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Preparation of Unsymmetrical 1,2,4,5-Tetrazines via a Mild Suzuki Cross-Coupling Reaction

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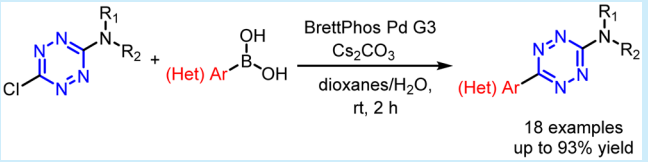
Supporting Information

ABSTRACT: *N*-Alkyl substituted chlorotetrazines were coupled with various boronic acids under Suzuki conditions in high yield at room temperature, giving a mild and straightforward synthetic route toward diverse unsymmetrical 1,2,4,5-tetrazines, a rare heteroarene. This chemistry not only expands the known substrate scope of tetrazine cross-coupling reactions but also allows for the synthesis of novel, tetrazine-containing biologically active molecules with improved DMPK properties.



1,2,4,5-Tetrazines, or *s*-tetrazines, were first reported toward the end of the 19th century, when Pinner found that combining equimolar quantities of hydrazine and benzonitrile produced 3,6-diphenyl-*s*-tetrazine after oxidation.[1] Since Pinner's discovery, this class of nitrogen-rich heterocycles has been of great interest to synthetic organic chemists and materials scientists alike, and many research groups have reported unique syntheses and applications of *s*-tetrazines.[2]

Carboni and Lindsey were the first to report that *s*-tetrazines could undergo cycloaddition reactions,[3] and a number of groups have utilized this inverse demand Diels–Alder cycloaddition chemistry toward the synthesis of novel 3,6-substituted pyridazines, a heterocyclic motif found in myriad biologically active molecules.[4] Contributions to this field by Boger and Sauer[5] have allowed for the Carboni–Lindsey cycloaddition to find use in a number of elegant total syntheses, including Ningalin B,[6] Lycogarubin C,[7] and *ent*-(−)-Roseophilin.[8] In addition to serving as an important building block in the total syntheses of natural products, the cycloaddition reactions of *s*-tetrazines have found applications toward the production of other complex, nitrogen-rich heterocycles, including highly substituted pyridazine boronic esters,[9] and pyrimido[4,5-*d*]pyridazines.[10] This unique *s*-tetrazine chemistry is also being developed as a rapid bioorthogonal coupling reaction.[11]

While the synthesis of symmetrical 3,6-substituted *s*-tetrazines is straightforward, the generation of 3,6-unsymmetrical analogs has proven challenging.[2b] A number of interesting synthetic approaches to unsymmetrical tetrazines have been reported. Smith and colleagues have reported unsymmetrical macropeptides with *s*-substituted tetrazine cores,[12] and Devaraj and colleagues have reported Ni-catalyzed cyclization of nitriles with hydrazine to give unsymmetrical tetrazines.[13] 3,6-Bis(methylthio)-1,2,4,5-tetrazine[14] and 3,6-bis(3,5-dimethylpyrazol-1-yl)-1,2,4,5-tetrazine[15] are widely used starting materials to generate unsymmetrical tetrazines, and such compounds are known to undergo nucleophilic aromatic substitution (S_NAr) with various amines and alkyl carbanions.[14−17] Despite the propensity of *s*-tetrazines to participate in S_NAr reactions, the generation of unsymmetrical *s*-tetrazines remains limited by the narrow scope of reactions that can be performed on the highly electron-deficient scaffold.

