Organic LETTERS

Letter

pubs.acs.org/OrgLett

Solid-Phase Synthesis of Oxetane Modified Peptides

Jonathan D. Beadle,[†] Astrid Knuhtsen,[‡] Alex Hoose,[‡] Piotr Raubo,[§] Andrew G. Jamieson,*[,‡] and Michael Shipman*[,†]

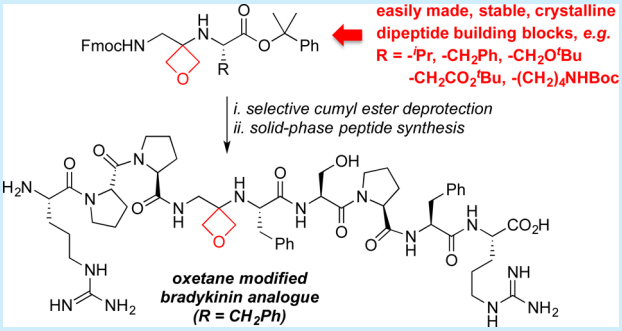
[†]Department of Chemistry, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, U.K.

[‡]School of Chemistry, University of Glasgow, Joseph Black Building, University Avenue, Glasgow G12 8QQ, U.K.

[§]IMED Oncology, AstraZeneca, 310 Cambridge Science Park, Milton Road, Cambridge CB4 0WG, U.K.

Supporting Information

ABSTRACT: Solid-phase peptide synthesis (SPPS) is used to create peptidomimetics in which one of the backbone amide C═O bonds is replaced by a four-membered oxetane ring. The oxetane containing dipeptide building blocks are made in three steps in solution, then integrated into peptide chains by conventional Fmoc SPPS. This methodology is used to make a range of peptides in high purity including backbone modified derivatives of the nonapeptide bradykinin and Met- and Leu-enkephalin.



Despite resurgent interest in the use of peptides as drugs,[1] their development is often hampered by their poor oral bioavailability and short plasma half-lives. As a consequence, there is intense interest in the design, synthesis, and study of peptidomimetics that mimic the structure and biological function of native peptides yet possess better drug-like properties.[2−4] Independently, Shipman[5] and Carreira[6] introduced a new type of peptide bond isostere in which the heterocyclic 3-aminooxetane unit was used as a replacement for one of the amide bonds (Figure 1).[7] Several features of these oxetane modified peptides (OMPs) make them of particular interest as peptidomimetics. First, deletion of one of the amide bonds can improve peptide half-lives through increased stability to proteases while retaining bioactivity.[6b] Second, oxetanes are known to make excellent bioisosteric replacements for C═O bonds, and are commonly used in conventional, small molecule drug discovery.[8] Third, the 3-aminooxetane unit can act as both a H-bond donor and acceptor, supporting the types of noncovalent interactions available to peptides. Finally, conformational changes arising from removal of the double bond character of the peptide bond change the conformational bias of OMPs,[5a] opening up new areas of peptide structural space to explore.

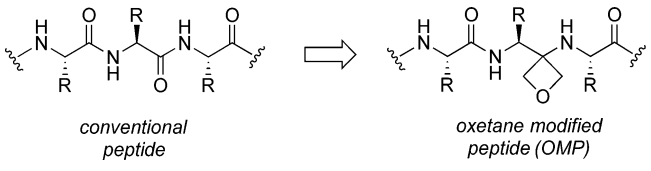
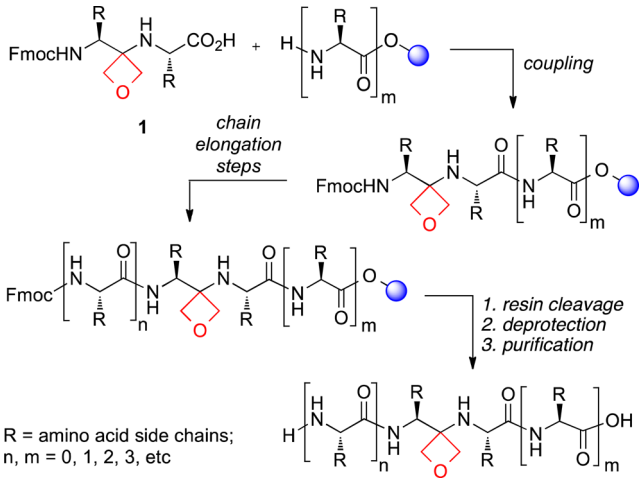


Figure 1. Oxetane based peptidomimetics.

