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Difluorobenzocyclooctyne: Synthesis, Reactivity, and Stabilization by β-Cyclodextrin

Ellen M. Sletten,[†] Hitomi Nakamura,[†] John C. Jewett,[†] and Carolyn R. Bertozzi*[,†,‡,§,||]

Departments of Chemistry and Molecular and Cell Biology and Howard Hughes Medical Institute, University of California, Berkeley, California 94720, and The Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, California 94720

Received June 8, 2010; E-mail: crb@berkeley.edu

Abstract: Highly reactive cyclooctynes have been sought as substrates for Cu-free cycloaddition reactions with azides in biological systems. To elevate the reactivities of cyclooctynes, two strategies, LUMO lowering through propargylic fluorination and strain enhancement through fused aryl rings, have been explored. Here we report the facile synthesis of a difluorobenzocyclooctyne (DIFBO) that combines these modifications. DIFBO was so reactive that it spontaneously trimerized to form two asymmetric products that we characterized by X-ray crystallography. However, we were able to trap DIFBO by forming a stable inclusion complex with β-cyclodextrin in aqueous media. This complex could be stored as a lyophilized powder and then dissociated in organic solvents to produce free DIFBO for in situ kinetic and spectroscopic analysis. Using this procedure, we found that the rate constant for the cycloaddition reaction of DIFBO with an azide exceeds those for difluorinated cyclooctyne (DIFO) and dibenzocyclooctyne (DIBO). Cyclodextrin complexation is therefore a promising approach for stabilizing compounds that possess the high intrinsic reactivities desired for Cu-free click chemistry.

Introduction

Growing interest in bioorthogonal reactions has prompted many chemists to revisit exotic structures previously considered to be of purely theoretical interest.[1] The roots of many bioorthogonal reactions are found in the physical organic chemistry literature from the middle of the 20th century, when chemists were studying the boundaries between reactivity and stability of molecules.[2] It was during this time that now-famous molecules such as tetrahedrane, cubane, and Dewar benzene were envisioned and many clever, strain-inducing reactions were developed.[3] Also of interest to physical organic chemists was cyclooctyne, the smallest readily isolable cycloalkyne.[4] Cyclooctynes possess ∼18 kcal/mol of strain energy, primarily as a result of the distorted bond angles surrounding their sp-hybridized carbon atoms.[5]

Interest in cyclooctynes has reemerged in recent years for reasons beyond their odd structures. The reagents engage in rapid and selective cyclooaddition reactions with azides,[6] enabling their use as substrates for "Cu-free click chemistry" in biological settings.[7] The applications of this chemistry have proven to be quite broad,[1] some examples being protein[8] and lipid[9] labeling and imaging of glycans in cells,[7c,d,f] *Caenorhabditis elegans*,[10] and developing zebrafish.[11]

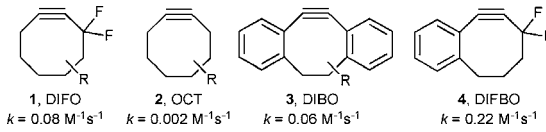

Figure 1. A panel of selected cyclooctynes and their second-order rate constants for the reaction with benzyl azide in acetonitrile-d_3 (**1**, **2**, **4**) or methanol-d_4 (**3**).

A theme that has emerged from these studies is the need for rapid kinetics to achieve the desired reaction under conditions where the azide-functionalized biological target is present in relatively low abundance. In earlier work, we found that the addition of a *gem*-difluoro group at the propargylic position, embodied in the compound we named DIFO (**1**; Figure 1A), increased the rate constant for the reaction with benzyl azide by almost 2 orders of magnitude relative to the parent cyclooctyne (OCT, **2**).[7a,c] Boons and co-workers achieved a similar rate enhancement by fusing two aryl rings to the cyclooctyne scaffold, as reflected in the compound named DIBO (**3**).[7d]

[†] Department of Chemistry, University of California, Berkeley.
[‡] Department of Molecular and Cell Biology, University of California, Berkeley.
[§] Howard Hughes Medical Institute.
[||] The Molecular Foundry.

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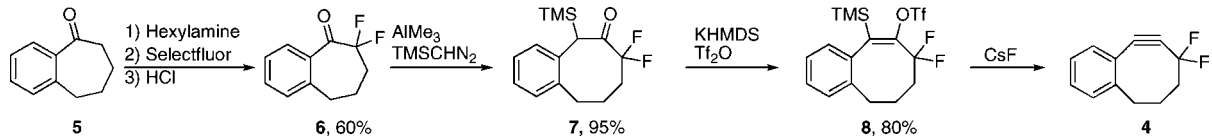
Scheme 1. Synthesis of DIFBO



In parallel with these experimental studies, theoretical work aimed at understanding the basis of the rate enhancements for DIFO and DIBO relative to OCT has been performed.[12] Density functional theory (DFT)-based methods have consistently predicted the rate-enhancing impact of fluorination, which has been attributed to its effects on transition-state distortion/interaction energies.[12a,b] The fused aryl rings of DIBO were proposed by Goddard and co-workers to exert two opposing effects on the rate of cycloaddition with azides. On the one hand, the aryl rings could promote the cycloaddition reaction by augmenting the ring strain in the cyclooctyne. However, this effect was predicted to be tempered by unfavorable steric interactions (i.e., A-1,3 strain) between the alkyl substituent of the azide and the ortho hydrogen atoms of the aryl rings. Goddard further speculated that removal of one aryl ring could enhance the cycloaddition rate relative to DIBO, at least for one triazole regioisomer.[12c]

Motivated by this growing body of experimental and theoretical work, we propose that compounds combining the rate-enhancing features of DIFO and DIBO might possess superior kinetics that are desirable for labeling of azides in biological systems. Here we report the synthesis and characterization of difluorobenzocyclooctyne (DIFBO) (**4**; Figure 1), a hybrid of DIFO and DIBO. This new cyclooctyne was more reactive than either parent compound, to an extent that it underwent spontaneous homotrimerization in solution. However, we were able to stabilize DIFBO in an inclusion complex with β-cyclodextrin, enabling long-term storage (on the benchtop) and use as a reagent for rapid cycloaddition with azides.