Our group became interested in *s*-tetrazines as a potential bioisostere for other six-membered nitrogen-containing heterocycles, and in particular pyridazines, as part of an ongoing medicinal chemistry effort. Starting from commercially available 3,6-dichloro-1,2,4,5-tetrazine, we speculated whether it would be possible to use transition metal cross-coupling reactions to generate unsymmetrical *s*-tetrazines. Very few successful reports of *s*-tetrazine cross-couplings currently exist in the literature, and it is known that the highly electron-deficient core must be stabilized with an electron-donating group if the reaction is to be successful.[16,18] Indeed, preliminary attempts in our own laboratory to perform Pd-catalyzed cross-coupling reactions on 3,6-dichloro-1,2,4,5-tetrazine were unsuccessful. In 2003, Novák and Kotschy reported the first cross-coupling reactions (Sonogashira and Negishi) on *s*-tetrazines, starting from various 3-amino-6-chloro-1,2,4,5-tetrazine scaffolds **1** to afford **3** (Figure 1).[18] Despite this notable advance, yields were moderate, and the authors were unable to isolate any desired products under Suzuki conditions. In 2007, Leconte and colleagues found that 3-methylthio-6-(morpholin-4-yl)-1,2,4,5-tetrazine **4** could readily undergo Suzuki and Stille couplings with displacement of a methylthio group to provide **6** (Figure 1), and to our knowledge this method provides the only examples in the literature of Suzuki cross-couplings directly into a tetrazine to give 3,6-unsymmetrical *s*-tetrazines.[10,16] Although encouraging as the first report of a successful Suzuki reaction on a tetrazine, yields remained moderate for this series, and the *s*-tetrazine system was limited to a morpholino group on one side.[16]

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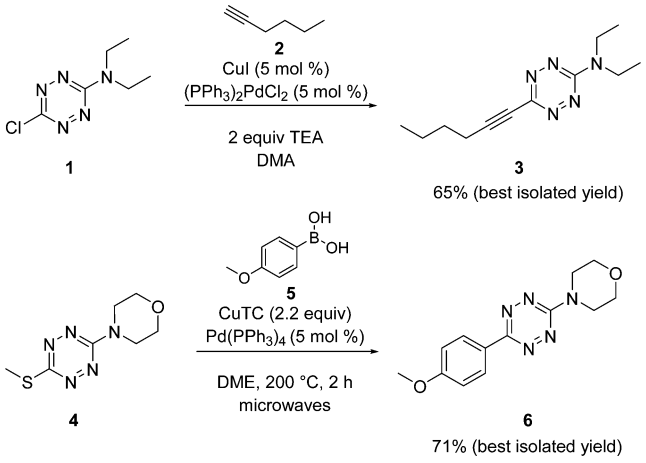
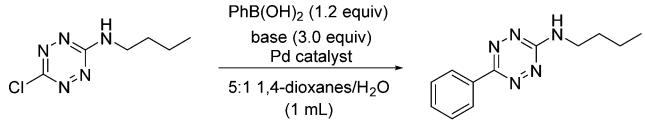


Figure 1. Previously reported Pd-catalyzed cross-coupling reactions of *s*-tetrazines.[16,18]

We therefore wondered if the scope of this reaction could be expanded to include other amino-substituted *s*-tetrazines, specifically amines not limited to a closed, tertiary ring system (i.e., morpholino). Additionally, we were interested in the optimization of milder reaction conditions, as yields in the previous report for Suzuki couplings were increased only through microwave synthesis at 200 °C.[16] For our optimization study, we therefore selected *N*-butyl-6-chloro-1,2,4,5-tetrazin-3-amine (**7**), synthesized under previously reported conditions from 3,6-dichloro-1,2,4,5-tetrazine (Table 1).[19] A screen of palladium catalysts (10% loading with phenylboronic acid and K_2CO_3 as base) encouragingly indicated several promising systems, including $Pd(dppf)Cl_2$ (entry 5) and the third generation BrettPhos palladacycle (entry 6),[20] both of which gave moderate yields of desired product **8** after 1 h at 100 °C. Increasing the reaction time to 2 h with both catalysts improved yield due to the complete consumption of starting material (entries 9 and 10), particularly in the case of the BrettPhos palladacycle. Changing the base to Cs_2CO_3 further improved yield using this catalyst (entry 11, 92% yield), and we were encouraged to find that this reaction could be run with comparable yields both at 70 °C and even at room temperature (entries 16 and 18) and at a reduced catalyst loading of 5% at room temperature (entry 19, 90% yield). A 1% catalyst loading at room temperature (entry 20) resulted in significantly decreased yield compared to entry 19, although a 1% catalyst loading at 70 °C proved successful (entry 21, 81% yield). These mild reaction conditions and high yields are a testament to the robustness of this and other Buchwald palladacycles, particularly for otherwise problematic or unreactive coupling partners, and the mild conditions reported for entry 19 were selected for further substrate scope studies. Utilizing these optimized reaction conditions, **8** was isolated in 93% yield after column chromatography.

