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Article

Acceleration of 1,3-Dipolar Cycloadditions by Integration of Strain and Electronic Tuning

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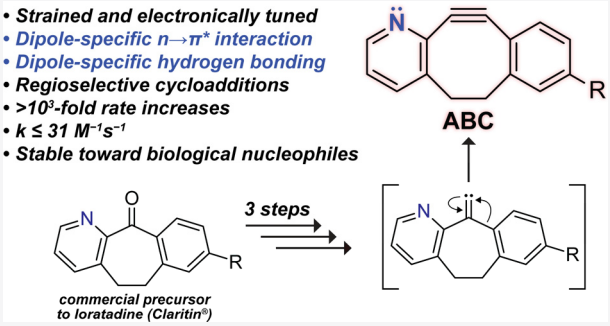
ABSTRACT: The 1,3-dipolar cycloaddition between azides and alkynes provides new means to probe and control biological processes. A major challenge is to achieve high reaction rates with stable reagents. The optimization of alkynyl reagents has relied on two strategies: increasing strain and tuning electronics. We report on the integration of these strategies. A computational analysis suggested that a CH → N aryl substitution in dibenzocyclooctyne (DIBO) could be beneficial. In transition states, the nitrogen of 2-azabenzo-benzocyclooctyne (ABC) engages in an n→π^* interaction with the C=O of α-azidoacetamides and forms a hydrogen bond with the N−H of α-diazoacetamides. These dipole-specific interactions act cooperatively with electronic activation of the strained π-bond to increase reactivity. We found that ABC does indeed react more quickly with α-azidoacetamides and α-diazoacetamides than its constitutional isomer, dibenzoazacyclooctyne (DIBAC). ABC and DIBAC have comparable chemical stability in a biomimetic solution. Both ABC and DIBO are accessible in three steps by the alkylidene carbene-mediated ring expansion of commercial cycloheptanones. Our findings enhance the accessibility and utility of 1,3-dipolar cycloadditions and encourage further innovation.



INTRODUCTION

The discovery of "spring-loaded"[1] but chemoselective reactions has transformed chemical biology, polymer chemistry, materials chemistry, and allied fields. In this realm, a particular 1,3-dipolar cycloaddition[2,3] —the strain-promoted azide−alkyne cycloaddition (SPAAC)[4−6] —has been at the forefront. Its preeminence is attributable to the attractive features of the azido group[7−9] along with the formation of an aromatic product,[10,11] enabling high chemoselectivity.[12,13]

Efforts to both understand[14−23] and optimize[24−29] SPAAC reactivity have focused on two strategies (Figure 1A): (1) increasing strain (i.e., predistortion), and (2) tuning electronics.[30−32] After the discovery of the reactivity of cycloalkynes in SPAACs in chemical contexts,[4−6] the utility of OCT was demonstrated in a biological context.[7] The installation of fluoro groups at the propargylic position via a 10-step synthetic route generated DIFO and further increased reaction rates[24] while compromising reagent stability.[33] Initial theoretical investigations attributed the higher SPAAC reactivity to LUMO-lowering.[15,19] More recently, specific orbital interactions that elicit a low-energy transition state (TS) have become apparent.[21,22] The exocyclic fluoro groups are *gauche* relative to the forming C−N bonds. In contrast, optimal orbital overlap (i.e., antiperiplanar) is achievable with endocyclic heteroatoms. Studies in model systems[34,35] and the subsequent substitution of heteroatoms into cyclooctynes such as diF-SNO-OCT and cyclononynes demonstrated the utility of this design principle.[21,22,27−29]

In parallel efforts, rate acceleration was pursued by increasing strain. In particular, benzannulation to give DIBO[36] and DIBAC[37,38] led to reaction rates comparable to those attained with electronic tuning and without compromise to reagent stability (Figure 1A). However, computations revealed steric repulsion between the incoming dipole and the "flagpole" C−H group (Figure 1B).[18,39]

Limited success has been achieved in the integration of electronic tuning with strain. The installation of remote heteroatoms has led to only incremental increases in reactivity[40] and compromised reagent stability.[26,40,41] Hence, reagents that harness both strategies are absent from the landscape.

We sought a hybrid cyclooctyne reagent for SPAAC. To begin, we performed computational analyses to guide reagent development. We discovered that a single C−H → N substitution at the "flagpole" position effects electronic activation in a dibenzocyclooctyne (Figure 1C). Notably, a C−N antibonding orbital of 2-azabenzo-benzocyclooctyne (2-ABC) lies *syn*-periplanar relative to forming bonds, effectively stabilizing the transition state. Additional stabilization can be

