Organic LETTERS

Letter

pubs.acs.org/OrgLett

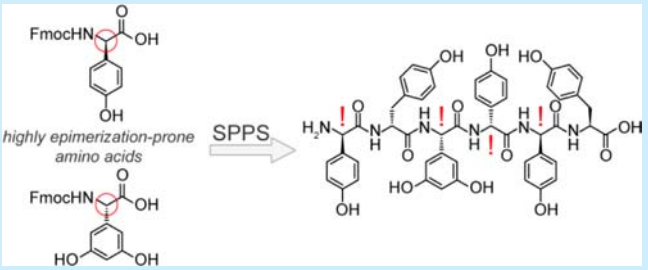
A Facile Fmoc Solid Phase Synthesis Strategy To Access Epimerization-Prone Biosynthetic Intermediates of Glycopeptide Antibiotics

Clara Brieke and Max J. Cryle*

Department of Biomolecular Mechanisms, Max-Planck Institute for Medical Research, Jahnstrasse 29, 69120 Heidelberg, Germany

Supporting Information

ABSTRACT: A rapid protocol based on Fmoc-chemistry for the solid phase peptide synthesis of vancomycin- and teicoplanin-type peptides is described. Epimerization of highly racemization-prone arylglycine derivatives is suppressed through optimized Fmoc-deprotection and coupling conditions. Starting from easily accessible Fmoc-protected amino acids, this strategy enables the enantioselective synthesis of peptides corresponding to intermediates found in vancomycin and teicoplanin biosynthesis with excellent purity and in high yields (38%−71%).



Vancomycin and teicoplanin belong to the class of glycopeptide antibiotics produced by various *Streptomyces* species. They act by inhibiting cell-wall biosynthesis of many Gram-positive bacteria and remain "last-resort" antibiotics against *Enterococci* or methicillin-resistant *Staphylococcus aureus* (MRSA) infections.[1] As bacteria have begun to evolve resistance to these compounds, there is a great need for novel, highly active derivatives of these antibiotics; despite impressive advances,[2] this is hampered by the highly complex total synthesis of these natural products.[3] An alternate approach therefore is to use semisynthetic or biotechnological approaches, which can help to develop modified derivatives.[4]

The unique structural framework (Figure 1) of these glycopeptides, which is crucial for their antibiotic activity,[4a] is biosynthetically derived from a nonribosomally produced heptapeptide precursor that is oxidatively cross-linked between the aromatic amino acids by Cytochrome P450 enzymes (Oxys):[5] the order of these cross-links and the respective Oxys have now been assigned for vancomycin[6] as well as for teicoplanin,[7] and several examples have been structurally characterized.[8] As the chemical synthesis of these cross-links remains as one of the great challenges in the synthesis of novel glycopeptide antibiotic derivatives, there is significant interest in understanding these enzymatic transformations in more detail, with an outlook to use the enzymes as biocatalysts for obtaining access to novel aglycone derivatives with the potential for improved antibiotic activity against resistant bacteria.

The group of Robinson has shown that it is possible to perform the first oxidative cross-linking reaction with OxyB to form the vancomycin C−O−D ring *in vitro* by using precursor hexa- and heptapeptides bound to peptidyl carrier protein domains (PCP) of the vancomycin NRPS.[9] However, this approach is currently limited by difficulties in the chemical synthesis of the linear peptide precursors, due to the high