With conditions optimized for **7** and phenylboronic acid, we next sought to expand the scope of the boronic acid coupling partner (Scheme 1). Under the optimized conditions, 2-, 3-, and 4-methoxyphenylboronic acid all proved high yielding as a coupling partner (**9a**–**c**), as did 4-methylphenylboronic acid (**9d**). In cases of electron-deficient aromatic rings such as 4-trifluoromethyl (**9e**) and 4-nitrile (**9f**), yields proved lower at room temperature (48% for **9e** and 18% for **9f**), although the total recovery of **9f** could be dramatically increased by running the reaction at 70 °C under otherwise identical conditions

Table 1. Optimization of the Suzuki Coupling Conditions[a]



entry	catalyst	catalyst loading	base	temp (°C)	time (h)	yield (%)[b]
1	RuPhos Pd G3	10	K_2CO_3	100	1	50
2	$Pd(OAc)_2$	10	K_2CO_3	100	1	12
3	$Pd(PPh_3)_4$	10	K_2CO_3	100	1	53
4	$Pd_2(dba)_3$	10	K_2CO_3	100	1	6
5	$Pd(dppf)Cl_2$·DCM	10	K_2CO_3	100	1	63
6	BrettPhos Pd G3	10	K_2CO_3	100	1	60
7	tBuXPhos Pd G3	10	K_2CO_3	100	1	3
8	Pd(amphos) Cl_2	10	K_2CO_3	100	1	59
9	$Pd(dppf)Cl_2$·DCM	10	K_2CO_3	100	2	71
10	BrettPhos Pd G3	10	K_2CO_3	100	2	85
11	BrettPhos Pd G3	10	Cs_2CO_3	100	2	92
12	BrettPhos Pd G3	10	K_3PO_4	100	2	89
13	BrettPhos Pd G3	10	DIPEA	100	2	65
14	BrettPhos Pd G3	10	CH_3COONa	100	2	31
15	BrettPhos Pd G3	10	Cs_2CO_3	130	1	63
16	BrettPhos Pd G3	10	Cs_2CO_3	70	2	97
17	BrettPhos Pd G3	10	Cs_2CO_3	50	2	94
18	BrettPhos Pd G3	10	Cs_2CO_3	rt	2	90
19	**BrettPhos Pd G3**	**5**	**Cs_2CO_3**	**rt**	**2**	**90**
20	BrettPhos Pd G3	1	Cs_2CO_3	rt	2	7
21	BrettPhos Pd G3	1	Cs_2CO_3	70	2	81

[a]Reaction conditions: **7** (30 mg, 0.16 mmol), $PhB(OH)_2$ (1.2 equiv), base (3.0 equiv), 5:1 1,4-dioxanes/H_2O (1.0 mL). [b]Yields determined by LCMS using anisole as internal standard.

(86%). Heteroaryl bicyclic ring systems (**9g** and **9h**) were also tolerated, albeit in diminished yield for 6-quinoline **9g**.

The coupling of **7** with potassium vinyltrifluoroborate (product **9i**) proved sluggish at room temperature, although this product could also be isolated in high yield by increasing the temperature to 70 °C (73%). This result was particularly encouraging, as vinyl-substituted *s*-tetrazines are of interest as a class of precursors to highly nitrogen-rich polymers, and to our knowledge there are no known previous examples of a successful cross-coupling between an *s*-tetrazine and a vinyl group.[21] This methodology should therefore prove straightforward for the generation of new vinyltetrazines. Furthermore, protic substrates such as 4-hydroxyphenylboronic acid (**9j**), smaller aromatic systems (methylpyrazole **9k**) as well as 4-Boc-aminophenylboronic acid (**9l**) also proved compatible under our conditions with substrate **7**, and all were isolated in

