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Synthesis of a reactive oxygen species responsive heterobifunctional thioketal linker

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ABSTRACT

A new heterobifunctional reactive oxygen species (ROS) responsive thioketal linker and its synthesis are described. This linker allows for developing new ROS-responsive agents with two distinct functionalities using universal bioconjugation methods. The reaction kinetics of the thioketal cleavage in the presence of ROS is also described.

Photodynamic therapy (PDT), a non-invasive method for diseases treatment, has gained substantial attention over the last decade.[1] In general, PDT utilizes light and photosensitizers to generate reactive oxygen species (ROS), which induce oxidative damage toward the target. Recently, Merlin and Murthy reported that thioketal can be selectively cleaved in the presence of ROS.[2] This structure was later utilized by Liu et al. as the key linker in a polyprodrug construct, which combines PDT and chemotherapy.[3] However, such a homobifunctional thioketal linker is limited in linking two distinct functionalities because byproducts with two molecules of the same functionality coupled to one linker are often collected (Scheme 1). Besides, these homobifunctional linkers restrict the functional group options of the molecules to be conjugated. Here, we report a heterobifunctional thioketal linker that allows the conjugation of two functionalities (such as a photosensitizer and a targeting ligand) with distinct functional groups. Such a linker will have great potential in developing ROS-responsive molecular agents for wide therapeutic and imaging applications.

Initially, we attempted to synthesize the heterobifunctinal thioketal linker using similar methods as those described for developing heterobifunctional *S*,*S*-thioketal. Early success of such a reaction was reported by Young et al.,[4–6] in which aldehydes or ketones were treated with equal amounts of thioacetic acid and thiols to yield a mixed acylthio-thioacetal/ketal convertable to asymmetric dithioacetal/ketals. Later on, McNamara explored a route for an unsymmetrical dithioacetal[7] by generating an *O*-trimethylsilyl hemithioacetal intermediate, which was later converted to the desired product through a low temperature transthioacetalization reaction catalyzed by boron trifluoride etherate. Additionally, Morton reported successful interconversion of methoxy acetal derivatives to thioacetals in good yield.[8] Based on these reports, we opted to prepare an *O*,*S*-thioketal as the key precursor of the heterobifunctional *S*,*S*-thioketal, as outlined in Scheme 2. 3-Mercaptopropionic acid (**1**) was reacted with excess amount of 2,2-dimethoxypropane in dichloromethane at 40 °C in the presence of catalytic amount of *p*-toluenesulfonic acid monohydrate. Three different ester products,[9] *O*,*S*-thioketal **2**, enol thioether **3**, and *S*,*S*-thioketal **4**, were separated from the reaction mixture (structure determined with ^{1}H NMR, data not shown) with the desired *O*,*S*-thioketal **2** as the major product in 90% yield.[10] However, the following transthioketalization reaction[8] with trifluoroacetamide protected *β*-mercaptoethylamine **5** was unsuccessful despite several attempts using various acids (*p*-toluenesulfonic acid, hydrochloric acid, and aluminum chloride).

In an effort to synthesize the heterobifunctional *S*,*S*-thioketal using an alternative but facile method, we chose to prepare the linker in a one-pot fashion (Scheme 3).[7,11] Stoichiometric amounts of methyl 3-mercaptopropionate (**7**), trifluoroacetamide protected *β*-mercaptoethylamine **5,** and acetone were mixed with boron trifluoride etherate[7] at 0 °C. The desired product **6** was isolated from byproducts **8** and **9** in 37% yield. Although this method caps the

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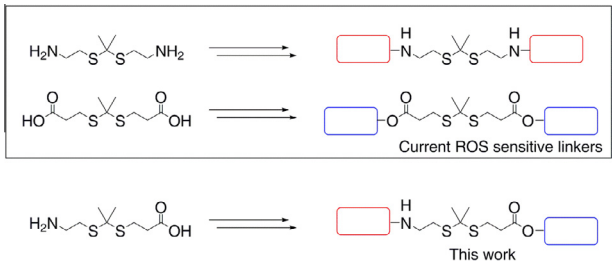
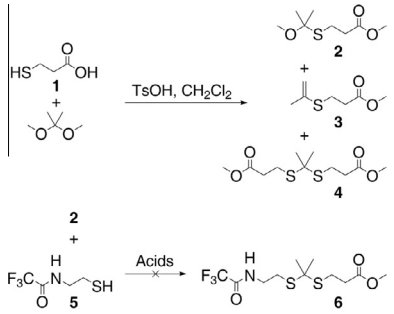
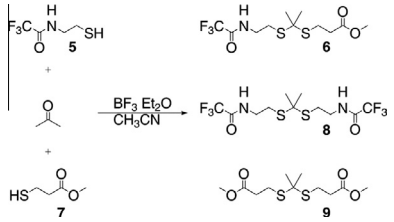


Scheme 1. Representative examples of ROS responsive linkers.



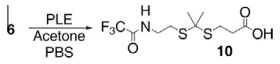
Scheme 2. Synthesis of thioketal **6** using transthioketalization.



Scheme 3. Synthesis of thioketal **6** using a one-pot method.

yield of the desired heterobifunctional *S*,*S*-thioketal **6** at 50%, as dictated by statistical distribution of the three possible combinations, it is a much more straightforward and easier method with an acceptable yield.

