Regioselective Inverse Electron Demand Diels−Alder Reactions of *N*-Acyl 6-Amino-3-(methylthio)-1,2,4,5-tetrazines

Dale L. Boger,* Robert P. Schaum, and Robert M. Garbaccio

Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

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The regioselective inverse electron demand Diels−Alder reactions of 6-[(*tert*-butyloxycarbonyl)-amino]-3-(methylthio)-1,2,4,5-tetrazine (**2**), 6-(acetylamino)-3-(methylthio)-1,2,4,5-tetrazine (**3**), and 6-(benzyloxycarbonyl)amino-3-(methylthio)-1,2,4,5-tetrazine (**4**) are disclosed. All three underwent regioselective [4 + 2] cycloaddition with electron-rich dienophiles to form the corresponding functionalized 1,2-diazines in excellent yields. An order of reactivity with electron-rich dienophiles was observed with both **2** and **3** being more reactive than 3,6-bis(methylthio)-1,2,4,5-tetrazine (**1**, i.e. **3** > **2** > **1**), and both **3** and **4** were shown to be more robust than **2** at the higher temperatures necessary for [4 + 2] cycloaddition with less reactive dienophiles. The cycloaddition regioselectivity is consistent with the polarization of the diene and the ability of the methylthio group to stabilize a partial negative charge at C-3, and the *N*-acylamino group to stabilize a partial positive charge at C-6. While intermolecular reactions of unactivated alkynes either did not proceed or required high temperatures and long reaction times, intramolecular Diels−Alder reactions utilizing tethered unactivated acetylenes led to five- and six-membered bicyclic 1,2-diazines under mild conditions.

The inverse electron demand Diels−Alder reactions of electron-deficient heterocyclic azadienes[1] with electron-rich dienophiles have proven useful in the total synthesis of a number of natural products[2] possessing highly functionalized heteroaromatic systems not easily accessed by conventional means. In particular, the well-defined participation of 1,2,4,5-tetrazines in [4 + 2] cycloadditions, and the ability of the resulting 1,2-diazines to participate in subsequent intramolecular Diels−Alder reactions[3] or a reductive ring-contraction reaction to provide the corresponding pyrrole,[4] has been enlisted in the total synthesis of PDE-I/PDE-II and CC-1065,[5] streptonigrin,[6] prodigiosin,[7] OMP,[4] trikentrin A,[8] and isochrysohermidin.[9] In each case, the reaction of a symmetrical 1,2,4,5-tetrazine with an electron-rich dienophile served as a key transformation. The extension of these studies to the regioselective cycloadditions of unsymmetrical 1,2,4,5-tetrazines would extend the flexibility of the approach. However, reports detailing the preparation and participation of such unsymmetrically substituted 1,2,4,5-tetrazines in regioselective Diels−Alder reactions are limited.[10−13] Herein, we detail the preparation of three such azadienes, 6-[(*tert*-butyloxycarbonyl)amino]-3-(methylthio)-1,2,4,5-tetrazine (**2**), 6-(acetylamino)-3-(methylthio)-1,2,4,5-tetrazine (**3**), and 6-[(benzyloxycarbonyl)amino]-3-(methylthio)-1,2,4,5-tetrazine (**4**), and an investigation of their inter- and intramolecular inverse electron demand Diels−Alder reactions which define their [4 + 2] cycloaddition regioselectivity and reactivity. The studies complement the recent disclosure of Panek and Zhu[10] enlisting analogous solid-supported tetrazines and our own report on 6-methoxy-3-(methylthio)-1,2,4,5-tetrazine.[11]

Synthesis of 1,2,4,5-Tetrazines 2−4. Tetrazines **2**−**4** were anticipated to be accessible through the selective displacement of one methylthio group of 3,6-bis(methylthio)-1,2,4,5-tetrazine **1**[3,8,10,11] with the anions of *tert*-butyl carbamate, acetamide, or benzyl carbamate in a one-step procedure. While this route proved successful, an alternative approach enlisting first the addition of ammonia and subsequent acylation of the resulting 6-amino-3-(methylthio)-1,2,4,5-tetrazine[14] was also examined thus providing two methods for their preparation applicable to other unsymmetrically substituted tetrazines (Scheme 1).

Thus, treatment of a solution of **1** in DMF cooled to −40 °C with *tert*-butyl carbamate (1.2 equiv, 16−18 h), acetamide (1.2 equiv, 12 h), or benzyl carbamate (1.2 equiv, 29 h) and NaH (1.8 equiv) cleanly provided **2**

(1) Boger, D. L.; Weinreb, S. M. *Hetero Diels−Alder Methodology in Organic Synthesis*; Academic: San Diego, 1987. Boger, D. L. *Tetrahedron* **1983**, *39*, 2869.
(2) Boger, D. L. *Chemtracts: Org. Chem.* **1996**, *9*, 149. Boger, D. L. *Bull. Soc. Chim., Belg.* **1990**, *99*, 599. Boger, D. L. *Chem. Rev.* **1986**, *86*, 781.
(3) Boger, D. L.; Sakya, S. M. *J. Org. Chem.* **1988**, *53*, 1415. Boger, D. L.; Coleman, R. S. *J. Org. Chem.* **1984**, *49*, 2240.
(4) Boger, D. L.; Coleman, R. S.; Panek, J. S.; Yohannes, D. *J. Org. Chem.* **1984**, *49*, 4405.
(5) Boger, D. L.; Coleman, R. S. *J. Org. Chem.* **1986**, *51*, 3250. Boger, D. L.; Coleman, R. S. *J. Am. Chem. Soc.* **1987**, *109*, 2717. Boger, D. L.; Coleman, R. S. *J. Am. Chem. Soc.* **1988**, *110*, 4796. Boger, D. L.; Coleman, R. S. *J. Am. Chem. Soc.* **1988**, *110*, 1321.
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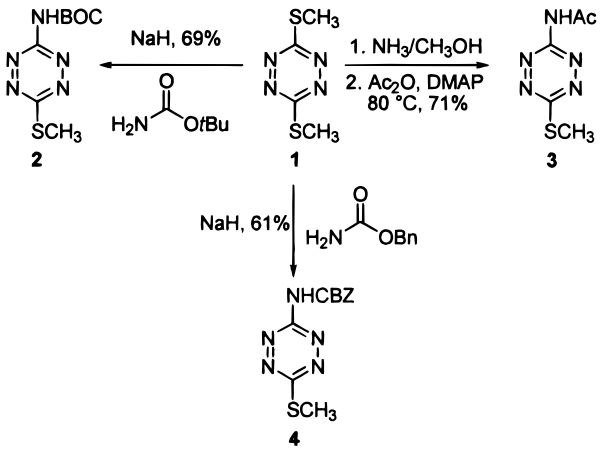
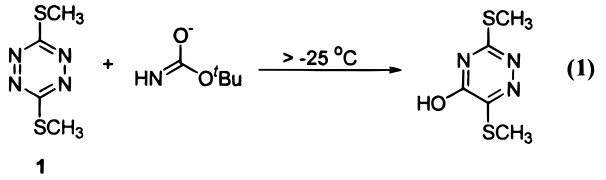
Scheme 1



(69%), **3** (65%), and **4** (61%), respectively, accompanied by lesser amounts of recovered **1**. In initial efforts conducted with the preparation of **2**, the use of higher reaction temperatures (−25 to 25 °C) and prolonged reaction times (48−72 h) provided only low yields of **2** (<15%) and predominantly 3,6-bis(methylthio)-4-hydroxy-1,2,4-triazine (48%)[15] derived from a formal [4 + 2] cycloaddition of the carbamate anion with **1** (eq 1). This competitive reaction was suppressed at the lower reaction temperatures (−40 °C) and with shortened reaction times. When subjected to typical workup conditions, **2**−**4** were found to be unstable to aqueous base extractions, but surprisingly stable to aqueous acid extractions and standard SiO_2 column chromatography. This practical stability of **2**−**4**, unlike the instability of the more electron-deficient 1,2,4,5-tetrazines,[16] simplified their preparation, purification, characterization, and subsequent study.



