**Title:** NEW S-ACYL-GLUTATHIONE DERIVATIVES, THEIR SYNTHESIS AND USE IN THE TREATMENT OF OXIDATIVE STRESS-RELATED DISEASES

**Abstract:** Novel S-acyl glutathione derivatives are described, in particular S-lauroyl GSH, S-palmitoleoyl GSH, S-linoleoyl GSH, S-linolenoyl GSH, S-arachidonoyl GSH, and a synthesis method thereof. Thanks to their hydrophobic properties, the new derivatives can cross the cell membranes to release, in the cell, following an enzymatic hydrolysis, GSH and corresponding fatty acids both exploiting protective activity against the cell oxidative stress.
NEW S-ACYL-GLUTATHIONE DERIVATIVES, THEIR SYNTHESIS AND USE IN THE TREATMENT OF OXIDATIVE STRESS-RELATED DISEASES

Field of the invention

The present invention generally relates to lipophilic derivatives of glutathione and in particular S-acyl-glutathione derivatives usable as effective agents in counteracting the cytotoxic effects from prefibrillar protein aggregates, such as those found in the early phases of important degenerative diseases such as Alzheimer disease, Huntington chorea, etc.. The invention also relates to a method for the synthesis of these derivatives and the pharmaceutical compositions containing the derivatives.

Background art

A number of works have shown a reduction of the level of intracellular glutathione (GSH) in some pathological conditions such as diabetes, hepatitis, ulcerative colitis, Crohn's disease, renal dysfunctions, myocardic ischemia, adult respiratory distress disease and Alzheimer disease. It is also known that, beside pathological causes, the glutathione levels progressively decrease with the age.

In particular, in the case of the Alzheimer disease as other neurological diseases, the loss of neuronal cells due to oxidative stress has been related to the glutathione decrease. As is known, Alzheimer's disease is a neurological disorder characterized by progressive dementia, extracellular amyloid plaques and intracellular neurofibrillary tangles. It has been suggested that β-amyloid (Aβ) peptide has a causal role in the development and progression of Alzheimer disease and that, among the mechanisms involved in the neurotoxicity mediated by this peptide, oxidative stress plays a pivotal role in the development of this disease. Recent data indicate that lymphoblasts and fibroblasts from familial AD patients carrying mutations in the APP and PS-1 genes display a significant impairment of the total antioxidant capacity, with altered GSH levels, a marked increase in membrane lipoperoxidation and a higher vulnerability to Aβ aggregates as compared to the same cells from age-matched healthy controls.

As is known, glutathione (GSH) is one of the most abundant intracellular non-protein thiols in the central nervous system where it plays a major antioxidant role within both neurons and non-neuronal cells. Glutathione (γ-L-glutamyl-L-cysteinyl-
glycine) is a tripeptide that, instead of the typical α-linkage, contains a γ-linkage which resists degradation by intracellular peptidase. GSH formula is the following:

![GSH formula](image)

GSH

As used herein, the term glutathione means the compound in its monomeric or reduced form, whilst the dimer of GSH is typically known as oxidated glutathione or glutathione disulphide.

GSH has a key role in the cellular defense as it exhibits a powerful antioxidant action due to its high electron-donating capacity combined with high intracellular concentration, often at the millimole level (ranging from 1 to 10mM depending on the type of cell). Thiol group is the active part of the molecule and serves as a reducing agent to prevent tissue oxidation.

The biological functions of GSH are well known. Besides its antioxidant function, GSH also exhibits a detoxification action against exogenous and endogenous toxins combining with them to form water-soluble conjugates excreted through urine and has an immune system modulating function and a cell regulatory function acting as redox regulator on the main cell's vital functions such as DNA synthesis and repair, protein synthesis, enzyme activation and regulation.

