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Note

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Oxidative formation of disulfide bonds by a chemiluminescent 1,2-dioxetane under mild conditions

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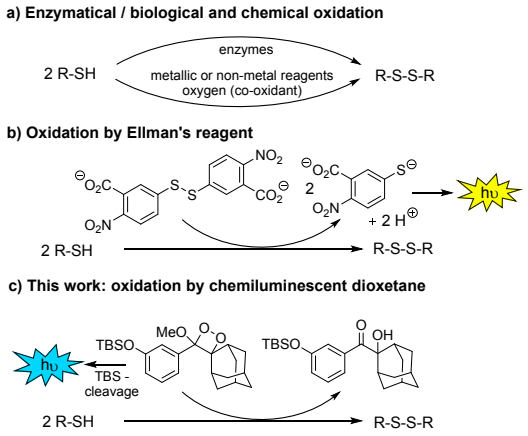
Supporting Information Placeholder

ABSTRACT: The oxidation of alkyl thiols to disulfides has been achieved under mild conditions using a chemiluminescent 1,2-dioxetane as stoichiometric oxidant. Besides the mild and biocompatible reaction conditions, this approach offers the possibility to monitor the presence of thiols through oxidation and chemiluminescence of the remaining dioxetane.

In general, and with cysteine as the most prominent example, thiols play an important role in nature ranging from the formation of disulfide bridges to induce the folding of peptides and proteins to the activation of signaling pathways to the binding of metal ions. Moreover, cysteines and their related thiolate anions may serve as nucleophiles in the active site of enzymes or may be involved in redox catalytic processes such as oxidations and reductions mediated by thioredoxins.[1] While the formation of disulfides from thiols in nature mainly occurs through enzymatic oxidations,[2, 3] a large and diverse number of synthetic methods have been developed to achieve this highly important transformation (Scheme 1a). Suitable oxidants include metal-based reagents such as permanganate salts, ferric chloride, cerium(IV) salts, copper(II) salts,[4] chromates or dichromates,[5] rhenium-sulfoxide complexes,[6, 7] as well as diverse non-metal reagents comprising sodium perborate, sodium chlorite, bromine on silica gel,[8] *N*-bromosuccinimide,[9] nitric oxide, sodium nitrite,[4] hydrogen peroxide in tetrafluoroethanol,[10] hydantoin derivatives,[11-13] sulfuryl chloride,[14] and peroxymonosulfate,[15] among others. Further methods involve oxygen as a co-oxidant combined with suitable catalysts, as for example a $FeCl_3$-NaI/air[16] or a CsF-Celite/air system.[17] In this context, particularly mild and biocompatible reaction conditions, such as oxidations under air[4, 18, 19] or by iodine,[20] are highly desirable since they can be potentially applied to complex biomolecules. In addition, the simultaneous visualization of disulfide bond formation can be a useful feature. So far, mainly mild oxidants such as Ellman's reagent[21] (5,5'-dithiobis-(2-nitrobenzoic acid), DTNB), solid-phase versions thereof,[22, 23] and related derivatives[24-28] have been used to determine the amount of free thiols groups along with oxidation (Scheme 1b). With regard to visualization, structural combinations of Ellman's reagent with chemiluminescent probes[29] or with fluorescent dyes[30] are particularly interesting, whereat both strategies have been applied to determine choline esterase activity.

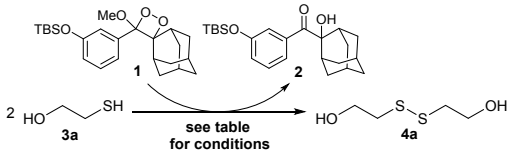
Scheme 1. Thiol oxidation in biology and synthetic chemistry.



In this work, we present a novel strategy for the mild formation of disulfides using a chemiluminescent 1,2-dioxetane as stoichiometric oxidant.[31] The reaction can then be further investigated by exploiting the light-emitting properties of the 1,2-dioxetane. Various derivatives of chemiluminescent dioxetanes have so far been reported and been used for diverse applications.[32-34] For the present work, the *tert*-butyldimethylsilyl (TBS) analog **1** was selected, from which chemically initiated electron-exchange luminescence[35] (CIEEL) can be easily triggered by the cleavage of the TBS group by fluoride ions[36], or by other conditions (Table 1).[37] For the synthesis of **1**, we followed the McMurry cross-coupling approach by Baader and co-workers[38] using TBS-protected methyl 3-hydroxybenzoate[39] and 2-adamantanone as starting materials.[40] Photooxygenation of the resulting methyl vinyl ether in the presence of methylene blue provided **1**.[41]

Initial experiments showed that the reductive ring-opening of the chemiluminescent 1,2-dioxetane **1** can be indeed coupled to the oxidation of 2-mercaptoethanol **3a** yielding disulfide **4a** along with the 2-hydroxy ketone **2**, which is most likely formed from **1** via an unstable α-hydroxy hemiacetal (Table 1). The structure of the so far unknown ketone **2** was confirmed after isolation using HRMS and NMR analysis, with a C_q signal at 205.1 ppm providing strong support.

