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Letter

Cross-Coupling Reactions of Monosubstituted Tetrazines

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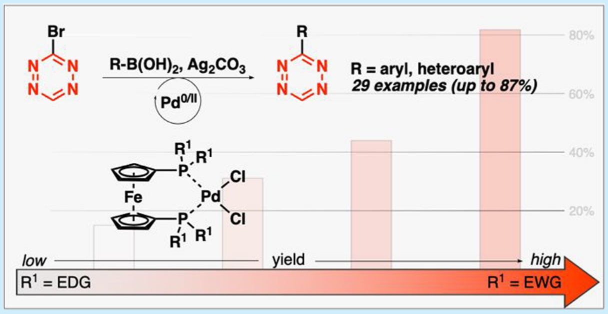
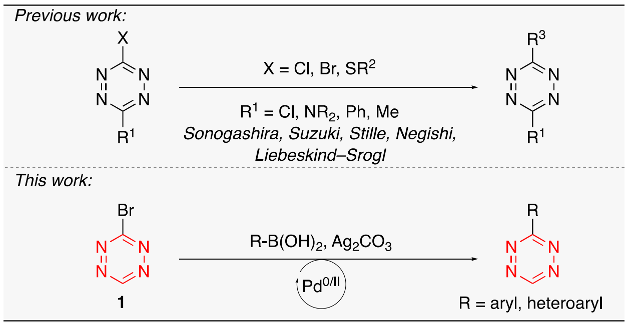
ABSTRACT: A Ag-mediated Pd-catalyzed cross-coupling method for 3-bromo-1,2,4,5-tetrazine with boronic acids is presented. Electronic modification of the 1,1′-bis(diphenylphosphine)ferrocene (dppf) ligand was found to be crucial for good turnover. Using this fast method, a variety of alkyl-, heteroatom-, and halide-substituted aryl- and heteroaryl-tetrazines were prepared (29 examples, up to 87% yield).



Tetrazines have become a frequently used motif in the field of chemical biology.[1,2] These heterocycles exhibit exceptionally fast kinetics in inverse electron-demand Diels–Alder (iEDDA) cycloadditions with strained alkenes and alkynes while only producing biologically benign N$_2$ as a side product.[3] To maximize their bio-orthogonal value, tetrazines should ideally be readily incorporated into target molecules without significantly impairing their physicochemical properties. However, many strategies for the implementation of tetrazines require the presence of bulky hydrophobic linkers and, frequently, large substituents at the 6-position.[4] Hence, methods for the preparation of smaller, nonsymmetric, and easily modifiable tetrazines have only been developed recently.[5−7] Such tetrazines have since been used for direct linker-free incorporation into target molecules by nucleophilic aromatic substitution,[5,6] although the introduction of an electron-donating heteroatom into the motif negatively impacts the kinetics of iEDDA reactions.[8] Hence, carbon–carbon cross-coupling reactions have attracted attention as a method for incorporating tetrazines into target molecules. After the first reported cross-coupling of tetrazines by Kotschy and co-workers in 2003,[9] only a few reports have been found in the literature to our knowledge (Scheme 1).[10−15] The early examples required an aryl moiety or an electron-donating substituent *para* to the newly formed bond.[9,13−15] While these substituents do enable the coupling, they also considerably slow the rates of the subsequent iEDDA reactions because of the additional bulk and inferior electronic properties.[2] Therefore, more recent work in this field has aimed to replace these undesirable substituents with smaller electronically neutral alternatives, such as 6-methyl-substituted tetrazines. Their ability to undergo various cross-coupling reactions was demonstrated by the groups of Wombacher,[10] Fox,[7,16] and Riera.[17] To date, 6-methyl-substituted tetrazines are the smallest tetrazines to be coupled. The transition-metal-catalyzed cross-coupling of monosubstituted tetrazines, however, has remained elusive.

Scheme 1. Previously Reported Tetrazine Cross-Couplings and the Method Presented in This Work



In this study, we report the first methodology for the Pd-catalyzed cross-coupling of monosubstituted *s*-tetrazines. The method utilizes 3-bromo-1,2,4,5-tetrazine (BrTet, **1**) under mild conditions and short reaction times to deliver a broad range of monofunctionalized products in yields of up to 87%.

