OL | Organic Letters

Cite This: Org. Lett. XXXX, XXX, XXX−XXX

Letter

pubs.acs.org/OrgLett

Chemoselective Synthesis of Lenalidomide-Based PROTAC Library Using Alkylation Reaction

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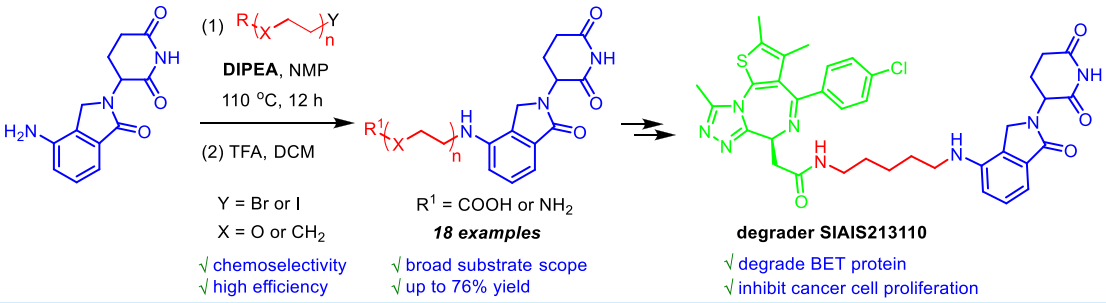
Supporting Information



ABSTRACT: An organic base-promoted chemoselective alkylation of lenalidomide with different halides was developed, which offers a novel approach to a highly functionalized lenalidomide-based PROTAC library under mild reaction conditions. DIPEA was found to act as an efficient base to trigger facile generation of arylamine alkylation products compared with inorganic bases. This library was successfully applied to BET PROTAC, which not only degraded BET protein but also effectively inhibited cancer cell proliferation.

Immunomodulatory drugs (Figure 1), including lenalidomide (**1a**), pomalidomide (**1b**), and thalidomide (**1c**), play an important role in the treatment, response, and prognosis of patients with multiple myeloma.[1] Cereblon (CRBN), a component of a cullin-RING ubiquitin ligase complex,[2,3] was identified as the target of **1c** and its derivatives, **1b** and **1a**. Mechanisms of action were confirmed by crystal structures of the DDB1−CRBN complex E3 ubiquitin ligase in complex with **1c**.[2] Recently, PROTAC-directed protein-specific degradation using bifunctional molecules (Figure 1) for the recruitment of E3 ubiquitin ligase complex was proven to be an efficacious therapeutic strategy, particularly, for cancer,[4] as illustrated by degradation of AR,[5] ERRα,[6] BET,[7] BCR-ABL,[8] BTK,[9] BRD9,[10] and EGFR.[11] CRBN ligands such as **1b** have been widely used as an E3 ubiquitin ligase ligand in PROTACs. It is reported that **1a** lenalidomide, as an E3 ligand in BRD9 degrader, displayed a better overall performance than **1b**.[10] Furthermore, the lack of the one phthalimide keto group makes **1a** more metabolically and chemically stable.[12] A powerful lenalidomide-based PROTAC library is therefore critical in identifying potent PROTAC degraders. However, no robust method has been reported to build such a quality library.

Recently, Bradner[10] reported a reductive amination of **1a** and alkyl aldehyde substituted amines to synthesize a lenalidomide-based CRBN ligand (**2a**), but the reactions require complex operations, lack generality, and suffer from low overall yield (46%). Li reported the synthetic method of **1a** derivative **3a** via

