(Z)-Oxopropene-1,3-diyl, a Linker for the Conjugation of the 
Thiol Group of Cysteine with Amino-Derivatized Drugs

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<td>Vilarrasa, Jaume; Universitat de Barcelona, Organic Chemistry Section Petit, Elena; Universitat de Barcelona, Organic Chemistry Section Bosch, Lluis; Universitat de Barcelona, Organic Chemistry Section Costa, Anna; Universitat de Barcelona, Organic Chemistry Section</td>
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**Supporting Information**

**ABSTRACT:** The handicaps of the maleimides, the most commonly used reagents to link thiol groups (of Cys) to drugs, fluorescent labels, etc., prompted us to revise and develop an alternative, based on the known thia-Michael addition to activated triple bonds, which could be useful under physiological conditions. A drug with an amino group was converted into its propynamide and, in aqueous media at 37 °C and pH 7.4, Cys derivatives were added, to afford rapidly the Z adduct. This chemical linker (the oxopropene-1,3-diyl group) showed advantages, in terms of following the reaction course: no diastereomers were formed; excellent selectivity (S⁻ vs. NH₂, even at pH 10.0 and 12.0), without secondary reactions; no exchange with other thiols; and no incorporation of deuterium at the linker when the samples were dissolved in D₂O to register their NMR spectra.

Conventional cancer treatments that use cytotoxic compounds cause serious side effects due to their lack of selectivity for tumor cells. As known, a very promising strategy to avoid or alleviate these drawbacks is to conjugate cytotoxic drugs with antibodies (targeted therapy). The preparation of antibody–drug conjugates (ADCs) requires efficient and reliable linkage protocols. Most ADCs currently on the market (4 are currently FDA-approved) and in clinical development use the Michael addition reaction of a native or genetically-engineered Cys in the antibody to a maleimide group to form the conjugate. This transformation is widely used because the reaction is quantitative and fast under physiological conditions; however, the adducts may undergo thiol exchange in vivo causing off-site cytotoxicity and compromising the efficacy of the conjugate. Other Cys conjugations have been reported (Scheme 1), including the addition to electron-deficient alkynes.

Our long-standing interest in the conjugate addition of several nucleophiles to activated triple bonds has recently led us to examine the possibility of going a step further: whether, under real physiological conditions, these reagents—specifically, propynamides—will or will not be useful to bind or link (as a linchpin or link pin) thiols and amines (Scheme 2). In other words, from our own work and from that of our colleagues Arjona and Plumet, we were aware that HC≡C–EWG compounds with strong EWGs such as SO₂R and COOR are capable of reacting at rt even with very weak nucleophiles, in the presence of suitable catalysts. We did not know, however, if the reactions of Cys with HC≡C–CONHR would be sufficiently quick, complete, and stereoselective in aqueous solvents, without any additive, or if the...
addition products (adducts) would be stable enough in physiological media and in the presence of other thiols. The carboxamido groups (CONHR and CONR₂), although weaker EWGs, are much more resistant to chemical hydrolysis than ester groups, and so could function as a suitable point of attachment for any drug "decorated" with amino groups. In this Note we report our results in this direction.

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**Scheme 1. Summary of Reactions for the Modification or Protection of Cysteine Units (Thiol Groups). EWG = Electron-Withdrawing Group**

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**Scheme 2. Reactions and Starting Materials Used as Models**

The acylation of a primary or secondary amine with commercially available reagents such as propynoic acid (also known as propiolic or propargylic acid) and 3-trimethylsilylpropynoic acid (followed by desilylation), in the presence of a coupling agent (for example, dicyclohexylcarbodiimide, DCC, or any of its analogs) are well-known reactions. In the direct reaction, variable yields are usually reported because the product may undergo, either in situ or during the workup, the addition of the amine and/or any nucleophile present. With the C-silylated derivative, two steps are necessary—coupling in the presence of any dehydrating agent, followed by smooth, well-known desilylation protocols—but both yields are excellent (>90%) and the process may be carried out in one pot. We
will not comment further on the standard preparations of the starting propynamides (Scheme 2), since this Note is tightly focused on their reactions with thiols in aqueous media.

