Synthesis and Biological Evaluation in Human Monocyte-Derived Macrophages of *N*-(*N*-Acetyl-L-cysteinyl)-*S*-acetylcysteamine Analogues with Potent Antioxidant and Anti-HIV Activities

Joël Oiry,*,† Patricia Mialocq,‡ Jean-Yves Puy,† Philippe Fretier,‡ Nathalie Dereuddre-Bosquet,§ Dominique Dormont,‡ Jean-Louis Imbach,† and Pascal Clayette*,§

Laboratoire de Chimie Organique Biomolécularie de Synthèse, UMR CNRS-UM II 5625, Université Montpellier II Sciences et Techniques du Languedoc, place Eugène Bataillon, 34095 Montpellier, France, Service de Neurovirologie, DRM/DSV, CEA/CRSSA/EPHE/University Paris XI, 18 route du Panorama, B.P. 6, 92265 Fontenay aux Roses Cedex, France, and SPI-BIO c/o Service de Neurovirologie, Fontenay aux Roses Cedex, France

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We synthesized a series of *N*-(*N*-acetyl-L-cysteinyl)-*S*-acetylcysteamine (**10**) analogues bearing various acyl groups on thiol cysteine or cysteamine residues, to investigate the structure−activity relationship for pro-GSH and anti-HIV properties in human macrophages. The S-substituents were ranked in the following order of efficacy: H ≥ acetyl > isobutyryl > pivaloyl > benzoyl. We found that none of these derivatives had pro-GSH or antiviral activities in vitro higher than that of **10**, but several displayed similar levels of anti-HIV activity, making them possible candidates for use as adjuvant therapies in conjunction with HAART, for treating neurological aspects of HIV infection.

Introduction

Oxidative stress plays a major role in various pathological disorders, including viral diseases, cardiovascular diseases, inflammation, cancer, neurological diseases, and septic shock.[1−9] Glutathione (GSH or L-γ-glutamyl-L-cysteinylglycine) is a tripeptide widely distributed in mammalian cells and tissues. Its reduced form is involved in various cell functions, including the biosynthesis of proteins and DNA precursors, amino acid transport, and the maintenance of sulfhydryl redox status. Indeed, GSH is a scavenger of NO and peroxinitrite species and acts as the substrate of GSH-peroxidases. These enzymes inactivate peroxides, which are in turn the substrate of GSH-dehydrogenases, which regenerate antioxidant molecules, such as ascorbate, and of GSH-S-transferases, which detoxify endogenous and exogenous compounds. GSH therefore plays an important role in the intracellular antioxidant defense system.[10]

HIV infection is associated with decreases in systemic and tissue GSH contents.[11,12] The molecular mechanisms responsible for these decreases are unclear. However, both oxidative stress and viral replication seem to be involved. The deficit in GSH aggravates oxidative stress and contributes to the pathophysiology of HIV infection by increasing viral replication,[13] CD4 T-lymphocyte apoptosis,[14,15] inflammatory syndrome,[16] chronic weight loss, drug toxicities, and neuroAIDS.[17] Indeed, GSH deficit has been associated with lower survival rates in HIV-infected patients and the administration of pro-GSH drugs, such as *N*-acetyl-L-cysteine (NAC), has been shown to decrease mortality.[18] In vitro, GSH and NAC inhibit viral replication in human monocyte-derived macrophages (MDM) and lymphocytes.[19−21] Since high concentrations of GSH and NAC are required to obtain biological effects, their efficacy is limited due to their low bioavailability.[22]

We previously reported that **10** (Scheme 1), a NAC-MEA (2-mercaptoethylamine, cysteamine) conjugate, strongly increases GSH levels in various cell lines.[23] Preliminary studies of the decomposition of **10** in cell extracts have shown that this compound is deacetylated, presumably upon esterase activation, to the corresponding dithiol derivative, which may be a key metabolite responsible for the in situ release of NAC and MEA.[23]

We therefore decided to synthesize this dithiol derivative and other **10** analogues bearing various *S*-acyl groups and to carry out a structure−activity relationship (SAR) analysis of this new class of antioxidants. We report here the synthesis of this class of compounds and their in vitro biological effects in MDM and show that several of these compounds display potent pro-GSH and anti-HIV effects.

Chemistry

The new compounds were synthesized from commercially available *N*-acetyl-*S*-trityl-L-cysteine, compound **1**. Scheme 1 outlines the synthesis of pseudodipeptide intermediates **6**−**9**, which were used to obtain the target compounds **10**−**13** and their corresponding *S*-acyl derivatives (**14**−**29**). We recently described some of the biological activities of **10**,[23] but only a summary of experiments carried out was given. The pseudodipeptides **6**−**9** were obtained by a two-step "one-pot" procedure, using **1**, isobutyl chloroformate, and 4-methylmorpholine (NMM) in EtOAc to form a mixed anhydride. This anhydride was added to *N*-hydroxysuccin-

* To whom correspondence should be addressed. J.O.: Tel: +33 467 14 38 37. Fax: +33 467 04 20 29. E-mail: oiryj@univ-montp2.fr. P.C.: Tel: +33 146 54 87 69. Fax: +33 146 54 77 26. E-mail: clayette@dsvidf.cea.fr.
† Université Montpellier II.
‡ CEA/CRSSA/EPHE.
§ SPI-BIO.

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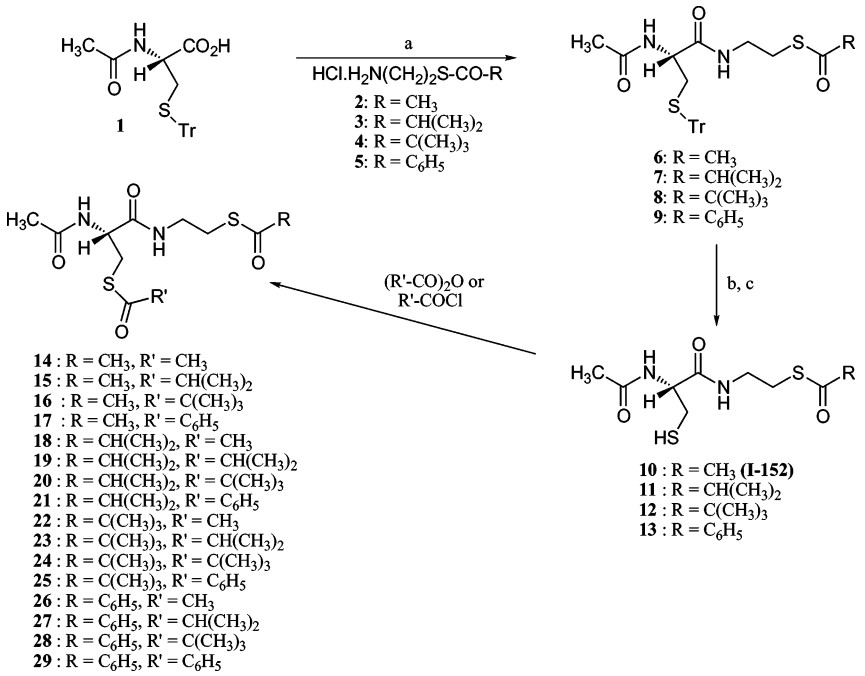
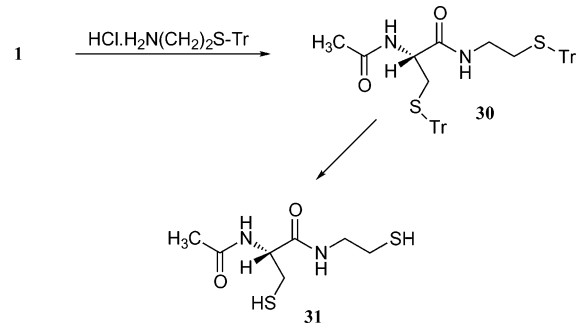
Scheme 1[a]



[a] Reagents and conditions: (a) (1) AcOEt, IBC, NMM, −15 °C, (2) HOSu, −15 °C, (3) **2**, **3**, **4**, or **5**, NMM, −15 °C-rt, 12 h; (b) MeOH, $CHCl_3$, mixture of MeOH/$AgNO_3$/pyridine, rt; (c) $CHCl_3$, HCl, 37%, rt.

