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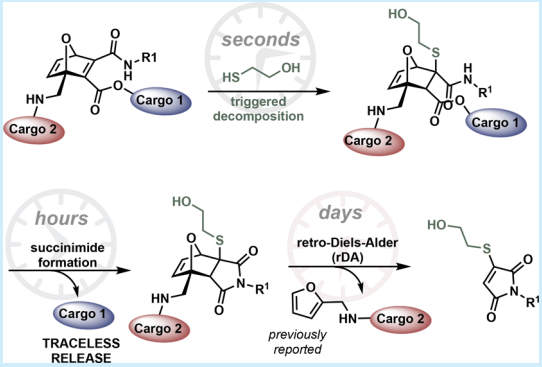
Traceless Release of Alcohols Using Thiol-Sensitive Oxanorbornadiene Linkers

Allison G. Aioub, Cody J. Higginson, and M. G. Finn*

School of Chemistry & Biochemistry, School of Biological Sciences, Georgia Institute of Technology, 901 Atlantic Drive, Atlanta, Georgia 30332, United States

Supporting Information

ABSTRACT: A class of ester−amide oxanorbornadiene (EA-OND) molecules was developed to release alcohol cargos by succinimide formation upon addition of a thiol reagent. The resulting ring-closed adducts undergo further fragmentation by retro-Diels−Alder reaction to release a furan moiety in a manner similar to oxanorbornadiene diesters. The rates of each of these fragmentation pathways in the same medium were found to be sensitive to the steric nature of the amide substituent. Alcohol release was much faster in protic solvents than in aprotic ones, suggesting that this system may be useful for rapid response to thiols in biological environments. Accordingly, the attachment and thiol-dependent release of cholesterol was characterized as an example of the manipulation of a drug-like cargo.



The controlled release of bioactive compounds from molecular modifiers or carrier scaffolds is a necessary component of many strategies to improve the site specificity, bioavailability, half-life, and other pharmacological features of drugs. We have previously identified electron-deficient oxanorbornadienes (ONDs, **1**) as reactive electrophiles toward thiols (k_1 > 50 M^{-1} s^{-1} under physiological conditions),[1,2] generating adducts that undergo retro-Diels−Alder cleavage with rates that vary over an extraordinarily large range ($k_2 \approx 3 \times 10^{-6}$ to 0.5 min^{-1}, Figure 1).[3,4] However, the utility of this cleavage process for drug delivery could be compromised in some cases by the fact that it leaves residual fragments−either furan or thiomaleate−attached to the released cargo. We elaborate here on another mode of reactivity of these connectors that allows for the rapid release of an alcohol moiety in a traceless fashion in response to the reaction with thiols via intramolecular cyclization to form a succinimde.

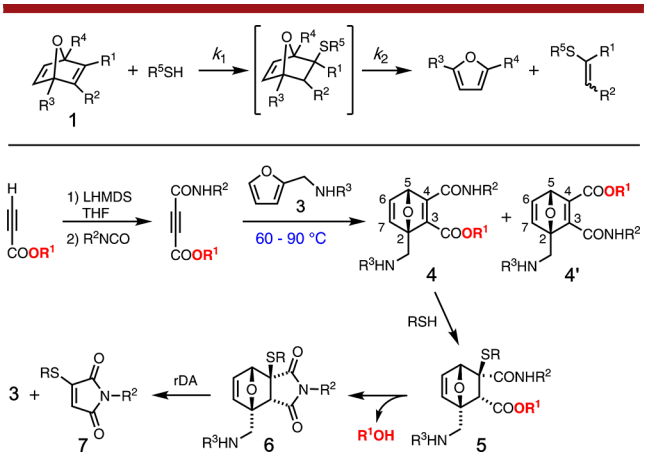


Figure 1. (Top) Thiol-triggered reactivity of standard diester OND linkers (**1**). (Bottom) Synthesis and thiol-triggered alcohol release from amide-ester OND electrophiles (**4**).

The majority of OND-based linkages described in our previous reports have been derived from acetylene dicarboxylic esters. Here, we focus on analogous monoamide monoester oxanorbornadienes (EA-ONDs, structures **4**, **4′** in Figure 1). These compounds were prepared from propynoate amides **2** (R^1 = CO_2R, R^2 = CONHR, obtained from the corresponding lithium propiolate and isocyanate) by a Diels−Alder reaction at slightly higher temperatures than previously employed for the diesters. A separable mixture of regioisomers was usually obtained (structure **4** = 4-amide-3-ester; **4′** = 3-ester-4-amide, referring to the position numbering shown in Figure 1).