To prepare the linker for conjugation, it is necessary to selectively remove either the methyl ester or the trifluoroacetamide protecting group. Both methyl ester and trifluoroacetamide are popular small molecule protecting groups in bioconjugation that are relatively stable in many conditions but may be readily hydrolyzed in alkaline conditions. Although reports[12–14] of selective deprotection of methyl ester in the presence of trifluoroacetamide are known, the reaction conditions are not universally transferable to other structures due to poor efficiency or selectivity.[15] Unfortunately, attempts using typical literature conditions (lithium hydroxide or triethylamine in aqueous methanol) were unsuccessful. To resolve this chemoselectivity problem, we decided to use porcine liver esterase (PLE) to remove the methyl ester (see Scheme 4). PLE earned most of its fame in enzymatic organic synthesis for its remarkable performance in asymmetric hydrolysis of methyl esters.[16] While enantioselectivity is not a concern in this ester hydrolysis, the PLE worked profoundly well due to the chemoselectivity associated with enzymatic reactions. Initial trial of adding PLE to a PBS buffer containing the thioketal linker precursor **6** in acetone gave the propionic acid product **10** in 32% yield. The reaction yield was later improved to 73% by carefully maintaining the pH of the reaction mixture around 7–8 with the addition of sodium hydroxide solution as the reaction



Scheme 4. Selective deprotection of **6** using PLE.

proceeded. The resulting carboxylic acid **10** can then be coupled with a desired moiety using classical procedure before the trifluoroacetamide protection is removed for the second conjugation.

To validate the linker may be cleaved by ROS, we assessed the reaction kinetics of ROS triggered linker cleavage of thioketal **6** by ^{1}H NMR using simulated ROS condition.[17] Thioketal linker **6** was dissolved in a 1:1 mixture of CD_3OD and D_2O containing a catalytic amount of copper(II) chloride and 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TMSP) as the internal reference. Upon addition of hydrogen peroxide solution, ROS was generated in the presence of copper(II) chloride.[18] The decomposition of the thioketal group and generation of acetone ($\delta = 1.60$ and 2.20 ppm, respectively) were observed (see Fig. SI-1) and quantified using TMSP as the internal reference at 37 °C (see Fig. 1(a)). During the first 1.9 h, limited amount of the thioketal was converted as the reaction was likely limited by the low concentration of ROS. This is supported by the slow broadening and disappearance of the hydrogen peroxide signal around 10.8 ppm (see Fig. SI-2) and poor ^{1}H NMR spectrum resolution which may be associated with the presence of the oxygen bubble (see Fig. SI-1, spectra between 0 and 111 min) during this time period. Over the next hour, the reaction started to accelerate as the concentration of ROS increased (see Fig. 1(b)), though the reaction kinetics were not accessible due to the dynamic change of the reaction conditions and limited data. After 2.8 h the concentration of ROS was presumably high enough that its reaction with the thioketal became the rate-determining step. Hereafter, this reaction followed pseudo-first order kinetics with a rate constant of $1.30(\pm0.01) \times 10^{-4}\ s^{-1}$ and a half-life of 1.5 h. After 10 h, over

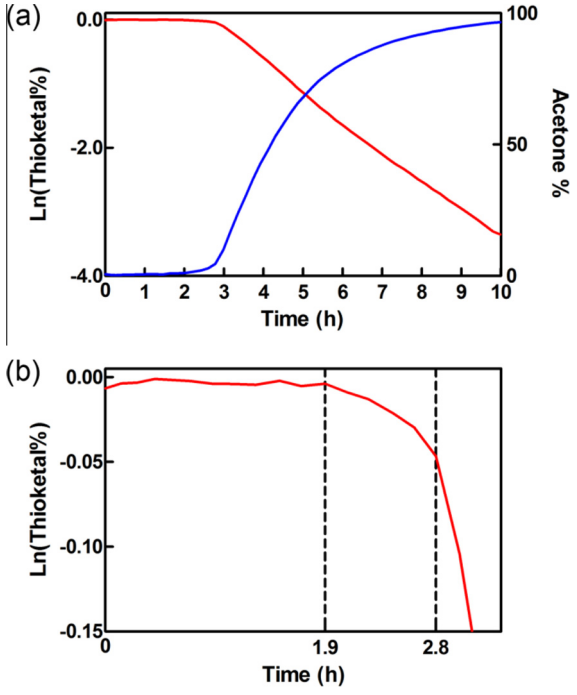


Figure 1. (a) The natural logarithm of the percentage of residual thioketal signal from **6** (red; left *y*-axis) and the percentage of acetone signal (blue; right *y*-axis) in simulated ROS environment plotted against time. (b) Zoomed view of the first 3 h of (a).

96% of thioketal was oxidized. Additional signals which may be related to reaction intermediates or other side reactions[19] were also observed; however, these did not influence the determination of the reaction rate.

In summary, we have developed a new heterobifunctional ROS-responsive thioketal linker with a carboxylic acid and a primary amine group as the conjugation sites. Such a heterobifunctional linker allows for developing ROS-responsive agents with two distinct functionalities, therefore, it has great promise in biomedical applications where on-demand photo activated bond cleavage is desirable. In addition, we found that PLE could be a useful tool to selectively remove methyl ester in the presence of trifluoroacetamide protecting group. Lastly, kinetic study showed that ROS effectively cleaved this thioketal linker and the cleavage reaction followed pseudo-first order kinetics in the presence of a sufficient amount of ROS.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.07.059.

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