Enhanced clickability of doubly sterically-hindered aryl azides

Suguru Yoshida[1], Akira Shiraishi[2], Kimiyuki Kanno[2], Takeshi Matsushita[3], Kohei Johmoto[4], Hidehiro Uekusa[4] & Takamitsu Hosoya[1,2]

[1]Laboratory of Chemical Biology, Graduate School of Biomedical Science, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan, [2]Department of Biological Information, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan, [3]Goi Research Center, Chisso Petrochemical Corporation, 5-1 Goikaigan, Ichihara, Chiba 290-8551, Japan, [4]Department of Chemistry and Materials Science, Graduate School of Science and Engineering, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro-ku, Tokyo, 152-8551, Japan.

Steric character is one of the most fundamental factors to determine the reactivity of the substrate in organic synthesis. In bimolecular reaction, the sterically-bulky group situated close to the reactive center generally prevents the approach of the reaction partner retarding the bond formation. This report describes, to the contrary, significantly enhanced reactivity of 2,6-disubstituted phenyl azides observed in catalyst-free 1,3-dipolar cycloaddition with alkynes, unexpectedly reacting faster than unsubstituted phenyl azide and even more faster than unhindered alkyl azide, despite the steric hindrance adjacent to the reactive azido group. Experimental and computational studies have indicated that the steric hindrance eliciting the inhibition of resonance between azido group and the aromatic ring is the primary cause of this apparently-paradoxical phenomenon. This is the first type of steric acceleration, indicating a possibility of designing a highly reactive functional group by strategically locating it in the sterically-congested environment.

Click reaction, epitomized by copper(I)-catalyzed azide–alkyne cycloaddition, has become one of the most reliable methods to connect molecules covalently in broad disciplines including materials chemistry and chemical biology.[1–5] In particular, strain-promoted click reaction, a copper-free variant exploiting a cyclooctyne derivative that reacts spontaneously with an azide, has realized harmless chemical modification of biomolecules in cultured cells and in living animals.[6–15] Recently, we have developed the "double-click" reaction to conjugate conveniently an azido-biomolecule with a small azido compound using Sondheimer diyne (1)[16] as a bis-dipolarophile (Fig. 1a).[17] In this reaction, an efficient assembly of two azides takes place by virtue of the two highly strained triple bonds of 1 providing bis-cycloadduct in high yield. The practical utility of the double-click strategy has been demonstrated by efficient labeling of azido-glycoconjugates on the cell surface as well as an azido-installed recombinant protein with a fluorescein-conjugated azide. While the double-click technique has been shown comparable in labeling efficiency to the single-click procedure, experimental and computational studies presented that the monoyne intermediate, considered as the initial cycloadduct of this reaction, is remarkably reactive than the starting diyne 1, which might pose an insufficient conjugation.

To make the double-click conjugation system more efficient, we have conceived the idea of performing a sequential double-click reaction[18] using diazidobenzene derivative 2, which bears two sterically-differentiated azido groups, with an anticipation to connect first alkyne at the less hindered side and then the second alkyne at the remaining sterically-hindered side (Fig. 1b). Contrary to our expectations, however, the click reaction of diazide 2 with strained alkyne 3a proceeded predominantly at the sterically-hindered side furnishing 4b in high yield (Fig. 1c, see Supplementary Information). This unexpected but very intriguing result prompted us to elucidate the origin of enhanced reactivity of sterically-hindered azido group.