Scheme 2[a]



[a] Reagents and conditions are identical to those for Scheme 1.

imide to generate the corresponding active *O*-succinimide ester, which was finally condensed with appropriate *S*-acylcysteamine hydrochlorides (**2**−**5**) in the presence of NMM, to give the expected compounds. We used this mixed method to optimize the yield of coupling reactions. *S*-Detritylations of **6**−**9** were performed with silver nitrate−pyridine in MeOH,[24] to generate the corresponding silver sulfides, which were treated with concentrated HCl in $CHCl_3$ to give **10**−**13**. Finally, these free thiols were S-acylated with an anhydride or acid chloride in pyridine to give the corresponding S,S′-diacylated derivatives, **14**−**29**.

Dithiol **31** (Scheme 2), which has been identified as a metabolite of **10**,[23] was also synthesized using commercially available **1** as the starting material. In a similar sequence to that used to obtain **10**−**13**, **1** was coupled with an *S*-alkylcysteamine (*S*-tritylcysteamine hydrochloride) to give **30**. Removal of the *S*-trityl groups, via the corresponding silver sulfides, gave the expected compound **31**.

The Experimental Section reports general synthetic procedures and characterization of all the new compounds based on the usual analytical data.

Table 1. ED_{50}, ED_{70}, and ED_{90} for the Anti-HIV-1/Ba-L Activity of **10**, **11**, **12**, **18**, **21**, and **31** in Human MDM

	10, μM	**11**, μM	**12**, μM	**18**, μM	**21**, μM	**31**, μM
ED_{50}	45 ± 10	110 ± 5	145 ± 15	45 ± 5	165 ± 25	40 ± 10
ED_{70}	80 ± 35	175 ± 15	185 ± 25	55 ± 15	205 ± 15	75 ± 10
ED_{90}	195 ± 135	380 ± 25	270 ± 30	80 ± 30	285 ± 15	200 ± 15

Results and Discussion

In human MDM, **10** has higher pro-GSH activity and anti-HIV activity than NAC and MEA.[23] In this study, the anti-HIV effects of **10** were confirmed with effective doses (ED_{50}, ED_{70}, and ED_{90}) of 45 ± 10 μM, 80 ± 35 μM, and 195 ± 135 μM, respectively (Table 1). With the aim of identifying analogues with more potent pro-GSH and antiviral activities than **10**, we synthesized a series of new molecules by (1) changing the nature of the *S*-acyl group on the MEA residue (**11**−**13**) and (2) *S*-acylation of the free thiol groups of **10**−**13** (**14**−**29**, Scheme 1). The resulting compounds, which were theoretically more lipophilic than **10**, might be expected to have higher pro-GSH and anti-HIV activities. We also assessed the pro-GSH effects of **31** the major intracellular metabolite of **10**.

We assessed potential GSH activity by determining GSH levels after treatment for 24 h with **10**, its thioester analogues **11**−**13**, with **14**, **15**, **16**, **18**, **20**−**24**, **26**−**28**; or with **31** the intracellular dithiol metabolite of **10** (Figure 1). The efficacy of **10** derivatives decreased with increasing size of R′ alkyl groups (steric hindrance). Indeed, compounds **10**−**13**, which had a free thiol group on a cysteine residue, displayed greater pro-GSH properties than the corresponding *S*-acetyl derivatives **14**, **18**, **22**, and **26**. Similarly, these *S*-acetyl derivatives were more efficient than the *S*-pivaloyl analogues **16**, **20**, **24**, and **28**. In contrast, the presence of an R radical on the MEA moiety had no significant effect on the pro-GSH activity of these compounds. Nevertheless, the

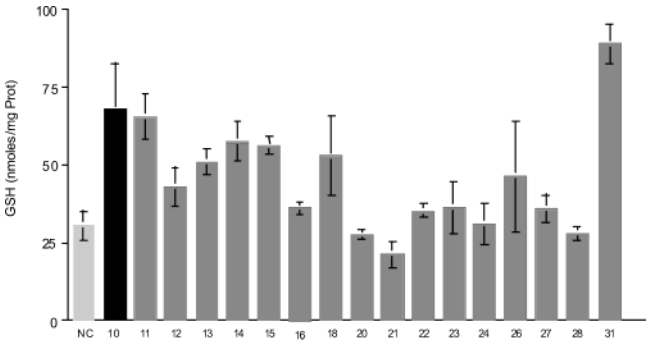


Figure 1. Pro-GSH activity of **10** and its analogues and derivatives at a concentration of 150 μM in uninfected MDM. Results are expressed as means ± SD. NC: Negative control corresponding to untreated and uninfected MDM.

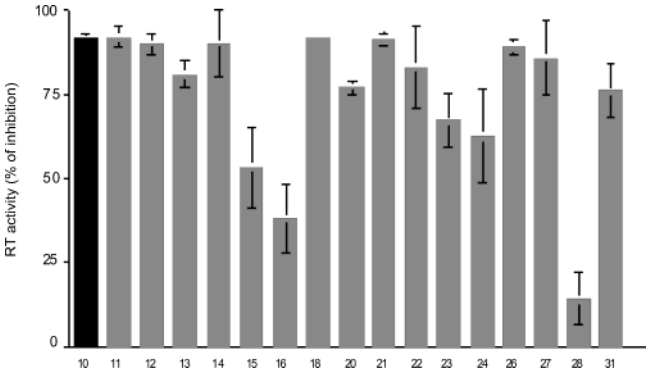


Figure 2. Anti-HIV effects of **10** and its analogues and derivatives at a concentration of 150 μM in HIV-1/Ba-L-infected MDM. Cumulative RT activities were used to calculate percentages with respect to untreated control, and the results are expressed as means ± SD.

dithiol **31** (Scheme 2) displayed stronger pro-GSH effects than **10** (Figure 1).

With the exception of **15**, **16**, and **28**, all the derivatives presented anti-HIV activities equivalent to that of **10** (Figure 2), with no cytotoxic events. The introduction of various R groups did not seem to affect the antiviral effects of the thioesters **10**−**13**. Similarly, the HIV-1/Ba-L activity of the dithiol **31** in human MDM was comparable to that of **10**. However, *S*-isobutyryl (**15**, **23**, **27**) and *S*-pivaloyl (**16**, **20**, **24**, **28**) groups on the cysteine residue seemed to decrease antiviral activity with respect to the corresponding free thiol precursors (**10**, **11**, **12**, **13**) and their *S*-acetyl derivatives (**14**, **18**, **22**, **26)**. We therefore carried out an experiment to determine more precisely the dose-dependent effects of the most effective compounds—**11**, **12, 18**, **21**, and **31**—comparing these effects with those of **10** (Table 1). We found that none of these derivatives was more efficient than **10** itself.

The most efficient derivatives tested appeared to be those with R radicals that were not too bulky. The size of the R radical seemed to be important for both pro-GSH and anti-HIV activities. Steric interactions linked to the size of the R radical may decrease the efficiency of thiol release by the enzyme, therefore reducing the ability of derivatives to increase GSH levels and to inhibit HIV replication. As expected, the dithiol **31** increased GSH content more efficiently than **10**. However, its anti-HIV activity was no higher than that of **10**. Indeed, no strict correlation between pro-GSH and anti-HIV effects was identified. We therefore decided to synthesize *N*-isobutyryl analogues and to assess their anti-HIV activity; these compounds should theoretically have no pro-GSH activity.[25] We also investigated the mode of action of **10**. Preliminary results showed that **10** interfered with both early and late steps in the HIV biological cycle.

In conclusion, although none of the new derivatives tested displayed pro-GSH and antiviral activities greater than those of **10**, several displayed similar anti-HIV activities. The S-substituents were ranked in order of efficacy as follows: H ≥ acetyl > isobutyryl > pivaloyl > benzoyl. As these compounds would be expected to be more lipophilic than **10**, they may be able to cross barriers such as the blood−brain barrier more easily than **10**. Such compounds may therefore be of value as adjuvant therapies to HAART (highly active antiretroviral therapy), targeting neurological aspects of HIV infection by interfering with inflammatory and oxidative processes.

