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Synthesis, characterization, and cycloaddition reactivity of a monocyclic aromatic 1,2,3,5-tetrazine

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ABSTRACT. Herein we disclose the synthesis and full characterization of the first monocyclic aromatic 1,2,3,5-tetrazine, 4,6-diphenyl-1,2,3,5-tetrazine. Initial studies of its cycloaddition reactivity, mode, regioselectivity, and scope illustrate that it participates as the 4π-component of well-behaved inverse electron demand Diels–Alder reactions where it preferentially reacts with electron-rich or strained dienophiles. It was found to exhibit an intrinsic reactivity comparable to that of the isomeric 3,6-diphenyl-1,2,4,5-tetrazine, display a single mode of cycloaddition with reaction only across C4/N1 (no N2/N5 cycloaddition observed), proceed with a predictable regioselectivity (dienophile most electron-rich atom attaches to C4), and manifest additional reactivity complementary to the isomeric 1,2,4,5-tetrazines. It not only exhibits a remarkable cycloaddition reactivity, surprisingly good stability (e.g., stable to chromatography, long term storage, presence of H_2O even as reaction co-solvent), and broad cycloaddition scope, but it also displays powerful orthogonal reactivity with the 1,2,4,5-tetrazines. Whereas the latter reacts at extraordinary cycloaddition rates with strained dienophiles (tetrazine ligation), the new and isomeric 1,2,3,5-tetrazine displays similarly remarkable cycloaddition rates and efficiencies with amidines (1,2,3,5-tetrazine/amidine ligation). The crossover reactivities (1,2,4,5-tetrazines with amidines vs 1,2,3,5-tetrazines with strained dienophiles) are sufficiently low to indicate they may be capable of use concurrently without competitive reactions.

INTRODUCTION

The inverse electron demand Diels–Alder cycloadditions of heterocyclic azadienes have provided powerful methodology for the synthesis of highly substituted and functionalized heterocycles[1] widely used in organic synthesis and the pharmaceutical industry, in the divergent construction of screening libraries[2] and in bioorthogonal conjugation.[3,4] Our past efforts have provided systematic explorations of the reactions of 1,2,4,5-tetrazines,[5] 1,2,4-triazines,[6] 1,3,5-triazines,[7] 1,3,4-oxadiazoles,[8] 1,2-diazines[9] and most recently

1,2,3-triazines.[10] Each heterocyclic azadiene was found to possess a unique reactivity toward different classes of dienophiles, display now predictable modes of cycloaddition, and exhibit substantial substituent electronic effects impacting their intrinsic reactivity and cycloaddition regioselectivity. These insights permitted their use as key steps in more than 50 natural products total syntheses[11] and helped inspire the development of the powerful 1,2,4,5-tetrazine based bioorthogonal conjugation and labeling technology (tetrazine ligation) widely used today. Consequently, it is remarkable in this day and age that no member of another fundamentally important heterocyclic azadiene ring system, the 1,2,3,5-tetrazines, has yet been reported (Figure 1A).

Limited attempts to prepare a 1,2,3,5-tetrazine have been described[14] and no monocyclic aromatic 1,2,3,5-tetrazine as a discrete 6π heterocycle has been reported. Stability profiles established in computational studies suggest a decreased kinetic stability for 1,2,3,5-tetrazine compared with the well-known and isomeric 1,2,4,5-tetrazine (ΔE_{act} 14 kcal/mol), but a greater thermodynamic stability than 1,2,4,5-tetrazine (7–8 kcal/mol).[12] The principal challenge in the synthesis of 1,2,3,5-tetrazines is the construction of two consecutive N–N bonds, which are not present in 1,2,4,5-tetrazines but are found in the 1,2,3-triazines. Although no synthesis of a monocyclic 1,2,3,5-tetrazine as a 6π aromatic system has been reported, limited numbers of heterocyclic compounds bearing the 1,2,3,5-tetrazine core have been disclosed (Figure 1B).[13-15] The nonaromatic 1,2,3,5-tetrazine core (**3**) was synthesized as part of fused heterocycles in the development of energetic materials,[13] monocyclic tetrazinones have been described that were prepared by a 6π-electrocyclization of acyclic triazene isocyanate precursors[14] and the synthesis of a library of ring-fused bicyclic tetrazinones has been reported of which a derivative (temozolomide, **4**) is used clinically.[15] The methods used in this work for the construction of the core ring system as well as those used for the preparation of 1,2,3-triazines[10,16] provided valuable insights into the possible synthesis of aromatic 1,2,3,5-tetrazines. With even their stable existence in question, we explored several approaches for their synthesis. As a result of these efforts, herein we report the synthesis, characterization, properties, and cycloaddition reactivity of the first member of this new and previously unknown class of heterocycles.

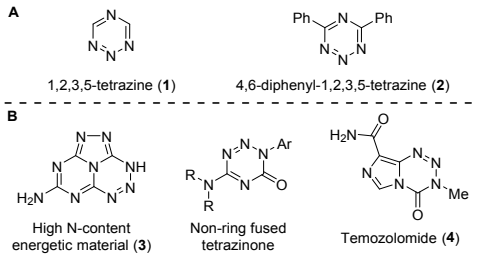
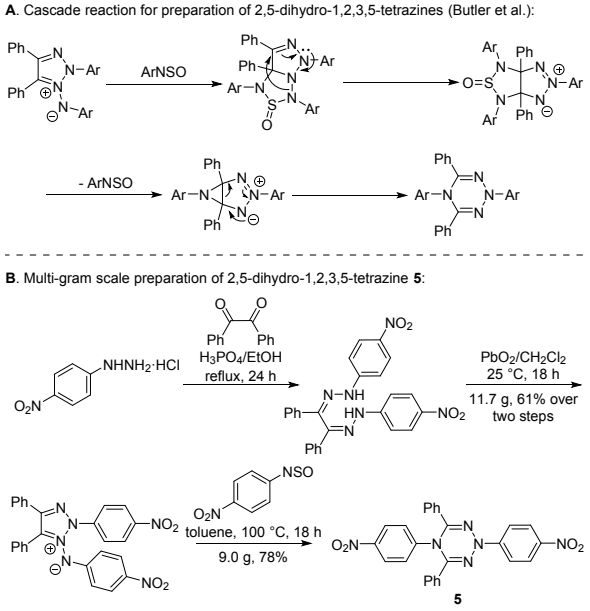


Figure 1. (A) Structure of 1,2,3,5-tetrazine (**1**) and 4,6-diphenyl-1,2,3,5-tetrazine (**2**). (B) Reported structures bearing a nonaromatic 1,2,3,5-tetrazine core.

RESULTS AND DISCUSSION

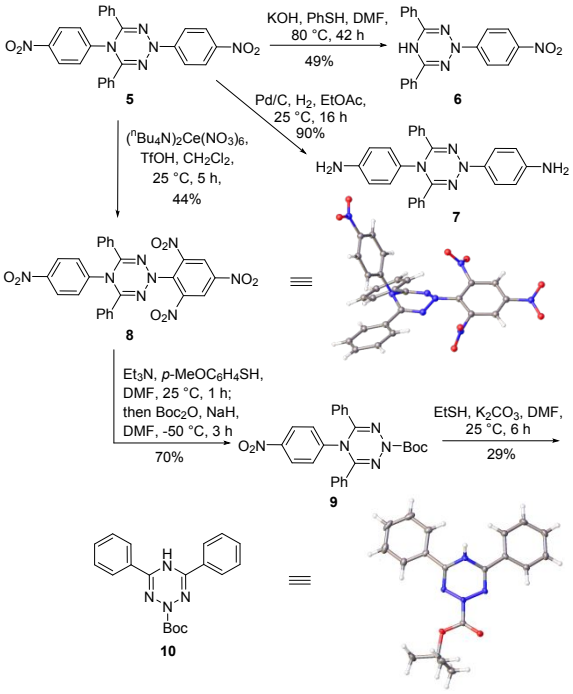
Synthesis and characterization of 4,6-diphenyl-1,2,3,5-tetrazine. The widely utilized syntheses of the isomeric 1,2,4,5-tetrazines employ an oxidative aromatization of dihydro-1,2,4,5-tetrazines as an effective final step,[17] suggesting that the preparation of the 1,2,3,5-tetrazine system from a non-aromatic dihydro precursor likely would be similarly successful. In a series of studies focused on the reactivity of 1,2,3-triazolium amides, Butler et al. reported a beautiful reaction cascade to furnish N^2,N^5-diaryl substituted 2,5-dihydro-1,2,3,5-tetrazines (Scheme 1A).[18] We envisioned that subsequent removal of the N-aryl substituents by either oxidation or S_NAr reactions, followed by a subsequent oxidation of the resulting dihydrotetrazine would be a promising approach to prepare 4,6-disubstituted 1,2,3,5-tetrazines.

