Click Chemistry Hot Paper

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Strain-Promoted Cycloaddition of Cyclopropenes with *o*-Quinones: A Rapid Click Reaction

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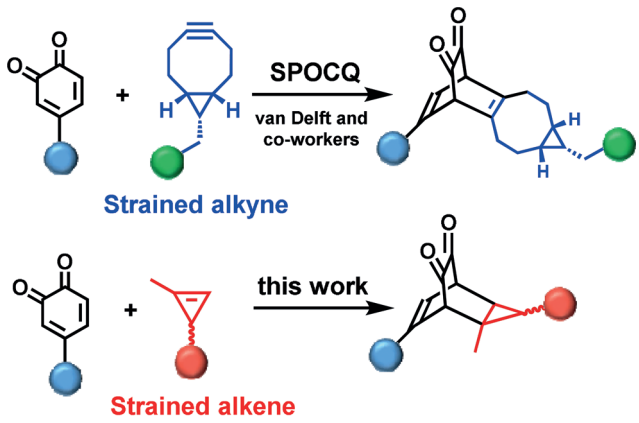
Abstract: *Novel click reactions are of continued interest in fields as diverse as bio-conjugation, polymer science and surface chemistry. Qualification as a proper "click" reaction requires stringent criteria, including fast kinetics and high conversion, to be met. Herein, we report a novel strain-promoted cycloaddition between cyclopropenes and o-quinones in solution and on a surface. We demonstrate the "click character" of the reaction in solution and on surfaces for both monolayer and polymer brush functionalization.*

The discovery and application of novel click reaction strategies is a growing domain[1] that has garnered significant interest amongst (bio-)organic[2] and material chemists.[3] Since the introduction of the copper-catalyzed azide alkyne cycloaddition (CuAAC),[4] major advances have been made in this regard. Specifically the development of metal-free click reactions[5] that are either strain-promoted or catalyzed by simple bases is noteworthy. Examples include the strain-promoted azide-alkyne cycloaddition (SPAAC)[5]—which uses a highly strained cyclooctyne motif—a range of inverse electron-demand Diels–Alder (IEDDA) reactions such as the tetrazine-*trans*-cyclooctene (TCO)/cyclopropene click,[6] and most recently a series of sulfur-fluoride exchange (SuFEx) reactions.[7] Most of these reactions involve strained reactants (alkyne or alkene) that accelerate the reaction or a highly facile bond exchange.[8] There also has been a growing increase in the use of photochemical click reactions in this regard.[9] The advantages of such reactions include metal-free conditions,[10] faster kinetics[11] and good-to-excellent yields.[12]

The strain-promoted oxidation-controlled cyclooctyne-1,2-quinone cycloaddition (SPOCQ)[11] shown in Scheme 1, is another example of such a click strategy. The fast kinetics of this reaction in solution ($k_2 = 496 \pm 70\,M^{-1}s^{-1}$) makes it amenable for example, accelerated site-specific protein conjugation,[11a,12] cell labelling[13] and hydrogel preparation.[14] An additional feature of this reaction is that quinone formation can be triggered by enzymatic[12] or electrochemical[15] oxidation, thus providing an inducible click handle. In addition, when used for surface functionalization, SPOCQ achieves a rarely obtained quantitative conversion[16] within 4 h with high surface-bound rates ($k_2 = 33 \pm 2\,M^{-1}s^{-1}$).[17] A distinct feature of this reaction is, however, the use of a relatively large, hydrophobic eight-membered ring, which in itself is not necessary for bio-conjugations and typically disadvantageous in sterically crowded environments such as polymers and surfaces. Thus, a smaller, yet fast, stable and easily synthesizable alternative is quite desirable. With this goal in mind, we hypothesized that a smaller dienophile such as a strained cyclopropene could meet these criteria.



Scheme 1. Scheme showing the inspiration for strain-promoted click reactions between quinones and cyclopropenes.

