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D 1074-1 EP

91-06-12

SD/EN/ie

Novel 3,3'-dithiobis(propionic acids) and esters thereof.

This Sheet Has Been Replaced



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Novel 3,3'-dithiobis (propionic acids) and esters thereof.

5 Field of the Invention

The present invention relates to new cystine derivatives with immunomodulating activity, processes for their preparation, pharmaceutical compositions containing them
10 and methods for their pharmacological use.

The object of the invention is to provide a cystine derivative with immunomodulating activity. Such a substance will be useful in the treatment of various
15 diseases.

Background of the Invention

N-Acetyl-L-cysteine is a compound widely used for treating
20 chronic obstructive airway diseases/chronic bronchitis (for further references see Multicentre Study Group. Long-term oral acetylcysteine in chronic bronchitis. A double-blind controlled study. Eur. J. Respir. Dis. 1980, 61 (suppl. 111), 93-108; Boman, G., Bäcker, U., Larsson, S.,
25 Melander, B., and Wåhlander, L. Oral acetylcysteine reduces exacerbation rate in chronic bronchitis. Report of a trial organized by the Swedish Society for Pulmonary Disease. Eur. J. Respir. Dis. 1983, 64, 405-415; and British Thoracic Society Research Committee. Oral N-
30 acetylcysteine and exacerbation rates in patients with chronic bronchitis and severe airway obstruction. Thorax 1985, 40, 832-835). The mechanism of action of the compound is not disclosed; its effect has been attributed to mucolytic properties (see Multicentre Study Group.
35 Long-term oral acetylcysteine in chronic bronchitis. A



double-blind controlled study. Eur. J. Respir. Dis. 1980, 61 (suppl. 111), 93-108; Boman, G., Bäckér, U., Larsson, S., Melander, B., and Wåhländer, L. Oral acetylcysteine reduces exacerbation rate in chronic bronchitis. Report of
 5 a trial organized by the Swedish Society for Pulmonary Disease. Eur. J. Respir. Dis. 1983, 64, 405-415; and British Thoracic Society Research Committee. Oral N-acetylcysteine and exacerbation rates in patients with chronic bronchitis and severe airway obstruction. Thorax
 10 1985, 40, 832-835), antioxidant properties (see Aruoma, O.I., Halliwell, B., Hoey, B.M., and Butler, J. Free Radical Biol. Med. 1989, 6, 593-597), and also immunomodulating properties (see Bergstrand, H., Björnson, A., Eklund, A., Hernbrand, R., Eklund, A.,
 15 Larsson, K., Linden M., and Nilsson, A. Stimuli-induced superoxide radical generation in vitro by human alveolar macrophages from smokers: Modulation by N-Acetylcysteine treatment in vivo. J. Free Radicals Biol. & Med. 2, 1986, 119-127).

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Also known is N,N'-diacetyl-L-cystine. This compound has previously shortly been described in the patent literature as well as in the scientific literature (US 4827016; EP 300100; US 4724239; US 4708965; DE 2326444; Wilson, I.D.,
 25 and Nicholson, J.K. Analysis of thiols and disulfides in Sulphur-containing drugs and related organic compounds. Chemistry, Biochemistry and Toxicology (ed L.A. Damani) Vol. 2A. Analytical, biochemical and toxicological aspects of sulphur xenobiochemistry. Ellis Horwood Series
 30 in Biochemical Pharmacology (Halsted Press: a division of John Wiley & Sons) Chichester 1989, p. 45; and Sjödin, K., Nilsson, E., Hallberg, A., and Tunek, A. Metabolism of N-Acetyl-L-cysteine. Some structural requirements for the deacetylation and consequences for the oral bioavail-
 35 ability. Biochem. Pharmacol. 1989, 38, 3981-3985). In US 4827016 the compound is claimed to be effective for

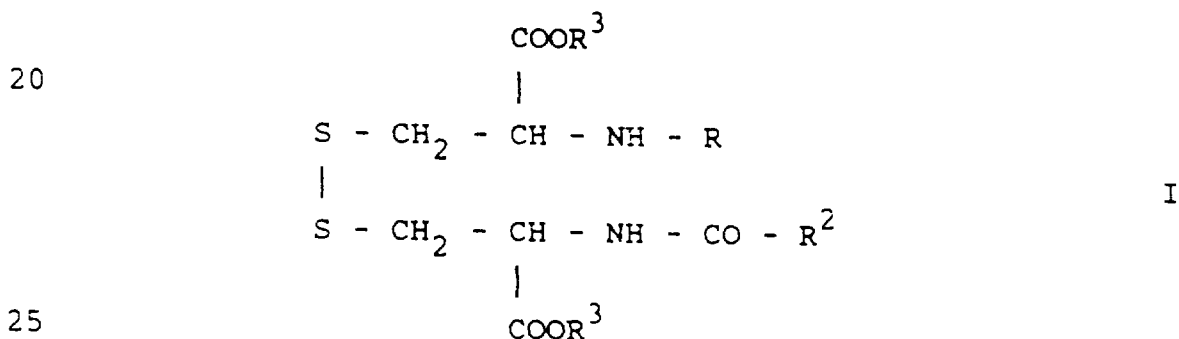


topical treatment of dermal inflammations which are induced and propagated by leukotrienes. However, nothing has been reported or generally known regarding its pharmacological and/or therapeutic properties with respect to immunological systems and inflammatory diseases of the lung such as chronic bronchitis.

Previously, immunostimulating properties have been reported for simple disulfides such as hydroxyethyl disulfide (HEDS, see: St. Georgiev, V. New synthetic immunomodulating agents. Trends in Pharmacological Science 1988, 446-51).

Disclosure of the Invention

According to the present invention it has been found that a compound of the general formula



wherein R is hydrogen or a moiety $-\text{CO}-\text{R}^1$ wherein R^1 is methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, iso-propyl, 1-methylpropyl, tert. butyl, 3-methylbutyl or 2-methylbutyl, R^2 is methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, iso-propyl, 1-methylpropyl, tert. butyl, 3-methylbutyl or 2-methylbutyl, and R^3 is hydrogen, methyl, ethyl, propyl, isopropyl, butyl or isobutyl, provided that



R^1 and R^2 are not simultaneously methyl and further provided that when R^3 is hydrogen R^1 and R^2 are not simultaneously n-propyl or n-heptyl, or a physiologically acceptable salt and/or a stereochemical isomer thereof, is
 5 an immunomodulating, particularly immunostimulating, agent.

Therefore, the compounds of the invention may be used for treatment of diseases where a defect in the immune system
 10 and/or an ineffective host defence is at hand or can be suspected.

Examples of such diseases are chronic bronchitis and other inflammatory diseases of the airways such as asthma and
 15 rhinitis but also certain forms of autoimmune diseases like diabetes and rheumatoid arthritis and/or various malignant diseases. HIV-infection or full blown AIDS may be treated with the compounds. Also atherosclerotic disease may be treated with the compounds.

20 Effective amounts of the compounds of the invention for use in the treatment of the above mentioned diseases are in the range 0.5-500 mg, preferably 5-50 mg, daily dose.

25 Preferred compounds of the formula I above are those wherein R is hydrogen or a moiety $-\text{CO}-R^1$ wherein R^1 is methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, iso-propyl, 1-methylpropyl, tert. butyl, 3-methylbutyl or 2-
 30 methylbutyl, R^2 is methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, iso-propyl, 1-methylpropyl, tert. butyl, 3-methylbutyl or 2-methylbutyl, and R^3 is hydrogen, methyl or ethyl, provided that R^1 and R^2 are not simultaneously
 35 methyl and further provided that when R^3 is hydrogen R^1 and R^2 are not simultaneously n-propyl or n-heptyl.



Preferred compounds of the formula I when R^3 is hydrogen are those wherein R^1 and R^2 are simultaneously isopropyl and tert. butyl, respectively.

Preferred compounds of the formula I above when $R^3 =$
5 hydrogen are those where R^1 and R^2 contain 3-7 carbon atoms.

Particularly, preferred compounds of the formula I above when $R^3 \neq$ hydrogen are those where R^1 and R^2 are
10 isopropyl and R^3 is methyl; R^1 and R^2 are n-pentyl and R^3 is methyl; R^1 and R^2 are n-heptyl and R^3 is methyl or ethyl.

The stereoisomeric form of the compounds of the invention
15 particularly preferred is the L-isomer, i.e. compounds derived from L-cystine.

A physiologically acceptable salt of the compounds of formula I when R^3 is hydrogen is e.g. a salt of sodium, ammonium, calcium or magnesium, together with the non-
20 toxic acid addition salts thereof. Also included are salts derived from arginine, lysine, histidine, ethanolamine, diethanolamine, ethylenediamine and choline, or other suitable organic amines.

25 A physiologically acceptable salt of the compounds of the formula I when $R^3 \neq$ hydrogen and $R =$ hydrogen is the hydrochloride, hydrobromide, hydrosulphate, oxalate, tartrate etc. The salts may also be in the form of solvates, e.g. hydrates.

30

Pharmaceutical formulations

The described active substances can be included in different dosage forms e.g. tablets, coated tablets,
35 gelatin capsules, solutions and aerosols.



For the preparation of tablets, coated tablets and gelatin capsules the active substance can be combined with pharmaceutically acceptable materials, e.g. lactose, starch, dicalcium phosphate, microcrystalline cellulose, polyvinylpyrrolidone, gelatin, cellulose derivatives, colloidal silicone dioxide, talc and stearic acid or its salts.

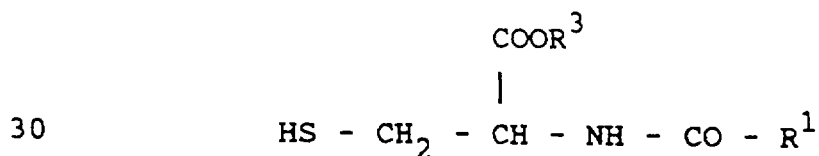
For the preparation of oral solutions suitable excipients are water, saccharose, glucose, sorbitol, fructose and xylitol.

The dosage forms can besides mentioned excipients contain preservatives, stabilizers, viscosity regulating agents, emulsifiers, sweetening agents, colouring agents, flavouring agents, tonicity regulating agents, buffers or antioxidants. They can also contain other therapeutically valuable substances.

20 Methods of Preparation

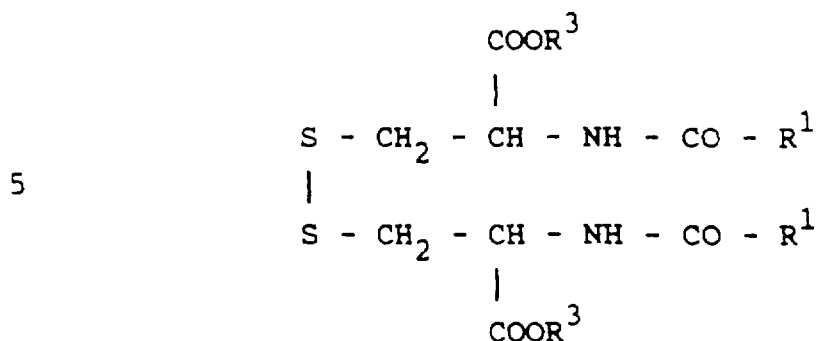
The compounds of the invention may be obtained by any of the following methods:

25 A. The oxidation of an N-acylcysteine derivative of the formula



wherein R^1 and R^3 are as defined above or when R^3 is hydrogen optionally salt thereof, to the formation of a compound of the formula





10 As oxidant may be used:

Hydrogen peroxide, air under alkaline conditions, alkylhydroperoxides, peracids, a halogen, nitrous or nitric oxide, oxidizing metals such as thallium (III), trialkylsulfonium salts, or selenium or tellurium oxides. The

15 oxidation can also be performed electrochemically.

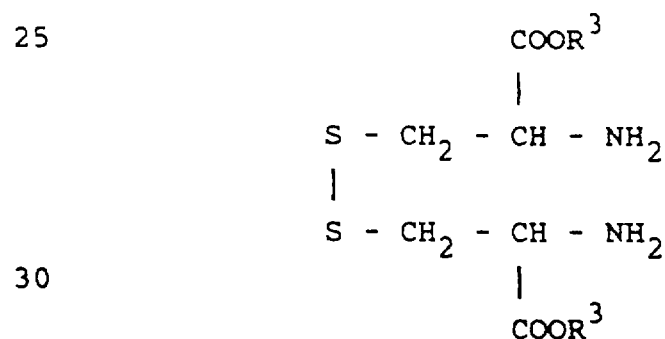
A halogen is for instance chlorine, bromine or iodine.

As salts in method A and the following may be used:

sodium, potassium, calcium, ammonium etc.

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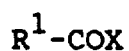
B. The reaction, in the presence of a suitable base, of a compound having the formula



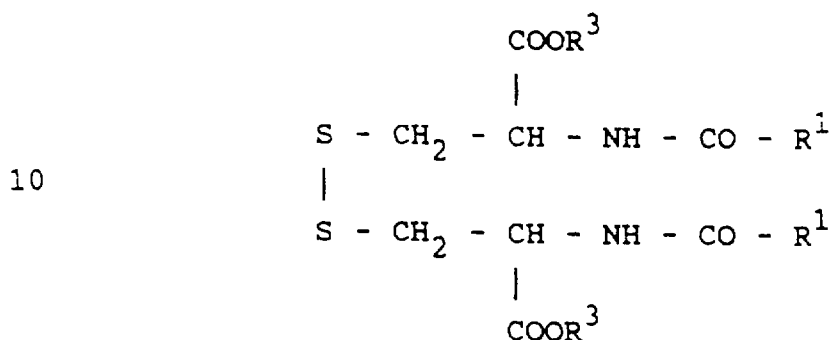
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or a salt thereof wherein R^3 is as defined above, with a compound of the formula

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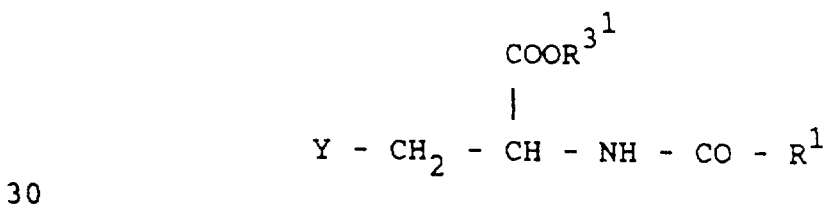
wherein R^1 is defined as above and COX is a reactive group capable of reacting with an amino group under formation of an amide moiety, to the formation of a compound of the
 5 formula



15 The acylating agent $R^1\text{COX}$ can for instance be an acid halide, anhydride, an amide, or an activated acid or ester.

