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Transient Protection of Strained Alkynes from Click Reaction via Complexation with Copper

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Supporting Information

ABSTRACT: A transient protection method of cyclooctynes from a click reaction with an azide through 1:1 complexation with a cationic copper(I) salt is reported. The application of the method to a cyclooctyne bearing a terminal alkyne enabled the selective copper-catalyzed click conjugation with an azide at the terminal alkyne moiety, which made cyclooctyne derivatives readily accessible.

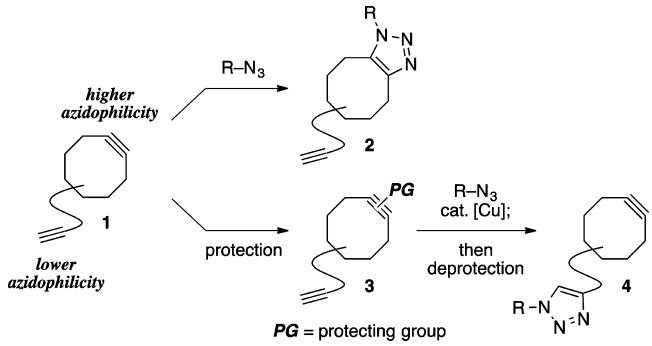
The click reaction,[1] notably the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC), has become one of the most reliable methods for connecting molecules in broad disciplines including materials chemistry and chemical biology.[2] Moreover, copper-free click reactions that exploit functionalized strained alkynes, and particularly cyclooctyne derivatives that spontaneously react with azides,[3] have enabled the chemical modification of azido-incorporated biomolecules in cultured cells and in living animals, greatly expanding the utility of click chemistry.[4] To make the method more practical, various types of cyclooctynes with improved properties, such as increased azidophilicity and reduced hydrophobicity, have been developed, and a variety of derivatives bearing functional moieties, such as fluorescent or biotinyl groups, have been prepared.[5] Some of these cyclooctynes are commercially available, but on-demand synthesis is required for those with a new functionality, which is not always easy because of the high reactivity of the strained alkyne moiety.[6]

Versatile functionalized cyclooctynes will be readily available if the CuAAC reaction can be performed selectively at the terminal alkyne moiety of diyne compounds such as **1** (Scheme 1). However, the catalyst-free click conjugation of diyne **1** with an azide proceeds only at the higher azidophilic strained alkyne side to afford undesired triazole **2**. To address this issue, the protection of the strained alkyne to allow the CuAAC reaction to proceed at the unprotected terminal alkyne moiety of **3** should be an effective solution for the preparation of functionalized cyclooctyne **4**. In this study, we present a novel method for the protection of strained alkynes that enables the facile preparation of cyclooctyne derivatives.

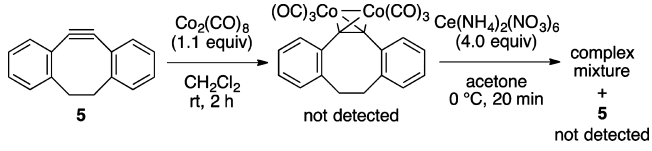
Our initial attempt to protect the strained alkyne moiety of dibenzo-fused cyclooctyne **5** with $Co_2(CO)_8$ was unfruitful (Scheme 2). This dinuclear cobalt complex is the most widely used reagent for alkyne protection due to its stable complexation ability with alkynes and the availability of known and reliable decomplexation methods.[7] While the protection/deprotection of some medium-sized cycloalkynes via cobalt complexation has been demonstrated, there is, to the best of our knowledge, no report on the successful regeneration of the alkyne moiety via the decomplexation of cycloalkyne–cobalt complexes when the ring size is less than nine.[8] Most of these complexes were used directly in valuable transformations, such as the Pauson–Khand reaction, without isolation of demetalated cycloalkynes.[9] In our case, even the complexation of **5** with $Co_2(CO)_8$ was unsuccessful (Scheme 2).

