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Aqueous-Phase Deactivation and Intramolecular [2+2+2] Cycloaddition of Oxanorbornadiene Esters

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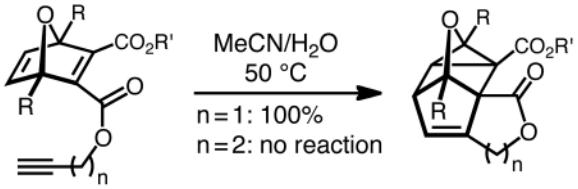
Abstract



Both inter- and intramolecular degradation pathways were identified for the aqueous phase deactivation of oxanorbornadiene (OND) electrophiles, and propargylic OND esters were found to undergo facile intramolecular [2+2+2] homo-Diels–Alder cycloaddition in polar media.

7-Oxabicyclo[2.2.1]hept-2,5-diene-2,3-dicarboxylates (oxanorbornadienedicarboxylates, OND) have recently attracted attention due to their potential as bioconjugation reagents. The unique electrophilicity of a maleate moiety embedded in a homoconjugated bicycle[1] is manifested by several processes more typical for electron-deficient alkynes than alkenes. These include facile Diels–Alder addition of a second furan[2], room temperature reactivity with organic azides ($k_2 \sim 10^{-3}$ $M^{-1}s^{-1}$)[3-6] and aliphatic thiols[7] ($k_2 \sim 10^2$ $M^{-1}s^{-1}$), and the ability to quench pendant dansyl fluorophore *via* photoinduced electron transfer.[7-8] Despite enhanced electrophilicity and formidable strain, OND-dicarboxylates are soft electrophiles that are remarkably stable under ambient conditions. High aqueous stability, exceptional reactivity towards thiols, and the simplicity of preparation make OND reagents viable competitors to traditional thiol-labeling reagents.

We previously reported the reactivity and stability of OND reagents to be inversely, but not linearly, correlated.[7] For instance, dimethyl ester **1** (Eq. 1) was slightly more reactive towards glutathione (k_2 = 104 M^{-1} s^{-1}) and less stable (half life in aqueous buffer = $t_{1/2}$ = 9.3 days) than diethyl ester **2** (k_2 = 63.4 $M^{-1}s^{-1}$, $t_{1/2}$ = 20.6 days).[7] The slightly more electron-deficient dipropargyl ester **3** was about twice as reactive as **1** (k_2 = 197 $M^{-1}s^{-1}$), yet dramatically less stable ($t_{1/2}$ = 1.8 days). Compound **4**, containing a bridgehead methyl group, was five-fold less reactive than **3** (k_2 = 39.9 $M^{-1}s^{-1}$), but the stability was improved only two-fold ($t_{1/2}$ = 3.2 days).[7] In order to make the best use of OND electrophiles, we investigated the pathways responsible for their deactivation, with the goal of finding effective compromises between reactivity and aqueous stability.

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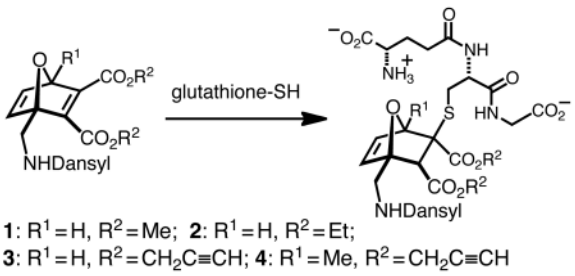
Supporting information. Experimental procedures and characterization data of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.



1: R^1 = H, R^2 = Me; **2**: R^1 = H, R^2 = Et;
3: R^1 = H, R^2 = $CH_2C{\equiv}CH$; **4**: R^1 = Me, R^2 = $CH_2C{\equiv}CH$

(1)

OND diesters such as **1**–**4** have two electrophilic sites. While soft nucleophiles such as thiolates attack the double bond, hard oxygen-centered nucleophiles such as hydroxide and alkoxides were expected to react with the ester groups.[3,7, 9,10]. Indeed, exposure of dialkyl OND diesters **1**–**4** to aqueous hydroxide ion led only to sequential saponifications (Figure 1, path A), selectively giving fluorescent monoacids **5** when one equivalent of base was used.[7] To study the stability of **1**–**4** in neutral aqueous buffers, we originally incubated 0.1 mM **1**–**4** in 1:9 DMSO:aqueous phosphate (0.1 M, pH 7.0). Interestingly, upon consumption of **1** and **2** the samples did not become fluorescent, but they gradually lost their turn-on capacity (*i.e.* addition of a thiol did not result in the appearance of fluorescence). Mass spectrometry revealed a single set of peaks with the same *m/z* values as the starting OND, suggesting an isomerization process.

