3,4-Epoxyprecocenes as Models of Cytotoxic Epoxides: Synthesis of the Cis Adducts Occurring in the Glutathione Metabolic Pathway

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The adducts of 3,4-epoxyprecocene II (**1b**) with *N*-acetyl-L-cysteine, L-cysteine, and glutathione (GSH), in which a formal syn addition of the thiol reagent to the oxirane has taken place, with the sulfur atom linked to the benzylic position of the benzopyranyl skeleton of **1b**, have been synthesized. A stererochemical correlation between the two sets of diastereomeric cis adducts **3**, **5**, and **6** formed, i.e. the 3*R*,4*S* and 3*S*,4*R* series, has been established by spectroscopic, chromatographic, and enzymatic means. To assign the absolute configuration of these adducts, a synthesis of chiral **1b** (3*R*,4*R*), using the cysteinyl moiety itself as reagent for resolution of the precursor racemic bromohydrin **7**, has been developed. Regarding the influence of the reaction conditions on the stereochemical course of the oxirane cleavage, the addition of methyl esters of L-cysteine or its *N*-acetyl derivative to **1b** in neutral organic solvents led to the corresponding cis adducts **4** and **2**, respectively, with a high degree of stereoselectivity. On the other hand, in aqueous media the stereochemical outcome of the reaction of GSH with epoxide **1b** depends on the solvent polarity and pH. Thus, while above pH 13 the trans diastereomeric pair of adducts is almost quantitatively formed, a significant proportion of the corresponding cis conjugates is obtained within the pH range 8.5–10.5 (up to 24%). This result suggests that the cis conjugates could also exert a role in the detoxification mechanisms of highly reactive epoxides.

Introduction

Epoxidation of carbon double bonds by cytochrome P-450 is usually the first step in the metabolic detoxification of olefinic xenobiotics.[1] However, in some cases this process can lead to undesirable cytotoxic effects. For example, epoxides generated from polycyclic aromatic hydrocarbons (PAH)[2] and aflatoxins[3] are toxic intermediates. The ability to anticipate the potential cytotoxicity of bioactive epoxides is a subject of increasing importance in predictive toxicology.

Our interest in bioactive epoxides is derived from our work on precocenes.[4] These compounds are insect juvenile hormone antagonists with a 2*H*-1-benzopyranyl structure, which exert a cytotoxic effect on the *corpora allata*, the glands where these hormones are biosynthesized.[5] In addition, the cytotoxicity of precocenes presents a broader spectrum since it has also been reported that these compounds are hepatotoxic[6] and nephrotoxic.[7] Thus, 3,4-epoxyprecocenes, the intermediates postulated as the species responsible for this cytotoxic activity, can be envisaged as useful models of bioactive epoxides for general xenobiochemical studies.

These intermediates are interesting because of their extremely high chemical reactivity, as was evidenced from the attempts carried out for their preparation.[8,9] Since then, several studies on the reactivity of 3,4-epoxyprecocenes with oxygen nucleophiles have been reported.[9-11] From the results obtained in these studies, it has been concluded that these oxiranes undergo hydrolysis under physiological conditions at higher rates than those exhibited by epoxides derived from selected cytotoxic PAH, affording an approximate 1:2 cis–trans mixture of 3,4-diols. In this context, Jerina and co-workers[12] recently studied the chemical hydrolysis of 3,4-epoxyprecocene I (**1a**) and reported a change in the rate-determining step with varying pH and buffer concentrations. They also provided kinetic evidence for the existence of a carbocationic intermediate.

Precocenes
(I: $R^1 = OCH_3$; $R^2 = H$)
(II: $R^1 = R^2 = OCH_3$)

1a ($R^1 = OCH_3$; $R^2 = H$)
1b ($R^1 = R^2 = OCH_3$)

Our previous studies with epoxyprecocene II (**1b**) pointed to the possibility that nonoxygenated nucleophiles present in biological matrices, such as thiols, could also interact with this type of epoxide. Thus, the synergistic effect caused by diethyl maleate, a glutathione (GSH) depletor, on the biological activity of precocenes in immature stages of the bug *Oxycarenus lavaterae*[13] and the extensive depletion of GSH levels in adults of the cockroach *Blattella germanica* after topical administration of precocene II[14] suggested the intervention of GSH in the metabolism of these toxins. In fact, it is well known that, together with hydration, the *S*-glutathione transferase catalyzed conjugation of epoxides is an effective detoxification pathway.[15]

In this context we have recently reported the stereoselective preparation of trans adducts of 3,4-epoxyprecocene II (**1b**) with GSH, L-cysteine, and *N*-acetyl-L-cysteine, in which the sulfur atom is linked to the benzylic position of the benzopyranyl moiety. The unambiguous structural

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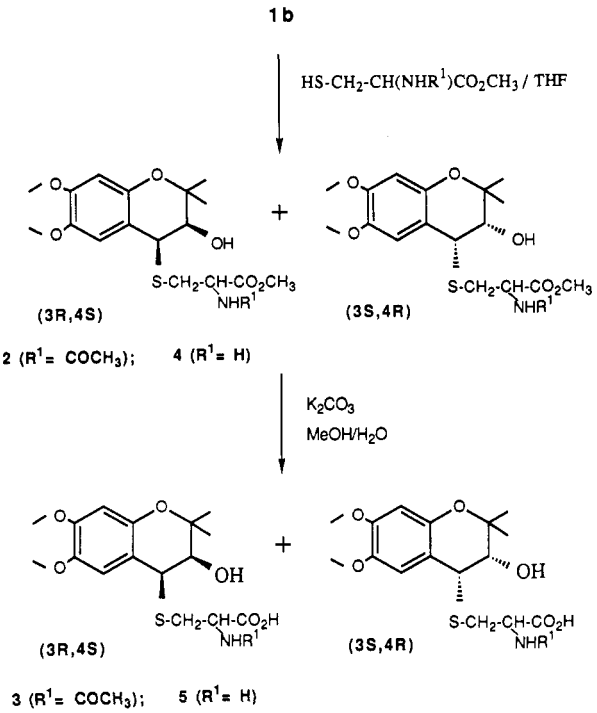
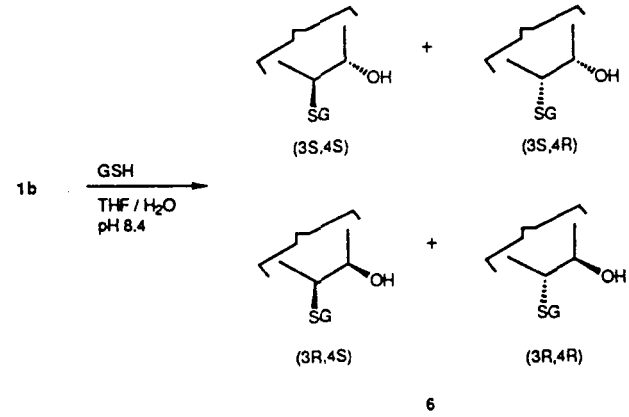
Scheme I



Scheme II



elucidation of both sets of possible diastereomeric adducts, i.e. the 3*S*,4*S* and the 3*R*,4*R* series, was carried out by X-ray diffraction analysis of the (3*S*,4*S*)-*N*-acetylcysteinyl adduct[16] and complementary stereochemical correlations using ^{1}H and ^{13}C NMR, HPLC, and enzymatic means.[17] Besides these studies, the only report to our knowledge on the reactivity of 3,4-epoxyprecocenes with thiol reagents is that of Aizawa et al.[18] on the isolation of trans adducts from the reaction of **1b** with thiophenol or L-cysteine methyl ester in neutral organic solvents.

In the present paper we report on the preparation and structural elucidation of the sulfur conjugates of epoxide **1b** related to the glutathione metabolic pathway in which a formal syn addition of the thiol reagent to the oxirane has taken place, with the sulfur atom linked to the benzylic position of the benzopyranyl skeleton. To accomplish the structural elucidation of the cis adducts, particularly to determine the absolute configuration of each diastereomer, a synthesis of chiral **1b** using the cysteinyl moiety itself as the resolution reagent, has been developed. This particular conjugation model has been studied in view of its potential relevance in the detoxification of bioactive epoxides, as a possible alternative to the known anti addition catalyzed by *S*-glutathione transferases that generally leads to the corresponding trans adducts.[19]

Results and Discussion

Preparation and Characterization of Cis *S*-Adducts (Scheme I). The reaction of L-cysteine and *N*-acetyl-L-cysteine with epoxide **1b** was carried out with the corresponding methyl ester derivatives to avoid the formation of side products derived from the presence of the free carboxylic acid group. Thus, the addition of *N*-acetyl-L-cysteine methyl ester to a tetrahydrofuran solution of **1b** led to a slow reaction with formation of a complex mixture of products, plausibly originated from a nonregioselective cleavage of the oxirane ring. Column chromatography of this crude reaction mixture afforded a major fraction (79%) containing two compounds in a 1:1 ratio (HPLC),[20] which after separation by semipreparative HPLC, were identified as the corresponding adducts **2** with a cis configuration at C3–C4.

