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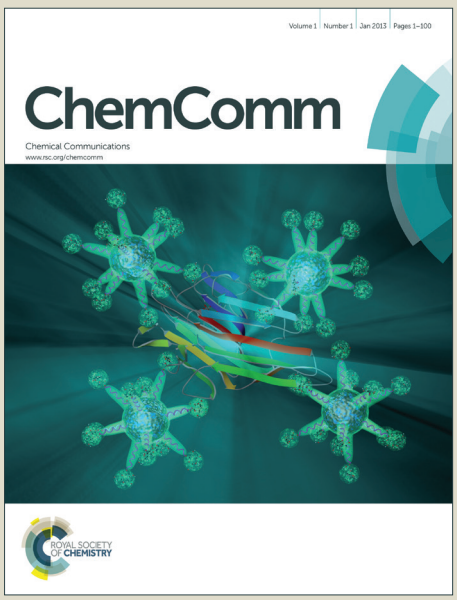

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ARTICLE TYPE

Expanding the Scope of Strained-Alkyne Chemistry: a Protection-Deprotection Strategy *via* the Formation of a Dicobalt-Hexacarbonyl Complex

Pierangelo Gobbo[a], Tommaso Romagnoli[a], Stephanie M. Barbon[a], Jacquelyn T. Price[a], Jennifer Keir[a], Joe B. Gilroy*[a] and Mark S. Workentin*[a]

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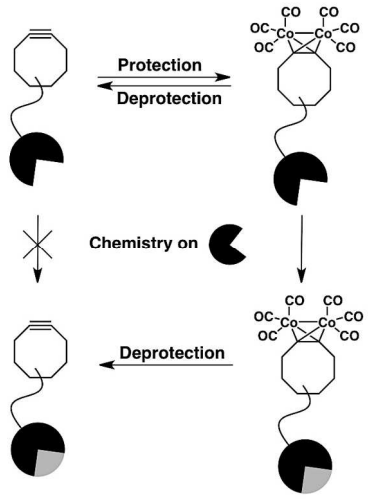
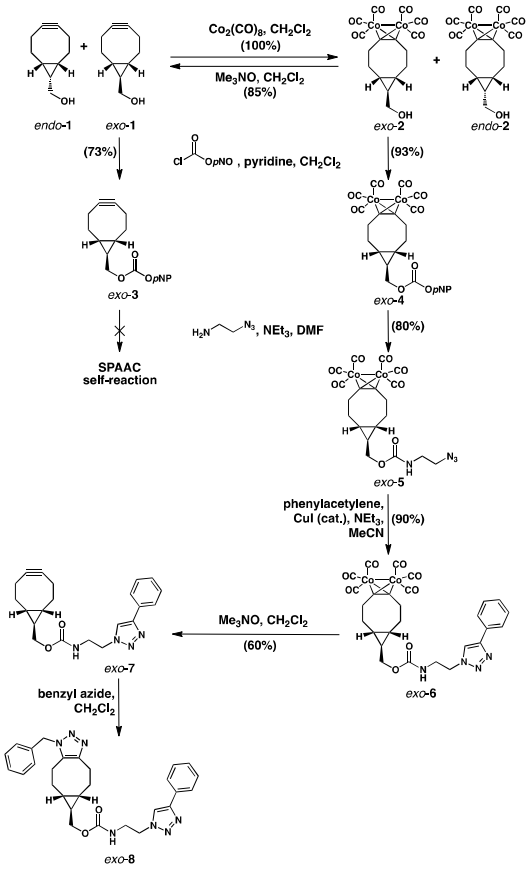
A protection-deprotection strategy for strained alkynes used for bioorthogonal chemistry is reported. A strained alkyne can be protected with dicobalt-octacarbonyl and we demonstrate for the first time that the strain alkyne can be re-formed and isolated under mild reaction conditions for further bioorthogonal reactivity. The protection-deprotection strategy herein reported will expand the versatility of strained alkynes for the preparation of substrates in chemical biology and materials applications.