Scheme 1

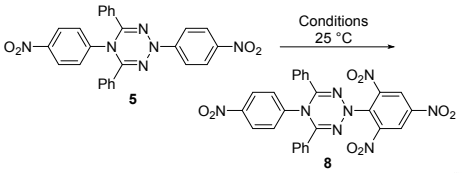


For initial studies, we chose to target the preparation of the substituted tetrazine **2**, 4,6-diphenyl-1,2,3,5-tetrazine. We began our study with development of a multigram scale preparation of **5**,[18] a reported dihydro-1,2,3,5-tetrazine bearing *p*-nitrophenyl groups as the N-substituents (Scheme 1B). In order to investigate the oxidative dearylation effected by reagents like cerium ammonium nitrate (CAN), hydrogenation of **5** was conducted to yield 2,5-dihydrotetrazine **7** in which the N-substituents were reduced to *p*-anilinyl as an appropriate reaction precursor (Scheme 2). The alternative S_NAr reaction of **5**, using thiophenol as the nucleophile, proceeded smoothly to yield the stable N^5-dearylated product **6**. However, the second dearylation of the remaining N^2-substituent was found to be difficult and has not yet been achieved even under harsher reaction conditions.

Scheme 2



A serendipitous finding was observed when the oxidative dearylations of **5**, **6**, and **7** were examined. CAN or 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) mediated oxidative dearylations of **6** and most notably **7** were not successful where oxidation and decomposition of the dihydrotetrazine system were observed prior to the desired N-dearylations. With **5**, however, treatment with $(^nBu_4N)_2Ce(NO_3)_6$, a CAN equivalent that is soluble in organic solvents, resulted in selective dinitration of the N^2-*p*-nitrophenyl substituent, affording **8** in 44% yield. The formation and structure of **8** were established by NMR and HRMS and unambiguously confirmed with a single crystal X-ray structure determination (Scheme 2).[19] CAN-mediated nitrations have been reported,[20] and the presence of stoichiometric acid was needed to effectively promote the reaction that provided **8**.[21] Notably, use of CAN instead of $(^nBu_4N)_2Ce(NO_3)_6$ either in acetonitrile or methanol resulted in little or no reaction product and CH_2Cl_2 was found to be the most effective solvent for the reaction of those examined (Figure 2). Although the attempted oxidation of **5** did not remove the aryl substituents, we viewed this transformation as an activation of the unreactive N^2-aryl moiety, permitting subsequent S_NAr dearylation.

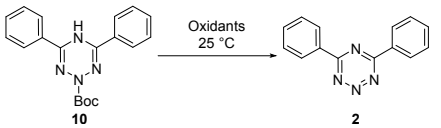


Entry	Reagent	Solvent	Time	Yield
1	$(^nBu_4N)_2Ce(NO_3)_6$	CH_2Cl_2	3 h	0%
2	$(^nBu_4N)_2Ce(NO_3)_6$+TfOH	CH_2Cl_2	3 h	40%
3	$(^nBu_4N)_2Ce(NO_3)_6$+TfOH	$CHCl_3$	18 h	0%
4	$(^nBu_4N)_2Ce(NO_3)_6$+TfOH	CH_3CN	24 h	17%
5	$(NH_4)_2Ce(NO_3)_6$ +TfOH	CH_3CN	3 h	2%
6	$(NH_4)_2Ce(NO_3)_6$	CH_3OH	3 h	0%
7	$(^nBu_4N)_2Ce(NO_3)_6$+TfOH	Acetone	24 h	20%
8	$(^nBu_4N)_2Ce(NO_3)_6$+TfOH	$ClCH_2CH_2Cl$	3 h	37%
9	$(^nBu_4N)_2Ce(NO_3)_6$+TfOH	THF	72 h	13%
10[a]	$(^nBu_4N)_2Ce(NO_3)_6$+TfOH	CH_2Cl_2	5 h	44%

[a]22 mmol scale

Figure 2. Discovery and optimization of the nitration reaction of **5**.

With **8** in hand, two sequential S_NAr dearylations were conducted. As expected, the N^2-picryl group is now more vulnerable to nucleophilic attack by an aryl thiol (*p*-$MeOC_6H_4SH$, Et_3N) under mild conditions (25 °C, 1 h), leaving the N^5-substituent intact. The released free amine was found to be unstable at 25 °C, which is in sharp contrast to its isomer **6** (stable at 80 °C). As a result, a subsequent in situ Boc-protection was performed to provide **9** in 70% overall yield. Interestingly, normal protection conditions using a weak base (Et_3N) were not effective, and a strong base (NaH) was required. Conducting the reaction at low temperature (–50 °C) was also found to be important in this protection step to avoid unproductive consumption of the free amine. The second nucleophilic substitution reaction was more challenging due to the instability of **9** (compared to **5**) in the presence of nucleophiles. Use of an alkyl thiol as a stronger nucleophile was found to promote the remaining S_NAr reaction under mild conditions (EtSH, K_2CO_3, DMF, 25 °C, 6 h), providing the N^2-Boc protected dihydrotetrazine **10** (29%, see Table S2 in Supporting Information for conditions surveyed). Because limited structural information was provided by NMR, the structure and solid-state conformation of **10** were unambiguously determined by X-ray crystallography (Scheme 2).[19] The final oxidation of **10** proceeded smoothly with a range of oxidants under mild reaction conditions, affording the desired 4,6-diphenyl-1,2,3,5-tetrazine (**2**). A spontaneous loss of the Boc group was observed to accompany the oxidation. Compared with other oxidants, including CAN or $(^nBu_4N)_2Ce(NO_3)_6$, MnO_2 was found to be the most effective and convenient reagent of those examined, providing **2** in high yield (80%) with simple filtration removal of the oxidant following completion of the reaction (Figure 3). Tetrazine **2** is semi-stable on a silica gel column, although a rapid column chromatography purification was important to provide the product in high yield.



10 2

Entry	Oxidant	Solvent[a]	Time	Yield
1	$(^nBu_4N)_2Ce(NO_3)_6$	CH_2Cl_2	5 min	34%
2	$(NH_4)_2Ce(NO_3)_6$	CH_3CN	10 min	45-69%
3	MnO_2	CH_2Cl_2	15 min	72%
4	MnO_2[b]	CH_2Cl_2	60 min	80%

[a]Anhydrous solvents. [b]Pre-mixed slurry of MnO_2 in CH_2Cl_2 was used.

Figure 3. Oxidation of dihydrotetrazine **10** to tetrazine **2**.