The C–S connectivity at the benzylic position of the benzopyranyl moiety was inferred from comparison of the ^{13}C NMR absorptions of the adducts of the above thiol ester and [4-^{13}C]-3,4-epoxyprecocene II with those of previously reported for structurally related compounds.[17]

The assignment of cis configuration was based upon the comparison of the coupling constants of protons at C3 and C4 (4.1 Hz) with those of the corresponding trans isomers (9.7 Hz).[17] These new adducts eluted in HPLC at lower retention times than the corresponding diastereomeric trans pair, in agreement with the observed relative order of elution of cis–trans mixtures of diols from hydrolysis of 3,4-epoxyprecocenes.[12] All other spectroscopic and analytical features of the isolated adducts were also consistent with the proposed structures. Finally, separate hydrolyses of each adduct with potassium carbonate in methanol/water afforded the corresponding cis mercapturic acids **3**.[21]

The minor fraction from chromatography of the original reaction mixture contained the corresponding trans adducts (12%) and four additional compounds (9%), whose structures were tentatively assigned as adducts with the sulfur atom linked to C-3 (cis–trans mixture). However, the small amounts isolated and the instability of these adducts, probably because of the presence of a hydroxyl group linked to C-4, precluded their complete identification. In short, reaction of **1b** with *N*-acetyl-L-cysteine methyl ester in a neutral organic solvent afforded good yields of a 79:12 mixture of cis and trans adducts with the sulfur atom linked to C-4.[22]

Reaction of the L-cysteine methyl ester with epoxide **1b** carried out under the same above conditions led to an even slower transformation of the starting oxirane; however, the

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(20) Unless otherwise stated, reversed phase was used in all HPLC analyses.
(21) Cis adducts **3** are stable enough to phosphate buffer (pH 7.2, 2 h at 100 °C) or to 5% CCl_3CO_2H (24 h at 25 °C) (HPLC monitoring with internal standard); hence, application of these conventional treatments to biological samples are not going to alter the conjugates potentially present therein.
(22) Under these conditions, the formation of trans adducts was favored by addition of acid. Thus, when a 10 or 25% molar amount of *p*-toluenesulfonic acid was added to the reaction medium the cis:trans ratios obtained were 65:35 and 43:57, respectively.

overall conversion yield (87%) and both chemoselectivity (>90% of sulfur insertion at C-4) and stereoselectivity (>95% of cis adducts) were higher than in the case of the N-acetylated thiol reagent. Adducts derived from amino group attack on the epoxide were not detected.[23] Separation of the diastereomeric pair of cis 3*R*,4*S* and 3*S*,4*R* adducts **4** was easily accomplished by conventional chromatographic procedures. When compared with other similar conjugates of known structures (see above), both compounds exhibited ^{1}H NMR spectra and HPLC retention times in concordance with the assigned cis configuration. The basic hydrolysis of these compounds afforded the corresponding cis 3*R*,4*S* and 3*S*,4*R* cysteinyl adducts **5** as free acids.

Finally, reaction of epoxide **1b** with GSH was carried out by using the free, unprotected tripeptide in a tetrahydrofuran–water mixture at pH 8.4 (Scheme II). Although these conditions would favor an opening of the oxirane with predominance of the trans adducts, they were selected due to its experimental simplicity. Thus, the reaction gave a 29:71 cis–trans isomeric mixture of adducts **6** with an overall conversion yield of 92%. The trans conjugates were separated from the mixture by reversed-phase column chromatography and final isolation of the diastereomeric pair of cis 3*R*,4*S* and 3*S*,4*R* adducts **6** was performed by semipreparative HPLC with good recovery and high purity. Characterization of these conjugates was carried out by comparing their spectroscopic and analytical data with those of the corresponding trans adducts and related derivatives such as **3** and **5**.

Stereochemical Correlation between Series 3*R*,4*S* and 3*S*,4*R*. Once the separation and identification of the three pairs of cis adducts of epoxide **1b** related to the GSH metabolic pathway, i.e. 3*R*,4*S* and 3*S*,4*R* **3**, **5**, and **6** had been accomplished, the stereochemical correlation between the diastereomeric GSH pair and those derived from L-cysteine and *N*-acetyl-L-cysteine was carried out as we have previously described for the trans series[17] (Figure 1).

When the cis diastereomer **6** with the lower t_R in HPLC was incubated with γ-glutamyl transpeptidase (EC 2.3.2.2.), it was stereospecifically converted into a compound with a longer retention time. This intermediate, assumed to be the corresponding glycylcysteinyl adduct, was not isolated, and the incubation was pursued under the same reaction conditions to give the corresponding cysteinyl adduct **5**. This cleavage occurred at a slower rate than the previous one and was attributed to a nonspecific protease activity exhibited by the enzyme preparation used. The stereochemical correlation was completed by acetylation of the above isomer **5** to give the corresponding diastereoisomeric mercapturic acid **3**. Likewise, the exclusive formation of the other cis diastereoisomer **3** was detected when the GSH adduct **6** eluting later was subjected to the same process.

From the results of these correlations a striking difference between the cis and trans series of adducts became apparent. Thus, whereas the trans group of diastereomers with the same configuration, i.e. 3*R*,4*R* or 3*S*,4*S*, always eluted in the same order in HPLC, in agreement with the chromatographic behavior observed for GSH conjugates of other related xenobiotics so far studied,[24] there was an elution inversion in the cis series. As shown in Figure 1, the cis GSH adduct **6** eluting first in HPLC was correlated

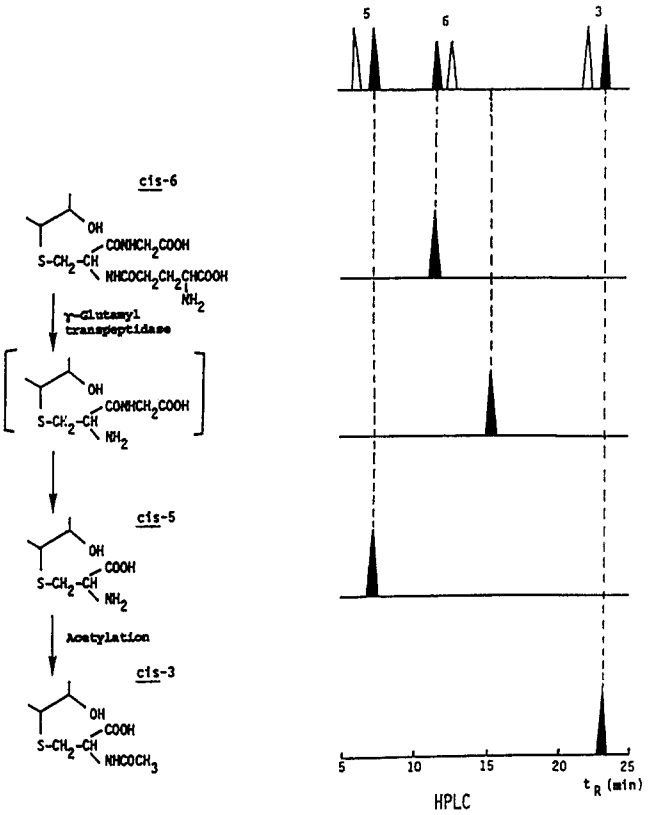

Figure 1. Stereochemical correlations of the different cis conjugates derived from the reaction of 3,4-epoxyprecocene II with glutathione, L-cysteine and *N*-acetyl-L-cysteine. The cis glutathione **6** with the lower t_R in HPLC was enzymatically hydrolyzed to the corresponding cis cysteinyl adduct **5**, with the higher t_R, which in turn was acetylated to give the cis mercapturic acid adduct **3** with the higher t_R whose absolute stereochemistry is known. The HPLC profile representation was obtained with a Hypersil ODS column (10 × 0.46 cm, 5 μ), eluting at 1.8 mL/min with 20% (v/v) H_2O–CH_3CN–THF (3:1:1 v/v) in 0.075 M formic acid buffered with triethylamine, pH3.1.

with the corresponding cysteinyl and *N*-acetylcysteinyl derivatives eluting in second place. In the absence of a bibliography on cis GSH conjugates derived from other bioactive epoxides, we cannot confirm whether this behavior is exceptional or not, but in any case it cautions against the general use of HPLC order of elution for establishing stereochemical correlations for this particular class of compounds.

