Organic LETTERS

Letter

pubs.acs.org/OrgLett

Peptide Macrocyclization Assisted by Traceless Turn Inducers Derived from Ugi Peptide Ligation with Cleavable and Resin-Linked Amines

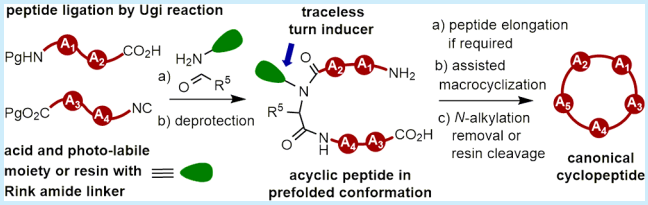
Alfredo R. Puentes,[†,‡,§] Micjel C. Morejón,[†,‡,§] Daniel G. Rivera,*[,†,‡] and Ludger A. Wessjohann*[,†]

[†]Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Weinberg 3, 06120 Halle/Saale, Germany

[‡]Center for Natural Products Research, Faculty of Chemistry, University of Havana, Zapata y G, 10400 Havana, Cuba

Supporting Information

ABSTRACT: A multicomponent approach enabling the installation of turn-inducing moieties that facilitate the macrocyclization of short and medium-size oligopeptides is described. The strategy comprises the Ugi ligation of peptide carboxylic acids and isocyanopeptides in the presence of aldehydes and acid or photolabile amines followed by cyclization and cleavage of the backbone *N*-substituents to render canonical cyclopeptides. Implementing the approach on solid phase with the use of Rink amide resins led to a new class of backbone amide linker strategy.



The head-to-tail cyclization of short peptides stands as one of the most challenging procedures in synthetic chemistry.[1] Typical problems encountered in this process are the *C*-terminal epimerization and cyclooligomerization.[1] Whereas new coupling reagents[2] and synthetic tools[1,3] have been developed to partially solve these issues, they are intrinsically dependent on both the sequence and the ring size and, therefore, difficult to generalize in short peptides. Besides the classic solutions of either conducting the peptide macrocyclization at extreme dilution (even 0.01 mM) or employing pseudo-dilution conditions,[4] the most successful strategy has been the incorporation of turn-inducing elements capable of facilitating the macrocyclic ring closure by bringing both termini closer.[1a]

Owing to their capacity to favor the *cis*-amide bond, proline and *N*-methyl amino acids are well-known turn-inducing elements that improve peptide cyclization yields when embedded midway along the acyclic precursor.[1,5] The incorporation of D-amino acids[6] and pseudo-prolines,[7] i.e., (4*S*)-oxazolidine-4-carboxylic acids derived from serine and threonine, into linear peptides also makes the macroyclization much more efficient. Nonetheless, the employment of these structural elements to assist the peptide cyclization is limited to the presence of such amino acids in the target cyclic peptide. A more versatile strategy is the use of a traceless turn inducer, i.e., a moiety that can be installed in the middle of any peptide to bend its backbone and be subsequently cleaved or transformed into a native peptide structure after cyclization. Remarkable examples are the use of the photolabile 2-hydroxy-6-nitrobenzyl[8] *N*-substituent, the acid-labile dimethoxybenzyl (DMB)[9] and *tert*-butyloxycarbonyl[10] (Boc) *N*-substituents, as well as dehydrophenylalanine[11] as traceless turn inducers capable of assisting the cyclization of short peptides.

