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# A Traceless Staudinger Reagent To Deliver Diazirines

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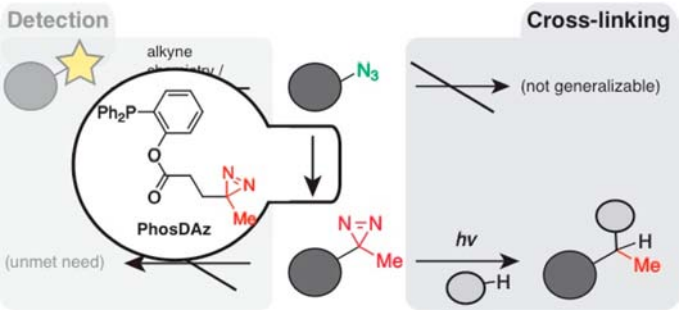
**ABSTRACT**




**A triarylphosphine reagent that reacts with organic azides to install amide-linked diazirines is reported. This traceless Staudinger reagent reacts with complex organic azides to yield amide-linked diazirines, thus expanding the scope of the utility of both azide and diazirine chemistry.**

The area of bioorthogonal chemistry relies on the ability of chemists to sneak unnatural reactivity past the defenses that exist in a biochemical system. The Trojan workhorse in this area is the azide, a small but highly energetic functionality that reacts selectively with several soft, non-natural nucleophiles.[1] The diminutive size of the azide, in particular, has allowed it to be incorporated into myriad biological macromolecules, providing an azide labeled biomolecular metabolic building block to the machinery present within living systems.

Our interest in studying host–pathogen interactions associated with dengue virus (DENV), a flavivirus that infects between 50 and 100 million people per year, led us to investigate compounds that can photo-cross-link pathogenic proteins to those found within the host.[2] We have recently found that various alkyl azides can be incorporated into DENV. Regrettably, unlike their aryl counterparts, alkyl azides are not easily degraded photochemically.[3] In lieu of re-engineering our incorporation strategy, we sought to convert our existing azides to something more susceptible to photochemical cross-linking. Fortunately, the azide's less stable chemical cousin, the diazirine, is readily degraded using conditions that have been shown to be relatively benign to living systems.[4] The resulting carbenes are highly reactive and cross-link with whatever is in close proximity. There are several unnatural diazirine-containing metabolites that have proven useful for biochemical photo-cross-linking studies, but these are often complicated by questions of levels and locations of incorporation.[5] These complications stem from the fact that probes that selectively react with diazirines to report on their presence have yet to be described. All of these issues are summarized in Figure 1. Upon searching for reagents that would selectively transform our azides into diazirines, either through direct functional group

(1) Sletten, E. M.; Bertozzi, C. R. *Acc. Chem. Res.* **2011**, *44*, 666.
(2) WHO, *Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control*; World Health Organization: Geneva, 2009.
(3) (a) Krieg, U. C.; Walter, P.; Johnson, A. E. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 8604. (b) Schnapp, K. A.; Poe, R.; Leyva, E.; Soundararajan, N.; Platz, M. S. *Bioconjugate Chem.* **1993**, *4*, 172.
(4) For a recent review on biologically relevant photo-cross-linking, see: Pham, N. D.; Parker, R. B.; Kohler, J. J. *Curr. Opin. Biol.* **2013**, *17*, 90.
(5) (a) Suchanek, M.; Radzikowska, A.; Thiele, C. *Nat. Methods* **2005**, *2*, 261. (b) MacKinnon, A. L.; Garrison, J. L.; Hegde, R. S.; Taunton, J. *J. Am. Chem. Soc.* **2007**, *129*, 14560. (c) Tanaka, Y.; Kohler, J. J. *J. Am. Chem. Soc.* **2008**, *130*, 3278. (d) Zhang, M.; Lin, S.; Song, X.; Liu, J.; Fu, Y.; Ge, X.; Fu, X.; Chang, Z.; Chen, P. R. *Nat. Chem. Biol.* **2011**, *7*, 671. (e) Li, Z.; Hao, P.; Li, L.; Tan, C. Y. J.; Chang, X.; Chen, G. Y. J.; Sze, S. K.; Shen, H.-M.; Yao, S. Q. *Angew. Chem., Int. Ed.* **2013**, *52*, 8551.
(6) The closest transformation is found in the conversion of organic azides to diazo compounds; see: Meyers, E. L.; Raines, R. T. *Angew. Chem., Int. Ed.* **2009**, *48*, 2359.

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interconversion or using the azide's reactivity to install a diazirine, we were surprised to find that there were none.[6] Herein we report a first generation compound that selectively reacts with azides to install a minimally perturbing diazirine functionality via a traceless Staudinger reaction.

