



An efficient and mild oxidant for the synthesis of s-tetrazines



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ABSTRACT

PhI(OAc)₂ serves as a mild and effective oxidant for the synthesis of s-tetrazine derivatives—molecules of emerging significance to the field of bioorthogonal chemistry. This reagent serves as a complementary oxidant to harsher nitrous reagents. The use of PhI(OAc)₂ improves the synthesis of 5-amino-di(pyridin-2-yl)-s-tetrazine, a molecule that has been broadly used for cellular imaging and nuclear medicine. The generality of PhI(OAc)₂ as the oxidant for tetrazine synthesis is demonstrated for nine tetrazines in 75–98% yield.

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Bioorthogonal reactions based on the cycloadditions of strained alkenes and cycloalkynes have emerged as powerful tools for chemical biology.^{1,2} In recent years, the bioorthogonal reactions of strained alkenes with s-tetrazines have served as tools for rapid bioorthogonal labeling, with applications that extend to cell biology,^{3–5} nuclear medicine,^{6–8} and materials science.^{9–12} In particular, the bioorthogonal reactions of s-tetrazines with *trans*-cyclooctene (TCO) derivatives have been shown to be exceptionally rapid, with rate constants in excess of $k_2 = 10^6 \text{ M}^{-1} \text{ s}^{-1}$.^{13,14}

The synthesis of s-tetrazines generally involves the preparation of 1,4-dihydro-s-tetrazine precursors, which are subsequently oxidized to provide s-tetrazine products. Commonly, nitrous reagents (e.g., HONO, NaNO₂, and isoamyl nitrite) are used to oxidize 1,4-dihydro-s-tetrazines to s-tetrazines. This method has good scope and proceeds with yields that are often excellent. However, moderate yields have been reported in several cases^{15–18} and nitrous reagents can fail for substrates with sensitive functionality.¹⁹ While oxidants such as chromium trioxide,²⁰ hydrogen peroxide,²¹ and DDQ^{19,22} have been used for s-tetrazine synthesis, there remains a need to develop general, reliable, and efficient methods for preparing s-tetrazines.²³

One example of an s-tetrazine that cannot be directly prepared via oxidation with nitrous reagents is the amino substituted di(pyridin-2-yl)-s-tetrazine (**2**) (Fig. 1). Compound **2** was prepared through a statistical combination of 2-cyanopyridine and 5-amino-2-cyanopyridine to give 1,4-dihydro-s-tetrazine **1**, which was subsequently oxidized to **2**. Attempts to use nitrous reagents were unsuccessful in this synthesis, possibly due to oxidation of the

amino functionality. While we found that DDQ served to oxidize **1** in good yield, the hydroquinone byproducts are difficult to remove, and the synthesis could not be readily scaled due to difficult chromatographic steps.¹⁹ Developing an improved synthesis of **2** was necessary, as acyl derivatives of **2** have been used in a number of applications (Fig. 1). An In(III)-DOTA derivative **3** was used by Robillard and co-workers²⁴ in pretargeted tumor imaging in live mice. Cyclic RGD analog **4** has been used in combination with ¹⁸F-labeled TCO **5** for PET imaging in live mice.⁶ Recently, a ¹¹C-labeled derivative of **2** for PET imaging applications has also been described.²⁵ Fluorescently labeled s-tetrazines such as **6** have been used for site specific labeling in live mammalian cells with proteins that contain either norbornene-¹⁵ or TCO-containing²⁶ amino acids. For the preparation of **6**, NaNO₂ mediated oxidation of a BOC-protected glycine derivative proceeded in moderate yield.²⁶

We sought an oxidant for 1,4-dihydro-s-tetrazine derivatives that would be mild, general, and produce readily separable byproducts. To develop a more scalable one-pot synthesis of **2** from 2-cyanopyridine (**8**) and 5-amino-2-cyanopyridine (**7**), a number of oxidants were surveyed as shown in Table 1. Attempts to use NBS, NCS, hydrogen peroxide, peracetic acid, and bromine to oxidize **1** were all unsuccessful, and gave only decomposition products. Compound **2** was formed when mCPBA was used as the oxidant in 27% yield over 2 steps, or 55% in the oxidation step. Both benzoquinone and benzoyl peroxide were more successful oxidants, providing **2** in 32% and 35% overall yields, respectively. As was the case for the DDQ-mediated oxidation, the purification of **2** from the benzoquinone mediated oxidation was complicated by difficult chromatography. Of the oxidants that were surveyed, PhI(OAc)₂ was the most successful, providing a yield of 42% over 2 steps (86% in the oxidation step). The byproduct of the

