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Boosting Fmoc Solid-Phase Peptide Synthesis by Ultrasonication

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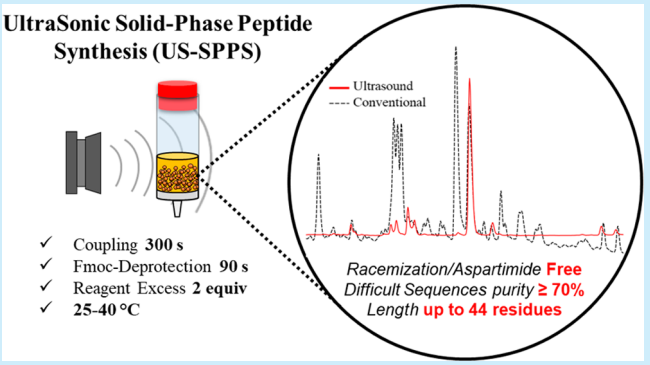
Supporting Information

ABSTRACT: We investigated the ultrasonication-mediated effects on the Fmoc-based solid-phase peptide synthesis (SPPS). Our study culminated with the development of an ultrasound-assisted strategy (US-SPPS) that allowed for the synthesis of different biologically active peptides (up to 44-mer), with a remarkable savings of material and reaction time. Noteworthy, ultrasonic irradiation did not exacerbate the main side reactions and improved the synthesis of peptides endowed with "difficult sequences", placing the US-SPPS among the current high-efficient peptide synthetic strategies.



The introduction of the solid-phase peptide synthesis (SPPS) by Merrifield[1] provided a tremendous contribution in understanding the potential of peptides in numerous scientific fields.[2−4] Based on the different strategies employed to protect the α-amino and the side-chain functional groups, two main SPPS approaches were established: the *tert*-butyloxycarbonyl/benzyl (Boc/Bzl) ones and the fluorenylmethoxycarbonyl/*tert*-butyl (Fmoc/*t*Bu) ones. Nowadays, most of the synthetic peptides are prepared by the Fmoc-SPPS, which allows the use of milder reaction conditions during the synthesis and the cleavage of peptides from the solid support.[5]

Nevertheless, the conventional Fmoc-SPPS is costly and time-consuming, and its performance can be affected by events such as sequence-dependent side-chain aggregations, occurring in the so-called "difficult sequences", which jeopardize the overall progress of the synthesis.[6] In this regard, a significant advancement came from the application of microwave heating (μW-SPPS), which reduces the occurrence of aggregation.[7] Unfortunately, most of the μW-SPPS protocols are efficient when high temperature is employed, limiting their use, in some cases.[8,9]

In parallel to the early reports of the μW-assisted reactions, numerous studies documented the use of ultrasonication (US) to activate reagents in both homogeneous and heterogeneous reactions, giving rise to a branch of chemistry called "sonochemistry".[10,11] The propagation of ultrasound waves in the fluids triggers the formation, growth, and implosion of bubbles, which is a phenomenon known as cavitation, which, in turn, produces a local increment of temperature and pressure.[10] Although the cavitation has been extensively studied in heterogeneous reactions, to the best of our knowledge, a very few examples of sonochemical-assisted transformations in SPPS have been reported to date.[12−15] To bridge this gap, in this work, we focused on the two main SPPS reaction steps: Fmoc-removal and amide bond formation, which are two examples of heterogeneous polar transformations. They are influenced by US mainly through a mechanical action (i.e., microstreams and dispersions),[16] which could substantially increase the number of efficient collisions between the reagents in solution and the resin-reactive sites, otherwise unachievable through conventional strategies (e.g., magnetic stirring and orbital shaking). First, considering that cavitation erodes the solid surface contacting the fluid,[10] we verified that the overall polymeric structure of the resin was not affected by US irradiation (see the Supporting Information). Moreover, ultrasonic propagation is also known to produce heat,[17] which could influence the reaction rate and eventually mislead the interpretation of the results. Thus, preliminary investigations that aimed to define optimal reaction time and reagent excess were conducted by setting the ultrasonic bath temperature at 25 °C and monitoring the temperature trend in the SPPS vessel.

