

Hot Paper

Copper-Catalyzed Sulfimideation in Aqueous Media: a Fast, Chemoselective and Biomolecule-Compatible Reaction

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Performing transition metal-catalyzed reactions in cells and living systems has equipped scientists with a toolbox to study biological processes and release drugs on demand. Thus far, an impressive scope of reactions has been performed in these settings, but many are yet to be introduced. Nitrene transfer presents a rather unexplored new-to-nature reaction. The reaction products are frequently encountered motifs in pharmaceuticals, presenting opportunities for the controlled, intracellular synthesis of drugs. Hence, we explored the transition metal-catalyzed sulfimideation reaction in water for future *in vivo*

application. Two Cu(I) complexes containing trispyrazolylborate ligands (Tp⁺) were selected, and the catalytic system was evaluated with the aid of three fitness factors. The excellent nitrene transfer reactivity and high chemoselectivity of the catalysts, coupled with good biomolecule compatibility, successfully enabled the sulfimideation of thioethers in aqueous media. We envision that this copper-catalyzed sulfimideation reaction could be an interesting starting point to unlock the potential of nitrene transfer catalysis *in vivo*.

Introduction

Catalysis proves a powerful tool to achieve otherwise synthetically challenging, or even impossible, transformations and is therefore naturally employed in various areas of chemistry. Transition metal-catalyzed reactions have even found successful applications in cells (*in vitro*) and living systems (*in vivo*) recently.^[1–3] Such applications range from cell surface labelling to the activation of prodrugs.^[4–6] To develop more tools for, e.g., live cell imaging or treatment, chemists have devoted much research to translate transition metal-catalyzed reactions from organic solvents to aqueous media.

Considerable efforts into developing 'new-to-nature' reactions have led to an impressive scope of transition metal-catalyzed transformations performed in biological settings.^[7,8] Key examples of these transformations are the copper-catalyzed azide-alkyne cycloadditions,^[9,10] and the ruthenium-catalyzed deallylation,^[11,12] (photocatalytic) azide reduction,^[13,14] and ring-

closing metathesis reactions.^[15,16] This scope of reactions is still rapidly expanding. For example, Mascareñas and co-workers showed the application of carbene transfer catalysis in mammalian cells.^[17] They performed copper-catalyzed N–H carbene insertions to afford bioactive benzoquinoxalines, which can alter the mitochondrial functions of cells, from simple ortho-amino arylamines and α -keto diazocarbene.

Although carbene transfer catalysis has been performed intracellularly,^[17,18] nitrene transfer catalysis to nonnatural substrates, such as prodrugs, has yet to be reported in biological settings to the best of our knowledge. Nonetheless, several promising studies in water as the reaction medium have been performed, mainly focused on C–H insertions and aziridination reactions.^[19–22] Sulfimideation reactions are somewhat less explored in water, while its new-to-nature reaction products, e.g., sulfimides (RN=SR'R''), only one report of a naturally occurring sulfimide moiety has been presented to date,^[23] and their oxidized analogs, sulfoximines (RN=SOR'R''),^[24,25] are also fre-

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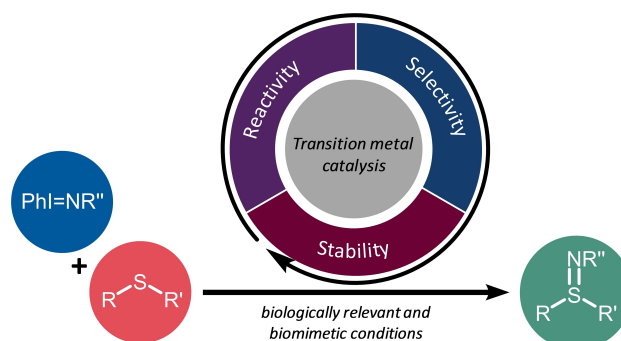


Figure 1. Graphical representation of this study, in which we set out to explore the transition metal-catalyzed sulfimideation reaction of thioethers in aqueous media and evaluate the potential of the catalytic system for future *in vivo* application with the focus on three fitness factors: reactivity, selectivity and stability.

quently encountered motifs in pharmaceuticals. For example, (*N*-arylsulfonyl)sulfimide-based drugs ($\text{ArSO}_2\text{N}=\text{SR}''$, with $\text{R}' = \text{alkyl}$ and $\text{R}'' = \text{aryl}$) were reported to inhibit osteoclastogenesis and bind to proteins (e.g., pirin), causing inhibition of melanoma cell migration.^[26,27] Hence, performing sulfimide reactions in biological settings presents opportunities for the controlled and on demand synthesis of drugs. Motivated by this potential application, we set out to explore transition metal-catalyzed sulfimide reactions in water for future *in vivo* application.

