Organic LETTERS

Letter

pubs.acs.org/OrgLett

Palladium-Catalyzed Aminocarbonylation in Solid-Phase Peptide Synthesis: A Method for Capping, Cyclization, and Isotope Labeling

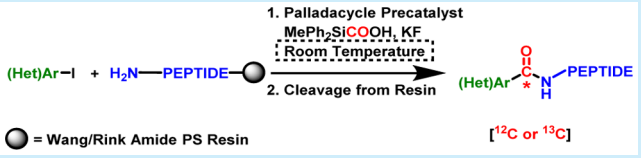
Anna Skogh,[†] Stig D. Friis,[‡] Troels Skrydstrup,[‡] and Anja Sandström*[,†]

[†]Department of Medicinal Chemistry, Organic Pharmaceutical Chemistry, BMC, Uppsala University, Box 574, SE-751 23 Uppsala, Sweden

[‡]Carbon Dioxide Activation Center (CADIAC), Interdisciplinary Nanoscience Center (iNANO) and Department of Chemistry, Aarhus University, Gustav Wieds Vej 14, 8000 Aarhus C, Denmark

Supporting Information

ABSTRACT: A new synthetic approach for introducing N-capping groups onto peptides attached to a solid support, combining aminocarbonylation under mild conditions using a palladacycle precatalyst and solid-phase peptide synthesis, is reported. The use of a silacarboxylic acid as an in situ CO-releasing molecule allowed the reaction to be performed in a single vial. The method also enables versatile substitution of side chains, side-chain-to-side-chain cyclizations, and selective [^{13}C] acyl labeling of modified peptides.



Peptides have been neglected in drug discovery for many years due to their generally poor oral bioavailability and limited stability. However, the situation is rapidly changing due to the comparatively higher approval rate of peptide-based drugs as compared to small-molecule drugs in recent years.[1−4] Indeed, peptides and peptidomimetics beyond the rule of five have been suggested as potential future therapeutics for addressing difficult targets and diseases with unmet medical needs.[1,2] Several chemical approaches have been used to improve bioavailability and stability of peptides, for example, *N*-methylation of the backbone,[5] various cyclizations strategies,[3,6] substitution by constrained and unnatural amino acids,[7] and modifications of the C- and N-terminal ends.[1] N-Terminal capping of peptides with acyl, aroyl, or heteroaroyl groups can, besides masking the basic amine, improve target interactions, selectivity, cell permeability, and plasma stability.[8−11] Aroyl N-capping groups are found in several pharmaceutically relevant peptide-based compounds (Figure 1) and have proven useful for ^{18}F-labeling and as photoactivatable probes in peptides.[12,13]

N-Capping groups are usually incorporated through acylation using carboxylic acid derivatives in solid-phase peptide synthesis (SPPS).[10,13,14] An alternative approach to amides is through the well-established, three-component, Pd-catalyzed coupling between (hetero)aryl halides, an amine, and carbon monoxide.[15] The development of CO-releasing molecules (CORMs) for in situ[16] and ex situ[17] generation of CO has improved handling and safety aspects significantly in carbonylation chemistry. To our knowledge, aminocarbonylation has so far never been applied in SPPS.

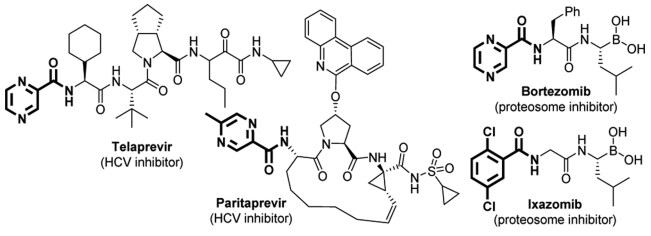


Figure 1. Examples of pharmaceuticals with N-capping groups marked in bold style.