In order to maintain or restore a proper GSH level in the interested cell compartments, therapeutic strategies have been developed aiming at increasing GSH levels by dietary or pharmacological intake of GSH precursors, GSH mimetics or substrates for GSH synthesis to protect the cells against the oxidative stress. Since GSH itself poorly penetrates the blood-brain barrier and doesn't freely cross cellular membranes due to its hydrophily, other treatment options including GSH carriers, analogs, mimetic or precursors have been used.

The cellular GSH concentration depends on cysteine availability, which is a function of dietary protein intake and methionine trans-sulfuration in liver. However,
cysteine cannot be administered directly due to quick metabolization and toxicity, whereby it was proposed the use of compounds which, once they are carried in the cells, can be converted into cysteine intracellularly, such as thiazolidine, or even acetyl-cysteine which, however, has a certain toxicity and in any case requires the action of an enzyme for being deacetylated.

A GSH precursor capable of enhancing the GSH level in the lens to prevent cataract onset is for example disclosed in US5624955. The precursor consists of a glutamyl cysteine derivative in which S is bond to an acyclic group R-CO, wherein R is an alkyl group CrC 2, aryl group C 6-Ci 2 or cycloalkyl group C 5-Ci 8. US7092695 discloses analogs of GSH for hemopoiesis modulation, in lipid formulation, e.g. liposomes, to enhance the bioavailability and reduce the toxicity.

With the same aim to enhance the cellular GSH level the use of GSH monoesters was also proposed. In particular, US5474825 discloses the use of an ethyl ester of N-acyl-GSH, in the place of the respective aminoacidic or dipeptidic sterates, as substances that can be administered per os and potentially suitable for releasing GSH following intracellular deesterification and deacylation. Such type of esters have the disadvantage of releasing the alcohol group in the cell following intracellular hydrolysis and their safety in the long run was not demonstrated.

Another compound used to achieve an increase in endogenous GSH level in the neuronal cells is tricyclodecan-9-yl-xanthogenate (D609) [see for instance, Perluigi, M et al., In vivo protection by the xanthate tricyclodecan-9-ylxanthogenate against amyloid β-peptide (142)-induced oxidative stress. Neuroscience 138:1 161-1 170; 2006]. Among the natural antioxidants, vitamin E, vitamin C, melatonin, ginkgo biloba, steroid hormones, etc have been used. However, many clinical trials are still unsuccessful because all the antioxidants tested difficulty cross the blood-brain barrier and do not readily enter the brain in the adult.

Recent attention has been paid to the possible role of dietary fatty acids in age-related cognitive impairment of degenerative diseases. At present, several studies suggest a clear reduction of risk of cognitive impairment with a high intake of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The protective effect of dietary unsaturated fatty acids could be related to the role of fatty
acids in maintaining the structural integrity of neuronal membranes, determining the fluidity of synaptosomal membranes and thereby regulating neuronal transmission. Furthermore, essential fatty acids can modify the activity of certain membrane-bound enzymes (phospholipase A2, protein kinase C, and acetyltransferase) and the function of membrane proteins including ion channels. Finally, an increase in MUFA content and a decrease in PUFA content, in neuronal membranes in advancing age has been demonstrated suggesting that in the aging process there is an increasing demand for MUFA.

Summary of the invention

The general object of the present invention is to provide new antioxidant compounds especially for use in the treatment of diseases related to reduced GSH levels, and in particular neurodegenerative diseases such as Alzheimer disease.

A particular object of the present invention is to provide S-acyl GSH derivatives, in particular long-chain fatty acids GSH thioesters, which, due to their hydrophobic nature, can easily cross plasma membrane and be internalized in cellular compartments.

Another object of the present invention is to provide a method for the synthesis of the long-chain fatty acid GSH thioester derivatives.

A further object of the present invention is to provide pharmaceutical compositions containing the long-chain fatty acid GSH thioester derivatives and the use of these derivatives to make easier the GSH intake in the cells, in particular neuronal cells.

