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Review

Design and synthesis of glutathione analogues☆

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Abstract

This review reports recent structural modifications (since 1989) performed on the glutathione molecule both in the oxidized and reduced form. Relevant chemical aspects, biochemical consequences and therapeutical implications are illustrated. Natural thiols related to glutathione are also considered [1]. © 1998 Elsevier Science S.A. All rights reserved.

Keywords: Cysteine; γ-Glutamyl peptides; Glutathione analogues; Thiols; Trypanothione

1. Introduction

There has been an increasing interest in glutathione as it is the most abundant non-protein thiol in mammalian cells and the preferred substrate for several enzymes in xenobiotic metabolism and antioxidant defense [1–3].

An excellent reference book on glutathione illustrates the topical interest of chemical, biochemical and medical research in this peptide [4]; in particular, it supplies a comprehensive entry into the chemical aspects concerning glutathione up to 1989 [5]. Since then, a variety of glutathione analogues and derivatives have been synthesized in view of their potential pharmacological properties and, more specifically, as probes of the structural features of the active site of glutathione-dependent enzymes. The present account is not meant to exhaustively cover all the structures reported in the last years, but to discuss the most representative recent examples of chemical modification on glutathione molecule.

Abbreviations: ααAA, α,α-disubstituted amino acid; Ac, acetyl; Ac_3c, 1-aminocyclopropane-1-carboxylic acid; Ac_5c, 1-aminocyclopentane-1-carboxylic acid (cycloleucine); Acm, acetamidomethyl; AcOH, acetic acid; Aib, 2-aminoisobutyric acid (2-methylalanine); β-Ala, β-alanine; Boc, *tert*-butyloxycarbonyl; $(n\text{-}Bu)_3P$, tri-*n*-butylphosphine; Bu^t, *tert*-butyl; Bzl, benzyl; Cle, cycloleucine; DCC, dicyclohexylcarbodiimide; DIEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DMSO-d_6, hexadeuterodimethyl sulfoxide; Et_3N, triethylamine; Et_2O, diethyl ether; EtOH, ethanol; GABA, γ-aminobutyric acid; γ-GT, γ-glutamyltransferase; GCSG, *N*,*N*-bis(γ-glutamyl)-L,L-cystathionine diglycine; GH, desthio glutathione (nor-ophthalmic acid); Glo, L-γ-oxaglutamic acid; $GluP^{\alpha}$, 4-amino-4-phosphonobutanoic acid; Glx, glyoxalase; GlyP, aminomethanphosphonic acid; Glz, L-azaglutamic acid; GOH, γ-glutamyl–seryl–glycine; GP, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GSH-ase, glutathione synthetase; GSNO, *S*-nitrosoglutathione; Gsp, glutathionyl-spermidine; GSSG, glutathione disulfide; GS_3G, glutathione trisulfide; GSSeSG, glutathione selenotrisulfide; GST, glutathione-*S*-transferase; HOBt, 1-ydroxy-benzotriazole; MeCys, α-methyl-cysteine; MeOH, methanol; NMM, *N*-methylmorpholine; Np, *p*-nitrophenyl; Pa, phenacyl; Pen, penicillamine; Pfp, pentafluorophenyl; *n*-PrOH, *n*-propanol; Suc, succinyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran; thioThr, thiothreonine; Tos, tosyl; TR, trypanothione reductase; TRNOESY, transferred nuclear Overhauser effect spectroscopy; TSA, transition-state analogue; $T(SH)_2$, trypanothione; TS_2, trypanothione disulfide; Z, benzyloxycarbonyl.

☆ Dedicated to Professor Dr Aurelio Romeo on the occasion of his 75th birthday.

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[1] Abbreviations follow the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature as given in Eur. J. Biochem. 138 (1984) 9–37.

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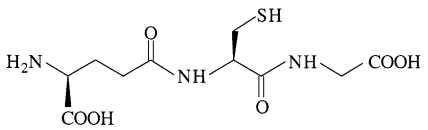


Fig. 1. Glutathione (GSH).

2. General aspects

Glutathione in its reduced form (GSH) is the tripeptide γ-L-glutamyl–L-cysteinyl–glycine (Fig. 1); it exists primarily as GSH but it is also present as the disulfide dimer (GSSG). The most interesting chemical features are the free SH group and the unusual main-chain structure containing a γ-glutamyl isopeptidic bond, which leaves an extra carboxyl group located near the amino terminal [6]. The isopeptidic nature of the γ-glutamyl linkage renders it resistant to cleavage by most peptidases. The electronic structure of the sulfur atom (available d-orbitals, permitting overlap during the formation of transition states and dissipation of electrons transferred from radicals) accounts for sulfhydryl high reactivity towards nucleophilic addition, oxireductive reactions (through ionic or radical mechanisms) and metal chelation. All these properties are connected with the antioxidant and detoxifying effects of SH-containing natural compounds; for a comprehensive review on this topic see Ref. [7].

Large scale synthesis of GSH suffers from difficulties due to the presence of four reactive functional groups. This has stimulated a number of exercise syntheses for the evaluation of new protective group strategies and coupling methods [8–10].

The interaction of glutathione with enzymes is of general biochemical [11,12] and particular medical [13,14] interest; thus, much attention has been focused on the GSH structural requirements which are essential in these interactions [15,16]. A brief list of the main enzymatic systems involving GSH, together with accepted abbreviations, is reported in Table 1.

The chemical modifications performed on the GSH molecule and reported in the present review are collected according to the involved amino acid residue (Glu, Cys, Gly); the remaining examples are described as modifications at more than one residue.

3. Strategies for the chemical modification

Most of the chemical variations follow the general approaches for structural modification of peptides (e.g. amino acid replacement, introduction of amide bond surrogates, cyclization, introduction of secondary structure mimetics) which are summarized and illustrated in Fig. 2. Excellent reviews are available on this topic [17–20].

3.1. Modification at the Glu moiety

3.1.1. Glu replacement

In order to elucidate the effect of individual changes on binding and catalytic processes, a variety of GSH analogues in which the γ-glutamyl residue is replaced by other amino acids has been designed. The approach of introducing D-, *N*-methyl- and α-methyl-Glu has been already exploited and covered [5].

Other modifications concern the synthesis of analogues in which the number and the nature of the two polar groups of the γ-glutamyl moiety have been modified in order to study the effect of individual charges on binding and catalysis involving the glutathione reductase (GR). For the introduction of glutaryl [21,22] or γ-aminobutyryl [23] residues, lacking the amino or the carboxy groups respectively, see com-

Table 1
Main GSH-dependent enzymes

Enzyme	Catalysed reaction	Biological implications
γ-Glutamyl transferase (γ-GT)	Transpeptidation Hydrolysis Autotransfer at the level of γ-Glu–Cys bond	Amino acid and peptide transport Processing of leukotrienes Renal ammoniagenesis
Glutathione-*S*-transferase (GST)	Nucleophilic addition of the Cys thiol group to electrophiles	Detoxification processes
Glutathione reductase (GR)	Reduction of the GSSG cystine distilfide bond	Maintaining of the GSH:GSSG ratio
Glyoxalase I (Glx I)	Isomerisation of the methylglyoxal–GSH hemithioacetalic adduct to *S*-D-lactoyl glutathione	Methylglyoxal detoxification
Glyoxalase I (Glx II)	Hydrolysis of *S*-D-lactoyl glutathione thioester group	Methylglyoxal detoxification
Glutathione peroxidase (GP)	Reduction of hydroperoxides to the corresponding alcohols	Prevention of oxidative damage

