The reversible oxidation of methionine residues in proteins has emerged as a biologically important post-translational modification. However, detection and quantitation of methionine sulfoxide in proteins is difficult. Our aim is to develop a method for specifically derivatizing methionine sulfoxide residues. We report a Pummerer rearrangement of methionine sulfoxide treated sequentially with trimethylsilyl chloride and then 2-mercaptoimidazole or pyridine-2-thiol to produce a dithioacetal product. This derivative is stable to standard mass spectrometry conditions, and its formation identified oxidized methionine residues. The scope and requirements of dithioacetal formation are reported for methionine sulfoxide and model substrates. The reaction intermediates have been investigated by computational techniques and by $^{13}$C NMR spectroscopy. These provide evidence for an $\alpha$-chlorinated intermediate. The derivatization allows for detection and quantitation of methionine sulfoxide in proteins by mass spectrometry and potentially by immunochemical methods.

Introduction

Oxidative modification of proteins by reactive oxygen species had long been viewed as an inevitable, negative by-product of aerobic metabolism. Over the past two decades, many investigators have established that cells produce reactive oxygen species in a controlled fashion. These reactive oxygen species mediate the reversible oxidative modification of specific proteins as an important mechanism of cellular regulation. Methionine in proteins is often thought to be a generic hydrophobic residue, functionally replaceable with another hydrophobic residue such as valine or leucine. However, this frequently is not the case because of the presence of sulfur in methionine. The methionine can be oxidized to methionine sulfoxide, and all aerobic organisms contain methionine sulfoxide reductases capable of reducing the sulfoxide back to the thiol ether. Moreover, the cycle also constitutes a reversible post-translational covalent modification analogous to phosphorylation that can regulate cellular metabolism. The reversible oxidative modification of methionine enables methionine residues to provide a catalytically efficient antioxidant defense that scavenges reactive oxygen species.

Progress in identifying the proteins and pathways affected by methionine oxidation and reduction has been slow because detection and quantitation of methionine sulfoxide in specific proteins is difficult. A general antibody capable of specifically binding to any methionine sulfoxide residue would be very useful, but such antibodies cannot be raised. Mass spectrometry would seem an ideal method for analysis, and various mass spectrometric methods have been proposed, but they often suffer from artifactual sulfoxide formation during sample preparation or analysis. A method for covalently and specifically derivatizing methionine sulfoxide residues could circumvent these issues. Such a method could be used in a variety of detection techniques, including one and two dimensional separations, mass spectrometry, and fluorescence. Modified proteins or their proteolytic digests could also be enriched by affinity techniques based on the covalent modification.

The Pummerer rearrangement is a reaction specific to sulfoxides. Sulfoxides can be O-alkylated or acylated with a strong electrophile or other acid, forming an adduct that then eliminates to give rise to a thionium ion. The thionium ion may undergo a variety of reactions: in the most common incarnation of the Pummerer rearrangement, an acetate ion or similar weak to moderate nucleophile adds to generate an $\alpha$-substituted sulfide as the product, essentially transferring the oxidation from the original sulfur atom to the adjacent carbon. Attack of water yields a hemithioacetal, which can decompose...
to the corresponding aldehyde and thiol. Other nucleophiles have included carboxylic acids, alcohols, phenyl rings, indoles, phenols, anilines and amides.\textsuperscript{[16,11]} Intramolecular Pummerer-like rearrangements have recently been reviewed.\textsuperscript{[12]} Sulfur nucleophiles, while known, are somewhat underrepresented in comparison, with just a few examples of conversion of a sulfoxide to a dithioacetal.\textsuperscript{[13,14]} We now report the application of Pummerer rearrangement using thiol nucleophiles to selectively transform oxidized methionine residues from sulfoxides into dithioacetals.