Reactions of a protected cysteine (1), cysteine itself (Cys, 2) and a cysteine-containing pseudo-tripeptide (the natural antioxidant glutathione, 3) with N-substituted propynamide a and N,N-disubstituted propynamide b were examined in water or in a mixture of water and a miscible organic solvent. "Water" means here a phosphate-buffered aqueous solution of pH 7.4. A summary is shown in Table 1. Although the reactions in buffered water were complete, the use of \( ^1\text{BuOH} - \text{H}_2\text{O} \), EtOH - H\(_2\)O, or THF - H\(_2\)O was also studied due to the poor solubility of a and b in buffered water. For the linkage of two water-soluble biomolecules, the addition of an alcohol or THF will be unnecessary.

![Diagram](image)

**Table 1. Screening the Reaction Conditions for the Addition of the Thiol Group of Cysteines to Propynamides a and b**

<table>
<thead>
<tr>
<th>entry</th>
<th>thiol</th>
<th>alkyne</th>
<th>solvent(s)</th>
<th>pH</th>
<th>T (°C)</th>
<th>t (min)</th>
<th>yield (%)</th>
<th>Z/E</th>
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<tr>
<td>1</td>
<td>1</td>
<td>a</td>
<td>H(_2)O or (^1\text{BuOH} - \text{H}_2\text{O})</td>
<td>6.0</td>
<td>rt</td>
<td>1,000</td>
<td>1a, 5</td>
<td>50:50</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>a</td>
<td>H(_2)O</td>
<td>7.4</td>
<td>rt</td>
<td>70</td>
<td>1a, 93</td>
<td>98:2</td>
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<td>a</td>
<td>H(_2)O</td>
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<td>37</td>
<td>60</td>
<td>1a, 94</td>
<td>98:2</td>
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<td>a</td>
<td>EtOH - H(_2)O, 1:1</td>
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<td>50</td>
<td>1b, 98</td>
<td>93:7</td>
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<td>&gt;99:1</td>
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<td>2a, 92</td>
<td>85:15</td>
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<td>95:5</td>
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<td>&gt;99:1</td>
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<td>10.0</td>
<td>37</td>
<td>40</td>
<td>3b, 93</td>
<td>95:5</td>
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\(^{a}\)Thiols 1–3 (0.20–0.50 mmol) were dissolved in phosphate-buffered solutions (2.5 mL) under \( \text{N}_2 \); in the cases of 2 and 3, solid \( \text{K}_2\text{HPO}_4 \) was added until the pH value was 7.40 (pHmeter). These solutions were then slowly added, over 10 min, to the propynamides (0.22–0.55 mmol) in 2.5 mL of either H\(_2\)O, EtOH, \(^1\text{BuOH}, \text{THF or aqueous THF. Phosphate buffer was not added in entry 1, but the pH was continuously measured (pHmeter, 6.0). In other cases the pH was adjusted to 10.0 by addition of \( \text{K}_2\text{CO}_3 \) and to 12.0 with \( \text{K}_3\text{PO}_4 \). Stirring under \( \text{N}_2 \) at rt or in a bath at 37 ± 1 °C was maintained for further 10–60 min. Isolated yields are given for 1a and 1b, before removing small amounts of isomers E (when necessary) by column chromatography. Yields before recrystallization are given for zwitterions 2a,b and 3a,b (see Experimental Section). \(^{b}\)Z/E ratios were determined by \( ^1\text{H} \) NMR before purification. \(^{c}\)The propynamide was dissolved in buffered water + THF to ensure that H\(_2\)O predominated in the medium from the very beginning. \(^{d}\)The minor isomer was not detected during and after the reaction, even when increasing the number of scans and expanding the spectra.
As shown in Table 1, the reactions of 1–3 with a and b were completed in 40–70 min even at concentrations of the reactants ≤ 0.1 M, either at rt or 37 °C. Excellent isolated yields of the desired adducts were obtained (by TLC chromatography and NMR the conversions were complete). At 0.5 M concentrations, only a few minutes were required, as expected, but we were interested in examining the reactions under dilute conditions for the potential application of the protocol to mAbs or to molecular scaffolds.

