OL | Organic Letters

Letter

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

pubs.acs.org/OrgLett

Benzimidazolinone-Free Peptide *o*-Aminoanilides for Chemical Protein Synthesis

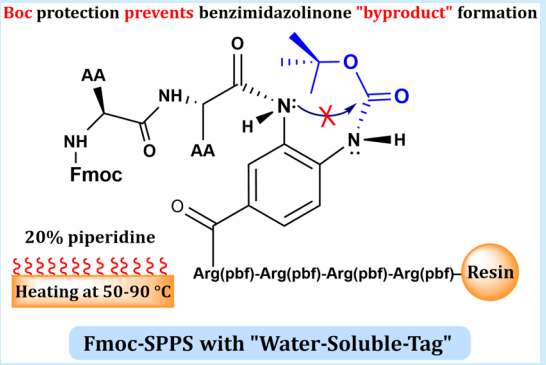
Jamsad Mannuthodikayil,[†,§] Sameer Singh,[†,§] Anamika Biswas,[†] Abhisek Kar,[†] Wahida Tabassum,[‡,§] Pratap Vydyam,[‡,§] Mrinal Kanti Bhattacharyya,[‡] and Kalyaneswar Mandal*[,†]

[†]TIFR Centre for Interdisciplinary Sciences, Tata Institute of Fundamental Research Hyderabad, 36/p Gopanpally, Hyderabad, Telangana 500107, India

[‡]Department of Biochemistry, School of Life Sciences, University of Hyderabad, Gachibowli, Hyderabad, Telangana 500046, India

Supporting Information

ABSTRACT: The thioester surrogate 3,4-diaminobenzoic acid (Dbz) facilitates the efficient synthesis of peptide thioesters by Fmoc chemistry solid phase peptide synthesis and the optional attachment of a solubility tag at the C-terminus. The protection of the partially deactivated *ortho*-amine of Dbz is necessary to obtain contamination-free peptide synthesis. The reported carbamate protecting groups promote a serious side reaction, benzimidazolinone formation. Herein we introduce the Boc-protected Dbz that prevents the benzimidazolinone formation, leading to clean peptide *o*-aminoanilides suitable for the total chemical synthesis of proteins.



Native chemical ligation (NCL) is a well-established protocol for the condensation of unprotected peptide segments in aqueous media.[1] NCL has been routinely used for the total synthesis of a wide variety of protein molecules over the past two and half decades.[2] In recent times, membrane-associated proteins have also become frequent targets for total chemical protein synthesis using NCL. The NCL polypeptide condensation reaction requires effective syntheses, handling, purification, and characterization of peptide thioester segments. Among the several methods reported to date,[3] the two most common strategies used to prepare the peptide thioester surrogate by Fmoc chemistry solid phase peptide synthesis (SPPS) involve the utilization of a 3,4-diaminobenzoic acid (Dbz) (**1**, Figure 1A) derivative (Dawson linker)[3a] and a hydrazide[3b] linker, respectively. Both of these linkers can be converted to a peptide thioester by $NaNO_2$-mediated oxidation under aqueous conditions.[3b,c]

The majority of the functional proteins contain a hydrophobic patch in their polypeptide sequence. Hydrophobic peptide segments are sometimes difficult to purify, even after their successful synthesis using advanced SPPS methods,[4] mainly due to their restricted solubility in the solvent systems generally used for purification by high-performance liquid chromatography (HPLC). The limited solubility of such peptides in ligation buffer can also contribute to difficulty in the NCL reaction. This poor solubility can be overcome by adding a "solubility tag", consisting of multiple Arg residues, at the C-terminus of the thioester or thioester surrogate peptide.[5,3c] The polyarginine "solubility tag" assists peptide purification and enhances peptide solubility in aqueous buffer in NCL reaction but does not end up in the ligation product. The hydrazide linker, however, does not allow the incorporation of a solubility tag at the peptide C-terminus. Therefore, the use of a Dbz linker is the method of choice for attaching a polyarginine tag when making peptide *o*-aminoanilides as a thioester surrogate.[3c]