We foresee several benefits to combining an aminocarbonylation protocol with SPPS. First, it would facilitate the possibility of using excess reagents to drive reactions to completion due to easy workup using filtration. Second, the use of aryl halides instead of reactive acyl-transfer reagents like acid chlorides allows mild conditions but also new opportunities for cyclization of peptides without the demand of orthogonally protected carboxylic acids. Moreover, such methods would enable ^{13}C- and potentially ^{11}C-isotope labeling of peptides, which would be useful in the study of bioactive peptides in modern drug discovery.[18−21]

Herein, we demonstrate for the first time the facile conversion of aryl and heteroaryl iodides into their corresponding amides using solid-phase bound amino acid/peptide nucleophiles and in situ generation of CO from a crystalline silacarboxylic acid[18] at room temperature.

In order to develop a safe, mild, and easy-to-execute aminocarbonylation compatible with the polystyrene resin the mode of mixing, choice of CORM, and reaction temperature were investigated. First, mixing by agitation rather than magnetic stirring was preferred in our SPPS protocol to reduce the risk of resin fragmentation. Second, during preliminary studies, silacarboxylic acid **3** proved to be a clean and efficient CO precursor for aminocarbonylations upon fluoride treatment at

Received: April 13, 2017

DOI: 10.1021/acs.orglett.7b01068
Org. Lett. XXXX, XXX, XXX−XXX

room temperature, even when used in situ. Third, a low reaction temperature was desired to avoid racemization of the amino acids. Previously, the employment of a XantPhos ligated palladacycle precatalyst[22] in aminocarbonylation reactions has been demonstrated to convert (hetero)aryl bromides to their corresponding amide in 14−16 h at only 45 °C due to the enhanced reactivity of the active Pd-catalyst generated.[23] We therefore envisioned that using the more reactive (hetero)aryl iodides could enable formation of the corresponding amides through agitation already at room temperature using this palladacycle precatalyst and silacarboxylic acid **3** even if the nucleophile is attached to a solid phase. Our investigation commenced with the reaction of 4-iodoanisole with preloaded phenylalanine Wang polystyrene (PS) resin (**1a**) in dry DMF in the presence of 2 mol % of XantPhos-ligated palladacycle precatalyst **2** and 2 equiv of $MePh_2SiCOOH$ (**3**) and KF (entry 1, Table 1). The solid-phase-bound amine **1a** was set as the limiting reagent, and the yield was calculated from the loading of the resin. Aminocarbonylation of 4-iodoanisole proceeded efficiently and afforded **4a** in 95% yield after 15 h with subsequent cleavage from the resin using 95% TFA in the presence of triethylsilane (TES) followed by precipitation in cold diethyl ether. Furthermore, the method was successfully applied to a range of other solid-phase-bound amino acid nucleophiles (entries 2−8). Thus, the secondary amine of proline (**1b**) (entry 2) gave **4b** in an isolated yield of 94%. Moreover, the *β*-branched amino acids **1c** and **1d** (entries 3 and 4) were transformed into products **4c** and **4d** in 90% and 95% yields, respectively.

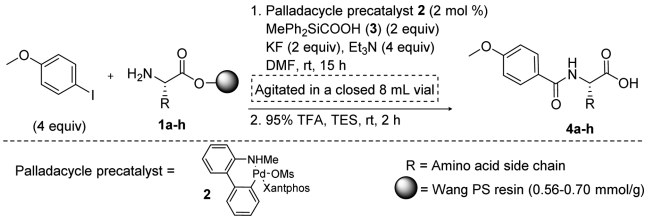
To investigate whether racemization had occurred, the optical rotation of selected products from Table 1 were compared with those synthesized from the conventional coupling of *p*-anisic acid using *N,N,N′,N′*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uranium hexafluorophosphate (HBTU) and *N,N*-diisopropylethylamine (DIPEA). In all cases, equivalent values for optical rotation were obtained, indicating the present method is performing equally well in this aspect. The fact that no epimers where formed in the synthesis of longer peptides (vide infra) further supports negligible racemization (see the Supporting Information).