The Z diastereomer was always obtained with excellent diastereoselectivity, except for two cases: in entries 9 and 14, when a non-hydroxylic solvent such as THF was used. However, it was sufficient to perform the addition with propynamides a and b dissolved in a mixture of THF and buffered water to avoid the formation of isomer E. In other words, in buffered water or a hydroxylic solvent as the medium throughout the reaction time, the protonation of the addition intermediate is so rapid that isomer Z largely predominates. The 1:1 'BuOH–buffered H₂O mixture was the medium of choice with both the N-substituted and N,N-disubstituted carboxamides.

No catalyst or additives were necessary for the reactions in Table 1. They did not progress in water at pH < 6, but in water at pH 7.4, the thiol protons are sufficiently acid (pKₐ of Cys = 8.18, general pKₐ range in proteins ≈ 7.4–9.1)¹¹ to be in equilibrium with a certain percentage of thiolate ion, which attacks the most electron-deficient carbon of the activated triple bond. The rapid protonation of the anionic adduct⁷ leads to a well-known trans-addition, in such a way that the substituents of the double bond turn out to be cis. This is an advantage of all thia-Michael additions in relation to other click reactions for thiols, since (potentially) toxic catalysts and/or water-insoluble additives can be avoided. This is in sharp contrast to parallel conjugate additions of thiols in organic solvents, which require catalysis by nucleophiles and which preferentially lead to E isomers. For example, 1 and a, treated with catalytic amounts of DABCO in anhydrous CH₂CN at rt, quantitatively gave the E isomer (data not included in Table 1, for the sake of simplicity), which showed olefin protons at δH 5.92 and 7.46 ppm, J = 14.8 Hz and olefin carbons at δC 118.1 and 141.6 ppm.

What matters here is that the competition between the thiol and the amino group of 2 or 3 was won by the first. It reacted with complete selectivity with the alkyne, even when using water at pH 10.0 (entries 11, 18, and 21); no products derived from the attack of the amino group were detected in any case.⁷ In one experiment at pH 12.0 with 2 and a (entry 12), we once again observed that only the thiolate ion was added to the triple bond (formation of 2a, no attack through the amino group). In summary, the thiolate ion is more reactive than the amino group, in conjugate additions to propynamides. The reaction of 2 with commercially available N-benzylmaleimide [N-(phenylmethyl)maleimide, e], at pH 10.0 and pH 12.0, also afforded only the products arising from the thiolate addition, but the product, 2e, underwent ring opening.

Isomers Z of 1a–3a and 1b–3b are readily characterized by the diagnostic NMR signals of the double bonds that are formed. In contrast, adducts of 1–3 with N-benzylmaleimide (e), which we prepared and isolated for the sake of comparison, were mixtures of diastereoisomers, as expected. This unnecessarily complicates the NMR spectra and chromatograms, as well as the future evaluation of the homogeneity of the samples arising from the functionalization of peptides and molecular scaffolds or platforms with several thiol groups (the characterization of which is then limited to the use of mass spectrometry). In Figure 1 representative spectra of 1a and 1c are compared (for expanded, high-quality spectra see Supporting Information). The spectra of 1a, in the left column, are very clear with relevant protons in the double bond region where overlap with

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other signals, apart from broad NH signals of CONH, seldom occurs in protein chemistry. Also, the relevant carbon atoms of the double bond appear downfield and upfield, respectively, to standard aromatic and olefinic carbons. In short, there are only two proton signals in the $^1$H NMR spectrum and only two signals in the $^{13}$C NMR spectrum. In sharp contrast, in the case of succinimide 1c, see the right column, there are two sets of 3'-CH signals while protons 4'-CH$_2$ (in duplicate, 4 diastereotopic hydrogens, 2 diastereotopic C atoms) appear in the same 3.5–2.5 ppm and 34–37 ppm regions as the signals of 3-CH$_2$ (of Cys). Thus, if we had to prepare a molecular scaffold with 4 thiol groups, which by treatment with maleimide-type acceptors could afford mixtures of up to 8 succinimides, NMR spectroscopy would be useless for the product characterization.