Compared to the highly controlled and adaptable conditions achieved in a flask, the conditions in cells and living systems are fairly different: water, oxygen, 37 °C (for mammalian cells), and pH 7. Moreover, the reaction components are typically present at low concentrations due to practical limitations such as solubility or non-specific toxicity, or the intrinsic metabolism of the biological system.^[28,29] Nonetheless, the catalytic reaction must still proceed at reasonable rates to ensure product formation.^[30,31] Furthermore, the inherent complexity of the cellular environment demands exquisite chemoselectivity.^[32] In the presence of various functionalities, the catalyst and reagents must react selectively with each other. Finally, an ideal catalyst should display high compatibility with salts and biomolecules bearing (coordinating) functional groups, such as thiols or basic amines.^[33]

To successfully apply a catalytic system in biological settings, the above-mentioned criteria, i.e., reactivity, selectivity, and stability, also known as 'fitness factors',^[32] have to be considered. In this study, we evaluate transition metal-catalyzed sulfimide reactions under biologically relevant (water, oxygen, 37 °C, pH 7) and biomimetic conditions (in the presence of salts and biomolecules) with the aid of these explorative fitness factors (Figure 1).

Results and Discussion

Reactivity

We initially sought a suitable transition metal catalyst for our studies. We came across a series of Tp^xCuL complexes ($\text{Tp}^x = \text{trispyrazolylborate}$ ligand), which presented excellent reactivity and selectivity in olefin aziridination and cyclopropanation reactions in aqueous and micellar media, employing diazo compounds and iminoiodinanes (PhINR , $\text{R} = \text{tosyl}$ or nosyl) as the respective *CR*- and *NR*-sources.^[20,21] In a separate study, it was disclosed that those complexes with electron-poor ligands disfavor a reaction with oxygen, being stable toward Cu(I) to Cu(II) oxidation.^[34] Given that the undesired formation of reactive oxygen species (ROS) is one of the toxic side reactions typically associated with the application of copper-based catalysts,^[9,35,36] intrinsic inertness towards oxygen is highly desirable. Hence, similar complexes have been employed in mammalian cells, indeed without presenting significant toxicities.^[37]

For the above-mentioned reasons, we chose those Tp^xCuL complexes to explore the transition metal-catalyzed sulfimida-

tion reaction in aqueous media; under biologically relevant and biomimetic conditions. Based on its previously reported activity for the aziridination reaction of styrene with iminoiodinanes,^[20] the well-defined $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$ complex was selected as the first candidate. This catalyst was subjected to thioanisole as the model substrate, employing PhINTs ($\text{Ts} = \text{tosyl}$) as the *NR*-source in a stock solution of DMSO. Both reagents were cleanly converted to *N*-(4-methylbenzenesulfonyl)-*S*-methyl-*S*-phenylsulfimide (**1**) in 78% yield under aerobic conditions in 2 h at 37 °C in water (5% DMSO) with 10 mol% catalyst (1 mM).

For intracellular applications, a catalyst is typically introduced at low concentrations: ~10–100 μM .^[38–40] We therefore gradually decreased the concentrations of the reaction components during optimization of the reaction conditions (Table S1 and Table S2). Under the optimized conditions, we observed that product **1** was still afforded in 72% yield using 10 mol% catalyst at a 100 μM concentration under aerobic conditions in 2 h at 37 °C in water (1% DMSO) (Table 1, entry 1). Decreasing the catalyst concentration to 50 μM (10 mol%) yielded 58% **1** (entry 2). In the absence of the copper-based complex, <1% product was obtained (entry 3), confirming the involvement of the complex as the catalyst.

Given that the formation of oxygen-containing side products is an often observed side reaction for nitrene transfer reactions in water,^[22] the potential formation of (methylsulfinyl)benzene (**2**) was carefully monitored (Table 1). Under the above-mentioned conditions, with 10 mol% catalyst at a 100 μM concentration, only 3% sulfoxide byproduct was formed with respect to the substrate (entry 1). In the absence of the catalyst, 10% of **2** was obtained, its formation resulting from a background reaction between the *NR*-source and substrate (entry 3). We assume that the hydrolysis of the iminoiodinane over time could afford iodosylbenzene (PhIO),^[41]

Table 1. Catalytic sulfimide with $\text{Tp}^x\text{Cu}(\text{NCMe})$ complexes.

