Article

Hydropersulfides: H-Atom Transfer Agents par Excellence

Jean-Philippe R. Chauvin, Markus Griesser, and Derek A. Pratt

J. Am. Chem. Soc., **Just Accepted Manuscript** • Publication Date (Web): 18 Apr 2017

Hydropersulfides: H-Atom Transfer Agents par Excellence

Jean-Philippe R. Chauvin, Markus Griesser and Derek A. Pratt*

Department of Chemistry and Biomolecular Sciences, University of Ottawa, Ottawa, Ontario K1N 6N5, Canada

ABSTRACT: Hydropersulfides (RSSH) are formed endogenously via the reaction of the gaseous biotransmitter hydrogen sulfide (H_2S) and disulfides (RSSR) and/or sulfenic acids (RSOH). RSSH have been investigated for their ability to store H_2S *in vivo* and as a line of defence against oxidative stress, from which it is clear that RSSH are much more reactive to two-electron oxidants than thiols. Herein we describe the results of our investigations into the H-atom transfer chemistry of RSSH, contrasting it with the well-known H-atom transfer chemistry of thiols. In fact, RSSH are excellent H-atom donors to alkyl ($k \sim 5 \times 10^8$ M^{-1} s^{-1}), alkoxyl ($k \sim 1 \times 10^9$ M^{-1} s^{-1}), peroxyl ($k \sim 2 \times 10^6$ M^{-1} s^{-1}) and thiyl ($k > 1 \times 10^{10}$ M^{-1} s^{-1}) radicals, besting thiols by as little as one and as much as four orders of magnitude. The inherently high reactivity of RSSH to H-atom transfer is based largely on thermodynamic factors; the weak RSS-H BDE (~ 70 kcal/mol) and associated high stability of the perthiyl radical make the foregoing reactions exothermic by 15 to 34 kcal/mol. Of particular relevance in the context of oxidative stress is the reactivity of RSSH to peroxyl radicals, where favourable thermodynamics are bolstered by a secondary orbital interaction in the transition state of the formal H-atom transfer that drives the inherent reactivity of RSSH to match that of α-tocopherol (α-TOH), Nature's premier radical-trapping antioxidant. Significantly, the reactivity of RSSH eclipses that of α-TOH in H-bond accepting media because of their low H-bond acidity ($\alpha_2^H \sim 0.1$). This affords RSSH a unique versatility compared to other highly reactive radical-trapping antioxidants (e.g. phenols, diarylamines, hydroxylamines, sulfenic acids), which tend to have high H-bond acidities. Moreover, the perthiyl radicals that result are highly persistent under autoxidation conditions and undergo very rapid dimerization ($k = 6.0 \times 10^9$ M^{-1} s^{-1}) in lieu of reacting with O_2 or autoxidizable substrates.

■ INTRODUCTION

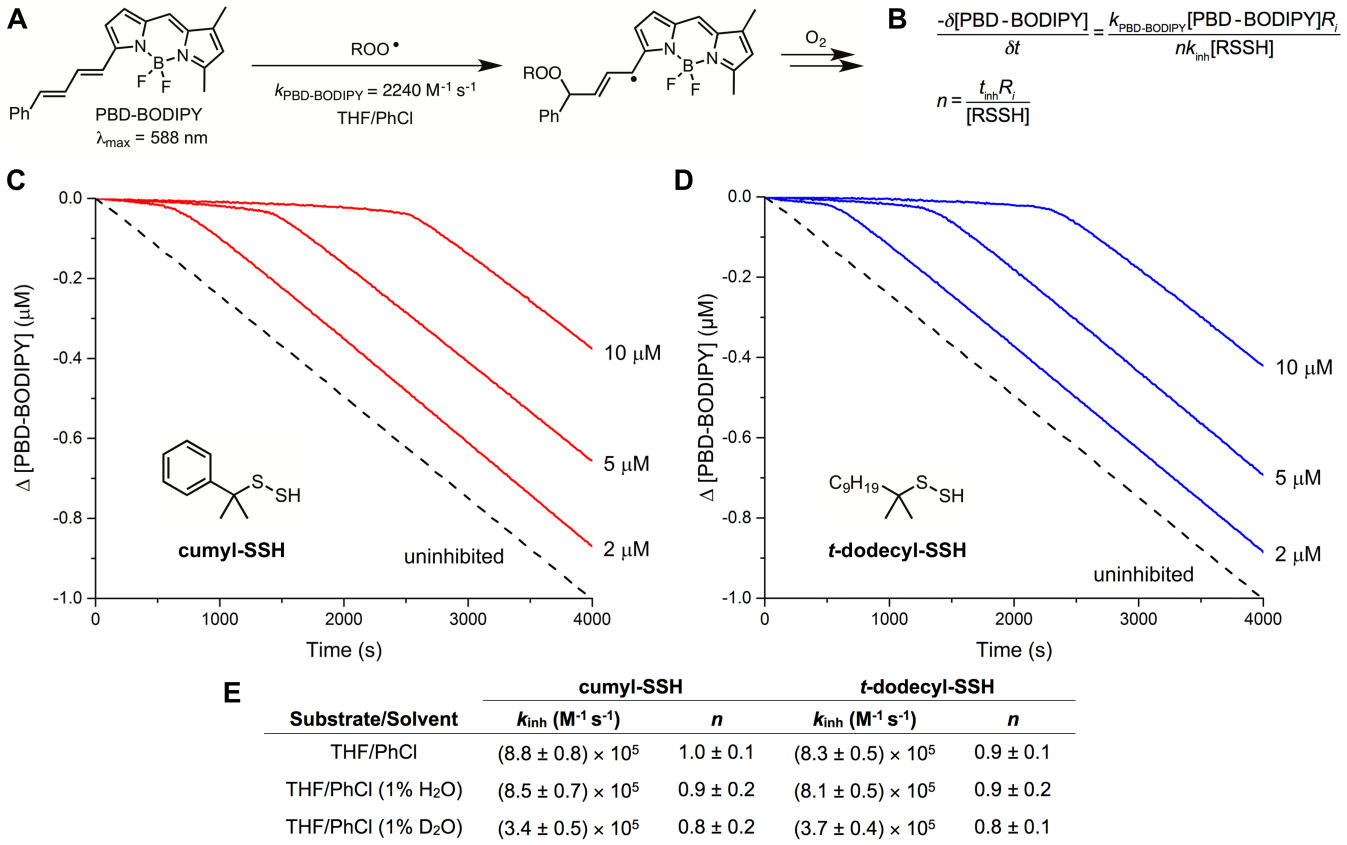
Considerable interest in hydropersulfides (RSSH) has emerged in recent years owing to their identification at significant levels in mammalian tissues.[1-3] They, and other polysulfide (RS_nR') species, have been proposed to be responsible for the cell signaling and/or redox modulatory effects of H_2S,[1,4] which is produced by cystathionine β-synthase (CBS),[5,6] cystathionine γ-lyase (CSE)[7] and 3-mercaptopyruvate sulfurtransferase (MST)[8,9] in response to various stimuli. Indeed, multiple biological functions have been ascribed to H_2S, including circulatory regulation,[10,11] energy production,[12] apoptotic gene regulation,[13] and protection from oxidative stress.[14-16]

It is widely believed that the various functions of H_2S are mediated by RSSH, which arise from its reaction with disulfides or other sulfur electrophiles.[9,17,18] Protein persulfides have been detected using various chemical probes such as cycloalkynes and iodoacetates.[2,3,19-23] Nevertheless, the chemical basis of the putative physiological effects of RSSH is unclear.[24,25] Recent work by Fukuto and co-workers[26] has provided insight into the ionic reactivity of RSSH toward oxidants and electrophiles, revealing that these are more reactive than the thiols from which RSSH are derived. This provides some support for the suggestion that H_2S is produced to augment the abilities of thiols to sense oxidants and electrophiles. Given these observations, it figures that the homolytic reactivity of RSSH may also be enhanced relative to that of thiols.

Thiols are well known to be useful H-atom donors in synthetic contexts, perhaps most prominently in the thiol-ene reaction and as polarity-reversal catalysts.[27] There is a widespread misconception that thiols are also useful as radical-trapping antioxidants (RTAs), which react with autoxidation chain-carrying peroxyl radicals by formal H-atom transfer (HAT). In fact, thiols react slowly with peroxyl radicals ($<10^3$ M^{-1} s^{-1})[28] relative to good RTAs, such as α-tocopherol (α-TOH), the most biologically active form of Vitamin E ($k > 10^6$ M^{-1} s^{-1}).[29] On the other hand, despite their apparently weak S-H BDEs (estimated to be ca. 70 kcal/mol – compare to thiols at 87 kcal/mol),[30,31] little is known of the H-atom transfer (HAT) reactivity of RSSH.[32] Herein we describe the results of our efforts to fill this void, and present data for the reactivity of RSSH toward a variety of oxidants, including peroxyl (ROO•), alkoxyl (RO•), alkyl (R•) and thiyl (RS•) radicals. In line with the trends in their ionic reactivity, we find the HAT reactivity of RSSH to be analogous – but greatly accelerated – relative to thiols.

■ RESULTS

Cumylhydropersulfide (cumyl-SSH) and 2-methylundecan-2-persulfide (*t*-dodecyl-SSH) were chosen for study owing to the greater persistence of hydropersulfides adjacent to tertiary centres, which slow their self-reaction to trisulfide and H_2S.[33] Both compounds (Figure 1) were synthesized by acidic methanolysis of the corresponding *S*-acetylalkyldisulfide, which was synthesized from the corresponding thiol and acetylsulfenyl chloride.[33]



E

	cumyl-SSH		*t*-dodecyl-SSH	
Substrate/Solvent	k_{inh} (M^{-1} s^{-1})	***n***	k_{inh} (M^{-1} s^{-1})	***n***
THF/PhCl	(8.8 ± 0.8) × 10^5	1.0 ± 0.1	(8.3 ± 0.5) × 10^5	0.9 ± 0.1
THF/PhCl (1% H_2O)	(8.5 ± 0.7) × 10^5	0.9 ± 0.2	(8.1 ± 0.5) × 10^5	0.9 ± 0.2
THF/PhCl (1% D_2O)	(3.4 ± 0.5) × 10^5	0.8 ± 0.2	(3.7 ± 0.4) × 10^5	0.8 ± 0.1

Figure 1. PBD-BODIPY serves as the signal carrier in THF autoxidations **(A)**, enabling determination of rate constants (k_{inh}) and reaction stoichiometries (*n*) for reactions of inhibitors with chain-carrying peroxyl radicals **(B)**. Co-autoxidations of THF (3.1 M) and PBD-BODIPY (10 μM) initiated by AIBN (6 mM) in chlorobenzene at 37 °C (dashed black trace) and inhibited by 2 μM, 5 μM and 10 μM of cumyl-SSH **(C)** and *t*-dodecyl-SSH **(D)**. Average inhibition rate constants and stoichiometry summarized in **(E)**. Reaction progress was monitored by absorbance at 588 nm (ε = 128,100 M^{-1} cm^{-1}).

