

β -Scission of the N–O Bond in Alkyl Hydroxamate Radicals: A Fast Radical Trap

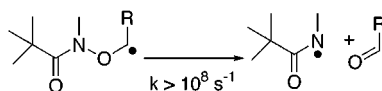
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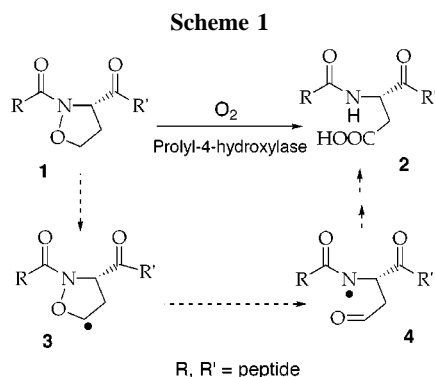
ABSTRACT



R = methyl, cyclopropyl or 2-phenylcyclopropyl

The rate of the β -scission of the N–O bond in the alkyl hydroxamate radical is faster than $2 \times 10^8 \text{ s}^{-1}$. This reaction may be useful as a radical trap.

During our studies on the mechanism of inactivation of prolyl-4-hydroxylase by 5-oxaproline-containing peptides, we identified **2** as the product of the enzyme catalyzed oxidation of **1**.¹ This suggested that N–O bond cleavage from the putative radical intermediate **3** had occurred (Scheme 1).



While N–O bond fragmentation reactions β to a radical center have been previously described,² we tested the plausibility of our proposal on a structure more closely

related to **3**. In this communication, we describe a small molecule model system **12** (Scheme 2) in which we have been able to duplicate the N–O bond fragmentation and estimate a lower limit for the rate of this reaction.

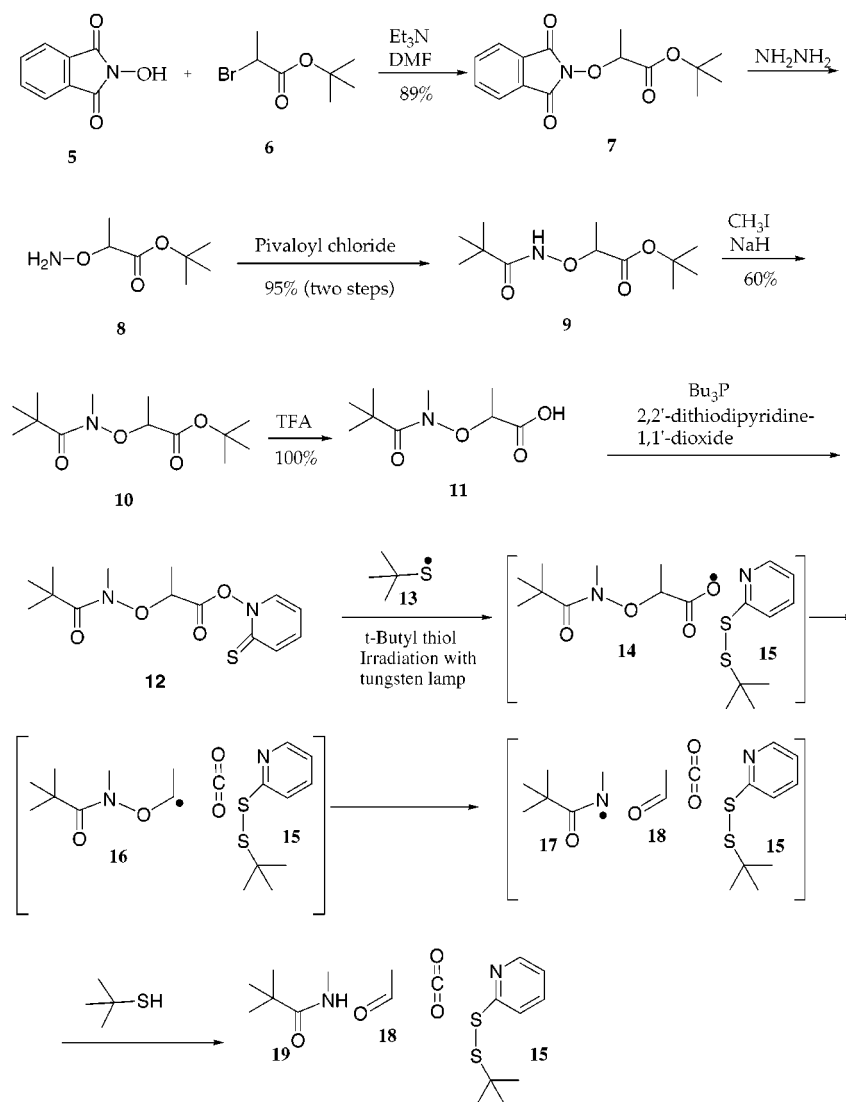
The synthesis of the model system **12** is outlined in Scheme 2. Irradiation of **12** with a tungsten lamp in the presence of *tert*-butyl thiol resulted in the formation of amide **19** which was isolated in 82% yield and the disulfide **15**. We suggest that these products were formed from radical **14**. Decarboxylation of **14** would give radical **16**, the analogue of the putative enzyme-generated radical **3**. Cleavage of the NO bond of **16** would give **17** which would then abstract a hydrogen atom from *tert*-butyl thiol to give amide **19**.