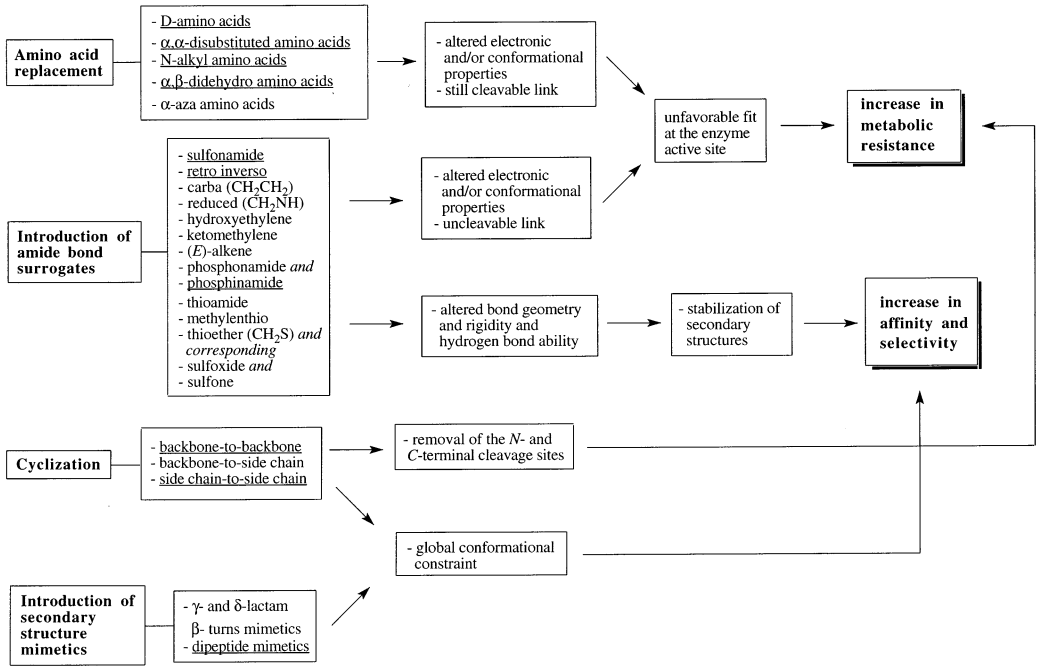


Fig. 2. General strategies for peptide modification (chemical modifications relative to GSH are underlined).

Table 2
Glu replacements for **X**–Cys–Gly

Compound	X	Ref.
1	HO_2C ⌒ CO—	[21,22]
2	H_2N ⌒ CO—	[23]
3	H_2N CO— (Ph)	[24]
4	H_2N CO— (indole)	[24]
5	H_2N CO— (PO_3H)	[28]
6	H_2N ⌒ CO— (CO_2H)	[29]
7 R, R′ = H	R, N, R′, CO—, CO_2H	[30]
8 R, R′ = CH_3		[30]
9 R = CH_3, R′ = C_5H_{11}		[30]

pounds **1** and **2** in Table 2. In the case of compounds **3** and **4** the less polar residues of Phe and Trp [24] have been introduced during studies aimed at obtaining trypanothione reductase inhibitors (see below). The biological activity shown by phosphono–oligopeptides as antimicrobials, antiviral, analgesic, fungicidal, herbicidal, etc. [25,26] has prompted the development of efficient synthetic routes for this class of compounds [27]. Classical solution methods were applied to the synthesis of GSH analogues in which the N-terminal glutamic acid and/or C-terminal glycine are replaced by their phosphonic counterparts [28]. In Table 2 is reported the γ-glutamyl-modified GSH **5** containing the phosphonic analogue of glutamic acid, i.e. 4-amino-4-phosphonobutanoic acid ($GluP^{\alpha}$).

Besides the already reviewed [5] approaches consisting in the exchange of Glu for Asp and γ-Glu–isopeptidic bond for α-Glu–peptidic bond, elongation has been performed with the introduction of δ-L-aminoadipic acid residue in compound **6** [29], which has been tested as potential GST inhibitor.

A new family of GSH derivatives with specific antioxidant properties has been prepared by transforming the amino group of GSH into 2,5-disubstituted or nonsubstituted pyrroles (Table 2, compounds **7**–**9**) [30]. Probably due to steric hindrance and/or hydrophobicity of the pyrrole ring, these GSH analogues are not inhibitors of glutathione peroxidase (GP) and GR, which represent the natural enzymatic defense against oxidative stress.

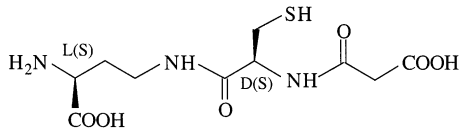
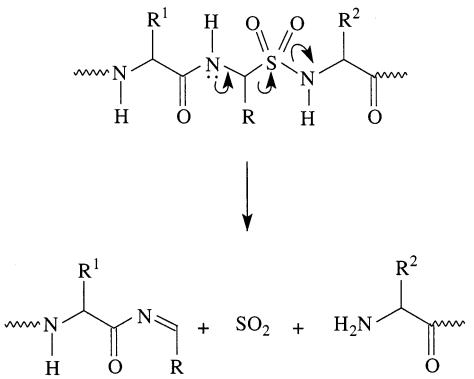


Fig. 3. The end-group modified retro-inverso isomer of GSH.

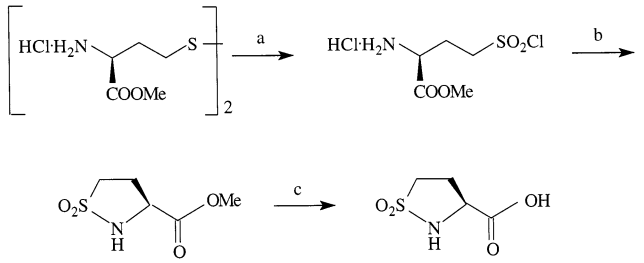


Scheme 1. Degradation of pseudopeptides containing the SO_2NH peptide bond surrogate.

3.1.2. γ-Glutamylic amide bond surrogates

Since the report [31,32] of the end-group modified retro-inverso isomers of GSH (Fig. 3) and its disulfide, very few chemical modifications involving the γ-glutamylic junction have been described.

Transition state analogues (TSA) of peptide bond hydrolysis have received widespread attention in the design of tight-binding protease inhibitors. One promising unit to be introduced is the sulfonamide junction, which shares with its phosphonamide counterpart good resemblance to the tetrahedral intermediate of peptide bond cleavage. Several interesting aspects connected with

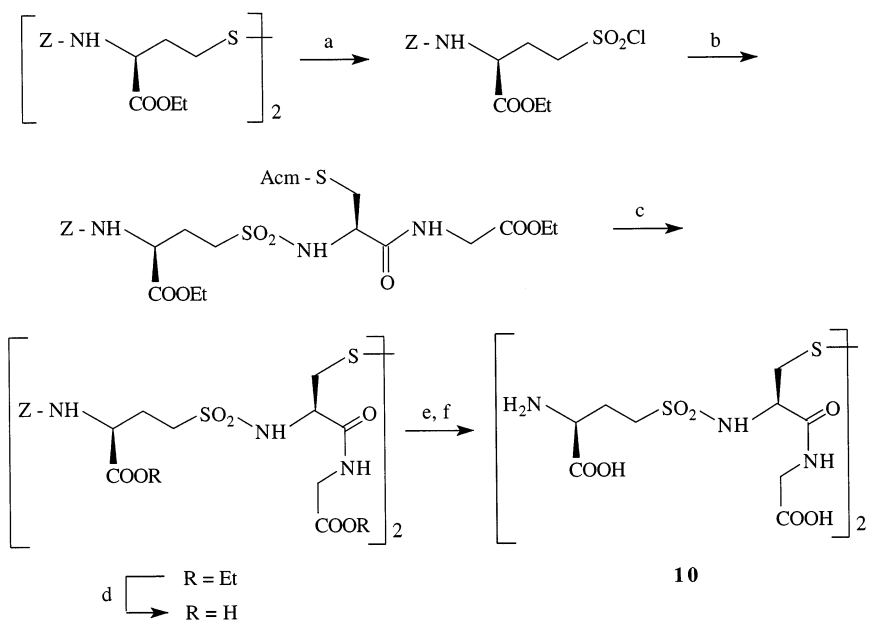


Scheme 3. Synthesis of the sulfonamide analogue of L-pyroglutamic acid. (a) Cl_2, $CHCl_3$–EtOH, 0°C, 20 min; (b) Et_3N, $CHCl_3$, −5°C, 20 min, then r.t., 48 h; (c) 1 N NaOH, r.t., 20 min.