Hydropersulfides Are Excellent Radical-Trapping Antioxidants. The reactivity of the hydropersulfides toward peroxyl radicals was determined using the venerable inhibited autoxidation approach.[34] The addition of PBD-BODIPY as co-substrate enables reaction monitoring simply by loss of the absorbance at 588 nm due to the addition of peroxyl radicals to the 1-phenylbutadiene moiety (Figure 1A).[35] THF is present in order to maintain a radical chain reaction, ensuring a steady-state concentration of peroxyl radicals and enabling derivation of the rate constant for the reaction of the hydropersulfide with peroxyl radicals (k_{inh}) from the initial rate of the inhibited reaction (Figure 1B). The stoichiometry (*n*) of the RTA-peroxyl reaction is determined from the duration of the inhibited period (t_{inh}, also in Figure 1B).[35] Representative results are shown for cumyl-SSH and *t*-dodecyl-SSH in Figures 1C and 1D, respectively.

The traces in Figures 1C and 1D clearly indicate that hydropersulfides are excellent inhibitors of autoxidation. The inhibition rate constants derived from the initial rates in the presence of each compound are essentially indistinguishable, with k_{inh} = (8.0 ± 0.8) × 10^5 M^{-1} s^{-1} and (8.3 ± 0.5) × 10^5 M^{-1} s^{-1} for cumyl-SSH and *t*-dodecyl-SSH, respectively. For comparison, α-TOH is characterized by k_{inh} = 7.1 × 10^5 M^{-1} s^{-1} under the exact same conditions (see Supporting Information for the data). The observed inhibition periods correspond to stoichiometries of *n* = 1.0 ± 0.1 and *n* = 0.9 ± 0.1 for the reactions of cumyl-SSH and *t*-dodecyl-SSH, respectively, suggesting that following H-atom transfer to peroxyl radicals, the resultant perthiyl radicals do not contribute to either chain-propagating or chain-breaking events. Indeed, we have previously shown that perthiyl radicals combine to give tetrasulfides with $k = 6 \times 10^9$ M^{-1} s^{-1}, and tetrasulfides are unreactive to peroxyl radicals under these conditions.[36]

In order to provide direct evidence that the reaction between the hydropersulfides and peroxyl radicals is a HAT process, corresponding inhibited autoxidations were carried out in the presence of 1% H_2O or 1% D_2O. Whilst the kinetic data in the presence of water were indistinguishable from those obtained in its absence, in the presence of D_2O the rate constants were 2.2 to 2.5-fold lower. This primary kinetic isotope effect suggests that an (exchangeable) H-atom is transferred in the reaction of the hydropersulfides with peroxyl radicals. The relatively small kinetic isotope effects are consistent with the significant exergonicity of the reaction (ca. 16 kcal/mol) and suggest a relatively early transition state (corroborated by computation, *vide infra*).

H-Atom Transfer from Hydropersulfides is Relatively Insensitive to H-Bonding Interactions. The kinetics of HAT reactions are known to vary systematically as a function of the H-bond donating ability of the H-atom donor and the H-bond accepting ability of the solvent according to the scheme in Figure 2A and associated kinetics in Figure 2B.[37] Given that thiols are poor H-bond donors whose HAT kinetics are insensitive to changes in reaction medium,[38] we wondered if hydropersulfides would be

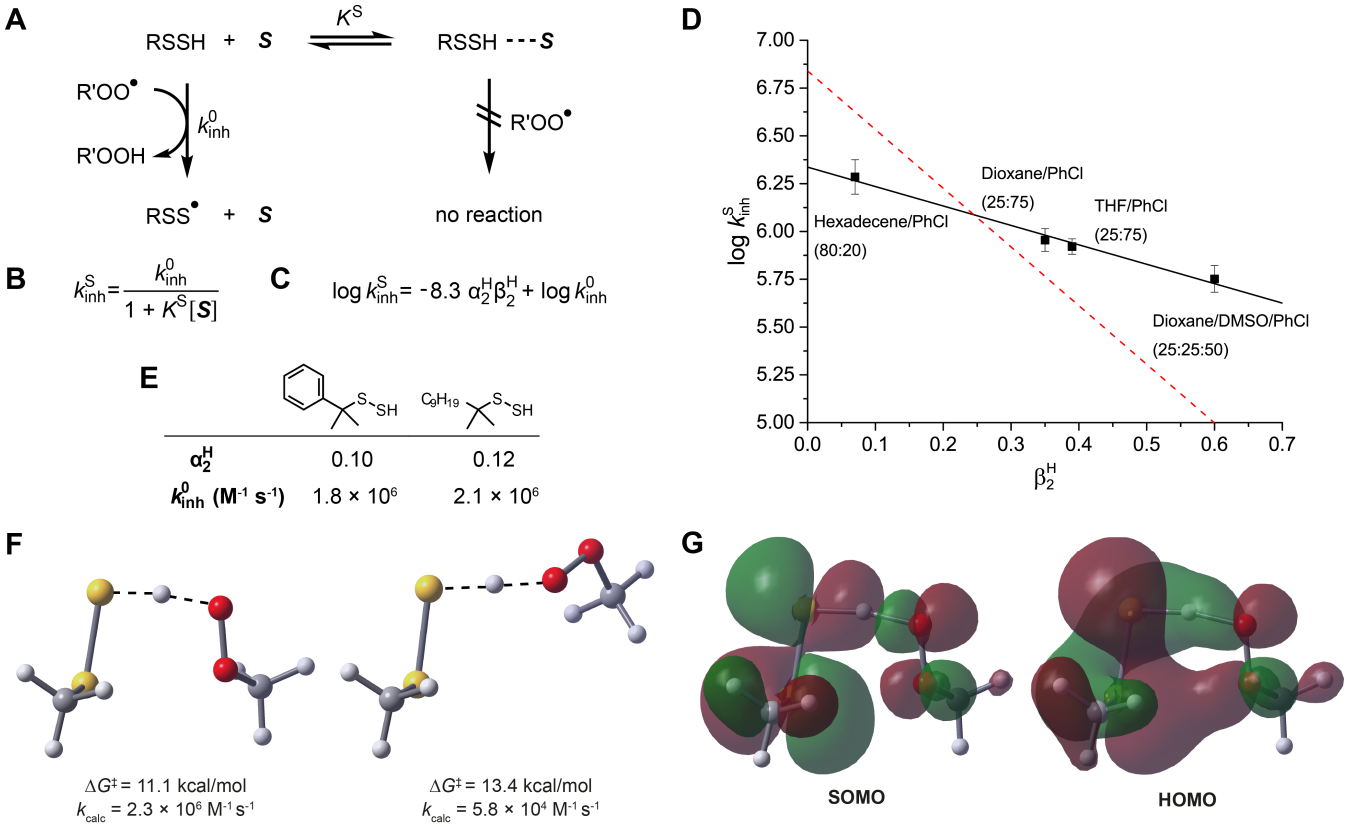


Figure 2. The kinetics of H-atom transfer reactions are slowed by H-bonding interactions with solvent, illustrated for hydropersulfides in (**A**), which can be quantified according to the expression in (**B**). The H-bonding equilibrium can be expressed in terms of Abraham's H-bond acidity and basicity parameters α_2^H and β_2^H as in (**C**). The inhibition rate constants for *t*-dodecyl-SSH, determined as in Figure 1 (see Supporting Information for the raw data) plotted as a function of medium β_2^H; the corresponding relationship for α-TOH is represented by the dashed red line (**D**). The H-bond acidity parameters for cumyl-SSH and *t*-dodecyl-SSH determined from the line of best fit of the data in (D) fit to the expression in (C), and estimated rate constants for the reaction in the absence of any H-bonding interactions (**E**). The calculated (CBS-QB3) transition state structures for HAT between a model hydropersulfide (MeSSH) and model peroxyl radical (MeOO•) and associated free energy barriers and rate constants estimated using transition state theory (**F**). The SOMO and HOMO of the *syn* transition state structure (**G**).

similarly poor H-bond donors with corresponding medium-independent HAT kinetics.

Using Abraham's NMR method,[39] we determined the H-bond acidities (on the α_2^H scale) of cumyl-SSH and *t*-dodecyl-SSH to be 0.10 and 0.09, respectively – slightly larger than those determined for the corresponding thiols (0.02 and 0.08, respectively) determined under identical conditions. For comparison, alcohols have α_2^H values that range from 0.4 to 0.8.[40] Even a somewhat hindered and electron rich phenol such as α-TOH has an α_2^H value of 0.37,[41] and since there is a logarithmic relationship between this parameter and the HAT kinetics (Figure 2C), its k_{inh} varies considerably with the H-bond accepting basicity of the solvent. For example, on going from pure chlorobenzene (β_2^H = 0.09) to 3:1 chlorobenzene:THF (β_2^H = 0.39), k_{inh} drops from 3.6 × 10^6 M^{-1} s^{-1} to 7.1 × 10^5 M^{-1} s^{-1}. In sharp contrast, and consistent with the values of α_2^H for cumyl-SSH and *t*-dodecyl-SSH we determined by NMR, autoxidations inhibited by the hydropersulfides were much less affected by changes in medium, as shown for *t*-dodecyl-SSH in Figure 2D. The slope of the correlation between logk_{inh} and the β_2^H of the media yielded an α_2^H value of 0.12 for *t*-dodecyl-SSH – in excellent agreement with the NMR data. Thus, while α-TOH is a better radical-trapping antioxidant than the hydropersulfides in simple hydrocarbons, the trend is reversed once H-bond acceptors are introduced to the medium.