Strain-promoted cycloaddition (SPC) reactions (alkyne-azide – SPAAC, and alkyne-nitrone – SPANC) are click reactions designed to occur with very fast reaction kinetics, high efficiency and chemoselectivity. Because of these characteristics they are now exploited in many fields of science. Since Bertozzi showcased the characteristics of SPAAC for the *in vivo* labeling of carbohydrates in the zebrafish,[1] the SPC reactions have been used to gain new insights into many biological processes (*e.g.*, activity-based protein profiling,[2] protein lipidation and lipid trafficking[3]), the synthesis of PET contrast agents,[4] and the modification of materials[5] and nanomaterials[6]. Currently, much effort is being devoted to the design and synthesis of stable strained alkynes with fast reaction kinetics.[7] However, the more reactive the strained alkyne the higher the chance of undesired side reactivity, especially addition-type reactions with nucleophiles.[8-10] This side reactivity makes their functionalization challenging because a very narrow range of reaction conditions are suitable for their attachment to a substrate (*e.g.*, biomolecule, fluorescent probe, material, etc.) while simultaneously preserving the integrity of the strained alkyne. As a consequence, the further development of this kind of technology has been hindered. In order to properly functionalize strained alkynes, it is therefore necessary to employ protection-deprotection strategies. For example, we have recently demonstrated the use of the SPAAC reaction for the bioconjugation of peptides onto azide–functionalized small, water-soluble gold nanoparticles. To achieve bioconjugation, it was necessary i) to protect the nucleophilic side chains of the peptide; ii) couple the strained-alkyne to the protected peptide; iii) click the strained alkyne-functionalized peptide onto the nanomaterial, and iv) deprotect the peptide residues.[11] This strategy proved to be reliable, but the development of a more facile and general protection-deprotection strategy is highly desirable in order to further expand the applications of SPC reactions.



Scheme 1. Schematic representation of our protection and deprotection strategy.

One of the most commonly used strategies in organic synthesis for the protection of linear alkynes involves the complexation of the alkyne with dicobalt-octacarbonyl. The linear alkyne can then be deprotected using different methods, for example by employing oxidizing or reducing agents.[12, 13] However, this strategy is well known to be challenging when applied to cyclic alkynes. In particular, the regeneration of the strained alkyne is extremely difficult to perform. The regenerated alkyne reacts *in situ* to yield cycloalkenes, anhydrides, diiodocycloalkenes, cycloalkanones, *etc.* as the major products.[14] In fact, Yoshida and coworkers recently reported a creative approach to protect strained alkynes reversibly via the formation of a copper complex because their attempts to apply the dicobaltoctacarbonyl-based strategy were unsuccessful.[15] They demonstrated that the Cu-protected strained alkyne could then be functionalized with a linear alkyne functionality that could undergo the copper-catalyzed alkyne azide-cycloaddition (CuAAC) reaction. The coordination of the



Scheme 2. Reaction scheme showing the different synthetic steps for the functionalization of the protected strained alkyne. The same transformations are also possible with the *endo* isomer.

strained alkyne to the copper center was reversed in excess NH_3. A strained-alkyne-$Co_2(CO)_6$ complex may be more desirable in many cases because it is stable under a wide variety of reaction conditions, and therefore would allow for a versatile and "orthogonal" functionalization somewhere else in the molecule. The strained alkyne can also be regenerated under much more mild conditions. Linear alkyne-$Co_2(CO)_6$ complexes are also gaining interest within the life sciences because they can be easily introduced into cells and biomolecules, and provide invaluable insights into biological processes due to their characteristic absorbances in infrared (carbonyls) and UV-visible regions.[16-19] Extending this to strained alkynes may be desirable as would be the ability to regenerate the strained alkyne *in situ*. More generally, given the growing application of strain promoted reactions in chemical biology (*vide supra*) developing additional protection-deprotection strategies for the strained alkyne to allow chemical modifications of molecules containing them is important, as highlighted by Yoshida and co-workers. Our group has been working on such a protection-deprotection strategy based on the reversible complexation of the strained alkyne for potential applications in (bio)materials chemistry. Herein we report our results showing that the complexation of a strained alkyne with $Co_2(CO)_8$ is possible, and that the resulting cobalt-hexacarbonyl complex is stable under different reaction conditions (including in the presence of Cu(I) for copper-catalyzed cycloaddition reactions) allows for an efficient and