Results

The distinguished reactivity of sterically-hindered aryl azide was demonstrated through the study on the substrate scope of the azide in double-click reaction with diyne 1. First, we compared the reactivities of two typical sterically-hindered azides, 1-adamantyl azide (5b) and 2,6-diisopropylphenyl azide (5c), with unhindered benzyl azide (5a) by monitoring the reaction with 1 in methanol-d_4 by ^{1}H NMR spectroscopy. As a result, while the reaction of 1 with bulky 5b was retarded drastically (18% yield of bis-cycloadducts at 4 h) compared to that with
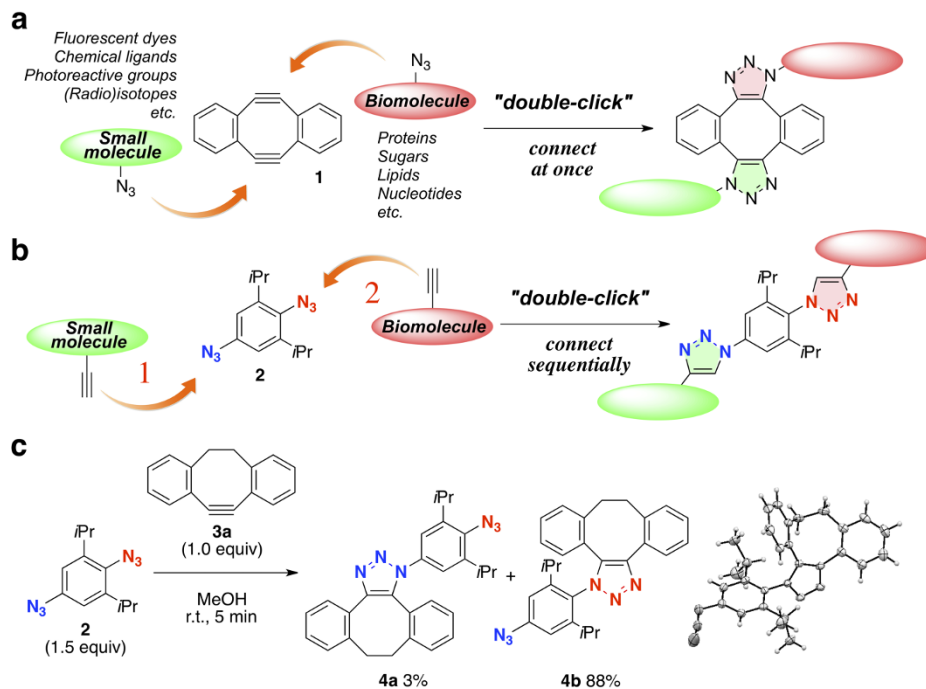
Figure 1 | Double-click reactions using a bis-reactive compound for efficient assembly of molecules. (a) The double-click method for convenient conjugation of an azido-biomolecule with a small azido compound mediated by the Sondheimer diyne (**1**). (b) An initial plan of sequential double-click conjugation by diazidobenzene derivative **2** bearing two sterically-differentiated azido groups. We envisaged that the first cycloaddition with an alkyne would proceed at the less hindered side and the remaining sterically-hindered azido group could be used for the second cycloaddition with another alkyne. (c) Click reaction of diazide **2** with strained alkyne **3a** unexpectedly affording **4b** as the major product. This result indicated that the reaction occurred predominantly at the more sterically-hindered azido group of **2**. The regiochemistry of **4b** was unequivocally determined by X-ray structure analysis (CCDC 810844).

benzyl azide (**5a**) (87%, 30 min), the reaction with **5c** was rather hastened under the same conditions affording the product quantitatively within 5 min (see Supplementary Information). To our surprise, the second-order rate constant (k) for the reaction of **1** with **5c** in methanol at 25 °C ($k = 6.7 \times 10^{-1}$ M^{-1} s^{-1}) was ten times as large as that for **5a** ($k = 6.3 \times 10^{-2}$ M^{-1} s^{-1}).[17]

Exploring further the double-click reaction with a focus on the substituent effect of aryl azides more clearly showed that the bulkiness around the azido group is the key factor to enhance the reaction rate (Fig. 2, Table 1). Although all of the reactions of diyne **1** with various aryl azides **5d**–**5j** afforded a regioisomeric mixture of bis-cycloadducts in an excellent yield, the reaction rates varied greatly depending on the substrate. Notably, the reaction with unsubstituted phenyl azide (**5d**) proceeded about seven times slower than **5a** and, more importantly, 76 times slower than **5c** (entry 1 vs 8, Table 1). *para*-Methoxy and *para*-trifluoromethyl groups showed only limited effects (entries 2 and 3, Table 1), indicating that the electronic nature of the substituent is not a decisive factor to affect the reaction rate. The reactivity of mono-*ortho*-substituted phenyl azides was neither enhanced nor diminished to a large extent (entries 4 and 5, Table 1). In contrast, the reactions with 2,6-disubstituted phenyl azides were dramatically accelerated, as the size of substituents became bulkier (entries 6–8, Table 1).