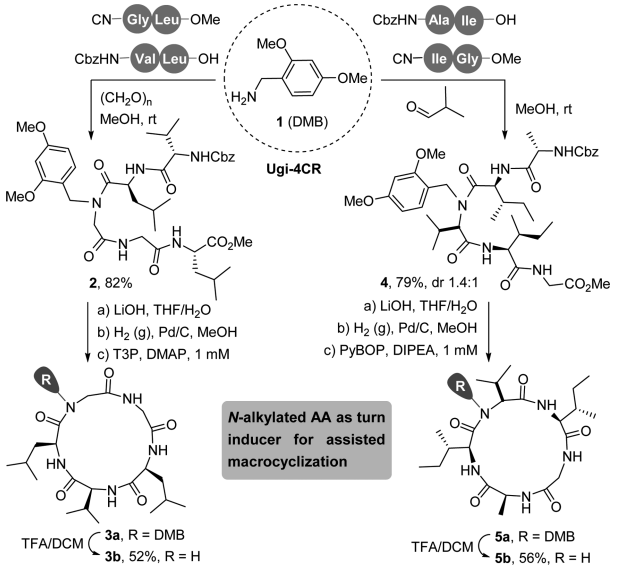
Herein, we describe a novel strategy for the solution- and solid-phase macrocyclization of peptides assisted by traceless turn inducers. The approach comprises the ligation of a peptide carboxylic acid and an isocyanopeptide by the Ugi four-component reaction (Ugi-4CR) using a cleavable amine (or a resin amine linker), thus leading to a peptide including an *N*-alkylated amino acid midway along the sequence. An aldehyde as fourth Ugi component defines the newly formed α-amino acid side chain. Our endeavor was to use the known turn-inducing capability of this *N*-alkylated peptide fragment to facilitate the cyclization both in solution and on-resin.

We have previously documented that *N*-alkylated pentapeptide derived from Ugi reactions are relatively easy to cyclize[12] and that, in the case of a bulk *N*-substituent, the acyclic peptide occurs in a ß-turn conformation.[12a] As a result, we envisioned that the Ugi reaction could be a straightforward way to install acid- and photolabile *N*-substituents[8,9] capable of improving the cyclization efficiency, thus acting as traceless macrocyclization assistants. Scheme 1 depicts the use of 2,4-dimethoxybenzylamine (**1**) in the multicomponent[13] ligation of dipeptide acids and isocyanides to produce *N*-alkylated pentapeptides **2** and **4** in very good yields. Subsequent deprotection of both termini followed by macrolactamization at 1 mM concentration with either propanephosphonic acid anhydride[14] (T3P, known for minimizing C-terminal epimerization) or PyBOP afforded cyclic peptides **3a** and **5a**. The DMB *N*-substituent was subsequently removed under acidic conditions to afford canonical peptides **3b** and **5b** in good yields of isolated products over four steps. The use of a prochiral oxo component leads to a ca. 1.5:1 mixture of diastereomers due to the poor stereoselectivity of the Ugi-4CR, which is perhaps the only drawback of this strategy.

Received: June 10, 2017

DOI: 10.1021/acs.orglett.7b01761
Org. Lett. XXXX, XXX, XXX−XXX

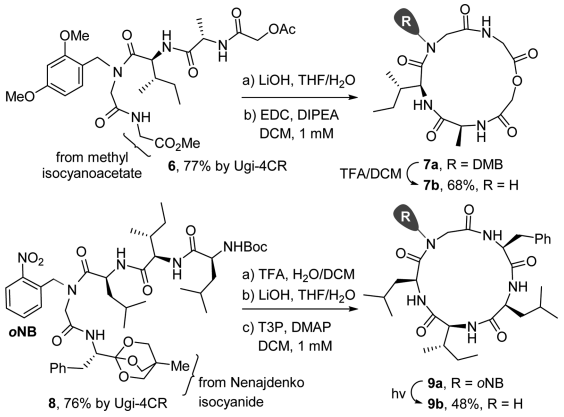
Scheme 1. Macrocyclization of Pentapeptides Assisted by a Traceless Turn-Inducing Core Derived from the Ugi Reaction



To assess the macrocyclization improvement provided by the *N*-substituted Ugi reaction fragment, we also carried out the T3P-mediated cyclization of the model peptide H-Val-Leu-Gly-Gly-Leu-OH and compared both results. This study proved that at 1 mM concentration and 12 h of reaction the cyclization of the non-*N*-alkylated peptide rendered 21% yield of cyclic peptide **3b**, while the deprotected peptide **2** led to 52% yield of isolated **3b**. HPLC/ESI-MS analysis of the crude product also showed the presence of ca. 8% of the unreacted linear precursor, but no dimer was detected.

