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FULL PAPER

A Buchwald-Hartwig Protocol to Enable Rapid Linker Exploration of Cereblon E3-Ligase PROTACs

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Abstract: A palladium-catalysed Buchwald-Hartwig amination for lenalidomide-derived aryl bromides was optimised using high throughput experimentation (HTE). The substrate scope of the optimised conditions was evaluated for a range of alkyl- and aryl-amines and functionalised aryl bromides. The methodology allows access to new cereblon-based bifunctional proteolysis targeting chimeras with a reduced step count and improved yields.

Introduction

Immunomodulatory imide drugs (IMiDs) such as thalidomide (**1**) have been used since the late 1950s, including the treatment of nausea in pregnant women.[1] While thalidomide was removed from the market in 1961 due to teratogenicity,[2] it was subsequently found to be a useful treatment for leprosy.[3] Interest in the immuno-modulatory properties of **1**[4] resulted in further clinical studies and it was shown to be effective in the treatment of multiple myeloma (MM) in 1999 and approved for use in MM by the US Food and Drug Administration in 2006.[2, 5] This success led to the development of more potent analogues such as lenalidomide (**2**) and pomalidomide (**3**), that were approved for MM in 2005 and 2013 respectively.[6]

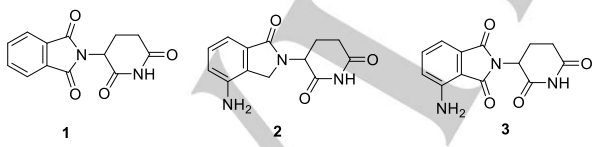

Figure 1. Structures of IMiD drugs thalidomide (**1**), lenalidomide (**2**) and pomalidomide (**3**).

The biological targets of the IMiD drugs were initially unknown. One protein that thalidomide binds to is cereblon (CRBN), which is believed to be responsible for the teratogenicity of the drug;[2] at least partially driven by the degradation of SALL4[7] and p63.[8] CRBN associates with DBB1-CUL4A protein complex, an E3 ubiquitin ligase.[9] One of the many functions of ubiquitin ligases is the labelling of proteins for degradation by the 26S proteosome through linear ubiquitination of K48.[10] In the last decade there has been considerable interest in hijacking the ubiquitination function of E3 ligases to target specific proteins for degradation. One widely used approach involves a heterobifunctional molecule joining an E3 ligase binder, such as CRBN, to another protein-of-interest (POI) binder, via a chemical linker. In many cases degradation of the POI is observed. These compounds are known as proteolysis targeting chimeras (PROTACs) and have used a number of different E3 ligases such as MDM2,[11] Von Hippel-Lindau (VHL)[12] and CRBN.[13] CRBN based PROTACs are of considerable interest since IMiDs have the smallest molecular weight and the fewest hydrogen-bond donors (HBD) of the E3 ligase binders disclosed, which may improve the oral exposure of these new modalities.[14].

CRBN-based PROTACs have been demonstrated to degrade a range of targets such as Brd4, for example dBET1 (**4**)[13] and ARV-825 (**5**),[15] in addition to multiple kinases with TL12-186 (**6**),[16] Bruton's tyrosine kinase with P13I (**7**),[17] and RIPK2 with PROTAC 3 (**8**) (Figure 2).[18]

