EDGE ARTICLE

Diazo compounds as highly tunable reactants in 1,3-dipolar cycloaddition reactions with cycloalkynes†

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Diazo compounds, which can be accessed directly from azides by deimidogenation, are shown to be extremely versatile dipoles in 1,3-dipolar cycloaddition reactions with a cyclooctyne. The reactivity of a diazo compound can be much greater or much less than its azide analog, and is enhanced markedly in polar-protic solvents. These reactivities are predictable from frontier molecular orbital energies. The most reactive diazo compound exhibited the highest known second-order rate constant to date for a dipolar cycloaddition with a cycloalkyne. These data provide a new modality for effecting chemoselective reactions in a biological context.

Introduction

The development of chemical reactions that are chemoselective[1–3] in the context of biological systems has become of paramount importance to chemical biology. A major challenge is to achieve high reaction rates without sacrificing reagent stability. One reaction that fulfills these criteria is the 1,3-dipolar cycloaddition reaction between an azide and a cycloalkyne. Early work by Wittig and Krebs[4] demonstrated that the strain energy[5] imparted to cyclooctynes was sufficient to obviate the need for elevated temperatures[6] or copper catalysts.[7,8] This reaction is especially attractive for chemical biology because the azido group is relatively small and inert, and is thus a well-tolerated pendant on biological molecules.[9–14]

Over the past decade, considerable effort has been directed at optimizing this transformation by tuning the electronics and strain energy of the cycloalkyne. As expected from frontier molecular orbital (FMO) theory,[15–19] the electron-withdrawing fluorine atoms in **DIFO**[20] (Fig. 1) lower the energy of its FMOs with respect to **OCT**, thereby increasing its reactivity with azides. Increasing ring strain[21,22] by fusing cyclopropane[23] or, more commonly, aryl rings onto the cyclooctyne (as in **DIBO**,[24] **DIBONE**,[25] and **BARAC**[26]) or by decreasing ring size through sulfur incorporation (as in **TMTH**[27]) provides an additional increase in reactivity. An alternative approach has been to use a nitrone as a surrogate for the azide to increase reactivity.[28–30] Still, most experimental[31–33] and computational[34–36] optimization

has focused on increasing the reactivity of the dipolarophile—the cycloalkyne.

Recently, we described a new transformation that utilizes an activated phosphinoester to convert an azide into a diazo compound under gentle conditions (Fig. 2).[37] 1,3-Dipolar cycloaddition reactions of diazo compounds with polarized alkenes and alkynes are well known.[38–40] Still, chemical biologists have not employed this strategy, presumably because polarized alkenes and alkynes lack chemoselectivity in a biological context and because known cycloaddition reactions of diazo compounds
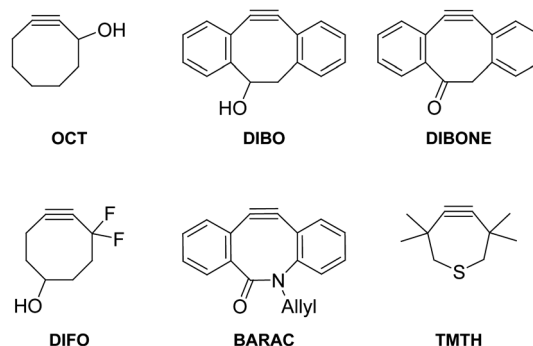

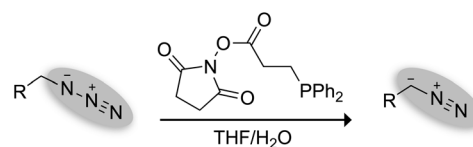**Fig. 1** Representative cycloalkynes developed for 1,3-dipolar cycloaddition reactions with azides.

Fig. 2 Isoelectronic 1,3-dipoles. The azide can be converted into the diazo compound with a phosphinoester like that shown.[37]

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involve either highly reactive diazoalkanes generated *in situ*,[41,42] or highly stabilized diazo compounds[43] requiring elevated reaction temperatures[44] or toxic metal catalysts to attain useful reaction rates.[45–50] Now that a wide variety of diazo compounds are readily accessible from azides in a single step, we sought to assess their reactivity in strain-promoted cycloaddition reactions with forefront cycloalkynes.