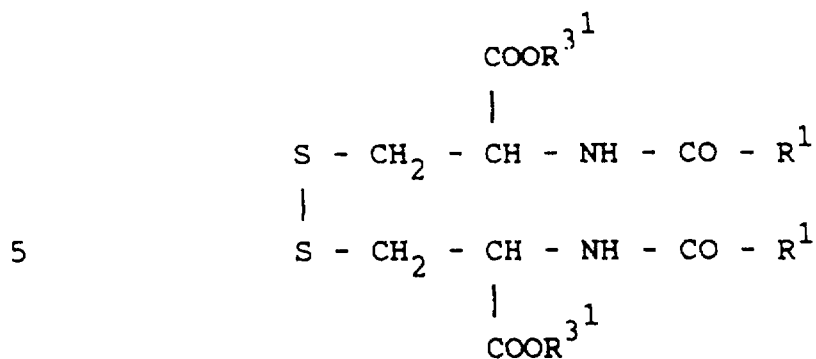
A salt in method B and in any following methods where a
 20 salt can be used can be an hydrochloride, hydrobromide, hydrosulphate, oxalate, tartrate etc. A salt in method B can also be any of the alkali salts mentioned in method A.

C. The reaction of a 2-(N-acylamino)-3-halopropionic acid
 25 derivative of the formula



wherein R^1 is as defined above, R^{3^1} is R^3 as defined above or an acid or base labile organic group and Y is a halogen atom, with sulphur or disulphide dianion in the presence of a base, to the formation of a compound of the formula
 35



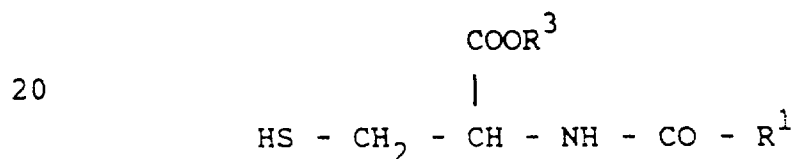


whereafter, if a compound wherein $\text{R}^3 = \text{H}$ is derived,
 10 removal of the protecting group R^{3^1}

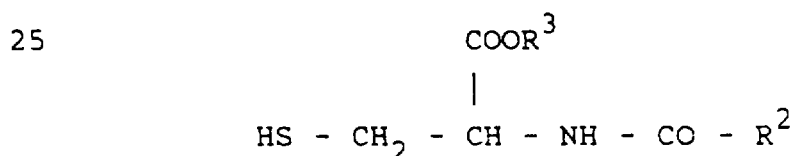
The protecting group, R^{3^1} , may be an acid or base labile
 organic group such as an alkyl, benzyl, aryl, vinyl or an
 allyl group.

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D. The oxidation of a mixture of N-acylcysteine
 derivatives of the formulas

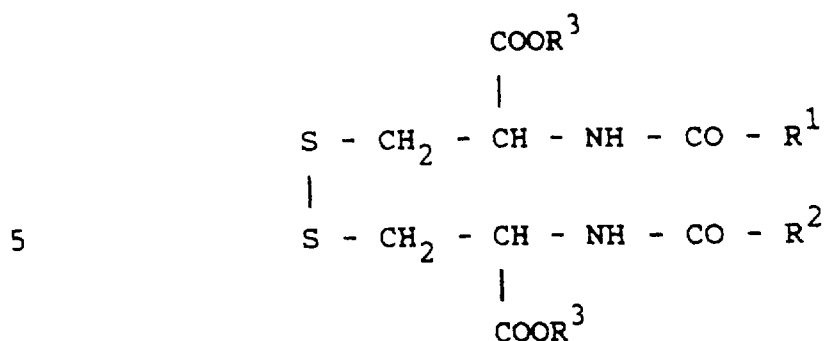


and



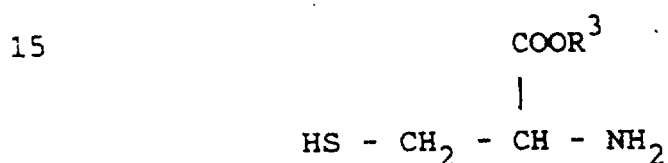
30 wherein R^1 , R^2 and R^3 are as defined above or when R^3 is
 hydrogen optionally alkali salts thereof, to the formation
 of a compound of the formula





10 The oxidising agents of method A are applicable.

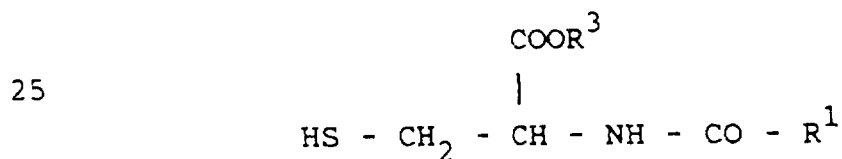
E. The oxidation of a mixture of cysteine or a cysteine ester and an N-acylcysteine derivative of the formulas



or a salt thereof

20

and

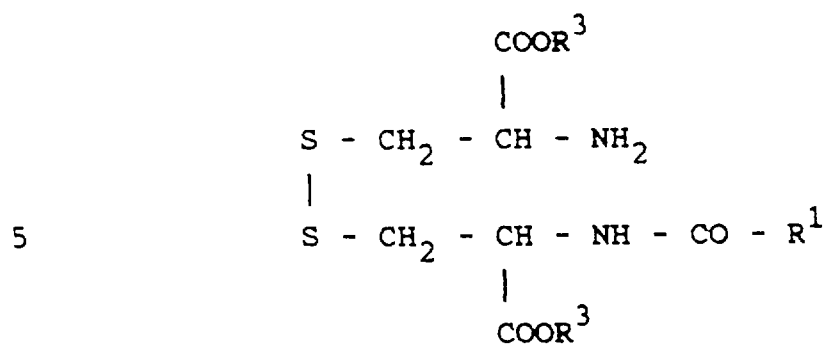


or alkali salts thereof,

wherein R^1 and R^3 are as defined above, to the formation

30 of a compound of the formula

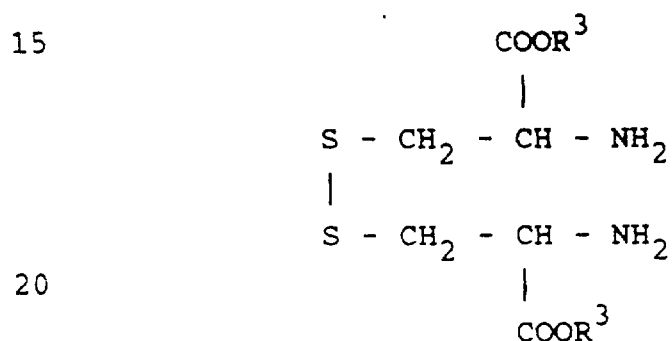




or a salt thereof.

10 The oxidising agents of method A may be used.

F. The reaction, in the presence of a suitable base, of an excess of cystine or a cystine diester having the formula

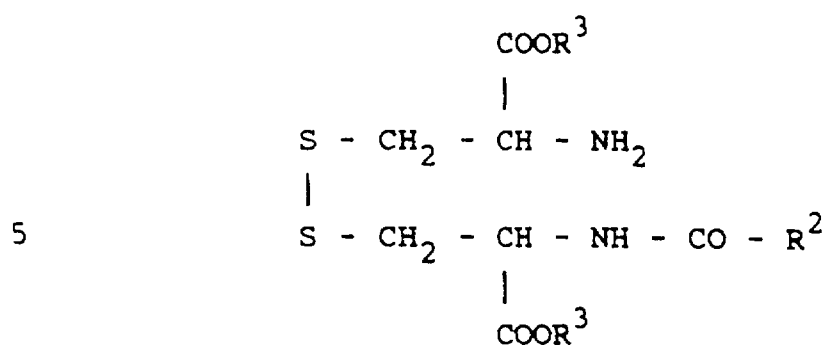


or a salt thereof wherein R^3 is as defined above, with a compound of the formula

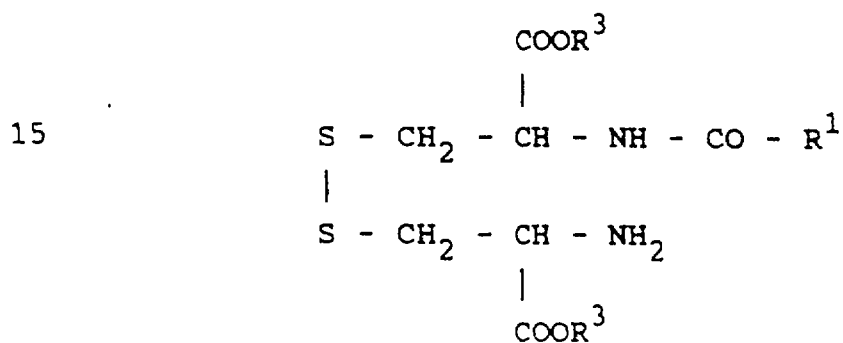


wherein R^2 and COX are as defined above, to the formation of a compound of the formula

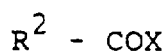




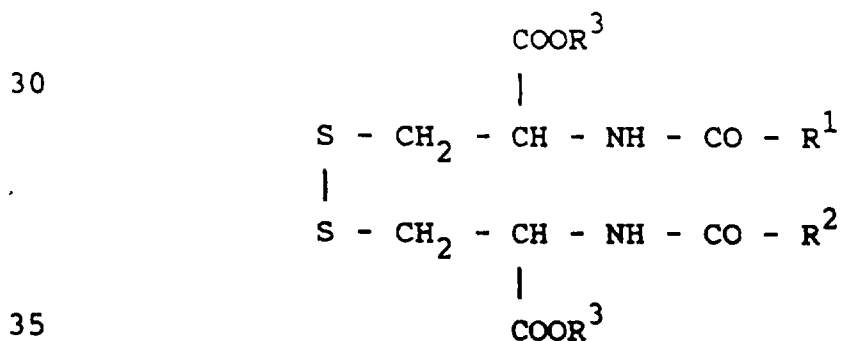
10 G. The reaction, in the presence of a suitable base, of a compound of the formula



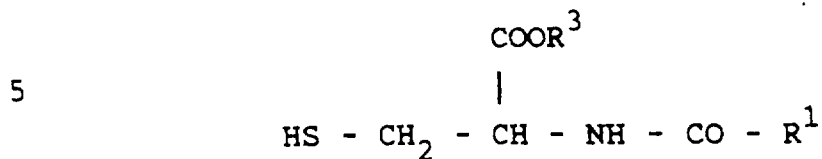
20 or a salt thereof wherein R^1 and R^3 are as defined above, with a compound of the formula



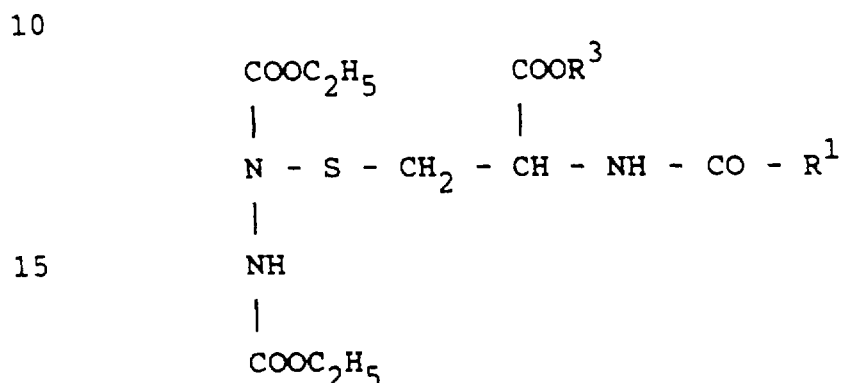
wherein R^2 and COX are as defined above, to the formation of a compound of the formula



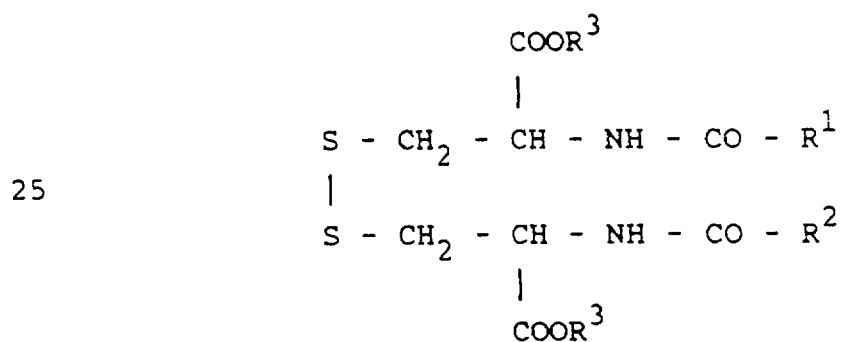
H. The reaction of an N-acylcysteine derivative of the formula



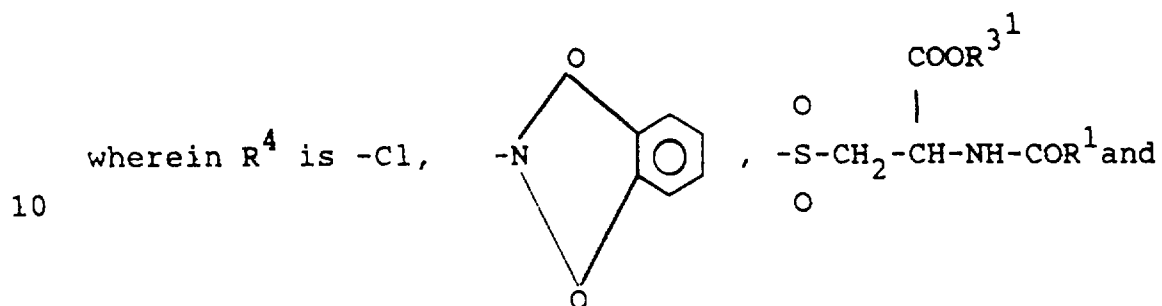
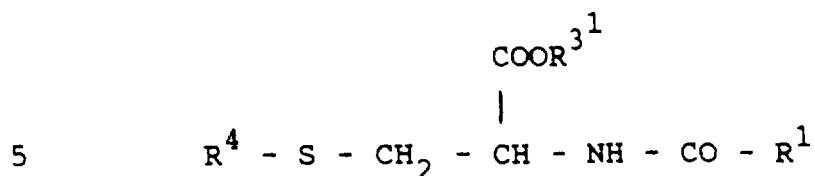
or a salt thereof with an activating reagent such as diethylazodicarboxylate to give an adduct of e.g. the formula