1-Methyl-3-substituted cyclopropenes have been recently reported for fast IEDDA cycloadditions with tetrazines.[18] This click reaction has been widely used for glycoprotein conjugation,[19] cell imaging,[20] and so forth.[21] Moreover, the higher stability of substituted cyclopropenes[18a] as compared to TCO, an alternative strained alkene, is an additional advantage. Based on these findings, we envisaged that

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cyclopropenes could serve as a potentially novel candidate for facile conjugation with *o*-quinones both in solution and on surfaces.

Herein, we report a novel click reaction between strained alkenes, namely 1-methyl-3-substituted cyclopropenes, and *o*-quinones. We determine the rate constants for the reaction in solution using UV spectroscopy and on a surface using DART-HRMS.[17] Furthermore, we also demonstrate the quantitative nature of the reaction on surfaces and demonstrate its versatility using anti-fouling polymer brush functionalization. Finally, we use density functional theory (DFT) to study the reaction mechanism in more detail. We believe that the high solution-phase yields, minute-scale completion times for monolayer modification coupled with the ability for polymer functionalization demonstrate the wide potential of this novel reaction.

The cyclopropene probes were designed based on the balance between reactivity and stability found by Devaraj and co-workers.[18b] To this end, we synthesized 1-methylcyclopropenes **2**, **5** and **7** (Scheme 2; see the Supporting Information for details). Compounds **5** and **7** were derived as fluorinated aromatic ester and carbamate, respectively, from cyclopropene alcohol **3**. The aromatic fluorinated head groups were chosen to ease visualization by XPS and DART-HRMS characterization after surface functionalization, based on previous experience.[22] Following the synthesis of the cyclopropene probes, we performed a reaction between **5** and t-butyl quinone (**8**), which proceeded with good yield in solution (75%) at room temperature within 4 h. The resulting cycloadducts (mixture of isomers) were isolated and thoroughly characterized by NMR (see the Supporting Information, section S2.5). Based on NOESY and COSY correlations, we deduced an *endo*-configuration of the resultant cycloadducts (three-membered ring formed away from quinone oxygen atoms; see section S5).

Next, we determined the reaction kinetics in solution for compounds **2**, **5** and **7** (see the Supporting Information for experimental conditions, section S3.1) by following the decay of the characteristic UV absorption signal for the quinone at 385 nm.[11a] In accordance with literature data,[18] **2** showed a sluggish kinetics ($k_2 = 0.20 \pm 0.04\,\text{M}^{-1}\text{s}^{-1}$; Figure S3.1) with reaction completion in about 1 h. In contrast, the fluorinated ester **5** and carbamate **7** showed rapid kinetics ($k_2 = 1.95 \pm 0.02\,\text{M}^{-1}\text{s}^{-1}$ and $1.70 \pm 0.01\,\text{M}^{-1}\text{s}^{-1}$; respectively) and the reaction is completed within 5 minutes (Figure 1 and Figure S3.1).

