JOC The Journal of Organic Chemistry

Note

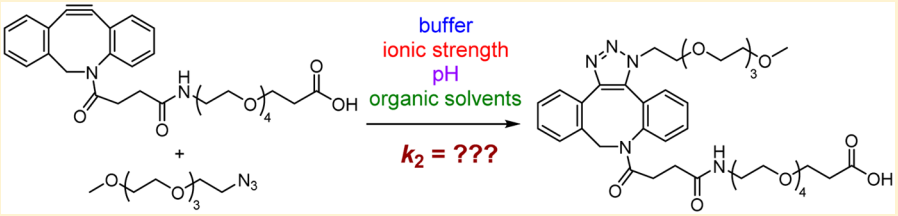
pubs.acs.org/joc

Effect of Buffer Conditions and Organic Cosolvents on the Rate of Strain-Promoted Azide–Alkyne Cycloaddition

Derek L. Davis,[‡] Erin K. Price,[‡] Sabrina O. Aderibigbe, Maureen X.-H. Larkin, Emmett D. Barlow, Renjie Chen, Lincoln C. Ford, Zackery T. Gray, Stephen H. Gren, Yuwei Jin, Keith S. Keddington, Alexandra D. Kent, Dasom Kim, Ashley Lewis, Rami S. Marrouche, Mark K. O'Dair, Daniel R. Powell, Mick'l H. C. Scadden, Curtis B. Session, Jifei Tao, Janelle Trieu, Kristen N. Whiteford, Zheng Yuan, Goyeun Yun, Judy Zhu, and Jennifer M. Heemstra*

Department of Chemistry, University of Utah, Salt Lake City, Utah 84112, United States

Supporting Information



ABSTRACT: We investigate the effect of buffer identity, ionic strength, pH, and organic cosolvents on the rate of strain-promoted azide–alkyne cycloaddition with the widely used DIBAC cyclooctyne. The rate of reaction between DIBAC and a hydrophilic azide is highly tolerant to changes in buffer conditions but is impacted by organic cosolvents. Thus, bioconjugation reactions using DIBAC can be carried out in the buffer that is most compatible with the biomolecules being labeled, but the use of organic cosolvents should be carefully considered.

Strain-promoted azide–alkyne cycloaddition (SPAAC) has emerged as a powerful tool for the modification of biomolecules in a variety of contexts, as an azide and cyclooctyne will react to form a linked triazole product, but this reaction is orthogonal to the other functional groups found in biomolecules and biological systems.[1−3] A number of cyclooctyne reagents have been reported, and aza-dibenzobicyclooctyne (DIBAC)[4] is arguably the most commonly used, as it has one of the highest reported rate constants and can be synthesized in a straightforward manner, and a number of DIBAC-functionalized fluorophores and chemical probes are commercially available.[5] Despite the wide use of DIBAC reagents, the kinetics of the reaction between DIBAC and organic azides has only been sparsely studied. The initial report of the DIBAC cyclooctyne reagent from van Delft and coworkers provided a rate constant for reaction with benzyl azide in methanol,[4] and this laboratory has recently reported data for five additional azide reagents and two different organic/aqueous solvent mixtures.[6] Taking a different approach, the ability of micelles to catalyze SPAAC has been demonstrated for DIBAC and the closely related DIBO cyclooctyne reagents.[7,8] However, DIBAC conjugation reactions are often carried out in aqueous buffers, as these are the most compatible conditions for biomolecules, yet there are no reports exploring the effect of buffer identity, ionic strength, or pH on reaction kinetics. Here, we report a systematic investigation into the effect of these parameters on reaction rate and further explore the effect of organic cosolvents. Using a water-soluble azide, we find that the kinetics of DIBAC reactions are highly tolerant to changes in buffer conditions but are impacted by organic cosolvents. Together, these data serve to guide the choice of solvent and conditions for researchers seeking to use SPAAC for labeling of proteins, nucleic acids, and other water-soluble molecules.

