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A Facile One-pot Synthesis of the Very Useful Building Blocks *N*-Boc-*S*-alkylatedcysteines

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Abstract: A convenient one-pot method for the synthesis of *N*-Boc protected *S*-alkylcysteines, useful intermediates for the solution-phase synthesis of glutathione-conjugates and modified peptides is presented, together with the reactivity of epoxides, halohydrins and α-halo carbonyl derivatives towards the sulfur nucleophile of the substrate. The reactions with α-halo esters lead to thiomorpholinocarboxylic acids which are interesting potential inhibitors of transport systems.

Keywords: Cysteine-conjugates, Glutathione-conjugates, Alkylation, S_N2 reactions

The interactions between electrophiles such as epoxides and alkyl halides and biogenic thiols is of paramount importance in many biosynthetic pathways catalyzed by different enzymes.[1] Glutathione (GSH) forms adducts with xenobiotic compounds,[2] usually under catalysis exerted by a pool of isozymes of glutathione transferase (GST).[3] The nucleophilicity of the cysteine sulfur atom is also exhibited in the formation of adducts with the α and β globins of haemoglobin during human exposure to carcinogens.[4] GSH itself induces drug resistance in some cancer treatments[5] by neutralization of electrophilic drugs and many cancer cells show a distribution of GST isozymes, which differs from that observed in normal cells.[6] GST transition state inhibitors could therefore be effective in increasing the bioavailability of chemotherapeutic agents.

Most of the syntheses of cysteine *S*-conjugates have been developed from the corresponding *N*-acetyl derivatives to afford mercapturates, where sulfur nucleophilicity was enhanced by using alkoxides[7] and cuprous[8] or palladium complexes.[9] The drawbacks associated with these procedures are represented by the low yields, or the occurrence of side reactions causing formation of complex mixtures. The efficient Michael addition of thiolates to 2-acetamidoacrylic acid gives racemic cysteines.[10] A suitable protection of the nitrogen site is also important when the *S*-conjugate cysteines have to be used as building blocks in the preparation of both modified GSH and peptides.[4] Indeed some of the functional groups linked to the sulfur atom could not survive the removal of the *N*-acetyl group.

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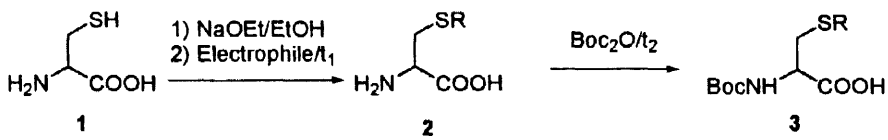
GSH-styrene oxide conjugates might act as transition state inhibitors of GST if the catalytic mechanism for similar systems is considered.[11] Moreover, *S*-benzyl analogues have shown a good specificity as inhibitors of α and π GST isozymes *in vitro*.[12]

An early attempt to form an unprotected cysteine-styrene oxide adduct did not provide full characterization of the products.[13] Recently, a two-step procedure, using the expensive 9-fluorenylmethoxycarbonylsuccinimate ester (Fmoc-OSu) to protect the cysteine amino function, was reported for the synthesis of GST selective inhibitors.[14]

We have developed a one-pot method, which smoothly leads to the synthesis of a pool of *N*-Boc protected *S*-conjugate cysteines, which can be employed in the preparation of biologically active compounds.

Table - Reaction of Cysteine with Electrophiles in Ethanol\Sodium Ethoxide Followed by Protection of the Amino Function with Boc_2O



