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Published in:
EPRINTS-BOOK-TITLE

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
1991

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Hummelen, J. C., Luider, T. M., Oudman, D., & Wynberg, H. (1991). 1,2-Dioxetanes: Luminescent and Nonluminescent Decomposition, Chemistry, and Potential Applications. In *EPRINTS-BOOK-TITLE* University of Groningen, Stratingh Institute for Chemistry.

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1,2-Dioxetanes: Luminescent and Nonluminescent Decomposition, Chemistry, and Potential Applications

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Although compounds having the strained four-membered heterocyclic 1,2-dioxetane ring as part of their structure were reported at the end of the nineteenth century [1] and a number of investigators in the fields of oxidation reactions and chemi- and bioluminescence later proposed such structures as intermediates in some of these reactions [2-7], Kopecky and Mumford [8] were the first to prove that such compounds could actually be prepared and characterized. Since that time, well over 200 different 1,2-dioxetanes have been synthesized. These compounds were subjected mainly to experimental research for the determination of thermal stability, quantum yields of singlet and triplet excited-state product formation, (nonchemiluminescent) chemical reactivity, rearrangements, and induced decomposition by catalysts. Using these results, but also by means of theoretical calculations, theories were developed concerning the mechanism of thermal decomposition.

Since the mechanism of decomposition is strongly related to the thermal and chemical stability of a 1,2-dioxetane and, moreover, to the yield of excited-state products, it is of great importance to have a detailed insight into these mechanisms and the factors ruling them, in order to adequately design 1,2-dioxetanes that are useful for practical application in, for example, analyses, biology, agriculture, and medicine, Furthermore, knowledge concerning the chemical stability of 1,2-dioxetanes is important because these

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molecules will have to survive in a variety of chemical and biological environments if they are to be of value as a scientific tool.

18.1. MECHANISMS OF DECOMPOSITION OF 1,2-DIOXETANES

18.1.1 Concerted and Biradical Mechanism

After almost 20 years of scientific debate, the two different mechanisms ("concerted" and "biradical") proposed for the unimolecular thermal decomposition of 1,2-dioxetanes having neutral (alkyl, alkoxy, aryl) substituents still stand. The concerted mechanism was proposed by McCapra [9, 10], followed by a more detailed picture given by Kearns [11, 12] and Turro and coworkers [13-15]. The concerted mechanism involves a thermally induced simultaneous and homolytic cleavage of the C-C and O-O bonds of the four-membered ring, resulting directly in the formation of two carbonyl products of which one is in the singlet or triplet excited state (Figure 1a).

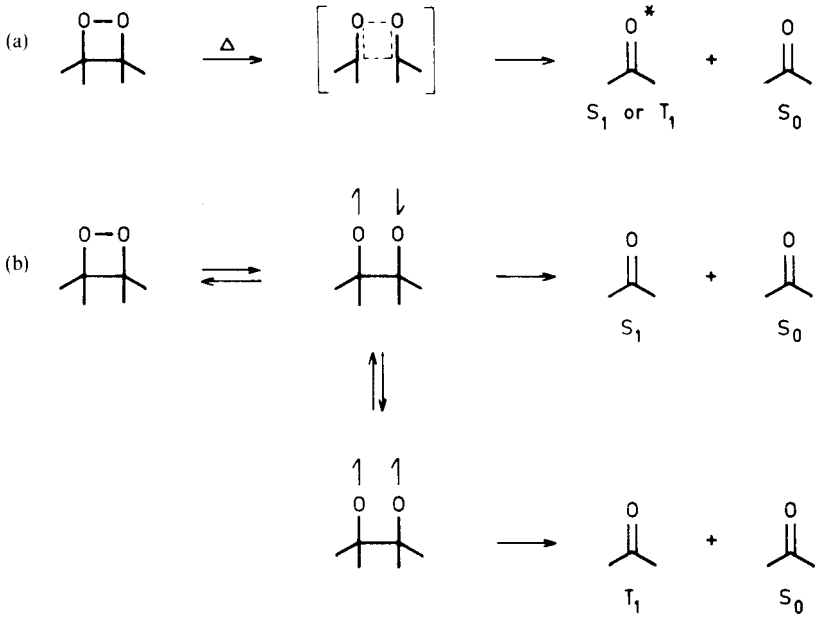

Figure 1 Mechanistic extremes proposed for the unimolecular thermal degradation of 1,2-dioxetanes having neutral substituents. (a) concerted process, (b) biradical process.

The biradical mechanism was postulated by Richardson and O'Neal [16-18]. In this mechanism the O-O bond stretches to form an intermediate singlet biradical in the rate-determining step. This singlet biradical can either close again to the 1,2-dioxetane, undergo C-C bond cleavage to form a singlet excited and a ground-state carbonyl product, or undergo (reversible) intersystem crossing to the triplet biradical. The latter biradical can undergo C-C bond cleavage to form a triplet excited and a ground-state carbonyl product (Figure 1b).

Quantum-mechanical calculations support either the concerted [19-22] or OCCO biradical pathway [23-26], depending on the type of calculation. Some calculations indicate only small differences in energy between the two reaction pathways [21, 27]. MINDO/3 calculation by Lechtken [21] indicates that primary O-O bond cleavage, concerted fragmentation, and even primary C-C bond cleavage are all possible pathways, depending on the substituents of the 1,2-dioxetanes.

Interestingly, experimental data in favor of the biradical mechanism have been accumulated using heteroatomic or aromatic substituted 1,2-dioxetanes, whereas mono-, di-, tri-, and tetraalkyl-substituted 1,2-dioxetanes (acyclic, alicyclic, and polyalicyclic) yield data that are rationalized most readily by a concerted mechanism.

18.1.2. Catalyzed 1,2-Dioxetane Decomposition

18.1.2.1. Chemically Initiated Electron-Exchange Luminescence

A third mechanism involves single electron transfer from a suitable donor (either inter- or intramolecular) to an organic peroxide, for example, a 1,2-dioxetane. After rearrangement or loss of part of the radical anion, a strongly reducing new radical anion is formed, leading to charge annihilation within the solvent cage. The latter step results in the formation of an electronically excited product. Although it was McCapra who first mentioned the possible involvement of charge separation for the oxidation of electronegatively substituted oxalate esters in the presence of a (donor) fluorescer [28], the importance of this mechanism has become fully recognized from the work of Schuster and co-workers [29-35]. The "chemically initiated electron exchange luminescence" (CIEEL) mechanism is now thought to be operating in almost all of the most efficient chemiluminescent reactions that involve peroxides. A general scheme for this mechanism is depicted in Figure 2.

The (thermal) stability of 1,2-dioxetanes that decompose via an intramolecular CIEEL mechanism is very low. On the other hand, the efficiency of singlet excited-state product formation is much higher. While aliphatic 1,2-dioxetanes yield singlet excited ketones with efficiencies (Φ_s) between 4×10^{-6} and 2×10^{-2}, 1,2-dioxetanes decomposing along the CIEEL

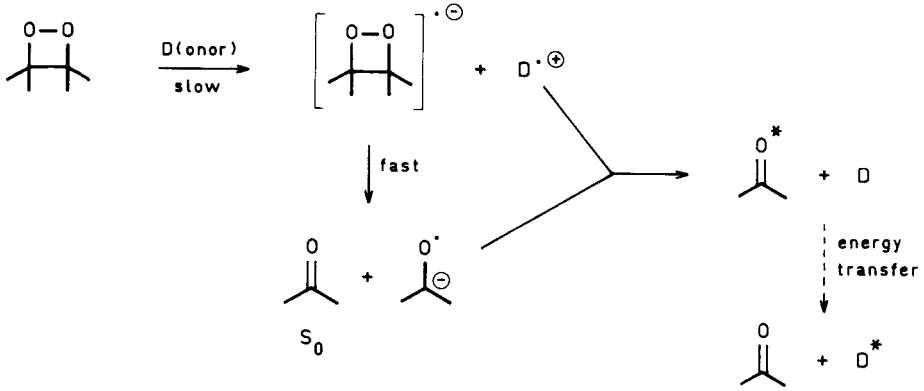

Figure 2 Scheme for 1,2-dioxetane decomposition by the chemically initiated electron exchange luminescence (CIEEL) mechanism.

pathway can reach Φ_s values of 5×10^{-2} to 0.57 [36-39]. Although more and more insight into this mechanism has been obtained during the last decade [40], from the recent work of Schaap and co-workers [41, 42] it has become clear that the exact geometric and electronic requirements for efficient CIEEL from a 1,2-dioxetane are still not well understood [41, 42]. From a pair of 1,2-dioxetanes having a meta-, or para-hydroxyphenyl substituent in the anionic form, only the meta-hydroxy-substituted phenyl-1,2-dioxetane showed efficient singlet product formation. From this result and the fact that the anions of a 2-(6-hydroxynaphthyl)-substituted, a 9-spiro (3-hydroxyxanthenyl)-substituted, and a 9-spiro (3-hydroxyfluorenyl)-substituted 1,2-dioxetane also showed low efficiencies for singlet excited-state product formation [41-43], one is tempted to conclude that efficient CIEEL is produced in systems where the 1,2-dioxetane is reduced by a (sufficiently strong reducing) *b*-carbanion (as opposed to an (*a*-carbanion). From molecular models it can be seen that close proximity between the aryl *b*-carbanion electron pair and the $s^*_{0\text{-}0}$ orbital can be reached in the case that the aryl group can rotate freely. Interestingly, in the case of highly efficiently chemiluminescent nitrogen-substituted 1,2-dioxetanes, in which the nitrogen atom plays a possible role in the electron transfer process, the possibility of formation of an (*a*-nitrogen anion (firefly luciferin, Cypridinia luciferin, Renilla luciferin), or the vinylogous (*a*-carbanion (spiroacridan, indolyl), appears to be present (as opposed to a *b*-nitrogen anion or a *b*-carbanion).