Experimental Section

Chemical Methods. Melting points were determined on a Büchi capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Brüker AC 250 or DRX 400 in $CDCl_3$. Chemical shifts are reported in ppm and given in δ units with respect to TMS, used as an internal standard. FAB mass spectra were recorded on a JEOL DX 300 mass spectrophotometer in the positive ion mode, using 1:1 glycerol/thioglycerol, unless otherwise stated. Specific optical rotations were recorded on a Perkin-Elmer 241 polarimeter. Elemental analysis was carried out by the Service Central de Microanalyze du CNRS de Vernaison (France), and the results were within ±0.4% of calculated values. Analytical thin-layer chomatography (TLC) was carried out on Merck silica gel 60 F_{254} plates. Spots were visualized by exposure to ultraviolet light (254 nm), iodine vapor, or by spraying with ninhydrin solution. Flash column chomatography was conducted with Merck silica gel 60 (230−400 mesh ASTM). All the commercial reagents and solvents were of analytical grade and were purchased from Aldrich or Fluka. *N*-Acetyl-*S*-trityl-L-cysteine was obtained from Bachem. *S*-Acetylcysteamine hydrochloride, *S*-benzoylcysteamine hydrochloride, and *S*-tritylcysteamine hydrochloride were prepared as previously described.[26,27]

General Coupling Procedure for 1 and *S*-Acylcysteamine. *N*-(*N*-Acetyl-*S*-trityl-L-cysteinyl)-*S*-acetylcysteamine (6). A stirred solution of *N*-acetyl-*S*-trityl-L-cysteine (**1**, 1.18 g, 2.91 mmol) in EtOAc (15 mL) was cooled to −15 °C and successively treated with isobutyl chloroformate (IBC, 402 μL, 3.09 mmol), 4-methylmorpholine (NMM, 352 μL, 3.25 mmol), and then (15 min) *N*-hydroxysuccinimide (HOSu, 350 mg, 3.04 mmol). After stirring for 30 min at −15 °C, *S*-acetylcysteamine hydrochloride (**2**,[26] 495 mg, 3.19 mmol) was added to the mixture, followed by NMM (351 μL, 3.19 mmol), which was added one drop at a time. The reaction mixture was then stirred for 1 h at 0 °C and at room temperature for 12 h. The insoluble salt was collected by filtration and washed with EtOAc (3 × 5 mL). The organic phases were pooled, washed [water (2 × 20 mL), ice-cold saturated aqueous $NaHCO_3$ (15 mL), water (2 × 20 mL), ice-cold aqueous 10% HCl (15 mL), and water (neutral pH)], dried over Na_2SO_4, collected by filtration, and evaporated to dryness under vacuum to give **6** as a white foam (1.15 g, 78%). TLC and NMR analysis indicated that the product was sufficiently pure and could be used without further purification.

An analytical sample was obtained by recrystallization from EtOAc and petroleum ether mixture to give white crystals: mp 111−113 °C. TLC (EtOAc/petroleum ether, 9:1) R_f = 0.41. $[\alpha]^{20}_D$ +10.5° (*c* 0.8, $CHCl_3$). ^{1}H NMR: 1.90 (s, 3H, NCOCH_3),

2.29 (s, 3H, SCOC*H*$_3$), 2.48 (dd, J = 5.7, 12.9 Hz, 1H, β *H*a cys), 2.82 (dd, J = 6.4, 12.9 Hz, 1H, β *H*b cys), 2.92−3.01 (m, 2H, NCH$_2$C*H*$_2$S), 3.32−3.42 (m, 2H, NC*H*$_2$CH$_2$S), 4.07−4.20 (m, 1H, α *H* cys), 5.70 (d, J = 7.6 Hz, 1H, N*H* cys), 6.34 (t, J = 5.5 Hz, 1H, N*H*CH$_2$), 7.19−7.35, 7.40−7.47 (2m, 15H, Ar*H*). MS m/z 507 (M + H)$^+$. Anal. (C$_{28}$H$_{30}$N$_2$O$_3$S$_2$) C, H, N.

General Procedure for the S-Detritylation of the Cysteine Residue. *N*-(*N*-Acetyl-L-cysteinyl)-*S*-acetylcysteamine (10). Compound **6** (1.15 g, 2.27 mmol) was dissolved in MeOH (18 mL) and CHCl$_3$ (1.2 mL). We added a mixture of AgNO$_3$ (465 mg, 2.73 mmol), pyridine (210 μL, 2.73 mmol), and MeOH (15 mL) at room temperature, in the dark. The reaction mixture was then stirred for 12 h. The resulting silver sulfide precipitate was collected by filtration, washed with MeOH (2 × 10 mL) and CHCl$_3$ (2 × 10 mL), and rapidly dried under vacuum.

This product was then taken up in CHCl$_3$ (15 mL) and placed in the dark under argon, and concentrated HCl (400 μL) was added dropwise. The mixture was stirred for 2 h at room temperature and then heated for 2 min at 30−35 °C. The reaction mixture was cooled to room temperature and diluted in CHCl$_3$ (70 mL), and the silver chloride precipitate was removed and washed with CHCl$_3$ (3 × 10 mL). The combined filtrates were rapidly washed with iced water (3 × 10 mL), dried over Na$_2$SO$_4$, filtered, and evaporated to dryness under vacuum to give a white solid that was crystallized from EtOAc and petroleum ether mixture to yield **10** (336 mg, 56%) as white crystals. The physicochemical characteristics of this molecule have been described elsewhere (mp, [α]$^{20}_D$ ^{1}H and ^{13}C NMR, MS, anal.).[23]

General Procedure for S-Acylation of the Cysteine Residue. *N*-(*N*,*S*-Diacetyl-L-cysteinyl)-*S*-acetylcysteamine (14). A stirred solution of **10** (83 mg, 314 μmol) in pyridine (1 mL) was cooled to 0 °C and treated with an excess of acetic anhydride (90 μL, 950 μmol). The reaction mixture was stirred for 1 h at 0 °C, allowed to warm to room temperature, and left to stand overnight. The solution was then evaporated to dryness under vaccum. The oily residue was taken up in CH$_2$Cl$_2$ (30 mL), washed [water (2 × 20 mL), ice-cold saturated aqueous NaHCO$_3$ (15 mL), water (2 × 20 mL), 1 N aqueous citric acid (15 mL), and water (neutral pH)], dried over Na$_2$SO$_4$, filtered, and evaporated to dryness under vaccum. The crude product was then recrystallized from EtOAc to give **14** as white crystals (73 mg, 75%): mp 153−154 °C. TLC (CH$_2$Cl$_2$/MeOH, 9.5:0.5) R_f = 0.46. [α]$^{20}_D$ −33.7° (c 0.8, CHCl$_3$). ^{1}H NMR: 2.02 (s, 3H, NCOC*H*$_3$), 2.37, 2.39 (2s, 2 × 3H, 2 × SCOC*H*$_3$), 2.93−3.12 (m, 2H, NCH$_2$C*H*$_2$S), 3.24 (dd, J = 7.2, 14.5 Hz, 1H, β *H*a cys), 3.31 (dd, J = 5.2, 14.5 Hz, 1H, β *H*b cys), 3.39−3.49 (m, 2H, NC*H*$_2$CH$_2$S), 4.54 (ddd, J = 5.3, 7.2, 7.3 Hz, 1H, α *H* cys), 6.44 (d, J = 7.3 Hz, 1H, N*H* cys), 6.83 (t, J = 5.1 Hz,1H, N*H*CH$_2$). MS m/z 613 (2M + H)$^+$, 307 (M + H)$^+$. Anal. (C$_{11}$H$_{18}$N$_2$O$_4$S$_2$) C, H, N, S.