Determination of the Absolute Configuration: Synthesis of Chiral Epoxide 1b. Unfortunately, in this case it was not possible to obtain appropriate crystals from any diastereomer of cis adducts **3**, **5**, or **6** for X-ray diffraction analysis.[17] Therefore, it was necessary to evaluate alternate methods for determining the absolute configuration of the compounds belonging to the cis series.

In this respect, all efforts to achieve a correlation with the trans series by specific inversion of the hydroxyl group at C3 of cis adducts using the Mitsunobo approach[25] were unsuccessful. Thus, neither diastereomer of **3** or **5** reacted at all, probably for steric reasons, as we previously observed in similar attempts performed with dimeric structures derived from **1b**.[26] Likewise, assays carried out to induce a specific epimerization of the C-3 hydroxyl group on any of the mercapturic acid derivatives **3** (cis or trans) in acid medium were also unfruitful, since a mixture of all four

(23) However, it has been observed that the addition through the amino group is possible under certain reaction conditions (Conchillo, A.; Camps, F.; Messeguer, A., unpublished results).

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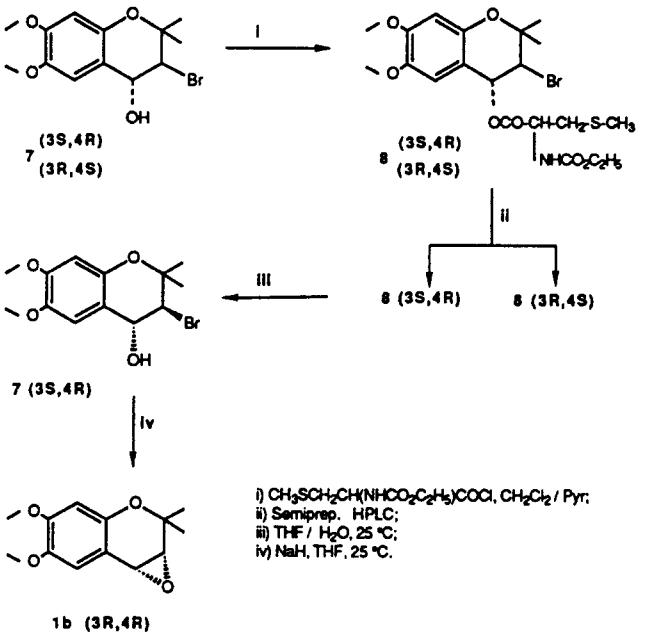
(26) Camps, F.; Coll, J.; Conchillo, A.; Messeguer, A. *Tetrahedron* **1985**, *41*, 5169–5174.

Scheme III



diastereomers was invariably obtained (HPLC monitoring), indicating that a concomitant epimerization at C4 had also taken place under these reaction conditions.

Therefore, we decided to synthesize a chiral epoxide **1b** of known configuration as the starting material for the preparation of the cis adducts. For this purpose, owing to the reactivity of precocenes and their corresponding epoxides, the resolution of an appropriate synthetic precursor of the oxirane, namely the bromohydrin,[9] was the method of choice. In this context, R. C. Jennings has reported the resolution of diols derived from precocene I through the formation of diastereomeric esters by using (−)-menthoxyacetic acid (MAA) as the chiral agent.[27] Likewise, Halpin et al. prepared the same enantiomeric diols by resolution of the corresponding bromohydrin with (+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) and hydrolysis of the isolated esters.[28] In our case, since we were planning to use the trans bromohydrin derived from precocene II as the starting material for preparation of the chiral epoxide, it was important not only to form the diastereomeric derivatives in good yields and with a good chromatographic resolution, but also to cleave the resolved compounds under mild enough conditions to prevent undesirable transformations, such as epimerization or dehydration. According to our previous experience, the lability of the hydroxyl group when located at the benzylic position facilitates these side reactions.

Unexpectedly, direct esterification of the bromohydrin **7** with MAA in the presence of *N,N'*-dicyclohexylcarbodiimide and (*N,N*-dimethylamino)pyridine led primarily to the formation of the 3-bromochromene derivative. Alternatively, reaction of **7** with the acid chloride of MAA in pyridine afforded the corresponding diastereomeric esters; however, these compounds could not be satisfactorily resolved by either chromatographic (TLC, HPLC) or fractional crystallization methods. Similar results were found when esters derived from MTPA were assayed.

Finally, we turned our attention to L-cysteine. Our guess was that this molecule, conveniently protected, could be an appropriate chiral resolution agent since it had quite

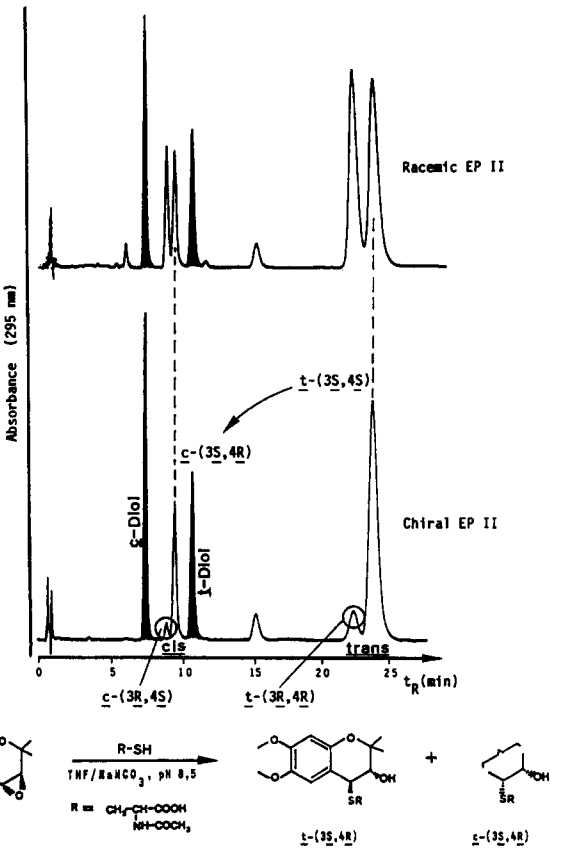


Figure 2. HPLC profiles of the adducts derived from reaction of *N*-acetyl-L-cysteine with racemic 3,4-epoxyprecocenene II (EP II, upper trace), and chiral EP II (lower trace), at pH 8.5. The chromatography was carried out by using a Hypersil ODS column (6 × 0.46 cm, 3 μ), eluting at 1.1 mL/min with 20% (v/v) H_2O–CH_3CN–THF (3:1:1 v/v) in 0.075 M formic acid buffered with triethylamine, pH 3.5.

different substituents on the asymmetric carbon atom and this atom would also be in proximity to that which would esterify the bromohydrin. After several preliminary attempts, reaction of **7** with the acid chloride of the *S*-methyl-*N*-(ethoxycarbonyl)-L-cysteine afforded the diastereomeric esters **8** in good yield (Scheme III).

These compounds could be separated by carefully controlled normal-phase semipreparative HPLC to give both diastereomers with lower and higher t_R, in 97% and 99% purity, respectively. These esters, although stable enough to be handled with minimum losses, exhibited sufficient lability to undergo hydrolysis to the bromohydrins in very mild conditions (1:1 tetrahydrofuran–water mixture at 25 °C).

Reaction of the enantiomeric bromohydrin **7**, with the highest t_R in normal-phase HPLC, in the presence of sodium hydride,[9] afforded the corresponding chiral epoxide **1b**. The absolute configuration of this epoxide and its enantiomeric purity were inferred from the stereoselective opening with *N*-acetyl-L-cysteine in basic medium[17] to give a trans mercapturic acid as major adduct. This conjugate was identified by HPLC as the 3*S*,4*S* isomer, which was contaminated by 7% of the corresponding 3*R*,4*R* diastereomer. Hence, the stereochemistry of the parent chiral epoxide had to be the 3*R*,4*R* and the 93:7 ratio of the sulfur adducts should reflect the enantiomeric purity of the synthesized epoxide.

Finally, as shown in Figure 2 for the reaction with *N*-acetyl-L-cysteine, HPLC monitoring of 3*R*,4*R* epoxide **1b** opening with sulfur nucleophiles under conditions which favor cis–trans mixtures (in sodium bicarbonate solution

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at pH 8.4), allowed the determination of the absolute configuration of the cis adducts, given that the configurations of the respective trans pairs were already known. Accordingly, the cis GSH adduct **6** eluting first in HPLC turned out to be the 3*S*,4*R* isomer and, as we have shown above, was stereochemically related to the corresponding second peaks of the conjugates derived from L-cysteine (**5**) and *N*-acetyl-L-cysteine (**3**), in agreement with the observed inversion in the elution order.