The US effects on the Fmoc-removal were assessed on a functionalized Fmoc-L-Asp(O*t*Bu)-Rink amide-AM PS resin upon treatments with 20% pip/DMF solution (procedure A, ultrasonic irradiation; procedure B, mechanical shaking) at various reaction times (1−6; see Figure 1a). The yields were extrapolated by ultraviolet (UV) monitoring of the dibenzofulvene−piperidine adduct formation at 301 nm.

As reported in Figure 1a, the ultrasound-assisted treatments 1−4 yielded quantitative amine deprotection (>99%). As

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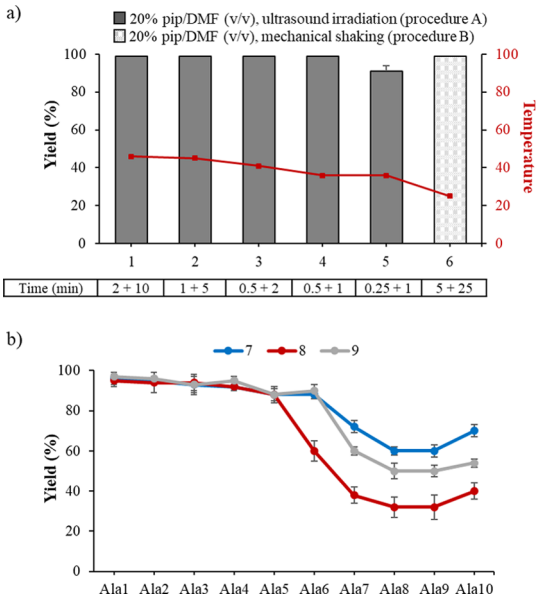

Figure 1. Fmoc-removal procedures: (A) 20% pip/DMF, ultrasonic irradiation, and (B) 20% pip/DMF, mechanical shaking. (a) Fmoc-removal yields and maximum temperature observed for each reaction time; (b) Fmoc-removal yields obtained by using 20% pip/DMF solution, via the following conditions: 0.5 + 1 min by ultrasonic irradiation (7) (denoted as condition i), 0.5 + 1 min by mechanical shaking (8) (denoted as condition ii), and 5 + 25 min by mechanical shaking (9) (denoted as condition iii). Experiments were performed in triplicate and the yields are expressed as a percentage (mean values ± standard error of measurement (SEM), $N = 3$).

expected, we observed a local moderate increment of temperature, with respect to the reaction times (see Figure 1a, as well as Table S1 in the Supporting Information). In the case of 3 and 4, the highest measured temperatures (40.0 and 35.1 °C, respectively) appeared significantly lower than those reported in the conventional or μW-heating SPPS procedures.[7,18] Noteworthy, US impacted the efficiency of Fmoc-deprotection, compared to treatments with 20% pip/DMF solution (7−9) during the synthesis of a 10-mer oligo-alanine peptide (Figure 1b), which is known to aggregate on solid supports.[6] Particularly, a progressive gap between the ultrasound-assisted and conventional performances arose from the sixth alanine, probably as consequence of the role of ultrasonication in reducing the peptide aggregation during the sequence growing. These results encourage the use of sonication during the Fmoc deprotection, especially in the synthesis of "difficult sequences".

Ideally, the application of an energy source to a chemical transformation should not prompt new undesired reactions or exacerbate the already-known ones. Hence, we started monitoring the base-related aspartimide formation during the 1−6 fragment of toxin II by *Scorpion Androctonus australis Hector* (VKDGYI) synthesis (Figure 2).[19] Three different deprotection protocols were employed: (a) 5 + 25 min, 20% pip/DMF solution, by mechanical shaking (entry 1); (b) 0.5 + 1 min, 20% pip/DMF solution, by ultrasonic irradiation (entry 2); and (c) 0.5 + 1 min, 20% pip/DMF solution added of 1% formic acid, by ultrasonic irradiation (entry 3).[20]

As depicted in Figure 2, the US process did not cause the formation of any additional undesired products, but especially led to a detectable decrement of the aspartimide byproduct (1 vs 2 and 3), promoting their use in aspartic acid-containing

t_R	MW calcd. $[M+H]^+$	MW found $[M+H]^+$	% peak		
			1	2	3
11.7	675.38	675.35	6.0	3.5	2.0