Table 1. Influence of base on the 1,2-dioxetane-induced oxidation of thiol 3a.



entry	solvent	K_2CO_3 (eq.)[g]	**4a** (%)[h]	**2** (%)[i]
1[a]	CD_3OD	-	< 5	0
2[b]	CD_3OD	0.1	33	35
3[b]	CD_3OD	0.2	60	61
4[b]	CD_3OD	0.4	72	80
5[b]	CD_3OD	1.0	76	88
6[b]	$(CD_3)_2SO$	1.0	63	66
7[c]	CD_3CN, D_2O, CD_3OD	1.0	75	84
8[d,e]	CD_3OD, Tris buffer	-	< 5	0
9[d,f]		-	23	< 15

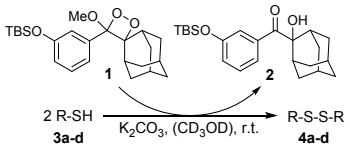
[a] Standard conditions (NMR tube): **1** (0.01 mmol), 2-mercaptoethanol (**3a**) (0.02 mmol) in solvent (700 µL). ^{1}H NMR spectrum recorded after 3 hours at r.t. [b] CD_3OD or $(CD_3)_2SO$ previously incubated with K_2CO_3 (15 min). [c] 10% CD_3CN, 10% D_2O, only CD_3OD incubated with K_2CO_3. [d] 10% Tris buffer (D_2O, pH = 8.6) in CD_3OD. [e] ^{1}H NMR spectrum recorded after 1.5 hours. [f] ^{1}H NMR spectrum recorded after 18 hours. [g] Equivalents refer to **3a**. [h] Yield of **4a** derived from ratio of **3a** and **4a**. [i] Yield of **2** derived from ratio of **1** and **2**.

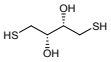
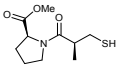
The reactions conducted for optimization were monitored by ^{1}H NMR spectroscopy in CD_3OD, whereas the emerging triplet signals of disulfide **4a** at 2.84 ppm and 3.79 ppm turned out as reliable indicators. Since major side products could not be detected, the yields were calculated from ratios of **1**:**2** and **3a**:**4a**. The experiments summarized in Table 1 show that the use of CD_3OD saturated with potassium carbonate (approx. 11 mM) (entry 5) provides the highest yield of disulfide **4a** (76%). While lower amounts of base (entries 1-4) and the change to $(CD_3)_2SO$ slowed down the reaction (entry 6), the mixture of CD_3OD, D_2O, and CD_3CN also provided disulfide **4a** in a good yield of 75% (entry 7). Finally, an attempt with alkaline Tris buffer[42] (entries 8 and 9) revealed that the desired oxidation proceeds much more readily in methanol (entry 5). In aqueous systems (entries 7-9), changes in the additives can lead to strong deviations, whereat the limited solubility of dioxetane **1** may also play a role. This assumption is in agreement with earlier results by Adam,[31] who achieved the oxidation of thiols with a structurally far more simple and more polar, but non-chemiluminescent 1,2-dioxetane in pure water.

While an equable, ideally 1:1-formation of **2** and **4a** would be advantageous regarding quantification of the oxidation by chemiluminescence, increased reaction times - on the other hand - might give rise to side reactions. Against this background, the dioxetane-mediated oxidation of thiol **3a** was evaluated in comparison with three further thiols **3b-d** (Table 2).

Except for the oxidation of *DL*-dithiothreitol (**3b**), the thiols **3a,c**,**d** were now converted with much lower deviations regarding the formation of disulfide **4a,c,d** and hydroxy ketone **2** (max. 7% for **3c**→**4c** after 1 h). We currently assign this to an at that time improved preparation of 1,2-dioxetane **1**, which provided the oxidant in higher purity than previously used for the experiments in Table 1. Two-fold analysis of dioxetane **1,** as obtained by the improved procedure, by ^{1}H NMR using 1,3,5-trimethoxybenzene as internal standard gave purities of 94% and 97%.[43, 44]

Table 2. Oxidation of thiols 3a-d.



entry	thiol **3a-d**		time (h)	**4a-d** (%)[c]	**2** (%)[d]
1[a]		**3a**	1	54	57
			3	82	87
2[b]		**3b**	1	78	57
			3	100	74
3[a]		**3c**	1	44	51
			3	80	84
4[a,e]		**3d**	1	53	53
			3	79	82

[a] Standard conditions (NMR tube): **1** (0.01 mmol) and thiol **3** (0.02 mmol) in CD_3OD (700 µL), previously incubated with K_2CO_3 (0.02 mmol, 15 min). ^{1}H NMR spectrum recorded after 1 hour and 3 hours at r.t. [b] Thiol **3b** (0.01 mmol). [c] The yield of **4a-d** derived from ratio of **3a-d** and **4a-d**. [d] Yield of **2** derived from ratio of **1** and **2**. [e] Transesterification ($-OCH_3 \rightarrow -OCD_3$) observed for **3d** to **4d**.

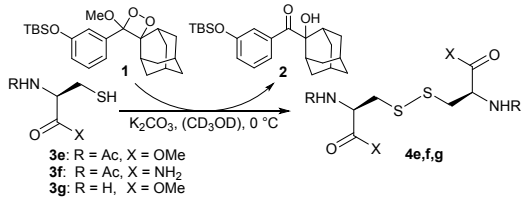
In the oxidation of **3b**, the formation of the cyclic disulfide **4b** occurred faster than the formation of **2**, with deviations of 21% after 1 h and 26% after 3 h. This is most likely due to the intramolecular reaction course, thereby opening an additional mechanistic pathway. As control reactions ruled out a dioxetane-independent oxidation as background reaction,[45] an alternative dioxetane-dependent oxidative mechanism appears plausible for thiol **3b**, which, however, does not follow the typical 1:2 stoichiometry of dioxetane to thiol.

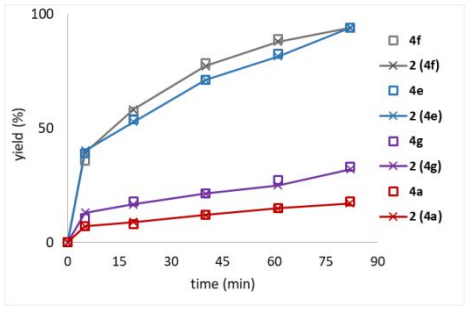
To investigate whether a disulfide **4** reacts further with dioxetane **1**, a control reaction between *trans*-4,5-dihydroxy-1,2-dithiane (**4b**) and **1** in basic methanol under standard reaction conditions was monitored by ^{1}H NMR spectroscopy. Under the conditions of Table 2, and over 24 hours, the cyclic disulfide **4b** was found to be stable. To demonstrate the synthetic applicability and reliability of the novel oxidation method, three disulfides were prepared on a larger than 0.01 mmol scale, followed by isolation and purification. These experiments gave **4a**, **4c**, and **4d** in yields of 81%, 83%, and 83%, respectively, which are well comparable to the NMR yields reported in Table 2 (**4a**: 82%, **4c**: 80%, **4d**: 79%).