Next, an array of (hetero)aryl iodides were explored to demonstrate the generality of this reaction (Scheme 1). The electron-rich aryl iodides showed good isolated yields using a low catalytic loading and only 2 equiv of **3** (**5a**,**b**, Scheme 1), while aminocarbonylation of iodobenzene to yield product **5c** required a higher catalytic loading of 5 mol % to reach full conversion of **1a** according to ^{1}H NMR analysis.

Electron-poor aryl iodides showed diminished levels of reactivity (**5d**−**f**, Scheme 1). Increasing the catalytic loading (**2**) was beneficial, but full conversion of the amine was reached only after the amount of **3** was increased to 3 equiv. This result is in agreement with the observation that CO insertion into electron-poor aryl palladium(II) intermediates is slower when compared to more electron-rich substrates.[24] Furthermore, the aminocarbonylation of 1-bromo-3-iodobenzene exhibited good chemoselectivity, which would allow further functionalization of the aryl bromide product **5g**.

In general, *meta*- and *para*-substituted aryl iodides were successfully transformed into their corresponding products **5a**−**g** (82−94% yields, Scheme 1). However, applying the reaction protocol to *ortho*-substituted substrates mainly resulted in recovery of unreacted **1a**. An increase in the reaction temperature to 45 °C, under ultrasonication, however, resulted in **5h** in 80% yield. The same was observed for *ortho*-substituted products **5i**−**k**, which all worked well at this temperature.

Table 1. Aminocarbonylation of 4-Iodoanisole with Solid-Phase-Bound Amino Acid Nucleophiles[a]



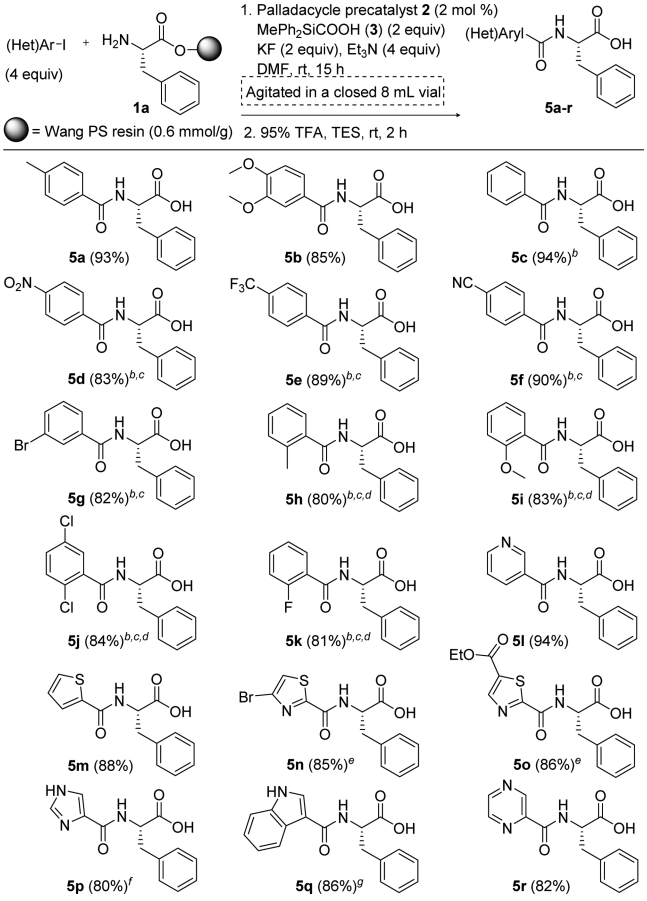
entry	amino acid	product	yield[b] (%)
1	Phe	**1a**	**4a**	95[c,d]
2	Pro	**1b**	**4b**	94[c]
3	Val	**1c**	**4c**	90
4	Thr	**1d**	**4d**	95[c]
5	Tyr	**1e**	**4e**	87[c]
6	Asn	**1f**	**4f**	93
7	Asp	**1g**	**4g**	90[c]
8	Lys	**1h**	**4h**	91[c]

[a] **1a**−**h** (0.3 mmol, 0.075 M). [b] Isolated yield after precipitation (purity ca. 87−100%). [c] Optical rotation of products was compared with the corresponding products synthesized from *p*-anisic acid, HBTU, and DIPEA. [d] 1.5 equiv (0.45 mmol) of $MePh_2SiCOOH$ did not give full conversion of the amino acid nucleophile, according to ^{1}H NMR analysis.