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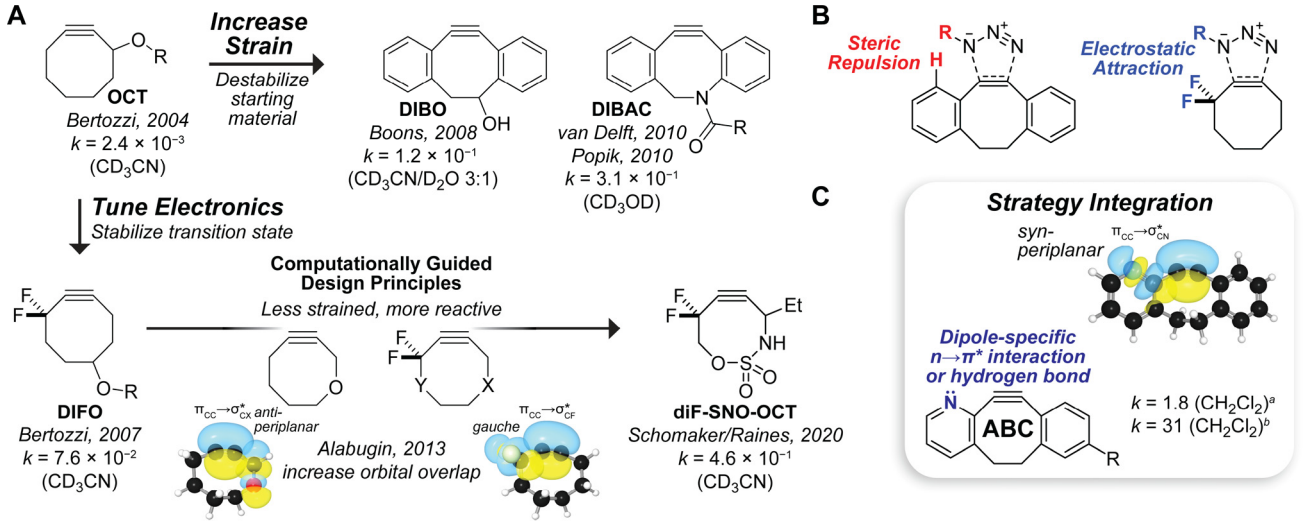


Figure 1. Optimization strategies employed to accelerate the strain-promoted cycloaddition reactivity of cyclooctynes. (A) Heteroatom-incorporation tunes alkyne electronics; benzannulation increases strain. (B) Cyclooctyne substitutions can engender steric repulsion or electrostatic attraction. (C) Strategic heteroatom placement in azabenzo-benzocyclooctyne (ABC) combines electronic-tuning with increasing strain while replacing steric repulsion with a potentially dipole-specific n→π^* interaction or hydrogen bond. Second-order rate constants (M^{-1} s^{-1}) are for the reaction with benzyl azide,[30] 2-azido-*N*-benzylacetamide,[a] or 2-diazo-*N*-benzylacetamide[b] (this work).

attained by dipole-specific interactions between substituents on the dipole and the azabenzo lone pair. This new class of cyclooctyne provides a rate for cycloadditions that surpasses those of commercially available cyclooctyne reagents with negligible impact on stability in the presence of biological nucleophiles.

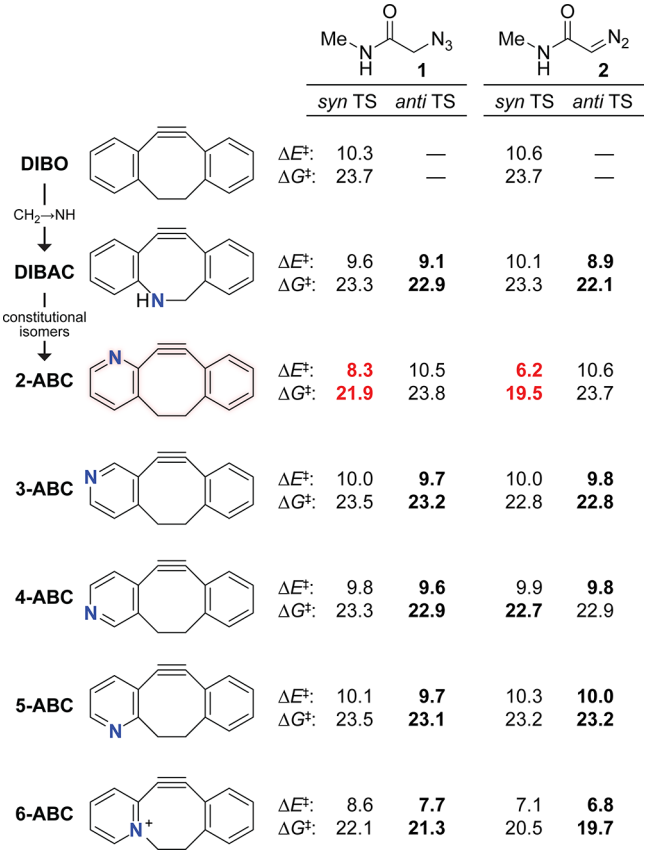
RESULTS AND DISCUSSION

Design of an Optimal Dipolarophile. Our goal was to identify a modification of the dibenzocyclooctyne scaffold to accelerate its 1,3-dipolar cycloaddition with azides and diazo compounds. We focused our computations on nitrogen substitution, as the valency of nitrogen enables an isologous C−H → N substitution within a benzo group. In accordance with recent studies,[23,42] we used Gaussian 16 to employ both the M06-2X level of theory[43,44] with the 6-311++G(d,p) basis set (including the IEFPCM solvation model) and the B97D level of theory[45] with the 6-311+G(d,p) basis set (including the CPCM solvation model). First, we modeled the 1,3-dipolar cycloaddition reactions of both *N*-methylazidoacetamide (**1**) and *N*-methyldiazoacetamide (**2**) with DIBO and DIBAC, as well as a series of constitutional isomers of DIBAC: azabenzo-benzocyclooctynes (ABCs; Tables 1 and S1).