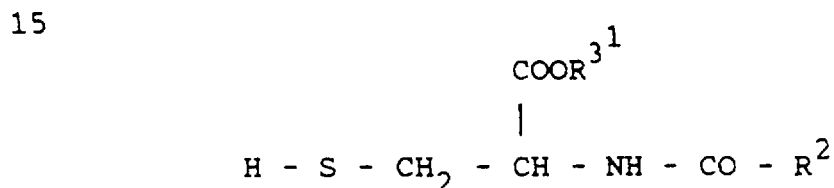
followed by reaction with a second, different or the same N-acylcysteine or N-acylcysteine ester to give a compound of the formula



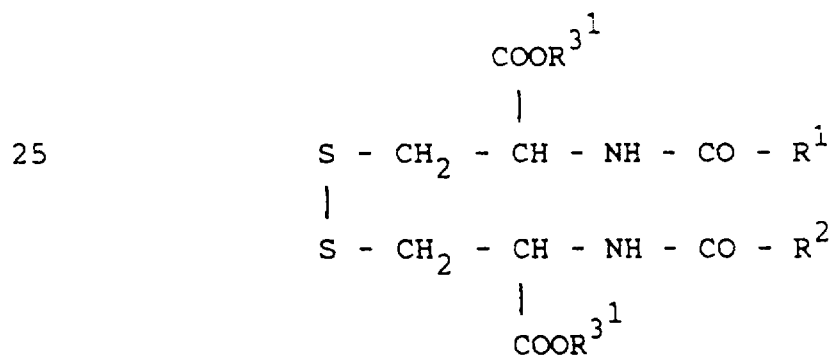
I. The reaction of a compound of the formula



R^1 and R^{3^1} are as defined above, with a compound of the formula



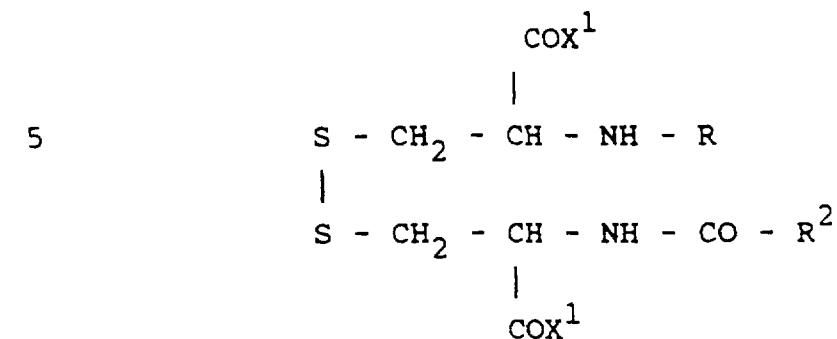
wherein R^2 and R^{3^1} are as defined above to the formation of a compound of the formula



whereafter, if a compound wherein $\text{R}^{3^1} = \text{H}$ is desired, removal of the protecting group R^{3^1} .



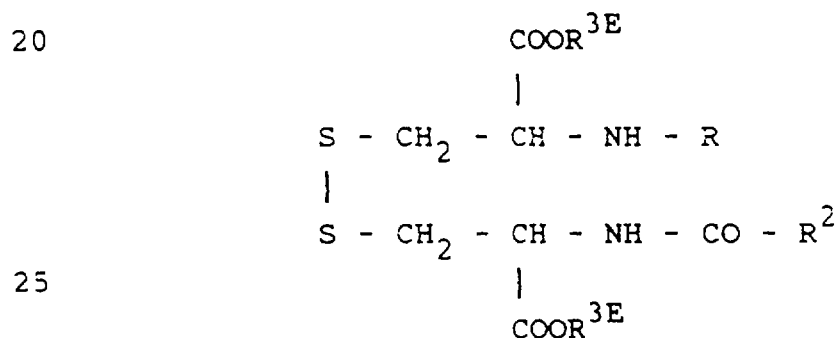
J. The esterification of a compound of the formula



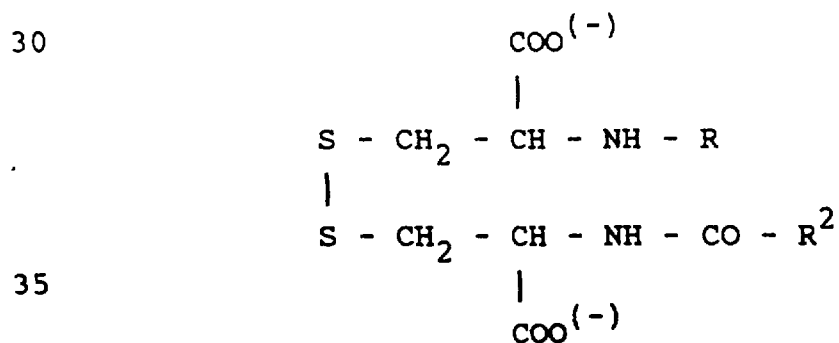
wherein R and R² are as defined above and X¹ is OH or a halogen atom, with a compound of the formula



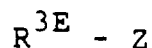
wherein R^{3E} is methyl, ethyl propyl, isopropyl, butyl or isobutyl, to the formation of a compound of the formula



K. The alkylation of a compound of the formula



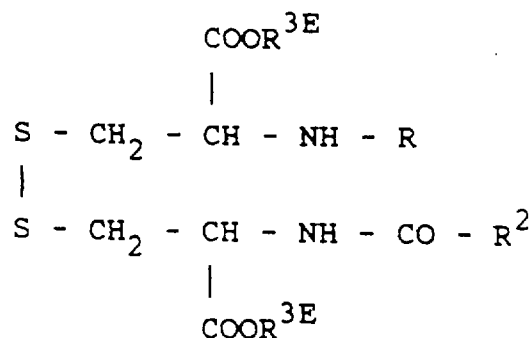
wherein R and R² are as defined above, with a compound of the formula



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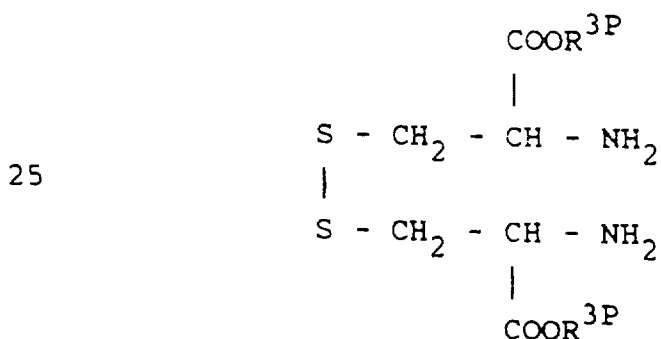
wherein R^{3E} is as defined above and Z is a halide, alkylsulphate, tosylate or another nucleofuge to the formation of a compound of the formula

10



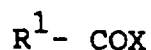
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L. The reaction, in the presence of a suitable base, of a carboxyl protected cystine derivative of the formula



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or a hydrochloride salt thereof, wherein R^{3P} is an acid or base labile organic group, with a compound of the formula

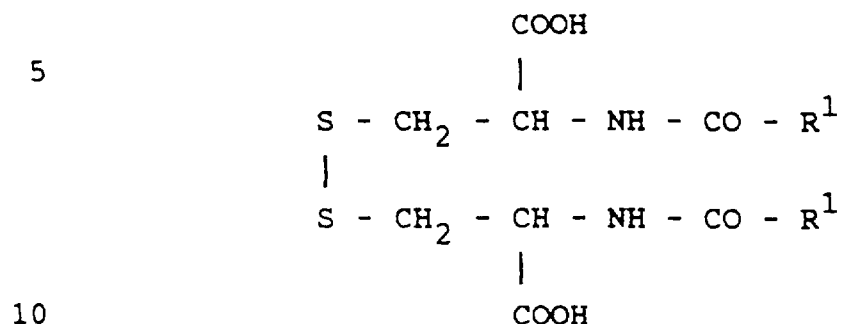


wherein R¹ is defined as above and COX is a reactive group capable of reacting with an amino group under formation of

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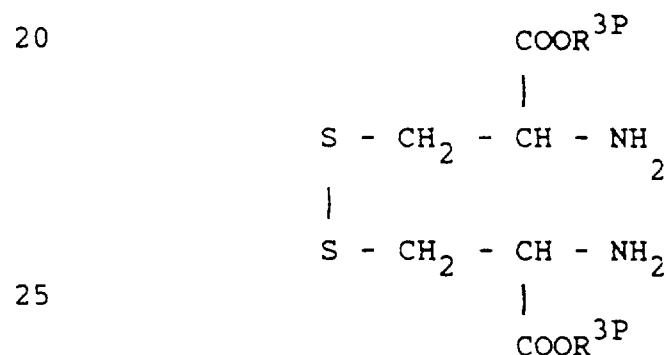


an amide moiety, and thereafter removal of the protecting group, R^{3P} to the formation of a compound of the formula



The acylating agent R^1COX is as defined under B. The protecting group, R^{3P} , may be an acid or base labile organic group such as an alkyl, benzyl, aryl, vinyl or allyl group.

M. The reaction, under alkaline conditions, of a carboxyl protected cystine derivative of the formula

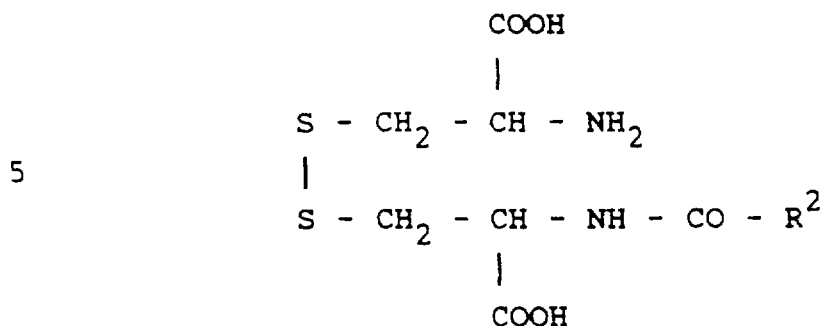


or a hydrochloride salt thereof, wherein R^{3P} is as defined above, with a compound of the formula

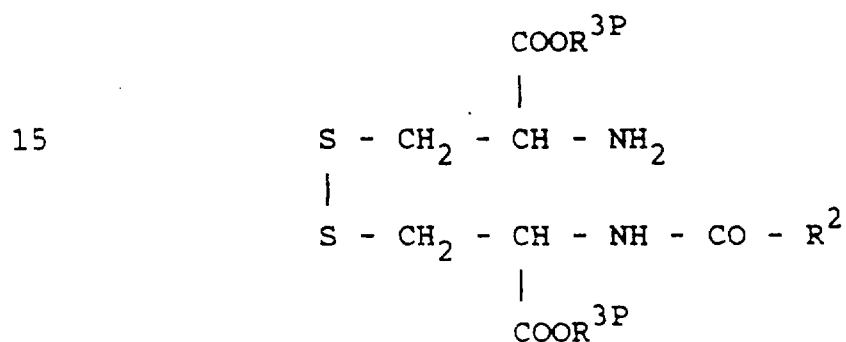


wherein R^2 and COX are as defined above and thereafter removal of the protecting group R^{3P} to the formation of a compound of the formula





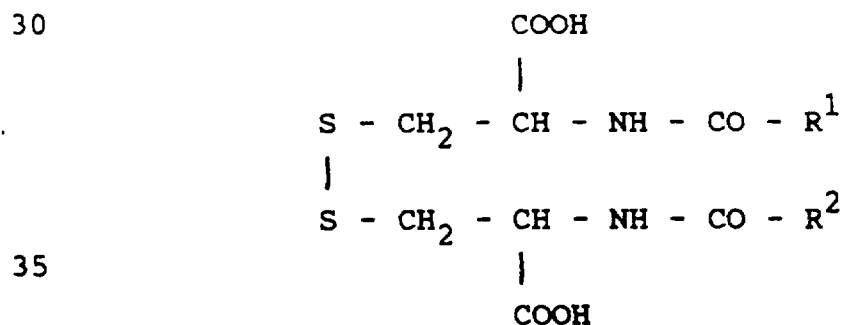
10 N. The reaction of a carboxyl protected cystine derivative of the formula



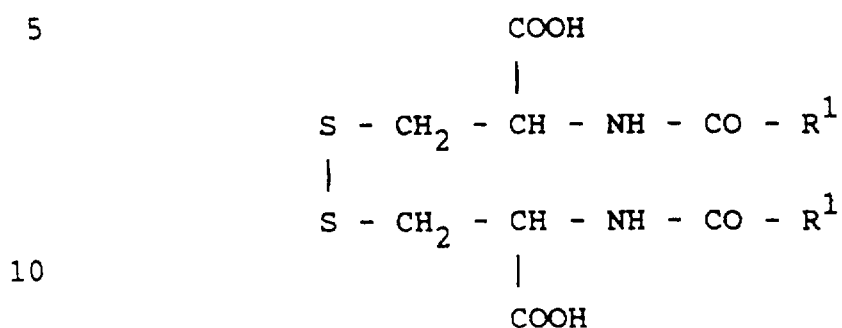
or a hydrochloride salt thereof, wherein R^2 and $\text{R}^{3\text{P}}$ are as defined above, with a compound of the formula



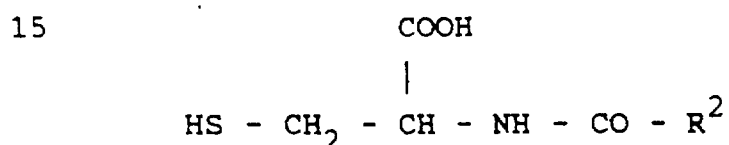
25 wherein R^1 and COX are as defined above and thereafter removal of the protecting group $\text{R}^{3\text{P}}$ to the formation of a compound of the formula