Results and Discussion

An attempt to quantitate methionine oxidation via the Pummerer rearrangement was reported in 1972 by Lunder.\textsuperscript{[15]} However, we have been unable to reproduce his results, and there are no published papers that used this protocol. Classical Pummerer protocols were often carried out in acetic acid or trifluoroacetic acid with acetic anhydride or trifluoroacetic anhydride as initiators.\textsuperscript{[11]} These harsh conditions usually covalently modify or degrade proteins. Trimethylchlorosilane (TMSCl) was an intriguing alternative to acetic anhydride or trifluoroacetic anhydride, particularly as TMSCl has been used as a cleavage and deprotection reagent in peptide synthesis\textsuperscript{[17]} and would be expected to cause minimal protein decomposition. In addition, TMSCl is often used for transient protection of sensitive groups, as it readily forms adducts with hydroxy groups or amines that are spontaneously removed during aqueous workup.\textsuperscript{[18,19]} We considered that TMSCl could transiently protect alcohols, amines, or other sensitive side chains during the activation step, an effect that should simultaneously increase solubility in organic solvents. Subsequent interception of the thionium ion or other reactive intermediate\textsuperscript{[20]} with an appropriate thiol could give rise to a dithioacetal adduct, as shown in Scheme 1. Dithioacetals are generally quite stable to all but forcing conditions,\textsuperscript{[21]} including mass spectrometry, and a methionine-based dithioacetal should be readily differentiated from a methionine thioether by MS or other analytical methods. Such an approach could offer the ability to both monitor total sulfoxide levels and determine the regiospecificity of oxidation; that is, which specific methionine residues within the protein of interest were in the sulfoxide oxidation state.

We chose the commercially available Fmoc-Met(O)-OH (1) as our initial test substrate, as the Fmoc group is both acid-stable and presents a useful UV handle for LC analysis studies. Initial reaction of 1 with TMSCl in THF was promising, as a mass of 370 corresponding to a putative thionium intermediate was observed by LCMS. Upon treatment with 2-mercaptoimidazole (2d, Table 1) robust signals with m/z 481 were observed, consistent with the desired adduct. With this preliminary result in hand, we set out to explore the scope and limitation of the reaction of 1 to form dithioacetal adducts.

An initial screen of small molecule thiols for adduct formation, with a focus on thiol-substituted heterocycles, suggested that the scope of effective nucleophiles was relatively narrow (Table 1). Most tested thiols formed adducts of 1 in very modest amounts, even when the structure was closely related to a thiol that formed the corresponding dithioacetal adduct in good yield. 4-Mercaptopyridine (2e) for example, yielded far less adduct than 2d, and 2-mercaptoimidazoline (2f) yielded very little adduct. 2-Mercaptomidazole (2a), in contrast, displayed adduct formation similar to that of 2d. Closer analysis suggested the formation of multiple regioisomeric products for most nucleophiles, presumably owing to formation of the thionium ion from either carbon adjacent to the unsymmetrical sulfoxide (Scheme 2).

Adducts of C3 produced two distinct diastereomers (4b–k). The dithioacetal adducts 3a and 4a, formed from reaction of 1 with 2a, eluted as a single slightly asymmetric peak in most cases, and this nucleophile was therefore used in reaction optimization screenings. While some conversion was observed with a single equivalent of 2a, the best yields were obtained with the addition of 4 equivalents or more. Estimated conversions (LCMS) of 1 to 3 + 4 for all nucleophiles are listed in Table 1. The formation of 2a dithioacetal adduct was also examined for simpler systems, using DMSO and methyl phenyl sulfoxide as substrates, to ensure a single product (Scheme 3). The expected adducts 5a and 6a were formed in 70 and 64% isolated yield after preparative HPLC. The thiol nucleophiles previously examined via HPLC analysis of their reaction with 1 were screened against a DMSO substrate in order to enable full characterization and yield analysis of the products 5a–k. The results are shown in Table 1.