4,6-Diphenyl-1,2,3,5 tetrazine **2** is a yellow crystalline solid at room temperature, and crystalline needles suitable for X-ray single crystal diffraction analysis were obtained by recrystallization from CH_2Cl_2, $CHCl_3$, or MeCN.[19] The crystal structure is presented in Figure 4, alongside the structure of 2,5-dihydro-1,2,3,5-tetrazine **10** for comparison. All atoms in **2** were found to be nearly coplanar and equalization of the bond lengths in the central 1,2,3,5-tetrazine core was observed, with C–N bond lengths of 1.338–1.356 Å and N–N bond lengths of 1.326–1.328 Å, depicting a clear aromatic character for the delocalized 6π-aromatic 1,2,3,5-tetrazine ring (typical bond lengths: C–N: 1.416 Å, C=N: 1.279 Å, N–N: 1.420 Å, N=N: 1.240 Å[22]). This is in sharp contrast to the dihydrotetrazine derivatives **8** and **10**, where the dihydro-1,2,3,5-tetrazine core adopt boat conformations, and bond lengths of 1.392–1.393 Å, 1.284–1.286 Å and 1.424–1.425 Å were found for the C–N bonds, C=N bonds and N–N bonds in **10**, respectively. The ^{1}H NMR spectrum of tetrazine **2** exhibited a single set of phenyl peaks, indicative of free rotation of the phenyl ring in the solution. Notably, the ortho-protons of the phenyl substituent exhibit a downfield chemical shift of 8.8 ppm (same set of protons in **10** has a chemical shift of 7.8 ppm), resulting from a combined inductive and deshielding effect of the aromatic tetrazine. The tetrazine was found to display a characteristic downfield carbon peak (163 ppm) in the ^{13}C NMR, like that of the carbon found in 3,6-diphenyl-1,2,4,5-tetrazine (164 ppm).

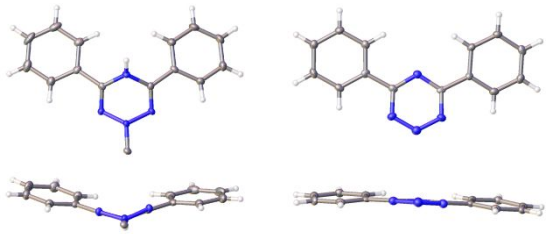


Figure 4. Top and side view of single crystal X-ray structures of **10** (left) and **2** (right). The Boc-group in **10** is omitted for clarity.

Tetrazine **2** was found to be stable at 25 °C in the crystalline state, in a chloroform solution, and even in a protic solvent (CD_3CN/D_2O, V/V = 7:3), all for at least 5 days after which its monitoring was discontinued. It was also found to be stable for at least 24 h at 25 °C in 70% H_2O/CH_3CN, 50% and 80% H_2O/DMSO, CH_3CN/PBS buffer (1:1), and hexafluoroisopropanol (HFIP). It can be stored at 4 °C for more than 50 days without observable decomposition. To obtain insights into its thermal stability, the kinetics of thermal consumption of tetrazine **2** were monitored by ^{1}H NMR at 80 °C, the lowest temperature at which measurable conversion was readily observed. A clean transformation of tetrazine **2** to benzonitrile and N_2 was found, with no observation of other byproducts or intermediates. First-order kinetics for the transformation were observed, with a rate constant of $k = 0.0293$ h^{-1} (8.15×10^{-6} s^{-1}) and half-life of $t_{1/2}$ = 23.6 h (8.50×10^4 s) (Supporting Information Figure S2). Although it is kinetically less stable than the corresponding 1,2,4,5-tetrazine in agreement with earlier computational studies,[12] the observed stability of tetrazine **2** permits easy handling under normal conditions and it can be warmed to elevated temperatures in solvent to promote cycloadditions with predictable limitations on the conditions used.[21]

We also examined the photophysical and electrochemical properties of **2**. The UV-Vis spectrum of **2** showed a strong absorption (molar extinction coefficient $\varepsilon = 2.52 \times 10^4$ L·mol^{-1}·cm^{-1}) peak at 275 nm, corresponding to the π-π* transition of the phenyl rings and tetrazine core (Figure 5). A much weaker absorption ($\varepsilon = 4.90 \times 10^2$ L·mol^{-1}·cm^{-1}) was observed at 396 nm, corresponding to the n-π* transition of the tetrazine. It displayed no fluorescence, being distinct from the isomeric and weakly fluorescent 3,6-diphenyl-1,2,4,5-tetrazine ($\phi = 1.5 \times 10^{-4}$).[23] Tetrazine **2** was found to undergo a quasi-reversible single-electron reduction at –1.37 V (vs Fc^+/Fc, –0.78 V vs N. H. E.) in cyclic voltammetry (CV), revealing its electron-deficient nature (Figure 5). In contrast to its reduction potential, no observable oxidation was observed when a positive scan was performed on **2**. The reduction potential of **2** is comparable but slightly lower than 3,6-diphenyl-1,2,4,5-tetrazine (–1.21 V vs Fc^+/Fc),[24] indicating a similar electron deficiency and likely similar reactivity in inverse electron demand Diels–Alder cycloadditions.

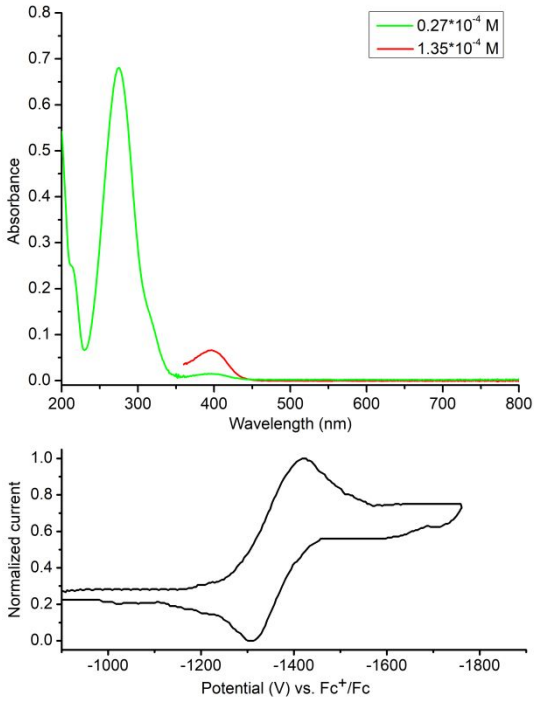


Figure 5. UV-Vis spectrum (top) and cyclic voltammetry data (bottom) of tetrazine **2**. UV-Vis spectrum was measured in CH_3CN. CV (scan rate, 40 mV/s) measurement was carried out in a solution of $^nBu_4NBF_4$ (100 mM) in CH_3CN.

Cycloaddition reactivity. We previously reported the discovery of the unusually rapid inverse electron demand Diels–Alder cycloadditions of a series of 1,2,3-triazines with amidines as heterodienophiles.[10] As a 5-aza-1,2,3-triazine, 1,2,3,5-tetrazines were anticipated to react with amidines to provide analogous cycloaddition products. When tetrazine **2** in acetonitrile (40 mM) was treated with benzamidine **11a**, complete conversion to **12a** was found after 2 h at 25 °C even at the dilute reaction concentrations. An exclusive C4/N1 versus N2/N5 mode of cycloaddition and a single reaction regioselectivity were observed, affording triphenyl-1,3,5-triazine **12a** in near quantitative yield (97%). The scope of this cycloaddition of tetrazine **2** with amidines was defined (Figure 6). All amidines, including aryl amidines (**11a**–**f**), heteroaryl amidines (**11g**–**j**), and aliphatic amidines (**11k**–**n**), provided the corresponding fully substituted 1,3,5-triazines (**12a**–**n**) in excellent yields (87–99%) under these remarkably mild reaction conditions (40 mM, 25 °C, 2 h). Notably, the reaction proceeded effectively even with electron-deficient (hetero)arylamidines (**11f**–**h**, **11j**). To examine the compatibility of this cycloaddition with protic solvents including water, the cycloaddition of tetrazine **2** with amidine **11l** was also conducted in CH_3CN/H_2O (V/V = 7:3, 2 h, 89% and 3:7, 24 h, 94%), where the reaction proceeded smoothly even in 70% H_2O/CH_3CN and provided triazine **12l** in the same yield observed in CH_3CN alone. In order to gain a deeper insight into the reaction mechanism and kinetics, ^1H NMR was used to monitor the reaction between tetrazine **2** (10 mM, CD_3CN) and amidine **11k** (2.0 equiv) at 25 °C. Fast consumption

of tetrazine **2** and accumulation of product **12k** were observed with appreciable product formation within 5 min, 90% conversion within 1 h, and full (>95%) conversion after 2 h. It is worth noting that ^{1}H NMR signals from only tetrazine **2**, amidine **11k**, and triazine **12k** were observed throughout the course of the reaction, without detection of any reaction intermediates or byproducts (Figure 7). Therefore, it is the cycloaddition reaction or addition-cyclization reaction between tetrazine and amidine that is the rate-determining step, which is followed by rapid sequential elimination of N_2 and NH_3 from the cycloadduct. From the NMR studies, the second-order rate constant of this reaction was determined to be k = (5.87±0.10)*10^{-2} M^{-1}·s^{-1} (Supporting Information Figure S3).