1 2 3

Entry	Electrophile	Product	R	t_1 (h)	t_2 (h)	Yield (%)
1	Ethylene oxide	**3a**	CH_2CH_2OH	6	3	27
2	$BrCH_2CH_2OH$	**3a**	CH_2CH_2OH	2	4	57
3	Styrene oxide	**3b**	$CH_2CH(OH)Ph$ +$CH(Ph)CH_2OH$[a]	2	4	75
4	$PhCH(Br)CH_2OH$	**3b**	$CH_2CH(OH)Ph$ +$CH(Ph)CH_2OH$[a]	4	3	76
5	$PhCH(OH)CH_2Br$	**3b**	$CH_2CH(OH)Ph$ +$CH(Ph)CH_2OH$[a]	5	4	90
6	$PhCH(Br)CH_2OTHP$	**3c**	$CH(Ph)CH_2OTHP$	24	3	66
7	$PhCH(Br)CH_2OTBDMS$	**3d**	$CH(Ph)CH_2OTBDMS$	9	3	77
8	$PhCH(OTHP)CH_2Br$	**3e**	$CH_2CH(OTHP)Ph$	36	4	traces
9	$PhCH(OTHP)CH_2Br$	**3e**	$CH_2CH(OTHP)Ph$	9[b]	4	59
10	$PhCH(OTBDMS)CH_2Br$	**3f**	$CH_2CH(OTBDMS)Ph$	9	3	80
11	$PhCOCH_2Br$	**3g**	CH_2COPh	4	3	36
12	PhCH(Br)COOMe	**3h**	CH(Ph)COOMe	4	3	0
13	PhCH(Br)COOH	**3i**	CH(Ph)COOH	4	3	55

[a] 2:1 mixture
[b] at reflux

RESULTS AND DISCUSSION

In a typical procedure cysteine was dissolved in a sodium ethoxide solution and the appropriate

electrophile was added. Ethanol was then removed and the crude mixture was dissolved in 1:2 water/dioxane before adding *t*-butylpyrocarbonate (Boc_2O). Finally the organic material was extracted with dichloromethane, dried and purified.

Both epoxides and halohydrins gave the corresponding *N*-Boc-protected cysteine conjugate in good to high yield. Gaseous epoxides are better replaced by the corresponding halohydrins owing to the easier handling of liquids (Table, entries 1, 2). It should be noted that Boc-protection can be performed on the crude mixture after a TLC-monitoring of the reaction, avoiding the tedious separation of the cysteine conjugate. *N*-Boc protected compounds can easily be extracted from water and removal of the organic solvent generally gave a product pure enough for the solution-phase synthesis of GSH-conjugates.

However, all compounds were submitted to purification on silica gel before submitting them to characterization. The pure samples were then analyzed by HPLC on a chiral stationary phase to determine the optical purity. Samples of **3a** and **3g** gave a single peak, while the diastereomeric mixtures of **3b,d,f,j** gave two peaks and the mixture of diastereomers of **3c,e** gave four peaks.

α,β-Epoxy carbonyl compounds are reported to be opened by sulfur nucleophiles at both carbon atoms.[15] It is known that carbanions, under appropriate experimental conditions, preferentially attack styrene oxide at the more substituted carbon terminus.[16] A reaction with styrene oxide was therefore carried out to establish the behavior of cysteine thiolate under the present reaction conditions (Table, entry 3). A mixture of regioisomers was recovered the most abundant product arising from attack at the less hindered carbon atom. The same result was obtained repeating the coupling between styrene oxide and cysteine under experimental conditions which should give a single product only according to a previously published procedure.[13]

To avoid tedious separation of the four isomers of **3b**, we prepared both regioisomeric bromohydrins of styrene oxide. Unfortunately, the reaction medium is too basic to allow the bromohydrins to survive. In fact both halo derivatives gave the same product distribution as styrene oxide (table, entries 4 and 5) demonstrating that they underwent intramolecular epoxide ring-closure before reaction with thiolate.

Other precursors were prepared starting from α–bromoacetophenone and methyl α–bromophenyl-acetate. The former, however, leads to the expected conjugates **3g** in low yields (Table, entry 11), whereas the latter did not give **3h** (Table, entry 12).

Finally, both regioisomers of **3b** can be obtained in a pure form by protecting the hydroxyl function of the bromohydrin as a *t*-butyldimethylsilyl ether or a tetrahydropyranyl derivative (Table, entries 6-10). Selective deprotection of the hydroxyl function of **3c, 3d, 3e** and **3f** was attempted to characterize of the mixture of **3b**. The TBAF-promoted[17] desilylation of **3d** and **3f** afforded the expected products in 70% and 65% yield respectively, while selective removal of the THP group was unsuccessful.

