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Article

Selective N1/N4 1,4-Cycloaddition of 1,2,4,5-Tetrazines Enabled by Solvent Hydrogen Bonding

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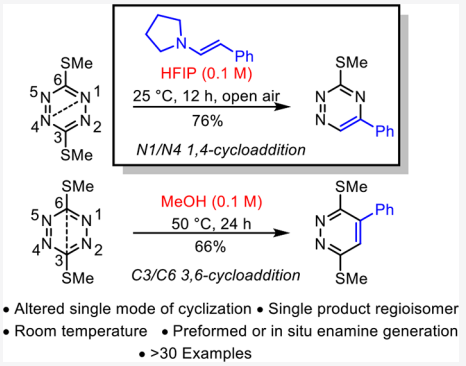
ABSTRACT: An unprecedented 1,4-cycloaddition (vs 3,6-cycloaddition) of 1,2,4,5-tetrazines is described with preformed or in situ generated aryl-conjugated enamines promoted by the solvent hydrogen bonding of hexafluoroisopropanol (HFIP) that is conducted under mild reaction conditions (0.1 M HFIP, 25 °C, 12 h). The reaction constitutes a formal [4 + 2] cycloaddition across the two nitrogen atoms (N1/N4) of the 1,2,4,5-tetrazine followed by a formal retro [4 + 2] cycloaddition loss of a nitrile and aromatization to generate a 1,2,4-triazine derivative. The factors that impact the remarkable change in the reaction mode, optimization of reaction parameters, the scope and simplification of its implementation through in situ enamine generation from aldehydes and ketones, the reaction scope for 3,6-bis(thiomethyl)-1,2,4,5-tetrazine, a survey of participating 1,2,4,5-tetrazines, and key mechanistic insights into this reaction are detailed. Given its simplicity and breath, the study establishes a novel method for the simple and efficient one-step synthesis of 1,2,4-triazines under mild conditions from readily accessible starting materials. Whereas alternative protic solvents (e.g., MeOH vs HFIP) provide products of the conventional 3,6-cycoladdition, the enhanced hydrogen bonding capability of HFIP uniquely results in promotion of the unprecedented formal 1,4-cycloaddition. As such, the studies represent an example of not just an enhancement in the rate or efficiency of a heterocyclic azadiene cycloaddition by hydrogen bonding catalysis but also the first to alter the mode (N1/N4 vs C3/C6) of cycloaddition.



INTRODUCTION

The inverse electron demand Diels−Alder reaction of electron-deficient heterocyclic azadienes is an effective method for the synthesis of highly functionalized heterocycles widely used in organic synthesis,[1] medicinal chemistry, and chemical biology.[2] Previously, we have reported systematic explorations and applications of the cycloaddition reactions of 1,2,4,5-tetrazines,[3] 1,2,4-triazines,[4] 1,3,5-triazines,[5] 1,3,4-oxadiazoles,[6] 1,2-diazines,[3b,7] 1,2,3-triazines,[8] and most recently a 1,2,3,5-tetrazine.[9] Among all heterocyclic azadienes, the readily available 1,2,4,5-tetrazines are the most widely used due to their superb cycloaddition reactivity with an unusually broad range of dienophiles (Figure 1A).[1,2] In the >60 years since its first disclosure and among the now countless examples, a single cycloaddition mode is observed that occurs across the two carbon atoms (3,6-cycloaddition) independent of the 1,2,4,5-tetrazine substitution pattern or nature of the dienophile.[10] To the best of our knowledge, no example of the alternative [4 + 2] cycloaddition across two nitrogen atoms (1,4-cycloaddition) of a 1,2,4,5-tetrazine has been disclosed. Best defined and articulated by Houk in computational studies,[11] the remarkable rate of cycloaddition, the preferential 3,6-cycloaddition mode, and lack of 1,4-cycloaddition can be attributed to orbital interactions and differential distortion energies en route to the transition state along with the energetically preferential formation of two C−C versus two C−N bonds and the subsequent release of N_2 rather than a nitrile.

Until recently and although examined for decades, no general approach to catalysis of the inverse electron demand Diels−Alder reactions of heterocyclic azadienes had been described.[12,13] Typically, additives such as Lewis acids lead to nonproductive consumption of the electron-rich dienophiles without productive activation of the electron-deficient heterocyclic azadienes. We found that heterocyclic azadienes can be activated for cycloaddition by H-bonding[14] with the non-nucleophilic solvents hexafluoroisopropanol (HFIP) and trifluoroethanol (TFE).[11] The H-bonding was established in mechanistic ^{1}H NMR studies, the catalysis was found to be unique to HFIP and TFE versus other protic solvents due to the reduced basicity of such heterocyclic azadienes, and our conclusions were verified in subsequent computational studies by Houk.[15] Since its discovery, we have continued to investigate the scope of solvent H-bonding assisted inverse

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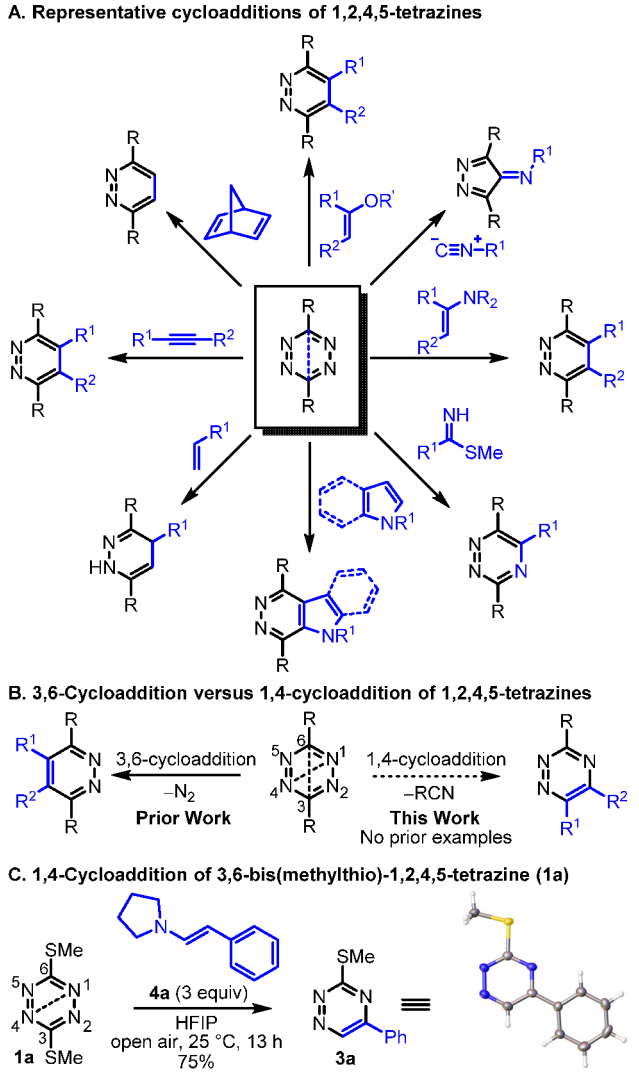