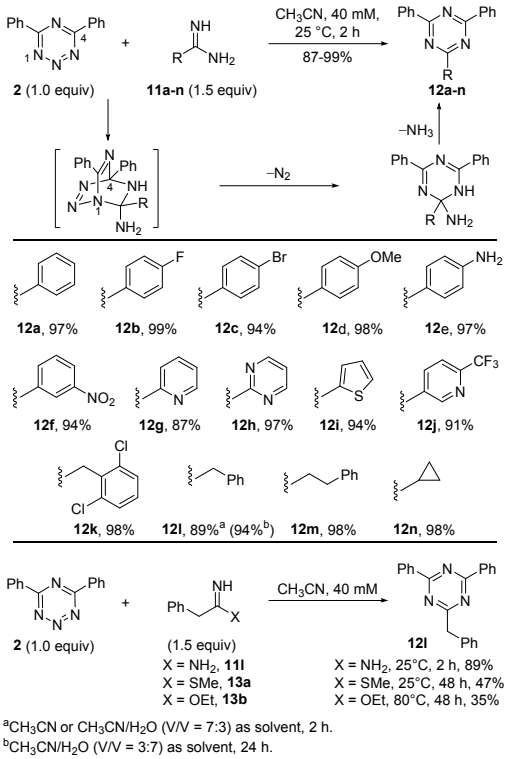


Figure 6. Scope of the cycloaddition between **2** and amidines, imidates and thioimidates.

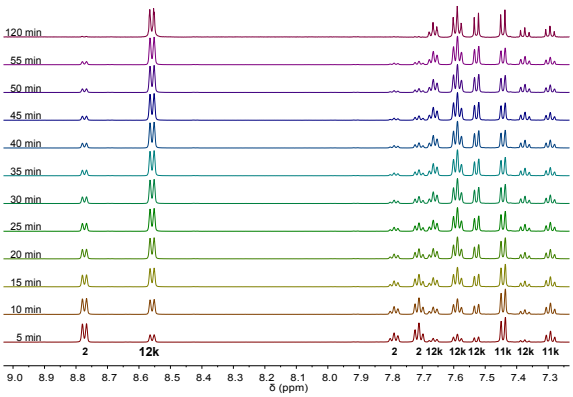


Figure 7. ^{1}H NMR study of the reaction between tetrazine **2** and amidine **11k** (2 equiv) over 120 min (10 mM, 25 °C, CD_3CN).

To further probe whether the reaction of amidines with 1,2,3,5-tetrazine **2** represents a single-step cycloaddition or a stepwise addition-cyclization, we conducted the reaction of **2** with amidine **11l** that was doubly labeled with ^{15}N (Figure 8). The reaction produced the 1,3,5-triazine product **12l** in superb yield (94%) with incorporation of a single ^{15}N label (1.07 ±0.01) as established by mass spectrometry (Supporting Information Table S3) and NMR experiments (Supporting Information Figure S4). This is consistent with a single-step cycloaddition and, while not ruling out a stepwise addition-cyclization mechanism, the latter would have been expected to provide a near equal mixture of singly and doubly ^{15}N labeled triazine products (ca. 1.5 vs 1 ^{15}N). If the cycloaddition does proceed in a non-concerted fashion with generation of a zwitterionic intermediate,[25] it would appear to require tight contact ion pair association following the initial addition with collapse to the [4+2] cycloadduct (vs proton transfer) prior to loss of nitrogen.

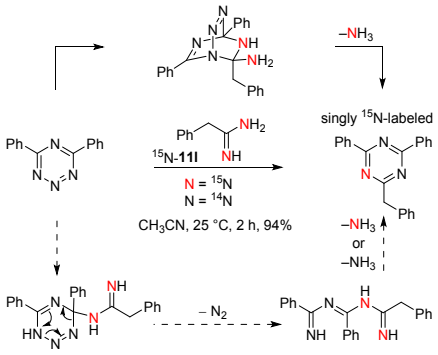


Figure 8. Reaction of **2** with doubly ^{15}N labeled **11l**.

Imidates and thioimidates are related heterodienophiles reported to react with some heterocyclic azadienes, but which typically display a lower reactivity than the corresponding amidines and a similar reactivity difference was observed with tetrazine **2** (Figure 6). Whereas the reaction of **2** with amidine **11l** proceeds smoothly under mild conditions (2 h, 25 °C, 89%), a longer reaction time was required for the corresponding thioimidate **13a** (48 h, 25 °C) and even then the formation of the same triazine **12l** was observed in a lower yield (47%). With an increase in the amount of dienophile (5 equiv) and reaction concentration (400 mM), requiring $CHCl_3$ as solvent for solubility purposes, the reaction provided **12l** in good yield (68%, 48 h, 25 °C). The corresponding imidate **13b** exhibited an even lower reactivity and the reaction between **2** and **13b** required an elevated temperature (35%, 48 h, 80 °C) or higher concentration (43%, 400 mM in $CHCl_3$, 5 equiv **13b**, 48 h, 25 °C) and longer reaction times, providing more modest yields of the triazine **12l**.

Additional dienophiles classes were examined, including electron-rich olefins and alkynes (Figure 9). The initial cycloaddition reaction between 4,6-diphenyl-1,2,3,5-tetrazine (**2**) with enamines **14a**–**d** in acetonitrile is fast even at room temperature and is accompanied by loss of N_2. This is followed by a slower amine elimination with rearomatization, requiring the longer reaction times and subsequent elevated

temperatures. The same exclusive C4/N1 (vs N2/N5) cycloaddition mode was observed and occurs with a predictable exclusive regioselectivity (dienophile most electron-rich atom attaches to C4), affording substituted pyrimidines (**15a**–**d**) in good yields. In the case of **14b**, the use of $CHCl_3$ (41%) versus CH_3CN (43%) as an alternative solvent provided similar yields of **15b**, although the impact of solvent on the slow and occasionally problematic final aromatization was not investigated in detail. A similar reactivity was observed for the cycloaddition of tetrazine **2** with ynamines (**16a**–**b**), where the reaction proceeded smoothly under mild conditions. Complete conversion of tetrazine **2** to pyrimidine **17a** (90% yield) was observed after 1 h (25 °C), whereas the reaction of the sterically more hindered ynamine **16b** was found to be slower, providing pyrimidine **17b** (92% yield) after 24 h.

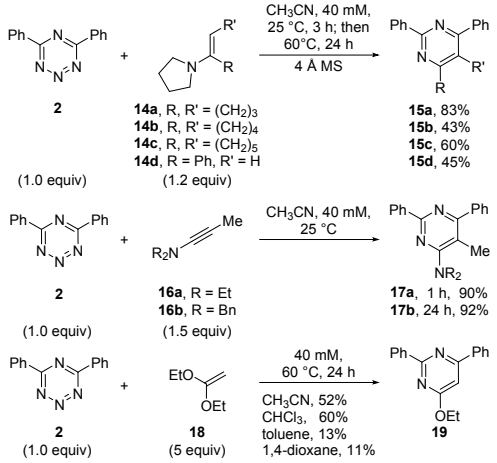


Figure 9. Reaction of **2** with enamines, ynamines and a ketene acetal.

Treatment of tetrazine **2** with ketene acetal **18** in warm acetonitrile (60 °C) provided the expected pyrimidine product **19** in good yield (52%) after 24 h (Figure 9). An interesting solvent effect was observed in this cycloaddition where the reaction in $CHCl_3$ gave a slightly improved yield (60%), but a slower reaction and incomplete conversion of the tetrazine **2** was observed when the reaction was conducted in toluene (13%) or 1,4-dioxane (11%) under otherwise identical conditions.