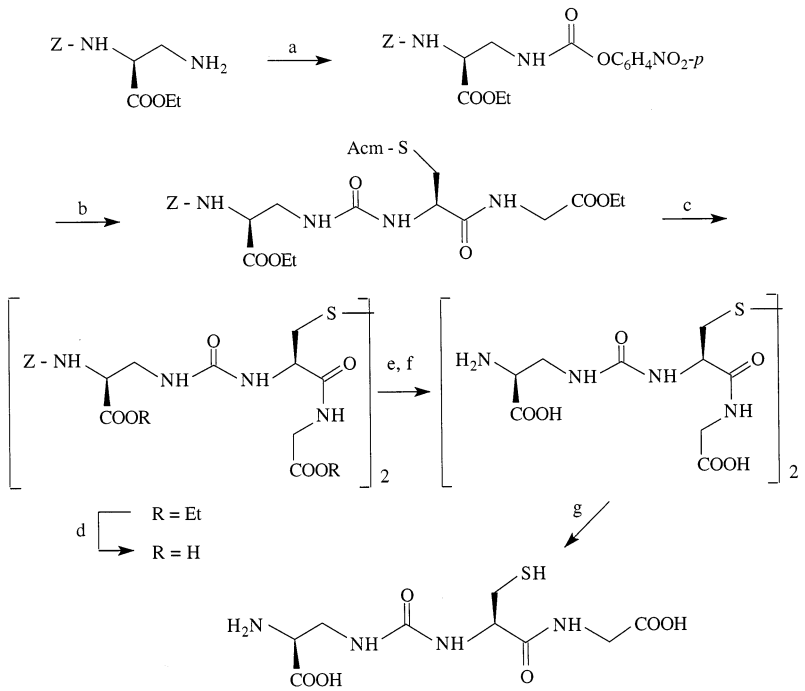
reactivity, structure and conformational preferences of the sulfonamide junction have been investigated [33–36]. However sulfonamidopeptides, bearing an amino group α to the internal sulfonamide moiety, are intrinsically unstable [37–44] (Scheme 1). As can be deduced from the Scheme, the fragmentation pattern is connected with the nature of 'carbonyl-addition compounds' that α-aminosulfonamidopeptides possess [41,43].

β-Aminosulfonic and γ-aminosulfonic acid amides are, on the other hand, generally stable; thus, γ-glutamyl peptides and GSH itself represent valuable models to evaluate the potentiality of the SO_2NH versus CONH replacement. This approach has been exploited with the synthesis of pseudoglutathione **10**, containing the sulfonamide junction in place of the γ-Glu–Cys scissile peptide bond [45] (Scheme 2).

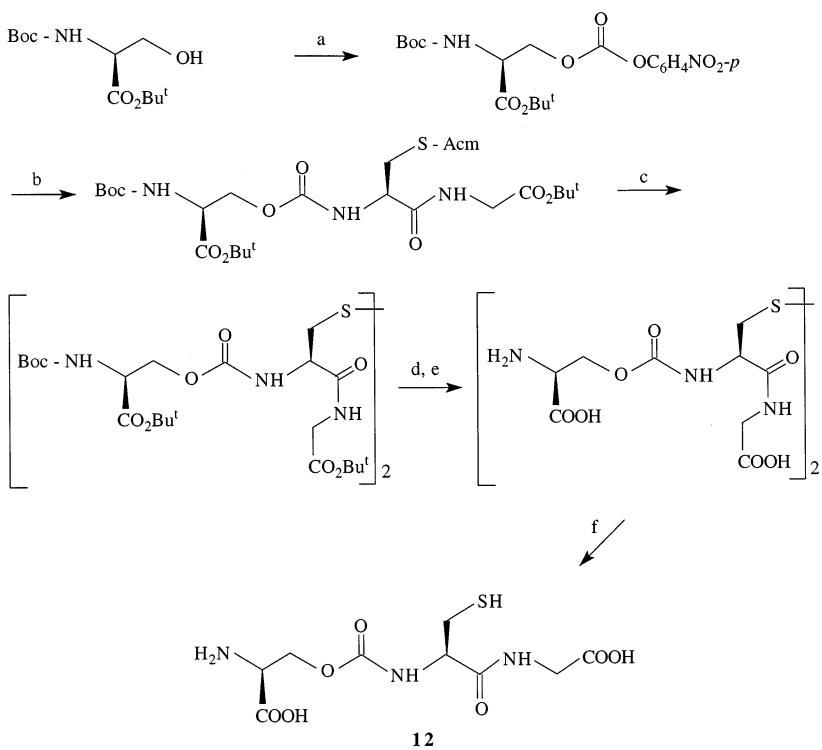
A derivative of the sulfonyl chloride reported in Scheme 2 has been used as key intermediate in the synthesis of the sulfonamide analogue of the biologically relevant residue of pyroglutamic acid [46] (Scheme 3).



Scheme 2. Synthesis of [γ-Glu–Ψ(SO_2NH)–Cys–Gly]$_2$ (**10**). (a) Cl_2, CCl_4–EtOH, 0°C, 2 h; (b) Cys(Acm)–Gly–OEt, Et_3N, $CHCl_3$, 5°C, 12 h; (c) I_2, MeOH, r.t., 3 h; (d) 1 N NaOH, DMF–H_2O–acetone (6:1:4), r.t., 1.5 h; (e) 4 N HBr–AcOH, r.t., 2 h; (f) NH_3, EtOH, r.t., 1 h.

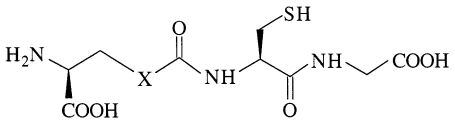


Scheme 4. Synthesis of γ-Glz–Cys–Gly (**11**). (a) pNO_2–C_6H_4OCOCl, THF, NMM, 0°C, 3 h; (b) TFA · Cys(Acm)–Gly–OEt, Et_3N, dioxane–$CHCl_3$ (2:1), 40°C, 24 h; (c) I_2, MeOH, r.t., 3 h; (d) 1 N NaOH, acetone–H_2O (5:3), r.t., 4 h; (e) HBr–AcOH, r.t., 2 h; (f) 1 N aq. NH_3, r.t., 30 min; (g) $(n\text{Bu})_3P$, nPrOH–H_2O (2:1), pH 8.5, r.t., 1 h.



Scheme 5. Synthesis of γ-Glo–Cys–Gly (**12**). (a) pNO_2–C_6H_4OCOCl, pyridine, 40°C, 24 h; (b) Cys(Acm)–Gly–OBu^t, dioxane, 80°C, 10 h; (c) I_2, MeOH, r.t., 4 h; (d) TFA, r.t., 4 h; (e) 1 N aq. NH_3, r.t., 30 min; (f) $(n\text{Bu})_3P$, nPrOH–H_2O (2:1), pH 8.5, r.t., 1 h.

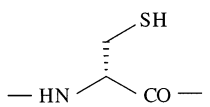
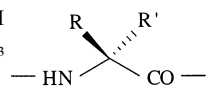
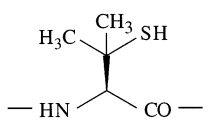
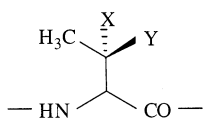
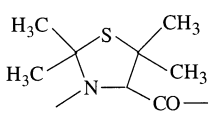
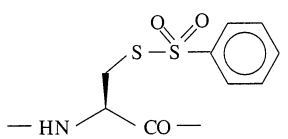
In the case of GSH, few mimetics of the amide bond, other than SO_2NH, have been studied. These are based on the replacement of the Glu γ-carbon atom, adjacent to the isopeptidic bond, with an NH or an oxygen atom. This approach gives rise to ureic [47] and urethanic [48] analogues (Fig. 4, compounds **11** and **12**,



11 X = NH γ-Glz-Cys-Gly
12 X = O γ-Glo-Cys-Gly

Fig. 4. Backbone-modified GSH analogues **11** and **12**.

