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Cross-coupling Reactions of Monosubstituted Tetrazines

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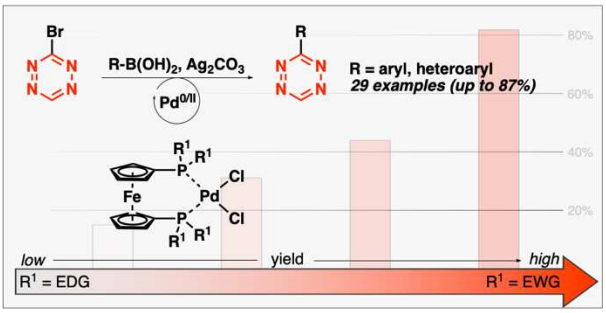


ABSTRACT: A Ag-mediated Pd-catalyzed cross-coupling method for 3-bromo-1,2,4,5-tetrazine with boronic acids is presented. Electronic modification of the 1,1′-bis(diphenylphosphine)ferrocene (dppf) ligand was found to be crucial for good turnover. Using this fast method, a variety of alkyl-, heteroatom-, 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, non-symmetric, and easily modifiable tetrazines have only been developed recently.[5–7] Such tetrazines have since been used for their direct, linker-free incorporation into target molecules by nucleophilic aromatic substitution,[5,6] although the introduction of an electron donating heteroatom onto 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 are 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 down the rates of subsequent iEDDA reactions, because of additional bulk and inferior electronic properties respectively.[2] Therefore, more recent work in this field has aimed at replacing 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. Transition metal catalyzed cross-coupling of monosubstituted tetrazines, however, remained elusive.

In this study, we report on 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 mono-functionalized products in yields of up to 87%.

Scheme 1. Previously reported tetrazine cross-couplings and the method presented in this work

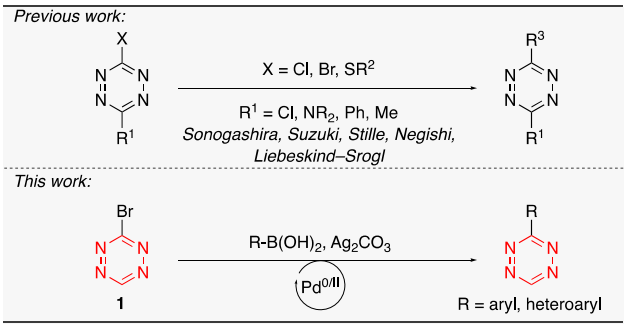
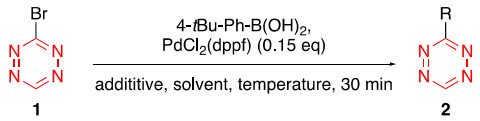


Table 1. Optimization of the solvent, silver source and temperature



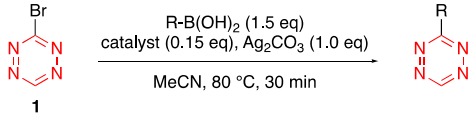
1 2

entry	boronic acid	additive (eq)	solvent (0.1 M)	temp	yield[a]
1	2.0 eq	Ag_2O (2.5)	DMF	60 °C	23%
2	2.0 eq	Ag_2O (2.5)	MeCN	60 °C	41%
3	2.0 eq	AgF (2.5)	MeCN	60 °C	41%
4	2.0 eq	Ag_2CO_3 (2.5)	MeCN	60 °C	71%
5	2.0 eq	Ag_2CO_3 (1.5)	MeCN	60 °C	72%
6	1.5 eq	Ag_2CO_3 (1.5)	MeCN	60 °C	75%
7	1.5 eq	Ag_2CO_3 (1.0)	MeCN	60 °C	72%
8	1.5 eq	Ag_2CO_3 (0.5)	MeCN	60 °C	41%
9	1.5 eq	Ag_2CO_3 (1.0)	MeCN	25 °C	traces
10	1.5 eq	Ag_2CO_3 (1.0)	MeCN	80 °C	80%
11	1.5 eq	Ag_2CO_3 (1.0)	MeCN	100 °C	61%