We were aware that treating diazoacetyl azide with dimethyl acetylenedicarboxylate leads to a reaction with the diazo component, leaving the azide intact.[51] This chemoselective reactivity—though not with biocompatible reactants—inspired us to carry out quantum mechanical calculations[52] to understand from an FMO perspective how a series of azides and their corresponding diazo compounds might compare in terms of their reactivity in strain-promoted cycloaddition reactions.

In normal electron demand (NED) cycloadditions, the reaction rate is governed largely by the interaction between the highest occupied molecular orbital (HOMO) of the dipole and the lowest unoccupied molecular orbital (LUMO) of the dipolarophile (ΔE_{NED}). Minimizing this energy difference allows for better overlap of the required orbitals and thus accelerates the reaction.[53] Inverse electron demand (IED) cycloadditions are more common when the dipole becomes sufficiently electron poor. Then, electron-withdrawing groups lower the FMO energies, creating better overlap between the LUMO of the dipole and the HOMO of the dipolarophile (ΔE_{IED}). Tuning the electronics of the 1,3-dipole is facilitated by converting an azide into the diazo compound, which moves the register of the 1,3-dipole and can bring otherwise distal electron-withdrawing groups into conjugation.

Results and discussion

We chose three pairs of 1,3-dipoles that vary in their extent of electron delocalization in an attempt to achieve a range in reactivity. Upon calculating the HOMO/LUMO energies of these dipoles along with those of some common cycloalkynes (Fig. 1) at the MP2/cc-PVTZ level of theory, we found that the greatest orbital overlap occurs with **DIBONE** (Table S1†). Hence, we chose **DIBONE** as the dipolarophile for our study.

In the electron-rich dipole pair (**1** and **2**), calculations showed an NED cycloaddition with the diazo compound being favored over that with the azide by >12 kcal mol^{-1} (Scheme 1). An NED cycloaddition is favored slightly for the intermediate group (**5** and **6**), but an IED cycloaddition favoring azide by >3 kcal mol^{-1} is predicted for the most electron-deficient pair (**9** and **10**).

To test the validity of these calculations, we treated each individual azide or diazo compound with an equimolar amount of **DIBONE** in acetonitrile. Our experimental results were in gratifying agreement with the calculations: diazo compounds **2** and **6** were the fastest to react, whereas diazoacetophenone **10** was, by far, the slowest (Scheme 1). To explore these differences in reactivity, each set of azide and diazo compounds was subjected to a direct competition experiment with **DIBONE**. In these experiments, the two dipoles and **DIBONE** were mixed at equimolar concentrations and allowed to react to completion, and the reaction mixtures were analyzed by ^{1}H-NMR spectroscopy (Fig. S1–S3†). As predicted from the large $\Delta\Delta E$ value,
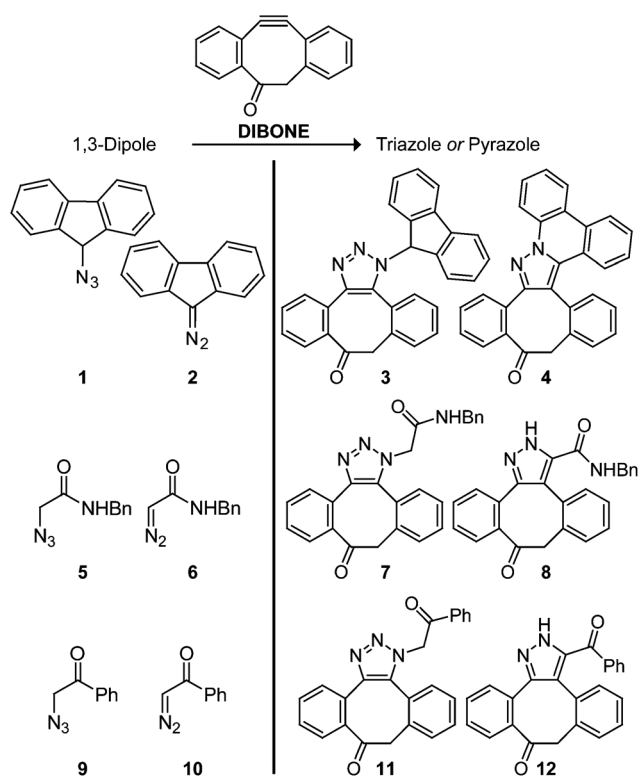
Dipole	ΔE_{NED}^{a} kcal mol^{-1}	ΔE_{IED}^{b} kcal mol^{-1}	Product^{c} (% yield)	Time (min)	$\Delta\Delta E$ kcal mol^{-1}	Product^{d} (% yield)
1	221.963	239.238	**3** (93)	35	12.481	**4** (100)
2	209.482	233.509	**4** (89)	<5		
5	245.927	259.996	**7** (89)	40	0.213	**7** (31), **8** (67)
6	245.714	248.036	**8** (94)	25		
9	256.881	228.865	**11** (92)	60	−3.125	**11** (100)
10	254.556	231.990	**12** (88)	1260		

