A Novel Substitution Process for the Preparation of Alkoxy-, Aryloxy-, and Acyloxy-Substituted 1,2-Dioxetanes

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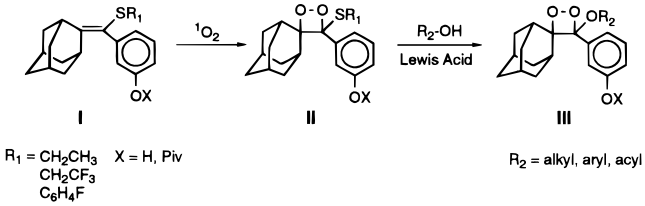
Stabilized 1,2-dioxetanes which are enzymatically triggered to undergo chemiluminescent decomposition are widely used in numerous applications including immunoassays, gene expression studies, Western blotting, Southern blotting, DNA sequencing, and the identification of infectious agents.[1] The dioxetane 4-methoxy-4-(3-phosphoryloxyphenyl)spiro[1,2-dioxetane-3,2′-tricyclo[3.3.1.1^{3,7}]decane], disodium salt, and analogous derivatives are chemiluminescent substrates for alkaline phosphatase. Common structural features of these dioxetanes include an alkoxy group, a spiroadamantyl group, and a protected aryl oxide group.

The preparation of these alkoxy-substituted dioxetanes has generally been accomplished by photooxygenation of the corresponding vinyl ether.[2] Other methods for preparing dioxetanes with alkoxy substituents from the corresponding vinyl ethers are known, e.g. electron-transfer oxidation with oxygen and triarylaminium cation radical salts,[3] oxidation by Cr(VI) or Mo(VI) oxide diperoxides,[4] and oxidation with triethylsilyl hydrotrioxide.[5] Nevertheless, there is no general method for synthesizing dioxetanes incorporating other types of groups in place of the alkoxy moiety. Synthetic methods are needed to prepare triggerable dioxetanes bearing groups which would be adversely affected during the oxidation of the vinyl ether or which would deactivate the vinyl ether toward $^1O_2$. We now report a general method for the preparation of a variety of alkoxy-, aryloxy-, and acyloxy-substituted dioxetanes from a common dioxetane intermediate. This process does not require the preparation of each individual vinyl ether precursor.

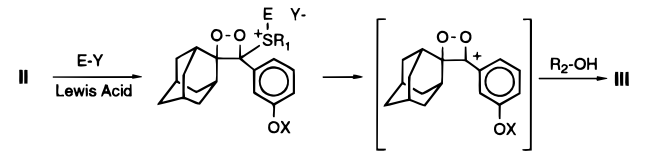
The key feature of this method is the replacement of a thiol group SR_1 of an alkylthio- or arylthio-substituted dioxetane by one of various oxygen nucleophiles (Scheme 1). Oxidation of vinyl sulfide **I** with $^1O_2$ at temperatures between 0 and −78 °C produces the sulfur-substituted dioxetane **II**. Treatment of **II** with a stoichiometric amount of a Lewis acid oxidant such as *N*-chlorosuccinimide (NCS), $Hg(OAc)_2$, or H_2O_2/I_2 and an excess of a nucleophilic oxygen compound leads to formation of oxygen-substituted dioxetanes **III** in moderate yields.

The direct replacement of a group on the dioxetane ring with another group is without precedent in dioxetane chemistry. Further, no reaction involving the direct introduction of substituents on a pre-formed dioxetane ring has been reported. This novel reaction sequence is best explained by an S_N1 mechanism involving a dioxetane carbocation (Scheme 2). Reaction of the

Scheme 1



Scheme 2



electrophile at sulfur converts the thioalkyl or thioaryl substituent into a good leaving group which ionizes to a benzylic carbocation. It seems likely that the cationic center is also stabilized by overlap with a lone pair on oxygen. No products resulting from carbocationic rearrangements were observed, although the presence of unidentified minor reaction products prevents us from definitively excluding their formation. The lack of significant amounts of rearranged products can be attributed to the thermodynamic stability of the adamantyl ring system. An S_N2 mechanism would appear unlikely on steric grounds. Recently, the nucleophilic substitution of a chloride leaving group on an ozonide has been reported.[6] Fluoride-, methoxy-, and acetoxy-substituted ozonides accompanied by ring-opened products were formed by an S_N1 reaction. It is particularly significant in the present case that the additional ring strain of the four-membered ring does not preclude substitution in favor of ring fragmentation as is the case with epoxides.[7]

Vinyl sulfides **I** (R_1 = C_2H_5, CH_2CF_3, or *p*-C_6H_4F) can be prepared by $TiCl_4$-initiated reaction of the corresponding aryl adamantyl ketone with the requisite mercaptan.[8] We have discovered that vinyl sulfides can also be formed by coupling adamantanone and a thioester with a low-valent titanium reagent prepared from $TiCl_3$ and $LiAlH_4$ in the presence of Et_3N in THF. The latter reaction achieves the direct formation of both carbon−carbon bonds of the vinyl sulfide. Although the formation of vinyl sulfides by reduction of vinyl sulfones with $LiAlH_4$−$TiCl_4$,[9] and the reductive elimination of β-halo sulfoxides with Zn−$TiCl_4$[10] have been reported, neither of these methods involves the direct formation of the sulfur-substituted C−C double bond from two carbonyl compounds.