The reaction was most effective when conducted in two steps: an initial activation step in ethereal solvent with 0.2–0.33 M TMSCl followed by condensation with a thiol, typically 2-mercaptoimidazole. The initial activation appeared to be a multi-step process itself, as premature addition of the thiol nucleophile resulted in the production of significant amounts of reduced Fmoc-Met-OH (7), presumably due to thiol attack on a

\[
\begin{align*}
\text{Thiol nucleophile} & \rightarrow \text{Dithioacetal adduct} \\
\text{Scheme 1. Proposed labelling approach.}
\end{align*}
\]
Allowing the reaction mixture to progress from a turbid and opaque mixture in the ethereal solvent to a translucent solution prior to addition of 2-mercaptoimidazole delivered consistent LCMS yields of approximately 80% with thioacetal adducts \(3a\) and \(4a\), as judged by absorbance at 280 nm. Reactions were analyzed at 280 nm in order to maximize the Fmoc absorbance signal while minimizing the imidazole contribution to dithioacetal absorbance.

The reaction was strongly dependent on solvent, as shown in Table 2, with ethereal solvents consistently producing optimal yields. Less polar solvents such as dichloromethane or chloroform were significantly less effective, while EtOAc and acetonitrile were moderately effective. Dimethylformamide, N-methylpyrrolidone and dimethylacetamide yielded varying amounts of reduction to the thioether 7. Acidic solvents such as trifluoroethanol or acetic acid tended to favor reduction to 7 over formation of \(3a\) and \(4a\). In the case of trifluoroethanol, it is possible that the well-precedented mechanism of alcohol oxidation by activated sulfoxide dominates, which would support the high efficiency of reduction to 7. Mixed-phase systems such as 1:1 water/THF or water/CH\(_2\)Cl\(_2\) were not effective.

A variety of activating agents were screened for effectiveness. Silyl chlorides were found to be generally effective, although increased bulk around the silane corresponded to a requirement for extended reaction times (Table 3). Indeed, TBDPSCI failed to generate any \(3a\) or \(4a\) products. Several traditional Pummerer electrophiles proved to be ineffective in the conversion into \(3a\) and \(4a\), as acetic or trifluoroacetic anhydrides yielded no adduct (Table S1 in the Supporting Information). Interestingly, chloride appears to be required for dithioacetal formation. While it is perhaps unsurprising that the strongly reactive TMSBr does not yield a great deal of desired products, or that the less reactive TMS-imidazole and TMS-polypophosphate are ineffective, it is telling that the addition of

### Table 1. Thiol nucleophiles

| Thiol name | Structure | Fmoc-Met adduct (3 + 4) \[^{[a]}\] | DMSO adduct \(^{[b]}\) |
|------------|-----------|---------------------------------|-----------------|
| 2a         | 2-mercaptoimidazole | 82 | 70 |
| 2b         | 3-carboxy-2-mercaptopyridine | 6 | 6.1 |
| 2c         | 3-carboxy-6-mercaptopyridine | 48 | 19 |
| 2d         | 2-mercaptopyridine | 81 | 78 |
| 2e         | 4-mercaptopyridine | 12 | 56 |
| 2f         | 6-amino-2-mercapto benzothiazole | 4 | 9 |
| 2g         | 2-mercaptobenzimidazole | 37 | 42 |
| 2h         | cysteamine | 5.2 | – |
| 2i         | 2-mercaptopyrimidine | 7 | 21 |
| 2j         | thiourea | 21 | – |
| 2k         | thiazolidine | 15 | 46 |

