Acknowledgment. We are grateful to the National Institutes of Health and National Science Foundation for support of this work. We also are grateful to Professor A. I. Meyers for stimulating discussion and spectral comparison, to Professor R. W. Gawley for a discussion of the stereochemistry of these reactions, and to Professor R. Hill for a sample of *trans*-2,6-dimethylpiperidine.

Supplementary Material Available: Experimental data for the preparations of **7**, **9**, **11**, **14–30**, **33**, **35–42**, and **44** and the X-ray structure of **40** (41 pages). Ordering information is given on any current masthead page.

Articles

Kinetics of the Aqueous Periodate Oxidation of Aliphatic Disulfides and Thioethers

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Water-soluble aliphatic disulfides are oxidatively cleaved by borate-buffered periodate at 23 °C. The reaction conditions were selected because they are used for the oxidation of methionine in protein modification, and we wanted to test the reactivity of the disulfide linkage in various bifunctional molecules under these conditions. A colorimetric method was developed which uses 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) to determine the concentrations of periodate. The γ-substituted amine–disulfide **1b** consumes 4 equiv of periodate at a rate which is accelerated 100-fold over that of 4,4′-dithiodibutanol (**1c**) and forms the cyclic sulfinamide **3** and sulfonamide **4**. To account for the stoichiometry and acceleration, we have proposed intermediates in which a nucleophilic sulfur atom attacks an oxygen atom of periodate to give an anhydride or complex rather than invoking direct oxygen atom transfer. The γ- and δ-hydroxy disulfides **1a** and **1c** consume 5 equiv of periodate and are oxidized to the sulfonic acids. The rate of DL-methionine (**2a**) oxidation in water is reported, along with the oxidations of dibutyl sulfide (**2c**) and of 1,5-dithiacyclooctane (**2d**) in 50% aqueous ethanol. The oxidation of **2d** is only 2.1 times faster than the oxidation of **2c**, showing that the transannular sulfur atom in **2d** does not participate in the oxidation. A comparison of the rate of periodate oxidation of disulfides, thioethers, and ethylene glycol under the same conditions shows that it is possible for these processes to be competitive.

Introduction

Periodate is an excellent reagent for the conversion of thioethers to sulfoxides,[1] and, for that reason, it is used in protein modification studies to convert methionine to methonine sulfoxide.[2] Although many of the functional groups present on side chains of amino acids were examined and shown to be resistant to periodate oxidation under the conditions used in protein modification studies, the disulfide group was not studied. Unsubstituted disulfides are rather resistant to oxidation by aqueous periodate[3] because of their insolubility in water, but substituted disulfides such as cystine have been shown to be oxidized slowly to sulfonic acids.[4-6] However, the oxidation of isolated disulfides and cystine itself may not be realistic models of the reactions of disulfides in proteins. For example, we have shown that the concomitant electrophilic–nucleophilic oxidation of aliphatic thioethers and disulfides with aqueous iodine is strongly enhanced by neighboring nucleophiles.[7-13] In order to determine whether the oxidative cleavage of disulfides by periodate can proceed at a rate which is comparable to the rate of oxidation of thioethers, the rate and products of the oxidation of a series of bifunctional, water-soluble, aliphatic disulfides were examined. The three disulfides used are 3,3′-dithiodipropanol (**1a**), bis(3-aminopropyl) disulfide (**1b**), and 4,4′-dithiodibutanol (**1c**).

$$[X(CH_2)_2S]_2$$

$$\mathbf{1}: X = CH_2OH\ (\mathbf{a}),\ CH_2NH_2\ (\mathbf{b}),\ (CH_2)_2OH\ (\mathbf{c})$$

Ruff and Kucsman[14,15] studied the periodate oxidation of aliphatic and aromatic thioethers in aqueous alcohol and concluded that oxidation proceeds by a one-step electrophilic oxygen transfer from periodate to the thioether

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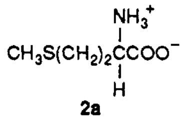
through a polar transition state. They attributed modest differences in the rates of ortho-substituted aryl thioethers to stabilization of the transition state by a polar neighboring group. We wanted to determine the rates of oxidation of aliphatic thioethers, especially methionine and those thioethers known to have enhanced rates of iodine oxidation due to neighboring group participation, and compare the rates with that of a simple thioether such as dibutyl sulfide.[14] Therefore, the rate of oxidation of the following thioethers, DL-methionine (**2a**), 3,3′-thiodipropanoic acid (**2b**), dibutyl sulfide (**2c**), and 1,5-dithiacyclooctane (1,5-DTCO, **2d**) were determined.



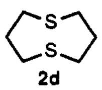
$$CH_3S(CH_2)_2\overset{NH_3^+}{\underset{H}{C}}COO^-$$

2a

RSR

2b: R = $(CH_2)_3COOH$

2c: R = $(CH_2)_3CH_3$



2d

Results and Discussion

Reaction Conditions. The reactions were carried out in solutions containing 0.5–5.0 mM of each of the reactants in pH 8.0 aqueous buffer at room temperature (23 °C) for 40 min. These conditions were used by Feeney et al.[2] for the oxidation of methionine residues in proteins. They are also similar to the ethanol–water solutions, buffered at pH 8 which Ruff and Kucsman[14,15] used for their kinetic studies of thioether oxidation with periodate. Under these conditions periodate exists mainly as tetrahedral IO_4^- as opposed to dimers or hydrates.[16–21] In view of the known reaction of iodine with these same compounds, it was important to us that no production of iodine, as an orange tint, was ever observed.

Product Runs. The products of the reaction of compounds **1a** and **1b** were determined by treating the disulfide with 5 equiv of $NaIO_4$ in 0.2 M borate buffer at pH 8.0. Compound **1a** was stirred at 70 °C for 4 h and then at room temperature for 12 h. Compound **1b** was stirred at room temperature for 3 days. After the reaction was complete, the solution from the oxidation of **1a** was evaporated and the residual solid was analyzed by ^1H NMR to reveal only the corresponding sulfonate. The yield was determined by integration of the ^1H NMR signals using added potassium hydrogen phthalate as a standard.