Previous work by our group has revealed the importance of secondary orbital interactions in reactions between H-atom donors and peroxyl radicals. CBS-QB3 calculations indicate that this contributes to the reactivity of hydropersulfides as well. As is the case with phenols,[42] diphenylamines,[43] and the more structurally-related sulfenic (RSOH)[44] and selenenic acids (RSeOH)[45], the lowest energy transition state (TS) structure for the reaction of hydropersulfides with peroxyl radicals is characterized by a *syn* relationship between the substituents on the atoms between which the H-atom is being transferred (Figure 2F). This maximizes overlap between the singly-occupied π* of the peroxyl radical and the lone-pair of the 'outer' sulfur atom of the hydropersulfide, as illustrated in Figure 2G. The free energy barrier calculated as this level of theory is 11.1 kcal/mol, which corresponds to a rate constant of k = 2.3 × 10^6 M^{-1} s^{-1} at 37 °C upon application of TS theory. This value is in excellent agreement with the experimental values derived in the absence of H-bonding (k^0 = 1.8-2.1 × 10^6 M^{-1} s^{-1} at 37 °C). The barrier to reaction via the *anti* TS structure is 2.3 kcal/mol higher. The TS is expectedly early (consistent with the KIEs, *vide supra*), with little S-H bond breakage

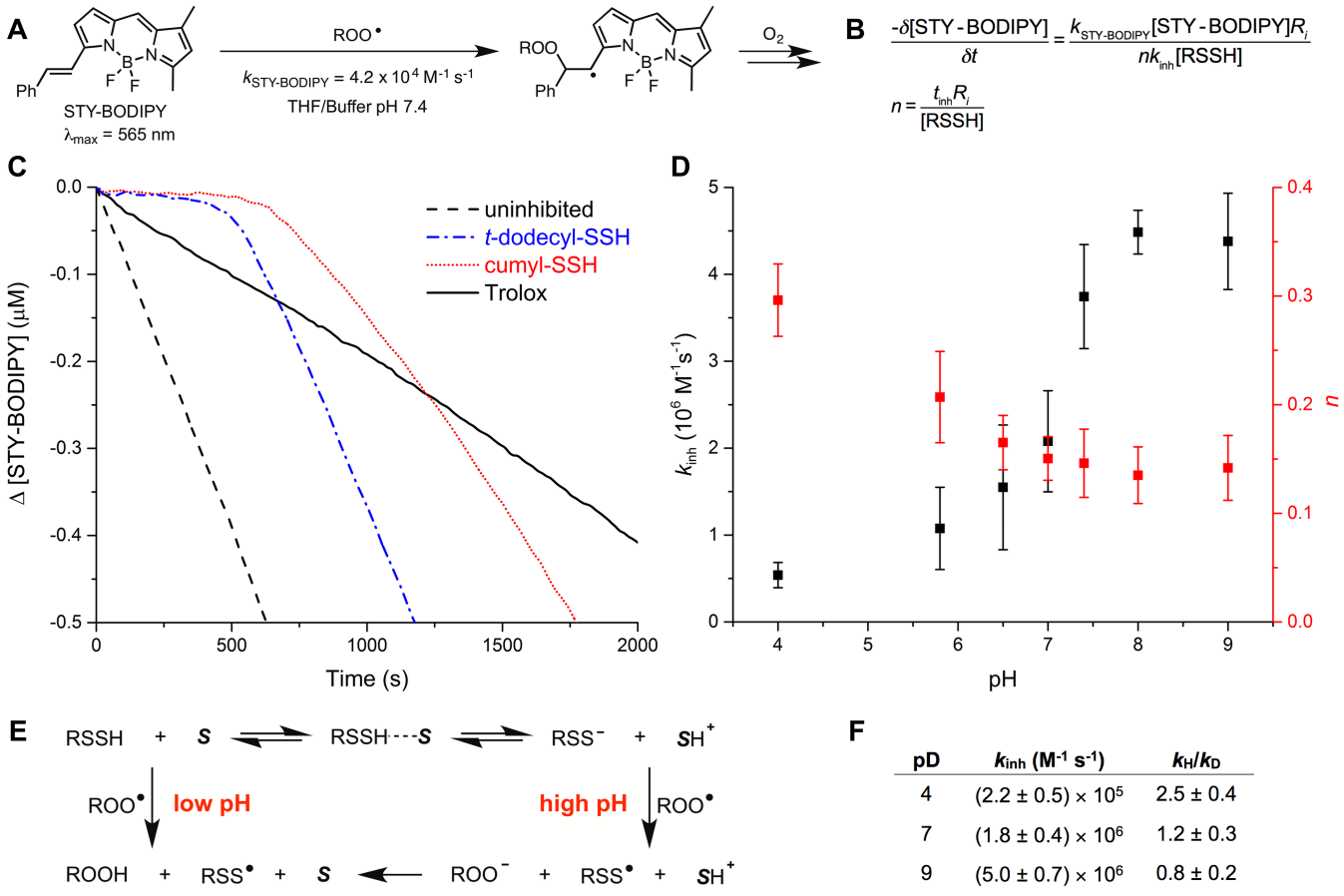


Figure 3. STY-BODIPY serves as the signal carrier in THF autoxidations (**A**), enabling determination of rate constants (k_{inh}) and reaction stoichiometries (n) for reactions of inhibitors with chain-carrying peroxyl radicals (**B**). Co-autoxidations of THF (3.1 M) and STY-BODIPY (10 μM) initiated by AAPH (1 mM) in phosphate-buffered saline (pH 7.4, 100 mM) at 37 °C (dashed black trace) and inhibited by 10 μM of cumyl-SSH (red), *t*-dodecyl-SSH (blue) and 4 μM of Trolox (black) (**C**). Inhibition rate constants and reaction stoichiometries determined as a function of pH (**D**). Reaction scheme illustrating the change in mechanism of the hydropersulfide/peroxyl radical reaction from H-atom transfer to sequential proton loss electron transfer (SPLET) as a function of pH (**E**). Inhibition rate constants determined as a function of pD and corresponding deuterium kinetic isotope effects (**F**).

(1.472 Å compared to 1.349 Å in the starting hydropersulfide and little O-H bond formation (1.402 Å compared to 0.967 Å in the product hydroperoxide). This is consistent with the significant thermodynamic driving force (ΔG° = –15.4 kcal/mol) owing to the difference in the MeSS-H and MeOO-H bond dissociation enthalpies (BDEs), which were calculated to be 70.8 and 86.2 kcal/mol, respectively.

Hydropersulfides React with Peroxyl Radicals via Two Distinct Mechanisms in Water. The HAT reactivity of the hydropersulfides was also investigated in aqueous solution. THF has been shown to be a useful autoxidizable substrate for monitoring peroxyl radical reactions in buffered solutions (pH 2-12),[46] and we have shown that STY-BODIPY, the more slowly oxidized analogue of PBD-BODIPY, is an effective signal carrier for monitoring reaction progress under these conditions (Figure 3A,B).[35] Representative results from co-autoxidations of THF and STY-BODIPY carried out at pH 7.4 are shown in Figure 3C.

Following the trend established in the previous section, the hydropersulfides were significantly more reactive to peroxyl radicals than α-TOH – or at least Trolox, a water-soluble version of α-TOH in which the phytyl sidechain has been replaced with a carboxylic acid. The inhibition rate constants determined for cumyl-SSH and *t*-dodecyl-SSH were 3.7 × 10^6^ and 3.2 × 10^6^ M^{-1} s^{-1}, respectively – almost an order of magnitude larger than that determined for Trolox (4.1 × 10^5^ M^{-1} s^{-1}).[46] Despite this exciting reactivity, the hydropersulfides suffer from poor stability in water, which facilitates their self-reactions (to mixtures of polysulfides and thiols). As such, the inhibition periods were much shorter than those observed in organic solution, corresponding to much smaller reaction stoichiometries of n = 0.15 ± 0.03 and 0.12 ± 0.05 for cumyl-SSH and t-dodecyl-SSH, respectively, as opposed to n ~ 1 as above.

Since cumyl-SSH has slightly better stability in aqueous solution than *t*-dodecyl-SSH, we expanded our studies with it to include inhibited autoxidations in buffers of varying pH ranging from 4 to 9. Interestingly, not only were the k_{inh} values dependent on pH, but a sigmoidal relationship was observed (Figure 3D). The reaction stoichiometries were also pH-dependent, increasing at lower pH presumably due to a slowing of their self-reactions, which are known to be faster at basic pH.[33] The sigmoidal curve was fit using a Boltzmann function, yielding a pK_a value of 7.0 corresponding to the inflection point at pH = 7, strikingly similar to pK_as estimated for hydropersulfides.[47,48]