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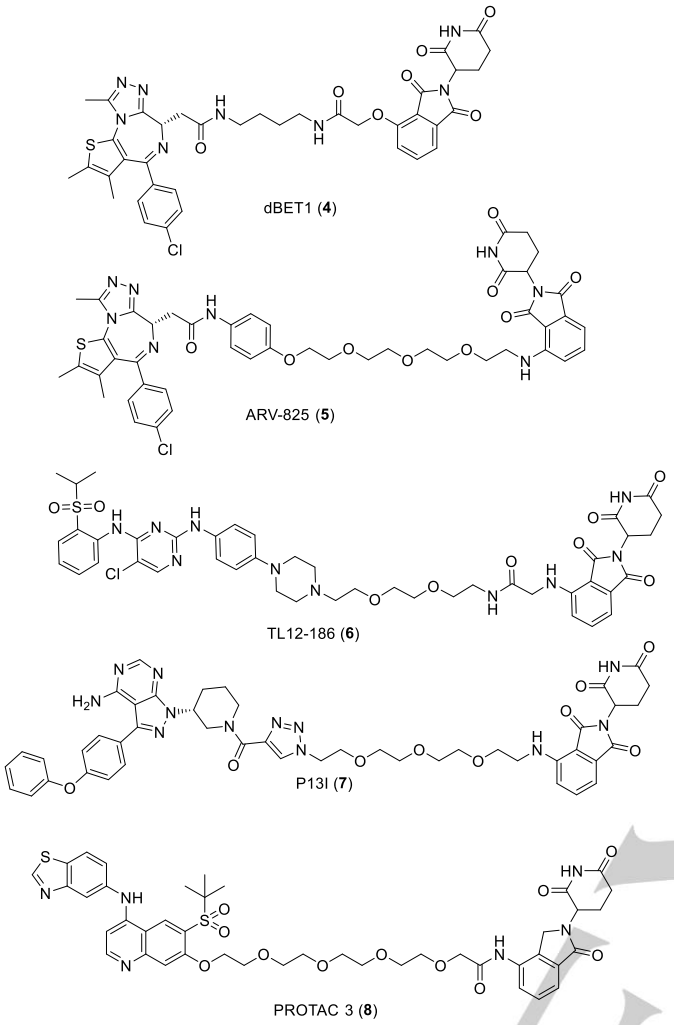


Figure 2. Structures of CRBN based PROTACs.

Published imide-based PROTACs include examples with linkers attached at either the 4- or 5-position of the phthalimide ring system. The compounds exemplified in Figure 2 have the linker attached at the 4-position of the IMiD. Within the patent literature there are an increasing number of examples with attachment at the 5-position of the IMiDs. Both C4 Therapeutics and Arvinas have attached the linkers of their PROTACs via the 5-position on both pomalidomide-based (**9**)[19] and lenalidomide-based CRBN binders (**10**[20] and **11**[21]) (Figure 3).

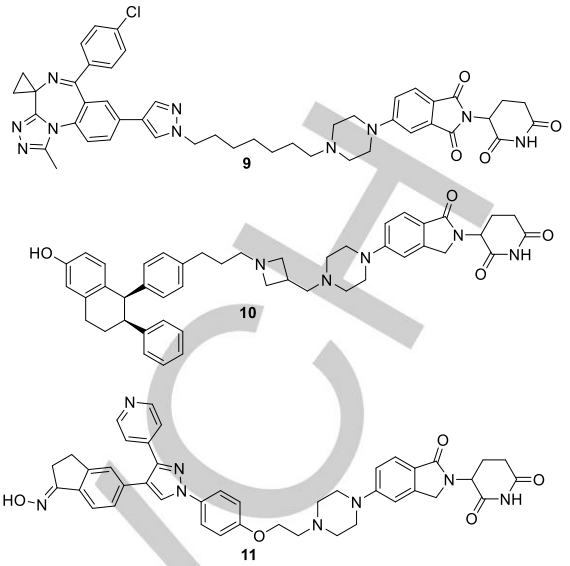
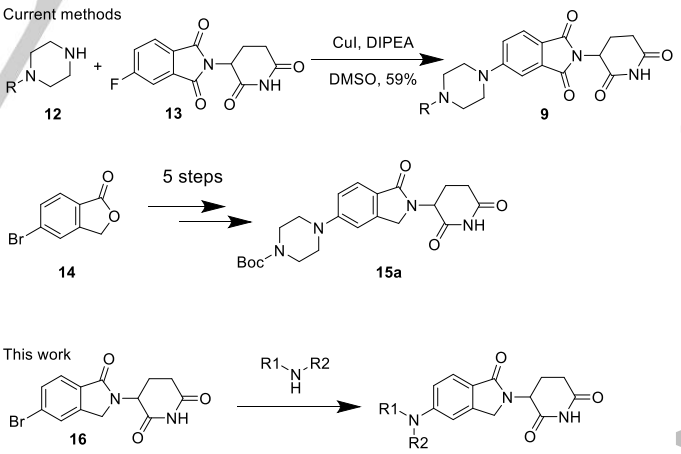


Figure 3. Structures of *N*-linked 5-substituted CRBN PROTACs.