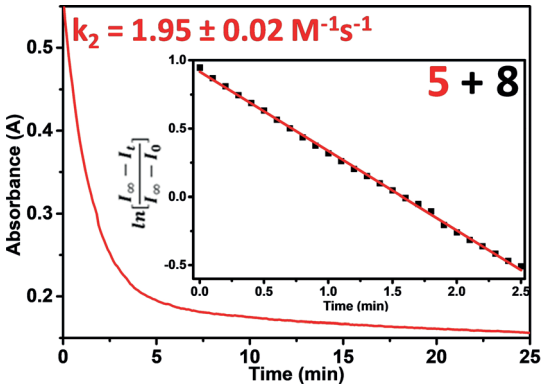
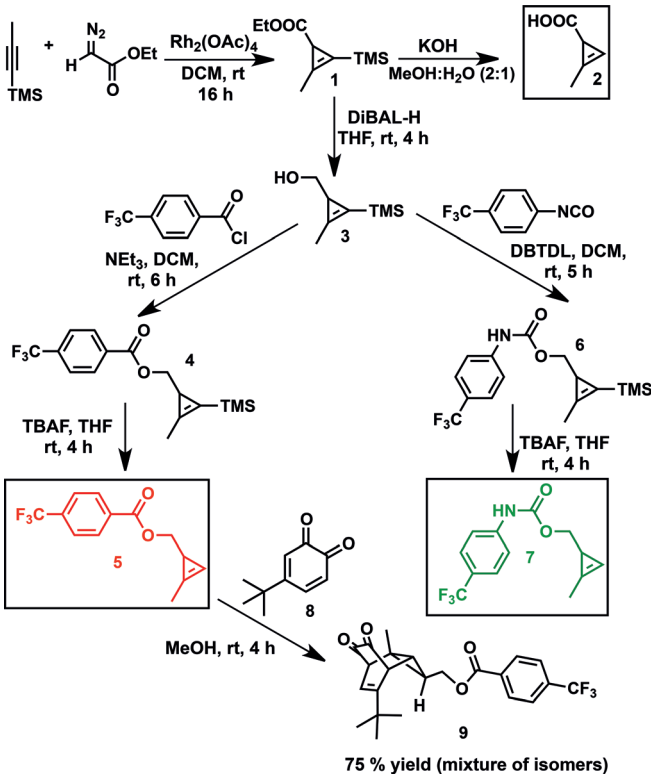


Figure 1. UV kinetics (at 385 nm) of the reaction between cyclopropenes **5** and *t*-butyl quinone **8** at 30 °C in methanol; inset: Linear plots of $ln\,[(I_\infty - I_t)/(I_\infty - I_0)]$ versus time (minutes), to obtain second-order constants; [**5**] = 5 mM.

These rates bring this reaction into the realm of potentially useful for in vivo application according to Houk's classification of metal-free click reactions.[23] As explained in their study, the orthogonal reactivity of 1,3 di-substituted cyclopropenes coupled with high reaction rates enables their application in multicomponent imaging as well.

Density functional theory (DFT) calculations were performed using Gaussian 16,[24] in order to study the reaction mechanism of this exothermic reaction ($\Delta H_{calc} = -31$ to -35 kcal mol^{-1}) in more detail. For that purpose, we used the dispersion-corrected B97D density functional, which has been proved to give accurate activation energies for SPOCQ cycloaddition reactions,[25] and the conductor-like polarizable continuum model (CPCM) to mimic methanol. These computations yield that the cycloaddition reaction proceeds through a non-synchronous transition state (TS), as shown by the distances for both new C–C bonds (see Figure 2). In addition, the activation free energies for the *endo*-cycloaddition of **5** and **8** are lower than that of the *exo*-approach (7.5 vs. 9.0 kcal mol^{-1}, respectively). A subsequent distortion analysis shows that this difference is largely caused by the smaller distortion energy that is required to obtain the *endo* TS compared to that for the *exo*-TS (22.1 vs. 22.6 kcal mol^{-1}).



Scheme 2. Synthesis of methyl-substituted cyclopropenes (**2**, **5** and **7**) and reaction with t-butyl quinone, **8**. See the Supporting Information for detailed synthetic procedures.

