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Reactions of 1,3-Diketones with a Dipeptide Isothiazolidin-3-one: Toward Agents That Covalently Capture Oxidized Protein Tyrosine Phosphatase 1B

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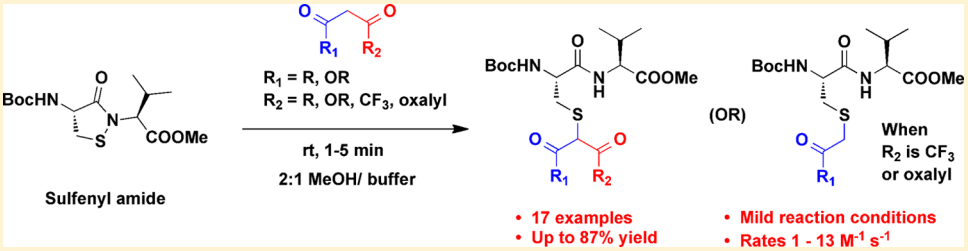
Supporting Information



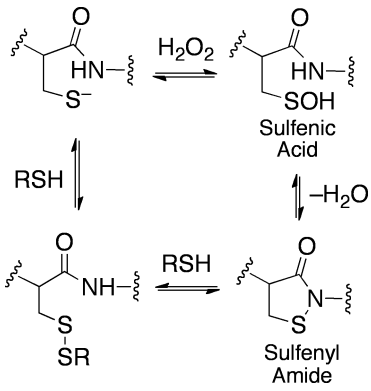
ABSTRACT: Protein tyrosine phosphatase 1B (PTP1B) is a validated therapeutic target for the treatment of type 2 diabetes; however, the enzyme has been classified by some as an "undruggable target". Here we describe studies directed toward the development of agents that covalently capture the sulfenyl amide "oxoform" of PTP1B generated during insulin signaling events. The sulfenyl amide residue found in oxidized PTP1B presents a unique electrophilic sulfur center that may be exploited in drug and probe design. Covalent capture of oxidized PTP1B could permanently disable the intracellular pool of enzyme involved in regulation of insulin signaling. Here, we employed a dipeptide model of oxidized PTP1B to investigate the nucleophilic capture of the sulfenyl amide residue by structurally diverse 1,3-diketones. All of the 1,3-diketones examined here reacted readily with the electrophilic sulfur center in the sulfenyl amide residue to generate stable covalent attachments. Several different types of products were observed, depending upon the substituents present on the 1,3-diketone. The results provide a chemical foundation for the development of agents that covalently capture the oxidized form of PTP1B generated in cells during insulin signaling events.

INTRODUCTION

The enzyme protein tyrosine phosphatase 1B (PTP1B) is a major negative regulator of the insulin signaling pathway by virtue of its ability to dephosphorylate the insulin receptor and insulin receptor substrates.[1−3] Inhibitors of PTP1B potentiate insulin signaling and decrease blood glucose levels.[3] Indeed, the enzyme PTP1B is considered a validated therapeutic target for the treatment of type 2 diabetes.[3,4] However, despite nearly 15 years of intense effort, no inhibitors of this enzyme are currently in clinical use or clinical trials.[4] This collective failure may be traced to the dual challenge of identifying bioavailable inhibitors that target the highly polar PTP active site and also display specificity among members of this highly homologous enzyme family.[4]

Detailed considerations of the insulin signaling pathway suggest an alternative to traditional reversible inhibitors for the chemical knockdown of PTP1B activity in cells. A burst of hydrogen peroxide induced by stimulation of the insulin receptor inactivates PTP1B via oxidation of the active site cysteine to an isothiazolidin-3-one residue, often termed a sulfenyl amide (Scheme 1).[5−9] Subsequent reactions with cellular thiols regenerate the active enzyme (Scheme 1).[8,10−12] The transient inactivation of PTP1B helps regulate the intensity and duration of cellular responses to insulin. The structure of

Scheme 1



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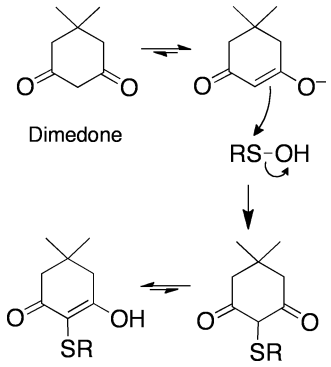
oxidized PTP1B ($PTP1B_{ox}$) is quite different from that of the native enzyme.[6,7] Perhaps more importantly, the sulfenyl amide residue in $PTP1B_{ox}$ presents a unique electrophilic sulfur center that may be exploited in drug and probe design.

An agent capable of irreversibly capturing $PTP1B_{ox}$ could selectively incapacitate the cellular pool of PTP1B involved in the regulation of insulin signaling. Along these lines, Tonks and co-workers showed that an antibody against $PTP1B_{ox}$, capable of preventing reductive regeneration of the catalytically active enzyme, potentiated phosphorylation of the insulin receptor and insulin signaling in H3T3 cells.[13] Despite increasing use of antibodies as therapeutic agents, it may be advantageous to identify small molecules that irreversibly capture $PTP1B_{ox}$.

Shiau et al. examined the covalent capture of $PTP1B_{ox}$ by various low-molecular weight thiols using a dipeptide model for the oxidized enzyme.[14] Presumably, however, most thiols will not irreversibly capture $PTP1B_{ox}$. Instead, treatment of the oxidized enzyme with a molar excess of thiol-containing agents is expected to simply regenerate the active enzyme via a labile disulfide intermediate as illustrated in Scheme 1.[8,10−12] While sulfur−sulfur bonds generated by the reaction of thiols with the sulfenyl amide are typically labile under physiological conditions, one can envision that carbon−sulfur bonds forged by the reaction of carbon acids with $PTP1B_{ox}$ might be relatively stable under physiological conditions. Along these lines, in the 1970s, Allison showed that 5,5-dimethyl-1,3-cyclohexadione (dimedone) generates stable adducts via reaction with the electrophilic sulfur center in protein sulfenic acid residues (Scheme 2).[15,16] Furdui's group used β-ketoesters

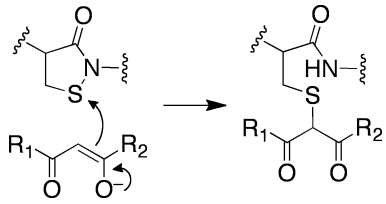
Scheme 2



to trap a sulfenic acid residue in the peroxidase AhpC.[17] Shiau et al. reported the reaction of 1,3-cyclopentadione with the dipeptide model for $PTP1B_{ox}$, though the stability of the resulting adduct was not characterized.[14] Finally, Carroll's group elegantly demonstrated the potential of dimedone derivatives for the covalent capture of $PTP1B_{ox}$ in proteomic analyses.[18,19]

In the work described here, we employed a dipeptide isothiazolidin-3-one as a low-molecular weight model for $PTP1B_{ox}$ to investigate fundamental chemical processes associated with the covalent capture of the sulfenyl amide residue. We examined the reaction of this model peptide with a series of structurally diverse 1,3-diketones and found that all reacted readily with the electrophilic sulfur center in the sulfenyl amide residue to generate stable covalent attachments (Scheme 3). Several different types of products were observed, depending upon the substituents present on the 1,3-diketone. The results provide a solid chemical foundation for the

Scheme 3



development of agents that covalently capture the oxidized form of PTP1B that is generated in cells during insulin signaling events.

RESULTS AND DISCUSSION

Synthesis and Characterization of the Dipeptide Sulfenyl Amide (4). We prepared the dipeptide sulfenyl amide **4** by the general route of Morin and co-workers involving coupling of *N*-Boc-protected cystine **2** with valine methyl ester, followed by treatment of the disulfide-bridged dipeptide **3** with Br_2 in dichloromethane containing pyridine (Scheme 4).[20] The desired sulfenyl amide **4** was separated by column chromatography in 72% yield from other oxidation products, including the sulfinyl and sulfonyl amides **5** and **6**, respectively (17 and 6% yields, respectively), and was characterized by ^{1}H NMR, ^{13}C NMR, high-resolution mass spectrometry, and single-crystal X-ray crystallography.[21]

Reaction with Excess Thiol Regenerates the Native Cysteine Residue in 4. Our previous work with PTP1B showed that cysteine is an effective reagent for conversion of $PTP1B_{ox}$ to the catalytically active enzyme containing a native thiol group at Cys215 within the active site.[10] To confirm that our model compound **4** recapitulated this important redox transformation of $PTP1B_{ox}$, we treated the dipeptide sulfenyl amide with 5 equiv of D,L-cysteine. For this reaction (and most of the reactions described in this work), we employed a 2:1 methanol/HEPES buffer (50 mM, pH 7) solvent system containing NaCl (100 mM) and EDTA (1 mM). Under these predominantly organic solvent conditions, the sulfenyl amide **4** persists for several hours. We found that the reaction of **4** with D,L-cysteine (5 equiv) rapidly (within ∼1 min) gave the reduced Cys-Val dipeptide **7**, in 85% yield (Scheme 5).

