-cooled and carefully acidified to pH 1 with concentrated HCl. The formed precipitate was filtred, washed with cold water, and lyophilized. The solid was then crystallized from chloroform to afford pure keto diacid **10a** as a beige solid (8.022 g, 64% yield). The product was further recrystallized in water: TLC (chloroform/acetic acid, 9/1) R_f 0.37; mp 102−103 °C (recrystallized from water); ^{1}H NMR (CD_3OD) δ 1.20−2.02 (m, 8H, $H_{3,7-9}$), 2.20−2.40 (m, 4H, $H_{2,10}$), 2.40−2.67 (m, 4H, $H_{4,6}$), 5.09 (s broad, 2H, OH); ^{13}C NMR (CD_3OD) δ 20.03, 24.45, 25.80, 29.68, 33.90, 34.70, 42.31, 43.21, 176.99, 177.50, 213.02; IR (KBr) ν 3400−2400, 1680 broad, 1400, 1260, 1090, 1070, 900; MS (GT) m/z 231 (M + H)$^+$, 213 (M − OH)$^+$, 185 (M − CO_2H)$^+$. Anal. ($C_{11}H_{18}O_5$) C, H.

4-Oxo-1,10-decanedioic Acid (10b). Prepared as **10a** starting from enamine **6** (13.00 mL, 77.35 mmol) and acid chloride **7** (10.50 mL, 85.08 mmol). After filtration the solid was crystallized from water and gave pure **10b** as a beige solid (8.600 g, 52% yield): TLC (chloroform/acetic acid, 9/1) R_f 0.43; mp 108−109 °C (recrystallized from water); ^{1}H NMR (CD_3OD) δ 1.22−1.78 (m, 6H, H_{6-8}), 2.29 (t, J = 7.0, 2H, H_9), 2.44−2.58 (m, 4H, $H_{2,5}$), 2.67−2.80 (m, 2H, H_3), 5.01 (s broad, 2H, OH); ^{13}C NMR (CD_3OD) δ 24.44, 25.82, 28.64, 29.67, 34.72, 37.90, 176.49, 177.53, 211.71; MS (GT) m/z 217 (M + H)$^+$, 199 (M − OH)$^+$. Anal. ($C_{10}H_{16}O_5$) C, H.

6-Oxo-1,12-dodecanedioic Acid (10c). Prepared as **10a** starting from enamine **6** (14.80 mL, 88.04 mmol) and acid chloride **9** (16.50 mL, 96.99 mmol). After filtration the solid was crystallized from chloroform and recrystallized from water and gave pure **10c** as a beige solid (14.130 g, 66% yield): TLC (chloroform/acetic acid, 9/1) R_f 0.47; mp 109−110 °C (recrystallized from water); ^{1}H NMR (CD_3OD) δ 1.20−1.70 (m, 10H, $H_{3,4,8-10}$), 2.29 (t, J = 7.3, 4H, $H_{2,11}$), 2.47 (t, J = 6.7, 4H, $H_{5,7}$), 4.92 (s broad, 2H, OH); ^{13}C NMR (CD_3OD) δ 25.14, 25.34, 26.41, 26.70, 30.59, 35.54, 35.59, 43.89, 44.04, 178.21, 178.38, 214.37; MS (GT) m/z 245 (M + H)$^+$, 227 (M − OH)$^+$. Anal. ($C_{12}H_{20}O_5$) C, H.

5-Oxo-1,10-decanedioic Acid (10d). Prepared as **10a** starting from enamine **5** (8.00 g, 52.21 mmol) and acid chloride **8** (7.95 mL, 57.53 mmol). After filtration the solid was crystallized from chloroform to afford pure keto diacid **10d** as a beige solid (6.950 g, 62% yield). The product was further recrystallized in water: TLC (chloroform/acetic acid, 9/1) R_f 0.39; mp 116−117 °C (recrystallized from water); ^{1}H NMR (CD_3OD) δ 1.59−1.68 (m, 4H, $H_{7,8}$), 1.82−1.93 (m, 2H, H_3), 2.29−2.42 (m, 4H, $H_{2,9}$), 2.50−2.54 (m, 2H, H_6), 2.57 (t, J = 7.2, 2H, H_4), 4.99 (s broad, 2H, OH); ^{13}C NMR (CD_3OD) δ 20.05, 24.25, 25.53, 33.92, 34.66, 42.29, 43.03, 177.03, 177.35, 212.73; IR (KBr) ν 3500−2500, 1700, 1660, 1420, 1400, 1270, 910; MS (NBA) m/z 239 (M + Na)$^+$, 217 (M + H)$^+$, 199 (M − OH)$^+$. Anal. ($C_{10}H_{16}O_5$) C, H.

6-Oxo-1,11-undecanedioic Acid (10e). Prepared as **10a** starting from enamine **5** (12.60 mL, 78.69 mmol) and acid chloride **9** (14.75 mL, 86.71 mmol). After filtration the solid was crystallized from chloroform to afford pure keto diacid **10e** as a beige solid (12.141 g, 67% yield). The product was further recrystallized in water: TLC (chloroform/acetic acid, 9/1) R_f 0.45; mp 110−111 °C (recrystallized from water); ^{1}H NMR (CD_3OD) δ 1.50−1.70 (m, 8H, $H_{3,4,8,9}$), 2.15−2.40 (m, 4H, $H_{2,10}$), 2.42−2.59 (m, 4H, $H_{5,7}$), 4.93 (s broad, 2H, OH); ^{13}C NMR (CD_3OD) δ 24.24, 25.52, 34.65, 42.97, 177.32, 213.23; IR (KBr) ν 3200−3000, 2940, 1680 broad, 1465, 1400, 1330, 1260, 1200, 900; MS (NBA) m/z 253 (M + Na)$^+$, 231 (M + H)$^+$, 213 (M − OH)$^+$. Anal. ($C_{11}H_{18}O_5$) C, H.

Dimethyl 5-Oxo-1,11-undecanedioate (11a). In a dried round-bottomed flask under argon were introduced the starting keto diacid **10a** (7.002 g, 30.41 mmol), DCC (13.817 g, 66.966 mmol), and DMAP (1.866 g, 15.274 mmol). The reaction was started with the rapid addition of freshly distilled CH_2Cl_2 (300 mL) and dry methanol (2.70 mL, 66.66 mmol). The mixture was vigorously stirred at room temperature for 18 h. Concentrated acetic acid was then added to the reaction (0.87 mL, 15.20 mmol) which was stirred for several hours to eliminate the remaining DCC (monitored by TLC, ca. 2 h). The formed DCU was then filtered off and washed with ethyl ether. The solvents were removed in vacuo, and the crude solid was taken up in ethyl ether (50 mL), filtered, and washed once more to eliminate the maximum amount of DCU. After removal of the solvent in vacuo, the crude material was chromatographed on a silica gel column (heptanes/EtOAc, 7/3) to give diester **11a** as a white solid (7.341 g, 94% yield): TLC (heptanes/EtOAc, 6/4) R_f 0.45; mp 37−38 °C (from column chromatography); ^{1}H NMR ($CDCl_3$) δ 1.10−1.69 (m, 6H, H_{7-9}), 1.70−2.00 (m, 2H, H_3), 2.19−2.52 (m, 8H, $H_{2,4,6,10}$), 3.62 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 18.75, 23.22, 24.55, 28.52, 32.93, 33.71, 41.38, 42.38, 51.37, 51.42, 173.50, 173.93, 209.88; IR (KBr) ν 2920, 2860, 1720, 1700, 1420, 1370, 1340, 1250, 1220, 1180, 1150, 1100, 965, 870; MS (GT) m/z 259 (M + H)$^+$, 227 (M − OCH_3)$^+$, 195 (M − 2OCH_3 − H)$^+$. Anal. ($C_{13}H_{22}O_5$) C, H.

Dimethyl 4-oxo-1,10-Decanedioate (11b). Prepared as **11a** starting from **10b** (7.490 g, 34.64 mmol). Purification by silica gel column chromatography (heptanes/EtOAc, 7/3) afforded **11b** as a colorless oil that solidified on cooling (6.815 g, 81% yield): TLC (heptanes/EtOAc, 7/3) R_f 0.33; ^{1}H NMR ($CDCl_3$) δ 1.24−1.70 (m, 6H, H_{6-8}), 2.31 (t, J = 7.7, 2H, H_9), 2.46 (t, J = 7.0, 2H, H_2), 2.48−2.76 (m, 4H, $H_{3,5}$), 3.66 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 23.26, 24.61, 27.65, 28.55, 33.78, 36.98, 42.40, 51.45, 51.74, 173.24, 174.02, 208.74; IR (film) ν 2930, 2850, 1715 broad, 1420, 1400, 1350, 1190, 1160, 1090, 1000, 975, 840; MS (GT) m/z 245 $(M + H)^+$, 213 $(M - OCH_3)^+$, 181 $(M - 2OCH_3 - H)^+$. Anal. ($C_{12}H_{20}O_5$) C, H.

