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Monitoring electrophilic intermediates in reactions of thiols in aqueous solution directly with ^{19}F NMR†

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Mechanistic studies of thiol reactivity can be challenging because electrophilic reaction intermediates, such as sulfenic acids (RSOH) and sulfenyl chlorides (RSCl), are generally too reactive to be observed directly. Herein we report the design and synthesis of a sterically-encumbered fluorinated triptycene thiol which enables direct observation of reaction intermediates in aqueous buffer by ^{19}F NMR, as demonstrated in reactions with hydrogen peroxide and hypochlorous acid. Reactions with H_2O_2 resulted in the formation of a persistent RSOH species, which was subsequently converted to a sulfinic acid (RSO_2H) and then a sulfonic acid (RSO_3H), while RSCl was found to be the intermediate in reactions with HOCl. Utilizing the same scaffold, reactions of thiol with thermally and photochemically generated singlet oxygen afforded RSO_2H as the primary product. The stark difference in product profile from sterically-unencumbered thiols – which yield disulfides – implies that the reaction proceeds through a sulfenyl hydroperoxide (RSOOH) intermediate. Sulfenic acids, which were not observed in reactions of thiols with singlet oxygen, were also found to rapidly react with singlet oxygen to afford sulfinic acids, which is proposed to involve initial formation of an analogous sulfinyl hydroperoxide (RS(O)OOH). The formation and reactions of RSOOH are explored by computations. Use of the water-soluble fluorinated triptycene scaffold to probe reductive processes on RSOH (*e.g.*, with ascorbate and/or iron) is also illustrated, wherein it was found that RSOH are surprisingly resistant to reductive heterolysis – in stark contrast with hydroperoxides – owing to their strong S–O bond.

1 Introduction

Thiol redox reactivity is central to many aspects of biology. Cysteine, the unique thiol-containing amino acid, provides proteins and peptides (*i.e.* glutathione) with unique catalytic,[1,2] structural,[3] signaling,[4,5] and antioxidant[6] functions. In many cases, these functions are underpinned by thiol/disulfide exchange reactions, while in others, oxidation of the thiol to a reactive intermediate is believed to be key. Of particular interest are the reactions of cysteine thiols with H_2O_2, the pervasive 'reactive oxygen species' that abounds the cell – and at elevated levels in pathological contexts. Disulfides are primarily formed in these reactions, but sulfinic acids (RSO_2H) and sulfonic acids (RSO_3H) can also be observed depending on conditions.[7] All of these products are believed to result from the initial formation of a sulfenic acid (RSOH) from substitution of the thiolate on H_2O_2; the sulfenic acid then either reacts as an electrophile, undergoing substitution by another equivalent of thiolate to yield disulfide, or as a nucleophile, where it is oxidized by another equivalent of H_2O_2 to yield RSO_2H and then RSO_3H. While thiol oxidation to RSO_2H is reversible in the cell, RSO_3H formation is irreversible, abolishing the function of the thiol.[8]

The reactivity of RSOH as both electrophile and nucleophile leads to their rapid self-reaction, making isolation and determination of accurate physicochemical properties and/or reaction kinetics challenging – especially under physiologically-relevant conditions (*i.e.* in aqueous solutions).[9] Although some protein sulfenic acids are stable and can be observed by mass spectrometry,[10] the protein structure can make mechanistic studies and generalizations about RSOH reactivity difficult.[11,12] To provide insight to their reactivity, several persistent small-molecule RSOH have been prepared,[13,14] including a sterically-encumbered cysteine sulfenic acid.[15] Unfortunately, the hydrophobicity of the scaffold restricted the study of its chemical properties to largely organic solutions (*i.e.* 20 : 1 THF/H_2O), which cannot be buffered for studies of the pH-dependence of the reactivity of thiols and sulfenic acids – a key consideration given that physiologically-relevant thiol chemistry is dominated by the more reactive conjugate base (thiolate), which is present to a substantial extent at pH 7.4 (thiol pK_as range from 5.0 to 8.8).[16]

We previously used a triptycene scaffold functionalized with a bridgehead fluorine atom to monitor the reaction of thiol and H_2O_2 in buffered methanol directly using ^{19}F NMR.[13] The

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triptycene moiety precludes electrophilic reactions of the sulfenic acid because a nucleophile cannot access the σ^*_{S-O} orbital localized on the sulfur atom, unequivocally confirming it as the initial product. This approach not only enabled detailed kinetic measurements and unambiguous characterization of the specific-base catalysis mechanism responsible, but also the successive oxidations to sulfinic and sulfonic acids which follow.[17] Unfortunately, studies of the reaction of thiols and sulfenic acids with stronger oxidants, including other biologically-relevant 'reactive oxygen species' such as hypochlorous acid (HOCl) and singlet oxygen ($^1O_2$), were not possible due to their competitive reactions with the (amine-based) buffers. Similarly to the reactions of thiols with H_2O_2, reactions of HOCl and $^1O_2$ have been shown to yield disulfides, but the mechanisms remain unclear due, in part, to the inability to directly observe reaction intermediates. We surmised that substitution of the 9-triptycenethiol scaffold to increase water solubility would enable direct observation of any intermediates formed in the reactions of thiols with HOCl, $^1O_2$ and/or other high energy oxidants. Moreover, it would permit analogous studies on sulfenic acids and the investigation of other reactions which have been proposed to involve RSOH intermediates. Herein we describe the preparation of such a scaffold, its use to provide insights to the reactions of thiols and sulfenic acids with H_2O_2, HOCl and $^1O_2$, and describe other applications to study sulfenic acid reactivity.

2 Results & discussion

2.1. Synthesis of thiol 1 and its oxidation products (6, 7, 8)

To improve the water-solubility of the fluorinated 9-triptycenethiol used in our previous work, we incorporated pendant tetraethylene glycol (TEG) units on the triptycene backbone as in **1**. The synthesis of **1** was carried out in 7 steps from 9-bromoanthracene with an overall yield of 9% (Fig. 1A). Lithium-bromide exchange on 9-bromo-10-fluorotriptycene (**2**)[17] and substitution of the resulting triptycenyl lithium on methyl disulfide afforded methyl sulfide **3** in 92% yield. Selective bromination of **3** was challenging; the Lewis base-catalyzed approach developed by Nishii and Miura[18] was most successful, leading to a mixture of dibrominated products **4a** (*meta*, *meta* to the bridgehead C–S bond) and **4b** (*meta*, *para*). Interestingly, despite its striking similarity, the fluorinated triptycene **3** did not assist the reaction on its own and the TpSMe used by Nishii and Miura[18] had to be added for the reaction to proceed to completion. Indeed, the presence of the fluorine atom also precluded access to the tribrominated triptycene, which was readily obtained under these reaction conditions in its absence. Both isomers of **4** were then subjected to Ullmann-type coupling conditions in the presence of $Cu(OAc)_2$ using TEG as both nucleophile and solvent, sluggishly affording a corresponding isomeric mixture of **5** in 78% yield. The methyl sulfide was then cleaved using freshly prepared lithium thioethoxide in absolute DMPU at 120 °C to obtain the target thiol **1** in 51% yield. Reverse phase HPLC could resolve **1a** from **1b**, enabling its unequivocal characterization, but most of our experiments were carried out on the mixture as it afforded a second data set in each independent experiment (*vide infra*).

The sulfenic acids (**6**) and sulfinic acids (**7**) derived from **1** were prepared in 46% and 22% yield, respectively, from the direct oxidation of thiols **1** by H_2O_2 (2 eq.) in H_2O/THF (1 : 1) containing K_2HPO_4 and separated by flash column chromatography. The sulfonic acids (**8**) were prepared similarly by the overnight reaction of **1** with 5 eq. of H_2O_2 (Fig. 1B).

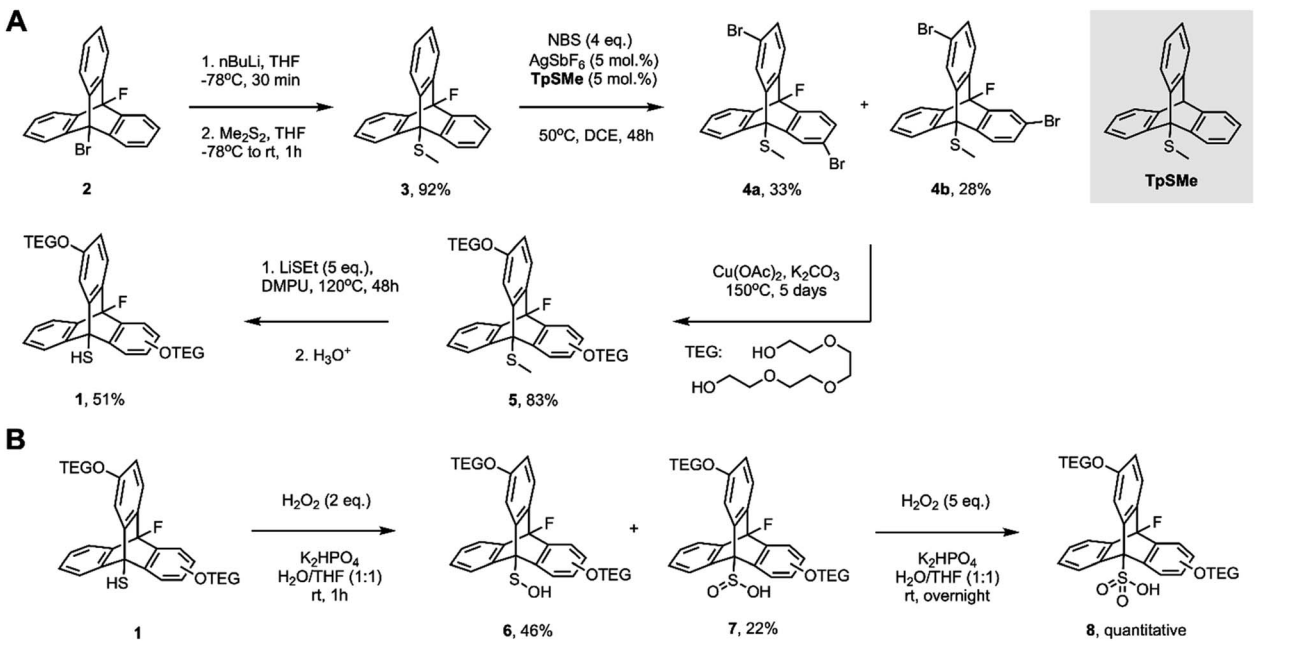

Fig. 1 (A) Preparation of thiols **1**, and (B) corresponding sulfenic acids **6**, sulfinic acids **7** and sulfonic acids **8**.