While somewhat less electron-deficient than the diesters, these ester−amide oxanorbornadiene reagents were found to be reasonably electrophilic. Each gave a single dominant adduct isomer (**5**) in aqueous media (slightly basic pH) or organic solvents (with activating base) within 15−20 min at room temperature and millimolar concentrations.[3] The regioselectivity of this addition was determined primarily by electronic factors which favor addition β to the more electron-withdrawing ester group. When steric and electronic factors conflict, as in thiol addition to isomer **4′**, regioselectivity was preserved but the addition rate was diminished (Supporting Information, SI).

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Rather than undergoing retro-Diels–Alder (rDA) cleavage directly upon thiol addition as for other OND derivatives, EA-OND adducts were found to first cyclize to the corresponding succinimides **5** (Figure 1). This ejects an alcohol from the adjacent ester, making this a traceless linkage if the alcohol is a desired cargo. The imide then decomposed via rDA reaction to the corresponding furan (**3**) and a thiomaleimide (**7**). Following up on our original observation of this process,[1] we describe here the dependence of each of these release steps on EA-OND structure.

The rates of succinimide formation and retro-Diels–Alder fragmentation were determined by ^{1}H NMR, beginning immediately after the addition of excess β-mercaptoethanol, as illustrated by a representative case (compound **4a**) in Figure 2. The well-resolved nature of resonances for each species allowed for concentration-vs-time measurements, which fit the first-order kinetic behavior expected for both succinimide formation and subsequent retro-Diels–Alder fragmentation. Experimental details of the syntheses and kinetics are given in the SI.

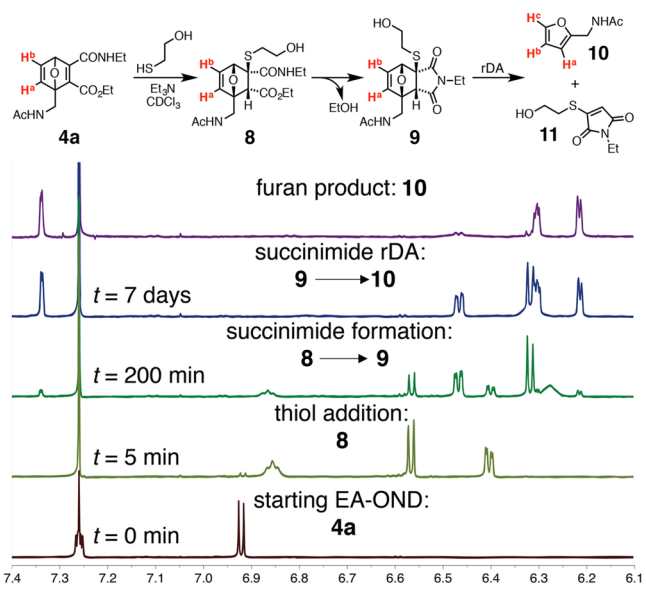

Figure 2. Representative ^{1}H NMR spectra for calculation of succinimide and furan product formation.

Reactions of different EA-OND compounds with β-mercaptoethanol were followed in this manner with the results summarized in Figure 3 for 3-amide-2-ester regioisomers. In organic solvent ($CDCl_3$), the structure–activity landscape proved to be fairly flat. Thus, variations in the groups attached to the furfurylamine nitrogen (acetyl, tosyl, mesyl, compounds **4a**, **4g**, **4h**) gave rise to similar rates of succinimide formation (half-lives approximately 1–3 h at ambient temperature) and rDA fragmentation (3–8 days). Capping the acetamide group with a methyl group (**4f** vs **4a**) also had little effect, suggesting that activation of the ester group by intramolecular H-bonding was not important in accelerating the succinimide ring closure reaction. Increasing the size of the amide group from primary (ethyl) to secondary (isopropyl) slowed ring closure by about a factor of 3 (**4a** vs **4f**). The nature of the ejected alcohol had a somewhat larger impact: formation of succinimide by release of a secondary alcohol (cyclohexanol, compound **4b**) was slower (half-life = 7.6 h), compared to primary alcohols (methyl, ethyl, benzyl, 3-butynyl; structures **4a**, **4d**, **4f–i**, half-lives = 1–3 h). Compound **4c**, bearing a pendant carboxylic acid, exhibited a slower rate of ring closure than other primary esters (half-life = 5.9 h), for reasons that are not understood at present. Bridgehead substitution (**4e**) induced the expected acceleration in the rDA rate (approximately 10-fold vs compound **4a**),[3,4] and also substantially faster succinimide formation (approximately 14-fold).