Because of the importance of heteroaryl cores in pharmaceutically relevant compounds, the use of heteroaryl halides as substrates was next examined. Both **5n** and **5o** were synthesized from the corresponding 4- or 5-substituted 2-bromothiazole. The aminocarbonylation of 2,4-dibromothiazole gave clean conversion with complete selectivity for the bromide positioned in the more electron poor 2-position, thus securing **5n** in an isolated yield of 85%. The ethyl ester containing product **5o** was successfully isolated in 86% yield and hence was unaffected by the treatment with aqueous TFA and TES to cleave the product from the solid-phase resin. Furthermore, due to the unfavorable

DOI: 10.1021/acs.orglett.7b01068
Org. Lett. XXXX, XXX, XXX−XXX

Scheme 1. Aminocarbonylation of a Variety of Aryl and Heteroaryl Iodides with Solid-Phase-Bound Phenylalanine[a]



[a]**1a** (0.3 mmol, 0.075 M). Isolated yield after precipitation (purity ca. 92–100%). [b]Five mol % of **2** was used. [c]Three equiv (0.6 mmol) of **3** and KF was used. [d]45 °C, under ultrasonication. [e]Synthesized from the corresponding heteroaryl bromide. [f]Synthesized from Trt-protected 4-iodoimidazole. [g]Synthesized from Boc protected 3-iodoindole.

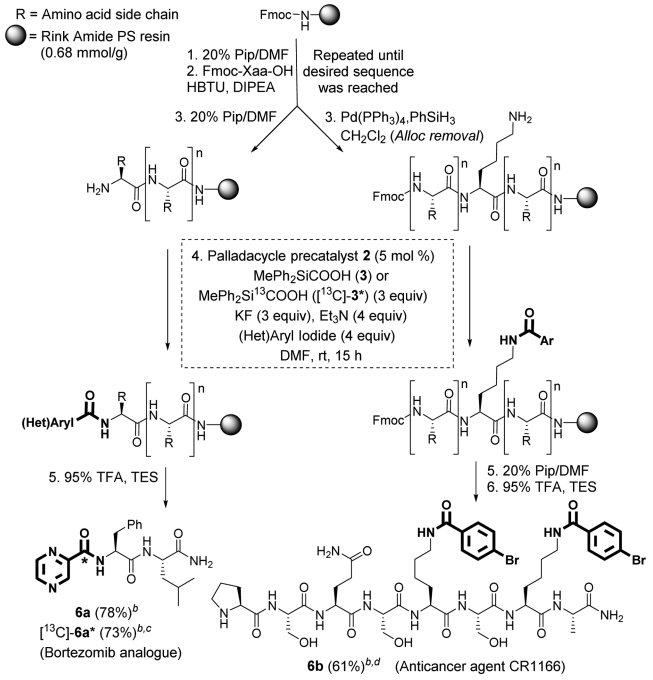
coordination of the unprotected basic nitrogen atoms in the palladium-catalyzed coupling reactions,[23,25] the acid-labile trityl (Trt)- and Boc-protecting groups, respectively, were used for the imidazole and indole substrates (to be simultaneous cleaved upon treatment with 95% TFA). This resulted in good isolated yields of **5p** and **5q** (80% and 86%, respectively).