Scheme 1. SPPS Route to Oxetane Modified Peptides

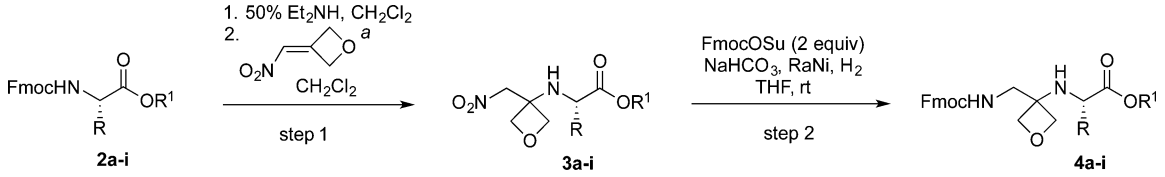


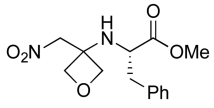
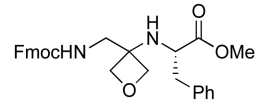
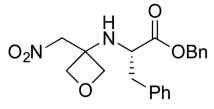
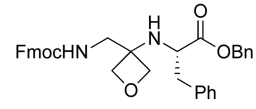
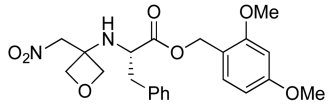
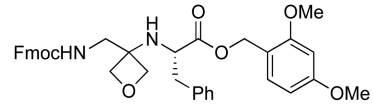
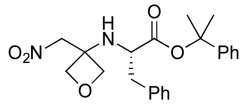
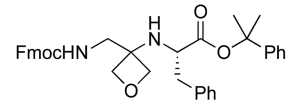
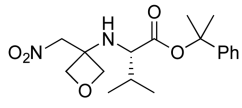
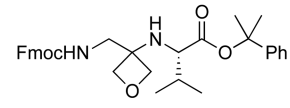
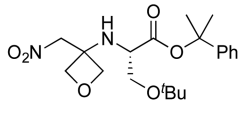
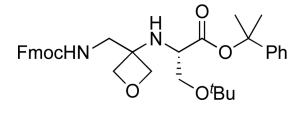
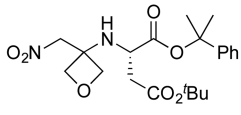
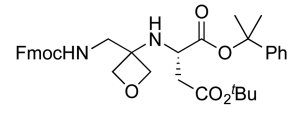
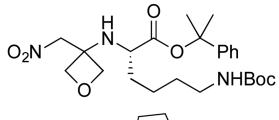
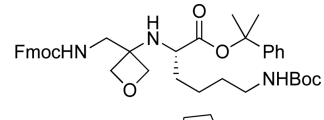
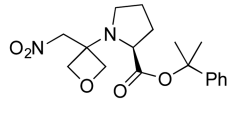
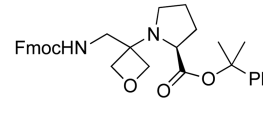
To date, access to OMPs has been by way of solution-based methods.[5,6] However, to study the impact of oxetane modification on the structure and properties of larger, biologically important peptides, we turned to solid-phase peptide synthesis (SPPS).[9] Our plan was to synthesize Fmoc-protected dipeptide building blocks such as **1**, then integrate them into a growing peptide chain using automated SPPS techniques (Scheme 1). Here, we describe the successful development of this methodology and use it to make a range of OMPs including novel enkephalin and bradykinin analogues.

Received: May 15, 2017
Published: June 6, 2017

DOI: 10.1021/acs.orglett.7b01466
Org. Lett. 2017, 19, 3303−3306

Table 1. Synthesis of Oxetane Containing Dipeptide Building Blocks



entry	product (step 1)	structure	yield (%)[b]	product (step 2)	structure	yield (%)[b]
1	**3a**		54[c,d]	**4a**		87[f]
2	**3b**		73[c,e]	**4b**		69[f]
3	**3c**		61	**4c**		63[f]
4	**3d**		57[g]	**4d**		58
5	**3e**		55	**4e**		76
6	**3f**		54	**4f**		78
7	**3g**		40	**4g**		77
8	**3h**		56	**4h**		71
9	**3i**		56[c]	**4i**		50