Table 3
Some cysteinyl-modified GSH analogues of γ-Glu–**X**–Gly

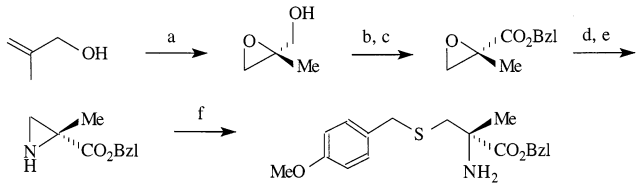
Compound	**X**	Ref.
13		[15]
14 R = CH_3, R′ = CH_2SH		[63]
15 R = CH_2SH, R′ = CH_3		[63]
16		[15]
17 X = SH, Y = H		[64]
18 X = H, Y = SH		[64]
19[a]		[65]
20		[67]

[a] Obtained as a diastereoisomeric mixture.

respectively) with chemical, conformational and biochemical properties well distinct from those of the parent GSH. Synthetic routes to compounds **11** and **12** are illustrated in Schemes 4 and 5, respectively.

3.2. Modification at the Cys moiety

An examination of the recent literature data reveals that cysteine is the prevailing site of intervention. Most of the modifications retain the cysteine skeleton and are centred on sulfur or α/β carbon atoms. Biological interest in derivatizing or substituting cysteine is connected with the possibility to modulate the interactions occurring at the active site of glutathione-*S*-transferase (GST), perhaps the most appealing enzyme target for therapeutical applications. Due to the crucial role of



Scheme 6. Synthetic route to α-methyl-cysteine via chiral aziridines. (a) Sharpless asymmetric oxidation (see Ref. [61]); (b) $RuCl_3$, $NaIO_4$; (c) DCC, DMAP, BzlOH; (d) NaN_3; (e) PPh_3; (f) $BF_3 \cdot Et_2O$, *p*-MeO–C_6H_4–CH_2–SH.

sulfhydryl group, the replacement of Cys with different amino acids has been scarcely developed.

3.2.1. Substitution at Cys C^{α}

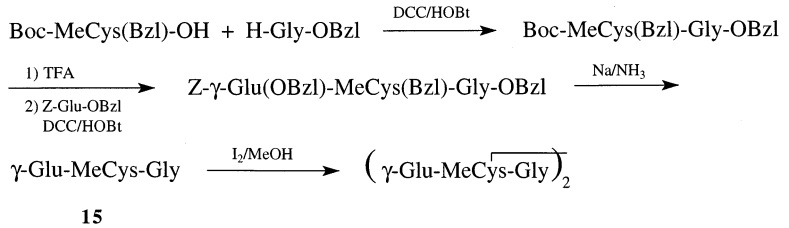
Recent interventions on C^{α} include inversion of the absolute configuration [15] (Table 3, compound **13**) and α,α-disubstitution.

Achiral [49–52] and chiral [53] α-amino acids disubstituted at the α carbon (ααAAs) are of special interest in peptide design since they induce severe restriction in the backbone conformation. In particular, C^{α}-methylation, due to the tendency to induce specific α-helical, 3_{10}-helical or β-turn conformations, has been widely used to explore the conformational requirements for bioactivity [54].

In the field of peptide analogues, α-methyl-L-cysteine (MeCys) [55,56] represents an attractive target molecule since, in addition to the above mentioned alteration of the backbone conformation, typical of ααAAs, it can give rise to constrained cyclic structures via disulfide bridge formation. Very few routes for the synthesis of chiral α-methyl-cysteine are available: Schöllkopf and co-workers have applied their method, based on metallation and alkylation of bis-lactim ethers, to synthesize *S*-benzyl and *S*-butyl α-methyl-cysteine esters [57]. Pattenden et al. [58] have prepared optically active MeCys through a modification of Seebach's procedure [59].

An efficient asymmetric synthesis of α-methyl-cysteine derivatives, based on chiral aziridine synthons, has been recently described by Goodman et al. [60]. This (Scheme 6) starts with the allylic alcohol 2-methyl-2-propen-1-ol, from which the (*R*)-2-methyl-glycidol was readily prepared in high enantiomeric purity by Sharpless asymmetric epoxidation [61]. Subsequent oxidation and oxirane-to-aziridine conversion led to the desired compounds in good yields. A related route has been recently followed by Wipf and co-workers for a new synthesis of α-methyl-L-serine by nucleophilic ring-opening of *N*-sulfonyl aziridines [62].

GSH analogues incorporating both enantiomers of α-methyl-cysteine (Table 3, compounds **14** and **15**) have been recently synthesized [63]; in Scheme 7 the synthesis of compound **15**, containing the L enantiomer of α-methyl-cysteine (MeCys), is illustrated; the synthetic pathway implies resolution of racemic



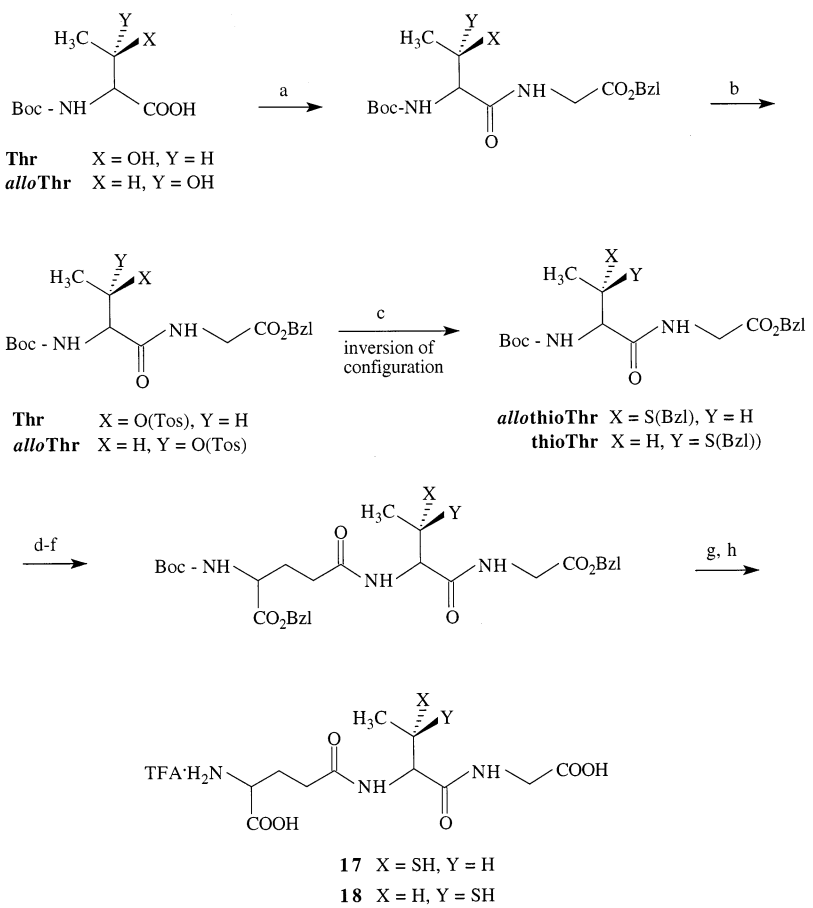
Scheme 7. Synthesis of the GSH analogue **15** containing L-MeCys.

Boc-DL-MeCys(Bzl)-OH obtained through the Bücherer–Bergs reaction. Synthesis of GSH analogues by using chiral MeCys or MeSer as starting material has not been described.

3.2.2. Substitution at Cys C^{β}

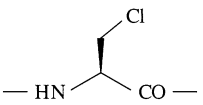
Both β-methyl-cysteine (thio-threonine, thioThr) [64] and β,β-dimethyl-cysteine (penicillamine, Pen) [15] have been used to synthesize GSH analogues (Table 3, compounds **16–18**). It is worth noting that, as compared with Cys and Pen, the thioThr residue contains an additional chiral centre and, when inserted into the GSH molecule, represents a valuable probe to acquire information on the steric environments of GSH-dependent enzymes. The two GSH analogues, γ-L-Glu-L-*allo*-thioThr-Gly (**17**) and its diastereoisomer γ-L-Glu-L-thioThr-Gly (**18**), reported in Table 3 have been designed as potential glyoxalase I (Glx I) inhibitors [64]. The interesting synthetic route (Scheme 8) implies the SN_2 displacement of the tosyl function from the protected *O*-tosyl L-threonyl- and L-*allo*-threonyl-glycine fragment by benzyl mercaptide ion. The reaction proceeds with complete inversion of configuration at C^{β} and leads to the corresponding *S*-benzyl derivatives.

