Application of the copper catalysed *N*-arylation of amidines in the synthesis of analogues of the chemical tool, blebbistatin†

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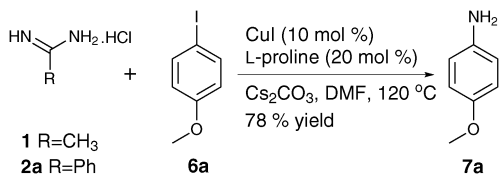
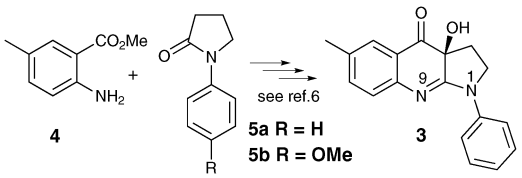
A robust protocol for the CuI-catalysed arylation of amidines is presented. Whilst the initially identified conditions were useful for benzamidine-derived substrates, difficulties were encountered with more complex substrates. This problem was overcome following a change in ligand type, enabling the synthesis of analogues of the chemical tool, blebbistatin.

Considerable advances have been made in the copper-catalysed Ullmann *N*-arylation reaction due to the large demand for *N*-arylated heterocycles in natural product synthesis, chemical biology and drug discovery. Whilst examples of the Ullmann *N*-arylation of amines, anilines, amides, carbamates and *N*-containing heterocycles abound,[1,2] there are relatively few reports of the use of simple amidines. Exceptions include elegant work on the copper-catalysed arylation of amidines en route to various heterocyclic systems[3] and the functionalisation of several amidine-containing heterocycles.[4]

A recent report[5] described the use of amidine hydrochlorides in the copper-catalysed synthesis of arylamines with the amidine salt serving as an ammonia surrogate (Scheme 1). This useful reaction was proposed to occur *via* initial *N*-arylation of the amidine hydrochloride (**1** or **2a**) in the presence of CuI, L-proline and Cs_2CO_3. Hydrolysis of the resulting *N*-arylamidine by water, produced in the initial reaction of the amidine salt with Cs_2CO_3, delivers the desired aniline. Based on this mechanistic proposal, we reasoned that if water was removed, either by use of the free base of the amidine or by addition of molecular sieves, it should be possible to isolate the *N*-arylated amidine. Here we report the results of our attempts to optimise the conditions required to *N*-arylate amidine-containing substrates using Ullmann-type procedures. Our studies culminate in the synthesis of novel analogues of blebbistatin (**3**) (Scheme 2), an increasingly popular chemical tool used to study myosin function.[6] We have previously reported the synthesis of **3** and its analogues in a sequence starting from anthranilates such as **4** and lactam **5a**.[7]



Scheme 1 Reported[5] conversion of amidine salts to anilines.



Scheme 2 Previous approach to (*S*)-(−) blebbistatin (**3**).[7]

Initial studies focused on the reaction of acetamidine hydrochloride (**1**) with *p*-iodoanisole (**6a**). The only product isolated was the corresponding aniline, anisidine **7a** (Scheme 1), albeit in lower yield than previously reported (48% *vs.* 78%,[5] Table 1, entry 1). Repeating the reaction with the sole change of the addition of predried 4 Å molecular sieves only resulted in a decrease in the yield of **7a** (entry 2) with no additional products being isolated.