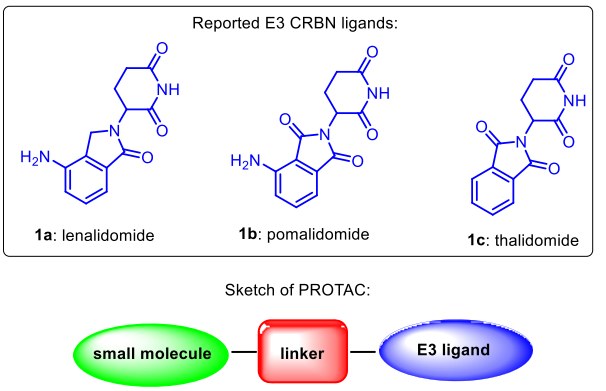


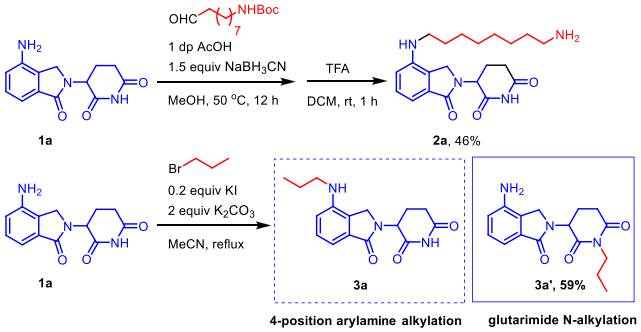
Figure 1. Reported E3 CRBN ligands and sketch of PROTACs

alkylation of **1a** and 1-bromopropane under inorganic base (K_2CO_3) conditions (Scheme 1).[13] To apply Li's alkylation method to efficiently construct the lenalidomide-based library, we repeated the reaction and identified that **3a′** was the major product instead of **3a** under the same conditions.[14a] Therefore, we attempted a new synthetic route using **4b** and different amine linkers as the starting materials for nucleophilic aromatic substitution reaction (S_NAr), which was successfully used to

Received: April 16, 2019

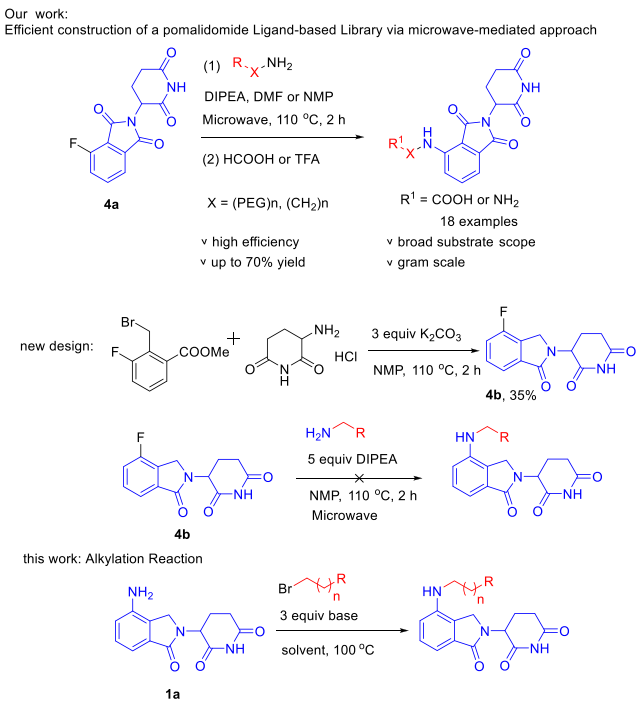
DOI: 10.1021/acs.orglett.9b01326
Org. Lett. XXXX, XXX, XXX−XXX

Scheme 1. Reported Methods for the Synthesis Lenalidomide Linkers



efficient construction of a pomalidomide-based library via a microwave-mediated approach (Scheme 2).[14b] Initially, we

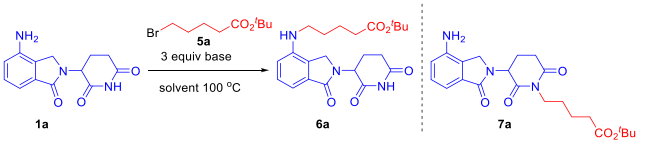
Scheme 2. Our Design and New Design



smoothly synthesized starting material **4b** by condensation reaction under K_2CO_3 conditions. However, in the next step, desired alkylation product was not observed under common S_NAr conditions, including microwave assistance and regular thermal conditions, and **4b** remained (Scheme 2). Furthermore, as **1a** is commercially available, we redesigned the conditions for optimizing alkylations to realize the construction of a variety of 4-position chemoselective alkylation instead of glutarimide N-alkylation products. Based on the above research, we designed a chemoselective synthesis of a lenalidomide-based PROTAC library using alkylation under organic base conditions, which led to a facile synthesis of PROTAC degraders. This is a generally efficient and useful method.