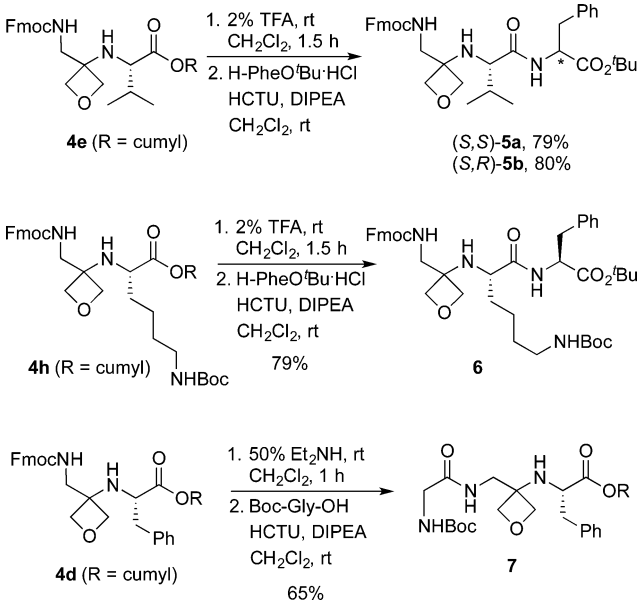
[a]Prepared *in situ* from oxetan-3-one and nitromethane (see Supporting Information). [b]After column chromatography. [c]Made directly from α-amino ester without Fmoc deprotection. [d]Reference 5b. [e]Reference 5a. [f]Using 1.2 equiv of FmocOSu. [g]60% yield on higher 3 mmol scale (see Supporting Information).

Initially, we sought a practical route to Fmoc-protected dipeptide building blocks in which the oxetane residue is based on glycine. Conjugate addition of the appropriate amino ester, made from **2a**–**i** by initial Fmoc cleavage, to 3-(nitromethylene)oxetane readily gave the addition products **3a**–**i** (Table 1).[5] Raney Ni reduction of **3a**–**i** in the presence of FmocOSu provided **4a**–**i** in good yields.[6] A range of protected side chain types were introduced namely: acidic, basic, polar, and nonpolar.

The last step prior to SPPS was selective removal of the ester group (R^1); a transformation that required considerable optimization to safeguard the Fmoc and side chain protecting groups. Initially, phenylalanine derivatives containing methyl (**4a**), benzyl (**4b**), 2,4-dimethoxybenzyl (**4c**), and 2-phenylisopropyl (cumyl, **4d**) esters were studied (Table 1, entries 1–4). Cleavage of the methyl ester from **4a** using LiOH-THF/H_2O led to unwanted *N*-Fmoc cleavage. Other conditions reported to leave Fmoc groups unscathed such as Me_3SnOH[10] and NaOH/$CaCl_2$[11] also proved unsuccessful. Hydrogenolysis of benzyl ester **4b** (H_2, 10% Pd/C, MeOH) did provide the corresponding carboxylic acid in an encouraging 50% yield. However, optimization of this transformation and its extension to derivatives containing other side chains proved impractical.[12] Poor results were also observed in the attempted removal of the 2,4-dimethoxybenzyl group from **4c** using mild acid (1% TFA-CH_2Cl_2, anisole).[13] Much better outcomes were achieved with

DOI: 10.1021/acs.orglett.7b01466
Org. Lett. 2017, 19, 3303–3306

Scheme 2. Selective Cumyl Ester Deprotection and Solution Phase Couplings

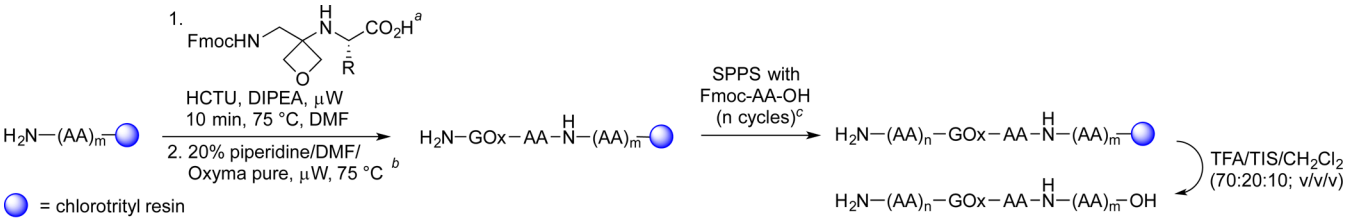


cumyl ester **4d**, which was quantitatively deprotected upon treatment with 2% TFA in CH_2Cl_2 for 1.5 h.[14a] On this basis, we focused our subsequent efforts on cumyl esters **4d–i**. From a practical standpoint, these derivatives proved attractive as they were bench stable, crystalline solids. Indeed, it proved most convenient to store the building blocks as the cumyl esters, then reveal the carboxylic acid using 2% TFA in CH_2Cl_2 immediately prior to SPPS. These materials were produced on up to a 3 mmol scale (see Supporting Information). Moreover, the starting cumyl esters **2d–i** were easily made from the corresponding Fmoc-protected amino acids by reaction with 2-phenylisopropyl-trichloroacetimidate (see Supporting Information).[14]