The pomalidomide-based PROTACs, such as **9**, can be accessed in one step from 2-(2,6-dioxo-3-piperidyl)-5-fluoro-isoindoline-1,3-dione (**13**) using S_NAr methodology. Access to related lenalidomide-based PROTACs is less straightforward as nucleophilic aromatic substitution is no longer viable to introduce amines due to the reduced electrophilicity of the bicyclic ring system. Current methods involve a 5-step synthesis to key intermediate **15a**, with Boc-piperazine introduced in the first step and these lengthy syntheses hamper SAR exploration (Scheme 1).



Scheme 1. Methods to synthesise CRBN binders.

To enable our medicinal chemistry exploration of lenalidomide based CRBN PROTACs, we aimed to develop a concise and robust synthetic route that would provide rapid access to a diverse set of PROTAC linkers for SAR development. Our strategy was to install the amine fragment using a Buchwald-Hartwig amination and crucially perform this key coupling as the last step of the sequence, rather than the first, to facilitate exploration of structural diversity.

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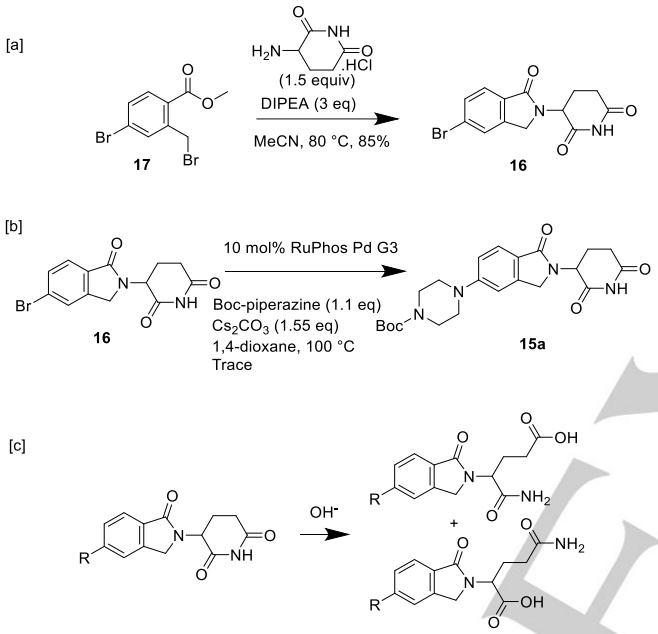
Results and Discussion

The key Buchwald-Hartwig precursor, bromide **16,** was prepared in a single step from commercially available starting material **17** (Scheme 2a). With this in hand, palladium-catalysed Buchwald-Hartwig coupling conditions were examined on **16** using Boc-piperazine (**18**), cesium carbonate and RuPhos Pd G3 but disappointingly only trace amounts of desired product were obtained (Scheme 2b). Accurate analysis of the reaction profiles by LCMS required the use of an acidic diluent, 5% acetic acid in acetonitrile. Dissolution of the analytical samples in methanol caused significant impurities with masses corresponding to hydrolysis of the glutarimide ring, presumably from trace amounts of aqueous base (Scheme 2c).



Scheme 2. [a] Synthesis of key bromide starting material **16** [b] Trial Buchwald-Hartwig conditions [c] Hydrolysis of glutarimide by aqueous base.

High throughput experimentation (HTE) was used for the rapid identification of improved reaction conditions, using minimal quantities of intermediates. A screen was run to compare the original RuPhos Pd G3 catalysed conditions with variation of catalyst and ligand, base, and solvent (Table 1, full screen data available in SI, Tables S1 and S2). For the HTE optimisation, conversion to **15a** (Conv) was determined by comparison of the peak areas of **15a** and **16** where 100 indicates complete consumption of **16** (Equation S1 in SI). The product to internal standard ratio (P/IS) was used to determine the degree of conversion to **15a.** P/IS was calculated by the peak area of **15a** being divided by the peak area of the internal standard, m-terphenyl, and normalised to 1 for the highest ratio per screen. In order to determine the success of a reaction both conversion of **16** and P/IS were considered. In examples where conversion was high and P/IS was poor, the major by-products observed were the hydrolysed glutarimides of both the starting bromide and the aminated product (Scheme 2c) in addition to unidentified minor impurities.