Entry	Catalyst	Yield 1 (%) ^[a]	Yield 2 (%) ^[a]
1	100 μM $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$	72	3
2	50 μM $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$	58	7
3	–	< 1	10
4	100 μM $\text{Tp}^{(\text{CF}_3)_2\text{Br}}\text{Cu}(\text{NCMe})$	8	33
5	100 μM $\text{Tp}^{\text{CF}_3}\text{Cu}(\text{NCMe})$	73	2

Conditions: 1.0 mM *NR*-source. [a] Yields are quantified by ^1H NMR integration with 1,3,5-tri-*tert*-butylbenzene as an external standard. Reactions (blanks excepted) were at least performed in duplicate, yields are averaged. Yields of **1** are *versus* PhINTs and of **2** *versus* substrate.

which yields minor amounts of **2** from a (background) reaction with thioanisole.

With the optimized reaction conditions at hand, two other copper-based complexes were evaluated as potential catalysts: $\text{Tp}^{(\text{CF}_3)_2\text{Br}}\text{Cu}(\text{NCMe})^{[21]}$ and $\text{Tp}^{\text{CF}_3}\text{Cu}(\text{NCMe})^{[42]}$ (Table 1). These complexes bear CF_3 groups, which may be helpful handles for characterization in complex reaction media while maintaining the electron-poor character of the Tp^x ligands (*vide supra*). Under the optimized reaction conditions, the $\text{Tp}^{(\text{CF}_3)_2\text{Br}}$ -based catalyst is less productive in the sulfimide reaction of thioanisole to product **1** (8%, entry 4). In contrast, the Tp^{CF_3} analog afforded 73% of **1** and only 2% of **2** (entry 5). This catalyst was therefore utilized in further studies.

Intracellular transition metal-catalyzed reactions need to proceed with reasonable rates to ensure product formation before the reaction components are metabolized.^[28,29] To have

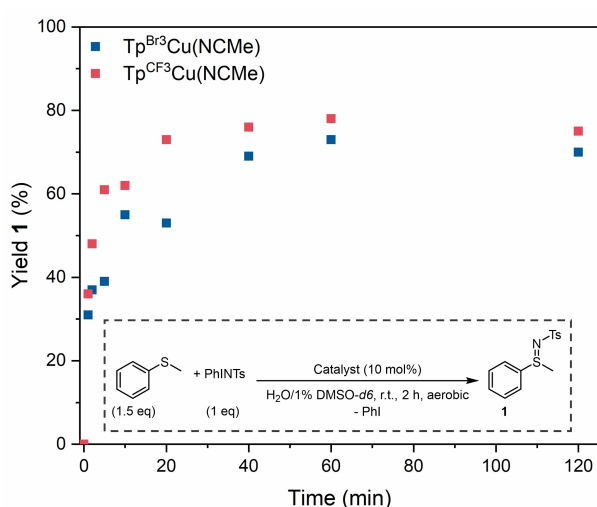
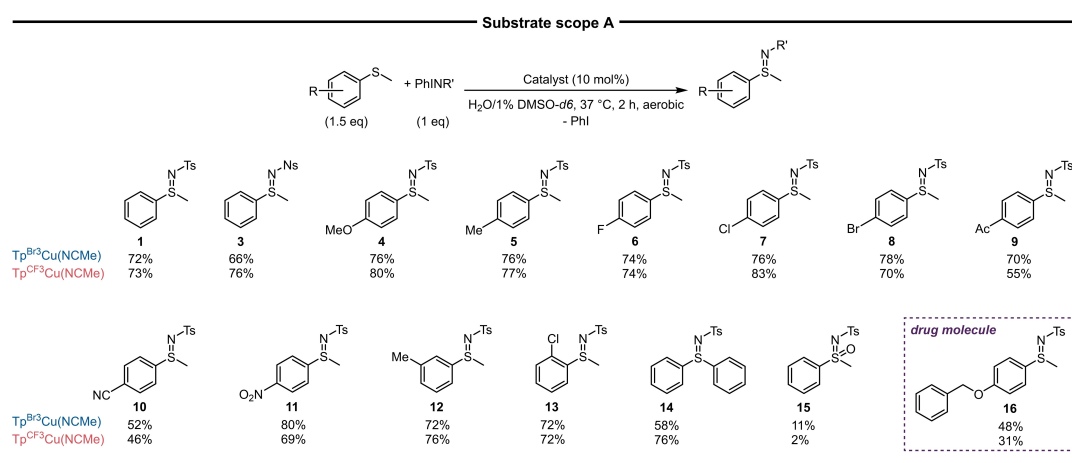