Reactions of Aliphatic 1,3-Diketones with 4. Treatment of **4** with the aliphatic 1,3-diketone carbon acids **A**, **B**, **C**, and **D** in the 2:1 methanol/buffer solvent mixture described above afforded thioether products arising from presumed ionization of the carbon acid to form the corresponding enolate, followed by attack of the carbon nucleophile on the electrophilic sulfur center of the sulfenyl amide (Table 1). Adducts **8a−c** generated by reaction of **4** with β-diketone acetylacetone **A**, the β-keto ester methyl acetoacetate **B**, and the β-diester dimethyl malonate **C** were produced in comparable yields (70−78%). The NMR spectra of **8a** and **8b** suggested that these products exist exclusively as the enol tautomers. For example, in the ^{1}H NMR spectrum of **8a** ($CDCl_3$), the enolic -OH proton was observed at ∼17 ppm, while no resonance corresponding to the C−H_α proton of the keto tautomer was seen.[22] Although β-keto esters typically exist in the keto form in organic solvents,[23] evidently the presence of the α-thio group in **8b** promotes enolization. On the other hand, the ^{1}H NMR spectrum of **8c** indicated that this product exists exclusively in keto form, as no signals downfield from the residual $CHCl_3$ in the $CDCl_3$ NMR solvent were observed. A resonance at ∼4.4 ppm was consistent

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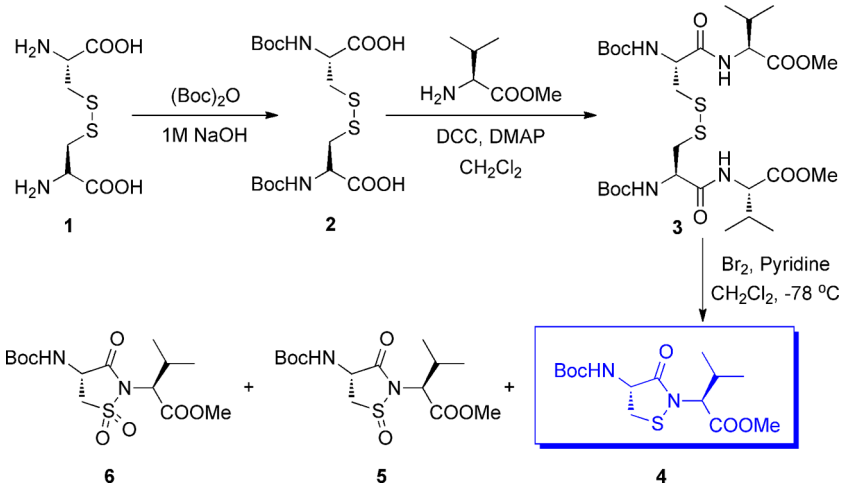
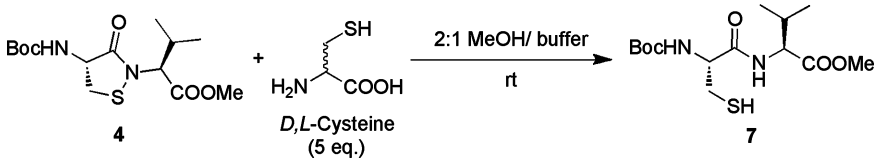
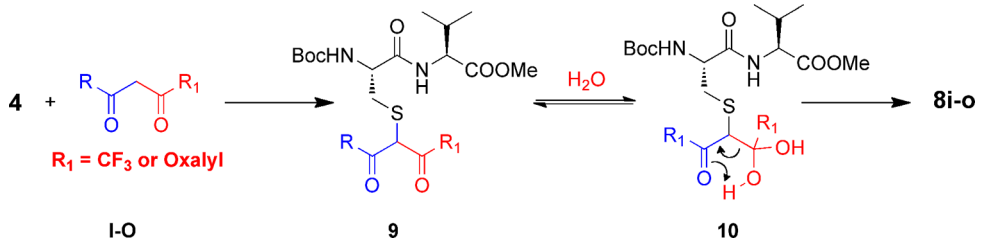
Scheme 4



Scheme 5



Scheme 6



with the presence of an α-proton. Peak splitting due to the presence of diastereomeric isomers of **8c** was observed, but signals were too poorly resolved to be integrated separately. The product **8d** resulting from the reaction of dimedone with **4** existed in the enol form. This was expected as dimedone exists preferentially as the enol in solution,[24] and previous work by Shiau et al. showed that the analogous product resulting from reaction of 1,3-cyclopentanedione with **4** existed in the enol form.[14]

Reactions of Aryl-Substituted 1,3-Diketones with Sulfenyl Amide 4. Treatment of **4** with aryl 1,3-diketones **E**, **F**, **G**, and **H** afforded the expected adducts **8e**−**h**, respectively, in very good yields [73−87% (Table 1)]. Products **8e** and **8f** exhibited complex ^{1}H NMR spectra in $CDCl_3$, consistent with the presence of both keto and enol forms. Product **8g** lacks an enolizable proton, and the doubling of resonances such as the methyl ester group in the spectra was consistent with a mixture of diastereomeric products. Overlap with neighboring peaks prevented reliable integration of these two methyl ester resonances, but the fact that the two peaks are comparable in height suggested that diastereomers of **8g** formed in a roughly 1:1 ratio.

Reactions of the Model Sulfenyl Amide with 1,3-Diketones Bearing α-Electron-Withdrawing Groups. We felt that it would be of interest to examine the reactivity of the model sulfenyl amide with stronger carbon acids that can afford greater fractions of reactive enolate at neutral pH. Toward this end, we studied the reactions of aryl 1,3-diketones bearing electron-withdrawing α-trifluoromethyl or oxalyl substituents with **4**. In general, 1,3-diketones bearing either a -CF_3 or oxalyl group have aqueous pK_a values between 5.0 and 6.0 (compared to pK_a values of 8.0−9.0 for acyclic 1,3-diketones). We found that the 1,3-diketone compounds **I**−**O** reacted readily with model sulfenyl amide **4** in the buffered methanol solvent system (Table 2). Interestingly, ^{1}H and ^{13}C NMR spectra of products **8i**−**o** were consistent with addition of the enolates to the electrophilic sulfur center of **4** but indicated the absence of the -CF_3 or oxalyl groups. For example, the characteristic splitting pattern associated with the -CF_3 group was absent in the ^{13}C NMR spectra of **8i**−**m**. The absence of the -CF_3 group also was evident in the mass spectra of these compounds.

The generation of products **8i**−**o** presumably involves initial formation of the intermediate **9** (Scheme 6), followed by hydration of the electron-deficient carbonyl. Electron-withdrawing groups are known to increase both the rate and extent of carbonyl hydration.[25−27] Collapse of the hydrated ketone group, in turn, leads to ejection of the enol leaving group (Scheme 6) and net loss of the -CF_3 or oxalyl group from the dipeptide unit. This proposed mechanism is somewhat reminiscent of the decarboxylation of β-keto acids. The 1,3-

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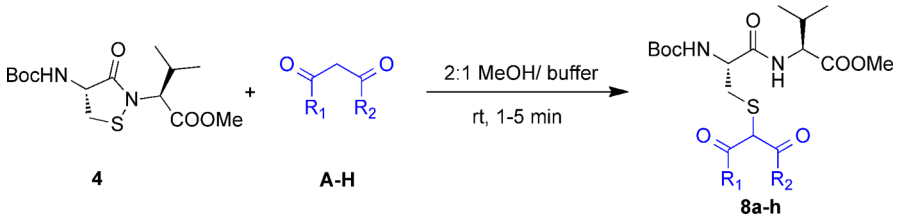
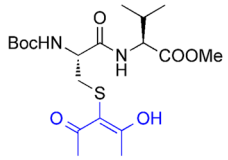
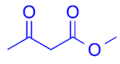
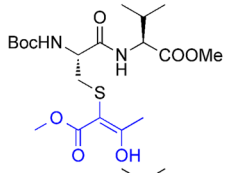
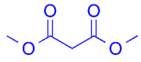
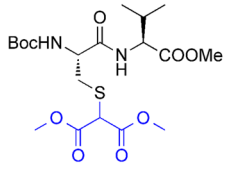
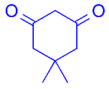
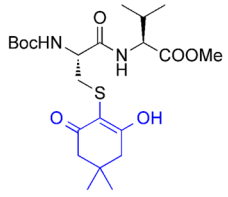
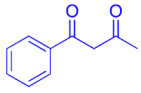
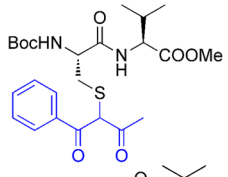
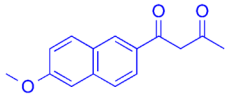
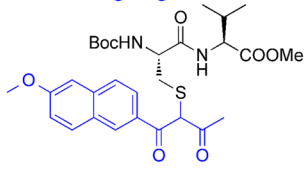
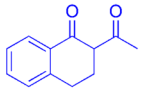
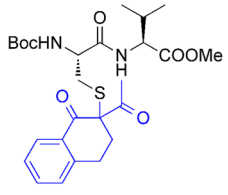
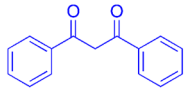
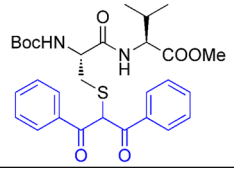
Table 1. Reaction of PTP1B$_{ox}$ Model Compound 4 with Various 1,3-Diketones[a]



4 **A-H** **8a-h**

Entry	Nucleophile		Product		Yield (%)[b]
1		**A**		**8a**	77
2		**B**		**8b**	78
3		**C**		**8c**	70
4		**D**		**8d**	71
5		**E**		**8e**	87
6		**F**		**8f**	81
7		**G**		**8g**	77
8		**H**		**8h**	73

[a]Reaction conditions: **4** (0.060 mmol, 1.0 equiv), **A**–**H** (0.066 mmol, 1.1 equiv) in 3.0 mL of a MeOH/HEPES mixture (2:1) at rt. [b]Yields refer to isolated yield; the compounds were characterized by NMR and mass spectrometry.

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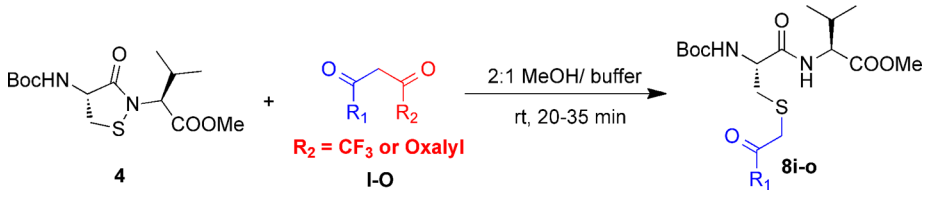
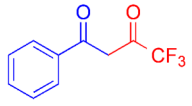
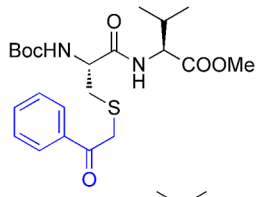
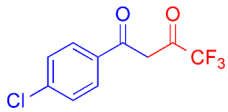
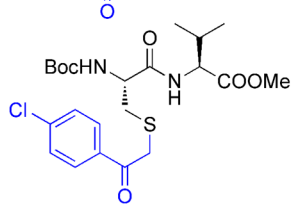
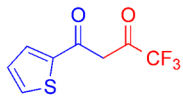
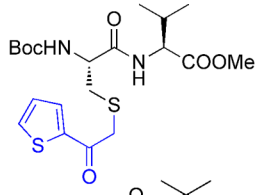
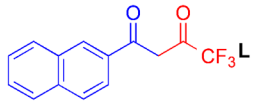
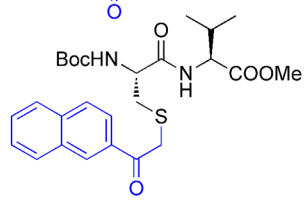
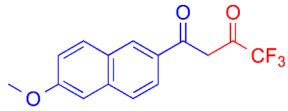
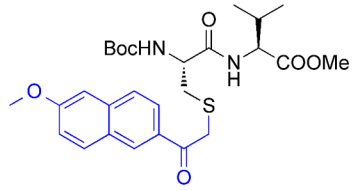
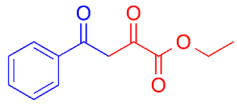
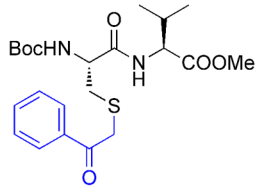
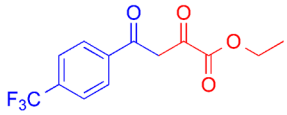
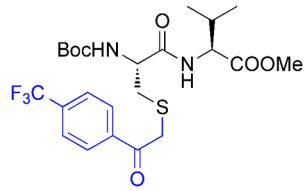
Table 2. Reaction of 4 with Trifluoroketone- and Oxalyl-Containing 1,3-Diketones[a]