Alternatively, addition of NH_3 to **1** (saturated NH_3−CH_3OH, 0 °C, 30 min, 100%)[14] followed by *N*-acetylation with Ac_2O (1.5 equiv, 0.1 equiv of DMAP, THF, reflux, 15 h) cleanly provided **3** (71%), unreacted 6-amino-3-(methylthio)-1,2,4,5-tetrazine (20%), and a trace amount of bisacetylated product **5** (5%). This provided the preferred method for the synthesis of **3**. Attempts to extend this approach to the preparation of **2** have not yet been successful, although it has not been extensively examined. 6-Amino-3-(methylthio)-1,2,4,5-tetrazine failed to react with di-*tert*-butyl dicarbonate (BOC_2O) in refluxing THF, and the addition of catalytic amounts of DMAP (0.1 equiv, 25 °C) or reactions in refluxing dioxane provided only mixtures of the bis *N*-acylated tetrazine **6**[17] and unreacted starting material with only traces of **2** detected in the reaction mixture.

Inverse Electron Demand Diels−Alder Reactions of 6-[(*tert*-Butyloxycarbonyl)amino]-3-(methylthio)-1,2,4,5-tetrazine (2). The electron-rich alkenes **7a**−**j** including enamines, ketene acetals, alkyl- and trimethylsilyl enol ethers, and enamides participated in regioselective inverse electron demand Diels−Alder reactions with **2** providing the 1,2-diazine products **8a**−**f** (Table 1). The cyclic enamines **7a**−**c** underwent rapid cycloaddition at 23 °C usually within 2 h, with concomitant loss of N_2. As with Diels−Alder cycloadditions of 3,6-bis-(methylthio)-1,2,4,5-tetrazine (**1**),[3] the slowest step of the reaction cascade was the aromatization step involving the elimination of the secondary amine. This could be facilitated by subsequent addition of an acid catalyst or, optimally, by treatment with 10% HOAc/C_6H_6 (v:v). For example, entry 1 (Table 1) required acidic conditions (v:v 10% HOAc/C_6H_6) to effect complete elimination of pyrrolidine while entry 2 did not require such acid catalysis to promote the elimination of morpholine. The Diels−Alder reactions with 1-morpholino-1-cyclohexene **7c** (entry 3, Table 1) and 3-morpholino-2-pentene **7d** (entry 4, Table 1) also proceeded with excellent yields and only one regioisomer of the 1,2-diazine product **8c** was obtained.

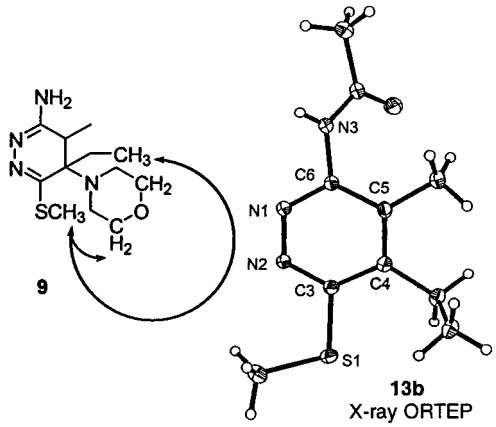


Figure 1.

The regioselectivity was initially established by 1D pulsed field gradient NOE ^1H NMR analysis of the isolable cycloadduct **9** obtained from the reaction of **2** with **7d** (dioxane, 70 °C, 2 h) (Figure 1). Strong NOE's were observed between the methylthio group and the methyl protons of the ethyl group as well as the methylene protons of the morpholine moiety, providing evidence of the cycloaddition regioselectivity. This was later unambiguously established with a single-crystal X-ray structure determination of **13b**.[18]

The [4 + 2] cycloadditions of **2** with the morpholino (**7e**, entry 5) and pyrrolidino (**7f**, entry 6) enamines of acetophenone required higher temperatures and longer reaction times (100 °C, 12 h) for completion of the initial cycloaddition step. In both cases, elimination of the secondary amine required acid catalysis (10% HOAc/C_6H_6, 23 °C, 14 h). As with entry 4, only one cycloaddi-

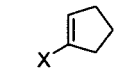
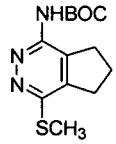
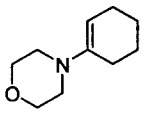
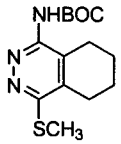
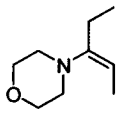
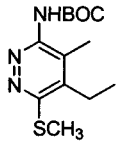
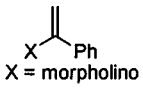
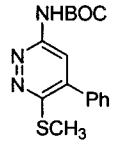
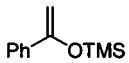
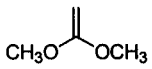
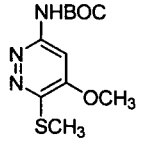
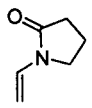
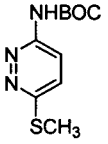
(15) For 5-hydroxy-3,6-bis(methylthio)-1,2,4-triazine: ^1H NMR (DMF-d_7, 250 MHz) δ 14.11 (s, 1H), 2.55 (s, 3H), 2.34 (s, 3H); FABHRMS (NBA-NaI) *m/z* 190.2612 (M + H^+, $C_5H_7N_3OS_2$ requires 190.2607).

(16) Boger, D. L.; Panek, J. S.; Patel, M. *Org. Synth.* **1991**, *70*, 79.

(17) For 6-[bis(*tert*-butyloxycarbonyl)amino]-3-(methylthio)-1,2,4,5-tetrazine (**6**): ^1H NMR ($CDCl_3$, 250 MHz) δ 2.76 (s, 3H), 1.43 (s, 18H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.5, 161.0, 149.2, 85.1, 27.7, 13.8; IR (film) ν_{max} 2981, 2935, 1809, 1771, 1734, 1367, 1279, 1243, 1152 cm^{-1}; FABHRMS (NBA-NaI) *m/z* 366.1212 (M + Na^+, $C_{13}H_{21}N_5O_4S$ requires 366.1242). Preliminary efforts to enlist **6** in Diels−Alder reactions with electron-rich dienophiles provided mixtures including cycloddition products bearing $N(BOC)_2$, NHBOC and NH_2 substituents.

(18) Details of the X-ray structure determination may be found in the Supporting Information and have been deposited in the Cambridge Crystallographic Data Centre.

Table 1. Inverse Electron Demand Diels–Alder Reactions of 6-[(*tert*-Butyloxycarbonyl)amino]-3-(methylthio)-1,2,4,5-tetrazine (2)

entry	dienophile	equiv	conditions: solvent, temperature, time	product	yield (%)
1	**7a**  X = pyrrolidino	1.5	$CHCl_3$, 23 °C, 1.5 h, then 10% HOAc/C_6H_6, 23 °C, 16 h	**8a** 	96
2	**7b** X = morpholino	1.5	$CHCl_3$, 23 °C, 0.25 h	**8a**	93
3	**7c** 	1.5	$CHCl_3$, 23 °C, 1.5 h, then 10% HOAc/C_6H_6, 23 °C, 16 h	**8b** 	79
4	**7d**[a] 	3	$CHCl_3$, 23 °C, 16 h	**8c** 	86
5	**7e**[a]  X = morpholino	1.5	dioxane, 100 °C, 12 h, then 10% HOAc/C_6H_6, 23 °C, 14 h	**8d** 	60
6	**7f**[a] X = pyrrolidino	4	dioxane, 100 °C, 12 h, then 10% HOAc/C_6H_6, 23 °C, 14 h	**8d**	55
7	**7g** 	3	dioxane, 60 °C, 84 h or dioxane 100 °C, 18 h	**8d** **8d**	56 53
8	**7h**[b] 	6	dioxane, 80 °C, 16 h	**8e** 	75
9	**7i** 	3	dioxane, 80 °C, 30 h	**8f** 	58
10	**7j** 	3	C_6H_6, 60 °C, 24 h, then 80 °C, 40 h	**8f**	50

a) Prepared as decribed by Stork, G.; Brizzolara, A.; Landesman, H.; Szmuszkovic, J.; Terrell, R. *J. Am Chem. Soc.* **1963**, *85*, 207. b) Prepared as described by Corey, E. J.; Bass, J. D.; LeMahieu, R.; Mitra, R. B. *J. Am. Chem. Soc.* **1964**, *86*, 5570.