We found that installing a nitrogen in DIBO can lower the predicted energy barriers for cycloaddition with dipoles **1** and **2** (Scheme 1). Negligible differences in activation energies ($\Delta E^{\ddagger}$) and free energies of activation ($\Delta G^{\ddagger}$) were observed for 3-, 4-, and 5-ABC in their reactions with dipoles **1** and **2**. In contrast, 2-ABC and 6-ABC were predicted to be more reactive than the other constitutional isomers. Notably, 2-ABC and 6-ABC contain a propargylic C−N bond that enables a direct interaction between the alkyne π-bond and the C−N antibonding orbital (σ^*_{CN}).[22] Interestingly, the preferred regioisomeric transition states generally favored the *anti* approach of substituents on dipoles **1** or **2** relative to the azabenzo group in all reactions except that with 2-ABC.

A comparison of optimized geometries and NBO charges on each alkyne carbon of DIBO, DIBAC, 2-ABC, and 6-ABC illustrates the effects of direct $\pi_{CC}\rightarrow\sigma^*_{CN}$ interactions between the alkyne and propargylic acceptors (Figure 2). Both 2-ABC and 6-ABC display greater asymmetry and polarization as a result of the proximal C−N bonds. In 2-ABC, the *syn*-periplanar

Scheme 1. Effect of Nitrogen Placement in Dibenzocyclooctynes[a]



		1 *syn* TS	1 *anti* TS	2 *syn* TS	2 *anti* TS
DIBO	$\Delta E^{\ddagger}$:	10.3	—	10.6	—
	$\Delta G^{\ddagger}$:	23.7	—	23.7	—
DIBAC	$\Delta E^{\ddagger}$:	9.6	**9.1**	10.1	**8.9**
	$\Delta G^{\ddagger}$:	23.3	**22.9**	23.3	**22.1**
2-ABC	$\Delta E^{\ddagger}$:	**8.3**	10.5	**6.2**	10.6
	$\Delta G^{\ddagger}$:	**21.9**	23.8	**19.5**	23.7
3-ABC	$\Delta E^{\ddagger}$:	10.0	**9.7**	10.0	**9.8**
	$\Delta G^{\ddagger}$:	23.5	**23.2**	22.8	**22.8**
4-ABC	$\Delta E^{\ddagger}$:	9.8	**9.6**	9.9	**9.8**
	$\Delta G^{\ddagger}$:	23.3	**22.9**	**22.7**	22.9
5-ABC	$\Delta E^{\ddagger}$:	10.1	**9.7**	10.3	**10.0**
	$\Delta G^{\ddagger}$:	23.5	**23.1**	23.2	**23.2**
6-ABC	$\Delta E^{\ddagger}$:	8.6	**7.7**	7.1	**6.8**
	$\Delta G^{\ddagger}$:	22.1	**21.3**	20.5	**19.7**

[a]Activation parameters (kcal/mol) were calculated at the M06-2*X*/6-311++G(d,p) employing the IEFPCM solvation model (water). Preferred regioisomers are indicated with energies in bold typeface.

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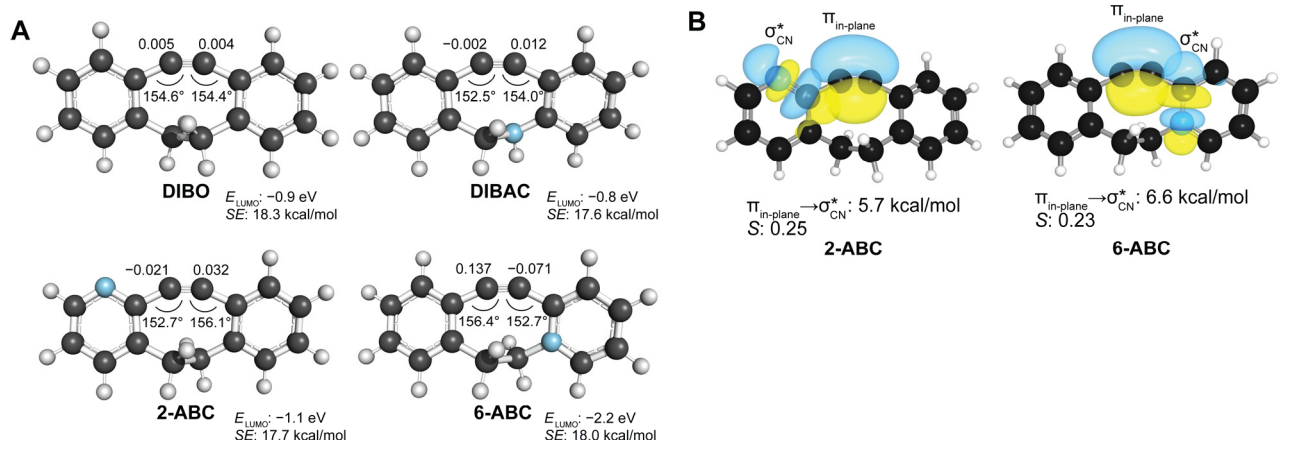