By this procedure, assignment of the absolute configurations for all possible conjugates of 3,4-epoxyprecocene II related to the GSH metabolic pathway was completed. In this context, the optimization of HPLC conditions for separation of the cis-trans diols and all the above conjugates in a single chromatogram would allow the unambiguous identification of these compounds in biological matrices, as we have recently shown with the cis and trans GSH adducts, in the incubation of epoxide **1b** with rat liver cytosolic fraction in the presence of exogenous GSH.[29]

Reaction of Epoxide 1b with Sulfur Nucleophiles at Different pH. From the results so far described it can be concluded that the reaction of epoxide **1b** with GSH related compounds in neutral organic media leads to predominant formation of the cis conjugates by slow opening of the oxirane ring, suggesting that under these conditions the attack of the sulfhydryl group takes place from the same side of the oxirane ring (syn addition). On the other hand, opening of **1b** in strong basic medium (0.1 N sodium hydroxide) gives stereoselectively the trans adducts in a few minutes;[17] in this case, the occurrence of a S_N2 type mechanism would rationalize the predominance of products arising from anti addition.

Consequently, it seemed interesting to study the influence of pH on the reaction of these thiols with epoxide **1b**, with a particular emphasis on the relative competition between oxygen and sulfur toward addition to the oxirane and also on the cis:trans ratio of the sulfur adducts formed. For this purpose, GSH was chosen as the model thiol, and the reactions were carried out in a 9:1 water–tetrahydrofuran mixture at the appropriate pH values. Phosphate, bicarbonate, or borate salts were used for the adjustment of pH. In this sense, pH variations due to acidity of GSH or addition of tetrahydrofuran to the reaction medium were systematically corrected. After completion, the reactions were monitored by HPLC and special care was taken to ensure that the corresponding amounts of sulfur adducts did not arise, even partially, from an attack of thiols on the 3,4-diol mixture concomitantly formed in these experiments.

Figure 3 shows the results from the reaction of **1b** with GSH within the pH range 6.5–13. As it can be seen, in the 8.5–10.5 pH interval the highest relative amount of cis GSH adducts (up to 24%) was obtained, and this percentage remained constant within these pH values. Conversely, under these conditions the relative proportions of trans GSH adducts followed a positive slope whereas that of 3,4-diols was negative. These results suggested that the increase in trans GSH conjugates was produced at the expense of the diol mixture and not of the corresponding cis adducts. A plausible explanation for this fact could be that the pH variation from 8.5 to 10.5 would cause a concomitant increase in the concentration of thiolate anions, which could compete advantageously with the aqueous nucleophilic reaction medium. However, this increase would not be strong enough to make an S_N2 mechanism predominant in the thiol addition thus favoring

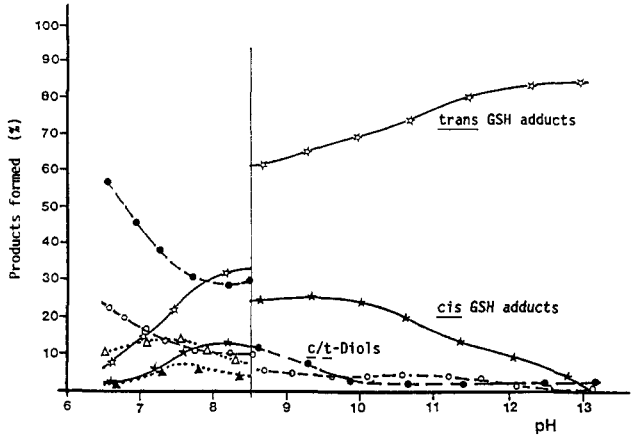

Figure 3. Effect of pH on the product distribution upon reaction of 3,4-epoxyprecocene II with glutathione (for details see the Experimental Section). Percentages of the different compounds were calculated from the corresponding peak areas on HPLC. The chromatographic conditions were: Hypersil ODS column (10 × 0.46 cm, 5 μ), eluting at 1.8 mL/min with 20% (v/v) H_2O–CH_3CN–THF (3:1:1 v/v) in 0.075 M formic acid buffered with triethylamine, pH 5.0. *trans*-3,4-Diols (●), *cis*-3,4-diols (○), trans glutathione adducts (☆), cis glutatione adducts (★), compounds tentatively identified as phosphate adducts, cis (▲) and trans (△).

the formation of the trans GSH adducts. In any case, these results are interesting, since it has been proposed that the *S*-glutathione transferase catalytic effect could be accounted for by the enzyme assisted creation of microenvironments in which the concentration of GSH in its thiolate form is increased.[30]

Within the 6.5–7.8 pH range (phosphate buffers) there was a clear predominance for the formation of the 3,4-diols over that of the sulfur adducts, probably due to a lower concentration of thiolate anions under these conditions. Then, from 7.8 to 8.5 pH values the relative proportions of sulfur adducts and 3,4-diols change and become more similar to those obtained with the bicarbonate buffered solutions (see above). Incidentally, assays in phosphate buffers led to the HPLC detection of two additional compounds, which according to their polarity and hydrolytic reactivity could be phosphate adducts also derived from epoxide **1b**. In fact, this type of intermediate had been tentatively reported by Hamnett et al.[10] in a study of the hydrolysis of epoxide **1a** in aqueous organic solvents. In this context, these authors suggested that this easy phosphorylation of the precocene epoxides could also be related to the macromolecular alkylation pathway which produces the observed cytotoxic effects.

The HPLC monitoring of the epoxide **1b** opening by reaction with GSH in the presence of borate solutions at pH 9.5 and 10.1 revealed the formation of the corresponding GSH adducts and 3,4-diols in less than 1% conversion yields. In addition, no methoxyhydrins were detected in a separate HPLC analysis, replacing our usual elution solvent mixture by methanol–water, which according to our previous experience was an unequivocal sign of absence of unreacted epoxide in the crude reaction mixture. However, analysis of this reaction mixture after 30 min showed the same minor amount of GSH adducts, but now the 3,4-diols abundance (90:10 cis:trans ratio) corresponded to an almost quantitative conversion of the starting epoxide. This result could be rationalized by assuming that once again the buffering species had interacted with the substrate, forming in this case an in-

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termediate borate which underwent a further easy hydrolytic cleavage in the reaction medium to give a high cis proportion of the 3,4-diol mixture.

In conclusion, it appears that the reaction of thiols with 3,4-epoxyprecocene II depends on the solvent polarity and pH. Several mechanisms could play an important role according to the conditions used. Although there is still a lack of accurate kinetic studies on this subject, it could be presumed that a combination of S_N1 and S_N2 mechanisms operate in the reactions carried out in aqueous media with strong pH dependency. Thus, while a S_N1 or a participation of both mechanisms would explain adducts ratios obtained below pH 10–11 values, over pH 13 it seems that S_N2 is the prevalent reaction pathway. The fact that within a wide pH range the opening of epoxide **1b** gives rise to significant amounts of GSH conjugates with a cis configuration offers an interesting perspective in xenobiochemical studies related to bioactive epoxides, which is of value because of the potential importance of this type of reactivity in biological matrices. Furthermore, the great stereoselectivity observed for a syn addition of GSH related thiols to epoxide **1b** when the reactions were carried out in neutral organic solvents, although with a biological significance that remains unexplored, also raised interesting questions from the mechanistic point of view, which are now under investigation in our laboratory.

Experimental Section

General Methods. Melting points (uncorrected) were determined with a Koffler apparatus. Elemental analyses were obtained with a Carlo Erba Model 1106 instrument. IR were performed with a Perkin-Elmer 399B apparatus. ^1H and ^{13}C NMR spectra were recorded on a Bruker WP-80 SY spectrometer in $CDCl_3$ solutions unless otherwise stated. Chemical shifts are reported in ppm downfield from internal $SiMe_4$. Apparent coupling constants (J) are given in hertz. The following descriptions are used: br = broad, ca = complex absorption, d = doublet, m = multiplet, q = quartet, s = singlet, t = triplet. FAB mass spectra were obtained on a VG-ZAB HF instrument and with an MS-9 VG updated spectrometer equipped with the VG 11/250 data system, using in both cases xenon as bombarding gas at 8 kV energy.