When moving to *N*-acetyl *L*-cysteine methyl ester (**3e**) and *N*-acetyl *L*-cysteine amide (**3f**), we found that these oxidations surprisingly proceeded faster than those summarized in Table

2, so that the reaction temperature had to be lowered to 0°C. For comparison, the oxidation of 2-mercaptoethanol **3a** to disulfide **4a**, which was repeated at 0 °C, was added as a reference reaction to Figure 1.

Figure 1. Oxidation of cysteine derivatives 3e,f,g, and thiol 3a at 0 °C.

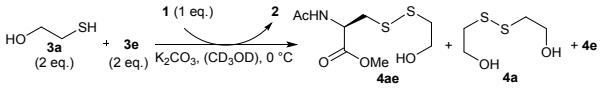


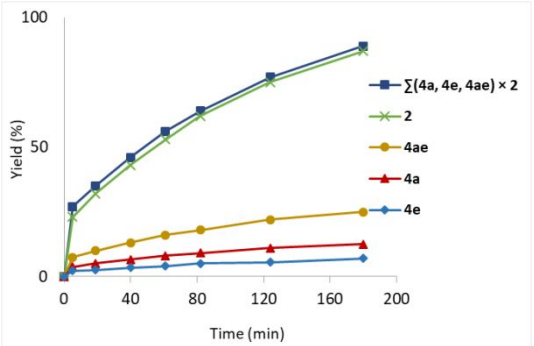


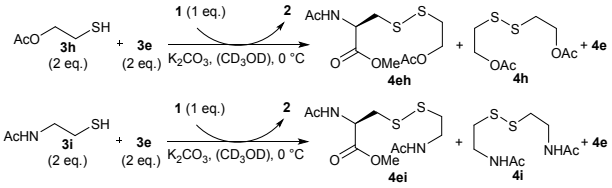
At 0 °C, very low deviations between the formation of disulfides **4a,e,f,g** and the hydroxy ketone **2** were found in each of the four experiments (see Table S1). Furthermore, a control experiment with cysteine **3f** ruled out a dioxetane-independent formation of **4f**. The clean formation of disulfide **4g** from the N-unprotected cysteine **3g** demonstrated that free amino groups do not interfere with the oxidation. Dioxetane **1** thus appears to be more stable towards nucleophiles than the sterically less hindered dioxetanes studied by Adam.[31, 46]

The faster oxidation of the cysteines **3e** and **3f** - compared to thiol **3a** - then turned our interest towards the question of whether the dioxetane **1** might be able to oxidize particular thiols selectively, which would be a unique feature (Figure 2).

Figure 2. Competitive oxidation of thiols 3a, h or i and cysteine derivative 3e.







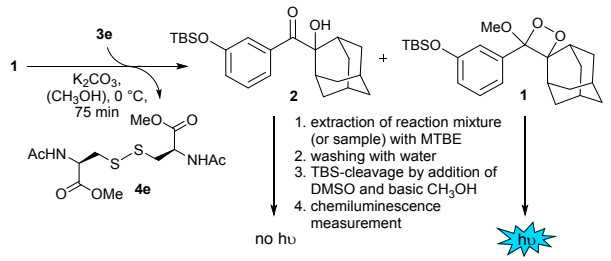
In the first competition experiment (Figure 2, top), it was surprising to see that the formation of the cysteine disulfide **4e** was now even slower than that of **4a**, whereas the mixed disulfide **4ae** arose as major product leading to a final product ratio of **4a**:**4e**:**4ae** = 28:16:56. The summarized yields of the disulfides **4a** (12.5%), **4e** (7%) and **4ae** (25%), when multiplied by a factor of 2 (89%), nicely correspond to the amount of hydroxy ketone **2** (87%), thereby further supporting the suitability of 1,2-dioxetane **1** as a clean 1:2 oxidant for thiols (see Table S2).

When 2-mercaptoethanol (**3a**) was replaced by its *O*-acetylated derivative **3h**, and competition was studied with cysteine **3e**, a final product ratio of **4h:4e:4eh** = 27:19:54 was obtained (Figure 2, bottom). This ratio is only slightly different from the product distribution resulting from the competition of **3a** and **3e**, as shown above. *N*-Acetyl cysteamine (**3i**), however, when competing with **3e**, led to an altered ratio of **4i:4e:4ei** = 42:16:42. In comparison to the thiols **3a**, **3e**, and **3h**, *N*-acetyl cysteamine (**3i**) thus shows the highest reactivity to form symmetric disulfides.

Although these competition experiments give interesting insights into the relative reactivity of particular thiols in combination with dioxetane **1** as mild oxidant, it is yet difficult to draw mechanistic conclusions.[47] In general, radical[31] as well as ionic mechanisms[46] have been described for the oxidation of thiols by 1,2-dioxetanes, whereat seminal and pioneering work on the reaction of 1,2-dioxetanes with thiols has been conducted by the Adam research group.[31]

Finally, we turned to evaluate whether the chemiluminescent properties of dioxetane **1** can be employed to monitor the thiol oxidation process. In a series of preliminary experiments (see Table S3 and Figure S1), it turned out that the dioxetane **1** remaining in the reaction mixture needs to be separated from the newly formed disulfide, as chemiluminescence is otherwise significantly quenched. Moreover, we found that a mixture of basic methanol and DMSO was well suited to cleave the TBS group from **2** and to trigger light emission. On this basis, a simple and practical general procedure could be developed. Accordingly, and as exemplified for the oxidation of cysteine **3e** (Scheme 2), remaining dioxetane **1** and hydroxy ketone **2** were extracted with MTBE from the reaction mixture after oxidation. Alternatively, samples may be taken from an ongoing oxidation. After washing with water, chemiluminescence was triggered by the addition of DMSO and basic CH_3OH.