Tetrazine **2** was also found to react with the electron-rich styrene **20** at an elevated temperature (80 °C), where cycloaddition is followed by an in situ oxidation to afford the aromatic pyrimidine product **21** (Figure 10). A slow conversion and low yield (9%) were found when the reaction was conducted in acetonitrile, whereas an improved yield (28%) was observed when **2** was warmed with neat **20**. Although the latter results might appear modest, they do highlight the exceptional reactivity that can be expected of the 1,2,3,5-tetrazines, rivaling that of the 1,2,4,5-tetrazines and exceeding that of the respective triazines.

Strained alkenes and alkynes are an especially interesting and important dienophile class. They serve as an excellent group of dienophiles and their reactions with 1,2,4,5-tetrazines proceed at remarkable

rates under extraordinarily mild conditions even at dilute reaction concentrations.[4] Consequently, variations on these reactions have been explored and adopted for powerful bioorthogonal conjugation and labeling techniques.[3,4] In order to investigate whether 1,2,3,5-tetrazine **2** possesses similar or a distinctive reactivity, the reaction of 1,2,3,5-tetrazine **2** with the strained alkyne **22** was examined. Efficient cycloaddition with formation of the fused pyrimidine **23** in excellent yield (97%) was observed under mild (25 °C) and modestly concentrated reaction conditions (400 mM **2**, 4 equiv dienophile) and progressively slower conversions and decreased yields were observed under increasingly more dilute reaction concentrations (40 mM **2**, 28%; 5 mM **2**, no reaction) with the balance of the materials being recovered starting tetrazine **2** and alkyne **22** (Figure 10). Again, only a single mode of cycloaddition was observed (C4/N1 vs N2/N5).

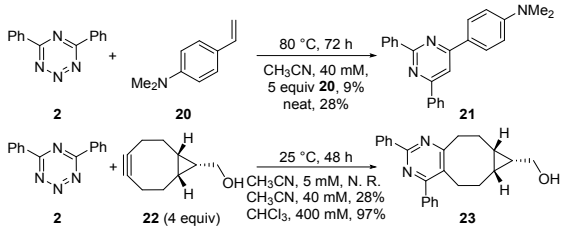


Figure 10. Reaction of **2** with an electron-rich styrene and a strained alkyne.

Reactivity comparison between two isomeric tetrazines: 4,6-diphenyl-1,2,3,5-tetrazine (2) versus 3,6-diphenyl-1,2,4,5-tetrazine (24). In order to compare what became evident as distinct cycloaddition behaviors, the reactivity of 4,6-diphenyl-1,2,3,5-tetrazine (**2**) and the well-known and similarly substituted 3,6-diphenyl-1,2,4,5-tetrazines (**24**) was compared side-by-side and in competition studies with representative members of the different dienophile classes. Notably, these would not be the most reactive members of either tetrazine class, but the comparisons do provide the first opportunity to define cycloaddition distinctions. Three dienophiles were chosen for study, amidine **11l**, ynamine **16a,** and cyclooctyne **22**, representing examples of a heterodienophile, an electron-rich dienophile, and a strained dienophile, respectively. Separate reactions between each of the tetrazines and dienophiles were conducted, followed by competition reactions where 1 equivalent of dienophile was added to an equimolar mixture of 1,2,3,5-tetrazine **2** and 1,2,4,5-tetrazine **24** (1 equiv each) (Figure 11).

Three different outcomes were observed for the three dienophiles. While amidine **11l** rapidly reacted with 1,2,3,5-tetrazine **2** under mild conditions (25 °C, 40 mM, CH_3CN, 2 h, 89%), no reaction between the same amidine **11l** and 1,2,4,5-tetrazine **24** was observed under the same reaction conditions, consistent with reports of the modest reactivity of 1,2,4,5-tetrazines toward amidines.[1a] Perhaps even more remarkable, 1,3,5-triazine **12l** was observed as the only product (92%) when an equimolar mixture of the two tetrazines was treated with amidine **11l**, reflecting the much greater reactivity of **2**. A completely reversed cycloaddition preference was observed when cyclooctyne was used as the dienophile. Consistent

with prior reports, 3,6-diphenyl-1,2,4,5-tetrazine (**24**) possessed an outstanding reactivity toward cyclooctyne **22**, rapidly forming the pyridazine **25** (25 °C, 5 mM, CH_3CN, 10 min, 95%). By contrast, the reaction of 1,2,3,5-tetrazine **2** with the strained cyclooctyne **22** did not produce any pyrimidine **23** under the same reaction conditions even after prolonged reaction times (48 h). Expectedly, only pyridazine **25** was isolated (98%) when an equimolar of mixture of the two tetrazines **2** and **24** were treated with cyclooctyne **22** (1 equiv), reflecting now the greater reactivity of **24**. Ynamine **16a** was found to react with either tetrazine individually (25 °C, 40 mM, 1 h), affording the corresponding pyrimidine **17a** (90%) or pyridazine **26** (95%) as products. The treatment of ynamine **16a** (1 equiv) with an equimolar mixture of the two tetrazines gave both **17a** (15%) and **26** (53%) as reaction products, revealing a competitive but slightly lower reactivity of 1,2,3,5-tetrazine **2** than 1,2,4,5-tetrazine **24** toward the ynamine dienophile (1:3.5).

These results, along with the observations on the reactivities of tetrazines with other dienophiles, indicates the dependence of inverse electron demand Diels–Alder reactivity on both the core structure of the tetrazine as well as the dienophile itself. Compared to the 1,2,4,5-tetrazine **24**, 1,2,3,5-tetrazine **2** exhibited an outstanding reactivity toward heterodienophiles (amidines), a comparable reactivity toward electron-rich dienophiles (ynamines, enamines, ketene acetal), and a lower reactivity toward strained olefins and alkynes (cyclooctyne). Houk et al. recently reported a comprehensive computational study of the reaction of the full and systematic series of heterocyclic azadienes with olefinic dienophiles.[26] A selectivity for C–C bond formation over C–N bond formation within a given azadiene was predicted, defining a preferred intrinsic mode of cycloaddition, and the cycloaddition reactivity was found to follow a general trend of two C–C bond > C–C and C–N bond > two C–N bond formations. Thus, in addition to the relative energies of the reacting LUMO energies (slightly lower LUMO for 1,2,4,5-tetrazine vs 1,2,3,5-tetrazine, ΔE = 0.03–0.04 eV, both of which are much lower than any of the isomeric triazines, ΔE = 0.5–0.8 eV; MNDO/AM1), this correspondence between cycloaddition reactivity and nature of the bond formation at the diene termini was attributed to both a lower distortion energy of the dienes/dienophiles and productive interaction energy (orbital overlap efficiency) in the reaction transition state, where both are more favorable for the cycloaddition of olefinic dienophiles (ethylene) with 1,2,4,5-tetrazine versus 1,2,3,5-tetrazine (ΔE_{act} = 9.4 kcal/mol). Consequently, a 1,2,4,5-tetrazine was predicted to be more reactive than a 1,2,3,5-tetrazine toward olefin/alkyne dienophiles. Whereas we found that the expected distinction was modest with an ynamine (electron-rich alkyne), we found that it was remarkably pronounced with a strained alkyne. However, the origin of the remarkably efficient and high reactivity of amidines with a 1,2,3,5-tetrazine, especially compared with a 1,2,4,5-tetrazine, is unclear. It is possible that the polarized nature of both the diene and dienophile and the polarity match of the reacting partners might result in a change in reaction mechanism, with the reaction proceeding now through a non-concerted cycloaddition or asynchronous