The expected benzylic acceleration[18] afforded a similar yield of **3d** and **3f** thus showing a similar reactivity of the benzylic and primary electrophiles. However, electronic effects alone cannot explain the peculiar behavior of the THP-protected isomers **2c** and **2e**, since the primary substrate did not undergo

nucleophilic displacement after 36 hours at ambient temperature (Table, entry 8); whereas satisfactory yields of the cysteine conjugate **3c** were obtained under the same experimental conditions starting from **2c**. A MM2 minimization[19] of the conformation of the protected bromohydrins shows that the opposite side of the primary bromine is much more hindered than that of the secondary (Figure).

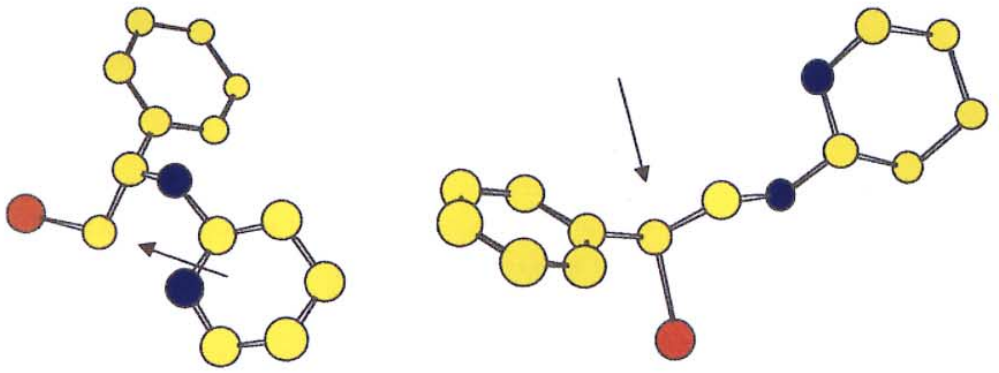
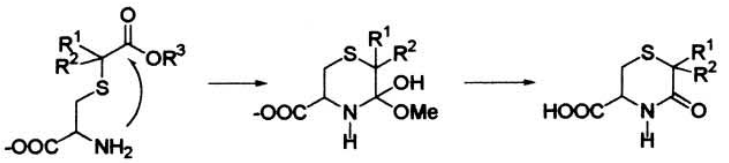


Figure: MM2 Minimized Structures of *O*-THP-2-Bromo-1-phenylethanol (Left) and *O*-THP-2-Bromo-2-phenylethanol (Right). Legend: Carbon (Yellow), Bromine (Red), Oxygen (Blue), Hydrogen (Hidden).

The most remarkable finding in the derivatization of cysteine is represented by the reaction with methyl α-bromophenylacetate (table, entry 12). The reaction product has been isolated and characterized as 4-phenyl-5-oxo-3-thiomorpholincarboxylic acid, thus accounts for the negative ninhydrin test after the first reaction step. It is likely, therefore, that, under the adopted basic reaction conditions, the amino moiety of cysteine attacks the ester group of the initially formed adduct, to give the cyclic compound (Scheme). The reaction is general and a satisfactory yield of cyclic compounds was obtained with other α-bromoesters.



2 **4** **5**

h. R^1=H; R^2=Ph; R^3=Me
i. R^1=R^3=H; R^*=Ph (two equivalents of base)
k. R^1=R^2=H; R^3=Me
l. R^1=R^2=R^3=Me

i. 67% (from **2h**)
i. 46% (from **2i**)
k. 68%
l. 96%

Scheme

This reaction pathway also accounts for the need of a third equivalent of base in the reaction of cysteine with bromophenylacetic acid, to prevent the formation of the cyclic thiomorpholine **4i**. In fact, in the absence

of base, the carboxylic acid-carboxylate equilibrium between the two acidic functions of the adduct **2i** can likely favor the intramolecular nucleophilic displacement. Cyclic compounds similar to **5** are known as moderate inhibitors of either A or L transport systems in the S37 tumor cells.[20]

In conclusion the formation of *S*-conjugates of cysteine can be better accomplished if the alkylated products are isolated directly as *N*-Boc protected species. The chemistry of styrene oxide and of related isomeric bromohydrins is quite interesting and mimics, to some extent, the behavior of similar species *in vivo* in the presence of GSH/GST system. Finally, the choice of proper experimental conditions can control the intramolecular interactions between the amino acidic nitrogen and the *S*-side chain.