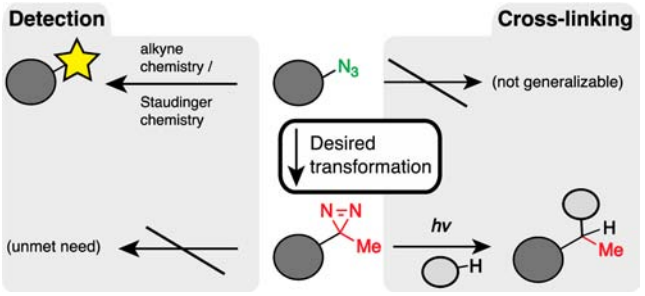



**Figure 1.** Extending the bioorthogonal utility of organic azides.

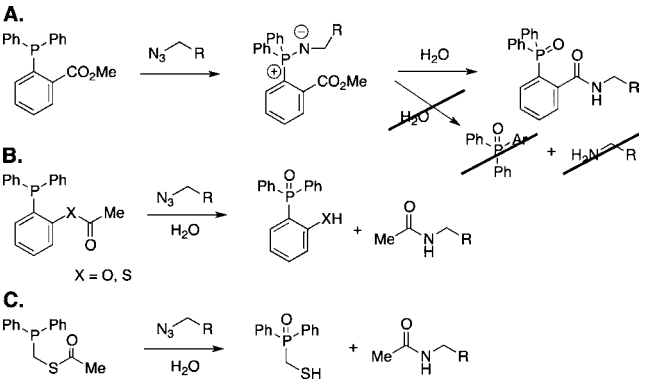
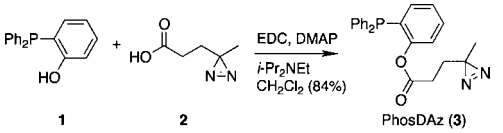



**Figure 2.** Staudinger ligations. (A) A generic example of Bertozzi's Staudinger ligation.[7] An acetate functionality can be delivered by traceless Staudinger ligations reported by either Bertozzi (B)[10b] or Raines (C).[10a]

The Staudinger ligation, developed by Bertozzi, takes advantage of the exquisite selectivity that phosphines have toward azides.[7,8] The nucleophilic aza-ylide that is formed upon loss of nitrogen gas can be trapped in an intramolecular fashion by a well-positioned reactive carbonyl as opposed to simply hydrolyzing as happens with a traditional Staudinger reaction (Figure 2A).[9] The chemistry of Staudinger reactions continued to progress, and later in 2000 the traceless Staudinger ligation, where the oxidized phosphine is not covalently bound to the resulting amide, was independently reported by both Raines and Bertozzi (Figure 2B–C).[10] We envisioned that a traceless Staudinger reagent with a small linker to a diazirine would serve our needs and be of use to the growing community of chemists and biochemists who use organic azides. For our application, the major limitation of Staudinger chemistry, namely the slow reaction rate, is greatly outweighed by its remarkable selectivity.[11] We were somewhat concerned that we did not find examples of a diazirine-containing compound that was subjected to compounds containing phosphorus at our desired oxidation state. Diazirines are somewhat electrophilic, and if they readily react with phosphines, that reactivity would terminate our quest at the onset. Certainly triphenylphosphine reacts with highly electron deficient diazo dicarboxylate compounds under mild conditions in the context of Mitsunobu chemistry, but the combination of a straightforward synthetic route, and the lack of precedent that clearly showed adverse phosphine-diazirine reactivity prompted us to continue with our strategy.[12]

**Scheme 1.** Synthesis of PhosDAz




With several traceless Staudinger platforms to choose from we weighed the options. Raines' thiol-ester (Figure 2C) has proven most efficient for appending amino acids with chirality α to the amide bond being formed,[13] but the requisite diazirine-containing portion to be ligated was sterically unencumbered. As such we reasoned that any traceless Staudinger scaffold that had been shown to efficiently transfer simple acyl groups to form amides from azides would serve well as proof of concept for further optimization studies. Starting with commercially available 2-hydroxydiphenylphosphinylbenzene (**1**, Scheme 1), we coupled diazirine acid **2** using standard coupling conditions to give PhosDAz (**3**) in excellent yield. Upon establishing that PhosDAz was not self-destructing or overly prone to oxidation it was treated with benzyl azide to cleanly provide amide **4** in 96% yield (Scheme 2). Indeed, PhosDAz is stable for months when stored in a freezer, and also stable at room temperature in a DMSO/water solution.[14]