Figure 2. Combining increased strain with electronic activation. (A) Optimized geometries and NBO charge on the alkynyl carbons of DIBO, DIBAC, 2-ABC, and 6-ABC calculated at the M06-2*X*/6-311++G(d,p) employing the IEFPCM solvation model (water). (B) Interactions of the distorted alkyne with the *syn*-periplanar C−N bond in 2-ABC and the antiperiplanar C−N bond in 6-ABC. Shown are natural bonding orbitals depicting $\pi_{CC}\rightarrow\sigma^*_{CN}$ interactions, second-order perturbations energies, and PNBO overlap integrals (*S*). Strain energies (*SE*) were calculated with the isodesmic equation in Figure S3.[17]

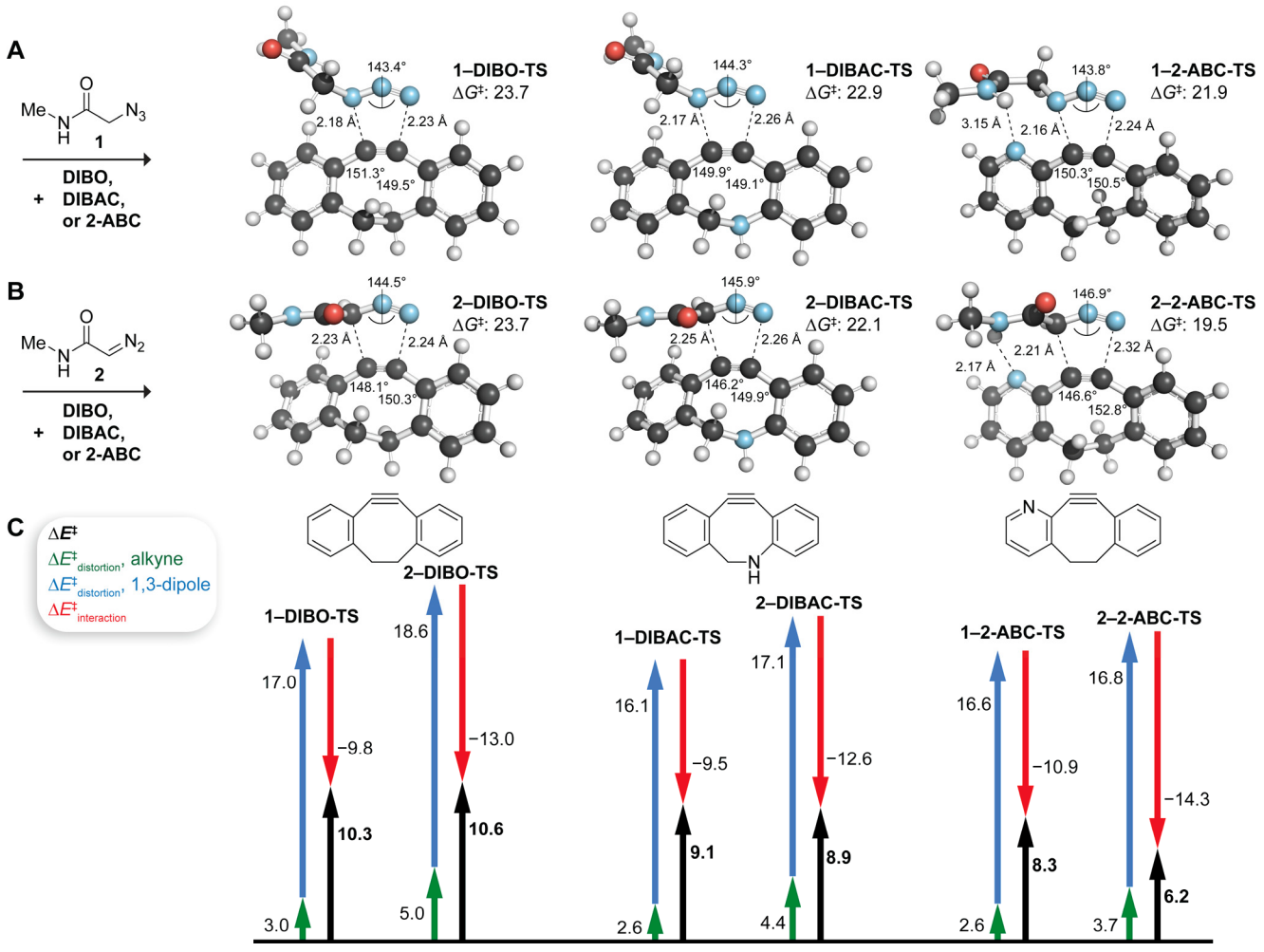

Figure 3. Computational analysis of cycloadditions with *N*-methylazidoacetamide (**1**) and *N*-methyldiazoacetamide (**2**). (A, B) Optimized transition state geometries and free energies of activation (kcal/mol) calculated at the M06-2*X*/6-311++G(d,p) level employing the IEFPCM solvation model (water). (C) Distortion/Interaction (Strain−Activation) analysis.