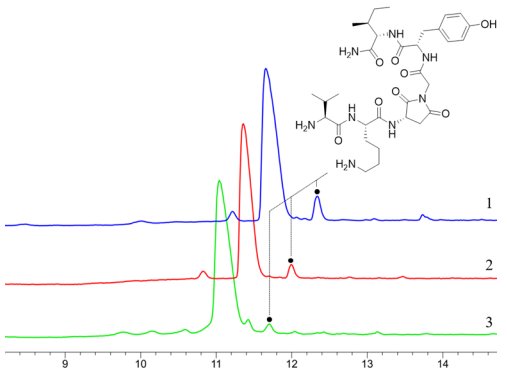

Figure 2. Comparison of chromatograms of 1−3. The main products purity was extrapolated considering the integration of peaks at t_R = 11.1 min.

peptides bearing a glycine as a flanking amino acid. As a result of all the reported studies, we adopted entry 4 (20% pip/DMF solution for 0.5 + 1 min) as standard US-assisted procedure for subsequent investigations.

To test ultrasonication on the amide bond formation, a model pentapeptide (Fmoc-KFRFD) was synthesized using a Rink amide-AM PS resin as a solid support and *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU)/1-hydroxybenzotriazole (HOBt) as a combination of activating/additive agents. The pentapeptides were assembled by varying the reaction times and stoichiometry of reactants. Again, during the synthesis, the temperature trend in the reaction vessels was monitored (Table 1). All peptides were then analyzed by HPLC (see Figures S11−S22 in the Supporting Information) to determine crude purity by peak integration.

Initially, the reaction time was reduced from 30 to 2 min (Table 1, entries 2−5) in the presence of a specific amount of reagents excess (4 equiv). As reported in Table 1, the ultrasonic treatments 2−4 enabled to efficiently accelerate the couplings, yielding higher final purities (≥90%), compared to the conventional synthesis (Table 1, entry 1). Next, we adopted the reaction time of entry 4 in the presence of 3, 2, and 1 reagent equiv (Table 1, entries 6−8) and with the sole exception of entry 8 (1 equiv), the syntheses resulted in final compounds with a high degree of purity (Table 1, entries 6 and 7). At this stage, taking into account the heat produced during the coupling steps (e.g., Table 1, entries 2−5), we asked whether or not the observed effects could be ascribed to the sole increment of temperatures. Thus, two experiments were designed for decoupling the roles of heat and sonication in accelerating the reaction rate. First, the model pentapeptide was synthesized by mechanical shaking at 45 °C (Table 1, entry 9) (the maximum temperature observed after 30 min coupling under US irradiation), adopting the reaction conditions used for entry 7. The comparison of the resulting chromatograms (7 vs 9; see Table 1) highlighted that the sole ultrasonication enhanced the crude purity by ∼25%. A further confirmation was provided by repeating the synthesis in the ultrasonic bath, thermostatically controlled by an external immersion cooler (Table 1, entries 10−12), which afforded the desired product with a purity comparable to entry 7, regardless the operational temperature. Altogether, these results provide

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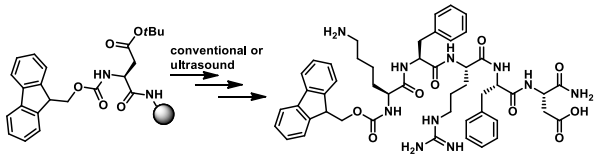
Table 1. Ultrasound-Assisted Couplings: Synthetic Protocols Used To Achieve the Model Pentapeptides[a]



entry	US irradiation	t (min)	equiv[b]	crude purity (%)	temperature (°C)[c]
1	no	60	4	84 ± 3	–
2	yes	30	4	93 ± 4	44.7 ± 0.9
3	yes	10	4	92 ± 3	41.8 ± 0.7
4	yes	5	4	90 ± 2	40.2 ± 1.2
5	yes	2	4	76 ± 4	38.0 ± 0.5
6	yes	5	3	89 ± 2	–
7	yes	5	2	88 ± 3	–
8	yes	5	1	65 ± 3	–
9	no	5	2	64 ± 5	45.0
10	yes	5	2	85 ± 2	13.7 ± 1.5[d]
11	yes	5	2	90 ± 3	24.7 ± 1.2[d]
12	yes	5	2	91 ± 4	29.0 ± 2.0[d]

