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Aminocatalysis-Mediated on-Resin Ugi Reactions: Application in the Solid-Phase Synthesis of *N*-Substituted and Tetrazolo Lipopeptides and Peptidosteroids

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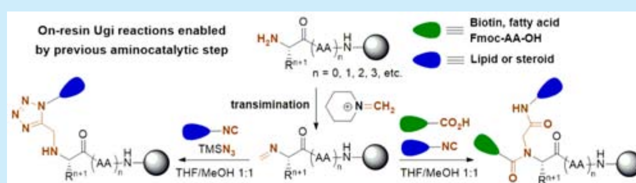
Supporting Information

ABSTRACT: A new solid-phase protocol for the synthesis of *N*-substituted and tetrazolo peptides is described. The strategy relies on the combination of aminocatalysis-mediated on-resin Ugi reactions and peptide couplings for the *N*-alkylation of peptides at selected sites, including the *N*-terminal double lipidation, the simultaneous lipidation/biotinylation, and the steroid/lipid conjugation via tetrazole ring formation. The solid-phase Ugi four-component reactions were enabled by on-resin transimination steps prior to addition of the acid and isocyanide components. The strategy proved to be suitable for the feasible incorporation of complex *N*-substituents at both termini and at internal positions, which is not easily achievable by other solid-phase methods.



The utilization of isocyanide-based multicomponent reactions (MCRs) for the synthesis of peptidomimetics[1] and naturally occurring peptides[2] has continuously grown over recent years. Among such a class of MCRs, the Ugi four-component reaction,[3] and its synthetic variants,[4] has exhibited the greatest applicability owing to the high chemical efficiency, complexity, and diversity-generating character. Another fact favoring applications in peptide chemistry is that the Ugi reaction employs the amino and carboxylic groups of peptides,[5] which upon reaction with an oxo-component and an isocyanide give rise to an *N*-substituted dipeptide moiety.[3] Recent applications of the Ugi reaction in the field of peptide chemistry include the synthesis of bioactive (natural product-like) peptides,[6] the development of peptide catalysts for asymmetric transformations,[7] and the implementation of multicomponent macrocyclization strategies[8] toward cyclic peptides[9] and peptoids.[10]

Literature survey reveals that solution-phase approaches have been typically used for either the synthesis or derivatization of peptides by means of the Ugi reaction. This is intriguing considering the fact that solid-phase protocols prevail in the area of peptide synthesis,[11] and that most relevant methods for peptide derivatization have been efficiently translated from solution to solid phase. One of the major limitations of peptide pharmaceuticals is their low metabolic stability and fast clearance, which is partially solved by derivatizations such as terminal capping, cyclization, and PEGylation,[12] albeit peptides remain cleavable by *endo*-peptidases. A natural solution to further improve proteolytic stability is partial *N*-alkylation (typically methylation) of internal amide bonds,[13] which can also be accomplished by the Ugi reaction. Accordingly, we envisioned that the utilization of this multicomponent reaction for the *N*-alkylation of amide bonds during solid-phase peptide synthesis (SPPS) would provide a powerful tool to the peptide chemistry community in the pursuit of peptide pharmaceuticals with better pharmacological properties.

This letter describes an efficient and reproducible method implementing on-resin Ugi reactions with peptides, and its utilization in combination with peptide couplings for the solid-phase synthesis of *N*-substituted and tetrazolo peptides. Various groups have previously described Ugi reactions on resin-bound amines,[14] albeit such protocols are not suitable for the Ugi derivatization of large peptides bound to resins. In such reports, pro-chiral aliphatic and aromatic aldehydes were utilized, which leads to two stereoisomers as a result of the low stereoselectivity of the Ugi reaction. In our hands, Ugi reactions with such aldehydes and resin-bound oligopeptides of variable sequences resulted in the formation of the desired *N*-substituted peptides, but with very low purity and as difficult-to-separate mixtures of diastereomers. Aiming at developing a general method leading to *N*-substituted peptides with actual pharmaceutical potential, we endeavored to carry out on-resin Ugi reactions with formaldehyde as an oxo component, so that a single stereoisomer is formed. However, new experimental difficulties showed up due to the inefficient imine formation using paraformaldehyde with either resin-bound oligopeptides