To test the feasibility of our hypothesis, *tert*-butyl 5-bromopentanoate **5a** was chosen as a model substrate for optimization of the reaction conditions. The results are shown in Table 1. Due to the efficient performance of DIPEA in various alkylation reactions, we first investigated the reaction of **1a** with **5a**

Table 1. Optimization of Reaction Conditions



entry	base	solvent	time (h)	yield of **6a** (%)[a]	yield of **7a** (%)[a]
1	DIPEA	NMP	12	74	7
2	TEA	NMP	12	40	7
3	NMM	NMP	12	32	2
4	DABCO	NMP	12	0	0
5	pyridine	NMP	12	9	1
6	DMAP	NMP	12	1	0
7	DBU	NMP	2	0	62
8	^{t}BuOK	NMP	2	0	89
9	^{t}BuONa	NMP	2	0	85
10	MeONa	NMP	2	0	86
11	LiHMDS	NMP	2	0	74
12	K_2CO_3	NMP	2	0	85
13	DIPEA	DMF	12	65	4
14	DIPEA	MeCN	12	65	5
15[b]	DIPEA	NMP	12	85	3

[a]HPLC yield. [b]reaction was carried out at 110 °C.

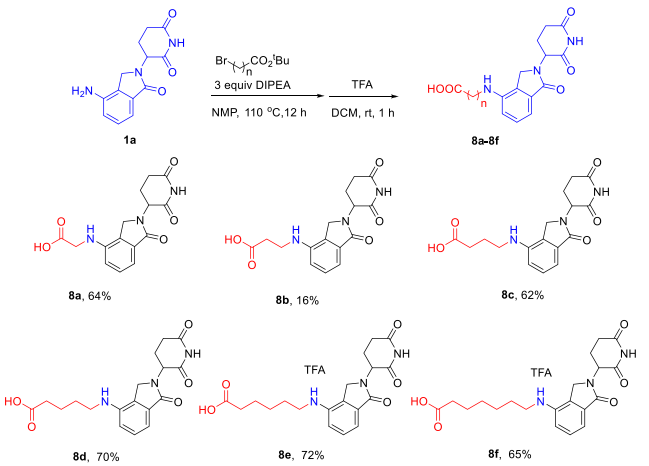
in the presence of 3 equiv of DIPEA in NMP at 100 °C for 12 h (Table 1, entry 1). Desired 4-position alkylation product **6a** was obtained in 74% isolated yield with 7% glutarimide N-alkylated **7a**. Other organic bases, such as Et_3N, NMM, DABCO, pyridine, and DMAP, were not effective in this reaction (entries 2−6); however, when DBU was used as the base, the major alkylation product of **7a** was gained in 62% yield, possibly due to the strong basicity of DBU (entry 7). Strong organic base or inorganic bases such as ^{t}BuOK, ^{t}BuONa, MeONa, LiHMDS, and K_2CO_3, which were proven to be efficient reagents in alkylation, afforded only traces of the desired product **6a** but with 74−89% yield of **7a** (entries 8−12). However, when MeCN or DMF was used as the solvent, only 65% of **6a** was formed (entries 13 and 14). Increasing the reaction temperature to 110 °C resulted in a higher product yield of 85% with a same reaction time (entry 15). It was then realized that the chemoselective favored 4-position instead of glutarimide alkylation used different amine linkers and **1a** as the materials under metal-free organic base conditions.

Having optimized the reaction conditions of the alkylation, we turned our attention to the scope of this method to build the lenalidomide-based PROTAC library. A broad range of alkyl bromides with different lengths bearing $-CO_2{}^t$Bu and −NHBoc functional groups were well-tolerated under the optimized reaction conditions (Table 1, entry 15), affording alkylated products in good yields. After hydrolysis under TFA conditions, a series of PROTAC precursors were efficiently synthesized (Schemes 3 and 4). **8b** was obtained in moderate yield due to the possibility of a competitive elimination reaction. Furthermore, when the desired product was given directly by freeze-drying, it forms a TFA salt; however, when the desired product was purified by preparative HPLC, it exists as a free base. A designed terminal acid and amine CLB (CRBN ligand-based) library can be used to react with various small molecule drugs for preparation of PROTAC degraders to degrade different oncogenic proteins.