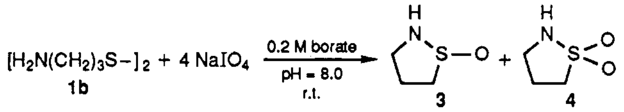
$$\underset{\mathbf{1a}}{[HO(CH_2)_3S\text{-}]_2} + 5NaIO_4 \xrightarrow[\substack{pH = 8.0 \\ r.t.}]{0.2\ M\ borate} 2HO(CH_2)_3SO_3^-$$

The reaction mixture from **1b** was continuously extracted into $CHCl_3$, dried, and column chromatographed. Two cyclic molecules, the sulfinamide **3** and the sulfonamide **4**, were isolated in approximately equal amounts. These products, which had been prepared previously,[22–28] were identified by low- and high-field ^1H NMR, GCMS, and index of refraction.

Table I. Kinetic Data for the IO_4^- Oxidations[a]

compound	10^3 ($[IO_4^-]_0$: $[compound]_0$)	k_2, $M^{-1}\ s^{-1}$[b]	stoichiometry found (assumed)
$[H_2N(CH_2)_3S]_2$, **1b**	2.00:0.500[c]	1.8 ± 0.1	(4:1)
	2.00:0.500	2.9 ± 0.5[d]	(4:1)
	2.50:0.500	2.1 ± 0.1[e]	3.96:1.00
	3.50:0.500	2.1 ± 0.3	4.17:1.00
$[HO(CH_2)_3S]_2$, **1a**	2.50:0.500	0.18 ± 0.02	(5:1)
	3.50:0.500	0.20 ± 0.04	5.05:1.00
	4.00:0.500	0.12 ± 0.01	4.93:1.00
$[HO(CH_2)_4S]_2$, **1c**	2.50:0.585	0.017 ± 0.001	(5:1)
methionine, **2a**	5.00:5.00	0.64 ± 0.07[f]	(1:1)
	10.0:5.00	0.69 ± 0.07[g]	1.00:1.09
	5.00:5.00[h]	1.66 ± 0.16[h]	(1:1)
$[HO_2C(CH_2)_2]_2S$, **2b**	0.50:0.50	0.53 ± 0.04	(1:1)
	1.00:1.00	0.40 ± 0.05	(1:1)
	5.00:5.00	0.18 ± 0.01	(1:1)
n-butyl sulfide, **2c**	5.0:5.0[i]	0.30 ± 0.02[i]	(1:1)
1,5-DTCO, **2c**	5.0:5.0[i]	1.3 ± 0.2[i]	(1:1)
ethylene glycol	2.5:2.5[j]	3.3 ± 0.5[j]	(1:1)

[a] T = 23.0 °C; 0.2 M borate buffer, pH 8.0. [b] Instantaneous second-order rate constants for the decay of organic compound, calculated using more than 6 points over at least 20–75% of the reaction, unless otherwise noted. The precision has been calculated from the k_2 values used. [c] Determined by continuously scanning with ultraviolet. [d] 40–80%. [e] 25–64%. [f] 34–60%. [g] 35–86%. [h] T = 23.0 °C; 0.2 M acetate buffer of pH 6.0. [i] T = 25.0 °C; 50% aqueous ethanol. [j] T = 25.0 °C; water, pH 5.7.



No special product runs were done on the thioethers since it has been previously demonstrated that under our conditions methionine[2] and dibutyl sulfide[14] are oxidized to their sulfoxides. Additionally, our standard procedure for the oxidation of 1,5-DTCO to the monosulfoxide[29] in 83% yield utilized $NaIO_4$ in aqueous methanol at 0–10 °C.

Kinetics. We adapted a colorimetric method in which 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) is used for periodate determination.[30] Two other methods have been used in the past for kinetic studies of periodate oxidations. We did not use these because the iodometric method[31] would produce reactive iodine when iodide ion was added, and the spectrophotometric method,[32] which measures the periodate ion concentration at λ = 222.5 nm, requires corrections for iodate, other products, and the starting substrate.

The general method for monitoring the reaction consisted of preparing separate solutions of the thioether or disulfide and periodate in 0.2 M borate buffer at pH 8.0 at 23 °C and mixing them to give the initial concentrations listed below. At various times, aliquots were taken and quenched in sufficient 0.1 M, pH 7.6 phosphate buffer to make the final concentration of periodate between 0.08 and 0.10 μmol/mL. Then, 1.0 mL of the final solution was treated with 2.0 mL of 0.137% ABTS in phosphate buffer and left for 30 min in the dark before the intensity of the absorption at λ = 418 nm was determined. Absorbance

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values were converted directly to periodate concentration by using a standardized plot of absorbance versus [IO_4^-].

The rate data, initial concentrations, and reaction stoichiometry for disulfides **1a–c** and thioethers **2a–d** are listed in Table I. The reaction stoichiometry of 5:1 for [IO_4^-]:[RSSR] was verified for disulfides **1a** and **1c**, of 4:1 for **1b**, and of 1:1 for thioethers **2a–d**.

To confirm the validity of the ABTS method, two literature values were reproduced, one for the oxidation of dibutyl sulfide[14] and the other for the oxidation of ethylene glycol.[33,34] Our value for dibutyl sulfide is 0.30 M^{-1} s^{-1} (lit.[14] 0.291 M^{-1} s^{-1}, determined as pseudo-first-order rates by UV) and for ethylene glycol is 3.3 M^{-1} s^{-1} (lit.[34] 2.50 M^{-1} s^{-1}).

At the outset of this research, the integrated equations for the determination of second-order rate constants were used.[35] However, there was always some question as to the percentage of the reaction to include to obtain meaningful second-order rate constants since second-order behavior was often not displayed throughout the entire reaction.[36] By use of the Master Fit computer program[37] for curve analysis and for derivative calculation, we were able to determine instantaneous second-order rate constants at a minimum of 6 points over at least 20–75% of the reaction (supplementary material available), except as noted in the footnotes to Table I. The precision is calculated from these instantaneous rate constants. In Table I are reported the "true" rate constants for disulfides, that is, k_2, from the relationship $d[IO_4^-]/dt = -ak_2[IO_4^-][RSSR]$, where $a = 5$ for disulfides **1a** and **1c** and $a = 4$ for disulfide **1b** where the value was determined by use of excess periodate in some runs. Thus, to calculate the rate of periodate loss, k_2 in Table I must be multiplied by the stoichiometric ratio given in the last column.

Although Feeney et al.[2] oxidized methionine (**2a**) to methionine sulfoxide with periodate, the rate of the reaction was not determined. We decided to determine the rate constant for this important reaction using the ABTS method for periodate analysis in borate buffer at a pH of 8.0. Doubling the [IO_4^-] did not change the value of the rate constant. The 1:1 stoichiometry was confirmed by the fact that the decay curve leveled off when 1 equiv of periodate is consumed. With acetate buffer at pH 6.0, the reaction is approximately twice as fast as in pH 8.0 phosphate.