\[^{[a]}\] Reactions were performed at 0.25 mmol scale (Fmoc-Met(O)-OH, 1) following the General Procedure for Dithioacetal Formation or 1 mmol scale (DMSO), following the General Procedure for Condensation of DMSO with Thiol Nucleophiles. \(^{[b]}\) \(R=\) Fmoc-alanine; estimated percent yield of adducts are reported based on integration of the 280 nm absorbance trace between 3 min and 5 min. No correction was made for the contribution of the nucleophile to the absorbance of 3 + 4. \(^{[c]}\) \(R=\) H; isolated percent yield.
LiCl to the TMS-polyphosphate reaction is sufficient to "rescue" this reaction and produce a significant amount of dithioacetal adduct. LiBr and LiI were not effective in this context, perhaps owing to the in situ generation of TMSBr or TMSI. Of the non-silyl-based activating agents tested, only those containing chloride were even modestly effective (Table S1). The Pummerer-like reaction of thioethers with N-chlorosuccinimide is known, and treatment of 1 with NCS followed by addition of 2a gave rise to a moderate yield of 3a + 4a as well. The addition of base was not productive as smaller amounts tended to promote thioether 7 formation and larger amounts suppressed Pummerer-like reactions altogether. Lower temperature promoted the side reduction to 7, while modestly heating the reaction to 40 or 60 °C accelerated the activation for adduct formation, with an optimal activation time of 2–4 h. Isolated yields of the product mixture 3a and 4a using 1 as a substrate were identical (77%) for the reaction with activation at RT (20 h) or 40 °C (3 h). The dithioacetal adduct of the terminal methyl group 3a was isolated and characterized. However, the adducts 4a of the internal C3 methylene were inseparable chromatographically and appeared to be less stable to prolonged exposure to aqueous TFA solution. This runs counter to other dithioacetals, which are typically stable to the mildly acidic conditions of preparative HPLC and could be largely

### Table 2. Effect of solvent on Fmoc-methionine sulfoxide Pummerer reaction.

| Solvent       | Dithioacetal adduct | Thiourea | Starting material 1 |
|---------------|---------------------|----------|---------------------|
| acetic acid   | 3a + 4a [a]         | 32       | 63                  |
| chloroform    | 7                   | 41       | –                   |
| dichloromethane | 6                  | 43       | –                   |
| diglyme       | 83                  | 1        | –                   |
| dioxane       | 82                  | 3        | –                   |
| dimethoxyethane | 71                 | 6        | –                   |
| triglyme      | 71                  | 3        | –                   |
| THF           | 79                  | 3        | –                   |
| ethyl acetate | 62                  | 8        | –                   |
| acetonitrile  | 43                  | 31       | –                   |
| dimethylacetamide | –                 | 32       | 63                  |
| dimethylformamide | –                 | 90       | 3                   |
| NMP           | –                   | 54       | 37                  |
| toluene       | –                   | 19       | 79                  |
| trifluoroethanol | –                 | 94       | –                   |

[a] Reaction as shown in Scheme 2; percent yields of each species are based on LCMS analysis. A 1 mmol portion of TMSCI at ambient temperature was added to a suspension of 0.25 mmol Fmoc-Met(OH) (1) in 3 mL of the indicated solvent and the mixture was stirred for a minimum of 16 h, followed by the addition of 2a and stirring for a further 24 h. [b] Adduct percent yields are reported as the sum of mixed isomers.
due to the relatively electron-poor nature of 2a, which is in resonance with a thiourea form.

We propose that the mechanism of dithioacetal formation involves a fast initial reaction of the sulfoxide 1 with TMSCl to generate the adduct 8 shown in Scheme 2, which could then either first undergo chloride exchange to form species 9 or lose TMSOH directly to form thionium ion 10. The dependence of dithioacetal 3a + 4a formation on the presence of a halide ion, preferably a chloride ion, suggests that the active species is either the chloride-thionium ion pair 10 or quite possibly the α-chloro thioether 11. The latter species has been invoked previously[16] and Jung et al observed incorporation of a chloride ion into an aromatic ring under Pummerer rearrangement conditions.[27] Both of these examples used the highly reactive thionyl chloride.