In line with the literature[5] we found that benzamidine hydrochloride (**2a**) was a less efficient substrate for the synthesis of aniline **7a** than **1** (entry 3). However, trace amounts of the desired amidine **8a** were also isolated from this reaction. Small molecule X-ray crystallographic and ^{1}H NMR analysis of **8a** was consistent with the tautomer shown in Table 1.† When predried 4 Å molecular sieves were added to this reaction, a 48% isolated yield of **8a** was achieved (entry 4). **8a** could also be prepared when commercially available benzamidine **2b** was used in place of its hydrochloride salt (entry 5). The presence of sieves improved the yield further to 45% (entry 6) and these conditions also enabled the synthesis of **8b** in 66% yield (entry 7). No reaction was observed in the absence of copper iodide, although a 24% yield of **8a** was obtained in the absence of ligand (entries 8 and 9). Changing the solvent used from DMF to toluene leads to a significant improvement in the reaction yields with **8a** and **8b** being prepared in 73% and 79%, respectively (entries 10 and 11). Attempts to use DMSO as the reaction solvent led to a decrease in yield of **8a** (entry 12). Finally, an attempt was made to reduce the catalyst loading. Whilst a reduction in the yield of **8a** from 73% (entry 10) to 60% (entry 13) was observed when 5 mol% copper iodide and 10 mol% L-proline were used, it was decided to explore the scope of the reaction by varying the aryl halide using this lower catalyst loading (entries 14–22). Under the chosen conditions, a preference for electron-donating groups at the 4-position of the iodide was observed. Strong electron-withdrawing groups such as 4-nitro were not well tolerated at this position,[3a] but substrates containing the relatively weakly electron-withdrawing chloro-group at either the 4- or 3-position

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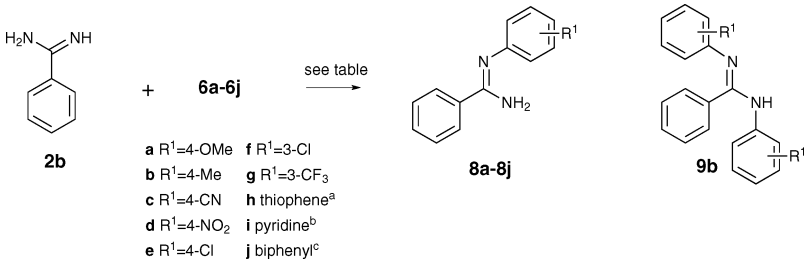
† Electronic supplementary information (ESI) available: Experimental procedures and spectroscopic characterisation for all new compounds are provided. CCDC 791559–791561 (**8a**, **8b**, **9b**). For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c0cc03624b