Dimethyl 6-Oxo-1,12-dodecanedioate (11c). Prepared as **11a** starting from **10c** (0.493 g, 2.018 mmol). Purification by silica gel column chromatography (heptanes/EtOAc, 7/3) afforded **11c** as a colorless oil that solidified when refrigerated (0.446 g, 81% yield): TLC (heptanes/EtOAc, 6/4) R_f 0.52; ^{1}H NMR ($CDCl_3$) δ 1.12−1.51 (m, 2H, H_9), 1.51−1.71 (m, 8H, $H_{3,4,8,10}$), 2.22−2.50 (m, 8H, $H_{2,5,7,11}$), 3.65 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 23.01, 23.16, 24.27, 24.49, 28.47, 33.63 (2C), 42.11, 42.28, 51.30 (2C), 173.61, 173.83, 210.23; IR (film) ν 2930, 2860, 1715 broad, 1425, 1365, 1185, 1160, 990; MS (GT) m/z 273 $(M + H)^+$, 241 $(M - OCH_3)^+$. Anal. ($C_{14}H_{24}O_5$) C, H.

Dimethyl 5-Oxo-1,10-decanedioate (11d). Prepared as **11a** starting from **10d** (2.002 g, 9.258 mmol). Purification by silica gel column chromatography (heptanes/EtOAc, 6/4) afforded **11d** as a colorless oil that solidifies when refrigerated (2.126 g, 94% yield): TLC (heptanes/EtOAc, 7/3) R_f 0.25; ^{1}H NMR ($CDCl_3$) δ 1.53−1.62 (m, 4H, $H_{7,8}$), 1.85 (tt, 2H, J = 7.2, H_3), 2.25−2.34 (m, 4H, $H_{2,9}$), 2.35−2.40 (m, 2H, H_6), 2.44 (t, J = 7.2, 2H, H_4), 3.63 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 18.75, 23.05, 24.32, 32.93, 33.70, 41.40, 42.23, 51.46 (2C), 173.52, 173.73, 209.56; IR (film) ν 2940, 2860, 1720, 1700, 1425, 1360, 1240, 1190, 1150, 1005; MS (NBA) m/z 267 $(M + Na)^+$, 245 $(M + H)^+$, 213 $(M - OCH_3)^+$, 181 $(M - 2OCH_3 - H)^+$. Anal. ($C_{12}H_{20}O_5$) C, H.

Dimethyl 6-Oxo-1,11-undecanedioate (11e). Prepared as **11a** starting from **10e** (6.530 g, 28.36 mmol). Purification by silica gel column chromatography (heptanes/EtOAc, 7/3) afforded **11e** as a colorless oil that solidifies when refrigerated (6.939 g, 95% yield): TLC (heptanes/EtOAc, 6/4) R_f 0.44; ^{1}H NMR ($CDCl_3$) δ 1.45−1.74 (m, 8H, $H_{3,4,8,9}$), 2.23−2.49 (m, 8H, $H_{2,5,7,10}$), 3.67 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 23.14, 24.40, 33.78, 42.29, 51.52, 173.82, 210.11; IR (film) ν 2940, 2860, 1725, 1700, 1425, 1405, 1360, 1235, 1190, 1160, 1000; MS (NBA) m/z 281 $(M + Na)^+$, 259 $(M + H)^+$, 227 $(M - OCH_3)^+$, 195 $(M - 2OCH_3 - H)^+$. Anal. ($C_{13}H_{22}O_5$) C, H.

Ethyl 7-Iodoheptanoate (16). Ethyl 7-bromoheptanoate (**15**) (10.0 mL, 51.32 mmol) was dissoved in dry acetone (100 mL) containing NaI (38.825 g, 259.02 mmol). The mixture was stirred under reflux for 20 h, cooled to room temperature, and allowed to stand for 20 h. The crystallized salts were filtered and washed with ethyl ether and CH_2Cl_2. Solvents were evaporated in vacuo, and ethyl ether (50 mL) was added to the crude residue. This organic phase was then washed with 10% $Na_2S_2O_4$ solution, water, and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The crude residue was rapidly chromatographed on a silica gel column (heptanes/CH_2Cl_2, 8/2 to 6/4) and the obtained product distilled with a Kugelrohr apparatus (130−140 °C, 0.5 mmHg) to give pure **16** as a slightly yellowish liquid (12.723 g, 87% yield): TLC (heptanes/ethyl ether, 8/2) R_f 0.51; ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J = 7.2, 3H, CH_3), 1.34−1.90 (m, 8H, H_{3-6}), 2.31 (t, J = 7.1, 2H, H_2), 3.19 (t, J = 6.9, 2H, H_7), 4.13 (q, J = 7.1, 2H, OCH_2); ^{13}C NMR ($CDCl_3$) δ 6.92, 14.21, 24.66, 27.95, 30.06, 33.19, 34.15, 60.19, 173.65; MS (GT) m/z 307 $(M + Na)^+$, 285 $(M + H)^+$, 239 $(M - OEt)^+$. Anal. ($C_9H_{17}IO_2$) C, H.

1-Methyl 12-Ethyl 5-Oxododecanedioate (11f). To a stirred suspension of zinc−copper couple (3.000 g, 45,893 mmol) in dry benzene (10.8 mL) was added the iodide compound **16** (8.803 g, 30.98 mmol) in a mixture of dry benzene (38.0 mL) and DMA (5.0 mL). The suspension was vigorously stirred at room temperature for 2 h and under reflux (bath temperature 90 °C) for 4 h. After the mixture was cooled to 60 °C, a solution of Pd(0) catalyst (0.3098 g, 0.2681 mmol) in dry benzene (8.2 mL) was added, and stirring was continued for 10 min at 60 °C. The heating bath was then removed, a solution of acid chloride **8** (3.70 mL, 26.77 mmol) in dry benzene (5.4 mL) was added dropwise to the reaction mixture, and stirring was continued for 1 h until the reaction was complete (TLC monitored). The suspension was filtered through a Celite pad which was washed with ethyl ether. The filtrate was then washed successively with 10% NH_4Cl, 10% $NaHCO_3$, and saturated NaCl solutions, dried over $MgSO_4$, and concentrated in vacuo. The crude brown oil was then purified on a silica gel chromatography column (heptanes/EtOAc, 7/3) followed by distillation in a Kugelrohr apparatus (160−180 °C, 0.5 mmHg) to give pure **11f** (6.149 g, 80% yield) as a white solid at room temperature: TLC (heptanes/EtOAc, 7/3) R_f 0.40; mp 37 °C (from column chromatography); ^{1}H NMR ($CDCl_3$) δ 1.23 (t, J = 6.2, 3H, CH_3C), 1.28−1.70 (m, 8H, H_{7-10}), 1.78−2.00 (m, 2H, H_3), 2.18−2.52 (m, 8H, $H_{2,4,6,11}$), 3.64 (s, 3H, OCH_3), 4.09 (q, J = 7.1, 2H, CH_2O); ^{13}C NMR ($CDCl_3$) δ 14.17, 18.79, 23.49, 24.67, 28.78 (2C), 32.96, 34.16, 41.39, 42.62, 51.46, 60.10, 173.54, 173.64, 210.10; MS (GT) m/z 287 $(M + H)^+$, 255 $(M - OCH_3)^+$, 210 $(M - OCH_3 - OEt - H)^+$. Anal. ($C_{15}H_{26}O_5$) C, H.

