Strained Molecules

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Heteroatom-embedded Medium-Sized Cycloalkynes: Concise Synthesis, Structural Analysis, and Reactions**

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Dedicated to Professor Keisuke Suzuki on the occasion of his 60th birthday

Abstract: *A variety of medium-sized cycloalkynes were efficiently synthesized by the double Nicholas reaction of cobalt complex and bis(hetero)substituted acyclic compound. The alkyne moiety within the ring has a unique bent structure and high reactivity toward cycloaddition reactions. Furthermore, preparation of multifunctionalized alkynes was achieved by embedding the cycloalkyne within a peptide chain.*

Alkyne is one of the most important functional groups in organic chemistry owing to its versatile synthetic utility. The reactivity of an alkyne heavily depends on the electronic and steric characteristics of the substituents.[1] It is also well known that the reactivity of an alkyne is influenced by structural strain. Indeed, bent medium-sized cycloalkynes such as cyclooctyne or cyclononyne show remarkably high reactivity in comparison with nonbent acyclic alkynes in a variety of addition reactions.[2] In the last decade, highly reactive medium-sized cycloalkynes have attracted considerable attention as smart ligation molecules in the field of chemical biology.[3] In 2004, Bertozzi and colleagues reported the pioneering work on the catalyst-free Huisgen reaction of cyclooctyne derivatives with azides and successfully utilized this reaction in a bioimaging technique based on the bioorthogonality.[3a] Thus, a variety of medium-sized bent cycloalkynes have since been synthesized. However, some of these are not applicable to chemical biological experiments because of their instability.[4,5] Hence, imparting a suitable reactivity to alkynes is an important issue not only for structural organic chemistry and reaction chemistry, but also for chemical biology.

To this end, we planned to synthesize the medium-sized cycloalkyne **1** with two heteroatom functionalities (X and Y) on the endocyclic propargylic positions (Figure 1). We expected the reactivity of the alkyne moiety to be adjustable by varying the ring size and heteroatom functionalities.[6,7] Furthermore, the heteroatom functionalities can be used as connection points for a variety of functional units (FU), such as tag molecules and probe molecules. The resulting multifunctionalized cycloalkynes are valuable as novel smart ligation molecules.

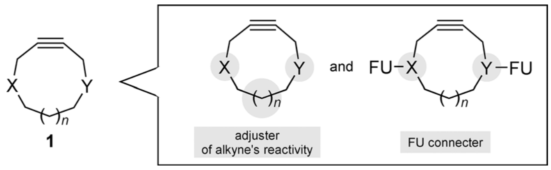
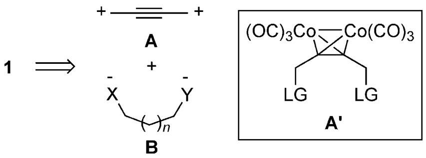


Figure 1. Heteroatom-embedded medium-sized cycloalkyne (**1**).

To realize the synthesis of **1**, an efficient approach which overcomes the inherent entropic disadvantages of cyclization is needed to construct the strained ring system. A double Nicholas reaction of the dicationic 2-butyne unit **A**, derived from the alkyne/cobalt complex **A′**, and the bis(hetero)substituted acyclic unit **B** would be the most straightforward approach to **1** [Eq. (1)],[8–10] even though related reports could not obtain satisfactory results for medium-sized cycloalkyne synthesis because of the difficulty in removal of the cobalt moiety.[11–13] Based on this idea, we investigated and found that the introduction of a sulfonamide moiety (X = NSO_2R) to **B** as a nucleophilic heteroatom functionality is the key to success. Herein, we report the details of the synthesis, structural analysis, and reactions of **1**.