Results and Discussion

Synthesis of DIFBO. In previous cyclooctyne syntheses, we found it prudent to install the alkyne toward the end of the synthetic route using mild conditions. Fluoride-mediated elimination of a silyl enol triflate precursor, which proceeds rapidly at room temperature and in high yield,[7f] seemed ideal for generating the alkyne in DIFBO. Thus, we envisioned trimethylsilyl ketone **7** as an intermediate in the synthesis (Scheme 1). Installing a silyl group α to a carbonyl functionality is notoriously difficult, as the conditions necessary for carbon−silicon bond formation often lead to silyl enol ether formation instead.[13] We therefore looked to disconnections other than carbon−silicon bond formation. This led us to propose a homologation strategy in which **7** could be produced by Lewis acid-mediated ring expansion of difluorobenzosuberone **6** with trimethysilyl diazomethane. Homologation with trimethylsilyl diazomethane is well-precedented,[14] although not with α-fluoro ketones.[15] Trimethylsilyl diazomethane is usually chosen as a diazomethane surrogate on the basis of safety considerations and not because the silyl functionality is desired.[16,17]

As shown in Scheme 1, **6** was synthesized by treatment of the hexylamine imine of 1-benzosuberone (**5**) with Selectfluor, a method reported by Stavber and co-workers.[18] Treatment of **6** with trimethylsilyl diazomethane and a stoichiometric amount of trimethylaluminum resulted in clean homologation to yield compound **7** with the trimethylsilyl group intact. Immediate enol triflate formation by deprotonation with KHMDS followed by trapping with trifluoromethane sulfonic anhydride yielded compound **8**. This intermediate was converted to DIFBO (**4**) by reaction with cesium fluoride (CsF); however, the desired product could not be isolated in pure form. Rather, DIFBO underwent spontaneous homotrimerization to form a mixture of two compounds, an early indication of its extreme reactivity.[4,19] After extensive NMR analysis (Figures S1 and S2 in the

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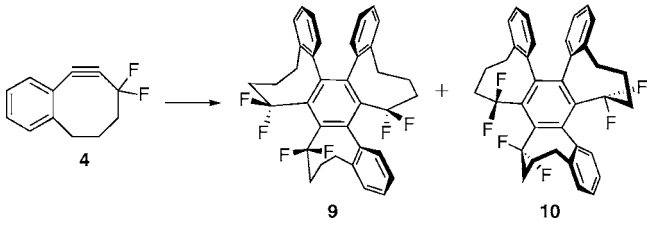
Scheme 2. Asymmetric Trimerization of DIFBO



Supporting Information) and further confirmation by X-ray crystallography (Figure 2), we determined that the two trimer products were asymmetric diastereomers **9** and **10** (Scheme 2). Interestingly, a trimeric product that would be derived from three DIFBO molecules coming together in a C_3-symmetric manner was not observed.

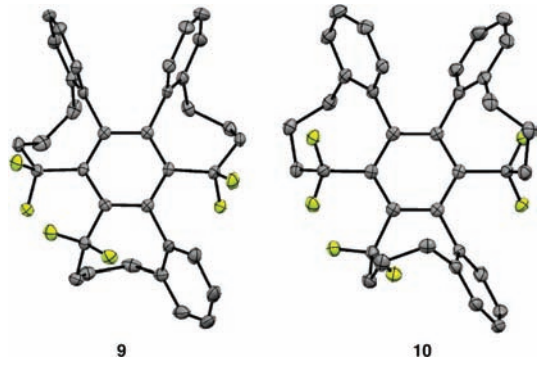
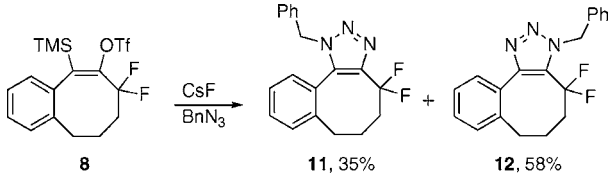


Figure 2. Thermal ellipsoid plots for trimer products **9** and **10** shown at 50% probability. Gray atoms correspond to carbon and green atoms to fluorine. Hydrogen atoms have been omitted for clarity. See Figures S12 and S13 for ORTEP diagrams with labeled atoms.

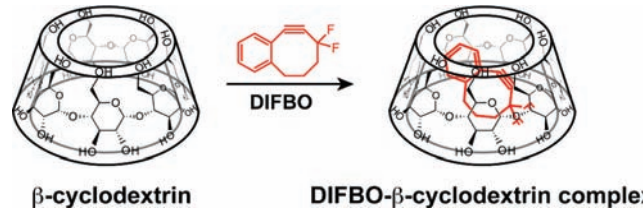
To confirm that DIFBO had indeed been formed prior to trimerization, we added benzyl azide to the reaction of compound **8** with CsF and obtained a mixture of triazole products **11** and **12** (1:1.6 ratio; Figures S3 and S4) in 93% yield (Scheme 3). Thus, the rate of reaction of DIFBO with benzyl azide appeared to be faster than that of trimerization.

