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New *N,N'*-bis(alkoxycarbonyl)-L-cystine bis(methylamides) **4a**, **4b** and *N,N'*-bis(benzyloxycarbonyl)-L-cystine bis(methylamide) **4c** have been synthesized by mixed anhydride method from the essential amino acid L-cystine **1** in good yield. These cystine bis(methylamides) **4a,b,c** have been cyclized with sulfuryl chloride. New 2-methyl-4-amino-3-isothiazolone and 5-chloro-2-methyl-4-amino-3-isothiazolone hydrobromide salts **7**, **8** have been obtained by deacylation of 2-methyl-4-(benzyloxycarbonyl)amino-3-isothiazolone **5c** and 5-chloro-2-methyl-4-(benzyloxycarbonyl)amino-3-isothiazolone **6c** with hydrogen bromide in acetic acid. The microbicidal effect of the new 2-methyl-3-isothiazolones **5a,b,c**; **6a,b,c**; **7** and **8** compounds obtained by the above method has been investigated.

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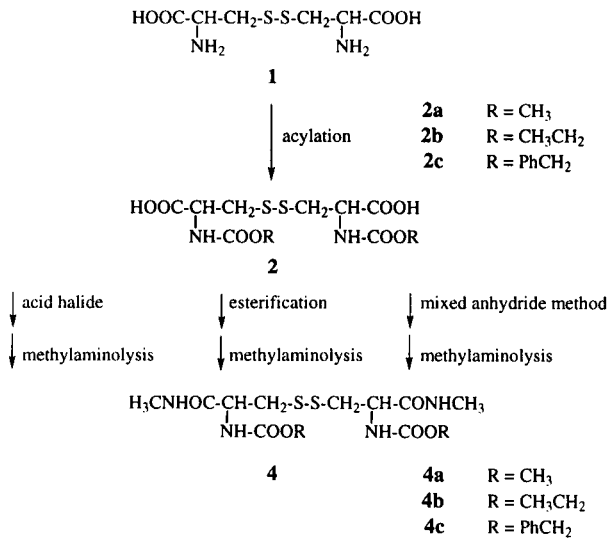
From the 2-methyl-4-substituted isothiazolones the compounds substituted with alkyl, halogen or cyano groups have been synthesized by now [1-2]. The antimicrobial, fungicidal and algicidal effects of 2-methyl-3-isothiazolones are well known. If these characteristics are combined with low toxicity, good biodegradability and compatibility, these compounds will be excellent for conservation. The methods for preparation of the already known isothiazoles can be found in the literature.

The object of our work was to study the synthesis of new 4-amino-substituted or 4-acylamino-substituted 2-methyl-3-isothiazolones, originating from (*R,R*)-3,3'-dithiobis(2-aminopropionic acid) *i.e.*, natural L-cystine **1**. The working hypothesis was to *N,N'*-diacylate **1** in the first step, then change the obtained (*R,R*)-*N,N'*-diacyl-3,3'-dithiobis(2-aminopropionic acids) **2a,b,c** into (*R,R*)-*N,N'*-diacyl-3,3'-dithiobis(2-aminopropionic acid) bis(methylamides) *i.e.*, *N,N'*-diacyl-L-cystine bis(methylamides) **4a,b,c** (Scheme 1).

Scheme 1



The **2a,b,c** derivatives had been prepared in aqueous alkali medium by the reaction of the appropriate chloroformate and L-cystine disodium salt. Theoretically the transformation of the amino-blocked cystines into methylamides **4** can be carried out by the methylaminolysis of amino-protected cystine diacyl chloride or cystine diester.

The bis(methylamide) **4** production through acid chloride is inexpedient. On one hand the amino acid chlorides are not stable, on the other hand in the presence of urethane protection group the so called 'Leuchs anhydride' [3] can be easily formed in the reaction. It is not desirable for the production of the blocked amino acid amides. The anhydride formation would not cause any harm in itself as it is an excellent acylating agent to the primary amines like the methylamine. In our case, however, its formation is disadvantageous as the aim is to produce blocked amino acid amides. The literature deals only with the production of **4c** out of the **4a,b,c** compounds. This has been produced earlier by Gustus [4] under extreme conditions (-78°, ether), through acid chloride.

We tried to produce **4c** starting from the diethyl ester of **2c**. Only the reactions carried out by methylamine gas in dry solvent were effective. Using aqueous methylamine, under the examined conditions, **4c** was obtained in a rather low yield. The following conclusion can be drawn from the data of Table 1 that presents the results of the experiments: 1) Elementary sulfur can be isolated from the obtained reaction mixture without the presence of water-fixing substance, with an equimolar amount of methylamine, at a reaction temperature higher than room temperature. It is explained by the cleavage of the disulfide bond because of an alkaline and/or thermal effect. At room temperature, with a shorter reaction time, the original ester is obtained, whereas with longer reaction time elementary sulfur was isolated. 2) In the presence of water-fixing substance with equimolar amount of methylamine at room temperature diacyl-cystine **2c** was isolated

from the reaction mixture, due to hydrolysis caused by the water. 3) To a certain extent the excess of methylamine helps the amide formation, but in any case the above described secondary reactions must be taken into consideration.