(1)

At the outset, we examined a $BF_3{\cdot}OEt_2$-promoted double Nicholas reaction of either the alkyne/cobalt complex **2a** or **2b** (**2a**: LG = OH, **2b**: LG = OMe; LG = leaving group) with the 1,3-diol **3a** (X, Y = O) and 1,3-bis(benzamide) **3b** (X, Y = NBz; Bz = benzoyl) [Eq. (2)]. However, the reactions afforded only complex mixtures including oligomers of **2**. In sharp contrast, similar reactions with the 1,3-bis(tosylamide) **3c** (X, Y = NTs; Ts = *p*-toluenesulfonyl) and either **2a** or **2b** provided the desired nine-membered **4c** (X, Y = NTs) in excellent yields (for **2a**: 94 %, for **2b**: 97 %).[14] The removal

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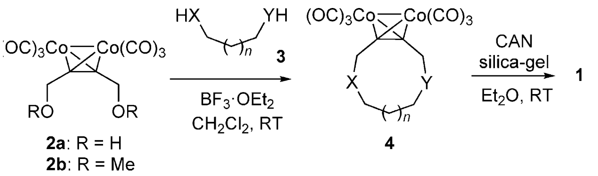
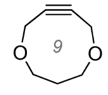
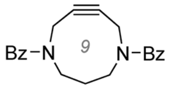
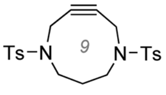
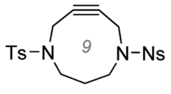
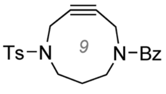
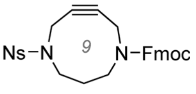
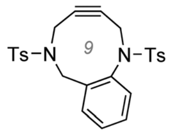
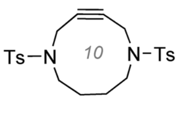
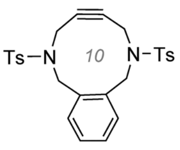
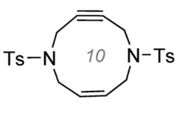
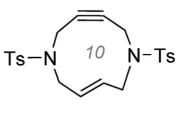
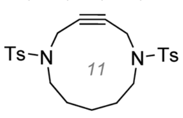
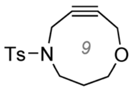
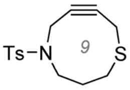
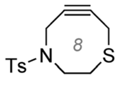


(2)

of the cobalt moiety from **4c** required modified reaction conditions to avoid side reactions owing to the high reactivity of the medium-sized ring structures of the cobalt complex **4c** and eliminated the cycloalkyne **1c**.[15] After several attempts, we found that treatment with ceric ammonium nitrate (CAN) in the presence of a good deal of silica gel efficiently afforded **1c** in excellent yield (87%).[16] The reasonable chemical stability of **1c** allowed concentration and purification with silica gel column chromatography to be performed without special care.[17]

This simple two-step approach is applicable to a variety of cycloalkynes (Table 1), including nine-membered cycloalkynes with two different nitrogen functionalities, that is, **1d** (X = NTs, Y = NNs), **1e** (X = NTs, Y = NBz), **1f** (X = NNs, Y = NFmoc), and the aniline ring-containing **1g**. The bis(tosylamide)-containing ten-membered alkyne **1h**, the benzene-ring- and alkene-containing ten-membered alkynes **1i** and **1j**, respectively, as well as the eleven-membered cycloalkyne **1k** were also afforded in excellent yields. Furthermore, ether and thioether congeners (**1l–n**) were successfully synthesized with similar reactions.

Table 1: Scope and limitations of the double Nicholas approach.