***N*-(*N*-Acetyl-*S*-isobutyryl-L-cysteinyl)-*S*-acetylcysteamine (15).** This compound was prepared according to the general procedure described for **14**, using **10** (85 mg, 322 μmol) and isobutyryl chloride (137 μL, 1.3 mmol). The crude product was purified by flash column chromatography (CH$_2$Cl$_2$/Et$_2$O, 8:2 to 8:3) to give a white solid. Recrystallization from EtOAc and petroleum ether mixture gave **15** as white crystals (54 mg, 50%): mp 116−118 °C. TLC (CH$_2$Cl$_2$/MeOH, 9.4:0.6) R_f = 0.6. [α]$^{20}_D$ −18.4° (c 0.87, CHCl$_3$). ^{1}H NMR: 1.20 (d, J = 6.9 Hz, 6H, C(C*H*$_3$)$_2$, 2.00 (s, 3H, NCOC*H*$_3$), 2.37 (s, 3H, SCOC*H*$_3$), 2.80 (hept, J = 6.9 Hz, 1H, C*H*(CH$_3$)$_2$, 2.93−3.12 (m, 2H, NCH$_2$C*H*$_2$S), 3.23−3.30 (m, 2H, β *CH*$_2$ cys), 3.38−3.49 (m, 2H, NC*H*$_2$CH$_2$S), 4.46−4.57 (m, 1H, α *H* cys), 6.42 (d, J = 7.3 Hz, 1H, N*H* cys), 6.80 (t, J = 5.2 Hz, 1H, N*H*CH$_2$). MS m/z 669 (2M + H)$^+$, 335 (M + H)$^+$. Anal. (C$_{13}$H$_{22}$N$_2$O$_4$S$_2$) C, H, N, S.

***N*-(*N*-Acetyl-*S*-pivaloyl-L-cysteinyl)-*S*-acetylcysteamine (16).** This compound was prepared according to the general procedure described for **14**, using **10** (95 mg, 360 μmol) and pivaloyl chloride (176 μL, 1.44 mmol). The crude product was purified by flash column chromatography (CH$_2$Cl$_2$/Et$_2$O, 1:1) to give a white solid. Recrystallization from EtOAc and petroleum ether mixture gave **16** as white crystals (64 mg, 51%): mp 92−94 °C. TLC (CH$_2$Cl$_2$/Et$_2$O, 4:6) R_f = 0.23. [α]$^{20}_D$ −11.1° (c 1.08, CHCl$_3$). ^{1}H NMR: 1.25 (s, 9H, C(C*H*$_3$)$_3$, 2.00 (s, 3H, NCOC*H*$_3$), 2.37 (s, 3H, SCOC*H*$_3$), 2.94−3.12 (m, 2H, NCH$_2$C*H*$_2$S), 3.25 (d, J = 6.5 Hz, 2H, β *CH*$_2$ cys), 3.38−3.49 (m, 2H, NC*H*$_2$CH$_2$S), 4.51 (td, J = 6.5, 7.3 Hz, 1H, α *H* cys), 6.42 (d, J = 7.3 Hz, 1H, N*H* cys), 6.80 (t, J = 5.2 Hz,1H, N*H*CH$_2$). MS m/z 697 (2M + H)$^+$, 349 (M + H)$^+$. Anal. (C$_{14}$H$_{24}$N$_2$O$_4$S$_2$) C, H, N, S.

***N*-(*N*-Acetyl-*S*-benzoyl-L-cysteinyl)-*S*-acetylcysteamine (17).** This compound was prepared according to the general procedure described for **14**, using **10** (110 mg, 420 μmol) and benzoyl chloride (120 μL, 1.03 mmol). The crude product was purified by flash column chromatography (CH$_2$Cl$_2$/MeOH, 9.85:0.15) to give a colorless gum. Recrystallization from CH$_2$Cl$_2$ and Et$_2$O mixture gave **17** as a white powder (75 mg, 48%): mp 157−158 °C. TLC (CH$_2$Cl$_2$/MeOH, 9.5:0.5) R_f = 0.44. [α]$^{20}_D$ +5.8° (c 1.03, CHCl$_3$). ^{1}H NMR: 2.00 (s, 3H, NCOC*H*$_3$), 2.33 (s, 3H, SCOC*H*$_3$), 2.98−3.08 (m, 2H, NCH$_2$-C*H*$_2$S), 3.41−3.51 (m, 2H, NC*H*$_2$CH$_2$S), 3.49 (overlapping d, J = 6.2 Hz, 2H, C*H*$_2$ cys), 4.58−4.69 (m, 1H, α *H* cys), 6.55 (d, J = 7.0 Hz, 1H, N*H* cys), 6.79−6.90 (m, 1H, N*H*CH$_2$), 7.41−7.52, 7.57−7.66, 7.92−8.01 (3m, 5H, Ar*H*). MS m/z 737 (2M + H)$^+$, 369 (M + H)$^+$. Anal. (C$_{16}$H$_{20}$N$_2$O$_4$S$_2$) C, H, N.

***N*-(*N*-Acetyl-*S*-trityl-L-cysteinyl)-*S*-isobutyrylcysteamine (7).** This compound was prepared according to the general procedure described for **6**, using **1** (1.82 g, 4.5 mmol) and *S*-isobutyrylcysteamine hydrochloride (**3**, obtained by the procedure described for S-acetylcysteamine hydrochloride,[26] mp 147−148 °C). The crude product was purified by flash column chromatography (CH$_2$Cl$_2$/Et$_2$O, 7:3) to give **7** as a colorless foam (1.92 g, 80%). TLC (EtOAc/petroleum ether, 8:2) R_f = 0.37. [α]$^{20}_D$ +10° (c 1.1, CHCl$_3$). ^{1}H NMR: 1.16 (d, J = 6.9 Hz, 6H, C(C*H*$_3$)$_2$), 1.90 (s, 3H, NCOC*H*$_3$), 2.49 (dd, J = 5.7, 12.9 Hz, 1H, β *H*a cys), 2.70 (hept, J = 6.9 Hz, 1H, C*H*(CH$_3$)$_2$), 2.79 (dd, J = 6.4, 12.9 Hz, 1H, β *H*b cys), 2.88−3.01 (m, 2H, NCH$_2$C*H*$_2$S), 3.29−3.41 (m, 2H, NC*H*$_2$CH$_2$S), 4.08−4.19 (m, 1H, α *H* cys), 5.76 (d, J = 7.7 Hz, 1H, N*H* cys), 6.36 (t, J = 5.5 Hz, 1H, N*H*CH$_2$), 7.15−7.35, 7.38−7.52 (2m, 15H, Ar*H*). MS m/z 535 (M + H)$^+$. Anal. (C$_{30}$H$_{34}$N$_2$O$_3$S$_2$) C, H, N.

***N*-(*N*-Acetyl-L-cysteinyl)-*S*-isobutyrylcysteamine (11).** This compound was prepared from **7** (1.27 g, 2.38 mmol) according to the general procedure described for **10**. The crude product was purified by flash column chromatography (CH$_2$Cl$_2$/MeOH; 9.85:0.15) to give **11** as a colorless gum (396 mg, 57%). TLC (CH$_2$Cl$_2$/MeOH, 9.5:0.5) R_f = 0.45. [α]$^{20}_D$ −26.6° (c 1.09, CHCl$_3$). ^{1}H NMR: 1.21 (d, J = 6.9 Hz, 6H, C(C*H*$_3$)$_2$), 1.61 (dd, J = 7.6, 10.3 Hz, 1H, S*H*), 2.09 (s, 3H, NCOC*H*$_3$), 2.70 (ddd, J = 6.4, 10.3, 13.8 Hz, 1H, β *H*a cys), 2.77 (hept, J = 6.9 Hz, 1H, C*H*(CH$_3$)$_2$), 2.99−3.08 (m, 2H, NCH$_2$C*H*$_2$S), 3.09 (ddd, J = 4.1, 7.6, 13.8 Hz, 1H, β *H*b cys), 3.41−3.53 (m, 2H, NC*H*$_2$-CH$_2$S), 4.60 (ddd, J = 4.1, 6.4, 7.5 Hz, 1H, α *H* cys), 6.48 (d, J = 7.5 Hz, 1H, N*H* cys), 6.68−6.88 (m, 1H, N*H*CH$_2$). MS m/z 585 (2M + H)$^+$, 293 (M + H)$^+$. Anal. (C$_{11}$H$_{20}$N$_2$O$_3$S$_2$) C, H, N.