Table 1

Examination of Methylaminolysis of *N,N'*-Bis(benzyloxylcarbonyl)-L-cystine Bis(ethylester) with Aqueous Solution of Methylamine

Methylamine/Ester Molar Ratio	Solvent	Water Fixant A [a]	Water Fixant B [b]	Reaction Temperature (°C)	Reaction Time (Hours)	Isolated Product	4c Yield (%)
2	Methanol or Benzene	–	–	25-60	6-120	Sulfur	–
2	Methanol	–	–	25	70	Ester	–
2	Methanol	5	–	25	24	**2c**	–
4,33	Methanol	10	–	25	24	**4c**	4
6	Methanol	10	–	25	48	**4c**	15,6
8	Methanol	10	–	25	48	Sulfur + **4c**	15,6
6	Methanol	10	–	25	70	Sulfur	–
4,33	Methanol	–	–	25	48	Ester + **2c**	–
6	Benzene or Ethyl Acetate	–	0,5	25	20-48	Ester	–

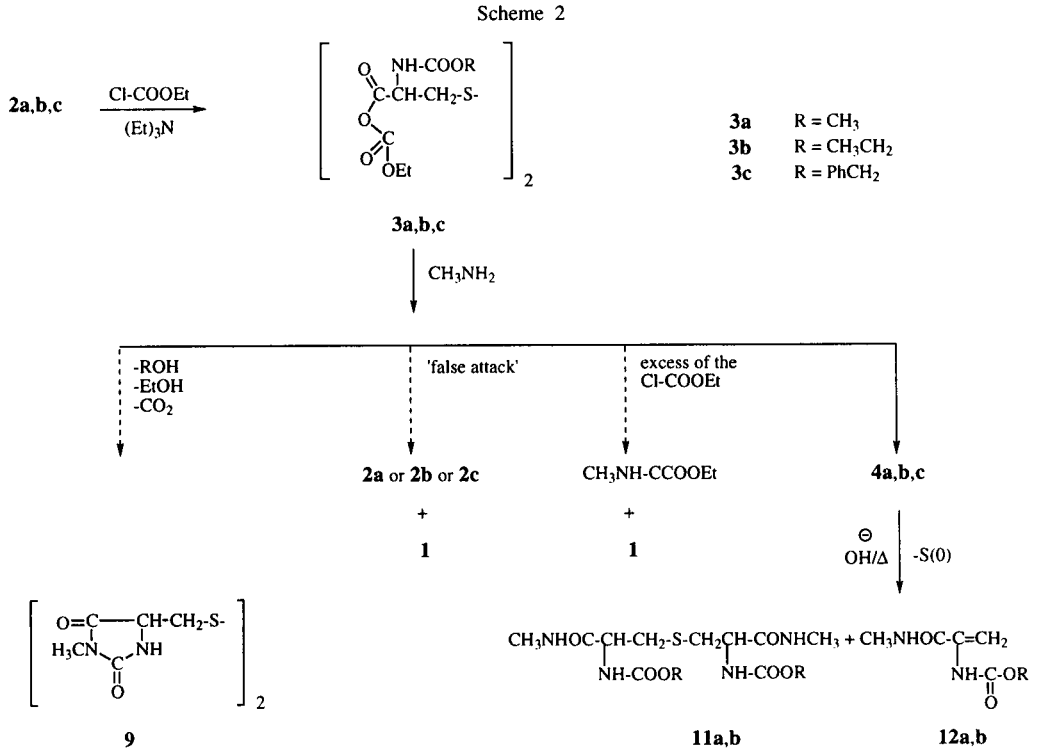
[a] **A**: Molselect 4A (Zeolite); [b] **B**: Kruppodex cross-linked dextran (120-320μ).

Surprisingly, the syntheses through mixed anhydride gave the best results. The blocked cystine bis(methylamides) **4a,b,c** have been produced by a 'test-tube reaction' in three steps, with good yields from **2a,b,c** compounds (Scheme 2).

The method was first described by Boissonnas [5]. This 'reaction' has been known for a long time in peptide chemistry. Though the mixed anhydride method is excellent for the primary amino group acylation even in aqueous medium, it is not widely used, because of the requirement of the racemization-free products and the possible undesired secondary reactions *i.e.*, a partial disproportionation of the mixed anhydride **3** that provides symmetric amino acid anhydride or symmetric carbonic acid semiester anhydride that changes into carbon dioxide and carbonic acid-diester at once [6].