EXPERIMENTAL

^{1}H-NMR and ^{13}C-NMR spectra were recorded at 300 MHz and 75 MHz respectively with a Brucker WM300 instrument. Chemical shifts are given in p.p.m. from Me_4Si. Coupling constants are given in Hertz. IR spectra were recorded with a Perkin-Elmer Paragon 1000 PC FTIR spectrometer. FAB-MS were recorded with a VG-ZAB spectrometer. Melting points are uncorrected and were determined with a Kofler hot desktop.

THF was dried by refluxing over sodium wires until the blue color of benzophenone ketyl persisted and then distilling into a dry receiver under nitrogen atmosphere immediately before use. Ethanol was dried by refluxing over magnesium turnings and then distilling into a dry receiver under nitrogen atmosphere immediately before use.

α-Bromophenylacetic acid, α-bromoacetophenone, methyl bromoacetate, methyl α-bromophenylacetate, ethyl α-bromoisobutyrate, ethylene oxide, 2-bromoethanol, styrene oxide are commercial products (Aldrich). 2-Bromo-2-phenylethanol[21] and 2-bromo-1-phenylethanol[22] were synthesized as reported in literature.

THP-protection: The appropriate alcohol (15 mmol) and 3,4-dihydropyrane (23 mmol) were dissolved in dry THF (10 mL) and catalytic amounts (0.2 mmol) of *p*-toluensulfonic acid added. The reaction mixture was stirred overnight at 0 °C, then poured into water, washed with $NaHCO_3$ (5%), brine and water and dried over Na_2SO_4. The O-THP protected alcohols were recovered almost quantitatively after solvent evaporation.

O-THP-2-bromo-1-phenylethanol. [Found C, 54.66; H, 6.00; Br 28.08. $C_{13}H_{17}BrO_2$ (285.18) requires C, 54.75; H, 6.01; Br 28.02%]; ν_{max} (liquid film) 1203 (CH_2Br), 1118 (CO ketal) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.30-1.90 (m, 6 H; $(CH_2)_3$), 3.20-3.60 (m, 4 H; CH_2O, CH_2Br); 4.51 (t, 1 H; J = 2.7, OCHO); 4.84 (dd, 1 H; J = 5.4, 8.1, CHPh,); 4.93 (dd, 1 H; J = 4.7, 8.8, CHPh); 5.01 (t, 1 H; J = 3, OCHO) 7.20-7.60 (m, 5 H; ArH). *m/z* (70eV, EI): 185-183 (M$^+$- THPO); 104 (base), 85.

O-THP-2-Bromo-2-phenylethanol. Mixture of diastereomers as a pale yellow oil. [Found C, 54.81; H, 6.00; Br, 28.00. $C_{13}H_{17}BrO_2$ (285.18) requires C 54.75, H 6.01, Br 28.02%]; ν_{max} (liquid film) 1201 (CBr), 1125 (CO ketal) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.30-1.80 (m, 6 H; $(CH_2)_3$), 3.40-3.55 (m, 2 H; $CH_2\underline{CH_2}O$), 3.66 (t, 1 H; J = 8.7, CHBr), 3.67 (t, 1 H; J = 8.7, CHBr), 3.80-4.00 (m, 2 H; $CH\underline{CH_2}O$), 4.05-4.25 (m, 2 H; $CH\underline{CH_2}O$), 4.55-4.80 (m, 1 H, OCHO), 4.95-5.15 (m, 1 H, OCHO), 7.20-7.60 (m, 5 H; ArH). *m/z* (70eV, EI): 185-183 (M$^+$- THPO), 104, 85(base).

