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Title

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The Influence of Substitution on Thiol-Induced Oxanorbornadiene Fragmentation

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Abstract

Oxanorbornadienes (ONDs) undergo facile Michael addition with thiols and then fragment by retro-Diels-Alder (rDA) reaction, a unique two-step sequence among electrophilic cleavable linkages. The rDA reaction rate was explored as a function of furan structure, with substituents at the 2- and 5-positions found to be the most influential and fragmentation rate to be inversely correlated with electron-withdrawing ability. Density functional theory calculations provided an excellent correlation with the experimentally measured OND rDA rates.
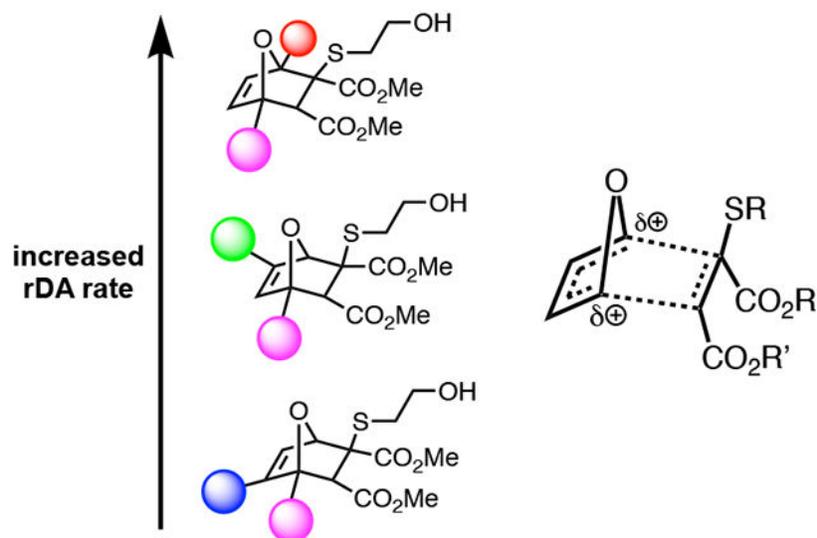
Graphical Abstract

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[†]Author Contributions
These authors contributed equally to this work. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

increased rDA rate

OH, S, CO₂Me, CO₂Me (as shown in figure)

SR, CO₂R', CO₂R', δ⊕, δ⊕

Covalent linkages that are detachable on demand have been used for a variety of applications including drug delivery,[1-2] proteomics,[3] materials development,[4-5] and solid phase synthesis.[6-7] Bond fragmentation can be induced by a variety of different stimuli, such as heat, light, nucleophiles, acids, bases and enzymes.[8] Oxanorbornadienes (ONDs) derived from electron-deficient alkynes are potent electrophiles that undergo Michael addition preferentially with thiols, and then fragment by retro-Diels-Alder (rDA) reaction, a unique two-step sequence among thiol-reactive linkages (Figure 1). The advantages of this system include mild cleavage conditions, highly tunable rates of fragmentation, and ease of synthesis. The rDA process of the thiol adduct gives furans and thiomaleates, whereas rDA cleavage of the starting OND occurs much more slowly in the absence of thiol.[9] Modifications of the OND structure can be made which change adduct stability toward rDA over a very wide range, with half-lives from minutes to months.[9-11] We have used the OND system for drug cargo delivery[12-13] and the generation of degradable hydrogels.[14-15]

The rDA rates of various mono- and 2,5-disubstituted OND have been previously reported.[9-10] Here we describe the effects of different patterns of disubstitution on the furan component including variations in steric and electronic properties, pairing these with dimethylacetylenedicarbocxylate in an exploration of the bis(dimethylester) OND motif. Details of synthesis and characterization are provided in Supporting Information.