During the transformation of the urethane type diacylcystines **2a,b,c** that we transformed practically without racemization into the amides **4a,b,c** by water-containing

Scheme 2



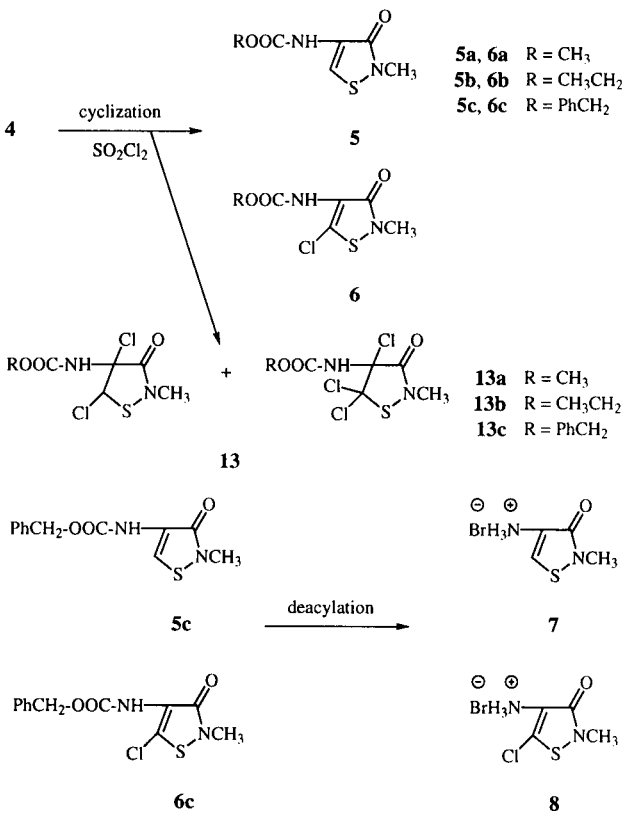
methylamine, there are also other secondary reactions (Scheme 2). Bis-hydantoin **9** is formed from the mixed dianhydride **3** by alcohol elimination. In the case of excess ethyl chloroformate *N*-methyl-urethane **10** is produced, and also the methylaminolytic cleavage of the mixed anhydrides on the 'false' carboxyl C atom must be taken into consideration [7-8]. From the target compounds of amidation **4a,b,c** due to excess base, or to a slight thermal effect, further secondary products **11a,b,c** and **12a,b,c** with β-elimination may be formed [9] (Scheme 2).

To suppress the secondary reactions we applied the so-called 'REMA' (Repetitive Excess Mixed Anhydride) method during the methylaminolysis [10]. The cleavage of the disulfide bond and the β-elimination, as well as the hydantoin formation can be suppressed by excess of the anhydrides. The connecting was carried out at -15 to -17°, thus both the disproportionation and the nucleophilic attack on the 'false' carboxyl group can be suppressed.

The optical purity of our compounds **4a,b,c** produced by the methylaminolysis of **3a,b,c** has not been examined because the optical isomers have no role during the cyclization reactions of these compounds. Compounds **5** and **6** were obtained from **4a,b,c** [11]. The ring has been closed by sulfuryl chloride in dry solvent at 20-60° (Scheme 3).

The molar ratio of the ring-closing agent to the original amides has been changed in 3,5-6,5-intervals. Apart from this parameter, it is the reaction temperature and medium, as well as the rate of feeding that can influence the quality of the products and the relative amount of the 2-methyl-4-(acyl)amino-4-isothiazolin-3-ones unsubstituted or chlorine-substituted on the 5-C (**5a-6a**, **5b-6b**, **5c-6c**). In case of applying higher amounts of sulfuryl chloride compounds **5**, **6** will be contaminated with the extremely well soluble, not separately isolated dichlorine- or trichlorine-isothiazolidin-3-ones **13** produced by further chlorination

Scheme 3



of the ring closers' intermediates [12-13] (Scheme 3). The deacylation of **5c** and **6c** have been carried out by hydrogen bromide in glacial acetic acid at room temperature (Scheme 3). The obtained 4-amino-4-isothiazolin-3-one hydrobromide salts are excellently soluble in water but their storage stability is low. The experiments are summarized in Table 2.

Table 2

Examination of Ring Closing Reactions of the Amino Blocked Cystine Bis(methylamides)

Original Amide	Solvent	SO_2Cl_2/Amide Molar Ratio	Rate of Feeding (ml/hour)	Temperature (°C)	Product
4a	CH_2Cl_2:Hexane (1:1)	4	9.6	35-40	**5a**
4a	Hexane	6	19.2	30	**6a**
4b	$CHCl_3$	3.5	3.4	Boiling	**5b**
4b	CCl_4	6.5	20.8	25	**6b**
4c	CH_2Cl_2:Hexane (1:1)	4	6.4	35-40	**5c**
4c	$CHCl_3$	6.5	31.2	50	**6c**
4a	CCl_4	7	20	30-35	**5a + 6a + 13a**
4b	CCl_4	7.5	20	20	**5b + 6b + 13b**
4c	CCl_4	7.5	25	55	**5c + 6c + 13c**

We examined the antimicrobial characteristics of the newly produced compounds. The results are summarized in Table 3. Data of Table 3 indicate that out of the newly produced compounds, **5c** and **6c** merit further investigations in respect of their antimicrobial effects. The toxicological tests of the new **5a,b,c** and **6a,b,c** derivatives are in progress.