According to the invention GSH thioester derivatives are provided of the formula

\[
\text{HO-}
\begin{array}{c}
\text{NHR}^1 \\
\text{SR}^1
\end{array}
\text{OH}
\]

wherein \(R^1\) is an aliphatic or aromatic acyl group, preferably an aliphatic group such as a saturated or an unsaturated \(C_2-C_{24}\) group, preferably a polyunsaturated \(C_{16}^N-C_{24}\) group, and \(R^2\) is hydrogen or an aliphatic or aromatic acyl group preferably an aliphatic group, and most preferably an acetyl group.
In a particularly preferred embodiment of the invention $R^1$ is a radical selected from the group consisting of lauroyl, palmitoleoyl, linoleoyl, linolenoyl, arachidonoyl groups.

Thanks to their lipophilic nature, the S-acyl GSH derivatives according to the invention easily cross the cell membrane and in the cell they are enzymatically hydrolysed by GSH-thioesterases and deacylated by cytoplasm esterases to release GSH and the carboxylic acids, in particular the unsaturated fatty acid. The ability to cross the cell membrane is due to the presence of the hydrocarbon chain of the apolar acyl group bond to the GSH thiol group. Once released in the cell by hydrolysis, the acyl group also produces a protective effect on cell membrane, in particular the neuronal cell membrane. The carboxylic group bond to the thiol group of GSH synergically acts both by making possible cell membrane crossing and, once released by hydrolysis in the cell, by exploiting a protective action on the cell.

In particular, according to the present invention S-lauroyl-GSH, S-palmitoleoyl-GSH, S-linoleoyl-GSH, S-linolenoyl-GSH and S-arachidonoyl-GSH were synthesized, and their protective effect on human neurotypic SH-SY5Y cells experiencing amyloid aggregate oxidative insult was tested. It was found that the S-acyl-GSH derivatives according to the present invention can easily cross plasma membrane and be internalized in cellular compartments. S-acyl-GSH thioesters can prevent Aβ42 (1-42 β-amyloid peptide) toxicity exhibiting a significant decrease of oxidative stress in neuroblastoma cells. In particular, these derivatives can account for a significant increase in the total cellular antioxidant defenses, a significant reduction in intracellular reactive oxygen species (ROS) production and a large inhibition of membrane lipoperoxidation. These results provide for a strong basis for the use of the S-acyl-GSH derivatives according to the present invention in the preparation of integrators and drugs useful in the prevention and treatment of cell oxidative stress-related disorders and in particular the Alzheimer disease.

According to another aspect of the present invention, the S-acyl GSH derivatives can be synthesized either enzymatically by transthioesterification by reacting GSH with the corresponding acyl esters of coenzyme A (CoA) or chemically by reacting GSH with the corresponding acyl halide.
Brief description of the drawings

Figures 1a, 1b, 2a, 2b, show the purification and characterization of S-lauroyl-GSH and S-palmitoleoyl-GSH;

Figure 3, sections a), b) and c), graphically shows the viability of SH-SY5Y cells a) as a function of the treatment time, b) and c) as a function of the thioester amount for cell exposed both to Aβ42 aggregate for 24 hours and to H2O2;

Figure 4 shows the antioxidant properties of S-lauroyl-GSH and S-palmitoleoyl-GSH as a function of the exposure time to a 1 μM of these derivatives (diagram a) and after treatment with 5 μM Aβ42 aggregates (diagram b) or 250 μM H2O2 for 10, 20, 30, 60, 180 min. at 37°C (diagram c);

Figure 5 shows the intracellular ROS production in SH-SY5Y cells exposed or not to 1 μM S-acyl-GSH thioesters and treated with 5 μM Aβ42 aggregates (diagram a) or 250 μM H2O2 for 10, 20, 30, 60 and 180 min. at 37°C (diagram b)

Figure 6 shows the MTT reduction vs. thioester concentration of pretreated human neuroblastoma SH-SY5Y cells treated with Aβ42 aggregates;

Figure 7 shows the MTT reduction vs. thioester concentration of pretreated human neuroblastoma SH-SY5Y cells treated with H2O2 as a positive control.