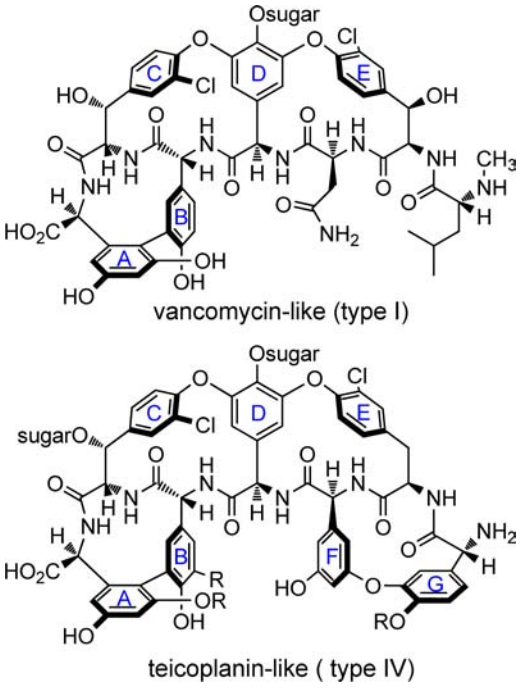


Figure 1. Core structures of different glycopeptide antibiotics.

sensitivity to epimerization of the arylglycine derivatives 4-hydroxyphenylglycine (Hpg) and especially 3,5-dihydroxyphenylglycine (Dpg) in the respective aglycones:[10] these residues have previously been thought to be incompatible with the two standard solid phase peptide synthesis (SPPS)