Thin-layer chromatography (TLC) analyses were performed on Merck silica gel 60 F-254 plates (0.25 mm, precoated on aluminium). HPLC analyses were carried out with a Waters modular system equipped with a U6 K injector, two Model 510 pumps, an automated gradient controller, and a Model 481 UV detector set at 295 nm. Columns and chromatographic conditions are indicated where appropriate.

Preparation of L-Cysteine Derivatives. *N*-Acetyl-L-cysteine Methyl Ester. $SOCl_2$ (1.0 mL, 13.5 mmol) was carefully added to a solution of *N*-acetyl-L-cysteine (2.0 g, 12.3 mmol) in MeOH (50 mL), and the mixture was stirred for 90 min at 25 °C. The residue obtained after elimination of solvent under vacuum was extracted with AcOEt (3 × 40 mL), and combined organic extracts were washed with brine and dried over $MgSO_4$. The new residue obtained after solvent elimination crystallized on standing to give the expected ester (1.7 g, 74% yield): mp 79–81 °C (AcOEt) (lit.[31] mp 79–80 °C); ^1H NMR δ 1.35 (t, 1 H, J = 9 Hz, SH), 2.07 (s, 3 H, CH_3CO), 2.8–3.3 (ca, 2 H, CH_2), 3.80 (s, 3 H, CO_2CH_3), 4.7–5.0 (ca, 1 H, CH), 6.40 (br, 1 H, NH).

***N*-(Ethoxycarbonyl)-*S*-methyl-L-cysteine.** This compound was prepared from *S*-methyl-L-cysteine (0.5 g, 3.7 mmol), 1 M Na_2CO_3 solution (10 mL), and ethyl chloroformate (0.48 g, 4.4 mmol), following the procedure reported by Cupps and co-workers for the case of methionine.[32] The compound was purified by bulb-to-bulb distillation (135–40 °C/0.15 Torr, 0.65 g, 85% yield): IR (film) ν_{max} 3330, 2984, 2923, 1478, 1426, 1340, 1244, 1061, 872, 779 cm^{-1}; ^1H NMR δ 1.26 (t, 3 H, J = 7.1 Hz, CH_3), 2.15 (s, 3 H, SCH_3), 2.99 (d, 2 H, J = 5.4 Hz, SCH_2), 4.15 (q, 2 H, J = 7.1 Hz, CH_2), 4.6 (ca, 1 H, CH), 5.6 (br, 1 H, NH), 9.87 (s, 1 H, CO_2H).

Anal. Calcd for $C_7H_{13}NO_4S$: C, 40.57; H, 6.32; N, 6.76; S, 15.47. Found: C, 40.65; H, 6.41; N, 6.69; S, 15.39.

Oxalyl chloride (0.14 mL, 1.6 mmol) and DMF (5 μL) were added to a solution of *N*-(ethoxycarbonyl)-*S*-methyl-L-cysteine (0.30 g, 1,4 mmol) in dichloromethane (5 mL), maintained under N_2 at 0 °C, and the crude reaction mixture was stirred for 1 h at 25 °C. After solvent removal under vacuum, the corresponding *N*-(ethoxycarbonyl)-*S*-methyl-L-cysteinyl chloride was obtained as a yellowish oil (0.30 g, 92% yield): IR (film) ν_{max} 3325, 2985, 2920, 2360, 1790, 1700, 1530, 1425, 1245, 1060, 945, 780 cm^{-1}.

***cis*-3,4-Dihydro-4-[*S*-(*N*-acetyl-*O*-methylcysteinyl)]-3-hydroxy-6,7-dimethoxy-2,2-dimethylbenzo-1*H*-pyran (2).** A solution of *N*-acetyl-L-cysteine methyl ester (0.30 g, 1.7 mmol) in THF (10 mL) was added to a solution of epoxide **1b** (0.20 g, 0.85 mmol) in the same solvent, and the mixture was stirred in the dark until TLC monitoring showed the absence of 3,4-diols (i.e. total conversion of starting **1**, 3 days). The residue obtained after solvent removal was purified by column chromatography through silica gel (30:25:4 hexane–AcOEt–iPrOH) to give two fractions (53 and 204 mg, respectively, 70% overall yield). An aliquot (40 mg) of the first fraction was purified by semipreparative HPLC (Spherisorb ODS-2, 10 μ, 30 × 0.78 cm, 40:60 MeOH–H_2O, 3 mL/min) to yield, after solvent elimination and further liophylization, four fractions (10, 5, 5, and 6 mg, respectively). The first one was identified as a mixture of trans adducts **2** (3*R*,4*R*) and (3*S*,4*S*),[17] while the others were tentatively identified as a cis–trans mixture of conjugates with the thiol moiety linked to C3. These compounds were unstable, and their structures could not be confirmed. C3 adducts: HPLC t_R 39.3 min; ^1H NMR δ 1.20 (s, 3 H, CH_3), 1.51 (s, 3 H, CH_3), 2.06 (s, 3 H, CH_3CO), 2.8–3.2 (ca, 2 H, CH_2), 3.7–4.0 (ca, 11 H, CH_3O, CO_2CH_3, CH-4, CH-3), 4.8–5.2 (ca, 1 H, CH), 6.15 (d, 1 H, J = 7 Hz, NHCO), 6.36 (s, 1 H, ArH), 6.97 (s, 1 H, ArH); HPLC t_R 43.5 min; ^1H NMR δ 1.20 (s, 3 H, CH_3), 1.52 (s, 3 H, CH_3), 2.06 (s, 3 H, CH_3CO), 3.21 (d, 2 H, J = 6 Hz, CH_2), 3.8–4.0 (ca, 11 H, CH_3O, CO_2CH_3, CH-4, CH-3), 4.7–5.0 (ca, 1 H, CH), 6.30 (br, 1 H, NHCO), 6.36 (s, 1 H, ArH), 6.96 (s, 1 H, ArH); HPLC t_R 45.4 min (2 isomers); ^1H NMR δ 1.27 (s, 3 H, 2 CH_3), 1.51 (s, 3 H, 2 CH_3), 2.04 (s, 3 H, CH_3CO), 2.06 (s, 3 H, CH_3CO), 3.2–3.5 (ca, 4 H, 2 CH_2), 3.7–3.9 (ca, 22 H, 4 CH_3O, 2 CO_2CH_3, CH-3, CH-4), 6.40 (br, 2 H, NHCO), 6.40 (s, 2 H, 2 ArH), 6.96 (s, 1 H, ArH), 6.97 (s, 1 H, ArH).

HPLC analysis of the second fraction (Nova-Pak C-18, 15 × 0.39 cm, 5 μ, 35:65 MeOH–H_2O, 1.6 mL/min) showed the presence of the expected cis diastereomeric adducts **2** (3*R*,4*S*) and (3*S*,4*R*) in a 1:1 ratio: IR (CCl_4) ν_{max} 3420, 3000, 2930, 1745, 1685, 1515, 1440, 1200, 1145, 1015 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 22.5 and 23.5 (CH_3), 24.5 and 25.5 (SCH_2), 33.06 (CH_3CO), 33.95 (CH_3CO), 47.20 (CH-4), 47.86 (CH-4), 52.54 (CHNH), 52.74 (CHNH), 55.83 (CH_3O), 56.67 (CH_3O), 69.37 (CH-3), 69.80 (CH-3), 76.85 (C-2), 76.95 (C-2), 101.41 (C-8), 108.49 (C-5), 108.83 (C-5), 112.54 (C-4a), 143.56 (C-6), 147.09 (C-7), 147.20 (C-8a), 170.19 (CONH), 171.14 (COO).

Anal. Calcd for $C_{19}H_{27}NO_7S$: C, 55.19; H, 6.58; N, 3.39; S, 7.71. Found: C, 55.34; H, 6.83; N, 2.93; S, 7.91.