The reactions of 1 with a and c were also followed by $^1$H NMR in D$_2$O (pH 7.4, by addition of a small amount of K$_2$HPO$_4$). They are summarized in Scheme 3 (top). Incorporation of deuterium in (Z)-1a was not surprising. Incorporation of deuterium in 1c (a nearly 1:1 diastereomeric mixture) was also evident: one of each 4'-CH$_2$ protons of the succinimide or pyrrolidine-2,5-dione rings "disappeared" and the $^{13}$C NMR spectra also confirmed the monodeuteration of the CH$_2$ groups. Few minutes later, a set of trideuterated isotopomers (1c-$d_3$) had been formed due to the exchange of the relatively more acidic hydrogen H3' with the solvent, as shown in Scheme 3 (bottom). This
exchange at position 3’ was also observed when 1c, prepared in 'BuOH–H2O, was simply dissolved in D2O to register its NMR spectra. This also happened when 1c was dissolved in hot CD3OD: 86% of deuteration on C3’ was noted within 1 h at 60 ºC without any additive. The deuteration of such a position was also very rapid with 2c and 3c, each a mixture of diastereomers as well, when dissolved in D2O, which was warmed for few minutes to favor the dissolution of the samples. However, in D2O with a drop of TFA no exchange was observed. In other words, not only are the succinimides such as 1c–3c labile in basic media, with ring opening, and sensitive to exchange reactions with other thiols (either by elimination–addition reactions or by direct substitutions), but there are rapid proton exchanges at the S–CH–CO moiety, probably via its enolate-like anion at pH ≥ 7.4.

Carboxamides 1a, 1b, 2a, 2b, 3a, and 3b are reported here for the first time. They have been fully characterized. Their 1H and 13C NMR spectra and relevant 2D NMR spectra are provided as Supporting Information.

Scheme 3. Deuterated Species Observed in D2O

An alternative to the preparation of the thia-Michael adducts just described is the addition–elimination reaction of thiols with β-halopropenamides (β-haloacrylamides), a known reaction, but not reported in aqueous solvents or with biologically relevant thiols. When 1–3 were treated with β-haloacrylamides d and e under physiological conditions, the expected adducts were obtained in excellent yields and with complete retention of the stereochemistry of the double bond (Scheme 4, top). Addition of 1–3 to propenamide (acrylamide) f also took place under the same conditions, but more slowly. In fact, the qualitative order of reactivity, as observed by 1H NMR, was: propynamide a >> (Z)-iodopropenamide d >> (E)-iodopropenamide e >> propynamide g (Scheme 4, bottom). Thus, activated triple bonds react with nucleophiles more rapidly than similarly activated double bonds, which is a textbook statement. Nevertheless, maleimide c (with two CO groups activating the double bond) reacted more quickly than propynamide a under identical physiological conditions.
In our hands, from qualitative competition experiments at pH 7.4 as always, in tBuOH–H2O to ensure that all the starting compounds remain soluble, maleimide c appeared to be more electrophilic than ethyl propynoate (HC≡C–COOEt) and morpholine amide of propynoic acid (b), which is slightly more reactive than a. However, maleimide c is less reactive than tosylacetylene 9a and ethynyl phenyl sulfone ("besylacetylene", HC≡C–SO2Ph).8f High reactivity is very often incompatible with the chemoselectivity that is desired, in agreement with the reactivity–selectivity principle, so we were not interested in examining a variety of HC≡C–EWG with very strong EWGs.

A final issue concerns the stability of the carbonylvinylsulfide moiety, that is, of the S–linker bond, in the presence of other thiols and under acidic or basic conditions. Several experiments were carried out in this regard. By addition to 1a of an equivalent amount of 1 in tBuOH–H2O at pH 7.4 and 37 ºC, with overnight stirring, no change was observed (no formation of dithioacetal, no isomerization at all to the E isomer).16 We had to add 4 equiv of 1, under these conditions, to observe a 25% of Z-to-E isomerization and nothing else. Furthermore, addition to (Z)-1b, again in tBuOH–H2O at pH 10.0 and 37 ºC, with overnight stirring, of 1 or 4 equiv of 12-dodecanethiol did not produce any change. Also, the S–CH=CH–CO linkage turned out to be stable in acidic media, since the dissolution of 1a–3a and 1b–3b in water with 10% TFA, again overnight in a bath at 37 ºC, did not cleave the linker (only hydrolysis of the ester group of 1a and 1b was observed). Some NMR spectra of 2a and 3a were registered in TFA without decomposition. Moreover, in a basic medium, addition of 10% of Et3N to tBuOH–H2O solutions of 1a and overnight stirring, as always at 37 ºC, did not affect the linker (only the ester group was saponified, as expected). This is not the case with 1c, the succinimide ring of which was cleaved under these conditions, in agreement with literature reports.4,5 We also observed that succinimide 2c, once dissolved in DMSO-d6, was cleaved within 1 h by addition of K2HPO4/D2O. To summarize, in contrast to the 3-(thioalkoxy)-succinimide moieties, the S–CH=CH–CO moiety appeared to be reasonably stable in the presence of thiols, acids and bases.