Detailed description of the invention

The enzymatic synthesis of the S-acyl-GSH derivatives according to the present invention comprises incubating reduced GSH with the corresponding acyl-CoA thioesters in sodium phosphate buffer at 37 °C and then purifying the product from the water phase with solvent by HPLC. The purity of S-acyl-GSH derivatives was confirmed through MALDI/TOF (Matrix-Assisted Laser Desorption/Ionization/Time-of-flight) mass spectrometry. The acyl-CoA thioesters reaction with GSH leading to the synthesis of S-lauroyl-GSH and S-palmitoleoyl-GSH resulted time-dependent and the synthesis reaction reached its maximum after 24 hours, in particular for S-lauroyl-GSH, while a lower production of S-palmitoleoyl-GSH was observed in the same period of time.

In particular, according to a non-limiting embodiment of the invention, 25 mM reduced GSH were incubated with 5 mM acyl-CoA thioesters of lauric acid or palmitoleic acid, in 50 mM sodium phosphate buffer, pH 7.5 at 37 °C.
The reaction of formation of the two S-acyl-GSH derivatives are:

a) S-lauroyl-GSH

\[ \text{Lauroyl CoA} + \text{GSH} \xrightarrow{\text{NaH}_2\text{PO}_4, 37 \, ^\circ\text{C}} \text{S-lauroyl-GSH} \]

b) S-palmitoleoyl-GSH

\[ \text{Palmitoleoyl CoA} + \text{GSH} \xrightarrow{\text{NaH}_2\text{PO}_4, 37 \, ^\circ\text{C}} \text{S-palmitoleoyl-GSH} \]

In a completely equivalent way the other S-acyl-GSH derivatives, wherein acyl is a linoleic, linolenic and arachidonic radical, can be synthesized.

S-lauroyl-GSH and S-palmitoleoyl-GSH derivatives were purified from the water phase with solvent A (0.1% TFA, v/v, in water). A gradient elution of 20-60% B (0.1% TFA, v/v, in acetonitrile) in 60 min for S-lauroyl-GSH derivative and of 20-70% B in 70 min for S-palmitoleoyl-GSH derivative was performed. The flow rate used was of 0.7 ml/min. Fractions containing S-acyl-GSH derivatives were collected, frozen, lyophilized to dryness and stored at -80 °C until use. The identity and the purity of S-acyl-GSH derivatives dissolved in water/acetonitrile (50:50) in the presence of formic acid were analysed by MALDI/TOF mass spectrometry on a OmniFlex-NT (Bruker Daltronics)
instrument. GSH, lauroyl-CoA, palmitoleoyl-CoA, and other chemicals were purchased from Sigma (Milan, Italy). The average process yield was 30% at the stated conditions.

Using the chemical synthesis an halide of the corresponding carboxylic acid is added to a solution of L-glutathione in trifluoroacetic acid in inert atmosphere. After stirring at ambient temperature and under heating conditions, the reaction is terminated by cold water addition. After further stirring trifluoroacetic acid is removed under vacuum, ethyl acetate is added and the reaction mixture is cooled. A salt of S-acyl-GSH trifluoroacetate is collected by filtration. The unsalified derivative can then be obtained by ionic exchange chromatography or by dialysis in physiological solution (NaCl 0.9%). The overall yield is about 40%.

By way of example, S-palmitoleoyl-GSH trifluoroacetate was prepared in the following way:

Palmitoleoyl chloride (2.4 ml, 7.8 mmol, 2.4 equiv.) was added dropwise to a solution of L-glutathione (1.0g, 3.25 mmol) in trifluoroacetic acid (11 ml) in argon atmosphere. After stirring at ambient temperature for 20 min and at 40°C for 30 min, the reaction was terminated by addition of cold water (0.25 ml, 14 mmol, 4.3 equiv.) and the reaction mixture was stirred for another hour at 40°C. Trifluoroacetic acid was removed under vacuum and ethyl acetate (20 ml) was added, and then the mixture was cooled to 10°C and precipitated glutathione trifluoroacetate collected by filtration.