To survey the effect of buffer conditions and cosolvents on SPAAC, we utilized commercially available DIBAC-PEG_4-CO_2H (**1**, Figure 1), which has a short PEG chain that provides water solubility and mimics the linker used on many DIBAC-labeled fluorophores and probes. While studies of SPAAC kinetics are typically carried out using benzyl azide, we instead chose to employ PEG_3-azide **2** (Figure 1), which is water-soluble and better mimics the aliphatic azide structures that are frequently employed for labeling of proteins, carbohydrates, or nucleic acids.[9−11] While many kinetic studies of SPAAC have been carried out using NMR, this method is not ideal for reactions with DIBAC, as the rate constants for these reactions are sufficiently high that at the mM concentrations required for NMR the reactions often go to completion more quickly than a sample can be inserted into an NMR and properly shimmed.[6] However, UV does provide a convenient method for measurement of rate constants, as DIBAC undergoes a decrease in absorbance at 309 nm upon

Received: May 11, 2016
Published: July 7, 2016

DOI: 10.1021/acs.joc.6b01112
J. Org. Chem. 2016, 81, 6816–6819

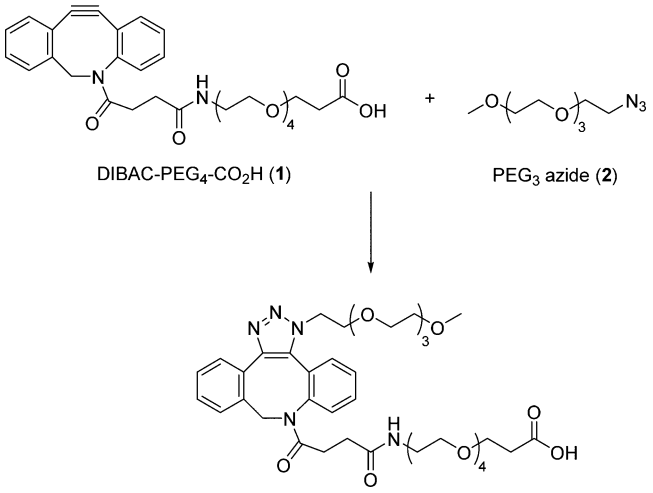


Figure 1. DIBAC cyclooctyne **1** and PEG_3-azide **2** react to provide a ligated triazole product.[4,7]

reaction with an azide to produce the triazole product.[7] Additionally, these reactions can be carried out at micromolar concentrations, which both conserves material and provides a longer window of time in which data can be collected before full conversion occurs.

To obtain second-order rate constants, we carried out all reactions under pseudo-first-order conditions using an excess of the azide. We monitored the absorbance at 309 nm and fit these data to the integrated rate law to provide k_{obs}, which in turn provides the second-order rate constant (k_2) using eq 1:

$$k_2 = \frac{k_{obs}}{[\text{azide}]} \quad (1)$$

For each reaction, we calculated k_{obs} and k_2 using at least three different concentrations of azide. We observed that the values of k_2 were independent of azide concentration over the range of concentrations tested, validating that the reactions are operating under second-order kinetics (see the Supporting Information).

We were first curious to survey the effect of buffer identity and ionic strength on reaction rate. Thus, we measured rate constants for the reaction of **1** + **2** in a wide variety of buffers including sodium phosphate (NaPi), tris(hydroxymethyl)-aminomethane (Tris), Tris/borate/EDTA (TBE), 2-(*N*-morpholino)ethanesulfonic acid (MES), 3-(*N*-morpholino)-propanesulfonic acid (MOPS), and 4-(2-hydroxyethyl)-piperazine-1-ethanesulfonic acid (HEPES), which together represent the buffers most commonly used with proteins and nucleic acids.[12] For each buffer, we measured k_2 in a solution having 100 mM of the buffer salt and either 0, 150, or 500 mM added NaCl (Table 1). The pH values for these buffers were all in the neutral range (pH 6.5−8.0) and were chosen on the basis of the mostly commonly used pH value for each buffer.

We initially hypothesized that increasing ionic strength might impact reaction rate, as the reactive portions of the DIBAC cyclooctyne and azide are relatively hydrophobic. Thus, we were surprised to observe nearly uniform rate constants of approximately 1 M^{-1} s^{-1} across all of the neutral pH buffers and ionic strengths. Interestingly, when we explored the use of 100 mM sodium acetate buffers having acidic pH values, we did observe a slight decrease in rate constant, as we obtained k_2 values of 0.748, 0.856, and 0.697 M^{-1} s^{-1} for buffers having pH 3.5, 4.5, and 5.5, respectively. However, these data together demonstrate that reaction of DIBAC with a water-soluble azide is highly tolerant to changes in buffer identity and ionic strength, which provides significant flexibility for bioconjugation reactions. Additionally, the rate constants observed in aqueous buffer solutions are 3- to 4-fold higher than the value of 0.33 M^{-1} s^{-1} reported using similar DIBAC and azide reactants in CD_3OD.[6] The lack of effect from buffer identity or ionic strength may be attributable to the fact that the cyclooctyne and azide are each functionalized with hydrophilic PEG chains, which we hypothesize significantly reduces the magnitude of hydrophobic effects that might be observed with less polar reagents such as benzyl azide.