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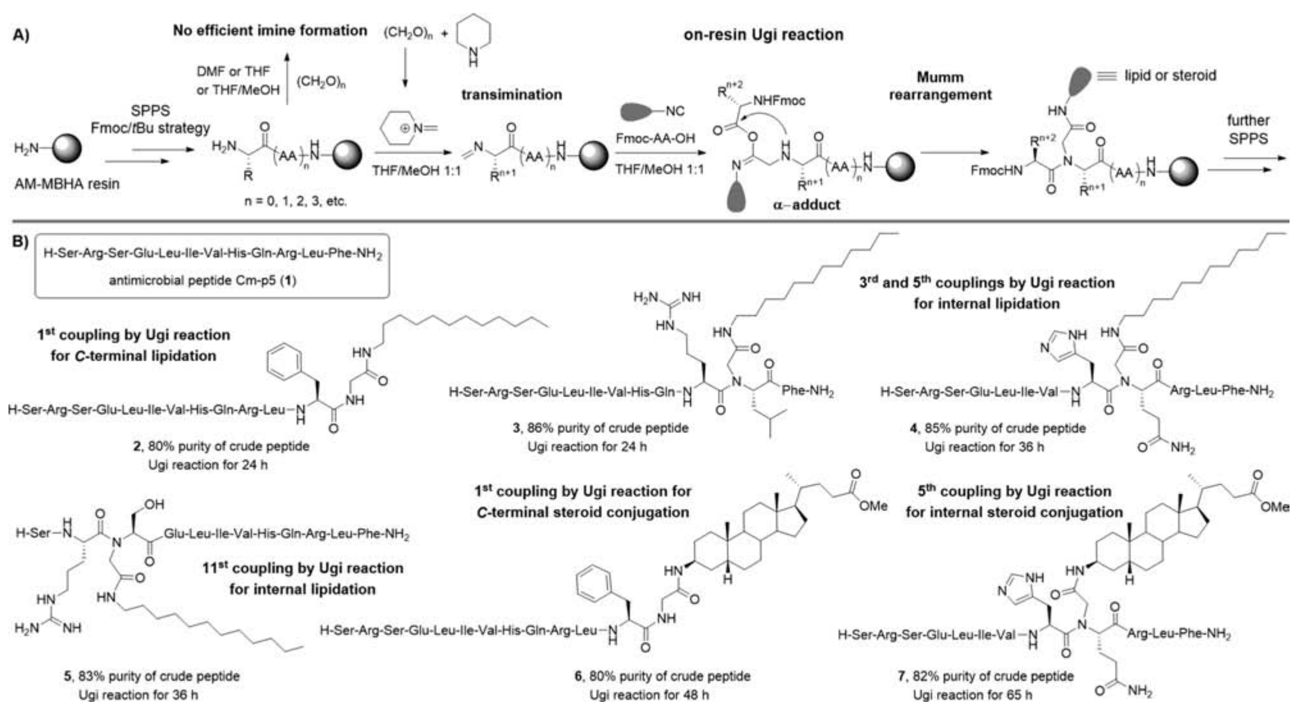
Scheme 1. (A) Solid-Phase Protocol for the Synthesis of *N*-Substituted Peptides by Combination of on-Resin Ugi Reaction and Peptide Couplings; (B) Structures of *N*-Lipidated and *N*-Steroidal Peptides Analogues of 1



(with free terminal amine) or resin-bound amines. As shown in Scheme 1, initial experiments were unsatisfactory in various solvents and led to almost no product formation upon addition of the Fmoc-amino acid and the isocyanide. To avoid the polymeric reagent, trioxane was employed as the carbonyl input, but results were also irreproducible and sequence dependent. After several attempts, the solution for this was the introduction of an aminocatalytic transimination step that ensures the efficient imine formation prior to addition of the acid and isocyanide. This process derives from a previous solution-phase procedure that uses secondary amine organocatalysts for the preparation of *N*-sulfinyl and *N*-sulfonyl imines.[15] However, this type of aminocatalytic approach has not been implemented either in solid phase or in a multicomponent reaction sequence.