Dimethyl 5-Methylene-1,11-undecanedioate (12a). In a dried round-bottomed flask under argon were introduced *t*-BuOK (1.700 g, 15.15 mmol) and Ph_3PCH_3Br (5.436 g, 15.22 mmol). The ylide formation was started with the addition of freshly distilled THF (60 mL). The yellow suspension was then stirred at room temperature for 3 h, and a solution of the keto diester **11a** (3.020 g, 11.69 mmol) in freshly distilled THF (12 mL) was then added with a syringe to the ylide. The reaction mixture was stirred for 16 h and quenched with 10% aqueous NH_4Cl (40 mL), and heptanes (120 mL) was added. The layers were separated, and the water phase was extracted with heptanes (2 × 50 mL). The combined heptanes extract was washed with water and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The crude product, containing the formed triphenylphosphine oxide, was taken up in ethyl ether (50 mL), filtered, and washed with ethyl ether. The collected filtrate was concentrated in vacuo, and the residue was purified by silica gel column chromatography (heptanes/EtOAc, 9/1 to 85/15) to afford **12a** (2.299 g, 77% yield) as a colorless liquid: (TLC heptanes/EtOAc, 85/15) R_f 0.35; ^{1}H NMR ($CDCl_3$) δ 1.27−1.85 (m, 8H, $H_{3,7-9}$), 1.86−2.10 (m, 4H, $H_{4,6}$), 2.30 (t, J = 7.2, 4H, $H_{2,10}$), 3.65 (s, 6H, OCH_3), 4.70 (s broad, 2H, CH_2=); ^{13}C NMR ($CDCl_3$) δ 22.80, 24.74, 27.22, 28.75, 33.44, 33.95, 35.16, 35.50, 51.38 (2C), 109.56, 148.39, 174.01, 174.14; IR (film) ν 3050, 2910, 2830, 1710, 1620, 1415, 1340, 1225, 1180, 1150, 1070, 990, 870; MS (GT) m/z 257 $(M + H)^+$, 243 $(M - CH)^+$, 225 $(M - OCH_3)^+$, 193 $(M - 2OCH_3 - H)^+$. Anal. ($C_{14}H_{24}O_4$) C, H.

Dimethyl 4-Methylene-1,10-decanedioate (12b). Prepared as **12a** starting from **11b** (5.832 g, 23.87 mmol). Purification by silica gel column chromatography (heptanes/ethyl ether, 8/2) afforded **12b** as a colorless liquid (4.561 g, 79% yield): TLC (heptanes/ethyl ether, 8/2) R_f 0.27; ^{1}H NMR ($CDCl_3$) δ 1.30−1.80 (m, 6H, H_{6-8}), 1.95−2.10 (m, 2H, H_3), 2.24−2.60 (m, 6H, $H_{2,5,9}$), 3.65 (2s, 6H, OCH_3), 4.71−4.74 (m, 2H, CH_2=); ^{13}C NMR ($CDCl_3$) δ 24.76, 27.25, 28.74, 30.72, 32.47, 33.99, 35.96, 51.45, 51.55, 109.29, 147.84, 173.75, 174.18; IR (film) ν 2920, 2850, 1725, 1630, 1425, 1345, 1240, 1185, 1155, 980; MS (GT) m/z 243 $(M + H)^+$, 211 $(M - OCH_3)^+$, 179 $(M - 2OCH_3 - H)^+$. Anal. ($C_{13}H_{22}O_4$) C, H.

Dimethyl 6-Methylene-1,12-dodecanedioate (12c). Prepared as **12a** starting from **11c** (6.020 g, 22.10 mmol). Purification by silica gel column chromatography (heptanes/EtOAc, 9/1) afforded **12c** as a colorless liquid (4.961 g, 83% yield): TLC (heptanes/EtOAc, 9/1) R_f 0.38; ^{1}H NMR ($CDCl_3$) δ 1.33−1.80 (m, 10H, $H_{3,4,8-10}$), 1.93−2.08 (m, 4H, $H_{5,7}$), 2.31 (t, J = 7.4, 4H, $H_{2,11}$), 3.67 (s, 6H, OCH_3), 4.70 (s broad, 2H, CH_2=); ^{13}C NMR ($CDCl_3$) δ 24.45, 24.64, 26.99, 27.15, 28.66, 33.73, 33.82, 35.38, 35.50, 51.22 (2C), 108.92, 148.90, 173.88, 173.96; IR (film) ν 3060, 2920, 2840, 1720 broad, 1625, 1420,

1340, 1180, 1150, 990, 870; MS (GT) *m/z* 271 $(M + H)^+$, 239 $(M - OCH_3)^+$, 207 $(M - 2OCH_3 - H)^+$. Anal. ($C_{15}H_{26}O_4$) C, H.

Dimethyl 5-Methylene-1,10-decanedioate (12d). Prepared as **12a** starting from **11d** (1.585 g, 6.488 mmol). Purification by silica gel column chromatography (heptanes/EtOAc, 85/15) afforded **12d** as a yellowish liquid (1.290 g, 82% yield): TLC (heptanes/EtOAc, 8/2) R_f 0.46; ^{1}H NMR ($CDCl_3$) δ 1.30−2.06 (m, 10H, $H_{3,4,6-8}$), 2.28 (t, J = 6.9, 4H, $H_{2,9}$), 3.63 (s, 6H, OCH_3), 4.69 (s broad, 2H, CH_2=); ^{13}C NMR ($CDCl_3$) δ 22.70, 24.44, 26.94, 33.34, 33.75, 35.02, 35.25, 51.31 (2C), 109.73, 147.93, 173.90 (2C); IR (film) ν 3070, 2940, 2850, 1725 broad, 1630, 1420, 1350, 1235, 1190, 1160, 1070, 1000, 880; MS (NBA) *m/z* 265 $(M + Na)^+$, 243 $(M + H)^+$, 211 $(M - OCH_3)^+$, 179 $(M - 2OCH_3 - H)^+$. Anal. ($C_{13}H_{22}O_4$) C, H.

Dimethyl 6-Methylene-1,11-undecanedioate (12e). Prepared as **12a** starting from **11e** (2.050 g, 7.94 mmol). Purification by silica gel column chromatography (heptanes/EtOAc, 85/15) afforded **12e** as a yellowish liquid (1.661 g, 82% yield): TLC (heptanes/EtOAc, 8/2) R_f 0.47; ^{1}H NMR ($CDCl_3$) δ 1.26−1.77 (m, 8H, $H_{3,4,8,9}$), 2.01 (t, J = 6.9, 4H, $H_{5,7}$), 2.33 (t, J = 7.2, 4H, $H_{2,10}$), 3.67 (s, 6H, OCH_3), 4.71 (s broad, 2H, CH_2=); ^{13}C NMR ($CDCl_3$) δ 24.54, 27.06, 33.86, 35.43, 51.40, 109.25, 148.66, 174.07; IR (film) ν 3070, 2940, 2860, 1730, 1635, 1430, 1360, 1230, 1190, 1165, 880; MS (NBA) *m/z* 279 $(M + Na)^+$, 257 $(M + H)^+$, 225 $(M - OCH_3)^+$, 193 $(M - 2OCH_3 - H)^+$. Anal. ($C_{14}H_{24}O_4$) C, H.

1-Methyl 12-Ethyl 5-Methylenedodecanedioate (12f). Prepared as **12a** starting from **11f** (6.131 g, 21.41 mmol). After the combined heptanes extracts were concentrated, the residue was taken up in a mixture of heptanes/EtOAc (9/1, 50 mL). Purification by silica gel column chromatography (heptanes/EtOAc, 85/15) afforded **12f** as a colorless liquid (5.066 g, 83% yield): TLC (heptanes/EtOAc, 85/15) R_f 0.45; ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J = 7.1, 3H, CH_3C), 1.32−1.87 (m, 10H, $H_{3,7-10}$), 1.90−2.11 (m, 4H, $H_{4,6}$), 2.30 and 2.31 (2t, J = 7.0, 7.4, 4H, $H_{4,6}$), 3.67 (s, 3H, OCH_3), 4.12 (q, J = 7.0, 2H, CH_2O), 4.72 (s broad, 2H, CH_2=); ^{13}C NMR ($CDCl_3$) δ 14.24, 22.89, 24.92, 27.50, 28.99 (2C), 33.54, 34.34, 35.25, 35.74, 51.45, 60.15, 109.51, 148.66, 173.84, 174.09; IR (film) ν 3060, 2960, 2920, 2840, 1730, 1710, 1630, 1420, 1350, 1250−1120, 1085, 1020, 880; MS (GT) *m/z* 285 $(M + H)^+$, 253 $(M - OCH_3)^+$, 239 $(M - EtO)^+$, 207 $(M - OCH_3 - EtO - H)^+$. Anal. ($C_{16}H_{28}O_4$) C, H.

