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A glucose oxidase-hemoglobin system for efficient oxysulfonylation of alkenes/alkynes in water

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ABSTRACT

Background: β-ketosulfones are important bioactive compounds that have been extensively studied in organic chemistry. In this work, a green and efficient process for the synthesis of β-ketosulfones from alkenes (**1**) or alkynes (**3**) with sodium benzenesulfinate (**2**) was developed.
Results: Under optimal conditions (alkenes (0.5 mmol) or alkynes (0.5 mmol), sodium benzenesulfinate (0.5 mmol), water (2 mL), hemoproteins (heme concentration: 0.06 mol%), GOX (42 U/ml), room temperature, 2 h), high yields of β-ketosulfones could be obtained when HgbRb (hemoglobin from rabbit blood) and GOX (glucose oxidase from *Aspergillus niger*) was used as the catalyst.
Conclusion: This enzymatic method demonstrates the great potential for the synthesis of β-ketosulfones and extends the application of dual protein systems in organic synthesis.

Introduction

Enzyme catalysis has attracted considerable interest due to its exceptional functionality, adaptivity and sustainability [1]. Multiple-enzyme catalysis plays a significant role in the production of many valuable materials/chemicals at the industrial level because they facilitate tandem or cascade reactions [2–4]. In the past decade, exploring novel non-natural reactions has increasingly gained attention in research [5–8]. Hemoproteins, especially hemoglobin, exhibit huge potential as catalysts for non-natural reactions due to their low costs, commercial availability, broad catalytic abilities and good stability [9–14]. More importantly, the combination of promiscuous catalytic and specific catalytic abilities of proteins can further expand the application range of multiple-enzyme catalysis in organic chemistry [15–17].

As important synthetic intermediates, β-ketosulfones have been widely used in the construction of natural products, polyfunctionalized 4H-pyrans, quinolines, allenes, ketones and vinylsulfones [18–21]. β-Ketosulfones can be used as antifungal and antibacterial drugs [22]. Given that β-ketosulfones are used in a wide range of applications, substantial effort has been devoted to their synthesis. Traditionally, the synthesis of β-ketosulfones is mainly performed through the direct alkylation of sodium sulfinates with phenacyl halides, the acylation of alkyl sulfones with acid chlorides [23–25], esters or N-acylbenzotriazoles [26] and the oxidation of β-ketosulfides or β-hydroxysulfones with stoichiometric inorganic oxidants [27,28]. The copper-catalyzed coupling of oxime acetates with sodium sulfinates is used in the synthesis of β-ketosulfones [29]. However, the limited availability of substrates and long reaction time limit the practical application of β-ketosulfones. In the last decade, as one of the promising synthesis strategies, the direct oxysulfonylation of alkenes or alkynes has attracted considerable attention. A variety of homogenous catalytic methods have been developed based on the sulfonation of alkenes for access of β-ketosulfones catalyzed by transition metal catalysts (Scheme 1, A) [30–33]. Lei et al. reported an aerobic oxidative difunctionalization of alkynes towards β-ketosulfones in the presence of 4 equiv. pyridine (Scheme 1, B1) [34]. However, when alkenes were employed as precursors under similar reaction conditions, the only products obtained were β-hydroxysulfones. Zhou et al. utilized iron salt to catalyze aerobic oxysulfonylation of terminal alkynes and sodium sulfinates in MeOH/H_2O (v : v = 3 : 1) at 50 °C (Scheme 1, B2) [35]. In 2016, Wang et al. reported the visible light-initiated direct oxysulfonylation of alkenes with sulfinic acids in the presence of TBHP (Scheme 1, C1) [36]. In