Scheme 3. Formation of DIFBO and In Situ Trapping with Benzyl Azide



Complexation with *β*-Cyclodextrin. The trimerization side reaction created several problems with regard to the characterization of DIFBO. The compound was impossible to isolate in pure form at convenient temperatures and could not be stored for future use. Thus, we sought a means of stabilizing DIFBO for detailed spectroscopic and kinetic characterization. Cyclodextrins, cyclic oligomers of α-1,4-linked glucose, are well-known for their ability to bind small, nonpolar molecules within their bowl-shaped cavities.[20] The three commonly used cyclodextrins, α, β, and γ, which comprise six, seven, and eight glucose residues, respectively, possess different cavity dimensions that can be selected to match the small-molecule guest of interest.[20] The inclusion complexes are stable in water and during freeze-drying but readily dissociate upon suspension in organic solvents. Cyclodextrin complexation has previously been exploited to enhance the stability of lipophilic drugs.[20,21] On the basis of this precedent, we sought to test whether cyclodextrins would form stable inclusion complexes with DIFBO, thereby permitting long-term storage and facilitating kinetic characterization (Scheme 4).

Scheme 4. Formation of an Inclusion Complex between DIFBO and β-Cyclodextrin



We combined a crude solution of DIFBO in acetonitrile, generated as shown in Scheme 1, with aqueous solutions of α-, β-, or γ-cyclodextrin. Thin-layer chromatography and mass spectrometry analyses showed the persistence of DIFBO in the β-cyclodextrin-containing mixture, whereas side products formed in the mixtures containing α- or γ-cyclodextrin (see below). Encouraged by this observation, we developed a procedure for generating and isolating the DIFBO−β-cyclodextrin complex. We found that the reaction solution in which DIFBO was generated could be directly loaded onto a silica gel column without concentration. Upon elution with hexanes, pure DIFBO was obtained. The DIFBO−β-cyclodextrin complex cannot be formed in the presence of hexanes, and consequently, we exchanged the solvent by dilution with acetonitrile followed by careful room-temperature evaporation of the hexanes. An aqueous solution of β-cyclodextrin was then added to the DIFBO/acetonitrile solution, and immediately a white precipitate formed. This mixture was lyophilized to afford a white powder.

^{1}H NMR analysis of the powder dissolved in DMSO-d_6 (Figure S5) confirmed the presence of pure DIFBO, providing strong evidence for a successful complexation process. However, an inclusion complex of this type would be expected to dissociate in DMSO,[22] and indeed, our NMR analysis suggested that β-cyclodextrin and DIFBO were both free in solution (Figure S5). To obtain direct spectral evidence of the DIFBO−β-cyclodextrin complex, we performed solid-state cross-polarization magic-angle-spinning (CPMAS) ^{13}C NMR analysis of the lyophilized powder in comparison with pure β-cyclodextrin (Figure S6). The resonance corresponding to the anomeric carbon of glucose in the putative DIFBO−β-cyclodextrin complex was shifted downfield from the corresponding resonance in pure β-cyclodextrin. In addition, all of the cyclodextrin ^{13}C resonances were broadened in the putative complex. Both of these effects have been previously observed in CPMAS ^{13}C NMR spectra of β-cyclodextrin inclusion complexes,[23] supporting our conclusion that DIFBO was indeed complexed inside the β-cyclodextrin cavity.

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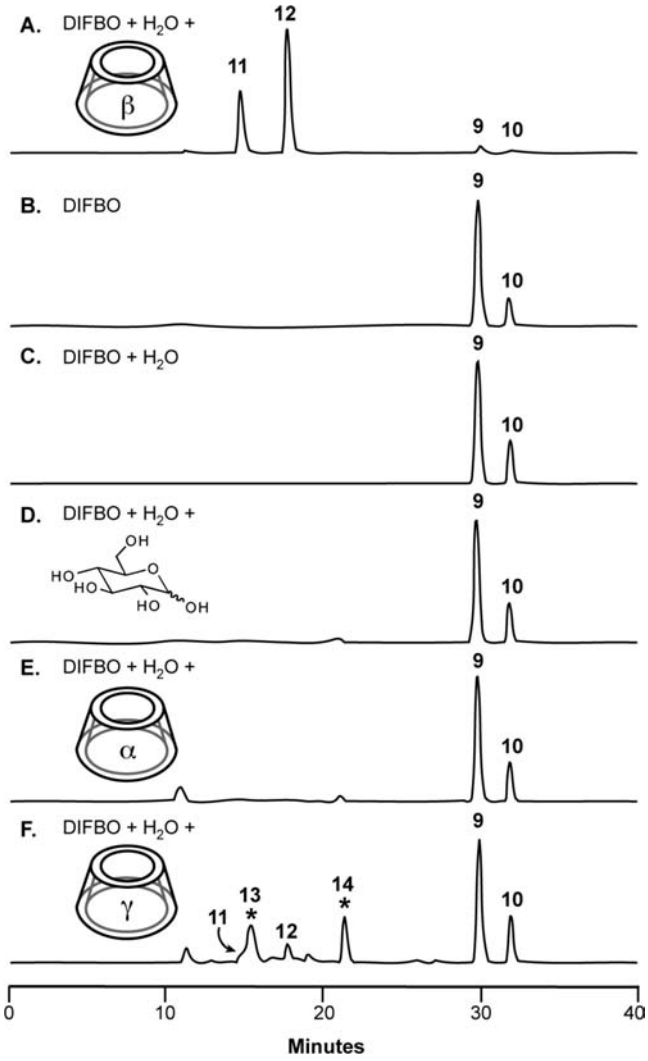


Figure 3. Reversed-phase HPLC analysis of lyophilized DIFBO mixtures reacted with benzyl azide. A solution of DIFBO in acetonitrile was combined with (A) β-cyclodextrin (1 equiv), (B) no additive, (C) water, (D) glucose (1 equiv), (E) α-cyclodextrin (1 equiv), or (F) γ-cyclodextrin (1 equiv) and concentrated. The resulting solids were resuspended in a 1:1 acetonitrile/water mixture containing 1 equiv of benzyl azide (final concentration 600 μM). The reaction mixture was analyzed by reversed-phase HPLC with detection by absorbance at 254 nm. Peak assignments were made through comparison to synthetic standards (Figure S7).