Dimethyl 5-[(Acetylthio)methyl]-1,11-undecanedioate (13a). In a pear-shaped flask flushed with argon were introduced the olefin **12a** (1.004 g, 3.92 mmol), freshly distilled thiolacetic acid (1.10 mL, 15.39 mmol), and AIBN (40 mg). The mixture was stirred at 90 °C for 6 h, then cooled to room temperature, and diluted with ethyl ether (15 mL). The organic phase was washed with water, 10% aqueous $NaHCO_3$, and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by two silica gel column chromatographies (heptanes/EtOAc, 8/2, and heptanes/ethyl ether, 7/3) to give pure **13a** as a yellowish oil (1.120 g, 86% yield): TLC (heptanes/EtOAc) 75/25, R_f 0.43; ^{1}H NMR ($CDCl_3$) δ 1.20−1.75 (m, 13H, H_{3-9}), 2.31 (t, J = 6.9, 4H, $H_{2,10}$), 2.33 (s, 3H, CH_3CO), 2.90 (d, J = 5.6, 2H, CH_2S), 3.67 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 21.92, 24.79, 26.15, 29.26, 30.67, 32.58, 32.84, 33.07, 33.98, 34.12, 37.60, 51.46 (2C), 173.94, 174.17, 195.83; IR (film) ν 2910, 2840, 1715, 1675, 1410, 1340, 1230−1085, 995, 935, 610; MS (GT) *m/z* 333 $(M + H)^+$, 301 $(M - OCH_3)^+$, 259 $(M - OCH_3 - CH_3CO + H$ or $M - CH_3COS + 2H)^+$, 227 $(M - 2OCH_3 - CH_3CO)^+$. Anal. ($C_{16}H_{28}O_5S$) C, H.

Dimethyl 4-[(Acetylthio)methyl]-1,10-decanedioate (13b). Prepared as **13a** starting from **12b** (2.324 g, 9.59 mmol) and AIBN (40 mg). Purification by two silica gel column chromatographies (heptanes/EtOAc, 8/2, and heptanes/ethyl ether, 7/3) afforded **13b** as a yellowish oil (2.562 g, 84% yield): TLC (heptanes/EtOAc, 8/2) R_f 0.29; ^{1}H NMR ($CDCl_3$) δ 1.31 (s broad, 6H, H_{6-8}), 1.57−1.70 (m, 5H, H_{3-5}), 2.24−2.40 (m, 4H, $H_{2,9}$), 2.34 (s, 3H, CH_3CO), 2.90 (d, J = 5.2, 2H, CH_2S), 3.67 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 24.76, 26.12, 28.10, 29.22, 30.68, 31.32, 32.74, 32.81, 33.97, 37.32, 51.45, 51.57, 173.94, 174.16, 195.71; IR (film) ν 2920, 2850, 1720, 1680, 1420, 1345, 1240, 1190, 1160, 1125, 1100, 1000, 945, 615; MS (GT) *m/z* 319 $(M + H)^+$, 287 $(M - OCH_3)^+$, 245 $(M - OCH_3 - CH_3CO + H$ or $M - CH_3COS + 2H)^+$, 213 $(M - 2OCH_3 - CH_3CO)^+$.

Dimethyl 6-[(Acetylthio)methyl]-1,12-dodecanedioate (13c). Prepared as **13a** starting from **12c** (2.517 g, 9.31 mmol) and AIBN (40 mg). Purification by two silica gel column chromatographies (heptanes/EtOAc, 85/15) afforded **13c** as a yellowish oil (2.680 g, 83% yield): TLC (heptanes/EtOAc, 8/2) R_f 0.35; ^{1}H NMR ($CDCl_3$) δ 1.23−1.73 (m, 15H, H_{3-10}), 2.21−2.35 (m, 4H, $H_{2,11}$), 2.30 (s, 3H, CH_3CO), 2.85 (d, J = 5.5, 2H, CH_2S), 3.64 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 24.72, 24.99, 26.02, 26.12, 29.20, 30.58, 32.72, 32.89, 33.10, 33.85, 33.90, 37.49, 51.35 (2C), 174.00, 174.08, 195.75; IR (film) ν 2920, 2850, 1725, 1680, 1425, 1355, 1235, 1185, 1160, 1125, 1095, 1000, 945, 610; MS (NBA) *m/z* 369 $(M + Na)^+$, 347 $(M + H)^+$, 315 $(M - OCH_3)^+$, 273 $(M - OCH_3 - CH_3CO + H$ or $M - CH_3COS + 2H)^+$, 241 $(M - 2OCH_3 - CH_3CO)^+$. Anal. ($C_{17}H_{30}O_5S$) C, H.

Dimethyl 5-[(Acetylthio)methyl]-1,10-decanedioate (13d). Prepared as **13a** starting from **12d** (1.276 g, 5.27 mmol) and AIBN (40 mg). Purification by two silica gel column chromatographies (heptanes/EtOAc, 8/2) afforded **13d** as a yellowish oil (1.466 g, 87% yield): TLC (heptanes/EtOAc, 8/2) R_f 0.29; ^{1}H NMR ($CDCl_3$) δ 1.23−1.31 (m, 6H, $H_{4,6,7}$), 1.52−1.61 (m, 5H, $H_{3,5,8}$), 2.22−2.26 (m, 4H, $H_{2,9}$), 2.28 (s, 3H, CH_3CO), 2.84 (d, J = 6.0, 2H, CH_2S), 3.62 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 21.98, 25.04, 26.05, 30.67, 32.57, 32.67, 33.01, 33.91, 34.10, 37.51, 51.46 (2C), 173.88, 174.04, 195.72; IR (film) ν 2930, 2880, 1725, 1680, 1430, 1350, 1240−1100, 950, 615; MS (NBA) *m/z* 341 $(M + Na)^+$, 319 $(M + H)^+$, 245 $(M - OCH_3 - CH_3CO + H$ or $M - CH_3COS + 2H)^+$, 213 $(M - 2OCH_3 - CH_3CO)^+$. Anal. ($C_{15}H_{26}O_5S$) C, H,O.

Dimethyl 6-[(Acetylthio)methyl]-1,11-undecanedioate (13e). Prepared as **13a** starting from **12e** (0.250 g, 0.975 mmol) and AIBN (15 mg). Purification by silica gel column chromatography (heptanes/EtOAc, 8/2) afforded **13e** as a yellowish oil (0.308 g, 95% yield): TLC (heptanes/EtOAc, 8/2) R_f 0.30; ^{1}H NMR ($CDCl_3$) δ 1.22−1.34 (m, 8H, $H_{4,5,7,8}$), 1.51−1.64 (m, 5H, $H_{3,9}$), 2.28 (t, J = 7.5, 4H, $H_{2,10}$), 2.29 (s, 3H, CH_3CO), 2.85 (d, J = 6.0, 2H, CH_2S), 3.64 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 25.03, 26.05, 30.65, 32.73, 33.05, 33.90, 37.44, 51.43, 174.06, 195.82; IR (film) ν 2910, 2840, 1720, 1675, 1420, 1340, 1230−1080, 940, 610; MS (NBA) *m/z* 355 $(M + Na)^+$, 333 $(M + H)^+$, 259 $(M - OCH_3 - CH_3CO + H$ or $M - CH_3COS + 2H)^+$, 227 $(M - 2OCH_3 - CH_3CO)^+$.

1-Methyl 12-Ethyl 5-[(Acetylthio)methyl]dodecanedioate (13f). Prepared as **13a** starting from **12f** (2.608 g, 10.17 mmol) and AIBN (80 mg). Purification by two silica gel column chromatographies (heptanes/EtOAc, 9/1, and heptanes/ethyl ether, 8/2) afforded **13f** as a yellowish oil (2.981 g, 81% yield): TLC (heptanes/EtOAc, 8/2) R_f 0.40; ^{1}H NMR ($CDCl_3$) δ 1.18−1.75 (m, 18H, H_{3-10}, CH_3C), 2.22−2.37 (m, 4H, $H_{2,11}$), 2.33 (s, 3H, CH_3CO), 2.90 (d, J = 5.6, 2H, CH_2S), 3.67 (s, 3H, OCH_3), 4.13 (q, J = 7.1, 2H, CH_2O); ^{13}C NMR ($CDCl_3$) δ 14.24, 22.04, 24.93, 26.36, 29.05, 29.45, 30.68, 32.64, 33.05, 33.17, 34.17, 34.33, 37.63, 51.48, 60.15, 173.82, 173.94, 195.83; MS (GT) *m/z* 360 $(M + H)^+$, 287 $(M - OCH_3 - CH_3CO + H)^+$, 273 $(M - EtO - CH_3CO + H)^+$, 241 $(M - OCH_3 - EtO - CH_3CO)^+$. Anal. ($C_{18}H_{32}O_5S$) C, H.