Among the variables tested, the reaction medium had the most dramatic effect on the rate of succinimde formation. In methanol-d_4, ring closure occurred within a few minutes upon the addition of thiol for all primary amide EA-OND variants tested (Figure 3, **4a–e**). The same was observed in D_2O for the aqueous-soluble carboxylic acid **4c**, along with a much greater sensitivity to the nature of the amide substituent, with the

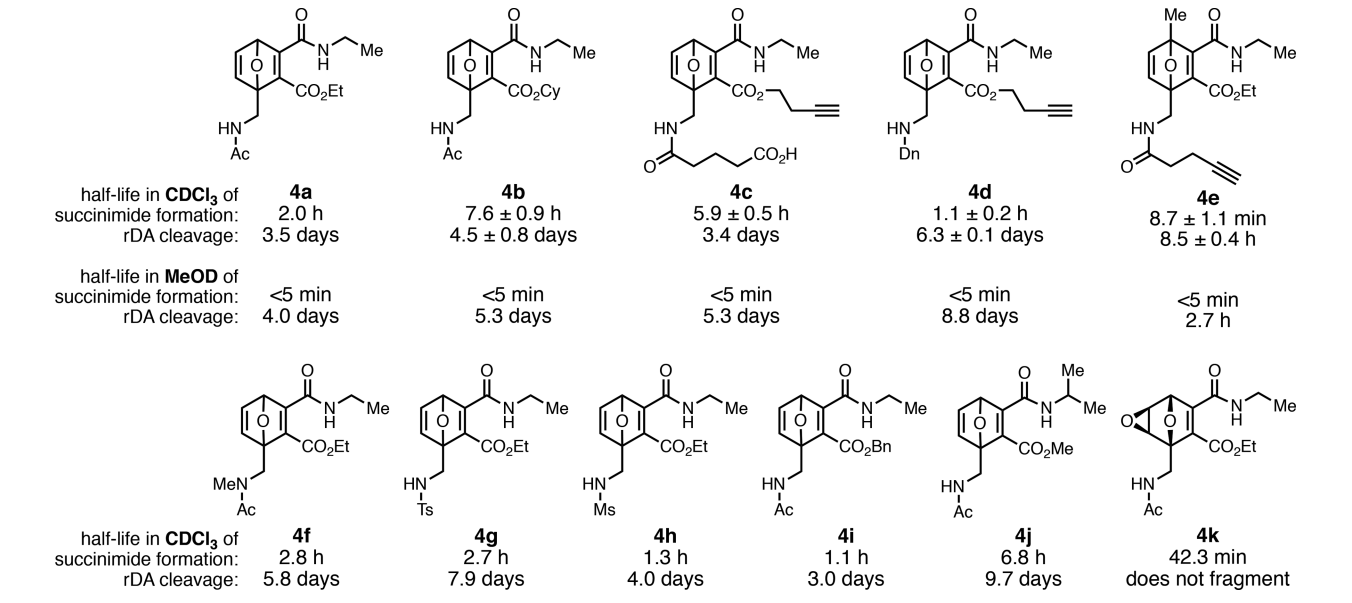

Figure 3. OND modifications and the half-life of both the succinimide and rDA transformations in $CDCl_3$ and CD_3OD (Dn = dansyl).

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isopropyl derivative cyclizing at least 50 times slower than the ethyl derivative (Figure 4, **4l** vs **4c**).