^{a}NED, Normal Electron Demand: LUMO$_{DIBONE}$ − HOMO$_{Dipole}$. ^{b}IED, Inverse Electron Demand: LUMO$_{Dipole}$ − HOMO$_{DIBONE}$. ^{c}Each reaction resulted in a mixture of both regioisomeric products, and yields refer to the mixture obtained after silica-gel chromatography. ^{d}Competition experiment in which equimolar dipoles were mixed with 1 equiv. of **DIBONE**. Reactions were performed in CD$_3$CN; yields were determined by ^{1}H-NMR spectroscopy.

Scheme 1 Calculated energies and experimental yields for reactions of azides or diazo compounds with **DIBONE**.

diazofluorene **2** was converted completely to pyrazole **4**, leaving azidofluorene **1** unreacted. In marked contrast, azidoacetophenone **9** was converted entirely to triazole **11** leaving diazoacetophenone **10** unaffected. Finally, 31% of azidocarboxamide **5** and 67% of diazocarboxamide **6** were converted to **7** and **8**, respectively. Thus, a calculated $\Delta\Delta E$ >3 kcal mol^{-1} for a dipole pair led to complete chemoselectivity.

To obtain a more precise measure of reactivity, the second-order rate constant for the reaction of each dipole with **DIBONE** was determined by ^{1}H-NMR spectroscopy (Fig. S4†). The relative rate constants were consistent with the chemoselectivity observed in the direct competition experiments (Fig. 3). Again, when the calculated $\Delta\Delta E$ for a dipole pair was >3 kcal mol^{-1} (**1/ 2**; **9/10**), the rate constants were orders of magnitude apart, but when $\Delta\Delta E$ was only 0.2 kcal mol^{-1} (**5/6**), the rate enhancement was merely 2.5-fold. Rate constants for the reaction of diazo

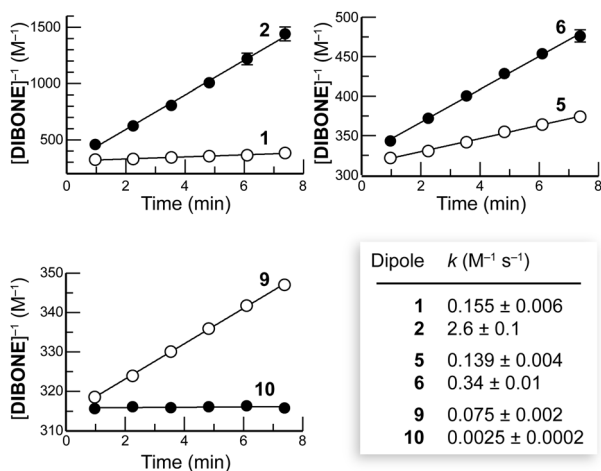**Fig. 3** Reaction rates of azides (○) or diazo compounds (●) with **DIBONE** as determined by ^{1}H-NMR spectroscopy. All reactants were at 3.2 mM in CD$_3$CN.

Fig. 4 Solvent effects on the reaction rate of azide **1** (top) or diazo compound **2** (bottom) with **DIBONE** as determined by ^{1}H-NMR spectroscopy. All reactants were at 3.2 mM.

compound **2** with three cycloalkynes (**OCT**, **DIBO**, and **DIBONE**) were likewise consistent with calculated FMO energies (Table S2†).