One of the difficulties in making peptide *o*-aminoanilides as thioester surrogates is the overacylation of the deactivated *ortho*-amine group of the Dbz linker. Substantial research efforts by several research groups around the world have been devoted to circumventing the overacylation issue (Figure 1A).[6] These include the utilization of 4-amino-3-nitrobenzoic acid (**2**; Figure 1A) as an alternative thioester surrogate,[6a] and the orthogonal carbamate protection of the deactivated amine group of Dbz by Alloc (**3**; Figure 1A),[6b] Proc (**4**; Figure 1A),[6c] or 2-ClZ (**5**; Figure 1A, designed specifically for Boc chemistry SPPS).[6d] However, these strategies suffered from either racemization at the C-terminal residue, restricting the applicability to the synthesis of peptides having a Gly residue only at the C-terminus,[6a] or the need for an additional step, as in case of Alloc or Proc, that involves palladium catalyst treatment[6b,c] for the removal of the protecting groups.

Nowadays, high-temperature Fmoc SPPS is very common. It is widely believed that peptide synthesis at an elevated temperature results in more efficient chain assembly by Fmoc chemistry SPPS. However, for the synthesis of peptide

Received: September 28, 2019

DOI: 10.1021/acs.orglett.9b03440
Org. Lett. XXXX, XXX, XXX−XXX

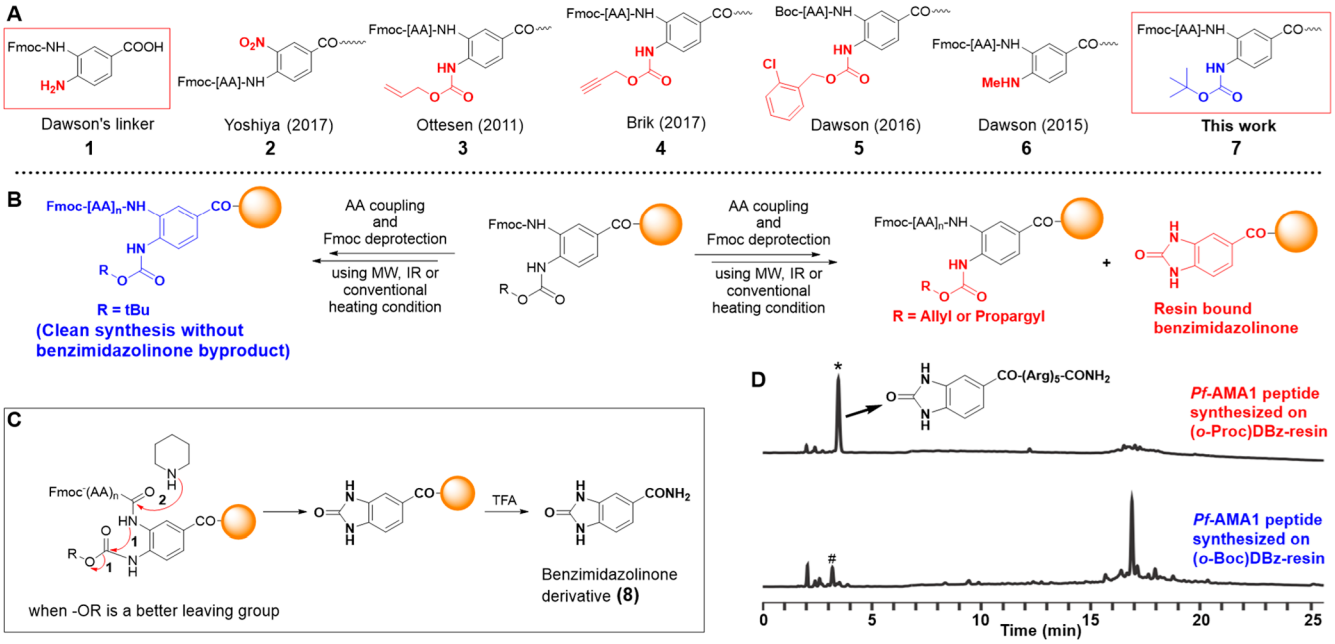


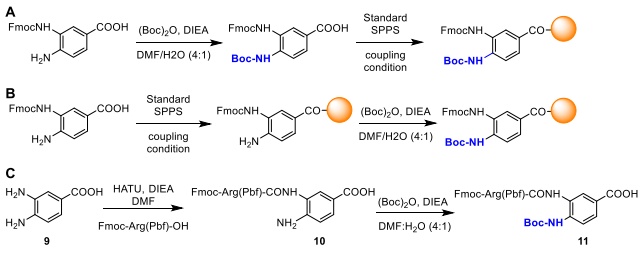
Figure 1. (A) Various 3,4-diaminobenzoic acid derivatives used as thioester surrogates over the years. (B) Benzimidazolinone byproduct (**8**) observed during Fmoc chemistry SPPS at elevated temperature when Alloc- or Proc-protected Dbz was used. The (*o*-Boc)Dbz linker gives clean synthesis without any peptide chain loss or benzimidazolinone formation. (C) Possible mechanism of the formation of **8**.[6a] Step 1: Peptide benzimidazolinone forms under basic conditions. Step 2: Nucleophilic attack by piperidine results in chain loss. (D) HPLC chromatogram of the crude Pf-AMA1(Cys149−Phe183) peptide having an $(Arg)_5$ tag at the C-terminus synthesized on Proc-protected Dbz-resin (top) and Boc-protected Dbz-resin (bottom). * indicates the benzimidazolinone-Arg_5-$CONH_2$ byproduct. # indicates the acetylated Dbz-Arg_5-$CONH_2$ obtained from the acetylation in the capping step after first residue coupling on (*o*-Boc)Dbz.