2.2. Reactions of 1 with H_2O_2

To obtain reaction kinetics for the oxidation of thiols **1** by H_2O_2 in water, **1** was treated with an excess of H_2O_2 in phosphate-buffered D_2O/THF (3 : 1, v/v) at pD 7.8 (pH 7.4),[19] and their consumption was monitored by ^{19}F NMR along with the corresponding appearance of product sulfenic, sulfinic and sulfonic acids (quantified relative to added 2,2,2-trifluoroethanol) as shown in Fig. 2. Since the two regioisomers of **1** and their corresponding sulfur oxyacids have distinct ^{19}F NMR shifts, two data sets were collected per experiment (Fig. 2B). These data sets were fit to a simple kinetic model for successive bimolecular reactions of **1**, **6** and **7** with H_2O_2 (Fig. 2C), from which observed rate constants (k_{obs}) of 0.10, 0.013 and 0.0014 M^{-1} s^{-1} were derived for the reactions of thiol, sulfenic and sulfinic acid, respectively. Not only is this the first example of these reactions being followed directly in aqueous solution but, as far as we are aware, these are the first rate constants for RSOH and sulfinic acid oxidation by H_2O_2 measured in aqueous solution.‡

Previously measured k_{obs} for the reactions of the water-insoluble triptycene thiol, sulfenic acid and sulfinic acid with H_2O_2 in methanol at $^{s}_{s}$pH = 9.9 (*ca.* pH = 7.66 in H_2O)[20] were 2.5 × 10^{-3}, 9.5 × 10^{-4} and 7.3 × 10^{-4} M^{-1} s^{-1}, respectively. Given that these reactions are specific-base catalyzed (Fig. 2A), we suspected that the origin of the large solvent effects on the reactions of the thiol (40×) and sulfenic acid (13×) is the more favourable pre-equilibrium in aqueous solution relative to methanol. To derive absolute rate constants, the pK_as of thiol **1** (8.3 ± 0.1) and sulfenic acid **6** (10.4 ± 0.1) were measured in

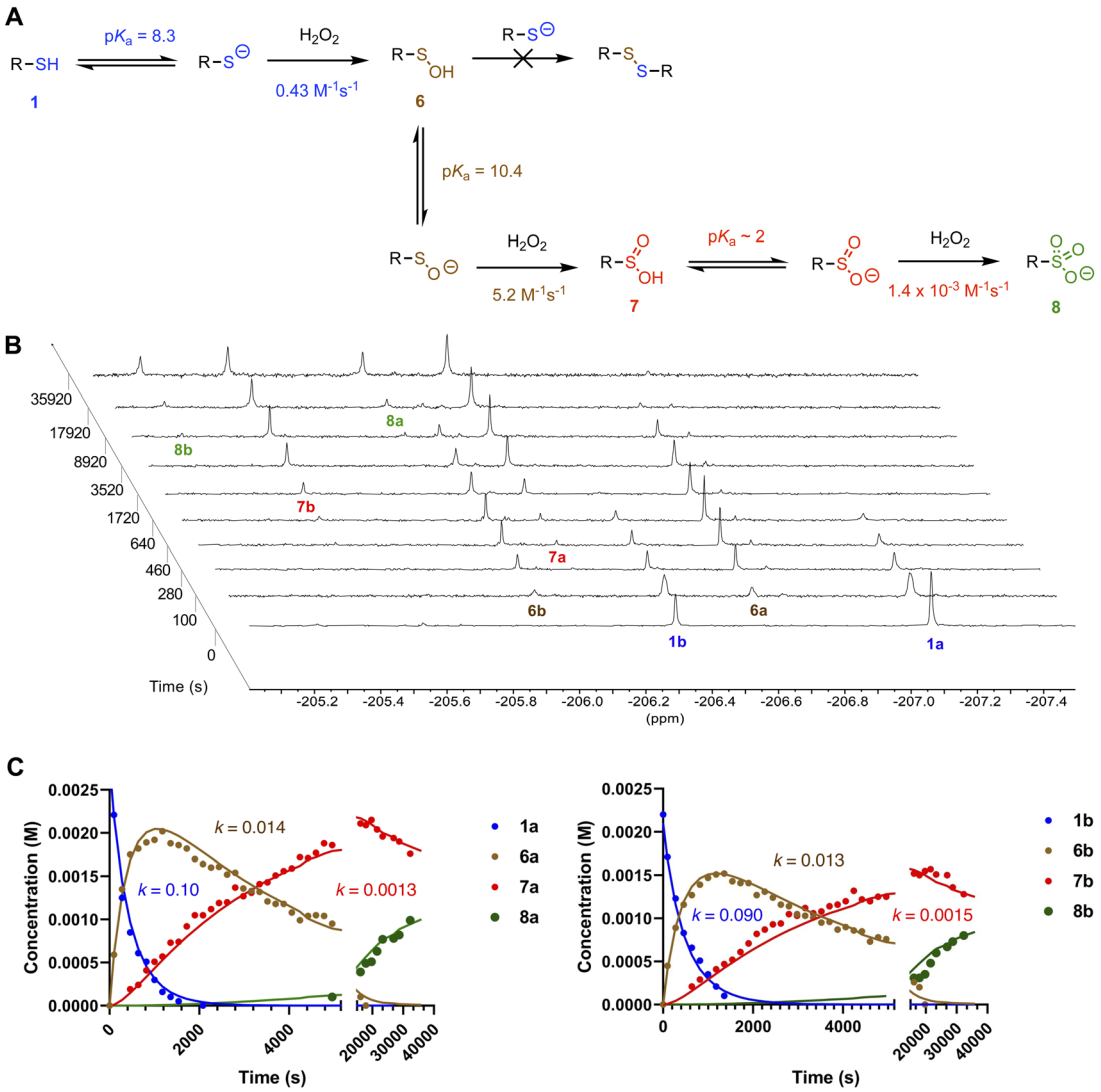

Fig. 2 (A) Reaction scheme for oxidation of thiols **1** by H_2O_2, including pK_a values determined for each of **1**, **6** and **7** and absolute rate constants obtained for the reactions of the conjugate bases of each of **1**, **6**, and **7** with H_2O_2 in 50 mM phosphate-buffered pD 7.8 D_2O/THF (3 : 1). The rate constants are derived from the mean of the individual rate constants determined for each of the **a** and **b** isomers (see ESI†). (B) Representative time-resolved ^{19}F NMR spectra obtained upon treatment of **1** (5 mM) with 25 mM H_2O_2 in 50 mM phosphate-buffered pD 7.8 D_2O/THF (3 : 1). (C) Representative reaction profiles of **1a** (2.8 mM) and **1b** (2.2 mM) with 5 eq. H_2O_2 in pD 7.8 D_2O/THF (3 : 1), fits of the data to a simple kinetic model for successive bimolecular reactions of **1**, **6** and **7** with H_2O_2 and the corresponding observed rate constants (in M^{-1} s^{-1}) derived from the fits.

D_2O/THF (3 : 1) (the values for **1a** and **6a** were within the margin of error on the measurements made with the mixtures and corresponding values in H_2O afforded 8.0 ± 0.1 and 10.1 ± 0.1, respectively). Expectedly, deprotonation of thiol and sulfenic acid were found to be much more favourable in water than in methanol (pK_a = 11.6 and 12.8, respectively).[17] With these pK_a values at hand, we derived absolute (pH-independent) rate constants for the reactions of thiolate (k = 0.43 M^{-1} s^{-1}, compared to 0.13 M^{-1} s^{-1} in methanol) and sulfenate (k = 5.2 M^{-1} s^{-1}, compared to 0.75 M^{-1} s^{-1} in methanol) with H_2O_2. As expected from the alpha effect[21] and our previous data,[17] sulfenate anions were found to be more reactive nucleophiles than thiolate anions. Sulfinate was found to be much less reactive as a nucleophile ($k = 1.4 \times 10^{-3}$ M^{-1} s^{-1} compared to 5.8×10^{-4} M^{-1} s^{-1} in methanol) consistent with the greater relative stability of the anion (pK_a ~2[17] *vs.* 10 for the corresponding RSOH). Overall, the rate constant determined for **1** is toward the low end of the range of those which have previously been reported (derived from consumption of thiol(ate)s),§ which span from 0.16 M^{-1} s^{-1} (for *N*-acetylcysteine) to 4.5 M^{-1} s^{-1} (for penicillamine) at pH 7.4,[12] which is presumably due to the electron-withdrawing effect of the 10-fluorotriptycenyl scaffold.

2.3. Reactions of 1 with HOCl

At physiological pH, thiols react rapidly with HOCl ($k > 10^8$ M^{-1} s^{-1}),[22] with sulfenyl chlorides being proposed as the initial products, but which are not observed due to their rapid reaction with thiols to yield disulfides.[23] We observed instant formation of a single product (**13a**) upon addition of limiting NaOCl to a solution of **1a** in pD 7.8 D_2O/THF (Fig. 3A). The ^{19}F NMR chemical shift (−205.9 ppm) did not correspond to the sulfenic acid **6a** (−206.6 ppm), implying formation of the sulfenyl chloride. However, when thiol **1a** was treated with *N*-chlorosuccinimide in chloroform, a product (**14a**) with a distinct ^{19}F NMR chemical shift (−206.4 ppm) was obtained (Fig. 3B).[24] High-resolution mass spectrometry indicated that **14a** was the sulfenyl chloride. While **14a** was stable over extended periods in pD 7.8 D_2O/THF buffer, addition of **1a** readily yielded **13a**, which we have characterized as the disulfide of **1a** (Fig. 3C). Since substitution of thiolate **1a** on sulfenyl chloride **14a** to form the disulfide is unlikely (the nucleophile cannot access the σ^*_{S-Cl} orbital localized on the sulfur atom), the thiolate presumably reacts with the sulfenyl chloride by electron transfer, resulting in the formation of thiyl radicals which dimerize to give the disulfide (Fig. 3B). An analogous mechanism has been proposed for the observation of disulfides from the reaction of sulfenyl chlorides and amines.[25] The intervention of thiyl radicals in the reaction of non-hindered thiols with HOCl has previously been demonstrated by Davies,[26] who characterized DMPO adducts of thiyl radicals by EPR, suggesting that this pathway is not unique to the reaction of 9-triptycenethiols.

