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PII: S0968-0896(16)30212-7
DOI: http://dx.doi.org/10.1016/j.bmc.2016.03.054
Reference: BMC 12903

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 8 February 2016
Revised Date: 16 March 2016
Accepted Date: 27 March 2016

Please cite this article as: Parsons, Z.D., Ruddraraju, K.V., Santo, N., Gates, K.S., Sulfone-stabilized carbanions for the reversible covalent capture of a posttranslationally-generated cysteine oxoform found in protein tyrosine phosphatase 1B (PTP1B), *Bioorganic & Medicinal Chemistry* (2016), doi: http://dx.doi.org/10.1016/j.bmc.2016.03.054

Sulfone-stabilized carbanions for the reversible covalent capture of a posttranslationally-generated cysteine oxoform found in protein tyrosine phosphatase 1B (PTP1B)

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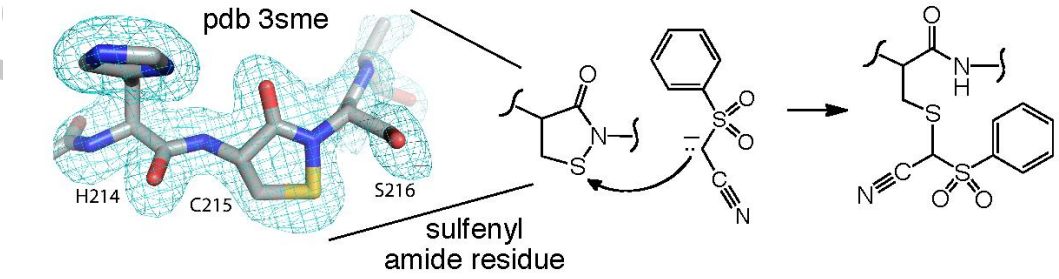
Abstract: Redox regulation of protein tyrosine phosphatase 1B (PTP1B) involves oxidative conversion of the active site cysteine thiolate into an electrophilic sulfenyl amide residue. Reduction of the sulfenyl amide by biological thiols regenerates the native cysteine residue. Here we explored fundamental chemical reactions that may enable covalent capture of the sulfenyl amide residue in oxidized PTP1B. Various sulfone-containing carbon acids were found to react readily with a model peptide sulfenyl amide via attack of the sulfonyl carbanion on the electrophilic sulfur center in the sulfenyl amide. Both the products and the rates of these reactions were characterized. The results suggest that capture of a peptide sulfenyl amide residue by sulfone-stabilized carbanions can slow, but not completely prevent, thiol-mediated generation of the corresponding cysteine-containing peptide. Sulfone-containing carbon acids may be useful components in the construction of agents that knock down PTP1B activity in cells via transient covalent capture of the sulfenyl amide oxoform generated during insulin signaling processes.

TOC Graphic



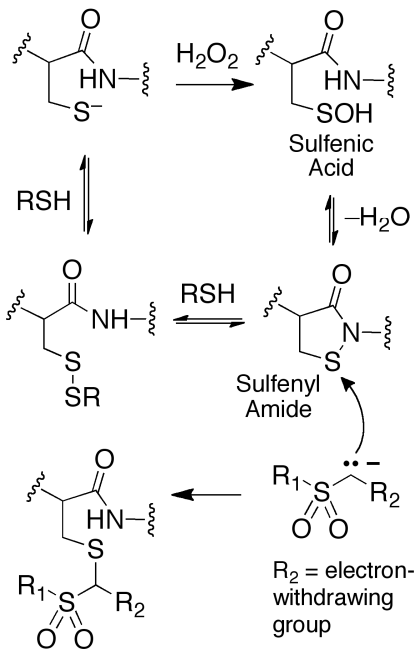
1. Introduction

Type 2 diabetes is an immense and growing worldwide health problem[1-5] and there is a need for new therapeutic agents in this area.[6,7] The enzyme protein tyrosine phosphatase 1B (PTP1B) is an important negative regulator of insulin signaling in mammalian cells[8,9] and studies with PTP1B knockout mice established this enzyme as a therapeutic target for the treatment of type 2 diabetes.[10-13] However, it has proven challenging to develop traditional reversible inhibitors that are both bioavailable and selective for PTP1B over other members of the highly homologous protein tyrosine phosphatase enzyme family.[14] More than ten years of intense effort has failed to produce successful clinical candidates.[14-19] As a result, some have classified PTP1B as an "undruggable target".[20,21] Here we describe chemical studies designed to explore an alternate approach that has been suggested for the chemical knockdown of PTP1B activity in cells involving covalent capture of a post-translationally modified, *oxidized* form of the enzyme that is generated transiently as part of the insulin signaling cascade.[22]

Binding of the peptide hormone insulin to the insulin receptor stimulates production of H_2O_2, which causes transient inactivation PTP1B via oxidation of the active site cysteine residue.[23-29] Multiple crystallographic analyses have shown that the catalytic cysteine in oxidized PTP1B exists as a cyclic, acyl sulfenamide (commonly referred to as a cysteine sulfenyl amide residue, Scheme 1).[24,30,31] Reactions of oxidized PTP1B with biological thiols regenerate the catalytically-active enzyme[25,32,33] that can terminate cellular responses to insulin via dephosphorylation of the insulin receptor and insulin receptor substrates.[8,15-19,34,35]

Importantly, the sulfenyl amide residue in oxidized PTP1B presents a unique electrophilic sulfur center that may be exploited in drug and probe design.[22,36,37] Covalent

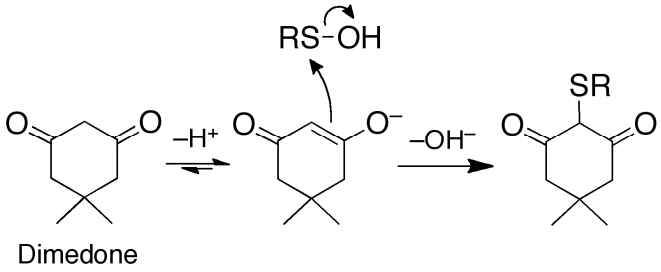
capture of oxidatively-inactivated PTP1B by an appropriate nucleophile has the potential to block regeneration of the catalytically active enzyme, thus knocking down cellular activity of this enzyme.[22] This strategy may yield selectivity arising from the insulin signaling process itself, whereby the nucleophilic agent reacts with only the small subset of PTP enzymes that are involved in, and oxidatively regulated by, insulin signaling.[38]



Scheme 1. Oxidative inactivation of PTP1B, thiol-mediated recovery of enzyme activity, and capture of the oxidized enzyme by a sulfone-stabilized carbanion.

The choice of nucleophile for trapping oxidized PTP1B may be crucial. For example, many common nucleophiles such as thiols, amines, and phosphines may *not* be ideal for capturing the sulfenyl amide because the S-S, S-N, and S-P bonds resulting from their reactions with the electrophilic sulfur center would be unstable in the presence of biological thiols or water.[33,39] Consequently, these nucleophiles may not effectively block regeneration of active enzyme under physiological conditions. On the other hand,

reactions of carbon nucleophiles with the sulfenyl amide would yield carbon-sulfur bonds, which are expected to be relatively stable under physiological conditions (Scheme 1). Along these lines, carbon nucleophiles derived from 1,3-diketones such as dimedone (5,5-dimethyl-1,3-cyclohexanedione) have long been used as tools for the covalent capture of the electrophilic sulfur center in peptide and protein sulfenic acids (Scheme 2).[37,40-46] Additionally, carbon nucleophiles derived from 1,3-diketones have been shown to capture of the electrophilic sulfur center in a dipeptide model of the protein sulfenyl amide.[36,47]



Scheme 2. Reaction of dimedone with a sulfenic acid residue.

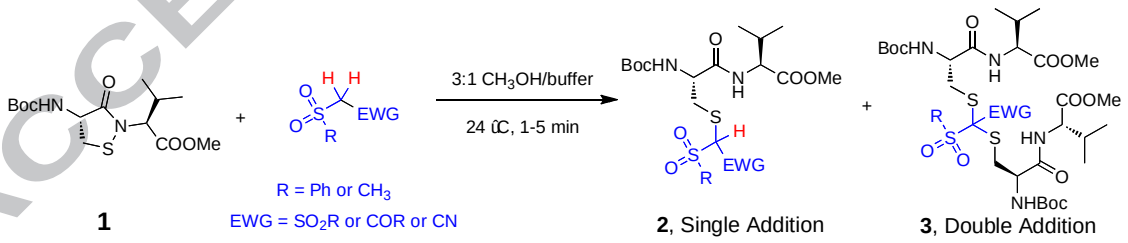
Sulfone-stabilized carbon anions are excellent nucleophiles whose properties are distinctly different from those of 1,3-diketone-derived enolates.[48-50] The sulfonyl group is a powerful electron-withdrawing group that stabilizes α-carbanions by an inductive effect.[48,51-53] Sulfone-stabilized carbanions are polarizable species that are, in some cases, more reactive in protic media than aprotic media.[51,54,55] These interesting chemical properties prompted us to examine the reactions of sulfone-stabilized carbanions with the synthetic dipeptide **1** containing a sulfenyl amide functional group.[56] We found that β-ketosulfones, β-disulfones, and β-cyanosulfones react readily with the electrophilic sulfur center in the sulfenyl amide to generate adducts containing the expected carbon-sulfur bonds. Our results reveal that the α-sulfonyl sulfide adducts **2** derived from the reaction

of **1** with sulfone-based carbanions slowed, but did not completely prevent, the ability of thiols to generate the reduced cysteine dipeptide **11**. Overall, these results suggest that covalent capture of the sulfenyl amide residue in PTP1B by sulfone-stabilized carbanions has the potential to transiently inhibit thiol-mediated regeneration of catalytic activity from oxidized PTP1B.

2. Results and Discussion

2.1 Reactions of sulfone-based carbon nucleophiles with the peptide sulfenyl amide 1.

The dipeptide sulfenyl amide **1** was prepared by the method of Morin and Gordon.[36,56,57] Reactions of **1** with the sulfone-containing carbon acids **A-I** were carried out in a solvent system composed of 3:1 methanol and HEPES buffer (50 mM, pH 7) containing NaCl (100 mM), and EDTA (1 mM). In this predominantly organic solvent mixture, the sulfenyl amide-containing dipeptide **1** is reasonably stable and, in the absence of added nucleophile, persists for several hours as judged by thin layer chromatographic analysis (TLC).