Incubation of dimethyl ester **1** on a preparative scale in 4:1 acetonitrile/phosphate buffer (pH 7) over several days did not result in its degradation, presumably due to a lower buffer strength (20 mM) or a smaller aqueous component in the reaction medium. This suggests that the degradation pathway(s) of **1** involves general acid or base, or has a negative volume of activation which responds well to the high cohesive energy density of water. At elevated temperatures (50 °C, 5 days) partial saponification to monoacid **5** was observed (Figure 1, path A). Interestingly, when excess acetylacetone (HAcAc) was added to reaction mixture, ester **1** gave the non-fluorescent isomeric (*E*)-olefin **7** in excellent yield. It is likely that the acetylacetone anion acts as a base and deprotonates the sulfonamide[11] of **1**, which undergoes intramolecular 4-*exo*-*trig* conjugate addition to give **6**. This highly strained azetidine should then be subject to fast retro-Diels–Alder (RDA) cycloreversion to form (*E*)-**7** (path B1). Alternative mechanisms involving intermolecular conjugate addition–RDA fragmentation sequences could not be ruled out. Plausible intermediates **8** and **9** formed by conjugate addition of water or methanol to **1** could not be detected.

Blocking the sulfonamide group (methylated compound **10**) diverted the pathway to simple conjugate addition of methanol (path B2), giving **12** in 75% yield. The addition of water (to give **11**) was not detected under these conditions, nor when acetonitrile was used in place of methanol as a co-solvent. Methanol adduct **12** was found to be much less susceptible to RDA fragmentation than thiol adducts, although furan **13** and methoxymaleate **14** could be detected in the reaction mixture by TLC and ESI-MS. Since the alternative degradation reaction of **10** took place at lower temperature than the decomposition of **1**, it is significant that the reaction of **1** did not follow the same course as **10**. In other words, despite having an additional decomposition route available (pathways B1 and B2), the NH-sulfonamide **1** was more stable than methylated **10** (only pathway B2).[7] This suggested the presence of a stabilizing hydrogen-bonding interaction available to **1** but not **10**.

Degradation of various OND derivatives in unbuffered aqueous solutions required more forcing conditions (80–100 °C). For example, benzamide **15** gave a mixture of furan **16** and

1:1 furan–OND adduct[2] **17**; the latter was the major product when the reaction was run at high concentration. This suggests that intermolecular conjugate addition of water does occur and is followed by rapid RDA fragmentation at high temperatures (path B2). A significant amount of polar compounds is formed under these conditions; mono- and diacids **18** could be detected in the reaction mixtures by ESI-MS. It is unlikely that retro-Diels-Alder cleavage occurs in **15** or any other OND derivatives described here since such fragmentations typically require very harsh conditions and favor the formation of acetylene and substituted furan[12] **19**, which was not detected.

Instead of the above hydrolysis, Michael, and retro-Diels-Alder pathways, the dipropargyl esters **3** and **4** were each found to give two fluorescent products, **20a/b** and **21a/b**, derived from cycloadditions on opposite sides of oxanorbornadiene core. This reaction occurred to approximately 50% completion over six months storage (room temperature, capped flask), and in only a few days in aqueous buffer at the same temperature. The structures were assigned on the basis of ^{1}H, ^{13}C, COSY, and ^{1}H–^{13}C HMBC NMR spectroscopy, and by X-ray crystallographic analysis of one of the products (**21a**) obtained from **4** (Supporting information).