18.1.2.2. Metal-Catalyzed Decomposition by CIEEL and Other Mechanisms

The majority of the reported metal-catalyzed 1,2-dioxetane decomposition reactions appear to be nonluminescent. In 1973, Turro and Lechtken [44, 45] reported that the rate of decomposition of tetramethyl-1,2-dioxetane (TMD) was strongly solvent-dependent. The relatively very fast reaction of TMD in ethanol and methanol was explained later to be the result of metal catalysis (via a nonchemiluminescent route), since the addition of EDTA or Chelex 100 resulted in normal decomposition rates [46, 47]. The catalytic effects of the chlorides of Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Mn^{2+}, and Cd^{2+} on the decomposition of TMD has been studied quantitatively. The kinetic data were found consistent with a coordination mechanism in which the transition metal ion acts as a Lewis acid [48].

The catalytic effects of two iridium and 13 rhodium complexes on the decomposition of TMD were studied by Bartlett and McKennis [49]. These complexes seem to cleave the 1,2-dioxetane ring by oxidative addition. Again, the catalytic reaction appeared to be nonchemiluminescent. The lanthanide (Eu^{III}) shift reagent-catalyzed decomposition of TMD was reported in 1979, but no details were given [50, 51].

The Eu^{III}-catalyzed decomposition of spiroacridane-substituted 1,2-dioxetane 1 (Figure 3), found by McCapra and Watmore [52], was the first

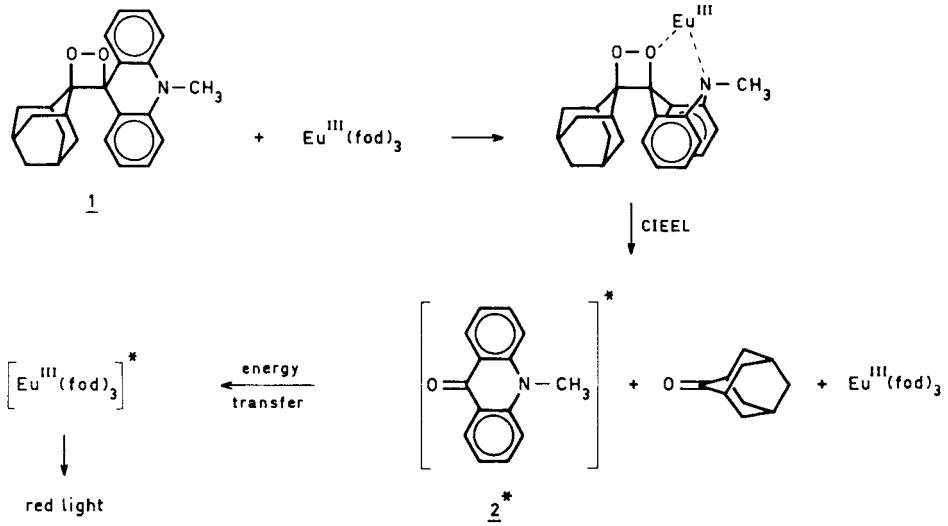

Figure 3 Luminescent Europium(III) catalyzed decomposition of a spiroacridane substituted 1,2-dioxetane (1).

chemiluminescent example of metal catalysis. The relative rate of decomposition of the dioxetane in the presence of $Pr(fod)_3$, $Eu(fod)_3$, $Dy(fod)_3$, and $Yb(fod)_3$ followed that expected for the decreasing Lewis acidity across the lanthanide series. Therefore, these authors state that the catalysis is the result of the increase in oxidation potential of the peroxide upon complexation with the Lewis acid. The process can, however, also be explained by supposing that the Eu^{III} ion complexes with both the electron donor (the acridane nitrogen atom) and the 1,2-dioxetane (in a Lewis acid fashion). Upon such complexation, both a favorable conformation and a favorable electronic situation are created for a CIEEL process. Subsequent efficient energy transfer from excited N-methyl acridone 2* to the fluorescent Eu^{III} complex [$Eu(fod)_3$] results in the emission of red light (Figure 3). In the case of a nonfluorescent lanthanide complex, energy transfer results in quenching.

Tolstikov and co-workers investigated the catalytic action of three organoaluminum compounds and complexes thereof with $Ru(bipy)_3Cl_2$ [53] and of $Tb(NO_3)_3 \cdot 5H_2O$, $Eu(fod)_3$, and $Ru(bipy)_3Cl_2 \cdot 6H_2O$ [54, 55] on the decomposition of adamantylideneadamantane 1,2-dioxetane 3 (Figure 4). The fast organoaluminum-catalyzed reaction is only weakly chemiluminescent ($\Phi_{CL} \approx 10^{-9}$). Furthermore, the identity of the emitter of the observed red light was not established. Upon the addition of $Ru(bipy)_3Cl_2$, the chemiluminescence intensity of this reaction was significantly enhanced.

The catalytic action of $Tb(NO_3)_3$ on adamantylideneadamantane 1,2-dioxetane was found to be negligible. Energy transfer from both triplet and singlet excited adamantanone to the lanthanide ion occurs in this case. The seemingly slightly decreased stability of the 1,2-dioxetane in the presence of the metal ion was explained by the shorter lifetime of T_1-adamantanone (T_1-4) at a higher temperature.

The action of Eu^{III} salts on this 1,2-dioxetane appeared to be more complicated [54]. $Eu(fod)_3$ accelerates the decomposition by hundreds of times. From the altered lifetime of excited $Eu(fod)_3$ in the presence of the 1,2-dioxetane it was concluded that complex formation occurs. Furthermore, the

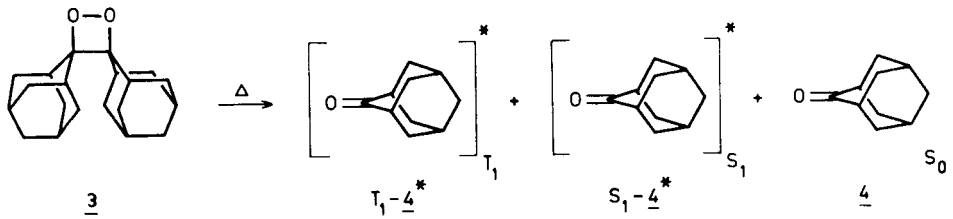

Figure 4 Thermal decomposition of adamantylideneadamantane 1,2-dioxetane (3) yielding adamantanone (4) in different electronic states.

adamantanone formed will compete for complexation with $Eu(fod)_3$, The net result of this set of interactions is a catalyzed 1,2-dioxetane decomposition with subsequent energy transfer of excited adamantanone to $Eu(fod)_3$, either complexed or not. The authors report that a similar catalytic reaction was found with TMD.

When $Ru(bipy)_3Cl_2 \cdot 6H_2O$ is added to a solution of adamantylideneadamantane 1,2-dioxetane in MeCN, another set of interactions, very different from that with Tb^{3+} and Eu^{3+}, rules the observed chemiluminescence. The luminescence is subject to temperature quenching, leading to an apparent strong drop in the activation energy (to ≈ 21 kcal/mol). Taking this into account, the efficiency of sensitization by Ru^{2+} exceeds the efficiency of energy transfer from both singlet and triplet excited adamantanone by one order of magnitude. The results were in accord with the assumption that $Ru(bipy)_3Cl_2$ plays a role in a chemiluminescent process, while the normal thermal decomposition takes place with a much higher rate constant and without emission of light because of efficient absorption quenching by ruthenium. Hence, the observed luminescence (with maximum intensity at 600 nm) originates exclusively from a minor (catalytic) process with a high chemiluminescence efficiency. A CIEEL type of process has been proposed [55] in which Ru^{2+} is first oxidized by the 1,2-dioxetane to Ru^{3+}, and reverse electron transfer from the adamantanone anion, formed upon fragmentation of the 1,2-dioxetane anion, leads to efficient production of excited Ru^{2+}.

Schuster and Schmidt [34, 56] reported the “unusual” catalytic activity of certain metalloporphyrins on the decomposition of dimethyl-1,2-dioxetanone. Thus, while with normal activators a good correlation of the initial chemiluminescence intensity (corrected for fluorescence efficiency and photomultiplier tube and monochromator response) with the oxidation potential of the activator was observed, such a correlation was not found for the tetraphenyl porphyrin (TPP) complexes Zn(TPP), Cd(TPP), Co(TPP), and Mg(TPP) and chlorophyll a. On the other hand, Ag(TPP) acted as a normal activator. On the basis of spectroscopic studies on mixtures of these metalloporphyrins and TMD, it was concluded that the unusually effective catalysts form ground-state complexes with the 1,2-dioxetane, while the other catalysts do not.