orientation of the C−N acceptor (relative to the obtuse lobe of the bent alkyne) provides 5.7 kcal/mol of $\pi_{CC}\rightarrow\sigma^*_{CN}$ stabilization, whereas the *anti*-periplanar orientation in 6-ABC provides 6.6 kcal/mol, quantified via the second-order perturbation of NBO analysis from NBO 7.0 software (Figure 2B).[46] The relatively minor difference in interaction energies,

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despite the formal charge on the nitrogen in 6-ABC, is in agreement with previous computational studies in which the *syn*-periplanar geometry was found to offset the energy cost of symmetric bending nearly as efficiently as the *anti*-periplanar geometry in 1-fluoro-2-butyne.[21] These direct interactions between the alkyne π-bond and the propargylic C−N bonds in 2-ABC and 6-ABC account for the asymmetry and alkyne polarization.

Although the propargylic C−N bonds in 2-ABC and 6-ABC lower the barriers to cycloaddition with azide **1** and diazo compound **2**, the two dipoles are affected differentially. For instance, the barrier for the reaction of azide **1** with 2-ABC is ~0.6 kcal/mol higher in energy than that for its reaction with 6-ABC (21.9 versus 21.3 kcal/mol), whereas the same comparison with diazo compound **2** shows a decrease of 0.2 kcal/mol (19.5 for 2-ABC versus 19.7 kcal/mol for 6-ABC).[47] Such differences can be exploited to develop chemoselective reactions between similar dipoles that are mutually orthogonal.[29,48,49]

To understand the reactivity of 2-ABC with the intent of exploiting differential reactivity toward different dipoles, we employed distortion/interaction (strain−activation) analysis.[14,16,50−52] In particular, we sought to compare the reactivity of 2-ABC with those of DIBO and DIBAC (Figure 3, Tables S2 and S3). We found that the transition states for the reaction of each dipole with 2-ABC display the strongest interactions (−10.9 and −14.3 kcal/mol for **1**−2-ABC-TS and **2**−2-ABC-TS, respectively). Diazoacetamide **2** also provides a decrease in distortion energies for both the dipole (16.8 kcal/mol) and the cyclooctyne (3.7 kcal/mol) in **2**−2-ABC-TS relative to both **2**−DIBO-TS and **2**−DIBAC-TS.[53] Meanwhile, azidoacetamide **1** displays both a dipole distortion energy (16.6 kcal/mol) and an alkyne distortion energy (2.6 kcal/mol) in **1**−2-ABC-TS that are similar to those in **1**−DIBAC-TS.

The origins of the favorable distortion and interaction energies for both 2-ABC transition states became apparent upon inspection of optimized geometries (Figures 3 and 4). The propargylic C−N bond within 2-ABC facilitates bond formation,[22] resulting in shortened incipient bonds at the internal N/C within the 2-ABC-TS relative to the corresponding DIBO-TS and DIBAC-TS, for each 1,3-dipole.

Interactions between the aryl nitrogen in 2-ABC and the acetamide in both dipoles **1** and **2** are evident from large interaction energies in optimized transition state geometries. In addition, 2-ABC is the sole constitutional isomer in which the *syn* approach of substituents on the incoming dipole relative to the azabenzo ring is favored for both azide **1** and diazo compound **2** (Scheme 1). Having found that interactions in both **1**−2-ABC-TS and **2**−2-ABC-TS enable each dipole to overcome alkyne polarization in 2-ABC (Figure 2), we next examined the nature of the interactions that enhance cycloaddition reactivity.

We found that azide **1** adopts a conformation containing an intramolecular N···H−N hydrogen bond within a 5-membered ring. That hydrogen bond is retained in **1**−2-ABC-TS (Figure 4). There, significant stabilization via an $n_N \rightarrow \pi^*_{C=O}$ interaction from the aryl nitrogen to the acetamide carbonyl of azide **1** is apparent from an N···C=O distance of 2.89 Å, N···C=O angle of $\theta = 105.2°$, and energy of 2.0 kcal/mol (Figure 4). Such an $n \rightarrow \pi^*$ interaction[54] is unprecedented in a SPAAC. In contrast to azide **1**, the diazo compound **2** does not form an intramolecular hydrogen bond. Instead, we found an intermolecular hydrogen bond with an N···H−N distance of 2.17 Å and energy of 3.9 kcal/mol in **2**−2-ABC-TS. That strong hydrogen bond leads to a lower activation barrier for diazoacetamide **2** than that for azide **1**.