We next evaluated the efficacy of a photolabile *N*-substituent and the scope of a macrolactonization protocol. As shown in Scheme 2, *N*-alkylated peptide **6** was produced by an Ugi

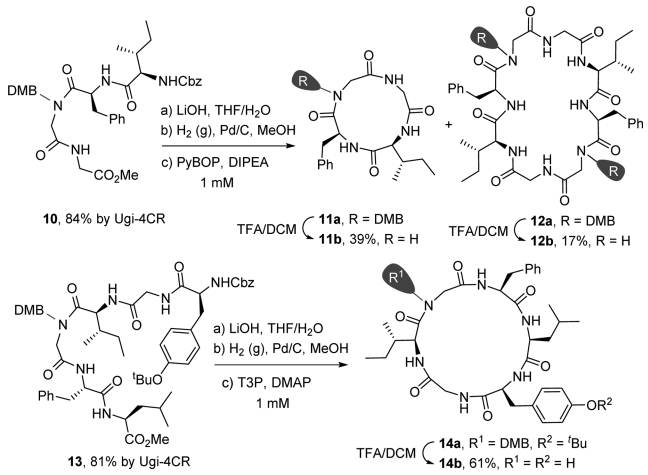
Scheme 2. Macrocyclization of *N*-Alkylated (Depsi)peptides Including Traceless Acid- and Photolabile *N*-Substituents



reaction in good yield and subsequently subjected to saponification and standard macrolactonization with EDC. Final DMB cleavage led to isolated cyclic depsipeptide **7b** in 68% yield over three steps. Alternatively, 2-nitrobenzylamine (*o*NB)[15] and phenylalanine-derived Nenajdenko isocyanide[16] were employed in the multicomponent synthesis of the *N*-alkylated peptide **8**, which was subsequently deprotected and macrocyclized with T3P for 12 h. In this case, the cyclization/oNB cleavage protocol produced the cyclic peptide **9b** in 48% yield after HPLC purification. HPLC/ESI-MS analysis after DMB cleavage showed 75% of purity for the cyclic monomer, with only 4% of the *C*-terminal epimer. On the other hand, HPLC traces of a parallel macrocyclization with the non-*N*-alkylated analogue showed 51% of purity of cyclopeptide **9b**, with 7% of the dimer and 9% of the *C*-terminal epimer. Again, the Ugi-derived fragment improved the cyclization efficiency as compared with the canonical peptide and reduces cyclodimerization. It should be mentioned that these examples encompass the ligation of tripeptide carboxylic acids and amino acid-derived isocyanides, which move the *N*-alkylation position away from the favorable middle and place it one amino acid toward the *C*-terminus. However, even if this is not the ideal positioning, the turn inducer produced better results than without it.

After proving the efficacy of the method with pentapeptides, we extended the scope to tetra- and hexapeptides, with the former ones being very challenging due to their cyclodimerization propensity. Scheme 3 depicts the cyclization of tetrapeptide

Scheme 3. Macrocyclization of Tetra- And Hexapeptides Assisted by a Traceless *N*-Alkylated Ugi Reaction Fragment