Received: March 20, 2014
Published: April 14, 2014

   dx.doi.org/10.1021/ol500840f | *Org. Lett.* 2014, 16, 2454−2457

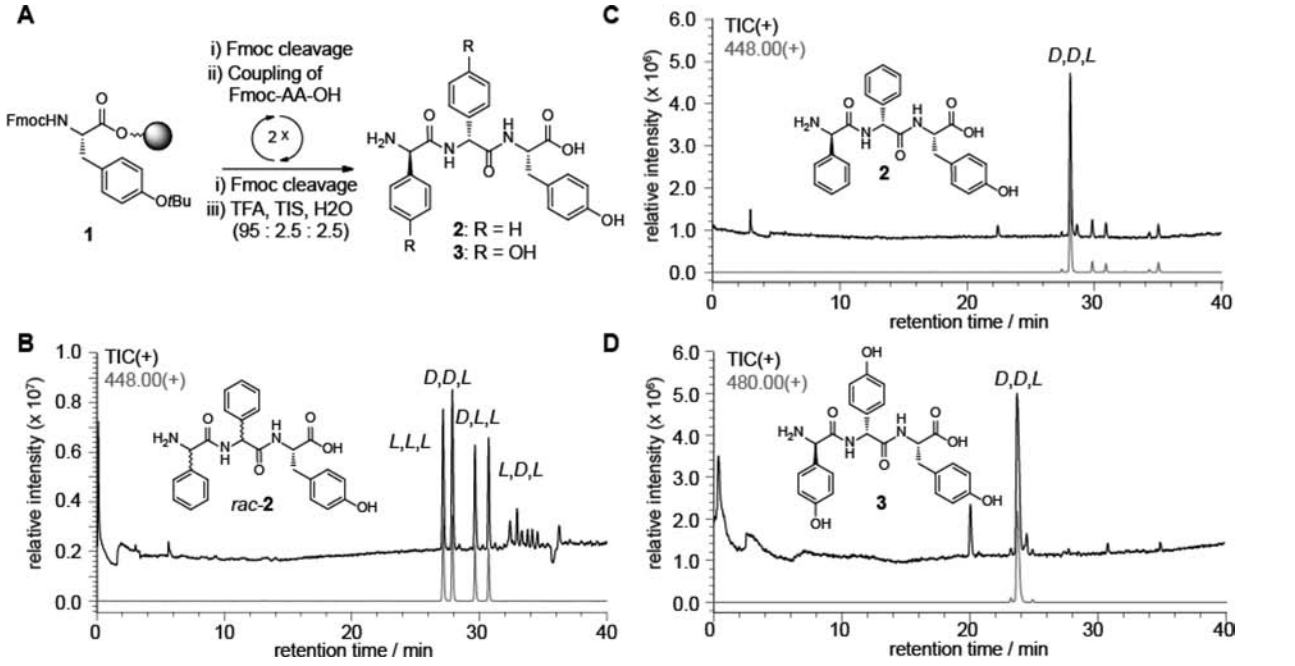


Figure 2. Optimization of Fmoc cleavage and coupling conditions for arylglycine derivatives: (A) General synthesis of tripeptides **2** and **3**; nonoptimized: (i) 20% piperidine/DMF, (ii) 4 equiv of Fmoc-D-Phg-OH **4**, 4 equiv of HCTU, 8 equiv of NMM; optimized: (i) 1% DBU/DMF, (ii) 4 equiv of **4** or Fmoc-D-Hpg-OH **5**, 4 equiv of COMU, 4 equiv of TEA. (B–D) LC-MS traces of crude synthesis mixtures of tripeptides **2** and **3** using nonoptimized (B) and optimized conditions (C and D). Total ion counts (TIC) and single ion counts of the respective peptides are shown. Stereochemistry of *rac*-**3** was assigned by synthesis of all four possible diastereomers (see SI).

strategies (Boc- and Fmoc-chemistry)[11,12] and required Alloc-chemistry.[11,13] Disadvantages of Alloc-chemistry are the use of a palladium-mediated cleavage protocol and the tedious synthesis of all protected amino acid building blocks, while, in contrast, Fmoc chemistry has the advantages of a large range of available building blocks, simple synthesis of such starting materials where this is not the case, and the possibility to monitor the cleavage step during synthesis using UV-absorption.

Therefore, we sought to develop a streamlined and efficient SPPS protocol for vancomycin- and teicoplanin-type biosynthetic precursor peptides using Fmoc-chemistry. For this a series of conditions were explored to investigate the susceptibility of phenylglycine residues to racemization, initially using the model tripeptide NH_2-Phg-Phg-Tyr-OH **2**, synthesized from preloaded Fmoc-Tyr-Wang resin **1**. An initial experiment, performed using standard SPPS conditions—20% piperidine for *N*-deprotection and 0.4 M NMM for amino acid activation—afforded an equimolar ratio of all four possible diastereomers, resulting from racemization of the phenylglycine residues (Figure 2B). To overcome the effect of piperidine different conditions for Fmoc-removal were tested, focusing on alternative reagents reported to be compatible with the solid phase synthesis of base-labile thioesters, such as TBAF[14] or DBU mixtures[15,16] (for the full range of conditions tested; see Table S1, SI). Experiments using DBU gave the best results. Use of HOBt as an additive[16] was equally effective as DBU alone; however, it had the disadvantage of not allowing UV-monitoring of the reaction due to the absorption of the DBU/HOBt solution. The addition of a secondary amine to scavenge the diphenylfulvene product of Fmoc deprotection was tested and had deleterious effects on the extent of racemization. Thus, a 1% solution of DBU in DMF was chosen for deprotection of the *N*-Fmoc group, which enables the effective deprotection of phenylglycine residues in less than 30 s with very little racemization of the C^{α}-carbon.

For the exploration of a coupling reagent,[17] alternative uronium-type reagents and the phosphorus reagent DEPBT were chosen (see Table S1, Supporting Information (SI)). DEPBT has been reported in the liquid phase coupling of arylglycine derivatives among others by Boger and co-workers.[2,12] From these reagents HATU[18] and COMU[19] in combination with TEA quickly proved to be superior in reducing epimerization during coupling, while a coupling time of 30 min was optimal to maximize coupling efficiency. Using more than 1 equiv of base to activator led to significant epimerization (entries 5–10, Table S1). HATU afforded slightly higher coupling yields compared to COMU (entry 11 vs 15), although the ratio between coupling efficiency and epimerization proved to be best using COMU. Also, we did not observe advantages of performing double coupling with half the equivalents of activator and base (entries 13/14). Thus, 4 equiv of COMU together with 4 equiv of TEA was chosen as an optimized Fmoc-SPPS coupling mixture (Figure 2C; Table S1, entry 15). Interestingly, during the course of investigation we observed a reduced tendency to racemization when substituting phenylglycine residues for 4-hydroxyphenylglycine resulting in tripeptide **3** (Figure 2D), most likely due to a stabilizing electron-donating mesomeric effect from the *para*-hydroxyl groups.

