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Facile and efficient Lewis acid catalyzed synthesis of an asymmetric tetrazine useful for bio-orthogonal click chemistry applications ☆

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ABSTRACT

Bio-orthogonal tetrazine click reactions have recently attracted significant interest for applications spanning biological imaging, cancer targeting, and biomaterials science. Here, we report a simple and efficient two-step scheme for the synthesis of an asymmetric tetrazine molecule containing a carboxylic acid handle for subsequent macromolecular conjugation. Yields as high as 75% were achieved using as little as 0.005 equiv of nickel triflate catalyst, which is a significant improvement over previous methodologies.

Introduction

The synthesis of bio-orthogonal click chemistry reagents has been of significant interest since the inception of the field. In addition to designing new reagents, developing synthetic routes that improve access to these broadly useful molecules is also important to the advancement of the field, as it facilitates the development of new applications. For tetrazine click chemistry specifically, which has emerged as a powerful tool for a range of applications (e.g., live cell imaging,[1,2] in vivo tumor targeting,[3,4] protein modification and conjugation,[5–7] post-synthetic modification of nucleic acids,[8,9] and polymer chemistry[10,11]), improved methods for the nontrivial synthesis of tetrazine heterocycles was recently identified as a key challenge.[12]

Although the mechanism by which the heterocyclic tetrazine ring is formed is not well understood, the synthesis of 1,2,4,5-tetrazines is generally achieved in two steps via the addition of hydrazine to an aromatic nitrile followed by oxidation of the resulting 1,2-dihydrotetrazine intermediate.[13] For bioconjugate chemistry applications, the nitrile typically must bear a reactive moiety such as a carboxylic acid or amine for subsequent coupling to biomacromolecules. However, to avoid crosslinking, the tetrazine molecule must be asymmetric and it must have only one reactive moiety. In general, asymmetric tetrazines are more difficult to synthesize than their symmetric counterparts, with typical yields reported in the range of 10–20%.[5,13,14]

We recently demonstratedproof of concept for hydrogel crosslinking and cellular encapsulation with tetrazine click chemistry.[15] However, despite the advantages of this bio-orthogonal hydrogel system, the low yield (~17%) and complex purification of the asymmetric tetrazine were recognized as important barriers to scale up for materials applications in future work. Motivated by the fact that biomaterials applications can require large amounts of material for characterization and testing, we began to explore methods to improve the yield and also simplify the synthesis, work up, and purification of the tetrazine molecule, 5-(4-(1,2,4,5-tetrazin-3-yl)benzylamino)-5-oxopentanoic acid. Of specific interest was the implementation of nickel triflate as a catalyst, as Yang et al. recently reported that Lewis acid transition metals like nickel can catalyze the 1,2,4,5-tetrazine heterocycle formation and significantly improve the yield, possibly by coordinating with the nitrile and promoting the nucleophilic addition of hydrazine.[13]

In this Letter we summarize our findings and report a facile and efficient approach that can be used to produce large quantities of 5-(4-(1,2,4,5-tetrazin-3-yl)benzylamino)-5-oxopentanoic acid, which reacts with dienophiles like norbornene and *trans*-cyclooctene via an inverse electron demand Diels–Alder mechanism. The effects of the nickel triflate concentration on the overall yield are reported over a range of molar equivalents.

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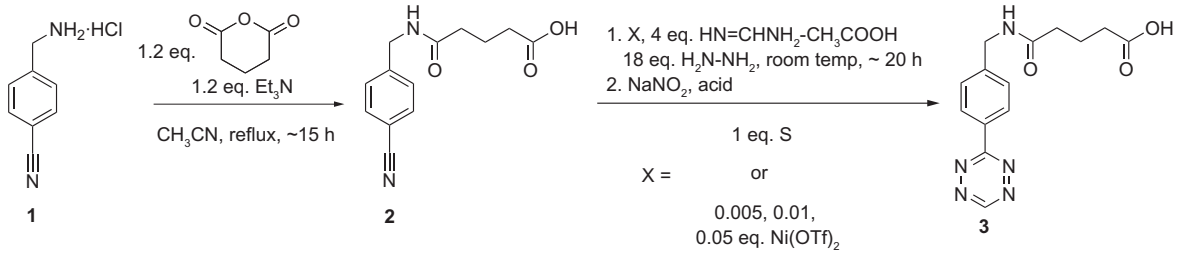

Scheme 1. Synthesis of 5-(4-(1,2,4,5-tetrazin-3-yl)benzylamino)-5-oxopentanoic acid.