5-(Mercaptomethyl)-1,11-undecanedioic Acid (14a). In an argon-flushed flask, compound **13a** (0,583 g, 1.752 mmol) was dissolved in a mixture of ethanol and water (1/1, 6 mL) containing KOH (85%, 0.990 g, 15.00 mmol). The reaction was heated under reflux (bath temperature 100 °C) for 3 h, cooled to room temperature, acidified with 3 N HCl (pH ca. 1), and extracted with EtOAc. Small quantities of saturated NaCl solution were added to resolve emulsions. The combined organic extracts were washed with saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (chloroform/methanol, 95/5) to give pure thiol **14a** as a colorless oil which was placed under vacuum for 24 h to eliminate trace amounts of solvents. The obtained oil was refrigerated and became a

white solid (0.292 g, 64% yield): TLC (chloroform/methanol, 9/1) R_f 0.45; mp 43−44 °C (from column chromatography); ^{1}H NMR (CD_3OD) δ 1.29−1.75 (m, 13H, H_{3-9}), 2.29 (t, J = 7.0, 4H, $H_{2,10}$), 2.50−2.60 (m, 2H, CH_2S), 4.94 (s broad, 3H, OH, SH); ^{13}C NMR (CD_3OD) δ 23.13, 26.04, 27.33, 28.66, 30.46, 32.84, 33.18, 34.92, 35.09, 41.17, 177.55, 177.72; IR (film) ν 3400−3020, 2910, 2840, 2660, 2560, 1690 broad, 1400, 1265, 1220, 920; MS (GT) m/z 285 $(M + Na)^+$, 263 $(M + H)^+$, 245 $(M - OH)^+$, 227 $(M - 2OH - H)^+$. Anal. ($C_{12}H_{22}O_4S$) C, H.

4-(Mercaptomethyl)-1,10-decanedioic Acid (14b). Prepared as **14a** starting from **13b** (2.346 g, 7.37 mmol). Purification by silica gel column chromatography (chloroform/methanol, 95/5) and vacuum treatment afforded **14b** as a white solid (1.075 g, 59% yield): TLC (chloroform/methanol, 9/1) R_f 0.45; mp 53−54 °C (from column chromatography); ^{1}H NMR ($CDCl_3$) δ 1.13−1.77 (m, 12H, H_{3-8}, SH), 2.29−2.61 (m, 6H, $H_{2,9}$, CH_2S), 10.75 (s broad, 2H, OH); ^{13}C NMR ($CDCl_3$) δ 24.49, 26.04, 27.04, 27.99, 29.10, 31.35, 31.68, 33.97, 39.21, 180.05, 180.24; MS (GT) m/z 249 $(M + H)^+$, 231 $(M - OH)^+$, 213 $(M - 2OH - H)^+$. Anal. ($C_{11}H_{20}O_4S$) C, H.

6-(Mercaptomethyl)-1,12-dodecanedioic Acid (14c). Prepared as **14a** starting from **13c** (0.662 g, 1.91 mmol). Purification by silica gel column chromatography (chloroform/methanol, 95/5) and vacuum treatment afforded **14c** as a white solid (0.346 g, 66% yield): TLC (chloroform/methanol, 9/1) R_f 0.40; mp 56−57 °C (from column chromatography); ^{1}H NMR (CD_3OD) δ 1.30−1.70 (m, 15H, H_{3-10}), 2.30 (t, J = 6.9, 4H, $H_{2,11}$), 2.48−2.58 (m, 2H, CH_2S), 4.95 (s broad, 3H, OH, SH); ^{13}C NMR (CD_3OD) δ 26.02, 26.29, 27.16, 27.33, 28.73, 30.45, 33.05, 33.22, 34.87, 34.91, 41.17, 177.65, 177.70; MS (GT) m/z 277 $(M + H)^+$, 259 $(M - OH)^+$, 241 $(M - 2OH - H)^+$. Anal. ($C_{13}H_{24}O_4S$) C, H.

5-(Mercaptomethyl)-1,10-decanedioic Acid (14d). Prepared as **14a** starting from **13d** (1.353 g, 4.25 mmol). Purification by silica gel column chromatography (chloroform/methanol, 95/5) and vacuum treatment afforded **14d** as a white solid (0.900 g, 85% yield): TLC (chloroform/methanol, 9/1) R_f 0.50; mp 60−62 °C (from column chromatography); ^{1}H NMR ($CDCl_3$) δ 1.18 (t, J = 8.0, 1H, SH), 1.30−1.45 (m, 6H, $H_{4,6,7}$), 1.48−1.56 (m, 1H, H_5), 1.58−1.65 (m, 4H, $H_{3,8}$), 2.34 (t, J = 7.3, 2H, H_2 or 9), 2.35 (t, J = 7.3, 2H, H_2 or 9), 2.51 (dd, J = 8.0, 5.5, 2H, CH_2S), 10.44 (s broad, 2H, OH); ^{13}C NMR ($CDCl_3$) δ 21.62, 24.76, 25.88, 28.22, 31.48, 31.70, 33.92, 34.13, 39.67, 179.67; IR (KBr) ν 3400 broad, 3040 broad, 2920, 2840, 1695, 1450, 1420, 1395, 1285, 1230, 1180, 920 broad; MS (NBA) m/z 271 $(M + Na)^+$, 249 $(M + H)^+$, 231 $(M - OH)^+$, 213 $(M - 2OH - H)^+$. Anal. ($C_{11}H_{20}O_4S$) C, H,O.

6-(Mercaptomethyl)-1,11-undecanedioic Acid (14e). Prepared as **14a** starting from **13e** (0.475 g, 1.429 mmol). Purification by silica gel column chromatography (chloroform/methanol, 95/5) and vacuum treatment afforded **14e** as a white solid (0.285 g, 76% yield): TLC (chloroform/methanol, 9/1) R_f 0.48; ^{1}H NMR ($CDCl_3$) δ 1.18 (t, J = 8.0, 1H, SH), 1.24−1.45 (m, 8H, $H_{4,5,7,8}$), 1.50−1.55 (m, 1H, H_6), 1.57−1.66 (m, 4H, $H_{3,9}$), 2.35 (t, J = 7.2, 4H, $H_{2,10}$), 2.50 (dd, J = 8.0, 5.7, 2H, CH_2S), 10.67 (s broad, 2H, OH); ^{13}C NMR ($CDCl_3$) δ 24.78, 25.88, 28.34, 31.72, 34.00, 39.62, 180.32; IR (film) ν 3400−3000, 2920, 2840, 2660, 1690 broad, 1450−1400, 1270 broad, 925 broad; MS (NBA) m/z 285 $(M + Na)^+$, 263 $(M + H)^+$, 245 $(M - OH)^+$, 227 $(M - 2OH - H)^+$. Anal. ($C_{12}H_{22}O_4S$) C, H.

5-(Mercaptomethyl)-1,12-dodecanedioic Acid (14f). Prepared as **14a** starting from **13f** (0.536 g, 1.487 mmol). Purification by silica gel column chromatography (chloroform/methanol, 95/5) and vacuum treatment afforded **14f** as a white solid (0.302 g, 74% yield): TLC (chloroform/methanol, 9/1) R_f 0.45; mp 44−45 °C (from column chromatography); ^{1}H NMR (CD_3OD) δ 1.28−1.73 (m, 15H, H_{3-10}), 2.29 (t, J = 6.8, 4H, $H_{2,11}$), 2.49−2.60 (m, 2H, CH_2S), 4.98 (s broad, 3H, OH, SH); ^{13}C NMR (CD_3OD) δ 23.13, 26.06, 27.47, 28.69, 30.16, 30.63, 32.85, 33.27, 34.95, 35.10, 41.19, 177.55, 177.74; IR (film) ν 3300−3000, 2900, 2820, 2640, 2240, 2040, 1685 broad, 1390, 1220, 1040, 910 broad; MS (GT) m/z 299 $(M + Na)^+$, 277 $(M + H)^+$, 259 $(M - OH)^+$, 241 $(M - 2OH - H)^+$. Anal. ($C_{13}H_{24}O_4S$) C, H.

Dimethyl 6,6-(1,2-Ethylenedioxy)-1,11-undecanedioate (17). A flask fitted with a Dean−Stark trap was charged with a solution of ketone **11e** (2.841 g, 11.00 mmol), ethylene glycol (7.40 mL, 132.69 mmol) and *p*-toluenesulfonic acid (0.377 g, 1.98 mmol) in benzene (750 mL). The reaction mixture was heated at reflux for 10 h, cooled to room temperature, washed with 10% aqueous $NaHCO_3$, water, and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (heptanes/EtOAc, 7/3) to afford **17** (2.486 g, 75% yield) as a colorless oil: TLC (heptanes/EtOAc, 6/4) R_f 0.48; ^{1}H NMR ($CDCl_3$) δ 1.25−1.77 (m, 12H, $H_{3-5,7-9}$), 2.31 (t, J = 6.9, 4H, $H_{2,10}$), 3.67 (s, 6H, OCH_3), 3.92 (s, 4H, CH_2O); ^{13}C NMR ($CDCl_3$) δ 23.21, 25.00, 33.86, 36.58, 51.31, 64.77, 111.16, 173.87; IR (film) ν 2930, 2860, 1740, 1420, 1355, 1230, 1185, 1160, 1060, 935; MS (NBA) m/z 325 $(M + Na)^+$, 303 $(M + H)^+$, 271 $(M - OCH_3)^+$, 187 $(M - (CH_2)_4COOCH_3)^+$. Anal. ($C_{15}H_{26}O_6$) C, H.

