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


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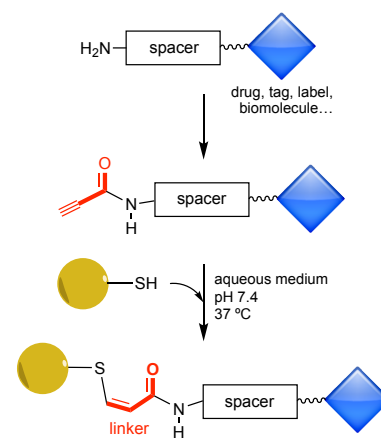
# (Z)-Oxopropene-1,3-diyl, a Linker for the Conjugation of the Thiol Group of Cysteine with Amino-Derivatized Drugs

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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.  $NH_2$ , 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  $D_2O$  to register their NMR spectra.

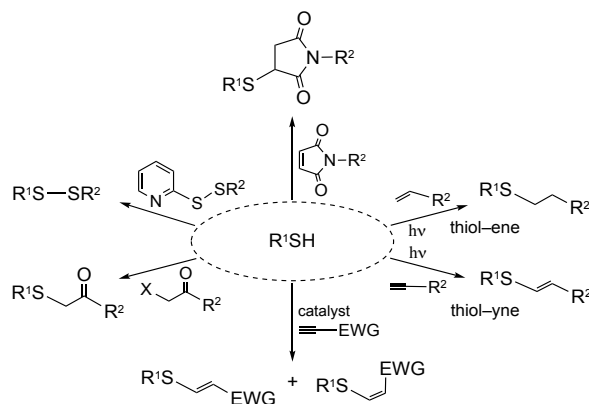


Conventional cancer treatments that use cytotoxic compounds cause serious side effects due to their lack of selectivity for tumor cells. As known, a very promising strategy to avoid or alleviate these drawbacks is to conjugate cytotoxic drugs with antibodies (targeted therapy).<sup>1</sup> The preparation of antibody–drug conjugates (ADCs) requires efficient and reliable linkage protocols.<sup>2</sup> 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.<sup>3</sup> 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.<sup>4,5</sup> Other Cys conjugations have been reported (Scheme 1)<sup>6</sup>, including the addition to electron-deficient alkynes.<sup>7</sup>

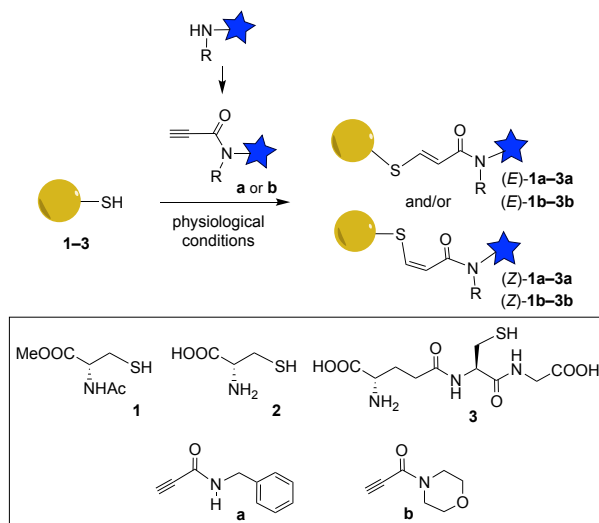
Our long-standing interest in the conjugate addition of several nucleophiles to activated triple bonds<sup>8</sup> has recently led us to examine the possibility of going a step further: whether, under real physiological conditions, these reagents—specifically, propynamides—will or will not be useful to bind or link (as a linchpin or link pin) thiols and amines (Scheme 2). In other words, from our own work<sup>8</sup> and from that of our colleagues Arjona and Plumet,<sup>9</sup> we were aware that  $HC\equiv C$ -EWG compounds with strong EWGs such as  $SO_2R$  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  $HC\equiv C$ -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 CONR<sub>2</sub>), 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.

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



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

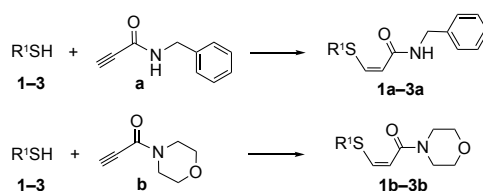


The acylation of a primary or secondary amine with commercially available reagents such as propynoic acid (also known as propiolic or propargylic acid) and 3-trimethylsilylpropynoic acid (followed by desilylation), in the presence of a coupling agent (for example, dicyclohexylcarbodiimide, DCC, or any of its analogs) are well-known reactions.<sup>10</sup> 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 <sup>t</sup>BuOH–H<sub>2</sub>O, EtOH–H<sub>2</sub>O, or THF–H<sub>2</sub>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.

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



entry	thiol	alkyne	solvent(s)	pH	T (°C)	t (min)	yield (%) <sup>a</sup>	Z/E <sup>b</sup>
1	<b>1</b>	<b>a</b>	H <sub>2</sub> O or <sup>t</sup> BuOH–H <sub>2</sub> O	6.0	rt	1,000	<b>1a</b> , 5	50:50
2	<b>1</b>	<b>a</b>	H <sub>2</sub> O	7.4	rt	70	<b>1a</b> , 93	98:2
3	<b>1</b>	<b>a</b>	H <sub>2</sub> O	7.4	37	60	<b>1a</b> , 94	98:2
4	<b>1</b>	<b>a</b>	EtOH–H <sub>2</sub> O, 1:1	7.4	37	50	<b>1b</b> , 98	93:7
5	<b>1</b>	<b>b</b>	H <sub>2</sub> O	7.4	37	50	<b>1b</b> , 98	>99:1 <sup>d</sup>
6	<b>1</b>	<b>b</b>	<sup>t</sup> BuOH–H <sub>2</sub> O, 1:1	7.4	37	40	<b>1b</b> , 98	>99:1 <sup>d</sup>
7	<b>2</b>	<b>a</b>	H <sub>2</sub> O	7.4	rt	70	<b>2a</b> , 92	98:2
8	<b>2</b>	<b>a</b>	<sup>t</sup> BuOH–H <sub>2</sub> O, 1:1	7.4	37	60	<b>2a</b> , 96	>99:1 <sup>d</sup>
9	<b>2</b>	<b>a</b>	THF–H <sub>2</sub> O, 1:1	7.4	37	60	<b>2a</b> , 92	85:15
10	<b>2</b>	<b>a</b>	THF–H <sub>2</sub> O, 1:3 <sup>c</sup>	7.4	37	60	<b>2a</b> , 94	98:2
11	<b>2</b>	<b>a</b>	<sup>t</sup> BuOH–H <sub>2</sub> O, 1:1	10.0	37	30	<b>2a</b> , 96	>99:1 <sup>d</sup>
12	<b>2</b>	<b>a</b>	H <sub>2</sub> O	12.0	37	30	<b>2a</b> , 92	88:12
13	<b>2</b>	<b>b</b>	H <sub>2</sub> O	7.4	37	40	<b>2b</b> , 99	95:5
14	<b>2</b>	<b>b</b>	THF–H <sub>2</sub> O, 1:1	7.4	37	40	<b>2b</b> , 96	65:35
15	<b>2</b>	<b>b</b>	THF–H <sub>2</sub> O, 1:3 <sup>c</sup>	7.4	37	40	<b>2b</b> , 97	>99:1 <sup>d</sup>
16	<b>2</b>	<b>b</b>	<sup>t</sup> BuOH–H <sub>2</sub> O, 1:1	7.4	37	40	<b>2b</b> , 97	>99:1 <sup>d</sup>
17	<b>3</b>	<b>a</b>	<sup>t</sup> BuOH–H <sub>2</sub> O, 1:1	7.4	37	60	<b>3a</b> , 93	>99:1 <sup>d</sup>
18	<b>3</b>	<b>a</b>	THF–H <sub>2</sub> O, 1:3 <sup>c</sup>	10.0	37	40	<b>3a</b> , 95	95:5
19	<b>3</b>	<b>b</b>	H <sub>2</sub> O	7.4	37	40	<b>3b</b> , 95	95:5
20	<b>3</b>	<b>b</b>	<sup>t</sup> BuOH–H <sub>2</sub> O, 1:1	7.4	37	40	<b>3b</b> , 98	>99:1 <sup>d</sup>
21	<b>3</b>	<b>b</b>	THF–H <sub>2</sub> O, 1:3 <sup>c</sup>	10.0	37	40	<b>3b</b> , 93	95:5