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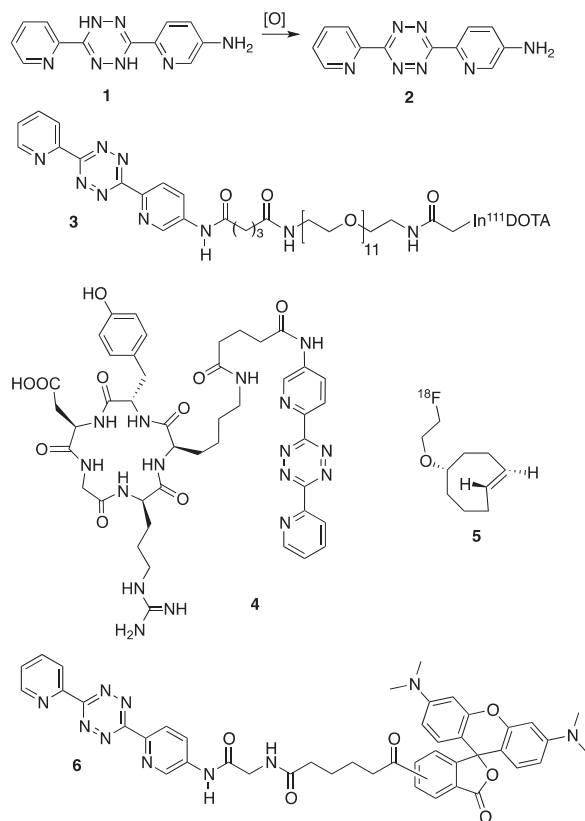


Figure 1. Amino-substituted di(pyridin-2-yl)-s-tetrazine (**2**) cannot be directly prepared from **1** by oxidation with nitrous reagents. Derivatives **3**, **4**, and **6** have been applied to radiochemical and cellular imaging.

Table 1
Optimization of the oxidant in the one-pot synthesis of tetrazine **2**

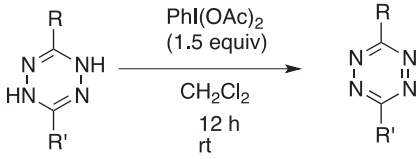
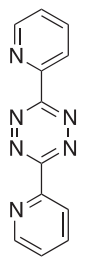
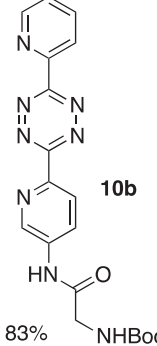
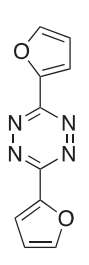
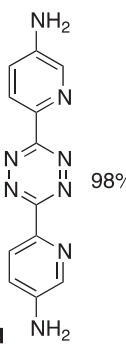
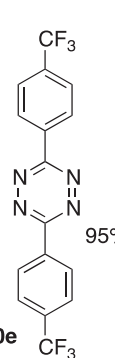
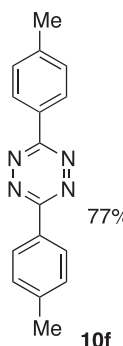
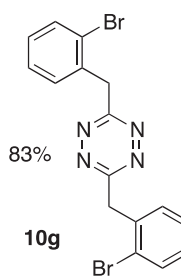
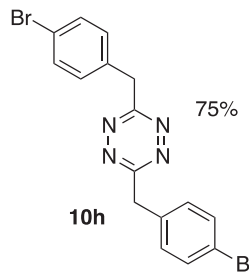
Oxidant	Yield of oxidation ^a (%)	Two-step yield (%)
<i>N</i> -Chlorosuccinimide	0	0
<i>N</i> -Bromosuccinimide	0	0
Hydrogen peroxide	0	0
Peracetic acid	0	0
Bromine	0	0
<i>m</i> -CPBA	55	27
Benzoyl peroxide	65	32
Benzoquinone	71	35
PhI(OAc) ₂	86	42

^a Yield of the oxidation step was based on the NMR yield of 49% for the first step.

reaction—iodobenzene—is readily removed at the stage of purification. Importantly, this process could be carried out without purification of the 1,4-dihydro-s-tetrazine intermediate **1**, and s-tetrazine **2** could be prepared on a 2 gram scale.

Having identified a suitable oxidant for the preparation of **2**, we explored the oxidation of a number of 1,4-dihydro-s-tetrazine derivatives (**9**) by PhI(OAc)₂ as shown in Table 2. Heteroaromatic derivatives **10a–d** were prepared in 83–98% yield. Also well

Table 2
Oxidation by PhI(OAc)₂ to give s-tetrazines

 9 10		
 10a 91%	 10b 83%	 10c 88%
 10d 98%	 10e 95%	 10f 77%
 10g 83%	 10h 75%	

tolerated were diphenyl-s-tetrazines with trifluoromethyl and methyl functionality, as compounds **10e–f** could be prepared in 77–95% yield. Dialkyl-s-tetrazines **10g–h** could be prepared in 75–83% yield.

In sum, PhI(OAc)₂ has been identified as a mild and effective oxidant for the synthesis of s-tetrazine derivatives, which are of emerging significance to the field of bioorthogonal chemistry. This reagent serves as a complementary method to harsher procedures for the oxidation of 1,4-dihydro-s-tetrazines.

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Supplementary data

Supplementary data (full experimental details, copies of ^1H NMR and ^{13}C NMR spectra) associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.tetlet.2014.07.012>.

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