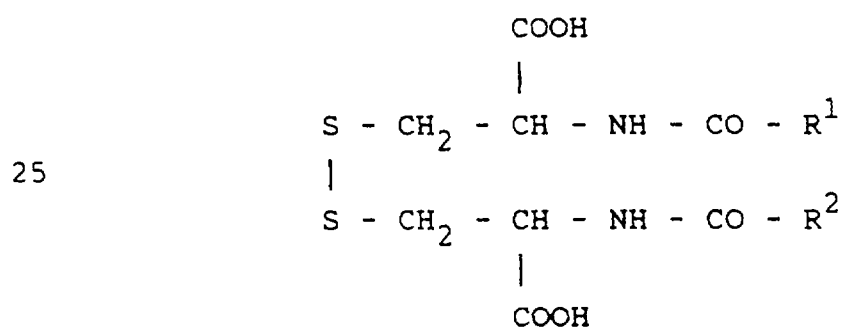
O. The equilibration, in alcohol or aqueous solution, of a mixture of a cystine derivative and a cysteine derivative having the formulas



and



or alkali salts thereof, wherein R^1 and R^2 are as defined
 20 above, to the formation of a compound of the formula



30 An optional step in all methods A-O is transferring of the obtained compound into a physiologically acceptable salt.



Working examples

Example 1. (R,R)-N,N'-dipentanoyl-3,3'-dithiobis(2-aminopropionic acid)dimethylester: A suspension of L-cystinethylester dihydrochloride (1.0 g, 3 mmol) in THF (20 mL) (white slurry) was stirred and cooled to 0°C. To the reaction mixture was added 4 equiv. (2.0 mL) of N-ethyl-diisopropylamine and 2.2 equiv. (0.8 mL, 6.6 mmol) of pentanoylchloride. The mixture was stirred for 4 h on the ice-bath and the white precipitate of N-ethyl-diisopropylammonium chloride was removed by filtration. The solvent was removed by evaporation and the crude product was redissolved in dichloromethane. After washing with water the organic phase was dried over sodium sulphate. Filtration and evaporation of the solvent gave the crude title product, which was recrystallised from ethyl acetate.

Physical data:

¹H-NMR (300 MHz, CDCl₃): δ 6.42 (2H, d, NH), 4.88 (2H, dt, NCH), 3.77 (6H, s, OCH₃), 3.21 (4H, m, SCH₂), 2.27 (4H, t, COCH₂), 1.63 (4H, m, CH₃CH₂CH₂), 1.36 (4H, m, CH₃CH₂), 0.92 (6H, t, CH₃). α_D²⁵ = -146° (C=0.490, MeOH). Mp=110-112°C. Anal. Calcd for C₁₈H₃₂O₆N₂S₂: C, 49.52; H, 7.39; N, 6.42; S, 14.69. Found: C, 49.40; H, 7.40; N, 6.40; S, 14.70.

Example 2. (R,R)-N,N'-dipropionyl-3,3'-dithiobis(2-aminopropionic acid)dimethylester: The compound was prepared according to the procedure described in Example 1 using propionylchloride as acylating reagent.

Physical data:

¹H-NMR (300 MHz, CDCl₃): δ 6.44 (2H, d, NH), 4.89 (2H, dt, NCH), 3.78 (6H, s, OCH₃), 3.22 (4H, m, SCH₂), 2.31 (4H, q, CH₃CH₂CH₂), 1.18 (6H, t, CH₃).



Example 3. **(R,R)-N,N'-dipentanoyl-3,3'-dithiobis(2-aminopropionic acid)diethylester:** The compound was prepared according to the procedure described in Example 1 starting from L-cystinediethylester dihydrochloride and
 5 using pentanoylchloride as acylating reagent.

Physical data:

¹H-NMR (300 MHz, CDCl₃): δ 6.43 (2H, d, NH), 4.85 (2H, dt, NCH), 4.23 (4H, m, OCH₂), 3.22 (4H, m, SCH₂), 2.27 (4H, t, COCH₂), 1.63 (6H, m, CH₃CH₂CH₂), 1.37 (4H, m, CH₃CH₂),
 10 1.30 (6H, t, OCH₂CH₃), 0.92 (6H, t, CH₃). α_D²⁸ = -130° (C=0.514, MeOH). Mp=109°C. Anal. Calcd for C₂₀H₃₆O₆N₂S₂: C, 51.70; H, 7.81; N, 6.03; S, 13.80. Found: C, 51.85; H, 7.75; N, 6.10; S, 13.60.

15 Example 4. **(R,R)-N,N'-dipropionyl-3,3'-dithiobis(2-aminopropionic acid)diethylester:** The compound was prepared according to the procedure described in Example 1 starting from L-cystine diethylester dihydrochloride and using propionylchloride as acylating reagent.

20

Physical data:

¹H-NMR (300 MHz, CDCl₃): δ 6.44 (2H, d, NH), 4.86 (2H, dt, NCH), 4.23 (4H, m, OCH₂), 3.22 (4H, m, SCH₂), 2.31 (4H, q, COCH₂), 1.30 (6H, t, CO₂CH₂CH₃), 1.18 (6H, t, CH₃).
 25

Example 5. **(R,R)-N,N'-dihexanoyl-3,3'-dithiobis(2-aminopropionic acid)diethylester:** The compound was prepared according to the procedure described in Example 1 starting from L-cystinediethylester dihydrochloride and using hexanoylchloride as acylating reagent.

Physical data:

¹H-NMR (300 MHz, CDCl₃): δ 6.44 (2H, d, NH), 4.86 (2H, dt, NCH), 4.23 (4H, m, OCH₂), 3.21 (4H, m, SCH₂), 2.26 (4H, t, COCH₂), 1.65 (4H, m, COCH₂CH₂), 1.31 (4H, m, CH₃CH₂CH₂),
 35



1.31 (4H, m, CH_3CH_2), 1.31 (6H, t, OCH_2CH_3), 0.90 (6H, t, CH_3).

- 5 Example 6. **(R,R)-N,N'-di(2-methylpropionyl)-3,3'-dithiobis(2-aminopropionic acid)diethylester:** The compound was prepared according to the procedure described in Example 1 starting from L-cystinediethylester dihydrochloride and using 2-methylpropanoylchloride as
10 acylating reagent.

Physical data:

- $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 6.42 (2H, d, NH), 4.84 (2H, dt, NCH), 4.23 (4H, m, OCH_2), 3.22 (4H, m, SCH_2), 2.46 (2H, m, $(\text{CH}_3)_2\text{CH}$), 1.30 (6H, t, OCH_2CH_3), 1.18 (12H, d, $(\text{CH}_3)_2$).
15 $\alpha_D^{25} = -127^\circ$ (C=0, 512, MeOH). Mp=140-141°C. Anal. Calcd for $\text{C}_{18}\text{H}_{32}\text{O}_6\text{N}_2\text{S}_2$: C, 49.52; H, 7.39; N, 6.42; S, 14.69. Found: C, 49.15; H, 7.40; N, 6.30; S, 14.75.

- Example 7. **(R,R)-N,N'-di(1-oxo-dodecanyl)-3,3'-dithiobis(2-aminopropionic acid) dimethylester:**
20

The compound was prepared according to the procedure described in Example 1, starting from (R,R)-3,3'-dithiobis(2-aminopropionic acid) dimethylester dihydro-
25 chloride and using dodecanoic acid chloride as the acylating agent.

Total yield: 40%

Physical data:

- Mp = 98-99°C. $[\alpha]_D^{25} = -93^\circ$ (c = 0.531, MeOH). $^1\text{H-NMR}$ (300
30 MHz CDCl_3) δ 6.42 (2H, d, NH) 4.88 (2H, dt, NCH) 3.77 (6H, s, OCH_3) 3.21 (4H, m, SCH_2) 2.26 (4H, t, OCH_2), 1.64 (4H, m, OCH_2CH_2), 1.26 (32H, m, $(\text{CH}_2)_8$), 0.88 (6H, t, CH_2CH_3).
Anal. Calcd for $\text{C}_{32}\text{H}_{60}\text{N}_2\text{O}_6\text{S}_2$: C, 60.72; H, 9.56; N, 4.43; S, 10.13.
35 Found: C, 60.4; H, 9.3; N, 4.5; S 10.1



Example 8. (S,S)-N,N'-di(2-methylpropionyl)-3,3'-dithiobis(2-aminopropionic acid)dimethylester:

N,N'-diisobutyryl-D-cystine (1.86 g, 4.9 mmol) was
 5 dissolved in 10 ml of methanol containing one drop of
 hydrochloric acid. Trimethylorthoformate (0.6 ml, 5.5
 mmol) was added and the reaction mixture was stirred at
 room temperature for 4 days. After evaporation of the
 solvent, the product was purified by column chromato-
 10 graphy (solvent heptane:ethyl acetate 1:5).
 Physical data: Mp 145.5-147.5°C. $^1\text{H-NMR}$ (300 MHz CDCl_3) δ
 6.39 (2H, d, NH) 4.86 (2H, dt, NCH) 3.78 (6H, s, OCH_3)
 3.21 (4H, m, SCH_2) 2.47 (2H, h, $\text{CH}(\text{CH}_3)_2$) 1.18 (12H, d,
 $\text{CH}(\text{CH}_3)_2$. $[\alpha]_{\text{D}}^{25} = +135.2$ (C = 0.42, MeOH)
 15

Example 9. (R,R)-N,N'-dihexanoyl-3,3'-dithiobis(2-aminopropionic acid)dimethylester: The compound was
 prepared according to the procedure described in Example 1
 using hexanoylchloride as acylating reagent.

20 Physical data:
 $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 6.41 (2H, d, NH), 4.88 (2H,
 dt, NCH), 3.76 (6H, s, OCH_3), 3.21 (4H, m, SCH_2), 2.26
 (4H, t, COCH_2), 1.65 (4H, q, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2$), 1.33 (4H, m,
 25 CH_3CH_2 , 1.33 (4H, m, CH_3CH_2), 0.89 (6H, t, CH_3). $\alpha_{\text{D}}^{25} = -$
 135° (C=0.486, MeOH). Mp=90-92°C. Anal. Calcd for
 $\text{C}_{20}\text{H}_{36}\text{N}_2\text{O}_6\text{S}_2$: C, 51.70; H, 7.81; N, 6.03; S, 13.80.
 Found: C, 51.90; H, 7.95; N, 6.10; S, 13.75.

30 Example 10. (R,R)-N,N'-di(1-oxo-octyl)-3,3'-dithiobis-
 (2-aminopropionic acid)dimethylester: The compound was
 prepared according to the procedure described in Example 1
 using octanoylchloride as acylating reagent.

Physical data:
 35 $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 6.41 (2H, d, NH), 4.88 (2H,
 dt, NCH), 3.77 (6H, s, OCH_3), 3.21 (4H, m, SCH_2), 2.26



(4H, t, COCH₂), 1.65 (4H, t, COCH₂CH₂), 1.29 (16H, m, CH₃(CH₂)₁₋₄), 0.88 (6H, t, CH₂CH₃). $\alpha_D^{25} = -119^\circ$ (C=0, 490, MeOH). Mp=90-91° Anal. Calcd for C₂₄H₄₄N₂O₆S₂: C, 55.36; H, 8.52; N, 5.38; S, 12.31. Found: C, 54.80; H, 8.45; N, 5.35; S, 11.80.

Example 11. (R,R)-N,N'-di(1-oxo-octyl-3,3'-dithiobis(2-aminopropionic acid)diethylester: The compound was prepared according to the procedure described in Example 1 starting from L-cystinediethylester dihydrochloride and using octanoylchloride as acylating reagent.

Physical data:

¹H-NMR (300 MHz, CDCl₃): δ 6.43 (2H, d, NH), 4.85 (2H, dt, NCH), 4.23 (4H, m, CH₃CH₂O), 3.21 (4H, m, SCH₂), 2.26 (4H, t, COCH₂), 1.65 (4H, t, COCH₂CH₂), 1.30 (6H, m, OCH₂CH₃), 1.30 (16H, m, CH₃(CH₂)₁₋₄), 0.88 (6H, t, CH₂CH₃).

Example 12. (R,R)-N,N'-di(2,2-dimethylpropionyl)-3,3'-dithiobis(2-aminopropionic acid)diethylester: The compound was prepared according to the procedure described in Example 1 starting from L-cystinediethylester dihydrochloride and using 2,2-dimethylpropanoylchloride as acylating reagent.

Physical data:

¹H-NMR (300 MHz, CDCl₃): δ 6.52 (2H, d, NH), 4.80 (2H, dt, NCH), 4.22 (4H, m, CH₃CH₂O), 3.22 (4H, m, SCH₂), 1.30 (6H, t, OCH₂CH₃), 1.23 (18H, s, C(CH₃)₃).

Example 13. (R,R)-N,N'-di(2,2-dimethylpropionyl)-3,3'-dithiobis(2-aminopropionic acid)dimethylester: The compound was prepared according to the procedure described in Example 1 using 2,2-dimethylpropanoylchloride as acylating reagent.

Physical data:

¹H-NMR (300 MHz, CDCl₃): δ 6.51 (2H, d, NH), 4.83 (2H,



dt, NCH), 3.78 (6H, s, OCH₃), 3.21 (4H, m, SCH₂), 1.23 (18H, s, C(CH₃)₃).