Table 1 Amidine *N*-arylation

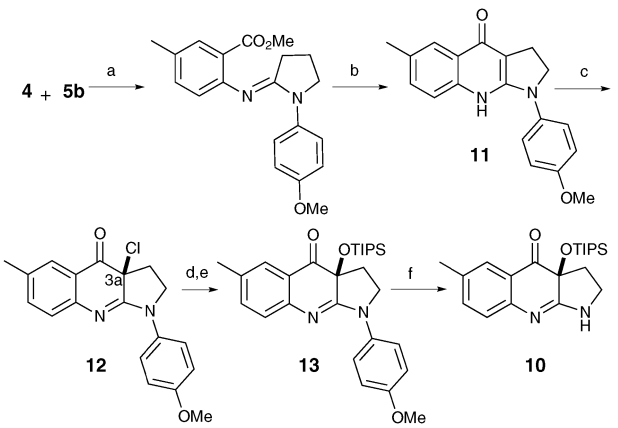


Entry	Amidine	Halide **6**[e]	CuI/mol%	L-proline[f]/mol%	Solvent	Yield of **8** (and **7a**)
1	**1**	**6a**	10	20	DMF	0 (48[d])
2	**1** + Sieves	**6a**	10	20	DMF	0 (28[d])
3	**2a**	**6a**	10	20	DMF	Trace (29[d])
4	**2a** + Sieves	**6a**	10	20	DMF	48
5	**2b**	**6a**	10	20	DMF	37
6	**2b** + Sieves	**6a**	10	20	DMF	45
7	**2b** + Sieves	**6b**	10	20	DMF	66
8	**2b** + Sieves	**6a**	0	20	DMF	0
9	**2b** + Sieves	**6a**	10	0	DMF	24
10	**2b** + Sieves	**6a**	10	20	Toluene	73
11	**2b** + Sieves	**6b**	10	20	Toluene	79
12	**2b** + Sieves	**6a**	10	20	DMSO	49
13	**2b** + Sieves	**6a**	5	10	Toluene	60
14	**2b** + Sieves	**6b**	5	10	Toluene	69
15	**2b** + Sieves	**6c**	5	10	Toluene	41
16	**2b** + Sieves	**6d**	5	10	Toluene	17
17	**2b** + Sieves	**6e**	5	10	Toluene	50
18	**2b** + Sieves	**6f**	5	10	Toluene	53
19	**2b** + Sieves	**6g**	5	10	Toluene	36
20	**2b** + Sieves	**6h**	5	10	Toluene	37
21	**2b** + Sieves	**6i**	5	10	Toluene	52
22	**2b** + Sieves	**6j**	5	10	Toluene	21
23	**2b** + Sieves	*p*-Bromo-toluene	5	10	Toluene	46
24	**2b** + Sieves	*p*-Chloro-toluene	5	10	Toluene	0

[a] 2-Iodothiophene. [b] 4-Iodopyridine. [c] 4-Phenyl-iodobenzene. [d] Isolated yield of **7a**. [e] 1.2 eq. with respect to amidine. [f] Difficulties in dissolving all the proline and base were experienced under the reaction conditions.

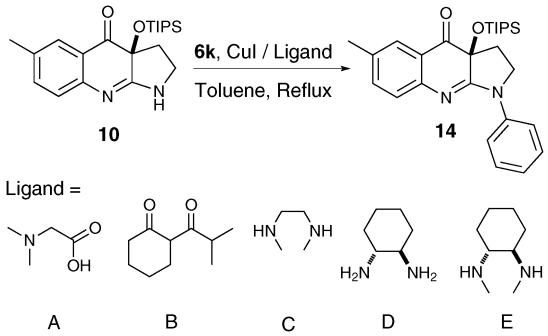
led to the desired amidines in moderate yields. Heteroaromatic iodides were also shown to work (entries 20 and 21). No attempt was made to optimise further the yields of these transformations. During the course of these experiments, a small amount of an additional product was frequently observed. In one case, this product was isolated and shown to be the diarylated amidine **9b** (Table 1) from the reaction with **6b**. The structure of **9b** was confirmed by X-ray crystallographic analysis.[3a] † The synthesis of **8b** using *p*-bromo-toluene was also successful although *p*-chloro-toluene did not work under these conditions (entries 23 and 24).

Having demonstrated that it was possible to prepare *N*-arylated benzamidines using this approach, our focus turned to the blebbistatin system (Schemes 2 and 3). Detailed inspection of the recently reported X-ray crystal structure of (*S*)-(−)-blebbistatin (**3**) bound to non-muscle myosin II from *D. discoideum* showed that whilst some 75% of **3** is enclosed within a glove like binding pocket, the *N*1-phenyl ring in **3** protrudes out of the main binding site.[8] The synthesis of alternative *N*1-arylated analogues of **3** may therefore lead to an increase in inhibitor potency or induce selectivity within the myosin family. In our previous approach to **3** and its analogues (Scheme 2), the *N*1-substitutent was incorporated *via* lactam **5** early in the synthesis. The potential for the late stage incorporation of a range of *N*1-aryl or heteroaryl groups was therefore appealing. The required substrate for these studies, amidine **10** (Scheme 3), was prepared in racemic form in 6 steps from methyl 4-methyl anthranilate (**4**). Key steps in this sequence included the C3a-chlorination[9] of quinolone **11** to



Scheme 3 *Reagents and conditions*: (a) **5b** (1.2 eq.), $POCl_3$ (1.2 eq.), DCM, RT, 3 h then **4** (1.0 eq.), reflux 18 h, 91%; (b) LiHMDS (2.5 eq.), THF, −78 °C to 0 °C, 3 h, 89%; (c) dichloroisocyanuric acid (0.5 eq.), THF : H_2O (1 : 1), RT, 4 h, 91%; (d) aq. NaOH (1.85 eq.), THF : H_2O (1 : 1), 18 h, 52%; (e) TIPSOTf (3.0 eq.), $^{i}Pr_2NEt$, (4.0 eq.), reflux, 6 h, 80%; (f) CAN (4.5 eq.), MeCN : H_2O (1 : 1), 8 h, 50%.