o-aminoanilides at elevated temperatures, a serious drawback of both Alloc and Proc protection has been the formation of a significant amount of benzimidazolinone byproduct (**8**; Figure 1B,C) in every Fmoc deprotection step during Fmoc chemistry SPPS.[6a,g] Interestingly, the most efficient strategy to date to prevent overacylation was reported by Dawson's group by the methylation of the deactivated amine of the DBz linker (MeDBz, **6**; Figure 1A) that delivered a benzimidazolinone-free peptide using Fmoc deprotection even at 90 °C under microwave irradiation conditions.[6e] However, MeDbz is resistant to $NaNO_2$-mediated oxidation. Hence, on-resin activation was obligatory for the peptides synthesized using the MeDBz linker.

Here we sought to introduce the *tert*-butyloxycarbonyl (Boc) group as a very simple, acid-labile, and efficient protecting group for the deactivated amine of Dbz. The Boc group has several advantages. It can be introduced easily by either on-resin or off-resin treatment of Fmoc-Dbz with Boc-anhydride in 20% water in dimethylformamide (DMF). Unlike Proc or Alloc, the Boc group can be removed by trifluoroacetic acid (TFA) during the global deprotection of the peptide from resin without the need for an additional step. Boc deprotection also avoids the usage of a Pd catalyst, thus preventing the possibility of the contamination of chemically synthesized peptides with toxic heavy-metal impurities.[7] Most importantly, being a poor leaving group, the sterically crowded *tert*-butyloxy group abrogates acylbenzimidazolinone formation during Fmoc chemistry SPPS under heating conditions, preventing undesired peptide chain loss during every Fmoc deprotection cycle and improving the purity and yield of the peptides.

The protection of the deactivated *ortho*-amine group of Dbz with a Boc group was readily performed either in solution or on a solid support. Solution phase synthesis of the building block Fmoc-(*o*-Boc)Dbz-OH was straightforward (Scheme 1A; Suppporting Information (SI) Section 2.2). The Boc protection of Fmoc-Dbz-OH with $(Boc)_2O$ in the presence of 20% water in DMF furnished Fmoc-(*o*-Boc)Dbz-OH with quantitative conversion (based on the UV intensity in the RP-HPLC chromatogram). The presence of a polar protic solvent (in this case, 20% water) in the reaction mixture has been found to reduce the unwanted side products drastically. The Fmoc-(*o*-Boc)Dbz-OH was then successfully coupled to the Rink Amide aminomethyl resin and used for further peptide chain assembly. We have also shown that the Boc protection can be carried out using the same reaction conditions on a solid support, if preferred (Scheme 1B, SI Section 2.5). However, because of the difficulty associated with the on-resin monitoring of Boc protection (see SI Section 2.5), we recommend using the solution phase synthesis of Fmoc-(*o*-Boc)Dbz-OH.