These optimized conditions (Figure 2) were then applied to the SPPS of vancomycin-type hexapeptide **6** (Figure 3), starting from **1** using commercially available building blocks and Fmoc-D-Hpg-OH **5**, prepared in one step from the free amino acid (SI). Following cleavage of **6** from the resin using a TFA/TIS/H_2O (95:2.5:2.5) mixture, analytical HPLC-MS analysis revealed one major product of correct mass (Figures 3 and S9): thus, essentially no side reactions or epimerization had

dx.doi.org/10.1021/ol500840f | *Org. Lett.* 2014, 16, 2454–2457

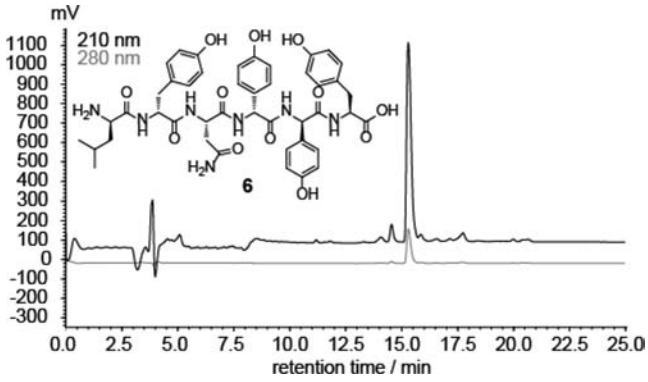


Figure 3. HPLC trace of crude product from the synthesis of vancomycin-type peptide **6**.

occurred during SPPS, global deprotection, and cleavage from the solid phase. Following HPLC-purification, hexapeptide **6** could be isolated in an overall yield of 71%, with the structure confirmed by MS and by the assignment of the ^{1}H and ^{13}C NMR spectra using 2D NMR experiments (COSY, ROESY, HSQC, HMBC). It should be emphasized that, with this optimized protocol for Fmoc-chemistry, not only the synthetic effort for the synthesis of vancomycin-type peptides is reduced but also, compared to a previously reported optimized protocol using Alloc-chemistry, where the coupling for each amino acid was performed overnight,[11] the speed of synthesis has also been increased tremendously.

In the next step, we focused on teicoplanin-type peptides. Teicoplanin is often a more potent antibiotic compared to vancomycin,[1] and it is structurally more complex. Additionally (and to the best of our knowledge) there have been no *in vitro* investigations of the respective Cytochromes P450 to date, likely due to the difficulties in preparing the required peptide substrates. From the synthetic point of view, the SPPS of teicoplanin-type peptides is yet more demanding than vancomycin-type peptides for two reasons: first, three Hpg residues have to be incorporated into the peptide as opposed to two Hpg residues for vancomycin-type peptides, and second, at position 3 of the peptide the highly racemization-prone amino acid 3,5-dihydroxyphenylglycine must be incorporated. As enantiomerically pure Dpg is very expensive to purchase or requires extensive synthesis, we first focused on the synthesis of peptide **7** having substituted Dpg with Phg at position 3 of the peptide (Figure 4). Using the same synthesis protocol as that for peptide **6**, teicoplanin-type hexapeptide **7** was obtained in high purity (Figures 4A and S11, SI) and could be isolated in 61% yield after HPLC purification.

As initial SPPS trials under the optimized conditions revealed a low coupling efficiency and high tendency for epimerization for Fmoc-Dpg-OH **8**, we next focused on a systematic comparison of different activating bases to alleviate this problem (see Table 1 and Figure S7). Thus, the less basic and sterically hindered bases 2,4,6-trimethylpyridine (TMP, $pK_a = 7.43$[20]),[21] 2,6-dimethylpyridine (2,6-DMP, $pK_a = 6.60$[20]), and the dibasic compound DABCO ($pK_a = 8.9$[20]) were chosen for the synthesis of model tripeptides NH_2-L-Phg-L-Dpg-L-Tyr-OH **9** and **2**. Best results for reducing epimerization and enhancing coupling efficiency were obtained with the least basic 2,6-DMP, yielding 96% of the correct enantiomer of Dpg-containing peptide **9**.