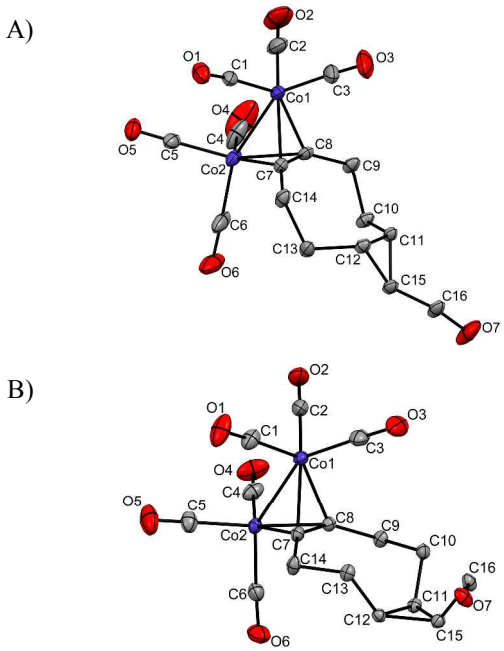


Fig. 1. Solid-state structures of A) *exo*-**2**, and B) *endo*-**2**. Thermal ellipsoids are shown at 50% probability and hydrogen atoms have been removed for clarity. For *exo*-**2** only one of two closely related molecules in the asymmetric unit are presented. For *endo*-**2** only one of three closely related molecules in the asymmetric unit are presented.

versatile functionalization elsewhere in the molecule. Most importantly, we demonstrate for the first time that the strained alkyne can be reformed and isolated in good yields under very mild reaction conditions using biocompatible trimethylamine *N*-oxide (Me_3NO) as the decomplexation agent (see Scheme 2).

To showcase our protection-deprotection strategy, we selected bicycle[6.1.0]-nonyne-methanol (BCN) as the strained alkyne because of its high stability, high 2nd order rate constant with azides or nitrones[20], simple molecular structure, and facile and cheap synthesis that allows production on the gram-scale. These characteristics make BCN a useful strained alkyne for materials chemistry applications. Bicycle[6.1.0]-nonyne-methanol *endo/exo*-**1** (see Scheme 2) was synthesized according to procedure reported by J. Dommerholt *et al*. (see ESI).[20] The two isomers, *endo*-**1** and *exo*-**1**, were synthesized by first separating the *endo* and *exo* isomers (64% *exo* and 36% *endo*) of the product formed during the cyclopropanation step of the synthesis and further reacting the separated isomers according to published procedures (see ESI).[20] In a typical strained alkyne protection reaction, *endo/exo*-**1** were dissolved in dry dichloromethane and 1.1 equivalents of dicobalt-octacarbonyl were added to the solution. The reaction was carried out for 60 min at room temperature and under inert atmosphere. $CO_{(g)}$ evolution was observed during the reaction, indicating a reaction between $Co_2(CO)_8$ and the alkyne. Purification of the target complex from the excess of $Co_2(CO)_8$ lead to the desired cobalt-hexacarbonyl-protected *endo/exo*-**2** in quantitative yield, as confirmed by ^1H and ^{13}C NMR spectroscopy, high-resolution mass spectrometry, UV-Vis and IR spectroscopy, and X-ray

crystallography (see ESI). In particular the IR spectrum shows the appearance of characteristic intense peaks at 2082, 2035 and 1982 cm^{-1} due to the stretching of the carbonyls.[21] X-ray diffraction studies showed that in both complexes the eight membered ring exists in a distorted chair conformation, and confirmed the structure of the two isomers, showing that in the *exo* isomer (see Figure 1A), the alcohol substituent is *anti* to the eight-member ring, while in the *endo* conformation (see Figure 1B) is *syn*. X-ray diffraction also demonstrated lengthening of the C-C triple bond by more than 0.1 Å, and narrowing of C-C-C angles around the protected alkyne triple bond by more than 16°, compared to free cyclooctyne.[22, 23] These characteristics are not commonly observed for other metal complexes of strained alkynes.[24]

Regeneration of the strained (*i.e.*, deprotected) alkyne was carried out under very mild reaction conditions by employing the biocompatible reagent trimethylamine-*N*-oxide (Me_3NO).[25] A sample of *endo/exo*-**2** was dissolved in dichloromethane in air and 5 equivalents of Me_3NO were added to the solution. In 4 h the decomplexation was complete, and pure *endo/exo*-**1** was recovered in 85% yield. It is noteworthy that, despite the unusual structural metrics of the protected alkyne, we retain the ability to re-establish the triple bond character of the strained alkyne.

To showcase the validity and generality of our new protection-deprotection strategy, we functionalized the alcohol group of *exo*-**2** with an azide (refer to Scheme 2). *Exo*-**2** was first reacted with 4-nitrophenyl chloroformate with a significantly higher reaction yield than the same reaction on the unprotected *exo*-**1** compound. The structure of *exo*-**4** was verified through X-ray crystallography (see ESI). Within experimental error, no appreciable changes to the bond length or angles of the nine-member ring were observed.