Table 1. Investigation of catalysts, solvents and bases

Entry [a]	Pd Catalyst / Ligand	Base[b]	Solvent [c]	Conv (%)	P/IS 17 h
1	RuPhos Pd G3	Cs_2CO_3	1,4-dioxane	22[d]	N/A
2	RuPhos Pd G3	Cs_2CO_3	^{t}AmOH	100	0.64
3	RuPhos Pd G3	Cs_2CO_3	DMF	62	0.61
4	BrettPhos Pd G3	Cs_2CO_3	DMF	0	0.00
5	DavePhos Pd G3	Cs_2CO_3	DMF	20	0.18
6	$Pd(OAc)_2$ / XantPhos	Cs_2CO_3	DMF	9	0.03
7	Pd-PEPPSI-IPent	Cs_2CO_3	DMF	100	1.00
8	RuPhos Pd G3	K_3PO_4	1,4-dioxane	0[d]	N/A
9	RuPhos Pd G3	NaO^{t}Bu[e]	1,4-dioxane	100	0.45
10	RuPhos Pd G3	LiHMDS[e]	1,4-dioxane	74	0.41

[a] ArBr (1 eq), *N*-Boc-piperazine (1.3 eq), cat / L (10 mol%), 80 °C, glove box [b] 3 eq unless otherwise stated, [c] 30 volumes, [d] 21 hours [e] 2 eq.

The initial reaction conditions afforded partial conversion to product after 21 hours (Entry 1). Switching to more polar solvents, *tert*-amyl alcohol and DMF, enabled more rapid consumption of bromide **16** than in 1,4-dioxane (Entries 2 and 3). Solubility studies revealed that **16** is fully soluble in DMF and sparingly soluble in *tert*-amyl alcohol at 80 °C in 30 volumes of solvent (SI Table S4). Solubility was likely not a contributing factor to the rate of reaction. Entries 3 – 7 compared a variety of catalysts and while most saw some conversion, BrettPhos Pd G3 was unable to catalyse the desired reaction (Entry 4). DavePhos Pd G3 (Entry 5) and $Pd(OAc)_2$ / Xantphos (Entry 6) showed less conversion after 17 hours than RuPhos Pd G3. The standout result used Pd-PEPPSI-IPent an *N*-heterocyclic carbene containing ligand,[22] which showed complete conversion after 17 hours and with the greatest P/IS (Entry 7). Using K_3PO_4 as a base gave no desired product (Entry 8). The use of stronger bases gave high levels of conversion compared to Cs_2CO_3, although modest P/IS (Entries 9 and 10).

Table 2. Optimisation with Pd-PEPPSI-IPent

Entry [a]	Base[b]	Solvent [c]	Conv 3 h (%)	P/IS 3 h	Conv 21 h (%)	P/IS 21 h
1	Cs_2CO_3	DMF	34	0.72	100	0.85
2[d]	Cs_2CO_3	DMF	31	0.70	100	0.96
3[e]	Cs_2CO_3	DMF	30	0.67	100	0.93
4	NaO^{t}Bu[f]	DMF	100	0.65	100	0.63
5	Cs_2CO_3	DMF[g]	89	0.96	100	0.57
6	Cs_2CO_3	DMF[h]	93	0.98	95	1.00

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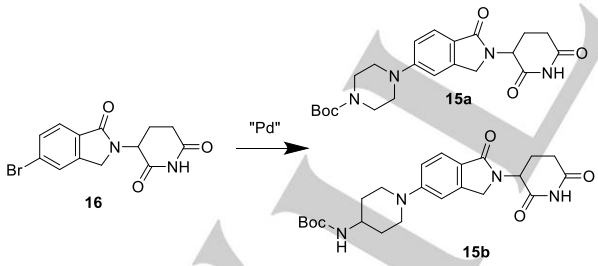
7	Cs_2CO_3	2-MeTHF	74	0.91	89	0.74
8	Cs_2CO_3	1,4-dioxane	73	1.00	81	0.63
9	Cs_2CO_3	CH_3CN	13	0.37	100	0.48
10	Cs_2CO_3	^{t}AmOH	100	0.93	100	0.41

[a] ArBr (1 eq), *N*-Boc-piperazine (1.3 eq), Pd-PEPPSI-IPent (10 mol%), 80 °C, glove box [b] 3 eq unless otherwise stated [c] 30 vols unless otherwise stated, [d] 2 mol% cat, [e] 5 mol% cat, [f] 2 eq NaO^{t}Bu, [g] 20 vols, [h] 10 vols.