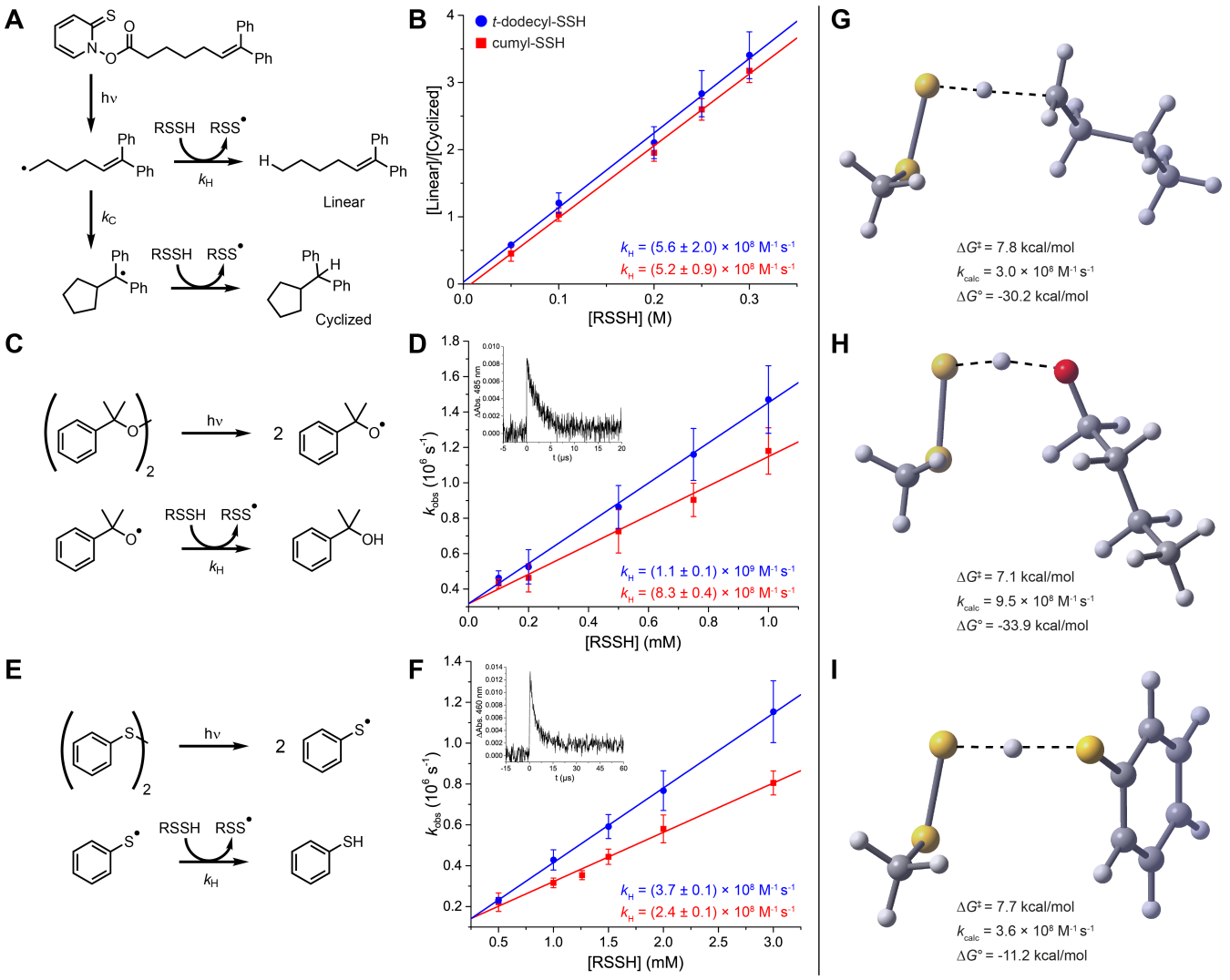


Figure 4. Photolytic generation of the 6,6-diphenylhexenyl radical and its use as a radical clock to obtain the rate constant for HAT from hydropersulfides to alkyl radicals (**A**). Ratio of linear and cyclized products as a function of the concentration of cumyl-SSH (red) or *t*-dodecyl-SSH (blue) during photocleavage of the radical precursor in benzene at 25 °C (**B**). Photolytic generation of cumyloxyl radicals from dicumylperoxide (**C**). Dependence of the pseudo-first order rate on the concentration of cumyl-SSH (red) or *t*-dodecyl-SSH (blue) upon photolysis of dicumylperoxide in benzene at 25 °C; inset: example decay of the cumyloxyl radical (**D**). Photolytic generation of phenylthiyl radicals from diphenyldisulfide (**E**). Dependence of the pseudo-first order rate on the concentration of cumyl-SSH (red) or *t*-dodecyl-SSH (blue) upon photolysis of diphenyldisulfide in benzene at 25 °C; inset: example decay of the phenylthiyl radical (**F**). Calculated (CBS-QB3) transition state structures and associated free energy barriers, estimated rate constants and reaction free energies for HAT between a model hydropersulfide (MeSSH) and a model alkyl radical (*n*-Bu•) (**G**), a model alkoxyl radical (*n*-BuO•) (**H**), and phenylthiyl radical (**I**).

Accordingly, we wondered if the pH dependence of k_{inh} could be attributed to a change in mechanism: as the pH of the medium increases and the concentration of the persulfide anion increases, the reactions takes place by electron transfer as opposed to the HAT that takes place from the hydropersulfide at lower pH (Figure 3E). This mechanism is sometimes referred to as sequential proton loss electron transfer or SPLET.[37] To provide additional insight on this apparent change in mechanism, deuterium kinetic isotope effects were determined at acidic pH (4), neutral pH (7) and basic pH (9) by running the inhibited autoxidations in buffers made in D_2O as opposed to H_2O (Figure 3F). In the event, the DKIE decreased from 2.5 at pH 4 (similar to the value obtained in chlorobenzene, *vide supra*) to 1.8 at pH 7, and then vanished at pH 9 (0.8 ± 0.2). These trends are fully consistent with an increasing contribution of the SPLET mechanism in the reaction of the hydropersulfide with peroxyl radicals on going from pH 4 to 7 and this mechanism operating exclusively at basic pH.

Hydropersulfides Have Enhanced Reactivity to Alkyl, Alkoxyl and Thiyl Radicals Compared to Thiols. The H-atom transfer chemistry of hydropersulfides was further investigated with alkyl, alkoxyl and thiyl radicals. Alkyl radicals were considered first, as they are known to react very quickly with thiols ($k \sim 10^6$ M^{-1} s^{-1}

^{1})[38,49] in one of the two propagating steps in the thiol-ene reaction. To do so, a classic alkyl radical clock approach was employed which utilized the 5-exo *trig* cyclization of the 6,6-diphenylhexenyl radical (k_c = 5.0 × 10^7 s^{-1}) as the reference reaction (Figure 4A).[50] The PTOC ester precursor to this radical was synthesized as described by Newcomb,[50,51] who had used it to 'clock' the reactions of selenols with alkyl radicals. Similarly, we photolyzed the ester (at 25 °C in benzene) and determined the ratio of the linear and cyclized hydrocarbons (by GC) as a function of hydropersulfide concentration in order to obtain the second order rate constant for trapping the primary alkyl radical (Figure 4B) using Eq. (1):

$$\frac{[\text{Linear}]}{[\text{Cyclized}]} = \frac{k_H}{k_C}[\text{RSSH}] \quad (1)$$

The reactions of the two hydropersulfides yielded essentially indistinguishable rate constants, *viz.* k_H = (5.2 ± 0.9) × 10^8 and (5.6 ± 2.0) × 10^8 M^{-1} s^{-1}, for cumyl-SSH and *t*-dodecyl-SSH, respectively. Corresponding calculations of the HAT transition state (Figure 4G) yielded a free energy barrier of 7.8 kcal/mol, which corresponds to a rate constant of 3.0 × 10^8 M^{-1} s^{-1}, in excellent agreement with experiment. Once again, the driving force for HAT is considerable (ΔG^0 = −30.2 kcal/mol), as a result of the weak RSS-H bond.

H-atom transfer kinetics to alkoxyl radicals were determined by transient absorption spectroscopy. Cumyloxyl radicals were generated by photolysis of dicumylperoxide (Figure 4C) with the 308 nm emission of a nanosecond-pulsed XeCl excimer laser and the rates of decay of their absorbance at 485 nm were determined as a function of hydropersulfide concentration (Figure 4D). The rate constants were expectedly greater than those measured above, at (8.3 ± 0.4) × 10^8 and (1.1 ± 0.1) × 10^9 M^{-1} s^{-1} for cumyl-SSH and *t*-dodecyl-SSH, respectively, consistent with the greater driving force for the H-atom transfer of −33.9 kcal/mol (calculated by CBS-QB3, Figure 4H). Again, computation predicts a barrier to reaction ($\Delta G^{\ddagger}$ = 7.1 kcal/mol) that yields a calculated rate constant in excellent agreement with experiment (9.5 × 10^8 M^{-1} s^{-1}). These rate constants are all significantly faster than those reported for the reactions of thiols with tertiary alkoxyl radicals (e.g. 6.5 × 10^7 M^{-1} s^{-1} for t-BuO• + *n*-C_6H_{13}SH).[52]

Transient absorption spectroscopy was also used to obtain kinetics for the reaction of the hydropersulfides with the triplet state of benzophenone (corresponding data can be found in the Supporting Information). We expected similar results to those obtained with cumyloxyl radicals since the $\boldsymbol{n} \rightarrow \boldsymbol{\pi}^*$ state of the carbonyl has an effectively unpaired electron on the oxygen atom. As for the reaction with cumyloxyl radicals, the experiment was performed under pseudo-first order conditions and the benzophenone triplet state monitored at 530 nm following photolysis at 308 nm. The resulting rate constants were determined to be (1.1 ± 0.1) × 10^9 and (1.5 ± 0.1) × 10^9 M^{-1}s^{-1} for cumyl-SSH and *t*-dodecyl-SSH, respectively. Once again, the results of CBS-QB3 calculations are in excellent agreement with experiment, predicting a slightly faster reaction than with alkoxyl radicals (k = 1.3 × 10^9 M^{-1} s^{-1}, $\Delta G^{\ddagger}$ = 6.9 kcal/mol) and a driving force of ΔG^0 = −36.5 kcal/mol. For comparison, thiols are reported to react with the benzophenone triplet much more slowly (e.g. 8.8 × 10^6 M^{-1} s^{-1} for *n*-C_5H_{12}SH).[53]

HAT between thiols and thiyl radicals, despite being a thermoneutral process, is a fast reaction. Literature reports offer rate constants that span 10^4 to 10^7 M^{-1} s^{-1}, and our own CBS-QB3 calculations suggest a rate constant of 2.2 × l0^7 M^{-1} s^{-1} for the reaction between *n*-butylthiyl radical and n-butylthiol.[54] Thus, the analogous reaction between hydropersulfides and thiyl radicals, which would have a considerable driving force, could be diffusion-controlled. Despite the fact that thiyl radicals are quite easily generated (by photolysis of disulfides), they are challenging species to follow by optical spectroscopy due to their lack of absorbance > 300 nm (aromatic thiyls being the exception, *vide infra*). Fortunately, the perthiyl radical has an absorbance at 375 nm – enabling monitoring of the reaction by growth of this signal rather than consumption of the thiyl. Regardless, when *n*-butyl disulfide was photolyzed in the presence of cumyl-SSH or *t*-dodecyl-SSH, we were unable to fully resolve the growth of the corresponding perthiyl radical. Considering the experimental conditions and the time resolution of our instrument, we estimate a lower-bounds for the rate constant of 1 × 10^{10} M^{-1} s^{-1}. Efforts to compute a rate constant using the same CBS-QB3 approach that has yielded highly accurate predictions with peroxyl, alkyl and alkoxyl radicals gave inconsistent results. Initially, when MeSSH was used as a simplified model, a predicted rate constant of 4.2 × 10^9 M^{-1} s^{-1} was obtained, but the larger *t*-BuSSH yielded k_H = 1.2 × 10^{10} M^{-1} s^{-1} ($\Delta G^{\ddagger}$ = 5.6 kcal/mol). The origins of this difference are unclear. Nevertheless, it seems reasonable to suggest that these reactions are indeed diffusion-controlled.