The rate constants for the reaction of the three azides differ by only two fold (Fig. 3). In contrast, those for the diazo compounds vary by a factor of a thousand. This high variation in diazo reactivity is consistent with the direct conjugation of the diazo dipoles with the carbonyl group or aryl rings in these reactants; that conjugation is broken by a carbon spacer in the azide dipoles. The ensuing tunable electronics of diazo compounds make them such highly versatile reagents for cycloaddition reactions with cycloalkynes.

The effect of solvent on rate can provide insight into the change in polarity upon passing from the ground state to the transition state of a reaction.[54,55] Extensive computational and experimental work has been devoted to illuminating the role of solvent in 1,3-dipolar cycloadditions.[56–63] Cycloadditions that show little or no preference for solvent polarity are considered to be concerted. On the other hand, large rate increases with solvent polarity indicate a more step-wise or asynchronous process in which the transition state is more zwitterionic than the starting materials.

We sought to compare the rate of cycloaddition of **DIBONE** with azidofluorene **1** and diazofluorene **2** in various solvents. A clear trend of increasing rate with solvent polarity was observed for both **1** and **2** (Fig. 4). Apparently, the transition state for these cycloadditions is more polarized than the ground state. Of particular interest, however, is the dramatic increase in rate with diazofluorene **2** upon switching to protic solvents, a trend not observed with azidofluorene **1**. Evidently, hydrogen bonding plays a significant role in the transition state for the diazo compound but not the azide, indicative of a more asynchronous mechanism for the diazo compound. This rate-enhancement could be of use in the highly protic medium of biological systems. Finally, we note that the second-order rate constant of 13.5 M^{-1} s^{-1} for the reaction of **DIBONE** with diazofluorene **2** is the highest known for a dipolar cycloaddition reaction with a cycloalkyne.
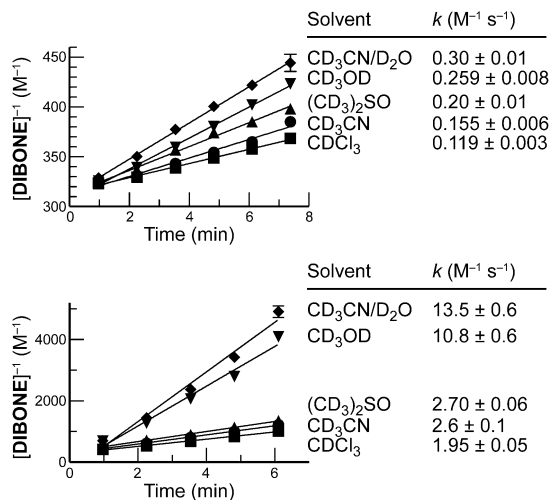
To explore the chemoselectivity of the most reactive diazo compound (**2**) in a biological context, we carried out stability and selectivity experiments in the presence of relevant reactive functionalities. We found diazo compound **2** to be unaffected (^{1}H-NMR) during an 18 h incubation in phosphate-buffered saline containing 10 mM cysteine. In addition, we treated an aqueous acetonitrile solution of **2** (3 equiv.) with two competing reactants: **DIBONE** (1 equiv.) and reduced glutathione (10 equiv.). After 5 min, **DIBONE** was converted completely to pyrazole **4** and the remaining **2** was intact (^{1}H-NMR). Thus, diazo compounds react selectively with cyclooctynes even in the presence of free amines, thiols, and carboxylic acids.

Concluding remarks

In summary, we have reported on foundational efforts to tune the reactivity of *both* reactants—the dipole and the dipolar-ophile—in a biocompatible 1,3-dipolar cycloaddition reaction. We found that the reaction with a diazo compound can be much faster, or much slower, than that with its azide analog and that polar-protic solvents greatly accelerate the reaction. The >10^3-fold range in the reactivity of diazo compounds stems from the extent of conjugation of the diazo group with a flanking group, and can be predicted *a priori* by calculating FMO energies. The versatile reactivity of diazo compounds in 1,3-dipolar cycloadditions could be of substantial utility in biological contexts. Efforts to explore and exploit those attributes are underway in our laboratory.

Acknowledgements

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