Dimethyl 5-Bromo-6,6-(1,2-ethylenedioxy)-1,11-undecanedioate (18). To a stirred solution of **17** (6.163 g, 20.38 mmol) in dry ethyl ether (35 mL) at 15 °C was slowly and carefully added bromine (1.05 mL, 19.79 mmol). The orange-red solution obtained was stirred for 1 h. Anhydrous Na_2CO_3 (4.759 g, 44.90 mmol) was then carefully added (portionwise) and vigorously stirred for 16 h to neutralize the reaction mixture. The white suspension was then filtered and washed with ethyl ether. The filtrate was concentrated in vacuo, and the resulting crude compound was purified by silica gel column chromatography (heptanes/CH_2Cl_2/EtOAc, 4/5/1) to afford **18** (7.012 g, 90% yield) as a slightly yellowish oil: TLC (heptanes/CH_2Cl_2/EtOAc, 4/5/1) R_f 0.40; ^{1}H NMR ($CDCl_3$) δ 1.32−1.43 (m, 2H, H_8), 1.57−1.74 (m, 5H, $H_{3a,4a,7a,9}$), 1.93−2.06 (m, 3H, $H_{3b,4b,7b}$), 2.26−2.37 (m, 4H, $H_{2,10}$), 3.64 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 3.89 (dd, J = 9.5, 1.8, 1H, H_5), 3.96−4.06 (m, 4H, CH_2O); ^{13}C NMR ($CDCl_3$) δ 22.36, 23.63, 24.95, 32.65, 33.29, 33.71, 34.01, 51.46, 51.55, 58.98, 66,00 (2C), 110.98, 173.53, 173.92; IR (film) ν 2940, 2880, 1725, 1425, 1355, 1240, 1190, 1165, 1050, 940; MS (NBA) m/z 383 and 381 $(M + H)^+$, 351 and 349 $(M - OCH_3)^+$, 267 and 265 $(M - (CH_2)_4COOCH_3)^+$, 187 $(M - (CH_2)_4COOCH_3 - Br + H)^+$. Anal. ($C_{15}H_{25}BrO_6$) C, H.

Dimethyl 6,6-(1,2-Ethylenedioxy)-4(*E*)-undecene-1,11-dioate (19). To a stirred solution of bromide **18** (7.012 g, 18.39 mmol) in anhydrous DMSO (185 mL) and under argon was added DBU (8.25 mL, 55.17 mmol). The solution was heated at 120 °C until total disappearance of the bromide **18** on TLC (around 20 h). The reaction was then cooled to room temperature, quenched with 10% aqueous NH_4Cl, and extracted with EtOAc. The organic extract was washed with water and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (heptanes/EtOAc, 7/3) to afford **19** (4.766 g, 86% yield) as a colorless oil: TLC (heptanes/CH_2Cl_2/EtOAc, 4/5/1) R_f 0.35; ^{1}H NMR ($CDCl_3$) δ 1.35−1.41 (m, 2H, H_8), 1.57−1.69 (m, 4H, $H_{7,9}$), 2.28 (t, J = 7.6, 2H, H_{10}), 2.33−2.41 (m, 4H, $H_{2,3}$), 3.65 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 3.81−3.91 (m, 4H, CH_2O), 5.36 (dt, J = 15.5, 1.3, 1H, H_5), 5.75 (dt, J = 15.4, 6.5, 1H, H_4); ^{13}C NMR ($CDCl_3$) δ 22.92, 24.90, 26.96, 33.48, 33.90, 37.83, 51.29, 51.39, 64.30 (2C), 108.52, 129.39, 130.63, 173.12, 173.90; IR (film) ν 2930, 2875, 1720, 1655, 1425, 1355, 1230, 1180, 1155, 1025, 960; MS (NBA) m/z 323 $(M + Na)^+$, 301 $(M + H)^+$, 269 $(M - OCH_3)^+$, 185 $(M - (CH_2)_4COOCH_3)^+$. Anal. ($C_{15}H_{24}O_6$) C, H.

Dimethyl 6-Oxo-4(*E*)-undecene-1,11-dioate (20). To a stirred suspension of silica gel (63−200 μm, 8.0 g) in CH_2Cl_2 (16 mL) was added a mixture of TFA (0.63 mL, 8.23 mmol) and water (0.63 mL). The suspension was vigorously stirred for 10 min at room temperature, then a solution of **19** (1.658 g, 5.52 mmol) in CH_2Cl_2 (5 mL) was added dropwise, and the mixture stirred for an additional 2 h. The reaction mixture was filtered and the silica gel washed with ethyl ether (4 × 30 mL). The organic filtrate was then washed with 10% aqueous $NaHCO_3$, water, and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (heptanes/ethyl

ether, 5/5) to afford **20** (1.272 g, 90% yield) as a colorless oil: TLC (heptanes/EtOAc, 4/6) R_f 0.40; ^{1}H NMR ($CDCl_3$) δ 1.58−1.63 (m, 4H, $H_{8,9}$), 2.22−2.30 (m, 2H, H_{10}), 2.44−2.52 (m, 6H, $H_{2,3,7}$), 3.62 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 6.08 (dt, J = 15.9, 1.5, 1H, H_5), 6.76 (dt, J = 15.9, 6.4, 1H, H_4); ^{13}C NMR ($CDCl_3$) δ 23.47, 24.49, 27.36, 32.33, 33.78, 39.73, 51.32, 51.61, 130.81, 144.29, 172.50, 173.65, 199.45; IR (film) ν 2950, 2870, 1725, 1685, 1660, 1620, 1430, 1360, 1245, 1190, 1160, 1040, 965, 720; MS (NBA) m/z 279 $(M + Na)^+$, 257 $(M + H)^+$, 225 $(M - OCH_3)^+$. Anal. ($C_{13}H_{20}O_5$) C, H; C: calcd, 60.92; found, 60.10.

Dimethyl 6-Methylene-4(*E*)-undecene-1,11-dioate (21). A −10 °C suspension of Ph_3PCH_3Br (1.842 g, 5.16 mmol) in freshly distilled THF (15 mL) under argon was treated with *n*-BuLi (2.0 mL, 2.5 M in hexanes, 5.00 mmol), stirred up to room temperature for 30 min, and recooled at −10 °C. To this orange suspension was added a solution of **20** (1.152 g, 4.49 mmol) in freshly distilled THF (4 mL) dropwise. The reaction mixture was stirred at −10 °C for 10 min and then at room temperature for 1 h. The reaction was quenched with 10% aqueous NH_4Cl (10 mL), ethyl ether (30 mL) was added, and the mixture was extracted twice with ethyl ether. The combined organic phase was washed with water and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The crude product, containing the formed triphenylphosphine oxide, was taken up in ethyl ether (20 mL), filtered, and washed with ethyl ether. The collected filtrate was concentrated in vacuo, and the residue was purified by silica gel column chromatography (heptanes/ethyl ether, 75/25) to afford **21** (0.709 g, 62% yield) as a colorless liquid: TLC (heptanes/ethyl ether, 75/25) R_f 0.32; ^{1}H NMR ($CDCl_3$) δ 1.41−1.50 (m, 2H, H_8), 1.57−1.65 (m, 2H, H_9), 2.12 (t, J = 7.5, 2H, H_7), 2.27 (t, J = 7.3, 2H, H_{10}), 2.36−2.37 (m, 4H, $H_{2,3}$), 3.61 (s, 3H, OCH_3), 3.62 (s, 3H, OCH_3), 4.84 (d, J = 15.8, 2H, CH_2=), 5.63 (td, J = 15.8, 6.6, 1H, H_4), 6.02 (d, J = 15.8, 1H, H_5); ^{13}C NMR ($CDCl_3$) δ 24.63, 27.42, 27.91, 31.54, 33.67, 33.73, 51.24, 51.33, 114.02, 127.35, 132.94, 145.24, 173.20, 173.85; IR (film) ν 3070, 2930, 2850, 1720, 1600, 1420, 1345, 1240, 1180, 1155, 955, 875; MS (NBA) m/z 277 $(M + Na)^+$, 255 $(M + H)^+$, 223 $(M - OCH_3)^+$, 191 $(M - 2OCH_3 - H)^+$. Anal. ($C_{14}H_{22}O_4$) C, H.