[a]Fmoc-deprotection procedure B (20% pip/DMF, mechanical shaking, 0.5 + 1 min) was used for entries 1 and 9; Fmoc-deprotection procedure A (20% pip/DMF, ultrasonic irradiation, 0.5 + 1 min) was used for entries 2–8 and 10–12. [b]Equivalents are referred to Fmoc-aa–OH, HBTU/HOBt, DIEA (equiv × 2). [c]Maximum temperature of the reaction mixture observed (temperature at time zero was 25 °C). [d]Maximum temperature of the reaction mixture (temperature at time zero was 5, 10, and 15 °C for entires 10, 11, and 12, respectively). Peptide sequences were synthesized three times and crude purities are expressed as percentage (mean values ± standard error of measurement (SEM), N = 3).

Table 2. Cysteine and Histidine Racemization Study: Comparison between Conventional and US-Assisted SPPS

entry	sequence	US irradiation[a]	L-isomer (%)	D-isomer (%)
1	GCF	no	96.5	3.5
2	GCF	yes	94.5	5.5
3	GHF	no	97.7	2.3
4	GHF	yes	98.5	1.5
5	GcF	no	2.7	97.3
6	GcF	yes	2.5	97.5
7	GhF	no	0	100
8	GhF	yes	0	100

[a]A response of "no" indicates the following: Fmoc-deprotection procedure B (20% pip/DMF, mechanical shaking, 5 + 25 min); coupling Fmoc-aa–OH, HBTU/HOBt/DIEA 0.13 M, 60 min. A response of "yes" indicates the following: Fmoc-deprotection procedure A (20% pip/DMF, ultrasonic irradiation, 0.5 + 1 min); coupling Fmoc-aa–OH, HBTU/HOBt/DIEA 0.13 M, 5 min.

Table 3. Library of Described Biologically Relevant Sequences

entry	ID[a]	size	crude purity (%)	t (min)
1	Kisspeptin-10	10-mer	85	66.5
2	Angiotensin-I[b]	10-mer	92	66.5
3	α-MSH	13-mer	80	91.0
4	PEPITEM[c]	14-mer	81	92.5
5	p53-TAD$_{15-29}$	15-mer	82	99.0
6	γ-endorphin[b]	17-mer	62	112.0
7	PAMP$_{1-20}$	20-mer	80	131.5
8	VIP	28-mer	63	183.5
9	GRF	44-mer	51	287.5

[a]For peptide sequences, see the Supporting Information. [b]Synthesis performed on a preloaded Wang resin. [c]Synthesis performed on a preloaded 2-CTC resin.

direct evidence that ultrasonic irradiation allowed for a substantial reduction of reagent excess and reaction time. Hence, entry 7 was applied as protocol to screen the most common activating/additive agents (see the Supporting Information for details). Among them, the combination of US and 1-cyano-2-ethoxy-2-oxoethylidenaminooxy)-dimethylamino-morpholino-carbenium hexafluorophosphate (COMU)/ethyl cyano(hydroxyimino)acetate (Oxyma) enabled the synthesis of the test peptide with a purity of 94% ± 2% (see entry 7c in Table S3 in the Supporting Information) and, thus, it was considered for all the subsequent experiments, including that aimed at showing the effect of US on the amino acid racemization (Table 2). To address this issue, we adopted the method developed by Barany and co-workers for the evaluation of cysteine racemization, which was extended to histidine-containing peptides as well.[21] Eight tripeptides were synthesized by ultrasound-assisted or conventional strategies (see Table 2, entries 1–8).

The resulting chromatograms (Figures S29–S36 in the Supporting Information) revealed no significant increase of the racemization for both cysteine and histidine enantiomers, indicating that the sonication can be used without any specific precautions also to build sequences containing amino acid residues prone to racemization.

These results encouraged us to test the sonication effects during the synthesis of longer and more-complex peptides. Biologically relevant sequences (Table 3, entries 1–9), ranging from 10 to 44 residues,[22−30] were prepared by adopting the reaction conditions mentioned for entry 7c. For this study, we extended the use of US to the synthesis of CO_2H-terminal peptides (Table 3, entries 2, 4, and 6). As shown by the HPLC profiles (see the Supporting Information for details), all the crude peptides were obtained in good purity (see Table 3), thereby proving the reliability of the low-frequency sonication on the overall synthetic process, regardless the size of the peptides and the type of resin-bound linkers.