cycloaddition rather than a synchronous concerted cycloaddition. However, we find no evidence to support a simple addition-cyclization reaction in direct reaction monitoring (Figure 7), ^{15}N-labeling studies (Figure 8), or through intercepted reaction byproducts. Alternatively, it is plausible that the interaction energy of an amidine with a 1,2,3,5-tetrazine is much more favorable than with a 1,2,4,5-tetrazine, where the latter but not the former would suffer destabilizing electrostatic interactions of its ring nitrogens with the amidine amine substituent in the [4+2] cycloaddition transition state. Regardless of the origin but as a result of the remarkable distinctions in cycloaddition reactivity, a new orthogonal reaction pair was discovered, the 1,2,3,5-tetrazine/amidine pair, complementing the powerful 1,2,4,5-tetrazine/strained alkyne pair. Whereas 1,2,4,5-tetrazine displays extraordinary cycloaddition rates with strained dienophiles (tetrazine ligation), the new and isomeric 1,2,3,5-tetrazine displays similarly remarkable cycloaddition rates and efficiencies with amidines (1,2,3,5-tetrazine/amidine ligation). When following the reactions of 4,6-diphenyl-1,2,3,5-tetrazine with amidines (^{1}H NMR), only starting material and product are observed and no intermediate(s) or byproducts are detected (see Figure 7). The crossover reactivities (1,2,4,5-tetrazines with amidines vs 1,2,3,5-tetrazines with strained dienophiles) are sufficiently low to indicate they may be used concurrently without competitive reactions. Both reactions proceed at low reaction concentrations under mild conditions and are compatible with H_2O as the reaction co-solvent, providing potential new applications in orthogonal or sequential ligation reactions. Such studies are presently under investigation.

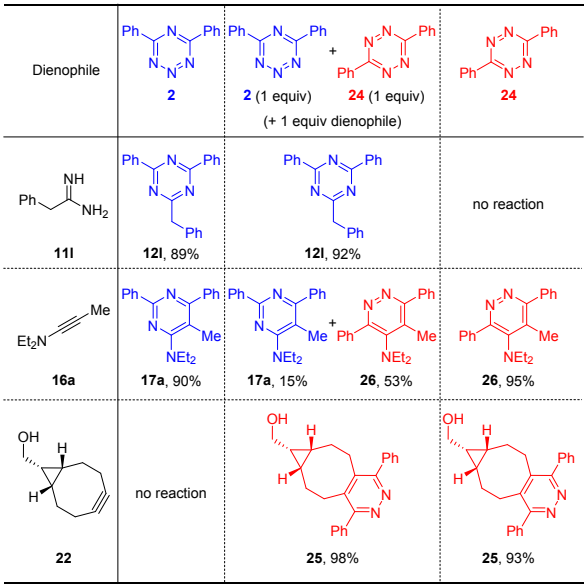


Figure 11. Comparison of the reactivity of 1,2,3,5-tetrazine **2** and 1,2,4,5-tetrazine **24** with different dienophile classes.

CONCLUSIONS

Herein we disclosed a novel synthesis and the full characterization of the first member of a monocyclic aromatic 1,2,3,5-tetrazine, 4,6-diphenyl-1,2,3,5-tetrazine. It is remarkable that no member of such a fundamental and important heterocyclic ring system had been previously described. Its synthesis and full characterization, including $^{1}H/^{13}C$ NMR, X-ray structure, intrinsic chemical stability, UV spectrum, and quasi reversible single-electron reduction (–1.37 V vs Fc^{+}/Fc) are reported. Although we continue to define its cycloaddition reactivity, mode, regioselectivity, and scope, we have found that: (1) its reactivity is comparable to that of the isomeric 3,6-diphenyl-1,2,4,5-tetrazine, (2) it displays a single mode of cycloaddition with reaction only across C4/N1 (no N2/N5 cycloaddition observed), (3) its cycloaddition occurs with a predictable regioselectivity (dienophile most electron-rich atom attaches to C4), and (4) it participates as the 4π-component of well-behaved inverse electron demand Diels–Alder reactions preferentially reacting with electron-rich or strained dienophiles. Although we are only now beginning to explore the full cycloaddition scope, we have already found that it displays additional reactivity complementary to the isomeric 1,2,4,5-tetrazines. It not only displays the expected remarkable cycloaddition reactivity, a surprisingly good stability (e.g., stable to chromatography, long term storage, presence of H_2O even as reaction co-solvent), and broad cycloaddition scope, but it also exhibits a powerful orthogonal reactivity with the 1,2,4,5-tetrazines that may be especially useful, including in the fields of click ligation and bioconjugation. Whereas the latter displays extraordinary cycloaddition rates with strained dienophiles (tetrazine ligation), the new and isomeric 1,2,3,5-tetrazine displays similarly remarkable cycloaddition rates and efficiencies with amidines (1,2,3,5-tetrazine/amidine ligation). The crossover reactivities (1,2,4,5-tetrazines with amidines vs 1,2,3,5-tetrazines with strained dienophiles) are sufficiently low to indicate they may be capable of concurrent use without competitive reactions. Extensions of the work to the study of additional substituted 1,2,3,5-tetrazines, the development of additional and needed synthetic approaches to this previously unknown class of heterocyclic azadienes, and their applications are in progress and will be reported in due time.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:10.1021/jacs.xxxxxxx. Full experimental details and characterization data (PDF)

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Notes

The authors declare no competing financial interests.

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REFERENCES

1. (a) Boger, D. L. Diels–Alder Reactions of Azadienes. *Tetrahedron* **1983**, *39*, 2869–2939. (b) Boger, D. L. Diels–Alder Reactions of Heterocyclic Azadienes. Scope and Applications. *Chem. Rev.* **1986**, *86*, 781–793.

2. (a) Moisan, L.; Odermatt, S.; Gombosuren, N.; Carella, A.; Rebek, J., Jr. Synthesis of an Oxazole–Pyrrole–Piperazine Scaffold as an α-Helix Mimetic. *Eur. J. Org. Chem.* **2008**, *10*, 1673–1676. (b) Volonterio, A.; Moisan, L.; Rebek, J., Jr. Synthesis of Pyridazine-Based Scaffolds as α-Helix Mimetics. *Org. Lett.* **2007**, *9*, 3733–3736. (c) Biros, S. M.; Moisan, L.; Mann, E.; Carella, A.; Zhai, D.; Reed, J.; Rebek, J., Jr. Heterocyclic α-Helix Mimetics for Targeting Protein–Protein Interactions. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4641–4645. (d) Lahue, B. R.; Lo, S.-M.; Wan, Z.-K.; Woo, G. H. C.; Snyder, J. K. Intramolecular Inverse-Electron-Demand Diels−Alder Reactions of Imidazoles with 1,2,4-Triazines: A New Route to 1,2,3,4-Tetrahydro-1,5-Naphthyridines and Related Heterocycles. *J. Org. Chem.* **2004**, *69*, 7171–7182.

3. (a) Devaraj, N. K.; Weissleder; Hilderbrand, S. A. Tetrazine-Based Cycloadditions: Application to Pretargeted Live Cell Imaging. *Bioconjugate Chem.* **2008**, *19*, 2297–2299. (b) Blackman, M. L.; Royzen, M.; Fox, J. M. Tetrazine Ligation: Fast Bioconjugation Based on Inverse-Electron-Demand Diels−Alder Reactivity. *J. Am. Chem. Soc.* **2008**, *130*, 13518–13519. (c) Devaraj, N. K.; Upadhyay, R.; Haun, J. B.; Hilderbrand, S. A.; Weissleder, R. Fast and Sensitive Pretargeted Labeling of Cancer Cells through a Tetrazine/trans-Cyclooctene Cycloaddition. *Angew. Chem., Int. Ed.* **2009**, *48*, 7013–7016. (d) Li, Z.; Cai, H.; Hassink, M.; Blackman, M. L.; Brown, R. C. D.; Conti, P. S.; Fox, J. M. Tetrazine–trans-Cyclooctene Ligation for the Rapid Construction of ^{18}F Labeled Probes. *Chem. Commun.* **2010**, *46*, 8043–8045. (e) Rossin, R.; Verkerk, P. R.; van den Bosch, S. M.; Vulders, R. C. M.; Verel, I.; Lub, J.; Robillard, M. S. In vivo Chemistry for Pretargeted Tumor Imaging in Live Mice. *Angew. Chem., Int. Ed.* **2010**, *49*, 3375–3378. (f) Haun, J. B.; Devaraj, N. K.; Hilderbrand, S. A.; Lee, H.; Weissleder, R. Bioorthogonal Chemistry Amplifies Nanoparticle Binding and Enhances the Sensitivity of Cell Detection. *Nat. Nanotechnol.* **2010**, *5*, 660–665. (g) McKay, C. S.; Finn, M. G. Click Chemistry in Complex Mixtures: Bioorthogonal Bioconjugation. *Chem. Biol.* **2014**, *21*, 1075–1101. (h) Denk, C.; Svatunek, D.; Filip, T.; Wanek, T.; Lumpi, D.; Fröhlich, J.; Kuntner, C.; Mikula, H. Development of a ^{18}F-Labeled Tetrazine with Favorable Pharmacokinetics for Bioorthogonal PET Imaging. *Angew. Chem., Int. Ed.* **2014**, *53*, 9655–9659. (i) Wu, H.; Devaraj, N. K. Advances in Tetrazine Bioorthogonal Chemistry Driven by the Synthesis of Novel Tetrazines and Dienophiles. *Acc. Chem. Res.* **2018**, *51*, 1249–1259.

