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Organic salts of N,N'-diacetyl cystine

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Organic salts of N,N'-diacetyl cystine**5 Field of the Invention**

The present invention relates to organic salts of N,N'-diacetyl cystine, in the following referred to as DiNAc, with immunomodulating activity as well as pharmaceutical compositions based on these salts and methods for their pharmacological use. The invention specifically concerns methods to obtain
10 crystalline, non-hygroscopic and chemically stable salts containing non-toxic organic cations, which are useful for therapy of diseases where a defect in the immune system is indicated.

Background Art

15 N-Acetyl-L-cysteine is a well known compound which is routinely used as a therapeutic agent against chronic obstructive diseases and chronic bronchitis. Although the first patent was filed in 1964 (GB 954268) the mechanism of action of the compound has not been established. It has recently been discovered that the corresponding disulfide of N-Acetyl-L-
20 cysteine, i.e. L-DiNAc, acts as a potent immunostimulator (Swedish patent application no SE 9002067-8), showing an activity comparable to contemporary immunostimulants such as sodium diethyl dithiocarbamate or 2,2'-dithiobisethanol.

25 Routes for the preparation of DiNAc have been reported in several patents (US Patent Nos 4,827,016, 4,724,239 and 4,708,965, European Patent No 300100 and German Patent No 2326444). DiNAc is, however, amorphous and hygroscopic, so that it is difficult to isolate and formulate into pharmaceutical compositions and its administration is normally only in the
30 form of aqueous solutions. Most salts of DiNAc with inorganic or organic cations share the same unfavourable physical properties with the free



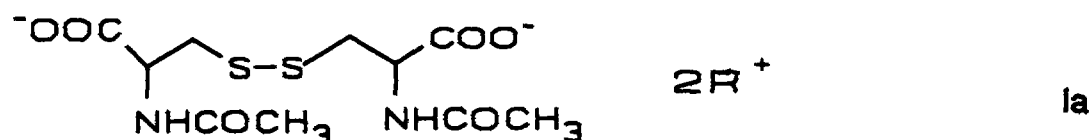
diacid. Examples of salts of other, sulfur-containing, amino acids have appeared in the patent literature (US patent No 3,647,834, JP patent No 56155298 and FR patent No 8106592)

5 Disclosure of the Invention

We have now surprisingly found that a few salts of DiNAc with organic bases exhibit a favourable combination of non-hygroscopicity and crystallinity which permits the isolation and formulation of these salts in solid form and allows them to be administered by inhalation in solid form, or in other dry
10 formulations, should this be clinically desirable.

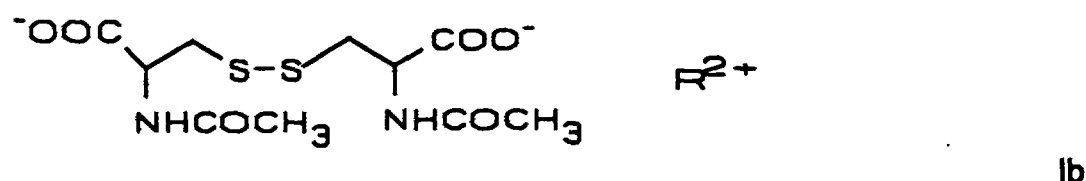
The present invention provides organic salts of DiNAc, having the formula Ia or Ib:

15



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or



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where R^+ and R^{2+} is a mono- or diprotonated organic amine respectively. The organic amine is selected from lysinium, ethylenediaminium, N,N'-dibenzylethylenediaminium, N-benzyl-2-phenylethylaminium, piperazinium and 1-adamantanaminium.

30

Lysinium can be in its D- or L-form. Most preferred is the L-form.



The invention includes hydrated and solvated salts, e.g. solvated with lower alkanols. The invention includes salts of DiNAc in its individual isomers, i.e. D-, L- and meso-forms as well as in its racemic form. Most preferred are the L-forms of these salts.

5

We have found that the new salts of the invention fulfil the requirements of ease of crystallization, non-hygroscopicity and chemical stability while still retaining the immunomodulating activity of DiNAc, and are thus medically useful.

10

This invention thus provides compounds with advantageous properties for the treatment of diseases where an anomaly of the immune response or an aberrant immune response or an ineffective host defence can be suspected. Such diseases include chronic bronchitis, where a reduction of the rate of exacerbations has previously been reported with immune response modifiers such as Biostim (Radermecker, M. et al. Int. J. Immunopharmac. 10, 913-917, 1988; Scheffer, J. et al. Arzneim. Forsch/Drug Res. 41, 815820, 1991), Ribomunyl and BronchoVaxom (Paupe, J. Respiration 58, 150-154, 1991) as well as with N-Acetylcysteine (See Bergstrand, H. et al J. Free Radic. Biol. Med. 2, 119-127, 1986).

20

Such diseases also include certain forms of malignant diseases. Thus, numerous research institutes round the world aim at finding ways of stimulating the immune response of patients with various forms of malignant diseases and numerous reviews in the literature deal with this approach (Stevenson, F.K. FASEB J 5: 2250-2257, 1991). To mention one example patients with intracranial tumours (gliomas) exhibit a profound decrease in immunity possibly due to a defect in the secretion of IL-2 as well as the expression of IL-2 receptors in T cells from such patients (Roszman, T. et al. Immunology Today 12, 370-374, 1991). Moreover, a significant adjuvant effect in immunotherapy of melanoma and colon carcinoma has been

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documented for the immunostimulator Levamisole (Van Wauwe, J. and Janssen, P.A.J. Int J. Immunopharmac. 13, 3-9, 1991) and immunotherapy with IL-2 in vivo or treatment of patients lymphokine activated killer cells with IL-2 ex vivo has caused the regression of cancer in selected patients

5 (Rosenberg, S.A. Immunology Today 9, 58-, 1988). The malignant diseases at which the compounds I can be expected to have advantageous effects include tumours of mesenchymal origin such as sarcomas like fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma or chordosarcoma, sarcomas like angiosarcoma, endotheliosarcoma,

10 lymphangiosarcoma, synoviosarcoma or mesotheliosarcoma, leukemias and lymphomas like granulocytic leukemia, monocytic leukemia, lymphocytic leukemia, malignant lymphoma, plasmocytoma, reticulum cell sarcoma or Hodgkins disease, sarcomas like leiomyosarcoma or rhabdomyosarcoma, tumours of epithelial origin (Carcinomas) like squamous cell carcinoma,

15 basal cell carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, adenocarcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, undifferentiated carcinoma, bronchogenic carcinoma, melanoma, renal cell carcinoma, hepatoma-liver cell carcinoma, bile duct carcinoma-cholangiocarcinoma, papillary carcinoma,

20 transitional cell carcinoma, squamous cell carcinoma, choriocarcinoma, seminoma or embryonal carcinoma, tumours of the central nervous system like glioma, meningoma, medulloblastoma, schwannoma or ependymoma.

Moreover, the compounds also have advantageous properties for treatment

25 of chronic infections such as herpes, aphthous stomatitis and minimal change syndrome where clinical improvement has previously been reported by treatment with an immunostimulator such as Levamisole as well as other chronic inflammatory diseases in the urinary tract or in ear, nose or throat, which benefit from treatment with immunostimulators such as Biostim,

30 Broncho-Vaxom and Ribomunyl, or at HIV infection or AIDS.



Moreover, an impairment, a defect or an imbalance of the immune response has also been postulated to exist at atopic diseases such as atopic dermatitis, rhinitis and asthma (Katz, D.H. Transplantation Reviews 41, 77-108, 1977). Since theoretical considerations suggest that stimulation of an immune response would possibly be the best way of restoring imbalances and autoimmunity (Varela, F.J. and Coutinho, A. Immunology Today 12, 159-166, 1991), the compounds can also be expected to have advantageous properties for treatment of asthma, rhinitis, atopic dermatitis and autoimmune diseases like non-obese diabetes, systemic lupus erythematosus, sclerodermia, Sjögren's syndrome, dermatomyositis or multiple sclerosis, rheumatoid arthritis and possibly psoriasis.

Moreover, the compounds, due to their immune stimulating properties, can be expected to have advantageous properties as adjuvants in various forms of vaccine preparations.

Finally, the compounds can be expected to have advantageous properties for treatment of atherosclerosis whether or not it will influence a putative inflammatory process in this condition (Hansson. G.K. et al. Proc. Nat. Acad. Sci. USA 88,10530, 1991).

The importance of immunostimulatory drugs on tumour outgrowth is illustrated in our relevant tumour outgrowth test systems. The experimental rat tumour models described below reflect very well the tumourostatic potency of the compounds of this invention in comparison with the well established immunostimulatory drug Levamisole, and they reflect clinically very well a fastly growing tumour, the mammary carcinoma, and a slowly progressively growing tumour, the glioma, and in both systems the drug has an excellent static effect on tumour outgrowth.

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Particularly suitable for treatment with the compounds of this invention are: Malignancies such as melanoma, mammary carcinoma, gastrointestinal carcinoma, glioma, bladder carcinoma and squamous cell carcinoma of the neck and head region;

5

Infections such as chronic bronchitis, hepatitis, post-infectious anergy and aquired immune deficiencies such as AIDS;

Posttraumatic immunological anergy, and

10

Purported autoimmune diseases such as rheumatoid arthritis, multiple sclerosis and psoriasis.

15

Effective doses for treatment of the diseases above are in the range of 0.1 - 100 mg, preferably 1.0 - 60 mg daily.

Methods of Preparation

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The organic salts of formula Ia and Ib are generally prepared by mixing DiNAc and the organic base, as defined above, each dissolved or dispersed in a solvent or solvent mixture. Solvents such as water, alcohols, glycols, ketones, amides, sulphoxides or other polar solvents or solvent mixtures may be used. The salt either precipitates directly from the reaction mixture, or is obtained by the addition of a less polar solvent or by evaporation or lyophilisation. The reaction is performed at elevated temperature or room

25 temperature, depending on the solubility in the medium. Alternatively, the salt can be prepared by oxidation of the appropriate N-acetyl cysteine salt in aqueous or alcoholic solution, followed by precipitation as above. The oxidation may be effected either chemically, using e.g. hydrogen peroxide or halogen, or electrochemically.

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Pharmaceutical Formulations

According to the present invention the compounds of the formula Ia and Ib can be formulated for administration by inhalation as well as by other routes, e.g. orally or topically with or without a pharmaceutically acceptable carrier.

5

The substance can be inhaled from a pressurized metered dose inhaler, from a dry powder inhaler, e.g. Turbuhaler® or from a dry powder inhaler utilizing gelatine, plastic or other capsules. Non-toxic and chemically inert substances e.g. lactose, trehalose, mannitol or glucose, can be added to the powdered substance.

10

The substance can also be administered orally in the form of capsules or tablets where the capsule, the tablet or the active substance itself can be coated or non-coated. This coating may consist of for example hydroxypropyl cellulose and aerosil in isopropanol or other suitable solvents. A non-toxic and chemically inert substance can be added to the powdered substance in order to obtain desirable physical or pharmaceutical properties.

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Parenteral administration of the new compounds is also possible.

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Pharmacological Experiments

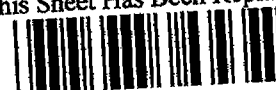
The ability of the compound di-L-lysiniun-N,N'-diacetyl-L-cystinate (Ia; R = lysine) to modulate immune responses is illustrated by its efficacy in the animal delayed type hypersensitivity (DTH) test in the mouse.

25

Both male and female Balb/c mice, obtained from Bomholtsgaard (Denmark), were used at the weight of 18-20 gram. 4-Ethoxymethylene-2-phenyloxazolin-5-one (OXA) was purchased from BDH (England) and served as the antigen in this test.

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The mice were sensitized, Day 0, by epicutaneous application of 150 μ l of an absolute ethanol-acetone (3:1) solution containing 3% OXA on the shaved abdomen. Treatment with the disulphide salt or vehicle (0.9% NaCl) was initiated by oral feeding immediately after sensitization and continued
5 once daily until Day 6. Seven days (Day 6) after the sensitization, both ears of all mice were challenged on both sides by topical application of 20 μ l 1% OXA dissolved in peanut oil. Ear thickness was measured prior to and 24 or 48 hours after challenge using an Oditest spring calliper. Challenges and measurements were performed under light pentobarbital anaesthesia.

10

The intensity of the DTH reactions was expressed according to the formula: $T_{t24/48} - T_{t0}$ μ m units, where t_0 , t_{24} and t_{48} represent the ear thickness before and 24 or 48 hours after challenge respectively, in individual tests (T). The results were expressed as the mean $\pm$ S.E.M. The level of significance
15 between means of the groups was obtained by Student's two-tailed t-test. Tables 1 and 2 show the results from 24 and 48 hours measurements respectively, from a representative experiment. The immunostimulating potency of the compound is reflected in a significant difference in the increase in ear thickness as compared to the control. Thus, in treated
20 animals, after 24 h the response was about twice that of the control animals (15 μ m compared to 8 μ m, Table 1).

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Table 1.

Ear thickness 24 hours after challenge of animals treated with the indicated doses of di-L-lysiniium-N,N'-diacetyl-L-cystinate or vehicle (NaCl).

5	Dose $\mu\text{mol/kg}$	N	Diff. $T_{t24}-T_{t0}$	S.E.M.	Sign.
	NaCl	10	8.25	0.56	
	0.03	10	15.00	0.42	***
10	3.0	10	15.80	0.77	***

*** $P < 0.001$.

Table 2.

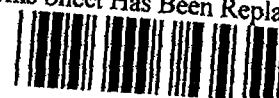
Ear thickness 48 hours after challenge of animals treated with the indicated doses of di-L-lysiniium-N,N'-diacetyl-L-cystinate or vehicle (NaCl).

20	Dose $\mu\text{mol/kg}$	N	Diff. $T_{t48}-T_{t0}$	S.E.M.	Sign.
	NaCl	10	8.83	0.31	
	0.03	10	12.55	0.41	***
	3.0	10	13.20	0.28	***

*** $P < 0.001$.

The ability of the same test compound (la, R = lysine) to prolong or increase the survival of rats suffering from tumours is illustrated by two different experiments with tumour inoculation in rats.

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Two groups each consisting of ten rats of the Wistar Furth strain were inoculated subcutaneously with 10^4 mammary carcinoma cells per rat and tumour size was measured twice a week. In one group the rats were drinking ordinary water and in the other group the test compound dissolved in water so as to result in a dose of $0,03 \mu\text{mol/kg/day}$ of animal weight. After 38 days one animal from the water-drinking group was still alive while from the group which was given the compound seven animals were alive.

In another experiment three groups each consisting of ten rats of the Lewis strain were inoculated subcutaneously with 10^6 glioma cells per rat and tumour size was measured twice a week. In one group the rats were drinking ordinary water and in the second group the test compound dissolved in water so as to give a dose of $0,03 \mu\text{mol/kg/day}$ of animal weight, while in the third group the rats were drinking water containing Levamisole, a well-known cancer therapeutic drug also at a dose of $0,03 \mu\text{mol/kg/day}$ of animal weight. After 119 days there was no surviving rat in the water-drinking group. In the group, which was given the test compound, six rats were still alive while in the group, which received Levamisole, three rats were alive after 119 days.

In both experiments with tumours there was a lower frequency of tumour positive animals and in both experiments there was an inhibition of tumour growth of the developed tumours in the groups receiving the compound solubilized in water.