The results of these experiments are summarized in Figure 2. Similar to previously reported structures,[9-11] these compounds were found to engage in rapid Michael addition with β-mercaptoethanol to form the corresponding adducts. In each case, this step was completed within 10-15 minutes at millimolar concentrations at room temperature in organic solvent and with equimolar activating base. We then measured the rates of retro-Diels-Alder fragmentation to the furan and corresponding thiomaleate by ¹H NMR. A representative example (**1a**) is shown in Figure 3, starting upon addition of excess β-mercaptoethanol and triethylamine. Following clean Michael addition, each adduct underwent a first-order rDA

process, chronicled by the integration of characteristic ^{1}H NMR resonances followed by an excellent fit to natural log vs time plots.

The 2,3-substituted ONDs (**1a-c**) were found to undergo very slow fragmentation, with room-temperature half-lives ranging from 16 to 34 days for compounds with methyl or benzyl substituents at the 3-position. Limited exploration of other disubstitution patterns (**2d** = 2,4; **2e** = 2,3) showed somewhat faster cycloreversion, with half-lives of 2-4 days. Bridgehead aromatic substitution produced strong acceleration of the rDA process, with half-lives of 2-14 hours for aromatic analogues **2f-2i**. While steric effects can contribute,[16] electronics plays a significant role as indicated by the apparent linear correlation of relative rate with Hammett σ^+ constant shown in the inset in Figure 2, with electron-donation stabilizing the Diels-Alder transition state.

Electronic effects at the bridgehead position were further explored with fluorinated OND derivatives **1j-1o**. As expected, fluorination gave rise to more stable thiol adducts, with trifluoromethyl (**2m**) having a greater effect than difluoromethyl (**2j** and **2k**, differing in the substituent at the other bridgehead position). While we did not make discrete comparisons to non-fluorinated analogues, the aryl-substituted compounds **2n** and **2o** were far more stable than other compounds having aryl-alkyl substitution at the bridgehead positions such as **7**.[10] Most striking was the great stability of the 5-fluoro derivative **2l,** which gave negligible amounts of rDA cleavage products after one month at room temperature. Interestingly, 5-cyclopropyl substitution provided enhanced stability relative to methyl (**2p** vs. **2q, 5, 6**); an oxetane group did not have a noticeable effect (**2q** vs **6**).

These structures were analyzed by density functional theory using the SMD(chloroform)-M06-2X-D3/6-311+G(d,p) level of theory (a detailed description of computational methods is provided in the Supporting Information). We found an excellent correlation between observed and calculated energetics over three orders of magnitude in cycloreversion rate, as shown in Figure 4. To gain further insight into the observed reactivities, the parent unsubstituted system (**16**) was analyzed in detail, focusing on Hirshfeld charges in both the reactant and the transition state (Figure 5a). In the reactant a charge of +0.06 e was assigned to the furan part, while a much larger charge separation with +0.26 e on the furan was calculated for the transition state. The biggest positive charges were seen at positions 2 and 5 of the furan, while positions 3 and 4 showed relatively low charges. Since stabilization of positive charges on the furan fragment should lead to a more stabilized transition state and therefore a lower reaction barrier, effects at the 2 or 5 positions of the furan should be stronger than at the 3 or 4 positions (Figure 5b).

Using CF_3 and phenyl (Ph) as stabilizing and destabilizing substituents, respectively, the effect of substitution in all four possible positions was investigated (Figure 5c). As expected, substitution at the 3 and 4 position provided only marginal stabilization or destabilization in the free energy of activation of the rDA reaction. However, substitution at the 2 or 5 position led to strong reductions in barrier height for phenyl substitution and strong increases in activation energy for trifluoromethyl.

A second important factor emerged in consideration of unsaturated carbon substituents such as Ph. When attached to furan positions 2 or 5, they are of course bound to a quaternary carbon in the oxanorbornadiene reactant. In the transition state, the conjugated system of the furan is mostly restored, allowing for additional stabilization by conjugation with the substituent. In contrast, such substituents in the 3 and 4 positions are already conjugated with an adjacent alkene in the reactant, leading to much less additional stabilization in the transition state. This difference is also reflected in the overall calculated driving force for rDA reactions: for example, the free energy of reaction for **9** (−6.8 kcal/mol) is significantly more favorable than for **11** (−2.7 kcal/mol).