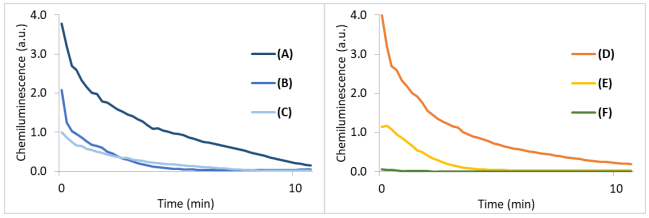
Scheme 2. Procedure for the oxidation of cysteine 3e combined with chemiluminescence read-out.



The chemiluminescence read-out curves depicted in Figure 3 were obtained according to the overall procedure described in Scheme 3. In the left part of Figure 3, the decay curves from three calibration experiments (**A**)-(**C**) are summarized. In the right part, the results of two dioxetane-mediated oxidations of cysteine **3e** with subsequent optical read-out are shown ((**E**),(**F**)). The experiment (**D**), which was performed in the absence of thiol **3e**, gives an impression on the good reproducibility, as it corresponds to the calibration experiment (**A**).

In the oxidation experiments, the ratio of **3e** to **1** of 2:1 led to a full consumption of **1** and basically no light emission (**F**). Starting with a ratio of **3e** to **1** of 4:3 (**E**), left one-fourth of dioxetane **1** unchanged, which should result in approximately 25% of the maximal light emission. As the read-out of experiment (**E**) is well comparable with calibration experiment (**C**), where 25% of the full amount of **1** was used, this underlines the applicability of the method.

Figure 3. Oxidation of varying amounts of cysteine 3e by dioxetane 1 combined with chemiluminescence read-out.[a]



[a] Calibration measurements (**A**)-(**C**): (**A**): 100% of **1**, (**B**): 50% of **1**, (**C**) 25% of **1**. Ratios of reactants in the experiments (**D**)-(**F**): (**D**): only **1** (no thiol added), (**E**): **1**:**3e** (3:4), (**F**): **1**:**3e** (1:2).

In addition to the monitoring of completed oxidation reactions, which allows determining the thiol content (Scheme 2 and Figure 3), the optical read-out can also be applied to follow the reaction course. For this purpose, the oxidation of cysteine **3e** to **4e** at 0°C was quenched after three different intervals. The amounts of remaining dioxetane **1** were determined by chemiluminescence and the values were compared to those measured by ^{1}H NMR (Figure 1 and Table S1). After reaction times of 22.5, 43.5, and 64.5 minutes, calculation of the amount of **1** from the chemiluminescence read-out gave values of 50%, 33%, and 23%, respectively (see Figure S2). Within typical error ranges, these values correspond well to those measured by ^{1}H NMR (47%, 29%, and 18%) after identical reaction times.

From the experiments combined with chemiluminescence measurements, it became obvious that the initial values recorded during the chemiluminescence read-out (performed in triplicates) are well suited for analysis and calculation of the dioxetane concentration. As a result, a recording of the whole decay curve (as shown in Figure 3) is not necessary, which adds to the attractiveness of the overall method.

In conclusion, we have shown that 1,2-dioxetane **1** can serve as a particularly mild oxidant for thiols in methanol to give disulfides. In single reactions and competition experiments, insights into the relative reactivities of the thiols were obtained. Furthermore, a first procedure has been developed, which allows linking the thiol oxidation to the highly sensitive chemiluminescent properties of 1,2-dioxetane **1**, so that the course of thiol oxidation or the thiol content of a sample can be monitored. Regarding the importance of thiols in chemistry and biology, these results represent a valuable starting point for future investigations. Experiments towards more polar and thus fully water-soluble chemiluminescent derivatives of dioxetane **1** are currently underway in our laboratory.

EXPERIMENTAL SECTION

General experimental. All reactions were carried out following common organic chemistry laboratory procedures. Chemicals and solvents were obtained from commercial sources and were used as received. Reactions with air- or moist-sensitive reagents were carried out in glass equipment, previously heated under vacuum and subsequently purged with nitrogen or argon gas. Air or water-sensitive reactions were performed in anhydrous solvents (indicated in each case). Potassium carbonate (anhydrous, free-flowing, Redi-Dri™, ACS reagent, ≥ 99%) was purchased from Sigma-Aldrich (product number: 791776). NMR spectra were recorded on a Bruker Avance III HD 400 (^{1}H-NMR: 400 MHz; DEPTQ: 101 MHz) and a Bruker Avance III HD 600 (^{1}H-NMR: 600 MHz; DEPTQ: 151 MHz) spectrometers. Chemical shifts (δ) are reported in parts per million (ppm). For ^{1}H NMR, $CDCl_3$, CD_3OD or $(CD_3)_2SO$ were used as solvents referenced to the internal standard tetramethylsilane (TMS, δ = 0.00 ppm) or the residue proton signal of the used solvents $CDCl_3$ (δ = 7.26 ppm), CD_3OD (δ = 3.31 ppm) or $(CD_3)_2SO$ (δ = 2.50 ppm). The DEPTQ spectra were calibrated to the characteristic signals of the solvents: $CDCl_3$ (δ = 77.0 ppm), CD_3OD (δ = 49.0 ppm) or $(CD_3)_2SO$ (δ = 39.4 ppm). The following abbreviations were used for the signal multiplicity: s (singlet), d (doublet), dd (doublet doublet), t (triplet), dt (doublet triplet), q (quadruplet), m (multiplet), br (broad). Coupling constants *J* were indicated in Hertz (Hz). The spectra were evaluated with the program MestReNova.