Table 1. Effect of Buffer Identity and Ionic Strength on Rate Constant for Reaction of 1 + 2[a]

	second-order rate constant (k_2, M^{-1} s^{-1})		
	0 mM NaCl	150 mM NaCl	500 mM NaCl
100 mM NaPi, pH 7.4	1.14 ± 0.19	0.967 ± 0.104	1.10 ± 0.06
100 mM Tris, pH 8.0	0.964 ± 0.092	1.03 ± 0.06	1.07 ± 0.10
100 mM TBE, pH 7.4	1.11 ± 0.11	1.18 ± 0.14	1.29 ± 0.03
100 mM MES, pH 6.5	0.974 ± 0.031	0.981 ± 0.046	1.02 ± 0.05
100 mM MOPS, pH 7.5	1.11 ± 0.10	1.38 ± 0.16	1.26 ± 0.03
100 mM HEPES, pH 7.5	1.18 ± 0.25	1.13 ± 0.06	1.28 ± 0.13

[a]Errors represent the standard deviation of three to four independent trials.

We next turned our attention to the effect of organic cosolvents, which may be utilized in SPAAC reactions to help solubilize more hydrophobic reagents such as fluorophores. Surveying the reaction of DIBAC with a variety of azides, van Delft and co-workers have demonstrated that use of 3:1 D_2O/CD_3CN as the solvent increases reaction rate relative to CD_3OD but use of 1:9 H_2O/THF decreases reaction rate.[6] Similarly, the ability of organic−aqueous mixtures to modulate reaction rate has been observed for bicyclononyne (BCN),[13] dibenzocyclooctyne (DIBO),[14] and oxa-dibenzocyclooctyne (ODIBO)[15] cyclooctynes.

To systematically explore the impact of organic cosolvents in SPAAC, we measured the k_2 values for reaction of **1** + **2** in aqueous solvent mixtures having either 10, 40, or 70 vol % of dimethyl sulfoxide (DMSO), acetonitrile (CH_3CN), *N*-methyl-2-pyrrolidone (NMP), methanol (MeOH), or ethanol (EtOH). As shown in Table 2, we generally observe lower rate constants at 70% organic cosolvent compared to 10% or 40% organic cosolvent. Interestingly, addition of MeOH or CH_3CN results in a steady decline in reaction rate as the volume % of solvent is

Table 2. Effect of Organic Co-solvents on Rate Constant for Reaction of 1 + 2[a]

	second-order rate constant (k_2, M^{-1} s^{-1})		
	10% solvent	40% solvent	70% solvent
DMSO	1.29 ± 0.02	1.56 ± 0.01	1.31 ± 0.21
CH_3CN	1.10 ± 0.11	0.813 ± 0.072	0.470 ± 0.076
NMP	1.11 ± 0.12	1.18 ± 0.16	0.704 ± 0.148
MeOH	1.38 ± 0.10	1.06 ± 0.26	0.853 ± 0.217
EtOH	1.13 ± 0.04	1.55 ± 0.18	0.811 ± 0.072

[a]Errors represent the standard deviation of three to four independent trials. Percentages of solvent represent vol %.