 **1a**	 **1b**	 **1c** (97%)[a], (87%)[b]
 **1d** (91%)[a], (quant)[b]	 **1e** (98%)[a], (87%)[b]	 **1f** (67%)[a], (65%)[b]
 **1g** (94%)[a], (67%)[b]	 **1h** (82%)[c], (94%)[b]	 **1i** (75%)[c], (99%)[b]
 (*Z*)-**1j** (86%)[c], (92%)[b]	 (*E*)-**1j** (quant)[c], (96%)[b]	 **1k** (74%)[a], (91%)[b]
 **1l** (90%)[c], (64%)[b]	 **1m** (79%)[c], (85%)[b]	 **1n** (76%)[c], (35%)[b]

[a] Yield of isolated **4** obtained by the double Nicholas reaction using **2b**. [b] Yield of the isolated **1** obtained by removal of the cobalt moiety. [c] Yield of the isolated **4** obtained by the double Nicholas reaction using **2a**. Ns = 2-nitrobenzenesulfonyl. Fmoc = 9-fluorenylmethyloxycarbonyl.

Next, we turned our attention to the structural analysis and reaction of representative cycloalkynes. Fortunately, the cycloalkynes **1c**, **1l**, **1m**, and **1n** afforded good single crystals suitable for the elucidation of their detailed structural features in the solid state by X-ray crystallographic analysis (Figure 2).[18] The bond angles of the sp-hybridized carbon atoms of **1c** [∠C(1)C(2)C(3) and ∠C(2)C(3)C(4)] are bent 18–19° from 180°, and the sum of the distortional angles (SDA) in **1c** is 37°. In **1l**, the ether side [∠C(2)C(3)C(4)] is more bent (21°) and the SDA is 40°. In contrast, the bending angles of **1m** [16° for ∠C(1)C(2)C(3), and 13° for ∠C(2)C(3)C(4); SDA is 29°] are substantially smaller than those of **1c** and **1l**. These significant differences in bond angles for the sp-hybridized carbon atoms of **1c**, **1l**, and **1m**, which depend on the embedded heteroatoms, are probably due to the synergetic effect of the C−Y bond length and electronic interaction with the sp-hybridized carbon atoms. In terms of the C−Y bond length, the order of the SDA for the sp-hybridized carbon atoms is consistent with the inverse order of the length of C−Y: **1l** [1.49 Å for N(1)-C(1) and N(1)-C(7), and 1.43–1.44 Å for O(1)-C(4) and O(1)-C(5)] < **1c** [1.49 Å for N(1)-C(1), N(1)-C(7), N(2)-C(4), and N(2)-C(5)] < **1m** [1.83 Å for S(1)-C(4) and S(1)-C(5), and 1.49–1.50 Å for N(1)-C(1) and N(1)-C(7)], which shows that a shorter C−Y bond causes a more bent structure of **1**.

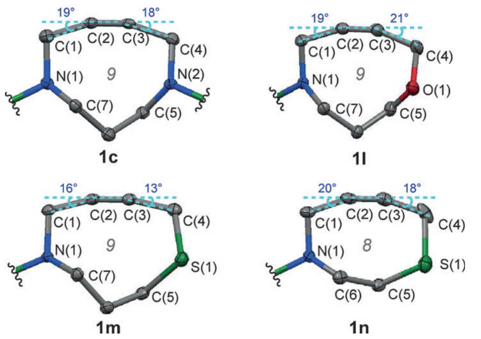


Figure 2. X-ray crystallographic analysis of the selected cycloalkynes **1**. Thermal ellipsoids shown at 50% probability.

In contrast, natural bond orbital (NBO) analysis of the optimized structures of **1c**, **1l**, and **1m** computed at the B3LYP/6-311G(d,p) level of theory showed the substantially high interaction energy in **1l** of $\sigma^*_{\text{C-O}}$ with $\pi_{\text{C}\equiv\text{C}}$ (interaction energy of the C−Y bonds; C−O of **1l**: 5.8 kcal mol^{-1}, C−S of **1m**: 5.1 kcal mol^{-1}, C−N of **1c**: 4.9 kcal mol^{-1}),[19] and allowed a more bent structure because of the decrease in the ring strain (Figure 3).[20]

As spectroscopic evidence of the substantial electronic effect of the C−O bond, the X-ray analyses showed that the SDA of the nine-membered **1l** is larger than that of **1n** [SDA: 38°, 1.82-1.84 Å for S(1)-C(4) and S(1)-C(5), and 1.48 Å for N(1)-C(1) and N(1)-C(6)], which has a smaller eight-membered ring (Figure 2). Assuming the reactivity for the cyclo-

