HHS Public Access

Author manuscript

Tetrahedron Lett. Author manuscript; available in PMC 2015 August 20.

Published in final edited form as:

Tetrahedron Lett. 2014 ; 55(34): 4795–4797. doi:10.1016/j.tetlet.2014.07.012.

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. 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.

Keywords

s-tetrazine; phenyliodonium diacetate; oxidation; bioorthogonal

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 $k_2 = 10^6\ M^{-1}s^{-1}$.[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, $NaNO_2$, isoamylnitrite) 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**) (Figure 1). Compound **2** was prepared through a statistical combination of 2-cyanopyridine and 5-amino-2-

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Supplementary Material

Full experimental details, copies of ^1H NMR and ^{13}C NMR spectra are provided.

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 (Figure 1). An In(III)-DOTA derivative **3** was used by Robillard and coworkers[24] in pretargeted tumor imaging in live mice. Cyclic RGD analog **4** has been used in combination with ^{18}F-labelled TCO **5** for PET imaging in live mice.[6] Recently, a ^{11}C-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–containing[26] amino acids. For the preparation of **6**, $NaNO_2$ 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 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 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 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge support of the National Science Foundation (NSF CHE1112409) and National Institutes of Health (R01 EB014354). For instrumentation support, we thank NIH P20RR017716, NIH S10 RR026962, NSF CHE0840401, CHE- 1229234.

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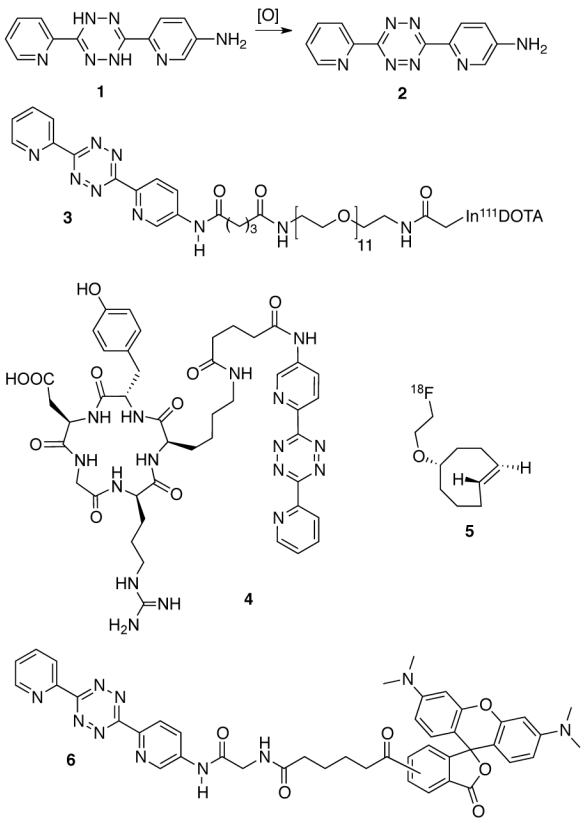
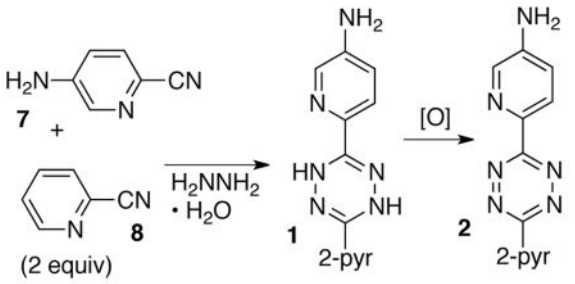


Figure 1.

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
N-chlorosuccinimide	0%	0%
N-bromoosuccinimide	0%	0%
hydrogen peroxide	0%	0%
peracetic acid	0%	0%
bromine	0%	0%
m-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.

Table 2

Oxidation by $PhI(OAc)_2$ to give *s*-tetrazines