Entry	Nucleophile		Product		Yield (%)[b]
1		I		8i	72
2		J		8j	68
3		K		8k	65
4		L		8l	63
5		M		8m	69
6[c]		N		8n	79
7[c]		O		8o	83

[a]Reaction conditions: **4** (0.060 mmol, 1.0 equiv), **A**–**H** (0.066 mmol, 1.1 equiv) in 3.0 mL of a MeOH/HEPES mixture (2:1) at rt. [b]Yields refer to the isolated yield; the compounds were characterized by NMR and mass spectrometry. [c]Reactions took ~6 h for completion.

diketone starting material does not undergo fragmentation under our reaction conditions. This suggests that the α-sulfur residue in the putative intermediate **10** permits the fragmentation reaction, perhaps via stabilization of the developing negative charge in the transition state of the reaction.[28]

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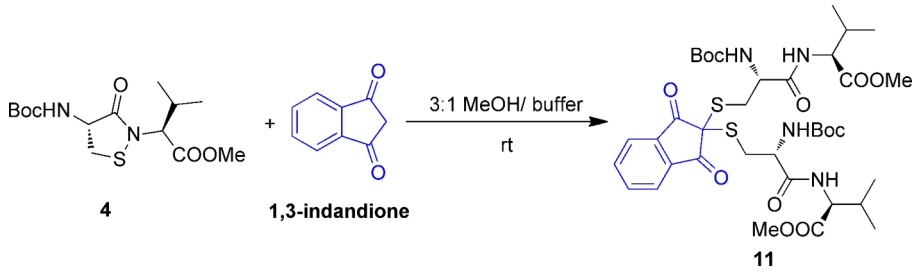
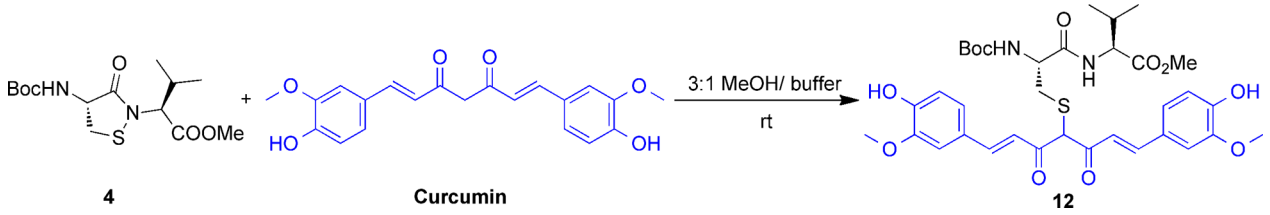
Scheme 7



Scheme 8



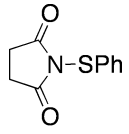
Reaction of 4 with 1,3-Indandione. Interestingly, reaction of **4** with 1,3-indandione exclusively produced a product resulting from addition of 2 equiv of the sulfenyl amide to the enolate [74% yield (Scheme 7)]. This is the only 1,3-diketone for which we observed double addition of sulfenyl amide. Less steric crowding at α-carbon may allow formation of this product in the case of indandione. In contrast, 2-acetyl-4-nitro-1,3-indandione gave no product after 8 h under our standard reaction conditions. This may reflect the highly stabilized and less reactive nature of this enolate.

Reaction of 4 with Curcumin. Reaction of **4** with curcumin (**Q**), a highly conjugated 1,3-diketone,[29] produced a monoadduct similar to those generated by nucleophiles **A**–**H** in 78% yield (Scheme 8). The reaction required 8 h to reach completion. Product **12** formed by the reaction of curcumin with **4** is highly fluorescent.

Reactions of 1,3-Diketones with 4 in Dry Organic Solvents. It is possible that, in the aqueous/organic solvent mixtures used here, reactions proceed via equilibrium amounts of the sulfenic acid generated by reaction of the sulfenyl amide **4** with water (see the equilibrium on the right side of Scheme 1). To address the fundamental reactivity of **4** under conditions under which formation of the corresponding sulfenic acid was unlikely, we examined the reaction of representative 1,3-diketones with **4** in dry organic solvents. We found that acetylacetone **A** (1.2 equiv) reacted cleanly with **4** in dry THF to give **8a** in quantitative yield within 5 min at 24 °C. Similarly, the reactions of **D**, **E**, and **H** (1.1 equiv) with **4** in dry acetone containing K_2CO_3 (0.2 equiv) for 5 min at 24 °C provided the expected products **8d**, **8e**, and **8h** in 97, 93, and 86% yields, respectively. The rapid reactions of **A**, **D**, **E**, and **H** with **4** in dry solvent to give the same adducts (**8a**, **8d**, **8e**, and **8h**, respectively) obtained in the aqueous solvent mixtures provide evidence that the sulfenic acid is not an obligate intermediate in the reactions described here, further suggesting that 1,3-diketone-derived nucleophiles can react directly with the electrophilic sulfur residue in **4**.

The Products Resulting from Covalent Capture of Sulfenyl Amide 4 by 1,3-Diketones Are Stable against Thiols. Because the cellular milieu contains millimolar concentrations of the thiols such as glutathione,[30] any molecular probe or therapeutic agent designed to covalently capture oxidized sulfur centers in proteins must forge a bond to sulfur that is resistant to thiol-mediated cleavage. Therefore, we tested representative members of each product class for stability against the potent thiol reducing agent 1,4-dithio-D-threitol (DTT). Products **8a**, **8e**, **8g**, **8i**, and **8k** were incubated with DTT (50 mM) at 45 °C for 6 h (2:1 methanol/buffer), and no reaction was observed as judged by thin layer chromatographic analysis (TLC). These results indicate that adducts formed by attack of 1,3-diketone-derived carbon nucleophiles on the sulfenyl amide group are stable in the presence of thiol-containing reagents.

Reaction of 1,3-Diketones with Other Sulfur Electrophiles. To assess the relative reactivity of the electrophilic sulfur center in the sulfenyl amide residue of **4**, we conducted parallel reactions of carbon acids **E**, **F**, **L**, and **N** with the electrophilic sulfur centers in disulfide **3** and *N*-(phenylthio)-succinimide **13**. The reactions of **3** with these 1,3-diketones showed no progress even after 6 h as judged by TLC. Similarly, the reaction of **13** with these nucleophiles did not occur after 30 min, although after 10 h small amounts of new products were evident. Interestingly, in the reaction of **D** with **13**, substantial amounts of product were observed after 6 h.



13

Determination of Kinetics for the Reaction of 4 with 1,3-Diketones. We next set out to determine rate constants associated with covalent capture of the dipeptide sulfenyl amide by various 1,3-diketones. Toward this end, we designed a colorimetric assay to monitor sulfenyl amide concentration spectrophotometrically. This assay employed 2-nitro-5-sulfanylbenzoic acid (TNB), the reduced form of Ellman's reagent, to capture unreacted **4**.[31] In this assay, the 1,3-diketone nucleophile and sulfenyl amide were allowed to react under conditions similar to those employed in the syntheses of the thioether adducts, consisting of a 1:1 (v/v) mixture of methanol

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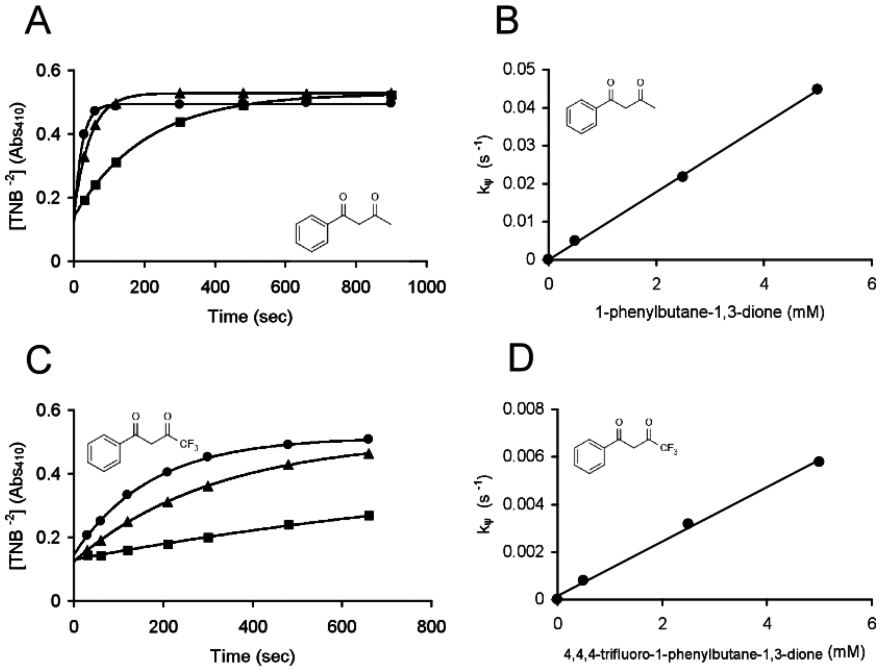

Figure 1. Reaction kinetics for trapping sulfenyl amide **4** by compounds **E** and **I**. Kinetic runs were conducted at 23 ± 2 °C in a 1:1 methanol/buffer B (50 mM Tris, 50 mM Bis-Tris, 10 mM DTPA, and 100 mM sodium acetate at pH 7.0) mixture under pseudo-first-order conditions (excess 1,3-diketone). (A and C) Sulfenyl amide **4** (50 μM) was allowed to react with either **E** or **I**. At various time points, aliquots were removed from the reaction mixture and assayed for remaining unreacted **4** via dilution into assay buffer containing TNB. Time-dependent consumption of **4** was measured at several different concentrations of 1,3-diketone [0.5 (■), 2.5 (▲), and 5 mM (●)]. (B and D) Pseudo-first-order rate constants depend linearly on the concentration of 1,3-diketone, consistent with a second-order process. Bimolecular rate constants were calculated by division of the corresponding pseudo-first-order rate constants (s^{-1}) by the molar concentration of nucleophile to afford the apparent second-order rate constants in units of M^{-1} s^{-1}. The apparent second-order rate constants calculated at each concentration of 1,3-diketone were averaged and the standard deviations computed: k_{trap} values for **E** of 9.2 ± 0.6 M^{-1} s^{-1} and for **I** of 1.24 ± 0.07 M^{-1} s^{-1}.

and buffer (50 mM Tris, 50 mM Bis-Tris, 100 mM sodium acetate, and 10 mM DTPA at pH 7.0 and 23 ± 2 °C). At selected times, aliquots were removed from the reaction mixture and diluted into an assay buffer (pH 5.0) containing TNB. Reaction of TNB with the remaining **4** afforded a decrease in the TNB absorbance at 410 nm corresponding to the amount of unreacted **4**. Thus, as the reactions of the 1,3-diketone with **4** progressed and the amount of unreacted **4** decreased, an increasing absorbance of TNB was observed in the kinetic assays.