We chose piperidine, a reagent already used in SPPS, as an organocatalyst to enable the on-resin transimination step via an iminium ion activation pathway. Typically, 4 equiv of paraformaldehyde and piperidine are added to the resin-bound peptide in 1:1 THF/MeOH and the heterogeneous mixture is stirred for 30 min. Subsequent washing of the beads with THF, followed by addition of the corresponding Fmoc-amino acid and isocyanide dissolved in 1:1 THF/MeOH, enabled the efficient on-resin Ugi reaction. The 1:1 THF/MeOH mixture turned out to be the best solvent, owing to the great swelling capacity of polystyrene-based resins in THF and the need for a polar protic solvent such as MeOH to favor the Ugi reaction. The 1:1 DCM/MeOH mixture also proved to be suitable, and it is the solvent of choice to introduce Fmoc-His(Trt)-OH due to insolubility in THF/MeOH. However, reactions performed in DMF (a solvent of good resin swelling capacity typically used for peptide coupling) were not satisfactory and only led to partial conversion after several days of reaction. MeOH as the only solvent was not considered owing to its poor swelling capacity for polystyrene-based resins.

To assess the scope of combining the on-resin multicomponent reaction with peptide coupling protocols, we chose the recently developed antimicrobial peptide *Cm*-p5 (**1**) as a model compound.[16] SPPS of *N*-alkylated derivatives of *Cm*-p5 were carried out manually on the Am-MBHA resin by a stepwise Fmoc/tBu strategy (see Supporting Information), as part of a program aiming for the development of more potent and metabolically stable products. As depicted in Scheme 1A, the Ugi reaction proceeds via the intermediate α-adduct, which next evolves through an intramolecular acylation step (Mumm rearrangement) to the final *N*-substituted dipeptide fragment. A remarkable feature of this on-resin Ugi protocol is that the reaction conversion can be followed using the ninhydrin test (i.e., Kaiser test),[17] in a similar way as in traditional SPPS. The ninhydrin test is typically performed after each coupling cycle to check either the presence or absence of free primary amino groups (i.e., a positive test is indicated by blue resin beads). We found that unreacted imine groups can be detected as well by a positive ninhydrin test, which is expected due to the instability of the imine under the test conditions. Thus, about 20–30 min after addition of the Fmoc-amino acid and isocyanide, the ninhydrin test turns negative for primary amino groups, indicating complete consumption of the imine. At this moment, the test gives a pale yellow solution and red resin beads, which is characteristic of imino acids such as proline, clearly showing the formation of the α-adduct bearing a secondary amino group. Varied comparative studies with different sequences and chain lengths proved that the Mumm rearrangement is the slow reaction step of on-resin Ugi reactions. Finally, completion of the Ugi reaction is communicated by a negative ninhydrin test wherein both the solution and the resin beads appear yellow.

Alternatively, we used RP-HPLC analysis to monitor this specific step. Several parallel syntheses followed by RP-HPLC and MS analysis of the released peptides were carried out with different reaction times (see Supporting Information), in most