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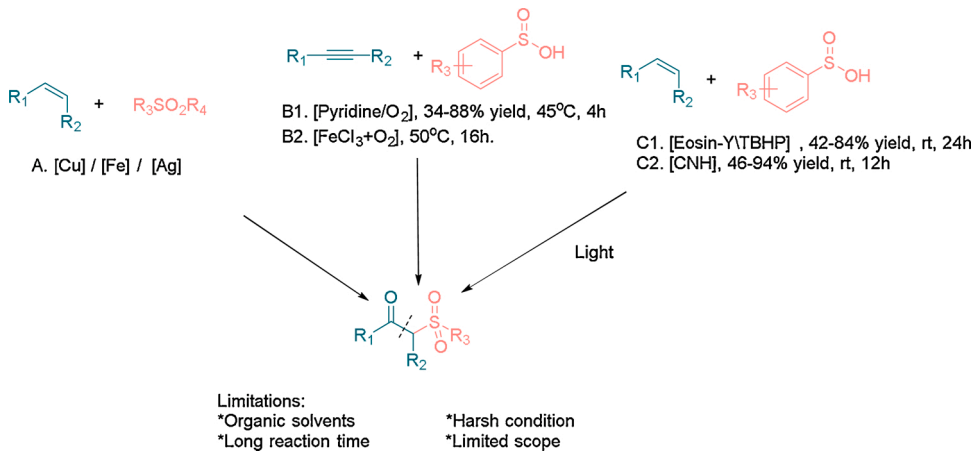
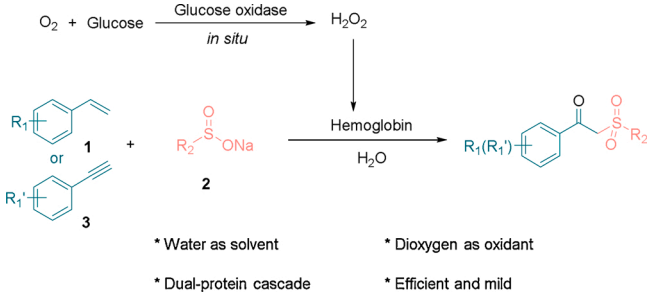


Scheme 1. Methods for oxysulfonylation of alkenes/alkynes.



Scheme 2. Oxysulfonylation of alkenes/alkynes catalyzed by dual-protein system.

2020, they developed a method for the visible light-promoted sulfonation of alkenes catalyzed by a biomimetic photocatalyst of a single-atom iron site prepared through coupling carbon nitride with hemin (Scheme 1, C2) [37]. However, the long reaction time, excess of oxidant and hazardous organic solvent limit the practical application of visible light-promoted methods. Therefore, demand for the development of mild, efficient and green methods for synthesizing β-ketosulfones has considerably increased

Herein, we designed a glucose oxidase (GOX)–hemoglobin (Hgb) system for the synthesis of β-ketosulfones in water through the oxysulfonylation of alkenes/alkynes. Scheme 2 shows that GOX catalyzes the oxidation of glucose into D-glucono-delta-lactone and hydrogen peroxide (H_2O_2) [38]. Hgb can catalyze the oxysulfonylation of alkenes/alkynes and sodium sulfinates with H_2O_2 generated *in situ*. To a great extent, this 'feed-on-demand' reaction system can prevent the use of excessive amounts of unstable oxidants. In addition, gluconolactone spontaneously reacts in water to gluconic acid, which is a high-value added product, and thus this method has potential practical applications. Notably, water is used in this reaction because of its low cost and safety. According to the principles of green chemistry, using water to replace hazardous solvents is another advantage of organic reactions [39]. Moreover, sodium sulfinates can be used as substrates to replace unstable sulfinic acids in this dual-protein system. Compared with previous methods, this dual-protein system is more efficient, milder and has higher tolerance to substrates. To the best of our knowledge, this is the first report on β-ketosulfone synthesis using proteins as catalysts.

Experimental

Chemicals

Myoglobin from equine heart, Hemoglobin from porcine blood, Hemoglobin from bovine blood, Horseradish peroxidase, Cytochrome c from bovine heart muscle, Glucose oxidase from *A. niger* (200 U/mg) was purchased from Shanghai Yuan Ye Biological Technology Company. Alkenes, alkynes, sodium benzenesulfinate, were purchased from Bide Pharmatech Ltd. (Shanghai, China). All the other chemical reagents were purchased from Shanghai Chemical Reagent Company (Shanghai, China). All the commercially available reagents and solvents were used without further purification. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a 400 MHz spectrometer in $CDCl_3$. Chemical shifts for protons are reported in parts per million downfield from tetramethylsilane (TMS) and are referenced to residual protium in the NMR solvent ($CHCl_3$ = δ 7.26 ppm). NMR data are presented as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant in Hertz (Hz), integration. Mass spectra were recorded on the Bruker MicrOTOF Q II and an Orbitrap Fusion™ Tribrid™ mass spectrometer (Thermo Scientific, San Jose, CA, U.S.A.) coupled with HESI ion source. The experiments were performed in triplicate, and all data were obtained based on the average values.