Given the excellent conversion and P/IS using Pd-PEPPSI-IPent, conditions were further optimised using this catalyst (Table 2, selected examples, full screen data available in SI Table S3). Reducing the level of catalyst loading from 10 mol% to 5 mol% or 2 mol% had no significant effect on reaction progression (Entries 1, 2 and 3). Use of strong base NaO^{t}Bu showed complete consumption of starting material within 3 hours, although at a reduced P/IS, suggesting side reactions also occuring (Entry 4). Running the reaction at higher concentration (Entries 5 and 6) increased conversion compared to 30 volumes at 3 hours (Entry 1). A variety of solvents afforded good consumption of starting material, although P/IS ratios were varied, especially on prolonged heating (Entries 7 – 10).

On scaling up the conditions optimised by HTE and performing the reaction outside of a glovebox, it was found that the temperature needed to be increased to >90 °C for efficient cross-coupling to take place. Additionally, using DMF as a solvent made extraction during aqueous work ups more difficult compared with 1,4-dioxane. Therefore, we sought to further optimise the amination with a focus on nucleophile diversity by assessing a range of similar, highly sterically hindered PEPPSI precatalysts (Scheme 4). Pd-PEPPSI-IHept,[23] Pd-PEPPSI-IPent^{Cl},[24] Pd-PEPPSI-IHept^{Cl},[25] and (DiMeIHept^{Cl})Pd(cinnamyl)Cl[26] with both Boc-piperazine (**18**) and (*N*-Boc-4-amino)piperidine (**19**) were evaluated (Table 3).



Scheme 3. Reaction with Boc-piperazine (**18**) and (*N*-Boc-4-amino)piperidine (**19**).

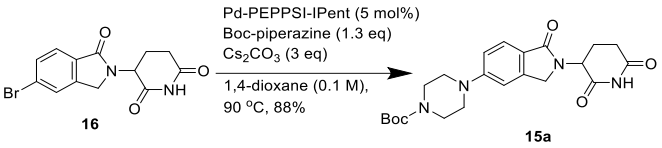
Table 3. Variation of PEPPSI catalyst and substrates

Entry[a]	Pd-PEPPSI Catalyst	Substrate	Product	Yield (%)[b]
1	IPent	**18**	**15a**	44
2	IPent	**19**	**15b**	51
3	IHept	**18**	**15a**	42
4	IHept	**19**	**15b**	33
5	IPent^{Cl}	**18**	**15a**	63
6	IPent^{Cl}	**19**	**15b**	67
7	IHept^{Cl}	**18**	**15a**	70
8	IHept^{Cl}	**19**	**15b**	76
9	IHept^{Cl}[c]	**18**	**15a**	29
10	DiMeIHept^{Cl}	**18**	**15a**	38
11	DiMeIHept^{Cl}	**19**	**15b**	35

[a] ArBr (1 eq), amine (1.3 eq), catalyst (5 mol%), cesium carbonate (3 eq) 1,4-dioxane (0.1 M), 100 °C, [b] Isolated yield, [c] 2.5 mol% catalyst.

These small scale reactions demonstrated the clear superiority of the more electron-deficient PEPPSI^{Cl} catalysts compared to the ligands without the Cl on the backbone with both amines (Entries 1 – 4 vs 5 – 8). We attribute the lower conversions using DiMeIHept^{Cl} (Entries 10 and 11) compared to the IPent^{Cl} and IHept^{Cl} to the increased and less flexible steric bulk of the ligand. On the basis of achieving higher isolated yields with Pd-PEPPSI-IHept^{Cl}, this catalyst was chosen for further investigation. Attempts were made to lower the catalyst loading from 5 mol%, and while desired products were observed using these conditions, they did not enable full consumption of the starting material (Entry 9). Therefore, we identified our optimised conditions as 5 mol% Pd-PEPPSI-IHept^{Cl}, 3 equivalents of dried cesium carbonate in degassed dioxane at 0.1 M with 1.3 equivalents of amine (Entries 7 and 8).