Dimethyl 6-(Hydroxymethyl)-4(*E*)-undecene-1,11-dioate (22). To a stirred solution of 9-BBN-H (1.006 g, 4.12 mmol) in freshly distilled THF (7 mL) at room temperature and under argon was added dropwise a solution of diene **21** (0.698 g, 2.75 mmol) in freshly distilled THF (2 mL). The reaction mixture was stirred for 2 h, then cooled to −0 °C, and hydrolyzed with the careful addition of NaOH (1.40 mL, 3 M in water, 4.20 mmol) followed by H_2O_2 (1.40 mL, 30 wt % in water). The reaction was stirred at room temperature overnight and diluted with ethyl ether (20 mL), and 10% aqueous $NaHCO_3$ (6 mL) was added. The layers were separated, and the water phase was extracted with ethyl ether. The conbined organic extract was washed with 10% aqueous $NaHCO_3$, water, and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (heptanes/ethyl ether, 3/7) to give **22** (0.439 g, 59% yield) as a colorless oil: TLC (heptanes/EtOAc, 5/5) R_f 0.42; ^{1}H NMR ($CDCl_3$) δ 1.10−1.67 (m, 6H, H_{7-9}), 2.02−2.40 (m, 8H, $H_{2,3,6,10}$, OH), 3.20−3.44 (m, 2H, CH_2OH), 3.60 (s, 6H, OCH_3), 5.14 (dd, J = 15.3, 8.4, 1H, H_5), 5.48 (td, J = 15.4, 5.9, 1H, H_4); ^{13}C NMR ($CDCl_3$) δ 24.76, 26.41, 27.86, 30.38, 33.79 (2C), 45.46, 51.30, 51.38, 65.66, 131.06, 132.63, 173.46, 174.01; IR (film) ν 3600−3240, 2930, 2860, 1725, 1430, 1360, 1245, 1195, 1160, 1040, 960; MS (GT) m/z 273 $(M + H)^+$, 255 $(M - OH)^+$, 241 $(M - OCH_3)^+$, 223 $(M - OH - OCH_3 - H)^+$.

6-(Hydroxymethyl)-4(*E*)-undecene-1,11-dioic Acid (23). Compound **22** (0.0609 g, 0.224 mmol) was dissolved in a mixture of ethanol (0.5 mL) and KOH solution (0.5 mL, 2 M in water, 1.0 mmol). The mixture was stirred at room temperature for 6 h, acidified with 1 N HCl (pH around 1), and extracted with EtOAc. Small quantities of saturated NaCl solution were added to resolve emulsions. The combined organic extracts were washed with saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (chloroform/methanol, 9/1 to 85/15) to give pure alcohol **23** as a colorless oil which was placed under vacuum for 24 h to eliminate trace amounts of solvents (0.0400 g, 73% yield): TLC (chloroform/methanol, 85/15) R_f 0.25; ^{1}H NMR (CD_3OD) δ 1.13−1.62 (m, 6H, H_{7-9}), 2.21−2.38 (m, 7H, $H_{2,3,6,10}$), 3.41 (d, J = 6.5, 2H, CH_2OH), 4.88 (s broad, 3H, OH), 5.12−5.60 (m, 2H, $H_{4,5}$); ^{13}C NMR (CD_3OD) δ 28.65, 30.17, 31.66, 34.34, 37.42, 37.57, 49.18, 69.42, 133.95, 136.38, 179.58, 180.23; MS (GT) m/z 267 $(M + Na)^+$, 245 $(M + H)^+$, 227 $(M - OH)^+$, 209 $(M - 2OH - H)^+$, 191 $(M - 3OH - 2H)^+$. Anal. ($C_{12}H_{20}O_5 \cdot 0.5H_2O$) C, H.

Dimethyl 6-[(Acetylthio)methyl]-4(*E*)-undecene-1,11-dioate (24). To a solution of triphenylphosphine (0.484 g, 1.84 mmol) in freshly distilled THF (4.6 mL) at 0 °C was added dropwise diisopropyl azodicarboxylate (DIAD; 0.36 mL, 1.83 mmol). The mixture was stirred at 0 °C for 30 min, and a white solid precipitated out. To this suspension, compound **22** (0.251 g, 0.922 mmol) dissolved in dry THF (2.30 mL) was added, followed by thiolacetic acid (0.13 mL, 1.83 mmol). The reaction was stirred at 0 °C for 1 h and at room temperature for an additional 1 h, diluted, and extracted with ethyl ether. The organic extract was washed with water and saturated NaCl, dried over $MgSO_4$, and concentrated. The crude material was purified by two column chromatographies on silica gel (heptanes/ethyl ether, 6/4, and petroleum spirit/ethyl ether, 8/2) to give pure **24** as a yellowish oil (0.251 g, 93% yield): TLC (heptanes/ethyl ether, 5/5) R_f 0.45; ^{1}H NMR ($CDCl_3$) δ 1.16−1.43 (m, 4H, $H_{7,8}$), 1.50−1.61 (m, 2H, H_9), 2.01−2.12 (m, 1H, H_6), 2.20−2.35 (m, 6H, $H_{2,3,10}$), 2.26 (s, 3H, CH_3CO), 2.74 (dd, J = 13.3, 7.9, 1H, CH_2S), 2.87 (dd, J = 13.3, 5.9, 1H, CH_2S), 3.61 (s, 3H, OCH_3), 3.62 (s, 3H, OCH_3), 5.14 (dd, J = 15.3, 8.7, 1H, H_5), 5.38 (td, J = 15.3, 6.3, 1H, H_4); ^{13}C NMR ($CDCl_3$) δ 24.76, 26.52, 27.72, 30.53, 33.78, 33.88, 33.92, 34.26, 42.42, 51.38, 51.43, 129.96, 133.26, 173.35, 173.98, 195.56; IR (film) ν 2930, 2840, 1720, 1680, 1425, 1340, 1240, 1180, 1160, 1120, 1095, 955, 610; MS (GT) m/z 353 $(M + Na)^+$, 331 $(M + H)^+$, 289 $(M - CH_3CO + 2H)^+$, 257 $(M - OCH_3 - CH_3CO + H)^+$, 243, 225 $(M - 2OCH_3 - CH_3CO)^+$. Anal. ($C_{16}H_{26}O_5S$) C, H.

6-(Mercaptomethyl)-4(*E*)-undecene-1,11-dioate (25). Prepared as **14a** starting from **24** (0.247 g, 0.748 mmol). Purification by silica gel column chromatography (chloroform/methanol, 95/5) and vacuum treatment afforded **27** as a white solid (0.125 g, 64% yield): TLC (chloroform/methanol, 9/1) R_f 0.38; ^{1}H NMR ($CDCl_3$) δ 1.15−1.63 (m, 7H, $H_{7,8,9}$, SH), 2.00−2.12 (m, 1H, H_6), 2.22−2.45 (m, 8H, $H_{2,3,10}$, CH_2S), 5.13 (dd, J = 15.3, 8.8, 1H, H_5), 5.43 (td, J = 15.3, 6.3, 1H, H_4), 10.29 (s broad, 2H, OH); ^{13}C NMR ($CDCl_3$) δ 24.43, 26.35, 27.48, 29.68, 33.30, 33.89, 33.94, 45.67, 130.17, 133.35, 179.37, 180.13; MS (GT) m/z 261 $(M + H)^+$, 219. Anal. ($C_{12}H_{20}O_4S$) C, H.

Biological Methods. 1. GST Expression. The recombinant GST was prepared as described by Smith and Corcozan[28] using an *Escherichia coli* XL1 blue strain transformed with the pGEX vector (Pharmacia Biotech).[12] Briefly, 5 L of TB/ampicillin medium in a 7.5-L tabletop fermentor was inoculated with an overnight starter culture. Expression of the recombinant protein was induced by adding IPTG to a final concentration of 0.1 mM. Induction was allowed to proceed for 2 h. The cells were then harvested by centrifugation (4000*g* for 30 min). After lysis of the bacteria, the recombinant GST protein was purified on a GSH-agarose (Sigma) column. The recovered protein was then desalted on a Sephadex G-50 column and freeze-dried.

2. Protein Concentration. The protein concentration used throughout this work was inferred from UV absorbance measurements at 276 nm (Cary 118 spectrophotometer) in the absence of aggregation effects (as determined in the 300−350-nm range). Molar absorptivities at 276 nm of the protein used in this work were determined by the procedure of Gill and von Hippel (1989) giving 35 050 L mol^{-1} cm^{-1}.