<sup>a</sup>Thiols **1–3** (0.20–0.50 mmol) were dissolved in phosphate-buffered solutions (2.5 mL) under N<sub>2</sub>; in the cases of **2** and **3**, solid K<sub>2</sub>HPO<sub>4</sub> 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<sub>2</sub>O, EtOH, <sup>t</sup>BuOH, 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 K<sub>2</sub>CO<sub>3</sub> and to 12.0 with K<sub>3</sub>PO<sub>4</sub>. Stirring under N<sub>2</sub> 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). <sup>b</sup>Z/E ratios were determined by <sup>1</sup>H NMR before purification. <sup>c</sup>The propynamide was dissolved in buffered water + THF to ensure that H<sub>2</sub>O predominated in the medium from the very beginning. <sup>d</sup>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  $\leq 0.1$  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.

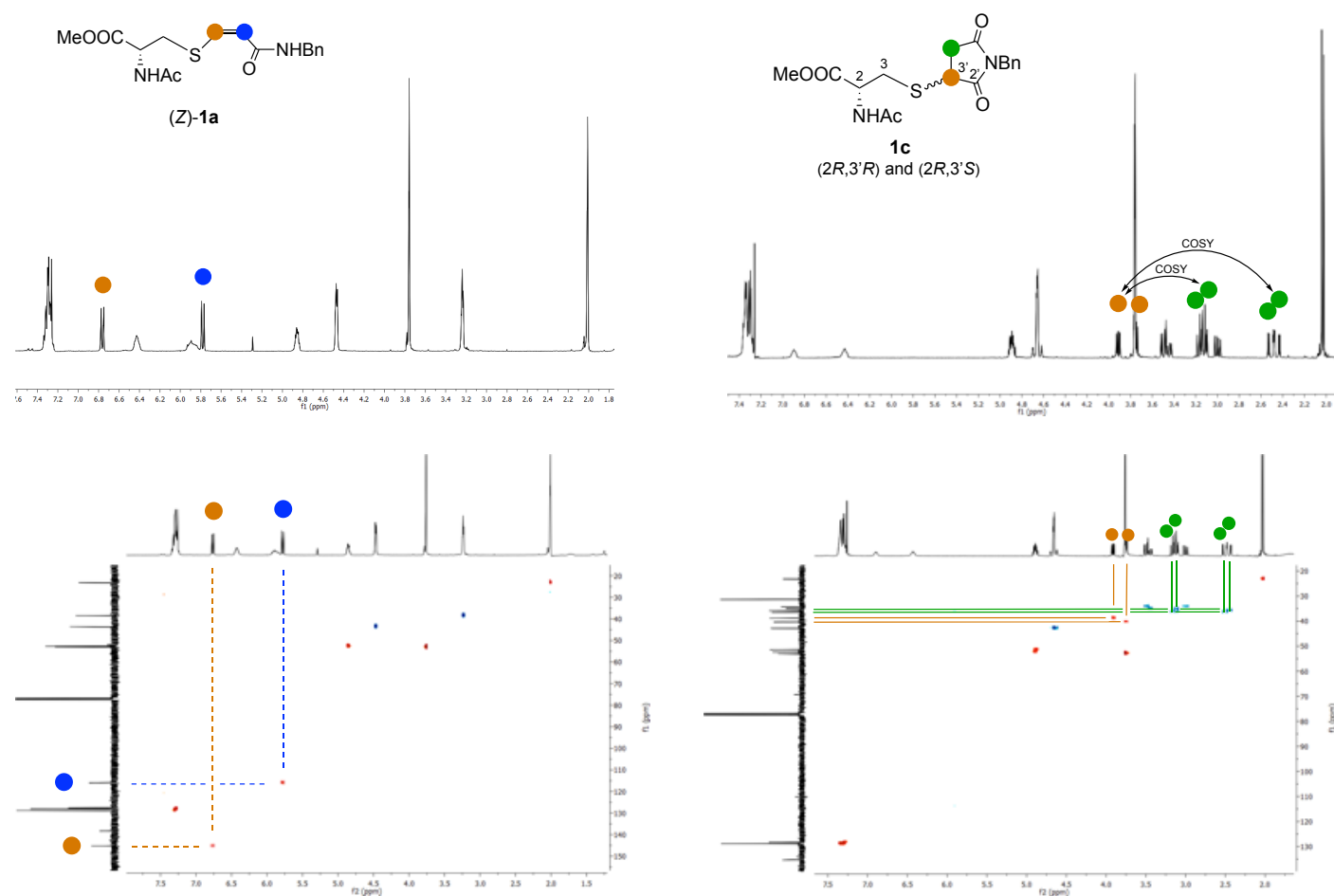
The *Z* diastereomer was always obtained with excellent diastereoselectivity, except for two cases: in entries 9 and 14, when a non-hydroxylic solvent such as THF was used. However, it was sufficient to perform the addition with propynamides **a** and **b** dissolved in a mixture of THF and buffered 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 <sup>t</sup>BuOH–buffered H<sub>2</sub>O 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 ( $pK_a$  of Cys = 8.18, general  $pK_a$  range in proteins  $\approx 7.4–9.1$ )<sup>11</sup> 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 adduct<sup>7a</sup> 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.<sup>8,9</sup> For example, **1** and **a**, treated with catalytic amounts of DABCO in anhydrous CH<sub>3</sub>CN at rt, quantitatively gave the *E* isomer (data not included in Table 1, for the sake of simplicity), which showed olefin protons at  $\delta$ H 5.92 and 7.46 ppm, <sup>3</sup>*J* = 14.8 Hz and olefin carbons at  $\delta$ C 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.<sup>7a</sup> 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, **c**], at pH 10.0 and pH 12.0, also afforded only the products arising from the thiolate addition, but the product, **2c**, 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 (**c**), 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

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  $^1\text{H}$  NMR spectrum and only two signals in the  $^{13}\text{C}$  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<sub>2</sub> (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<sub>2</sub> (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.



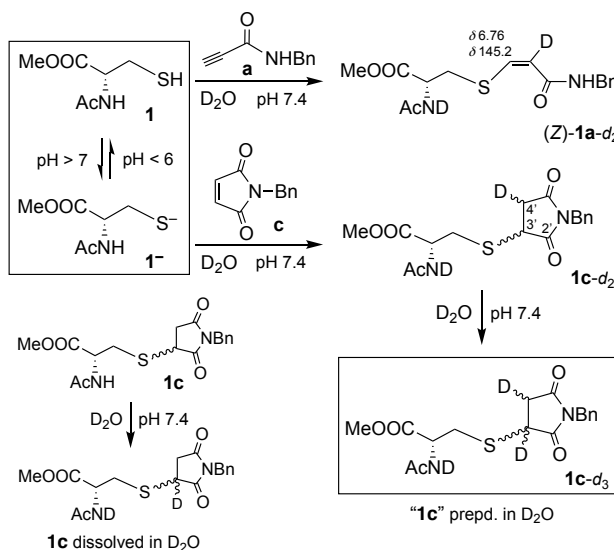
**Figure 1.**  $^1\text{H}$  and HSQC NMR Spectra of **1a** (Left Column) and **1c** (Right Column)

The reactions of **1** with **a** and **c** were also followed by  $^1\text{H}$  NMR in  $\text{D}_2\text{O}$  (pH 7.4, by addition of a small amount of  $\text{K}_2\text{HPO}_4$ ). 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<sub>2</sub> protons of the succinimide or pyrrolidine-2,5-dione rings "disappeared" and the  $^{13}\text{C}$  NMR spectra also confirmed the monodeuteration of the CH<sub>2</sub> groups.<sup>12</sup> Few minutes later, a set of trideuterated isotopomers (**1c-d<sub>3</sub>**) 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 <sup>t</sup>BuOH–H<sub>2</sub>O, was simply dissolved in D<sub>2</sub>O to register its NMR spectra. This also happened when **1c** was dissolved in hot CD<sub>3</sub>OD: 86% of deuteration on C3' was noted within 1 h at 60 °C 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 D<sub>2</sub>O, which was warmed for few minutes to favor the dissolution of the samples. However, in D<sub>2</sub>O 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<sup>4,5</sup> (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 <sup>1</sup>H and <sup>13</sup>C NMR spectra and relevant 2D NMR spectra are provided as Supporting Information.