- 5 Example 14. (R,R)-N,N'-di(2-methylpropionyl)-3,3'-dithiobis(2-aminopropionic acid)dimethylester: The compound was prepared according to the procedure described in Example 1 but with 2-methylpropionyl chloride replacing pentanoyl chloride.
- 10 Physical data:
Mp 142-5°C. δ 6.40 (2H,d,NH), 4.86(2H,dt, NCH), 3.78(6H, s, OCH₃), 3.21 (4H,m,SCH₂), 2.47 (2H,h,CH(CH₃)₂), 1.18(12H,d,CH(CH₃)₂).
- 15 Example 15. (R,R)-N-acetyl-N'-hexanoyl-3,3'-dithiobis-(2-aminopropionic acid)dimethylester: (R,R)-3,3'-dithiobis(2-aminopropionic acid)dimethylester dihydrochloride (690 mg, 2 mmol) was stirred together with 1.39 ml (10 mmol) of triethylamine in 20 mL of THF in a 100 mL round bottomed flask. The turbid solution was
- 20 cooled to 0°C on an ice bath before a solution of acetyl chloride (142 µL, 2 mmol) and hexanoylchloride (276 µL, 2 mmol) in 3 mL of THF was added dropwise. The turbid solution was stirred for 1 h at 0°C. A white precipitate of 1 g (100%) Et₃NHCl was filtered off and the filtrate
- 25 was evaporated to give an oily residue, which was partitioned between 10 mL of H₂O and 10 mL of CHCl₃. After separating the phases, the aqueous phase was extracted with 3x10 mL of CHCl₃. After evaporating the combined CHCl₃-phases the crude product (containing the desired
- 30 product and the symmetrical derivatives) was separated by Flash chromatography according to Still et al. (J. Org. Chem., 1978, 43, 2923) on silica gel 60 (E. Merck 5735, 230-400 mesh ASTM), with EtOAc/Heptane/MeOH 6:3:1 as the eluent. The separation was monitored by thin layer
- 35 chromatography (plastic sheets silica gel 60 WF₂54s, E. Merck 16483, with EtOAc/Heptane/MeOH 6:3:1 as eluent and I₂ as visualization agent). The fractions with acceptable



purity were pooled and evaporated to give an oily residue. This residue was dissolved in acetone and the solution was again evaporated to give the title product as a colourless solid.

5 Yield 184 mg, 25%.

Physical data:

TLC: R_f =0.30 (EtOAc/Heptane/MeOH = 6/3/1).

$^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 6.53 (1H, d, NH), 6.43 (1H, d, NH), 4.88 (2H, m, NCH), 3.78 (6H, s, CO_2CH_3), 3.22 (4H, m, SCH_2), 2.26 (2H, t, COCH_2), 2.07 (3H, s, COCH_3), 1.65 (2H, m, CH_2), 1.32 (4H, m, CH_2), 0.90 (3H, t, CH_3). FAB-MS (m/z): 431 $[\text{MNa}]^+$, 409 $[\text{MH}]^+$, 311 $[\text{MH}-\text{C}_7\text{H}_{11}\text{O}]^+$.

15 Example 16. (R,R)-N,N'-di(2-methylpropionyl)-3,3'-dithiobis(2-aminopropionic acid): N-Isobutyryl-L-cysteine (9.5 g, 50 mmol) was dissolved in 50 mL of water. Hydrogen peroxide (30%, 3.1 mL, 30 mmol) was added and the reaction mixture was stirred at room temperature for 6
20 hours. After evaporation of the solvent under reduced pressure, a white crystallised oil (9.8 g) was obtained. Recrystallisation from ethyl acetate furnished the title compound as a white solid which was dried in vacuo.
Yield: 4.8 g (50%). Physical data: Mp. 143-5°C; $^1\text{H-NMR}$ (300 MHz, $\text{DMSO}-d_6$), δ (2H, b, CO_2H), 8.16 (2H, d, NH), 4.47 (2H, m, CHN), 3.15 (2H, dd, CH_2S , $J=14\text{Hz}$, 5Hz), 2.92 (2H, dd, CH_2S , $J=14\text{Hz}$, 9Hz), 2.43 (2H, h, $\text{CH}(\text{CH}_3)_2$, $J=7\text{Hz}$), 1.01 (12H, d, CH_3 , $J=7\text{Hz}$). $[\delta]_D^{25} = -169.8$ (MeOH, $C=0.510$)
25
30

Example 17. (R,R)-N-Acetyl-3,3'-dithiobis(2-aminopropionic acid): L-cysteine (2.42 g, 20 mmol) and N-acetyl-L-cysteine (3.26 g, 20 mmol) were dissolved in 25 mL of water. The pH of this solution was 2.6 according to
35 litmus paper. Aqueous hydrogen peroxide (30%, 2.3 mL, 21 mmol) was added, and the reaction mixture was allowed to stand at room temperature overnight. A white precipitate



was filtered off (1.26 g). This material was shown to be L-cystine by comparison of spectral data with an authentic sample. The yellowish filtrate, containing the desired compound and the two symmetrical compounds (R,R)-N,N'-diacetylcystine and (minor amounts of) cystine was separated on a preparative HPLC using a Dynamax C₁₈-column (8 μ m, 60 Å, 21.4 x 250 mm) with a Dynamax C₁₈-guard column (8 μ m, 21.4 x 50 mm) and with Gilson dual solvent delivery system (305 pump, pumphead 100 SC acting as a solvent delivery pump, pumphead 5 SC acting as a sample injector, 806 manometric module, 811B dynamic mixer, 115 UV detector, 201 fraction collector, 201-202 fraction controller). The solvents used were A=10 mM HOAc/H₂O and B=MeOH, with a 95:5 ratio of A:B. The solvent flow was 10 mL/min and the separation was recorded at 230 nm. After detection of each fraction on TLC (Merck 16483, plastic sheets silica gel 60 WF 254s, eluent ⁿBuOH/HOAc/H₂O 1:1:1, I₂ as visualization agent), the fractions with acceptable purity were pooled and evaporated to give an oily residue. This residue was dissolved in acetone (pa) and the solution was evaporated to give the title product as white crystals. Yield: 10%.

Physical data:

TLC: R_f=0.69 (ⁿBuOH/HOAc/H₂O=1/1/1). ¹H-NMR (300 MHz, D₂O): δ 4.73 (1H, dd, NCH), 4.18 (1H, dd, NCH), 3.37 (2H, m, SCH₂), 3.10 (2H, m, SCH₂), 2.06 (3H, s, CH₃). TSP-MS (m/z): 283 [MH]⁺, 164 [MH-C₃H₅NO₂]⁺.

30

Example 18. (R,R)-N-Acetyl-N'-(2-methylpropionyl)-3,3'-dithiobis(2-aminopropionic acid): A mixture of N-acetyl-L-cysteine (0.652 g, 4 mmol) and N-isobutyryl-L-cysteine (0.764 g, 4 mmol) in 10 mL of MeOH was stirred while hydrogen peroxide (30%, 0.60 mL, 5 mmol) was added dropwise. The stirring was continued for 3 hours at room



temperature, after which the solvent was removed on the rotary evaporator. Addition of 25 mL of acetone and repeated evaporation afforded 1.5 g of crude material as an oil which solidified on standing. The desired title
 5 compound was isolated from this mixture by preparative HPLC as described in Example 17. Yield: 31%.

Physical data:

HPLC elution with 60% A (isocratic, for solvents see Example 17). TLC: $R_f=0.76$ (n BuOH/ H_2O /HOAc=1/1/1). 1H -NMR
 10 (300 MHz, D_2O): δ 4.73 (2H, m, NCH), 3.33 (2H, m, SCH_2), 3.03 (2H, m, SCH_2), 2.59 (1H, n, H), 2.06 (3H, s, COH_3), 1.13 (3H, d, CH_3), 1.11 (3H, s, CH_3). FAB-MS (m/z): 376 $[MNa]^+$, 353 $[MH]^+$.

15

Example 19. (R,R)-N-(2-methylpropionyl)-3,3'-dithiobis-(2-aminopropionic acid): The compound was prepared by the procedure given in Example 17, starting from L-
 20 cysteine and N-isobutyryl-L-cysteine. Yield: 8%.

Physical data: HPLC elution with 55% A (isocratic, see Example 17 for solvents). TLC: $R_f=0.67$ n BuOH/ H_2O /HOAc = 1/1/1). 1H -NMR (300 MHz, D_2O): δ 4.73 (1H, dd, NCH), 4.14 (1H, dd, NCH), 3.38 (2H, n, SCH_2), 3.08 (2H, m, SCH_2),
 25 2.06 (1H, n, CH), 1.15 (3H, d, CH_3), 1.13 (3H, d, CH_3). TSP-MS (m/z): 311 $[MH]^+$.

Example 20. (R,R)-N-Acetyl-N'-(2,2-dimethylpropionyl)-
 30 3,3'-dithiobis(2-aminopropionic acid): The compound was obtained following the procedure given in Example 18, starting from N-acetyl-L-cysteine and N-pivaloyl-L-cysteine. Yield: 21%.

Physical data:

35 HPLC elution gradient: 50% A/15 min, 50 30% A/5 min, 30% A/ isocratic (for solvents see Example 17). TLC:



$R_f=0.78$ $n\text{-BuOH}/\text{H}_2\text{O}/\text{HOAc} = 1/1/1$.

$^1\text{H-NMR}$ (300 MHz, D_2O): δ 4.72 (2H, m, NCH), 3.35 (2H, m, CH_2), 3.04 (2H, m, SCH_2), 2.06 (3H, s, CH_3), 1.21 (9H, s, CCH_3).

5 FAB-MS (m/z): 411 $[\text{MNa}]^+$, 389 $[\text{MNa}]^+$, 367 $[\text{MH}]^+$, 349 $[\text{MH}-\text{H}_2\text{O}]^+$.

Example 21. (R,R)-N,N'-di(2,2-dimethylpropionyl)-3,3'-
10 dithiobis(2-aminopropionic acid): The compound was isolated from the crude material of Example 20. Yield: 15%.

Physical data:

HPLC elution gradient: 50% A/15 min, 50 30% A/15 min,
15 30%. A/isocratic (see Example 17 for solvents). TLC:

$R_f=0.88$ $n\text{-BuOH}/\text{H}_2\text{O}/\text{HOAc} = 1/1/1$.

$^1\text{H-NMR}$ (300 MHz, D_2O): δ 4.72 (2H, dd, NCH), 3.37 (2H, dd, SCH_2), 3.06 (2H, dd, SCH_2), 1.21 (18H, s, CCH_3).

FAB-MS (m/z): 431 $[\text{MNa}]^+$, 409 $[\text{MH}]^+$, 391 $[\text{MH}-\text{H}_2\text{O}]^+$.

20

Example 22. (R,R)-N-Acetyl-N'-pentanoyl-3,3'-dithiobis-
(2-aminopropionic acid): This material was prepared as in Example 18, starting from N-acetylcysteine and N-pentanoylcysteine. Yield: 23%.

25 Physical data:

HPLC elution gradient: 50% A/20 min, 50 25% A/5 min,
25%

A/isocratic (see Example 17 for solvents). TLC: $R_f=0.84$
 $n\text{-BuOH}/\text{H}_2\text{O}/\text{HOAc} = 1/1/1$.

30 $^1\text{H-NMR}$ (300 MHz, D_2O): δ 4.70 (2H, m, NCH), 3.30 (2H, n, SCH_2), 2.99 (2H, m, SCH_2), 2.30 (2H, t, COCH_2), 2.04 (3H, s, COCH_3), 1.57 (2H, m, CH_2), 1.30 (2H, m, CH_2), 0.86 (3H, t, CH_3).

FAB-MS (m/z): 389 $[\text{MNa}]^+$, 367 $[\text{MH}]^+$, 349 $[\text{MH}-\text{H}_2\text{O}]^+$.

35

Example 23. (R,R)-N,N'-dihexanoyl-3,3'-dithiobis(2-



aminopropionic acid):

A suspension of 1 equiv. 3,3'-dithiobis(2-aminopropionic acid) dimethylester dihydrochloride in tetrahydrofuran (white slurry) was stirred and cooled to 0°C. To the reaction mixture was added 4 equiv. of N-ethyldiisopropylamine and 2.2 equiv. of hexanoylchloride. The mixture was stirred for 4 hrs on an ice bath and the white precipitate of N-ethyldiisopropylammonium chloride was filtered off. The solvent was removed by evaporation under reduced pressure and the crude product was redissolved in dichlormethane. After washing with water, the organic phase was dried over sodium sulphate. Filtration and evaporation of the solvent gave crude (R,R)-N,N'-dihexanoyl-3,3'-dithiobis(2-aminopropionic acid) dimethylester, which was recrystallized from methanol/water and triturated with diethylether.

A white slurry (0.1 M) of the above formed dimethylester and 0.5 M sodium hydroxide in 10% methanol was stirred vigorously at room temperature. After about 48 hrs the pH of the clear solution was adjusted to 2, and the formed white precipitate of crude product was filtered off and recrystallized from acetone/hexane to give the title compound as white crystals. Total yield: 28%.

Physical data:

Mp: 132-135°C. $[\alpha]_D^{25}$: -164° (c=0.501, MeOH). $^1\text{H-NMR}$ (300 MHz, DMSO- d_6), δ 8.22 (2H, d, NH), 4.49 (2H, m, CHN), 3.14 (2H, dd, CH_2S), 2.91 (2H, dd, CH_2S), 2.12 (4H, t, CH_2CO), 1.50 (4H, p, $\text{CH}_2\text{CH}_2\text{CO}$), 1.26 (8H, m, $(\text{CH}_2)_2$), 0.87 (6H, t, CH_3). Anal. Calcd for $\text{C}_{18}\text{H}_{32}\text{N}_2\text{O}_6\text{S}_2$: C, 49.5; H, 7.4; N, 6.4; S, 14.7. Found: C, 49.4; H, 7.2; N, 6.2; S, 14.3.



Example 24 (R,R)-N,N'-di(1-oxo-octyl)-3,3'-
dithiobis(2-aminopropionic acid):

The compound was prepared according to the procedure described in Example 23, using octanoic acid chloride as the acylating agent.

The initially formed (R,R)-N,N'-di(1-oxo-octyl)-3,3'-dithiobis(2-aminopropionic acid) dimethylester was recrystallized from ethyl acetate and the title compound was recrystallized from acetone/heptane and triturated with diethylether to give white crystals. Total yield: 20%
Physical data:

Mp: 105-107°C. $[\alpha]_D^{25}$: -147° (c = 0.541, MeOH). $^1\text{H-NMR}$ (300 MHz, DMSO- d_6), δ 8.18 (2H, d, NH), 4.48 (2H, m, CHN), 3.17 (2H, dd, CH_2S), 2.89 (2H, dd, CH_2S), 2.12 (4H, t, CH_2CO), 1.49 (4H, m, $\text{CH}_2\text{CH}_2\text{CO}$), 1.26 (16H, m, $(\text{CH}_2)_4$), 0.87 (6H, t, CH_3). Anal. Calcd for $\text{C}_{22}\text{H}_{40}\text{N}_2\text{O}_6\text{S}_2$: C, 53.6; H, 8.2; N, 5.7; S, 13.0. Found: C, 53.4; H, 8.5; N, 5.7; S, 12.6.