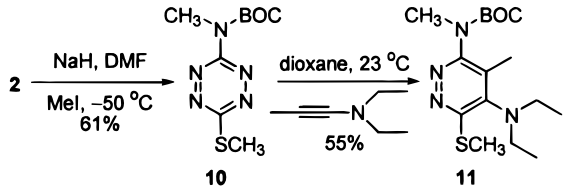
tion regioisomer, **8d**, was observed. Significantly longer reaction times (60 °C, 86 h) were also required to effect the Diels–Alder addition of the trimethylsilyl enol ether **7g**, and it also provided **8d** as a single regioisomer. Similarly, 1,1-dimethoxyethylene (**7h**) underwent efficient cycloaddition with **2** requiring elevated temperatures (80 °C, 16 h) to provide **8e** as a single regioisomer. Reactions with *N*-vinyl pyrrolidinone (**7i**, entry 9) and ethyl vinyl ether (**7j**, entry 10) proceeded effectively but required more extended reaction times (30–60 h) at temperatures between 60 and 80 °C. Higher reaction temperatures (e.g., 100 °C) sustained for prolonged times resulted in competitive thermal removal of the BOC group deactivating the tetrazine from further reaction.[19,20]

Despite their well-behaved participation in inverse electron demand Diels–Alder reactions with other electron-deficient 1,2,4,5-tetrazines, the activated acetylenes 1-(diethylamino)-1-propyne and ethoxy acetylene failed to give the desired [4 + 2] cycloaddition products when treated with tetrazine **2**. The initial attempts with

(19) 3-Amino-6-(methylthio)-1,2,4,5-tetrazine did not react even with enamine **7b** at 100 °C.

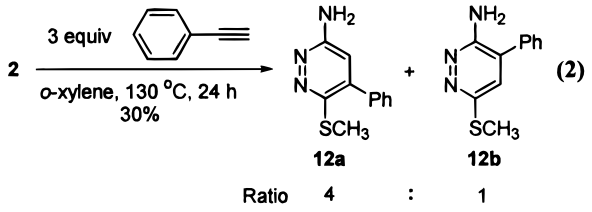
(20) The half-life of **2** in dioxane at 120 °C was 1 day.

Scheme 2



1-(diethylamino)-1-propyne led to a color change, but upon workup provided starting tetrazine. Moreover, modifications enlisting excess dienophile and variations in the reaction temperatures failed to provide the desired products. Recognizing the utility of this ynamine as a peptide coupling reagent and its potential basicity, a deprotonation and inactivation of the tetrazine was suspected to be competitive with the desired [4 + 2] cycloaddition. Consistent with this interpretation, *N*-methylation of **2** to provide **10**, followed by treatment with the ynamine, cleanly gave the expected cycloadduct **11** in 55% (23 °C, 16 h, dioxane) as a single regioisomer (Scheme 2). Just as importantly, the successful reaction of **10** also indicates that the [4 + 2] cycloaddition reactions of **2**−**4** do not proceed through the tautomeric *N*-acylimines with simple nucleophilic attack by the electron-rich dienophile, and subsequent collapse of the zwitterionic intermediates to the formal products of a [4 + 2] cycloaddition.

Typically, **2** was found to be unreactive toward unactivated acetylenic dienophiles (diphenylacetylene, 2-butyne) with the exception of phenylacetylene (eq 2) which provided a 4:1 mixture of diazines **12a** and **12b** (30% combined yield). The thermal lability of the *tert*-butyloxycarbonyl precludes its effective use with such unactivated dienophiles which require sustained reaction times at the higher reaction temperatures (≥100 °C).[20]



[4 + 2] Cycloaddition Reactions of 6-(Acetylamino)-3-(methylthio)-1,2,4,5-tetrazine (3). The Diels−Alder reactions of **3** with electron-rich dienophiles also proceeded effectively providing the 1,2-diazine cycloadducts in good to excellent yields (Table 2). The [4 + 2] cycloaddition with enamine **7b** occurred rapidly (23 °C, 0.1 h) and gave **13a** in high yield (88%). Similarly, the enamine **7d** also reacted with **3** at room temperature (2 h) to provide the single regioisomer of 1,2-diazine **13b** (74%). Higher reaction temperatures were required to effect cycloaddition with 1-(trimethylsilyloxy)-1-cyclopentene (**7k**, 110 °C, 48 h), 1,1-dimethoxyethylene (**7h**, 80 °C, 4 h), and trimethylsilyl enol ether **7g** (100 °C, 24 h) to provide **13a**, **13c**, and **13d**, respectively. Finally, [4 + 2] cycloaddition with *N*-vinyl pyrrolidinone (**7i**) provided **13e** in good yield (100 °C, 24 h, 73%) even when conducted at 160 °C (3 h, 82%) without evidence of deacylation.

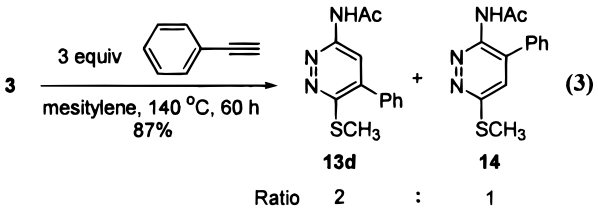
As with **2**, tetrazine **3** was found to be essentially unreactive toward diphenylacetylene at temperatures up to 130 °C but did undergo Diels−Alder addition with

Table 2. Inverse Electron Demand Diels−Alder Reactions of 6-(Acetylamino)-3-(methylthio)-1,2,4,5-tetrazine (3)

entry	dienophile[a]	equiv	conditions: solvent, temperature, time	product	yield (%)
1	**7b**	1.5	$CHCl_3$, 23 °C, 0.1 h	**13a**	88
2	TMSO **7k**[b]	4	dioxane, 110 °C, 48 h	**13a**	61
3	**7d**	3	$CHCl_3$, 23 °C, 2 h	**13b**	74
4	**7h**	5	dioxane, 80 °C, 4 h	**13c**	78
5	**7g**	3	dioxane, 100 °C, 24 h	**13d**	84
6	**7i**	3	dioxane, 100 °C, 24 h	**13e**	73
			or dioxane, 160 °C, 3h	**13e**	82

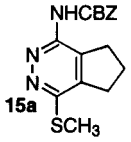
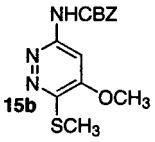
a) See Table 1 for structures. b) Prepared from a procedure adpated from Lin, J. M.; Liu, B. S. *Synth. Commun.* **1997**, *27*, 739.

phenylacetylene to provide a 2:1 mixture of regioisomers **13d**:**14** in 87% yield (eq 3). Unlike **2**, the thermal stability of the *N*-acetyl group allows its use for sustained reaction times at such elevated temperatures.



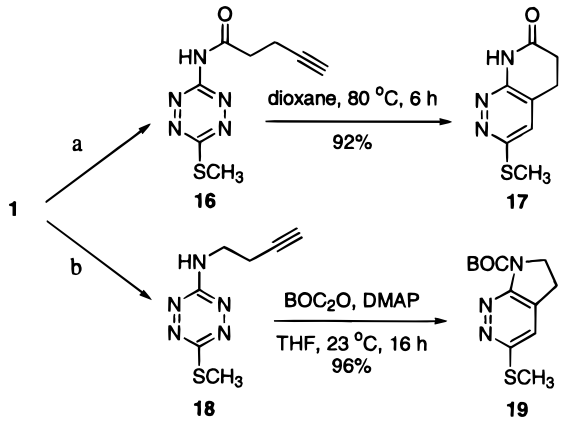
[4 + 2] Cycloaddition Reactions of 6-[(Benzyloxycarbonyl)amino]-3-(methylthio)-1,2,4,5-tetrazine (4). Recognizing the thermal instability of **2** and the shortcomings of acetyl as a protecting group for further functionalization of **3**, we prepared a third unsymmetrically substituted tetrazine **4** to combine the robust thermal stability of **3** with the synthetic versatility of **2** (Scheme 1). The participation of **4** in [4 + 2] cycloadditions was examined with three representative dienophiles **7b**, **7h**, and **7i**. Tetrazine **4** behaved in a manner analogous to **3**, and the reaction with *N*-vinyl pyrrolidinone (**7i**, 2.0 equiv, 72%) to provide **15c** could be carried out at 120 °C with no detectable decomposition or deacylation of the tetrazine (Table 3). However, when this same reaction was conducted at 160 °C (3 h), 6-amino-3-(methylthio)-1,2,4,5-tetrazine (29%) resulting from deacylation of **4** was isolated along with **15c** (60%).

