Enhanced clickability of doubly sterically-hindered aryl azides

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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 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.

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-d₄ by ¹H 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

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alkyne. (c) Click reaction of diazide would proceed at the less hindered side and the remaining sterically-hindered azido group could be used for the second cycloaddition with another azide. The reactivity of mono-caffeine was not a decisive factor to affect the reaction rate. Effects (entries 2 and 3, Table 1), indicating that the electronic nature 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 ($\lambda_{\text{max}} = 248$ 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 supported this implication indicating that the azido group of 5c lies coplanar with the benzene ring, while that of 5c is largely twisted out of the plane, forced by the bulky 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 5c and, more importantly, 76 times slower than 5c (entry 1 vs 8, Table 1).

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).

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

| Entry | 5 | R′ | R2 | R3 | Yield [%] | k [M⁻¹ s⁻¹] | krel |
|-------|---|----|----|----|---------|------------|------|
| 1     | 5d| H  | H  | H  | 94 [43/57]* | 8.8 x 10⁻³ | 1    |
| 2     | 5e| H  | H  | OMe| 89 [36/64]* | 3.3 x 10⁻² | 3.8  |
| 3     | 5f| H  | H  | CF₃| 97 [50/50] | 7.9 x 10⁻³ | 1.9  |
| 4     | 5g| Me | H  | H  | 98 [52/48]* | 1.2 x 10⁻² | 1.4  |
| 5     | 5h| iPr| H  | H  | 95 [60/40] | 8.9 x 10⁻³ | 1.0  |
| 6     | 5i| Me | Me | H  | 92 [64/36] | 3.2 x 10⁻¹ | 36   |
| 7     | 5j| Et | Et | H  | 93 [73/27] | 3.8 x 10⁻¹ | 43   |
| 8     | 5c| iPr| iPr| H  | 95 [96/4] | 6.7 x 10⁻¹ | 76   |

*Isolated yield as a mixture of regioisomers. Ratios were determined based on ¹H 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.

Figure 2 | Reaction scheme for Table 1. The double-click reaction of Sondheimer diyne (1) with various aryl azides 5 was examined.
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 resonance20 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 orbitals21 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 hyper-conjugation 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.

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**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. \(\theta\) 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.
than those of 5c. 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-disopropophenyl 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, CH₂Cl₂/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 94/6 based on 'H NMR analysis. The isomers were separated by flash column chromatography (silica-gel 10 g, CH₂Cl₂/MeOH = 100/1) and recrystallized. The geometry of each isomer was confirmed by X-ray crystallographical analysis (CCDC 810931 (trans-6c) and CCDC 810838 (cis-6c)).

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