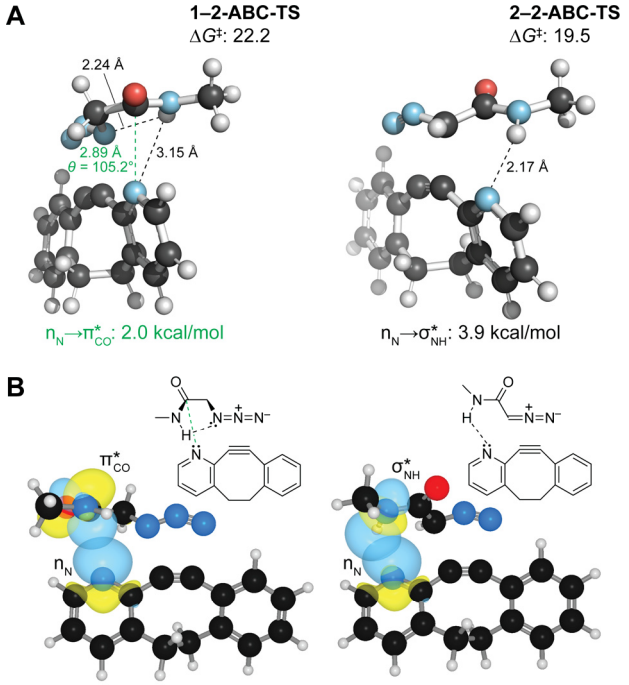

Figure 4. Comparison of interactions in 2-ABC cycloadditions with *N*-methylazidoacetamide (**1**) and *N*-methyldiazoacetamide (**2**). (A) Second-order perturbations obtained from an NBO analysis. (B) Key stabilizing orbital interactions: N···C=O n→π^* interaction with azide **1** and N···H−N hydrogen bond with diazo compound **2**.

Synthesis of ABC. We sought to experimentally test the computational results. When considering the available synthetic methods to access strained alkynes, we were challenged by the inherent limitations posed by an azabenzo group. Common routes to cyclooctynes rely on the synthesis of parent alkenes, dibromination, and subsequent elimination of HBr (2 ×), often requiring extended synthetic routes.[30,37,38] A possible circumvention is the Friedel−Crafts reaction of electron-rich benzyl phenyl ethers with tetrachlorocyclopropene, followed by hydrolysis to generate a biaryl cyclopropenone and UV irradiation to form a cycloalkyne.[26] Unfortunately, an azabenzo group is not compatible with a Friedel−Crafts reaction.

To overcome these challenges, we reasoned that we could harness the high energy that is inherent in an alkylidene carbene to accomplish a [1,2]-rearrangement that yields strained alkynes (Scheme 2). In analogy to the Fritsch−Buttenberg−Wiechell rearrangement[55−57] (which is the second step of the Corey−Fuchs reaction[58]), we sought to enlist the dehydrative fragmentation of a 5-hydroxyalkyl-1*H*-tetrazole, accessed via *N*-morpholinomethyl-5-lithiotetrazole that is generated *in situ*.[59,60] Specifically, we found that the nucleophilic addition of *N*-morpholinomethyl-5-lithiotetrazole to commercially available ketone **3**, which is a precursor to the antihistamine loratadine (Claritin),[61] proceeded smoothly in THF and afforded tetrazole **4** after acid hydrolysis. Dehydration with EDC in THF gave a tetraazafulvene intermediate, which expelled dinitrogen to generate an unstable alkylidene carbene.[62] Its [1,2]-rearrangement[63] afforded the desired cyclooctyne. Adventitiously, this opportunistic route to ABC affords a chloro group that is an ideal handle for functionalization through well-established aryl chloride coupling chem-

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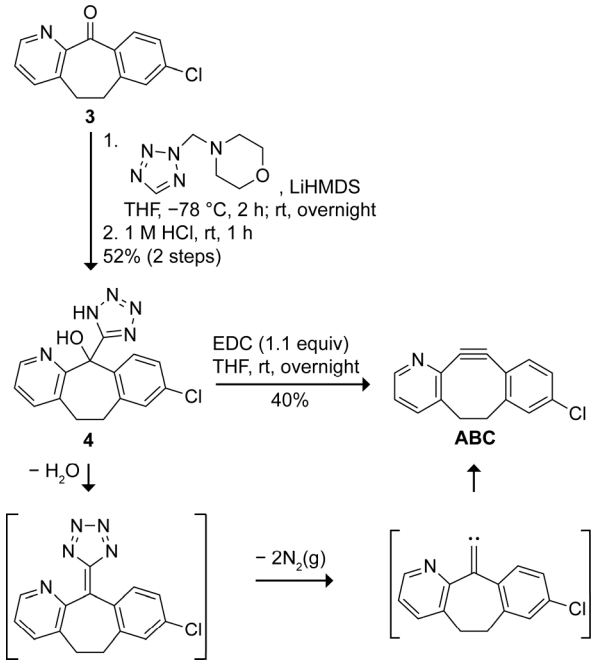
Scheme 2. Synthesis of ABC and Putative Mechanism for the Alkylidene Carbene Ring-Expansion of the Intermediate



istry.[64,65] We also applied the alkylidene carbene ring-expansion strategy for the expedient synthesis of DIBO, and we anticipate its utility in the synthesis of other cycloalkynes as well.