An aliquot part (40 mg) of this mixture was purified by semipreparative HPLC (same column as above, 45:55 MeOH–H_2O, 1.5 mL/min, 3 mg per injection) to yield **2** (3*R*,4*S*) [(14 mg, HPLC t_R 29.7 min, 99% diastereomeric purity): mp 66–8 °C; ^1H NMR δ 1.23 (s, 3 H, CH_3), 1.51 (s, 3 H, CH_3), 2.06 (s, 3 H, CH_3CO), 2.95 (br, 1 H, OH), 2.8–3.5 (ca, 2 H, SCH_2), 3.65 (d, 1 H, J = 4.1 Hz, CH-3), 3.8–3.9 (2 s, 9 H, 2 CH_3O, CO_2CH_3), 4.27 (d, 1 H, J = 4.1 Hz, CH-4), 4.90 (m, 1 H, CHNH), 6.39 (s, br, 2 H, ArH, NHCO), 7.01 (s, 1 H, ArH)] and **2** (3*S*,4*R* [(11 mg, HPLC t_R 31.8 min, 100% diastereomeric purity): mp 70–73 °C; ^1H NMR δ 1.25 (s, 3 H, CH_3), 1.48 (s, 3 H, CH_3), 2.07 (s, 3 H, CH_3CO), 2.90 (br, 1 H, OH), 2.9–3.4 (ca, 2 H, SCH_2), 3.65 (d, 1 H, J = 4.1 Hz, CH-3), 3.8–3.9 (2 s, 9 H, 2 CH_3O, CO_2CH_3), 4.22 (d, 1 H, J = 4.1 Hz, CH-4), 4.95 (m, 1 H, CHNH), 6.38 (s, 1 H, ArH), 6.70 (d, 1 H, J = 8 Hz, NHCO), 7.00 (s, 1 H, ArH); HPLC/MS[29] m/z 414 (M + H)$^+$, 431 (M + NH4)$^+$, 237 (M − cysteinyl fragment)$^+$.

***cis*-[4-^{13}C]-3,4-Dihydro-4-[*S*-(*N*-acetyl-*O*-methylcysteinyl)]-3-hydroxy-6,7-dimethoxy-2,2-dimethylbenzo-1*H*-pyran.** *N*-acetyl-L-cysteine methyl ester (0.15 g, 0.8 mmol) and

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p-toluenesulfonic acid (18 mg, 0.1 mmol) were added to a solution of [4-^{13}C]-3,4-epoxyprecocene II[17] (0.10 g, 0.4 mmol) in THF (12 mL), and the mixture was allowed to react in the darkness for 24 h at 25 °C. The residue obtained after solvent removal was purified by preparative TLC (silica gel, two elutions with 30:25:4 hexane–AcOEt–iPrOH) to give a fraction (33 mg, R_f 0.20) containing the diastereomeric pair of cis adducts **2**: ^{13}C NMR δ 48.12 and 47.43 (SCH-4). The corresponding pair of trans adducts (63 mg, R_f 0.10) was also isolated. ^{13}C NMR δ 49.84 and 49.04 (SCH-4). All structures were confirmed by HPLC comparison with their respective nonlabeled compounds.

***cis*-3,4-Dihydro-4-[*S*-(*N*-acetylcysteinyl)]-3-hydroxy-6,7-dimethoxy-2,2-dimethylbenzo-1*H*-pyran (3).** A solution of the corresponding adduct **2** (10 mg) in MeOH (1 mL) was treated with a 0.2 M K_2CO_3 aqueous solution. When the reaction was completed (30 min at 25 °C, TLC monitoring), the crude reaction mixture was acidified with 0.1 N HCl (8 mL) and extracted with AcOEt (2 × 10 mL), and the combined organic extracts were washed with brine and dried over $MgSO_4$. The new residue obtained after solvent elimination afforded the respective mercapturic acid **3**.

3 (3*R*,4*S*): 7 mg; ^{1}H NMR δ 1.26 (s, 3 H, CH_3), 1.49 (s, 3 H, CH_3), 2.09 (s, 3 H, CH_3CO), 3.09 (d, 2 H, J = 5.1 Hz, SCH_2), 3.82 (s, 3 H, CH_3O), 3.85 (s, 3 H, CH_3O), 3.7–4.0 (ca, 1 H, CH-3), 4.30 (d, 1 H, J = 4.1 Hz, CH-4), 4.7–5.0 (ca, 1 H, CHNH), 6.40 (2 H, ArH, NHCO), 7.02 (s, 1 H, ArH).

3 (3*S*,4*R*): 6 mg; ^{1}H NMR δ 1.27 (s, 3 H, CH_3), 1.49 (s, 3 H, CH_3), 2.11 (s, 3 H, CH_3CO), 2.8–3.4 (ca, 2 H, SCH_2), 3.71 (d, 1 H, J = 4.1 Hz, CH-3), 3.83 (s, 3 H, CH_3O), 3.85 (s, 3 H, CH_3O), 4.27 (d, 1 H, J = 4.1 Hz, CH-4), 4.7–5.0 (ca, 1 H, CHNH), 6.40 (s, 1 H, ArH), 7.06 (s, 1 H, ArH), 7.29 (d, 1 H, J = 6.7 Hz, NHCO). MS (m/z, both isomers) 399 $(M)^+$, 237 (M – cysteinyl fragment)$^+$.

The structures of these compounds were confirmed by comparison with the acetylation products of the stereochemically related cis L-cysteine adducts **5** (see below).

***cis*-3,4-Dihydro-4-[*S*-(*O*-methylcysteinyl)]-3-hydroxy-6,7-dimethoxy-2,2-dimethylbenzo-1*H*-pyran (4).** A solution of L-cysteine methyl ester (0.23 g, 1.7 mmol) in THF (5 mL) was added to a solution of epoxide **1b** (0.20 g, 0.85 mmol) in the same solvent (10 mL), and the mixture was stirred in the dark until TLC monitoring showed the absence of 3,4-diols (i.e. total conversion of starting **1**, 4 days). The residue obtained after solvent removal was acidified (0.5 N HCl, 15 mL) and extracted with AcOEt (2 × 15 mL) to remove neutral compounds. Then, the aqueous fraction was neutralized with 2 N NaOH and extracted with AcOEt (2 × 15 mL); the combined organic extracts were washed with $NaHCO_3$ saturated solution and brine and dried over $MgSO_4$. The new residue obtained after solvent removal was redissolved in $CHCl_3$ to give, on standing, a crystalline compound (120 mg) which was identified as one cis diastereomer. The rest of the crude was purified by preparative TLC (4:0.1 $CHCl_3$–MeOH), affording 20 mg of the above compound (R_f 0.25) and 135 mg of the second diastereomer (R_f 0.40).

4 (3*R*,4*S*): mp 138–9 °C; IR (KBr) ν_{max} 3360, 3300, 2970, 1730, 1620, 1510, 1440, 1400, 1240, 1200, 1140, 1120, 1100, 1020, 980, 870, 830, 765 cm^{-1}; ^{1}H NMR δ 1.25 (s, 3 H, CH_3), 1.49 (s, 3 H, CH_3), 2.65 (br, 3 H, OH, NH_2), 2.8–3.1 (dd, 2 H, J_1 = 8 Hz, J_2 = 4 Hz, SCH_2), 3.6–3.9 (11 H, CH-3, $CHNH_2$, CO_2CH_3, CH_3O), 4.26 (d, 1 H, J = 4 Hz, CH-4), 6.40 (s, 1 H, ArH), 7.07 (s, 1 H, ArH).

4 (3*S*,4*R*): mp 132–3 °C; IR (KBr) ν_{max} 3460, 3400, 2980, 1730, 1615, 1510, 1440, 1400, 1235, 1200, 1140, 1120, 1095, 1020, 870, 830, 765 cm^{-1}; ^{1}H NMR δ 1.24 (s, 3 H, CH_3), 1.49 (s, 3 H, CH_3), 2.70 (br, 3 H, OH, NH_2), 2.8–3.2 (ca, 2 H, SCH_2), 3.6–3.9 (11 H, CH-3, $CHNH_2$, CO_2CH_3, CH_3O), 4.21 (d, 1 H, J = 4 Hz, CH-4), 6.41 (s, 1 H, ArH),7.09 (s, 1 H, ArH); HPLC/MS analysis (both isomers)[29] m/z 372 $(M + H)^+$, 413 $(M + H + CH_3CN)^+$, 237 (M – cysteinyl fragment)$^+$.

Anal. Calcd for $C_{17}H_{25}NO_6S$: C, 54.97; H, 6.78; N, 3.77. Found: C, 54.77; H, 6.78; N, 3.61.

***cis*-3,4-Dihydro-4-(*S*-cysteinyl)-3-hydroxy-6,7-dimethoxy-2,2-dimethylbenzo-1*H*-pyran (5).** A solution of the corresponding adduct **4** (15 mg) in MeOH (1 mL) was treated with a 0.2 M K_2CO_3 aqueous solution, and the reaction was completed after 30 min at 25 °C (TLC monitoring). The residue obtained after solvent removal was redissolved in H_2O (0.5 mL) and passed through a Sep-Pak C-18 cartridge and washed with H_2O (5 mL). The free acids were eluted with MeOH (5 mL).