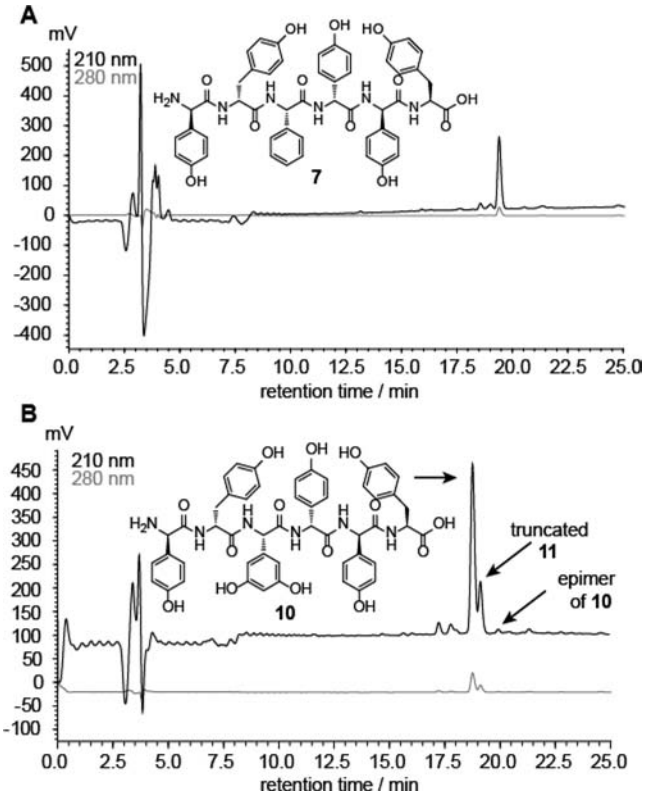
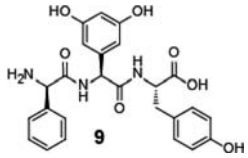


Figure 4. HPLC traces of crude synthesis mixtures for teicoplanin-type peptides **7** (A) and **10** (B) after cleavage from the solid phase. In the synthesis of **10**, incomplete coupling of **8** resulted in truncated peptide **11**; epimerized side product of **10** is highlighted.

Table 1. Yields and Epimerization Rates for Model Peptides 2 and 9 Using Different Activating Bases[a]



peptide	base	equiv	tri- vs dipeptide [%][b]	correct diastereomer [%][b]
9	TEA	4	75	57
2	TEA	4	94	90
2	TMP	4	92	90
2	2,6-DMP	4	94	94
2	DABCO	2	64	96
9	2,6-DMP	4	88	96

[a]Fmoc cleavage: 1% DBU in DMF, 0.5 min. Coupling: COMU (4 equiv), 30 min, in DMF. [b]Yields were determined by HPLC analysis.

Thus, for the SPPS of teicoplanin-type peptide **10** with Dpg at position 3, the coupling of Fmoc-L-Dpg-OH **8** was performed using the specific Dpg-conditions with 2,6-DMP as the base. Despite a significantly reduced coupling efficiency of **8**, which resulted in a 36% yield of truncated peptide NH_2-D-Hpg-D-Tyr-D-Hpg-D-Hpg-L-Tyr-OH **11**, only minimal amounts of epimerization were detected. By performing double coupling with 3 equiv of **8**, the amount of truncated peptide **11** could be reduced to 22% (Figure 4B and Figure S13) resulting in an overall yield of 38% of peptide **10** following HPLC purification. In contrast to double coupling, no reduction of truncation was

dx.doi.org/10.1021/ol500840f | *Org. Lett.* 2014, 16, 2454−2457

observed by increasing the excess of **8**. Thus, it is likely that truncation could be reduced yet further, if desired, by executing multiple coupling cycles with **8** as the combination of COMU together with 2,6-DMP, for the coupling shows almost complete suppression of epimerization of Dpg.