Preparation of apo Hgb

To the ice-cold, salt-free Hgb solution containing sufficient 0.1 N HCl to give pH 2, is added an equal volume of ice-cold methyl ethyl ketone, and the mixture is shaken for a short time. On standing in the cold, separation takes place into a ketonic supernatant containing all the heme, and the aqueous layer containing all the protein, which is dialysed against water to remove the dissolved ketone.

Table 1
Synthesis of β-ketosulfones by a dual-protein system (GOX and hemoprotein) [a].

Entry	GOX	Hemoprotein	Isolated yield (%)
1	GOX	HgbRb	91
2	GOX	HgbBv	61
3	GOX	HgbSw	52
4	GOX	MYO	69
5	GOX	Cyt C. Bv	75
6	GOX	HRP	71
7	GOX	Hemin[b]	9
8	GOX	Apo-HgbRb	ND[c]
9	GOX	–	ND
10	–	HgbRb	ND
11	H_2O_2 (stepwise)[d]	HgbRb	41
12	H_2O_2 (continuous)[d]	HgbRb	66
13	GOX[e]	HgbRb	62
14	GOX[f]	HgbRb	10
15	GOX[g]	HgbRb	Trace

[a] **1a** (0.5 mmol), **2a** (0.5 mmol), H_2O (2.0 mL), glucose (1.1 mmol), GOX (42 U/mL), hemoprotein (heme concentration: 0.06 mol %), room temperature, oxygen, 2 h.
[b] Using 0.6 % mmol.
[c] Not detected.
[d] H_2O_2 (10 % aqueous solution, 1.0 mmol).
[e] Air.
[f] N_2 instead O_2.
[g] Added 3 equiv. TEMPO; Abbreviation: MYO (Myoglobin from equine heart); HgbSw (Hemoglobin from swine blood); HgbBv (Hemoglobin from bovine blood); HgbRb (Hemoglobin from rabbit blood); Cyt.C Bv (Cytochrome C from bovine heart); GOX (Glucose oxidase from *A. Niger*); HRP (Horseradish peroxidase).

General procedure for the synthesis of β-ketosulfones

To a mixture of alkenes/alkynes (0.5 mmol), sodium benzenesulfinate (0.5 mmol), glucose (1.1 mmol) in water (2 ml), hemoproteins (heme concentration: 0.06 mol%), GOX (42 U/ml), was added. The reaction mixture was then stirred at room temperature in a round bottom flask for 2 h, with oxygen added at a rate of 1 mL/min. The reaction was monitored by TLC. When the reaction was complete, the crude mixture was extracted with ethyl acetate. Then the organic phase was dried over sodium sulfate and concentrated under reduced pressure. Finally, the desired product was obtained by flash column chromatography with petroleum ether/ethyl acetate (4/1) as an eluent. All the isolated products were well characterized by their 1H spectral analysis.