Phenyldisulfide was also used as a thiyl radical precursor (Figure 4E). We reasoned that the lower S-H BDE in thiophenol (83 kcal/mol)[55] would render the reaction of phenylthiyl radicals with the hydropersulfides less exergonic and this may afford the opportunity to measure its kinetics. Moreover, the phenylthiyl radical is characterized by a broad absorption band centered around 460 nm such that its decay in the presence of hydropersulfides could be followed directly by LFP (Figure 4F). Indeed, the rate constants for HAT could be determined as (2.4 ± 0.1) × 10^8 and (3.7 ± 0.1) × 10^8 M^{-1} s^{-1} for cumyl-SSH and *t*-dodecyl-SSH, respectively. (Similar data were obtained for reactions of the hydropersulfides with 2-pyridinethiyl radicals, see the Supporting Information for the data). In this case it is difficult to compare these results to the kinetics of the corresponding reaction of a thiol since it would be endothermic. Interestingly, the calculated transition state structure for the reaction between the model hydropersulfide (MeSSH) and phenylthiyl radical (Figure 4I) suggests some interaction between the SOMO of the phenylthiyl radical and the HOMO of the hydropersulfide – a secondary orbital interaction not unlike that seen in the reaction with peroxyl radicals (*cf.* Figure 2G). The associated free energy barrier is computed to be $\Delta G^{\ddagger}$ = 7.7 kcal/mol, equivalent to a rate constant of 3.6 × 10^8 M^{-1} s^{-1}, in excellent agreement with experiment. Expectedly, the driving force for HAT from the hydropersulfide to phenylthiyl (ΔG^0 = −11.2 kcal/mol) is predicted to be smaller than to *n*-butylthiyl (ΔG^0 = −15.4 kcal/mol).

■ DISCUSSION

Thiols are often described as 'antioxidants', but this can be misleading. Their ionic reactions with H_2O_2 and hydroperoxides are not particularly fast (k ~ 1 M^{-1} s^{-1} at neutral pH),[56] so they are used

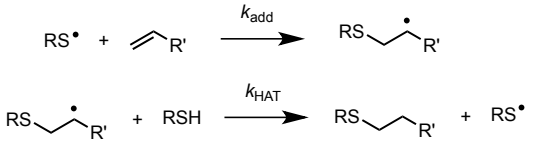
instead as reducing co-factors for enzymes (e.g. glutathione peroxidases) that catalyze reactions with these oxidants with kinetics that are greater by several orders of magnitude. Likewise, HAT from thiols to peroxyl radicals, the key chain-carrying radicals in lipid peroxidation, are quite slow ($<10^3$ M^{-1} s^{-1}),[28,57] making it impossible for them to compete with propagation of the peroxidation chain reaction. In a biological context, this task is largely the responsibility of α-tocopherol (α-TOH), owing to its much greater reactivity to peroxyl radicals ($> 10^6$ M^{-1} s^{-1}).[29]

According to the Evans-Polanyi principle and estimates of the RSS-H bond strength (70 kcal/mol),[30] hydropersulfides should undergo HAT reactions more readily than thiols (87 kcal/mol).[31] The foregoing results establish that this is indeed true for HAT to peroxyl radicals as well as alkyl, alkoxyl and thiyl radicals. The inherent reactivity of hydropersulfides to peroxyl radicals is particularly striking because it is 4 orders of magnitude greater than for thiols, and essentially the same as that of α-TOH, long considered the gold-standard against which other radical-trapping antioxidants are measured.[58,59] In fact, upon transitioning from hydrocarbons to media containing H-bond accepting functionality, hydropersulfides become superior to α-TOH, owing to the fact that they do not engage in strong H-bonds. The weak H-bond donating acidities of hydropersulfides ($\alpha_2^H \sim 0.1$) make them ideal HAT agents to peroxyl radicals, as all other classes that have similarly high reactivity ($k_{inh} > 10^6$ M^{-1} s^{-1}), which include some phenols,[42] diarylamines,[60] sulfenic acids[45,61] and hydroxylamines[62] are characterized by significantly higher H-bond acidities (generally $\alpha_2^H \geq 0.4$). Moreover, the perthiyl radicals that arise upon HAT from hydropersulfides persist until they encounter another perthiyl radical with which they form tetrasulfides rather than carry on the oxidation via reactions with either O_2 or the substrate. These results suggest that unlike thiols, hydropersulfides, produced when cells synthesize H_2S, prevent lipid peroxidation.

In order to operate effectively in this capacity, the disulfides or other electrophilic thiol derivatives from which the hydropersulfides are produced should be relatively lipophilic. This ensures they have access to lipid regions where they are more likely to encounter lipid-derived peroxyl radicals, and because the hydropersulfides are much less effective as H-atom donors in aqueous media. It is important to make the distinction between *effective* and *reactive*, since the hydropersulfides are highly reactive to peroxyl radicals in aqueous solution. In fact, over a range of pH from 4 to 10, k_{inh} varied from 0.5 to 4.4 × 10^6 M^{-1} s^{-1} – greatly exceeding the corresponding rate constants obtained with Trolox, a water-soluble derivative of α-TOH (k = 4.1 × 10^5 M^{-1} s^{-1}), which varies little in the same pH range (k = 2.1 × 10^5 M^{-1} s^{-1} and k = 6.8 × 10^5 M^{-1} s^{-1} at pH 4 and 9, respectively).[46] The pH dependence of hydropersulfide reactivity results from the increasing contribution of a faster electron transfer reaction between the persulfide anion and a peroxyl radical as the pH approaches – and then exceeds – the estimated pK_a of the hydropersulfide (ca. 7).[47,48] It is noteworthy that the position of the inflection point in the sigmoidal relationship between the inhibition rate constant and the pH nicely corroborate estimated pK_a values reported in the literature.

The difficulty associated with measuring a reliable pK_a value for hydropersulfides underlies why they are relatively ineffective radical-trapping antioxidants in aqueous solutions: they decompose too readily. As such, where the hydropersulfides each cleanly trap one peroxyl radical by HAT in organic solution, the reaction stoichiometry decreases sharply in aqueous solution (0.3 at pH 4) and is further eroded with increasing pH where decomposition is accelerated.[33] Hydropersulfides are known to decompose to yield mixtures of polysulfides and thiols,[33] which are unreactive to peroxyl radicals.[36]

In contrast to the insignificant role of HAT reactions between thiols and peroxyl radicals, thiols are important reagents in a variety of alkyl radical-mediated reactions, perhaps most importantly in the thiol-ene reaction (Scheme 1).



Scheme 1. The radical chain propagation steps of a generalized thiol-ene reaction.

A corresponding hydropersulfide-ene reaction would, in principle, provide a route to unsymmetric disulfides. Although hydropersulfides are two orders of magnitude more reactive to (primary) alkyl radicals than thiols, and it is often the HAT step that is rate-controlling in the propagation sequence, it is likely that the perthiyl addition would be too thermodynamically unfavourable (CBS-QB3 predicts the reaction with styrene to have ΔG° = +9.4 kcal/mol). Indeed, while we have estimated that styrene reacts with *t*-butylperthiyl radicals generated by laser flash photolysis of *t*-BuSSSS*t*-Bu with k = 1.1 × 10^4 M^{-1} s^{-1} (see Supporting Information) this was only observed in the presence of O_2, which presumably trapped the intermediate benzylic radical precluding the reverse reaction (fragmentation).[63] This can be compared to the addition of thiyl radicals to styrene, which proceeds 200-fold faster, and is slightly exothermic (CBS-QB3 predicts the reaction to have ΔG° = −4.5 kcal/mol).[64] Although it seems unlikely that disulfides can be prepared via a hydropersulfide-ene reaction, hydropersulfides may yet find use in place of thiols for other transformations.