2.4. Reactions of 1 with $^1O_2$

Similarly to their reactions with H_2O_2 and HOCl, thiols have been shown to yield disulfides upon reaction with $^1O_2$.[27] To provide insight to the mechanism of this reaction, we investigated the reaction of **1** with $^1O_2$ derived from decomposition of the endoperoxide of sodium naphthalene-1,4-dipropionate ($NDPO_2$) at 37 °C ($t_{1/2}$ = 23 min).[28,29] The reactions were again monitored by ^{19}F NMR at 37 °C in pD 7.8 phosphate buffered D_2O/THF (3 : 1, v/v), but now in the presence of 4 eq. of $NDPO_2$ *in lieu* of either H_2O_2 or HOCl. After 33 minutes, the conversion of thiol was ~45%, giving primarily sulfinates **7** (~25%). Importantly, disulfide **13a** was not observed. Other products included two presumed pairs of unknown isomers (~10%) downfield of **8** at δ = −204.55 ppm and −205.25 ppm (Fig. 4A). Control experiments suggest that the formation of these unknowns can be attributed to a reaction occurring in THF in the presence of $^1O_2$ (see ESI Fig. S6†). The missing ~10% in the mass balance suggests that some signal suppression from the formation of persistent paramagnetic species is taking place (see ESI Fig. S3†).[30,31]

Consistent with the foregoing experiments with **1**, when thiol **9** was subjected to Rose Bengal-photosensitized oxygenation in 15 : 1 CD_3OD : H_2O containing 3 eq. NaOH, the corresponding sulfinate **11** and sulfonate **12** were formed (Fig. 4B) (see Fig. S4 of the ESI† for corresponding ^1H NMR spectra). Traces of disulfide **15** could be detected under these conditions (as a precipitate), suggesting that thiyl radical formation is possible under the reaction conditions, albeit as a minor reaction pathway.¶ Likewise, while RSOH **6** was not detected in D_2O/THF, a miniscule amount of **10** could be detected in CD_3OD/D_2O (Fig. 4B).

The drastically different product distribution arising from the reaction of **1** (and **9**) with $^1O_2$ compared to the precedent[32] suggests that nucleophilic substitution on an oxidized sulfur intermediate is responsible for disulfide formation from reactions of thiols and $^1O_2$, in general. While at first glance it would seem that RSOH is that intermediate, and although we found that RSOH **10** was readily photooxidized to RSO_2H **11** (see ESI Fig. S7†), it is difficult to provide a compelling mechanistic rationale for the direct formation of RSOH from RSH and $^1O_2$. Instead, we posit that a sulfenyl hydroperoxide (RSOOH) is the immediate product, and when the reaction with a thiolate cannot take place at sulfur to give a disulfide (*i.e.* for **1** and **9**), the reaction takes place at oxygen to give two equivalents of RSOH. Alternatively, RSOOH can rearrange to RSO_2H. If formed, RSOH can react with $^1O_2$ to afford a sulfinyl hydroperoxide (RS(O)OOH) which can oxidize another equivalent of RSOH to yield two equivalents of RSO_2H (Fig. 4C).

Formation of $RSOO^-$ from thiolate (RS^-) and $^1O_2$ is predicted by CBS-QB3 (ref. 33) to be highly exergonic (ΔG° = −25.1 kcal mol^{-1} for *t*-BuS^- as a model thiolate). No TS could be computed for the nucleophilic addition pathway, presumably due to the use of a single determinant wavefunction approach. A TS could, however, be computed for the addition of RSH to $^1O_2$, which was associated with a barrier of $\Delta G^\ddagger$ = 14.7 kcal mol^{-1} (Fig. 4D). This may be taken as an upper bound for the thiolate reaction since the barrier to addition of the better nucleophile is sure to be lower. Another possibility involves electron transfer from RS^- to $^1O_2$ to form a radical pair that can combine or, if cage escape occurs, oxygenation of the thiyl radical to yield

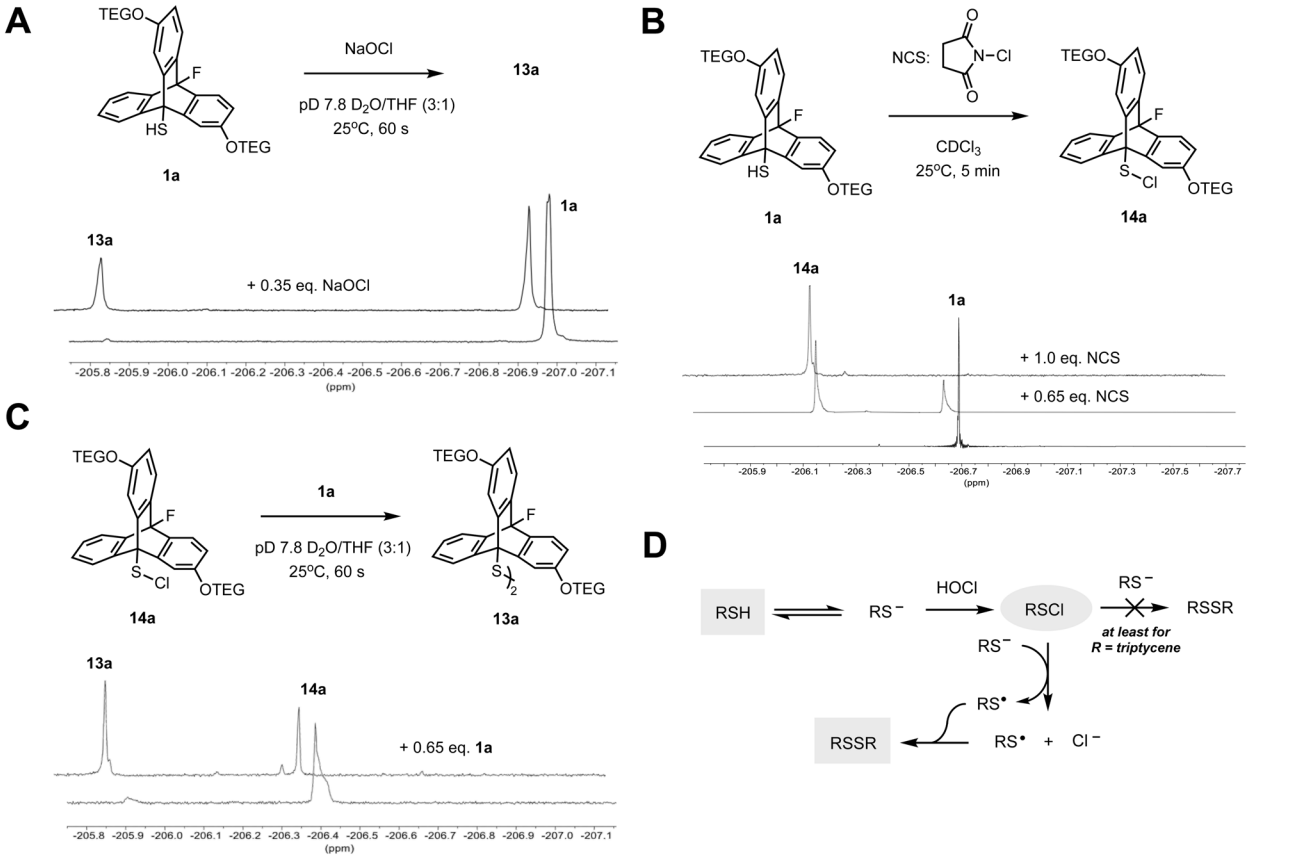

Fig. 3 (A) Representative ^{19}F NMR spectra obtained before and after treatment of **1a** (17 mM) with 0.35 eq. of NaOCl in 50 mM phosphate-buffered pD 7.8 D_2O/THF (3 : 1). (B) Preparation of sulfenyl chloride **14a** from **1a** and representative ^{19}F NMR in $CDCl_3$. (C) Representative ^{19}F NMR spectra obtained before and after treatment of **14a** (17 mM) with 0.65 eq. of **1a** in 50 mM phosphate-buffered pD 7.8 D_2O/THF (3 : 1). (D) Proposed mechanism of disulfide formation upon thiol oxidation by hypochlorous acid.

a thiylperoxyl radical (RSOO$^\bullet$) that could be subsequently reduced *via* either H-atom transfer or electron transfer. H-atom transfer from a thiol to RSOO$^\bullet$ is predicted to be exergonic ($\Delta G^\circ = -4.1$ kcal mol^{-1}) and proceed *via* an accessible barrier ($\Delta G^\ddagger =$ 18.8 kcal mol^{-1}). However, our calculations indicate that the highly exergonic ($\Delta G^\circ = -76.8$ kcal mol^{-1}) rearrangement of RSOO$^\bullet$ to a sulfonyl radical ($RSO_2^\bullet$) is much more likely. Earlier calculations had suggested this would require traversing a prohibitively high barrier ($\Delta G^\ddagger = 45$ kcal mol^{-1}),[34] but we have found this barrier to be significantly lower ($\Delta G^\ddagger =$ 13.7 kcal mol^{-1}) using the higher accuracy CBS-QB3 methodology. Regardless, the lack of disulfide formed in our experiments argues that thiyl radicals are not key intermediates.