Figure 2. Kinetic profiles for the formation of **1** in water, catalyzed by $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$ (blue) and $\text{Tp}^{\text{CF}_3}\text{Cu}(\text{NCMe})$ (red). Conditions: 1.0 mM *NR*-source. Yields are quantified by ^1H NMR integration using 1,3,5-*tert*-butylbenzene as an external standard and are *versus* PhINTs. Reactions were performed in duplicate, yields are averaged.

an idea of the reaction progress of the copper-catalyzed sulfimide reaction under the applied biologically relevant conditions, we obtained kinetic profiles (Figure 2) for the formation of **1** with both catalysts at room temperature (see the Supporting Information for more details; Table S3). Based on these kinetic profiles, we observed that the Tp^{Br_3} -based catalyst reached the maximum yield after 40 minutes, whereas the Tp^{CF_3} analog reached the maximum yield after 20 minutes.

Next, we evaluated the general suitability of both catalysts for the sulfimide reaction of thioethers under biologically relevant conditions (Scheme 1 and Table S4). First, the influence of the nitrene precursor was investigated by using a more electron-withdrawing *NR*-source: PhINNs (Ns = nosyl). The reaction proceeded equally well for both catalysts with either PhINNs or PhINTs as the *NR*-source, affording products **1** and **3** in good yields. The catalysts were also subjected to a series of thioanisole derivatives to examine the compatibility of the reaction with different arene substituents. Substrates with electron-donating ($-\text{OMe}$, $-\text{Me}$) and -withdrawing ($-\text{F}$, $-\text{Cl}$, $-\text{Br}$, $-\text{Ac}$, $-\text{CN}$, $-\text{NO}_2$) substituents at the *para*-position of the arene ring were tolerated. Products **4** and **5**, and **6–11**, respectively, were obtained in good yields. Substitutions at the *ortho*- and *meta*-positions were also tolerated (**12–13**). Furthermore, two more classes of sulfides were investigated. Diphenylsulfane was cleanly converted to **14** in 58% and 76% yield with the Tp^{Br_3} - and Tp^{CF_3} -based catalysts, respectively. Notably, the former displayed some activity towards (methylsulfonyl)benzene affording **15**, albeit with poor yield. Lastly, we explored the transformative potential of the copper-catalyzed sulfimide reaction for potential intracellular drug synthesis applications. (4-(Benzyloxy)phenyl)(methyl)sulfane was employed as a substrate to access (*N*-arylsulfonyl)sulfimide-based drug **16**. This compound has been studied in the context of cancer treatment and inhibits melanoma cell migration (*vide supra*).^[26,27] Both catalysts selectively provided the drug after clean conversion of the substrate with moderate to good yields (up to 48%).



Scheme 1. Substrate scope A for the sulfimide reaction catalyzed by $\text{Tp}^x\text{Cu}(\text{NCMe})$ complexes. Conditions: 1.0 mM *NR*-source. Yields are quantified by ^1H NMR integration using 1,3,5-*tert*-butylbenzene as an external standard and are *versus* PhINTs.

Chemoselectivity

A myriad of chemical functional groups can be found in the cellular environment.^[43] To afford the formation of the desired product, the reaction compounds must be somewhat inert towards these functionalities and react selectively with each other. In other words, the catalytic system must possess excellent chemoselectivity. To investigate the chemoselectivity of the copper-based catalysts for nitrene transfer reactions, we first performed intermolecular competition experiments (Table 2).^[44] Nitrene transfer catalysis is not limited to thioethers. Nitrenes and their transition metal complexes can also add to double bonds and insert into C–H bonds; both are functionalities present in nature, generating aziridines and amines, respectively. Hence, the copper-catalyzed sulfidation reaction of thioanisole was performed in the presence of styrene or ethylbenzene as representative substrates. After 2 h, we observed 85–90% selectivity for sulfimination in the presence of styrene and >99% selectivity in the presence of ethylbenzene. In the absence of thioanisole, styrene aziridination is strongly favored over C–H amination of ethylbenzene (>99%) but is less productive than the sulfimination reaction.