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

Initial experiments with 4-*tert*-butylphenylboronic acid in the presence of $Pd(dppf)Cl_2$ and Ag_2O afforded the desired product **2** in 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 similar trends to those noted by Fox and co-workers[7] and found that Ag_2O (41%), AgF (41%) or Ag_2CO_3 (71%) (Table 1, entries 2 – 4) 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 literature.[18] The amounts of Ag_2CO_3 and boronic acid were minimized and 1.0 eq and 1.5 eq, respectively, 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-fluorophenyl- (**3**, 32%) and other boronic acids identified electron poorer 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 side reactions.[18,20] We envisioned optimization of the temperature and the catalytic system to 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 80% yield. Performing the reaction at even higher temperatures (100 °C) resulted in 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). Hence, we hypothesized that 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 electron-deficient character.[23] In contrast, 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 posed viable substrates, while their electron-poorer alternatives resulted in diminished yield. With this in mind, we hypothesized that for a series of dppf analogs, which vary electronically, a clear trend in reactivity should be observed.

Table 2. Investigation of the catalytic system



1

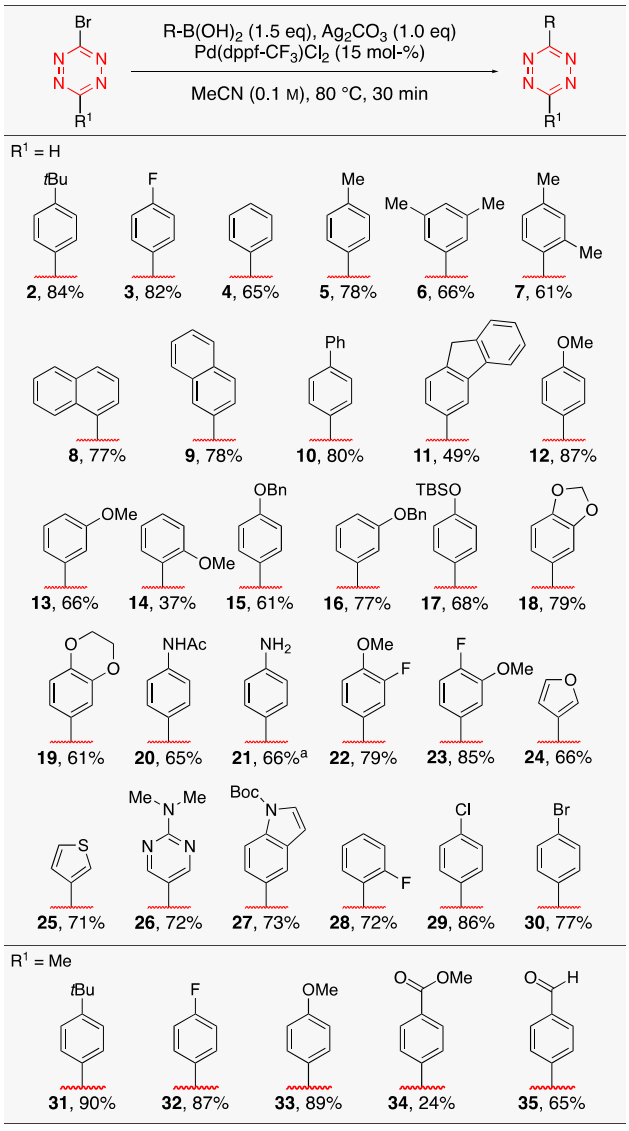
entry	R	catalyst	yield[a]
1	4-*t*Bu-Ph-	$Pd(PPh_3)_2Cl_2$	9%
2	4-*t*Bu-Ph-	DPEphos,$PdCl_2$	48%
3	4-*t*Bu-Ph-	BINAP,$PdCl_2$	41%
4	4-*t*Bu-Ph-	$Pd(dtbpf)Cl_2$	34%
5	4-*t*Bu-Ph-	$Pd(dippf)Cl_2$	85%
6	4-*t*Bu-Ph-	$Pd(dppf\text{-}CF_3)Cl_2$	84%
7	4-F-Ph-	$Pd(dtbpf)Cl_2$	15%
8	4-F-Ph-	$Pd(dippf)Cl_2$	31%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