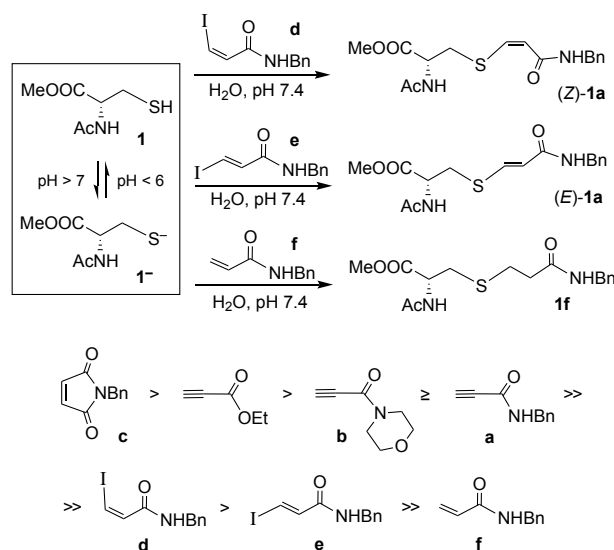
**Scheme 3. Deuterated Species Observed in D<sub>2</sub>O**



An alternative to the preparation of the thia-Michael adducts just described is the addition–elimination reaction of thiols with  $\beta$ -halopropenamides ( $\beta$ -haloacrylamides), a known reaction,<sup>13</sup> but not reported in aqueous solvents or with biologically relevant thiols.<sup>14</sup> When **1–3** were treated with  $\beta$ -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 <sup>1</sup>H NMR, was: propynamide **a** >> (*Z*)-iodopropenamide **d** > (*E*)-iodopropenamide **e** >> propenamide **f** (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.<sup>15</sup>

In our hands, from qualitative competition experiments at pH 7.4 as always, in <sup>t</sup>BuOH–H<sub>2</sub>O 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<sup>9a</sup> and ethynyl phenyl sulfone ("besylacetylene", HC≡C–SO<sub>2</sub>Ph).<sup>8f</sup> 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.

**Scheme 4. Evaluation of Alternatives to Propynamides **a** and **b****

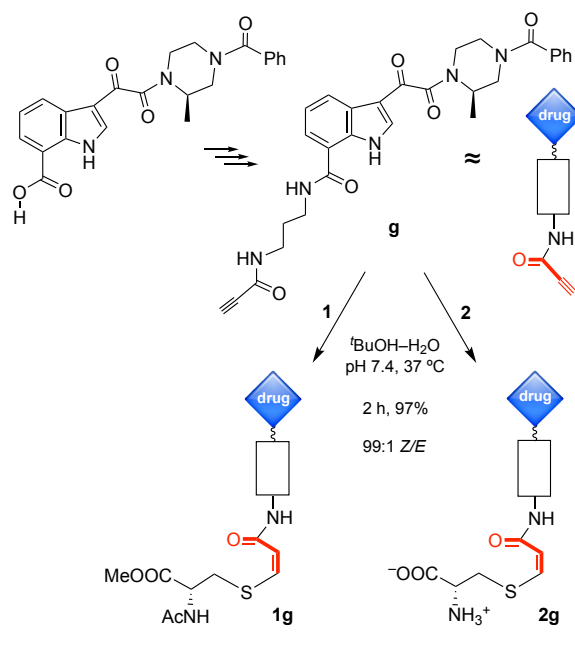


A final issue concerns the stability of the carbonylvinylsulfide moiety, that is, of the S–linker bond, in the presence of other thiols and under acidic or basic conditions. Several experiments were carried out in this regard. By addition to **1a** of an equivalent amount of **1** in <sup>t</sup>BuOH–H<sub>2</sub>O at pH 7.4 and 37 °C, with overnight stirring, no change was observed (no formation of dithioacetal, no isomerization at all to the *E* isomer).<sup>16</sup> 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 <sup>t</sup>BuOH–H<sub>2</sub>O at pH 10.0 and 37 °C, 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 °C, 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 Et<sub>3</sub>N to <sup>t</sup>BuOH–H<sub>2</sub>O solutions of **1a** and overnight stirring, as always at 37 °C, 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.<sup>4,5</sup> We also observed that succinimide **2c**, once dissolved in DMSO-*d*<sub>6</sub>, was cleaved within 1 h by addition of K<sub>2</sub>HPO<sub>4</sub>/D<sub>2</sub>O. 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.*



Last but not least, as a proof of concept, we started synthesis of a more complex model from a known carboxylic acid,<sup>17</sup> 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.<sup>17</sup> Although the details will be reported elsewhere, we can advance that we attached this carboxyl derivative to commercially available 3-azidopropylamine (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<sup>18</sup>), 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 <sup>t</sup>BuOH–H<sub>2</sub>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 bioconjugation 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

1  
2 according to standard procedures. Analytical thin-layer chromatography (TLC) was performed on 0.25 mm silica gel plates (F<sub>254</sub>). Retention factors (*R<sub>f</sub>*)  
3  
4 are approximate. Flash column chromatography was performed on silica gel 60 (35–70 μm). Melting points or decomposition temperatures were  
5  
6 obtained with a Gallenkamp apparatus. <sup>1</sup>H NMR spectra were recorded in CDCl<sub>3</sub> on 400 MHz spectrometers; chemical shifts are given in ppm with the  
7  
8 solvent resonance as the internal standard (residual CHCl<sub>3</sub> in CDCl<sub>3</sub>, δ 7.26 ppm; residual CD<sub>3</sub>SOCHD<sub>2</sub> in DMSO-*d*<sub>6</sub>, δ 2.50 ppm; DOH in D<sub>2</sub>O, δ 4.79  
9  
10 ppm). Data are reported as usual: chemical shift in δ, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, br s = broad singlet, m  
11  
12 = multiplet), coupling constants in Hz, and integration. For cysteine derivatives **2a–c** and **3a–c** the solvent was D<sub>2</sub>O, but one or two drops of TFA had to  
13  
14 be added to help solubilize **2a–c** and to avoid deuteration at C3' in the case of **3c**, with a glass capillary filled with CHCl<sub>3</sub> inside, that is, with CHCl<sub>3</sub> as  
15  
16 external reference. <sup>13</sup>C NMR spectra were recorded in CDCl<sub>3</sub>, DMSO-*d*<sub>6</sub>, or D<sub>2</sub>O at 100.6 MHz with proton decoupling. Chemical shifts are reported in  
17  
18 ppm (CDCl<sub>3</sub>, δ 77.0 ppm; DMSO-*d*<sub>6</sub>, δ 39.5 ppm). For **2a–c** and **3a–c** the solvent was D<sub>2</sub>O, plus one or two drops of TFA in the cases of **2a–c** and **3c**,  
19  
20 with an internal capillary filled with CDCl<sub>3</sub> [TFA in CDCl<sub>3</sub>, δ 116.5 (q, *J* = 290.4 Hz), 164.4 (q, *J* = 36.6 Hz)], as external reference. Where necessary,  
21  
22 2D NMR experiments (HSQC and NOESY, mainly) were carried out to assist in structure elucidation and signal assignments. FTIR spectra were  
23  
24 registered with an ATR accessory; only the more relevant frequencies (cm<sup>-1</sup>) are reported. HRMS were obtained by using ESI–TOF techniques.