Example 25 (R,R)-N,N'-di(1-oxo-dodecanyl)-3,3'-
dithiobis(2-aminopropionic acid):

The compound was prepared according to the procedure described in Example 23, using dodecanoic acid chloride as the acylating agent. The hydrolysis was carried out in 20% methanol.

The initially formed (R,R)-N,N'-di(1-oxo-dodecanyl)-3,3'-dithiobis(2-aminopropionic acid) dimethylester was recrystallized from ethyl acetate. The title compound was recrystallized from toluene/dichloromethane to give white crystals.

Total yield: 23%

Physical data:

Mp: 110-112°C. $[\alpha]_D^{25}$: -113° (c=0.507, MeOH). $^1\text{H-NMR}$ (300 MHz, DMSO- d_6), δ 8.19 (2H, d, NH), 4.48 (2H, m, CHN), 3.15 (2H, dd, CH_2S), 2.89 (2H, dd, CH_2S), 2.11 (4H, t, CH_2CO), 1.49 (4H, m,



CH₂CH₂CO), 1.25 (32H, m, (CH₂)₈), 0.87 (6H, t, CH₃). Anal.
Calcd for C₃₀H₅₆N₂O₆S₂: C, 59.6; H, 9.3; N, 4.6; S, 10.6.
Found: C, 59.2; H, 9.4; N, 4.6; S, 10.3.

5 Example 26 (S,S)-N,N'-di(2-methylpropionyl)-3,3'-
dithiobis(2-aminopropionic acid):

Potassium carbonate (17.6 g, 127 mmol) was dissolved in 40
ml of water and 40 ml of methylene chloride under
10 nitrogen. The solution was cooled (-10°C) and 2-methyl-
propionoyl chloride (5.6 ml, 40 mmol) and D-cysteine
hydrochloride monohydrate (8.7 g, 49.5 mol) was added
quickly. The reaction mixture was stirred at room
temperature for 4 hours, after which hydrochloric acid was
15 added until the pH of the mixture became less than 1
according to lithmus paper. The aqueous layer was
discarded and light petroleum (40-60°) was added to the
organic layer whereupon N-isobutyryl-D-cysteine
precipitated as white crystals.

20

N-isobutyryl-D-cysteine (1 g, 5.2 mmol) was dissolved in
10 ml of methanol. Hydrogen peroxide (30%, 0.3 ml, 2.6
mmol) was added and the reaction mixture was stirred at
room temperature for 6 hours. After evaporation of the
25 solvent under reduced pressure, a white crystallised oil
was obtained. Recrystallisation from ethyl acetate
furnished the title compound as a white solid which was
dried in vacuo.

Yield: 0.55 g (55%) Physical data: Mp 135-137°C. ¹H-NMR
30 (300 MHz, DMSO-d₆) δ 8.16 (2H, d, NH) 4.47 (2H, m, CHN)
3.15 (2H, dd, CH₂S, J = 14 Hz, 5 Hz) 2.93 (2H, dd, CH₂S, J
= 14 Hz, 9 Hz) 2.43 (2H, h, CH(CH₃)₂ J = 7 Hz) 1.01 (12H,
d, CH₃, J = 7 Hz).

[α]_D²⁵ = +167.6 (c = 0.516, MeOH).

35



Example 27.

Formulation A

5 Tablet containing 10 mg of active substance per tablet:

	Active substance	10 mg
	Lactose	100 mg
	Potato starch	50 mg
10	Polyvinylpyrrolidone	5 mg
	Microcrystalline cellulose	15 mg
	Magnesium stearate	1 mg

15 Formulation B

Direct compression tablet containing 5 mg of active substance per tablet:

20	Active substance	5 mg
	Lactose, anhydrous	150 mg
	Microcrystalline cellulose	50 mg
	Colloidal silicon dioxide	1 mg
	Magnesium stearate	2 mg

25

If desired, the obtained tablets can be film coated with e.g. hydroxypropyl methylcellulose, hydroxypropyl cellulose or dimethylaminoethyl methacrylate methacrylic acid ester copolymer.

30



Formulation_C

Solution for injection containing active substance 1 mg/ml

5

Active substance	1.0 mg
Sodium chloride	8.8 mg
Water for injection	to 1 ml

10 Formulation_D

Oral solution containing active substance 1 mg/ml

15 Active substance	1.0 mg
Sorbitol	150 mg
Glycerin	100 mg
Disodium edetate	0.5 mg
Preservative	q.s.
20 Flavour	q.s.
Water, purified	to 1 ml

Formulation_E

25

Powder aerosol giving 1 mg per dose

The micronized active substance can be filled into a powder inhaler device e.g. Turbuhaler^R giving 1 mg/dose.

30

Effects of the compounds of the invention in a model of delayed type hypersensitivity in the mouse.

The property of the compounds of the invention to
 35 stimulate immune responses are illustrated by their efficacy in a model in the mouse of the delayed type



hypersensitivity (DTH) reaction.

Both male and female Balb/c mice obtained from Bomhotsgaard (Denmark) and Charlie Rivers (England), were
5 used at the weight of 18-20 gram. 4-ethoxymethylene-2-phenyloxazolone (OXA) was purchased from BDH (England) and served as an antigen in this test.

The mice were sensitized, Day 0, by epicutaneous
10 application of 150 ul absolute ethanol-acetone (3:1) solution containing 3% OXA on the shaved thorax and abdomen. Treatment with the compound under examination, DiNAC (as a positive control) or vehicle (phosphate buffer, pH 7.0) was initiated by oral feeding immediately
15 after sensitization and continued once to Day 6. Seven days (Day 6) after the sensitization both ears of all mice were challenged on both sides by topical application of 20 ul 1% OXA dissolved in olive oil. Ear thickness was measured prior to and 24 or 48 hours after challenge using
20 an Oditest spring calliper. Challenges and measurements were performed under light pentobarbital anaesthesia. The intensity of the DTH reactions was expressed according to the formula: $T_{t24/48} - T_{t0}$ um units, where t_0 and $t_{24/48}$ represent the ear thickness before and 24 or 48 hours
25 after challenge, respectively, in individual test (T). The results were expressed as the mean+/-S.E.M. The level of significance between means of the groups was obtained by Student's two-tailed t-test. Table 1 shows representative results from 24 and 48 hours measurements expressed as %
30 increase in ear thickness relative to that of the non-challenged reference ear. A figure of 100 thus indicates a doubled ear thickness.



Table 1

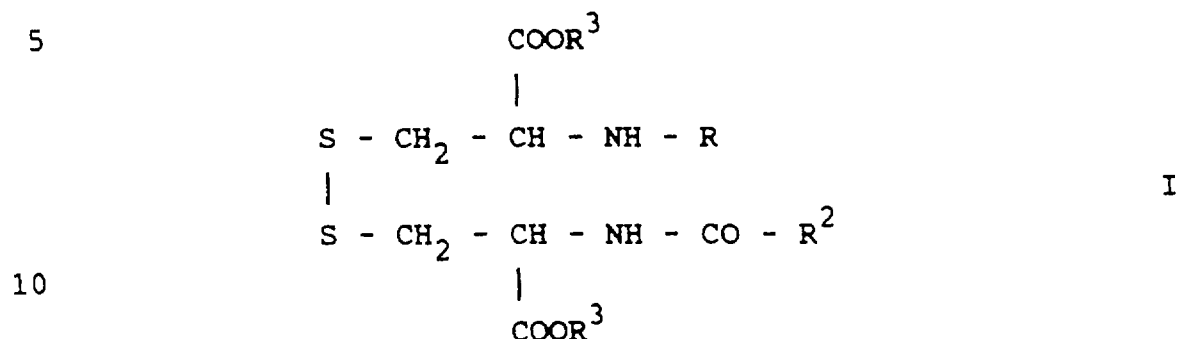
Immunostimulatory action

R	R ²	R ³	% Increase, 24h		% Increase, 48	
			0.03 $\mu\text{mol/kg}$	3.0 $\mu\text{mol/kg}$	0.03 $\mu\text{mol/kg}$	3.0 $\mu\text{mol/kg}$
$\text{C}(\text{CH}_3)_2$	$\text{C}(\text{CH}_3)_2$	CH^3	110***	92**	17*	27***
$\text{n-C}_5\text{H}_{11}$	$\text{n-C}_5\text{H}_{11}$	CH_3	73**	86**	51***	45***
$\text{n-C}_7\text{H}_{15}$	$\text{n-C}_7\text{H}_{15}$	CH_3	58***	52***	28**	20*
$\text{n-C}_7\text{H}_{15}$	$\text{n-C}_7\text{H}_{15}$	C_2H_5	49***	7	0	0
$\text{COC}(\text{CH}_3)_3$	$\text{C}(\text{CH}_3)_3$	H	14*		26*	27*
$\text{COCH}(\text{CH}_3)_2$	$\text{CH}(\text{CH}_3)_2$	H	17	18	27**	62***
COCH_3	$\text{C}(\text{CH}_3)_3$	H	24*	26*	14	17*
H	$\text{CH}(\text{CH}_3)_2$	H	37**	31***		

*, **, ***: $P < 0.05, 0.01, 0.001$ 

CLAIMS

1. Compounds of the general formula



wherein R is hydrogen or a moiety $-\text{CO}-\text{R}^1$ wherein R^1 is methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, iso-propyl, 1-methylpropyl, tert. butyl, 3-methylbutyl or 2-methylbutyl, R^2 is methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, iso-propyl, 1-methylpropyl, tert. butyl, 3-methylbutyl or 2-methylbutyl and R^3 is hydrogen, methyl, ethyl, propyl, isopropyl, butyl or isobutyl, provided that R^1 and R^2 are not simultaneously methyl and further provided that when R^3 is hydrogen R^1 and R^2 are not simultaneously n-propyl or n-heptyl or a physiologically acceptable salt and/or a stereochemical isomer thereof.

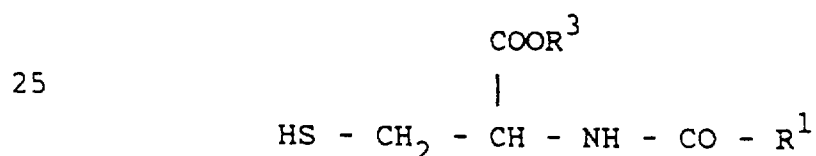
2. A compound according to claim 1 wherein R is hydrogen or a moiety $-\text{CO}-\text{R}^1$ wherein R^1 is methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, iso-propyl, 1-methylpropyl, tert. butyl, 3-methylbutyl or 2-methylbutyl, R^2 is methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, iso-propyl, 1-methylpropyl, tert. butyl, 3-methylbutyl or 2-methylbutyl and R^3 is hydrogen, methyl or ethyl, provided that R^1 and R^2 are not simultaneously methyl and further provided that when R^3 is hydrogen R^1 and R^2 are not simultaneously n-propyl or n-





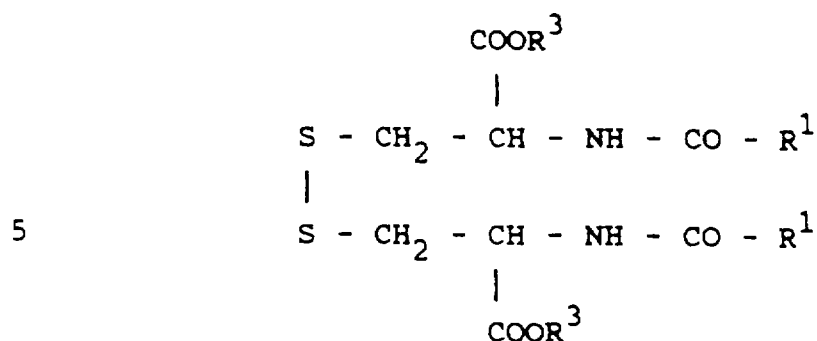
heptyl.

3. A compound according to claim 1 wherein R^1 and R^2 are isopropyl and R^3 is methyl.
- 5 4. A compound according to claim 1 wherein R^1 and R^2 are n-pentyl and R^3 is methyl.
- 10 5. A compound according to claim 1 wherein R^1 and R^2 are n-heptyl and R^3 is methyl or ethyl.
6. A compound according to claim 1 wherein R^1 and R^2 are simultaneously isopropyl and R^3 is hydrogen.
- 15 7. A compound according to claim 1 wherein R^1 and R^2 are simultaneously tert. butyl and R^3 is hydrogen.
8. A process for the preparation a compound of the general formula I as defined in claim 1, characterized by
- 20 a) the oxidation of an N-acylcysteine derivative of the formula



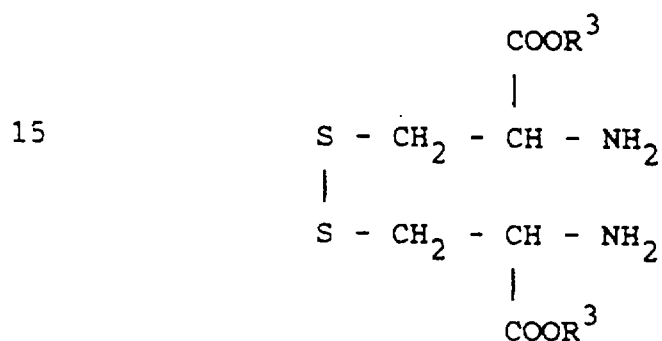
- wherein R^1 and R^3 are as defined above or when R^3 is
- 30 hydrogen optionally an alkali salt thereof, to the formation of a compound of the formula



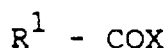


or

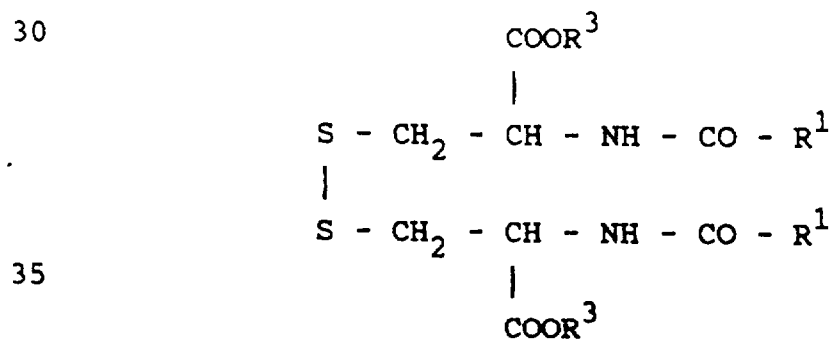
- 10 b) the reaction, in the presence of a suitable base, of a compound having the formula