Both **H** and **E** captured the sulfenyl amide in a time- and concentration-dependent manner (Figure 1). The observed pseudo-first-order rate constants (k_{ψ}, s^{-1}) depended linearly on diketone concentration in each case (Figure 1B,D), and both reactions were found to be first-order in nucleophile, consistent with an overall second-order process for which the relevant rate law is

$$\text{rate} = k_{trap}[\mathbf{4}][\text{Nu}] \quad (1)$$

where k_{trap} is the apparent second-order rate constant, [**4**] is the concentration of sulfenyl amide, and [Nu] is the concentration of the carbon nucleophile. The rate constants measured for these processes were 9.2 ± 0.6 M^{-1} s^{-1} for **E** and 1.24 ± 0.07 M^{-1} s^{-1} for **I**. Approximate second-order rate constants were also determined for **A** (12 M^{-1} s^{-1}), **D** (6 M^{-1} s^{-1}), **L** (2 M^{-1} s^{-1}), and two additional 1,3-diketones, **N** (13 M^{-1} s^{-1}) and **O** [3 M^{-1} s^{-1} (Supporting Information)]. In these cases, pseudo-first-order rate constants were determined at a single concentration of 1,3-diketone.

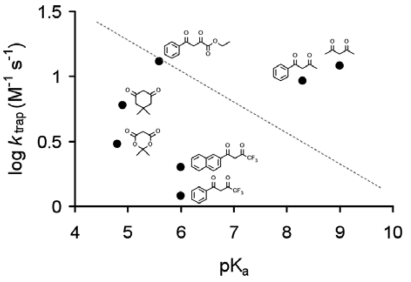

Figure 2. There is no clear correlation between log k_{trap} and 1,3-diketone pK_a (the dashed line represents the type of trend that would be observed if more acidic 1,3-diketones displayed higher reaction rates with **4**).

To explore potential quantitative structure−activity relationships in the reactions of **4** with 1,3-diketones, we measured the aqueous acidity constants for a set of 1,3-diketones (Table 3). We found that the aqueous acidities of the 1,3-diketones measured here spanned a range of roughly 4 orders of magnitude (pK_a = 4.8−9.0). In contrast, the rate constants measured for the reaction of various 1,3-diketones with **4** span only 1 order of magnitude (1.3−13 M^{-1} s^{-1}). A plot of 1,3-diketone pK_a values versus log k_{trap} revealed no correlation between the acidity of the diketone and reaction rate (Figure 2).

■ CONCLUSIONS

Our studies exploited compound **4** as a dipeptide model of the sulfenyl amide residue in PTP1B$_{ox}$. This compound was first

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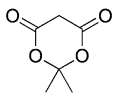
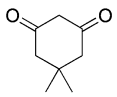
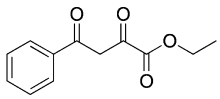
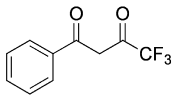
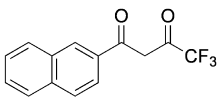
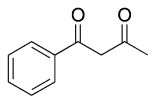
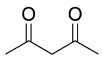
Table 3. Aqueous pK_a Values for Diketones Examined in Kinetic Studies[a]

1,3-diketone	Structure	pK_a
R		4.8
D		4.9
N		5.6
I		6.0
L		6.0
E		8.3
A		9.0

[a]Values were determined spectrophotometrically, as described in the Experimental Section.

synthesized more than 40 years ago by Morin and co-workers as part of early efforts in the synthesis of the cephalosporin antibiotics.[20] We showed that **4** can be readily reduced to the Cys-Val dipeptide **7** by reactions with the biological thiol cysteine. This confirmed that **4** recapitulates central reactions involved in the redox regulation of PTP activity.[10]

All of the structurally diverse 1,3-diketones examined here reacted readily with the electrophilic sulfur center in **4**. The reaction products were consistent with nucleophilic attack of the corresponding enolates on the electrophilic sulfenyl amide residue of **4** (Scheme 3). Four different types of products were generated in these reactions, depending upon the structure of the 1,3-diketone starting material. The 1,3-diketones **C**, **E**–**H**, and **Q** gave cysteine thioether adducts that existed in the diketo form, while the 1,3-diketones **A**, **B**, and **D** gave cysteine thioether adducts that existed in the enol form (Table 1). In the case of 1,3-diketones bearing a trifluoromethylor oxalyl group, we obtained α-thioether monoketone products (Table 2). These products were consistent with initial formation of the expected enolate adducts (similar to those seen in Table 1), followed by hydration of the electron-poor trifluoromethyl ketone or oxalyl ketone and collapse of the hydrated ketone to eject the dipeptide enol thioether (Scheme 6). The compound 1,3-indandione was the only 1,3-diketone in which the major product arose from addition of 2 equiv of sulfenyl amide (Scheme 7).

As noted in the Introduction, biomedical applications require that nucleophilic capture of the sulfenyl amide residue in PTP1B$_{ox}$ generate a bond that is stable under physiological conditions. Critical in this regard is the fact that the bond forged at the electrophilic sulfur atom of the sulfenyl amide must be stable against millimolar concentrations of thiol that are present in the cellular milieu.[30] This requirement argues against the utility of many common sulfur, nitrogen, and trivalent phosphorus nucleophiles that might be used in aqueous media (e.g., thiols, amines, and phosphines). The S–S, S–N, and S–P bonds generated by such nucleophiles are expected to be unstable against thiols and/or water.[10,32] On the other hand, the sulfur–carbon bonds generated by the reaction of 1,3-diketones are expected to be relatively stable. Indeed, we found that products **8a**, **8e**, **8g**, **8i**, and **8k** were stable when they were incubated with the strong thiol reducing agent DTT (50 mM) at 45 °C in a 2:1 methanol/buffer mixture.

We measured the rates at which 1,3-diketones reacted with the sulfenyl amide **4**. Apparent rate constants ranged from 1.3 to 13 M^{-1} s^{-1}. There was no clear correlation between the pK_a of the 1,3-diketone and the observed rates of reaction with **4**. Rather, the reaction rates may reflect a complex interplay of the amount of enolate in solution alongside the steric features and thiophilicity of the enolate. In the case of trifluoromethyl and oxalyl ketones, carbonyl hydration likely serves to decrease the fraction of reactive enolate in solution.[25−27] It is well-established that α,α,α-trifluoromethyl ketones are extensively hydrated (K_{eq} values for trifluoroacetone and trifluoroacetophenone are 32 and 80, respectively).[25−27,33] Hydration of the electron-poor ketones in these compounds dramatically diminishes the acidity of α-protons. On the other hand, methyl ketones are poorly hydrated in solution. For example, the equilibrium constants for hydration of acetone and acetophenone are 10^{-3} and $10^{-5.2}$, respectively.[25−27,33]

We showed that, in the buffered aqueous methanol mixture used for these studies, the 1,3-diketones react selectively with the sulfenyl amide residue over other types of electrophilic sulfur centers. Specifically, the 1,3-diketones **E**, **F**, **L**, and **N** did not react with the disulfide **3** and reacted only slowly with the synthetically used sulfenylating agent *N*-(phenylthio)-succinimide (**13**). It may be important to note that there is no reason to expect that the agents examined here would display chemical selectivity for the sulfenyl amide residue over a sulfenic acid residue. Indeed, the work of Allison and others indicates that 1,3-diketones react readily with the electrophilic sulfur center in protein sulfenic acids to generate a stable sulfur–carbon bond.[15,16] In fact, it is possible that the reactions of **4** in aqueous solution proceed via the equilibrium amounts of the sulfenic acid (see the left side of Scheme 1). Along these lines, it is similarly not known whether the cyclic sulfenyl amide residue at the active site of PTP1B exists in equilibrium with a ring-opened sulfenic acid.[7] Regardless of the exact mechanism, the reactions between **4** and the 1,3-diketones studied here are clearly relevant to the covalent capture of PTP1B$_{ox}$ by small organic molecules.

There are approximately 80 PTPs encoded by the human genome, and developing traditional reversible inhibitors that selectively target only PTP1B among this highly homologous

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family of enzymes has proven to be difficult.[4,34] The approach considered here seeks to exploit specificity that is inherent to cellular signaling processes. The insulin signaling process results in the selective generation of $PTP1B_{ox}$.[5−9] Thus, an agent that is able to covalently capture sulfenyl amide (and possibly sulfenic acid) residues has the potential to selectively target the oxidized cysteine residues at the active sites of PTP enzymes involved in the regulation of insulin-stimulated glucose uptake and utilization. Our results show that the 1,3-diketone unit may offer a versatile platform for the development of agents that irreversibly capture $PTP1B_{ox}$ in cells. Screening of structurally diverse 1,3-diketones ultimately may identify compounds that exploit noncovalent binding interactions to facilitate the covalent capture of $PTP1B_{ox}$.