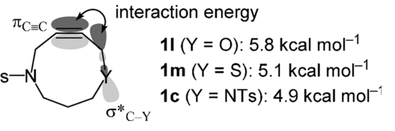
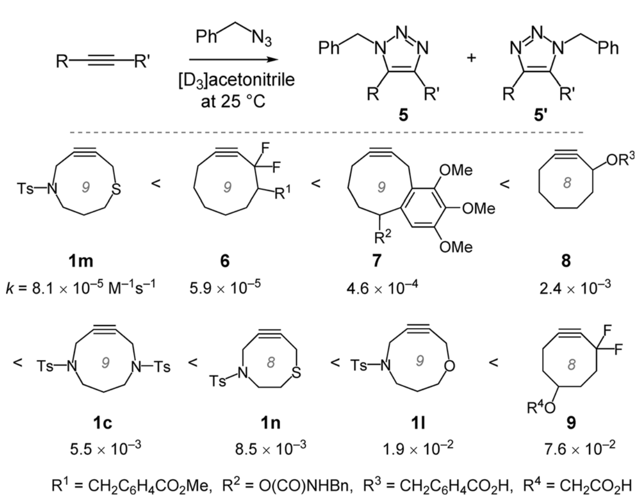


Figure 3. NBO analysis on the electronic effect of the embedded heteroatoms on structural distortion of **1**.

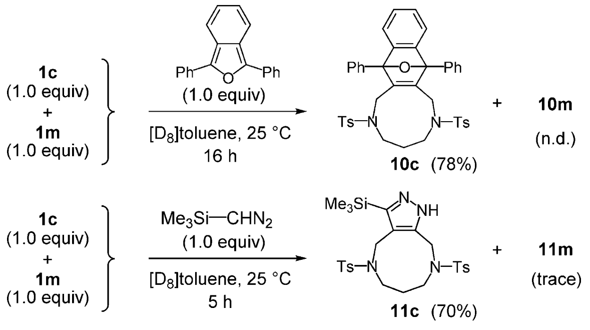
addition reactions of alkynes is dependent on the bend angles of the sp-hybridized carbon atoms, the order of reactivity is estimated to be **1m** < **1c** < **1n** < **1l**.[21]

To demonstrate the effect of the embedded heteroatoms on the reactivity of **1**, the second-order rate constants for the catalyst-free Huisgen reaction with benzyl azide, providing triazole **5**, were measured in [D$_3$]acetonitrile (5 mM of **1** and benzyl azide) at 25 °C using NMR experiments (Scheme 1). A rate constant of $(5.5\pm0.1)\times10^{-3}\,\text{M}^{-1}\,\text{s}^{-1}$ was determined for **1c**, and is substantially larger than that of the recently reported functionalized cyclononyne **6**[22] and **7**.[23] It is noteworthy that the rate constant of **1c** is more than two times larger than that of cyclooctyne derivative **8**, reported by Bertozzi and co-workers.[3a] In line with the reactivity we predicted, a similar reaction of **1l** proceeded approximately three times faster than the reaction of **1c** [**1l**: $(1.88\pm0.08)\times10^{-2}\,\text{M}^{-1}\,\text{s}^{-1}$], and is comparable to that of the difluoronated cyclooctyne **9**,[3b] whereas the equivalent reaction of **1m** was three orders of magnitude slower than that of **1l** [**1m**: $(8.1\pm0.4)\times10^{-5}\,\text{M}^{-1}\,\text{s}^{-1}$]. N,S-embedded eight-membered alkyne **1n** also showed higher reactivity than **8** [**1n**: $(8.5\pm0.4)\times10^{-3}\,\text{M}^{-1}\,\text{s}^{-1}$], and the rate constant was smaller than that of **1l**, as was expected from the SDA value.[24] These results clearly show that the reactivity of the cycloalkynes is related to their structural distortion, which is controlled by the embedded heteroatoms.