Benzyl azide does not suffer from steric congestion, and its simplicity makes it an ideal model azide for testing general reactivity. To get a better sense of the utility of PhosDAz we sought to test it on more complex, congested

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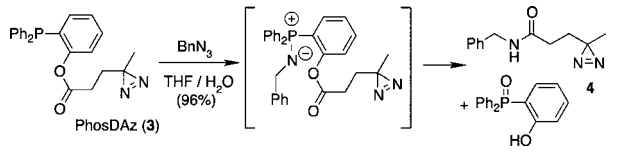
(8) Schilling, C. I.; Jung, N.; Biskup, M.; Schepers, U.; Bräse, S. *Chem. Soc. Rev.* **2011**, *40*, 4840.
(9) (a) Staudinger, H.; Meyer, J. *Helv. Chim. Acta* **1919**, *2*, 635. (b) Gololobov, Y. G. *Tetrahedron* **1981**, *37*, 437.
(10) (a) Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett.* **2000**, *2*, 1939. (b) Saxon, E.; Armstrong, J. I.; Bertozzi, C. R. *Org. Lett.* **2000**, *2*, 2141.
(11) (a) Lin, F.; Hoyt, H. M.; van Halbeek, H.; Bergman, R. G.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2005**, *127*, 2686. (b) Soellner, M. B.; Nilsson, B. L.; Raines, R. T. *J. Am. Chem. Soc.* **2006**, *128*, 8820.
(12) (a) Swamy, K. C. K.; Kumar, N. N. B.; Balaraman, E.; Kumar, K. V. P. P. *Chem. Rev.* **2009**, *109*, 2551. (b) During the course of this work, a paper wherein diazirines were submitted to conditions that contained triphenyl phosphine demonstrated that the diazirine functionality remained intact; see ref 5e.
(13) Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett.* **2001**, *3*, 9.

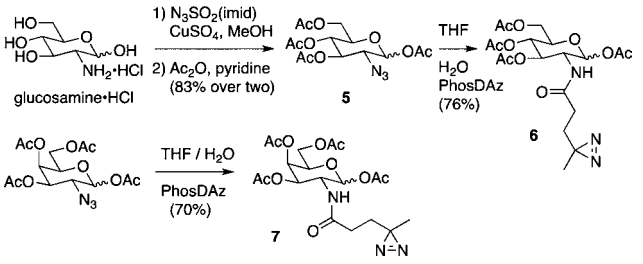
**Scheme 2.** Reaction of PhosDAz with Benzyl Azide




substrates. Hydrolysis of the aza-ylide (bracketed in Scheme 2) instead of ligation is always a concern with Staudinger ligations, and if the chemical environment is too congested then the desired amide bond can fail to form resulting in an amine and oxidized phosphine.

To test more complex, congested azides we focused our effort on the synthesis of two diazirinyl sugars (Scheme 3). These sugars had been previously synthesized by Kohler, and she has shown them to be useful for the study of glycosylation in living cells.[15] The requisite peraceylated 2-azido-2-deoxy-glucose, **5**, was synthesized in two steps from glucosamine hydrochloride using diazotransfer[16] chemistry followed by peracetylation of the remaining alcohols. We noted that the reaction of **5** with PhosDAz proceeded more slowly than that with benzyl azide, but upon consumption of **5**, the resulting sugar, $Ac_4$GlcNDAz (**6**), was obtained in 76% yield. $Ac_4$GalNDAz (**7**) was also synthesized using an analogous route, with the final coupling step providing the desired diazirine-containing sugar in 70% yield.

**Scheme 3.** Syntheses of $Ac_4$GlcNDAz (**6**) and $Ac_4$GalNDAz (**7**)




We expect that PhosDAz will find general utility among those who wish to add the delicate diazirine functionality to an azide-containing complex molecule, ubiquitous in this age of click chemistry, without the need to perform functional group interconversions. For our research purposes, attempts to test PhosDAz in a purely aqueous environment were thwarted by poor water solubility, something that we are currently addressing with new versions of the reagent. The initial approach we are pursuing is analogous to that of Raines', who developed a water-soluble traceless Staudinger reagent.[17] We expect that these next generation probes will further expand the utility of this transformation and facilitate our work with DENV.