EXPERIMENTAL SECTION

Materials and Methods. Unless otherwise noted, all reagents were purchased from commercial suppliers and used without further purification. Tetrahydrofuran was distilled under a nitrogen atmosphere over sodium metal with benzophenone ketyl as an indicator. Organic solvents were evaporated using rotary evaporation at 40−45 °C. Column chromatography was performed using silica gel (60 Å, 40−63 μm particle size) as the stationary phase. Thin layer chromatography (TLC) was performed using glass plates precoated with silica gel (1.0 mm, 60 Å pore size) impregnated with a fluorescent indicator (254 nm). Compounds on TLC plates were visualized by exposure to ultraviolet (UV) light or by exposure to a ninhydrin stain solution followed by warming with a heat gun for 10−20 s. NMR chemical shifts (δ) are reported in parts per million relative to TMS as internal reference. The ^{13}C NMR spectra employed $CDCl_3$ as an internal reference. FT-IR spectra were obtained on NaCl plates. Compound **M** was prepared according to the method of Zhang et al.[35] Compound **F** was prepared via methodology identical to that for compound **M**, except using ethyl acetate instead of trifluoromethyl ethyl acetate. Spectral data for **F** matched that previously reported.[36] Compound **13** was prepared by a literature protocol.[38]

Synthesis of (*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-({(*R*)-2-[(*tert*-butoxycarbonyl)amino]-2-carboxyethyl}disulfanyl)-propanoic Acid (2). Compound **2** was prepared according the procedure described in the literature starting with L-cystine (500 mg) to give a final yield of 660 mg (72%) of a white solid:[39] mp 144−146 °C; R_f = 0.22 (2:8 methanol/dichloromethane); ^1H NMR (MeOH-d_4, 500 MHz) δ 4.97 (br, 2H), 4.43 (br, 2H), 3.25 (dd, J = 14.0, 4.0 Hz, 2H), 2.99 (dd, J = 14.0, 9.0 Hz, 2H), 1.45 (s, 18H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.2, 157.8, 80.7, 54.2, 41.5, 28.7; HRMS (ESI-TOF, $[M + Na]^+$) m/z calcd for $C_{16}H_{28}N_2O_8S_2Na$ 463.1185, found 463.1176.

Synthesis of (2*S*,2′*S*)-Dimethyl 2,2′-[((2*R*,2′*R*)-3,3′-Disulfanediylbis{2-[(*tert*-butoxycarbonyl)amino]propanoyl})-bis(azanediyl)]bis(3-methylbutanoate) (3). To a stirred solution of L-valine methyl ester (3.2 g, 24.96 mmol) in 100 mL of anhydrous CH_2Cl_2 was added *N*,*N*-dicyclohexylcarbodiimide (DCC) (5.1 g, 24.96 mmol) followed by *N*,*N*-di-Boc-L-cystine (5.0 g, 11.35 mmol) and 4-dimethylaminopyridine (DMAP) (0.30 g, 2.27 mmol). The resulting solution was stirred at room temperature overnight. The precipitate that formed was filtered off, and the filtrate was evaporated in vacuo. The residue was dissolved in 50 mL of ethyl acetate and washed with 40 mL of brine. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated. Column chromatographic purification of the residue using silica and 40% ethyl acetate in hexanes as an eluent afforded the product as a white powder (6.0 g, 79% yield): mp 128−130 °C; R_f = 0.46 (4:6 ethyl acetate/hexanes); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.37 (s, 1H), 5.56 (d, J = 8.5 Hz, 1H), 4.69 (s, 1H), 4.48 (dd, J = 8.5, 6.5 Hz, 1H), 3.71 (s, 3H), 3.05 (m, 2H), 2.18 (m, 1H), 1.45 (s, 9H), 0.95 (t, J = 6.5 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.8, 170.4, 155.5, 79.9, 57.6, 53.9, 51.9, 44.1, 30.6, 28.1, 18.9, 18.1; IR 3417, 3332, 2981, 1742, 1703, 1676, 1503, 1267, 1155, 735, 700 cm^{-1}; HRMS (ESI-TOF, $[M + H]^+$) m/z calcd for $C_{28}H_{51}N_4O_{10}S_2$ 667.3047, found 667.3044.

Synthesis of (*S*)-Methyl 2-{(*R*)-4-[(*tert*-Butoxycarbonyl)-amino]-3-oxoisothiazolidin-2-yl}-3-methylbutanoate (4). To a solution of L-valine ester of *N*,*N*-di-*tert*-butyloxycarbonyl-L-cystine (1.0 g, 1.50 mmol) in 50 mL of anhydrous CH_2Cl_2 was added pyridine (20 equiv). The solution was cooled to −78 °C under N_2 and stirred for 15 min, and bromine (135 μL, 2.62 mmol) in dry CH_2Cl_2 was added slowly over a period of 30 min. The solution was allowed to warm to 0 °C over 1 h, and then CH_2Cl_2 was evaporated in vacuo to afford the crude material. Flash chromatography (1:1 ethyl acetate/hexanes) of crude material afforded the desired sulfenylamide (**4**) as a white solid. Sulfoxide (**5**) and sulfone (**6**) were also isolated as pure white solids (360 mg, 72% yield): mp 90−93 °C; R_f = 0.67 (4:6 ethyl acetate/hexanes); ^1H NMR ($CDCl_3$, 300 MHz) δ 5.37 (s, 1H), 4.60 (d, J = 8.5, 1H), 4.51 (t, J = 5.5 Hz, 1H), 3.80 (t, J = 9.5 Hz, 1H), 3.66 (s, 3H), 3.31 (t, J = 11.5 Hz, 1H), 2.16 (m, 1H), 1.38 (s, 9H), 0.90 (dd, J = 6.5, 5.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.1, 169.4, 155.4, 80.1, 61.8, 53.9, 52.2, 51.9, 37.4, 29.0, 28.1, 19.2, 18.9; IR 3332, 2969, 2930, 2873, 1746, 1715, 1688, 1510, 1368, 1167, 1020 cm^{-1}; HRMS (ESI-TOF, $[M + H]^+$) m/z calcd for $C_{14}H_{25}N_2O_5S$ 333.1484, found 333.1483.

(2*S*)-Methyl 2-{(4*R*)-4-[(*tert*-Butoxycarbonyl)amino]-1-oxido-3-oxoisothiazolidin-2-yl}-3-methylbutanoate (5). White solid (90 mg, 17% yield): mp 109−111 °C; R_f = 0.34 (4:6 ethyl acetate/hexanes); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.54 (d, J = 8.5 Hz, 1H), 4.90 (s, 1H), 4.53 (d, J = 9.5 Hz, 1H), 3.77 (s, 3H), 3.43 (dd, J = 13.5, 8.0 Hz, 1H), 3.03 (dd, J = 13.5, 2.0 Hz, 1H), 2.38 (m, 1H), 1.43 (s, 9H), 1.01 (d, J = 6.5 Hz, 3H), 0.88 (d, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.3, 169.5, 154.8, 80.8, 62.0, 54.3, 52.6, 49.4, 30.1, 28.2, 19.4, 19.3; IR (cm^{-1}) 3405, 3345, 3054, 2973, 2930, 1730, 1711, 1650, 1503, 1264, 1163, 1090, 739, 700; HRMS (ESI-TOF, $[M + H]^+$) m/z calcd for $C_{14}H_{25}N_2O_6S$ 349.1433, found 349.1433.

(*S*)-Methyl 2-{(*R*)-4-[(*tert*-Butoxycarbonyl)amino]-1,1-dioxido-3-oxoisothiazolidin-2-yl}-3-methylbutanoate (6). White solid (35 mg, 6% yield): mp 97−99 °C; R_f = 0.51 (4:6 ethyl acetate/hexanes); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.38 (s, 1H), 4.81 (dd, J = 7.5, 1.5 Hz, 1H), 4.26 (d, J = 9.0 Hz, 1H), 4.09 (dd, J = 12.0, 8.0 Hz, 1H), 3.75 (s, 3H), 3.65 (t, J = 11.5 Hz, 1H), 2.69 (m, 1H), 1.45 (s, 9H), 1.13 (d, J = 6.5 Hz, 3H), 1.02 (d, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 168.1, 166.5, 154.9, 81.6, 60.5, 52.9, 52.7, 51.1, 28.2, 28.1, 20.6, 19.6; IR (cm^{-1}) 3412, 3050, 2979, 2931, 1747, 1712, 1501, 1346, 1263, 1239, 1151, 734, 702; HRMS (ESI-TOF, $[M + Na]^+$) m/z calcd for $C_{14}H_{24}N_2O_7SNa$ 387.1202, found 387.1198.

General Procedure for the Synthesis of Compounds 8a−o, 11, and 12. To a stirred solution of the sulfenyl amide **4** (20 mg, 0.060 mmol) in 3 mL of a 2:1 methanol/buffer A (50 mM HEPES, 100 mM NaCl, and 1 mM EDTA at pH 7.0) mixture was added the 1,3-diketone nucleophile (1.1 equiv), and the resulting mixture was stirred at room temperature. Reactions were monitored by TLC. After complete consumption of **4** was observed [1−5 min, except for reactions involving **I**−**O** (Table 2), which required 30 min], methanol was completely removed by evaporation under a stream of nitrogen gas and the remaining aqueous mixture was extracted with CH_2Cl_2 (2 × 1 mL). Products were then isolated by column chromatography on silica gel eluted with either ethyl acetate/hexane or methanol/dichloromethane mixtures. For products **8i**−**o**, the solution developed a yellow-green color immediately upon addition of the nucleophile; the color disappeared as the final reaction product was generated (after approximately 30 min). For reactions with 1,3-indandione and curcumin, a 3:1 methanol/buffer A mixture was used as the solvent system for the reaction to ensure the solubility of the 1,3-diketone reactant.

*(E)-Methyl 2-{2-[(tert-Butoxycarbonyl)amino]-3-[(2-hydroxy-4-oxopent-2-en-3-yl)thio]propanamido}-3-methylbutanoate (**8a**).* White powder (20 mg, 77% yield): mp 109−112 °C; R_f = 0.56 (4:6 ethyl acetate/hexanes); ^1H NMR ($CDCl_3$, 500 MHz) δ 17.11 (s, 1H), 6.83 (d, J = 5.5 Hz, 1H), 5.18 (d, J = 7.5 Hz, 1H), 4.50 (dd, J = 8.5, 4.5 Hz, 1H), 4.17 (d, J = 5.0 Hz, 1H), 3.73 (s, 3H), 2.97 (dd, J = 13.0, 5.5 Hz, 1H), 2.82 (dd, J = 13.5, 8.0 Hz, 1H), 2.16 (m, 1H), 1.44 (s, 9H),

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0.90 (dd, J = 13.5, 6.5 Hz 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 197.5, 171.9, 170.2, 155.6, 103.3, 80.8, 57.1, 53.7, 52.2, 37.7, 31.3, 28.2, 24.4, 18.9, 17.5; IR (cm^{-1}) 3425, 3350, 2970, 2931, 1737, 1695, 1681, 1495, 1264, 1161, 1021, 906, 722, 645; HRMS (ESI-TOF, $[M + H]^+$) m/z calcd for $C_{19}H_{33}N_2O_7S$ 433.2008, found 433.2013.