![Scheme 4. Evaluation of Alternatives to Propynamides a and b](image-url)
Last but not least, as a proof of concept, we started synthesis of a more complex model from a known carboxylic acid,\textsuperscript{17} a derivative of a drug candidate (see Scheme 5) that was available in our lab in connection with a different project and which we prepared in accordance with the reported procedure.\textsuperscript{17} Although the details will be reported elsewhere, we can advance that we attached this carboxyl derivative to commercially available 3-azidopropylamina (3-azido-1-propanamine), which may be considered the simplest model of a spacer. After reduction of the azido group to amine (by catalytic hydrogenation, but other already classical routes were also successful\textsuperscript{18}), conversion to its propynamide g (see Scheme 5) was effected by standard procedures mentioned in the introduction. We treated 1 and 2 with g in tBuOH–H\textsubscript{2}O at pH 7.4 and 37 °C, also at approximately 0.1 M concentrations of the reactants. The reactions were completed within 2 h, to afford enantiopure 1g and 2g, which were characterized by NMR and MS as usual (see Experimental Section).

**Scheme 5. Synthesis of 1g and 2g**

In conclusion, from a simple conjugate addition—a known thia-Michael reaction—we have developed a practical linker for the bio-conjugation of Cys derivatives under physiological conditions, without additives, with substrates containing a propynamide group (that is, a moderately activated triple bond, which has advantages, as shown herein). It can be considered another click reaction. We recognize that the application of these new methodological results, which complement those reported in the chemistry literature, to real ADCs is still far off and that the stability in vivo of the linker has yet to be studied; these studies were outside the scope of the present work, but they are our next objective.

**EXPERIMENTAL SECTION**

**General Methods.** Unless specified otherwise, all starting materials and reagents were obtained from commercial suppliers and used without further purification. All reactions were conducted in oven-dried glassware, under nitrogen, in anhydrous solvents, which were dried and distilled before use.
General Procedure for the Addition of Thiols 1–3 to Propynamides (Propiolamides) a and b in Aqueous Media. Thiols 1–3 (in general, 0.20–0.50 mmol) were dissolved in phosphate-buffered solutions (2.5 mL) under N₂; in the cases of 2 and 3, solid K₂HPO₄ was added until the pH value was 7.40 (pH meter). These solutions were slowly added, over 10 min, to the propynamides (0.22–0.55 mmol) in 2.5 mL of H₂O, ¹BuOH, EtOH, THF, or THF–buffered water, either at rt or 37 ºC. The reactions were stirred until TLC analysis indicated complete consumption of the substrate (usually for further 10–60 min, see Table 1). The conversion yields were 100% in all cases, at pH ≥ 7.4.