Next, two combination of reaction times and reagent equiv were probed for assembling a set of peptides known as "difficult sequences", such as the Aib-Enkephaline Aib-Enk (Y-Aib-Aib-FL),[31] the Acyl Carrier Protein fragment ACP$_{65-74}$ (VQAAIDYING),[18] the Jung–Redemann peptide JR 10-mer (WFTTLISTIM),[32] and the amyloid peptide Aβ 1–42 (DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA) (see Figure 3, as well as Table S4 in the Supporting Information).[32,33]

All the ultrasound-assisted syntheses produced final peptides endowed with a degree of purity that is comparable to those elsewhere reported with the use of μW.[31,33] The effects of US in the synthesis of the difficult sequences were also estimated through preparation of the peptides by stirring the resin at 45 °C and adopting the following reaction conditions: 20% pip/DMF, 0.5 + 1 min, for Fmoc-deprotections; and Fmoc-aa–OH (8 equiv), COMU/Oxyma (8 equiv), DIEA (16 equiv), 10 min, for couplings. The HPLC profiles of the so-obtained peptides (see the Supporting Information for chromatograms), showed a significant difference in terms of crude quality (~20% for Aib-Enk, ~31% for ACP$_{65-74}$, ~48% for JR 10-mer,

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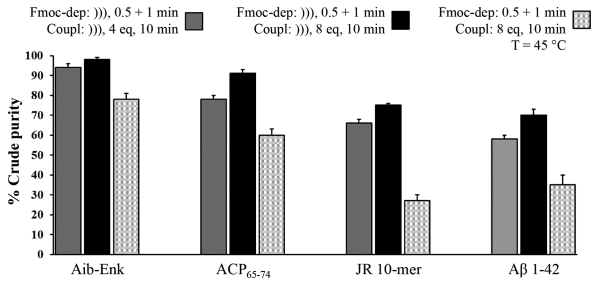

Figure 3. Optimization study for the synthesis of "difficult sequences": Aib-Enk, ACP_{65-74}, JR 10-mer, and Aβ 1−42 peptides.

and ∼35% for Aβ 1−42), representing strong evidence of the substantial effects of US, especially for aggregation-prone sequences.

In conclusion, the present study describes an unprecedented method for the SPPS (US-SPPS), which can be placed among the current highly efficient peptide synthetic ones. These data set the stage for extensively applying low-frequency US to SPPS, encouraging future studies to fully unveil the potential of their cooperation. For instance, the replacement of the solid supports, as well as the use of canonical and not canonical solvents for SPPS, could allow for probing the effects of different features, such as alternative polymeric composition and/or size of the beads and the solvent viscosity and density, on the US-SPPS performance. The optimization of these parameters will provide a powerful and accessible method not solely for the main peptide modifications, including the introduction of nonpeptidic moieties (e.g., fatty acids, nucleobases, fluorophores) and conformational constrains (e.g., cyclization, *N*-alkylation), but also for the solid-phase organic synthesis (SPOS), increasing the strategies for the synthesis of small molecules for medical and biological application.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b02283.

Detailed experimental procedures; additional optimization data; representative HPLC chromatograms and mass spectra of all described peptides (PDF)