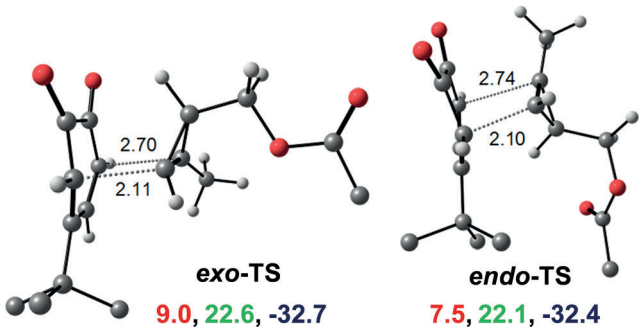
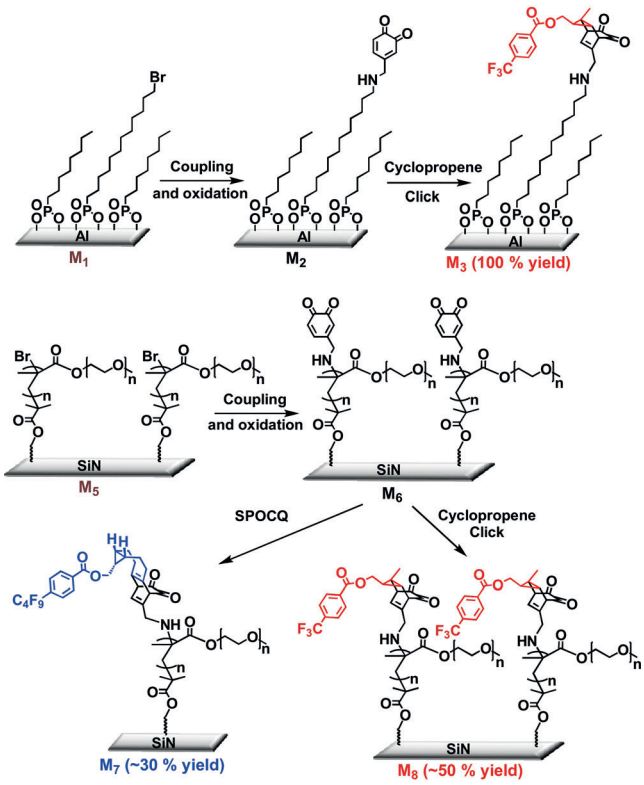


Figure 2. B97D/6–311+G(d,p) transition state geometries for the *exo*- and *endo*-attacks of **5**+**8**. Bond lengths reported in Å. Solution-based activation free energies (red), distortion energies (green) and reaction free energies (blue) are in kcal mol^{-1}.

The TS geometries also suggest that the cycloaddition is favored on the face away from the 3-methyl substituent of the cyclopropene ring. These activation energies are higher than those calculated for bicyclo[6.1.0]non-4-yne and cyclooctyne, with barriers of 4.9 and 6.9 kcal mol^{-1}, respectively, and rate constants of 838 and 51 M^{-1}s^{-1}, but lower than that of dibenzoazacyclooctyne (12.1 kcal mol^{-1}, with k_2 = 0.51 M^{-1}s^{-1}),[25] and those reported for the Diels–Alder reaction of cyclopropenes and butadiene (21–27 kcal mol^{-1}).[22] The marginally slower reaction of **7** and **8**, the cycloaddition proceeds similarly via a non-synchronous *endo*-TS with an activation barrier of 7.9 kcal mol^{-1} (vs. 7.5 kcal mol^{-1} for **5**; vide supra). In this case, the distortion energies for the cyclopropene and *o*-quinone were found to be higher (26.3 kcal mol^{-1}, respectively).

The potential of this novel click reaction should become evident in crowed environments, where the small size of cyclopropene is of relevance. Thus, we tested the applicability of this click reaction for surface functionalization. Surface functionalization provides difficult reaction conditions due to the steric constraints and immobility of one of the reaction partners. A 100% reaction efficiency is specifically in high demand, as purification after covalent on-surface reactions is simply not possible. We envisaged that the highly efficient and fast nature of our novel reaction would also translate on a surface. For this purpose, activated aluminum (Al) surfaces (**M$_0$**) were modified with dodecyl (C_{12}) Br-terminated phosphonic acids diluted with octyl chains in a 3:1 ratio, to get **M$_1$** surfaces (section S1). This was followed by coupling with 3,4-dihydroxybenzylamine hydrobromide and oxidation to *o*-quinones with $NaIO_4$ to yield **M$_2$** surfaces (Scheme 3).