18.1.2.3. Other Catalytic Decomposition Reactions

In degassed solutions, TMD decomposes by an autocatalytic process in which the triplet excited acetone acts as the sensitizer [46, 57].

The addition of 9,10-dibromoanthracene (DBA) to a solution of 3,4-diethoxy-1,2-dioxetane (DED) has a similar effect on the rate of decomposition. The decomposition is catalyzed (in a nonluminescent way) by triplet excited DBA, which is formed by energy transfer from triplet excited ethylformate [58].

The decomposition of DED is also catalyzed via a competitive dark pathway by amines such as Et_3N, Et_2NH, and 1,4-diazabicyclo[2.2.2]octane (DABCO) [59]. Some other 1,2-dioxetanes, but not TMD, were also found susceptible to (dark) amine catalysis [60].

One example of electron-rich alkene-catalyzed decomposition (of DED) has been reported [59]. A mechanism involving charge transfer was proposed.

In general, simple 1,2-dioxetanes, that is, 1,2-dioxetanes without electronegative substituents, appear to be less susceptible to catalytic decomposition modes induced by electron-rich molecules.

Quenching phenomena, encountered during the high-temperature decomposition of functionalized adamantylideneadamantane 1,2-dioxetanes in aqueous solutions of a series of metal ions and other compounds [61] are efficiently inhibited upon the addition of cyclodextrins [61, 62]. In the stable inclusion compounds formed by these 1,2-dioxetanes and b- or g-cyclodextrin, the 1,2-dioxetane moiety is perfectly protected.

18.2 REACTIONS OF 1,2-DIOXETANES LEADING TO PRODUCTS OTHER THAN TWO CARBONYL FRAGMENTS

In addition to the various modes of typical 1,2-dioxetane decomposition, described above, these molecules undergo a series of chemical reactions in which they act similarly to other organic peroxides. Although the circumstances and the reagents used in the majority of these reactions will perhaps seldom be encountered during the possible applications of 1,2-dioxetanes in analysis, agriculture, and medicine, the results give further insight into the behavior of these compounds.

Triphenylphosphine and derivatives react quickly and quantitatively with TMD in C_6D_6 to yield phosphoranes [63]. The phosphoranes decompose upon heating to triphenylphosphine oxide and tetramethyl oxirane. When the insertion reaction was performed in CCl_4, a mixture of products was obtained [64].

The reaction of TMD with methyl diphenylphosphinite, trimethyl phosphite, and triethyl phosphite in C_6D_6 also yields phosphoranes in reasonable yields [65]. Kinetic studies using TMD and 3,4-diphenyl-1,2-dioxetane and a series of substituted triphenylphosphines have given more insight into the mechanism of insertion by the trivalent phosphor compounds [64, 66-71]. 3,3-Dimethyl-1,2-dioxetane was used in the preparation of a caged phosphorane [72]. The reactions of trimethylphosphite with an indolyl-1,2-dioxetane [73] and of triphenylphosphine with an imino-1,2-dioxetane [74] follow a different course after possible initial insertion.

Triphenylarsine and triphenylantimony also yield insertion products with TMD, although in the latter case a competitive catalytic decomposition of the 1,2-dioxetane occurs. Triphenylbismuth only catalyzes the decomposition of TMD [75].

The reaction of 1,2-dioxetanes (3,3-dimethyl- and trimethyl-) with organic sulfides yielding epoxides and sulfoxides [76] or other products [77] is of importance with respect to application of 1,2-dioxetanes in the presence of, for example, peptides and proteins. Such reactions have never been observed during the preparation and use of proteins labeled with derivatives of adamantylideneadamantane 1,2-dioxetane [78-80]. Recently an a-sulfur-substituted 1,2-dioxetane was prepared that was stable enough to be isolated and fully characterized [81]. The redox reaction may be a serious threat during the application of 1,2-dioxetanes that are of moderate stability.

Dimethyl and dipropyl sulfoxylates react with 1,2-dioxetanes to yield tetraalkoxysulfuranes [76].

Treatment of TMD with BF_3 results in a rearrangement to tert-butyl methyl ketone [82].

Kregs and co-workers found an acid-catalyzed rearrangement of an a,b-unsaturated 1,2-dioxetane to a homoallylic hydroperoxide [83].

Stannous chloride reacts with TMD to yield pinacol and acetone, as a result of insertion and catalytic decomposition, respectively [51].

The reduction of 1,2-dioxetanes to 1,2-diols can be accomplished more effectively using either Zn/HOAc [84, 85] or $LiAlH_4$ [64]. In the case of adamantylideneadamantane 1,2-dioxetane, Zn-HOAc is needed, since $LiAlH_4$ causes only slow decomposition to adamantanone and subsequent reduction to adamantanol. Upon hydrogenation with Pd/C as the catalyst, this 1,2-dioxetane yields the same products as a mixture. It was found that Pd/C acts as catalyst for decomposition of this 1,2-dioxetane [85] and trimethyl-1,2-dioxetane [64].

Like other peroxides, 1,2-dioxetanes are reduced quantitatively with aqueous KI-HOAc, yielding the corresponding 1,2-diols [64]. Adamantylideneadamantane 1,2-dioxetane is not readily reduced by this reagent.

The reduction of trimethyl-1,2-dioxetane with aqueous sulfite yields trimethyl oxirane and sulfate [64].

Jefford and co-workers [86] reported the reaction of aldehydes with para substituted aryloxy-spiroadamantyl-1,2-dioxetones to yield trioxanes.

There have been only a few reports on the reactivity of 1,2-dioxetanes toward nucleophiles [87, 88]. The reaction of NaN_3 with 3,3-dimethyl-1,2-dioxetane yielding acetone and N_2 is of importance because NaN_3 is widely used as a preservative for protein solutions. The reaction involves nucleophilic attack on the secondary 1,2-dioxetane carbon atom. Only a few 3,3-substituted 1,2-dioxetanes have been prepared. No such reactions were

reported for these other compounds. Most probably, only mono- and gem-disubstituted 1,2-dioxetanes are susceptible to S_N2-type reactions (on the unsubstituted carbon atom).

18.3. FUNCTIONAL GROUP TRANSFORMATION LEAVING THE 1,2-DIOXETANE MOIETY INTACT

For many types of applications of 1,2-dioxetanes, it is necessary to design and prepare derivatives having, for example, a functional group by which they can be attached to other molecules (1,2-dioxetane labels), a group that can be removed chemically or enzymatically (1,2-dioxetane enzyme substrates and the like), or a substructure that will interact specifically with other molecules [DNA-intercalators, amphiphiles, enzyme inhibitors, (antigenic) epitopes, liquid crystal building blocks, etc.]. It is not always possible to prepare a complete precursor that upon for example, photooxygenation, yields the desired 1,2-dioxetane in the last step of the synthetic route. Hence it may be necessary to perform some functional group transformations and/or derivatization reactions after the 1,2-dioxetane moiety has been constructed.

Well over 40 derivatives of adamantylideneadamantane 1,2-dioxetane have been prepared since 1977 by Wynberg and co-workers [78, 89-96]. During the course of these investigations, a variety of reactions were performed, leaving the 1,2-dioxetane moiety intact.

The first example was the quantitative Jones oxidation of a mixture of syn- and anti-4-equatorial-hydroxy adamantylideneadamantane 1,2-dioxetane 5a and 5b to the corresponding syn- and anti-4-keto compounds 6a and 6b (Figure 5) [91].

A subsequent ketalization of the ketones with ethylene glycol with a catalytic amount of p-TsOH in boiling benzene failed. The ketal of adamantanone and the mono- and diketals of adamantan-2,4-dione were isolated [91].

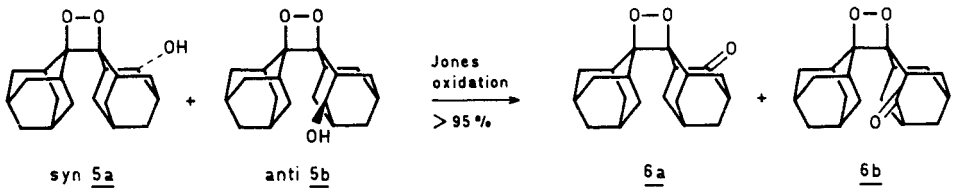

Figure 5 The first example of a functional group transformation leaving the 1,2-dioxetane moiety intact: Jones oxidation of *g*-hydroxy-1,2-dioxetanes to *g*-keto-1,2-dioxetanes.