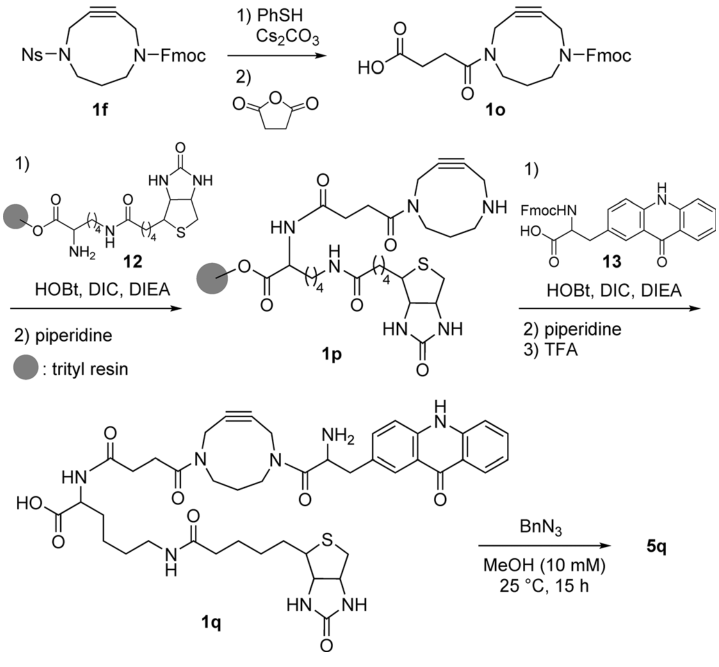
Scheme 1. Kinetic study on the Huisgen reaction of **1**.

Substantial effects of embedded heteroatoms on the reactivity of alkynes **1** were also observed in other cycloaddition reactions (Scheme 2). For example, the reaction of an equimolar mixture of **1c**, **1m**, and 1,3-diphenyl isobenzofuran at 25 °C only provided **10c**, the Diels–Alder product of **1c**, in 78 % yield, and no **10m** (product of **1m**) was formed.[2b,25] The greater reactivity of **1c** compared with **1m** was also shown for a similar competitive reaction using trimethylsilyldiazomethane, in which the pyrazole **11c** was provided in 70 % yield with only trace amounts of **11m** (product of **1m**).[2e,25]



Scheme 2. Competitive reactions of **1c** and **1m**. n.d. = not detected.

These newly designed medium-sized cycloalkynes **1** have the advantage of availability, chemical stability, and adjustable reactivity. Medium-sized cycloalkynes with nitrogen functionalities can also be derivatized by introducing diverse functional units onto the nitrogen atoms. For example, the alkyne **1f** can be easily converted into the Fmoc-protected amino acid **1o** in 43 % yield by removal of the Ns group using PhSH and condensation with succinic acid (Scheme 3).[26] By using the Fmoc chemistry of solid-phase synthesis,[27] **1o** was converted into the tri-peptide **1q** having a different functional



Scheme 3. Preparation of amino-acid- and peptide-containing medium-sized alkyne moiety. Bn = benzyl, Bt = benzotriazole, DIC = *N*,*N'*-diisopropylcarbodiimide, DIEA = *N*,*N*-diisopropylethylamine, TFA = trifluoroacetic acid.

unit on each nitrogen functionality. Namely, **1o** was condensed with a biotin-linked amino acid **12**, supported on a trityl resin, followed by removal of the Fmoc group to afford **1p**. Then **1p** was condensed with the alanine derivative **13**, containing a fluorescent 9-acridinone moiety, and removed from the resin following the removal of the Fmoc group. The Huisgen reaction of **1q** with benzyl azide (10 mM of **1q** and benzyl azide) in methanol at 25 °C proceeded smoothly and almost all **1q** was converted into the corresponding triazole **5q** within 15 hours.[28] This protocol allows access to a wide range of biological applications which require highly functionalized medium-sized cycloalkynes, and are otherwise difficult to obtain.