Initially, the use of the building blocks in solution-phase couplings was established (Scheme 2). Cleavage of the cumyl ester from **4e** followed by coupling with L-phenylalanine *tert*-butyl ester with O-(1*H*-6-chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU) and diisopropylethylamine (DIPEA) gave (*S,S*)-**5a** in excellent yield. Using D-phenylalanine *tert*-butyl ester in the same sequence provided (*S,R*)-**5b**. Analysis by ^{1}H NMR confirmed that no detectable epimerization arose during these couplings (see Supporting Information). Subjecting **4h** bearing a Boc-protected lysine to the same cleavage/coupling conditions cleanly gave **6** without evidence of side chain deprotection. Additionally, **4d** was converted to **7** by chain extension at the N-terminus. In this transformation, no products derived from acylation of the secondary amine of the 3-aminooxetane unit were isolated despite the use of 4 equiv of the coupling partners. This observation encouraged us to explore SPPS without recourse to protection of the oxetane nitrogen atom.

Next, we examined the use of **4d–i** in SPPS. These experiments were conducted on a 0.1 mmol scale in a Biotage Alstra microwave synthesizer using preloaded chlorotrityl resin and HCTU activation (for full details, see Supporting Information). Initially, six tetrapeptides **8–13** were synthesized using each of the building blocks, **4d–i** (Table 2, entries 1–6). Two equivalents of the building blocks were used, with a pretreatment with 2% TFA in CH_2Cl_2 under anhydrous conditions to cleave the cumyl ester, immediately prior to coupling. Standard conditions for Fmoc removal (20% piperidine, 0.1 M Oxyma pure, DMF),[15] coupling [Fmoc-Trp-OH, HCTU, DIPEA, DMF], and resin cleavage/deprotection [TFA/triisopropylsilane(TIS)/CH_2Cl_2

Table 2. Solid-Phase Synthesis of Oxetane Modified Peptides



entry	building block[a]	peptide sequence[d]	purity (%)[e]	mass, mg (yield, %)	peptide content (%)[f]	HRMS calculated	HRMS observed
1	**4d**	W-*GOx-F*-A, **8**	89	10.7 (21)	75	508.2554 $[M + H]^+$	508.2531 $[M + H]^+$
2	**4e**	W-*GOx-V*-A, **9**	93	8.4 (18)	55	460.2554 $[M + H]^+$	460.2544 $[M + H]^+$
3	**4f**	W-*GOx-S*-A, **10**	81 (90)[g]	12.0 (27)	73	448.2191 $[M + H]^+$	448.2190 $[M + H]^+$
4	**4g**	W-*GOx-D*-A, **11**	81 (91)[g]	13.7 (29)	75	476.2140 $[M + H]^+$	476.2129 $[M + H]^+$
5	**4h**	W-*GOx-K*-A, **12**	93	5.0 (10)	48	489.2820 $[M + H]^+$	489.2818 $[M + H]^+$
6	**4i**	W-*GOx-P*-A, **13**	94	14.6 (32)	62	480.2217 $[M + Na]^+$	480.2213 $[M + Na]^+$
7	**4d**	Y-G-*GOx-F*-M, **14**	91	17.0 (28)	81	602.2643 $[M + H]^+$	602.2659 $[M + H]^+$
8	**4d**	Y-G-*GOx-F*-L, **15**	91	16.4 (28)	79	584.3079 $[M + H]^+$	584.3056 $[M + H]^+$
9	**4d**	R-P-P-*GOx-F*-S-P-F-R, **16**	94	25.3 (46)	63	544.8036 $[M + 2H]^{2+}$	544.8014 $[M + 2H]^{2+}$

[a]Treated with 2% TFA in CH_2Cl_2 for 2 h to reveal the free carboxylic acid from **4d–i** prior to coupling with the resin-bound peptide. [b]30 s, then 3 min with fresh reagents. [c]All couplings performed at 75 °C for 10 min except arginine (60 min at rt, then 5 min at 75 °C, repeated with fresh reagents). [d]Italicized residues derived from the oxetane containing dipeptide building block. [e]By reverse-phase HPLC (see Supporting Information). [f]Determined by UV spectroscopy (at 280 nm) except **16** (at 214 nm). [g]Improved to >90% by second purification.