Next, we examined the intramolecular chemoselectivity for the sulfimination reaction in the presence of alkenes and weak

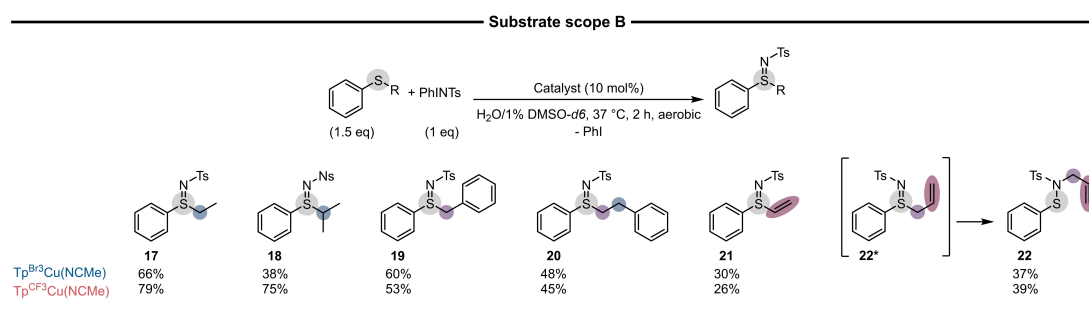
C–H bonds (Scheme 2, Table S4).^[44] Those with a bond dissociation energy (BDE) of ≤ 85 kcal mol^{−1} or $85 < (\text{BDE}) \leq 95$ kcal mol^{−1} are highlighted in purple and blue, respectively. Alkene fragments prone to aziridination are highlighted in red. The same conditions as for the intermolecular competition experiments were employed in these studies.

Based on ¹H NMR spectroscopy, no transformations of the relatively stronger C–H bonds (highlighted in blue) were observed. Substitution of the methyl group in thioanisole for ethyl or *iso*-propyl afforded **17** and **18** selectively and in moderate to good yields (Scheme 2, Table S4). Side reactions with the relatively weaker α -CH₂ bonds of benzyl(phenyl)sulfane and phenethyl(phenyl)sulfane (highlighted in purple) were not observed either. Both substrates were selectively converted to **19** and **20** in moderate yields. The corresponding ¹H NMR spectra after reactions performed with alkene functionalized substrates, phenyl(vinyl)sulfane and allyl(phenyl)sulfane (highlighted in red), did not indicate aziridine formation. The former substrate led to the clean formation of **21**, albeit with poor yields. The latter substrate afforded product **22** in moderate yields via a [2,3]-sigmatropic rearrangement of the initially formed *S*-allyl-sulfimide, as reported in literature.^[44,45] This rearrangement does, however, support the initial *S*-imidation of allyl(phenyl)sulfane (product **22***). Also, no reaction with the

Table 2. Intermolecular competition experiments to investigate the chemoselectivity for sulfimination in the presence of C=C and weak C–H bonds.

Catalyst (10 mol%)					
Sub ^A + Sub ^B + PhINTs			H ₂ O/1% DMSO- <i>d</i> 6, 37°C, 2 h, aerobic -PhI		Prod ^{A-R} + Prod ^{B-R}
1.5 eq 1.5 eq 1 eq					
Entry	Substrate A	Substrate B	Yield Prod ^{A-R} (%) ^[a]	Yield Prod ^{B-R} (%) ^[a]	Selectivity Prod ^{A-R} (%) ^[a]
Tp ^{Br3} Cu(NCMe)					
1	Thioanisole	Styrene	63	5	85
2	Thioanisole	Ethylbenzene	33	0	> 99
3	Styrene	Ethylbenzene	28	0	> 99
Tp ^{CF3} Cu(NCMe)					
4	Thioanisole	Styrene	73	2	90
5	Thioanisole	Ethylbenzene	75	0	> 99
6	Styrene	Ethylbenzene	23	0	> 99

Conditions: 1.0 mM NR-source. [a] Yields and selectivities are quantified by ¹H NMR integration with 1,3,5-tri-*tert*-butylbenzene as an external standard and are *versus* PhINTs. Reactions were performed in duplicate, yields are averaged.



Scheme 2. Substrate scope B for the sulfimination reaction catalyzed by Tp^{Br3}Cu(NCMe) and Tp^{CF3}Cu(NCMe). Grey: desired position for nitrene transfer (sulfimination). Blue: C–H bonds with $85 < (\text{BDE}) \leq 95$ kcal mol^{−1}. Purple: relatively weak C–H bonds ($\text{BDE} \leq 85$ kcal mol^{−1}). Red: alkene prone for aziridination. Conditions: 1.0 mM NR-source. Yields are quantified by ¹H NMR integration using 1,3,5-tri-*tert*-butylbenzene as an external standard and are *versus* PhINTs.

relatively weak C–H bonds could be observed for these two substrates.