We next performed a more detailed study of DIFBO's stability and reactivity with azides in the presence of various additives, including the three cyclodextrins as well as monomeric glucose and water alone. A solution of DIFBO in acetonitrile was combined with each of the above additives, and the aqueous mixtures were lyophilized. The resulting powders were resuspended in a solution of benzyl azide in acetonitrile, and the products were analyzed by HPLC (Figure 3) alongside synthetic standards (Figure S7).

The reaction containing β-cyclodextrin produced two major products, which we identified as triazoles **11** and **12**, with only trace amounts of trimer isomers **9** and **10** (Figure 3A). This result further confirms that β-cyclodextrin prevents trimerization of DIFBO. We then investigated the long-term stability of the DIFBO−β-cyclodextrin complex. The dry powder was periodically suspended in 1:1 acetonitrile/water containing benzyl azide, and the integrity of DIFBO was determined indirectly by monitoring the formation of triazoles **11** and **12** as well as the absence of trimers **9** and **10**. We observed neither significant trimer formation nor any other degradation products over the course of 2 months during storage on the benchtop at room temperature and open to air (Figures S8 and S9). Therefore, β-cyclodextrin appears to be a convenient host for stabilization and storage of DIFBO. DIFBO can be readily extracted from the cyclodextrin simply by suspending the complex in an organic solvent and removing the insoluble free cyclodextrin by filtration.

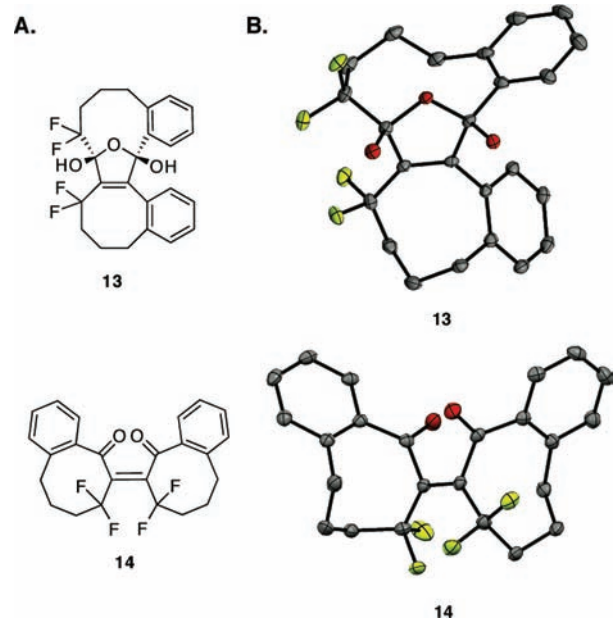
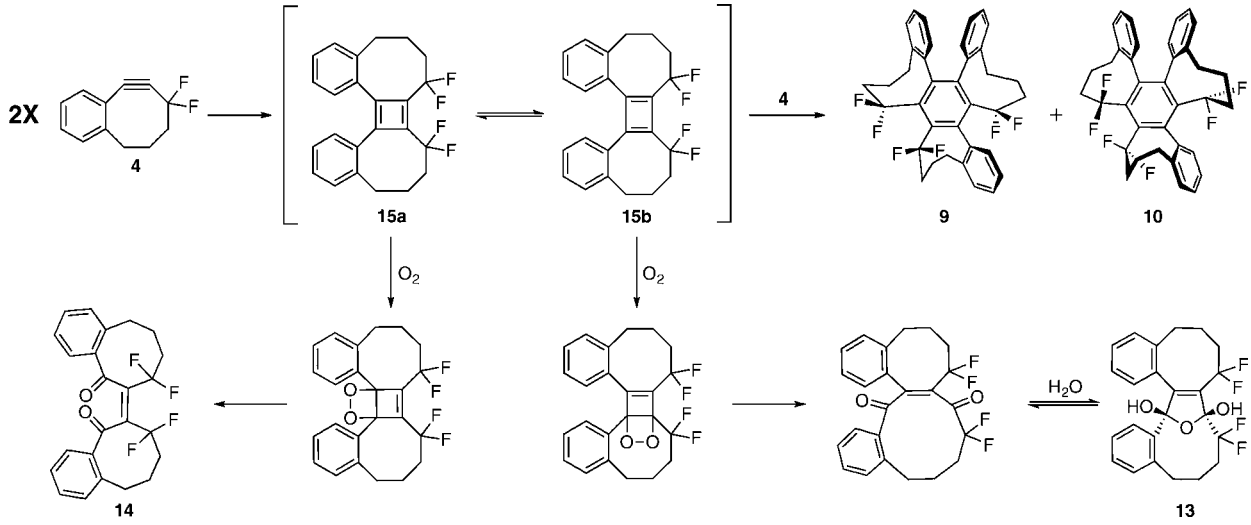


Figure 4. (A) Chemical structures of dimers **13** and **14**. (B) Thermal ellipsoid plots of dimers **13** and **14** shown with 50% probability. Gray atoms correspond to carbon, green atoms to fluorine, and red atoms to oxygen. Hydrogen atoms have been omitted for clarity. See Figures S14 and S15 for ORTEP diagrams with labeled atoms.

In contrast, in the absence of any additive (Figure 3B) or in the presence of water (Figure 3C) or glucose (Figure 3D), DIFBO formed trimers **9** and **10** prior to resuspension with benzyl azide. Similarly, addition of α-cyclodextrin had no stabilizing effect on DIFBO, and only trimers were observed, indicating either that DIFBO cannot fit into the smaller cyclodextrin cavity or that its complex with α-cyclodextrin is relatively unstable (Figure 3E).