- 20 or a salt thereof wherein R^3 is as defined above, with a compound of the formula



- 25 wherein R^1 is defined as above and COX is a reactive group capable of reacting with an amino group under formation of an amide moiety, to the formation of a compound of the formula

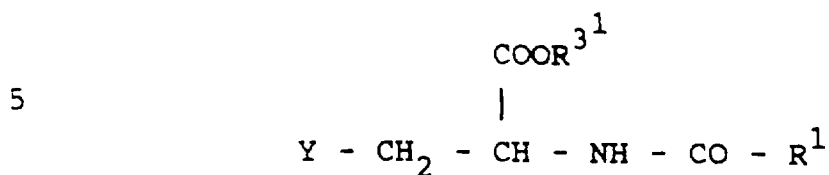


or

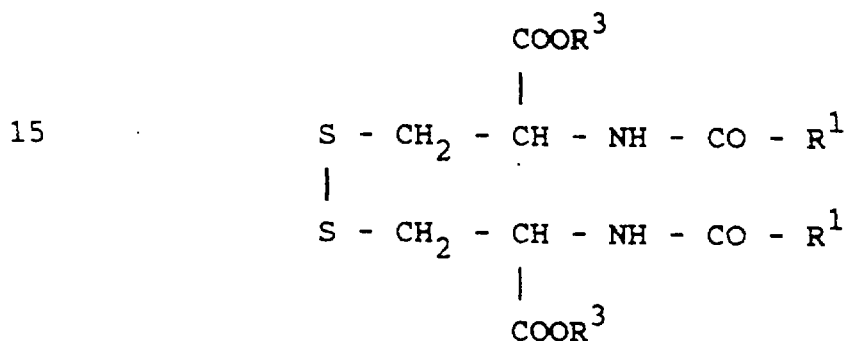




c) the reaction of 2-(N-acylamino)-3-halopropionic acid derivative of the formula

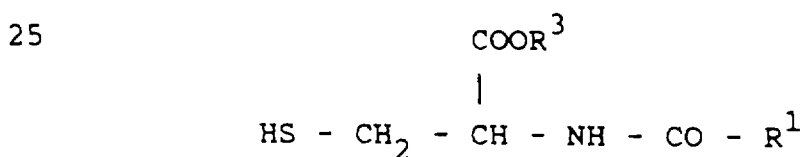


wherein R^1 is as defined above, R^{3^1} is R^3 as defined above or an acid or base labile organic group and Y is a halogen atom, with sulphur or disulphide dianion in the presence of a base, to the formation of a compound of the formula

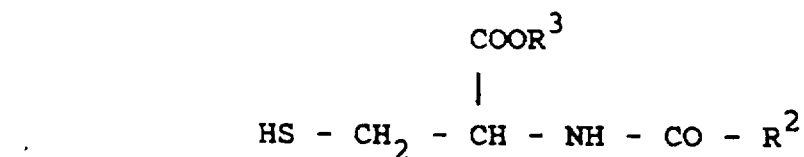


or

d) the oxidation of a mixture of N-acylcysteine ester derivatives of the formulas



and

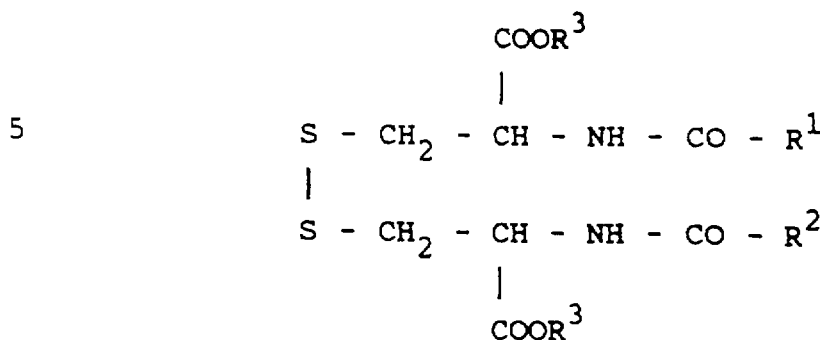


wherein R^1 , R^2 and R^3 are as defined above or when R^3 is hydrogen optionally alkali salts thereof, to the formation



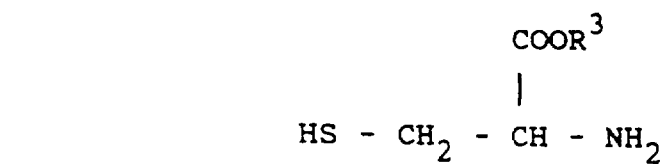


of a compound of the formula

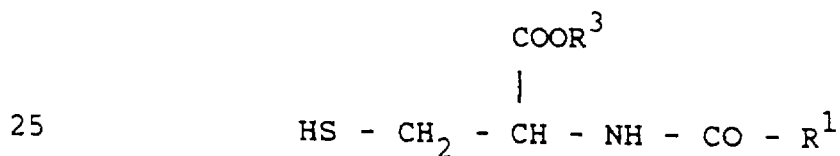


or

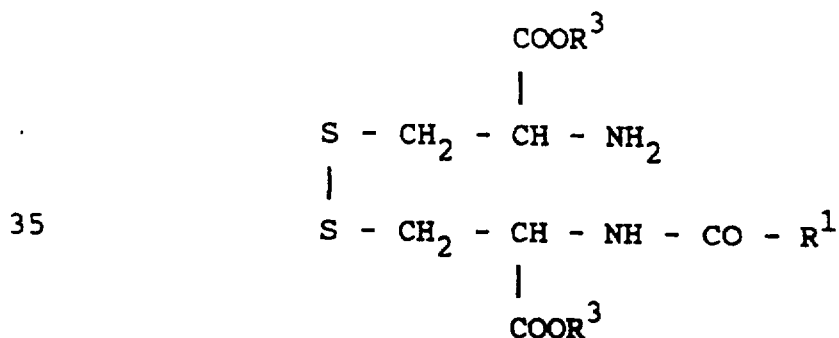
e) the oxidation of a mixture of cysteine or a cysteine ester and an N-acylcysteine derivative of the formulas



or a salt thereof and

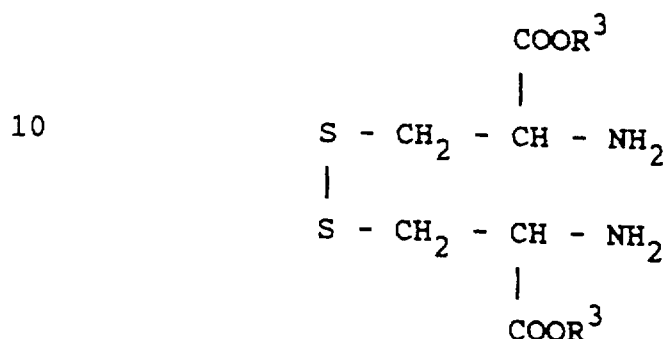


or an alkali or hydrochloride salts thereof, wherein R^1 and R^3 are as defined above, to the formation of a compound of the formula

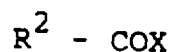


or a salt thereof,
or

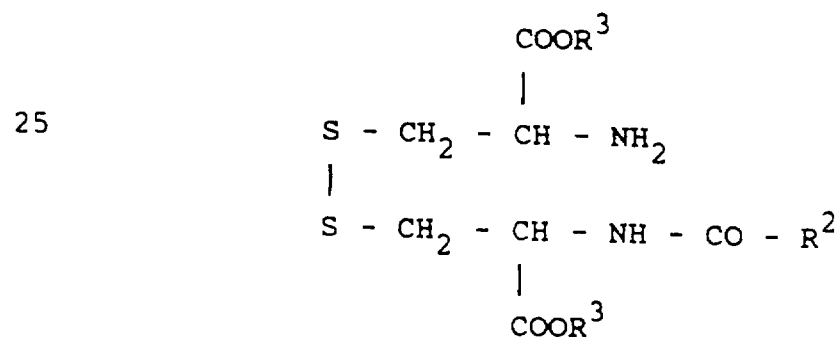
- 5 f) the reaction, in the presence of a suitable base, of an excess of cysteine or a cystine diester having the formula



- 15 or a salt thereof wherein R^3 is as defined above, with a compound of the formula



- 20 wherein R^2 and COX are as defined above, to the formation of a compound of the formula



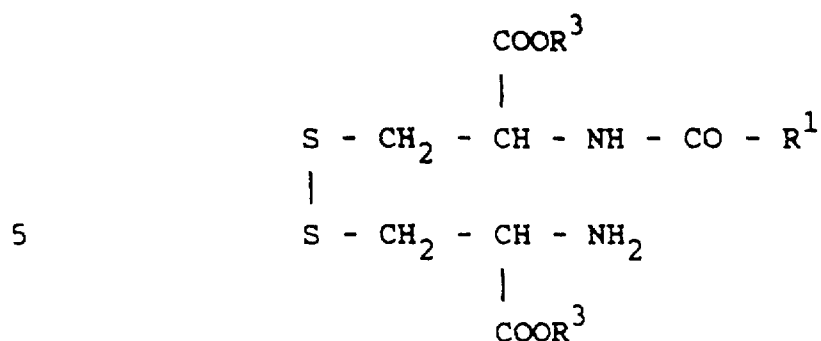
30

or

- g) the reaction, in the presence of a suitable base, of a compound of the formula

35

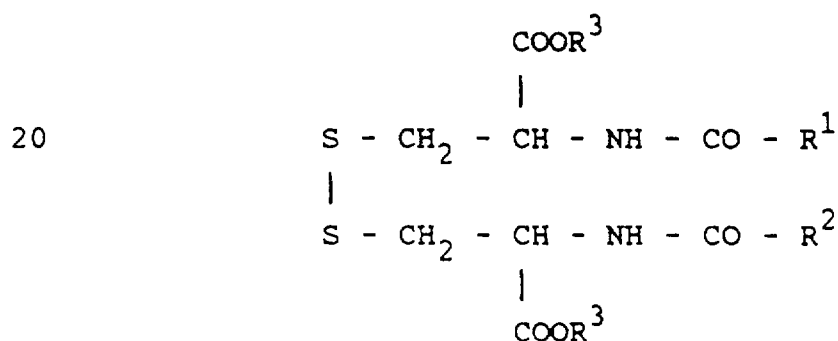




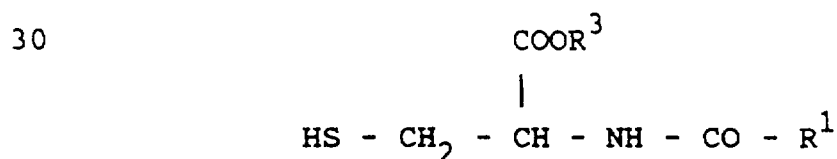
10 or a salt thereof wherein R^1 and R^3 are as defined above,
with a compound of the formula



15 wherein R^2 and COX are as defined above, to the formation
of a compound of the formula

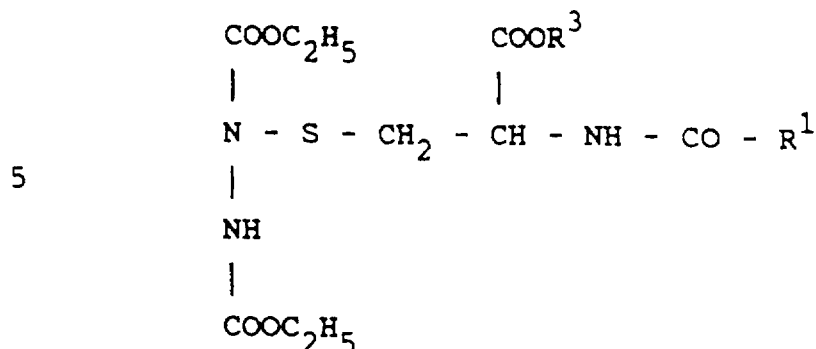


h) the reaction of an N-acylcysteine derivative of the
formula

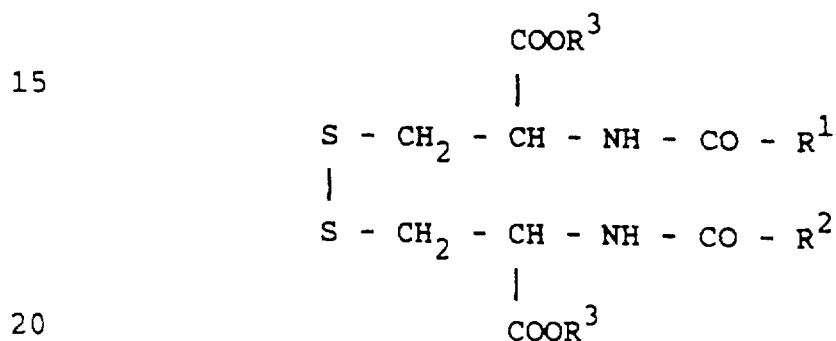


or a salt thereof,
35 wherein R^1 and R^3 are as defined above, with an activating
reagent to the formation of an adduct of the formula

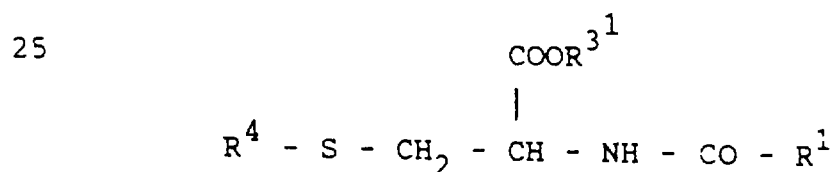




10 followed by reaction with a second, different or the same
N-acylcysteine or cysteine ester derivative, to give a
compound of the formula



i) the reaction of a compound of the formula



30

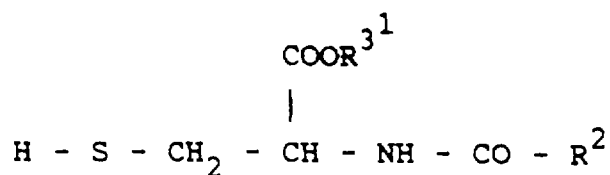
wherein R^4 is -Cl or -N

$$\begin{array}{c}
 \text{O} \qquad \qquad \text{O} \qquad \text{COOR}^{3^1} \\
 \qquad \qquad \qquad | \qquad \qquad | \\
 \qquad \qquad \qquad \text{S} - \text{CH}_2 - \text{CH} - \text{NH} - \text{CO} - \text{R}^1 \text{ and} \\
 \qquad \qquad \qquad \text{O} \qquad \qquad \qquad \text{O}
 \end{array}$$