In conclusion, density functional theory calculations were found to correlate well with experimental measurements of retro-Diels-Alder reactions of OND-thiol adducts over a wide range. These studies illuminated two electronic and orbital concepts for the control of OND rDA rates: the electronic nature of substituents which stabilize or destabilize positive charge at the furan 2 and 5 positions, and the ability of substituents to engage in π-overlap with the furan fragment, also much more important at the 2 and 5 positions. These insights can be used to design OND linkers for a variety of drug delivery and materials applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding Sources

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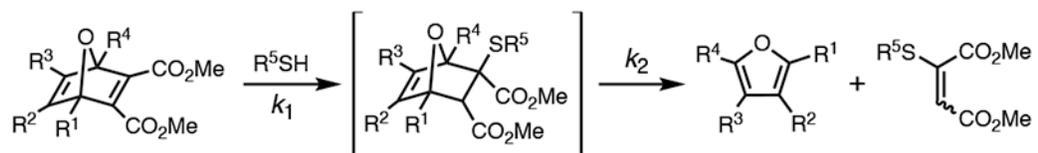
Figure 1.
Michael addition of a free thiol onto OND followed by retro-Diels-Alder rearrangement to yield furan and thiomaleate.
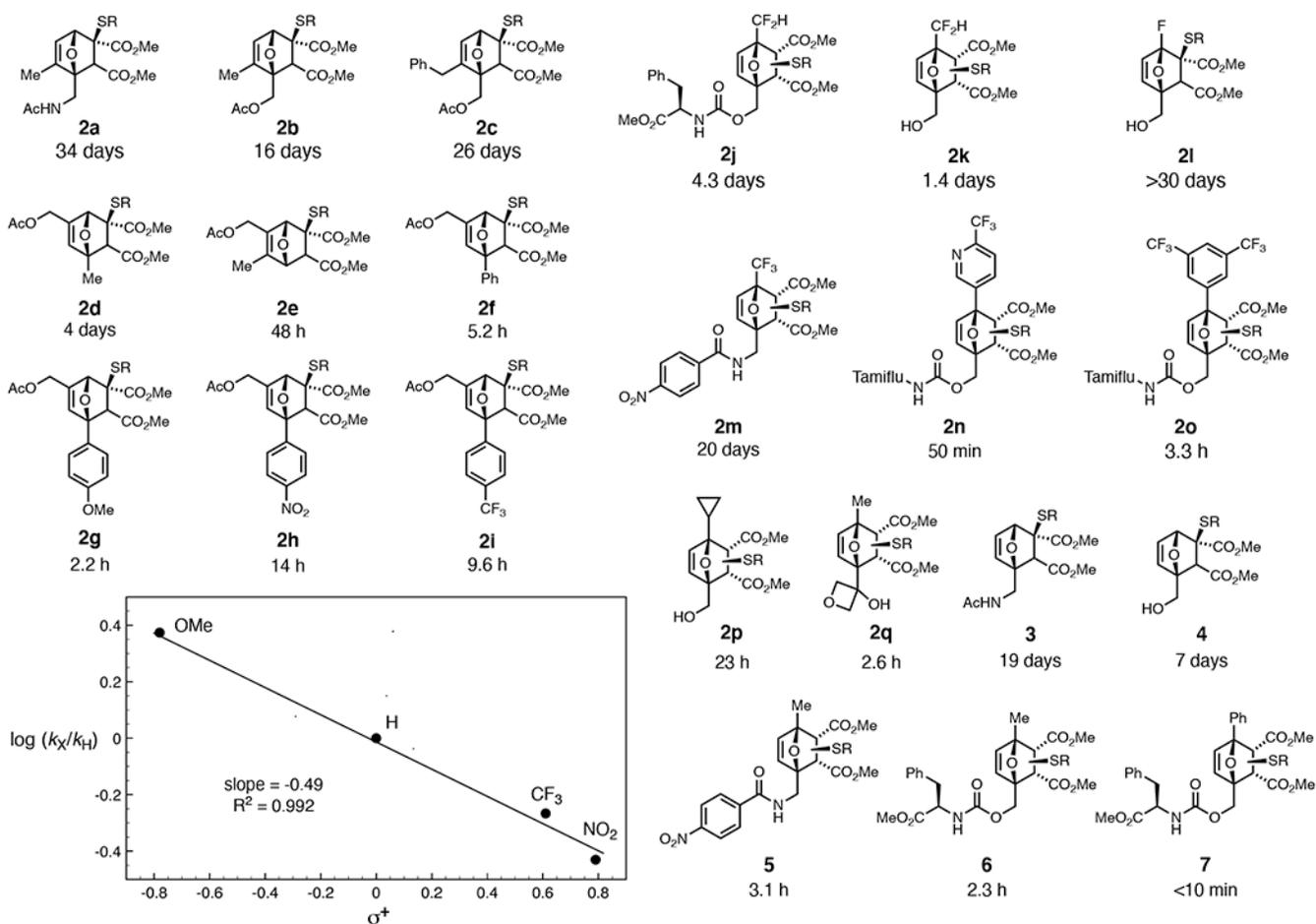
Figure 2.
Structures of OND-thiol adducts explored in this study with corresponding rDA rates at room temperature in CDCl$_3$. Experimental error in rate constants is ±10%. Compounds **3**, **4**, and **5** are from reference 