The use of unprotected amino acid building blocks raised the question of acylation of such residues on capping and/or N-terminal modification with acetylating reagents (Figure S8, SI). While partial acylation of Hpg residues was observed, this did not interfere with the peptide synthesis when acylation was used for capping and could be selectively removed postsynthesis without inducing racemization of the peptide using a $NaHCO_3/H_2O$/MeOH solution (Figure S8, SI). Thus, both capping and N-terminal acylation can be incorporated in the peptide synthesis route if desired.

In conclusion, we have developed a protocol enabling the SPPS assembly of vancomycin- or teicoplanin-type peptides possessing multiple epimerization-prone arylglycine derivatives using Fmoc-chemistry. This approach is significantly simplified over previously reported methods[11] and shows a reduction in time of synthesis. Thus, this protocol facilitates the preparation of peptide substrates that we currently are using for the investigation of the later stages of glycopeptide biosynthesis. As arylglycine derivatives are widespread components of peptidic natural products, we anticipate that this approach will also aid in the exploration of other biosynthetic pathways, such as those of β-lactam antibiotics[22] or other peptide antibiotics.[12,23]

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedure, HPLC-MS chromatograms, and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: Max.Cryle@mpimf-heidelberg.mpg.de.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to acknowledge the Deutsche Forschungsgemeinschaft for an Emmy-Noether Fellowship (CR 392/1-1, M.J.C.) and the Max Planck Society for financial support. M.J.C. is also grateful for the constant support of I. Schlichting (MPI-Hd).

■ REFERENCES

(1) Williams, D. H.; Bardsley, B. *Angew. Chem., Int. Ed.* **1999**, *38*, 1172.

(2) Xie, J.; Okano, A.; Pierce, J. G.; James, R. C.; Stamm, S.; Crane, C. M.; Boger, D. L. *J. Am. Chem. Soc.* **2012**, *134*, 1284.

(3) Boger, D. L. *Med. Res. Rev.* **2001**, *21*, 356.

(4) (a) Nicolaou, K. C.; Boddy, C. N. C.; Bräse, S.; Winssinger, N. *Angew. Chem., Int. Ed.* **1999**, *38*, 2096. (b) Wolter, F.; Schoof, S.; Süssmuth, R. D. In *Glycopeptides and Glycoproteins*; Wittmann, V., Ed.; Topics in Current Chemistry; Springer: Berlin, Heidelberg, 2007; pp 143−185. (c) Crane, C. M.; Boger, D. L. *J. Med. Chem.* **2009**, *52*, 1471. (d) Fowler, B. S.; Laemmerhold, K. M.; Miller, S. J. *J. Am. Chem. Soc.* **2012**, *134*, 9755. (e) Pathak, T. P.; Miller, S. J. *J. Am. Chem. Soc.* **2012**, *134*, 6120. (f) Pathak, T. P.; Miller, S. J. *J. Am. Chem. Soc.* **2013**, *135*, 8415.

(5) (a) Cryle, M. J. *Metallomics* **2011**, *3*, 323. (b) Cryle, M. J.; Brieke, C.; Haslinger, K. In *Amino Acids, Peptides and Proteins*; Farkas, E., Ryadnov, M., Eds.; Royal Society of Chemistry: Cambridge, 2014; Vol. *38*, pp 1−36.

(6) (a) Bischoff, D.; Pelzer, S.; Holtzel, A.; Nicholson, G. J.; Stockert, S.; Wohlleben, W.; Jung, G.; Süssmuth, R. D. *Angew. Chem., Int. Ed.* **2001**, *40*, 1693. (b) Bischoff, D.; Pelzer, S.; Bister, B.; Nicholson, G. J.; Stockert, S.; Schirle, M.; Wohlleben, W.; Jung, G.; Süssmuth, R. D. *Angew. Chem., Int. Ed.* **2001**, *40*, 4688.