The sulfinate observed when disulfide formation is suppressed could also arise from concerted rearrangement of RSOOH – a highly exergonic reaction ($\Delta G^\circ = -60.6$ kcal mol^{-1}) with an easily accessible barrier ($\Delta G^\ddagger = 13.6$ kcal mol^{-1}). Again, this barrier is likely an upper bound given that the H-bond between the migrating OH group and an explicit solvent molecule will become stronger in the TS (this cannot be captured in the polarizable solvent continuum employed in the calculation). Alternatively, a step-wise rearrangement can be envisioned. The calculated O–O BDE of RSOOH was found to be a meagre 10.2 kcal mol^{-1}, driven by the stability of the sulfinyl radical, suggesting O–O bond homolysis followed by in-cage recombination to form a sulfinic acid is possible. Earlier spin-trapping studies during thiol photooxygenation suggested the formation of $^\bullet$OH, presumably due to cage-escape following RSOOH thermolysis (Fig. 4E).[30] The lack of significant signal suppression in our NMR experiments, suggests that homolysis does not occur to a significant extent and that nucleophilic chemistry predominates. Regardless, the predicted rapid rearrangement chemistry presumably prevents the direct observation of RSOOH on the timescale of the NMR experiments.

The implication of RSOOH in disulfide formation from thiols exposed to $^1O_2$ compels consideration that RSOOH could also be intermediates in disulfide formation in the radical-mediated oxidation (autoxidation) of thiols, given the rapid reaction of thiyl radicals with $^3O_2$ ($\sim 10^8$ M^{-1} s^{-1}).[35] Indeed, sulfur oxyacids are major products under some conditions,[36,37] and may derive from RSOOH as a common intermediate to disulfide formation. However, as indicated above, given that the (unimolecular) rearrangement of RSOO$^\bullet$ to $RSO_2^\bullet$ is predicted to proceed with a smaller barrier than the (biomolecular) H-atom transfer to RSOO$^\bullet$ from RSH (13.7 kcal mol^{-1} *vs.* 18.8 kcal mol^{-1}, respectively), the formation of RSOOH under

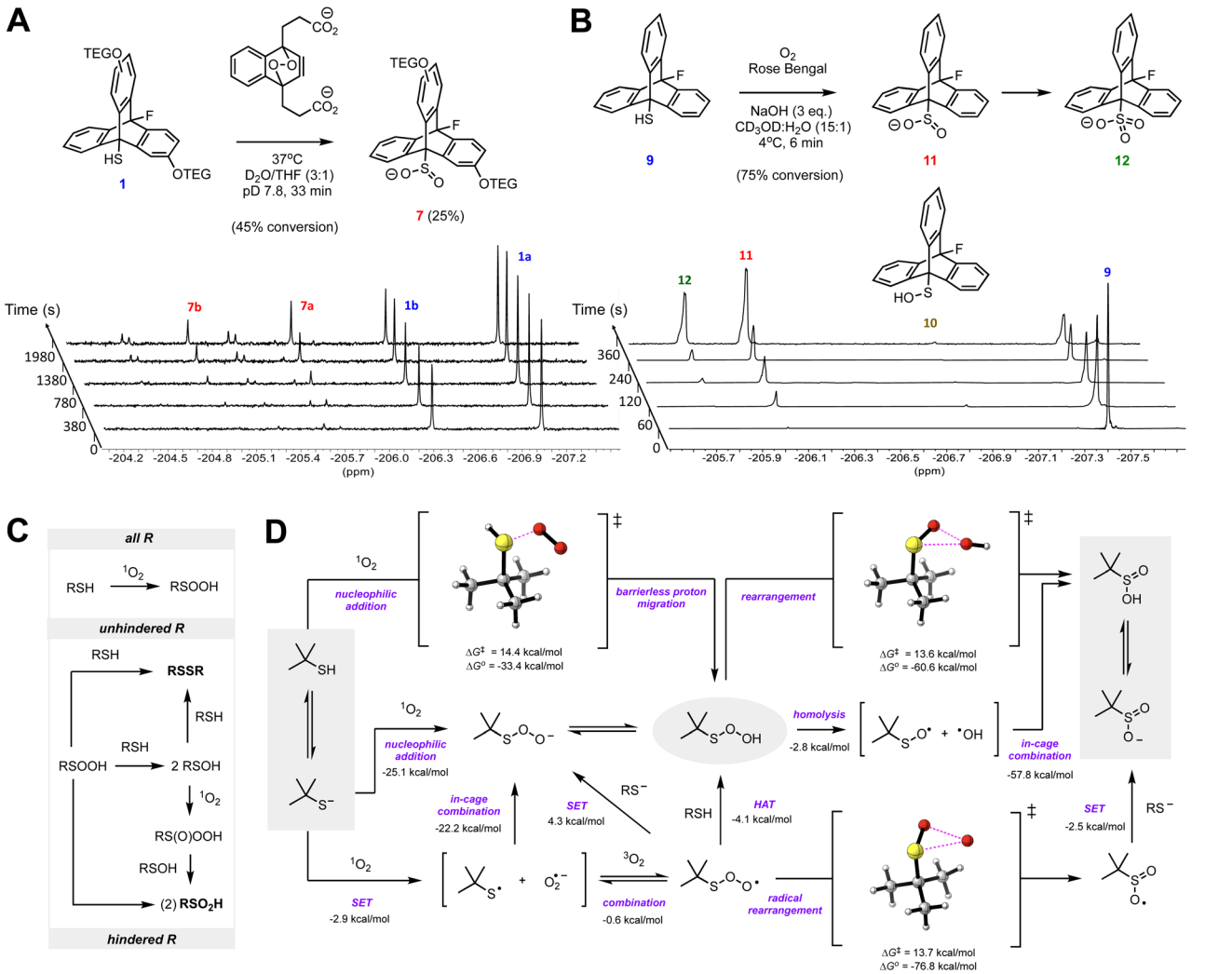

Fig. 4 (A) Representative time-resolved ^{19}F NMR spectra obtained upon treatment of **1** (5 mM) with 4 eq. of $NDPO_2$ in 50 mM phosphate-buffered pD 7.8 D_2O/THF (3 : 1). (B) Representative time-resolved ^{19}F NMR spectra obtained of the reaction of **9** (20 mM) with photochemically generated $^1O_2$ in CD_3OD/H_2O (15 : 1) containing 60 mM NaOH. (C) Scheme accounting for the different product distributions arising from reaction of hindered and unhindered thiols with $^1O_2$. (D) Mechanistic possibilities and CBS-QB3 calculated free energies (298 K) of the reaction of a model (*t*-butyl) thiol with $^1O_2$ (SET = single electron transfer; HAT = hydrogen atom transfer). Data calculated using a polarizable continuum model using the integral equation formalism (IEFPCM) parameterized for water are given; associated gas phase values are given in the ESI.†

radical conditions is unlikely. Instead, the partitioning of RS$^\bullet$ and RSOO$^\bullet$ between thiyl dimerization and thiylperoxyl rearrangement pathways is likely key in determining whether disulfides or sulfur oxyacids are the major products.

2.5. Other applications of the new scaffold

Since comparably very little remains known of RSOH reactivity (relative to RSH, RSO_2H and RSO_3H), we surmised that **6** may prove generally useful as a mechanistic probe. It has been shown previously that ascorbate acts as the cofactor for 1-Cys peroxiredoxins (Prx), and it was proposed to reduce the RSOH of 1-Cys Prx back to its active thiol form after oxidation.[38] However, other protein sulfenic acids are resistant to reduction by ascorbate.[39] Previously employed indirect bifunctional assays estimated rate constants ranging from 2.2 to 4 × 10^2 M^{-1} s^{-1}, depending on the protein RSOH. To provide additional insight to this reactivity, we incubated **6** with 3 eq. of ascorbate‖ in pD 7.8 D_2O/THF (3 : 1) and monitored the reaction by ^{19}F NMR (Fig. 5A). No reaction was observed over 72 hours, suggesting an amino acid sidechain in the Prx active site is required for activation of the sulfenic acid to reduction (the conjugate acid of an essential histidine residue is a possibility), or that a more sophisticated redox cascade is at play.

Labile iron(II) is often invoked as key to the auto-initiation of cellular (phospho)lipid peroxidation and associated ferroptosis due to its ability to reduce lipid hydroperoxides to form initiating lipid alkoxyl radicals.[40,41] Since thiyl radicals are significantly more thermodynamically stable than alkoxyl radicals (*i.e.* *t*-BuSH and *t*-BuOH BDEs are 86 and 106 kcal mol^{-1}, respectively),[42] we anticipated that RSOH would readily undergo the same reaction (Fig. 5B). To our great surprise, **10** did not react with Fe(II) (or Fe(III) in the presence of ascorbic acid) in methanol. At first glance, we surmised that this may have to do with

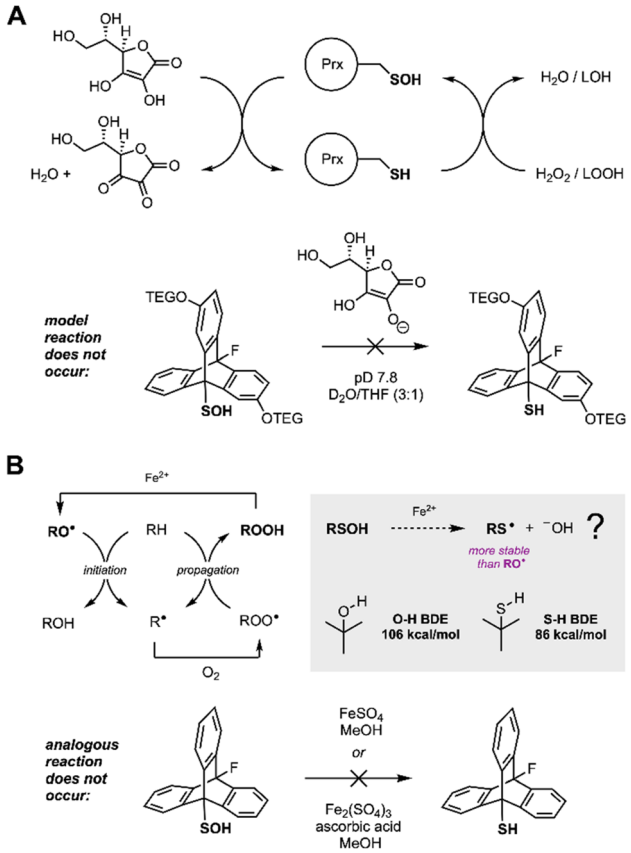

Fig. 5 (A) 1-Cys peroxiredoxins (Prx) reduce hydrogen peroxide and hydroperoxides by nucleophilic substitution by the cysteine thiol(ate). The resultant sulfenic acids are converted back to the starting thiol by ascorbate to complete the catalytic cycle. The lack of reactivity of RSOH **6** to ascorbate implies that active site residues are essential to enzyme turnover. (B) Lipid peroxidation is auto-initiated in the presence of Fe^{2+} due to reductive cleavage of the O–O bonds in product hydroperoxides, which yields initiating radicals. No reaction is observed for RSOH **10**, implying that the process is less favourable for RSOH than it is for the corresponding ROOH.