Table 3. Diels–Alder Reactions of 6-[(Benzyloxycarbonyl)amino]-3-(methylthio)-1,2,4,5-tetrazine (4)

entry	dienophile[a]	equiv	conditions: solvent, temperature, time	product	yield (%)
1	**7b**	1.5	$CHCl_3$, 23 °C, 0.1 h	 **15a**	98
2	**7h**	6	dioxane, 80 °C, 36 h	 **15b**	64
3	**7l**	3	dioxane, 120 °C, 24 h or dioxane, 160 °C, 3h	 **15c**	72 60

a) See Table 1 for structures

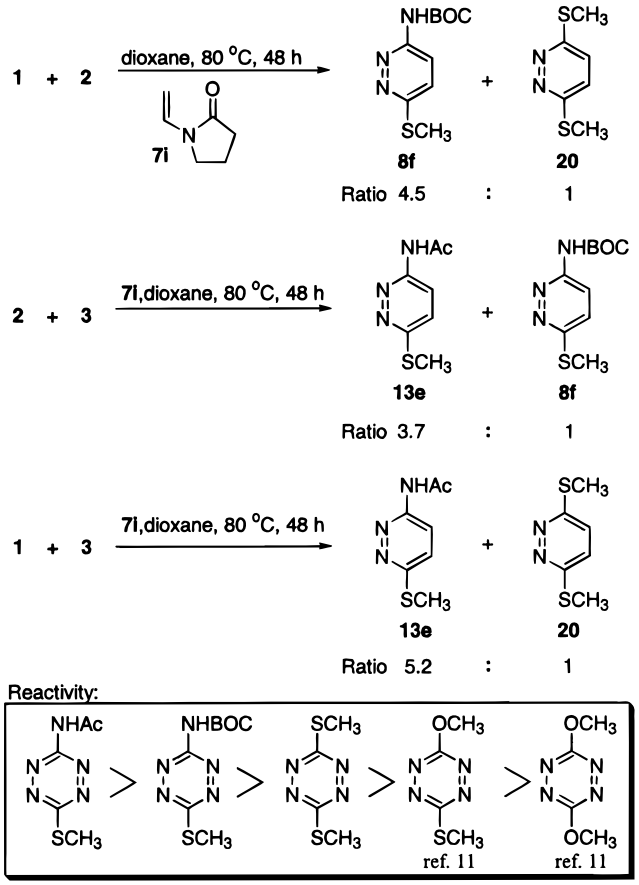
Scheme 3



a) NaH, DMF, -10 °C, 4-pentynoylamide (**20**). b) 1-amino-3-butyne, CH_3OH, 23 °C.

Intramolecular Diels–Alder Reactions of *N*-Acyl 6-Amino-3-(methylthio)-1,2,4,5-tetrazines. Although the intermolecular Diels–Alder reactions of **2**–**4** were found to proceed sluggishly with unactivated dienophiles including simple alkynes, the intramolecular variant was found to proceed under mild conditions (23–80 °C, 6–16 h, Scheme 3). The 6-(acylamino)-3-(methylthio)-1,2,4,5-tetrazine **16** possessing a terminal acetylene linked by a three-carbon tether underwent near quantitative conversion to the bicyclic 1,2-diazine **17** (92%) with formation of a fused six-membered ring when warmed in dioxane at 80 °C for 6 h. Likewise, the tethered alkyne **18** underwent a sequential acylation and [4 + 2] cycloaddition with formation of a fused five-membered ring at 23 °C (16 h) when exposed to BOC_2O and catalytic DMAP in THF affording **19** in superb yield (96%). In this latter case, *N*-acylation of the C-6 amino group was sufficient to activate the tetrazine for room temperature cycloaddition. Thus, unactivated acetylenes are able to smoothly undergo [4 + 2] cycloaddition when tethered to the tetrazine,[21–23] and parallel observations have been made with alternative dienophiles linked to unsymmetrical 6-amino-3-(methylthio)-1,2,4,5-tetrazines which have been shown to proceed sluggishly[21] or not at all, unless the amine is *N*-acylated.[21,22]

Scheme 4



Reactivity. A study of the relative reactivity of tetrazines **1**–**3** toward *N*-vinyl pyrrolidinone was undertaken by competitively subjecting 1 equiv of each component to the usual reaction conditions and analyzing the crude reaction mixture by ^1H NMR (Scheme 4). A product ratio of 4.5:1 **8f**:**20** was obtained when tetrazines **1** and **2** competed for the available dienophile, indicating that tetrazine **2** is slightly more reactive than **1**. Similarly, the competitive Diels–Alder reaction of **3** and **2** indicated that the reactivity of tetrazine **3** is greater than that of **2** by a factor of 3.7 (Scheme 4).

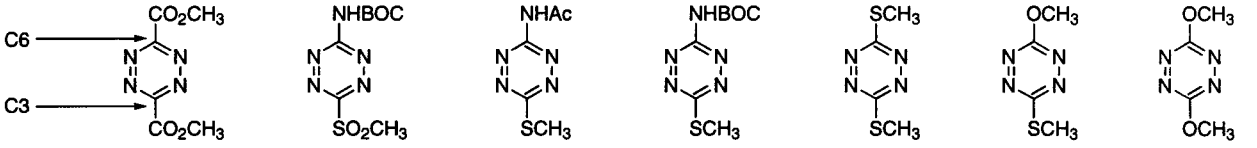
This proved consistent with AM1 computational studies of a full range of substituted 1,2,4,5-tetrazines where the LUMO of both **2** and **3** are at a higher energy than that of 3,6-dicarbomethoxy-1,2,4,5-tetrazine but lower than those of 6-methoxy-3-(methylthio)- or 3,6-dimethoxy-1,2,4,5-tetrazine accurately reflecting their relative reactivities (Table 4).[11] Similarly, the LUMO for **3** was significantly lower than that of **2** also accurately reflecting their relative reactivities. In addition, while the LUMO of **3** was lower than that of **1** mirroring the experimental observations on relative rate, those of **2** and **1** were not significantly different, and these results less

(21) Seitz, G.; Dietrich, S.; Görge, L.; Richter, J. *Tetrahedron Lett.* **1986**, *27*, 2747.

(22) Benson, S. C.; Lee, L.; Snyder, J. K. *Tetrahedron Lett.* **1996**, *37*, 5061.

(23) For examples of intramolecular Diels–Alder additions with other heteroatom-tethered alkynes, see: Sietz, G.; Görge, L.; Dietrich, S. *Tetrahedron Lett.* **1985**, *26*, 4355.

Table 4. AM1 Computational Results (LUMO) of 1,2,4,5-Tetrazines



LUMO (EV)	-2.1489	-2.11670[a]	-1.38922	-1.36422[a]	-1.36527	-1.13952	-0.91775
C-3 coefficient	0.591	0.513[a]	0.538	0.549[a]	0.584	0.571	0.585
C-6 coefficient	0.591	0.626[a]	0.598	0.596[a]	0.584	0.596	0.585
C-3 net charge	-0.102	-0.485[a]	-0.308	-0.301[a]	-0.277	-0.300	-0.015
C-6 net charge	-0.102	0.079[a]	0.025	0.022[a]	-0.277	0.010	-0.015

a. BOC was replaced with CO_2CH_3 for the calculations

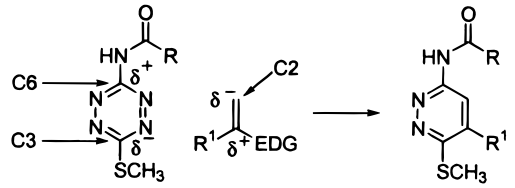


Figure 2.

closely parallel the experimental observations where **2** was found to be slightly more reactive (ca. 4.5×).

Regioselectivity. The regioselectivity of the cycloadditions are consistent with the expectation that the methylthio group controls the orientation by stabilizing a partial negative charge at C-3 (Figure 2). The dienophile addition follows an approach predicted by this stabilization and by the added ability of the acylamino group to stabilize a partial positive charge on C-6 of the electron-deficient tetrazine. These intuitive predictions of the observed regioselectivity are supported by the AM1 computational studies (Table 4). The C-3 position of both **2** and **3** bears a significant partial negative charge (−0.308 to −0.301) while C-6 is slightly electropositive (+0.025 to +0.022). Moreover, C-6 bears the largest LUMO orbital coefficient indicating it dominates the regioselectivity by preferentially combining with the dienophile C-2 center which possesses the largest HOMO orbital coefficient.

Conclusions

1,2,4,5-Tetrazines **2**−**4** undergo well-behaved and regioselective inverse electron demand Diels−Alder reactions with a variety of electron-rich dienophiles to provide the corresponding 1,2-diazines in good to excellent yields. Thus, simple *N*-acylation of the unreactive 6-amino-3-(methylthio)-1,2,4,5-tetrazine provides sufficient activation to permit the effective usage of these unsymmetrically substituted 1,2,4,5-tetrazines. A limitation of tetrazine **2** is the thermal instability of the *N*-BOC group at temperatures ≥100 °C for extended periods of time.[20] Tetrazines **3** and **4** (**3** > **4** > **2**) were found to be much more stable to such vigorous conditions required for reaction with less reactive dienophiles and, typically, no deacylation products were observed. The comparative reactivity of the tetrazines illustrated in Scheme 4 highlights an unusual reactivity pattern where **2**−**4** (**3** > **2**, **4**) are more reactive than **1** and possess a reactivity amenable to widespread utilization.