Results and discussion

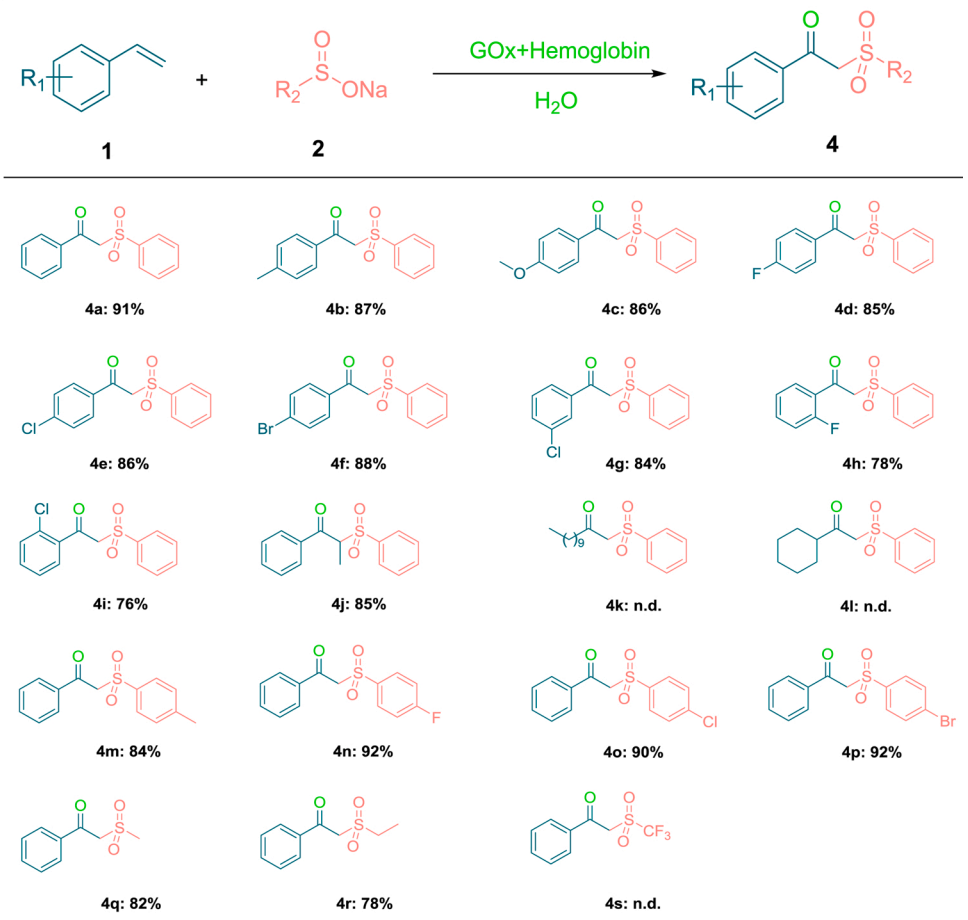
Initially, styrene **(1a)** and sodium benzenesulphinate **(2a)** were adopted as model substrates for the synthesis of β-ketosulfones in the GOX-Hgb system. Generally, GOX from *A. niger* is well known for its broad applicability as H_2O_2 producer [40]. Therefore, we attempted to investigate the effect of hemoprotein under an oxygen atmosphere. As shown in Table 1, all the hemoproteins possessed catalytic ability to a certain extent, and hemoglobin from rabbit blood (HgbRb) exhibited the highest catalytic performance in this reaction (Entry 1). Notably, when hemin and GOX were combined as catalysts, only a 9 % yield was obtained (Entry 7). No corresponding product was obtained when hemoglobin, GOX or apo-HgbRb was used as the catalyst (Entry 8–10). These results indicate that the active conformation and heme center of hemoglobin play key roles in the synthesis of β-ketosulfones. Compared with the *in situ* feeding mode of H_2O_2 generated by GOX, the stepwise or continuous addition of H_2O_2 combined with HgbRb in this reaction resulted in a low yield of β-ketosulfone **4a** (Entry 11–12). When the reaction was performed in air, yield decreased to 62 % (Entry 13) and a low yield was obtained when the reaction was conducted under a nitrogen atmosphere in the presence of H_2O_2 (Entry 14). This result indicates that oxygen is necessary not only for the generation of H_2O_2 catalyzed by GOX but also for the oxdative steps in the synthesis of β-ketosulfones catalyzed by Hgb. Hence, oxygen was used to improve the catalytic efficiency of this reaction system for further investigation. To determine the reaction involving a radical pathway, we added TEMPO, a radical scavenger, to this reaction (Entry 15). Accordingly, only trace product was determined. We optimized the reaction conditions, such as the substrate ratio, protein concentration and glucose dosage (data shown in Table S1).

Under optimal conditions, the scope and limitations of the dual-protein system were evaluated. The result is summarized in Table 2. A variety of aromatic alkenes containing electron-donating (Me, OMe) or electron-withdrawing groups (F, Cl, Br) on aryl rings reacted with sodium benzenesulphinate efficiently and afforded the corresponding products with satisfactory yields. The reaction was not obviously affected by the electronic effects of the substituents in substituted styrenes, and steric hindrances influenced the yields. Compared with the yields obtained after *meta*- and *para*-substituted counterparts were used (**4b-4 g**), a lower yield was obtained when *ortho*-substituted styrene (**4h-4i**) was selected as substrate. Internal alkyne (β-methylstyrene) reacted with sodium benzenesulphinate (**4 j**) in a higher yield than those obtained by other methods [33,34]. Unfortunately, aliphatic alkenes, such as dodec-1-ene and vinylcyclohexane, did not work in the reaction (**4k-4l**). Subsequently, some substituted sodium benzenesulphinates bearing either electron-donating groups (Me) or electron-withdrawing groups (F, Cl, Br) were used as substrates for the production of the desired β-ketosulfones in high yields (4m-4p). Moreover, when sodium aliphatic sulfinates (Me, Et) were used as substrates, the reaction proceeded smoothly and generated high yields (4q-4 r). However, no expected product was obtained when sodium trifluomethanesulfonate was selected as the substrate (**4 s**).