To empirically examine the nature of the active intermediate, we carried out the reaction on 1 that had been 13C-labeled at the methyl group of the side chain and monitored the reaction by 13C NMR spectroscopy. The parent sulfoxide 13C-enriched resonances appeared at 38.86 and 38.78 ppm presumably owing to diastereomeric oxidation of the parent 7. Treatment with excess TMSCl in deuterated THF for 2 h at 40°C yielded roughly equivalent new signals at 50.6, 13.5, and 14.1 ppm. This result is consistent with formation of α-chlorinated thioethers 11a + 11b as a statistical mixture either at the 13C-enriched methyl (50.6 ppm) or at the interior C3 methylene (13.50, 14.1 ppm). A trace of presumed thioether 7 was also observed at 15.3 ppm. Subsequent condensation of the reaction with excess 2a saw these peaks migrate to 40.2, 13.8, and 14.5 ppm, respectively; consistent with formation of one primary (3a) and two secondary (diastereomeric, 4a) dithioacetals. A similar experiment was carried out using unlabeled DMSO as a substrate. The initial 13C NMR showed a single signal at 42.0 ppm, which gave rise to a split signal at 53.00/ 52.99 ppm as well as a signal at 14.5 ppm. We first investigated the initial step, reaction of DMSO with TMSCl, in order to elucidate: 1) The highest energy barriers, or "bottlenecks," that must be overcome, and 2) which active species product, chloride–thionium ion pair or α-chloro thioether, is produced. Geometry optimizations and subsequent vibrational frequency calculations (to confirm true minima and transition states) were performed in gas and tetrahydrofuran (THF) solvent phases. Figure 1 illustrates the full computed energy profile for this initial activation process. Note that gas-phase structures are shown where transition states have been defined (transition states) were performed in gas and tetrahydrofuran (THF) solvent phases. Figure 1 illustrates the full computed energy profile for this initial activation process. Note that gas-phase structures are shown where transition states have been defined.
The activation process is observed to proceed through three intermediates, and thus four distinct transition states. The final energetically most stable product is the α-chlorinated thioether. This computed mechanism affirms that shown in Scheme 2; however, important details are garnered from the quantum chemistry results. One of these is that the rate-limiting step, or bottleneck, is the proton transfer, shown as TS2 in Figure 1, with computed barriers of 37 (gas) and 38 (THF) kcal mol\(^{-1}\) (relative to separated reactants DMSO and TMSCl). The best experimental thioacetal yields were obtained with aprotic ethereal solvents (Table 2) which cannot participate directly in proton exchange. While ethereal solvents may participate in hydrogen bonding, thereby slightly lowering the energy of the proton transfer transition state, stabilization of minima is also likely. The slight increase in energy barrier (one kcal mol\(^{-1}\)) when going from gas to THF-solvent phase suggests that the hydrogen bonding capabilities of the solvent do not appreciably facilitate the proton transfer step.

Thus, the only real way to go from TMSO-S(+)Me\(_2\) to TMSO+ + MeS(+) = CH\(_2\) is via intramolecular proton transfer, as exemplified by TS2. We also find that for the portion of the activation process leading to TMSOH-MeS(+) = CH\(_2\) (I2), solvent effects (THF) are negligible for four of the stationary points, including the rate-limiting proton transfer step, but stabilize the other species (I1a, TS1b, I1b) by 5 to 12 kcal mol\(^{-1}\).

The other key point is that upon formation of the chloride-thionium ion pair, conversion into the substantially more stable α-chlorothioether (MeSCH\(_2\)Cl) should readily occur. The computed gas-phase barrier of 34 kcal mol\(^{-1}\) is lowered to 18 kcal mol\(^{-1}\) when modeled in THF solvent. This step is illustrated at far right of Figure 1 where TMSOH was considered a spectator species. We speculate that if the proton transfer energy barrier can be overcome, then formation of α-chlorothioether (MeSCH\(_2\)Cl) product is almost certain. Because the separated products TMSOH + MeSCH\(_2\)Cl are computed to be more stable than the separated reactants by 17 (gas) and 14 (THF) kcal mol\(^{-1}\), the complete exothermic activation process is highly unlikely to be reversible because the backward proton transfer energy barrier is over 50 kcal mol\(^{-1}\). These quantum chemistry results nicely account for the experimental observations, particularly the slowness of reaction and irreversibility. They also indicate that the α-chloro thioether is produced, rather than ion pair, in line with the \(^{35}\)Cl NMR results.