Recently Martens et al. [65] described an unusual route to the totally protected GSH analogue **19** (see Table 3). This compound, incorporating a DL-Pen residue, protected as diacetone derivative, has been obtained in a Ugi four-component condensation (4CC-reaction) from isocyanacetic acid methyl ester, tetra-



Scheme 8. Synthesis of γ-Glu–*allo*-thioThr–Gly (**17**) and γ-Glu–thioThr–Gly (**18**). (a) Gly–Obz, DCC, Et_3N, CH_2Cl_2; (b) TosCl, pyridine; (c) $BzlS^-Na^+$, DMF; (d) TFA; (e) Et_3N; (f) Boc–Glu–Obzl, DCC, Et_3N, CH_2Cl_2; (g) Na/NH_3; (h) TFA.

Table 4
Cys replacements in γ-Glu–**X**–Gly

Compound	**X**	Ref.
21		[67]
22		[67]

methyl-3-thiazoline, and Boc–Glu–OCH_2Ph. In the same paper the authors describe the synthesis of an analogous modification leading to a GSH hydantoin derivative.

3.2.3. S-Derivatization

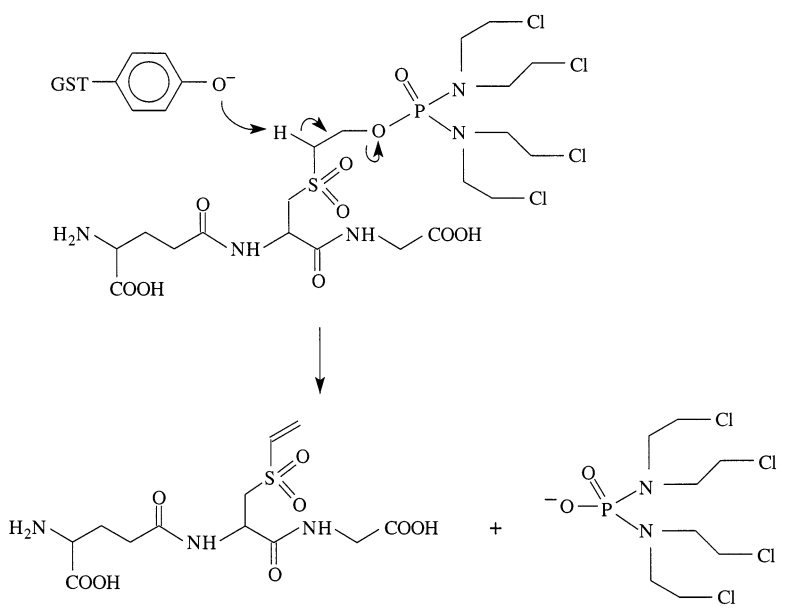
Many *S*-alkyl, *S*-aryl and *S*-acyl glutathione derivatives have been described [29,66].

Adang and co-workers reported a series of thirteen GSH derivatives containing a reactive group designed to react covalently with the GST active site [67]. The general structure of these GSH derivatives can be denoted as G–S–R, in which the S–R bond is susceptible to nucleophilic attack. Depending on the nature of the R group, the attack can be directed towards the sulfur atom or towards the R group itself. Along with the first three classes of compounds (i.e. sulfonium derivatives, thioesters and mixed disulfides), which showed only modest activity as GST inhibitors, a last group of miscellaneous compounds (Table 3, compound **20**; Table 4, compounds **21**, **22**) has been considered by the authors. The phenyl-thiosulfonate derivative of GSH **20** proved to be an efficient GST inhibitor. Compounds **21** and **22** will be discussed below (see Cys replacements).

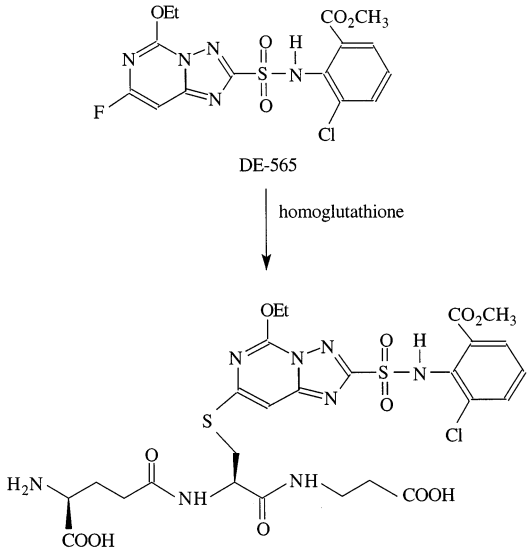
Synthesis and characterization of *S*-(*N*-aryl-*N*-hydroxycarbamoyl) glutathione derivatives have been described [68]; such compounds have proved to be powerful inhibitors of Glx I.

A novel field of opportunities in the therapy of cancer is offered by the design of latent alkylating agents activated by GST [69,70]. This approach is based on the observation that GST levels have frequently been shown to be elevated in many tumours, relative to surrounding healthy tissues. On this basis site-specific activation of the latent drugs to potently cytotoxic species, like phosphorodiamidate and urethane mustards, can be achieved. This drug activation approach (illustrated in Scheme 9, where the proton-abstracting enzyme moiety is a tyrosine hydroxyl group) may be extended to other disease-associated enzymes, providing a substantial advantage over prodrugs that are activated in a manner unrelated to the diseased tissue's properties.

GSH-conjugation with electrophiles represents a well established detoxication mechanism and several papers deal with glutathionyl-derivatives of xenobiotics utilized in agriculture as pesticides. In order to get more insights into relations between structure and toxicity of these xenobiotics, some glutathione *S*-conjugates of the general fungicide chlorothalonil (2,4,5,6-tetrachloro-1,3-dicyanobenzene) have been synthesized and their spectral properties have been determined [71]. A mixture of mono-, di- and tri-glutathionyl conjugates is obtained, whose composition depends on molar ratio of GSH in the substitution reaction. A further *S*-conju-



Scheme 9. GST-mediated cleavage mechanism of new alkylating agents.

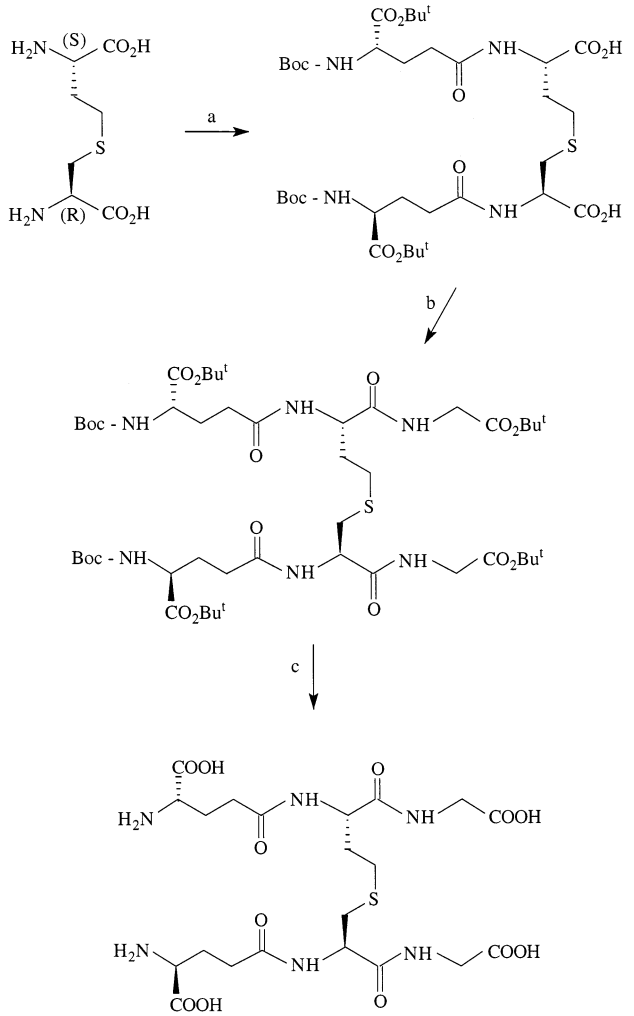


Scheme 10. Homoglutathione conjugate of sulfonamide pesticide DE-565.

gate, formed in leguminous plants, has been recently described [72]; in this case the scavenger biomolecule is the homoglutathione (γ-Glu–Cys–β-Ala) and the electrophilic species is the sulfonamide herbicide DE-565 (Scheme 10). Synthesis of this *S*-conjugate by direct coupling of protected thiol tripeptide with DE-565 failed, presumably due to the unreactive nature of the electrophile; the Authors circumvented the problem by reacting DE-565 with the appropriate dipeptide, followed by incorporation of the final glutamyl residue. This represents a useful method which can be applied to the synthesis of a variety of biologically significant conjugates of electrophiles.