3,3-Thiodipropanoic acid (**2b**), a thioether carboxylic acid, was oxidized under the same conditions used for methionine and reacts at about the same rate. With **2b**, however, the value of k_2 is lower at the highest concentrations studied. Critical studies of the aqueous periodate oxidation of glycol by Duke[38,39] and by Taylor[34] reveal that there is a decrease in the second-order rate constant as the concentration of glycol or periodate is increased. This variation in the value of the second-order rate constants was attributed to the formation of octahedral complexes between periodate and glycol. Treatment of our data by the method used by Duke[38] and Taylor[34] did not give consistent values for complex formation. We also showed that this aberration in rate constants was not eliminated when the reaction was run in the dark, when oxygen was bubbled through the reaction mixture, or when various sources of water were used.

In order to determine whether appreciable anchimeric assistance occurs in thioether oxidation by periodate, 1,5-DTCO was oxidized under the same conditions as *n*-butyl sulfide (**2c**). Since neither of these compounds has high water solubility, they were oxidized in 50% aqueous ethanol. A rate constant of 1.3 M^{-1} s^{-1} for 1,5-DTCO was determined. Thus, 1,5-DTCO is oxidized only 4.3 times faster than **2c**. Taking into account the fact that 1,5-DTCO has a statistical advantage which is a factor of 2, a single sulfur atom on a 1,5-DTCO molecule is oxidized only 2.1 times faster than the sulfur atom in **2c**. In contrast, the oxidation of 1,5-DTCO by aqueous iodine is accelerated by a factor of 10^6 relative to the cyclic monothioether, thiocane.[9] This enormous rate enhancement with iodine is attributable to neighboring group participation by the transannular nucleophilic sulfur atom on the iodine/1,5-DTCO complex to give the 1,5-DTCO dication. Thus, we find no evidence for anchimeric assistance in the periodate oxidation of aliphatic thioethers. This result also rules out a ligand coupling mechanism[40] in which the thioether sulfur coordinates to give a hypervalent periodate ion followed by oxygen atom transfer to give sulfoxide and iodate. If a ligand coupling mechanism had occurred, one would have expected significant neighboring group participation, since the transannular sulfur would have interacted with the coordinated sulfur to give the dication in the same manner as observed when iodine was used as the oxidant.[9]

The rate constants for the oxidation of 3,3′-thiodipropanoic acid and methionine in water and those of *n*-butyl sulfide and 1,5-DTCO in 50% aqueous ethanol all range between 0.2 and 1.7 M^{-1} s^{-1}. We were surprised to find that the thioethers oxidized so slowly in water since Ruff and Kucsman[14] have shown that the rate of periodate oxidation of phenyl methyl sulfide increases with increasing percentage of water, $\log k_2 = 0.722Y$. According to this relationship, the rate of periodate oxidations should be 40 times faster in water ($Y = 3.49$) than in 50% aqueous ethanol ($Y = 1.604$). This difference may be related to the fact that the reaction between the very nonpolar phenyl methyl sulfide with the very polar periodate could be highly dependent on the bulk property of the solvent, whereas the reaction of 3,3′-thiodipropanoic acid and methionine with periodate involves the interaction of two highly solvated polar species, and any variation in solvent composition effects both species in the same way. Our observations are consistent with the low value of ρ measured by Ruff and Kucsman; however, unlike these authors, we would attribute the small differences in rate constants to differences between solvation of the ground states and transition states.

The kinetic studies of the periodate oxidation of the functionalized disulfides **1a–c** employed the ABTS method under the conditions used for thioethers **2a** and **2b**. When the alcohol disulfide **1a** is treated with 5 molar equiv of $NaIO_4$, all the periodate is consumed. The formation of sulfonic acid in high yield does not proceed through the corresponding five-membered sultine intermediate since the sultine[41] and sultone[42] are known to be stable under

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the conditions of the reaction. When excess periodate was used, only 4.93 ± 0.08 equiv of periodate reacts. There is a variation in the values of the k_2 from 0.12 to 0.20 M^{-1} s^{-1} as the concentration of periodate changes, with the lower value being obtained at the highest concentration of periodate.

The amine disulfide **1b** consumes only the 4 equiv of periodate required to form an equimolar mixture of cyclic sulfinamide **3** and sulfonamide **4**. The values of k_2 determined by the ABTS method are summarized in Table I and have values 2.1–2.7 M^{-1} s^{-1}, which compare favorably with the value of 1.8 M^{-1} s^{-1} determined by continuous scanning of the periodate absorbance at λ = 221 nm. When excess periodate is used, only 4 equiv is consumed in the reaction.

As an example of a disulfide with decreased solubility in water and with lower probability of anchimeric assistance, the extended alcohol **1c** was studied at one concentration. The value of k_2, the "true" second-order rate constant, was about one-tenth of the value of its homologue, **1a**.

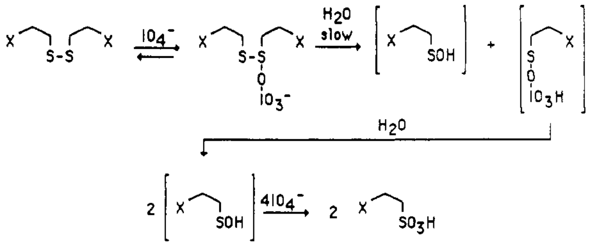
Relative Reactivities of Disulfides and Thioethers. When water-soluble thioethers were oxidized, the values of k_2 ranged from 0.18 to 0.84 M^{-1} s^{-1}. The "true" rate constants (k_2) for the oxidative cleavage of water-soluble disulfides covered a wide range from 0.017 to 2.7 M^{-1} s^{-1}, and, since the actual consumption of periodate is measured by the relationship $d[IO_4^-]/dt = -ak_2[IO_4^-][RSSR]$, where a = number of equivalents of IO$_4^-$ per equivalent of disulfide, the values of ak_2 ranged from 0.09 to 11 M^{-1} s^{-1}. Since the two ranges overlap, it cannot be taken for granted that periodate will preferentially oxidize a thioether rather than a disulfide as had been thought.[43,44]

Although there is no evidence for anchimeric assistance in the oxidation of the thioethers, the acceleration of the rate of the amine disulfide, **1b**, over the alcohol disulfide, **1c**, is 100-fold. Furthermore, the products of the oxidation of **1b** are the cyclic sulfinamide and sulfonamide. These two observations indicate that the oxidative cleavage of **1b** is anchimerically assisted.