The applicability of the method was later demonstrated in the preparation of biologically and pharmaceutically relevant peptides (Scheme 2).[10,26] The peptides were synthesized using standard Fmoc-SPPS from Rink Amide PS resin on a 0.1 mmol scale. The N-capping group in the bortezomib analogue **6a** was incorporated using the optimized aminocarbonylation protocol as the last step before final cleavage from the resin and resulted in the product as the major component in an isolated yield of 78% after preparative RP-HPLC. To avoid C-terminal amide hydrolysis it is important to not prolong the cleavage time in TFA further.[27]

The method was further extended to selective [^{13}C] acyl labeling using silacarboxylic acid [^{13}C]-**3***, thus securing peptide [^{13}C]-**6a*** in an isolated yield of 73% (Scheme 2).

In order to functionalize the nucleophilic lysine in the precursor peptide to **6b** (peptide part of the anticancer agent CR1166) the orthogonal protecting group Alloc, which is selectively deprotected using $Pd(PPh_3)_4$ and $PhSiH_3$ in dry

Scheme 2. Application of the Optimized Combined Aminocarbonylation and Fmoc-SPPS Method[a]



[a]See the Supporting Information for experimental details. [b]Isolated yields after preparative RP-HPLC. Isolated yields not compensated for possible water content. [c]$MePh_2Si^{13}COOH$ ([^{13}C]-**3***) was used for the isotopic labeling. [d]8 equiv of 1-bromo-4-iodobenzene and 4 equiv of **3** and KF were used in the aminocarbonylation.

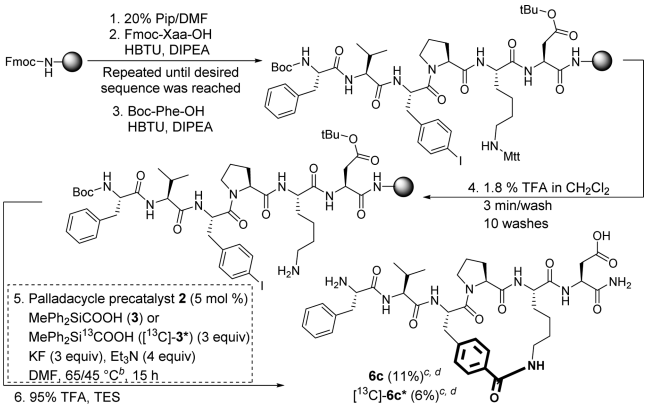
CH_2Cl_2, was chosen. In order to ensure complete conversion of the amines to their corresponding amides, 8 equiv of 1-bromo-4-iodobenzene were used together with an increased amount of the CORM. Peptide **6b** was isolated using preparative RP-HPLC in a yield of 61%.[28]

4-Iodophenylalanine and lysine protected with the highly acid-labile *N*-methyltrityl (Mtt) group were incorporated into the precursor peptides to **6c** and [^{13}C]-**6c*** in order to achieve an intramolecular aminocarbonylation after selective deprotection. Indeed, the side-chain-to-side-chain cyclized products **6c** and [^{13}C]-**6c*** were formed as major components (approximately 70% HPLC purity) and isolated in 11% and 6% yields, respectively (Scheme 3). Elevated temperature was needed for the cyclization to occur, in accordance to the synthesis of a cyclic RGD peptide in solution using a Pd-catalyzed aminocarbonylation protocol.[29] In agreement with the RGD case and with our previously reported aminocarbonylations of peptide substrates no epimerization was observed, even if heating and long reaction times were required in these cases.[21,29,30] However, N-terminal Fmoc proved to be unstable during these conditions, necessitating an exchange to Boc protection. Minor amount of intermolecular cyclization was observed, possibly as a result of the relatively high loading of the Rink amide resin (0.68 mmol/g) (see the Supporting Information).[31]

In conclusion, a mild and efficient protocol for the conversion of aryl and heteroaryl iodides to their corresponding amides through Pd-catalyzed aminocarbonylation using solid-phase-bound amino acid/peptide nucleophiles has been developed. The use of a palladacycle precatalyst enabled the reaction to be performed at room temperature, while the use of a silacarboxylic acid as an in situ CO source, compatible with the resin, enables