TBDMS-protection: The appropriate alcohol (15 mmol), *t*-butyldimethylchlorosilane (16.5 mmol) and imidazole (37.5 mmol) were dissolved in dry THF (10 mL). The reaction mixture was stirred 2 h at room temperature, then acidified with citric acid to pH=5 and extracted with diethyl ether. The organic layer was washed with water, brine and dried over Na_2SO_4. The O-TBDMS protected alcohols were recovered after solvent evaporation.

O-TBDMS-2-Bromo-1-phenylethanol. 94% of a pale yellow oil. [Found C, 53.40; H, 7.37; Br, 25.30, Si, 8.88. $C_{14}H_{23}BrOSi$ (315.33) requires C, 53.33; H, 7.35; Br, 25.34; Si, 8.91%]; ν_{max} (liquid film) 1118 (COSi) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 0.20 (s, 6 H; Me_2Si), 0.99 (s, 9 H; t-Bu), 3.50-3.62 (ABX, 2 H; CH_2Br),

4.94 (dd, 1 H; J = 4.9, 7.3, CHO), 7.40-7.60 (m, 5 H, ArH). *m/z* (70eV, EI): 259-257 (M^+ - t-Bu); 177 (base), 139, 137, 104, 75.

O-TBDMS-2-Bromo-2-phenylethanol. 97% of a pale yellow oil. [Found C, 53.30; H, 7.35; Br, 25.32; Si, 8.90. $C_{14}H_{23}BrOSi$ (315.33) requires C, 53.33; H, 7.35; Br, 25.34; Si, 8.91%]; ν_{max} (liquid film) 1121 (COSi) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 0.05 (s, 6 H; Me_2Si), 0.87 (s, 9 H; t-Bu), 4.00-4.40 (ABX, 2 H; CH_2O), 4.97 (t, 1 H; J = 7; CHBr), 7.30-7.50 (m, 5 H; ArH). *m/z* (70eV, EI): 259-257 (M^+ - t-Bu); 177 (base), 139, 137, 104, 73.

Synthesis of S-Alkylated N-Boc-cysteines. General Procedure. In a two-necked round-bottomed flask L-cysteine (10 mmol) was dissolved in dry ethanol (30 mL) under nitrogen at room temperature. Sodium chips (21 mmol) were added during 15 min. When the solution became clear the bromo derivative (10 mmol) was added dropwise. The mixture was stirred for the appropriate time (t_1, Table) and then carefully acidified with HCl (4%) until pH=3-4. The crude mixture was evaporated under reduced pressure and dissolved in dioxane/water (2:1, 30 mL). NaOH 1 N (20 mL, with α-bromophenylacetic acid 30 mL) was added at 0 °C, and stirring was continued for 30 min. Boc_2O (11 mmol) was added dropwise at room temperature. The mixture was stirred for the appropriate time (t_2, Table) and then partially evaporated, extracted twice with diethyl ether, carefully acidified with citric acid (5%) until pH=4 and extracted again three times with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The products are pure enough to be used in solution-phase peptide synthesis, however they were flash-chromatographed on a short silica gel column (chloroform:methanol 9:1 as eluant) before characterization. Yields are collected in Table. Physical data follow and refer to mixture of diastereomers when possible. Melting point and $[\alpha]_D^{20}$ of mixtures (all are not 0) are not given, since they have no significance.

N-Boc-*S*-(2-hydroxyethyl)-L-cysteine (**3a**). Pale yellow oil. [Found C, 45.30; H, 7.20; N, 5.24; S, 12.10. $C_{10}H_{19}NO_5S$ (265.32) requires C, 45.27; H, 7.22; N, 5.28; S 12.08%]; $[\alpha]_D^{20}$ -64.50 (*c* 3.3, MeOH); ν_{max} (liquid film) 3388 (OH), 1736 and 1710 (CO) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.45 (s, 9 H, t-Bu), 2.78 (t, 2 H; J = 6, $S\underline{CH_2}CH_2O$); 2.90-3.10 (m, 2 H; C*- CH_2S); 3.78 (t, 2 H; J = 5.8, $SCH_2\underline{CH_2}O$); 4.56 (dd, 1 H; J = 5.4, 13, CH); 5.69 (d, 1 H; J = 6.8, NH); 7.28 (brs, 2 H; OH). *m/z* (FAB ν^+, glycerol) 266 (MH^+) 210, 166.