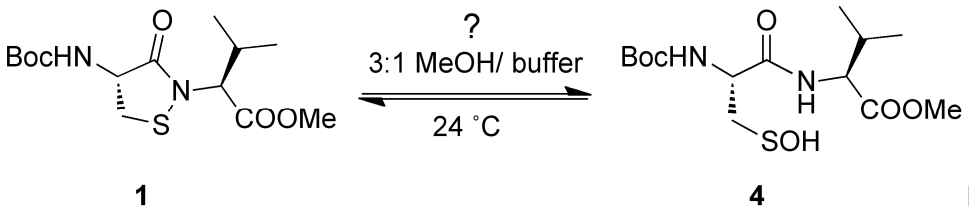


Scheme 3. Reactions of sulfone-derived carbanions with the sulfenyl amide residue in the dipeptide **1**.

The reactions of the sulfones **A-I** (22 mM) with the dipeptide **1** (20 mM) were rapid, with all starting sulfenyl amide consumed within 10 min as judged by TLC. In

each case, two major products were isolated by column chromatography on silica gel. Spectroscopic analyses indicated that these products corresponded to the single and double addition products **2** and **3** (Scheme 3). The single-addition products **2** were obtained as mixtures of diastereomers. For example, product **2a** resulting from reaction of **1** with 1.1 equiv methanesulfonylacetone **A** was a 2:1 mixture of the two possible diastereomers (Figure 1). The double-addition products **3b-i** were characterized by a doubling of the integrals for peptide-associated resonances and the absence of a resonance for the α-proton in the ^{1}H-NMR (resonance "k" in Figure 1). Mass spectrometric analyses were also consistent with the proposed structures.

Presumably, these reactions proceed via an initial ionization of the parent carbon acids to afford the sulfone-stabilized carbanions. Then, nucleophilic attack of an equivalent of sulfonyl carbanion on the electrophilic sulfur center in **1** affords the observed single-addition products. Subsequently, double-addition products may form after loss of the remaining acidic α-proton present in the single-addition products **2a-i**, followed by reaction with a second equivalent of electrophile **1**. The mass balances in these reactions were generally good, with the isolated products accounting for 69-93% of the input dipeptide **1**. In general, reactions leading to the double-addition products **3b-i** seemed to be faster than the initial reactions leading to the single-addition products **2a-i**. This speculation is based on the observation that double-addition products tended to predominate even though a slight excess of sulfone over **1** was employed in the reactions. A similar propensity for the generation of double addition products was reported previously in the alkylation of sulfone-stabilized anions.[58]



1 **4**

Scheme 4. No evidence was observed for generation of significant concentrations of sulfenic acid **4** under the reaction conditions employed in these studies.

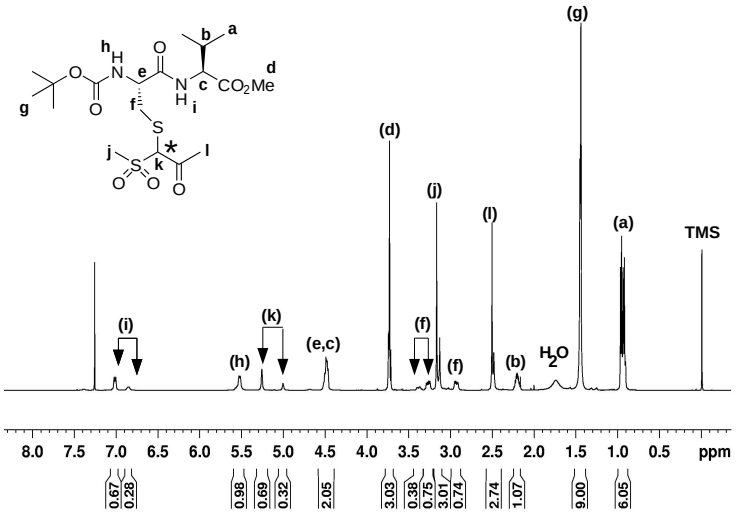


Figure 1. ^{1}H-NMR spectrum of **2a**.

It was recently proposed that the sulfenyl amide **1** exists in equilibrium with the ring-opened sulfenic acid **4** (Scheme 4), when dissolved in a 2:1 (v/v) aqueous-organic solvent mixture containing acetonitrile and phosphate buffered saline (phosphate, 10 mM, pH 7; NaCl, 137 mM; KCl, 2.7 mM).[46] This suggestion was based on the observed rapid decomposition (< 15 min) of **1** in this solvent system affording products reasonably expected to arise from dimerization of the putative sulfenic acid **4**. With this in mind, it was interesting for us to consider whether the sulfenic acid **4** might be a significant

contributor to the reactivity of **1** in our solvent mixture containing a relatively small fraction of aqueous buffer (3:1 methanol and HEPES buffer).

We made several observations suggesting that, in our solvent mixture, significant concentrations of the sulfenic acid **4** are *not* generated. First, as noted above, we did not observe significant decomposition of **1** over the course of several hours when carbon nucleophiles were omitted from the reaction mixture. Second, we observed no reaction when **1** (12 mM) was stirred with methyl iodide (2 M) at 24 ˚C for 8 h. This result is significant because we and others have shown that methyl iodide, in this concentration range, effectively traps sulfenic acid intermediates to generate the corresponding methyl sulfoxide.[46,59] Finally, we showed that **1** reacted rapidly with the carbon nucleophiles **D** and **F** in dry organic solvent (acetone), where generation of significant concentrations of the sulfenic acid were not possible. The reactions in dry solvent were complete in less than 5 min and gave the same products observed in our standard 3:1 organic-aqueous solvent mixture. Specifically, the reaction of **1** with **D** (1.1 eq.) in dry acetone containing K_2CO_3 (0.5 eq.) provided **2d** and **3d** in 83% and 8% yields respectively (5 min at 24 ˚C). Similarly, reaction of **1** with **F** (1.1 eq.) in dry acetone yielded **2f** and **3f** in 16% and 78% yields at 24 ˚C in 5 min. These reactions in dry solvent confirm that the sulfenyl amide group is a competent electrophile, capable of reacting directly with sulfone-stabilized carbanions. While it is not possible to rule out some involvement of the sulfenic acid **4** (or an analogous sulfenyl methyl ester) in our 3:1 methanol-buffer system, the evidence suggests: (1) that substantial amounts of the sulfenic acid are not present in our reaction mixtures and, (2) that the sulfenic acid is not an obligate intermediate in the reaction of sulfenyl amide **1** with sulfonyl-stabilized carbanions. Our 3:1 (v/v) organic-aqueous solvent conditions may be complementary to the 1:2 (v/v) organic-aqueous system used

by Gupta and Carroll.[46] Dissolution of **1** in the 1:2 organic-aqueous system of Gupta and Carroll may enable access to significant concentrations of the sulfenic acid **4**, while the 3:1 organic-aqueous solvent condition used here may allow **1** to remain largely intact, thus serving as a faithful chemical model for the active site of oxidized PTP1B where, at least under the conditions used for X-ray crystallographic analyses, the sulfenyl amide form predominates over the sulfenic acid (Scheme 1).[24,30,31] The equilibrium between cysteine sulfenyl amide and sulfenic acid in peptides and proteins under various aqueous solvent conditions and protein environments deserves further study.

Table 1. Products obtained from the reaction of the sulfenyl amide-containing dipeptide 1 with various sulfone-stabilized carbon nucleophiles. (The dipeptide is truncated in these structures. Complete structures of the side chains in the single addition and double addition products are shown in Scheme 3).

2.2 Reaction of sulfone-based carbon nucleophiles with other electrophilic sulfur compounds. For the purposes of comparison, we examined the reactions of the sulfone-based carbon nucleophiles **B**, **C**, **D**, and **F** with the electrophilic sulfur centers in the cysteine disulfide **5**. Additionally, we examined the reactivity of **C**, **E**, and **F** with the synthetically-used[60] sulfenylating agent *N*-(phenylthio)succinimide **6**. Stirring the disulfide **5** with 1.1 equiv of the carbon acids **B**, **C**, **D**, or **F** in our standard 3:1 methanol and HEPES buffer gave no reaction over the course of 8 h, as judged by TLC. On the other hand, the acyl sulfenamide **6** displayed reactivity that was quite similar to the sulfenyl amide residue in **1**. For example, stirring **6** with 1.1 equiv **E** for 30 min produced a mixture of the single addition and double addition products **8** and **9** in 34% and 51% yields, respectively **(**Table 2**)**. Sulfones **E** and **F** gave exclusively the double-addition products **7** and **10**. The results suggest that, for some applications, **6** might provide a reasonable model for the reactivity of the electrophilic sulfur center in the sulfenyl amide residue.

Table 2. Products resulting from the reaction of sulfone-stabilized carbanions with **6**.

2.3 Kinetics of reactions between sulfone-based carbon nucleophiles and 1. We employed a spectrophotometric assay[36] to measure the rates at which sulfone-based carbon nucleophiles reacted with **1**. This assay exploits the rapid reaction of **1** with the highly colored thiol, 5-thio-2-nitrobenzoic acid (TNB, the reduced form of Ellman's reagent) and enabled us to monitor the amount of **1** remaining as a function of time, following addition of a large molar excess of the carbon nucleophile (10-fold or greater) in a solvent mixture composed of 1:1 (v/v) methanol and buffer (Tris, 50 mM, Bis-Tris, 50 mM, sodium acetate, 100 mM, and diethylenetriaminepentaacetic acid DTPA, 10 mM, pH 7.0). Under these conditions, we found that **1** was stable over the time-course of the kinetic assays (10 min, Fig. S1 panel A). We determined the reaction rates of **1** with three representative sulfones for which reaction products had been spectroscopically characterized: β-ketosulfone **A**, β-sulfonylsulfone **D**, and β-cyanosulfone **F** (Fig. S1). To

further explore structure-activity relationships, we also examined the reaction of **1** with the β-ketosulfones **J**, **K**, **L**, and **M** (Fig. S1).