Experimental Section

6-[(*tert*-Butyloxycarbonyl)amino]-3-(methylthio)-1,2,4,5-tetrazine (2). *tert*-Butyl carbamate (323 mg, 2.75 mmol) in 46.0 mL of anhydrous DMF at 0 °C under Ar was treated with NaH (80% dispersion, 124 mg, 4.1 mmol) in several portions, and the mixture was stirred for 30 min. The suspension was cooled to −40 °C, treated with **1**[3] (400 mg, 2.3 mmol) in 7.6 mL of anhydrous DMF dropwise, and stirred for 18 h at −40 °C. The reaction mixture was diluted with Et_2O (150 mL), washed sequentially with 1 N aqueous HCl (150 mL) and saturated aqueous NaCl (150 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash chromatography (SiO_2, 3 × 15 cm, 30% EtOAc−hexane) provided recovered **1** (90.0 mg, 23%) and **2** (384 mg, 69%) as a red oil which slowly crystallized: mp 65−66 °C (red needles, EtOAc); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.76 (s, 1H), 2.69 (s, 3H), 1.54 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.8, 158.6, 149.5, 83.2, 28.0, 13.5; IR (film) ν_{max} 3225, 2979, 1732, 1537, 1368 cm^{-1}; FABHRMS (NBA-NaI) *m*/*z* 244.0860 (M + H$^+$, $C_8H_{13}N_5O_2S$ requires 244.0868). Anal. Calcd for $C_8H_{13}N_5O_2S$: C, 39.49; H, 5.39; N, 28.79; S, 13.18. Found: C, 39.83; H, 5.45; N, 28.79; S, 12.98.

6-(Acetylamino)-3-(methylthio)-1,2,4,5-tetrazine (3). 3,6-Bis(methylthio)-1,2,4,5-tetrazine (**1**,[3] 400 mg, 2.28 mmol) in 22.0 mL anhydrous CH_3OH at 0 °C under Ar was treated with 22.0 mL of saturated NH_3−CH_3OH and stirred for 30 min[14] before the solvents were removed under reduced pressure. The orange solid was dissolved in 12.0 mL of anhydrous THF and treated with Ac_2O (320 μL, 3.4 mmol) and DMAP (28.0 mg, 0.2 mmol), and the mixture was warmed at reflux under Ar for 15 h. The reaction mixture was concentrated under reduced pressure. Flash chromatography (SiO_2, 3 × 15 cm, 0−60% EtOAc−hexane gradient) provided recovered **1** (66.0 mg, 20%), the diacetate **5** (18 mg, 5%) as a bright red oil, and **3** (300 mg, 71%) as a bright red solid: mp 131−133 °C (red needles, EtOAc); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.93 (s, 1H), 2.70 (s, 3H), 2.50 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.9, 169.4, 158.3, 25.0, 13.6; IR (film) ν_{max} 3188, 3054, 1694, 1556, 1347 cm^{-1}; FABHRMS (NBA-NaI) *m*/*z* 186.0453 (M + H$^+$, $C_5H_7N_5OS$ requires 186.0450). Anal. Calcd for $C_5H_7N_5OS$: C, 32.42; H, 3.81; N, 37.81; S, 17.31. Found: C, 32.78; H, 4.11; N, 37.73; S, 17.55.

For 6-bis(acetylamino)-3-(methylthio)-1,2,4,5-tetrazine (**5**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.78 (s, 3H), 2.36 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.1, 171.3, 161.8, 26.2, 13.7; IR (film) ν_{max} 1732, 1418, 1350 cm^{-1}; FABHRMS (NBA-NaI) *m*/*z* 250.0367 (M + Na$^+$, $C_7H_9N_5O_2S$ requires 250.0375).

6-[(Benzyloxycarbonyl)amino]-3-(methylthio)-1,2,4,5-tetrazine (4). Benzyl carbamate (208 mg, 1.38 mmol) in 23.0 mL of anhydrous DMF at 0 °C under Ar was treated with NaH (80% dispersion, 62.0 mg, 2.06 mmol) in several portions and stirred for 30 min. The suspension was cooled to −40 °C, treated dropwise with **1**[3] (200 mg, 1.15 mmol) in 3.8 mL of anhydrous DMF, and stirred for 29 h at −40 °C. The reaction mixture was diluted with Et_2O (80 mL), washed sequentially with 1 N aqueous HCl (80 mL) and saturated aqueous NaCl (80 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash chromatography (SiO_2, 3 × 15 cm, 30% EtOAc−hexane) provided recovered **1** (28.0 mg, 14%) and **4** (195.0 mg, 61%) as a red solid: mp 90−92 °C (red needles, EtOAc); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.30 (s, 1H), 7.29−7.39 (m, 5H), 5.27 (s, 2H), 2.67 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz)

δ 172.7, 158.3, 150.7, 134.9, 128.7, 128.6, 128.5, 68.3, 13.5; IR (film) ν_{max} 3234, 3063, 1755, 1557 cm^{-1}; FABHRMS (NBA-NaI) *m/z* 278.0718 (M + H$^+$, $C_{11}H_{11}N_5O_2S$ requires 278.0712). Anal. Calcd for $C_{11}H_{11}N_5O_2S$: C, 47.64; H, 4.00; N, 25.26; S, 11.56. Found: C, 48.04; H, 3.81; N, 24.88; S, 11.29.

General Procedure for the Diels−Alder Reaction of 6-[(*tert*-Butyloxycarbonyl)amino]-3-(methylthio)-1,2,4,5-tetrazine (2), 6-(Acetylamino)-3-(methylthio)-1,2,4,5-tetrazine (3), and 6-[(Benzyloxycarbonyl)amino]-3-(methylthio)-1,2,4,5-tetrazine (4) with Representative Dienophiles 7a−7j. According to the conditions listed in Tables 1−3, **2**, **3**, or **4** was dissolved in the appropriate solvent (0.5 M) under N_2. Dienophile was added, and the reaction was run at the temperature and for the duration indicated. The solvent was removed under reduced pressure, and for the indicated enamines only, the residue was redissolved in 10% HOAc−benzene and stirred for 14−16 h as indicated to induce aromatization before reconcentration. Flash chromatography provided the corresponding 1,2-diazine(s).

1-[(*tert*-Butyloxycarbonyl)amino]-4-(methylthio)-6,7-dihydro-5*H*-cyclopenta[*d*]pyridazine (8a). Reaction of **2** (27.0 mg, 0.11 mmol) with 1-pyrrolidino-1-cyclopentene (**7a**, 24 μL, 0.16 mmol) followed by flash chromatography (SiO_2, 1.5 × 10 cm, 20% EtOAc−hexane) provided **8a** (30.0 mg, 96%) as a light tan solid: mp 97−99 °C (tan prisms, EtOAc); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 3.03 (t, *J* = 7.6 Hz, 2H), 2.81 (t, *J* = 7.6 Hz, 2H), 2.69 (s, 3H), 2.08−2.17 (m, 2H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 156.7, 152.8, 150.4, 145.4, 138.1, 81.2, 32.2, 31.0, 28.1, 23.3, 12.7; IR (film) ν_{max} 3181, 2975, 1566, 1493, 1366 cm^{-1}; FABHRMS (NBA-NaI) *m/z* 282.1272 (M + H$^+$, $C_{13}H_{19}N_3O_2S$ requires 282.1276). Anal. Calcd for $C_{13}H_{19}N_3O_2S$: C, 55.49; H, 6.81; N, 14.93; S, 11.40. Found: C, 55.43; H, 6.89; N, 14.98; S, 11.30.

Similarly, reaction of **2** (54.0 mg, 0.22 mmol) with 1-morpholino-1-cyclopentene (**7b**, 53 μL, 0.33 mmol) provided **8a** (58.1 mg, 93%).