DOI: 10.1021/acs.orglett.7b01068
Org. Lett. XXXX, XXX, XXX–XXX

Scheme 3. Intramolecular Cyclization Using the Optimized Aminocarbonylation Method[a]



[a]See the Supporting Information for experimental details. Synthesized from Rink Amide PS resin (0.68 mmol/g). [b]**6c** was run at 65 °C and [^{13}C]-**6c*** at 45 °C, under ultrasonication. [c]Isolated yields after preparative RP-HPLC. [d]Isolated as cis/trans rotamers.

the reaction to be agitated in a single vial. The presented method is versatile, high yielding, has a broad scope, and comprises a new solid-phase synthetic approach to *N*-modifications, isotopic labeling, and cyclization of peptides. Altogether, because of its easy-to-execute and efficient nature, we foresee that this strategy will be useful in peptide-related drug discovery.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b01068.

Experimental procedures, NMR spectra, and RP-HPLC chromatograms (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: anja.sandstrom@orgfarm.uu.se.

ORCID

Troels Skrydstrup: 0000-0001-8090-5050

Anja Sandström: 0000-0001-5720-2904

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge financial support from the Anna Maria Lundin Travel Grant (Smålands Nation), the Danish National Research Foundation (Grant No. DNRF118), the Villum Foundation, and the Danish Council for Independent Research: Technology and Production Sciences. The authors thank Alfred Svan for assistance with the HRMS analysis.

■ REFERENCES

(1) Vlieghe, P.; Lisowski, V.; Martinez, J.; Khrestchatisky, M. *Drug Discovery Today* **2010**, *15*, 40.

(2) Doak, B. C.; Over, B.; Giordanetto, F.; Kihlberg. *Chem. Biol.* **2014**, *21*, 1115.

(3) Craik, D. J.; Fairlie, D. P.; Liras, S.; Price, D. *Chem. Biol. Drug Des.* **2013**, *81*, 136.

(4) Uhlig, T.; Kyprianou, T.; Martinelli, F. G.; Oppici, C. A.; Heiligers, D.; Hills, D.; Calvo, X. R.; Verhaert, P. *EuPa Open Proteomics* **2014**, *4*, 58.

(5) Chatterjee, J.; Rechenmacher, F.; Kessler, H. *Angew. Chem., Int. Ed.* **2013**, *52*, 254.

(6) Hill, T. a; Shepherd, N. E.; Diness, F.; Fairlie, D. P. *Angew. Chem., Int. Ed.* **2014**, *53*, 13020.

(7) Hanessian, S.; McNaughton-Smith, G.; Lombart, H. G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789.

(8) Lin, K. C.; Ateeq, H. S.; Hsiung, S. H.; Chong, L. T.; Zimmerman, C. N.; Castro, A.; Lee, W. C.; Hammond, C. E.; Kalkunte, S.; Chen, L. L.; Pepinsky, R. B.; Leone, D. R.; Sprague, A. G.; Abraham, W. M.; Gill, A.; Lobb, R. R.; Adams, S. P. *J. Med. Chem.* **1999**, *42*, 920.

(9) Zhou, H. J.; Aujay, M. A.; Bennett, M. K.; Dajee, M.; Demo, S. D.; Fang, Y.; Ho, M. N.; Jiang, J.; Kirk, C. J.; Laidig, G. J.; Lewis, E. R.; Lu, Y.; Muchamuel, T.; Parlati, F.; Ring, E.; Shenk, K. D.; Shields, J.; Shwonek, P. J.; Stanton, T.; Sun, C. M.; Sylvain, C.; Woo, T. M.; Yang, J. *J. Med. Chem.* **2009**, *52*, 3028.