The products formed from the DIFBO/γ-cyclodextrin mixture were more complicated (Figure 3F). Trimers **9** and **10** were the major species observed, although the presence of small amounts of triazoles **11** and **12** indicated that some DIFBO had been preserved through the lyophilization process. However, multiple new peaks in the HPLC trace suggested that γ-cyclodextrin alters the reactivity of DIFBO. We isolated the two major new species (HPLC peaks designated with asterisks in Figure 3F) and used mass spectrometry, NMR spectroscopy and X-ray crystallography to identify them as oxidized DIFBO dimers **13** and **14** (Figure 4). Both **13** and **14** comprise two DIFBO moieties as well as a molecule of oxygen, with **14** being hydrated as well. We speculate that the two compounds are derived from a common intermediate, cyclobutadiene **15**, formed by combination of two DIFBO molecules (Scheme 5).[24] This unstable antiaromatic intermediate[25] presumably reacts rapidly with molecular oxygen, resulting either in exocyclic (**15a** leading to **14**) or endocyclic (**15b** leading to **13**) C−C bond cleavage with respect to the cyclooctyne rings (Scheme 5). Compound

Scheme 5. Proposed Mechanism of DIFBO Dimerization and Trimerization

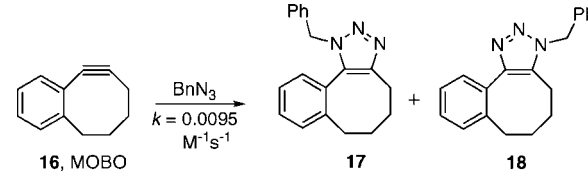


15 may also be an intermediate in the formation of trimers **9** and **10**, which constitute the two regioisomers formed by addition of another molecule of DIFBO. Indeed, rapid formation of **15** as the first step in the trimerization process would explain why the C_3-symmetric homotrimer was not observed.

The formation of dimers **13** and **14** only in the presence of γ-cyclodextrin raises an interesting question regarding the participation of this host molecule. It is possible that the γ-cyclodextrin cavity can accommodate two DIFBO molecules and thereby facilitate the dimerization reaction. Also, the host might uniquely bind intermediate **15**, thereby preventing its reaction with another large DIFBO molecule but not with molecular oxygen.

Reactivity of DIFBO in Comparison with Other Cyclooctynes. The ability to extract pure DIFBO from the β-cyclodextrin complex allowed us to test how the combination of fluorination and a fused aryl ring affects the cycloaddition rate relative to that for either rate-enhancing feature alone. The DIFBO−β-cyclodextrin complex was dissolved in acetonitrile-d_3, and the free β-cyclodextrin was removed by filtration. A stoichiometric amount of benzyl azide was added, and the reaction was monitored by ^{1}H NMR spectroscopy. We measured the second-order rate constant to be 0.22 ± 0.01 M^{-1} s^{-1} (Figure S10), which is larger than those for DIFO ($k = 0.08$ M^{-1} s^{-1}) and DIBO ($k = 0.06$ M^{-1} s^{-1}).[26] These results demonstrate that fusion of an aryl ring to the DIFO scaffold enhances its reactivity with azides by a significant margin and suggest that addition of electron-withdrawing groups to the DIBO scaffold would augment its reactivity as well. However, DIFBO and DIBO cannot be directly compared, since according to Goddard's proposal,[12c] the second aryl ring may actually retard the reaction rate as a result of unfavorable steric interactions between its ortho hydrogen atom and the azide substrate in the transition state. Thus, we synthesized monobenzocyclooctyne (MOBO, **16**; Scheme 6)[27] as a better substrate against which to judge the relative contribution of fluorination to the reactivity of DIFBO. Unlike DIFBO, MOBO was readily isolable and displayed only minimal decomposition upon extended storage. We measured a rate constant of 0.0095 ± 0.0003 M^{-1} s^{-1} for its cycloaddition reaction with benzyl azide, which affords cycloadducts **17** and **18** in a 1:2 ratio (Scheme 6, Figure S11). This rate constant is 23-fold smaller than that observed for DIFBO and also almost an order of magnitude lower than those for DIFO and DIBO. This result confirms that both the electronic effects of fluorination and the enhanced ring strain created by the fused aryl ring contribute to DIFBO's reactivity, though the electronic effects seem to predominate. Also noteworthy is the fact that DIBO reacts with benzyl azide considerably faster than MOBO, contrary to the previous DFT-based predictions.[12c]

Scheme 6. Reaction of Monobenzocyclooctyne (MOBO) with Benzyl Azide



To further elucidate the rate-enhancing effects in Cu-free click chemistry, we attempted to synthesize other substituted monobenzocycloalkynes using the synthetic strategy developed for DIFBO.[28] Unfortunately, homologation with trimethylsilyl diazomethane did not prove to be a general strategy for cycloalkyne synthesis (see the Supporting Information for further details).

Conclusions

The expanding field of bioorthogonal chemistry has derived considerable inspiration from classic chemistries that were first

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(26) The rate for DIBO was determined in MeOD and is not directly comparable to the rates measured for DIFBO and DIFO. For a rate constant for DIBO that is consistent with other experimental results, see: Poloukhtine, A. A.; Mbua, N. E.; Wolfert, M. A.; Boons, G.-J.; Popik, V. V. *J. Am. Chem. Soc.* **2009**, *131*, 15769–1171. For a direct comparison of DIBO and DIFO, see ref 7f.