To estimate the rate of the N–O bond cleavage, the methyl group of **12** was replaced with a cyclopropyl group. For this compound (**20**), we expected that ring opening of

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Scheme 2

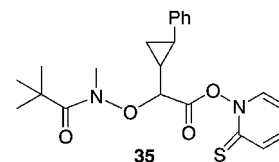


the cyclopropyl group of **21** would compete with the N–O bond fragmentation and that the ratio of **19** to **25** would allow us to estimate the N–O bond fragmentation rate (Scheme 3).

The synthesis of **20** is outlined in Scheme 4. Compound **20** was photolyzed in CD_2Cl_2 under the same conditions used for the photolysis of **12**. Analysis of the photolysis mixture by NMR and by GC/MS demonstrated the formation of

aldehyde **23** and amide **19**. The product **25** resulting from opening of the cyclopropyl ring of **21** was not detected (detection limit $< 10\%$). The product **22** resulting from direct hydrogen atom abstraction by **21** was also not detected. The ring-opening rate of the trimethylsilyloxycyclopropylmethyl radical is $2 \times 10^7 \text{ s}^{-1}$.³ If we assume that this is a reasonable approximate model for the rate of the cyclopropyl ring opening in **21**, we can estimate the rate of the N–O bond cleavage in **21** to be greater than $2 \times 10^8 \text{ s}^{-1}$.

The methyl group of **12** has also been replaced with a phenyl-substituted cyclopropyl group (**35**). Photolysis of this compound resulted in a more complex reaction mixture than



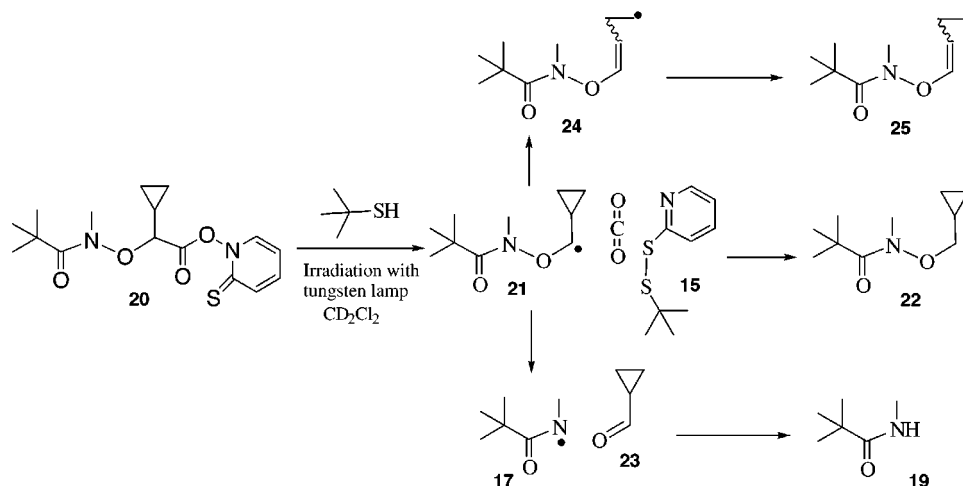
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Scheme 3