Although cyclooctynes readily coordinate to metals and are known to form complexes with transition metals, including platinum, gold, silver, and copper,[10] the synthetic utility of these complexes has not been explored. We therefore searched for an appropriate metal that forms a complex with cyclo-

Scheme 1. Synthetic Plan for Functionalized Cyclooctynes via Click Conjugation



Scheme 2. Attempted Protection of Cyclooctyne 5 with a Dinuclear Cobalt Reagent



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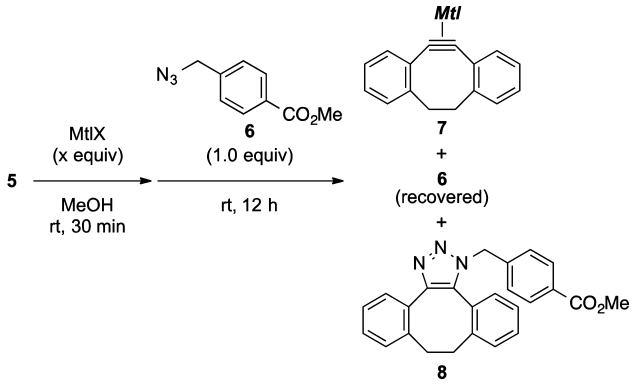
octynes, sufficiently stable to prevent the reaction with an azide, and also capable of dissociating under mild conditions to regenerate the highly reactive strained alkyne moiety.

After extensive studies, the complexation of cyclooctyne **5** with a copper salt was found to meet these criteria (Table 1).

Table 1. Screening of Metals To Mask the Clickability of Cyclooctyne 5



entry	MtlX	*x* (equiv)	**6** (%)[a]	**8** (%)[a]
1	none	–	0	97[b]
2	$AgBF_4$	1.0	18	77
3	AuCl	1.0	11	61
4	AuCl + $AgBF_4$	1.0	40	50
5	CuCl	1.0	12	78
6	$(MeCN)_4CuBF_4$	1.0	quant.	0
7	$(MeCN)_4CuBF_4$	2.0	quant.	0
8	$(MeCN)_4CuBF_4$	0.5	46	47

[a]Yields based on ^{1}H NMR analysis by using 1,1,2,2-tetrachloroethane as an internal standard, unless otherwise noted. [b]Isolated yield.

Transition metal salts were screened by pretreating them with **5** in methanol for 30 min, followed by addition of azide **6**. After stirring each of the mixtures for 12 h, the quantities of the click product **8** and recovered **6** were determined. Without the addition of metal under these conditions, all of the azide **6** was consumed to furnish triazole **8** nearly quantitatively (entry 1). Several coinage metal salts were found to be effective at inhibiting the triazole formation (entries 2–6). In particular, the cationic copper(I) salt $(MeCN)_4CuBF_4$ provided an excellent result: the cycloaddition reaction was completely inhibited and azide **6** was completely recovered (entry 6). This result suggested the in situ formation of cyclooctyne–metal complex **7**. Notably, the use of one-half of a molar equivalent of the copper reagent led to the protection of only half of the starting cyclooctyne **5** (entry 8), indicating that more than an equimolar amount of the copper reagent is required to completely protect the strained alkyne from cyclization with the azide.

NMR studies then provided valuable insights into the formation of the cycloalkyne–copper complex (Figure 1). Addition of an equimolar amount of $(MeCN)_4CuBF_4$ to a methanol-d_4 solution of cyclooctyne **5** caused a slight downfield shift in the signals for the aromatic protons in the ^{1}H NMR spectrum, suggesting the formation of complex **9** (Figure 1A). Addition of azide **6** to this mixture clearly demonstrated the independent existence of **9** and **6**, which was in good agreement with the experimental results (Table 1, entry 6). In addition, a single set of peaks was observed in the ^{1}H and ^{13}C NMR spectra when cyclooctyne **5** was treated with 0.1–2.0 equiv of