Reactivity of ABC. With ABC in hand, we examined its 1,3-dipolar cycloaddition with common dipoles. We focused on 2-azido-*N*-benzylacetamide (**5**) and 2-diazo-*N*-benzylacetamide (**6**).[48,49,53,66−68] We measured the rates for the reaction of dipoles **5** and **6** with ABC in both aprotic (CH_2Cl_2) and protic solvents (MeOH and PBS containing 2% v/v DMSO).[69] We assessed the depletion of ABC by using HPLC and calculated second-order rate constants from the slope of a plot of $[ABC]^{-1}$ versus time. As a benchmark, we also measured the rate of the reaction of DIBO with each dipole.[30]

We found the reaction rates with ABC were exceptionally high (Figure 5). In all solvent conditions, each acetamide dipole displayed rate constants with ABC that exceed those attainable with commercially available cyclooctyne reagents. In CH_2Cl_2, the rate constants are among the highest reported for both SPAAC and the analogous reaction with diazo compounds. The strategic CH → N substitution that converts DIBO to ABC leads to 30- and 1200-fold rate increases with 2-azido-*N*-benzylacetamide (**5**) and 2-diazo-*N*-benzylacetamide (**6**), respectively.

A significantly larger rate constant for diazoacetamide **6** over azidoacetamide **5** provides experimental corroboration of the computational predictions (Scheme 1 and Figure 3). With DIBO, 2-azido-*N*-benzylacetamide reacts 2- to 3-fold faster than 2-diazo-*N*-benzylacetamide, consistent with reactions of DIBAC[9b] and DIBONE.[66] Thus, the ∼20-fold rate increase of the diazoacetamide over the azidoacetamide with ABC exceeds the difference observed with both DIBAC and DIBONE as well as with SNO-OCTs.[28,29]

To provide a benchmark for the general use of ABC in a SPAAC, we performed a competition experiment. Specifically, we mixed 1 equiv of ABC with 1 equiv of both azide **5** and benzyl azide. The product ratios indicated a preferential reaction with azide **5** (71:29 in CH_2Cl_2 and 65:35 in MeOH), consistent with a favorable n→π^* interaction in its TS (Figure 4) that is not

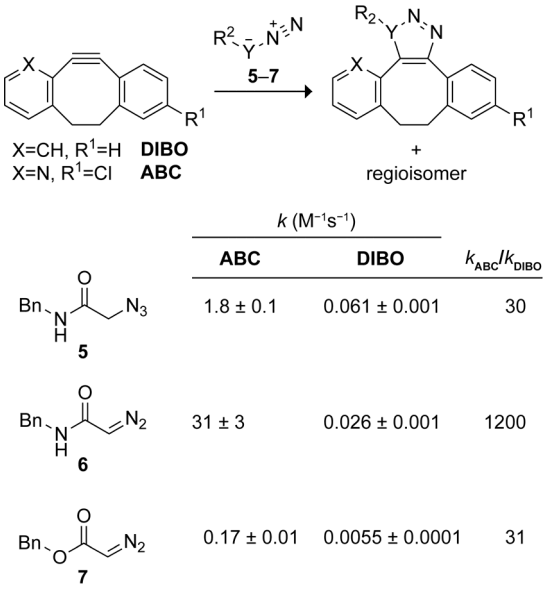


Figure 5. Second-order rate constants for the 1,3-dipolar cycloaddition of DIBO or ABC with dipoles **5**−**7** in CH_2Cl_2. Values are the mean ± SE from triplicate experiments.

accessible by benzyl azide. We conclude that α-azidoacetamides (like azide **5**) are ideal for a SPAAC with ABC but other azides can react rapidly as well. Moreover, ABC avails of tunability that is atypical for SPAAC reactivity.[66,70,71]

Basis for ABC Reactivity. We sought the basis for the high reactivity of ABC beyond electronic tuning of the LUMO energy via direct orbital interactions (Figure 2). We began by corroborating the existence of an intramolecular hydrogen bond in an α-azidoacetamide (Figure 4). We found that azide **5** does indeed adopt a conformation containing an N···H−N hydrogen bond within a 5-membered ring. That hydrogen bond is evident from the large downfield ^{1}H NMR chemical shift of the donor proton (6.64 ppm in $CDCl_3$) compared with the analogous H−N proton in *N*-benzylacetamide (5.87 ppm in $CDCl_3$), which lacks an azido group.

Next, we probed for the formation of an intermolecular n→π^* interaction and hydrogen bond upon cycloaddition with ABC (Figure 4) by ascertaining the regiochemistry of cycloadducts. Because *N*-methylation of the amide in **5** and **6** should impede intermolecular interactions in both **1**−2-ABC-TS and **2**−2-ABC-TS (Figure 4), regioselectivity should report on their importance. As expected, ^{1}H NMR spectra revealed that regioselectivity is attained only when these interactions are accessible in the two transition states (Figure 6A,B).

To further assess the effect of the putative hydrogen bond in **2**−2-ABC-TS (Figure 4), we tested the reactivity of ABC and DIBO with benzyl 2-diazoacetate (**7**), which lacks a hydrogen bond donor. We found that the rate constant for the reaction of ABC with ester **7** was nearly 200-fold lower than that with amide **5**. This decrease is substantially greater than the 5-fold decrease in rate constant for the reaction of these same dipoles with DIBO (Figure 5).