9. Compound **6** and **7** are from reference 10. (Lower left) Linear free energy relationship between relative rDA rate and σ$^+$ parameter[17] for **2f-2i**. R = CH$_2$CH$_2$OH; all compounds were racemic. For compounds derived from 2,5-disubstituted furans, thiol addition occurs to either of two positions, as indicated. rDA rates for these compounds were measured for the mixture.
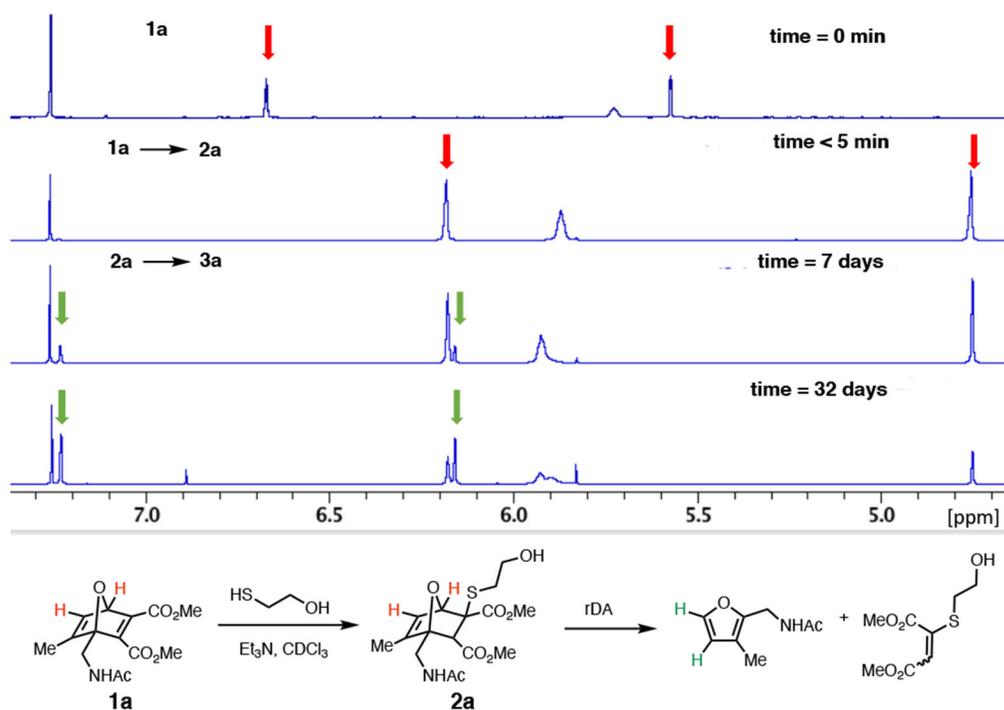
Figure 3.
Addition and rDA fragmentation reactions of OND **1a** as followed by ^{1}H NMR (CDCl$_3$, 25°C, showing 4.5-7.4 ppm).
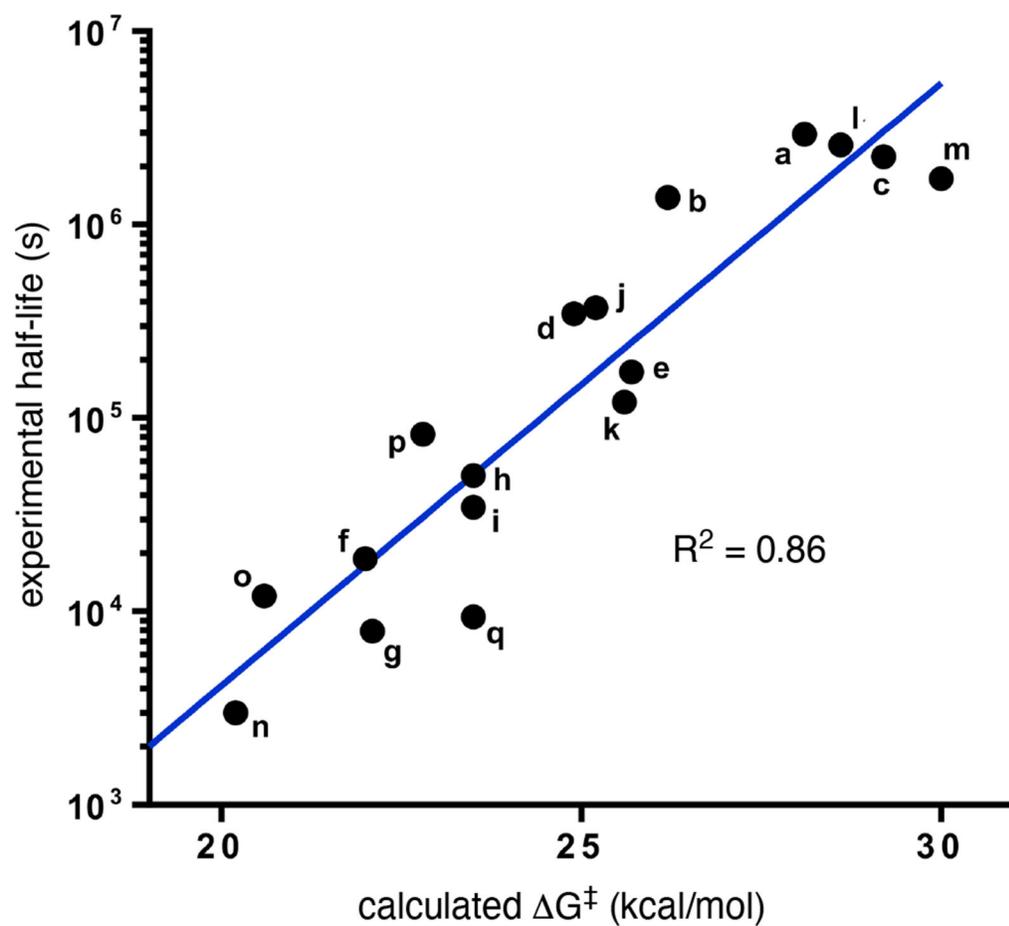