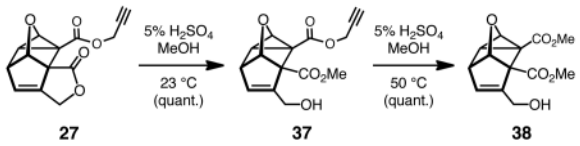
The intramolecular [2+2+2] transformations of propargylic OND substrates proceeded under mild conditions and were accelerated in aqueous acetonitrile, presumably by virtue of the standard hydrophobic effect common for cycloaddition reactions (Table 2).[13-16] Heating at reflux was found to be best for preparative-scale reactions (entry 4). OND diester **26** gave unsubstituted pentacycle **27** *in situ* under the conditions of Diels–Alder reaction (entry 5), or after isolation and heating (entry 6). As expected, internal propargylic alkynes underwent the same reaction, compound **28** providing pentacycles **29a,b** in good yield (entry 7).

The cyclization rate was quite sensitive to linker length. Homopropargyl ester **30** was much more stable than the analogous propargyl esters **3** and **4**, and the expected δ-lactone product could not be detected (entry 8). The use of copper(I) to catalyze the reaction of **30** was not effective. Similarly, dihomopropargyl ester **31** and bis(pent-4-yn-1-yl) ester **33** gave only trace amounts of the corresponding δ- (**32**) and ε-lactones (**34**) (entries 9, 10). Dihomopropargyl esters **30** and **31** were recovered largely unchanged, while diester **33** was completely degraded after heating at 100 °C, presumably by hydrolysis and Michael addition at the higher temperature. N-Propargylamide **35** (entry 11) also did not cyclize, presumably because a strong intramolecular hydrogen bond locks the amide moiety in a planar conformation.[17] Attempted formation of saturated oxadeltacyclanes from diallyl ester **36** was also unsuccessful (entry 12).

The oxadeltacyclene products are formed by intramolecular homo-Diels–Alder (HDA) reaction, or [2+2+2] cycloaddition. This process is well precedented with norbornadienes and oxanorbornadienes, but proceeds only under harsh conditions (>90 °C, several days) with poor chemoselectivity, unless catalyzed by cobalt[18] or ruthenium[19] complexes. Intramolecular, intermolecular and asymmetric versions are known.[20] Deltacyclenes prepared from norbornadienes are useful building blocks for synthesis of polycyclic natural products,[19] but reports of HDA adducts of 7-oxanorbornadienes are relatively rare. The Diels–Alder adduct between 2,5-dimethylfuran and ethyl propiolate has been reported to undergo an HDA reaction with itself, producing oxadeltacyclenes as well as many other by-products;[21] bis-furan adducts of electron-deficient dialkynylcarbinols undergo this formal dimerization intramolecularly.[22]

Unlike related and more strained oxaquadricyclanes,[23] the pentacyclic oxahemiquadricyclane lactones produced here proved to be quite stable. Compounds **21a** and **27** were unchanged after heating at 120 °C for several days. Attempted acid-catalyzed

rearrangement of **27** performed under conditions that cleaves three out of four cycles in oxaquadricyclanes[24] (5% methanolic H_2SO_4) resulted only in sequential lactone opening (**37**) and transesterification (**38**) (Eq. 2).



(2)