Our expectations for the greater HAT reactivity of hydropersulfides compared to thiols are based on the S-H BDE estimates that were made on the basis of Benson's group additivity scheme.[30,47,65] These estimates have been corroborated by the high accuracy quantum chemical calculations (CBS-QB3) we have made use of here, which suggest BDEs of 70.8, 70.4 and 70.4 kcal/mol in MeSSH, *t*-BuSSH and cumyl-SSH, respectively. Given the similarity of the hydropersulfide S-H BDE with that of the hydroxylamine derived from the persistent nitroxide TEMPO (69.6 kcal/mol),[66] we attempted experimental confirmation of a hydropersulfide S-H BDE by measuring the equilibrium constant for eq 2 by EPR – an approach used to obtain many X-H BDEs (making the reasonable assumption that $\Delta S \sim 0$ for the HAT process).[67,68]

$$\text{RSSH} + \text{TEMPO}\bullet \leftrightarrows \text{RSS}\bullet + \text{TEMPOH} \qquad (2)$$

Sadly, despite extensive efforts, we were unable to resolve a signal for the perthiyl radical. Expecting that this may be due to its rapid dimerization to the tetrasulfide (k = 6.0 × 10^9 M^{-1} s^{-1}),[69] we also carried out the reaction under continuous photolysis, but to no avail (see Supporting Information). It is important to note that Fukuto, Toscano and co-workers recently reported similar

results.[70] This led us to suspect that the nitroxide and perthiyl may react with each other. Indeed, perthiyl radicals generated by laser flash photolysis of solutions of di-*tert*-butyl tetrasulfide gave clean pseudo-first order decay kinetics in the presence of TEMPO that afforded a second order rate constant of (1.9 ± 0.1) × 10^8 M^{-1} s^{-1}. The formation of the perthiyl-TEMPO adduct is readily reversible since the central S-O bond is very weak (ΔG° = 6.0 kcal/mol, by CBS-QB3), but competitive N-O bond cleavage of the adduct (which requires only 4.2 kcal/mol more free energy, by CBS-QB3) leads to reactive products, precluding the reverse reaction. This would account for the inability to observe the perthiyl radical in the radical equilibration experiment.[71]

■ CONCLUSIONS

Hydropersulfides are excellent H-atom donors towards alkyl (k ~ 5 × 10^8 M^{-1} s^{-1}), alkoxyl (k ~ 1 ×10^9 $M^{-1}s^{-1}$), peroxyl (k ~ 2 × 10^6 M^{-1} s^{-1}) and thiyl (k > 1 × 10^{10} M^{-1} s^{-1}) radicals – besting thiols in all cases by at least one, and up to four, orders of magnitude. The inherently high reactivity of hydropersulfides to H-atom transfer is based largely on thermodynamic factors; the weak RSS-H BDE (~ 70 kcal/mol) and associated high stability of the perthiyl radical makes the foregoing reactions exothermic by 15 to 34 kcal/mol. Given the implication of hydropersulfides in redox biology, their (peroxyl) radical-trapping antioxidant activity may be the most relevant reactivity studied here. The favourable thermodynamics of the reaction are bolstered by secondary orbital interactions in the HAT transition state that enhance the kinetics. Furthermore, the persistence of the product perthiyl radicals – toward both O_2 and the substrate undergoing autoxidation – is key, as is their near-diffusion-controlled combination to give innocuous tetrasulfide products. A highly unique attribute of hydropersulfides as radical-trapping antioxidants is their low H-bond acidity, which makes their reactivity relatively insensitive to changes in solvent/medium. The reactivity of well-established radical-trapping antioxidants, such as phenols and diarylamines, are highly medium-dependent owing to strong H-bond interactions of the H-atom undergoing HAT. The radical-trapping antioxidant activity of hydropersulfides may be exploited by amphiphilic disulfides (thiols) in small molecules and/or proteins exposed to exogenous H_2S, which is known to readily transverse and accumulate in lipid bilayers.[72-74] Whether this reactivity could be exploited in the design of small molecule preventive and/or therapeutic agents for diseases wherein lipid peroxidation (ferroptosis)[75-77] has been implicated remains to be explored.

■ EXPERIMENTAL SECTION

General. Reagents were obtained from commercial suppliers and used as is, unless indicated otherwise. Column chromatography was carried out with 40-63 μm, 230-400 mesh silica gel. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE spectrometer at 400 MHz and 101 MHz, respectively, unless indicated otherwise. High-resolution mass spectra were obtained on a Kratos Concept Tandem mass spectrometer (EI) and Micromass Q-TOF (ESI). PBD-BODIPY and STY-BODIPY were synthesized following our previously reported procedure.[35] Chlorobenzene was dried over 3 Å molecular sieves before use. UV-visible spectra were measured with a Cary 100 spectrophotometer equipped with a thermostatted 6 x 6 multi-cell holder. pH measurements were carried out using an Accument Electrode referenced to Ag/AgCl. pD were measured by adding a correction factor of 0.4 to the pH readings (pD = pH_{read} + 0.4).[78] Unless otherwise indicated, the errors which are reported were obtained from the standard deviation of at least three experimental trials.

Synthetic Procedures. The syntheses of cumyl-SSH and *t*-dodecyl-SSH have been achieved following a similar approach to Pluth's reported methods.[33] For the complete synthetic procedure of the intermediates see supporting information.

Cumylhydropersulfide. Cumylacetyldisulfide (0.10 g, 0.44 mmol) was dissolved in methanol under nitrogen at 0 °C. In a separate flask, a 5 M methanolic solution of HCl was made by the addition of acetyl chloride (0.89 mL) to methanol (1.61 mL) at 0 °C. The HCl solution (0.30 mL, 1.50 mmol) was added dropwise to the cumylacetyldisulfide solution after which the flask was equipped with a nitrogen balloon directly on the septum, without the use of a needle to avoid any metal from making contact in the flask. The reaction was stirred overnight at room temperature and the solvent was evaporated by rotary evaporation with a water bath at room temperature to yield the product as a clear colorless oil (67 mg, 83%). ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.52-7.49 (m, 2H), 7.38-7.33 (m, 2H), 7.28-7.23 (m, 1H), 2.62 (s, 1H), 1.76 (s, 6H). ^{13}C NMR (101 MHz; $CDCl_3$): δ 144.4, 128.4, 127.2, 126.8, 51.3, 28.0. HRMS (ESI, [M-H$^+$]): m/z calc. for $C_9H_{11}S_2$: 183.0307, found 183.0302.

t-Dodecylhydropersulfide. *t*-Dodecylacetyldisulfide (0.10 g, 0.36 mmol) was dissolved in isopropanol under nitrogen at 0 °C. In a separate flask, a 5 M methalonic solution of HCl was made by the addition of acetyl chloride (0.89 mL) to methanol (1.61 mL) at 0 °C. The HCl solution (0.25 mL, 1.24 mmol) was added dropwise to the *t*-butyldodecylacetyldisulfide solution after which the flask was equipped with a nitrogen balloon directly on the septum, without the use of a needle to avoid any metal from making contact in the flask. The reaction was stirred overnight at room temperature and the solvent was evaporated by rotary evaporation with a water bath at room temperature to yield the product as a clear colorless oil (74 mg, 87%). ^{1}H NMR (300 MHz; $CDCl_3$): δ 2.63 (s, 1H), 1.57-1.52 (m, 2H), 1.37-1.32 (m, 3H), 1.27 (br s, 17H), 0.88 (t, J = 6.7 Hz, 3H). ^{13}C NMR (76 MHz; $CDCl_3$): δ 48.9, 40.5, 32.0, 30.2, 29.8, 29.5, 26.8, 24.8, 22.8, 14.3. HRMS (ESI, [M-H$^+$]): m/z calc. for $C_{12}H_{25}S_2$: 233.1398, found 233.1394.

General Procedure for Inhibited Autoxidations. Inhibited autoxidations were carried out following our recently published method.[35] Similar procedures were used for all autoxidations reported herein, the procedure for THF autoxidation in chlorobenzene is given as an example. For detailed autoxidation procedures of other substrates in various solvents, see supporting information. A 3.5 mL quartz cuvette was loaded with unstabilized THF (0.625 mL) along with PhCl (1.805 mL), such that the final reaction volume is 2.50 mL. The cuvette was then pre-heated in a thermostatted sample holder of a UV-vis spectrophotometer and allowed to equilibrate to 37 °C for approximately 15 minutes. A small volume (12.5 μL) of a 2.00 mM solution of the PBD-BODIPY probe in 1,2,4-trichlorobenzene was added, followed by 50 μL of a 300 mM solution of AIBN in PhCl. The solution was thoroughly mixed and the absorbance at 588 nm was monitored for 10 minutes to ensure that the reaction was proceeding at a constant rate, after which 10.0 μL of a solution of the test antioxidant was added. The solution was thoroughly mixed and the absorbance readings resumed. The resulting data

was processed as previously reported.[35] The rate of initiation (R_i = 3.2×10^{-9} M s^{-1}) and propagation rate constant for the dye ($k_{PBD\text{-}BODIPY}$ = 2240 M^{-1} s^{-1}) necessary to derive stoichiometric data and inhibition rate constants, were determined using PMC as a standard, which has an established stoichiometry of 2.[79]

Alkyl Radical Clock Kinetics. Alkyl radical clock kinetics were determined using a previously reported procedure by Newcomb.[50,51] The PTOC ester and the standards were synthesized according to the procedures described therein. Briefly, vials equipped with a septum and wrapped in aluminum foil were loaded with the appropriate amount of hydropersulfides (50-300 mM) and PTOC ester clock (20 mM) in THF (1 mL). The vials were thoroughly purged with nitrogen for 10 minutes before the aluminum foil was removed and the vials exposed to a high pressure sodium lamp (400 W) at a distance of 50 cm for 1 h. A 50 µL aliquot was taken from each vial and added to a GC vial containing 50 µL of a hexylbenzene standard solution (40 mM). The solution was diluted by adding 900 µL of benzene for a total volume of 1 mL per vial. The samples (4 µL splitless injections) were analyzed by GC-MS equipped with an Agilent HP-5 ms column (30 m x 0.25 mm x 0.25 µm) with a constant He flow of 1.2 mL min^{-1} using the following temperature profile (inlet temperature was set at 260 °C): 100 °C hold 3 min, 8 °C min^{-1} to 260 °C, hold 12 min. The method yielded retention times of 7.1, 15.9, and 16.7 min for hexylbenzene, linear and cyclized products, respectively.

Laser Flash Photolysis. Nanosecond transient absorption experiments were performed on an LFP-112 spectrometer (Luzchem, Canada). Irradiation source was an EX10 (GAM Laser, USA) XeCl Excimer laser (308 nm, ca. 10 mJ/pulse, ca. 12 ns pulse width). The transient absorption data were recorded in a quartz cuvette (1 cm x 1 cm) equipped with a septum. Sample concentrations in benzene/PhCl were adjusted to yield an absorbance of 0.2-0.3 at 308 nm and the solutions were bubbled with nitrogen (or oxygen) for 10 minutes before measurement. The rate constants for hydrogen abstraction (k_H) were determined in pseudo-first-order according to the following equation $k_{obs} = k_0 + k_H$[RSSH]. Each plotted data point is the average of 6-12 individual traces (corresponding standard deviation plotted as error bars).