Thin-layer chromatography (TLC) analyses were performed on Merck 60 F254 aluminum sheets (0.25 mm silica gel) and visualized by fluorescent detection using UV light (254 nm and 360 nm) or the following TLC stain: $KMnO_4$ [3.0 g $KMnO_4$, 20 g K_2CO_3, 5 mL NaOH (5% w/w) in 300 mL H_2O]. Flash chromatography was carried out using silica gel 60 (40 – 63 µm, 230 – 400 mesh ASTM) as stationary phase and under pressure of 1.0 – 1.5 bar. The used solvent mixtures are listed at the respective procedures. Liquid chromatography – mass spectrometry (LC-MS) was carried out on a Waters e2695 HPLC system with a photodiode array detector for detection at λ = 220 and 240 nm and a quantum diode array detector for mass analysis. A binary solvent gradient of acetonitrile and Milli-Q® water was applied on a C18 column (2.1 × 50 mm × 2.6 µm). High resolution mass spectrometry (HRMS) was carried out on a timsTOF Pro from the company Brucker with ESI source. A warm-white LED lamp (PAR38, 2800K, 18 W) was used for light irradiation. Measurements of the luminescence intensities were carried out with a Victor^3 V 1420 Multilabel Plate Counter from the company Perkin Elmer in triplicates. The operation "Chemolumineszenz" was applied and the delay between repeats was 2 seconds. 96-well white clear-

bottom microtest plates were used for the measurement of the probes.

Among the starting materials **1**, **3a-i**, the compounds **3a-c**,**e**,**g,h** and **3i** were commercially available. Dioxetane **1**[39-41] and thiols **3d**[48] and **3f**[49] were prepared according to known methods. Among the disulfides **4a-i**, **4a,c**,**e,g** were commercially available and were used as authentic samples for comparison. Compounds **4b,d,f,h,i** were prepared according to known methods to be available for comparison.[20, 50, 51]

Preparation and characterization of compounds.

Preparation of **1** was carried out according to known methods.[39-41]

4-[(3-tert-Butyldimethylsilyloxy)phenyl]-4-methoxyspiro[1,2-dioxetane-3,2'-adamantane] (**1**): ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.19 (s, 6 H), 0.98 (s, 9 H), 1.23 – 1.90 (m, 12 H), 2.24 (br s, 1 H), 3.02 (br s, 1 H), 3.24 (s, 3 H), 6.93 (dd, *J* = 2.4 Hz, *J* = 9.1 Hz, 1 H), 7.38 (t, *J* = 7.6 Hz, 1 H). 2 signals are missing. ^{1}H-NMR (600 MHz, CD_3OD): δ (ppm) = 0.21 (s, 6 H), 1.00 (s, 9 H), 1.08 (dt, *J* = 2.7 Hz, *J* = 13.1 Hz, 1 H), 1.26 (dd, *J* = 2.9 Hz, *J* = 13.1 Hz, 1 H), 1.47 – 1.93 (m, 10 H), 2.17 (br s, 1 H), 2.97 (br s, 1 H), 3.20 (s, 3 H), 6.93 (ddd, *J* = 1.0 Hz, *J* = 2.5 Hz, *J* = 8.1 Hz, 1 H), 7.35 (t, *J* = 7.8 Hz, 1 H). 2 signals are missing. DEPTQ (151 MHz, CD_3OD): δ (ppm) = -4.2 (2 × CH_3), 19.1 (C_q), 26.1 (3 × CH_3), 27.4 (CH), 27.6 (CH), 32.6 (CH_2), 33.0 (CH), 33.3 (CH_2), 34.0 (CH_2), 34.7 (CH), 35.7 (CH_2), 37.4 (CH_2), 50.1 (CH_3), 96.5 (C_q), 113.1 (C_q), 122.6 (br, CH), 130.6 (br, CH), 137.6 (C_q), 157.2 (C_q). 2 signals are missing. The analytical data are in agreement with those reported in literature.[38]

Procedures used for optimization (Table 1).

The equivalents of K_2CO_3 refer to 2-mercaptoethanol (**3a**).

Entry 1: Dioxetane **1** (5.00 mg, 0.01 mmol) and 2-mercaptoethanol (**3a)** (1.40 µL, 0.02 mmol) were dissolved in CD_3OD (700 µL) in a NMR tube. After 3 hours at room temperature, a ^{1}H NMR spectrum was recorded at room temperature.

Entries 2-6: CD_3OD or $(CD_3)_2SO$ (700 µL) was previously incubated with K_2CO_3 (0.1 – 1.0 eq.) for 15 minutes at room temperature. Subsequently, dioxetane **1** (5.00 mg, 0.01 mmol) and 2-mercaptoethanol (**3a)** (1.40 µL, 0.02 mmol) were dissolved in the CD_3OD supernatant in a NMR tube. After incubation of 3 hours at room temperature, a ^{1}H NMR spectrum was recorded at room temperature.

Entry 7: CD_3OD (700 µL) was previously incubated with K_2CO_3 (1.0 eq.) for 15 minutes at room temperature. Subsequently, dioxetane **1** (5.00 mg, 0.01 mmol) and 2-mercaptoethanol (**3a)** (1.40 µL, 0.02 mmol) were dissolved in a solvent mixture, comprising of 80% CD_3OD supernatant (560µL), 10% D_2O (70 µL) and 10% CD_3CN (70 µL) in a NMR tube. After incubation of 3 hours at room temperature, a ^{1}H NMR spectrum was recorded at room temperature.

Entries 8 and 9: Dioxetane **1** (5.00 mg, 0.01 mmol) and 2-mercaptoethanol (**3a**) (1.40 µL, 0.02 mmol) were dissolved in a solvent mixture, comprising of CD_3OD (600 µL) and Tris buffer in D_2O (60 µL, 100 mM, pH = 8.6) in a NMR tube. After incubation of 1.5 and 18 hours at room temperature, a ^{1}H NMR spectrum was recorded at room temperature, respectively.