*(E)-Methyl 2-({2-[(tert-Butoxycarbonyl)amino]-3-[(1-methoxy-3-methyl-1-oxobutan-2-yl)amino]-3-oxopropyl}thio)-3-hydroxybut-2-enoate (**8b**).* Colorless oil (21 mg, 78% yield): R_f = 0.52 (4:6 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 13.45 (s, 1H), 7.10 (d, J = 7.0 Hz, 1H), 5.57 (s, 1H), 4.50 (dd, J = 8.5, 5.0 Hz, 1H), 4.19 (br, 1H), 3.86 (s, 3H), 3.73 (s, 3H), 3.04 (br, 1H), 2.81 (dd, J = 14.0, 6.0 Hz, 1H), 2.35 (s, 3H), 2.17 (m, 1H), 1.45 (s, 9H), 0.92 (dd, J = 12.0, 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 183.9, 172.9, 171.8, 170.5, 155.6, 93.2, 80.5, 57.1, 53.1, 52.6, 52.0, 37.5, 31.2, 28.2, 20.9 18.8, 17.5; IR 3420, 3369, 3052, 2967, 1736, 1691, 1688, 1623, 1262, 1164, 911, 727, 645 cm^{-1}; HRMS (ESI-TOF, $[M + H]^+$) m/z calcd for $C_{19}H_{33}N_2O_8S$ 449.1958, found 449.1964.

*Dimethyl 2-({2-[(tert-Butoxycarbonyl)amino]-3-[(1-methoxy-3-methyl-1-oxobutan-2-yl)amino]-3-oxopropyl}thio)malonate (**8c**).* Colorless oil (20 mg, 70% yield): R_f = 0.60 (4:6 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.11 (s, 1H), 5.60 (d, J = 5.0 Hz, 1H), 4.49 (dd, J = 8.5, 5.0 Hz, 1H), 4.36 (s, 2H), 3.81 (s, 3H), 3.80 (s, 3H), 3.73 (s, 3H), 3.16 (dd, J = 14.0, 6.0 Hz, 1H), 2.98 (dd, J = 14.0, 6.5 Hz, 1H), 2.20 (m, 1H), 1.45 (s, 9H), 0.94 (dd, J = 11.5, 6.5 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.8, 170.2, 167.7, 167.3, 155.6, 80.4, 57.4, 53.5, 53.4, 52.1, 51.1, 33.9, 31.0, 28.3, 18.9, 17.6; IR 3316, 2963, 2931, 1735, 1699, 1683, 1656, 1517, 1274, 1246, 1163, 1016 cm^{-1}; HRMS (ESI-TOF, $[M + H]^+$) m/z calcd for $C_{19}H_{33}N_2O_9S$ 465.1907, found 465.1902.

*Methyl 2-{2-[(tert-Butoxycarbonyl)amino]-3-[(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)thio]propanamido}-3-methylbutanoate (**8d**).* Colorless oil (20 mg, 71% yield): R_f = 0.45 (1:9 methanol/CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.74 (d, J = 8.0 Hz, 1H), 5.96 (d, J = 7.0 Hz, 1H), 4.49 (dd, J = 8.5, 5.0 Hz, 1H), 4.11 (d, J = 5.5 Hz, 1H), 3.73 (s, 3H), 2.97 (dd, J = 13.5, 5.0 Hz, 1H), 2.61 (dd, J = 13.5, 7.5 Hz, 1H), 2.33−2.59 (br, 4H), 2.23 (m, 1H), 1.43 (s, 9H), 1.10 (s, 6H), 0.98 (dd, J = 9.0, 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.9, 170.4, 155.5, 106.8, 80.2, 57.8, 53.9, 52.2, 37.5, 38.3, 31.6, 30.9, 28.3, 19.0, 17.9; IR 3390, 2965, 2359, 2336, 1698, 1684, 1649, 1634, 1559, 1505, 1159 cm^{-1}; HRMS (ESI-TOF, $[M + H]^+$) m/z calcd for $C_{22}H_{37}N_2O_7S$ 473.2321, found 473.2317.

*(2S)-Methyl 2-{(2R)-2-[(tert-Butoxycarbonyl)amino]-3-[(1,3-dioxo-1-phenylbutan-2-yl)thio]propanamido}-3-methylbutanoate (**8e**).* Yellow gum (26 mg, 87% yield) as an inseparable mixture of isomers (1:1): R_f = 0.47 (4:6 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.97 (m, 2H), 7.74 (m, 2H), 7.61 (m, 1H), 7.47 (m, 5H), 7.13 (s, 1H), 7.03 (d, J = 7.5 Hz, 1H), 6.62 (s, 1H), 5.71 (s, 1H), 5.63 (s, 1H), 5.32 (m, 1H), 4.68 (s, 1H), 4.45 (m, 2H), 4.31 (m, 1H), 3.93 (s, 1H), 3.70 (m, 6H), 2.99 (m, 1H), 2.84 (m, 2H), 2.64 (m, 1H), 2.54 (s, 3H), 2.29 (m, 3H), 2.13 (m, 3H), 1.44 (m, 18H), 0.96 (m, 3H), 0.88 (m, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 200.9, 200.3, 200.0, 192.4, 191.5, 171.8, 171.7, 170.2, 170.0, 155.7, 155.3, 136.2, 135.0, 134.2, 131.0, 128.9, 128.7, 127.9, 103.2, 80.5, 62.7, 62.5, 57.5, 57.4, 57.1, 53.9, 53.6, 53.1, 52.1, 38.2, 33.9, 32.9, 31.2, 31.0, 30.9, 29.6, 28.2, 28.2, 27.5, 27.3, 25.3, 19.0, 18.9, 18.8, 17.6; IR 3412, 3050, 2984, 1735, 1695, 1675, 1417, 1262, 1151, 893, 742, 702 cm^{-1}; HRMS (ESI-TOF, $[M + H]^+$) m/z calcd for $C_{24}H_{35}N_2O_7S$ 495.2165, found 495.2169.

*(2S)-Methyl 2-((2R)-2-[(tert-Butoxycarbonyl)amino]-3-{[1-(6-methoxynaphthalen-2-yl)-1,3-dioxobutan-2-yl]thio}propanamido)-3-methylbutanoate (**8f**).* Yellow gum (28 mg, 81% yield) as an inseparable mixture of isomers (2:1): R_f = 0.42 (4:6 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.49 (s, 1H), 8.45 (s, 1H), 8.29 (s, 1H), 7.99 (m, 2H), 7.88 (m, 3H), 7.79 (m, 4H), 7.19 (m, 6H), 7.04 (d, J = 8.0 Hz, 1H), 6.58 (s, 1H), 5.74 (d, J = 6 Hz, 1H), 5.62 (s, 1H), 5.45 (d, J = 16.0 Hz, 1H), 4.47 (m, 2H), 4.38 (m, 3H), 3.95 (m, 9H), 3.69 (m, 9H), 3.04 (m, 2H), 2.88 (m, 2H), 2.77 (m, 1H), 2.66 (m, 1H), 2.56 (s, 3H), 2.32 (s, 3H), 2.30 (s, 3H), 2.22 (m, 1H), 2.15 (m, 1H), 2.01 (m, 1H), 1.45 (s, 9H), 1.44 (s, 9H), 1.35 (s, 9H), 0.97 (dd, J = 10.5, 7.0 Hz, 6H), 0.88 (t, J = 6.5 Hz, 6H), 0.81 (d, J = 7.0 Hz, 3H), 0.76 (d, J = 7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 201.2, 200.3, 200.1, 192.0, 191.9, 191.0, 171.8, 171.7, 170.3, 170.0, 160.4, 159.3, 155.7, 155.5, 155.2, 137.9, 137.8, 136.0, 131.6, 131.5, 131.3, 131.2, 131.1, 130.8, 130.4, 129.8, 127.7, 127.6, 127.5, 126.2, 124.8, 124.7, 120.1, 120.0, 119.5, 105.8, 105.7, 103.0, 80.5, 80.3, 62.9, 62.7, 57.6, 57.4, 57.1, 55.5, 55.4, 54.0, 53.6, 53.2, 52.1, 52.0, 38.1, 34.0, 33.1, 31.1, 31.0, 30.9, 28.2, 28.1, 27.3, 27.1, 25.3, 19.0, 18.9, 18.8, 17.64, 17.5; IR 3412, 3336, 3054, 2971, 2927, 2871, 1739, 1707, 1672, 1620, 1481, 1370, 1266, 1207, 1167, 1024, 857, 734, 702 cm^{-1}; HRMS (ESI-TOF, $[M + H]^+$) m/z calcd for $C_{29}H_{39}N_2O_8S$ 575.2427, found 575.2415.

*(2S)-Methyl 2-{(2R)-3-[(2-Acetyl-1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl)thio]-2-[(tert-butoxycarbonyl)amino]propanamido}-3-methylbutanoate (**8g**).* Colorless liquid (24 mg, 77% yield) as an inseparable mixture of isomers (1:1): R_f = 0.35 (4:6 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.07 (dd, J = 7.0, 1.0 Hz, 2H), 7.51 (t, J = 7.5 Hz, 2H), 7.34 (t, J = 7.5 Hz, 2H), 7.22 (d, J = 7.5 Hz, 2H), 7.10 (m, 2H), 5.94 (s, 1H), 5.61 (s, 1H), 4.60 (m, 2H), 4.26 (s, 2H), 3.71 (s, 3H), 3.69 (s, 3H), 3.17 (m, 2H), 2.97 (m, 3H), 2.86 (d, J = 6.0 Hz, 2H), 2.75 (m, 3H), 2.31 (s, 3H), 2.28 (s, 3H), 2.18 (m, 5H), 1.45 (s, 9H), 1.44 (s, 9H), 0.91 (m, 12H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 202.0, 201.4, 192.2, 171.8, 171.7, 170.4, 170.3, 156.0, 155.7, 142.6, 142.5, 134.2, 130.9, 130.7, 128.7, 128.3, 127.1, 80.3, 67.4, 67.1, 57.3, 53.8, 52.0, 31.1, 30.9, 30.7, 30.4, 28.2, 26.5, 26.2, 25.8, 25.7, 18.9, 17.6; IR 3318, 3001, 2962, 2930, 1740, 1701, 1697, 1670, 1513, 1164, 1024, 757 cm^{-1}; HRMS (ESI-TOF, $[M + H]^+$) m/z calcd for $C_{26}H_{37}N_2O_7S$ 521.2321, found 521.2322.