Table 3

Microbiological Activity [a]

Compound		Minimum Inhibitory Concentration MIC (μg/ml) [b] *Staph* [c]	*E.Coli* [c]	*Pseud* [c]	*C.Alb* [c]	*A.niger* [c]
	A [c]	250	250	250	120	1000
5c	B [c]	1	120	120	60	120
	A [c]	250	250	250	120	500
6c	B [c]	30	120	120	2	250
	A [c]	1000	1000	1000	500	>1000
6b	B [c]	30	250	120	60	1000
	A [c]	>1000	1000	>1000	1000	>1000
5b	B [c]	60	1000	500	500	500
	A [c]	1000	1000	>1000	1000	>1000
5b + **6b** (1:3)	B [c]	8	500	250	250	1000
	A [c]	>1000	1000	1000	500	>1000
6a	B [c]	8	500	500	250	>1000
	A [c]	>1000	1000	>1000	1000	>1000
5b + **6b** (1:1)	B [c]	60	500	500	250	>1000
	A [c]	>500	1000	1000	250	>1000
5a	B [c]	120	500	500	120	>1000
	A [c]	>1000	>1000	>1000	>1000	>1000
7	B [c]	1000	1000	1000	1000	>1000
	A [c]	>1000	500	500	500	>1000
8	B [c]	250	120	120	500	1000

[a] The examinations were carried out in bacterium cultures of 10^6 germs/ml or fungus cultures of 10^6 spores/ml; [b] The minimal microbicide/fungicide concentrations of the ethanol solution of the compounds under examination are given in μg/ml; [c] Abbreviations used in the table: **A**: treatment time, 30 minutes; **B**: treatment time, 72 hours; *Staph* = *Staphylococcus aureus*; *E.coli* = *Escherichia coli*; *Pseud* = *Pseudomonas aeruginosa*; *C.alb* = *Candida albicans*; *A.niger* = *Aspergillus niger*.

EXPERIMENTAL

General.

Melting points (uncorrected) were taken on a Boëtius instrument. The ir spectra were obtained on a Pye Unicam Sp 1100 spectrophotometer using KBr pellets. Mass spectra were recorded on a Jeol JMS-01SG2 mass spectrometer, the ionization energy was 75 eV. The nmr measurements were carried out on a FX-100 multinuclear instrument. Chemical shift are given relative to $\delta_{TMS} = 0.00$.

(*R,R*)-*N,N'*-Dimethoxycarbonyl-3,3'-dithiobis(2-aminopropionic acid) (**2a**).

L-Cystine **1** (24 g, 0.1 mole) was dissolved under continuous stirring in 200 ml aqueous solution of 1*M* sodium hydroxide (0.2 mole) in a 2 liter four-neck round-bottom flask equipped with a stirrer, refluxing cooler, thermometer and dropping funnel. Subsequently the solution was cooled to 0-5° and 31 ml (0.4 mole) of methyl chloroformate (d = 1.223) and the solution of 48.4 g (0.456 mole) of sodium carbonate in 730 ml water were added alternately dropwise to it, so that the *p*H value of the reaction mixture was kept between 8-9 and the temperature below 0-5°. After that the reaction mixture was warmed up to room temperature and stirred for an additional 2 hours. Then the mixture was acidified to a *p*H value of 1 by adding 1*M* hydrocloric acid. The volume of the reaction mixture was concentrated to one-third *in vacuo* and the residue was extracted 3 times by 400 ml of ethyl acetate. The organic phases were collected, dried over anhydrous sodium sulfate, filtered and the filtrate was evaporated *in vacuo*. The residue was dried until a constant weight was reached in a vacuum desiccator over phosphorus (V) oxide to yield 35.6 g (100%) of **2a**, mp 68-70°; ir (potassium bromide) ν 3350 (NH), 2950 (CH_2, CH_3), 2600 (SCH_2), 1710 (C=O) cm^{-1}; ^1H nmr (60 MHz) (deuteriochloroform): δ 3.79 (d, 2H, S-CH_2), 4.05 (s, 3H, O-CH_3), 5.65 (br, 1H, NH), 8.27 ppm (s, 1H, COOH).

Anal. Calcd. for $C_{10}H_{16}N_2O_8S_2$: C, 33.70; H, 4.52; N, 7.86; O, 35.91. Found: C, 33.59; H, 4.47; N, 7.73; O, 35.77.

(*R,R*)-*N,N'*-Diethoxycarbonyl-3,3'-dithiobis(2-aminopropionic acid) (**2b**).