***N*-(*N*,*S*-Diacetyl-L-cysteinyl)-*S*-isobutyrylcysteamine (18).** This compound was prepared according to the general procedure described for **14**, using **11** (93.4 mg, 320 μmol) and acetic anhydride. The crude product was purified by flash column chromatography (CH$_2$Cl$_2$/Et$_2$O; 4.5:5.5) to give a colorless gum. Trituration with hexane gave **18** as a white powder (65 mg, 61%): mp 115−117 °C. TLC (CH$_2$Cl$_2$/MeOH, 9.5:0.5) R_f = 0.58. [α]$^{20}_D$ −20.2° (c 1.04, CHCl$_3$). ^{1}H NMR: 1.20 (d, J = 6.9 Hz, 6H, C(C*H*$_3$)$_2$), 2.02 (s, 3H, NCOC*H*$_3$), 2.38 (s, 3H, SCOC*H*$_3$), 2.78 (hept, J = 6.9 Hz, 1H, C*H*(CH$_3$)$_2$), 2.96−3.06 (m, 2H, NCH$_2$C*H*$_2$S), 3.19−3.36 (m, 2H, C*H*$_2$ cys), 3.38−3.48 (m, 2H, NC*H*$_2$CH$_2$S), 4.47−4.60 (m, 1H, α *H* cys), 6.37 (d, J = 7.1 Hz, 1H, N*H* cys), 6.70−6.83 (m, 1H, N*H*CH$_2$). MS m/z 669 (2M + H)$^+$, 335 (M + H)$^+$. Anal. (C$_{13}$H$_{22}$N$_2$O$_4$S$_2$) C, H, N.

***N*-(*N*-Acetyl-*S*-isobutyryl-L-cysteinyl)-*S*-isobutyrylcysteamine (19).** This compound was prepared according to the general procedure described for **14**, using **11** (93.4 mg, 320 μmol) and isobutyryl chloride. The crude product was purified by flash column chromatography (CH$_2$Cl$_2$/Et$_2$O, 1:1) to give a colorless gum. Trituration with hexane gave **19** as a white powder (70 mg, 60%): mp 99−100 °C. TLC (CH$_2$Cl$_2$/Et$_2$O, 3.5:

6.5) $R_f = 0.34$. $[\alpha]^{20}_D$ −9.1° (*c* 0.88, $CHCl_3$). ^{1}H NMR: (1.20 (d, *J* = 6.9 Hz, 12H, C(C*H*$_3$)$_2$), 2.01 (s, 3H, NCOC*H*$_3$), 2.78 (hept, *J* = 6.9 Hz, 2H, C*H*(CH$_3$)$_2$), 2.92−3.10 (m, 2H, NCH$_2$C*H*$_2$S), 3.26 (d, *J* = 6.4 Hz, 2H, C*H*$_2$ cys), 3.37−3.48 (m, 2H, NC*H*$_2$-CH$_2$S), 4.46−4.58 (m, 1H, α *H* cys), 6.38 (d, *J* = 7.3 Hz, 1H, N*H* cys), 6.73−6.83 (m, 1H, N*H*CH$_2$). MS *m*/*z* 725 (2M + H)$^+$, 363 (M + H)$^+$. Anal. ($C_{15}H_{26}N_2O_4S_2$) C, H, N.

***N*-(*N*-Acetyl-*S*-pivaloyl-L-cysteinyl)-*S*-isobutyrylcysteamine (20).** This compound was prepared according to the general procedure described for **14**, using **11** (93.4 mg, 320 μmol) and pivaloyl chloride. The crude product was purified by flash column chromatography (CH_2Cl_2/Et_2O; 1:1) to give a colorless gum. Recrystallization from EtOAc and petroleum ether mixture gave **20** as white needles (77 mg, 64%): mp 103−104 °C. TLC (CH_2Cl_2/Et_2O, 1:1) $R_f = 0.27$. $[\alpha]^{20}_D$ −8.3° (*c* 0.97, $CHCl_3$). ^{1}H NMR: 1.20 (d, *J* = 6.9 Hz, 6H, C(C*H*$_3$)$_2$), 1.25 (s, 9H, C(C*H*$_3$)$_3$), 2.01 (s, 3H, NCOC*H*$_3$), 2.77 (hept, *J* = 6.9 Hz, 1H, C*H*(CH$_3$)$_2$), 2.94−3.08 (m, 2H, NCH$_2$C*H*$_2$S), 3.25 (d, *J* = 6.4 Hz, 2H, C*H*$_2$ cys), 3.37−3.49 (m, 2H, NC*H*$_2$CH$_2$S), 4.44−4.57 (m, 1H, α *H* cys), 6.35 (d, *J* = 7.3 Hz, 1H, N*H* cys), 6.69−6.80 (m, 1H, N*H*CH$_2$). MS *m*/*z* 753 (2M + H)$^+$, 377 (M + H)$^+$. Anal. ($C_{16}H_{28}N_2O_4S_2$) C, H, N.

***N*-(*N*-Acetyl-*S*-benzoyl-L-cysteinyl)-*S*-isobutyrylcysteamine (21).** This compound was prepared according to the general procedure described for **14**, using **11** (93.4 mg, 320 μmol) and benzoyl chloride. The crude product was purified by flash column chromatography (CH_2Cl_2/Et_2O; 6.5:3.5) to give a colorless gum. Trituration with hexane gave **21** as a white powder (76 mg, 60%): mp 137−138 °C. TLC (CH_2Cl_2/Et_2O, 1:1) $R_f = 0.27$. $[\alpha]^{20}_D$ +2.8° (*c* 1.08, $CHCl_3$). ^{1}H NMR: 1.18 (d, *J* = 6.9 Hz, 6H, C(C*H*$_3$)$_2$), 2.02 (s, 3H, NCOC*H*$_3$), 2.73 (hept, *J* = 6,9 Hz, 1H, C*H*(CH$_3$)$_2$), 2.96−3.06 (m, 2H, NCH$_2$C*H*$_2$S), 3.39−3.49 (m, 2H, NC*H*$_2$CH$_2$S), 3.50 (d, *J* = 6.2 Hz, 2H, C*H*$_2$ cys), 4.60−4.72 (m, 1H, α *H* cys), 6.59 (d, *J* = 7.3 Hz, 1H, N*H* cys), 6.85−6.97 (m, 1H, N*H*CH$_2$), 7.41−7.52, 7.57−7.65, 7.93−8.01 (3m, 5H, Ar*H*). MS *m*/*z* 793 (2M + H)$^+$, 397 (M + H)$^+$. Anal. ($C_{18}H_{24}N_2O_4S_2$) C, H, N.

***N*-(*N*-Acetyl-*S*-trityl-L-cysteinyl)-*S*-pivaloylcysteamine (8).** This compound was prepared according to the general procedure described for **6**, using **1** (3 g, 7.41 mmol) and *S*-pivaloylcysteamine hydrochloride (**4**, obtained by the procedure described for *S*-acetylcysteamine hydrochloride,[26] mp 212−213 °C). The crude product was purified by flash column chromatography (CH_2Cl_2/Et_2O, 7:3) to give **8** as a colorless foam (3.49 g, 86%). TLC (EtOAc/petroleum ether, 7:3) $R_f = 0.5$. $[\alpha]^{20}_D$ +8.5° (*c* 1.29, $CHCl_3$). ^{1}H NMR: 1.21 (s, 9H, C(C*H*$_3$)$_3$), 1.90 (s, 3H, NCOC*H*$_3$), 2.49 (dd, *J* = 5.9, 12.9 Hz, 1H, β *H*a cys), 2.78 (dd, *J* = 6.5, 12.9 Hz, 1H, β *H*b cys), 2.88−2.98 (m, 2H, NCH$_2$C*H*$_2$S), 3.28−3.40 (m, 2H, NC*H*$_2$CH$_2$S), 4.06−4.19 (m, 1H, α *H* cys), 5.77 (d, *J* = 7.6 Hz, 1H, N*H* cys), 6.27−6.41 (m, 1H, N*H*CH$_2$), 7.16−7.35, 7.40−7.48 (2m, 15H, Ar*H*). MS *m*/*z* 549 (M + H)$^+$. Anal. ($C_{31}H_{36}N_2O_3S_2$) C, H, N.