1-[(*tert*-Butyloxycarbonyl)amino]-4-(methylthio)-5,6,7,8-tetrahydrophthalazine (8b). Reaction of **2** (25.0 mg, 0.10 mmol) and 1-pyrrolidino-1-cyclohexene (**7c**, 25 μL, 0.15 mmol) followed by flash chromatography (SiO_2, 1.5 × 10 cm, 20% EtOAc−hexane) provided **8b** (24.0 mg, 79%) as a tan solid: mp 101−103 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.63−2.72 (m, 2H), 2.63 (s, 3H), 2.49−2.60 (m, 2H), 1.80−1.90 (m, 2H), 1.69−1.71 (m, 2H), 1.48 (s, 9H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 153.4, 136.8, 132.7, 81.1, 28.2, 24.8, 24.2, 21.3, 13.1; IR (film) ν_{max} 3201, 2934, 1726, 1484, 1366 cm^{-1}; FABHRMS (NBA-NaI) *m/z* 296.1442 (M + H$^+$, $C_{14}H_{21}N_3O_2S$ requires 296.1442).

6-[(*tert*-Butyloxycarbonyl)amino]-4-ethyl-5-methyl-3-(methylthio)-1,2-diazine (8c). Reaction of **2** (15.0 mg, 0.06 mmol) with 3-morpholino-2-pentene (**7d**, 29.0 mg 0.18 mmol) followed by flash chromatography (SiO_2, 1.5 × 10 cm, 20% EtOAc−hexane) provided **8c** (15.0 mg, 86%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.05 (s, 1H), 2.68 (dd, *J* = 7.6 Hz, 2H), 2.65 (s, 3H), 2.02 (s, 3H), 1.48 (s, 9H), 1.16 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.5, 151.9, 141.2, 131.1, 81.3, 28.2, 28.1, 22.2, 13.6, 13.4, 11.5; IR (film) ν_{max} 3193, 2975, 2931, 1728, 1488, 1392, 1367 cm^{-1}; FABHRMS (NBA-NaI) *m/z* 306.1246 (M + Na$^+$, $C_{13}H_{21}N_3O_2S$ requires 306.1252).

6-[(*tert*-Butyloxycarbonyl)amino]-3-(methylthio)-4-phenyl-1,2-diazine (8d). Reaction of **2** (22.0 mg, 0.09 mmol) with 1-morpholino-1-phenylethene (**7e**, 26.0 mg, 0.14 mmol) followed by flash chromatography (SiO_2, 1.5 × 10 cm, 20% EtOAc−hexane) provided **8d** (17 mg, 60%) as a pale yellow solid: mp 139−141 °C (pale yellow prisms, EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 (s, 1H), 7.57 (s, 1H), 7.45 (m, 5H), 2.61 (s, 3H), 1.51 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.6, 152.9, 152.2, 141.3, 135.4, 129.4, 128.7, 116.6, 81.9, 28.1, 14.0; IR (film) ν_{max} 3189, 3083, 2973, 2927, 1738, 1716, 1574, 1538, 1484 cm^{-1}; FABHRMS (NBA-NaI) *m/z* 318.1286 (M + H$^+$, $C_{16}H_{19}N_3O_2S$ requires 318.1276). Anal. Calcd for $C_{16}H_{19}N_3O_2S$: C, 60.54; H, 6.03; N, 13.24; S, 10.10. Found: C, 60.54; H, 6.10; N, 13.20; S, 10.21.

Similarly, reaction of **2** (18 mg, 0.07 mmol) with 1-pyrrolidino-1-phenylethene (**7f**, 51.0 mg, 0.30 mmol) provided **8d** (13.0 mg, 55%).

Similarly, reaction of **2** (54 mg, 0.22 mmol) with 1-(trimethylsilyloxy)-1-phenylethene (**7g**, 136 μL, 0.67 mmol) provided **8d** (37.0 mg, 53%).

6-[(*tert*-Butyloxycarbonyl)amino]-4-methoxy-3-(methylthio)-1,2-diazine (8e). Reaction of **2** (31.0 mg, 0.13 mmol) with 1,1-dimethoxyethylene (**7h**, 67 μL, 0.77 mmol) followed by flash chromatography (SiO_2, 1.5 × 10 cm, 20% EtOAc−hexane) provided **8e** (26 mg, 75%) as a white solid: mp 161−162 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.58 (s, 1H), 7.50 (s, 1H), 3.95 (s, 3H), 2.60 (s, 3H), 1.50 (s, 9H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 158.3, 156.5, 153.9, 152.4, 95.7, 81.8, 55.8, 28.2, 12.3; IR (film) ν_{max} 3190, 2973, 1723, 1592, 1513, 1462, 1372 cm^{-1}; FABHRMS (NBA-NaI) *m/z* 272.1077 (M + H$^+$, $C_{11}H_{17}N_3OS$ requires 272.1069). Anal. Calcd for $C_{11}H_{17}N_3OS$: C, 48.79; H, 6.59; N, 15.17; S, 12.11. Found: C, 48.69; H, 6.32; N, 15.49; S, 11.82.

6-[(*tert*-Butyloxycarbonyl)amino]-3-(methylthio)-1,2-diazine (8f). Reaction of **2** (47.0 mg, 0.19 mmol) with *N*-vinyl pyrrolidinone (**7i**, 65 μL, 0.58 mmol) followed by flash chromatography (SiO_2, 1.5 × 10 cm, 20% EtOAc−hexane) provided **8f** (27.0 mg, 58%) as a pink solid: mp 112−114 °C (pink needles, EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.03 (d, *J* = 7.6 Hz, 1H), 7.67 (s, 1H), 7.27 (d, *J* = 7.6 Hz, 1H), 2.64 (s, 3H), 1.50 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.6, 152.7, 152.2, 127.5, 117.2, 81.9, 28.0, 13.4; IR (CCl_4) ν_{max} 3421, 2981, 2931, 1736, 1492 cm^{-1}; FABHRMS (NBA-NaI) *m/z* 242.0958 (M + H$^+$, $C_{10}H_{15}N_3O_2S$ requires 242.0963). Anal. Calcd for $C_{10}H_{15}N_3O_2S$: C, 49.77; H, 6.27; N, 17.41; S, 13.29. Found: C, 49.54; H, 6.50; N, 17.24; S, 13.11.

Similarly, reaction of **2** (40.0 mg, 0.16 mmol) with ethyl vinyl ether (**7j**, 47 μL, 0.49 mmol) provided **8f** (20.0 mg, 50%) and 6-amino-3-(methylthio)-1,2-diazine (2.8 mg, 9%).

6-[(*tert*-Butyloxycarbonyl)methylamino]-3-(methylthio)-1,2,4,5-tetrazine (10). A solution of **2** (12 mg, 0.05 mmol) in anhydrous DMF (200 μL) at −40 °C under N_2 was treated with NaH (3.0 mg of 60% in oil, 0.07 mmol) and stirred for 30 min. The resulting suspension was treated with CH_3I (9 μL, 0.15 mmol) and stirred for an additional 30 min before the reaction was quenched with the addition of 5% aqueous HCl. The reaction mixture was extracted with Et_2O (3 × 5 mL), and the combined organic layers were washed with H_2O (5 mL) and saturated aqueous NaCl (5 mL). The solvent was removed under reduced pressure, and flash chromatography (SiO_2, 1.5 × 5 cm, 10% EtOAc−hexane) provided **10** (8.0 mg, 61%) as a red oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.53 (s, 3H) 2.72 (s, 3H), 1.51 (s, 9H); IR ($CHCl_3$) ν_{max} 3690, 1719, 1601, 1382 cm^{-1}; FABHRMS (NBA-NaI) *m/z* 280.0839 (M + Na$^+$, $C_9H_{15}N_5O_2S$ requires 280.0844).

6-[(*tert*-Butyloxycarbonyl)methylamino]-4-(diethylamino)-5-methyl-3-(methylthio)-1,2-diazine (11). Reaction of **10** (6.0 mg, 24 μmol) with 1-(diethylamino)-1-propyne (5.0 mg, 47 μmol) followed by flash chromatography (SiO_2, 1.5 × 10 cm, 10% EtOAc−hexane) provided **11** (4.5 mg, 55%) as a light yellow oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 3.20 (s, 3H), 3.12 (q, *J* = 8.4 Hz, 4H) 2.68 (s, 3H) 2.18 (s, 3H), 1.40 (s, 9H), 1.05 (t, *J* = 8.6 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.3, 155.1, 154.2, 144.9, 132.4, 80.8, 45.4, 36.2, 29.7, 28.3, 14.9, 14.1, 13.8; IR (film) ν_{max} 2973, 2928, 1709, 1530, 1429, 1367 cm^{-1}; FABHRMS (NBA-NaI) *m/z* 363.1844 (M + Na$^+$, $C_{16}H_{28}N_4O_2S$ requires 363.1831).