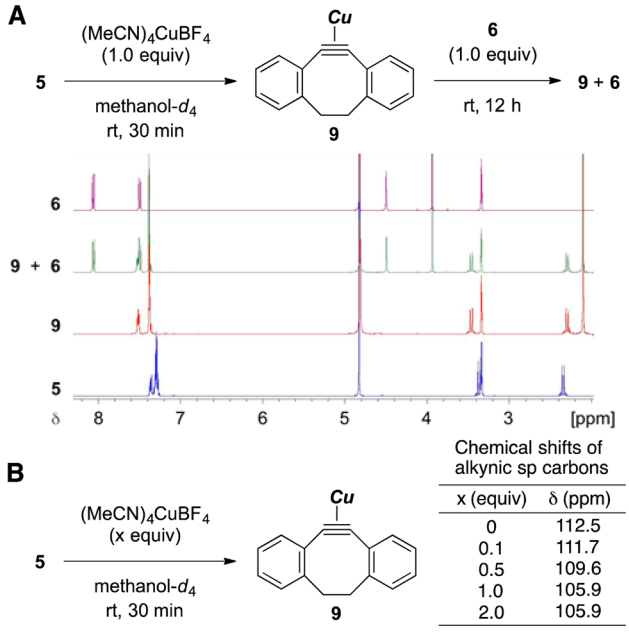


Figure 1. NMR studies of cyclooctyne **5** following the addition of a copper salt. (A) ^{1}H NMR spectra of cyclooctyne **5**, azide **6**, and **5** with an equimolar amount of copper salt in the presence and absence of **6**. (B) ^{13}C chemical shifts of the alkyne carbons in **5** following the addition of the copper salt.

the copper reagent. This result indicated that complex **9** and uncomplexed alkyne **5** rapidly exchanged the copper, which is similar to the behavior observed for the previously reported cyclooctyne–coinage metal complexes.[10f,11] Furthermore, a gradual upfield shift of the signals for the alkyne carbons of **5** was observed in the ^{13}C NMR spectrum as the amount of added copper salt was increased from 0.1 to 1.0 equiv (Figure 1B). Further addition of copper, however, did not induce this ^{13}C chemical shift, supporting the 1:1 complexation of the cyclooctyne **5** with the copper salt.

X-ray crystallographic analysis also suggested a 1:1 complexation. The recrystallization of the cycloalkyne–copper complex from CH_2Cl_2–hexane provided a single crystal that was found to be the tricoordinate cycloalkyne–copper(I) complex **10**, containing acetonitrile and water as ligands (Figure 2).[12] The C–C≡C angle of **10** was decreased by 4° compared to that of

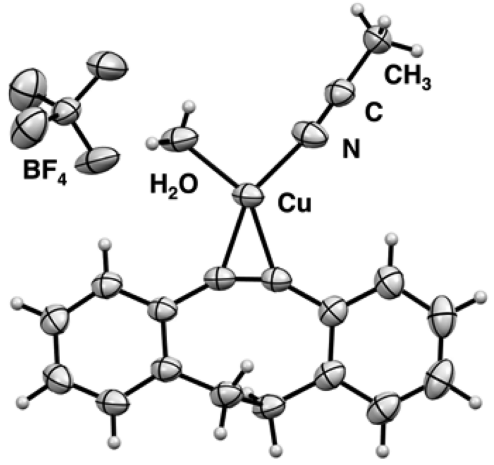


Figure 2. X-ray structure of cyclooctyne–copper complex **10**.

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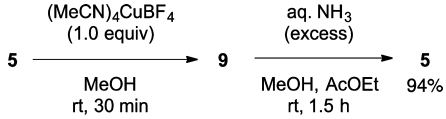
uncoordinated cyclooctyne **5** as found for its optimized structure using the density functional theory (DFT) method at the B3LYP/6-31G(d) level.[13] Notably, this result is in good agreement with the angles observed for the previously reported cyclooctyne–copper complexes.[10c]

Fortunately, the treatment with aqueous ammonia[14] led to the successful dissociation of the cycloalkyne–copper complex **9** and cyclooctyne **5** was regenerated with high efficiency (Scheme 3). These results clearly confirmed the smooth