*(S)-Methyl 2-{(R)-2-[(tert-Butoxycarbonyl)amino]-3-[(1,3-dioxo-1,3-diphenylpropan-2-yl)thio]propanamido}-3-methylbutanoate (**8h**).* Colorless oil (24 mg, 73% yield): R_f = 0.59 (3:7 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.04 (d, J = 8.0 Hz, 2H), 7.96 (d, J = 7.5 Hz, 1H), 7.59 (t, J = 7.5 Hz, 1H), 7.56 (t, J = 7.5 Hz, 1H), 7.47 (t, J = 8.0 Hz, 2H), 7.43 (t, J = 7.5 Hz, 2H), 7.30 (s, 1H), 6.14 (s, 1H), 6.02 (s, 1H), 4.43 (dd, J = 8.5, 5.0 Hz, 1H), 4.35 (d, J = 5.0 Hz, 1H), 3.17 (d, J = 10.5 Hz, 1H), 2.88 (dd, J = 13.5, 6.5 Hz, 1H), 2.17 (m, 1H), 1.46 (s, 9H), 0.96 (d, J = 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 192.8, 192.0, 171.7, 170.5, 155.9, 135.1, 134.9, 133.9, 133.8, 130.1, 130.0, 129.0, 128.9, 128.8, 80.3, 58.0, 57.5, 53.8, 52.0, 33.3, 30.9, 28.3, 28.2, 18.9, 17.7; IR 3411, 3338, 3051, 2966, 2929, 1736, 1691, 1670, 1495, 1446, 1364, 1262, 1209, 1164, 735, 702 cm^{-1}; HRMS (ESI-TOF, $[M + Na]^+$) m/z calcd for $C_{29}H_{36}N_2O_7SNa$ 579.2141, found 579.2137.

*(S)-Methyl 2-{(R)-2-[(tert-Butoxycarbonyl)amino]-3-[(2-oxo-2-phenylethyl)thio]propanamido}-3-methylbutanoate (**8i**).* Yellow gum (19.5 mg, 72% yield): R_f = 0.47 (4:6 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.97 (d, J = 7.5 Hz, 2H), 7.59 (t, J = 7.0 Hz, 1H), 7.47 (t, J = 7.5 Hz, 2H), 7.26 (s, 1H), 5.59 (s, 1H), 4.48 (dd, J = 8.5, 5.0 Hz, 1H), 4.36 (d, J = 5.0 Hz, 1H), 4.08 (d, J = 15.5 Hz, 1H), 4.08 (d, J = 15.5 Hz, 1H), 3.70 (s, 3H), 2.97 (dd, J = 14.0, 6.5 Hz, 1H), 2.86 (dd, J = 14.0, 6.5 Hz, 1H), 2.21 (m, 1H), 1.43 (s, 9H), 0.96 (t, J = 7.5 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 195.3, 171.8, 170.5, 155.5, 135.2, 133.7, 128.7, 128.6, 80.2, 57.5, 53.3, 52.1, 38.3, 35.0, 30.9, 28.2, 18.9, 17.6; IR 3412, 3324, 3058, 2967, 2931, 2871, 1739, 1675, 1525, 1278, 1171, 1016, 913, 726, 686 cm^{-1}; HRMS (ESI-TOF, $[M + H]^+$) m/z calcd for $C_{22}H_{33}N_2O_6S$ 453.2059, found 453.2051.

*(S)-Methyl 2-((R)-2-[(tert-Butoxycarbonyl)amino]-3-{[2-(4-chlorophenyl)-2-oxoethyl]thio}propanamido)-3-methylbutanoate (**8j**).* Colorless oil (20 mg, 68% yield): R_f = 0.46 (4:6 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.91 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 9.0 Hz, 2H), 7.18 (d, J = 6.0 Hz, 1H), 5.54 (d, J = 5.5 Hz 1H), 4.48 (dd, J = 8.5, 4.5 Hz, 1H), 4.35 (d, J = 6.0 Hz, 1H), 4.03 (d, J = 15.0 Hz, 1H), 3.96 (d, J = 15.0 Hz, 1H), 3.71 (s, 3H), 2.94 (dd, J = 14.0, 6.5 Hz, 1H), 2.85 (dd, J = 14.0, 6.5 Hz, 1H), 2.20 (m, 1H), 1.43 (s, 9H), 0.95 (t, J = 7.5 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 194.0, 171.8, 170.4, 155.5, 140.2, 133.6, 130.1, 129.1, 80.3, 57.5, 53.3, 52.1, 38.1, 34.9, 30.9, 28.2, 18.9, 17.6; IR 3519, 3324, 3054, 2967, 2931, 2871, 1739, 1687, 1660, 1584, 1521, 1274, 1167, 1087, 1012, 734 cm^{-1}; HRMS (ESI-TOF, $[M + H]^+$) m/z calcd for $C_{22}H_{32}ClN_2O_6S$ 487.1670, found 487.1672.

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*(S)-Methyl 2-((R)-2-[(tert-Butoxycarbonyl)amino]-3-{[2-oxo-2-(thiophen-2-yl)ethyl]thio}propanamido)-3-methylbutanoate (**8k**).* Colorless oil (18 mg, 65% yield): R_f = 0.47 (4:6 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.78 (d, J = 4.0 Hz, 1H), 7.69 (d, J = 5.0 Hz, 1H), 7.24 (s, 1H), 7.15 (t, J = 4.0, 1H), 5.60 (s, 1H), 4.48 (dd, J = 8.5, 5.0 Hz, 1H), 4.37 (d, J = 5.5 Hz, 1H), 3.99 (d, J = 15.0 Hz, 1H), 3.91 (d, J = 15.0 Hz, 1H), 3.71 (s, 3H), 3.00 (dd, J = 14.0, 6.0 Hz, 1H), 2.89 (dd, J = 14.0, 6.0 Hz, 1H), 2.21 (m, 1H), 1.44 (s, 9H), 0.97 (dd, J = 9.0, 7.0 Hz 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 188.5, 171.8, 170.4, 155.5, 142.3, 134.8, 133.2, 128.3, 80.2, 57.6, 53.4, 52.1, 38.7, 35.3, 30.9, 28.3, 19.0, 17.7; IR 3416, 3336, 3050, 2967, 2931, 1735, 1714, 1692, 1671, 1651, 1504, 1261, 1162, 906, 735, 646 cm^{-1}; HRMS (ESI-TOF, [M + H]$^+$) m/z calcd for $C_{20}H_{31}N_2O_6S_2$ 459.1624, found 459.1619.

*(S)-Methyl 2-((R)-2-[(tert-Butoxycarbonyl)amino]-3-{[2-(naphthalen-2-yl)-2-oxoethyl]thio}propanamido)-3-methylbutanoate (**8l**).* White solid (19 mg, 63% yield): mp 97−99 °C; R_f = 0.44 (4:6 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.51 (s, 1H), 8.02 (dd, J = 8.5, 1.5 Hz, 1H), 7.97 (d, J = 8.0 Hz, 1H), 7.89 (d, J = 9.0 Hz, 1H), 7.87 (d, J = 8.5 Hz, 1H), 7.61 (t, J = 8.0 Hz, 1H), 7.56 (t, J = 7.5 Hz, 1H), 7.29 (d, J = 6.0 Hz, 1H), 5.62 (d, J = 5.0 Hz, 1H), 4.49 (dd, J = 8.5, 5.0 Hz, 1H), 4.39 (s, 1H), 4.21 (d, J = 15.5 Hz, 1H), 4.12 (d, J = 15.5 Hz, 1H), 3.70 (s, 3H), 3.01 (dd, J = 14.0, 6.5 Hz, 1H), 2.90 (dd, J = 14.0, 6.5 Hz, 1H), 2.21 (m, 1H), 1.43 (s, 9H), 0.96 (t, J = 7.5 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 195.3, 171.8, 170.5, 155.5, 135.8, 132.6, 132.4, 130.65, 129.7, 128.8, 128.6, 127.8, 126.9, 124.1, 80.2, 57.5, 53.4, 52.0, 38.4, 35.0, 30.9, 28.2, 28.1, 18.9, 17.7; IR 3413, 3332, 3054, 2958, 2923, 2869, 1739, 1734, 1714, 1683, 1673, 1518, 1365, 1263, 1165, 745 cm^{-1}; HRMS (ESI-TOF, [M + H]$^+$) m/z calcd for $C_{26}H_{35}N_2O_6S$ 503.2216, found 503.2222.

*(S)-Methyl 2-((R)-2-[(tert-Butoxycarbonyl)amino]-3-{[2-(6-methoxynaphthalen-2-yl)-2-oxoethyl]thio}propanamido)-3-methylbutanoate (**8m**).* Yellow gum (22 mg, 69% yield): R_f = 0.66 (4:6 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.43 (s, 1H), 7.99 (dd, J = 9.0, 2.0 Hz, 1H), 7.85 (d, J = 8.5 Hz, 1H), 7.77 (d, J = 9.0 Hz, 1H), 7.34 (d, J = 7.0 Hz, 1H), 7.20 (dd, J = 9.0, 2.5 Hz, 1H), 7.15 (d, J = 2.5 Hz, 1H), 5.64 (d, J = 5.5 Hz, 1H), 4.49 (dd, J = 8.5, 4.5 Hz, 1H), 4.40 (d, J = 5.0 Hz, 1H), 4.19 (d, J = 15.0 Hz, 1H), 4.09 (d, J = 15.0 Hz, 1H), 3.95 (s, 3H), 3.70 (s, 3H), 3.00 (dd, J = 14.0, 6.0 Hz, 1H), 2.90 (dd, J = 14.0, 6.5 Hz, 1H), 2.21 (m, 1H), 1.43 (s, 9H), 0.97 (t, J = 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 195.0, 171.8, 170.5, 160.0, 155.5, 137.6, 131.3, 130.6, 130.5, 127.7, 127.3, 124.9, 119.9, 105.7, 80.2, 57.5, 55.42, 53.4, 52.0, 38.2, 35.1, 30.9, 28.2, 28.1, 18.9, 17.7; IR 3416, 3324, 3006, 2967, 2931, 1739, 1668, 1620, 1505, 1473, 1278, 1203, 1171, 1024, 754, 662 cm^{-1}; HRMS (ESI-TOF, [M + H]$^+$) m/z calcd for $C_{27}H_{37}N_2O_7S$ 533.2321, found 533.2330.