Initial experiments with 4-*tert*-butylphenylboronic acid in the presence of Pd(dppf)Cl$_2$ and Ag$_2$O afforded the desired product **2** in a 23% yield after 30 min (Table 1, entry 1). An evaluation of classical cross-coupling solvents (Table S1) identified the superiority of acetonitrile (41%) (Table 1, entry 2). Regarding the Ag(I) source, we experienced trends similar

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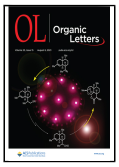

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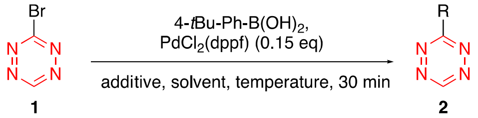
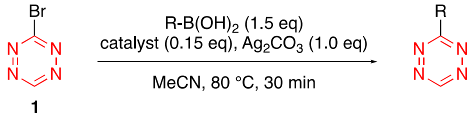
Table 1. Optimization of the Solvent, Silver Source, and Temperature



entry	additive (equiv)	solvent (0.1 M)	temp	yield[a]
1[b]	Ag_2O (2.5)	DMF	60 °C	23%
2[b]	Ag_2O (2.5)	MeCN	60 °C	41%
3[b]	AgF (2.5)	MeCN	60 °C	41%
4[b]	Ag_2CO_3 (2.5)	MeCN	60 °C	71%
5[b]	Ag_2CO_3 (1.5)	MeCN	60 °C	72%
6[c]	Ag_2CO_3 (1.5)	MeCN	60 °C	75%
7[c]	Ag_2CO_3 (1.0)	MeCN	60 °C	72%
8[c]	Ag_2CO_3 (0.5)	MeCN	60 °C	41%
9[c]	Ag_2CO_3 (1.0)	MeCN	25 °C	traces
10[c]	Ag_2CO_3 (1.0)	MeCN	80 °C	80%
11[c]	Ag_2CO_3 (1.0)	MeCN	100 °C	61%

[a] Yields refer to isolated material after column chromatography. [b] Reactions performed with 2.0 equiv of boronic acid. [c] Reactions performed with 1.5 equiv of boronic acid.

Table 2. Investigation of the Catalytic System



entry	R	catalyst	yield[a]
1	4-*t*Bu-Ph	$Pd(PPh_3)_2Cl_2$	9%
2	4-*t*Bu-Ph	DPEphos, $PdCl_2$	48%
3	4-*t*Bu-Ph	BINAP, $PdCl_2$	41%
4	4-*t*Bu-Ph	Pd(dtbpf)Cl_2	34%
5	4-*t*Bu-Ph	Pd(dippf)Cl_2	85%
6	4-*t*Bu-Ph	$Pd(dppf{-}CF_3)Cl_2$	84%
7	4-F-Ph	Pd(dtbpf)Cl_2	15%
8	4-F-Ph	Pd(dippf)Cl_2	31%
9	4-F-Ph	Pd(dppf)Cl_2	44%
10	4-F-Ph	$Pd(dppf{-}CF_3)Cl_2$	82%

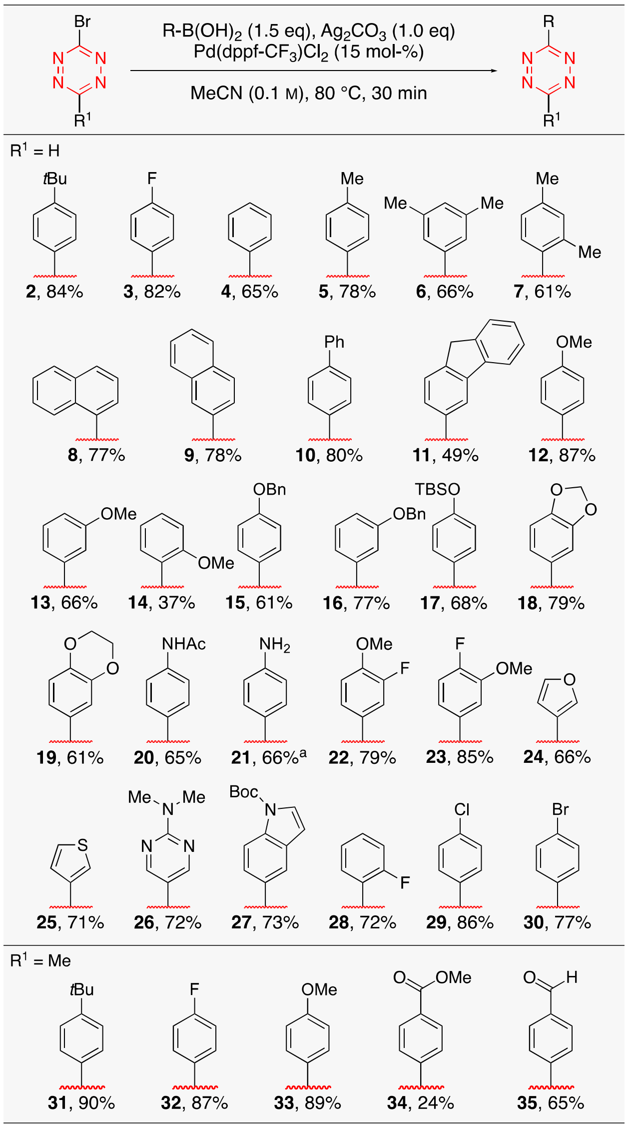
[a] Yields refer to isolated material after column chromatography.