L-Cystine **1** (12 g, 0.05 mole) was dissolved under continuous stirring in 50 ml aqueous solution of 2*M* sodium hydroxide (0.1 mole). The solution of the L-cystine disodium salt was cooled to 0° and a solution of 6.63 g (0.0625 mole) of sodium carbonate in 100 ml of water plus 12.4 ml (0.129 mole) of ethyl chloroformate (d = 1.135) were added alternately dropwise to the solution. The *p*H value of the reaction mixture was kept between 8-9, and the temperature was kept at 0°. The reaction mixture was stirred for 1.5 hours at room temperature, then it was acidified to a *p*H value of 1 by adding hydrochloric acid diluted to 1:1 with water. The volume of the obtained reaction

mixture was concentrated to one-third *in vacuo* and the residue was extracted 3 times by 100 ml of ethyl acetate. The organic phases were collected, dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. The residue was dried until a constant weight was reached in a vacuum desiccator over phosphorus (V) oxide, to yield 1.8 g (98%) of **2b**, mp 42°; ir (potassium bromide) ν 3320 (NH), 2980 (CH_2CH_3), 1700 (C=O), 1520 (amide II band), 1220 (amide III band) cm^{-1}; ^1H nmr (60 MHz) (deuteriochloroform): δ 1.25 (t, 3H, CH_3), 3.30 (br, 2H, S-CH_2), 4.10 (qa, 2H, O-CH_2), 4.79-4.67 (m, 1H, CH), 5.85 (br, 1H, NH), 8.81 ppm (s, 1H, COOH).

Anal. Calcd. for $C_{12}H_{20}N_2O_8S_2$: C, 37.49; H, 5.24; N, 7.28; O, 33.29. Found: C, 37.35; H, 5.17; N, 7.13; O, 33.18.

(*R,R*)-*N,N'*-Dibenzyloxycarbonyl-3,3'-dithiobis(2-aminopropionic acid) (**2c**).

L-Cystine **1** (12 g, 0.05 mole) was dissolved under continuous stirring in 50 ml aqueous solution of 2*M* sodium hydroxide (0.1 mole) and it was diluted with 25 ml water. The solution was cooled to 0° and 20 ml of benzene was added. The solution of 21.6 ml (0.151 mole) of benzyl chloroformate (d = 1.195) in 170 ml benzene and 86 ml aqueous solution of 2*M* sodium hydroxide (0.172 mole) were added then to the reaction mixture alternately dropwise. The temperature of the reaction mixture was kept at 0°, the *p*H value between 8-9. The reaction mixture was stirred for an additional 1 hour at 5-10°, and for 3 hours at room temperature. The reaction mixture was spontaneously separated into two phases overnight. The phases were separated in a separating funnel. The aqueous phase was washed twice with 50 ml of benzene, diluted with 300 ml of water and added dropwise into 13.2 ml of hydrochloric acid diluted to 1:1 with water. After acidification, the reaction mixture was stirred for 1 hour, then cooled to 15°. The precipitated product was filtered off, washed on the filter with a small amount of water, dried to yield 23.9 g of **2c** (94%), mp 80-90°. The crude product was dissolved in 300 ml of ethyl acetate, the non-soluble mono-acyl-L-cystine was filtered on a Seitz-sheet, the filtrate was evaporated *in vacuo*. The residue was dissolved in 250 ml of chloroform:hexane (3:5) mixture and cooled in a refrigerator. The crystalline product was filtered and dried to yield 18 g (71%) of **2c**, mp 114-117°, (softens at 80°); ir (potassium bromide) ν 3350 (NH), 3080 (CH=), 2950 (CH_2), 1730 (C=O), 1530 (amide II band), 1270 (amide III band), 730 (γ CH=) cm^{-1}; ^1H nmr (60 MHz) (dimethyl sulfoxide-d_6): δ 2.96 (d, 2H, S-CH_2), 4.18-3.92 (m, 1H, CH), 4.89 (s, 2H, Ar-CH_2O), 6.51 (br, 1H, NH-urethane) 7.18 (s, 5H, Ar-H), 7.86 ppm (s, 1H, COOH).

Anal. Calcd. for $C_{22}H_{24}N_2O_8S_2$: C, 51.95; H, 4.75; N, 5.50; O, 25.16. Found: C, 51.82; H 4.69; N, 5.47; O, 25.08.

(*R,R*)-*N,N'*-Dimethoxycarbonyl-3,3'-dithiobis(2-aminopropionic acid) Bis(methylamide) (**4a**).