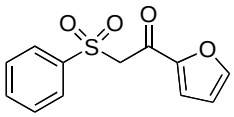
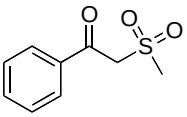
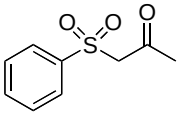
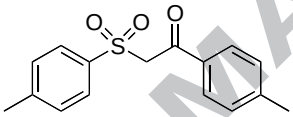
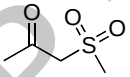
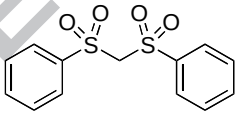
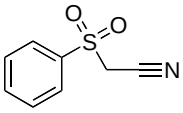
We first confirmed the molecularity of these reactions using pseudo-first order kinetic analysis. To that end, we employed a large molar excess of •-ketosulfone **L** over sulfenyl amide **1** and determined the pseudo first-order rate constants associated with capture of **1** by **L** at various molar excesses of **L** (Fig. 2A). A replot of these reaction rate constants versus molar concentration of **L** revealed a straight line (r^2 = 0.9998), consistent with an overall second-order process. The rate constant for this process was 15.8 ± 0.5 M^{-1} s^{-1}. It is worth noting that in these experiments, because a *minimum* 10-fold molar excess of sulfone over **1** was employed, these kinetic data likely reflect the rate of monoadduct formation – that is, the rate of initial nucleophilic attack of carbanion on sulfenyl amide. We then determined approximate second-order rate constants for capture of **1** by the remaining sulfones **A**, **D**, **F**, **J**, **K**, and **M** (Fig. S1). In those experiments, we employed a single concentration of carbon nucleophile under pseudo first-order conditions and calculated apparent second-order rate constants from the data. All kinetic data fit well to first-order processes, and the apparent second-order rate constants measured for these sulfones ranged from 9 to 64 M^{-1} s^{-1} (Table 3).

To probe the origins of the observed kinetic trends, we spectrophotometrically determined the pK_a values of sulfones **A**, **D**, **F**, **J**, **K**, **L** and **M** (Fig. S3). Interestingly there was no clear correlation between the acidity of the sulfone-derived carbon acids and the rates at which they reacted with **1** (Table 3). For example, although disulfone **D** and the β-cyanosulfone **F** have nearly identical aqueous pK_a values (10.9 and 11.0, respectively), the β-cyanosulfone **F** was found to react with **1** approximately 5-fold faster than disulfone **D** (approximate second-order rate constants 12 and 64 M^{-1} s^{-1},

respectively). In general, previous work has shown that the nucleophilicities of carbanions and enolates do not correlate strongly with the pK_a of the corresponding carbon acids.[51] This observation was recapitulated in our recent work describing covalent capture of **1** by 1,3-diketone-derived carbon acids[36] as well as recent work from Carroll's group.[46]

Figure 2. Kinetics of capture of sulfenyl amide **1** by sulfone **L**.

Table 3. Reaction rates of sulfone-stabilized carbanions with **1**.

Compound	Structure	k_{trap} (M^{-1} s^{-1})[a]	pK_a[b]
J		31[c]	9.2
K		18[c]	9.3
L		15.8 ± 0.5	9.3
M		20[c]	9.7
A		9[c]	10.0
D		12[c]	10.9
F		64[c]	11.0

[a] Second-order rate constants for trapping the model sulfenyl amide in 1:1 CH_3OH:Buffer B (pH 7.0), 23 ± 2 °C.
[b] Aqueous pK_a values were determined as reported in the text.
[c] Approximate second-order rate constants for trapping the model sulfenyl amide in 1:1 CH_3OH:Buffer B (pH 7.0), 23 ± 2 °C.

2.4. Reaction of products 2a, 2d, and 2f with 1,4-dithio-D-threitol (DTT). The stability of the series of adducts **2** resulting from covalent capture of **1** by the sulfone-stabilized nucleophiles is an important consideration (in the Conclusions, we provide

some discussion regarding why the monoadducts **2** may be more relevant to the enzyme). In the context of the enzyme, conversion of the oxidized PTP1B sulfenyl amide to a *stable* sulfone adduct would prevent regeneration of the catalytically active form of the enzyme. Of special concern is whether reactions of the adducts **2** with thiols can regenerate the native cysteine residue, analogous to catalytically active PTP1B. Therefore, we exposed the sulfone-dipeptide adducts **2a**, **2d**, and **2f** to the thiol-containing reducing agent, 1,4-dithio-D-threitol **(**DTT, 20 mM) in our standard solvent mixture, composed of 3:1 methanol and buffer (HEPES 50 mM, NaCl 100 mM, and EDTA 1 mM, pH 7) at 37 ˚C (Scheme 5). Under these conditions, the β-cyanosulfone adduct **2f** released the cysteine-containing peptide **11** in a modest 24% yield after 10 min. The β-ketosulfone adduct **2a** and β-disulfone adduct **2d** gave only traces of **11** after a reaction time of 10 min. On the other hand, the unmodified sulfenyl amide-containing dipeptide **1** afforded a 92% yield of **11** after 10 min under these conditions. However, following extended incubation with DTT (5 h), adducts **2f**, **2d** and **2a** gave 94%, 77% and 58% yields of **11**, respectively. Overall, these results show that covalent capture of **1** by sulfone-derived carbon nucleophiles may impede, but not completely prevent, thiol-dependent generation of the cysteine dipeptide **11**. From the data, it is possible to estimate a half-life of approximately 25 min for **2f** at 37 ˚C in the presence of 25 mM DTT. These results stand in contrast to our earlier results with diketone adducts of **1**, where enolization may have stabilized the diketone-peptide adducts against thiol-mediated reversion to the cysteine dipeptide.[36]

Scheme 5. Reaction of adducts **2** with DTT.

3. Conclusions

The sulfone-stabilized carbanions examined here reacted readily with the sulfenyl amide residue in the dipeptide **1**. The products were consistent with a mechanism involving ionization of the carbon acid, followed by nucleophilic attack of the resulting carbanion on the electrophilic sulfur center in the sulfenyl amide residue of **1**. These monoadduct products **2** may then react with a second equivalent of **1** to give the double-addition products **3**. The double-addition reaction is probably not relevant to the enzyme PTP1B, as steric constraints seem unlikely to permit two molecules of the enzyme to be linked via their occluded active site cysteine residues[30] through a single carbon atom.

Rate constants for the reaction of the sulfones with **1** ranged from 9–64 M^{-1} s^{-1}. These rate constants are larger than those reported recently for the reaction of 1,3-diketone-derived nucleophiles with **1**[57] and are comparable to the rates ascribed to the reaction of cyclic sulfone-stabilized nucleophiles with the sulfenic acid **4**.[46] By way of further comparison, rates reported for the reaction of dimedone with sulfenic acid residues are in the range of 0.03[61] to 12 M^{-1} s^{-1}.[46] Importantly, rate constants in the range of 9–64 M^{-1} s^{-1} may allow micromolar concentrations of these agents to compete

effectively against millimolar concentrations of cellular thiols for reaction with oxidized PTPs.[33] We also examined the stabilities of the adducts **2** against thiols. Cells contain approximately 1-5 mM glutathione (E_0' = –0.207 V).[62,63] We found that, under our standard solvent conditions, the reactions of the adducts **2** with glutathione (5 mM) were sluggish (data not shown). Therefore, in order to measure the relative reactivity of the different adducts **2**, we employed more stringent reaction conditions involving an increased concentration (20 mM) of the strongly-reducing thiol DTT (E_0' = –0.327 V).[64] Our results showed that conversion of the sulfenyl amide **1** to the sulfone adducts **2** slowed, but did not completely prevent, the ability of thiols to generate the reduced cysteine dipeptide **11**. The polarizable nature of sulfone-stabilized carbanions[51,54,55] that makes them excellent nucleophiles may also make them reasonably good leaving groups in reactions of the adducts with thiols. From a pharmacological perspective, the thiol-labile nature of the sulfone adducts **2** could be viewed as an advantage, given that some degree of reversibility may be desirable in covalent drugs.[65] At the same time, in the context of PTP1B, the sulfone-cysteine adducts modeled by **2** may enjoy greater stability if sequestered within the rather deep active site of the enzyme where the attachment to the enzyme is less accessible to thiols.[66]

The pharmacological pairing explored in this work, involving reaction of an electrophilic protein target with a nucleophilic small molecule is quite unusual. Among the few such examples in medicinal chemistry is the use of 2-pyridine aldoxime methyl chloride (PAM) as an antidote to regenerate catalytic activity of acetylcholinesterases inactivated by organophosphorus nerve agents.[67,68] This reaction involves nucleophilic attack of PAM on the electrophilic adducts formed by nerve agents at the active site serine residue of acetylcholinesterases. A second example involves the nucleophilic

attack of the arylamine group of sulfa drugs on 6-hydroxymethyl-7,8-dihydropterin pyrophosphate bound at the active site of dihydropteroate synthase.[69] The sulfur center in the oxidized, sulfenyl amide form of PTP1B generated during insulin signaling may represent a unique endogenous electrophilic protein target in the cell. Our results show that carbon nucleophiles do not react with the electrophilic sulfur centers in disulfides.[36] As a result, the reaction of nucleophilic agents with oxidized PTP1B has the potential to be an inherently selective process, with off-target attack on other redox-regulated proteins being one obvious concern. Overall, our studies alongside other recent work[37,47,57,70] suggest that covalent capture of oxidized PTP1B by carbon nucleophiles may offer a viable alternative to traditional inhibitors for the chemical knockdown of this enzyme activity in cells.

4. Experimental

4.1. General

Unless otherwise noted, all reagents were purchased from commercial vendors and used without further purification. Analytical thin layer chromatography was performed on silica gel plates with UV indicator. Flash chromatography was carried out using 230-400 mesh silica gel. ^{1}H NMR spectra were recorded on either a Bruker DRX-500 (500 MHz) or a DRX-600 (600 MHz) spectrometer with chemical shifts reported in δ ppm. ^{13}C NMR spectra were obtained on the same instruments at 125 and 150 MHz, respectively. Melting points were recorded on a Unitemp capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a FT-IR spectrometer

using NaCl plates. High-resolution mass spectrometry (HRMS) was performed using electrospray ionization (ESI) and time-of-flight (TOF) mass analysis.