***N*-(*N*-Acetyl-L-cysteinyl)-*S*-pivaloylcysteamine (12).** This compound was prepared from **8** (2.5 g, 4.57 mmol) according to the general procedure described for **10**. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH, 9.85:0.15) to give **12** as a colorless gum (839 mg, 60%). TLC (CH_2Cl_2/MeOH, 9.5:0.5) $R_f = 0.49$. $[\alpha]^{20}_D$ −20.2° (*c* 0.94, $CHCl_3$). ^{1}H NMR: 1.24 (s, 9H, C(C*H*$_3$)$_3$), 1.61 (dd, *J* = 7.6, 10.3 Hz, 1H, S*H*), 2.08 (s, 3H, NCOC*H*$_3$), 2.70 (ddd, *J* = 6.4, 10.3, 13.9 Hz, 1H, β *H*a cys), 2.97−3.09 (m, 2H, NCH$_2$C*H*$_2$S), 3.08 (overlapping ddd, *J* = 4.1, 7.6, 13.9 Hz, 1H, β *H*b cys), 3.40−3.52 (m, 2H, NC*H*$_2$CH$_2$S), 4.60 (ddd, *J* = 4.1, 6.4, 7.8 Hz, 1H, α *H* cys), 6.49 (d, *J* = 7.8 Hz, 1H, N*H* cys), 6.69−6.82 (m, 1H, N*H*CH$_2$). MS *m*/*z* 613 (2M + H)$^+$, 307 (M + H)$^+$. Anal. ($C_{12}H_{22}N_2O_3S_2$) C, H, N.

***N*-(*N*,*S*-Diacetyl-L-cysteinyl)-*S*-pivaloylcysteamine (22).** This compound was prepared according to the general procedure described for **14**, using **12** (101 mg, 330 μmol) and acetic anhydride. The crude product was purified by flash column chromatography (CH_2Cl_2/Et_2O, 4.5:5.5) to give a colorless gum. Recrystallization from EtOAc and petroleum ether mixture gave **22** as white needles (77 mg, 67%): mp 112−114 °C. TLC (CH_2Cl_2/MeOH, 9.5:0.5) $R_f = 0.58$. $[\alpha]^{20}_D$ −13.8° (*c* 0.94, $CHCl_3$). ^{1}H NMR: 1.24 (s, 9H, C(C*H*$_3$)$_3$), 2.03 (s, 3H, NCOC*H*$_3$), 2.38 (s, 3H, SCOC*H*$_3$), 2.94−3.04 (m, 2H, NCH$_2$C*H*$_2$S), 3.18−3.36 (m, 2H, C*H*$_2$ cys), 3.36−3.48 (m, 2H, NC*H*$_2$CH$_2$S), 4.48−4.60 (m, 1H, α *H* cys), 6.40 (d, *J* = 7.5 Hz, 1H, N*H* cys), 6.71−6.83 (m, 1H, N*H*CH$_2$). MS *m*/*z* 697 (2M + H)$^+$, 349 (M + H)$^+$. Anal. ($C_{14}H_{24}N_2O_4S_2$) C, H, N.

***N*-(*N*-Acetyl-*S*-isobutyryl-L-cysteinyl)-*S*-pivaloylcysteamine (23).** This compound was prepared according to the general procedure described for **14**, using **12** (98 mg, 320 μmol) and isobutyryl chloride. The crude product (gum) was triturated with hexane to give pure **23** (67 mg, 56%) as a white powder: mp 101−102 °C. TLC (CH_2Cl_2/Et_2O, 3.5:6.5) $R_f = 0.46$. $[\alpha]^{20}_D$ −5.7° (*c* 1.05, $CHCl_3$). ^{1}H NMR: 1.20 (d, *J* = 6.9 Hz, 6H, C(C*H*$_3$)$_2$), 1.25 (s, 9H, C(C*H*$_3$)$_3$), 2.01 (s, 3H, NCOC*H*$_3$), 2.79 (hept, *J* = 6.9 Hz, 1H, C*H*(CH$_3$)$_2$), 2.90−3.08 (m, 2H, NCH$_2$C*H*$_2$S), 3.25 (d, *J* = 6.3 Hz, 2H, C*H*$_2$ cys), 3.35−3.47 (m, 2H, NC*H*$_2$CH$_2$S), 4.46−4.58 (m, 1H, α *H* cys), 6.38 (d, *J* = 7.1 Hz, 1H, N*H* cys), 6.71−6.81 (m, 1H, N*H*CH$_2$). MS *m*/*z* 753 (2M + H)$^+$, 377 (M + H)$^+$. Anal. ($C_{16}H_{28}N_2O_4S_2$) C, H, N.

***N*-(*N*-Acetyl-*S*-pivaloyl-L-cysteinyl)-*S*-pivaloylcysteamine (24).** This compound was prepared according to the general procedure described for **14**, using **12** (104 mg, 340 μmol) and pivaloyl chloride. The crude product (gum) was triturated with hexane to give a white powder. Recrystallization from EtOAc and petroleum ether mixture gave **24** as white crystals (80 mg, 60%): mp 109−111 °C. TLC (CH_2Cl_2/Et_2O, 1:1) $R_f = 0.36$. $[\alpha]^{20}_D$ −4.4° (*c* 0.91, $CHCl_3$). ^{1}H NMR: 1.24 (s, 18H, C(C*H*$_3$)$_3$), 2.00 (s, 3H, NCOC*H*$_3$), 2.90−3.08 (m, 2H, NCH$_2$C*H*$_2$S), 3.24 (d, *J* = 6.4 Hz, 2H, C*H*$_2$ cys), 3.36−3.47 (m, 2H, NC*H*$_2$CH$_2$S), 4.44−4.57 (m, 1H, α *H* cys), 6.38 (d, *J* = 7.4 Hz, 1H, N*H* cys), 6.68−6.88 (m, 1H, N*H*CH$_2$). MS *m*/*z* 781 (2M + H)$^+$, 391 (M + H)$^+$. Anal. ($C_{17}H_{30}N_2O_4S_2$) C, H, N.

***N*-(*N*-Acetyl-*S*-benzoyl-L-cysteinyl)-*S*-pivaloylcysteamine (25).** This compound was prepared according to the general procedure described for **14**, using **12** (104 mg, 340 μmol) and benzoyl chloride. The crude product was purified by flash column chromatography (CH_2Cl_2/Et_2O; 6.5:3.5) to give a colorless gum. Trituration with hexane gave **25** as a white powder (89 mg, 64%): mp 133−134 °C. TLC (CH_2Cl_2/Et_2O, 1:1) $R_f = 0.33$. $[\alpha]^{20}_D$ +5.1° (*c* 0.98, $CHCl_3$). ^{1}H NMR: 1.21 (s, 9H, C(C*H*$_3$)$_3$), 2.01 (s, 3H, NCOC*H*$_3$), 2.90−3.08 (m, 2H, NCH$_2$C*H*$_2$S), 3.33−3.52 (m, 4H, NC*H*$_2$CH$_2$S, C*H*$_2$ cys), 4.64−4.77 (m, 1H, α *H* cys), 6.76 (d, *J* = 7.4 Hz, 1H, N*H* cys), 7.06−7.22 (m, 1H, N*H*CH$_2$), 7.40−7.50, 7.55−7.63, 7.91−8.0 (3m, 5H, Ar*H*). MS *m*/*z* 821 (2M + H)$^+$, 411 (M + H)$^+$. Anal. ($C_{19}H_{26}N_2O_4S_2$) C, H, N.