To the mixture of *N,N'*-dimethoxycarbonyl-L-cystine **2a** 17.65 g (0.0482 mole) and 300 ml of dichloromethane was added triethylamine (13.5 g, 0.0964 mole) (d = 0.73; 99% purity) under continuous stirring. The mixture was cooled -15°, into it was dropped ethyl chloroformate 10.15 ml (0.106 mole) at such a rate as to keep the reaction temperature below -14°. Then aqueous methylamine solution 6.55 ml (0.0925 mole) (c = 50.3 weight-%, d = 0.87) was added to it at -15° and the reaction mixture was warmed to room temperature followed by 1.5 hours stirring. The mixture was cooled to 0°, the *p*H was adjusted to 8 with saturated aqueous potassium hydrogen carbonate solution, stirred for another 30 minutes, and was allowed to warm up to room temperature. The solvent was evaporated *in vacuo*, the residue was triturated with 200 ml absolute ethanol and was crystallized in a refrigerator for a few hours. The precipitated crystalline product was filtered, washed on the filter with 100 ml of cooled ethanol in several parts, then was dried. The product of **4a** was obtained 12.45 g, (68%) mp 190-191°; ir (potassium bromide) ν 3300 (NH), 2950 (CH_2, CH_3), 1680 (C=O), 1645 (amide I band), 1510 (amide II band), 1265 (amide III band) cm^{-1}; ^1H nmr (60 MHz) (dimethyl sulfoxide-d_6 + deuteriochloroform): δ 2.75 (d, 3H, N-CH_3), 3.17 (s, 2H, S-CH_2), 3.65 (s, 3H, O-CH_3), 6.75 (d, 1H, NH-urethane), 7.64 ppm (s, 1H, NH); ms: m/z 386 (M^+) (14), 324 (14), 224 (18), 192 (58), 191 (12), 190 (12), 159 (100), 134 (94), 117 (99,5), 58 (60).

Anal. Calcd. for $C_{12}H_{22}N_4O_6S_2$: C, 37.68; H, 5.79; N, 14.65. Found: C, 37.64; H, 5.73; N, 14.51.

(*R,R*)-*N,N'*-Diethoxycarbonyl-3,3'-dithiobis(2-aminopropionic acid) Bis(methylamide) (**4b**).

To the mixture of 17.1 g (0.0446 mole) *N,N'*-diethoxycarbonyl-L-cystine **2b** and 350 ml of dichloromethane was added 12.5 ml (0.0892 mole) of triethylamine and it was cooled to -15° under continuous stirring. Ethyl chloroformate 8.54 ml (0.892 mole) was dropped to the mixture at such a rate as to keep the reaction temperature below -14°. After that 6.31 ml (0.0892 mole) of methylamine solution was added dropwise at -15°. The reaction mixture was allowed to warm to room temperature followed by 1.5 hours of stirring. The solvent was evaporated *in vacuo*, the residue was triturated with 150 ml of water and was crystallized in a refrigerator for a few hours. The precipitate was filtered, washed on the filter by some cooled ethyl acetate, and was dried. The product of **4b** was obtained 12.62 g, (69%) mp 200-201°; ir (potassium bromide) ν 3305 (NH), 2950 (CH_2, CH, CH_3), 1680 (C=O), 1645 (amide I band), 1520 (amide II band), 1260 (amide III band) cm^{-1}; ^1H nmr (60 MHz) (dimethyl sulfoxide-d_6): δ 1.10 (t, 3H, CH_3), 2.34 (d, 3H, N-CH_3), 3.04 (s, 2H, S-CH_2), 3.80 (qa, 2H, O-CH_2), 7.04 (d, 1H, NH-urethane), 7.65 ppm (m, 1H, NH).

Anal. Calcd. for $C_{14}H_{26}N_4O_6S_2$: C, 40.96; H, 6.38; N, 13.65. Found: C, 40.98; H, 6.24; N, 13.47.

(*R,R*)-*N,N'*-Dibenzyloxycarbonyl-3,3'-dithiobis(2-aminopropionic acid) Bis(methylamide) (**4c**).

To the mixture of 10.7 g (0.021 mole) *N,N'*-dibenzyloxycarbonyl-L-cystine **2c** and 150 ml of anhydrous ethyl acetate was added 5.9 ml (0.042 mole) of triethylamine and cooled to -17° under continuous stirring. Ethyl chloroformate 4.22 ml (0.0441 mole) was added to the mixture at such a rate as to keep the temperature of the reaction mixture below -16°. Then 2.38 ml (0.0336 mole) of methylamine was added and allowed to warm to room temperature. The reaction mixture was stirred for 1.5 hours, then it was cooled to 0°. The *p*H was adjusted to 8 by saturated aqueous potassium hydrogen carbonate solution, the mixture was stirred for 30 minutes, and the solvent was evaporated *in vacuo*. The obtained crude product was recrystallized from 450 ml hot mixture of isopropanol:methanol:water (10:10:3), filtered and dried to yield 6.55 g (73%) of **4c**, mp 195-196°; ir (potassium bromide) ν 3350 (NH), 1685 (C=O), 1645 (amide I band), 1520 (amide II band), 1260 (amide III band) cm^{-1}; ^1H nmr (60 MHz) (deuteriochloroform): δ 2.78 (d, 6H, N-CH_3), 2.96 (d, 2H, S-CH_2),

4.82 (m, 1H, CH-NH), 5.11 (s, 2H, Ar-CH_2O), 5.85 (d, 1H, NH-CO_2), 7.32 ppm (s, 5H, Ar-H).