N-Boc-*S*-(2-phenyl-2-hydroxyethyl)-L-cysteine (**3b'**). Mixture of two diastereomers as a pale yellow oil. [Found C, 56.22; H, 6.81; N, 4.10; S, 9.35. $C_{16}H_{23}NO_5S$ (341.42) requires C, 56.29; H, 6.79; N, 4.10; S 9.39%]; ν_{max} (liquid film) 3346 (NH); 1738 and 1681 (CO) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.44 (s, 9 H, t-Bu); 2.90-3.10 (m, 4 H, CH_2SCH_2); 4.5-4.65 (m, 1 H, C*); 4.75-4.85 (m, 1 H, CHOH); 5.58 (d, 1 H, J =7.9, NH 1° diast.) 5.72 (d, 1 H J = 8.5, NH 2° diast.) 6.4 (brs, 2 H, COOH + OH) 7.20-7.50 (m, 5 H; ArH). *m/z* (FAB ν^+, glycerol) 342 (MH^+) 268, 264.

N-Boc-*S*-(1-phenyl-2-hydroxyethyl)-L-cysteine (**3b''**). Mixture of two diastereomers as a pale yellow oil. [Found C, 56.33; H, 6.77; N, 4.12; S, 9.41. $C_{16}H_{23}NO_5S$ (341.42) requires C, 56.29; H, 6.79; N, 4.10; S 9.39%]; ν_{max} (liquid film) 3346 (NH); 1738 and 1681 (CO) cm^{-1}. δ_H (300 MHz, $CDCl_3$) 1.44 (s, 9 H, t-Bu); 2.70-3.00 (m, 2 H, CH_2S); 3.75-3.90 (m, 2 H, CH_2OH); 4.06 (t, 1 H, J =5.2, PhCHS); 4.4-4.5 (m, 1 H, C*); 5.42 (d, 1 H, J =8.5, NH 1° diast.); 5.51 (d, 1 H, J =7.9, NH 2° diast.), 6.45 (brs, 2 H, COOH + OH) 7.20-7.50 (m, 5 H; ArH). *m/z* (FAB ν^+, glycerol) 342 (MH^+) 268, 264.

N-Boc-*S*-(2-*O*-THP-1-phenylethyl)-L-cysteine (**3c**). Mixture of four diastereomers as a white powder. [Found C, 59.24; H, 7.36; N, 3.33; S, 7.49. $C_{21}H_{31}NO_6S$ (425.54) requires C, 59.27; H, 7.34; N, 3.29; S, 7.53%]; ν_{max} (KBr) 2938 (NH) 1720-1700 (CO) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.30-1.90 (m, 15 H; t-Bu + $(CH_2)_3$); 2.70-3.00 (m, 2 H; C*CH_2S); 3.40-4.70 (m, 7 H); 5.70 (brs, 2 H; NH + COOH); 7.20-7.50 (m, 5 H; ArH). *m z* (FAB ν^+, glycerol) 426 (MH^+).

N-Boc-*S*-(2-*O*-TBDMS-1-phenylethyl)-L-cysteine (**3d**). Mixture of two diastereomers as a white powder.[Found C, 58.04; H, 8.15; N, 3.03; S, 7.05 Si, 6.14. $C_{22}H_{37}NO_5SSi$ (455.68) requires C, 57.99; H, 8.18; N, 3.07; S, 7.04; Si, 6.16%]; ν_{max} (KBr) 3400 (NH + OH), 1716 (CO), 1254 (CSi) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 0.08 (s, 6 H; Me_2Si), 0.90 (s, 9 H; t-BuSi), 1.51 and 1.53 (s, 9 H; t-BuO), 2.90-3.10 (m, 2 H; SCH_2), 3.90-4.10 (m, 3 H; CH_2O and CHS), 4.40-4.60 (m, 1 H; CH), 5.48 (brs, 1 H; NH), 7.20-7.40 (m, 5 H; ArH), 8.40 (brs, 1 H; OH). *m z* (FAB ν^+, glycerol) 456 (MH^+).