Figure 1. Cycloadditions of 1,2,4,5-tetrazines (C3/C6 cycloaddition) and the unprecedented formal 1,4-cycloaddition (N1/N4) detailed herein.

electron demand Diels–Alder cycloadditions.[13] In these studies, which have focused on defining the rate accelerations, improved conversions, regioselectivity enhancements or alterations, and expansion of the productive reactive diene/dienophile pairs, we discovered an unprecedented formal 1,4-cycloaddition of 3,6-bis(methylthio)-1,2,4,5-tetrazine (**1a**) with the enamine **4a**, 1-styrylpyrrolidine. When HFIP was used as the solvent at room temperature open to air, the reaction provided the 1,2,4-triazine **3a** in 75% yield, the structure of which was established with a single-crystal X-ray structure determination (Figure 1C).[16] Given the unprecedented nature of the reaction coupled with the importance of the 1,2,4-triazine core in drugs and biologically active molecules (Figure 2),[17] we examined and herein detail features that impact this change in the cycloaddition mode, optimization of the reaction parameters, the scope and further simplification of the reaction as it relates to the 1,2,4,5-tetrazine and preformed or in situ generated enamine, and mechanistic insights into this remarkable reaction. Given its breath, it establishes a new simple method for the efficient one-step synthesis of 1,2,4-triazines under mild reaction conditions from easily accessible starting materials.

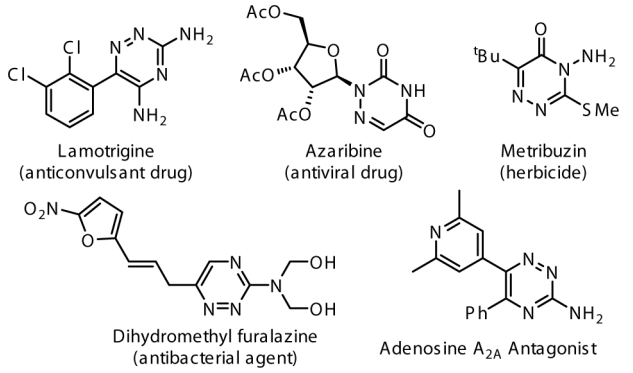
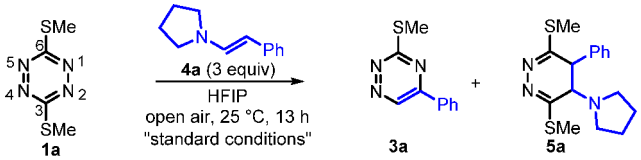


Figure 2. Selected biologically active 1,2,4-triazines.

RESULTS AND DISCUSSION

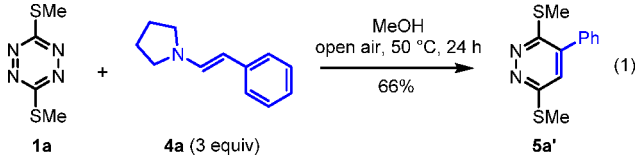
Reaction Discovery, Key Parameters, and Optimization. In order to better understand the role of each factor, especially the solvent, a series of experiments was conducted (Figure 3). An increase in the reaction temperature to 60 °C



entry	Change from standard conditions[a]	3a	5a
1	None	75%[b]	0%
2	60 °C instead of 25 °C.	71%	0%
3	22 h instead of 13 h	76%	0%
4	0.2 M instead of 0.1 M	71%	0%
5	$CHCl_3$ instead of HFIP	0%	32%
6	MeOH instead of HFIP	0%	79%[b]
7	$CHCl_3$/HFIP (1:1) instead of HFIP	34%	0%
8	1 equiv TFA in $CHCl_3$ instead of HFIP	0%	0%

Figure 3. Reaction parameters. [a]Unless otherwise noted, all the reactions were run with **1a** (0.05 mmol) in 0.5 mL solvent. [b]Isolated yield, other yields were established by NMR.

(entry 2, vs 25 °C), an increase in the reaction time to 22 h (entry 3, vs 13 h), and an increase in the reaction concentration (entry 4) had little impact on the yield of product. Importantly, only the conventional 3,6-cycloaddition product **5a**, without elimination of pyrrolidine, was generated when the reaction was conducted in nonfluorinated solvents, including $CHCl_3$ (entry 5) and methanol (entry 6). The reaction in methanol was found to generate the aromatic pyridazine product **5a′** (66%) with elimination of pyrrolidine when it was further warmed to 50 °C for 24 h (eq 1). The use



of HFIP as solvent was found to be essential for the altered 1,4-cycloaddition, although use of mixed solvent of $CHCl_3$–HFIP (1:1) led to the same product **3a**, albeit in lower yield (entry 7). Finally, addition of strong acid, TFA (1 equiv), and conducting the reaction in $CHCl_3$ resulted in no cycloaddition products, likely due to protonation of the enamine **4a** (entry 8). The unique behavior of HFIP arises from its ability to H-

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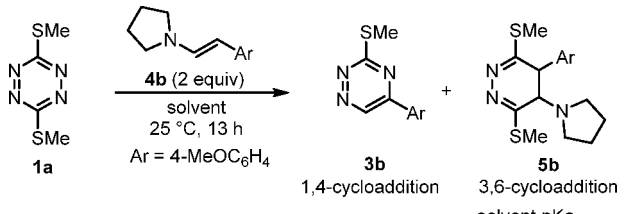
bond the 1,2,4,5-tetrazine, thereby activating it for reaction, and yet not consume either the starting 1,2,4,5-tetrazine because of the attenuated nucleophilic character of HFIP or the conjugated enamine through protonation because of its weakly acidic nature (pK_a = 9.3).