Next, we determined reaction energies in THF solvent for the final step, formation of thioacetals: RS\(_2\) + MeSCH\(_2\)Cl → RSH + MeSCH\(_2\)Cl. We investigated the four thiols 2-mercaptoimidazole (2a), 2-mercaptopyrindine (2d), 4-mercaptopyrindine (2e), and 2-mercaptopirimidine (2f). Full potential energy surface (PES) scans were performed using the cc-pVDZ basis sets to discover lowest-energy structures for separated reactants and products. Their geometries were then optimized and vibrational frequencies computed using the cc-pVTZ basis sets. The results are shown in Figure 2 where electronic reaction energies (ΔE), lying between −6.5 and −5.3 kcal mol\(^{-1}\), indicate favorable thioacetal formation. However, free energies of reaction (ΔG\(_{\text{free}}\)) are less exothermic, with products computed to be only slightly more stable than reactants by 3.8 to 2.4 kcal mol\(^{-1}\). There are many complex factors that can shift these reaction energies up/down by a few kcal mol\(^{-1}\), and thus cause experimental yields to vary significantly, as seen in Table 1.
computational structures and energetics for conversions of four thiols to dithioacetals: $\text{RSH} + \text{MeSCl} \rightarrow \text{RSCH}_2\text{Cl} + \text{HCl}$. The M11(THF)/cc-pVTZ level of theory was used, electronic ($\Delta E$) and free ($\Delta G_{\text{st}}$) energies of reaction are given in kcal mol$^{-1}$.

Figure 2. Computed structures and energetics for conversions of four thiols to dithioacetals: $\Delta E = -6.6$, $\Delta G_{\text{st}} = -3.8$ for 2-mercaptoimidazole; $\Delta E = -6.1$, $\Delta G_{\text{st}} = -3.3$ for 2-mercaptopyrimidina; $\Delta E = -6.1$, $\Delta G_{\text{st}} = -3.1$ for 2-mercaptopyridine; $\Delta E = -5.3$, $\Delta G_{\text{st}} = -2.4$ for 4-mercaptopyridine.

These factors include reaction conditions, explicit solvent interactions, and reactant-reactant/product-product complex formations. As such, all we can really surmise from the computed results is that thioacetol formation is barely exothermic and that actual experimental yields may vary due to other factors that are difficult to model with kcal mol$^{-1}$ accuracy.

Having investigated the optimal conditions for dithioacetol formation in small molecules, we turned our attention to using the optimized method for labeling methionine sulfoxide residues in peptides. Conversion of sulfoxides to dithioacetols offers a stable mass tag to differentiate oxidized methionine residues definitively from the thioether state. We chose the peptide Met-enkephalin (H-Tyr-Gly-Gly-Phe-Met-OH, 12) for our study. The peptide was oxidized with NaOCl to the sulfoxide form 13. The sulfoxide-containing peptide 13 was then activated for a minimum of two days in 10% (v/v) TMSCI in dioxane, followed by addition of solid 2-mercaptoimidazol(2a) and stirring for at least 24 h. In the case of the oxidized peptide, more than 70% of peptide-associated absorbance at 220 nm corresponded to a mass of 672, consistent with the expected dithioacetol adduct 14a + 15a. Approximately 17% of the signal corresponded to reduction to methionine thioether form 12. In a control reaction using the parent peptide, >90% of 220 nm absorbance corresponded to a mass of 574 consistent with the starting peptide 12 (Figure S2). Approximately 6% of peptide absorbance corresponded to dithioacetol adducts 14a + 15a, presumably formed after air oxidation of 12 to 13. The use of an inert atmosphere was found to be useful in suppressing background dithioacetol formation. The requirement for ethereal solvents may not be compatible with the solubility of some proteins. However, prior fragmentation with trypsin, pepsin, or other proteases commonly employed in protein chemistry may enable Pummerer-based dithioacetol formation of the resulting fragments. In addition, TMSCI is expected to transiently protect many polar groups, such that minimal initial solubility may be sufficient to ultimately enable reaction. Although total conversion of the peptide will be less than indicated by the absorbance percentage, owing to contribution of the thioimidazole substituent at 220 nm, our protocol presents clearly differentiable results between otherwise-identical peptides containing either sulfoxide or thioether functionality.