XPS wide scan analysis (Br/P = 1:4 for **M$_1$** and N/P = 1:4 for **M$_2$** surfaces, Figure S4.2–4.4) coupled with the disappearance of the Br3d signal (at 67.0 eV, Figure S4.5) in XPS narrow scan for **M$_2$** confirmed formation of surface-bound *o*-quinones. **M$_2$** surfaces were then subject to a 5 mM solution of **5**, to yield clicked **M$_3$** surfaces (Scheme 3). F/P ratios (3:4) in the XPS wide (Figure 3 and Figure S4.6) and narrow scan F1s and P2s analysis (Figure 4) confirmed a quantitative click reaction (100±3% yield) within 20 minutes. The standard deviation of the reaction yield was determined over a hexaplet of independent samples prepared on different days to ensure rigorous reproducibility of the reaction.



Scheme 3. Scheme for preparation of quinone-terminated surfaces (**M$_3$**) and reaction with **5**. See the Supporting Information for more detail.

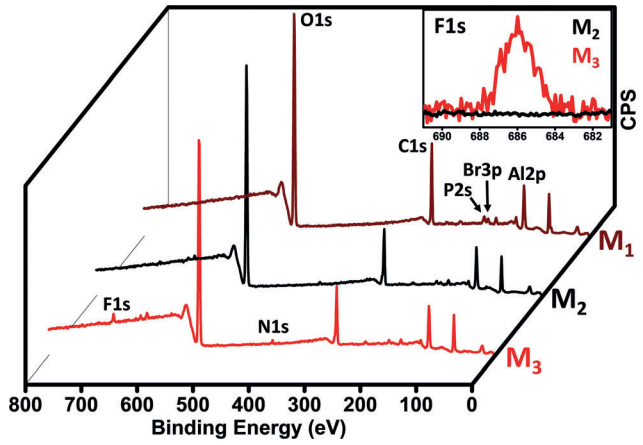


Figure 3. Stacked XPS wide scan spectra of **M$_1$**–**M$_3$** surfaces. Inset: F1s narrow range spectra of **M$_2$** and **M$_3$** surfaces.

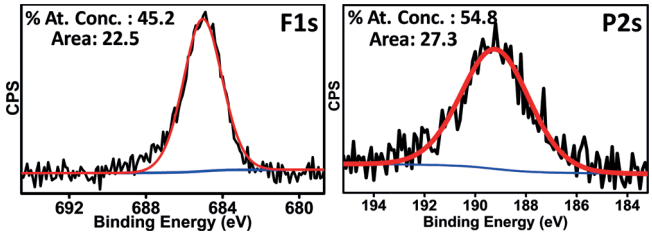


Figure 4. XPS F1s and P2s narrow scan spectra of **M$_3$** surfaces showing the atomic concentration of the two elements, respectively.

For testing polymer brush functionalization, we used poly(MeOEGMA) brushes that have been shown to possess good anti-fouling properties.[26] Bromine-ended polymer brushes ($\mathbf{M_5}$) were prepared by surface-initiated atom transfer radical polymerization (Supporting Information, section 1) on silicon nitride (SiN) surfaces and analyzed by XPS (Figures S4.8–S4.13) and AFM (thickness = 11 ± 1 nm, roughness = 2.2 nm, Figure S4.14). This was followed by coupling and subsequent oxidation steps to yield *o*-quinone-terminated brushes $\mathbf{M_6}$ (Scheme 3), as shown by the disappearance of the Br3d signal in the XPS wide and narrow scan analysis (Figure 3c and Figures S4.15–S4.17). To compare the clickability of strained alkyne (BCN derivative) versus strained alkene (cyclopropene) on polymer brushes, we performed both the reactions on $\mathbf{M_6}$ surfaces and calculated the approximate conversion via the ratio of F1s (686.0 eV)/N1s (ca. 400.7 eV) signals from narrow scan analysis (Figures S4.19 and S4.23). With a BCN-C_4F_9 analog,[17] the reaction yielded a 30% yield, while the sterically advantageous cyclopropene provided 60% conversion (each averaged over 6 samples). This further shows the wide applicability of this approach for polymer modification.