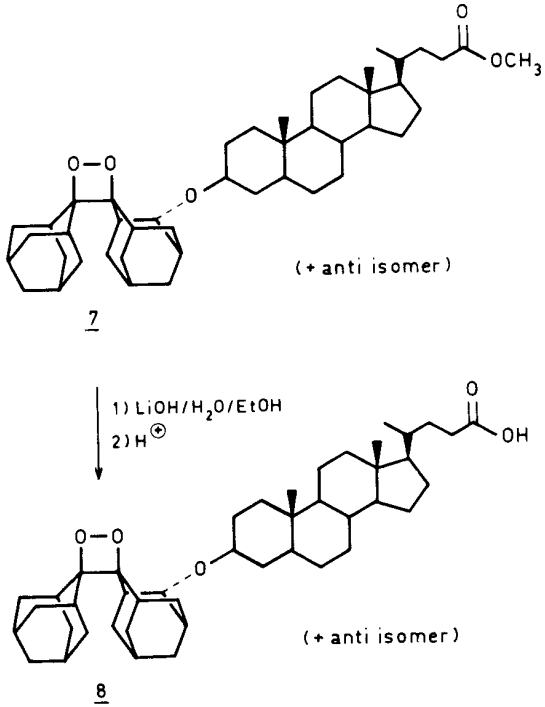

Figure 6 Hydrolysis of a methyl ester of a 1,2-dioxetane-labeled steroid.

Hydrolysis of the methyl ester of O-(4-adamantylideneadamantane 1,2-dioxetane)lithocholic acid 7 with LiOH in EtOH/H_2O for 12 hr at room temperature afforded the corresponding acid 8 in 98% yield (Figure 6) [93].

Esterification of iodoacetic acid with anti-4-equatorial-hydroxy-adamantylideneadamantane 1,2-dioxetane 5b using dicyclohexyl carbodiimide (DCC) and pyridine in CH_2Cl_2 yielded the first 1,2-dioxetane label 9 (for molecules containing a free thiol group) in 71% as a white crystalline product that can be kept for years in the dark without decomposition (Figure 7). This compound could not be prepared by a route comprising the photooxygenation of the iodoacetate of 4-hydroxyadamantylidene-adamantane because this homoallylic ester hydrolyzes readily.

Subsequent reaction of the 1,2-dioxetane label 9 with either 3-carboxy-4-nitrothiophenol 10 (reduced DTNB), reduced glutathione 11, or bovine serum albumin (BSA) in phosphate-buffered pH 7.0 EtOH-H_2O mixtures yielded the expected thiol coupling products without significant decom-

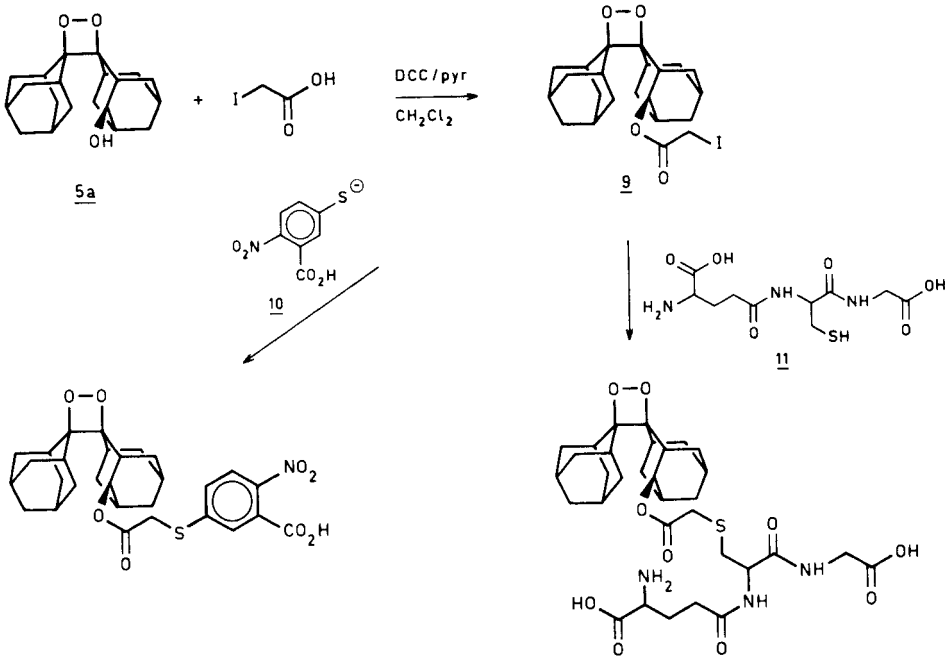

Figure 7 Esterification of a *g*-hydroxy-1,2-dioxetane and subsequent nucleophilic substitution reactions.

position of the 1,2-dioxetane (Figure 7) [78, 94, 95]. The BSA-1,2-dioxetane conjugate was chromatographed over Sephadex G-10, dialyzed against distilled water, and lyophilized to leave the protein as a white powder showing bright blue chemiluminescence upon heating. After storage at –20°C for 4 years, the material still showed the original chemiluminescence specific activity in a specially designed robotic "thermochemiluminescence" reader [79]. The specificity of this label for free thiol groups in proteins was established by amino acid analysis of 6 N HCl-hydrolyzed labeled BSA showing both the absence of cysteine and the presence of S-carboxymethyl cysteine, while the lysine content was not altered [94].

In the four-step synthesis of a homologous series of three 1,2-dioxetane labels starting from 4-bromoadamantylideneadamantane 12 (Figure 8), it appeared advantageous to construct the 1,2-dioxetanes (14a, b, c) in the second step. One of the reasons was that, in contrast to the alkene, the 1,2-dioxetane survives permanganate oxidation [94, 95]. The oxidation of the primary hydroxyl groups in 14a, b, c to the corresponding carboxylic acids

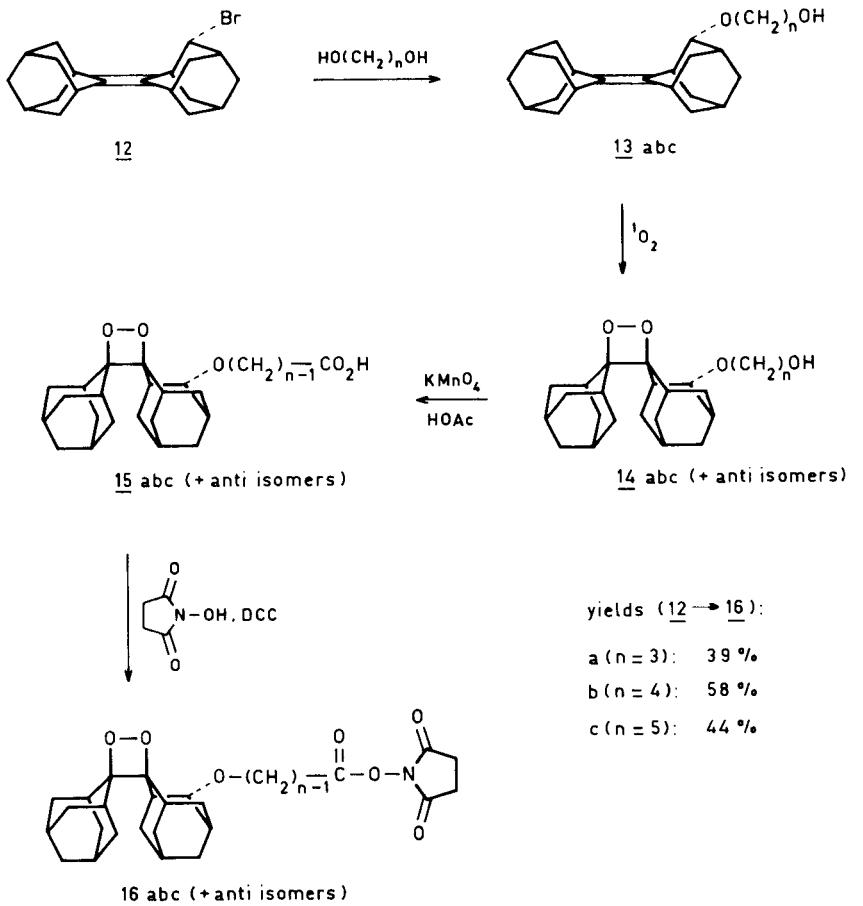

Figure 8 Synthetic scheme for the preparation of a homologous series of 1,2-dioxetane labels. Two subsequent functional group transformations are performed on the 1,2-dioxetanes.

15a, b, c was first attempted using pyridinium chlorochromate, pyridinium dichromate, or acidic chromium trioxide, but the yields of oxidation products were very poor. The 1,2-dioxetane, however, survived these conditions. Upon oxidation with $KMnO_4$-HOAc at room temperature, a clean and complete conversion was observed within a few hours. Excess $KMnO_4$ was removed by reduction with aqueous $NaHSO_3$ without traceable destruction of the 1,2-dioxetane (S_N2-type reactions on this 1,2-dioxetane are completely blocked by the adamantyl groups). The homologous carboxylic acids 15a, b, c could

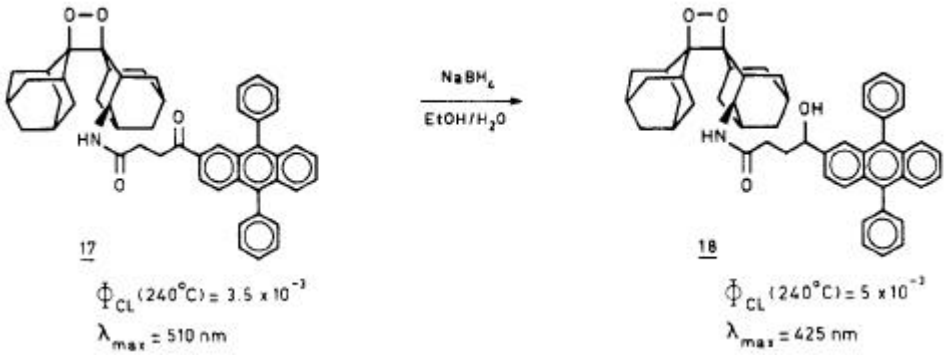

Figure 9 An aroyl substituted adamantylideneadamantane 1,2-dioxetane surviving a sodium borohydride reduction.

be isolated as their ammonium salts from dry NH_3-saturated ether-pentane solutions. Thereafter, they were converted to the free acids again by treatment with dilute mineral acid (2 N HCl) in an overall yield of 48-76% [94].