Conclusion

We have demonstrated that the Pummerer rearrangement can be used to covalently label oxidized methionine side chains with good efficiency. We have described the use of silyl chlorides, especially TMSCI, to convert alkyl sulfoxides into intermediate α-chlorinated thioethers, which subsequently undergo chlorine displacement by thiol nucleophiles. The efficacy of a variety of thiol nucleophiles has been investigated, and 2-mercaptoimidazole and 2-mercaptopyridine proved to be most effective. Ethereal solvents were shown to be optimal for dithioacetol formation. The α-chlorinated intermediates were investigated by computation and by $^{13}$C NMR spectroscopy. Finally, we have demonstrated the use of our optimized conditions to selectively label a peptide containing an oxidized methionine residue while the corresponding unoxidized peptide remained essentially unlabeled. It should be possible to raise an antibody specific for the dithioacetol derivative. Such an antibody could be used to detect and quantitate methionine sulfoxide in proteins by immunochemical methods or for affinity purification of derivatized peptides prior to mass spectroscopic sequencing. We anticipate that this approach can be used to identify peptides with oxidized methionine residues by tagging them with a thio nucleophile functionalized with mass or other reporter groups.

Experimental Section

General: Reagents were purchased from commercial sources and used without further purification. Fmoc-methionine $^{13}$C-methyl was purchased from Cambridge Isotope Laboratories. Met-Enkephalin was obtained from Chempep Inc. NMR spectra were obtained on a 400 MHz Varian NMR and processed using MestReNova software. LCMS data for small molecules were acquired on an Agilent Technologies 1290 Infinity HPLC system using a 6130 quadrupole LC/MS detector and a Poroshell 120 SB-C18 2.7 um column (4.6 x 50 mm). Peptides were analyzed using an Agilent 1200 series HPLC system with an LC/MS Trap XCT detector and a Zorbax 300SB-C18 3.5 um column (4.6 x 50 mm). Preparative HPLC chromatography was performed on a Shimadzu system using a 30 mm x 150 mm Zorbax SB C18 column (Agilent) or a 19 mm x150 mm Xbridge C4 column (Waters), Flash chromatography was performed on a Teledyne Isco CombiFlash Rf + instrument using hexane/ethyl acetate gradients. HRMS data were acquired on a Waters XEVO G2-XS QTOF running MassLynx version 4.1.

Quantum chemistry: The computations in this study were executed using the GAMESS package and molecular structures were
illustrated using MacMolPlt. We used the density functional theory (DFT) method with the hybrid meta-GGA M11 functional. Calculations were performed in gas and tetrahydrofuran (THF) solvent phases, and the latter was accomplished using the Polarizable Continuum Model (PCM) with a high density of tesseractia: NTSALL = 960 in STECAV. Geometries were optimized (maximum Cartesian gradient < 10⁻⁴ Hartree/Bohr) using analytic gradients and Hessians were computed seminumerically (double differences) using analytic gradients. The cc-pVQZ basis sets were used to probe potential energy surfaces for locations of lowest-energy conformations and transition state structures (having exactly one imaginary frequency). Refined optimized geometries and subsequent Hessians were computed using the cc-pVTZ basis sets and minimum energy paths connecting transition states to corresponding reactant/product minima were determined using the second-order intrinsic reaction coordinate (IRC) method of González and Schlegel. All data presented and discussed in the manuscript represent the M11/cc-pVTZ level of theory. Structures and energies (electronic (E) and free (G_0) of all stationary points described are given in the Supporting Information.