In conclusion, we have developed a concise synthetic method for a variety of heteroatom-embedded medium-sized cycloalkynes (**1**) and revealed the effect of the heteroatoms on the bent structure and reactivity of the cycloalkynes. This newly developed approach using the double Nicholas reaction is highly efficient for the synthesis of nitrogen-containing cycloalkynes, and these nitrogen functionalities are useful as connection points for functional units. Further studies on the cycloalkynes **1** from the viewpoint of fundamental structural organic chemistry along with their application for biochemical analysis are in progress.

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Keywords: alkynes · cycloaddition · heterocycles · peptides · strained molecules

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[10] The application of double Nicholas reactions for the synthesis of medium-sized or macrocyclic alkyne/cobalt complexes has been reported, see: a) A. Gelling, G. F. Mohmand, J. C. Jeffery, M. J. Went, *J. Chem. Soc. Dalton Trans.* **1993**, 1857; b) E. Álvaro, M. C. de La Torre, M. A. Sierra, *Chem. Commun.* **2006**, 985; c) L. J. Hope-Weeks, M. J. Mays, G. A. Solan, *Eur. J. Inorg. Chem.* **2007**, 3101; d) V. B. Golovko, M. J. Mays, G. A. Solan, *Polyhedron* **2008**, *27*, 167; e) D. D. Díaz, J. P. Ceñal, V. S. Martín, *Molbank* **2008**, M562; f) V. B. Golovko, M. J. Mays, G. A. Solan, *J. Organomet. Chem.* **2008**, *693*, 2683.

[11] A part of this work was presented at the annual meeting of the Chemical Society of Japan in March 2013, see: R. Ni, K. Igawa, K. Tomooka, *Abstracts of Papers*, 93rd Annual Meeting of The Chemical Society of Japan, The Chemical Society of Japan, Tokyo, **2013**, 1E6–49.

[12] The application of double Nicholas reactions for the synthesis of a twenty-membered alkyne crown ether has been reported, see: a) N. Kihara, K. Kidoba, *Org. Lett.* **2009**, *11*, 1313; b) B. Gold, P. Batsomboon, G. B. Dudley, I. V. Alabugin, *J. Org. Chem.* **2014**, *79*, 6221.

[13] During the course of our study, Hagendorn and Bräse reported the double Nicholas approach to dithia- and thiooxacyclooctyne, and it shows modest chemical yields (13–26 %). See: T. Hagendorn, S. Bräse, *R. Soc. Chem. Adv.* **2014**, *4*, 15493.

[14] The tosylamide moiety has been well utilized as good nucleophile in cyclization reactions for construction of medium-sized rings. For selected examples, see: a) H. Stetter, *Chem. Ber.* **1953**, *86*, 197; b) H. Stetter, E. E. Roos, *Chem. Ber.* **1954**, *87*, 566; c) J. E. Richman, T. J. Atkins, *J. Am. Chem. Soc.* **1974**, *96*, 2268; d) H. Koyama, T. Yoshino, *Bull. Chem. Soc. Jpn.* **1972**, *45*, 481; e) F. Bottino, M. D. Grazia, P. Finocchiaro, F. R. Fronczek, A. Mamo, S. Pappalardo, *J. Org. Chem.* **1988**, *53*, 3521.

[15] A significant amount of oligomers and maleic anhydride-fused derivatives of **1c** were obtained as by-products. A similar side reaction has been reported by Tanino and Kuwajima: a) K. Tanino, T. Shimizu, M. Miyama, I. Kuwajima, *J. Am. Chem. Soc.* **2000**, *122*, 6116; b) M. Kudo, F. Kondo, H. Maekawa, T. Shimizu, M. Miyashita, K. Tanino, *Tetrahedron Lett.* **2014**, *55*, 1192. In contrast, our modified procedure efficiently avoided the generation of the by-products. Presumably, silica gel would work as an absorber of cobalt residues and a water source for the acceleration of the reaction.