For interfacial kinetics determination, we followed the course of the reaction on $\mathbf{M_2}$ surfaces the growth of an MS-ionizable tag (*p*-CF_3 benzoate anion [*m*/*z* 189.016]) obtained by aromatic ester fragmentation from $\mathbf{M_3}$ surfaces by DART-HRMS (Figure 5).[22a] Given the relatively short reaction time of 20 minutes (cf. 4 h for the previously reported SPOCQ reaction) we could follow the kinetics over the entire kinetic regime. As also confirmed by XPS results, the reaction tends to an asymptotic limit within 20 minutes, signaling completion. The second-order rate constant (k_2) was found to be $0.50 \pm 0.01\,\mathrm{M^{-1}s^{-1}}$ at 30 °C. Despite the slightly reduced rate, it is quite remarkable that the reaction still achieved complete conversion within 20 minutes. In contrast, the SPOCQ reaction ($k_2 = 3.3\,\mathrm{M^{-1}s^{-1}}$ for first 70% conversion; afterwards more complex and slower kinetics) achieves quantitative conversion only in 4 h. This further emphasizes the positive role played by smaller sterics of the cyclopropene motif as compared to a bulky cyclooctyne. We believe the "clean kinetics" of this reaction to be a significant benefit over its predecessor interfacial SPOCQ. These kinetics also reveal a more general point on the difference between dilute solution data and those relevant in crowded environments. The surface-bound cyclopropene–*o*-quinone click is only four times slower than in solution. In contrast, the SPOCQ reaction is about 150 times slower on the surface than in solution. In other words: the solution-based kinetics, but also quantum chemical data mimicking solutions, provide an important first indication on the relative rate in crowded environments—however, these data may still be up to two orders of magnitude off when predicting relative rates and efficiencies of different click or coupling reactions under the conditions where these reactions are actually most useful, namely in crowded environments. One-on-one transposition of solution data to for example, surface modification, polymer modification or bio-conjugation efficacy is therefore not generally allowed, and more detailed considerations and/or calculations are in order. Surface-bound rates might more closely mimic the rates relevant in those situations.

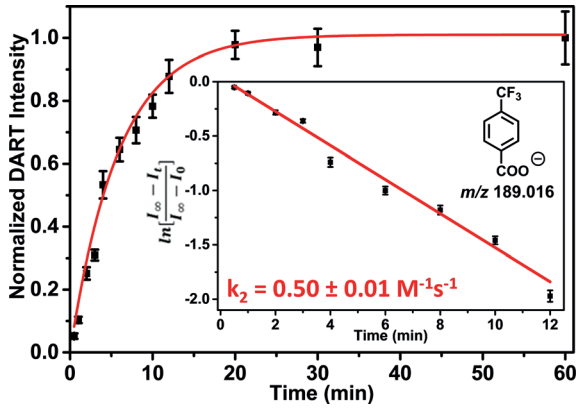


Figure 5. Normalized DART-HRMS intensity versus time (minutes) for reaction between $\mathbf{M_3}$ surfaces and **5**. Inset: Linear plots of $\ln[(I_\infty - I_t)/(I_\infty - I_0)]$ versus time to obtain the pseudo-first-order constants, from which k_2 was determined.

As suggested by Sharpless, Barner-Kowollik and others,[4a,9] click reactions have to fulfill stringent criteria of fast rate, high efficiency, modularity and orthogonality. We demonstrate that indeed our reaction proceeds with fast kinetics and high efficiencies both in solution and on surfaces for two distinct examples, monolayers and polymer brushes, thus validating its click character. Finally, the very slow reactivity of 1,3-disubstituted cyclopropenes towards for example, azides and nitrile imines[23] should allow a preferential and orthogonal reactivity towards quinones as it does towards tetrazines.[23]