A more refined solvent survey for the reaction of **1a** with enamine **4b** in a series of perfluoroalcohols was conducted. Despite the variations in yields, the ratio of formal 1,4-cycloaddition versus the conventional 3,6-cycloaddition that provides **5b** and p*K*a of the perfluoroalcohols, which is a measure of their H-bonding capability,[18] were found to correlate exceptionally well (Figure 4). In fact, a clean switch



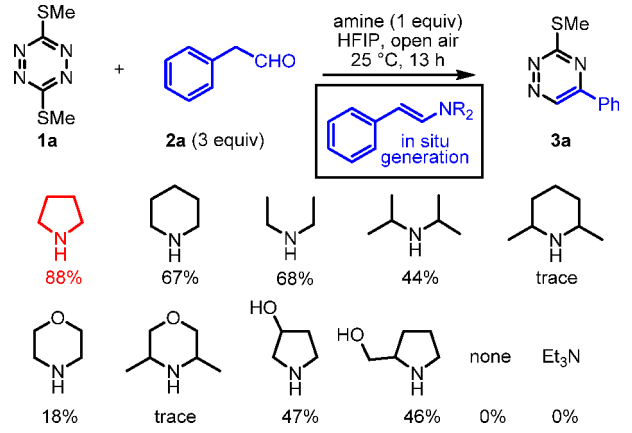
entry	solvent	3b:5b	yield	solvent pKa (in H_2O)
1	MeOH	only **5b**	46%	15.54
2	2,2,3,3-tetrafluoropropanol	1 : 1.2	55%	12.74
3	2,2,2-trifluoroethanol (TFE)	2.5 : 1	52%	12.37
4	hexafluoro-2-methylisopropanol	3 : 1	41%	9.55
5	HFIP	only **3b**	64%	9.30

Figure 4. Refined solvent survey. Yields were established by NMR.

from exclusive 3,6-cycloaddition to exclusive formal 1,4-cycloaddition was observed as the p*K*a of the solvent decreased from 15.5 (MeOH) to 9.3 (HFIP). As such, the results highlight the unique behavior of HFIP and indicate that the extent of the H-bonding interaction between the tetrazine and solvent is the feature controlling the 1,4- versus 3,6-cycloaddition selectivity. Experimentally, we observed that the HFIP alcohol proton exhibits a pronounced downfield chemical shift upon titration with 3,6-bis(thiomethyl)-1,2,4,5-tetrazine (**1a**, Δ0.53 ppm, 0−2 equiv) consistent with this H-bonding interaction with **1a** (Supporting Information Figure S1). It is possible that the selectivity is altered due to change in the LUMO molecular orbital distribution that is induced by solvent H-bonding, leading to a conjugated nitrogen now more susceptible to nucleophilic attack than carbon. Simple AM1 computation of the LUMO energy of free and protonated **1a** (−1.93 eV vs −6.17 eV) and sum of squared coefficients as they relate to tetrazine carbons (C3, C6) and nitrogens (N1, N2, N4, N5) (free **1a**, 0.62 for C and 0.26 for N; protonated **1a**, 0.43 for C and 0.39 for N; see Supporting Information Figure S2 for details) supports both the enhanced reactivity (rel E_{LUMO}) and a shift from C to N for attack of a nucleophile.

On the basis of the precedent that we first introduced[4b] and with recognition that a preformed enamine may not always be readily available, easily prepared and stored, or stable in open air, we examined whether the enamine could be generated in situ from the corresponding aldehyde and amine. To our delight, replacement of enamine **4a** (3 equiv) with phenylacetaldehyde (**2a**, 3 equiv) and pyrrolidine (1 equiv) provided **3a** in an improved yield (88%) under otherwise identical conditions (0.1 M HFIP, 25 °C, 13 h, open flask) (Scheme 1). A screen of alternative secondary amines revealed that the in situ generated pyrrolidine enamine provided the highest conversion to **3a** of those examined (Scheme 1). No reaction was observed either when a tertiary amine such as Et_3N was

Scheme 1. Screen of Secondary Amines



used or in the absence of an added secondary amine, and both the 1,2,4,5-tetrazine **1a** and aldehyde **2a** were recovered unchanged. Use of 0.5 equiv of pyrrolidine (with 1 equiv of **1a**/3 equiv of **2a**) as above provided similar results (79% yield) indicating productive turnover, although use of ≤0.25 equiv pyrrolidine resulted in lower yields even with extended reaction times (Supporting Information Figure S4). This substoichiometric use of pyrrolidine with **2b** improved with the faster reaction of in situ generated **4b**, where good conversion was observed even with 0.25 equiv and dropped off only at 0.1 equiv of pyrrolidine (Supporting Information Figure S4). The limited pyrrolidine turnover is possibly due to acid-promoted self-condensation of the in situ generated enamine (Supporting Information Figure S5).

Substrate Scope. The carbonyl substrate scope for this transformation was explored (Scheme 2). With the 1,2,4,5-tetrazine **1a** as the diene, 2-arylacetaldehyde substrates bearing either electron-rich (**3b**, **3f**, **3h**, **3i**, **3k**, **3l**) or electron-deficient (**3c−e**, **3g**, **3j**, **3m−o**) arenes are well tolerated, all participating in the reaction effectively, although electron-deficient arenes were found to display a lower reactivity. As a result of the benign reaction conditions, a wide range of functional groups are expected to be well tolerated, including those illustrated herein, consisting of methoxy (**3b**), halides (**3c−e**, **3j**), phenyl (**3f**), trifluoromethyl (**3g**), ester (**3m**), and nitrile (**3o**) substituents. Arenes with *ortho*-substitution (**3i**, **3k**, **3n**) that might suffer steric issues also provide the 1,2,4-triazines in satisfactory yields. Heterocyclic as well the all-carbon arenes are also compatible, including thiophene (**3h**), indole (**3k**), and quinoline (**3n**). Nonconjugated enamines (e.g., 1-pyrrolidinocyclopentene) did not react with **1a** under current reaction conditions likely due to their protonation by HFIP (for unreactive substrates, see Supporting Information Figure S6). Although the ketone 1-phenylacetone was unreactive, conjugated cyclic ketones (see below) and two related substrates containing five-membered heterocycles, 1-(thiophen-2-yl)propan-2-one (**2p**) and 1-(furan-2-yl)propan-2-one (**2q**), were found to be suitable substrates under the current reaction conditions, providing the 1,2,4-triazines **3p** (79%) and **3q** (26%), respectively. This differentiated behavior can be attributed to a lower steric barrier (Me/O repulsion vs Me/C−H repulsion) to achieving a conjugated coplanar enamine conformation, increasing the enamine stability toward nonproductive HFIP protonation and self-condensation (Figure 5). This conclusion was further supported by computation (AM1, Gaussian 09) of the C1−C2−C3−C4