In order to achieve specific enzyme inhibitors, other analogues containing an *S*-derivatizated Cys residue have been examined. The analogue of glutathione disulfide, *N*,*N*′-bis(γ-L-glutamyl)-L,L-cystathionine diglycine (GCSG), containing the L-cystathionine sulfide system $-CH_2-S-$ in place of the cystine scissile disulfide bridge, has been synthesized from L-cystathionine according to Scheme 11 [73]. GCSG was found to be a linear competitive inhibitor of GR. An interesting investigation of the GR bound conformation of this glutathione disulfide analogue has been undertaken with the aid of transferred nuclear Overhauser effect spectroscopy (TRNOESY) [73].

Glutathione trisulfide (GS_3G) was synthesized from glutathione disulfide and elemental sulfur [74]. It has been found that GS_3G is still recognized and reduced by GR. The reduction mechanism has been studied and the results compared with those obtained for the reduction of the selenotrisulfide derivative of GSH (GSSeSG) [75].

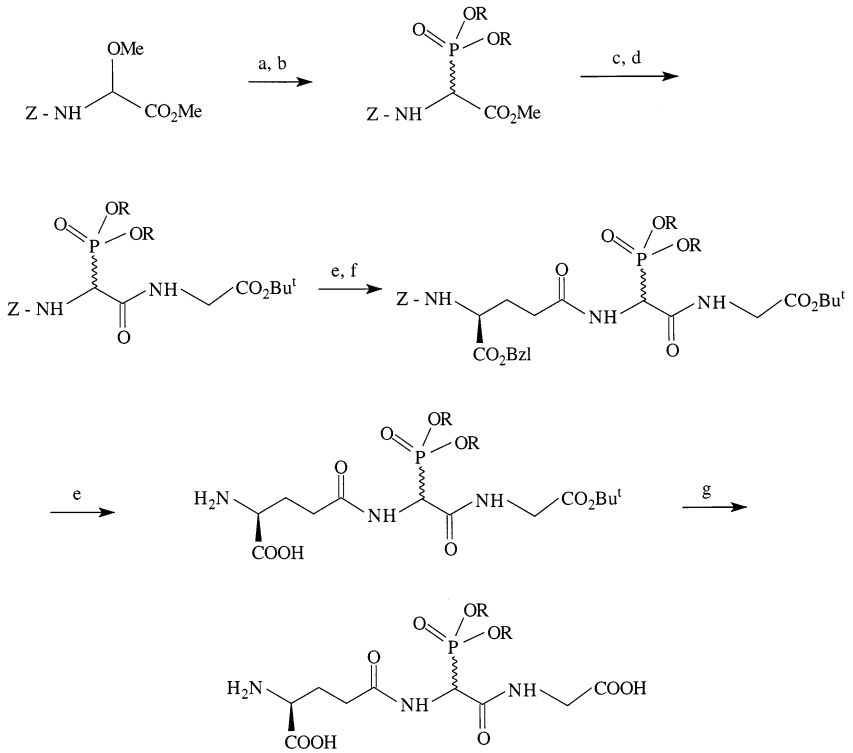


Scheme 11. Synthesis of the GSSG analogue containing the L-cystathionine sulfide system. (a) Boc–Glu(Suc)–OBu′, $NaHCO_3$–H_2O–THF, r.t., 7 h; (b) HCl · Gly–OBu′, DCC, HOBt, Et_3N, doxane, r.t., 26 h; (c) TFA, CH_2Cl_2, Et_3SiH, r.t., 7.5 h.

Currently there is considerable interest in the chemistry and biochemistry of *S*-nitrosothiols, since they are being examined as possible drugs to effect vasodilation and to reduce platelet aggregation. A comprehensive account, reviewing classes of nitric oxide (NO)-donors (including *S*-nitrosothiols) is now available [76]. *S*-Nitrosoglutathione (GSNO) (Fig. 5) is a solid compound which can be isolated and characterized; synthetic pathways to GSNO have been already reviewed [5]. The reactions governing formation and decomposition of *S*-nitrosothiols have been recently investigated [77,78]. In particular it has been suggested that the bioactivity of *S*-nitrosothiols (including GSNO) may be associated with both heterolytic and homolytic mechanisms of decomposition [78].

3.2.4. Cys replacement

In the context of the synthesis of potential GST inhibitors designed to react covalently at the active site



Scheme 12. Synthesis of phosphono-analogues of GSH. (a) PCl_3, 70°C, 18 h; (b) $P(OR)_3$, 70°C, 2 h; (c) 2 N NaOH, dioxane, 0°C; (d) Gly–OBu′, DCC, CH_3CN, 0°C, 1 h, then r.t., 14 h; (e) H_2, 10% Pd–C, MeOH, 3 atm., 20°C, 2–4 h; (f) Z–Glu–Obzl, DCC, CH_3CN, 0°C, 1 h, then r.t., 14 h; (g) 0.25 N HBr in AcOH, 4°C, 10–15 h.

Currently there is considerable interest in the chemistry and biochemistry of *S*-nitrosothiols, since they are being examined as possible drugs to effect vasodilation and to reduce platelet aggregation. A comprehensive account, reviewing classes of nitric oxide (NO)-donors (including *S*-nitrosothiols) is now available [76]. *S*-Nitrosoglutathione (GSNO) (Fig. 5) is a solid compound which can be isolated and characterized; synthetic pathways to GSNO have been already reviewed [5]. The reactions governing formation and decomposition of *S*-nitrosothiols have been recently investigated [77,78]. In particular it has been suggested that the bioactivity of *S*-nitrosothiols (including GSNO) may be associated with both heterolytic and homolytic mechanisms of decomposition [78].

3.2.4. Cys replacement

In the context of the synthesis of potential GST inhibitors designed to react covalently at the active site of the enzyme, Adang and co-workers [67] prepared two GSH analogues (Table 4, compounds **21** and **22**) containing β-Cl–Ala and 1-aminocyclopropane-1-carboxylic acid (Ac_3c) as Cys replacements, respectively. Each compound possesses an electrophilic centre of a different nature, a chloride and a cyclopropyl group, able to react with a nucleophilic centre in the catalytic site. It should be noted that cycloaliphatic achiral α-amino acids (Ac_nc) are the subject of current interest for conformational studies [79–81]. There are a few examples of GSH analogues incorporating in the central position ααAAs not related to Cys. The insertion of cycloleucine (Cle or Ac_5c) and of the simplest C^{α}-branched residue, i.e. the natural α-aminoisobutyric acid (Aib or αMeAla), has been previously examined [82].

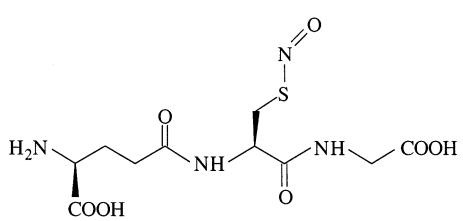
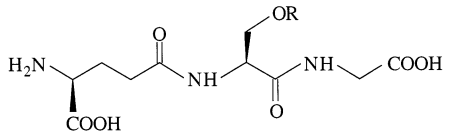


Fig. 5. *S*-Nitroso-glutathione (GSNO).