DOI: 10.1021/acs.joc.6b01112
J. Org. Chem. 2016, 81, 6816−6819

increased, but the remaining solvents show the highest rate constants at the intermediate solvent composition of 40 vol %. In the cases of CH_3CN and NMP, addition of the organic cosolvent has an overall negligible or negative impact on reaction rate compared to the k_2 value of 1.18 M^{-1} s^{-1} measured for the same reactants in water.[7] However, in the cases of EtOH and DMSO, the addition of 40% organic cosolvent slightly increases the reaction rate to 1.55 and 1.56 M^{-1} s^{-1}, respectively. We were most intrigued by the reaction kinetics in DMSO, as all of the cosolvent ratios tested provided a reaction rate comparable to or higher than that measured in water. Thus, we surveyed additional cosolvent ratios and, as shown in Figure 2, found that rate constants as high as 1.8 M^{-1} s^{-1} could be achieved using either 50% or 60% DMSO. These results are in agreement with the observations outlined above from other laboratories,[6] as small to moderate quantities of organic cosolvent can increase reaction rate but large quantities of organic cosolvent are generally deleterious to the reaction. The slower reaction rates at high organic cosolvent ratios can be rationalized by the loss of hydrogen bonding interactions from water, which have been reported to accelerate 1,3-dipolar cycloaddition reactions.[16] However, the higher reaction rates at low to moderate cosolvent ratios, especially in the case of DMSO, are more challenging to explain. One possibility is that certain organic cosolvents may promote reaction between **1** and **2** by increasing the solubility of the DIBAC reagent, and at low to moderate ratios, this outweighs the loss of beneficial hydrogen bonding interactions with water. We note that while the changes in reaction kinetics are in some cases minor, the fact that we observe a nearly 4-fold variation in rate between the slowest and fastest reactions indicates that the use of organic cosolvents can significantly impact SPAAC, and use of these solvents should be carefully considered when reaction kinetics are of import.

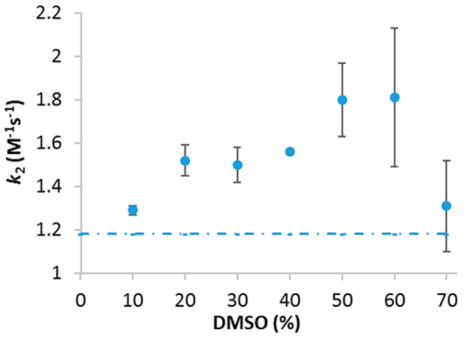


Figure 2. Effect of DMSO cosolvent percentage on rate constant for **1** + **2**. Error bars represent the standard deviation of three to four independent trials. Dashed line represents the rate constant in water. Percentages of solvent represent vol %.

Here, we report a systematic investigation into the effect of buffer identity, ionic strength, and organic cosolvents on the kinetics of SPAAC between DIBAC cyclooctyne and a water-soluble azide. We find that the reaction rate is highly tolerant to changes in buffer conditions, though a slight decrease in reactivity is observed at acidic pH values. Interesting, we find that the addition of organic cosolvents can significantly impact reaction rate, with some solvents slowing the reaction and others providing acceleration. van Delft and co-workers have found that the trends in rate constant observed when using CD_3CN and THF cosolvents are similar for both DIBAC and BCN cyclooctynes. Thus, we hypothesize that our observations regarding the effect of buffer identity, ionic strength, and organic cosolvents will likely translate to additional cyclooctyne reagents. Together, these results represent the first thorough investigation of SPAAC kinetics under the aqueous conditions typically used for bioconjugation reactions, and provide guidance to researchers utilizing SPAAC for labeling of biomolecules such as proteins and nucleic acids.

EXPERIMENTAL SECTION

General Procedures. All chemicals were purchased from commercial sources and used without further purification. UV measurements were acquired at room temperature (20−25 °C) using a quartz cuvette having a 1 cm path length.

Kinetics Measurement. In all experiments, the concentration of DIBAC **1** was 50 μM, and the concentration of azide was varied from 0.25 to 2.0 mM. Using a large excess of azide enabled calculation of reaction rates using the pseudo-first-order assumption. For each reaction, a mixture was prepared containing **1** and the appropriate buffer or organic−aqueous solvent, and this was transferred to a quartz cuvette. The appropriate volume of azide **2** as a 10 mM solution in water was then added. The cuvette was capped, and the solution mixed by inversion to start the reaction. The delay time between addition of the azide and the start of data acquisition was noted. The absorbance at 309 nm was recorded at intervals of 3−15 s for a total time of 10 min. For reactions that proceeded to completion within <20 min, the absorbance at 309 nm was monitored until no change was observed, and this value was used as the final absorbance. For reactions that did not proceed to completion within this time, one trial of the reaction was monitored for 1−2 h to ensure completion, and the final A_{390} value was used for all other trials using that buffer or solvent mixture.