*(S)-Methyl 2-{(R)-2-[(tert-Butoxycarbonyl)amino]-3-[(2-oxo-2-phenylethyl)thio]propanamido}-3-methylbutanoate (**8n**).* Yellow gum (21 mg, 79% yield). This product is the same as **8i** (Table 2).

*(S)-Methyl 2-[(R)-2-[(tert-Butoxycarbonyl)amino]-3-({2-oxo-2-[4-(trifluoromethyl)phenyl]ethyl}thio)propanamido]-3-methylbutanoate (**8o**).* Colorless oil (26 mg, 83% yield): R_f = 0.50 (4:6 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.09 (d, J = 8.0 Hz, 2H), 7.75 (d, J = 8.0 Hz, 2H), 7.12 (s, 1H), 5.50 (s, 1H), 4.49 (dd, J = 9.0, 5.0 Hz, 1H), 4.36 (d, J = 6.0 Hz, 1H), 4.08 (d, J = 15.5 Hz, 1H), 4.02 (d, J = 15.5 Hz, 1H), 3.72 (s, 3H), 2.96 (dd, J = 14.0, 6.5 Hz, 1H), 2.88 (dd, J = 14.0, 6.5 Hz, 1H), 2.21 (m, 1H), 1.44 (s, 9H), 0.96 (dd, J = 8.0, 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 194.1, 171.8, 170.3, 155.5, 137.9, 134.9, 134.7, 129.1, 126.7, 125.8, 125.7, 124.5, 122.3, 80.3, 57.5, 53.3, 52.1, 38.3, 34.9, 30.9, 28.2, 18.9, 17.6; IR 3409, 3328, 3054, 2969, 2930, 2869, 1734, 1680, 1650, 1503, 1325, 1267, 1167, 1132, 1067, 1009, 735 cm^{-1}; HRMS (ESI-TOF, [M + H]$^+$) m/z calcd for $C_{23}H_{32}F_3N_2O_6S$ 521.1933, found 521.1942.

*(2S,2′S)-Dimethyl 2,2′-[((2R,2′R)-3,3′-[(1,3-Dioxo-2,3-dihydro-1H-indene-2,2-diyl)bis(sulfanediyl)]bis{2-[(tert-butoxycarbonyl)amino]-propanoyl})bis(azanediyl)]bis(3-methylbutanoate) (**11**).* Colorless oil (18 mg, 74% yield): R_f = 0.27 (3:7 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.96−7.98 (m, 2H), 7.88−7.90 (m, 2H), 7.21 (s, 1H), 5.51 (s, 2H), 4.52 (s, 4H), 3.74 (s, 6H), 3.36 (m, 4H), 2.17−2.21 (m, 2H), 1.43 (s, 18H), 0.96 (t, J = 8.0 Hz, 12H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 192.8, 188.5, 171.9, 171.8, 170.1, 155.4, 138.3, 137.5, 137.4, 137.3, 137.2, 136.7, 125.4, 125.3, 124.8, 80.3, 60.6, 57.5, 57.4, 53.8, 52.2, 32.2, 31.2, 31.1, 28.3, 19.0, 18.9, 17.9; IR 3416, 3052, 2983, 2361, 2300, 1716, 1683, 1499, 1266, 1164, 894, 739, 702 cm^{-1}; HRMS (ESI-TOF, [M + H]$^+$) m/z calcd for $C_{37}H_{55}N_4O_{12}S_2$ 811.3258, found 811.3251.

*Methyl S-[(1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dien-4-yl]-N-(tert-butoxycarbonyl)-L-cysteinyl-L-valinate (**12**).* Orange, light-sensitive, gum (33 mg, 78% yield): R_f = 0.34 (4:6 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.72 (s, 4H), 7.54 (d, J = 8.4 Hz, 0.5H), 7.20−7.23 (m, 4H), 6.94 (d, J = 8.4 Hz, 2H), 6.85 (d, J = 8.4 Hz, 0.5H), 6.73 (d, J = 8.4 Hz, 0.5H), 5.94 (s, 2H), 5.20 (br, 1H), 4.47−4.49 (m, 1H), 4.29 (br, 1H), 4.00 (s, 6H), 3.69 (s, 3H), 3.06−3.11 (m, 1H), 3.02 (br, 1H), 2.11−2.17 (m, 1H), 1.37 (s, 3H), 1.36 (s, 6H), 0.87−0.92 (m, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 187.3, 171.8, 170.1, 158.1, 155.4, 148.3, 146.8, 143.7, 130.7, 127.9, 123.7, 118.7, 115.9, 114.8, 110.4, 104.2, 80.7, 57.3, 56.2, 54.1, 52.2, 39.3, 31.4, 28.2, 28.1, 18.9, 17.7; IR 3518, 3412, 3351, 3056, 2970, 2934, 1732, 1683, 1600, 1585, 1512, 1266, 1209, 1164, 743, 702 cm^{-1}; HRMS (ESI-TOF, [M + H]$^+$) m/z calcd for $C_{35}H_{45}N_2O_{11}S$ 701.2744, found 701.2734.

Reduction of the Model Sulfenyl Amide 4 with Excess Cysteine. To a stirred solution of D,L-cysteine (36 mg, 5 equiv) dissolved in a 2:1 methanol/buffer A (4 mL) mixture was added 20 mg (1 equiv) of the model sulfenyl amide. The reaction mixture was stirred at room temperature for 1 min, after which time complete consumption of the starting sulfenyl amide **4** was observed by TLC. Methanol was evaporated from the reaction mixture under a stream of nitrogen gas and the remaining aqueous layer extracted with CH_2Cl_2 (2 × 1.5 mL). The organic layer was then dried over Na_2SO_4 and filtered and the solvent removed by rotary evaporation to afford pure cysteine-containing dipeptide **7** in 85% yield.

*(S)-Methyl 2-{(R)-2-[(tert-Butoxycarbonyl)amino]-3-mercaptopropanamido}-3-methylbutanoate (**7**).* White powder (17 mg, 85% yield): mp 100−102 °C; R_f = 0.65 (4:6 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.83 (d, J = 8.0 Hz, 1H), 5.48 (d, J = 8.0 Hz, 1H), 4.49 (dd, J = 8.5, 5.0 Hz, 1H), 4.33 (d, J = 6.0 Hz, 1H), 3.72 (s, 3H), 3.06 (m, 1H), 2.72 (m, 1H), 2.17 (m, 1H), 1.67 (t, J = 9.0 Hz, 1H), 1.44 (s, 9H), 0.91 (t, J = 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 168.1, 166.5, 154.9, 81.6, 60.5, 52.9, 52.7, 51.1, 28.2, 28.1, 20.6, 19.6; IR 3412, 3332, 3050, 2979, 2927, 1735, 1695, 1679, 1652, 1501, 1263, 1159, 738, 698 cm^{-1}; HRMS (ESI-TOF, base peak [M − CO_2 − C_4H_8 + H]$^+$) m/z calcd for $C_9H_{19}N_2O_3S$ 235.1116, found 235.1113.

Stability of Products against Thiol. To stirred solutions of *threo*-1,4-dimercapto-2,3-butanediol (DTT, in 3 mL of a 50 mM solution in a 2:1 methanol/buffer A mixture) was added either compound **8a**, **8e**, **8g**, **8i**, or **8k** (10 mg, to give final concentrations of approximately 10 mM). The solutions were then warmed to 45 °C and allowed to stir for 6 h. The reaction was periodically monitored by TLC. No changes in TLC of the starting material were observed over the course of 6 h.

Spectrophotometric Determination of Carbon Acid Ionization Constants. A series of buffers composed of glycine, sodium acetate, and sodium phosphate (50 mM each) were prepared at half-pH unit intervals between pH 3 and 13 in distilled, deionized H_2O. Small aliquots of concentrated carbon acid in DMSO were added to these buffered solutions to afford solutions containing 4% (v/v) DMSO. For each 1,3-diketone, the concentration of carbon acid was held constant in each buffered solution, with final concentrations ranging from 50 to 250 μM, depending on individual molar absorptivities. Finally, wavelengths found to vary with buffer pH were plotted as a function of solution pH, and the data were fit with the following equation using Microsoft Excel:

$$A_{pH} = A_{min} + \frac{A_{max} - A_{min}}{1 + 10^{pK_a - pH}} \quad (2)$$

where A_{min}, A_{max}, and A_{pH} are the absorbances at minimum pH, maximum pH, and intermediate pH values, respectively, at the wavelength(s) monitored for a given carbon acid (Supporting Information). During data fitting, only the pK_a was optimized without constraint, and A_{min} and A_{max} were subject to constrained

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optimizations (±10% of values measured at pH 3 and 13).[37] This procedure was validated using several weak acids of known pK_a values (Supporting Information).

Determination of Rate Constants of Reactions between 1,3-Diketo Nucleophiles and the Model Sulfenyl Amide (4). Briefly, we developed a spectrophotometric assay for monitoring the covalent capture of **4** using a "reverse Ellman's method" approach (Supporting Information). First, sulfenyl amide **4** and excess (≥10-fold) 1,3-diketo nucleophile were allowed to react in 1:1 methanol/buffer (50 mM Tris, 50 mM Bis-Tris, 10 mM DTPA, and 100 mM sodium acetate at pH 7.0) mixture at 23 ± 2 °C. At various times during the reaction, aliquots (0.8 mL) were removed and diluted into premade solutions (0.2 mL) of reduced Ellman's reagent [2-nitro-5-mercaptobenzoic acid (TNB)] in pH 5.0 buffer (0.5 M sodium acetate, 0.1 M Bis-Tris, and 75 mM DTPA). In this assay mixture, sulfenyl amide that had not been covalently captured by 1,3-diketo enolate immediately reacted with the aryl thiolate of TNB, affording a decrease in Abs_{410} proportional to the amount of remaining sulfenyl amide. Plotting A_{410} versus reaction time afforded kinetic data, to which the (pseudo) first-order rate equation was fit:

$$\frac{A_t - A_\infty}{A_o - A_\infty} = e^{-k_\psi t} \tag{3}$$

where A_o, A_∞, and A_t are the Abs_{410} initially, finally, and intermediately during the reaction, respectively, and k_ψ is the pseudo-first-order rate constant for reaction at a given concentration of carbon nucleophile. Full experimental details for the kinetics assays are available in the Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b01949.

Descriptions of ^{1}H and ^{13}C NMR spectra for all compounds, a detailed description of the kinetic assays, and UV−vis data for kinetic assays and pK_a determinations (PDF)

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Notes

The authors declare no competing financial interest.

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