**General Procedure for the Addition of Thiols 1–3 to Propynamides (Propiolamides) a and b in Aqueous Media.** Thiols **1–3** (in  
25  
26 general, 0.20–0.50 mmol) were dissolved in phosphate-buffered solutions (2.5 mL) under N<sub>2</sub>; in the cases of **2** and **3**, solid K<sub>2</sub>HPO<sub>4</sub> was added  
27  
28 until the pH value was 7.40 (pHmeter). These solutions were slowly added, over 10 min, to the propynamides (0.22–0.55 mmol) in 2.5 mL of  
29  
30 H<sub>2</sub>O, <sup>t</sup>BuOH, EtOH, THF, or THF–buffered water, either at rt or 37 °C. The reactions were stirred until TLC analysis indicated complete  
31  
32 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.

33  
34 The reaction mixtures from **1** were diluted with H<sub>2</sub>O and extracted three times with CH<sub>2</sub>Cl<sub>2</sub>. The organic layers were collected, dried over  
35  
36 anhydrous MgSO<sub>4</sub>, filtered, concentrated under vacuum, and analyzed by NMR (last column in Table 1). The residues were usually purified by  
37  
38 column chromatography on silica gel (to remove small amounts of the *E* isomers) and the solids were then stored in vacuum desiccators over P<sub>4</sub>O<sub>10</sub>  
39  
40 until constant weight (isolated yields, Table 1). The reaction mixtures from **2** were concentrated under vacuum (to remove <sup>t</sup>BuOH) and the  
41  
42 precipitates that appeared were filtered, rinsed with water, dried under vacuum over P<sub>4</sub>O<sub>10</sub>, weighted, and analyzed by TLC and NMR (yields and  
43  
44 percentages given in Table 1). The crude products from most experiments were chromatographically and spectroscopically pure (only the *Z* isomers  
45  
46 of **2a** and **2b**); no recrystallization was needed. The reaction mixtures from **3** were acidified to pH 3.5 with NaH<sub>2</sub>PO<sub>4</sub> (plus a drop of H<sub>3</sub>PO<sub>4</sub>) and a  
47  
48 similar volume of EtOH was added and separated (three times), the moist EtOH solutions were evaporated to dryness, the residues were treated  
49  
50 with 4:1 <sup>i</sup>PrOH–H<sub>2</sub>O, the solutions were filtered through Celite® (to remove remaining amounts of the insoluble phosphates) and rinsed with the  
51  
52 same mixture (<sup>i</sup>PrOH–H<sub>2</sub>O), evaporated to dryness, stored in the desiccator, weighted (yield in Table 1), and analyzed by NMR (last column in  
53  
54 Table 1). In two experiments (in which a mixture of stereoisomers was formed), the crude products were recrystallized from <sup>i</sup>PrOH–H<sub>2</sub>O (80–85%  
55  
56 recovery) to remove the minor *E* isomers.

57  
58 We proceeded similarly with the other commercially available Michael acceptors: *N*-(phenylmethyl)maleimide, or *N*-benzylmaleimide, **c**;  
59  
60 *N*-benzyl-3-iodopropenamides **d** and **e**; and *N*-benzylpropenamide **f**.

**Methyl *N*-acetyl-S-[(*Z*)-3-phenylmethylamino]-3-oxo-1-propen-1-yl]-L-cysteinate, (*Z*)-**1a**.** Yield: 94% (91 mg). White solid; mp 123–125 °C; *R<sub>f</sub>* = 0.30  
(CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 98:2). <sup>1</sup>H NMR (CDCl<sub>3</sub>) 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

s, 1H), 6.43 (br s, 1H), 6.76 (d,  $J = 9.9$ , 1H), 7.29 (m, 5H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 23.2, 38.5, 43.6, 53.0, 53.0, 116.0, 127.7, 128.1, 128.8, 138.3, 145.2, 166.0, 170.1, 170.6. FTIR 3303, 3271, 3069, 2917, 1738, 1660, 1632. HRMS (ESI+)  $m/z$  calcd for  $\text{C}_{16}\text{H}_{21}\text{N}_2\text{O}_4\text{S}^+$   $[\text{M} + \text{H}]^+$  337.1217, found 337.1221.

**Methyl N-acetyl-S-[(Z)-3-N-morpholino-3-oxo-1-propen-1-yl]-L-cysteinate, (Z)-1b.** Yield: 98% (89 mg). White solid; mp 177–178 °C;  $R_f = 0.28$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 98:2).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 2.03 (s, 3H), 3.25 (d,  $J = 4.5$ , 2H), 3.49 (br s, 2H), 3.68 (br s, 6H), 3.77 (s, 3H), 4.88 (m, 1H), 6.16 (d,  $J = 9.9$ , 1H), 6.36 (br s, 1H), 6.90 (d,  $J = 9.9$ , 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100.6 MHz)  $\delta$  23.2, 38.5, 41.9, 46.1, 52.7, 53.0, 66.8, 67.0, 111.8, 147.6, 165.5, 170.0, 170.6. FTIR 3278, 3069, 2914, 2850, 1721, 1639, 1625, 1614. HRMS (ESI+)  $m/z$  calcd for  $\text{C}_{13}\text{H}_{21}\text{N}_2\text{O}_5\text{S}^+$   $[\text{M} + \text{H}]^+$  317.1166, found 317.1171.

**S-[(Z)-3-Phenylmethylamino-3-oxo-1-propen-1-yl]-L-cysteine, (Z)-2a.** Yield: 96% (56 mg). White solid; dec. 215–217 °C;  $R_f = 0.71$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{TFA}$ , 80:15:5).  $^1\text{H}$  NMR ( $\text{D}_2\text{O} + \text{TFA}$ , ref. to  $\text{CHCl}_3$ ) 3.41 (m, 3H), 4.40 (s, 2H), 6.09 (d,  $J = 10.0$ , 1H), 7.01 (d,  $J = 10.0$ , 1H), 7.35 (m, 5H).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O} + \text{TFA}$ , ref. to  $\text{CDCl}_3$ ) 35.5, 43.0, 53.0, 117.4, 127.5, 129.0, 129.4, 138.3, 143.6, 170.0, 170.4. HRMS (ESI-)  $m/z$  calcd for  $\text{C}_{13}\text{H}_{15}\text{N}_2\text{O}_3\text{S}^-$   $[\text{M} - \text{H}]^-$  279.0809, found 279.0815.

**S-[(Z)-3-Morpholino-3-oxo-1-propen-1-yl]-L-cysteine, (Z)-2b.** Yield: 97% (65 mg). White solid, dec. 198–200 °C;  $R_f = 0.62$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{TFA}$ , 80:15:5).  $^1\text{H}$  NMR ( $\text{D}_2\text{O} + \text{TFA}$ , ref. to  $\text{CHCl}_3$ ) 3.55 (m, 3H), 3.83 (m, 2H), 3.97 (m, 6H), 6.66 (d,  $J = 10.1$ , 1H), 7.37 (d,  $J = 10.1$ , 1H).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O} + \text{TFA}$ , ref. to  $\text{CDCl}_3$ ) 32.6, 36.1, 42.3, 54.3, 63.8, 66.6, 113.9, 146.0, 167.1, 172.1. HRMS (ESI-)  $m/z$  calcd for  $\text{C}_{10}\text{H}_{15}\text{N}_2\text{O}_4\text{S}^-$   $[\text{M} - \text{H}]^-$  259.0758, found 259.0766.