Scheme 1[a]



[a](A) Off-resin chemical synthesis and coupling of Fmoc-(*o*-Boc)Dbz linker on to the resin. (B) On-resin Boc protection of Fmoc-Dbz-resin. (C) Solution-phase coupling of Fmoc-Arg(Pbf)-COOH on Dbz, followed by Boc protection.

DOI: 10.1021/acs.orglett.9b03440
Org. Lett. XXXX, XXX, XXX−XXX

Boc protection of one of the amines was expected to reduce the reactivity of the remaining amine on (*o*-Boc)Dbz after Fmoc deprotection. Therefore, it was imperative to check the efficiency of the first amino acid coupling on the Boc-protected Dbz linker. We selected 10 representative amino acids to study the coupling efficiency onto (*o*-Boc)Dbz-resin (Table S1; see SI Section 3.1) using high-temperature coupling conditions. DIC/Oxyma (0.1 equiv *N*,*N*-diisopropylethylamine (DIEA)) activation with 0.5 M amino acid provided near-quantitative conversion for most of the amino acids in a single coupling cycle at 75 °C (Figure S5). To avoid racemization, His and Cys were coupled at room temperature (Figure S6). Attempts to couple Fmoc-Arg(Pbf)-OH at 75 °C gave very poor (<20%) conversion. It is well known that during the activation and coupling step, the nucleophilic side chain of arginine is susceptible to δ-lactam formation.[8a] This side reaction is known to be favored at elevated temperatures[8b] and effectively reduces the activated arginine concentration during high-temperature coupling. In contrast, room-temperature coupling of arginine does not lead to significant δ-lactam formation because the rate of coupling to form a peptide bond would be higher than the δ-lactam formation. DIC/Oxyma (0.1 equiv DIEA) activation with 0.5 M Fmoc-Arg(Pbf)-OH at room-temperature triple coupling resulted in ∼90% conversion (Figure S7C).

To avoid multiple coupling cycles for Arg residues on-resin, we preferred the coupling of a preformed Fmoc-Arg(Pbf)Dbz-(*o*-Boc)-OH. We prepared the Fmoc-Arg(Pbf)Dbz(*o*-Boc)-OH in only two simple steps by coupling Fmoc-Arg(Pbf)-OH to commercially available DBz, followed by the protection of the deactivated amine by a Boc group using the same protocol as previously discussed (Scheme 1C; SI Section 3.2). Thus our result represents an opportunity to choose either an on-resin or an off-resin route for the efficient coupling of any amino acid on the Boc-protected Dbz linker.

To demonstrate the feasibility of the synthesis of a large peptide segment on Fmoc-(*o*-Boc)Dbz-$(Arg(Pbf))_5$-Rink-Amide aminomethyl resin, we carried out the synthesis of a 35-residue hydrophobic peptide segment, (Cys149-Phe183), associated with *Plasmodium falciparum* protein apical membrane antigen 1 (*Pf*-AMA1 (3D7 strain)), with a poly-Arg tag at the C-terminus, which had defied our repeated synthesis attempts with a Proc-protected Dbz derivative under heating conditions. The high-temperature peptide chain assembly (coupling at 60 °C and Fmoc deprotection at 50 °C) on Fmoc-(*o*-Proc)Dbz-$(Arg(Pbf))_5$-Rink-Amide aminomethyl resin resulted in significant peptide chain loss during every Fmoc deprotection cycle, leaving virtually no desired target peptide (*o*-Proc)aminoanilide (Figure 1D, top). The mechanism of the peptide chain loss most likely involves the formation of peptide−benzimidazolinone in basic medium under thermal conditions and the subsequent cleavage of the peptide−benzimidazolinone by the nucleophilic attack of piperidine used for Fmoc deprotection (Figure 1C).[6a,g] The formation of benzimidazolinone is reminiscent of the aspartimide formation often observed during Fmoc chemistry SPPS. Because of the very early retention time shift in the standard HPLC gradient, the byproduct **8** usually remains unnoticed and is frequently ignored. In contrast, peptide synthesis at an elevated temperature using Fmoc-(*o*-Boc)Dbz-$(Arg(Pbf))_5$-Rink-Amide aminomethyl resin produced a clean synthesis of the crude peptide without the formation of "branched" byproducts and benzimidazolinone derivatives, demonstrating the extremely high thermal stability of the Boc group compared with the Proc group (Figure 1D, bottom; see SI Section 4).