Calculations. Calculations were carried out using the CBS-QB3[80] complete basis set method as implemented in the Gaussian 09[81] suite of programs. Rate constants were calculated via transition state theory at 25 °C, except for peroxyls (37 °C).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website. NMR spectra of new compounds, KIE experiments, details of LFP experiments, and optimized geometries and energies for computational results. (PDF)

■ AUTHOR INFORMATION

Corresponding Author

* dpratt@uottawa.ca

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada and the Canada Foundation for Innovation, and through generous access to the computational resources of the Centre for Advanced Computing (cac.queensu.ca). DAP and JPRC also acknowledge the support of the Canada Research Chairs and Ontario Graduate Scholarships programs, respectively. The authors thank Luke Farmer for assistance with the standardization of autoxidation kinetics in dioxane/PhCl and dioxane/DMSO/PhCl as well as the β_2^H values for hexadecene/PhCl, THF/PhCl, dioxane/PhCl and dioxane/DMSO/PhCl solvent systems.

■ REFERENCES

(1) Ida, T.; Sawa, T.; Ihara, H.; Tsuchiya, Y.; Watanabe, Y.; Kumagai, Y.; Suematsu, M.; Motohashi, H.; Fujii, S.; Matsunaga, T.; Yamamoto, M.; Ono, K.; Devarie-Baez, N. O.; Xian, M.; Fukuto, J. M.; Akaike, T. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111*, 7606.

(2) Dóka, É.; Pader, I.; Bíró, A.; Johansson, K.; Cheng, Q.; Ballagó, K.; Prigge, J. R.; Pastor-Flores, D.; Dick, T. P.; Schmidt, E. E.; Arnér, E. S. J.; Nagy, P. *Sci. Adv.* **2016**, *2*, e1500968.

(3) Longen, S.; Richter, F.; Köhler, Y.; Wittig, I.; Beck, K.-F.; Pfeilschifter, J. *Sci. Rep.* **2016**, *6*, 29808.

(4) Mustafa, A. K.; Gadalla, M. M.; Sen, N.; Kim, S.; Mu, W.; Gazi, S. K.; Barrow, R. K.; Yang, G.; Wang, R.; Snyder, S. H. *Sci. Signal.* **2009**, *2*, ra72.

(5) Abe, K.; Kimura, H. *J. Neurosci* **1996**, *16*, 1066.

(6) Chen, X.; Jhee, K.-H.; Kruger, W. D. *J. Biol. Chem.* **2004**, *279*, 52082.

(7) Swaroop, M.; Bradley, K.; Ohura, T.; Tahara, T.; Roper, M. D.; Rosenberg, L. E.; Kraus, J. P. *J. Biol. Chem.* **1992**, *267*, 11455.

(8) Shibuya, N.; Tanaka, M.; Yoshida, M. *Antioxid. Redox. Signal.* **2009**, *11*, 703.

(9) Paul, B. D.; Snyder, S. H. *Nat. Rev. Mol. Cell Biol.* **2012**, *13*, 449.

(10) Yang, G.; Wu, L.; Jiang, B.; Yang, W.; Qi, J.; Cao, K.; Meng, Q.; Mustafa, A. K.; Mu, W.; Zhang, S.; Snyder, S. H.; Wang, R. *Science* **2008**, *322*, 587.

(11) Morikawa, T.; Kajimura, M.; Nakamura, T.; Hishiki, T.; Nakanishi, T.; Yukutake, Y.; Nagahata, Y.; Ishikawa, M.; Hattori, K.; Takenouchi, T.; Takahashi, T.; Ishii, I.; Matsubara, K.; Kabe, Y.; Uchiyama, S.; Nagata, E.; Gadalla, M. M.; Snyder, S. H.; Suematsu, M. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 1293.

(12) Fu, M.; Zhang, W.; Wu, L.; Yang, G.; Li, H.; Wang, R. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 2943.

(13) Sen, N.; Paul, B. D.; Gadalla, M. M.; Mustafa, A. K.; Sen, T.; Xu, R.; Kim, S.; Snyder, S. H. *Molecular Cell* **2012**, *45*, 13.

(14) Kimura, Y.; Kimura, H. *FASEB J.* **2004**, *18*, 1165.

(15) Yang, G.; Zhao, K.; Ju, Y.; Mani, S.; Cao, Q.; Puukila, S.; Khaper, N.; Wu, L.; Wang, R. *Antioxid. Redox. Signal.* **2013**, *18*, 1906.

(16) Mishanina, T. V.; Libiad, M.; Banerjee, R. *Nat. Chem. Biol.* **2015**, *11*, 457.

(17) Nishida, M.; Sawa, T.; Kitajima, N.; Ono, K.; Inoue, H.; Ihara, H.; Motohashi, H.; Yamamoto, M.; Suematsu, M.; Kurose, H.; van der Vliet, A.; Freeman, B. A.; Shibata, T.; Uchida, K.; Kumagai, Y.; Akaike, T. *Nat. Chem. Biol.* **2012**, *8*, 714.

(18) Yadav, P. K.; Martinov, M.; Vitvitsky, V.; Seravalli, J.; Wedmann, R.; Filipovic, M. R.; Banerjee, R. *J. Am. Chem. Soc.* **2016**, *138*, 289.

(19) Pan, J.; Carroll, K. S. *ACS Chem. Biol.* **2013**, *8*, 1110.

(20) Zhang, D.; Macinkovic, I.; Devarie-Baez, N. O.; Pan, J.; Park, C.-M.; Carroll, K. S.; Filipovic, M. R.; Xian, M. *Angew. Chem. Int. Ed.* **2014**, *53*, 575.

(21) Galardon, E.; Padovani, D. *Bioconjugate Chem.* **2015**, *26*, 1013.

(22) Han, X.; Yu, F.; Song, X.; Chen, L. *Chemical Science* **2016**, *7*, 5098.

(23) Wedmann, R.; Onderka, C.; Wei, S.; Szijártó, I. A.; Miljkovic, J. L.; Mitrovic, A.; Lange, M.; Savitsky, S.; Yadav, P. K.; Torregrossa, R.; Harrer, E. G.; Harrer, T.; Ishii, I.; Gollasch, M.; Wood, M. E.; Galardon, E.; Xian, M.; Whiteman, M.; Banerjee, R.; Filipovic, M. R. *Chem. Sci.* **2016**, *7*, 3414.

(24) Ono, K.; Akaike, T.; Sawa, T.; Kumagai, Y.; Wink, D. A.; Tantillo, D. J.; Hobbs, A. J.; Nagy, P.; Xian, M.; Lin, J.; Fukuto, J. M. *Free Radic. Biol. Med.* **2014**, *77* (C), 82.

(25) Saund, S. S.; Sosa, V.; Henriquez, S.; Nguyen, Q. N. N.; Bianco, C. L.; Soeda, S.; Millikin, R.; White, C.; Le, H.; Ono, K.; Tantillo, D. J.; Kumagai, Y.; Akaike, T.; Lin, J.; Fukuto, J. M. *Arch. Biochem. Biophys.* **2015**, *588* (C), 15.

(26) Millikin, R.; Bianco, C. L.; White, C.; Saund, S. S.; Henriquez, S.; Sosa, V.; Akaike, T.; Kumagai, Y.; Soeda, S.; Toscano, J. P.; Lin, J.; Fukuto, J. M. *Free Radic. Biol. Med.* **2016**, *97* (C), 136.

(27) Dénès, F.; Pichowicz, M.; Povie, G.; Renaud, P. *Chem. Rev.* **2014**, *114*, 2587.

(28) Chenier, J. H. B.; Furimsky, E.; Howard, J. A. *Can. J. Chem.* **1974**, *52*, 3682.

(29) Burton, G. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1981**, *103*, 6472.

(30) Benson, S. W. *Chem. Rev.* **1978**, *78*, 23.

(31) Janousek, B. K.; Reed, K. J.; Brauman, J. I. *J. Am. Chem. Soc.* **1980**, *102*, 3125.

(32) H-atom abstraction from RSSH by an alkyl radical (propan-2-ol-2-yl) generated using pulse radiolysis at pH 4 yielded a rate constant of $k = 2.4 \times 10^9$ M^{-1} s^{-1}. See Everett, S. A.; Folkes, L. K.; Wardman, P.; Asmus, K. D. *Free Radic. Res.* **1994**, *20*, 387.

(33) Although these might be the initial reaction products, polysulfides and thiols are generally the end products.[33a-b] (a) Bailey, T. S.; Zakharov, L. N.; Pluth, M. D. *J. Am. Chem. Soc.* **2014**, *136*, 10573. (b) Bailey, T. S.; Pluth, M. D. *Free Radic. Biol. Med.* **2015**, *89*, 662.

(34) Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1962**, *40*, 1851.

(35) Haidasz, E. A.; Van Kessel, A. T. M.; Pratt, D. A. *J. Org. Chem.* **2016**, *81*, 737.

(36) Chauvin, J.-P. R.; Haidasz, E. A.; Griesser, M.; Pratt, D. A. *Chem. Sci.* **2016**, *7*, 6347.

(37) Litwinienko, G.; Ingold, K. U. *Acc. Chem. Res.* **2007**, *40*, 222.

(38) Tronche, C.; Martinez, F. N.; Horner, J. H.; Newcomb, M.; Senn, M.; Giese, B. *Tetrahedron Lett.* **1996**, *37*, 5845.

(39) Abraham, M. H.; Abraham, R. J.; Byrne, J.; Griffiths, L. *J. Org. Chem.* **2006**, *71*, 3389.

(40) Abraham, M. H.; Grellier, P. L.; Prior, D. V.; Duce, P. P.; Morris, J. J.; Taylor, P. J. *J. Chem. Soc., Perkin Trans. 2* **1989**, 6, 699.

(41) Snelgrove, D. W.; Lusztyk, J.; Banks, J. T.; Mulder, P.; Ingold, K. U. *J. Am. Chem. Soc.* **2001**, *123*, 469.