to those noted by Fox and co-workers[7] and found that Ag_2O (41%), AgF (41%), and Ag_2CO_3 (71%) (Table 1, entries 2−4, respectively) are superior to other silver salts and additives (Table S2). These observations are in agreement with intermediary silver aryl species, which have been described in the literature.[18] The amounts of Ag_2CO_3 and boronic acid were minimized to 1.0 and 1.5 equiv, respectively, which provided the best results (Table 1, entries 5−8). When Ag_2CO_3 was omitted from the cross-coupling reactions, or when it was exchanged with Na_2CO_3, no product was formed (Table S6). Preliminary control experiments with 4-fluorophenylboronic acid (**3**, 32%) and other boronic acids identified electron-poor substrates as unsuitable coupling partners (Table S6). As a potential cause, we hypothesized that the electronic nature of these boronic acids impedes reductive elimination, as has previously been described.[19] Consequently, an imbalance between the silver-mediated activation of the boronic and the palladium cycle leads to an increase in the number of side reactions.[18,20] We envisioned that the optimization of the temperature and the catalytic system would be feasible handles for addressing this issue. While at 25 °C, only traces of the desired product were isolated, increasing the temperature to 80 °C resulted in an 80% yield. Performing the reaction at even higher temperatures (100 °C) resulted in a lower yield (61%) (Table 1, entries 9−11). Next, we investigated the role of the catalyst. Other frequently used Pd-based systems with monodentate ($Pd(PPh_3)_2Cl_2$, 9%) or bidentate (DPEphos, 48%, and BINAP, 41%) ligands devoid of the ferrocene moiety afforded the desired product in inferior yields (Table 2, entries 1−3, respectively). Hence, we hypothesized that the electronic optimization of the dppf ligand might be superior to making drastic structural changes. For many metal complexes, electron density and ease of oxidative addition coincide,[21,22] especially when the aryl halide has an electron-deficient character.[23] In contrast, the reductive elimination of electron-poor ligands in the presence of electron-donating ancillary ligands is difficult.[24−26] Therefore, boronic acids bearing electron-donating substituents were viable substrates, while their electron-poorer alternatives resulted in diminished yields. With this in mind, we hypothesized that a clear trend in reactivity should be observed for a series of dppf analogs, which vary electronically.

Thus, we investigated the di-*tert*-butylphosphino (dtbpf), diisopropylphosphino (dippf), and di(CF_3)phenylphosphino (dppf-CF_3) variants of dppf for both 4-*tert*-butylphenyl- and 4-fluorophenylboronic acid (Table 2, entries 4−10). While good yields were generally observed for the electron-rich acid (80−85%), the yields obtained with the electron-poorer derivative (15−82%) exhibited the postulated trend. Here, the electron-poor dppf-CF_3 system greatly increased the yield (from 44% to 82%). Next, the reduction of the catalyst loading was investigated (Table S5). As expected, the coupling of the more electron-rich 4-*tert*-butylphenylboronic acid tolerated a lower catalyst loading (5 mol %, 83% yield) than the coupling of the electron-poorer 4-fluorophenylboronic acid (10 mol %, 79% yield). Finally, at a 1.24 mmol scale and a 5 mol % catalyst loading, the method produced the desired product in an 83% yield (Table S5). However, when the scope was investigated, properly estimating the lowest tolerated catalyst loading was found to be nontrivial. Hence, all presented substrates were prepared using 15 mol % catalyst.