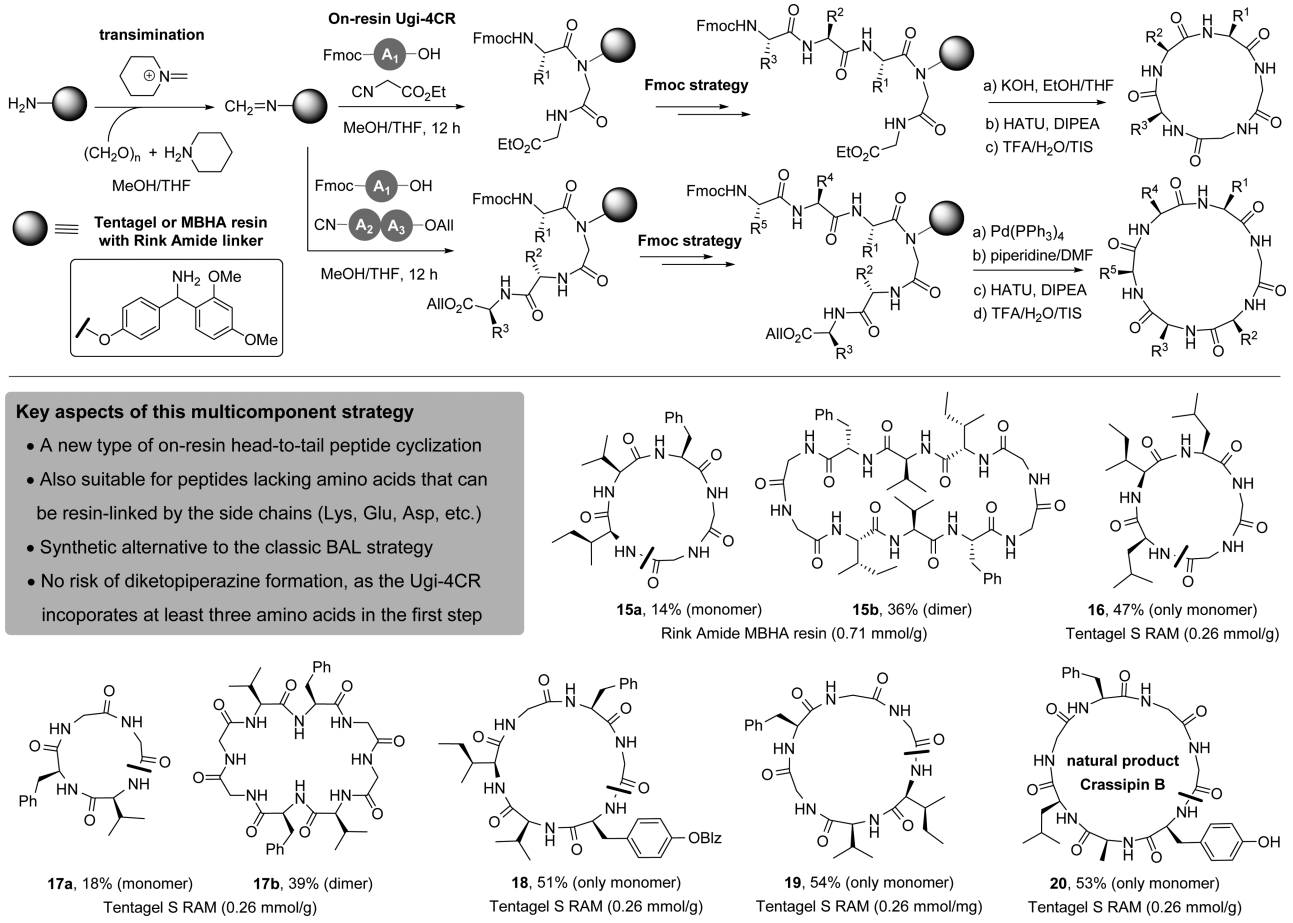
10 and hexapeptides **13**, previously produced in good yields by Ugi-4CR. As peptide **10** has two Gly in its sequence, we intentionally introduced the bulky Ile at the *N*-terminus so that the cyclization would not be so much facilitated by sequence and an accurate assistant effect could be assessed. Cyclization of **10** followed by DMB cleavage rendered cyclic tetrapeptide **11b** and the cyclodimer **12b** in 39% and 17% yield, respectively, after HPLC purification (see the Supporting Information). Nonetheless, our method did prove much more effective than the cyclization of the analogous model peptide H-Ile-Phe-Gly-Gly-OH, which led almost exclusively to the formation of the dimer in its acyclic and cyclic variants. Analytical HPLC traces of the crude cyclization showed a monomer/dimer ratio of 2.6:1 for *N*-alkylated peptide **10** and a ratio of 1:19 for cyclization of the non-*N*-alkylated one.

As expected, cyclization of deprotected hexapeptide **13** proceeded smoothly to furnish cyclic peptide **14b** in 61% yield after DMB/*t*Bu cleavage and purification. Only 4% of the *C*-terminal epimer was detected by analytical HPLC/ESI-MS.

In our opinion, the highest potential of the multicomponent/turn induction strategy to assist peptide cyclization lies on its possible adaptation to the solid phase. Over the years, much

DOI: 10.1021/acs.orglett.7b01761
Org. Lett. XXXX, XXX, XXX−XXX

Scheme 4. Solid-Phase Synthesis of Cyclic Peptides by a Multicomponent Backbone Amide Linker (BAL) Strategy



effort has been devoted to develop effective protocols for the on-resin head-to-tail cyclization of peptides that do not have trifunctional amino acids such as Glu, Asp, Lys, His, and Ser, whose side chain are commonly linked to the resin leaving the two termini available for cyclization.[17] A major advance in this field was the development of the so-called backbone amide linker (BAL) strategy, introduced independently by Albericio and Barany[18] and Ellman.[19] These approaches enable the on-resin *C*-terminal modification and cyclization of peptides anchored to the resin by an internal amide *N*-substituent.[20] The protocol comprises the attachment of an acid-labile substituted benzaldehyde to the polymeric support, followed by reductive amination and acylation to incorporate two amino acids.