In conclusion, we report a novel strain-promoted reaction between *o*-quinones and strained alkenes (1-methyl-3-substituted cyclopropenes) with reaction rates paralleling that of click reactions of cyclopropenes with unsymmetrical tetrazines. In addition, we show that reaction is quantitative for monolayer functionalization and high yielding for polymer brushes. Finally we show that the small size of the cyclopropene moiety is highly advantageous in crowded environments, as present in for example, polymer and bio-conjugation reactions, and on surfaces. Furthermore, we believe that the use of 1-methyl-3-substituted cyclopropenes will therefore also be highly useful for bio-conjugations that require small reagents.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: kinetics · mass spectrometry · metal-free click reactions · monolayers · polymer brushes

[1] J. E. Moses, A. D. Moorhouse, *Chem. Soc. Rev.* **2007**, *36*, 1249 – 1262.
[2] a) P. Thirumurugan, D. Matosiuk, K. Jozwiak, *Chem. Rev.* **2013**, *113*, 4905 – 4979; b) V. Castro, H. Rodríguez, F. Albericio, *ACS Comb. Sci.* **2016**, *18*, 1 – 14.
[3] a) C. Barner-Kowollik, F. E. Du Prez, P. Espeel, C. J. Hawker, T. Junkers, H. Schlaad, W. Van Camp, *Angew. Chem. Int. Ed.* **2011**, *50*, 60 – 62; *Angew. Chem.* **2011**, *123*, 61 – 64; b) J. Escorihuela, A. T. M. Marcelis, H. Zuilhof, *Adv. Mater. Interfaces* **2015**, *2*, 1500135.
[4] a) H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2001**, *40*, 2004 – 2021; *Angew. Chem.* **2001**, *113*, 2056 – 2075; b) C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057 – 3064.
[5] S. T. Laughlin, J. M. Baskin, S. L. Amacher, C. R. Bertozzi, *Science* **2008**, *320*, 664 – 667.
[6] A. C. Knall, C. Slugovc, *Chem. Soc. Rev.* **2013**, *42*, 5131 – 5142.
[7] J. Dong, L. Krasnova, M. G. Finn, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2014**, *53*, 9430 – 9448; *Angew. Chem.* **2014**, *126*, 9584 – 9603.
[8] a) S. Li, P. Wu, J. E. Moses, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2017**, *56*, 2903 – 2908; *Angew. Chem.* **2017**, *129*, 2949 – 2954; b) B. Gao, L. Zhang, Q. Zheng, F. Zhou, L. M. Klivansky, J. Lu, Y. Liu, J. Dong, P. Wu, K. B. Sharpless, *Nat. Chem.* **2017**, *9*, 1083.
[9] a) C. D. McNitt, H. Cheng, S. Ullrich, V. V. Popik, M. Bjerknes, *J. Am. Chem. Soc.* **2017**, *139*, 14029 – 14032; b) H. Frisch, D. E. Marschner, A. S. Goldmann, C. Barner-Kowollik, *Angew. Chem. Int. Ed.* **2018**, *57*, 2036 – 2045; *Angew. Chem.* **2018**, *130*, 2054 – 2064, and references in there.
[10] H. L. Kim, K. Sachin, H. J. Jeong, W. Choi, H. S. Lee, D. W. Kim, *ACS Med. Chem. Lett.* **2015**, *6*, 402 – 407.
[11] a) A. Borrmann, O. Fatunsin, J. Dommerholt, A. M. Jonker, D. W. P. M. Löwik, J. C. M. van Hest, F. L. van Delft, *Bioconjugate Chem.* **2015**, *26*, 257 – 261; b) J. J. Bruins, B. Albada, F. L. van Delft, *Chem. Eur. J.* **2018**, https://doi.org/10.1002/chem.201703919.