**S-[(Z)-3-Phenylmethylamino-3-oxo-1-propen-1-yl]glutathione, (Z)-3a.** Yield (entry 18): 93% (152 mg). White solid; dec. 236–237 °C;  $R_f = 0.55$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{TFA}$ , 80:15:5).  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ ) 2.13 (m, 2H), 2.50 (t,  $J = 7.1$ , 2H), 3.08 (dd,  $J = 13.8$ , 9.8, 1H), 3.29 (d,  $J = 13.9$ , 1H), 3.75 (m, 3H), 4.40 (s, 2H), 4.66 (m, 1H), 6.03 (d,  $J = 10.0$ , 1H), 7.03 (d,  $J = 10.0$ , 1H), 7.40 (m, 5H).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ , ref. to  $\text{CDCl}_3$ ) 26.4, 31.7, 37.1, 43.0, 43.6, 54.0, 54.3, 116.3, 127.5, 127.6, 129.0, 138.5, 145.2, 168.6, 171.6, 174.1, 175.0, 176.4. HRMS (ESI+)  $m/z$  calcd for  $\text{C}_{20}\text{H}_{27}\text{N}_4\text{O}_7\text{S}^+$   $[\text{M} + \text{H}]^+$  467.1595, found 467.1596; HRMS (ESI-)  $m/z$  calcd for  $\text{C}_{20}\text{H}_{25}\text{N}_4\text{O}_7\text{S}^-$   $[\text{M} - \text{H}]^-$  465.1449, found 465.1463.

**S-[(Z)-3-N-Morpholino-3-oxo-1-propen-1-yl]glutathione, (Z)-3b.** Yield: 95% (146 mg). White solid; dec. 220–222 °C;  $R_f = 0.55$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{TFA}$ , 80:15:5).  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ ) 2.13 (m, 2H), 2.49 (m, 2H), 3.07 (dd,  $J = 14.4$ , 8.8, 1H), 3.28 (dd,  $J = 14.3$ , 5.0, 1H), 3.59 (m, 4H), 3.71 (m, 7H), 4.63 (dd,  $J = 8.8$ , 5.0, 1H), 6.36 (d,  $J = 10.1$ , 1H), 7.13 (d,  $J = 10.2$ , 1H).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ , ref. to  $\text{CDCl}_3$ ) 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+)  $m/z$  calcd for  $\text{C}_{17}\text{H}_{27}\text{N}_4\text{O}_8\text{S}^+$   $[\text{M} + \text{H}]^+$  447.1544, found 447.1545. HRMS (ESI-)  $m/z$  calcd for  $\text{C}_{17}\text{H}_{25}\text{N}_4\text{O}_8\text{S}^-$   $[\text{M} - \text{H}]^-$  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. Yellowish oil;  $R_f = 0.34$  (hexanes/EtOAc, 20:80).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 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).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 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, 128.9, 135.3, 135.4, 170.2, 170.3, 171.0, 171.2, 173.8, 174.1, 176.6, 176.8. FTIR 3303, 2960, 1741, 1697, 1670, 1626. HRMS (ESI+)  $m/z$  calcd for  $\text{C}_{17}\text{H}_{21}\text{N}_2\text{O}_5\text{S}^+$   $[\text{M} + \text{H}]^+$  365.1166, found 365.1171.

**S-[(3RS)-1-Phenylmethyl-2,5-dioxopyrrolidin-3-yl]-L-cysteine, 2c.** Yield: 94% (80 mg); nearly 1:1 diastereomeric mixture. White solid,  $R_f = 0.63$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{TFA}$ , 80:15:5).  $^1\text{H}$  NMR ( $\text{D}_2\text{O} + \text{TFA}$ , ref. to  $\text{CHCl}_3$ ) 2.69 (br d,  $J = 18.8$ , 1H), 2.70 (br d,  $J = 18.8$ , 1H), 3.17 (dd,  $J = 15.0$ , 8.1, 1H), 3.31 (m, 3H), 3.43 (dd,  $J = 15.2$ , 4.4, 1H), 3.49 (dd,  $J = 15.0$ , 4.4, 1H), 4.05 (dd,  $J = 9.8$ , 4.2, 1H), 4.08 (dd,  $J = 9.8$ , 4.0, 1H), 4.27 (dd,  $J = 8.1$ ,

4.5, 1H), 4.41 (dd,  $J = 7.6, 4.5$ , 1H), 4.66 (m, 4H), 7.32 (m, 10H).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O} + \text{TFA}$ , ref. to  $\text{CDCl}_3$ ) 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, 129.1, 135.2, 162.5, 162.9, 170.0, 170.1, 177.6, 178.9, 179.5. HRMS (ESI $^-$ )  $m/z$  calcd for  $\text{C}_{14}\text{H}_{15}\text{N}_2\text{O}_4\text{S}^- [\text{M} - \text{H}]^-$  307.0758, found 307.0763.

***S*-[*(3RS)*-1-Phenylmethyl-2,5-dioxopyrrolidin-3-yl]glutathione, 3c.**<sup>19</sup> Yield: 92% (161 mg); almost 1:1 diastereomeric mixture. White solid;  $R_f = 0.54$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{TFA}$ , 80:15:5).  $^1\text{H}$  NMR ( $\text{D}_2\text{O} + \text{TFA}$ , ref. to  $\text{CHCl}_3$ ) 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).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O} + \text{TFA}$ , ref. to  $\text{CDCl}_3$ ) 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*-[*(E)*-3-phenylmethylamino-3-oxo-1-propen-1-yl]-*L*-cysteinate, 1e [(*E*)-1a].** Yield: 95% (70 mg). White solid; mp 105-107 °C;  $R_f = 0.30$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 98:2).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 2.00 (s, 3H), 3.23 (dd,  $J = 14.1, 4.8$ , 1H), 3.32 (dd,  $J = 14.1, 5.0$ , 1H), 3.77 (s, 3H), 4.46 (d,  $J = 5.6, 2\text{H}$ ), 4.88 (dt,  $J = 7.3, 4.9$ , 1H), 5.92 (d,  $J = 14.9, 1\text{H}$ ), 6.15 (br s, 1H), 6.57 (br d,  $J = 7.3, 1\text{H}$ ), 7.27 (m, 5H), 7.50 (d,  $J = 14.9, 1\text{H}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 23.0, 34.6, 43.7, 52.0, 53.0, 117.7, 127.5, 127.8, 128.7, 138.1, 141.8, 164.1, 170.2, 170.4. HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{16}\text{H}_{21}\text{N}_2\text{O}_4\text{S}^+ [\text{M} + \text{H}]^+$  337.1215, found 337.1221.

**Methyl *N*-acetyl-*S*-[3-phenylmethylamino-3-oxopropyl]-*L*-cysteinate, 1f.** Yield: 91% (99 mg). Oil;  $R_f = 0.66$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 99:1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 1.99 (s, 3H), 2.48 (m, 2H), 2.86 (t,  $J = 7.0, 2\text{H}$ ), 2.96 (d,  $J = 5.1, 2\text{H}$ ), 3.73 (s, 3H), 4.43 (d,  $J = 5.7, 2\text{H}$ ), 4.80 (m, 1H), 6.35 (br s, 1H), 6.72 (br s, 1H), 7.28 (m, 5H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 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 $^+$ )  $m/z$  calcd for  $\text{C}_{16}\text{H}_{23}\text{N}_2\text{O}_4\text{S}^+ [\text{M} + \text{H}]^+$  339.1373, found 339.1379.

**Propanamide g.** Yield: 43% (330 mg). Colorless oil;  $R_f = 0.47$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 95:5). Mixture of rotamers (piperazine ring):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 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, 1H), 7.39 (m, 5H), 7.63 (d,  $J = 7.4, 1\text{H}$ ), 7.69 (br s, 1H), 8.01 (s, 1H), 8.42 (br s, 1H), 11.42 (br s, 1H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 15.3, 29.5, 36.0, 37.0, 40.1, 45.0, 50.1, 74.1, 77.4, 114.3, 116.9, 121.8, 122.9, 126.1, 127.0, 127.1, 128.8, 130.2, 135.1, 135.2, 136.4, 153.3, 166.2, 167.5, 171.5, 185.6. HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{29}\text{H}_{30}\text{N}_5\text{O}_5^+ [\text{M} + \text{H}]^+$  528.2241, found 528.2242.