some impairment of the reaction by the triptycene backbone. However, CBS-QB3 calculations predict that the S–O bond in a model (*t*-butyl) RSOH is 27 kcal mol^{-1} stronger than the O–O bond in the corresponding hydroperoxide – despite the 20 kcal mol^{-1} difference in the stabilities of the product thiyl and alkoxyl radicals. The significant stabilization of RSOH relative to ROOH can be rationalized by lesser lone pair/lone pair repulsion in RSOH due to the longer S–O bond and more diffuse S lone pairs and also greater lone-pair/σ^*_{O-H} hyperconjugation in RSOH compared to ROOH. Based on these calculated thermodynamics and the lack of reactivity of **6** and ascorbate with or without added iron, it is clear that reductive heterolysis of RSOH is a highly unfavourable process in instances where there is no acid available to activate the RSOH.

3 Conclusions

A newly synthesized 10-fluorotriptycene thiol enables visualization of transient reactive intermediates (*e.g.*, RSOH and RSCl) and can be used to directly observe transformations of thiols and sulfenic acids in aqueous buffer by ^{19}F NMR. Reactions of thiols and sulfenic acids with H_2O_2 were found to proceed with kinetics that were slightly faster than in methanol, largely due to more favourable ionization to the reactive conjugate bases. Reaction of thiol with HOCl did not yield a sulfenic acid, but presumably a sulfenyl chloride. The sulfenyl chloride could be independently prepared by reaction of the thiol with *N*-chlorosuccinimide and was found to rapidly yield disulfide when treated with thiol(ate) in buffer – attributed to dimerization of thiyl radicals formed upon electron transfer from thiolate to sulfenyl chloride. Product distributions of the reaction of thiols with $^1O_2$ enable us to propose sulfenyl and sulfinyl hydroperoxides as intermediates en route to disulfides, sulfinic and sulfonic acids. Through product analyses and CBS-QB3 calculated reaction energetics, the myriad of one- and two-electron processes that these short-lived intermediates undergo are herein summarized. A lack of reactivity of RSOH **6** and **10** to one-electron reduction strongly supports that reductive heterolysis of RSOH does not occur to a significant extent without enzyme (acid) catalysis and highlights the energetic contribution of lone pair repulsion in hydroperoxides to their relative instability. We anticipate that this scaffold will be a useful tool compound for mechanistic investigations of thiol and sulfenic acid reactivity.

4 Experimental section

4.1. General

Reagents and solvents were obtained from commercial suppliers and used as received, unless indicated otherwise. Hydrogen peroxide concentration was determined by manganometric titration. Sodium hypochlorite concentration was determined by iodometry. ^1H, ^{13}C and ^{19}F NMR spectra were recorded on a Bruker AVANCE spectrometer at the specified frequency. ^{19}F NMR spectra in D_2O were referenced using 2,2,2-trifluoroethanol (TFE) (δ −77.0 ppm) as an internal standard, ^{19}F NMR spectra in $CDCl_3$, CD_3OD, and C_6D_6 were referenced to trifluorotoluene (δ −63.72 ppm). Product ratios were obtained through integration of their ^{19}F NMR signals. For quantification, ^{19}F NMR spectra were phased and baseline corrected. For NMR characterization of isomeric mixtures, the signals corresponding to each isomer were assigned based on those observed for HPLC-purified isomer **1a**, and the corresponding signals observed when it was oxidized with H_2O_2. Preparative HPLC was carried on a Waters 2996 HPLC system, using a preparative Waters XBridge Prep C18 5 μm (19 × 150 mm) column. Column chromatography was carried out with 40–63 μm, 230–400 mesh silica gel purchased from SiliCycle. All calculations were carried out using the Gaussian 09 quantum chemistry package using the CBS-QB3 complete basis set approach.[33]

4.2. Synthesis

4.2.1 10-Fluoro-9,10-[1′,2′]benzenoanthracen-9(10*H*)-yl(methyl)sulfane (3). Warning: a significant amount of

methanethiol is produced in this reaction. To an oven-dried flask charged with a solution of 9-bromo-10-fluorotriptycene[17] (3.0 g, 8.57 mmol) in neat THF (40.0 mL) and cooled to −78 °C, was slowly added *n*BuLi (4.12 mL, 10.28 mmol) as a 2.5 M solution in hexanes. The reaction was stirred at −78 °C for 30 minutes, after which methyl disulfide (0.91 mL, 10.28 mmol) was added and the suspension was left to warm up to room temperature, upon which it became a homogeneous solution. After 1 h, the reaction was quenched with a saturated solution of NH_4Cl. The crude product was extracted using EtOAc (3 × 50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was then purified by column chromatography using hexanes as the eluent to yield **3** (2.51 g, 92%) as a colourless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65–7.59 (m, 6H), δ 7.17–7.09 (m, 6H), δ 2.58 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.2, 144.0, 141.7, 141.6, 125.7, 125.6, 122.5, 118.7, 97.6, 60.5, 14.1. HRMS (EI) *m*/*z* calculated for [M] $C_{21}H_{15}FS$ 318.0879, found 318.0866.

4.2.2 Dibromo-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10*H*)-yl(methyl)sulfane (4). An oven-dried Schlenk tube was charged with **3** (1.74 g, 5.47 mmol), TpSMe[18] (82 mg, 0.274 mmol) and DCE (25 mL). Under a flow of nitrogen, *N*-bromosuccinimide (3.51 g, 19.69 mmol) and $AgSbF_6$ (93 mg, 0.274 mmol) were both added as solids. The reaction was stirred for 48 h at 50 °C before being poured into a saturated $NaHCO_3$ solution (50 mL). The product was extracted using $CHCl_3$ (3 × 30 mL), concentrated *in vacuo*, and dried over $MgSO_4$. The residue was purified by column chromatography using hexanes as the eluent to give a mixture of dibrominated isomers as an off-white foam (1.58 g, 61% total yield). The monobrominated derivative was also isolated.

*4.2.2.1 2,7-Dibromo-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10H)-yl(methyl)sulfane (**4a**).* 33% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.74–7.71 (m, 2H), δ 7.61–7.57 (m, 2H), δ 7.47–7.43 (m, 2H), δ 7.31–7.24 (m, 2H), δ 7.19–7.13 (m, 2H), δ 2.56 (s, 3H) ^{13}C NMR (151 MHz, $CDCl_3$) δ 145.9, 143.6, 143.2, 142.9, 142.8, 142.7, 140.7, 140.5, 128.9, 126.2, 126.1, 122.8, 122.4, 120.5, 119.0, 97.8, 59.9, 14.1. HRMS (EI) *m*/*z* calculated for [M] $C_{21}H_{13}Br_2FS$ 473.9087, found 473.9089.

*4.2.2.2 2,6-Dibromo-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10H)-yl(methyl)sulfane (**4b**).* 28% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.74–7.71 (m, 2H), δ 7.61–7.57 (m, 2H), δ 7.47–7.43 (m, 2H), δ 7.31–7.24 (m, 2H), δ 7.19–7.13 (m, 2H), δ 2.54 (s, 3H) ^{13}C NMR (151 MHz, $CDCl_3$) δ 145.7, 143.4, 143.1, 142.6, 140.7, 140.5, 128.9, 126.2, 126.1, 124.4, 122.8, 122.4, 120.5, 119.9, 118.9, 97.0, 59.9, 14.0. HRMS (EI) *m*/*z* calculated for [M] $C_{21}H_{13}Br_2FS$ 473.9087, found 473.9089.

4.2.3 Di(tetraethylene glycol)-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10*H*)-yl(methyl)sulfane (5). To an oven-dried Schlenk tube was added a mixture of the isomers **4** (1.10 g, 2.32 mmol) suspended in tetraethylene glycol (20.0 mL). Copper(II) acetate hydrate (0.91 g, 4.58 mmol) and potassium carbonate (3.20 g, 23.15 mmol) were then both added as solids. The tube was sealed, degassed, and refilled with nitrogen. The blue solution was heated at 150 °C for 5 days, during which the reaction became brown. Once the reaction mixture was cooled to ambient temperature, it was poured into distilled water (100 mL) and extracted (3 × 25 mL) with CH_2Cl_2. The organic layer was washed with water (3 × 100 mL) to remove excess TEG, dried over $MgSO_4$, and concentrated *in vacuo* to obtain the crude product as a dark-orange oil, which was then purified by column chromatography (gradient 1–3% MeOH/CH_2Cl_2) to obtain the mixture of isomers **5** (**5a** : **5b** = 57 : 43) as a viscous yellow oil (1.27 g, 1.81 mmol, 78% total yield).

*4.2.3.1 2,7-Di(tetraethylene glycol)-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10H)-yl(methyl)sulfane (**5a**).* ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58–7.52 (m, 2H), 7.46–7.40 (m, 2H), 7.43 (d, J = 8.2 Hz, 2H), 7.23–7.16 (m, 2H), 7.14–7.06 (m, 2H), 6.64–6.57 (m, 2H), 4.08 (t, J = 4.8 Hz, 4H), 3.80 (t, J = 4.8 Hz, 4H), 3.73–3.61 (m, 20H), 3.57 (t, J = 4.5 Hz, 4H), 2.56 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 156.9, 143.3, 141.5, 137.0, 136.1, 125.7, 125.5, 122.5, 122.2, 119.3, 118.2, 111.3, 110.8, 110.2, 106.2, 97.0, 72.5, 70.8, 70.6, 70.3, 69.7, 67.8, 61.7, 60.6, 60.0, 14.2. HRMS (ESI+) *m*/*z* calculated for $[M + Na]^+$ $C_{36}H_{45}FNaO_{10}S$ 725.2772, found 725.2767.