Photooxygenation of vinyl sulfide **I** in the presence of either methylene blue or Rose Bengal is readily monitored by thin layer chromatography (TLC) or ^{1}H NMR spectroscopy by observing the disappearance of the vinyl sulfide. Additionally, heating a small portion of the reaction solution leads to easily detectable chemiluminescence indicating formation of the sulfur-substituted dioxetane. Treatment of sulfur-substituted dioxetanes **II** with F^- in DMSO produces yellow or orange-red chemiluminescence. Sulfur-substituted dioxetanes of relatively lower thermal stability (R_1 = C_2H_5) are not isolated at this point but instead reacted at −78 °C with a compound containing a hydroxyl group or its salt. Sulfur-substituted dioxetanes of higher thermal stability (R_1 = CH_2CF_3 and *p*-C_6H_4F) can be isolated first or reacted at 0 to −78 °C. A Lewis acid oxidant

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Table 1. Dioxetanes **III** Prepared (X = COt-Bu)

dioxetane	R_2	oxidant	yield (%)
1	CH_3	NCS	52
2	CH_2CF_3	NCS	21
3	$CH_2CH{=}CH_2$	NCS	19
4	C_6H_5	NCS	56
5	$COCH_3$	$Hg(OAc)_2$	52

(1−1.5 equiv based on **I**) is added to the sulfur-substituted dioxetane and the solution maintained at 0 to −78 °C for 10−30 min. The oxygen nucleophile (typically, 1−1.5 equiv based on **I**) is then added at low temperature and the reaction mixture warmed to room temperature. Alternately, the oxygen nucleophile may be present during the photooxygenation. Dioxetanes **III** were purified by silica gel chromatography.

The generality of the substitution process appears to be limited only by the availability of the oxygen nucleophile. Pivalate-protected dioxetanes **1**−**5** which have been prepared from the corresponding pivalate-protected ethylthio vinyl sulfide via the corresponding sulfur-substituted dioxetane **6** (**II**, X = COt-Bu, $R_1 = C_2H_5$) illustrate the scope of the reaction as shown in Table 1. Yields reported are after isolation and chromatographic purification and are unoptimized. Numerous other dioxetanes have been prepared, the details of which will be reported in a full paper. These include dioxetanes **III** in which X = H and R_2 = alkyl, aryl, acetyl, allyl, substituted alkyl, and polyhaloalkyl groups. In general, substitution reactions using sulfur-substituted dioxetanes derived from vinyl sulfides where X = COt-Bu were somewhat slower than those where the phenol was unprotected. The rates of substitution were improved by using a larger excess of the oxygen nucleophiles in those cases.

Since the oxygen-containing group OR_2 is not introduced until after formation of the dioxetane ring, it is possible to prepare dioxetanes which would be problematic to prepare by photooxygenation of the vinyl ether precursor. This process is amenable to the preparation of dioxetanes with R_2 groups such as alkenes and dienes which themselves react with $^1O_2$ (dioxetane **3**) or groups which could interfere with the photooxygenation either by quenching of singlet oxygen or by quenching or bleaching the photosensitizer. It is also amenable to the preparation of dioxetanes with groups which make the vinyl ether electron-deficient and therefore less reactive or unreactive to singlet oxygen. An aryloxy-substituted dioxetane **4** and an acyloxy-substituted dioxetane **5** have been prepared for the first time by this reaction.

The stable 1,2-dioxetanes **1**−**5** have long half-lives at room temperature, but can be triggered by an activating agent to decompose rapidly with half-lives ranging from seconds to a few minutes depending on the reaction medium. These dioxetanes are useful in their own right and as precursors to the phosphate and other derivatives which can be used in enzyme-linked assays.

The surprising stability of thioalkyl- and thioaryl-substituted dioxetanes **II** also deserves comment in view of the known thermal instability of other sulfur-substituted dioxetanes.[11] The most stable sulfur-substituted dioxetanes previously reported are derived from 4,5-dialkyl-2,3-dihydrothiophene and decompose with a half-life of a few minutes at room temperature.[12] Even incorporation of a spiroadamantyl group in other sulfur-containing dioxetanes has been insufficient to achieve a significant degree of stabilization. Two such dioxetanes bearing one and two sulfur substituents, respectively, on the dioxetane ring have been reported to rapidly and completely decompose on attempted isolation at room temperature.[11a] In contrast, sulfur-substituted dioxetanes used in the present study can be manipulated at temperatures of −10 to 25 °C. Dioxetanes bearing a (*p*-fluorophenyl)thio or (trifluoroethyl)thio group can be chromatographically purified at room temperature. Reaction of each of the sulfur-substituted dioxetanes with the triggering agent 0.1 M *n*-Bu_4NF in DMSO cleaves the O−X bond in seconds and induces a chemiluminescent decomposition at rates several orders of magnitude more rapid than thermal decomposition. The progress of this reaction can be monitored by eye in a darkened room. In our experience, the reagent *n*-Bu_4NF/DMSO is both a strong base and a powerful nucleophile and cleaves various types of phenol protecting groups including carboxylic esters, glycosides, and phosphate esters as well as silyl ethers.

A more detailed study of the thermal stability and triggered decomposition of the sulfur-substituted dioxetanes, including the unexpected stability of a (trifluoroethyl)thio-substituted dioxetane in alkaline amine buffers, will be presented in a subsequent publication. The full scope of the generality of this nucleophilic substitution method with regard to the electrophilic reagent, other oxygen nucleophiles, and stabilizing groups is being explored and will be detailed in a full report of this work.

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Supporting Information Available: Synthetic procedures and analytical data (7 pages). See any current masthead page for ordering and Internet access instructions.

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