The reaction mixtures from 1 were diluted with H₂O and extracted three times with CH₂Cl₂. The organic layers were collected, dried over anhydrous MgSO₄, filtered, concentrated under vacuum, and analyzed by NMR (last column in Table 1). The residues were usually purified by analytical thin-layer chromatography (TLC) on silica gel 60 (35–70 µm) eluted with CH₂Cl₂/MeOH, 98:2. Retention factors (Rf) are approximate. Flash column chromatography was performed on silica gel 60 (35–70 µm). Melting points or decomposition temperatures were obtained with a Gallenkamp apparatus. ¹H NMR spectra were recorded in CDCl₃ on 400 MHz spectrometers; chemical shifts are given in ppm with the solvent resonance as the internal standard (residual CHCl₃ in CDCl₃, δ 7.26 ppm; residual CD₃SOCHD₂ in DMSO-d₆, δ 2.50 ppm; DOH in D₂O, δ 4.79 ppm). Data are reported as usual: chemical shift in δ, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, br s = broad singlet, m = multiplet), coupling constants in Hz, and integration. For cysteine derivatives 2a–c and 3a–c the solvent was D₂O, but one or two drops of TFA had to be added to help solubilize 2a–c and to avoid deuteration at C3' in the case of 3c, with a glass capillary filled with CHCl₃ inside, that is, with CHCl₃ as external reference. ¹³C NMR spectra were recorded in CDCl₃, DMSO-d₆, or D₂O at 100.6 MHz with proton decoupling. Chemical shifts are reported in ppm (CDCl₃, δ 77.0 ppm; DMSO-d₆, δ 39.5 ppm). For 2a–c and 3a–c the solvent was D₂O, plus one or two drops of TFA in the cases of 2a–c and 3c, with an internal capillary filled with CDCl₃ [TFA in CDCl₃, δ 116.5 (q, J = 290.4 Hz), 164.4 (q, J = 36.6 Hz), as external reference. Where necessary, 2D NMR experiments (HSQC and NOESY, mainly) were carried out to assist in structure elucidation and signal assignments. FTIR spectra were registered with an ATR accessory; only the more relevant frequencies (cm⁻¹) are reported. HRMS were obtained by using ESI–TOF techniques.

**Methyl N-acetyl-S-[(Z)-3-phenylmethlamino]-3-axo-1-propen-1-yl-L-cysteinate, (Z)-1a.** Yield: 94% (91 mg). White solid; mp 123–125 ºC; R₇ = 0.30 (CH₂Cl₂/MeOH, 98:2). ¹H NMR (CDCl₃) 2.01 (s, 3H), 3.24 (d, J = 4.5, 2H), 3.76 (s, 3H), 4.47 (d, J = 5.8, 2H), 4.85 (m, 1H), 5.78 (d, J = 9.9, 1H), 5.89 (br
[48x163](dd, J = 8.8, 5.0, 1H), 6.36 (d, J = 10.1, 1H), 7.13 (d, J = 10.2, 1H). 13C NMR (D2O, ref. to CDCl3) 26.3, 31.5, 36.8, 42.0, 43.5, 46.1, 53.8, 54.1, 66.4, 112.9, 147.0, 167.0, 171.4, 174.0, 174.8, 176.2. HRMS (ESI+) m/z calec for C25H23N4O8S+ [M + H]+ 467.1595, found 467.1596; HRMS (ESI−) m/z calec for C25H23N4O8S− [M−H]− 465.1449, found 465.1463.

S-(3RS)-1-Phenylmethyl-2,5-dioxopyrrolidin-3-yl-L-cysteine, 1c. Yield: 98% (61 mg); ca. 1:1 diastereomeric mixture.

Yellowish oil; Rf = 0.34 (hexanes/EtOAc, 20:80). 1H NMR (CDCl3) 2.02 (s, 3H), 2.04 (s, 3H), 2.46 (dd, J = 18.8, 3.9, 1H), 2.50 (dd, J = 18.8, 4.1, 1H), 2.99 (dd, J = 14.0, 7.5, 1H), 3.14 (m, 3H), 3.45 (dd, J = 14.4, 5.2, 1H), 3.49 (dd, J = 14.0, 4.4, 1H), 3.75 (dd, J = 9.2, 4.1, 1H), 3.76 (s, 6H), 3.91 (dd, J = 9.2, 3.9, 1H), 4.66 (m, 4H), 4.89 (m, 2H), 6.43 (br s, 1H), 6.90 (br s, 1H), 7.32 (m, 10H). 13C NMR (CDCl3) 23.2, 23.2, 34.3, 35.0, 35.8, 36.5, 38.9, 40.4, 42.8, 42.9, 51.4, 52.4, 52.9, 53.0, 128.2, 128.3, 128.7, 128.8, 128.9, 135.3, 135.4, 170.2, 170.3, 171.0, 171.2, 173.8, 174.1, 176.6, 176.8. FTIR 3278, 3069, 2914, 2850, 1721, 1639, 1625, 1614. HRMS (ESI+) m/z calec for C17H16N2O6S+ [M + H]+ 445.1399, found 445.1413.