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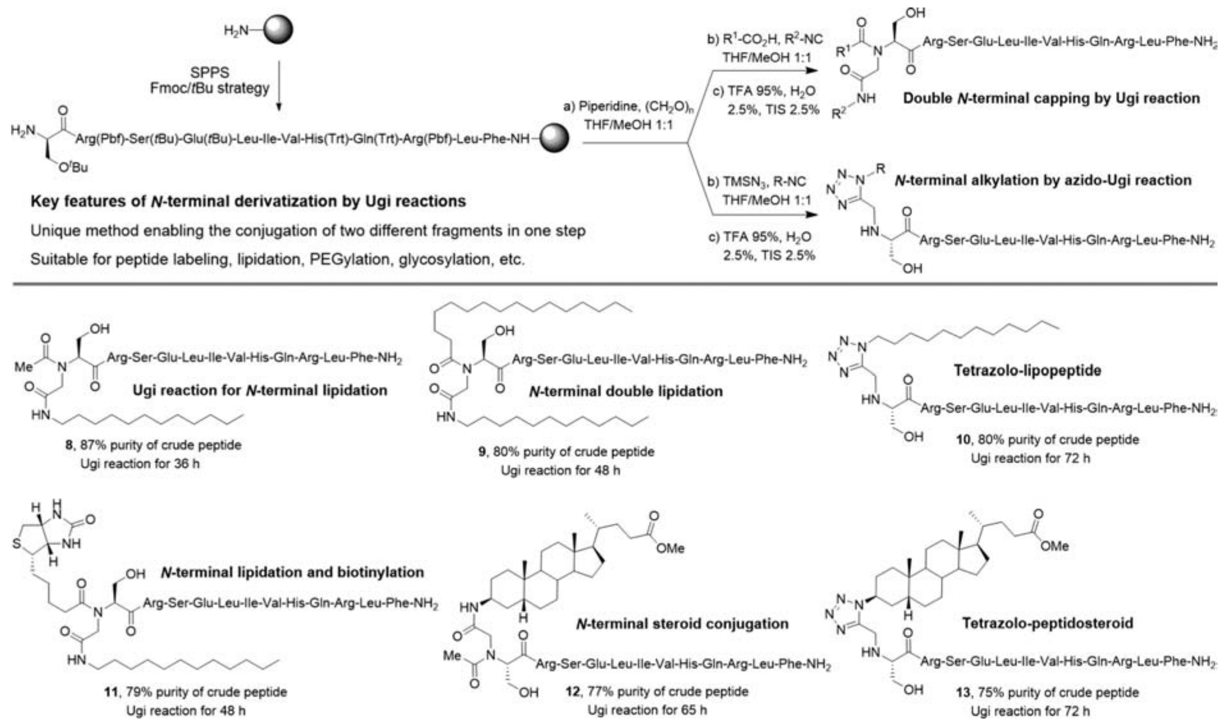
Scheme 2. On-Resin Ugi Reactions for the *N*-Terminal Derivatization of Peptide 1 with Lipids, Steroids, and Biotin



cases proving the truthful character of the ninhydrin test to follow the on-resin Ugi reaction. An exception is when the bulky isocyanosteroid is employed, which usually provides a negative test (i.e., both solution and beads yellow) just after a few hours of reaction, but in contrast RP-HPLC indicates uncompleted conversion. Nonetheless, most RP-HPLC analyses of peptides produced relying on the result of the ninhydrin test led to the absence of the peak corresponding to the unreacted peptide precursor. On the other hand, peptides with the complete amino acid sequence but lacking the *N*-alkylation moiety were frequently detected. Although they were found in a trace amount (ca. 5%), this conveys the existence of a parallel isocyanide-mediated mechanism that activates the Fmoc-amino acid enabling the amidation step.[18]

In SPPS, the incorporation of *N*-methyl amino acids frequently requires more than one coupling cycle and expensive coupling agents.[19] This becomes even more challenging for the introduction of bigger *N*-alkyl groups. In this regard, the current protocol provides an advantage over known methods in terms of easy generation of molecular complexity via *N*-substitution. To prove this, we synthesized analogues of peptide **1** with lipidic chains and a bulky steroidal skeleton as *N*-substituents at different positions of the peptide backbone.

Scheme 1B shows *N*-lipidated and *N*-steroidal peptides synthesized in solid-phase by the combination of on-resin Ugi reactions and peptide couplings. Despite their complex structures, these *N*-substituted peptides were produced with a purity that is either higher than or about 80%, as determined by analytical RP-HPLC. All compounds were characterized by MS and purified by preparative RP-HPLC to >95% purity. Lipopeptide **2** and peptidosteroid **6** were produced by carrying out the Ugi reaction for the incorporation of the first amino acid. As a result, these peptides include at the *C*-terminus an additional Gly residue functionalized with the lipidic and steroidal tails. This new and simple way of *C*-terminal derivatization shows great potential in SPPS, as this type of modification is known to require ingenious design of linkers and cleavable functional groups, as well as challenging manipulation of the resins.[20] Alternatively, peptides **3**, **4**, and **5** were designed to be *N*-lipidated at internal positions, while peptide **7** also includes an internal steroidal *N*-substituent. It is worth noting that peptide **7** is a novel class of *N*-steroidal peptide[21] not easily available in such a direct manner by other peptide coupling procedures.