6-Amino-3-(methylthio)-4-phenyl-1,2-diazine (12a and 12b). Reaction of **2** (56.0 mg, 0.23 mmol) with phenylacetylene (76 μL, 0.69 mmol) followed by flash chromatography (SiO_2, 2.5 × 10 cm, 30−100% EtOAc−hexane gradient) provided **12a** and **12b** (15.0 mg, 30%) as an inseparable mixture (4:1 by NMR) and 6-amino-3-(methylthio)-1,2,4,5-tetrazine (18 mg, 54%). For **12a** and **12b**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40−7.50 (m, 5H), 7.00 (s, 1H), 6.60 (s, 1H), 2.59 (s, 3H), 2.49 (s, 3H); FABHRMS (NBA-NaI) *m/z* 218.0752 (M + H$^+$, $C_{11}H_{11}N_3S$ requires 218.0759).

1-(Acetylamino)-4-(methylthio)-6,7-dihydro-5*H*-cyclopenta[*d*]pyridazine (13a). Reaction of **3** (15.0 mg, 0.08

mmol) with 1-(trimethylsilyloxy)-1-cyclopentene (**7k**, 50.0 mg, 0.32 mmol) followed by flash chromatography (SiO_2, 1.5 × 10 cm, 40% EtOAc−hexane) provided **13a** (11.0 mg, 61%) as a white solid: mp 181−182 °C (white needles, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.01 (t, J = 7.5 Hz, 2H), 2.84 (t, J = 7.5 Hz, 2H), 2.67 (s, 3H), 2.35 (s, 3H), 2.11 (t, J = 7.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 169.8, 156.9, 150.8, 145.3, 138.7, 32.6, 31.1, 23.8, 23.2, 12.7; IR ($CHCl_3$) ν_{max} 3182, 2932, 1696, 1560, 1507, 1481 cm^{-1}; FABHRMS (NBA-NaI) m/z 246.0685 (M + Na$^+$, $C_{10}H_{13}N_3OS$ requires 246.0677).

Similarly, reaction of **3** (14.0 mg, 0.08 mmol) with 1-morpholino-1-cyclopentene (**7b**, 18 μL, 0.11 mmol) provided **13a** (14.8 mg, 88%).

6-(Acetylamino)-4-ethyl-5-methyl-3-(methylthio)-1,2-diazine (13b). Reaction of **3** (10.0 mg, 0.05 mmol) with 3-morpholino-2-pentene (**7d**, 25.0 mg, 0.16 mmol) followed by flash chromatography (SiO_2, 1.5 × 10 cm, 20% EtOAc−hexane) provided **13b** (9.0 mg, 74%) as a pale yellow solid: mp 125−127 °C (white prisms, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.72 (q, J = 7.6 Hz, 2H), 2.47 (s, 3H), 2.29 (s, 3H), 2.22 (s, 3H), 1.18 (t, J = 7.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.8, 160.0, 152.5, 141.6, 131.7, 29.7, 23.5, 22.3, 13.7, 13.6, 11.4; IR (film) ν_{max} 3221, 2972, 2931, 1698, 1548, 1494, 1433, 1392 cm^{-1}; FABHRMS (NBA-NaI) m/z 248.0830 (M + Na$^+$, $C_{10}H_{15}N_3OS$ requires 248.0834).

Anal. Calcd for $C_{10}H_{15}N_3OS$: C, 53.31; H, 6.71; N, 18.65; S, 14.23. Found: C, 53.62; H, 7.11; N, 18.28; S, 13.90.

A single-crystal X-ray structure determination conducted on crystals grown from $CHCl_3$ unambiguously established the structure of **13b** and the regioselectivity of the cycloaddition reaction.[18]

6-(Acetylamino)-4-methoxy-3-(methylthio)-1,2-diazine (13c). Reaction of **3** (22.0 mg, 0.12 mmol) with 1,1-dimethoxyethylene (**7h**, 50.0 mg, 0.60 mmol) followed by simple filtration with an EtOAc wash provided **13c** (20.0 mg, 78%) as a white solid: mp 253 °C (decomp); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.01 (s, 1H), 3.98 (s, 3H), 2.57 (s, 3H), 2.44 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.0, 156.6, 154.9, 150.7, 97.5, 56.0, 25.0, 16.4; IR ($CHCl_3$) ν_{max} 3209, 3029, 2943, 1694, 1587, 1489, 1400, 1373 cm^{-1}; FABHRMS (NBA-NaI) m/z 306.1246 (M + Na$^+$, $C_{13}H_{13}N_3OS$ requires 306.1252).

6-(Acetylamino)-3-(methylthio)-4-phenyl-1,2-diazine (13d) and 3-(Acetylamino)-6-(methylthio)-4-phenyl-1,2-diazine (14). Reaction of **3** (32.0 mg, 0.17 mmol) with phenylacetylene (57 μL, 0.52 mmol) followed by flash chromatography (SiO_2, 1.5 × 15 cm, 0−2% CH_3OH−$CHCl_3$ gradient) afforded **13d** (26.0 mg, 58%) as a white solid and **14** (13.0 mg, 29%) as a yellow oil. For **13d**: mp 188−190 °C (white needles, EtOAc); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.36 (s, 1H), 8.39 (s, 1H), 7.47 (s, 5H), 2.58 (s, 3H), 2.47 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.7, 157.1, 154.0, 141.9, 135.1, 130.0, 128.5, 118.6, 29.7, 24.9, 14.0; IR ($CHCl_3$) ν_{max} 3204, 3089, 2930, 1696, 1567, 1544, 1480, 1397 cm^{-1}; FABHRMS (NBA-NaI) m/z 260.0855 (M + H$^+$ $C_{13}H_{13}N_3OS$ requires 260.0858). Anal. Calcd for $C_{13}H_{13}N_3OS$: C, 60.21; H, 5.05; N, 16.20; S, 12.37. Found: C, 60.50; H, 4.94; N, 15.90; S, 12.01.

For **14**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40−7.50 (m, 5H), 7.28 (s, 1H), 2.69 (s, 3H), 2.30 (s, 3H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 170.4, 160.3, 150.0, 134.4, 131.4, 130.3, 129.5, 129.2, 127.9, 127.6, 127.4, 24.0, 13.4; IR (film) ν_{max} 3174, 3000, 1683, 1538, 1489, 1393 cm^{-1}; FABHRMS (NBA-NaI) m/z 260.0860 (M + H$^+$, $C_{13}H_{13}N_3OS$ requires 260.0858).

Similarly, reaction of **3** (17.0 mg, 0.09 mmol) with 1-(trimethylsilyloxy)-1-phenylethene (**7g**, 56 μL, 0.27 mmol) followed by flash chromatography (SiO_2, 1.5 × 10 cm, 50% EtOAc−hexane) provided only **13d** (20.0 mg, 84%).

6-(Acetylamino)-3-(methylthio)-1,2-diazine (13e). Reaction of **3** (31.0 mg, 0.17 mmol) with *N*-vinyl pyrrolidinone (**7i**, 39 μL, 0.34 mmol) followed by flash chromatography (SiO_2, 1.5 × 10 cm, $CHCl_3$) provided **13e** (22.3 mg, 73%) as a yellow solid: mp 208−210 °C (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.90 (s, 1H), 8.43 (d, J = 9.4 Hz, 1H), 7.38 (d, J = 9.4 Hz, 1H), 2.62 (s, 3H), 2.41 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.8, 158.5, 153.2, 128.6, 119.6, 24.8, 13.4; IR (CCl_4) ν_{max} 3035, 2928, 1711, 1685, 1506, 1487, 1410 cm^{-1}; FABHRMS (NBA-NaI) m/z 184.0551 (M + H$^+$, $C_7H_9N_3OS$ requires 184.0545).

1-[(Benzyloxycarbonyl)amino]-4-(methylthio)-6,7-dihydro-5*H*-cyclopenta[*d*]pyridazine (15a). Reaction of **4** (9.1 mg, 33.0 μmol) with 1-morpholino-1-cyclopentene (**7b**, 7.8 μL, 49.0 μmol) followed by flash chromatography (SiO_2, 1.5 × 10 cm, 10−25% EtOAc−hexane gradient) provided **15a** (10.1 mg, 98%) as a white film: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.31−7.39 (m, 5H), 5.20 (s, 2H), 3.06 (t, J = 7.5 Hz, 2H), 2.83 (t, J = 7.5 Hz, 2H), 2.66 (s, 3H), 2.12 (p, J = 7.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.8, 154.0, 150.0, 146.0, 138.0, 135.5, 128.6, 128.4, 128.3, 67.7, 32.2, 31.1, 23.2, 12.8; IR (film) ν_{max} 3170, 2928, 1732, 1504 cm^{-1}; FABHRMS (NBA-NaI) m/z 338.0949 (M + Na$^+$, $C_{16}H_{17}N_3O_2S$ requires 338.0939).