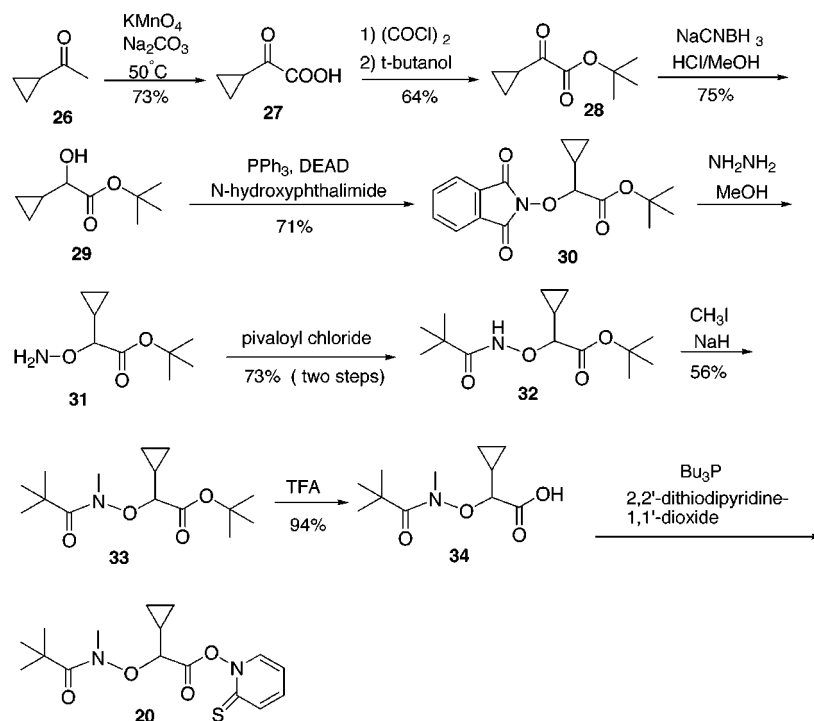


was obtained for the photolysis of **20**. NMR analysis of the crude reaction mixture demonstrated that amide **19** and the phenyl-substituted analogue of **23** were the major products of the radical fragmentation reaction. However, several minor components in the vinylic region that did not correspond with the expected ring-opened compound were observed and additional experiments will be needed before this system can be used to calibrate the rate of the N–O bond fragmentation. While not allowing for a precise determination, this experiment demonstrates that the NO bond cleavage is competitive

with the ring opening of the (2-phenylcyclopropyl)methyl radical ($k = 1.6 \times 10^{11} \text{ s}^{-1}$).⁴

The ring opening of the cyclopropyl carbinyl radical is the most widely used radical trap in mechanistic enzymology³ and has been used for example to probe for radical intermediates in the reactions catalyzed by monoamine oxidase,⁵ isopenicillin N synthase,⁶ deacetoxyacetylcephalosporin C synthase,⁷ methane monooxygenase,⁸ cytochrome P450,⁹ and acyl CoA dehydrogenase.¹⁰ However, in many cases the addition of a cyclopropyl group or a phenyl-

Scheme 4



substituted cyclopropyl group to the substrate for an enzymatic reaction is too large a perturbation on the structure and such substrate analogues cannot bind at the active site of the enzyme.¹¹ In addition, the synthesis involved in the introduction of the cyclopropyl group into the substrate can be difficult. For such cases, the alkyl hydroxamate based radical probe described here may be a useful alternative.

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Supporting Information Available: The synthetic and photolytic procedures for compounds **12** and **20**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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