To demonstrate the utility of the Buchwald-Hartwig approach to produce large-scale quantities of intermediates, the coupling was performed on 19 g of bromide **16,** affording **15a** in an excellent 88% isolated yield (Scheme 4).[27]



Scheme 4. Large scale preparation of **15a**.

With our newly developed conditions in hand, we were keen to expand the substrate scope of the amination beyond **15a** and **15b**. Using the optimised Pd-PEPPSI-IHept^{Cl} conditions a wide variety of amines were found to be competent in the cross-coupling with bromide **16**. Complete consumption of the starting material usually allowed isolation of the clean products by trituration after 3 hours (Figure 4).

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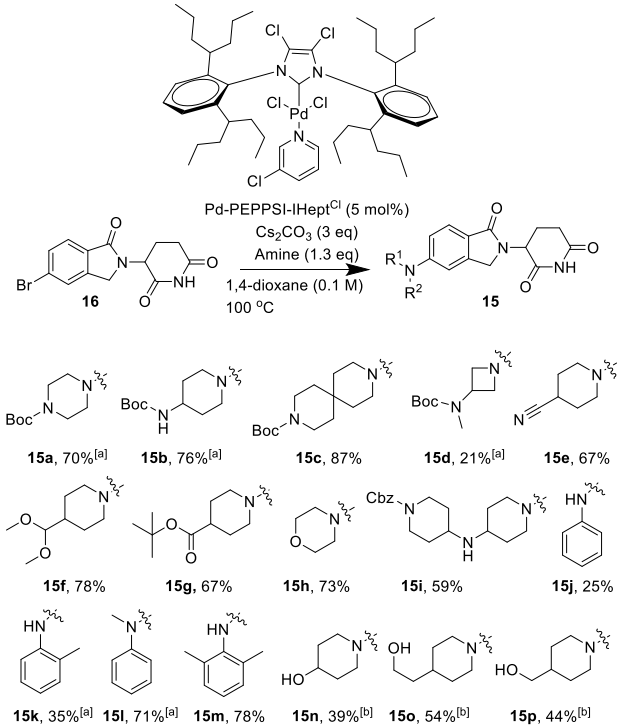


Figure 4. Substrates allowing full conversion, isolated by trituration unless otherwise stated. [a] Isolated by column chromatography, [b] 3 eq of amine.

Beyond Boc-protected amines (**15a-d**), amines bearing nitrile (**15e**), acetal (**15f**), ester (**15g**) and ether (**15h**) substituents were excellent coupling partners in this transformation. A di-basic substrate formed desired product (**15i**) coupling *via* the less sterically hindered nitrogen. In addition, anilines, including primary anilines and *ortho*-substituted anilines (**15j-m**) resulted in full conversion of bromide **16**. Alcohol containing amines proved more challenging. However, increasing the amine loading from 1.3 to 3 equivalents allowed for full conversion to the alcohol containing desired products (**15n-p**).

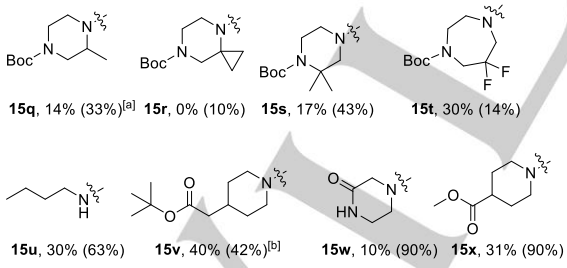


Figure 5. Substrates showing partial conversion. First value is isolated yield, value in parentheses is LCMS conversion [a] 3 eq of amine, Pd-PEPPSI-IPent^{Cl} (5 mol%), [b] Pd-PEPPSI-IHept^{Cl} (10 mol%).

More sterically hindered amines also proved challenging to couple. The alpha-methylpiperazine **15q** underwent poor conversion using Pd-PEPPSI-IHept^{Cl} and was eventually made in sufficient quantities for isolation by increasing the amine to 3 equivalents and using Pd-PEPPSI-IPent^{Cl}, consistent with our hypothesis that the increased conversion of the bulky amine is facilitated by using a less sterically hindered catalyst. Bulky substitution around the piperazine ring greatly reduced the conversion of bromide **16** to the desired products (**15r** and **15s**). Boc-3,3-difluorohomopiperazine underwent limited coupling to **15t**, presumably affected by the reduced nucleophilicity of the amine. Butylamine allowed partial conversion to **15u**. Unexpectedly, the *tert*-butyl ester gave only 42% conversion to **15v**, significantly lower than the other ester examples **15g** and **15x**. The coupling with piperazinone demonstrated a single product **15w**, coupling *via* the amine moiety rather than the amide. The conversion of **16** to **15w** was efficient, if not complete; isolation of the final product was difficult due to the hydrophilicity of the compound. Similarly, the final yield of **15x** was lower than expected given the conversion from **16**.