Methyl N-acetyl-S-(3RS)-1-phenylmethyl-2,5-dioxopyrrolidin-3-yl-L-cysteinate, 1c. Yield: 98% (61 mg); ca. 1:1 diastereomeric mixture.
4.5, 1H), 4.41 (dd, J = 7.6, 4.5, 1H), 4.66 (m, 4H), 7.32 (m, 10H). 13C NMR (D2O + TFA, ref. to CDCl3) 31.0, 32.3, 35.6, 36.0, 40.0, 41.3, 42.7, 42.8, 52.3, 53.0, 128.0, 128.0, 128.4, 129.1, 130.2, 162.5, 162.9, 170.0, 170.1, 177.6, 178.9, 179.5. HRMS (ESI–) m/z cale for C32H30N5O3S [M–H]– 307.0758, found 307.0763.

S-[(3RS)-1-Phenethylmethyl-2,5-dioxopyrroloidin-3-yl]glutathione, 3c. Yield: 92% (161 mg); almost 1:1 diastereomeric mixture. White solid; Rf = 0.54 (CH2Cl2/Methanol/TFA, 80:15:5). 1H NMR (D2O + TFA, ref. to CHCl3) 2.20 (m, 4H), 2.54 (m, 4H), 2.63 (dd, J = 9.9, 3.8, 1H), 2.68 (dd, J = 9.9, 3.9, 1H), 2.94 (dd, J = 14.0, 8.6, 1H), 3.10 (dd, J = 14.0, 7.8, 1H), 3.11 (dd, J = 14.0, 5.6, 1H), 3.27 (dd, J = 19.0, 6.6, 1H), 3.28 (dd, J = 19.0, 6.5, 1H), 3.95 (s, 2H), 3.97 (s, 2H), 4.01 (dd, J = 9.0, 3.9, 1H), 4.03 (dd, J = 8.9, 3.7, 1H), 4.06 (dd, J = 5.0, 1.7, 1H), 4.07 (dd, J = 6.5, 3.3, 1H), 4.59 (d, J = 5.6, 1H), 4.61 (d, J = 5.3, 1H), 4.63 (s, 4H), 7.30 (m, 10H). 13C NMR (D2O + TFA, ref. to CDCl3) 25.4, 25.4, 30.9, 32.5, 32.7, 35.8, 36.0, 39.9, 40.6, 41.1, 42.4, 52.2, 52.6, 53.1, 127.7, 128.1, 128.9, 135.1, 171.4, 172.2, 172.3, 172.8, 172.8, 174.2, 174.2, 177.8, 177.8, 179.0, 179.1.

Methyl N-acetyl-S-[1-(E)-3-phenethylamino]-3-oxo-1-propenyl-L-cysteinate, 1e [(E)-1a].

Methyl N-acetyl-S-(3-phenethylamino-3-oxopropyl)-L-cysteinate, 1f.

Propynamide g. Yield: 43% (330 mg). Colorless oil; Rf = 0.47 (CH2Cl2/Methanol, 95:5). Mixture of rotamers (piperazine ring); 1H NMR (CDCl3) 1.25 (br s, 3H), 1.75 (m, 2H), 2.83 (s, 1H), 3.39 (s, 2H), 3.49 (s, 2H), 4.80–3.11 (m, 7H), 6.97 (br s, 1H), 7.28 (m, 5H). 13C NMR (CDCl3) 23.0, 28.6, 34.5, 36.5, 43.7, 52.3, 52.7, 127.5, 127.8, 128.7, 138.1, 141.8, 164.1, 170.2, 170.4. FTIR 3275, 3033, 1744, 1639. HRMS (ESI+) m/z cale for C19H22N2O4S [M + H]+ 337.1215, found 337.1221.