Subsequently, we determined whether alkynes can be reacted with sodium aryl sulphinates to produce corresponding β-ketosulfones (Table 3). As expected, substituted phenylethyne can be reacted with sodium benzenesulphinate in moderate yields under the dual-protein system (**4b-4i**). However, all the used alkynes exhibited lower reactivity in this dual-protein-catalyzed reaction. Aliphatic alkynes did not yield corresponding products (**4k-4l**). Compared with reactions of styrene in Table 2, similar phenomena were observed when sodium aryl sulphinates (**4m-4p**) and sodium aliphatic sulfinates (**4q-4 s**) were reacted with phenylethyne. Considering the good substrate generality and tolerance to various functional groups, this dual-protein-catalyzed method is more attractive in the practical synthesis of β-ketosulfones

To gain insight into the reaction mechanism, ^{18}O-labeling experiment was performed to elucidate the origin of the carbonyl oxygen atom of β-ketosulfones (Scheme 3). As demonstrated in eqn.1, the reaction of **1a** and **2a** under $^{18}O_2$ for 2 h and ^{18}O-**4a** were detected. In contrast, only ^{16}O-**4a** was obtained when the reaction was conducted in $H_2^{18}O$ (eqn.2). The experimental result shows that the carbonyl oxygen atom of β-ketosulfone is derived from dioxygen (MS spectrum, see Fig S1). To determine the kinetic parameters for HgbRb activity on this protein-catalyzed reaction, we performed the steady-state reactions under the following conditions (HgbRb heme concetration: 0.02 mol%, 100 mM **1a**, phosphate buffered solution (pH 7.4), 100 mM H_2O_2, air, 25 °C) by

Table 2
Oxysulfonylation of alkenes catalyzed by dual-protein system[a].



[a] **1** (0.5 mmol), **2** (0.5 mmol), H_2O (2.0 mL), glucose (1.1 mmol), GOX (42 U/mL), HgbRb (heme concentration: 0.06 mol %), room temperature, oxygen, 2 h.

varying the concentration of **2a**. The results are shown in Fig S2-S3, and the obtained kinetic parameters, V_{max} and K_m are listed in Fig S3.

On the basis of our preliminary results and previous reports [41–43], we propose a reasonable mechanism for this dual-protein-catalyzed reaction (Scheme 4). Initially, H_2O_2 is formed from the reaction of dioxygen with the FAD (flavin adenine dinucleotide) cofactor of GOX. Then, the heme of hemoglobin is converted to compound I in the presence of H_2O_2, which can produce a sulfonyl radical (II). Thereafter, sulfonyl radical (II) is added to alkenes or alkynes, generating a reactive radical (III or III'). Subsequently, peroxy radical (IV or IV') is generated from radical (III or III') captured by dioxygen. The peroxy radical (IV) can react with radical (III) to produce oxyl radical (V). Finally, the hydrogen radical abstraction of radical V furnishes corresponding β-keto sulfone 4. Peroxy radical (IV') is converted to an intermediate VI, then undergoes reduction and isomerisation to produce β-ketosulfone 4.

Overall, we successfully constructed an efficient dual-protein system for the synthesis of β-ketosulfones in water. This method has several distinct advantages over non-enzymatic methods, such as strong oxidant-free, high efficiency, broad substrate scope, mild and environmentally-benign reaction conditions. Therefore, this dual-protein system offers great prospects for organic chemistry applications. To enhance the reusability of protein and decrease the cost of this dual-protein system, the co-immobilization of these two proteins is ongoing in our laboratory and will be reported in the future.