In another experiment two groups each consisting of eight SCID mice (mice with the immune defect Severe Combined Immunodeficiency Disease) were inoculated with $2 \cdot 10^3$ mammary carcinoma cells and outgrowth was measured twice a week. In one group the mice were drinking ordinary water and in the second group the test compound dissolved in water so as to give a dose of $0.03 \mu\text{mol/kg/day}$ of animal weight. There were no significant



differences between the groups concerning growth rate and incidence. This indicates that the compound has no direct effect on the tumour cells but acts via the immune apparatus.

- 5 In an animal model of lupus, experimental murine systemic lupus erythematosus (SLE), MRL lpr/lpr mice spontaneously develop lymphoid hyperplasia, dermatitis, arthritis and glomerulonephritis. The effect of di-L-lysinium-N,N'-diacetyl-L-cystinate on glomerulonephritis as proteinuria and hematuria and also the survival rate of the animals, has been studied in this
- 10 murine lupus model. Di-L-lysinium-N,N'-diacetyl-L-cystinate was administrated in the drinking water and the mean dosage was calculated to 0.03 $\mu\text{mol/kg/day}$. The control mice received tap water. Both groups had free access to the drinking water. The treatment started when the animals reached 8 weeks of age and continued until death or 46 weeks of age, when
- 15 the study was ended.

The assessment of proteinuria and haematuria was performed by the usage of reagent strips, Eour-Test*, Boehringer Mannheim.

- 20 Upon administration of di-L-lysinium-N,N'-diacetyl-L-cystinate the survival rate was significantly improved compared to the control mice. The mortality rate for the untreated group (21 animals) reached 50% around 25 weeks of age. For the di-L-lysinium-N,N'-diacetyl-L-cystinate treated MRL-lpr/lpr mice (12 animals) this mortality rate was not reached until week 44.

25

This form of treatment also significantly improved the score for both proteinuria and haematuria measured as arbitrary units of the reagent strips, compared to the untreated group.

- 30 These results show the immunomodulating potency of the compounds of this invention, particularly with regard to SLE.

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Working Examples

Pharmaceutical Formulations

The following examples are representative of pharmaceutical formulations intended for different modes of local and systemic administration.

Example 1 Pressurised aerosol for inhalation.

The aerosol system is arranged so that each metered dose contains 0.1 - 1.0 mg.

10

Compound Ia or Ib, micronized	1.0 % w/w
Sorbitan trioleate	0.7 % w/w
Trichloromonofluoromethane	24.4 % w/w
Dichlorotetrafluoroethane	24.4 % w/w
Dichlorodifluoromethane	49.5 % w/w

Example 2. Powder aerosol for inhalation of pure substance.

Pure substance prepared for inhalation from Turbuhaler.

Each single dose contains 0.1 - 1.0 mg.

20

Compound Ia or Ib, processed	0.1 - 1.0 mg
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Example 3. Powder aerosol for inhalation.

Each single dose contains 0.1 - 1.0 mg in a capsule.

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Compound I, micronized	0.1 - 1.0 mg
Lactose	50 mg

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Example 4. Solution for nebulising.

The solution contains 1.0 - 10.0 mg/mL and 1 - 3 mL may be administered in a single dose.

5	Compound I	1.0 - 10.0 mg
	Water for injection	to 1.0 mL

Example 5. Tablets.

Each tablet contains:

10	Compound I	0.1 - 100 mg
	Maize starch	50 mg
	Lactose	150 mg
	Polyvidone	7 mg
15	Microcrystalline cellulose	20 mg
	Magnesium stearate	2 mg

Example 6. Oral solution.

A single dose of 10 mL contains 10 - 100 mg.

20	Compound I	1 - 10 mg
	Sorbitol 70 %	150 mg
	Glycerol	100 mg
	Sodium benzoate	1 mg
25	Flavour	q.s.
	Water purified	to 1.0 mL

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Example 7. Tablet for controlled release

1 tablet:

	Compound I	1-100 mg
5	Paraffin Special	145 mg
	Lactose Powder	50 mg
	Colloidal Silicon Dioxide	5 mg
	Ethylcellulose 10 cps	13 mg
	Ethanol 99,5 vol%	85 mg
10	Magnesium Stearate	2,5 mg

Example 8. Granulate for controlled release

1 g of granulate:

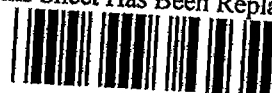
15	Compound I	1-100 mg
	Ethylcellulose Dispersion	10 mg
	Acetyltributylcitrate	0,5 mg
	Eudragit L 100-55	55 mg
	Triethylcitrate	5 mg
20	Talc	30 mg
	Water newly distilled	350 mg
	Pellets, neutral	to 1000 mg

Example 9. Solution for injection.

25 1 mL in a single dose contains 1.0 - 10.0 mg

	Compound Ia or Ib	1.0 - 10.0 mg
	Sodium chloride	8.9 - 7.7 mg
30	Water for injection	to 1.0 mL

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Example 10. Cream for topical application

1 g of cream contains:

	Compound I	0.1 - 1 mg
5	White soft paraffin	75 mg
	Liquid paraffin	10 mg
	Cetostearyl alcohol	75 mg
	Cetomacrogol 1000	20 mg
	Metagin	0.8 mg
10	Propagin	0.2 mg
	Water, purified	to 1.0 g

Example 11. Ointment for topical application

1 g of ointment contains:

15	Compound I	0.1 - 1 mg
	Liquid paraffin	150 mg
	White soft paraffin	to 1.0 g

20 Example 12. Ophthalmic solution

One dose of 2 drops contains 0.01-0.1 mg of compound I

	Compound I	0.1-1 mg
	Benzalkonium chloride	0.1 mg
25	Sodium chloride	9.0 mg
	Water, sterile	to 1.0 mL

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ChemistryExample 13

Di-L-lysiniun-N,N'-diacetyl-L-cystinate (Ia; R =

5 $\text{H}_2\text{N}(\text{COOH})\text{CH}(\text{CH}_2)_4\text{NH}_3$):

N-Acetyl-L-cysteine (22 mol, 3.59 kg) was dissolved in 2.6 L of deionized water. Sodium hydroxide 45% in water (22 mol, 1.92 kg) was added with stirring and temperature kept below 20°C. After adjusting the temperature to
 10 5°C, hydrogen peroxide (11.0 mol, 0.95L) was carefully added dropwise during 90 min. The temperature was not allowed to exceed 10°C during this addition. To the resulting solution solution was added 9 L of activated strongly acidic cation exchanger. After stirring for 10 minutes the pH was 2.0 and the ion exchanger was filtered off. The filtrate contained 9.65 mol of
 15 N,N'-diacetyl-L-cystine, as determined by HPLC using a standard prepared from the pure substance. To this crude solution was added L-lysine (19.3 mol, 3.17 kg). The thick solution formed, was slowly added to 50 L of refluxing ethanol containing 0.23 kg of crystalline di-L-lysiniun-N,N'-diacetyl-L-cystinate. After the addition, the slurry was allowed to cool and the crystals
 20 were filtered off. Washing with ethanol (8 L) and drying (vacuum, 40°C) for 12 hours afforded 5.36 kg (90%) of the title substance as a white crystalline solid.

Physical data: Mp: 210°C (dec); $[\alpha]_{\text{D}}^{25} = -70^\circ$ (c=0.54, H₂O) ; ¹H-NMR
 25 (D₂O) δ: 1.36-1.60 [4H, m, Lys γ CH₂], 1.73 [4H, p, Lys δ CH₂], 1.84-1.96 [4H, m, Lys β CH₂], 2.05 [6H, s, CH₃], 2.95 [2H, dd, CH₂S], 3.02 [4H, t, Lys ε CH₂], 3.25 [2H, dd, CH₂S], 3.76 [2H, t, Lys α CH], 4.50 [2H, dd, CHN]
¹³C-NMR (D₂O δ: 24.27; 24.80; 29.24; 32.72; 41.89; 42.78; 52.96; 57.30; 176.53; 177.41; 179.74; Analysis: Calcd for C₂₂H₄₄O₁₀N₆S₂, C:42.8 H:7.2
 30 N: 13.6 Found, C: 42.6 H: 7.4 N: 13.7; MS m/z = 325 (MH⁺), m/z = 471 (MLysH⁺), m/z = 617 (MLys₂H⁺).

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Example 14**Ethylenediaminium-N,N'-diacetyl-L-cystinate (lb; R = H₃NCH₂CH₂NH₃):**

To N,N'-Diacetyl-L-cystine (30.9 mmol, 10 g) dissolved in 20 mL of water was added ethylenediamine (61.8 mmol, 3.73 g) and ethanol (30 mL). The solution was concentrated to a thick paste which was redissolved in 80 mL of ethanol. Crystallisation occurred after 2 hours stirring at 10°C. Filtration and drying gave 6.2 g (45%) of the title compound.

Physical data: Mp 185.2-192.4°C. ¹H-NMR (D₂O) δ: 2.06 [6H, s, CH₃], 2.96[2H, dd, CH₂S], 3.26[2H, dd, CH₂S], 3.28 [4H, s, H₃N⁺CH₂CH₂N⁺H₃], 4.50 [2H, dd, CHN].
¹³C-NMR (D₂O) δ: 24.78; 39.99; 42.74; 56.98; 176.55; 179.82; Analysis: Calcd C: 37.5, H: 6.3, N: 14.6, S: 16.7. Found, C: 37.3, H: 6.8, N: 15.3, S: 15.2; MS m/z = 385 [M(H₂NCH₂CH₂NH₂)H⁺]

Example 15**N,N'-dibenzylethylenediaminium-N,N'-diacetyl-L-cystinate (lb; R = PhCH₂NH₂CH₂CH₂NH₂CH₂Ph)**

To a 63% solution of N,N'-diacetyl-L-cystine in water (67 mmole, 21.8 g) was added N,N'-dibenzylethylenediamine (67 mmole, 16.0 g). The exothermic reaction gave a slightly oily product which could be recrystallised from water to give 12.0 g (32%) of the title substance as white crystalline needles.

Physical data: Mp 163.8-165.3°C. ¹H-NMR (D₂O) δ: 2.04 [6H,s,CH₃], 2.93 [2H,dd,CH₂S], 3.22 [2H,dd,CH₂S], 3.44 [4H,s,CH₂NBn], 4.27 [4H,s,PhCH₂N], 4.47 [2H,dd,CHN], 7.44-7.54 [10H,m,Ph]. ¹³C-NMR (D₂O) δ: 24.82, 42.82, 45.71, 54.47, 56.99, 132.22, 132.58, 132.67, 133.52, 176.56, 179.80. Analysis (monohydrate): Calcd, C: 53.6, H: 6.6, N: 9.6, S: 11.0. Found, C: 54.5, H: 6.6, N: 9.6, S: 11.2.

Example 16**DI-(1-adamantanaminium)-N,N'-diacetyl-L-cystinate (la; R=[C(1) CH(3,5,7) CH₂(2,4,6,8,9,10) NH₃])**

To N,N'-diacetyl-L-cystine (5.35 mmole, 1.73 g) dissolved in 5 mL of water was added 1-adamantanamine (10.7 mmole, 1.61 g). To the solution was dropwise added 60 mL of acetone. The resulting crystalline salt was filtered and dried in vacuo, yielding 2.3 g (67%) of the title compound.

- 5 Physical data: Mp 162°C, $^1\text{H-NMR}$ (D_2O) δ : 1.71 [12H, broad dd, $\text{CH}_2(4,6,10)$], 1.87 [12H,d, $\text{CH}_2(2,8,9)$], 2.05 [6H,s, CH_3], 2.17 [6H, broad s, $\text{CH}(3,5,7)$], 2.96 [2H,dd, CH_2S], 3.26 [2H,dd, CH_2S], 4.50 [2H,dd,CHN]

Example 17

- 10 **Di-(N-benzyl-2-phenylethylaminium)-N,N'-L-cystinate**
(1a; $\text{R}=\text{PhCH}_2\text{CH}_2\text{NH}_2\text{CH}_2\text{Ph}$)

To N,N'-diacetyl-L-cystine (28.7 mmole, 9.3 g) dissolved in 20 mL of water was added N-benzyl-2-phenylethylamine (57.4 mmole, 12.1 g). The solution was concentrated to a thick paste, from which the salt slowly crystallized.

- 15 The crystalline title compound was isolated and dried.

Physical data: Mp 87°C. $^1\text{H-NMR}$ (D_2O) δ : 2.05 [6H,s, CH_3], 2.95 [2H,dd, CH_2S], 3.04 [4H,t, PhCH_2C], 3.24 [2H,dd, CH_2S], 3.33 [4H,t, CH_2NBn], 4.25 [4H,s, PhCH_2N], 4.49 [2H,dd,CHN], 7.30-7.52 [20H,m,Ph]

20

Example 18

Piperazinium-N,N'-diacetyl-L-cystinate
(1b; $\text{R}=\text{NH}_2(1,4) \text{CH}_2(2,3,5,6)$)

- 25 To N,N'-diacetyl-L-cystine (4.60 mmole, 1.49 g) dissolved in 5 mL of water was added piperazine (4.60 mmole, 0.90 g). To the solution was added enough isopropanol to cause formation of an oil, which slowly solidifies.

The salt was isolated and dried.

- Physical data: Mp >170°C (dec.) $^1\text{H-NMR}$ (D_2O) δ : 2.05 [6H,s, CH_3], 2.96
30 [2H,dd, CH_2S], 3.26 [2H,dd, CH_2S], 3.42 [8H,s, $\text{CH}_2(2,3,5,6)$], 4.49 [2H,dd,CHN]

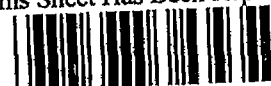
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Example 19**Di-L-lysinium-N,N'-diacetyl-L-cystinate (Ia;** **$R=H_2N(COOH)CH(CH_2)_4NH_3$).**

- 5 N-acetyl-L-cysteine (37 mmol, 6.0 g) and L-lysine (37 mmol, 5.4 g) were dissolved in 10 mL of deionized water. Hydrogen peroxide (18 mmol, 1.5 mL) was added dropwise while stirring, and the temperature was kept below 25°C. The solution was stirred for an additional 4 h. The viscous solution was slowly added to 150 mL of refluxing ethanol containing 0.50 g of
- 10 crystalline di-L-lysinium-N,N'-diacetyl-L-cystinate. After addition the solution was allowed to cool, and the crystals were filtered off. Washing with ethanol (20 mL), and drying (vacuum, 45°C) for 24 h afforded 10.0 g (84%) of the title substance as a white crystalline solid.
- 15 Physical data: Mp: 208°C; $[\alpha]_D^{25} = -73^\circ$ (c=0.54, H₂O)

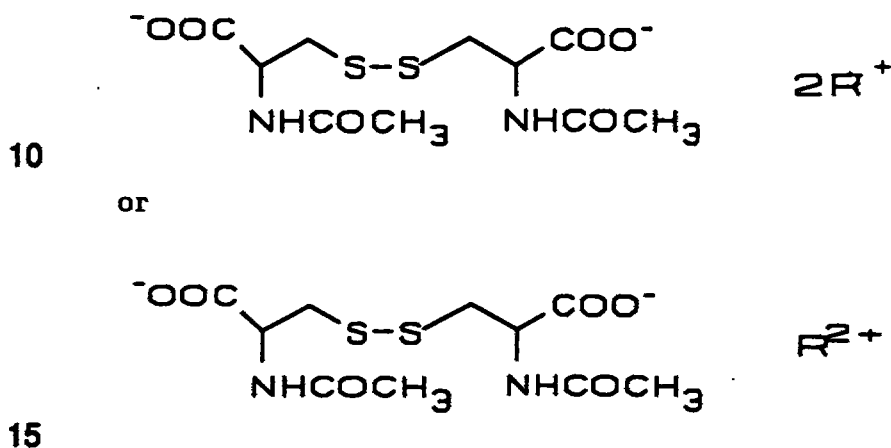
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Claims

1. A salt of an organic base and N,N'-diacetyl cystine in its individual isomers, i.e. D-, L- and meso-forms as well as its racemic forms [DiNAc]
5 characterized by the general formula



wherein R^+ and R^{2+} is the cation of the organic base lysine, ethylenedi-
amine, N,N'-dibenzylethylenediamine, adamantanamine, N-benzyl-2-
20 phenylethylamine or piperazine.