A valuable hint to understand the role of bulky substituents in enhancing the reactivity of sterically-surrounded azido group was provided from UV absorption spectra of the azides (Fig. 3a). The intensity of the peak at long-wavelength region observed for **5d** (λ_{max} = 248 nm) decreased considerably in **5c**, suggesting that the conjugated state of the azido group with the aromatic ring between these azides differs substantially. The stationary structure of azides at the ground state optimized by a density functional theory (DFT) (B3LYP/6-31G(d)) method[19] supported this implication indicating that the azido group of **5d** lies coplanar with the benzene ring, while that of **5c** is largely twisted out of the plane, forced by the bulky
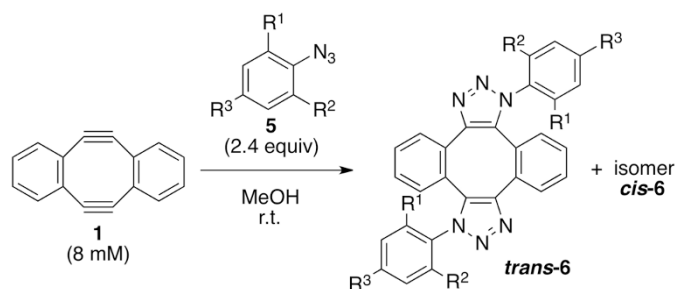

Figure 2 | Reaction scheme for Table 1. The double-click reaction of Sondheimer diyne (**1**) with various aryl azides **5** was examined.

Table 1 | Double-click reaction of diyne 1 and aryl azide 5.

Entry	5	R^1	R^2	R^3	6	Yield (%)* (*trans/cis*)†	k (M^{-1} s^{-1})	k_{rel}
1	**5d**	H	H	H	**6d**	94 (43/57)§	8.8×10^{-3}	1
2	**5e**	H	H	OMe	**6e**	89 (36/64)	3.3×10^{-2}	3.8
3	**5f**	H	H	CF$_3$	**6f**	97 (50/50)	7.9×10^{-3}	0.9
4	**5g**	Me	H	H	**6g**	98 (52/48)	1.2×10^{-2}	1.4
5	**5h**	iPr	H	H	**6h**	95 (60/40)	8.9×10^{-3}	1.0
6	**5i**	Me	Me	H	**6i**	92 (64/36)	3.2×10^{-1}	36
7	**5j**	Et	Et	H	**6j**	93 (73/27)	3.8×10^{-1}	43
8	**5c**	iPr	iPr	H	**6c**	95 (96/4)	6.7×10^{-1}	76

*Isolated yield as a mixture of regioisomers.
†Ratio was determined based on ^{1}H NMR analysis of isolated regioisomeric mixture.
‡Stereochemistry of **6** was unequivocally determined by X-ray analysis of purified regioisomer (CCDC 810837–810843 for *cis*-**6b**, **6c**, **6e**, **6g**, **6h**, **6i**, **6j** and 810930–810936 for corresponding *trans*-isomers) except **6f**.
§Data from ref. 17.

substituents at both *ortho*-positions (Fig. 3c). The calculation of rotation energy of the azido group also exhibited that **5d** takes predominantly the highly-conjugated structure, showing a sharp contrast with **5c**, which rather prefers the markedly-twisted conformation (Fig. 3b). Interestingly, the rotational barrier of the sterically-hindered azido group of **5c** was significantly lower than that of **5d**. These data have implied that the reactivity enhancement could be attributed to the inhibition of resonance[20] between the aromatic ring and the azido group lowering its motional energy.