4.2. General procedure for synthesis of adducts 2a-2i and 3a-3i.

To a stirred solution of the sulfenyl amide peptide **1** (20 mg, 0.060 mmol) in 3 mL of 3:1 methanol:HEPES buffer (HEPES, 50 mM, NaCl, 100 mM, EDTA, 1 M, pH 7.0) was added the sulfone-containing nucleophile (1.1 equiv) and the mixture stirred at room temperature (24 ˚C). When the reaction was judged complete by TLC analysis, methanol was completely removed by blowing a stream of nitrogen gas on the solution, and the resulting aqueous solution extracted with ethyl acetate or dichloromethane (2 × 2 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated by rotary evaporation. The products were isolated by column chromatography on silica gel eluted with mixtures of either ethyl acetate-hexane or methanol-dichloromethane.

4.2.1. (2*S*)-methyl 2-((2*R*)-2-((*tert*-butoxycarbonyl)amino)-3-((1-(methylsulfonyl)-2-oxopropyl)thio)propanamido)-3-methylbutanoate (2a)

Yellow oil (19.5 mg, 69%) as an inseparable mixture of diastereomers (70:30). R_f = 0.37 (40% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.01 (d, *J* = 8.5 Hz, 1H), δ 6.85 (br, 0.5H), δ 5.52 (d, *J* = 7.0 Hz, 1H), δ 5.26 (s, 0.5H), δ 5.00 (s, 0.5H), δ 4.46-4.50 (m, 2H), δ 3.73 (s, 2H), δ 3.71 (s, 1H), δ 3.38 (br, 0.5H), δ 3.26 (dd, *J* = 14.0, 7.0 Hz, 0.5H), δ 3.17 (s, 2H), δ 3.13 (s, 1H), δ 2.92 (dd, *J* = 14.2, 6.5 Hz, 1H), δ 2.50 (s, 2H), δ 2.48 (s, 1H), δ 2.16-2.24 (m, 1H), δ 1.45 (s, 4H), δ 1.44 (s, 5H), δ 0.94 (dd, *J* = 16.5, 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 198.3, 198.1, 172.1, 171.9, 170.3,

169.9, 155.8, 155.7, 80.9, 80.6, 73.8, 72.4, 57.7, 57.6, 54.2, 52.4, 52.3, 52.2, 37.3, 37.1, 36.6, 36.1, 31.0, 30.9, 30.8, 28.4, 19.3, 19.2, 17.7, 17.6; IR (cm^{-1}) 3416, 3054, 2979, 1735, 1715, 1675, 1497, 1366, 1310, 1262, 1159, 1107, 734, 698; HRMS (ESI-TOF, [M + H]$^+$) m/z calcd for $C_{18}H_{32}N_2O_8S_2$ 469.1683, found 469.1678.

4.2.2. (2*S*)-methyl 2-((2*R*)-2-((*tert*-butoxycarbonyl)amino)-3-((2-methoxy-1-(methylsulfonyl)-2-oxoethyl)thio)propanamido)-3-methylbutanoate (2b)

Colorless oil (17 mg, 58%) R_f = 0.32 (30% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.16 (d, *J* = 8.5 Hz, 0.5H), δ 6.84 (d, *J* = 8.5 Hz, 0.5H), δ 5.49-5.54 (m, 1H), δ 4.86 (s, 0.5H), δ 4.80 (s, 0.5H), δ 4.48-4.53 (m, 2H), δ 3.86 (s, 1H), δ 3.85 (s, 2H), δ 3.74 (s, 1H), δ 3.73 (s, 2H), δ 3.41-3.45 (m, 0.5H), δ 3.28 (s, 1.5H), δ 3.24 (s, 1.5H), δ 3.07-3.18 (m, 1H), δ 2.17-2.24 (m, 1H), δ 1.46 (s, 5H), δ 1.45 (s, 4H), δ 0.90-0.96 (m, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.9, 169.9, 169.8, 165.4, 164.9, 155.5, 155.1, 80.8, 80.5, 69.2, 57.4, 57.3, 54.3, 54.0, 53.9, 53.1, 52.3, 52.2, 37.2, 36.9, 36.5, 36.3, 30.9, 29.7, 28.3, 28.2, 18.9, 17.5; IR (cm^{-1}) 3357, 3016, 2769, 2929, 1742, 1677, 1487, 1377, 1315, 1217, 1162, 756, 667; HRMS (ESI-TOF, [M + H]$^+$) m/z calcd for $C_{18}H_{33}N_2O_9S_2$ 485.1627, found 485.1635.

4.2.3. (3*S*,6*R*,12*R*,15*S*)-trimethyl 6,12-bis((*tert*-butoxycarbonyl)amino)-2,16-dimethyl-9-(methylsulfonyl)-5,13-dioxo-8,10-dithia-4,14-diazaheptadecane-3,9,15-tricarboxylate (3b)

Colorless oil (3 mg, 12%) R_f = 0.24 (30% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.22 (br, 1H), δ 5.69 (d, *J* = 8.0 Hz, 1H), δ 5.55 (d, *J* = 8.0 Hz, 1H), δ 4.51-4.55 (m, 4H), δ 3.92 (s, 3H), δ 3.75 (s, 6H), δ 3.36 (s, 3H), δ 3.23-3.32 (m, 4H), δ 2.15-2.21 (m, 2H), δ 1.46 (s, 18H), δ 0.93 (dd, J = 13.0 Hz, 6.5 Hz, 12H); ^{13}C NMR

($CDCl_3$, 125 MHz) δ 172.3, 172.2, 172.0, 170.0, 169.9, 165.2, 155.8, 155.5, 84.4, 80.7, 57.6, 57.5, 55.0, 53.6, 53.5, 52.4, 38.9, 34.9, 34.8, 31.4, 31.3, 28.5, 19.1, 17.9; IR (cm^{-1}) 3412, 3356, 3015, 2971, 2931, 2871, 1740, 1676, 1497, 1366, 1314, 1215, 1159, 754, 666; HRMS (ESI-TOF, $[M + H]^+$) m/z calcd for $C_{32}H_{57}N_4O_{14}S_3$ 817.3033, found 817.3028.

4.2.4. (2'*S*)-dimethyl 2,2'-(((2*R*,2'*R*)-3,3'-((1,1-dioxido-3-oxo-2,3-dihydrobenzo[b]thiophene-2,2-diyl)bis(sulfanediyl))bis(2-((*tert*-butoxycarbonyl)amino)propanoyl))bis(azanediyl))bis(3-methylbutanoate) (3c)

Colorless oil (20 mg, 78%) R_f = 0.25 (40% ethyl acetate/hexanes). ^1H NMR ($CDCl_3$, 500 MHz) δ 8.05 (d, *J* = 7.5 Hz, 1H), δ 7.92-7.97 (m, 2H), δ 7.83-7.86 (m, 1H), δ 5.60 (s, 1H) δ 5.55 (d, *J* = 7.5 Hz, 1H), δ 4.53-4.56 (m, 4H), δ 3.76 (s, 3H), δ 3.75 (s, 3H), δ 3.16-3.31 (m, 4H), δ 2.15-2.22 (m, 2H), δ 1.45 (s, 9H), δ 1.43 (s, 9H), δ 0.92-0.95 (m, 12H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 183.4, 172.1, 169.8, 169.7, 155.5, 155.4, 142.0, 137.2, 134.7, 130.1, 126.5, 122.4, 80.5, 80.4, 79.2, 57.6, 57.5, 53.3, 52.4, 52.3, 34.0, 33.8, 31.2, 29.7, 28.3, 19.0, 17.9; IR (cm^{-1}) 3408, 3354, 3058, 2969, 2933, 2872, 1716, 1680, 1504, 1363, 1262, 1212, 1158, 1021, 737, 705; HRMS (ESI-TOF, $[M + H]^+$) m/z calculated for $C_{36}H_{55}N_4O_{13}S_3$:847.2928, found 847.2911.

4.2.5. (*S*)-methyl 2-((*R*)-3-((bis(phenylsulfonyl)methyl)thio)-2-((*tert*-butoxycarbonyl)amino)propanamido)-3-methylbutanoate (2d)

White solid (19.5 mg, 51%). Mp: 65-73 °C. R_f = 0.42 (40% ethyl acetate/hexanes). ^1H NMR ($CDCl_3$, 500 MHz) δ 8.16 (d, *J* = 7.5 Hz, 2H), δ 8.03 (d, *J* = 7.5 Hz, 2H), δ 7.73 (t, *J* = 7.5 Hz, 1H), δ 7.67 (t, *J* = 7.0 Hz, 1H), δ 7.61 (t, *J* = 7.5 Hz, 2H), δ 7.52 (t, *J* = 7.5 Hz, 2H), δ 7.20 (d, *J* = 8.5 Hz, 1H), δ 6.16 (s, 1H), δ 5.71 (d, *J* =

6.5 Hz, 1H), δ 4.46 (dd, *J* = 8.0, 4.5 Hz, 1H), δ 4.37-4.41 (m, 1H), δ 3.74 (s, 3H), δ 2.65 (br, 2H), δ 2.20-2.29 (m, 1H), δ 1.48 (s, 9H), δ 0.96 (dd, *J* = 12.0, 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.7, 169.7, 155.4, 137.7, 137.2, 135.0, 134.8, 130.4, 130.2, 129.1, 128.9, 85.6, 80.7, 58.0, 53.8, 52.3, 37.2, 30.7, 28.4, 19.2, 17.6; IR (cm^{-1}) 3413, 3369, 3055, 2972, 2361, 2322, 1678, 1265, 1154, 747, 703; HRMS (ESI-TOF, $[M + H]^+$) m/z calcd for $C_{27}H_{36}N_2O_9S_3$ 629.1661, found 629.1670.

4.2.6. (2*S*,5*R*,11*R*,14*S*)-dimethyl 5,11-bis((*tert*-butoxycarbonyl)amino)-2,14-diisopropyl-4,12-dioxo-8,8-bis(phenylsulfonyl)-7,9-dithia-3,13-diazapentadecane-1,15-dioate (3d)

White solid (11.5 mg, 40%). Mp: 108-113 °C. R_f = 0.26 (40% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.13 (d, *J* = 7.5 Hz, 4H), δ 7.70 (t, *J* = 7.5 Hz, 2H), δ 7.56 (t, *J* = 8.0 Hz, 4H), δ 7.16 (br, 2H), δ 5.59 (br, 2H), δ 4.43-4.50 (m, 4H), δ 3.74 (s, 6H), δ 3.40 (br, 2H), δ 3.28 (dd, *J* = 13.5, 7.5 Hz, 2H), δ 2.11-2.20 (m, 2H), δ 1.45 (s, 18H), δ 0.90 (dd, *J* = 12.5, 7.0 Hz, 12H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.9, 169.7, 155.4, 135.6, 135.9, 132.0, 128.3, 99.9, 80.3, 57.2, 53.8, 52.1, 34.4, 31.1, 28.1, 18.8, 17.6; IR (cm^{-1}) 3364, 2967, 2360, 1706, 1660, 1520, 1327, 1246, 1158, 1076, 1018, 855, 732; HRMS (ESI-TOF, $[M + H]^+$) m/z calcd for $C_{41}H_{60}N_4O_{14}S_4$: 961.3067, found 961.3054.