[16] The remaining cobalt residues in the crude reaction mixtures were easily removed by filtration through a short column of aminopropylated silica gel. See the Supporting Information.

[17] No reaction of **1c** was observed by heating in toluene at 80 °C for two weeks.

[18] Selected crystallographic data are provided in the Supporting Information. CCDC 1011586 (**1c**), 1011587 (**1l**), 1011588 (**1m**), and 1011589 (**1n**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

[19] NBO analyses were performed with Gaussian NBO version 3.1 using Gaussian09 package: Gaussian09 (Revision C.01), M. J. Frisch, et al., Gaussian, Inc. **2009**.

[20] Quite recently, Alabugin and Dudley reported the Huisgen reaction of a macrocyclic alkyne crown ether to demonstrate the acceleration effect of the propargylic oxygen atom. The work clearly elucidated the stereoelectronic effect of $\sigma^*_{\text{C-O}}$ in the transition-state geometries of the Huisgen reaction on eliminating the distortional effect of the alkyne moiety by experimental and theoretical studies, see: Ref. [12b].

[21] Houk and colleagues reported that the bending of an alkyne lowers the energy required to distort the alkyne and azide into the transition-state geometry (distortion energy) for the Huisgen reaction. See: a) D. H. Ess, G. O. Jones, K. N. Houk, *Org. Lett.* **2008**, *10*, 1633; b) F. Schoenebeck, D. H. Ess, G. O. Jones, K. N. Houk, *J. Am. Chem. Soc.* **2009**, *131*, 8121. In terms of the distortional energy, Hosoya and colleagues reported that the steric hindrance in aromatic azides lowers the distortional energy for the Huisgen reaction and accelerates the reaction. See: c) S. Yoshida, A. Shiraishi, K. Kanno, T. Matsushita, K. Johmoto, H. Uekusa, T. Hosoya, *Sci. Rep.* **2011**, *1*, 82.

[22] E. M. Sletten, G. deAlmeida, C. R. Bertozzi, *Org. Lett.* **2014**, *16*, 1634.

[23] J. Tummatorn, P. Batsomboon, R. J. Clark, I. V. Alabugin, G. B. Dudley, *J. Org. Chem.* **2012**, *77*, 2093.

[24] DFT calculations showed that SDA of the cyclooctyne **8** is about 46°, which is bigger than that of **1l** having higher reactivity than **8** in Huisgen reaction. The inconsistency of the order of SDA and reactivity suggests that the reactivity of **1** for Huisgen reaction should be strongly depending on not only the structural distortion but also stereoelectronic effect of $\sigma^*_{\text{C-Y}}$ in the transition state. Regarding the stereoelectronic effects of the propargylic heteroatoms on the transition state of the Huisgen reaction, a discussion by Alabugin and colleagues can be found in recent reports: Refs. [7] and [12b].

[25] The yields of **10c** and **11c** were determined by ^{1}H NMR analysis using 1,3,5-trimethoxybenzene as an internal standard. See the Supporting Information.

[26] No PhSH adduct of **1f** was observed in the removal of the Ns group, and strongly suggests that the bis(nitrogen)-embedded nine-membered cycloalkynes have high bioorthogonality for bioimaging technique in the presence of intravital thiols. See: Ref. [5].

[27] E. Atherton, H. Fox, D. Harkiss, C. J. Logan, R. C. Sheppard, B. J. Williams, *J. Chem. Soc. Chem. Commun.* **1978**, 537.

[28] The conversion of **1q** and the formation of **5q** in the Huisgen reaction were established by MALDI-TOF mass spectrometry with the same laser intensity and the same delay time. See the Supporting Information.