(27) MOBO (**16**) was synthesized previously via selenadiazole degradation. See: Meier, H.; Layer, M.; Zetzche, A. *Chem.-Ztg* **1974**, *98*, 460–461. We synthesized MOBO through syn elimination of an enol triflate (see Scheme S1).

reported as mechanistic curiosities. This work has expanded on that theme with the development of a new substituted cyclooctyne, DIFBO, whose reactivity profile provides insight into how 1,3-dipolar cycloaddition kinetics can be modulated. DIFBO is highly reactive with azides ($k = 0.22\ M^{-1}\ s^{-1}$) but also prone to decomposition via trimerization. X-ray crystallography revealed that the two trimer products were asymmetric diastereomers **9** and **10**, which are likely formed from a common cyclobutadiene intermediate, **15**.

To control the reactivity of DIFBO, we looked to another classic molecule used in host/guest chemistry: cyclodextrin. β-Cyclodextrin was most effective in complexing and stabilizing DIFBO, presumably because of its appropriate cavity dimensions, enabling the isolation of a stable inclusion complex that we characterized by CPMAS ^{13}C NMR spectroscopy. β-Cyclodextrin protected DIFBO from oligomerization and allowed it to be stored as a lyophilized powder. Interestingly, our attempts to form an inclusion complex of DIFBO and the larger γ-cyclodextrin promoted the formation of two dimer products (compounds **13** and **14**) in addition to trimer products **9** and **10**. The formation of these dimer products can also be rationalized on the basis of the proposed intermediate **15**. We suspect that DIFBO's reactivity is rooted in its propensity to form this putatively antiaromatic compound. Thus, in addition to enhancing our understanding of cyclooctyne−azide cycloaddition reactions, DIFBO offers a new tool for studying the classical chemical principle of (anti)aromaticity, which is still a topic of discussion today.[29] Additionally, the use of β-cyclodextrin to control the reactivity of DIFBO demonstrates a promising strategy for harnessing highly reactive small-molecule components in bioconjugation reactions, opening the door to new, rapid bioorthogonal chemistries.

Experimental Procedures

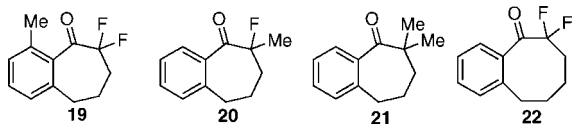
2,2-Difluoro-1-benzosuberone (6).[18] 1-Benzosuberone (**5**, 1.0 mL, 6.7 mmol, 1 equiv) was dissolved in cyclohexane (13.5 mL), after which hexylamine (1.2 mL, 9.1 mmol, 1.4 equiv) and trifluoroacetic acid (5 drops) were added. The reaction mixture was heated to reflux overnight with azeotropic removal of water (Dean−Stark trap). The following morning, the reaction mixture was evaporated to dryness, dissolved in Et_2O (25 mL), and washed with saturated $NaHCO_3$ (1 × 15 mL) and brine (1 × 15 mL). The organic solution was dried over $MgSO_4$ and evaporated to dryness. The resulting crude imine (1.8 g) was dissolved in CH_3CN (67 mL). To this solution were added Selectfluor (5.01 g, 14.2 mmol, 2.1 equiv) and Na_2SO_4 (680 mg, 4.9 mmol). The reaction mixture was heated to reflux overnight. The following morning, 3 M HCl (4.5 mL) was added to hydrolyze the imine. After 10 min at reflux, the solution was cooled to room temperature (rt) and evaporated to dryness. The residue was dissolved in Et_2O (25 mL) and then washed with saturated $NaHCO_3$ (1 × 15 mL) and brine (1 × 15 mL). The organic solution was dried over $MgSO_4$ and evaporated to dryness. The crude mixture was purified by flash chromatography with a hexane/EtOAc solvent system (30:1 to 15:1). This procedure yielded pure difluorobenzosuberone **6** as a clear oil (0.92 g, 4.7 mmol, 70%). $R_f = 0.4$ in 4:1 hexane/EtOAc. ^1H NMR (400 MHz, $CDCl_3$): δ 7.72 (dd, $J = 7.7$, 1.3 Hz, 1H), 7.49 (td, $J = 6.5$, 1.4 Hz, 1H), 7.36 (td, $J = 8.4$, 0.9 Hz, 1H), 7.27 (dd, $J = 7.7$, 0.6 Hz, 1H), 3.04−3.07 (m, 2H), 2.35−2.46 (m, 2H), 2.04 (apparent quin, $J = 6.4$ Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 194.2 (t, $J = 29$ Hz), 141.7, 134.8, 133.0, 130.3, 129.9, 126.9, 119.0 (t, $J = 250$ Hz), 34.4 (t, $J = 24$ Hz), 33.6, 22.0 (t, $J = 5$ Hz). ^{19}F NMR (376 MHz, $CDCl_3$): δ −99.3 (t, $J = 15$ Hz, 2F). HRMS (EI): calcd for $C_{11}H_{10}OF_2^+$ $[M]^+$, 196.0700; found, 196.0705.