4.2.7. (*S*)-methyl 2-((*R*)-2-((*tert*-butoxycarbonyl)amino)-3-((ditosylmethyl)thio)propanamido)-3-methylbutanoate (2e)

Colorless oil (25 mg, 64%) R_f = 0.46 (30% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.03 (d, *J* = 8.0 Hz, 2H), δ 7.91 (d, *J* = 8.0 Hz, 2H), δ 7.39 (d, *J* = 8.0 Hz, 2H), δ 7.31 (d, *J* = 8.0 Hz, 2H), δ 7.24 (d, *J* = 8.5 Hz, 1H), δ 6.05 (s, 1H), δ 5.73

(d, *J* = 6.5 Hz, 1H), δ 4.47 (dd, *J* = 8.5, 4.5 Hz, 1H), δ 4.39 (t, *J* = 6.5 Hz, 1H), δ 3.74 (s, 3H), δ 2.64-2.71 (m, 2H), δ 2.48 (s, 3H), δ 2.45 (s, 3H), δ 2.21-2.28 (m, 1H), δ 1.49 (s, 9H), δ 0.97 (dd, *J* = 11.0, 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ171.6, 169.6, 155.2, 146.0, 145.8, 134.5, 134.1, 130.3, 130.1, 129.6, 129.4, 85.6, 80.5, 57.8, 53.6, 52.1, 37.1, 30.54, 28.3, 21.8, 21.7, 19.1, 17.5; IR (cm^{-1}) 3412, 3370, 3052, 2970, 2929, 2872, 1736, 1675, 1593, 1483, 1332, 1258, 1158, 1077, 734; HRMS (ESI-TOF, $[M + H]^+$) m/z calculated for $C_{29}H_{41}N_2O_9S_3$: 657.1974, found 657.1969.

4.2.8. (2*S*,5*R*,11*R*,14*S*)-dimethyl 5,11-bis((*tert*-butoxycarbonyl)amino)-2,14-diisopropyl-4,12-dioxo-8,8-ditosyl-7,9-dithia-3,13-diazapentadecane-1,15-dioate (3e)

Colorless oil (8.5 mg, 29%) R_f = 0.31 (30% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.99 (d, *J* = 8.5 Hz, 2H), δ 7.35 (d, *J* = 8.5 Hz, 2H), δ 7.17 (br, 1H), δ 5.64 (br, 1H), δ 4.43-4.49 (m, 2H), δ 3.74 (s, 3H), δ 3.40-3.42 (m, 1H), δ 3.28 (dd, *J* = 13.5, 7.5 Hz, 1H), δ 2.46 (s, 3H), δ 2.12-2.19 (m, 1H), δ 1.45 (s, 9H), δ 0.91 (dd, *J* = 12.0, 6.5 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.9, 169.9, 155.6, 146.4, 132.7, 132.2, 129.2, 80.45, 57.4, 54.0, 52.2, 34.6, 31.3, 28.3, 21.8, 18.9, 17.7; IR (cm^{-1}) 3359, 3050, 2973, 2929, 2359, 2333, 1733, 1718, 1697, 1682, 1504, 1337, 1262, 1152, 737, 701; HRMS (ESI-TOF, $[M + H]^+$) m/z calculated for $C_{43}H_{65}N_4O_{14}S_4$: 989.3362, found 989.3358.

4.2.9. (2*S*)-methyl 2-((2*R*)-2-((*tert*-butoxycarbonyl)amino)-3-((cyano(phenylsulfonyl)methyl)thio)propanamido)-3-methylbutanoate (2f)

Inseparable mixture of two isomers in 1:1 ratio. Colorless oil (7 mg, 22%) R_f = 0.47 (30% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.06-8.09 (m, 2H), δ 7.77-7.80 (m, 1H), δ 7.64-7.68 (m, 2H), δ 7.029 (d, *J* = 7.0 Hz, 0.5H), δ 6.86 (d, *J* = 8.5

Hz, 0.5H), δ 5.61 (d, *J* = 6.5 Hz, 0.5H), δ 5.42-5.49 (m, 1H), δ 4.48-4.54 (m, 2H), δ 3.76 (s, 1.5H), δ 3.75 (s, 1.5H), δ 3.50 (dd, *J* = 15.0, 5.0 Hz, 0.5H), δ 3.22-3.23 (m, 1H), δ 3.13-3.16 (m, 0.5H), δ 2.17-2.27 (m, 1H), δ 1.48 (s, 4.5H), δ 1.47 (s, 4.5H), δ 0.91-0.99 (m, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.8, 171.7, 170.1, 169.9, 155.7, 135.7, 135.6, 134.8, 134.7, 132.2, 132.1, 130.4, 130.3, 129.7, 129.6, 129.5, 112.2, 111.9, 81.4, 81.1, 57.9, 57.7, 57.6, 57.4, 56.9, 54.3, 53.6, 52.5, 52.4, 36.1, 35.6, 31.1, 30.9, 28.4, 28.3, 19.2, 19.1, 17.8, 17.7; IR (cm^{-1}) 3420, 3018, 2971, 2251, 1735, 1675, 1485, 1211, 1155, 905, 758, 734, 650; HRMS (ESI-TOF, $[M + H]^+$) m/z calculated for $C_{22}H_{31}N_3O_7S_2$: 514.1682, found 514.1688.

4.2.10. (2*S*,5*R*,11*R*,14*S*)-dimethyl 5,11-bis((*tert*-butoxycarbonyl)amino)-8-cyano-2,14-diisopropyl-4,12-dioxo-8-(phenylsulfonyl)-7,9-dithia-3,13-diazapentadecane-1,15-dioate (3f)

White solid (18 mg, 70%). Mp: 45-48 °C. R_f = 0.22 (30% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.05 (d, *J* = 7.5 Hz, 2H), δ 7.77 (t, *J* = 7.5 Hz, 1H), δ 7.62 (t, *J* = 7.5 Hz, 2H), δ 7.14 (br, 2H), δ 5.59-5.56 (m, 2H), δ 4.59 (br, 1H), δ 4.52-4.56 (m, 3H), δ 3.76 (s, 3H), δ 3.75 (s, 3H), δ 3.35-3.40 (m, 4H), δ 2.13-2.23 (m, 2H), δ 1.46 (s, 10H), δ 1.45 (s, 8H), δ 0.90-0.94 (m, 12 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.2, 169.6, 155.5, 135.8, 133.0, 131.8, 129.3, 112.3, 80.8, 72.5, 57.6, 53.5, 53.4, 52.5, 52.4, 36.6, 36.1, 31.4, 28.4, 19.0, 17.8; IR (cm^{-1}) 3323, 3048, 2973, 2133, 1718, 1660, 1514, 1400, 1240, 1146, 1070, 861; HRMS (ESI-TOF, $[M + H]^+$) m/z calcd for $C_{36}H_{55}N_5O_{12}S_3$ 846.3071, found 846.3088.

4.2.11. (2*S*)-methyl 2-((2*R*)-2-((*tert*-butoxycarbonyl)amino)-3-((cyano(tosyl)methyl)thio)propanamido)-3-methylbutanoate (2g)

Colorless oil (11 mg, 34%) R_f = 0.42 (30% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.96 (d, *J* = 8.5 Hz, 1H), δ 7.93 (d, *J* = 8.5 Hz, 1H), δ 7.43 (t, *J* = 8.0 Hz, 2H), δ 7.05 (d, *J* = 8.0 Hz, 1H), δ 6.87 (d, *J* = 8.0 Hz, 1H), δ 5.62 (d, *J* = 7.0 Hz, 1H), δ 5.45 (s, 1H), δ 4.48-4.54 (m, 2H), δ 3.76 (s, 1.5H), δ 3.75 (s, 1.5H), δ 3.49 (dd, *J* = 15.0, 5.0 Hz, 0.5H), δ 3.22 (d, *J* = 6.0 Hz, 1H), δ 3.14 (dd, *J* = 15.0, 6.0 Hz, 0.5H), δ 2.50 (s, 1.5H), δ 2.49 (s, 1.5H), δ 2.17-2.27 (m, 1H), δ 1.48 (s, 4H), δ 1.47 (s, 5H), δ 0.91-0.99 (m, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.9, 171.8, 170.1, 169.9, 155.7, 147.3, 147.2, 131.7, 131.6, 130.5, 130.4, 130.3, 130.2, 112.4, 112.1, 81.3, 81.0, 57.9, 57.7, 57.4, 57.0, 54.2, 53.6, 52.5, 52.4, 36.1, 35.5, 31.1, 31.0, 28.4, 28.3, 22.0, 19.2, 19.1, 17.8, 17.7; IR (cm^{-1}) 3347, 3052, 2965, 2929, 2871, 2241, 1738, 1677, 1594, 1518, 1363, 1334, 1262, 1151, 1083, 1018, 816, 737, 701; HRMS (ESI-TOF, $[M + H]^+$) m/z calculated for $C_{23}H_{34}N_3O_7S_2$:528.1838, found 528.1841.

4.2.12. (2*S*,5*R*,11*R*,14*S*)-dimethyl 5,11-bis((*tert*-butoxycarbonyl)amino)-8-cyano-2,14-diisopropyl-4,12-dioxo-8-tosyl-7,9-dithia-3,13-diazapentadecane-1,15-dioate (3g)

Colorless oil (14 mg, 53%) R_f = 0.42 (30% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.92 (d, *J* = 8.0 Hz, 2H), δ 7.40 (d, *J* = 8.0 Hz, 2H), δ 7.13 (br, 1H), δ 5.58-5.62 (m, 2H), δ 4.58 (br, 1H), δ 4.51-4.55 (m, 2H), δ 3.76 (s, 3H), δ 3.75 (s, 3H), δ 3.33-3.39 (m, 4H), δ 2.49 (s, 3H), δ 2.14-2.21 (m, 2H), δ 1.46 (s, 18H), δ 0.90-0.94 (m, 12H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.2, 169.7, 155.5, 147.5, 131.8, 130.0, 129.9, 112.5, 80.8, 72.6, 57.6, 53.6, 53.4, 52.5, 52.4, 36.6, 36.2, 31.4, 28.4, 22.1, 19.1, 17.9; IR (cm^{-1}) 3318, 2965, 2929, 2871, 2360, 1741, 1659, 1515, 1367, 1270, 1248, 1151, 1018,

734, 650; HRMS (ESI-TOF, $[M + H]^+$) m/z calculated for $C_{37}H_{58}N_5O_{12}S_3$:860.3244, found 860.3231.