We sought to further exploit the multicomponent nature of the Ugi reaction for the introduction of more than one substituent at the *N*-terminus and to implement other variations of the Ugi reaction. Scheme 2 illustrates this endeavor with the synthesis of *N*-substituted and tetrazolo peptides derived from Ugi reactions with resin-bound (fully protected) peptide **1**. Initially, we produced peptides **8** and **12** using acetic acid and *n*-dodecylisocyanide[22] and methyl 3-isocyanolitocholanoate, respectively. As expected, the Ugi reaction with the linear isocyanide was faster and more efficient than that with the bulky one, although the former proved to be slower than that with peptides having shorter sequences (see compounds **2**–**5**). Similarly to peptide couplings, we found that the longer the sequence is, the slower the on-resin Ugi reaction takes place. Importantly, synthesis of peptides **9** and **11** exemplifies the potential of this method to allow for the double lipidation and the simultaneous lipidation/biotinylation in one step, a key feature that differentiates this protocol from other solid-phase approaches.

Lipopeptide **10** and peptidosteroid **13** were prepared by the modified Ugi reaction based on the use of hydrazoic acid,[23] leading to formation of a 1,5-disubtituted tetrazole ring at the *N*-terminus. This type of Ugi reaction, taking place by an electrocyclic ring closure of the α-adduct, is slower than the classic Ugi and certainly requires RP-HPLC monitoring to assess its completion. The reason for this is that the ninhydrin test is not effective for conversion analysis because it gives the same result for both the final product and the intermediate α-

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adduct, as both bear a secondary amine. However, this reaction has the advantage of not annulling the basic character of the terminal residue while introducing a metabolically stable tetrazole ring as linkage between the peptide and either the lipidic or steroidal fragment.

In conclusion, we have proven that the combination of aminocatalysis-mediated Ugi reactions and peptide coupling is a robust strategy for the *N*-alkylation of peptides in solid phase. The development of the on-resin transimination step was the key to enabling the multicomponent reaction and therefore the incorporation of structurally complex *N*-substituents that are not easily introduced by other solid-phase methods. We hope this strategy opens up a venue of possibilities for the derivatization of biologically relevant peptides, including crucial modifications such as fluorescent labeling, PEGylation, glycosylation, etc.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures, RP-HPLC chromatograms and MS spectra of all final compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01147.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) (a) Rotstein, B. H.; Zaretsky, S.; Rai, V.; Yudin, A. K. *Chem. Rev.* **2014**, *114*, 8323. (b) Koopmanschap, G.; Ruijter, E.; Orru, R. V. A. *Beilstein J. Org. Chem.* **2014**, *10*, 544. (c) Gulevich, A. V.; Zhdanko, A. G.; Orru, R. V. A.; Nenajdenko, V. G. *Chem. Rev.* **2010**, *110*, 5235. (d) Wessjohann, L. A.; Rhoden, C. R. B.; Rivera, D. G.; Vercillo, O. E. *Top. Heterocycl. Chem.* **2010**, *23*, 19. (e) Dömling, A. *Chem. Rev.* **2006**, *106*, 17.

(2) (a) Slobbe, P.; Ruijter, E.; Orru, R. V. A. *Med. Chem. Commun.* **2012**, *3*, 1189. (b) Touré, B. B.; Hall, D. G. *Chem. Rev.* **2009**, *109*, 4439.

(3) Ugi, I.; Meyr, R.; Fetzer, U.; Steinbrücker, C. *Angew. Chem.* **1959**, *71*, 386.