Lastly, we found that the use of protic solvents has little effect on the rate constant for the reaction of ABC with azide **5** but lowers the rate constant for the reaction with diazo compound **6** (Figure 6C). These data are consistent with reports that protic solvents both strengthen and weaken aspects of an n→π^* interaction,[72,73] like that in **1**−2-ABC-TS, but weaken hydrogen

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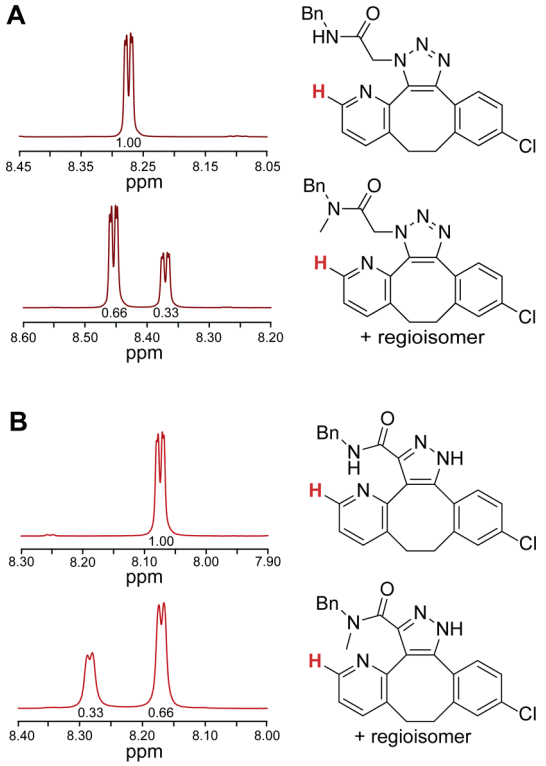

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solvent	k ($M^{-1}s^{-1}$) 5	6
CH_2Cl_2	1.8 ± 0.1	31 ± 3
MeOH	0.98 ± 0.01	4.9 ± 0.1
PBS	1.3 ± 0.1	2.1 ± 0.1

Figure 6. Effect of an intramolecular n→π^* interaction and hydrogen bond on the 1,3-dipolar cycloaddition of ABC with 2-azido-*N*-benzylacetamide (**5**) and 2-diazo-*N*-benzylacetamide (**6**). (A) ^{1}H NMR shifts of the C3−H (red) proton in the product of the cycloaddition of ABC with dipole **5** or its *N*-methyl derivative in CH_2Cl_2. (B) ^{1}H NMR shifts of the C3−H (red) proton in the product of the cycloaddition of ABC with dipole **6** or its *N*-methyl derivative in CH_2Cl_2. (C) Second-order rate constants for the reaction of ABC with dipoles **5** and **6** in CH_2Cl_2 (as in Figure 5), MeOH, and PBS containing DMSO (2% v/v). Values are the mean ± SE from triplicate experiments.

bonding,[74,75] like that in **2**−2-ABC-TS (Figure 4B). Still, the reaction rates observed in protic solvents are among the highest reported for 1,3-dipolar cycloadditions.[30]

Stability of ABC. Only a stable alkyne has utility for a SPAAC in a physiological context. Accordingly, we determined the stability of ABC in the presence of biological nucleophiles. To do so, we employed the nucleophiles within glutathione,[76] which contains amino, carboxyl, and sulfhydryl groups. As a comparator, we used DIBAC. Specifically, we incubated ABC and DIBAC in a solution of reduced glutathione (1 mM) and oxidized glutathione (0.2 mM) in PBS containing DMSO (2% v/v) at 37 °C (Figure S7). The rates of degradation of ABC and DIBAC under these conditions were comparable, with $t_{1/2}$ = 1.9 h and $t_{1/2}$ = 3.8 h, respectively.

CONCLUSIONS

In summary, computations were successful in guiding the design of ABC, which is the first known heterobiarylcyclooctyne. Its combination of alkynyl strain and electronic tuning provides rate constants that are among the highest reported for a SPAAC. Moreover, the reaction of ABC with a diazo compound can be >10-fold faster than that with an azido analog, further expanding utility. The three-step synthetic route to ABC (and DIBO) is the first example of accessing a strained alkyne using an alkylidene carbene-mediated ring expansion. This route is shorter than that for accessing any other cyclooctyne used for SPAACs.[30] Finally, ABC could harbinger a new class of dipolarophiles that exploit cooperative elements of molecular recognition (e.g., an n→π^* interaction or hydrogen bond).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.1c03133.

Synthetic methods and analytical data along with computational methods and additional computational data (PDF)

Cartesian coordinates, energies, and imaginary frequencies (for TSs) of optimized structures (ZIP)

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Complete contact information is available at: https://pubs.acs.org/10.1021/jacs.1c03133

Notes

The authors declare the following competing financial interest(s): The Massachusetts Institute of Technology has applied for a patent on technology described in this article.

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