For each buffer or solvent mixture, three to four independent trials were carried out using varying concentrations of azide **2**. Comparison of rate constants obtained using different azide concentrations enabled validation that the reactions were functioning under second-order kinetics. For each reaction, the first 4−6 data points were extrapolated over the recorded delay time to provide the initial absorbance (A_o). The final absorbance (A_f) was obtained as described above. Using the absorbance data over the course of the reaction (A), the fraction of starting material remaining was calculated according to eq 2

$$\frac{[\mathrm{DIBAC}]}{[\mathrm{DIBAC}]_o} = \frac{A - A_f}{A_o - A_f} \quad (2)$$

in which $[\mathrm{DIBAC}]_o$ is the initial concentration of DIBAC and [DIBAC] is the concentration remaining at a given time point. In accordance with the integrated first-order rate law, we plotted $\ln([\mathrm{DIBAC}]_o/[\mathrm{DIBAC}])$ vs time and obtained k_{obs} as the slope of this plot. For reactions that proceeded to more than 50% completion in 10 min, we only utilized data up to the point of 50% conversion.[7] Finally, we converted k_{obs} into k_2 using eq 1. For each of the buffers or solvent mixtures, the k_2 values from the three to four independent trials were averaged to provide the values reported in Tables 1 and 2. Reported error values represent the standard deviation.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b01112.

Comprehensive kinetics data for all buffers and solvent mixtures (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: heemstra@chem.utah.edu.

Author Contributions

‡D.L.D. and E.K.P. contributed equally.

DOI: 10.1021/acs.joc.6b01112
J. Org. Chem. 2016, 81, 6816−6819

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Victoria Russell, Sven Miller, Tsz Ho Tsang, and Rameshu Rallabandi for helpful discussions and Agnes Szarzec-Larsen for assistance with lab supplies and instrumentation. This work was supported by the Research Corporation for Science Advancement (Cottrell Scholar Award to J.M.H.), the National Science Foundation (CBET 1552961 to J.M.H), and the University of Utah.

■ REFERENCES

(1) Sletten, E. M.; Bertozzi, C. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 6974.
(2) Sletten, E. M.; Bertozzi, C. R. *Acc. Chem. Res.* **2011**, *44*, 666.
(3) Debets, M. F.; van Berkel, S. S.; Dommerholt, J.; Dirks, A. J.; Rutjes, F. P. J. T.; van Delft, F. L. *Acc. Chem. Res.* **2011**, *44*, 805.
(4) Debets, M. F.; van Berkel, S. S.; Schoffelen, S.; Rutjes, F. P. J. T.; van Hest, J. C. M.; van Delft, F. L. *Chem. Commun.* **2010**, *46*, 97.
(5) Commercially available analogues of DIBAC are named using the acronym DBCO.
(6) Dommerholt, J.; Rutjes, F. P. J. T.; van Delft, F. L. *Top.Curr. Chem.* **2016**, DOI: 10.1007/s41061-016-0016-4.
(7) Anderton, G. I.; Bangerter, A. S.; Davis, T. C.; Feng, Z.; Furtak, A. J.; Larsen, J. O.; Scroggin, T. L.; Heemstra, J. M. *Bioconjugate Chem.* **2015**, *26*, 1687.
(8) Tian, H.; Sakmar, T. P.; Huber, T. *ChemBioChem* **2015**, *16*, 1314.
(9) Link, A. J.; Vink, M. K. S.; Agard, N. J.; Prescher, J. A.; Bertozzi, C. R.; Tirrell, D. A. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 10180.
(10) Chang, P. V.; Prescher, J. A.; Sletten, E. M.; Baskin, J. M.; Miller, I. A.; Agard, N. J.; Lo, A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 1821.
(11) Sharma, A. K.; Heemstra, J. M. *J. Am. Chem. Soc.* **2011**, *133*, 12426.
(12) Rédei, G. P. *Encyclopedia of Genetics, Genomics, Proteomics, and Informatics*, 3rd ed.; Springer, 2008.
(13) Dommerholt, J.; Schmidt, S.; Temming, R.; Hendriks, L. J.; Rutjes, F. P.; van Hest, J. C.; Lefeber, D. J.; Friedl, P.; van Delft, F. L. *Angew. Chem., Int. Ed.* **2010**, *49*, 9422.
(14) Ning, X.; Guo, J.; Wolfert, M. A.; Boons, G. J. *Angew. Chem., Int. Ed.* **2008**, *47*, 2253.
(15) McNitt, C. D.; Popik, V. V. *Org. Biomol. Chem.* **2012**, *10*, 8200.
(16) Butler, R. N.; Cunningham, W. J.; Coyne, A. G.; Burke, L. A. *J. Am. Chem. Soc.* **2004**, *126*, 11923.

DOI: 10.1021/acs.joc.6b01112
J. Org. Chem. 2016, 81, 6816−6819