The last value in Table I is the Malaprade reaction of ethylene glycol. Comparison of this rate constant with ak_2 for disulfides **1a** and **1b** and thioethers **2a** and **2b** show that these reactions can all occur competitively in the presence of a limiting amount of periodate. When McCormick and McElhinney[45] oxidized *trans*-tetrahydrothiophene-3,4-diol with 1 equiv of periodate, they were able to obtain only 50–60% of the dialdehyde (as the disemicarbazone), even when the reaction was left for an extended period of time. When 2 equiv of periodate was added, the product after 1 h was virtually pure sulfoxide–dialdehyde. From this, they deduced that the 1 equiv of periodate yielded some sulfoxide–diol along with 50–60% of the dialdehyde. Presumably, in this molecule, thioether oxidation is competitive with the Malaprade reaction of the diol with periodate.

In studies of periodate oxidations of molecules which contain both thioether and disulfide groups, the sulfoxide product of thioether oxidation is usually found to dominate, but the yields are not quantitative.[43,44] Because it is easier to isolate and quantify the sulfoxide by extraction from the aqueous solvent than to separate and purify any sulfonic acid from iodate and periodate which remain in the aqueous phase, we feel that the products of disulfide oxidation may have been overlooked. For example, if k_2 for thioether oxidation and ak_2 for disulfide cleavage were equal and equimolar amounts of IO$_4^-$ were used, the sulfoxide would form in 50% yield, the disulfide would form in 10% yield, and 40% of the starting material would remain.

Scheme I. Periodate Oxidation of Disulfides



With respect to protein modification studies using NaIO$_4$[2], the rates of periodate oxidation of methionine and of functionalized disulfides are both at least on the same order of magnitude, and thus the oxidation of disulfide linkages as well as methionine residues must be considered.

As regards to water-insoluble disulfides, we were unable to detect any oxidation of *n*-butyl disulfide even when a suspension was vigorously stirred and heated for 12 h in borate buffered solution.

Mechanisms of Periodate Oxidative Cleavage. On the basis of the kinetic results and the overall stoichiometry, a general scheme can be proposed for the reaction of disulfides with periodate. All that can be observed are the two reagents coming together prior to the rate-determining step and attack by a nucleophile, either water or the neighboring amine. Since the rate-determining step is early in the reaction, we cannot propose a mechanism in which all the steps are known in detail, but we can propose a series of stepwise processes which account for the products of the reaction. Alternative mechanisms were considered but we were unable to account for the rate acceleration and all the products by any other sequence of steps. In this and other studies of periodate oxidations of disulfides[46] we have been able to write more consistent mechanisms by forming intermediates in which a nucleophilic sulfur atom attacks an oxygen atom of periodate to give an anhydride rather than invoking direct oxygen atom transfer. In previous discussions of the mechanism of periodate oxidations of sulfur compounds, a direct transfer of oxygen from periodate to sulfur is normally proposed while $-S-O-IO_3H$ or $-S-IO_4H$ intermediates were rejected.[14] We have previously shown[13] that $-S-O-C(O)^-$ or $-S-O-P(O)_2^{2-}$ anhydrides are obligatory intermediates in the oxidative cleavage of both carboxylic acid and phosphonic acid disulfides. Thus the inclusion of an anhydride intermediate in the mechanism of periodate oxidations should not be unexpected. Since periodate is reduced to iodate on complexation, we will normally refer to these molecules as anhydrides between iodic acid and the appropriate sulfur-containing acid (e.g., sulfenic/iodic acid anhydride). When periodate interacts with a disulfide, we refer to this species as a periodate/disulfide complex since the disulfide is not an acid.

For disulfides which consume 5 equiv of periodate, the initial step consists of the reaction of 1 equiv of periodate to give a periodate/disulfide complex (Scheme I). In the absence of anchimeric assistance, cleavage of the sulfur–sulfur bond in the periodate/disulfide complex must be

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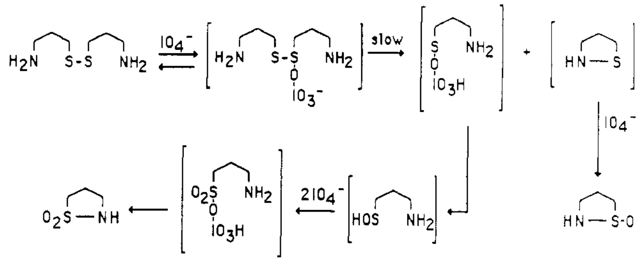
Scheme II. Periodate Oxidation of Bis(3-aminopropyl) Disulfide (1b)



caused by water in the rate-limiting step. Thus 2 equiv of sulfenic acid are produced after hydrolysis of the resulting sulfenic/iodic acid anhydride, and complete oxidation to sulfonic acid is accomplished by 2 more equiv of periodate.

The oxidation of bis(3-aminopropyl) disulfide (**1b**) consumes only 4 equiv of periodate, and the observed stoichiometry of 4:1 requires that the products consist of a 50/50 mixture of sulfinamide and either the sulfonamide or the sulfonic acid. Only the sulfinamide and sulfonamide were isolated, but traces of the sulfonic acid were observed by ^{1}H NMR in the evaporated aqueous layer. In addition, the reaction is accelerated by a factor of about 100 over that of **1c**. To account for both the stoichiometry and acceleration, the following mechanism is proposed. One of the sulfur atoms of the disulfide interacts with periodate to give a complex which is then cleaved by attack of the neighboring amine at the adjacent sulfur in a rate-limiting step (Scheme II). Neighboring group participation by the amine is also observed in the aqueous iodine oxidation of **1b**.[22] The sulfenamide is then oxidized by a second equivalent of periodate to the sulfinamide, and this half of the reaction stops at this step. The sulfinamide is stable to oxidation so the sulfonamide must come directly from further reactions of the sulfenic/iodic acid anhydride. The only time the neighboring amine group can interact with sulfur to give a sulfonamide is with a sulfonic/iodic acid anhydride. If cyclization occurs at any time prior to the interaction with a sulfonic/iodic acid anhydride, the sulfinamide would form and the reaction would stop there. Therefore, we envision that the lone pair of the sulfinic acid interacts with an oxygen atom of periodate to give a sulfonic/iodic acid anhydride, and then the neighboring amine attacks the sulfur to give the sulfonamide and iodate. We feel that the reason that the amine does not attack either the sulfenic/iodic acid anhydride or the sulfinic/iodic acid anhydride is that the sulfur atom is still too soft an electrophile. Only when there is no lone pair left on the sulfur atom does the amine attack.