To gain a mechanistic insight, we initially calculated and compared the frontier molecular orbitals[21] of **1**, **5c**, and **5d**, which, however, did not afford a reasonable explanation (see Supplementary Information). Fortunately, instead, the distortion/interaction model, a generalized theory for 1,3-dipolar cycloadditions recently proposed by Houk and coworkers,[22,23] led us to a comprehensive understanding. They elegantly explained the enhanced clickability of strained cycloalkynes by dividing the activation energy into distortion and interaction energies, demonstrating that the energy required to distort the 1,3-dipole and dipolarophile into their transition-state geometries is the crucial factor as well as the frontier molecular orbital interaction energy. To apply this theory, the transition state (TS) structures for the first cycloaddition of **1** with **5d** and **5c**, **TS-d1** and **TS-c1**, were also obtained at the same level of the theory (Fig. 3d). The activation energy for the reaction of **1** with **5c** was estimated to be 2.5 kcal mol^{-1} lower than that with **5d**, providing a good agreement with the experimental result (Fig. 3e). The difference in distortion energies, unexpectedly, was almost equal to that of the activation energies indicating that there is little difference in interaction energies, which must include the factor of steric repulsion arising between the reactants. Considering that the difference in individual distortion energy of diyne **1** between each reaction was comparatively smaller (0.4 kcal mol^{-1}) than that between azides (2.3 kcal mol^{-1}), the

enhanced reactivity of **5c** can be mostly attributed to its decreased distortion energy compared with **5d**.

The generality on the higher reactivity of bulky **5c** over unhindered **5d** was easily examined by reacting them with other simple alkynes. As a result, not only strained cyclooctyne derivative **3a** but also unstrained alkyne such as dimethyl acetylenedicarboxylate (**3b**), under the Huisgen reaction conditions,[24] predominantly afforded **5c**-derived cycloadducts in the reaction with an equimolar mixture of **5c** and **5d**, clearly demonstrating the prominent clickability of doubly sterically-hindered aryl azides despite the steric barrier (Fig. 4a). On the other hand, an inverted selectivity was observed in the reaction of aryl azides with an acetylide (Fig. 4b).[25] The preferred formation of **5d**-derived triazole **8dc** agrees well with the proposed stepwise mechanism, which should be disadvantageous for hindered substrates.