Determination of the amount of dissolved K_2CO_3 in CD_3OD.

K_2CO_3 (37.9 mg, 0.27 mmol) was incubated in CH_3OH (10.0 mL) for 15 minutes at room temperature. The solvent of the CH_3OH supernatant was removed under reduced pressure to yield the K_2CO_3 (15.2 mg). Referring to the NMR experiment (Table 1, Entry 5), this means that in 700 µL CD_3OH 1.03 mg K_2CO_3 is dissolved. This corresponds to a molarity of 10.6 mM.

[(3-tert-Butyldimethylsilyloxy)phenyl]-2-hydroxyadamantan-2-yl]methanone (**2**): To confirm the structure of hydroxy ketone **2**, an experiment was performed on a larger scale to isolate the product **2**. CH_3OH (3.36 mL) was previously incubated with K_2CO_3 (12.7 mg, 0.92 mmol) for 15 minutes at room temperature. The supernatant solution was transferred to a tube and used to dissolve dioxetane **1** (20.0 mg, 0.05 mmol) and 2-mercaptoethanol (**3a**) (7.00 µL, 0.10 mmol). After stirring for 3 hours at room temperature, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (*iso*-hexane / ethyl acetate = 20:1) to yield **2** as a clear oil. R_f value: 0.2 (*iso*-hexane / ethyl acetate = 20:1) [UV]. ^{1}H-NMR (400 MHz, CD_3OD): δ (ppm) = 0.22 (s, 6 H), 1.00 (s, 9 H), 1.55 – 1.82 (m, 10 H), 2.29 (br s, 2 H), 2.38 (d, *J* = 12.6 Hz, 2 H), 6.99 (ddd, *J* = 1.0 Hz, *J* = 2.5 Hz, *J* = 8.1 Hz, 1 H), 7.28 (t, *J* = 7.7 Hz, 1 H), 7.55 (ddd, *J* = 1.1 Hz, *J* = 1.6 Hz, *J* = 7.7 Hz, 1 H), 7.56 – 7.62 (m, 1 H). DEPTQ (151 MHz, CD_3OD): δ (ppm) = −4.3 (2 × CH_3), 19.1 (C_q), 26.2 (3 × CH_3), 28.5 (CH), 28.6 (CH), 33.4 (CH_2), 35.6 (CH_2), 35.9 (2 × CH), 38.6 (CH_2), 39.3 (2 × CH_2), 81.0 (C_q), 121.8 (CH), 123.3 (CH), 124.3 (CH), 130.1 (CH), 140.0 (C_q), 156.4 (C_q), 205.1 (C_q). HRMS (ESI) m/z: $[M + H]^+$ calcd. for $C_{23}H_{35}O_3Si$ 387.2350; Found: 387.2349.

2-Hydroxyethyl disulfide (**4a**): ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 2.84 (t, *J* = 6.5 Hz, 4 H), 3.79 (t, *J* = 6.5 Hz, 4 H). This data is in agreement with those obtained from an authentic sample under identical conditions.

General Procedure for Table 2.

CD_3OD (700 µL) was previously incubated with K_2CO_3 (0.02 mmol) for 15 minutes at room temperature. The supernatant solution was transferred to a NMR tube and used to dissolve dioxetane **1** (5.00 mg, 0.01 mmol) and the respective thiol **3a-d** (0.01 or 0.02 mmol). After incubation for 1 hour and 3 hours at room temperature, a ^{1}H NMR spectrum was recorded at room temperature, respectively.

Trans-4,5-dihydroxy-1,2-dithiane (**4b**): ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 2.87 (dd, *J* = 9.9 Hz, *J* = 13.4 Hz, 2 H), 3.03 (d, *J* = 13.3 Hz, 2 H), 3.46 – 3.54 (m, 2 H). This data is in agreement with those obtained from an authentic sample under identical conditions.

Dibenzyl disulfide (**4c**): ^{1}H NMR (600 MHz, CD_3OD): δ (ppm) = 3.61 (s, 4 H), 7.21 – 7.33 (m, 10 H). This data is in agreement with those obtained from an authentic sample under identical conditions.

Captopril methyl ester disulfide (**4d**): R_f value: 0.2 (dichloromethane / methanol = 40:1) [$KMnO_4$]. ^{1}H-NMR (400 MHz, CD_3OD): δ (ppm) = 1.21 (d, *J* = 6.9 Hz, 6 H), 1.91 – 2.09 (m, 6 H), 2.19 – 2.31 (m, 2 H), 2.72 (dd, *J* = 5.1 Hz, *J* = 13.3 Hz, 2 H)., 2.93 (dd, *J* = 9.0 Hz, *J* = 13.3 Hz, 2 H), 3.11 – 3.20 (m, 2 H), 3.70 (s, 6 H), 3.72 – 3.79 (m, 4 H), 4.54 (dd, *J* = 4.1 Hz, *J* = 8.7 Hz, 2 H). DEPTQ (151 MHz, CD_3OD): δ (ppm) = 17.1 (2 × CH_3), 25.7 (2 × CH_2), 30.1 (2 × CH_2), 39.0 (2 × CH), 42.1 (2 × CH_2), 48.5 (2 × CH_2), 52.7 (2 × CH_3), 60.3 (2 × CH), 174.2 (2 × C_q), 175.9 (2 × C_q). MS (ESI) m/z: $[M + H]^+$ 461.23. HRMS (ESI) m/z: $[M + H]^+$ calcd. for $C_{20}H_{33}N_2O_6S_2$ 461.1775; Found: 461.1776.

Procedures for large scale reactions to disulfides 4a,c,d.