**Adduct of methyl *N*-acetyl-*L*-cysteinate and propanamide g (1g).** Yield: 98% (80 mg). Yellowish oil;  $R_f = 0.19$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 95:5). Mixture of rotamers:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 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,  $J = 9.9, 1\text{H}$ ), 6.48 (br s, 1H), 6.72 (d,  $J = 9.7, 1\text{H}$ ), 7.29 (m, 1H), 7.39 (m, 5H), 7.64 (d,  $J = 7.3, 1\text{H}$ ), 7.73 (d,  $J = 6.9, 1\text{H}$ ), 7.92 (br s, 1H), 8.02 (m, 1H), 8.42 (br s, 1H), 11.33 (s, 1H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 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, 136.2, 136.6, 144.7, 167.2, 167.4, 167.5, 170.4, 170.5, 170.8, 185.6. HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{35}\text{H}_{41}\text{N}_6\text{O}_8\text{S}^+ [\text{M} + \text{H}]^+$  705.2701, found 705.2699.

**Adduct of *L*-cysteine and propanamide g (2g).** Yield: 97% (103 mg). Yellowish oil;  $R_f = 0.33$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{TFA}$ , 80:15:5). Mixture of rotamers:  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ) 1.21 (br s, 3H), 1.72 (quin,  $J = 6.9, 2\text{H}$ ), 2.71–4.60 (m, 13H), 4.22 (s, 1H), 5.95 (d,  $J = 9.8, 1\text{H}$ ), 6.90 (d,  $J = 10.0, 1\text{H}$ ), 7.40 (m, 6H), 7.82 (d,  $J = 7.6, 1\text{H}$ ), 8.05 (d,  $J = 3.3, 1\text{H}$ ), 8.29 (br s, 1H), 8.75 (t,  $J = 5.6, 1\text{H}$ ), 12.20 (d,  $J = 12.9, 1\text{H}$ ).  $^{13}\text{C}$  NMR

(DMSO-*d*<sub>6</sub>) 14.9, 16.0, 28.8, 29.3, 32.2, 36.8, 36.9, 37.0, 37.4, 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<sub>32</sub>H<sub>35</sub>N<sub>6</sub>O<sub>7</sub>S<sup>-</sup> [M – H]<sup>-</sup> 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.....

<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of the new compounds and 2D NMR spectra of **1c**

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### Notes

The authors declare no competing financial interest.

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## REFERENCES

(1) For very recent reviews, see: (a) Tsuchikama, K.; An, Z. Antibody-Drug Conjugates: Recent Advances in Conjugation and Linker Chemistries. *Protein Cell* **2018**, *9*, 33–46. (b) Rodrigues, T.; Bernardes, G. J. L. Development of Antibody-Directed Therapies: Quo Vadis? *Angew. Chem., Int. Ed.* **2018**, *57*, 2032–2034. (c) Lambert, J. M.; Berkenblit, A. Antibody–Drug Conjugates for Cancer Treatment. *Annu. Rev. Med.* **2018**, *69*, 191–207. (d) Beck, A.; Goetsch, L.; Dumontet, C.; Corvaia, N. Strategies and Challenges for the Next Generation of Antibody–Drug Conjugates. *Nat. Rev. Drug Discov.* **2017**, *16*, 315–337. (e) Chudasama, V.; Maruani, A.; Caddick, S. Recent Advances in the Construction of Antibody–Drug Conjugates. *Nat. Chem.* **2016**, *8*, 114–119.

(2) For reviews, see: (a) Sletten, E. M.; Bertozzi, C. R. Bioorthogonal Chemistry: Fishing for Selectivity in a Sea of Functionality. *Angew. Chem., Int. Ed.* **2009**, *48*, 6974–6998. (b) Kalia, J.; Raines, R. T. Advances in Bioconjugation. *Curr. Org. Chem.* **2010**, *14*, 138–147. (c) Yousuke, T.; Akio, O.; Itaru, H. Protein Organic Chemistry and Applications for Labeling and Engineering in Live-Cell Systems. *Angew. Chem., Int. Ed.* **2013**, *52*, 4088–4106. (d) Tang, W.; Becker, M. L. “Click” Reactions: A Versatile Toolbox for the Synthesis of Peptide-Conjugates. *Chem. Soc. Rev.* **2014**, *43*, 7013–7039. (e) Krall, N.; Cruz, F. P.; Boutureira, O.; Bernardes, G. J. L. Site-Selective Protein-Modification Chemistry for Basic Biology and Drug Development. *Nat. Chem.* **2015**, *8*, 103–113.

(3) For reviews, see: (a) Chalker, J. M.; Bernardes, G. J.; Lin, Y. A.; Davis, B. G. Chemical Modification of Proteins at Cysteine: Opportunities in Chemistry and Biology. *Chem. Asian J.* **2009**, *4*, 630–640. (b) Jain, N.; Smith, S. W.; Ghone, S.; Tomczuk, B. Current ADC Linker Chemistry. *Pharm. Res.* **2015**, *32*, 3526–3540. (c) Jackson, D. Y. Processes for Constructing Homogeneous Antibody Drug Conjugates. *Org. Proc. Res. Dev.* **2016**, *20*, 852–866.

(4) (a) Baldwin, A. D.; Kiick, K. L. Tunable Degradation of Maleimide–Thiol Adducts in Reducing Environments. *Bioconjug. Chem.* **2011**, *22*, 1946–1953. (b) Shen, B.-Q.; Xu, K.; Liu, L.; Raab, H.; Bhakta, S.; Kenrick, M.; Parsons-Reponte, K. L.; Tien, J.; Yu, S.-F.; Mai, E.; Li, D.; Tibbits, J.; Baudys, J.; Saad, O. M.; Scales, S. J.; McDonald, P. J.; Hass, P. E.; Eigenbrot, C.; Nguyen, T.; Solis, W. A.; Fujii, R. N.; Flagella, K. M.; Patel, D.; Spencer, S. D.; Khawli, L. A.; Ebens, A.; Wong, W. L.; Vandlen, R.; Kaur, S.; Sliwkowski, M. X.; Scheller, R. H.; Polakis, P.; Junutula, J. R. Conjugation Site Modulates the in Vivo Stability and Therapeutic Activity of Antibody–Drug Conjugates. *Nat. Biotechnol.* **2012**, *30*, 184–189. (c) Ponte, J. F.; Sun, X.; Yoder, N. C.; Fishkin, N.; Laleau, R.; Coccia, J.; Lanieri, L.; Bogalhas, M.; Wang, L.; Wilhelm, S.; Widdison, W.; Pinkas, J.; Keating, T. A.; Chari, R.; Erickson, H. K.; Lambert, J. M. Understanding How the Stability of the Thiol–Maleimide Linkage Impacts the Pharmacokinetics of Lysine-Linked Antibody–Maytansinoid Conjugates. *Bioconjug. Chem.* **2016**, *27*, 1588–1598, and references therein. Also see: (d) Raycroft, M. A. R.; Racine, K. É.; Rowley, C. N.; Keillor, J. W. Mechanisms of Alkyl and Aryl Thiol Addition to *N*-Methylmaleimide. *J. Org. Chem.* **2018**, *83*, 11674–11685, and references therein.

(5) Several ingenious alternatives (dibromomaleimides, in situ transformations of the adducts, etc.) have been developed to overcome the maleimide drawbacks—retro-Michael/thiol exchange, partial hydrolysis, etc.—and to improve the homogeneity of drug–antibody ratios and site-specific loading: (a) Robin, M. P.; Wilson, P.; Mabire, A. B.; Kiviaho, J. K.; Raymond, J. E.; Haddleton, D. M.; O'Reilly, R. K. Conjugation-Induced Fluorescent Labeling of Proteins and Polymers Using Dithiomaleimides. *J. Am. Chem. Soc.* **2013**, *135*, 2875–2878. (b) Castaneda, L.; Maruani, A.; Schumacher, F. F.; Miranda, E.; Chudasama, V.; Chester, K. A.; Baker, J. R.; Smith, M. E. B.; Caddick, S. Acid-Cleavable Thiomaleamic Acid Linker for Homogeneous Antibody–Drug Conjugation. *Chem. Comm.* **2013**, *49*, 8187–8189. (c) Lyon, R. P.; Setter, J. R.; Bovee, T. D.; Doronina, S. O.; Hunter, J. H.; Anderson, M. E.; Balasubramanian, C. L.; Duniho, S. M.; Leiske, C. I.; Li, F.; Senter, P. D. Self-Hydrolyzing Maleimides Improve the Stability and Pharmacological Properties of Antibody-Drug Conjugates. *Nat. Biotechnol.* **2014**, *32*, 1059–1065. (d) Tumey, L. N.; Charati, M.; He, T.; Sousa, E.; Ma, D.; Han, X.; Clark, T.; Casavant, J.; Loganzo, F.; Barletta, F.; Lucas, J.; Graziani, E. I. Mild Method for Succinimide Hydrolysis on ADCs: Impact on ADC Potency, Stability, Exposure, and Efficacy. *Bioconjug. Chem.* **2014**, *25*, 1871–1880. (e) Fontaine, S. D.; Reid, R.; Robinson, L.; Ashley, G. W.; Santi, D. V. Long-Term Stabilization of Maleimide–Thiol Conjugates.