S-acyl-GSH thioesters were added to cell culture of human SH-SY5Y neuroblastoma cells (AT. C.C, Manassas, VA) at differing final concentrations for differing incubation times. Then, cells were exposed to 5 µM Aβ42 prefibrillar aggregates obtained according to Lambert's protocol.

The protective effect of S-acyl-GSH derivatives against amyloid aggregate cytotoxicity was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay by exposing treated and untreated cells with these derivatives to 5.0 µM Aβ42 aggregates in a 96-well plate. Parallel experiments were performed using 250 µM H₂O₂ as positive control.

In a series of experiments cells were incubated with S-lauroyl-GSH and S-palmitoleoyl-GSH thioesters (1, 3 and 5 hours) and without them for the same times. In another set of experiments, differing final concentrations of S-acyl-GSH thioesters (0.2,
1.0 and 5 µM) were added to cell culture media for 3 hours at 37 °C. Comparative experiments with 1 µM acyl-CoA thioesters of lauric and palmitoleic acids and GSH alone were conducted.

After washing with PBS, cells were exposed to 5.0 µM Aβ42 aggregates or to 250 µM H₂O₂ for 24 h in culture medium without S-acyl-GSH derivatives of the invention. Then, 100 µl of 0.5 mg/ml MTT solution in PBS were added to the cell cultures and the samples were incubated for 4.0 h at 37 °C. Finally, 100 µl of cell lysis buffer (20% SDS, 50% N,N-dimethylformamide, pH 4.7) were added to each well and the samples were incubated for at least 3 h at 37 °C in a humidified incubator, before absorbance values determination of blue formazan at 590 nm with an ELISA plate reader. Cell viability was expressed as percent of MTT reduction in aggregate or H₂O₂ exposed cells compared to untreated cells (taken as 100%).

Test results are shown in Figure 3a), b), c). A significant impairment (about 35%) in control cells viability was evident following exposure to Aβ42 aggregates for 24 hours. A significant recover in cell viability was evident when cell were pre-treated with S-lauroyl-GSH and much more with S-palmitoleoyl-GSH thioesters before exposure to Aβ42 aggregates (Figure 3a). In particular, the more prolonged S-acyl-GSH treatment time, the higher protection against aggregate toxicity appeared. The protective ability of S-palmitoleoyl-GSH thioester was higher than S-lauroyl-GSH thioester at differing conjugate concentrations (from 0.2 to 5 µM) (Figure 3b). In particular, at 1 µM a significant difference in the protective effect of S-palmitoleoyl-GSH thioester respect to S-lauroyl-GSH was observed. Similar results were obtained in H₂O₂ exposed cells, in which S-palmitoleoyl-GSH ability to counteract H₂O₂ oxidative injury overcame that of S-lauroyl-GSH thioester at all investigated concentrations (Figure 3c).

The same MTT test has been used to assess the protective action of linoleoyl -GSH and arachidonoyl-GSH against the cytotoxicity induced by Aβ42 prefibrillar aggregates in human neuroblastoma SH-SY5Y cells and compared with the protective action of 1.0 µM palmitoleoyl-GSH and with untreated Aβ42 exposed cells. SH-SY5Y cells were pretreated for 3 hours in dose-dependence (0.1, 1.0, 10 µM) with linoleoyl -GSH and arachidonoyl-GSH, and then treated for 24 hours with 5 µM Aβ42. The results were compared with the treatment with 1.0 µM palmitoleoyl-GSH for 3 hours
and with cells treated with 5 µM Aβ42 only for the same time. The results of the
comparison are shown in figure 6. An higher protective action of linoleoyl-GSH and
arachidonoyl-GSH is clearly shown.