6-[(Benzyloxycarbonyl)amino]-4-methoxy-3-(methylthio)-1,2-diazine (15b). Reaction of **4** (14.1 mg, 53.2 μmol) with 1,1-dimethoxyethylene (**7h**, 30 μL, 320 μmol) followed by flash chromatography (SiO_2, 1.5 × 10 cm, 30% EtOAc−hexane) provided **15b** (10.8 mg, 64%) as a white solid: mp 134−136 °C (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.77 (s, 1H), 7.61 (s, 1H), 7.36 (m, 5H), 5.23 (s, 2H), 3.96 (s, 3H), 2.61 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.5, 153.5, 153.1, 150.9, 135.4, 128.7, 128.2, 95.7, 67.5, 55.8, 12.3; IR (film) ν_{max} 3410, 2976, 2930, 1735, 1587, 1554, 1500, 1462, 1401, 1380 cm^{-1}; FABHRMS (NBA-NaI) m/z 306.0923 (M + H$^+$, $C_{14}H_{15}N_3O_3S$ requires 306.0912).

6-[(Benzyloxycarbonyl)amino]-3-(methylthio)-1,2-diazine (15c). Reaction of **4** (15.8 mg, 57.0 μmol) with *N*-vinyl pyrrolidinone (**7i**, 19 μL, 171 μmol) followed by flash chromatography (SiO_2, 1.5 × 10 cm, 10−20% EtOAc−hexane gradient) provided **15c** (11.3 mg, 72%) as a white solid: mp 134−136 °C (peach-colored needles, EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.23 (br s, 1H), 8.11 (d, J = 9.4 Hz, 1H), 7.29−7.38 (m, 5H), 7.31 (d, J = 9.4 Hz, 1H), 5.22 (s, 2H), 2.63 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.3, 153.0, 152.3, 135.3, 128.7, 128.6, 128.3, 128.1, 117.5, 67.7, 13.4; IR (film) ν_{max} 3171, 2963, 1712, 1531 cm^{-1}; FABHRMS (NBA-NaI) m/z 276.0813 (M + H$^+$, $C_{13}H_{13}N_3O_2S$ requires 276.0807).

3-Methylthio-6-(4-pentynoylamino)-1,2,4,5-tetrazine (16). 4-Pentynoyl amide[24] (55.0 mg, 0.57 mmol) in 4.0 mL of anhydrous DMF at −10 °C under N_2 was treated with NaH (18.0 mg of 60% in oil, 0.614 mmol), and the resulting suspension was warmed to 0 °C over 1 h. The suspension was recooled to −10 °C, **1** (82.0 mg, 0.472 mmol) was added, and the reaction mixture was stirred 18 h at 25 °C. Flash chromatography (SiO_2, 2.5 × 15 cm, 40% EtOAc−hexane) provided **16** (18.0 mg, 14% unoptimized) as a red solid, and recovered **1** (35.0 mg, 43%). For **16**: mp 133−135 °C (EtOAc−hexane); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.57 (s, 1H), 3.00 (t, J = 7.1 Hz, 2H), 2.71 (s, 3H), 2.65 (dt, J = 2.4, 7.1 Hz, 2H), 2.03 (t, J = 2.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.1, 158.1, 82.1, 69.8, 36.4, 29.7, 14.1, 13.6; IR (film) ν_{max} 3194, 2923, 1687, 1455, 1345 cm^{-1}; FABHRMS (NBA-NaI) m/z 246.0434 (M + Na$^+$, $C_8H_9N_5OS$ requires 246.0426).

3-(Methylthio)-5,8-dihydro-6*H*-pyrido[2,3-*c*]pyridazin-7-one (17). A solution of **16** (11.0 mg, 0.05 mmol) in 3.0 mL of dioxane under N_2 was warmed at 80 °C for 18 h. Flash chromatography (SiO_2, 1.5 × 10 cm, 30% EtOAc−hexane) provided **17** (9.0 mg, 92%) as a light tan solid: mp 225 °C (decomp); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.27 (s, 1H), 7.14 (s, 1H), 2.95 (t, J = 7.4 Hz, 2H), 2.70−2.67 (m, 2H), 2.67 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.6, 158.4, 151.6, 125.1, 124.8, 29.5, 23.6, 13.5; IR (film) ν_{max} 3111, 2924, 1726, 1486, 1435, 1364, 1321 cm^{-1}; FABHRMS (NBA-NaI) m/z 196.0551 (M + H$^+$, $C_8H_9N_3OS$ requires 196.0545). Anal. Calcd for $C_8H_9N_3OS$: C, 49.21; H, 4.65; N, 21.52; S, 16.42. Found: C, 49.04; H, 4.24; N, 21.32; S, 16.81.

6-(But-3-yn-1-ylamino)-3-(methylthio)-1,2,4,5-tetrazine (18). 3,6-Bis(methylthio)-1,2,4,5-tetrazine (**1**, 200 mg, 1.15 mmol) was added to a solution of 4-amino-1-butyne (95.0 mg, 1.4 mmol) in 5.0 mL of CH_3OH and stirred at 23 °C for 24

(24) Prepared by adding NH_3(l) to the mixed anhydride of 4-pentynoic acid ($ClCO_2iBu$) in THF at −78 °C.

h under N_2. The solvents were removed under reduced pressure, and flash chromatography (SiO_2, 3.0 × 15 cm, 20% EtOAc−hexane) yielded **18** (116 mg, 52%) as a red solid: mp 75−76 °C (red needles, EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.16 (s, 1H), 3.73 (dt, J = 6.4, 6.4 Hz, 2H), 2.64 (s, 3H), 2.57 (dt, J = 2.6, 6.4 Hz, 2H), 2.03 (t, J = 2.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.5, 160.8, 80.9, 70.7, 39.9, 18.9, 13.6; IR (film) ν_{max} 3298, 2928, 2855, 1699, 1574, 1557, 1435, 1347 cm^{-1}; FABHRMS (NBA-NaI) m/z 196.0659 (M + H$^+$, $C_7H_9N_5S$ requires 196.0657). Anal. Calcd for $C_7H_9N_5S$: C, 43.06; H, 4.65; N, 35.87; S, 16.42. Found: C, 43.37; H, 4.60; N, 35.73; S, 16.59.

7-(*tert*-Butyloxycarbonyl)-3-(methylthio)-5,6-dihydropyrrolo[2,3-*c*]pyridazine (19). A solution of **18** (22.0 mg, 0.113 mmol) in 1.0 mL of anhydrous THF at 23 °C under N_2 was treated with BOC_2O (98.0 mg, 0.45 mmol) followed by DMAP (2.0 mg, 0.016 mmol) and the mixture was stirred for 18 h at 23 °C. Removal of solvents under reduced pressure followed by flash chromatography (SiO_2, 1.5 × 15 cm, 20% EtOAc−hexane) provided **19** (33.0 mg, 96%) as a pale tan oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.00 (s, 1H), 3.98 (t, J = 8.0 Hz, 2H), 3.01 (t, J = 8.0 Hz, 2H), 2.64 (s, 3H), 1.56 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.7, 155.9, 150.8, 132.3, 122.4, 82.2, 46.0, 28.2, 24.4, 13.5; IR (film) ν_{max} 2977, 2927, 1728, 1703, 1622, 1538, 1484, 1418 cm^{-1}; FABHRMS (NBA-NaI) m/z 290.0948 (M + Na$^+$, $C_{12}H_{17}N_3O_2S$ requires 290.0939). Anal. Calcd for $C_{12}H_{17}N_3O_2S$: C, 53.91; H, 6.41; N, 15.72; S, 11.99. Found: C, 54.23; H, 6.29; N, 15.95, S, 11.75.

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Supporting Information Available: Copies of ^{1}H NMR spectra of 5-hydroxy-3,6-bis(methylthio)-1,2,4-triazine, **5**, **6**, **8b**, **8c**, **10**, **11**, **12a** + **12b** mixture, **13a**, **13c**, **13e**, **14**, **15a**−**c**, **16** and details of the X-ray structure of **13b** (29 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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