4.2.13. (2*S*)-methyl 2-((2*R*)-3-((((4-bromophenyl)sulfonyl)(cyano)methyl)thio)-2-((*tert*-butoxycarbonyl)amino)propanamido)-3-methylbutanoate (2h)

Colorless oil (6.5 mg, 18%) R_f = 0.51 (30% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.91-7.96 (m, 2H), δ 7.78-7.81 (m, 2H), δ 6.99 (d, *J* = 8.0 Hz, 1H), δ 6.86 (d, *J* = 8.5 Hz, 1H), δ 5.55-5.60 (m, 1H), δ 5.46 (br, 1H), δ 4.48-4.54 (m, 2H), δ 3.76 (s, 1.5H), δ 3.75 (s, 1.5H), δ 3.54 (dd, *J* = 15.0, 4.0 Hz, 0.5H), δ 3.13-3.26 (m, 1.5H), δ 2.17-2.27 (m, 1H), δ 1.48 (s, 4.5H), δ 1.47 (s, 4.5H), δ 0.91-0.98 (m, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.7, 171.6, 170.0, 169.8, 155.6, 155.5, 133.4, 133.3, 133.2, 133.0, 132.9, 132.8, 131.7, 131.6, 131.5, 112.0, 111.8, 81.3, 81.0, 57.7, 57.6, 57.5, 57.3, 56.8, 54.1, 53.5, 52.4, 52.3, 36.1, 35.6, 30.9, 30.8, 28.3, 28.2, 19.1, 19.0, 18.9, 17.6, 17.5; IR (cm^{-1}) 3404, 3048, 2969, 2929, 2872, 1734, 1677, 1569, 1500, 1389, 1342, 1259, 1158, 1064, 1007, 823, 737, 701; HRMS (ESI-TOF, $[M + H]^+$) m/z calculated for $C_{22}H_{31}BrN_3O_7S_2$: 592.0787, found 592.0793.

4.2.14. (2*S*,5*R*,11*R*,14*S*)-dimethyl 8-((4-bromophenyl)sulfonyl)-5,11-bis((*tert*-butoxycarbonyl)amino)-8-cyano-2,14-diisopropyl-4,12-dioxo-7,9-dithia-3,13-diazapentadecane-1,15-dioate (3h)

Colorless oil (20.5 mg, 73%) R_f = 0.48 (30% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.90 (d, *J* = 8.0 Hz, 2H), δ 7.76 (d, *J* = 8.0 Hz, 2H), δ 7.13 (br, 1H), δ 5.58 (br, 2H), δ 4.60 (br, 2H), δ 4.52-4.56 (m, 2H), δ 3.77 (s, 3H), δ 3.76 (s, 3H), δ 3.34-3.39 (m, 4H), δ 2.15-2.21 (m, 2H), δ 1.46 (s, 18H), δ 0.92 (dd, *J* = 14.0, 7.0 Hz, 12H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.0, 169.5, 169.4, 155.4, 132.9, 132.5, 131.8,

131.7, 112.0, 80.7, 72.5, 57.4, 53.4, 53.2, 52.4, 52.3, 36.5, 36.0, 31.2, 28.3, 18.9, 17.7; IR (cm^{-1}) 3401, 3340, 3055, 2965, 2929, 2868, 2303, 1738, 1677, 1572, 1518, 1389, 1367, 1262, 1158, 1064, 737, 704; HRMS (ESI-TOF, $[M + H]^+$) m/z calculated for $C_{36}H_{54}BrN_5O_{12}S_3$: 924.2193, found 924.2184.

4.2.15. (2*S*)-methyl 2-((2*R*)-2-((*tert*-butoxycarbonyl)amino)-3-((((4-chlorophenyl)sulfonyl)(cyano)methyl)thio)propanamido)-3-methylbutanoate (2i)

Colorless oil (5 mg, 15%) R_f = 0.30 (20% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.03 (d, *J* = 9.0 Hz, 1H), δ 7.95 (d, *J* = 8.5 Hz, 1H), δ 7.61-7.64 (m, 2H), δ 6.99 (d, *J* = 7.5 Hz, 0.5H), δ 6.86 (d, *J* = 8.0 Hz, 0.5H), δ 5.46-5.57 (m, 2H), δ 4.47-4.54 (m, 2H), δ 3.76 (s, 2H), δ 3.75 (s, 1H), δ 3.54 (dd, *J* = 14.5, 4.0 Hz, 0.5H), δ 3.13-3.26 (m, 1.5H), δ 2.17-2.27 (m, 1H), δ 1.48 (s, 4H), δ 1.47 (s, 5H), δ 0.91-0.99 (m, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.7, 171.6, 170.0, 169.8, 155.6, 155.5, 142.8, 142.7, 132.9, 132.8, 131.7, 131.6, 130.0, 129.9, 112.0, 111.8, 81.3, 81.0, 57.7, 57.6, 57.4, 56.8, 54.1, 53.5, 52.4, 52.3, 36.1, 35.6, 31.0, 30.8, 28.3, 28.2, 19.0, 18.9, 17.6, 17.5; IR (cm^{-1}) 3416, 3032, 2982, 2945, 2862, 2304, 1741, 1662, 1581, 1372, 1351, 1260, 1152, 1069, 823, 734, 708; HRMS (ESI-TOF, $[M + Na]^+$) m/z calculated for $C_{22}H_{30}ClN_3O_7S_2$: 570.1111, found 570.1104.

4.2.16. (2*S*,5*R*,11*R*,14*S*)-dimethyl 5,11-bis((*tert*-butoxycarbonyl)amino)-8-((4-chlorophenyl)sulfonyl)-8-cyano-2,14-diisopropyl-4,12-dioxo-7,9-dithia-3,13-diazapentadecane-1,15-dioate (3i)

Colorless oil (18 mg, 78%) R_f = 0.28 (20% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.98 (d, *J* = 7.0 Hz, 2H), δ 7.59 (dt, *J* = 7.5, 2.0 Hz, 2H), δ 7.16 (br, 1H), δ 5.59 (br, 2H), δ 4.60 (br, 2H), δ 4.52-4.56 (m, 2H), δ 3.77 (s, 4H), δ 3.76 (s, 2H), δ

3.34-3.39 (m, 4H), δ 2.15-2.21 (m, 2H), δ 1.46 (s, 9H), δ 1.45 (s, 9H), δ 0.89-0.94 (m, 12H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 172.1, 169.6, 169.5, 155.4, 142.9, 132.9, 131.2, 129.5, 112.1, 80.7, 72.5, 57.4, 53.3, 53.2, 52.4, 52.3, 36.5, 36.0, 31.2, 28.3, 18.9, 17.7; IR (cm^{-1}) 3409, 3321, 3034, 2968, 2924, 2312, 1756, 1674, 1558, 1368, 1271, 1159, 1058, 736, 704; HRMS (ESI-TOF, $[M + H]^+$) m/z calculated for $C_{36}H_{55}ClN_5O_{12}S_3$: 880.2698, found 880.2686.

4.3. Synthesis of *N*-(phenylthio)-succinamide (6). Our preparation of 6 was carried out by a modification of the method developed by Henke and Srogl.[71] To a stirred suspension of *N*-chlorosuccinimide (133mg, 0.99 mmol) in benzene (5 mL) was added thiophenol (92 µL, 0.91 mmol) as a solution in benzene (2 mL) and the reaction stirred at 24 ˚C for 15 min. During this time, the mixture turned from colorless to orange. A solution of triethylamine (153 µL, 1.09 mmol) in benzene (2 mL) was added dropwise over a period of 15 min and the resulting mixture stirred for 30 min at 24 ˚C. The reaction mixture was washed with 1 M HCl (1 × 20 mL), DI water (1 × 15 mL) and brine (1 × 15 mL) and then dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to afford an orange solid. The crude mixture was triturated with diethyl ether to leave pure **6** as a colorless crystalline solid (165 mg, 88%) mp: 112-114 ^{o}C (lit. 113-113.5 ^{o}C).[71] R_f = 0.26 (30% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.56-7.58 (m, 2H), δ 7.28-7.34 (m, 3H), δ 2.78 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.4, 133.9, 132.1, 129.8, 129.3, 28.6; IR (cm^{-1}) 3082, 2996, 2943, 2848, 1729, 1594, 1582; HRMS (ESI-TOF, $[M + H]^+$) m/z calcd for $C_{10}H_{10}NO_2S$ 208.0432, found 208.0425.

4.4. Synthesis of 2,2-bis(phenylthio)benzo[b]thiophen-3(2H)-one 1,1-dioxide (7). To a stirred solution of *N*-(phenylthio)-succinamide (20 mg, 0.10 mmol) in 3 mL of 3:1 methanol:HEPES buffer (50 mM HEPES, 100 mM NaCl and 1mM EDTA) was added **C** (19 mg, 0.11 mmol) and the reaction stirred at 24 ˚C for 4 h. The solid formed during the reaction was collected on a sintered glass funnel and washed with ice cold water (2 × 2 mL) to afford **7** as a white powder (17.5mg, 91% yield) mp: 134-136 ^{o}C R_f = 0.70 (30% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.95 (d, *J* = 7.5 Hz, 1H), δ 7.86-7.89 (m, 1H), δ 7.79 (d, *J* = 7.5 Hz, 1H), δ 7.71-7.74 (m, 1H), δ 7.65-7.67 (m, 4H), δ 7.38-7.42 (m, 2H), δ 7.38 (t, *J* = 7.5 Hz, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 183.2, 142.8, 137.0, 136.7, 134.3, 131.0, 130.5, 128.9, 127.6, 125.4, 122.3, 82.9; IR (cm^{-1}) 3056, 2982, 1724, 1328, 1270, 1160, 739, 702; HRMS (ESI-TOF, $[M + H]^+$) m/z calcd for $C_{20}H_{15}O_3S_3$ 399.0183, found 399.0169.