The conversion of eight different carboxylic acid derivatives of adamantylideneadamantane 1,2-dioxetane to the corresponding N-hydroxysuccinimide esters (among them compounds 16a, b, c in Figure 8) has been achieved by reaction with dry N-hydroxysuccinimide and DCC in dry 1,4-dioxane at room temperature for 12-24 hr [94, 97]. The preparation of such "active esters," used as thermochemiluminescent labels for proteins and for coupling to amines, was always done as the last step in a synthetic route (i.e., on the 1,2-dioxetanes), because these esters are very sensitive to moisture. The yield of this reaction was 90-100%. Concurrent degradation of the 1,2-dioxetane has never been observed.

Clean and quantitative reduction of an aryl ketone moiety of a 1,2-dioxetane-containing compound 17 (Figure 9) was achieved with $NaBH_4$ in EtOH-H_2O. This conversion is of interest because the maximum wavelength of light emission (and fluorescence) by these thermochemiluminescent compounds changes from 510 nm in 17 to 425 nm in 18 upon reduction [97]. (See also next paragraph.)

A mixture of syn-4-equatorial- and syn-4-axial-hydroxyadamantylideneadamantane 1,2-dioxetanes was obtained in 80% yield from the syn-4-keto compound 6a by similar reduction with $NaBH_4$.

Recently, Adam and co-workers [98-102] reported on the chemical transformations of 3-(hydroxymethyl)-3,4,4-trimethyl-1,2-dioxetane 19 and derivatives thereof.

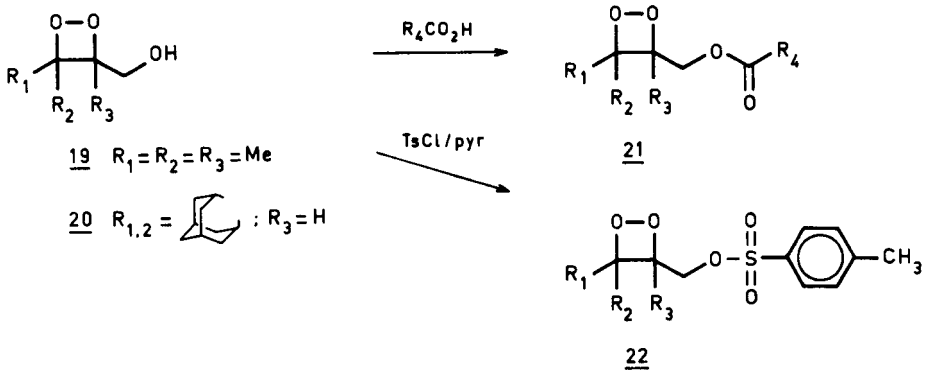

Figure 10 Esterification and tosylation of 1,2-dioxetanes by the Mitsunobu and the Brewster-Ciotti procedures, respectively.

A number of carboxylic esters 21 were prepared from 19 as well as from 2,3'-spiroadamantyl-4'-(hydroxymethyl)-[1,2]-dioxetane 20 (Figure 10) using either the Brewster-Ciotti or the Mitsunobu procedure (RCO_2H-tosyl chloride in pyridine at 0-13°C, or triphenylphosphine-ethyl azodicarboxylate-RCO_2H in diethyl ether, respectively). By leaving out the carboxylic acid in the Brewster-Ciotti procedure, tosylates 22 were prepared from both hydroxymethyl-1,2-dioxetanes [98]. Later [100], a simpler esterification procedure [RCO_2H-DCC-4-(dimethylamino)pyridine] analogous to that followed before for 1,2-dioxetanes [94, 95] was used.

A series of carbamates 24-27 derived from 3-hydroxymethyl-3,4,4-trimethyl-1,2-dioxetane 19 were prepared either by a direct reaction with isocyanates using DABCO or trifluoroacetic acid as a catalyst or by a two-step procedure via chloroformate 23 [99]. Chloroformate 23 was stable enough to allow isolation and purification (Figure 11). Using 23 a series of bis-carbamate-linked bis-1,2-dioxetanes 27 and carbamate-linked amino acid and dipeptide 1,2-dioxetane derivatives 25 were synthesized in 47-95% yield [101].

Carbonates 28a and 28b were obtained from the reaction of sodium or lithium salts of the hydroxymethyl-1,2-dioxetane with chloroformate derivatives of methanol and cholesterol (Figure 12). The yields of these reactions were very poor, however [100]. A better result was obtained when the chloroformate 23 was allowed to react with methanol-pyridine or the lithium salt of lauryl alcohol to yield 28a and 28c [101]. Carbonate derivatives of cholesterol (28b) and phenol (28d) were obtained analogously. Even the phenylthiocarbonate 29 could be prepared by this method,

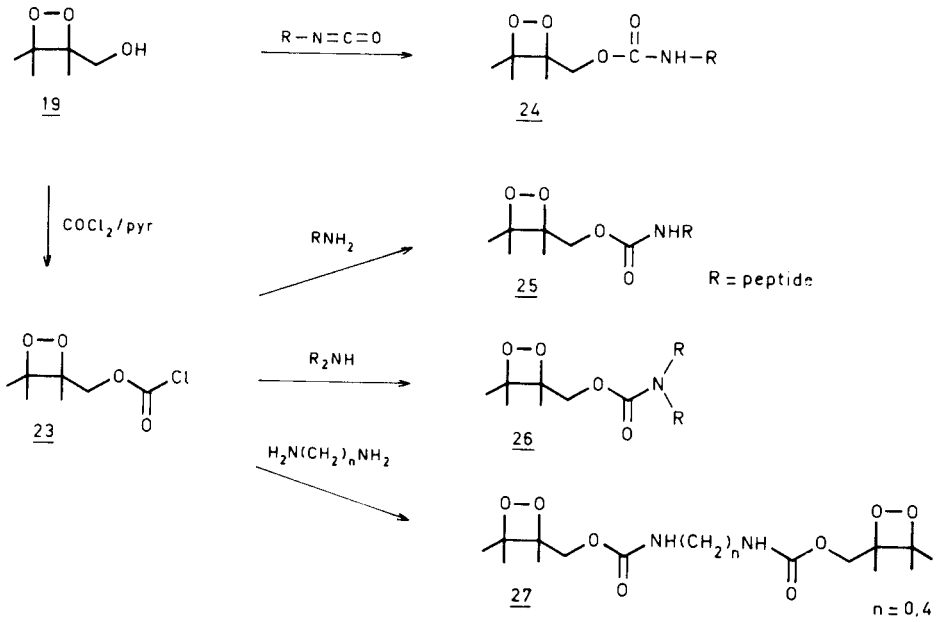

Figure 11 The direct and indirect derivatization of a *b*-hydroxy-1,2-dioxetane to carbamates.

although rapid workup and purification appeared obligatory to avoid destruction of the 1,2-dioxetane by unreacted thiol. Furthermore, the carbonate derivatives 30 of two oximes were prepared in moderate yield by means of a two-phase reaction between the chloroformate and sodium salts of the oximes.

The preparation of benzofuran, coumarin, and psoralen derivatives 28e, f, g (Figure 13) of 3-(hydroxymethyl)-3,4,4-trimethyl-1,2-dioxetane through a carbonate linkage was also achieved using chloroformate 23 [102]. These compounds were prepared in 26-64% yield.

The reaction of 19 with chlorosulfonyl isocyanate in diethyl ether (Figure 13) yielded an interesting functionalized 1,2-dioxetane 31 that can possibly be used as a 1,2-dioxetane label [100].

Formation of the ethyl ether 32 was achieved upon reaction of the 1,2-dioxetane alcohol with triethyl oxonium tetrafluoroborate in methylene chloride at 20°C. Trimethylsilyl ether 33 was prepared by using trimethylsilyl chloride and pyridine-Et_2O at 0°C [100] (see Figure 14).