2. A salt according to Claim 1 of N,N'-diacetyl-L-cystine.
3. A process for preparing a salt of a compound as defined in Claim 1 or 2
25 by treating DiNAc with the organic base or a salt containing these cations of the organic base, reactants being dissolved or dispersed in a solvent or a solvent mixture and isolating the salt which may optionally be in hydrated or solvated form.
- 30 4. A process for preparing a salt as defined in Claim 1 or 2 by oxidizing a salt of N-acetylcysteine and the organic base and isolating the DiNAc salt





which may optionally be in hydrated or solvated form.

5. A pharmaceutical composition comprising as active ingredient a salt of DiNAc according to Claim 1 or 2.

5

6. A pharmaceutical composition as claimed in Claim 5 for administration from a pressurized metered dose inhaler or from a dry powder inhaler or from a dry powder utilizing gelatine, plastic or other capsules.

10 7. A pharmaceutical composition as claimed in Claim 5 or 6 including a pharmaceutically acceptable carrier is added to the composition.

8. A pharmaceutical composition as claimed in Claim 5 in the form of capsules or tablets.

15

9. A pharmaceutical composition as claimed in Claim 8 including a pharmaceutically acceptable carrier.

10. A pharmaceutical composition as claimed in claim 5 for administration in a solution by nebulization.

20

11. A salt according to Claim 1 or 2 for use as a therapeutically active substance.

12. A salt according to Claim 1 or 2 for use in the treatment of malignant diseases.

25

13. Use of a salt according to Claim 1 or 2 for the preparation of a medicament with immunomodulating activity.

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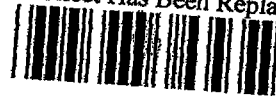


14. Use of a salt according to Claim 1 or 2 for the preparation of a medicament for use in the treatment of malignant diseases.

5 15. A method for the treatment of diseases which are due to defects in the immune system in mammals including man, characterized by the administration to a host in need of such treatment of an effective amount of a salt of DiNAc according to Claim 1 or 2.

10 16. A method for the treatment of malignant diseases in mammals including man, characterized by the administration to a host in need of such treatment of an effective amount of a salt of DiNAc according to Claim 1 or 2.

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Abstract

New crystalline organic salts of N,N'-diacetyl cystine with immunomodulating effect, processes for their preparation, pharmaceutical compositions
5 containing them and methods of their pharmacological use.

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Aktiebolaget Astra
Södertälje/Sweden

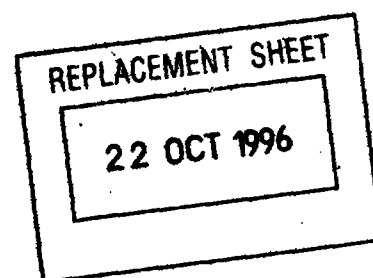
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☒	Abstract : paged ...23... to paged ...25...	
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Inventors: Carl-Magnus Andersson, Håkan Bergstrand, Edib Jakupovic,
Bo-Göran Josefsson, Magnus Lindvall, Bengt Sämstrand,
Eric Teneberg

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Organic salts of N,N'-diacetyl cystine



Organic salts of N,N'-diacetyl cystine

Field of the Invention

The present invention relates to organic salts of N,N'-diacetyl cystine, in the following referred to as DiNAc, with immunomodulating activity as well as pharmaceutical compositions based on these salts and methods for their pharmacological use. The invention specifically concerns methods to obtain crystalline, non-hygroscopic and chemically stable salts containing non-toxic organic cations, which are useful for therapy of diseases where a defect in the immune system is indicated.

Background Art

N-Acetyl-L-cysteine is a well known compound which is routinely used as a therapeutic agent against chronic obstructive diseases and chronic bronchitis. Although the first patent was filed in 1964 (GB 954268) the mechanism of action of the compound has not been established. It has recently been discovered that the corresponding disulfide of N-Acetyl-L-cysteine, i.e. L-DiNAc, acts as a potent immunostimulator (Swedish patent application no SE 9002067-8), showing an activity comparable to contemporary immunostimulants such as sodium diethyl dithiocarbamate or 2,2'-dithiobisethanol.

Routes for the preparation of DiNAc have been reported in several patents (US Patent Nos 4,827,016, 4,724,239 and 4,708,965, European Patent No 300100 and German Patent No 2326444). DiNAc is, however, amorphous and hygroscopic, so that it is difficult to isolate and formulate into pharmaceutical compositions and its administration is normally only in the form of aqueous solutions. Most salts of DiNAc with inorganic or organic cations share the same unfavourable physical properties with the free



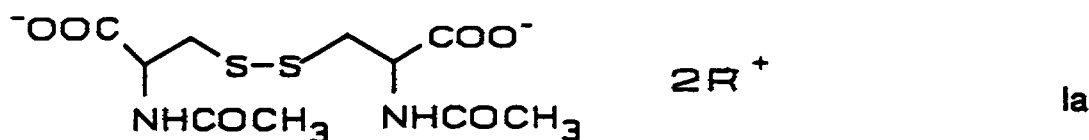
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diacid. Examples of salts of other, sulfur-containing, amino acids have appeared in the patent literature (US patent No 3,647,834, JP patent No 56155298 and FR patent No 8106592)

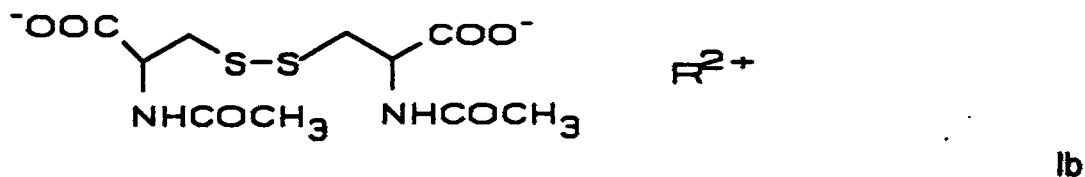
Disclosure of the Invention

We have now surprisingly found that a few salts of DiNAc with organic bases exhibit a favourable combination of non-hygroscopicity and crystallinity which permits the isolation and formulation of these salts in solid form and allows them to be administered by inhalation in solid form, or in other dry formulations, should this be clinically desirable.

The present invention provides organic salts of DiNAc, having the formula Ia or Ib:



or



where R^+ and R^{2+} is a mono- or diprotonated organic amine respectively. The organic amine is selected from lysinium, ethylenediaminium, N,N'-dibenzylethylenediaminium, N-benzyl-2-phenylethylaminium, piperazinium and 1-adamantanaminium.

Lysinium can be in its D- or L-form. Most preferred is the L-form.

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The invention includes hydrated and solvated salts, e.g. solvated with lower alkanols. The invention includes salts of DiNAc in its individual isomers, i.e. D-, L- and meso-forms as well as in its racemic form. Most preferred are the L-forms of these salts.

We have found that the new salts of the invention fulfil the requirements of ease of crystallization, non-hygroscopicity and chemical stability while still retaining the immunomodulating activity of DiNAc, and are thus medically useful.

This invention thus provides compounds with advantageous properties for the treatment of diseases where an anomaly of the immune response or an aberrant immune response or an ineffective host defence can be suspected. Such diseases include chronic bronchitis, where a reduction of the rate of exacerbations has previously been reported with immune response modifiers such as Biostim (Radermecker, M. et al. Int. J. Immunopharmac. 10, 913-917, 1988; Scheffer, J. et al. Arzneim. Forsch/Drug Res. 41, 815820, 1991), Ribomunyl and BronchoVaxom (Paupe, J. Respiration 58, 150-154, 1991) as well as with N-Acetylcysteine (See Bergstrand, H. et al J. Free Radic. Biol. Med. 2, 119-127, 1986).

Such diseases also include certain forms of malignant diseases. Thus, numerous research institutes round the world aim at finding ways of stimulating the immune response of patients with various forms of malignant diseases and numerous reviews in the literature deal with this approach (Stevenson, F.K. FASEB J 5: 2250-2257, 1991). To mention one example patients with intracranial tumours (gliomas) exhibit a profound decrease in immunity possibly due to a defect in the secretion of IL-2 as well as the expression of IL-2 receptors in T cells from such patients (Roszman, T. et al. Immunology Today 12, 370-374, 1991). Moreover, a significant adjuvant effect in immunotherapy of melanoma and colon carcinoma has been



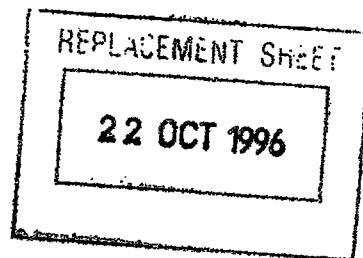
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documented for the immunostimulator Levamisole (Van Wauwe, J. and Janssen, P.A.J. Int J. Immunopharmac. 13, 3-9, 1991) and immunotherapy with IL-2 in vivo or treatment of patients lymphokine activated killer cells with IL-2 ex vivo has caused the regression of cancer in selected patients (Rosenberg, S.A. Immunology Today 9, 58-, 1988). The malignant diseases at which the compounds I can be expected to have advantageous effects include tumours of mesenchymal origin such as sarcomas like fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma or chordosarcoma, sarcomas like angiosarcoma, endotheliosarcoma, lymphangiosarcoma, synoviosarcoma or mesotheliosarcoma, leukemias and lymphomas like granulocytic leukemia, monocytic leukemia, lymphocytic leukemia, malignant lymphoma, plasmacytoma, reticulum cell sarcoma or Hodkins disease, sarcomas like leiomyosarcoma or rhabdomyosarcoma, tumours of epithelial origin (Carcinomas) like squamous cell carcinoma, basal cell carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, adenocarcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, undifferentiated carcinoma, bronchogenic carcinoma, melanoma, renal cell carcinoma, hepatoma-liver cell carcinoma, bile duct carcinoma-cholangiocarcinoma, papillary carcinoma, transitional cell carcinoma, squamous cell carcinoma, choriocarcinoma, seminoma or embryonal carcinoma, tumours of the central nervous system like glioma, meningoma, medulloblastoma, schwannoma or ependymoma.

Moreover, the compounds also have advantageous properties for treatment of chronic infections such as herpes, aphthous stomatitis and minimal change syndrome where clinical improvement has previously been reported by treatment with an immunostimulator such as Levamisole as well as other chronic inflammatory diseases in the urinary tract or in ear, nose or throat, which benefit from treatment with immunostimulators such as Biostim, Broncho-Vaxom and Ribomunyl, or at HIV infection or AIDS.

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Moreover, an impairment, a defect or an imbalance of the immune response has also been postulated to exist at atopic diseases such as atopic dermatitis, rhinitis and asthma (Katz, D.H. Transplantation Reviews 41, 77-108, 1977). Since theoretical considerations suggest that stimulation of an immune response would possibly be the best way of restoring imbalances and autoimmunity (Varela, F.J. and Coutinho, A. Immunology Today 12, 159-166, 1991), the compounds can also be expected to have advantageous properties for treatment of asthma, rhinitis, atopic dermatitis and autoimmune diseases like non-obese diabetes, systemic lupus erythematosus, sclerodermia, Sjögren's syndrome, dermatomyositis or multiple sclerosis, rheumatoid arthritis and possibly psoriasis.

Moreover, the compounds, due to their immune stimulating properties, can be expected to have advantageous properties as adjuvants in various forms of vaccine preparations.

Finally, the compounds can be expected to have advantageous properties for treatment of atherosclerosis whether or not it will influence a putative inflammatory process in this condition (Hansson. G.K. et al. Proc. Nat. Acad. Sci. USA 88,10530, 1991).

The importance of immunostimulatory drugs on tumour outgrowth is illustrated in our relevant tumour outgrowth test systems. The experimental rat tumour models described below reflect very well the tumourostatic potency of the compounds of this invention in comparison with the well established immunostimulatory drug Levamisole, and they reflect clinically very well a fastly growing tumour, the mammary carcinoma, and a slowly progressively growing tumour, the glioma, and in both systems the drug has an excellent static effect on tumour outgrowth.

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Particularly suitable for treatment with the compounds of this invention are: Malignancies such as melanoma, mammary carcinoma, gastrointestinal carcinoma, glioma, bladder carcinoma and squamous cell carcinoma of the neck and head region;

Infections such as chronic bronchitis, hepatitis, post-infectious anergy and acquired immune deficiencies such as AIDS;

Posttraumatic immunological anergy, and

Purported autoimmune diseases such as rheumatoid arthritis, multiple sclerosis and psoriasis.

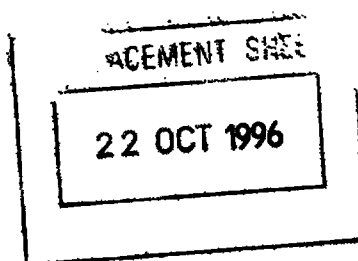
Effective doses for treatment of the diseases above are in the range of 0.1 - 100 mg, preferably 1.0 - 60 mg daily.

Methods of Preparation

The organic salts of formula Ia and Ib are generally prepared by mixing DiNAc and the organic base, as defined above, each dissolved or dispersed in a solvent or solvent mixture. Solvents such as water, alcohols, glycols, ketones, amides, sulfoxides or other polar solvents or solvent mixtures may be used. The salt either precipitates directly from the reaction mixture, or is obtained by the addition of a less polar solvent or by evaporation or lyophilisation. The reaction is performed at elevated temperature or room temperature, depending on the solubility in the medium. Alternatively, the salt can be prepared by oxidation of the appropriate N-acetyl cysteine salt in aqueous or alcoholic solution, followed by precipitation as above. The oxidation may be effected either chemically, using e.g. hydrogen peroxide or halogen, or electrochemically.

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Pharmaceutical Formulations

According to the present invention the compounds of the formula Ia and Ib can be formulated for administration by inhalation as well as by other routes, e.g. orally or topically with or without a pharmaceutically acceptable carrier.

The substance can be inhaled from a pressurized metered dose inhaler, from a dry powder inhaler, e.g. Turbuhaler® or from a dry powder inhaler utilizing gelatine, plastic or other capsules. Non-toxic and chemically inert substances e.g. lactose, trehalose, mannitol or glucose, can be added to the powdered substance.

The substance can also be administered orally in the form of capsules or tablets where the capsule, the tablet or the active substance itself can be coated or non-coated. This coating may consist of for example hydroxypropyl cellulose and aerosil in isopropanol or other suitable solvents. A non-toxic and chemically inert substance can be added to the powdered substance in order to obtain desirable physical or pharmaceutical properties.