Synthesis of the oxygen analogue of GSH, γ-Glu–Ser–Gly (GOH) and of its *O*-acetyl, propionyl, butyryl and valeryl esters has been described [83] (Fig. 6). Oxygen esters are generally more stable to hydrolysis than thioesters; this fact may provide improved specificity for potent inhibitors of Glx II with respect to Glx I. A simple synthesis of GOH and of the desthio analogue of GSH, γ-Glu–Ala–Gly (GH), a natural product known as nor-ophthalmic acid, has been previously reported [84].

A recent report describes phosphono-analogues of GSH, containing the $O{=}P(OR)_2$ moiety in place of the Cys CH_2SH group (Scheme 12), designed in order to



GOH: R = H
GOH esters: R = CH_3CO, CH_3CH_2CO, $CH_3(CH_2)_2CO$, $(CH_3)_2CHCH_2CO$

Fig. 6. γ-Glu–Ser–Gly (GOH) and its esters.

obtain novel GST inhibitors [85]. The rationale behind this modification is the assumption that analogues containing the $O{=}P(OR)_2$ group would bind to the GSH-binding domain (G-site) in the enzyme through hydrogen bond formation. The inhibition tests clearly show that the formal substitution of the GSH thiol function by phosphonic acid esters leads to a new class of GST inhibitors.

3.2.5. S-Complexes

GSH can act as a polydentate ligand and has been frequently used as a model system for the binding of metal ions by larger peptides and proteins. Its deprotonated thiol group has high affinity for 'soft' metal ions resulting in the formation of GS–metal complexes which can play a major role in the mobilization and transport of metal ions across membranes. References and discussion on this point can be found in the recent paper of Sadler and co-workers [86], in which the authors report studies on the interactions of the antiulcer drug ranitidine bismuth citrate with GSH and formation of a $[Bi(GS)_3]$ complex by citrate displacement.

Table 5
Some glycyl-modified GSH analogues for γ-Glu–Cys–**X**

X	Ref.
–Val	[15]
–Asp	[15,88]
–PhGly	[15,66,88]
–D-PhGly	[66]
–Phe	[15]
–GABA	[15,88]
–Lys	[15]
–His	[15]
–Ala	[15,88]
–D-Ala	[15]
–β-Ala	[15,66,88]

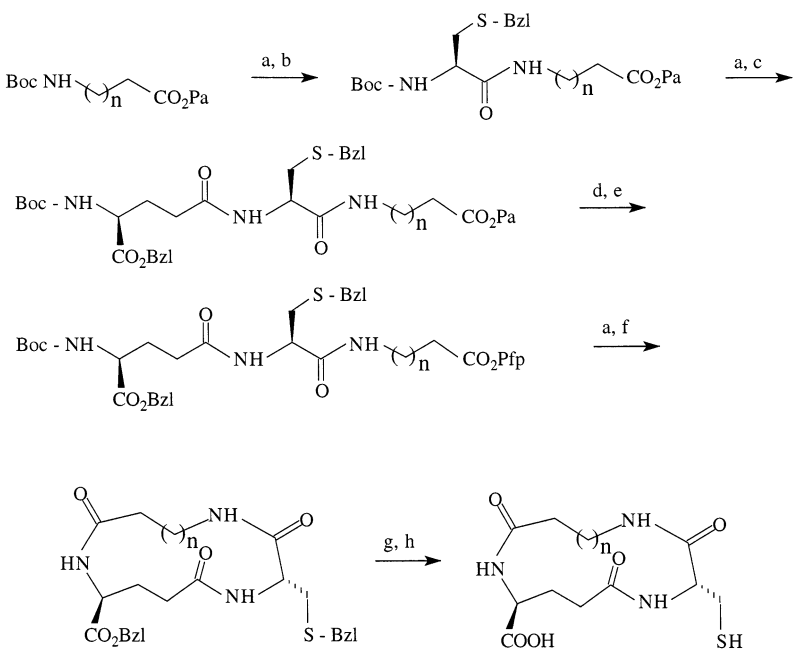
3.3. Modification at the Gly moiety

The new thiol γ-glutamyl–cysteinyl–serine has been isolated from graminaceous plants [87], where it seems to fulfill the same functions as does GSH in other plants.

A series of glutathione analogues in which glycine is replaced with different amino acids was synthesized to optimize chemical features responsible for GST isozyme specificity [15,66,88] (Table 5).

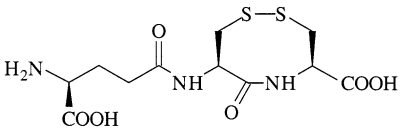
Derivatization of the Gly carboxyl group to esters [15] and amides [15,22–24] was also achieved.

The approach of substituting the carboxylic acid function of the C-terminal glycine with a phosphonic or a phosphinic group has been exploited. The GSH analogue containing aminomethanphosphonic acid (GlyP)



n = 1, 2 (OPa = phenacyl ester, OPfp = pentafluorophenyl ester)

Scheme 13. Synthesis of cyclo(-γ-Glu–Cys–β-Ala-) and cyclo(-γ-Glu–Cys–GABA-). (a) TFA, CH_2Cl_2; (b) Boc–Cys(Bzl)–OH, DCC/HOBt, DIEA, CH_2Cl_2; (c) Boc–Glu–Obzl, DCC/HOBt, DIEA, CH_2Cl_2; (d) Zn/AcOH; (e) C_6F_5OH, DCC, CH_2Cl_2; (f) dioxane–pyridine, EtOH, 85°C; (g) 0.5 N NaOH, MeOH–THF; (h) Na/NH_3.



23

Fig. 7. Glutathione analogue **23** incorporating the Cys–Cys dyad in the oxidized form.

in place of C-terminal glycine has been obtained by standard procedures; this modification can be found in the already cited report describing changes at the Glu moiety [28].

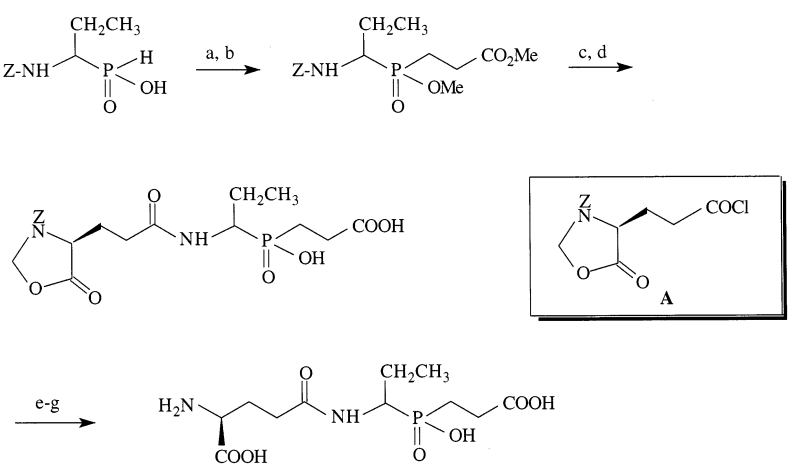
To improve GSH stability towards proteolysis and absorption through membranes, some useful strategies, such as the introduction of conformational constraint and the increase in lipophilicity, have been developed. Covalent cyclization introduces global constraint and eliminates at the same time peptide C- and N-termini; these are points of attack of exopeptidases and with their ionic charges contribute to reduce bioavailability. Thus, synthesis of cyclic analogues of GSH has been attempted with the objective of obtaining new stable GSH-like antitumour agents [89] (Scheme 13). Since cyclization of GSH molecule as such was found to be difficult, the Authors adopted the approach of replacing Gly with βAla or GABA in order to relieve the strain associated with a small ring. The two cyclic peptides obtained, i.e. cyclic homoglutathione cyclo(-γ-Glu–Cys–β-Ala-), and its homologue cyclo(-γ-Glu–Cys–GABA-), show only weak citotoxic activity. The potential conformational interest present in these cyclic tripeptide homologues in terms of ability to give intramolecular H-bond, have stimulated the Authors to undertake a conformational analysis in DMSO-d_6 solution.