(4) Dömling, A.; Ugi, I. *Angew. Chem., Int. Ed.* **2000**, *39*, 3168.

(5) Ugi, I.; Marquarding, D.; Urban, R. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Weinstein, B., Ed.; Marcel Dekker: New York, 1982; pp 246−289.

(6) (a) Pando, O.; Stark, S.; Denkert, A.; Porzel, A.; Preusentanz, R.; Wessjohann, L. A. *J. Am. Chem. Soc.* **2011**, *133*, 7692. (b) Tanino, T.; Ichikawa, S.; Shiro, M.; Matsuda, A. *J. Org. Chem.* **2010**, *75*, 1366. (c) Znabet, A.; Polak, M. M.; Janssen, E.; de Kanter, F. J. J.; Turner, N. J.; Orru, R. V. A.; Ruijter, E. *Chem. Commun.* **2010**, *46*, 7918. (d) Socha, A. M.; Tan, N. Y.; LaPlante, K. L.; Sello, J. K. *Bioorg. Med. Chem.* **2010**, *18*, 7193. (e) Dömling, A.; Beck, B.; Eichelberger, U.; Sakamuri, S.; Menon, S.; Chen, Q.-Z.; Lu, Y.; Wessjohann, L. A. *Angew. Chem., Int. Ed.* **2006**, *45*, 7235.

(7) (a) Scatena, G. S.; de la Torre, A. F.; Cass, Q. B.; Rivera, D. G.; Paixão, M. W. *ChemCatChem* **2014**, *6*, 3208. (b) de la Torre, A. F.; Rivera, D. G.; Ferreira, M. A. B.; Corrêa, A. G.; Paixão, M. W. *J. Org. Chem.* **2013**, *78*, 10221. (c) Znabet, A.; Ruijter, E.; de Kanter, F. J. J.; Köhler, V.; Helliwell, M.; Turner, N. J.; Orru, R. V. A. *Angew. Chem., Int. Ed.* **2010**, *49*, 5289.

(8) (a) White, C. J.; Yudin, A. K. *Nat. Chem.* **2011**, *3*, 509. (b) Masson, G.; Neuville, L.; Bughin, C.; Fayol, A.; Zhu, J. *Top. Heterocycl. Chem.* **2010**, *25*, 1. (c) Wessjohann, L. A.; Rivera, D. G.; Vercillo, O. E. *Chem. Rev.* **2009**, *109*, 796.

(9) (a) Ricardo, M. G.; Vicente, F. M.; Garay, H.; Reyes, O.; Wessjohann, L. A.; Rivera, D. G. *Org. Biomol. Chem.* **2015**, *13*, 438. (b) White, C. J.; Hickey, J. L.; Scully, C. C. G.; Yudin, A. K. *J. Am. Chem. Soc.* **2014**, *136*, 3728. (c) Scully, C. C. G.; Rai, V.; Poda, G.; Zaretsky, S.; Burns, D. C.; Houliston, R. S.; Lou, T.; Yudin, A. K. *Chem.—Eur. J.* **2013**, *19*, 17668. (d) Hili, R.; Rai, V.; Yudin, A. K. *J. Am. Chem. Soc.* **2010**, *132*, 2889.

(10) (a) 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. (b) Vercillo, O. E.; Andrade, C. K. Z.; Wessjohann, L. A. *Org. Lett.* **2008**, *10*, 205. (d) Rivera, D. G.; Vercillo, O. E.; Wessjohann, L. A. *Org. Biomol. Chem.* **2008**, *6*, 1787. (c) Rivera, D. G.; Wessjohann, L. A. *J. Am. Chem. Soc.* **2006**, *128*, 7122.

(11) Sewald, N.; Jakubke, H.-D. *Peptides: Chemistry and Biology*; Wiley-VCH: Mannheim, 2002.

(12) (a) Hamley, I. W. *Biomacromolecues* **2014**, *15*, 1543. (b) Marsault, E.; Peterson, M. L. *J. Med. Chem.* **2011**, *54*, 1961. (c) Hruby, V. J. *Nat. Rev. Drug Discovery* **2002**, *1*, 847.