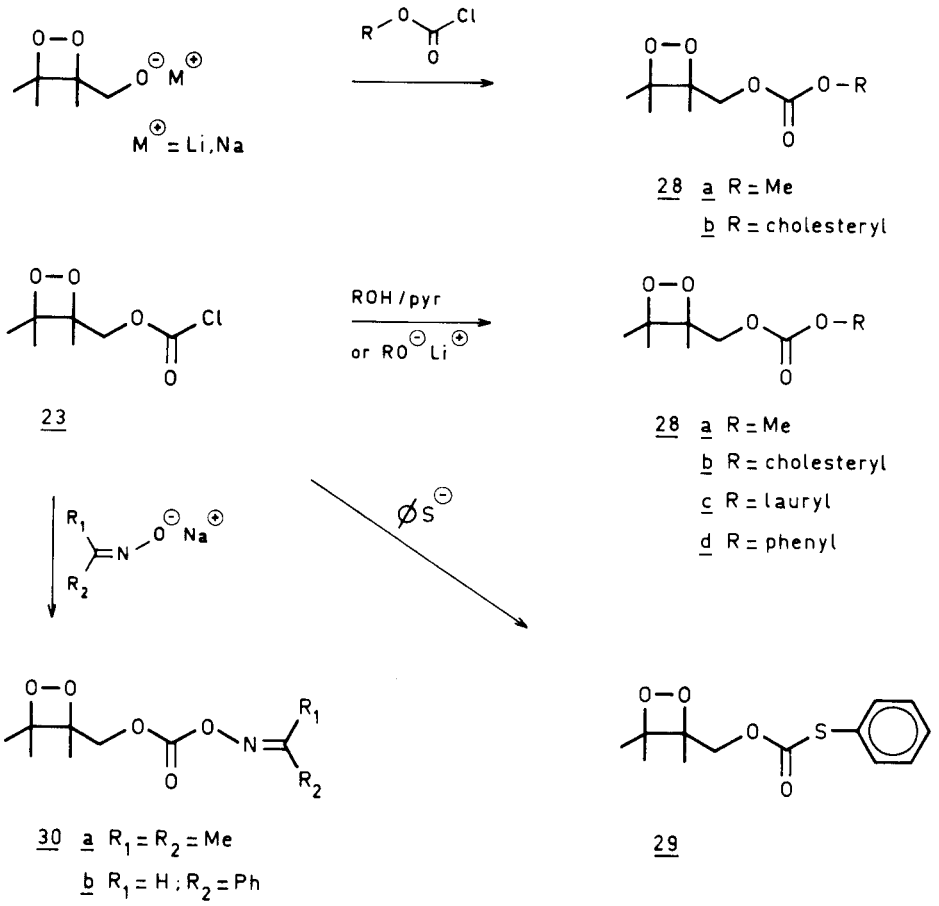

Figure 12 Preparation of carbonates, oximecarbonates, and a thiocarbamate from a hydroxy-1,2-dioxetane and a chloroformato-1,2-dioxetane.

From the above-mentioned reactions it becomes clear that although chemical modifications have been described only for the tetraalkyl-1,2-dioxetanes derived from TMD and adamantylideneadamantane 1,2-dioxetane, if the reaction conditions are chosen properly-moderate temperatures, no strong mineral acid, no strong reducing agents-and other more obvious precautions are taken, 1,2-dioxetanes can be functionalized and derivatized to a great variety of potentially interesting and useful compounds.

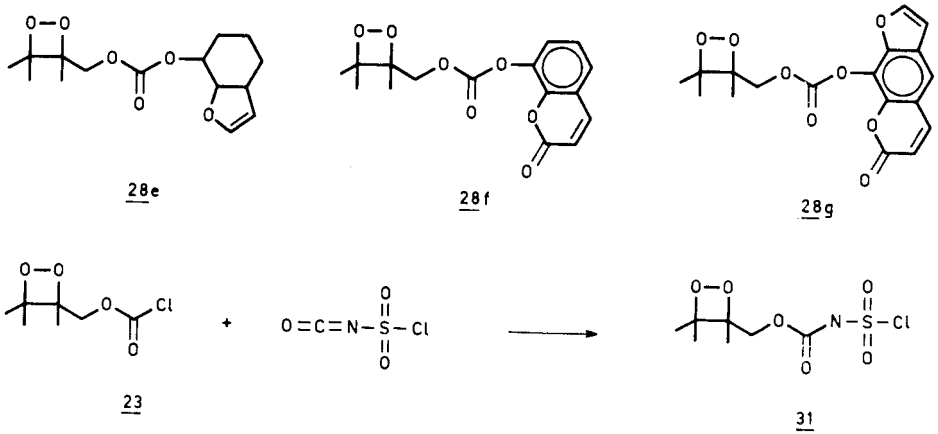

Figure 13 Tetrametyl-1,2-dioxetane linked by a carbonate bridge to benzofuran, coumarin, and psoralen (28e-g). Preparation of a chlorosulfonylcarbamate (31) from a *b*-chloroformato-1,2-dioxetane.

18.4 APPLICATION OF 1,2-DIOXETANES

18.4.1. Applicability in General; Miscellaneous Uses of 1,2-Dioxetanes

Although from the start of the research on 1,2-dioxetane chemistry and physics many workers speculated about the possible uses of these compounds, it is only recently that actual application in other disciplines of science has become reality. (1,2-Dioxetanedione, the compound that is likely to be the intermediate in the chemiluminescent reactions of oxalate esters, is left out of consideration, since it is not a 1,2-dioxetane that can be isolated.) Considering the rather sudden burst of papers that have appeared during the last few years, especially from the groups of Adam, Schaap, and Wynberg, it seems that we are only at the beginning of harvesting the utilities of 1,2-dioxetanes. Since most 1,2-dioxetanes are rather precious compounds that are synthesized without much emphasis on cost and waste, and since 1,2-dioxetanes are explosive, they seem unattractive for use in bulk products of the kind that Rauhut foresaw for the application of oxalate esters [103]. Until now, most studies were on the feasibility of 1,2-dioxetanes of various sorts to play a key role as fine chemicals for use in analytical techniques and medicine.

The first applications of 1,2-dioxetanes were quite different, however. Since chemiluminescence reactions are splendid examples of visual demonstrations of chemical reactions, they are of great educational value [104-106].

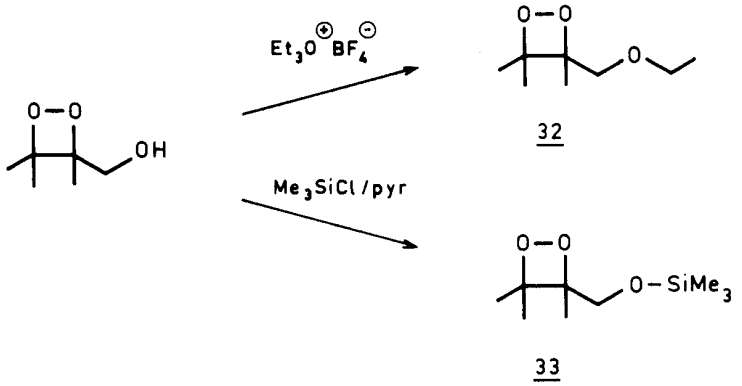

Figure 14 Etherification of a beta-hydroxy-1,2-dioxetane.

Turro and others [105] recognized the enormous value of 1,2-dioxetanes in the study of chemical and (photo)physical processes:

> Study of the mechanism of the $\Delta H \rightarrow *$ step (conversion of exothermicity of a chemical reaction into electronic excitation energy of a reaction product) in the thermolysis of 1,2-dioxetanes allows a blending of the ideas of classical kinetics and molecular spectroscopy. Also it provides insight concerning the mechanism and concepts of nonadiabatic reactions, spin-flipping processes, cycloelimination reactions, and intramolecular energy exchanges which are all interwoven in the $\Delta H \rightarrow *$ step in the 1,2-dioxetane decompositions. Moreover, we show how appropriate coupling of the high selectivity and efficiency of the chemiexcitation step with the experimental simplicity and sensitivity of the luminescence step allows us to utilize the chemiluminescence of 1,2-dioxetanes: (a) to discover new processes such as quantum chain reactions, anti-Stokes sensitization processes, and excitation energy hopping from one solvent molecule to another solvent molecule; (b) to develop new concepts for photochemistry, such as masked excited state; and (c) to study in detail mechanisms of electronic energy transfer, diffusion of mass and diffusion of excitation in fluid solvents, and cage effects.

The same authors also imagined the use of 1,2-dioxetanes as “chemical pumps” for lasers that emit visible light [105]. More recently [107, 108], the infrared laser-induced explosive decomposition of solid TMD yielding a blue flash (with potential lasing properties) was described. The results were consistent with a two-step process in which the infrared laser heats the skin of

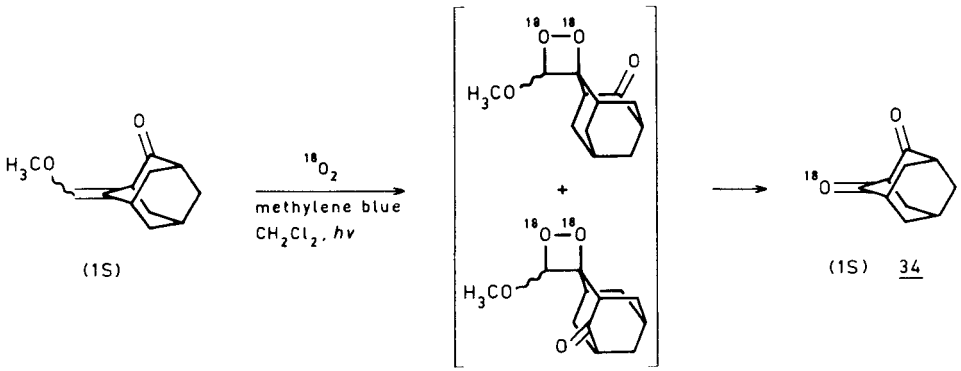

Figure 15 1,2-dioxetanes serving as intermediates in the synthesis of a 1,3-dione, chiral solely due to the presence of different isotopes of oxygen.

the TMD crystal, resulting in autocatalytic decomposition of the rest of the crystal.