Using H2O2 as a positive control (treatment with 250 µM for 24 hours after 3
hours treatment with 1.0 µM S-acyl GSH of the invention) the same comparative results
are obtained, as shown in figure 7.

As a further test, changes in non-enzymatic, hydrophilic total antioxidant capacity
(TAC) following exposure to amyloid aggregates or H2O2 in SH-SY5Y control or S-acyl-
GSH thioesters pre-treated cells were measured. As shown in Figure 4a, cells
underwent a significant increase in TAC in the presence of both 1 µM S-Lauroyl-GSH
and S-palmitoleoyl-GSH thioesters in the cell culture media, reaching a maximum after
3 hours of exposure compared to the basal TAC of untreated cells. In particular, an
higher S-palmitoleoyl-GSH thioester antioxidant property compared to S-Lauroyl-GSH
derivative was evident just after 20 min of GSH thioester treatment.

The ability of the S-acyl-GSH thioesters of the invention to counteract the free
radicals (ROS) production was also measured. Intracellular ROS levels were detected
by using the ROS-sensitive fluorescent probe CM-H2, DCFDA esterified derivative by
which SH-SY5Y cells exposed to S-acyl-GSH thioesters of the invention and treated
with Aβ42 aggregates or H2O2 were incubated. CM-H2, DCFDA fluorescence was
measured using a Perkin Elmer LS 55 spectrofluorimeter at 485 nm excitation and 538
nm emission wavelengths.

As shown in figure 5, S-acyl-GSH thioesters pre-treated cells were able to
counteract the early rise in intracellular ROS despite of control cells. In particular, S-
palmitoleoyl-GSH appeared more effective than S-lauroyl-GSH in facing both
aggregate (Figure 5A) and H2O2 (Figure 5B) oxidative injury.

S-acyl-GSH thioesters protective effect against amyloid aggregate- or H2O2-
induced lipid peroxidation was investigated by confocal scanning microscopy analysis,
using the fluorescent probe BODIPY. The BODIPY red fluorescence observed in
untreated control cells shifted to green following 3 hours of exposure to the aggregates
and more evidently to H2O2. On the other hand, the fluorescence signals of cells pre-
treated with both S-lauroyl-GSH and S-palmitoleoyl-GSH thioesters, exposed to Aβ42
aggregates or to \( \text{H}_2\text{O}_2 \), did not significantly differ from their respective in untreated cells.

Finally, the preventive activity of S-acyl-GSH derivatives of the present invention against the apoptotic program activation was confirmed by using caspase-3 as apoptotic bioindicator (Caspase-3 & -7 FLICA kit (FAM-DEVD-FMK), Immunochemistry Technologies, LLC, Bloomington, MN) by assessing the caspase-3 activity through confocal microscopy analysis. The result was that caspase-3 activity was significantly increased after three hours treatment with amyloid aggregate or \( \text{H}_2\text{O}_2 \), whilst cells pre-treated with S-acyl-GSH thioesters underwent a significant reduction in caspase-3 activity.

These results confirm that it is possible to use S-acyl-GSH thioesters of the invention, particularly those in which the acyl group is a saturated or unsaturated fatty acid radical, in particular S-lauroyl-GSH, S-palmitoleoyl-GSH, S-linoleyl-GSH, S-linolenoleyl-GSH and S-arachidonoyl-GSH, as Aβ42 cytotoxicity modulators and hence for the use in the prevention and treatment of the Alzheimer disease and other oxidative stress related diseases or diseases which can gain benefit from a cellular intake of GSH.

The present invention also provides for pharmaceutical compositions comprising, as active ingredient, a compound of formula (I) or a pharmaceutically acceptable salt thereof, taken alone or in association with a pharmaceutically acceptable excipient, such as a carrier or a diluent. Preferably the compositions are prepared in a suitable form for *per os* or injectable administration. The pharmaceutically acceptable excipients which are mixed with the active compound or their salts to form the compositions according to the invention are well known and their selection depends, *inter alia*, from the administration method of the compositions.