3. Binding Assays. A protein affinity chromatography method was used to evaluate binding of the GSH analogues to the recombinant GST protein. The recombinant GST was dialyzed in a PBS buffer (137 mM NaCl, 2.7 mM KCl, 4.3 mM

Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.3) and bound to a glutathione-agarose slurry (Qiagen) equilibrated in the same buffer. The mixture was left for 2 h at room temperature under smooth agitation. The GSH analogue was dissolved in the same buffer and then added at increasing concentrations to the GSH-agarose−GST mixture. After a 2-h incubation, the mixture was loaded in a column (1 × 2.5 cm) and washed with 4 mL of PBS buffer. The unbound GST protein was recovered in the fractions eluted during the washing steps. Its concentration was calculated from its UV absorption at 276 nm using the extinction coefficient determined as described above. The percentage of GST−GSH-agarose complex was then plotted as a function of the analogue concentration. The typical dissociation plots obtained were used to determine the IC_{50} values.

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Supporting Information Available: ^{1}H NMR,^{13}C NMR, and MS spectral data of the final analogues listed in Table 1, completed with some additional 2D NMR (COSY and carbon−proton heterocorrelation) spectral data (30 pages). Ordering information is given on any current masthead page.

References

(1) Meister, A.; Anderson, M. GLUTATHIONE. *Annu. Rev. Biochem.* **1983**, *52*, 711−760.
(2) Lomaestro, B. M.; Malone, M. Glutathione in health and disease: pharmacotherapeutic issues. *Ann. Pharmacother.* **1995**, *29*, 1263−1273.
(3) Schröder, C. P.; Godwin, A. K.; O'Dwyer, P. J.; Tew, K. D.; Hamilton, T. C.; Ozols, R. F. Glutathione and Drug Resistance. *Cancer Invest.* **1996**, *14*, 158−168.
(4) Ouwerkerk-Mahadevan, S.; van Boom, J. H.; Dreef-Tromp, M. C.; Ploemen, J. H. T. M.; Meyer, D. J.; Mulder, G. J. Glutathione analogues as novel inhibitors of rat and human glutathione S-transferase isoenzymes, as well as of glutathione conjugation in isolated rat hepatocytes and in the rat in vivo. *Biochem. J.* **1995**, *308*, 283−290.
(5) Lyttle, M. H.; Hocker, M. D.; Hui, H. C.; Caldwell, C. G.; Aaron, D. T.; Engqvist-Goldstein, A.; Flatgaard, J. E.; Bauer, K. E. Isozyme-Specific Glutathione-S-Transferase Inhibitors: Design and Synthesis. *J. Med. Chem.* **1994**, *37*, 189−194.
(6) Calcagni, A.; Duprè, S.; Lucente, G.; Luisi, G.; Pinnen, F.; Rossi, D.; Spirito, A. Synthesis and Activity of the Glutathione Analogue γ-(L-γ-Oxaglutamyl)-L-cysteinyl-glycine. *Arch. Pharm. Pharm. Med. Chem.* **1996**, *329*, 498−502.
(7) Adang, A. E. P.; Brussee, J.; van der Gen, A.; Mulder, G. J. Inhibition of Rat Liver Glutathione S-Transferase Isoenzymes by Peptides Stabilized against Degradation by γ-Glutamyl Transpeptidase. *J. Biol. Chem.* **1991**, *266*, 830−836.
(8) Daly, M. J.; Ward, R. A.; Thompson, D. F.; Procter, G. Allylsilanes in Organic Synthesis; Stereoselective Synthesis of *trans*-Alkene Peptide Isosteres. *Tetrahedron Lett.* **1995**, *41*, 7545−7548 and references therein.
(9) Niel, G.; Escale, R.; Vidal, J.-P.; Rechencq, E.; Girard, J.-P.; Rossi, J.-C. Synthesis of a New Pseudopeptidoleukotriene: The First LTD_4 Analogue with Potent Agonist Activity. *Tetrahedron Lett.* **1991**, *32*, 2613−2614.
(10) Niel, G.; Rechencq, E.; Muller, A.; Vidal, J.-P.; Escale, R.; Durand, T.; Girard, J.-P.; Rossi, J.-C.; Bonne, C. Synthesis and contractile activity of new pseudopeptido and thioaromatic analogues of leukotriene D_4. *Prostaglandins* **1992**, *43*, 45−54.
(11) Adang, A. E. P.; Brussee, J.; van der Gen, A.; Mulder, G. J. The glutathione-binding site in glutathione S-transferases. *Biochem. J.* **1990**, *269*, 47−54.
(12) Banères, J.-L.; Klotz, Ph.; Parello, J.; Rossi, J.-C. A rapid and efficient method to evaluate the binding ability of Pseudoglutathione analogues to a Glutathione S-Transferase. *Biochem. Biophys. Res. Commun.* **1998**, in press.
(13) Watson, S.; Girdlestone, D. NC-IUPHAR, 1996 *TiPS* Receptor and Ion Chanel Nomenclature Supplement. *Trends Pharmacol. Sci.* **1998** (Suppl.), 50−51.
(14) Labat, C.; Ortiz, J. L.; Norel, X.; Gorenne, I.; Verley, J.; Abram, T. S.; Cuthbert, N. J.; Tudhope, S. R.; Norman, P.; Gardiner, P.; Brink, C. A Second Cysteinyl Leukotriene Receptor in Human Lung. *J. Pharmacol. Exp. Ther.* **1992**, *263*, 800−805.
(15) Gorenne, I.; Norel, X.; Brink, C. Cyteinyl leukotriene receptors in the human lung: what is new? *Trends Pharmacol. Sci.* **1996**, *17*, 342−345.
(16) Stork, G.; Brizzolara, A.; Laudesman, H.; Szmuszkovicz, J.; Terrell, R. The Enamine Alkylation and Acylation of Carbonyl Compounds. *J. Am. Chem. Soc.* **1963**, *85*, 207−222.
(17) Hünig, S.; Lücke, E.; Brenninger, W. 1-Morpholino-1-cyclohexene. *Org. Synth.* **1961**, *41*, 65−66.
(18) Xu, X.-X.; Dong, H.-Q. Enantioselective Total Syntheses and Stereochemical Studies of All Four Stereoisomers of Yingzhaosu C. *J. Org. Chem.* **1995**, *60*, 3039−3044.
(19) Bordwell, F. G.; Hewett, W. A. Synthesis from Thiolacetates. I. Synthesis of Alkanesulfonyl Chlorides. *J. Org. Chem.* **1957**, *22*, 980−981.
(20) Tamaru, Y.; Ochiai, H.; Nakamura, T.; Yoshida, Z.-I. Ethyl 5-oxo-6-methyl-6-heptenoate from methacryloyl chloride and ethyl 4-iodobutyrate. *Org. Synth.* **1989**, *67*, 98−104.
(21) Paquette, L. A.; Watson, T. J.; Friedrich, D.; Bishop, R.; Bacqué, E. Is Through-Bond Dihydroaromaticity Attainable? Preparation of [4,5]Dihomotropone, Investigation of Its Ground-State Properties, and an Attempt To Generate the Dihomotropylium Cation. *J. Org. Chem.* **1994**, *59*, 5700−5707.
(22) Brown, H. C.; Liotta, R.; Kramer, G. W. Hydroboration. 49. Effect of Structure on the Selective Monohydroboration of Representative Conjugated Dienes by 9-Borabicyclo[3.3.1]nonane. *J. Org. Chem.* **1978**, *43*, 1058−1063.
(23) Volante, R. P. A new, highly efficient methode for the conversion of alcohols to thioesters an thiols. *Tetrahedron Lett.* **1981**, *22*, 3119−3122.
(24) Muehldorf, A. V. Preparation of 11-Substituted Linoleic Acids. *Tetrahedron Lett.* **1994**, *35*, 6851−6852.
(25) Dirr, H.; Reinemer, P.; Huber, R. X-ray crystal structures of cytosolic glutathione S-transferases. Implication for protein architecture, substrate recognition and catalytic function. *Eur. J. Biochem.* **1994**, *220*, 645−661.
(26) Hammarström, S. LEUKOTRIENES. *Annu. Rev. Biochem.* **1983**, *52*, 355−377.
(27) Lewis, R. A.; Lee, C. W.; Levine, L.; Morgan, R. A.; Weiss, J. W.; Drazen, J. M.; Oh, H.; Hoover, D.; Corey, E. J.; Austen, K. F. Biology of the C6-sulfidopeptide leukotriene. *Adv. Prostaglandin Thromboxane Leukotriene Res.* **1983**, *11*, 15−26.
(28) Smith, D. B.; Corcoran, L. M. In *Current Protocols in Molecular Biology*; Ausubel, F. M., Brent, R., Kingstone, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., Struhl, K. Eds.; John Wiley & Sons: New York, 1994.

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