7,7-Difluoro-5-(trimethylsilyl)-7,8,9,10-tetrahydrobenzo[8]annulen-6(5*H*)-one (7). Difluorobenzosuberone **6** (4.0 mmol, 1 equiv) was dissolved in CH_2Cl_2 (50 mL), and the solution was cooled to −78 °C, after which trimethylaluminum (2 M solution in toluene, 2.0 mL, 4.0 mmol, 1 equiv) was added. After 15 min, trimethylsilyl diazomethane (2 M solution in hexanes, 2.0 mL, 4.0 mmol, 1 equiv) was added, and 5 min later the reaction was quenched with saturated aqueous NH_4Cl and warmed to 0 °C. Rochelle's salt was added to complex the aluminum salts. This solution was stirred at rt for 15 min and then extracted with CH_2Cl_2 (3 × 75 mL). The organic layers were combined, dried with $MgSO_4$, decanted, and evaporated to dryness. This procedure afforded compound **7** with 33% toluene remaining (1.2 g, 3.9 mmol, 97%). $R_f = 0.85$ in 4:1 hexane/EtOAc. ^1H NMR (600 MHz, $CDCl_3$): δ 7.17−7.22 (m, 2H), 7.07 (dd, $J =$ 6.8, 2.0 Hz, 1H), 7.04 (dd, $J = 6.9$, 1.9 Hz, 1H), 3.67 (s, 1H), 2.75 (dt, $J = 14.7$, 4.6 Hz, 1H), 2.45−2.51 (m, 1H), 2.04−2.12 (m, 1H), 1.90−1.97 (m, 1H), 1.64−1.76 (m, 1H), 1.56−1.63 (m, 1H), 0.12 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 201.8 (t, $J = 27$ Hz), 137.7, 135.1, 130.4, 130.1, 126.9, 126.7, 119.2 (t, $J = 252$ Hz), 48.6, 33.1 (t, $J = 25$ Hz), 30.2, 23.6 (t, $J = 5$ Hz), −1.6. ^{19}F NMR (564 MHz, $CDCl_3$): δ −100.95 (apparent t, $J = 17$ Hz, 1F), −100.99 (dd, $J = 23$, 17 Hz, 1F). HRMS (EI): calcd for $C_{15}H_{20}OF_2Si^+$ $[M]^+$, 282.1253; found, 282.1255.

7,7-Difluoro-5-(trimethylsilyl)-7,8,9,10-tetrahydrobenzo[8]annulen-6-yl Triflate (8). A solution of compound **7** (8.5 mmol, 1 equiv) in THF (110 mL) was cooled to −78 °C, and potassium bis(trimethylsilyl)amide (0.5 M solution in toluene, 20.4 mL, 10.2 mmol, 1.2 equiv) was added, after which the solution turned a dark orange/brown color. After 1 h, triflic anydride (2.07 mL, 10.2 mmol, 1.2 equiv) was added, and the solution turned a lighter yellow color. The solution was stirred for 3 h, warming to roughly −45 °C, at which point it was quenched with MeOH and evaporated to dryness. (CAUTION: Use of a large excesss of Tf_2O or warming this reaction to room temperature before quenching results in the formation of a gel from which it is very difficult to separate compound **8**.) The crude product was purified via silica gel chromatography with a hexane/toluene solvent system (75:1, 50:1, 25:1). This procedure resulted in pure trimethylsilyl enol triflate **8** in 80% yield (2.8 g, 6.8 mmol). $R_f = 0.8$ in 6:1 hexane/EtOAc. ^1H NMR (400 MHz, $CDCl_3$): δ 7.24−7.29 (m, 2H), 7.16−7.19 (m, 1H), 7.03−7.07 (m, 1H), 2.77 (dt, $J = 13.1$, 4.4 Hz, 1H), 2.56 (td, $J = 5.3$, 1.3 Hz, 1H), 1.91−2.13 (m, 2H), 1.49−1.69 (m, 2H), 0.19 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 144.9 (t, $J = 28$ Hz), 142.7 (t, $J = 4$ Hz), 136.2 (t, $J = 3$ Hz), 135.8, 128.7, 128.4, 126.7, 126.5, 119.0 (q, $J = 321$ Hz), 118.3 (dd, $J = 246$, 241 Hz), 32.6 (t, $J = 25$ Hz), 30.2, 23.9 (dd, $J = 7$, 3 Hz), −0.62. ^{19}F NMR (376 MHz, $CDCl_3$): δ −70.1 (t, $J = 13$ Hz, 3F), −84.5 (dsex, $J = 277$, 11 Hz, 1F), −92.0 (dm, $J = 278$ Hz, 1F). HRMS (EI): calcd for $C_{16}H_{19}O_3F_5SiS^+$ $[M]^+$, 414.0744; found, 414.0642.

Difluorobenzocyclooctyne (DIFBO, 4). Trimethylsilyl enol triflate **8** (34 mg, 0.082 mmol, 1 equiv) was dissolved in CD_3CN (3 mL), and CsF (75 mg, 0.50 mmol, 6 equiv) was added. The reaction was stirred at rt for ∼30 min (the reaction mixture turned yellow and had a foul odor), at which point it was transferred directly onto a plug of silica gel and eluted with CD_3CN (0.75 mL). A portion of this solution was taken for NMR analysis. $R_f = 0.75$ in 4:1 hexane/EtOAc. ^1H NMR (500 MHz, CD_3CN): δ 7.38 (t, J = 8.1 Hz, 1H), 7.33 (t, $J = 7.3$ Hz, 1H), 7.26−7.31 (m, 2H), 2.97

(28) The ketones shown below were synthesized, but their conversion to cyclooctynes was prevented because they did not undergo homologation with $TMSCHN_2$ (see the Supporting Information for further details).



(29) Bally, T. *Angew. Chem., Int. Ed.* **2006**, *45*, 6616–6619.

(apparent t, $J = 5.0$ Hz, 2H), 2.57−2.65 (m, 2H), 1.89 (bs, 2H). **4** was too unstable for ^{13}C NMR analysis. ^{19}F NMR (564 MHz, CD_3CN): δ −87.5 (bs, 2F). HRMS (EI): calcd for $C_{12}H_{10}F_2^+$ $[M]^+$, 192.0751; found, 192.0754.