N-Boc-*S*-(2-*O*-THP-2-phenylethyl)-L-cysteine (**3e**). Mixture of four diastereomers as a pale yellow oil. [Found C, 59.35; H, 7.35; N, 3.26; S, 7.51. $C_{21}H_{31}NO_6S$ (425.54) requires C, 59.27; H, 7.34; N, 3.29; S,

7.53%]; ν_{max} (liquid film) 3323 (NH) 3400-2800 (COOH) 1680-1740 (CO) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.45 (s, 9 H; t-Bu), 1.40-1.70 (m, 6 H; $(CH_2)_3$), 2.70-3.60 (m, 6 H; C*CH_2S, CH_2S, CH_2O), 4.00-5.10 (m, 3 H; CH, OCHO, CHPh), 5.40-5.70 (m, 1 H, NH); 7.20-7.40 (m, 5 H; ArH), 7.68 (brs, 1 H, COOH). *m/z* (FAB ν^+, glycerol) 426 (MH^+), 342, 268, 224, 135.

N-Boc-*S*-(2-*O*-TBDMS-2-phenylethyl)-L-cysteine (**3f**). Mixture of two diastereomers as a white powder. [Found C, 57.89; H, 8.20; N, 3.07; S, 7.01 Si, 6.20. $C_{22}H_{37}NO_5SSi$ (455.68) requires C, 57.99; H, 8.18; N, 3.07; S, 7.04; Si, 6.16%]; ν_{max} (KBr) 3440 and 3330 (NH + OH), 1716 and 1708 (CO), 1252 (CSi) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 0.18 (s, 6 H; Me_2Si), 0.99 (s, 9 H; t-BuSi), 1.45 (s, 9 H; t-BuO), 2.80-3.10 (m, 4 H; C*CH_2S, CH_2S); 4.40-4.60 (m, 1 H; C*H), 4.88 (t, 1 H; J = 4.31, CHPh), 5.53 (s, 1 H; NH or COOH), 5.60 (s, 2 H; NH or COOH); 7.40-7.60 (m, 5 H; ArH). *m/z* (FAB ν^+, glycerol) 456 (MH^+).

N-Boc-*S*-(2-oxo-2-phenylethyl)-L-cysteine (**3g**). Pale yellow oil. [Found C, 56.58; H, 6.20; N, 4.16; S, 9.48. $C_{16}H_{21}NO_5S$ (339.41) requires C 56.62, H 6.24, N 4.13, S 9.45%]; $[\alpha]_D^{20}$ -5.00 (*c* 1.0, MeOH); ν_{max} (liquid film) 3345 (NH + OH); 1670-1720 (CO) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.33 (s, 9 H; t-Bu); 2.80-3.10 (m, 2 H; C*CH_2S), 3.94 (s, 2 H; SCH_2CO), 4.30-4.50 (m, 1 H; C*H), 6.00 (brs, 2 H; NH + COOH), 7.30-7.50 (m, 3 H; ArH), 7.80-7.90 (m, 2 H; ArH). *m/z* (FAB ν^+, glycerol) 340 (MH^+).

2-(*N*-Boc-L-cystein-*S*-yl)-2-phenylacetic acid (**3i**). Mixture of two diastereomers as a pale yellow oil. [Found C, 54.00; H, 6.00; N, 3.91; S, 9.02. $C_{16}H_{21}NO_6S$ (355.41) requires C 54.07, H 5.96, N 3.94, S 9.02%]; ν_{max} (liquid film) 3362 (NH) 3200-2800 (broad COOH), 1704 (CO) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.42 (s, 9 H; t-Bu); 2.80-3.20 (m, 2 H; CH_2S); 4.50-4.70 (m, 1 H; CHNH), 4.72 (s, 1 H; CHPh); 5.53 (brs, 1 H; NH); 7.2-7.5 (m, 5 H; ArH); 9.80 (brs, 2 H; COOH). *m/z* (FAB ν^-, glycerol) 354 ($M\text{-}H^+$), 235.

