Synthetic Methods

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Metal-Catalyzed One-Pot Synthesis of Tetrazines Directly from Aliphatic Nitriles and Hydrazine**

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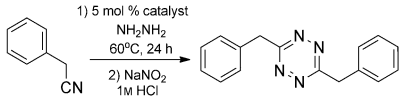
There is rapidly growing interest in the use of 1,2,4,5-tetrazines as bioorthogonal coupling agents.[1–3] Recent applications of tetrazine cycloadditions include intracellular small-molecule imaging, genetically targeted protein tagging, post-synthetic DNA labeling, nanoparticle-based clinical diagnostics, and in-vivo imaging.[4–7] In addition, tetrazines have seen significant use in materials science,[8,9] coordination chemistry,[10,11] and specialty explosives research.[12,13] They are also valuable synthetic intermediates, and have been elegantly deployed on route to several natural product syntheses.[14–16] Despite the promise of tetrazines, the lack of convenient synthetic methods is a significant roadblock to their broader use and study by the scientific community.[17] Here we report that Lewis acid transition metal catalysts, most notably divalent nickel and zinc salts, can catalyze the formation of 1,2,4,5-tetrazines directly from nitriles. To our knowledge, this is the first method utilizing homogenous catalysis to directly synthesize tetrazines from a wide range of unactivated aliphatic nitriles and hydrazine. Symmetric and asymmetric tetrazines were conveniently prepared from multiple precursors including alkyl nitriles, aromatic nitriles, and formamidine salts. This methodology should greatly improve the accessibility of tetrazines and lead to further exploration of their applications, particularly with respect to bioorthogonal conjugations.

The most convenient route to 1,2,4,5-tetrazines is by addition of hydrazine to aromatic nitriles followed by oxidation of the 1,2-dihydrotetrazine product.[18] Unfortunately, this strategy is not viable for producing tetrazines from unactivated nitriles such as alkyl nitriles. Earlier reports claiming to access tetrazines directly from alkyl nitriles have proven difficult to reproduce, likely due to confusion between the intermediate 1,2-dihydrotetrazines and isomeric 4-amino-1,2,4 -triazoles.[19–21] There have been several reported methods to access dialkyl tetrazines from alternative precursors, such as imidates, amidine salts, and aldehydes, but these methods suffer from low yields, limited substrate scope, and the requirement of additional synthetic steps.[22–24] For these reasons, a general and robust method to prepare symmetric and asymmetric 1,2,4,5-tetrazines directly from unactivated nitriles would be highly desirable.

Though the mechanism of tetrazine synthesis has been debated, it is generally agreed that reaction begins with nucleophilic attack of the nitrile by hydrazine forming an amidrazone.[25,26] We speculated that the addition of Lewis acid catalysts might promote this reaction by binding to the nitrile and/or hydrazine. Metal ions have long been known to activate nitriles to nucleophilic addition.[27–29] However, there has not been a report of using homogenous transition metal catalysis to promote the formation of 1,2,4,5-tetrazines.[21,30] We used the reaction of benzyl cyanide with neat hydrazine to survey a range of Lewis acid catalysts at 5 mol% loading (Table 1).[31] In the absence of catalyst, tetrazine products could not be isolated.[20] Remarkably, the addition of 5 mol% nickel triflate ($Ni(OTf)_2$) led to near quantitative yield of 3,6-dibenzyl-1,2,4,5-tetrazine. Zinc salts also gave good yields, with addition of 5 mol% zinc triflate ($Zn(OTf)_2$) leading to 70% yield of the desired tetrazine. Nickel and zinc salts possessing stronger coordinating anions gave lower yields, possible due to the lowered solubility of these salts in aprotic media and the decreased Lewis acid strength compared to the triflates.[32]

Given the high yields obtained with nickel and zinc triflates, we tested their effect on the yields of several other tetrazine syntheses where at least one component was an alkyl nitrile (Table 2). In each instance we tested either $Ni(OTf)_2$ or

Table 1: Survey of metal catalysts.



Catalyst	Yield [%][a]	Catalyst	Yield [%][a]	Catalyst	Yield [%][a]
none	0	$Cu(OAc)_2$	59	$NiCl_2$	73
$Zn(OAc)_2$	38	$MnBr_2$	55	NiI_2	93
$ZnCl_2$	11	$CuBr_2$	23	$Ni(OTf)_2$	**95**
$ZnBr_2$	46	$CoCl_2{\cdot}6H_2O$	13	CuOAc	53
ZnI_2	68	$MgCl_2$	63	CuCl	12
$Zn(OTf)_2$	**70**	$Yb(OTf)_3$	31	CuBr	42
$Cu(OTf)_2$	11	$Sc(OTf)_3$	26	CuI	50
$MgBr_2$	15	$Ni(acac)_2$	10	Cu(OTf)	57

[a] Yields reported after isolation by silica flash chromatography.