4.5. Synthesis of (ditosylmethyl)(phenyl)sulfane (8) and bis(phenylthio)ditosylmethane (9). To a stirred solution of *N*-(phenylthio)-succinamide (50 mg, 0.24 mmol) in 6 mL of 3:1 methanol:HEPES, was added **E** (86 mg, 0.27 mmol) and the reaction stirred at 24 ˚C for 30 min. Methanol was completely removed by blowing a stream of nitrogen gas on the reaction mixture and the aqueous layer extracted with ethyl acetate (2 × 3 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. Column chromatography on silica gel eluted with 30% ethyl acetate/hexanes gave **8** (35 mg, 34 %) and **9** (33 mg, 51). Compound **8**: Colorless oil (35 mg, 34 %) R_f = 0.43 (30% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.92 (d, *J* = 8.0 Hz, 4H), δ 7.36 (d, *J* = 8.5 Hz, 4H), δ 7.28-7.31 (m, 1H), δ 7.16-7.22 (m, 4H), δ 5.10 (s, 1H), δ 2.48 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 146.1,

134.3, 133.4, 132.0, 130.5, 129.7, 129.5, 129.4, 91.3, 21.8; IR (cm^{-1}) 3060, 2921, 1593, 1442, 1340, 1303, 1172, 1156,1078, 817, 735, 698; HRMS (ESI-TOF, $[M + Na]^+$) m/z calcd for $C_{21}H_{20}O_4S_3Na$ 455.0421, found 455.0421. Compound **9**: Colorless oil (33 mg, 51%) R_f = 0.51 (30% ethyl acetate/hexanes). ^1H NMR ($CDCl_3$, 500 MHz) δ 8.08 (d, *J* = 8.0 Hz, 4H), δ 7.55 (d, *J* = 7.5 Hz, 4H), δ 7.39 (t, *J* = 7.5 Hz, 2H), δ 7.34 (d, *J* = 8.5 Hz, 4H), δ 7.27 (t, *J* = 8.0 Hz, 4H), δ 2.49 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 145.7, 138.3, 134.1, 133.1, 130.8, 128.6, 128.5, 126.9, 21.8; IR (cm^{-1}) 3056, 2921, 1593, 1442, 1332, 1303, 1168, 1152, 1074, 817, 735, 698; HRMS (ESI-TOF, $[M + Na]^+$) m/z calcd for $C_{27}H_{24}O_4S_4Na$ 563.0455, found 563.0450.

4.6. Synthesis of 2-(phenylsulfonyl)-2,2-bis(phenylthio)acetonitrile (10). To a stirred solution of *N*-(phenylthio)-succinamide (20 mg, 0.10 mmol) in 3 mL of 3:1 methanol:HEPES, was added **F** (19 mg, 0.11 mmol) and the reaction stirred at 24 ˚C for 10 min. Methanol was completely removed by blowing a stream of nitrogen gas on the mixture and the resulting aqueous layer extracted with ethyl acetate (2 × 1.5 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in vacuo. Column chromatography on silical gel eluted with 30% ethyl acetate-hexane gave **10** as a white solid, mp: 161-163 ^oC (16.5 mg, 86%), R_f = 0.53 (30% ethyl acetate/hexanes). ^1H NMR ($CDCl_3$, 500 MHz) δ 8.11 (d, *J* = 7.5 Hz, 2H), δ 7.78 (t, *J* = 7.5 Hz, 1H), δ 7.63 (t, *J* = 7.5 Hz, 2H), δ 7.54 (d, *J* = 7.5 Hz, 4H), δ 7.48 (t, *J* = 7.5 Hz, 2H), δ 7.37 (t, *J* = 7.5 Hz, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 137.7, 135.4, 134.3, 131.8, 131.5, 129.2, 129.0, 128.1, 111.6; IR (cm^{-1}) 3052, 2982, 2300, 1266, 1156, 898, 745, 706; HRMS (ESI-TOF, $[M + Na]^+$) m/z calcd for $C_{20}H_{15}NO_2S_3Na$ 420.0163, found 420.0156.

Supplementary data

Kinetics assays for the reaction of sulfone-stabilized nucleophiles with **1** and data for spectrophotometric determination of pK_a values for selected compounds.

References and notes

(1) WHO *http://www.who.int/mediacentre/factsheets/fs312/en/index.html* **2011**, Accessed Jan 1, 2012.

(2) CDC *http://www.cdc.gov/diabetes/pubs/factsheet11.htm?utm_source=WWW&utm_medium=ContentPage&utm_content=CDCFactsheet&utm_campaign=CON* **2011**, Accessed Jan 1, 2012.

(3) Phung, O. J.; Sood, N. A.; Sill, B. E.; Coleman, C. I. *Diabet. Med.* **2011**, *28*, 948-964.

(4) Ross, S. A.; Gulve, E. A.; Wang, M. *Chem. Rev.* **2004**, *104*, 1255-1282.

(5) Brownlee, M. *Nature* **2001**, *414*, 813-820.

(6) Phillippe, J.; Raccah, D. *Int. J. Clin. Pract.* **2009**, *63*, 321-332.

(7) DeFronzo, R. A.; Triplitt, C. L.; Abdul-Ghani, M.; Cersosimo, M. D. *Diabetes Spectr.* **2014**, *27*, 100-112.

(8) Cheng, A.; Dubé, N.; Gu, F.; Tremblay, M. L. *Eur. J. Biochem.* **2002**, *269*, 1050-1059.

(9) Tonks, N. K. *FEBS Lett.* **2003**, *246*, 140-148.

(10) Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; al., e. *Science* **1999**, *253*, 1544-1548.

(11) Klaman, L. D.; Boss, O.; Peroni, O. D.; Kim, J. K.; Martino, J. L.; al., e. *Mol. Cell Biol.* **2000**, *20*, 5479-5489.

(12) Rondinone, C. M.; Trevillyan, J. M.; Clampit, J.; Gum, R. J.; Berg, C.; Kroeger, P.; Frost, L.; Zinker, B. A.; Reilly, R.; Ulrich, R.; Butler, M.; Monia, B. P.; Jirousek, M. R.; Waring, J. F. *Diabetes* **2002**, *51*, 2405-2411.

(13) Zinker, B. A.; Rondinone, C. M.; Trevillyan, J. M.; Gum, R., J.; Clampit, J. E.; Waring, J. F.; Xie, N.; Wilcox, D.; Jacobson, P.; Frost, L.; Kroeger, P. E.; Reilly, R. M.; Koterski, S.; Opgenorth, T. J.; Ulrich, R. G.; Crosby, S.; Butler, M.; Murray, S. F.; McKay, R. A.; Bhanot, S.; Monia, B. P.; Jirousek, M. R. *Proc. Nat. Acad. Sci. USA* **2002**, *99*, 11357-11362.

(14) Combs, A. P. *J. Med. Chem.* **2010**, *53*, 2333-2344.

(15) Johnson, T. O.; Ermolieff, J.; Jirousek, M. R. *Nature Rev. Drug Discov.* **2002**, *1*, 696-709.

(16) Zhang, S.; Zhang, Z.-Y. *Drug Disc. Today* **2007**, *12*, 373-381.

(17) Hooft van Huijsduijnen, R.; Bombrun, A.; Swinnen, D. *Drug Disc. Today* **2002**, *7*, 1013-1019.

(18) Hooft van Huijsduijnen, R.; Sauer, W. H. B.; Bombrun, A.; Swinnen, D. *J. Med. Chem.* **2004**, *47*, 4142-4146.

(19) Nichols, A. J.; Mashal, R. D.; Balkan, B. *Drug Dev. Res.* **2006**, *67*, 559-566.

(20) Cheng, A. C.; Coleman, R. G.; Smyth, K. T.; Cao, Q.; Soulard, P.; Caffrey, D. R.; Salzberg, A. C.; Huang, E. S. *Nat. Biotech.* **2007**, *25*, 71-75.

(21) Lazo, J. S.; Sharlow, E. R. *Annu. Rev. Pharmacol. Toxicol.* **2016**, *56*, 24.1-24.18.

(22) Haque, A.; Andersen, J. N.; Salmeen, A.; Barford, D.; Tonks, N. K. *Cell* **2011**, *147*, 185-198.

(23) LaButti, J. N.; Chowdhury, G.; Reilly, T. J.; Gates, K. S. *J. Am. Chem. Soc.* **2007**, *129*, 5320-5321.

(24) Zhou, H.; Singh, H.; Parsons, Z. D.; Lewis, S. M.; Bhattacharya, S.; Seiner, D. R.; LaButti, J. N.; Reilly, T. J.; Tanner, J. J.; Gates, K. S. *J. Am. Chem. Soc.* **2011**, *132*, 15803–15805.

(25) Denu, J. M.; Tanner, K. G. *Biochemistry* **1998**, *37*, 5633-5642.

(26) Meng, T.-C.; Buckley, D. A.; Galic, S.; Tiganis, T.; Tonks, N. K. *J. Biol. Chem.* **2004**, *279*, 37716-37725.

(27) Mahedev, K.; Motoshima, H.; Wu, X.; Ruddy, J. M.; Arnold, R. S.; Cheng, G.; Lambeth, J. D.; Goldstein, B. J. *Mol. Cell Biol.* **2004**, *24*, 1844-1854.

(28) Mahedev, K.; Zilbering, A.; Zhu, L.; Goldstein, B. J. *J. Biol. Chem.* **2001**, *276*, 21938-21942.

(29) Chen, K.; Kirber, M. T.; Yang, Y.; Keaney, J. F. J. *J. Cell. Biol.* **2008**, *181*, 1129-1139.

(30) van Montfort, R. L. M.; Congreeve, M.; Tisi, D.; Carr, R.; Jhoti, H. *Nature* **2003**, *423*, 773-777.

(31) Salmeen, A.; Anderson, J. N.; Myers, M. P.; Meng, T.-C.; Hinks, J. A.; Tonks, N. K.; Barford, D. *Nature* **2003**, *423*, 769-773.

(32) Schwertassek, U.; Haque, A.; Krishnan, N.; Greiner, R.; Weingarten, L.; Dick, T. P.; Tonks, N. K. *FEBS J.* **2014**, *281*, 3545-3548.