Herein, we extend this multicomponent strategy to the synthesis of backbone amide-linked peptides and their subsequent derivatization by peptide growth and final cyclization. As illustrated in Scheme 4, our approach does not require the initial incorporation of a cleavable aldehyde linker and subsequent reductive amination and acylation, but instead it comprises the direct incorporation of an Fmoc-amino acid and an isocyanide peptide or amino acid to the polymer support having the Rink amide linker. To carry out the on-resin Ugi-4CR, an aminocatalytic transimination step using paraformaldehyde and piperidine is required prior addition of the acid and the isocyanide.[21] Other aldehydes can be employed without such transimination step but may result in a final mixture of two diastereomers. As at least a tripeptide is attached to the resin in the first step, this enables bypassing the difficult acylation step of the secondary amine and, even more important, avoids diketopiperazine (DKP) formation, which is common in BAL approaches if Fmoc deprotection is used at the dipeptide stage.[18] In addition, our multicomponent strategy preserves high flexibility for protecting groups, as either ethyl or allyl esters can be used at the *C*-terminus and Boc/*t*Bu groups at the side chains, being orthogonal to the Fmoc methodology employed for peptide elongation.

The scope of this multicomponent BAL strategy was assessed using oligopeptides ranging from four to seven amino acids. As shown in Scheme 4, the use of the Rink amide polystyrene resin MBHA with a loading of 0.71 mmol/g led to the cyclic pentapeptide **15a** in only 14% yield, while the dimer **15b** was isolated in 36% yield. Alternatively, when a Tentagel S RAM resin with a lower loading (0.26 mmol/g) was used, only the cyclic pentapeptide (monomer) **16** was isolated in good overall yield. However, using the same Tentagel S RAM resin, a mixture of cyclic tetrapeptide (monomer) **17a** (18%) and the dimer **17b** (39%) was obtained, proving the difficulty of cyclizing tetrapeptides without cyclodimerization. To avoid that, perhaps a resin with a much lower loading is required, as it has been proven in previously reported works.[17]

The on-resin macrocyclization of hexapeptides proceeded smoothly to furnish cyclic peptides **18** and **19** in good overall yield and without cyclodimerization. Peptide **18** derives from the initial incorporation of Fmoc-Ile-OH and isocyanide CN-Phe-Gly-OMe, while the synthesis of **19** begins with Fmoc-Phe-OH and ethyl isocyanoacetate. To further prove the scope of this method, we undertook the total synthesis of the natural product crassipin B.[22] This cyclic heptapeptide was produced in overall

DOI: 10.1021/acs.orglett.7b01761
Org. Lett. XXXX, XXX, XXX−XXX

53% yield through the initial multicomponent attachment of Fmoc-Phe-OH and ethyl isocyanoacetate to the resin, followed by peptide growing using the Fmoc strategy and consecutive macrocyclization and acidic cleavage from resin. In summary, this new multicomponent BAL strategy can be considered as a valuable and efficient improvement in peptide synthesis. Several factors such as the low synthetic cost for incorporating the first three or four amino acids into the resin, the great tolerance of protecting groups, and the complete absence of DKP formation, among others, make it a plausible alternative to classic BAL approaches. It is especially valuable for cyclic peptides having at least one Gly or Aib residue or for the construction of D/L peptide library including natural and non-natural amino acids, as higher aldehydes can be used leading to the two diastereomers.

In conclusion, we have developed an efficient strategy to assist peptide macrocyclization both in solution and on the solid phase. The approach uses the versatile Ugi-4CR for the ligation of peptides and the simultaneous incorporation of a removable *N*-alkyl substituent that serves as a turn-inducing moiety and facilitates the macrocyclic ring closure. Its assistance to the macrocyclization was proven with a variety of tetra- to heptapeptides. An extension of this concept to the solid phase led to the development of a new BAL strategy, relying for the first time on the multicomponent incorporation of at least three amino acids in one step instead of the three on-resin steps required in traditional BAL protocols for attaching a dipeptide fragment. Owing to its synthetic prospects, this concept can be very useful for the peptide, combinatorial, and medicinal chemistry communities.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b01761.