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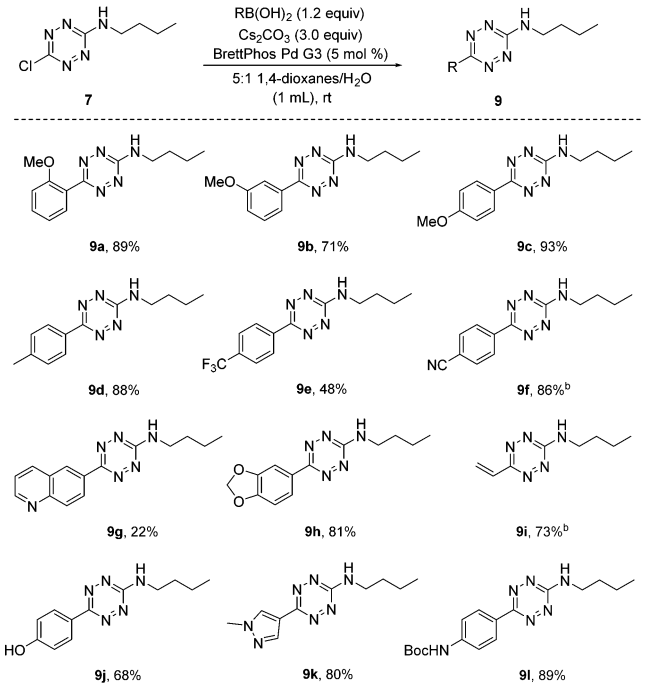
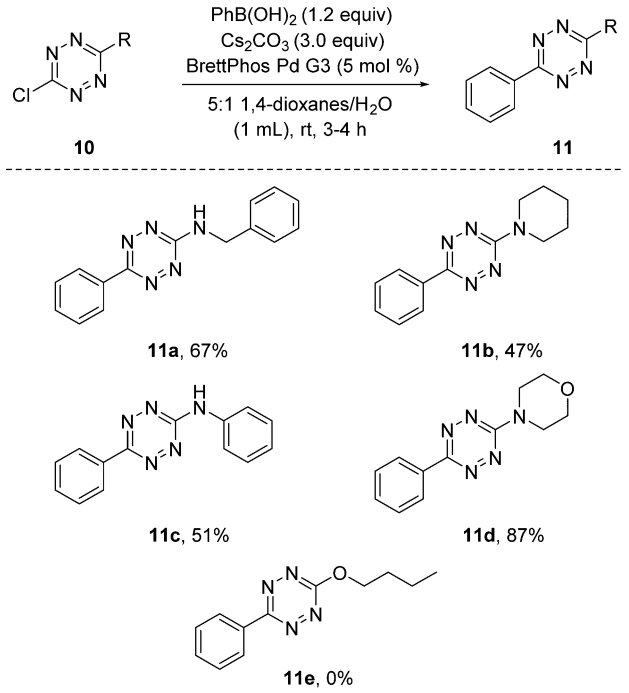
Scheme 1. Scope of the Suzuki Coupling with 7[a]



[a]Standard reaction conditions: **7** (30 mg, 0.16 mmol), $RB(OH)_2$ (1.2 equiv), base (3.0 equiv), 5:1 1,4-dioxanes/H_2O (1.0 mL). Isolated yields after column chromatography. [b]Reaction run at 70 °C.

Scheme 2. Scope of the Suzuki Coupling with 10[a]



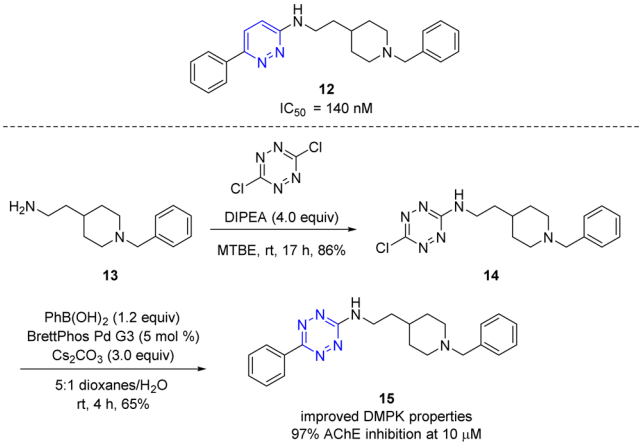
[a]Standard reaction conditions: **10** (30 mg, 0.16 mmol), $RB(OH)_2$ (1.2 equiv), base (3.0 equiv), 5:1 1,4-dioxanes/H_2O (1.0 mL). Isolated yields after column chromatography.

moderate to high yield. Thus, the optimized conditions afforded broad scope and general applicability to aryl, heteroaryl, and vinyl boronic acid coupling partners to deliver analogs **9**.