Conclusions

This work establishes the first evidence for the oxidative cleavage of water-soluble aliphatic disulfides by periodate. The γ-substituted amine–disulfide **1b** forms the cyclic sulfinamide and the sulfonamide at an accelerated rate. A comparison of the rate of periodate oxidation of disulfides, thioethers, and ethylene glycol under the same conditions shows that it is possible for these processes to be competitive under some conditions. Since the rate of periodate oxidation of methionine and of functionalized disulfides are both of the same order of magnitude, we suggest that, under the same conditions, the oxidative cleavage of disulfide linkages in proteins must also be considered. In this and other studies of periodate oxidations we have been able to write more consistent mechanisms by forming intermediates in which a nucleophile sulfur atom of the disulfide attacks an oxygen atom of periodate to give mixed anhydrides rather than invoking direct oxygen atom transfer.

Experimental Section

General. Physical Measurements. Melting points were obtained uncorrected on a Thomas-Hoover "Uni-Melt" capillary melting point apparatus. Low-field (90 MHz) ^{1}H NMR were recorded on a Varian 390 90-MHz continuous wave spectrophotometer. High-field ^{1}H NMR were recorded at ambient temperature on a Nicolet 360 FT spectrometer at 360 MHz with a 8.46-T superconducting magnet. All chemical shift values are listed in ppm referenced downfield to internal tetramethylsilane in the indicated deuterated solvent. Infrared spectra were obtained on an IBM FTIR-32 spectrometer. Samples were prepared either neat on NaCl plates or as KBr pellets, and absorptions were recorded in cm^{-1}. Ultraviolet–visible spectra (nonkinetic studies) were taken on a Hewlett-Packard Model 8450A UV–Vis spectrophotometer. Solution spectra were obtained using 1-cm matched quartz cells purchased from Helma Optics. Solvents used were of HPLC quality. Thin-layer chromatograms were obtained using either Eastman Kodak Silica Gel F_{254} TLC sheets or DC-Plastikfolien Kieselgel 60 F_{254}, both of 0.2 mm thickness using the indicated solvent systems. Column chromatography was performed using Silica Gel 60–200 M Bar (0.060- to 0.200-mm particle size) from J.T. Baker. Refractive indices were obtained from a Bausch and Lomb refractometer with the temperature held constant by a circulating water bath. Low-resolution gas chromatograph mass spectra were obtained on a Finnegan 3200 spectrometer. All pH values were obtained on a Radiometer Copenhagen PHM 82 standard pH meter using a Corning Scientific Semi-Micro electrode. The pH meter was referenced each time before use with buffered standard solutions at pH 4.00, 7.00, and 10.00 purchased from VWR Scientific, Inc. Elemental analyses were performed by the Microanalytical Laboratory, 20 Lewis Hall, University of California, Berkeley.

Kinetic Measurements. Solutions were thermostated to a constant temperature in a Wilkens-Anderson Co. lo-temp bath with an attached Precision Scientific Co. stir pump to achieve constant circulation. The temperature was recorded to an accuracy of ± 0.03 °C with a Messgeräte-Werk Lauda/Tauber thermometer calibrated in ice water to 0.00 °C. Reaction times were measured using a Cramer stopclock accurate to ±0.05 min. Visible absorbance values were recorded using a Beckman DU quartz monochromator equipped with a Gilford Instrument 2451A automatic cuvette positioner and photometer, Model 252, using 1-cm matched quartz cells. Ultraviolet absorbance values were also recorded using the Beckman DU quartz monochromator equipped with the Gilford Instrument and an attached chart recorder, Model 6051. Kinetic reactions monitored in the UV were accomplished in cuvette cells thermostated within a Brinkmann Instrument Lauda Type K-2/R constant-temperature bath accurate to ±0.03 °C. Volume measurements for kinetic aliquots were taken using Finnpipette autopipets of 1.00–5.00 mL or 50–200 μL calibrations. Temperature determinations inside the equilibrated cuvette cells were determined with a negative temperature coefficient thermistor attached to a Keithley Model 192 programmable digital multimeter readable to ±0.0002 ohm.

Reagents. Except as noted below, all commercial solvents used were analytical reagent grade and used without further purification. Analytical grade sodium metaperiodate, boric acid, sodium acetate, potassium phosphate monobasic, sodium phosphate dibasic, sodium acetate, and potassium hydroxide used were from Mallinckrodt. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt was purchased from Sigma Chemical Co. D,L-Methionine (Sigma Chemical Co.) was recrystallized from water, mp 278.0–279.5 °C (lit.[46] 279–280 °C). 3,3′-Thiodipropanoic acid (Evans Chemetics, Inc.) was further purified by recrystallization from water and dried over anhydrous P_2O_5 under reduced pressure at 65 °C, mp 131–134 °C (lit.[47] mp 131–134 °C). *n*-Butyl sulfide

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(Aldrich Chemical Co.) was distilled at 88.5 °C (42 Torr); $n^{24.5}_{D}$ = 1.4521 (lit.[47] n^{20}_{D} = 1.4530).

Synthetic Preparations. 1,5-Dithiacyclooctane (1,5-DTCO, 2d). Compound **2d** was prepared by the method of Roush,[49] which was a modification of the procedure of Meadow and Reid[50] and Leonard et al.,[51] and distilled twice: bp 102–103 °C (4 Torr), (lit. 43–46 °C (0.1 Torr),[51] 96–98 °C (3.5 Torr)[49]); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.811 (m, 8.0, $-C\mathit{H}_2S-$), 2.055 (m, 4.0, $-CH_2C\mathit{H}_2CH_2-$), (lit.[49] (CCl_4, 60 MHz) δ 2.8 (m, 8), 2.1 (m, 4); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 30.494 (double intensity, $-CH_2\mathit{C}H_2CH_2-$), 29.855 ($-\mathit{C}H_2S-$), (lit.[52] (CD_3CN, 25 MHz) δ 30.6 (double intensity, C-2), 30.0 (C-3)).