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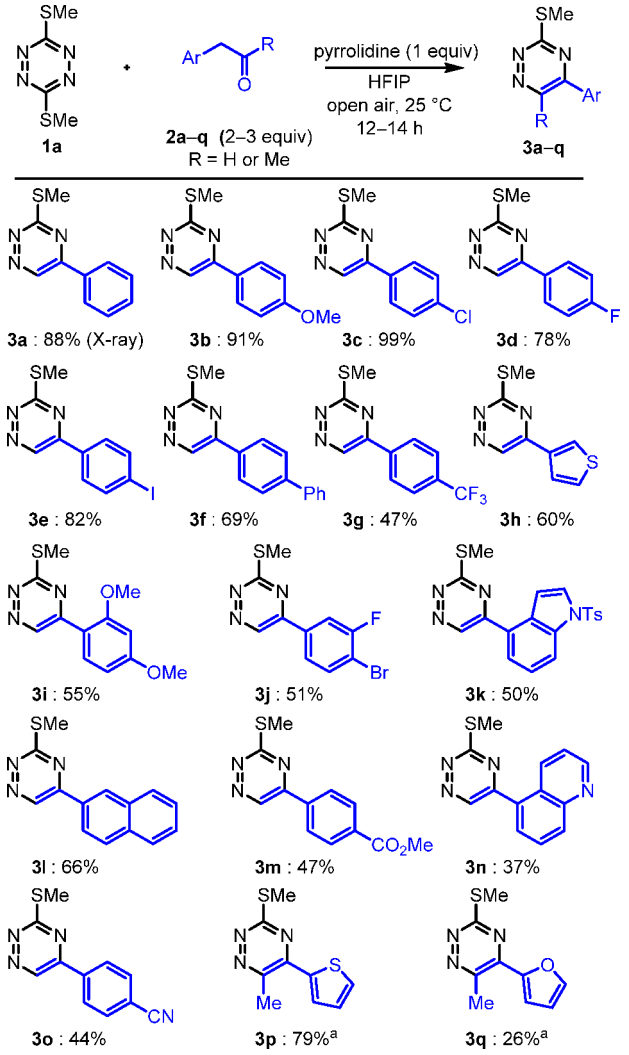
Scheme 2. Acyclic Substrates



[a]Reaction conducted at 60 °C versus 25 °C.

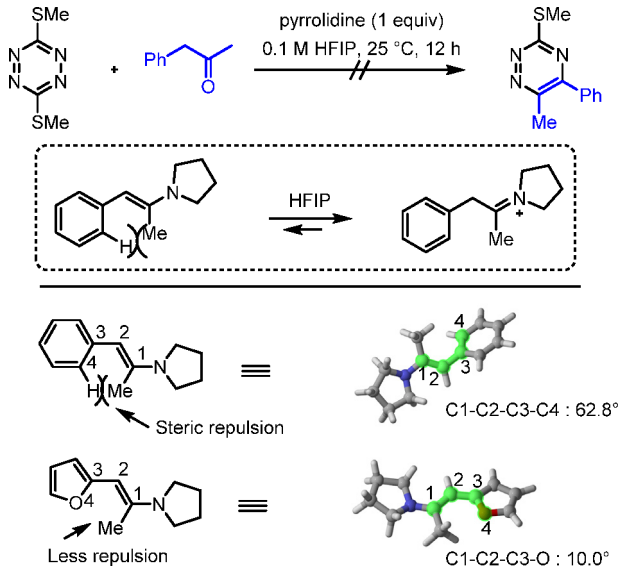
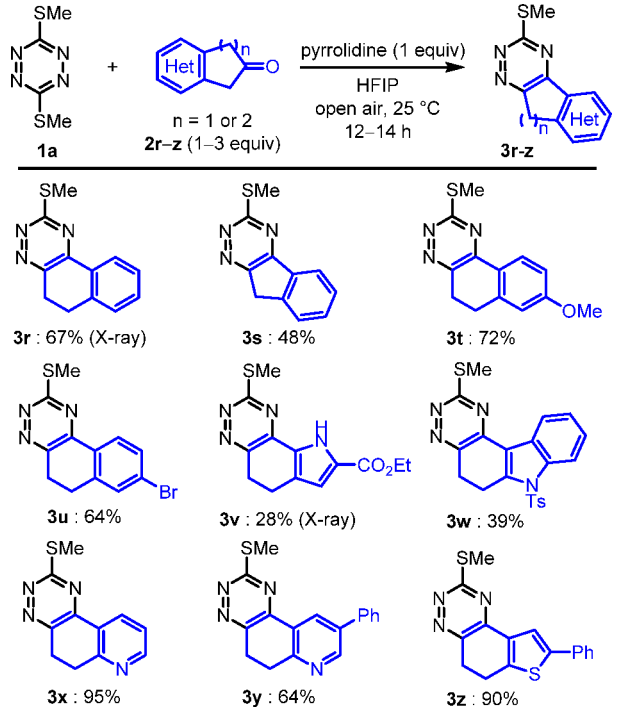


Figure 5. Steric repulsion of the pyrrolidine enamine of 1-phenylacetone versus the in situ generated enamine of **2p**.

dihedral angle of 1-phenylacetone (62.8°) and C1−C2−C3−O dihedral angle of **2p** (10.0°).

Significantly, conjugated cyclic ketones of which 2-tetralones and 2-indanones are prototypical members participate effectively in the reaction under the current reaction conditions (Scheme 3). In addition to the parent 2-tetralone (**3r**) and 2-indalone (**3s**), derivatives bearing methoxy (**3t**) and bromine (**3u**) substituents were also suitable substrates for this reaction. Cyclic ketones that form conjugated enamines with fused heterocycles, such as pyrrole (**3v**), indole (**3w**), pyridine (**3x**, **3y**), and thiophene (**3z**), all provided the 1,2,4-triazine products in good to excellent yield. The structures of **3r** and **3v** were confirmed by single-crystal X-ray structure determinations.[16]

Scheme 3. Conjugated Cyclic Ketone Substrates



The substrate scope as it relates to symmetrical 1,2,4,5-tetrazines was also briefly explored (Scheme 4). This was not done with the intent of comprehensively defining the scope and was conducted without alteration of the reaction parameters or optimization of the conditions (temp, time) including choice of perfluoroalcohol. Rather, it was done to establish whether the 1,4-addition extends beyond **1a** and to define the range of productive substituents, and the results portend a broad tetrazine scope. 3,6-Bis(benzylthio)-1,2,4,5-tetrazine (**1a′**) displayed a reactivity similar to that of **1a** toward the in situ generated pyrrolidine enamine of phenacetaldehyde, providing the corresponding 1,2,4-triazine **6a** in excellent yield (78%). Notably, the nonvolatile benzylthiocyanate was observed and characterized by NMR (76%) as a released product in this reaction. As such and while the reaction is unlikely to represent a true cycloaddition across the tetrazine N1/N4, the products are the same as those expected of such a reaction. More remarkable and without an effort at optimization, 1,2,4,5-tetrazines that are less reactive or unreactive in traditional cycloaddition reactions also provided the corresponding 1,2,4-triazines, including 3,6-dimethoxy