***N*-(*N*-Acetyl-*S*-trityl-L-cysteinyl)-*S*-benzoylcysteamine (9).** This compound was prepared according to the general procedure described for **6**, using **1** (3 g, 7.41 mmol) and *S*-benzoylcysteamine hydrochloride (**5**).[26] The crude product was purified by flash column chromatography (CH_2Cl_2/Et_2O, 8.5:1.5) to give **9** as a colorless foam (2.61 g, 62%). TLC (AcOEt/petroleum ether, 7:3) $R_f = 0.42$. $[\alpha]^{20}_D$ +10.8° (*c* 1.11, $CHCl_3$). ^{1}H NMR: 1.86 (s, 3H, NCOC*H*$_3$), 2.47 (dd, *J* = 5.7, 13.0 Hz, 1H, β *H*a cys), 2.82 (dd, *J* = 6.4, 13.0 Hz, 1H, β *H*b cys), 3.08−3.27 (m, 2H, NCH$_2$C*H*$_2$S), 3.41−3.53 (m, 2H, NC*H*$_2$-CH$_2$S), 4.08−4.21 (m, 1H, α *H* cys), 5.67 (d, *J* = 7.7 Hz, 1H, N*H* cys), 6.34−6.46 (m, 1H, N*H*CH$_2$), 7.17−7.32, 7.37−7.45, 7.54−7.61, 7.89−7.96 (4m, 20H, Ar*H*). MS *m*/*z* 569 (M + H)$^+$. Anal. ($C_{33}H_{32}N_2O_3S_2$) C, H, N.

***N*-(*N*-Acetyl-L-cysteinyl)-*S*-benzoylcysteamine (13).** This compound was prepared from **9** (2.52 g, 4.44 mmol) according to the general procedure described for **10**. The crude product was purified by flash column chromatography (EtOAc/petroleum ether, 1:1) to give **13** as a white solid (912 mg, 63%): mp 128−130 °C. TLC (CH_2Cl_2/MeOH, 9.5:0.5) $R_f = 0.38$. $[\alpha]^{20}_D$ −24.7° (*c* 1.01, $CHCl_3$). ^{1}H NMR: 1.59 (dd, *J* = 7.6; 10.2 Hz, 1H, S*H*), 2.04 (s, 3H, NCOC*H*$_3$), 2.71 (ddd, *J* = 6.5, 10.2, 13.8 Hz, 1H, β *H*a cys), 3.06 (ddd, *J* = 4.3, 7.6, 13.8 Hz, 1H, β *H*b cys), 3.20−3.31 (m, 2H, NCH$_2$C*H*$_2$S), 3.52−3.64 (m, 2H, NC*H*$_2$-CH$_2$S), 4.61 (ddd, *J* = 4.3, 6.5, 7.4 Hz, 1H, α *H* cys), 6.51 (d, *J* = 7.4 Hz, 1H, N*H* cys), 6.83−7.00 (m, 1H, N*H*CH$_2$), 7.43−7.52, 7.56−7.65, 7.92−8.00 (3m, 5H, Ar*H*). MS *m*/*z* 653 (2M + H)$^+$, 327 (M + H)$^+$. Anal. ($C_{14}H_{18}N_2O_3S_2$) C, H, N.

***N*-(*N*,*S*-Diacetyl-L-cysteinyl)-*S*-benzoylcysteamine (26).** This compound was prepared according to the general procedure described for **14**, using **13** (111 mg, 340 μmol) and acetic anhydride. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH, 9.85:0.15) to give a colorless gum. Recrystallization from EtOAc and petroleum ether mixture gave **26** as white needles (88 mg, 70%): mp 166−168 °C. TLC (CH_2Cl_2/MeOH, 9.5:0.5) R_f = 0.44. $[\alpha]^{20}_D$ −14.3° (*c* 0.98, $CHCl_3$). ^{1}H NMR: 1.99 (s, 3H, NCOC*H*$_3$), 2.32 (s, 3H, SCOC*H*$_3$), 3.18−3.31 (m, 4H, NCH$_2$C*H*$_2$S, C*H*$_2$ cys), 3.48−3.61 (m, 2H, NC*H*$_2$CH$_2$S), 4.49−4.62 (m, 1H, α *H* cys), 6.38 (d, *J* = 7.6 Hz, 1H, N*H* cys), 6.79−6.92 (m, 1H, N*H*CH$_2$), 7.42−7.51, 7.55−7.64, 7.93−8.01 (3m, 5H, Ar*H*). MS *m*/*z* 737 $(2M + H)^+$, 369 $(M + H)^+$. Anal. ($C_{16}H_{20}N_2O_4S_2$) C, H, N.

***N*-(*N*-Acetyl-*S*-isobutyryl-L-cysteinyl)-*S*-benzoylcysteamine (27).** This compound was prepared according to the general procedure described for **14**, using **13** (98 mg, 300 μmol) and isobutyryl chloride. The crude product was purified by flash column chromatography (CH_2Cl_2/Et_2O, 6:4) to give a colorless gum. Recrystallization from EtOAc and petroleum ether mixture gave **27** as white crystals (88 mg, 74%): mp 135−136 °C. TLC (CH_2Cl_2/Et_2O, 3:7) R_f = 0.2. $[\alpha]^{20}_D$ −6.7° (*c* 1.2, $CHCl_3$). ^{1}H NMR: 1.17 (d, *J* = 6.9 Hz, 6H, C(C*H*$_3$)$_2$), 1.97 (s, 3H, NCOC*H*$_3$), 2.75 (hept, *J* = 6.9 Hz, 1H, C*H*(CH$_3$)$_2$), 3.19−3.30 (m, 4H, NCH$_2$C*H*$_2$S, C*H*$_2$ cys), 3.46−3.62 (m, 2H, NC*H*$_2$-CH$_2$S), 4.49−4.61 (m, 1H, α *H* cys), 6.41 (d, *J* = 7.4 Hz, 1H, N*H* cys), 6.85−6.96 (m, 1H, N*H*CH$_2$), 7.41−7.52, 7.55−7.64, 7.90−8.02 (3m, 5H, Ar*H*). MS *m*/*z* 793 $(2M + H)^+$, 397 $(M + H)^+$. Anal. ($C_{18}H_{24}N_2O_4S_2$) C, H, N.

***N*-(*N*-Acetyl-*S*-pivaloyl-L-cysteinyl)-*S*-benzoylcysteamine (28).** This compound was prepared according to the general procedure described for **14**, using **13** (98 mg, 300 μmol) and pivaloyl chloride. The crude product was purified by flash column chromatography (CH_2Cl_2/Et_2O, 5.5:4.5) to give a colorless gum. Recrystallization from EtOAc and petroleum ether mixture gave **28** as white crystals (95 mg, 77%): mp 101−103 °C. TLC (CH_2Cl_2/Et_2O, 3:7) R_f = 0.3. $[\alpha]^{20}_D$ −3.8° (*c* 1.05, $CHCl_3$). ^{1}H NMR: 1.22 (s, 9H, C(C*H*$_3$)$_3$), 1.96 (s, 3H, NCOC*H*$_3$), 3.19−3.30 (m, 4H, NCH$_2$C*H*$_2$S, C*H*$_2$ cys), 3.46−3.62 (m, 2H, NC*H*$_2$CH$_2$S), 4.47−4.58 (m, 1H, α *H* cys), 6.38 (d, *J* = 7.2 Hz, 1H, N*H* cys), 6.81−6.92 (m, 1H, N*H*CH$_2$), 7.42−7.52, 7.55−7.65, 7.90−8.02 (3m, 5H, Ar*H*). MS *m*/*z* 821 $(2M + H)^+$, 411 $(M + H)^+$. Anal. ($C_{19}H_{26}N_2O_4S_2$) C, H, N.