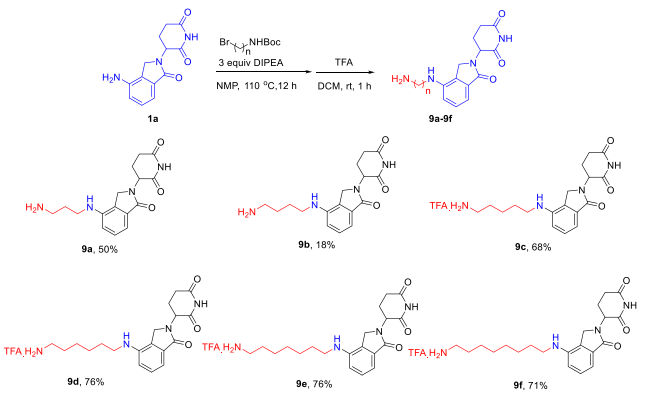
As the CLB library containing PEG parts is very useful in PROTAC degraders,[15] lenalidomide-based PROTAC precursors linked by $(PEG)_n$ were also designed. We first investigated the

DOI: 10.1021/acs.orglett.9b01326
Org. Lett. XXXX, XXX, XXX−XXX

Scheme 3. Synthesis of PROTAC Carboxylic Acid Precursors

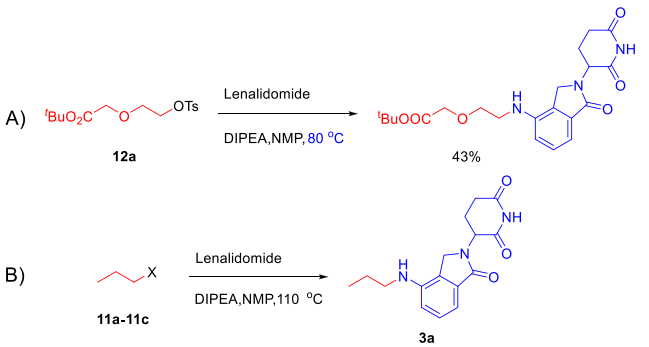


Scheme 4. Synthesis of PROTAC Amine Precursors



reactivity of 4-methylbenzenesulfonate **12a**. Only a trace of desired product was observed under the standard reaction condition, and 43% of desired product was obtained when the reaction was carried out at 80 °C (Scheme 5A). Alkyl iodide **11a** and alkyl bromide **11b** underwent smooth alkylation to furnish the desired product in excellent yields (Scheme 5B). Guided by these observations, we chose to convert OTs to I to facilitate the following alkylation, which turned out to be a robust protocol for the synthesis of PEG-containing PROTAC precursors **10a**–**10e**. Finally, a new lenalidomide-based PROTAC library containing PEG parts was efficiently constructed (Scheme 6).

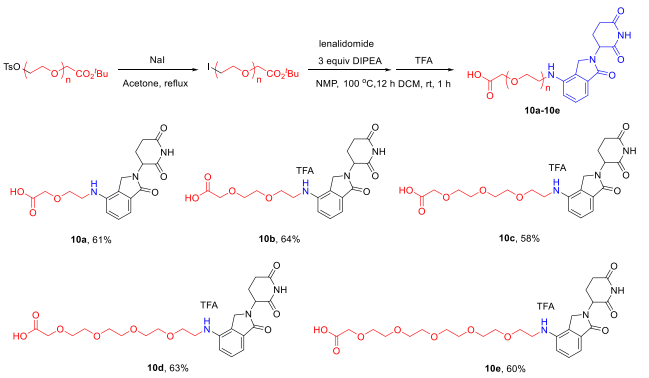
Scheme 5. Optimized Reaction Conditions for Lenalidomide-Based PROTAC Precursors Linked by $(PEG)_n$



entry	reactant	yield of **3a**(%)[a]
1	**11a** (X = I)	75
2	**11b** (X = Br)	84
3	**11c** (X = Cl)	no reaction.

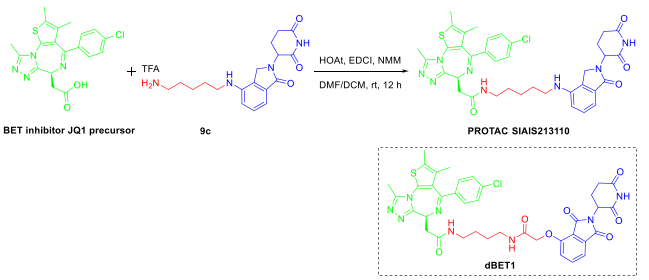
[a]HPLC yield.