Preparation of disulfide **4a**: CH_3OH (4.20 mL) was previously incubated with K_2CO_3 (15.9 mg, 0.12 mmol.) for 15 minutes at room temperature. The supernatant solution was transferred to a tube and used to dissolve dioxetane **1** (25.0 mg, 0.06 mmol) and 2-mercaptoethanol (**3a**) (8.40 µL, 0.12 mmol). After gentle stirring (250 rpm) of 3 hours at room temperature, the solvent was removed under reduced pressure under nitrogen atmosphere. The crude

product was purified by column chromatography (100% dichloromethane → dichloromethane / methanol = 40:1→30:1) to give **4a** (7.50 mg, 48.6 µmol, 81%) as a clear oil.

Preparation of disulfide **4c**: CH_3OH l (16.8 mL) was previously incubated with K_2CO_3 (66.6 mg, 0.48 mmol) for 15 minutes at room temperature. The supernatant solution was transferred to a tube and used to dissolve dioxetane **1** (100 mg, 0.24 mmol) and phenylmethanethiol (**3c**) (56.4 µL, 0.48 mmol). After gentle stirring (250 rpm) of 3 hours at room temperature, the solvent was removed under reduced pressure under nitrogen atmosphere. The crude product was purified by column chromatography (100% *iso*-hexane → *iso*-hexane / ethyl acetate = 40:1) to give **4c** (49.2 mg, 0.20 mmol, 83%) as a white solid.

Preparation of disulfide **4d**: CH_3OH (4.20 mL) was previously incubated with K_2CO_3 (15.9 mg, 0.12 mmol.) for 15 minutes at room temperature. The supernatant solution was transferred to a tube and used to dissolve dioxetane **1** (25.0 mg, 0.06 mmol) and captopril methyl ester (**3d**) (27.8 mg, 0.12 mmol). After gentle stirring (250 rpm) of 3 hours at room temperature, the solvent was removed under reduced pressure under nitrogen atmosphere. The crude product was purified by column chromatography (*iso*-hexane / ethyl acetate = 1:1→1:2→ 100% ethyl acetate) to give **4d** (23.2 mg, 0.05 mmol, 83%) as a clear oil.

General Procedure for Figure 1.
Cold CD_3OD (700 µL) was previously incubated with K_2CO_3 (0.02 mmol) for 15 minutes. The supernatant solution was transferred to a NMR tube and used to dissolve dioxetane **1** (5.00 mg, 0.01 mmol) and the respective thiol **3a,e,f,g** (0.02 mmol). The ^{1}H NMR spectra were recorded continuously at 0 °C.

N,N'-Diacetyl-L-cystine dimethyl ester (**4e**): ^{1}H NMR (400 MHz, CD_3OD): δ (ppm): 2.00 (s, 6 H), 2.97 (dd, J = 8.7 Hz, J = 14.0 Hz, 2 H), 3.21 (dd, J = 5.0 Hz, J = 14.0 Hz, 2 H), 3.75 (s, 3 H), 4.74 (dd, J = 5.0 Hz, J = 8.6 Hz, 2 H). This data is in agreement with those obtained from an authentic sample under identical conditions.

N,N'-Diacetyl-L-cystine bisamide (**4f**): ^{1}H NMR (600 MHz, CD_3OD): δ (ppm): 2.01 (s, 6 H), 2.91 (dd, J = 9.2 Hz, J = 13.9 Hz, 2 H), 3.22 (dd, J = 4.9 Hz, J = 13.9 Hz, 2 H), 4.71 (dd, J = 4.9 Hz, J = 9.2 Hz, 2 H). This data is in agreement with those obtained from an authentic sample under identical conditions.

L-cystine dimethyl ester (**4g**): ^{1}H NMR (400 MHz, CD_3OD): δ (ppm): 2.97 (dd, J = 6.8 Hz, J = 13.8 Hz, 2 H), 3.11 (dd, J = 5.4 Hz, J = 13.8 Hz, 2 H), 3.75 (s, 3 H), 3.79 (dd, J = 5.5 Hz, J = 6.8 Hz, 2 H). This data is in agreement with those obtained from an authentic sample under identical conditions.

General Procedure for competition experiments (Figure 2).
Cold CD_3OD (700 µL) was previously incubated with K_2CO_3 (0.02 mmol) for 15 minutes. The supernatant solution was transferred to a NMR tube and used to dissolve dioxetane **1** (5.00 mg, 0.01 mmol), thiol **3a**, and cysteine **3e** (each 0.02 mmol). Additional K_2CO_3 (1.03 mg, 7.45 µmol) was added to the solution in the tube. The ^{1}H NMR spectra were recorded continuously at 0 °C. Analysis of the product distribution is based on ^{1}H NMR data of previously prepared **4a** and **4e**. The same reaction conditions were used for the competition experiment with thiol **3h** and **3i**, respectively.

Bis(2-acetoxyethyl) disulfide (**4h**): ^{1}H NMR (600 MHz, CD_3OD): δ (ppm) = 2.05 (s, 6 H), 2.96 (t, J = 6.5 Hz, 4 H), 4.32 (t, J = 6.5 Hz, 4 H). This data is in agreement with those obtained from an authentic sample under identical conditions.

N,N'-Diacetylcystamine (**4i**): ^{1}H NMR (600 MHz, CD_3OD): δ (ppm) = 1.94 (s, 6 H), 2.82 (t, J = 6.7 Hz, 4 H), 3.47 (t, J = 6.7 Hz, 4 H). This data is in agreement with those obtained from an authentic sample under identical conditions.

Chemiluminescence measurement with 1 in the absence of thiols (reference reaction).
CH_3OH (700 µL) was previously incubated with K_2CO_3 (0.02 mmol) for 15 minutes at room temperature. The supernatant solution was transferred to a NMR tube and used to dissolve dioxetane **1** (5.00 mg, 0.01 mmol). The solution was transferred into a centrifuge tube and methyl *tert*-butyl ether (1.40 mL), water (3.00 mL), and aqueous barium chloride solution (0.5 M, 20 drops) were added. The organic phase (200 µL) was diluted with methyl *tert*-butyl ether (1/10) and was subsequently washed with water (2 mL). The chemiluminescent decay of **1** was triggered by the addition of the MTBE phase (7.16 µL) to a solution of dimethyl sulfoxide (183 µL) and potassium carbonate saturated methanolic solution (10 µL) in a well. Light emission was measured as described above. The basic methanolic solution was prepared as mentioned above and the final concentration of **1** was 25 µM per well.