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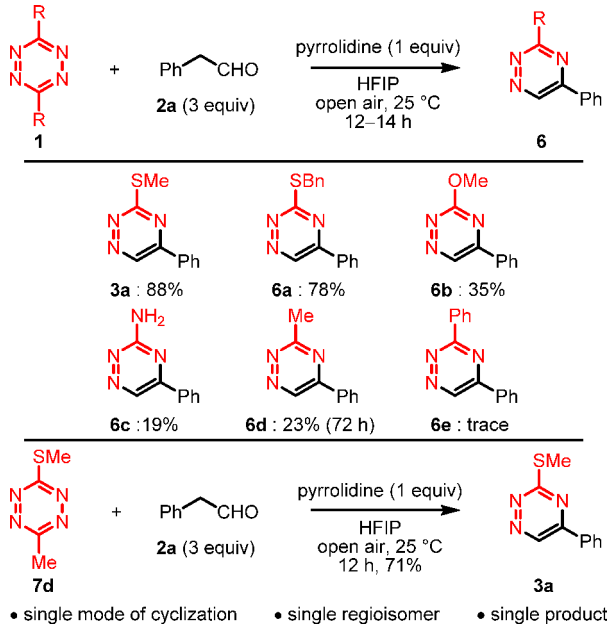
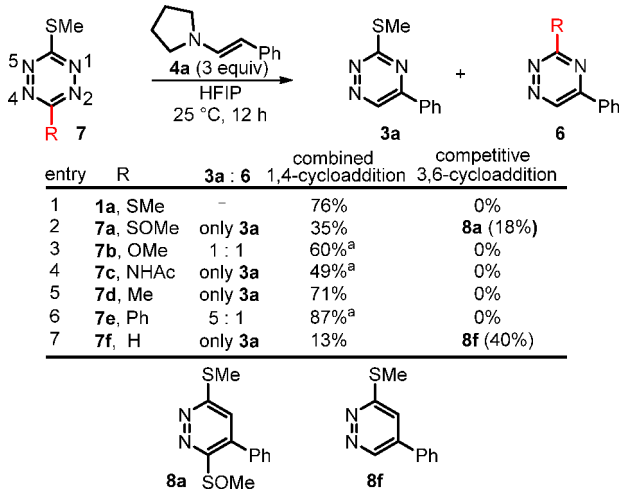
Scheme 4. Brief Survey of 1,2,4,5-Tetrazine Scope



(**6b**), diamino (**6c**), dimethyl (**6d**), and diphenyl (**6e**) 1,2,4,5-tetrazines, although in more modest conversions under the present reaction conditions. Unsubstituted 1,2,4,5-tetrazine (*s*-tetrazine) and dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate were found to undergo the conventional 3,6-cycloaddition without observation of the formal 1,4-cycloaddition product under current conditions (Supporting Information Figure S7). A series of unsymmetrical 1,2,4,5-tetrazines were also examined as is shown below, some of which displayed a remarkable regioselectivity for the 1,4-cycloaddition. Representative of this, the reaction of 4-methyl-1-thiomethyl-1,2,4,5-tetrazine (**7d**) with the in situ generated pyrrolidine enamine of phenylacetaldehyde under the standard reaction conditions (HFIP, 25 °C, 12 h) provided exclusively the 1,2,4-triazine **3a** (71%) with retention of the thiomethyl group and loss of acetonitrile.

In addition to the symmetrical 1,2,4,5-tetrazine substrates presented in Scheme 4, a larger representative series of unsymmetrical 1,2,4,5-tetrazines (**7a–f**) were prepared and examined, with one site substituted with a methylthio group and the remaining site bearing an electron-withdrawing (**7a**, S(O)Me), electron-donating (**7b** and **7c**, OMe and NHAc), neutral (**7d**, Me), conjugated (**7e**, Ph), or no substituent (**7f**, H). They were allowed to react directly with enamine **4a** in HFIP (0.1 M, 25 °C) to further probe factors impacting the mode of cycloaddition, its regioselectivity, and plausible mechanisms (Figure 6). The results revealed that only two, tetrazine **7b** (R = OMe, entry 3, 1:1) and **7e** (R = Ph, entry 6, 5:1), led to two 1,2,4-triazine products that bear each substituent (**3a**:**6**) in reactions that display the 1,4 mode of cycloaddition but represent two regioselectivities for the formal 1,4-cycloaddition. Like the symmetrical tetrazine **1a**, the remaining tetrazines provided the single 1,2,4-triazine **3a** in a regioselective 1,4-cycloaddition. In addition, the reactions of the electron-deficient tetrazine **7a** (R = S(O)Me, entry 2, 2:1) and the unsubstituted tetrazine **7f** (R = H, entry 7, 1:3) exhibited competitive 1,4-cycloaddition and 3,6-cycloaddition. Aside from the observation that an electron-withdrawing substituent increases competitive 3,6-cycloaddition as does



entry	R	3a : 6	combined 1,4-cycloaddition	competitive 3,6-cycloaddition
1	**1a**, SMe	–	76%	0%
2	**7a**, SOMe	only **3a**	35%	**8a** (18%)
3	**7b**, OMe	1 : 1	60%[a]	0%
4	**7c**, NHAc	only **3a**	49%[a]	0%
5	**7d**, Me	only **3a**	71%	0%
6	**7e**, Ph	5 : 1	87%[a]	0%
7	**7f**, H	only **3a**	13%	**8f** (40%)

Figure 6. Reaction of unsymmetrical 1,2,4,5-tetrazines. [a]Yield established by NMR. Others are isolated yields.

removal of a substituent, **3a** is exclusively (**7a,c,d,f**) or predominately (**7e**) formed in all cases with the exception of **7b**, bearing a methoxy substituent.

Mechanistic Insights. The unique 1,4-cycloaddition was examined in greater detail to gain insights into the potential reaction mechanism. Given that no prior example of cycloaddition across two nitrogen atoms (N1/N4) of 1,2,4,5-tetrazines has been described, it is unlikely that a concerted [4 + 2] cycloaddition followed by retro [4 + 2] nitrile extrusion is operative in this transformation. Moreover, analysis of the reaction indicates that not only is a concerted cycloaddition unlikely but even a stepwise addition–cyclization prior to nitrile loss is not able to account for formation of a single 1,2,4-triazine product rather than a mixture of two isomeric products (Figure 7). Further reinforcing this analysis is the observation

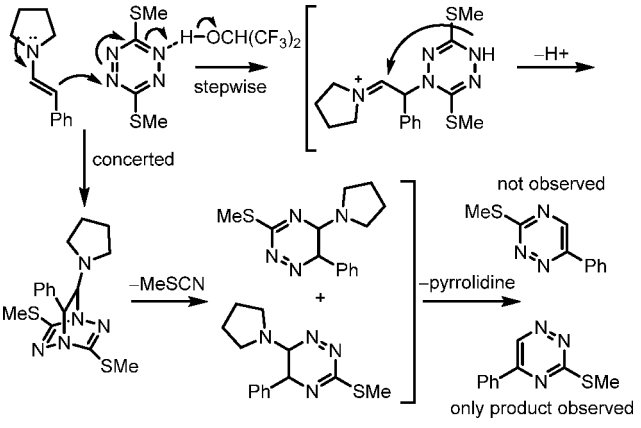


Figure 7. Plausible but improbable mechanisms.