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Author Contributions

All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Merrifield, R. B. Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. *J. Am. Chem. Soc.* **1963**, *85* (14), 2149−2154.
(2) Milroy, L. G.; Grossmann, T. N.; Hennig, S.; Brunsveld, L.; Ottmann, C. Modulators of protein-protein interactions. *Chem. Rev.* **2014**, *114* (9), 4695−4748.
(3) Hamley, I. W. Small bioactive peptides for biomaterials design and therapeutics. *Chem. Rev.* **2017**, *117* (24), 14015−14041.
(4) Henninot, A.; Collins, J. C.; Nuss, J. M. The current state of peptide drug discovery: back to the future? *J. Med. Chem.* **2018**, *61* (4), 1382−1414.
(5) Behrendt, R.; White, P.; Offer, J. Advances in Fmoc solid-phase peptide synthesis. *J. Pept. Sci.* **2016**, *22* (1), 4−27.
(6) Paradís-Bas, M.; Tulla-Puche, J.; Albericio, F. The road to the synthesis of "difficult peptides. *Chem. Soc. Rev.* **2016**, *45* (3), 631−654.
(7) Pedersen, S. L.; Tofteng, A. P.; Malik, L.; Jensen, K. J. Microwave heating in solid-phase peptide synthesis. *Chem. Soc. Rev.* **2012**, *41* (5), 1826−1844.
(8) Nissen, F.; Kraft, T. E.; Ruppert, T.; Eisenhut, M.; Haberkorn, U.; Mier, W. Hot or not—the influence of elevated temperature and microwave irradiation on the solid phase synthesis of an affibody. *Tetrahedron Lett.* **2010**, *51* (48), 6216−6219.
(9) Palasek, S. A.; Cox, Z. J.; Collins, J. M. Limiting racemization and aspartimide formation in microwave-enhanced Fmoc solid phase peptide synthesis. *J. Pept. Sci.* **2007**, *13* (3), 143−148.
(10) Mason, T. J. Ultrasound in synthetic organic chemistry. *Chem. Soc. Rev.* **1997**, *26* (6), 443−451.
(11) Puri, S.; Kaur, B.; Parmar, A.; Kumar, H. Applications of ultrasound in organic synthesis - a green approach. *Curr. Org. Chem.* **2013**, *17* (16), 1790−1828.
(12) Perez, J.; Wilhelm, E. J.; Sucholeiki, I. The use of power ultrasound coupled with magnetic separation for the solid phase synthesis of compound libraries. *Bioorg. Med. Chem. Lett.* **2000**, *10* (2), 171−174.
(13) Takahashi, S.; Shimonishi, Y. Solid phase peptide synthesis using ultrasonic waves. *Chem. Lett.* **1974**, *3* (1), 51−56.
(14) Bray, A. M.; Lagniton, L. M.; Valerio, R. M.; Maeji, N. Sonication-assisted cleavage of hydrophobic peptides. application in multipin peptide synthesis. *Tetrahedron Lett.* **1994**, *35* (48), 9079−9082.
(15) Anuradha, M.; Ravindranath, B. Ultrasound in peptide synthesis. 4: rapid cleavage of polymer-bound protected peptides by alkali and alkanolamines. *Tetrahedron* **1995**, *51* (19), 5675−5680.
(16) Cravotto, G.; Cintas, P. Power ultrasound in organic synthesis: moving cavitational chemistry from academia to innovative and large-scale applications. *Chem. Soc. Rev.* **2006**, *35* (2), 180−196.
(17) Legay, M.; Gondrexon, N.; Le Person, S.; Boldo, P.; Bontemps, A. Enhancement of heat transfer by ultrasound: review and recent advances. *Int. J. Chem. Eng.* **2011**, *2011*, 1−17.
(18) Varanda, L. M.; Miranda, M. T. M. Solid-phase peptide synthesis at elevated temperatures: a search for an optimized synthesis condition of unsulfated cholecystokinin-12. *J. Pept. Res.* **1997**, *50* (2), 102−108.