1  
2 *Bioconjug. Chem.* **2015**, *26*, 145–152. (f) Nunes, J. P. M.; Morais, M.; Vassileva, V.; Robinson, E.; Rajkumar, V. S.; Smith, M. E. B.; Pedley, R.  
3 B.; Caddick, S.; Baker, J. R.; Chudasama, V. Functional Native Disulfide Bridging Enables Delivery of a Potent, Stable and Targeted Antibody-  
4 Drug Conjugate (ADC). *Chem. Comm.* **2015**, *51*, 10624–10627. (g) Kalia, D.; Malekar, P. V.; Parthasarathy, M. Exocyclic Olefinic Maleimides:  
5 Synthesis and Application for Stable and Thiol-Selective Bioconjugation. *Angew. Chem., Int. Ed.* **2016**, *55*, 1432–1435. (h) Kalia, D.; Pawar, S.  
6 P.; Topate, J. S. Stable and Rapid Thiol Bioconjugation by Light-Triggered Thiomaleimide Ring Hydrolysis. *Angew. Chem., Int. Ed.* **2017**, *56*,  
7 1885–1889. (i) Chen, Z.; Boyd, S. D.; Calvo, J. S.; Murray, K. W.; Mejia, G. L.; Benjamin, C. E.; Welch, R. P.; Winkler, D. D.; Meloni, G.;  
8 D'Arcy, S.; Gassensmith, J. J. Fluorescent Functionalization across Quaternary Structure in a Virus-Like Particle. *Bioconjug. Chem.* **2017**, *28*,  
9 2277–2283. (j) Morais, M.; Nunes, J. P. M.; Karu, K.; Forte, N.; Benni, I.; Smith, M. E. B.; Caddick, S.; Chudasama, V.; Baker, J. R.  
10 Optimisation of the Dibromomaleimide (DBM) Platform for Native Antibody Conjugation by Accelerated Post-Conjugation Hydrolysis. *Org.*  
11 *Biomol. Chem.* **2017**, *15*, 2947–2952. (k) Levengood, M. R.; Zhang, X.; Hunter, J. H.; Emmerton, K. K.; Miyamoto, J. B.; Lewis, T. S.; Senter,  
12 P. D. Orthogonal Cysteine Protection Enables Homogeneous Multi-Drug Antibody–Drug Conjugates. *Angew. Chem., Int. Ed.* **2017**, *56*, 733–  
13 737, and references therein.

14  
15 (6) (a) Truong, V. X.; Dove, A. P. Organocatalytic, Regioselective Nucleophilic "Click" Addition of Thiols to Propiolic Acid Esters for  
16 Polymer–Polymer Coupling. *Angew. Chem., Int. Ed.* **2013**, *52*, 4132–4136. (b) Badescu, G.; Bryant, P.; Swierkosz, J.; Khayrzad, F.; Pawlisz,  
17 E.; Farys, M.; Cong, Y.; Muroli, M.; Rumpf, N.; Brocchini, S.; Godwin, A. A New Reagent for Stable Thiol-Specific Conjugation. *Bioconjug.*  
18 *Chem.* **2014**, *25*, 460–469. (c) Nair, D. P.; Podgórski, M.; Chatani, S.; Gong, T.; Xi, W.; Fenoli, C. R.; Bowman, C. N. The Thiol-Michael  
19 Addition Click Reaction: A Powerful and Widely Used Tool in Materials Chemistry. *Chem. Mater.* **2014**, *26*, 724–744. (d) McKay, C. S.;  
20 Finn, M. G. Click Chemistry in Complex Mixtures: Bioorthogonal Bioconjugation. *Chem. Biol.* **2014**, *21*, 1075–1101. (e) Li, X.; Li, H.;  
21 Zhuang, J.; Li, H.; Wang, W. A Mild and Selective Protecting and Reversed Modification of Thiols. *Tetrahedron Lett.* **2016**, *57*, 2660–2663.  
22 (f) Liu, Y.; Lai, Z.; Yang, P.; Xu, Y.; Zhang, W.; Liu, B.; Lu, M.; Chang, H.; Ding, T.; Xu, H. Thio-Michael Addition of  $\alpha,\beta$ -Unsaturated  
23 Amides Catalyzed by Nmm-Based Ionic Liquids. *RSC Adv.* **2017**, *7*, 43104–43113. (g) Sun, Y.; Zeng, X.; Xiao, Y.; Liu, C.; Zhu, H.; Zhou, H.;  
24 Chen, Z.; Xu, F.; Wang, J.; Zhu, M.; Wu, J.; Tian, M.; Zhang, H.; Deng, Z.; Cheng, Z.; Hong, X. Novel Dual-Function Near-Infrared II  
25 Fluorescence and PET Probe for Tumor Delineation and Image-Guided Surgery. *Chem. Sci.* **2018**, *9*, 2092–2097. And references cited therein.

26  
27 (7) (a) Crisp, G. T.; Millan, M. J. Conjugate Addition of Amino Acid Side Chains to Alkynes and Alkynoic Acid Derivatives.  
28 *Tetrahedron* **1998**, *54*, 637–648. (b) Owen, T. C. Thiol Detection, Derivatization and Tagging at Micromole to Nanomole Levels Using  
29 Propiolates. *Bioorg. Chem.* **2008**, *36*, 156–160. (c) Arcadi, A.; Alfonsi, M.; Marinelli, F. Facile Reaction of Thiols and Amines with Alkyl 4-  
30 Hydroxy-2-Alkynoates in Water under Neutral Conditions and Ultrasound Irradiation. *Tetrahedron Lett.* **2009**, *50*, 2060–2064. (d) Trofimov,  
31 B. A.; Mal'kina, A. G.; Shemyakina, O. A.; Nosyreva, V. V.; Borisova, A. P.; Khutsishvili, S. S.; Krivdin, L. B. Synthesis of Functionalized L-  
32 Cysteine and L-Methionine by Reaction with Electron-Deficient Acetylenes. *Synthesis* **2009**, 3136–3142. (e) Shiu, H.-Y.; Chan, T.-C.; Ho, C.-  
33 M.; Liu, Y.; Wong, M.-K.; Che, C.-M. Electron-Deficient Alkynes as Cleavable Reagents for the Modification of Cysteine-Containing  
34 Peptides in Aqueous Medium. *Chem. Eur. J.* **2009**, *15*, 3839–3850. (f) Randive, N. A.; Kumar, V.; Nair, V. A. A. Facile Approach to  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2 Substituted Acrylates by Regioselective and Stereoselective Addition of Thiols and Amines to an Alkynyl Ester in Water. *Monatsh. Chem.*  
3  
4 **2010**, *141*, 1329–1332. (g) Koniev, O.; Leriche, G.; Nothisen, M.; Remy, J.-S.; Strub, J.-M.; Schaeffer-Reiss, C.; Van Dorselaer, A.; Baati, R.;  
5  
6 Wagner, R. A. Selective Irreversible Chemical Tagging of Cysteine with 3-Arylpropionitriles. *Bioconjug. Chem.* **2014**, *25*, 202–206.