that room temperature retro [4 + 2] nitrile extrusion from an initial bicyclic intermediate is unlikely since related isolated bicyclic intermediates obtained from the cycloadditions of 2,4,6-tris(methylthio)-1,3,5-triazine require elevated reaction temperatures, extended reaction times, and often additional acid catalysis for nitrile extrusion.[19]

First and foremost, to determine whether a fragmented tetrazine, potentially releasing an acyclic azadiene, might be responsible for the observed reaction, we examined the fate of 1,2,4,5-tetrazine **1a** in HFIP alone (25 °C, 12 h), in the

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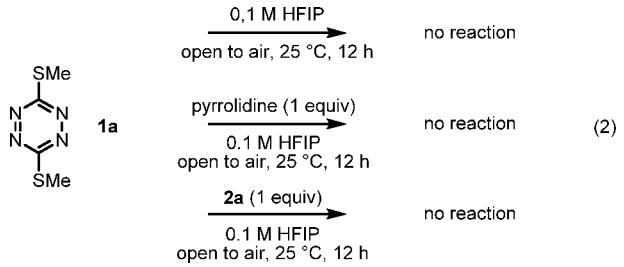
presence of pyrrolidine (1 equiv), and in the presence of phenylacetaldehyde (**2a**, 1 equiv) (eq 2). Under all conditions, tetrazine **1a** was recovered quantitatively without change, verifying that **1a**, and not a more reactive fragment, is the compound undergoing reaction.



(2)

We then undertook the detection, trap, or isolation of a reaction intermediate to help clarify the origin of the altered cycloaddition mode and the overall reaction process. After extensive attempts on intermediate capture, compound **3a′** was isolated in 36% yield when **1a** and enamine **4a** were allowed to react in a MeOH/HFIP (1:1) mixed solvent system for 2 h (Figure 8A). The structure of **3a′** was unambiguously

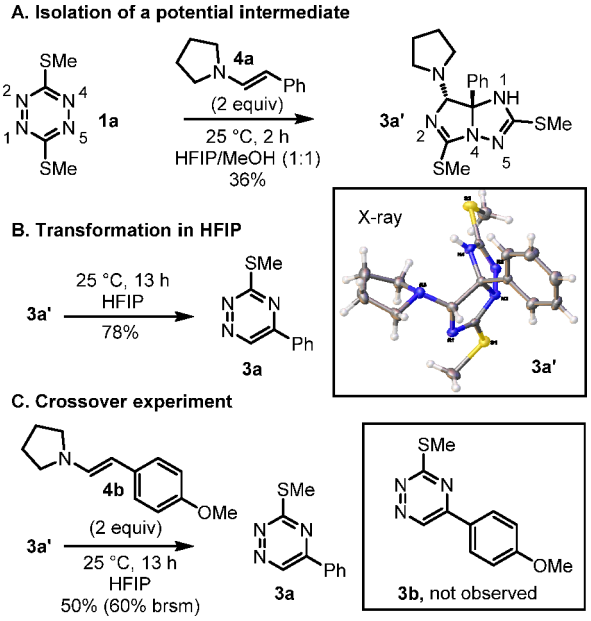


Figure 8. Isolation and studies of compound **3a′**.

established with a single crystal X-ray structure determination.[16] Compound **3a′** converts to product **3a** in 78% yield when subjected to the standard reaction conditions (HFIP, 25 °C, 13 h; Figure 8B). A crossover experiment where **3a′** was mixed with the enamine **4b** under the standard reaction conditions afforded **3a** as the sole product without observation of **3b**, indicating that at least one step in the generation of **3a′** from starting materials is not reversible (Figure 8C). Notable in this structure is a connectivity that indicates enamine attack on a tetrazine nitrogen (N1) as well as the necessary cleavage of a N–N bond (N1/N2) for 1,2,4-triazine formation prior to methylthiocyanate loss and that it is the alkylated, not distal, N–N bond (N4/N5) that is cleaved.

The reaction between **1a** (0.1 M, HFIP-d_2) and enamine **4a** (2 equiv, phenacetaldehyde pyrrolidine enamine) was also monitored by ^{1}H NMR (Supporting Information Figure S8) as was that with **4b** (4-methoxyphenacetaldehye pyrrolidine enamine). For **4a**, the consumption of **1a** and accumulation of product **3a** were directly observed as the reaction proceeded, monitoring the emerging diagnostic C6–H signal of **3a** and a discernible chemical shift in the SMe signal of **3a** versus **1a**. In addition, a diagnostic signal for **3a′** (aminal CH, δ 5.48 in HFIP-d_2) was observed to rise as the reaction progressed. No other prominent signals attributable to a thiomethyl group of other potential intermediates were observed to accumulate over the course of the reaction. Notably, enamine **4a** was not observed at any stage of the reaction. Instead, its deuterated iminium ion (**4a**-iminiun) was observed, suggesting that the enamine is in rapid equilibration with the protonated iminium ion and the former rapidly reacts. With the concentration of all major components able to be quantified by ^{1}H NMR (Supporting Information Table S1), a reaction time course profile was plotted and is depicted in Figure 9. As the reaction proceeds and as the concentration of

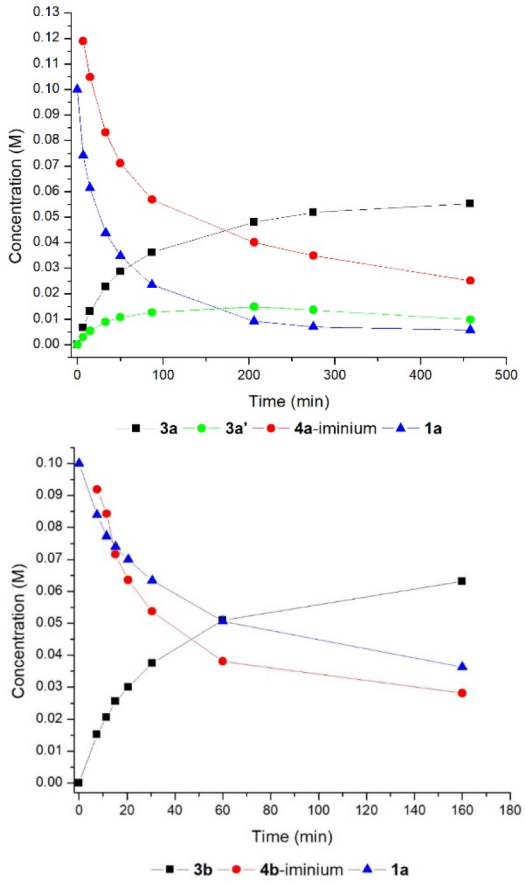


Figure 9. Reaction profile over time for reaction of **1a** with **4a** (top) and **4b** (bottom).