[12] J. J. Bruins, A. H. Westphal, B. Albada, K. Wagner, L. Bartels, H. Spits, W. J. H. van Berkel, F. L. van Delft, *Bioconjugate Chem.* **2017**, *28*, 1189 – 1193.
[13] A. George, G. Krishna Priya, M. Ilamaran, N. R. Kamini, S. Ganesh, S. Easwaramoorthi, N. Ayyadurai, *ChemistrySelect* **2017**, *2*, 7117 – 7122.
[14] A. M. Jonker, A. Borrmann, E. R. H. van Eck, F. L. van Delft, D. W. P. M. Löwik, J. C. M. van Hest, *Adv. Mater.* **2015**, *27*, 1235 – 1240.
[15] D. Nematollahi, S. Dehdashtian, *Tetrahedron Lett.* **2008**, *49*, 645 – 649.
[16] D. Gahtory, R. Sen, S. Pujari, Q. Zheng, J. E. Moses, K. B. Sharpless, H. Zuilhof, submitted to Angew. Chem. **2018**.
[17] R. Sen, J. Escorihuela, F. L. van Delft, H. Zuilhof, *Angew. Chem. Int. Ed.* **2017**, *56*, 3299 – 3303; *Angew. Chem.* **2017**, *129*, 3347 – 3351.
[18] a) J. Yang, J. Šečkutė, C. M. Cole, N. K. Devaraj, *Angew. Chem. Int. Ed.* **2012**, *51*, 7476 – 7479; *Angew. Chem.* **2012**, *124*, 7594 – 7597; b) J. Yang, Y. Liang, J. Šečkutė, K. N. Houk, N. K. Devaraj, *Chem. Eur. J.* **2014**, *20*, 3365 – 3375.
[19] A.-K. Späte, V. F. Schart, J. Häfner, A. Niederwieser, T. U. Mayer, V. Wittmann, *Beilstein J. Org. Chem.* **2014**, *10*, 2235 – 2242.
[20] A.-K. Späte, H. Bußkamp, A. Niederwieser, V. F. Schart, A. Marx, V. Wittmann, *Bioconjugate Chem.* **2014**, *25*, 147 – 154.
[21] D. M. Patterson, L. A. Nazarova, B. Xie, D. N. Kamber, J. A. Prescher, *J. Am. Chem. Soc.* **2012**, *134*, 18638 – 18643.
[22] a) R. Sen, D. Gahtory, J. Escorihuela, J. Firet, S. P. Pujari, H. Zuilhof, *Chem. Eur. J.* **2017**, *23*, 13015 – 13022; b) D. Gahtory, R. Sen, M. M. J. Smulders, H. Zuilhof, *Faraday Discuss.* **2017**, *204*, 383 – 394.
[23] F. Liu, Y. Liang, K. N. Houk, *Acc. Chem. Res.* **2017**, *50*, 2297 – 2308.
[24] M. J. Frisch et al. Gaussian 16, Revision A.03, Gaussian, Inc., Wallingford CT, **2016**.
[25] J. Escorihuela, A. Das, J. Looijen, F. L. van Delft, A. Aquino, H. Lischka, H. Zuilhof, *J. Org. Chem.* **2018**, *83*, 244 – 252.
[26] A. R. Kuzmyn, A. de los Santos Pereira, O. Pop-Georgievski, M. Bruns, E. Brynda, C. Rodriguez-Emmenegger, *Polym. Chem.* **2014**, *5*, 4124 – 4131.

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Communications

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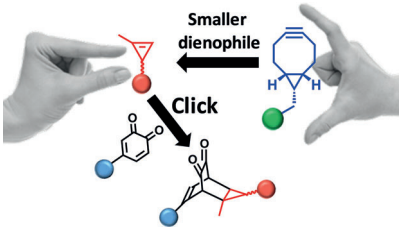
Strain-Promoted Cycloaddition of Cyclopropenes with *o*-Quinones: A Rapid Click Reaction



Strain-promoted reactions: Methyl-substituted cyclopropenes have been used for conjugation with *o*-quinones in solution and on a surface. By taking advantage of the small size of cyclopropene reactants a conjugation system with fairly rapid kinetics is built.