In summary, analysis of the aqueous deactivation of OND electrophiles revealed base-catalyzed isomerization of dansylamide-bearing bicycles and a facile intramolecular homo-Diels–Alder process. Since sulfonamidomaleate **7**, the degradation product of the simplest dimethyl ester **1**, is not fluorescent, partial decomposition should not be problematic for the detection and fluorogenic labeling of thiols. The somewhat limited stability of diesters **1** and **2** is only a minor drawback, given that they are the easiest to prepare among any fluorogenic thiol-reactive reagents. Furthermore, the cyclization-resistant (and therefore stable) acetylenic esters are highly useful as “clickable” connectors, since they retain their high reactivity toward thiols. For example, the dihomopropargyl ester **30** reacted with glutathione with a second order rate constant of $57.6 \pm 2.7\ M^{-1}s^{-1}$, comparable to the diethyl ester **2**, and no degradation over extended exposure (several months at room temperature) to aqueous buffers was observed. *N*-Propargylamide **35** was successfully used as a linker for attaching BSA (single thiol group) to an azide-functionalized Qβ protein nanoparticle.[25] Further investigations of the performance and utility of OND reagents are in progress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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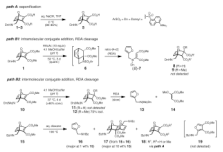
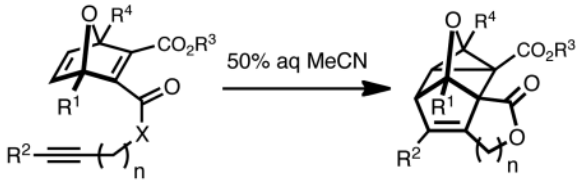
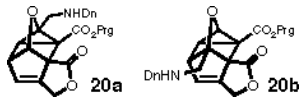
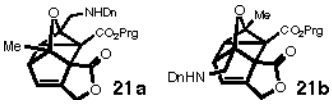
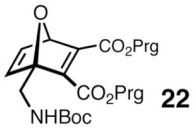
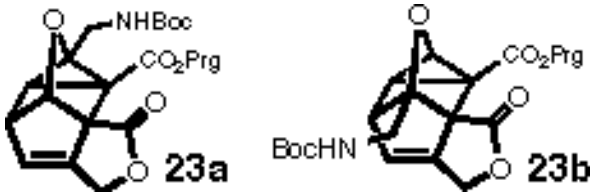
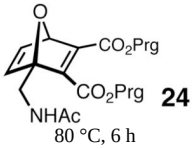
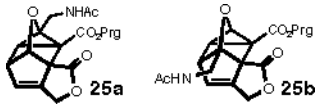
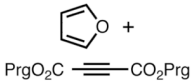
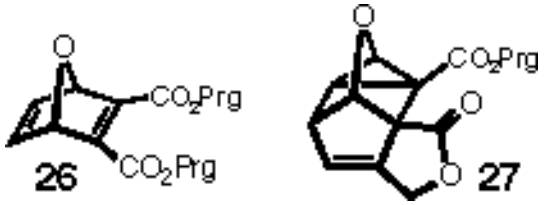
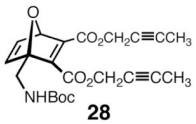
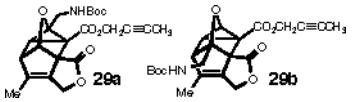
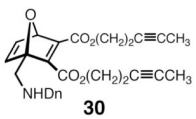


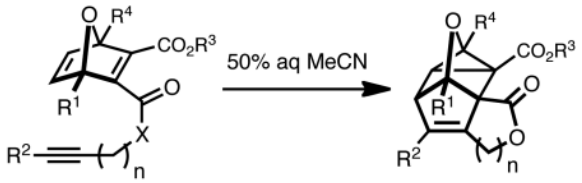
Figure 1.
Degradation pathways of OND electrophiles.

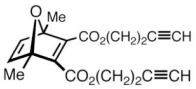
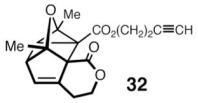
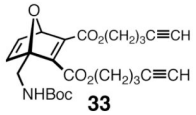
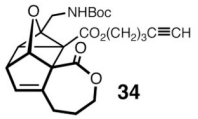
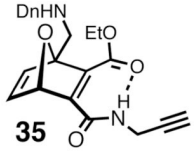
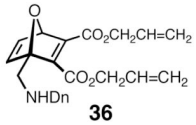
Table 1

Homo-Diels–Alder reaction of OND alkynes.[a]



entry	conditions	product(s)	yield, % (ratio)
1	**3**, 50 °C, 24 h	 **20a** **20b**	100 (1:1)
2	**4**, 50 °C, 24 h	 **21a** **21b**	100 (5:1)
3	 **22** 50 °C, 24 h	 **23a** **23b**	100 (1:2.3)
4	 **24** 80 °C, 6 h	 **25a** **25b**	91[b] (1:1.4)
5	 (1.2 equiv) neat, 50 °C, 5 d	 **26** **27**	47 (**26**) 21 (**27**)
6	**26**, 50 °C, 12 h	**27**	100
7	 **28** 50 °C, 24 h	 **29a** **29b**	90 (1:1.5)
8	 **30**	no reaction	85% recd **30**



entry	conditions	product(s)	yield, % (ratio)
	50 °C, 20 d		
9	 **31** 80 °C, 5 d	 **32**	trace (90% recd **31**)
10	 **33** 100 °C, 3 d	 **34**	trace
11	 **35** 50 °C, 5 d	no reaction	0
12	 **36** 50 °C, 20 d	no reaction	0

[a] Prg = propargyl;

[b] 0.7 mmol scale