starting **1a** and **4a**-iminiun decrease, the concentration of **3a′** increases until it reached the maximum concentration of 0.015 M, from where it slowly decreases. The appearance of product **3a** increases faster than the appearance of **3a′** for the first 200 min (2.2- to 2.7-fold), at which point nearly all **1a** is consumed, followed by a slower increase in product that now nearly matches the rate of disappearance of **3a′**. The decrease in the **4a**-iminiun was found to be faster than **1a**, likely attributable to nonproductive competitive consumption

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of **4a**-iminium by acid-promoted enamine self-condensation. A more refined treatment of the data, plotting the relative concentration of **3a** and **3a′** over time for the first 20 min, revealed that the **3a′**:**3a** ratio (0.45, 1:2.2) was time-independent. This conclusively establishes that **3a** and **3a′** are formed in parallel reactions derived from a mutual intermediate (Supporting Information Figure S9). This is consistent with **3a′** serving as an off route reversibly generated compound rather than being a primary reaction intermediate.

Further consistent with this, the reaction profile of the reaction of **1a** with enamine **4b** (4-methoxyphenacetaldehyde pyrrolidine enamine) was much more straightforward (Figure 9). The analogous intermediate **3b′** was not observed in detectable amounts, indicating that it is not generated in significant amounts under the conditions of the reaction or is much more rapidly converted to product precluding detection. To address this question, we identified conditions that allowed the isolation of **3b′** (Figure 10) and measured the rate at which

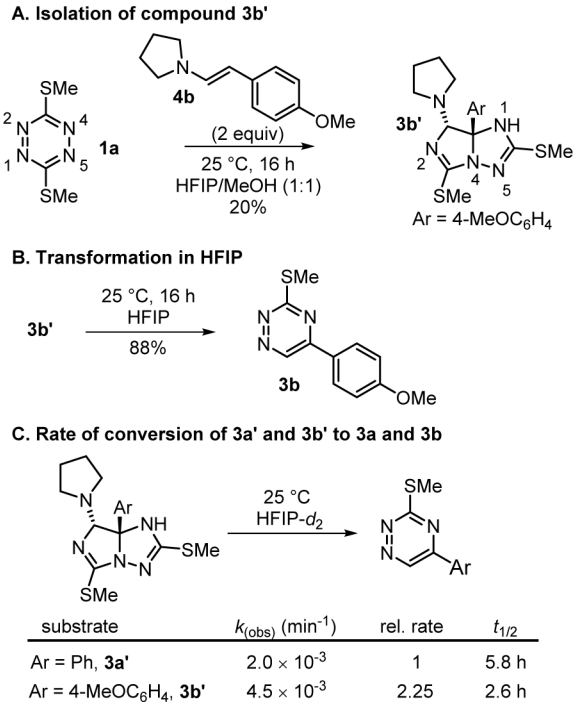

Figure 10. Related studies of compound **3b′**.

it and **3a′** convert to the triazine products under the reaction conditions (25 °C, HFIP-d_2). Both **3a′** and **3b′** converted to the triazine products at slow and near equivalent rates (apparent first order $k_{(obs)}$ = 2.0 × 10^{-3} min^{-1} (**3a′**) and 4.5 × 10^{-3} min^{-1} (**3b′**)), requiring approximately 25.0 h (**3a′**, $t_{1/2}$ = 5.8 h) and 11.1 h (**3b′**, $t_{1/2}$ = 2.6 h) to run to completion (reach 95% yield). Thus, the lack of detection of **3b′** in the reaction of **1a** with **4b** is not due to a much more rapid conversion to **3b**. Rather, it can be attributed to **3b′** not being generated in detectable amounts under the conditions of the faster direct reaction of **1a** with **4b** to provide **3b**. Together, the combined observations indicate that compound **3a′** is and **3b′** would be reversibly generated off route compounds that ultimately also convert to the triazine products but do not appear to be on the direct pathway to the products. Finally, the extent of consumption of **1a** nearly matches the accumulation of product **3b**, indicating that essentially no nonproductive consumption of tetrazine **1a** occurs in the first 160 min.

With these key observations in hand, a plausible pathway from **1a** and **4a** to **3a** and **3a′** is summarized in Figure 11. H-

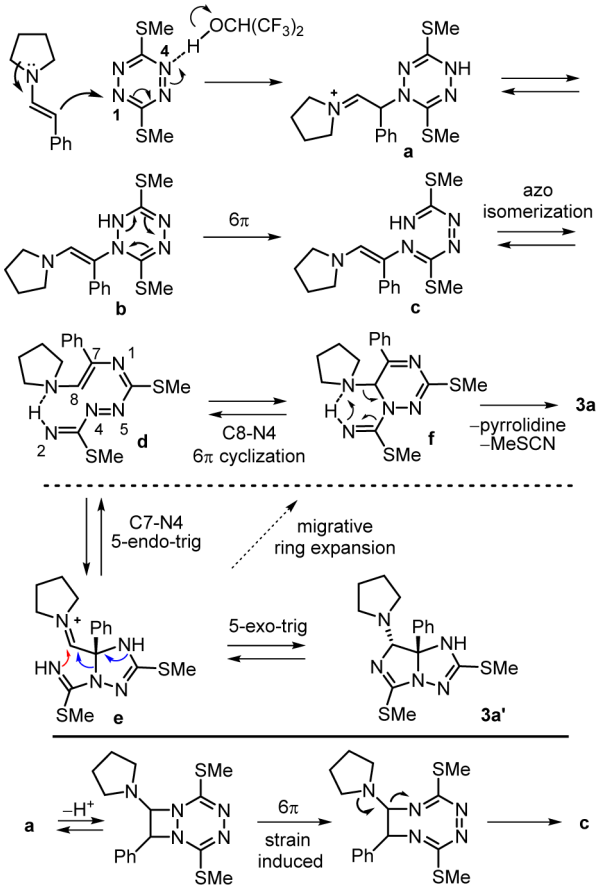

Figure 11. Plausible reaction pathway.