(13) (a) Chatterjee, J.; Rechenmacher, F.; Kessler, H. *Angew. Chem., Int. Ed.* **2013**, *52*, 254. (b) Chatterjee, J.; Chaim, G.; Hoffman, A.; Kessler, H. *Acc. Chem. Res.* **2008**, *41*, 1331. (c) Wessjohann, L. A.; Andrade, C. K. Z.; Vercillo, O. E.; Rivera, D. G. *Targets Heterocycl. Syst.* **2006**, *10*, 24.

(14) (a) Banfi, L.; Guanti, G.; Riva, R.; Basso, A. *Curr. Opin. Drug Discovery Dev.* **2007**, *10*, 704. (b) Constabel, F.; Ugi, I. *Tetrahedron* **2001**, *57*, 5785. (c) Suda, A.; Sudoh, A.; Tsukuda, T.; Shimma, N. *Heterocycles* **2001**, *55*, 1023. (d) Oertel, K.; Zech, G.; Kunz, H. *Angew. Chem., Int. Ed.* **2000**, *39*, 1341. (e) Li, Z.; Yeo, S. L.; Pallen, C. J.; Ganesan, A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2443. (f) Hulme, C.; Peng, J.; Morton, G.; Salvino, J. M.; Herpin, T.; Labaudiniere, R. *Tetrahedron Lett.* **1998**, *39*, 7227. (g) Sutherlin, D. P.; Stark, T. M.; Hughes, R.; Armstrong, R. W. *J. Org. Chem.* **1996**, *61*, 8350.

(15) Morales, S.; Guijarro, F. G.; García Ruano, J. L.; Cid, M. B. *J. Am. Chem. Soc.* **2014**, *136*, 1082.

(16) López-Abarrategui, C.; McBeth, C.; Mandal, S. M.; Zhenyu, J. S.; Heffron, G.; Alba-Menéndez, A.; Migliolo, L.; Reyes-Acosta, O.; García-Villarino, M.; Nolasco, D.; Falcão, R.; Cherobim, M. D.; Dias, S. C.; Brandt, W.; Wessjohann, L.; Starnbach, M.; Franco, O. L.; Otero-González, A. J. *FASEB J.* **2015**, *29*, doi: 10.1096/fj.14-269860.

(17) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.

(18) Wilson, R. M.; Stockdill, J. L.; Wu, X.; Vadoa, P. A.; Park, P. K.; Wang, D.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2012**, *51*, 2834.

(19) (a) Rodríguez, H.; Suarez, M.; Albericio, F. *J. Pept. Sci.* **2010**, *16*, 136. (b) Teixidó, M.; Albericio, F.; Giralt, E. *J. Pept. Res.* **2005**, *65*, 153. (c) Falb, E.; Yechezkel, T.; Salitra, Y.; Gilon, C. *J. Pept. Res.* **1999**, *53*, 507. (d) Reichwein, J. F.; Liskamp, R. M. J. *Tetrahedron Lett.* **1998**, *39*, 1243. (e) Carpino, L. A.; Elfaham, A.; Albericio, F. *J. Org. Chem.* **1995**, *60*, 3561. (f) Tung, R. D.; Rich, D. H. *J. Am. Chem. Soc.* **1985**, *107*, 4342.

(20) Alsina, J.; Albericio, F. *Biopolymers (Pept. Sci.)* **2003**, *71*, 454.

(21) For related *N*-steroidal peptides derived from solution-phase Ugi reactions, see: (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) Rivera, D. G.; León, F.; Concepción, O.; Morales, F. E.; Wessjohann, L. A. *Chem.—Eur. J.* **2013**, *19*, 6417.

(22) Pérez-Labrada, K.; Brouard, I.; Mendez, I.; Rivera, D. G. *J. Org. Chem.* **2012**, *77*, 4660.

(23) Ugi, I.; Bodesheim, F. *Chem. Ber.* **1961**, *94*, 2797.

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