Anal. Calcd. for $C_{24}H_{30}N_4O_6S_2$: C, 53.91; H, 5.65; N, 10.49. Found: C, 53.54; H, 5.80; N, 10.25.

4-(Methoxycarbonyl)amino-2-methyl-4-isothiazolin-3-one (**5a**).

To the mixture of 3.82 g (0.01 mole) *N,N'*-dimethoxycarbonyl-L-cystine bis(methylamide) **4a**, 50 ml of dichloromethane and 50 ml of hexane was added dropwise at 35-40° under continuous stirring 3.2 ml (0.04 mole) of sulfuryl chloride (d = 1.68) over 20 minutes. The mixture was stirred for 4 hours, cooled to 15° and filtered. The product was washed on the filter with a small amount of cooled 2-propanol and dried to yield 1.6 g (43%) of **5a**, mp 166-167°; ^{1}H nmr (500 MHz) (deuteriochloroform): δ 3.39 (s, 3H, N-CH_3), 3.79 (s, 3H, O-CH_3), 7.43 (br, 1H, NH), 8.05 ppm (s, 1H, 5-CH=).

Anal. Calcd. for $C_6H_8N_2O_3S$: C, 38.29; H, 4.28; N, 14.88; S, 17.04. Found: C, 37.96; H, 4.32; N, 14.53; S, 16.66.

4-(Ethoxycarbonyl)amino-2-methyl-4-isothiazolin-3-one (**5b**).

To the mixture of 4.1 g (0.01 mole) *N,N'*-diethoxycarbonyl-L-cystine bis(methylamide) **4b** and 70 ml of chloroform was added dropwise 2.8 ml (0.035 mole) of sulfuryl chloride under continuous stirring and boiling over 50 minutes. The mixture was stirred and boiled for 2.5 hours, cooled to room temperature and extracted three times with 50 ml water in a separating funnel. The organic phase was dried on anhydrous sodium sulfate, filtered and the filtrate was evaporated *in vacuo*. The obtained yellowish crystalline material was triturated in 10 ml acetone, cooled, then filtered and dried to yield 2.45 g (61%) of **5b**, mp 168.5-171.5°; ^{1}H nmr (80 MHz) (deuteriochloroform): δ 1.26 (t, 3H, CH_2CH_3), 3.40 (s, 3H, N-CH_3), 4.20 (qa, 2H, O-CH_2), 7.28 ppm (br, 1H, NH).

Anal. Calcd. for $C_7H_{10}N_2O_3S$: C, 41.55; H, 4.98; N, 13.85; O, 23.73; S, 15.86. Found: C, 41.38; H, 4.97; N, 13.67; O, 23.63; S, 16.01.

4-(Benzyloxycarbonyl)amino-2-methyl-4-isothiazolin-3-one (**5c**).

To a mixture of 5.34 g (0.01 mole) *N,N'*-dibenzyloxycarbonyl-L-cystine bis(methylamide) **4c**, 50 ml of hexane and 50 ml of dichloromethane was added 3.2 ml (0.04 mole) of sulfuryl chloride at 35-40° under continuous stirring over 30 minutes. The reaction mixture was stirred for 4 hours, cooled to room temperature, and the solvent was evaporated *in vacuo*. The obtained crude product was dissolved in 20 ml 96% ethanol, crystallized in a deep-freezer for several hours. After that the precipitated product was filtered and dried to yield 3.3 g (63%) of **5c**, mp 119-121°; ^{1}H nmr (80 MHz) (deuteriochloroform): δ 3.36 (s, 3H, N-CH_3), 5.23 (s, 2H, O-CH_2), 7.39 (s, 5H, Ar-H), 8.05 ppm (s, 1H, 5-CH=).

Anal. Calcd. for $C_{12}H_{12}N_2O_3S$: C, 54.53; H, 4.57; N, 10.59; O, 18.16. Found: C, 54.88; H, 4.97; N, 10.32; O, 18.33.

5-Chloro-4-(methoxycarbonyl)amino-2-methyl-4-isothiazolin-3-one (**6a**).

To the mixture of 3.82 g (0.01 mole) *N,N'*-dimethoxycarbonyl-L-cystine bis(methylamide) **4a** and 200 ml of hexane was added dropwise 4.8 ml (0.06 mole) of sulfuryl chloride at 30° over 15 minutes under continuous stirring. The mixture was stirred for 4 hours at 50-55°, cooled to room temperature and filtered. The product was washed on the filter with a small amount of hexane and dried to yield 2.3 g (52%) of **6a**, mp 154-155°; ^{1}H nmr (80 MHz) (deuteriochloroform): δ 3.33 (s, 3H, N-CH_3), 3.73 (s, 3H, O-CH_3), 7.12 ppm (br, 1H, NH).