Catalyst stability

The chemical stability of the catalytic system is of equal importance as its reactivity and selectivity.^[32] An ideal catalyst for *in vivo* applications displays high compatibility with biomolecules bearing (coordinating) functional groups, such as thiols or basic amines. In practice, however, catalyst stability tends to be one of the most critical challenges within the field of *in vivo* catalysis as such functional groups typically poison catalysts.^[33] A systematic evaluation of the catalyst performance in the presence of biomolecules is therefore indispensable and fundamental for further development of a catalytic system. To examine the compatibility of the copper-catalyzed sulfimidation reaction with various biomolecules, we employed an adapted version of the bio-additive-based screening protocol developed by the Glorius group (see the Supporting Information for more details, Table S5).^[46]

The copper-catalyzed nitrene transfer reaction to thioanisole with PhINTs as the *NR*-source was performed in the presence of sulfur- and basic amine-containing biomolecules, sugars, acids, and amino acids (1 equiv *versus* the catalyst, Figure 3). The presence of sugars, and amino acids did not affect the reaction outcome (~70–80% yield). The copper-catalyzed sulfimidation reaction also proceeded well in the presence of cytosine and adenosine, which belong to the basic-amine-containing class of

biomolecules. The presence of histidine and adenine does, however, affect the catalytic system to varying extents. In the presence of histidine, the $\text{Tp}^{\text{Br}^3}\text{Cu}(\text{NCMe})$ -based catalyst afforded only 18% product, whereas the $\text{Tp}^{\text{CF}_3}\text{Cu}(\text{NCMe})$ analog still afforded product **1** in 63% yield. The yields varied to 15% and 40% yield, respectively, in the presence of adenine. Based on these results, the fluorinated catalyst thus seems affected to a lesser extent than the brominated one. A similar trend was observed when acids were introduced to the reaction mixture. For example, in the presence of ascorbic acid, the $\text{Tp}^{\text{CF}_3}\text{Cu}(\text{NCMe})$ complex (53% yield) outperformed the $\text{Tp}^{\text{Br}^3}\text{Cu}(\text{NCMe})$ complex (37% yield). Finally, the effect of sulfur-containing biomolecules was evaluated. When employing the Tp^{Br^3} -based catalyst, the yield drops significantly in the presence of glutathione (GSH, 18%), whereas the biomolecules cysteine and methionine only have a moderate to negligible effect on the reaction outcome (42% and 64%, respectively). A similar trend was observed for the Tp^{CF_3} analog, which still affords product **1** in 36% yield in the presence of GSH. This class of sulfur-containing biomolecules thus has a negligible to significant effect on the catalytic systems. Nonetheless, the catalytic system seems compatible with most biomolecules. It is also worth mentioning that no C–H or S–H bond insertions were observed in the presence of (sulfur-containing) biomolecules.

In an effort to mimic the cellular environment, the copper-catalyzed sulfimidation reaction was performed in water containing 10% cell culture medium (i.e., DMEM) or cell lysates. Under these conditions, both catalysts still afford nitrene transfer to thioanisole, albeit with a significantly reduced product yield (Figure 3). Finally, the reaction was performed in PBS buffer as the reaction media. Although this medium affects the performance of the brominated catalyst (33% yield), the fluorinated one performs equally well as in water (72% yield).