Cycloadducts of Benzyl Azide and DIFBO (11 and 12). Compound **8** (202 mg, 0.49 mmol, 1 equiv) and CsF (440 mg, 2.9 mmol, 5.9 equiv) were combined in CH_3CN (10 mL). After 15 min, BnN_3 (66 μL, 0.53 mmol, 1.1 equiv) was added. This mixture was allowed to stir overnight at rt. The following morning, the reaction mixture was evaporated to dryness and purified by silica gel chromatography (hexane/EtOAC system), affording two triazole products, **11** (55 mg, 0.17 mmol, 35%) and **12** (92 mg, 0.28 mmol, 58%). Compound **11**: $R_f = 0.2$ in 6:1 hexane/EtOAc. ^1H NMR (600 MHz, CD_3CN): δ 7.48 (td, $J = 7.5$, 1.5 Hz, 1H), 7.44 (dd, $J = 7.7$, 1.3 Hz, 1H), 7.39 (apparent td, $J = 7.5$, 1.3 Hz, 1H), 7.30 (d, $J = 7.6$ Hz, 1H), 7.20−7.22 (m, 3H), 6.88−6.90 (m, 2H), 5.56 (apparent d, $J = 3.1$ Hz, 2H), 2.57 (apparent dd, $J = 13.4$, 6.2 Hz, 1H), 2.06−2.19 (m, 3H), 1.73−1.85 (m, 1H), 1.61−1.67 (m, 1H). ^{13}C NMR (150 MHz, CD_3CN): δ 143.1 (t, $J = 30$ Hz), 141.1, 136.6, 135.3 (t, $J = 6$ Hz), 132.0, 130.5, 130.0, 129.7, 129.1, 128.2, 127.9, 126.6, 119.1 (dd, $J = 235$, 231 Hz), 53.0, 31.9 (t, $J = 24$ Hz), 30.6, 25.9 (d, $J = 9$ Hz). ^{19}F NMR (564 MHz, CD_3CN): δ −91.8 (ddd, $J = 271$, 34, 6 Hz, 2F). HRMS (ESI): calcd for $C_{19}H_{18}N_3F_2^+$ $[M + H]^+$, 326.1463; found, 326.1461. Compound **12**: $R_f = 0.6$ in 6:1 hexanes/EtOAc. ^1H NMR (600 MHz, CD_3CN): δ 7.72 (dd, $J = 7.6$, 1.3 Hz, 1H), 7.45 (td, $J = 7.5$, 1.5 Hz, 1H), 7.36−7.41 (m, 3H), 7.32−7.35 (m, 4H), 5.80 (s, 2H), 2.50−2.68 (bm, 2H), 1.62−2.21 (bm, 4H). ^{13}C NMR (150 MHz, CD_3CN): δ 145.8 (t, $J = 6$ Hz), 139.5, 137.0, 131.5, 130.8, 130.7 (t, $J = 131$ Hz), 130.4, 130.2, 129.7, 129.2, 128.7, 127.8, 119.2 (t, $J = 235$ Hz), 54.5 (t, $J = 3$ Hz), 32.4 (t, $J = 24$ Hz), 30.8, 26.5 (t, $J = 5$ Hz). ^{19}F NMR (564 MHz, CD_3CN): δ −74.1 (d, $J = 270$ Hz, 1F), −89.8 (d, $J = 265$ Hz, 1F). HRMS (ESI): calcd for $C_{19}H_{18}N_3F_2^+$ $[M + H]^+$, 326.1463; found, 326.1460.

Generation of the DIFBO−β-Cyclodextrin Complex. DIFBO precursor **8** (256 mg, 0.618 mmol, 1 equiv) was dissolved in CH_3CN (26 mL), and CsF (573 mg, 3.77 mmol, 6.1 equiv) was added. This mixture was allowed to stir at rt until all of the **8** was converted into DIFBO (∼25 min; the reaction mixture turned slightly yellow in color and had a foul odor). This solution was directly loaded onto silica gel and eluted with hexane. The fractions containing DIFBO were combined with ∼75 mL of acetonitrile, and the hexane was removed by careful rotary evaporation at rt. The remaining DIFBO/acetonitrile solution was added to a solution of β-cyclodextrin (recrystallized, 702 mg, 0.618 mmol, 1 equiv, in 50 mL of deionized H_2O). The solution turned cloudy. The acetonitrile was removed by rotary evaporation, and the resulting aqueous solution was flash-frozen in liquid N_2 and lyophilized to a white powder (688 mg, 0.518 mmol, 84%).

Analysis of DIFBO−Cyclodextrin Complexes (Figure 3). DIFBO was generated, purified, and transferred into acetonitrile as described above. The resulting DIFBO/acetonitrile solution was divided into six portions (A−F) that were treated in different manners. Portion B was evaporated to dryness and placed on the lyophilizer. Portion C was combined with H_2O (4 mL), after which the acetonitrile was removed by rotary evaporation and the resulting aqueous solution flash-frozen and lyophilized. Portion D was combined with 1 equiv of glucose in H_2O (4 mL) and treated in the same manner as portion C. Portions A, E, and F were treated in the same manner as portion C except that the glucose was replaced by β-cyclodextrin, α-cyclodextrin, and γ-cyclodextrin respectively.

For HPLC analysis of the resulting products, 0.6 μmol of each powder was dissolved in 1:1 CH_3CN/H_2O (1 mL). To this solution was added 1 equiv of BnN_3 (15 μL of 0.04 mM solution in 1:1 CH_3CN/H_2O), and the mixture was allowed to react overnight. The following morning, 150 nmol (250 μL) was analyzed by reversed-phase HPLC (C-18 column) using a gradient of 50% CH_3CN/50% H_2O to 100% CH_3CN over 25 min followed by isocratic elution with 100% CH_3CN for 10 min before re-equilibration to 50% CH_3CN/50% H_2O. The chromatography was monitored by absorbance at 210 and 254 nm.

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Supporting Information Available: Figures S1−S15; Scheme S1; characterization data and CIF files for oligomer products **9**, **10**, **13**, and **14**; general experimental procedures; CPMAS NMR procedure; synthesis of MOBO (**16**) and ketones **19**−**22**; determination of the second-order rate constants for DIFBO and MOBO; NMR spectra for all reported compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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