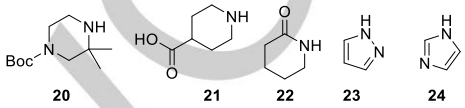
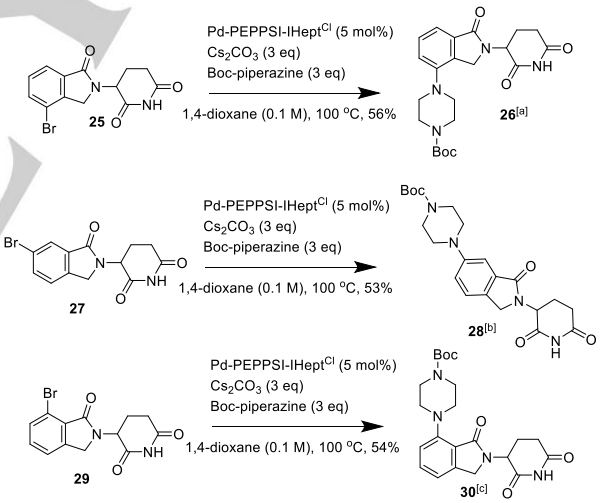


Figure 6. Substrates that gave no desired product.

Not all substrates showed conversion or gave desired products. Gem-dimethyl piperazine (**20**) was too sterically hindered to undergo reaction. Similarly, Buchwald-Hartwig couplings with piperidine-4-carboxylic acid (**21**), piperidinone (**22**), pyrazole (**23**) and imidazole (**24**) showed no evidence for the desired products.



Scheme 5. Reaction of other bromoisoindolinones. [a] 16 hours, [b] 5 hours, [c] recharged with catalyst after 24 hours and full conversion 2 hours subsequently.

Next, we investigated the Pd-catalysed coupling of Boc-piperazine to other bromoisoindolinones (Scheme 5). These gave desired products in moderate yields using our standard conditions and 3 equivalents of the amine, allowing exploration of vectors from all of the aromatic positions of the isolindolinone (**26**, **28** and **30**).

As a final test of the reaction conditions, we sought to make a potential PROTAC by introducing the isoindolinone E3-binder as a final step. A complex functionalised amine linker attached to JQ1(+)[28] **31** was prepared. Using our standard conditions, coupling of **31** with bromoisoindoline **16** was undertaken in 3.5

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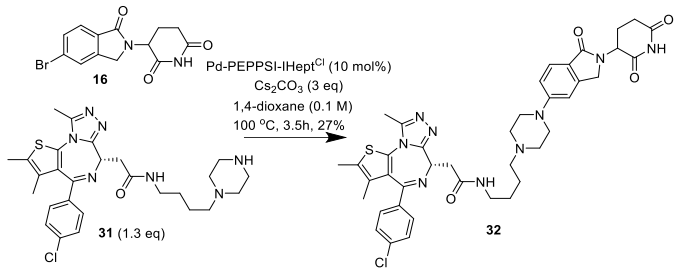
hours to provide a complete PROTAC-like molecule **32**.[29] This, to the best of our knowledge, is the first example of introducing a lenalidomide-like E3 ligase binder as a final step using a palladium-catalysed Buchwald-Hartwig amination protocol (Scheme 6).



Scheme 6. Buchwald-Hartwig coupling as a final step to synthesise a complete PROTAC.

Conclusion

We have reported a general method for the palladium-catalysed Buchwald-Hartwig coupling of amines to 3-(5-bromo-1-oxoisoindolin-2-yl)piperidine-2,6-dione (**16**) with a wide amine substrate scope. Additionally, we have demonstrated the ability to substitute all positions around the aromatic ring of the isoindolinone. Using these reaction conditions, we introduced for the first time a lenalidomide-based CRBN-binding moiety as a final step in the preparation of a potential PROTAC. We believe this methodology has utility in allowing rapid access to increasingly varied linkers to help identify PROTACs for *in-vitro* and *in-vivo* degradation of proteins through late stage diversification.