(42) Valgimigli, L.; Amorati, R.; Petrucci, S.; Pedulli, G. F.; Hu, D.; Hanthorn, J. J.; Pratt, D. A. *Angew. Chem. Int. Ed.* **2009**, *48*, 8348.

(43) Hanthorn, J. J.; Valgimigli, L.; Pratt, D. A. *J. Am. Chem. Soc.* **2012**, *134*, 8306.

(44) Vaidya, V.; Ingold, K. U.; Pratt, D. A. *Angew. Chem. Int. Ed.* **2009**, *48*, 157.

(45) Zielinski, Z.; Presseau, N.; Amorati, R.; Valgimigli, L.; Pratt, D. A. *J. Am. Chem. Soc.* **2014**, *136*, 1570.

(46) Amorati, R.; Baschieri, A.; Morroni, G.; Gambino, R.; Valgimigli, L. *Chem. Eur. J.* **2016**, *22*, 7924.

(47) Everett, S. A.; Folkes, L. K.; Wardman, P.; Asmus, K. D. *Free Radic Res* **1994**, *20*, 387.

(48) Cuevasanta, E.; Lange, M.; Bonanata, J.; Coitiño, E. L.; Ferrer-Sueta, G.; Filipovic, M. R.; Alvarez, B. *J. Biol. Chem.* **2015**, *290*, 26866.

(49) Newcomb, M.; Glenn, A. G.; Manek, M. B. *J. Org. Chem.* **1989**, *54*, 4603.

(50) Ha, C.; Horner, J. H.; Newcomb, M.; Varick, T. R.; Arnold, B. R.; Lusztyk, J. *J. Org. Chem.* **1993**, *58*, 1194.

(51) Newcomb, M.; Choi, S.-Y.; Horner, J. H. *J. Org. Chem.* **1999**, *64*, 1225.

(52) Denisov, E.; Chatgilialoglu, C.; Shestakov, A.; Denisova, T. *Int. J. Chem. Kinet.* **2009**, *41*, 284.

(53) Inbar, S.; Linschitz, H.; Cohen, S. G. *J. Am. Chem. Soc.* **1982**, *104*, 1679.

(54) The rate constant for the reaction of $C_6H_{13}S\bullet$ and $C_8H_{17}SH$ has been estimated to be $k = 2.7 \times 10^4$ M^{-1} s^{-1},[54a] while in aqueous medium the rate constant for the reaction of 2-hydroxyethanethiyl radical with 1,4-dithiothreitol has been measured at 1.7×10^7 M^{-1} s^{-1}.[54b] (a) Alnajjar, M. S.; Garrossian, M. S.; Autrey, S. T.; Ferris, K. F.; Franz, J. A. *J. Phys. Chem.* **1992**, *96*, 7037. (b) Akhlaq, M. S.; Sonntag, von, C. *Z. Naturforsch. C Bio. Sci.* **1987**, *42*, 134.

(55) There has been a range of experimental values reported, from 77[55a] to 83 kcal/mol.[55b] High level calculations are in the range of 81 (the CBS-QB3 method employed here) to 83 kcal/mol.[56c] (a) Lim, I. S.; Lim, J. S.; Lee, Y. S.; Kim, S. K. *J. Chem. Phys.* **2007**, *126*, 034306. (b) Borges dos Santos, R. M.; Muralha, V. S. F.; Correia, C. F.; Guedes, R. C.; Costa Cabral, B. J.; Martinho Simões, J. A. *J. Phys. Chem. A* **2002**, *106*, 9883. (c) Cabral do Couto, P.; Costa Cabral, B. J.; Martinho Simões, J. A. *Chem. Phys. Lett.* **2006**, *421*, 504.

(56) Winterbourn, C. C.; Metodiewa, D. *Free Radic. Biol. Med.* **1999**, *27*, 322.

(57) Alkylthiols react so slowly that they cannot compete with propagation, precluding a determination of this rate constant. CBS-QB3 calculations predict a rate constant of $k = 190$ M^{-1} s^{-1} for the reaction of *n*-butylthiol with methylperoxyl radical.

(58) Ingold, K. U.; Pratt, D. A. *Chem. Rev.* **2014**, *114*, 9022.

(59) Valgimigli, L.; Pratt, D. A. *Acc. Chem. Res.* **2015**, *48*, 966.

(60) Hanthorn, J. J.; Pratt, D. A. *J. Org. Chem.* **2012**, *77*, 276.

(61) Amorati, R.; Lynett, P. T.; Valgimigli, L.; Pratt, D. A. *Chem. Eur. J.* **2012**, *18*, 6370.

(62) Amorati, R.; Pedulli, G. F.; Pratt, D. A.; Valgimigli, L. *Chem. Commun. (Camb.)* **2010**, *46*, 5139.

(63) Chatgilialoglu, C.; Ferreri, C. *Acc. Chem. Res.* **2005**, *38*, 441.

(64) Northrop, B. H.; Coffey, R. N. *J. Am. Chem. Soc.* **2012**, *134*, 13804.

(65) Everett, S. A.; Wardman, P. *Meth. Enzymol.* **1995**, *251*, 55.

(66) McGrath, A. J.; Garrett, G. E.; Valgimigli, L.; Pratt, D. A. *J. Am. Chem. Soc.* **2010**, *132*, 16759.

(67) Lucarini, M.; Pedulli, G. F.; Cipollone, M. *J. Org. Chem.* **1994**, *59*, 5063.

(68) Lucarini, M.; Pedrielli, P.; Pedulli, G. F.; Cabiddu, S.; Fattuoni, C. *J. Org. Chem.* **1996**, *61*, 9259.

(69) The rate constants were determined to be $(5.3 \pm 0.9) \times 10^9$ M^{-1} s^{-1} and $(3.0 \pm 0.3) \times 10^9$ M^{-1} s^{-1} for the cumyl and *t*-dodecyl perthiyl radicals, respectively. We previously reported a recombination rate for *t*-butyl perthiyl radicals of $(6.0 \pm 0.5) \times 10^9$ M^{-1} s^{-1}. See ref 36.

(70) Bianco, C. L.; Chavez, T. A.; Sosa, V.; Saund, S. S.; Nguyen, Q. N. N.; Tantillo, D. J.; Ichimura, A. S.; Toscano, J. P.; Fukuto, J. M. *Free Radic. Biol. Med.* **2016**, *101*, 20.

(71) The cross-reactivity between TEMPO and perthiyl radicals also suggests that the results of experiments wherein TEMPO has been employed as the oxidant to produce perthiyl radicals from hydropersulfides must be interpreted with some caution. In particular, the lack of reactivity of perthiyl radicals to O_2 and NO, which was inferred on the basis that neither O_2 nor NO were consumed when a hydropersulfide was exposed to an excess of TEMPO could simply be explained by the very rapid reaction of the perthiyl radical with the excess TEMPO.

(72) Mathai, J. C.; Missner, A.; Kuegler, P.; Saparov, S. M.; Zeidel, M. L.; Lee, J. K.; Pohl, P. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 16633.

(73) Cuevasanta, E.; Denicola, A.; Alvarez, B.; Möller, M. N. *PLoS ONE* **2012**, *7*, e34562.

(74) Riahi, S.; Rowley, C. N. *J. Am. Chem. Soc.* **2014**, *136*, 15111.

(75) Dixon, S. J.; Lemberg, K. M.; Lamprecht, M. R.; Skouta, R.; Zaitsev, E. M.; Gleason, C. E.; Patel, D. N.; Bauer, A. J.; Cantley, A. M.; Yang, W. S.; Morrison, B., III; Stockwell, B. R. *Cell* **2012**, *149*, 1060.

(76) Friedmann Angeli, J. P.; Schneider, M.; Proneth, B.; Tyurina, Y. Y.; Tyurin, V. A.; Hammond, V. J.; Herbach, N.; Aichler, M.; Walch, A.; Eggenhofer, E.; Basavarajappa, D.; Radmark, O.; Kobayashi, S.; Seibt, T.; Beck, H.; Neff, F.; Esposito, I.; Wanke, R.; Forster, H.; Yefremova, O.; Heinrichmeyer, M.; Bornkamm, G. W.; Geissler, E. K.; Thomas, S. B.; Stockwell, B. R.; O'Donnell, V. B.; Kagan, V. E.; Schick, J. A.; Conrad, M. *Nat Cell Biol* **2014**, *16*, 1180.

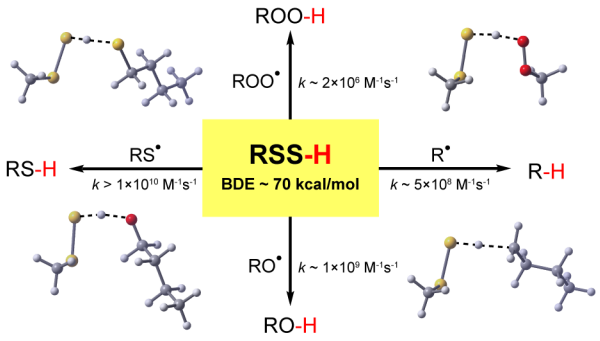
(77) Zilka, O.; Shah, R.; Li, B.; Friedmann Angeli, J. P.; Griesser, M.; Conrad, M.; Pratt, D. A. *ACS Cent. Sci.* **2017**, acscentsci.7b00028.

(78) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188.

(79) Burton, G. W.; Doba, T.; Gabe, E.; Hughes, L.; Lee, F. L.; Prasad, L.; Ingold, K. U. *J. Am. Chem. Soc.* **1985**, *107*, 7053.

(80) Montgomery, J. A., Jr; Frisch, M. J.; Ochterski, J. W.; Petersson, G. A. *J. Chem. Phys.* **1999**, *110*, 2822.

(81) Gaussian 09, Revision E.01. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian, Inc.: Wallingford, CT, USA 2009.


ROO-H
ROO•
k ~ 2×10⁶ M⁻¹s⁻¹
RS•
RS-H
k > 1×10¹⁰ M⁻¹s⁻¹
RSS-H
BDE ~ 70 kcal/mol
R•
k ~ 5×10⁸ M⁻¹s⁻¹
R-H
RO•
k ~ 1×10⁹ M⁻¹s⁻¹
RO-H