Chemiluminescence measurement with dioxetane 1 and disulfide 4e (verification of extraction procedure).
CH_3OH (700 µL) was previously incubated with K_2CO_3 (0.02 mmol) for 15 minutes at room temperature. The supernatant solution was transferred to a NMR tube and used to dissolve dioxetane **1** (5.00 mg, 0.01 mmol) and *N,N'*-diacetyl-*L*-cystine dimethyl ester (**4e**) (0.01 mmol). The solution was transferred into a centrifuge tube and methyl *tert*-butyl ether (1.40 mL), water (3.00 mL), and aqueous barium chloride solution (0.5 M, 20 drops) were added. The organic phase (200 µL) was diluted with methyl *tert*-butyl ether (1/10) and was subsequently washed with water (2 mL). The chemiluminescent decay of **1** was triggered by the addition of the MTBE phase (7.16 µL) to a solution of dimethyl sulfoxide (183 µL) and potassium carbonate saturated methanolic solution (10 µL) in a well. Light emission was measured as described above. The basic methanolic solution was prepared as mentioned above.

General procedure for Figure 3.
The chemiluminescence measurement with **1** in the absence of thiols was carried out for three different concentrations (reference reactions used for calibration).
CH_3OH (700 µL) was previously incubated with K_2CO_3 (0.02 mmol) for 15 minutes at room temperature. The supernatant solution was transferred to a NMR tube and used to dissolve dioxetane **1** (5.00 mg, 0.01 mmol) The solution was transferred into a centrifuge tube and methyl *tert*-butyl ether (1.40 mL), water (3.00 mL) and aqueous barium chloride solution (0.5 M, 20 drops) were added. The organic phase (200 µL) was diluted with methyl *tert*-butyl ether (1/10) and was subsequently washed with water (2 mL). The organic phase was used unchanged or was partly further diluted (1/1 and 1/4) to give the three desired concentrations. The chemiluminescent decay of **1** was triggered by the addition of the MTBE phase (7.16 µL) to a solution of dimethyl sulfoxide (183 µL) and potassium carbonate saturated methanolic solution (10 µL) in a well. Light emission was measured as described above. The basic methanolic solution was prepared as mentioned above.

Chemiluminescence measurement with 1 and 3e at ratios of 3:4 and 1:2.

Cold CH_3OH (700 µL) was previously incubated with K_2CO_3 (0.02 mmol) for 15 minutes. The supernatant solution was transferred to a NMR tube and used to dissolve dioxetane **1** (5.00 mg, 0.01 mmol) and *N*-acetyl-*L*-cysteine methyl ester (**3e**) (0.015 mmol or 0.02 mmol). The solution was incubated for 75 minutes at 0 °C. The solution was transferred into a centrifuge tube and methyl *tert*-butyl ether (1.40 mL), water (3.00 mL), and aqueous barium chloride solution (0.5 M, 20 drops) were added. The organic phase (200 µL) was diluted with methyl *tert*-butyl ether (1/10) and was subsequently washed with water (2 mL). The chemiluminescent decay of **1** was triggered by the addition of the MTBE phase (7.16 µL) to a solution of dimethyl sulfoxide (183 µL) and potassium carbonate saturated methanolic solution (10 µL) in a standard 96-well plate. Light emission was measured as described above. The basic methanolic solution was prepared as mentioned above.

Monitoring of the reaction course: comparison of NMR yields and chemiluminescence read-out after quenching.
Cold CH_3OH (700 µL) was previously incubated with K_2CO_3 (0.02 mmol) for 15 minutes. The supernatant solution was transferred to a NMR tube and used to dissolve dioxetane **1** (5.00 mg, 0.01 mmol) and *N*-acetyl-*L*-cysteine methyl ester (**3e**) (0.02 mmol). The solution was incubated for 22.5, 43.5 or 64.5 minutes at 0 °C. After incubation for the time indicated, the solution was transferred into a centrifuge tube and methyl *tert*-butyl ether (1.40 mL), water (3.00 mL), and aqueous barium chloride solution (0.5 M, 20 drops) were added. The organic phase (200 µL) was diluted with methyl *tert*-butyl ether (1/10) and was subsequently washed with water (2 mL). The chemiluminescent decay of **1** was triggered by the addition of the MTBE phase (7.16 µL) to a solution of dimethyl sulfoxide (183 µL) and potassium carbonate saturated methanolic solution (10 µL) in a standard 96-well plate. Light emission was measured as described above. The basic methanolic solution was prepared as mentioned above.

ASSOCIATED CONTENT

Supporting Information

Preliminary results and NMR spectra. The Supporting Information is available free of charge on the ACS Publications website.

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Notes

The authors declare no competing financial interests.

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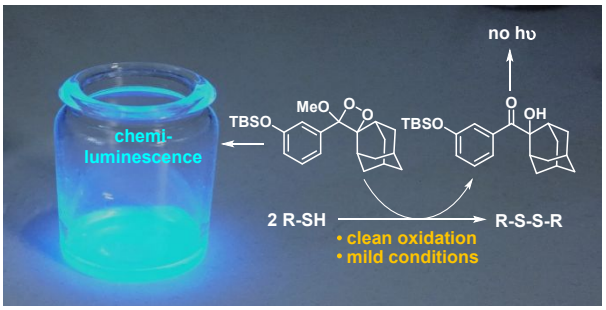
no hυ
chemi-
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TBSO
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2 R-SH
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