Methyl N-acetyl-S-[3-(phenethylamino-3-oxopropyl)-L-cysteinate, 1f. Yield: 91% (99 mg). Oil; Rf = 0.66 (CH2Cl2/Methanol, 99:1). 1H NMR (CDCl3) 1.99 (s, 3H), 2.48 (m, 2H), 2.86 (t, J = 7.0, 2H), 2.96 (d, J = 5.1, 2H), 3.73 (s, 3H), 4.43 (d, J = 5.7, 2H), 4.80 (m, 1H), 6.35 (br s, 1H), 6.72 (br s, 1H), 7.28 (m, 5H). 13C NMR (CDCl3) 23.0, 28.6, 34.5, 36.5, 43.7, 52.3, 52.7, 127.5, 127.8, 128.7, 138.1, 170.3, 170.8, 171.2. FTIR 3275, 3033, 1744, 1639. HRMS (ESI+) m/z cale for C19H22N2O4S [M + H]+ 339.1373, found 339.1379.

Adduct of methyl N-acetyl-L-cysteinate and propanamide g (1g). Yield: 98% (80 mg). Yellowish oil; Rf = 0.19 (CH2Cl2/Methanol, 95:5). Mixture of rotamers: 1H NMR (CDCl3) 1.28 (br s, 3H), 1.73 (m, 2H), 2.04 (s, 3H), 2.98–3.47 (m, 12H), 3.77 (s, 4H), 4.84 (br s, 1H), 5.79 (d, J = 9.9, 1H), 6.48 (br s, 1H), 6.72 (d, J = 9.7, 1H), 7.29 (m, 1H), 7.39 (m, 5H), 7.64 (d, J = 7.3, 1H), 7.73 (d, J = 6.9, 1H), 7.92 (br s, 1H), 8.02 (m, 1H), 8.42 (br s, 1H), 11.33 (s, 1H). 13C NMR (CDCl3) 15.2, 16.4, 23.3, 29.4, 29.8, 36.3, 36.4, 36.7, 38.4, 45.0, 52.7, 52.9, 114.2, 116.4, 117.4, 122.2, 122.9, 125.9, 126.7, 127.1, 128.8, 130.2, 135.1, 135.2, 136.4, 144.7, 167.2, 167.4, 167.5, 170.4, 170.5, 170.8, 185.6. HRMS (ESI+) m/z cale for C29H30N6O5S [M + H]+ 528.2241, found 528.2242.

Adduct of L-cysteine and propanamide g (2g). Yield: 97% (103 mg). Yellowish oil; Rf = 0.33 (CH2Cl2/Methanol/TFA, 80:15:5). Mixture of rotamers: 1H NMR (DMSO-d6) 1.21 (br s, 3H), 1.72 (quin, J = 6.9, 2H), 2.71–4.60 (m, 13H), 4.22 (s, 1H), 5.95 (d, J = 9.8, 1H), 6.90 (d, J = 10.0, 1H), 7.40 (m, 6H), 7.82 (d, J = 7.6, 1H), 8.05 (d, J = 3.3, 1H), 8.29 (br s, 1H), 8.75 (t, J = 5.6, 1H), 12.20 (d, J = 12.9, 1H). 13C NMR...
(DMSO-$d_6$) 14.9, 16.0, 28.8, 29.3, 32.2, 36.8, 36.9, 37.0, 44.3, 51.4, 112.7, 117.9, 118.5, 122.3, 122.4, 124.4, 126.3, 127.1, 128.6, 129.8, 135.2, 135.6, 138.0, 142.3, 165.8, 165.9, 166.1, 169.4, 186.0. HRMS (ESI−) m/z calcd for C$_{32}$H$_{35}$N$_6$O$_7$S− [M − H]− 647.2293, found 647.2296.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc........

$^1$H NMR and $^{13}$C NMR spectra of the new compounds and 2D NMR spectra of 1c

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Notes

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(12) When 1c (a nearly 1:1 diastereomeric mixture) was prepared in CD₃OD at rt, the solvent was evaporated under vacuum without heating, and the residue was analyzed by NMR spectroscopy in CDCl₃ (including NOE experiments), both 4’-CH₂ protons of both diastereoisomers appeared to have been partially exchanged with deuterium (see 1c-d₂).


(16) It has been reported (Ref 7e) that, for the cleavage of SCH=CHCOPh adducts, 30 equiv of Cys or of PhSH were required, but it did not work with SCH=CHCONHPh adducts.