Discussion

We have unexpectedly found that sterically-congested azido group of 2,6-disubstituted phenyl azides, despite the steric hindrance, reacts significantly faster than unsubstituted phenyl azide, as well as unhindered alkyl azide in catalyst-free 1,3-dipolar cycloaddition with an alkyne. Although a similar trend was previously reported in iron-catalyzed hydrogenation of sterically-hindered aryl azides, no mechanistic explanation has been given so far.[26] Our studies on substrate scope and computations have clearly shown that the enhanced reactivity of 2,6-disubstituted phenyl azides can be attributed to the increased distortability of the azido group elicited by the inhibition of resonance with the aromatic ring. The slower reaction observed for benzyl azide (**5a**) than 2,6-disubstituted phenyl azides can be reasonably explained by taking the contribution of the hyperconjugation between azido group and the hydrogen of alkyl chain into account. Indeed, the calculated distortion and activation energies of methyl azide in the cycloaddition with diyne **1** are also larger
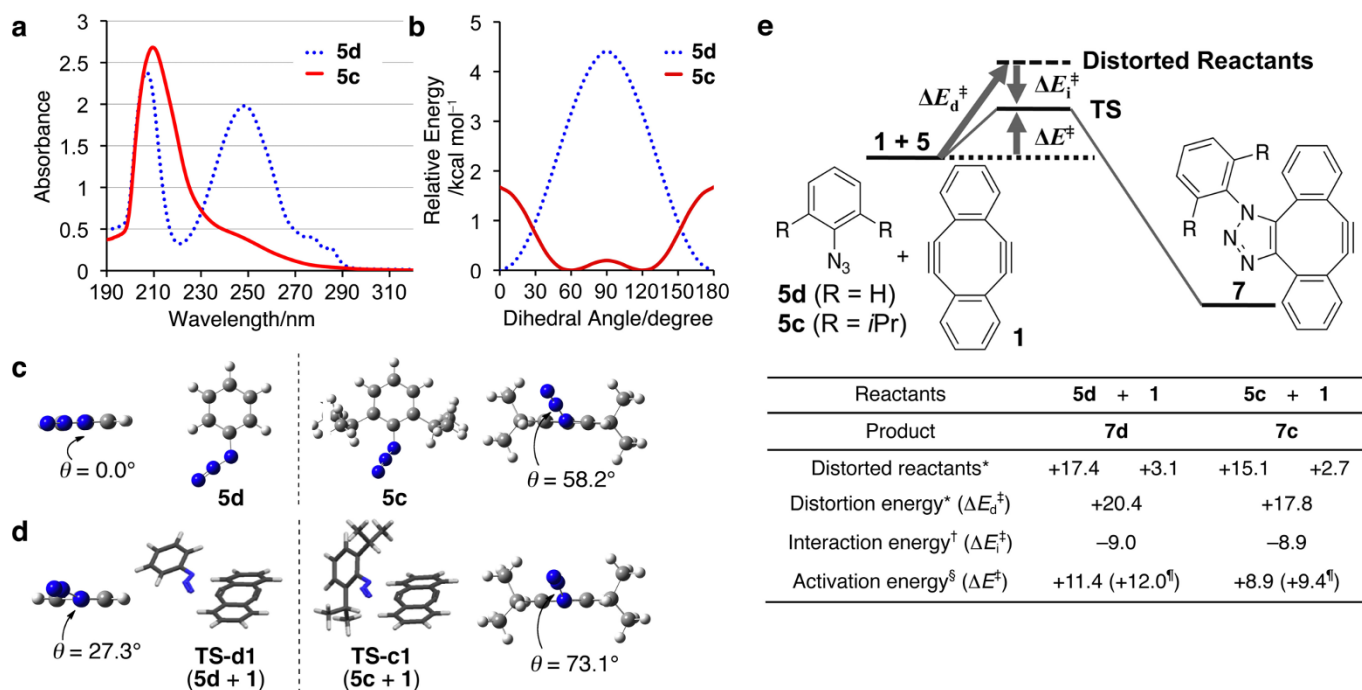

Figure 3 | Twisted-conformation of 2,6-diisopropylphenyl azide (5c) enhancing its clickability. (a) Absorption spectra of **5d** and **5c** in MeOH (100 μM). (b) Calculated rotation energy for azido group of **5d** and **5c**. (c) Side and overhead views of the global minima on the potential energy surface obtained for **5d** and **5c**. (d) Calculated transition state (TS) structures for the first cycloaddition of **1** with **5d** and **5c** and the side views of azides at the TS. θ indicates the rotational angle of the azido group from the aromatic plane. (e) Distortion, interaction and activation energies (in kcal mol^{-1}) for the first cycloaddition at the B3LYP/6-31G(d). *The energy required to distort the geometry of each reactant to the transition state (TS). †The interaction energy between the distorted fragments at the TS. §The energy difference of each fragment between the optimized and the TS geometries. ¶The values including zero-point corrections (ZPCs). All calculations were performed by a density functional theory (DFT) method (B3LYP/6-31G(d)) with a GAMESS suite of program codes on a TSUBAME 2.0 system at Tokyo Institute of Technology.
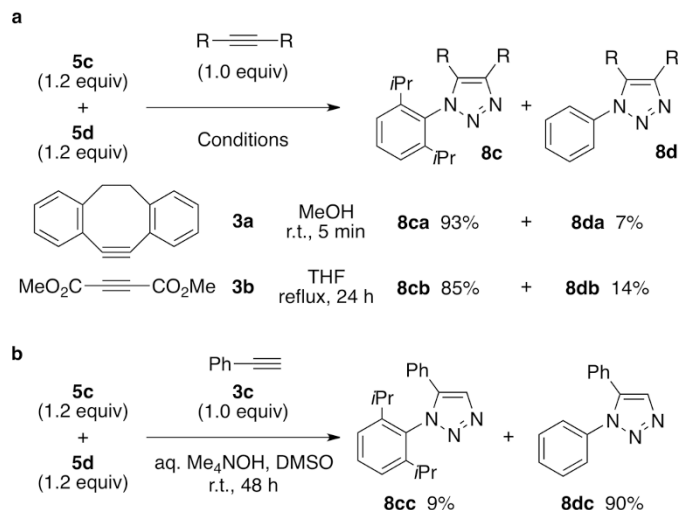
Reactants	5d	+	1	5c	+	1
Product		7d			7c	
Distorted reactants*	+17.4		+3.1	+15.1		+2.7
Distortion energy* ($\Delta E_d^{\ddagger}$)		+20.4			+17.8	
Interaction energy† ($\Delta E_i^{\ddagger}$)		−9.0			−8.9	
Activation energy§ ($\Delta E^{\ddagger}$)		+11.4 (+12.0¶)			+8.9 (+9.4¶)	