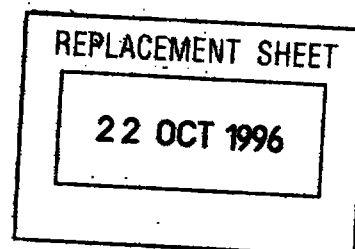
Parenteral administration of the new compounds is also possible.

Pharmacological Experiments

The ability of the compound di-L-lysiniun-N,N'-diacetyl-L-cystinate (Ia; R = lysine) to modulate immune responses is illustrated by its efficacy in the animal delayed type hypersensitivity (DTH) test in the mouse.

Both male and female Balb/c mice, obtained from Bomholtsgaard (Denmark), were used at the weight of 18-20 gram. 4-Ethoxymethylene-2-phenyloxazolin-5-one (OXA) was purchased from BDH (England) and served as the antigen in this test.

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The mice were sensitized, Day 0, by epicutaneous application of 150 μ l of an absolute ethanol-acetone (3:1) solution containing 3% OXA on the shaved abdomen. Treatment with the disulphide salt or vehicle (0.9% NaCl) was initiated by oral feeding immediately after sensitization and continued once daily until Day 6. Seven days (Day 6) after the sensitization, both ears of all mice were challenged on both sides by topical application of 20 μ l 1% OXA dissolved in peanut oil. Ear thickness was measured prior to and 24 or 48 hours after challenge using 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: $T_{t24/48} - T_{t0}$ μ m units, where t_0 , t_{24} and t_{48} represent the ear thickness before and 24 or 48 hours after challenge respectively, in individual tests (T). The results were expressed as the mean $\pm$ S.E.M. The level of significance between means of the groups was obtained by Student's two-tailed t-test. Tables 1 and 2 show the results from 24 and 48 hours measurements respectively, from a representative experiment. The immunostimulating potency of the compound is reflected in a significant difference in the increase in ear thickness as compared to the control. Thus, in treated animals, after 24 h the response was about twice that of the control animals (15 μ m compared to 8 μ m, Table 1).

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Table 1.

Ear thickness 24 hours after challenge of animals treated with the indicated doses of di-L-lysiniium-N,N'-diacetyl-L-cystinate or vehicle (NaCl).

Dose $\mu\text{mol/kg}$	N	Diff. $T_{t24}-T_{t0}$	S.E.M.	Sign.
NaCl	10	8.25	0.56	
0.03	10	15.00	0.42	***
3.0	10	15.80	0.77	***

*** $P < 0.001$.

Table 2.

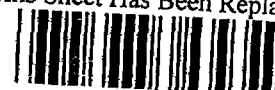
Ear thickness 48 hours after challenge of animals treated with the indicated doses of di-L-lysiniium-N,N'-diacetyl-L-cystinate or vehicle (NaCl).

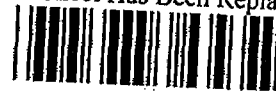
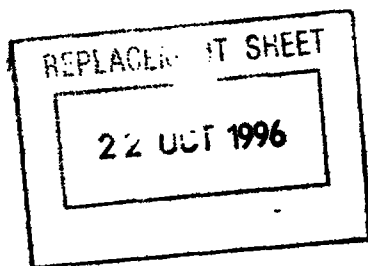
Dose $\mu\text{mol/kg}$	N	Diff. $T_{t48}-T_{t0}$	S.E.M.	Sign.
NaCl	10	8.83	0.31	
0.03	10	12.55	0.41	***
3.0	10	13.20	0.28	***

*** $P < 0.001$.

The ability of the same test compound (Ia, R = lysine) to prolong or increase the survival of rats suffering from tumours is illustrated by two different experiments with tumour inoculation in rats.

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Two groups each consisting of ten rats of the Wistar Furth strain were inoculated subcutaneously with 10^4 mammary carcinoma cells per rat and tumour size was measured twice a week. In one group the rats were drinking ordinary water and in the other group the test compound dissolved in water so as to result in a dose of $0,03 \mu\text{mol/kg/day}$ of animal weight. After 38 days one animal from the water-drinking group was still alive while from the group which was given the compound seven animals were alive.

In another experiment three groups each consisting of ten rats of the Lewis strain were inoculated subcutaneously with 10^6 glioma cells per rat and tumour size was measured twice a week. In one group the rats were drinking ordinary water and in the second group the test compound dissolved in water so as to give a dose of $0,03 \mu\text{mol/kg/day}$ of animal weight, while in the third group the rats were drinking water containing Levamisole, a well-known cancer therapeutic drug also at a dose of $0,03 \mu\text{mol/kg/day}$ of animal weight. After 119 days there was no surviving rat in the water-drinking group. In the group, which was given the test compound, six rats were still alive while in the group, which received Levamisole, three rats were alive after 119 days.

In both experiments with tumours there was a lower frequency of tumour positive animals and in both experiments there was an inhibition of tumour growth of the developed tumours in the groups receiving the compound solubilized in water.

In another experiment two groups each consisting of eight SCID mice (mice with the immune defect Severe Combined Immunodeficiency Disease) were inoculated with $2 \cdot 10^3$ mammary carcinoma cells and outgrowth was measured twice a week. In one group the mice were drinking ordinary water and in the second group the test compound dissolved in water so as to give a dose of $0.03 \mu\text{mol/kg/day}$ of animal weight. There were no significant

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differences between the groups concerning growth rate and incidence. This indicates that the compound has no direct effect on the tumour cells but acts via the immune apparatus.

In an animal model of lupus, experimental murine systemic lupus erythematosus (SLE), MRL lpr/lpr mice spontaneously develop lymphoid hyperplasia, dermatitis, arthritis and glomerulonephritis. The effect of di-L-lysinium-N,N'-diacetyl-L-cystinate on glomerulonephritis as proteinuria and hematuria and also the survival rate of the animals, has been studied in this murine lupus model. Di-L-lysinium-N,N'-diacetyl-L-cystinate was administered in the drinking water and the mean dosage was calculated to 0.03 $\mu\text{mol/kg/day}$. The control mice received tap water. Both groups had free access to the drinking water. The treatment started when the animals reached 8 weeks of age and continued until death or 46 weeks of age, when the study was ended.

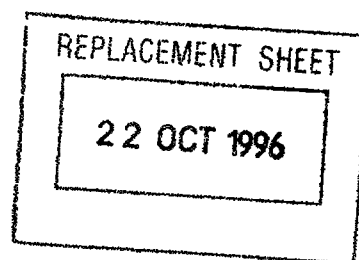
The assessment of proteinuria and haematuria was performed by the usage of reagent strips, Eour-Test*, Boehringer Mannheim.

Upon administration of di-L-lysinium-N,N'-diacetyl-L-cystinate the survival rate was significantly improved compared to the control mice. The mortality rate for the untreated group (21 animals) reached 50% around 25 weeks of age. For the di-L-lysinium-N,N'-diacetyl-L-cystinate treated MRL-lpr/lpr mice (12 animals) this mortality rate was not reached until week 44.

This form of treatment also significantly improved the score for both proteinuria and haematuria measured as arbitrary units of the reagent strips, compared to the untreated group.

These results show the immunomodulating potency of the compounds of this invention, particularly with regard to SLE.

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Working Examples

Pharmaceutical Formulations

The following examples are representative of pharmaceutical formulations intended for different modes of local and systemic administration.

Example 1 Pressurised aerosol for inhalation.

The aerosol system is arranged so that each metered dose contains 0.1 - 1.0 mg.

Compound Ia or Ib, micronized	1.0 % w/w
Sorbitan trioleate	0.7 % w/w
Trichloromonofluoromethane	24.4 % w/w
Dichlorotetrafluoroethane	24.4 % w/w
Dichlorodifluoromethane	49.5 % w/w

Example 2. Powder aerosol for inhalation of pure substance.

Pure substance prepared for inhalation from Turbuhaler.

Each single dose contains 0.1 - 1.0 mg.

Compound Ia or Ib, processed	0.1 - 1.0 mg
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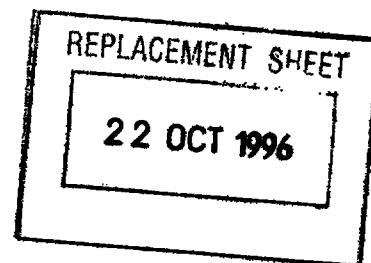
Example 3. Powder aerosol for inhalation.

Each single dose contains 0.1 - 1.0 mg in a capsule.

Compound I, micronized	0.1 - 1.0 mg
Lactose	50 mg

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**Example 4. Solution for nebulising.**

The solution contains 1.0 - 10.0 mg/mL and 1 - 3 mL may be administered in a single dose.

Compound I	1.0 - 10.0 mg
Water for injection	to 1.0 mL

Example 5. Tablets.

Each tablet contains:

Compound I	0.1 - 100 mg
Maize starch	50 mg
Lactose	150 mg
Polyvidone	7 mg
Microcrystalline cellulose	20 mg
Magnesium stearate	2 mg

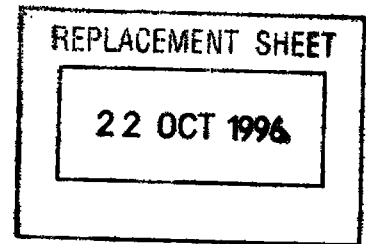
Example 6. Oral solution.

A single dose of 10 mL contains 10 - 100 mg.

Compound I	1 - 10 mg
Sorbitol 70 %	150 mg
Glycerol	100 mg
Sodium benzoate	1 mg
Flavour	q.s.
Water purified	to 1.0 mL

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**Example 7. Tablet for controlled release**

1 tablet:

Compound I	1-100 mg
Paraffin Special	145 mg
Lactose Powder	50 mg
Colloidal Silicon Dioxide	5 mg
Ethylcellulose 10 cps	13 mg
Ethanol 99,5 vol%	85 mg
Magnesium Stearate	2,5 mg

Example 8. Granulate for controlled release

1 g of granulate:

Compound I	1-100 mg
Ethylcellulose Dispersion	10 mg
Acetyltributylcitrate	0,5 mg
Eudragit L 100-55	55 mg
Triethylcitrate	5 mg
Talc	30 mg
Water newly distilled	350 mg
Pellets, neutral	to 1000 mg

Example 9. Solution for injection.

1 mL in a single dose contains 1.0 - 10.0 mg

Compound Ia or Ib	1.0 - 10.0 mg
Sodium chloride	8.9 - 7.7 mg
Water for injection	to 1.0 mL

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Example 10. Cream for topical application

1 g of cream contains:

Compound I	0.1 - 1 mg
White soft paraffin	75 mg
Liquid paraffin	10 mg
Cetostearyl alcohol	75 mg
Cetomacrogol 1000	20 mg
Metagin	0.8 mg
Propagin	0.2 mg
Water, purified	to 1.0 g

Example 11. Ointment for topical application

1 g of ointment contains:

Compound I	0.1 - 1 mg
Liquid paraffin	150 mg
White soft paraffin	to 1.0 g

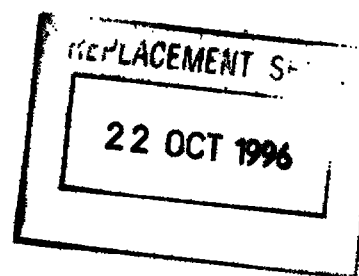
Example 12. Ophthalmic solution

One dose of 2 drops contains 0.01-0.1 mg of compound I

Compound I	0.1-1 mg
Benzalkonium chloride	0.1 mg
Sodium chloride	9.0 mg
Water, sterile	to 1.0 mL

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Chemistry

Example 13

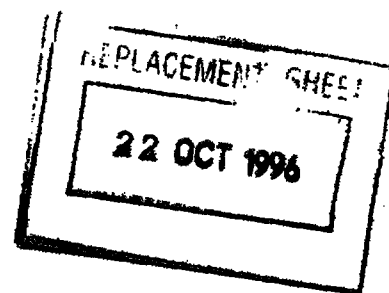
Di-L-lysinium-N,N'-diacetyl-L-cystinate (Ia; R = $\text{H}_2\text{N}(\text{COOH})\text{CH}(\text{CH}_2)_4\text{NH}_3$):

N-Acetyl-L-cysteine (22 mol, 3.59 kg) was dissolved in 2.6 L of deionized water. Sodium hydroxide 45% in water (22 mol, 1.92 kg) was added with stirring and temperature kept below 20°C. After adjusting the temperature to 5°C, hydrogen peroxide (11.0 mol, 0.95L) was carefully added dropwise during 90 min. The temperature was not allowed to exceed 10°C during this addition. To the resulting solution solution was added 9 L of activated strongly acidic cation exchanger. After stirring for 10 minutes the pH was 2.0 and the ion exchanger was filtered off. The filtrate contained 9.65 mol of N,N'-diacetyl-L-cystine, as determined by HPLC using a standard prepared from the pure substance. To this crude solution was added L-lysine (19.3 mol, 3.17 kg). The thick solution formed, was slowly added to 50 L of refluxing ethanol containing 0.23 kg of crystalline di-L-lysinium-N,N'-diacetyl-L-cystinate. After the addition, the slurry was allowed to cool and the crystals were filtered off. Washing with ethanol (8 L) and drying (vacuum, 40°C) for 12 hours afforded 5.36 kg (90%) of the title substance as a white crystalline solid.

Physical data: Mp: 210°C (dec); $[\alpha]_{\text{D}}^{25} = -70^\circ$ (c=0.54, H_2O) ; $^1\text{H-NMR}$ (D_2O) δ : 1.36-1.60 [4H, m, Lys γ CH_2], 1.73 [4H, p, Lys δ CH_2], 1.84-1.96 [4H, m, Lys β CH_2], 2.05 [6H, s, CH_3], 2.95 [2H, dd, CH_2S], 3.02 [4H, t, Lys ϵ CH_2], 3.25 [2H, dd, CH_2S], 3.76 [2H, t, Lys α CH], 4.50 [2H, dd, CHN] $^{13}\text{C-NMR}$ (D_2O) δ : 24.27; 24.80; 29.24; 32.72; 41.89; 42.78; 52.96; 57.30; 176.53; 177.41; 179.74; Analysis: Calcd for $\text{C}_{22}\text{H}_{44}\text{O}_{10}\text{N}_6\text{S}_2$, C:42.8 H:7.2 N: 13.6 Found, C: 42.6 H: 7.4 N: 13.7; MS $m/z = 325$ (MH^+), $m/z = 471$ (MLysH^+), $m/z = 617$ (MLys_2H^+).

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**Example 14**

Ethylenediaminium-N,N'-diacetyl-L-cystinate (Ib; R = H₃NCH₂CH₂NH₃):
 To N,N'-Diacetyl-L-cystine (30.9 mmol, 10 g) dissolved in 20 mL of water was added ethylenediamine (61.8 mmol, 3.73 g) and ethanol (30 mL). The solution was concentrated to a thick paste which was redissolved in 80 mL of ethanol. Crystallisation occurred after 2 hours stirring at 10°C. Filtration and drying gave 6.2 g (45%) of the title compound.