*4.2.3.2 2,6-Di(tetraethylene glycol)-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10H)-yl(methyl)sulfane (**5b**).* ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58–7.52 (m, 2H), 7.46–7.40 (m, 2H), 7.43 (d, J = 8.2 Hz, 2H), 7.23–7.16 (m, 2H), 7.14–7.06 (m, 2H), 6.64–6.57 (m, 2H), 4.08 (t, J = 4.8 Hz, 4H), 3.80 (t, J = 4.8 Hz, 4H), 3.73–3.61 (m, 20H), 3.57 (t, J = 4.5 Hz, 4H), 2.54 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.0, 156.9, 143.3, 137.0, 125.7, 125.5, 123.6, 122.5, 122.2, 119.5, 119.3, 118.3, 111.3, 110.8, 110.0, 97.0, 72.5, 70.8, 70.6, 70.3, 69.7, 67.8, 61.7, 60.6, 60.0, 14.2. HRMS (ESI+) *m*/*z* calculated for $[M + Na]^+$ $C_{36}H_{45}FNaO_{10}S$ 725.2772, found 725.2767.

4.2.4 Di(tetraethylene glycol)-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10*H*)-ylmercaptan (1). In a sealable tube, methyl sulfide **5** (349 mg, 0.497 mmol) was dissolved in anhydrous DMPU (2.0 mL), and the solution was purged with N_2 for 2 minutes before freshly prepared lithium ethylmercaptide[43] (169 mg, 2.485 mmol) was quickly added and the tube sealed. The reaction was heated at 120 °C for 2 days. Once cooled to room temperature, it was poured into 0.1 M aqueous HCl (100 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic fraction was then washed with 0.5 M NaOH (3 × 75 mL), the aqueous washings were collected and acidified to pH ∼2 using 12 M HCl, and extracted with CH_2Cl_2 (3 × 50 mL), dried over $MgSO_4$, and concentrated *in vacuo* to give the crude product as a red oil that was purified by column chromatography (gradient elution, 2–4% MeOH/$CHCl_3$) to give the mixture of thiols **1** (**1a** : **1b** = 57 : 43) as a viscous pale-yellow oil (174 mg, 51% total yield). The **1a** isomer could be separated by reverse phase HPLC using 3 : 1 MeOH : H_2O as the mobile phase.

*4.2.4.1 2,7-Di(tetraethylene glycol)-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10H)-ylmercaptan (**1a**).* δ 156.9 (s), 144.6 (d, J = 6.0 Hz), 143.8 (d, J = 20 Hz), 136.2 (d, J = 20 Hz), 126.1 (s), 125.5 (s), 121.4 (d, J = 2 Hz), 119.1 (d, J = 6.0 Hz), 118.0 (d, J = 6 Hz), 110.4 (s), 110.2 (s), 97.5 (d, J = 205 Hz), 72.5 (s), 70.8 (s), 70.6 (s), 70.3 (s), 69.7 (s), 67.8 (s), 61.7 (s), 57.5 (d, J = 2 Hz). ^{19}F NMR (471 MHz, D_2O/THF) δ −207.1. HRMS (ESI+) *m*/*z* calculated for $[M + Na]^+$ $C_{36}H_{45}FNaO_{10}S$ 711.2632, found 711.2615.

*4.2.4.2 2,6-Di(tetraethylene glycol)-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10H)-ylmercaptan (**1b**).* ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.63 (m, 1H), 7.57–7.49 (m, 2H), 7.43 (m, 1H), 7.31 (m,

1H), 7.18 (d, J = 2.4 Hz, 1H), 7.17–7.04 (m, 2H), 6.62 (dd, J = 8.4, 2.4 Hz, 1H), 6.58 (dd, J = 8.4, 2.4 Hz, 1H), 4.09 (t, J = 4.8 Hz, 4H), 3.80 (t, J = 4.8 Hz, 4H), 3.73–3.61 (m, 20H), 3.57 (t, J = 4.5 Hz, 4H), 2.42 (s, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.1 (s), 157.0 (s), 145.0 (d, J = 20 Hz), 143.5 (d, J = 20 Hz), 143.2 (d, J = 6 Hz), 135.4 (d, J = 20 Hz), 125.8 (s), 125.7 (s), 122.5 (d, J = 2 Hz), 121.1 (d, J = 2 Hz), 119.3 (d, J = 2 Hz), 118.2 (d, J = 2 Hz), 110.6 (s), 110.5 (s), 110.3 (s), 110.0 (d, J = 2 Hz), 97.5 (d, J = 205 Hz), 72.5 (s), 70.8 (s), 70.6 (s), 70.3 (s), 70.3 (s), 69.7 (s), 67.8 (s), 61.7 (s), 57.5 (d, J = 2 Hz). ^{19}F NMR (471 MHz, D_2O/THF) δ −206.3. HRMS (ESI+) m/z calculated for $[M + Na]^+$ $C_{36}H_{45}FNaO_{10}S$ 711.2632, found 711.2615.

4.2.5 Di(tetraethylene glycol)-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10*H*)-ylsulfenic acid (6a) and potassium di(tetraethylene glycol)-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10*H*)-ylsulfinate (7). Thiols **1** (60 mg, 0.087 mmol, as the isomeric mixture of **1a : 1b** 57 : 43) were dissolved in THF (1 mL) and added to a solution of dibasic potassium phosphate (30.4 mg, 0.194 mmol) in HPLC-grade H_2O (900 μL). The mixture was thoroughly mixed to ensure homogeneity before 100 μL of H_2O_2 as a 1.94 M solution in D_2O/H_2O was slowly added while stirring. Progress of the reaction was monitored by ^{19}F NMR. Upon complete consumption of the starting material the reaction was concentrated *in vacuo* until almost no water remained. The residue was purified by column chromatography (gradient elution 4–10% MeOH/$CHCl_3$) to obtain the isomeric sulfenic acids **6** (28.2 mg, 46% total yield, **6a : 6b** = 58 : 42) and sulfinates **7** (19.1 mg, 22% total yield, **7a : 7b** = 57 : 43). Isomerically pure **6a** was obtained using the same protocol starting from thiol **1a**. To obtain isomerically pure **7a**, sulfenic acid **6a** (5.8 mg, 0.00823 mmol) was dissolved in a 10 mM solution of NaOH in CD_3OD (0.75 mL) in an NMR tube. 25 μL of H_2O_2 as a 300 mM solution in CD_3OD was then added in 5 μL portions until quantitative conversion of **6a** to **7a** was observed by ^{19}F NMR.

*4.2.5.1 2,7-Di(tetraethylene glycol)-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10H)-ylsulfenic acid (**6a**).* ^{1}H NMR (600 MHz, CD_3OD) δ 7.53–7.48 (m, 2H), 7.41–7.38 (d, J = 8.2 Hz, 2H), 7.23 (t, J = 2.1 Hz, 2H), 7.14–7.05 (m, 2H), 6.67 (dd, J = 8.2, 2.1 Hz, 2H), 4.08 (m, 4H), 3.77 (m, 4H), 3.67–3.63 (m, 4H), 3.63–3.57 (m, 12H), 3.56–3.52 (m, 4H), 3.50–3.46 (m, 4H). ^{13}C NMR (151 MHz, CD_3OD) δ 158.4 (s), 146.2 (d, J = 20 Hz), 144.5 (d, J = 6 Hz), 142.7 (d, J = 6 Hz), 138.3 (d, J = 20 Hz), 128.8 (s), 126.6 (d, J = 6 Hz), 123.3 (d, J = 2.5 Hz), 119.8 (d, J = 6 Hz), 118.8 (d, J = 6 Hz), 112.2 (d, J = 2.5 Hz), 111.4 (s), 111.4 (s), 99.0 (d, J = 200 Hz), 73.6 (s), 71.7 (s), 71.6 (s), 71.5 (s), 71.3 (s), 71.3 (s), 70.8 (s), 68.9 (s), 64.5 (d, J = 2.5 Hz), 62.2 (s). ^{19}F NMR (471 MHz, D_2O/THF) δ −206.65. HRMS (ESI+) m/z calculated for $[M + Na]^+$ $C_{36}H_{45}FNaO_{11}S$ 727.2564, found 727.2592.

*4.2.5.2 2,6-Di(tetraethylene glycol)-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10H)-ylsulfenic acid (**6b**).* ^{1}H NMR (600 MHz, CD_3OD) δ 7.53–7.47 (m, 2H), 7.50–7.43 (m, 2H), 7.31–7.27 (m, 1H), 7.17–7.05 (m, 3H), 6.68 (dd, J = 8.2, 2.4 Hz, 1H), 6.62 (dd, J = 8.2, 2.4 Hz, 1H) 4.16 (m, 4H), 3.85 (m, 4H), 3.74–3.57 (m, 20H), 3.54 (t, J = 4.5 Hz, 4H). ^{13}C NMR (151 MHz, CD_3OD) δ 158.6 (s), 158.3 (s), 147.5 (d, J = 20 Hz), 145.7 (d, J = 20 Hz), 145.0 (d, J = 6 Hz), 143.2 (d, J = 6 Hz), 137.8 (d, J = 6 Hz), 135.3 (d, J = 20 Hz), 126.8 (s), 126.5 (s), 125.4 (s), 124.5 (d, J = 2.5 Hz), 123.1 (d, J = 2.5 Hz), 120.0 (d, J = 6 Hz), 119.0 (d, J = 6 Hz), 111.9 (d, J = 2.5 Hz), 111.7 (s), 111.3 (s), 99.0 (d, J = 200 Hz), 74.5 (s), 72.6 (s), 72.4 (s), 72.2 (s), 71.7 (s), 69.8 (s), 64.1 (d, J = 2.5 Hz), 63.1 (s). ^{19}F NMR (471 MHz, D_2O/THF) δ −205.95. HRMS (ESI+) m/z calculated for $[M + Na]^+$ $C_{36}H_{45}FNaO_{11}S$ 727.2564, found 727.2592.