Figure 4 | Competition between doubly sterically-hindered azide 5c and unhindered azide 5d in the reaction with various alkynes. (a) Competition between **5c** and **5d** in the cycloaddition reaction with alkyne **3**. (b) Competition between **5c** and **5d** in the reaction with acetylide generated in situ. Yields were determined based on ^{1}H NMR analysis.

than those of **5c**.[17] All of these results suggest that the extended conjugation containing azido group makes it hard to deform to the TS structure, thus decreasing the reactivity. In contrast, the sterically-demanding aromatic azido group, in which the resonance is cancelled to some extent, achieves increased distortability, thereby exerting the high reactivity. Not only in the reaction with strained alkyne, but also with simple alkyne such as dimethyl acetylenedicarboxylate (**3b**), the decrease of distortion energy of doubly sterically-hindered aromatic azido group largely eclipses the steric repulsion between the substrates, thereby, in total, significantly lowers the activation energy. To our knowledge, this is the first report demonstrating the enhanced reactivity of sterically-hindered group by the effect of steric inhibition of resonance. There are some reactions accelerated by the steric assistance, but this is a novel type of steric acceleration in that the intrinsic reactivity of the azido group has been invoked by the steric participation of neighboring groups. This work indicates, though it may sound paradoxical, a possibility of designing a highly reactive functional group by strategically locating it in an appropriate sterically-congested environment.

Methods

Representative procedure for the double-click reaction. To a solution of diyne **1** (10.0 mg, 50.0 μmol) in MeOH (5.00 ml) was added a solution of 2,6-diisopropylphenyl azide (**5c**) (24.4 mg, 120 μmol) in MeOH (1.25 ml) at room temperature. After stirring for 30 min at the same temperature, the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica-gel 10 g, CH_2Cl_2/MeOH = 100/1) to give a mixture of **trans-6c** and **cis-6c** (28.9 mg, 47.6 μmol, 95.4%). The ratio of **trans-6c** and **cis-6c** was determined to be 96/4 based on the ^{1}H NMR analysis. The isomers were separated by flash column chromatography (silica-gel 10 g, CH_2Cl_2/MeOH = 100/1) and recrystallized. The geometry of each isomers was confirmed by X-ray crystallographical analysis (CCDC 810931 (**trans-6c**) and CCDC 810838 (**cis-6c**)).

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Author contributions

T.H. directed the study. S.Y. and T.H. conceived the experiment and wrote the paper. A.S. and K.K. performed the experiments. S.Y. and T.M. performed computational studies. K.J. and H.U. carried out X-ray structure analyses.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

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