Physical data: Mp 185.2-192.4°C. ¹H-NMR (D₂O) δ: 2.06 [6H, s, CH₃], 2.96[2H, dd, CH₂S], 3.26[2H, dd, CH₂S], 3.28 [4H, s, H₃N⁺CH₂CH₂N⁺H₃], 4.50 [2H, dd, CHN].

¹³C-NMR (D₂O) δ: 24.78; 39.99; 42.74; 56.98; 176.55; 179.82; Analysis: Calcd C: 37.5, H: 6.3, N: 14.6, S: 16.7. Found, C: 37.3, H: 6.8, N: 15.3, S: 15.2; MS m/z = 385 [M(H₂NCH₂CH₂NH₂)H⁺]

Example 15

N,N'-dibenzylethylenediaminium-N,N'-diacetyl-L-cystinate (Ib; R = PhCH₂NH₂CH₂CH₂NH₂CH₂Ph)

To a 63% solution of N,N'-diacetyl-L-cystine in water (67 mmole, 21.8 g) was added N,N'-dibenzylethylenediamine (67 mmole, 16.0 g). The exothermic reaction gave a slightly oily product which could be recrystallised from water to give 12.0 g (32%) of the title substance as white crystalline needles.

Physical data: Mp 163.8-165.3°C. ¹H-NMR (D₂O) δ: 2.04 [6H,s,CH₃], 2.93 [2H,dd,CH₂S], 3.22 [2H,dd,CH₂S], 3.44 [4H,s,CH₂NBn], 4.27 [4H,s,PhCH₂N], 4.47 [2H,dd,CHN], 7.44-7.54 [10H,m,Ph]. ¹³C-NMR (D₂O) δ: 24.82, 42.82, 45.71, 54.47, 56.99, 132.22, 132.58, 132.67, 133.52, 176.56, 179.80. Analysis (monohydrate): Calcd, C: 53.6, H: 6.6, N: 9.6, S: 11.0. Found, C: 54.5, H: 6.6, N: 9.6, S: 11.2.

Example 16

Di-(1-adamantanaminium)-N,N'-diacetyl-L-cystinate

(Ia; R=[C(1) CH(3,5,7) CH₂(2,4,6,8,9,10) NH₃])





To N,N'-diacetyl-L-cystine (5.35 mmole, 1.73 g) dissolved in 5 mL of water was added 1-adamantanamine (10.7 mmole, 1.61 g). To the solution was dropwise added 60 mL of acetone. The resulting crystalline salt was filtered and dried in vacuo, yielding 2.3 g (67%) of the title compound.

Physical data: Mp 162°C, $^1\text{H-NMR}$ (D_2O) δ : 1.71 [12H, broad dd, $\text{CH}_2(4,6,10)$], 1.87 [12H, d, $\text{CH}_2(2,8,9)$], 2.05 [6H, s, CH_3], 2.17 [6H, broad s, $\text{CH}(3,5,7)$], 2.96 [2H, dd, CH_2S], 3.26 [2H, dd, CH_2S], 4.50 [2H, dd, CHN]

Example 17

Di-(N-benzyl-2-phenylethylaminium)-N,N'-L-cystinate

(Ia; R=PhCH₂CH₂NH₂CH₂Ph)

To N,N'-diacetyl-L-cystine (28.7 mmole, 9.3 g) dissolved in 20 mL of water was added N-benzyl-2-phenylethylamine (57.4 mmole, 12.1 g). The solution was concentrated to a thick paste, from which the salt slowly crystallized.

The crystalline title compound was isolated and dried.

Physical data: Mp 87°C. $^1\text{H-NMR}$ (D_2O) δ : 2.05 [6H, s, CH_3], 2.95 [2H, dd, CH_2S], 3.04 [4H, t, PhCH_2C], 3.24 [2H, dd, CH_2S], 3.33 [4H, t, CH_2NBn], 4.25 [4H, s, PhCH_2N], 4.49 [2H, dd, CHN], 7.30-7.52 [20H, m, Ph]

Example 18

Piperazinium-N,N'-diacetyl-L-cystinate

(Ib; R=NH₂(1,4) CH₂(2,3,5,6))

To N,N'-diacetyl-L-cystine (4.60 mmole, 1.49 g) dissolved in 5 mL of water was added piperazine (4.60 mmole, 0.90 g). To the solution was added enough isopropanol to cause formation of an oil, which slowly solidifies. The salt was isolated and dried.

Physical data: Mp >170°C (dec.) $^1\text{H-NMR}$ (D_2O) δ : 2.05 [6H, s, CH_3], 2.96 [2H, dd, CH_2S], 3.26 [2H, dd, CH_2S], 3.42 [8H, s, $\text{CH}_2(2,3,5,6)$], 4.49 [2H, dd, CHN]

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Example 19

**Di-L-lysini-um-N,N'-diacetyl-L-cystinate (Ia;
R=H₂N(COOH)CH(CH₂)₄NH₃).**

N-acetyl-L-cysteine (37 mmol, 6.0 g) and L-lysine (37 mmol, 5.4 g) were dissolved in 10 mL of deionized water. Hydrogen peroxide (18 mmol, 1.5 mL) was added dropwise while stirring, and the temperature was kept below 25°C. The solution was stirred for an additional 4 h. The viscous solution was slowly added to 150 mL of refluxing ethanol containing 0.50 g of crystalline di-L-lysini-um-N,N'-diacetyl-L-cystinate. After addition the solution was allowed to cool, and the crystals were filtered off. Washing with ethanol (20 mL), and drying (vacuum, 45°C) for 24 h afforded 10.0 g (84%) of the title substance as a white crystalline solid.

Physical data: Mp: 208°C; $[\alpha]_D^{25} = -73^\circ$ (c=0.54, H₂O)

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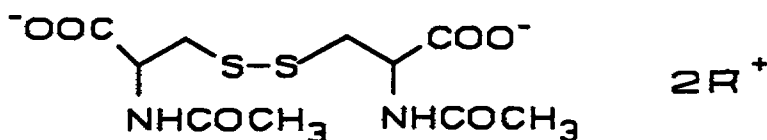
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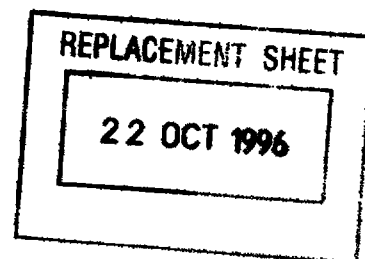
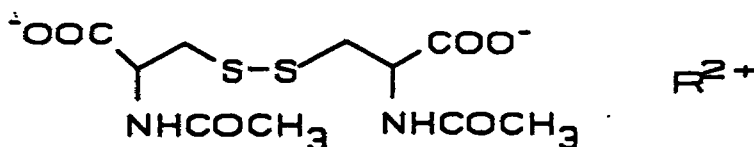


Claims

1. A salt of an organic base and N,N'-diacetyl cystine in its individual isomers, i.e. D-, L- and meso-forms as well as its racemic forms [DiNAc] characterized by the general formula

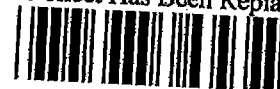


or



wherein R^+ and R^{2+} is the cation of the organic base lysine, ethylenediamine, N,N'-dibenzylethylenediamine, adamantanamine, N-benzyl-2-phenylethylamine or piperazine.

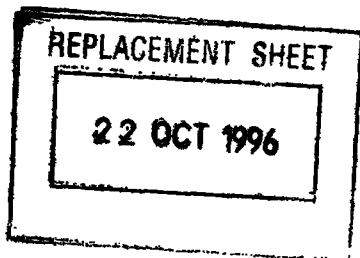
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2. A salt according to Claim 1 of N,N'-diacetyl-L-cystine.

3. A process for preparing a salt of a compound as defined in Claim 1 or 2 by treating DiNAc with the organic base or a salt containing these cations of the organic base, reactants being dissolved or dispersed in a solvent or a solvent mixture and isolating the salt which may optionally be in hydrated or solvated form.

4. A process for preparing a salt as defined in Claim 1 or 2 by oxidizing a salt of N-acetylcysteine and the organic base and isolating the DiNAc salt



which may optionally be in hydrated or solvated form.

5. A pharmaceutical composition comprising as active ingredient a salt of DiNAc according to Claim 1 or 2.

6. A pharmaceutical composition as claimed in Claim 5 for administration from a pressurized metered dose inhaler or from a dry powder inhaler or from a dry powder utilizing gelatine, plastic or other capsules.

7. A pharmaceutical composition as claimed in Claim 5 or 6 including a pharmaceutically acceptable carrier is added to the composition.

8. A pharmaceutical composition as claimed in Claim 5 in the form of capsules or tablets.

9. A pharmaceutical composition as claimed in Claim 8 including a pharmaceutically acceptable carrier.

10. A pharmaceutical composition as claimed in claim 5 for administration in a solution by nebulization.

11. A salt according to Claim 1 or 2 for use as a therapeutically active substance.

12. A salt according to Claim 1 or 2 for use in the treatment of malignant diseases.

13. Use of a salt according to Claim 1 or 2 for the preparation of a medicament with immunomodulating activity.

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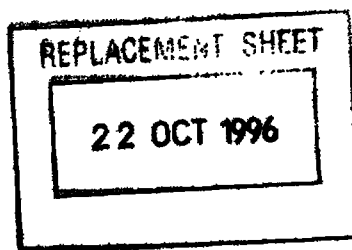




14. Use of a salt according to Claim 1 or 2 for the preparation of a medicament for use in the treatment of malignant diseases.

15. A method for the treatment of diseases which are due to defects in the immune system in mammals including man, characterized by the administration to a host in need of such treatment of an effective amount of a salt of DiNAc according to Claim 1 or 2.

16. A method for the treatment of malignant diseases in mammals including man, characterized by the administration to a host in need of such treatment of an effective amount of a salt of DiNAc according to Claim 1 or 2.



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ORGANIC SALTS OF N,N'-DIACETYL CYSTINE

Abstract

New crystalline organic salts of N,N'-diacetyl cystine with immunomodulating effect, processes for their preparation, pharmaceutical compositions containing them and methods of their pharmacological use.

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ORGANIC SALTS OF N,N'-DIACETYL

CYSTINE

Description

Field of the Invention

The present invention relates to organic salts of N,N'-diacetyl cystine, in the following referred to as DiNac, with immunomodulating activity as well as pharmaceutical compositions based on these salts and methods for their pharmacological use. The invention specifically concerns methods to obtain crystalline, non-hygroscopic and chemically stable salts containing non-toxic organic cations, which are useful for therapy of diseases where a defect in the immune system is indicated.

Background Art

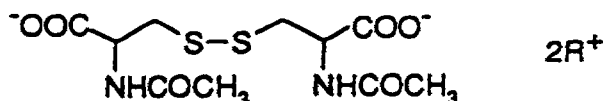
N-Acetyl-L-cysteine is a well known compound which is routinely used as a therapeutic agent against chronic obstructive diseases and chronic bronchitis. Although the first patent was filed in 1964 (GB 954268) the mechanism of action of the compound has not been established. It has recently been discovered that the corresponding disulfide of N-Acetyl-L-cysteine, i.e. L-DiNac, acts as a potent immunostimulator (Swedish patent application no SE 9002067-8), showing an activity comparable to contemporary immunostimulants such as sodium diethyl dithiocarbamate or 2,2'-dithiobisethanol.

Routes for the preparation of DiNac have been reported in several patents (US Patent Nos 4,827,016, 4,724,239 and 4,708,965, European Patent No 300100 and German Patent No 2326444). DiNac is, however, amorphous and hygroscopic, so that it is difficult to isolate and formulate into pharmaceutical compositions and its administration is normally only in the form of aqueous solutions. Most salts of DiNac with inorganic or organic cations share the same unfavourable physical properties with the free diacid. Examples of salts of other, sulfur-containing, amino acids have appeared in the patent literature (US patent No 3,647,834, JP patent No 56155298 and FR patent No 8106592).

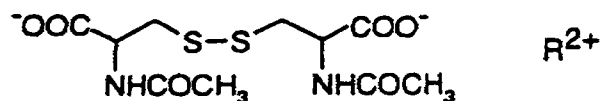
Disclosure of the Invention

We have now surprisingly found that a few salts of DiNac with organic bases exhibit a favourable combination of non-hygroscopicity and crystallinity which permits the isolation and formulation of these salts in solid form and allows them to be administered by inhalation in solid form, or in other dry formulations, should this be clinically desirable.

The present invention provides organic salts of DiNac, having the formula Ia or Ib:



or



Ia

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Ib

where R⁺ and R²⁺ is a mono- or diprotonated organic amine respectively. The organic amine is selected from lysinium, ethylenediaminium, N,N'-dibenzylethylenediaminium, N-benzyl-2-phenylethylaminium, piperazinium and 1-adamantanaminium.

Lysinium can be in its D- or L-form. Most preferred is the L-form.

The invention includes hydrated and solvated salts, e.g. solvated with lower alkanols. The invention includes salts of DiNac in its individual isomers, i.e. D-, L- and meso-forms as well as in its racemic form. Most preferred are the L-forms of these salts.

We have found that the new salts of the invention fulfil the requirements of ease of crystallization, non-hygroscopicity and chemical stability while still retaining the immunomodulating activity of DiNac, and are thus medically useful.

This invention thus provides compounds with advantageous properties for the treatment of diseases where an anomaly of the immune response or an aberrant immune response or an ineffective host defence can be suspected. Such diseases include chronic bronchitis, where a reduction of the rate of exacerbations has previously been reported with immune response modifiers such as Biostim (Radermecker, M. et al. Int. J. Immunopharmac. 10, 913-917, 1988; Scheffer, J. et al. Arzneim. Forsch/Drug Res. 41, 815820, 1991), Ribomunyl and Broncho Vaxom (Paupe, J. Respiration 58, 150-154, 1991) as well as with N-Acetylcysteine (See Bergstrand, H. et al J. Free Radic. Biol. Med. 2, 119-127,

1986).

Such diseases also include certain forms of malignant diseases. Thus, numerous research institutes round the world aim at finding ways of stimulating the immune response of patients with various forms of malignant diseases and numerous reviews in the literature deal with this approach (Stevenson, F.K. FASEB J 5, 2250-2257, 1991). To mention one example patients with intracranial tumours (gliomas) exhibit a profound decrease in immunity possibly due to a defect in the secretion of IL-2 as well as the expression of IL-2 receptors in T cells from such patients (Roszman, T. et al. Immunology Today 12, 370-374, 1991). Moreover, a significant adjuvant effect in immunotherapy of melanoma and colon carcinoma has been documented for the immunostimulator Levamisole (Van Wauwe, J. and Janssen, P.A.J. Int J. Immunopharmac. 13, 3-9, 1991) and immunotherapy with IL-2 in vivo or treatment of patients lymphokine activated killer cells with IL-2 ex vivo has caused the regression of cancer in selected patients (Rosenberg, S.A. Immunology Today 9, 58, 1988). The malignant diseases at which the compounds of the formula Ia and Ib can be expected to have advantageous effects include tumours of mesenchymal origin such as sarcomas like fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma or chordosarcoma, sarcomas like angiosarcoma, endotheliosarcoma, lymphangiosarcoma, synoviosarcoma or mesotheliosarcoma, leukemias and lymphomas like granulocytic leukemia, monocytic leukemia, lymphocytic leukemia, malignant lymphoma, plasmacytoma, reticulum cell sarcoma or Hodgkin's disease, sarcomas like leiomyosarcoma or rhabdomyosarcoma, tumours of epithelial origin (Carcinomas) like squamous cell carcinoma, basal cell carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, adenocarcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, undifferentiated carcinoma, bronchogenic carcinoma, melanoma, renal cell carcinoma, hepatoma-liver cell carcinoma, bile duct carcinoma-choleangiocarcinoma, papillary carcinoma, transitional cell carcinoma, squamous cell carcinoma, choriocarcinoma, seminoma or embryonal carcinoma, tumours of the central nervous system like glioma, meningoma, medulloblastoma, schwannoma or ependymoma.