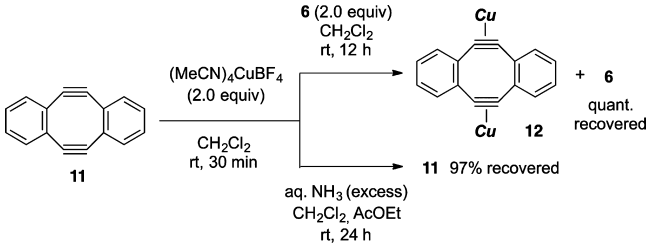
Scheme 3. Regeneration of Cyclooctyne from the Copper Complex



reversibility of the complexation of the strained alkyne by the copper salt and indicated that this approach should be suitable as a protection method for cycloalkynes.

Furthermore, we demonstrated that complexation using the copper salt prevents the Sondheimer diyne[15] (**11**) from participating in click reactions (Scheme 4). We previously

Scheme 4. Masking the Clickability of Sondheimer Diyne with Copper

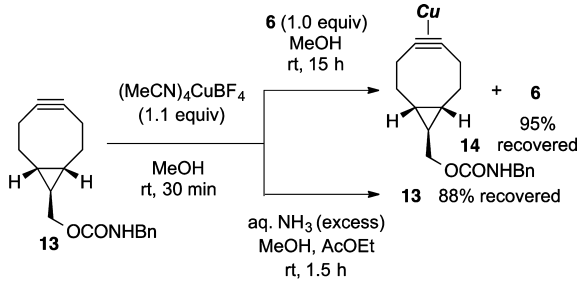


reported that diyne **11** spontaneously reacts with two azides to afford the bistriazole in high yield.[6a] However, the pretreatment of **11** with 2 molar equiv of the copper salt completely protected the two strained alkyne moieties from cyclization with the azides. In addition, the assumed diyne–dicopper complex **12** was uneventfully returned to diyne **11** by treatment with aqueous ammonia.

In addition to dibenzo-fused cyclooctynes, bicyclo[6.1.0]-nonyne (BCN) derivative[5e] **13** was similarly protectable with copper, expanding the scope of the method (Scheme 5).

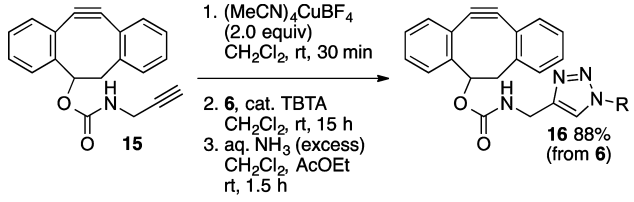
The utility of copper complexation as a method for the protection of strained alkynes was then demonstrated in the selective click conjugation of diyne **15** at the terminal alkyne

Scheme 5. Protection of BCN with Copper



moiety, which was achieved using a simple one-pot three-step procedure (Scheme 6). Diyne **15** was first treated with an

Scheme 6. Synthesis of a Click-Modified Cyclooctyne via Transient Protection of a Strained Alkyne



excess of the cationic copper salt for complexation, and then azide **6** and a catalytic amount of tris(benzyltriazolylmethyl)-amine (TBTA)[16] were added to perform the CuAAC reaction. Finally, aqueous ammonia was added to dissociate the copper, furnishing the desired terminal alkyne-modified cyclooctyne **16** in high overall yield.[17] The key to the success of this reaction must be the moderate strength of the coordination bond between the strained alkyne and the copper,[11] which must be sufficiently strong to withstand the CuAAC conditions but readily dissociable under specific mild conditions. Note that the same copper salt played a contrasting double role for the two alkynes in this transformation: the prevention of the clickability of the strained alkyne as a protective group and the promotion of the click reaction of the terminal alkyne as a catalyst.[2]