3,3′-Dithiodipropanol (1a).[53,54] 3-Chloropropanol (9.46 g, 0.100 mol, Aldrich Chemical Co.) was refluxed in a solution of $Na_2S_2O_3$ (17.40 g, 0.110 mol) in 120 mL of 50% v/v aqueous methanol with stirring for 1.8 days.[55–57] A solution of I_2 (14.0 g, 0.055 mol) in 144 mL of methanol was added slowly dropwise over a period of 3.5 h to the refluxing solution. The resulting mixture was filtered and the filtrate was evaporated under vacuum to remove most of the methanol. The aqueous solution was continuously extracted into diethyl ether, and the ether layer was decolorized with 0.6 M aqueous $Na_2S_2O_3$ and dried with $MgSO_4$. The ether was removed by rotary evaporation to yield 8.13 g (89%) of **1a**: TLC R_f = 0.13 (5% $EtOH/CHCl_3$); ^{1}H NMR (90 MHz, $CDCl_3$) δ 3.69 (t, 4, $HOC\mathit{H}_2-$), 2.83 (t, 4, $-C\mathit{H}_2S-$), 1.96 (pentet, 4, $-CH_2C\mathit{H}_2CH_2-$); 1.66 (s, 2, $-O\mathit{H}$) (lit.[53] (90 MHz, $CDCl_3$) δ 3.7 (t, 4), 2.7 (t, 4), 1.9 (m, 4).

Bis(3-aminopropyl) Disulfide (1b). Compound **1b** was prepared as described by Doi[22] and others,[55–57] but the pale yellow residue was precipitated as the HCl salt by the addition of Et_2O to yield 3.13 g of product (50%): mp 204–215 °C (lit.[58] 217–218 °C); ^{1}H NMR (90 MHz, D_2O) δ 2.95 (t, 4, $ClH_3NC\mathit{H}_2-$), 2.66 (t, 4, $-C\mathit{H}_2S-$), 1.90 (pentet, 4, $-CH_2C\mathit{H}_2CH_2-$). The salt was further purified by careful recrystallization from 98% aqueous EtOH and dried over anhydrous P_2O_5 under reduced pressure at 111 °C for 1 day to yield a yellow-white solid mp 215–217 °C (sealed under N_2, dec); TLC R_f = 0.12 (5 *n*-BuOH:1HOAc:4H_2O). The purified HCl salt was treated with 2.5 equiv of NaOH, and the free base was extracted into $CHCl_3$. The solvent was evaporated to provide the free amine in >95% yield: ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.80 (m, 8, $H_2NC\mathit{H}_2-$ and $-C\mathit{H}_2S-$), 1.87 (pentet, 4, $-CH_2C\mathit{H}_2CH_2-$), 1.14 (s, 2, H_2N-); (lit.[22] (90 MHz, $CDCl_3$) δ 2.7 (m, 8), 1.7 (m, 4)).

4,4′-Dithiodibutanol (1c).[53] 4-Chloro-1-butanol (8.39 g, 0.773 mol, Eastman Kodak) was added dropwise to a refluxing solution of $Na_2S_2O_3$ (13.44 g, 0.8502 mol) in 95 mL of 50% aqueous methanol and refluxed for 2.3 days. A solution of I_2 (10.79 g, 0.4251 mol) in 111 mL of methanol was slowly added dropwise to the refluxing solution. The solution was decolorized with a 1.0 M aqueous $Na_2S_2O_3$ solution, the cloudy white mixture was filtered, and the methanol was removed by rotary evaporation. The resulting aqueous solution was continuously extracted into $CHCl_3$, dried, and evaporated, and the resulting oil was column chromatographed with 5% $EtOH/CHCl_3$ to yield 0.23 g (5%) of **1c**: TLC R_f = 0.10 (5% $EtOH/CHCl_3$) (lit.[53] R_f = 0.13 (5% $EtOH/CHCl_3$)); ^{1}H NMR (90 MHz, $CDCl_3$) δ 3.67 (t, 4, $-C\mathit{H}_2OH$), 2.74 (t, 4, $-C\mathit{H}_2S-$), 1.90 (s, 2 $-O\mathit{H}$ which disappears upon shaking with 2 drops of D_2O), 1.70 (m, 8, $-CH_2C\mathit{H}_2C\mathit{H}_2CH_2-$) (lit.[53] (90 MHz, $CDCl_3$) δ 3.4 (m, 2), 3.2 (s, 1), 2.5 (t, 2), 1.4 (m, 4); the small difference in chemical shift was due to spectrometer error; actual spectra were identical).

3-Sulfopropanol from 3,3′-Dithiodipropanol (1a). To 100 mL of a 46 mM solution of disulfide **1a** was added 100 mL of 230 mM $NaIO_4$, both in 0.2 M borate buffer, pH 8.0. The solution was stirred at 70 °C for 4 h, followed by 12 h at room temperature. The water was evaporated under reduced pressure and the white solid was extracted into MeOH. After the MeOH was evaporated, a white solid resulted in 86% yield (NMR yield was based on added potassium acid phthalate): FTIR (KBr window) 3375 (very broad), 2940 (m), 1590 (w), 1385 (m), 1196 (s), 1060 (s) cm^{-1} (lit.[59] Nujol, 3314 (broad), 1281 (w), 1192 (s), 1120 (w), 1058 (s), 799 (w), 736 (w), 617 (w) cm^{-1}); ^{1}H NMR (90 MHz, D_2O) δ 3.56 (t, 2, $HOC\mathit{H}_2-$), 2.81 (t, 2,$-C\mathit{H}_2SO_3-$), 1.95 (pentet, 2, $-CH_2C\mathit{H}_2CH_2-$) (lit.[60] δ 3.65 (m), 2.98 (m), 2.00 (m)).