*4.2.5.3 2,7-Di(tetraethylene glycol)-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10H)-ylsulfinate (**7a**).* ^{1}H NMR (500 MHz, CD_3OD) δ 8.22 (br, 2H), 7.96 (br, 1H), 7.46 (m, 1H), 7.37 (d, J = 8.2 Hz, 2H), 7.11–7.04 (m, 2H), 6.67 (dd, J = 8.2 Hz, 2.1 Hz, 2H), 4.15 (m, 4H), 3.81 (m, 4H), 3.71–3.56 (m, 20H), 3.54–3.49 (m, 4H). ^{13}C NMR (126 MHz, CD_3OD) δ 157.3 (s), 157.2 (s), 149.1 (d, J = 20 Hz), 147.7 (d, J = 6 Hz), 143.8 (d, J = 6 Hz), 142.6 (d, J = 6 Hz), 140.2 (d, J = 20 Hz), 128.8 (s), 126.0 (d, J = 20 Hz), 125.4 (d, J = 2.5 Hz), 119.7 (d, J = 6 Hz), 118.6 (d, J = 6 Hz), 113.9 (d, J = 2.5 Hz), 111.1 (s), 99.1 (d, J = 200 Hz), 72.3 (d, J = 2 Hz), 72.1 (s), 70.3 (s), 70.2 (s), 70.1 (s), 70.0 (s), 69.3 (s), 67.5 (s), 60.6 (s). ^{19}F NMR (471 MHz, D_2O/THF) δ −206.1. HRMS (ESI−) m/z calculated for $[M–H]^-$ $C_{36}H_{44}FO_{12}S$ 719.2538, found 719.2526.

*4.2.5.4 2,6-Di(tetraethylene glycol)-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10H)-ylsulfinate (**7b**).* ^{1}H NMR (500 MHz, CD_3OD) δ 8.50 (br, 1H), 7.97 (br, 1H), 7.47 (m, 1H), 7.36 (m, 1H), 7.14–7.04 (m, 3H), 6.71–6.62 (m, 2H), 4.15 (m, 4H), 3.81 (m, 4H), 3.71–3.56 (m, 20H), 3.54–3.49 (m, 4H). ^{13}C NMR (126 MHz, CD_3OD) δ 157.5 (s), 157.4 (s), 145.1 (d, J = 20 Hz), 144.6 (d, J = 20 Hz), 142.0 (d, J = 6 Hz), 141.6 (d, J = 6 Hz), 137.1 (d, J = 20 Hz), 126.1 (s), 125.1 (s), 125.0 (d, J = 2.5 Hz), 119.4 (d, J = 6 Hz), 118.0 (d, J = 6 Hz), 117.0 (d, J = 6 Hz), 116.8 (d, J = 6 Hz), 114.3 (d, J = 2.5 Hz), 113.1 (d, J = 2.5 Hz), 110.5 (s), 110.3 (s), 99.9 (d, J = 200 Hz), 72.4 (d, J = 2.5 Hz), 72.1 (s), 70.3 (s), 70.2 (s), 70.1 (s), 70.0 (s), 69.3 (s), 67.5 (s), 60.6 (s). ^{19}F NMR (471 MHz, D_2O/THF) δ −205.4. HRMS (ESI−) m/z calculated for $[M–H]^-$ $C_{36}H_{44}FO_{12}S$ 719.2538, found 719.2526.

4.2.6 Potassium di(tetraethylene glycol)-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10*H*)-ylsulfonate (8). Thiols **1** (30 mg, 0.044 mmol, as a 53 : 47 isomeric mixture of **1a : 1b**) or purified **1a** were dissolved in THF (1 mL) and added to a solution of dibasic potassium phosphate (15 mg, 0.086 mmol) in HPLC-grade H_2O (900 μL). 114 μL of H_2O_2 as a 1.94 M solution in D_2O/H_2O was added all at once. After stirring overnight, the reaction was concentrated *in vacuo* until almost no water remained. The residue was purified by column chromatography (10% MeOH/$CHCl_3$) to obtain the isomeric sulfonates **8** (30 mg, quantitative yield, **8a : 8b** = 53 : 47).

*4.2.6.1 2,7-Di(tetraethylene glycol)-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10H)-ylsulfonate (**8a**).* ^{1}H NMR (500 MHz, CD_3OD) δ 8.23 (m, 1H), 7.91 (t, J = 2.1 Hz, 2H), 7.51–7.45 (m, 1H), 7.41–7.35 (m, 2H), 7.14–7.04 (m, 2H), 6.71–6.63 (m, 2H), 4.15 (m, 4H), 3.81 (m, 4H), 3.71–3.56 (m, 20H), 3.54–3.49 (m, 4H). ^{13}C NMR (126 MHz, CD_3OD) δ 156.5 (s), 145.1 (d, J = 20 Hz), 141.5 (d, J = 6 Hz), 140.0 (d, J = 6 Hz), 137.5 (d, J = 20 Hz), 125.1 (s), 124.9 (d, J = 2 Hz), 124.8 (s), 117.9 (d, J = 6 Hz), 116.9 (d, J = 6 Hz), 113.0 (d, J = 2 Hz), 110.4 (s), 97.5 (d, J = 200 Hz), 73.6 (d, J = 2 Hz), 73.4 (s), 71.6 (s), 71.5 (s), 71.4 (s), 71.3 (s), 71.2 (s), 68.9 (s), 62.2 (s), 62.0 (s). ^{19}F NMR (471 MHz, D_2O/THF) δ −205.9. HRMS (ESI−) m/z calculated for $[M–H]^-$ $C_{36}H_{44}FO_{13}S$ 735.2503, found 735.2491.

*4.2.6.2 2,6-Di(tetraethylene glycol)-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10H)-ylsulfonate (**8b**).* ^{1}H NMR (500 MHz, CD_3OD) δ 8.23 (m, 1H), 8.12 (dd, J = 8.4, 1.4 Hz, 1H), 7.89 (t, J = 2.1 Hz, 1H), 7.51–7.45 (m, 1H), 7.41–7.35 (m, 1H), 7.14–7.04 (m, 3H), 6.71–6.63 (m, 2H), 4.15 (m, 4H), 3.81 (m, 4H), 3.71–3.56 (m, 20H), 3.54–3.49 (m, 4H). ^{13}C NMR (126 MHz, CD_3OD) δ 156.7 (s), 156.6 (s), 146.4 (d, J = 20 Hz), 144.5 (d, J = 20 Hz), 142.0 (d, J = 6 Hz), 140.0 (d, J = 6 Hz), 137.0 (d, J = 20 Hz), 132.5 (d, J = 6 Hz), 126.2 (d, J = 2 Hz), 125.0 (s), 124.9 (d, J = 2 Hz), 124.7 (d, J = 2 Hz), 118.0 (d, J = 6 Hz), 117.0 (d, J = 6 Hz), 112.7 (d, J = 2 Hz), 110.4 (s), 110.0 (s), 97.5 (d, J = 200 Hz), 73.6 (d, J = 2 Hz), 73.4 (s), 71.6 (s), 71.5 (s), 71.4 (s), 71.3 (s), 71.2 (s), 68.9 (s), 62.2 (s), 62.0 (s). ^{19}F NMR (471 MHz, D_2O/THF) δ −205.25. HRMS (ESI−) m/z calculated for $[M–H]^-$ $C_{36}H_{44}FO_{13}S$ 735.2503, found 735.2487.

4.2.7 2,7-Di(tetraethylene glycol)-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10*H*)-yl disulfide (13a). In an NMR tube, thiol **1a** (8.2 mg, 11.9 μmol) was dissolved in THF (175 μL) containing 240 mM of TFE (internal standard). Phosphate-buffered saline (490 μL of a 50 mM pD 7.8 solution in D_2O) was then added. Upon reaching homogeneity, 17 μL of NaOCl (as a 700 mM solution in D_2O/H_2O) was added portion-wise, monitoring the consumption of **1a**. The tube was mixed, and the reaction was complete upon acquiring the next set of scans (*ca.* 45 s). The contents of the NMR tube were diluted with distilled H_2O (10 mL) and extracted with $CHCl_3$ (3 × 5 mL), dried over $MgSO_4$, and concentrated *in vacuo* to give **13a** (8.2 mg, 11.9 μmol). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11 (d, J = 7.5 Hz, 2H), 7.55 (d, 2H), 7.39 (d, J = 8.2 Hz, 4H), 7.33 (t, J = 2.1 Hz, 4H), 7.13 (td, J = 7.5 Hz, 1.3 Hz, 2H), 7.04 (td, J = 7.5 Hz, 1.3 Hz, 2H), 6.56 (d, J = 8.2 Hz, 2.1 Hz, 4H), 4.15 (m, 8H), 3.81 (m, 8H), 3.71–3.56 (m, 40H), 3.54–3.49 (m, 4H), 2.78 (br s, exchangeable Hs). ^{13}C NMR (151 MHz, $CDCl_3$) δ 156.4 (s), 144.7 (d, J = 20 Hz), 142.8 (d, J = 6 Hz), 141.5 (s), 136.3 (d, J = 20 Hz), 126.1 (s), 125.3 (s), 123.1 (s), 119.0 (d, J = 2.5 Hz), 118.1 (d, J = 2.5 Hz), 118.1 (d, J = 2.5 Hz), 111.7 (s), 111.4 (s), 97.2 (d, J = 200 Hz), 72.5 (s), 70.6 (s), 70.5 (s), 70.3 (s), 69.5 (s), 67.0 (s), 62.8 (d, J = 2.5 Hz), 61.7 (s). ^{19}F NMR (471 MHz, D_2O/THF) δ −205.9. HRMS (ESI+) m/z calculated for $[M + Na]^+$ $C_{72}H_{88}F_2O_{20}NaS_2$ 1397.5176, found 1397.5193.