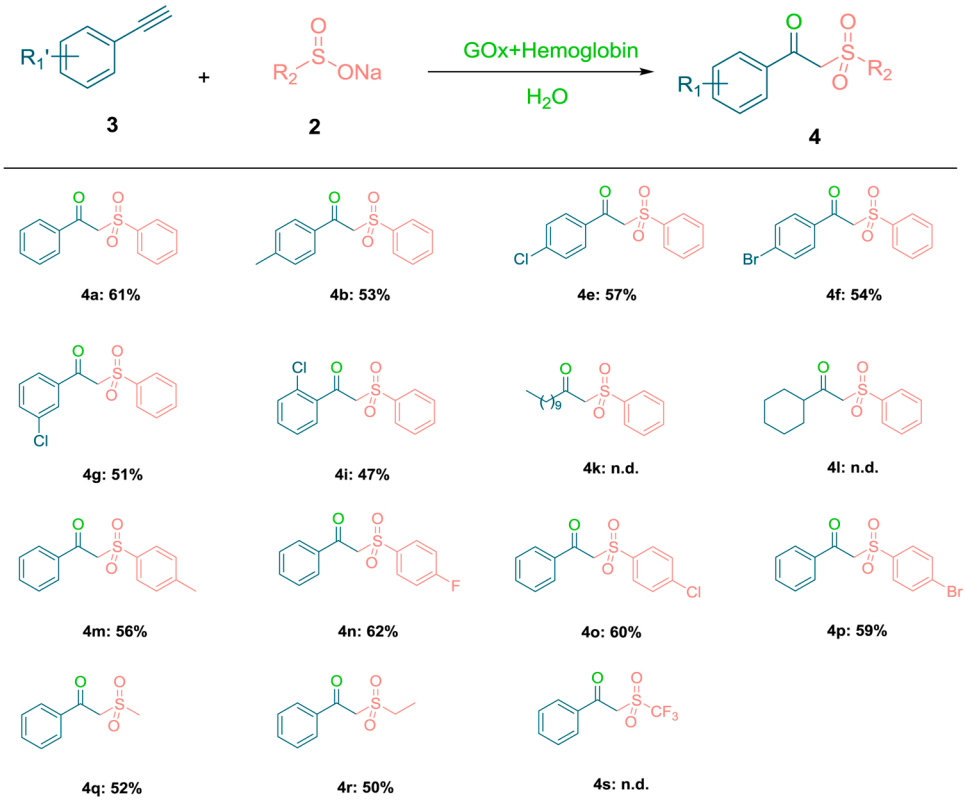
CRediT authorship contribution statement

Fengxi Li: Conceptualization, Methodology, Formal analysis, Software. **Jiali Su:** Investigation, Resources. **Yaning Xu:** Visualization, Data curation. **Jiaxu Liu:** Writing - original draft. **Yue Yu:** Writing - review & editing. **Chunyu Wang:** Software. **Zhengqiang Li:** Software. **Chen Li:** Supervision. **Lei Wang:** Supervision.

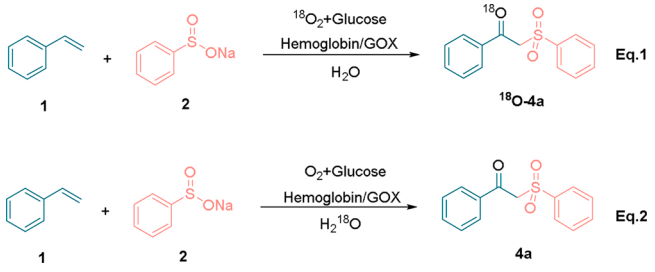
Declaration of Competing Interest

The authors report no declarations of interest.

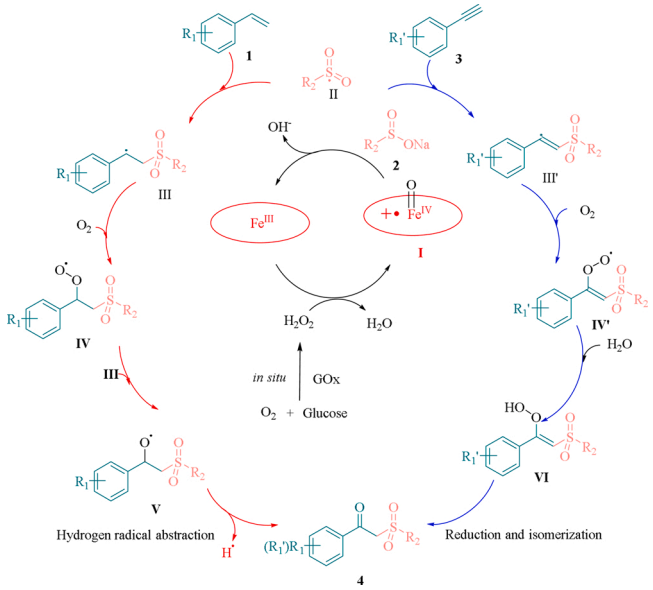
Table 3
Oxysulfonylation of alkynes catalyzed by dual-protein system[a].



[a] **2** (0.5 mmol), **3** (0.5 mmol), H_2O (2.0 mL), glucose (1.1 mmol), GOX (42 U/mL), HgbRb (heme concentration: 0.06 mol %), room temperature, oxygen, 2 h.



Scheme 3. Labelling experiments.



Scheme 4. The plausible mechanism of this dual-protein system.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mcat.2020.111336.

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