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

Thus, we investigated the di-*tert*-butylphosphino- (dtbpf), diisopropylphosphino- (dippf), and di(CF_3)phenylphosphino (dppf-CF_3) variants of dppf for both the 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, reduction of the catalyst loading was investigated (Table S5). As expected, coupling of the electron-richer 4-tert-butylphenylboronic acid tolerated lower catalyst loading (5 mol-%, 83% yield) than coupling of the electron-poorer 4-fluorophenylboronic acid (10 mol-%, 79% yield). Finally, at 1.24 mmol scale and 5 mol-% catalyst loading, the method produced the desired product in 83% yield (Table S5). However, when the scope was investigated, it was found non-trivial to properly estimate the minimal tolerated catalyst loading. Hence,

all presented substrates were prepared using 15 mol-% of catalyst.

Scheme 2. Conditions and scope of the cross-coupling reaction



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

With 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 cross-coupling cleanly and tolerated substituents in the *para*- (**2** (84%), **5** (78%)), *meta*- (**6** (66%)), and *ortho*-positions (**7** (61%)). Polyaromatic systems, such as naphthyl- (**8** (77%), **9** (78%)), biphenyl- (**10** (80%)), and fluorenyl-substituted tetrazines (**11**, (49%)) were obtained in good to excellent yields. Various methoxy- (**12** (87%), **13** (66%), **14** (37%)) and benzyloxyphenylboronic acids (**15** (61%), **16** (77%)) posed 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, reduction of the nucleophilic character, *i.e.* by Boc protection or acetylation (20 (65%)) was found crucial. This strategy allowed, after Boc-deprotection with TFA, the preparation of free amine **21** (66%). Further, substituents of opposite electronic nature were also well tolerated and produced tetrazines **22** and **23** in 79% and 85% yield, 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 prepared in synthetically useful yields. Along the same lines, fluoro- (**3** (82%), **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 viable substrates. The coupling between **1** and 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%), **33** (89%)[7]). Even more demanding substrates afforded the respective products, such as methyl ester **34** (24%) and aldehyde **35** (65%).[7]

We then investigated the rates of mono- vs. disubstituted tetrazines in iEDDA reactions. To follow the progress of the iEDDA reaction, we monitored the disappearance of the typical tetrazine absorbance band around 540 nm.[27] This band is not present in the corresponding iEDDA products and thus the accompanying color change from pink/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 non-linear least squares fit method. For the monosubstituted tetrazine **2**, a value for $k = 143.6\ M^{-1}s^{-1}$ was measured, while the disubstituted tetrazine **31** was roughly 70-fold slower ($k = 2.097\ M^{-1}s^{-1}$). For a proper comparison, **3** and **32** were analyzed by the same method. A 60-fold faster rate constant of $k = 153.9\ M^{-1}s^{-1}$ was measured for the monosubstituted tetrazine and $k = 2.484\ M^{-1}s^{-1}$ for the disubstituted reference. Therefore, a significant acceleration of the rate for mono- versus disubstituted tetrazines is observed for the iEDDA reactions, already for small substituents such as a Me group at the 6-position.

In conclusion, a method for the mild and fast Pd-catalyzed carbon-carbon bond formation between a mono-substituted tetrazine and an array of aryl boronic acids is reported. 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 mono-functionalized tetrazines via mild and selective cross-coupling. Kinetic measurements of the iEDDA reaction of these monosubstituted tetrazines and comparison to 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 on the ACS Publications website.

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

CCDC-2080087-2080090 contain the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/structures.

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Notes

The authors declare no competing financial interest.

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