4. (a) Han, H.; Devaraj, N. K.; Lee, J.; Hilderbrand, S. A.; Weissleder, R.; Bawendi, M. G. Development of a Bioorthogonal and Highly Efficient Conjugation Method for Quantum Dots Using Tetrazine−

Norbornene Cycloaddition. *J. Am. Chem. Soc.* **2010**, *132*, 7838–7839. (b) Schoch, J.; Weissler, M.; Jäschke, A. Post-Synthetic Modification of DNA by Inverse-Electron-Demand Diels−Alder Reaction. *J. Am. Chem. Soc.* **2010**, *132*, 8846–8847. (c) Pipkorn, R.; Waldemar, W.; Didinger, B.; Koch, M.; Mueller, G.; Wiessler, M.; Braun, K. Inverse-Electron-Demand Diels−Alder Reaction as a Highly Efficient Chemoselective Ligation Procedure: Synthesis and Function of a BioShuttle for Temozolomide Transport into Prostate Cancer Cells. *J. Pept. Sci.* **2009**, *15*, 235–241.

5. Initial report: (a) Carboni, R. A.; Lindsey, R. V. Reactions of Tetrazines with Unsaturated Compounds. A New Synthesis of Pyridazines. *J. Am. Chem. Soc.* **1959**, *81*, 4342–4346. (b) Boger, D. L.; Coleman, R. S.; Panek, J. S.; Yohannes, D. Thermal Cycloaddition of Dimethyl 1,2,4,5-Tetrazine-3,6-dicarboxylate with Electron-Rich Olefins: 1,2-Diazine and Pyrrole Introduction. Preparation of Octamethylporphin (OMP). *J. Org. Chem.* **1984**, *49*, 4405–4409. (c) Boger, D. L.; Sakya, S. M. Inverse Electron Demand Diels–Alder Reactions of 3,6-Bis(methylthio)-1,2,4,5-tetrazine. 1,2-Diazine Introduction and Direct Implementation of a Divergent 1,2,4,5-Tetrazine → 1,2-Diazine → Benzene (Indoline/Indole) Diels–Alder Strategy. *J. Org. Chem.* **1988**, *53*, 1415–1423. (d) Boger, D. L.; Schaum, R. P.; Garbaccio, R. M. Regioselective Inverse Electron Demand Diels−Alder Reactions of N-Acyl 6-Amino-3-(methylthio)-1,2,4,5-tetrazines. *J. Org. Chem.* **1998**, *63*, 6329–6337.

6. Initial report: (a) Steigel, A.; Sauer, J. (4+2)-Cycloadditionen 6-Gliedriger Heterocyclen mit Inaminen. *Tetrahedron Lett.* **1970**, *11*, 3357–3360. (b) Boger, D. L.; Panek, J. S. Diels–Alder Reaction of Heterocyclic Azadienes. I. Thermal Cycloaddition of 1,2,4-Triazine with Enamines: Simple Preparation of Substituted Pyridines. *J. Org. Chem.* **1981**, *46*, 2179–2182. (c) Boger, D. L.; Panek, J. S.; Meier, M. M. Diels–Alder Reaction of Heterocyclic Azadienes. 2." Catalytic" Diels–Alder Reaction of in situ Generated Enamines with 1,2,4-Triazines: General Pyridine Annulation. *J. Org. Chem.* **1982**, *47*, 895–897.

7. Initial report: (a) Neunhoeffer, H.; Bachmann, M. Cycloadditionen mit Azabenzolen, X. Cycloadditionen mit 1,3,5-Triazinen. *Chem. Ber.* **1975**, *108*, 3877–3882. (b) Boger, D. L.; Schumacher, J.; Mullican, M. D.; Patel, M.; Panek, J. S. Thermal Cycloaddition of 1,3,5-Triazine with Enamines: Regiospecific Pyrimidine Annulation. *J. Org. Chem.* **1982**, *47*, 2673–2675.

8. (a) Wilkie, G. D.; Elliott, G. I.; Blagg, B. S. J.; Wolkenberg, S. E.; Soenen, D. R.; Miller, M. M.; Pollack, S.; Boger, D. L. Intramolecular Diels−Alder and Tandem Intramolecular Diels−Alder/1,3-Dipolar Cycloaddition Reactions of 1,3,4-Oxadiazoles. *J. Am. Chem. Soc.* **2002**, *124*, 11292–11294. (b) Elliott, G. I.; Fuchs, J. R.; Blagg, B. S. J.; Ishikawa, H.; Tao, H.; Yuan, Z.-Q.; Boger, D. L. Intramolecular Diels−Alder/1,3-Dipolar Cycloaddition Cascade of 1,3,4-Oxadiazoles. *J. Am. Chem. Soc.* **2006**, *128*, 10589–10595. (c) Sears, J. E.; Boger, D. L. Tandem Intramolecular Diels–Alder/1,3-Dipolar Cycloaddition Cascade of 1,3,4-Oxadiazoles: Initial Scope and Applications. *Acc. Chem. Res.* **2016**, *49*, 241–251.

9. Initial report: (a) Neunhoeffer, H.; Werner, G. Reaktion von Pyridazinen mit 1-Diäthylamino-propin. *Liebigs Ann. Chem.* **1973**, 437–442. (b) Boger, D. L.; Coleman, R. S. Intramolecular Diels–Alder Reactions of 1,2-Diazines: General Indoline Synthesis. Studies on the Preparation of the Central and Right-hand Segments of CC-1065. *J. Org. Chem.* **1984**, *49*, 2240–2245. (c) Kessler, S. N.; Wegner, H. A. Lewis Acid Catalyzed Inverse Electron-Demand Diels−Alder Reaction of 1,2-Diazines. *Org. Lett.* **2010**, *12*, 4062–4065.

10. (a) Anderson, E. D.; Boger, D. L. Inverse Electron Demand Diels–Alder Reactions of 1,2,3-Triazines: Pronounced Substituent Effects on Reactivity and Cycloaddition Scope. *J. Am. Chem. Soc.* **2011**, *133*, 12285–12292. (b) Glinkerman, C. M.; Boger, D. L. Catalysis of Heterocyclic Azadiene Cycloaddition Reactions by Solvent Hydrogen Bonding: Concise Total Synthesis of Methoxatin. *J. Am. Chem. Soc.* **2016**, *138*, 12408–12413.

11. Zhang, J.; Shukla, V.; Boger, D. L. Inverse Electron Demand Diels–Alder Reactions of Heterocyclic Azadienes, 1-Aza-1,3-Butadienes, Cyclopropenone Ketals and Related Systems. A Retrospective. *J. Org. Chem.* **2019**, *84*, 9397–9445.

12. (a) Thomas, J. R.; Quelch, G. E.; Schaefer, H. F. The Unknown Unsubstituted Tetrazines: 1,2,3,4-Tetrazine and 1,2,3,5-Tetrazine. *J. Org. Chem.* **1991**, *56*, 539–543. (b) Fabian, J.; Lewars, E. Azabenzenes (Azines) – The Nitrogen Derivatives of Benzene with One to Six N Atoms: Stability, Homodesmotic Stabilization Energy, Electron Distribution, and Magnetic Ring Current; a Computational Study. *Can. J. Chem.* **2004**, *82*, 50–69.