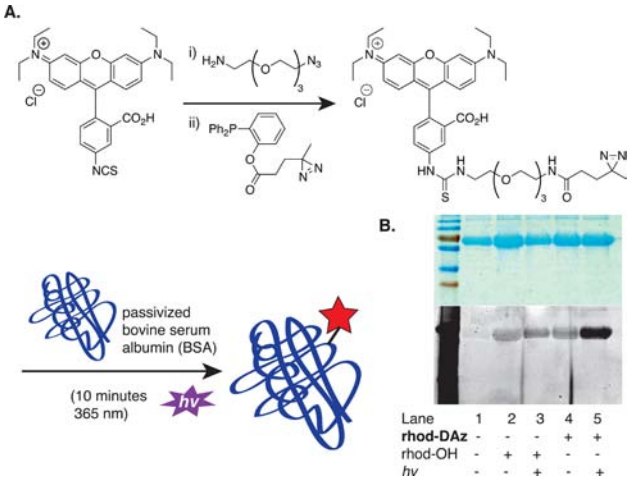



**Figure 3.** (A) One-drop synthesis of Rhodamine B conjugated to a diazirine (Rhod-DAz) and photo-cross-linking to bovine serum albumin (BSA). (B) SDS-PAGE gel showing passivized/photobleached BSA[18] treated with Rhod-DAz and either not-exposed (−) or exposed (+) to 365 nm light for 10 min (Lanes 4 and 5 respectively). A control rhodamine conjugated to ethanolamine (rhod-OH) showed minimal light-dependent labeling (Lanes 2 and 3). The fluorescent signal in the BSA sample exposed to photo-cross-linking conditions is significantly more intense (bottom gel scan). The coomassie stained gel (top gel scan) shows that lanes were loaded with similar amounts of protein.

In the meantime, in spite of its poor water solubility, we sought to explore the utility of PhosDAz in a more complex reaction environment. In the event, we used PhosDAz to functionalize an in situ synthesized azide-containing fluorophore and took that compound forward without purification to photo-cross-link the fluorophore to a model protein of interest (Figure 3A). For our fluorophore we chose rhodamine B isothiocyanate because of its ease of conjugation and absorption at a wavelength of light that is red-shifted compared with the optimal diazirine photo-cross-linking wavelength. A primary amine linked to an azide via a short PEG chain to confer additional water solubility served as a spacer between the fluorophore and diazirine. The conjugation reactions were performed in a drop of DMSO and monitored using ReactIR, where the disappearance of initially the isothiocyanate and subsequently the azide were followed. For our model protein of interest we selected bovine serum albumin (BSA) because of its ability to bind small organic compounds.

(14) A solution of PhosDAz in degassed DMSO-$d_6$/$D_2O$ (80:20) was monitored for degradation by NMR. After 24 h at room temperature no degradation was observed by proton or phosphorus NMR. This is longer than a typical traceless Staudinger reaction is allowed to proceed.

(15) (a) Bond, M. R.; Zhang, H.; Vu, P. D.; Kohler, J. J. *Nat. Protoc.* **2009**, *4*, 1044. (b) Yu, S.-H.; Boyce, M.; Wands, A. M.; Bond, M. R.; Bertozzi, C. R.; Kohler, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 4834. (c) Bond, M. R. Ph.D. Thesis, Stanford University, 2010.

(16) Ye, H.; Lui, R.; Li, D.; Liu, Y.; Yuan, H.; Guo, W.; Zhou, L.; Cao, X.; Tian, H.; Shen, J.; Wang, P. G. *Org. Lett.* **2013**, *15*, 18.

(17) Tam, A.; Soellner, M. B.; Raines, R. T. *J. Am. Chem. Soc.* **2007**, *129*, 11421.

(18) Significant background photo-cross-linking was observed when the BSA had not been previously exposed to 365 nm in the presence of a thiol scavenger.

This binding was expected to provide a sufficiently tight interaction to trap the highly reactive carbene generated upon liberation of nitrogen from the diazirine. We employed a UV-LED emitting at 365 nm as our light source, chosen for its narrow peak width in the lower energy region of wavelengths that cleave diazirines. Light-dependent fluorescent labeling of BSA was confirmed by SDS-PAGE analysis. Due to the intrinsic ability of BSA to bind to small molecules we always observed a small amount of background labeling, even in the absence of photo-cross-linking conditions, but irradiation for 10 min provided a BSA sample with significantly enhanced fluorescence compared with the no-light control (Figure 3B).

In conclusion, we reported a reagent, PhosDAz, that bridges the gap in reactivity between azides and diazirines. We expect that it will be of general use to any research that seeks to confer photo-cross-linking ability to otherwise photo-unresponsive alkyl azides in complex samples. The ease of azide incorporation into organic compounds and complex biological macromolecules makes the azide a powerful functional group, and a one-step process to convert those azides to amide-containing diazirines will prove broadly useful. Finally, the remarkable simplicity by which this reagent is made, from a commercially available phosphine and diazirine, makes it accessible to a wide spectrum of chemists and biochemists.

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**Supporting Information Available.** The preparation and characterization of the compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.