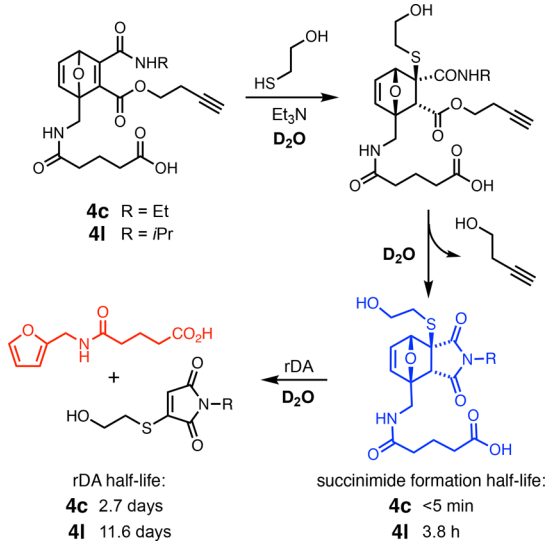
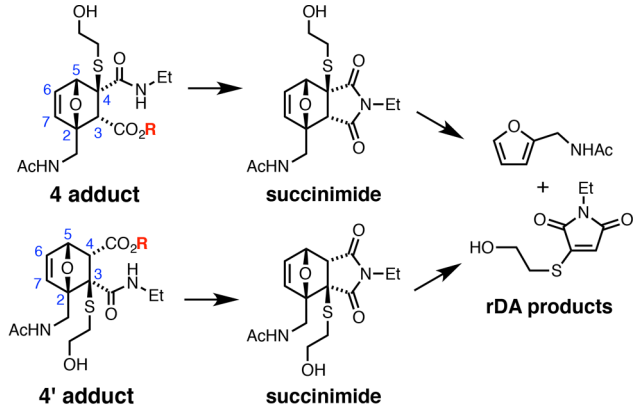


Figure 4. Rates of succinimide formation and rDA cleavage in D_2O with different amide groups.

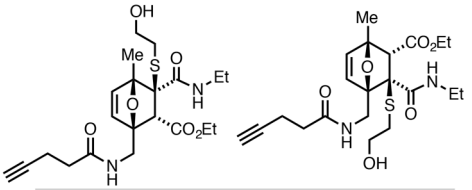
The two EA-OND regioisomers derived from the Diels–Alder reaction of alkyne and substituted furan behaved much differently in thiol-triggered reactions, as shown in Figure 5. Five derivatives were studied in each case, including primary, benzylic, and secondary esters, one substrate containing a bridgehead methyl group, and another containing an epoxide (providing for facile conjugate addition but no rDA fragmentation). Thiol addition occurred primarily β to the ester substituent, as expected from stereoelectronic considerations of the Michael reaction and supported by the observation of expected ^{1}H NMR splitting between the C–H moiety introduced in this process and the bridgehead C–H unit when present (SI).

In general, adducts from isomers **4** (amide in the 4-position, and bridgehead-H at position 5; see Figure 5) underwent much slower succinimide-forming ring closure and rDA cleavage than the regioisomeric adducts from **4′** (amide in the 3-position). For example, in nonprotic solvent ($CDCl_3$), succinimide formation was approximately 10–100 times faster for the more crowded (**4′**) adducts, and retro-Diels–Alder fragmentations were even more sensitive, with rate differences of approximately 175-fold (**4b′ adduct** vs **4b adduct**) to 230–300-fold (adducts of **4a′** and **4i′** vs adducts of **4a** and **4i**). While a significant number of publications have appeared in which the retro-Diels–Alder cleavage of furan-maleimide adducts is used,[5−9] we are not aware of relevant information in the literature on cases such as this. In analogy to previous calculations,[4] we suggest that the large rDA effect may derive from a difference in transition state symmetry caused by the differing substitution patterns of the thiol-succinimides, with the faster compounds (from **4′**) having adjacent quaternary centers. As before, succinimide ring closure was very fast for both regioisomers in protic (CD_3OD) solvent, but the same trend in rDA rates was observed, in which **4a′** cleaved faster than **4a** (60-fold) and **4b′** faster than **4b** (173-fold).

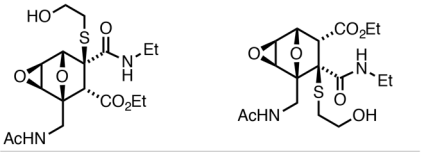
To demonstrate the ability of the EA-OND linkage to release a biologically active cargo, we turned to cholesterol as an example of a hydrophobic molecule requiring controlled release



		half-life in $CDCl_3$ of:		half-life in CD_3OD of:	
R	adduct	succinimide formation	rDA cleavage	succinimide formation	rDA cleavage
Et	**4a**	2.0 h	3.5 days	<5 min	4.0 days
	4a'	9.4 min	16.6 min	<5 min	1.6 h
Cy	**4b**	7.6 ± 0.9 h	4.5 ± 0.8 days	<5 min	5.3 days
	4b'	<5 min	3.1 h	<5 min	44.2 min
CH_2Ph	**4i**	1.1 h	3.0 days		
	4i'	<5 min	18.4 min		