Finally, we demonstrated the general utility of Fmoc-(*o*-Boc)Dbz chemistry by the total chemical synthesis of two proteins, ubiquitin and the extra cellular domain of the membrane protein "rhoptry neck protein 2" (RON2), a key protein associated with the human red blood cell invasion by malaria parasites.

Ubiquitin is an important protein marker that is covalently conjugated with other proteins and modulates their biochemical properties, triggering unique cellular functions, such as proteasomal degradation, the regulation of the chromatin structure, and protein localization.[9a,b] Several researchers have chemically synthesized the ubiquitin protein to investigate its biochemical and biophysical properties.[9c−e] The nonaromatic hydrophobic cluster located at the N-terminus of ubiquitin makes the segment spanning the first two beta strands sparingly soluble in aqueous solvent[9f,g] and may require a solubility tag for the easy handling and purification; hence we selected ubiquitin as a suitable protein target to demonstrate the applicability of Fmoc-(*o*-Boc)Dbz-linker.

Human ubiquitin contains a 76-residue polypeptide chain having no cysteines (Figure 2A). We prepared ubiquitin from three unprotected peptide segments using two consecutive N-to-C ligation reactions, followed by desulfurization (Figure 2B). We synthesized the N-terminal hydrophobic peptide segment, Met1-Lys27-Dbz-$(Arg)_4$-$CONH_2$ (**12**), with a poly-

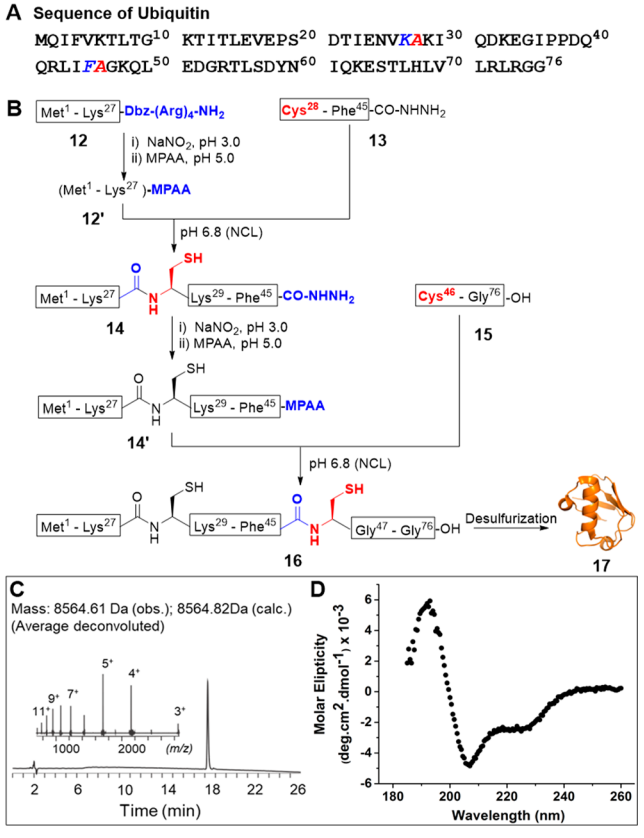


Figure 2. Total chemical synthesis of ubiquitin. (A) Sequence and (B) synthetic strategy. The cartoon representation of **17** was taken from PDB ID 1YIW. (C) LC−MS data of purified ubiquitin and (D) CD of the folded ubiquitin.

DOI: 10.1021/acs.orglett.9b03440
Org. Lett. XXXX, XXX, XXX−XXX

Arg tag at the C-terminus on Fmoc-(*o*-Boc)Dbz-$(Arg(Pbf))_4$-Rink-Amide aminomethyl resin with excellent crude purity (Figure S16). As described in the synthetic strategy, the N-terminal peptide segment was first activated at low temperature with $NaNO_2$ (20 mM) and thioesterified with 4-mercaptophenylacetic acid (MPAA) (100 mM), giving the peptide-C^{α}-MPAA-thioester (**12′**), which upon reaction with the second segment Cys28-Phe45-$CONHNH_2$ (**13**) at pH 6.8 gave the NCL product Met1-Phe45-$CONHNH_2$ (**14**) within 15 h. The purified polypeptide **14** (64% based on the limiting peptide **12**) was then further activated by $NaNO_2$ and exchanged with MPAA to give peptide C^{α}-MPAA-thioester (**14′**). The C-terminus segment Cys1-Gly76 (**15**) was then added, and the pH was adjusted to 6.8. Within 3 h, the NCL was complete, furnishing the target full-length polypeptide Met1-Gly76 (**16**) in 79.4% isolated yield (based on the limiting peptide **14**).