Bonding activation of 3,6-dimethylthio-1,2,4,5-tetrazine and irreversible enamine nucleophilic attack at N1, either para (N4, shown) or ortho (N2) to the site of H-bonding (interconvertible by tautomerization), generate intermediate **a** or its 2*H* tautomer. Regeneration of the enamine from the iminium ion and 6π electrocyclic rearrangement with required cleavage of the N1/N2 bond lead to **c**,[20,21] followed by facile cis to trans azo-isomerization that provides **d**. Intermediate **d** may undergo a 6π electrocyclic cyclization (C8−N4 bonding) to generate **f** or a reversible 5-endo-trig cyclization[22] (C7−N4 bonding) followed by reversible 5-exo-trig ring closure to generate compound **3a′** (red arrow). Final aromatization of **f** with loss of pyrrolidine and MeSCN through a six-membered transition state, of which the latter two steps both may benefit from an intramolecular H-bond, provides the products of the reaction (**3a** and MeSCN). Direct generation of **f** from **3a′** via **e** through a migrative ring expansion (blue arrows) is also possible. In this mechanism, **3a′** is also eventually converted to **3a**, although the possibility of direct transformation of **3a′** to **3a** without passing through **e** cannot be ruled out. A related alternative route to intermediate **c** involves an initial addition to provide **a** and its stepwise cyclization to the [2 + 2] adduct[23] followed by a strain induced 6π electrocyclic rearrangement with cleavage of the N1/N2 bond, followed by aminal cleavage and conversion to **c** (Figure 10). In addition to accounting for

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the generation of single 1,2,4-triazine product (3-methylthio-5-phenyl-1,2,4-triazine but no 3-methylthio-6-phenyl-1,2,4-triazine, Figure 5), key elements of the mechanism are a solvent H-bonding activation of the 1,2,4,5-tetrazine for enamine nucleophilic addition to N1 and a 6π electrocyclic rearrangement for the needed cleavage of the N1/N2 bond, representing a pathway to **3a** where overall stepwise 1,4-cycloaddition occurs prior to elimination of thiomethylcyanate (MeSCN).

Application Potential. The unprecedented 1,4-cycoladdition has provided rapid access to a suite of analogs of **3a**, which itself has been used both to access functionalized 1,2,4-triazines and as a key intermediate in the synthesis of biologically active compounds (Figure 12). Thus, the thiomethyl group in the

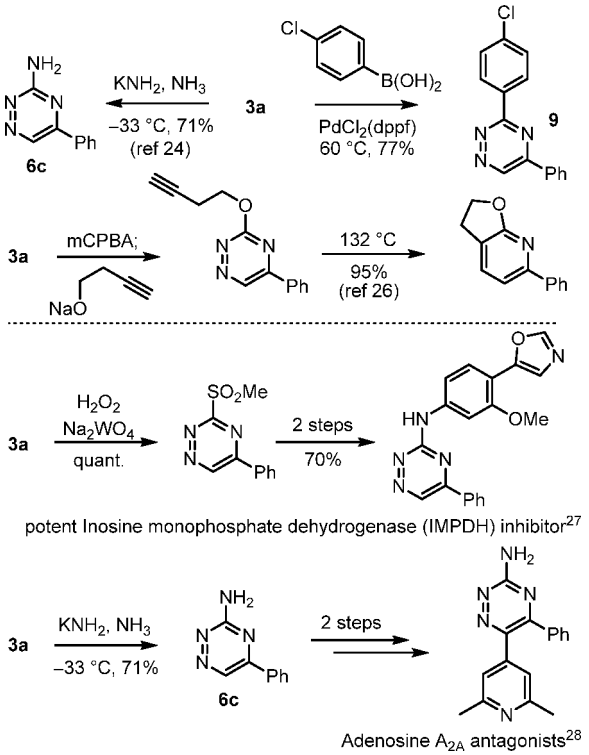

Figure 12. Representative diversification of **3a** and its use in the synthesis of biologically active compounds.

product 1,2,4-triazines **3** can be used as an effective functional group for further diversification of the product 1,2,4-triazine.[24,25] This serves to complement the use of alternatively substituted 1,2,4,5-tetrazines (e.g., **6c**). An example that we would like to especially highlight is the use of **3a** as a precursor for a second heterocyclic azadiene Diels–Alder reaction,[26] setting the foundation for sequential cycloaddition strategies for rapidly accessing a diverse set of highly substituted or fused pyridines that are challenging to prepare by other means.[1b] Similarly, representative examples of the direct use of **3a** in the preparation of biologically active compounds can be highlighted with the preparation of inosine monophosphate dehydrogenase (IMPDH) inhibitors[27] and adenosine A_{2A} antagonists,[28] for which the work herein provides a simple synthesis of a suite of **3a** analogs.

CONCLUSIONS

An unprecedented 1,4-cycloaddition of 1,2,4,5-tetrazines with enamines has been discovered. The reaction is conducted under mild reaction conditions (0.1 M HFIP, 25 °C, 12-14 h) with a broad scope of in situ generated acyclic and cyclic aryl-conjugated enamines to provide 5-aryl-1,2,4-triazines in good yields. Factors that impact this unprecedented change in the reaction mode (N1/N4 vs C3/C6 cycloaddition), optimization of the reaction conditions, the substrate scope of the reaction partners, simplification of its implementation with preformed, in situ formed, or substoichiometrically generated enamines, and mechanistic insights into this remarkable cycloaddition are detailed. Given its breath, it establishes a new method for the simple and efficient one-step synthesis of 1,2,4-triazines under mild conditions from readily accessible starting materials. Mechanistic studies revealed several important features of the reaction and highlight the unique behavior of HFIP, where the strength or extent of its H-bonding interaction with the tetrazines is responsible for the alteration of the cycloaddition mode from the typical 3,6-cycloaddition to 1,4-cycloaddition. As such, the studies represent the first example of not just an enhancement in the rate and efficiency of a heterocyclic azadiene cycloaddition by H-bonding catalysis but an alteration in the mode of cycloaddition as well. Key elements of a plausible stepwise addition–cyclization mechanism that accounts for the generation of a single 1,2,4-triazine product (3-methylthio-5-phenyl-1,2,4-triazine and no 3-methylthio-6-phenyl-1,2,4-triazine) and other observations to date are a solvent H-bonding activation of the 1,2,4,5-tetrazine that promotes enamine nucleophilic addition to N1 and a 6π electrocyclic rearrangement for the needed cleavage of the N1/N2 bond, providing a pathway to the products where overall 1,4-cycloaddition occurs prior to elimination of a nitrile. Continued efforts that examine and further expand solvent hydrogen bonding catalysis of other heterocyclic and acyclic azadienes are ongoing and will be reported in due course.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.0c09775.

Materials and methods, full experimental details for studies of substrate scope, mechanistic insights, AM1 studies, and ^{1}H, ^{13}C, and ^{19}F NMR spectra (PDF)
Crystallographic information (CIF)
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Notes

The authors declare no competing financial interest.

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