5 (3*R*,4*S*): mp 187–9 °C dec; IR (KBr) ν_{max} 3600–2600 (br), 3420, 1635, 1510, 1405, 1200, 1150, 1010 cm^{-1}; ^{1}H NMR (D_2O) δ 1.14 (s, 3 H, CH_3), 1.37 (s, 3 H, CH_3), 2.0–2.4 (ca, 2 H, SCH_2), 3.72 (s, 3 H, CH_3O), 3.75 (s, 3 H, CH_3O), 3.80 (d, 1 H, J = 3.9 Hz, CH-3), 4.00 (ca, 1 H, CHNH), 4.42 (d, 1 H, J = 4 Hz, CH-4), 6.40 (s, 1 H, ArH), 7.13 (s, 1 H, ArH).

5 (3*S*,4*R*): mp 173–5 °C dec; IR (KBr) ν_{max} 3600–2600 (br), 1620, 1510, 1405, 1200, 1140, 1125, 1010 cm^{-1}; ^{H} NMR (D_2O) δ 1.22 (s, 3 H, CH_3), 1.43 (s, 3 H, CH_3), 2.0–2.5 (ca, 2 H, SCH_2), 3.79 (s, 3 H, CH_3O), 3.82 (s, 3 H, CH_3O), 3.88 (d, 1 H, J = 4 Hz, CH-3), 4.00 (ca, 1 H, CHNH), 4.31 (d, 1 H, J = 3.9 Hz, CH-4), 6.67 (s, 1 H, ArH), 7.21 (s, 1 H, ArH); HPLC/MS analysis (both isomers)[29] m/z 358 $(M + H)^+$, 399 $(M + H + CH_3CN)^+$, 314 $(M + H - CO_2)^+$, 237 (M – cysteinyl fragment)$^+$.

A 0.1 mL solution of **5** (3*R*,4*S*) (1 mg in 10 mL 1:1 THF–H_2O) maintained at 5 °C was treated with 50% NaOAc solution (0.1 mL) and Ac_2O (3 μL), and the mixture was allowed to react for 15 min at 5 °C. The crude reaction mixture was diluted with MeOH (0.2 mL) and analyzed by HPLC (Hypersil ODS 6 × 0.46 cm, 3 μ, 80:20 0.075 M HCO_2H–Et_3N buffer (pH 3.1):(3:1:1 H_2O–CH_3CN–THF), 1.1 mL/min) for establishing the correlation with the corresponding mercapturic acid **3** (3*R*,4*S*) (both compounds exhibiting t_R 3.0 and 8.9 min, respectively). The same procedure was applied on the second diastereomer **5** (3*S*,4*R*) to give the corresponding cis adduct **3** (3*S*,4*R*) (t_R 3.2 and 9.4 min, respectively). Isolation of pure mercapturic acids **3** was carried out by performing the above reaction in greater scale, followed by purification of the crude reaction mixture through Sep-Pak cartridges as described above for the L-cysteine adducts.

***cis*-3,4-Dihydro-4-(*S*-glutathionyl)-3-hydroxy-6,7-dimethoxy-2,2-dimethylbenzo-1*H*-pyran (6).** A solution of GSH (0.52 g, 1.7 mmol) at pH 8.4 was prepared by adjusting 50 mL of a 0.05 M $NaHCO_3$ solution to pH 11 with 0.1 N NaOH (total volume 70 mL), followed by addition of the thiol. Then, a solution of epoxide **1b** (0.2 g, 0.85 mmol) in THF (20 mL) was added to the GSH solution, and the mixture was stirred for 30 min at 25 °C (final pH 8.8) to give cis adducts **6** (27%), trans adducts **6** (65%), *cis*-3,4-diol (3%), and *trans*-3,4-diol (5%) (HPLC under the conditions described above for acids **5**, total conversion yield from **1**, 92%). The crude reaction mixture was concentrated to 20 mL under vacuum and extracted with AcOEt (3 × 20 mL) to remove the diols. Then, a fraction containing 270 mg of the adducts mixture was dissolved in 0.1 M HCO_2H–Et_3N buffer, pH 3.1, and subjected to flash chromatography through C-18 reversed phase (55–105 μ), eluting with a gradient of the above buffer and the standard organic modifier (3:1:1 H_2O–CH_3CN–THF) from 95:5 to 70:30. By this procedure 60 mg of the pure cis adducts mixture **6** was isolated. Finally, the diastereomeric pair of cis adducts was separated by semipreparative HPLC (Spherisorb ODS-2, 15 × 1 cm, 5 μ, eluting with a 85:15 mixture of pH 3.1 buffer and the same organic modifier, 4 mL/min), to give **6** (3*S*,4*R*) (21 mg, t_R 23.2 min, 100% purity by HPLC) and **6** (3*R*,4*S*) (23 mg, t_R 24.4 min, 97% purity by HPLC).

6 (3*S*,4*R*): mp 155–8 °C; IR (KBr) ν_{max} 3640–2800 (br), 2920, 1650, 1510, 1405, 1200, 1140, 1080, 1005 cm^{-1}; ^{1}H NMR (D_2O) δ 1.21 (s, 3 H, CH_3), 1.42 (s, 3 H, CH_3), 2.14 (ca, 2 H, CH_2-Glu), 2.54 (ca, 2 H, CH_2-Glu), 3.1 (ca, 2 H, SCH_2), 3.78 (s, 3 H, CH_3O), 3.80 (s, 3 H, CH_3O), 3.7–3.9 (2 H, CH-3, $CHNH_2$), 3.93 (s, 2 H, CH_2-Gly), 4.31 (d, 1 H, J = 3.8 Hz, CH-4), 4.68 (m, 1 H, CHNH-Cys), 6.45 (s, 1 H, ArH), 7.18 (s, 1 H, ArH); FAB-MS m/z 544 (M + 1, positive)$^+$, 237 (M – GSH residue, positive)$^+$, 542 (M – 1, negative)$^-$, 306 (M – benzopyranyl residue)$^-$.

6 (3*R*,4*S*): mp 160–5 °C; IR (KBr) ν_{max} 3640–2800 (br), 2920, 1650, 1510, 1405, 1200, 1140, 1080, 1005 cm^{-1}; ^{1}H NMR (D_2O) δ 1.23 (s, 3 H, CH_3), 1.45 (s, 3 H, CH_3), 2.15 (ca, 2 H, CH_2-Glu), 2.55 (ca, 2 H, CH_2-Glu), 2.9–3.3 (ca, 2 H, SCH_2), 3.81 (s, 3 H, 2 CH_3O), 3.7–3.8 (br, 1 H, $CHNH_2$), 3.87 (d, 1 H, J = 3.8 Hz, CH-3), 3.94 (s, 2 H, CH_2-Gly), 4.31 (d, 1 H, J = 3.8 Hz, CH-4), 4.71 (m, 1 H, CHNH-Cys), 6.47 (s, 1 H, ArH), 7.14 (s, 1 H, ArH); FAB-MS m/z 544 $(M^+ + 1$, positive)$^+$, 237 (M – GSH residue, positive)$^+$, 542 (M – 1, negative)$^-$, 306 (M – benzopyranyl residue)$^-$.

Stereochemical Correlation of Cis Adducts 3, 5, and 6. The correlation was carried out according to the procedure described for the corresponding trans adducts.[17] Thus, a solution of γ-glutamyl transpeptidase (0.5 mg, 7.65 units, Sigma) in 0.5 mL of

a 40 mM Tris-HCl buffer at pH 8.0 containing $MgCl_2$ was added to a solution of the corresponding cis GSH adduct **6** (0.5 mg, 0.9 μmol) in the same buffer (0.5 mL), which had been warmed up for 30 min at 37 °C. The final concentration of the GSH adduct was 0.9 mM. The progress of successive degradations was followed by HPLC (Hypersil ODS-2, 5 μ, 80:20 mixture of buffer at pH 3.0 and standard organic modifier, 1.1 mL/min, see above). When all the starting adduct had been converted into the corresponding L-cysteine adduct **5**, an aliquot fraction of the crude reaction mixture (100 μL containing 0.09 μmol of **5**) was evaporated under N_2 and treated with Ac_2O (3 μL) in 50% NaOAc (1 mL) for 10 min at 5 °C. In both cases, HPLC analyses showed the formation of the corresponding mercapturic acid derivative **3** in almost quantitative yield. The stereochemical correlation obtained is depicted in Figure 1.