The radical mediated desulfurization[10] of **16** in the presence of VA044 (100 mM), 150 mM tris(2-carboxyethyl)phosphine (TCEP), and MESNa (75 mM) at 42 °C produced the native ubiquitin polypeptide. The purified ubiquitin had the desired mass (Figure 2C) and was obtained in very good yield (5.5 mg, 60% based on **16**). Circular dichroism (CD) data in phosphate buffer revealed the presence of the characteristic secondary structural elements of the folded ubiquitin protein molecule (Figure 2D).

As a part of our ongoing research using phage display to design protein inhibitors to interfere with the *P. falciparum* parasite invasion of human red blood cells, we undertook the total chemical synthesis of a biotinylated analogue of the membrane-associated extracellular domain of the parasite protein rhoptry neck protein 2 (here designated as *Pf*-RON2ed). We selected RON2ed as our target protein because the sequence is highly conserved among all of the available strains of *P. falciparum*. The sequence of *Pf*-RON2ed is shown in Figure 3A. We attached a biotin tag at the N-terminus, keeping a 38-atom spacer and a Gly residue to facilitate the immobilization of the target protein for phage screening in the future. The chemical structure of the biotin tag is shown in Figure 3B.

We synthesized *Pf*-RON2ed by the NCL of two unprotected peptide segments, followed by folding under air oxidation conditions, as shown in the synthetic strategy (Figure 3C). Because the N-terminus segment was found to be poorly soluble in aqueous solvents, we synthesized the N-terminus segment with an Arg_4 tag attached at the C-terminus preceding the (*o*-Boc)Dbz linker. The Arg_4-tagged peptide was obtained in very high crude purity, as evidenced from the chromatogram shown in Figure S24. The low-temperature activation of the purified N-terminal segment **19** by $NaNO_2$ at pH 3.0, followed by MPAA exchange gave the corresponding thioester **19′**, which upon reaction with the Cys peptide **20** at pH 6.8 furnished the desired biotin-tagged full-length polypeptide **21** in 35% yield. The purified full-length polypeptide was then folded under air oxidation conditions with the concomitant formation of the disulfide bond. The folded and purified protein had a mass of 4949.4 Da (Figure 3D). The chemically synthesized biotin-tagged RON2ed inhibited the growth of the *P. falciparum* (3D7) parasite in the growth inhibition activity (GIA) assay in vitro (Figure 3E).

In summary, we have demonstrated the use of the Boc group as a very simple and efficient protection strategy for the deactivated *ortho*-amine of the Dbz thioester equivalent used in Fmoc chemistry SPPS. The Boc-proteced Dbz derivative,