**Synthesis**

**General procedure for dithioacetal formation:** TMSCl 127 μL (108 mg, 1 mmol) was added to a stirred solution of sulfonamide (0.25 mmol) in 3 mL of dioxane. The reaction was stirred for at least 24 h at RT, at which point the thiol nucleophile (1.04 mmol) was added. The reaction was stirred for an additional 24 h at RT. Neutralization with 1 m triethylammonium bicarbonate solution was followed by either preparative HPLC or extraction and flash chromatography purification to afford the pure material.

6-(((methylthio)methyl)thio)nicotinic acid (5c): 10.3 mg white solid, 19%; 1H NMR (CD3CN): δ = 8.96 (dd, J = 2.2, 0.9 Hz, 1H), 8.08 (dd, J = 8.4, 2.2 Hz, 1H), 7.35 (dd, J = 8.4, 0.9 Hz, 1H), 4.39 (s, 2H), 2.21 ppm (3H); 13C[H] NMR (CD3CN): δ = 166.6, 164.7, 151.6, 138.1, 123.3, 122.6, 36.1, 15.7 ppm; HRMS (ESI/QTOF) m/z: calcd for C11H8NO2S: 216.0153 [M+H]+; found 216.0152.

**General procedure for condensation of DMSO with thiol nucleophiles:** TMSCl 127 μL (108 mg, 1 mmol) was added to a stirred solution of dimethyl sulfide (19.5 mg, 17.7 μL, 0.25 mmol) in 3 mL of dioxane. The reaction was stirred for 24 h at RT, and the thiol nucleophile 2 (1.04 mmol) was then added. The reaction was stirred for an additional 24 h at RT. Neutralization with 1 m triethylammonium bicarbonate solution was followed by either preparative HPLC or extraction and flash chromatography purification to afford the pure material.

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were dried over Na$_2$SO$_4$ and evaporated to yield $^{13}$C-methyl Fmoc-methionine oxide (white solid, 10.7 mg, quantitative).

$^{13}$C NMR experiment

$^{13}$C Methyl Fmoc-methionine oxide (10.7 mg, 27.7 μmol) was dissolved in D$_2$THF (1 mL) and the $^{13}$C NMR spectrum was acquired. TMSCl (50 μL, 13.9 equiv) was added, and the reaction mixture was heated at 40 °C. After 2 h, the reaction mixture was cooled, and the $^{13}$C NMR spectrum was acquired. After returning the NMR sample to the reaction, mercaptoimidazole (39.8 mg, 14 equiv) was added and the reaction was stirred at RT for 24 h. The $^{13}$C NMR spectrum of the reaction was then acquired once again.

Met-enkephalin sulfoxide (13): Met-Enkephalin (12, 20 mg, 35 μmol) was dissolved in 1 mL of deionized water, and sodium periodate (9.0 mg, 42 μmol) was added. The reaction mixture was stirred overnight, then neutralized with AcOH. The desired oxidized peptide was purified by preparative HPLC on a Waters C4 column using a gradient of 10—45 % MeCN (0.05% TFA) in water (0.05% TFA); 13.3 mg of pure material was obtained after lyophilization.

Pummerer reaction of met-enkephalin: Met-enkephalin sulfoxide (13, 0.6 mg, 1 μmol) and met-enkephalin (12, 0.6 mg, 1 μmol) were each suspended in 0.1 mL of deionized water, and sodium periodate (9.0 mg, 42 μmol) was added. The reaction mixture was stirred overnight, then neutralized with AcOH. The desired oxidized peptide was purified by preparative HPLC on a Waters C4 column using a gradient of 10—45 % MeCN (0.05% TFA) in water (0.05% TFA); 13.3 mg of pure material was obtained after lyophilization.

Acknowledgements

We are grateful to Burchelle N. Blackman for performing high-resolution mass spectrometry. This research was supported by the NIH Intramural Research Program and NHLBI. S.M. and R.L.L. were supported by the Intramural Research Program of the National Heart, Lung and Blood Institute (grant ZIA HL000225). J.J. was supported with federal funds from the National Cancer Institute, National Institutes of Health, under contract no. HHSN 261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: oxidoreductases · protein modifications · Pummerer rearrangement · reaction mechanism · sulfoxides

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