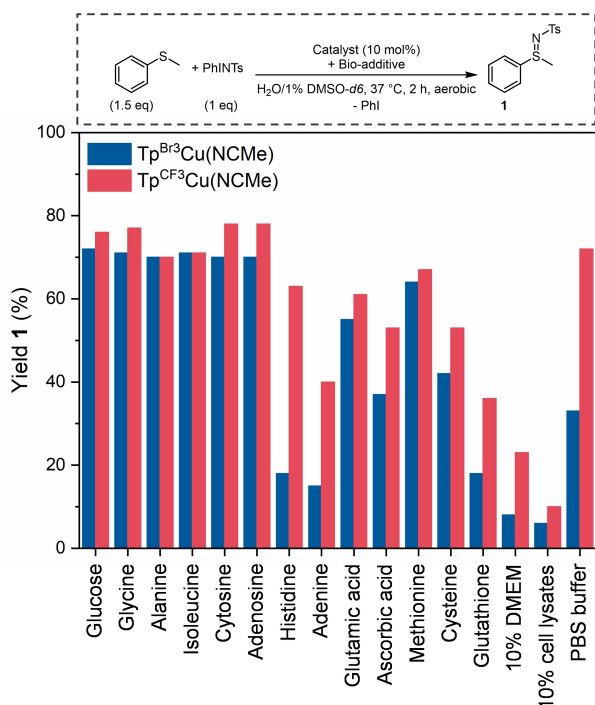


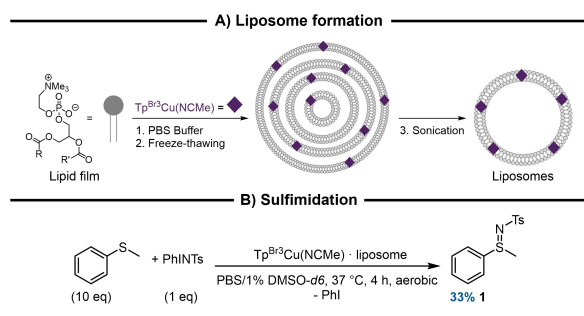
Figure 3. Screening of the compatibility of the copper-catalyzed sulfimidation reaction in the presence of biomolecules. Conditions: 1.0 mM *NR*-source, 1 equiv biomolecule *versus* catalyst. Yields are quantified by ^1H NMR integration using 1,3,5-tri-*tert*-butylbenzene as an external standard and are *versus* PhINTs.

Catalyst solubility

The reduced performance of the $\text{Tp}^{\text{Br}^3}\text{Cu}(\text{NCMe})$ -based catalyst in PBS buffer may be explained by poor solubility of the catalyst in the presence of substantial salt concentrations, as we observed some precipitation throughout the reaction (which was not observed for the $\text{Tp}^{\text{CF}_3}\text{Cu}(\text{NCMe})$ analog). With solubility being a critical physicochemical property for *in vivo* applications, we sought a general solution to overcome this issue.

Motivated by the previously reported application of the $\text{Tp}^{\text{Br}^3}\text{Cu}(\text{NCMe})$ complex in micellar catalysis,^[20] we investigated the potential use of liposomes. Liposomes are tunable spherical vesicles with both hydrophobic and hydrophilic domains due to the amphipathic nature of the phospholipids they are assembled from.^[47,48,49] Hence, hydrophobic compounds can be solubilized upon encapsulation within the hydrophobic domain: the lipid bilayer. This property has been beneficial for drug delivery applications, where the lipid bilayer ‘dissolves’ and protects its cargo.^[50,51]

Catalyst-liposome solutions were prepared in PBS buffer with the aid of passive loading^[52–54] and subsequently filtered to remove undissolved catalyst (Scheme 3A, see the Supporting



Scheme 3. $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$ encapsulation in liposomes to enhance its solubility in buffered medium. (A) Schematic representation of the formation of $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe}) \cdot \text{liposome}$ solutions via passive loading. (B) Catalysis experiment with a 10 mol % $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe}) \cdot \text{liposome}$ solution. Reaction conditions: 1.0 mM PhINTs. The yield is quantified by ^1H NMR integration using 1,3,5-tri-*tert*-butylbenzene as an external standard and is *versus* PhINTs.

Information for more details). Dynamic light scattering (DLS) measurements supported the self-assembly of the vesicles in the presence of $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$ (95 or 223 μM , Table S6). These measurements indicated that monodispersed solutions (PDI < 0.4^[55]) of particles with an average diameter of 108 to 126 nm were obtained. ICP measurements were performed to further investigate the inclusion of the $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$ complex in the lipid bilayer (Table S7), which does otherwise not dissolve in water or PBS buffer without a co-solvent. Based on these measurements, an average encapsulation efficiency of 89% was calculated for the $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$ complex, supporting the formation of aqueous $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe}) \cdot \text{liposome}$ solutions.

To investigate if the encapsulated catalyst retains its reactivity for nitrene transfer catalysis, the obtained $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe}) \cdot \text{liposome}$ solutions were subjected to catalysis experiments following a slightly altered procedure (see the Supporting Information for more details). Under the standard reaction conditions (Table 3, entry 1), product 1 was obtained, albeit with poor yield (13%) and significant formation of 2. Modifying the reaction conditions by increasing the thioanisole concentration (5–10 mM) afforded the product in up to 33% yield and with less byproduct formation (entries 2 and 3; Scheme 3B).

Also, increasing the catalyst loading to 23 mol % had a positive effect on the catalysis outcome at lower thioanisole concentration (5 mM); product 1 was formed in 36% yield (entry 4). No product formation was observed when the reaction was performed with 'empty' liposomes (entry 5), highlighting the indispensability of the copper-based catalyst. It should however be emphasized that the sulfimination reaction performed with the encapsulated catalyst is significantly less productive than the copper-catalyzed reaction in the absence of liposomes. We propose the following potential reasons for this: 1) diffusion limitations of the reaction components, 2) coordination of phospholipid moieties to the Cu(I) center, or 3) side reactions between the nitrene intermediate and the phospholipid moieties.