Synthetically, 1,2-dioxetanes have been used as intermediates in the preparation of isotopically labeled (^{18}O) compounds. Both for a mechanistic study in which $^{18}O_2$-cis-1,2-diols were obtained [109] and for the preparation of (1S)-2,4-adamantanedione-4-^{18}O 34, a molecule solely chiral due to the presence of an oxygen isotope (Figure 15) [110], the 1,2-dioxetanes were prepared by photooxygenation with $^{18}O_2$.

18.4.2. Analytical Applications

Parallel with the applications of chemiluminescent and bioluminescent reactions in general, the most eminent field for application of 1,2-dioxetanes is that of analytical chemistry. Such applications, with special emphasis on the development of chemiluminescent labels for immunoassay-type analysis, have been studied since 1979 by Wynberg and co-workers [61, 62, 78-80, 92-97]. The recent work of Schaap's group could also lead to the use of chemically or enzymatically triggerable 1,2-dioxetanes in analysis, for example, as substrates for the end-point determination as part of an enzyme immunoassay [39, 41-43, 111].

18.4.2.1. 1,2-Dioxetanes as Chemiluminescent Labels

The rather unique property of 1,2-dioxetanes of being inherently luminescent makes these compounds suitable as thermochemiluminescent (TCL) labels [the prefix "thermo" is used because the chemiluminescent signal is obtained

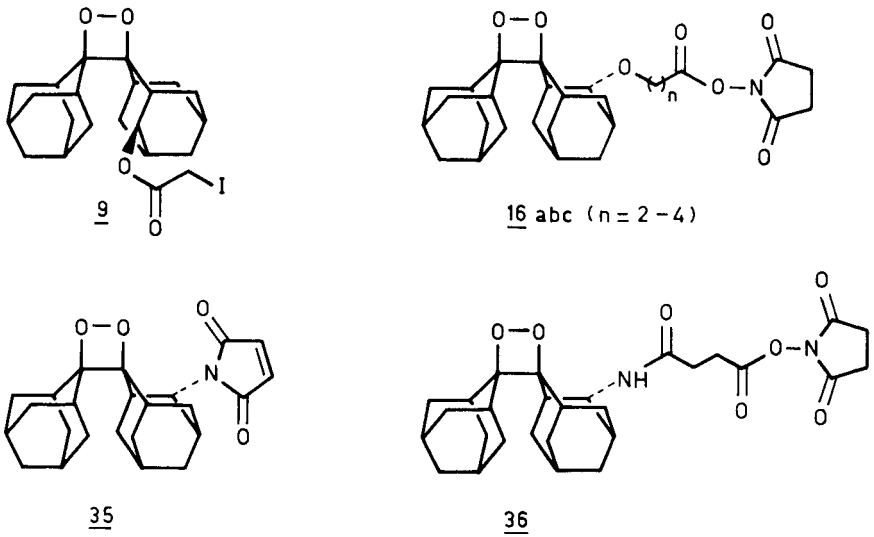

Figure 16 Thermochemiluminescent labels derived from adamantylidene-adamantane 1,2-dioxetane.

simply by heating a sample, in contrast to chemiluminogenic compounds, which are luminescent upon the addition of (mostly oxidizing) reagents], provided that the 1,2-dioxetane is stable enough to allow (1) storage of the label and labeled compounds, (2) purification of labeled compounds, (3) normal assay protocol procedures (e.g., the use of a variety of aqueous buffers in the range of pH 5-9, incubation temperatures up to 40°C, the presence of metal ions that are normal ingredients of biological fluids), and (4) reproducible and reliable quantitation within a large range of concentrations in the presence of biological material.

The only 1,2-dioxetanes that have been used for this purpose until now are those derived from adamantylideneadamantane 1,2-dioxetane (Figure 16). The thermal and chemical stability of these compounds indeed allow virtually all biochemical and immunological procedures that are commonly used in the field of immunoassay.

TCL-labeled proteins (e.g., antibody conjugates) can be stored for long periods of time without detectable loss of TCL specific activity. Solid-phase sandwich immunoassays can be performed by using antibodies labeled with 5-15 1,2-dioxetane molecules [61]. However, the sensitivity of such assays is not comparable to that of the corresponding enzyme immunoassay (EIA), since the specific activity of the labeled antibody is relatively low. This is due

to the fact that the adamantylideneadamantane 1,2-dioxetane-based labels have a chemiluminescence efficiency of "only" about 1×10^{-4}.

A great enhancement of the CL efficiency (and the corresponding specific activity of the labeled antibody) is obtained upon labeling the antibody with both the 1,2-dioxetane label and the fluorescent label 37, derived from 9,10-diphenylanthracene (Figure 15) [61, 78-80, 94]. Such dually labeled (monoclonal) antibody conjugates are still immunologically active and can be used to perform immunoassays (e.g., for hCG) with a sensitivity and accuracy only slightly inferior to those of the corresponding commercial EIA [80]. The detection limit of hCG in human serum was ~5mIU/mL. When BSA was labeled with excess TCL label and the fluorescent label, a soluble protein with a chemiluminescence efficiency of maximally 4% was obtained. The efficiency of intramolecular energy transfer in the labeled protein was found to be quantitatively consistent with a Förster-type process [62]. After coupling of an antibody with such heavily labeled BSA, a protein-protein conjugate was obtained that could also be used in an immunoassay [78].

When TCL labels derived from adamantylideneadamantane 1,2-dioxetane are complexed with *b*- or *g*-cyclodextrin, extremely reproducible quantitation is possible in solutions containing 5×10^{-15} to 5×10^{-10} mol of inclusion compound per sample [61, 62, 78]. Quenching by metal ions and some other

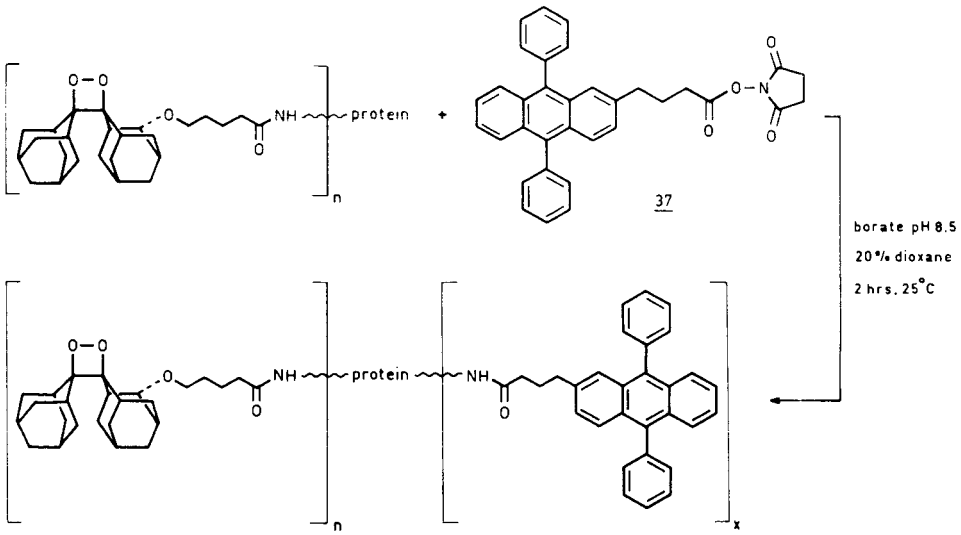

Figure 17 Dual labeling of a protein with a TCL-label and a fluorescent label that serves as efficient energy acceptor and fluorescer at temperatures up to 250°C.

compounds of the TCL of 1,2-dioxetane-labeled proteins at 240°C (e.g., up to 1 % solutions of $CuCl_2$, $CoCl_2$, $FeCl_2$, $MnCl_2$, hydroquinone, p-iodophenol, NaN_3, or bis-*t*-butyl-p-cresol) is effectively inhibited upon complexation with cyclodextrins [61].

The main obstacles for routine application of the TCL immunoassay technique, using the extremely stable adamantylideneadamantane 1,2-dioxetanes as labels, are (1) the special apparatus needed for fast and accurate quantitation and (2) the temperature that is needed for a fast reading of a sample (above 200°C), making it impossible to measure liquid aqueous solutions at atmospheric pressure.

18.4.2.2. 1,2-Dioxetanes as Enzyme Substrates

Based on the finding that deprotonated hydroxyphenyl-substituted 1,2-dioxetanes are unstable compared to the corresponding phenol and derivatives thereof [112], a series of 1,2-dioxetanes were prepared that are reasonably thermally stable but can be converted chemically or enzymatically into labile 1,2-dioxetanes [39, 41-43, 111]. As a result, an almost instantaneous chemiluminescence is observed following the kinetics of the chemical or enzymatic reaction and/or the kinetics dictated by the (low) stability of the chemical or enzymatic 1,2-dioxetane product. Thus, upon chemical (or enzymatic) conversion, the 1,2-dioxetane changes from the kind that decomposes by a biradical or concerted mechanism into one of the kind that decomposes by a CIEEL mechanism. Examples of such 1,2-dioxetanes are shown in Figure 18.