With the optimized conditions in hand, we set out to explore the scope of the reaction (Scheme 2). Phenylboronic acid (**4** (65%)) and alkyl-substituted variants thereof underwent the cross-coupling cleanly and tolerated substituents in the *para*- (**2** (84%) and **5** (78%)), *meta*- (**6** (66%)), and *ortho*-positions (**7** (61%)). Polyaromatic systems, such as naphthyl- (**8** (77%) and **9** (78%)), biphenyl- (**10** (80%)), and fluorenyl-substituted tetrazines (**11**, (49%)) were obtained in good to excellent yields. Various methoxy- (**12** (87%), **13** (66%), and **14** (37%)) and benzyloxyphenylboronic acids (**15** (61%) and **16** (77%)) were suitable substrates. Similarly, TBS-protected phenol **17** (68%) and bridged catechols **18** (79%) and **19** (61%) were produced in good yields. For the preparation of nitrogen-containing derivatives, a reduction of the nucleophilic character, i.e., by Boc protection or acetylation (20 (65%)), was found to be crucial. This strategy allowed, after Boc deprotection with TFA, the preparation of free amine **21** (66%). Further, substituents of an opposite electronic nature were also well tolerated and produced tetrazines **22** and **23** in 79% and 85% yields, respectively. Besides phenylboronic acids, heterocyclic boronic acids were investigated. Furanyl- (**24** (66%)), thienyl- (**25** (71%)), and pyrimidinyltetrazine (**26** (72%)), as well as Boc-protected indole (**27** (73%)), were all

Scheme 2. Conditions and Scope of the Cross-Coupling Reaction



[a] Anilinyltetrazine **21** was prepared by the cross-coupling of the Boc-protected derivative, followed by deprotection with TFA (yield is reported over two steps).

prepared in synthetically useful yields. Along the same lines, fluoro- (**3** (82%) and **28** (72%)), 4-chloro (**29** (86%)), and 4-bromophenylboronic acid (**30** (77%)), which did not produce the desired coupling products in useful amounts with the dppf system, were found to be viable substrates. The coupling between **1** and the boronic acids of benzoates, benzaldehydes, benzonitriles, and similar electron-deficient benzene derivatives remains as a synthetic challenge. When **1** was exchanged with 3-bromo-6-methyl-1,2,4,5-tetrazine, the corresponding known products were obtained in excellent yields (**31** (90%), **32** (87%), and **33** (89%)[7]). Even more demanding substrates afforded the respective products, such as methyl ester **34** (24%) and aldehyde **35** (65%).[7]

We then compared the iEDDA reaction rates induced by mono- and disubstituted tetrazines. To follow the progress of the iEDDA reaction, we monitored the disappearance of the typical tetrazine absorbance band at around 540 nm.[27] This band is not present in the corresponding iEDDA products and thus the accompanying color change from pink or purple to colorless correlates with the progress of the transformation. First, the reaction of 4-*t*Bu-Ph-Tet (**2**) and its Me analog **31** with TCO-PNB ester was performed in acetonitrile. The second-order rate constant was extracted from the obtained data by means of a nonlinear least-squares fit method. For the monosubstituted tetrazine **2**, a value of $k = 143.6\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$ was measured, while the reaction involving the disubstituted tetrazine **31** was roughly 70-fold slower ($k = 2.097\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$). For a proper comparison, **3** and **32** were analyzed by the same method. A 60-fold faster rate constant of $k = 153.9\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$ was measured for the monosubstituted tetrazine, and $k = 2.484\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$ was measured for the disubstituted reference. Therefore, a significant acceleration of the reaction rates of monosubstituted tetrazines versus those of disubstituted tetrazines was observed for the iEDDA reactions, albeit for small substituents such as a Me group at the 6-position.

In conclusion, a method for mild and fast Pd-catalyzed carbon−carbon bond formation between a monosubstituted tetrazine and an array of aryl boronic acids has been reported. The addition of Ag_2CO_3 enabled the target reaction without the need for strong bases or high temperatures. Screening analogs of the ferrocene-based dppf scaffold identified the electron-poorer dppf-CF_3 variant of the catalyst as the optimal mediator. These results open the way for the tailored synthesis of monofunctionalized tetrazines via mild and selective cross-coupling reactions. Kinetic measurements of the iEDDA reactions of these monosubstituted tetrazines and a comparison to those of their disubstituted methyl analogs revealed a significant increase in rate for the monosubstitued tetrazines, which warrants their further use in biorthogonal chemistry.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.1c01813.

Detailed experimental procedures and characterization data together with the crystallographic data (PDF)

Accession Codes

CCDC 2080087−2080090 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via https://www.ccdc.cam.ac.uk/structures.

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Notes

The authors declare no competing financial interest.

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