(10) Patra, C. R.; Rupasinghe, C. N.; Dutta, S. K.; Bhattacharya, S.; Wang, E.; Spaller, M. R.; Mukhopadhyay, D. *ACS Chem. Biol.* **2012**, *7*, 770.

(11) Lim, H. A.; Ang, M. J. Y.; Joy, J.; Poulsen, A.; Wu, W.; Ching, S. C.; Hill, J.; Chia, C. S. B. *Eur. J. Med. Chem.* **2013**, *62*, 199.

(12) Xu, T.; Khanna, H.; Coward, J. K. *Bioorg. Med. Chem.* **1998**, *6*, 1821.

(13) Sutcliffe-Goulden, J. L.; O'Doherty, M. J.; Marsden, P. K.; Hart, I. R.; Marshall, J. F.; Bansal, S. S. *Eur. J. Nucl. Med. Mol. Imaging* **2002**, *29*, 754.

(14) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149.

(15) Brennführer, A.; Neumann, H.; Beller, M. *Angew. Chem., Int. Ed.* **2009**, *48*, 4114.

(16) Wannberg, J.; Larhed, M. *J. Org. Chem.* **2003**, *68*, 5750.

(17) Friis, S. D.; Lindhardt, A. T.; Skrydstrup, T. *Acc. Chem. Res.* **2016**, *49*, 594.

(18) Friis, S. D.; Taaning, R. H.; Lindhardt, A. T.; Skrydstrup, T. *J. Am. Chem. Soc.* **2011**, *133*, 18114.

(19) Cornilleau, T.; Audrain, H.; Guillemet, A.; Hermange, P.; Fouquet, E. *Org. Lett.* **2015**, *17*, 354.

(20) Friis, S. D.; Andersen, T. L.; Skrydstrup, T. *Org. Lett.* **2013**, *15*, 1378.

(21) Andersen, T. L.; Nordeman, P.; Christoffersen, H. F.; Audrain, H.; Antoni, G.; Skrydstrup, T. *Angew. Chem., Int. Ed.* **2017**, *56*, 4549.

(22) Bruno, N. C.; Niljianskul, N.; Buchwald, S. L. *J. Org. Chem.* **2014**, *79*, 4161.

(23) Friis, S. D.; Skrydstrup, T.; Buchwald, S. L. *Org. Lett.* **2014**, *16*, 4296.

(24) Wu, X. F.; Neumann, H.; Spannenberg, A.; Schulz, T.; Jiao, H.; Beller, M. *J. Am. Chem. Soc.* **2010**, *132*, 14596.

(25) Düfert, M. A.; Billingsley, K. L.; Buchwald, S. L. *J. Am. Chem. Soc.* **2013**, *135*, 12877.

(26) Teicher, B. A.; Ara, G.; Herbst, R.; Palombella, V. J.; Adams, J. *Clin. Cancer Res.* **1999**, *5*, 2638.

(27) Samaritoni, J. G.; Copes, A. T.; Crews, D. K.; Glos, C.; Thompson, A. L.; Wilson, C.; O'Donnell, M. J.; Scott, W. L. *J. Org. Chem.* **2014**, *79*, 3140.

(28) Isolated yield was affected by LC−MS analysis of resin aliquots to follow each step of the synthesis and also due to poor solubility of the crude peptide.

(29) Doi, T.; Kamioka, S.; Shimazu, S.; Takahashi, T. *Org. Lett.* **2008**, *10*, 817.

(30) Skogh, A.; Fransson, R.; Sköld, C.; Larhed, M.; Sandström, A. *J. Org. Chem.* **2013**, *78*, 12251.

(31) The isolated yields of **6c** and [13C]-**6c*** were affected by LC−MS analysis of resin aliquots to follow each step of the synthesis and by successive HPLC runs in order to separate the desired product from the coeluting side product, formed from intermolecular cyclization, as well as minor loss during Mtt cleavage. See the Supporting Information.

DOI: 10.1021/acs.orglett.7b01068
Org. Lett. XXXX, XXX, XXX−XXX