Besides the ability to solubilize hydrophobic compounds, liposomes can be employed because of their protective function.^[47,48,50] We were curious to see if the biomolecule compatibility of the brominated catalyst could also be improved by encapsulation in these vesicles. During the bio-additive-based screening (*vide supra*), we observed that the presence of histidine induced a significant drop in the yield of product 1 (from 68% to 18%) when using the above-mentioned catalyst. We therefore selected this biomolecule for further initial studies on this topic. The sulfimination reaction of thioanisole with PhINTs as the *NR*-source was performed with the $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe}) \cdot \text{liposome}$ solutions in the absence or presence of histidine (1 equiv *versus* the catalyst; Table 4, entries 1 and 2). To monitor the effect of the presence of histidine, we used a higher catalyst loading to reach significant product formation as we wanted to keep the substrate concentration relatively low (1.5 equiv). Gratifyingly, similar yields were obtained in the presence and absence of histidine (~15%), indicating a positive effect of catalyst encapsulation. To support this hypothesis further, we reproduced the reactions with the non-encapsulated $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$ complex in PBS buffer (entries 3 and 4). In the absence of the liposomes, a significant drop in the yield of 1 was observed in the presence of histidine (from 28% to 13%).

Table 3. Catalytic sulfimination with $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe}) \cdot \text{liposome}$ solutions.

Entry	Catalyst (μM)	Catalyst loading (mol %)	Thioanisole (equiv)	Yield 1 (%) ^[a]	Yield 2 (%) ^[a]
1	95	10	1.5	13	18
2	95	10	5	21	11
3	95	10	10	33	7
4	227	23	5	36	9
5	–	–	10	< 1	8

Conditions: 1.0 mM *NR*-source. [a] Yields are quantified by ^1H NMR integration with durene as an external standard. Reactions (blanks excepted) were at least performed in duplicate, yields are averaged. Yields of 1 are *versus* PhINTs and of 2 *versus* substrate.

Table 4. Catalysis experiments with $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$ -liposome solutions and the $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$ complex in PBS buffer, in the absence and presence of histidine.

Entry	Catalyst (μM)	Histidine (equiv)	Yield 1 (%) ^[a]	Yield 2 (%) ^[a]
1	$\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$ -liposome	–	17	18
2	$\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$ -liposome	1	15	16
3	$\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$	–	28	13
4	$\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$	1	13	4

Conditions: 1.0 mM NR-source. [a] Yields are quantified by ^1H NMR integration with durene as an external standard. Reactions (blanks excepted) were at least performed in duplicate, yields are averaged. Yields of 1 are versus PhINTs and of 2 versus substrate.

Conclusions

In this study, we explored the copper-catalyzed sulfimination reaction in aqueous media and evaluated its potential for future *in vivo* application with the aid of three fitness factors: reactivity, selectivity, and stability. Catalysts $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$ and $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$ presented good reactivity and chemoselectivity for the desired nitrene transfer reaction at low concentrations of catalyst (50–100 μM) and under biologically relevant conditions. Maximum yields are reached in short reaction times: 20–40 minutes, with minimal byproduct formation ($\leq 3\%$). Late stage sulfimination of (4-benzyloxyphenyl)(methyl)sulfane afforded a reported drug molecule in good yields, providing proof of principle for potential intracellular drug synthesis applications. Furthermore, both catalysts proved compatible with most biomolecules selected from different classes. Even in the presence of coordinating biomolecules, such as GSH and histidine, the sulfimination product was formed, whereby the $\text{Tp}^{\text{CF}_3}\text{Cu}(\text{NCMe})$ complex seems more robust than $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$. Similar observations were made when the reaction was carried out in PBS buffer, cell culture medium, and in the presence of cell lysates, highlighting the potential of the Tp^{CF_3} -based catalyst for future *in vivo* application. Solubility limitations of the $\text{Tp}^{\text{Br}_3}\text{Cu}(\text{NCMe})$ complex in buffered media could be countered via the encapsulation of the catalyst in liposomes, which were also observed to have a positive effect on the biomolecule compatibility of the catalyst. Overall, copper-catalyzed sulfimination is a fast, chemoselective, and biomolecule-compatible reaction, which could be an interesting starting point to unlock the potential of nitrene transfer catalysis *in vivo*.

Supporting Information

Experimental details, ^1H NMR spectra (Figure S1–S21) and DLS spectra (Figure S22–S51) are reported in the Supporting Information. The authors have also cited additional references within the Supporting Information.^[56–68]

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

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