***N*-(*N*-Acetyl-*S*-benzoyl-L-cysteinyl)-*S*-benzoylcysteamine (29).** This compound was prepared according to the general procedure described for **14**, using **13** (98 mg, 300 μmol) and benzoyl chloride. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH, 9.9:0.1) to give a colorless gum. Recrystallization from EtOAc gave **29** as white crystals (85 mg, 66%): mp 188−190 °C. TLC (CH_2Cl_2/MeOH, 9.5:0.5) R_f = 0.66. $[\alpha]^{20}_D$ +4.1° (*c* 0.98, $CHCl_3$). ^{1}H NMR: 1.99 (s, 3H, NCOC*H*$_3$), 3.19−3.28 (m, 2H, NCH$_2$C*H*$_2$S), 3.49 (d, *J* = 6.1 Hz, 2H, C*H*$_2$ cys), 3.52−3.61 (m, 2H, NC*H*$_2$CH$_2$S), 4.63−4.71 (m, 1H, α *H* cys), 6.56 (d, *J* = 7,2 Hz, 1H, N*H* cys), 6.92−7.08 (m, 1H, N*H*CH$_2$), 7.38−7.48, 7.52−7.62, 7.88−7.97 (3m, 10H, Ar*H*). MS *m*/*z* 861 $(2M + H)^+$, 431 $(M + H)^+$. Anal. ($C_{21}H_{22}N_2O_4S_2$) C, H, N.

***N*-(*N*-Acetyl-*S*-trityl-L-cysteinyl)-*S*-tritylcysteamine (30).** This compound was prepared according to the general procedure described for **6**, using **1** (1 g, 2.47 mmol) and *S*-tritylcysteamine hydrochloride.[27] Trituration of the crude product (foam) with EtOAc at 0 °C gave pure **30** (1.26 g, 72%) as a white powder: mp 193−195 °C. TLC (CH_2Cl_2/MeOH, 9.8:0.2) R_f = 0.5. $[\alpha]^{20}_D$ +7.2° (*c* 0.97, $CHCl_3$). ^{1}H NMR: 1.87 (s, 3H, NCOC*H*$_3$), 2.33 (t, *J* = 6.6 Hz, 2H, NCH$_2$C*H*$_2$S) 2.47 (dd, *J* = 6.0, 13.0 Hz, 1H, β *H*a cys), 2.68 (dd, *J* = 6.7, 13.0 Hz, 1H, β *H*b cys), 2.90−3.01 (m, 2H, NC*H*$_2$CH$_2$S), 3.92−4.06 (m, 1H, α *H* cys), 5.74 (d, *J* = 7.6 Hz, 1H, N*H* cys), 5.88 (t, *J* = 5.5 Hz, 1H, N*H*CH$_2$), 7.18−7.32, 7.34−7.46 (2m, 30H, Ar*H*). MS (NBA) *m*/*z* 707 $(M + H)^+$. Anal. ($C_{45}H_{42}N_2O_2S_2$) C, H, N.

***N*-(*N*-Acetyl-L-cysteinyl)cysteamine (31).** This compound was prepared from **30** (1.26 g, 1.78 mmol) and 2.1 mol equiv of $AgNO_3$ and pyridine in MeOH, according to the general procedure described for **10**. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH, 9.8:0.2) to give **31** as a white powder (70 mg, 18%): mp 133−135 °C. TLC (CH_2Cl_2/MeOH, 9.7:0.3) R_f = 0.32. $[\alpha]^{20}_D$ −50.0° (*c* 1.2, $CHCl_3$). ^{1}H NMR: 1.44 (t, *J* = 8.5 Hz, 1H, S*H* cysteamine), 1.71 (dd, *J* = 7.8, 10.0 Hz, 1H, S*H* cys), 2.07 (s, 3H, NCOC*H*$_3$), 2.62−2.82 (m, 3H, β *H*a cys, NCH$_2$C*H*$_2$S), 3.06 (ddd, *J* = 4.5, 7.6, 13.8 Hz, 1H, β *H*b cys), 3.36−3.59 (m, 2H, NC*H*$_2$CH$_2$S), 4.53−4.66 (m, 1H, α *H* cys), 6.53 (d, *J* = 7.0 Hz, 1H, N*H* cys), 6.77−6.91 (m, 1H, N*H*CH$_2$). MS (NBA) *m*/*z* 445 $(2M + H)^+$, 223 $(M + H)^+$. Anal. ($C_7H_{14}N_2O_4S_2$) C, H, N, S.

Biological Methods. Isolation of Monocyte-Derived Macrophages (MDM). Human peripheral blood mononuclear cells (PBMC) were obtained from healthy HIV-, HCV-, and HBV-seronegative donors by Ficoll-Hypaque density gradient centrifugation (MSL 2000, Eurobio, Les Ulis, France). Monocytes were isolated from PBMC by countercurrent centrifugal elutriation, as previously described, with a degree of enrichment ≥95%.[28] Freshly isolated human monocytes were resuspended in medium A: Dulbecco's Modified Eagle Medium (DMEM) Glutamax (Roche Products, Meylan, France) supplemented with 10% heat-inactivated (56 °C for 30 min) fetal calf serum (FCS) (Roche Products), and 1% antibiotic cocktail (penicillin, streptomycin, neomycin, PSN; InVitroGen). Cells were dispensed (one million cells per well) into 48-well plates (BD BioSciences, Lincoln Park, NJ) and maintained for 7 days in a humidified 5% CO_2 atmosphere to allow their differentiation into MDM. Cell culture media were endotoxin-free, as shown by the Limulus Amebocyte Lysate test (LAL; Sigma Chemical Co, Saint Quentin-Fallavier, France). Differences in morphological appearance and the cell surface expression of various molecules, such as HLA-DR and CD16, have been described elsewhere.[29]

Drugs. NAC and MEA (Sigma), **10**, and its derivatives or analogues were immediately dissolved in dimethyl sulfoxide (DMSO, Sigma) and stored at −80 °C. Since several of the molecules (**17**, **19**, **25**, **29**) were insoluble, they were not subjected to biological testing. The pH of drug solutions was adjusted to 7.0 with 3 M NaOH if necessary.

Total Intracellular GSH Levels. Total intracellular glutathione levels were determined by the Griffith's colorimetric method (Cayman Chemical Company, Ann Arbor, MI)[30] in uninfected MDM, after treatment for 24 h. In parallel, we determined the protein content of cell lysates by Bradford's method (BioRad, Marnes la Coquette, France). One million uninfected MDM were treated for 24 h, washed with PBS, and lysed by incubation with 150 μL of 0.1% Tween 20 in PBS for 1 h.

Virus. MDM were infected with the reference macrophage-tropic HIV-1/BaL strain.[31] This virus was propagated in vitro in human phytohemagglutinin (PHA)-P-activated umbilical blood mononuclear cells (UBMC). To eliminate soluble factors such as cytokines, the cell-free UMBC culture supernatant was ultracentrifuged at 360 000*g* for 5 min and the pellet resuspended in DMEM. This viral preparation was titrated on PHA-P-activated-PBMC. The 50% tissue culture infectious dose (TCID50) and the multiplicity of infection (moi) were calculated using Kärber's formula[32] and Poisson's law, respectively.

Antiviral Assay. One million MDM were pretreated for 1 h with various concentrations of compounds and infected with 10 000 TCID50 (0.01 multiplicity of infection) of the HIV-1/Ba-L strain. The MDM were then incubated for 24 h at 37 °C, washed once to eliminate excess virus, and fed with fresh medium A. Twice per week we removed supernatants, which were then frozen and stored at −20 °C for later evaluation of viral replication. Cell culture medium and drugs were then renewed, and cells were observed under a microscope to assess the possible cytotoxicity of the drugs. HIV replication was measured by quantifying reverse transcriptase (RT) activity in cell culture supernatants. RT activity was determined using the RT RetroSys kit, according to the manufacturer's (Innovagen) instructions. The kinetics of RT activity in the cell culture supernatants of HIV-infected MDM were as previously reported.[33,34]

Data Analysis. All experiments were performed in triplicate and repeated with cells isolated from a second blood donor. Results are expressed as the mean ± standard deviation (SD). For the antiviral assay, cumulative RT activity − the sum of RT activity obtained for each time-point in each individual cell culture well was used to calculate percentages with respect to untreated control and 50, 70, and 90% effective doses. To calculate the percentages with respect to the untreated control, the results obtained for treated MDM cultures were divided by the untreated control value. Effective doses were determined on the basis of cumulative RT activity using computer software (J. and T. C. Chou, Biosoft, Cambridge, UK). For the GSH assay, intracellular GSH contents were expressed in nanomoles per milligram of protein.

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