Experimental Section

Exemplar synthesis of *tert*-butyl (1-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-5-yl)piperidin-4-yl)carbamate (**15b**):

Pd-PEPPSI-IHept^{Cl} (0.030 g, 0.030 mmol) was added to 3-(5-bromo-1-oxoisoindolin-2-yl)piperidine-2,6-dione (**16**) (0.2 g, 0.62 mmol), cesium carbonate (0.605 g, 1.86 mmol) and 4-(*N*-Boc-amino)piperidine (**19**) (0.161 g, 0.80 mmol) in 1,4-dioxane (6 mL) at 20 °C under nitrogen. The resulting mixture was vacuum degassed, backfilling with nitrogen and stirred at 100 °C for 3 hours. The reaction mixture was diluted with DCM (15 mL) and washed sequentially with 5% AcOH in water (10 mL) and saturated brine (10 mL). The organic layer was dried with $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 5% MeOH in DCM. Pure fractions were evaporated to dryness to afford the title compound (0.208 g, 76%) as a beige powder. ^{1}H NMR (500 MHz, DMSO-d6): δ= 10.93 (s, 1H), 7.49 (d, *J*=8.5 Hz, 1H), 7.09 – 6.98 (m, 2H), 6.84 (d, *J*=7.5 Hz, 1H), 5.04 (dd, *J*=13.3, 5.1 Hz, 1H), 4.31 (d, *J*=16.9 Hz, 1H), 4.19 (d, *J*=16.9 Hz, 1H), 3.82 (d, *J*=13.1 Hz, 2H), 3.53 – 3.41 (m, 1H), 2.90 (t, *J*=12.7 Hz, 3H), 2.58 (dd, *J*=14.4, 3.1 Hz, 1H), 2.41 –2.30 (m, 1H), 1.99 –1.92 (m, 1H), 1.79 (d, *J*=10.4 Hz, 2H), 1.47 – 1.35 (m, 11H); ^{13}C NMR (126 MHz, DMSO-d6): δ=172.9, 171.3, 168.3, 154.9, 153.4, 144.1, 123.8, 120.9, 114.8, 108.4, 77.5, 51.4, 47.4, 46.9, 46.9, 31.3, 31.0, 28.3, 22.6; IR (ATR): 3361 (br), 3213 (br), 1676 (s), 1662 (s), 1614 (m), 1318 (m), 1225 (m), 770 (w) cm^{-1}; HR-MS(ESI): *m/z* calculated for $C_{23}H_{30}N_4O_5$ [M+H]+:443.2289, found: 443.2279.

Full experimental details can be found in the Supporting Information.

Acknowledgements

The authors are thankful for the advice of Prof Michael G. Organ on the storage and handling of PEPPSI catalysts. We express our gratitude to the analytical and structural chemistry group within AstraZeneca Oncology Chemistry, in particular to Paul Davey for the high resolution mass spectrometry, Eva Lenz for assistance with NMR spectrometry, and Daniel Rhodes and Joanna Raubo for the optical rotations.

Conflict of interest

The authors declare no conflict of interest.

Keywords: Amination • Cereblon • Drug Discovery • PEPPSI • PROTAC

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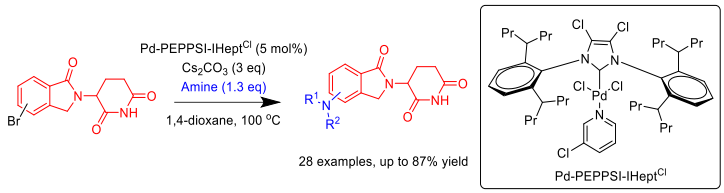

PROTAC assembly: A method to introduce diverse amines to lenalidomide-like scaffolds is described. The products from the Buchwald-Hartwig cross couplings could be used to access a range of PROTACs in a short number of subsequent steps. The coupling is effective from milligram to multigram scale.

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[29] The experiment was terminated prior to complete consumption of **16**, as analysis of the reaction mixture by LCMS revealed the formation of undesired by-products.