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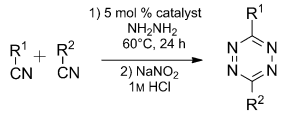
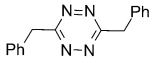
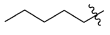
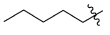
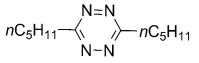
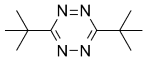
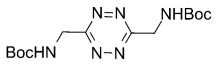
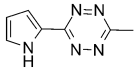
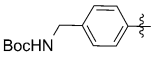
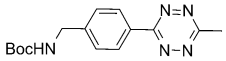
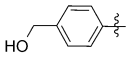
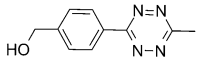
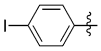
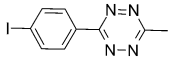
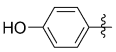
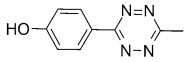
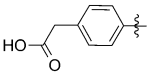
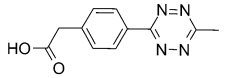
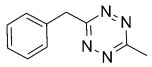
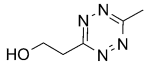
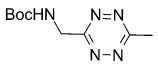
Table 2: Synthesis of 1,2,4,5-tetrazines directly from nitriles catalyzed by $Ni(OTf)_2$ or $Zn(OTf)_2$.



Entry	R^1	R^2	Cat.	Product	Yield [%][a]
1			Ni		95
2			Zn		59
3			Zn		24
4			Zn		32
5[b]			Ni		58
6			Ni		68
7			Ni		66
8			Ni		41
9			Zn		43
10			Ni		70
11			Zn		40
12[c]			Zn		36
13			Ni		36
14			Zn		40
15			Zn		12
16[b]			Zn		30

[a] Yields reported after isolation by silica flash chromatography. [b] The protective groups were lost during oxidative workup. [c] Reaction required 36 h.

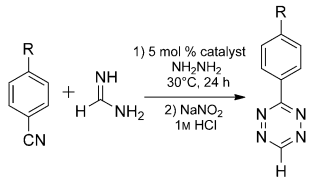
$Zn(OTf)_2$ for catalytic effect. In general we observed that zinc salts gave higher yields for less active nitriles such as those that were sterically hindered or affected by electron-donating groups. On the other hand, more reactive nitriles benefited from the use of nickel salts. However, there were exceptions and it is suggested that both catalysts be tried when attempting synthesis of new tetrazines. For the synthesis of symmetric 3,6-dialkyl 1,2,4,5-tetrazines, yields ranged from 95% for 3,6-dibenzyl-1,2,4,5-tetrazine (entry 1) to 24% for the sterically hindered 3,6-di-*tert*-butyl-1,2,4,5-tetrazine (entry 3). The moderate yield for the latter tetrazine is impressive given that all previous attempts to synthesize the molecule have only led to trace isolated yield.[24,33]

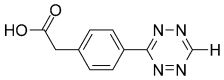
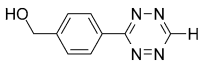
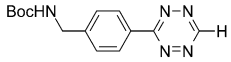
The scope of this method extends to asymmetric 3,6-disubstituted 1,2,4,5-tetrazines, which are among the most challenging tetrazines to synthesize.[17,25,34] Metal ions could readily promote the formation of 6-methyl-terminated tetrazines from acetonitrile and aromatic nitriles in 40–70% yield (entries 5–10). Alkyl nitriles and acetonitrile could also combine with hydrazine to yield 6-methyl-terminated alkyl tetrazines in 36–40% yield (entries 11–14). Several of these tetrazines possess functional group handles to facilitate their use in biological applications. For instance, it has recently been demonstrated that methyl-terminated tetrazines are highly stable partners in bioorthogonal cycloadditions and can be used in a mutually orthogonal fashion with azide–alkyne cycloadditions.[6,35] 6-Methyl-terminated tetrazines were previously only accessible from reactive precursors such as imidates and amidine salts and in lower yield.[23,35] Dialkyl asymmetric tetrazines with bulkier substituents are extremely difficult to isolate, even from imidates and amidines. In contrast, we were able to isolate 3-benzyl-6-pentyl-1,2,4,5-tetrazine (entry 15) from benzyl cyanide and excess hexanenitrile, albeit in lower yield (12%).