Experimental procedures, NMR, and ESI-MS spectra of selected intermediates and final cyclic peptides (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: dgr@fq.uh.cu.
*E-mail: wessjohann@ipb-halle.de.

ORCID

Daniel G. Rivera: 0000-0002-5538-1555

Author Contributions

§A.R.P. and M.C.M. contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

D.G.R. is grateful to the Alexander von Humboldt Foundation for an Experienced Researcher Fellowship.

■ REFERENCES

(1) (a) White, C. J.; Yudin, A. K. *Nat. Chem.* **2011**, *3*, 509. (b) Lambert, J. N.; Mitchell, J. P.; Roberts, K. D. *J. Chem. Soc. Perkin Trans. 1* **2001**, 471.

(2) (a) El-Faham, A.; Albericio, F. *Chem. Rev.* **2011**, *111*, 6557. (b) Montalbetti, C. A. G. N.; Falque, V. *Tetrahedron* **2005**, *61*, 10827.

(3) (a) Davies, J. S. *J. Pept. Sci.* **2003**, *9*, 471. (b) Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev.* **1997**, *97*, 2243.

(4) Macrocyclizations under pseudo-dilution conditions comprise the use of syringe pumps; see, for example: (a) Malesevic, M.; Strijowski, U.; Bächle, D.; Sewald, N. *J. Biotechnol.* **2004**, *112*, 73. (b) Wessjohann, L. A.; Kreye, O.; Rivera, D. G. *Angew. Chem., Int. Ed.* **2017**, *56*, 3501.

(5) (a) Chatterjee, J.; Mierke, D. F.; Kessler, H. *Chem. - Eur. J.* **2008**, *14*, 1508. (b) Favre, M.; Moehle, K.; Jiang, L.; Pfeiffer, B.; Robinson, J. A. *J. Am. Chem. Soc.* **1999**, *121*, 2679. (c) Takeuchi, Y.; Marshall, G. R. *J. Am. Chem. Soc.* **1998**, *120*, 5363. (d) Rothe, M.; Steffen, K.-D.; Rothe, I. *Angew. Chem., Int. Ed. Engl.* **1965**, *4*, 356.

(6) (a) Kessler, H.; Haase, B. *Int. J. Pept. Protein Res.* **1992**, *39*, 36. (b) Yongye, A. B.; Li, Y.; Giulianotti, M. A.; Yu, Y.; Houghten, R. A.; Martínez-Mayorga, K. *J. Comput.-Aided Mol. Des.* **2009**, *23*, 677.

(7) (a) Dumy, P.; Keller, M.; Ryan, D. E.; Rohwedder, B.; Wöhr, T.; Mutter, M. *J. Am. Chem. Soc.* **1997**, *119*, 918. (b) Skropeta, D.; Jolliffe, K. A.; Turner, P. *J. Org. Chem.* **2004**, *69*, 8804. (c) Sayyadi, N.; Skropeta, D.; Jolliffe, K. A. *Org. Lett.* **2005**, *7*, 5497. (d) Fairweather, K. A.; Sayyadi, N.; Luck, I. J.; Clegg, J. K.; Jolliffe, K. A. *Org. Lett.* **2010**, *12*, 3136.

(8) Meutermans, W. D. F.; Bourne, G. T.; Golding, S. W.; Horton, D. A.; Campitelli, M. R.; Craik, D.; Scanlon, M.; Smythe, M. L. *Org. Lett.* **2003**, *5*, 2711.

(9) (a) El Haddadi, M.; Cavelier, F.; Vives, E.; Azmani, A.; Verducci, J.; Martinez, J. *J. Pept. Sci.* **2000**, *6*, 560. (b) van Maarseveen, J. H.; Horne, W. S.; Ghadiri, M. R. *Org. Lett.* **2005**, *7*, 4503.

(10) Cavelier-Frontin, F.; Achmad, S.; Verducci, J.; Jacquier, R.; Pepe, G. *J. Mol. Struct.: THEOCHEM* **1993**, *286*, 125.

(11) Le, D. N.; Riedel, J.; Kozlyuk, N.; Martin, R. W.; Dong, V. M. *Org. Lett.* **2017**, *19*, 114.