Isothiazolidine 1-Oxide (3) and Isothiazolidine 1,1-Dioxide (4) from Bis(3-aminopropyl) Disulfide (1b). To 100 mL of a 46 mM solution of disulfide **1b** was added 100 mL of a 230 mM solution of $NaIO_4$ in 0.2 M borate buffer at pH 8.0; the resulting solution was stirred for 3 days at room temperature. The mixture was filtered and the aqueous filtrate was continuously extracted into $CHCl_3$, dried, and rotary evaporated. The yellow liquid was chromatographed on silica gel with 10% $EtOH/CHCl_3$ to yield 0.24 g (50%) of the sulfinamide **3** [TLC R_f = 0.25 (10% $EtOH/CHCl_3$); FTIR (neat on NaCl), 3225 (very broad), 2950, 2880, 1400, 1300, 1200, 1050 cm^{-1} (lit.[22] (neat) 3225, 1048, 1014, 640 cm^{-1}); ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.58 (s, broad, 1), 3.70 (t, 1), 3.28 (q, 1), 2.80 (m, 2), 2.44 (m, 1) (lit.[22] (90 MHz, $CDCl_3$) δ 4.5 (broad, 1), 3.7 (m, 1), 3.1 (m, 1), 2.8 (m, 2), 2.3 (m, 2)); n^{25}_{D} = 1.5284] and 0.21 g (38%) of the sulfonamide 4 [TLC R_f = 0.37 (10% $EtOH/CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$), δ 4.51 (s, broad, 1, $-N\mathit{H}-$), 3.40 (q, 2, J = 6.4 Hz, $-NHC\mathit{H}_2CH_2-$), 3.07 (t, 2, J = 7.5 Hz, $-O_2SC\mathit{H}_2CH_2-$), 2.44 (pentet, 2, J = 7.0 Hz, $-CH_2C\mathit{H}_2CH_2-$), (lit.[23] (DMSO–d_6) δ 4.40 ($-N\mathit{H}-$), 3.39 ($-NHC\mathit{H}_2CH_2-$), 3.10 (J = 7.8 Hz, $-CH_2C\mathit{H}_2SO_2-$), 2.48 (J = 6.8 Hz, $-CH_2C\mathit{H}_2CH_2-$)); FTIR (neat) 3600, 3300 (broad), 2950, 1400, 1380, 1125, 1050 cm^{-1} (lit.[24] 3250 (vs), 2940 (m), 1390 (m), 1320 (vs), 1180 (vs), 1140 (vs), 1040 (m), 998 (m), 925.9 (m), 730 cm^{-1}; weak absorptions occurred at 2870, 1450 and 881.1 cm^{-1}); n^{25}_{D} = 1.4892 (lit. n^{25}_{D} = 1.4826,[24] 1.4815[28]); gas chromatograph mass spectrum, *m/e* 121.036, calcd, 121.020].

The aqueous layer was evaporated under reduced pressure to yield a small amount of white solid: ^{1}H NMR (D_2O) δ 3.10 (apparent q (2 overlapping t), 4, $^+H_3NC\mathit{H}_2-$ and $-C\mathit{H}_2SO_3^-$), 2.10 (pentet, 2, $-CH_2C\mathit{H}_2CH_2-$).

Attempted Oxidation of *n*-Butyl Disulfide with $NaIO_4$. A 50 mM solution of *n*-butyl disulfide in 0.2 M borate buffer (100 mL), pH 8.0, was emulsified with vigorous stirring with an overhead stirrer, in a Morton-type flask fitted with a reflux condenser. An equal volume of a 250 mM solution of sodium periodate in 0.2 M borate buffer, pH 8.0, was added and the mixture was heated to 70 °C for 12 h. The mixture was filtered and extracted three times with 50 mL of Et_2O; the water was removed under reduced pressure to yield a white solid which contained no cyclized or cleaved products nor any starting material as determined by ^{1}H NMR.

Attempted Oxidation of Isothiazolidine 1-Oxide (3) with Sodium Metaperiodate. Ten (10.00) milliliter solutions of 1.26 × 10^{-3} M **3** and 3.78 × 10^{-3} M $NaIO_4$, both in 0.2 M borate buffer, were adjusted to pH 8.0 and then maintained at 23.0 °C in a circulating temperature bath. The artificial initial IO_4^- concentration (time = 0.00 min) and concentrations after mixing were obtained as described under Kinetic Runs below. After 106.0 min, there was a change of only 5% in absorbance: initial absorbance = 1.144 (1.932 × 10^{-3} M) and final absorbance = 1.080 (1.82 × 10^{-3} M). Therefore, essentially no change in IO_4^- concentration was seen.

Colorimetric Determination of Periodate Using 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS); Standardized Plot. Sodium periodate solutions with concentrations of 0.025, 0.050, 0.075, and 0.100 μmol/mL were prepared and to 1.0 mL of each was added 2.0 mL of 0.137% ABTS, in 0.1 M pH 7.6 phosphate buffer which had been previously degassed with Ar. This solution was then allowed to stand for 30 min in

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the dark according to the procedure for periodate concentration determination.[30] The value of λ_{max} was 418 nm for each solution, and their absorbances, subtracted from an ABTS solution blank, were plotted against $[NaIO_4]$ in μmol/mL. The molar extinction coefficient (ϵ) was found to be 1.48×10^4 M^{-1} cm^{-1} with a correlation coefficient of 0.9996.

For use in kinetic runs, fresh 100 mL solutions of 0.137% ABTS in 0.1 M pH 7.6 phosphate buffer were prepared, degassed with Ar for 30 min, and kept in the dark and used within 12 h.

Kinetics of the Oxidation of Thioethers and Disulfides. Aqueous Solution: Borate Buffer. All kinetic solutions were maintained at the designated temperature within ±0.1 °C at 23.0 °C using a circulating constant-temperature water bath. Separate 50.0 or 100.0 mL solutions of thioether or disulfide and $NaIO_4$ were prepared in 0.2 M borate buffer, previously degassed with Ar and the final pH adjusted to 8.0 using small drops of 6 M aqueous KOH or HCl; 10-mL volumes were equilibrated at 23.0 °C and mixed at time zero by pouring 10.0 mL of each solution into an empty temperature-equilibrated reaction flask, briefly swirling, and then immediately replacing the flask into the constant-temperature bath. Mixing was also accomplished by the simultaneous injection from syringes, using 6.0 mL of each solution. Aliquots of 0.20 mL were removed during experiments in which the initial concentration of periodate was 2.0 mM. Proportionately sized aliquots were used for other periodate concentrations. The aliquots were quenched by 5.0 mL of 0.1 M phosphate buffer, pH 7.6, to give periodate solutions in the range of 0.08 to 0.10 μmol/mL. Then, according to the procedure of Mahuzier et al.[30] 1.0 mL of the solution was mixed with 2.0 mL of ABTS in 0.1 M phosphate buffer, pH 7.6, and developed in the dark for 30 min. The absorbance was read at 418 nm, and an ABTS solution blank was subtracted.

Aqueous Solution: Acetate Buffer. The same procedure was used with 0.2 M acetate buffer adjusted to pH 6.0.