Moreover, the compounds also have advantageous properties for treatment of chronic infections such as herpes, aphthous stomatitis and minimal change syndrome where clinical improvement has previously been reported by treatment with an immunostimulator such as Levamisole as well as other chronic inflammatory diseases in the urinary tract or in ear, nose or throat, which benefit from treatment with immunostimulators such as Biostim, BronchoVaxom and Ribomunyl, or at HIV infection or AIDS.

Moreover, an impairment, a defect or an imbalance of the immune response has also been postulated to exist at atopic diseases such as atopic dermatitis, rhinitis and asthma (Katz, D.H. Transplantation Reviews 41, 77-108, 1977). Since theoretical considerations suggest that stimulation of an immune response would possibly be the best way of restoring imbalances and autoimmunity (Varela, F.J. and Coutinho, A. Immunology Today 12, 159-166, 1991), the compounds can also be expected to have advantageous properties for treatment of asthma, rhinitis, atopic dermatitis and autoimmune diseases like non-obese diabetes, systemic lupus erythematosus, scleroderma, Sjögren's syndrome, dermatomyositis or multiple sclerosis, rheumatoid arthritis and possibly psoriasis.

Moreover, the compounds, due to their immune stimulating properties, can be expected to have advantageous properties as adjuvants in various forms of vaccine preparations.

Finally, the compounds can be expected to have advantageous properties for treatment of atherosclerosis whether or not it will influence a putative inflammatory process in this condition (Hansson, G.K. et al. Proc. Nat. Acad. Sci. USA 88, 10530, 1991).

The importance of immunostimulatory drugs on tumour outgrowth is illustrated in our relevant tumour outgrowth test systems. The experimental rat tumour models described below reflect very well the tumourostatic potency of the compounds of this invention in comparison with the well established immunostimulatory drug Levamisole, and they reflect clinically very well a fastly growing tumour, the mammary carcinoma, and a slowly progressively growing tumour, the glioma, and in both systems the drug has an excellent static effect on tumour outgrowth.

Particularly suitable for treatment with the compounds of this invention are:

Malignancies such as melanoma, mammary carcinoma, gastrointestinal carcinoma, glioma, bladder carcinoma and squamous cell carcinoma of the neck and head region:

Infections such as chronic bronchitis, hepatitis, post-infectious anergy and acquired immune deficiencies such as AIDS:

Posttraumatic immunological anergy, and

Purported autoimmune diseases such as rheumatoid arthritis, multiple sclerosis and psoriasis.

Effective doses for treatment of the diseases above are in the range of 0.1 - 100 mg, preferably 1.0 - 60 mg daily.

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Methods of Preparation

The organic salts of formula Ia and Ib are generally prepared by mixing DiNAc and the organic base, as defined above, each dissolved or dispersed in a solvent or solvent mixture. Solvents such as water, alcohols, glycols, ketones, amides, sulfoxides or other polar solvents or solvent mixtures may be used. The salt either precipitates directly from the reaction mixture, or is obtained by the addition of a less polar solvent or by evaporation or lyophilisation. The reaction is performed at elevated temperature or room temperature, depending on the solubility in the medium. Alternatively, the salt can be prepared by oxidation of the appropriate N-acetyl cysteine salt in aqueous or alcoholic solution, followed by precipitation as above. The oxidation may be effected either chemically, using e.g. hydrogen peroxide or halogen, or electrochemically.

Pharmaceutical Formulations

According to the present invention the compounds of the formula Ia and Ib can be formulated for administration by inhalation as well as by other routes, e.g. orally or topically with or without a pharmaceutically acceptable carrier.

The substance can be inhaled from a pressurized metered dose inhaler, from a dry powder inhaler, e.g. Turbuhaler® or from a dry powder inhaler utilizing gelatine, plastic or other capsules. Non-toxic and chemically inert substances e.g. lactose, trehalose, mannitol or glucose, can be added to the powdered substance.

The substance can also be administered orally in the form of capsules or tablets where the capsule, the tablet or the active substance itself can be coated or non-coated. This coating may consist of for example hydroxypropyl cellulose and aerosil in isopropanol or other suitable solvents. A non-toxic and chemically inert substance can be added to the powdered substance in order to obtain desirable physical or pharmaceutical properties. Parenteral administration of the new compounds is also possible.

Pharmacological Experiments

The ability of the compound di-L-lysine-N,N'-diacetyl-L-cystinate (Ia: R = lysine) to modulate immune responses is illustrated by its efficacy in the animal delayed type hypersensitivity (DTH) test in the mouse.

Both male and female Balb/c mice, obtained from Bomholtsgaard (Denmark), were used at the weight of 18-20 gram. 4-Ethoxymethylene-2-phenyloxazolin-5-one (OXA) was purchased from BDH (England) and served as the antigen in this test.

The mice were sensitized, Day 0, by epicutaneous application of 150 µl of an absolute ethanol-acetone (3:1) solution containing 3% OXA on the shaved abdomen. Treatment with the disulphide salt or vehicle (0.9% NaCl) was initiated by oral feeding immediately after sensitization and continued once daily until Day 6. Seven days (Day 6) after the sensitization, both ears of all mice were challenged on both sides by topical application of 20 µl 1% OXA dissolved in peanut oil. Ear thickness was measured prior to and 24 or 48 hours after challenge using 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: $T_{24/48} - T_{10}$ µm units, where T_{10} , T_{24} and T_{48} represent the ear thickness before and 24 or 48 hours after challenge respectively, in individual tests (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. Tables 1 and 2 show the results from 24 and 48 hours measurements respectively, from a representative experiment. The immunostimulating potency of the compound is reflected in a significant difference in the increase in ear thickness as compared to the control. Thus, in treated animals, after 24 h the response was about twice that of the control animals (15 µm compared to 8 µm, Table 1).

Table 1.

Ear thickness 24 hours after challenge of animals treated with the indicated doses of di-L-lysine-N,N'-diacetyl-L-cystinate or vehicle (NaCl).				
Dose µmol/kg	N	Diff. S.E.M. $T_{24} - T_{10}$		Sign.
NaCl	10	8.25	0.56	
0.03	10	15.00	0.42	***
3.0	10	15.80	0.77	***

*** P < 0.001.

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Table 2.

Ear thickness 48 hours after challenge of animals treated with the indicated doses of di-L-lysiniun-N,N'-diacetyl-L-cystinate or vehicle (NaCl).				
Dose $\mu\text{mol/kg}$	N	Diff. S.E.M. $T_{148}-T_{10}$		Sign.
NaCl	10	8.83	0.31	
0.03	10	12.55	0.41	***
3.0	10	13.20	0.28	***

*** $P < 0.001$.

The ability of the same test compound (Ia, R = lysine) to prolong or increase the survival of rats suffering from tumours is illustrated by two different experiments with tumour inoculation in rats.

Two groups each consisting of ten rats of the Wistar Furth strain were inoculated subcutaneously with 10^4 mammary carcinoma cells per rat and tumour size was measured twice a week. In one group the rats were drinking ordinary water and in the other group the test compound dissolved in water so as to result in a dose of $0.03 \mu\text{mol/kg/day}$ of animal weight. After 38 days one animal from the water-drinking group was still alive while from the group which was given the compound seven animals were alive.

In another experiment three groups each consisting of ten rats of the Lewis strain were inoculated subcutaneously with 10^6 glioma cells per rat and tumour size was measured twice a week. In one group the rats were drinking ordinary water and in the second group the test compound dissolved in water so as to give a dose of $0.03 \mu\text{mol/kg/day}$ of animal weight, while in the third group the rats were drinking water containing Levamisole, a well-known cancer therapeutic drug also at a dose of $0.03 \mu\text{mol/kg/day}$ of animal weight. After 119 days there was no surviving rat in the water-drinking group. In the group, which was given the test compound, six rats were still alive while in the group, which received Levamisole, three rats were alive after 119 days.

In both experiments with tumours there was a lower frequency of tumour positive animals and in both experiments there was an inhibition of tumour growth of the developed tumours in the groups receiving the compound solubilized in water.

In another experiment two groups each consisting of eight SCID mice (mice with the immune defect Severe Combined Immunodeficiency Disease) were inoculated with 2×10^3 mammary carcinoma cells and outgrowth was measured twice a week. In one group the mice were drinking ordinary water and in the second group the test compound dissolved in water so as to give a dose of $0.03 \mu\text{mol/kg/day}$ of animal weight. There were no significant differences between the groups concerning growth rate and incidence. This indicates that the compound has no direct effect on the tumour cells but acts via the immune apparatus.

In an animal model of lupus, experimental murine systemic lupus erythematosus (SLE), MRL lpr/lpr mice spontaneously develop lymphoid hyperplasia, dermatitis, arthritis and glomerulonephritis. The effect of di-L-lysiniun-N,N'-diacetyl-L-cystinate on glomerulonephritis as proteinuria and haematuria and also the survival ratio of the animals, has been studied in this murine lupus model. Di-L-lysiniun-N,N'-diacetyl-L-cystinate was administered in the drinking water and the mean dosage was calculated to $0.03 \mu\text{mol/kg/day}$. The control mice received tap water. Both groups had free access to the drinking water. The treatment started when the animals reached 8 weeks of age and continued until death or 46 weeks of age, when the study was ended.

The assessment of proteinuria and haematuria was performed by the usage of reagent strips. Eour-Test[®]. Boehringer Mannheim.

Upon administration of di-L-lysiniun-N,N'-diacetyl-L-cystinate the survival ratio was significantly improved compared to the control mice. The mortality ratio for the untreated group (21 animals) reached 50% around 25 weeks of age. For the di-L-lysiniun-N,N'-diacetyl-L-cystinate treated MRL-lpr/lpr mice (12 animals) this mortality rate was not reached until week 44.

This form of treatment also significantly improved the score for both proteinuria and haematuria measured as arbitrary units of the reagent strips, compared to the untreated group.

These results show the immunomodulating potency of the compounds of this invention, particularly with regard to SLE.

Working Examples

Pharmaceutical Formulations

The following examples are representative of pharmaceutical formulations intended for different modes of local

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and systemic administration.

Example 1 Pressurised aerosol for inhalation.

The aerosol system is arranged so that each metered dose contains 0.1 - 1.0 mg.

Compound Ia or Ib, micronized	1.0 % w/w
Sorbitan trioleate	0.7 % w/w
Trichloromonofluoromethane	24.4 % w/w
Dichlorotetrafluoroethane	24.4 % w/w
Dichlorodifluoromethane	49.5 % w/w

Example 2. Powder aerosol for inhalation of pure substance.

Pure substance prepared for inhalation from Turbuhaler.
Each single dose contains 0.1 - 1.0 mg.

Compound Ia or Ib, processed	0.1 - 1.0 mg
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Example 3. Powder aerosol for inhalation.

Each single dose contains 0.1 - 1.0 mg in a capsule.

Compound Ia or Ib, micronized	0.1 - 1.0 mg
Lactose	50 mg

Example 4. Solution for nebulising.

The solution contains 1.0 - 10.0 mg/mL and 1 - 3 mL may be administered in a single dose.

Compound Ia or Ib	1.0 - 10.0 mg
Water for injection	to 1.0 mL

Example 5. Tablets.

Each tablet contains:

Compound Ia or Ib	0.1 - 100 mg
Maize starch	50 mg
Lactose	150 mg
Polyvidone	7 mg
Microcrystalline cellulose	20 mg
Magnesium stearate	2 mg

Example 6. Oral solution.

A single dose of 10 mL contains 10 - 100 mg.

Compound Ia or Ib	1 - 10 mg
Sorbitol 70 %	150 mg
Glycerol	100 mg
Sodium benzoate	1 mg
Flavour q.s.	

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(continued)

Water purified	to	1.0 mL
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Example 7. Tablet for controlled release

1 tablet:

Compound Ia and Ib	1-100 mg
Paraffin Special	145 mg
Lactose Powder	50 mg
Colloidal Silicon Dioxide	5 mg
Ethylcellulose 10 cps	13 mg
Ethanol 99.5 vol%	85 mg
Magnesium Stearate	2.5 mg

Example 8. Granulate for controlled release

1 g of granulate:

Compound Ia or Ib	1-100 mg
Ethylcellulose Dispersion	10 mg
Acetyltributylcitrate	0.5 mg
Eudragit L 100-55	55 mg
Triethylcitrate	5 mg
Talc	30 mg
Water newly distilled	350 mg
Pellets, neutral	to 1000 mg

Example 9. Solution for injection.

1 mL in a single dose contains	1.0 - 10.0 mg
Compound Ia or Ib	1.0 - 10.0 mg
Sodium chloride	8.9 - 7.7 mg
Water for injection	to 1.0 mL

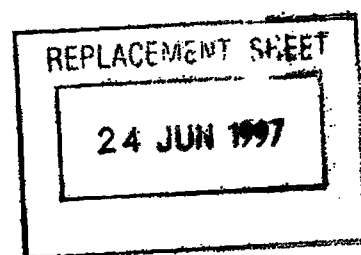
Example 10. Cream for topical application

1 g of cream contains:

Compound Ia or Ib	0.1 - 1 mg
White soft paraffin	75 mg
Liquid paraffin	10 mg
Cetostearyl alcohol	75 mg
Cetomacrogol 1000	20 mg
Metagin	0.8 mg
Propagin	0.2 mg
Water, purified	to 1.0 g

Example 11. Ointment for topical application

1 g of ointment contains:

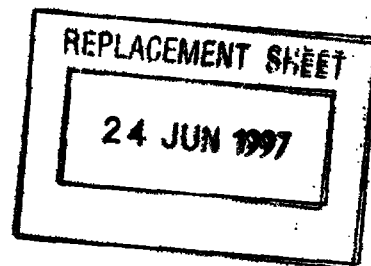


Compound Ia or Ib	0.1 - 1 mg
Liquid paraffin	150 mg
White soft paraffin	to 1.0 g

Example 12. Ophthalmic solution

One dose of 2 drops contains 0.01-0.1 mg of compound Ia or Ib

Compound Ia or Ib	0.1-1 mg
Benzalkonium chloride	0.1 mg
Sodium chloride	9.0 mg
Water, sterile	to 1.0 mL



Chemistry

Example 13

Di-L-lysiniun-N,N'-diacetyl-L-cystinate (Ia; R = H₂N(COOH)CH(CH₂)₄NH₃):

N-Acetyl-L-cysteine (22 mol, 3.59 kg) was dissolved in 2.6 L of deionized water. Sodium hydroxide 45% in water (22 mol, 1.92 kg) was added with stirring and temperature kept below 20°C. After adjusting the temperature to 5°C, hydrogen peroxide (11.0 mol, 0.95L) was carefully added dropwise during 90 min. The temperature was not allowed to exceed 10°C during this addition. To the resulting solution was added 9 L of activated strongly acidic cation exchanger. After stirring for 10 minutes the pH was 2.0 and the ion exchanger was filtered off. The filtrate contained 9.65 mol of N,N'-diacetyl-L-cystine, as determined by HPLC using a standard prepared from the pure substance. To this crude solution was added L-lysine (19.3 mol, 3.17 kg). The thick solution formed, was slowly added to 50 L of refluxing ethanol containing 0.23 kg of crystalline di-L-lysiniun-N,N'-diacetyl-L-cystinate. After the addition, the slurry was allowed to cool and the crystals were filtered off. Washing with ethanol (8 L) and drying (vacuum, 40°C) for 12 hours afforded 5.36 kg (90%) of the title substance as a white crystalline solid.