(12) (a) Rivera, D. G.; Vasco, A. V.; Echemendía, R.; Concepción, O.; Pérez, C. S.; Gavín, J. A.; Wessjohann, L. A. *Chem. - Eur. J.* **2014**, *20*, 13150. (b) Barreto, A. F. S.; Vercillo, O. E.; Birkett, M. A.; Caulfield, J. C.; Wessjohann, L. A.; Andrade, C. K. Z. *Org. Biomol. Chem.* **2011**, *9*, 5024. (c) Vercillo, O. E.; Andrade, C. K. Z.; Wessjohann, L. A. *Org. Lett.* **2008**, *10*, 205.

(13) For the use of DMB amine in Ugi reactions, see: (a) Shaw, A. Y.; Xu, Z.; Hulme, C. *Tetrahedron Lett.* **2012**, *53*, 1998. (b) Plant, A.; Thompson, P.; Williams, D. M. *J. Org. Chem.* **2009**, *74*, 4870.

(14) T3P is a registered trademark. Original report: (a) Wissmann, H.; Kleiner, H.-J. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 133. (b) Review: Llanes García, A. L. *Synlett* **2007**, *2007*, 1328.

(15) Sung, K.; Chen, F.-L.; Huang, P.-C. *Synlett* **2006**, *2006*, 2667.

(16) Zhdanko, A. G.; Nenajdenko, V. G. *J. Org. Chem.* **2009**, *74*, 884.

(17) (a) de Leon Rodriguez, L.; Weidkamp, A. J.; Brimble, M. A. *Org. Biomol. Chem.* **2015**, *13*, 6906. (b) Alcaro, M. C.; Sabatino, G.; Uziel, J.; Chelli, M.; Ginanneschi, M.; Rovero, P.; Papini, A. *J. Pept. Sci.* **2004**, *10*, 218. (c) Alsina, J.; Rabanal, F.; Giralt, E.; Albericio, F. *Tetrahedron Lett.* **1994**, *35*, 9633. (d) Kates, S. A.; Solé, N. A. *Tetrahedron Lett.* **1993**, *34*, 1549. (e) Mazur, S.; Jayalekshmy, P. *J. Am. Chem. Soc.* **1979**, *101*, 677.

(18) (a) Jensen, K. J.; Songster, M. F.; Vagner, J.; Alsina, J.; Albericio, F.; Barany, G. In *Peptides – Chemistry, Structure and Biology: Proceedings of the Fourteenth American Peptide Symposium (1995)*; Kaumaya, P. T. P., Hodges, R. S., Eds.; Mayflower Scientific: Kingswinford, U.K., 1996; pp 30–32. (b) Jensen, K. J.; Alsina, J.; Songster, M. F.; Vagner, J.; Albericio, F.; Barany, G. *J. Am. Chem. Soc.* **1998**, *120*, 5441. (c) Alsina, J.; Yokum, T. S.; Albericio, F.; Barany, G. *J. Org. Chem.* **1999**, *64*, 8761.

(19) (a) Boojamra, C. G.; Burow, K. M.; Ellman, J. A. *J. Org. Chem.* **1995**, *60*, 5742. (b) Boojamra, C. G.; Burow, K. M.; Thompson, L. A.; Ellman, J. A. *J. Org. Chem.* **1997**, *62*, 1240.

(20) For reviews, see: (a) Góngora-Benítez, M.; Tulla-Puche, J.; Albericio, F. *ACS Comb. Sci.* **2013**, *15*, 217. (b) Boas, U.; Brask, J.; Jensen, K. J. *Chem. Rev.* **2009**, *109*, 2092. (c) Alsina, J.; Jensen, K. J.; Albericio, F.; Barany, G. *Chem. - Eur. J.* **1999**, *5*, 2787.

(21) (a) Morales, F. E.; Garay, H. E.; Muñoz, D. F.; Augusto, Y. E.; Otero-Gonzalez, A. J.; Reyes Acosta, O.; Rivera, D. G. *Org. Lett.* **2015**, *17*, 2728. (b) Morejón, M. C.; Laub, A.; Westermann, B.; Rivera, D. G.; Wessjohann, L. A. *Org. Lett.* **2016**, *18*, 4096.

(22) Jia, A. Q.; Tan, N. H.; Yongping, Y.; Wu, S. G.; Wang, L. Q.; Zhou, J. *Act. Bot. Sin.* **2004**, *46*, 625–630.

DOI: 10.1021/acs.orglett.7b01761
Org. Lett. XXXX, XXX, XXX–XXX