In conclusion, we demonstrated that the strained alkyne moiety of a cyclooctyne can be transiently masked via 1:1 complexation with a cationic copper(I) salt. Furthermore, this method enabled the facile modification of a cyclooctyne derivative bearing a terminal alkyne moiety via the CuAAC reaction. Investigation of the scope of alkyne substrates, including acyclic ones,[18] that can be protected by this method, evaluation of the stability of the strained alkyne–copper complex under various reaction conditions, and application of the method to the synthesis of diverse cyclooctyne derivatives with various functional groups are now in progress.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and characterization for new compounds including copies of NMR spectra and the X-ray crystallographic data for cycloalkyne–copper(I) complex **10** (CCDC 1016209). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004−2021. (b) Lahann, J. *Click Chemistry for Biotechnology and Materials Science*; John Wiley & Sons: West Sussex, 2009.

(2) (a) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057−3064. (b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596−2599. (c) Meldal, M.; Tornøe, C. W. *Chem. Rev.* **2008**, *108*, 2952−3015.

(3) Wittig, G.; Krebs, A. *Chem. Ber.* **1961**, *94*, 3260−3275.

(4) (a) Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2004**, *126*, 15046−15047. (b) Laughlin, S. T.; Baskin, J. M.; Amacher, S. L.; Bertozzi, C. R. *Science* **2008**, *320*, 664−667. For reviews, see: (c) Sletten, E. M.; Bertozzi, C. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 6974−6998. (d) Debets, M. F.; van der Doelen, C. W. J.; Rutjes, F. P. J. T.; van Delft, F. L. *ChemBioChem* **2010**, *11*, 1168−1184. (e) Jewett, J. C.; Bertozzi, C. R. *Chem. Soc. Rev.* **2010**, *39*, 1272−1279.

(5) Selected examples: (a) Codelli, J. A.; Baskin, J. M.; Agard, N. J.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2008**, *130*, 11486−11493. (b) Ning, X.; Guo, J.; Wolfert, M. A.; Boons, G.-J. *Angew. Chem., Int. Ed.* **2008**, *47*, 2253−2255. (c) Poloukhtine, A. A.; Mbua, N. E.; Wolfert, M. A.; Boons, G.-J.; Popik, V. V. *J. Am. Chem. Soc.* **2009**, *131*, 15769−15776. (d) Jewett, J. C.; Sletten, E. M.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2010**, *132*, 3688−3690. (e) Dommerholt, J.; Schmidt, S.; Temming, R.; Hendriks, L. J. A.; Rutjes, F. P. J. T.; van Hest, J. C. M.; Lefeber, D. J.; Friedl, P.; van Delft, F. L. *Angew. Chem., Int. Ed.* **2010**, *49*, 9422−9425.

(6) (a) Kii, I.; Shiraishi, A.; Hiramatsu, T.; Matsushita, T.; Uekusa, H.; Yoshida, S.; Yamamoto, M.; Kudo, A.; Hagiwara, M.; Hosoya, T. *Org. Biomol. Chem.* **2010**, *8*, 4051−4055. (b) Yoshida, S.; Shiraishi, A.; Kanno, K.; Matsushita, T.; Johmoto, K.; Uekusa, H.; Hosoya, T. *Sci. Rep.* **2011**, *1*, 82.

(7) Hegedus, L. S.; Söderberg, B. C. G. *Transition Metals in the Synthesis of Complex Organic Molecules*, 3rd ed.; University Science Books: Sausalito, CA, 2009; pp 321−326.

(8) Selected examples: (a) Magnus, P.; Carter, R.; Davies, M.; Elliott, J.; Pitterna, T. *Tetrahedron* **1996**, *52*, 6283−6306. (b) Teobald, B. J. *Tetrahedron* **2002**, *58*, 4133−4170. (c) Young, D. G. J.; Burlison, J. A.; Peters, U. *J. Org. Chem.* **2003**, *68*, 3494−3497. (d) Yang, Z.-Q.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 9602−9603. (e) Díaz, D. D.; Betancort, J. M.; Martín, V. S. *Synlett* **2007**, 343−359.