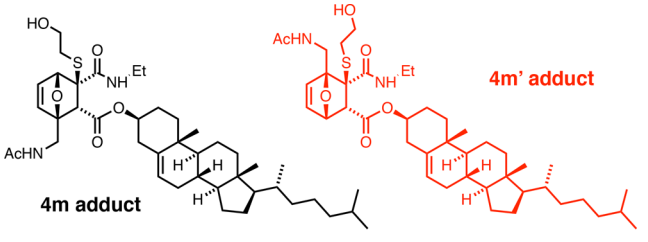
half-life in $CDCl_3$ of	4e adduct	4e' adduct
succinimide formation:	8.7 ± 1.1 min	< 5 min
rDA cleavage:	8.5 ± 0.4 h	1.5 h



half-life in $CDCl_3$ of	4k adduct	4k' adduct
succinimide formation:	42.3 min	32.8 min
rDA cleavage:	no rDA	no rDA

Figure 5. Rates of succinimide formation and rDA cleavage comparing different regioisomers of thiol addition. Position numbering used in the text appears in blue on the adduct structures.

for some therapeutic applications.[10] The properties of cholesterol ester derivatives **4m** and **4m′** (Figure 6) were



half-life of	$CDCl_3$	MeOD	$CDCl_3$
succinimide formation:	15.0 h	< 5 min	< 5 min
rDA cleavage:	4.2 days	4.8 days	31.0 min

Figure 6. Rates of succinimide formation and rDA cleavage for an EA-OND designed to release cholesterol.

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consistent with the above studies: both the release of cholesterol by succinimide formation (at least 180-fold) and rDA fragmentation of the resulting succinimide (195-fold) were much faster from **4m** than from **4m′** in $CDCl_3$. Also as before, succinimide formation (alcohol release) was very fast even from **4m** in protic solvent (CD_3OD).

While the ester linkage provides a means for hydrolytic release of alcohols or carboxylic acids in biological systems or other environments, nonenzymatic cleavage of the ester bond is often slow or difficult to control. We describe here a triggered process by which an oxanorbornadiene Michael acceptor takes on a thiol nucleophile, forming an intermediate adduct that undergoes intramolecular ester cleavage by an amide group to form a cyclic imide. In essence, this transduces the rapid addition of thiol into the rapid release of alcohol. The resulting succinimide undergoes further fragmentation by a retro-Diels–Alder pathway, unless prevented from doing so by epoxidation of the remaining OND double bond.

The alcohol-releasing step was much faster in protic media than organic solvent, an observation that we find reasonable in principle, but surprising in magnitude. In addition to a large body of research on cyclization of aspartate residues to form cyclic byproducts or intermediates in the context of peptide synthesis[11] and protein stability,[12] a variety of reports of the conversion of 1,2-amidoesters to succinimides have appeared involving acceleration by base,[13−16] protic acid,[17,18] Lewis acid,[19] and activation of the leaving group.[20,21] To our knowledge, none that release nonactivated alcohols occurs at rates approaching those observed here in methanol solvent.

This work expands the scope and utility of OND molecules for the delivery of molecular cargo. In addition to the extreme sensitivity to the nature of the solvent, the rate of succinimide formation was most sensitive in aprotic media to the nature of the alcohol (primary ≫ secondary) and was accelerated by the presence of two alkyl bridgehead substituents on the oxanorbornadiene core. However, in alcohol or aqueous solvent, succinimde formation was rapid in all cases studied, suggesting that this mode of release will be fast in biological applications outside of lipophilic environments.

The retro-Diels–Alder cleavage step also provided an unexpected result, in which succinimides derived from thiol adducts bearing bridgehead substituents in adjacent positions (the **4′** series in Figure 5) underwent fragmentation up to hundreds of times faster than the less sterically crowded regioisomer. For regioisomers **4**, on the other hand, rDA cleavage of thiol-succinimides was much slower than their formation in any solvent tested, with bridgehead substitution being the most important rate-controlling structural variable. The OND family of linkages therefore continues to provide different elements of control of fragmentation and release, which we believe will have useful application in a number of different fields.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b01093.

Methods, chemical syntheses and characterization data, and data for all kinetic experiments (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: mgfinn@gatech.edu.

ORCID

M. G. Finn: 0000-0001-8247-3108

Notes

The authors declare no competing financial interest.

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