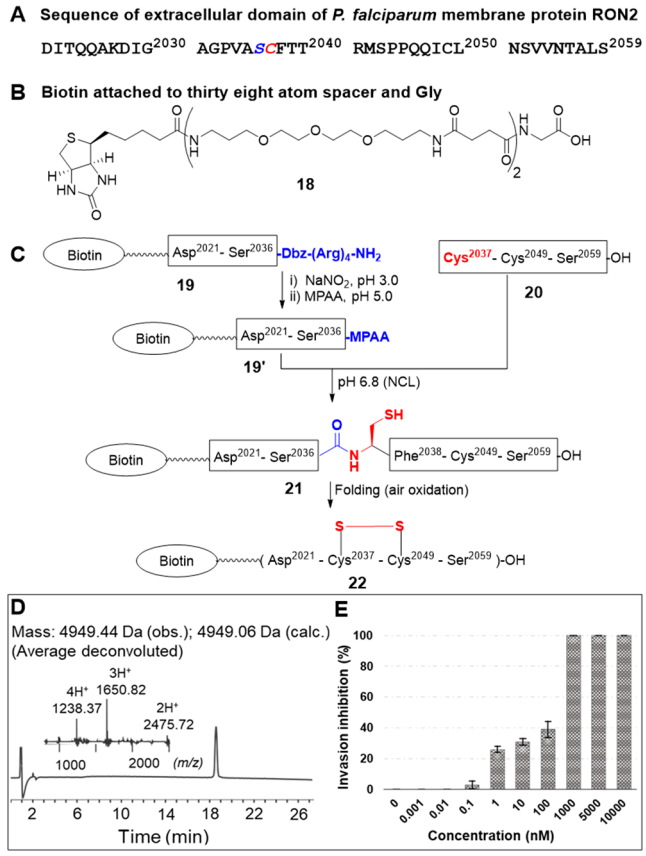


Figure 3. Total chemical synthesis of the biotinylated analogue of the extracellular domain of *Pf*-RON2. (A) Sequence, (B) chemical structure of the biotin tag with spacer, (C) synthetic strategy, (D) LC−MS data of the purified chemically synthesized biotinylated ectodomain of RON2, and (E) in vitro parasite growth inhibition activity (GIA) of the chemically synthesized biotinylated analogue of the extracellular domain of *Pf*-RON2 protein (3D7 strain).

Fmoc-(*o*-Boc)Dbz-OH, can be utilized as an effective linker for attaching a solubility tag at the C-terminus when synthesizing peptide *o*-aminoanilides as thioester surrogates using Fmoc chemistry SPPS under heating conditions. The protection of the Fmoc-Dbz *ortho*-amine by the Boc group has been found to be superior to other reported protecting groups, such as Alloc and Proc. Boc protection precludes the possibility of the formation of an undesired benzimidazolinone byproduct under standard high-temperature Fmoc chemistry SPPS. The Boc group removal does not need any additional step and is removed using TFA during the global deprotection of the peptide. Boc deprotection also avoids the usage of toxic heavy-metal catalysts frequently used for Alloc or Proc group removal. Therefore, we believe that the (*o*-Boc)Dbz linker will find many applications in the field of chemical protein synthesis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b03440.

Protocols for the synthesis and purification of Dbz derivatives and peptides; HPLC, LC−MS, ^1H NMR, and ^{13}C NMR data; protocols for the chemical synthesis of

DOI: 10.1021/acs.orglett.9b03440
Org. Lett. XXXX, XXX, XXX−XXX

ubiquitin and RON2ed; and protocols for parasite growth inhibition assay (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: kmandal@tifrh.res.in.

ORCID

Kalyaneswar Mandal: 0000-0002-3194-1378

Author Contributions

§J.M. and S.S. contributed equally. W.T. and P.V. contributed equally.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by TIFR and the Wellcome Trust/DBT India Alliance Grant (grant no. IA/I/15/1/501847) awarded to K.M. We acknowledge the biophysics core facility (for the CD instrument), TIFR Hyderabad.

REFERENCES

(1) Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. *Science* **1994**, *266*, 776−9.

(2) (a) Kent, S. B. H. *Protein Sci.* **2019**, *28*, 313−328. (b) Kent, S. B. H. *Bioorg. Med. Chem.* **2017**, *25*, 4926−4937.

(3) (a) Blanco-Canosa, J. B.; Dawson, P. E. *Angew. Chem., Int. Ed.* **2008**, *47*, 6851−5. (b) Fang, G. M.; Li, Y. M.; Shen, F.; Huang, Y. C.; Li, J. B.; Lin, Y.; Cui, H. K.; Liu, L. *Angew. Chem., Int. Ed.* **2011**, *50*, 7645−9. (c) Wang, J. X.; Fang, G. M.; He, Y.; Qu, D. L.; Yu, M.; Hong, Z. Y.; Liu, L. *Angew. Chem., Int. Ed.* **2015**, *54*, 2194−2198. (d) Li, H. X.; Dong, S. W. *Sci. China: Chem.* **2017**, *60*, 201−213. (e) Agouridas, V.; El Mahdi, O.; Diemer, V.; Cargoet, M.; Monbaliu, J. C. M.; Melnyk, O. *Chem. Rev.* **2019**, *119*, 7328−7443.