We next turned our attention to the replacement of the *N*-butyl substitution (**10**) (Scheme 2) and synthesized *N*-benzyl-6-chloro-1,2,4,5-tetrazin-3-amine (**10a**), 3-chloro-6-(1-piperidyl)-1,2,4,5-tetrazine (**10b**), 6-chloro-*N*-phenyl-1,2,4,5-tetrazin-3-amine (**10c**), 4-(6-chloro-1,2,4,5-tetrazin-3-yl)morpholine (**10d**), and 3-butoxy-6-chloro-1,2,4,5-tetrazine (**10e**) in conditions similar to those for the synthesis of **7** (see Supporting Information). These substrates were selected in order to probe the electronic requirements of the group donating electron density to the chloro-*s*-tetrazine core. Our conditions proved successful for benzylamine **11a**, piperidine **11b**, aniline **11c**, and morpholine **11d**, although no evidence of desired product could be detected by LCMS in the case of butoxy-*s*-tetrazine **11e** (Scheme 2). These results indicate that our Suzuki conditions can tolerate a variety of different amino-*s*-tetrazines while maintaining good yields, and optimization efforts toward conditions for ether-containing tetrazine substrates are ongoing in our laboratory.

There are few reported examples of tetrazine motifs in biologically active molecules,[22] and structure−activity relationship (SAR) studies that include this unusual heterocycle have historically been limited largely due to an inability to rapidly and consistently generate unsymmetrical substituted *s*-tetrazines. We therefore became interested in utilizing our optimized Suzuki conditions to generate a direct *s*-tetrazine analog of an existing molecule (**12**) and were aware of a report describing 3,6-substituted pyridazines as acetylcholinesterase (AChE) inhibitors, structurally related to minaprine (Scheme 3).[23] Using a simple, two-step S_NAr/Suzuki-coupling sequence starting from amine **13** (Scheme 3), we were able to generate a

Scheme 3. Synthesis of AChE Inhibitor Analog 15



direct *s*-tetrazine analog (**15**) of the existing molecule (**12**) in high yield under mild conditions and to compare the human hepatic microsomal intrinsic clearance (CL_{int}) and plasma protein binding (PPB) as the fraction unbound (f_u) of each (Table 2), conducted as previously described.[24] The tetrazine analog **15** displayed greater than 3-fold improvement (i.e.,

Table 2. Comparison of Human CL_{int}, Predicted CL_{hep}, PPB, and cLogP for Analogs 12 and 15

compd	CL_{INT} (hum) mL/min/kg	CL_{HEP} (hum) mL/min/kg	f_u (plasma) (%)	cLogP[a]
12	46	14	2.5	5.5
15	14	8.3	2.6	4.4

[a]Calculated using ChemDraw Professional version 16.0.

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reduction) in human hepatic intrinsic clearance compared to the pyridazine analog **12**, while both showed comparable f_u values in the PPB assay. Compound **15** was also found to maintain activity as an AChE inhibitor (Scheme 3).[25] Incorporation of the *s*-tetrazine moiety can therefore maintain certain desirable drug metabolism and pharmacokinetic (DMPK) properties in existing molecules, and in this case even reduced the rate of metabolic clearance (*in vitro*). Additionally, replacement of the pyridazine with the tetrazine moves the molecule into an improved drug-like space in terms of cLogP (5.5 vs 4.4).

In conclusion, the facile cross-coupling chemistry described herein should allow for the generation of new, drug-like, tetrazine-containing molecules and enable further SAR and DMPK studies around such scaffolds, as well as open avenues for the further incorporation and study of tetrazine cores as replacements for pyridazines, pyrimidines, and pyrazines in pharmaceuticals, agrochemicals, and for the material sciences.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b02868.

Experimental procedures, characterization data, and ^{1}H and ^{13}C NMR spectra for new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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