(9) Direct transformations of medium-ring cycloalkyne−dicobalt complexes have been developed for the synthesis of complex molecules. For selected examples, see: (a) Jamison, T. F.; Shambayati, S.; Crowe, W. E.; Schreiber, S. L. *J. Am. Chem. Soc.* **1997**, *119*, 4353−4363. (b) Tanino, K.; Shimizu, T.; Miyama, M.; Kuwajima, I. *J. Am. Chem. Soc.* **2000**, *122*, 6116−6117. (c) Baba, T.; Huang, G.; Isobe, M. *Tetrahedron* **2003**, *59*, 6851−6872. (d) Iwasawa, N.; Inaba, K.; Nakayama, S.; Aoki, M. *Angew. Chem., Int. Ed.* **2005**, *44*, 7447−7450.

(10) (a) Wittig, G.; Dorsch, H.-L. *Liebigs Ann. Chem.* **1968**, *711*, 46−54. (b) Wittig, G.; Fischer, S. *Chem. Ber.* **1972**, *105*, 3542−3552. (c) Gröger, G.; Behrens, U.; Olbrich, F. *Organometallics* **2000**, *19*, 3354−3360. (d) Shelbourne, M.; Chen, X.; Brown, T.; El-Sagheer, A. H. *Chem. Commun.* **2011**, *47*, 6257−6259. (e) Das, A.; Dash, C.; Yousufuddin, M.; Celik, M. A.; Frenking, G.; Dias, H. V. R. *Angew. Chem., Int. Ed.* **2012**, *51*, 3940−3943. (f) Das, A.; Dash, C.; Celik, M. A.; Yousufuddin, M.; Frenking, G.; Dias, H. V. R. *Organometallics* **2013**, *32*, 3135−3144. For a review of metal complexes of cycloalkynes, see: (g) Bennett, M. A.; Schwemlein, H. P. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1296−1320.

(11) Given that cycloaddition with azide did not proceed, the equilibrium between the complexed and uncomplexed cyclooctyne would be dominated by the side of the 1:1 cycloalkyne−copper complex even in the presence of a terminal alkyne, although the details are yet to be investigated.

(12) The recrystallization of copper complex **10** was performed using solvents from a bottle without special drying.

(13) We optimized the structure of dibenzo-fused cyclooctyne **5** using the DFT method at the B3LYP/6-31G(d) level in order to compare the results with the experimental data obtained for the crystal structure of cycloalkyne−copper(I) complex **10**. See the Supporting Information.

(14) Fields, E. K. Arynes. In *Organic Reactive Intermediates*; McManus, S. P., Ed.; Academic Press: New York, 1973; p 475.

(15) Wong, H. N. C.; Garratt, P. J.; Sondheimer, F. *J. Am. Chem. Soc.* **1974**, *96*, 5604−5605.

(16) Rodionov, V. O.; Presolski, S. I.; Díaz, D. D.; Fokin, V. V.; Finn, M. G. *J. Am. Chem. Soc.* **2007**, *129*, 12705−12712.

(17) The structure of triazole **16** was confirmed by further transformation. The reaction of **16** with ethyl azidoacetate without the copper catalyst afforded bistriazole in 89% yield, which was different from the product obtained from unprotected diyne **15** by the sequential double-click reaction, i.e. strain-promoted cycloaddition with azide **6** followed by CuAAC reaction with ethyl azidoacetate. See the Supporting Information for details.

(18) Preliminary studies suggested the applicability of the protection method to unstrained internal alkynes. For example, pretreatment of a mixture of dimethyl acetylenedicarboxylate (DMAD) and phenylacetylene with $(MeCN)_4CuBF_4$ in THF followed by the addition of benzyl azide and TBTA and heating the reaction mixture at 65 °C for 24 h afforded the CuAAC product, 1-benzyl-4-phenyl-1*H*-1,2,3-triazole (quant.), with recovery of DMAD (52%). In this case, Huisgen cycloaddition between DMAD and benzyl azide did not proceed, although the cycloadduct was obtained in 61% yield when the reaction was performed without premixing with copper. See the Supporting Information for details.