(4) (a) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149−2154. (b) Schnolzer, M.; Alewood, P.; Jones, A.; Alewood, D.; Kent, S. B. H. *Int. J. Pept. Res. Ther.* **2007**, *13*, 31−44. (c) Mijalis, A. J.; Thomas, D. A.; Simon, M. D.; Adamo, A.; Beaumont, R.; Jensen, K. F.; Pentelute, B. L. *Nat. Chem. Biol.* **2017**, *13*, 464−466.

(5) Johnson, E. C.; Kent, S. B. *Tetrahedron Lett.* **2007**, *48*, 1795−1799.

(6) (a) Tsuda, S.; Uemura, T.; Mochizuki, M.; Nishio, H.; Yoshiya, T. *Synlett* **2017**, *28*, 1956−1960. (b) Mahto, S. K.; Howard, C. J.; Shimko, J. C.; Ottesen, J. J. *ChemBioChem* **2011**, *12*, 2488−94. (c) Bondalapati, S.; Eid, E.; Mali, S. M.; Wolberger, C.; Brik, A. *Chem. Sci.* **2017**, *8*, 4027−4034. (d) Weidmann, J.; Dimitrijevic, E.; Hoheisel, J. D.; Dawson, P. E. *Org. Lett.* **2016**, *18*, 164−167. (e) Blanco-Canosa, J. B.; Nardone, B.; Albericio, F.; Dawson, P. E. *J. Am. Chem. Soc.* **2015**, *137*, 7197−7209. (f) Pala-Pujadas, J.; Albericio, F.; Blanco-Canosa, J. B. *Angew. Chem., Int. Ed.* **2018**, *57*, 16120−16125. (g) Hong, Z. Z.; Yu, R. R.; Zhang, X.; Webb, A. M.; Burge, N. L.; Poirier, M. G.; Ottesen, J. J. *bioRxiv* **2019**.

(7) (a) Liu, T. Z.; Lee, S. D.; Bhatnagar, R. S. *Toxicol. Lett.* **1979**, *4*, 469−473. (b) Liu, T. Z.; Khayambashi, H.; Bhatnagar, R. S. *Clin. Chem.* **1977**, *23*, 1119−1119a. (c) Chen, M. L.; Chen, S. X.; Du, M.; Tang, S. H.; Chen, M.; Wang, W.; Yang, H.; Chen, Q. Y.; Chen, J. M. *Aquat. Toxicol.* **2015**, *159*, 208−216.

(8) (a) Cezari, M. H.; Juliano, L. *Pept. Res.* **1996**, *9*, 88−91. (b) White, P.; Collins, J.; Cox, Z. J. Comparative Study of Conventional and Microwave-Assisted Synthesis. In *19th American Peptide Symposium*, San Diego, CA, 2005.

(9) (a) Debelouchina, G. T.; Gerecht, K.; Muir, T. W. *Nat. Chem. Biol.* **2017**, *13*, 105−110. (b) Komander, D.; Rape, M. *Annu. Rev. Biochem.* **2012**, *81*, 203−229. (c) Bang, D.; Makhatadze, G. I.; Tereshko, V.; Kossiakoff, A. A.; Kent, S. B. *Angew. Chem., Int. Ed.* **2005**, *44*, 3852−3856. (d) Pan, M.; Gao, S.; Zheng, Y.; Tan, X. D.; Lan, H.; Tan, X. L.; Sun, D. M.; Lu, L. N.; Wang, T.; Zheng, Q. Y.; Huang, Y. C.; Wang, J. W.; Liu, L. *J. Am. Chem. Soc.* **2016**, *138*, 7429−7435. (e) Kumar, K. S. A.; Bavikar, S. N.; Spasser, L.; Moyal, T.; Ohayon, S.; Brik, A. *Angew. Chem., Int. Ed.* **2011**, *50*, 6137−6141. (f) Riemen, A. J.; Waters, M. L. *Biopolymers* **2008**, *90*, 394−398. (g) We encountered extreme difficulty in solubilizing a synthetic peptide segment containing the first 17 residues of ubiquitin in aqueous solution (data not shown).

(10) Wan, Q.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2007**, *46*, 9248−52.

DOI: 10.1021/acs.orglett.9b03440
Org. Lett. XXXX, XXX, XXX−XXX