Figure 4.
Observed half-life vs. calculated (DFT) activation energy for retro-Diels-Alder reactions; letter designations refer to the mercaptoethanol adducts of structures **2a-2q** shown in Figure 2.
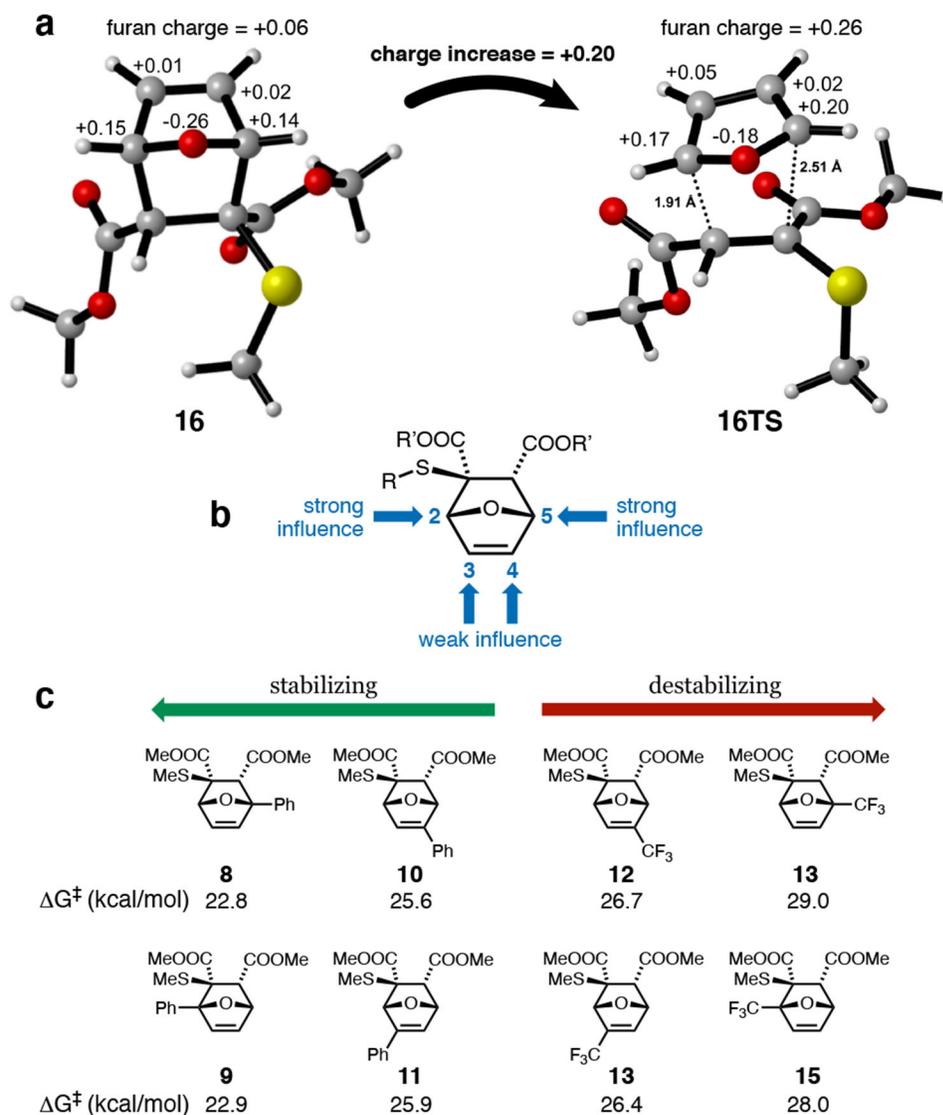
Figure 5.
a) Analysis of Hirshfeld charges in the furan part of reactant **16** and transition state **16TS**. Charges in electrons with hydrogens summed into heavy atoms. b) Predicted influence on retro Diels-Alder reactivity. c) Effect of stabilizing and destabilizing substituents on the free energy of activation for the retro-Diels-Alder reaction.