Scheme 6. Synthesis of PROTAC $(PEG)_n$ Carboxylic Acid Precursors



We applied our library to produce PROTAC first to target the BET (bromodomain and extra-terminal) protein. BET is an epigenetic "reader" by binding to acetyl lysine residues in the histone tail and regulate gene expression and has been implicated in a variety of human diseases to drive cancer formation.[16] In this study, we mainly use JQ1-acid (generated from JQ1 by hydrolysis of *tert*-butyl ester) as the small molecule binder and **9c** as the linker to highly screen PROTAC SIAIS213110 to specifically target the BET protein (Scheme 7).

Scheme 7. Synthesis of BET PROTAC Degrader



Next, three cell lines (multiple myeloma cell line MM.1S, leukemia cell line MV-4-11, triple-negative breast cancer cell line MDA-MB-468) which overexpressed the BET protein were chosen to test the biological efficacy of the synthesized SIAIS213110 (Table 2). SIAIS213110 shows better inhibition of the proliferation compared to reported PROTAC dBET1[7a] and JQ1 in three different cancer cell lines. Moreover, SIAIS213110 degraded the target BET protein in a dose-dependent manner, 10-fold (DC_{50} < 0.5 nM vs 5.0 nM) better than with dBET1. As c-Myc

Table 2. Cell Proliferation Assay in Three Cell Lines

	IC_{50}[a] (nM) (mean ± SD)		
cell line	MM.1S	MV-4-11	MDA-MB-468
JQ1	54.2 ± 17.1	75.8 ± 12.7	501.8 ± 68.1
dBET1	115.3 ± 45.9	11.5 ± 1.6	863.7 ± 201.0
SIAIS213110	6.7 ± 0.5	0.9 ± 0.01	125.4 ± 11.6

[a]IC_{50} values were obtained from three independent experiments.

DOI: 10.1021/acs.orglett.9b01326
Org. Lett. XXXX, XXX, XXX−XXX

protein expression is regulated by BET proteins, the potent BET degrader SIAIS213110 downregulates the levels of c-Myc protein (Figure 2). This evidence collectively demonstrates that the BET-

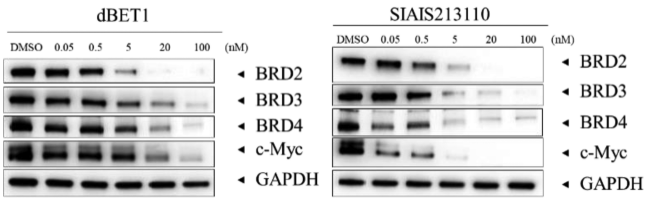


Figure 2. SIAIS213110 effectively degraded BET protein (Western blot is representative of two independent experiments).

targeting PROTAC molecule SIAIS213110, which was synthesized based on our described method, shows great advantages over the reported dBET1. Therefore, SIAIS213110 could be clinically significant in the treatment of diseases related to BET overexpression.

In summary, we developed a highly efficient protocol for the synthesis of a lenalidomide-based PROTAC library through organic base-promoted chemoselective alkylation. Three new CRBN ligand-based libraries were successfully constructed with this established method. Base played a very important role in this chemoselective alkylation, and different bases led to different alkylation products. For example, an organic base such as DIPEA favored the formation of arylamine alkylation products, and an inorganic base such as K_2CO_3 favored the formation of glutarimide alkylation products instead. This method offers several advantages such as having easily accessible starting materials, high efficiency, and wide functional group compatibility. This library has been successfully used to identify BET PROTAC degraders, providing potential drug candidates for cancer therapy. Further studies to extend the application of this lenalidomide-based PROTAC library toward PROTAC drugs are in progress in our laboratory.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b01326.

Experimental details, characterization data, and NMR spectra for all new compounds (PDF)

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Author Contributions

§X.Q. and N.S. contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the National Natural Science Foundation of China (Grant No. 21502114 to B.J.), China Postdoctoral Science Foundation (2018M632181 to N.S. and 2018M642110 to L.L.), and Jing Medicine for financial support.

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(14) (a) Li's reported alkylation product **3a** is corrected to **3a′** by analyzing the NMR spectra. It is confirmed that the characteristically shifted glutarimide NH proton peak (δ = 11 ppm, DMSO-d_6) was lost. (b) Please see Supporting Information for the construction of a pomalidomide-based library via a microwave approach.

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DOI: 10.1021/acs.orglett.9b01326
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