13. William, M. K.; Michael, E. S. Low-Smoke Gas Generating Low Order Pressure Pulse Compositions. US Patent 7220328B1.

14. Baydar, A. E.; Boyd, G. V.; Lindley, P. F.; Walton, A. R. Approaches to 1,2,3,5-Tetrazines. Synthesis of 1,2,3,5-Tetrazinones and the Formation of Zwitterionic 1,2,4-Triazolin-3-ones and 1-Arylimino-1,2,4-triazolium Salts. *J. Chem. Soc. Perkin Trans. 1* **1985**, 415–418.

15. (a) Stevens, M. F. G.; Hickman, J. A.; Langdon, S. P.; Chubb, D.; Vickers, L.; Stone, R.; Baig, G.; Goddard, C.; Gibson, N. W.; Slack, J. A.; Newton, C.; Lunt, E.; Fizames, C.; Lavelle, F. Antitumor Activity and Pharmacokinetics in Mice of 8-Carbamoyl-3-methyl-imidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (CCRG 81045; M & B 39831), a Novel Drug with Potential as an Alternative to Dacarbazine. *Cancer Res.* **1987**, *47*, 5846–5852. (b) Stevens, M. F. G.; Hickman, J. A.; Stone, R.; Gibson, N. W.; Baig, G. U.; Lunt, E.; Newton, C. G. Antitumor Imidazotetrazines. 1. Synthesis and Chemistry of 8-Carbamoyl-3-(2-chloroethyl)imidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one, a Novel Broad-Spectrum Antitumor Agent. *J. Med. Chem.* **1984**, *27*, 196–201.

16. (a) Ohsawa, A.; Heihachiro, A.; Hidefumi, O.; Hiroshi, I. Synthesis, Oxidation, and Reduction of Monocyclic 1,2,3-Triazines. *J. Chem. Soc. Chem. Commun.* **1980**, *24*, 1182–1183. (b) Okatani, T.; Koyama, J.; Tagahara, K. Modified Synthesis of Monocyclic 1,2,3-Triazine and Cycloaddition Reaction with Enamine: The Application to the Synthesis of Alkaloids, Tortuosamine, N-Formyltortuosamine and N-Acetyltortuosamine. *Heterocycles* **1989**, *29*, 1809–1814. (c) Sugimura, H.; Takeuchi, R.; Ichikawa, S.; Nagayama, E.; Sasaki, I. Synthesis of 1,2,3-Triazines Using the Base-Mediated Cyclization of (*Z*)-2,4-Diazido-2-alkenoates. *Org. Lett.* **2018**, *20*, 3434–3437.

17. (a) Sauer, J.; Heldmann, D. K.; Hetzenegger, J.; Krauthan, J.; Sichert, H.; Schuster, J. 1,2,4,5-Tetrazine: Synthesis and Reactivity in [4+2] Cycloadditions. *Eur. J. Org. Chem.* **1998**, *12*, 2885–2896. (b) Karver, M. R.; Weissleder, R.; Hilderbrand, S. A. Synthesis and Evaluation of a Series of 1,2,4,5-Tetrazines for Bioorthogonal Conjugation. *Bioconjugate Chem.* **2011**, *22*, 2263–2270. (c) Selvaraj, R.; Fox, J. M. An Efficient and Mild Oxidant for the Synthesis of s-Tetrazines. *Tetrahedron Lett.* **2014**, *55*, 4795–4797.

18. (a) Butler, R. N.; Cunningham, D.; McArdle, P.; O'Halloran, G. A. 1,2,3,5-Tetrazines and 1,2,3-Triazaspiro[4.4]nonanes: Remarkable Products from 1,3-Dipolar Cycloadditions of N-sulphinylamines with Substituted Triazolium Imides. *J. Chem. Soc. Chem. Commun.* **1988**, *3*, 232–234. (b) Butler, R. N.; Evans, A. M.; McNeela, E. M.; Cunningham, D.; McArdle, P. 8π-Six-Atom Rings: 1,3,4,5-Oxa- and -Thia-triazines and 1,2,3,5-Tetrazines from an Extended Tandem Reaction: Reactions of 1,2,3-Triazolium-1-imides with (*E*)-Cinnamaldehyde, Methyl Cyanodithioformate, and Aryl-N-sulphinylamines: New Tetrahydro-oxazolo[4,5-*d*]-1,2,3-Triazoliumides and Triazaspiro-[4.4]nonanes. Azolium 1,3-Dipoles. Part 4. *J. Chem. Soc. Perkin Trans. 1* **1990**, 2527–2536.

19. The structures of **2** (CCDC 1941636), **8** (CCDC 1941634), and **10** (CCDC 1941635) were established and confirmed in single-crystal X-ray structure determinations conducted on crystals grown from CH_2Cl_2 (**2**, yellow needles) or $CHCl_3$/hexanes (**8**, orange block; **10**, yellow block) and have been deposited with the Cambridge Crystallographic Data Center.

20. Yang, X.; Xi, C.; Jiang, Y. Regioselective Nitration of N,N-Dialkylanilines Using Cerium (IV) Ammonium Nitrate in Acetonitrile. *Tetrahedron Lett.* **2005**, *46*, 8781–8783.

21. Differential scanning calorimetric measurements (Supporting Information page S29) at 5 °C min^{-1} indicate that **2** does not melt prior to decomposition, has an onset decomposition temperature of 138 °C, and does so with a moderate thermal potential (0.177 kcal/g, 744 J/g). Intermediate **8** melts at 233 °C then displays an onset decomposition temperature at 242 °C, decomposes over a broader temperature range (242–400 °C, peak at 305 °C), and releases 0.657 kcal/g (2756 J/g) of energy over the full temperature range of decomposition. Safety precautions should be exercised especially when **8** is prepared on large scale and conducting chemistry with **8** within 100 °C of the onset temperature should be avoided.

22. Allen, F. H.; Kennard, O.; Watson, D. G.; Brammer, L.; Orpen, A. G.; Taylor, R. Tables of Bond Lengths Determined by X-ray and Neutron Diffraction. Part 1. Bond Lengths in Organic Compounds. *J. Chem. Soc. Perkin Trans. 2* **1987**, *12*, S1–S19.

23. Plugge, M.; Alain-Rizzo, V.; Audebert, P.; Brouwer, A. M. Excited State Dynamics of 3,6-Diaryl-1,2,4,5-tetrazines. Experimental and Theoretical Studies. *J. Photochem. Photobiol. A* **2012**, *234*, 12–20.

24. Fischer, H.; Müller, T.; Umminger, I.; Neugebauer, F. A.; Chandra, H.; Symons, M. C. Radical Cations and Anions of 1,2,4,5-Tetrazines: An Electron Spin Resonance and Cyclic Voltammetric Study. *J. Chem. Soc. Perkin Trans. 2* **1988**, 3413–3421.

25. Yang, Y. F.; Yu, P.; Houk, K. N. Computational Exploration of Concerted and Zwitterionic Mechanisms of Diels–Alder Reactions Between 1,2,3-Triazines and Enamines and Acceleration by Hydrogen-Bonding Solvents. *J. Am. Chem. Soc*. **2017**, *139*, 18213–18221.

26. Yang, Y.-F.; Liang, Y.; Liu, F.; Houk, K. N. Diels–Alder Reactivities of Benzene, Pyridine, and Di-, Tri-, and Tetrazines: The Roles of Geometrical Distortions and Orbital Interactions. *J. Am. Chem. Soc.* **2016**, *138*, 1660–1667.

Table of Contents Graphic

The first monocyclic aromatic 1,2,3,5-tetrazine:

- Synthesis and full characterization
- Reactive diene in inverse electron demand Diels-Alder reactions
- Single mode of cycloaddition (C4/N1) and predictable regioselectivity
- Orthogonal reactivity with 1,2,4,5-tetrazine without competitive reactions