(33) Parsons, Z. D.; Gates, K. S. *Biochemistry* **2013**, *52*, 6412-6423.

(34) Saltiel, A. R.; Pessin, J. E. *Trends Cell Biol.* **2002**, *12*, 65-71.

(35) Saltiel, A. R.; Kahn, C. R. *Nature* **2001**, *414*, 799-806.

(36) Ruddraraju, K. V.; Parsons, Z. D.; Barnes, C. L.; Gates, K. S. *J. Org. Chem.* **2015**, *80*, doi:10.1021/acs.joc.5b01949.

(37) Leonard, S. E.; Garcia, F. J.; Goodsell, D. S.; Carroll, K. S. *Angew. Chem. Int. Ed. Eng.* **2011**, *50*, 4423-4427.

(38) Meng, T.-C.; Fukada, T.; Tonks, N. K. *Mol. Cell* **2002**, *9*, 387-399.

(39) Yu, B.; Reynisson, J. *Eur. J. Med. Chem.* **2011**, *46*, 5833-5837.

(40) Yang, J.; Gupta, V.; Tallman, K. A.; Porter, N. A.; Carroll, K. S.; Liebler, D. C. *Nat. Protocols* **2015**, *10*, 1022-1037.

(41) Allison, W. S. *Accounts Chem. Res.* **1976**, *9*, 293-299.

(42) Carballal, S.; Radi, R.; Kirk, M. C.; Barnes, S.; Freeman, B. A.; Alvarez, B. *Biochemistry* **2003**, *42*, 9906-9914.

(43) Gupta, V.; Carroll, K. S. *Biochim. Biophys. Acta* **2014**, *1840*, 847-875.

(44) Qian, J.; Wani, R.; Klomsiri, C.; Poole, L. B.; Tsang, A. W.; Furdui, C. M. *Chem. Comm.* **2012**, *48*, 4091-4093.

(45) Benitez, L. V.; Allison, W. S. *J. Biol. Chem.* **1974**, *249*, 6234-6243.

(46) Gupta, V.; Carroll, K. S. *Chem. Sci.* **2015**, *6*, DOI:10.1039/c5sc02569a.

(47) Shiau, T. P.; Erlanson, D. A.; Gordon, E. M. *Org. Lett.* **2006**, *8*, 5697-5699.

(48) Binkowska, I. *C. R. Chimie* **2015**, *18*, 898-908.

(49) House, H. O.; Larson, J. K. *J. Org. Chem.* **1968**, *33*, 61-65.

(50) Truce, W. E.; Bannister, W. W.; Knospe, R. H. *J. Org. Chem.* **1962**, *27*, 2821-2828.

(51) Mayr, H.; Ofial, A. R. *J. Phys. Org. Chem.* **2008**, *21*, 584-595.

(52) Zimmerman, H. E.; Thyagarajan, B. S. *J. Am. Chem. Soc.* **1960**, *82*, 2505-2511.

(53) Holst, E. H.; Fernelius, W. C. *J. Org. Chem.* **1958**, *23*, 1881-1883.

(54) Berger, S. T. A.; Ofial, A. R.; Mayr, H. *J. Am. Chem. Soc.* **2007**, *129*, 9753-9761.

(55) Terrier, F.; Magnier, E.; Kizilian, E.; Wakselman, C.; Buncel, E. *J. Am. Chem. Soc.* **2005**, *127*, 5563-5571.

(56) Morin, R. B.; Gordon, E. M. *Tet. Lett.* **1973**, *24*, 2159-2162.

(57) Ruddraraju, K. V.; Hillebrand, R.; Barnes, C. L.; Gates, K. S. *Acta Cryst.* **2015**, *E71*, 741-743.

(58) Pine, S. H.; Shen, G.; Bautista, J.; Sutton, C. J.; Yamada, W.; Apodaca, L. *J. Org. Chem.* **1990**, *55*, 2234-2237.

(59) Sivaramakrishnan, S.; Keerthi, K.; Gates, K. S. *J. Am. Chem. Soc.* **2005**, *127*, 10830-10831.

(60) Blanco, G. A.; Baumgartner, M. T. *Tet. Lett.* **2011**, *52*, 7061-7063.

(61) Turell, L.; Botti, H.; Carballal, S.; Ferrer-Sueta, G.; Souza, J. M.; Durán, R.; Freeman, B. A.; Radi, R.; Alvarez, B. *Biochemistry* **2008**, *47*, 358-367.

(62) Szajewski, R. P.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 2011-2026.

(63) Meister, A.; Anderson, M. E. *Ann. Rev. Biochem.* **1983**, *52*, 711-760.

(64) Lees, W. J.; Whitesides, G. M. *J. Org. Chem.* **1993**, *58*, 642-647.

(65) Krishnan, S.; Miller, R. M.; Tian, B.; Mullins, R. D.; Jacobson, M. P.; Taunton, J. *J. Am. Chem. Soc.* **2014**, *136*, 12624-12630.

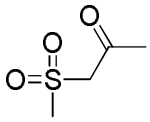
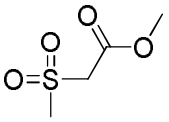
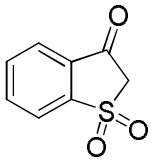
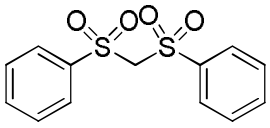
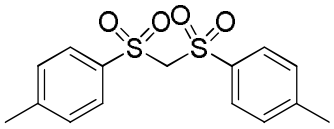
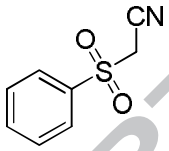
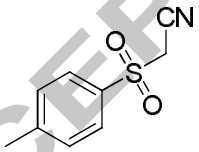
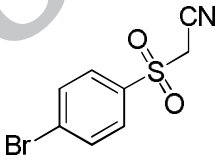
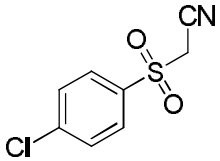
(66) Liu, S.; Yang, H.; He, Y.; Jiang, Z.-H.; Kumar, S.; Wu, L.; Zhang, Z.-Y. *J. Am. Chem. Soc.* **2008**, *130*, 8251-8260.

(67) Mercey, G.; Verdelet, T.; Renou, J.; Kliachyna, M.; Baati, R.; Nachon, F.; Jean, L.; Renard, P.-Y. *Acc. Chem. Res.* **2012**, *45*, 756-766.

(68) Kalisiak, J.; Ralph, E. C.; Cashman, J. R. *J. Med. Chem.* **2011**, *54*, 3319-3330.

(69) Yun, M. K.; Wu, Y.; Li, Z.; Zhao, Y.; Waddell, M. B.; Ferreira, A. M.; Lee, R. E.; Bashford, D.; White, S. W. *Science* **2012**, *335*, 1110-1114.
(70) Garcia, F. J.; Carroll, K. S. *Eur. J. Med. Chem.* **2014**, *88*, 28-33.
(71) Henke, A.; Srogl, J. *J. Org. Chem.* **2008**, *73*, 7783-7784.

Table 1. Reactions of 1 with various beta ketosulfones, beta disulfones and beta cyanosulfones.[a]

Entry	Nucleophile	Mono Adduct	Yield (%)[b]	Di Adduct	Yield (%)[b]
A		2a	69	N/I[c]	--
B		2b	58	3b	12
C		N/D[d]	--	3c	78
D		2d	51	3d	40
E		2e	64	3e	29
F		2f	22	3f	70
G		2g	34	3g	53
H		2h	18	3h	73
I		2i	15	3i	78

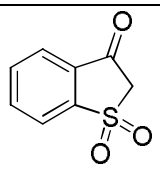
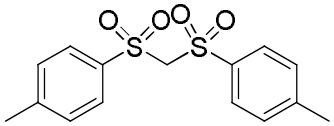
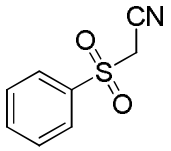
[a] Conditions: 1 (0.060 mmol, 1.0 eq.), A-I (0.066 mmol, 1.1 eq.) in 3.0 ml of MeOH: HEPES buffer (3:1) at room temperature.

[b] Isolated yields

[c] N/I = low yields may have been formed, but have not isolated.

[d] N/D = not detected

Table 2. Reactions of 5 with various sulfone based carbon nucleophiles[a]

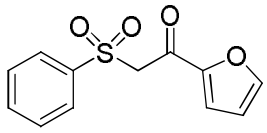
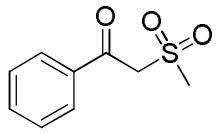
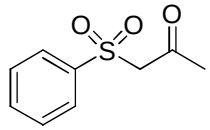
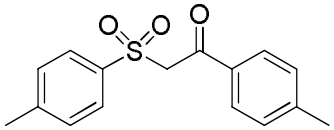
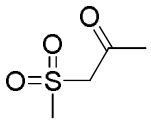
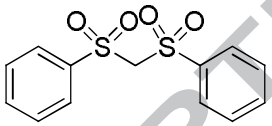
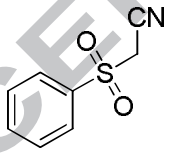
Entry	Nucleophile	Mono Adduct	Yield (%)[b]	Di Adduct	Yield (%)[b]
C		N/D[c]	--	6	91
E		7	34	8	51
F		N/D[c]	--	9	86

[a] Conditions: 1 (0.060 mmol, 1.0 eq.), A-I (0.066 mmol, 1.1 eq.) in 3.0 ml of MeOH: HEPES buffer (3:1) at room temperature.

[b] Isolated yields

[c] N/D = not detected in the reaction

Table 3. Aqueous pK_a values of several sulfone based carbon nucleophiles.

Entry	Nucleophile	k_{trap} (M^{-1} s^{-1})[a]	pK_a[b]
J		31[c]	9.2
K		18[c]	9.3
L		15.8 ± 0.5	9.3
M		20[c]	9.7
A		9[c]	10.0
D		12[c]	10.9
F		64[c]	11.0

[a] Second-order rate constants for trapping the model sulfenyl amide in 1:1 MeOH : Buffer B (pH 7.0), 23 ± 2 °C.

[b] Aqueous pK_a values were determined as reported in the text.

[c] Approximate second-order rate constants for trapping the model sulfenyl amide in 1:1 MeOH : Buffer B (pH 7.0), 23 ± 2 °C.