4.2.8 2,7-Di(tetraethylene glycol)-10-fluoro-9,10-[1′,2′]benzenoanthracen-9(10*H*)-ylsulfenyl chloride (14a). In an NMR tube, thiol **1a** (6.9 mg, 10.0 μmol) was dissolved in $CDCl_3$ (700 μL) containing TFE (internal standard). *N*-Chlorosuccinimide as a 200 mM solution in $CDCl_3$ was added portion-wise every 5 minutes until the starting thiol was completely consumed (*ca.* 50 μL, 1 eq.). The contents of the NMR tube were washed with distilled H_2O (5 mL) and extracted with $CHCl_3$ (3 × 3 mL), dried over $MgSO_4$, and concentrated *in vacuo* to give **14a** (6.8 mg, 94% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 (dd, J = 7.4 Hz, 1.0 Hz, 2H), 7.45 (d, J = 8.2 Hz, 2H), 7.39 (d, J = 8.2 Hz, 2H), 7.23 (br m, 2H), 7.17–7.07 (m, 2H), 6.65 (d, J = 8.2 Hz, 2.1 Hz, 2H), 4.09 (m, 4H), 3.81 (m, 4H), 3.72–3.61 (m, 20H), 3.58–3.54 (m, 4H), 2.61 (br s, exchangeable H's). ^{13}C NMR (151 MHz, CD_3OD) δ 156.9 (s), 144.4 (d, J = 20 Hz), 141.3 (d, J = 6 Hz), 139.8 (d, J = 6 Hz), 136.6 (d, J = 20 Hz), 126.2 (s), 125.6 (s), 122.1 (br), 119.4 (d, J = 6 Hz), 118.4 (d, J = 6 Hz), 110.8 (s), 97.4 (d, J = 200 Hz), 72.5 (s), 70.8 (s), 70.7 (s), 70.6 (s), 70.3 (s), 69.6 (s), 67.8 (s), 63.8 (d, J = 2 Hz), 61.7 (s). ^{19}F NMR (471 MHz, D_2O/THF) δ −206.4. HRMS (ESI+) m/z calculated for $[M + Na]^+$ $C_{36}H_{44}FClO_{10}S$ 745.2225, found 745.2249.

4.2.9 10-Fluoro-9,10-[1′,2′]benzenoanthracen-9(10H)-yl disulfide (15). Thiol **9** (ref. 17) (30 mg, 0.1 mmol) was suspended in MeOH (5 mL), potassium carbonate (45 mg, 0.3 mmol) was added as a solid, and the mixture was stirred for 10 minutes at room temperature. Iodine (50 mg, 0.2 mmol) was then added directly and the reaction was stirred for 2 hours. The reaction was then poured into an aqueous solution (50 mL) of sodium thiosulfate pentahydrate (100 mg, 0.4 mmol), and subsequently extracted with EtOAc (3 × 25 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (10% EtOAc/hexanes) to obtain disulfide **15** (11 mg, 37%). Although conversion looked to be complete by TLC, the isolated yield was reduced due to low solubility of the compound and corresponding losses up during chromatography. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.99 (dd, J = 7.6, 1.1 Hz, 6H), 7.61 (dd, J = 7.6, 1.1 Hz, 6H), 7.11 (td, J = 7.6, 1.1 Hz, 6H), 6.94 (td, J = 7.6, 1.1 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 146.1, 143.5, 143.3, 141.5, 126.0, 125.5, 123.3, 118.0, 96.6, 62.8. ^{19}F NMR (282 MHz, C_6D_6) δ −206.6. HRMS (EI) m/z calculated for $[M]^+$ $C_{40}H_{24}F_2S_2$ 606.1288, found 606.1287.

4.3 ^{19}F NMR-monitored reactions

4.3.1 Buffer preparation. Mixtures of K_2HPO_4 and KH_2PO_4 were dissolved in D_2O to a final concentration of 50 mM. Either KOH or HCl was added to adjust the pH to the desired value. Observed pH (pD) was converted to pH by subtracting 0.4.[19]

4.3.2 Oxidation of 1 with H_2O_2. Solutions of **1** (typically 15–20 mM) in THF containing 240 mM TFE were prepared before each experiment. 175 μL of this solution was transferred to a NMR tube. 525 μL of phosphate-buffered D_2O was added to the NMR tube and the mixture was vortexed to homogeneity. An initial set of scans was acquired and then H_2O_2 was added as a 1.94 M solution in D_2O/H_2O. The NMR tube was mixed before the reaction was monitored for 15 hours. COPASI[44] was used to fit the data to a simple kinetic scheme for successive bimolecular reactions of thiol, sulfenic and sulfinic acids with H_2O_2, which yielded the observed rate constants given in the text.

4.3.3 Oxidation of 1a with HOCl. A solution of **1a** (68 mM) in THF containing 240 mM TFE was prepared. An aliquot (175 μL) of this solution was transferred to an NMR tube and 490 μL of phosphate-buffered D_2O (pD 7.8) was added. An initial spectrum was recorded, after which 0.35 eq. of NaOCl (as a solution in D_2O) was added at once. The tube was mixed and the spectrum was recorded, revealing immediate quantitative conversion to the disulfide **13a**.

4.3.4 Reduction of sulfenyl chloride 14a with 1a. A solution of **14a** (50 mM) in THF containing 240 mM TFE was prepared. An aliquot (175 μL) of this solution was transferred to an NMR tube and 490 μL of phosphate-buffered D_2O (pD 7.8) was added. The NMR tube was left overnight under ambient conditions to rule out hydrolysis as a decomposition pathway. After 18 h an initial set of spectra was acquired and a solution of **1a** (20 mM)

in D_2O/THF (3 : 1) was added dropwise, while mixing, revealing stoichiometric conversion of **14a** to **13a**.

4.3.5 Oxidation of 1 with thermally generated $^1O_2$. A 20 mM solution of **1** in THF containing 240 mM TFE was prepared before the experiment. 175 μL of this solution was transferred to an NMR tube and 525 μL of an ice-cooled freshly prepared 27 mM solution of $NDPO_2$ (prepared using a reported procedure[45]) in phosphate-buffered D_2O (pD 7.8) was added to the NMR tube. The NMR tube was mixed and an initial set of scans at 25 °C was acquired before the NMR spectrometer was heated to 37 °C using a thermostat. The reaction was then monitored for 30 min. Due to significant deviation in chemical shift values from the conditions involving oxidation of **1** by H_2O_2, a reaction mixture obtained by reacting thiol **1** with H_2O_2 was added to the reaction mixture after completion to identify the observed products.

4.3.6 Oxidation of 9 with photochemically generated $^1O_2$. In an NMR tube, **9** (6 mg, 0.02 mmol) was suspended in CD_3OD (690 mL), to this suspension was added sodium hydroxide as a 1 M solution in water (60 μL, 0.06 mmol, 3 eq.) and the mixture became homogeneous. 10 μL of a 1.5 mM aqueous solution of Rose Bengal (0.0002 mmol. 0.01 eq.) was added and the reaction mixture was cooled to *ca.* 4 °C. Trifluorotoluene (10 mM) was used as an internal standard for NMR quantification. Oxygen was bubbled through the solution after which the reaction vessel was irradiated with a 400 W HPS lamp. Time points were taken to analyze reaction progress. The reaction was continued to the point that **9** was fully consumed, after which the reaction was concentrated *in vacuo*. Following this, the crude product was concentrated *in vacuo*, suspended in C_6D_6, and analyzed by ^{19}F NMR, which resulted in the observation of disulfide **14** as a trace reaction product, **11** and **12** were not observed despite being major products as they are insoluble in C_6D_6 (Fig. S5†). Products were assigned after adding authentic **12** to the NMR tube (CD_3OD). Monitoring of reaction progress is shown in Fig. 3B of the manuscript. ^1H NMR spectra of the initial and final (after 6 min. of irradiation) time points are shown in Fig. S4.†

4.3.7 Reduction of sulfenic acid 6 with ascorbate. A 5 mM solution of **6** in THF containing 10 mM TFE was prepared before the experiment. 175 μL of this solution was transferred to an NMR tube and 300 μL of phosphate-buffered pD 7.8 D_2O was added to the NMR tube before an initial set of scans was acquired. No spectroscopic changes were observed after 24 h. An additional time point was taken after 3 days, and no changes were observed.

4.3.8 Reduction of sulfenic acid 10 with ascorbate and iron. Sulfenic acid **10** (6.4 mg, 0.02 mmol) was dissolved in MeOH (8 mL). 1 mL of a freshly prepared 200 mM solution of ascorbic acid in MeOH (1 eq.) was then added, followed by addition of a 20 mM solution of $Fe_2(SO_4)_3$ in MeOH (1 mL, 0.2 eq.). The mixture was stirred for 24 hours, after which it was diluted with CH_2Cl_2 (50 mL), filtered through a silica plug and washed with 20% MeOH/CH_2Cl_2 (50 mL). The filtrate was concentrated *in vacuo* and analyzed by ^{19}F NMR. No changes were observed, with the mass balance indicating that no reaction had occurred.

Data availability

Additional experimental procedures and reaction monitoring data, NMR spectra and computational data are available in the ESI.†

Author contributions

D. D. S. designed and executed the experiments and carried out quantum chemical calculations. D. A. P. conceived the project and contributed to experimental design. D. D. S. and D. A. P. wrote the manuscript.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

‡ Kinetic studies on reactions of protein sulfenic acids have been conducted, however, the approach did not involve direct visualization of the RSOH intermediate. See ref. 39.

§ It was assumed that the sulfinic acid is fully deprotonated at this pD, based on previously measured pK_a values.[17]

¶ Reduction potentials in water ($E^0(RS^{\bullet}/RS^-) = +0.84$ V *vs.* NHE[46] and $E^0(^1O_2/O_2^{\bullet-})$ = +0.81 *vs.* NHE[47]) suggest that the reaction of $^1O_2$ and RS^- is essentially thermoneutral. This may indicate an even stronger oxidant than $^1O_2$ is formed in the reaction. Earlier studies support this notion, as it has been shown that thiols significantly exacerbate the damage to DNA caused by $^1O_2$ and that hydroxyl radicals may be formed in the reaction.[48]

‖ Three equivalents of ascorbate were selected for this reaction based on the reported rate constants.[49] If reduction were to occur, it would be possible to monitor by ^{19}F NMR under these conditions.

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