Physical data: Mp: 210°C (dec); [α]_D²⁵ = -70° (c=0.54, H₂O); ¹H-NMR (D₂O) δ: 1.36-1.60 [4H, m, Lys γ CH₂], 1.73 [4H, p, Lys δ CH₂], 1.84-1.96 [4H, m, Lys β CH₂], 2.05 [6H, s, CH₃], 2.95 [2H, dd, CH₂S], 3.02 [4H, t, Lys ε CH₂], 3.25 [2H, dd, CH₂S], 3.76 [2H, t, Lys α CH], 4.50 [2H, dd, CHN]; ¹³C-NMR (D₂O) δ: 24.27: 24.80: 29.24: 32.72: 41.89: 42.78: 52.96: 57.30: 176.53: 177.41; 179.74; Analysis: Calcd for C₂₂H₄₄O₁₀N₆S₂, C:42.8 H:7.2 N: 13.6 Found: C: 42.6 H: 7.4 N: 13.7; MS m/z = 325 (MH⁺), m/z = 471 (MLysH⁺), m/z = 617 (MLys₂H⁺).

Example 14

Ethylenediaminium-N,N'-diacetyl-L-cystinate (Ib; R = H₂NCH₂CH₂NH₃):

To N,N'-Diacetyl-L-cystine (30.9 mmol, 10 g) dissolved in 20 mL of water was added ethylenediamine (61.8 mmol, 3.73 g) and ethanol (30 mL). The solution was concentrated to a thick paste which was redissolved in 80 mL of ethanol. Crystallisation occurred after 2 hours stirring at 10°C. Filtration and drying gave 6.2 g (45%) of the title compound.

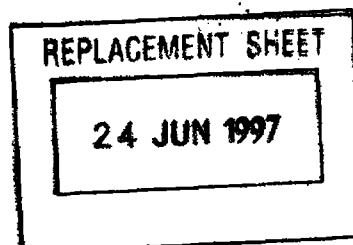
Physical data: Mp 185.2-192.4°C. ¹H-NMR (D₂O) δ: 2.06 [6H, s, CH₃], 2.96[2H, dd, CH₂S], 3.26[2H, dd, CH₂S], 3.28 [4H, s, H₃N⁺CH₂CH₂N⁺H₃], 4.50 [2H, dd, CHN]. ¹³C-NMR (D₂O) δ: 24.78: 39.99: 42.74: 56.98: 176.55: 179.82: Analysis: Calcd C: 37.5, H: 6.3, N: 14.6, S: 16.7. Found: C: 37.3, H: 6.8, N: 15.3, S: 15.2; MS m/z = 385 [M (H₂NCH₂CH₂NH₂)H⁺]

Example 15

N,N'-dibenzylethylenediaminium-N,N'-diacetyl-L-cystinate (Ib; R = PhCH₂NH₂-CH₂CH₂NH₂CH₂Ph)

To a 63% solution of N,N'-diacetyl-L-cystine in water (67 mmole, 21.8 g) was added N,N'-dibenzylethylenediamine (67 mmole, 16.0 g). The exothermic reaction gave a slightly oily product which could be recrystallised from water to give 12.0 g (32%) of the title substance as white crystalline needles.

Physical data: Mp 163.8-165.3°C. ¹H-NMR (D₂O) δ: 2.04 [6H, s, CH₃], 2.93 [2H, dd, CH₂S], 3.22 [2H, dd, CH₂S], 3.44



[4H.s.CH₂NBn], 4.27 [4H.s,PhCH₂N], 4.47 [2H.dd.CHN], 7.44-7.54 [10H.m,Ph]. ¹³C-NMR (D₂O) δ: 24.82, 42.82, 45.71, 54.47, 56.99, 132.22, 132.56, 132.67, 133.52, 176.56, 179.80. Analysis (monohydrate): Calcd, C: 53.6, H: 6.6, N: 9.6, S: 11.0. Found, C: 54.5, H: 6.6, N: 9.6, S: 11.2.

Example 16

Di-(1-adamantanaminium)-N,N'-diacetyl-L-cystinate (Ia; R=[C(1) CH(3,5,7) CH₂(2,4,6,8,9,10) NH₂])

To N,N'-diacetyl-L-cystine (5.35 mmole, 1.73 g) dissolved in 5 mL of water was added 1-adamantanamine (10.7 mmole, 1.61 g). To the solution was dropwise added 60 mL of acetone. The resulting crystalline salt was filtered and dried in vacuo, yielding 2.3 g (67%) of the title compound.

Physical data: Mp 162°C. ¹H-NMR (D₂O) δ: 1.71 [12H, broad dd, CH₂(4,6,10)], 1.87 [12H.d.CH₂(2,6,9)], 2.05 [6H, s,CH₃], 2.17 [6H, broad s, CH(3,5,7)], 2.96 [2H.dd,CH₂S], 3.26 [2H.dd,CH₂S], 4.50 [2H.dd,CHN]

Example 17

Di-(N-benzyl-2-phenylethylaminium)-N,N'-L-cystinate (Ia; R=PhCH₂CH₂NH₂CH₂Ph)

To N,N'-diacetyl-L-cystine (25.7 mmole, 9.3 g) dissolved in 20 mL of water was added N-benzyl-2-phenylethylamine (57.4 mmole, 12.1 g). The solution was concentrated to a thick paste, from which the salt slowly crystallized. The crystalline title compound was isolated and dried.

Physical data: Mp 87°C. ¹H-NMR (D₂O) δ: 2.05 [6H,s,CH₃], 2.95 [2H.dd,CH₂S], 3.04 [4H.t,PhCH₂C], 3.24 [2H.dd,CH₂S], 3.33 [4H.t,CH₂NBn], 4.25 [4H.s,PhCH₂N], 4.49 [2H.dd,CHN], 7.30-7.52 [20H.m,Ph]

Example 18

Piperazinium-N,N'-diacetyl-L-cystinate [Ib; R=NH₂(1,4) CH₂(2,3,5,6)]

To N,N'-diacetyl-L-cystine (4.60 mmole, 1.49 g) dissolved in 5 mL of water was added piperazine (4.60 mmole, 0.90 g). To the solution was added enough isopropanol to cause formation of an oil, which slowly solidifies. The salt was isolated and dried.

Physical data: Mp >170°C (dec.) ¹H-NMR (D₂O) δ: 2.05 [6H.s,CH₃], 2.96 [2H.dd,CH₂S], 3.26 [2H.dd,CH₂S], 3.42 [8H.s,CH₂(2,3,5,6)], 4.49 [2H.dd,CHN]

Example 19

Di-L-lysiniun-N,N'-diacetyl-L-cystinate (Ia; R=H₂N(COOH)CH(CH₂)₄NH₂).

N-acetyl-L-cysteine (37 mmol, 6.0 g) and L-lysine (37 mmol, 5.4 g) were dissolved in 10 mL of deionized water. Hydrogen peroxide (18 mmol, 1.5 mL) was added dropwise while stirring, and the temperature was kept below 25°C. The solution was stirred for an additional 4 h. The viscous solution was slowly added to 150 mL of refluxing ethanol containing 0.50 g of crystalline di-L-lysiniun-N,N'-diacetyl-L-cystinate. After addition the solution was allowed to cool, and the crystals were filtered off. Washing with ethanol (20 mL), and drying (vacuum, 45°C) for 24 h afforded 10.0 g (84%) of the title substance as a white crystalline solid.

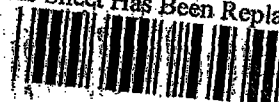
Physical data: Mp: 208°C: [α]_D²⁵ = -73° (c=0.54, H₂O)

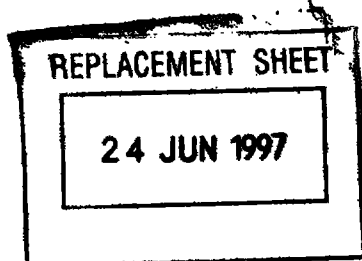
Claims

Claims for the following Contracting States : AT, BE, CH, DE, DK, FR, GB, GR, IT, LI, LU, MC, NL, PT, SE, IE

1. A salt of an organic base and N,N'-diacetyl cystine in its individual isomers, i.e. D-, L- and meso-forms as well as its racemic forms [DiNac] characterized by the general formula

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[4H,s,CH₂NBn], 4.27 [4H,s,PhCH₂N], 4.47 [2H,dd,CHN], 7.44-7.54 [10H,m,Ph]. ¹³C-NMR (D₂O) δ: 24.82, 42.82, 45.71, 54.47, 56.99, 132.22, 132.58, 132.67, 133.52, 176.56, 179.80. Analysis (monohydrate): Calcd, C: 53.6, H: 6.6, N: 9.6, S: 11.0. Found, C: 54.5, H: 6.6, N: 9.6, S: 11.2.

Example 16

Di-(1-adamantanaminium)-N,N'-diacetyl-L-cystinate (Ia; R=[C(1) CH(3,5,7) CH₂(2,4,6,8,9,10) NH₂])

To N,N'-diacetyl-L-cystine (5.35 mmole, 1.73 g) dissolved in 5 mL of water was added 1-adamantanamine (10.7 mmole, 1.61 g). To the solution was dropwise added 60 mL of acetone. The resulting crystalline salt was filtered and dried in vacuo, yielding 2.3 g (67%) of the title compound.

Physical data: Mp 162°C. ¹H-NMR (D₂O) δ: 1.71 [12H, broad dd, CH₂(4,6,10)], 1.87 [12H,d,CH₂(2,8,9)], 2.05 [6H,s,CH₃], 2.17 [6H, broad s, CH(3,5,7)], 2.96 [2H,dd,CH₂S], 3.26 [2H,dd,CH₂S], 4.50 [2H,dd,CHN]

Example 17

Di-(N-benzyl-2-phenylethylaminium)-N,N'-L-cystinate (Ia; R=PhCH₂CH₂NH₂CH₂Ph)

To N,N'-diacetyl-L-cystine (28.7 mmole, 9.3 g) dissolved in 20 mL of water was added N-benzyl-2-phenylethylamine (57.4 mmole, 12.1 g). The solution was concentrated to a thick paste, from which the salt slowly crystallized. The crystalline title compound was isolated and dried.

Physical data: Mp 87°C. ¹H-NMR (D₂O) δ: 2.05 [6H,s,CH₃], 2.95 [2H,dd,CH₂S], 3.04 [4H,t,PhCH₂C], 3.24 [2H,dd,CH₂S], 3.33 [4H,t,CH₂NBn], 4.25 [4H,s,PhCH₂N], 4.49 [2H,dd,CHN], 7.30-7.52 [20H,m,Ph]

Example 18

Piperazinium-N,N'-diacetyl-L-cystinate (Ib; R=NH₂(1,4) CH₂(2,3,5,6))

To N,N'-diacetyl-L-cystine (4.60 mmole, 1.49 g) dissolved in 5 mL of water was added piperazine (4.60 mmole, 0.90 g). To the solution was added enough isopropanol to cause formation of an oil, which slowly solidifies. The salt was isolated and dried.

Physical data: Mp >170°C (dec.) ¹H-NMR (D₂O) δ: 2.05 [6H,s,CH₃], 2.96 [2H,dd,CH₂S], 3.26 [2H,dd,CH₂S], 3.42 [8H,s,CH₂(2,3,5,6)], 4.49 [2H,dd,CHN]

Example 19

Di-L-lysiniun-N,N'-diacetyl-L-cystinate (Ia; R=H₂N(COOH)CH(CH₂)₄NH₂).

N-acetyl-L-cysteine (37 mmol, 6.0 g) and L-lysine (37 mmol, 5.4 g) were dissolved in 10 mL of deionized water. Hydrogen peroxide (18 mmol, 1.5 mL) was added dropwise while stirring, and the temperature was kept below 25°C. The solution was stirred for an additional 4 h. The viscous solution was slowly added to 150 mL of refluxing ethanol containing 0.50 g of crystalline di-L-lysiniun-N,N'-diacetyl-L-cystinate. After addition the solution was allowed to cool, and the crystals were filtered off. Washing with ethanol (20 mL), and drying (vacuum, 45°C) for 24 h afforded 10.0 g (84%) of the title substance as a white crystalline solid.

Physical data: Mp: 208°C; [α]_D²⁵ = -73° (c=0.54, H₂O)

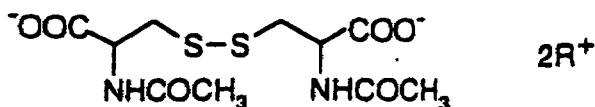
Claims

Claims for the following Contracting States : AT, BE, CH, DE, DK, FR, GB, GR, IT, LI, LU, MC, NL, PT, SE, IE

1. A salt of an organic base and N,N'-diacetyl cystine in its individual isomers, i.e. D-, L- and meso-forms as well as its racemic forms [DiNAc] characterized by the general formula

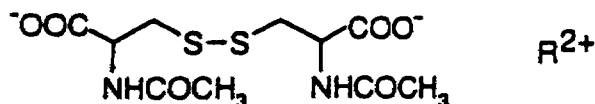
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Ia

or



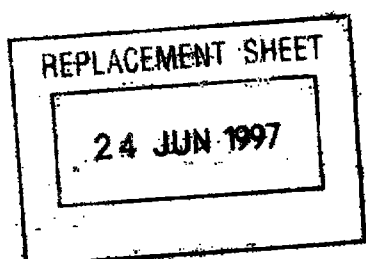
Ib

wherein R^+ and R^{2+} is the cation of the organic base lysine, ethylenediamine, N,N'-dibenzylethylenediamine, adamantanamine, N-benzyl-2-phenylethylamine or piperazine.

2. A salt according to Claim 1 of N,N'-diacetyl-L-cystine.
3. A process for preparing a salt of a compound as defined in Claim 1 or 2 by treating DiNAC with the organic base or a salt containing these cations of the organic base, reactants being dissolved or dispersed in a solvent or a solvent mixture and isolating the salt which may optionally be in hydrated or solvated form.
4. A process for preparing a salt as defined in Claim 1 or 2 by oxidizing a salt of N-acetylcysteine and the organic base and isolating the DiNAC salt which may optionally be in hydrated or solvated form.
5. A pharmaceutical composition comprising as active ingredient a salt of DiNAC according to Claim 1 or 2.
6. A pharmaceutical composition as claimed in Claim 5 for administration from a pressurized metered dose inhaler or from a dry powder inhaler or from a dry powder inhaler utilizing gelatine, plastic or other capsules.
7. A pharmaceutical composition as claimed in Claim 5 or 6 including a pharmaceutically acceptable carrier is added to the composition.
8. A pharmaceutical composition as claimed in Claim 5 in the form of capsules or tablets.
9. A pharmaceutical composition as claimed in Claim 8 including a pharmaceutically acceptable carrier.
10. A pharmaceutical composition as claimed in claim 5 for administration in a solution by nebulization.
11. A salt according to Claim 1 or 2 for use as a therapeutically active substance.
12. A salt according to Claim 1 or 2 for use in the treatment of malignant diseases.
13. Use of a salt according to Claim 1 or 2 for the preparation of a medicament with immunomodulating activity.
14. Use of a salt according to Claim 1 or 2 for the preparation of a medicament for use in the treatment of malignant diseases.