Several groups have measured the rate of cycloaddition between various tetrazines and strained dienophiles such as norbornene and *trans*-cyclooctene.[1,2,5,36] The substituents on the 3 and 6 positions of 1,2,4,5-tetrazines have a significant effect on the kinetics of the reaction.[35] While 6-methyl-terminated tetrazines benefit from stability, tetrazines terminated with hydrogen at the 6 position react much faster and have proven utility in live cell and live animal applications where lowered concentrations of labeling agent are typically used.[2,7,36] With this in mind, we examined if metal catalysis could improve synthetic routes to hydrogen-terminated monoaryl tetrazines. We found that an excess of trimethylsilyl cyanide can be used along with an aromatic nitrile to yield a hydrogen-terminated asymmetric tetrazine (entry 16). This is the first example of using trimethylsilyl cyanide to synthesize tetrazines and is possible due to the addition of nitrile-activating metal catalysts. Additionally, we explored the effect of $Ni(OTf)_2$ and $Zn(OTf)_2$ on the synthesis of tetrazine from aromatic nitriles and formamidine salts (Table 3). Although these reactions do not require catalysis, yields are typically low, between 10–20%.[35] Interestingly, we found that metal ions could promote the reaction and significantly increase the yield of tetrazine, which was 60–74% depending on the precursors and catalyst used. This improved methodology will be highly useful to researchers interested in performing rapid bioorthogonal couplings.

Given the lack of clarity on the mechanism of tetrazine formation, and the large number of metal-coordinating species involved such as hydrazine, nitriles, and amidrazones, it is difficult to confidently propose the precise role of the metal ion. It is likely that the metal acts as a Lewis acid by coordinating to the nitrile and promoting nucleophilic addition by hydrazine.[28,29,37,38] It is also plausible that the

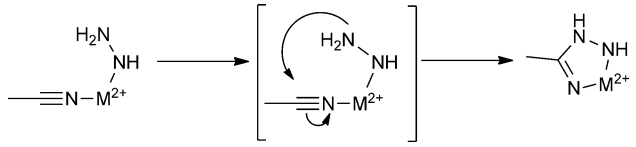
Table 3: Metal-catalyzed synthesis of tetrazines from aromatic nitriles and formamidine.



Entry	R	Cat.	Product	Yield [%][a]
17		Ni		74
18		Ni		64
19[b]		Zn		70

[a] Yields reported after isolation by silica flash chromatography.
[b] Required use of DMF as cosolvent and 36 h of reaction.

metal binds both the nitrile and hydrazine, promoting synthesis of the amidrazone intermediate (Scheme 1).[39] Recent elegant work has demonstrated that a similar mechanism is likely responsible for the nickel-catalyzed addition of pyrazole to acetonitrile.[37] Clearly future studies are required to refine our understanding of tetrazine synthesis.



Scheme 1. Plausible catalytic role of the metal during synthesis.

In conclusion we have discovered that metal salts such as nickel and zinc triflates can be used to catalyze the one-pot synthesis of 1,2,4,5-tetrazines from unactivated nitriles. Previously, these tetrazines were either not practically accessible or could be obtained in low yield after multistep syntheses. The ability to conveniently obtain a range of symmetric and asymmetric tetrazines directly from commercially available nitriles should encourage further use of tetrazines as bioorthogonal coupling agents. Furthermore, this finding will benefit important niche applications of tetrazines, for instance in coordination chemistry, explosives research, materials science, and total synthesis.[8,11,12,15,16,40] We believe that this methodology will increase the accessibility of substituted tetrazines to a broader range of researchers, allow syntheses of compounds that were previously unobtainable in useful quantities, and help remove a synthetic roadblock to studying the unique properties and applications of these heterocycles.

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Keywords: bioorthogonal synthesis · cycloaddition · heterocycles · metal catalysis · tetrazines

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Communications

Synthetic Methods

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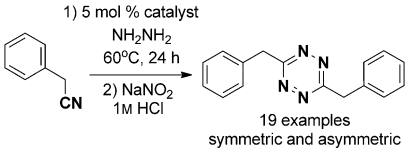
Metal-Catalyzed One-Pot Synthesis of Tetrazines Directly from Aliphatic Nitriles and Hydrazine



Paving the way: The lack of convenient synthetic methods is a significant roadblock to the broader use of 1,2,4,5-tetrazines in bioorthogonal chemistry and functional materials. Lewis acid metal catalysts—most notably divalent nickel and zinc salts— are described to catalyze the one-pot synthesis of 1,2,4,5-tetrazines directly from aliphatic nitriles (see scheme).