Anal. Calcd. for $C_6H_7ClN_2O_3S$: C, 32.37; H, 3.17; N, 12.58; O, 21.56; S, 14.40. Found: C, 32.90; H, 3.31; N, 12.52; O, 21.91; S, 14.20.

5-Chloro-4-(ethoxycarbonyl)amino-2-methyl-4-isothiazolin-3-one (**6b**).

To the mixture of 4.1 g (0.01 mole) N,N'-diethoxycarbonyl-L-cystine bis(methylamide) **4b** and 100 ml of carbon tetrachloride was added dropwise 5.2 ml (0.065 mole) of sulfuryl chloride under continuous stirring at 25° over 15-20 minutes. The reaction mixture was warmed to 40° and stirred for 2 hours, then cooled to room temperature and filtered. The filtrate was evaporated. A small amount of cooled acetone was added to the distillation residue, the precipitated product was filtered and dried to yield 3.1 g (66%) of **6b**, mp 146-149°; ^{1}H nmr (80 MHz) (deuteriochloroform): δ 1.26 (t, 3H, CH_2CH_3), 3.30 (s, 3H, N-CH_3), 4.20 (qa, 2H, CH_2CH_3), 7.20 ppm (br, 1H, NH).

Anal. Calcd. for $C_7H_9ClN_2O_3S$: C, 35.53; H, 3.83; N, 11.84; O, 20.28; S, 13.55. Found: C, 35.59; H, 3.91; N, 11.77; O, 20.31; S, 13.52.

4-(Benzyloxycarbonyl)amino-5-chloro-2-methyl-4-isothiazolin-3-one (**6c**).

To the mixture of 5.34 g (0.01 mole) *N,N'*-dibenzyloxycarbonyl-L-cystine bis(methylamide) **4c** and 100 ml of chloroform was added dropwise 5.2 ml (0.065 mole) of sulfuryl chloride under continuous stirring at 50° over 10 minutes. The reaction mixture was stirred for 5 hours, then cooled to room temperature and filtered. The solvent was evaporated from the filtrate *in vacuo*. The residue was dissolved in 25 ml absolute ethanol and cooled in a refrigerator for a few hours. The obtained crystalline product was filtered and dried to yield 3.15 g (53%) of **6c**, mp 111.5-113°; ^{1}H nmr (80 MHz) (deuteriochloroform): δ 3.2 (s, 3H, N-CH_3), 5.1 (s, 2H, O-CH_2), 7.3 ppm (s, 5H, ArH).

Anal. Calcd. for $C_{12}H_{11}ClN_2O_3S$: C, 48.24; H, 3.71; N, 9.38; S, 10.73. Found: C, 48.24; H, 3.77; N, 9.44; S, 10.41.

4-Amino-2-methyl-4-isothiazolin-3-one Hydrobromide (**7**).

In a flask equipped with a mechanical stirrer and dropping funnel was placed 2.64 g (0.01 mole) of 4-(benzyloxycarbonyl)-amino-2-methyl-4-isothiazolin-3-one **5c**. To it was added dropwise 9 ml (0.05 mole) of hydrogen bromide solution in glacial acetic acid (33 weight-percent, d = 1.38) at room temperature. A precipitate was observed after 10-15 minutes. The reaction mixture was stirred for 2-3 hours, then the precipitated product was filtered, washed on the filter with a small amount of carbon tetrachloride, dried to yield 1.65 g (78%) of **7**, mp 187-193° (dec); ^{1}H nmr (80 MHz) (deuteriochloroform + tetradeuteriomethanol): δ 3.36 (s, 3H, N-CH_3), 7.56 ppm (br, NH_3).

Anal. Calcd. for $C_4H_7BrN_2OS$: C, 22.76; H, 3.34; N, 13.27; S, 15.19. Found: C, 22.56; H, 3.20; N, 13.08; S, 15.07.

5-Chloro-4-amino-2-methyl-4-isothiazolin-3-one Hydrobromide (**8**).

A solution of hydrogen bromide in glacial acetic acid (18 ml, 0.1 mole) was added to 2.98 g (0.01 mole) of 5-chloro-4-(benzyloxycarbonyl)amino-2-methyl-4-isothiazolin-3-one **6c** at

room temperature over a few minutes under continuous stirring. A clear solution was obtained for a short while, then a precipitation was observed. The reaction was completed after 3 hours stirring at 45-50°. The mixture was cooled to room temperature, the precipitated product was filtered, washed on the filter with a small amount of cooled absolute ethanol, dried to yield 1.6 g (65%) of **8**, mp *ca.* 200° (dec); ^{1}H nmr (80 MHz) (deuteriochloroform + tetradeuteriomethanol): δ 3.41-3.33 (m, N-CH_3), 7.50 (s, 2H, NH_2), 8.42 ppm (br, NH_3).

Anal. Calcd. for $C_4H_6BrClN_2OS$: C, 19.56; H, 2.46; N, 11.41; S, 13.06. Found: C, 20.02; H, 2.51; N, 11.80; S, 13.01.

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