DOI: 10.1021/acs.orglett.9b02283
Org. Lett. XXXX, XXX, XXX−XXX

(19) Nicolás, E.; Pedroso, E.; Girald, E. Formation of aspartimide peptides in Asp-Gly sequences. *Tetrahedron Lett.* **1989**, *30* (4), 497−500.
(20) Michels, T.; Dölling, R.; Haberkorn, U.; Mier, W. Acid-mediated prevention of aspartimide formation in solid phase peptide synthesis. *Org. Lett.* **2012**, *14* (20), 5218−5221.
(21) Han, Y.; Albericio, F.; Barany, G. Occurrence and minimization of cysteine racemization during stepwise solid-phase peptide synthesis. *J. Org. Chem.* **1997**, *62* (13), 4307−4212.
(22) Ohtaki, T.; Shintani, Y.; Honda, S.; Matsumoto, H.; Hori, A.; Kanehashi, K.; Terao, Y.; Kumano, S.; Takatsu, Y.; Masuda, Y.; Ishibashi, Y.; Watanabe, T.; Asada, M.; Yamada, T.; Suenaga, M.; Kitada, C.; Usuki, S.; Kurokawa, T.; Onda, H.; Nishimura, O.; Fujino, M. Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature* **2001**, *411* (6837), 613−617.
(23) Peach, M. J. Renin-angiotensin system: biochemistry and mechanisms of action. *Physiol. Rev.* **1977**, *57* (2), 313−370.
(24) Hadley, M. E.; Dorr, R. T. Melanocortin peptide therapeutics: historical milestones, clinical studies and commercialization. *Peptides* **2006**, *27* (4), 921−930.
(25) Chimen, M.; Mcgettrick, H. M.; Apta, B.; Kuravi, S. J.; Yates, C. M.; Kennedy, A.; Odedra, A.; Alassiri, M.; Harrison, M.; Martin, A.; Barone, F.; Nayar, S.; Hitchcock, J. R.; Cunningham, A. F.; Raza, K.; Filer, A.; Copland, D. A.; Dick, A. D.; Robinson, J.; Kalia, N.; Walker, L. S. K.; Buckley, C. D.; Nash, G. B.; Narendran, P.; Rainger, G. E. Homeostatic regulation of T cell trafficking by a B cell-derived peptide is impaired in autoimmune and chronic inflammatory disease. *Nat. Med.* **2015**, *21* (5), 467−475.
(26) Chi, S.-W.; Lee, S.-H.; Kim, D.-H.; Ahn, M.-J.; Kim, J.-S.; Woo, J.-Y.; Torizawa, T.; Kainosho, M.; Han, K.-H. Structural details on MDM2-p53 interaction. *J. Biol. Chem.* **2005**, *280* (46), 38795−38802.
(27) Ling, N.; Burgus, R.; Guillemin, R. Isolation, primary structure, and synthesis of α-endorphin and γ-endorphin, two peptides of hypothalamic-hypophysial origin with morphinomimetic activity. *Proc. Natl. Acad. Sci. U. S. A.* **1976**, *73* (11), 3942−3946.
(28) Kitamura, K.; Kangawa, K.; Ishiyama, Y.; Washimine, H.; Ichiki, Y.; Kawamoto, M.; Minamino, N.; Matsuo, H.; Eto, T. Identification and hypotensive activity of proadrenomedullin N-terminal 20 peptide (PAMP). *FEBS Lett.* **1994**, *351* (1), 35−37.
(29) Nicole, P.; Lins, L.; Rouyer-Fessard, C.; Drouot, C.; Fulcrand, P.; Thomas, A.; Couvineau, A.; Martinez, J.; Brasseur, R.; Laburthe, M. Identification of key residues for interaction of vasoactive intestinal peptide with human VPAC1 and VPAC2 receptors and development of a highly selective VPAC1 receptor agonist. *J. Biol. Chem.* **2000**, *275* (31), 24003−24012.
(30) Ling, N.; Esch, F.; Bohlen, P.; Brazeau, P.; Wehrenberg, W. B.; Guillemin, R. Isolation, primary structure, and synthesis of human hypothalamic somatocrinin: growth hormone-releasing factor. *Proc. Natl. Acad. Sci. U. S. A.* **1984**, *81* (14), 4302−4306.
(31) Subiros-Funosas, R.; Acosta, G. A.; El-Faham, A.; Albericio, F. Microwave irradiation and COMU: a potent combination for solid-phase peptide synthesis. *Tetrahedron Lett.* **2009**, *50* (45), 6200−6202.
(32) Carpino, L. A.; Krause, E.; Sferdean, C. D.; Schümann, M.; Fabian, H.; Bienert, M.; Beyermann, M. Synthesis of 'difficult' peptide sequences: application of a depsipeptide technique to the Jung−Redemann 10- and 26-mers and the amyloid peptide Aβ(1−42). *Tetrahedron Lett.* **2004**, *45* (40), 7519−7523.
(33) Collins, J. M.; Porter, K. A.; Singh, S. K.; Vanier, G. S. High-efficiency solid phase peptide synthesis (HE-SPPS). *Org. Lett.* **2014**, *16* (3), 940−943.

DOI: 10.1021/acs.orglett.9b02283
Org. Lett. XXXX, XXX, XXX−XXX