Recently Lucente et al. [90] reported the synthesis of a novel glycyl-modified GSH analogue (Fig. 7, compound **23**) obtained through introduction of the cyclic cysteinyl–cysteine fragment in place of the native C-terminal dipeptide unit –Cys–Gly. This disulfide-bridge containing dipeptide introduces a 2-fold constraint which involves both the GSH backbone and the Cys side chain. The backbone conformation is altered as a consequence of the strong tendency of the endocyclic amide bond to adopt a *cis*-configuration [91], while the rotation of the Cys side chain is severely limited by the incorporation into an eight-membered ring. Conformational features of compound **23** are expected to influence binding at active sites; furthermore, the disulfide bridge could participate to redox equilibria which, in contrast with GSSG, may involve a monomeric form.

3.4. Modification at more than one site

Recently, Sheh and co-workers extended their investigation to another cyclic GSH analogue modified at both Cys and Gly sites [92]. This new analogue contains a lysine residue in the central positions and β-Ala as C-terminal amino acid; the aim was to gain information on the biochemical consequences of the introduction of a supplementary positive charge. This cyclic peptide has been examined for its ability to induce interleukin-1 production, which is an important factor for cell proliferation.

A potent inactivation of GSH synthetase has been recently realized by Oda et al. [93] with the synthesis of the phosphinic acid T.S. analogue **24**, which is illustrated in Scheme 14.



24

Scheme 14. Synthesis of the phosphinic acid transition state analogue of GSH **24**. (a) CH_2N_2, Et_2O; (b) NaOMe, methyl acrylate, MeOH, 0→25°C; (c) H_2, 10% Pd–C, MeOH; (d) **A**, HOBt, Net_3, CH_2Cl_2, 0→25°C; (e) NaOH, THF–H_2O (1:1), 25°C; (f) H_2, 10% Pd–C, MeOH–AcOH–H_2O; (g) Dowex 50W X-8 (H^+), eluted with H_2O.

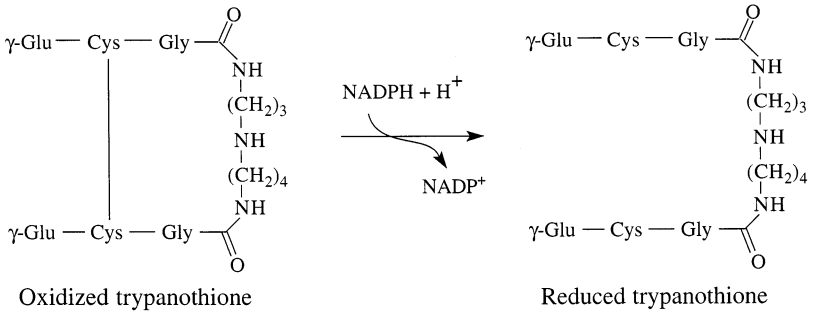


Fig. 8. Tripanothione [$T(SH)_2$].

The role of glutathione diethyl ester as delivery agent for both the monoester and GSH in human cells has been recently evidenced [94].

4. Glutathione-related natural thiols

Healthy ocular lenses contain the highest concentrations of GSH of any other mammalian tissue. A marked age-related decrease in GSH levels as well as depression of γ-Glu–Cys synthetase activity are believed to render the aged lens more susceptible to oxidative stress and, therefore, to cataractogenesis [95]. Prodrugs of γ-Glu–Cys (e.g. γ-Glu–Cys(Ac)–OEt) have been recently synthesized and proved to be effective in raising human lenticolar GSH concentration in vitro.

A natural substance, the tripeptide *S*-(1,2-dicarboxyethyl)glutathione (DCE-GS), was found to be present in considerable amounts in rat liver, heart and lens; its potential as anti-inflammatory and anti-anaphylaxis agent has been investigated [96].

In leguminous plants, GSH is partially or totally replaced by the tripeptide γ-Glu–Cys–β-Ala, which is called homoglutathione (see also Ref. [72]), while the thiol γ-Glu–Cys–Ser is found in graminaceous plants, as already mentioned [87].

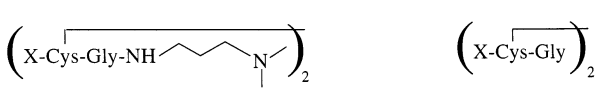
Several pathogenic protozoa of the *Trypanosoma* and *Leishmania* genera do not depend on the GSH for their thiol redox balance and antioxidant defense, but utilize a specific GSH derivative i.e. N^1,N^8-diglutathionylspermidine, named trypanothione (Fig. 8). The two biosynthetic steps leading from GSH to glutathionylspermidine (Gsp) and then to trypanothione [$T(SH)_2$] are catalysed by two pathogen specific ligases, named Gsp-synthetase and [$T(SH)_2$]-synthetase, respectively. Both Gsp and [$T(SH)_2$] are maintained in the reduced state by another specific enzyme which is the trypanothione reductase (TR) [97–99]. Since the trypanothione system is unique to the parasites, all these enzymes represent an obvious choice for antiprotozoal drug design [100]. Recently Douglas et al. [24] synthesized two groups of symmetrical disulfides as potential alternative substrates for $T(SH)_2$ and GSH reductase. In a group of compounds (Fig. 9, compounds **25** and **26**) the native spermidine was replaced by 3-dimethylaminopropylamine and the γ-Glu moiety by the nonpolar residues of Phe or Trp. The same modifications were applied by the Authors to the γ-Glu moiety of the GSSG, thus obtaining a second group of derivatives (Fig. 9, compounds **3** and **4**; see also Table 2).

By adopting the approach of phosphorylated peptides as TSA inhibitors of ATP-dependent ligases (see also the related GSH-synthetase inhibitors in Ref. [93]), Verbruggen and co-workers reported a series of phosphonic and phosphinic acid derivatives of GSH synthesized as potential inhibitors of glutathionylspermidine synthetase [101]. These compounds possess the general structure γ-Glu–Leu–XaaP or γ-Glu–Val–XaaP where XaaP represents an amino acid residue containing a phosphonic or phosphinic acid function. The most active derivative is γ-Glu–Leu–GlyP ($K_i = 60 \pm 9$ mM, linear non-competitive inhibitor) which may be useful as a lead compound for further more potent inhibitors.

Recently, Coward et al. reported the synthesis of the phosphapeptides **27–29**, mimics of the Gsp, which were evaluated for their activity as Gsp-synthetase/amidase inhibitors [102,103] (Fig. 10).

5. Conclusions

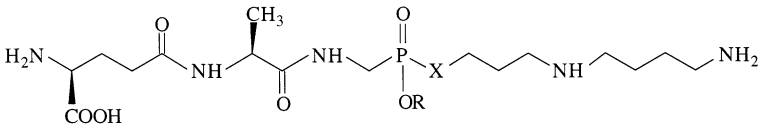
Due to the crucial role evidenced for sulfhydryl-containing compounds, particularly GSH, much attention



25 (X = Phe)
26 (X = Trp)

3 (X = Phe)
4 (X = Trp)

Fig. 9. $T(SH)_2$/GSH analogues.



27 X = O, R = H

28 X = NH, R = CH_3

29 X = CH_2, R = H

Fig. 10. Glutathionyl–spermidine analogues.

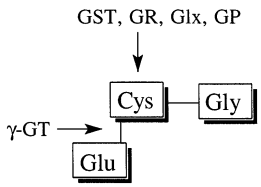


Fig. 11. GSH interaction sites with enzymes.

has been devoted to elucidation of structural and functional aspects of enzymes involved in GSH-related detoxifying pathways. It is not surprising that so many analogues and derivatives of such a small molecule as GSH have been designed, since this tripeptide presents several sensitive points which may be attacked by different enzymes (Fig. 11); thus, enzymatic processes may be selectively blocked or modulated through the structural modification of single moieties of the molecule. Without underestimating the relevance of the biochemical implications, the present overview has been focused on the chemical aspects of GSH modification. The structural modifications discussed may be usefully applied to the search for new GSH analogues.

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