DOI: 10.1021/acs.orglett.7b01466
Org. Lett. 2017, 19, 3303–3306

(70:20:10, v/v/v)] were adopted. After reverse-phase HPLC, these tetrapeptides were isolated in high purity and acceptable yields. Pleasingly, the oxetane did not undergo ring opening under the harsh acidic conditions required for release from the resin and concomitant side chain deprotection. Encouraged by these results, we prepared oxetane modified analogues of biologically relevant peptides. First, we made **14** and **15**, analogues of opioid-binding Met- and Leu-enkephalin, respectively, in which the central glycine was replaced (Table 2, entries 7 and 8). Similarly, **16** was produced in good yield and excellent purity as an analogue of the vasodilator bradykinin. No erosion in peptide yield or purity was seen in the preparation of this nonapeptide, indicating that the 3-aminooxetane residue is well tolerated in repetitive rounds of coupling/Fmoc deprotection. The synthesis of Leu-enkephalin analogue **15** highlights the benefits of the SPPS approach; although this material has previously been made by a solution-phase strategy, it required multiple steps and chromatographic purifications.[6b]

In summary, we have developed a practical route to oxetane modified peptides using solid-phase peptide synthesis techniques. Key findings include: (i) cumyl-protected dipeptide building blocks **4d−i** are easily made in three simple steps and the ester selectively cleaved with 2% TFA in CH_2Cl_2; (ii) efficient amide couplings using these building blocks can be achieved in solution or on solid-phase without protecting the secondary amine of the 3-aminooxetane residue; (iii) oxetane containing peptides (up to 9 amino acids) can be produced in high purity using conventional SPPS methods for coupling, resin cleavage, deprotection, and purification, suggesting the broad applicability of this modification in peptide science. Current work is focused on using this new methodology to produce a variety of OMPs so that the impact of this backbone modification on the secondary structure and physicochemical and biological properties of the peptides can be systematically explored.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b01466.

Experimental procedures and characterization data for all new compounds, copies of ^1H and ^{13}C NMR spectra for building blocks, and analytical HPLC traces for peptides (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: andrew.jamieson.2@glasgow.ac.uk.
*E-mail: m.shipman@warwick.ac.uk.

ORCID

Andrew G. Jamieson: 0000-0003-1726-7353
Michael Shipman: 0000-0002-3349-5594

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank AstraZeneca for generous financial support.

■ REFERENCES

(1) (a) Fosgerau, K.; Hoffmann, T. *Drug Discovery Today* **2015**, *20*, 122. (b) Nevola, L.; Giralt, E. *Chem. Commun.* **2015**, *51*, 3302. (c) Kaspar, A. A.; Reichert, J. M. *Drug Discovery Today* **2013**, *18*, 807.

(2) Trabocchi, A.; Guarna, A. *Peptidomimetics in Organic and Medicinal Chemistry: The Art of Transforming Peptides in Drugs*; John Wiley & Sons Ltd: Chichester, 2014.

(3) For reviews, see (a) Qvit, N.; Rubin, S. J. S.; Urban, T. J.; Mochly-Rosen, D.; Gross, E. R. *Drug Discovery Today* **2017**, *22*, 454. (b) Wang, Z. A.; Ding, X. Z.; Tian, C.-L.; Zheng, J.-S. *RSC Adv.* **2016**, *6*, 61599. (c) Klinker, K.; Barz, M. *Macromol. Rapid Commun.* **2015**, *36*, 1943. (d) Jayatunga, M. K. P.; Thompson, S.; Hamilton, A. D. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 717. (e) Avan, I.; Hall, C. D.; Katritzky, A. R. *Chem. Soc. Rev.* **2014**, *43*, 3575. (f) Liskamp, R. M. J.; Rijkers, D. T. S.; Kruijtzer, J. A. W.; Kemmink, J. *ChemBioChem* **2011**, *12*, 1626. (g) Grauer, A.; König, B. *Eur. J. Org. Chem.* **2009**, 5099. (h) Vagner, J.; Qu, H.; Hruby, V. J. *Curr. Opin. Chem. Biol.* **2008**, *12*, 292.