Aqueous Ethanol: Standard Plot. A standardized plot was prepared and a new extinction coefficient was determined in 50% aqueous ethanol. Solutions of $NaIO_4$ dissolved in 50% aqueous ethanol were prepared with concentrations of 1.00, 1.25, 1.50, 1.75, and 2.00×10^{-3} M. Aliquots of 6.00 mL were removed and extracted (by vigorous vortexing) into 15 mL of $CHCl_3$ and a 3.00-mL aliquot of the aqueous layer was removed and diluted into 2.00 mL of 0.1 M phosphate buffer to give a solution with a concentration range of 0.6–1.2 μmol/mL. These solutions were then allowed to react with the ABTS solution, as previously described. Plotting the absorbance at 418 nm versus the IO_4^- concentration yielded a molar extinction coefficient, ϵ, of 1.87×10^3 M^{-1} cm^{-1} with a correlation coefficient of 0.998.

Aqueous Ethanol. Separate 100.0-mL aqueous solutions of 2.00×10^{-3} M thioether and $NaIO_4$ in 50% $EtOH–H_2O$ were prepared; 50.0 mL of each reagent was equilibrated at 25.0 °C for 1.5 h, then either poured into an equilibrating empty reaction flask and briefly swirled at time zero or simultaneously injected into an equilibrating empty reaction flask. Aliquots of 4.0 mL were removed and extracted with 10 mL of $CHCl_3$; a 1.5-mL aliquot of the aqueous layer was immediately removed and diluted into 1.0 mL of the ice-cold 0.1 M phosphate buffer of pH 7.6 to give a solution containing ≤0.6 μmol/mL. This was then developed colorimetrically as before by mixing 1.0 mL of the solution with 2.0 mL of the ABTS solution, developing 30 min in the dark and observing the absorbance at 418 nm.

Kinetics of the Oxidation of Ethylene Glycol. A mixture of 100.0 mL of aqueous 5.00×10^{-3} M $NaIO_4$ (pH 5.66) and 250.0 mL of aqueous 5.00×10^{-3} M ethylene glycol (pH 5.73) was prepared; 10.0 mL of each reagent was equilibrated at 25.0 °C for 1 h and then poured into an empty temperature-equilibrated reaction flask and swirled immediately. At appropriate intervals, aliquots of 0.20 mL, were removed and quenched to 5.0 mL in ice-cold 0.1 M phosphate buffer of pH 7.6. This solution was developed with ABTS solution as previously described and the absorbance at 418 nm was noted.

Determination of Reaction Rates by Ultraviolet Spectrophotometry. Temperature Determination. The temperature inside a thermostated 1-cm quartz cell in the spectrophotometer was determined to 23.00 °C (12.2321 $k\Omega$) by use of a negative temperature coefficient thermistor and a standardized plot of log $k\Omega$ versus temperature.

Kinetic Runs. Equal volumes of separate solutions of 4.00×10^{-3} M $NaIO_4$ in 0.2 M borate buffer (pH adjusted to 8.0) and 1.00×10^{-3} M bis(3-aminopropyl) disulfide (**1b**) in 0.2 M borate buffer (pH adjusted to 8.0) were thermostated at 23.0 °C in a constant-temperature bath; 1.00 mL of the 4.00×10^{-3} M $NaIO_4$ solution was further thermostated in the spectrophotometer in a 1-cm quartz cell at 23.0 °C. At time zero 1.00 mL of the equilibrated disulfide solution was immediately pipeted into the cell; the cell was then capped, inverted one time to mix, and immediately placed back into the spectrophotometer. The rates of oxidation were followed spectrophotometrically by monitoring the decrease in absorbance of IO_4^- in solution at 221 nm monitored against a solution blank which contained 0.2 M borate buffer, pH 8.0, and also against a standard solution of 2.00×10^{-3} M $NaIO_4$ in 0.2 M borate buffer at pH 8.0, both of which retained a constant absorbance. The decay of the IO_4^- absorbance was recorded versus time through 74% of the reaction. The base line at infinity was greater than zero; thus it was necessary to correct for IO_3^- formation and RSSR decay at time t by the relationship:

$$A_t = [IO_4^-]_t\epsilon(IO_4^-)b + [IO_3^-]_t\epsilon(IO_3^-)b + [RSSR]_t\epsilon(RSSR)b$$

where A = absorbance, [ ] = concentration at time t, ϵ = extinction coefficient, b = cell path length (1 cm); $\epsilon(IO_4^-) = 7870$, $\epsilon(IO_3^-) = 2225$, and $\epsilon(RSSR) = 2000$.

The extinction coefficients were determined from the absorbance of each reagent individually at 221 nm in a 1-cm quartz cell. A 2.00×10^{-3} M solution of $NaIO_4$ in 0.2 M pH 8.0 borate buffer provided an extinction coefficient, $\epsilon(IO_4^-)$ of 7870 (A = 1.574) (lit. λ = 222 nm, 2.0×10^{-4} M KIO_4, pH 6.0 in $MeOH–H_2O$, $\epsilon(IO_4^-) = 10\,700$[17]; 10^{-2}–10^{-4} M $NaIO_4$ in H_2O, $\epsilon(IO_4^-) = 10\,200$[61]). A 2.00×10^{-3} M solution of KIO_3 in 0.2 M pH 8.0 borate buffer provided an $\epsilon(IO_3^-)$ of 2225 (A = 0.445) (lit. λ = 220 nm, 1.05×10^{-3}–1.05×10^{-2} M KIO_3 in H_2O, $\epsilon(IO_3^-) = 1670$[61]). A 0.50×10^{-3} M solution of the amine disulfide **1b** in 0.2 M borate buffer of pH 8.0 provided an $\epsilon(RSSR)$ of 2000 (A = 0.100). Since $[IO_3^-]_t = [IO_4^-]_i - [IO_4^-]_t$ and, from the stoichiometry of the reaction, $[RSSR]_t = [IO_4^-]_t/4$, $[IO_4^-]_t = (A_t - 0.445)/614.5$.

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Registry No. **1a**, 30453-21-5; **1b**, 463-22-9; **1b**·HCl, 125829-10-9; **1c**, 30453-20-4; **2a**, 63-68-3; **2b**, 111-17-1; **2c**, 626-85-7; **2d**, 125829-09-6; **3**, 93531-48-7; **4**, 5908-62-3; ABTS, 28752-68-3; 3-chloropropanol, 627-30-5; 4-chloro-1-butanol, 928-51-8; *n*-butyl disulfide, 629-45-8; sodium metaperiodate, 7790-28-5; periodate, 15056-35-6; ethylene glycol, 107-21-1; 3-sulfopropanol, 15909-83-8.

Supplementary Material Available: Examples of the use of Master Fit to determine rate constants for selected reactions (tables and graphs; S1 and S2) (2 pages). Ordering information is given on any current masthead page.

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