Results and discussion

1,2,4,5-Tetrazines terminated with a hydrogen at the 6th position have previously been shown to have a combination of good stability and high reactivity,[1,14] making these molecules of high interest for bio-orthogonal click chemistry and biomaterials. Syntheses starting from 4-(aminomethyl) benzonitrile (**1**) have previously been published.[1,14,16] Thus, our approach (Scheme 1) begins with the modification of **1** with a pentanoic acid handle by reacting with glutaric anhydride in refluxing acetonitrile. This reaction proceeded to near quantitative yield at large scale, and 5-(4-(cyano)benzylamino)-5-oxopentanoic acid (**2**) was isolated simply by extraction with ethyl acetate. In addition to providing a carboxylic acid functional group for convenient coupling to amine bearing macromolecules, we hypothesized that this modification would facilitate work up by improving the solubility of the target molecule in organic solvents after tetrazine heterocycle formation. Indeed, after reacting **2** with hydrazine and formamidine acetate in the presence of elemental sulfur and then oxidizing with sodium nitrite, extraction with dichloromethane produced a crude mixture of product and starting material, as determined by thin layer chromatography. Subsequent recrystallization from ethanol yielded 5-(4-(1,2,4,5-tetrazin-3-yl)benzylamino)-5-oxopentanoic acid (**3**) as a pink solid that was >95% pure by analytical HPLC (see Supplementary information). Typical yield from recrystallization was 14%, which is only slightly lower than what we previously achieved by flash chromatography.[15]

Despite the low yield, the one-pot reaction for tetrazine formation was amenable to scale up. Starting with 60.9 mmol of **2**, 2.5 g of **3** (8.3 mmol; 14% yield) was obtained after recrystallization, which was viewed as impressive considering the simplicity of this approach. Nevertheless, we were interested in further improving the efficiency and yield. Yang et al. previously showed that the addition of 0.05 equiv of nickel or zinc triflate catalysts could improve the tetrazine yield by 3–4 fold over catalyst free reactions.[13] Thus, to explore the effects of Lewis acid catalysis in our scheme, we eliminated sulfur and instead reacted **2** with hydrazine and formamidine acetate in the presence of 0.005, 0.01, and 0.05 equiv of nickel triflate. Following oxidation with sodium nitrite in acidic conditions, the reaction was extracted with dichloromethane, as before. In contrast to the uncatalyzed reaction, unreacted **2** was not detected by TLC or ^{1}H NMR in the organic extract for any of the nickel catalyzed reactions, suggesting improved efficiency of these reactions. Nevertheless, impurities were observed by analytical HPLC. Thus, for the nickel catalyzed reactions, **3** was purified by flash chromatography.

Overall, nickel triflate catalysis was highly effective, and yields as high as 75% were obtained. These results are presented in Table 1. Each reaction was performed several times and multiple scales were used. Thus, to be conservative, the yields are reported as a range rather than as a single value. Interestingly, there was no clear trend in yield with the amount of catalyst. At 0.005 and 0.01 equiv of nickel triflate, yields were in the 65–75% range, suggesting that very little catalyst is actually required to boost the efficiency of the reaction.

Table 1
Effect of nickel triflate catalysis on tetrazine yield

Entry	Molar equivalents of $Ni(OTf)_2$	Yield of **3**[a] (%)
1	0	14
2	0.005	65–75
3	0.01	65–75
4	0.05	50–60

[a] Yields are reported for multiple reactions and reaction scales.

Furthermore, there appeared to be no benefit to increasing the equivalents of catalyst. At 0.05 equiv of nickel triflate, the yield was 50–60%. While this slight reduction in yield was reproducibly observed, the practical implications may actually be minimal as this is still a 3–4 fold increase over the noncatalyzed reaction. Nevertheless, the apparent reduced catalyst requirement over what has previously been reported[13] is certainly attractive for future work.

To our knowledge, the only previous report of Lewis acid catalysis of tetrazine synthesis is the study by Yang et al.[13] While our results support their finding that Lewis acid transition metal catalysis can significantly improve tetrazine yield, we show here that excellent yields can be obtained with a 10-fold reduction in the amount of catalyst. It will be interesting to see if this result can be extended to the synthesis of other tetrazine molecules. Regardless, we are now able to quickly and efficiently access gram scale quantities of **3**, circumventing an important barrier to our work with this powerful bio-orthogonal chemistry in the design of monomers for polymeric material development. We believe that the reported synthetic schemes may be of interest to those developing bio-orthogonal hydrogel crosslinking for encapsulation of cells and biologics, as well as to the broader biomaterials and bio-orthogonal click chemistry communities.

Conclusions

Here, we report a simple, high yielding synthetic scheme for a carboxylic acid functionalized asymmetric tetrazine. This tetrazine molecule can be readily coupled to other biomacromolecules of interest using standard acid–amine coupling chemistries, making it of broad interest for bio-orthogonal click chemistry applications. The Lewis acid catalyzed synthesis of this tetrazine, however, is a significant improvement over previously reported methods, with yields as high as 75% being achieved with only 0.005 equiv of nickel triflate. This new methodology should enable broader adoption of tetrazine click chemistry and facilitate the development of new applications of tetrazine chemistry in materials design.

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Supplementary data

Supplementary data (synthetic methods, HPLC chromatograms, NMR spectra) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.08.010.

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