(4) For illustrative examples, see (a) Nadon, J.-F.; Rochon, K.; Grastilleur, S.; Langlois, G.; Dao, T. T. H.; Blais, V.; Guérin, B.; Gendron, L.; Dory, Y. L. *ACS Chem. Neurosci.* **2017**, *8*, 40. (b) Busschaert, N.; Thompson, S.; Hamilton, A. D. *Chem. Commun.* **2017**, *53*, 313. (c) Krishnan, B. P.; Rai, R.; Asokan, A.; Sureshan, K. M. *J. Am. Chem. Soc.* **2016**, *138*, 14824. (d) Lee, K. J.; Lee, W. S.; Yun, H.; Hyun, Y.-J.; Seo, C. D.; Lee, C. W.; Lim, H.-S. *Org. Lett.* **2016**, *18*, 3678. (e) Wu, H.; Qiao, Q.; Teng, P.; Hu, Y.; Antoniadis, D.; Zuo, X.; Cai, J. *Org. Lett.* **2015**, *17*, 3524. (f) Barnard, A.; Long, K.; Martin, H. L.; Miles, J. A.; Edwards, T. A.; Tomlinson, D. C.; Macdonald, A.; Wilson, A. J. *Angew. Chem., Int. Ed.* **2015**, *54*, 2960. (g) Newberry, R. W.; VanVeller, B.; Raines, R. T. *Chem. Commun.* **2015**, *51*, 9624. (h) Doan, N.-D.; Hopewell, R.; Lubell, W. D. *Org. Lett.* **2014**, *16*, 2232. (i) Traoré, M.; Doan, N.-D.; Lubell, W. D. *Org. Lett.* **2014**, *16*, 3588.

(5) (a) Powell, N. H.; Clarkson, G. J.; Notman, R.; Raubo, P.; Martin, N. G.; Shipman, M. *Chem. Commun.* **2014**, *50*, 8797. (b) Beadle, J. D.; Powell, N. H.; Raubo, P.; Clarkson, G. J.; Shipman, M. *Synlett* **2016**, *27*, 169.

(6) (a) McLaughlin, M.; Yazaki, R.; Fessard, T. C.; Carreira, E. M. *Org. Lett.* **2014**, *16*, 4070. (b) Möller, G. P.; Müller, S.; Wolfstädter, B. T.; Wolfrum, S.; Schepmann, D.; Wünsch, B.; Carreira, E. M. *Org. Lett.* **2017**, *19*, 2510.

(7) Oxetane modification of proteins through side chain functionalization has also recently been described, see Boutureira, O.; Martínez-Sáez, N.; Brindle, K. M.; Neves, A. A.; Corzana, F.; Bernardes, G. J. L. *Chem. - Eur. J.* **2017**, *23*, 6483.

(8) (a) Bull, J. A.; Croft, R. A.; Davis, O. A.; Doran, R.; Morgan, K. F. *Chem. Rev.* **2016**, *116*, 12150. (b) Burkhard, J. A.; Wuitschik, G.; Rogers-Evans, M.; Müller, K.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2010**, *49*, 9052.

(9) Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer-Verlag: Berlin, 1994.

(10) Nicolaou, K. C.; Estrada, A. A.; Zak, M.; Lee, S. H.; Safina, B. S. *Angew. Chem., Int. Ed.* **2005**, *44*, 1378.

(11) Pascal, R.; Sola, R. *Tetrahedron Lett.* **1998**, *39*, 5031.

(12) When the side chain of **4b** (R = Bn) was replaced with other substituents (R = H or ^{i}Pr), cleavage of the Fmoc group was seen during hydrogenolysis. We speculate that the success witnessed with **4b** (R = Bn) arises from its insolubility in MeOH. During hydrogenolysis, the carboxylic acid rapidly precipitates preventing further Fmoc cleavage.

(13) McMurray, J. S. *Tetrahedron Lett.* **1991**, *32*, 7679.

(14) (a) Yue, C.; Thierry, J.; Potier, P. *Tetrahedron Lett.* **1993**, *34*, 323. (b) Respondek, T.; Cueny, E.; Kodanko, J. J. *Org. Lett.* **2012**, *14*, 150.

(15) Subirós-Funosas, R.; El-Faham, A.; Albericio, F. *Biopolymers* **2012**, *98*, 89.

DOI: 10.1021/acs.orglett.7b01466
Org. Lett. 2017, 19, 3303−3306