Subsequently, we reacted 2-azidoethanamine with *exo*-**4** to append the azide, forming *exo*-**5**. Compound *exo*-**5** cannot be synthesized in the absence of the protecting group, as it would self-react. The azide end of the protected strained alkyne *exo*-**5** can now be used for the facile introduction of the strained alkyne to other functionalities, biomolecules, molecular probes or materials. As a proof of concept, *exo*-**5** was reacted through copper-catalyzed alkyne-azide cycloaddition reaction with phenylacetylene as a model alkyne. The cycloaddition product, *exo*-**6**, was formed in 90% yield, indicating that the cobalt protecting group is stable in presence of Cu(I). It is expected that *exo*-**5** can safely react also through other click reactions that involve an azide as a reactant, for example with other strained alkynes or modified triphenylphosphines for the Staudinger-Bertozzi ligation. Each of the reactions described above are not possible if the strained alkyne was not protected.

Compounds *exo*-**4**, -**5**, and -**6** were characterized through ^1H NMR, ^{13}C NMR, UV-Vis and IR spectroscopies (see ESI). In particular, UV-Vis spectra of the different cobalt complexes (*exo*-**4** to -**6**) showed minimal changes during the different synthetic steps, while the IR spectra consistently showed the typical cobalt carbonyl peaks at 2082, 2035 and 1982 cm^{-1}. The IR spectrum of compound *exo*-**5** also showed the presence of the asymmetrical azide stretch at 2103 cm^{-1}. All of the ^{13}C NMR spectra showed a broad peak at 200.4 ppm related to the cobalt-bound carbonyl ligands.

The cobalt compounds *exo*-**2** to *exo*-**6** exhibits the typical stability of alkyne-hexacarbonyl complexes.[14, 17] In particular they could be handled under normal atmosphere. However, oxygen and moisture can cause a slow loss of $CO_{(g)}$ from the metal centers, resulting in an appreciable amount of decomposed compound observed after 2-3 days. They can be stored indefinitely at -20 ºC and under normal atmosphere. Nucleophiles, such as primary amines, thiols or sulfides, are also known to displace carbonyls and coordinate the metal centers resulting in slow formation of byproducts.[13, 26, 27]

Exo-**6** was deprotected using Me_3NO in 60% yield, and the resulting strained alkyne *exo*-**7** was finally reacted through the SPAAC reaction with benzyl azide to yield *exo*-**8** in essentially quantitative yield. After the deprotection reaction, the IR spectrum of compound *exo*-**7** showed, as expected, the disappearance of the carbonyl stretching bands, while the ^{13}C NMR spectrum of compound *exo*-**6** showed the disappearance of the peak at 200.4 ppm (see ESI). After the SPAAC reaction, the ^1H NMR spectrum of compound *exo*-**8** showed the appearance of the typical peaks associated with cycloaddition products (1,4-substituted and 1,5-substituted regioisomers) with the benzyl azide as previously reported by J. Dommerholt *et al*.[20]

In conclusion, we have demonstrated that strained alkynes can be protected by forming the corresponding $Co_2(CO)_6$ complexes easily and quantitatively through reaction with $Co_2(CO)_8$. The resulting cobalt-protected-strained-alkynes can be deprotected under very mild reaction conditions in air and at room temperature by using biocompatible trimethylamine *N*-oxide to regenerate the strained alkyne in good to high yields for further modifications. We do not exclude that other harsher oxidative or reductive deprotection methods (*e. g.* ceric ammonium nitrate, ceric sulfate, lithium in liquid ammonia, tributyltin hydride in benzene *etc.*[12]) may successfully be used to regenerate the strained alkyne but may more likely interfere with the stability of the substrate to which the strained alkyne is attached. The protection-deprotection method here described will allow for the facile attachment of strained alkynes to a variety of potential substrates (biomolecules, fluorescent probes, material, etc.) under a wide range of reaction conditions, allowing for the further expansion of the potential applications of the SPC reactions. We are currently exploring the potential of this novel protection-deprotection strategy in materials chemistry.

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Notes and references

[a] *Department of Chemistry and the Centre for Materials and Biomaterials Research (CAMBR), The University of Western Ontario, 1151 Richmond St., London, Ontario, Canada, N6A 5B7.*
Prof. Mark S. Workentin
E-mail: mworkent@uwo.ca
Tel: +1 519-661-2111 ext. 86319
Prof. Joe B. Gilroy
E-mail: joe.gilroy@uwo.ca
Tel: +1 519-661-2111 ext. 81561

† Electronic Supplementary Information (ESI) available: details of synthetic procedures, characterization data for all the compounds, crystal structure of compound *exo*-**4**. See DOI: 10.1039/b000000x/

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