(7) Hadatsch, B.; Butz, D.; Schmiederer, T.; Steudle, J.; Wohlleben, W.; Süssmuth, R.; Stegmann, E. *Chem. Biol.* **2007**, *14*, 1078.

(8) (a) Zerbe, K.; Pylypenko, O.; Vitali, F.; Zhang, W.; Rouset, S.; Heck, M.; Vrijbloed, J. W.; Bischoff, D.; Bister, B.; Süssmuth, R. D.; Pelzer, S.; Wohlleben, W.; Robinson, J. A.; Schlichting, I. *J. Biol. Chem.* **2002**, *277*, 47476. (b) Pylypenko, O.; Vitali, F.; Zerbe, K.; Robinson, J. A.; Schlichting, I. *J. Biol. Chem.* **2003**, *278*, 46727. (c) Cryle, M. J.; Staaden, J.; Schlichting, I. *Arch. Biochem. Biophys.* **2011**, *507*, 163.

(9) (a) Zerbe, K.; Woithe, K.; Li, D. B.; Vitali, F.; Bigler, L.; Robinson, J. A. *Angew. Chem., Int. Ed.* **2004**, *43*, 6709. (b) Woithe, K.; Geib, N.; Zerbe, K.; Li, D. B.; Heck, M.; Fournier-Rousset, S.; Meyer, O.; Vitali, F.; Matoba, N.; Abou-Hadeed, K.; Robinson, J. A. *J. Am. Chem. Soc.* **2007**, *129*, 6887. (c) Schmartz, P. C.; Wölfel, K.; Zerbe, K.; Gad, E.; El Tamany, E. S.; Ibrahim, H. K.; Abou-Hadeed, K.; Robinson, J. A. *Angew. Chem., Int. Ed.* **2012**, *51*, 11468.

(10) Smith, G. G.; Sivakua, T. *J. Org. Chem.* **1983**, *48*, 627.

(11) Li, D. B.; Robinson, J. A. *Org. Biomol. Chem.* **2005**, *3*, 1233.

(12) Dettner, F.; Hänchen, A.; Schols, D.; Toti, L.; Nusser, A.; Süssmuth, R. D. *Angew. Chem., Int. Ed.* **2009**, *48*, 1856.

(13) Freund, E.; Robinson, J. A. *Chem. Commun.* **1999**, 2509.

(14) Ueki, M.; Amemiya, M. *Tetrahedron Lett.* **1987**, *28*, 6617.

(15) Wade, J. D.; Bedford, J.; Sheppard, R. C.; Tregear, G. W. *Pept. Res.* **1991**, *4*, 194.

(16) Clippingdale, A. B.; Barrow, C. J.; Wade, J. D. *J. Pept. Sci.* **2000**, *6*, 225.

(17) El-Faham, A.; Albericio, F. *Chem. Rev.* **2011**, *111*, 6557.

(18) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.

(19) El-Faham, A.; Funosas, R. S.; Prohens, R.; Albericio, F. *Chem.—Eur. J.* **2009**, *15*, 9404.

(20) (a) International Union of Pure and Applied Chemistry; Commission on Electroanalytical Chemistry; Perrin, D. D. *Dissociation constants of organic bases in aqueous solution*; Butterworths: London, 1965. (b) Benoit, R. L.; Lefebvre, D.; Fréchette, M. *Can. J. Chem.* **1987**, *65*, 996.

(21) Carpino, L. A.; Ionescu, D.; El-Faham, A. *J. Org. Chem.* **1996**, *61*, 2460.

(22) Kelly, W. L.; Townsend, C. A. *J. Am. Chem. Soc.* **2002**, *124*, 8186.

(23) Liu, W. T.; Kersten, R. D.; Yang, Y. L.; Moore, B. S.; Dorrestein, P. C. *J. Am. Chem. Soc.* **2011**, *133*, 18010.