Although all of the 1,2-dioxetanes 38-46 have a thermal stability that is expected to be sufficient for their use as an enzyme substrate without giving a high (thermal) background signal, only compound 41 has a (very) high CIEEL efficiency (upon treatment with fluoride). All other compounds, among which the enzyme-cleavable ones 38, 43 (cleavable by aryl esterase), and 45 (cleavable by alkaline phosphatase) show CIEEL efficiencies ranging from 1×10^{-6} to 4×10^{-5}. Hence, the most promising candidate for a useful enzyme substrate, a 1,2-dioxetane with a structure analogous to that of 41, has yet to be prepared. The combination of such compounds, if efficiently chemiluminescent, with enzymes having a high turnover rate can probably compete with the enhanced peroxidase-luminol chemiluminescent system and the very efficient but fragile firefly (*Photinus pyralis*) luciferin-luciferase bioluminescent system for sensitivity and applicability in immunoassays and the like. An example of an enzyme-triggered 1,2-dioxetane decomposition is shown in Figure 19.

The chemiluminescence efficiency of the triggering reactions using compounds 43-45 appears to be equal, that is, the efficiency is dictated solely by the nature ofthe 1,2-dioxetane after chemical or enzymatic removal of the oxygen protecting group [42, 97].

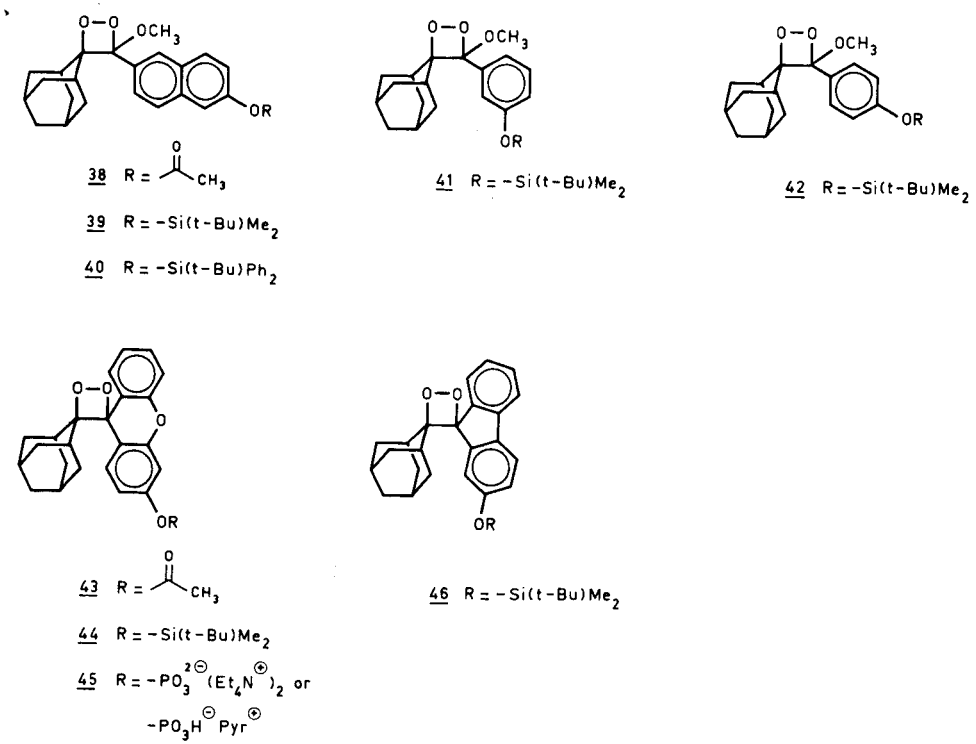

Figure 18 1,2-dioxetanes that decompose by a CIEEL mechanism upon chemical or enzymatic removal of a group that masks an intramolecular electron donor-moiety.

Interestingly, the thermal decomposition of compounds 38-46 yields electronically excited adamantanone and ground-state neutral aromatic esters or ketones, whereas the triggered reactions result in the formation of ground-state adamantanone and the electronically excited anions of the hydroxyaryl esters and ketones.

The rate of the base-triggered chemiluminescence from an acetate-substituted 1,2-dioxetane as well as that of the fluoride-triggered reaction of silyloxy dioxetanes is effectively enhanced upon the addition of cationic surfactants such as CTAB [111]. The micellar catalysis was explained as being the result of electrostatic attraction between the cationic head group and the anionic reagent.

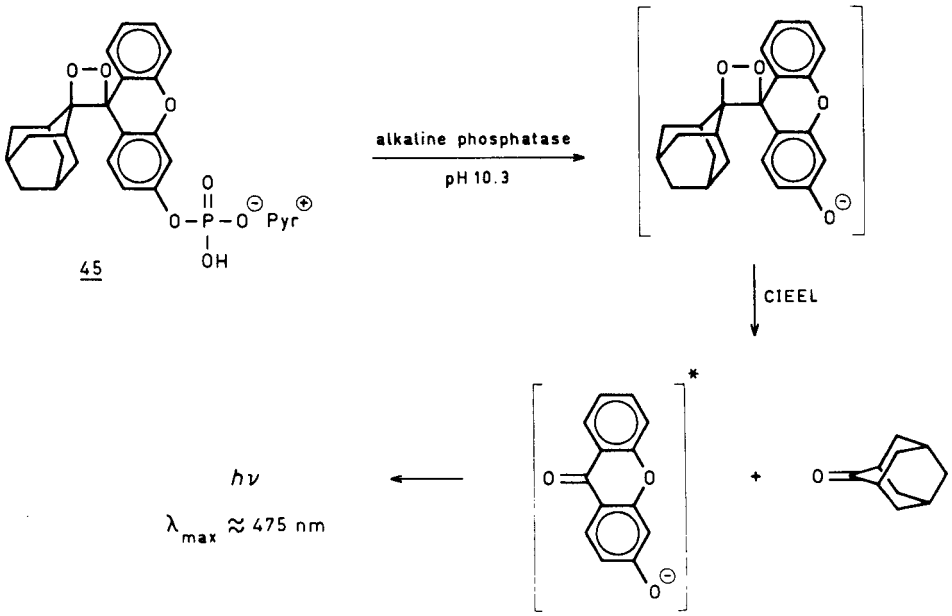

Figure 19 Enzyme triggered 1,2-dioxetane decomposition.

18.4.3. Application of 1,2-Dioxetanes in Medicine and Biology

In 1969-1970, White et al. [113-115] showed that the thermal decomposition of trimethyl-1,2-dioxetane can induce “photochemical reactions without light” by transfer of electronic energy from the excited acetone to various acceptor molecules. One year later, Lamola [116] showed that the same 1,2-dioxetane can induce the production of pyrimidine dimers in DNA in vitro. These results gave rise to much speculation about the possible applications of 1,2-dioxetanes in research dealing with photobiological problems and the possibility of using biologically generated 1,2-dioxetanes as sources of electronic energy in living matter [117-119]. Recently, Kopecky et al. [120] reported that trimethyl-1,2-dioxetane induces lesions in DNA. The 1,2-dioxetane had several effects on DNA, including photodimerization of adjacent pyrimidine bases, local denaturation, and interstrand cross-linking. The lesions were detected and quantified by using ethidium binding assays in conjunction with repair enzymes.

Adam, Cilento and co-workers [98, 121] proposed the possible use of 1,2-dioxetanes as a local source of electronic energy for photoaffinity labeling “in the dark.”

Adam et al. [98-100] also proposed that functionalized 1,2-dioxetanes may be useful as chemotherapeutic or phototherapeutic agents. 1,2-Dioxetane-substituted carbamates, carbonates, esters, and ethers, described in Section 18.3, were synthesized as a start for this type of research.

Other functionalized 1,2-dioxetanes were prepared and tested for their photogenotoxic action [101, 102, 122-124].

18.5 CONCLUSIONS

During the period of about 20 years of 1,2-dioxetane research, over 800 papers have appeared in the open literature. Many of the questions that rose at the beginning of the preparation and study of these compounds have been answered in part or in detail. Especially fruitful developments are that 1,2-dioxetanes showing high chemiluminescence efficiencies and molecular complexity can be prepared. The thermal stability of a newly made 1,2-dioxetane can nowadays be roughly estimated in advance from the data obtained from a great number of 1,2-dioxetanes bearing a still increasing variety of substituents. Hence, the state of the art allows the design of 1,2-dioxetanes that combine the property of being chemiluminescent (either thermally or after the removal of a chemical substituent that masks an intramolecular CIEEL electron donor) with properties desired for special types of application.

Several review articles on the subject of 1,2-dioxetanes have appeared through the years [36, 40, 51, 60, 70, 121, 125-131]. We hope that this chapter will be the first in a series of review articles in which the exciting properties of 1,2-dioxetanes can be summarized along with the (potentially) very exciting ways of application of these molecules in science and health care. We believe that just thinking of the applicability of 1,2-dioxetanes will inspire scientists to design and prepare novel and ingenious chemical structures to shine light on their area of research.

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