3,4-Dihydro-3-bromo-4-[*O*-(*N*-(ethoxycarbonyl)-*S*-methylcysteinyl)]-6,7-dimethoxy-2,2-dimethylbenzo-1*H*-pyran (8). *N*-(Ethoxycarbonyl)-*S*-methyl-L-cysteinyl chloride (80 mg, 0.36 mmol) was added to a solution of bromohydrin **7** (75 mg, 0,24 mmol)[9] in CH_2Cl_2 (3 mL) containing 0.1 mL of pyridine. The mixture was stirred at 25 °C until the reaction was completed (15 h, TLC monitoring). Then the crude reaction mixture was diluted with CH_2Cl_2 and washed with 0.5 N HCl, $NaHCO_3$ saturated solution and brine and dried over $MgSO_4$. The residue obtained after solvent removal was purified by filtration through silica gel, affording the diastereomeric mixture of esters **8** (90 mg). This mixture was separated by semipreparative direct-phase HPLC (Porasil, 7.8 × 3 cm, 10 μ, 34:66 THF (containing 0.5% iPrOH)–hexane, 3 mL/min).

8 (t_R 11.7 min, 40 mg, 97% HPLC purity): IR (KBr) ν_{max} 3360, 2925, 1720, 1620, 1510, 1260, 1200, 1130, 1055 cm^{-1}; ^1H NMR δ 1.28 (t, 3 H, J = 8 Hz, CH_3), 1.49 (s, 3 H, CH_3), 1.57 (s, 3 H, CH_3), 2.14 (s, 3 H, SCH_3), 3.0–3.1 (ca, 2 H, SCH_2), 3.82 (s, 3 H, CH_3O), 3.84 (s, 3 H, CH_3O), 4.18 (q, 2 H, J = 8 Hz, CH_2), 4.26 (d, 1 H, J = 8 Hz, CHBr), 4.70 (ca, 1 H, CHNH), 5.56 (br, 1 H, NH), 6.32 (d, 1 H, J = 8 Hz, CH-4), 6.39 (s, 1 H, ArH), 6.68 (s, 1 H, ArH).

8 (t_R 10.6 min, 20 mg, 99% HPLC purity): IR (KBr) ν_{max} 3410, 1750, 1720, 1620, 1510, 1200, 1055 cm^{-1}; ^1H NMR δ 1.27 (t, 3 H, J = 8 Hz, CH_3), 1.49 (s, 3 H, CH_3), 1.58 (s, 3 H, CH_3), 2.14 (s, 3 H, SCH_3), 2.9–3.2 (ca, 2 H, SCH_2), 3.84 (s, 3 H, CH_3O), 3.86 (s, 3 H, CH_3O), 4.18 (q, 2 H, J = 8 Hz, CH_2), 4.28 (d, 1 H, J = 8 Hz, CHBr), 4.80 (ca, 1 H, CHNH), 5.52 (br, 1 H, NH), 6.30 (d, 1 H, J = 8 Hz, CH-4), 6.39 (s, 1 H, ArH), 6.69 (s, 1 H, ArH); MS (*m/z*, both isomers) 507 and 505 $(M)^+$, 301 and 299 $(M - \text{ester moiety})^+$, 220.

Anal. Calcd for $C_{20}H_{28}BrNO_7S$: C, 47.43; H, 5,58; N, 2.76. Found: C, 47.50; H, 5.71; N, 2.61.

(3*R*,4*R*)-3,4-Epoxy-6,7-dimethoxy-2,2-dimethylbenzo-1*H*-pyran (1b). A solution of ester **8** (highest t_R, 20 mg) in 1:1 THF–H_2O (1 mL) was allowed to stand at 25 °C. HPLC monitoring (under the same conditions as above but using a Porasil analytical column, 1.5 mL/min and external standard) showed the almost quantitative conversion of the starting diastereomeric ester into the corresponding bromohydrin **7** (24 h). After removal of THF under N_2, the crude reaction mixture was diluted with H_2O (4 mL) and extracted with ether (2 × 5 mL). The combined organic fractions were washed with $NaHCO_3$ saturated solution and brine and dried over $MgSO_4$ to give, after solvent removal, the expected bromohydrin (12 mg), identified by comparison with the racemic sample.

A solution of the enantiomeric bromohydrin (12 mg, 0.04 mmol) in anhydrous THF (0.5 mL) was added dropwise, under N_2, to a suspension of NaH (1.8 mg, 0.08 mmol) in the same solvent (0.5 mL), and the mixture was stirred for 15 h at 25 °C.[17] GLC monitoring of the crude reaction mixture (3% OV-101, 220 °C, 35 mL/min) showed the complete conversion of **7** into the corresponding epoxide **1b** (concentration was estimated by ^1H NMR to be 8.9 mg/mL). The absolute configuration of the epoxide and its enantiomeric purity (93%) were inferred from its stereoselective opening with *N*-acetyl-L-cysteine in strong basic medium (see Results and Discussion).

Reaction of 1b (3*R*,4*R*) with L-Cysteine, *N*-Acetyl-L-cysteine, and GSH: Cis–Trans Correlation and Absolute Stereochemistry of Cis Adducts 3, 5, and 6. General Procedure. A solution of the above chiral epoxide **1b** (10 μL) was added to a solution of the corresponding thiol reagent (18 μL, 45 mM in 1:4 THF–buffer (0.05 M $NaHCO_3$, pH 10.5) diluted with 100 μL of the same buffer. For control purposes, a parallel reaction was carried out in each case with racemic epoxide **1b**. The correaltion was performed by HPLC analysis of the corresponding crude reaction mixtures (Hypersil ODS-2, 6 × 0.4 cm, 3 μ, 80:20 0.075 M HCO_2H-Et_3N buffer pH 3.1; standard organic modifier, 1.1 mL/min) and results obtained for *N*-acetyl-L-cysteine are depicted in Figure 2.

Reactions of 1b with GSH at Different pH. GSH (35 mg) was dissolved in a mixture of THF (0.5 mL) and the appropriate phosphate, bicarbonate, or borate buffer (2.5 mL), and an aliquot (0.8 mL) was diluted with more buffer (4 mL), so that concentration in GSH was 6.3 mM. Finally, a THF amount corresponding to that containing the epoxide **1b** (0.35 mL) was added to the above solution, and pH was measured to obtain the corrected values. Under these conditions, KH_2PO_4, Na_2HPO_4, $NaHCO_3$, or $Na_2B_4O_7$ solutions gave pH ranges comprised between 4.0–7.9, 7.9–12.2, 8.5–11.7, or 9.5–10.2, respectively. For obtaining pH values over 12, the appropriate sodium hydroxide solution was used.

A solution of epoxide **1b** (7.5 μL, 42 mM) in THF was added to 100 μL of GSH solution in the appropriate pH. When the reaction was completed, the crude reaction mixture was analyzed by HPLC (Hypersil ODS-2, 10 × 0.46 cm, 5 μ, 80:20 0.075 M HCO_2H-Et_3N buffer pH 5.0; standard organic modifier, 1.8 mL/min). The results obtained are depicted in Figure 3.

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Registry No. (±)-**1b**, 124605-61-4; (3*R*,4*R*)-**1b**, 124649-77-0; [4-^{13}C]-(±)-**16**, 124605-63-6; (3*R*,4*R*)-**2**, 124605-62-5; (3*S*,4*S*)-**2**, 124649-68-9; (3*R*,4*S*)-**2**, 124649-69-0; (3*S*,4*R*)-**2**, 124649-70-3; [4-^{13}C]-(3*R*,4*R*)-**2**, 124605-64-7; [4-^{13}C]-(3*S*,4*S*)-**2**, 124649-71-4; [4-^{13}C]-(3*R*,4*S*)-**2**, 124649-72-5; [4-^{13}C]-(3*S*,4*R*)-**2**, 124649-73-6; (3*R*,4*S*)-**3**, 119433-44-2; (3*S*,4*R*)-**3**, 119433-40-8; (3*R*,4*S*)-**4**, 124649-74-7; (3*S*,4*R*)-**4**, 124649-75-8; (3*R*,4*S*)-**5**, 119433-43-1; (3*S*,4*R*)-**5**, 119433-39-5; (3*S*,4*S*)-**6**, 119434-29-6; (3*S*,4*R*)-**6**, 119341-98-9; (3*R*,4*S*)-**6**, 119433-42-0; (3*R*,4*R*)-**6**, 119433-41-9; *trans*-(±)-**7**, 124605-65-8; (3*S*,4*R*)-**7**, 124649-76-9; (3*S*,4*R*)-**8**, 124751-27-5; (3*R*,4*S*)-**8**, 124605-66-9; GSH, 70-18-8; Ac-Cys-OH, 616-91-1; Ac-Cys-OMe, 7652-46-2; H-Cys(Me)-OH, 1187-84-4; ClCOOEt, 541-41-3; EtOCO-Cys(Me)-OH, 4026-53-3; EtOCO-Cys(Me)-Cl, 124605-60-3; H-Cys-OMe, 2485-62-3; H-Cys-OH, 52-90-4.