35

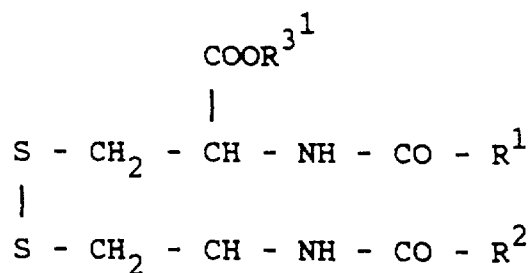
R^1 and R^{3^1} are as defined above, with a compound of the
formula





5

wherein R^2 and R^{3^1} are as defined above to the formation of a compound of the formula

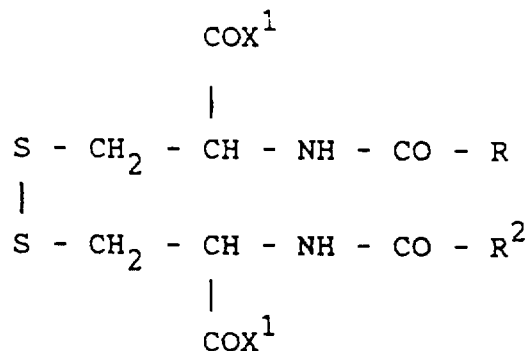


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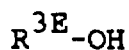
whereafter, if a compound wherein $\text{R}^{3^1} = \text{H}$ is desired, removal of the protecting group R^{3^1} , or

20 j) the esterification of a compound of the formula



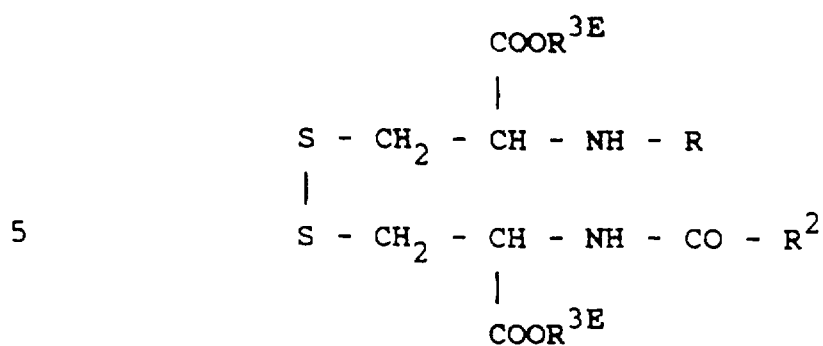
25

wherein R and R^2 are as defined above, and X^1 is OH or a
30 halogen atom with a compound of the formula



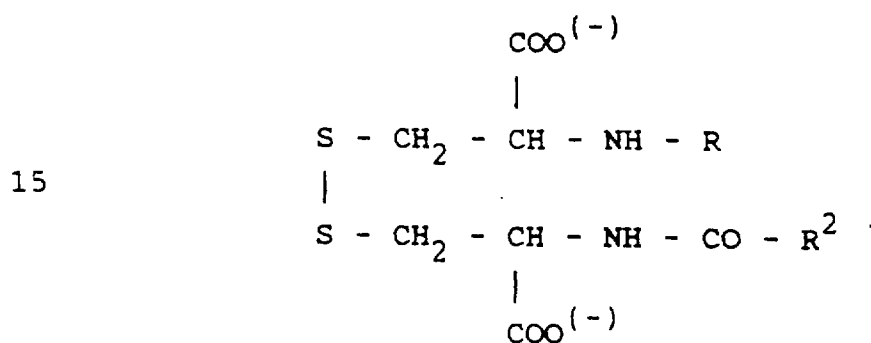
wherein $\text{R}^{3\text{E}}$ is methyl, ethyl, propyl, isopropyl, butyl or
35 isobutyl, to the formation of a compound of the formula



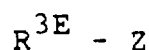


or

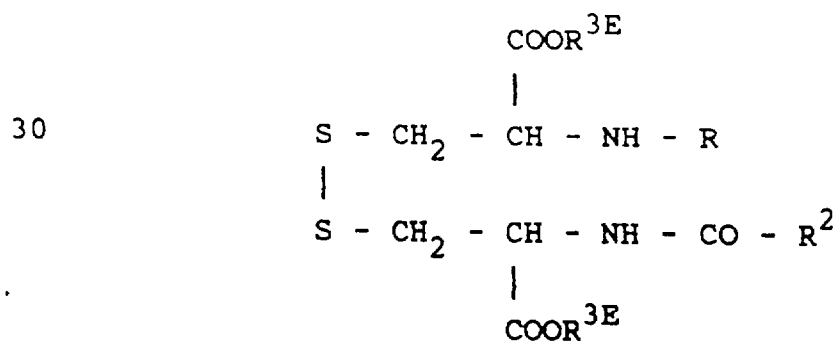
10 k) the alkylation of a compound of the formula



20 wherein R and R² are as defined above, with a compound of the formula



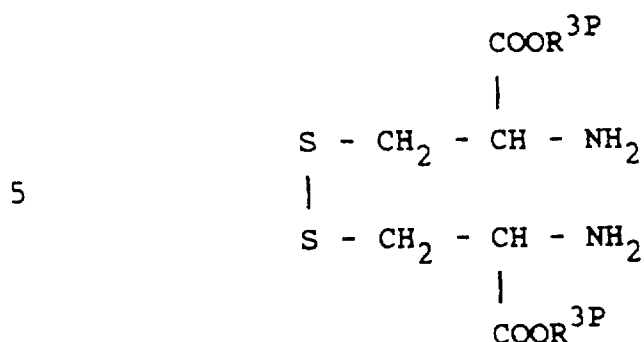
Wherein R^{3E} is as defined above and Z is halogen,
 25 alkylsulphate, tosylate or another nucleofuge to the formation of a compound of the formula



35

1) the reaction, in the presence of a suitable base, of a carboxyl protected cystine derivative of the formula



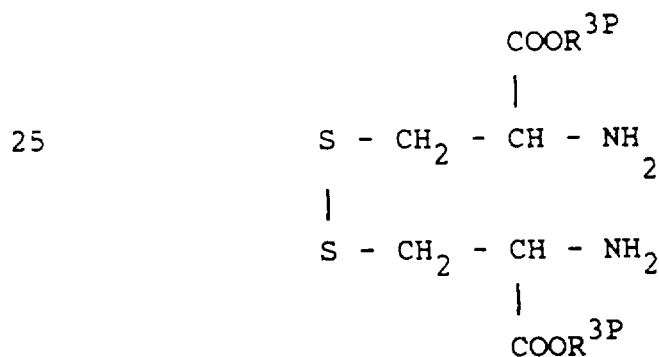


10 or a hydrochloride salt thereof, wherein $\text{R}^{3\text{P}}$ is an acid or base labile organic group, with a compound of the formula



15 wherein R^1 is defined as above and COX is a reactive group capable of reacting with an amino group under formation of an amide moiety, and thereafter removal of the protecting group $\text{R}^{3\text{P}}$ to the formation of a compound of the formula

20 m) the reaction, under alkaline conditions, of a carboxyl protected cystine derivative of the formula



30

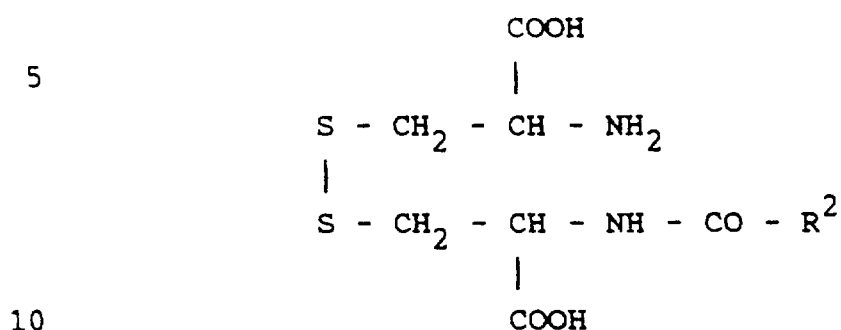
or a hydrochloride salt thereof, wherein $\text{R}^{3\text{P}}$ is as defined above, with a compound of the formula



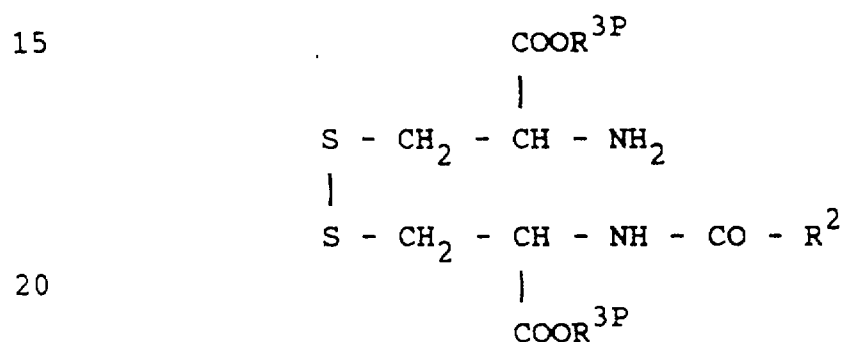
wherein R^2 and COX are as defined above and thereafter



removal of the protecting group R^{3P} to the formation of a compound of the formula



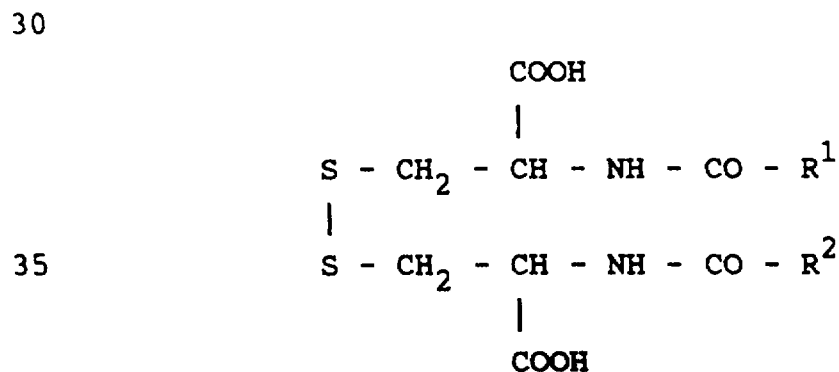
n) the reaction of a carboxyl protected cystine derivative of the formula



or a hydrochloride salt thereof, wherein R^2 and R^{3P} are as defined above, with a compound of the formula

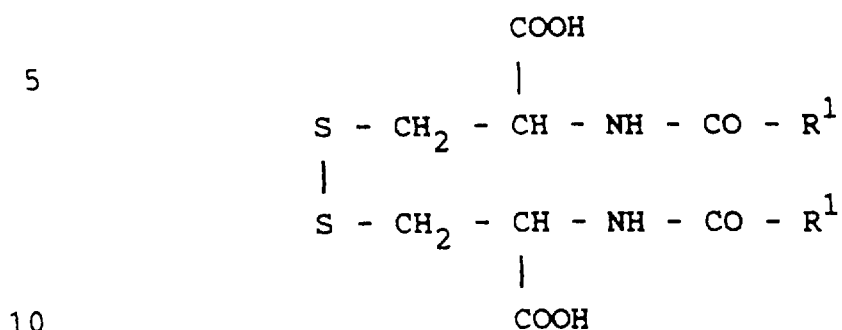


wherein R^1 and COX are as defined above and thereafter removal of the protecting group R^{3P} to the formation of a compound of the formula

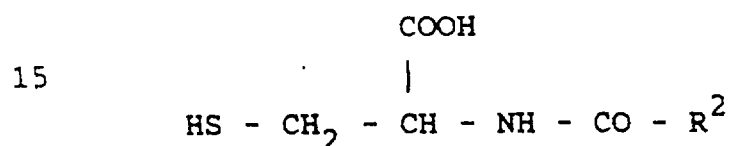




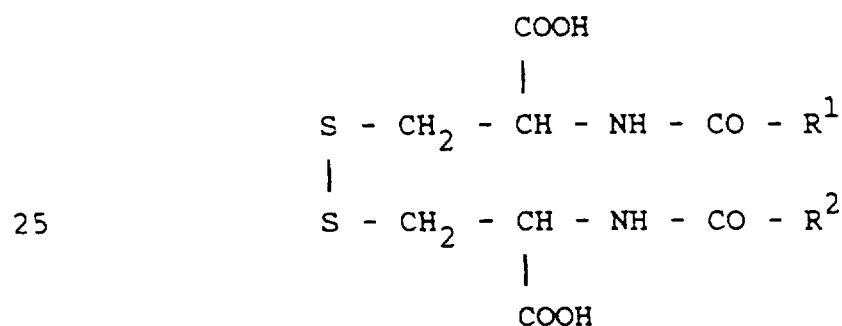
o) the equilibration, in alkaline aqueous solution of a mixture of a cystine derivative and a cysteine derivative having the formulas



and



or alkali salts thereof, wherein R^1 and R^2 are as defined above, to the formation of a compound of the formula



and finally, if desired, transferal of the compounds obtained by any of the methods a) - o) into a physiologically acceptable salt.

9. A pharmaceutical preparation comprising as active ingredient a compound according to any of claims 1-7.

10. A pharmaceutical preparation according to claim 9 in dosage unit form.





11. A pharmaceutical preparation according to claims 9-10 comprising the active ingredient in association with a pharmaceutically acceptable carrier.
- 5 12. A compound according to any of claims 1-7 for use as a therapeutically active substance.
13. Use of a compound according to any of claims 1-7 for the preparation of medicaments with immunomodulating
10 action.
14. A method for the treatment of diseases due to defects in the immune system, in mammals including man, characterized by the administration to a host in need of
15 such treatment of an effective amount of a compound according to any of claims 1-7.