The compositions for oral administration may take the form of tablets, retard tablets, sublingual tablets, capsules and the like, or elixirs, syrups or suspensions, all containing the compound of the invention; such preparations may be made by methods well-known in the art. Compositions for injectable use may be prepared from soluble salts which may be dissolved in an appropriate fluid for parenteral injection.
CLAIMS

1. S-acyl-GSH derivatives of the formula (I)

   \[
   \text{HO-} \quad \text{NHR}^2 \quad \text{SR}^3 \quad \text{OH}
   \]

wherein \( R^1 \) is an aliphatic or aromatic acyl group, preferably an aliphatic group such as a saturated or unsaturated \( \text{C}_{12}-\text{C}_{24} \) group, preferably a polyunsaturated \( \text{C}_{16}-\text{C}_{24} \) group, and \( R^2 \) is hydrogen or an aliphatic or aromatic acyl group preferably an aliphatic group, and most preferably an acetyl group.

2. S-acyl-GSH derivatives according to claim 1, wherein \( R^1 \) is an aliphatic acyl group selected from the lauric, palmitoleic, linoleic, linolenic, arachidonic acid radicals.

3. S-acyl-GSH derivatives according to claim 2, which are S-lauroyl-GSH, S-palmitoleoyl-GSH, S-linoleoyl-GSH, S-linolenoyl-GSH, S-arachidonoyl-GSH.

4. S-acyl-GSH derivatives according to any one of the previous claims, wherein \( R^2 \) is an acetyl group.

5. S-acyl-GSH derivatives according to claim 4, wherein \( R^2 \) is an halogen substituted acetyl group.

6. Method for the synthesis of an S-acyl-GSH derivative according to any one of the claims 1 to 5, characterized in that it comprises incubating reduced GSH with the corresponding acyl thioesters of coenzyme A in phosphate buffer at 37°C and purifying the derivative from the aqueous phase with a solvent by HPLC.

7. Method for the synthesis of an S-acyl-GSH derivative according to any one of the claims 1 to 5, characterized in that it comprises the steps of

   - reacting an halide of the corresponding carboxylic acid with a solution of L-GSH in trifluoroacetic acid and inert atmosphere,
   - removing trifluoroacetic acid under vacuum,
   - adding ethyl acetate, and
   - collecting the precipitated salt.
8. Pharmaceutical composition for prevention and treatment of degenerative diseases associated to a cellular glutathione level reduction, such as diabetes, hepatitis, ulcerative colitis, Crohn's disease, renal dysfunctions, myocardic ischemia, adult respiratory distress disease, Huntington's disease and Alzheimer's disease characterized in that it comprises a S-acyl-GSH derivative according to any one of claims 1 to 5 as an active ingredient.

9. Use of a S-acyl-GSH derivative according to any one of claims 1 to 5 for the preparation of a medicament useful in the prevention and treatment of degenerative diseases associated to a cellular glutathione level reduction, such as diabetes, hepatitis, ulcerative colitis, Crohn's disease, renal dysfunctions, myocardic ischemia, adult respiratory distress disease, Huntington's disease and Alzheimer's disease.
Fig. 1
Fig. 2

(a) Chromatograms at different time points:
- Time (h) 0
- Time (h) 2
- Time (h) 24

(b) Mass spectrum with peak at 544.53
Fig. 3
Fig. 4
Fig. 5
**Fig. 6**

MTT reduction (% vs untreated cells) for Aβ42 treated with different thioesters:
- Treated
- Linoleoyl-GSH
- Arachidonoyl-GSH
- Palmitoleoyl-GSH

**Fig. 7**

MTT reduction (% vs untreated cells) for H2O2 treated with different thioesters:
- Control cells
- Palmitoleoyl-GSH
- Linoleoyl-GSH
- Arachidonoyl-GSH