Table 2 *N*1-arylation of **10**: ligand screen



Entry	Solvent	Ligand type	CuI/ mol%	Ligand/ mol%	Temperature/ °C	Yield (%)
1	Toluene	L-Proline	10	20	110	—
2	DMSO	L-Proline	10	20	80	—
3	Toluene	A	10	20	110	—
4	Toluene	B	10	20	110	—
5	Toluene	C	10	20	110	7
6	Toluene	D	10	20	110	25
7	Toluene	E	10	20	110	80
8	Toluene	E	5	10	110	47

give **12** and the CAN-mediated PMP deprotection of **13** to give **10**, both of which proceeded in reasonable yields. Unfortunately, all our attempts to *N*1-arylate **10** using the conditions optimised for synthesis of, for example, **8b** (Table 1, entry 11), failed to give **3** or its *N*9-arylated isomer. In the light of this result, it was decided to resume our search for a catalyst system that would deliver successful *N*-arylation of both benzamidine **2b** and **10**. Table 2 summarises the results of a limited ligand screen carried out on the reaction of **10** with iodobenzene (**6k**). The ligands were selected based on their use in previous Ullmann-type procedures.[10–12]

Whilst none of the desired product **14** was observed with amino acid-based ligands[10] (Table 2, entries 1–3) or the β-diketone ligand B[11] (entry 4), limited success was observed with the diamine ligand C[12] (entry 5). Use of *trans*-diaminocyclohexyl-based ligands[12] resulted in further improvements in yield (entries 6 and 7) enabling the synthesis of **14** as a single regioisomer in 80% yield with a catalyst loading of 10 mol%. A reduction in yield was observed when a lower catalyst loading was used (entry 8). The protocol using ligand E followed by desilylation of the resulting crude products was then applied to the synthesis of blebbistatin analogues **3a,b,e,g–j** by reaction of **10** with a range of aryliodides (Table 3).

The newly developed protocol proved reasonably robust with a series of novel analogues being prepared in moderate to good yields. Again, a drop off in the yields at lower catalyst loading and a preference for the use of electron-rich iodides were observed. To date no evidence for the formation of the *N*9-isomer of **3a,b,e,g–j** has been observed in these reactions. When this protocol was applied to the synthesis of a number of the benzamidines described in Table 1 (see Table S1, ESI† for details) analogous reactivity was observed although the reaction yields were lower.

Table 3 *N*1-arylation of **10**: halide screen

Entry	Halide[a]	CuI/mol%	Ligand E/mol%	Product	Yield (%)
1	**6a**	10	20	**3a**	66
2	**6b**	10	20	**3b**	63
3	**6e**	10	20	**3e**	66
4	**6g**	5	10	**3g**	19
5	**6h**	10	20	**3h**	53
6	**6i**	10	20	**3i**	71
7	**6j**	5	10	**3j**	34

[a] See Table 1 for structures of aryliodides.

In summary, a novel approach to analogues of the small molecule tool blebbistatin **3** has been developed. This approach enables the facile introduction of *N*1-aryl substituents providing a flexible and rapid route to modifying a key region of this bioactive molecule. Whilst initial studies focused on the modification of a literature protocol,[5] these conditions proved applicable only to reactions involving simple amidine synthesis. A change in the catalyst ligand was required to enable successful reaction of the more complex substrate **10**. Whilst it is difficult to provide a clear rationalisation as to why the two systems behaved so differently it is possible that the steric environment around the amide functional group in **10** plays a role. Assessment of the biological activity of the new analogues of **3** is ongoing and will be reported in the near future.

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