7  
8 (8) (a) Faja, M.; Ariza, X.; Gálvez, C.; Vilarrasa, J. Reaction of Uridines and Thymidines with Methyl Propynoate. A New N-3 Protecting  
9  
10 Group. *Tetrahedron Lett.* **1995**, *36*, 3261–3264. (b) Ariza, X.; Costa, A. M.; Faja, M.; Pineda, O.; Vilarrasa, J. New Protecting Groups for 1,2-  
11  
12 Diols (Boc- and Moc-ethylidene). Cleavage of Acetals with Bases. *Org. Lett.* **2000**, *2*, 2809–2811. (c) Ariza, X.; Pineda, O.; Vilarrasa, J.;  
13  
14 Shipps, G. W.; Ma, Y.; Dai, X. Bocdene and Mocdene Derivatives of Catechols and Catecholamines. *Org. Lett.* **2001**, *3*, 1399–1401. (d)  
15  
16 Arjona, O.; Medel, R.; Rojas, J.; Costa, A. M.; Vilarrasa, J. Chemoselective Protection of Thiols versus Alcohols and Phenols. The Tosvinyl  
17  
18 Group. *Tetrahedron Lett.* **2003**, *44*, 6369–6373. (e) Mola, L.; Font, J.; Bosch, L.; Caner, J.; Costa, A. M.; Etxebarria-Jardí, G.; Pineda, O.;  
19  
20 Vicente, D.; Vilarrasa, J. Nucleophile-Catalyzed Additions to Activated Triple Bonds. Protection of Lactams, Imides, and Nucleosides with  
21  
22 MocVinyl and Related Groups. *J. Org. Chem.* **2013**, *78*, 5832–5842. (f) Petit, E.; Bosch, L.; Font, J.; Mola, L.; Costa, A. M.; Vilarrasa, J.  
23  
24 Tosvinyl and Besvinyl as Protecting Groups of Imides, Azinones, Nucleosides, Sultams, and Lactams. Catalytic Conjugate Additions to  
25  
26 Tosylacetylene. *J. Org. Chem.* **2014**, *79*, 8826–8834.

27  
28 (9) (a) Arjona, O.; Iradier, F.; Medel, R.; Plumet, J. *p*-Toluenesulfonylacetylene as Thiol Protecting Group. *J. Org. Chem.* **1999**, *64*, 6090–  
29  
30 6093; also see Ref. 8d. (b) Medel, R.; Monterde, M. I.; Plumet, J.; Rojas, J. K. Total Stereochemical Control in the Addition of Thiols to *p*-  
31  
32 Toluenesulfonylacetylene. Synthesis of *Z*- and *E*-2-Sulfanylvinylsulfonyl Derivatives. *J. Org. Chem.* **2005**, *70*, 735–738. (c) Medel, R.; Plumet,  
33  
34 J. Addition of Thiols to Sulfonylacetylenes: Synthetic Applications. *Synthesis* **2006**, 1339–1342.

35  
36 (10) (a) Coppola, G. M.; Damon, R. E. Acetylenic Amides. 1. Synthesis of *N*-Substituted-2-propynamides, *Synth. Commun.* **1993**, *23*,  
37  
38 2003–2010. (b) Safronova, L. P.; Medvedeva, A. S.; Klyba, L. V.; Bochkarev, V. N.; Andreev, M. V. Synthesis and Mass-Spectrometric  
39  
40 Investigation of *N*-Aryl-3-trimethylsilyl-2-propyne-1-carboxamides. *Russ. J. Org. Chem.* **2000**, *36*, 184–190.

41  
42 (11) (a) Awoonor-Williams, E.; Rowley, C. N. Evaluation of Methods for the Calculation of the  $pK_a$  of Cysteine Residues in Proteins. *J.*  
43  
44 *Chem. Theory Comput.* **2016**, *12*, 4662–4673. (b) Bulaj, G.; Kortemme, T.; Goldenberg, D. P. Ionization–Reactivity Relationships for  
45  
46 Cysteine Thiols in Polypeptides. *Biochemistry* **1998**, *37*, 8965–8972.

47  
48 (12) When **1c** (a nearly 1:1 diastereomeric mixture) was prepared in CD<sub>3</sub>OD at rt, the solvent was evaporated under vacuum without  
49  
50 heating, and the residue was analyzed by NMR spectroscopy in CDCl<sub>3</sub> (including NOE experiments), both 4'-CH<sub>2</sub> protons of both  
51  
52 diastereoisomers appeared to have been partially exchanged with deuterium (see **1c-d**<sub>2</sub>).

53  
54 (13) (a) Kissane, M.; Murphy, M.; O'Brien, E.; Chopra, J.; Murphy, L.; Collins, S. G.; Lawrence, S. E.; Maguire, A. R. Addition-  
55  
56 Substitution Reactions of 2-Thio-3-Chloroacrylamides with Carbon, Nitrogen, Oxygen, Sulfur and Selenium Nucleophiles. *Org. Biomol.*  
57  
58 *Chem.* **2011**, *9*, 2452–2472, and references therein. (b) Pil'ov, S. G.; Brovarets, V. S.; Vinogradova, T. K.; Golovchenko, A. V.; Drach, B. S.  
59  
60 Synthesis of New 5-Mercapto-1,3-Oxazole Derivatives on the Basis of 2-Acylamino-3,3-Dichloroacrylonitriles and Their Analogs. *Russ. J.*  
*Gen. Chem.* **2002**, *72*, 1714–1723.



1  
2 (14) For quite different alternatives to the use of maleimides and other activated double bonds, see: Toda, N.; Asano, S.; Barbas, C. F.  
3  
4 Rapid, Stable, Chemoselective Labeling of Thiols with Julia–Kocienski-Like Reagents: A Serum-Stable Alternative to Maleimide-Based  
5  
6 Protein Conjugation. *Angew. Chem., Int. Ed.* **2013**, *52*, 12592–12596.

7  
8 (15) For related comparisons, see: Palkowitz, M. D.; Tan, B.; Hu, H.; Roth, K.; Bauer, R. A. Synthesis of Diverse *N*-Acryloyl Azetidines  
9  
10 and Evaluation of Their Enhanced Thiol Reactivities. *Org. Lett.* **2017**, *19*, 2270–2273.

11  
12 (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  
13  
14 work with SCH=CHCONHPh adducts.

15 (17) (a) Blair, W. S.; Deshpande, M.; Fang, H.; Lin, P.-F.; Spicer, T. P.; Wallace, O. B.; Wang, H.; Wang, T.; Zhang, Z.; Yeung, K.-S.  
16  
17 Preparation of Antiviral Indoleoxoacetyl Piperazine Derivatives. PCT Int. Appl. WO 2000076521 A1 20001221. (b) Wallace, O. B.; Wang, T.;  
18  
19 Yeung, K.-S.; Pearce, B. C.; Meanwell, N. A.; Qiu, Z.; Fang, H.; Xue, Q. M.; Yin, Z. Preparation and Antiviral Activity of Substituted  
20  
21 Piperazinyloxoacetylindole Derivatives. US Patent 20030069245 A1 20030410.

22 (18) For comparisons of azide reduction methods, see: (a) Bartra, M.; Romea, P.; Urpí, F.; Vilarrasa, J. A Fast Procedure for the Reduction  
23  
24 of Azides and Nitro Compounds Based on the Reducing Ability of Sn(SR)<sub>3</sub><sup>-</sup> Species. *Tetrahedron* **1990**, *46*, 587–594. (b) Amantini, D.;  
25  
26 Fringuelli, F.; Pizzo, F.; Vaccaro, L. Selected Methods for the Reduction of the Azido Group. *Org. Prep. Proc. Int.* **2002**, *34*, 109–147.

27 (19) This compound is known. See the Supporting Information of: Healy, J.; Ekkerman, S.; Pliotas, C.; Richard, M.; Bartlett, W.;  
28  
29 Grayer, S. C.; Morris, G. M.; Miller, S.; Booth, I. R.; Conway, S. J.; Rasmussen, T. Understanding the Structural Requirements for  
30  
31 Activators of the Kef Bacterial Potassium Efflux System. *Biochemistry* **2014**, *53*, 1982–1992.  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
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