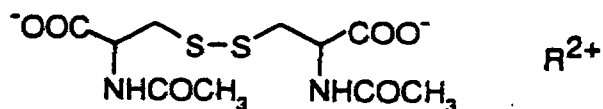
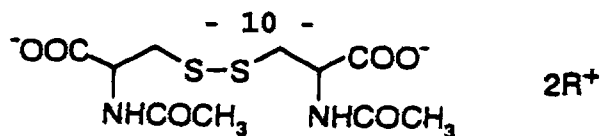
Claims for the following Contracting State : ES

1. A salt of an organic base and N,N'-diacetyl cystine in its individual isomers, i.e. D-, L- and meso-forms as well as its racemic forms [DiNAC] characterized by the general formula



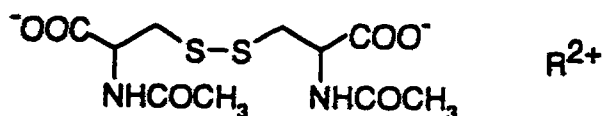
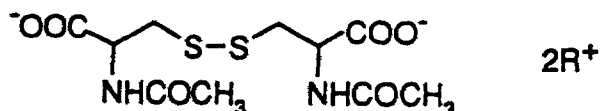
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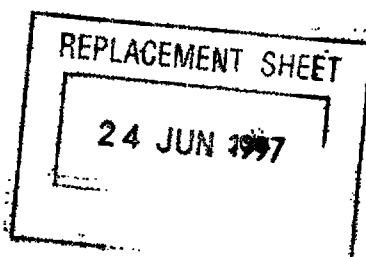
wherein R^+ and R^{2+} is the cation of the organic base lysine, ethylenediamine, N,N'-dibenzylethylenediamine, adamantanamine, N-benzyl-2-phenylethylamine or piperazine.

2. A salt according to Claim 1 of N,N'-diacetyl-L-cystine.
3. A process for preparing a salt of an organic base and N,N'-diacetyl cystine in its individual isomers, i.e. D-, L- and meso-forms as well as its racemic forms [DiNac] of the general formula



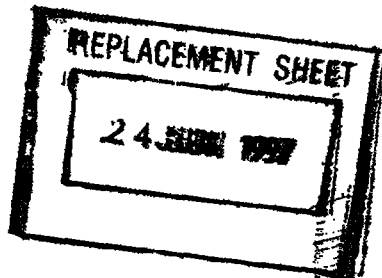
wherein R^+ and R^{2+} is the cation of the organic base lysine, ethylenediamine, N,N'-dibenzylethylenediamine, adamantanamine, N-benzyl-2-phenylethylamine or piperazine by treating DiNac with the organic base or a salt containing these cations of the organic base, reactants being dissolved or dispersed in a solvent or a solvent mixture and isolating the salt which may optionally be in hydrated or solvated form.

4. A process for preparing a salt of a compound as defined in claim 3 by oxidizing a salt of N-acetylcysteine and the organic base and isolating the DiNac salt which may optionally be in hydrated or solvated form.
5. A pharmaceutical composition comprising as active ingredient a salt of DiNac according to Claim 1 or 2.
6. A pharmaceutical composition as claimed in Claim 5 for administration from a pressurized metered dose inhaler or from a dry powder inhaler or from a dry powder inhaler utilizing gelatine, plastic or other capsules.
7. A pharmaceutical composition as claimed in Claim 5 or 6 including a pharmaceutically acceptable carrier is added to the composition.
8. A pharmaceutical composition as claimed in Claim 5 in the form of capsules or tablets.
9. A pharmaceutical composition as claimed in Claim 8 including a pharmaceutically acceptable carrier.
10. A pharmaceutical composition as claimed in claim 5 for administration in a solution by nebulization.
11. A salt according to Claim 1 or 2 for use as a therapeutically active substance.



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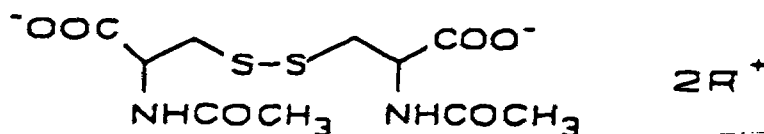


12. A salt according to Claim 1 or 2 for use in the treatment of malignant diseases.
13. Use of a salt according to Claim 1 or 2 for the preparation of a medicament with immunomodulating activity.
14. Use of a salt according to Claim 1 or 2 for the preparation of a medicament for use in the treatment of malignant diseases.
15. A process for the preparation of a pharmaceutical composition comprising as active ingredient a salt of DiNac as defined in claim 3 optionally with a pharmaceutically acceptable carrier for administration from a pressurized metered dose inhaler or from a dry powder inhaler or from a gelatin, plastic or other capsule characterized in that a dry powder of DiNac is filled on a metered dose inhaler or on a dry powder inhaler or into a gelatin, plastic or other capsule.
16. A process for the preparation of a pharmaceutical composition comprising as active ingredient a salt of DiNac as defined in claim 3 and a pharmaceutically acceptable carrier for formulation as a tablet characterized in that a salt of DiNac is mixed with the carrier and formulated to a tablet.
17. A process for the preparation of a pharmaceutical composition comprising as active ingredient a salt of DiNac as defined in claim 3 and a pharmaceutically acceptable carrier for formulation as granules characterized in that a salt of DiNac is mixed with the carrier and formulated to granules.
18. A process for the preparation of a pharmaceutical composition comprising as active ingredient a salt of DiNac as defined in claim 3 optionally with a pharmaceutically acceptable carrier for administration in a solution by nebulization characterized in that DiNac and the carrier is dissolved in water and filled on a container for nebulization.
19. A process for the preparation of a pharmaceutical composition comprising as active ingredient a salt of DiNac as defined in claim 3 optionally with a pharmaceutically acceptable carrier for administration as an ophtalmic solution or an oral solution characterized in that DiNac and the carrier is dissolved in water and filled on a container.
20. A process for the preparation of a pharmaceutical composition comprising as active ingredient a salt of DiNac as defined in claim 3 optionally with a pharmaceutically acceptable carrier for administration by injection characterized in that DiNac and the carrier is dissolved in water for injection and filled on a container.
21. A process for the preparation of a pharmaceutical composition comprising as active ingredient a salt of DiNac as defined in claim 3 and a pharmaceutically acceptable carrier for topical administration characterized in that a salt of DiNac as described in any of claims 1 or 2 is mixed with the carrier and formulated as an ointment.
22. A process for the preparation of a pharmaceutical composition comprising as active ingredient a salt of DiNac as defined in claim 3 and a pharmaceutically acceptable carrier for topical administration characterized in that a salt of DiNac as described in any of claims 1 or 2 is mixed with the carrier and formulated as a cream.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, DK, FR, GB, GR, IT, LI, LU, MC, NL, PT, SE, IE

1. Salz von einer organischen Base und N,N'-Diacetylcystin in seinen einzelnen Isomeren, d.h. D-, L- und meso-Formen, sowie seinen racemischen Formen [DiNac], gekennzeichnet durch die allgemeine Formel



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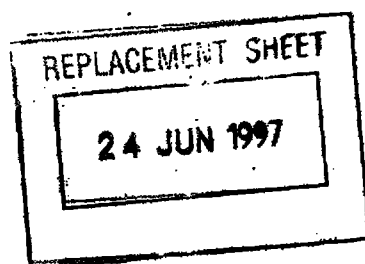
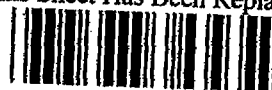


ORGANIC SALTS OF N,N'-DIACETYL CYSTINE

Abstract

New crystalline organic salts of N,N'-diacetyl cystine with immunomodulating effect, processes for their preparation, pharmaceutical compositions containing them and methods of their pharmacological use.

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☒ Claim(s) : paged ... to paged ...
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[4H, s, CH₂NBn], 4.27 [4H, s, PhCH₂N], 4.47 [2H, dd, CHN], 7.44-7.54 [10H, m, Ph], ¹³C-NMR (D₂O) δ: 24.82, 42.82, 45.71, 54.47, 56.99, 132.22, 132.58, 132.67, 133.52, 176.56, 179.80. Analysis (monohydrate): Calcd, C: 53.6, H: 6.6, N: 9.6, S: 11.0. Found, C: 54.5, H: 6.6, N: 9.6, S: 11.2.

Example 16

DI-(1-adamantanaminium)-N,N'-diacetyl-L-cystinate (1a: R=[C(1) CH(3,5,7) CH₂(2,4,6,8,9,10) NH₂])

To N,N'-diacetyl-L-cystine (5.35 mmole, 1.73 g) dissolved in 5 mL of water was added 1-adamantanamine (10.7 mmole, 1.51 g). To the solution was dropwise added 80 mL of acetone. The resulting crystalline salt was filtered and dried in vacuo, yielding 2.3 g (67%) of the title compound.

Physical data: Mp 162°C. ¹H-NMR (D₂O) δ: 1.71 [12H, broad dd, CH₂(4,6,10)], 1.97 [12H, d, CH₂(2,8,9)], 2.05 [6H, s, CH₃], 2.17 [6H, broad s, CH(3,5,7)], 2.96 [2H, dd, CH₂S], 3.26 [2H, dd, CH₂S], 4.50 [2H, dd, CHN]

Example 17

DI-(N-benzyl-2-phenylethylaminium)-N,N'-L-cystinate (1a: R=PhCH₂CH₂NH₂CH₂Ph)

To N,N'-diacetyl-L-cystine (29.7 mmole, 9.3 g) dissolved in 20 mL of water was added N-benzyl-2-phenylethylamine (57.4 mmole, 12.1 g). The solution was concentrated to a thick paste, from which the salt slowly crystallized. The crystalline title compound was isolated and dried.

Physical data: Mp 87°C. ¹H-NMR (D₂O) δ: 2.05 [6H, s, CH₃], 2.95 [2H, dd, CH₂S], 3.04 [4H, t, PhCH₂C], 3.24 [2H, dd, CH₂S], 3.33 [4H, t, CH₂NBn], 4.25 [4H, s, PhCH₂N], 4.49 [2H, dd, CHN], 7.30-7.52 [20H, m, Ph]

Example 18

Piperazinium-N,N'-diacetyl-L-cystinate (1b: R=NH₂(1,4) CH₂(2,3,5,6))

To N,N'-diacetyl-L-cystine (4.60 mmole, 1.49 g) dissolved in 5 mL of water was added piperazine (4.60 mmole, 0.90 g). To the solution was added enough isopropanol to cause formation of an oil, which slowly solidifies. The salt was isolated and dried.

Physical data: Mp >170°C (dec.) ¹H-NMR (D₂O) δ: 2.05 [6H, s, CH₃], 2.96 [2H, dd, CH₂S], 3.26 [2H, dd, CH₂S], 3.42 [8H, s, CH₂(2,3,5,6)], 4.49 [2H, dd, CHN]

Example 19

DI-L-lysiniun-N,N'-diacetyl-L-cystinate (1a: R=H₂N(COOH)CH(CH₂)₄NH₂).

N-acetyl-L-cysteine (37 mmol, 6.0 g) and L-lysine (37 mmol, 5.4 g) were dissolved in 10 mL of deionized water. Hydrogen peroxide (18 mmol, 1.5 mL) was added dropwise while stirring, and the temperature was kept below 25°C. The solution was stirred for an additional 4 h. The viscous solution was slowly added to 150 mL of refluxing ethanol containing 0.50 g of crystalline di-L-lysiniun-N,N'-diacetyl-L-cystinate. After addition the solution was allowed to cool, and the crystals were filtered off. Washing with ethanol (20 mL), and drying (vacuum, 45°C) for 24 h afforded 10.0 g (84%) of the title substance as a white crystalline solid.

Physical data: Mp: 208°C; [α]_D²⁵ = -73° (c=0.54, H₂O)

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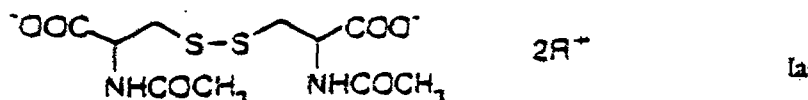
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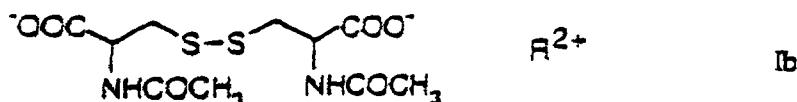
Claims

Claims for the following Contracting States : AT, BE, CH, DE, DK, FR, GB, GR, IT, LI, LU, MC, NL, PT, SE, IE

1. A salt of an organic base and N,N'-diacetyl cystine in its individual isomers, i.e. D-, L- and meso-forms as well as its racemic forms (DiNAC) characterized by the general formula

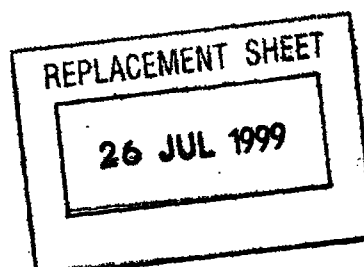


or



wherein R⁺ and R²⁺ is the cation of the organic base lysine, ethylenediamine, N,N'-dibenzylethylenediamine, adamantanamine, N-benzyl-2-phenylethylamine or piperazine.

2. A salt according to Claim 1 of N,N'-diacetyl-L-cystine.
3. A process for preparing a salt of a compound as defined in Claim 1 or 2 by treating DiNAC with the organic base or a salt containing these cations of the organic base, reactants being dissolved or dispersed in a solvent or a solvent mixture and isolating the salt which may optionally be in hydrated or solvated form.
4. A process for preparing a salt as defined in Claim 1 or 2 by oxidizing a salt of N-acetylcysteine and the organic base and isolating the DiNAC salt which may optionally be in hydrated or solvated form.
5. A pharmaceutical composition comprising as active ingredient a salt of DiNAC according to Claim 1 or 2.
6. A pharmaceutical composition as claimed in Claim 5 for administration from a pressurized metered dose inhaler or from a dry powder inhaler or from a dry powder inhaler utilizing gelatine, plastic or other capsules.
7. A pharmaceutical composition as claimed in Claim 5 or 6 including a pharmaceutically acceptable carrier is added to the composition.
8. A pharmaceutical composition as claimed in Claim 5 in the form of capsules or tablets.
9. A pharmaceutical composition as claimed in Claim 5 including a pharmaceutically acceptable carrier.
10. A pharmaceutical composition as claimed in claim 5 for administration in a solution by nebulization.
11. A salt according to Claim 1 or 2 for use as a therapeutically active substance.
12. A salt according to Claim 1 or 2 for use in the treatment of malignant diseases.
13. Use of a salt according to Claim 1 or 2 for the preparation of a medicament with immunomodulating activity.
14. Use of a salt according to Claim 1 or 2 for the preparation of a medicament for use in the treatment of malignant diseases.



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