Synthesis of thiomorpholincarboxylic acids. General Procedure. In a two-necked round-bottomed flask L-cysteine (10 mmol) was dissolved in dry ethanol (30 mL) under nitrogen at room temperature. Sodium chips (21 mmol) were added during 15 min. When the solution became clear the bromo ester derivative (10 mmol) was added dropwise. The mixture was stirred for the appropriate time (t_1, Table) and then carefully acidified with HCl (4%) until pH=3-4. The crude mixture was partially evaporated, extracted three times with ethyl acetate. The organic layers were collected washed with brine and water, dried over Na_2SO_4 and evaporated under reduced pressure. The following products were isolated. Melting point and $[\alpha]_D^{20}$ of mixtures (all are not 0) are not given, since they have no significance.

5-phenyl-5-oxo-3-thiomorpholincarboxylic acid (**5i**): 68% of a 1/1 diastereomeric mixture as a pale yellow foam. [Found C, 55.62; H, 4.69; N, 5.88; S, 13.48. $C_{11}H_{11}NO_3S$ (237.27) requires C 55.68, H 4.67, N 5.90, S 13.51 %]; ν_{max} (KBr) 3250 (NH), 3300-2800 (broad COOH), 1724 (CONH), 1634 (COOH) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 2.95-3.10 (m, 1 H; H-2); 3.23-3.36 (m, 1 H; H-2); 4.21 (dd, 1 H; J = 9.0, 2.0, H-1); 4.50 (dd, 1 H; J = 10.0, 2.0, H-1), 4.61 (s, 1 H; H-5), 4.70 (s, 1 H; H-5), 7.20-7.60 (m, 6 H; ArH + NH); 9.30 (brs, 1 H; COOH), 9.40 (brs, 1 H; COOH). *m/z* (FAB ν^+, glycerol) 238 (MH^+).

5-oxo-3-thiomorpholincarboxylic acid (**5k**). 47% as a pale yellow syrup. [Found C, 37.30; H, 4.41; N, 8.68; S, 19.84. $C_5H_7NO_3S$ (161.18) requires C 37.26, H 4.38, N 8.69, S 19.89%]; $[\alpha]_D^{20}$ -39.70 (*c* 3.4, MeOH); ν_{max} (liquid film) 3500-2800 (broad, COOH), 1732 (CONH), 1632 (COOH) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 2.94 (dd, 1 H; J = 5.0, 13.3, H-2), 2.50-3.50 (AB, 2 H, H-4), 3.33 (d, 1 H; H-2), 4.28 (d, 1 H; H-1), 7.84 (brs, 2 H; NH + COOH). *m/z* (FAB ν^+, glycerol) 162 (MH^+).

4,4-dimethyl-5-oxo-3-thiomorpholincarboxylic acid (**5l**). 96% of a white solid, m.p. 199-201 °C. [Found C, 44.48; H, 5.84; N, 7.40; S, 16.90. $C_7H_{11}NO_3S$ (189.23) requires C 44.43, H 5.86, N 7.40, S 16.94%]; $[\alpha]_D^{20}$ -41.19 (*c* 3.6, MeOH); ν_{max} (KBr) 3282 (N-H), 3300-2800 (broad COOH), 1731 (CONH), 1633 (COOH) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.36 (s, 3 H; Me), 1.48 (s, 3 H; Me), 2.95 (dd, 1 H; J = 5.0, 14.0, H-2); 3.23 (dd, 1 H; J = 3.5, 14.0, H-2), 4.25 (dd, 1 H; H-1), 7.60 (brs, 2,H, NH + COOH). *m/z* (FAB ν^+, glycerol) 190 (MH^+).

HPLC experiments Chiral purity of all compounds was performed by running out HPLC chromatograms on a HP series 1100 liquid chromatograph equipped with a CHIREX® (D)-penicillamine column isocratically eluted with H_2O ($CuSO_4$ 2 mM)/MeOH 85:15.

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